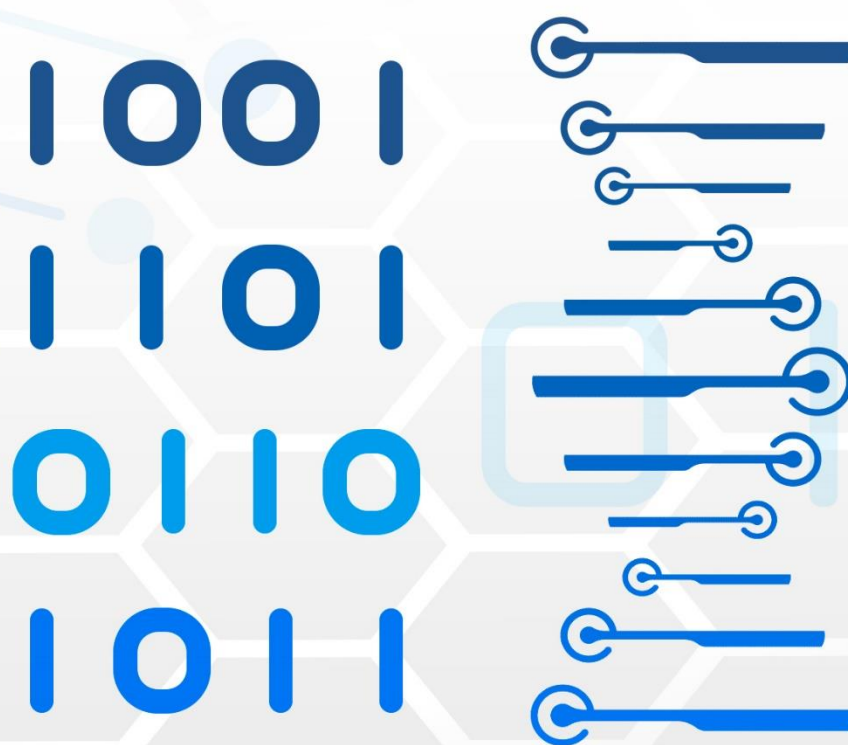


XXXII Symposium on Bioinformatics
and Computer-Aided Drug Discovery

PROCEEDINGS BOOK



Institute of Biomedical Chemistry
Moscow, Russia (Virtual), October 19-21 2026

**Russian Academy of Sciences
Ministry of Science and Higher Education of Russian Federation
Institute of Biomedical Chemistry
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry
of the Russian Academy of Sciences**

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PROCEEDINGS BOOK OF THE XXXII SYMPOSIUM
“BIOINFORMATICS AND COMPUTER-AIDED DRUG
DISCOVERY” – Moscow: Institute of Biomedical Chemistry, 2026

The materials of the XXXII International Symposium “Bioinformatics and Computer-Aided Drug Discovery” (Virtual, October 19–21, 2026) are presented. The Symposium is dedicated to addressing the emerging challenges and opportunities in *in silico* drug discovery. It covers contemporary areas of biomedical science focused on analyzing normal and pathological states of the organism and elucidating pathological processes at the cellular and molecular levels.

The main topics include the development and practical application of computational methods for identifying and validating new pharmacological targets, *in silico* design of potent and safe pharmaceutical agents, optimization of the structure and properties of drug-like compounds, and rational approaches to the use of pharmacotherapeutic agents in medical practice.

This information will be valuable to researchers developing computational methods and applying them to drug research and development using bioinformatics and chemoinformatics approaches based on post-genomic technologies. It may also be of interest to undergraduate, graduate, and postgraduate students specializing in related fields.

Responsible editors: Member of Rus. Acad. Sci. V.V. Poroikov, Prof. R.G. Efremov



Dear Colleagues,

on behalf of the Organizing Committee and the Administration of the Institute of Biomedical Chemistry, it is my great pleasure and a heartfelt honor to welcome you to the XXXII Symposium on Bioinformatics and Computer-Aided Drug Discovery (BCADD-2026).

It is especially moving to open this Symposium as we continue a tradition that began in 1995, when my teacher, Alexander Archakov, Member of the Russian Academy of Sciences (RAS), initiated it as part of the “Man and Drugs” Congress. Since then, it has been held annually under the leadership of Vladimir Poroikov (Member of the RAS), a leading Russian scientist in digital pharmacology and computer-aided drug discovery. The event was co-organized by Nikolay Zefirov (Member of the RAS) and, since 2018, jointly with Professor Roman Efremov. Their dedication reminds us how much of science is built not only on ideas, but on generosity, mentorship, and friendship.

BCADD-2026 highlights the enduring importance and rapid progress of biomedical science in the discovery of new medicines. Large-scale biomedical data analysis, advances in artificial intelligence and machine learning, and the development of modern bioinformatics and cheminformatics methods are laying the groundwork for a deeper understanding of pathological processes and the identification of promising biomarkers and therapeutic targets. The widespread adoption of *in silico* approaches not only saves time and resources in the search for safer, more effective drugs but also provides a robust foundation for integrating existing knowledge and generating new insights in this multidisciplinary field.

More than 300 researchers from 40 countries have registered to participate in the BCADD-2026 Symposium. A total of 52 abstracts have been submitted for 24 oral presentation slots, while 49 abstracts and posters have been submitted for 18 flash presentation slots in the Young Scientists Contest. The International Scientific Committee, comprising distinguished scientists from Brazil, Germany, Greece, India, Israel, Mexico, and Russia, has evaluated the submissions and selected the most promising candidates.

I especially welcome the young scientists taking part in the Young Scientists Contest. You are not only the future of this field, you are already its living present, and your courage and curiosity give us all hope.

To foster better communication and overcome time zone challenges, the Organizing Committee has introduced an extended online E-Poster Session, which will run for 20 days. This format offers greater flexibility for our international participants.

The Symposium brings together researchers from across the globe to share pioneering ideas, address pressing challenges in bioinformatics, cheminformatics, medicinal chemistry, and pharmacology, and forge collaborations that will shape the future of these fields. But behind every dataset, every algorithm, every molecule, there are people: mentors who believed in us, colleagues who became friends, and patients who wait for safer and more effective medicines. Your presence, from 40 countries and across many time zones, reminds us that our community is united not only by professional interests but by hope, curiosity, and care for others. This is what makes our work truly meaningful.

By honoring the Symposium's traditions while embracing new perspectives, we strengthen a shared commitment to scientific excellence and build a foundation for future discoveries. I extend my heartfelt thanks to all participants. May your lectures be inspiring, your discussions stimulating, and your connections sincere and lasting. And may you leave this Symposium with not only new ideas and partnerships, but also with warmth in your hearts and the quiet joy of belonging to a community that works for the good of people.

With warmest wishes and deep gratitude,

Director of the Institute of Biomedical Chemistry,
Corresponding Member of the Russian Academy of Sciences



Elena Ponomarenko



Dear Colleagues!

We are delighted to welcome you to the XXXII Symposium “Bioinformatics and Computer-Aided Drug Discovery” (BCADD-2026).

Our annual symposium series provides a forum for exploring emerging challenges and opportunities in drug discovery and development through modern in silico technologies. Since its inception in 1995, the series has brought together researchers to advance scientific dialogue at the interface of bioinformatics, computational chemistry, and pharmaceutical research.

The inaugural symposium was held as part of the Second Russian National Congress “Man and Drugs.” The initiative was launched by Academician Alexander Archakov, with Professor Vladimir Poroikov serving as co-chair. Professor Alexis Ivanov made an invaluable contribution to the organization of the earliest symposia.

From 1996 to 2017, the symposia were co-chaired by Academician Nikolay Zefirov and Professor Vladimir Poroikov. Professor Oleg Raevsky also played an important role in the subsequent development of the series and spearheaded the establishment of the Russian Section of the International QSAR Society.

Since 2018, Professors Vladimir Poroikov and Roman Efremov have jointly led the organization of the symposia, continuing this longstanding tradition of scientific exchange and collaboration.

Many world-wide famous researchers presented their lectures at the past symposia including Per Artursson (Uppsala University, Sweden), Igor Baskin (Lomonosov Moscow State University, Russia), Artem Cherkasov (University of British Columbia, Canada), Alexey Egorov (Lomonosov Moscow State University, Russia), Frank Eisenhaber (A*STAR Bioinformatics Institute, Singapore), Alexey Finkelstein (Institute of Protein Research, Russia), Viktor Finn (VINITI, Russia), Alexander Gabibov (Institute of Bioorganic Chemistry, Russia), Mikhail Gelfand (Institute for Information Transmission Problems, Russia), Jerome Golebiowski (CNRS GDR “Odorant Odor Olfaction”, France), Viktor Kuzmin (Bogatsky Physico-Chemical Institute, Ukraine), José Medina-Franco (National Autonomous University of Mexico, Mexico), Alexander Nemukhin (Lomonosov Moscow State University, Russia), Kyoung Tai No (Yonsei University, Republic of Korea), Oleg Raevsky (Institute of Physiologically Active Compounds, Russia), Narahari G. Sastry (CSIR-North East Institute of Science and Technology, India), Hanoch Senderowitz (Bar-Ilan University, Israel), Alexander Sobolevsky (Columbia University, New York, USA), Oliver Steck and Andreas Vitte (Tropis, Germany), Igor Tetko (Institute of Structural Biology, Helmholtz Zentrum München, Germany), Vladimir Tumanyan (Institute of Molecular Biology, Russia), Alexandre Varnek (University of Strasbourg, France), Gennady Verkhivker (Chapman University, Irvine, USA), Erik Weber (Environmental Protection Agency, USA), Robert Woods (University of Georgia, Athens, USA), and others.

The upcoming XXXII Symposium will bring together established experts and emerging researchers from across the world for an inspiring program of plenary and keynote lectures and oral presentations. Speakers will represent Algeria, Argentina, Bangladesh, Brazil, China, Costa Rica, Germany, India, Iran, Italy, Japan, Mexico, Morocco, Peru, Poland, Portugal, Russia, Serbia, Spain, South Africa, Sweden, the United Kingdom, and the United States. The scientific program will span a broad range of topics, highlighting advances in the development and application of in silico methods for drug discovery and development.

The traditional Young Scientists Contest (YSC) has attracted considerable interest, with 49 abstracts submitted by undergraduate and graduate students, as well as researchers under the age of 30 who do not yet hold a scientific degree. The submissions were evaluated by the members of the International Scientific Committee (ISC), including distinguished scientists from Brazil, Germany, India, Israel, Mexico, and Russia. Based on

the ISC members' votes, and with due consideration given to the geographical diversity of participants, 18 abstracts have been selected for flash presentations. The most outstanding presentations will receive first-, second-, and third-place diplomas.

To foster broader participation and sustained scientific exchange, the Organizing Committee has arranged an extended E-Poster Session spanning 20 days, rather than the conventional two to three hours. This format accommodates the demanding schedules of researchers and bridges time-zone differences across continents, giving participants more opportunities to explore the posters and engage in meaningful discussion. Outstanding posters will be recognized with special diplomas.

The Symposia on Bioinformatics and Computer-Aided Drug Discovery are organized by scientists, for scientists. No commercial entities are involved in organizing the meetings, and participation is free of registration fees.

We invite all participants to use the Symposium's discussion platform to exchange original scientific ideas, share innovative methodological approaches, and explore potentially transformative multidisciplinary technologies. Such dialogue is especially valuable in a global environment that increasingly challenges international scientific and educational cooperation and the effective exchange of information and data. Open communication and collaboration remain fundamental to scientific creativity and progress, particularly in biomedical research.

Recent years have underscored the urgent challenges posed by emerging and re-emerging infectious diseases, most notably the COVID-19 pandemic caused by SARS-CoV-2. These threats have reinforced the need for coordinated international action and highlighted the important contributions of bioinformatics and computer-aided drug design to the search for effective interventions. Against this backdrop, the scientific themes at the heart of our Symposia have become increasingly relevant - not only to advancing drug discovery, but also to strengthening our collective capacity to respond to global health challenges.

By bringing together scientists from many countries, our Symposium seeks to strengthen scientific diplomacy, sustain and deepen professional and personal ties, and inspire new collaborations across disciplines and borders. Through this exchange of knowledge, ideas, and expertise, we aim to advance the discovery of new medicines and unlock the full potential of computational approaches to drug discovery. We also hope that the Symposium will contribute, in its own way, to mutual understanding and dialogue at a time of global tension. Its online format broadens opportunities for international participation and enables distinguished colleagues from around the world to share their insights with our scientific community.

Welcome to the XXXII Symposium on Bioinformatics and Computer-Aided Drug Discovery. We wish you inspiring lectures, stimulating discussions, and fruitful exchanges that lead to lasting connections and new scientific opportunities!



Vladimir Poroikov

Member of the Russian

Academy of Sciences, Prof. Dr.



Roman Efremov

Prof. Dr.

Scientific Program

XXXII Symposium on Bioinformatics and Computer-Aided Drug Discovery (BCADD-2026)

Scheduled time - Moscow (UTC+3)

Chairpersons: Vladimir Poroikov and Roman Efremov	
Monday October 19, 2026	
08:30	Opening of the Symposium
Plenary lectures	
09:00	MACHINE LEARNING AND AI APPROACHES IN BIOCATALYSIS AND IMMUNOCHEMISTRY Alexander G. Gabibov Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia 
Oral presentations	
10:00	PREDICTION OF T-CELL EPITOPES INTERACTIONS WITH MAJOR HISTOCOMPATIBILITY COMPLEX II USING THEIR STRUCTURAL FORMULAS Anton Smirnov Pirogov Russian National Research Medical University, Moscow, Russia 
10:20	MOLECULAR MODELING OF INTERACTIONS BETWEEN S-GATIFLOXACIN ANTIBODY AND DIFFERENT FLUOROQUINOLONES Yulia Meteleshko Chemistry Department, M.V. Lomonosov Moscow State University, Moscow, Russia 
10:40	COMPUTATIONAL TARGETING OF IMMUNE AND GLYCO-IMMUNE CHECKPOINTS IN CAR T-CELL THERAPY Patricia Sobral NOVA FCT, Caparica, Lisbon, Portugal 
Keynote lectures	
11:00	A DYNAMIC QSPR FRAMEWORK FOR UNDERSTANDING DRUG RESISTANCE Maria Khrenova Department of Chemistry, M. V. Lomonosov Moscow State University, Moscow, Russia 

11:30	GENERATIVE DRUG DESIGN: FROM FRAGMENT SEEDS TO SMALL MOLECULE LEADS Jordi Mestres Chemotargets, Barcelona, Spain 
Oral presentations	
12:00	GENOMIC AND THERAPEUTIC INSIGHTS INTO MULTIDRUG-RESISTANT VIBRIO PARAHAEMOLYTICUS BD001 STRAIN ISOLATED FROM SHRIMP (PENAEUS MONODON) AHPND: IN VITRO, IN VIVO, AND IN SILICO APPROACHES Md Iftehimul Institute of Biotechnology, Bangladesh Agricultural University, Chapai Nawabganj, Bangladesh 
12:20	A MACHINE LEARNING-DRIVEN BIOPROSPECTING OF CHOLINERGIC MEDICINAL PLANTS FOR PUTATIVE LEADS AGAINST BUTYRYLCHOLINESTERASE TOWARD ALZHEIMER'S CARE Gyebi Gideon Durban University of Technology, Durban, South Africa 
12:40	ELUCIDATING THE MOLECULAR CROSSTALK BETWEEN PARKINSON'S DISEASE AND LEVODOPA-INDUCED DYSKINESIA: A NETWORK PHARMACOLOGY APPROACH Anchal Arora Punjabi University, Patiala, India 
lunch break 13:00-15:00	
Chairpersons: Jose Medina-Franco and Oxana Galzitskaya	
Keynote lectures	
15:00	AN INTERPRETABLE MACHINE LEARNING FRAMEWORK FOR ADVERSE DRUG REACTION PREDICTION FROM DRUG-TARGET INTERACTIONS Alan M. Jones School of Pharmacy, University of Birmingham, Birmingham, UK 
15:30	AI IN DRUG TARGET DISCOVERY: HOPES, HYPE, AND REALITY Alexander Kel geneXplain GmbH, Wolfenbuettel, Germany 
Oral presentations	
16:00	EXPLAINABLE AI GUIDES LEAD OPTIMIZATION OF HUMAN DHFR INHIBITORS USING A PUBCHEM FINGERPRINT-BASED QSAR MODEL Ibrahim Maattallaoui Abdelmalek Essaadi University, Tangier, Morocco 

16:20	ACTION-BASED HYBRID KNOWLEDGE GRAPH LEARNING FOR DRUG REPURPOSING AND FUTURE DIRECTIONS TOWARD EXPLAINABLE AI Marzieh Khodadadi AghGhaleh Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran 
16:40	DIRECTED IN-SILICO SEARCH FOR SPECIFIC BINDING WARHEADS TARGETING CEREBLON-CONTAINING E3 LIGASES Ekaterina Grigoreva Moscow Institute of Physics and Technology (National Research University), Moscow, Russia 
Keynote lectures	
17:00	TREATING SOLUTE POLARIZATION IN MD SIMULATIONS USING PRE-POLARIZED ATOMIC CHARGES: CARBOHYDRATES Robert Woods Complex Carbohydrate Research Center, University of Georgia, Athens, USA 
17:30	CONTEXT-AWARE BIOACTIVITY MODELING: FROM REPRESENTATION TO PREDICTIVE SPACE Karina Martinez Mayorga National Autonomous University of Mexico, Mexico City, Mexico 
Oral presentations	
18:00	INTEGRATING PASS, LLM ANALYSIS, AND MOLECULAR DOCKING TO UNCOVER A TARGET SWITCH IN PYRIMIDINE DERIVATIVES Tatyana Grigoreva St. Petersburg State Institute of Technology (Technical University), St. Petersburg, Russia 
18:20	LITBIOME: AN INTEGRATED WEB SERVICE FOR BIOMEDICAL TEXT MINING Nadezhda Biziukova Institute of Biomedical Chemistry, Moscow, Russia 
18:40	AN INTEGRATED BIBLIOMETRIC AND HUMAN TRANSCRIPTOMIC WORKFLOW FOR DRUG REPURPOSING IN PARKINSON'S DISEASE Saahithi Poluri Round Rock High School, Round Rock, USA 

Tuesday October 20, 2026

Chairpersons: Rajesh Goel and Dmitry Shulga

Keynote lectures

09:00	TOWARDS DATA-DRIVEN DESIGN OF VISIBLE-LIGHT PHOTOSWITCHES AS PHOTOPHARMACOLOGICAL AGENTS Pavel Sidorov Hokkaido University, Japan 
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09:30	LIGAND-MEDIATED CONFORMATIONAL PLASTICITY OF THE PORE DOMAIN OF TRPV6 ION CHANNEL Irina I. Veretenenko Research Institute for Systems Biology and Medicine, Moscow, Russia 
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Oral presentations

10:00	SPELL: AN INTERACTIVE PLATFORM FOR STRUCTURE-GUIDED LIGAND DESIGN AND FRAGMENT REPLACEMENT Daria Ryabchenko Ligand Pro, Moscow, Russia 
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10:20	THE NATURE OF NON-ARRHENIUS KINETICS IN THE HEAT DENATURATION OF PROTEINS Alexey Baklanov Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russia 
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10:40	PREDICTOR-GUIDED DE NOVO DESIGN OF NDM-1 INHIBITORS FEATURING REWARD-DRIVEN ZINC-BINDING MOTIFS Ivan Burov Lomonosov Moscow State University, Moscow, Russia 
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11:00	A POCKET-CENTRIC TRAIN-TEST SPLIT REDUCES DATA LEAKAGE IN STRUCTURE-BASED DEEP LEARNING Sergei Evteev P. Hertsen Moscow Oncology Research Institute, Moscow, Russia 
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11:20	STRUCTURE-BASED PRIORITIZATION OF DIKETOPIPERAZINE SCAFFOLDS FOR CLASS A GPCR RECOGNITION Sepideh Jafari Institute of General and Ecological Chemistry, Faculty of Chemistry, University of Technology, Lodz, Poland 
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11:40	INTEGRATIVE QSAR, STRUCTURAL BIOPHYSICS, AND NETWORK PHARMACOLOGY PIPELINE FOR MULTITARGET ANTICANCER DRUG DISCOVERY Abdelouahab Dehimat University of M'sila, M'sila, Algeria 
Keynote lectures	
12:00	LIGAND-RECEPTOR INTERACTION INSIGHTS FROM IN SILICO REVERSE FRAGMENT BASED DRUG DISCOVERY (R-FBDD) FOR KNOWN DRUGS AND LEAD COMPOUNDS Dmitry Shulga Department of Chemistry, M. V. Lomonosov Moscow State University, Moscow, Russia 
12:30	CRUSH: A CHEMISTRY-AWARE MOLECULAR FRAGMENTATION ALGORITHM FOR EXPANDED CHEMICAL SPACE EXPLORATION Edgar Lopez-Lopez DIFACQUIM Research Group, Department of Pharmacy, School of Chemistry, National Autonomous University of Mexico, Mexico; Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden 
lunch break 13:00-15:00	
Chairpersons: Athina Geronikaki and Alexey Lagunin	
Young Scientists flash presentations	
15:00	INTEGRATED QSAR RISK ASSESSMENT AND TRANSCRIPTOMIC EVALUATION OF CHEMICAL SAFETY: APPLICATIONS TO FOOD CONTACT SUBSTANCES AND CHRONIC ULTRA-LOW-DOSE ROUNDUP EXPOSURE Ankur Kumar Jadavpur University, Kolkata, India 
15:10	COMPUTATIONAL DESIGN OF TRASTUZUMAB CONJUGATES WITH HIGH CHELATOR-TO-ANTIBODY RATIOS Anastasiya Elizarova HSE University, St. Petersburg, Russia 
15:20	MECHANISTIC DECODING OF TARGET PHOTOTOXICITY: A COMPUTATIONAL STUDY POWERED BY ESIGNS Sofia Sousa Almeida NOVA FCT, Lisbon, Portugal 

15:30	COMPARATIVE EXPERIMENTAL AND COMPUTATIONAL ANALYSIS OF AAV9 AND AAVRH.10 TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER Viktoria Feoktistova ITMO University, St. Petersburg, Russia 
15:40	COMPUTATIONAL IDENTIFICATION OF STRUCTURAL DETERMINANTS ASSOCIATED WITH TRANSESTERIFICATION ACTIVITY IN MICROBIAL LIPASES Sahana Harisha School of Life Sciences Mysuru, JSS Academy of Higher Education & Research, Karnataka, India 
15:50	THE YTHDC1 GLUTAMATE-RICH DOMAIN DOCKS TO THE ADAR1 ZA DOMAIN, LINKING THE N6-METHYLADENOSINE MODIFICATION OF PRE-MRNAS TO DSRNA EDITING Dmitrii Gromak HSE University, Moscow, Russia 
16:00	WHICH OXIRANE CARBON DOES ASP12 ATTACK? QM/MM FREE-ENERGY PROFILES OF THE COVALENT INHIBITION OF KRAS(G12D) BY YK-8S Ekaterina Ilyukhina Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia 
16:10	CHEMOMETRIC MODELLING FOR TOXICITY ASSESSMENT OF DIVERSE CHEMICALS IN STONEFLY: A STEP TOWARDS ENHANCED RISK EVALUATION Disha Mahapatra Jadavpur University, Kolkata, India 
16:20	CHATGPT-LIKE TOOLS IN BIOINFORMATICS: ARCHITECTURAL APPROACHES AND FUNCTIONALITY OF EXPASYGPT, STRING-CHAT, AND MEGA-GPT Yana Syrovatskaya Sechenov First Moscow State Medical University, Moscow, Russia 
16:30	THE COMPARATIVE ANALYSIS OF 2D AND 3D STRUCTURAL REPRESENTATIONS FOR AI-QSAR MODELING OF HUMAN CYP24A1 INHIBITORS Alexandra Yakusheva Belarusian State University, Minsk, Belarus 
16:40	DIMENSIONALITY REDUCTION AND CLUSTERING FRAMEWORK FOR MAPPING HYDROLASE ACTIVE SITE DYNAMICS Mikhail Kuznetsov Lomonosov Moscow State University, Moscow, Russia 

16:50	C3-FUNCTIONALIZED ANDROSTANE HYDRAZONO-HYDRAZIDES: NEW CANDIDATES FOR AKR1C3 INHIBITION Djordje Jankovic University of Novi Sad, Novi Sad, Serbia 
17:00	PCA-BASED ASSESSMENT OF CONFORMATIONAL ENSEMBLES OF AChE AND BChE FOR SELECTIVE CHOLINESTERASE INHIBITOR SEARCH Elizaveta Shikina Lomonosov Moscow State University, Moscow, Russia 
17:10	ASSAY-AWARE LEARNING-TO-RANK FOR ROBUST MOLECULAR ACTIVITY PREDICTION FROM HETEROGENEOUS BIOACTIVITY DATA Luis Melo Federal University of Paraiba, João Pessoa, Brazil 
17:20	RETRIEVAL-AUGMENTED LLM SYSTEMS FOR BIOMEDICAL QUESTION ANSWERING AND PREDICTION INTERPRETATION Rustam Taktashov Institute of Biomedical Chemistry, Moscow, Russia 
17:30	IN SILICO EVALUATION OF MULTITARGET CANDIDATES AS POTENTIAL INHIBITORS OF ESSENTIAL DENGUE VIRUS TYPE 2 (DENV-2) PROTEINS USING MOLECULAR DOCKING, QUANTUM CHEMISTRY AND MOLECULAR DYNAMICS Andrey Justin Mayo Cesar Universidad Científica del Sur, Lima, Peru 
17:40	MANIA: A REPRODUCIBLE RESIDUE INTERACTION NETWORK PIPELINE FOR MOLECULAR DYNAMICS ANALYSIS OF THE TUMOR-ASSOCIATED TRANSPORTER NaPi2b (SLC34A2) Andrey Illarionov Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia 
17:50	DESIGN OF POTENTIAL DNA METHYLTRANSFERASE 1 INHIBITORS USING GENERATIVE MODELS Erik Diaz-Dionisio DIFACQUIM Research Group, School of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico 
Keynote lectures	
18:00	MACHINE LEARNING METHODOLOGIES AND THE FUTURE OF DRUG DISCOVERY Pedro J. Ballester Department of Bioengineering, Imperial College London, London, UK 

18:30	ACCELERATING BIOLOGICAL DISCOVERY WITH AI Cesar de la Fuente-Nunez University of Pennsylvania, USA 
Wednesday October 21, 2026	
Chairpersons: Kunal Roy and Irina Panina	
Keynote lectures	
09:00	ATOMISTIC INSIGHTS INTO A LANTIBIOTIC'S MECHANISM: AN INTEGRATED MOLECULAR DYNAMICS AND BIOPHYSICAL STUDY Irina Panina Research Institute for Systems Biology, Rospotrebnadzor, Moscow, Russia 
09:30	DELTA: DATABASE FOR EXTENDED LIGAND-TARGET ANALYSES - A NEW BALANCED RESOURCE FOR AI APPLICATIONS IN DRUG DISCOVERY Giulio Vistoli Università degli Studi di Milano, Milano, Italy 
Oral presentations	
10:00	BIOMARKER-GUIDED Q-RASAR MODELING OF ANTIBIOTICS-MICROPLASTICS MIXTURES: TOWARDS PREDICTIVE AQUATIC TOXICOLOGY AND RESISTANCE RISK ASSESSMENT Shubha Das Jadavpur University, Kolkata, India 
10:20	ADMETOX-AI: MULTI-TASK DEEP LEARNING PLATFORM FOR COMPREHENSIVE ADMET PROPERTY PREDICTION Rodion Bykadorov ADMETox.AI, Rostov-on-Don, Russia 
10:40	MULTIVARIATE QSAR MODELING FOR IDENTIFYING STRUCTURAL DRIVERS AND PRIORITIZING TOXIC CHEMICALS AFFECTING CRASSOSTREA VIRGINICA Prodipta Bhattacharyya Jadavpur University, Kolkata, India 
Keynote lectures	
11:00	AI-BASED REDESIGN OF A PERTUSSIS TOXIN PT-A PROTEIN FOR A VACCINE USE Anton Chugunov Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia 
11:30	METRICS FOR UNBIASED SELECTION OF MACHINE LEARNING REGRESSION MODELS

	Kunal Roy Jadavpur University, India 
Oral presentations	
12:00	IMPROVING THE ACCURACY AND GENERALIZABILITY OF MOLECULAR PROPERTY REGRESSION MODELS WITH A SUBSTRUCTURE-SUBSTITUTION-RULE-INFORMED FRAMEWORK Boxue Tian Tsinghua University, Beijing, China 
12:20	A WORKFLOW FOR BUILDING (Q)SAR MODELS WITH IMBALANCED AND HETEROGENEOUS DATA Leonid Stolbov Institute of Biomedical Chemistry, Moscow, Russia 
12:40	ARTIFICIAL INTELLIGENCE IN DRUG DISCOVERY: FROM DRUG-TARGET INTERACTIONS AND SYNERGISTIC DRUG COMBINATIONS TO GENERATIVE MOLECULAR DESIGN Sajjad Gharaghani Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran 
lunch break 13:00-16:00	
Chairpersons: Roman Zubarev and Dmitry Osolodkin	
Keynote lectures	
16:00	LIPOPHILICITY IN DRUG DESIGN: EVOLUTION FROM THE RULE OF FIVE TO AI-DRIVEN DISCOVERY William Zamora University of Costa Rica, San Pedro, San José, Costa Rica; National Advanced Computing Collaboratory (CNCA), National High Technology Center (CeNAT), Costa Rica 
16:30	DATASET CONSTRAINTS: A CASE OF PHARMACOLOGICALLY STRATIFIED MULTI-TARGET QSAR BENCHMARK FOR OPIOID RECEPTOR FUNCTIONAL ACTIVITY Khaled M. Elokely Division of Pharmaceutical Sciences, School of Pharmacy, University of Wyoming, United States 
17:00	GRAND CHALLENGES FOR PREDICTIVE MODELING IN SMALL MOLECULE DRUG DISCOVERY Woody Sherman Psivant Therapeutics, Boston, MA, United States 
17:30	WEB SERVICES FOR IN SILICO TOXICITY PREDICTION IN PRECLINICAL DRUG DEVELOPMENT

	Alexey A. Lagunin Institute of Biomedical Chemistry, Moscow, Russia 
Plenary lectures	
18:00	DECRYPTING DRUG MECHANISM OF ACTION BY PROTEOMICS Bernhard Küster Technical University of Munich, Freising, Germany 
19:00	Closure of the XXXII Symposium on Bioinformatics and Computer-Aided Drug Discovery

PLENARY LECTURES

MACHINE LEARNING AND AI APPROACHES IN BIOCATALYSIS AND IMMUNOCHEMISTRY

A. Gabibov

IBCH RAS RU, Moscow, Russia

The AI and ML approaches became powerful tools for improving biocatalitical processes. Predicting the substrate reactivity strength for a given biocatalyst remains a central challenge in computational biocatalysis. Here, we present Subdate, a modular workflow that combines descriptor-guided organization of substrate analogs with ab initio metadynamics simulations to prioritize reactive candidates. The workflow integrates i) substrate library construction, ii) conformer generation and descriptors set definition, iii) library clustering, iv) representative-substrate selection, and v) reaction-barrier quantitative prediction. Applied to selected biocatalysts (human butyrylcholinesterase and the catalytic antibody A17) sharing an SN2 reaction mechanism, Subdate quantitatively identifies reactivity trends that match experimental kinetic measurements. The developed workflow provides a mechanism-aware strategy for reactive substrate prioritization for efficient sampling through the chemical library in biocatalysis. This approach was extended for manipulation with protein matrix, enzymes and abzymes. At the first stage we succeeded in using ML approach to make effective virus-neutralizing antibodies. Despite substantial progress in single-cell screening techniques, antibody (Ab) repertoire still remains enigmatic. Here we show that Ab sequences can be linked to their functionality by using big data obtained from high-throughput sequencing. Using the expansive SARS-CoV-2 pandemic data, we develop an AI-based method to reveal the potential of Ab repertoires. We employ machine learning to process public 3D structural data of Ab-RBD complexes and create a comprehensive tool, RBD-AIM (<https://rbdaim.2a2i.org/>), for high-throughput prediction of structural Ab epitopes based on Ab sequence. Using RBD-AIM, we analyze the local big data sources to evaluate the functional biodiversity of native B cell repertoires raised after vaccination and reconstructed in a yeast display system using single-cell microfluidics. This pipeline allows for rapid isolation of neutralizing Abs that promote the survival of transgenic hACE2+ mice in lethal models of SARS-CoV-2 infection. We believe that the AI-guided sequence-functionality link can be successfully employed for further high-throughput discovery of therapeutic Abs and functional analysis of Ab repertoires.

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1. Terekhov, S.S., Ivanisenko, N.V., Zhang, N. (2026). *Nat. Commun.*, 17(1), 4009.
2. Solovev, Y.V., Kostin, N.N, Massson, P., ... Gabibov, A.G (2026). *Proc. Natl .Acad. Sci. USA*, 123(24), e2535430123.

DECRYPTING DRUG MECHANISM OF ACTION BY PROTEOMICS

B. Küster

Technical University of Munich, Munich, Germany

Almost all drugs exert their effects in a dose-dependent fashion, but a central challenge in drug discovery and pharmacology is to bridge the gap between observed phenotypic and the often complex underlying molecular mechanisms. Important questions to answer are: which proteins are physically bound by the compound, which pathways are engaged in the cell and how is the cell molecularly and physiologically reprogrammed en route to its eventual, drug-determined fate? In light of the advances in quantitative mass spectrometry speed and sensitivity over the past decade, it has become feasible to perform systematic full dose-response experiments at the level of: (1) target deconvolution; (2) pathway engagement; (3) proteome reprogramming; and (4) cellular consequences. Each enables the extraction of potency and effect size information for thousands of proteins and post-translational modification sites in parallel. In this presentation, the conceptual framework of system-level dose-response measurements is outlined and key published and unpublished studies are used to illustrate how such data inform successive layers of drug mechanisms of action.

KEYNOTE LECTURES

MACHINE-LEARNING SCORING FUNCTIONS FOR STRUCTURE-BASED BINDING AFFINITY PREDICTION A 15-YEAR PERSPECTIVE

P. Ballester

Department of Bioengineering, Imperial College London, London, UK

Molecular docking predicts whether and how small molecules bind to a macromolecular target from its 3D atomic-resolution structure. Docking supports two central problems in structure-based drug discovery. The first is virtual screening, which aims at discovering drug leads in compound libraries. The second is lead optimisation, which improves the properties of a drug lead, especially its binding affinities against the considered targets and off-targets. The success in tackling them depends, however, on how accurate is the scoring function applied to the docked molecules.

Over more than fifteen years, machine-learning approaches to scoring have produced large measurable improvements in binding affinity prediction. In this talk, I will review such machine-learning scoring functions and discuss the key lessons learned about how to enhance their performance for this application. In particular, I will discuss:

- the key role of training data augmentation.
- when and how performance can be improved by building target-specific scoring function.
- the shortcomings of current benchmarks and how they are currently being mitigated.
- the impact of docking pose errors on binding affinity prediction.
- the magnitude of the improvement introduced by deep learning algorithms.
- the need for releasing the code of scoring functions to reproduce them and further evaluate them.
- what is currently possible in binding affinity prediction guided by these tools.

AI-GUIDED REDESIGN OF THE PERTUSSIS TOXIN PT-A SUBUNIT FOR VACCINE DEVELOPMENT

A.O. Chugunov, V.A. Matskevich, V.M. Govorun, R.G. Efremov

Scientific Research Institute for Systems Biology and Medicine, Moscow, Russia

Whooping cough, a highly contagious respiratory infection caused by the bacterium *Bordetella pertussis*, was long regarded as a disease that, if not fully conquered, at least posed little cause for concern. Over the past two decades, however, the infection has been resurging at an accelerating pace, particularly in the post-COVID era. One driver of this “pertussis renaissance” is the comparatively limited duration of immunity conferred by acellular vaccines (such as Pentaxim), which in many countries (the United States, Western Europe) have supplanted whole-cell formulations (such as the DTP vaccine still used in Russia). Against this backdrop, the development of new subunit vaccines that combine the efficacy of whole-cell preparations with the safety profile of acellular ones remains an urgent task, both worldwide and within Russia.

The aim of the present work is to computationally redesign the pertussis toxin PT — the principal virulence factor of *B. pertussis* — so as to abolish its toxicity while preserving immunogenicity and enhancing solubility, thereby laying the groundwork for a new subunit vaccine against whooping cough based on the engineered protein.

PT is a hexameric AB₅-type protein, in which the catalytic PT-A subunit is responsible for the toxin’s biological activity (blockade of intracellular G-protein-mediated signaling), while the pentameric B-ring serves to stabilize PT-A and mediate its delivery into the target cell. PT-A is the most attractive candidate antigen for vaccine purposes, yet outside the hexameric complex it is inherently unstable and prone to spontaneous aggregation — a limitation that necessitates protein redesign.

Using neural-network tools such as ESM-2 and Boltz2, together with molecular dynamics simulations, we uncovered a complex internal architecture of PT-A: whereas its structured N-terminal domain mediates catalysis and is recognized by neutralizing antibodies, the 55 C-terminal residues of PT-A are intrinsically disordered, display high conformational entropy, and serve exclusively to mediate association with the B-ring and to enable “escape” from the endosome via ER-associated degradation (ERAD), through unfolding and threading across protein-conducting channels in the organellar membrane. Following refolding of recombinant PT-A expressed in *E. coli*, washed and resolubilized from inclusion bodies, these 55 C-terminal residues drive irreversible aggregation of the protein. By engineering a truncated form of the detoxified pertussis antigen (gdPT-A Δ 55), we achieved a marked increase in protein yield per liter of culture (approximately tenfold) and improved stability, while fully retaining its antigenic properties.

We further propose an original protocol for neural-network-guided protein sequence optimization, which identifies so-called “hotspots” for targeted substitutions that further enhance (thermo)stability and the biotechnological properties of the antigen. The resulting “upgraded” PT-A will be incorporated into a chimeric neoantigen that additionally comprises a fragment of *B. pertussis* hemagglutinin and a self-adjuvanting moiety.

The antigen under development may form the basis of the first Russian subunit vaccine against whooping cough, complementing the existing DTP vaccine to strengthen population-level protection against this re-emerging disease.

CAN MACHINES DISCOVER FUNCTION IN THE CODE OF LIFE?

C. de la Fuente

University of Pennsylvania, Philadelphia, USA

For centuries, machines have extended the reach of human understanding. Telescopes illuminated the cosmos; microscopes revealed the world of the invisible. Today, intelligent machines are emerging as a new kind of scientific instrument—one capable of searching the molecular sequences in which life is written and uncovering the principles that connect sequence to function. Yet the meaning of most of this vast information remains unknown.

Evolution is the only optimization process we know to have produced proteins, cells, multicellular life, organisms, brains, and ultimately intelligence. Over billions of years, it explored biological sequence space and preserved molecular solutions capable of performing useful tasks. AI now offers a way to learn from and compress that search, turning patterns shaped across deep time into experimentally testable hypotheses.

In this talk, I will describe our efforts to build AI systems that navigate biological sequence space to discover functional matter—molecules that perform useful biological tasks—and translate those discoveries into new therapeutics and tools for responding to emerging pathogens. Peptides are central to this effort. They are compact enough to synthesize and test at scale, yet diverse enough to encode a remarkable range of activities, providing a tractable bridge between computation and experiment. By integrating deep learning, large-scale computational mining, standardized datasets, molecular design, and laboratory validation, we have discovered previously unknown antimicrobial peptides across the tree of life, from the human microbiome and extinct organisms to archaea and venom proteomes. These studies have revealed that bioactive molecules can be encrypted within larger proteins, preserved across evolutionary time, or hidden in genomic regions overlooked by conventional annotation. Beyond uncovering molecules already encoded by nature, we are developing increasingly general AI systems that can predict activity and safety, prioritize candidates for experimental testing, and design entirely new peptides with desired properties. Our standardized dataset hypothesis proposes that high-quality, experimentally consistent data are essential for models to learn generalizable principles connecting sequence to function.

These findings suggest that biological sequences are not merely records of life's history, but an immense and largely unexplored reservoir of functional information. Machines can formulate hypotheses at a scale beyond unaided human analysis, but experiments remain essential for determining which predictions correspond to biological reality. By coupling computation and experimentation in a continuous cycle, we are developing new ways to read, test, and ultimately rewrite the information of life—to discover what life can do and use its underlying rules to create new medicines, molecules, and tools for confronting emerging biological threats.

DATASET CONSTRAINTS: A CASE OF PHARMACOLOGICALLY STRATIFIED MULTI-TARGET QSAR BENCHMARK FOR OPIOID RECEPTOR FUNCTIONAL ACTIVITY

M.A. Nael^{1,2}, L.M. Alakonda², K.M. Elokely²

¹*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Tanta University, Tanta, Egypt*

²*Division of Pharmaceutical Sciences, School of Pharmacy, University of Wyoming, Laramie, USA*

Objective. Public bioactivity databases are heterogeneous not only in measurement type, where affinities and functional potencies sit on different scales, but in pharmacology: one compound and target can carry agonist, antagonist, or inhibitor records measured by radioligand displacement, cAMP, β -arrestin recruitment, or [35S]GTP γ S readouts that quantify different biological events. Pooling them yields models detached from any coherent pharmacological claim. This study treats pharmacological mechanism and assay readout as a primary curation axis applied before modeling, and asks what a pooled functional QSAR model can legitimately claim.

Advancement over previous studies. Large-scale efforts have harmonized structures, units, and activity types, and the noise from combining IC₅₀ or K_i values across sources is quantified; these address the measurement-type and chemical-representation axes. Pharmacological mechanism and assay-readout class have not been treated as a primary stratification axis applied before modeling. We introduce that axis as an auditable, OECD-anchored dataset contract that composes with existing standardization, the pharmacology supplied as a versioned ontology rather than embedded in code.

Results. Four public sources (ChEMBL, BindingDB, PubChem, IUPHAR/BPS) were reconciled for MOR, DOR, KOR, and NOP: 115,232 raw records into 72,148 merged records and 50,977 curated measurements across 19,585 compounds, each carrying source agreement, endpoint meaning, pharmacology class, assay readout, and trust tier. Seventy-one endpoints were carried to scaffold-locked external validation. Receptor-level binding was compact and mature (KOR pK_i $R^2 = 0.79$, $n = 798$; MOR pK_i 0.75 , $n = 1,227$). Stratification exposed functional strata that pooled labels obscure: MOR antagonist functional inhibition ($R^2 = 0.86$, $n = 110$) and agonist potency for DOR, KOR, and MOR ($R^2 0.79$ – 0.81). Against a fully pooled baseline under the identical protocol the gain was not uniform. Where pools are already coherent (agonist-dominated EC₅₀, 23–53% binding-displacement) pooled and stratified models matched (MOR 0.829 vs 0.795 ; KOR 0.818 vs 0.832). Where heterogeneity exists the pooled number misleads: functional-IC₅₀ pools were 60–93% binding-displacement, so pooled DOR reached $R^2 = 0.825$ while predicting affinity rather than the labeled functional inhibition; AC₅₀ pools were entirely binding-displacement and collapsed once isolated (DOR 0.007); and the stratified MOR antagonist endpoint (0.856) beat its 80%-contaminated pool (0.764).

Significance. Performance cannot certify a claim; only the dataset definition can. A model pooled across agonist and antagonist records and across distinct readouts predicts a quantity corresponding to no single assay outcome, and cannot tell a medicinal chemist whether a scaffold is a G-protein agonist or an arrestin recruiter, the distinction that pathway-selective opioid design depends on. Stratification by pharmacology class and assay readout is therefore a precondition for a defensible functional claim, not a metric optimization. Curation logic, ontology, and frozen data are released as CURATO-OPIOID.

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AN INTERPRETABLE MACHINE LEARNING FRAMEWORK FOR ADVERSE DRUG REACTION PREDICTION FROM DRUG-TARGET INTERACTIONS

J. Roberts-Nuttall¹, A.M. Jones^{1,2}, M. Castellani¹, D. Pham¹

¹*School of Mechanical Engineering, University of Birmingham, Edgbaston, United Kingdom*

²*School of Pharmacy, University of Birmingham, Edgbaston, United Kingdom*

Adverse drug reactions (ADRs) remain a major challenge for patient safety and healthcare systems, yet their mechanistic causes are often unclear. Existing pharmacovigilance approaches, such as spontaneous reporting systems (SRS), provide valuable but noisy and incomplete post-marketing data, while many machine learning (ML) models for ADR prediction function as black boxes and offer limited pharmacological insight. Leveraging drug target interaction information within interpretable ML frameworks could improve ADR prediction while revealing biologically meaningful relationships between drugs, targets, and clinical outcomes.

In this work our objective was to develop and evaluate an interpretable machine learning framework that predicts statistically significant ADRs from drug target interaction data, to identify pharmacologically meaningful relationships that can inform drug safety.

Unlike prior ADR prediction work that often relies on opaque models or clinical-trial data alone, this study integrates large-scale drug–target interaction scores from STITCH with real-world post-marketing reports from the UK Yellow Card Scheme. The framework uses Random Forest classifiers with SMOTE + Tomek resampling and Bayesian hyperparameter optimisation and explicitly emphasises interpretability through feature importance analysis and external validation of top targets using DisGeNET plus comparison with the SIDER clinical-trial database.

In this study¹ across 21 MedDRA System Organ Class categories, the models achieved ROC AUC scores of 0.64–0.94 and accuracies of 0.81–0.94, indicating moderate to strong ability to distinguish significant from non-significant ADR signals despite substantial class imbalance. Feature-importance analysis highlighted targets with known pharmacological links to psychiatric and nervous-system disorders, with nine of the top ten targets in a psychiatric disorders case study matching established disease associations in DisGeNET. Most drugs interacting with these targets belonged to the ATC nervous-system class, reinforcing the biological plausibility of the predictions. These findings show that interpretable, target-based ML can extract clinically relevant ADR signals from noisy, imbalanced, real-world data, offering a promising tool for predictive pharmacovigilance and safer drug development.

1. Roberts-Nuttall, J., Jones, A. M., Castellani, M., Pham, D. (2026). *PLOS ONE*, 21(1), e0340900.

AI IN DRUG TARGET DISCOVERY: HOPES, HYPE, AND REALITY

G. Khegai¹, A. Kalmykov¹, D. Stelmashenko¹, R. Zubare², J. Borlak³, A. Kel¹

¹*geneXplain GmbH, Wolfenbüttel, Germany*

²*Science for Life Laboratory (SciLifeLab), Stockholm, Sweden*

³*Hannover Medical School, Hannover, Germany*

How many potential drug targets do we have? Thousands? Millions? Maybe the number is astronomical. A single nucleotide can change transcription factor binding, an amino acid can determine a protein–protein interaction, and each connection in a signaling network can potentially become a therapeutic intervention point. So, the real targets are often not individual genes or proteins, but molecular interactions which control disease mechanisms. How can we find the right targets among this enormous number of possibilities?

