XXXI Symposium on Bioinformatics and Computer-Aided Drug Discovery (BCADD-2025)

Emerging Challenges and Opportunities for In Silico Drug Discovery

COMPUTER-AIDED ANTIMICROBIAL DISCOVERY

Structure–Antimicrobial Activity Relationships of Recombinant Host Defense Peptides Against Drug-Resistant Bacteria

Dr. William J. Zamora R

21 October, 2025

UCR

Bio^3 COMPUTACIONAL BIOPHYSICS, BIOLOGY AND BIOINFORMATICS GROUP

aToxCIA

Laboratorio de Toxicología Computacional e Inteligencia Artificial

LEBI Laboratorio de Ensayos Biológicos

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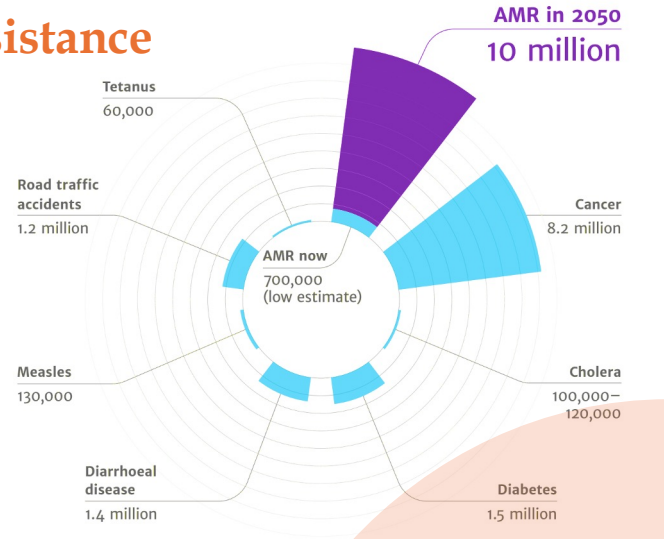
Introduction

○ Antimicrobial Resistance



World Health Organization

Global research agenda for antimicrobial resistance in human health

Policy brief

June 2023

45% related to treatment and development of new antibiotics

O'Neill J. (2016). Tackling drug-resistant infections globally: final report and recommendations. London: Review on Antimicrobial Resistance.
Laxminarayan R. (2022). The overlooked pandemic of antimicrobial resistance. Lancet (London, England), 399(10325), 606–607.

Introduction

Drug Design: A Difficult and Costly Process

frontiers | Frontiers in Drug Discovery

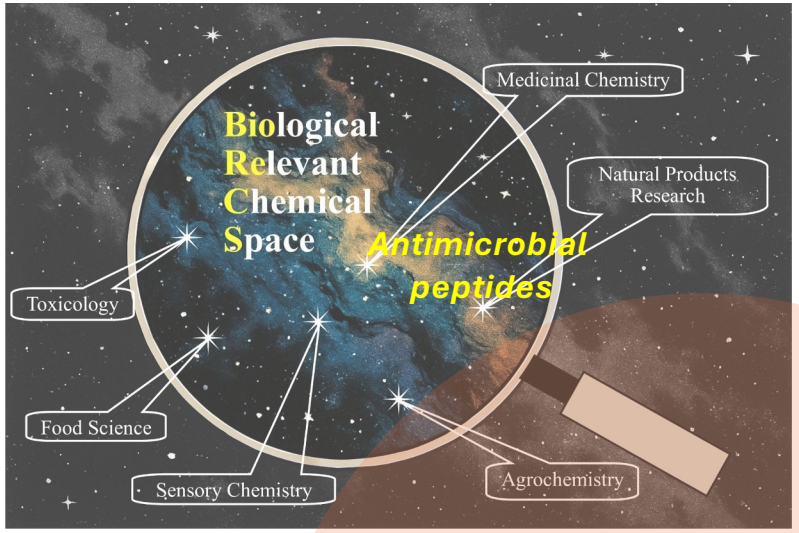
On the biologically relevant chemical space: BioReCS

José L. Medina-Franco[1]*, Edgar López-López[1,2], Juan F. Avellaneda-Tamayo[1] and William J. Zamora[3,4]

[1]DIFACQUIM Research Group, Department of Pharmacy, School of Chemistry, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Mexico City, Mexico, [2]Department of Chemistry and Graduate Program in Pharmacology, Center for Research and Advanced Studies of the National Polytechnic Institute, Section 14-740, Mexico City, Mexico, [3]CBio3 Laboratory, School of Chemistry, University of Costa Rica, San José, Costa Rica, [4]Laboratory of Computational Toxicology and Biological Testing Laboratory (LEBi), University of Costa Rica, San José, Costa Rica

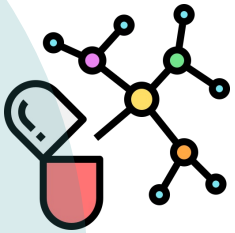
KEYWORDS

chemoinformatics, dark chemical matter, de novo design, food chemicals, metallodrugs, natural products, odor chemicals, peptides



Introduction

- Drug Development



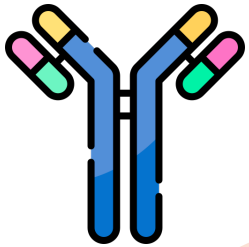
Small molecules

MW < 500 Da

Peptides

MW ~ 500–5000 Da

Antimicrobial peptides



Therapeutic proteins

MW > 5000 Da

Introduction

Small molecules

MW < 500 Da

The Rule of 5

1. 5 H-bond donors
2. 10 H-bond acceptors
3. Molecular weight < 500 Da
4. **Log *P* < 5**.

Drug Development

Acceptable level of in vivo **clearance** for a drug is **log*D* of 0–3**

log *P* < 3.25 provided the best odds for obtaining >100 μM **solubility** for a typical drug molecule (melting point of 150 °C)

$$\log(\mathrm{Sol}_{aq}) = 0.5 - \log P - 0.01(\mathrm{MP\text{-}25})$$

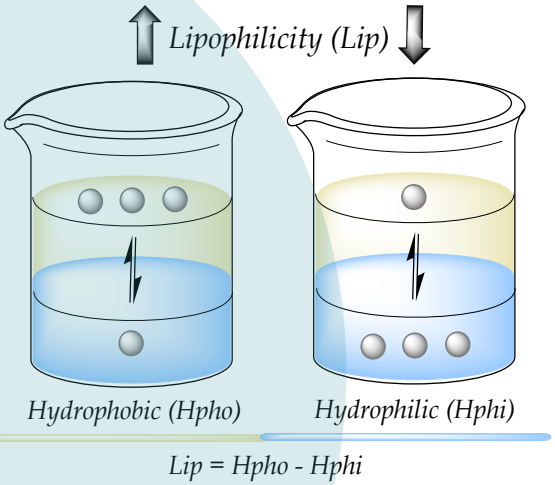
Acceptable **permeability log *D* of 1–3.5**

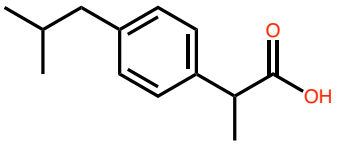
LipE should be prioritized based on the importance of **enthalpic optimization**

$$\mathrm{LipE} = \mathrm{LLE} = -\log(\mathrm{potency}) - \log D$$

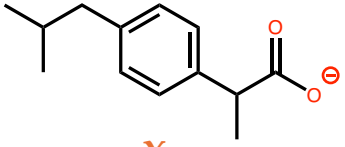
to achieve **low dose** and **dosing frequency**

