Evolutionary principles of antimicrobial peptide resistance

Balázs Papp

Hungarian Centre of Excellence for Molecular Medicine
& Biological Research Centre, Szeged

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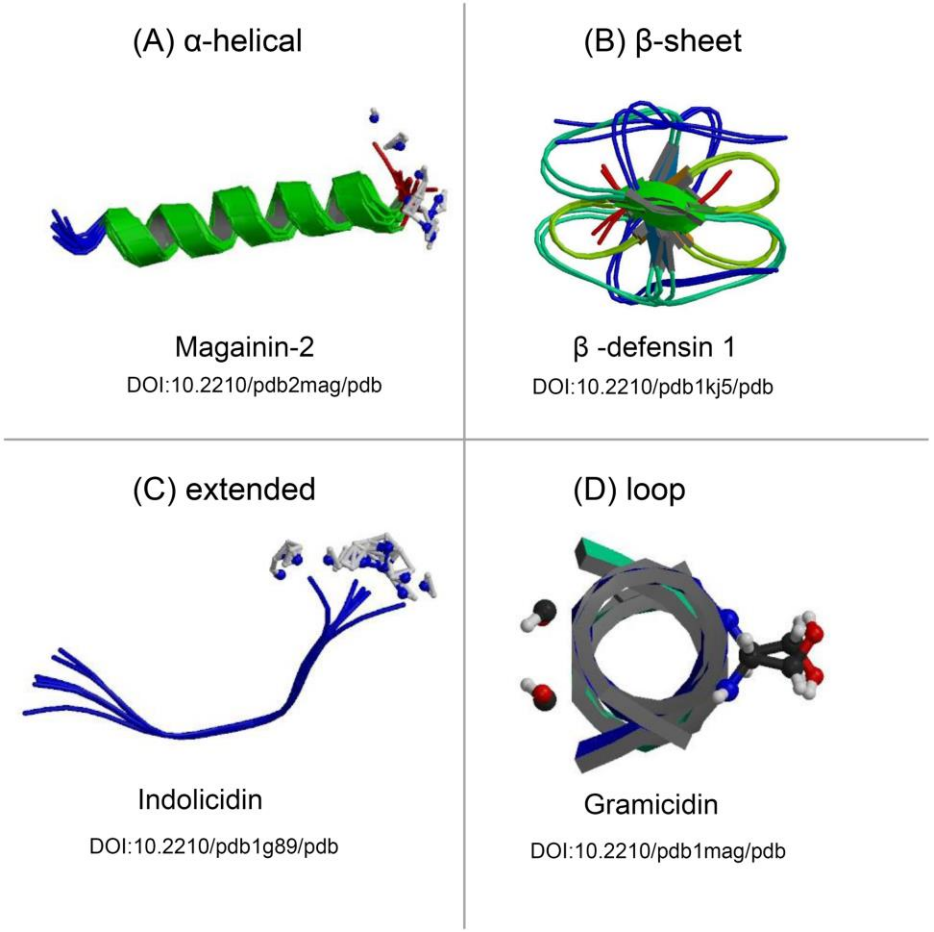
Antimicrobial peptides (AMP)



Short and diverse

Broad spectrum of bactericidal activity

Found in bacteria and eukaryotes alike

Part of innate immune system in vertebrates

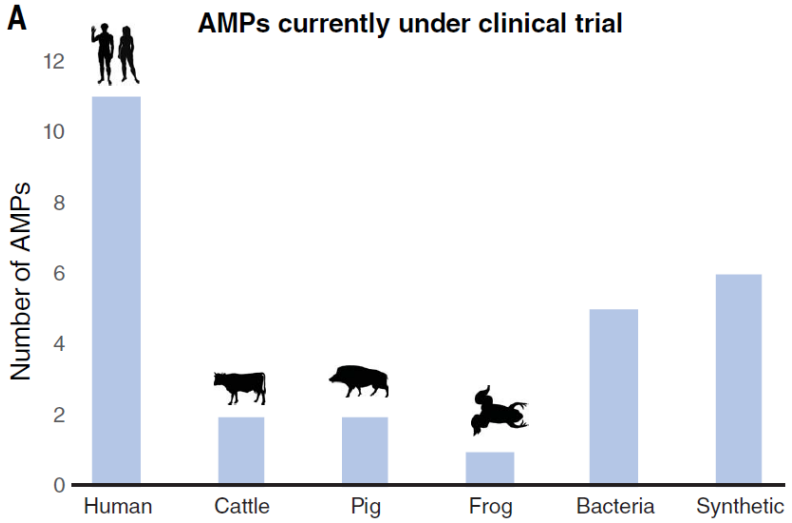
Some used in the clinic

A last-resort antibiotics against multi-drug resistant Gram-negatives

Main concerns

1) Resistance to certain AMPs evolves in the laboratory and in nature

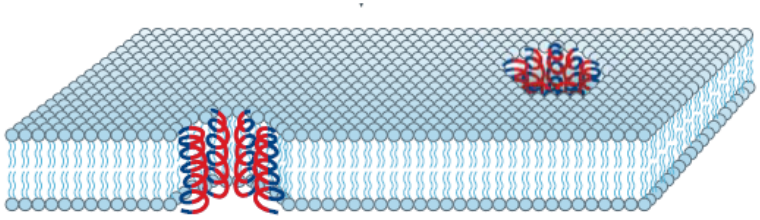
2) Resistance may compromise natural immunity (i.e. cross-resistance to human peptides)



