HBP-5: A Leading Heparin-Binding Peptide with Remarkable Activity against Gram-negative Bacteria

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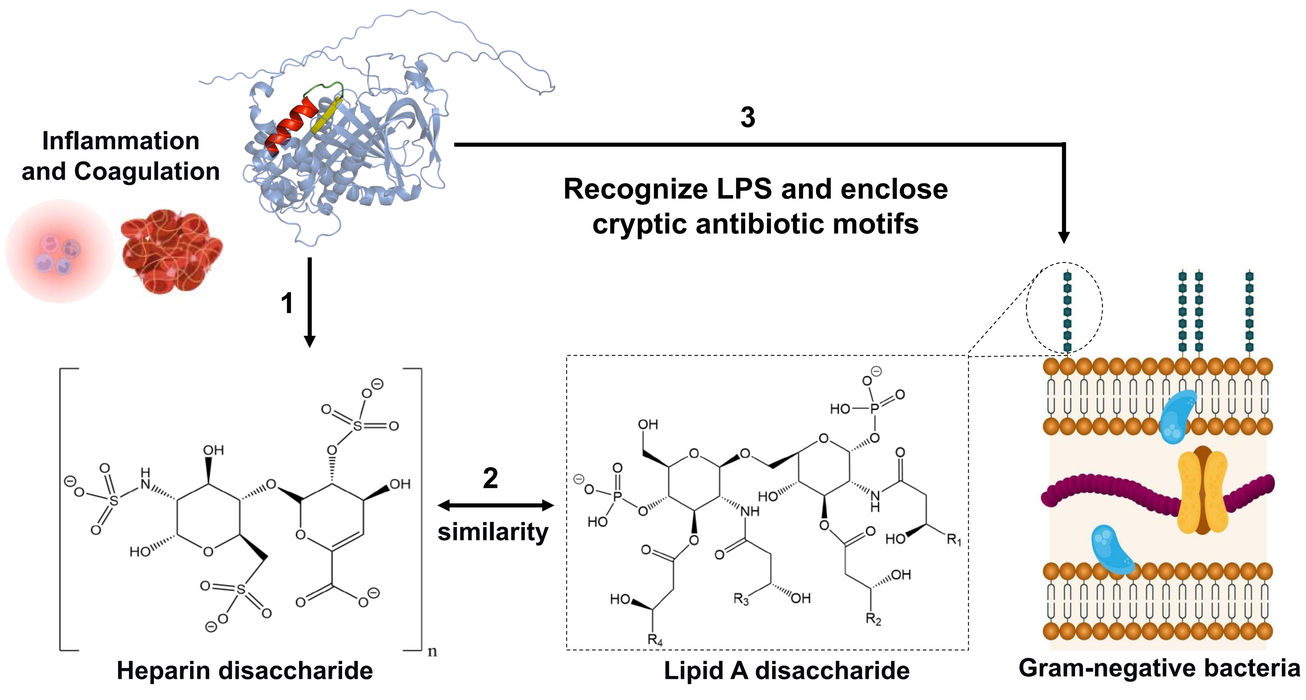
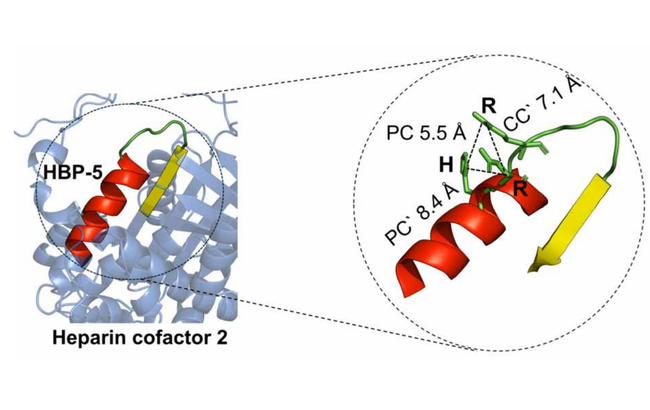
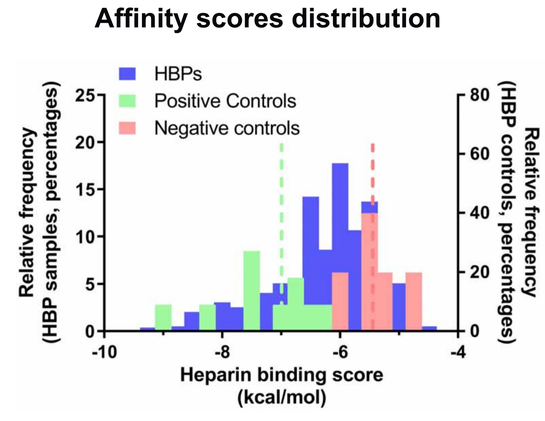
BACKGROUND



METHODOLOGY

- Use the AMPA predictor to identify antimicrobial regions in Heparin-Binding Proteins (HBPs).
- Identification of Heparin Binding sites (CPC` motif) in cryptic peptides and evaluation of their affinity by molecular docking.
- Synthesis and validation of the best candidates corresponding to the regions with the highest AMPA and Heparin-binding score.