AI brings great hopes, but also a lot of hype. Recent examples, such as TNIK in pulmonary fibrosis and CHK2 in Parkinson’s disease, demonstrate interesting progress in AI-assisted target discovery. However, these successes also show the problem: AI benefits enormously from the biological knowledge if it used on the stage of AI model building. We call it - “Big Knowledge”, which includes experimentally supported transcription factor binding, protein interactions, signaling reactions and disease mechanisms, provides the biological context which cannot be easily learned from omics data alone. Without this knowledge, even powerful AI models may find statistical associations but fail to explain the mechanisms behind them.

The real challenge is to move from correlations to mechanistic models. Finding genes associated with a disease is not enough. We need to understand how their interactions produce a pathological phenotype and predict what will happen if we change a particular interaction. As an example, we will present our PISA proteomics and single-cell RNA-seq analysis of breast cancer cells treated with a novel selective thioredoxin reductase inhibitor (LCTA2940). Comparing sensitive and partially resistant cells, we used machine learning (Genome Enhancer) to combine upstream analysis with knowledge-based reconstruction of regulatory and signaling networks. This revealed different response mechanisms, including NRF2-controlled antioxidant defense and a small adapted cell population with specific survival signaling pathways involving interactions of SYK, PRKCZ, IGFBP2 and mTORC2. The reconstructed mechanisms suggest possible intervention points to overcome adaptation, suggested a combinatory treatment that significantly reduced the treatment resistance of the tumor cells.

New agentic AI systems open especially interesting perspectives. They can autonomously generate hypotheses, collect biological evidence, build mechanistic models, predict interventions and propose experiments. But can they really discover new mechanisms, or do they mainly reproduce what is already known? We will discuss the hopes, hype and current reality of these approaches.

We believe that the most promising future is in combining Big Data, Big Knowledge and AI reasoning to reconstruct and test mechanistic models of disease. The real breakthrough will come when AI can tell us not only which molecules are involved in disease, but which interactions we need to change to reverse it.

A DYNAMIC QSPR FRAMEWORK FOR UNDERSTANDING DRUG RESISTANCE

M.G. Khrenova^{1,2}

¹Chemistry Department, Moscow State University, Moscow, Russia

²Federal Research Centre of Biotechnology of RAS, Moscow, Russia

Molecular modeling is a powerful complement to experimental research that provides an understanding of molecular mechanisms at atomic resolution. Drug resistance remains one of the most serious challenges in antiviral and antimicrobial therapy. Resistance typically arises when mutations in a drug target reduce the binding affinity or efficacy of an inhibitor while preserving the biological function of the target protein. In many cases, resistance cannot be explained by static crystal structures alone. Mutations may alter protein flexibility, conformational equilibria, dynamic interactions with inhibitor molecules, or the electronic environment of the active site. Thus, molecular dynamics (MD) simulations can clarify many experimentally observed drug resistance mechanisms.

In this study, we present results related to bacterial proteins, β -lactamases and penicillin-binding proteins, as well as viral proteins with resistance related to point mutations in corresponding genes leading to amino acid substitutions. We demonstrate how interactions of the substrate with the active site of the enzyme, studied using QM/MM MD, can be quantitatively described and how this knowledge can be utilized to predict inhibition potency. Using classical MD simulations, we show how dimension reduction techniques extract biologically important motions to disclose interaction mechanisms. Also, the power of dynamic network analysis is demonstrated with respect to the interactions of inhibitors and target proteins in non-covalent complexes. This method identifies communities, allosteric pathways, and long-range communication within a protein. In studying drug resistance, network analysis is especially useful because mutations far from the binding site can disrupt allosteric coupling and thereby reduce drug efficacy. Network analysis reveals that strong inhibition requires the bound inhibitor and neighboring residues to move together. Mutations that abrogate this correlated motion lead to escape from inhibition.

WEB SERVICES FOR IN SILICO TOXICITY PREDICTION IN PRECLINICAL DRUG DEVELOPMENT

***A. Lagunin^{1,2}, A. Rudik¹, P. Pogodin¹, L. Stolbov¹, A. Dmitriev¹, S. Ivanov^{1,2},
D. Druzhilovskiy¹, D. Filimonov¹, V. Poroikov¹***

¹Institute of Biomedical Chemistry, Moscow, Russia

²Pirogov Russian National Research Medical University, Moscow, Russia

The discovery and development of new drugs, especially within the paradigm of personalized medicine, has become an increasingly significant issue. Most pharmaceuticals interact with multiple molecular targets, exhibiting complex profiles of biological activity, cytotoxicity, acute or organ-specific toxicity, and adverse effects. Evaluating these toxicological profiles is a crucial part of the preclinical assessment process. Innovative approaches are finding widespread application [1], with computational (*in silico*) methods, particularly machine learning (ML), playing a steadily growing role. One of the key advantage of *in silico* approaches is their ability to perform simultaneous, comprehensive analyses of multiple types of biological activity, potential side effects, and toxicities. Crucially, these methods can be applied to virtual chemical structures prior to their chemical synthesis [2].

The aim of this study is to develop new and enhance previously established computational methods for modeling “structure-toxicity” and “structure-property” relationships of drug-like organic compounds. We have integrated these methods into the PRIS (*Preclinical Researches In Silico*) web platform, using the PASS, GUSAR, and SARMath software packages. Utilizing only the structural formula of a chemical compound, PRIS enables the prediction of acute toxicity, carcinogenicity, mutagenicity, reproductive toxicity, genotoxicity, endocrine disruption, cytotoxicity across various cell lines, cardiotoxicity, hepatotoxicity and immunotoxicity. Additionally, it evaluates effects on macromolecular targets associated with severe adverse reactions. To the best of our knowledge, this platform currently has no direct analogues, as existing resources predict only isolated types of toxicity or side effects.

Here, we present the intermediate results of this development. The current version of PRIS (<https://way2drug.com/pris/>) provides predictions for Ames mutagenicity across 69 different strains [3]; cytotoxic IC₅₀ and GI₅₀ values on 10 non-tumor and 10 tumor cell lines commonly used in toxicology [4]; LD₅₀ values for mice, rats, and rabbits via different routes of administration; more than 50 GHS Hazard Statements; and several major organ toxicities [5].

Thus, the developed web application serves as a comprehensive tool for the *in silico* estimation of a significant portion of the most critical endpoints required in preclinical toxicological studies.

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1. Wadman, M. (2023). *Science*, 379(6628), 127–128
2. Hong, H. (2023). QSAR in Safety Evaluation and Risk Assessment, Ed. Academic Press, 543 pp.
3. Dmitriev, A.V., Lagunin, A.A., Rudik, A.V., et al., (2025). *Pharmaceuticals (Basel)*, 18(12), 1853.
4. Lagunin, A.A., Lisitsa, E.Y., Rudik, A.V., et al. (2025). *Int. J. Mol. Sci.*, 26(24), 12063.
5. Ivanov, S.M., Lagunin, A.A., Rudik, A.V., et al., (2018). *J. Chem. Inf. Model.*, 58(1), 8-11.

CRUSH: A CHEMISTRY-AWARE MOLECULAR FRAGMENTATION ALGORITHM FOR EXPANDED CHEMICAL SPACE EXPLORATION

E. López-López^{1,2}, J.L. Medina-Franco², F. Miljković^{1,3}

¹*Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden*

²*DIFACQUIM Research Group, Department of Pharmacy,*

School of Chemistry, National Autonomous University of Mexico

³*Biopharma Chemistry, Discovery Sciences, Biopharmaceuticals R&D, AstraZeneca, Mölndal, Sweden*

Molecular fragmentation is a cornerstone of fragment-based drug discovery, virtual screening, and *de novo* molecular design. Established methods such as RECAP and BRICS, while widely adopted, are constrained by classical retrosynthetic rules that limit chemical space exploration beyond traditional medicinal chemistry contexts. This poses a significant challenge as chemical databases increasingly encompass structurally diverse compounds, including natural products, peptides, macrocycles, and food-related chemicals — all of which may benefit from more fine-grained fragmentation strategies.

Here we introduce CRUSH (Cleavage Rules Using SMIRKS Heuristics), a chemistry-aware molecular fragmentation algorithm comprising 33 curated SMIRKS-based rules that exhaustively applies bond disconnection heuristics across all eligible bonds at each fragmentation step. CRUSH explicitly encodes a broader range of chemical bond environments compared to RECAP and BRICS, including aliphatic and aromatic C–C bonds, heteroatom–carbon linkages, exocyclic aromatic disconnections, quaternary ammonium centers, alkynes, disulfides, and sulfonate esters, while carefully distinguishing ring from non-ring and saturated from unsaturated chemical contexts.

CRUSH was benchmarked against RECAP and BRICS using a unified evaluation framework across five chemically diverse datasets: small-molecule drugs (ChemDiv, $n=41,068$), natural products (LANaPDB v.2, $n=13,578$), antimicrobial peptides (anti-MRSA, $n=223$), macrocycles (Macrocyclic-DB, $n=46,089$), and food-related chemicals (FoodDB, $n=70,477$). Across all datasets, CRUSH consistently generated the highest fragment yield and accessed exclusive regions of chemical space not covered by either RECAP or BRICS. CRUSH-derived fragments were consistently smaller and less complex, with physicochemical property distributions more compliant with the Rule of Three ($MW \leq 300$ Da, $HBD \leq 3$, $HBA \leq 3$, $ClogP \leq 3$), making them particularly well-suited for fragment-based discovery workflows.

These results propose CRUSH as a robust, versatile, and open-source fragmentation framework well-positioned for applications in fragment-based drug discovery, virtual screening, *de novo* design, and chemoinformatic library generation across diverse domains of chemistry. The CRUSH implementation is freely available at <https://github.com/EdgL2/CRUSH>.

This study was supported by the Swedish Foundation for Strategic Environmental Research (Mistra SafeChem, project number 2018/11).

1. López-López, E., Medina-Franco, J.L., Miljković, F. (2026). *Journal of Chemoinformatics*, In press.
2. Lewell, X.Q., Judd, D.B., Watson, S.P., Hann M.M. (1998). *Journal of Chemical Information and Computer Sciences*, 38, 511–522.
3. Degen, J., Wegscheid-Gerlach, C., Zaliani, A., Rarey, M. (2008). *ChemMedChem*, 3, 1503–1507.

RETHINKING BIOACTIVITY MODELING: REPRESENTATION, CONTEXT, AND PREDICTIVE SPACES

K. Martinez-Mayorga, D. Ramirez-Palma

Institute of Chemistry, Campus Merida, National Autonomous University of Mexico, Mexico

Predictive models in cheminformatics commonly assume that bioactivity varies smoothly with molecular structure. However, changes in experimental context, such as dose range and data distribution, can lead to abrupt losses of predictivity even when the underlying chemical space remains unchanged. This work examines the distinction between chemical and predictive space and the roles of molecular representation and experimental context in bioactivity modeling.

Rather than distinguishing chemical and predictive spaces solely through unsupervised and supervised methods, we propose that their fundamental difference lies in scientific intent. Chemical space is primarily constructed to organize and interpret molecular relationships, whereas predictive space is constructed to predict a specific property or biological endpoint. Within this framework, descriptor selection actively defines the resulting space, and representations informed or optimized by endpoint information serve different purposes from those designed primarily to describe molecular structure.

Dose-dependent disruptions in bioactivity further illustrate that molecular structure alone may be insufficient to represent biological responses under changing experimental conditions. Incorporating contextual information, including dose and experimental conditions, provides a route toward context-aware predictive spaces. This framework highlights the importance of explicitly defining representation objectives and contextual information in QSAR, machine learning, and AI-driven drug discovery.

1. Ramírez-Palma, D., Martínez-Mayorga, K. (2025). *Drug Discovery Today*, 104472.
2. Ramírez-Palma, D., Martínez-Mayorga, K. (2026). *Chemical and Predictive Spaces: Convergent Constructs, Distinct Intent*. In: Medina-Franco, J.L., López-López, E. (Eds.), *Chemical Space*, Wiley, in press.

GENERATIVE DRUG DESIGN: FROM FRAGMENT SEEDS TO SMALL MOLECULE LEADS

X. Jalencas, R. Garcia-Serna, J. Cabot-March, L. Menestrina, M. Gorostiola, J. Mestres

Chemotargets SL, Barcelona, Spain

Structure-based generative design is emerging as a competitive tool in AI drug discovery. Instead of performing virtual screening on pre-enumerated ultra-large virtual libraries, it is the protein cavity that guides the growing of molecules that best interact with it and have the adequate safety profile. In this talk, we will introduce our Deep Drug Discovery (D3) platform, an unsupervised ultrafast fragment-to-drug generation engine designed to rapidly diversify chemical structures from initial seeds. Starting with an ensemble of protein structures obtained by clustering the trajectories of molecular dynamics simulations of an available X-ray structure or a model structure, a virtual fragment screening is performed on each protein cavity based on a database of chemical fragments interacting with protein environments (SAFR). [1] All fragments mapped inside the target cavity are then rank ordered by steer molecular dynamics.

Then, D3 takes the best fragment seeds and initiates a fragment evolution process. Within D3, efficient exploration of chemical space is guided by ChemBang [2], a computational engine that grows small molecules based on chemical transformations extracted by matched molecular pair analysis of all structures available in catalogues of synthesized molecules. Each chemical transformation is mapped onto its associated atomic environment defined as the substructure within a three-atom radius from the transformation site. Unsupervised chemical evolution is then performed in cycles by systematically applying chemical transformations inside the protein cavity to all exposed atomic environments present in a seed structure. The new molecules built are then processed against a list of 118 property, pharmacology, and safety models [3] designed to bias the selection of generated molecules towards those having the adequate safety profile as defined by the therapeutic area of the target. Finally, all molecules built are allowed to better accommodate within the cavity using tethered docking. Only the best molecules are ultimately allowed to progress to the next generation.

In a retrospective validation study, we will show D3's ability to generate drugs from a constituting fragment. D3 is agnostic on the binding cavity (orthosteric or allosteric) and has already proven its value in generating novel chemical matter when prospectively applied to both hot and cold targets.

1. Cabot-March, J., Jalencas, X., Mestres, J. (2026). *J. Chem. Inf. Model*, 66, 4848-4862.
2. Montes-Grajales, D., Menestrina, L., Garcia-Serna, R., Mestres, J. (2026). *Mol. Inform.*, 45, e70036.
3. Menestrina, L., Parrondo-Pizarro, R., Gómez, I., Garcia-Serna, R., Boyer, S., Mestres, J. (2025). *Pharmaceutics*, 17, 308.

ATOMISTIC INSIGHTS INTO A LANTIBIOTIC'S MECHANISM: AN INTEGRATED MOLECULAR DYNAMICS AND BIOPHYSICS STUDY

L.S. Panina^{1,2}, V.P. Shendrikov³, A.O. Chugunov^{1,2}, I.A. Boldyrev³, R.G. Efremov^{1,2}

*¹Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences,
Moscow, Russia*

²Scientific Research Institute for Systems Biology and Medicine, Moscow, Russia

*³Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences,
Moscow, Russia*

The rapid global spread of multidrug-resistant bacterial pathogens has created an urgent need for new antibiotic classes that act *via* novel molecular mechanisms. Lantibiotics, ribosomally synthesized and post-translationally modified peptides such as nisin, are promising candidates because they remain potent at low concentrations and often act against strains resistant to conventional antibiotics. A key feature of many lantibiotics is their high-affinity recognition of the cell-wall precursor lipid II, which both blocks peptidoglycan biosynthesis and promotes membrane permeabilization by pore formation. [1]. Despite extensive biochemical work, there is still no experimentally resolved atomistic structure of the full nisin–lipid II complex embedded in a native-like phospholipid bilayer. To date, the only high-resolution complex structure is a solution NMR model of nisin bound to a truncated lipid II analogue in DMSO, which revealed a “pyrophosphate cage” formed by the N-terminal lanthionine rings of nisin. Subsequent solid-state NMR and membrane studies showed that lipid environment and bilayer insertion significantly alter complex geometry, but without delivering a complete biomembrane-embedded complex structure [2].

The objective of this work is to build and validate an atomistic model of nisin–lipid II recognition in a model gram-positive membrane by integrating molecular dynamics simulations with *in vitro* black lipid membrane experiments and available NMR data. Starting from proposal of a structure of the truncated nisin recognition module (a.a. 1–12) interacting with a pyrophosphate in solution, we extend these simulations to full-length nisin and lipid II at the membrane–water interface in a POPG:POPE bilayer. The model is supported by solution NMR observations of the interaction between nisin and a newly synthesized lipid II mimetic, as well as functional parameters from black lipid membrane recordings of lipid II mimetic -dependent nisin pore diameters and lifetimes.

Our simulations reproduce a stable trap-like arrangement of N-terminal backbone amides around the lipid II pyrophosphate group, both in solution and at the membrane surface, consistent with the NMR data on nisin₁₋₁₂ in solution [3]. The model rationalizes how nisin selectively captures lipid II pyrophosphate group, despite the presence of chemically similar phosphate groups in surrounding membrane lipids. Moreover, biophysical studies indicate that the pyrophosphate moiety represents the minimal structural element required for specific recognition.

These atomistic insights advance previous work by embedding the nisin–lipid II complex in a realistic membrane context, thereby offering a blueprint for rational design and engineering of next-generation lantibiotics that exploit lipid II targeting to combat antibiotic-resistant bacteria.

1. Draper, L.A., Cotter, P.D., Hill, C., Ross, R.P. (2015). *Microbiol Mol Biol Rev.*, 79(2), 171-191.
2. Medeiros-Silva, J., Jekhmane, S., Paioni, A.L., et al. (2018). *Nat Commun.*, 9(1), 3963.
3. Dickman, R., Danelius, E., Mitchell, S.A., et al. (2019). *Chemistry.*, 25(64), 14572-14582.

METRICS FOR UNBIASED SELECTION OF MACHINE LEARNING REGRESSION MODELS

A. Banerjee, K. Roy

*Drug Theoretics and Cheminformatics Laboratory (DTC Lab), Department of Pharmaceutical Technology,
Jadavpur University, Kolkata, India*

Machine learning-based models are now widely used in predictive cheminformatics and data gap filling. Selecting a single best machine learning regression model from a set of competing models can be challenging. While models selected based on cross-validation performance do not guarantee good predictions on external data, models selected solely on external validation performance do not ascertain precise predictions for other external sets. Therefore, we propose three quantitative metrics to guide modelers in selecting the best model [1]. Three quantitative datasets of varying sizes and complexities were considered. Each dataset was randomly split into a modeling set and an independent external test set. The modeling set was further split thrice to generate training and validation sets. Various machine learning models were developed and validated against the validation sets. Our proposed metrics were computed for each model using only training and validation set performances. The novel metric values guided the selection of the best models, which also demonstrated expected performance on the independent external test set.

1. Banerjee A., Roy K., (2026). *ChemRxiv*

WHAT AI STILL NEEDS TO SOLVE IN DRUG DISCOVERY

W. Sherman

Psivant Therapeutics, Boston, MA, United States

Artificial intelligence has transformed how we search vast chemical spaces, automate workflows, and write code. Yet many of the most important problems in drug discovery remain unsolved. Models must determine not simply which molecules resemble previous successes, but how new molecules will behave in complex physical and biological environments. This talk will examine several unresolved challenges: molecular representations that capture 3D structure, electronic properties, dynamics, and environment; generalization of models under limited experimental data regimes; modeling of coupled properties such as potency, selectivity, permeability, metabolism, and toxicity; and prospective validation against decisions that matter in real discovery programs. I will argue that progress will increasingly require integration of machine learning with physics-based modeling, richer experimental data, and closed-loop learning from experiment. The central challenge for AI in drug discovery is moving from learning correlations in existing data to predicting the consequences of molecular interventions with sufficient accuracy and reliability to change experimental decisions.

LIGAND-RECEPTOR INTERACTION INSIGHTS FROM *IN SILICO* REVERSE FRAGMENT BASED DRUG DISCOVERY (R-FBDD) FOR KNOWN DRUGS AND LEAD COMPOUNDS

D. Shulga

Department of Chemistry at Moscow State University, Moscow, Russia

The Reverse Fragment Based Drug Discovery (R-FBDD) approach [1,2,3] is the *in silico* based counterpart of Fragment Based Drug Discovery (FBDD) [4]. R-FBDD aims at estimating the relative contribution of each ligand fragment into entire ligand-receptor interaction energy. Despite the fact that such a partitioning does not have a strict physical basis, it is a very useful tool at early stages of drug discovery, where near linear free energy relationships are often held and being utilized. Previously, we have shown that R-FBDD can be fruitfully used to find “anchor” and “ballast” groups of the ligand in terms of their contribution to interaction to the target [1,2]. Recently, we showed how R-FBDD can be used to select a promising series of ligands sharing the same scaffold during the virtual screening stage, which helps to minimize risks at later stages of drug discovery [5].

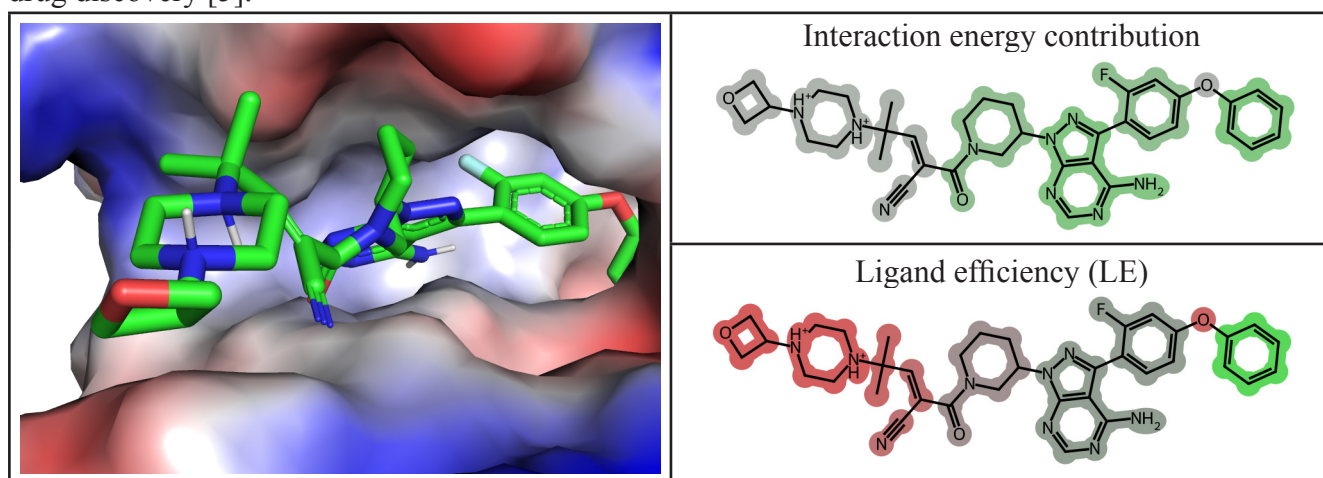


Figure 1. Non covalent interactions of complex of rilzabrutinib with BTK (PDB:7L5O). Interaction energy contributions colored from green (high) to grey (low), whereas LE contributions range from green (close to 0.3 kcal/mol) to red (much lower). Groups necessary for non covalent binding differ from groups necessary for covalent binding (cyanoacrylamide) and solubility (piperazine).

In the presentation it will be shown how R-FBDD can be successfully applied to the example analysis of interactions of the recent known drugs with their molecular targets in order to derive crucial insights on the importance of different fragments of the drug under study. It is shown also how the results of such analysis can be directly translated into an iterative cycle of a typical drug discovery project relying on CADD for hypothesis generation, followed by new compounds synthesis and experimental validation of their activity.

Thus it is shown that R-FBDD approach is a new versatile tool for *in silico* part of drug discovery, capable of providing additional valuable information out of the regular data, and especially applicable for lean drug discovery projects, in which the discovery risks are minimized via judicious re-use of the scaffolds and aid of *in silico* tools.

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1. Shulga, D.A., Ivanov, N.N., Palyulin, V.A. (2021). *Mend. Comm.*, 31(3), 291–293.
2. Shulga, D.A., Ivanov, N.N., Palyulin, V.A. (2022). *Molecules*, 27(6), 1985.
3. Ivanov, N.N., Shulga, D.A., Palyulin, V.A. (2023) *Biophysica*, 3(2), 362–372.
4. Erlanson, D.A., Fesik, S.W. et al. (2016). *Nature Reviews Drug Discovery*, 15(9), 605–619.
5. Myazin, V.D., Shulga, D.A., Ivanov, N.N., Palyulin, V.A. (2026). *Molecular Diversity*, 1-16.

TOWARDS DATA-DRIVEN DESIGN OF VISIBLE-LIGHT PHOTOSWITCHES AS PHOTOPHARMACOLOGICAL AGENTS

K. Prasad^{1,2}, P. Gantzer², P.K. Hashim³, P. Sidorov²

¹*Graduate School of Chemical Sciences & Engineering, Hokkaido University, Sapporo, Japan*

²*Institute for Chemical Reaction Design and Discovery, Hokkaido University, Sapporo, Japan*

³*Department of Applied Chemistry for Environment, Tokyo Metropolitan University, Tokyo, Japan*

Photoswitches are a category of compounds whose chemical structures and properties can be changed by light irradiation [1]. In the field of photoswitches, the wavelength with the maximum light absorption (λ_{max}) and the thermal half-life of a metastable photoisomer ($t_{1/2}$) decide their use in various applications in material science and biology [2]. Specifically, for biological applications, visible-light responsive photoswitches with long half-life are required. So far, experimentalists heavily rely on the time-consuming density functional theory calculations to estimate the λ_{max} and $t_{1/2}$ properties of photoswitches, although these approaches cannot be applied to large collections of compounds due to their prohibitive cost. Moreover, studies involving these approaches often neglect the solvent effects which play a critical role in determining photoswitch behaviour. Here, we present a global, multi-solvent machine learning framework for the simultaneous prediction of three key photoswitch properties – the π - π^* absorption maximum ($\lambda_{\pi-\pi^*}$), the n - π^* absorption maximum ($\lambda_{n-\pi^*}$), and the thermal half-life ($t_{1/2}$) – across seven solvents, as well as an outlook to the biological mechanism of their action.

We address the limited data challenge by compiling molecules from literature sources and experiments. Solvent-dependent data were also compiled, and associated physicochemical descriptors were utilized to examine solvatochromic shifts in the studied systems. Multi-solvent ML models based on fragment count descriptors and empirical solvent parameters achieve substantial improvements in predictive performance, most notably for $t_{1/2}$ ($R^2=0.774$, RMSE=0.782 log units), representing a significant advance over the prior state of the art ($R^2=0.583$) [3], and provide the first solvent-informed ML model for the n - π^* absorption band ($R^2=0.793$). We also performed Leave-One-Solvent-Out as the strictest validation approach to assess if the model has learned how solvent influences the property or if it is merely memorizing the solvent-molecule pairs. It was found that the models were successful in learning the trends and extrapolating them to novel solvents.

For external validation, we utilized novel azoheteroaryl derivatives synthesized from corresponding phenyl hydrazine chlorides via cyclisation or oxidation. Their spectroscopic and thermal isomerization studies were performed in acetonitrile and DMSO at 25°C. The experimental values were then compared with the model predictions. We also analyze the predictions via ColorAtom and SHAP analysis to confirm the models' inner workings, identifying specific structural motifs, including ortho-fluorine substitution and bis-heteroaryl scaffolds, and solvent parameters that govern photoswitch kinetics and spectra. We can observe that the models learned the motifs that lead to increased stability and absorption, which would be useful for the design of photoswitches with desired properties, although it can also be seen that the commonly encountered inverse relationship between these two properties is retained from the dataset. Nevertheless, these results establish a practical, interpretable framework for data-driven photoswitch design with explicit environmental sensitivity.

Moreover, docking and molecular dynamics study allow us to identify potential targets for photoswitchable molecules in Wnt pathway. We have investigated the family of FZD receptors to establish the potential targets for photoswitchable analogs of drug molecules, as these have shown good potential as light-controlled pharmaceutical agents. The analysis highlights the most likely targets within this family, although experimental confirmation is still required.

1. Göstl, R., Senf, A., Hecht, S. (2014). *Chem. Soc. Rev.*, 43, 1982.
2. Beharry, A. A., Woolley, G. A. (2011). *Chem. Soc. Rev.*, 40, 4422.
3. Byadi, S., Hashim, P. K., Sidorov, P. (2025). *J. Cheminform.*, 17(1), 42.

LIGAND-MEDIATED CONFORMATIONAL PLASTICITY OF THE PORE DOMAIN OF TRPV6 ION CHANNEL

I.I. Veretenenko^{1,2}, Yu.A. Trofimov², R.G. Efremov^{1,2,3}

¹Research Institute for Systems Biology and Medicine, Moscow, Russia

²Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

³National Research University «Higher School of Economics», Moscow, Russia

The calcium-selective ion channel TRPV6 is a key regulator of Ca^{2+} transport in epithelial tissues of the intestine, placenta, pancreas, mammary and prostate glands, controlling calcium absorption, bone mineralization and male fertility [1]. Reduced TRPV6 activity is linked to hereditary calcium-homeostasis disorders, while its hyperactivation drives Ca^{2+} -dependent proliferative signaling and tumor progression, making TRPV6 a promising anticancer target. TRPV6 is a constitutively active channel whose ion-conducting pore is highly conformationally plastic and responds to a variety of endogenous and exogenous ligands. This work aimed to characterize this plasticity *in silico*, focusing on the mechanisms of two endogenous inhibitors – the polyamine spermine and intracellular Mg^{2+} – combining molecular dynamics (MD) simulations and free-energy calculations with cryo-EM and electrophysiology data.

The conformational plasticity of the TRPV6 pore domain is governed by transitions of the S6 helices between a fully α -helical conformation and a π -helical segment at the hinge residue A566, giving rise to three conserved pore states: π -open (conducting), π -closed and α -closed [2]. Using molecular dynamics (MD) simulations, we characterized how two endogenous ligands act on this pore. Spermine, a natural polyamine present in the cytoplasm, was shown to enter the π -open pore from the intracellular side and bind within the selectivity filter, physically occluding the conducting pore without altering its open conformation; this binding mode was confirmed by cryo-EM and site-directed mutagenesis, establishing spermine as a direct open-channel blocker [3]. In contrast, intracellular Mg^{2+} acts through a distinct “locking” mechanism: well-tempered metadynamics (WTMetaD) free-energy calculations revealed a highly Mg^{2+} -selective site formed by residues D489 and D580 (S6 helix), where Mg^{2+} binding locks the S6 helix in the α -helical conformation, stabilizing the pore in the non-conducting α -closed state [4]. Thus, spermine and magnesium inhibit TRPV6 through mechanistically distinct strategies – open-pore block versus locked closed-state stabilization – illustrating how the conformational plasticity of the TRPV6 pore underlies diverse modes of ligand-mediated regulation and providing a basis for the rational design of new selective TRPV6 inhibitors for anticancer therapy.

The study was supported by the MSHE Agreement № 075-15-2024-536.

1. Yelshanskaya, M.V., Nadezhdin, K.D., Kurnikova, M.G., Sobolevsky, A.I. (2020). *The Journal of Physiology*, 599, 2673–2697.
2. Trofimov, Y.A., Krylov, N.A., Minakov, A.S., Nadezhdin, K.D., Neuberger, A., Sobolevsky, A.I., Efremov, R.G. (2024). *Communications Chemistry*, 7, 119.
3. Neuberger, A., Veretenenko, I.I., Shalygin, A., Trofimov, Y.A., Gudermann, T., Chubakov, V., Efremov, R.G., Sobolevsky, A.I. (2026). *Nature Communications*, 17, 4720.
4. Neuberger, A., Shalygin, A., Veretenenko, I.I., Trofimov, Y.A., Gudermann, T., Chubakov, V., Efremov, R.G., Sobolevsky, A.I. (2025). *Nature Communications*, 16, 9826.

DELTA: DATABASE FOR EXTENDED LIGAND-TARGET ANALYSES - A NEW BALANCED RESOURCE FOR AI APPLICATIONS IN DRUG DISCOVERY

A. Pisati, A. Pozzi, F. Bilancia, A. Pedretti, A. Zaliani, G. Vistoli

Department of Pharmaceutical Sciences, University of Milan, Milan, Italy

Reliable prediction of ligand-target interactions remains one of the major challenges in computational drug discovery. Machine learning approaches are increasingly employed to accelerate compound activity, however, their performance is fundamentally constrained by the quality and the consistency of the underlying data. Here, we present DELTA (Database for Extended Ligand-Target Analyses), a curated and standardized resource specifically designed for compound activity prediction across a broad range of therapeutically relevant protein targets [1]. DELTA was constructed through a unified pipeline for data collection and processing, ensuring consistency across heterogeneous experimental sources. In detail, DELTA comprises balanced annotated and curated datasets of ligands for more than 400 therapeutically relevant human proteins

The datasets contained in DELTA were then utilized to generate independent ML classifiers for each target by systematically evaluating multiple algorithms. Different dataset splitting strategies were investigated to assess model robustness and generalization. All stages, from data preparation to model development and evaluation were implemented using open-source software within a fully transparent and reproducible computational workflow

The so obtained models were finally exploited to develop DITO (DELTA Interpretable Target activity Outcomes) fingerprints, a target-space representation in which small molecules are encoded by their predicted activity across more than 400 human protein targets from the DELTA database.

We show that machine learning models built on these target-informed fingerprints predict cytotoxicity in HEK293 cells with MCC > 0.6, achieving comparable performance to models based on Morgan fingerprints while relying on a substantially smaller and biologically interpretable feature space [2]. Feature importance analysis identifies key targets whose roles are consistent with known cytotoxicity mechanisms. Overall, these results suggest that cellular phenotypes related to protein interactions can be learned from a compact representation of predicted target activities that retain both predictive power and mechanistic interpretability.

1. Pisati, A., Pozzi, A., Giuntini, F., Pedretti, A., Vistoli, G. (2025). *bioRxiv*, 2025, 05.

2. Feitosa, F. L., Cabral, V. F., Sanches, I. H., Silva-Mendonca, S., Borba, J. V., Braga, R. C., Andrade, C. H. (2024). *Journal of Chemical Information and Modeling*, 64(24), 9056.

TREATING SOLUTE POLARIZATION IN MD SIMULATIONS USING PRE-POLARIZED ATOMIC CHARGES: CARBOHYDRATES

X. Wang^{1,2}, S. Holmes¹, L. Newman^{3,4}, J. Nash⁵, T. Barnes⁵, D. Crawford^{5,6}, V. Welborn^{3,4}, R.J. Woods^{1,7}

¹*Complex Carbohydrate Research Center, University of Georgia, Athens, USA*

²*Hubei Key Laboratory of Agricultural Bioinformatics, College of Informatics, Huazhong Agricultural University, Wuhan, Hubei, China*

³*Department of Chemistry, Virginia Tech, Blacksburg, Virginia, USA*

⁴*Macromolecules Innovation Institute, Virginia Tech, Blacksburg, Virginia, USA*

⁵*Department of Chemistry, Virginia Tech, Blacksburg, Virginia, USA*

⁶*Molecular Sciences Software Institute, Blacksburg, Virginia, USA*

⁷*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, USA*

The characteristic presence of multiple hydroxyl groups in a carbohydrate ensures that sugars are hydrophilic and relatively polar. These hydroxyl groups can form hydrogen bonds between their neighbors, as well as with water, and the balance of forces between internal and external hydrogen bonds determines many of their physical properties, as well as the strengths of their interactions with other molecules, such as proteins. It is therefore essential that the electrostatic forces, which underlie these interactions, should be accurately modeled in force fields designed for simulating carbohydrates in solution.

Fully-polarizable force fields exist for carbohydrates, however, such MD simulations remain 20 – 40 fold slower than classical MD simulations. Here we introduce a method for generating solvent-polarized partial atomic charges for carbohydrates, and evaluate the ability of MD simulations employing these pre-polarized charges to reproduce critical properties of aqueous carbohydrates, including dipole moment, diffusion coefficients, aggregation propensities, and bulk water structure in the vicinity of the solute. We conclude that the accuracy of this approach is sensitive to the theoretical water model, with OPC and TIP5P being superior to TIP3P, and that such pre-polarized charges offer significant improvements in property predictions relative to non-polarized variants, without sacrificing simulation throughput. This feature is particularly critical for simulations of highly plastic polysaccharides and glycoconjugates, where simulation convergence requires MD timescales that extend well into the μ s timescale.

This work was supported by GlycoMIP, a National Science Foundation Materials Innovation Platform funded through Cooperative Agreement DMR-1933525

LIPOPHILICITY IN DRUG DESIGN: EVOLUTION FROM THE RULE OF FIVE TO AI-DRIVEN DISCOVERY

W.J. Zamora^{1,2,3}

¹*CBio3 Laboratory, School of Chemistry, University of Costa Rica, San Pedro, San José, Costa Rica*

²*Laboratory of Computational Toxicology and Artificial Intelligence (LaToxCIA), Biological Testing Laboratory (LEBi), University of Costa Rica, San Pedro, San José, Costa Rica*

³*National Advanced Computing Collaboratory (CNCA),
National High Technology Center (CeNAT), San José, Costa Rica*

Lipophilicity has long been recognized as one of the central physicochemical properties governing molecular recognition, membrane permeability, solubility, distribution, and ultimately the pharmacological and toxicological behavior of bioactive compounds. For decades, its interpretation in drug discovery has been strongly influenced by the Rule of Five, with conventional descriptors such as logP and logD providing valuable guidance for the optimization of small, drug-like molecules. However, the increasing exploration of chemical space beyond the Rule of Five including peptides, macrocycles, PROTACs, and other large and flexible molecules, challenges the assumption that lipophilicity can be adequately represented by a single, structure-independent molecular descriptor.

In this talk, I will present the evolution of lipophilicity research, from the development of machine-learning models for lipophilicity prediction and their successful performance in the international SAMPL blind challenges to the development of structure- and pH-dependent lipophilicity scales of amino acids based on continuum solvation models to their application in peptides, non-standard amino acids, antimicrobial molecules, and complex molecular systems. Emphasis will be placed on how ionization, molecular conformation, intramolecular hydrogen bonding, and the three-dimensional chemical environment can substantially alter the effective lipophilic character of a molecule and biomolecules.

Finally, I will introduce LipbRo5 a new computational approach for estimating lipophilicity in compounds beyond the Rule of Five (bRo5). This framework provides a new perspective on how lipophilicity can be computed and interpreted in emerging drug modalities, opening opportunities for more realistic physicochemical profiling and, ultimately, AI-driven molecular design beyond traditional drug-like chemical space.

ORAL PRESENTATIONS

ELUCIDATING THE MOLECULAR CROSSTALK BETWEEN PARKINSON'S DISEASE AND LEVODOPA-INDUCED DYSKINESIA: A NETWORK PHARMACOLOGY APPROACH

A. Arora, R.K. Goel

Department of Pharmaceutical Sciences & Drug Research, Punjabi University, Patiala, India

Background: Parkinson's disease (PD) and levodopa-induced dyskinesia (LID) are biologically interconnected disorders involving complex interactions among dopaminergic signaling, mitochondrial dysfunction, neuroinflammation, and synaptic remodelling. Although the molecular mechanisms underlying LID remain incompletely understood, a systems-level approach may help identify shared molecular components and potential therapeutic candidates.

Objective: The present study aimed to identify common molecular targets linking PD and LID and to discover multitarget phytochemicals using an integrated network pharmacology and molecular docking approach.

Methods: Disease-associated genes were retrieved from DisGeNET, OMIM, and Ensembl databases. Shared genes were identified through venn analysis, followed by protein-protein interaction (PPI) network construction, MCODE module detection, Gene Ontology (GO), KEGG pathway enrichment, and CytoHubba-based hub gene analysis. Phytochemicals from selected medicinal plants were prioritized using PASS prediction and ADMET screening before molecular docking against top hub genes.

Results: A total of 85 shared genes were identified between 2381 PD-associated and 213 LID-associated genes. The PPI network comprised 85 nodes and 241 edges with highly significant enrichment ($p < 1 \times 10^{-16}$). Functional enrichment revealed three interconnected pathological modules involving dopaminergic-synaptic cluster, mitochondrial-inflammatory cluster and lysosomal cluster. Fifteen hub genes, including PINK1, NLRP3, EIF2AK2, TH, IL10, NLRC4, NTRK1, ATP13A2, IRS1, CASP8, DRD1, DRD2, SNCA, PARK2, and FOSB, were identified as key regulatory nodes. Following PASS and ADMET screening, fourteen phytoconstituents were selected for molecular docking. Among them, IMPHY014895, IMPHY000253, and IMPHY000342 showed favourable predicted interactions with multiple targets, particularly toward NTRK1 (-10.6 kcal/mol), TH (-10.2 kcal/mol), and DRD2 (-10.4 kcal/mol), suggesting potential modulation of dopaminergic signaling, neuroinflammation, and mitochondrial homeostasis.

THE NATURE OF NON-ARRHENIUS KINETICS IN THE HEAT DENATURATION OF PROTEINS

A.V. Baklanov¹, A.O. Yanshin^{1,2}

¹*Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russia*

²*Department of Physics, Novosibirsk State University, Novosibirsk, Russia*

Protein denaturation results in the loss of the native structure and protein functionality. Temperature is a frequent factor involved in the loss of functionality. Heating leads to thermal inactivation of microorganisms (bacteria, viruses, etc.), which is used for food and biomaterial processing. Heat and cold are used in medicine as a thermal therapy (thermo- and cryotherapy). These applications have led to academic interest in the temperature dependence of protein denaturation kinetics. The data for protein denaturation demonstrate non-Arrhenius behavior, which usually corresponds to a decrease in the Arrhenius parameters with increasing temperature, resulting in convex Arrhenius plots [1]. The presence of a “convex” shape in the Arrhenius plots in nonbiological and biochemical kinetics was previously generally attributed to protein unfolding proceeding via complex (multistep) mechanisms [2], which are usually not specified. In our current work, we present an approach which allows us to calculate the temperature dependence of the rate constants of protein denaturation and compare it with the experimental data, obtained in the temperature interval 270–370 K of biological relevance and at higher temperature values up to 600 K, achieved recently with the technique of nanosecond plasmonic heating [1].

In the suggested approach we consider the protein unfolding as a process with reaction coordinate corresponding to simultaneous breaking of the manifold of interchain H-bonds, providing the secondary structure of protein. Free-energy profiles along the reaction coordinate of protein unfolding are built in a wide temperature interval. The built profiles reveal the temperature-dependent contribution of the “loose” intermediate which is assigned to be the dry molten globule (DMG) state, stabilized by the entropy gain provided by the loose framework of extended hydrogen bonds. The revealed DMG state with a “loose” pseudo-secondary structure of protein provides a funnel-shaped free-energy landscape, which is a central point of the folding mechanism, rationalizing Levinthal’s paradox. The rate constants of the elementary steps of the unfolding process are calculated according to Transition State Theory. The strong temperature dependence of the Arrhenius parameters for the rate constants of the elementary steps of the unfolding process, and the negative activation energy of the folding process are explained. The main factor influencing the non-Arrhenius behavior of the rate constants is the strong temperature-dependent shift in the location of the DMG and Transition State along the reaction coordinate. The Arrhenius plot for the calculated rate constant for heat denaturation of the protein is compared with experimental data obtained in a wide temperature range (270–600 K). The “convex” shape and the sharp drop in the values of the Arrhenius parameters at high temperatures, predicted by our model, are in a very good agreement with the experimentally observed dependencies.

The results of current work are presented in paper [3].

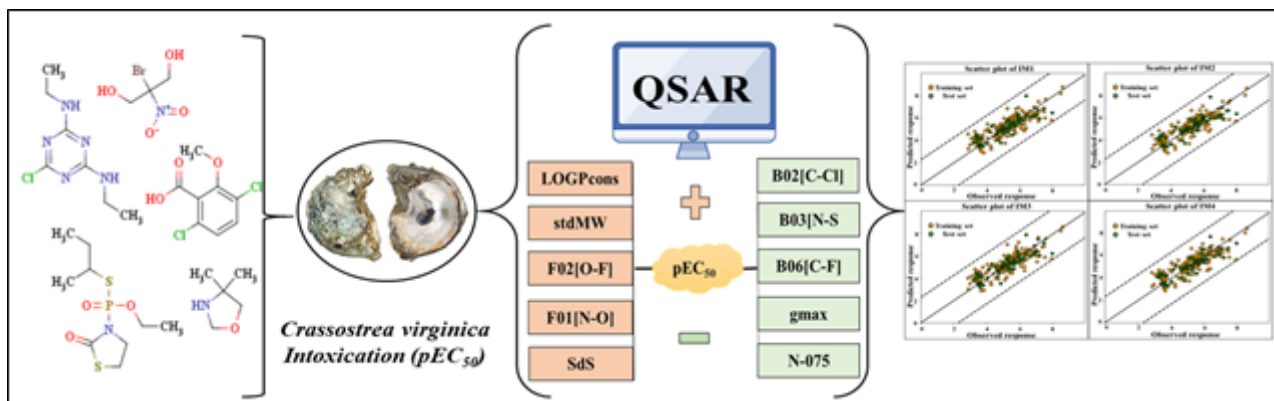
1. Sarkar, D., Kang, P., Nielsen, S.O., Qin, Z. (2019). *ACS Nano*, 13, 8669–8679.
2. Wizer, D.J., Jackson, C.M.J. (2006). *Mol. Recognit.*, 19, 389–407.
3. Baklanov, A.V., Yanshin, A.O. (2026) *Int. J. Mol. Sci.*, 27, 6449.