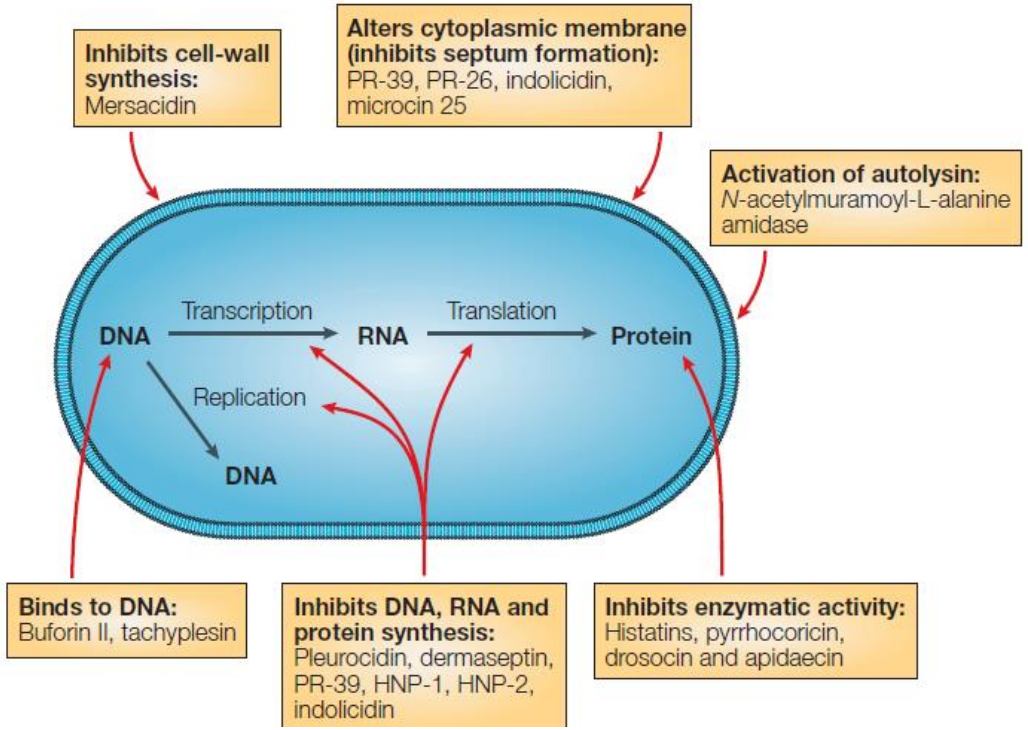
Lazzaro et al. (2020) Science

AMPs have diverse modes of action

Membrane targeting / pore formers

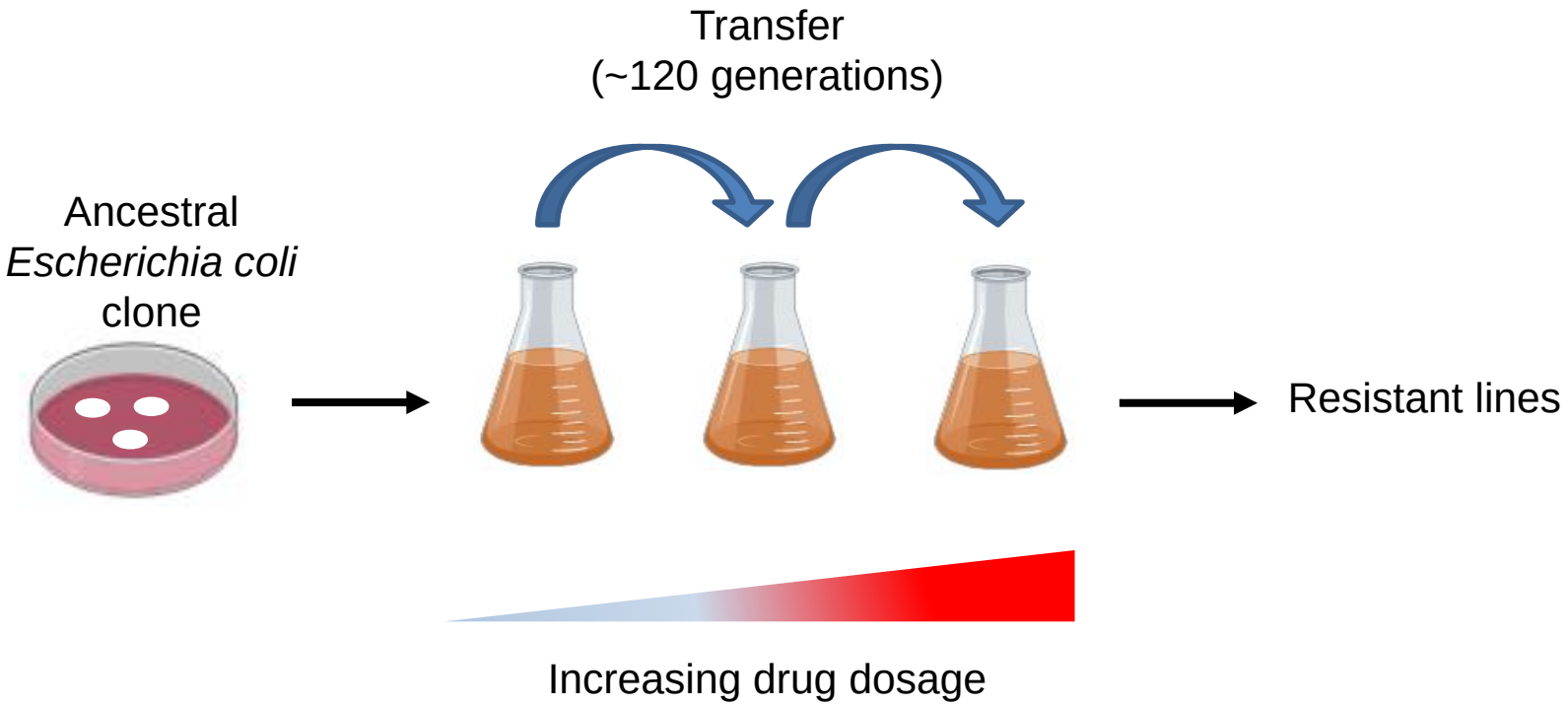


Intracellular targeting



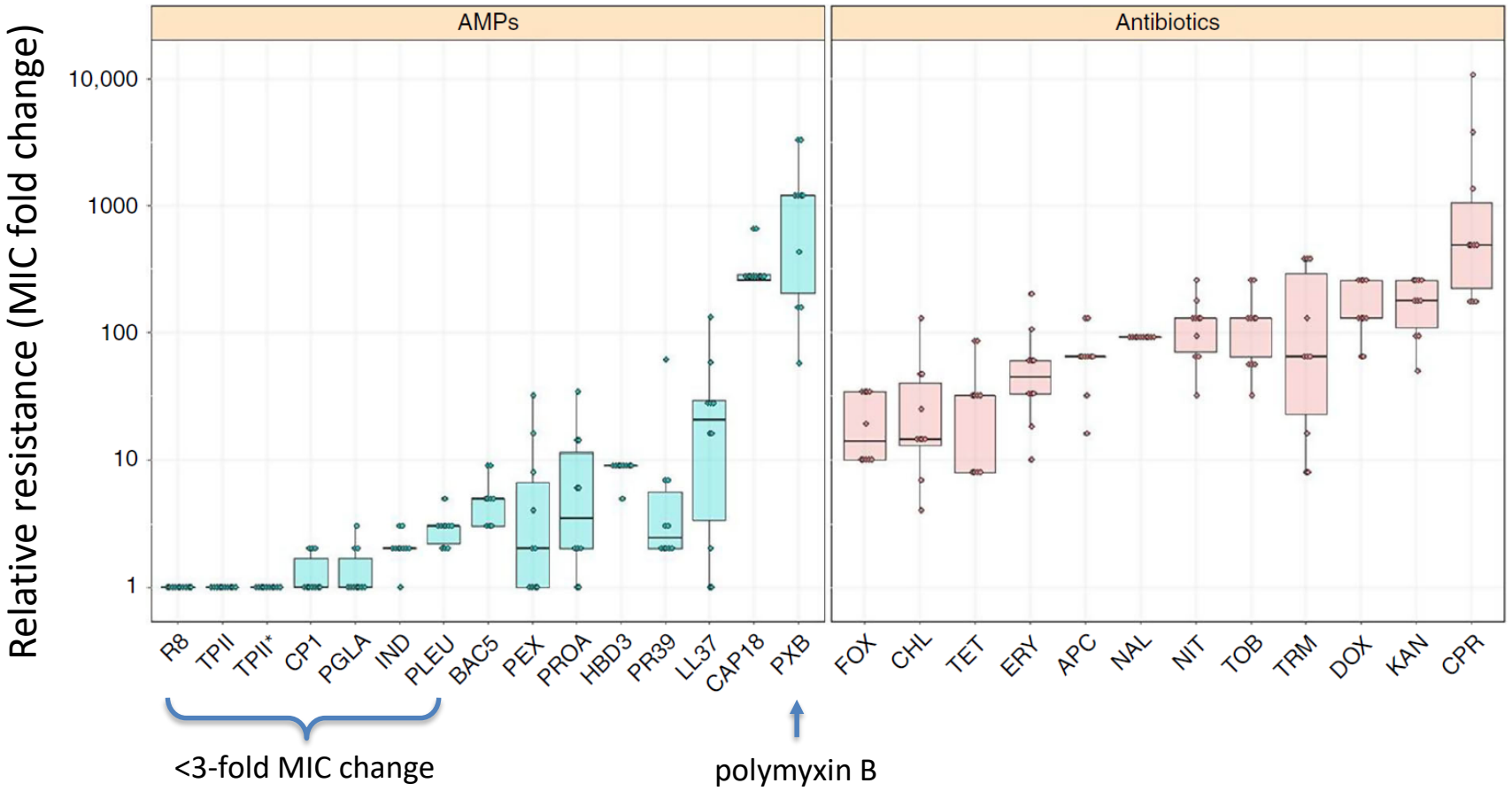
Brogned (2005) *Nat Rev Microbiol*

Laboratory evolution of resistance



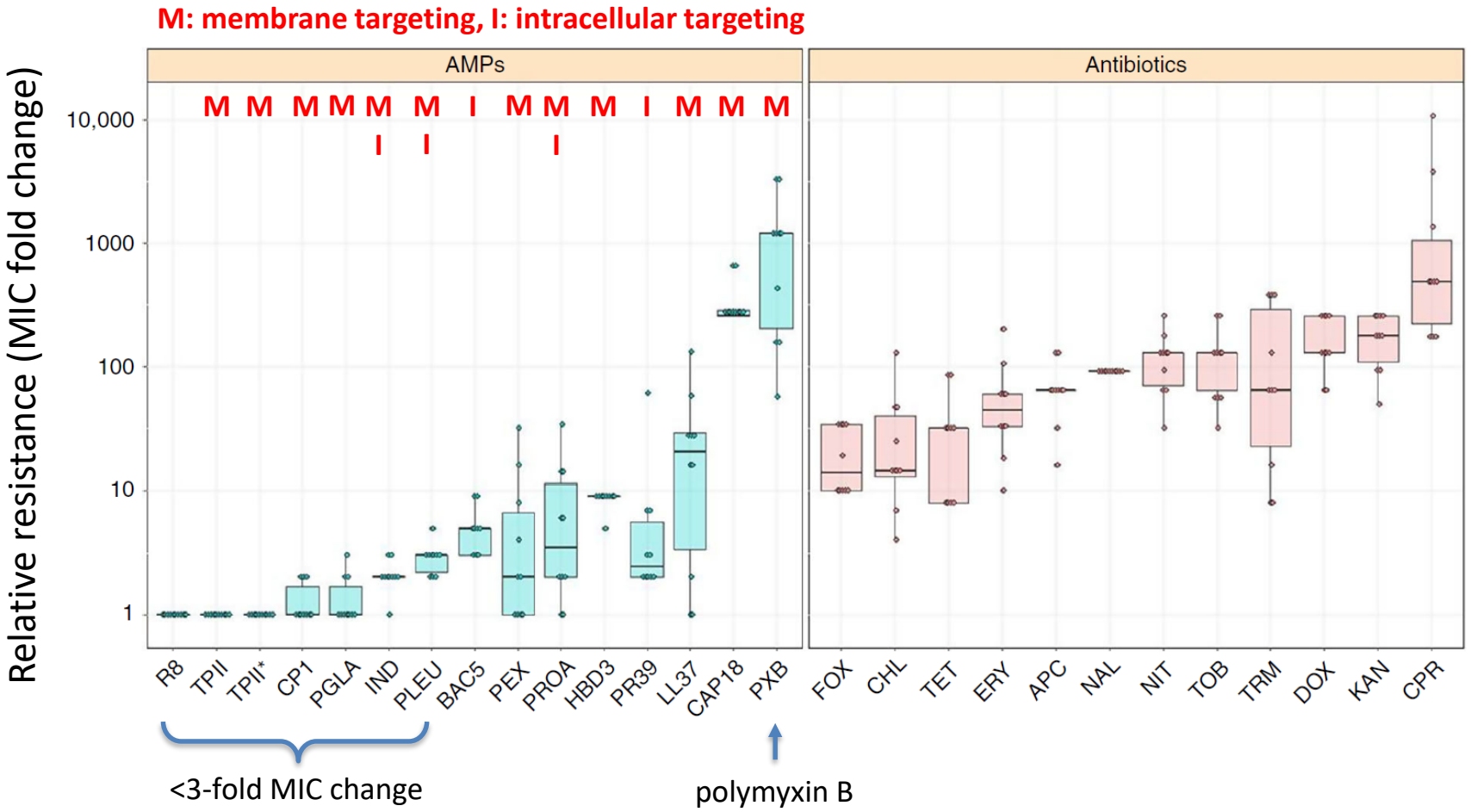
10 parallel lines per drug
14 AMPs with diverse structures and mechanisms

