

## Design of the heteroepitaxial vertical assembly of asymmetric protein needles

(<sup>1</sup>*School of Life Science and Technology, Tokyo Institute of Technology*)

○Kosuke Kikuchi,<sup>1</sup> Takafumi Ueno<sup>1</sup>

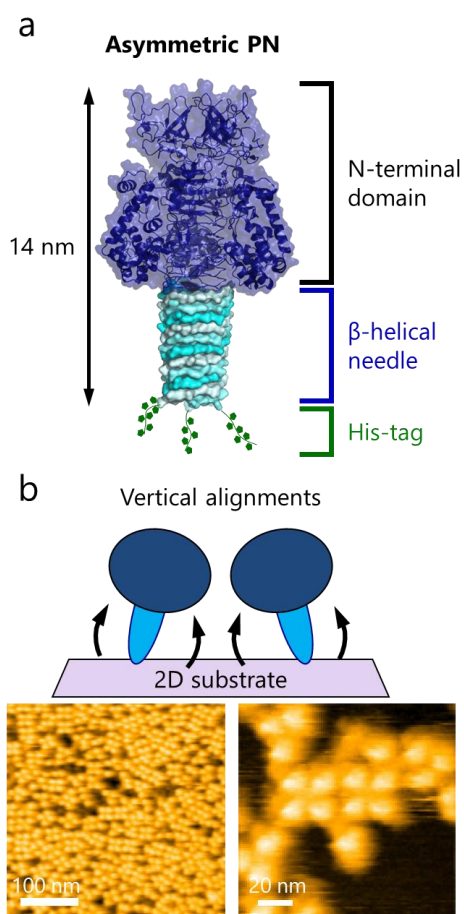
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Proteins assemble into oligomers then form highly ordered one-, two-, or three-dimensional nanostructures. Due to their biocompatibility, spontaneous organization, and spatial periodicity, protein assemblies have attracted significant attention for developing bio-nanomaterials.<sup>1</sup> The functionality of assemblies is deeply associated with the alignments of protein units. However, it is still challenging to manipulate the alignments of each protein unit in assembly structures because of the complexity and asymmetry of protein-protein interactions.

Recently, we reported the modulation of two-dimensional assembly patterns of protein needles (PNs).<sup>2</sup> PN is a robust trimer-dimer protein with an end-to-end symmetry.<sup>3</sup> By engineering the distal ends of PNs, we changed their alignments in the horizontal plane and constructed different two-dimensional patterns. In this study, we construct the vertical assembly of protein needles on a substrate. We designed asymmetric PNs so that PNs stand on a substrate with one end is immobilized on a surface while the other end faces upward (**Figure 1a**). The high-speed atomic force microscopy (HS-AFM) observations revealed that the designed asymmetric PNs vertically stood on mica and formed tetrameric assembly despite its unfavorable aspect ratio (**Figure 1b**).

Currently, we are investigating the protein-protein interactions and the driving force of the vertical conformations.

1) J. Zhu, *et al.*, *Chem Rev* **2021**, *121*, 13701. 2) K. Kikuchi, *et al.*, *Small* (in press). 3) N. Yokoi, *et al.*, *Small* **2010**, *6*, 1873-1879.



**Figure 1** | The structure of the asymmetric PN (a) and the results of HS-AFM observations (b).