Introduction

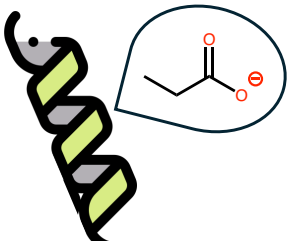


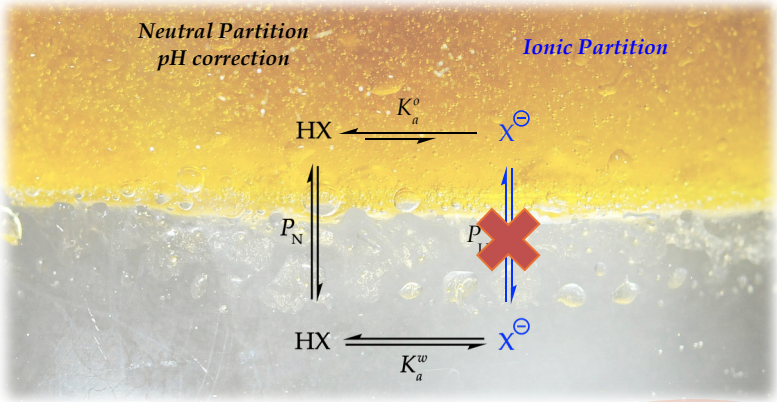


HX



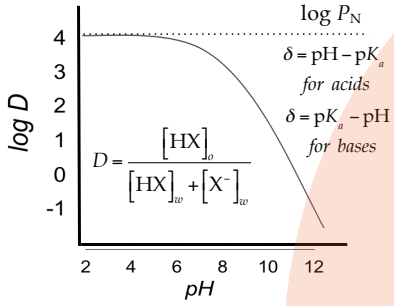
X-





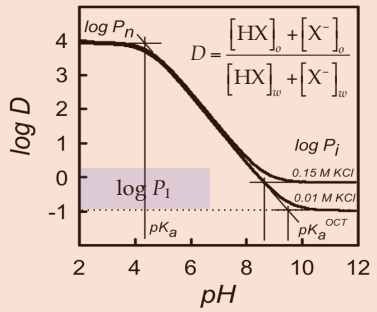
Model 1. pH Correction

$$\log D = \log P_N - \log(1+10^{\delta})$$

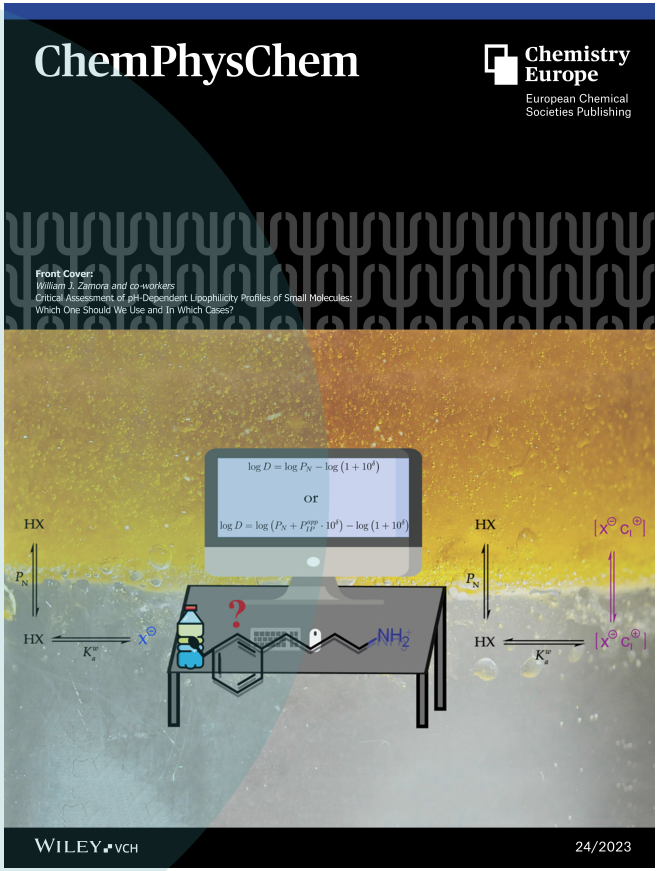


Model 2. Ionic Partition

$$\log D = \log\left(P_N + P_I \cdot 10^{\delta}\right) - \log(1+10^{\delta})$$



Introduction



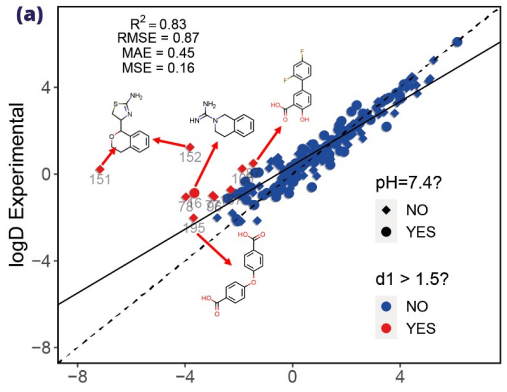
ChemPhysChem
Chemistry Europe
European Chemical Societies Publishing

Research Article

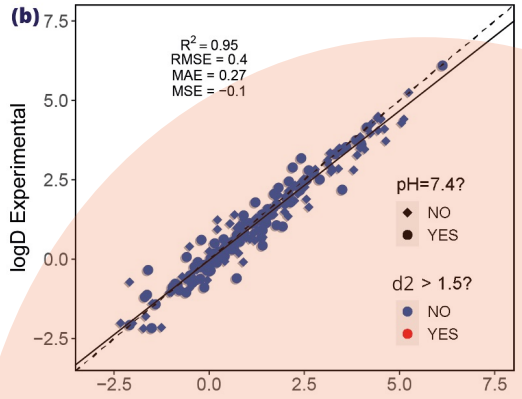
Critical Assessment of pH-Dependent Lipophilicity Profiles of Small Molecules: Which One Should We Use and In Which Cases?

Esteban Bertsch, Sebastián Suñer, Dr. Silvana Pinheiro, Prof. Dr. William J. Zamora

First published: 03 October 2023 | https://doi.org/10.1002/cphc.202300548



$$\log D = \log P_N - \log(1+10^{\delta})$$



$$\log D = \log\left(P_N + P_I \cdot 10^{\delta}\right) - \log(1+10^{\delta_7})$$

Introduction

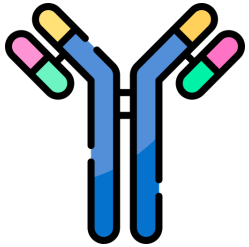
- **Beyond the Rule of 5 (bRo5) Chemical Space**

Peptides

MW ~ 500–5000 Da

Rapid clearance, short half-life

Low membrane permeability (chemically unmodified peptides)



Therapeutic proteins

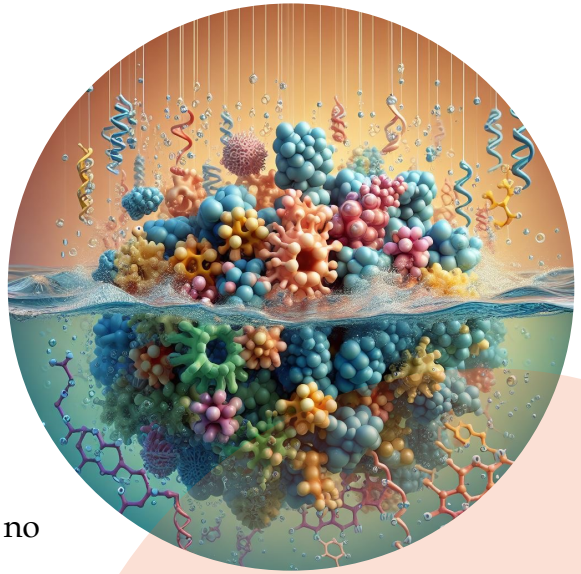
MW > 5000 Da

Complex (often recombinant) production, no easy chemical modification

Immunogenicity

Low membrane permeability due to size

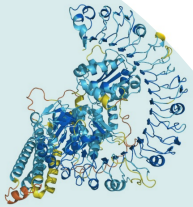
Only extracellular or surface-exposed targets

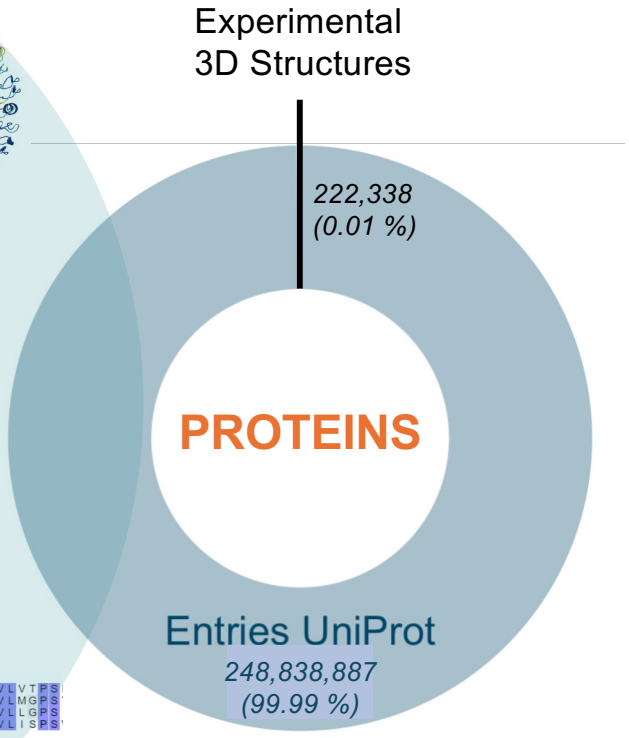


No clear guidelines for rational drug design!!!