MULTIVARIATE QSAR MODELING FOR IDENTIFYING STRUCTURAL DRIVERS AND PRIORITIZING TOXIC CHEMICALS AFFECTING CRASSOSTREA VIRGINICA

P. Bhattacharyya, P.K. Ojha

Drug Discovery and Development Laboratory (DDD Lab), Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

Crassostrea virginica, a bivalve mollusc, is considered a keystone species that serves as an “ecosystem engineer,” preserving the health and structural integrity of estuarine habitats. Its filter-feeding characteristics make it well-suited for investigating the impact of pollutants on aquatic organisms. They serve as a critical bio-indicator for monitoring environmental quality in coastal and marine ecosystems. Thus, they are widely used as sentinel species in ecotoxicological studies. The shellfish is also valued for its significant dietary importance to humans. It contains substantial amounts of protein, vitamins such as B12 and D, minerals, essential amino acids and omega-3 fatty acids, while having relatively low fat and calorie contents. However, according to 2014 data, this species is considered globally vulnerable, with a rounded global status of G3 as assessed by NatureServe. The Chesapeake Bay, which previously had a high abundance of *C. virginica* habitats, is now experiencing a sharp decline in its population, to less than 1% of its historical levels [1]. This is due to the combined effects of overharvesting, water pollution, reef habitat degradation, climate change, and disease outbreaks. Environmental hazards pose a severe threat to aquatic species. Therefore, assessing the ecotoxicological risks posed by various pollutants is urgently needed to safeguard these species. Despite this serious concern, conducting experimental tests in marine organisms is quite challenging. This is due to the need for specialized laboratory conditions, including maintaining appropriate salinity and ionic composition for testing, as well as cost constraints and ethical considerations. In this context, adopting *in silico* strategies to assess the toxicity of diverse chemicals in marine organisms will be a promising alternative to address the limitations of traditional testing methods. To the best of our knowledge, no quantitative structure-activity relationship (QSAR) framework has previously been reported for *C. virginica*. The objective of the present work is to predict the acute toxicity (pEC_{50}) of diverse organic chemicals in this species by developing chemometric models. We used a dataset of 226 unique chemicals to generate QSAR models. Furthermore, the implementation of the intelligent consensus prediction (ICP) approach improved prediction quality by overcoming the estimation bias of individual models for a particular test compound. Consensus model 2 (CM2) was the best model, with lower prediction error ($MAE_{95\%(\text{test})} = 0.381$) and higher predictive performance ($Q^2_{F1} = 0.761$, $Q^2_{F2} = 0.759$). The study revealed that hydrophobicity is the most impactful property contributing to intoxication. The crucial toxicophores and non-toxicophores identified by the models will provide deep insights into environmental risk assessment. Screening the Pesticides Properties Database (PPDB) helped to prioritize the potential toxicants. The study thus provides a sustainable framework for the early identification of potential hazards to safeguard this sentinel species and, consequently, the marine ecosystems.



1. Allen, K.L., Ihde, T., Knoche, S. et al. (2023). *Estuarine, Coastal and Shelf Science*, 292, 108465.

LITBIOME: AN INTEGRATED WEB SERVICE FOR BIOMEDICAL TEXT MINING

N.Y. Biziukova, A.V. Rudik, O.A. Tarasova

Institute of Biomedical Chemistry, Moscow, Russia

The growing amount of biomedical literature makes manual extraction and integration of published knowledge increasingly difficult. Existing text-mining tools commonly address individual natural language processing tasks, whereas biomedical research requires several consecutive stages, including entity recognition, relation extraction, entity normalization, evidence aggregation, and interpretation of the resulting associations. The objective of this study was to develop Litbiome, a web-based service integrating these stages into a unified workflow for automated analysis of user-defined biomedical text collections.

The Litbiome workflow combines machine learning and rule-based approaches. HunFlair2 [1] and scispaCy [2] are used to recognize chemicals, genes/proteins, diseases, organisms, cell lines, tissue and organ mentions. Regular expression modules recognize miRNAs and genetic variants, including SNP identifiers, star alleles, deletions, insertions, and other mutation expressions. Relation extraction uses manually defined lexical and semantic triggers: words, word forms, and short phrases, most commonly verbs and verbal constructions, used by authors to indicate a relationship between biomedical terms. The detected trigger and entity types determine the predicate assigned to the entity pair. Contextual patterns additionally identify statement modality, distinguishing positive, negative, and probabilistic associations. Each extracted relation receives a distance-based score: relations are ranked higher when the linked entities and the trigger occur closer to one another in the sentence. The score is used to prioritize stronger textual evidence and reduce the contribution of distant, potentially less reliable matches.

Recognized entities are normalized to records in entity-specific biomedical databases. Candidate identifiers are retrieved using complementary query strategies and evaluated against the recognized mention and database synonyms; exact and near lexical matches are prioritized, while semantic similarity is used when lexical matching is insufficient. Identical normalized relations extracted from different sentences and publications are subsequently aggregated. The amount of supporting evidence, relation-level and normalization estimates are combined to the so-called confidence score to prioritize recurrent and consistently supported associations. Source publications and sentence-level evidence remain linked to every relation, enabling traceability and interpretability.

Unlike our previous task-specific implementations, Litbiome integrates these procedures in a generalized web environment and extends the range of supported biomedical entities and relations. Earlier versions of the approach for the automated analysis of text were applied to the extensive literature-based search of pharmacological effects, drug combinations, and repurposing opportunities of ACE inhibitors [3], virus-host-drugs interactions [4], and identification of molecular associations involving the Hedgehog signaling pathway in neoplastic processes [5]. These applications demonstrate transferability across pharmacological, infectious-disease, and molecular-pathway research tasks. Litbiome consolidates the developed methods into a reusable platform for systematic exploration of biomedical literature and generation of traceable, structured data for further computational analysis.

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1. Sanger, M., Garda, S., Wang, X.D. et al. (2024). *Bioinformatics*, 40, btae564.
2. Neumann, M., King, D., Beltagy, I., Ammar, W. (2019). *Proceedings of the 18th BioNLP Workshop and Shared Task*, 319–327.
3. Biziukova, N.Y., Savosina, P.I., Druzhilovskiy, D.S. et al. (2026). *International Journal of Molecular Sciences*, 27, 2044.
4. Tarasova, O.A., Biziukova, N.Y., Stolbova, E.A. et al. (2024). *Biomeditsinskaya Khimiya*, 70, 469–474.
5. Biziukova, N.Y., Ivanov, S.M., Tarasova, O.A. (2024). *Big Data Mining and Analytics*, 7, 107–130.

PREDICTOR-GUIDED DE NOVO DESIGN OF NDM-1 INHIBITORS FEATURING REWARD-DRIVEN ZINC-BINDING MOTIFS

I.A. Burov¹, D.D. Kholmanskikh^{1,2}, M.Yu. Shneider¹, M.G. Khrenova^{1,3}

¹Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia

²N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia

³A. N. Bach Institute of Biochemistry, Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Moscow, Russia

New Delhi metallo- β -lactamase 1 (NDM-1) hydrolyzes most β -lactam antibiotics, including last-line carbapenems. Its active site contains two zinc ions; therefore, optimization of predicted affinity alone does not ensure plausible binding. Candidate inhibitors must coordinate zinc while retaining drug-like properties and synthetic accessibility. We developed a predictor-guided de novo design workflow that generates molecules with a prescribed number of canonical zinc-binding groups (ZBGs) and prioritizes them using metal-aware ensemble docking.

A protein–ligand neural network provided predicted pIC_{50} values to optimize a variational autoencoder (VAE) in three independent modes with exactly zero, one, or two predefined zinc-binding groups (tetrazole, carboxylate, sulfonamide, and thiol). Accepted molecules satisfied predicted $\text{pIC}_{50} \geq 8.0$, quantitative estimate of drug-likeness (QED) > 0.60 , retrosynthesis-based synthetic accessibility (BR-SA) < 4.0 , the requested ZBG count, and no pan-assay interference compound (PAINS) alerts. REINVENT was evaluated as a baseline with a threshold-aligned reward, three seeds, and 3,000 optimization steps per seed and mode. Chemical space was compared with 2,207 ChEMBL metallo- β -lactamase compounds and 100 compounds reported active in curated direct NDM-1 PubChem assays.

We selected 1000 unique accepted molecules from each generator for each ZBG mode, balancing chemical-space coverage against the cost of docking. Every candidate was docked with AutoDock4Zn against four NDM-1 conformations from molecular dynamic simulation. Candidates were ranked by their mean ligand efficiency and further evaluated for steric clashes and Bemis–Murcko scaffold diversity. Predicted pIC_{50} and docking score showed weak rank correlation across the three ZBG modes (Spearman $\rho = 0.07$ – 0.13), confirming that the two models provide complementary selection signals.

Ligand efficiencies and docking scores are reported as medians [Q1–Q3]. In ZBG=0, the VAE achieved higher ligand efficiency than REINVENT (0.713 [0.674–0.761] versus 0.696 [0.647–0.752]), while their docking scores were comparable (-18.8 [-19.6 to -17.9] versus -18.6 [-19.3 to -17.9] kcal mol⁻¹). The VAE advantage increased in ZBG=1: ligand efficiency reached 0.758 [0.716–0.806] versus 0.710 [0.671–0.756], with docking scores of -18.4 [-19.1 to -17.7] and -18.3 [-19.0 to -17.7] kcal mol⁻¹, respectively. REINVENT performed better in ZBG=2, reaching a ligand efficiency of 0.739 [0.705–0.774] and a docking score of -19.8 [-20.5 to -19.1] kcal mol⁻¹, compared with 0.704 [0.655–0.759] and -18.0 [-18.6 to -17.6] kcal mol⁻¹ for the VAE. The PubChem-active reference set had a ligand efficiency of 0.773 [0.709–0.823] and a docking score of -18.0 [-18.8 to -17.4] kcal mol⁻¹. In ZBG=2, the VAE retained 423 Bemis–Murcko scaffolds versus 297 for REINVENT, while the dominant scaffold represented 9.6% and 31.4% of the respective pools. The VAE covered seven of 41 PubChem-active scaffolds, compared with one for REINVENT. Of the 100 PubChem-active compounds, 15 had a VAE-generated analogue with Tanimoto similarity ≥ 0.5 , whereas none had a REINVENT analogue above this threshold.

The developed workflow integrates de novo generation, predicted NDM-1 activity, medicinal-chemistry filters, and ensemble docking. Relative to REINVENT, the VAE produced higher ligand efficiency in ZBG=0 and ZBG=1 and greater scaffold diversity and coverage of PubChem-active chemical space in ZBG=2. The resulting prioritized compounds are ready for synthesis and biochemical testing against NDM-1.

ADMETOX-AI: MULTI-TASK DEEP LEARNING PLATFORM FOR COMPREHENSIVE ADMET PROPERTY PREDICTION

R. Bykadorov

ADMETox.AI, Rostov-on-Don, Russia

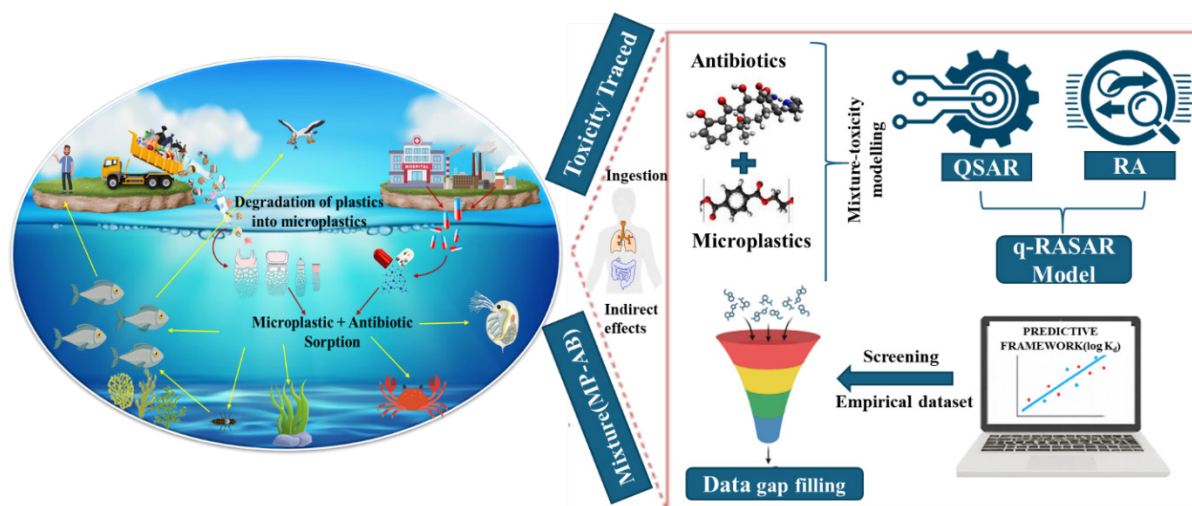
Accurate ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) prediction is critical for reducing late-stage drug attrition, yet existing computational tools offer limited accuracy and narrow applicability. Experimental profiling remains costly (\$350K per 1,000 compounds for a full panel) and low-throughput, preventing routine early-stage screening. We present ADMETox-AI, a deep learning platform combining graph attention networks (4 layers, 8 attention heads) with transformer encoders (6 layers, 8 heads, dim 512) for simultaneous prediction of 35 ADMET endpoints. Multi-task learning with uncertainty-weighted loss optimization enables effective knowledge sharing across endpoints. Training used 2.1M compounds from public (PubChem, ChEMBL, Tox21) and proprietary sources. ADMETox-AI achieves mean AUC 0.945 across all endpoints, significantly outperforming reference tools (RDKit 0.71, DeepChem 0.85, ChemProp 0.88). Per-endpoint AUC ranges from 0.91 (plasma protein binding) to 0.97 (AMES mutagenicity). Throughput reaches 1,000 compounds in 26 seconds on a single GPU, enabling million-compound library screening in under 8 hours at \$2 per 1,000 compounds - a 99.9% cost reduction versus experimental assays. Prospective validation on 50 clinical candidates achieved 92% accuracy. ADMETox-AI demonstrates that modern deep learning architectures can deliver production-ready ADMET prediction at scale, making comprehensive profiling accessible throughout the drug discovery pipeline. The platform is available for collaborative evaluation.

BIOMARKER-GUIDED Q-RASAR MODELING OF ANTIBIOTICS-MICROPLASTICS MIXTURES: TOWARDS PREDICTIVE AQUATIC TOXICOLOGY AND RESISTANCE RISK ASSESSMENT

S. Das, P.K. Ojha

Drug Discovery and Development Laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

Microplastics (MPs) and antibiotics are emerging pollutants that frequently co-occur in aquatic environments, where their interactions intensify ecotoxicological risks and may accelerate the spread of antibiotic resistance [1]. Experimental assessment of such sorption-driven environmental behavior of mixtures is costly, time-intensive, and ethically constrained, underscoring the need for predictive computational approaches. We have reported a Partial Least Squares (PLS)-based quantitative Read-Across Structure-Activity Relationship (q-RASAR) framework to evaluate the sorption-driven toxicity of antibiotics-microplastics mixtures. This study presents the first framework tailored to estimate the combined toxicity of antibiotics-microplastic mixtures, representing a methodological and conceptual advance in environmental toxicology. The distribution coefficient ($\log K_d$) was selected as the endpoint, reflecting partitioning between aqueous and plastic phases, a key determinant of environmental persistence and bioavailability. A curated dataset of antibiotics-microplastics mixtures was used to generate mixture descriptors based on additivity, squared, and norm-based rules. Descriptor reduction and q-RASAR integration yielded a final model with five hybrid descriptors (structural + similarity-based). The model exhibited strong internal robustness ($Q^2_{\text{LOO}} = 0.761$) and high external predictivity ($Q^2_{\text{F1}} = 0.832$; $\text{MAE}_{\text{test}} = 0.152$). Applicability domain and Y-randomization confirmed statistical soundness. Mechanistic analysis indicated that hydrogen-bond donors, oxygen-based polar functionalities, and terminal unsaturation enhance adsorption, while steric hindrance and charge asymmetry reduce binding affinity. To address limited experimental data, we further designed 111 hypothetical mixtures and validated predictions using the Prediction Reliability Indicator (PRI) tool. Most predictions were classified as “Good” and within the applicability domain, confirming the framework’s scalability and reliability. By combining mechanistic interpretability with strong predictive power, the framework enables high-throughput virtual screening and supports early-stage environmental risk assessment, data-gap filling, and regulatory decision-making in line with OECD guidelines. This predictive framework can also help to mitigate antibiotic resistance by identifying high-risk antibiotic-microplastic associations early, thereby informing regulatory controls and pollutant management strategies.



1. Wang, Y., Yang, Y., Liu, X., Zhao, J., Liu, R., Xing, B., (2021). *Environmental Science & Technology*, 55(23), 15579-15595.

INTEGRATIVE QSAR, STRUCTURAL BIOPHYSICS, AND NETWORK PHARMACOLOGY PIPELINE FOR MULTITARGET ANTICANCER DRUG DISCOVERY

A. Dehimat

*Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila,
University Pole, M'sila, Algeria*

The exponential expansion of commercially available chemical libraries renders exhaustive high-throughput docking computationally prohibitive. Moreover, the multifactorial nature of oncogenic signaling necessitates multitarget strategies, yet conventional 2D-QSAR approaches often fail to capture the steric and electrostatic requirements of protein–ligand recognition. This study introduces a pragmatic, fully integrated computational pipeline that synergistically combines 3D-QSAR modeling, machine learning (ML) pre-filtering, molecular docking, and explicit-solvent molecular dynamics (MD) to prioritize anticancer leads with significant efficiency gains. Network pharmacology analysis (STRING/Cytoscape) identified EGFR, Akt-1, and STAT3 as hub targets in tumor progression and resistance. A comparative 3D-QSAR workflow (CoMFA and CoMSIA) was established using a curated set of 1,800 bioactivity data points from the ChEMBL database, yielding robust external predictive performance (CoMFA $q^2 = 0.68$, $r^2_{\text{pred}} = 0.81$). Subsequently, a Gradient Boosting Machine (GBM) classifier trained on extended-connectivity fingerprints achieved a ROC-AUC of 0.91 and an external accuracy of 86% (Kappa = 0.72), enabling rapid pre-screening of the ZINC15 library (12 million compounds) to isolate 2,000 predicted actives. These hits underwent flexible molecular docking (AutoDock Vina) against the target binding pockets (EGFR-PDB:5HG8, Akt-1-PDB:1UNQ), with protocol validation via co-crystal redocking (RMSD = 1.3 Å). The top-ranked candidates were advanced to 200 ns explicit-solvent MD simulations (GROMACS/OPLS-AA), with binding free energies rigorously estimated via MM-PBSA and MM-GBSA cross-validation. Prioritization identified a pyrimidine-benzimidazole scaffold as the most promising lead, maintaining stable interactions with the EGFR hinge residue Met793 (H-bond occupancy > 85%) and the Akt-1 catalytic lysine. The cross-validated MM-PBSA binding free energy for this hit was -10.2 kcal/mol, a physically realistic value consistent with low-micromolar affinity. ADMET profiling (SwissADME, ProTox-II) predicted favorable oral bioavailability (Lipinski violations = 0), low hepatotoxicity, and the absence of PAINS alerts. Critically, absolute binding free energy perturbation (FEP) calculations and extended conformational analyses are currently ongoing; the definitive thermodynamic and kinetic profiles for the prioritized lead series will be delivered at the time of the oral presentation. The complete findings are intended for submission as a full research article to an international peer-reviewed computational journal. This integrated ML/3D-QSAR-to-Dynamics workflow demonstrates that intelligent pre-filtering substantially accelerates hit identification and de-risks downstream validation, offering a rapid, cost-effective, and physically sound platform for multitarget antitumor drug discovery.

1. Nilewar, S.S., Chobe, S., Dudhe, P., Kumar, P.K., Lodha, S., Raut, A.D., Fernández-Conde, D., Farhan, M., Muteeb, G., Pawar, T.J. (2026). *Pharmaceuticals*, 19(2), 249.
2. van Pinxteren, D.J.M., Jespers, W. (2025) *Journal of Chemical Information and Modeling*, 65(19), 9856-9864.
3. Sharma, D., Arumugam, S. A (2025). *Computers in Biology and Medicine*, 197(Pt B), 111068.
4. Das, U., Chanda, T., Kumar, J., Peter, (2025). *Computational Biology and Chemistry*, 117, 108427.
5. El Rhabori, S., Alaqarbeh, M., Naanaai, L., EL Allouche, Y., El Aissouq, A., Bouachrine, M., Zaitan, H., Chtita, S., Khalil, F. (2025) *New Journal of Chemistry*, 49, 14748-14768.
6. Verma, J., Khedkar, V.M., Coutinho, E.C. (2010). *Current Topics in Medicinal Chemistry*, 10(1), 95-115.

A POCKET-CENTRIC TRAIN-TEST SPLIT REDUCES DATA LEAKAGE IN STRUCTURE-BASED DEEP LEARNING

S. Evteev^{1,2}, A. Ereshchenko^{1,2}, D. Adjugim³, Ya. Ivanenkov^{1,2}

¹P. Hertsen Moscow Oncology Research Institute, Moscow, Russia

²MED CHEM Ltd., Moscow, Russia

³Yandex School of Data Analysis, Moscow, Russia

Machine learning is increasingly used in structural biology and structure-based drug design for binding-site identification [1, 2], molecular docking [3, 4], and ligand–protein affinity prediction [5, 6]. However, the reliability of these models depends critically on the way datasets are divided into training and test subsets. Random or release-date-based partitioning may place highly similar proteins, ligands, or binding environments in both subsets, resulting in data leakage and an overestimation of model performance. Sequence- and ligand-based approaches reduce particular forms of overlap but do not directly assess the structural redundancy of ligand-binding pockets.

We systematically evaluated commonly used train-test splitting strategies and developed a binding-site similarity method for constructing structurally nonredundant datasets. We analyzed 30 recent studies published between 2023 and 2025 that applied machine learning to structural biology and structure-based drug design. In parallel, we assembled a dataset of 48,177 ligand-protein complexes from the Protein Data Bank and compared random, release-date, protein sequence identity, ligand similarity, combined sequence-ligand, and binding-site similarity partitioning strategies.

For the proposed pocket-centric method, each binding site was defined as the set of amino acid residues located within 7 Å of the ligand. The local environment was represented by an 840-dimensional vector describing residue composition and pairwise spatial relationships, with separate descriptors for main-chain and side-chain arrangements. Similarity between binding sites was then quantified using an extended Tanimoto coefficient, without requiring sequence or structural alignment.

Half of the reviewed publications did not apply explicit similarity filtering when constructing training and test sets. In the benchmark analysis, random and release-date splits retained extensive overlap between the two subsets. Sequence-based splitting reduced global target similarity but did not reliably identify structurally conserved pockets. Ligand-based splitting minimized chemical overlap but left substantial redundancy at the protein and binding-site levels. The combined sequence-ligand strategy improved control of several leakage metrics but remained computationally demanding and did not completely eliminate pocket-level similarity. By contrast, pocket-centric partitioning reduced the mean binding-site similarity (26% vs. 81%) compared to random splitting.

The proposed pocket-centric representation provides a fast, interpretable, and alignment-free approach for reducing local structural redundancy in machine-learning datasets. Binding-site similarity splitting may therefore support more realistic evaluation of model generalizability and applicability domains in structural biology and structure-based drug design.

1. Evteev S.A., Ereshchenko A.V., Ivanenkov Ya.A. (2023). *J. Chem. Inf. Model.*, 63, 1124.
2. Jeevan K., Palistha S., Tayara H., Chong K.T. (2024). *J. Cheminform.*, 16, 66.
3. Lai H., Wang L., Qian R., et al. (2024). *Nat. Commun.*, 15, 10223.
4. Thaingtamtanha T., Preto J., Gentile F. (2025). *Chem. Sci.*, 16, 19876.
5. Evteev S., Ereshchenko A., Adjugim D., et al. (2025). *Proteins*, 93, 1269.
6. Mqawass G., Popov P. (2024). *J. Chem. Inf. Model.*, 64, 2323.

ARTIFICIAL INTELLIGENCE IN DRUG DISCOVERY: FROM DRUG–TARGET INTERACTIONS AND SYNERGISTIC DRUG COMBINATIONS TO GENERATIVE MOLECULAR DESIGN

S. Gharaghani

Department of Bioinformatics, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

The rapid growth of chemical, structural and biological data is creating new opportunities for artificial intelligence (AI) in computer-aided drug discovery. However, effective drug discovery requires integration of complementary computational strategies rather than reliance on a single predictive model. This presentation highlights an integrated AI-driven framework spanning drug–target interaction (DTI) prediction, synergistic drug combination prediction and generative molecular design.

Our research has combined chemoinformatics, quantitative structure–activity relationship modeling, molecular docking and molecular dynamics with machine learning, deep learning, biological network analysis and knowledge-based approaches. Particular emphasis is placed on DTI prediction, where representation learning, autoencoder-based models, contrastive learning and graph-oriented strategies can integrate heterogeneous information on drugs and biological targets. These approaches provide a basis for moving from isolated activity prediction toward a broader understanding of polypharmacology and target–pathway relationships. Building on this perspective, AI models can also be used to identify potentially synergistic drug combinations by integrating molecular, pharmacological and biological-context information, thereby supporting more rational combination therapy and drug repurposing.

A further step is generative molecular design, in which AI models are used not only to predict properties of existing compounds but also to generate new candidate molecules with desired biological and physicochemical characteristics. The presentation will discuss how these complementary approaches can be organized into an end-to-end workflow connecting target and pathway analysis, DTI prediction, combination prioritization, molecular generation and multi-parameter optimization. Particular attention will be given to realistic model evaluation, including external and scaffold-aware validation, prevention of data leakage, uncertainty assessment and experimental validation.

Together, these approaches illustrate how AI can transform computer-aided drug discovery from prediction of individual molecular interactions toward integrated, data-driven strategies for discovering, combining and designing therapeutic molecules.

A MACHINE LEARNING-DRIVEN BIOPROSPECTING OF CHOLINERGIC MEDICINAL PLANTS FOR PUTATIVE LEADS AGAINST BUTYRYLCHOLINESTERASE TOWARD ALZHEIMER'S CARE

G.A. Gyebi¹, O.O. Ones², S. Sabiu¹

¹Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, Durban, South Africa

²Department of Biochemistry, Faculty of Science and Technology, Bingham University, Karu, Nigeria

Butyrylcholinesterase (BChE) plays a key role in preserving appropriate cholinergic neurotransmission that is essentially altered in the brains of advanced Alzheimer's disease (AD), hence a therapeutic target. This study employed a machine learning bioactivity predictive model to explore the chemical space of potential BChE inhibitors. A cheminformatics pipeline was explored to create a machine learning (ML) model for BChE inhibition using structural insights complemented with a comprehensive variance importance plot (VIP) and correlation matrix analysis. Specifically, a compiled library of 2,179 secondary metabolites (SMs) from 50 Nigerian medicinal plants with reported cholinergic activity was investigated using the ML model. After which, molecular modelling was used to further screen the active SMs (827). The final predicted models demonstrated significant robustness, with a correlation coefficient of 0.8981. Molecular docking investigation of the 827 SMs identified the top five candidates based on their scores: three triterpenoids (adipedatol, lupenone, β -amyrin), one steroid (9(11)-dehydroergosteryl benzoate), and one flavonoid (tiliroside). These leads exhibited favourable ADMET properties and promising safety profiles. Among these five, lupenone (-48.78 kcal/mol), β -amyrin (-49.19 kcal/mol), and adipedatol (-48.87 kcal/mol), from *Peltophorum pterocarpum*, *Bryophyllum pinnatum* and *Alcyone laxiflora* respectively, were the most promising leads with significant binding free energy compared to decamethonium (-17.64 kcal/mol) and more favourable van der Waals, electrostatics and non-polar solvation energetics. Furthermore, the binding of these leads resulted in optimised interaction profiles that preserved the structural integrity of the BChE and aligned well with desirable drug-like characteristics. These findings position the leads as promising candidates for therapeutic applications targeting BChE for Alzheimer's disease management, subject to further in vitro and in vivo validation investigations.

DIRECTED IN-SILICO SEARCH FOR SPECIFIC BINDING WARHEADS TARGETING CEREBLON-CONTAINING E3 LIGASES

E. Grigoreva¹, D. Shulga^{1,2}

¹*Moscow Institute of Physics and Technology (National Research University), Moscow, Russia*

²*Chemical Department, Lomonosov Moscow State University, Moscow, Russia*

Targeted protein degradation has emerged as a powerful therapeutic strategy that exploits the ubiquitin–proteasome system (UPS) to selectively eliminate disease-associated proteins [1]. Cereblon (CRBN), a substrate receptor of the CRL4^{CRBN} E3 ubiquitin ligase complex, has attracted considerable attention as a key component of this approach due to its ability to mediate the degradation of proteins involved in cancer progression, including transcription factors and signaling regulators [2]. CRBN containing E3 ligases are one of the most widely used E3 ligases in the design of proteolysis-targeting chimeras (PROTACs), bifunctional molecules that recruit target proteins to the ubiquitination machinery and promote their proteasomal degradation. Compared with conventional inhibitors, PROTACs offer the potential for sustained target removal and may help overcome mechanisms of drug resistance [3]. However, currently available CRBN recruiters are largely limited to thalidomide-derived scaffolds. Despite their success, these compounds possess several drawbacks, including restricted chemical diversity, suboptimal physicochemical properties, and the potential for off-target effects, highlighting the need for novel CRBN-binding ligands with improved properties [4].

The aim of this work was to find novel chemical moieties capable of specifically binding to CRBN-containing E3 ligases using computational approaches. To achieve this goal, structures of CRBN complexes available in the Protein Data Bank were analyzed, including complexes with thalidomide, lenalidomide, pomalidomide and other ligands. Comparative analysis of known CRBN ligands revealed common interaction patterns within the binding pocket, including a combination of hydrophobic contacts and hydrogen-bonding interactions. Based on these observations, virtual screening experiments were performed using a focused CELMoD compound library and a fragment-based diversity screening library obtained from ChemDiv and Life Chemicals respectively. The docking experiments enabled prioritization of compounds according to predicted binding affinity and ligand efficiency. Several candidates demonstrated favorable docking scores, with the best-ranked molecules exhibiting predicted binding affinities below –9 kcal/mol and high ligand-efficiency values.

The obtained results indicate that the CRBN binding site can accommodate structurally diverse chemotypes while maintaining key intermolecular interactions required for stable complex formation. The identified compounds represent promising starting points for the development of novel CRBN warheads. However, molecular dynamics simulations are crucial to evaluate the stability of the predicted complexes, which we are going to show in the nearest future. The proposed computational workflow provides an efficient strategy for discovering new CRBN-targeting ligands and may contribute to the expansion of the chemical space available for targeted protein degradation technologies.

1. Zhao, L., et al. (2022). *Signal transduction and targeted therapy*, 7, 113.
2. Kim, H.K., et al. (2016). *Pflügers Archiv-European Journal of Physiology*, 468: 1299-1309.
3. Békés, M., Langley D.R., Crews C. M. (2022). *Drug discovery*, 21, 181-200.
4. Ito, T. et al. (2010). *Science*, 327, 1345-1350.

INTEGRATING PASS, LLM ANALYSIS, AND MOLECULAR DOCKING TO UNCOVER A TARGET SWITCH IN PYRIMIDINE DERIVATIVES

T.A. Grigoreva^{1,2}, A.S. Skrylkova³, D.M. Egorov³, V.G. Tribulovich^{1,2}

¹Laboratory of Molecular Pharmacology, St. Petersburg State Institute of Technology (Technical University), St. Petersburg, Russia

²Department of Medicinal Chemistry, St. Petersburg State Institute of Technology (Technical University), St. Petersburg, Russia

³Department of Organic Chemistry, St. Petersburg State Institute of Technology (Technical University), St. Petersburg, Russia

3,4-Dihydropyrimidine-2(1H)-thiones (DHPMs) are widely recognized as a privileged structural scaffold, classically known for their allosteric inhibition of the mitotic kinesin Eg5 (e.g., monastrol). In this study, we designed novel structural analogs through cyclization into thiazolo[3,2-a]pyrimidines and the introduction of a phosphonate moiety. This structural evolution allowed us to transform the flexible scaffold into a planar system with a hydrophobic anchor and polar cap.

To evaluate the biological potential of the designed compounds, we employed a multi-tiered computational pipeline. Initially, the PASS software was utilized for probabilistic activity prediction, revealing strong antimitotic and pro-apoptotic potential ($P_a > 0.55$, $P_i < 0.05$). Subsequently, a Large Language Model (LLM) was used for deep structural analysis, cross-referencing chemical features with biological targets, and hypothesis generation. The protein target selection was guided by two complementary criteria: consistency with PASS predictions and structural compatibility with the key pharmacophoric features of the designed molecules. Following manual validation of each proposed target, five targets were selected for molecular docking validation: Eg5, Mcl-1, MDM2, Akt1, and PTP1B, covering both the predicted primary mechanisms and potential off-target liabilities.

Finally, molecular docking (Schrödinger, Glide SP/XP, OPLS3e force field) was performed against a panel of predicted targets. For phosphonate-containing compounds, two protonation states were prepared: neutral dimethyl esters and hydrolyzed OH-forms to model prodrug activation. Docking studies revealed high affinity of the designed compounds for Mcl-1 and MDM2 (up to -8.5 and -7.8 kcal/mol, respectively), supporting their potential as PPI-targeting anticancer agents. The proposed compounds demonstrate a pronounced selectivity profile, exhibiting low affinity for the compact catalytic sites of classical kinases such as Akt1 and phosphatases such as PTP1B.

Remarkably, the structural modification triggered a switch in Eg5 inhibition mode: unlike parent DHPMs binding to the allosteric site, the hydrolyzed phosphonates simultaneously occupy both catalytic and allosteric regions, improving the score by 3.0 kcal/mol (from -6.1 to -9.4 kcal/mol).

Subsequent *in vitro* evaluation on tumor cell lines confirmed the enhanced cytotoxic potential of the designed compounds. Combined with the computational results, these findings validate the novel thiazolopyrimidine phosphonates as promising scaffolds for the development of effective antineoplastic agents.

GENOMIC AND THERAPEUTIC INSIGHTS INTO MULTIDRUG-RESISTANT *VIBRIO PARAHAEMOLYTICUS* BD001 STRAIN ISOLATED FROM SHRIMP (*PENAEUS MONODON*) AHPND: *IN VITRO*, *IN VIVO*, AND *IN SILICO* APPROACHES

Md. Iftehimul¹, S.A. Pranto³, N.A. Hasan², M.M. Haque⁴, M.T. Hossain³, Md.S. Islam¹

¹Institute of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh

²Department of Fisheries and Marine Bioscience, Gopalganj Science and Technology University, Gopalganj, Bangladesh

³Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh

⁴Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

The isolated multidrug-resistant (MDR) *Vibrio parahaemolyticus* strain BD001 from acute hepatopancreatic necrosis disease (AHPND) infected shrimp (*Penaeus monodon*) poses a major threat to aquaculture sustainability and public health due to its broad antibiotic resistance and horizontal gene transfer potential. The BD001 strain was characterized by PCR targeting the *groEL* gene, antibiotic susceptibility testing, and whole-genome sequencing (WGS) to determine genome size, ARGs, and MGEs of BD001. To explore an alternative treatment, the antibacterial activity of Chinese Rangan (*Ixora chinensis*) extract was assessed via *in vitro* and *in vivo* Swiss albino mice model. GC-MS analysis identified bioactive phytochemicals, while *in silico* pharmacophore modeling, molecular docking, ADMET profiling, and molecular dynamics simulations evaluated the bioactivity and stability of the key compounds. Among 150 collected shrimp samples, 8% tested positive for *V. parahaemolyticus*, with strain BD001 showing resistance to 14 antibiotics, including complete resistance to doxycycline. The assembled genome was 5.1 Mbp, with 149.33x coverage, 45.21% GC content, 6 ARGs, 4 Vir genes, 1 plasmid and bacteriocins, 54 bacteriophages, and a pan-core genome ratio of 27.84%. The *I. chinensis* extract exhibited strong antibacterial activity with inhibition zones up to 23 mm against the BD001 strain, and GC-MS identified 41 phytochemicals in the extract. *In vivo* treatment lowered bacterial load and normalized liver enzymes and ALP levels towards normal in infected mice. *In silico* analysis further identified six top phytochemicals with high pharmacophore fit scores (78.08-78.69) and strong docking affinities (up to -8.9 kcal/mol) against *bla**VEB-1*. ADMET analysis predicted favorable drug-like properties and low toxicity. Molecular dynamics simulations confirmed stable protein-ligand complexes. These findings suggest that phytochemicals from *I. chinensis*, particularly compounds such as olean-12-ene and β -amyrone, hold strong potential as alternative therapeutic agents against MDR *V. parahaemolyticus*, offering promise for novel antibacterial resistance drugs to enhance aquaculture health and reduce public health risks.

STRUCTURE-BASED PRIORITIZATION OF DIKETOPIPERAZINE SCAFFOLDS FOR CLASS A GPCR RECOGNITION

S. Jafari, J.Bojarska

*Institute of General and Ecological Chemistry, Faculty of Chemistry,
University of Technology, Lodz, Poland*

Diketopiperazines (DKPs) are conformationally restricted cyclic peptide-derived scaffolds that combine a defined three-dimensional geometry with hydrogen-bonding, hydrophobic and aromatic interaction features. Although DKP derivatives have been investigated against individual biological targets, systematic comparison of structurally distinct DKP scaffold classes across different G protein-coupled receptors (GPCRs) remains limited.

In this study, three structurally different DKP/DKP-like scaffold groups derived from experimentally determined crystal structures were screened against a panel of Class A GPCRs using molecular docking and protein–ligand interaction analysis. The aim was not simply to identify the lowest docking score, but to determine how scaffold architecture influences receptor-pocket recognition and to prioritise receptor–ligand systems for subsequent molecular dynamics simulations.

The preliminary screening revealed clear receptor-dependent differences in DKP recognition. Among the investigated systems, the adenosine A1 receptor (A1AR) emerged as a particularly promising target for classical DKP scaffolds, with JUNYAV showing a docking score of -9.08 kcal/mol. The closely related A2AR was selected as a potential subtype comparator, while structurally distinct DKP-like scaffolds provide an additional means of assessing the influence of scaffold architecture.

The next stage focuses on membrane molecular dynamics and advanced sampling of selected complexes to determine whether the docking-derived recognition patterns remain dynamically stable and whether alternative or metastable binding states occur. This workflow provides a structure-based strategy for prioritising GPCR targets and DKP scaffolds for further computational and experimental investigation.

1. Jafari, S., Bojarska, (2025). J. TYGIEL, Scientific Publishing House.
2. Bojarska, J., et al. (2026). *CrystEngComm*, 28(34), 4834–4846
3. Bojarska, et al. (2021). *Biomolecules*, 11, 1515.
4. Spackman, P.R., et al. (2019). *Angew. Chem. Int. Ed.*, 58, 16780–16784
5. Adessi, C., Soto, C. (2002). *Curr. Med. Chem.*, 9, 963–978.
6. Morris, G.M., et al. (1998). *J. Comput. Chem.*, 19, 1639–1662
7. Roe, D.R., Cheatham, T. E. J. (2013). *Chem. Theory Comput.*, 9, 3084–3095

ACTION-BASED HYBRID KNOWLEDGE GRAPH LEARNING FOR DRUG REPURPOSING AND FUTURE DIRECTIONS TOWARD EXPLAINABLE AI

M. Khodadadi^{1,2}, S. Gharaghani¹

¹Laboratory of Bioinformatics and Drug Design (LBD), Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

²Pharmaceutical Engineering Research Laboratory, School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran

Drug repurposing has emerged as a promising strategy for accelerating therapeutic discovery by identifying new applications for existing drugs while reducing time and cost compared with traditional drug development. However, the heterogeneity of biomedical data remains major challenge for accurately predicting novel drug–disease associations. In this study, we present ConvAHKG, an action-based hybrid knowledge graph framework that integrates diverse biological relationships among compounds, proteins, diseases, and molecular features to improve drug repurposing prediction. By incorporating biologically meaningful relation types and leveraging a dual-channel convolutional architecture for feature learning, ConvAHKG captures complex patterns within heterogeneous biomedical networks and addresses challenges associated with data imbalance. The proposed framework demonstrated strong predictive performance, achieving an AUC of 0.9836 and an AUPRC of 0.9686, and successfully identified potential therapeutic candidates for non-small cell lung cancer. Despite the high predictive performance of AI models, their black-box nature remains a major challenge in biomedical applications, where understanding the reasoning behind predictions is essential. In the future perspective of this presentation, we will discuss emerging explainable artificial intelligence (XAI) approaches for knowledge graph-based drug repurposing, including attention-based, post-hoc, rule-based, path-based, and subgraph-based methods. These approaches aim to explain why a specific drug–disease association is predicted by revealing the underlying biological mechanisms, including relevant proteins, genes, and pathways contributing to the prediction. Such explanations can provide clinicians and pharmacists with meaningful biological evidence to evaluate AI-generated hypotheses, improve trust in model outcomes in therapeutic discovery.

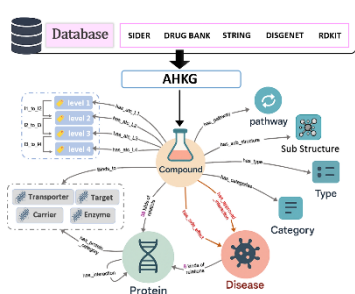


Figure 1. Overview of the action-based hybrid knowledge graph framework for drug repurposing

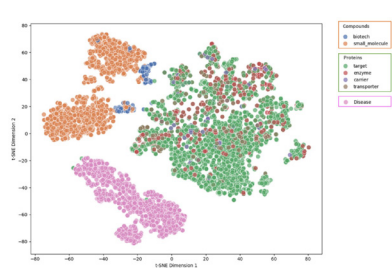


Figure 2. t-SNE representation demonstrates the distribution and separation of different entity types, including compounds, proteins, and diseases

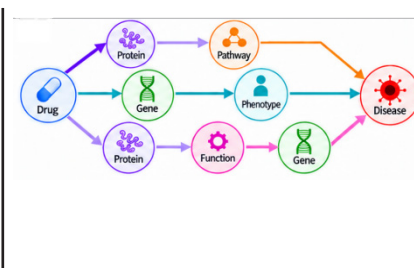


Figure 3. Path-based explanation strategy: identifying meaningful biological paths between predicted drugs-disease pairs

1. Khodadadi AghGhaleh, M., et al. (2026) *Scientific Reports*, 16(1), 7592.

EXPLAINABLE AI GUIDES LEAD OPTIMIZATION OF HUMAN DHFR INHIBITORS USING A PUBCHEM FINGERPRINT-BASED QSAR MODEL

I. Maattallaoui, N.E. Aouad

Faculty of Medicine and Pharmacy of Tangier, Abdelmalek Essaadi University, Tangier, Morocco

Proteins play essential roles in virtually all biological processes and represent major targets for therapeutic intervention in a wide range of diseases [1]. Among these targets, human dihydrofolate reductase (hDHFR) is a clinically important enzyme involved in folate metabolism and remains of considerable interest for the development of anticancer, antimicrobial, and antiprotozoal agents [2]. This study aimed to elucidate the molecular determinants underlying the predictions of our previously developed and externally validated PubChem fingerprint-based Random Forest QSAR model for hDHFR inhibitors [3].

Building upon this framework, the present work advances the model toward an interpretable medicinal chemistry approach using Explainable Artificial Intelligence (XAI) and SHapley Additive ExPlanations (SHAP), which quantify the contribution of individual features to model predictions [4].

Global SHAP analysis identified PubChemFP17 (mean absolute SHAP value = 0.11) as the most influential descriptor, followed by PubChemFP12 (0.10), PubChemFP528 (0.09), and PubChemFP659 (0.08). These fingerprints encode nitrogen- and carbon-rich patterns, an amino-type N–C–H₂ environment, and an O–C–C–N–C connectivity pattern, respectively. FP17 and FP12 predominantly contributed positively to predicted activity, whereas FP420, representing a carbonyl group, and FP607, corresponding to an N–C–C–C–aromatic carbon connectivity pattern, showed predominantly unfavorable contributions.

Local SHAP analysis further illustrates the contribution of these features to individual predictions. For the analyzed molecule, the predicted activity was 5.505 pIC₅₀ compared with an expected model output of 5.993 pIC₅₀. The absence of FP17, together with FP145, FP488, FP146, and FP593, predominantly decreased the prediction, whereas FP12 and FP659 contributed favorably.

The obtained results provide actionable SAR insights for preserving favorable structural features and modifying unfavorable patterns during lead optimization. This study extends the previously validated PubChem QSAR model from a predictive study into an explainable medicinal chemistry tool, highlighting the potential of XAI to support hDHFR inhibitor discovery and optimization.

1. Huang, Q., Mu, Q., Liu, N., Liu, G., Liu, C. (2026). *Advanced Interventional Materials*, 1(1), 100015.
2. Abali, E.E., Skacel, N.E., Celikkaya, H., Hsieh, Y. (2008). *Vitamins and Hormones*, 79, 267–292.
3. Maattallaoui, I., Sakho, M., Maattallaoui, A., Catalán, E.B., Aouad, N.E. (2025). *Molecules* 2025, 30, 4618.
4. Gangwal, A., Lavecchia, A.E (2026). *Computer Science Review*, 61, 100943.

MOLECULAR MODELING OF INTERACTIONS BETWEEN SGATIFLOXACIN ANTIBODY AND DIFFERENT FLUOROQUINOLONES

Y. Meteleshko^{1,2}, M. Khrenova^{1,2}, N. Byzova², A. Zherdev², B. Dzantiev², O. Hendrickson²

¹Chemistry Department, M.V. Lomonosov Moscow State University, Moscow, Russia

²A.N. Bach Institute of Biochemistry, Federal Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

Antibodies are widely used in different analytical systems for medical diagnostics, quality control and environmental monitoring due to their ability to selectively bind diverse targets with high affinity. However, antibodies against low-molecular-weight molecules (haptens) demonstrate cross-reactivity with structurally similar analogs. This complicates antibody applications when several derivatives of the antigen molecule are present. Therefore, it is important to find criteria which would allow us to predict if the molecule will be cross-reactive to the antibody against its structural analog.

In our work we investigated interactions between monoclonal antibody against s-gatifloxacin (SGAT-specific Mab) and a set of molecules from fluoroquinolone family of antibiotics (cinoxacin, ciprofloxacin, clinafloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, flumequine, garenoxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, nalidixic acid, ofloxacin, orbifloxacin, oxolinic acid, pazufloxacin, pefloxacin, pipemidic acid, rufloxacin, sarafloxacin, sparfloxacin, tosufloxacin). It was shown that some antibiotics from this set – ciprofloxacin, lomefloxacin and sarafloxacin are cross-reactive to SGAT-specific Mab while others are not [1]. The aim of our work was to find a descriptor which would allow us to differentiate between cross-reactive and non-cross-reactive fluoroquinolones.

Initially, the molecular docking simulations for SGAT-specific Mab and studied haptens were carried out. The obtained binding energies were too similar for cross-reactive and some non-cross-reactive haptens to be used to separate them. Then we performed classical molecular dynamics simulations for complexes of a part of SGAT-specific Mab with studied haptens to find out differences in their dynamical behavior. However, not only S-GAT and cross-reactive haptens were stably bound to the antibody during molecular dynamics trajectories, but most of non-cross-reactive haptens were stably bound to the antibody as well. There were no noticeable visible differences between complexes with cross-reactive and some non-cross-reactive haptens. Therefore, dynamical network analysis was applied to the molecular dynamic trajectories. To perform this analysis every hapten was divided into the nodes where one of the nodes contains atoms of quinolone ring. It was found that the sum of betweenness (betweenness centrality, a descriptor, which measures the importance of a node in the whole network) between a node corresponding to the quinolone ring and nodes representing amino acids of protein is higher for cross-reactive haptens and in most cases lower for non-cross-reactive ones. No significant quantitative correlation was found between cross-reactivities and the sum of betweenness for the quinolone ring node. But we were able to propose a cut-off criterion for separating non-cross-reactive haptens from cross-reactive ones (with two false positive results for difloxacin and orbifloxacin). We believe that proposed cut-off criterion is suitable for screening purposes to exclude haptens that will not be cross-reactive to SGAT-specific Mab.

