Supplementary Information for

Force spectra of single bacterial amyloid CsgA nanofibers

Jingqi Lv[1#], Yingfeng Li[2#], Kai Zhou[1#], Pei Guo[1], Yang Liu[1], Ke Ding[1], Ke Li[2], Chao Zhong[2,3,4] *, Botao Xiao[1,5]*

[1] Joint International Research Laboratory of Synthetic Biology and Medicine, School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510006, China

[2] Materials and Physical Biology Division, School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China

[3] Present address: Center for Materials Synthetic Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

[4] Present address: CAS Key Laboratory of Quantitative Engineering Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

[5] School of Physics, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

*Corresponding authors: xiaob@scut.edu.cn, chao.zhong@siat.ac.cn

Materials and Methods

CsgA-His protein purification and nanofiber production

The recombinant plasmid pET-11d/CsgA was transformed in NEB C3016 *E. coli*. The strain was grown to OD_{600}=1 in LB broth containing 50 mg/mL ampicillin at 37°C. Protein expression was induced with 1mM IPTG at 37°C for 1 h. Cells were collected by centrifugation at 4,000g. Every 5g cell pellet was resuspended and lysed by 50 mL extraction solution (8 M guanidine hydrochloride, in KPI buffer, 300mM NaCl, 50mM K_2HPO_4/KH_2PO_4, pH 7.2 pH=8). Lysates were incubated for 24h at room temperature. The insoluble portions of the lysates were removed by centrifuging at 10,000 g for 30 min before incubating with His-Select Ni-NTA resin for 1 h at room temperature. The mixed solutions were then loaded on the gravity column. Guanidine hydrochloride was further washed away by adding another 20mL KPI buffer. The CsgA proteins were then eluted with 4mL 300mM imidazole KPI buffer after washing with 20mL 40mM imidazole KPI buffer. Purified CsgA proteins self-assembled into nanofibers in 300mM imidazole KPI buffer in 24h. After that, the solution containing CsgA nanofibers was dialyzed with ddH_2O for 72h to remove salts completely.

Establishment of a single molecule system

One end of the fiber was bound to an amine-coated 2.7 μm diameter paramagnetic bead (Dynabeads, Invitrogen), and the other end bound to a glutaraldehyde-coated glass surface as the following steps. Briefly, the amine microbeads were washed using PBS twice, then suspended in the 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH=6, PolyLink Coupling Buffer, Polysciences, Inc.). The nanofiber with a concentration of 0.134 mg/mL was incubated with 10 mg/ml EDAC (Invitrogen) in MES buffer for two hours at room temperature. Next, the diluted nanofiber and magnetic beads were mixed and incubated on a rotor for two hours at room temperature for the carboxylate-amine reaction (Fig. 1b). The product was washed with and then suspended in MES buffer, and stored at 4 ^{0}C before use.

Cover glasses were coated with 2% (3-Aminopropyl)-trimethoxysilane and 8%

glutaraldehyde. The cover glass was used as the upper plate to make a flow chamber. The magnetic beads with fibers were added into the flow chamber which was inverted for 8-20 minutes to allow the beads to fall onto and bind to the glass. Therefore, the amino groups of the fibers on beads have plenty of chances to bind to the glutaraldehyde on the glass. Control experiments were performed for the cross linking. The EDAC solution was replaced with water, while the cover glasses were not coated with glutaraldehyde and the remaining steps remained the same.

Stretching experiments on magnetic tweezers

The flow chamber was put on a homemade magnetic tweezers for stretching experiments. As previously described, four permanent magnets are hold by a step motor and a rotation motor below the stage [1].

The vertical bead position was tracked by a piezoelectric objective positioner and the extension was determined using an automatic focusing algorithm. Fiber extension was monitored by real-time analysis of ~100 measurements per second. Data measured by the two methods check with each other. The stretching force was calculated using the equi-partition theorem. In addition, we calibrated the force by a 16.5 μm-long lambda DNA for different magnet positions as described by Zhao *et. al* [2].

Between adjacent steps, the magnet remained stationary from tens of seconds to minutes to allow the collection of sufficient extension data. The change of extension was used to check that the molecule was tethering to the bead, because the extension of the vast majority (99%) of beads tethered by single molecules was not affected by rotation of the magnets. All magnetic tweezers experiments were carried out at room temperature (~298 K). Constant force experiments were measured by fast-z measurement at ~ 100 frames per second [3].

