Structure and dynamics of pH-responsive ribbon-like protein analyzed by atomic force microscopy

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Proteins form hierarchical structures equipped with unique functions through self-assembly. Type 51 Refractile body (R-bodies) is a ribbon-like protein assembly and punctures cell membranes by its pH-responsive extension.[1,2] R-bodies possess 400-nanometers-wide coil-like morphologies at neutral pH and turn into 20-micrometers-long spiral-like morphologies at acidic pH (Figure 1a, b). This transformation is reversible and happens in less than one second. These features inspire us to use R-bodies as mechanochemical actuators, however, the structural and mechanical properties of R-bodies remain largely unclear.

In this study, we aimed to characterize R-bodies using an atomic force microscope (AFM) to quantify their structural and mechanical properties. We directly observed the spiral morphology in solution on mica and measured their size and stiffness. We also established the methodology to prepare fragmented R-body sheets (Figure 1c), which allowed us to investigate the precise thickness and curling of sheets by AFM. Furthermore, the synthesized R-body mutants, exhibited different properties compared to the wild-type, providing the underlying structural and mechanical insights of R-bodies.

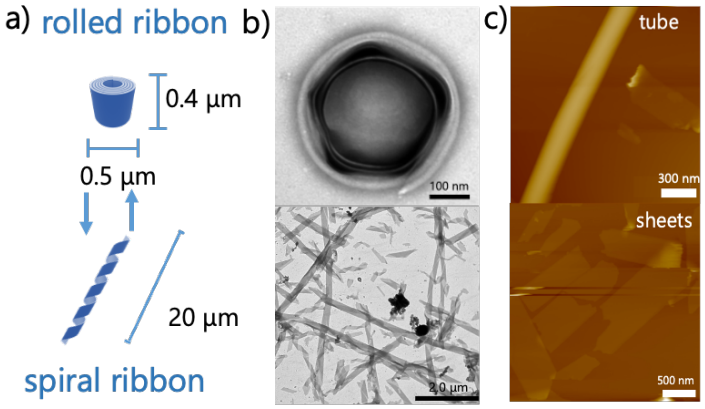

Figure 1. R-body coil and spiral morphologies. a) Schematic map, b) TEM and c) AFM.

1) Pond, F. R, *Microbiol. Rev.* **1989**, 53, 25–67.
2) Polka, J. K., *ACS Synth. Biol.* **2016**, 5, 303–311.