

IBCH RAS RU

Way2Drug
Understanding Chemical-Biological Interactions
PREDICTIVE SERVICES

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



MSU

MACHINE LEARNING AND AI APPROACHES IN BIOCATALYSIS AND IMMUNOCHEMISTRY

Director of IBCH RAS RU

Full member of Russian Academy of Sciences of Russia

GABIBOV ALEXANDRE

October 19 2026,

Big Data

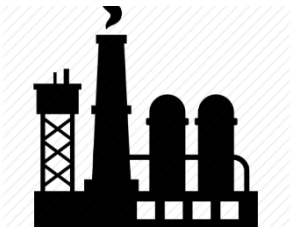
Finance



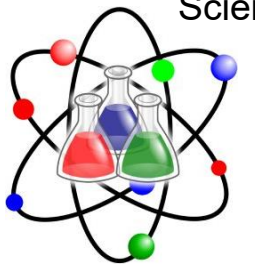
IT-Technologies

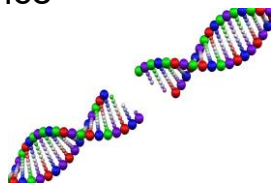


Industry



Science



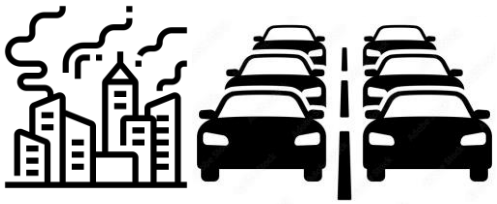


Life Science

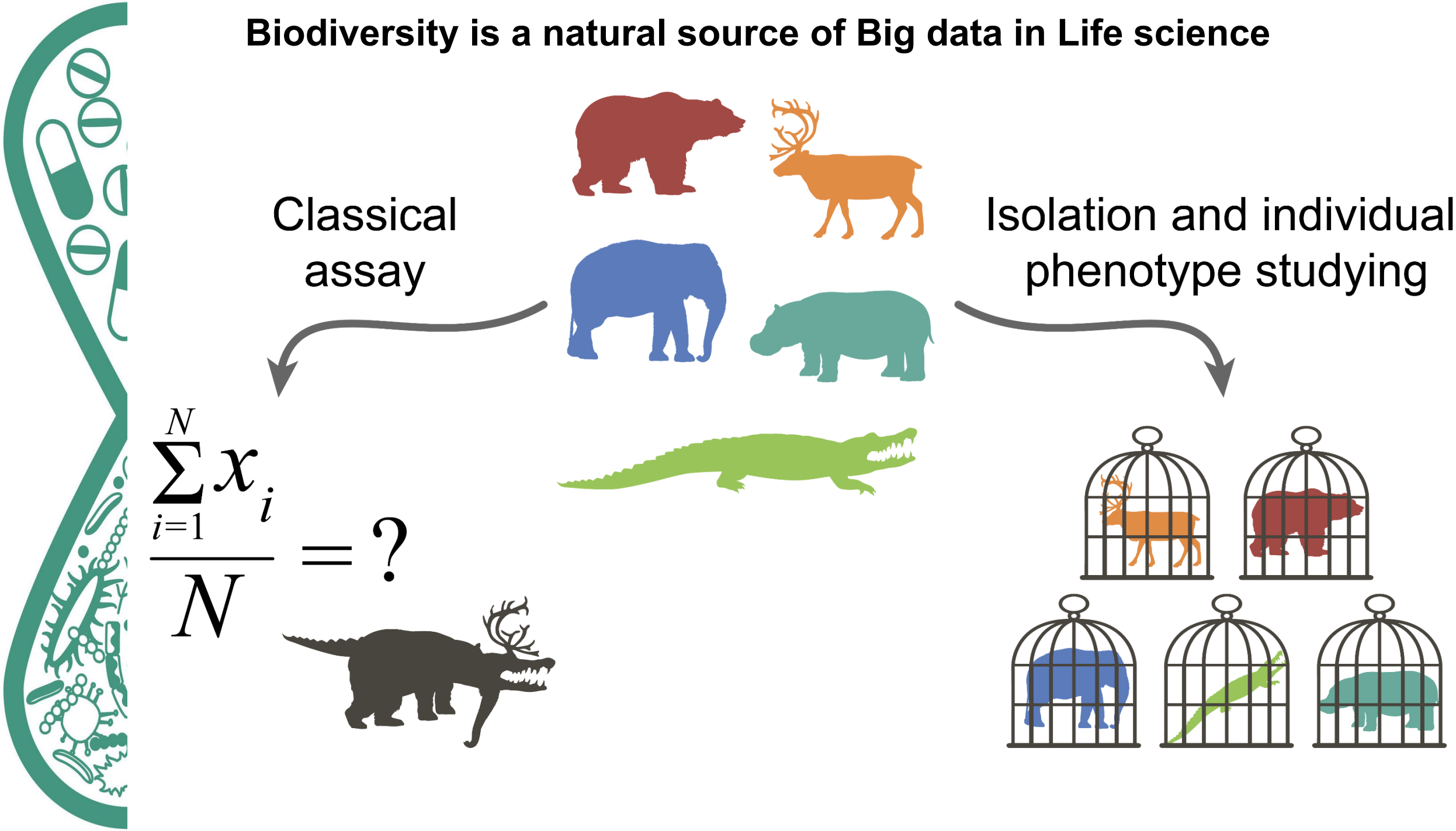
Medicine



City Environment



Biodiversity is a natural source of Big data in Life science



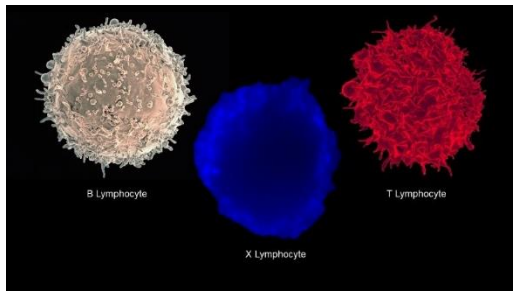
Big data in Life Science

Natural cell communities

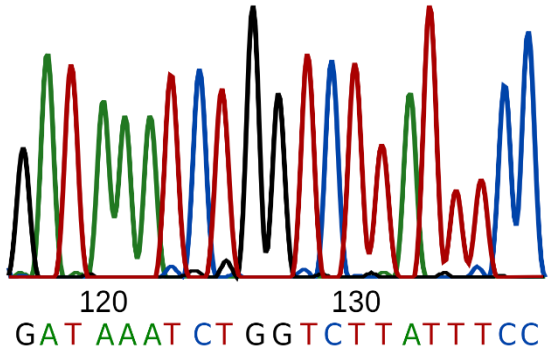
- Microbiome



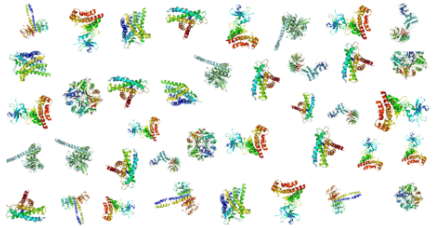
- Immune cell

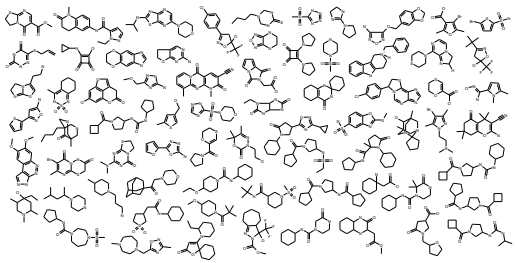


Genome databases (Genbank, NCBI, etc.)



Metabolome and proteome databases





Chemical and genome libraries

The era of big data in life sciences

Genomics

Proteomics

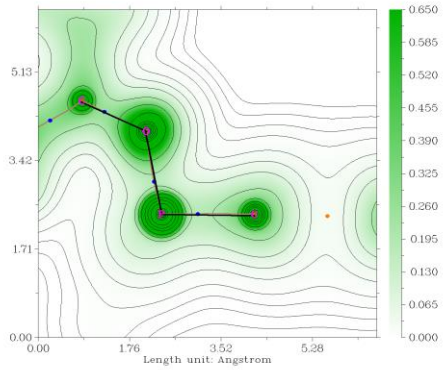
Structural biology

RCSB PDB PROTEIN DATA BANK

CoV-AbDab The Coronavirus Antibody Database

Metabolomics

Quantum chemical calculations

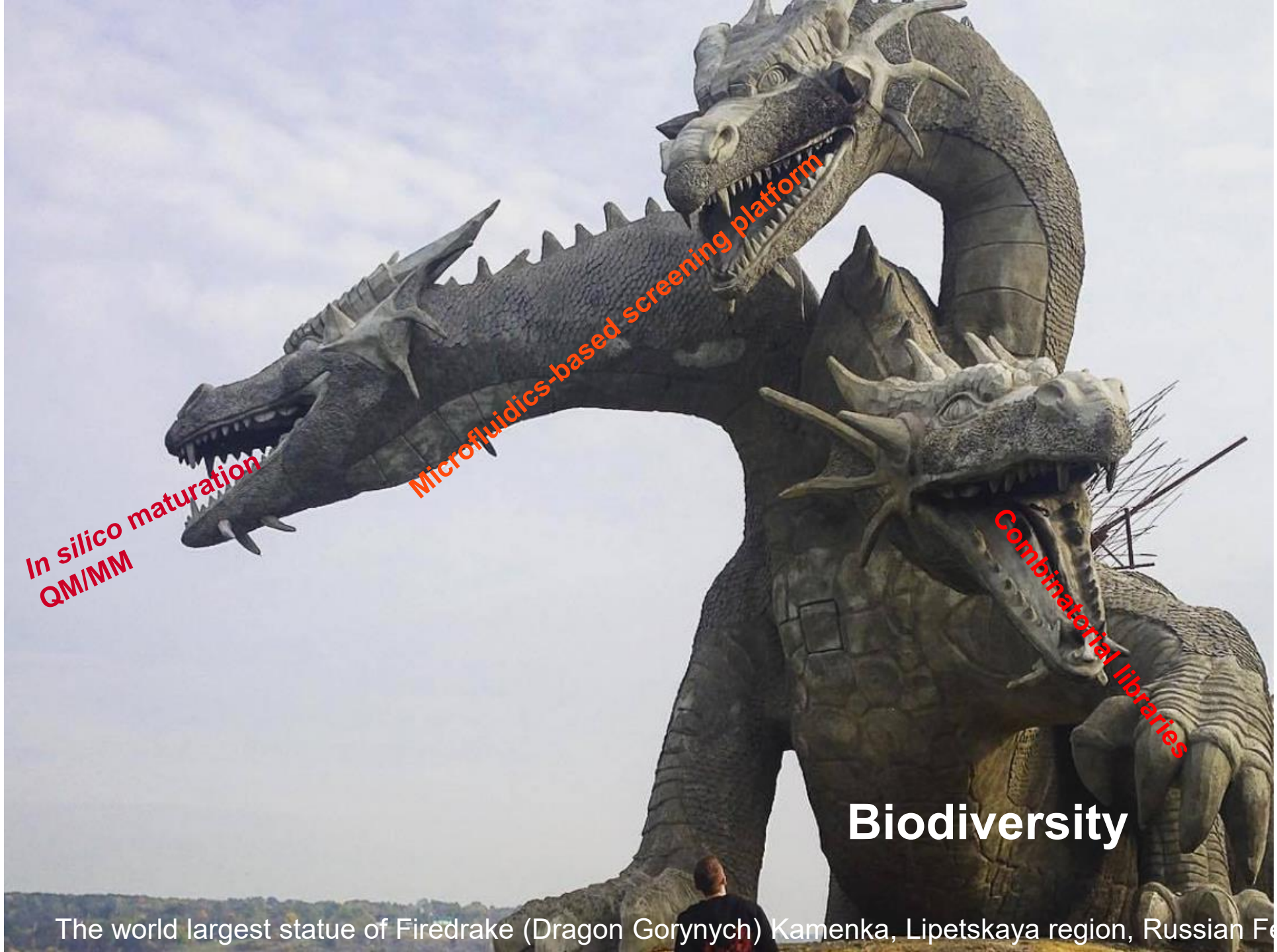


Electronic medical records (EMR)

Imaging: medical imaging data (MRI, CT, X-ray)

MESSAGE

- **THE ERA OF BIG DATA**
- **Why we need more species than exist in nature (the reason to develop synthetic biology)?**
- **How to produce expanded repertoires with pre-designed novel functionalities?**
- **How proceed screening of existing huge functional repertoires with demanded properties?**
- **Answer: Library screening & Compartmentalization & ML, AI**



The world largest statue of Firedrake (Dragon Gorynych) Kamenka, Lipetskaya region, Russian Fe

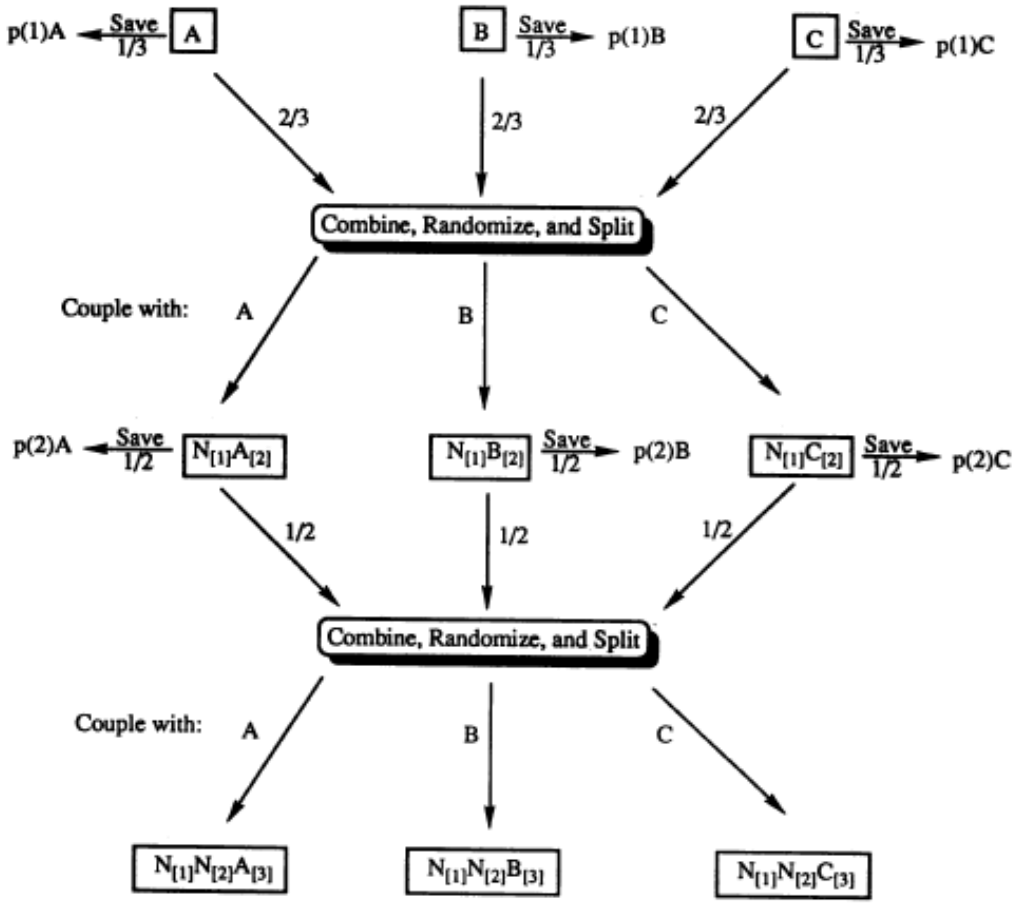
BioCat Lab

MESSAGE

Combinatorial Chemistry and Biology a hallmark of XXI century

Chemical Libraries

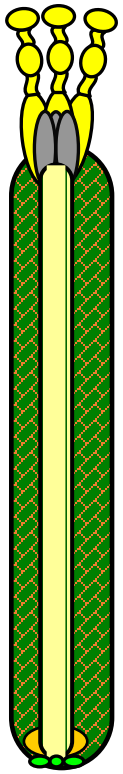
- ✓Mimotope Strategy
- ✓Parallel Synthesis of Combinatorial libraries
- ✓One-Bead, One-Peptide Solid-Support Technology
- ✓Positional Scanning, and Robotics Library Technology



"Biological libraries"

Tagged Methodologies:

- ✓Phage Technology
- ✓Peptides on Plasmids
- ✓Peptide coded Libraries
- ✓Electrophoric Polyhalobenzene Coded Libraries
- ✓Encoded Combinatorial Libraries



Filamentous phage

Based on a physical link between a protein and its encoded gene – the best known system is phage display.

The product of gene 8 (g8p) – is a small protein that forms the cylinder of the capsid; its number of copies (2700 for the wild-type phage). The other coat proteins (g3p, g6p, g7p and g9p) close the extremities of the cylinder. The product of gene 3 – present in three to five copies – is responsible for phage infectivity.
Proteins of interest are usually fused by their C-terminal to g3p or to g8p.

DNA-encoded chemical libraries,DECL

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5381–5383, June 1992
Chemistry

Encoded combinatorial chemistry

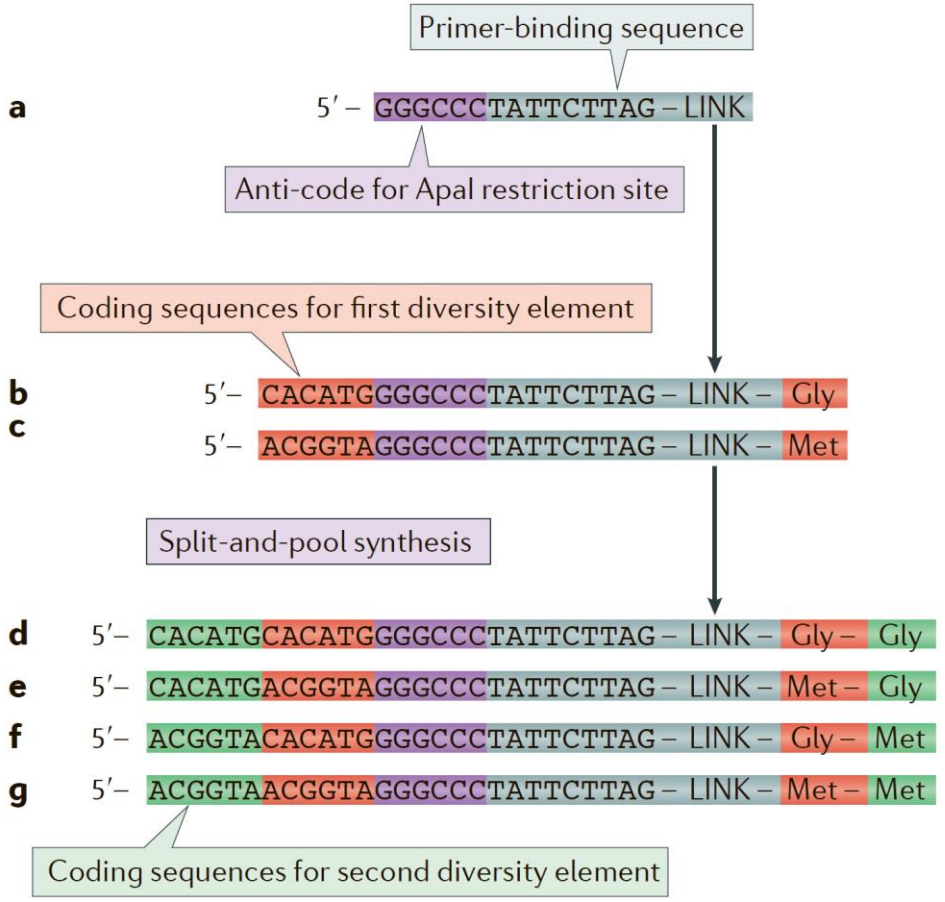
(chemical repertoire/encoded libraries/commaless code)

SYDNEY BRENNER AND RICHARD A. LERNER

Departments of Chemistry and Molecular Biology, The Scripps Research Institute

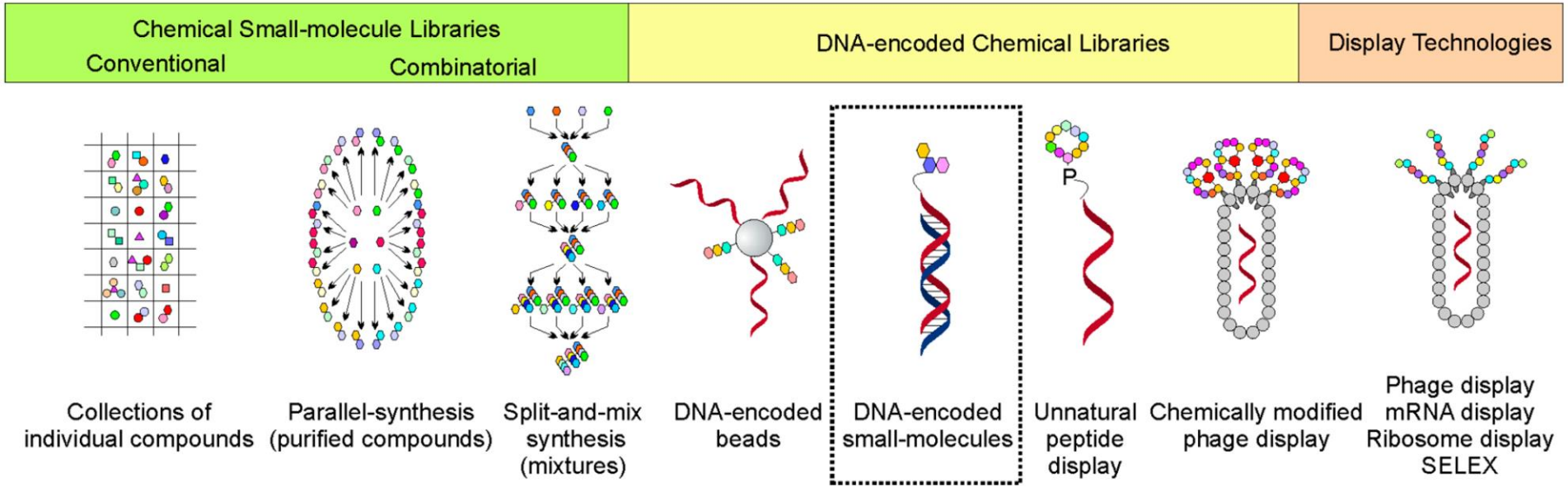
Contributed by Sydney Brenner, March 3, 1992

ABSTRACT **The diversity of chemical synthesis and the power of genetics are linked to provide a powerful, versatile method for drug screening. A process of alternating parallel combinatorial synthesis is used to encode individual members of a large library of chemicals with unique nucleotide sequences. After the chemical entity is bound to a target, the genetic tag can be amplified by replication and utilized for enrichment of the bound molecules by serial hybridization to a subset of the library. The nature of the chemical structure bound to the receptor is decoded by sequencing the nucleotide tag.**



DECL:
Between Chemistry and Biology

Chemistry ⟷ **Biology**



Franzini et al., Acc Chem Res., 2014

Chemical space

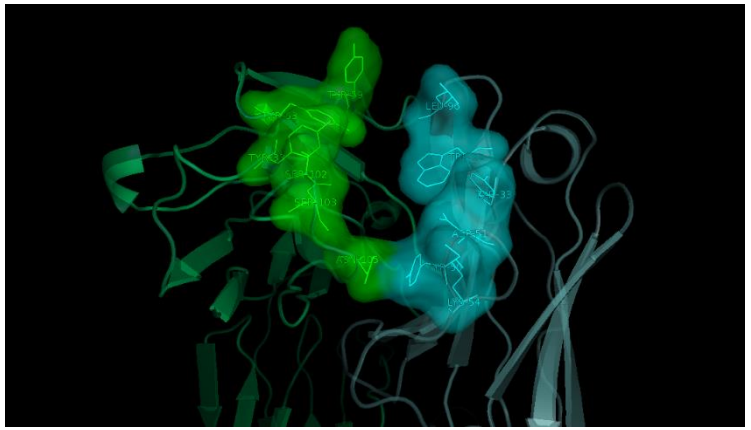
DECL:
Between Chemistry and Biology

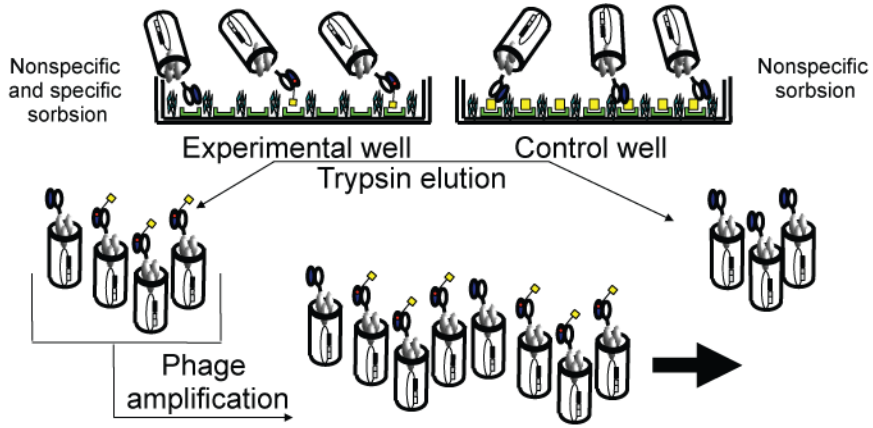


Directed evolution

Rational design

Combinatorial approach





High resolution 3D

Effective screening

MESSAGE

- **New approaches to solve old problems:**
- **-To create optimal binding/biocatalytic protein platform using different protein folds (enzymes, antibodies)**
- **To speed up chemical evolution of low molecular weight compounds (substrates, inhibitors, effectors)**



MESSAGE

Immunology

gives us a huge number of structural combinations

MESSAGE

BioCat Lab

Immune system is the main defender of organism.

How may we use principles of immune system machinery to improve the defense using combinatorial chemistry & biology?

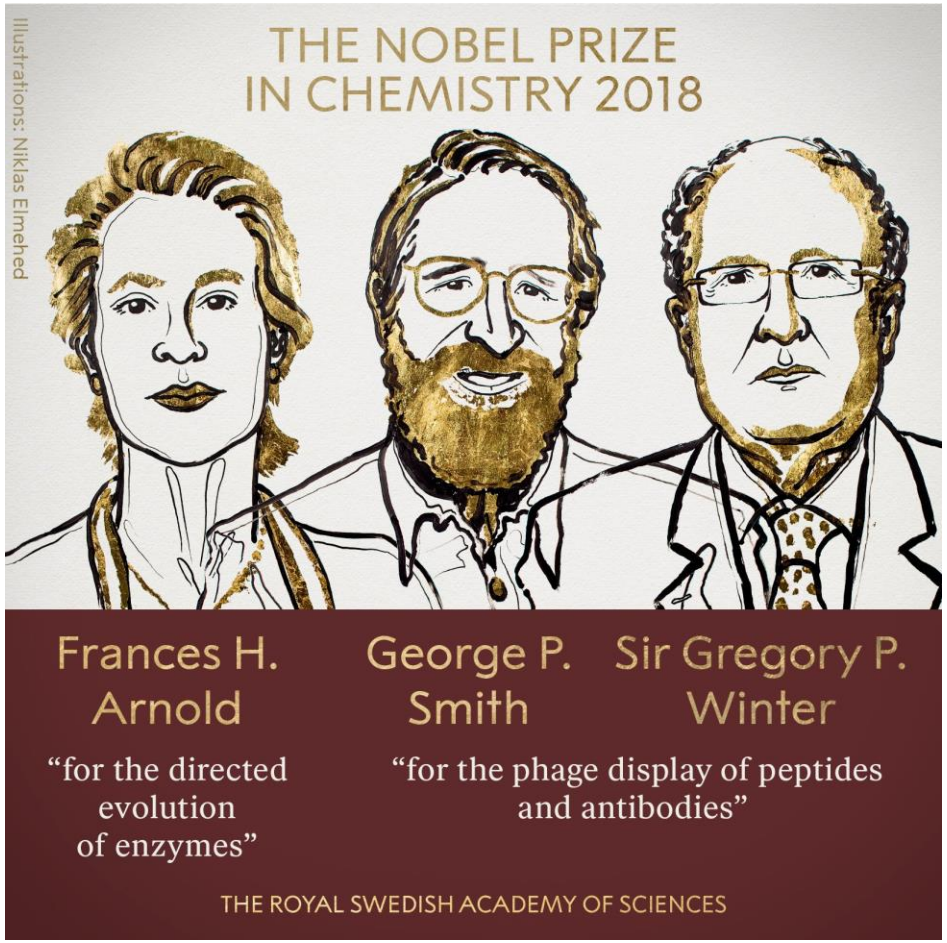
MESSAGE

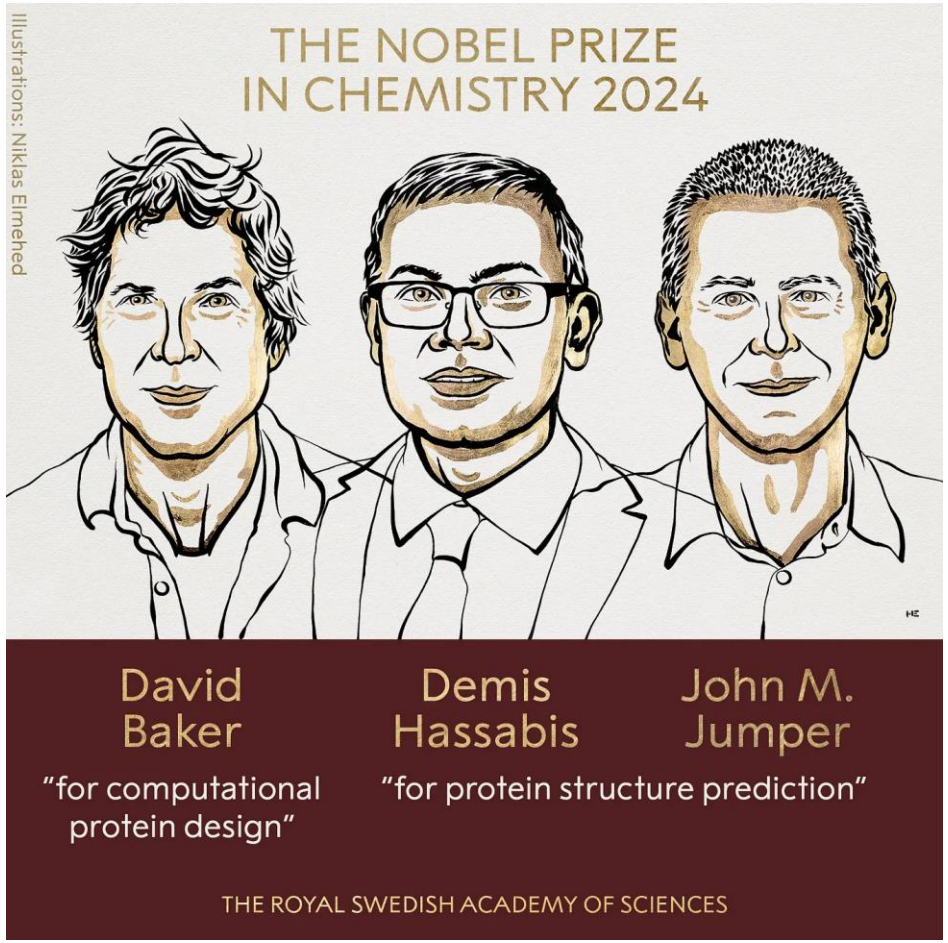
BioCat Lab

B cell diversity allows to design proteins with novel functionality:

Evolution in test –tube

To analyze Phenotype - Genotype

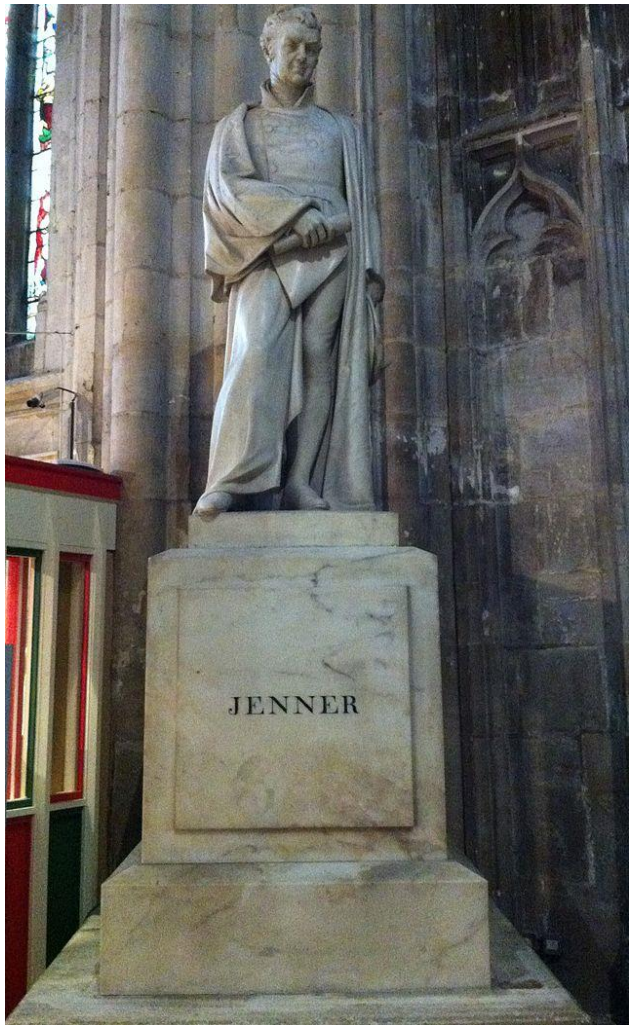




From human-guided *in vitro* evolution to ML-guided *in silico* evolution

Edward Jenner – father of immunology





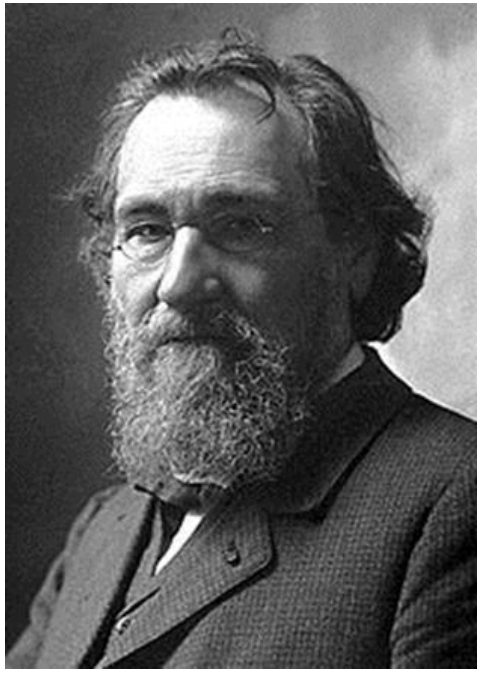
smallpox vaccine, the world's first vaccine.



Nobel Prize of 1908
Physiology or Medicine

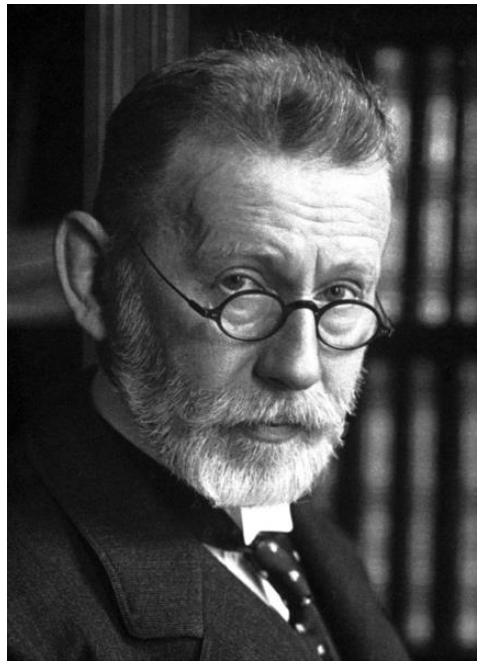


Ilya Mechnikov
1845-1916

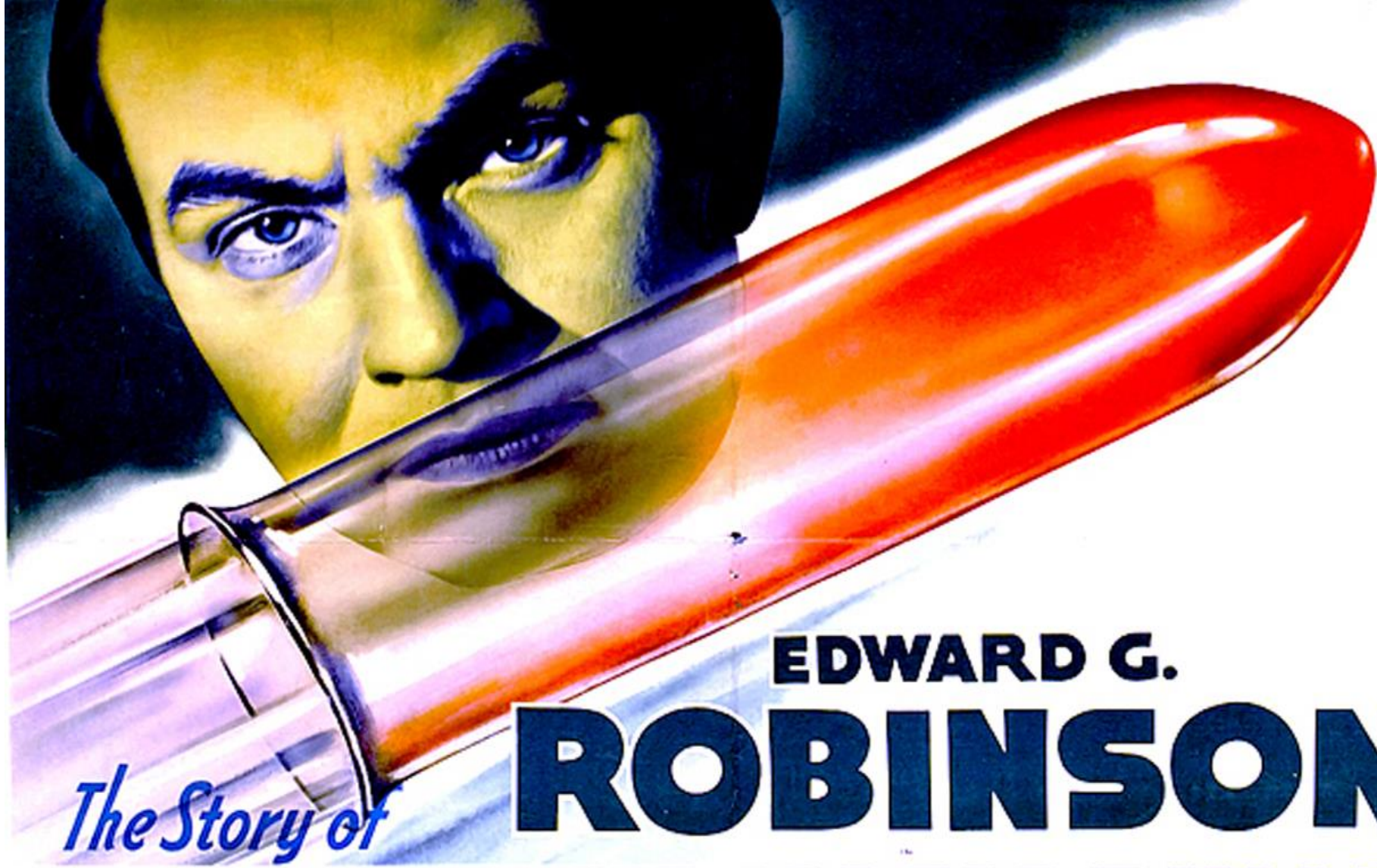


Discovered macrophage in 1882
Grand father of Modern
"Innate Immunity"

Paul Ehrlich
1854-1915



Grand father of Modern hematology, immunology and chemotherapy & pharmacoloy.



EDWARD G.

ROBINSON

The Story of

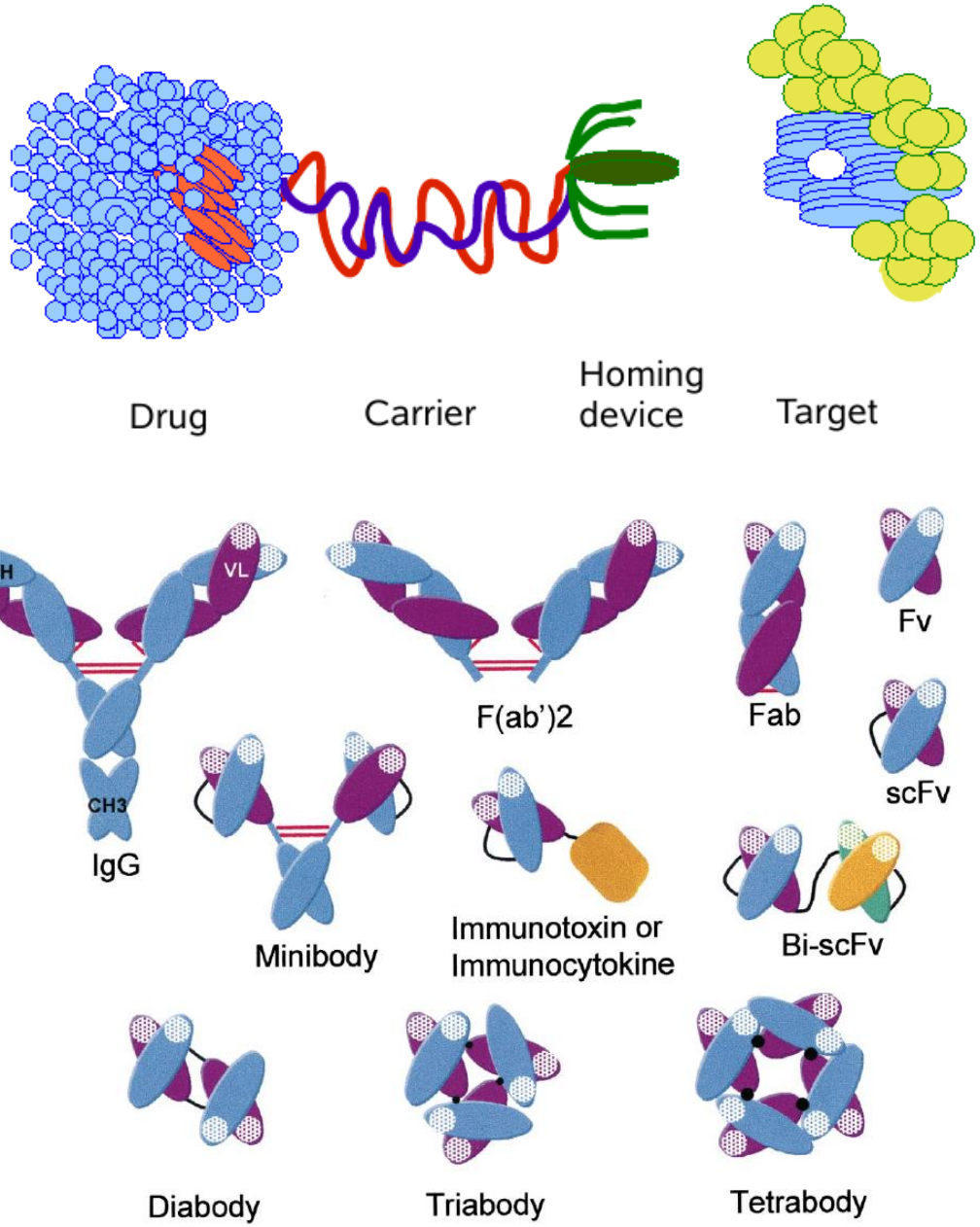
Dr. EHRLICH'S MAGIC BULLET

with RUTH GORDON · OTTO KRUGER · DONALD CRISP

Directed by WILLIAM DIETERLE • A WARNER BROS.-First National Picture

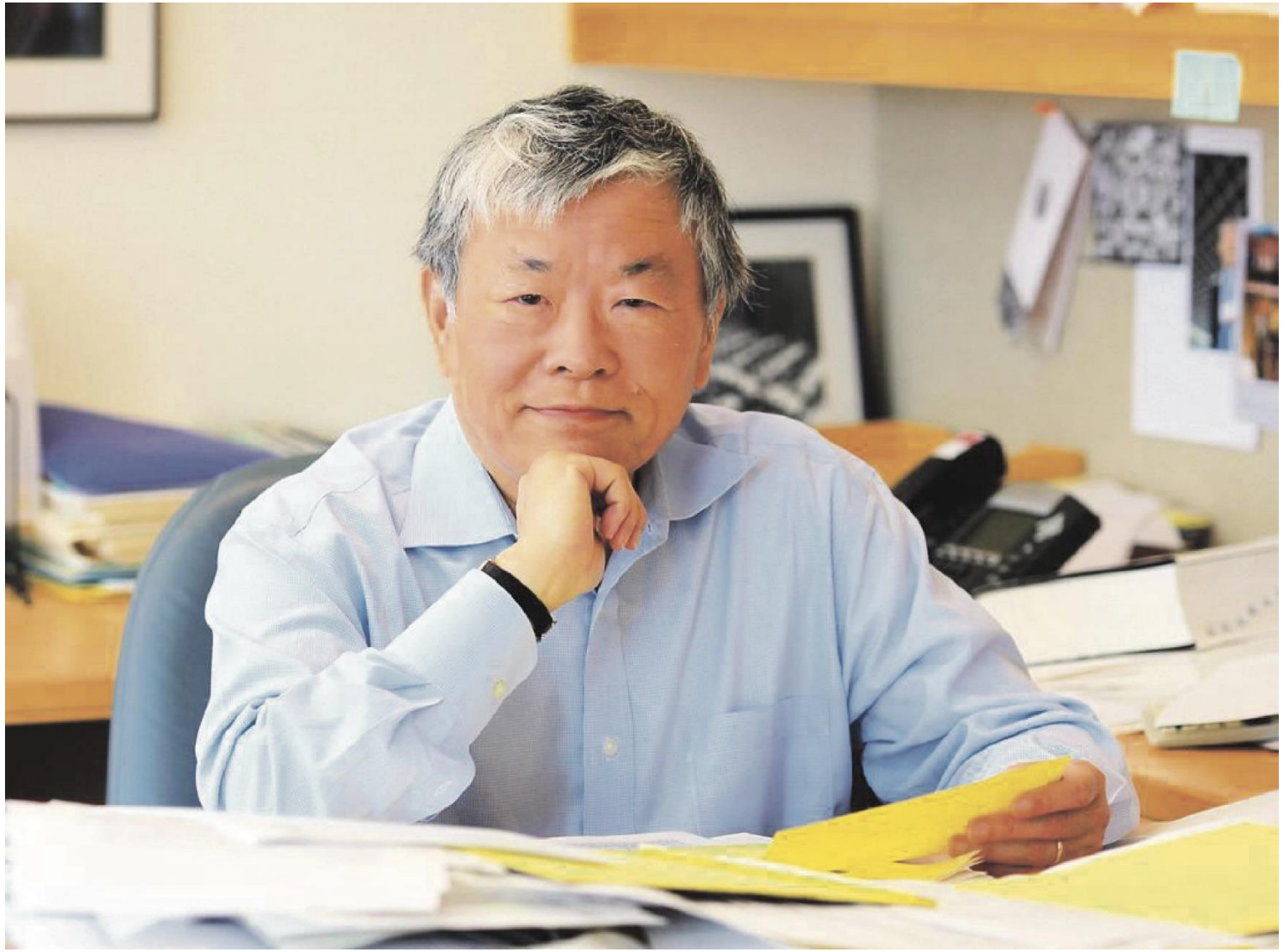
Presented by

WARNER BROS.