Lower resistance potential for AMPs than for antibiotics



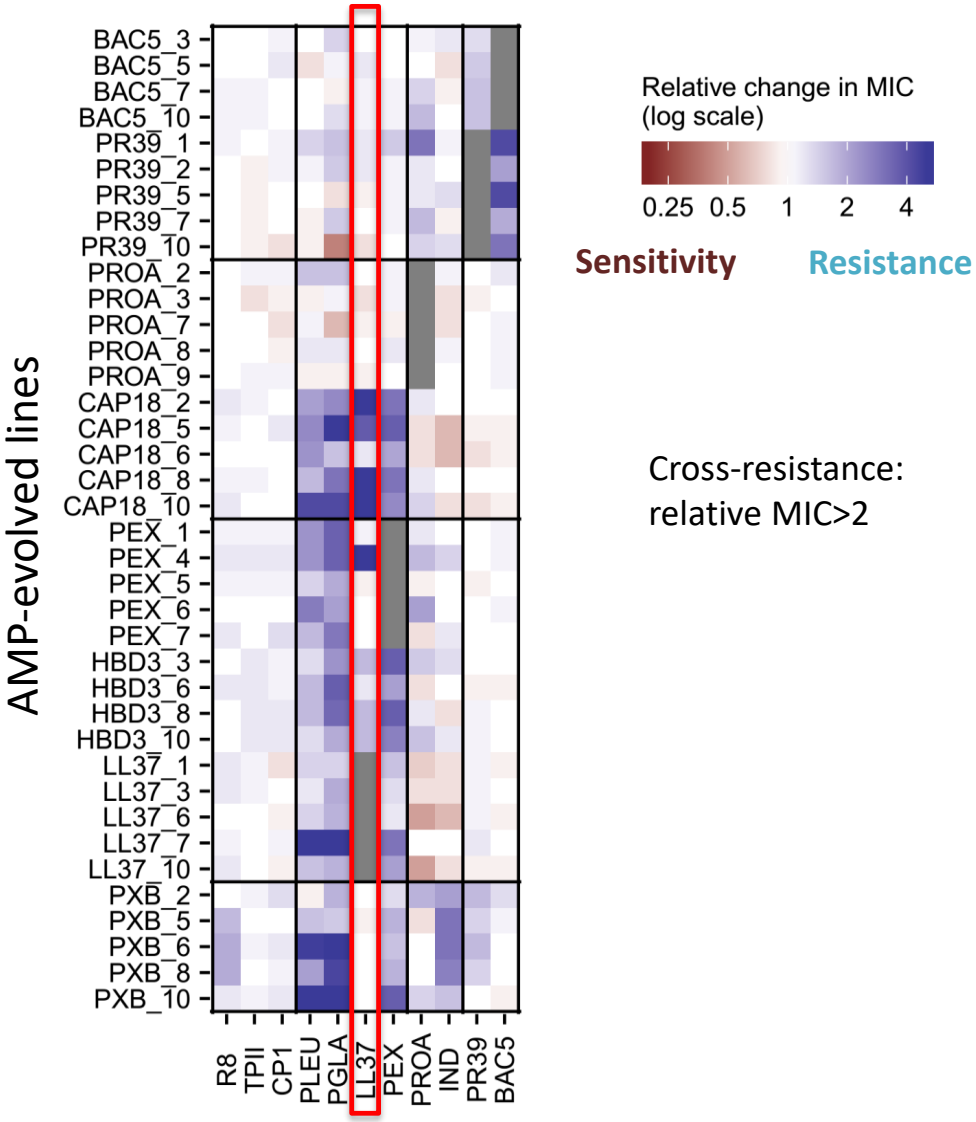
Spohn et al. *Nat Comm* 2019

Membrane targeting AMPs are not universally resistance-free



Spohn et al. *Nat Comm* 2019

Cross-resistance is relatively rare



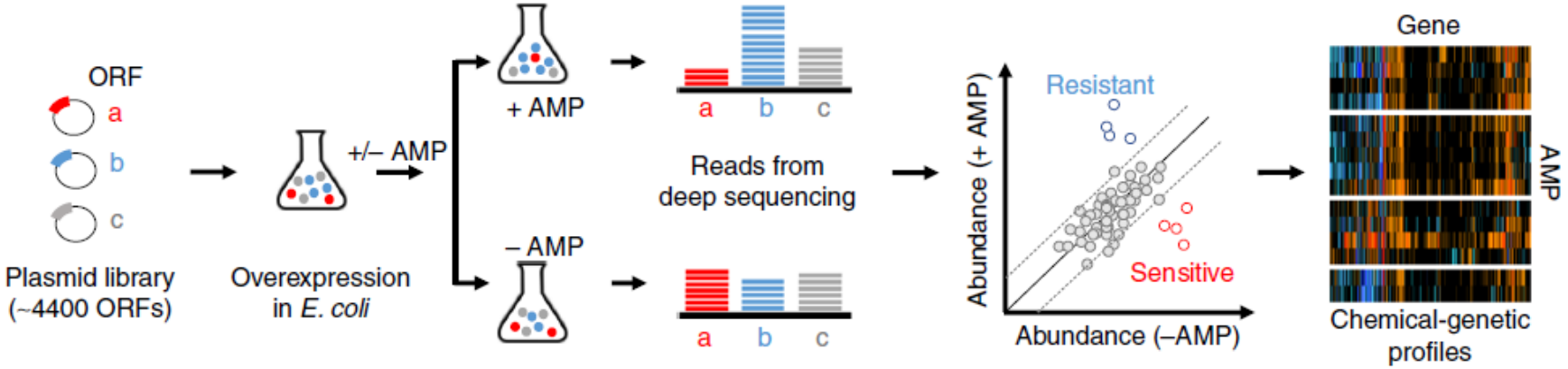
Cross-resistance:
relative MIC>2

Only 16.5% of combinations show cross-resistance

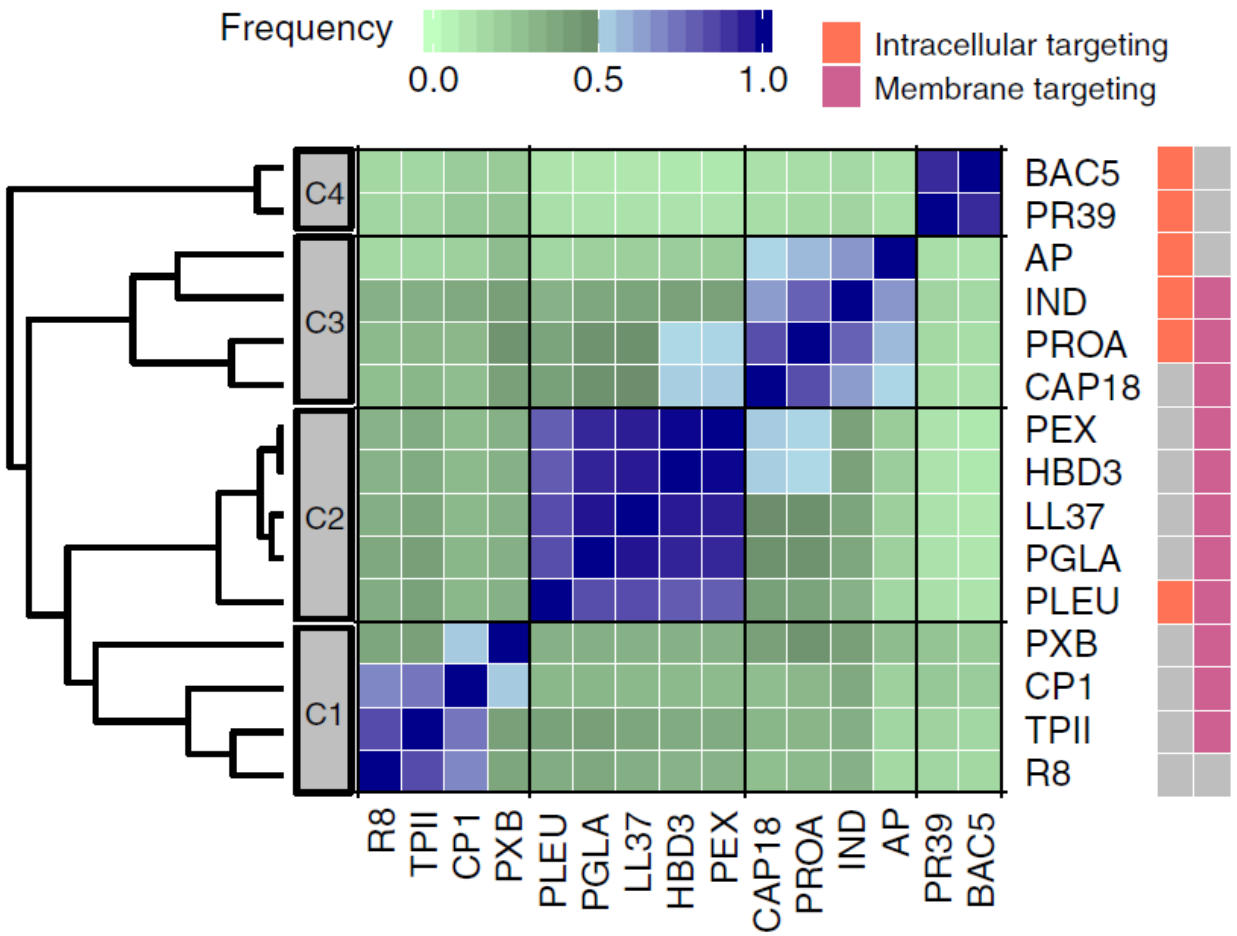
