Spatial integration of antimicrobial peptides controls release and activity in oral delivery systems

Cesar Augusto Roque-Borda, Ph.D.

Why oral AMPs fail

- ❑Proteolysis
- ❑Acid degradation
- ❑Burst release
- ❑Poor intestinal targeting
- ❑Electrostatic destabilization
- ❑Loss of bioactivity before reaching site

Most delivery systems treat AMPs as passive cargo.

Aim:
We investigated whether the AMP itself could become a structural component of the matrix.

Strategy A — Protected encapsulation
(core-shell double coating)

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Chitosan and HPMCAS double-coating as protective systems for alginate microparticles loaded with Ctx(Ile^{21})-Ha antimicrobial peptide to prevent intestinal infections

Cesar Augusto Roque-Borda [a,**], Mauro de Mesquita Souza Saraiva [b], Wagner Dias Macedo Junior [c], José Carlos Estanislao Márquez Montesinos [d], Andréia Bagliotti Meneguin [a], Anna Beatriz Toledo Borges [a,1], Edson Crusca Junior [e], Saulo Santesso Garrido [e], Adriana Maria de Almeida [b], Reinaldo Marchetto [e], Marlus Chorilli [a], Angelo Berchieri Junior [b], Silvio Rainho Teixeira [c], Fernando Rogério Pavan [a], Eduardo Festozo Vicente [f,*]

Strategy B — Electrostatic peptide integration
(self-assembled nano-in-micro structures)

Food Hydrocolloids 167 (2025) 111454

Contents lists available at ScienceDirect

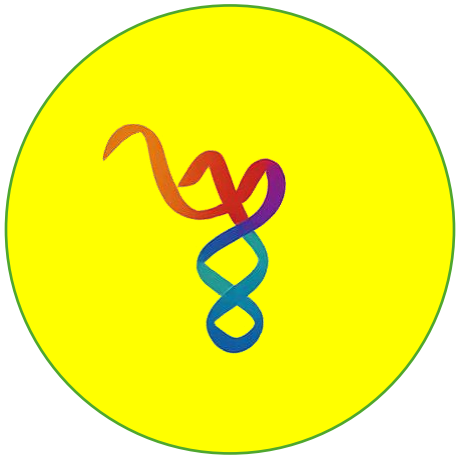
Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

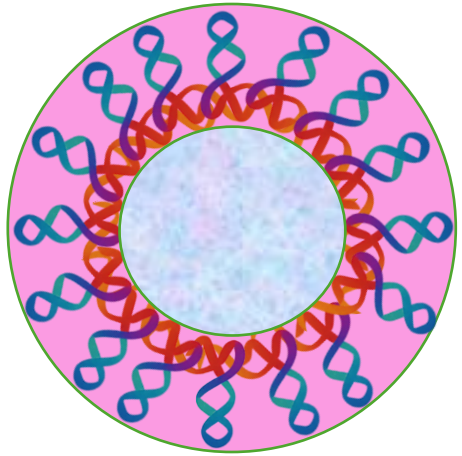
Alginate-pectin microparticles embedding self-assembling antimicrobial peptides and resveratrol for antimicrobial and anti-inflammatory applications

Cesar Augusto Roque-Borda [a,*], Marco Roberto Chávez-Morán [b], Laura Maria Duran Gleriani Primo [c], José C.E. Márquez Montesinos [d,i], Vinicius Martinho Borges Cardoso [c], Mauro M.S. Saraiva [e], Caroline Maria Marcos [c], Marlus Chorilli [c], Fernando Albericio [f,g,**], Beatriz G. de la Torre [h], Fernando Rogério Pavan [c], Andréia Bagliotti Meneguin [c,***]

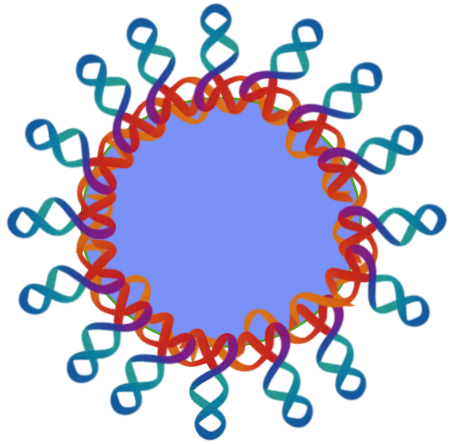
Micro/Nanoparticles



Core: Peptide



Core: Drug/Bioactive compound



Core: Drug/Bioactive compound

Knowing the systems

International Journal of Biological Macromolecules 183 (2021) 1236–1247

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Alginate-based microparticles coated with HPMCP/AS cellulose-derivatives enable the Ctx(Ile^{21})-Ha antimicrobial peptide application as a feed additive

