

## Extension and retraction cycles of giant protein self-assemblies visualized by phase contrast microscopy.

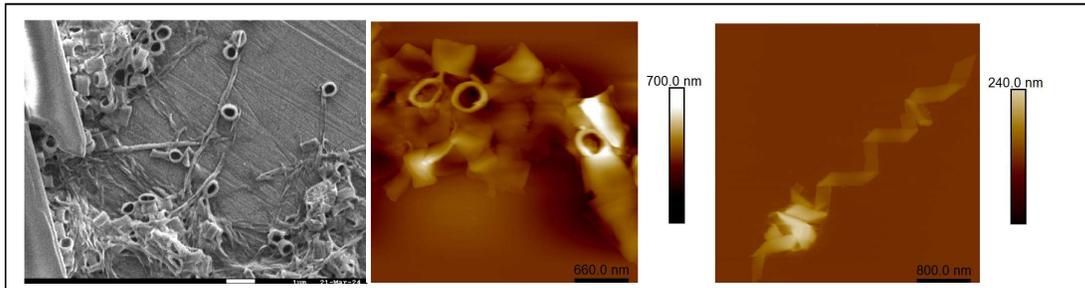
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R-bodies are giant proteinaceous self-assemblies that have the remarkable ability to switch from a 500nm sized scroll into a 20 $\mu$ m long, sharp needle that can puncture membranes.

R-bodies are assembled by the co-polymerization of 4 small proteins (RebA, B, C and D). The co-expression of these proteins in *E. coli* leads to the assembly of functional R-Bodies that can be easily purified through sedimentation and wash cycles.

R-bodies can extend and retract in response to small pH changes: when pH decreases below 5.7, scroll-form R-bodies extend to the needle form and revert to their initial contracted form when the pH rises again. This depends on the salt concentration. At high salt concentrations, the highest pH at which R-bodies are completely extended is approximately 5.4, whereas at very low ionic strength, R-bodies remain completely extended above pH 6.2<sup>1</sup>. They can go through hundreds of extension-retraction cycles without any loss of their ability to respond to pH.



*Figure1: EM and AFM images showing the different conformation of the Rbodies.*

In this internship we will combine a microfluidic setup designed for pH changes, with high resolution phase contrast visualisation to monitor cycles of extension and retractions. Microfluidic devices are commonly used in biophysics or active matter fields to study bacteria swimming. However, the subjects of study are rather large and mid-range magnification is enough to follow and track the movements. Here we will push to its limits the optic resolution to track a ribbon which is only 13 nm thick, 400 nm wide and 10-20  $\mu$ m long in its extended form. The challenge, here will be to use 100X magnification which require an oil immersion objective, that couples the sample with the objective and the table. The first part of the internship will be working on the optimisation of the setup in order to reduce vibrations from the flow and manage to records long enough videos without the need of re-focusing. A second part will be finding a proper way to confine R bodies in the vicinity of the surface. In order to do this, flow mechanics and physical chemistry techniques may be useful. Depending on the profile of the student, parts of the program may be implemented with more physics, physical chemistry or biophysics.

Ideally the student will come from a master in physics, material sciences or biological physics. He will be talented, motivated, patient, and dedicated to fix things in order to get reproducible measurements. For this internship the student will be expected to work full time in Strasbourg for 4 to 6 months. This subject is part of a collaboration in the framework of the ITI- Hi Fun mat including Laurent Pieuchot (biologist) from IS2M Mulhouse and Igor Kulic (theoretical physics) from ICS Strasbourg. A strong taste for interdisciplinarity is thus required.

[1]Polka, J. K. & Silver, P. A. A Tunable Protein Piston That Breaks Membranes to Release Encapsulated Cargo. *ACS Synth. Biol.* 5, 303–311 (2016).