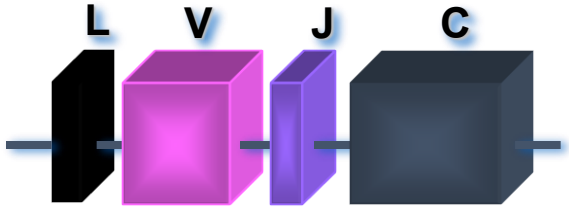
Magic bullet

Ehrlich reasoned that if a compound could be made that selectively targeted a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity. Hence, a "magic bullet" (*magische Kugel*, his term for an ideal therapeutic agent) would be created that killed only the organism targeted. The concept of a "magic bullet" was to some extent realized by the invention of monoclonal antibodies as they provide a very specific binding affinity.

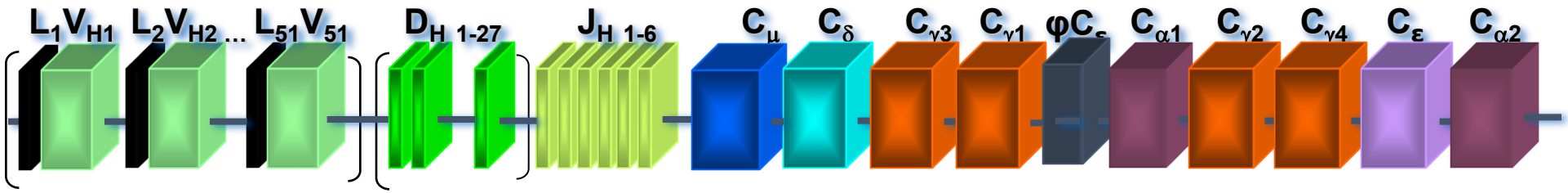


Susumu Tonegawa
Nobel prize 1987

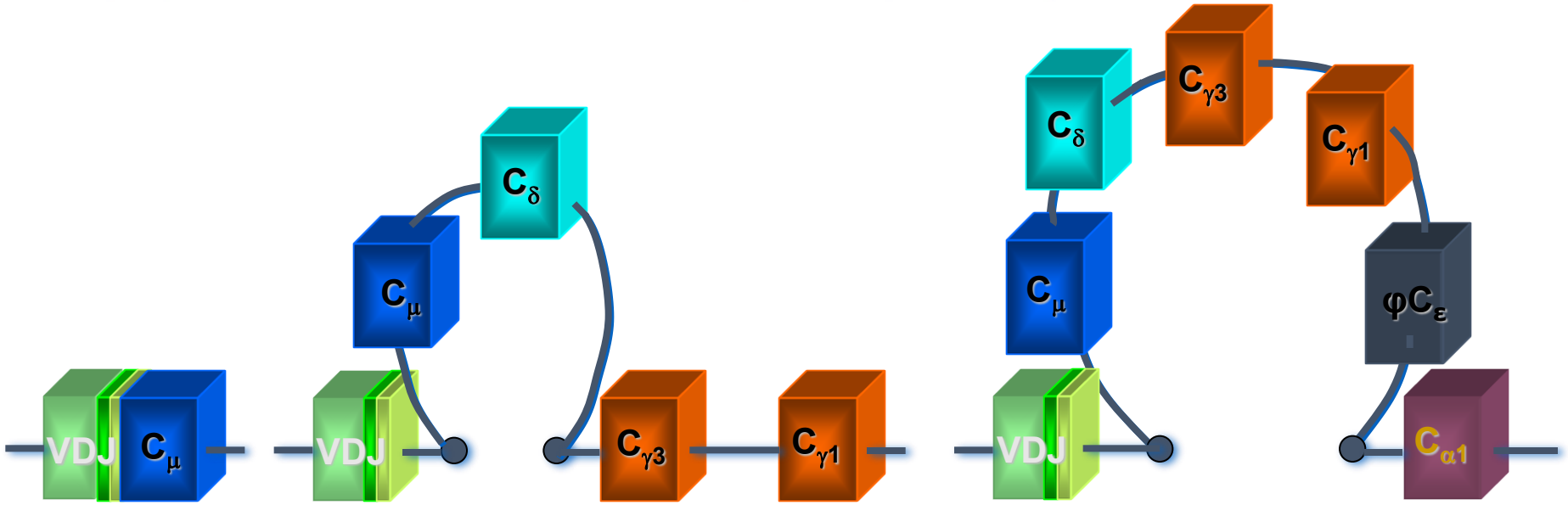
Genomic organization of immunoglobulins



Структура генов легкой (L) цепи иммуногло-булинов (у человека 2-я и 22-я хромосомы)



Структура генов тяжелой (H) цепи иммуноглобулинов (у человека 14-я хромосома)



Синтез IgM

Переключение с синтеза IgM и IgD на IgG3

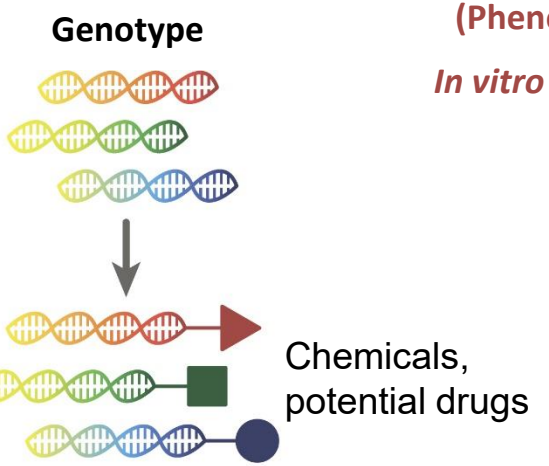
Переключение с синтеза IgG1 на IgA1

Universal screening technologies

Binding

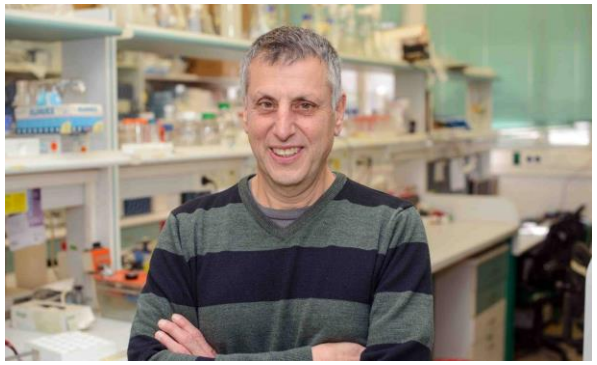
(Phenotype – formation of complex)

1. SELEX
2. Ribosome display
3. mRNA display
4. Phage display
5. Cell display

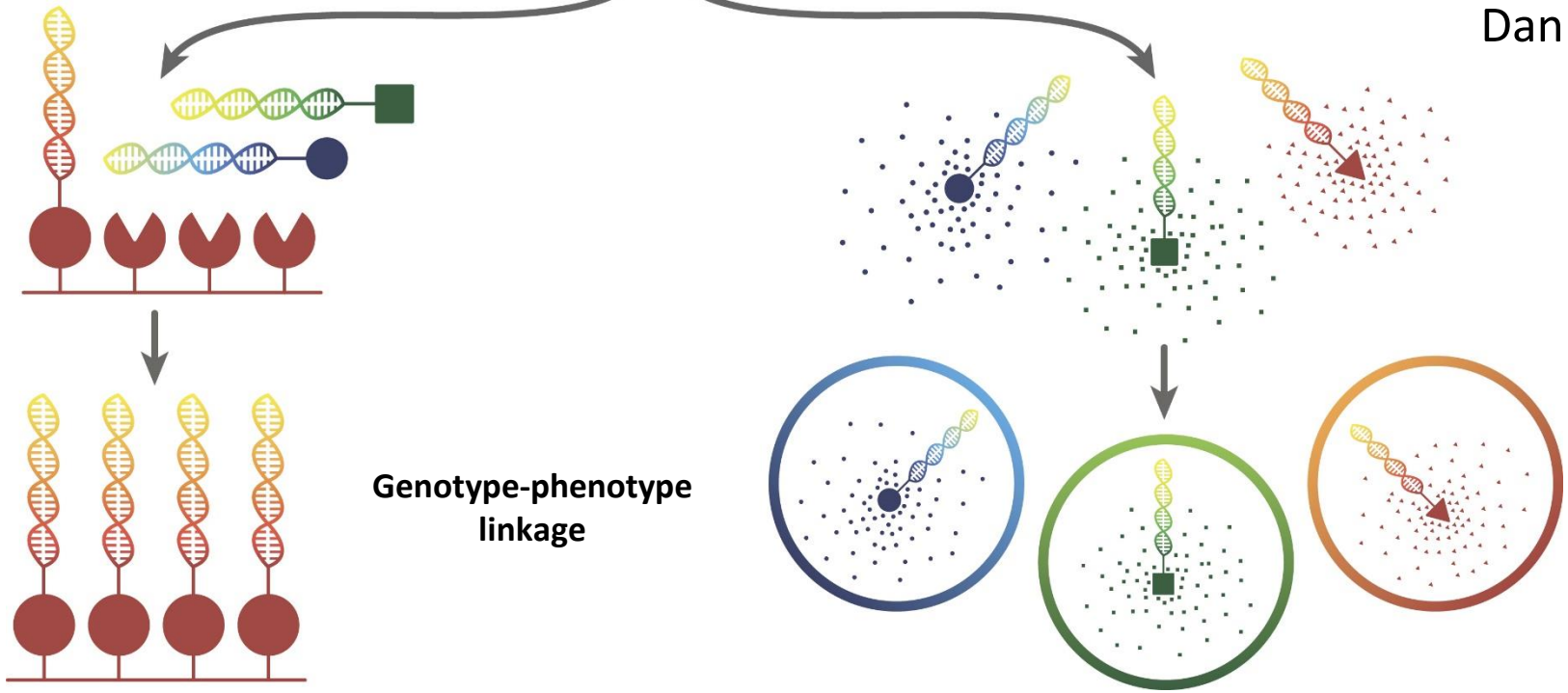


(Phenotype – any product)

***In vitro* compartmentalization**



Dan Tawfik



MESSAGE

BioCat Lab

Ultrahigh-throughput screening (uHTS) techniques can identify unique functionality from millions of variants.

Compartmentalization

is a powerful tool in screening technologies

Human-guided *in vitro* direct screening of big data

Single cell encapsulation is the key step of the uHTS

BioCat Lab

Microbiota from wild animals



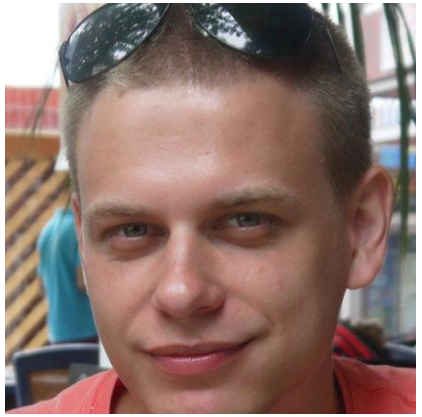
Soil microbiota

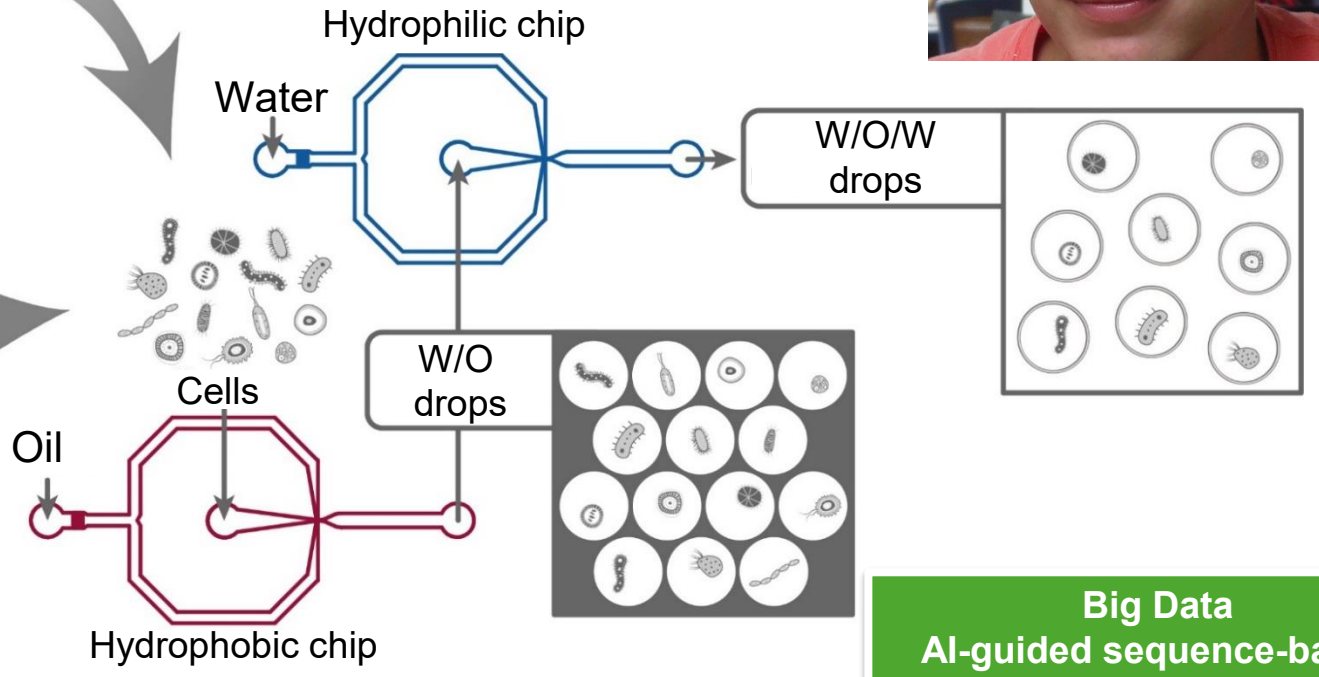


Microbiota from exotic sources



Stas Terekhov

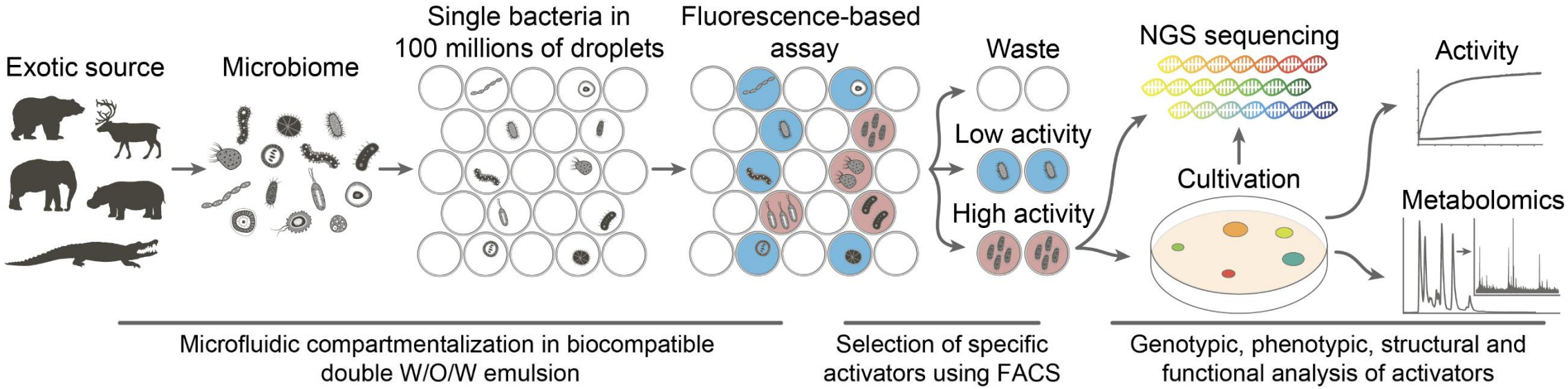




Big Data
AI-guided sequence-based search of antibiotic producers

Terekhov, Smirnov et al., PNAS 2017

Microfluidic platform for ultrahigh-throughput screening (uHTS microdroplet emulsion (MDE)-Platform)



Terekhov, Smirnov et al., PNAS 2017

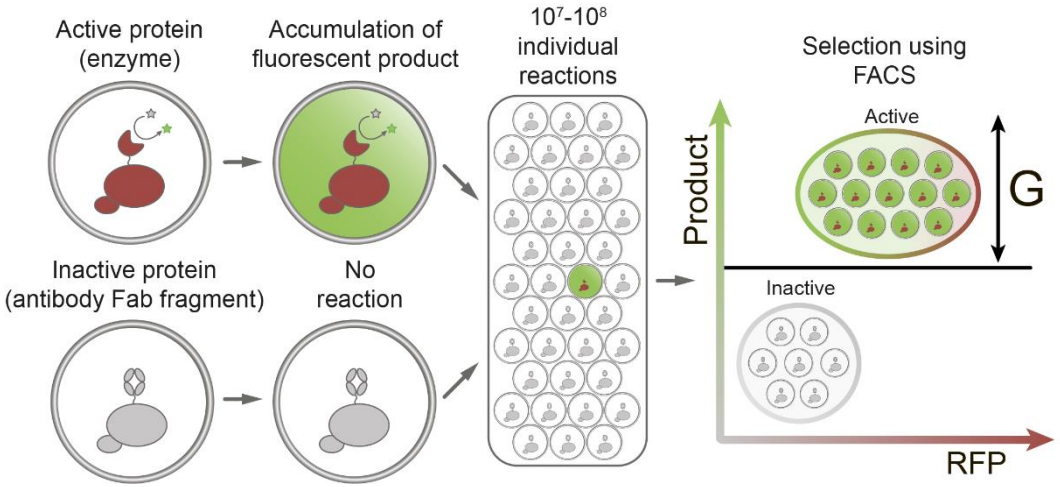
	Robot	Microfluidic drops
Total reactions	5×10^7	5×10^7
Reaction volume	100 μL	6 pL
Total volume	5,000 L	150 μL
Reactions/day	73,000	1×10^8
Total time	~2 years	~7 h
Number of plates/devices	260,000	2
Cost of plates/devices	$520,000	$1.00
Cost of tips	$10 million	$0.30
Amortized cost of instruments	$280,000	$1.70
Substrate	$4.75 million	$0.25
Total cost	$15.81 million	$2.50

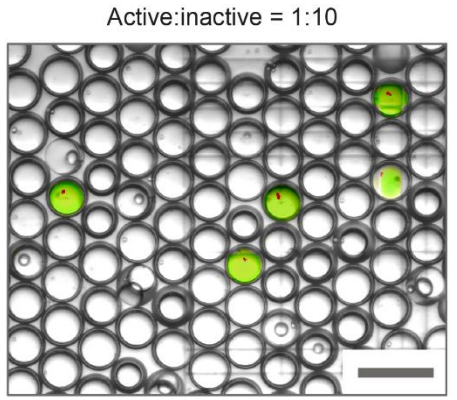
Time and costs for the screening using traditional methods and in microfluidic emulsions

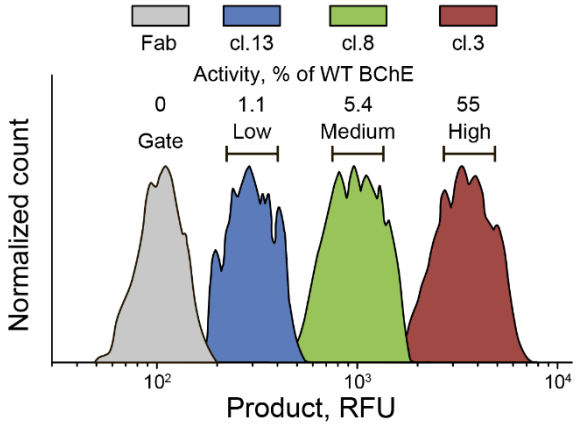
Agresti et al., PNAS 2010

Ultrahigh-throughput single cell screening

Direct evolution of enzyme: Selection of enzymes with different specificity and level of activity







What is it for?

- ✓ Specificity of enzyme: Optimization of therapeutics recombinant protein production
- ✓ Activity of enzyme: Rate of garbage destruction, effectivity of biofuel production
- ✓ Inhibitors of enzyme: Stop of pathological processes cancer/autoimmunity/infection

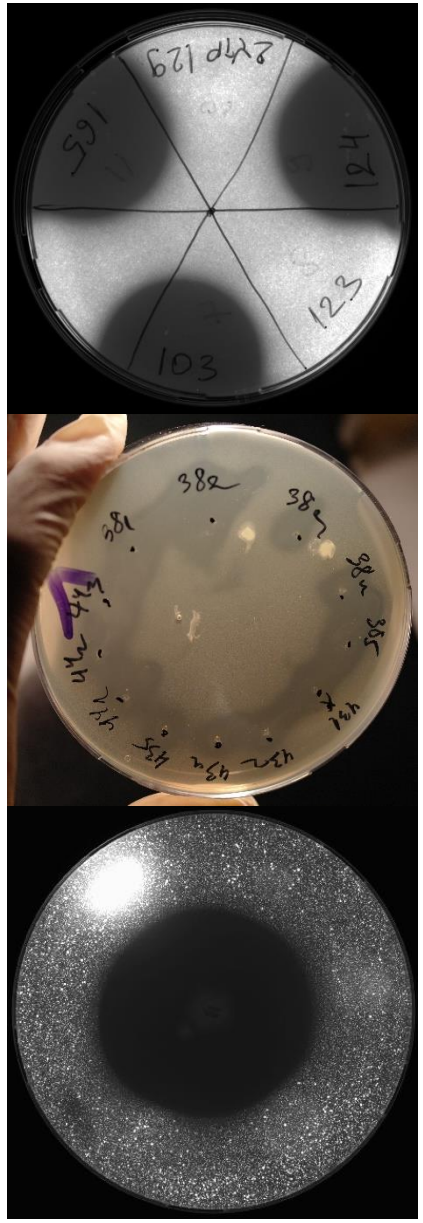
Terekhov, Smirnov et al., PNAS 2017

Oral microbiota of Siberian bear as an alternative source of *S. aureus* killers and antibiotic producers

BioCat Lab



New bacterial strains efficiently inhibiting *S. aureus* growth were selected using the developed microfluidic platform

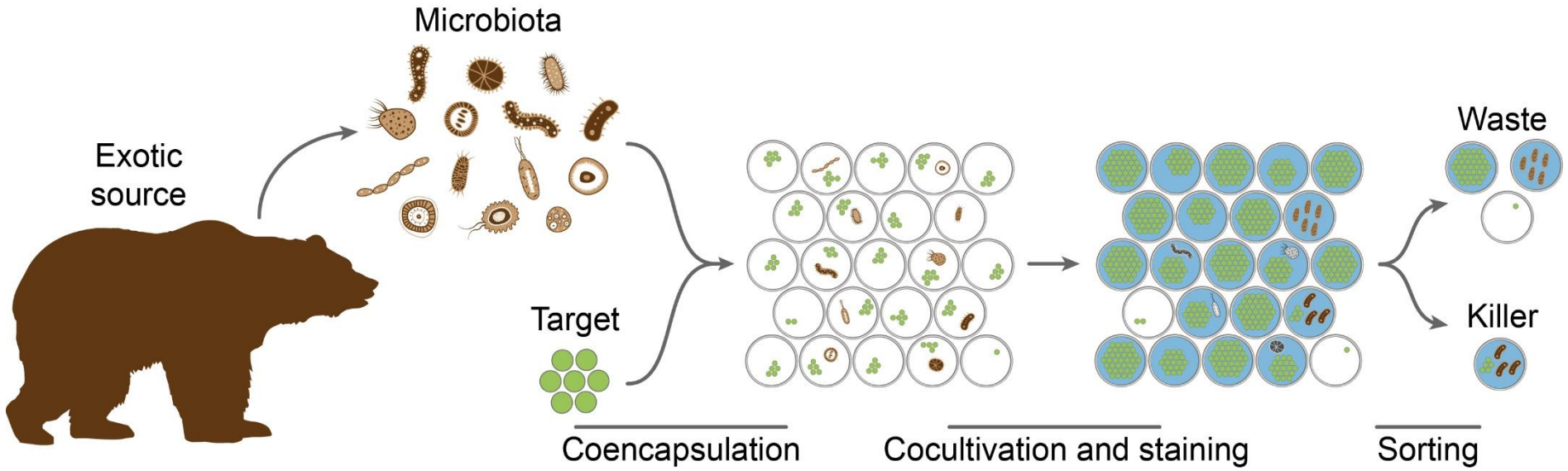


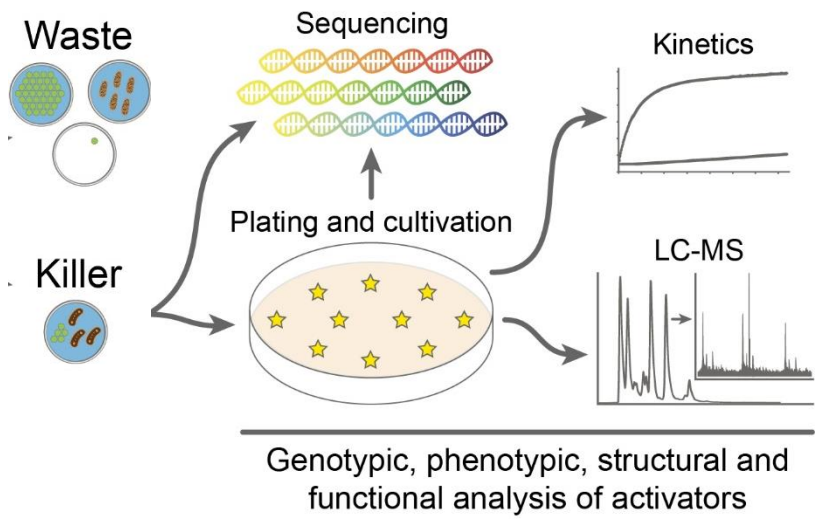
Oral microbiota of Siberian bear as an alternative source of *S. aureus* killers and antibiotic producers

BioCat Lab



Valentin Vlassov





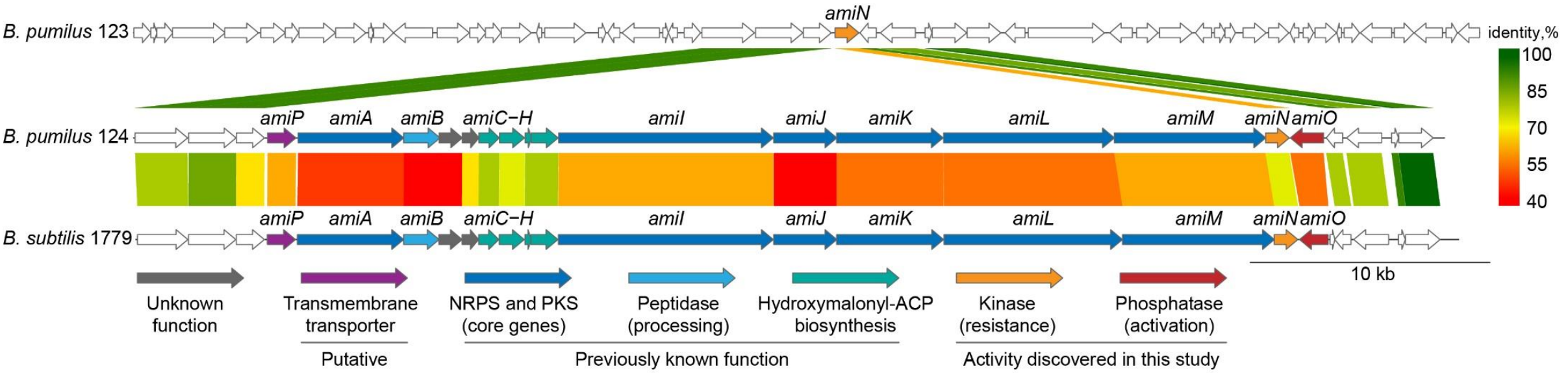


Terekhov, Smirnov et al., PNAS 2018

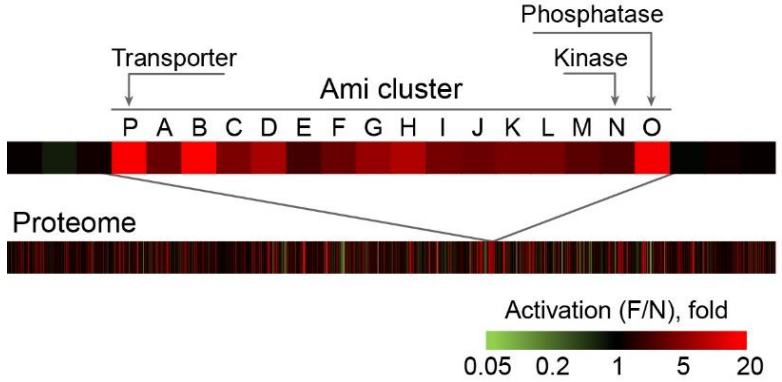
Multi-omics approach that was applied for discovery of regulation of Ami production

BioCat Lab

Genome analysis



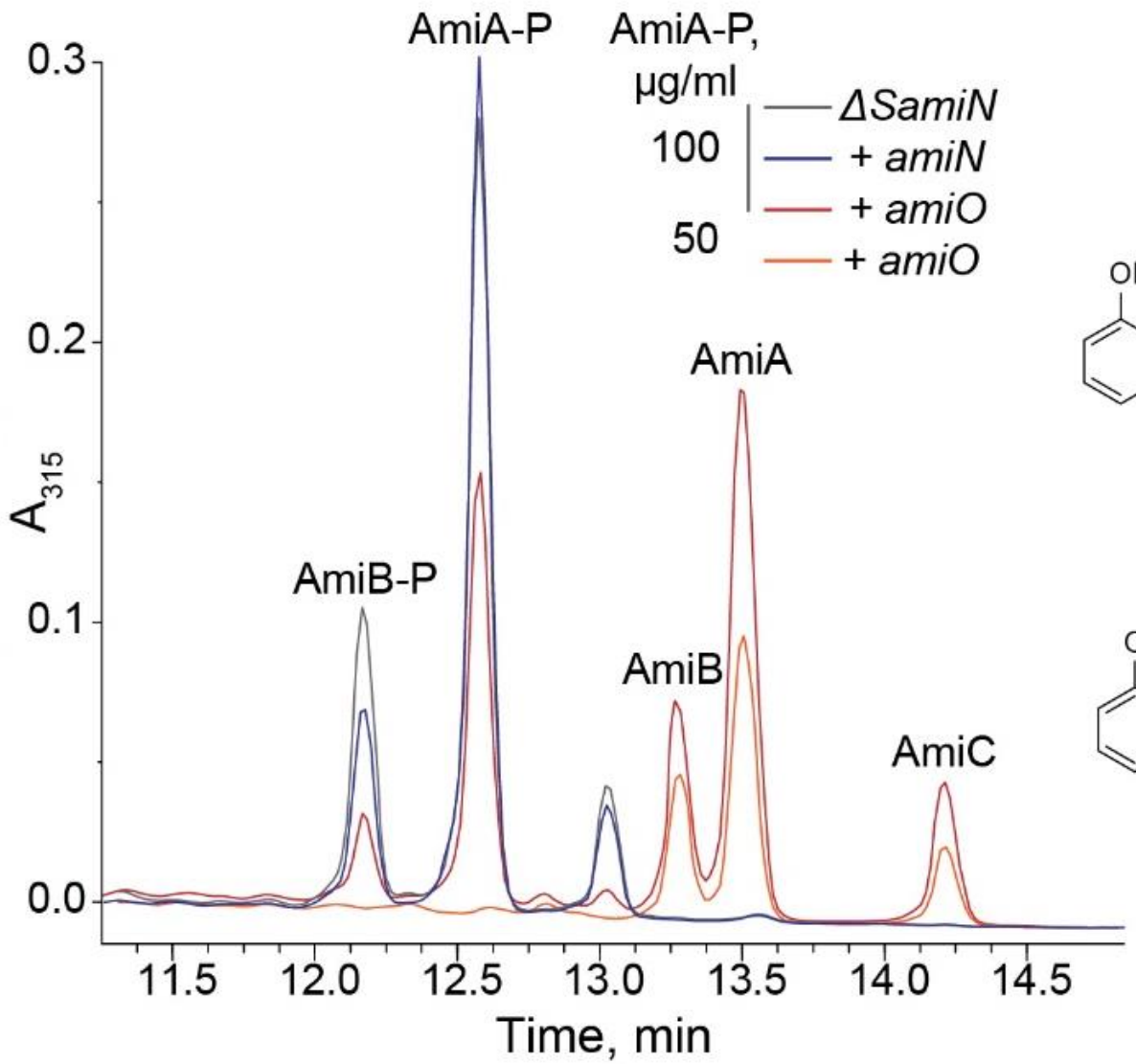
Proteome analysis

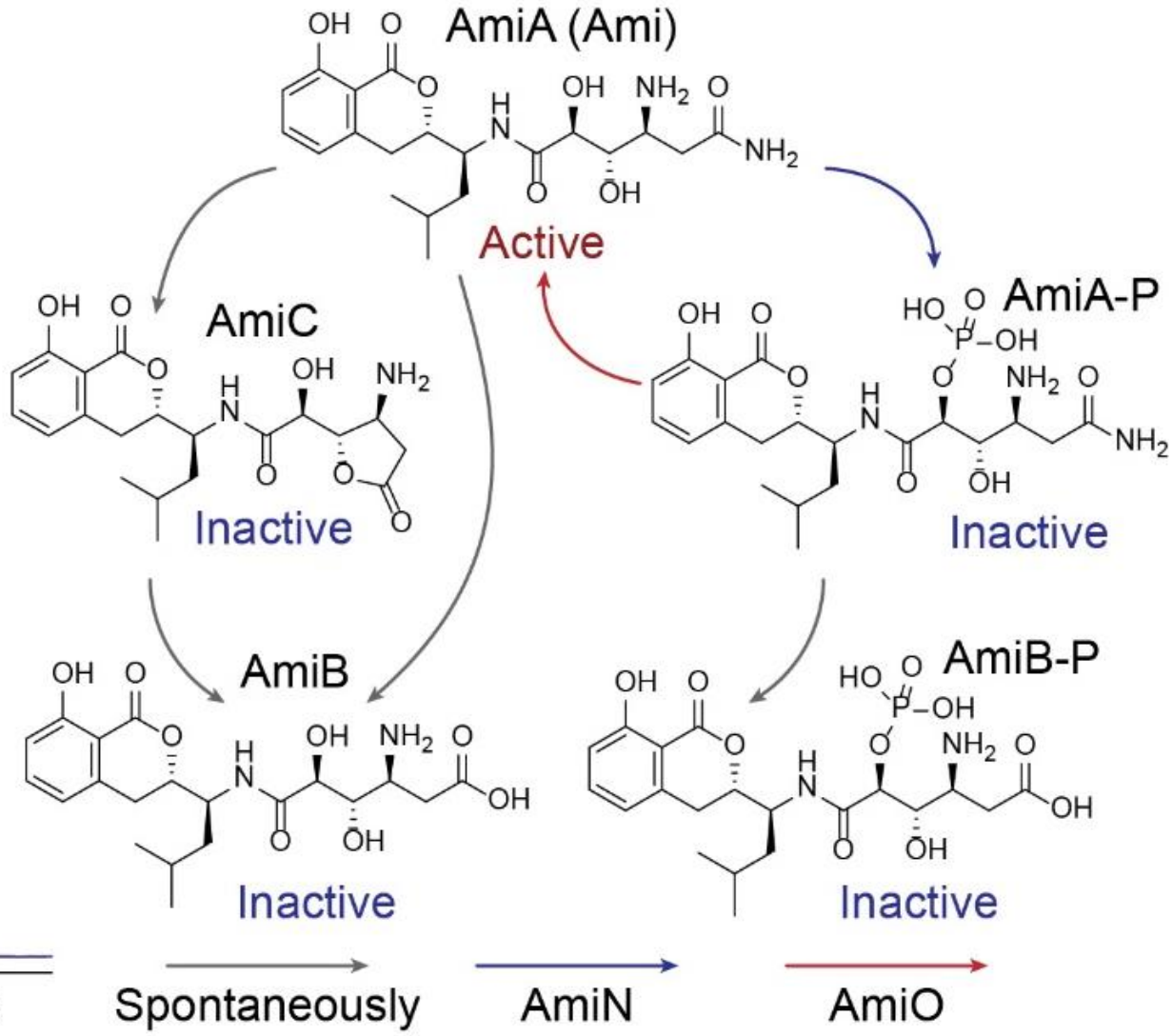


Operon mining
ML-guided genome data discrimination

Terekhov, Smirnov et al., PNAS 2018

Amicoumacin A activity regulation





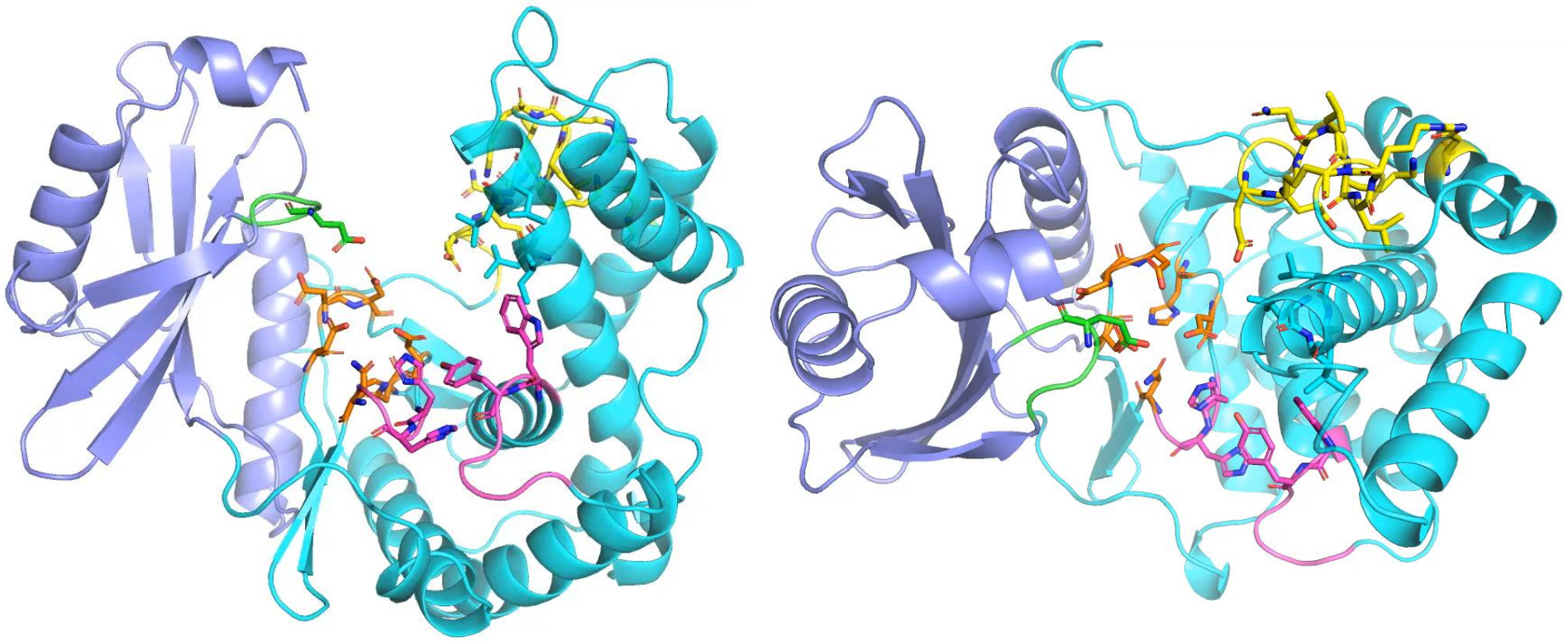
Terekhov, Smirnov et al., PNAS 2018

Reaction of amicoumacin kinases with substrate leads to close conformation of enzyme

BioCat Lab

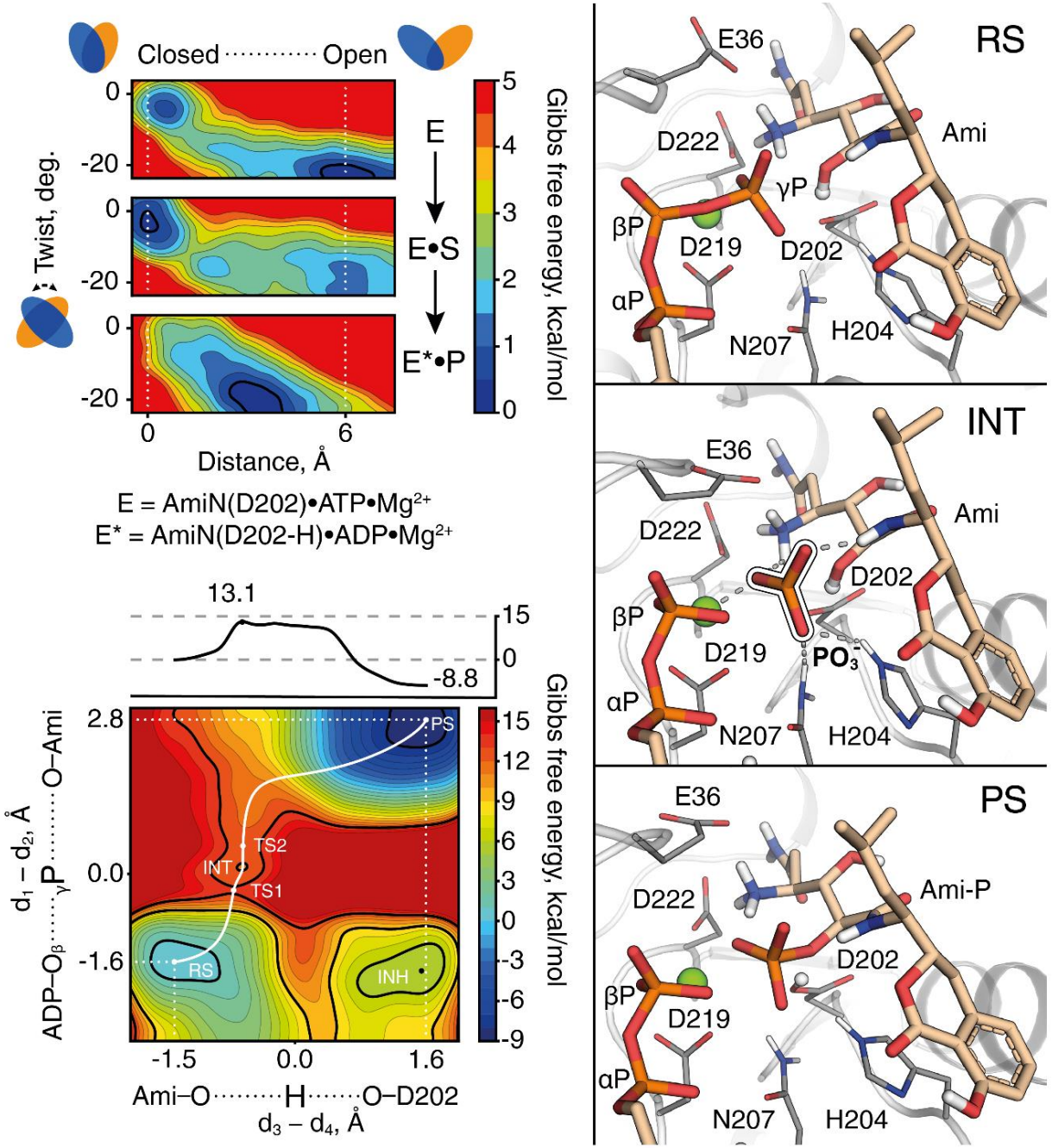
Front

Up



Terekhov et al., Science Adv. 2020

Atomistic details of the reaction



Terekhov et al., Science Adv. 2020

Analysis of specificity of AmiN kinase

BioCat Lab

AmiN, hAmiN, SubAmiN were inactive against:

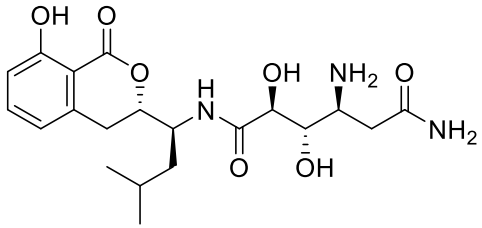
<u>Aminoglycosides</u>
- Kanamycin
- Gentamicin

<u>Macrolides</u>
- Erythromycin
- Tylosin

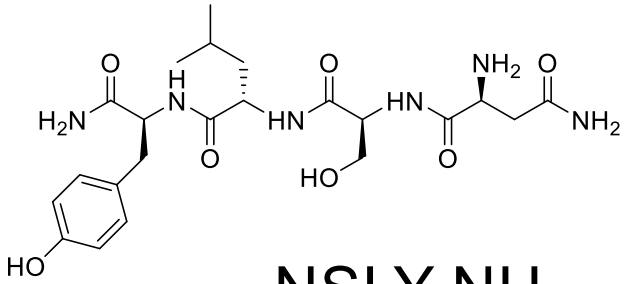
<u>Tetracycline class</u>
- Tetracycline
- Doxycycline

AmiN derived from protein kinase?