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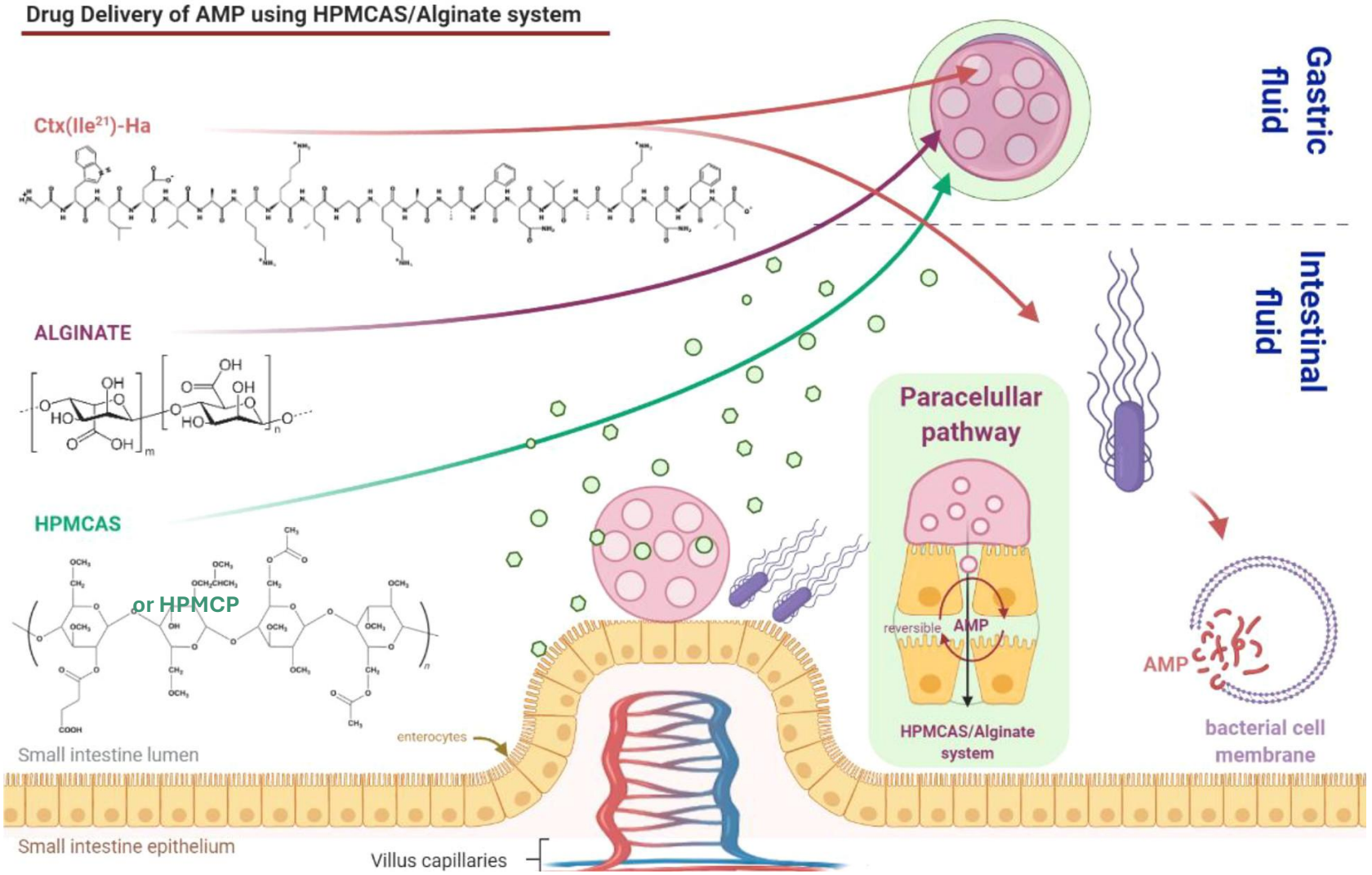
- Alginate = ionic gelation
- Chitosan = mucoadhesion
- HPMCAS/HPMCP = enteric protection

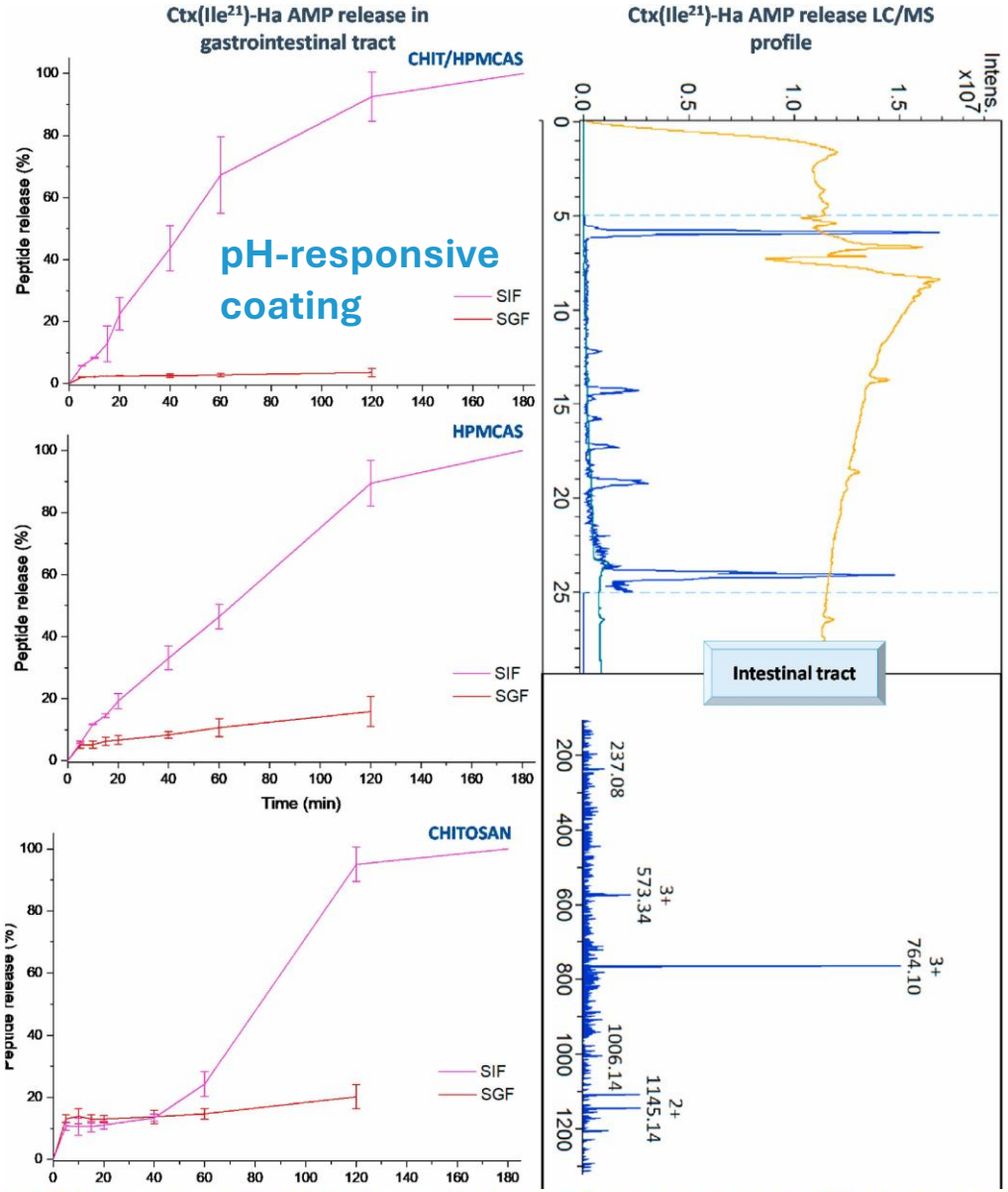


Why double coating?

