

Supplementary Material

Arginine-Rich Self-Assembling Peptides as Potent Antibacterial Gels

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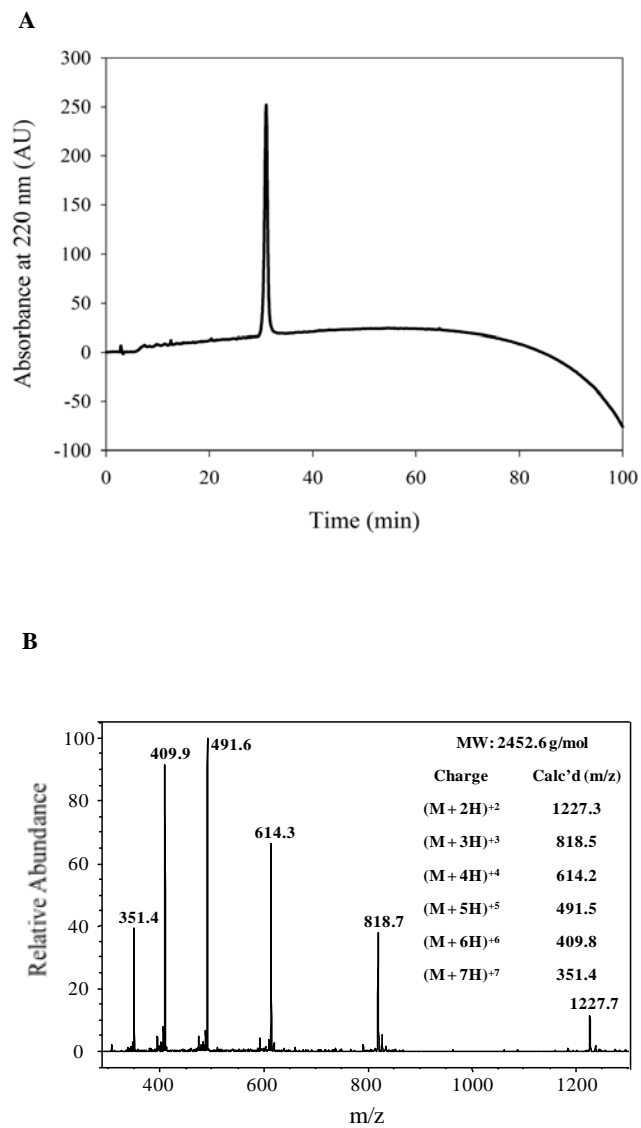


Figure S1 – (A) Analytical HPLC (Vydac C18) of purified PEP8R. 0% to 100% B over 100 minutes. (B) ESI (+) mass spectrum of purified PEP8R.