Peptides



Ami



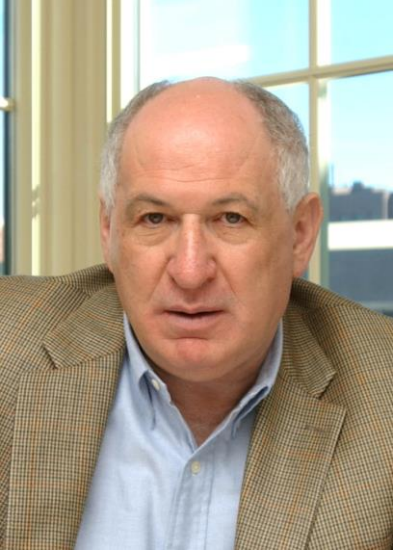
NSLY-NH_2

AmiN

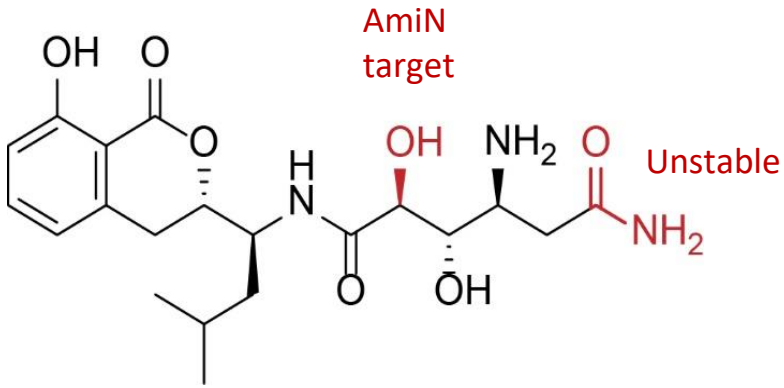
No phosphorylation of

NSLY-NH_2 NSL-NH_2 SLY-NH_2 SL-NH_2

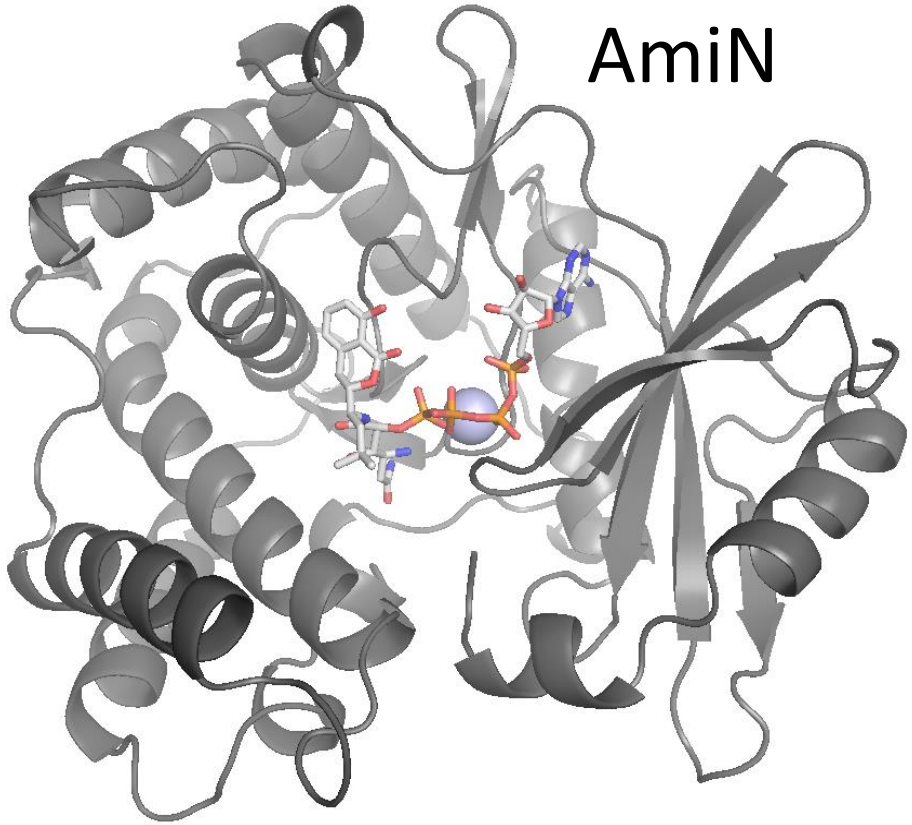
Rational design of semisynthetic antibiotics



Joseph Schlessinger



Identification of Ami weak points



MESSAGE

BioCat Lab

COVID 19
Serology of the 21st century

We need to work with individual Immune cells

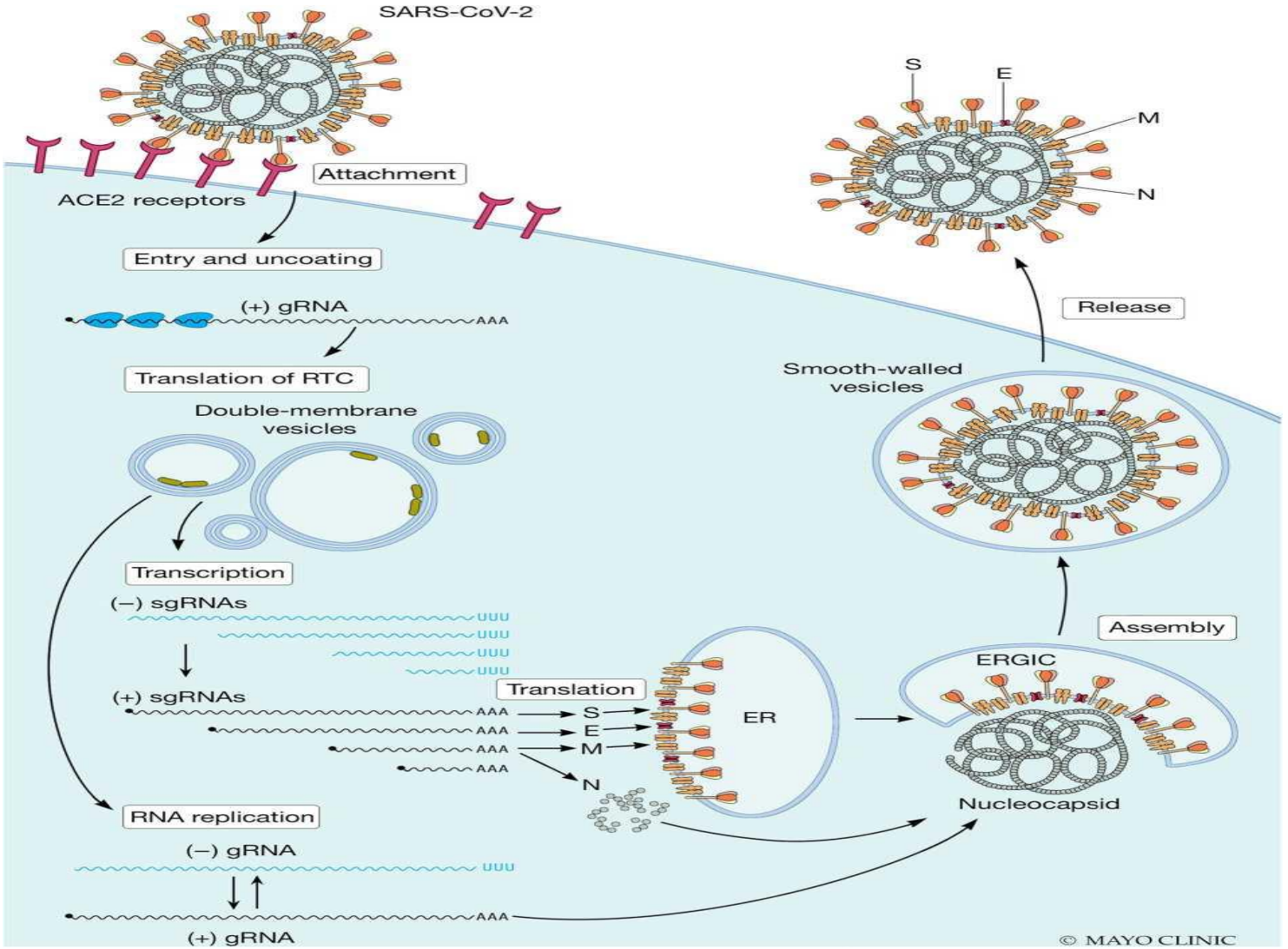
Nobel Prize 1984 "for the theory of specificity in the development and control of the immune system and the discovery of the principle of the formation of monoclonal antibodies."

César Milstein
1927-2002



Georges J.F. Köhler
1946-1995



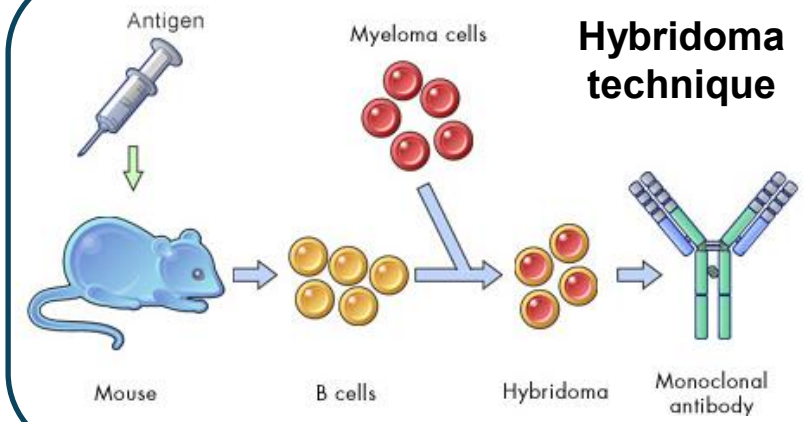


Viral replication pathway of Covid-19. The virus first attaches to the ACE2 receptor and internalizes into the respiratory epithelial cell and causes the release of its genome. The S protein (spikes on the viral surface responsible for attachment to host cell receptors), M protein (shapes the virion, promotes membrane curvature and binds to the nucleocapsid), E protein (helps with viral assembly and release)

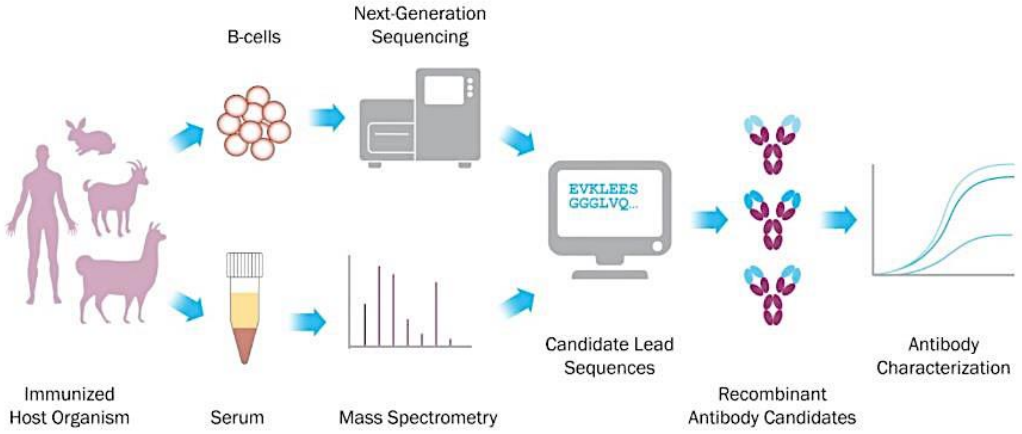
The production of monoclonal antibodies

BioCat Lab

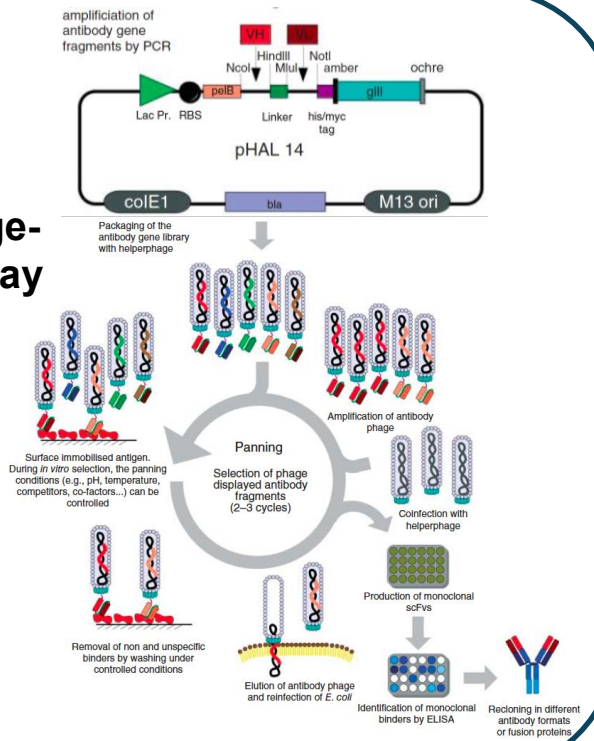
Hybridoma technique



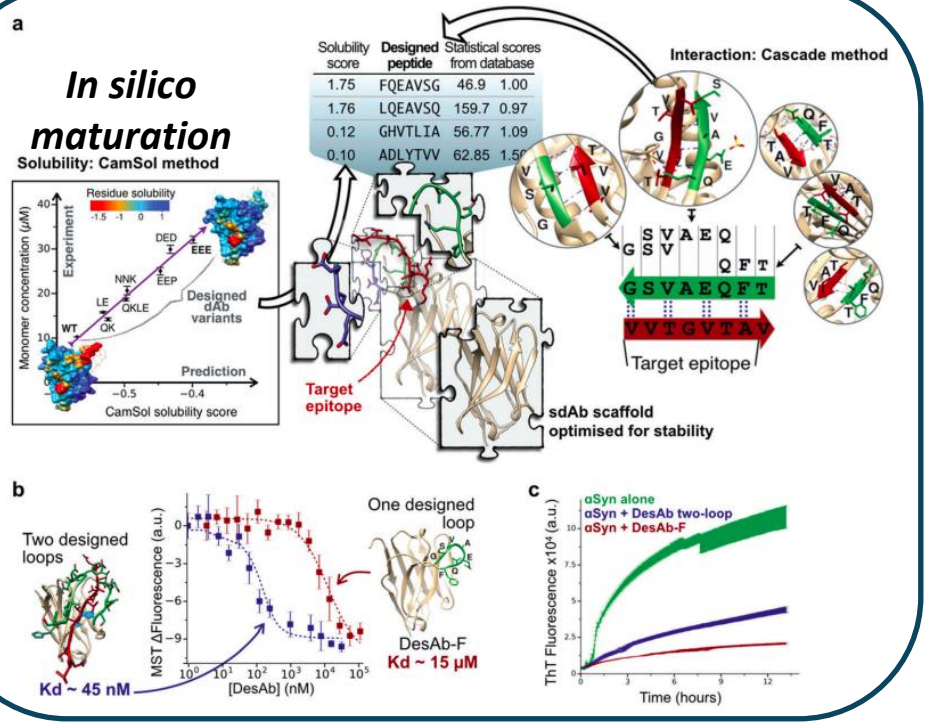
Single B-cell approach



Phage-display



In silico maturation



FDA gives emergency OK to Lilly's antibody treatment for Covid-19

The NEW ENGLAND JOURNAL of MEDICINE



ORIGINAL ARTICLE

SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19

Peter Chen, M.D., Ajay Nirula, M.D., Ph.D., Barry Heller, M.D., Robert L. Gottlieb, M.D., Ph.D., Joseph Boscia, M.D., Jason Morris, M.D., Gregory Huhn, M.D., M.P.H.T.M., Jose Cardona, M.D., Bharat Mocherla, M.D., Valentina Stosor, M.D., Imad Shawa, M.D., Andrew C. Adams, Ph.D., Jacob Van Naarden, B.S., Kenneth L. Custer, Ph.D., Lei Shen, Ph.D., Michael Durante, M.S., Gerard Oakley, M.D., Andrew E. Schade, M.D., Ph.D., Janelle Sabo, Pharm.D., Dipak R. Patel, M.D., Ph.D., Paul Klekotka, M.D., Ph.D., and Daniel M. Skovronsky, M.D., Ph.D., for the BLAZE-1 Investigators*

ABSTRACT

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (Covid-19), which is most frequently mild yet can be severe and life-threatening. Virus-neutralizing monoclonal antibodies are predicted to reduce viral load, ameliorate symptoms, and prevent hospitalization.

METHODS

In this ongoing phase 2 trial involving outpatients with recently diagnosed mild or moderate Covid-19, we randomly assigned 452 patients to receive a single intravenous infusion of neutralizing antibody LY-CoV555 in one of three doses (700 mg, 2800 mg, or 7000 mg) or placebo and evaluated the quantitative virologic end

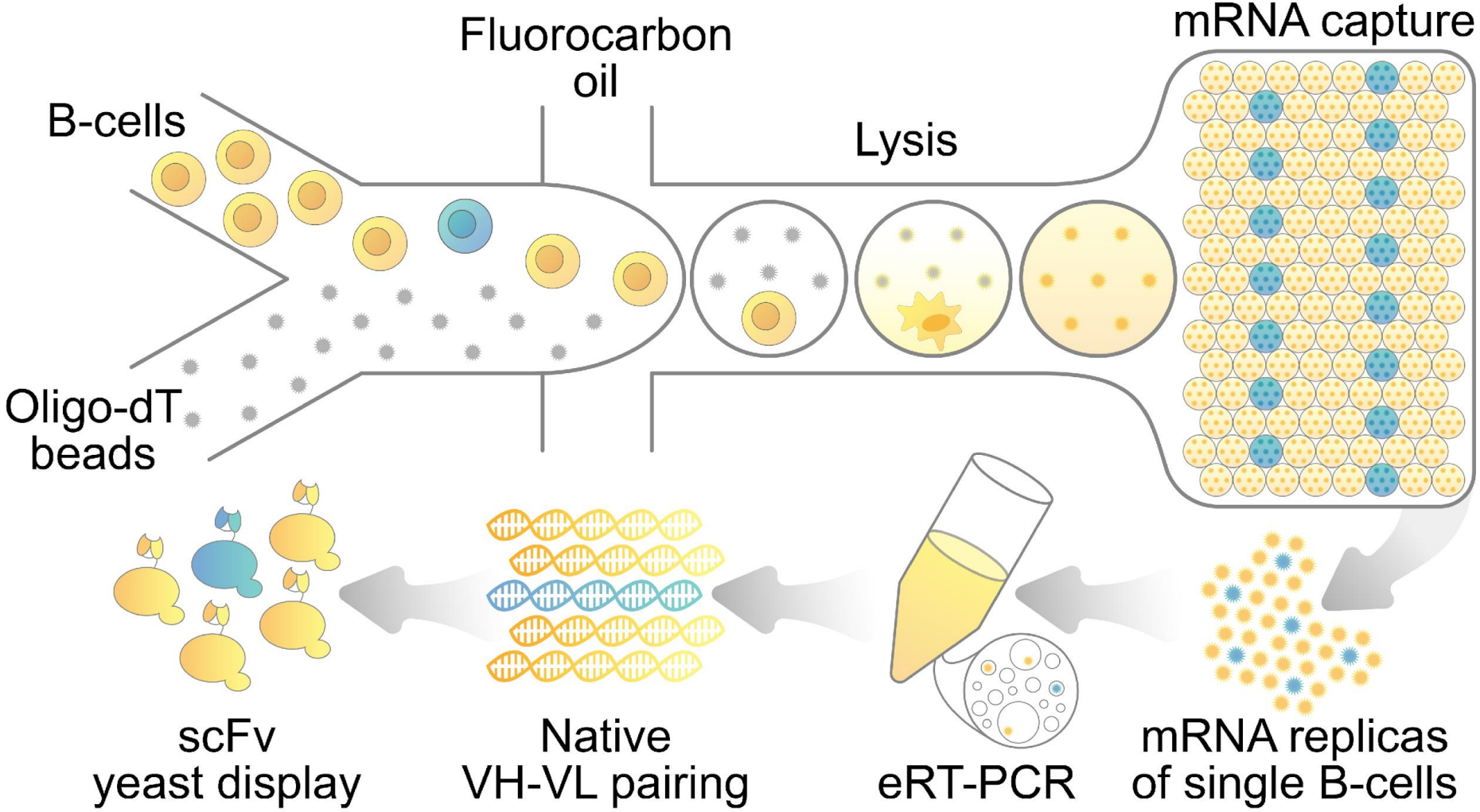
From the Department of Medicine, Women's Guild Lung Institute, Cedars–Sinai Medical Center, Los Angeles (P.C.), and Long Beach Clinical Trials, Long Beach (B.H.) — both in California; Eli Lilly, Indianapolis (A.N., A.C.A., J.V.N., K.L.C., L.S., M.D., G.O., A.E.S., J.S., D.R.P., P.K., D.M.S.), and Franciscan Health, Greenwood (I.S.) — both in Indiana; Baylor University Medical Center and Baylor Scott and White Research Institute, Dallas (R.L.G.); Vitalink Research, Union, SC (J.B.); Imperial Health, Lake

MESSAGE

Ultrahigh-throughput screening (uHTS) techniques can identify unique functionality from millions of variants of eucariotic cells.

Reconstruction of the natural B-cell repertoire

BioCat Lab



Single-cell droplet microfluidics enables preserving the native pairing of human immunoglobulin chains

Guo Y. et al. Nature Communications. 2021.
Lomakin Y. A. et al. Communications Biology. 2024.

MESSAGE

BioCat Lab

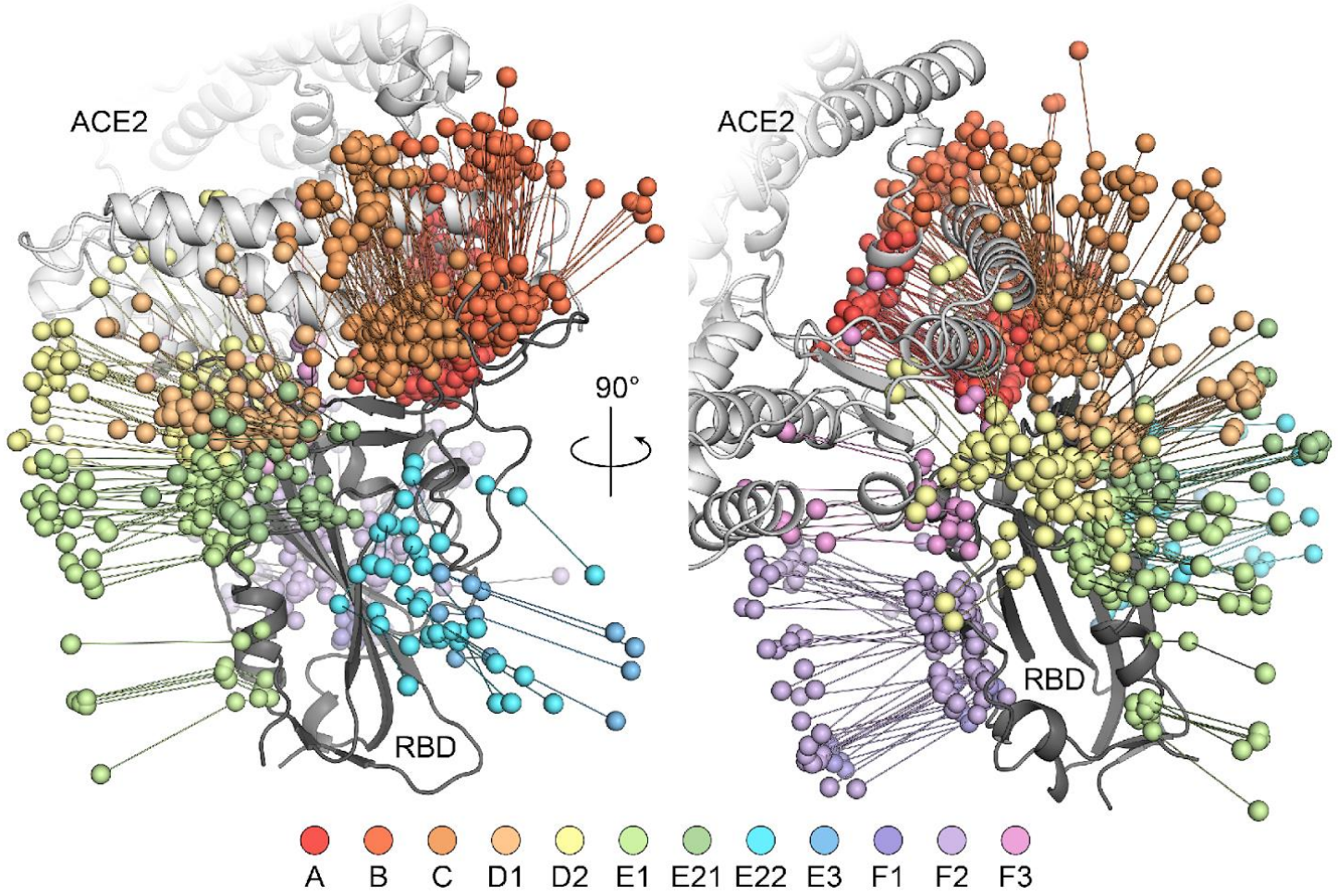
Why we need Artificial intelligence and Machine learning to solve immunological problems?

Antibody Epitope Prediction

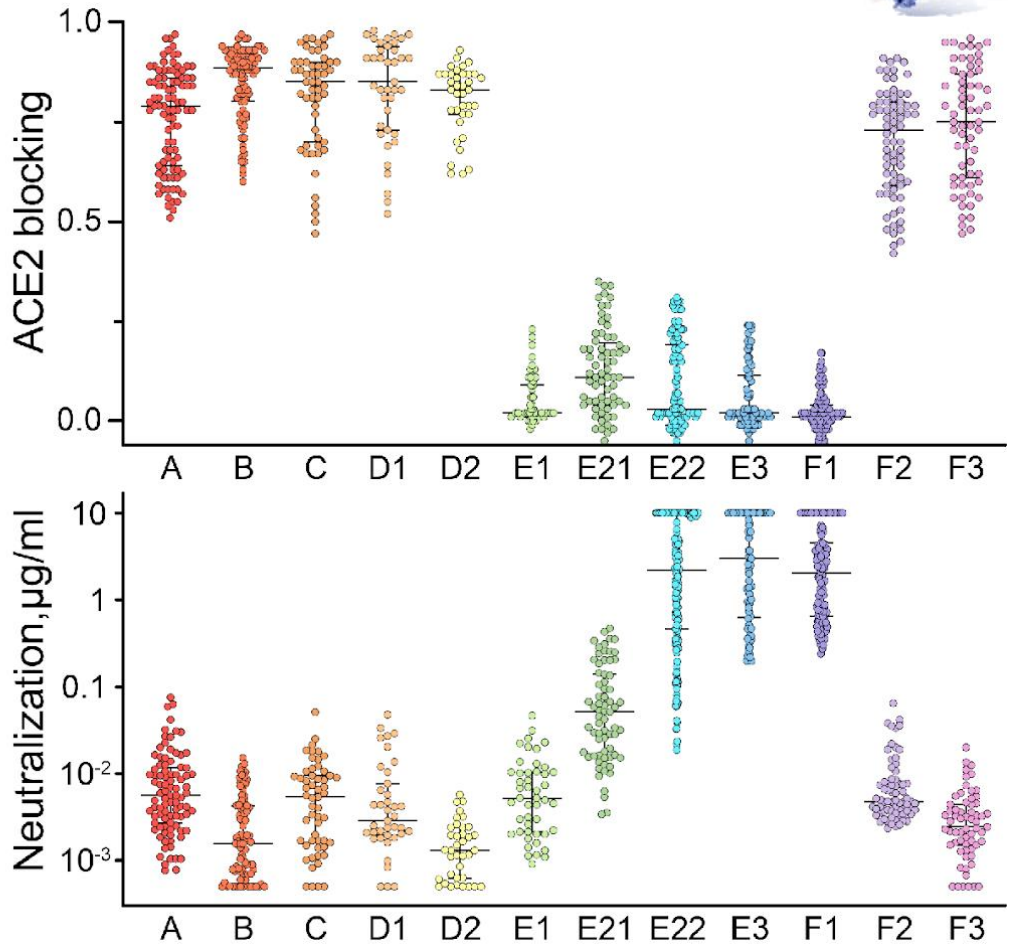
Functional Profiling of Antibody Repertoires

Identification of novel viral neutralization mechanisms

Experiment-driven target **ML application**



A visual representation of the 12-class model used for **antibody dataset** classification



ACE2-blocking and SARS-CoV-2 neutralizing properties distribution

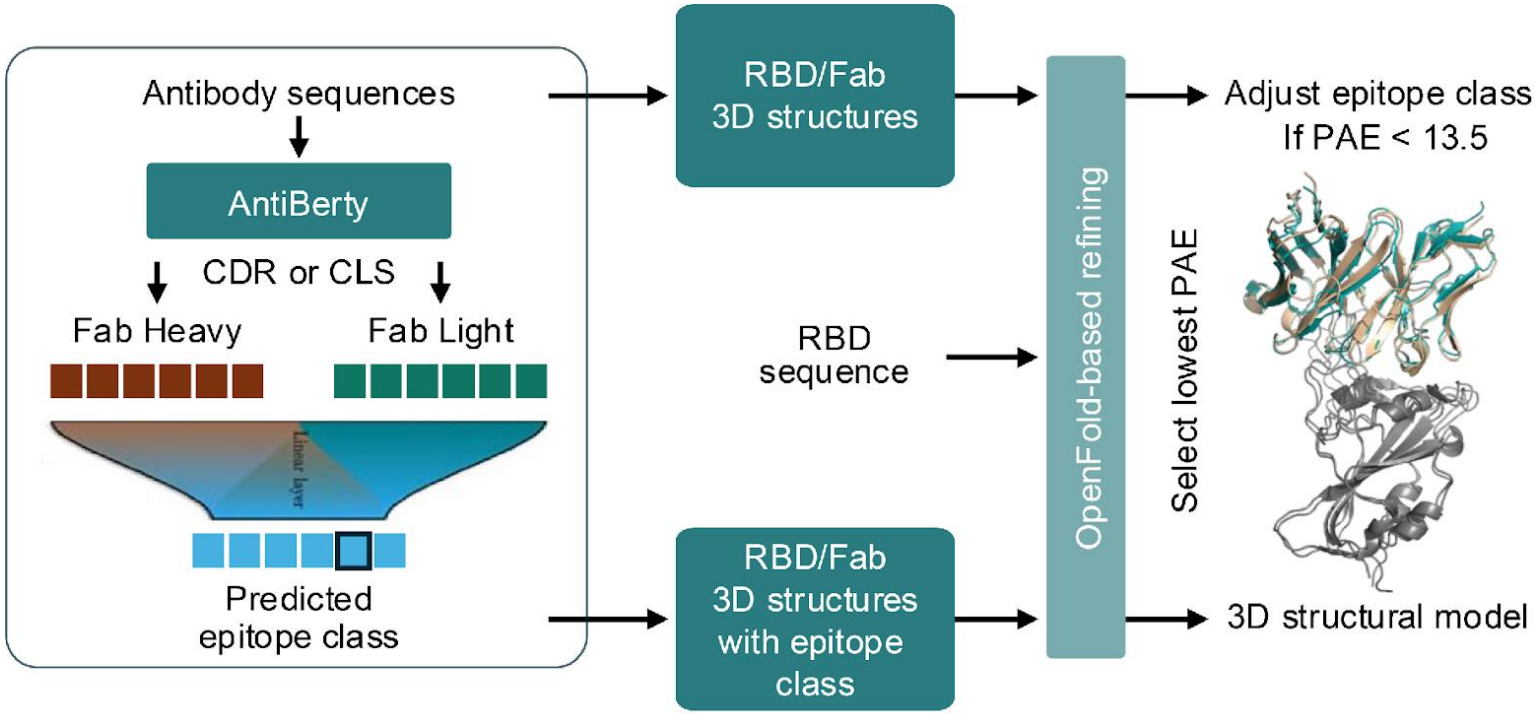
Trained on 450+ experimental crystal structures

Terekhov et al., Nature Communications, 2026

Prediction of antibody epitopes

BioCat Lab

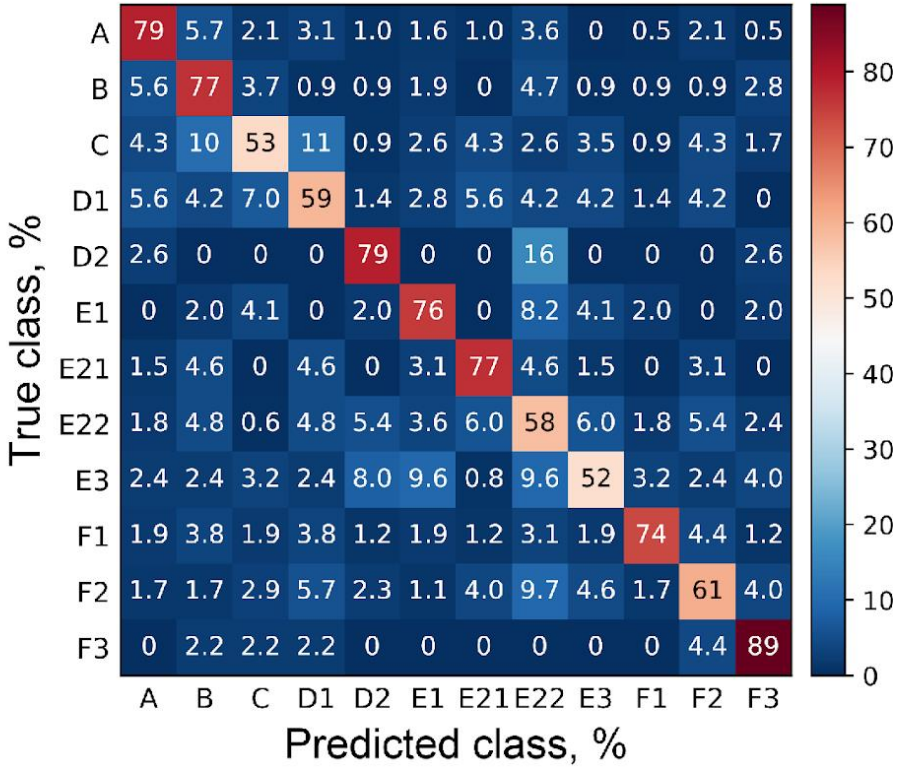
RBD-AIM computational pipeline





Hermann Gref

AntiBerty is model to predict the epitope class from the input light and heavy chains of an antibody



The confusion matrix for the predicted antibodies across epitope classes calculated using the RBD-AIM pipeline

Terekhov et al., Nature Communications, 2026

https://rbdaim.2a2i.org/

Home About

RBD-AIM

RBD Antibody Interaction Mapper

Heavy chain ? paste example

EVQLVESGGGLVQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWV
SVIYSGGSTFYADSVKGRFTISRDNSMNTLFLQMNSLRAEDTAVYYCARV
LPMYGDYLDYWGQGTLVTVSS

Light chain ?

DIVMTQSPSSLSASVGDRVTITCRASQSISRYLNWYQQKPGKAPKLLIYAA
SSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPEYTFG
QGTKLEIK

Select SARS-COV2 strains ?

Wuhan

Predicted binding score

Low (-5.8) High (2.8)

A	B	C	D1	D2	E1	E21	E22	E3	F1	F2	F3
2.8	-3.6	-4.3	-4.4	-5.3	-5.3	-4.6	-4.9	-4.8	-3.2	-5.5	-5.8

Predicted epitope class: A

Structure-based prediction

Description

Predicted epitope class: A

Predicted binding score: 7.14

Predicted 3D structure of the antibody-RBD complex

Show all epitope classes Show ACE2-binding interface

NLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADY
NYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYR
VVVLSFELLHAPATVCGP

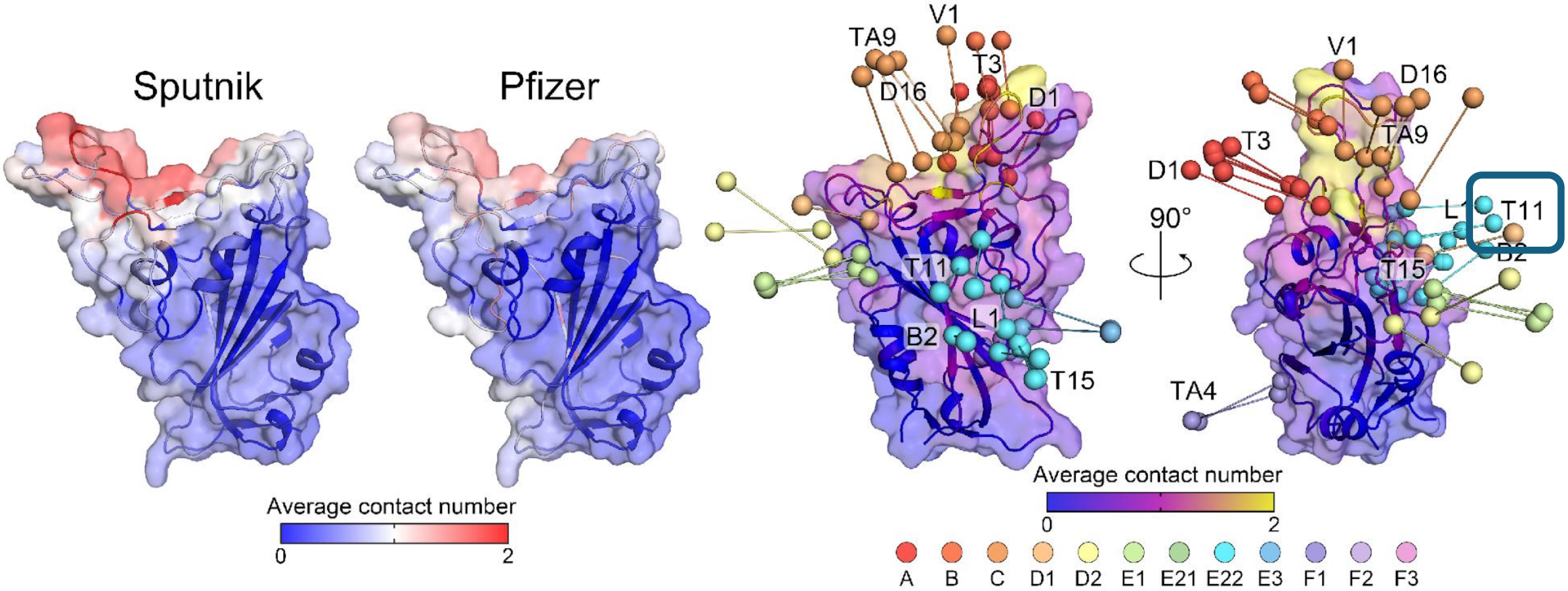


Escape mutations

Heatmap shows number of escape mutations between analyzed RBD sequence and RBD of SARS-COV2 strains.

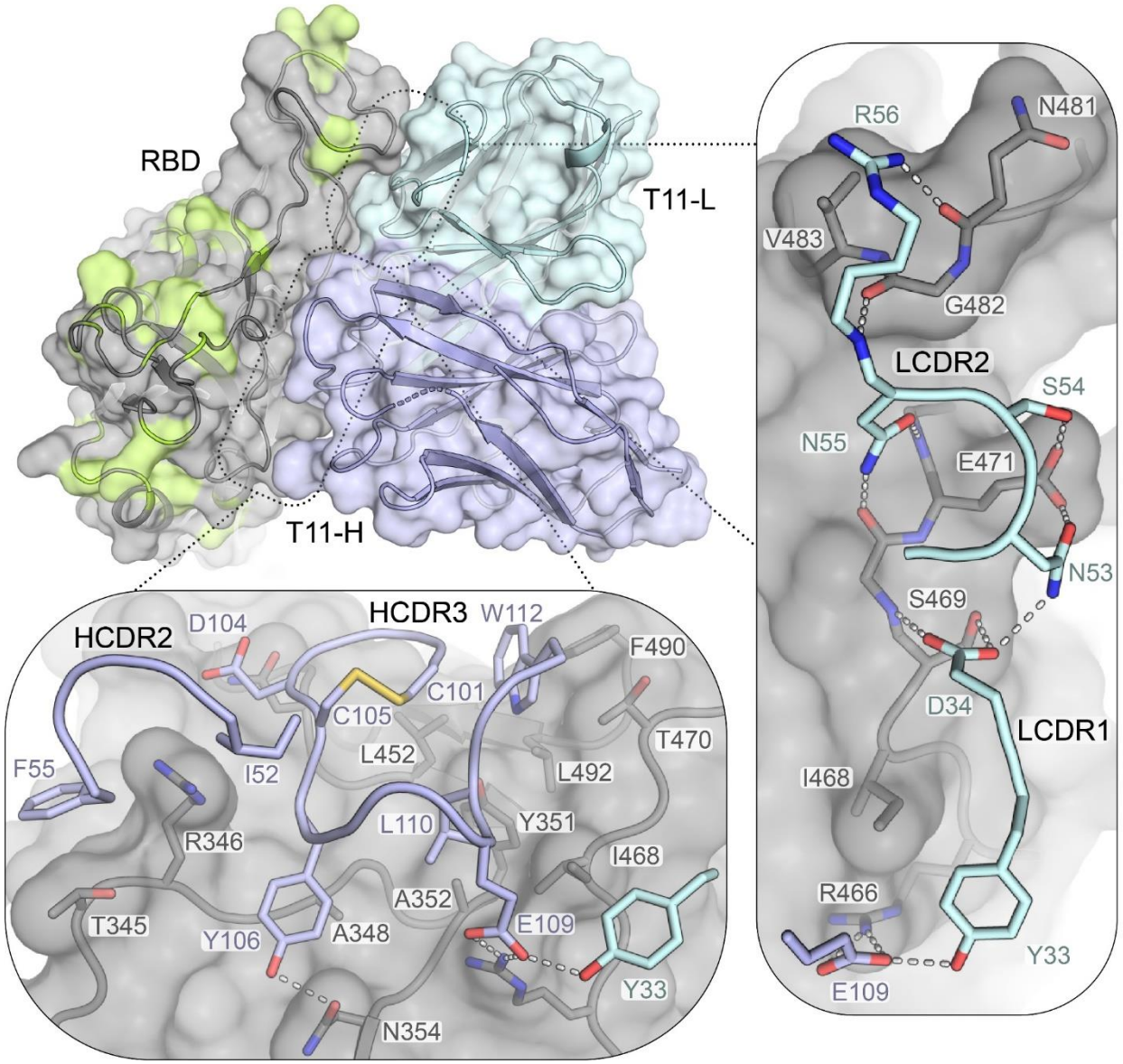
Wuhan	24A_(JN.1)	21C_(Epsilon)	20J_(Gamma)	23L_(BA.2.86)	23H_(HK.3)	21A_(Delta)	21L_(BA.2)	22B_(BA.5)	23F_(EG.5.1)	23E_(XBB.2.3	22D_(BA.2.75	21K_(BA.1)	23B_(XBB.1.16	20H_(Beta)	21G_(Lambda	21D_(Eta)	21F_(Iota)	23A_(XBB.1.5)	23D_(XBB.1.9.	20I_(Alpha)	22E_(BQ.1.1)	21H_(Mu)	21B_(Kappa)	23G_(XBB.1.5.	22F(XBB.1)	24B_(JN.1.11.1	22C_(BA.2.12.	22A_(BA.4)
0	16	0	3	15	15	2	10	11	15	13	11	10	13	3	2	1	1	13	13	1	12	2	2	15	13	17	11	11

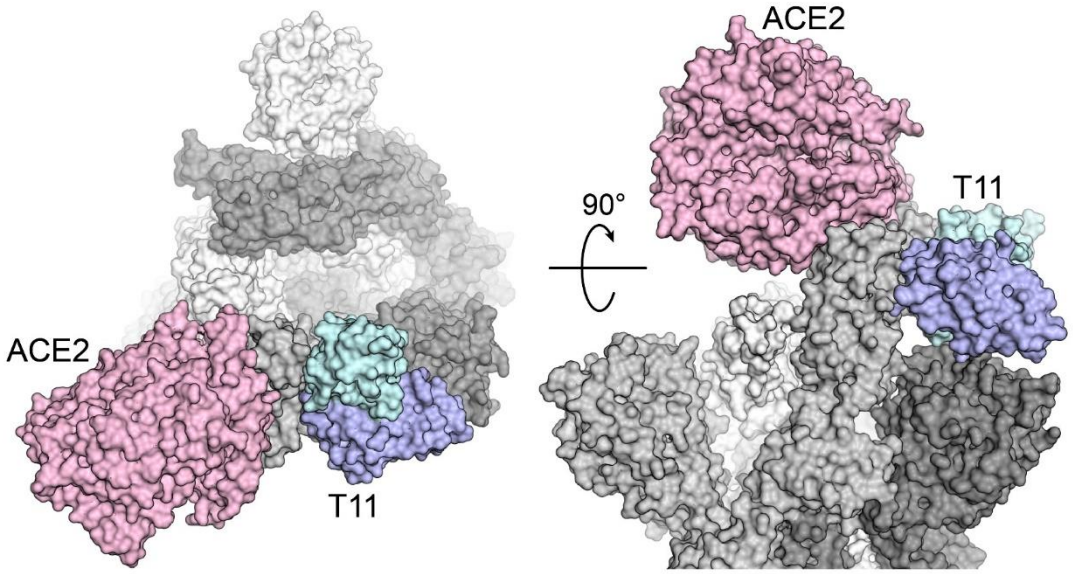
Functional profiling of antibody repertoires

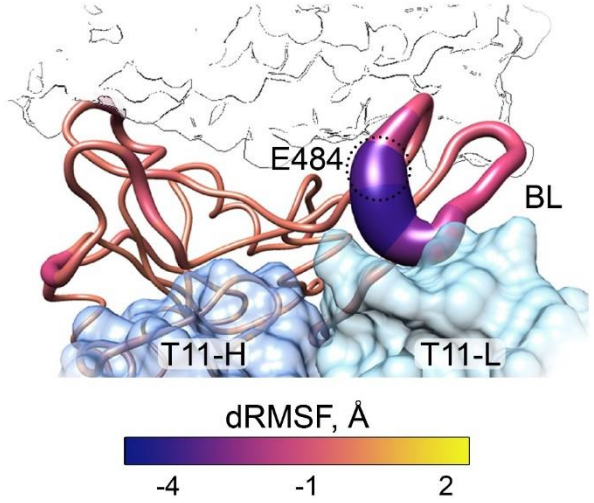


Terekhov, Smirnov et al., Nature Communications 2026

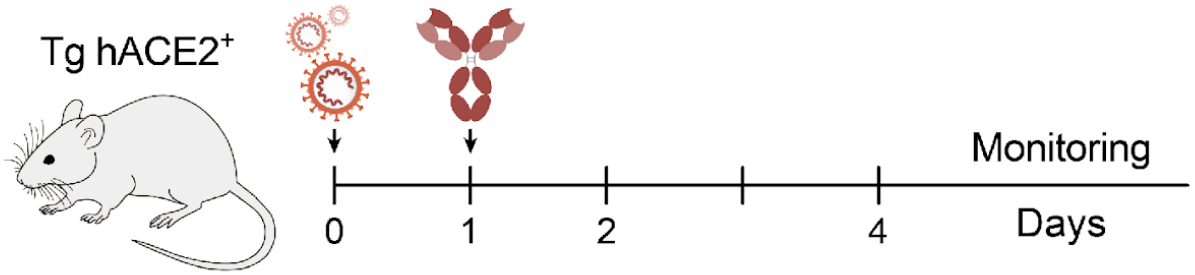
Identification of Novel Mechanisms of Virus Neutralization