intestinal-triggered release

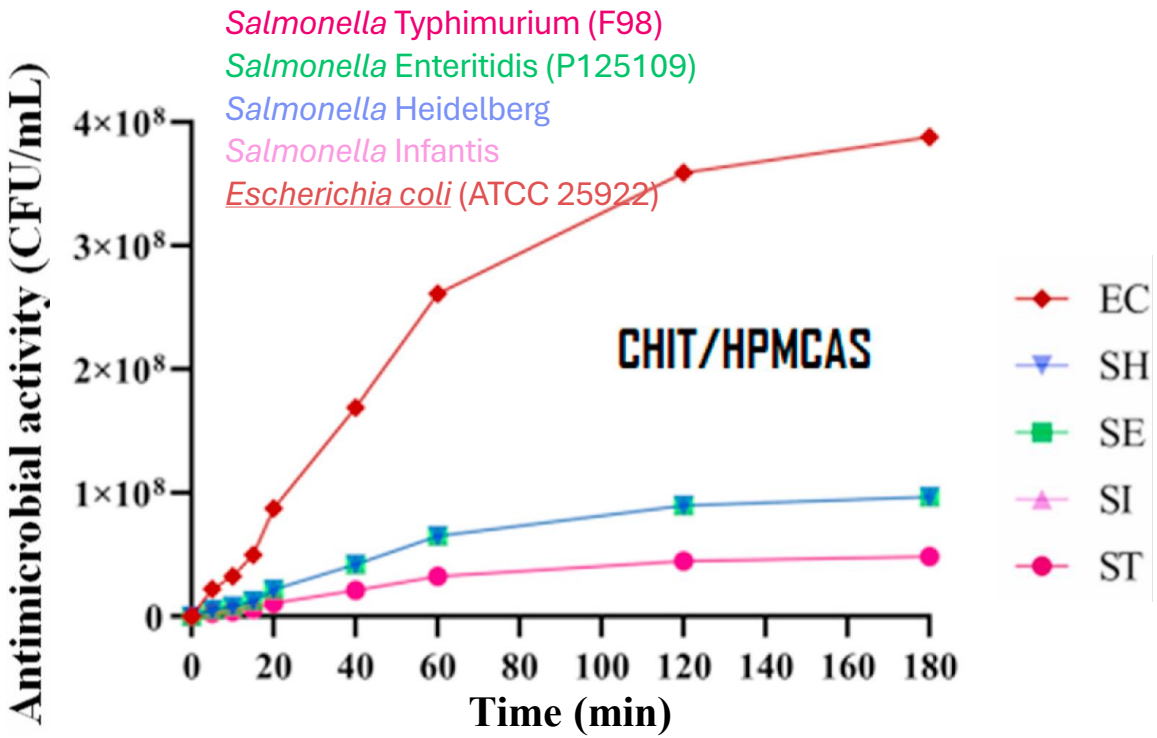
- protect AMP in gastric environment
- release in intestine
- low release at gastric pH
- higher release at intestinal pH

The system delayed premature peptide exposure.



Biological outcome

- ❑ antimicrobial activity preserved
- ❑ peptide survives GI simulation
- ❑ intestinal pathogen control



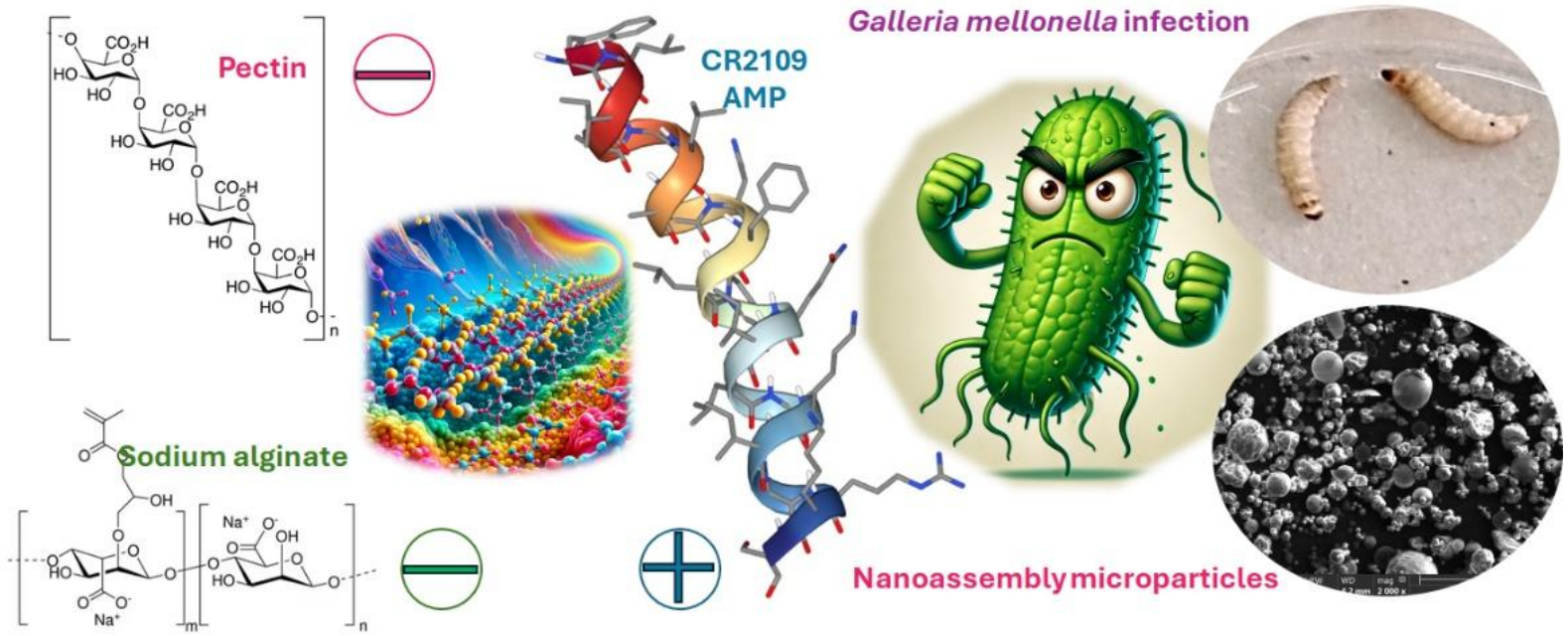
Encapsulation preserved functionality but limited immediate interaction with the environment.

