Design and investigation of proteins inspired by natural adhesive matrices

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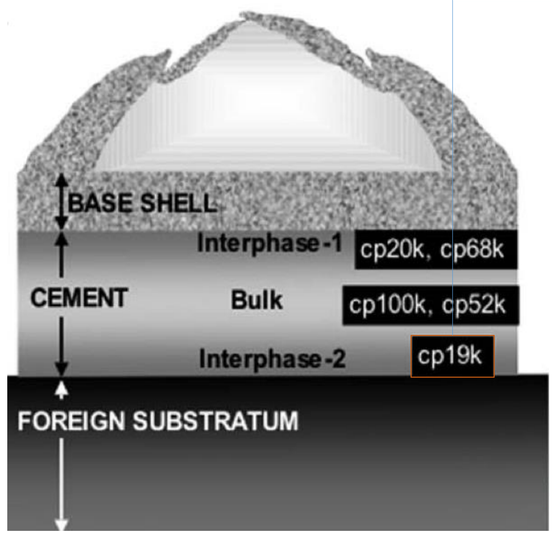
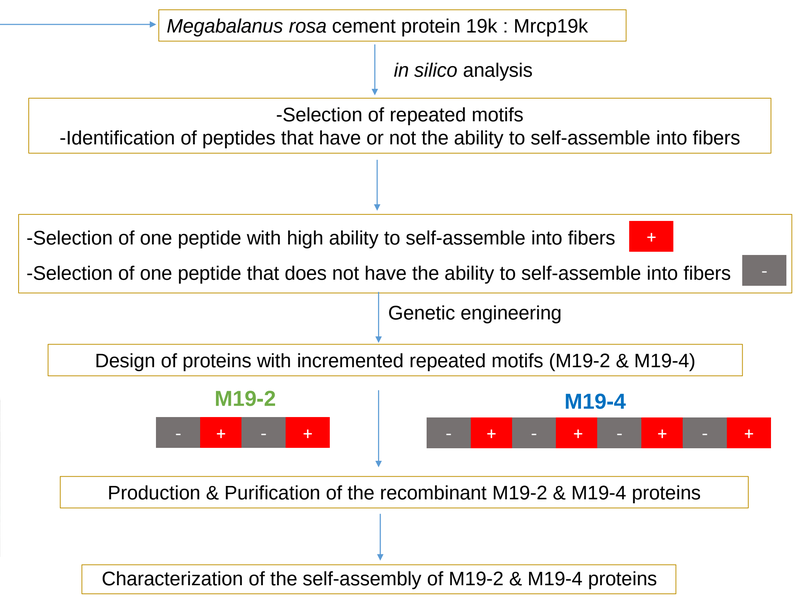
Introduction

Sessile organisms can adhere to diverse surfaces underwater. Among them, barnacles secrete an adhesive matrix, named cement, composed of proteins which are able to self-assemble into fibers to successfully achieve the adhesion under immersed environments [1]. The proteins forming the adhesive matrix of the barnacle *Megabalanus rosa* have been previously identified, sequenced and named Mrcp [2]. Mrcp19k sequence is particularly rich in repetitions [3]. The aim of the present study focus on the self-assembly of Mrcp19k inspired-proteins. We investigated the optimal conditions for their self-assembly (pH, contact surfaces...), their secondary structure and their adsorption capacity onto surfaces.



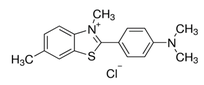
Barnacle attached to a substrate [2]