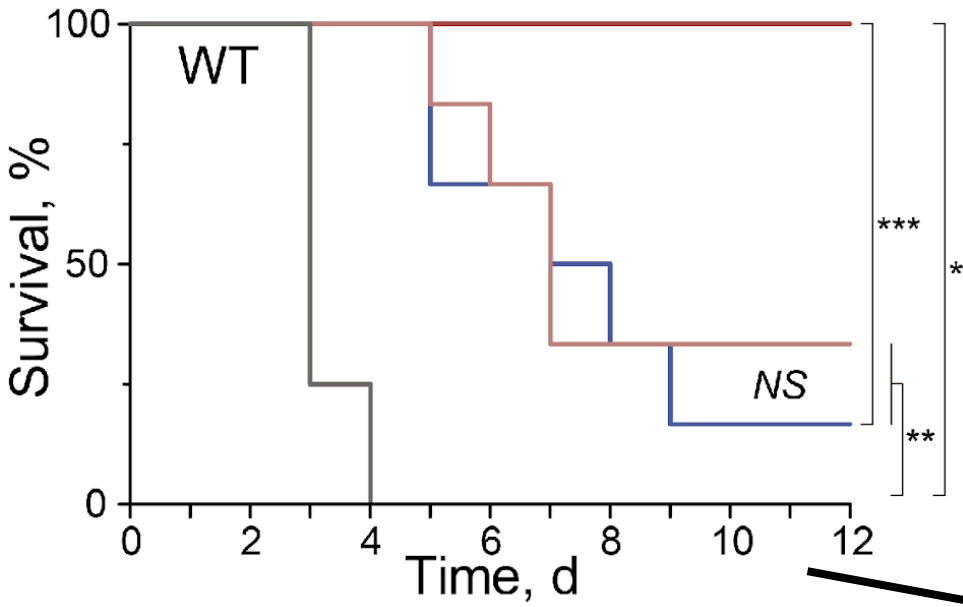




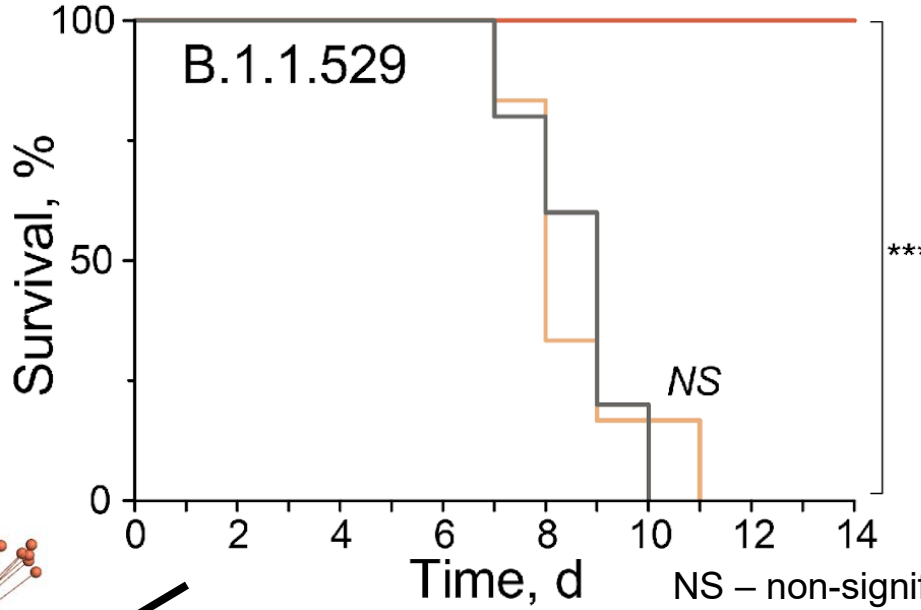


Development of virus-neutralizing antibodies



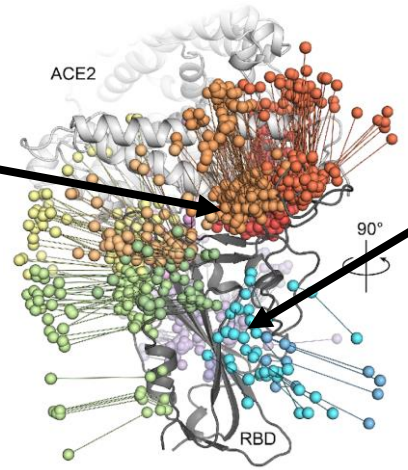


Therapy | Ab dose, mg/kg
D16 | 3
0.3
P4A1 | 10
PBS | –



Therapy | Ab dose, mg/kg
T11 | 3
0.3
PBS | –

NS – non-significant

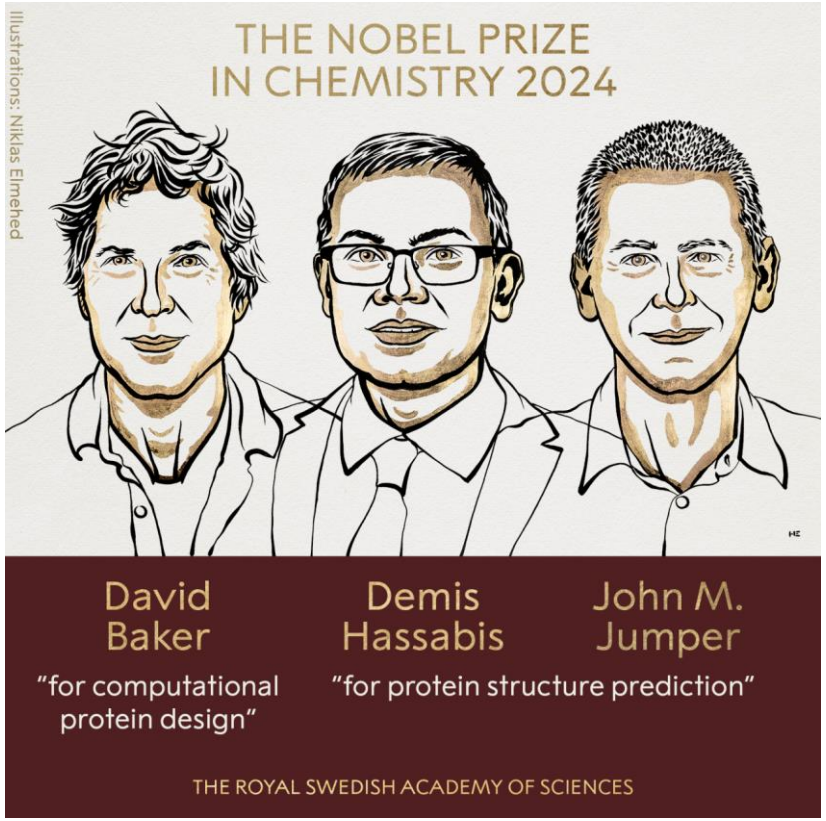


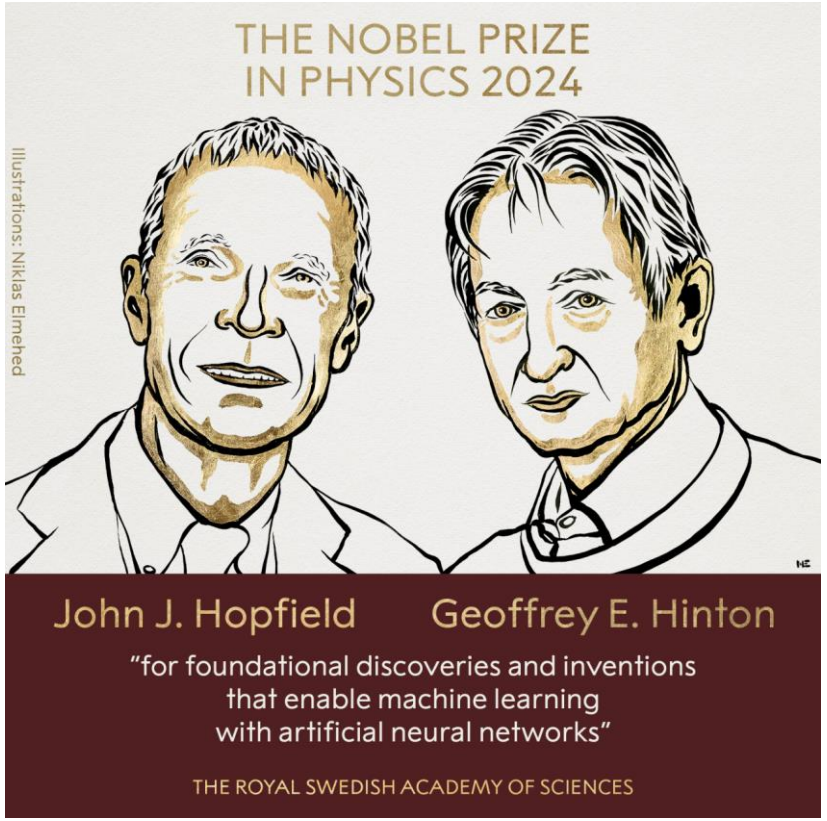
C-class

E22-class

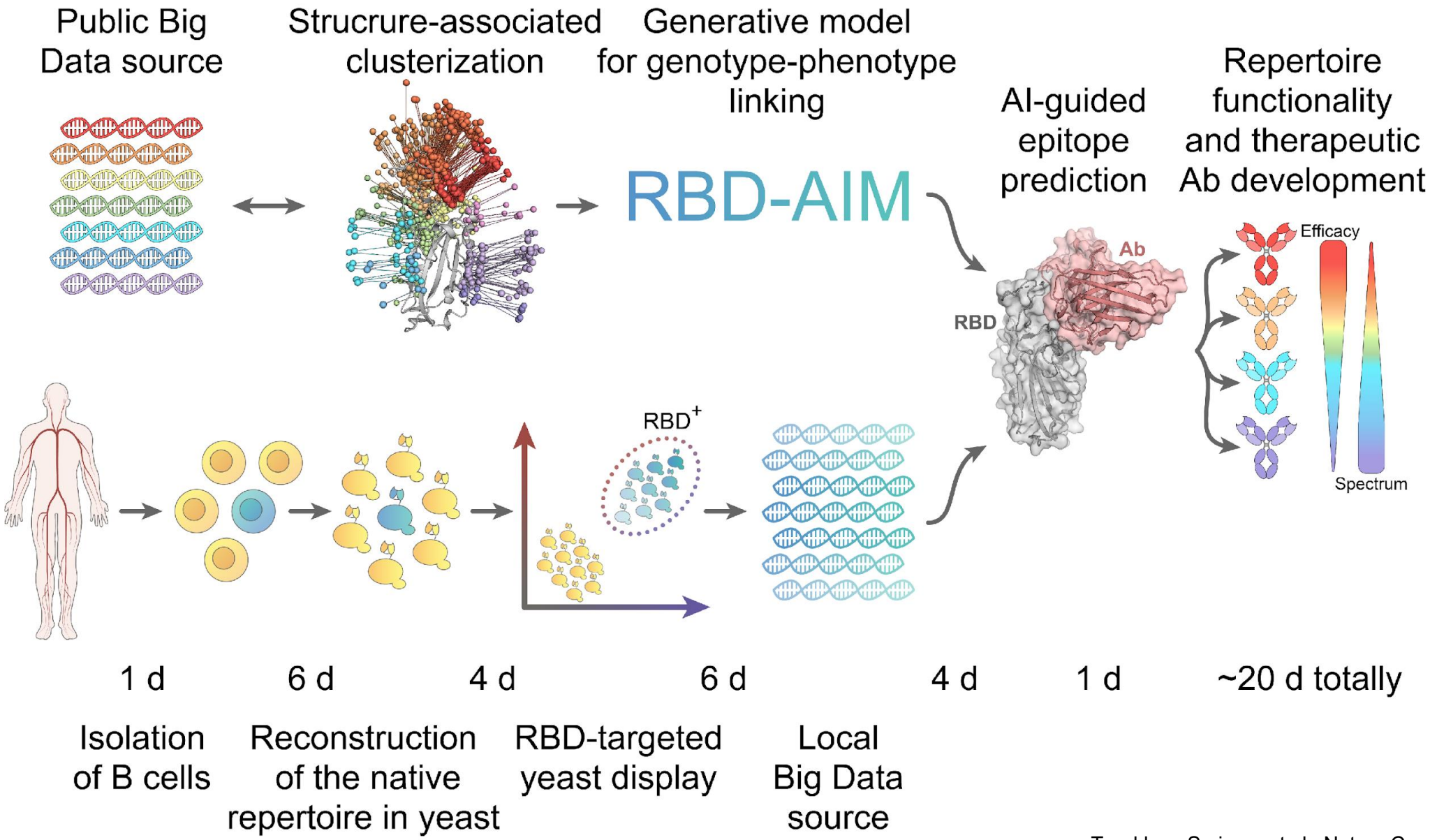
Terekhov, Smirnov et al., Nature Communications 2026

Predicting Antibody Antigen Determinants Using Artificial Intelligence





Searching for antibodies with specific properties using artificial intelligence



Terekhov, Smirnov et al., Nature Communications 2026

MESSAGE

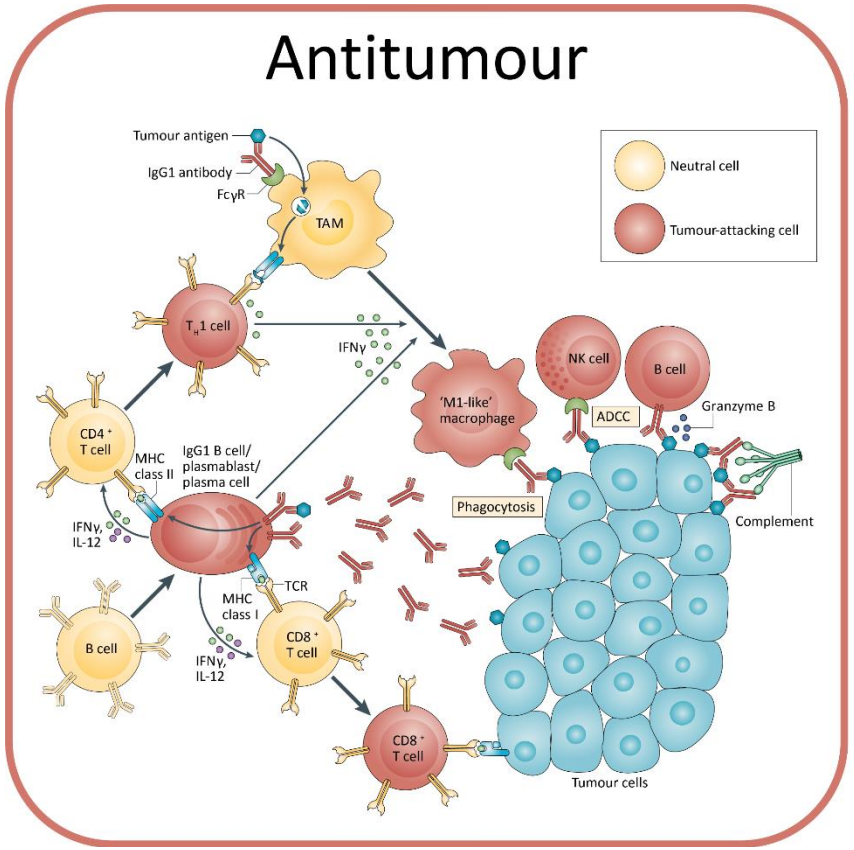
BioCat Lab

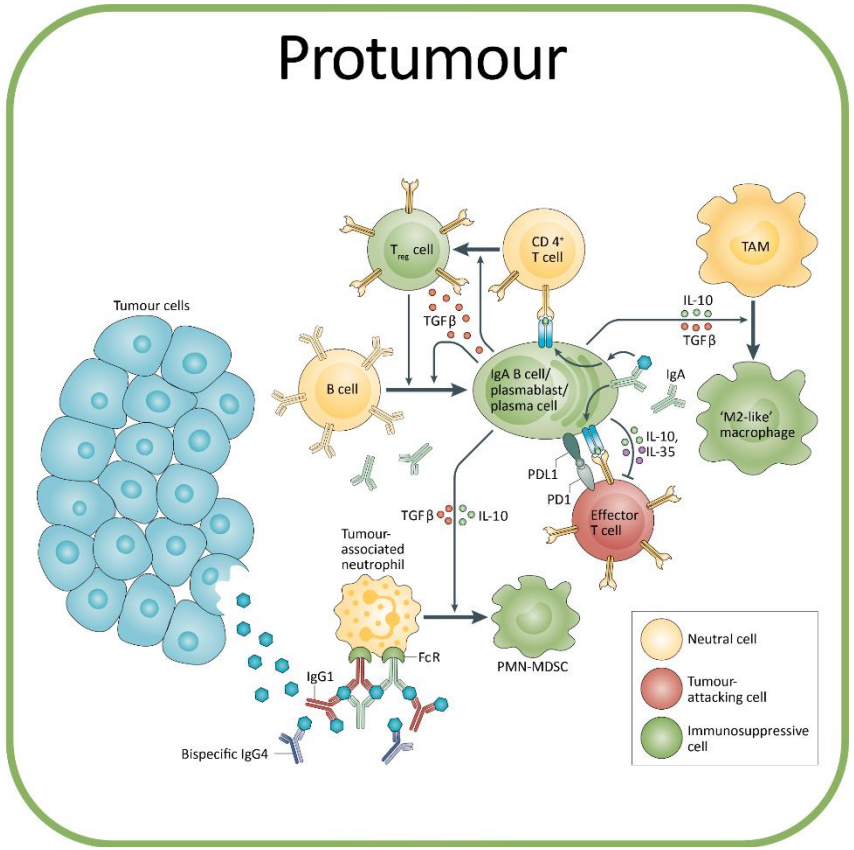
AI & ML Studies of Tumor infiltrating lymphocytes.

Discovery of novel antigens, epitopes for development of therapeutic antibodies and CARs

BioCat Lab

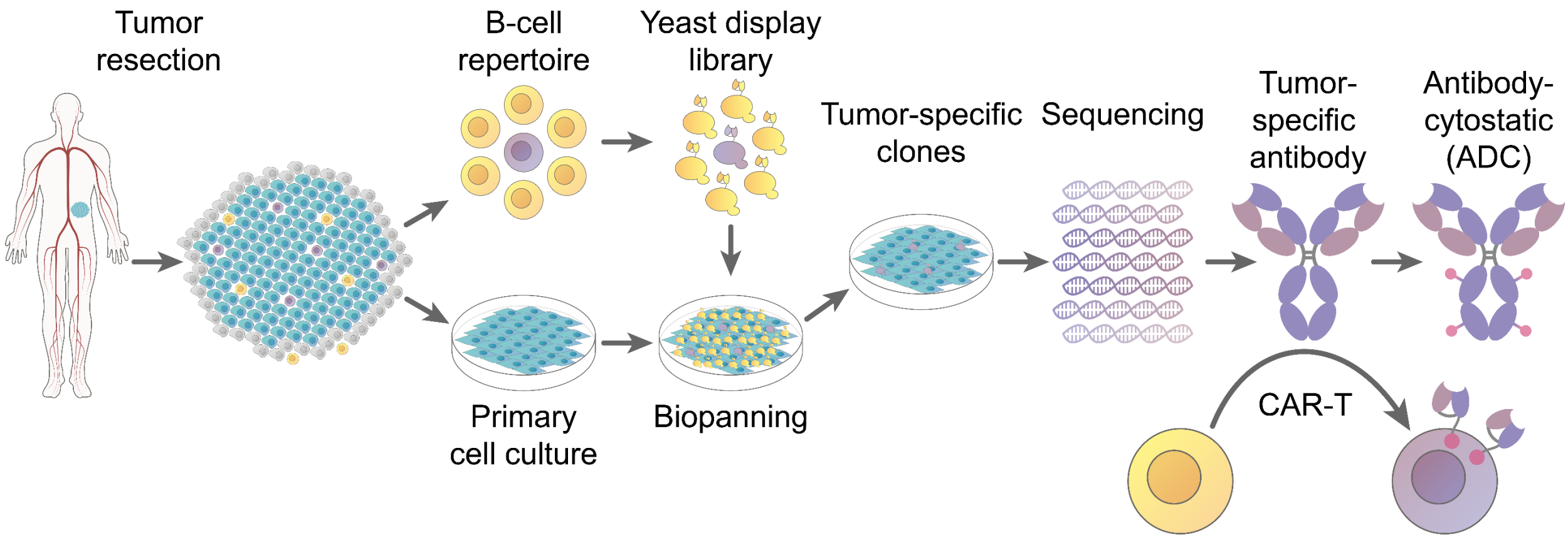
Tumor-infiltrating B lymphocytes



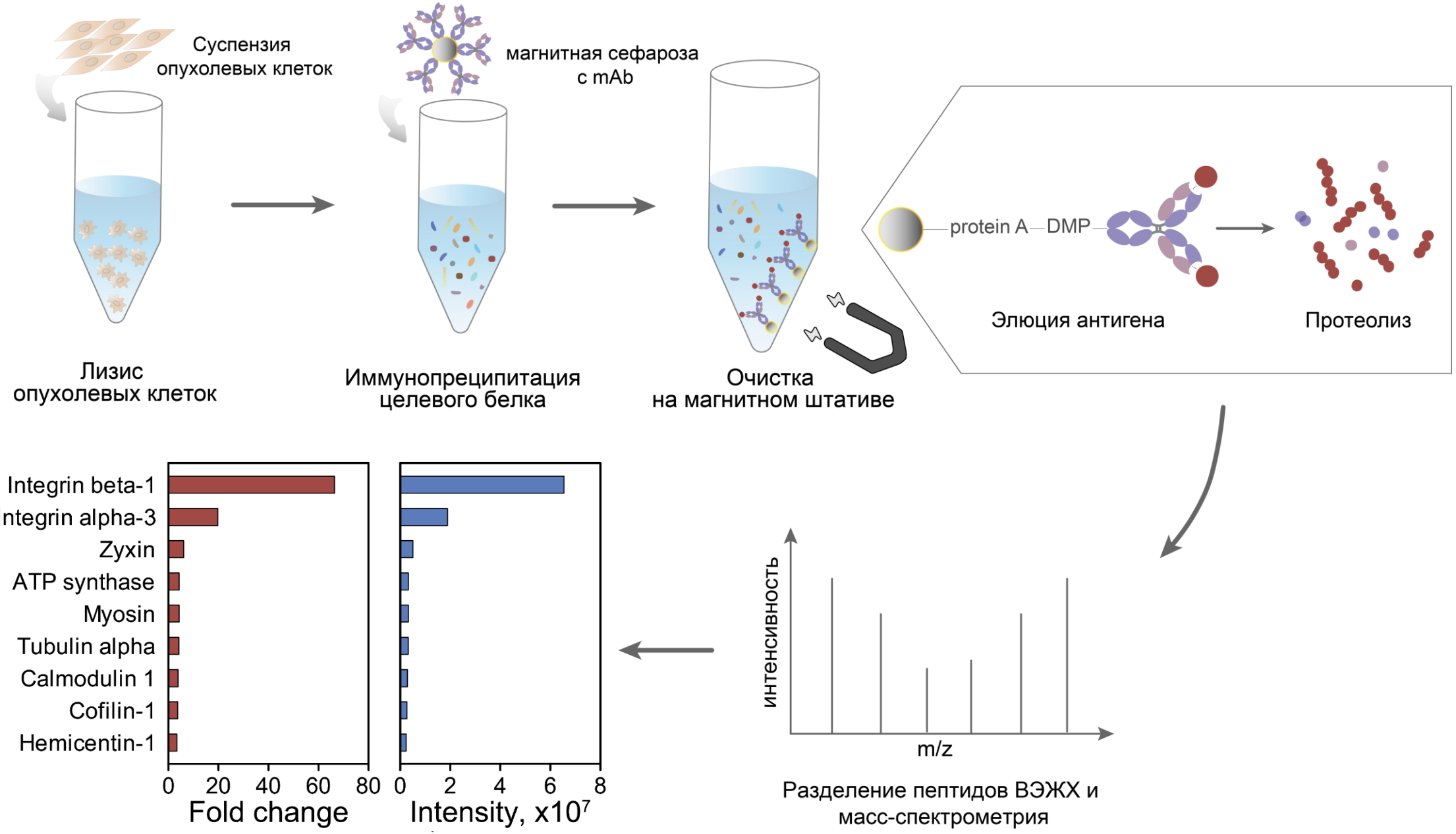


Sharonov et al., Nat Rev Immunol. 2020

Screening TIB repertoires for tumor-specific ABs



Identification of antigen of 3.4

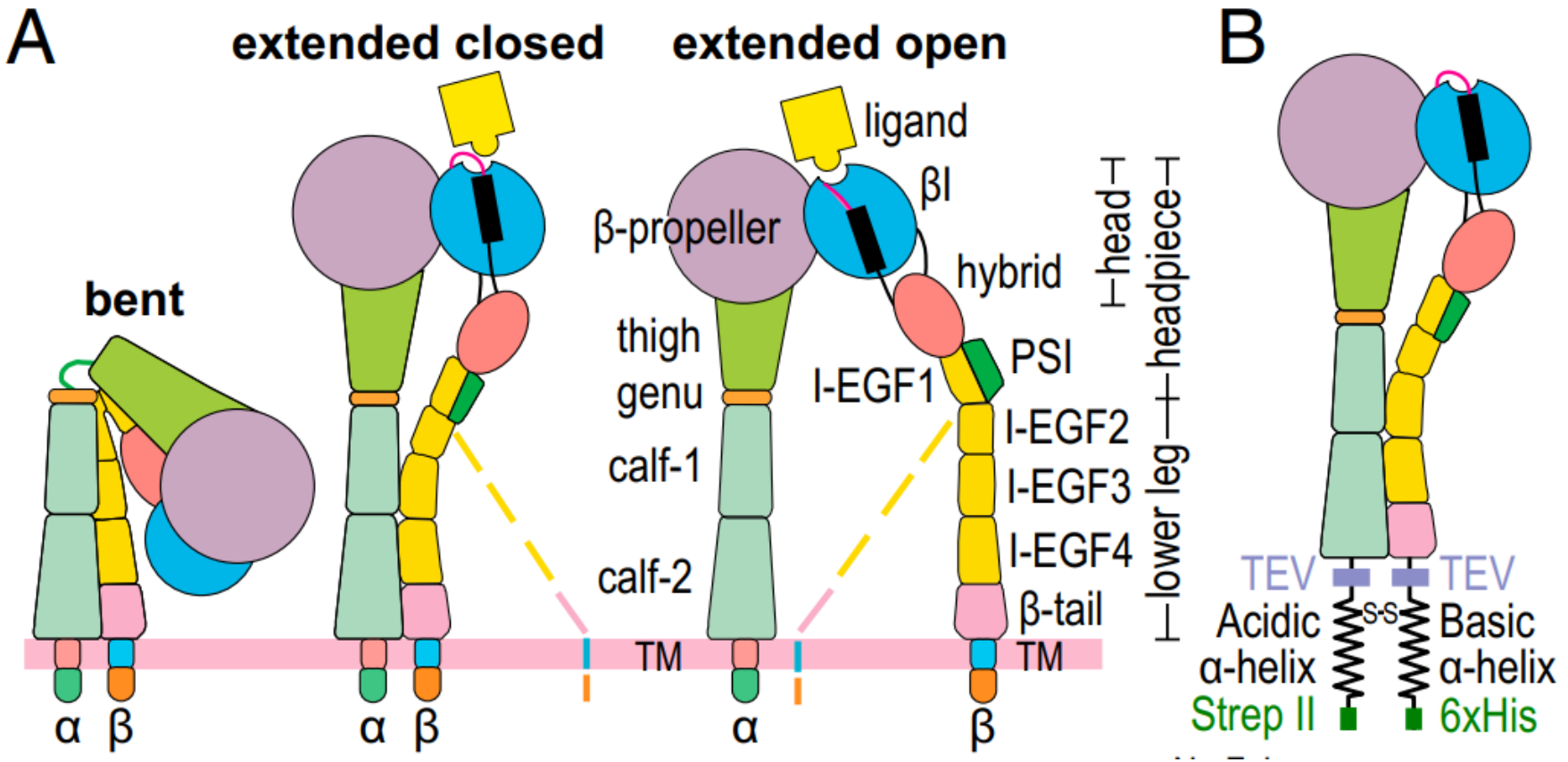


Relating conformation to function in integrin $\alpha_5\beta_1$

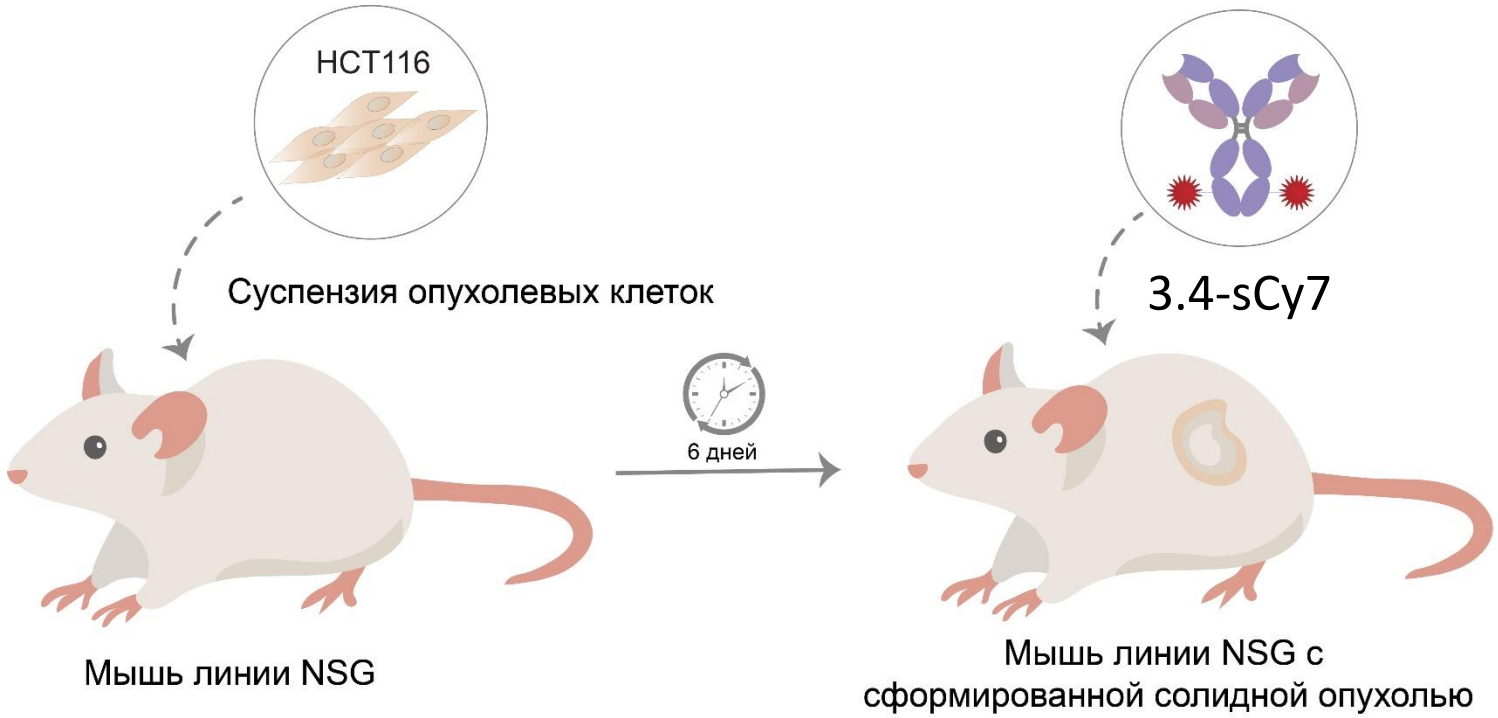
Yang Su[a,b,1], Wei Xia[a,b,1], Jing Li[a,b], Thomas Walz[c], Martin J. Humphries[d], Dietmar Vestweber[e], Carlos Cabañas[f], Chafen Lu[a,b], and Timothy A. Springer[a,b,2]

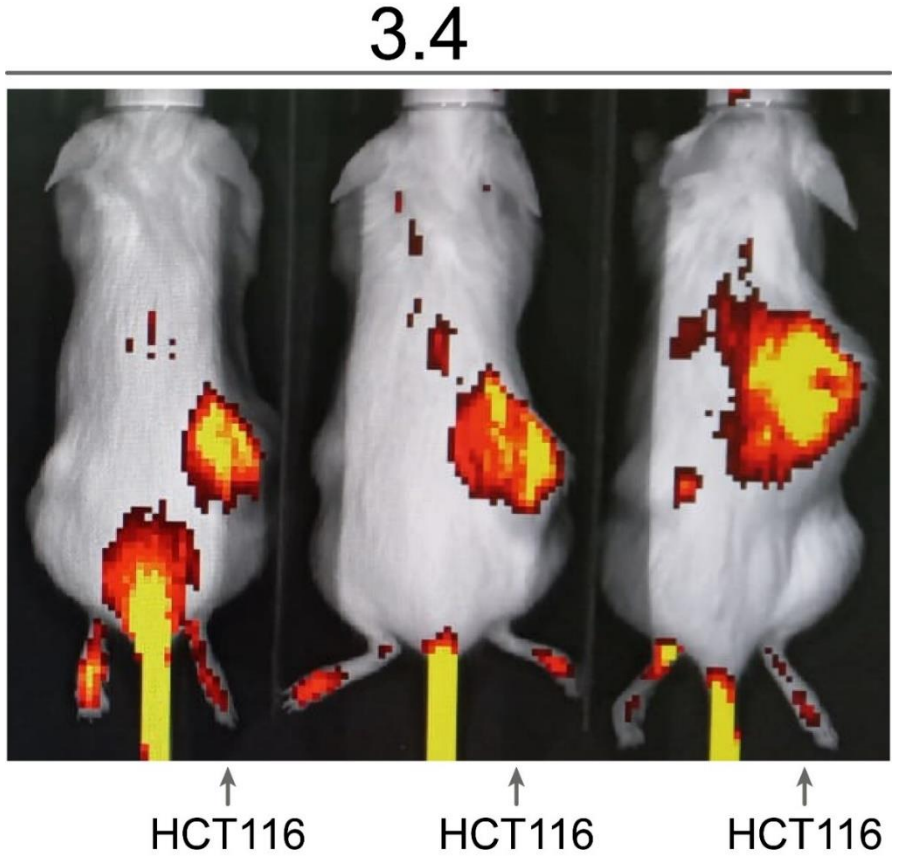
[a]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA 02115; [b]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115; [c]Rockefeller University, New York, NY 10065; [d]Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom; [e]Max Planck Institute of Molecular Biomedicine, 48149 Muenster, Germany; and [f]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), 28049 Madrid, Spain

Contributed by Timothy A. Springer, March 30, 2016 (sent for review March 21, 2016; reviewed by Michael L. Dustin and Kenneth M. Yamada)

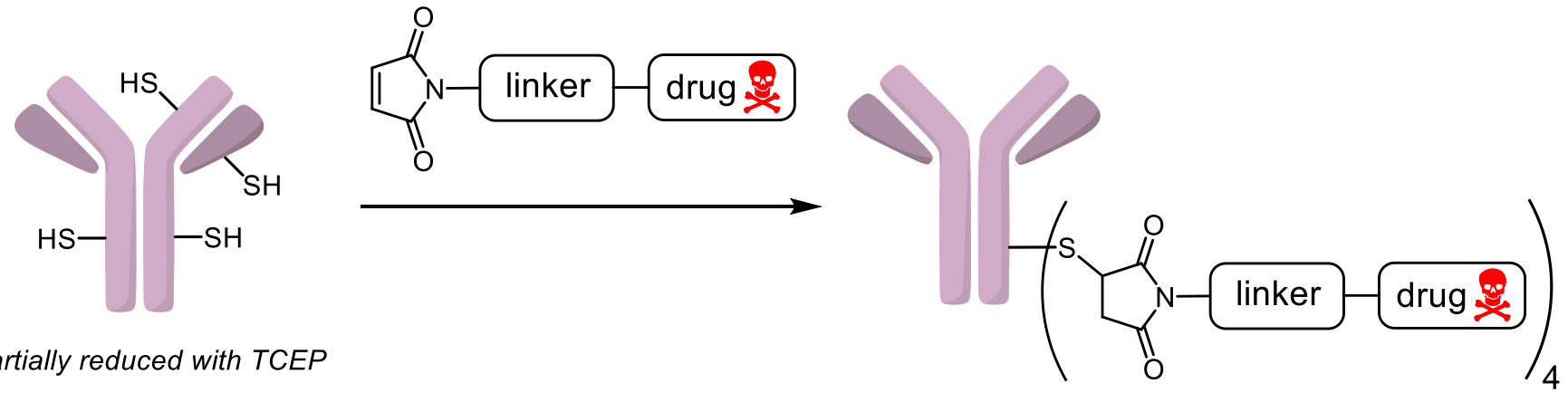


Human xenograft visualization using 3.4-sCy7 conjugates



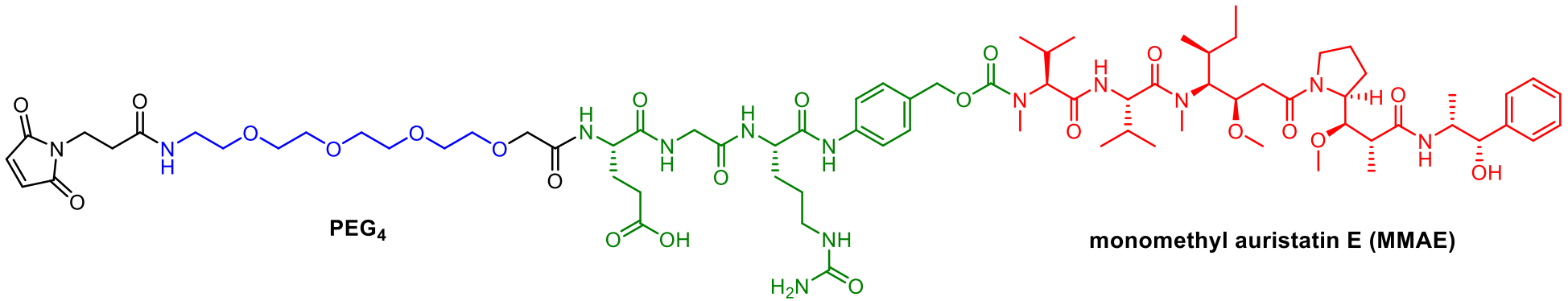


Standard maleimide-thiol ADC



partially reduced with TCEP

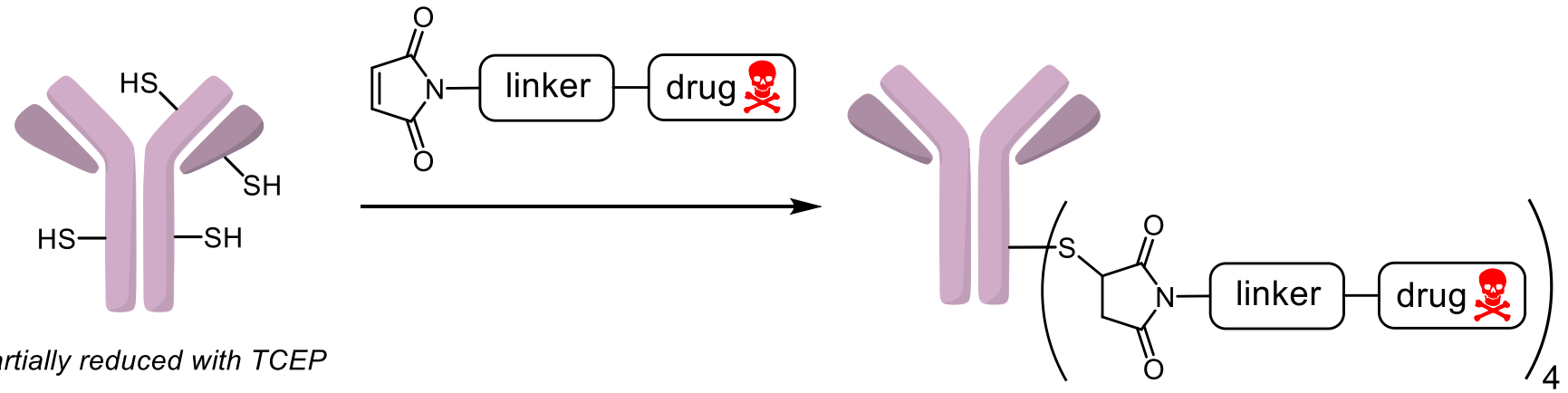
heterogeneous
optimal average drug-to-antibody ratio (DAR) ~ 4



GluGlyCitPAB
stable in mouse plasma, resistant to neutrophil proteases
(unlike the classic ValCitPAB)

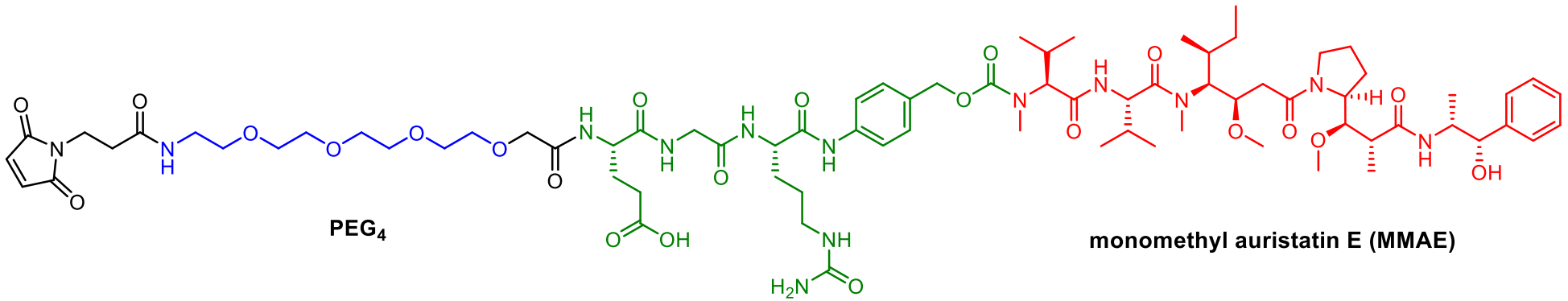
Mol. Cancer Ther. **2022**, *21*, 1449.

Standard maleimide-thiol ADC



partially reduced with TCEP

heterogeneous
optimal average drug-to-antibody ratio (DAR) ~ 4



GluGlyCitPAB
stable in mouse plasma, resistant to neutrophil proteases
(unlike the classic ValCitPAB)

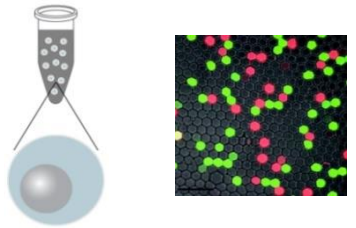
Mol. Cancer Ther. **2022**, *21*, 1449.

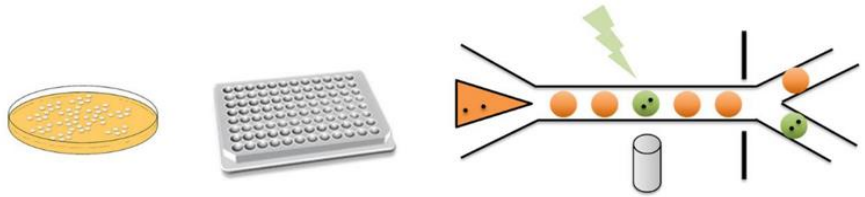
Technology for highly efficient screening of functionally active single cells

Стандартные методы



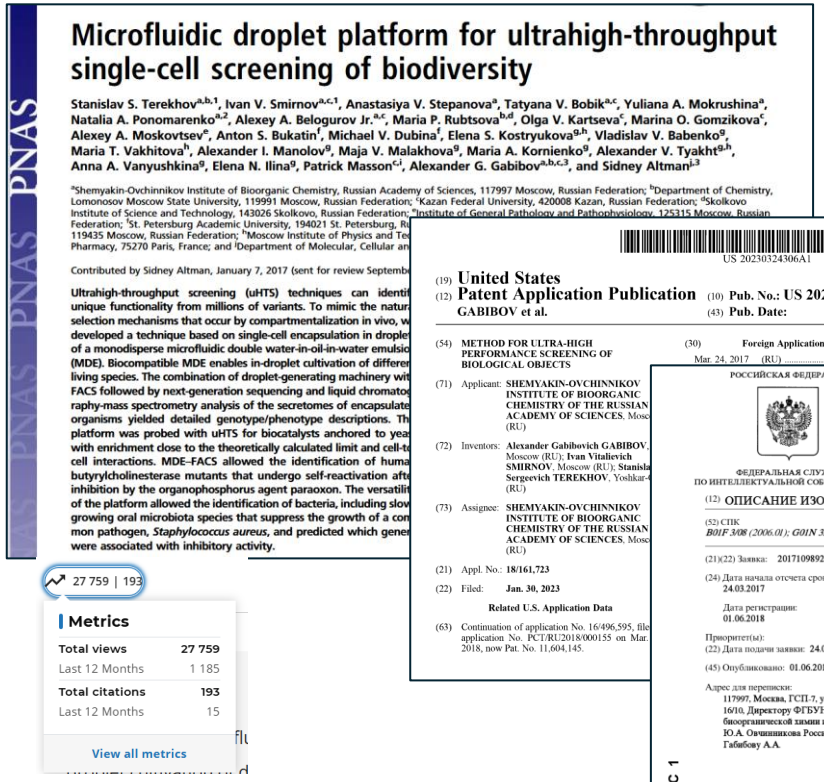
Эмульсионные методы





	Agar plate	Microtiter plate	FACS	Droplet microfluidics
Type of sample	Solid; Individual colonies;	Liquid; Sets of cell variants;	Liquid; Sets of cell variants in droplets or Individual cells;	
Library size	10^2 - 10^5	10^4	10^8	10^9
Advantages	Simple; Visual detection by direct phenotypical detection, heterologous complementation and induced gene expression;	Compatible with multiple analytical techniques; High sensitivity; Large dynamic range; Compatible with robotic workstations;	Pico- to femtolitre scale; Low sample and reagent consumption; High-throughput; Continuous operation; Can perform automated sorting;	Compatible with multiple analytical techniques and sensor types; Can perform automated directed evolution;
Disadvantages	Low dynamic range; Labour intensive; Usually requires another method (e.g. microtiter plates) for quantification;	Labour intensive; Works with lysed cells; Small library size; Might require quantification with time-consuming analytics (e.g. GC, MS);	Technnically challenging Limited to fluorescent detection or cell characteristics measured by light scattering;	Requires optimization of emulsions or polyelectrolyte shells used;

Gantz et al., Faraday Discuss., 2024



Microfluidic droplet platform for ultrahigh-throughput single-cell screening of biodiversity

Stanislav S. Terekhov[a,b,1], Ivan V. Smirnov[a,c,1], Anastasiya V. Stepanova[a], Tatyana V. Bobik[a,c], Yuliana A. Mokrushina[a], Natalia A. Ponomarenko[a,2], Alexey A. Belogurov Jr.[a,c], Maria P. Rubtsova[b,d], Olga V. Kartseva[c], Marina O. Gomzikova[c], Alexey A. Moskovtsev[e], Anton S. Bukatin[f], Michael V. Dubina[f], Elena S. Kostryukova[g,h], Vladislav V. Babenko[g], Maria T. Vakhitova[h], Alexander I. Manolov[g], Maja V. Malakhova[g], Maria A. Kornienko[g], Alexander V. Tyakht[g,h], Anna A. Vanyushkina[g], Elena N. Ilina[g], Patrick Masson[c,i], Alexander G. Gabibov[a,b,c,3], and Sidney Altman[j,3]

[a]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russian Federation; [b]Department of Chemistry, Lomonosov Moscow State University, 119991 Moscow, Russian Federation; [c]Kazan Federal University, 420008 Kazan, Russian Federation; [d]Skolkovo Institute of Science and Technology, 143026 Skolkovo, Russian Federation; [e]Institute of General Pathology and Pathophysiology, 125315 Moscow, Russian Federation; [f]St. Petersburg Academic University, 194021 St. Petersburg, Ru… 119435 Moscow, Russian Federation; [h]Moscow Institute of Physics and Te… Pharmacy, 75270 Paris, France; and [i]Department of Molecular, Cellular an…

Contributed by Sidney Altman, January 7, 2017 (sent for review Septembe…

Ultrahigh-throughput screening (uHTS) techniques can identif… unique functionality from millions of variants. To mimic the natur… selection mechanisms that occur by compartmentalization in vivo, w… developed a technique based on single-cell encapsulation in droplet… of a monodisperse microfluidic double water-in-oil-in-water emulsio… (MDE). Biocompatible MDE enables in-droplet cultivation of differen… living species. The combination of droplet-generating machinery wit… FACS followed by next-generation sequencing and liquid chromatog… raphy-mass spectrometry analysis of the secretomes of encapsulate… organisms yielded detailed genotype/phenotype descriptions. Th… platform was probed with uHTS for biocatalysts anchored to yea… with enrichment close to the theoretically calculated limit and cell-t… cell interactions. MDE–FACS allowed the identification of huma… butyrylcholinesterase mutants that undergo self-reactivation afte… inhibition by the organophosphorus agent paraoxon. The versatilit… of the platform allowed the identification of bacteria, including slow… growing oral microbiota species that suppress the growth of a com… mon pathogen, *Staphylococcus aureus*, and predicted which genes… were associated with inhibitory activity.

PNAS

27 759 | 193

Metrics

Total views	27 759
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Total citations	193
Last 12 Months	15

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US 20230324306A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2023/0324306 A1
GABIBOV et al. (43) Pub. Date: Oct. 12, 2023

(54) METHOD FOR ULTRA-HIGH PERFORMANCE SCREENING OF BIOLOGICAL OBJECTS

(71) Applicant: SHEMYAKIN-OVCHINNIKOV INSTITUTE OF BIOORGANIC CHEMISTRY OF THE RUSSIAN ACADEMY OF SCIENCES, Mosc… (RU)

(72) Inventors: Alexander Gabibovich GABIBOV, Moscow (RU); Ivan Vitalievich SMIRNOV, Moscow (RU); Stanisla… Sergeevich TEREKHOV, Yoshkar-… (RU)

(73) Assignee: SHEMYAKIN-OVCHINNIKOV INSTITUTE OF BIOORGANIC CHEMISTRY OF THE RUSSIAN ACADEMY OF SCIENCES, Mosc… (RU)

(21) Appl. No.: 18/161,723

(22) Filed: Jan. 30, 2023

Related U.S. Application Data

(63) Continuation of application No. 16/496,595, file… application No. PCT/RU2018/000155 on Mar. … 2018, now Pat. No. 11,604,145.