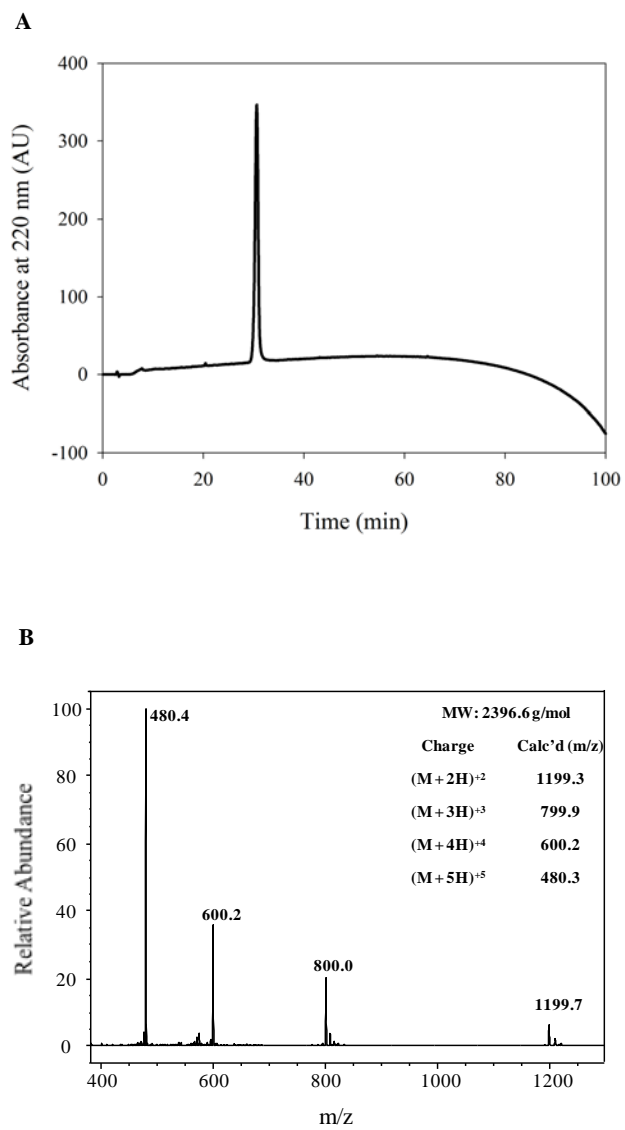


Figure S2 – (A) Analytical HPLC (Vydac C18) of purified PEP6R. 0% to 100% B over 100 minutes. (B) ESI (+) mass spectrum of purified PEP6R

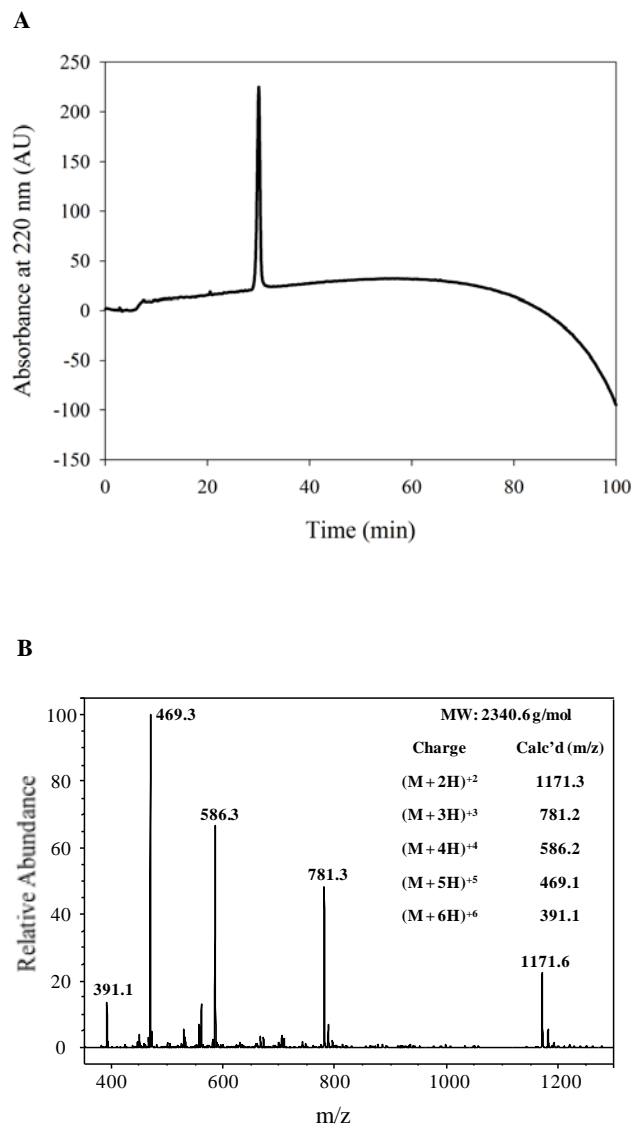


Figure S3 – (A) Analytical HPLC (Vydac C18) of purified PEP4R. 0% to 100% B over 100 minutes. (B) ESI (+) mass spectrum of purified PEP4R.