Strategy



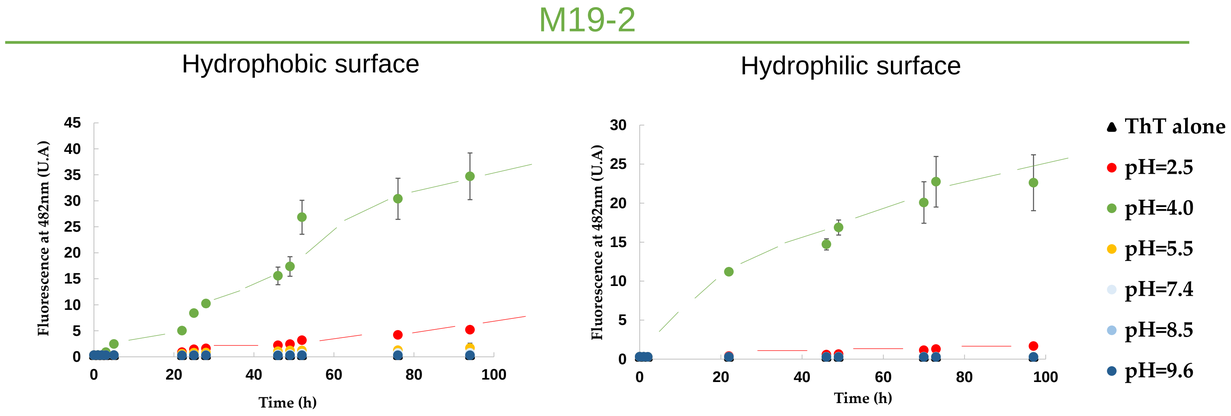
Self-assembly of the proteins by Fluorescence spectroscopy

Using Thioflavin T to detect β-sheet structure of proteins

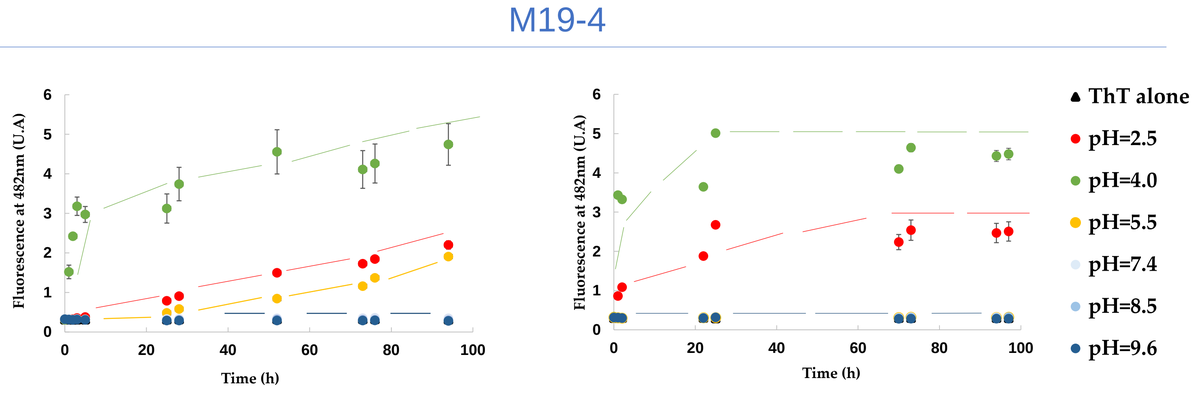


The fluorophore Thioflavin T is able to bind specifically to the amyloid β-sheet structure of the fibers and leads to an increase of fluorescence at 482 nm.
Proteins are incubated at 37°C under different pH conditions on hydrophobic or hydrophilic surfaces