(30) Foreign Application Priority Data

Mar. 24, 2017 (RU) 2017109892

РОССИЙСКАЯ ФЕДЕРАЦИЯ

(19) RU (11) 2 656 216 (13) C1

(51) МПК
B01F 3/08 (2006.01)
G01N 33/48 (2006.01)
C12Q 1/02 (2006.01)
C12Q 1/04 (2006.01)

ФЕДЕРАЛЬНАЯ СЛУЖБА ПО ИНТЕЛЛЕКТУАЛЬНОЙ СОБСТВЕННОСТИ

(12) ОПИСАНИЕ ИЗОБРЕТЕНИЯ К ПАТЕНТУ

(52) СПК
B01F 3/08 (2006.01); G01N 33/48 (2006.01); C12Q 1/02 (2006.01)

(21)(22) Заявка: 2017109892, 24.03.2017

(24) Дата начала отсчета срока действия патента: 24.03.2017

Дата регистрации: 01.06.2018

Приоритет(ы):
(22) Дата подачи заявки: 24.03.2017

(45) Опубликовано: 01.06.2018 Бюл. № 16

Адрес для переписки: 117997, Москва, ГСП-7, ул. Миклухо-Маклая, 16/10, Директору ФГБУН Институт биоорганической химии им. М.М. Шемякина и Ю.А. Овчинникова Российской академии наук Габибову А.А.

(72) Автор(ы): Габибов Александр Габибович (RU), Смирнов Иван Витальевич (RU), Терехов Станислав Сергеевич (RU)

(73) Патентообладатель(и): Федеральное государственное бюджетное учреждение науки Институт биоорганической химии им. М.М. Шемякина и Ю.А. Овчинникова Российской академии наук (RU)

(56) Список документов, цитированных в отчете о поиске: YAN J., BAUER W.C. et al., Monodisperse Water-in-Oil-in-Water (W/O/W) Double Emulsion Droplets as Uniform Compartments for High-Throughput Analysis via Flow Cytometry // Micromachines, 2013, 4, стр.402-413. WO 2015/103190 A1, 09.07.2015. US 2014/0179544 A1, 26.06.2014. US 9513207 B2, 06.12.2016. JPH 07313858 A, 05.12.1995. ROMANOWSKY M.B. et al., (см. прод.)

(54) Способ ультравысокопроизводительного скрининга клеток или микроорганизмов и средство для ультравысокопроизводительного скрининга клеток или микроорганизмов

RU 2656216 C1



MESSAGE

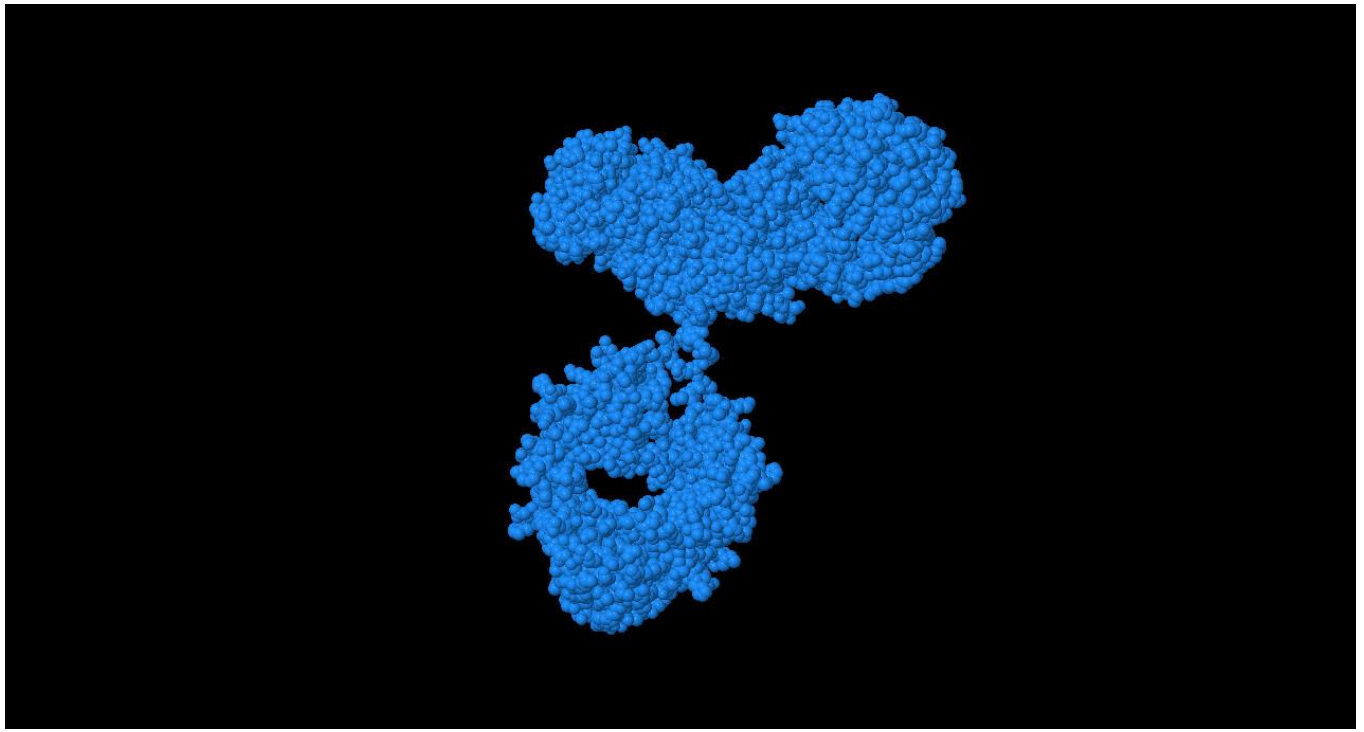
What antibody may do?

-To bind antigen/pathogen

-To destroy

Catalytic antibodies

BioCat Lab

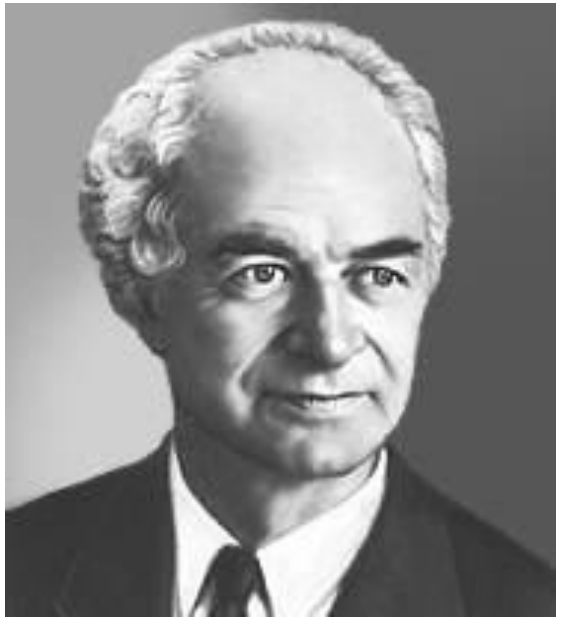


AntiBodies

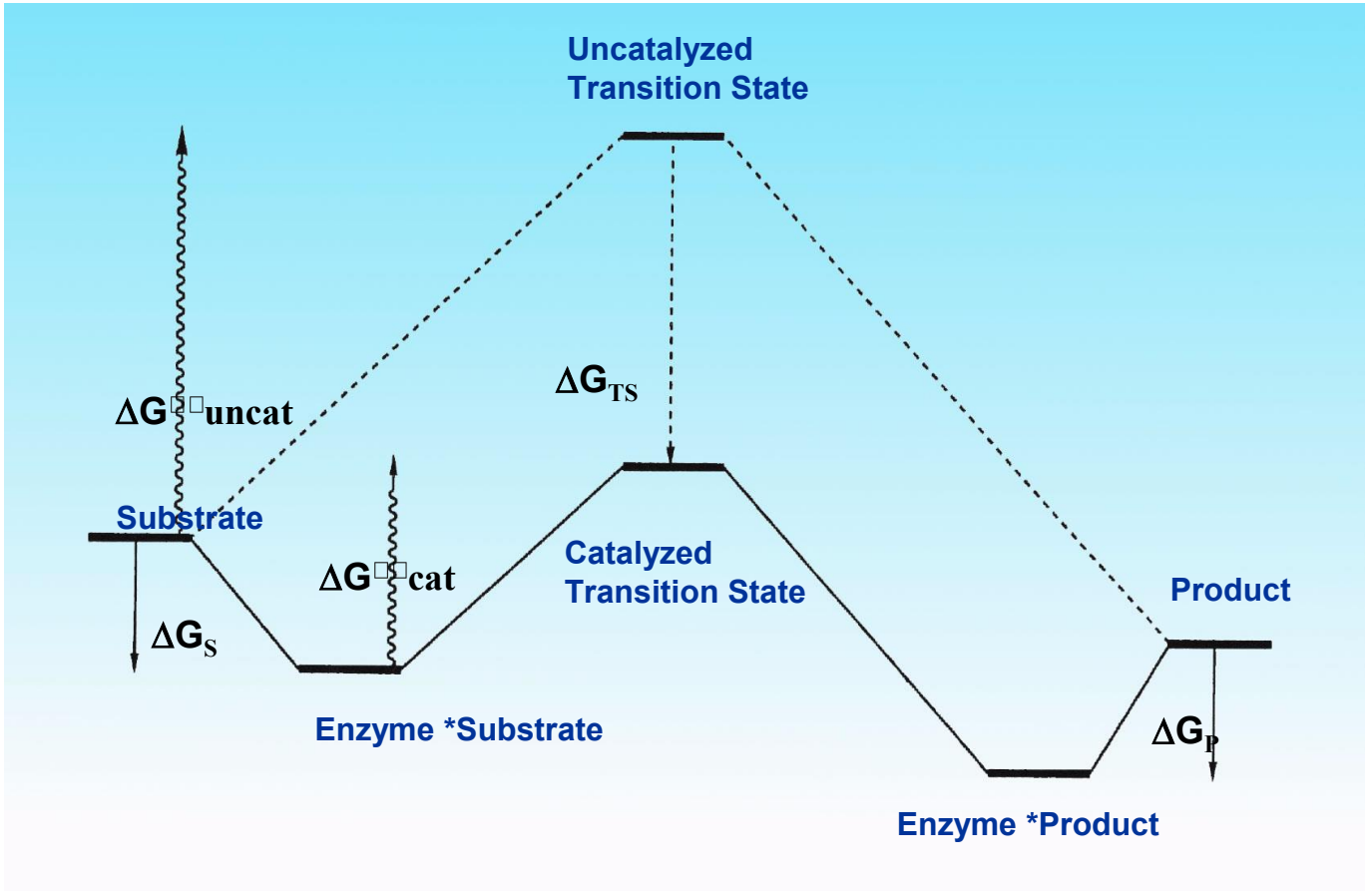
ENzymes

ABzymes

Transition state theory



Linus Pauling
Nobel prize 1954, 1962



DG= uncat-DG‡ cat = DG_{TS}-DG_S

or $k_{cat}/k_{uncat} = K_M/K_i$

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 254–257, January 1995
Biochemistry

Cleavage of supercoiled plasmid DNA by autoantibody Fab fragment: Application of the flow linear dichroism technique

(catalytic antibodies/nucleases/autoimmunity)

GENNADY V. GOLOLOBOV, ELENA A. CHERNOVA, DMITRY V. SCHOUROV, IVAN V. SMIRNOV, IRINA A. KUDELINA, AND ALEXANDER G. GABIBOV*

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Street 32, 117984 Moscow, Russia

Communicated by William P. Jencks, Brandeis University, Waltham, MA, October 3, 1994 (received for review May 24, 1994)

ABSTRACT **A highly effective method consisting of two affinity chromatography steps and ion-exchange and gel-filtration chromatography steps was developed for purification of autoantibodies from human sera with DNA-hydrolyzing activity. Antibody Fab fragment, which had been purified 130-fold, was shown to catalyze plasmid DNA cleavage. The flow linear dichroism technique was used for quantitative and qualitative studying of supercoiled plasmid DNA cleavage by these autoantibodies in comparison with DNase I and *Eco*RI restriction endonuclease. The DNA autoantibody Fab fragment was shown to hydrolyze plasmid DNA by Mg^{2+}-dependent single-strand multiple nicking of the substrate. Kinetic properties of the DNA autoantibody Fab fragment were evaluated from the flow linear dichroism and agarose gel electrophoresis data and revealed a high affinity (K_m^{obs} = 43 nM) and considerable catalytic efficiency (k_{cat}^{app}/K_m^{obs} = 0.32 $min^{-1} \cdot nM^{-1}$) of the reaction.**

Antibodies able to catalyze a variety of chemical transformations were developed in the last decade, using the strategy of raising antibodies to haptens that resemble the transition state of reactions, as was suggested by Jencks (1) (for review see refs. 2 and 3). An increasing number of autoantibodies with catalytic activity toward natural substrates (4–6), as well as anti-idiotypic antibodies exhibiting a catalytic function (7), have also been described recently. To date, however, the antibody-mediated catalysis is usually characterized by relatively low rate enhancements, indicating the existence of at least two very important problems. The first one, especially important in the case of naturally occurring antibodies, is the purity of the antibody preparation used for the assay, since even a trace enzyme contaminant may mimic a high antibody turnover number (8). The second problem is the development of sensitive, continuous, and accurate methods for detection and monitoring of such low activity (9, 10). Here we describe a reproducible method of purification of DNA-hydrolyzing autoantibodies from human sera. We also demonstrate the advantages of the flow linear dichroism (FLD) technique for quantitative and qualitative characterization of the interaction of these catalytic antibodies with supercoiled (sc) plasmid DNA.

EXPERIMENTAL PROCEDURES

Chemicals. All chemicals were from Sigma and Merck. Enzymes were obtained from Boehringer Mannheim.

Plasmid DNA. Plasmid pUC19 was isolated as described elsewhere (11). More than 95% of the isolated plasmid DNA was in the sc form, judging by 1% agarose gel electrophoresis (AGE).

To estimate the FLD magnitudes for different forms of plasmid DNA, the standard samples of relaxed circular and linear forms were obtained by incubation of sc plasmid DNA with DNase I and *Eco*RI, as described below, and further purification of products by centrifugation in a linear CsCl density gradient at 40,000 rpm at 4°C for 36 hr in a Beckman SW41 rotor. The purity of standard samples was checked by 1% AGE.

Glutaraldehyde-Modified DNA-Cellulose. This material was obtained as described in ref. 12. Briefly, denatured DNA solution (10 mg/ml) was heated to 96°C and AE-cellulose (Serva) was added. The mixture was stirred for 30 min at 96°C and glutaraldehyde was added to 0.5% concentration; the reaction mixture was incubated for 15 min, cooled, and washed with 1 M KCl on a glass filter until no absorption at 260 nm could be detected. The preparation was kindly provided by G. A. Nevinsky (Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences).

Patient Analysis. Patients were analyzed at the Institute of Rheumatology, Russian Academy of Medical Sciences, Moscow, by T. B. Prokaeva and Z. S. Alekberova. The diagnosis was confirmed and its reliability was checked according to the criteria developed by the American Rheumatoid Association. The serum of 1 of 10 patients with systemic lupus erythematosus was selected for further investigation as the most active in DNA hydrolysis, judging by AGE (see below).

Antibody Isolation. Antibodies in 5 ml of serum were precipitated twice with 50% saturated ammonium sulfate; this was followed by chromatography on a Pharmacia HR 5/5 staphylococcal protein A fast-performance liquid chromatography (FPLC) column, as described in ref. 13 (p. 310).

Antibody Purification. Samples isolated with protein A were dialyzed twice for 4 hr against 500 vol of buffer A (20 mM Tris·HCl, pH 9.0) at 4°C and applied to a Pharmacia HR 5/5 Mono Q FPLC column that was equilibrated with buffer A. The column was washed with buffer A and then developed with a linear NaCl gradient (0–1 M) in the same buffer. Samples were dialyzed twice for 4 hr against 500 vol of buffer B (20 mM Mops, pH 7.0/50 mM NaCl) at 4°C and loaded onto a glutaraldehyde-modified DNA-cellulose affinity column, which had been equilibrated with buffer B. The column was washed with the same buffer and then antibodies were eluted with a linear gradient of KCl (0–1 M) in buffer B. Collected fractions were dialyzed twice for 4 hr against 500 vol of buffer B at 4°C, and DNA-hydrolyzing activity toward sc plasmid DNA was measured by using AGE (see below). Active fractions were pooled and concentrated by ultrafiltration on Centricon-10 concentrators (Amicon), and 0.4-ml aliquots were loaded onto a Pharmacia HR 10/30 Superose-12 FPLC column that had been equilibrated with buffer C (1 M acetic acid/100 mM NaCl). Separation was performed in buffer C ("acid shock" conditions) at a flow rate of 0.5 ml/min. Fractions, corresponding by their molecular weight to IgG, were collected, immediately neutralized with 3 M Tris·HCl

Abbreviations: AGE, agarose gel electrophoresis; FLD, flow linear dichroism; FPLC, fast-performance liquid chromatography; LD, linear dichroism; sc, supercoiled.

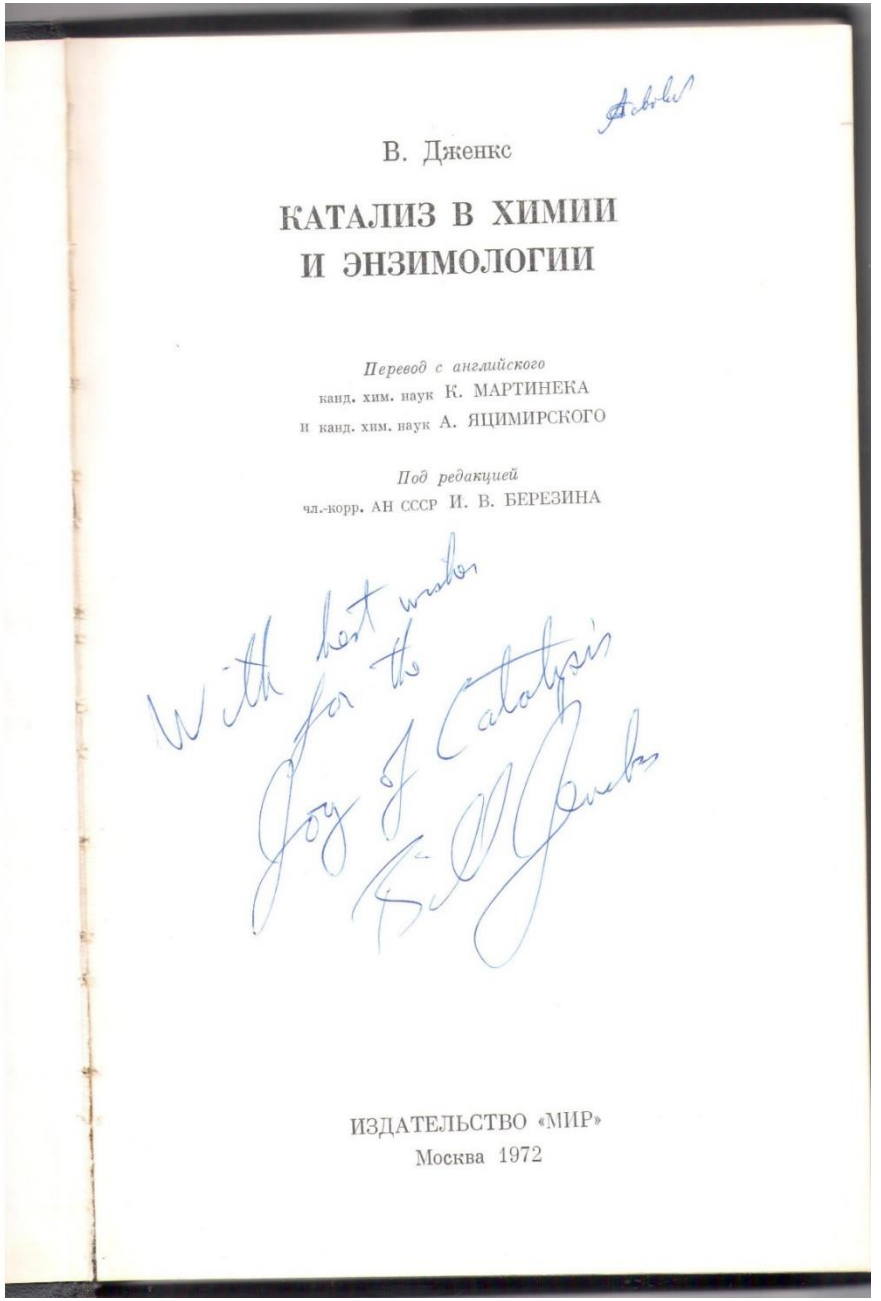
*To whom reprint requests should be addressed.

Downloaded from https://www.pnas.org by 195.178.197.57 on June 1, 2023 from IP address 195.178.197.57.



William P. Jencks

1927 —2007





Michael Blackburn and Richard Lerner
Saint-Petersburg, 2013
38 FEBS Congres

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2145–2149, March 1995
Immunology

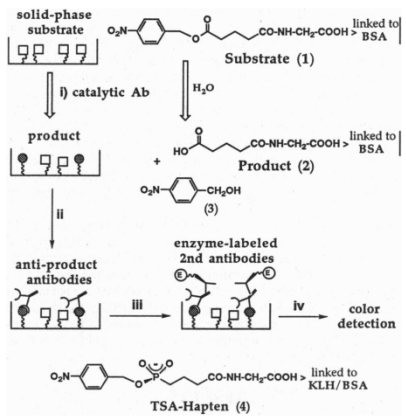
Unexpectedly high occurrence of catalytic antibodies in MRL/*lpr* and SJL mice immunized with a transition-state analog: Is there a linkage to autoimmunity?

DAN S. TAWFIK*†, RACHEL CHAP†, BERNARD S. GREEN†, MICHAEL SELA*, AND ZELIG ESHHAR*‡

*Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100 Israel; and †Department of Pharmaceutical Chemistry, The Hebrew University, Faculty of Medicine, P.O. Box 12065, Jerusalem 91120, Israel

Contributed by Michael Sela, November 14, 1994

ABSTRACT **Upon testing the ability of several strains of mice to elicit esterolytic antibodies after immunization with a *p*-nitrobenzyl phosphonate hapten, we have found that the occurrence of catalytic antibodies in SJL and MRL/*lpr* autoimmune mice is dramatically higher than in normal mouse strains (e.g., the wild-type MRL/++ or BALB/c). Fewer than 10 catalytic clones are usually obtained from a single fusion of lymphocytes taken from normal mice, whereas several hundred catalytic clones are obtained in SJL or MRL/*lpr* mice. Differences in the numbers of hapten-binding clones do not account for the high occurrences of catalytic clones in these strains. This phenomenon prevailed in the early responses; in both SJL and MRL/*lpr* mice a significant decline in the appearance of catalytic clones was observed after multiple immunizations. Esterolytic antibodies were not found in MRL/*lpr* mice immunized with haptens that do not mimic the transition state for the hydrolysis of the ester substrate (e.g., with a substrate analog). The catalytic antibodies manifest high specificity to the antigen and variability in their binding and catalytic properties. The use of autoimmunity-prone mice may greatly expand the repertoire of catalytic clones elicited against a transition-state analog hapten. More intriguing is the possible linkage between autoimmunity and the appearance of catalytic antibodies. These results suggest that there is normally a selection against the expression of certain variable genes encoding antibodies with catalytic activity.**



esterase and amidase

drolyzing Autoantibodies

Shuster, Gennady V. Gololobov, ashuk, Anastasiya E. Bogomolova, irnov, Alexander G. Gabibov*

tected in the sera of patients with various autoimmune be a property of autoantibodies. The DNA hydrolyzing affinity and high-performance liquid chromatography, cor- lobulin M (IgM) and IgG and had a positive response to NA hydrolyzing autoantibodies were stable to acid shock pattern that was different from that of deoxyribonuclease

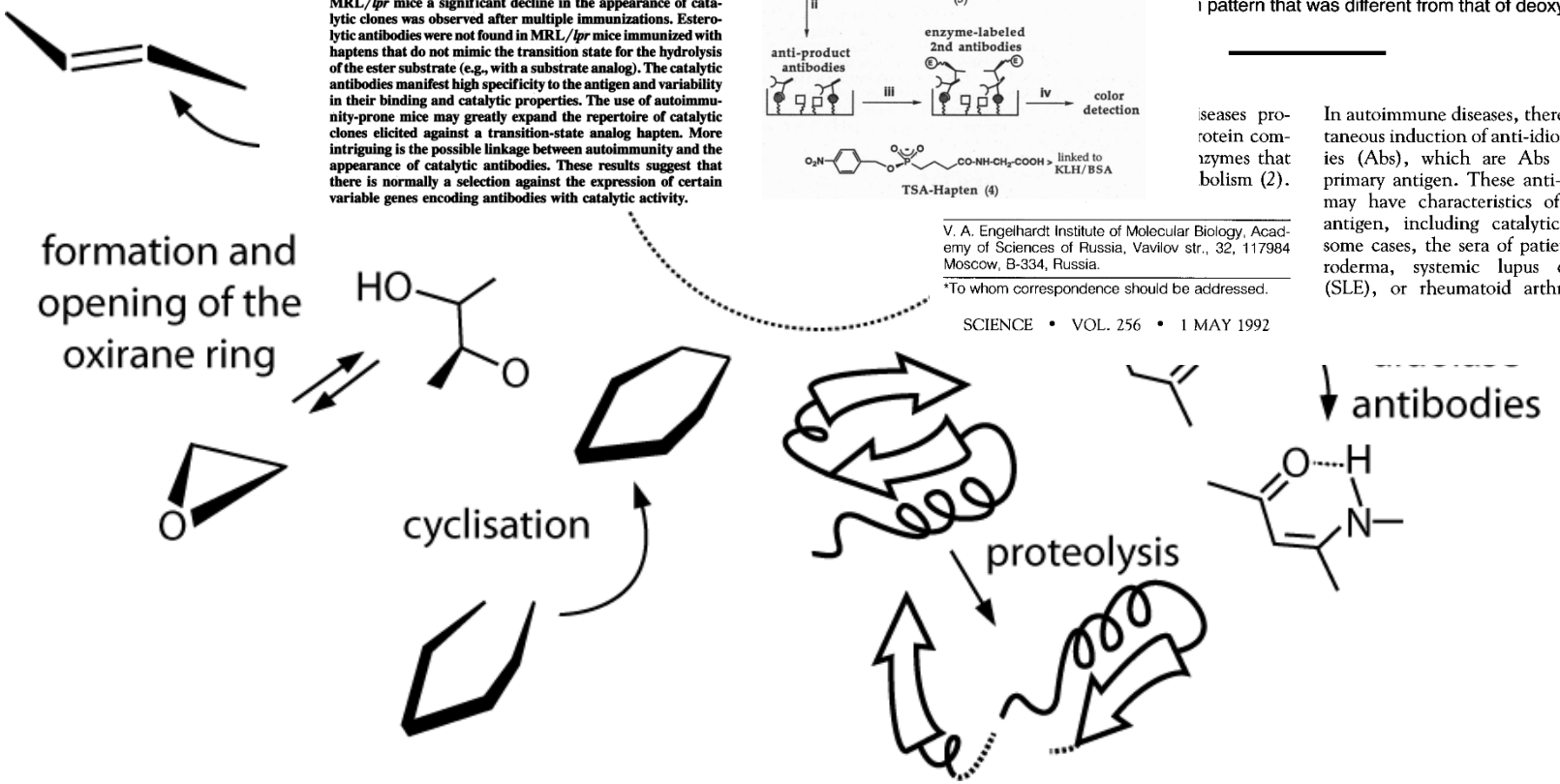
iseases pro- rotein com- nzymes that bolism (2).

In autoimmune diseases, there can be spontaneous induction of anti-idiotypic antibodies (Abs), which are Abs elicited by a primary antigen. These anti-idiotypic Abs may have characteristics of the primary antigen, including catalytic activity. In some cases, the sera of patients with scleroderma, systemic lupus erythematosus (SLE), or rheumatoid arthritis have an

V. A. Engelhardt Institute of Molecular Biology, Academy of Sciences of Russia, Vavilov str., 32, 117984 Moscow, B-334, Russia.

*To whom correspondence should be addressed.

β-elimin



Enzyme Catalysis in Organic Synthesis. Chapter 14. Catalytic antibodies. Smirnov I., Belogurov A., Gabibov A. Wiley-VCH, 2012



Michael Sela



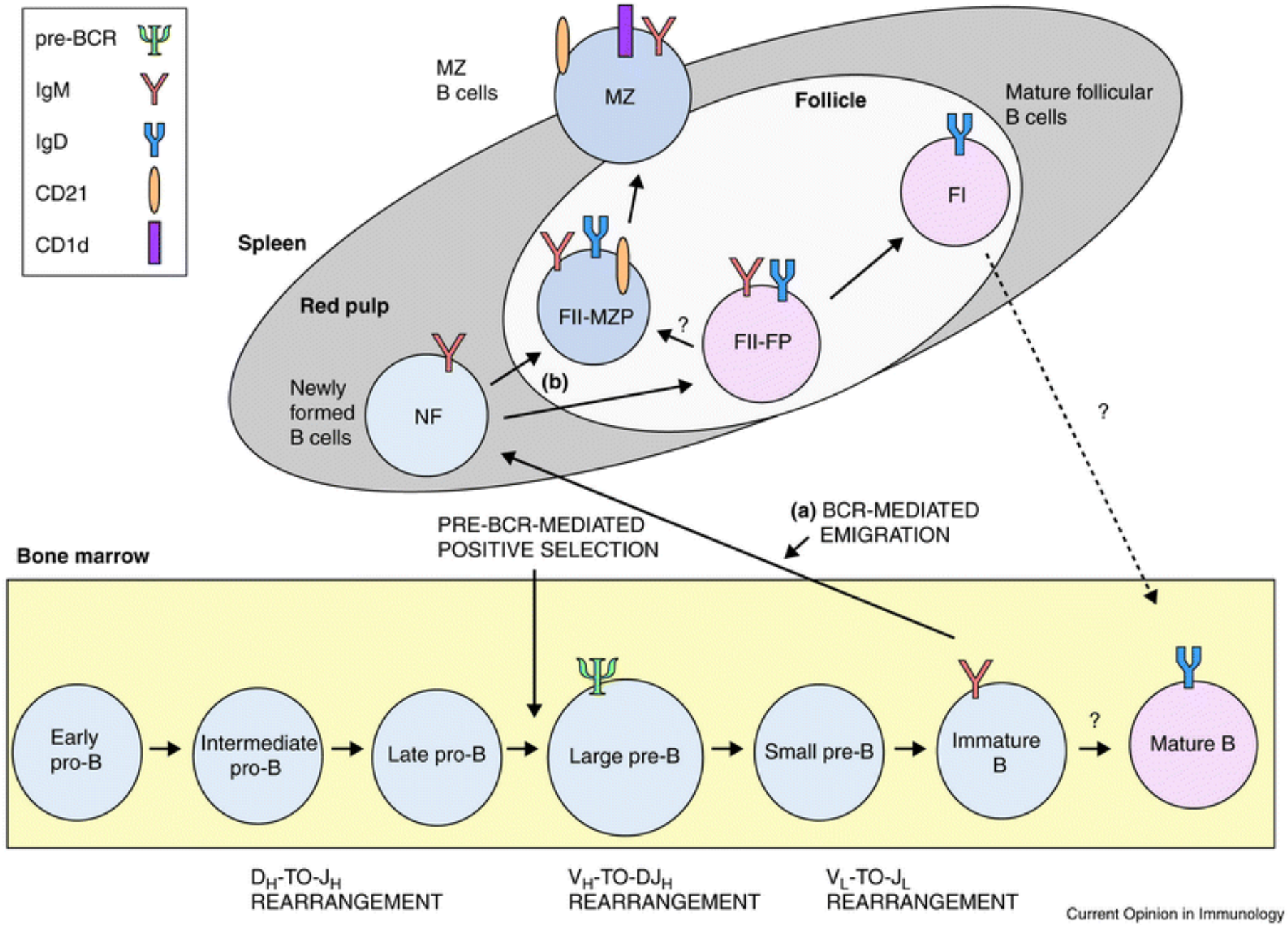
MESSAGE

Limited opportunity to accelerate reactions by mimicking unique transition state to generate active site



MATURATION
IN SILICO

BioCat Lab


pre-BCR
IgM
IgD
CD21
CD1d
MZ
B cells
MZ
Follicle
Mature follicular
B cells
FI
Spleen
Red pulp
FII-MZP
?
FII-FP
Newly
formed
B cells
NF
(b)
?
(a) BCR-MEDIATED
EMIGRATION
PRE-BCR-MEDIATED
POSITIVE SELECTION
Bone marrow
Early
pro-B
Intermediate
pro-B
Late pro-B
Large pre-B
Small pre-B
Immature
B
?
Mature B
D_H-TO-J_H
REARRANGEMENT
V_H-TO-DJ_H
REARRANGEMENT
V_L-TO-J_L
REARRANGEMENT
Current Opinion in Immunology

MESSAGE

BioCat Lab

Expanded opportunity to mimic several transition states and select optimal substrate and product orientations

MESSAGE:

Combination of instrumental and computational methods may be regarded as an efficient strategy to obtain artificial biocatalysts/binders *de novo*





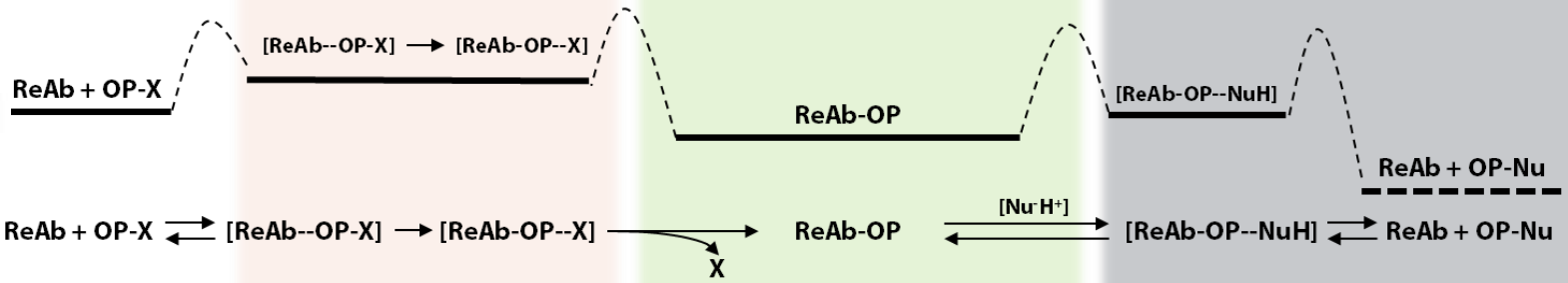




Modification

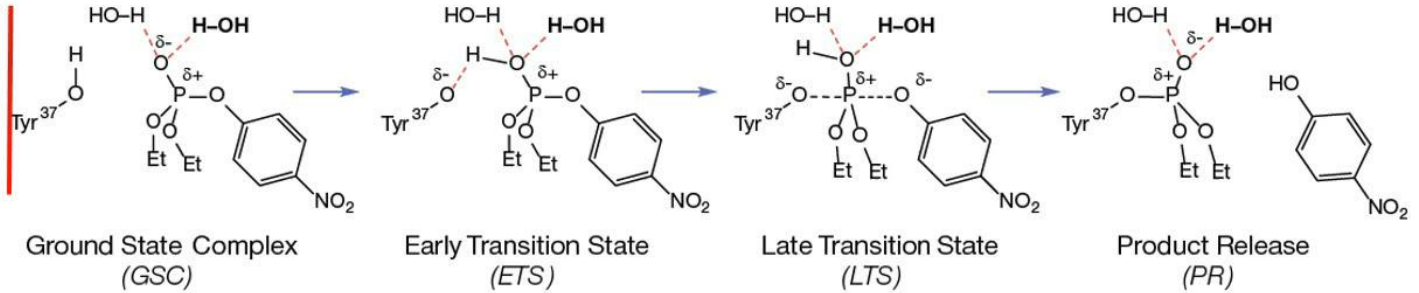
Covalent intermediate

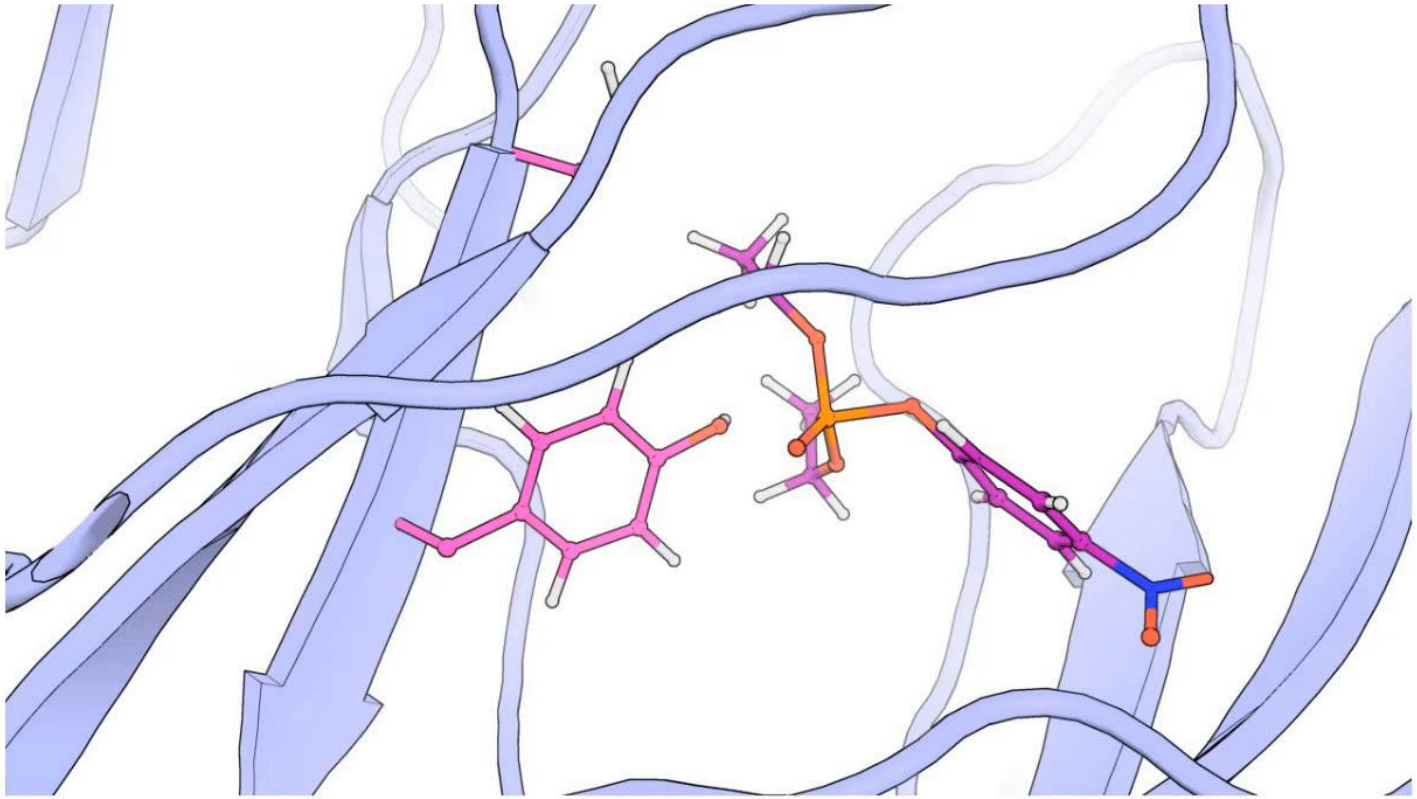
Turnover



Hybrid QM/MM and MD approach

Mechanism of paraoxon hydrolysis by Ig-paraoxonase.



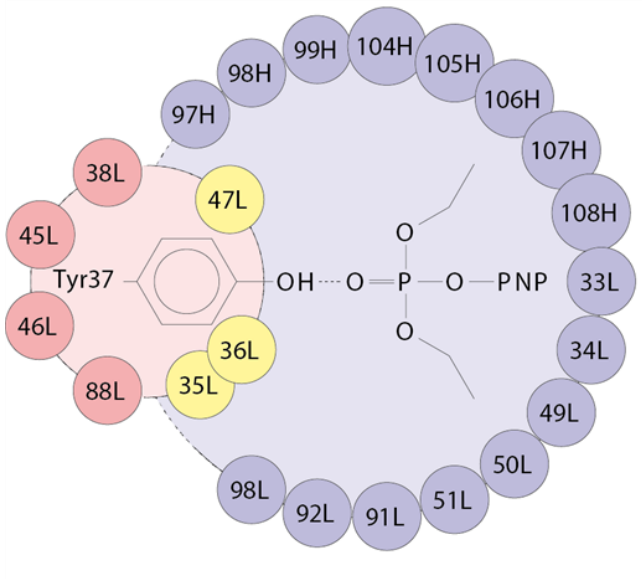


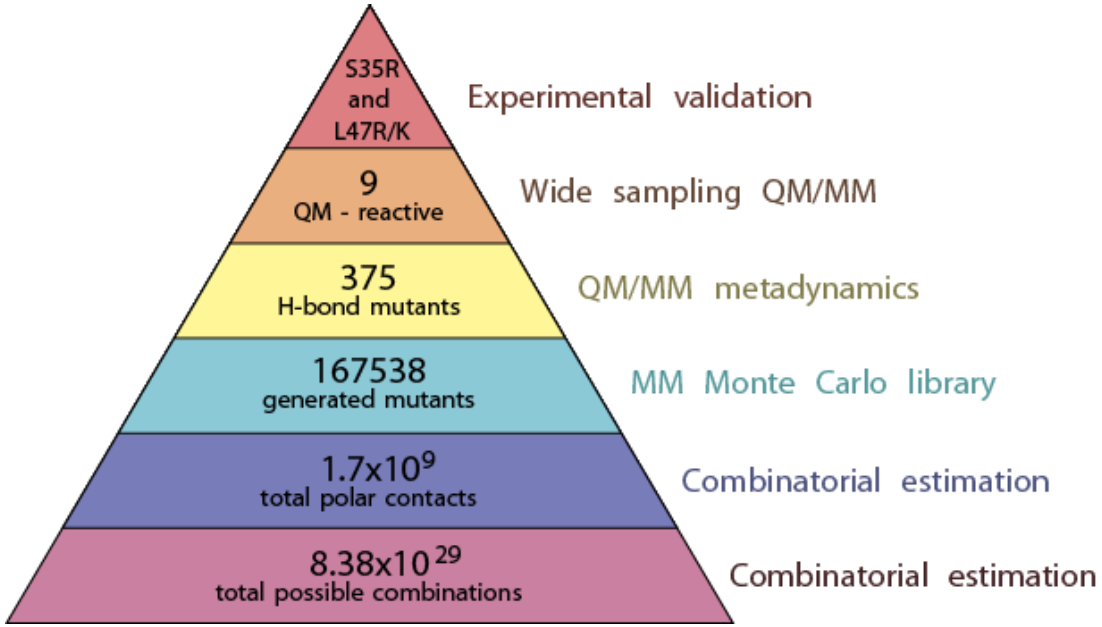
The QM computed mechanism of TS development for step 1 of WTIgP reaction with paraoxon; the classic $S_N2(P)$ mechanism with tbp geometry is initiated by early proton transfer followed in 5-20 fs by late TS O–P bond formation.

The *in silico* maturation scheme

BioCat Lab

We selected 23 amino acids which when mutated to Arg could form a H-bond (≤3.2 Å) with the phosphate moiety of paraoxon





Stages of rational restriction of library size:

1. Limiting to 11 polar residues and restricting permutations to four-at-a-time
2. Programming of 7 H-bond-donor amino acids (Arg, His, Lys, Ser, Thr, Trp, Tyr) and 3 of Glu, Asp, and Ser mutations to provide general acid-base catalysis for Tyr37

The QM/MM calculations revealed:

(i) systems with an anionic amino acid mutation did not deliver covalent reaction

(ii) the best successful runs resulted from Arg, Lys and His in positions 35, 47 of the light chain

Smirnov et al. Science Adv. 2016

MESSAGE

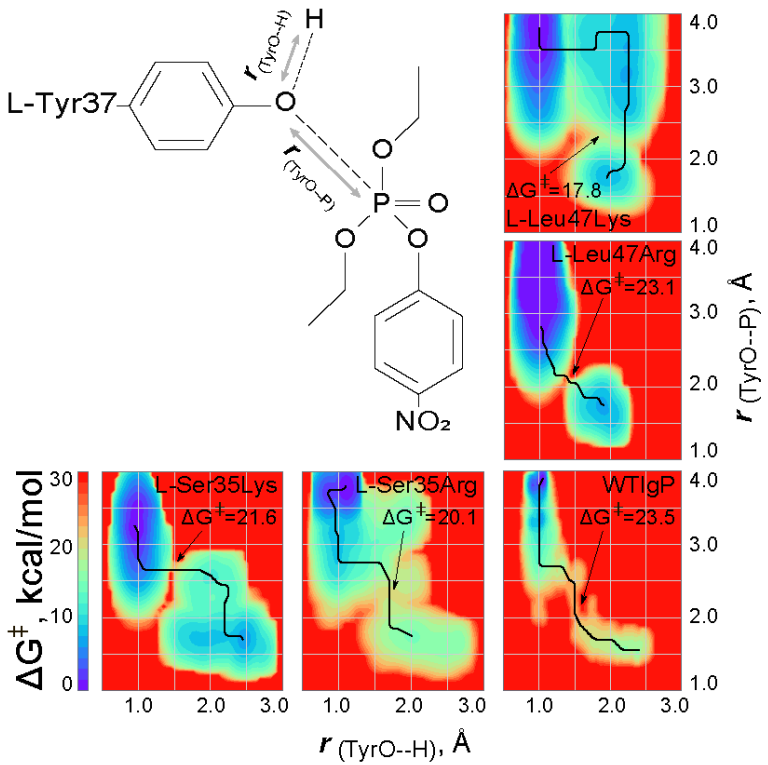
BioCat Lab

How to estimate the stereoselectivity of catalysis *in silico*?