But human peptide LL37 shows cross-resistance to several lines

Spohn et al. *Nat Comm* 2019
Kintses et al. *Nat Comm* 2019

Mapping resistance-modulating genes through chemical genetics



Chemical-genetic profiles group AMPs with similar mode of action…

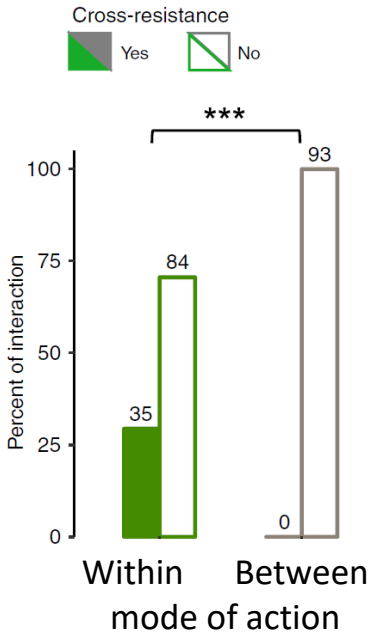


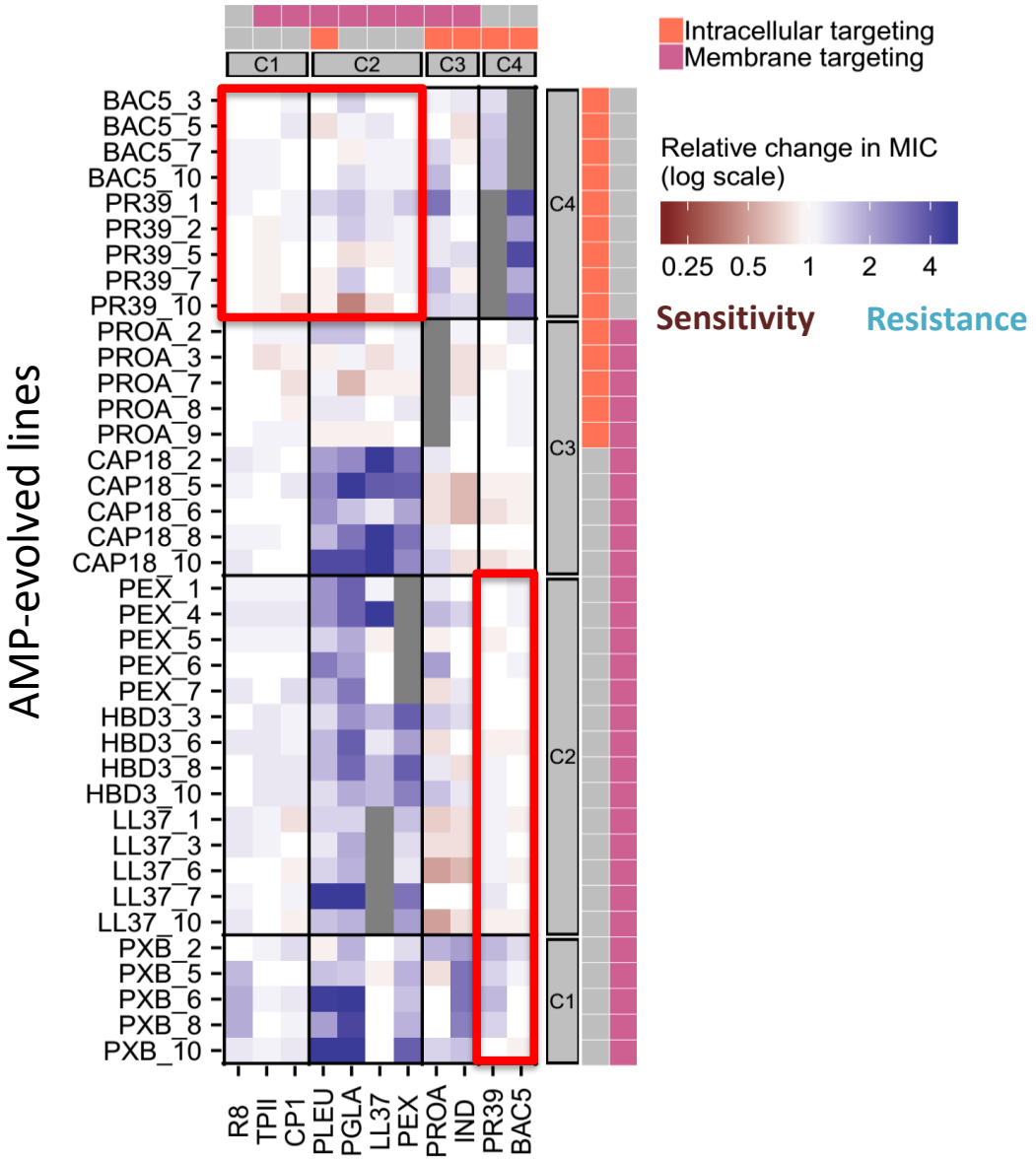
Both membrane and intracellular targeting activities