What if the peptide is not only protected… but structurally integrated?

Electrostatic self-assembly concept

- ❑ cationic peptide
- ❑ anionic alginate/pectin
- ❑ spontaneous nanoassembly

Peptide became part of matrix organization

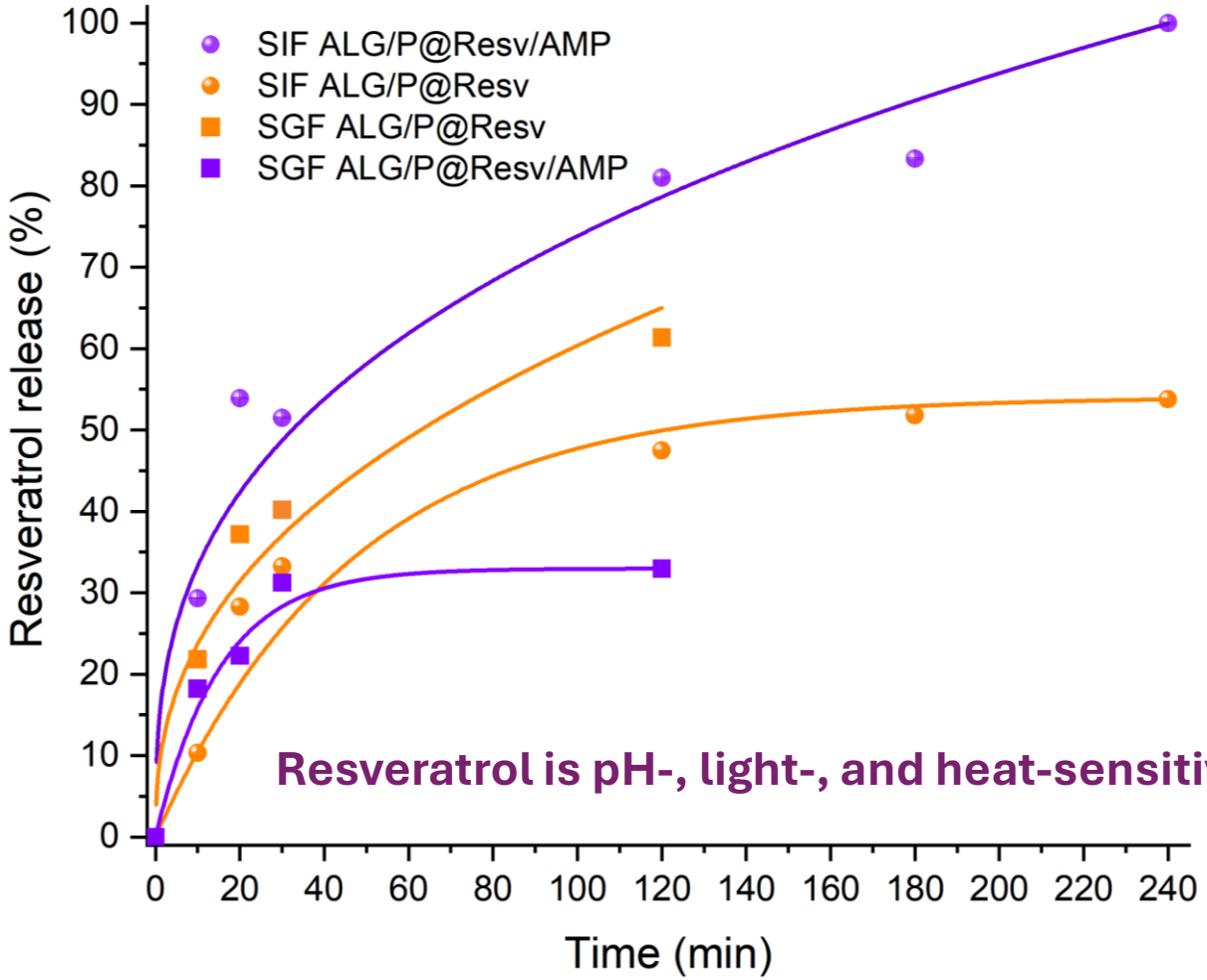


Self-assembly mechanism

Possible driving forces

- ❑ electrostatic interactions
- ❑ hydrophobic interactions
- ❑ hydrogen bonding
- ❑ polymer chain rearrangement

The AMP altered diffusional behavior of the matrix.



Resveratrol is pH-, light-, and heat-sensitive.