M19-2



M19-4

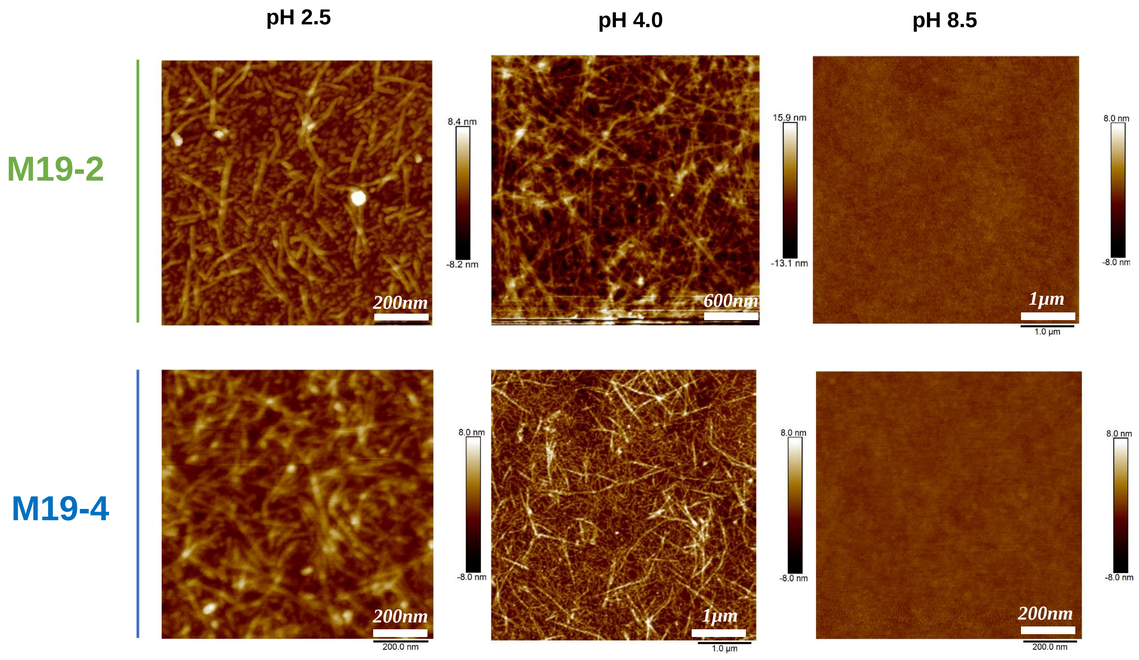


-M19-2 & M19-4 proteins seem to self-assemble into amyloid β-sheet structure on hydrophobic and hydrophilic surfaces at pH 4.0 & pH 2.5
-Hydrophobic surfaces seem to have a positive impact on the fibers formation

✓ Self-assembly of M19-2 & M19-4 seem to be optimal at pH 4 on both hydrophilic and hydrophobic surfaces
✓ M19-2 seems to have a higher self-assembly capacity into fibers compared to M19-4

Morphology of the fibers of M19-2 & M19-4 by AFM

Self-assembled proteins were imaged *in vitro* by Atomic force microscopy (AFM)



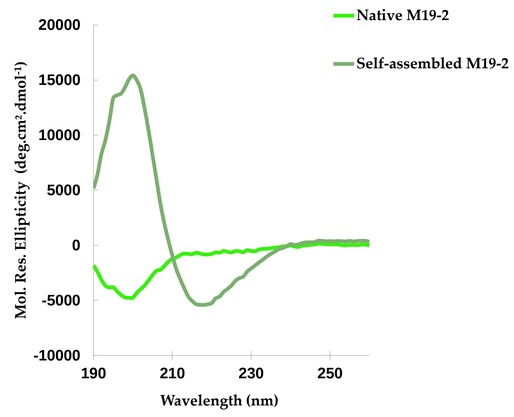
✓ At pH 2.5 and 4.0, different fiber sizes are observed, from 200 nm to 5 µm, with an approximate diameter of 10 nm, which is characteristic of amyloid fibers
✓ At pH 8.5, no structure is observed

Secondary structure of the proteins by CD

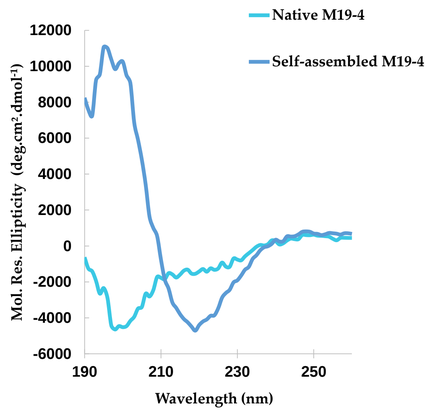
Circular Dichroïsm

The structure of native and self-assembled proteins at pH 4.0 was studied by circular dichroïsm