Kintses et al. *Nat Comm* 2019

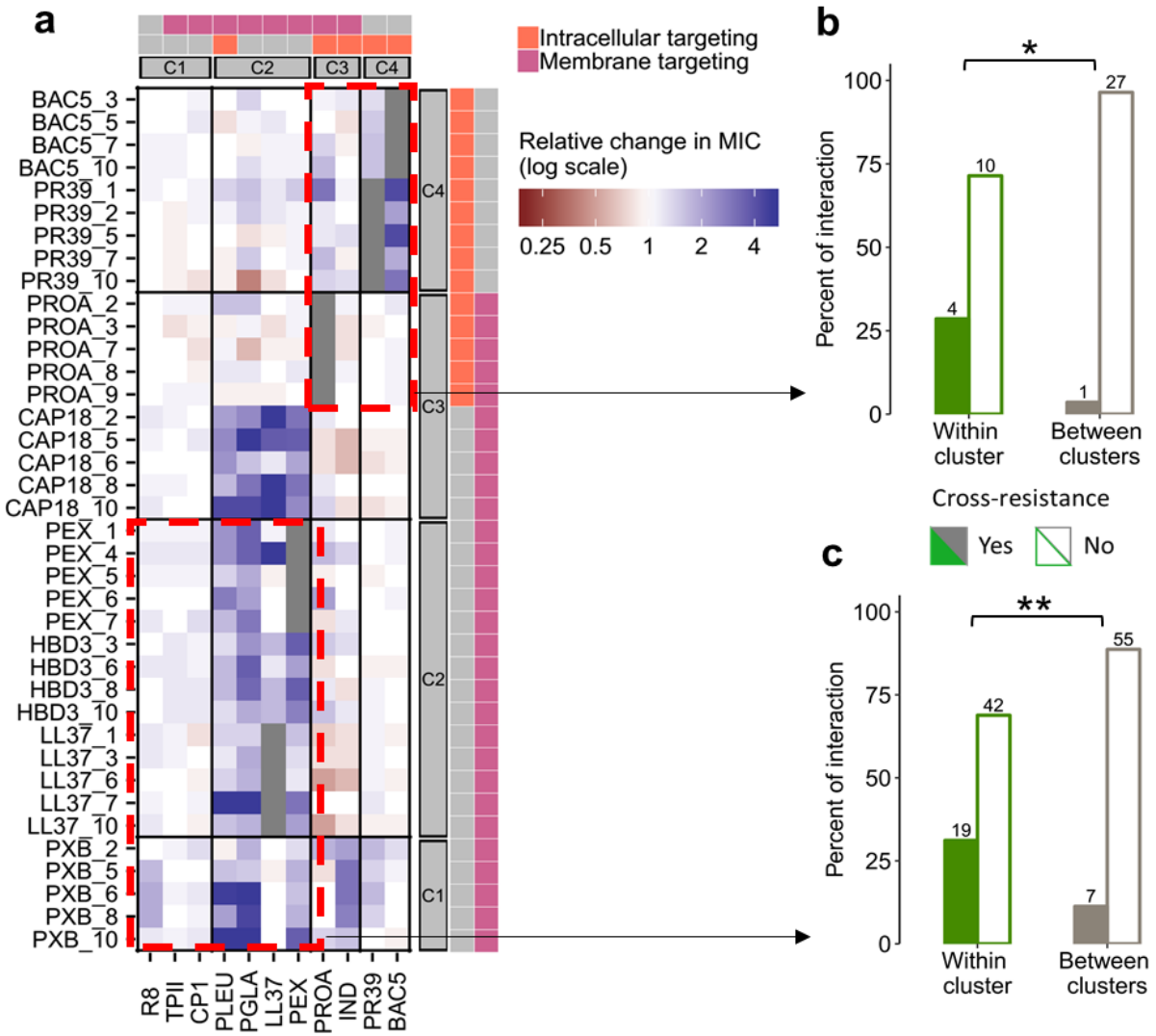
…and informs on cross-resistance

1) No cross-resistance between strictly intracellular and membrane targeting AMPs





2) Chemical-genetic profiles also predicts cross-resistance patterns within mode of action



Conclusions

1) AMPs differ greatly in resistance propensity

2) Cross-resistance to human peptides could be minimized by deploying intracellular targeting AMPs

Acknowledgements

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MAGYAR TUDOMÁNYOS AKADÉMIA 1825

Table 1 List and characteristics of AMPs used in this study. Their abbreviation, described mode of action, and clinical relevance (for details see Supplementary Data 7).

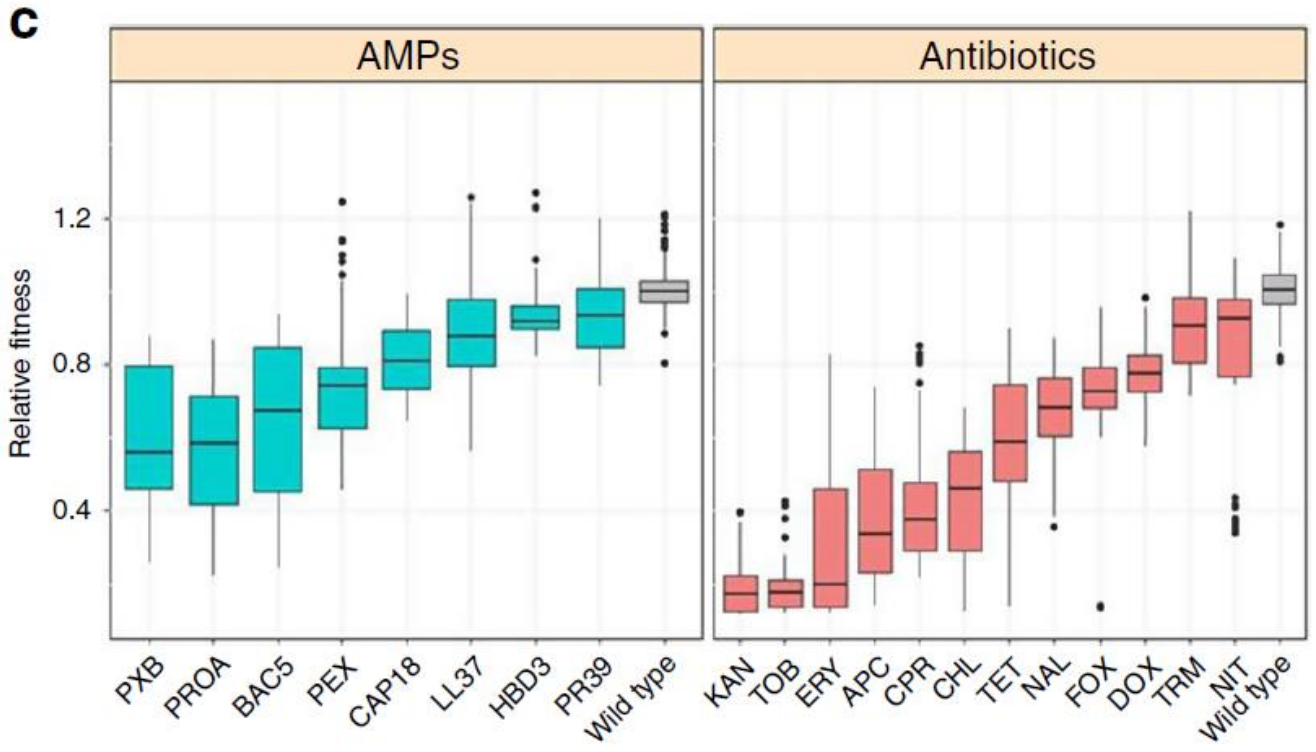
Name of AMP	Abbreviation	Mode of action	Clinical relevance
Apidaecin IB	AP	Inhibits protein biosynthesis by targeting ribosomes; Interacts with DnaK, GroEL/GroES, FtsH	Yes
Bactenecin 5	BAC5	Inhibits protein and RNA synthesis	n.a.
CAP18	CAP18	Disrupts cell membrane	Yes
Cecropin P1	CP1	Disrupts cell membrane	n.a.
Human beta-defensin-3	HBD-3	Disrupts cell membrane; Inhibits lipid II in peptidoglycan biosynthesis	n.a.
Indolicidin	IND	Inhibits DNA and protein synthesis; Disrupts cell membrane; Inhibits septum formation	Yes
LL-37 human cathelicidin	LL37	Disrupts cell membrane; Induces ROS formation	Yes
Peptide glycine-leucine amide	PGLA	Disrupts cell membrane	n.a.
Pexiganan	PEX	Disrupts cell membrane	Yes
Pleurocidin	PLEU	Disrupts cell membrane; Induces ROS formation; Inhibits protein and DNA synthesis	n.a.
Polymyxin B	PXB	Disrupts cell membrane; Induces ROS formation	Yes
PR-39	PR39	Inhibits protein and DNA synthesis	n.a.
Protamine	PROA	Affects cellular respiration and glycolysis; Disrupts cell envelop	n.a.
R8	R8	n.a.	n.a.
Tachyplesin II	TPII	Disrupts cell membrane	n.a.