Introduction

Drug Design: A Difficult and Costly Process





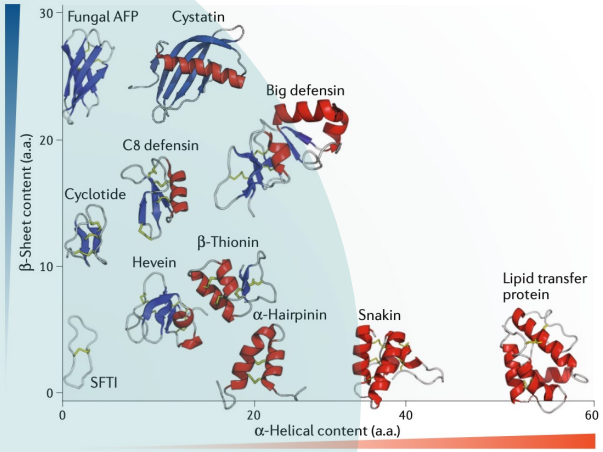
The gap has widened considerably in recent years (advances in DNA sequencing)

Experimental structure determination can not keep up with the pace of sequencing

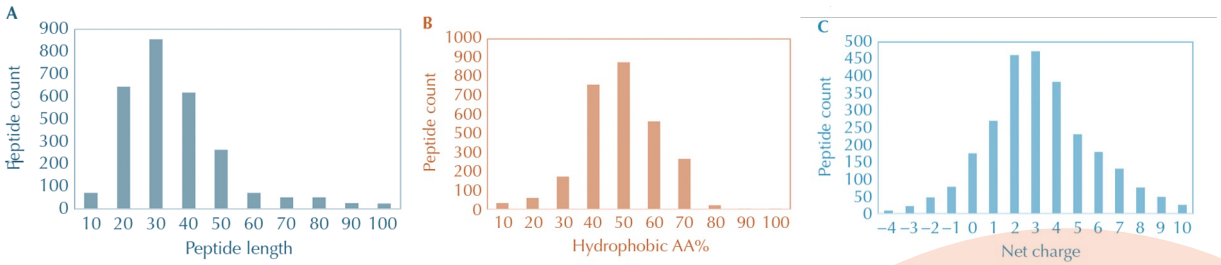
There have been hundreds of millions of known proteins with unknown structures.

Introduction

- Cationic Host Defense Peptides (**HDPs**)



Mookherjee, N., Anderson, M. A., Haagsman, H. P., & Davidson, D. J. (2020). Antimicrobial host defence peptides: functions and clinical potential. Nature reviews. Drug discovery, 19(5), 311-332.



Hydrophobicity/Lipophilicity

Amphipathicity

Introduction

○ Structure-Activity Relationship

Table 2. Correlation between the biological effective concentrations and biophysical descriptors.

		MIC	EC50
		E.Coli.	Red Blood Cell
		N_{pept} = 44	N_{pept} = 19
n_{charge}	The net charge of the peptides.	−0.46 (1.7E-03)	−0.13 (5.9E-01)
n_{res}	Number of residues.	−0.36 (1.3E-02)	−0.23 (3.4E-01)
H	Hydrophobicity.	−0.09 (5.8E-01)	−0.08 (7.4E-01)
μ_H	Hydrophobic moment.	−0.45 (2.4E-03)	−0.56 (1.3E-02)
Q_H	Hydrophobic quadrupole moment.	−0.39 (6.2E-03)	−0.45 (5.1E-02)
ΔW	Transfer energy of peptide from water to membrane surface.	0.46 (1.7E-03)	0.30 (2.1E-01)

He Y, Lazaridis T. Activity determinants of helical antimicrobial peptides: a large-scale computational study. PLoS One. 2013 Jun 12;8(6):e66440.

Journal of Medicinal Chemistry
pubs.acs.org/jmc Article

Hydrophobicity Determines the Bacterial Killing Rate of α-Helical Antimicrobial Peptides and Influences the Bacterial Resistance Development

Minghui Zhang,[¶] Jianhong Ouyang,[¶] Lei Fu,[¶] Cheng Xu, Yuke Ge, Shuqing Sun, Xiangyuan Li, Shian Lai, Hengte Ke, Bing Yuan, Kai Yang, Haining Yu,* Lianghui Gao,* and Yipeng Wang*

Cite This: J. Med. Chem. 2022, 65, 14701−14720 Read Online

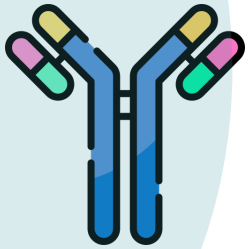
Antimicrobial activity increased with amphipathicity, but unfortunately, so did toxicity

Antimicrobial activity trends correlated with peptide amphipathicity and, to a lesser extent, with overall hydrophobicity