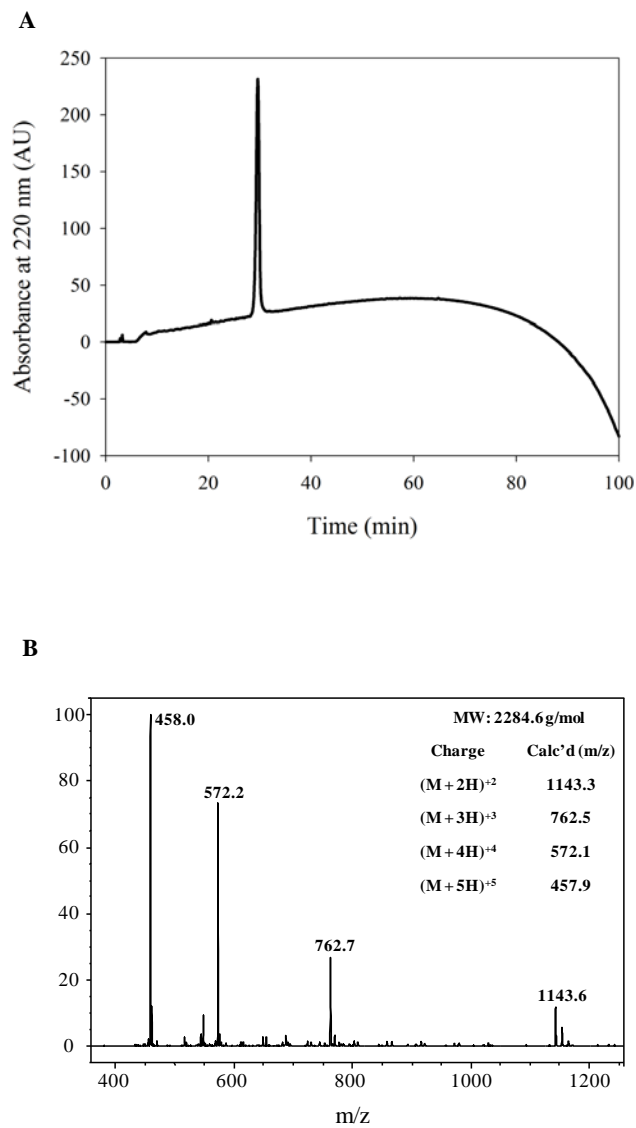


Figure S4 – (A) Analytical HPLC (Vydac C18) of purified PEP2R. 0% to 100% B over 100 minutes. (B) ESI (+) mass spectrum of purified PEP2R.

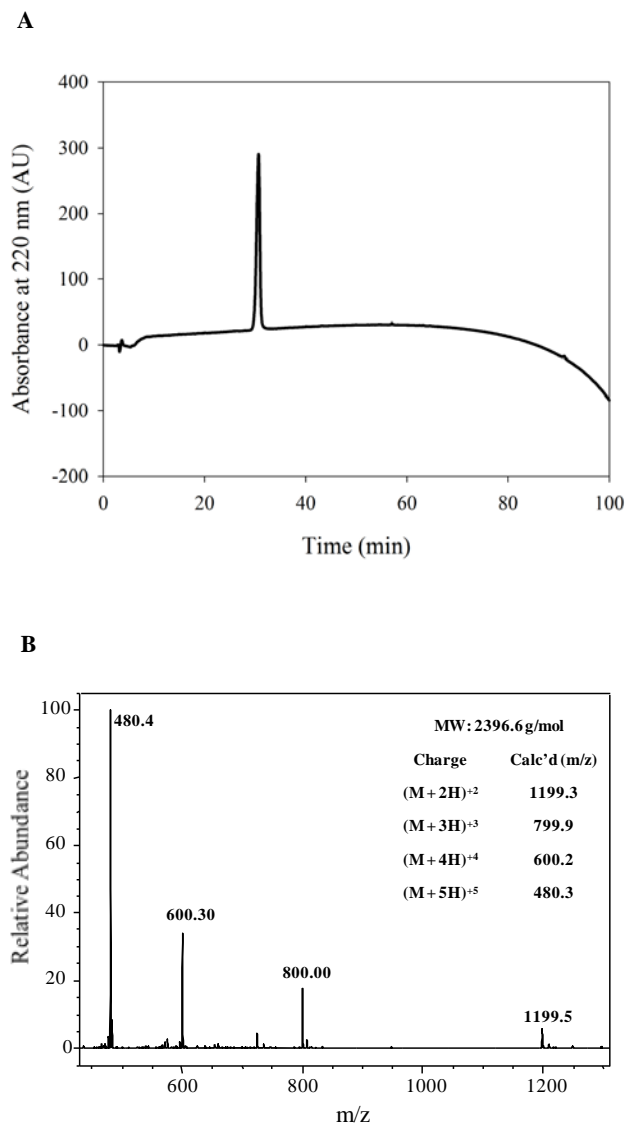


Figure S5 – (A) Analytical HPLC (Vydac C18) of purified D-PEP6R. 0% to 100% B over 100 minutes. (B) ESI (+) mass spectrum of purified D-PEP6R.