n.a. no data available

Kintses et al. Nature Microbiology 2019

Lower resistance potential of AMPs is not explained by high cost of resistance

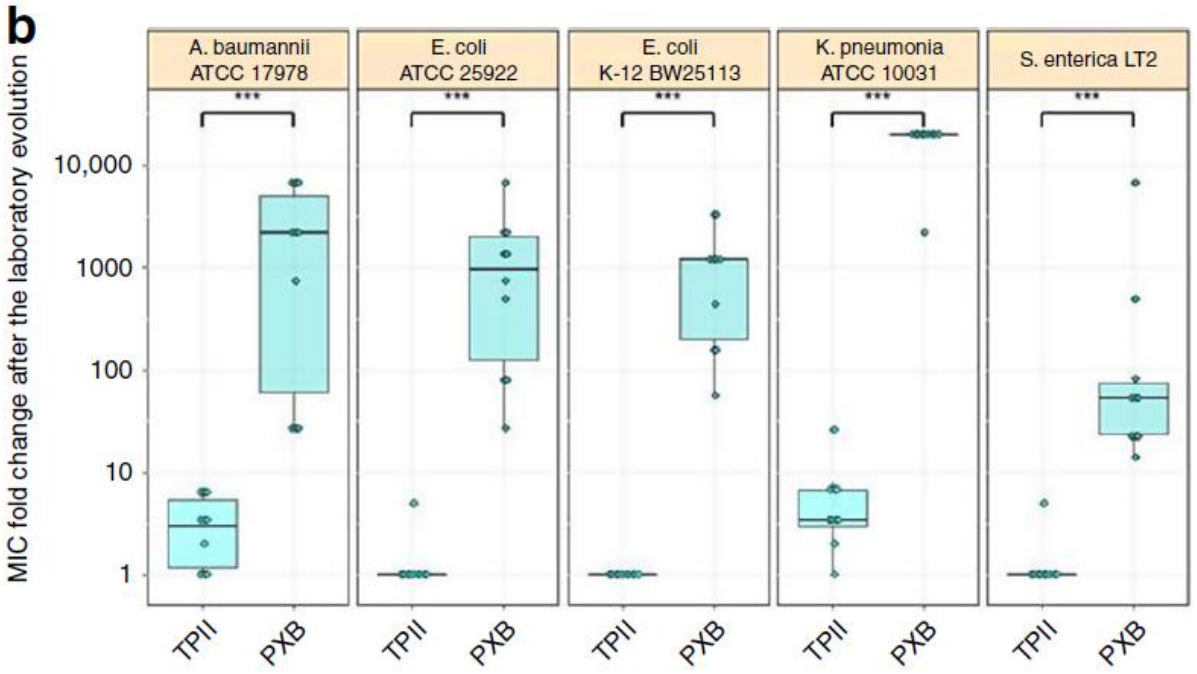
In general, AMP evolved lines show lower fitness costs

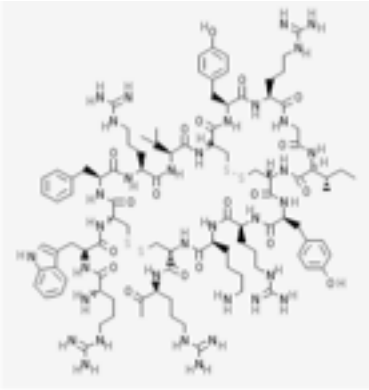


Spohn et al. *Nat Comm* 2019

Tachyplesin II: a cationic β-hairpin antimicrobial peptide discovered in horseshoe crab

Appears resistance-free when evolution is conducted in in different clinical isolates