Lipophilicity and Amphipathicity

Peptides

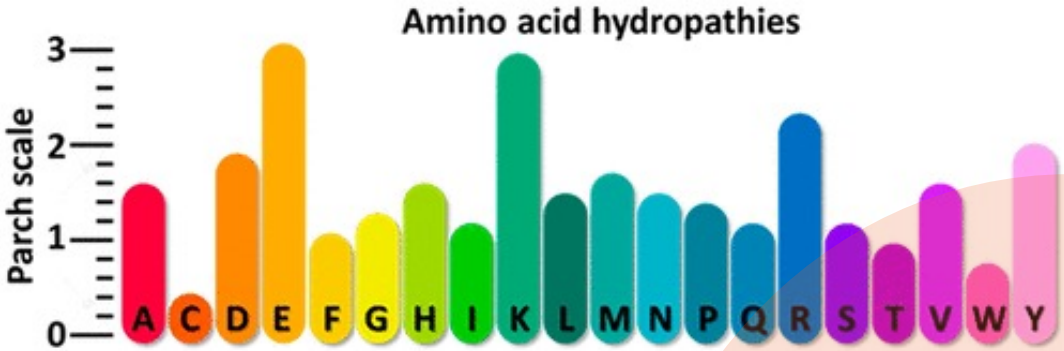
MW ~ 500–5000 Da



Therapeutic proteins

MW > 5000 Da

- **Computational**



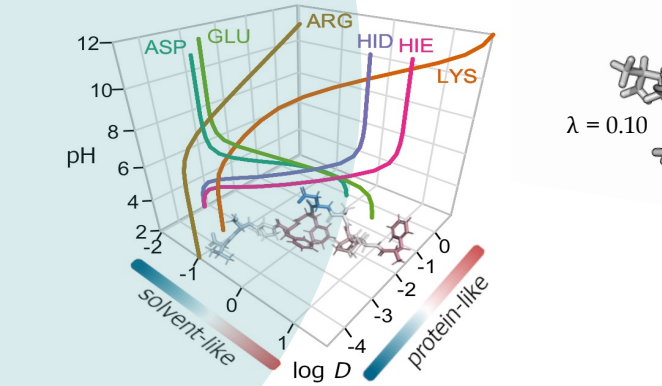
AKLDR
RDLKA

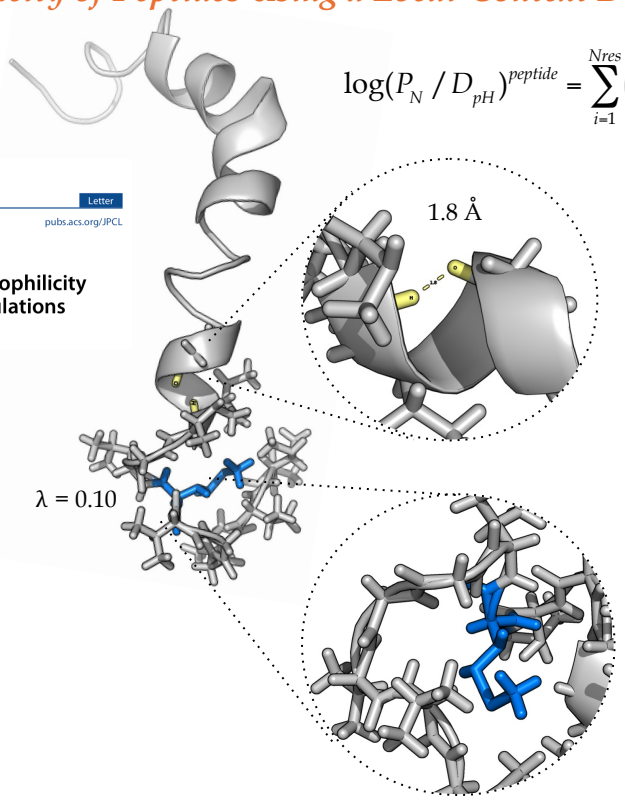
LDRKA

Same log*P* or log*D* values!!!

Methods

Application: Lipophilicity of Peptides Using a Local-Context Dependent Lipophilicity





$$\log(P_N / D_{pH})^{peptide} = \sum_{i=1}^{Nres} \left(\lambda^i \cdot \log(P^i_N / D^i_{pH})^{bb+sc} + \lambda^i \cdot \log(P^i_N / D^i_{pH})^{cg} + \alpha^i + \beta^i \right)$$

1. Parameter λ

$$\lambda^i = \frac{S^i_{peptide}}{\left\langle S^i_{\text{AA model}} \right\rangle}$$

2. Parameter α

0.73 (log *P* units) per HB

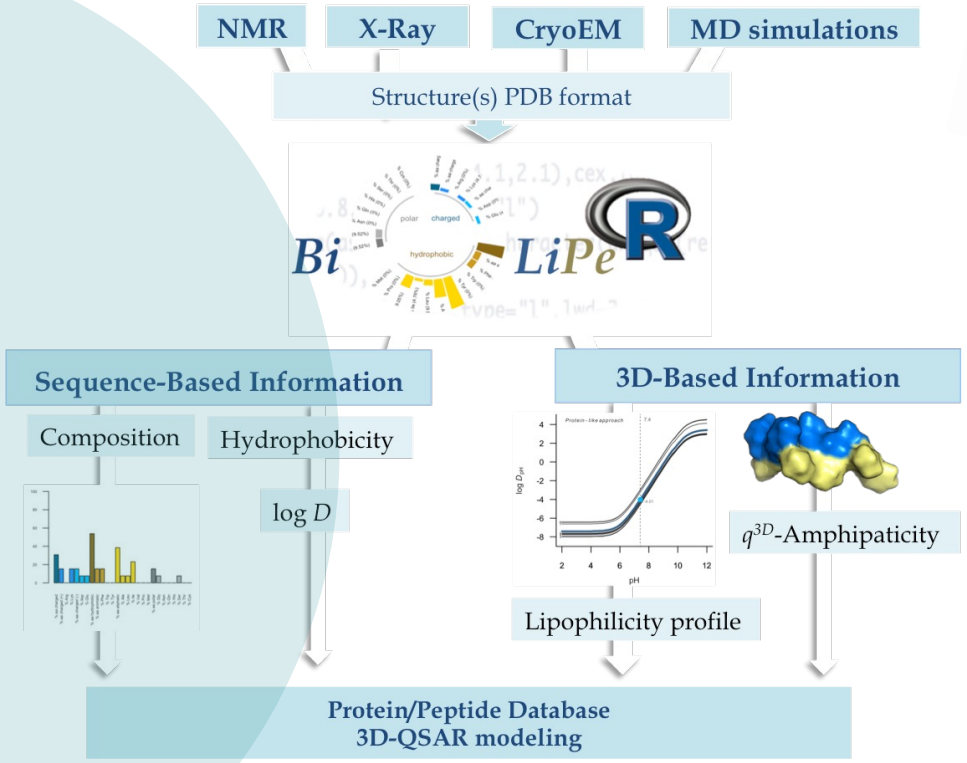
3. Parameter β

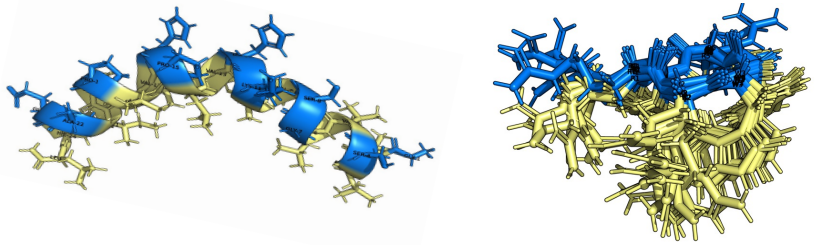
$$\beta^i = H^i_{res} \cdot (1 - \lambda^i)^{sc} \qquad H^i_{res} = \frac{0.023 \cdot \text{SASA}^{sc}_{res}}{2.303RT}$$

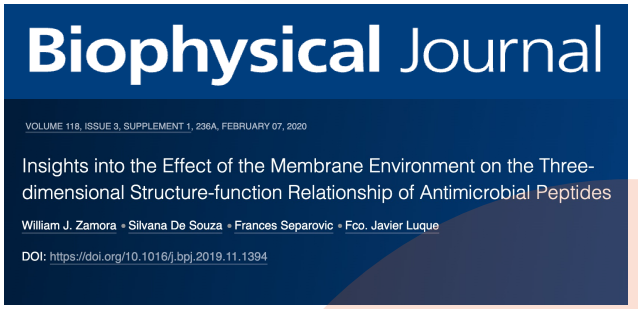
Table 7. Average Solvent Accessible Surface Area (SASA) for the Side Chain of the Hydrophobic Residues and the Hydrophobic Effect Contribution Value when the Side Chain is Fully Buried.

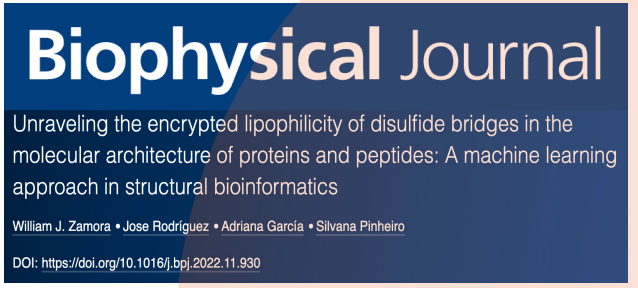
Residue	Average SASA (Å²)	H^i_{res} (log *P* units)
Ala	69	1.2
Val	130	2.2
Leu	158	2.7
Ile	157	2.6
Met	166	2.8
Pro	115	1.9
Phe	188	3.2
Trp	232	3.9
Tyr	201	3.4

Methods

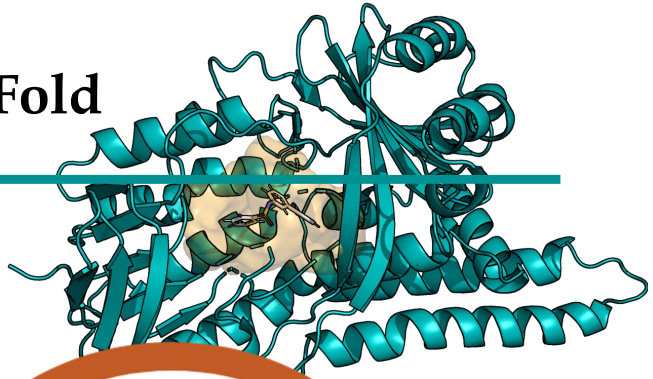

NMR
X-Ray
CryoEM
MD simulations
Structure(s) PDB format
Bi
LiPe
R
Sequence-Based Information
3D-Based Information
Composition
Hydrophobicity
log D
q^{3D}-Amphipaticity
Lipophilicity profile
Protein/Peptide Database
3D-QSAR modeling