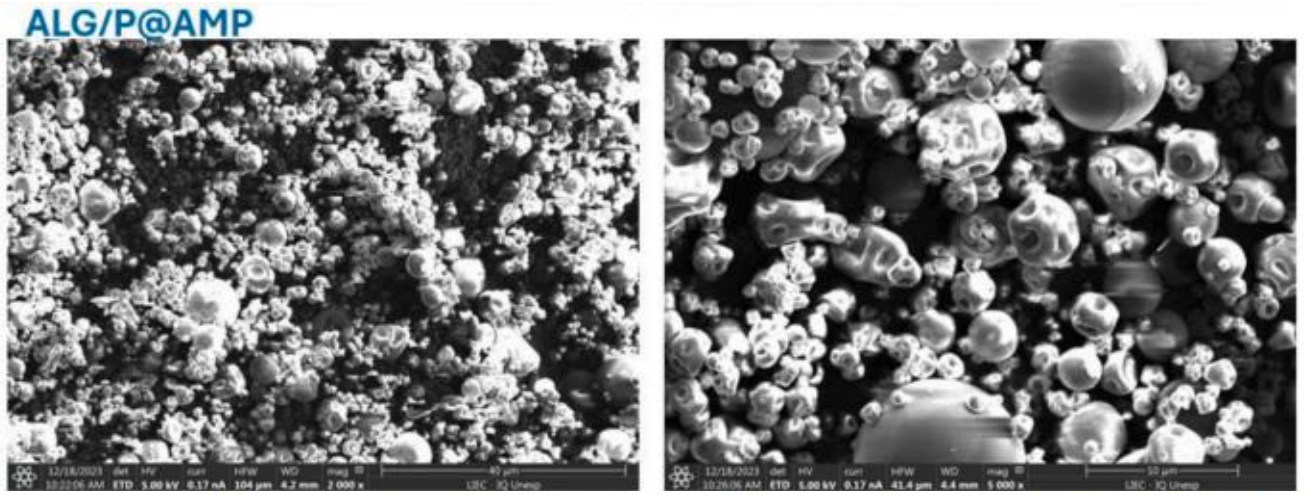
Microparticle architecture

The peptide reorganized the colloidal environment before spray drying

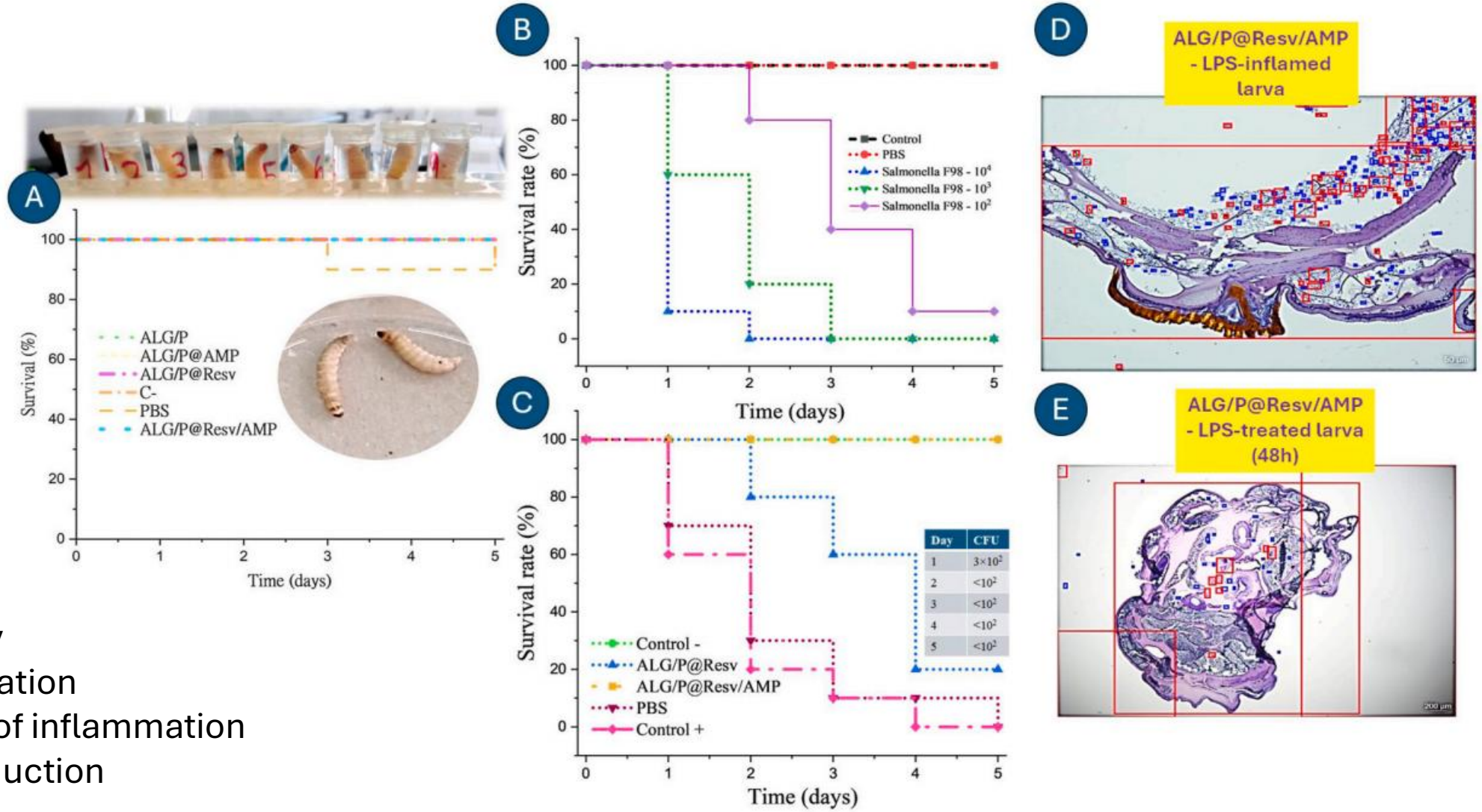
Table 2
Characteristics of nano-in-microparticles formed with peptide and resveratrol.