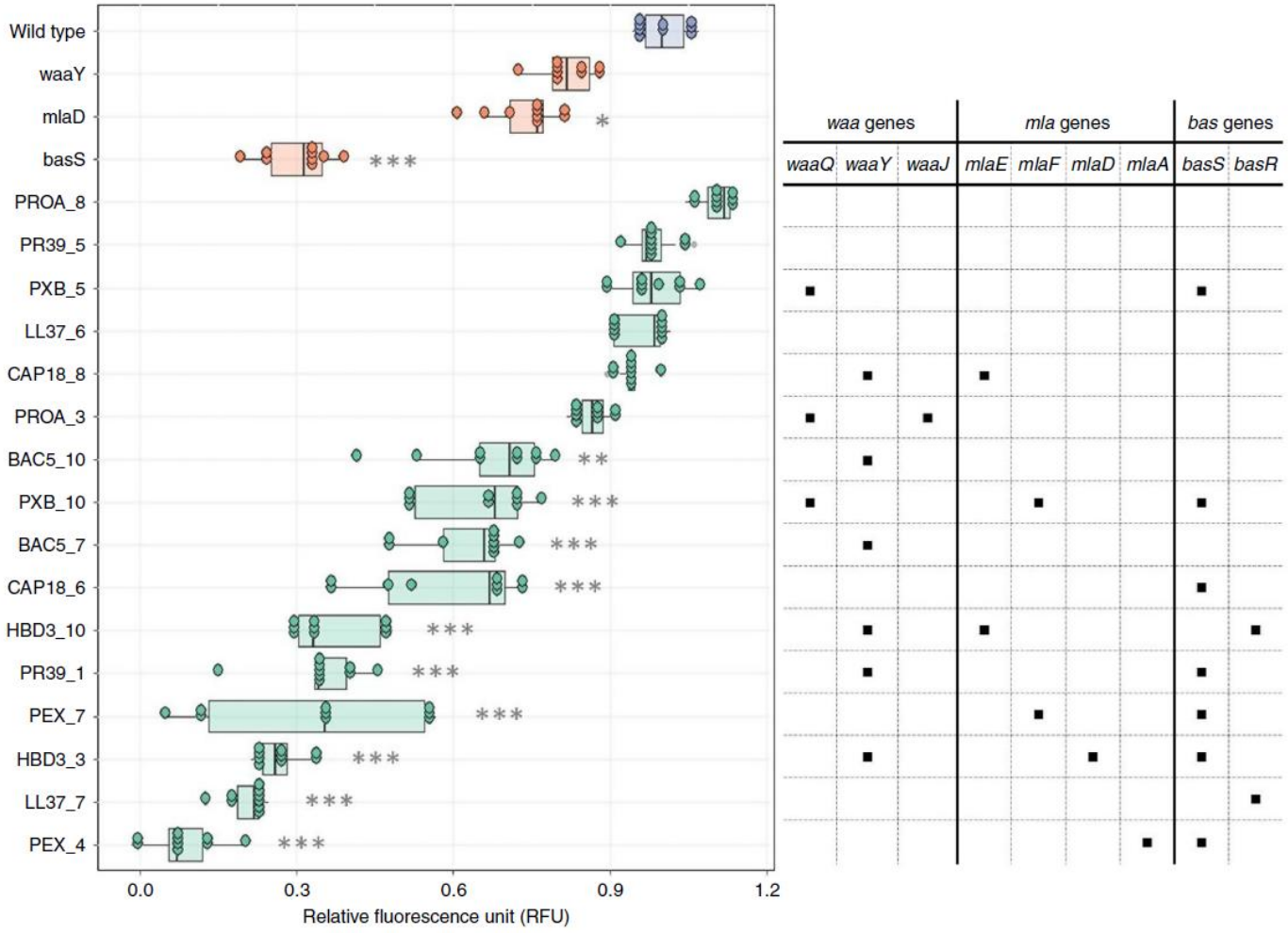



Tachyplesin II

Spohn et al. *Nat Comm* 2019

Surface charge changes underlie resistance

- 13 out of 16 tested evolved lines show a reduced net negative surface charge
- Carry mutations in BasR-BasS two-component system, a regulator of LPS pathway / phospholipid trafficking



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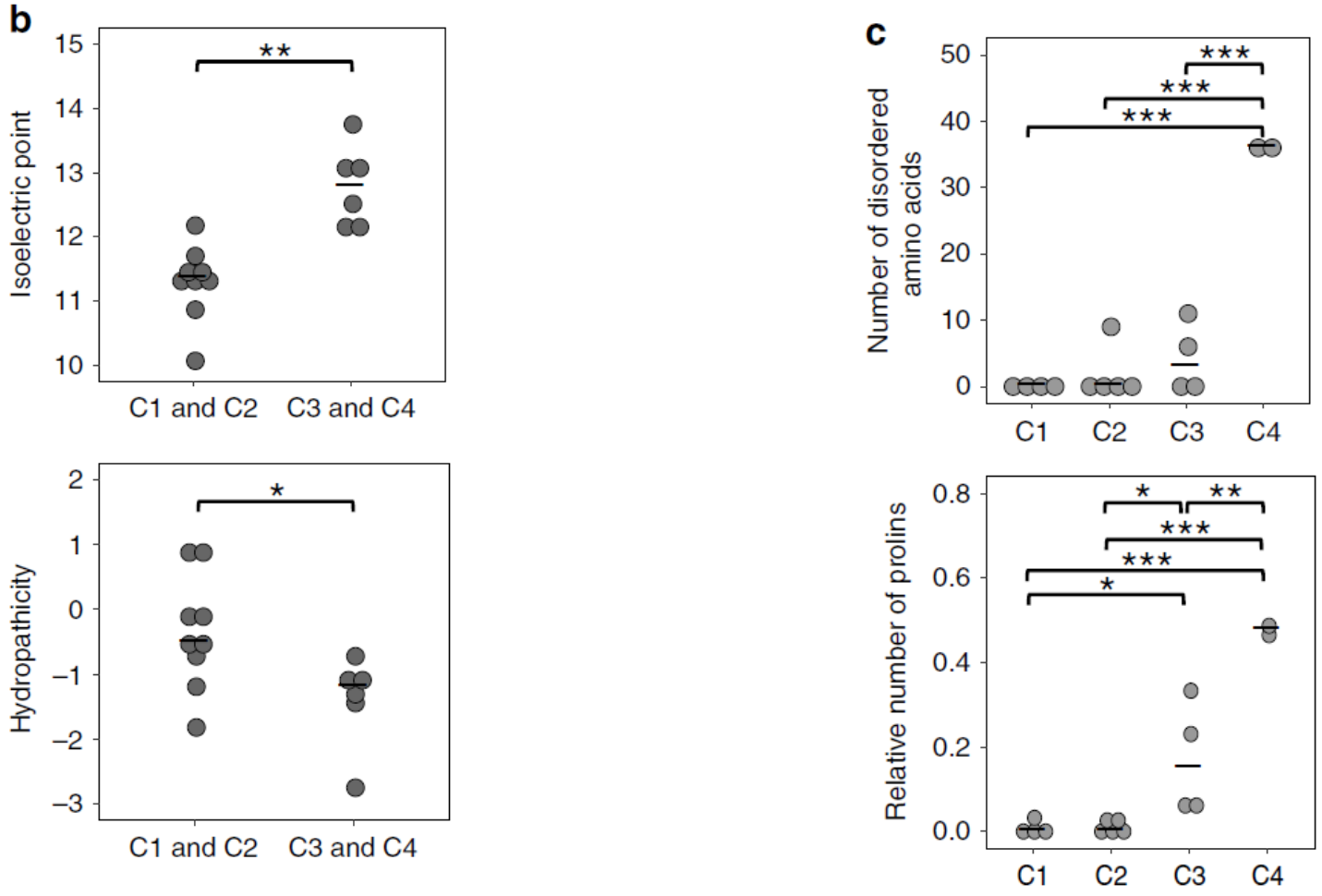
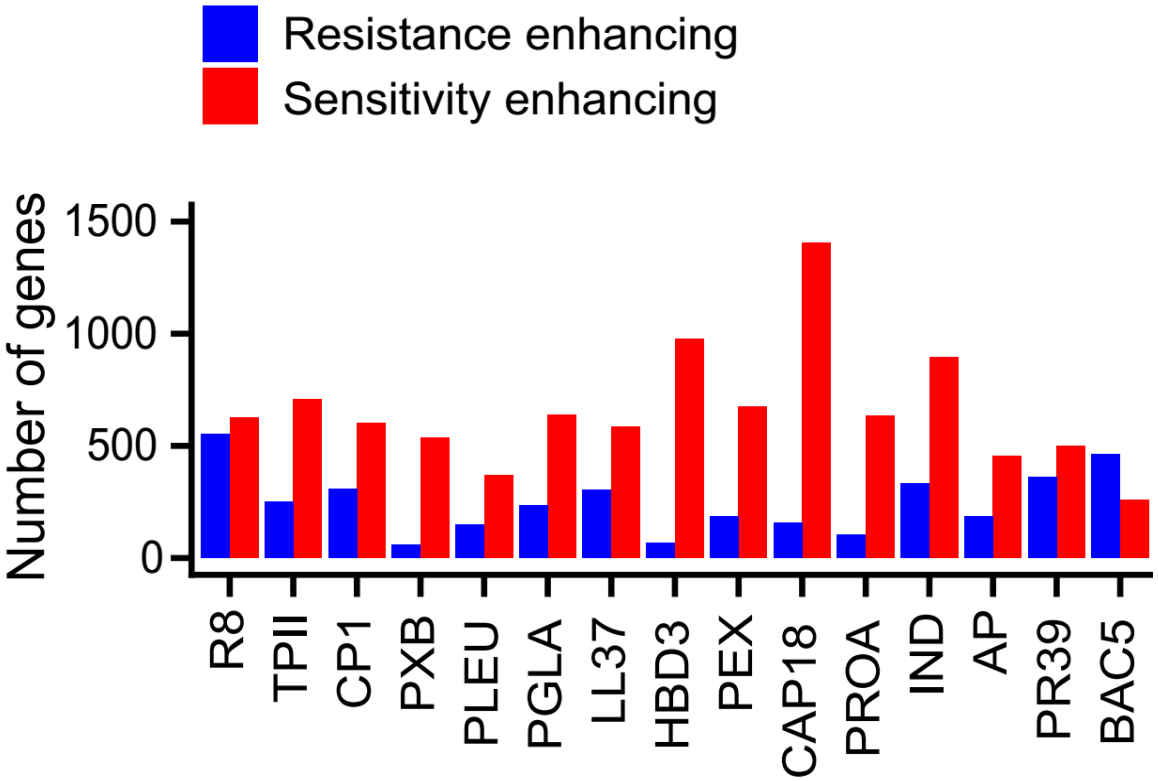
Chemical-genetic profiling distinguish AMPs with different physicochemical properties

