P430: BIFUNCTIONAL *DE NOVO* PEPTIDES TO TACKLE ANTIMICROBIAL RESISTANCE

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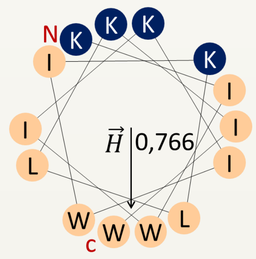
Context

Nearly 5 **million deaths** were associated to **antimicrobial resistance** worldwide in 2021, driving the urgent need for both new antimicrobial **therapeutics** and rapid **diagnostic** tools to reduce antibiotic misuse and improve patient outcomes.

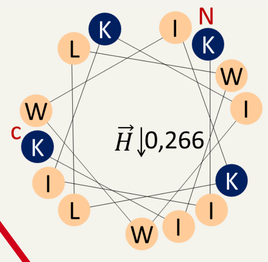
In this context, we designed ***de novo* peptides,** PACHA01 and PACHA02 with the dual aim of developing **new antimicrobial therapeutics** and bacterial **detection probes**. PACHAs were produced by solid-phase peptide synthesis with identical amino acid compositions but different primary sequences:

Peptide design and characterisation

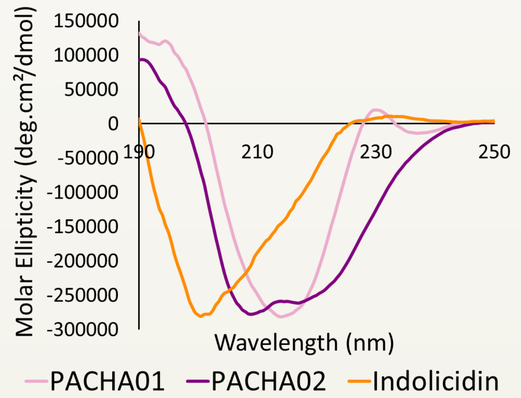
PACHA01 :



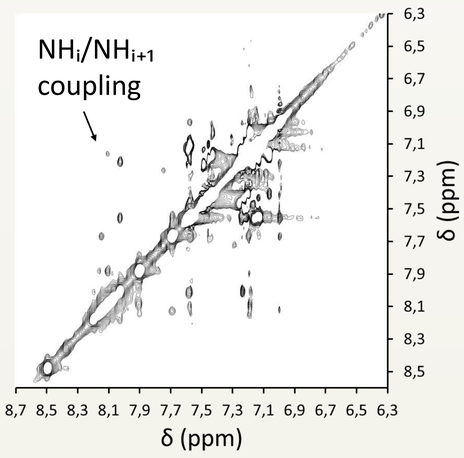
PACHA02 :



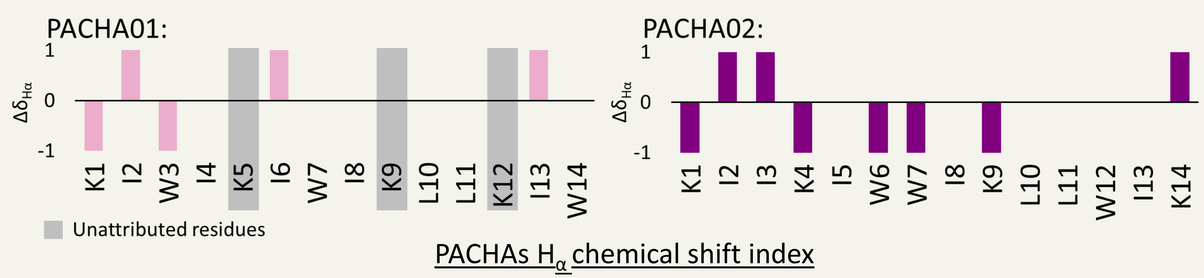
PACHAs ...



Circular dichroism of PACHAs compared with indolicidin



PACHA02 NOESY experiment, 600 MHz NMR

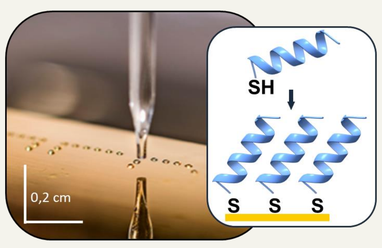


PACHAs H_α chemical shift index

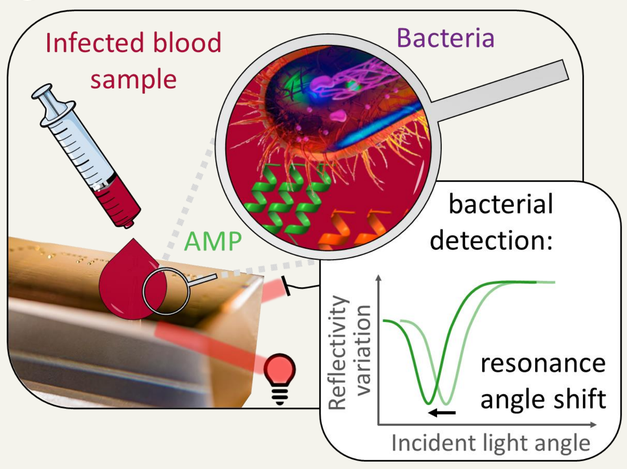
➥ PACH01 is less structure than PACH02 which folds into a partial α-helix at pH 7.