System/dispersion	EE (%)		DC (%)		Yield (%)	PDI	Zeta potential (mV)	Size (nm)
	Resv	AMP	Resv	AMP				
Blank	–	–	–	–	33.22	0.552	−25.0	894.5
ALG/P@Resv	78.21	–	39.10	–	40.87	0.456	−17.5	362.7
ALG/P@AMP	–	66.39	–	0.259	14.44	0.364	−7.4	220.6
ALG/P@Resv/AMP	76.11	72.84	89.23	2.73	4.06	0.185	−18.2	152.8
AMP	–	–	–	–	–	0.338	+10.6	–
Resv	–	–	–	–	–	0.570	−5.8	880.2

Zeta potential shifts suggested peptide–polymer nanoassembly prior to spray drying



Anti-inflammatory and antimicrobial effects



- ❑ sustained activity
- ❑ prolonged modulation
- ❑ in vivo reduction of inflammation
- ❑ bacterial load reduction

A third possibility: surface-exposed AMPs

Why graft the peptide?

The AMP no longer behaves as cargo, but as a functional nanointerface.

Carbohydrate Polymers 323 (2024) 121449

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antimicrobial peptides grafted onto the surface of *N*-acetylcysteine-chitosan nanoparticles can revitalize drugs against clinical isolates of *Mycobacterium tuberculosis*

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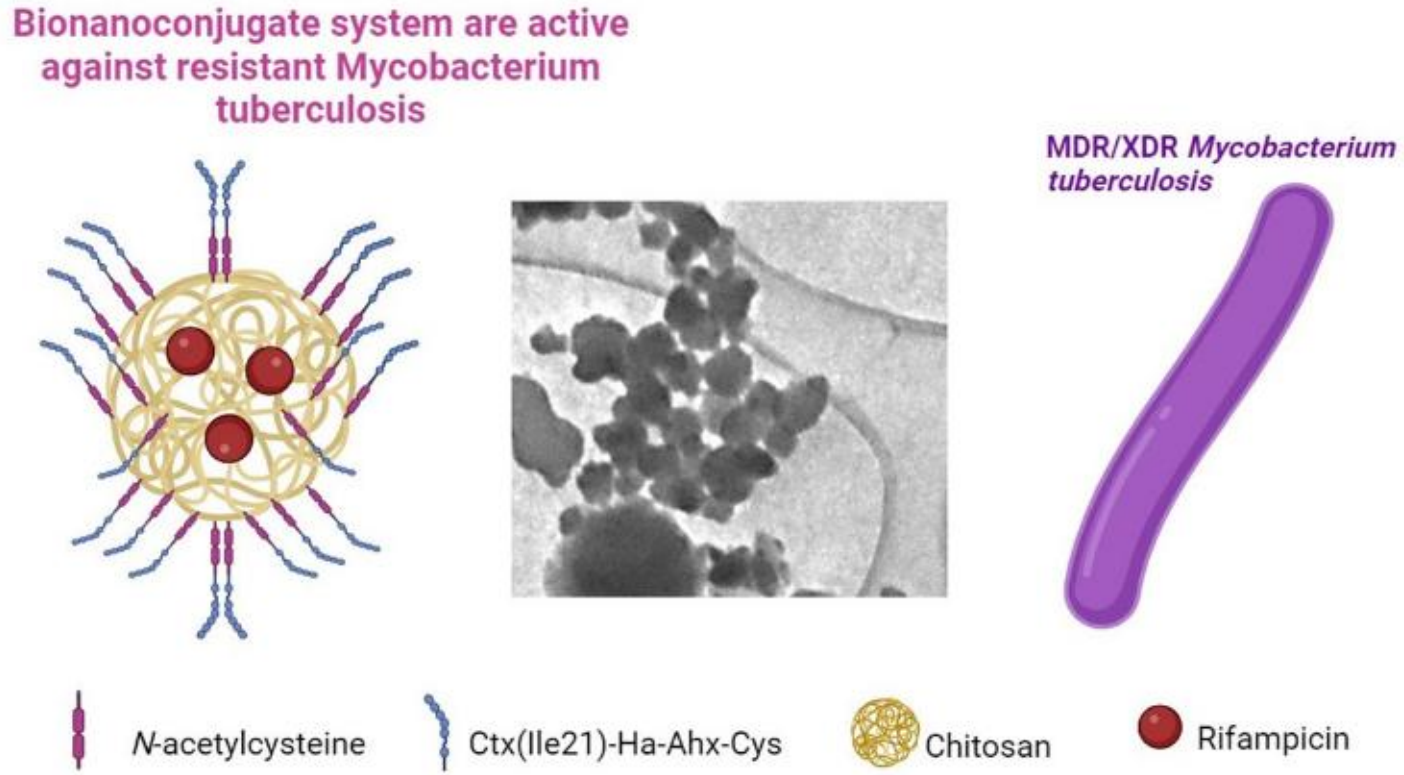
Multiple attack concept

NP core:
❑rifampicin delivery

Chitosan:
❑cationic destabilization

NAC:
❑biofilm-related modulation

Surface AMP:
❑membrane interaction
❑receptor interaction
❑possible efflux modulation