C1 + C2 = membrane targeting

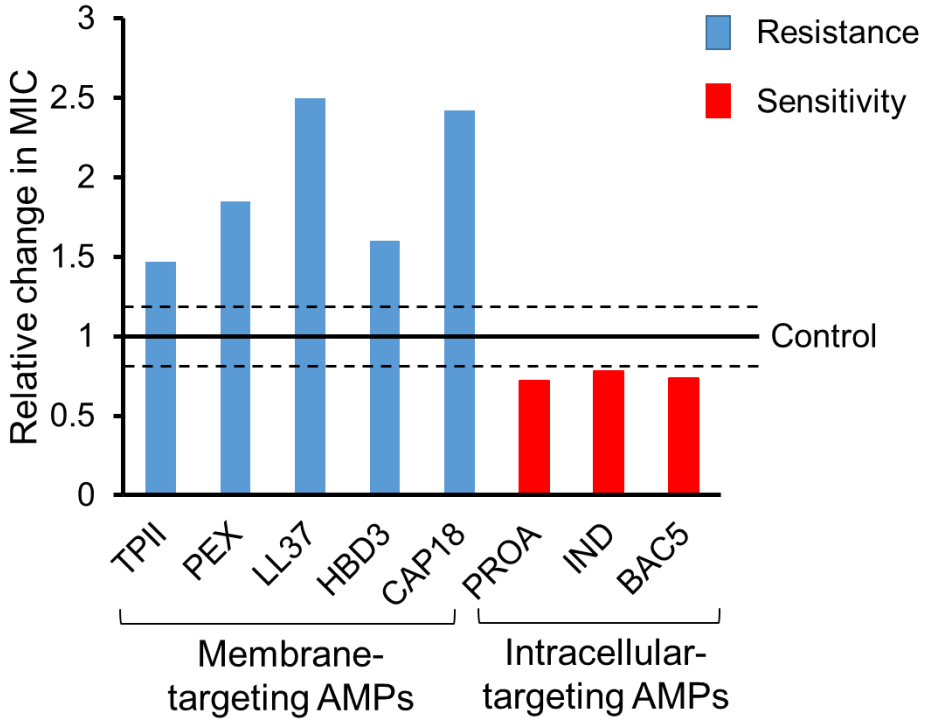
C3 and C4 intracellular targeting AMPs differ: C4 is more prone to disorder



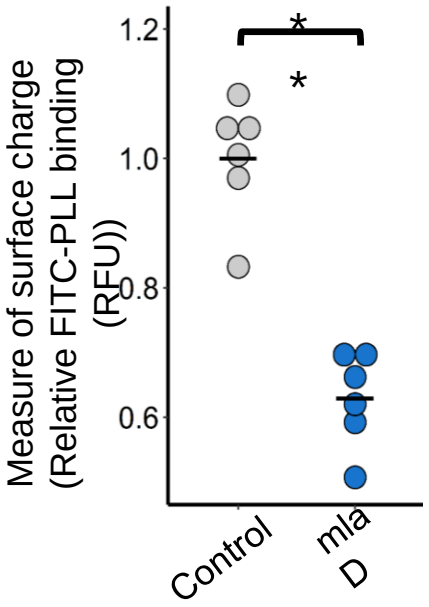
A large set of genes modulate AMP susceptibility

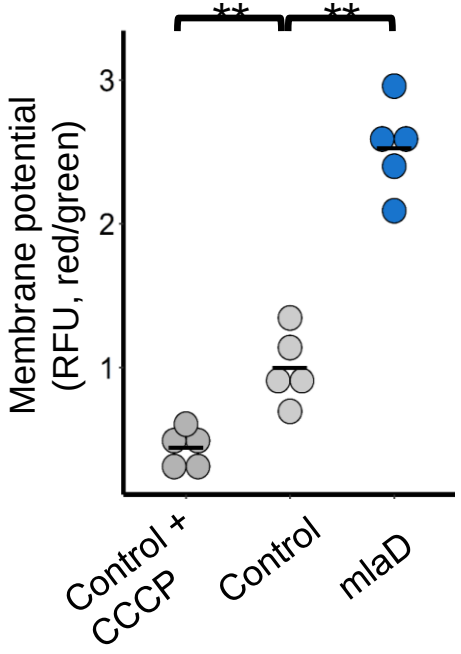


Perturbation of *mlaD* confers resistance to membrane-targeting AMPs and sensitivity to intracellular-targeting AMPs

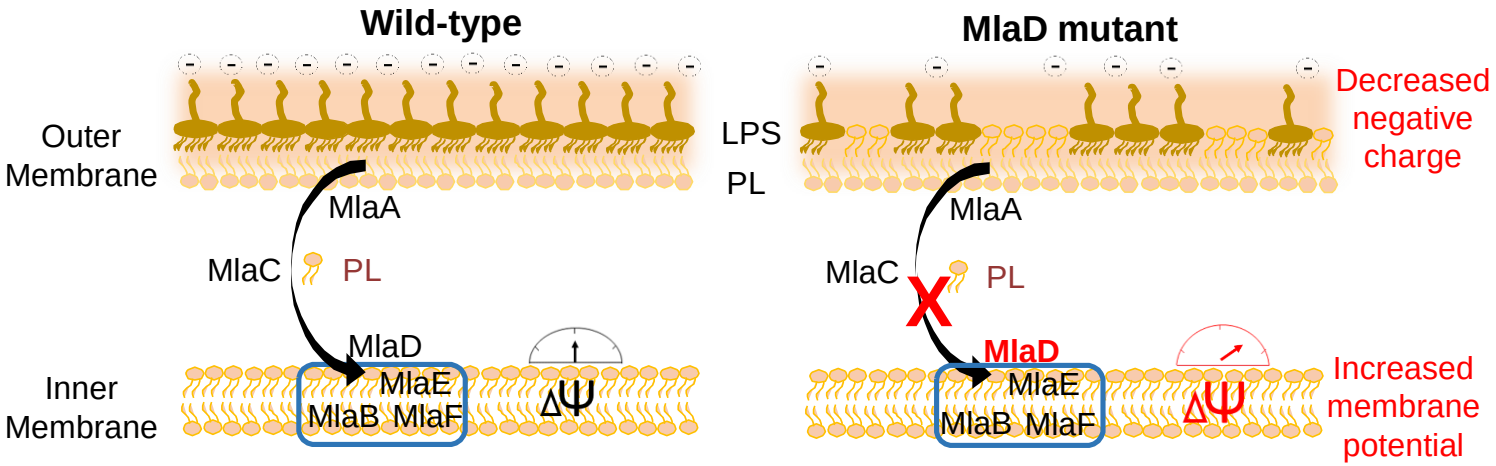


Perturbation of *mlaD* decreases membrane surface charge and increases membrane potential





Molecular basis of the collateral sensitivity interaction



Functionally diverse AMPs and antibiotics used

AMPs		Antibiotics	
Polymyxin B	(cyclic bacterial)	Trimetoprin	(FolA inhibitor)
PGLa	(pore former, amphibian)	Ampicillin	(beta lactam)
LL37	(pore former, human)	Tetracycline	(translation inhibitor)
Tachniplesin II	(pore former, crab)	Doxycyclin	(translation inhibitor)
Omiganan	(LL37 derivative, in clinical trials)	Streptomycin	(aminoglycoside)
Cecropin 1	(pore former, Ascaris suum)	Tobramicin	(aminoglycoside)
R8	(synthetic)	Cefoxitin	
Protamine	(arginine rich, herring)	Erythromcin	(macrolid)
Bactenectin 5	(proline rich, bovine)	Cloramphenicol	
PR39	(proline rich, pig)	Nalidixic acid	(gyrase inhibitor)
Pexiganan	(pore former, amphibian, in clinical trials)	Ciprofloxacin	
Indolicidin	(bovine)		

Lazar et al. Nature Microbiology 2018, Kintses et al. Nature Microbiology 2019