This study was funded by the Russian Science Foundation (project 24-46-00026).

1. Meteleshko, Y., Khrenova, M., Byzova, N. et al. (2025). *Int. J. Mol. Sci.*, 26, 11862.

AN INTEGRATED BIBLIOMETRIC AND HUMAN TRANSCRIPTOMIC WORKFLOW FOR DRUG REPURPOSING IN PARKINSON'S DISEASE

E. Zhao¹, S. Poluri², A. Bodla³, E. Govil⁴, Y. Song⁵, A. Kato⁶, J. Kam⁷, A. Kura⁸, A. Ogbeide⁹, P. Yadlapalli¹⁰, C. DiPrima¹¹, R. George¹²

¹Northeastern University, Boston, USA

²Round Rock High School, Round Rock, USA

³Downingtown STEM Academy, Downingtown, USA

⁴Briar Woods High School, Ashburn, USA

⁵International Academy East, Troy, USA

⁶Flower Mound High School, Flower Mound, USA

⁷Westwood High School, Austin, USA

⁸Rock Hill High School, Frisco, USA

⁹Franklin High School, Reistertown, USA

¹⁰Del Norte High School, San Diego, USA

¹¹Loyola Academy, Wilmette, USA

¹²University of Houston, Houston, USA

Dysfunction of PINK1/Parkin-mediated mitophagy contributes to mitochondrial pathology in Parkinson's disease (PD). Pathogenic variants in PINK1 and PRKN, which encodes Parkin, cause autosomal recessive early-onset PD. To characterize translational gaps in the PINK1/Parkin mitophagy literature, a PRISMA-guided Web of Science search [1] identified the 100 most-cited studies investigating PINK1/Parkin-mediated mitophagy. Among 63 primary studies, only 2 directly investigated human brain tissue, highlighting a translational gap. Consequently, our workflow explicitly prioritized human-derived datasets to identify FDA-approved drug repurposing candidates. Differential expression of USP30, PINK1, PRKN, and SNCA was assessed using web-facing GEO2R, utilizing limma in R across two Gene Expression Omnibus (GEO) datasets: human PD vs. control dopaminergic neurons (GSE20141; primary analysis) and human PD vs. control substantia nigra tissue (GSE7621; specificity assessment). None of the four assessed genes reached an FDR-adjusted $p < 0.05$. This finding is consistent with substantial post-translational regulation of PINK1/Parkin activity, including phosphorylation and ubiquitination [2], and prompted expansion to an iLINC signature-reversal analysis, in which the top 250 differentially expressed genes were defined as the disease-associated transcriptional signature to identify FDA-approved compounds predicted to reverse it. Three compounds, galantamine, albendazole, and lorazepam, demonstrated significant predicted signature reversal ($FDR < 0.05$) and were assessed for translational feasibility based on literature evidence of blood-brain barrier penetration and oral bioavailability. Galantamine demonstrated the strongest predicted signature reversal ($FDR = 0.010$) and was further supported by literature evidence of BBB penetration, oral bioavailability, autophagy induction, and reduced α -synuclein aggregation [3]. This integrated workflow combines bibliometric assessment, human transcriptomic analysis, and computational drug repurposing to address translational gaps in PD research. Galantamine emerged as a high-priority candidate for future experimental efficacy testing, while the limited representation of human brain tissue underscores the need for human-tissue validation to improve translational relevance and inform therapeutic development.

1. Page, M.J., McKenzie, J.E., Bossuyt, P.M., et al. (2021). *BMJ*, 372, 71.
2. Kane, L.A., Lazarou, M., Fogel, A.I., et al. (2014). *Journal of Cell Biology*, 205(2), 143-153.
3. Nozaki, S., Hijioka, M., Wen, X., et al. (2024). *Journal of Pharmacological Sciences*, 156(2), 102–114.

SPELL: AN INTERACTIVE PLATFORM FOR STRUCTURE-GUIDED LIGAND DESIGN AND FRAGMENT REPLACEMENT

D. Ryabchenko^{1,2}, P. Gurevich^{1,2}, S. Kadyrov¹, S. Nikolenko¹, D. Frolova^{1,2}, A. Shapeev², M. Pak¹

¹Ligand Pro, Moscow, Russia

²Skolkovo Institute of Science and Technology, Moscow, Russia

A medicinal chemist working on a bound ligand usually needs a local change: replace one fragment, keep the rest of the molecule, and stay compatible with the pocket [1]. Most generative models do the opposite. They sample new molecules from a learned distribution [2, 3], often without the protein condition, without a fixed scaffold, and without a 3D pose that can be inspected in the same session. The chemist then has to dock, filter and redraw the analogue by hand. ShEPHERD can generate 3D molecules conditioned on shape, electrostatic potential (ESP), and pharmacophores [4], but in its original form it is a research notebook, not a design tool. Therefore, there is no simple way to open a PDB complex, point to the atoms that should change, and get new 3D variants back in the pocket.

SPELL (Shape-Pharmacophore-Electrostatic Ligand Learner) is built to close that gap. It is a browser-based editor on Mol* [5] with a stable generation API. The model under the hood is interchangeable. Today the default engine is ShEPHERD, and we are working on plugging in other 3D generators behind the same interface, but the design loop does not depend on one network. The user loads a structure, selects a ligand and either generates a whole-ligand analogue or marks a fragment for replacement - by clicking atoms or by built-in lasso. The unmarked part of the ligand is kept as a scaffold, and only the selected atoms are regenerated, with exit vectors as attachment points. Before generation, SPELL shows the pocket conditioning used by the model, including surface shape, ESP, using xTB [6] or MMFF [7], and pharmacophores. Each channel can be turned on or off. Generated poses return into the same viewer as a trajectory, so the chemist compares them in place rather than in a separate docking program. After generation, SPELL checks chemical validity with RDKit [8], ranks candidates by 3D similarity to the reference (shape, ESP, and pharmacophore scores) and shows protein-ligand contacts with ProLIF fingerprints [9]. ESP maps of the parent ligand and a generated analogue can be overlaid.

The point of the platform is a working loop that makes it available for a medicinal chemist to see the complex, choose what to change, generate in 3D under pocket constraints, and decide from the pose and the interactions.

1. Erlanson, D.A., Fesik, S.W., et al. (2016). *Nature Reviews Drug Discovery*, 15, 605–619.
2. Loeffler, H.H., He, J., et al. (2024). *Journal of Cheminformatics*, 16(1), 20.
3. Zou, Y., Guo, T., et al. (2025). *Communications Biology*, 8(1), 422.
4. Adams, K., Abeywardane, K., et al. (2025) *The Thirteenth International Conference on Learning Representations*.
5. Sehnal, D., et al. (2021). *Nucleic Acids Research*, 49(W1), W431–W437.
6. Bannwarth, C., Ehlert, S., Grimme, S. (2019). *Journal of Chemical Theory and Computation*, 15, 1652–1671.
7. Halgren, T. A. (1996). *Journal of Computational Chemistry*, 17(5-6), 490-519.
8. Landrum, G., et al. (2025). RDKit. <https://www.rdkit.org>.
9. Bouysset, C., Fiorucci, S. (2021). *Journal of Cheminformatics*, 13, 72.

PREDICTION OF T-CELL EPITOPES INTERACTIONS WITH MAJOR HISTOCOMPATIBILITY COMPLEX II USING THEIR STRUCTURAL FORMULAS

A.S. Smirnov^{1,2}, D.A. Filimonov³, A.A. Lagunin^{1,3}

¹Pirogov Russian National Research Medical University, Moscow, Russia

²Research Centre for Medical Genetics, Moscow, Russia

³Institute of Biomedical Chemistry, Moscow, Russia

T-cell receptors recognize their ligands, known as epitopes, only bound with major histocompatibility complex (MHC). The MHC classifies in two classes: the first one is representing on cytoplasmic membranes almost all cell in mammals' organisms, the second is representing only on membranes of antigen-presenting cells (macrophages, dendritic cells, B-lymphocytes). A binding site of MHC I is small and formed only one protein chain. The task of prediction of epitope binding with MHC I is solved good by current state-of-art models and with our models based on structural formulas as well. But two chains are formed MHC II binding site and conformation is opened. So, there are a lot of combination of binding sites and peptide length and amino acid composition can vary. The state-of-art models, such as NetMHCIIpan, DeepMHCII, MHCAtnNet, PUFFIN, still have moderate accuracy [1]. They use different approaches for target and feature representation from standard one-hot or BLOSUM encoding to large language models embeddings. Our work aims to introduce the decision of this task through peptide representation using their structural formulas.

In this work, the structure-activity relationship (SAR) approach was used for building classification models predicting epitope-MHCII allele interactions. The epitopes are represented by their structural formulae instead letter sequences. The SAR models were created and validated by the modified version of Prediction of Activity Spectra for Substances (MultiPASS) software, which allows using different levels of multilevel neighborhoods of atoms (MNA) descriptors to describe the peptide's structural formula. The MultiPASS uses modified naïve Bayes algorithm for revealing relationships between structural formulae and interaction with MHCII alleles. The two datasets were used for training and validating models. The first was used for training NetMHCIIpan-3.2 and DeepMHCII and called BD2016. The second was used for training MHCAtnNet and called BD2020. Both datasets are provided strict partitioning for 5-fold cross-validation. BD2016 contains 134 281 records over 80 different MHC-II alleles (72 human and 8 mouse alleles) [1]. BD2020 consists of 65 954 records for 49 HLA-DRB alleles, where 36 035 ones are positive and 28 919 are negative. The MultiPASS training was carried out with MNA descriptors levels from 3 to 11 inclusive. DeepMHCII and MHCAtnNet were retrained on own datasets according to the instructions from their GitHub repositories. The computational part of this study was carried out using the Aldan3 cluster at the Institute of Translational Medicine, Pirogov Russian National Research Medical University [2].

The MultiPASS has inferior performance on BD2016 in comparison with DeepMHCII. The mean area under receiver operation curve (AUROC) for MultiPASS is 0.77 versus 0.87 for DeepMHCII. The mean area under precision-recall curve (AUPRC) for MultiPASS is 0.68 versus 0.80 for DeepMHCII. But on BD2020 MultiPASS shows superior performance in comparison with MHCAtnNet. The mean AUROC for MultiPASS is 0.84 versus 0.64 for MHCAtnNet. The mean AUPRC for MultiPASS is 0.83 versus 0.61 for MHCAtnNet. And obtained metrics are higher than reported in literature for BD2020 (DeepMHCII AUROC is 0.781, MHCAtnNet AUROC is 0.758) [1]. Although the MultiPASS doesn't show the best performance, but it can compete with state-of-art approaches and we recommend to choose model according to performance metric for alleles of interest.

This study was supported by the grant of Russian Science Foundation No. 25-25-01171, <https://rscf.ru/project/25-25-01171/>.

1. You, R., Wei Qu, W., Mamitsuka, H., Zhu, S. (2022). *Bioinformatics*, 38, Supplement_1.
2. Kotliar, V/, Bolotin, D., Syrko, D. (2024). *Phys. Part.Nucl.*, 55(3).

COMPUTATIONAL TARGETING OF IMMUNE AND GLYCO-IMMUNE CHECKPOINTS IN CAR T-CELL THERAPY

P.S. Sobral^{1,2,3}, D.I. Veiga^{2,3}, D.F. Tomás^{2,3,4}, Z. Silva^{2,3}, R.L. Paterson⁵, P.A. Videira^{2,3,4}, F. Pereira¹

¹LAQV-REQUIMTE, Department of Chemistry, NOVA FCT, Caparica, Portugal

²UCIBIO, Department of Life Sciences, NOVA FCT, Caparica, Portugal

³Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA FCT, Caparica, Portugal

⁴CDG & Allies, Department of Life Sciences, NOVA FCT, Caparica, Portugal

⁵Stemmatters, Biotechnologia e Medicina Regenerativa SA, Guimarães, Portugal

Chimeric antigen receptor (CAR) T-cell therapy has transformed the treatment of hematological malignancies, but immunosuppression within the tumor microenvironment can limit its efficacy. PD-1/PD-L1 signaling and glycan-mediated regulation through inhibitory Siglecs contribute to immune suppression and represent attractive targets for pharmacological modulation. Small molecules may provide a synergistic strategy to overcome inhibitory pathways and enhance CAR T-cell function [1].

Building on our previous computational work on the PD-1/PD-L1 axis [1], we are developing an integrated drug discovery strategy combining cheminformatics, machine learning, virtual screening, and molecular docking. A previously developed Random Forest model was used to explore new chemical space and prioritize candidate compounds. Two hits were synthesized and experimentally evaluated using ELISA-based PD-1/PD-L1 binding assays and microscale thermophoresis (MST), supporting their ability to modulate the PD-1/PD-L1 interaction.

This strategy is now being expanded to glyco-immune checkpoints, particularly Siglec-7 and Siglec-9. Computational screening and structure-based approaches are being applied to identify small molecules targeting these complementary pathways.

Overall, this work integrates computational drug discovery and experimental evaluation to identify small-molecule modulators of complementary mechanisms affecting CAR T-cell efficacy.

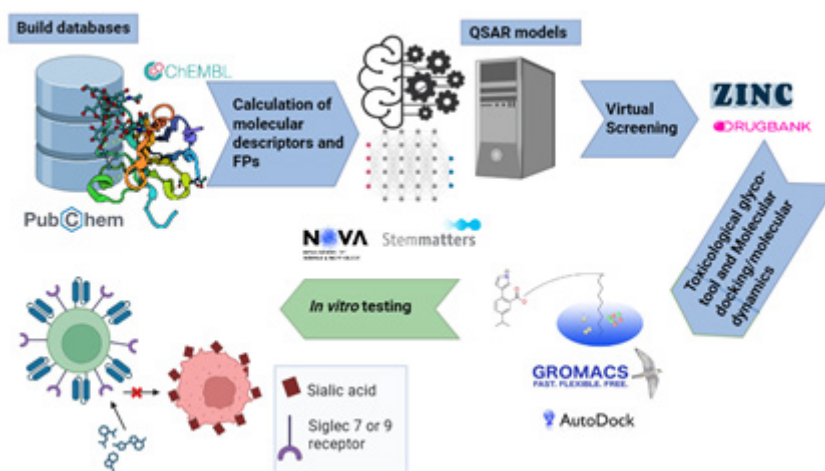


Figure 1. Computational drug discovery workflow.

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1. Sobral, P.S., Carvalho, T., Izadi, S., Castilho, A., Silva, Z., Videira, P.A.; Pereira, F. (2025). *RSC Adv.*, 15, 2298–2316.

A WORKFLOW FOR BUILDING (Q)SAR MODELS WITH IMBALANCED AND HETEROGENEOUS DATA

L.A. Stolbov, D.S. Druzhilovsky, A.V. Rudik, D.A. Filimonov, V.V. Poroikov

Institute of Biomedical Chemistry, Moscow, Russia

The development of reliable QSAR models is complicated by the heterogeneity of structural data, class imbalance, and redundancy of descriptor spaces. To overcome these issues, a robust methodological pipeline is proposed. It includes: (i) chemical standardization and harmonization of structural records – unification of aromatic systems (aromatic/Kekulé forms), normalization of explicit hydrogens, tautomeric forms and charge states, and standardization of functional groups (nitro, amide, sulfoxide, etc.) according to recommended practices [1]; (ii) descriptor calculation ensuring chemical consistency of the data; (iii) statistical regularization and iterative selection of relevant features; (iv) weight correction of class imbalance based on an extreme loss function (Gumbel distribution); and (v) training a Self-Consistent Extreme Classifier (SCEC) [2], which integrates these steps. SCEC reduces the number of descriptors by 3–4 times without loss of accuracy, improving the interpretability and generalization ability of the models.

The effectiveness of the approach was confirmed on three heterogeneous tasks:

(1) QSAR classification of HIV-1 inhibitors for three targets (protease, integrase, reverse transcriptase) on datasets of >4000 compounds – accuracy at the level of SVM and neural networks with substantially fewer features.

(2) Ternary classification of HIV-1 drug resistance for 19 inhibitors (8 protease, 6 NRTI, 3 NNRTI, 2 integrase) using sequence data. The Self-Consistent Extreme Classifier (SCEC) was applied in one-versus-rest (OVR) and one-versus-one (OVO) designs [3]. The final OVR-based classifiers achieved average sensitivity 0.913, specificity 0.894, precision 0.741, and AUC-ROC 0.953. Prospective validation from treatment-naive HIV-positive patients showed general agreement with therapeutic outcomes. The model was implemented in a freely available web application for clinical decision support.

(3) Prediction of cytotoxicity of ionic liquids using a dataset of 3837 records. The model achieved ROC-AUC 0.875 and PR-AUC 0.955 on validation. Key structural factors influencing cytotoxicity were identified: alkyl substituent length and anion nature. This enables targeted synthesis of safer ionic liquids.

To support the workflow, we implemented SarMath software as a full cycle of QSAR model creation tool, enabling the construction of QSAR models suitable for virtual screening and rational design under imbalanced and heterogeneous data including, substructure searches, analysis of chemical space, model development (Figure 1) and biological activities evaluation for selected structure.

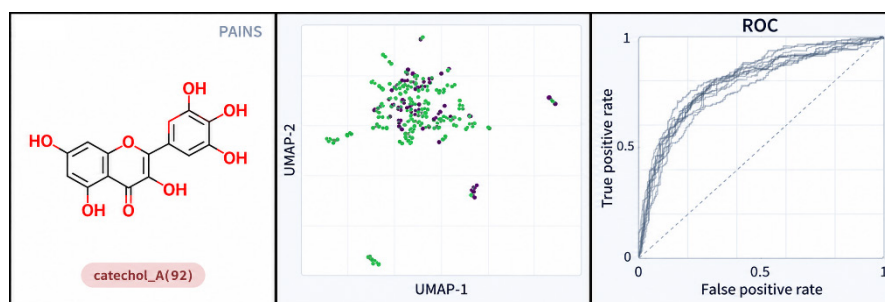


Figure 1. Elements of the SarMath interface for fragment analysis, chemical space visualization, and model metric evaluation.

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1. Fourches, D., et al. (2016). *J. Chem. Inf. Model.*, 56, 1243.
2. Stolbov, L.A., Filimonov, D.A., Poroikov, V.V. (2022). *SAR QSAR Environ. Res.*, 33, 793–804.
3. Stolbov, L.A., et al. (2026). *Computer Methods and Programs in Biomedicine*, 278.

IMPROVING THE ACCURACY AND GENERALIZABILITY OF MOLECULAR PROPERTY REGRESSION MODELS WITH A SUBSTRUCTURE-SUBSTITUTION-RULE-INFORMED FRAMEWORK

X. Fan, L. Guo, R. Jia, Y. Tian, Z. Yang, W. Li, B. Tian

*MOE Key Laboratory of Bioinformatics, State Key Laboratory of Molecular Oncology,
Beijing Frontier Research Center for Biological Structure, School of Pharmaceutical Sciences,
Tsinghua University, Beijing, China*

Artificial Intelligence (AI)-aided drug discovery is an active research field, yet AI models often exhibit poor accuracy in regression tasks for molecular property prediction, and perform catastrophically poorly for out-of-distribution (OOD) molecules. Here, we present MolRuleLoss, a substructure-substitution-ruleinformed framework that improves the accuracy and generalizability of multiple molecular property regression models (MPRMs) such as GEM and UniMol for diverse molecular property prediction tasks. MolRuleLoss incorporates partial derivative constraints for substructure substitution rules (SSRs) into an MPRM's loss function. When using GEM models for predicting lipophilicity, water solubility, and solvation-free energy (using lipophilicity, ESOL, and freeSolv datasets from MoleculeNet), the root mean squared error (RMSE) values with and without MolRuleLoss were 0.587 vs. 0.660, 0.777 vs. 0.798, and 1.252 vs. 1.877, respectively, representing 2.6–33.3% performance improvements. We show that both the number and the quality of SSRs contribute to the magnitude of prediction accuracy gains obtained upon adding MolRuleLoss to an MPRM. MolRuleLoss yielded relative improvements on activity-cliff molecules and on a melting-point property-OOD task; however, the absolute prediction error in the OOD setting remained high, indicating that property-OOD generalization remains an open challenge. In a controlled molecular-weight extrapolation experiment, MolRuleLoss reduced the test RMSE of a GEM model from 29.507 to 0.007; because molecular weight is an exact linear function of atomic composition, this result should not be extrapolated to noisy experimental properties. We also provide a formal demonstration that the upper bound of the variation for property change of SSRs are positively correlated with an MPRM's error. Together, we show that using the MolRuleLoss framework as a bolt-on boosts the prediction accuracy and generalizability of multiple MPRMs, supporting diverse applications in areas like cheminformatics and AI-aided drug discovery.

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POSTERS

NETWORK PHARMACOLOGY-GUIDED MOLECULAR DOCKING OF 7-PHENYL-5-(P-TOLYL)PYRIDO[2,3-D]PYRIMIDIN-4-AMINE: INSIGHTS INTO ITS MULTITARGET ANTICANCER ACTIVITY

B. Addoum¹, M. Chebaibi², I. Chaoui¹, M. El mzibri¹, M. Attaleb¹, M. Dakir³, A. Elmakssoudi³, L. Benbacer¹

¹National Center for Energy, Nuclear Sciences and Techniques (CNESTEN), Biology and Medical Research Unit - Life Sciences Division, Rabat, Morocco

²Ministry of Health and Social Protection, Higher Institute of Nursing Professions and Health Techniques, Fez, Morocco

³Laboratory of Organic Chemistry, Materials, Electrochemistry, and Environment, Faculty of Sciences Ain Chock, Hassan II University of Casablanca, Casablanca

Background: Cancer remains a major cause of morbidity and mortality worldwide, and current therapies often fail due to drug resistance and limited efficacy. Multi-target drug design has therefore emerged as a promising strategy to simultaneously modulate multiple pathways involved in tumor progression.

Objective: This study aimed to investigate the potential of a novel pyrido[2,3-d]pyrimidine derivative, 7-phenyl-5-(p-tolyl)pyrido[2,3-d]pyrimidin-4-amine, as a multitarget inhibitor against key proteins implicated in cancer development.

Methods: An integrated computational approach was employed to evaluate the interaction of the compound with four major cancer-related targets: Telomerase Reverse Transcriptase (TERT), E3 Ubiquitin-Protein Ligase MDM2, Epidermal Growth Factor Receptor (EGFR), and Cyclin-Dependent Kinase 2 (CDK2). Pharmacokinetic and toxicity properties were further predicted using ADMET analysis.

Results: Docking results revealed strong binding affinities for the compound across all targets (−8.1 to −9.7 kcal/mol), comparable or superior to those of reference inhibitors. Our results confirmed the stability of the ligand–protein complexes throughout the simulation period. ADMET predictions indicated favorable drug-likeness and acceptable pharmacokinetic properties.

Conclusion: These findings suggest that 7-phenyl-5-(p-tolyl)pyrido[2,3-d]pyrimidin-4-amine may represent a promising scaffold for multitarget anticancer drug development. However, further in vitro and in vivo studies are required to validate its biological activity and safety.

MOLECULAR MODELING OF TELOMERIC DNA–PROTEIN INTERACTIONS: COMPARING PALINDROMIC AND NON-PALINDROMIC SEQUENCE RECOGNITION BY hnRNP A2/B1

S.O. Afolabi¹, C.Y. Förster², E.V. Skorb¹, S. Shityakov¹

*¹Laboratory of Chemoinformatics, Infochemistry Scientific Center, ITMO University,
Saint Petersburg, Russia*

*²Department of Anaesthesiology, Intensive Care, Emergency and Pain Medicine, Würzburg University,
Würzburg, Germany*

DNA sequence, symmetry, and structural flexibility influence recognition by nucleic-acid-binding proteins involved in genome regulation, host defense, and disease. Heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1) has been implicated in nucleic-acid sensing and may distinguish self from non-self nucleic acids through sequence-dependent structural recognition. However, the molecular determinants that govern its differential affinity for palindromic versus non-palindromic single-stranded DNA remain insufficiently characterized. This study critically aimed to determine whether DNA sequence symmetry alone produces measurable differences in hnRNP A2/B1 binding stability and energetics, and to identify structural features that could be exploited for therapeutic modulation of protein-DNA recognition.

Crystallographic structures of hnRNP A2/B1 complexed with single-stranded palindromic DNA (pIDNA; PDB ID: 7WM3) and non-palindromic DNA (npIDNA; PDB ID: 8HNI) were refined, minimized, and evaluated using FoldX, followed by 500 ns molecular-dynamics simulations. Protein-DNA binding energies were subsequently estimated using MM/GBSA and MM/PBSA approaches. FoldX predicted stronger affinity for npIDNA than pIDNA, yielding binding energies of -22.59 and -17.40 kcal/mol, respectively. Both complexes remained intact over the simulation period, confirming persistent association between hnRNP A2/B1 and each DNA substrate. Nevertheless, npIDNA showed more favorable binding free energies, with MM/GBSA and MM/PBSA values of -108.76 and -112.92 kcal/mol, compared with -103.65 and -112.87 kcal/mol for pIDNA. These results indicate that npIDNA forms a more stable and comparatively rigid binding complex with hnRNP A2/B1, whereas pIDNA recognition is weaker and may depend on conformational distortions that enhance complementarity with the protein-binding interface. The agreement between FoldX and simulation-derived energetic estimates theoretically strengthens this interpretation.

Generally, the study identifies sequence-dependent structural recognition as a potential determinant of hnRNP A2/B1 selectivity. The npIDNA-hnRNP A2/B1 interface provides a rational starting point for virtual screening and design of small molecules or oligonucleotide mimetics that modulate DNA recognition, with potential applications in antiviral immune regulation and other diseases involving dysregulated nucleic-acid sensing.

MECHANISTIC DECODING OF TARGET PHOTOTOXICITY: A COMPUTATIONAL STUDY POWERED BY ESIGNS

A.S. Almeida^{1,2}, F. Pereira¹, P.E.M. Lopes²

¹Department of Chemistry, LAQV-REQUIMTE, NOVA School of Science and Technology
NOVA FCT, Portugal

²FastCompChem, Portugal

Drug-induced photosensitivity affects over half of marketed drugs [1], with nearly 400 compounds linked to severe clinical outcomes [2]. This PhD project investigates the mechanistic photophysical pathways driving drug photoactivation using Time-Dependent Density Functional Theory (TD-DFT) [3], converting excited-state deactivation profiles into mechanism-based phototoxicity descriptors for ESIGNS, the quantum-derived fingerprint developed in QUANTUM-TOX, enabling data-efficient, animal-free AI prediction of photosafety [4,5].

At the current project stage, a benchmark set of 39 photosensitizing drugs was assembled and classified into five mechanistic chromophore families (diaryl ketones, halogenated aryls, substituted alkenes/polyenes, nitrogen-rich heteroaromatics, and aryl-amine donor-acceptor systems). From this dataset, key mechanistic principles emerged:

- (1) Photoproducts, not parent drugs, often act as the true phototoxins (*e.g.*, diaryl ketone photodecarboxylation, statin 6 π -electrocyclization).
- (2) Structural alerts are context-dependent, with aryl halogens switching between reactive and inert roles depending on excited-state competition.
- (3) Microenvironmental factors dominate, with protein binding, pH, and oxygen tension selecting the operative pathway (*e.g.*, HSA Site 3 binding shifts simprevir from Type II to Type I).
- (4) Non-degrading ROS catalysts such as adapalene and rucaparib generate singlet oxygen while remaining intact under ICH photolysis.

These mechanistic insights provide the molecular foundation for the next project phase, where TD-DFT-computed photophysical parameters will be integrated into ESIGNS descriptors to train the QUANTUM-TOX AI engine and benchmark predictive performance against conventional QSAR models using fingerprints, 2D, and 3D molecular descriptors, advancing early-stage photosafety assessment in drug discovery.

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1. Hofmann, G., Gradl, G., Schulz, M., Haidinger, G., Tanew, A., Weber, B. (2020). *J. Eur. Acad. Dermatol. Venereol.*, 34, 589-600.
2. Hofmann, G., Weber, B. (2021). *J. Dtsch. Dermatol. Ges.*, 19, 19-29.
3. Stratmann, R., Scuseria, G., Frisch, M. (1998). *J. Chem. Phys.*, 109, 8218-8224.
4. Publications Office of the European Union. *QUANTUM-TOX - Revolutionizing Computational Toxicology with Electronic Structure Descriptors and Artificial Intelligence*; 2024. <https://cordis.europa.eu/project/id/101130724> DOI: <https://doi.org/10.3030/101130724>.
5. *QUANTUM TOX*. 2026. <https://quantum-tox.eu/> (accessed 16/09/2026).

DE NOVO DESIGN OF SMALL-MOLECULE LIGANDS FOR SPECIFIC BINDING SITES ON TARGET PROTEINS BASED ON EQUIVARIANT FLOW MATCHING

P. Savel'ev, A. Anashkina

Engelhardt Institute of Molecular Biology, Moscow, Russia

The aim of this work was to develop and implement a computationally efficient method for the *de novo* design of small-molecule ligands within a static target protein binding site, utilizing Equivariant Flow Matching combined with an integrated cascade of physicochemical heuristic filters. The method addresses the high computational cost and limited physical plausibility associated with stochastic diffusion models, which require hundreds to thousands of neural network inferences and often produce distorted covalent geometries, steric clashes, and topological artifacts.

The novelty of this approach, compared to previous studies, lies in replacing stochastic diffusion with deterministic conditional optimal transport flow matching. The mixed discrete-continuous nature of molecular graphs is handled by projecting categorical atom-type features onto a probability simplex and employing linear interpolation with a dynamically weighted loss function. A bipartite $E(n)$ -equivariant graph neural network (BipartiteEGNN) predicts target coordinates and atom types while the receptor remains rigidly fixed. Numerical ODE integration is augmented with a differentiable van der Waals repulsion gradient for dynamic steric control, while a multi-stage deferred filtering cascade (PoseBusters, QED, SA, Lipinski's Rule, AutoDock Vina) is employed for post-generation validation. A key feature is position-constrained energy relaxation, which prevents the collapse of bioactive conformations often observed during standard UFF optimization *in vacuo*.

The EquiLigandFM model was trained on CrossDocked2020 using LMDB. A weighted loss function with $w(t)=1/(1-t)^2$ biased training toward local molecular geometry and prevented the generation of three-membered carbon rings and invalid bond lengths. "Raw" generated outputs passed PoseBusters in only ~7% of cases; however, the proposed constrained relaxation increased the proportion of physically valid conformations by a factor of 7.5. For the clinically significant *Mycobacterium tuberculosis* target InhA (enoyl-ACP reductase; PDB ID: 2H91), the *de novo* generated scaffolds exhibited predicted micromolar affinity (Vina scores ranging from -5.375 to -6.239 kcal/mol), QED values of 0.768–0.864, SA scores of 3.889–4.761, compliance with Lipinski's Rule of Five, and successful PoseBusters validation. ADMET profiling via SwissADME demonstrated high gastrointestinal absorption, blood-brain barrier permeability, an absence of PAINS/Brenk alerts, and a Fraction Csp3 of 1.00, indicating complex aliphatic 3D scaffolds suitable for further optimization using medicinal chemistry methods.

The developed standalone, high-performance software suite EquiLigandFM features an open API and can be deployed on local server infrastructure; it reduces the computational costs of initial *de novo* ligand generation by orders of magnitude through the early filtering of invalid structures while safeguarding commercial intellectual property. This approach paves the way for the targeted discovery of hits and can be integrated into hybrid, human-in-the-loop rational design pipelines.

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IDENTIFICATION OF MOLECULAR TARGETS FOR URACIL-BASED COMPOUNDS IN VARIOUS BACTERIAL PATHOGENS USING COMPUTATIONAL MODELING METHODS

N. Malakhovskiy^{1,2}, E. Matyugina¹, A. Khandzhinskaya¹, A. Anashkina¹

¹*Engelhardt Institute of Molecular Biology, Moscow, Russia*

²*Sechenov University, Moscow, Russia*

The aim of this study was to identify molecular targets for uracil-based compounds in various bacterial pathogens and to select candidate compounds for the rational design of novel antibacterial agents. Modified uridines represent a promising class of compounds active against both Gram-positive and Gram-negative bacteria; however, their molecular targets remain a subject of debate. This study integrates data on the biological activity of uracil derivatives against three clinically significant pathogens: *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Acinetobacter baumannii*.

For *S. aureus* and *M. tuberculosis*, two binary QSAR models (utilizing Bayesian and principal component analysis methods) were constructed and validated based on experimental binary activity data and calculated 2D molecular descriptors. For *A. baumannii*, the analysis relied on existing experimental data. A list of potential protein targets associated with uridine metabolism and nucleotide synthesis was compiled for each pathogen. Molecular docking of the active compounds was performed using MOE 2024 (Triangle Matcher protocol, GBVI/WSA dG scoring).

The QSAR models for *S. aureus* and *M. tuberculosis* demonstrated high predictive capability (85.7–87% accuracy upon cross-validation, $p < 0.001$) and successfully reproduced known structural patterns, such as the optimal C10–C14 alkyl chain length and the importance of oxygen-containing linkers. Docking analysis revealed that the most promising targets for *A. baumannii* are TMK (thymidylate kinase), MraY (phospho-N-acetylmuramoyl-pentapeptide-translocase), and PglF (a glycosylation enzyme), all of which exhibit high binding-site specificity. For *S. aureus* and *M. tuberculosis*, the selected targets—MurA and MurB (cell wall synthesis), ThyX (a flavin-dependent thymidylate synthase absent in humans), TMPKmt (thymidylate kinase), and RNR (ribonucleotide reductase)—offer a multi-target mechanism of action.

It has been demonstrated that the activity of uracil-based compounds is linked to the inhibition of several independent metabolic pathways, potentially reducing the risk of resistance development. TMK, MraY, and PglF were identified for the first time as probable targets for uracil derivatives in *A. baumannii*. A polypharmacological profile involving effects on both cell wall synthesis and nucleotide metabolism was proposed for *S. aureus* and *M. tuberculosis*. Compounds combining high predicted activity with affinity for multiple key targets simultaneously were deemed the most promising candidates for synthesis and biological testing. These findings help narrow the scope of the search for new active antibacterial compounds against various pathogens.

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IDENTIFICATION OF DOWNREGULATED HUB GENES IN GASTRIC CANCER FROM RNA-SEQ DATA USING NETWORK TOPOLOGY AND SURVIVAL ANALYSIS

E.R. Andrews, L.A. Prasad

Department of Life Sciences, Garden City University, Bengaluru, India

Gastric cancer is a leading cause of cancer mortality, and reliable molecular markers for its early detection and prognosis are still limited. The objective of this study was to identify hub genes that drive the transcriptional changes in gastric tumours and to assess their relevance to patient outcome. Previous transcriptomic studies have mostly listed differentially expressed genes or concentrated on upregulated oncogenic hubs. Here we combine differential expression, functional enrichment, protein–protein interaction (PPI) network topology and survival analysis in a single workflow, with particular attention to downregulated hub genes, which are less often examined.

RNA-Seq data from the public dataset PRJNA721740 were analysed. Differentially expressed genes between tumour and normal tissue were identified with DESeq2. Gene Ontology and KEGG pathway enrichment was performed with Enrichr. A PPI network was built from the differentially expressed genes using STRING and visualised in Cytoscape, and hub genes were ranked with the Maximal Clique Centrality (MCC) algorithm of CytoHubba. Prognostic relevance of the hub genes was evaluated using GEPIA2 survival analysis.

The analysis identified five downregulated hub genes, KCNH2, SCN2B, CACNA1H, NLGN1 and CNTN4, which occupy central positions in the PPI network. These genes are linked to ion-channel activity, membrane excitability and cell adhesion, suggesting that loss of these functions is an important feature of gastric tumour biology. The results provide a shortlist of candidate biomarkers and therapeutic targets for gastric cancer. As the findings are computational, they require validation in independent cohorts and by experimental studies.

DESIGN OF ANTHRANILIC ACID DERIVATIVES WITH ANTI-INFLAMMATORY ACTIVITY AND MOLECULAR DOCKING AGAINST CYCLOOXYGENASE-1

K.V. Andryukov

Perm state pharmaceutical academy, Perm, Russia

Directed search is fundamentally linked to the molecular design of a chemical structure and the fragments it contains. Derivatives of anthranilic acid belong to a promising class of biologically active compounds. In previous studies of molecular docking by cyclooxygenase 1 (COX1), fragments of compounds characterized by high affinity with high anti-inflammatory activity of over 40%, determined on the model of carrageenan edema in %, were isolated [1].

The aim of the work is to carry out molecular design of potentially active anthranilic acid derivatives based on a previously obtained fragmentary model and to evaluate them using molecular docking with COX1. The fragmentary model from two fragments contains the following substituents at position 1: allyl ($\text{NHCH}_2\text{CH}=\text{CH}_2$), amino group (NH_2), cyclohexyl ($\text{NHC}_6\text{H}_{11}$), and benzyl ($\text{NHCH}_2\text{C}_6\text{H}_5$). Position 2 includes two structural fragments: 2-furanoyl and phthalyl. Using the search for "active" structures with anti-inflammatory activity as design fragments, we have constructed four compounds derived from anthranilic acid: benzylamide N-2-furanoyl anthranilic acid (1), N-2-furanoyl 5-bromanthranilic acid amide (2), benzylamide N-2-furanoyl 5-iodoanthranilic acid (3), and N-2-furanoyl 3,5-dibromanthranilic acid amide (4). The predicted values of anti-inflammatory activity (AIA) were calculated using the AK_QSAR program (anti-inflammatory activity) [2]: 74,01 % (1), 48,06 % (2), 78,09 % (3) and 54,38 % (4).

The modeling of ligand–receptor interactions was carried out using the AutoDock 4.0 program as part of the MGL Tools 1.5.6 software suite, employing the Lamarckian genetic algorithm. During molecular docking, we used a three-dimensional model of the COX1 molecule, the information about which was obtained from the RCSB Protein Data Bank database: PDB ID code: 3N8X [3]. The inhibitory activity of compounds 1 – 4 against COX1 has been studied. The binding site has been determined using the Molecular Operating Environment program for conformations with the maximum binding energy (Be max). The binding site contains amino acid residues with three types of interaction: hydrophobic (methionine 113 (Met113), valine 115 (Val115), valine 116 (Val116), valine 349 (Val349), leucine 352 (Leu352), leucine 359 (Leu359), phenylalanine 381 (Phe381), leucine 384 (Leu384), tryptophan 387 (Trp387), isoleucine 517 (Ile517), phenylalanine 518 (Phe518), methionine 522 (Met522), isoleucine 523 (Ile523), alanine 527 (Ala527), leucine 531 (Leu531)), acid-base (histidine 90 (His90), arginine (Arg120)) and polar (tyrosine 348 (Tyr348), serine 353 (Ser353), tyrosine 355 (Tyr355), tyrosine 385 (Tyr385), glycine 526 (Gly526), serine 530 (Ser530)).

The maximum Be max values were obtained for the compounds: Be max = -8,86 (1) and -7,91 kcal/mol (3). Compounds 2 and 4 showed binding values of -7,24 and -6,95 kcal/mol, respectively. The obtained molecular design result, determined with respect to COX1, is confirmed by the maximum predicted AIA values for compounds 1 and 3.

1. Andryukov, K., Korkodinova, L. (2018). *Pharm. Chem. J.*, 52(5), 411-414.
2. Andryukov, K., Korkodinova, L. (2019). *Chronicles of the United Fund of Electronic Resources: Science and Education*, 07(122), 28-29.
3. Sidhu, R. S., Lee, J. Y., Yuan, C., et al., (2010). *Journal Biochemistry*, 49, 7069-7079.

A COMPUTER-AIDED DRUG DESIGN (CADD) APPROACH TO THE OPTIMIZATION OF CAR T- CELL THERAPY THROUGH THE MODELLING OF IL-6 INHIBITION

V.P. Karkala, M. Ashri, F. Pereira

LAQV REQUIMTE - Lisboa Campus, Chemistry Department, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, Portugal

Chimeric antigen receptor (CAR) T-cell therapy is a transformative treatment for haematological malignancies, but its clinical use is frequently limited by cytokine release syndrome (CRS), which is a severe inflammatory toxicity driven largely by interleukin-6 (IL-6) signalling [1]. Targeted IL-6 inhibition offers a route to controlling CRS without suppressing antitumor CAR T-cell activity [2]. This work presents a computer-aided drug design (CADD) pipeline to identify FDA-approved drugs with IL-6 (STAT3-pathway) inhibitory potential for drug repurposing. Using bioactivity data from a PubChem high-throughput screen (AID 862), molecular structures were encoded as Morgan fingerprints and used to train the models and the balanced Random Forest model was selected for its superior performance for the training dataset in a ten-fold cross-validation estimation (72.1% accuracy, 73.5% recall). This model was applied to virtually screen 1,441 FDA-approved drugs, identifying 294 candidates (20.4%) with predicted activity. The top 20 hits were subjected to molecular docking against STAT3 (PDB 6NJS) using AutoDock Vina, revealing Cilostazol as the strongest binder (-7.22 kcal/mol) and Rufinamide as the most conformationally stable pose (RMSD 0.095 Å), among five compounds with binding affinities below -6.0 kcal/mol.

This integrated QSAR-screening and molecular docking approach successfully prioritized a small, chemically credible set of FDA-approved candidates for IL-6 inhibition, demonstrating a cost-effective, rapid strategy for drug repurposing to mitigate CAR T-cell-associated toxicity [3].

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1. Mian, A., et al. (2025). *Blood* 146, 2397
2. Nada, H., et al. (2023). Perspective for Discovery of Small Molecule IL-6 Inhibitors through Study of Structure–Activity Relationships and Molecular Docking. *J. Med. Chem.* 66, 4417–4433
3. Tran, Q.-H., et al. (2023). *Mol. Divers.* 27, 2315–2330

IN SILICO EVALUATION OF THE ANTINEOPLASTIC POTENTIAL AND MOLECULAR SIMILARITY OF SYNTHETIC METHOXYLATED CHALCONES

L.S. Batista, V. Z. Majeski, R.R. Kitagawa, R.C.R. Gonçalves

Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Espírito Santo, Vitória, Brasil

Chalcones are flavonoid precursors widely studied in medicinal chemistry due to their structural versatility and significant cytotoxic potential, where methoxylation plays a crucial role by modulating lipophilicity, target-binding affinity, and antiproliferative activity. Among chalcones with reported antineoplastic activity, cardamonin, licochalcone A, and xanthohumol have been extensively investigated and shown to modulate cancer-related mechanisms, including NF- κ B and EGFR signaling, endoplasmic reticulum stress, apoptosis, and the Wnt/ β -catenin pathway. The aim of this study was to perform an *in silico* evaluation of the antineoplastic properties and structural similarity of 13 synthetic methoxylated chalcones compared to commercial chalcones with well-established biological profiles in the literature. Initially, the molecular structures of 13 synthetic methoxylated chalcones were analyzed using the *PASS Online* (*Prediction of Activity Spectra for Substances*) to predict their potential biological activities. The *PASS* analysis was based on the probability coefficients of being active (P_a) and inactive (P_i), where all synthetic structures exhibited elevated P_a values (high similarity to known active compounds) and reduced P_i values (low similarity to proven inactive molecules). The results revealed a promising pharmacological profile for all tested molecules, highlighting attributes such as antileukemic activity showed P_a values ranging from 0,736 to 0,869, antineoplastic activity presents values ranging from 0,671 to 0,801, anti-inflammatory activity ranged from 0,589 to 0,723, and effectiveness in the treatment of preneoplastic conditions, 0,691 to 0,763. Based on these predictions, molecular similarity analysis was performed using the Tanimoto coefficient in a Python environment via Google Colab. Molecular fingerprints were generated using fingerprint/method, and the structures of the 13 synthetic methoxylated chalcones were compared with three reference chalcones with reported antineoplastic activity: cardamonin, licochalcone A, and xanthohumol. The Tanimoto coefficient was calculated based on the intersection and union of structural features represented in the molecular fingerprints. Tanimoto coefficients ranged from 0.2045 to 0.4762, with chalcone 7 showing the highest similarity to cardamonin (0.4762). According to the similarity ranges adopted from Bero et al. (2018), most compounds showed moderate structural similarity to the reference chalcones. Despite the moderate structural similarity to the reference compounds, *PASS Online* independently predicted antineoplastic-related activities for the synthetic methoxylated chalcones. In conclusion, the 13 synthetic methoxylated chalcones showed a promising predicted biological activity profile, including antineoplastic-related activities, despite their moderate structural similarity to the reference chalcones. These findings support further investigation of these compounds through *in vitro* and *in vivo* studies.

1. Bero, S., et al. (2018). *arXiv*: 1806.05237
2. Budziak-Wieczorek, I., Kamiński, D., Skrzypek, A., et al. (2023). *Molecules*, 28(8), 3412.
3. Constantinescu, T., Lungu, C.N. (2021). *International Journal of Molecular Sciences*, 22(21), 11306,

OPTIMIZING CAR T-CELL THERAPY THROUGH COMPUTATIONAL DESIGN OF IL-1 INHIBITION STRATEGIES

I. Carrapato^{1,2}, P. Videira², F. Pereira¹

¹LAQV REQUIMTE - Lisboa Campus, Chemistry Department, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, Portugal

²UCIBIO, Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

Chimeric antigen receptor T-cell (CAR T-cell) therapy has become an important immunotherapy for some types of leukaemia and lymphoma treatments, and it has demonstrated clinical efficacy and durable remissions, nonetheless, it presents immune toxicity correlated with Cytokine Release Syndrome (CRS) and Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) [1, 2]. The antibody, Tocilizumab, showed effectiveness in the treatment of CRS by inhibiting IL-6, but it was incapable of avoiding neurotoxicity, causing the mice to die [3]. In contrast, when they analysed and tried to inhibit IL-1 with anakinra, a recombinant protein, the results showed that this drug could avoid both CRS and ICANS [3]. Using a computeraided drug design workflow combined with a Quantitative Structure–Activity Relationship (QSAR) strategy, this project aims to identify additional small molecules capable of inhibiting the IL1 pathway, thereby mitigating CRS and ICANS and reducing CARTcell-associated side effects. The work is part of the CART MATTERS project and is structured into six main stages, currently at the beginning of Phase-1:

1. Compilation of a curated database of IL1 smallmolecule modulators
2. Development of machine learning (ML) classification and regression models to predict inhibition against IL-1.
3. Assessment of the Applicability Domain (AD) of the IL-1 models
4. Virtual screening of FDA-approved drugs
5. Molecular Docking
6. *In vitro* testing of candidate molecules against IL-1.

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1. Bonifant, C.L., Jackson, H.J., Brentjens, R.J., . Curran, K.J (2016). *Mol. Ther. Oncolytics*, 3, 16011.
2. Goto, H. (2025). *J. Clin. Exp. Hematop.*, 65(4), 268–275.
3. Norelli, M., et al. (2018). *Nat. Med.*, 24(6), 739–748.