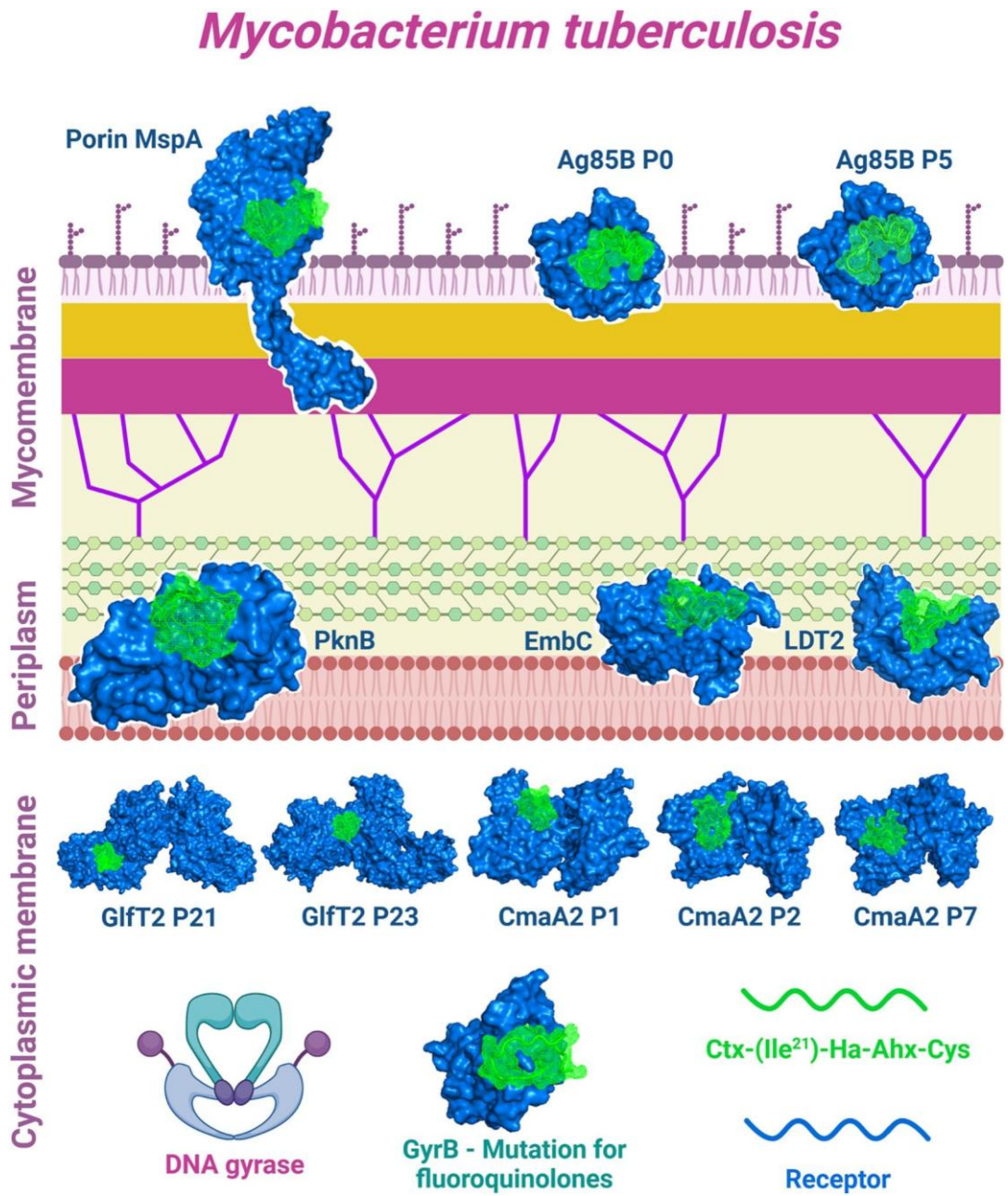
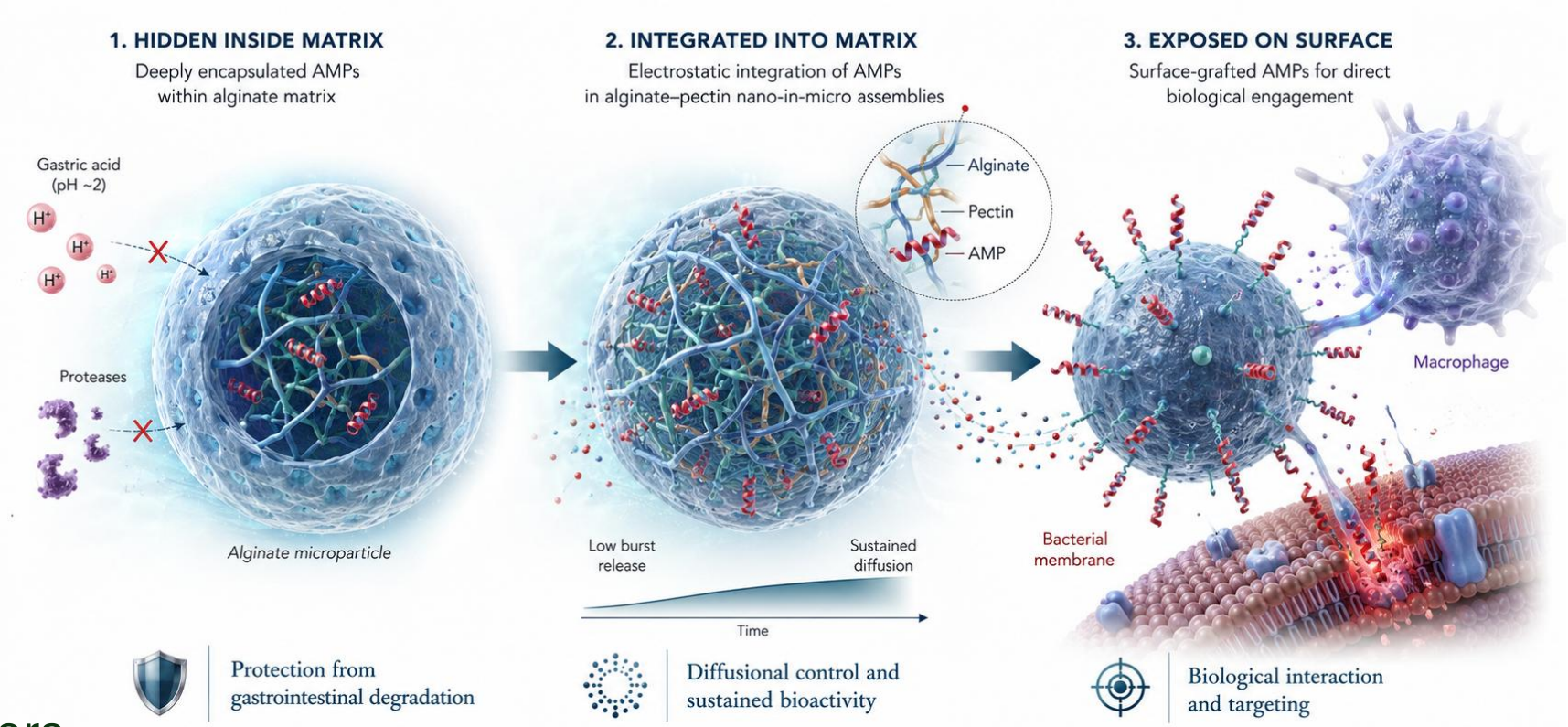


Table 3
MIC values of antibiotics and NP formulations by the REMA method after 7 days of exposure.