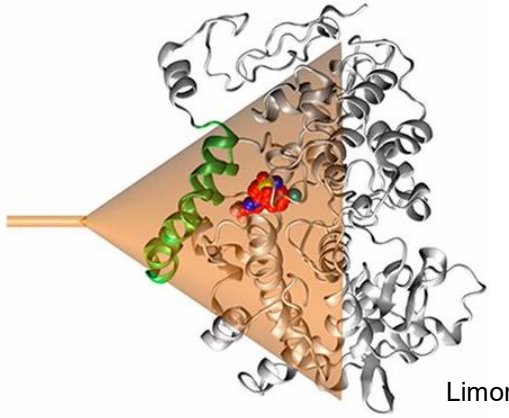
Synergy of QMMM and funnel metadynamics allows to generate catalytic antibody variants with high reactivity and stereospecificity

BioCat Lab

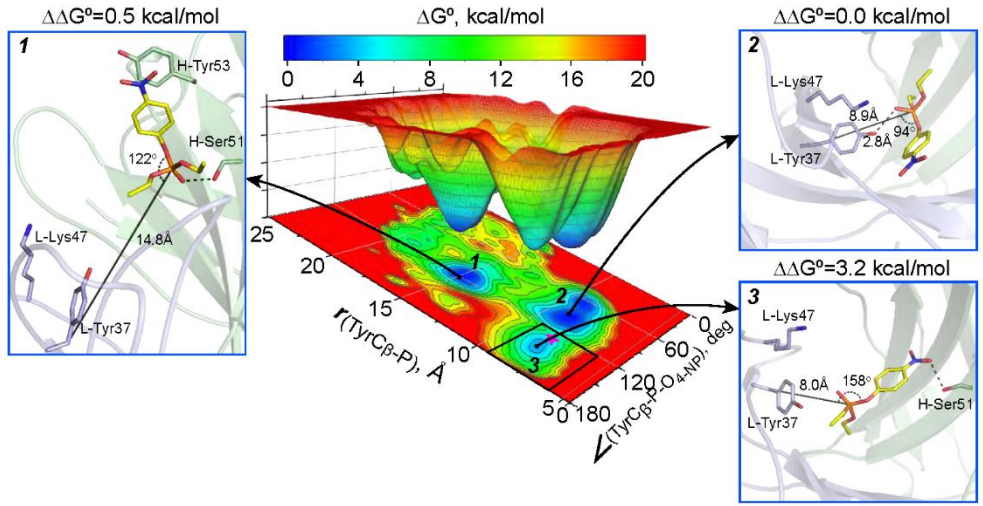
QM/MM



Funnel metadynamics



Limongelli et al., PNAS 2013



Mokrushina et al., PNAS, 2020

Novel enzyme development strategies

Discovery

- Sequence and structure mining
- **EC number prediction**
- **Parameter prediction**



Design

- **Structure prediction**
- Diffusion models
- Inverse folding
- **Protein language models**

Engineering

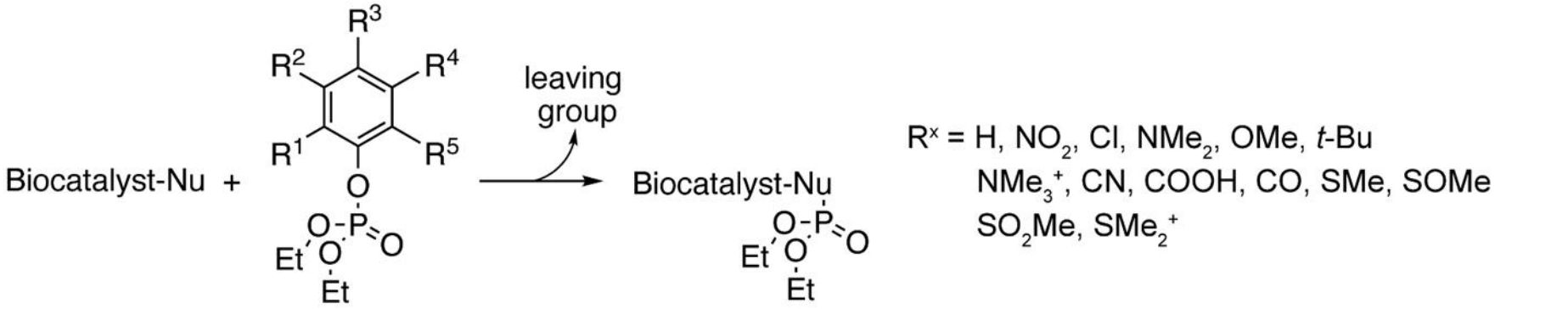
- **Zero-shot prediction**
- **MLDE**
- **Active learning**
- **Transfer learning**

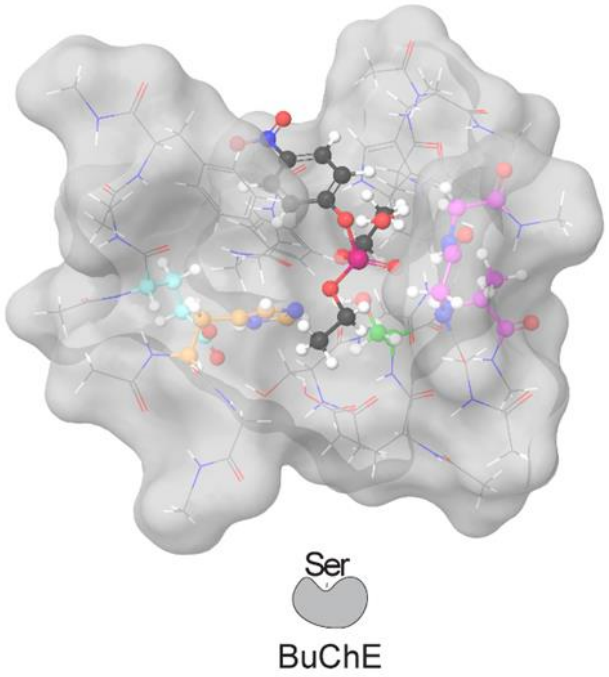
Experiments

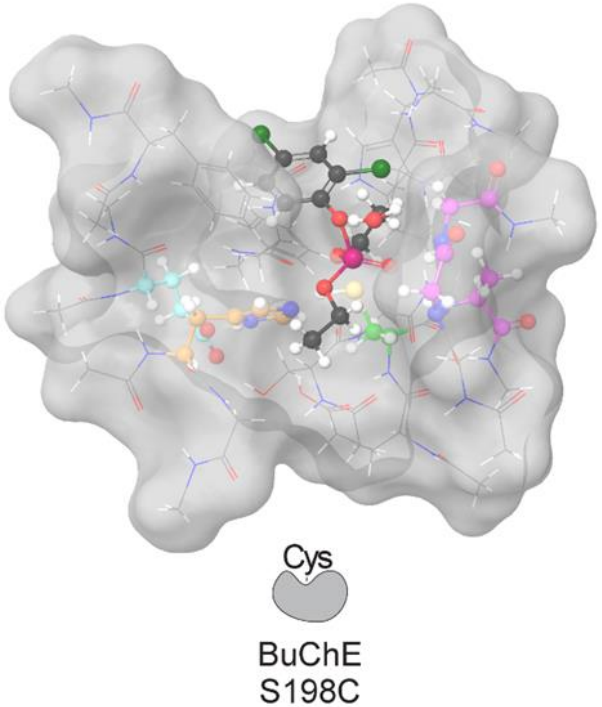
- Low-cost DNA synthesis & sequencing
- High-throughput screening
- Automation

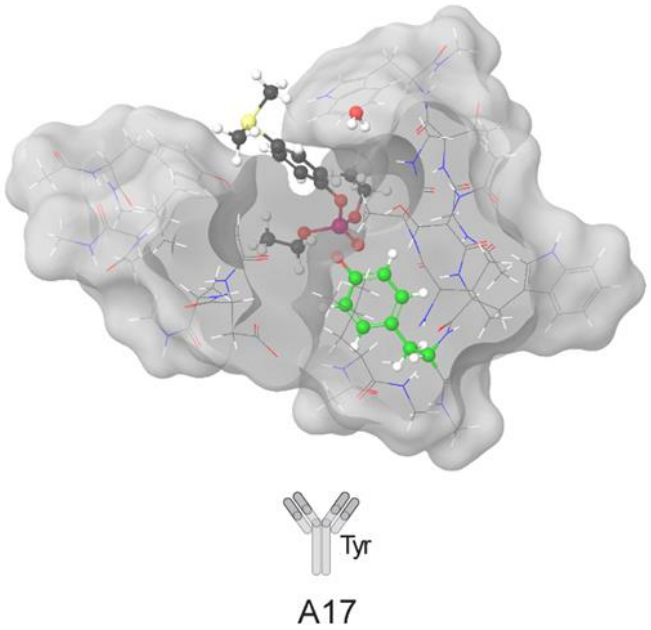
Docter, S. et al (2026). Current Opinion in Biotechnology, 97, 103393.

Reaction scheme and theozyme active site geometries for 3 examined biocatalysts



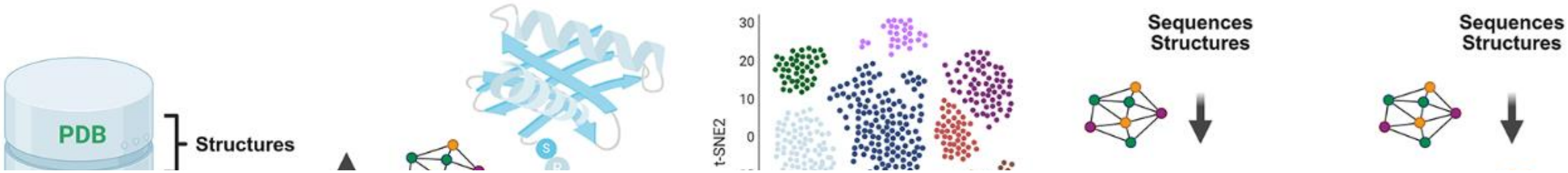






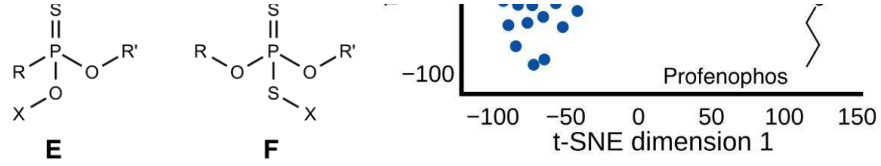
Solovev et al. "Chemical neighborhood exploration for substrate discovery in biocatalysis." *Proceedings of the National Academy of Sciences* 123.24 (2026): e2535430123.

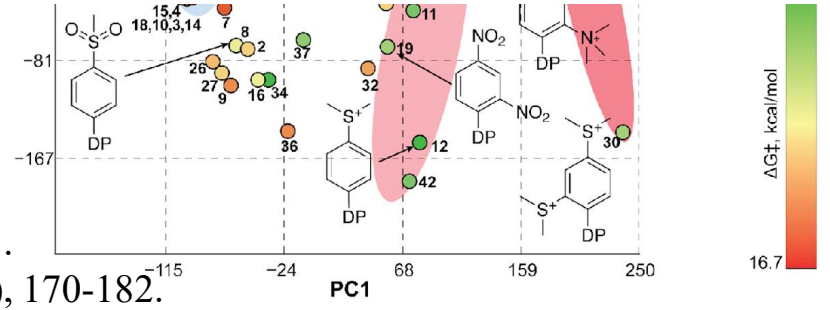
ML-assisted discovery of novel enzymes and substrates across large space

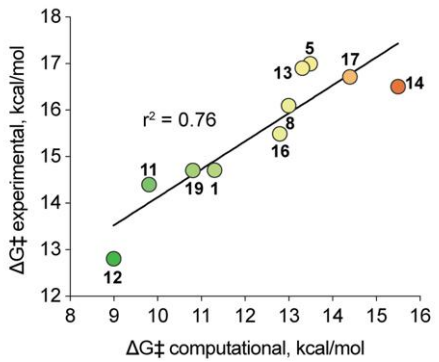


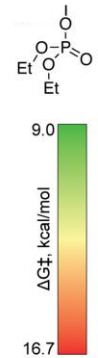
Описание рабочего датасета начинается с оценки распределения и группировки его элементов. Анализ ключевых различий в векторе признаков важен для понимания адекватности разделения выборки на группы и анализа причин различий в активности между кластерами.

Дальнейшая работа с отдельными группами позволяет существенным образом экономить времязатраты, концентрируя вычислительные ресурсы на наиболее перспективных кластерах.









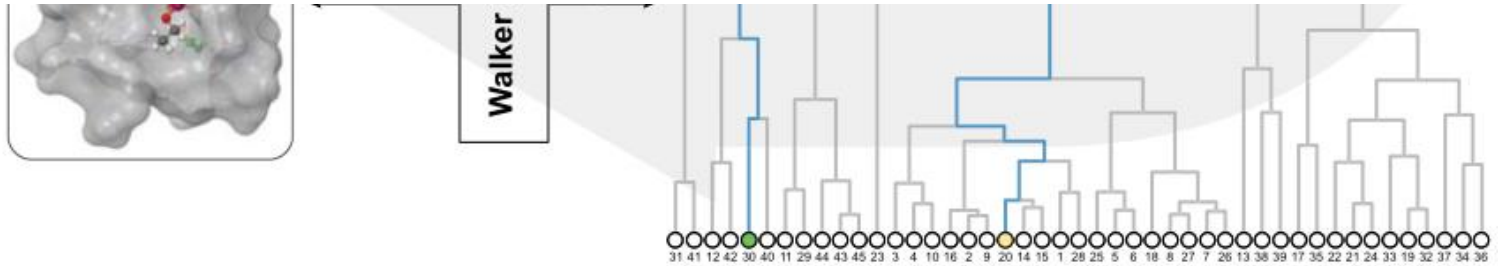
Docter, S. et al (2026). Current Opinion in Biotechnology, 97, 103393.
Petrova, V. V. et al (2024) Journal of Computational Chemistry, 45(3), 170-182.
Solovev Y.V. et al. (2026) PNAS, Jun 16;123(24):e2535430123

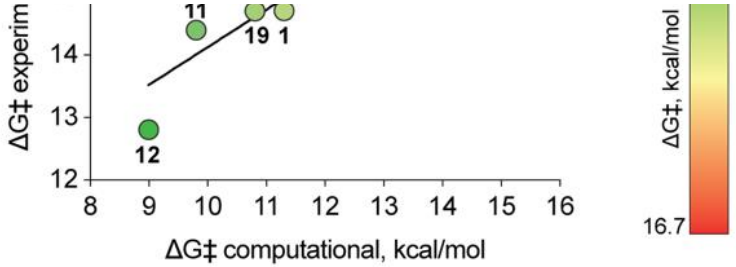
Bader theory and COnductorSolventMOdel(COSMO) surface descriptors





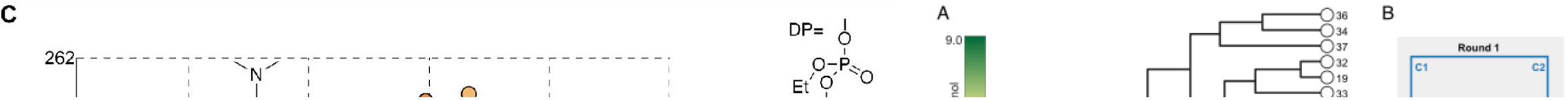
Использование физичных дескрипторов позволяет добиться наиболее релевантного описания рабочего датасета: выявить закономерности распределения ключевых признаков и построить карты схожести элементов выборки





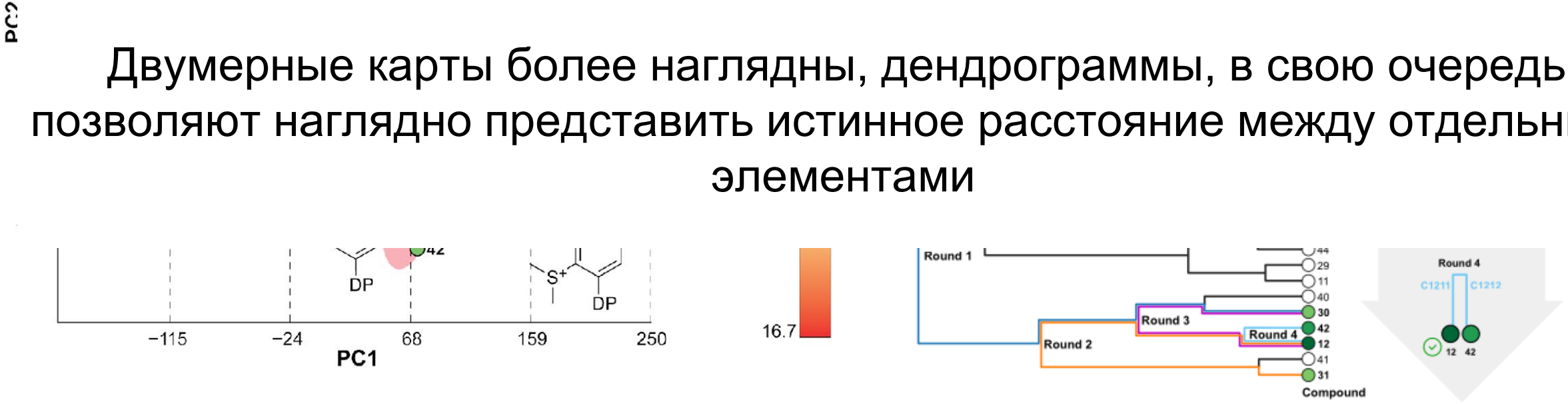
Solovev et al. "Chemical neighborhood exploration for substrate discovery in biocatalysis." *Proceedings of the National Academy of Sciences* 123.24 (2026): e2535430123.

Chemical space representation and discovery strategies



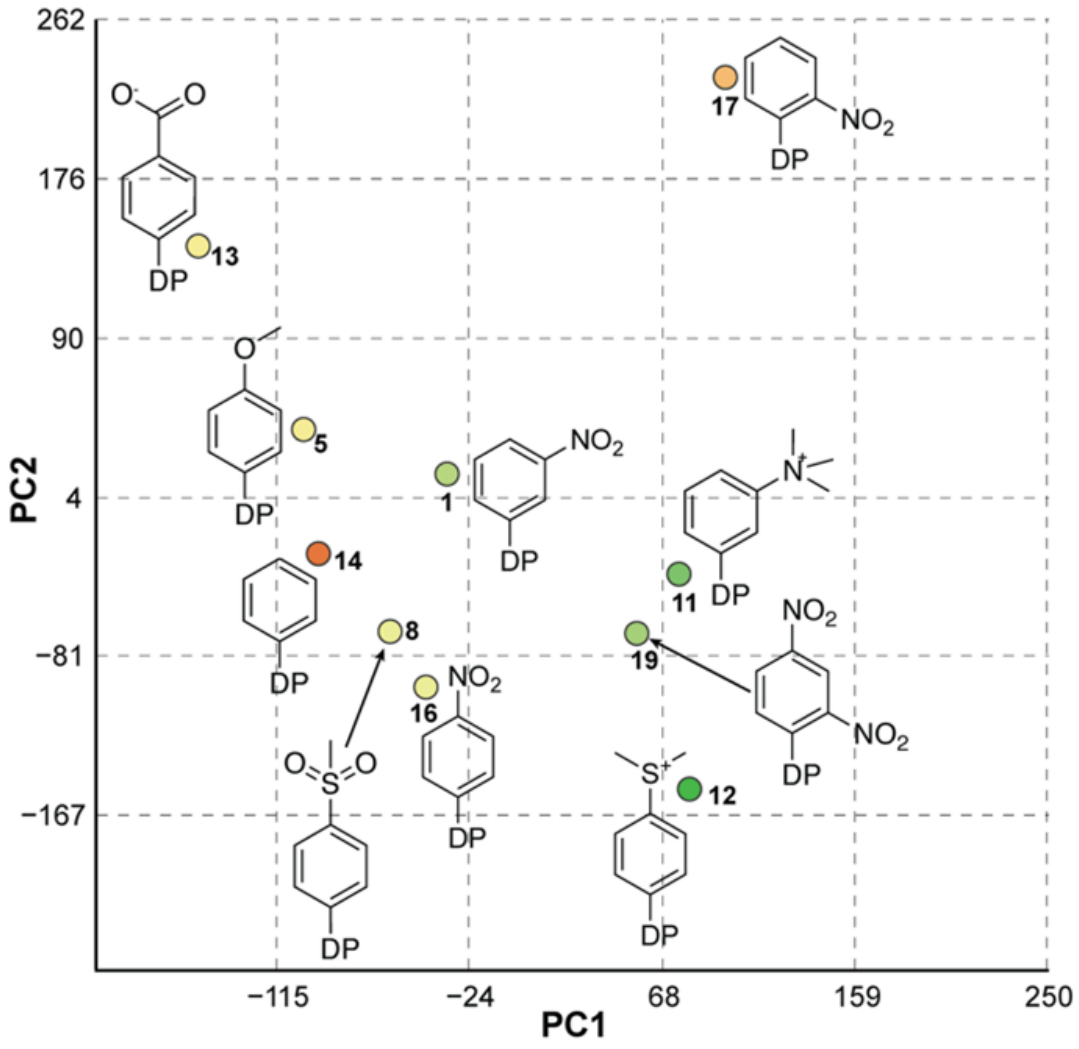
Использование разных алгоритмов кластеризации важно как для выявления наиболее релевантных групп (кластеров), так и для обнаружения уникальных элементов датасета.

Двумерные карты более наглядны, дендрограммы, в свою очередь, позволяют наглядно представить истинное расстояние между отдельными элементами



Solovev et al. "Chemical neighborhood exploration for substrate discovery in biocatalysis." *Proceedings of the National Academy of Sciences* 123.24 (2026): e2535430123.

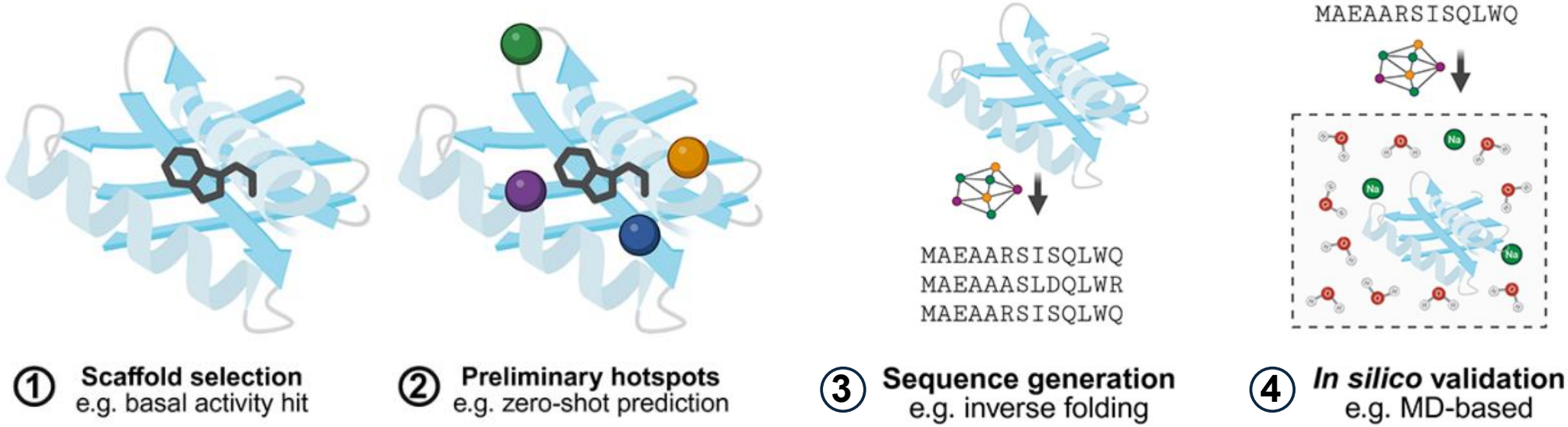
Prediction and experimental validation of kinetic parameters

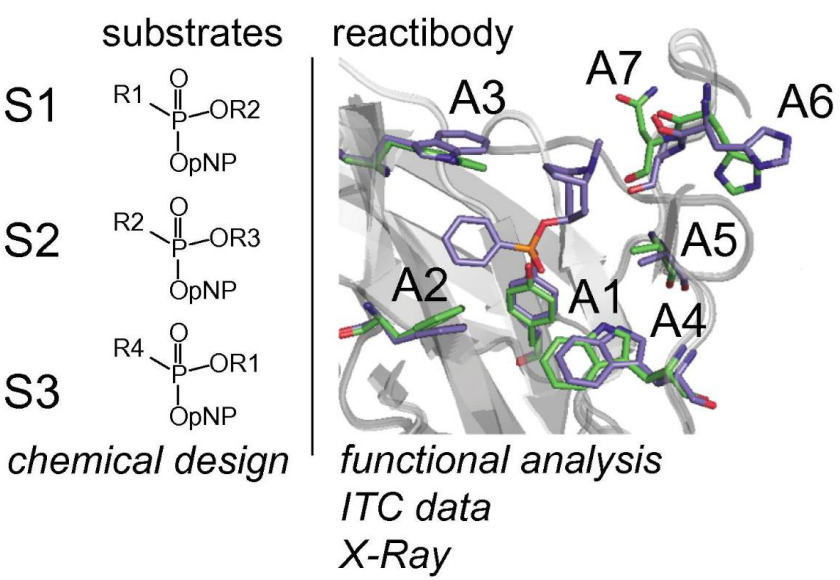


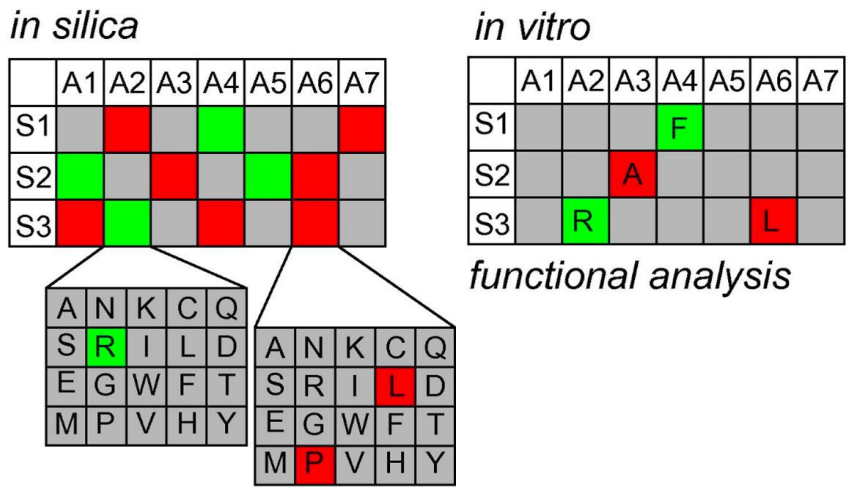
Substrates	ΔG‡comp kcal/mol	ΔG‡exp kcal/mol	k_2 $M^{-1}*s^{-1}$	Kd M	Ki $M^{-2}*s^{-1}$
Compound 1	11,3	14,7	$3*10^{-2}$	$3,1*10^{-8}$	10^{6}
Compound 5	13,5	17,0	$6,5*10^{-4}$	10^{-9}	$0,6*10^{6}$
Compound 8	13.0	16,1	$1,5*10^{-2}$	$3,6*10^{-7}$	$0,4*10^{5}$
Compound 11	9,8	14,4	$4,8*10^{-2}$	$9,2*10^{-8}$	$0,5*10^{6}$
Compound 12	9,0	12,8	$7,2*10^{-1}$	$4,7*10^{-8}$	$1,5*10^{7}$
Compound 13	13,3	16,9	$7,6*10^{-4}$	$1,2*10^{-8}$	$0,7*10^{6}$
Compound 14	15,5	16,5	$1,2*10^{-3}$	$1,8*10^{-7}$	$0,6*10^{5}$
Compound 16	12,8	15,5	$7,7*10^{-3}$	$8,4*10^{-8}$	$0,9*10^{6}$
Compound 17	14,4	16,7	$9,8*10^{-4}$	$1,6*10^{-9}$	$0,6*10^{6}$
Compound 19	10,8	14,7	$3*10^{-2}$	$2,7*10^{-9}$	10^{7}
Compound 23	15,3	X	X	X	X

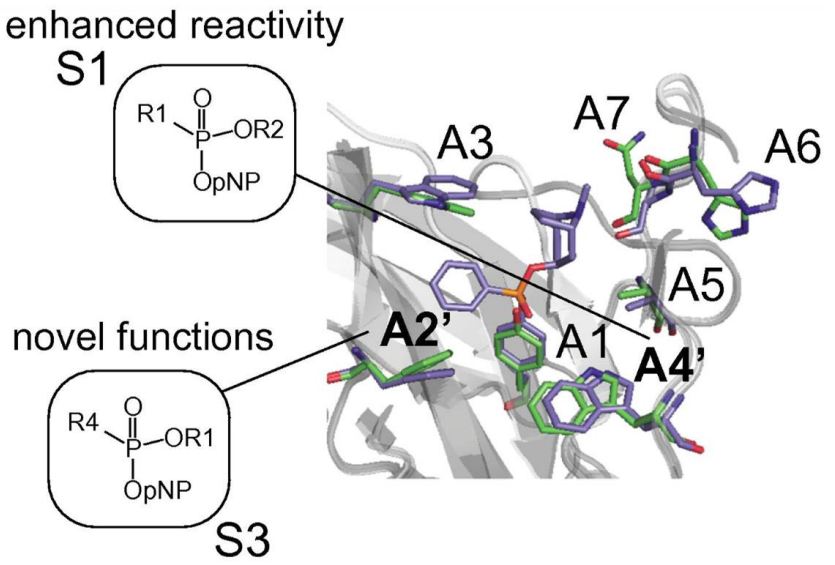
Solovev et al. "Chemical neighborhood exploration for substrate discovery in biocatalysis." *Proceedings of the National Academy of Sciences* 123.24 (2026): e2535430123.

ML-assisted *de novo* enzyme optimization



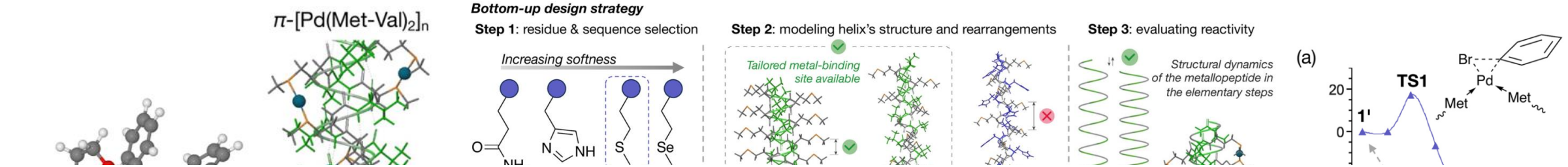




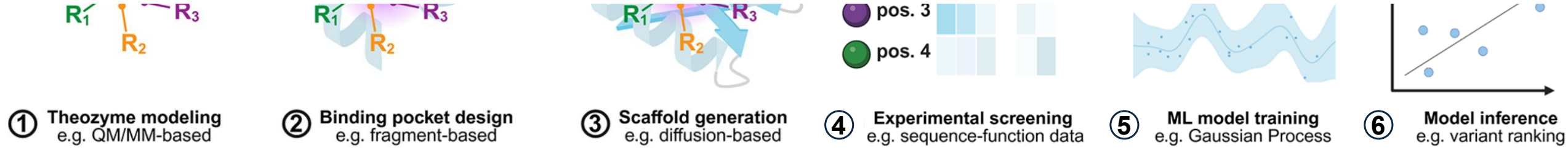


Docter, S. et al (2026). Current Opinion in Biotechnology, 97, 103393.
Belogurov Jr, A. et al (2012). FEBS letters, 586(18), 2966-2973.

ML-assisted *de novo* bottom-up enzyme engineering strategy

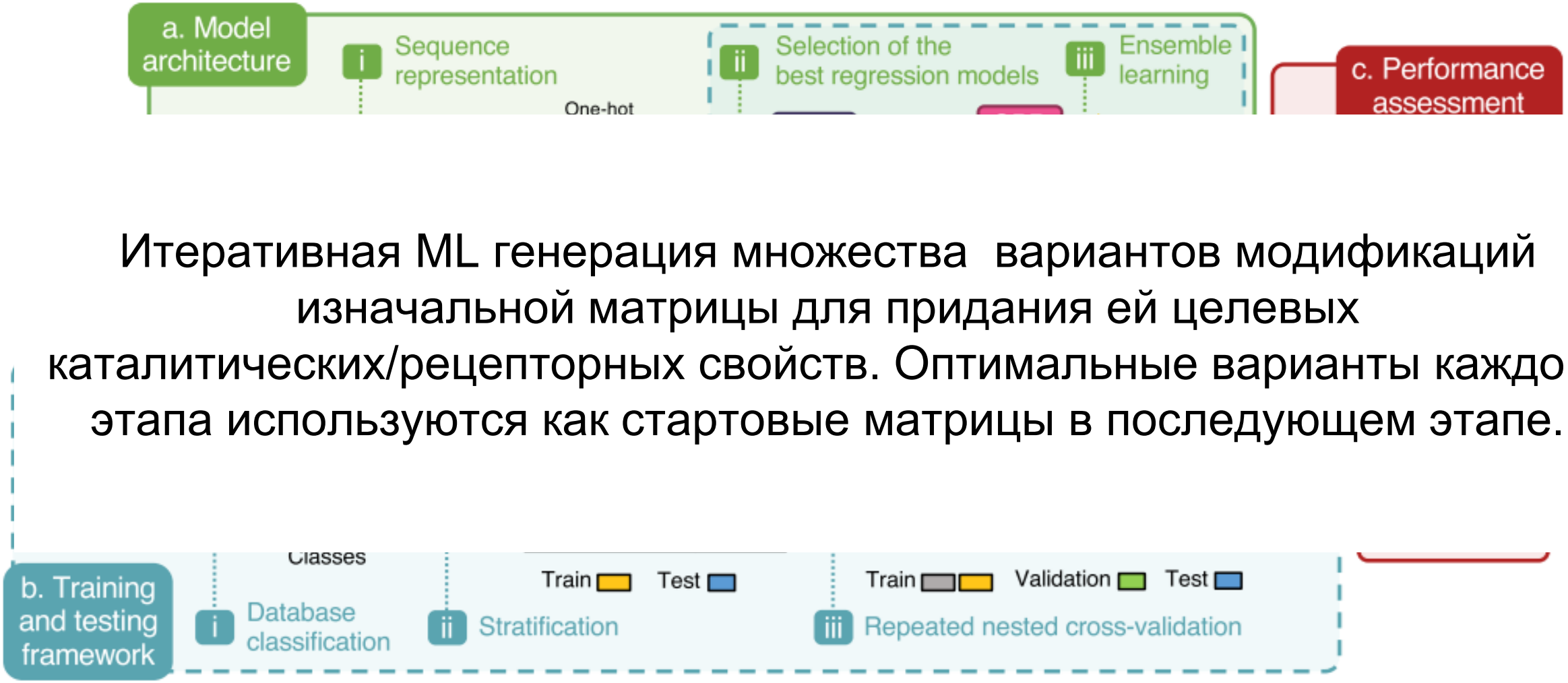


Экспертный и/или ML дизайн и *in silico* валидация активного центра катализатора с последующей встройкой оптимальной конфигурации в целевые матрицы



Docter, S. et al (2026). Current Opinion in Biotechnology, 97, 103393.
Petrova, V. V. et al (2024). The Journal of Organic Chemistry, 89(12), 8478-8485.
Domnin, A. V. et al (2026). Inorganic Chemistry Frontiers, 13(1), 246-259.

ML-assisted *de novo* sequence-based top-down enzyme engineering strategy



Итеративная ML генерация множества вариантов модификаций изначальной матрицы для придания ей целевых каталитических/рецепторных свойств. Оптимальные варианты каждого этапа используются как стартовые матрицы в последующем этапе.

Ramon, Aubin, et al. "Prediction of protein biophysical traits from limited data: a case study on nanobody thermostability through NanoMelt." *MAbs*. Vol. 17. No. 1. Taylor & Francis, 2025.

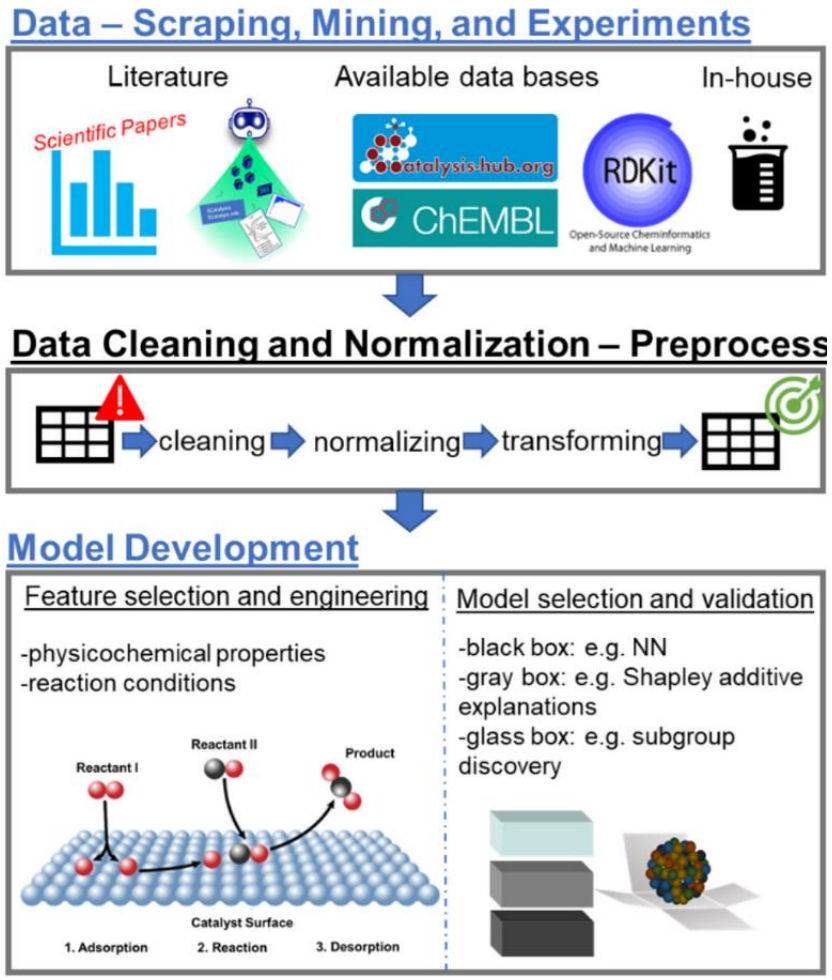
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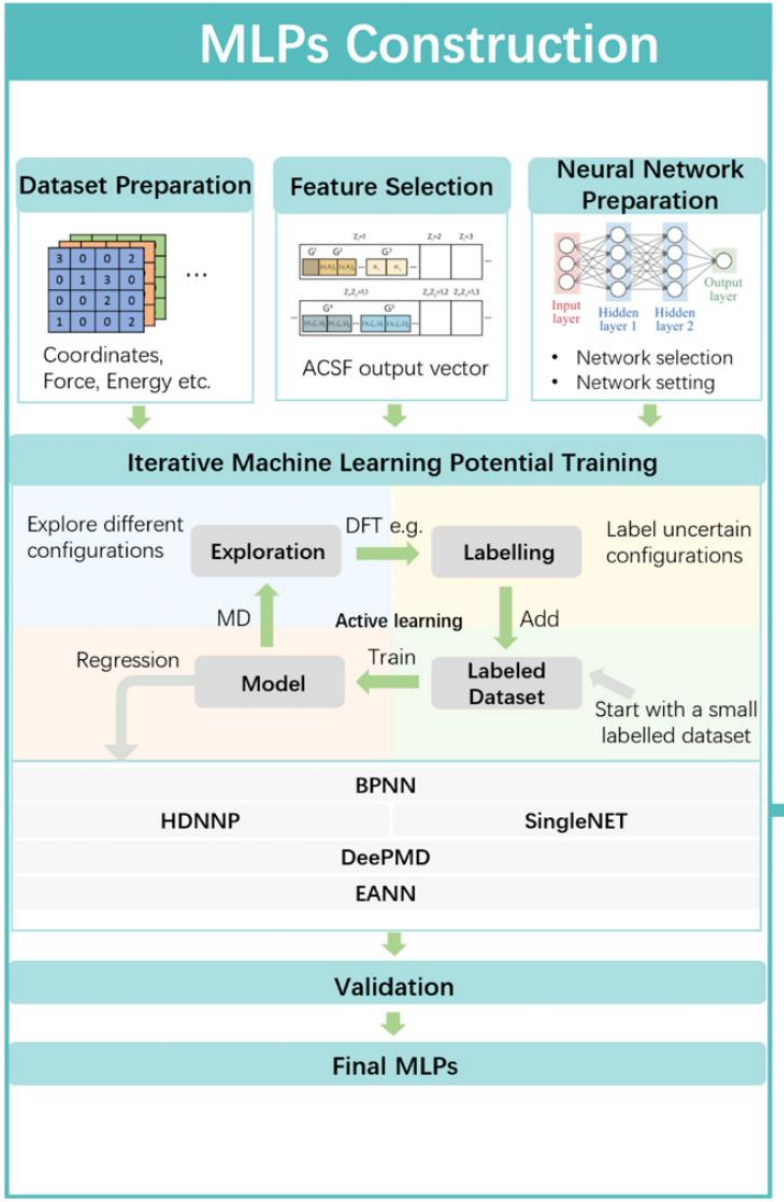
BioCat Lab

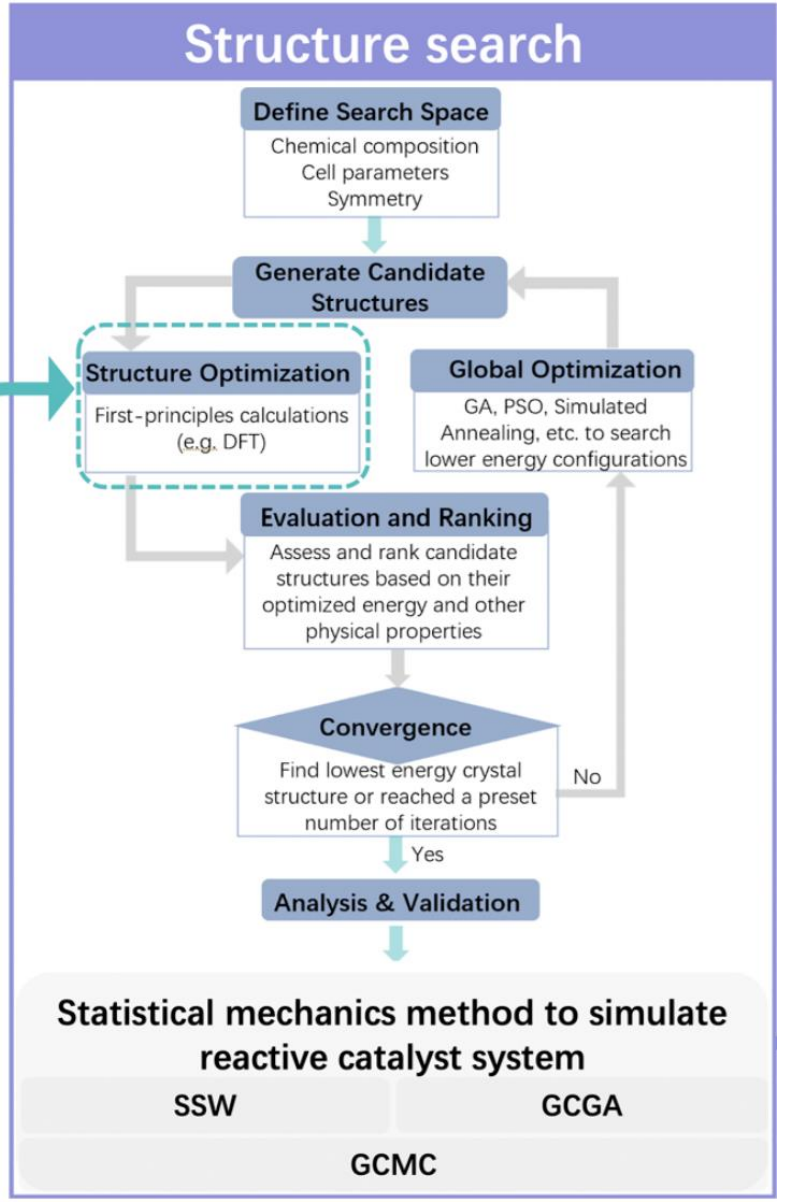
-How to automatically generate potential catalytic matrices with desired stereoselectivity?

-uMLIP (universal ML interatomic potentials) approach

universal ML interatomic potentials (uMLIP): basics concepts







de Araujo, Leandro Goulart, et al. "Recent developments in the use of machine learning in catalysis: A broad perspective with applications in kinetics." *Chemical Engineering Journal* (2025): 160872

Cheng, Xiran, et al. "Leveraging machine learning potentials for in-situ searching of active sites in heterogeneous catalysis." *Precision Chemistry* 2.11 (2024): 570-586.

Who is currently developing uMLIP?

Model	Org	r_{cut}	Links	Date Added	Targets	Params	Training Set
eSEN-30M-OAM		6 Å		2025-03-17	EFS_G	30.2M	6.6M (113M) OMat24+MPtrj+sAlex
EquFlash		6 Å		2025-06-23	EFS_G	28.7M	6.6M (113M) OMat24+MPtrj+sAlex
Nequip-OAM-L		6 Å		2025-09-08	EFS_G	9.6M	6.6M (113M) OMat24+sAlex+MPtrj
GRACE-2L-OAM-L	ICAMS	6 Å		2025-09-09	EFS_G	26.4M	6.6M (113M) OMat24+sAlex+MPtrj
ORB v3		6 Å		2025-04-05	EFS_G	25.5M	6.47M (133M) MPtrj+Alex+OMat24
SevenNet-MF-ompa		6 Å		2025-03-13	EFS_G	25.7M	6.6M (113M) OMat24+sAlex+MPtrj
Allegro-OAM-L		7 Å		2025-09-08	EFS_G	9.7M	6.6M (113M) OMat24+sAlex+MPtrj
GRACE-2L-OAM	ICAMS	6 Å		2025-02-06	EFS_G	12.6M	6.6M (113M) OMat24+sAlex+MPtrj
DPA-3.1-3M-FT		6 Å		2025-06-05	EFS_G	3.27M	163M OpenLAM

For **Training set** the number of unique compounds in the set is indicated without parentheses, while the number of *ab initio* molecular dynamics steps for these str

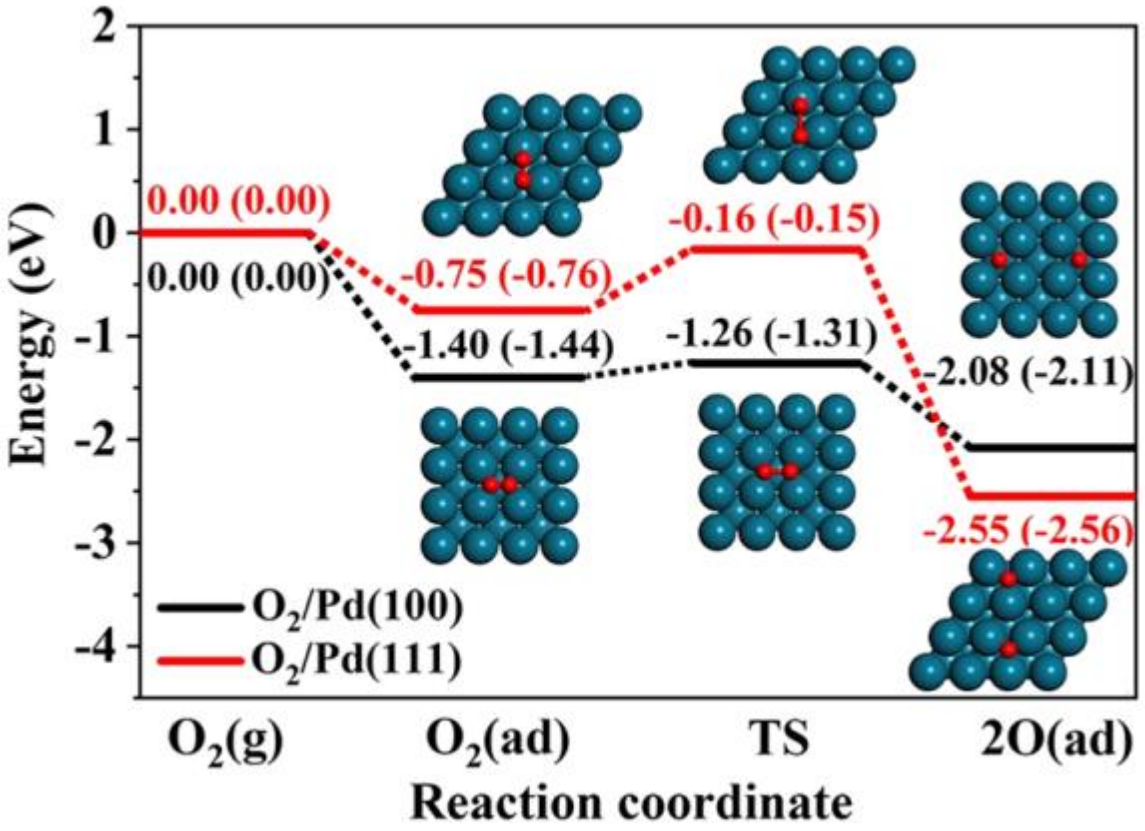
SAMSUNG
Advanced Institute of Technology AI Lab Montreal

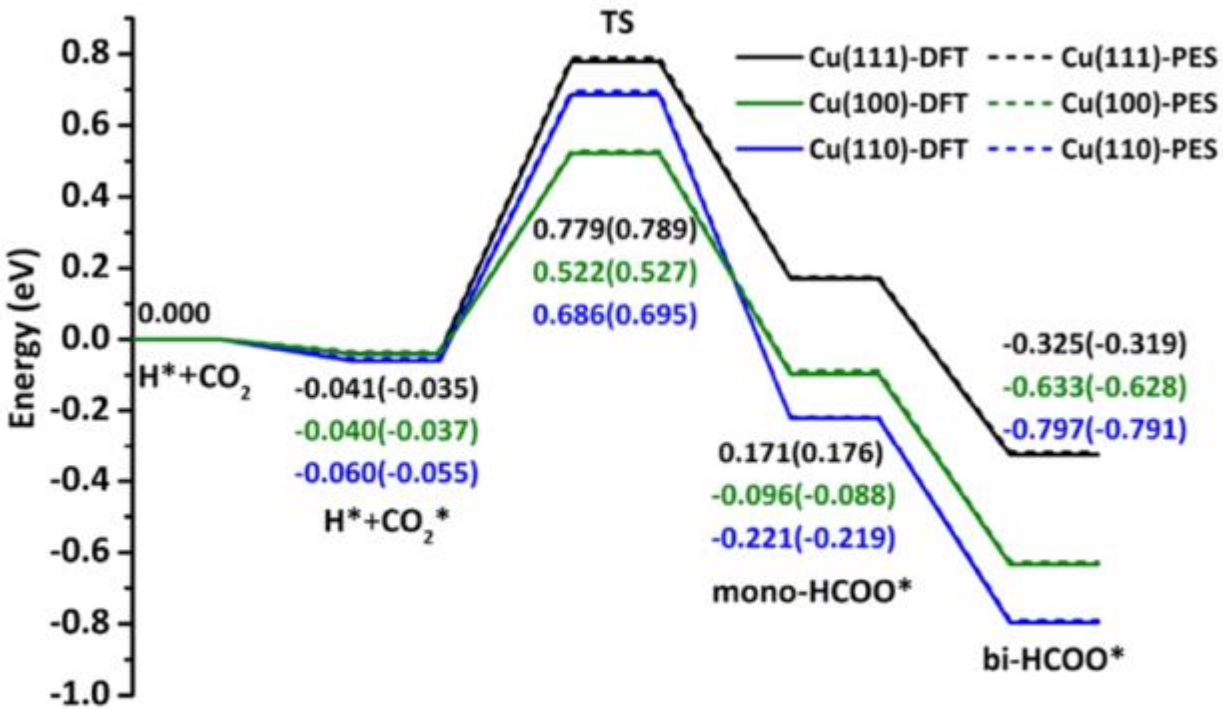
Orbital Materials

ICAMS

NequIP

uMLIP in tandem with DFT: reaction profile description





JLin, Qidong, and Bin Jiang. "Modeling Equilibration Dynamics of Hyperthermal Products of Surface Reactions Using Scalable Neural Network Potential with First-Principles Accuracy." *The Journal of Physical Chemistry Letters* 14.33 (2023): 7513-7518.