INVESTIGATION OF THE ADMET PROPERTIES OF SOME GINKGOLIDE A, B, AND C DERIVATIVES AS POTENTIAL PHARMACEUTICAL ACTIVE INGREDIENTS

R. Demir, F. İslamoğlu

Department of Chemistry, Recep Tayyip Erdoğan University, Rize, Türkiye

Ginkgolides, secondary metabolites with a diterpene trilactone structure isolated from *Ginkgo biloba* L. leaves, are notable for their potent neuroprotective, anti-inflammatory, and PAF (Platelet-Activating Factor) antagonist effects [1]. In this study, the drug potential of 30 different ginkgolide A, B, and C derivatives (Ginkgolide A1–Ginkgolide A10, Ginkgolide B1 – Ginkgolide B10, Ginkgolide C1– Ginkgolide C10) was comprehensively investigated using in silico methods via the ADMETlab 3.0 platform [2]. The molecules were evaluated under eight main headings: physicochemical properties, medicinal chemistry, absorption, distribution, metabolism, excretion, organ/system toxicity, and environmental toxicity. As a result of the multi-criteria decision analysis, the top three candidate molecules exhibiting the most balanced pharmacokinetic profile, high oral bioavailability, and low toxicity across the entire series were identified as B8, A5, and C8, respectively.

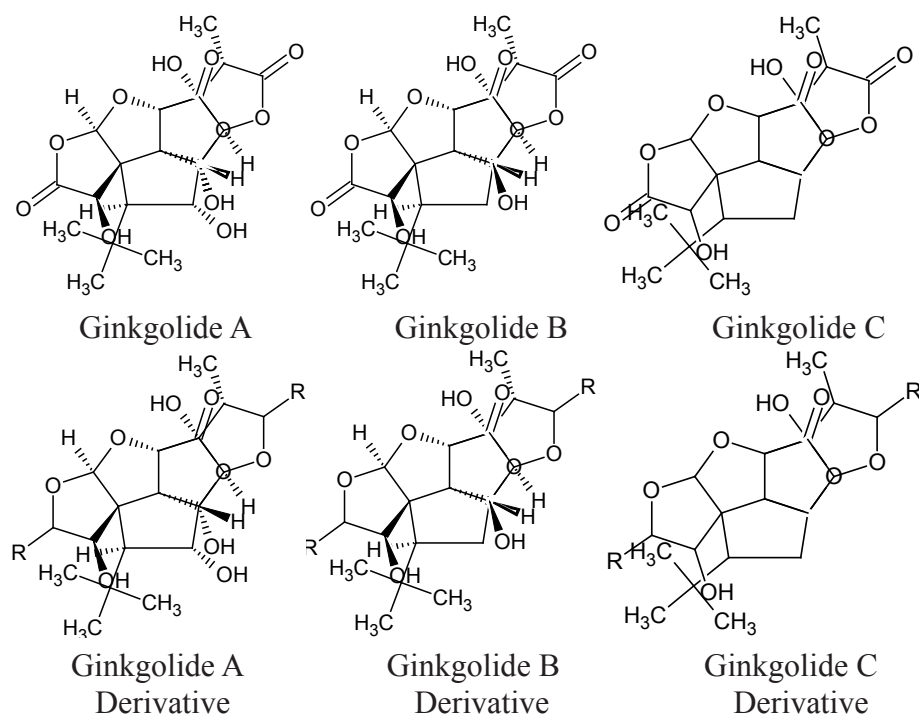


Figure 1. Ginkgolide derivative molecules

1. Li, C., Liu, K., Liu, S., Aerqin, Q., Wu, X. (2020). *Frontiers in Systems Neuroscience*, 14, 45.
2. Fu, L., Shi, S., Yi, J., Wang, N., He, Y., Wu, Z., Peng, J., Deng, Y., Wang, W., Wu, C., Lyu, A., Zeng, X., Zhao, W., Hou, T., Cao, D. (2024). *Nucleic Acids Research*, 52, W422–W431.

DESIGN OF POTENTIAL DNA METHYLTRANSFERASE 1 INHIBITORS USING GENERATIVE MODELS

E.D. Díaz-Dionisio, J.L. Medina-Franco

*DIFACQUIM Research Group, Department of Pharmacy, School of Chemistry,
National Autonomous University of Mexico*

DNMT1 inhibition induces DNA hypomethylation and reactivates silenced tumor suppressor genes, but FDA-approved nucleoside analog inhibitors incorporate into DNA, causing off-target genotoxicity. SAM-competitive inhibitors avoid this mechanism, yet their development remains largely confined to scaffold modification of existing leads and virtual screening of chemical libraries with compounds available, limiting exploration of novel chemical space. This study addresses that gap through *de novo* generation of SAM-competitive DNMT1 inhibitors using a variational autoencoder (VAE) coupled with an activity-guided classifier, extending virtual screening and docking strategies previously applied to DNMT1 inhibitor design.

A SELFIES-based VAE (512-dimensional latent space) was pretrained on ~296,000 ChEMBL36 molecules and fine-tuned via sequential word dropout, achieving 100% chemical validity, a 62.9% round-trip consistent exact reconstruction rate on a 10,000-molecule reference set, and minimal macrocyclic systems (4.7%). Latent space perturbation confirmed a smooth, continuous embedding, with Tanimoto similarity decaying systematically from 0.77 to 0.15 with perturbation magnitude. Principal component analysis based on ECFP4 fingerprints showed that generated molecules occupy the same chemical space as the training distribution, and that a curated DNMT1 dataset (n=259; 97 active, 162 inactive) falls within this generated space. A multilayer perceptron trained on frozen latent representations discriminated active from inactive SAM-competitive compounds with consistent performance across 5-fold cross-validation (AUC=0.884±0.040, MCC=0.656±0.071), despite class imbalance (AP=0.835±0.057, above baseline).

Together, these results establish a validated generative and discriminative framework for targeted molecule generation. Guided sampling from the active latent region, filtered by applicability domain and round-trip consistency, yielded 1,643 candidate molecules. Ongoing work focuses on consensus docking within the SAM catalytic pocket, candidate analogue search, and prioritization of top-ranked hits for future synthesis and *in vitro* validation.

COMPREHENSIVE ANALYSIS OF THE DRUG CANDIDATE POTENTIAL OF GINKGOLIT J AND M DERIVATIVES VIA IN SILICO ADMET PROFILING

D. Ekinçi, F. İslamoğlu

Department of Chemistry, Recep Tayyip Erdoğan University, Rize, Türkiye

In this study, 20 new bioactive compound candidates derived from two important diterpene lactone skeletons of Ginkgo biloba, Ginkgolide J (J1–J10) and Ginkgolide M (M1–M10), were comprehensively investigated [1, 2] using the ADMETlab 3.0 platform [3]. Ginkgolide J and M derivatives were evaluated for physicochemical parameters, medicinal chemistry compliance, membrane permeability (Caco-2/MDCK), oral bioavailability ($F_{50\%}$), plasma protein binding (PPB), cytochrome P450 (CYP) inhibition, plasma clearance (CL_{plasma}), elimination half-life ($T_{1/2}$), hERG cardiotoxicity, hepatotoxicity (DILI), and ecotoxicology. As a result of the comprehensive analytical scoring, the following from the Ginkgolide M series emerged as the three most promising drug candidates: M4 (superior performance in 7 out of 8 categories), M5 (highest QED and environmental safety), and M9 (longest half-life, $T_{1/2} = 4.044$ hours, and successful clearance). The Ginkgolide J series, meanwhile, demonstrated specific advantages, particularly in intestinal absorption and metabolic profile stability.

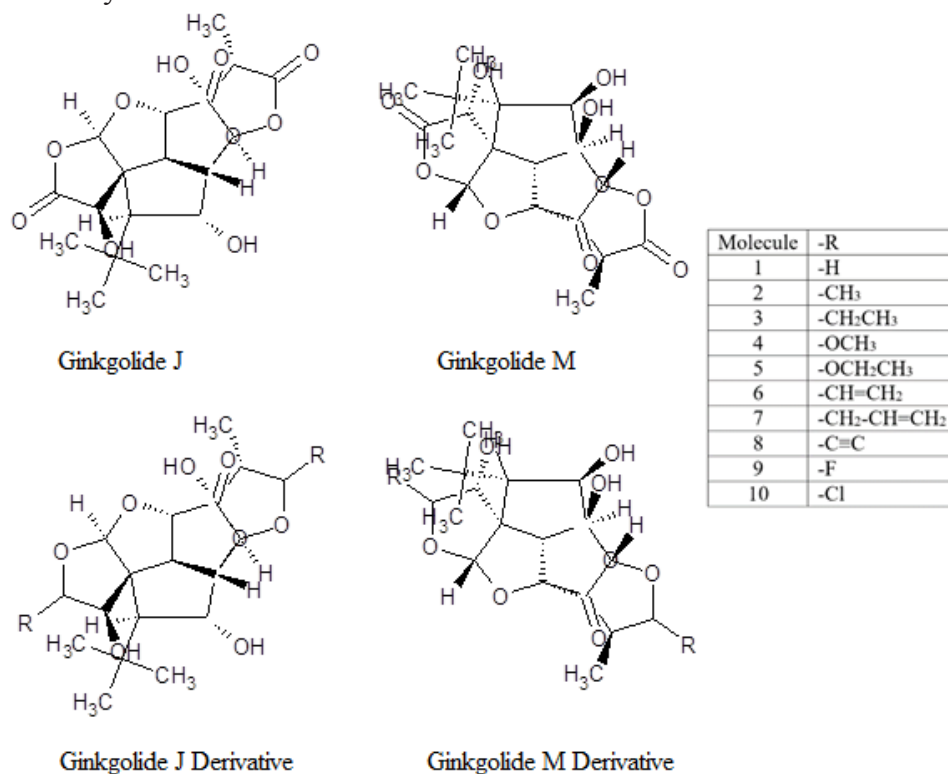


Figure 1. Ginkgolide J and M derivative molecules

1. Strømgaard, K., Nakanishi, K. (2004). *Angewandte Chemie International Edition*, 43, 1640–1658.
2. Vitolo, O., Gong, B., Cao, Z., Ishii, H., Jaracz, S., Nakanishi, K., Arancio, O., Dzyuba, S.V., Lefort, R., Shelanski, M. (2009). *Neurobiology of Aging*, 30, 257–265.
3. Fu, L., Shi, S., Yi, J., Wang, N., He, Y., Wu, Z., Peng, J., Deng, Y., Wang, W., Wu, C., Lyu, A., Zeng, X., Zhao, W., Hou, T., Cao, D. (2024). *Nucleic Acids Research*, 52, W422–W431.

COMPUTATIONAL DESIGN OF TRASTUZUMAB CONJUGATES WITH HIGH CHELATOR-TO-ANTIBODY RATIOS

A. Elizarova¹, M. Gureev¹, S. Tsymbal^{1,2}, A. Novikov^{1,2}, Yu. Porozov¹

¹*HSE University, St. Petersburg, Russia*

²*ITMO University, St. Petersburg, Russia*

Current treatment of metastatic HER2-positive breast cancer does not prevent the risk of relapse of the disease due to the development of drug resistance. We propose a new strategy to overcome the resistance: oxidative therapy based on copper(II)-chelating antibody-drug conjugates (ADCs). This approach combines antibody-targeted delivery of metals that generate reactive oxygen species (ROS) and cancer cell membrane damage.

Our study aims to design immunoconjugate with the highest number of copper ions per antibody (chelator-to-antibody ratio, CAR) without worsening its physicochemical properties, in order to ensure optimal antitumor efficacy at minimal dose. Two trastuzumab-CB-TE2A-copper(II) ADCs were designed *in silico* using combined site-specific conjugation methods. The ADCs have CAR = 4 (Fig. 1; **K207**, **K188**) and 6 (Fig. 1; **K207**, **K188**, **K288**). Complexes of trastuzumab conjugates with target receptor HER2 were created using protein-protein docking (RMSD < 2.5 Å, reference PDB: 1N8Z).

MD analysis indicates reduced flexibility upon conjugation without substantial changes in overall structural stability. Although AggScore [1] predictions indicate a lower overall aggregation profile, the CAR 6 conjugate shows increased aggregation potential in the light chain CDRs and CH1 domain, which may require antibody optimization. MM-GBSA calculations suggest pH-dependent differences in receptor binding. Binding energies for the tumor-associated HER2 model at pH 6.5 (trastuzumab: -108.25 kcal/mol, CAR 4: -169.06 kcal/mol, CAR 6: -150.33 kcal/mol) were significantly more favorable than for the healthy-tissue model at pH 7.4 (-55.15, -57.82, and -63.91 kcal/mol, respectively).

Both conjugates exhibit higher binding affinity to the receptor compared to unmodified trastuzumab. Increasing CAR from 4 to 6 did not improve predicted HER2 binding but increased propensity for aggregation in the light chain CDR/CH1 domain region. Our *in silico* analysis confirms both immunoconjugates as promising candidates for further *in vitro* studies.

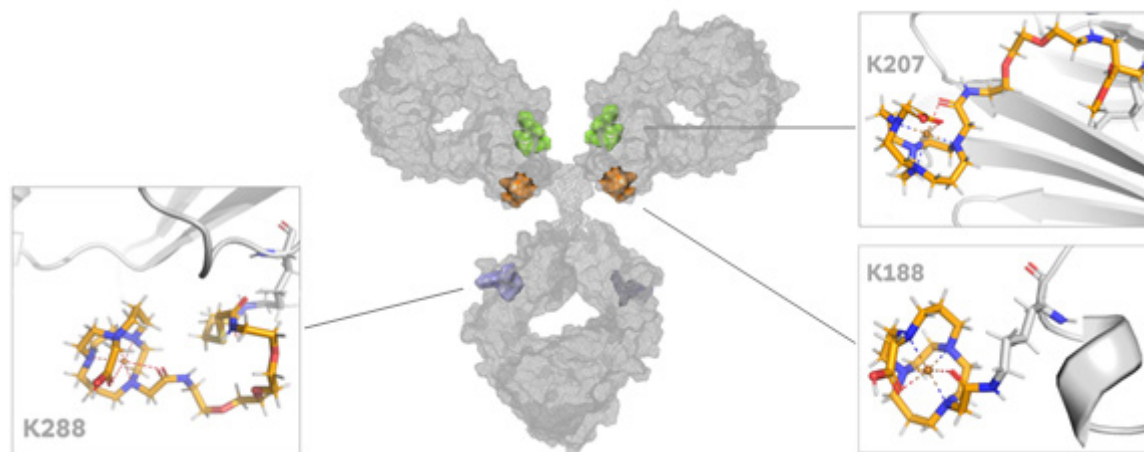


Figure 1. Site-specific lysine conjugation methods used in our study: K207 modification via sulfonyl acrylate [2], K188 modification via fluorophenyl ester [3], and K288 modification via second-generation AJICAP technology [4] using iodoacetamide linker.

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1. Matos, M.J., Oliveira, B. L., Martínez-Sáez, N. et al. (2018). *J. Am. Chem. Soc.*, 140, 4004–4017.
2. Pham, G.H., Ou, W., Bursulaya, B., et al. (2018). *ChemBioChem*, 19, 799–804.
3. Fujii, T., Matsuda, Y., Seki, T., et al. (2023). *Bioconjugate Chem.*, 34, 728–738.
4. Sankar, K., Krystek, S.R., Jr, Carl, S.M., et al. (2018). *Proteins*, 86, 1147–1156.

***IN SILICO* MOLECULAR DOCKING AND ADMET EVALUATION OF CAPPARIS DECIDUA PHYTOCHEMICALS AGAINST DLT A OF ENTEROCOCCUS FAECIUM**

A. Farooq

Department of Biochemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

The emergence of antimicrobial resistance in *Enterococcus faecium* highlights the need for new molecular targets and candidate inhibitors. DltA, the D-alanine-activating component of the dlt pathway involved in teichoic-acid modification, represents a biologically relevant target for exploratory inhibitor discovery. This study aimed to identify phytochemicals associated with *Capparis decidua* that show favorable predicted interactions with DltA and to determine whether the strongest docking candidates also possess acceptable computational drug-likeness, absorption and toxicity profiles. A 12-compound phytochemical library was assembled from traceable chemical-structure sources. The selected DltA structure was obtained through the UniProt/AlphaFold workflow, cleaned and assessed using structural-validation tools, and a plausible binding pocket was predicted with PrankWeb. Molecular docking was performed using AutoDock Vina in Google Colab, followed by pose visualization and integrated ADMET analysis using SwissADME, pkCSM and ProTox 3.0; PRODRIGY-LIG was used as an additional protein-ligand assessment. Rutin showed the strongest predicted binding affinity among the 12 compounds (−10.562 kcal/mol), followed by Quercetin (−9.458 kcal/mol). However, Rutin displayed low predicted gastrointestinal absorption, three Lipinski violations and a low predicted human intestinal absorption of 23.446%. Quercetin showed high predicted gastrointestinal absorption, no Lipinski violations and a predicted intestinal absorption of 77.207%. The available ProTox 3.0 prediction for Quercetin indicated an acute oral LD50 of approximately 159 mg/kg (toxicity class 3), which represents a computational warning rather than experimental evidence of human toxicity. PRODRIGY-LIG gave closely similar ΔG_{noelec} values for Rutin (−5.46 kcal/mol) and Quercetin (−5.44 kcal/mol). The advancement of this work is the integration of target-focused virtual screening with post-docking pharmacokinetic and toxicity assessment, demonstrating that docking affinity alone may not identify the most balanced candidate. Overall, Quercetin emerged as the more balanced computational lead, while Rutin remains a strong predicted binder requiring further validation. These findings provide a reproducible computational prioritization framework for *C. decidua* phytochemicals and support subsequent molecular dynamics, validated free-energy analysis and experimental biochemical testing.

COMPARATIVE EXPERIMENTAL AND COMPUTATIONAL ANALYSIS OF AAV9 AND AAVrh.10 TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER

V.A. Feoktistova¹, O.V. Morozova², I.A. Yakovlev², R.V. Deev³, I.S. Limaev³, A.M. Emelin³,
E.V. Skorb¹, S. Shityakov^{1,4}

¹*Infochemistry Scientific Center, ITMO University, Saint Petersburg, Russia*

²*Orphagenex LLP, Moscow, Russia*

³*Petrovsky National Research Center of Surgery, Moscow, Russia*

⁴*Department of Biology and General Genetics, Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia*

Adeno-associated viruses (AAVs) are widely used as gene delivery vectors due to their low immunogenicity and favorable safety profile [1]. AAV9 and AAVrh.10 serotypes are of particular interest, as they have been shown to cross the blood-brain barrier (BBB) [2, 3]. This characteristic makes them promising vectors for delivering therapeutic genes to the central nervous system. However, the molecular mechanisms underlying the BBB penetration of these vectors are not fully understood. The adeno-associated virus receptor (AAVR) is considered a key player in this process, as it facilitates AAV entry into cells.

The aim of this study was to investigate the interaction of AAV9 and AAVrh.10 with the AAVR receptor using both in vivo and in silico approaches. The experimental phase involved assessing the ability of AAV9 and AAVrh.10 to cross the BBB following systemic administration in mice. Computational analysis utilized experimentally determined structures of the AAV9 and AAVrh.10 VP1 proteins in complex with an AAVR fragment. The resulting complexes underwent energy minimization and protein-protein docking analysis, followed by molecular dynamics simulations.

The experiment demonstrated that, of the two serotypes analyzed, AAV9 exhibited a greater capacity to cross the BBB. In silico analysis was consistent with the experimental data: docking revealed a more favorable binding profile for AAV9, characterized by a larger buried surface area and more advantageous contributions from van der Waals and electrostatic interactions. Molecular dynamics simulations further corroborated these findings, indicating the formation of a more stable complex for AAV9. It can be hypothesized that differences in AAVR receptor recognition contribute to the varying efficiency of BBB transport for these two serotypes, and this study provides deeper insight into the mechanisms of AAV9 and AAVrh.10 interaction with the receptor. The findings underscore the role of AAVR and may facilitate the rational design of improved AAV-based viral vectors for gene therapy of central nervous system disorders.

1. Pupo, A. *et al.* (2022). *Molecular Therapy*, 30, 3515–3541.
2. Gray, S. J. *et al.* (2011). *Molecular Therapy*, 19, 1058–1069
3. Zhang, H. *et al.* (2011). *Molecular Therapy*, 19, 1440–1448.

ARTIFICIAL INTELLIGENCE IN IMMUNOREGULATORY GENE EXPRESSION ANALYSIS IN MULTIPLE SCLEROSIS

V.M. Gevorkian, I.I. Cherkashin

Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

Multiple sclerosis (MS) is a chronic immune-mediated disease characterised by inflammation, demyelination and neuroaxonal damage. Variability in disease course and treatment response complicates the identification of reliable molecular biomarkers. The objective of this work was to analyse the potential of artificial intelligence (AI) and machine learning methods for detecting clinically relevant patterns in immunoregulatory gene expression in MS.

The work integrates findings from blood and cerebrospinal fluid transcriptomic studies with single-cell data and compares their relevance for AI-based analysis. Particular attention was given to genes involved in immune regulation, including HLA-DRB1, IL2RA, IL7R, CTLA4 and CD40, as well as to analytical approaches such as regularised regression, support vector machines, random forests, boosting and deep learning. In comparison with considering individual transcriptomic studies separately, this approach emphasises the importance of biological compartment, cellular composition and methodological limitations when interpreting AI-derived gene-expression signatures.

Published studies demonstrate that blood transcriptomic profiles can contain information useful for classification of MS clinical stages, diagnosis and prediction of conversion from clinically isolated syndrome. Single-cell analyses of blood and cerebrospinal fluid additionally show substantial differences in immune-cell composition between biological compartments, indicating that changes in cellular proportions may influence bulk gene-expression signatures.

AI therefore represents a promising tool for the identification and interpretation of molecular biomarkers in MS. However, small cohorts, overfitting, treatment effects and technical variability currently limit generalisability. Further clinical application requires independent prospective validation and integration of transcriptomic information with clinical and MRI data. Such multimodal approaches may improve the reliability and clinical relevance of predictive models in multiple sclerosis.

CHRONOBIOTICSDB V3 AI: LLM-BASED MULTIAGENT SYSTEM FOR CLOCK-MODULATING DRUG SCREENING AND DISCOVERY

D.A. Golubev, I.A. Solovev, G.R. Kabachevskiy, A.I. Yagovkina, N.O. Kotelina

Pitirim Sorokin Syktyvkar State University, Medical Institute, Syktyvkar, Russia

Chronobiotics are compounds that modulate circadian rhythms by changing their phase, period, amplitude or related physiological outputs [1]. Their discovery remains difficult because known clock-modulating compounds are pharmacologically diverse and experimental information on molecular targets, mechanisms and circadian effects is fragmented. We developed three complementary components: ChronobioticsDB, a curated database of clock-modulating compounds [2]; a DeepSeek-based assistant for database exploration and literature-grounded support; and a SMILES-Transformer model for molecular representation and virtual screening of synthetically accessible compounds [3]. The objective of the present work is to integrate these components into ChronobioticsDB v3 AI, a local LLM-based multiagent system for reproducible screening and discovery of new chronobiotics.

The main advancement over previous stages is the transition from a database and AI-assisted analysis toward a domain-specific research environment coordinating several specialized agents. Following the three-stage framework of AI development in chemistry, from AI Assistant to AI Analyst and then to AI Researcher [4], the project has implemented the first two stages and has entered the initial stage of the third. The proposed architecture combines an agent orchestrator with database/RAG, evidence, chemoinformatics, validation and reporting agents. These modules are intended to decompose research questions, retrieve curated data and literature evidence, invoke molecular prediction workflows, assess candidate structures and produce traceable research reports.

ChronobioticsDB provides structured annotations of experimentally studied circadian rhythm modulators, including chemical structures in SMILES format, pharmacological classes, biological targets, mechanisms of action and reported circadian effects [2]. The SMILES-Transformer component extends this resource toward large-scale chemical-space screening and candidate prioritization [3]. In the integrated workflow, the LLM layer is expected to connect evidence retrieval with molecular screening, mechanistic interpretation and the formulation of experimentally testable hypotheses.

ChronobioticsDB v3 AI is conceived as research infrastructure rather than a conventional chatbot. A local multiagent deployment is required for reproducible orchestration, private LLM inference, vector search, model serving and controlled execution of computational tools. The next development stage therefore requires a sufficiently powerful local GPU server. Such infrastructure would support progression from database consultation and analytical assistance toward semi-autonomous prioritization of clock-modulating candidates and preparation of hypotheses for experimental validation.

1. Solovev, I.A., Golubev, D.A. (2024). *Biomeditsinskaya Khimiya*, 70(6), 381-393.
2. Solovev, I.A., Golubev, D.A., Yagovkina, A.I., Kotelina, N.O. (2025). *Clocks & Sleep*, 7(3), 30.
3. Solovev, I.A., Kabachevskiy, G.R., Golubev, D.A., Yagovkina, A.I., Kotelina, N.O. (2026). Preprints, 202604.2062.v1.
4. Ananikov, V.P. (2025). *Analitika*, 15(4), 246-260.

IN SILICO EVALUATION OF NATURALLY OCCURRING MOLECULES WITH CHEMICAL SIMILARITY TO A1AN8 AS INHIBITORS OF THE ANTIBIOTIC RESISTANCE MECHANISM THROUGH THE ACRB EFFLUX PUMP

M. Gonzalez-Quintana¹, C.A. Lobato-Tapia ²

¹*Instituto Politécnico Nacional, Unidad Profesional de Ingeniería Campus Guanajuato, Guanajuato, Mexico*

²*Universidad Politécnica Metropolitana de Puebla, Puebla, Mexico*

Multidrug-resistant (MDR) bacteria represent a major global health problem, since antibiotics that were once used as first-line treatments are losing their effectiveness due to the adaptation of these resistance mechanisms.[1] One of the mechanisms that enables this resistance is efflux pumps, which expel a wide range of structurally diverse antibiotics. In addition to their role in antimicrobial resistance, they are involved in bacterial virulence, as they affect host physiology, the bacterium's stress response, and biofilm formation. [2] One of these pumps is known as AcrB, which is present in pathogenic microorganisms such as *Salmonella enterica* and *Escherichia coli*. [3]

Inhibitors for these pumps are generally natural or synthetic compounds, or small peptides, that target a critical point in the pump's structure, affecting an important aspect of its function, such as the synthetic compound (2S)-1-[(3R)-3-aminopyrrolidin-1-yl]-3-(3,4-dichlorophenoxy) propan-2-ol (A1AN8). [4] Naturally occurring compounds are promising candidates, since they can be produced at large scale through biotechnological techniques with less environmental impact than conventional chemical synthesis. Therefore, the purpose of this project is to use *in silico* methods such as fragment-based virtual screening (FBVS) [5] to identify naturally occurring compounds as potential inhibitors of the pumping activity of the AcrB efflux pump system.

To this end, a total of 10 SMILES codes of compounds were obtained from databases such as NPASS and COCONUT, which were selected by using pubchem-881 fingerprint to identify compounds with structural similarity to A1AN8. An ADMET analysis was performed on these compounds using ADMET Lab 3.0 to assess the toxicity and carcinogenicity of each compound. Subsequently, the binding affinity energy of the 10 compounds was calculated using AUTODOCK VINA. The three compounds with the highest affinity were 3-[3-(3,4-dihydroxyphenyl) prop-2-enoyloxy]-1,4,5-trihydroxycyclohexane-1-carboxylic acid (chlorogenic acid) (-8.4 kcal/mol), amitriptyline (-8.3 kcal/mol), and nortriptyline (-8.3 kcal/mol), which were higher than the binding affinity energy obtained during redocking using the A1AN8 compound (-7.2 kcal/mol). These results demonstrate that these naturally occurring compounds have potential to inhibit the AcrB efflux pump mechanism.

With this progress, it is possible to determine which compounds are candidates for experimental validation, based on the results obtained through chemoinformatic tools.

1. Klenotic, P. A., Moseng, M. A., Morgan, C. E., et al. (2021). *Chem. Rev.*, 121(9), 5378-5402.
2. Gaurav, A., Bakht, P., Saini, M., et al. (2023). *Microbiology (Reading)*, 169(5).
3. World Health Organization. (2025). *WHO News Release*, Oct 13.
4. Allgood, S.C., Su, C.C., Crooks, A.L., et al. (2023) *mBio*, 14(6), e02492-23.
5. Menke, J., Massa, J., Koch, O. (2021). *Comput. Struct. Biotechnol. J.*, 19, 4593-4602.

THE YTHDC1 GLUTAMATE-RICH DOMAIN DOCKS TO THE ADAR1 Z α DOMAIN, LINKING THE N⁶-METHYLADENOSINE MODIFICATION OF PRE-MRNAs TO DSRNA EDITING

D. Gromak¹, A.K. Shaytan^{1,2}, A. Herbert^{1,3}, M. Poptsova¹

¹HSE University, Moscow, Russia;

²Lomonosov Moscow State University, Moscow, Russia;

³InsideOutBio, Charlestown, MA, USA

The p150 isoform of the double-stranded RNA editing enzyme ADAR1 binds Z-DNA and Z-RNA through the conserved winged helix-turn-helix Z α domain. Here, we describe an inverse computational design strategy to map protein interactors of Z α . We used RFdiffusion and ProteinMPNN to generate ~10,000 synthetic binders optimized for the Z α recognition surface, then used their sequences as structural templates for BLASTp searches against the human proteome. Multi-stage screening of ~1,200 candidate regions from 298 proteins via ColabFold pDockQ identified 79 candidates for high-resolution AlphaFold3 modeling, which revealed the m6A reader YTHDC1 as the top-ranked interactor. AlphaFold3 predicts that a glutamate-rich poly-E disordered region of YTHDC1 (residues 199–254) docks into the basic recognition pocket of Z α through a charge-complementary mechanism that mimics the phosphate backbone of Z-RNA. Microsecond molecular dynamics simulations confirmed stability of the binary ADAR1p150–YTHDC1 complex, with the Z α –poly-E interface maintaining RMSD below 3 Å throughout. Ternary complex simulations showed that dsRNA acts as a co-anchoring scaffold stabilizing simultaneous engagement of both proteins in a catalytically dormant conformation. YTHDC1 localizes to transcription-associated YT bodies where nascent RNAs undergo m6A modification and negative supercoiling promotes Z-DNA formation, suggesting that YTHDC1 recruits ADAR1p150 to promote editing of intron-containing substrates prior to splicing.

IN SILICO INVESTIGATION OF THE CYTOTOXIC AND MUTAGENIC POTENTIAL OF TRIMETHYLBENZOYL DIPHENYLPHOSPHINE OXIDE

N.M. Güven Aksu, S. Yılmaz Sarıaltın

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ankara University, Ankara, Türkiye

Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (TPO) is a Norrish type I photoinitiator [1] used in UV-curable coatings, printing inks, artificial nail systems, three-dimensional printing, and dental resins [2]. Upon irradiation, it generates trimethylbenzoyl and diphenylphosphinoyl radicals through α -cleavage, initiating polymerization without a co-initiator [1,3]. TPO is classified in the EU as a reproductive toxicant (Repr 1B) and skin sensitizer (Skin Sens 1B) [4] and has been prohibited in cosmetics since September 2025 [5]. TPO was detected in 27 of 30 gel nail polishes purchased online in China at concentrations of 1.42–6.43% (w/w), despite absent product labelling [6]. The aim of our study is to investigate the potential toxic effects of TPO, with a particular focus on its cytotoxicity and mutagenicity, using *in silico* models. For this purpose, the toxicological profile, cytotoxic potential, and mutagenicity of TPO were comprehensively evaluated from its SMILES representation employing PASS Online 2.0, CLC-Pred 2.0, and Ames mutagenicity prediction tools (2026), respectively [7]. PASS Online 2.0 yielded the highest P_a values for neutrophilic dermatosis ($P_a = 0.761$, $P_i = 0.033$) and testosterone 17 β -dehydrogenase (NADP⁺) inhibition ($P_a = 0.735$, $P_i = 0.043$). Nail discoloration ($P_a = 0.562$, $P_i = 0.077$) was also predicted with $P_a > 0.5$, while lower-probability predictions of cleft palate ($P_a = 0.348$, $P_i = 0.080$) and spermicidal activity ($P_a = 0.305$, $P_i = 0.102$) represented developmental and reproductive endpoints, respectively. CLC-Pred 2.0 predicted TPO to have IC_{50} values ranging from 3.51 to 44.42 μ M across non-tumor cell models and a GI_{50} of 3.27 μ M for human umbilical vein endothelial cells (HUVECs). TPO was predicted inactive among OECD TG 471 specified strains, *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA102 ($P_a = 0.023$ – 0.094 ; $P_i = 0.177$ – 0.627), indicating a lack of both frameshift and base-pair substitution mutagenic activity. Overall, the findings indicated cell-specific cytotoxic potential and several toxicity-related signals on TPO, while mutagenicity was predominantly predicted as inactive across OECD TG 471 recommended strains. Although our computational outcomes possess valuable insights, these findings are preliminary and require experimental validation.

1. Popal, M., Volk, J., Leyhausen, G., et al. (2018). *Dental Materials*, 34, 1783–1796.
2. NICNAS (2019). Phosphine oxide, diphenyl(2,4,6-trimethylbenzoyl)-: Human health tier II assessment.
3. Kowalska, A., Sokolowski, J., Bociong, K. (2021). *Polymers*, 13, 470.
4. European Commission (2024). *Commission Delegated Regulation (EU) 2024/197*. *Off J Eur Union*, L 2024/197.
5. European Commission (2025). *Commission Regulation (EU) 2025/877*. *Off J Eur Union*, L 2025/877.
6. Gong, X., Sun, J., Li, X. et al. (2025). *Chinese Journal of Chromatography*, 43(12), 1380–1388.
7. Filimonov, D.A., Lagunin, A.A., Glorizova, T.A. et al. (2014)/ *Chemistry of Heterocyclic Compounds*, 50, 444–457.

INTEGRATIVE TRANSCRIPTOMIC AND MACHINE LEARNING ANALYSIS OF ECDNA-ASSOCIATED FEATURES FOR STUDYING CHEMOTHERAPY RESISTANCE IN TNBC

Md. Iftehimul, D. Saha

Department of Biology, North Carolina Agricultural and Technical State University, Greensboro, NC, USA

Extrachromosomal DNA (ecDNA) has emerged as a critical mediator of oncogene amplification and transcriptional dynamics in aggressive cancers. Investigating the role of ecDNA in chemotherapy resistance *in vivo* will provide mechanistic insight into the evolvability of aggressive cancers and may further help guide potential treatment options. This study evaluates the association between ecDNA and predictive chemotherapy resistance in TNBC. We analyzed RNA-seq data from 4T1 TNBC cells and 4T1 bulk tumors at different growth stages (1-, 3-, and 6-week) to identify differentially expressed ecDNA alterations. We then used computationally modeled molecular docking to predict ecDNA protein-drug interactions and employed machine learning (ML) models to predict ecDNA-associated therapeutic resistance. Our results revealed changes in global gene expression, including expression of ecDNA-associated genes, that continued over time, with significant molecular remodeling observed at six weeks. Additionally, we found a gradual accumulation of mutations in ecDNA genes, which may have contributed to reduced drug binding affinity, suggesting potential resistance. ML models generated stable, high-confidence classification of resistant phenotypes, consistently identifying ecDNA burden and prevalence as dominant predictive features of drug resistance. Drug-specific predictions further highlighted elevated resistance probabilities for paclitaxel and doxorubicin, whereas hydroxyurea, which depletes ecDNA, showed reduced resistance probabilities, indicating a role for ecDNA in chemoresistance. This study provides new insights into the temporal remodeling of ecDNA in TNBC tumors and their potential association with drug resistance.

MANIA: A REPRODUCIBLE RESIDUE INTERACTION NETWORK PIPELINE FOR MOLECULAR DYNAMICS ANALYSIS OF THE TUMOR-ASSOCIATED TRANSPORTER NaPi2b (SLC34A2)

A.A. Illarionov, N.I. Akberova, R.A. Vlasenkova

Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia

Molecular dynamics (MD) is widely used to characterize conformational ensembles relevant to structure-based drug discovery, but reproducible interpretation of trajectories remains difficult because preprocessing, software versions and comparison assumptions are often incompletely recorded. The objective of this study was to develop and validate MANIA, a deterministic Python workflow that converts MD trajectories into auditable residue interaction network (RIN) descriptors, and to demonstrate it on NaPi2b (SLC34A2), a sodium-dependent phosphate transporter investigated as a tumor-associated cell-surface target [1].

Established tools construct dynamic residue networks and analyze protein structural ensembles [2, 3]. MANIA advances this workflow at the level of traceability rather than by introducing a new network metric. Preprocessing, static and sliding-window RIN analysis, binary contact fingerprints, principal component analysis (PCA), deterministic k-means clustering and pairwise condition comparison are integrated in one pipeline. Each requested output is assigned an explicit computed, skipped or unavailable status, while a provenance manifest records inputs and parameters. Contacts are defined between heavy atoms using a 4.5 Å cutoff, and stable residue identity is checked before cross-condition aggregation. The scientific layer reports degree, strength, betweenness, closeness, eigenvector centrality, PageRank and k-core, greedy-modularity communities and temporal network metrics. Trajectory processing is implemented with MDAnalysis [4].

Proof-of-concept used 30 ns all-atom GROMACS trajectories of two previously prepared NaPi2b systems labeled NORM and TUMOR (CHARMM36, TIP3P). The 5-10 ns interval was analyzed: 101 frames, 690 residues and approximately 5500 contacts per frame in each system. All graph diagnostics passed; key global metrics are summarized in Table 1. Residues 530 and 606 had the highest betweenness in both systems, whereas N-terminal residues 46, 47 and 51 ranked more prominently in TUMOR. Sliding-window density (~0.013) and modularity (~0.63) were stable. PCA showed no dominant component (PC1 ≈ 6%), and two-cluster solutions were weak (silhouette 0.046 and 0.054), consistent with gradual drift rather than well-separated states in this short single-replica pilot.

Table 1. Key global RIN metrics for NaPi2b (5-10 ns, 101 frames)

Metric	NORM	TUMOR
Static RIN edges	3719	3689
Communities	5	4
Modularity	0.617	0.602
k (silhouette)	2 (0.046)	2 (0.054)

Critically, MANIA did not compute aggregate cross-condition statistics: the comparison received `skipped_identity_conflict` because His358 and His491 had different protonation labels (HSD/HSP), while residue-level outputs were retained. This demonstrates the practical significance of the status model: incompatible inputs are surfaced instead of being converted into apparently valid biological differences. The present single-replica pilot therefore validates a reproducible and diagnosable baseline for target-focused MD analysis; biological condition effects require longer replicated simulations with uncertainty estimates and false-discovery-rate-controlled inference.

1. Lin, K., Rubinfeld, B., Zhang, C., et al. (2015). *Clin. Cancer Res.*, 21, 5139-5150.
2. Brown, D.K., Penkler, D.L., Sheik Amamuddy, O., et al. (2017) *Bioinformatics*, 33, 2768-2771.
3. Sora, V., Tiberti, M., Beltrame, L., et al. (2023) *J. Chem. Inf. Model.*, 63, 4237-4245.
4. Michaud-Agrawal, N., Denning, E.J., Woolf, T.B., Beckstein, O. (2011). *J. Comput. Chem.*, 32, 2319-2327.

WHICH OXIRANE CARBON DOES ASP12 ATTACK? QM/MM FREE-ENERGY PROFILES OF THE COVALENT INHIBITION OF KRAS(G12D) BY YK-8S

E.M. Ilyukhina², A.M. Kulakova¹

¹*Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia*

²*Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia*

KRAS mutations at codon 12 drive about a quarter of human cancers, and the most frequent variant, KRAS(G12D), lacks the cysteine exploited by the approved covalent drugs. The ligand YK-8S covalently modifies both KRAS(G12C) and KRAS(G12D) through an oxirane warhead [1]. The oxirane ring is asymmetric, so the Asp12 carboxylate can open it either at the CH carbon (α to the amide carbonyl) or at the terminal CH₂ carbon, while the crystal structure of the adduct (PDB 8JHL) shows the CH product. The objective of this work was to obtain the free-energy profiles of both pathways and to identify the factors that control the regioselectivity of the ring opening.

The model of KRAS(G12D)·GDP·Mg²⁺ with YK-8S was built from PDB 8JHL (CHARMM36/CGenFF/TIP3P) and equilibrated by classical MD in NAMD. QM/MM MD simulations were performed with the NAMD–TeraChem interface (PBE0-D3/6-31G**, electrostatic embedding, link atoms). Umbrella sampling was carried out along $\xi = d(\text{C}-\text{O}_{\text{oxirane}}) - d(\text{C}-\text{O}\delta_{\text{Asp12}})$; the profiles were reconstructed by WHAM and umbrella integration, and the rate constants were estimated with the Eyring equation.

With a 51-atom QM region (warhead, Asp12 side chain and the nearest waters) the activation free energies are 29.0 (CH) and 25.0 kcal/mol (CH₂), the reaction free energies +17 and +0.6 kcal/mol, and $k = 3.3 \cdot 10^{-9}$ and $2.9 \cdot 10^{-6} \text{ s}^{-1}$, respectively; i.e., the model favours the CH₂ attack, in contradiction with the experimentally observed product. The advancement of the present study is an enlarged, 138-atom QM region that includes Lys16 and Ser17 of the P-loop, Glu37, Thr58–Gln61 of Switch II, Arg68, the GDP phosphates, Mg²⁺ and 17 water molecules. Analysis of the umbrella-sampling trajectories showed that the backside position of the attacked CH carbon is occupied by Lys16 N ζ (85–99% of frames), which is anchored to the GDP β -phosphate and the Ala11 carbonyl. As a result, the attack angle does not exceed 159° and the transition state is loose (both C···O distances $\approx 1.9 \text{ \AA}$). Ring opening is coupled to proton transfer from a QM water molecule to the leaving oxygen, whereas the CH₂ pathway requires an unfavourable approach of the forming hydroxyl to the Gly60 backbone of Switch II.

With the enlarged QM region the activation free energies are 33.9 (CH) and 20.7 kcal/mol (CH₂), and the reaction free energies are +10.9 and –4.8 kcal/mol, respectively. Thus, enlarging the QM region increases the preference for the CH₂ attack, and the experimentally observed regioselectivity is not reproduced with a one-dimensional reaction coordinate. The coupling of ring opening with proton transfer and the blocking role of Lys16 indicate that two-dimensional umbrella sampling including the proton-transfer coordinate is required; these calculations are in progress.

1. Yu, Z., He, X., Wang, R., Xu, X., Zhang, Z., Ding, K., Zhang, Z.-M., Tan, Y., Li, Z. (2023). *J. Am. Chem. Soc.*, 145, 20403–20411.

MULTI-TARGET EVALUATION OF SOME EPICATECHIN DERIVATIVES AGAINST CRITICAL BIOLOGICAL TARGETS: MOLECULAR DOCKING, EFFICIENCY METRICS, AND STRUCTURAL DYNAMICS

F. İslamoğlu

Department of Chemistry, Recep Tayyip Erdoğan University, Rize, Türkiye

Epicatechin and its structurally modified derivatives have attracted considerable interest as potential bioactive scaffolds because of their diverse molecular recognition properties [1-3]. In the present study, twelve epicatechin derivatives were subjected to a comprehensive in silico investigation against a panel of pharmacologically relevant protein structures identified by Protein Data Bank (PDB) codes 6Y3C, 1UV5, 3IAI, 4RE3, 4D07, and 2OYE. Molecular docking calculations were performed using AutoDock Vina to comparatively evaluate the predicted binding affinities of the derivatives toward the selected protein targets. Among the investigated complexes, epicatechin derivative 8 ((2R,3R)-4-allyl-2-(3,4-dihydroxyphenyl) chroman-3,5,7-triol) exhibited the most favorable docking score against 4RE3, with a predicted binding affinity of $-11.2 \text{ kcal mol}^{-1}$. The docking results were further subjected to a multidimensional ligand-efficiency assessment, including estimated inhibitory constants, ligand efficiency (LE), lipophilic ligand efficiency (LLE/LipE), size-independent ligand efficiency (SILE), Fit Quality, binding efficiency index (BEI), and surface efficiency index (SEI). These complementary metrics provided a more comprehensive evaluation of the relationship between molecular size, lipophilicity, structural characteristics, and predicted binding affinity than docking scores alone. The highest-ranked 8–4RE3 complex was subsequently investigated through two- and three-dimensional Ramachandran analyses and normal mode analysis to further characterize the conformational and structural properties of the docked protein–ligand system. In addition, bioavailability radar profiles were generated for molecule 8 epicatechin derivative, while SwissADME-based Boiled-Egg plots were used to assess their predicted gastrointestinal absorption and blood–brain barrier permeability characteristics. Collectively, the results provide an integrated computational profile of the investigated epicatechin derivatives and identify derivative 8 as the most promising compound within the studied series based on its predicted binding affinity and complementary ligand-efficiency characteristics.

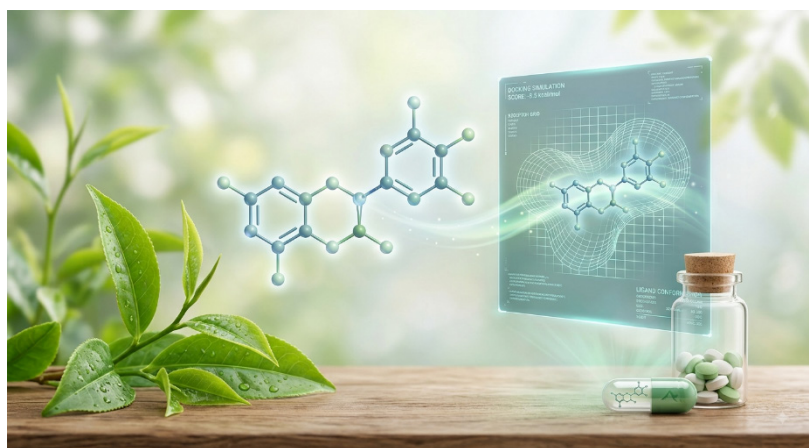


Figure 1. A visual representation of the potential of an epicatechin derivative as a drug candidate

1. Shay, J., Elbaz, H. A., Lee, I., et al. (2015). *Oxidative Medicine and Cellular Longevity*, 2015, 181260.
2. Miranda Buendia, E., González-Gómez, G. H., Maciel-Cerda, A. et al. (2025). *Tissue Engineering Part B: Reviews*, 31, 504-516.
3. Al-Shuhaib, M.B.S., Hashim, H.O., Al-Shuhaib, J.M.B. (2022). *Computers in Biology and Medicine*, 141, 105155.