Biosensor development

① Peptide arraying

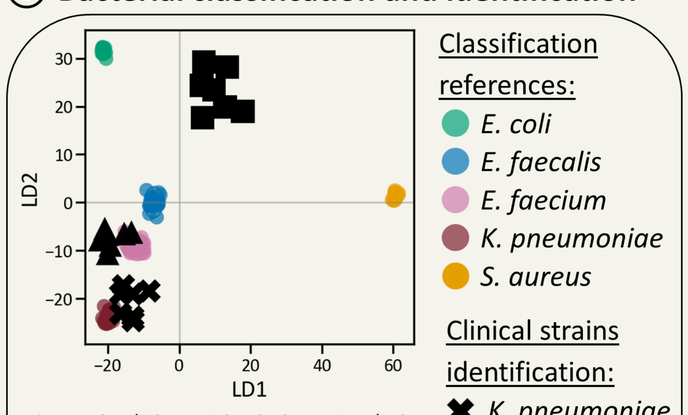


② SPRi monitoring : bacterial detection



... as probes for biosensor

③ Bacterial classification and identification



Supervised Linear Discriminant Analysis (3 replicates per specie microarray with 7 replicates per probe)

Accuracy : 93,3 ± 25,8%

➥ The method enables bacterial detection under 15 hours.

➥ The selected AMP panel generates distinct affinity profiles for each bacterial species. It enables species discrimination within the same genus with few experiments.

➥ The LDA model enable bacterial identification of clinical strains with limits.

Therapeutic development

... as antimicrobial agent

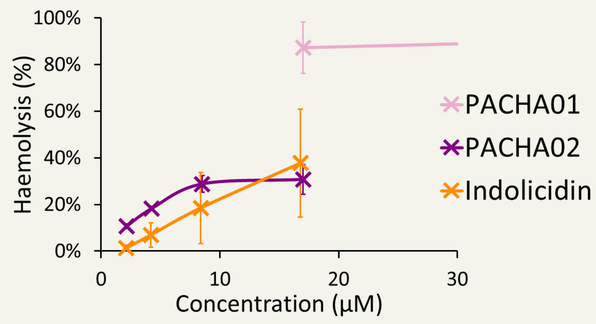
Antimicrobial activity:

MIC (mg/L / µM)	PACHA01	PACHA02	Indolicidin
S. aureus	4 2	4 2	8 4
E. coli	64 34	16 4	8 4
A. baumannii	N. D.	8 4	4 2
P. aeruginosa	N. D.	4 2	4 2
K. pneumoniae	N. D.	2 to 128 1 to 68	8 4

➥ Despite identical amino acid composition PACHA01 is less active than PACHA02 against *E. coli.*

➥ The antimicrobial activity of PACHA02 against a broad spectrum of bacterial species is comparable to that of a well-established AMP.

Toxicity:



Hemolytic ratio on human erythrocytes

IC_{50} (mg/L / µM)	PACHA01	PACHA02	Indolicidin
A-549	P	>256 >136	>64 >34
THP-1	P	>128 >68	>32 >17

P: precipitation prevents reading

Cytotoxicity toward epithelial and circulating cells

➥ PACHA02 is non-toxic to tested cells, except for RBCs, where it shows toxicity similar to Indolicidin but nearly 3-fold lower than PACHA01.

Optimisation:

		KIIKIAAIKLLAIK	KIIKIAAIKLLWIK	KIIKIWAIKLLAIK	KIIKIAWIKLLAIK
MIC (mg/L / µM)	*E. coli*	4 2	4 2	4 2	4 2
	S. aureus	4 2	4 2	4 2	4 2
IC_{50} (mg/L / µM)	A-549	>256 >167	>256 >155	>256 >155	>256 >155
	THP-1	>256 >167	>256 >155	>256 >155	>256 >155

➥ Reducing the tryptophan proportion of PACH02 sequence does not affect its antimicrobial activity and toxicity.

Conclusion & Perspectives

➥ PACHA02 is as promising an antimicrobial candidate as Indolicidin.

➥ Ongoing optimization shows highly encouraging results.

➥ AMP micro-array enables rapid bacterial detection and identification.

- Further optimization of PACHA02, including additional toxicity testing.
- Dataset expansion and enhanced data analysis.
- Method validation using relevant clinical samples, such as blood cultures.

New !

Check our last accepted results: É. Pardoux, L. Bastien, R. Mathey, A. Buhot, S. Boisset, D. Boturyn, Y. Roupioz, **Rapid classification of live bacteria in blood using antimicrobial peptides micro-arrays** *J. Mater. Chem. B*

New !