Yin, Rongrong, et al. "Theoretical insights into structure sensitivity in formate decomposition dynamics on Cu surfaces." *ACS Catalysis* 13.21 (2023): 14103-14111.

Cheng, Xiran, et al. "Leveraging machine learning potentials for in-situ searching of active sites in heterogeneous catalysis." *Precision Chemistry* 2.11 (2024): 570-586.

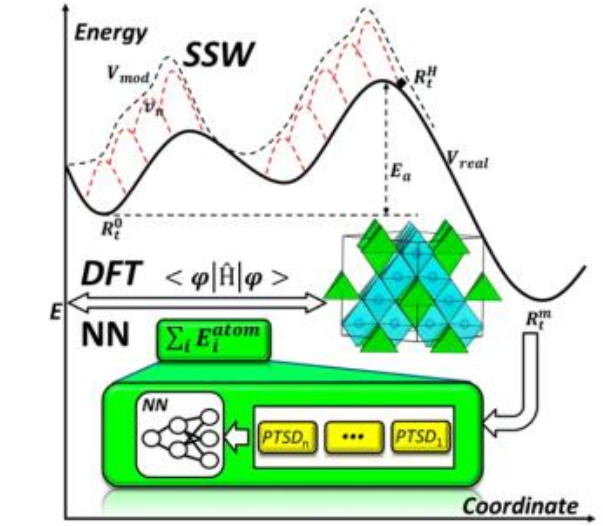
Active learning strategies for *ad hoc* uMLIPs improvement

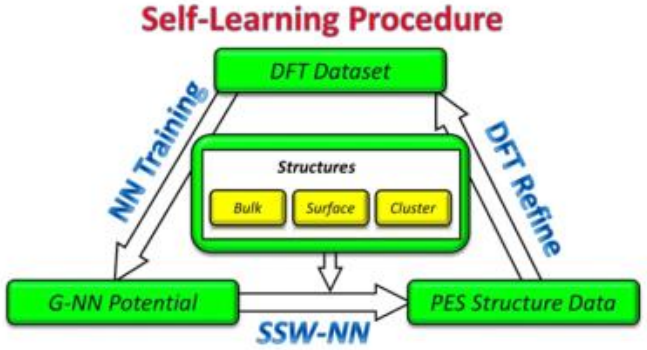
How the system identifies poorly covered areas?

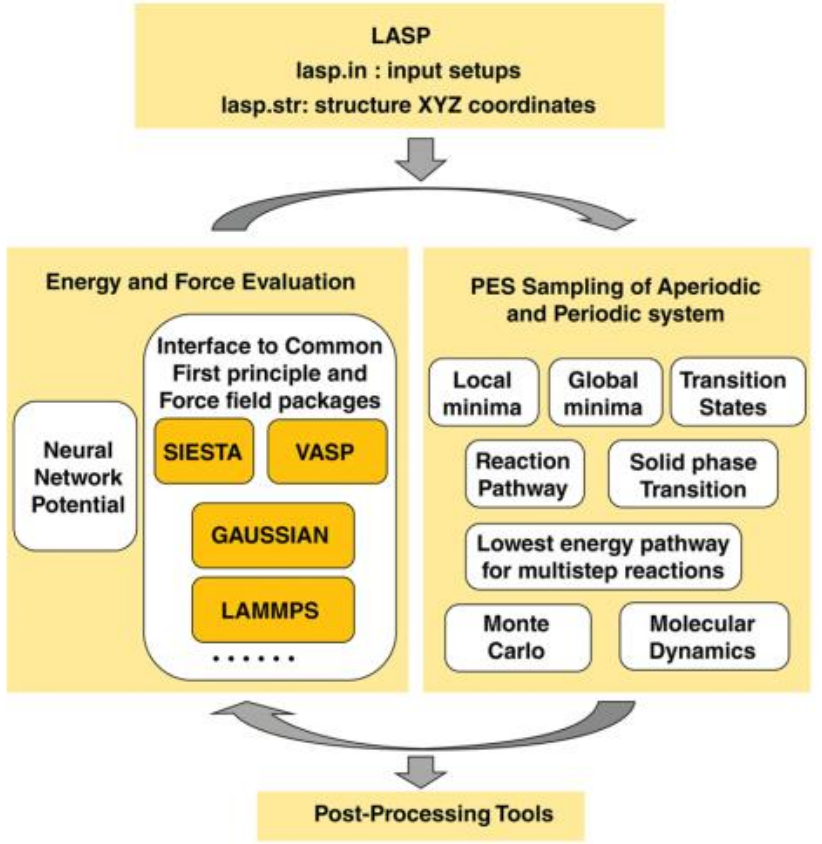
1) Large variance in predictions among multiple MLP models members indicates high uncertainty
2) Random neuron deactivation leads to variance across dropout iterations
3) Large distance to nearest training examples indicates extrapolation necessity

High uncertainty markers:

1) New element combinations or stoichiometries
2) Unusual bond lengths or angles not seen in training
3) New coordination numbers or chemical motifs
4) High-energy barriers
5) Saddle points and reaction pathways



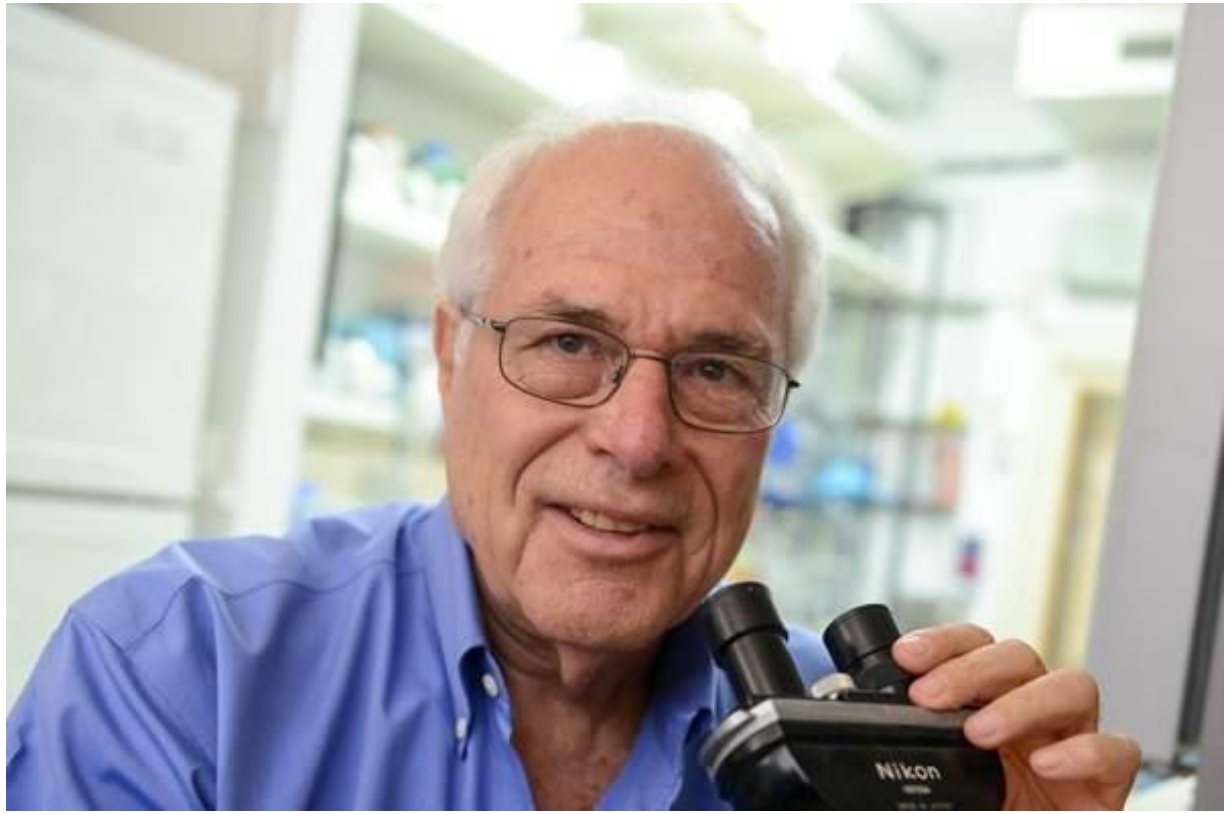




Huang, Si-Da, et al. "LASP: Fast global potential energy surface exploration." *Wiley Interdisciplinary Reviews: Computational Molecular Science* 9.6 (2019): e1415.
Cheng, Xiran, et al. "Leveraging machine learning potentials for in-situ searching of active sites in heterogeneous catalysis." *Precision Chemistry* 2.11 (2024): 570-586.

MESSAGE

Reengineering T cells using combinatorial approaches

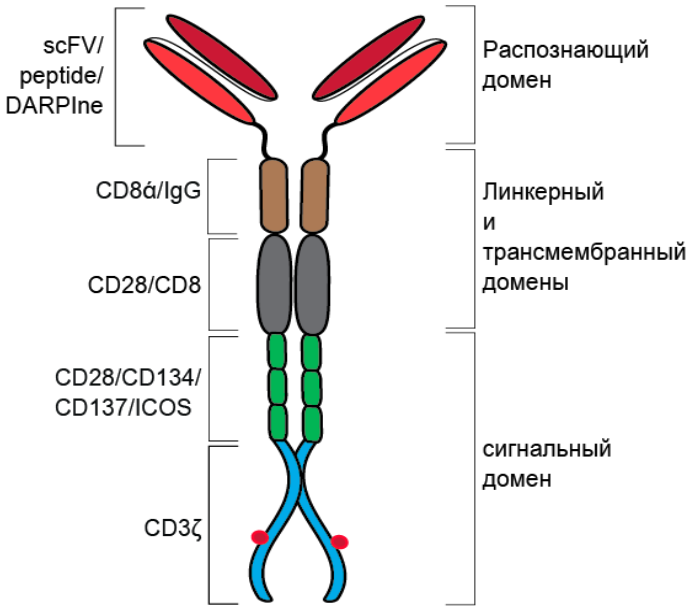


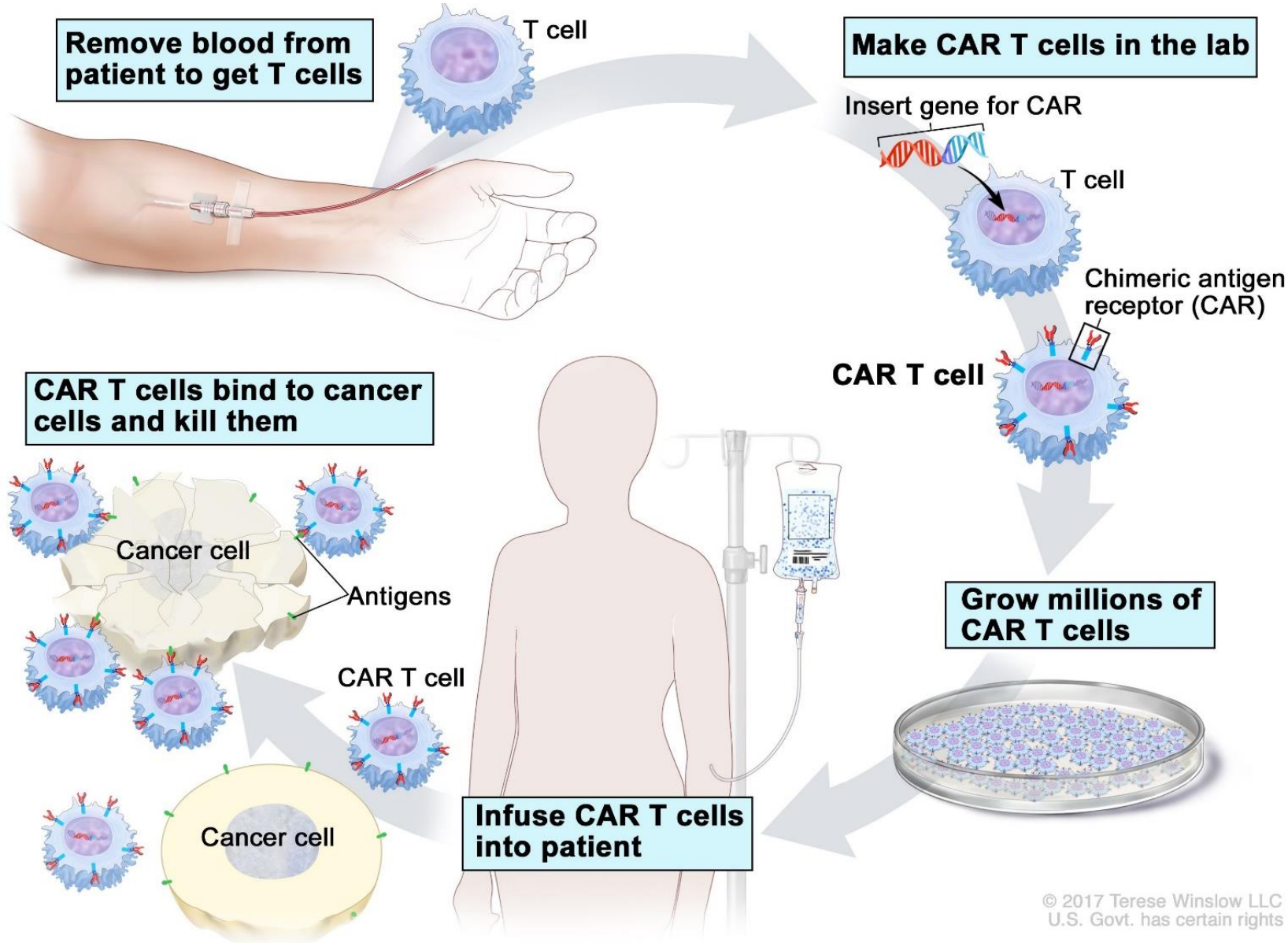
Prof. Zelig Eshhar
Weizmann Institute of science

CAR T cell therapy

BioCat Lab

CAR T structure





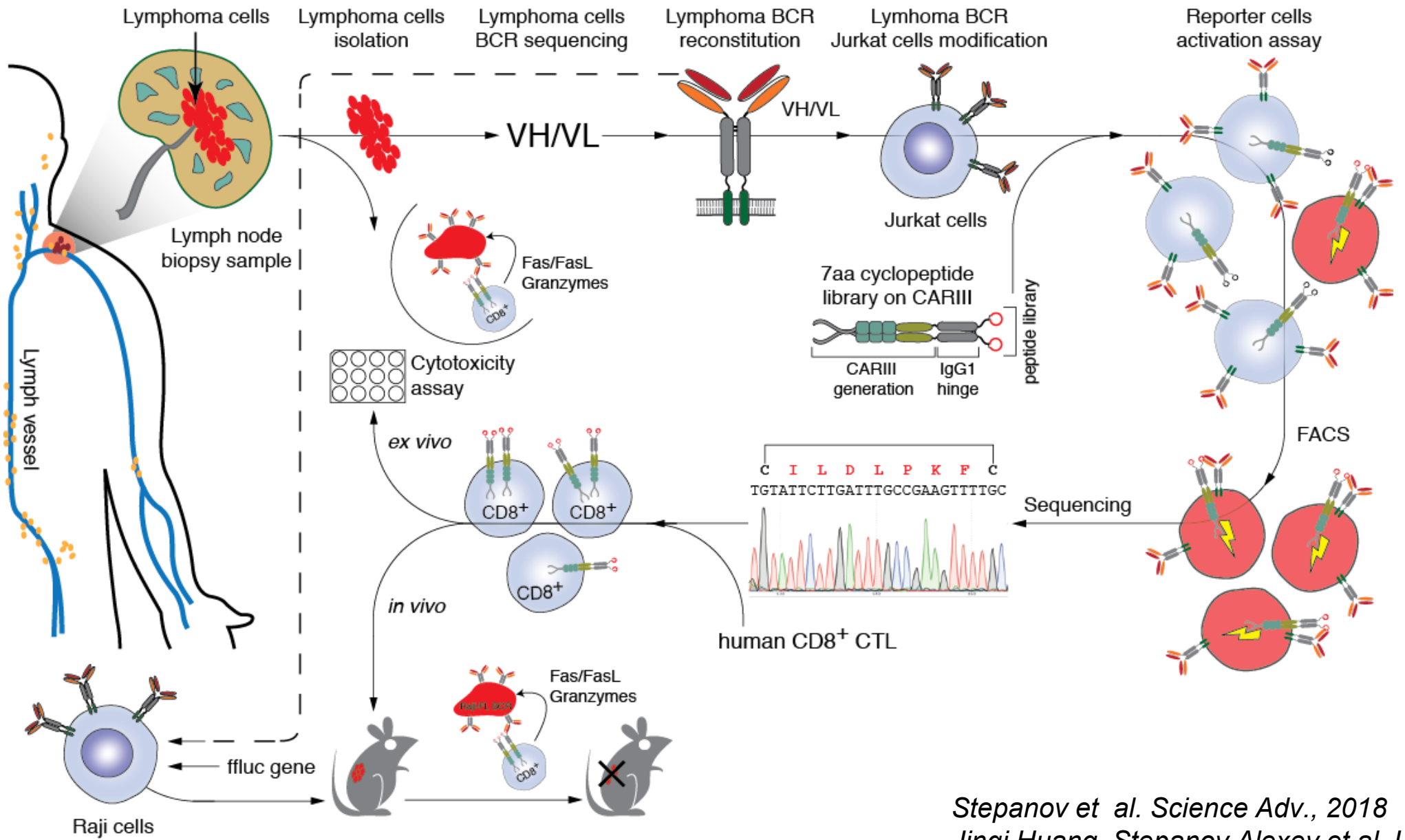
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MESSAGE

Combinatorial selection of peptides to develop novel CAR-mediated technology

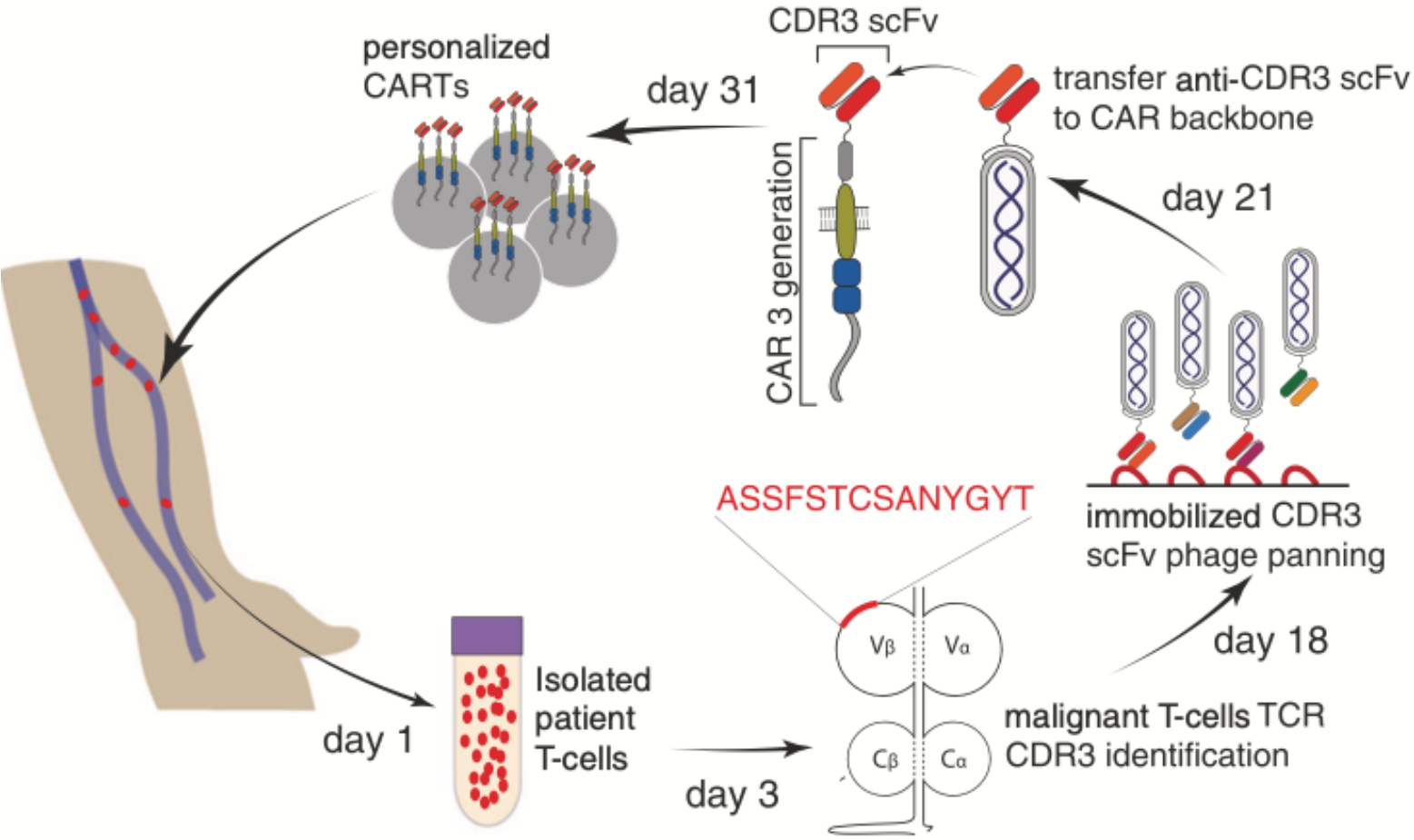
Autocrine-based selection of ligands that target lymphoma cells utilizing redirected CTLs

BioCat Lab



Stepanov et al. Science Adv., 2018
Jinqi Huang, Stepanov Alexey et al. Leukemia, 2019

Unique CDR3 epitope targeting by CAR-T cells is a viable approach for treating T-cell malignancies

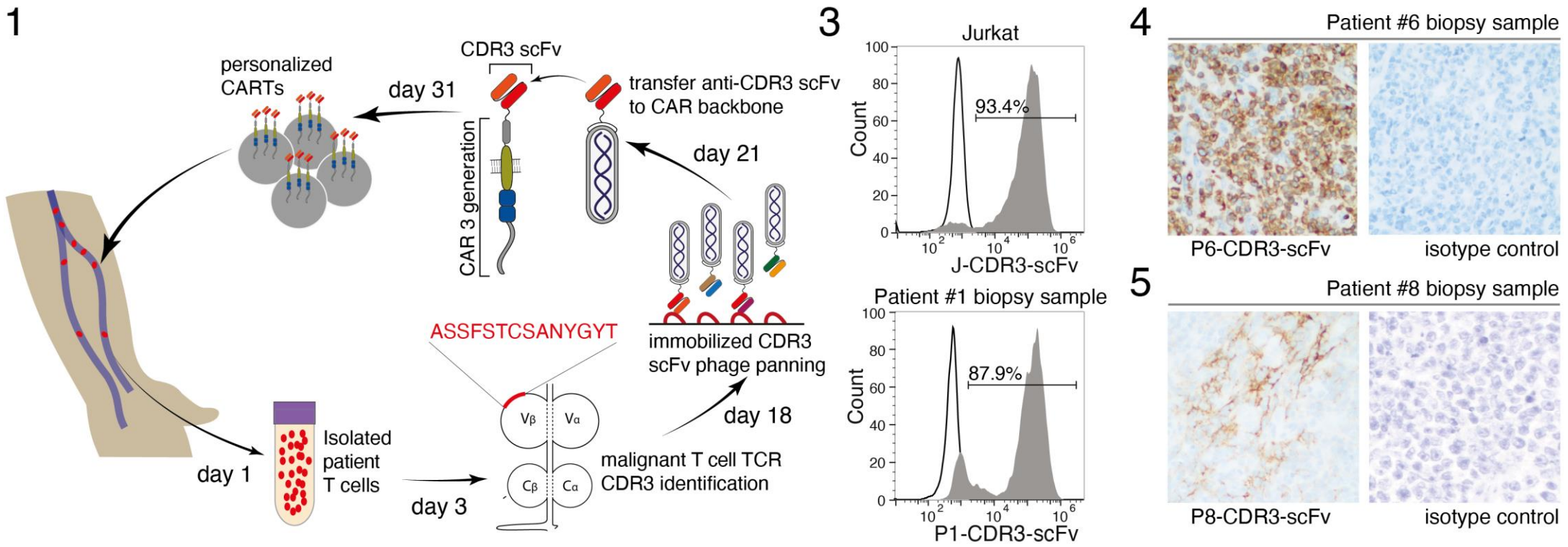


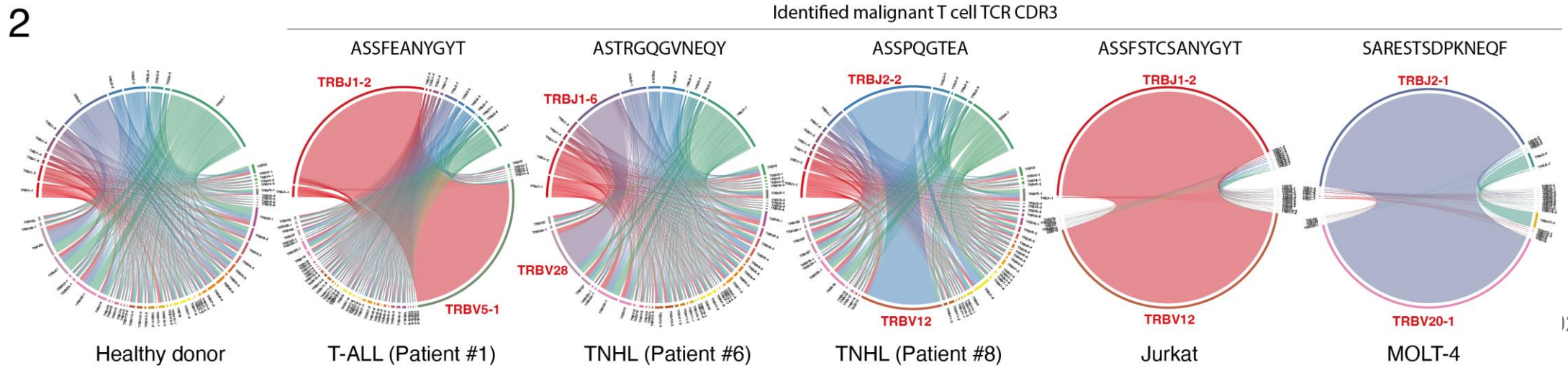
Workflow for the selection of ligands for personalized CDR3-selective CART therapy for lymphoma and leukemia:

- A biopsy sample from a patient with lymphoma or leukemia was isolated, and the collected tumor cells were utilized for identification of the TCR CDR3 sequence on malignant T cells.
- The identified CDR3 sequences were chemically synthesized and used for scFv phage panning.
- Selected scFv clones were sequenced and transferred to the CAR backbone.
- Autologous T cells were modified by personalized patient CDR3-selective CARs.

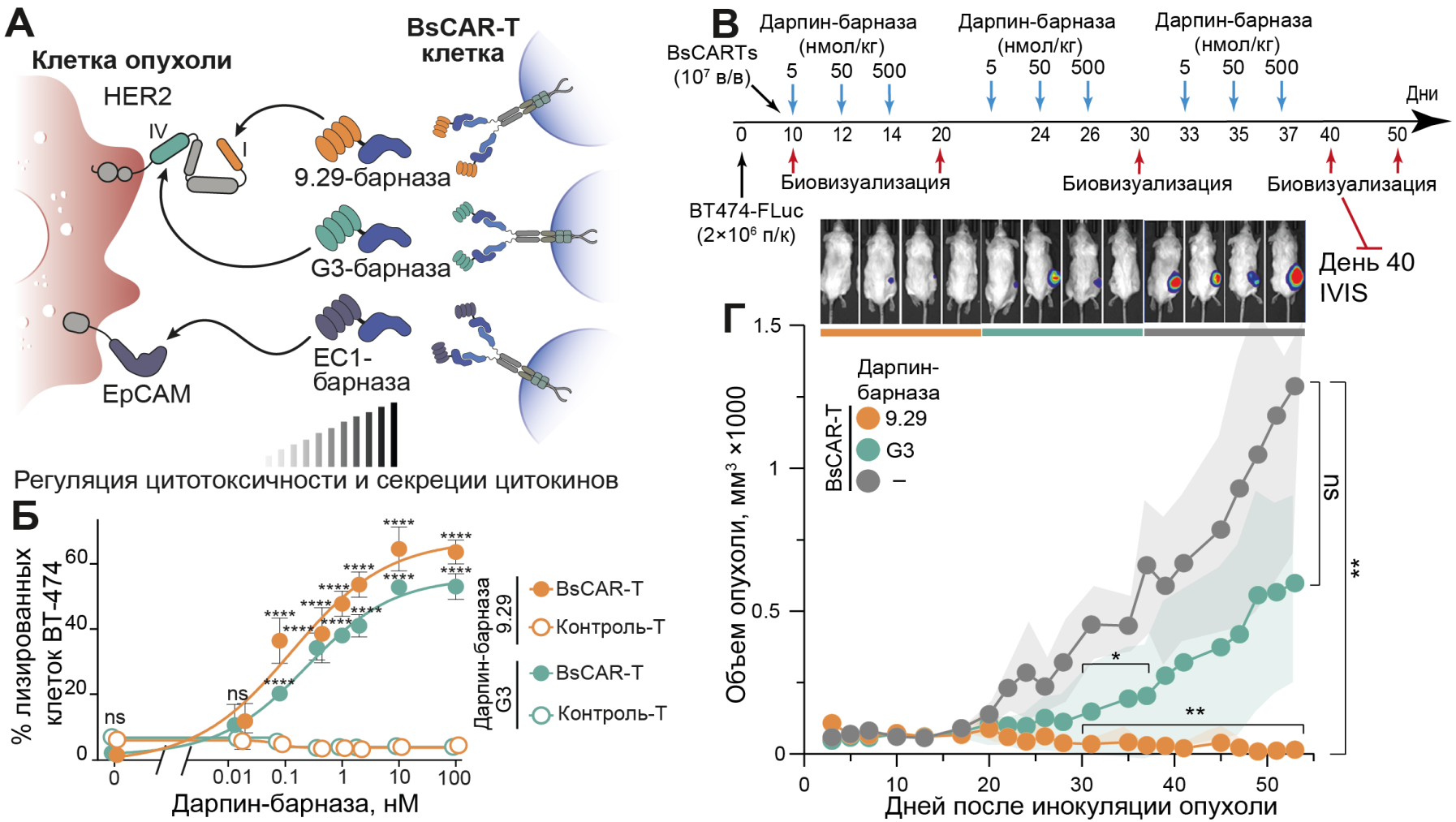
Leukemia 2019

Unique CDR3 epitope targeting by CAR-T cells is a viable approach for treating T-cell malignancies





Monitoring CAR-T pharmacokinetics.Switchable CARs based on the Barnaza-Barstar system



Stepanov et al., PNAS, 2022

Catalytic Chimeric Antigen Receptor for the Remote Control Over Therapeutic T Cells

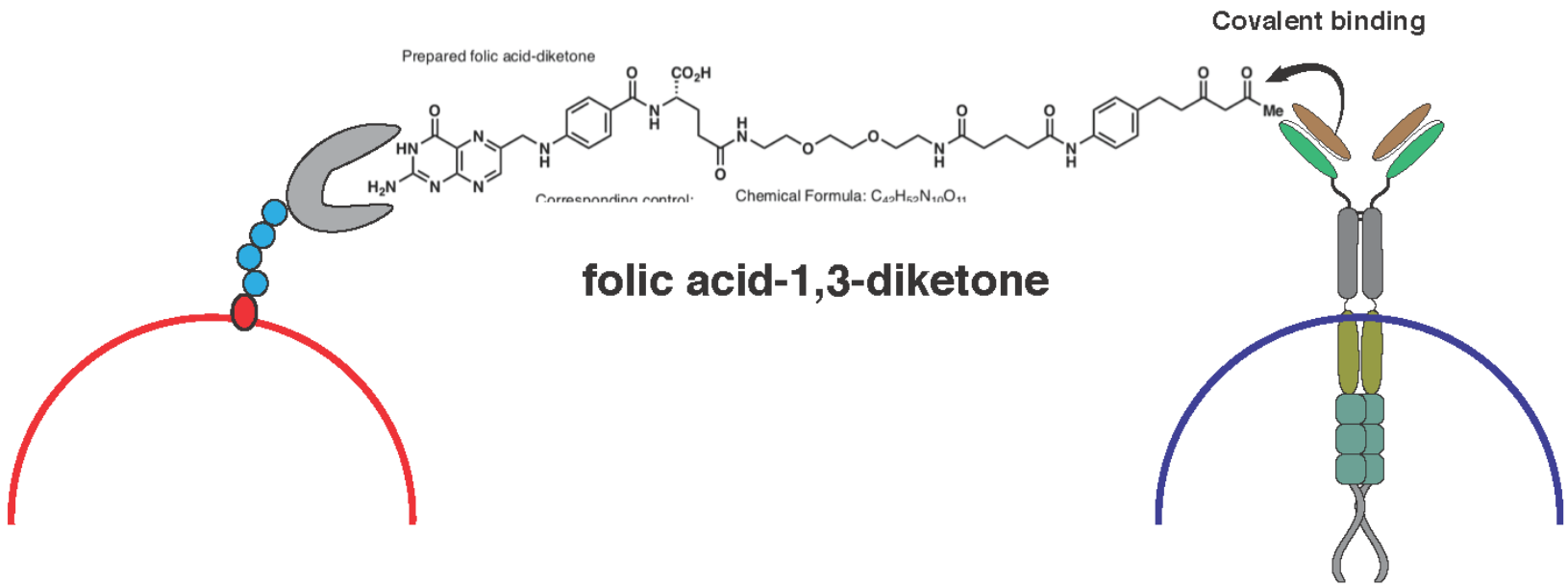
BioCat Lab

OFF

ON

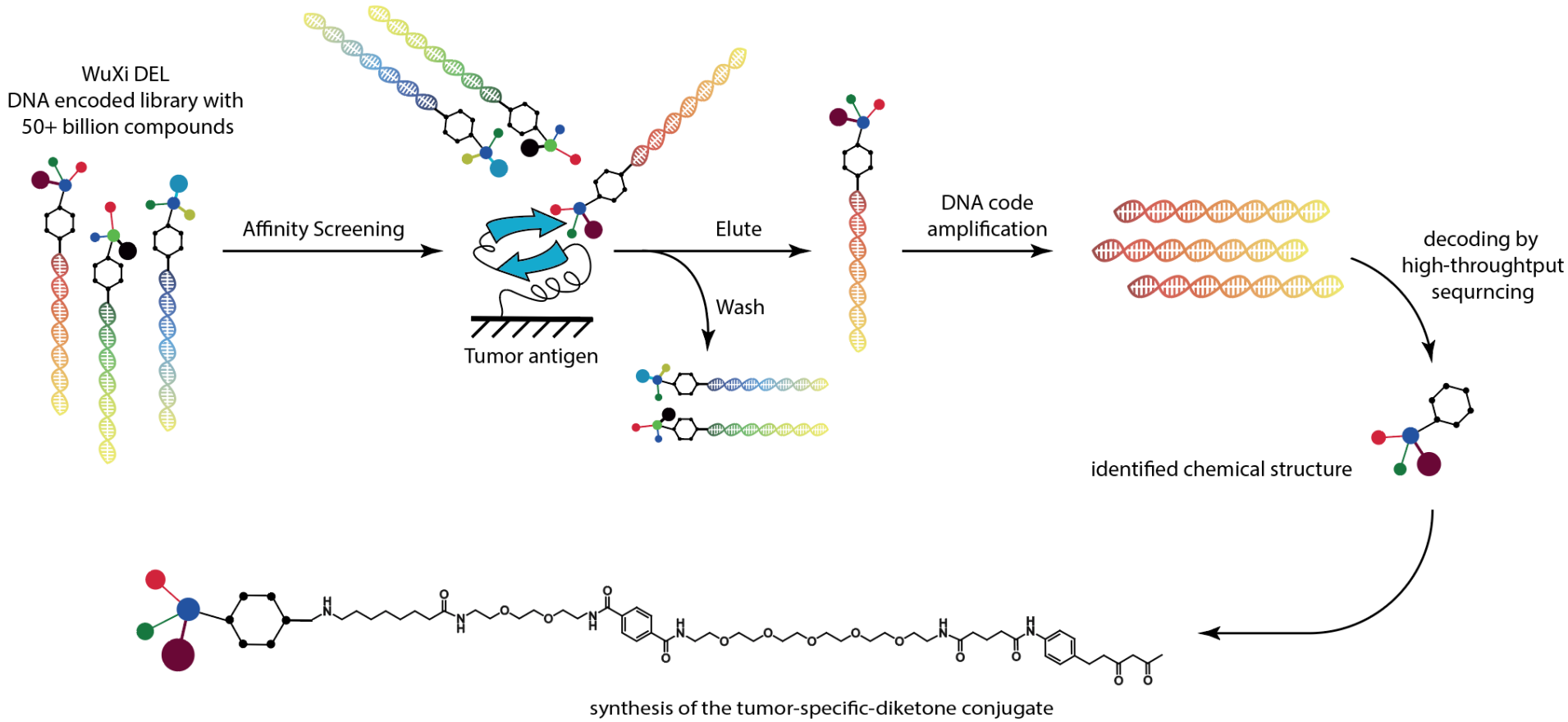
60% efficiency

tunable



Stepanov et al., Nature Biomed. Eng., 2023

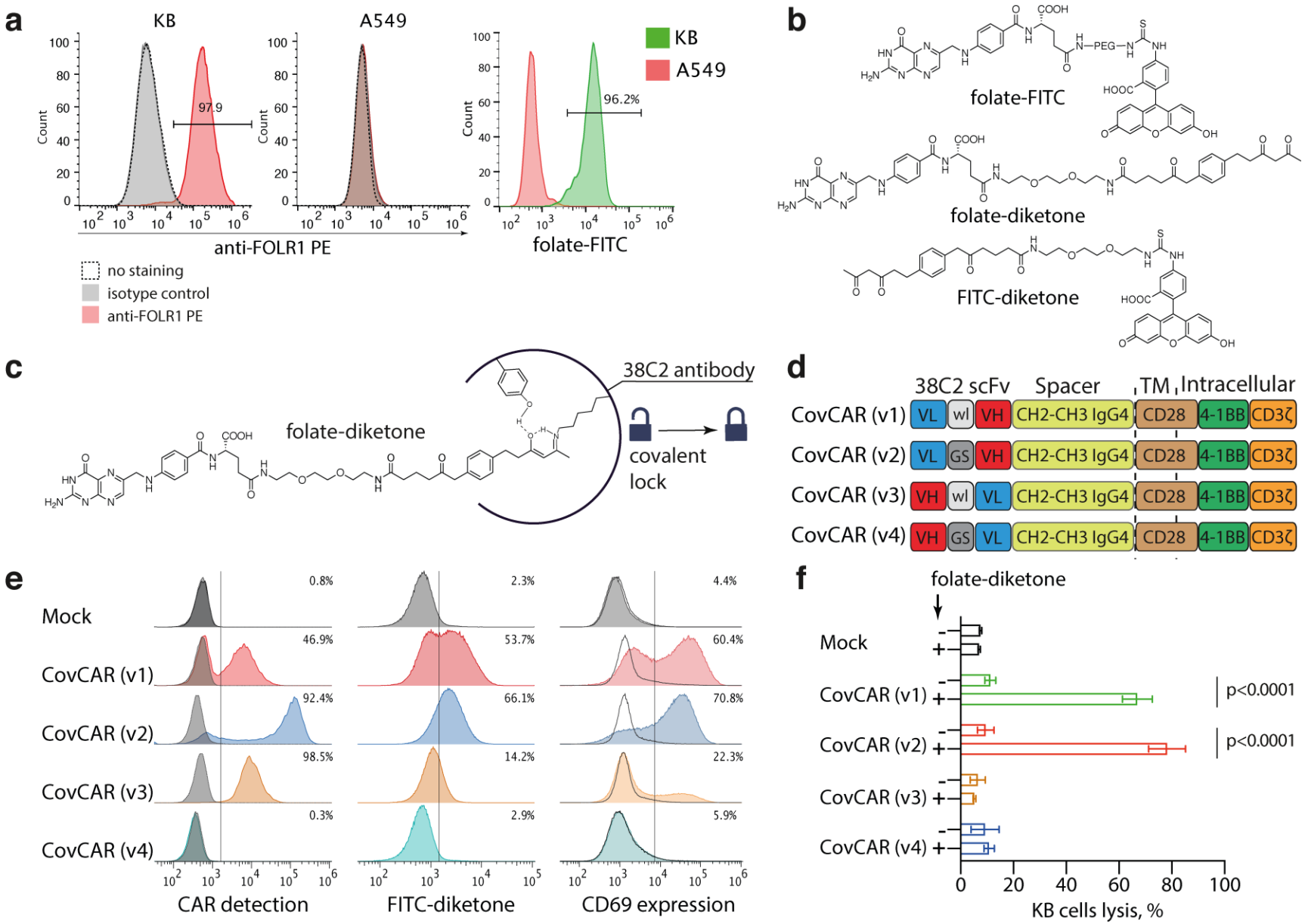
Generation of covalent CAR-Ts using organic adaptors selected from DNA-encoded libraries



Since the covalent bond-forming element of the CovCAR-T is universal, it can be engaged anew by simply using the power of DELs to change the small molecule portion of the adaptor. This could be very useful clinically. For example, within the therapeutic course of a single patient this might be necessary if the original surface protein is down-regulated, if metastatic cells express a different surface protein or, less likely, if the patient develops a different type of cancer. Of course, the T cell component of a CovCAR-T is still subject to the usual histocompatibility constraints, however the bi-functional adaptor controlling covalent bond formation and tumor cell specificity is truly universal.

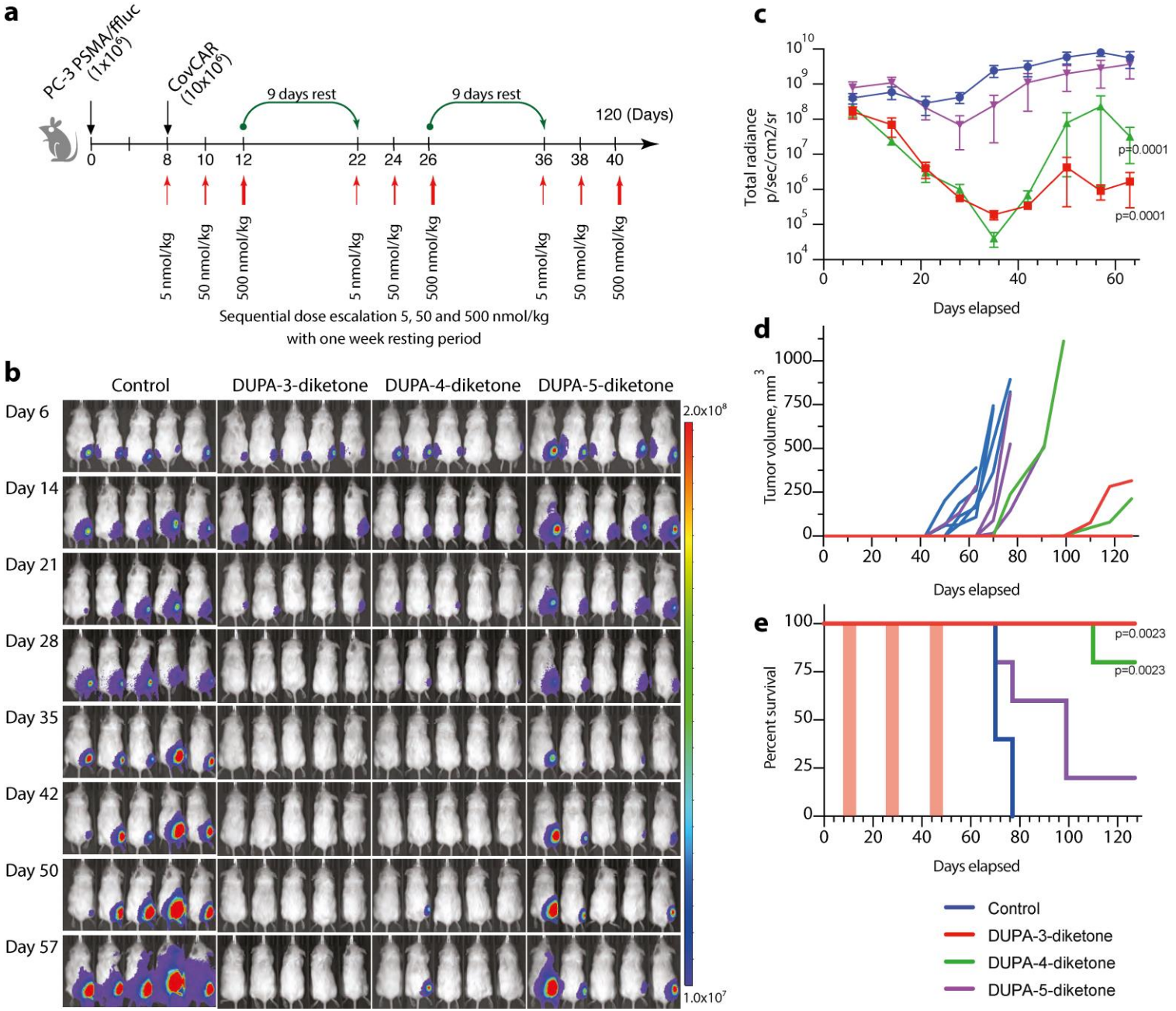
Nature Biomedical Engineering 2023

Control of the antitumour activity and specificity of CAR T cells via organic adapters covalently tethering the CAR to tumour cells.