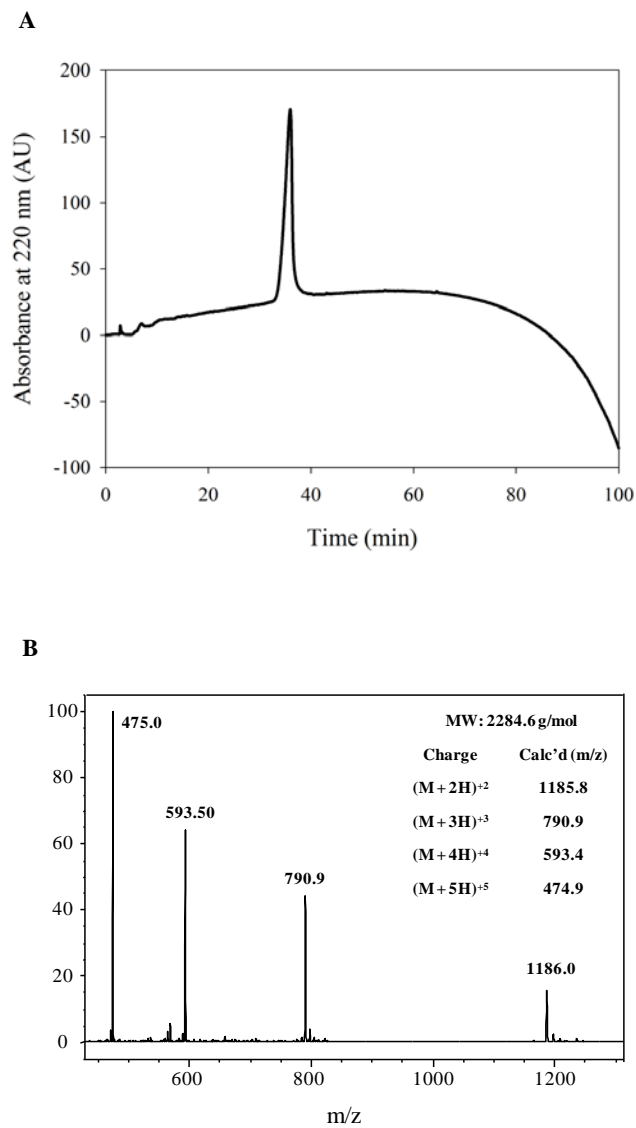


Figure S6 – (A) Analytical HPLC (Vydac C18) of purified PEP6RE. 0% to 100% B over 100 minutes. (B) ESI (+) mass spectrum of purified PEP6RE.

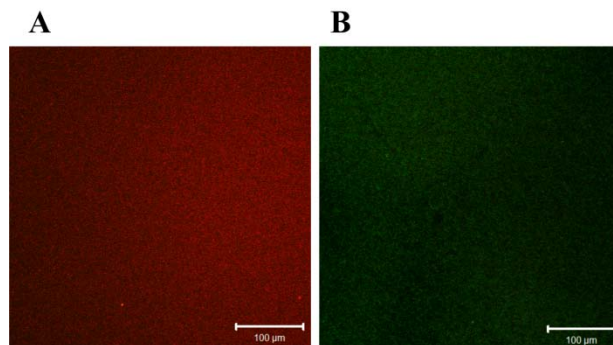


Figure S7 - LSCM xy projection taken of 10^5 CFU/dm² *P. aeruginosa* incubated on (A) 2 wt% PEP6R hydrogels and (B) a borosilicate control surface, after 24 h incubation. Green and red fluorescence denotes, respectively, live and dead cells with compromised membranes. (Scales bars: 100 μm)

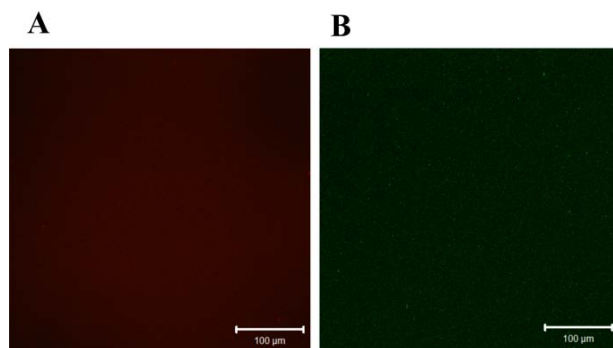


Figure S8 - LSCM xy projection taken of 10^5 CFU/dm² *P. aeruginosa* incubated on (A) 2 wt% PEP6R hydrogels and (B) a borosilicate control surface, after 2 h incubation. Green and red fluorescence denotes, respectively, live and dead cells with compromised membranes. (Scales bars: 100 μ m)