M19-2



M19-4



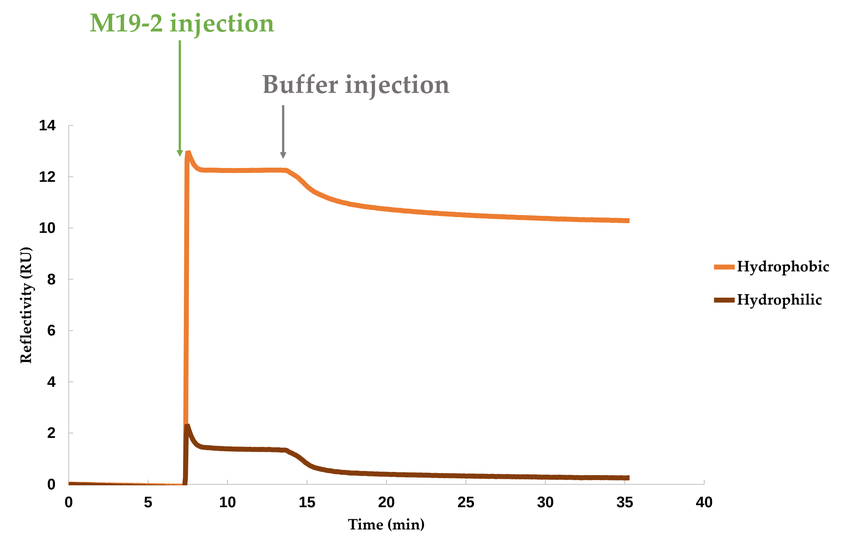
✓ Native proteins are intrinsically disordered : the negative peak is at 200nm
✓ Proteins self-assemble into β-sheet structures : the negative peak is at 215 nm and the positive peak at 200 nm

Adsorption of the proteins by SPR

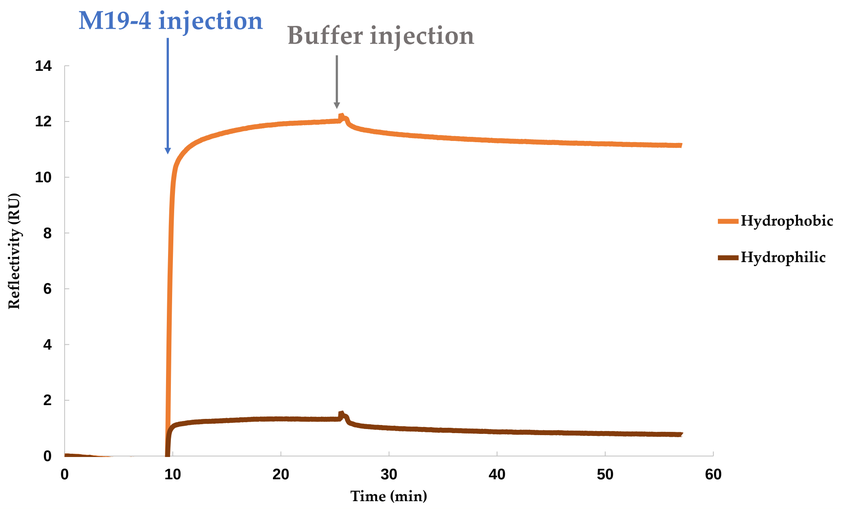
Surface Plasmon Resonance

Experiments have been performed using gold prisms whose surface has been chemically modified to be either hydrophobic or hydrophilic. A kinetic of absorption and desorption of M19-2 and M19-4 proteins at pH 4.0 was performed.

M19-2



M19-4



✓Native M19-2 & M19-4 proteins adsorb on a hydrophobic surfaces
✓Native M19-2 & M19-4 have no affinity for hydrophilic surfaces

Conclusions & perspectives

In our study, we designed proteins with repeatitive motifs inspired by barnacle cement proteins. We produced the biomimetic proteins M19-2 and M19-4 in *Escherichia coli* bacterial cells and purified them. We were able to determine in a first step the optimal conditions of self-assembly of these proteins in beta-sheet structure. The pH and the surface influence the self-assembly of both proteins M19-2 and M19-4. ThT fluorescence data reveal that the amount of M19-4 fibers formed is lower than the amount of M19-2 fibers at pH 4.0. The supramolecular structure was confirmed by circular dichroïsm and imaged by AFM. The high affinity of M19-2 and M19-4 proteins for hydrophobic surfaces was shown by SPR measurements.
We aim at analyzing further the interactions between M19-2 and M19-4 proteins and their cross-influence on their self-assembly and adsorption on different surfaces contact. We aim at investigating the properties of longer proteins such as M19-8 & M19-16 in order to understand the impact of repetitive motives on the self-assembly.

Acknowledgments
The authors give sincere thanks to Dr. Sébastien PERALTA (LPPI,CYU Cergy Paris University) for the help for AFM, Saqline MOSTAQ (LMGP) for the SPR measurements and LCBM (CEA, Grenoble) for the CD measurements.
This project is funded by ANR (ANR-15-CE08-0003).

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