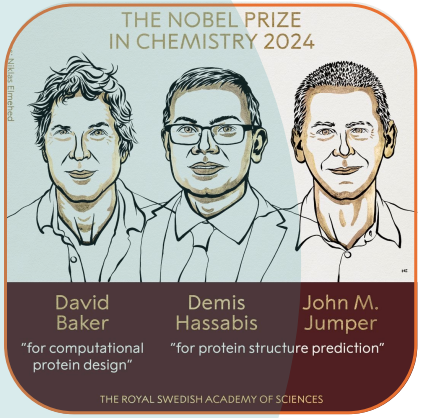

Biophysical Journal
VOLUME 118, ISSUE 3, SUPPLEMENT 1, 236A, FEBRUARY 07, 2020
Insights into the Effect of the Membrane Environment on the Three-dimensional Structure-function Relationship of Antimicrobial Peptides
William J. Zamora • Silvana De Souza • Frances Separovic • Fco. Javier Luque
DOI: https://doi.org/10.1016/j.bpj.2019.11.1394


Biophysical Journal
Unraveling the encrypted lipophilicity of disulfide bridges in the molecular architecture of proteins and peptides: A machine learning approach in structural bioinformatics
William J. Zamora • Jose Rodríguez • Adriana García • Silvana Pinheiro
DOI: https://doi.org/10.1016/j.bpj.2022.11.930

Predicting the Protein Structure: AlphaFold

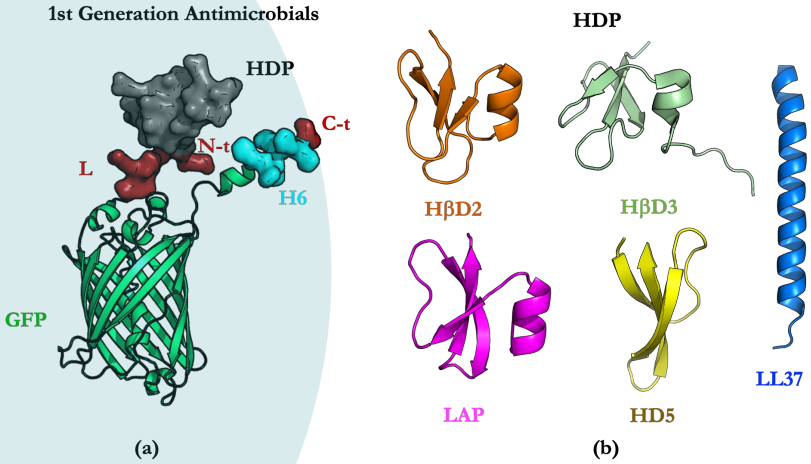


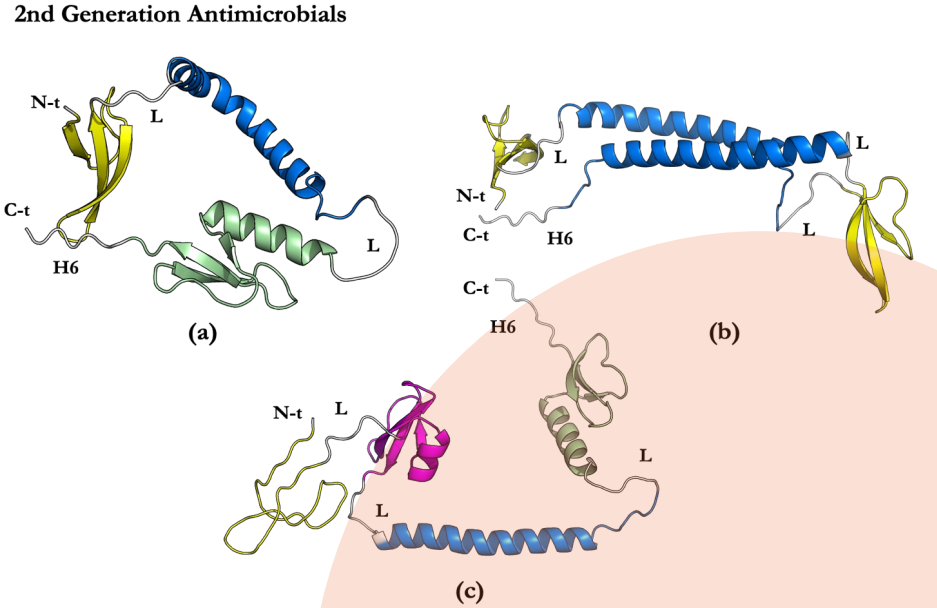
Proteins in the Hall of Fame



Methods

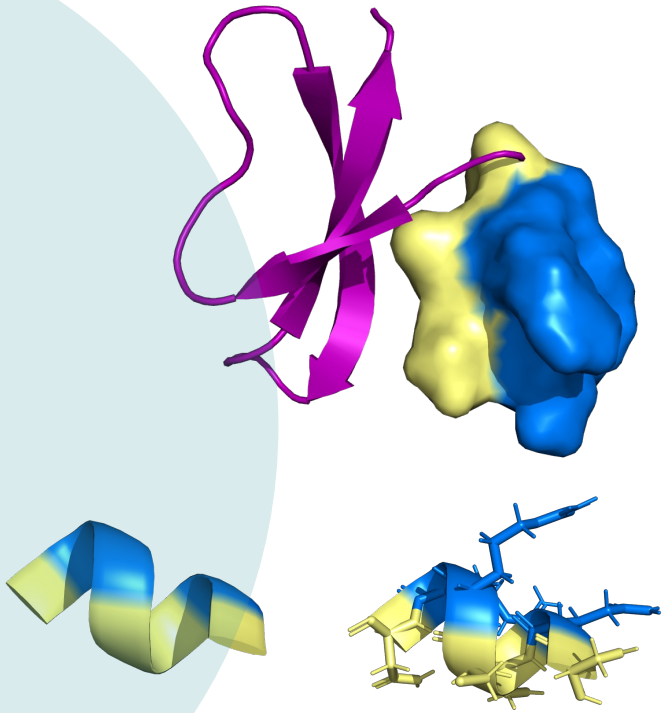
Structure–Antimicrobial Activity Relationships of Recombinant Host Defense Peptides Against Drug-Resistant Bacteria



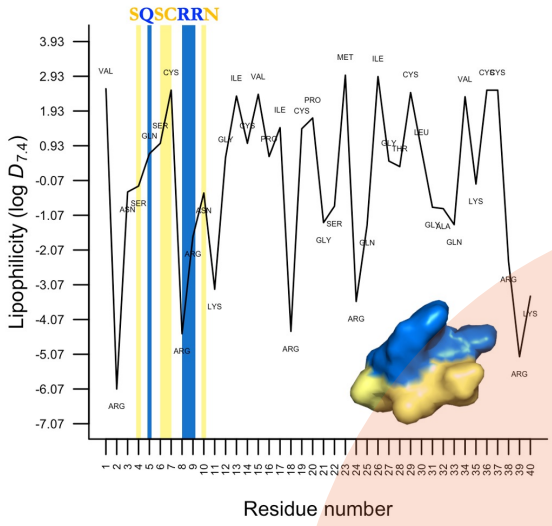


IRTA Institute of Agrifood Research and Technology

Methods



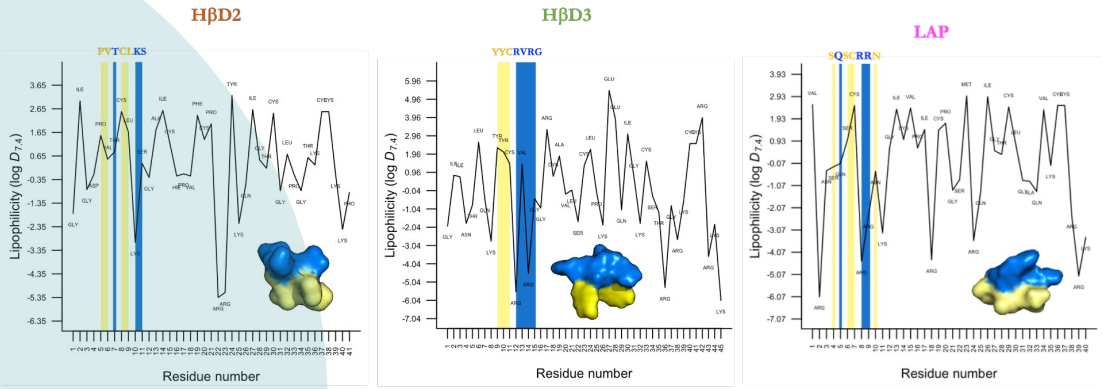
$$Amp = \sum_{i}^{n} \log D_i - \sum_{j}^{n} \log D_j$$