Soluble peptide antibacterial activity

PEP6R hydrogels were prepared in separate wells of 96-well tissue cultured-treated polystyrene plates at two different weight percentages (0.5 and 2 wt%) as described before. After the overnight equilibration, the BTP buffer was removed from the top of the hydrogels. Next 100 μL of TSB was added to the surface of the gels and incubated for 2 h at 37°C. After the 2 h the TSB supernatant was removed from the top of the gels and placed on different wells of 96-wells polypropylene plates (Costar 3879). An *E. coli* (ATCC 25922) solution of 10^6 CFU/mL was prepared as described before. A 1 μL aliquot of the bacterial stock solution was introduced to the 100 μL of TSB supernatant resulting in a final bacterial concentration of 10^4 CFU/mL. Bacteria were incubated for 24 h on the TSB supernatant, as well as a control at 37°C. The following day, 150 μL of bacteria-free TSB was added to remove the assay solution for measurement. Bacterial growth was monitored by measuring the $\text{OD}_{625\text{nm}}$ of the solution. Corrected $\text{OD}_{625\text{nm}}$ were calculated to account for dilution and to normalize the scattering for the bacterial strain.¹⁹ The assay was performed in triplicate. Antibacterial activity is represented as percent non-viable bacteria, where % non-viable bacteria = $[1 - (\text{OD}_{625\text{ surface}}/\text{OD}_{625\text{ control}})] \times 100$.

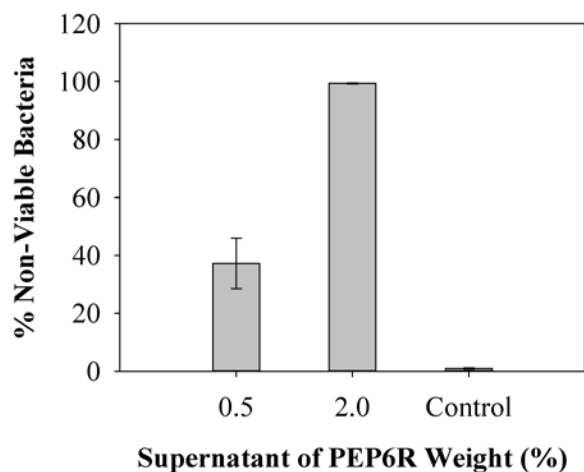


Figure S9 - Antibacterial activity of hydrogel TSB supernatant against *E. coli* after 24 h incubation at 37°C. Percent non-viable bacteria is reported for 0.5 and 2 wt% hydrogels supernatant (n=3).

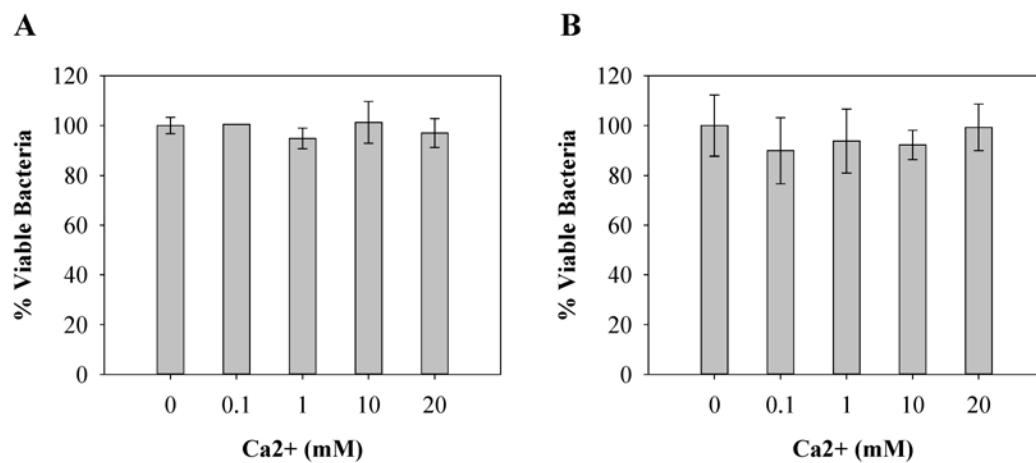


Figure S10 – Viability of *E. coli* (A) and *S. aureus* (B) on TCTP control surface in the presence of Ca²⁺, after 24 h incubation at 37°C (n=3).