Code	Formulation	H37RV (μg/mL)	CF 169 (μg/mL)	CF 110 (μg/mL)
INH	Isoniazid	0.5–1.0	>50	>50
RIF	Rifampicin	0.5	>25	16.29
GAT	Gatifloxacin	0.03–0.25	1.2	<0.05
MOX	Moxifloxacin	0.03–0.5	1.6	<0.05
OFL	Ofloxacin	0.71	11.5	0.3
AMK	Amicacin	0.5–1.0	0.6	0.5
CAP	Capreomycin	2.0	1.4	3.0
KAN	Kanamycin	2.0	1.5	2.8
NP1	NAC-CS	NA	NA	NA
NP2	CS@RIF[a]	<0.977	NA	NA
NP3	Ctx-NAC-CS	NA	NA	NA
NP4	NAC-CS@RIF[a]	1.47	199.05	NA
NP5	CS	NA	NA	NA
NP6	Ctx-CS	NA	NA	NA
NP7	Ctx-CS@RIF[a]	<0.977	104.34	NA
NP8	Ctx-NAC-CS@RIF[a]	<0.977	0.977	<0.977
Ctx	Ctx(Ile[21])-Ha-Ahx-Cys	7.57	12.24	>250
Ctx wild-type	Ctx(Ile[21])-Ha	14.0	NT	NT
NAC	*N*-acetylcysteine	NA	NA	NA

NA: no activity. NT: not tested.
CF169 strain: XDR MTB. CF110 strain: MDR MTB.
[a] MIC values based on RIF concentration. All NPs were tested based on their weight.

Conceptual convergence



- ❑Peptide localization matters
- ❑Matrix interactions shape bioactivity
- ❑Electrostatic integration alters diffusion
- ❑Self-assembly modulates biological output
- ❑Nano-in-micro systems enable sustained AMP action
- ❑AMP–matrix interactions are an overlooked design parameter

Spatial positioning of AMPs may be as important as peptide sequence itself.

CRITICAL ANALYSIS IN PEPTIDE CHEMISTRY

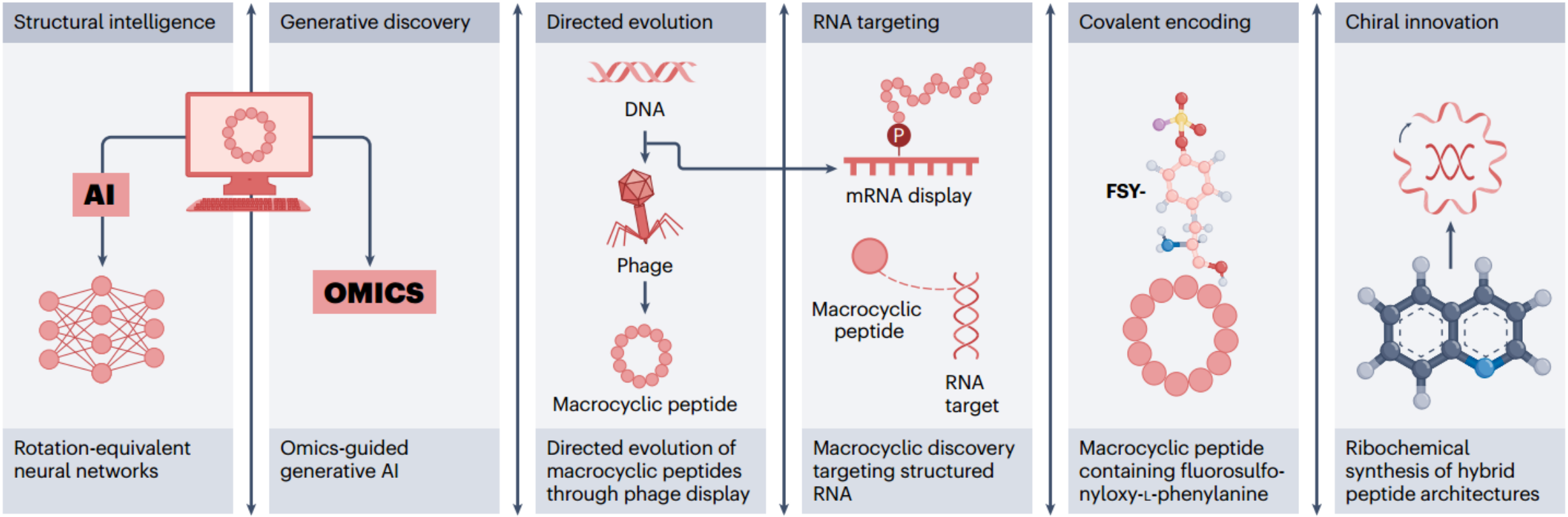
Ref:

Year in review
nature reviews chemistry
Peptide chemistry
https://doi.org/10.1038/s41570-025-00786-4

Redefining peptide chemistry beyond accumulating analogues

Cesar Augusto Roque-Borda, Fernando Rogério Pavan, Beatriz G. de la Torre & Fernando Albericio

Peptide design enters the generative age



Acknowledgments

Thank You For Your Attention

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