$$\log(D_{pH}/P_N)^{peptide} = \sum_{i=1}^{N} \left(\lambda^i \cdot \log(D_{pH}/P_N)^{bb+sc} + \lambda^i \cdot \log(D_{pH}/P_N)^{cg} + \alpha^i + \beta^i + \gamma^i\right)$$

Methods

Physicochemical properties of 1st generation antimicrobials



Host Defense Peptide (HDP)	Amphipathicity (*Amp*)
HβD2	8.08
HβD3	15.02
LAP	9.83
HD5	18.78
LL37	0.16

Methods

Antimicrobial activity

Reduction of colony-forming units (log10 cfu/mL)

Gram-positive strains

Strain	Host Defense Peptide (1st generation) (reduction of log10 cfu/mL)				
	LL37-GFP	LAP-GFP	HβD2-GFP	HβD3-GFP	HD5-GFP
Methicillin sensitive *S. aureus (MSSA)*	0.00	5.00	5.00	3.50	3.50
Methicillin resistant *S. aureus (MRSA)*	1.00	2.00	2.00	3.00	1.50
Methicillin resistant *S. epidermidis (MRSE)*	0.00	1.50	1.50	5.00	5.00
Average (log10 cfu/mL)	***0.33***	***2.83***	***2.83***	***3.83***	***3.33***

Gram-negative strain

Strain	Host Defense Peptide (1st generation) (reduction of log10 cfu/mL)				
	LL37-GFP	LAP-GFP	HβD2-GFP	HβD3-GFP	HD5-GFP
P. aeruginosa	1.00	5.00	5.00	5.00	5.00

Minimal inhibitory concentration (MIC, μM)

Gram-positive strains

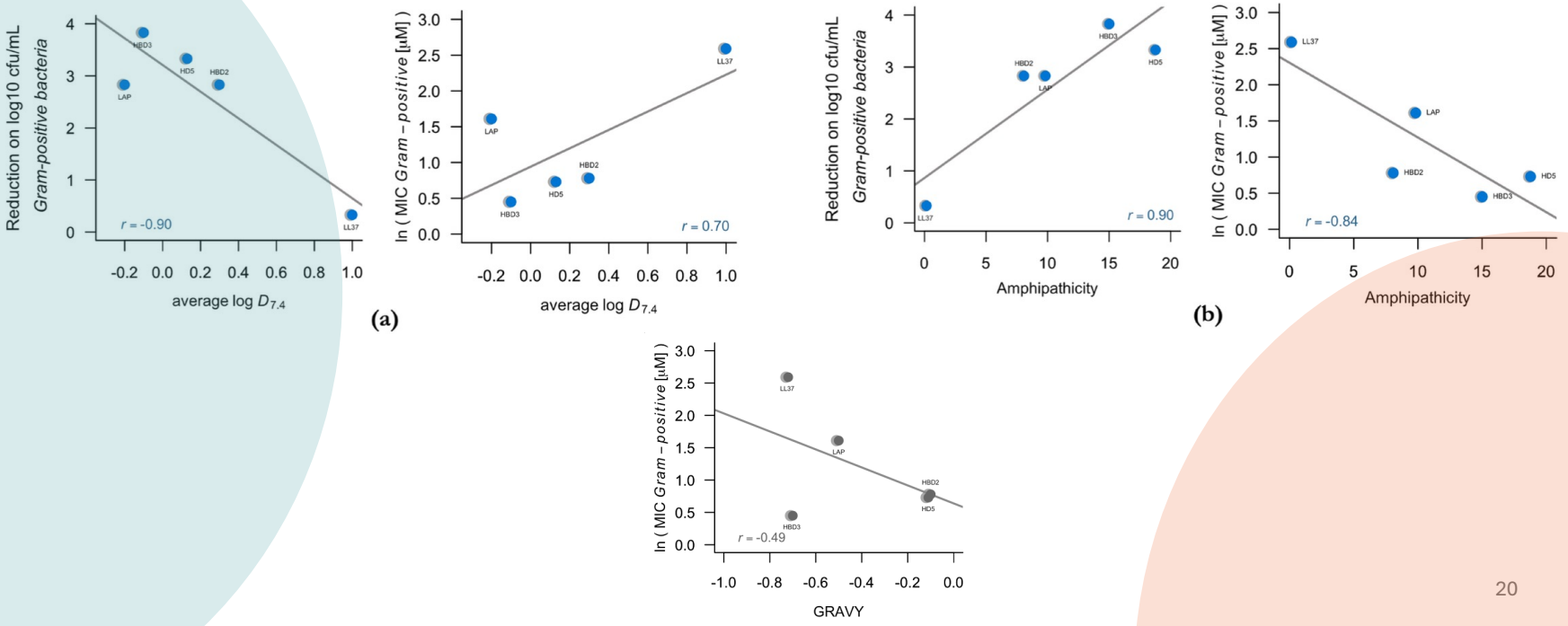
Strain	Host Defense Peptide (1st generation) (MIC, μM)				
	LL37-GFP	LAP-GFP	HβD2-GFP	HβD3-GFP	HD5-GFP
Methicillin sensitive *S. aureus (MSSA)*	>16	3.75	0.94	1.88	2.50
Methicillin resistant *S. aureus (MRSA)*	>16	7.50	1.88	0.94	2.50
Methicillin resistant *S. epidermidis (MRSE)*	8.00	3.75	3.75	1.88	1.25
Average (MIC, μM)	***>13.33***	***5.00***	***2.19***	***1.57***	***2.08***

Gram-negative strain

Strain	Host Defense Peptide (1st generation) (MIC, μM)				
	LL37-GFP	LAP-GFP	HβD2-GFP	HβD3-GFP	HD5-GFP
P. aeruginosa	>16	3.75	3.75	7.50	1.25

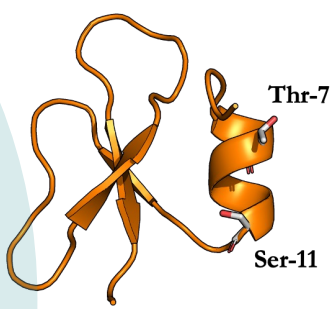
Results

Structure–antimicrobial activity relationships of 1st generation host defense peptides against Gram-positive

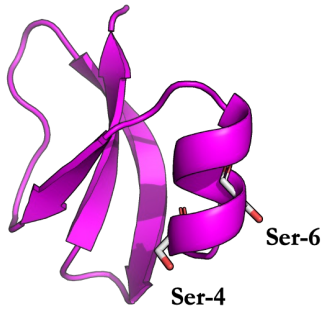


Results

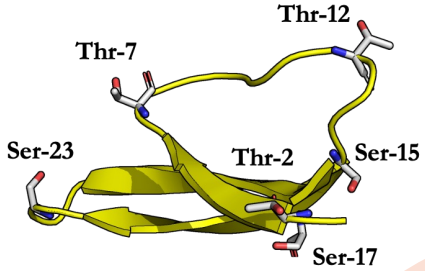
Structure–antimicrobial activity relationships of 1st generation host defense peptides against Gram-negative