IN SILICO DISCOVERY OF EPICATECHIN DERIVATIVES AS MULTI-TARGET KINASE INHIBITORS FOR CANCER THERAPY: INTEGRATING DOCKING, ADMET PROFILING, AND NORMAL MODE ANALYSIS

Z.N. İslamoğlu

*Department of Molecular Biology and Genetics, Faculty of Life and Natural Sciences,
Abdullah Gül University, Kayseri, Türkiye*

Epicatechin derivatives represent a promising class of bioactive polyphenols with notable anticancer potential [1]. In this study, twelve epicatechin derivatives were systematically evaluated through a comprehensive in silico workflow to identify potent lead candidates against six key oncological protein targets (PDB IDs: 1M17, 5IKQ, 1IEP, 1AQ1, 1JD0, and 5M8L). Molecular docking simulations executed via AutoDock Vina [2] revealed that compound 11 ((2R,3S)-4-chloro-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol) (Figure 1) exhibited the highest binding affinity toward the 5IKQ target, achieved docking score of -10.1 kcal/mol. In order to rigorously verify the binding quality beyond crude binding energy, along with the Half maximal inhibitory concentration (IC_{50}), critical ligand efficiency metrics, including Ligand Efficiency (LE), Lipophilic Efficiency (LipE/LLE), Size Independent Ligand Efficiency (SILE), Fit Quality (FQ), Binding Efficiency Index (BEI) and Surface Efficiency Index (SEI), were calculated using physicochemical parameters (molecular weight, number of heavy atoms, TPSA and LogP) derived from ADMET [3]. Pharmacokinetic evaluation via ADMETlab 3.0 [4] confirmed that compound 11 possesses drug-likeness and optimal ADMET profiles, which were further validated through a 13-parameter bioavailability radar map. Stereochemical reliability and backbone conformational stability of the top-performing 5IKQ-compound 11 complex were verified using 2D and 3D Ramachandran plots generated via Ramplot. Furthermore, Normal Mode Analysis (NMA) performed via the iMODS server demonstrated low deformability, favorable structural flexibility, and robust dynamic stability of the docked complex. Overall, these integrative computational findings strongly highlight compound 11 as a bioavailable, thermodynamically stable, and structurally optimal lead candidate for cancer therapy, offering a solid theoretical foundation for future in vitro and in vivo oncological validations.

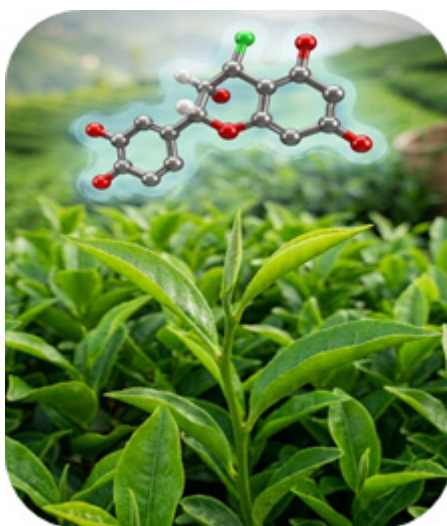


Figure 1. Epicatechin derivative ((2R,3S)-4-chloro-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol)

1. Shay, J., Elbaz, H.A., Lee, I., et al. (2015) *Oxidative Medicine and Cellular Longevity*, 2015, 181260.
2. Trott, O., Olson, A.J. (2010). *Journal of Computational Chemistry*, 31, 455-461.
3. Hopkins, A.L., Groom, C.R., Alex, A. (2004). *Drug Discovery Today*, 9, 430-431.
4. Fu, L., Shi, S., Yi, J. et al. (2024). *Nucleic Acids Research*, 52, W422-W431.

C3-FUNCTIONALIZED ANDROSTANE HYDRAZONO-HYDRAZIDES: NEW CANDIDATES FOR AKR1C3 INHIBITION

Đ. Janković¹, S. Bekić¹, M. Marinović², M. Savić¹, A. Čelić², E. Petri², M. Stevanović¹, J. Ajduković¹

¹University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia

²University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia

Steroidal compounds represent an important class of biologically active molecules that interact with molecular targets involved in physiological and pathological processes [1]. Among these, aldo-keto reductases (AKRs) are NAD(P)-dependent enzymes involved in the metabolism of steroid hormones, prostaglandins, xenobiotics, and therapeutic agents. AKR1C3 is of particular interest as a potential anticancer target because it is expressed in several tumor types and is involved in steroid hormone metabolism and therapy resistance. At the same time, AKR1C4 is predominantly expressed in healthy tissues, particularly the liver [2]. Hydrazono-hydrazide derivatives represent promising scaffolds with diverse biological activities, including antitumor effects [3].

This study aimed to synthesize two novel androstane hydrazono-hydrazide derivatives modified at position 3 using thiocarbohydrazide and phenylthiosemicarbazide, and to evaluate their potential as AKR1C3 inhibitors. Their inhibitory activity toward recombinant AKR1C3 was evaluated *in vitro*, while selectivity was assessed using AKR1C4. Both compounds demonstrated moderate to good inhibitory activity against AKR1C3, with the phenylthiosemicarbazone derivative exhibiting improved selectivity. Molecular docking was performed using AutoDock Vina against the AKR1C3 structure 1ZQ5. The synthesized compounds showed comparable docking scores of −9.72 and −10.43 kCal/mol, respectively, although both were less favorable than the reference molecule (−15.30 kCal/mol). Analysis of the predicted binding poses revealed key hydrogen-bonding and hydrophobic interactions within the active site. In addition, the pharmacokinetic properties and potential toxicity of the compounds were predicted using SwissADME and ProTox 3.0. Overall, the obtained results support further investigation of C3-hydrazono-hydrazide-functionalized androstanes as potential AKR1C3-targeting compounds.

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1. Singh, Bansal (2025). *RSC Med. Chem.*, 16, 2902.
2. Nagini, et al. (2024). *Oncol. Res.*, 32, 1287.
3. Murugappan, et al. (2024). *J. Mol. Struct.*, 1307, 138012.

IN SILICO DESIGN AND MOLECULAR MODELLING OF IL-13 TARGETTING DNA APTAMER CANDIDATES FOR ECZEMA-RELATED STUDIES

M.M.N. Juraidi¹, W.S.F.A. Wan Ibrahim¹, L. Rozano²

¹Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Malaysia

²Malaysian Agricultural Research and Development, Serdang, Selangor, Malaysia

Atopic dermatitis (eczema) is a chronic inflammatory skin condition involving multiple immune mediators, including interleukin-13 (IL-13), which plays an important role in disease progression. DNA aptamers are promising molecular recognition elements that can adopt specific three-dimensional structures to bind target molecules. This study have three main objectives: (1) to identify and select a suitable IL-13 protein structure, (2) design and prioritize IL-13-targeting DNA aptamer candidates based on sequence, and (3) structural characteristics and evaluate IL13-DNA aptamer complex predicted binding potential through molecular docking.

Conventional aptamer discovery commonly relies on experimental approaches such as Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which involves repeated cycles of selection and amplification. As an advancement, this study explores a structure-guided *in silico* strategy to computationally generate, screen, and prioritize aptamer candidates before experimental validation. The computational workflow included six steps: IL-13 PDB selection, generation of aptamer library, screening of the structure, molecular docking, analysis of the interface residues and final selection of candidates. Structural analysis of IL-13 structure was done in PDB and PyMOL and binding-site analysis was done in RING. In order to create a library of aptamers, a set of five anchor sequences were used to generate aptamer libraries in silico with python-based scripts and filtered with biopython and fold-and-filter. Moreover, structural screening included 3DNA for secondary-structure and hairpin evaluation, QGRS for hairpin screening of G-quadruplexes, and MolProbity for structural quality evaluation, followed by Python-based minimization. On the other hand, three aptamers were selected and used for molecular docking using the HDock Web Server and compared to the benchmark molecule, 5UC6, with a specified binding site of IL-13. Prioritizing representative candidates, predicted interfaces were analysed using HDock and PyMOL, comparing predicted and experimental binding residues.

Among the evaluated candidates, Aptamer 13 Model 4 achieved the lowest docking score of -130.250 , with a confidence score of 0.4025 and an overlap percentage of 80.95% of known binding residue in IL-13. Aptamer 1 Model 2 and Aptamer 15 Model 1 recorded docking scores of -87.660 and -70.670 , respectively. Overall, the findings demonstrate the feasibility of an integrated computational workflow for prioritizing IL-13-targeting DNA aptamer candidates. The resulting structurally characterized candidates provide a foundation for subsequent experimental validation and further investigation of IL-13-targeting aptamers for eczema-related research.

SYNTHESIS OF NEW FUNCTIONALIZED 1,4-DIHYDROPYRIDINE DERIVATIVES AND STUDY OF THEIR OPTICAL PROPERTIES

E.A. Karymova¹, M.I. Fokina¹, A.V. Egorova², D.M. Egorov²

¹*ITMO University, St. Petersburg, Russia*

²*Saint-Petersburg State Institute of Technology, St. Petersburg, Russia*

1,4-Dihydropyridine (1,4-DHP) derivatives hold a prominent position in medicinal chemistry. Their antihypertensive, vasodilatory, neuroprotective, and antioxidant properties have been reported in the scientific literature; these effects are attributed to the blockade of L-type voltage-gated calcium channels, the ability to scavenge reactive oxygen species, and the capacity to affect multiple pathological targets simultaneously. The aim of this study was to synthesize novel functionalized 1,4-DHPs and to predict their potential biological applications. The synthesis was carried out via a modified three-component Hantzsch reaction: condensation of an aromatic aldehyde, ethyl acetoacetate, and ammonium acetate in a molar ratio of 1:2:3.

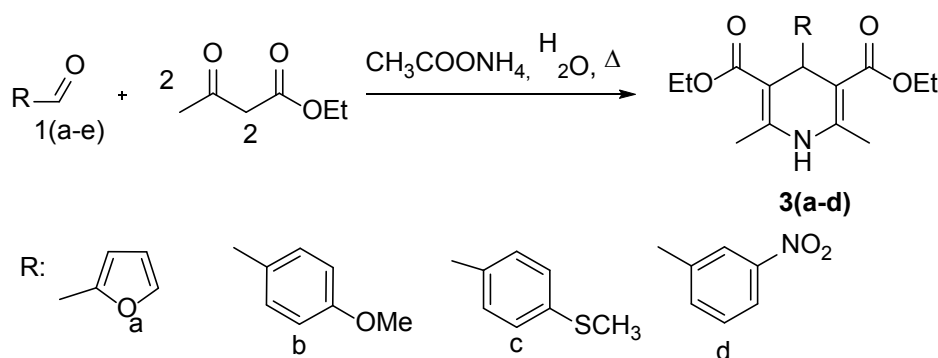


Figure 1. Scheme for the synthesis of novel 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives.

The PASS and MolPredict web services were used to evaluate possible biological activity. For **3b**, the following activities were predicted: membrane-stabilizing activity (0.909), antihypertensive activity (0.878), coronary vasodilation (0.877), interaction with voltage-gated calcium channels (0.822), among others. For **3c**, the predicted activities included antihypertensive activity (0.843), coronary vasodilation (0.826), and antihistamine activity (0.779), among others. MolPredict indicated a high probability of activity for **3b** against NADPH-dependent targets associated with Alzheimer's disease (Probability Active = 1.0, reliable).

In addition, analysis of the pharmacokinetic properties of compound **3b** using SwissADME revealed high gastrointestinal absorption, blood–brain barrier permeability, absence of P-glycoprotein substrate activity, compliance with Lipinski's rule (0 violations), and a bioavailability score of 0.55.

Collectively, literature data and the results of PASS, MolPredict, and SwissADME analyses suggest that compound **3b** warrants further investigation as a potential orally administered neuroprotective agent.

COMPUTATIONAL PROFILING OF PHYTOCHEMICAL-BASED MODULATORS TARGETING THE IMMUNOMETABOLIC AXIS IN THE TUMOR MICROENVIRONMENT

S. Kharade, S.L. Gaonkar, R.S. Bilachi

Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka, India

Cancer is a complex and dynamic disease characterized by extensive molecular interactions and substantial tumor microenvironment (TME) heterogeneity. Immunometabolism has emerged as an important concept in cancer biology, describing the interplay between metabolic pathways and immune-cell functions within the tumor immune microenvironment (TIME). Therapeutic modulation of immunometabolic pathways may help alleviate immunosuppression by influencing tumor, immune, and stromal cell functions. However, metabolic heterogeneity and tumor-specific metabolic reprogramming within the TME remain major challenges for the development of effective multitarget therapeutic strategies.

In the present study, an integrated computational approach incorporating molecular docking, interaction energy calculations, quantum mechanical analyses, and molecular dynamics (MD) simulations was employed to investigate CID 5481224 (GUA) and its rationally designed heterocyclic derivatives against selected targets associated with the immunometabolic axis. Notably, GUA and its derivatives have not previously been reported or systematically evaluated against the selected targets, providing a basis for their computational investigation in the context of tumor immunometabolism. GUA was identified as a potential multitarget scaffold, while incorporation of heterocyclic moieties generated derivatives that showed improved predicted binding interactions with several targets, particularly GS12, GS13, and GS7. DFT analysis revealed reduced ΔE values for the optimized derivatives relative to the parent compound. MM/GBSA calculations further indicated favorable binding energetics, particularly for GS6 against key metabolic and inflammatory targets. Molecular dynamics simulations demonstrated comparatively greater conformational stability of the GS7–GLUT1 complex relative to the parent compound. These findings demonstrate the utility of an integrated computational strategy for exploring structurally modified natural-product scaffolds and provide a rationale for further investigation of GUA-derived heterocyclic compounds as potential multitarget modulators of tumor immunometabolism.

INVERSE VARIANCE WEIGHTING CONCENTRATES INFLUENCE IN THE FREESOLV BENCHMARK

M.G. Kinandityo, A.E.R. Christsanda

Independent Researcher, Indonesia

Hydration free-energy benchmarks contain experimental uncertainty values with different origins. We examined how uncertainty-based weighting changes evaluation of the 642-compound FreeSolv database [1]. This analysis adds a compound-influence audit to aggregate error reporting, using the archived experimental values and calculated hydration free energies. Records carrying a default-uncertainty note were identified from the provenance field. We compared unweighted root mean squared error (RMSE) with RMSE weighted by the inverse sum of experimental and calculated variances. Weight concentration was measured using the Kish effective sample size. Experimental uncertainty floors were applied as sensitivity scenarios.

Default-uncertainty notes occurred in 583 records (90.8%). Unweighted RMSE was 1.542 kcal/mol, compared with 1.608 kcal/mol under inverse variance weighting. The effective sample size fell to 88.1 despite all 642 compounds contributing. The ten most heavily weighted compounds received 17.0% of the total weight but accounted for only 0.9% of unweighted squared error. Setting an experimental uncertainty floor of 0.2 kcal/mol produced a weighted RMSE of 1.765 kcal/mol; a 0.6 kcal/mol floor produced 1.437 kcal/mol.

These changes arise from scoring choices applied to unchanged predictions. They show why benchmark reports should retain uncertainty provenance and present weight concentration alongside weighted errors. The floors are analytical scenarios, not corrected measurements. This audit evaluates one archived prediction set and does not establish which uncertainty convention is physically preferable.

1. Mobley D.L., Guthrie J.P. (2014). *J. Comput. Aided Mol. Des.*, 28, 711–720.

MITOCHONDRIAL MEMBRANE POTENTIAL ASSAY ACTIVITY TRACKS OTHER STRESS RESPONSES IN TOX21

A.E.R. Christsanda, M.G. Kinandityo

Independent Researcher, Indonesia

A positive mitochondrial membrane potential assay can accompany other cellular stress responses, complicating interpretation of screening hits. We assessed this overlap in the public MoleculeNet Tox21 dataset [1], adding a scaffold-aware analysis of coactivity to its use as a prediction benchmark. Of 7,831 molecular records, 7,823 had structures that RDKit could parse; canonicalisation identified no duplicate structures. We calculated complete-case odds ratios between the mitochondrial membrane potential assay (SR-MMP) and four other stress-response assays. Missing labels were left missing. Confidence intervals were estimated from 5,000 bootstrap samples of Bemis–Murcko scaffold clusters, with each acyclic compound treated as a separate cluster.

Among 4,455 compounds with both SR-MMP and antioxidant response element (SR-ARE) labels, ARE activity occurred in 55.7% of MMP-active compounds and 8.7% of MMP-inactive compounds. The odds ratio was 13.17 (95% scaffold-bootstrap interval 10.62–16.84). For p53, the odds ratio was 15.65 (11.94–21.74; 5,434 compounds). Associations were also positive for ATAD5 and heat-shock response, with odds ratios of 9.20 and 9.42. Removing the largest scaffold cluster left the ARE and p53 odds ratios at 13.43 and 16.74.

MMP activity therefore frequently accompanies broader stress-assay activity in this dataset. Interpreting an isolated MMP hit without checking available companion assays may overlook that overlap. These cross-assay associations do not establish a causal pathway or clinical toxicity. Concentration effects and assay interference remain possible explanations, and the findings concern this processed screening dataset.

1. Wu Z., Ramsundar B., Feinberg E.N., et al. (2018). *Chem. Sci.*, 9, 513–530.

MOLECULAR MODELING OF NAD: STRUCTURAL DYNAMICS AND UV/VISIBLE SPECTRA

V.B. Krapivin^{1,2}, M.G. Khrenova¹

¹Lomonosov Moscow State University, Moscow, Russia;

²Ioffe Institute, Saint Petersburg, Russia.

Nicotinamide adenine dinucleotide (NAD) in its oxidized (NAD⁺) and reduced (NADH) forms is an important coenzyme involved in many biochemical reactions. The oxidized and reduced forms have different spectral characteristics. In the UV/visible spectrum of NAD⁺, the absorption band maximum is observed at 260 nm, which corresponds to the excitation of adenine and nicotinamide fragments. In contrast, the NADH spectrum has a second maximum at 340 nm, corresponding to the reduced nicotinamide fragment. Since the absorption bands of both fragments merge into a single peak in NAD⁺, precise measurement of the NAD⁺/NADH ratio remains an actual problem, and biochemical reactions are monitored by measuring the accumulation of the NADH form. This study focuses on detailed molecular mechanical and quantum chemical modeling of excited states to determine the relative positions of the nicotinamide and adenine bands in NAD⁺ and comparison with NADH. The obtained results can be useful for direct experimental determination of the NAD⁺/NADH ratio in cells.

It is known that the position and brightness of the spectrum lines are influenced by the conformational mobility of the molecule. Thus, the mobility of the nicotinamide and adenine fragments can shift the absorption maxima both to the red and blue regions [1], and also determine the difference in the fluorescence spectra [2]. Therefore, detailed conformational analysis is necessary for an accurate interpretation of the experimental spectra. In this study, the conformational mobility of NAD was provided using classical molecular dynamics simulation (MD) with CHARMM force field and molecular dynamics with combined quantum-chemical/molecular-mechanical potentials (QM/MM) in aqueous solution, taking into account explicit water molecules. Three types of conformations were identified based on the analysis of intramolecular contacts between the adenine (Adn) and nicotinamide (Nam) fragments: (1) open, without intramolecular interactions; (2) stack with close contacts between fragments; (3) stack conformations with π - π stacking interactions between And and Nam aromatic rings. The criterion for π -stacking was the distance between the aromatic rings centers $d(\text{And-Nam}) < 5 \text{ \AA}$ and the angle between them $\theta < 30^\circ$. The total fraction of stack conformations according to the classical MD trajectory analysis is 10-18 %, and the fraction of π -stacking conformations is less than 3 %. Absorption band calculations were performed for each type of conformation based on a 5 ps QM/MM dynamics trajectory using the method described in [3]. The absorption bands of the oxidized and reduced forms of NAD were calculated using the TD-DFT method with wB97XD functional. This functional contains a long-range correction and allows to provide the qualitative excited states calculation of molecules. The modeling of the individual adenine and nicotinamide revealed a systematic shift of 45 nm relative to the experimental absorption bands. For reduced form of NAD, the π -stacking conformation shifts the absorption band toward the red region compared to the open conformation. This effect is more pronounced for the nicotinamide fragment. If this effect can be determined for the oxidized form of NAD (and NADP), it may allow direct measurement of the NAD⁺/NADH ratio in cells.

This study was supported by the Russian Science Foundation (grant no. 26-75-31002).

1. Cao, X., Wu, L., Zhang, J., Dolg, M. (2020). *Journal of Computational Chemistry*, 41, 305.
2. Blacker, T. S., Duchon, M. R., Bain, A. J. (2023). *Biophysical journal*, 122, 1240.
3. Volkov, D. A., Gorbunova, I. A., Yashkov, D. V. et al. (2025). *Journal of Photochemistry and Photobiology A: Chemistry*, 461, 116155.

INTEGRATED QSAR RISK ASSESSMENT AND TRANSCRIPTOMIC EVALUATION OF CHEMICAL SAFETY: APPLICATIONS TO FOOD CONTACT SUBSTANCES AND CHRONIC ULTRA-LOW-DOSE ROUNDUP EXPOSURE

A. Kumar, P.K. Ojha

*Drug Discovery and Development Laboratory, Department of Pharmaceutical Technology,
Jadavpur University, Kolkata, India*

The widespread use of chemicals in food systems, food contact materials, and agricultural products has increased the need for reliable approaches to identify substances with potential adverse health effects. In the present study, Quantitative Structure–Activity Relationship (QSAR) modeling was integrated with transcriptomic evidence to evaluate chemical safety and investigate molecular mechanisms associated with chronic ultra-low-dose Roundup exposure. Toxicological endpoints including human exposure (mg/day), carcinogenic potency (TD_{50}), and No/Lowest Observed Adverse Effect Level (N/LOAEL) were analyzed to identify structural features and physicochemical characteristics associated with toxicity. QSAR analysis generated robust predictive models for human exposure and carcinogenic potency. The human exposure model achieved an R^2 of 0.835 and Q^2_{LOO} of 0.705, while the TD_{50} model yielded an R^2 of 0.827 and Q^2_{LOO} of 0.715. External validation further confirmed their predictive reliability, with Q^2_{F1} values of 0.847 and 0.883, respectively. The N/LOAEL model showed moderate predictive performance ($R^2 = 0.686$; $Q^2_{F1} = 0.628$), reflecting the complexity of dose-dependent toxicological responses. Structural interpretation of the models revealed that chemicals possessing chlorinated molecular fragments, nitrogen-containing functional groups, and unsaturated structural regions were more frequently associated with elevated toxicity. These features are known to influence chemical persistence, electrophilic reactivity, metabolic activation, and interactions with biological macromolecules. Several physicochemical properties appeared to contribute to the observed toxicological behavior. Increased lipophilicity and hydrophobicity may enhance membrane permeability, tissue accumulation, and bioavailability, while the presence of halogenated substituents, particularly chlorine atoms, can increase environmental persistence and resistance to metabolic degradation. Nitrogen-containing moieties may alter molecular polarity, hydrogen-bonding capacity, and protein-binding affinity, thereby affecting biological activity and toxic potential. Unsaturated chemical structures may facilitate oxidative reactions and the formation of reactive intermediates capable of inducing cellular damage. Transcriptomic evidence from chronic ultra-low-dose Roundup exposure studies identified molecular biomarkers associated with liver and kidney injury. Differentially expressed genes were predominantly involved in oxidative stress regulation, mitochondrial function, lipid and energy metabolism, xenobiotic detoxification, inflammatory signaling, apoptosis, and cellular stress-response pathways. These molecular alterations suggest disruption of metabolic homeostasis and cellular defense mechanisms, consistent with early stages of hepatorenal toxicity. Biomarkers related to oxidative stress and mitochondrial dysfunction were particularly prominent, indicating that reactive oxygen species generation and impaired energy metabolism may represent key mechanisms of toxicity. The integration of QSAR-derived structural alerts with transcriptomic biomarkers provides a comprehensive framework for chemical risk assessment. The results suggest that chlorinated fragments, nitrogen-containing functionalities, increased lipophilicity, hydrophobic character, and unsaturated structural features are important determinants of toxicological activity, while oxidative stress-, metabolism-, and inflammation-related biomarkers serve as sensitive indicators of adverse biological effects. This combined approach strengthens the mechanistic interpretation of toxicity and supports the prioritization of potentially hazardous chemicals used in food-related applications and environmental exposure settings.

1. European Food Safety Authority (EFSA). (2016). *EFSA Journal*, 14(1), 4357.
2. Ames BN, Magaw R, Gold LS. (1987). *Science*, 236(4799), 271–280.

DIMENSIONALITY REDUCTION AND CLUSTERING FRAMEWORK FOR MAPPING HYDROLASE ACTIVE SITE DYNAMICS

M.E. Kuznetsov^{1,2}, I.V. Polyakov^{1,2}

¹*Lomonosov Moscow State University, Moscow, Russia*

²*Emanuel Institute of Biochemical Physics RAS, Moscow, Russia*

Characterizing the conformational landscape of enzyme-substrate (ES) complexes is important for computer-aided drug design. Classical molecular dynamics (MD) simulations generate extensive trajectories, and it is hard to extract only a few relevant structures [1]. Here we present a universal and scalable analysis pipeline designed to systematically map the structural and solvation dynamics of serine hydrolase [2] active sites.

The proposed framework was tested on the *Candida antarctica* lipase B (CALB) complexed with the p-nitrophenyl benzoate (pNPB) substrate. The initial system was constructed based on the PDB entry 6TP8[3], employing the CHARMM36m force field for the protein and CGenFF for pNPB. Enhanced sampling was achieved with Gaussian accelerated Molecular Dynamics (GaMD) [4] for 200 ns to ensure thorough equilibration. Multiple production runs of 100 ns each were performed under NPT conditions (300 K, 1 atm, Langevin dynamics) using a 2 fs timestep and SHAKE/SETTLE algorithms. The first 10 ns of each production run were discarded as equilibration overhead, and the remaining trajectories were utilized for further data analysis.

To capture the ES behavior, an extensive set of 77 physical descriptors was extracted from the trajectories. These descriptors as follows: (1) ligand conformational and binding, including internal flexibility (torsions, radius of gyration, RMSD) and overall translation relative to the catalytic triad; (2) active site geometry and solvation- local hydrogen-bonding networks, active site water position, oxyanion hole stability etc. MDAAnalysis suite [5] was utilized for feature extraction. Subsequent calculations were carried out using scikit-learn [6] and other packages.

Given that PCA proved inadequate, retaining less than 50% of the explained variance even at 10 dimensions, Uniform Manifold Approximation and Projection (UMAP) was employed. UMAP reduced the 77-dimensional space into 4 prominent dimensions while maintaining high trustworthiness scores (>0.92). Subsequent clustering via Density-Based Spatial Clustering of Applications with Noise (HDBSCAN) identified 4 distinct, populated structural clusters. A comparative analysis of the cluster centroids and feature distributions within each cluster revealed significant structural divergence in the arrangement of the ligand pocket (including oxyanion hole residues) caused by change in positions of nearby protein structural elements (helices and loops)

The proposed pipeline offers a semi-automated, and suitable workflow for processing MD trajectories of serine hydrolase ES complexes. By efficiently filtering through the flexible ES landscape and identifying stable, highly populated conformations, developed tool is useful to select representative starting points for subsequent high-level QM/MM simulations and covalent inhibitor design.

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1. Long, J.Z., Cravatt, B.F. (2011). *Chem. Rev.*, *111*(10), 6022–6063.
2. Macke, A.C., Stump, J.E., Kelly, M.S., et al. (2024) *J. Chem. Inf. Model.* *64*(2), 470–482.
3. Silvestrini, L., Cianci, M. (2020). *Int. J. Biol. Macromol.*, *158*, 358–363.
4. Miao, Y., Feher, V.A., McCammon, J.A. (2015). *J. Chem. Theory Comput.*, *11*(8), 3584–3595.
5. Michaud-Agrawal, N., Denning, E.J., Woolf, T.B., Beckstein, O. (2011). *J. Comput. Chem.*, *32*(10), 2319–2327.
6. Pedregosa, F., Varoquaux, G., Gramfort, A., et al. (2011). *J. Mach. Learn. Res.*, *12*, 2825–2830.

VIRTUAL SCREENING OF THE BrNPDB AGAINST VEGFR2, MMP-9, AND THYMIDYLATE SYNTHASE IDENTIFIES TWO MULTI-TARGET ANTITUMORAL CANDIDATES

R.A. Romagna, D.N. Loose, E.S. Tonini, R.C.R. Gonçalves, R.R. Kitagawa

Graduate Program in Pharmaceutical Sciences, Federal University of Espírito Santo, Vitória, ES, Brazil

Cancer is a major social, economic and public health problem, considered one of the leading causes of premature death worldwide. An estimated 34.4 million new cases are projected for 2050, representing a 66.7% increase compared to 2024. Alongside rising incidence rates, tumor cell resistance to chemotherapy and the lack of specificity in current therapies pose major challenges in oncology. In this context, the search for new therapeutic alternatives is crucial, highlighting medicinal plants as a promising source of bioactive compounds, particularly for multi-target approaches capable of modulating various cellular processes. Specifically, endothelial growth factor receptor 2 (VEGFR-2) drives tumor angiogenesis, matrix metalloproteinase 9 (MMP-9) degrades the extracellular matrix to enable metastasis; and thymidylate synthase (TS) is essential for DNA replication. Therefore, targeting these proteins simultaneously might block blood supply, tissue invasion, and tumor growth. This study aimed to screen Brazilian Natural Products Database (BrNPDB) to identify potential multi-target compounds against VEGFR2, MMP-9, and TS through molecular docking using AutoDock Vina and QuickVina2-GPU 2.1. Protein structures were obtained from Protein Data Bank (PDB IDs: 2XIR, 6ESM and 6QXG) and protonated at physiological pH using AMBER force field on Poisson-Boltzman solver. The Scrubber tool was used to prepare three-dimensional structures for the 16,279 BrNPDB compounds, including protonation at pH 7.4 and energy minimization. For molecular docking calculations, grid boxes were centered at respective active sites covering all important residues, and protocols were validated by redocking co-crystallized ligand (RMSD < 2 Å). Finally, the pharmacokinetic properties of selected compounds were predicted using ADMETlab 3.0 and evaluated according to Lipinski's rule of five. To identify potential multi-target candidates, compounds were ranked according to their predicted binding affinities. The top 50 hits per protein were cross-referenced to isolate consensus ligands common to all three-target top 50 subsets, and two substances were found. The first one, 2-(4-phenylphenyl)-1H-naphtho[3,2-e]benzimidazole-6,11-dione is a semisynthetic derived of *Tabebuia sp.* heartwood compounds that achieved 20th, 1st and 8th best position for VEGFR2 (−12.4 kcal/mol), MMP-9 (−12.6 kcal/mol) and TS (−10.6 kcal/mol), respectively. The second molecule, 3-(1,3-benzodioxol-5-yl)-6-(1H-indol-3-ylmethylideneamino)chromen-2-one, is a coumarin ranked as 1st, 7th and 43th positions for VEGFR2 (−13.4 kcal/mol), MMP-9 (−11.6 kcal/mol) and TS (−10.1 kcal/mol), respectively. In the ADMETlab predictions suggest that both compounds complied with Lipinski's rule of five and showed favorable predicted gastrointestinal absorption, while they were predicted not to cross the blood-brain barrier. Overall, these results suggest favorable drug-likeness and pharmacokinetic profiles for both compounds. These finding require further experimental validation to confirm cytotoxic effect and to evaluate safety profile.

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CHEMOMETRIC MODELLING FOR TOXICITY ASSESSMENT OF DIVERSE CHEMICALS IN STONEFLY: A STEP TOWARDS ENHANCED RISK EVALUATION

D. Mahapatra, P.K. Ojha

*Drug Discovery and Development Laboratory, Department of Pharmaceutical Technology,
Jadavpur University, Kolkata, India*

The widespread use of pesticides in agriculture significantly increases toxicological risks to humans and other aquatic and terrestrial organisms. Traditional experimental methods are rarely used because they are costly, time-consuming, and pose ethical questions concerning animal sacrifice. In this regard, *in silico* predictions for the toxicological evaluation of specific toxicants are increasingly gaining interest. Quantitative Structure-Activity Relationship (QSAR) is such a chemo-metric modelling approach that offers accurate and standardized estimation of toxicity by developing statistical models. Using LC_{50} as a predefined endpoint and adhering to OECD guidelines, the current study develops a multiple linear regression (MLR)-based q-RASAR model for forecasting the acute lethality of diverse organic chemicals against the Stonefly (*Pteronarcys californica*) species. It is considered an important aquatic bio-indicator that plays a vital role in monitoring freshwater ecosystems and indicates water pollution caused by hazardous chemicals, pesticides, microplastics, heavy metals, and industrial waste [1]. They break down organic matter (shredders) and cycle nutrients through the food web, thereby maintaining biodiversity. Their unique size, physiology, short breeding life, and high sensitivity towards pollutants make them ideal species for eco-toxicological risk assessment and management of toxicants. The final q-RASTR model is robust and of good quality, according to the results of the internal ($R^2 = 0.775$, $Q^2_{(LOO)} = 0.705$) and external validation parameters ($Q^2_{F1} = 0.732$, $Q^2_{F2} = 0.731$, $MAE_{test} = 0.465$), producing better predictivity than the corresponding QSTR model ($R^2 = 0.754$, $Q^2_{(LOO)} = 0.684$, $Q^2_{F1} = 0.626$, $Q^2_{F2} = 0.625$, $MAE_{test} = 0.563$). The performed study revealed that the presence of electronegative hetero atoms, a higher number of ether-type oxygen atoms, and vinyl groups are some key fragments responsible for chemical toxicity. To ensure the real-world applicability and feasibility of the developed model, the Pesticides Properties Database (PPDB) was screened as an external dataset, which ranks all the toxicants according to their ecotoxicity and status of AD. Additionally, we have created a Python-based tool, '*StoneTox_Predictor-v1.0*', to predict the lethal concentration of any external set consisting of diverse chemicals in just one click and classify them according to the GHS and EPA toxicity standards. Furthermore, the colored auto-generated bar, pie, and doughnut charts illustrated the degree of toxicity of all compounds easily, thus emphasizing the overall novelty of this study.

In a nutshell, this work facilitates regulatory decision-making, helps design eco-friendly chemicals associated with the aquatic environment, and contributes to building a sustainable Earth.

1. Feeley, H.B., Baars, J.R., Kelly-Quinn, M. and Nelson, B., (2020). Ireland Red List No. 13: Stoneflies (Plecoptera). *National Parks and Wildlife Service, Department of Culture, Heritage and the Gaeltacht, Dublin, Ireland*.

IN SILICO SCREENING OF SILYMARIN COMPOUNDS AS POTENTIAL BACTERIAL HISTIDINE KINASES INHIBITORS

V. Z. Majeski, P.H. Tregnago, L.S. Batista, R.R. Kitagawa, R.C.R. Gonçalves

Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Espírito Santo, Vitória, Brasil

Histidine kinases (HKs) are key components of bacterial two-component systems (TCSs), signal transduction pathways widely distributed among bacteria typically composed of an HK and its cognate response regulator (RR). These systems allow bacteria to sense environmental signals and trigger adaptive physiological responses, including the regulation of virulence factor expression. HKs are known to possess highly conserved active motifs among different strains and has been considered the main target for the inhibitory activity over TCSs, which can lead to the increase of bacterial susceptibility to antibiotics or even compromise its pathogenicity as a whole [1]. Silymarin, a complex mixture of bioactive compounds obtained from *Silybum marianum*, has previously demonstrated antibacterial activity [2]. Moreover, some flavonoids have been reported to inhibit bacterial HKs in vitro, suggesting that silymarin constituents may represent potential candidates for investigation as HK inhibitors.

In order to evaluate the possibility of silymarin flavonolignans inhibiting HK activity, six of its main compounds, silybinin, isosilybinin, silychristin, isosilychristin, silydianin and taxifolin, and five of its minor compounds, silandrin, silyhermin, neosilyhermin, silymonin and silyamandin, were subjected to in silico activity prediction on the *PASS Online (Prediction of Activity Spectra for Substances)*, where the inhibition over the protein was evaluated. It was found that all tested compounds possess Pa values (probability «to be active») between 0.703 (for silyamandin) and 0.895 (for taxifolin), while no compound expressed Pi values (probability «to be inactive») above 0.008 (for silyamandin). These results demonstrate a favorable profile of activity, highlighting innovative potential.

Molecular similarity between the silymarin compounds and thirty-two HK inhibitors selected from literature was then evaluated using Tanimoto coefficients based on Morgan fingerprints (radius 2, 2048 bits length) generated with RDKit. Overall, the compounds demonstrated low similarity towards most of the reference molecules, which can be explained by the absence of nitrogen, sulfur and halogen atoms in their structure while being present in most of the reference molecules. The highest similarity values were obtained towards the reference compound luteolin by taxifolin (0.5652), silandrin (0.5500) and silyhermin (0.5323). Luteolin is a flavonoid reported to inhibit several bacterial HKs, including HK853 from *Thermotoga maritima*, VicK from *S. pneumoniae*, and CheA from *E. coli*, through interactions with the conserved catalytic ATP-binding domain [3]. The structural similarity observed between luteolin and some silymarin constituents, particularly taxifolin, suggests that these compounds may share structural features relevant to HK inhibition, supporting their further investigation as potential HK inhibitors. Notably, taxifolin showed both the highest predicted activity (Pa = 0.895) and the highest structural similarity to luteolin (Tc = 0.5652), supporting its prioritization for further experimental investigation.

Together, these results suggest that silymarin constituents may represent potential candidates for bacterial histidine kinase inhibition, with taxifolin emerging as a particularly promising compound based on both activity prediction and molecular similarity analyses. Further in vitro and in vivo studies are required to experimentally validate these findings.

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1. Ahsan R., et al. (2024). *Antibiotics*, 13.
2. Majeski VZ, et al. (2026). *Frontiers in Natural Products*.
3. Chen H, et al. (2022). *Frontiers in Chemistry*, 10.

THE M66I MUTATION REDUCES THE AFFINITY OF LENACAPAVIR FOR THE HIV-1 CAPSID PROTEIN

A.E. Makhmedova¹, P.V. Kazimirov², N.I. Akberova¹

¹Institute of Fundamental Medicine and Biology, Kazan Federal University, Russia

²State Autonomous Institution of Healthcare “Republican Center for the Prevention and Control of AIDS and Infectious Diseases of the Ministry of Health of the Republic of Tatarstan”, Kazan, Russia

The HIV-1 capsid protein plays a role in numerous processes of the viral life cycle and serves as a promising target for the development of antiretroviral drugs. The conical HIV capsid is composed of protein units formed by five or six monomers, each consisting of 231 amino acid residues. Lenacapavir is the first drug in the capsid inhibitor class used for cases of multidrug resistance. By binding within a hydrophobic pocket formed between two adjacent monomers, it acts by inhibiting immature Gag as well as the stages of maturation and nuclear import. Despite lenacapavir's success in clinical practice, mutations leading to resistance against the drug have been observed. The aim of this study is to evaluate the effect of the K70H, M66I, L56I, and N74D mutations on the interaction of lenacapavir within the hydrophobic pocket of HIV-1 capsid dimers. To obtain HIV-1 capsid protein dimers, the 8G6K structure was used, with missing amino acids added. Optimization of the dimer structure via a 2 ns equilibrium molecular dynamics simulation and a 0.0475 ns steered dynamics simulation were performed using NAMD Multicore CUDA 2.14 and the CHARMM36 force field. Amino acid substitutions were introduced using ChimeraX. Molecular docking was performed using AutoDock Vina 1.2.7. Docking results were visualized in PyMOL 3.1.6.1. Interactions were identified using the PLIP server. A Tanimoto coefficient was calculated for each pose, enabling the clustering of the obtained poses based on their similarity to the first pose in the wild-type. Interactions between the protein and all docking poses were analyzed via the fingerprint method using ProLIF. Analysis of the SMD trajectory revealed that the geometry of the hydrophobic pocket allows lenacapavir to rotate significantly and adopt various positions (RMSD = 5 Å). This mobility enabled the ligand to exit the hydrophobic pocket at 0.03 ns of the SMD simulation under the applied parameters. The primary contribution to the overall ligand fluctuation arises from the vibrations of hydrogen, fluorine, carbon, and oxygen atoms, whereas chlorine, nitrogen, and sulfur atoms exhibit significantly lower mobility. Binding affinities (ΔG) of lenacapavir within the dimer's hydrophobic pocket were determined via molecular docking: the median ΔG was -8.5 kcal/mol for the wild-type structure and the N74D substitution, -8.1 kcal/mol for the K70H mutation, -7.85 kcal/mol for the M66I mutation, and -8.48 kcal/mol for the L56I mutation. Moreover, in both the wild-type dimer and all substitutions, different poses of lenacapavir with high affinity energy in the hydrophobic pocket of the dimer were observed, with RMSD values reaching 5 Å. Hydrophobic and hydrogen-bonding interactions between ligand atoms and capsid protein amino acids were identified in both the wild-type and mutant structures. π -Cation interactions were detected in the wild-type and in structures containing the N74D and L56I mutations. Halogen bonds are formed in all complexes except the one with the N74D mutation. It should be noted that the same types of non-covalent interactions are formed by different ligand atoms in the mutant and non-mutant structures. The substitution of methionine at position 66 with isoleucine leads to the disappearance of hydrophobic interactions with this residue and the emergence of van der Waals interactions for certain poses. Thus, among the four amino acid substitutions associated with lenacapavir resistance examined in this study, only the M66I mutation results in reduced lenacapavir affinity for the capsid protein. Among the 30 poses obtained via molecular docking with the M66I mutant dimer, poses 5 and 9 are structurally closest to the wild-type reference, with RMSD values of 0.553 Å and 0.442 Å, respectively. In contrast, poses 4 and 12 for this mutant variant show less structural similarity to the reference (RMSD values of 4.295 Å and 4.125 Å, respectively). Interaction analysis revealed that poses 4 and 12 do not form π -cation interactions with the protein's amino acids. These structural analysis findings will help accelerate the development of new HIV-1 capsid inhibitors and provide insights into the emergence of mutations.

The data obtained provide some insight into how amino acid substitutions in the capsid protein affect its interaction with the lenacapavir molecule and could serve as a structural basis for accelerating the development of new HIV-1 capsid inhibitors.

STRUCTURE-BASED DESIGN OF NOVEL ISOXAZOLINE INSECTICIDES BASED ON THE ANALYSIS OF MOLECULAR MECHANISMS OF BINDING TO THE INSECT GABA RECEPTOR

N. Malakhovskii^{1,2}, A. Anashkina¹

¹Engelhardt Institute of Molecular Biology, Moscow, Russia

²Sechenov University, Moscow, Russia

The aim of this study was to conduct a structural analysis of the isoxazoline binding site within the insect GABA receptor and to rationally design new potential insecticidal compounds using computational modeling methods. Isoxazoline insecticides act on the NCA-II site and are effective against resistant pest populations; however, until recently, the lack of an experimentally determined insect GABA receptor structure limited the structure-based design of new compounds. The recently published cryo-electron microscopy structure of the honeybee RDL GABA receptor (PDB: 9SHO) provided, for the first time, an experimental structure for docking and pharmacophore analysis, thereby overcoming the limitations associated with homology modeling.

Molecular docking of 11 active isoxazolines (fluralaner, afoxolaner, sarolaner, lotilaner, isocycloseram, fluxametamide, and the research compounds D14, 10o, DP-9, M31, and E3) was performed using the MOE 2024 software and the activated 9SHO structure. Docking was conducted via the Triangle Matcher method, followed by Induced Fit refinement and final GBVI/WSA dG scoring. Binding poses were clustered based on protein-ligand interaction fingerprints (PLIF). Pharmacophore models were generated for each binding hypothesis. A binary QSAR model was trained on a dataset of 217 compounds (89 active, 128 inactive) using 31 molecular descriptors and the principal component method. New structures were generated using the “Add Group to Ligand” protocol and filtered based on the pharmacophore, QSAR (predicted activity > 0.3), binding energy, and synthetic accessibility (rsynth > 0.5).

The isoxazoline binding site is located at the inter-subunit interface of the M1–M3 transmembrane domains. Gln258, Asn303, Phe323, and Met299 were identified as key residues critical for inhibitory activity. Docking analysis revealed five reproducible binding hypotheses for the common isoxazoline scaffold, potentially reflecting the receptor’s conformational flexibility upon activation. Calculated binding energies for fluralaner ranged from –8.64 to –9.98 kcal/mol, depending on the hypothesis. The QSAR model demonstrated an overall accuracy of 85.7% on the training set and 81.1% upon cross-validation. Following the generation and sequential filtering of over 2,000 new structures, 16 candidate compounds were selected that met all criteria: presence in multiple binding hypotheses, GBVI/WSA dG < –9 kcal/mol, and rsynth > 0.5.

This study advances the rational design of isoxazoline insecticides by integrating experimental insect GABA receptor structures with docking, pharmacophore, and QSAR modeling. The identified key residues and binding modes provide a basis for selecting compounds with enhanced insecticidal activity and potentially reduced toxicity to non-target organisms. Sixteen selected candidates are recommended for chemical synthesis and further testing of insecticidal activity.

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NEW HYDRAZIDES OF COUMARIN-3-CARBOXYLIC ACIDS WITH A PYRIMIDINONE FRAGMENT: SYNTHESIS AND COMPUTATIONAL PREDICTION OF ANTIBACTERIAL ACTIVITY

V.A. Matveikov^{1,2}, G.A. Gordunov^{1,2}, I.V. Mineeva^{1,2}

¹*Faculty of Chemistry, Belarusian State University, 14 Leningradskaya str., Minsk, Belarus*

²*Research Institute for Physical and Chemical Problems of the Belarusian State University, Minsk, Belarus*

According to WHO data, the list of life-threatening fungal pathogens is expanding every year, antibiotic resistance in many bacteria is increasing, and the incidence of oncological diseases is growing, which calls for the development of new drugs as well as reagents for biochemical research in this field. Among the latter, particular attention is being given to the design of hybrid molecules that combine several pharmacophoric groups and potentially possess a new, combined mechanism of biological action. In this regard, based on previously obtained 5-cyano-6-oxo-1,6-dihydropyrimidine-2-hydrazides **3a,b**, as well as several representatives of coumarin acid chlorides, an approach was developed for the synthesis of hybrid structures of this type (compounds **4a-d** and **5a-d**) in good to moderate yields (Fig. 1).