Scanning electron microscopy

The fiber was bound firstly to a 2.7 μm magnetic bead and then to a round cover glass as the steps for magnetic tweezers. The glass was kept horizontal. A permanent magnet

was placed above the sample to stretch the beads. The samples were rinsed with pure water twice. The samples were consequently fixed with glutaraldehyde, washed gently by pure water. The magnet is moved from above the sample to the side of the sample and used to align the molecules approximately parallel to the surface of the glass. The sample was dried in a desiccator. A Splutter Coater was used to coat platinum film onto the glass surfaces for 60 seconds in vacuum. SEM images were obtained on a Zeiss Merlin field emission scanning electron microscope operated at 5 kV accelerating voltage with MPSE signal.

References

[1] B. Xiao, M.M. McLean, X. Lei, J.F. Marko, R.C. Johnson, Sci. Rep. 6 (2016) 23697.

[2] X. Zhao, X. Zeng, C. Lu, J. Yan, Nanotechnology 28 (2017) 414002.

[3] B. Xiao, B.S. Freedman, K.E. Miller, R. Heald, J.F. Marko, Mol. Biol. Cell 23 (2012) 4864-4871.

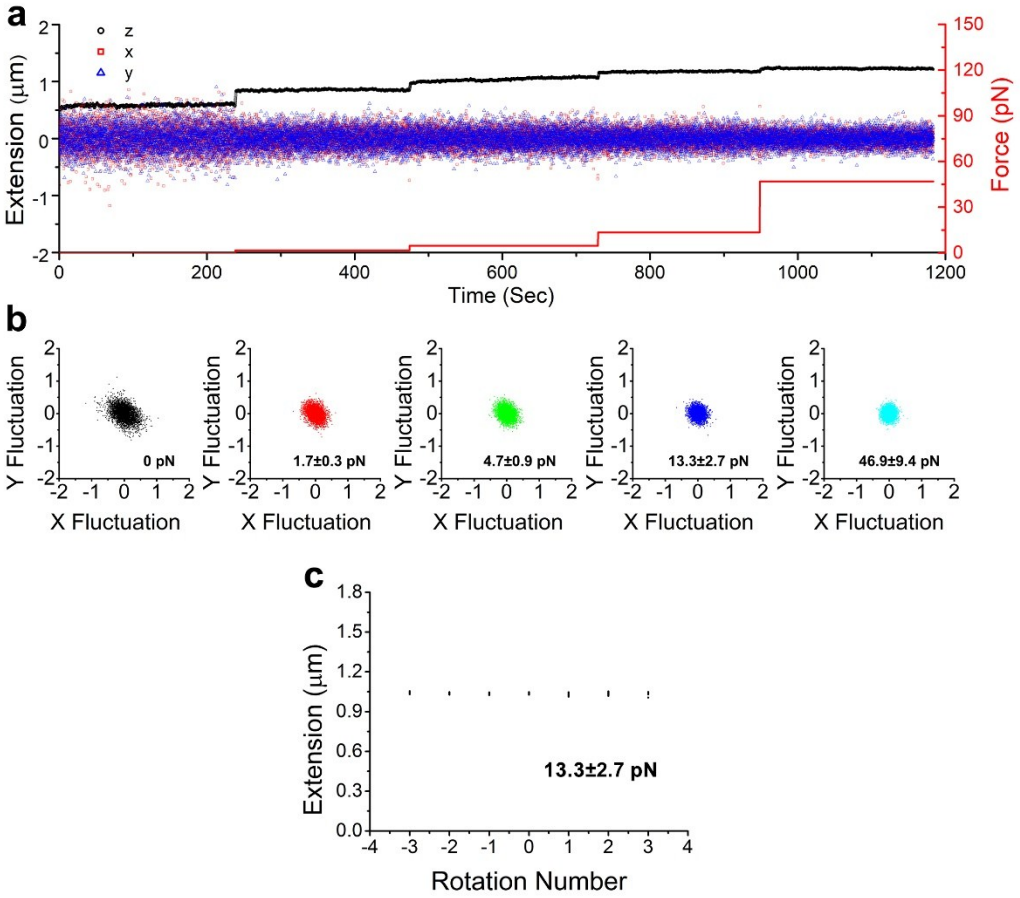

Fig. S1. Single molecule calibrated by two approaches. (a) The fluctuation of a tethered microbead measured in the x, y, and z directions simultaneously. The extension (in z direction) increased with the force applied in the z direction. The magnitudes of x and y fluctuations decreased with the force. (b) The fluctuations in x and y directions of the nanofiber in Figure 1a at 0, 1.7, 4.7, 13.3, and 42.1 pN. An ellipsoid pattern was constantly observed as the force increased, indicating the tether was single molecule instead of multiple molecules. (c) Screening for single molecule by rotation. For the same magnetic bead in Figure S1a, the extension of the tether did not change when it was rotated. This indicates that the magnetic bead had a single tether to the glass as shown in Figure 1b.