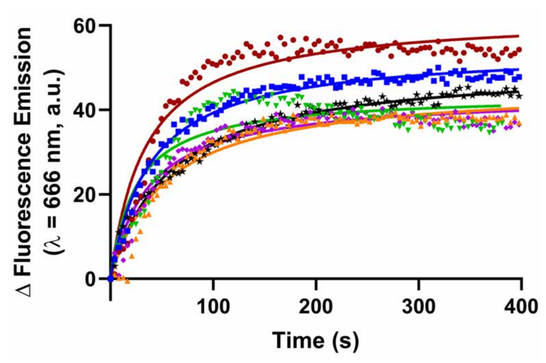
RESULTS

1 Antimicrobial Activity, Heparin-LPS Affinity and Toxicity of HBPs peptides

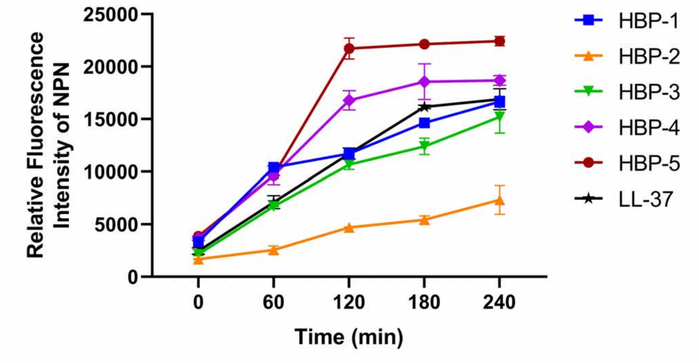
	Minimum Inhibitory Concentration (μM)						Heparin, LPS Affinity and Toxicity			
	E. coli	*A. baumannii*	*P. aeruginosa*	*S. aureus*	*E. faecium*	*L. monocytogenes*	Heparin Affinity (% Elution buffer)	LPS Affinity (EC50, μM)	Hemolysis % (125 μM of peptide)	MRC-5 cytotoxicity (IC50, μM)
HBP-1	1.6	1.6	3.1	50	100	6.3	60	42 ± 16	4.6 ± 0.6	>200
HBP-2	12.5	50	25	>100	>100	37.5	59	1500 ± 600	2 ± 1	>200
HBP-3	3.1	0.8	6.3	25	>100	6.3	68	7 ± 5	4 ± 1	>200
HBP-4	0.2	0.8	0.8	3.1	12.5	1.6	82	0.7 ± 0.6	15.5 ± 0.1	35 ± 1
HBP-5	0.4	0.2	0.8	6.3	25	1.6	98	0.9 ± 0.7	10.3 ± 0.2	69 ± 2
LL-37	1.6	6.3	0.8	25	0.8	0.8	50	0.9 ± 0.8	32.7 ± 0.7	26 ± 3