HβD2



LAP

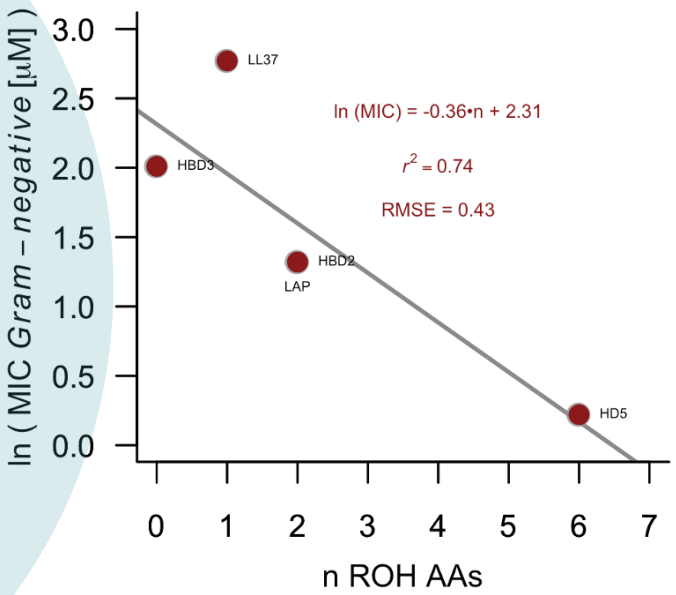


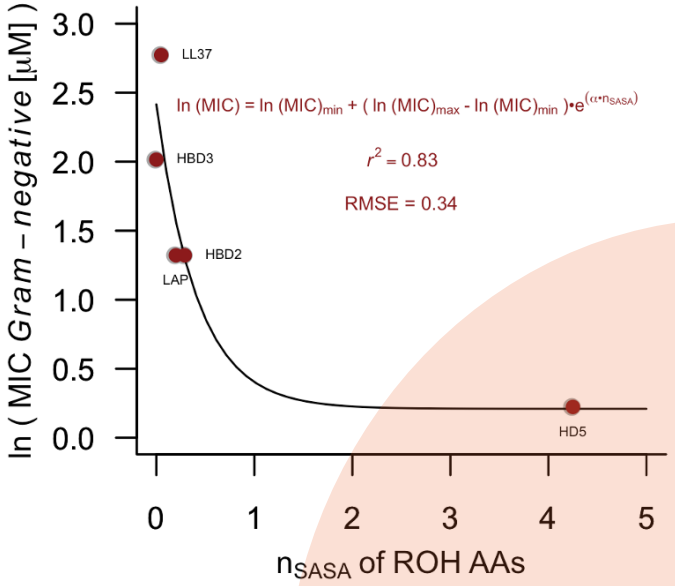
HD5

LL37

Results

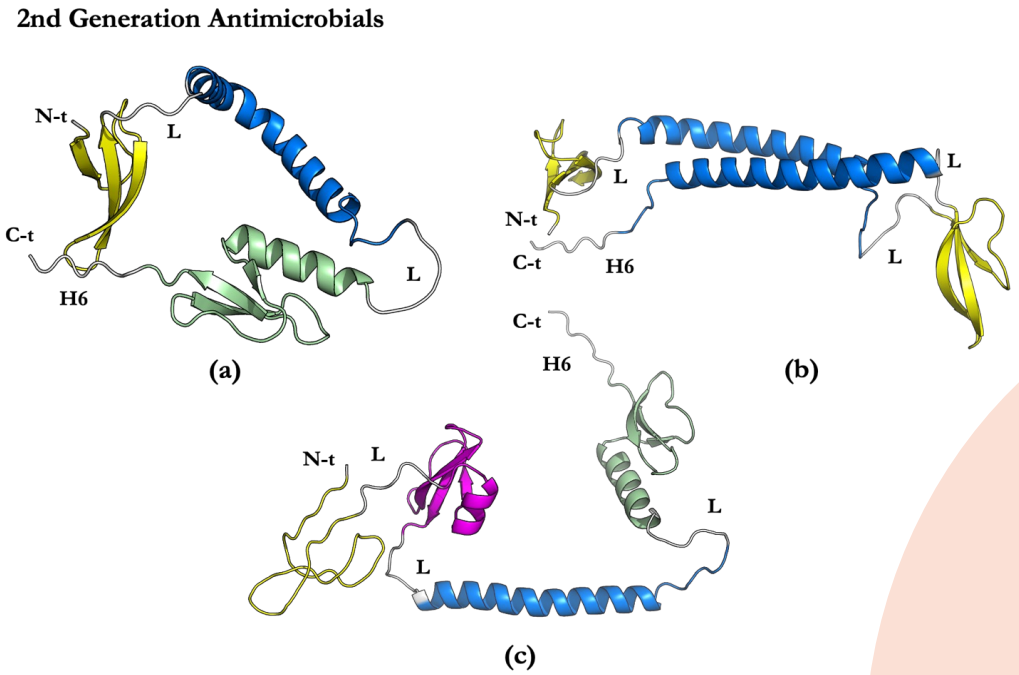
*Structure–antimicrobial activity relationships of 1st generation host defense peptides against **Gram-negative***





Results

Structure–antimicrobial activity relationships of 2nd generation host defense peptides against Gram-positive strains

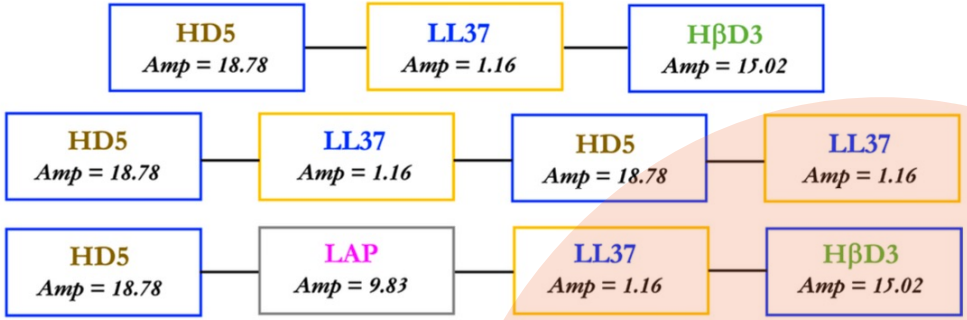


Results

Structure–antimicrobial activity relationships of 2nd generation host defense peptides against Gram-positive and Gram-negative strains

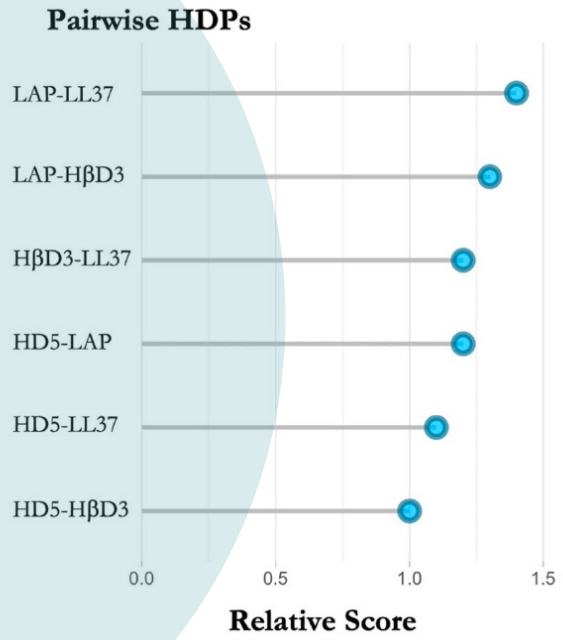
Strain	Host Defense Peptides (2nd generation) (MIC, μM)		
	HD5-LL37-HβD3	HD5-LL37-HD5-LL37	HD5-LAP-LL37-HβD3
Methicillin sensitive *S. aureus (MSSA)*	2.16	1.19	>8.10
Methicillin resistant *S. aureus (MRSA)*	4.32	1.19	>8.10
Methicillin resistant *S. epidermidis (MRSE)*	4.32	3.75	7.50
Average (MIC, μM)	***3.60***	***2.04***	***> 7.90***