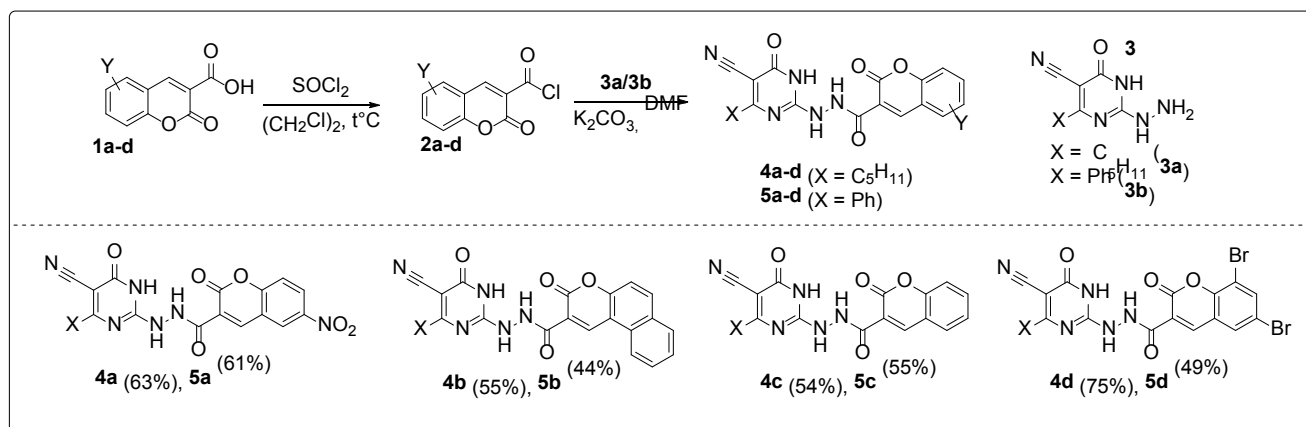


Figure 1. Synthesis scheme of conjugates **4a-d**, **5a-d**

Subsequently, using the PassOnline platform (<http://way2drug.com/passonline/predict.php>) we performed an *in silico* prediction of the antibacterial activity of the final synthesized compounds. The calculation analysis showed that for the majority of derivatives of 2-oxo-*N'*-(6-oxo-1,6-dihydropyrimidin-2-yl)-2*H*-chromene-3-carbohydrazides the most probable bacterial target is a resistant strain of the spiral-shaped Gram-negative bacterium *Helicobacter pylori* ($\text{Pa} = 0.18\text{--}0.34$). The maximum Pa values with respect to *H. pylori* (0.33–0.34) were obtained for compounds **4c** and **5c**, containing an unsubstituted coumarin fragment, whereas the introduction of a benzocoumarin moiety, combined with a phenyl fragment within the pyrimidinone core, expands the predicted spectrum of action to seven bacterial cell targets simultaneously. Such a broad range of effects may indicate less selective binding to the target sites and, accordingly, a different mechanism of biochemical action. The results obtained allow the conjugates **4c**, **5c** and **4d** to be regarded as promising for further evaluation of their activity against resistant *H. pylori* and Gram-positive pathogens *in vitro*.

This work was carried out with the financial support of the State Program of Scientific Research (SPSR) “New polyfunctional 3-hydroxy-2-oxoindoles and heterocyclic compounds based on them with high potential biological activity for the development of drug prototypes” (state registration No. 20261331).

IN SILICO EVALUATION OF MULTITARGET CANDIDATES AS POTENTIAL INHIBITORS OF ESSENTIAL DENGUE VIRUS TYPE 2 (DENV-2) PROTEINS USING MOLECULAR DOCKING, QUANTUM CHEMISTRY AND MOLECULAR DYNAMICS

A. Mayo¹, J. Collachagua^{1,2}, C. Reyes³, P. Ruiz¹

¹Universidad Científica del Sur, Lima, Perú

² Instituto Nacional de Salud, Lima, Perú

³ Universidad Internacional de la Rioja, Logroño, España

Dengue is a viral infection that has become an increasingly important global public health concern. The WHO estimates that 100–400 million infections occur annually, with approximately half of the world's population at risk. Its main vector is the *Aedes aegypti* mosquito, and the disease can be caused by four different serotypes (DENV-1 to DENV-4). Currently, there is no specific antiviral treatment for dengue; therefore, clinical management is primarily symptomatic and supportive, highlighting the need to investigate new therapeutic candidates.¹ Accordingly, this study aimed to design and evaluate potential DENV-2 inhibitors capable of maintaining favorable interactions with three viral targets: NS2B-NS3 protease, NS5 RNA polymerase, and NS5 methyltransferase. Although previous studies have focused on inhibitors of DENV-2 viral proteins, this work integrated an iterative process of structural substitution, large-scale virtual screening, and multitarget selection, followed by quantum chemical characterization, ADME evaluation, and molecular dynamics simulations.

Initially, a group of known drugs was evaluated against the three proteins using molecular docking and electrostatic complementarity (EC) analysis. After applying Lipinski's rule, nitazoxanide and niclosamide were selected according to the established criteria. Using Spark, approximately 7.71 million chemical fragments were explored, yielding 18 structural substitutions. A library of approximately 18,000 molecules was generated, of which 17,869 were evaluated against each target by molecular docking using FRED. The top 50 compounds for each target were reevaluated by docking in Flare and EC analysis. The selected compounds underwent further substitutions, generating 121 combinations that were reevaluated by docking and EC, ultimately yielding the eight molecules with the most favorable simultaneous profiles across the three targets. These candidates underwent geometric optimization using Avogadro and ORCA, followed by evaluation of their ADME properties. The prioritized molecule was subjected to 100 ns molecular dynamics simulations to assess the conformational stability of its protein–ligand complexes across the three viral targets.

Molecular docking results showed that although 3_18_D exhibited greater affinity for the RNA polymerase (5K5M), with an LF dG of -11.560 kcal/mol, its interactions with the methyltransferase (2P3L) and protease (PDB 6MO0) were less favorable, reducing its potential as a multitarget candidate. Among the eight candidates, Molecula_3_30_D displayed the most balanced profile across the three proteins, with LF dG values of -10.246 kcal/mol for methyltransferase, -10.517 kcal/mol for RNA polymerase, and -10.244 kcal/mol for protease. EC analysis supported this profile, showing a more favorable balance between molecular affinity and energetic stability across the three targets. Quantum chemical analyses showed geometrically stable structures, while ADME evaluation indicated that all eight molecules complied with Lipinski's rule and exhibited favorable properties for further evaluation as potential antiviral candidates. Furthermore, during 100 ns of molecular dynamics simulations, 3_30_D maintained internal conformational stability in all three complexes, with target-dependent structural fluctuations. Overall, Molecula_3_30_D was prioritized as a multitarget candidate against DENV-2 based on its balanced molecular affinity, energetic stability, and simulated dynamic behavior.

1. World Health Organization. Dengue and severe dengue. Geneva: World Health Organization (2025). Available from: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue>

ASSAY-AWARE LEARNING-TO-RANK FOR ROBUST MOLECULAR ACTIVITY PREDICTION FROM HETEROGENEOUS BIOACTIVITY DATA

M.T. Scotti, L. Melo

Federal University of Paraiba (UFPB), João Pessoa, Brazil

Pharmacological activity data aggregated from multiple assays often exhibit substantial inter-assay variability, with reported IC₅₀ or K_i values differing due to experimental conditions, protocols, and other sources of assay-specific variation [1]. When such heterogeneous measurements are pooled for conventional QSAR modeling, models may learn assay-specific effects rather than transferable molecular–activity relationships, potentially reducing predictive performance on previously unseen assays. Moreover, standard random cross-validation can provide overly optimistic estimates in this setting because measurements from the same assays may be simultaneously represented in training and test sets. An alternative is to focus on relative molecular activity, as the ranking of compounds within an assay may provide a more stable learning signal than their absolute activity values. We therefore investigated two complementary strategies for addressing assay heterogeneity: leave-one-assay-out (LOAO) validation to directly evaluate generalization to unseen assays, and assay-aware learning-to-rank to learn molecular activity relationships while explicitly preserving assay boundaries.

Data were retrieved from ChEMBL by selecting single-protein targets with IC₅₀ measurements reported in nM and at least three assays containing a minimum of ten molecules each, resulting in 1,097 targets. For each target, inter-assay duplicate molecule pairs were identified to quantify the consistency of relative activity relationships across assays. Three XGBoost-based models were evaluated: an absolute activity regression model (Reg), an assay-aware learning-to-rank model (Hetero), and a homogeneous ranking model that disregarded assay boundaries (Hom). The Hetero model used assay identifiers as ranking groups, allowing relative comparisons to be optimized within individual assays. Models were evaluated using LOAO validation, with pairwise accuracy, Kendall's tau, and Spearman's rho as performance metrics.

Across the filtered dataset, relative activity relationships between duplicated molecules were consistent between assays in approximately 86% of comparisons, indicating that substantial cross-assay ranking information is preserved despite differences in absolute activity measurements. Performance analysis further showed that the advantage of assay-aware ranking increased with the number of assays available for a target. Among targets with at least 100 assays, the Hetero model outperformed the regression model in 100% of cases for both pairwise accuracy and Spearman's rho, and in 96% of cases for Kendall's tau. Mean improvements were 0.08, 0.06, and 0.04 for pairwise accuracy, Spearman's rho, and Kendall's tau, respectively.

These results indicate that relative activity relationships can provide a useful learning signal for heterogeneous pharmacological datasets and that explicitly accounting for assay boundaries can improve generalization to unseen assays, particularly when sufficient assay diversity is available. However, the observed improvements were moderate, suggesting that assay-aware learning-to-rank does not fully resolve inter-assay heterogeneity and that further methodological developments are warranted.

1. Landrum, G. A., Riniker, S. (2024). *Journal of Chemical Information and Modeling*, 64(5),1560.

THE ANALYSIS OF DE NOVO PBOV1 PROTEIN: DETERMINATION OF STRUCTURAL AND FUNCTIONAL QUALITIES

M.I. Meshechko, P.A. Isaeva, R.I. Al-Shekhadat

ITMO University, Faculty of Biotechnology, St. Petersburg, Russia

Objective of the study. The purpose of the present study is a bioinformatic evaluation of the human de novo protein PBOV1 (Prostate and Breast Cancer Overexpressed 1). The study includes prediction of key post-translational modifications, functional and structural motives. The evolutionary origins, structural dynamics, and interaction with biochemical paths were also examined in purpose to determine its clinical significance and potential validity as an oncofetal biomarker.

Advancement over previous studies. Prior publications ewere restricted investigations exclusively to prostate or mammary tumors. This research includes modern, high-precision, gapless assemblies (including T2T-level data) to accurately evaluate synthenic gene clusters across 13 distinct mammalian lines. Furthermore, study involves analysis of PBOV1 transcription by transcriptomic databases (GTEx, GEPIA3, Expression Atlas, cBioPortal) to investigate tissue-dependent regulatory patterns. Tertiary structure was also analyzed by a comprehensive 500-ns all-atom molecular dynamics trajectory.

Results and significance. Evolutionary mapping confirmed that the PBOV1 open reading frame originated de novo in the primate lineage through specific frameshift mutations, developing into a conservative protein strictly bounded to hominids (*Homo sapiens*, *Pan troglodytes*, *Pongo abelii*). Integrative data synthesis revealed a dualistic tissue behavior: PBOV1 experiences extreme transcription upregulation in glandular epithelial malignancies — showing a 32-fold elevation in HER2-positive breast carcinoma and a 181-fold spike under reactive TNF- α stimulation — it aligns with high cellular proliferation and dedifferentiation (G3 WHO grade or pattern 4 Gleason score). In contrast, PBOV1 levels decrease during in skin squamous cell carcinomas and ovarian cancer progression.

Structural multi-consensus topology models (Phobius, Quick2D) identified PBOV1 as a single-pass membrane scaffold with a high-confidence mid-chain transmembrane α -helix separating a short non-cytoplasmic N-terminal sequence from an extended cytoplasmic tail. Local structure alignment using PyMOL revealed solid structural modules composed of four major α -helices with remarkable conformational congruence (local RMSD $\sim 0,81$ Å). The 500-ns molecular dynamics simulation demonstrated that in aqueous microenvironments, the extended regulatory C-terminal region acts as an intrinsically disordered region (IDR) rather than a rigid globule. This extreme dynamic flexibility exposes five highly reliable, solvent-accessible phosphorylation sites (10Y, 62S, 82T, 94S, 127S) predicted by NetPhos tool which are regulated by key oncogenic and mitotic kinases (EGFR, Src, PKC, p38MAPK, CDK1/cdc2. Proactive functional network modeling with STRING-db linked PBOV1 into a highly specialized nuclear-cytoplasmic signaling pathway with key chromatic cohesion acetyltransferases and developmental factors (ESCO1/2, FOXA1, CEBPB, EYA4), highlighting its prospective role as a membrane-anchored adapter integrating cellular microenvironmental stress with internal replication machineries.

NEW 3,5-DIARYL-4-BROMOPYRAZOLES WITH HALOGENCYCLOPROPYL FRAGMENTS

D.I. Frantau^{1,2}, I.V. Mineyeva^{1,2}

¹ Faculty of Chemistry, Belarusian State University, Minsk, Belarus

² Research Institute for Physical Chemical Problems, Belarusian State University, Minsk, Belarus

In this work, we synthesized 3,5-diaryl-4-bromopyrazoles **1a-d**, containing halocyclopropyl fragments in the benzene rings, in satisfactory yields (52-82%) from the corresponding chalcones **2a-d** (Fig. 1). Initially, pyrazolines **3a-d** were obtained from chalcones, which were oxidized with bromine without isolation.

The key step in the synthesis was the conversion of chalcones to pyrazoline via reaction with hydrazine hydrate. The use of chalcones with electron-withdrawing substituents on the benzene ring significantly slowed the rate of formation of cyclic pyrazoline, and in some cases, it did not form at all. Addition of catalytic amounts of acetic acid did not affect the degree of pyrazoline formation, as it was consumed in the formation of N-acetylpyrazolines. The use of solvents other than alcohols also did not result in the formation of the desired pyrazolines. Isolation of pyrazolines from solution was undesirable due to their rapid oxidation by atmospheric oxygen, forming a mixture of products of unknown composition.

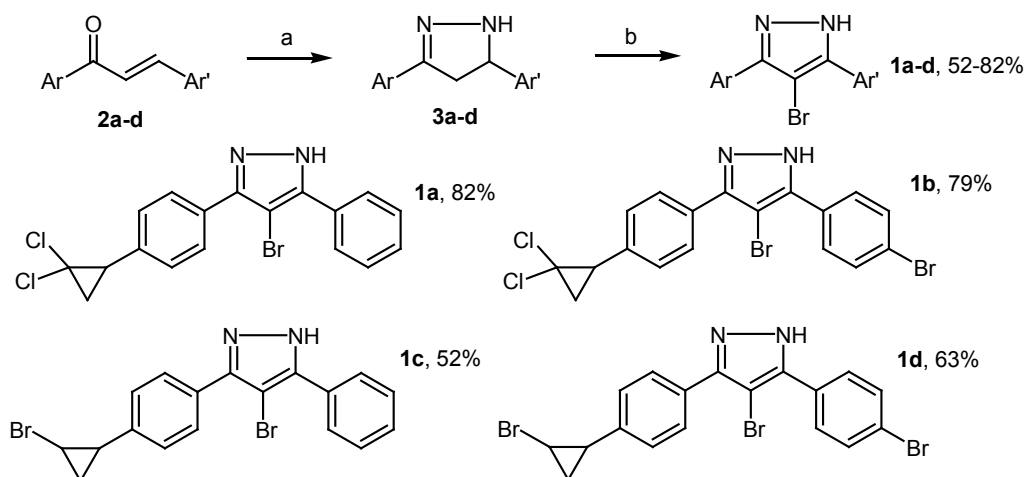


Figure 1. Scheme of synthesis of compounds **1a-d**. Reagents and conditions: (a) $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, MeOH, 65 °C, 8 h.; (b) Br_2 , 0°C, 1 h., after aq. NaOH

The analysis of pharmacological activity of obtained compounds was carried out using computer technologies *in silico* on the PassOnline platform (<http://way2drug.com/passonline/predict.php>). Calculations of acute rat toxicity using GUSAR, rodent organ-specific carcinogenicity using ROSC-Pred, *in silico* prediction of adverse drug effects ADVERPred, prediction of interaction with the undesirable targets (antitarget prediction), prediction of drug-induced changes of gene expression profile using DIGEP-Pred, prediction of interaction of pharmacological substances with human kinome using KinScreen, prediction of toxicity taking into account the metabolism of drug using MetaTox, prediction of interaction with molecular targets, prediction of substrate/metabolite specificity were performed. Possible effective interactions with molecular targets are calculated and predictions of various types of activity are made. A prediction of interaction with tumor and non-tumor cell lines has been made.

The results obtained indicate the possibility of obtaining new heterocyclic for the purpose of obtaining new molecular tools and drug prototypes.

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PREDICTION OF THE LAPLACIAN OF ELECTRON DENSITY AT THE CLEAVING PHOSPHORUS-OXYGEN BOND DURING ENZYMATIC PHOSPHATE HYDROLYSIS USING MACHINE LEARNING METHODS

K.D. Miroshnichenko^{1,2}, M.E. Kuznetsov^{1,2}, A.M. Kulakova¹, I.V. Polyakov^{1,2}, M.G. Khrenova¹

¹Lomonosov Moscow State University, Faculty of Chemistry, Moscow, Russia

²N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia

Adenosine triphosphate (ATP) is an essential component of cellular metabolism and energy storage. In metabolic processes, ATP can be converted to adenosine diphosphate (ADP), a transformation catalyzed by various specific enzymes. Myosin is a motor protein that plays a key role in converting the chemical energy released by ATP hydrolysis into the mechanical energy of muscle contraction. Therefore, ATP hydrolysis is an important reaction occurring in the human body.

The aim of this work is to investigate the relationship between the geometry of the active site of the myosin enzyme-substrate complex and the Laplacian of electron density at the cleaving PG-O3B bond using machine learning methods.

For this purpose, molecular dynamics simulations were performed using combined quantum mechanics/molecular mechanics (QM/MM) potentials, yielding a trajectory dataset of the enzyme-substrate complex. Geometric parameters used as machine-learning features were selected based on the reaction mechanism and the roles of amino acid residues in the active site. These parameters included the distances associated with nucleophilic attack and cleavage of the bond, distances corresponding to proton-transfer pathways, lengths of covalent bonds formed by phosphorus atoms, and intermolecular distances between ATP and neighboring groups. In total, 18 distances and 15 angles in the active site were selected. The dataset therefore contained 33 parameters extracted from each molecular dynamics frame and was intentionally redundant to account, where possible, for parameters that might not be expected to have a direct effect. In addition, the Laplacian of electron density at the PG-O3B bond was calculated for every trajectory frame, resulting in a dataset representing 104 distinct system-behavior patterns. These data were combined into a Pandas DataFrame.

The model describing the behavior of the electron-density Laplacian along the bond line includes a description of a hump-like feature in the Laplacian profile. A dataset was prepared for classification into three categories: “0” for electron-density depletion, “1” for profiles in which the hump crosses zero, and “2” for electron-density concentration. The machine-learning results showed that these three categories can be distinguished fairly well using the selected geometric parameters.

The work was performed using the facilities of the Shared Research Facilities Center for Supercomputing Resources at Lomonosov Moscow State University, including the Istok system (agreement No. 075-15-2025-541).

NETWORK PHARMACOLOGY AND MOLECULAR DOCKING OF FATTY ACIDS FROM NIPA PALM VINEGAR IN TYPE 2 DIABETES MELLITUS

N.A. Mohd Zamri, N. A. Yusoff, N. Yahaya, N. Mohamad Ansor

Pusat Kanser Tun Abdullah Ahmad Badawi, Universiti Sains Malaysia, Penang, Malaysia

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by impaired glucose metabolism, dyslipidemia, and insulin resistance, involving various molecular targets and signalling pathways. Nipa palm vinegar is a traditional fermented product containing diverse bioactive compounds, including fatty acids, which may contribute to its biological activities. However, the potential of fatty acids from nipa palm vinegar in the context of T2DM has not yet been extensively studied. This study aimed to identify potential target genes and molecular pathways influenced by these fatty acids, followed by molecular docking analysis to predict interactions between fatty acids identified from LC-MS/QTOF and target proteins. Five fatty acids, namely palmitic acid, stearic acid, oleic acid, margaric acid, and myristic acid, were identified using LC-MS/QTOF. Potential targets associated with the fatty acids were predicted using SwissTargetPrediction and the Similarity Ensemble Approach, while T2DM-related targets were obtained from DisGeNet and Open Targets database. Target overlap analysis identified 138 targets shared between fatty acid related and T2DM related targets. Protein-protein interaction analysis generated a network comprising 138 nodes and 110 edges, followed by GO and KEGG enrichment analyses. Network analysis identified ten hub genes with PTGS2 showing the highest degree value. The enrichment analysis indicated that the identified targets were primarily involved in lipid metabolic processes, fatty acid metabolism, lipid responses, and metabolic pathways, including the PPAR signaling pathway. Molecular docking of the five fatty acids against the hub genes demonstrated favourable binding affinities, with RXRA and PTGS2 showing more negative affinities, particularly towards oleic acid. Overall, this computational approach provides molecular insights into potential fatty acid-target interactions and the biological pathways associated with T2DM. The identified hub targets and interactions provide a basis for further experimental validation regarding the effects of fatty acids in nipa palm vinegar on T2DM.

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CHEMOINFORMATICS EVALUATION OF NATURAL COMPOUNDS FROM *DYSPHANIA AMBROSIoidES*, *TAGETES LUCIDA*, AND *USTILAGO MAYDIS* AS POTENTIAL INHIBITORS OF THE CTX-M-14 β -LACTAMASE ASSOCIATED WITH ANTIBIOTIC RESISTANCE

N.P. Muñoz-Granados¹, C.A. Lobato-Tapia², J.M. Falcón-González¹

¹*Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato, Instituto Politécnico Nacional, Silao, Guanajuato, México*

²*Universidad Politécnica Metropolitana de Puebla, Puebla, México*

The CTX-M-14 β -lactamase is one of the most prevalent variants of extended-spectrum β -lactamases (ESBLs) in clinical settings and represents a major public health challenge due to its ability to confer resistance to β -lactam antibiotics [1]. Despite the availability of clinically approved β -lactamase inhibitors, the continued emergence and dissemination of CTX-M-producing bacteria highlight the need for the discovery of new inhibitory compounds with improved pharmacological properties. Therefore, the aim of this study was to evaluate natural compounds from *Dysphania ambrosioides*, *Tagetes lucida*, and *Ustilago maydis* as potential inhibitors of the CTX-M-14 β -lactamase using chemoinformatics tools.

A database of natural compounds was compiled from specialized databases and curated. After curation, the compounds were assessed through an integrated chemoinformatics workflow combining ADMET prediction, molecular docking using AutoDock Vina through UCSF Chimera, and docking validation using the crystal structure of CTX-M-14 (PDB ID: 9ORH). The molecular docking protocol was validated by re-docking the co-crystallized ligand, avibactam.

A total of 109 compounds were retained, of which 22 were selected for molecular docking simulations. Among the evaluated species, *Tagetes lucida* exhibited the most favorable pharmacokinetic and toxicological profile. In contrast, *Dysphania ambrosioides* and *Ustilago maydis* showed greater toxicological limitations. Nevertheless, compounds from *Dysphania ambrosioides* displayed the highest binding affinities toward CTX-M-14. Kaempferol-7-O- α -L-rhamnoside displayed the highest binding affinity (-8.133 kcal/mol), followed by compound CID 11968950 (-7.822 kcal/mol) and oroselol (-7.562 kcal/mol), all of which exhibited higher binding affinities than the co-crystallized reference inhibitor avibactam (-6.503 kcal/mol).

These findings suggest that kaempferol-7-O- α -L-rhamnoside, CID 11968950, and oroselol are promising candidates for further development as CTX-M-14 β -lactamase inhibitors, as interaction analysis revealed binding to key active-site residues (Asn104, Tyr105, Ser130, Thr216, and Ser237). In addition, the RMSD value of 0.778 Å obtained from re-docking avibactam confirmed the reliability of the molecular docking protocol [2], supporting future experimental validation of these compounds as potential inhibitors of CTX-M-14-mediated antibiotic resistance.

1. Yoon, E., Choi, Y. J., Kim, D., Won, D., Choi, J. R., Jeong, S. H. (2022). *Microbiol. Spectr.*, 10, e00319-22.
2. Yusuf, D., Davis, A. M., Kleywegt, G. J., Schmitt, S. (2008) *J. Chem. Inf. Model.*, 48, 1411–1422.

EXPLORING THE ALLOSTERIC INHIBITION OF ZIKV NS2B–NS3 PROTEASE BY SELENOFLAVONOIDS: INSIGHTS FROM MOLECULAR DOCKING AND DYNAMICS

T. Nobre¹, T. do Valle Moreira², A. Rustick Athaide, M. Scheide², T. Schirmeister², L. Pergaud Sandjo³, M. Tullius Scotti¹

¹Federal University of Paraiba, João Pessoa, Brazil

²Johannes-Gutenberg University Mainz, Mainz, Germany

³Federal University of Santa Catarina, Florianópolis, Brazil

Zika virus (ZIKV) is a mosquito-borne flavivirus associated with severe neurological complications and remains a relevant public health concern, particularly in tropical regions. In the absence of specific approved antiviral therapies, the viral NS2B–NS3 protease represents an attractive therapeutic target due to its essential role in viral polyprotein processing. In this context, allosteric inhibition may provide an alternative to targeting the highly conserved catalytic site. Selenium incorporation into natural-product scaffolds has emerged as a strategy to modulate their physicochemical and biological properties, and selenium-functionalized flavonoids have previously demonstrated enhanced antiviral activity. However, the potential of selenium substitution across different flavonoid scaffolds remains underexplored.

Here, three novel selenoflavonoids, OMN-47, OMN-54, and OMN-62, inhibited ZIKV NS2B–NS3 protease with IC_{50} values of 1.36 ± 0.20 , 2.53 ± 0.22 , and 8.68 ± 0.88 μ M, respectively. Substrate competition assays indicated a non-competitive inhibition mechanism, prompting investigation of their potential binding to an experimentally characterized allosteric pocket. Consensus molecular docking was performed using Molegro Virtual Docker against experimentally validated orthosteric (PBD: 5LC0) and allosteric (PBD: 9TPG) pockets of ZIKV NS2B–NS3 protease, followed by 100-ns molecular dynamics (MD) simulations using GROMACS 2024.2. Redocking of the crystallographic ligands validated the docking protocols, yielding RMSD values of 0.43 and 0.22 Å for the orthosteric and allosteric structures, respectively. Docking poses were evaluated through an absolute consensus score (ACS) integrating PLANTS, MolDock, and Rerank scores.

At the orthosteric pocket, OMN-47, OMN-54, and OMN-62 yielded ACS values of 0.56, 0.48, and 0.51, respectively, compared with 1.00 for the crystallographic ligand. Despite interactions with functionally relevant residues, several unfavorable contacts were observed, suggesting less favorable accommodation within the orthosteric site. Conversely, docking at the allosteric pocket resulted in ACS values of 0.73, 0.58, and 0.44 for OMN-47, OMN-54, and OMN-62, respectively, compared with 0.93 for the crystallographic ligand. The compounds established hydrophobic and hydrogen-bond interactions with residues involved in recognition of the crystallographic ligand, supporting their accommodation within this pocket. During the 100-ns MD simulations at 9TPG, the protein–ligand systems remained structurally stable, with protein backbone RMSD values predominantly ranging from 0.35 to 0.60 nm, while ligand RMSDs remained below 0.40 nm, indicating limited displacement from their initial docked configurations. RMSF of residues shown no conformational changes for the residues located at the binding site of the allosteric pocket. Overall, the experimental and computational findings support a non-competitive inhibitory mechanism and suggest the experimentally characterized NS2B–NS3 allosteric pocket as a plausible binding site for these selenoflavonoids, providing a structural basis for their further investigation as anti-ZIKV agents.

MOLECULAR DYNAMICS STUDY OF KRAS DIMERS AS POTENTIAL SYSTEMS FOR GAP-FREE GTP HYDROLYSIS

D. Kholmanskikh^{1,2}, A. Obukhova¹, M. Khrenova^{1,3}

¹*Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia*

²*N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia*

³*A. N. Bach Institute of Biochemistry, Federal Research Centre “Fundamentals of Biotechnology” of the Russian Academy of Sciences, Moscow, Russia*

KRAS is a molecular switch that cycles between an active GTP-bound state and an inactive GDP-bound state. GTP hydrolysis is slow in KRAS alone and is strongly accelerated by GTPase-activating proteins (GAPs), which contribute an arginine finger to the active site. The reaction also depends on the organization of the native catalytic environment: Gln61 is associated with the positioning and stabilization of the attacking water, while Tyr32 can interact with the γ -phosphate directly or through an active-site water network [1]. Their orientations are therefore relevant when assessing whether a dimer forms a potentially reactive configuration. Previous molecular-dynamics studies suggested that KRAS dimerization can bring basic residues of one protomer close to the nucleotide bound to the other. Here, we investigated four KRAS dimer models—R68, R149, elevated, and $\alpha 4$ – $\alpha 5$ —obtained from the work of Ngo and Garcia [2].

For each model, we analyzed 10,000 frames from a 1- μ s molecular-dynamics trajectory. Production simulations were performed at 300 K under semi-isotropic NPT conditions using a 2-fs timestep and rigid-bond constraints. Temperature was controlled with a Langevin thermostat, while pressure was maintained at 1 atm with a Langevin piston; periodic boundary conditions and particle-mesh Ewald electrostatics were applied. To distinguish overall dimer motions from local rearrangements of the catalytic pocket, we performed both whole-protein clustering and clustering of the environment within 15, 10, and 5 Å of the GTP γ -phosphate. Side-chain torsion analysis and principal-component-based k-means clustering were used to identify representative conformations. These frames were then evaluated for Mg²⁺ coordination, Gln61 and Tyr32 orientations, and contacts between the GTP phosphates and Arg/Lys residues of the opposite protomer.

A direct interprotomer basic-residue contact was present in three of the four models. Arg68 in R68 and Arg149 in R149 were within 3.5 Å of the γ -phosphate/ β – γ bridge region in every saved frame; contacts with both the terminal and bridging oxygens each occurred in more than 90% of frames. In elevated, Lys176 contacted these oxygens in approximately 70–80% of frames, while Lys175 and Lys178 made less frequent contacts. No direct interprotomer Arg/Lys–phosphate contact was detected in $\alpha 4$ – $\alpha 5$.

The models also differed in their Gln61 and Tyr32 conformations. Gln61 χ_1 favored a rotamer near +60° in R68 (65%) and near –60° in elevated (99%). Tyr32 showed the reverse preference: near –60° in R68 (96%) and near +60° in elevated (87%). The latter state included one uninterrupted interval of approximately 0.72 μ s. Representative structures spanning these contact patterns and rotamers were selected for QM/MM calculations to test their possible roles in GAP-free GTP hydrolysis.

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1. Menyhárd, D. K., Pálffy, G., Orgován, Z., et al. (2020). *Chemical Science*, 11, 9272–9289.
2. Ngo, V. A., Garcia, A. E. (2022). *Biophysical Journal*, 121, 3730–3744.

IN SILICO INVESTIGATION OF THE METABOLIC AND TOXICITY PROFILE OF BUTYLPHENYL METHYLPROPIONAL

F.B. Oguzer^{1,2}, S. Yilmaz Sarialtin³

¹Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Baskent University, Ankara, Turkiye

²Graduate School of Health Sciences, Ankara University, Ankara, Turkiye

³Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ankara University, Ankara, Turkiye

Butylphenyl methylpropional (BMHCA) is a synthetic aromatic aldehyde widely used as a floral fragrance in cosmetics and household products. While banned in cosmetics in the European Union as a Category 1B reproductive toxicant (Repr. 1B, H360Fd), the US Food and Drug Administration lists it merely as a contact allergen. Inconsistent toxicological classifications across regulatory jurisdictions underscore the urgent need for new investigations into BHCA toxicity, especially concerning sensitive subgroups. Therefore, this study aims to evaluate the toxicity potentials of BMHCA and its metabolites regarding mutagenicity, genotoxicity, carcinogenicity, reproductive/developmental, and endocrine system endpoints using *in silico* models. Physicochemical and pharmacokinetic profiles were analyzed via SwissADME and pkCSM tools. All Phase 1 and Phase 2 reactions in MetaTox (v2.0) have been run, and a total of 11 metabolites have been predicted. To focus on plausible structures, a 0.7 probability cut-off was applied, retaining 7 primary metabolites for evaluation. Toxicity potential was evaluated using VEGA HUB (v1.2.4) and US EPA T.E.S.T. (v4.2.1) platforms and Way2Drug tools including Ames Mutagenicity Predictor, ROSC-Pred, hERG-Pred, ADVERPred, PASS Targets, and PASS Online (v.2.0). VEGA and EPA T.E.S.T. consensus models predicted BMHCA as non-mutagenic with a consensus and prediction scores of 0.675 and 0.05, respectively. These predictions were supported by probability to be active (Pa) scores lower than 0.5 across all strains evaluated using the Way2Drug Ames Predictor tool. PASS Online identified high probability for superoxide dismutase inhibition (Pa=0.847) supporting an oxidative stress-mediated toxicity rather than direct mutagenicity. Moreover, the compound was estimated to be respiratory toxic, eye and skin irritant with Pa values of 0.890, 0.727, and 0.693, respectively. ROSC-Pred provided organ-level carcinogenic signals in male subjects (mice stomach Pa=0.557, mice lung Pa=0.545, rat hematopoietic system Pa=0.502). VEGA and EPA T.E.S.T. models predicted the compound as a developmental toxicant; however, it was generally considered inactive in nuclear receptor models like estrogen, androgen, thyroid, and glucocorticoid based on highly reliable predictions and experimental data. PASS Online predicted it to be a testosterone 17-dehydrogenase (NADP⁺) inhibitor and to have reproductive dysfunction potential (Pa=0.892 and 0.692, respectively). Not only the parent compound, but also its predicted metabolites exhibited high electrophilicity capable indicating inhibition of antioxidant mechanisms. Since the mutagenicity predictions indicated no mutagenic potential for BMHCA, any observed carcinogenic risk is more likely driven by oxidative stress and endocrine-mediated pathways. Integrating diverse *in silico* platforms enhances both the reliability and mechanistic depth of safety assessments. While concordance among these model outputs reinforces their use in risk assessment, experimental validation remains essential, particularly for complex endpoints such as carcinogenicity.

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IN SILICO EVALUATION OF NATURALLY OCCURRING MOLECULES AS POTENTIAL INHIBITORS OF ANTIBIOTIC RESISTANCE MEDIATED BY THE MPH(2')-I ENZYME OF *ESCHERICHIA COLI*

L. Pérez-Valdivia¹, C.A. Lobato-Tapia², J.M. Falcón-González¹

¹Instituto Politécnico Nacional, Guanajuato, México

²Universidad Politécnica Metropolitana de Puebla, Puebla, México

Antibiotic resistance constitutes one of the main threats to global public health [1]. Among the priority microorganisms is *Escherichia coli*, whose ability to develop resistance mechanisms has limited the efficacy of various antimicrobial treatments [2]. One such mechanism involves the enzyme MPH(2')-I, which is responsible for inactivating macrolides such as erythromycin [3]. In this context, the objective of the present study was to identify potential inhibitors of the MPH(2')-I enzyme using computational tools.

To achieve this, a database of secondary metabolites reported for *Cineraria spp.*, *Eucalyptus globulus*, and *Hylocereus undatus* was compiled using the KNApSAcK Core System platform. The retrieved compounds underwent a screening process based on pharmacokinetic and toxicological properties using the ADMETlab 3.0 platform. Out of the 79 initial metabolites, 18 met the selection criteria and were evaluated via molecular docking using AutoDock Vina. Additionally, the co-crystallized ligand reported for the enzyme (ZIO) was included as a control. Subsequently, protein-ligand interactions were analyzed by generating interaction maps in BIOVIA Discovery Studio, and the methodology was validated by calculating the RMSD using Open Babel.

The results identified Macrocarpal A, Groenlandicine, and Eucalyptal C as the most promising compounds. These metabolites exhibited favorable binding affinity energies toward MPH(2')-I of -7.861 , -7.398 , and -7.385 kcal/mol, respectively—values close to that obtained for the control (-8.385 kcal/mol). Furthermore, they interacted with 14, 12, and 14 of the 18 residues forming the binding site of the co-crystallized ligand, suggesting high structural complementarity with the enzyme's active cavity.

Overall, the obtained results indicate that Macrocarpal A, Groenlandicine, and Eucalyptal C represent promising candidates for the development of MPH(2')-I inhibitors. Nevertheless, experimental studies are required to validate their inhibitory activity and evaluate their potential as adjuvant compounds in the treatment of infections caused by macrolide-resistant bacteria. Finally, the RMSD value was 0.3404 Å, which supports the reliability and precision of the methodology used.

1. Peña, Y.P., Hernández, M.E., Castillo, V.L. (2011). *Panorama. Cuba y Salud*, 6(1), 30-38.
2. Ayzanoa B, Cuicapuza D, Tsukayama P. (2024). *Investig Innov Clin Quir Pediatr.*, 2(1), 14-9.
3. Noguchi, N., Takada, K., Katayama, J., Emura, A., Sasatsu, M. (2000). *Journal of Bacteriology*, 182(18), 5052–5058.

INSIGHTS INTO HYDROLASE–LIGAND INTERACTIONS: A QM/MM CLUSTERING APPROACH

I.V. Polyakov^{1,2}, M.G. Khrenova¹

¹*Lomonosov Moscow State University, Moscow, Russia*

²*Emanuel Institute of Biochemical Physics RAS, Moscow, Russia*

Evaluating protein-ligand interactions between a target enzyme and potential drug molecules involves various computational frameworks. Among these, quantum mechanics-based approaches are particularly indispensable for investigating the behavior of covalent inhibitors. [1]. Combined quantum and molecular mechanics (QM/MM) methods are routinely used to study mechanisms of chemical reactions in proteins and protein complexes [2]. The SARS-CoV-2 main protease (M^{pro}) interaction with the drug molecules was recently simulated with different QM/MM protocols [3, 4] to uncover the inhibition mechanism by the PF-07321332 drug molecule (nirmatrelvir). The mechanism is as follows: the ion pair intermediate is formed by Cys145 side chain deprotonated by the His41 side chain, next, the covalent C-S bond is formed, followed by a proton transfer. The reported free energy profiles [3, 4] were in a striking disagreement of up to 11 kcal/mol in the reaction barrier height which translates into enormous discrepancies of the catalytic rate constant.

Such a discrepancy can be attributed to ES complex flexibility and conformational diversity, because the QM/MM modelling is usually on the scale of 1-10ps per trajectory, the system stays near the starting conformation of the ES complex which can fluctuate significantly on a larger timescale. On the other hand, the widely applied classic MD approaches (CHARMM, AMBER) fail to capture conformational landscape in the ES structures because of inability to properly describe polarization of the ligand by the protein surrounding and vice versa which are important in the ES complexes of hydrolases such as M^{pro}.

In this work we report the results of 0.8ns QM/MM simulation of the nirmatrelvir-M^{pro} ES-complex. The PBE0-D3/6-31G**//CHARMM36 level of theory was employed, and the quantum part comprised 132 atoms. The relaxed ion-pair structure from the initial QM/MM trajectory was chosen as the starting point for 9 independent QM/MM trajectories to enable better sampling of the conformational space. The resulting ~16000 trajectory frames were analyzed both through extracting a set of important descriptors and QT clustering approach implemented in the VMD software. We were able to identify 4 major clusters that differ significantly in ligand conformation/position in the active site and in the arrangement of the active site itself, involving reactive and non-reactive catalytic dyad (Cys145 and His41) side chain positions. Our results underline that significant caution is required when conducting computational research of covalent inhibitors in the active sites of such hydrolases.

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1. Sadybekov, A.V., Katritch, V. (2023). *Nature*, 616, 673–685.
2. Khrenova, M.G., Mulashkina, T.I., Kulakova, A.M., et al. (2024). *Moscow Univ. Chem. Bull.*, 79, 86–92.
3. Grigorenko, B.L., Polyakov I.V., Khrenova, M.G., et al. (2023). *J. Am. Chem. Soc.*, 145(24), 13204-13214.
4. Ramos-Guzmán, C.A., Ruiz-Pernia, J.J., Tunon, I. (2021). *Chem. Commun.* 57, 9096. 5. Lu, Y., Sen, K., Yong, C., et al. (2023) *Phys. Chem. Chem. Phys.*, 25, 21816-21835.

APPLICATION OF f_1 AND f_2 SIMILARITY FACTORS IN DISSOLUTION PROFILE COMPARISON AND ANALYTICAL METHOD VALIDATION OF ETORICOXIB

B. Radnović^{1,2}, J. Jeremić¹, A. Doban¹, K. Bondokić¹, S.J. Armaković^{2,3}, M.M. Savanović^{2,3}

¹Hemofarm A.D., Vršac, Serbia

*²University of Novi Sad, Faculty of Sciences, Department of Chemistry,
Biochemistry and Environmental Protection, Novi Sad, Serbia*

*³Association for the International Development of Academic and Scientific Collaboration (AIDASCO),
Novi Sad, Serbia*

The f_1 and f_2 similarity factors are widely used statistical tools for comparative evaluation of dissolution profiles, assessment of batch-to-batch consistency, and support of analytical method validation. The study included three dose strengths of etoricoxib (60 mg, 90 mg, and 120 mg), with one reference batch (I) and two validation batches (II and III) analyzed for each strength.

Etoricoxib is a highly selective COX-2 (cyclooxygenase-2) inhibitor commonly prescribed for managing pain and inflammatory disorders [1]. Etoricoxib has a pK_a of 4.6 and exhibits pH-dependent solubility, being highly soluble in acidic gastric media (low pH) and progressively less soluble as the pH increases [2].

The dissolution testing was performed using a paddle apparatus operated at 50 rpm and maintained at 37.0 ± 0.5 °C in 900 cm³ of dissolution medium. Samples were taken at defined time points (5, 10, 15, 30, and 45 min), and dissolution profiles were generated using 12 vessels per medium for each strength of film-coated etoricoxib tablets.

Dissolution media of different physiological relevance were made: 0.1 mol/dm³ HCl (pH 1.2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8), in order to simulate gastrointestinal conditions and evaluate the pH-dependent solubility of the active pharmaceutical compound. An isocratic HPLC method was used for quantitative analysis with a mobile phase consisting of ammonium citrate buffer and acetonitrile (50:50, v/v). Detection was performed at 235 nm using UV detection.

The obtained results confirmed the pronounced pH-dependent solubility profile of etoricoxib, which influences both the kinetics and extent of drug release. In acidic medium (pH 1.2), all tested dose strengths showed rapid and complete dissolution, reaching more than 85% etoricoxib release within 15 min, with high inter-batch similarity confirmed by f_1 and f_2 values within regulatory acceptance criteria. In acetate buffer (pH 4.5) and phosphate buffer (pH 6.8), incomplete dissolution and lower drug release were noticed, indicating formulation- and medium-dependent differences in release kinetics. The study demonstrates that f_1 and f_2 similarity factors provide a reliable statistical framework for dissolution profile comparison and support the validity of the applied analytical approach for assessing the dissolution behavior of etoricoxib under different pH conditions.

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1. Syed, H.M., Muhammad, I.N., Siddiqui, F., Muhammad, T.S., Muhammad, L.R., (2026). . *Pakistan Journal of Pharmaceutical Sciences*, 39(5), 1401.

2. Ashokraj, Y., Daroi, A., Gupta, R., Khanolkar, A., Kulkarni, A., Laud, S., Pokale, M., Shedge, S., Date, P., (2016). *Dissolution Technology*, 23(2), 30-34.

TARGETING *HELICOBACTER PYLORI* UREASE: ENSEMBLE DOCKING-BASED VIRTUAL SCREENING OF THE BRAZILIAN NATURAL PRODUCTS DATABASE

R.A. Romagna, D.N. Loose, E.S. Tonini, R.C.R. Gonçalves, R.R. Kitagawa

Graduate Program in Pharmaceutical Sciences, Federal University of Espírito Santo, Vitória, ES, Brazil

Urease is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea into ammonia and carbamic acid, playing a central role in nitrogen metabolism. It is essential for *Helicobacter pylori*, whose survival in the acidic gastric environment critically depends on this enzyme. Its active site is covered by a mobile flap whose opening and closing gates substrate access and is directly coupled to catalysis. Accordingly, inhibitors act either by coordinating the binuclear nickel center or by targeting the flap residues. Despite continuous efforts, the discovery of new drugs faces significant challenges, including chemical and metabolic instability and toxicity. A critical limitation lies in translating *in vitro* potency into whole cells, where permeation, efflux, and stability are crucial. Moreover, many inhibitors provide only short-term efficacy, allowing microbial adaptation, and the most potent compounds often exhibit mixed or noncompetitive inhibition rather than the competitive mechanism originally expected. Standalone clinical success has not been achieved, and the only approved drug, acetohydroxamic acid, has its use limited by the side effects. In this context, virtual screening of new chemical scaffolds, particularly those derived from natural products, emerges as a promising strategy to expand chemical diversity and identify urease inhibitors with improved potency and stability. Here, we screened the Brazilian Natural Products Database (BrNPDB) for potential *H. pylori* urease (PDB: 1E9Y) inhibitors through ensemble docking using QuickVina2-GPU-2.1. Water molecules and the crystallized ligand were removed, and both chains (A and B) were maintained. Residue pKa values were assigned with PDB2PQR, and the protein was prepared in GROMACS 2025.4 using the CHARMM36 forcefield. A 100 ns MD simulation was run after minimization and equilibration (NVT, NPT). A distance constraint was applied between the nickel atoms, and umbrella restraints ($5000 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{nm}^{-2}$) were applied to their coordinating atoms (OQ1 of Kcx219, Nε2 of His274, Nδ1 of His248, Nε2 of His136, Nε2 of His138, OQ2 of Kcx219, and OD2 of Asp362) throughout all steps. From the trajectory, 11 clusters were obtained and used to screen the BrNPDB. PDB files were converted to PDBQT using Meeko. Molscrub generated three-dimensional structures for the 16,279 BrNPDB compounds, yielding 23,303 ligand structures after tautomer/protonation enumeration at pH 7.4 and energy minimization, subsequently converted to PDBQT using Meeko. Molecular docking was performed with QuickVina2-GPU-2.1, with the grid box set to englobe all protein structures. Docking results were filtered (score $\leq -8.5 \text{ kcal/mol}$; score deviation < 1 ; frequency in best 10% scores $> 80\%$). Of 55 hits that passed the filter, 32 parent ligands were retained after consolidation by physicochemical descriptors (MW, LogP, TPSA, HBD, HBA, QED, Lipinski), conservation of contacts with active-site core residues, and preservation across the 11 clusters. Interaction fingerprint analysis of the top 15 hits revealed conserved contact residues; All 32 parent ligands interacted with Arg338 across the 11 conformations, showing a high frequency of hydrogen bonds and suggesting an anchor role near the active site. Compound **7367** presented the lowest mean docking score across the entire BrNPDB library. The pharmacophore converged on a hydrophobic point oriented toward the flap and a H-bond-acceptor point near the nickel-coordinating residues, matching the two known inhibitor mechanisms: nickel chelation and flap targeting. Compound **7367** shares an anthraquinone-fused benzimidazole scaffold with three other top hits (**6341**, **7722**, **8087**). Across the 11 conformations, this core displayed consistent interaction profiles. Although its molecular weight (411–447 g/mol) and H-bond count exceed the optimal ranges for urease inhibitors (near 350 g/mol; 2–4 H-bonds), the anthraquinone-benzimidazole framework is a promising starting point for fragment-based optimization, aiming to reduce molecular size while preserving interactions with the catalytic nickel-coordinating residues and improving synthetic accessibility.

