β-HAIRPIN PEPTIDE FIBRILLATION AND BACTERIA TRAPPING IN HYDROGEL

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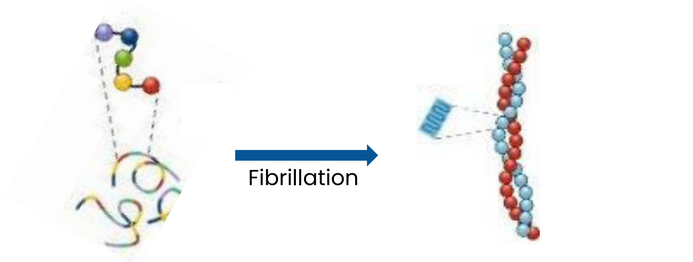
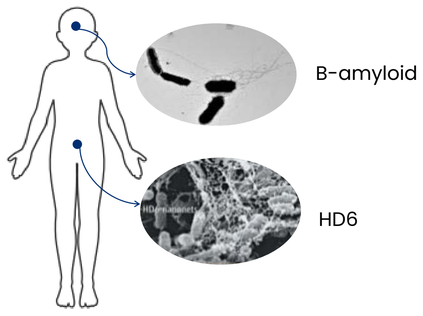
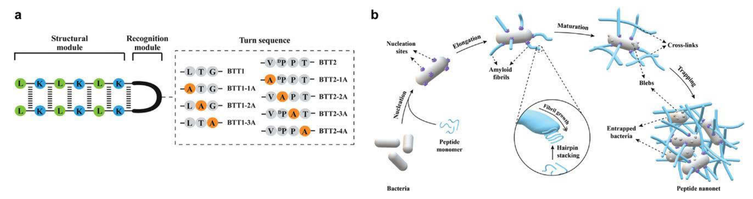
Peptide fibrillation protects organisms against bacterial infections



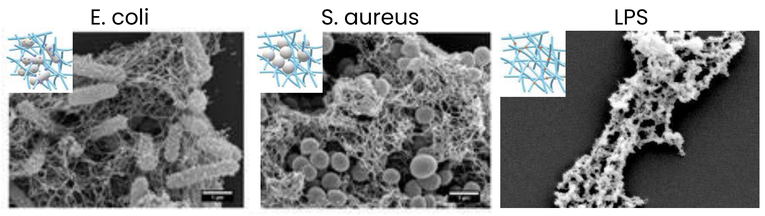


Peptide fibrillation is a process in which short chains of peptides self-assemble into organized structures. Originally studied in the context of misfolding that can lead to human disorders, increasing evidence now suggests that controlled fibrillation is involved in normal physiological processes. For example, the secretion of fibrillating human α-defensin 6 (HD6) by intestinal Paneth cells which fights infection by forming nanonets that entrap the microbial cells; and amyloid-β peptide whose expression protects against fungal and bacterial infections in animal models of Alzheimer's disease.

Bacteria-responsive nanonet forming peptides (BTT peptide)



Inspired by nature, we have designed a library of β-hairpin peptides (BTT). The sequences of BTT peptides are rationally designed with two functional modules: (i)a hairpin turn as the "recognition module"; and (ii) side stands as the "structure module".



BTT peptides are capable of self-assembling into nanonets in response to bacteria, and form nanonets that trap and kill bacteria.

How does BTT peptide interact, fibrillate and trap bacteria in hydrogels?