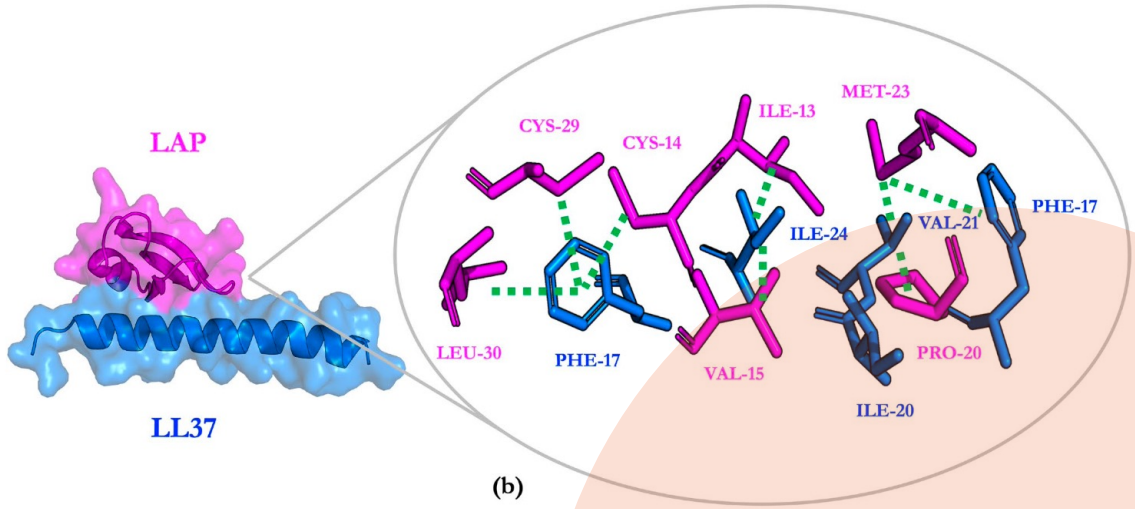
Strain	Host Defense Combined Peptides (2nd generation) (MIC, μM)		
	HD5-LL37-HβD3	HD5-LL37-HD5-LL37	HD5-LAP-LL37- HβD3
P. aeruginosa	3.75	1.19	>8.10



Results

Structure–antimicrobial activity relationships of 2nd generation host defense peptides against Gram-positive and Gram-negative strains

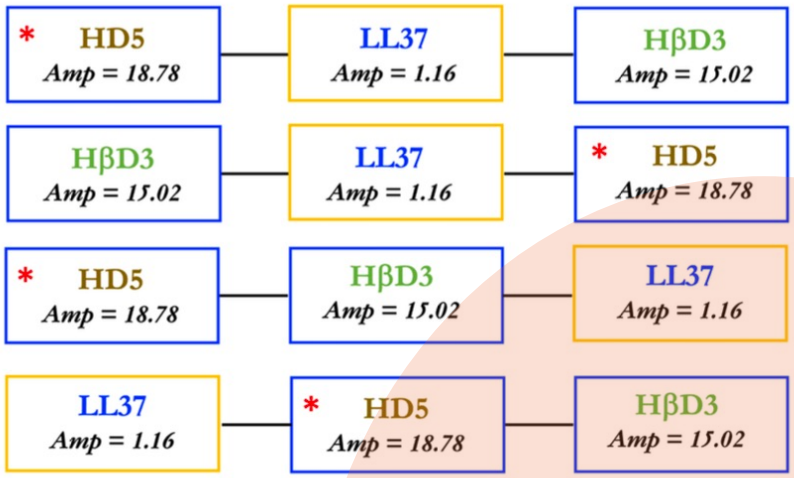




Results

Structure–antimicrobial activity relationships of 2nd generation host defense peptides against Gram-positive and Gram-negative strains

Strain	Host Defense Combined Peptides (2nd generation) (MIC, μM)			
	HD5-LL37-HβD3	HβD3-LL37-HD5	HD5-HβD3-LL37	LL37-HD5-HβD3
MRSE	4.17	4.17	4.17	8.33
P. aeruginosa	3.75	3.75	3.75	3.75



Results

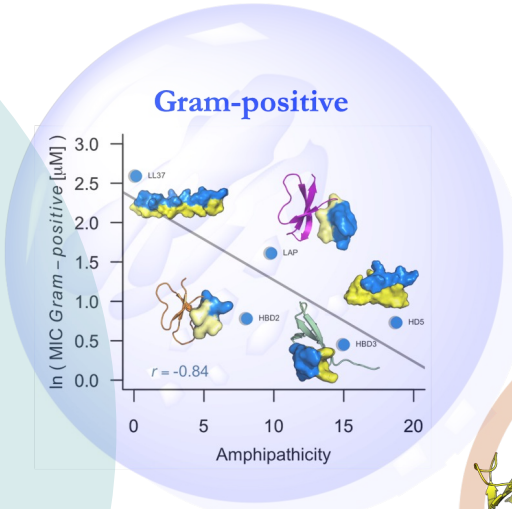
RESEARCH ARTICLE | Open Access

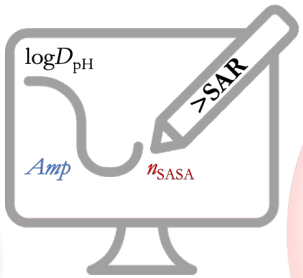
Structure–Antimicrobial Activity Relationships of Recombinant Host Defence Peptides Against Drug-Resistant Bacteria

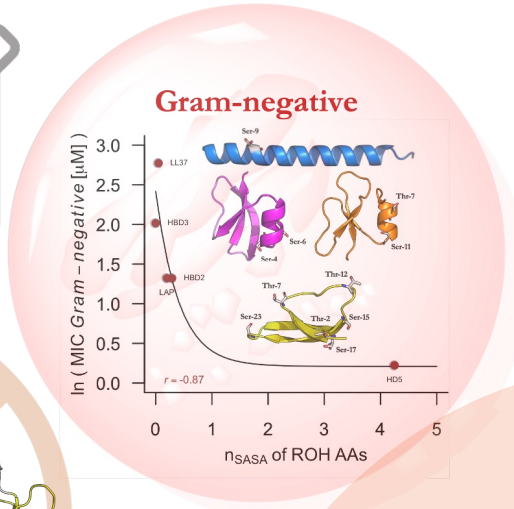
Sergi Travé-Asensio, Aida Tort-Miró, Silvana Pinheiro, Elena Garcia-Fruitós, Anna Arís, William J. Zamora

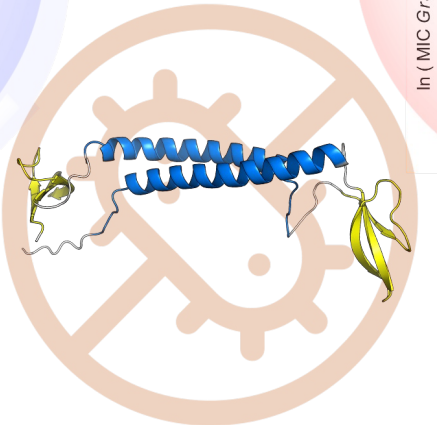
First published: 23 September 2025 | https://doi.org/10.1111/1751-7915.70204

Conclusions and Challenges









Acknowledgments

7TH INTERNATIONAL SYMPOSIUM ON ANTIMICROBIAL PEPTIDES – AMP2022

AMP2024
8th International Symposium on Antimicrobial Peptides

CBio3 Laboratory, LaToxCIA and CNCA members
Dra Silvana Pinheiro

Bio3 COMPUTATIONAL BIOPHYSICS, BIOLOGY AND BIOINFORMATICS GROUP

UCR UNIVERSIDAD DE COSTA RICA

aToxCIA EBi
oratorio de Toxicología Computacional e Inteligencia Artificial
LEBI Laboratorio de Ensayos Biológicos

VICERRECTORÍA DE INVESTIGACIÓN

EQ Escuela de Química

CNCA Colaboratorio Nacional de Computación Avanzada

SFM Société Française de Microbiologie

Sergio Travé-Asensio

IRTA Institute of Agrifood Research and Technology

Aida Tort-Miró

Thank you for your attention

Graciès!

Merci!

Gracias!

Thanks!

спасибо

william.zamoraramirez@ucr.ac.cr