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COMPUTATIONAL IDENTIFICATION OF STRUCTURAL DETERMINANTS ASSOCIATED WITH TRANSESTERIFICATION ACTIVITY IN MICROBIAL LIPASES

H. Sahana, V.S. Vidya, P. Saravanan

*Department of Biotechnology & Bioinformatics, School of Life Sciences Mysuru,
JSS Academy of Higher Education & Research, Karnataka, India*

Triacylglycerol acylhydrolases (lipases; EC 3.1.1.3) are versatile enzymes capable of catalysing both hydrolysis and synthetic reactions, including transesterification. Their broad catalytic properties make them valuable biocatalysts for various industrial and pharmaceutical applications. However, despite the availability of thousands of annotated lipase sequences in public databases, the functional characteristics and transesterification potential of many lipases remain poorly understood. Identifying the molecular and structural features associated with transesterification activity is therefore important for improving the functional characterization and prioritization of lipases for further investigation.

In the present study, an integrated computational approach was employed to characterize lipases and identify candidates with potential transesterification activity. Curated lipases with available experimental information were collected and analysed to investigate sequence motifs, conserved residues, and structural characteristics. Comparative analysis of representative lipases was performed to identify functionally important residues, substrate-binding features, and structural architectures associated with transesterification activity. These characteristics were subsequently used to investigate and prioritize poorly characterized lipase candidates. Furthermore, a candidate lipase exhibiting favourable sequence and structural characteristics, particularly substrate-binding features, was identified as a promising candidate for further investigation.

Overall, this study demonstrates that the integration of sequence and structural analyses provides an effective computational strategy for characterizing poorly understood lipases and identifying candidates with potential transesterification activity. The findings contribute to a better understanding of sequence–structure–function relationships in lipases and provide a basis for the computational prioritization of promising biocatalysts for subsequent experimental validation and potential industrial applications.

PREDICTING ANTIMICROBIAL PEPTIDE ACTIVITY USING MACHINE LEARNING AND PHYSICOCHEMICAL FEATURE IMPORTANCE

M. Sakho, I. Maattallaoui, N. El Aouad

*Laboratory of Life and Health Sciences, Faculty of Medicine and Pharmacy,
Abdelmalek Essaâdi University, Tangier, Morocco*

Antimicrobial peptides (AMPs) are promising candidates for the development of new therapeutic and food-preservation strategies, but experimental screening remains slow and costly. Many computational predictors are trained with synthetic inactive sequences or datasets in which peptide length differs between classes, creating a risk of biased evaluation. This study aimed to develop an interpretable machine-learning workflow using experimentally supported inactive peptides while controlling sequence length. Active peptides were collected from APD3 and BIOPEP-UWM, whereas inactive peptides were identified from DBAASP using explicit MIC and IC₅₀ criteria. Evidence aggregation, unit conversion and conflict removal produced a curated pool of 3,811 peptides. Exact-length matching yielded 1,278 unique sequences, equally divided between active and inactive classes, with identical class-specific length distributions. XGBoost, Random Forest and Support Vector Machine classifiers were compared using a 1,150-peptide development set and a separate held-out set of 128 peptides. A compact 200-tree XGBoost model was selected from development-set results using a one-standard-error rule and model-complexity criteria before held-out evaluation. It achieved a ROC-AUC of 0.8101 (95% CI 0.7270–0.8783), an F1-score of 0.7154 and an accuracy of 0.7266. Permutation importance identified aromaticity, charge density, lysine proportion and instability index as the leading descriptors, although the corresponding univariate effects were negligible to small and were interpreted as exploratory. The final model was deployed through AMP-Predictor, a public web application that reports activity predictions, sequence-similarity context and peptide-specific SHAP explanations. This workflow provides a transparent screening tool and shows that useful predictive performance can be retained after eliminating length-only discrimination.

SYSTEMS-LEVEL PROFILING OF *EMILIA SONCHIFOLIA* PHYTOCHEMICALS: FROM MOLECULAR TARGETS TO FUNCTIONAL NETWORKS

D. Samad, R. Shakya, Deepali

Department of Botany, Miranda House, University of Delhi, Delhi, India

Emilia sonchifolia (L.) DC. Is a medicinal plant traditionally used for conditions including inflammation, wounds, fever and gastrointestinal disorders. Several pharmacological activities have also been reported for this plant, but the molecular basis of these effects is still not well understood. In the present study, we examined the reported phytochemicals of *E. sonchifolia* and explored their possible molecular targets and associated biological pathways using a combination of computational approaches.

A total of 58 phytochemicals reported from *E. sonchifolia* were collected from the literature and evaluated for drug-likeness and ADMET properties using SwissADME. Based on their physicochemical and pharmacokinetic profiles, 9 compounds were selected for further analysis. Pharmacophore-based target prediction using PharmMapper resulted in 90 predicted target associations, corresponding to 41 unique human protein targets. Gene Ontology and KEGG enrichment analyses indicated that these targets were associated with processes involving apoptosis, inflammation, lipid metabolism, oxidative stress, endocrine regulation and cancer-related signaling. A protein–protein interaction network generated using STRING followed by analysis in Cytoscape identified 10 hub proteins, among which CASP3, PPARG, GSK3B and BRAF were prioritized for further analysis. Molecular docking of the 9 selected phytochemicals against these four proteins resulted in 36 ligand–protein interactions, with Vina scores ranging from -6.2 to -8.8 kcal/mol. Among the compounds examined, α -cadinol showed particularly favorable predicted interactions with PPARG (-8.8 kcal/mol) and BRAF (-8.7 kcal/mol).

The study brings together phytochemical screening, target prediction and network analysis to provide a clearer picture of the molecular interactions that may contribute to the pharmacological activity of *E. sonchifolia*. The identified lead compounds, hub proteins and associated pathways provide useful candidates for understanding the plant's multi-target pharmacological profile.

WHEN MODELING MEETS EXPERIMENTAL QCM: THE CASE OF ANTICANCER COMPOUNDS AND AN ONCOGENIC PROTEIN

A. Serova, O. Volkova, E. Smirnov, E. Skorb

ITMO University, Infochemistry Scientific Center, Saint-Petersburg, Russia

RNA-binding proteins (RBPs), particularly heterogeneous nuclear ribonucleoproteins (hnRNPs), regulate the RNA life cycle, controlling everything from transcription and splicing to transport and degradation. Thus, disruption of their functions leads to serious diseases, including various forms of cancer and neurodegenerative disorders. Among the proteins of this family, hnRNPA2B1 is a potential anticancer target: not only does it ensure RNA stability and transport, but it contributes to maintaining genome integrity and organizing the antiviral immune response.

Molecular modeling has been employed to enable cost-effective assessment of therapeutic strategies and to ease this task by predicting the behavior of a protein and its ligand-bound complex, allowing the best candidate to be selected from thousands of molecules. The ligand set was composed of FDA-approved drugs obtained from the open database ChEMBL. For each compound, the Open Babel package was used to generate tautomeric forms, optimize geometry, and create an ensemble of conformations to account for the conformational mobility of the ligands. Because of the conformational flexibility of the RRM1 domain of hnRNPA2B1, ensemble docking was performed to produce a set of representative target conformations. Molecular dynamics and HDBSCAN algorithm were applied to detect the more stable 4 conformations of RNA-binding motive of hnRNPA1B2 in GROMACS 2025.2 using the CHARMM36 force field. A total of 32,000 protein–ligand complexes were screened with AutoDock Smina. Ligand affinity was estimated from the binding energy parameter (ΔG , kcal/mol). Subsequently, the anticancer drugs with the best binding energies were subjected to molecular dynamics simulations in GROMACS 2025.2 to calculate the binding thermodynamics and assess the time-dependent stability of the complexes. Three molecules, each simulated in three parallel independent runs, formed stable complexes over 125 ns. For these complexes, in contrast to docking, which yields only a single estimated binding energy, RMSD (Å), RMSF (Å), and enthalpy (ΔH , kcal/mol) were calculated as time-dependent quantities across the trajectory, thereby capturing the dynamic behavior of the complexes in solution.

In the course of this work, the binding of more than two thousand FDA-approved drug compounds to the RNA-binding domain of the hnRNPA2B1 protein was evaluated. Molecular docking was used primarily as a screening tool to narrow down the compound set and exclude low-affinity molecules from further consideration. Based on the molecular dynamics results, specifically stable binding, low RMSD values, and favorable binding free energies across the trajectory, three ligands – Topotecan, Alectinib, and Tucatinib – were selected as promising binders. For Topotecan, the binding free energy calculated by the MM/PBSA method yielded an averaged enthalpy ΔH of -22.09 ± 4.23 kcal/mol; for Tucatinib, -20.42 ± 1.76 kcal/mol; and for Alectinib, -27.37 ± 3.74 kcal/mol. The obtained data allows the selected compounds to be considered as candidates for further experimental validation and for the development of new therapeutic strategies targeting hnRNPA2B1 in oncological and neurodegenerative diseases.

1. Gebauer, F., Schwarzl, T., Valcárcel, J., Hentze, M.W. (2021). *Nature Review Genetics*, 22, 185–198
2. Hentze, M. W., Castello, A., Schwarzl, T., Preiss, T. (2018). *Nature Reviews Molecular Cell Biology*, 19, 327–341
3. He, S., Valkov, E., Cheloufi, S., Murn, J. (2023). *Nature Review Genetics*, 24, 276–294.

COMPARATIVE BIOINFORMATIC, STRUCTURAL, AND DYNAMIC ANALYSIS OF RECOMBINANT *SERRATIA MARCESCENS* NUCLEASE WITH ALTERNATIVE SIGNAL PEPTIDES

A. Shevchenko, R. Al-Shekhadat

ITMO University, Saint Petersburg, Russia

Recombinant *Serratia marcescens* nuclease (BNZ) is of interest as an enzyme for DNA and RNA removal in biotechnological processes. Four BNZ constructs expressed in *Escherichia coli* share an identical 248-amino-acid mature nuclease domain but differ in their N-terminal signal peptides (PelB, OmpA, DsbA, or FhuD) and show different cellular and extracellular accumulation patterns. The aim of this study was to identify the molecular features associated with these phenotypes by comparing the properties of signal peptides, as well as the structural and dynamic characteristics of mature BNZ. To relate the observed phenotype to molecular features, signal-peptide properties were analyzed together with structural modeling and redox-state molecular dynamics of mature BNZ.

Densitometric analysis showed extracellular BNZ fractions of 58.8% for PelB-BNZ, 75.2% for OmpA-BNZ, and 67.7% for DsbA-BNZ, whereas extracellular FhuD-BNZ was not detected. SignalP 6.0 classified PelB, OmpA, and DsbA as Sec/SPI signal peptides and FhuD as Tat/SPI [1]. The FhuD signal peptide was additionally distinguished by its RRR motif, a more positive N-region charge (+3), and lower H-region hydrophobicity than the other signal peptides.

AlphaFold2 modeling showed that the mature BNZ domain retained a highly similar predicted geometry in all four precursor constructs [2]. Mean mature-domain pLDDT values ranged from 96.08 to 96.33, and all pairwise C α -RMSD values for the structural core were below 0.5 Å. Thus, the distinct FhuD-BNZ phenotype was not accompanied by a predicted FhuD-specific rearrangement of the mature nuclease domain.

Because the Tat pathway can transport folded substrates and is associated with folding quality control before export, the redox-dependent dynamics of mature BNZ were additionally examined. BNZ contains two disulfide bonds, Cys9–Cys13 and Cys201–Cys243, which have been shown to be important for nuclease activity [3–4]. Three independent 20-ns molecular dynamics trajectories were performed for each redox state. The two states showed similar global structural characteristics, whereas disulfide-bond reduction increased local flexibility. RMSF increased from 0.0490 ± 0.0032 to 0.0663 ± 0.0018 nm for Cys201 and from 0.0583 ± 0.0042 to 0.1041 ± 0.0093 nm for Cys243, with the most pronounced changes occurring in the C-terminal region.

FhuD-BNZ differed from the other constructs primarily in signal-peptide features, whereas no FhuD-specific change in mature-domain geometry was detected. Redox-state molecular dynamics revealed local, rather than global, changes in BNZ flexibility, supporting further investigation of the protein state in the context of Tat-dependent transport.

1. Teufel, F., Almagro Armenteros, J.J., Johansen, A.R., et al. (2022). *Nat. Biotechnol.*, 40(7), 1023–1025.
2. Jumper J., Evans R., Pritzel A., et al. (2021). *Nature*, 596(7873), 583–589.
3. DeLisa M.P., Tullman D., Georgiou G. (2003). *Proc. Natl. Acad. Sci. USA*, 100(10), 6115–6120.
4. Ball T.K., Suh Y., Benedik M.J. (1992). *Nucleic Acids Res.*, 20(19), 4971–4974.

PCA-BASED ASSESSMENT OF CONFORMATIONAL ENSEMBLES OF AChE AND BChE FOR SELECTIVE CHOLINESTERASE INHIBITOR SEARCH

E. Shikina, D. Shulga, V. Palyulin

*Laboratory of Medical Chemistry, Faculty of Chemistry,
Lomonosov Moscow State University, Moscow, Russia*

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder characterized by progressive cognitive decline and loss of functional independence. Acetylcholinesterase (AChE) inhibitors remain a standard pharmacological therapy for AD, while butyrylcholinesterase (BChE) has emerged as a promising target for therapeutic intervention at later disease stages. Selective inhibition of BChE is nevertheless complicated by the high structural similarity between the two cholinesterases. Furthermore, the dynamic nature of their active-site gorges may influence ligand recognition, whereas a single protein structure cannot fully capture the conformational states accessible under physiological conditions [1]. MD-based ensemble approaches offer a means to account for this conformational heterogeneity in molecular docking.

The aim of this study was to characterize the conformational space of human AChE and BChE and to assess the relevance of an ensemble representation for subsequent ensemble docking. Apo-initial and holo-initial structures were used to generate 4 all-atom MD systems: AChE 7XN1 and 6O4W, and BChE 2PM8 and 5K5E. Principal component analysis (PCA) were used to characterize structural stability, flexibility and collective motions.

The analysis revealed pronounced differences in the dynamic behavior of AChE and BChE. Increased mobility was particularly evident in the peripheral anionic site and gorge. Projection of experimentally determined structures onto the PCA spaces showed that available crystal structures covered only a subset of the states sampled by MD. Analysis of distinct PCA regions further revealed a substantial change in the BChE Asp70–Tyr332 distance, from approximately 8.5 to 4.1 Å.

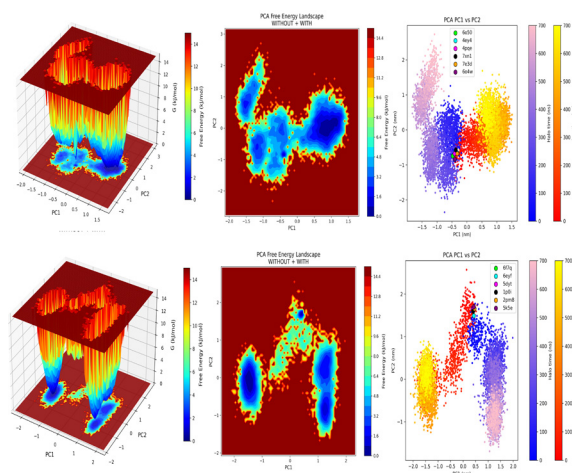


Figure 1. PCA-based free-energy landscape of the combined apo-initial and halo-initial trajectories projected onto PC1 and PC2. PCA space with crystallographic structures coordinates projection.

Taken together, these findings demonstrate marked differences in the conformational landscapes of AChE and BChE. The greater conformational heterogeneity of BChE and the limited coverage of MD-derived states by available crystal structures support the usefulness an ensemble representation for subsequent docking studies for ration inhibitor design.

1. Silva M.A. (2020). *Journal of Chemical Information and Modeling*, 60, 7.

IN SILICO STUDIES TO UNDERSTAND STRUCTURE AND FUNCTIONAL ACTIVITIES OF BEHAVIOURAL PROTEIN HUNTINGTIN (HTT) FROM *APIS MELLIFERA*

A. Singh, K.V. Ramesh

*Department of Biotechnology & Genetics, School of Sciences, JAIN (Deemed-to-be University),
Bengaluru, Karnataka, India*

Social animals such as *Apis mellifera* engage in a range of complex behaviours such as defending their hive against intruders and mite grooming behaviour against pest threats. Several critical genes, such as huntingtin (*htt*), code for essential neurological molecules involved in neuronal and central nervous system (CNS) development of *Apis mellifera*. Under evolutionary constraints, “critical residues” maintain symmetry of behavioural proteins at the structural level across a wide spectrum of social animals. Conversely, loss of certain “critical residues” implies the onset of asymmetry in the protein’s structure. Based on these assumptions, a homology model of HTT from *Apis mellifera* was constructed using the SWISS MODEL server. Analysis of this model revealed critical residues involved in defensive mechanisms, neuronal and CNS development of *Apis mellifera*. The qualitative modelling parameter, quality factor**: 90.5 hints toward functional readability of the constructed model. It is speculated that alpha helical regions of HTT have been influenced by the majority of amino acid residues that could be buried inside the three-dimensional structure of this protein. Moreover, binding site analysis of HTT from *Apis mellifera* revealed volume [$\text{\AA}^3$] 914.18, surface [$\text{\AA}^2$] 1385.61 and Drug Score 0.82. Comparative analysis of the predicted *Apis mellifera* HTT and experimentally resolved *Homo sapiens* HTT (PDB ID: 9PMW) indicates that both proteins have large, accessible ligand-binding cavities. Therefore, *Apis mellifera* HTT protein model demonstrates remarkable computational druggability, revealed through pocket geometry and physicochemical characteristics. Comparative analysis of these two molecules indicates that functionally critical amino acids were consistently conserved throughout evolution. However, docking-based studies and experimental validation will provide ligands / druggable molecules for biological studies of HTT in the defence mechanism of *Apis mellifera*.

CHATGPT-LIKE TOOLS IN BIOINFORMATICS: ARCHITECTURAL APPROACHES AND FUNCTIONALITY OF EXPASYGPT, STRING-CHAT, AND MEGA-GPT

Y. Syrovatskaya¹, Y. Orlov^{1,2}

¹*Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia*

²*Novosibirsk State University, Novosibirsk, Russia*

This work aimed to comparatively analyze the architectural features and functionality of three ChatGPT-like tools — ExpasyGPT, STRING-Chat, and MEGA-GPT — as a new group of large language model-based tools offering novel approaches to biological data, and to assess their prospects in bioinformatics, systems biology, and biomedical research.

The key challenge addressed by modern ChatGPT-like tools in bioinformatics is related to data heterogeneity. Life science data differ in format, storage, and acquisition methods, which makes their integration a complex task [1]. One solution has been the creation of SPARQL endpoints, enabling complex and federated queries across multiple distributed sources simultaneously. However, constructing such queries requires specialized knowledge, creating a significant technical barrier for many users [1]. The tools analyzed in this work employ different architectural approaches to overcome this barrier. Unlike existing reviews that consider LLM tools in bioinformatics in general, this work is the first to systematically compare three specific tools based on an analysis of their documentation and test queries. The analysis shows that the identified problem is solved through fundamentally different architectural approaches: Retrieval-Augmented Generation (RAG) in ExpasyGPT [1], Model Context Protocol (MCP) in STRING-Chat [2], and RAG-augmented ChatGPT in MEGA-GPT [3]. This not only reduces the technical barrier for researchers but also allows the tools to be classified by their functional purpose.

The analysis revealed that ExpasyGPT and STRING-Chat act as “translators”, converting natural language queries into structured formats (SPARQL queries [1] and MCP tool calls [2], respectively), which provides access to the integrated knowledge graphs of the Swiss Institute of Bioinformatics (SIB) (UniProt, OMA, Bgee, etc.) [1] and molecular interaction networks [2] without the need to master specialized syntax. MEGA-GPT performs the function of an “instructor”, providing detailed guidance for phylogenetic analysis in the MEGA software [3]. All three tools use large language models to create intuitive interfaces that make complex bioinformatics resources accessible to a wide range of researchers. A promising direction is the integration of such tools into unified systems and improving the reproducibility of generated results, especially in bioinformatics, systems biology, and biomedical research — from analyzing molecular interactions to identifying drug targets.

1. Bolleman, J., Emonet, V., Altenhoff, A. et al. (2025). *Gigascience*, 14, giaf045.
2. Szklarczyk, D., Kirsch, R., Koutrouli, M. et al. (2023). *Nucleic Acids Res.*, 51(D1), 638–646.
3. Allard, J.B., Kumar, S. (2025). *Mol. Biol. Evol.*, 42(6), msaf101.

RETRIEVAL-AUGMENTED LLM SYSTEMS FOR BIOMEDICAL QUESTION ANSWERING AND PREDICTION INTERPRETATION

R. Taktashov, N. Biziukova, A. Dmitriev, O. Tarasova

Institute of Biomedical Chemistry, Moscow, Russia

Large language models (LLMs) generate fluent text that is often unsupported by any source, which is unacceptable in biomedical research. The aim of this work is to build fully local and reproducible retrieval-augmented generation (RAG) systems in which every statement is conditioned on retrieved evidence, and to extend this architecture from question answering to the interpretation of experimental data. We have developed a multi-hop biomedical question answering system, MedHopper, for the MedHopQA benchmark in the BioCreative IX challenge [1]. Our previous work, DecompRAG, split a question into sub-questions before any retrieval, so an error in the first sub-question propagated into the final answer. MedHopper replaces prompt decomposition with a bounded finite state machine [2]. A question is routed into one of four strategies (direct, definition, intersection, multi-hop) together with a predicted answer type; dense retrieval with cross-encoder reranking supplies at most 20 passages; up to three intermediate entities are extracted, validated and locked into subsequent queries; a validation stage checks answer type, self-reference and grounding in the retrieved text and, upon failure, triggers a bounded query-repair loop.

On the MedHopQA test set (N = 1000) MedHopper achieves 0.55 exact match under the official CodaBench evaluation, against 0.43 for DecompRAG and 0.59 for the zero-shot GPT-4o baseline. The system runs on a single consumer GPU (RTX 5080, LLM Qwen2.5-7B) without cloud API calls, at zero marginal cost per query, and is fully deterministic (five seeds, standard deviation of exact match 0.000). Ablation attributes the largest contribution to capped multi-hop execution (0.05 exact match lost when disabled), followed by reranking and query repair (0.03 each). Orphanet-based query expansion improves accuracy on rare disease questions (55.3% against 42.6%, N = 47). Residual errors are dominated by answer-type and surface-form mismatch rather than by missing knowledge.

For compositional biomedical queries, performance is governed by the orchestration of retrieval and verification rather than by model scale. Systems of this kind run inside a laboratory with full reproducibility, data confidentiality and no recurring cost.

The developed approach can interpret the output of PASS-based predictive models available in the PRIS web application for *in silico* preclinical research (<https://way2drug.com/pris/>). This output currently reaches the user as a dozen independent tables containing terms and values. The LLM-based system aggregates them with explicit attribution of each number to its source model, and provides a dedicated biochemistry-oriented LLM that explains the proposed mechanism and analyses structures that have not yet been synthesized, for which no reference records exist. In this system, abstention is designed together with the answer: contradictory or insufficient evidence yields an explicit refusal rather than a confident statement.

This study was supported by the Russian Science Foundation, project No. 25-15-00300, <https://rscf.ru/project/25-15-00300/> (Preclinical toxicology *in silico*).

1. Islamaj, R., Leaman, R., Chan, J., et al. (2026). *Database*, 2026, baag057.

2. Taktashov, R.R., Biziukova, N.Yu., Dmitriev, A.V., Tarasova, O.A. (2026). *Database*, 2026, baag047.

IN SILICO IDENTIFICATION OF NATURAL ACRB EFFLUX PUMP INHIBITORS TARGETING THE HYDROPHOBIC TRAP: COMPARATIVE DOCKING AND ADMET ANALYSIS

S. Toydemir

Master's Degree Student, Department of Biochemistry, Institute of Health Sciences, Marmara University, Maltepe/Istanbul, Türkiye

The emergence of multidrug-resistant Gram-negative bacteria has posed a significant challenge for the effective treatment of bacterial infections. Acriflavine Resistance Protein B (AcrB), an RND-family transporter, is the main component of the AcrAB-TolC multidrug efflux pump. The AcrAB-TolC efflux system plays a central role in this resistance by actively expelling a wide range of antibiotics and other antimicrobial compounds from bacterial cells. AcrB contains a distal binding pocket (DBP) featuring a phenylalanine-rich hydrophobic trap (HT) that contributes to substrate recognition and binding. Previous studies have demonstrated that the HT is a promising target for efflux pump inhibition, and several synthetic inhibitors, including PA β N, D13-9001, and MBX2319, have been reported to interact with this region. However, the effects of mutations within the HT on ligand binding and interaction patterns remain insufficiently elucidated. This study aimed to identify natural compounds capable of targeting the AcrB hydrophobic trap and to evaluate their binding behaviors in both wild-type and HT-mutant structures.

The *E. coli* AcrB crystal structure (PDB: 5EN5)¹ was analyzed, and alanine mutants—F610A, F615A, and F628A, representing key residues of the hydrophobic trap—were generated. Natural compounds structurally similar to the known AcrB efflux pump inhibitors PA β N, D13-9001, and MBX2319 were selected from the LOTUS natural products database² based on a Tanimoto similarity threshold of ≥ 0.60 (300 ligands). The selected compounds were subjected to molecular docking³ analysis against the distal binding pockets of wild-type and mutant AcrB structures; subsequently, their binding interactions and drug-like properties were evaluated through ADMET⁴ analysis.

This analyses identified several natural compounds exhibiting favorable and mutation-tolerant binding profiles. Among the D13-9001-like compounds, LTS0102454 and LTS0153430 displayed strong and relatively stable binding profiles across the mutant structures. In the MBX2319-like group, LTS0157136 maintained conserved π - π , π -alkyl, and hydrophobic interactions across the analyzed structures, presenting one of the most stable overall interaction profiles. Among the PA β N-like compounds, LTS0012626 showed conserved π - π and hydrophobic interactions across the analyzed structures and retained a strong interaction profile despite HT mutations. Although its interaction pattern was significantly affected by the F615A mutation, LTS0089464 exhibited the strongest docking score (-13.1 kcal/mol) against the F610A mutant. Notably, several compounds were able to compensate for specific mutation-induced losses in interactions by establishing alternative aromatic or hydrophobic contacts within the binding pocket.

Overall, findings indicate that the AcrB hydrophobic trap is a promising target for the development of natural efflux pump inhibitors. The results further suggest that ligand interaction stability and the ability to accommodate mutation-induced structural changes may be more informative for candidate prioritization than docking affinity alone. LTS0012626, LTS0102454, LTS0153430, and LTS0157136 were identified as priority candidates for further experimental validation. This computational approach provides a framework for identifying and prioritizing natural compounds targeting the AcrB hydrophobic trap and may contribute to the development of novel strategies to overcome efflux-mediated antibiotic resistance.

1. Sjuts, H., Vargiu, A.V., Kwasny, S.M., Nguyen, et al. (2016). *Proc. Natl. Acad. Sci. USA*, 113, 3509–3514.
2. Rutz, A., Sorokina, M., Galgonek, J., Mietchen, D., et al. (2022). *eLife*, 11, e70780.
3. Trott, O., Olson, A.J. (2010). *Journal of Computational Chemistry*, 31, 455–461.
4. Daina, A., Michielin, O., Zoete, V. (2017). *Scientific Reports*, 7, 42717.

EVALUATION OF THE ACTIVITY OF CHALCONE-DERIVED PYRAZOLINES ON *HELICOBACTER PYLORI* AND UREASE

P.H. Tregnago¹, A.L.O. Fonseca¹, E.S. Tonini¹, L.A. Marquezini², V.Z. Majeski¹, R.B. dos Santos¹, R.C.R. Gonçalves¹, R.R. Kitagawa¹

¹Federal University of Espírito Santo, Vitória, ES - Brazil

²Universidade Vila Velha, Vila Velha, ES - Brazil

Helicobacter pylori is a bacterium characterized by its ability to colonize the human stomach and its high global prevalence, being associated with diseases such as peptic ulcers, gastritis, and gastric cancer. Currently, the treatment of *H. pylori* infection faces high rates of antimicrobial resistance, requiring the development of new therapeutic alternatives. An important target of interest is the enzyme urease, one of the major virulence factors of *H. pylori*, which catalyzes the hydrolysis of urea into ammonia and carbon dioxide, creating a microenvironment favorable to bacterial colonization. Chalcones are compounds that can be obtained naturally or synthetically, demonstrating several biological properties, including antimicrobial, anti-inflammatory, and antitumor activities. Molecular docking represents a useful strategy for investigating the potential interactions of bioactive compounds with *H. pylori* urease and identifying structural features associated with target binding.

Therefore, aiming to address the demand for new therapeutic alternatives for the eradication of this bacterium, this study evaluated the anti-*H. pylori* activity of 12 chalcone-derived pyrazolines (Figure 1), as well as their inhibitory potential against the urease enzyme. Anti-*H. pylori* activity was determined using the broth microdilution technique with ATCC strains. Urease inhibition was evaluated *in silico* by molecular docking using the GOLD program and the crystal structure of *H. pylori* urease (PDB: 1E9Y), with the ChemPLP scoring function and a 7 Å radius and *in vitro* using Jack bean urease.

Among the 12 pyrazolines evaluated, compounds AL008, AL018, AL029, and particularly AL013 showed the lowest minimum inhibitory concentration (MIC), corresponding to 256, 16, 32, and 8 µg/mL, respectively, with minimum bactericidal concentration (MBC) values of 256, 32, 32, and 8 µg/mL, respectively. The remaining compounds showed MIC and MBC values greater than 256 µg/mL. Following validation of the docking protocol by redocking the cocrystallized ligand with an RMSD of 0.8606 Å, the highest docking scores were obtained for AL029, AL013, and AL021, with scores of 68.83, 68.30, and 66.38, respectively. Analysis of the interactions between AL029 and urease revealed interactions with key residues within the active site, including a hydrogen bond with ASP362 and interactions with the nickel atoms, which were also observed for acetohydroxamic acid, the crystallographic ligand. However, in the *in vitro* assays, none of the pyrazolines evaluated was able to inhibit Jack bean urease activity. Nevertheless, the lack of inhibition of Jack bean urease does not necessarily exclude activity against *H. pylori* urease, considering differences between the enzymes. Although the results demonstrated anti-*H. pylori* activity for some of the pyrazolines and predicted favorable interactions of selected compounds with the *H. pylori* urease active site, the antibacterial activity did not correlate directly with the predicted urease binding profile. In particular, AL013 showed the highest antibacterial activity, whereas AL029 exhibited the highest docking score. Therefore, further studies, particularly *in vitro* assays using *H. pylori* urease, are necessary to determine whether these compounds exhibit inhibitory activity against the target enzyme.

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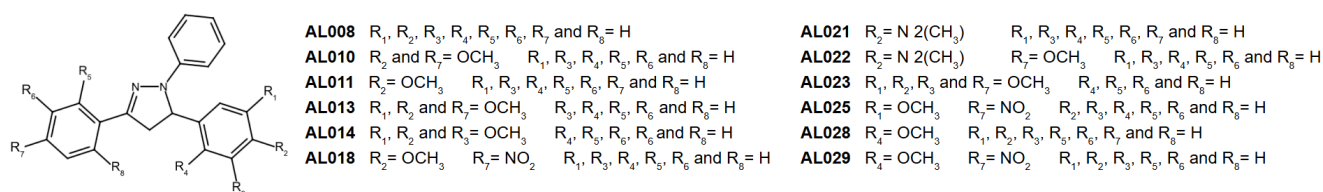


Figure 1. Structure of chalcone-derived pyrazolines

CONFORMATION-DEPENDENT MODELING OF HGF–c-MET INTERACTIONS IN 1:1 AND 2:1 COMPLEXES

V.A. Tsepkova, N.I. Akberova

Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

Hepatocyte growth factor (HGF) and its receptor c-MET form a signaling system involved in the regulation of cell proliferation, migration, morphogenesis, and survival. Under physiological conditions, HGF/c-MET signaling contributes to tissue regeneration and the limitation of fibrosis, whereas its dysregulated activation is associated with tumor progression, invasion, and metastasis, making this signaling axis an important therapeutic target. Structural studies have demonstrated that the active HGF–c-MET complex contains an asymmetric 2:1 core, in which one HGF molecule interacts with two c-MET molecules through a major multidomain interface and an additional K1–SEMA contact [1]. However, experimental structures predominantly represent fixed conformational states, while previous computational studies have mainly focused on individual regions of the HGF–c-MET interface [2]. Therefore, the effect of conformational variability of HGF and the extracellular region of c-MET on interface organization remains insufficiently characterized. The aim of this study was to investigate the conformational dynamics of HGF and c-MET and to characterize their interactions in complexes with 1:1 and 2:1 stoichiometries.

Structural models of HGF and the extracellular region of c-MET were generated using AlphaFold2 and SWISS-MODEL and subjected to structural validation. Selected models were studied by molecular dynamics simulations followed by RMSD, RMSF, radius of gyration, and principal component analyses. Representative conformations were used for information-driven protein–protein docking with HADDOCK 2.4, and the resulting interfaces were additionally analyzed using PISA and PRODIGY.

Molecular dynamics simulations revealed pronounced interdomain mobility of HGF, mainly associated with reorientation of the PAN and K1 domains; the first principal component accounted for 42.81% of the total variance. In c-MET, the dominant collective motion involved the flexible IPT/TIG domains, with PC1 accounting for 32.50% of the variance. Open and closed HGF states and early and late c-MET conformations were selected for docking. In the 1:1 complexes, PRODIGY-predicted binding free energies ranged from -9.5 to -10.1 kcal/mol, while the maximum interface area calculated by PISA reached 1033 Å². Depending on the conformational state, the HGF interface predominantly involved the K2/K4 or K3/SPH regions.

Targeted docking demonstrated the feasibility of an additional K1–SEMA interface. In the 2:1 c-MET–HGF–c-MET complexes, the predicted binding free energy ranged from -8.8 to -10.4 kcal/mol for the primary interface and from -8.1 to -10.2 kcal/mol for the K1–SEMA interface. Transition from 1:1 to 2:1 complexes resulted in a conformation-dependent redistribution of interface contributions: the primary interface was strengthened in models containing the closed HGF state, whereas in one complex containing open HGF the stabilizing contribution shifted toward the K1–SEMA contact with the second c-MET molecule. These results indicate that HGF–c-MET recognition is a conformation-dependent process and that stabilization of the 2:1 complex involves coordinated contributions from multiple interface regions. The obtained models may provide a structural basis for further evaluation of complex stability and for the identification of molecules capable of modulating extracellular HGF–c-MET interactions.

1. Uchikawa, E., Chen, Z., Xiao, G.Y., Zhang, X., Bai, X.-C. (2021). *Nature Communications*, 12, 4074.
2. Winter, A., Sigurdardottir, A.G., DiCara, D., Valenti, G., Blundell, T.L., Gherardi, E. (2016). *Molecular Cancer Therapeutics*, 15, 3–13.

STRUCTURE-BASED DISCOVERY AND PRIORITIZATION OF SELECTIVE TRYPTOPHAN HYDROXYLASE 1 INHIBITORS THROUGH DRUG REPURPOSING FOR HEPATOCELLULAR CARCINOMA

V.S. Vidya, H. Sahana, P. Saravanan

*Department of Biotechnology & Bioinformatics,
School of Life Sciences, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India*

TPH1 is the rate-limiting enzyme responsible for peripheral serotonin biosynthesis. TPH1 is an established therapeutic target for several serotonin-associated disorders and has emerged as a novel drug target to treat hepatocellular carcinoma. However, its high structural similarity to other aromatic amino acid hydroxylases presents a major challenge in the development of TPH1-specific therapeutics. The current study employed an integrative structure-based drug-repurposing strategy to identify selective TPH1 inhibitors through computational selectivity profiling against closely related paralogous enzymes.

Four experimentally determined structures of human TPH1, representing distinct inhibitor-binding pockets, were used to screen potential inhibitors against FDA-approved and investigational drugs from commercial drug competitive intelligence databases. The docking protocol was validated by successfully docking the co-crystallized ligands before conducting high-throughput virtual screening and Glide Standard Precision docking with the Schrodinger suite. Top-ranked compounds were subsequently subjected to selectivity profiling against paralogous enzymes, followed by Prime MM/GBSA binding free-energy calculations to prioritize selective TPH1 binders. Molecular dynamics simulations of the candidate hits revealed variable ligand-binding stability, emphasizing the importance of receptor flexibility in accurate binding-mode prediction. Collectively, the study identified promising candidate compounds with potential TPH1 selectivity, providing a foundation for further structural optimization and experimental validation toward the development of TPH1-targeted therapeutics for HCC.

RESIDUE-CONTACT NETWORK DYNAMICS REVEAL CONFORMATIONAL DIVERGENCE OF NAPI2B UNDER TUMOR-LIKE CONDITIONS

R.A. Vlasenkova, R.G. Kiyamova, N.I. Akberova

Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

Malignant transformation reshapes the tumor microenvironment through acidification and altered membrane lipid composition, both of which may reorganize the residue-contact network of membrane proteins without large-scale backbone rearrangements. For NaPi2b (*SLC34A2*), a sodium-phosphate cotransporter overexpressed in ovarian carcinoma and a target of antibody-based therapy directed against its extracellular epitope, we suppose that such subtle network-level rearrangements may modulate epitope accessibility and, consequently, treatment efficacy. Conventional trajectory-analysis approaches, such as RMSD-based clustering or Cartesian-coordinate PCA, are known to have limited sensitivity to this class of topological change [1,2]. We therefore asked whether a graph neural network (GNN) built on residue-contact graphs can distinguish normal from tumor-associated conformational states of NaPi2b directly from molecular dynamics (MD) trajectories, as a pilot step toward a scalable classification pipeline.

The AlphaFold model of NaPi2b (UniProt O95436) was simulated in GROMACS for 100 ns under two conditions: a normal-membrane system at pH 7.4, and a tumor-like system with altered lipid composition at pH 6.8 with protonation of pH-sensitive residues. Trajectories were partitioned into non-overlapping temporal blocks to reduce frame autocorrelation. For each frame, a heterogeneous residue-contact graph was built from non-covalent interactions (hydrogen bonds, ionic and hydrophobic contacts, aromatic stacking, salt bridges, cation- π and protein-lipid contacts) and encoded by a graph autoencoder with latent-norm control (target 1.0, observed ~ 0.9), showing no evidence of latent-space collapse. A downstream GNN classifier, combining graph structure and temporal block order, was trained across two independent block-based runs of five seeds each, alongside a label-permutation control with identical architecture.

Across ten real-label and ten permutation runs, the true-label models reached a mean MCC of 0.50 (95% CI 0.20–0.80), AUC of 0.665 (0.416–0.896) and accuracy of 0.752 (0.602–0.901), whereas permuted labels gave MCC = 0.00 and near-chance AUC. Notably, outcomes across seeds were strongly bimodal: some initializations achieved near-perfect class separation (MCC ≈ 1.0 , AUC ≈ 1.0), while others performed at chance level, with no intermediate cases. Classical classifiers trained on the same latent features occasionally reached MCC up to 0.98, suggesting a strong linearly separable component and possible leakage of temporal block structure into the latent space.

These pilot results confirm that the graph-based pipeline reliably separates true from permuted labels, and that the observed bimodality likely reflects heterogeneity across trajectory blocks rather than pipeline instability alone. Validation on expanded fold sets and independent MD replicates is required before biological conclusions on NaPi2b conformational divergence between normal and tumor states can be drawn.

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1. Grewal R.K., Roy S. (2015). *Protein Pept. Lett.*, 22(10), 923-933.
2. Di Paola L., Giuliani A. (2015). *Curr. Opin. Struct. Biol.*, 31, 43-48.

DE NOVO DESIGN OF PEPTIDE LIGANDS FOR A TUMOUR-EXPOSED EPITOPE OF HUMAN HSPA1A

N. Vyatkin, Y. Porozov

HSE University, Russia

The stress-inducible chaperone HSPA1A is presented on the plasma membrane of many malignant cells but not on the corresponding normal tissues, making membrane-bound Hsp70 an attractive anchor for tumour targeting. Most peptide work on Hsp70 has addressed the substrate-binding cleft, a groove that is competitively occupied in the cell, changes affinity with the nucleotide state, and is the most conserved surface of the chaperone family. A second site suits a recognition ligand better: the monoclonal antibody cmHsp70.1 maps to NLLGRFEL (aa 454-461) of the substrate-binding domain (SBD), and the Hsp70-derived 14-mer TKDNNLLGRFELSG (aa 450-463) binds membrane-Hsp70-positive tumour cells and is internalised [1, 2]. This patch is also the region of the SBD in which HSPA1A differs from the constitutive paralog HSPA8, at Arg458 and Ser462, making it the rational site for engineering isoform selectivity.

We generated linear peptides of 12-24 canonical residues against aa 450-463 and asked which computational criteria discriminate among them. The target was prepared from the human HSPA1A SBD bound to the substrate heptapeptide NRLLLTG (PDB 4PO2), retaining the substrate so that the conserved channel stayed occluded. Designs were produced with BoltzGen, an all-atom generative model conditioned on a specified binding site, under the compositional and N-terminal constraints imposed by downstream conjugation chemistry. Candidates were ranked by self-consistency refolding in complex with the target and by geometric epitope adherence, then assessed by FoldX interface energies against the wild-type and an HSPA8-mimetic target carrying R458K and S462T, and finally by explicit-solvent molecular dynamics in triplicate.

Of 14 000 designs, 370 passed all filters and a diversity-optimised set of 200 was carried forward; every one contacted Arg458, and ten of the twelve designs taken to simulation shared a single C-terminal motif. The triage criteria differed sharply in usefulness. The predicted HSPA8 penalty did not scale with Arg458 contact, consistent with the charge-conserving nature of the R458K substitution and the limited sensitivity of the empirical energy function to this change. In simulation no design dissociated and Arg458 contact was continuous throughout, so an occupancy therefore provided no discrimination among the simulated designs. We concluded that discrimination came instead from the fraction of interface contacts retained on the epitope (0.56-0.77) and from peptide displacement relative to the target. All designs also engaged residues outside the epitope, and the two most epitope-specific were the shortest, INDICATING that a 20-24-mer over-extends a 14-residue patch. Static confidence and energy scores correlated only weakly with these dynamic measures ($|r| < 0.4$).

Five peptides of 17-23 residues were selected for synthesis and N-terminal fluorophore conjugation; measurement of affinity and HSPA1A/HSPA8 selectivity is in progress. Beyond the designs, generative confidence metrics and empirical interface energies proved weak predictors of simulated interface behaviour for short linear peptides, stability criteria require grading to be informative, and binder length should be matched to the epitope footprint. Alchemical free-energy calculation of the R458K/S462T conversion is planned as the appropriate treatment of selectivity.

1. Gehrman, M., et al. (2014). *PLoS One*, 9(8), e105344.
2. Shevtsov, M., et al. (2024). *Cancer Research Communications* 4(8), 2025-2044.

THE COMPARATIVE ANALYSIS OF 2D AND 3D STRUCTURAL REPRESENTATIONS FOR AI-QSAR MODELING OF HUMAN CYP24A1 INHIBITORS

A. Yakusheva¹, M. Shaladonova²

¹*Belarusian State University, Minsk, Belarus*

²*Institute of Bioorganic Chemistry of National Academy of Sciences of Belarus, Minsk, Belarus*

Human cytochrome P450 24A1 (CYP24A1, 25-hydroxyvitamin D3–24-hydroxylase) is a mitochondrial enzyme responsible for the catabolism of active vitamin D3 (1,25-dihydroxyvitamin D3). Overexpression of CYP24A1 can lead to altered vitamin D levels, calcium metabolism disorders and tumor progression, which highlights CYP24A1 as an important drug target in both oncology and metabolic diseases. Therefore, CYP24A1 inhibitors represent prospective frontier in the development of targeted therapies for various health conditions, particularly for cancer treatment [1]. Artificial intelligence (AI) has recently been increasing in the drug development, utilizing the algorithms of machine learning (ML), deep learning (DL) and combined approaches. Traditional quantitative structure activity relationship (QSAR) AI-models rely on 2D structural fingerprints and descriptors, which are computationally efficient and perform well on congeneric series. However, these features lack explicit information about 3D shape and conformational flexibility [2]. DL models, such as the Uni-Mol 3D transformer, may overcome this limitation by learning spatial features directly from 3D conformations without 2D features calculation [3]. Thus, whether 3D deep learning models actually outperform 2D descriptors for human CYP24A1 ligands is still an open question.

The objective of our study was to conduct a comparative analysis between 2D molecular features (MACCS keys, Morgan fingerprints, and RDKit physicochemical descriptors) and 3D deep learning embeddings for CYP24A1 ligands QSAR classification. Bioactivity data were retrieved and curated from the ChEMBL database; compounds with $IC_{50} \leq 1000$ nM or $K_d \leq 1000$ nM were labeled as inhibitors, while those with higher values served as non-inhibitors in a binary way. Three modeling strategies were benchmarked: (1) Random Forest on 2D features; (2) transfer learning with 512-dimensional Uni-Mol 3D embeddings followed by Random Forest; and (3) fine-tuning of the Uni-Mol classificational head [2, 4]. Hyperparameters were optimized *via* Optuna within a stratified 5-fold cross-validation scheme and evaluated across ROC-AUC, PR-AUC, MCC, F1-score, recall, and precision metrics.

The obtained results demonstrated the performance differences between the approaches. On the balanced CYP24A1 dataset (N = 88, 1:1), the Random Forest model, trained on 2D features achieved the highest performance (ROC-AUC = 0.932, MCC = 0.76), closely followed by the fine-tuned Uni-Mol model (ROC-AUC = 0.919, MCC = 0.69). Transfer learning was notably less effective (ROC-AUC = 0.885, MCC = 0.64). Despite this, all models maintained high PR-AUC (> 0.90), confirming their ability to rank active and inactive molecules. The higher MCC achieved by 2D+RF points to a more balanced class separation with fewer false-positive and false-negative predictions. This difference likely results from conformational noise present in 3D embeddings. Meanwhile, 2D fingerprints capture core substructural motifs that determine ligand activity regardless of spatial orientation. The overall evaluation reveals that 2D molecular representations remain superior in efficiency and accuracy for CYP24A1 ligands dataset, suggesting that 3D deep learning may require larger datasets and refined conformer generation protocols to realize its potential in stereochemistry-driven tasks.

1. Milan, K.L., Ramkumar, K.M. (2024). *Pathology - Research and Practice*, 264, 155684.
2. Cherkasov, A., Muratov, E.N., Fourches, D. et al. (2014). *J. Med. Chem.*, 57, 4977–5010.
3. Zhou, G., Gao, Z., Ding, Q. et al. (2022). *ChemRxiv*.
4. Lu, S., Gao, Z., He, D. et al. (2024). *Nature Communications*, 15, 7104.

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