Materials

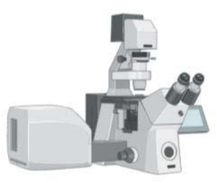

GFP-labelled PAO1


Agarose gel

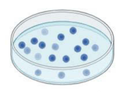

BTT1-3A peptide

Techniques & Methods


Dynamic rheology physical property of agarose gel

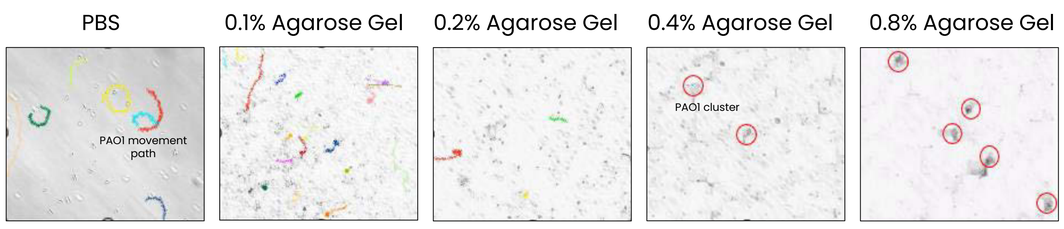

CLSM Time-lapse Bacterial movement live tracking

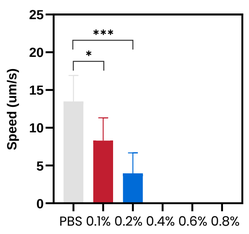

SEM gel porosity and in-gel peptide fibrillation

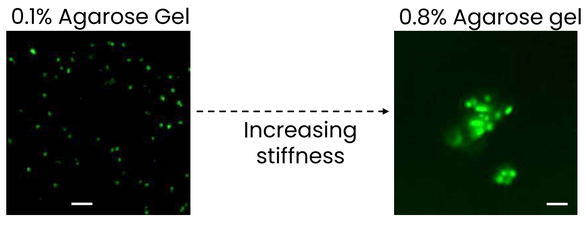

CFU counting


Soft agar motility check

1 Bacteria motility is negatively affected by hydrogel stiffness

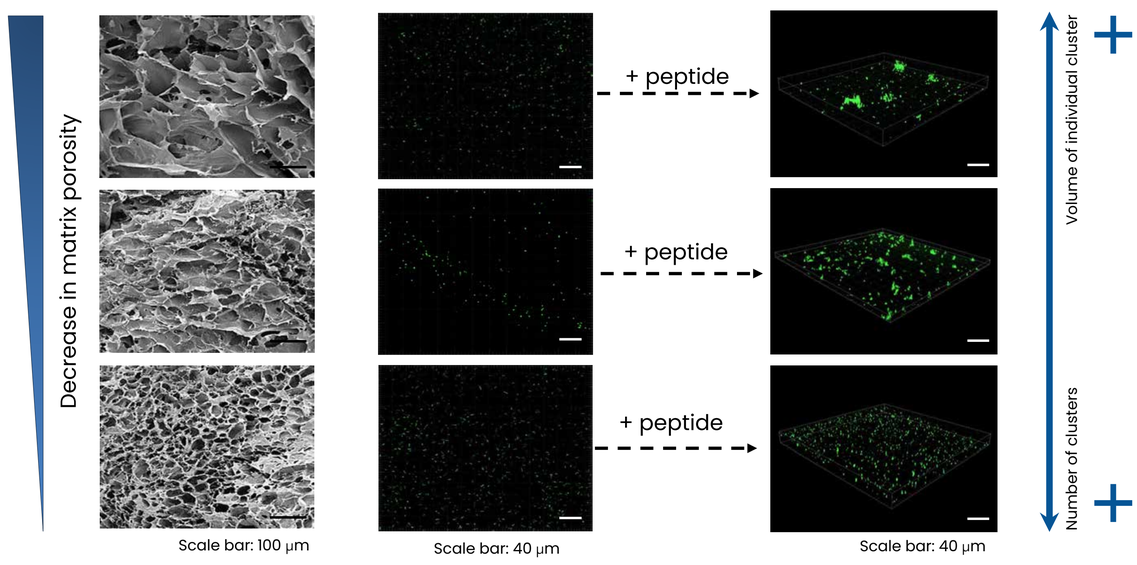






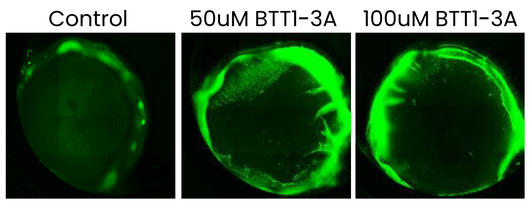
PAO1 cells swim freely in PBS solution but adopt curved paths and become transiently trapped when they are in a gel matrix. Moreover, as gel stiffness increases, the swimming path lengths decrease to the point where cells are locally confined and restricted to rotational movements.

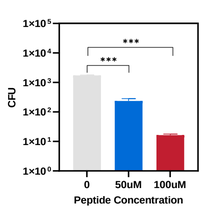
2 Matrix porosity affects the extensiveness and distribution of BTT-bacteria clusters

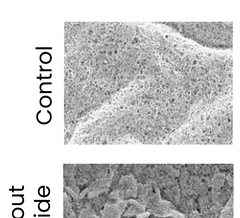


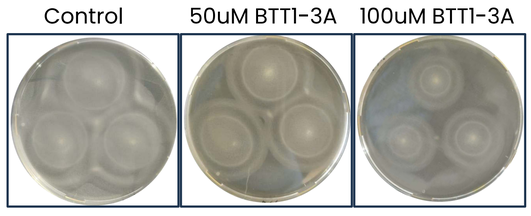
Without BTT peptide, PAO1 cells are uniformly distributed in agarose. Under the action of BTT peptide, PAO1 cells form clusters within the agarose matrix. The size of bacteria cluster is positively correlated with the pore size, while the number of observable clusters increases as pore size decreases.

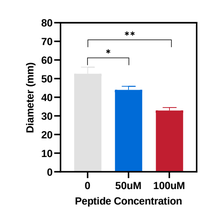
3 BTT peptide-laden hydrogel attracts and entraps bacteria











When BTT peptide is loaded in agarose gel, more PAO1 cells are attracted to and entrapped within the gel matrix. Moreover, BTT peptide forms fibrils around PAO1 cells inside the gel, restricting their motility.

4 Conclusion and Perspectives

By using agarose as a model material, we have demonstrated that BTT peptide can attract and entrap bacteria by forming nanonets within hydrogel. The stiffness and pore size of the matrix affect the motility of bacterial cells and the morphology of BTT-bacteria clusters. Our ongoing work has two focus:

- To investigate the effect of matrix charges on BTT fibrillation.
- To evaluate the *in vivo* anti-microbial efficacy of in-gel nanonets in animal model.

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