2 Mechanism of Action of HBP-derived Antimicrobial Peptides

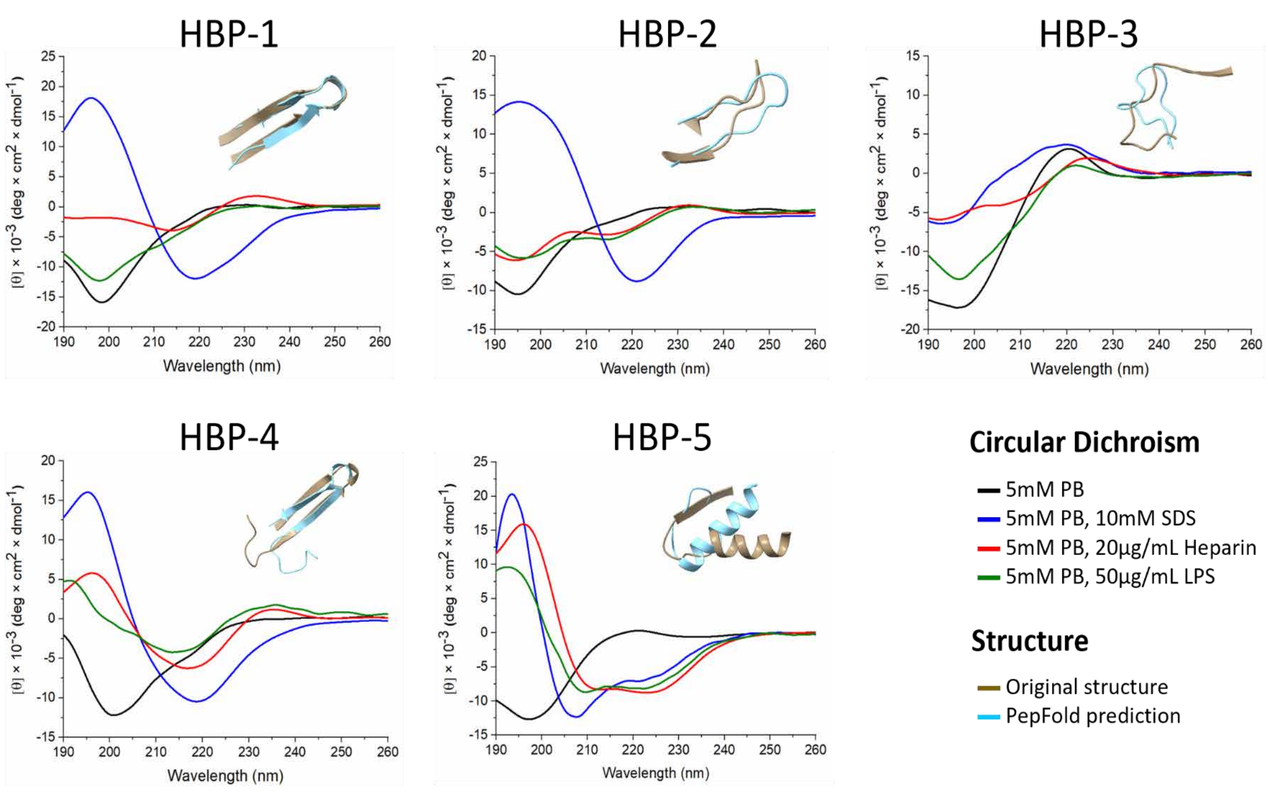
Cell Membrane depolarization



Outer Membrane permeabilization

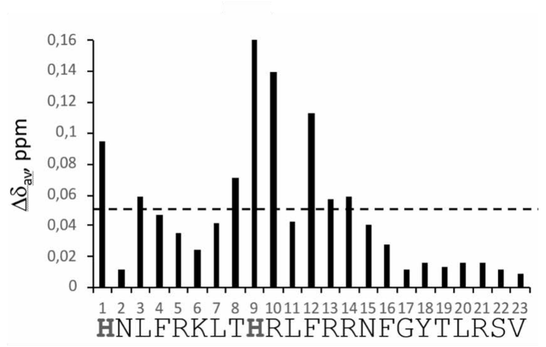


3 Structural Analysis by Circular Dichroism

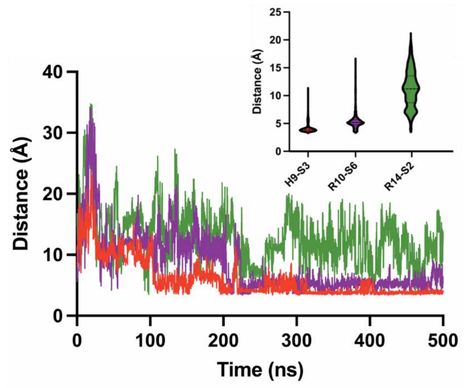


4 Characterization of HBP-5 peptide

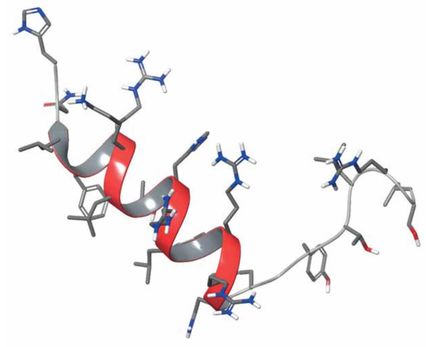
Chemical shifts induced by Heparin



Distances between CPC' residues involved in Heparin binding



Why HBP-5 is a leading peptide ?



- Broad spectrum of antibacterial activity
- Non-cytotoxic and non-hemolytic
- Bacterial cell depolarization and permeabilization
- High affinity for LPS

Conclusions

- We have shown that **Glycosaminoglycans-binding proteins can be a source of new AMPs**, some with remarkable activity, **particularly against Gram-negative bacteria**.
- The fact that **these peptides can bind to both Heparin and LPS** is consistent with the **structural similarity hypothesis.**

What`s next

- Investigate the structural determinants of HBP-5 and their implication on the antibacterial mechanism.
- Optimize and evaluate HBP-5 peptide in an *in vivo* infection model.