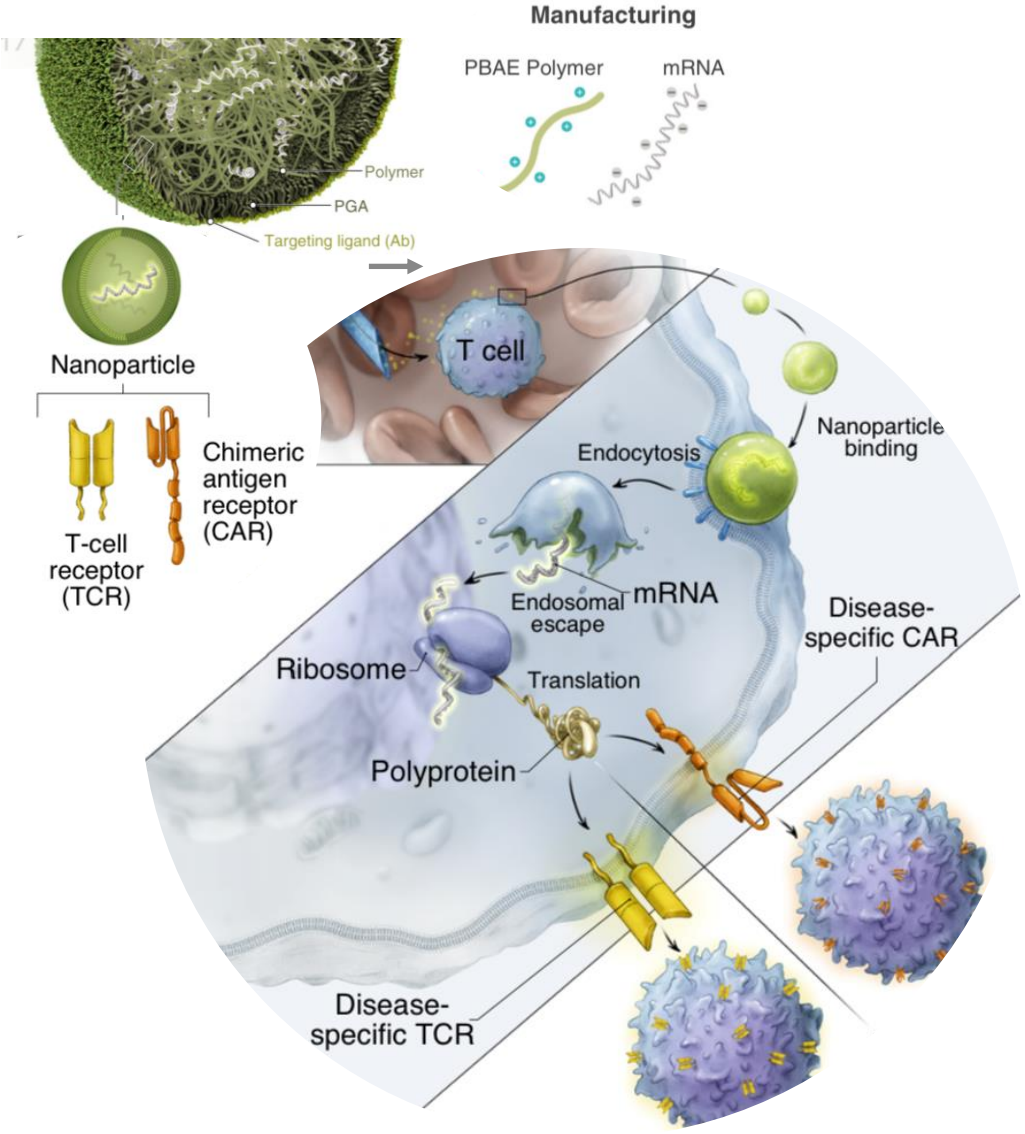
Nature Biomedical Engineering 2023

In vivo activity of CovCAR-T cells and DUPA-diketone conjugates in prostate cancer model



Nature Biomedical Engineering 2023

Genetic modification of CAR-T cells *in vivo*

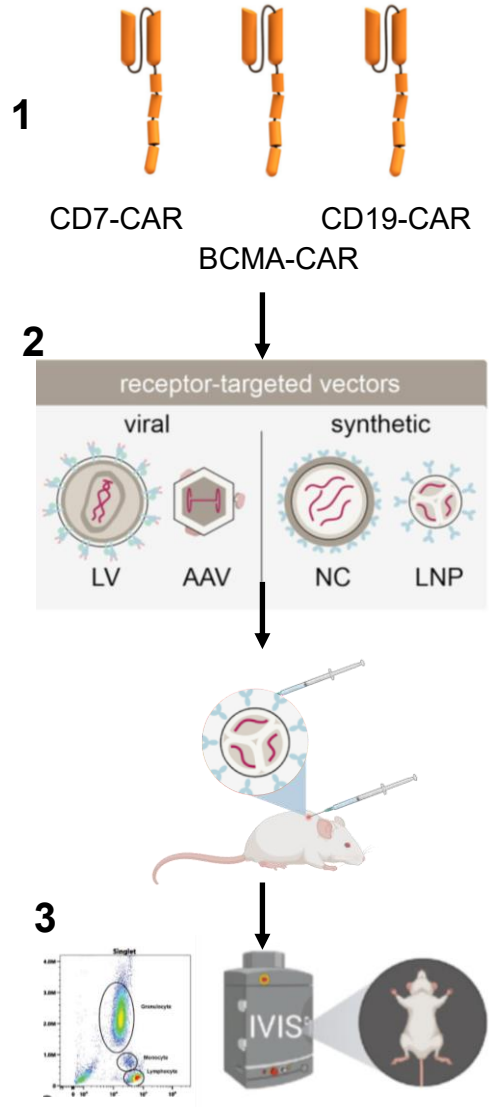


Major steps:

1. Creation of CAR constructs and synthesis of CAR-containing mRNA

2. Characterization of mRNA-containing LNPs/viruses *in vivo*

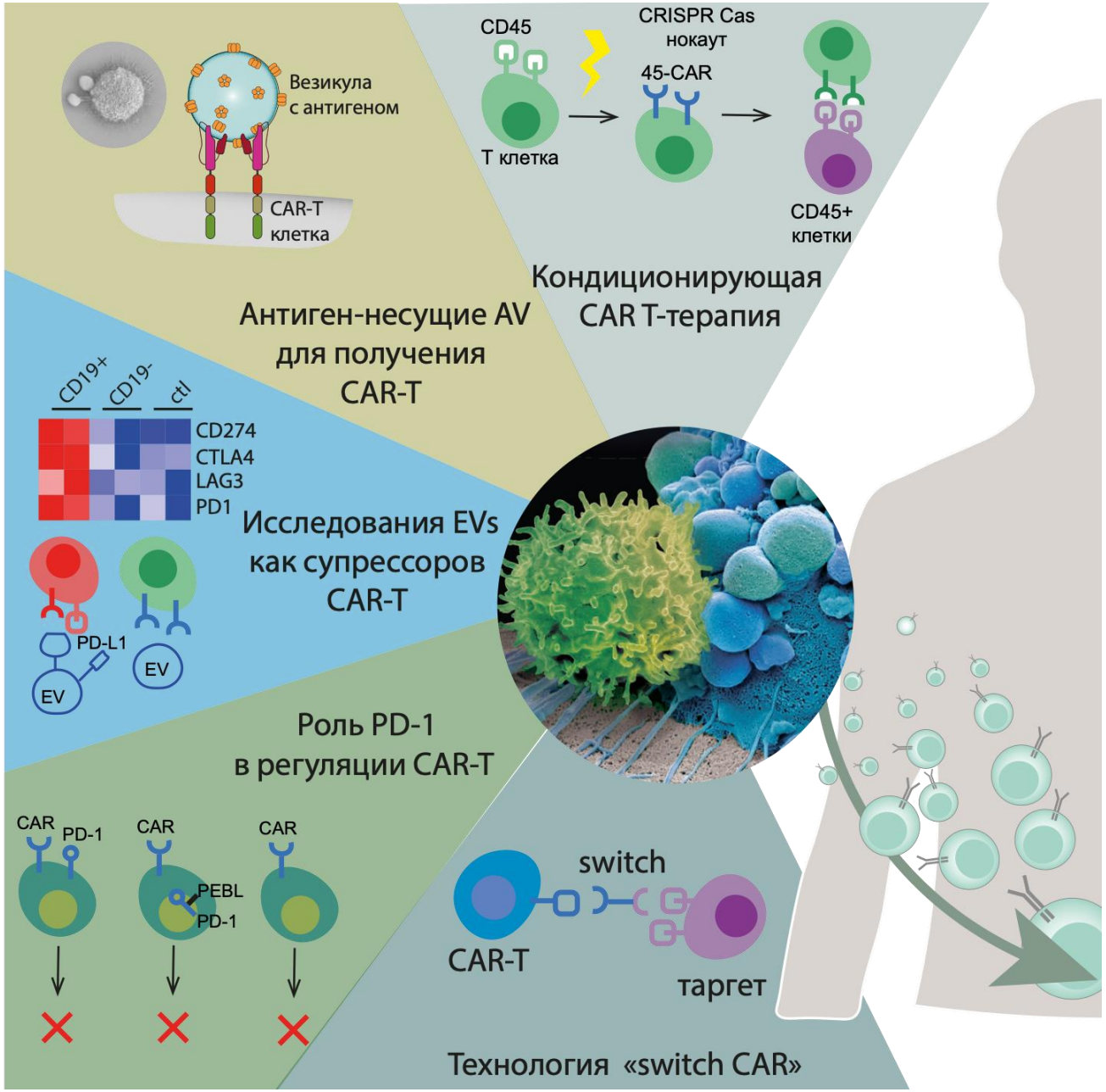
3. Evaluation of the safety, efficacy and cytotoxic potential of CAR T cells (preclinical studies)



AI-guided mRNA structure generation

https://doi.org/10.1016/j.ymthe.2022.05.018
https://doi.org/10.1038/s41467-020-19486-2
https://doi.org/10.1016/j.tips.2024.03.004

Результаты, полученные ранее в ИБХ РАН



Science Advances AAAS

Stepanov AV et al. *Science Adv* 2019

Leukemia

Stepanov AV et al. *Leukemia* 2019

nature biomedical engineering

Stepanov AV et al. *Nat Biomed Eng* 2023

Molecular Therapy Oncology

Stepanova VM et al. *Mol Ther Onc* 2024

PNAS

Stepanov AV et al. *PNAS* 2022

small science Open Access

Ukrainskaya VM et al. *Small* 2021

blood advances

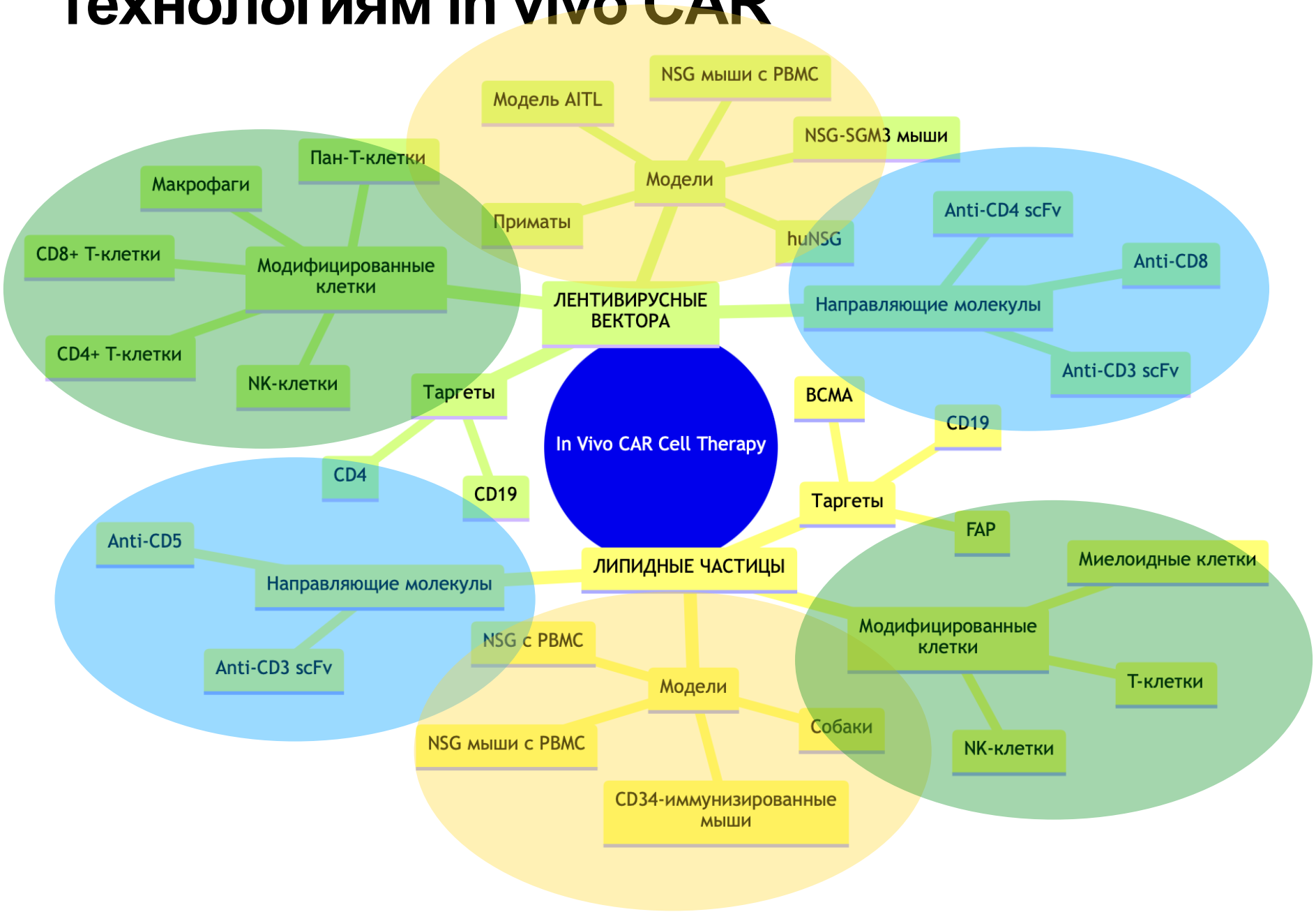
Ukrainskaya VM et al. *Blood Adv* 2020

frontiers

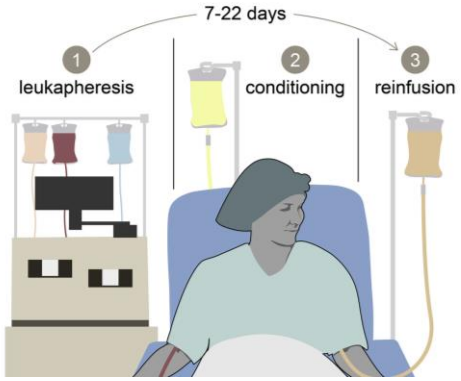
Kalinin RS et al. *Frontiers* 2021

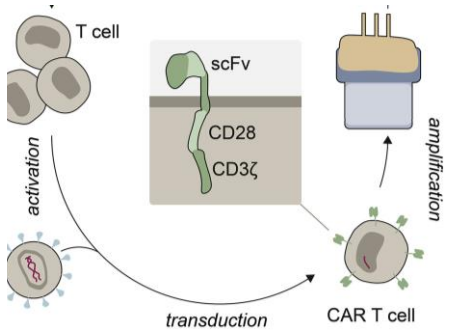
Clinical Infectious Diseases

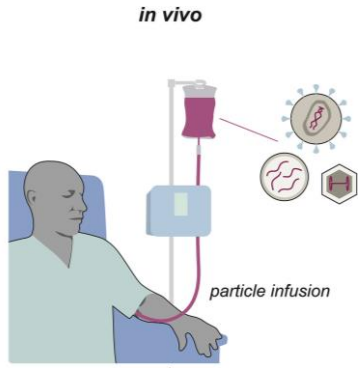
Mind-map по существующим технологиям in vivo CAR

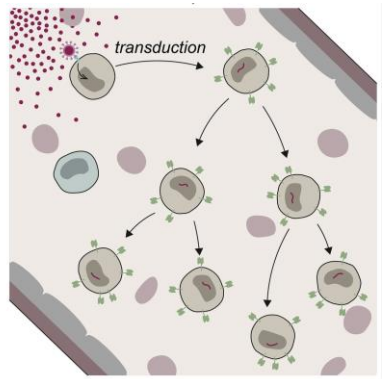


Ex vivo в сравнении с in vivo









+	−
Возможность создания терапии на основе клеток здорового донора	
Возможность ex vivo контроля клеточного продукта	Сложность при использовании для солидных опухолях
Есть препараты, прошедшие клинические испытания	Проблема с контролем качества
Более доступная процедура модификации Т-клеток	Долго, сложно и дорого

+	−
Одновременная модификация нескольких типов клеток	Низкая эффективность трансдукции
Физиологичная активация	Возможность модификации только Т-клеток пациента
Создание универсальной терапии на локальных площадках	Сложность дизайна вирусов/ частиц - синтез белков/мРНК
Быстрый доступ к терапии	Недоступность различных видов рака (Т-клеточные лимфомы)

nature medicine

Article

https://doi.org/10.1038/s41591-026-04244-6

In vivo generation of anti-BCMA CAR-T cells in relapsed or refractory multiple myeloma: a phase 1 study

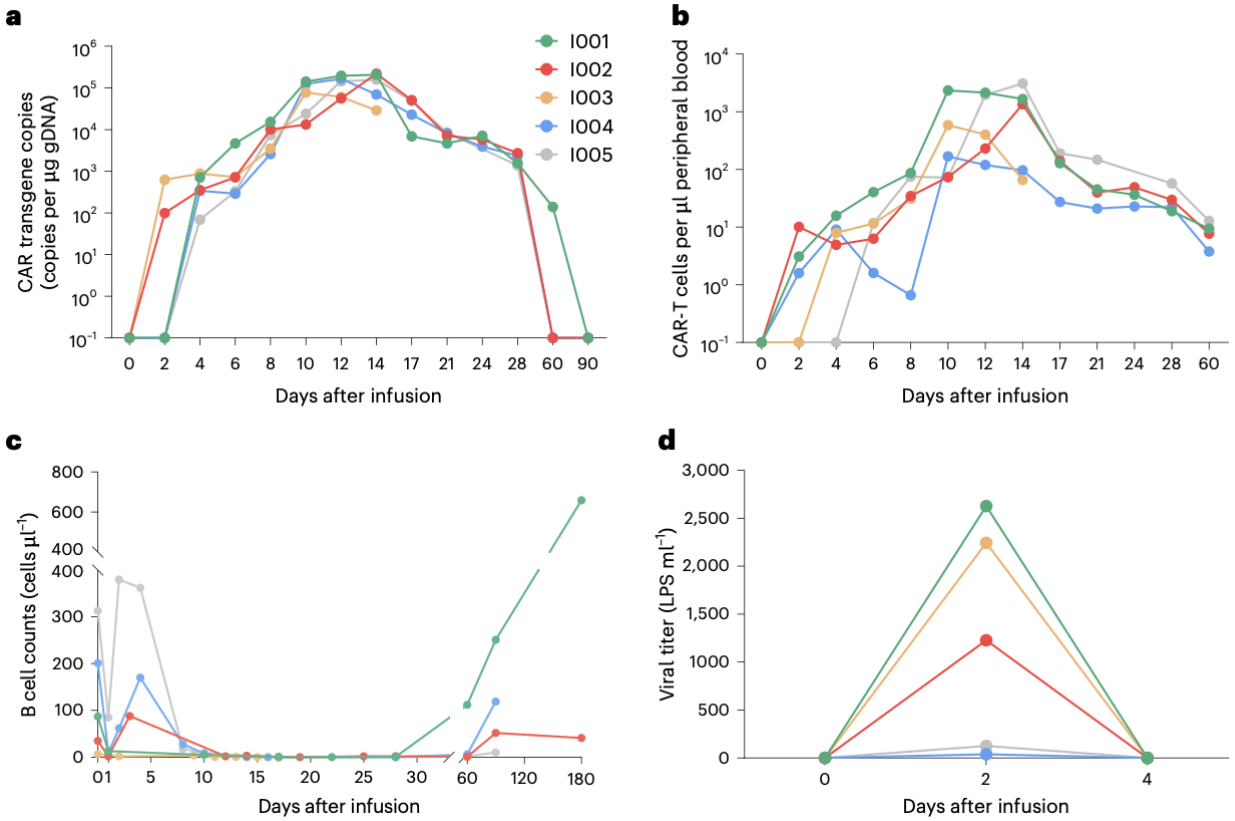
Received: 21 July 2025

Accepted: 26 January 2026

Published online: 25 March 2026

Ning An[1,6], Di Wang[1,6], Peiling Zhang[1,6], Jishuai Zhang[2,6], Philippe Parone[3,6], Jianlin Hu[1], Yuhan Bao[1], Li Xu[1], Haitao Ruan[1], Ying Wan[1], Xinyu Wen[1], Yang Gao[1] & Chunrui Li[1,4,5]

- 5 пациентов с множественной миеломой
- Лентивирус с VSV-G мутантным белком основанный на VHH к Pan-T (anti-TCR)
- anti-BCMA CAR терапия (однократная инфузия вируса)

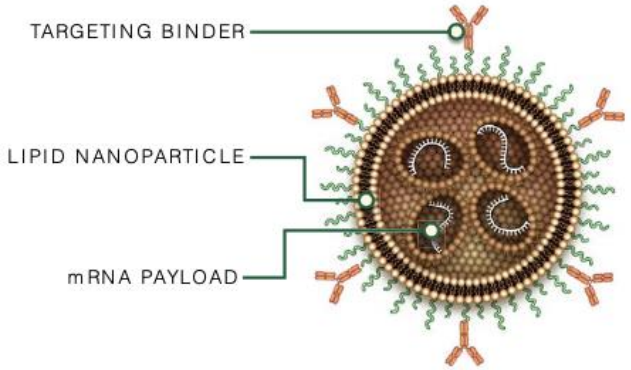


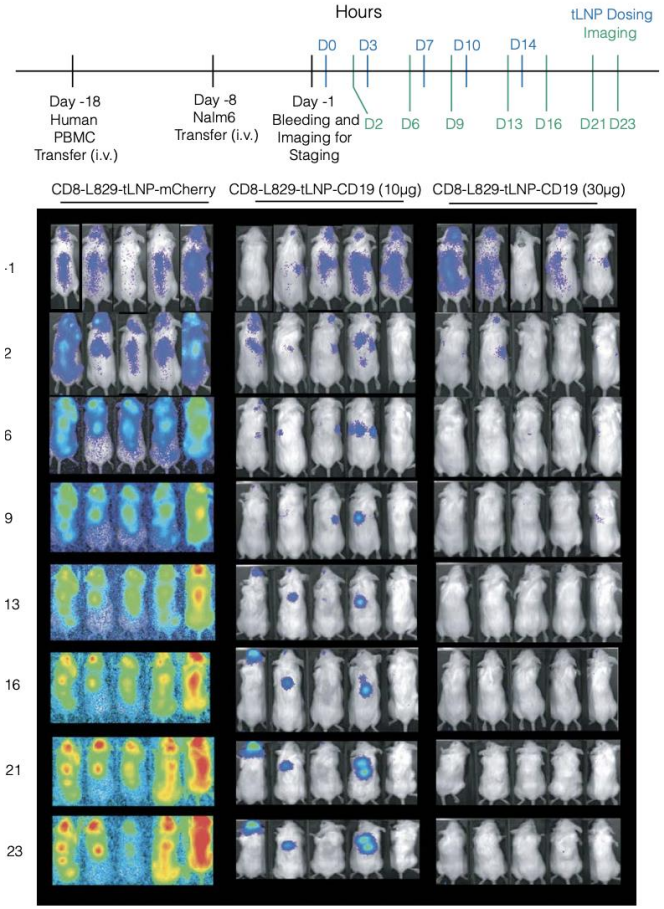
IMMUNOTHERAPY

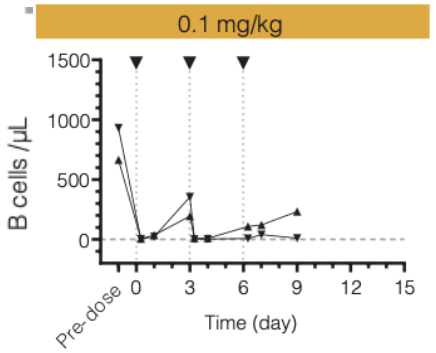
In vivo CAR T cell generation to treat cancer and autoimmune disease

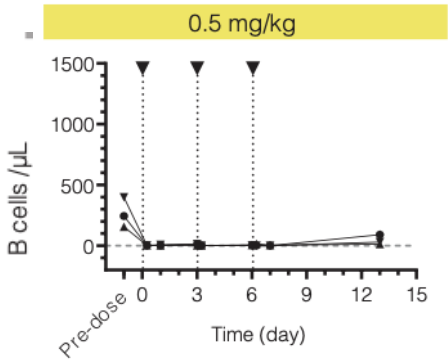
Theresa L. Hunter[1], Yanjie Bao[1], Yan Zhang[1], Daiki Matsuda[1], Romina Riener[1], Annabel Wang[1], John J. Li[1], Ferran Soldevila[1], David S. H. Chu[1], Duy P. Nguyen[1], Qian-Chen Yong[1], Brittany Ross[1], Michelle Nguyen[1], James Vestal[1], Scott Roberts[1], Diana Galvan[1], Jerel Boyd Vega[1], Donald Jhung[1], Matthew Butcher[1], Josephine Nguyen[1], Stanley Zhang[1], Claudia Fernandez[1], Jeffrey Chen[1], Carolina Herrera[1], Yi Kuo[1], E. Michael Pica[1], Goutam Mondal[1], Andrew L. Mammen[2], John Scholler[3], Steven P. Tanis[1], Stuart A. Sievers[1], Aric M. Frantz[1], Gregor B. Adams[1], Laura Shawver[1], Ramin Farzaneh-Far[1], Michael Rosenzweig[1], Priya P. Karmali[1], Adrian I. Bot[1], Carl H. June[3,4,5]*, Haig Aghajanian[1,3]*

- Доклиническое исследование mRNA LNP CAR терапии при аутоиммунных заболеваниях на мышах и обезьянах
- Таргетирование CD8+ клеток частицами
- anti-CD19 CAR терапия (многократная инфузия mRNA LNP)

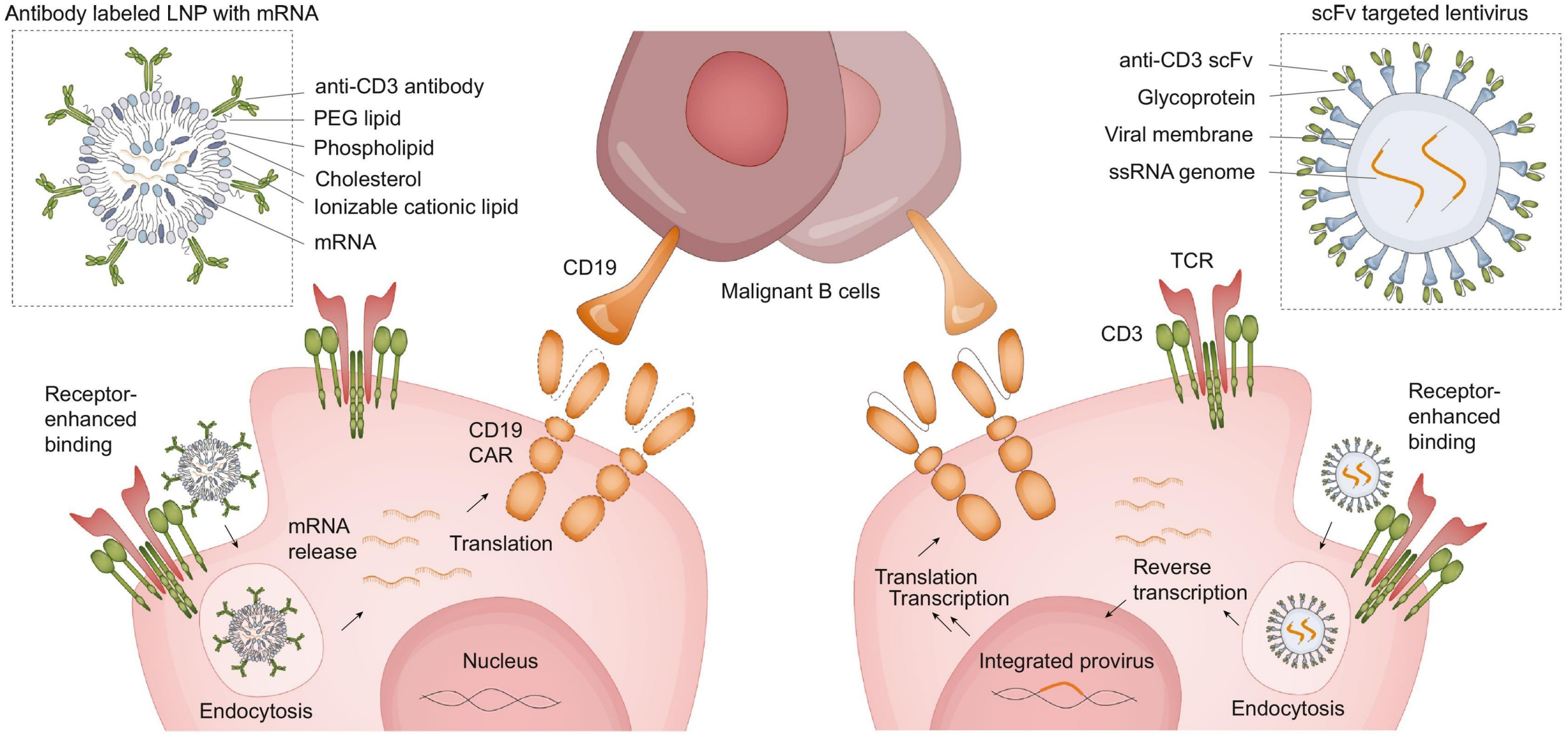








Methods of transgene delivery



https://doi.org/10.1016/j.tips.2024.03.004

Компании на рынке in vivo препаратов

In Vivo CAR-T Competitive Landscape

Company	Intended Target Cells	CAR Targets	Route of Administration
Lentiviral			
ABINTUS BIO	CD4+ T cells, CD8+ T cells, NK cells, NKT cells, and γδ T cells	CD19	IV
EXUMA Biotech	CD3+ T cells	Undisclosed	ILN
interius	CD7+ cells (T cells and NK cells)	CD20 BCMA CD19 BCMA + undisclosed target	IV
kelonia	T cells	BCMA	IV
Sana Biotechnology	CD8+ T cells CD4+ T cells	CD19 CD22 BCMA	IV
Umoja	CD3+ T cells	CD19 PSMA CA IX FAP	ILN
MUSTANGBIO	Activated T cells	CD20	ID+ILN
Lipid Nanoparticle (LNP)			
capstan therapeutics	CD8+ T cells CD2+ cells (T and NK cells) CD5+ cells (T, NK, and Macs)	CD19 CD20 BCMA FAP Other undisclosed targets	IV
carisma THERAPEUTICS moderna	Macrophages	Undisclosed	Undisclosed
ORNA™	Multiple immune cells	Undisclosed	Undisclosed
TESSERA	CD4+ and CD8+ T cells	Undisclosed	IV
MYELOID Therapeutics, Inc.	Myeloid cells	TROP2 GPC3	Undisclosed
Non-Lipid Nanoparticle			
ixaka	CD3+ cells	CD19	Undisclosed
TIDAL sanofi	Immune cells	Undisclosed	Undisclosed
Adenovirus-Based Particles			
Vector BioPharma	Undisclosed	Undisclosed	Undisclosed
ensoma	Undisclosed	Undisclosed	Undisclosed
Other/Undisclosed Technology			
EsoBiotec	Undisclosed	Undisclosed	Undisclosed
GIGAMUNE	Undisclosed	Undisclosed	Undisclosed
ImmTune Therapies	Undisclosed	Undisclosed	Undisclosed

FAP, fibroblast activation protein; FR, folate receptor; TROP, troponin; BCMA, B-cell maturation antigen; GPC3, glypican-3

Sources: Company reports; William Blair Equity Research

Первый препарат - Interius
NCT06539338

INT2104 specifically targets CD7-positive T and NK cells and delivers a CAR (chimeric antigen receptor) transgene to create effector CAR-T and CAR-NK cells *in vivo*. The CAR cells are then intended to target CD20-positive B cells for the treatment of B cell malignancies.

Возможно ли использовать in vivo CAR-T против глиобластомы?

Внутривенно

Интратекально

Интравентрикулярно

Интрапаренхиматозно

1. Повышение проницаемости ГЭБ

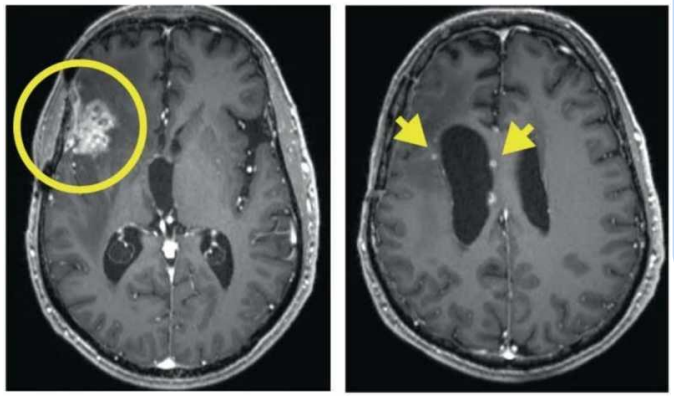
   Focused ultrasound (FUS)

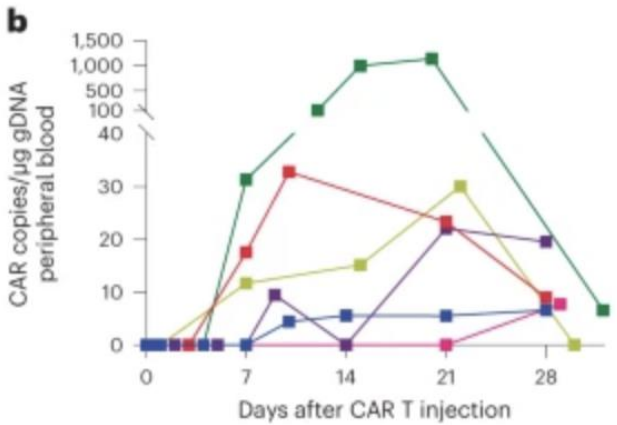
2. Изменение миграции CAR-T клеток

   CAR-T + Хемокины (CXCR2 , CCR4 , CCR2b , CXCR3)
   CAR-T + Ninjurins
   CAR-T + IL-7

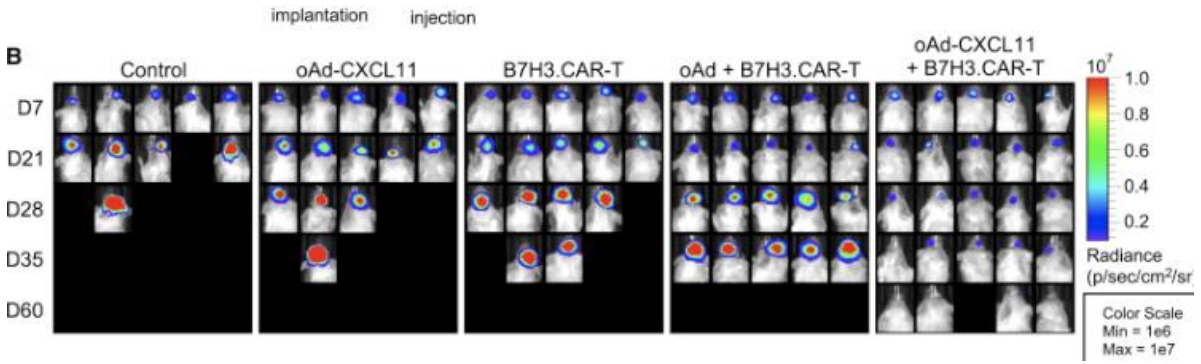
3. NK-CAR, “Armored” CAR

4. CXCL11+ онкологические аденовирусы



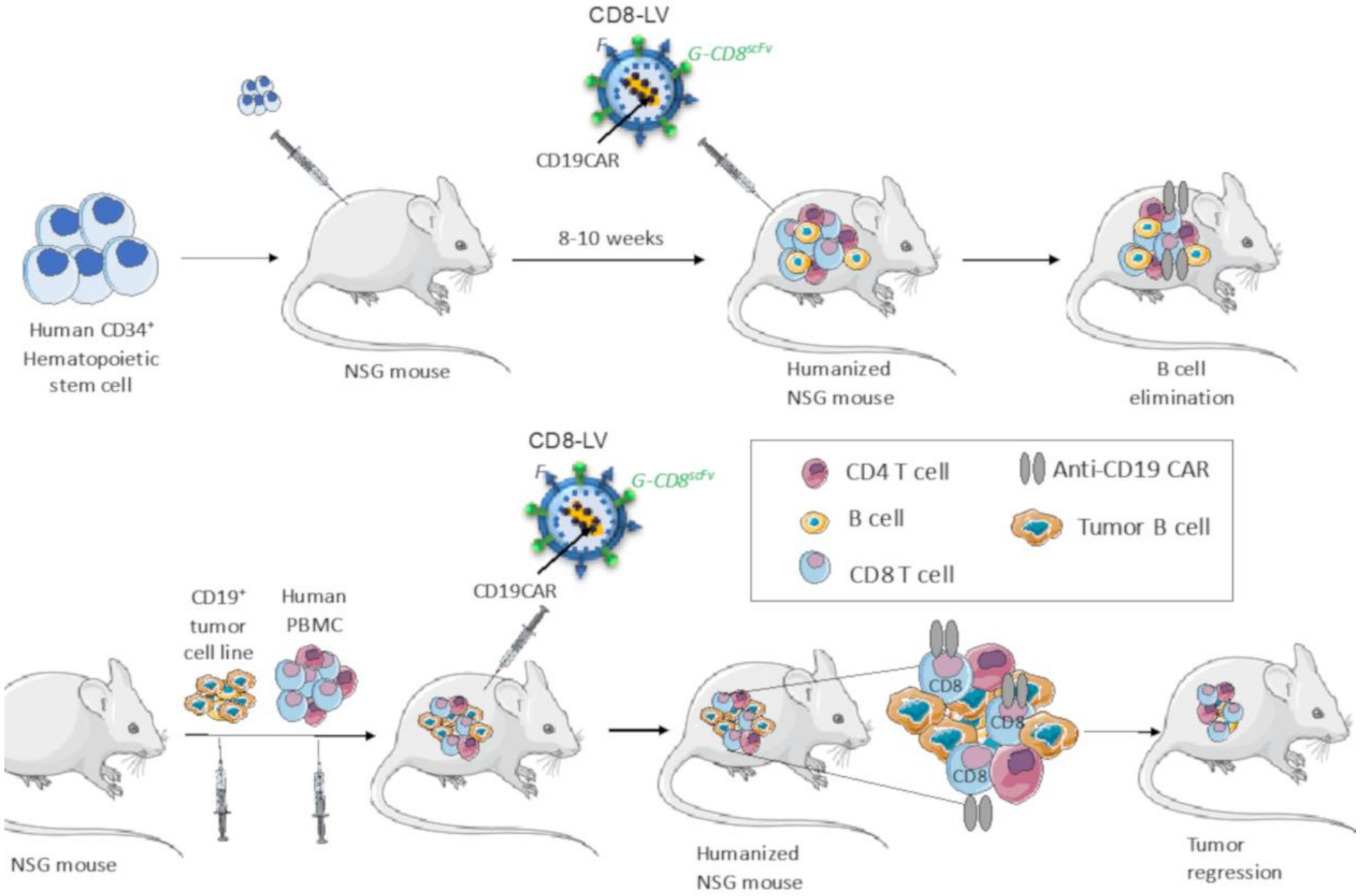


DOI: 10.1038/s41591-024-02893-z



DOI: 10.1016/j.ymthe.2022.08.021

Какую in vivo модель выбрать для in vivo CAR терапии?



doi:10.3390/cancers12071915

Использование приматов для исследования иммунотерапии



Macaca fascicularis

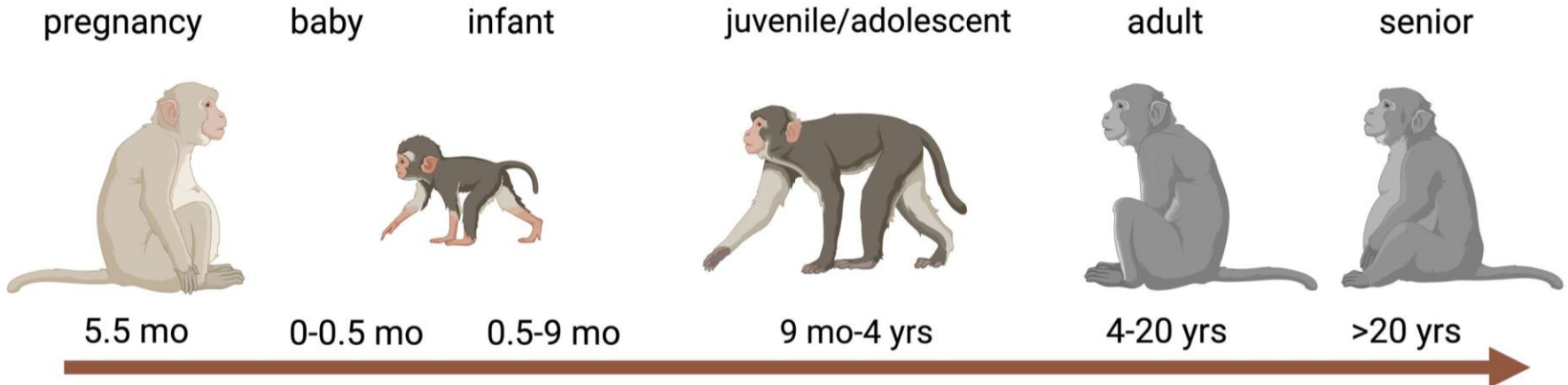


Macaca resus

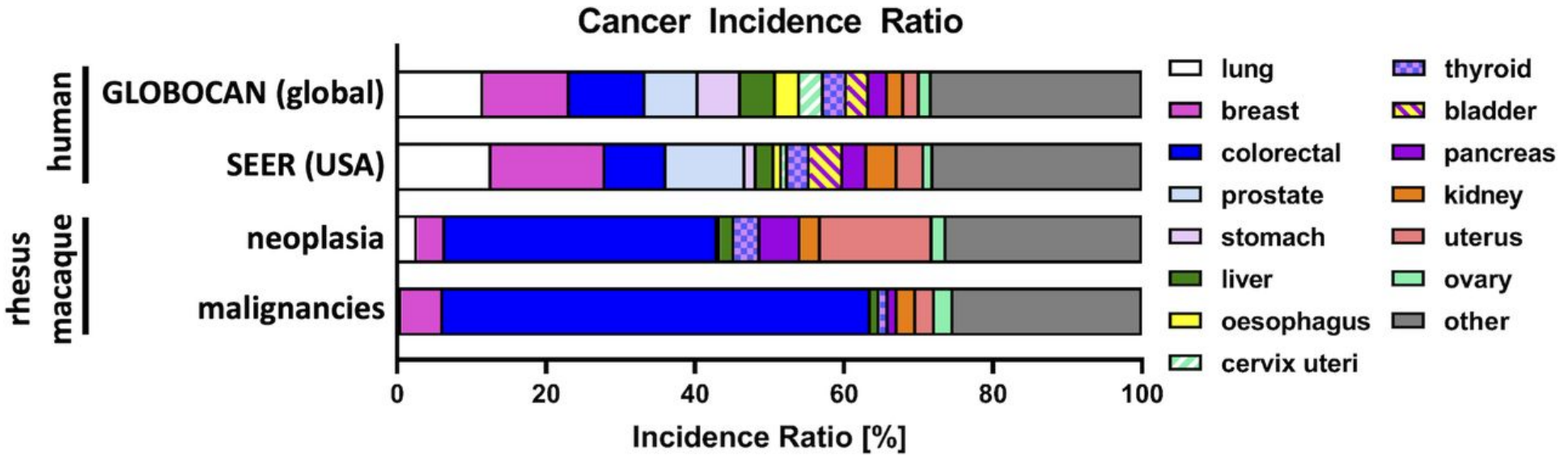


Marmosets

Аспект	Человек	Приматы (макаки-резус / макаки-крабоеды)
MHC класс I гены	HLA-A HLA-B HLA-C	Mamu-A, Mamu-B (нет аналога HLA-C)
Многообразие MHC класса I	Один аллель на локус	Несколько аллелей на хромосому → более высокое разнообразие гаплотипов
TLR-сигнальный путь в макрофагах	Активирует рецептор витамина D → синтез кателицидина (CAMP) через VDRE	То же самое (присутствует VDRE в промоторе CAMP)
Фенотип макрофагов	Разнообразен по тканям	То же: фенотипически и функционально разнообразны
NK-клетки	Экспрессируют NKG2 CD16 KIRs	
Подтипы Т-клеток	CD4+ CD8+ Treg Th1/Th2/Th17	
Спонтанные опухоли	Частые, особенно при старении	Развиваются редко



Спонтанно возникающие опухоли у приматов



Deycmar S, Gomes B, Charo J, et al Spontaneous, naturally occurring cancers in non-human primates as a translational model for cancer immunotherapy Journal for ImmunoTherapy of Cancer 2023;11:e00







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Institute of Bioorganic Chemistry,
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Anton Domnin



Yaroslav Solovev



Nikita Kostin

RUSSIA

Smirnov Ivan, Stepanov Alexey, Belogurov Alexey,
Zakharova Maria, Kostin Nikita,
Bobik Tatiana, Lomakin Yakov,
Mokrushina Yuliana, Ponomarenko Natalia,
Mamedov Azad,
Knorre Vera, Kurbatskaya Inna.
Siberian division, RAS .

Dmitry Genkin, Dobroslav Melamed

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Département de Toxicologie – CRSSA Institut de Recherche Biomédicale des Armées