

Single molecule study of initial structural features on the amyloidosis process

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Supplementary Information

1. Method

Reagent and chemicals

α -Hemolysin (α -HL) wild-type D8H6 was produced by expression in BL21 (DE3) pLysS *Escherichia coli* cells and then purified on a Ni column.¹ The monomer and heptamer proteins were separated via 8% SDS-PAGE. The heptamer band was cut from the gel. The purified heptamer was conserved in 10 mM Tris-HCl and 1.0 mM EDTA at pH 8.0 and stored at -80 °C.

A β 35-25 and A β 25-35 powder with a purity of 98% by HPLC was purchased from GL Biochem (Shanghai, China) Ltd. and used as received. The amino acid sequence of A β 25-35 is NH₂-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-COOH. A β 35-25 is a mutant of A β 25-35 with reversible amino acid sequence. A β 25-35 and A β 35-25 powder was placed at -20 °C in the refrigerator before used. Diphytanoyl-phosphatidyl-choline in CHCl₃ was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Unless otherwise noted, all other chemicals were of reagent grade. All solutions were prepared by using ultrapure water (EMD Millipore, Billerica, MA, USA).

Nanopore Electrical Recording

The lipid bilayers were formed by applying 30 mg/mL diphytanoyl-phosphatidyl-choline in decane (≥ 99 , Sigma-Aldrich St. Louis, MO, USA) to a 50 μ m orifice in a 1 mL Delrin cup integrated into a lipid bilayer chamber (Warner Instruments, Hamden, CT, USA) filled with Tris-KCl buffer (1M KCl, 10 mM Tris, 10 mM EDTA, pH = 8). A bilayer was deemed stable by monitoring its resistance and capacitance. The two compartments of the bilayer cell are termed *cis* and *trans* chambers, where the *trans* chamber is defined as virtual ground. The α -HL was injected adjacent to the aperture in the *cis* chamber, and pore insertion was determined by a well-defined jump in current value. Once a stable single α -HL pore was formed, 50 μ L A β 25-35 or A β 35-25 solution (Tris-HCl, pH = 8) were added in the *trans* chamber and the final concentration is 500 μ M. Data were filtered at 5 kHz by Axon 200B and acquired at a sampling rate of 100 kHz by using a DigiData 1440A converter and a PC running PClamp 10.4 (Axon Instruments, Forest City, CA, USA). Data analysis was performed using a home-designed

software (<http://people.bath.ac.uk/yl505/nanoporeanalysis.html>) and OriginLab 9.0 (OriginLab Corporation, Northampton, MA, USA). Nanopore measurements were conducted at 24 ± 2 °C.

Calculation of aggregation rate

The aggregation rate k can be obtained by fitting growth phase into a single-exponential function. Therefore, We calculated the aggregation rate by fitting a single-exponential function $f = A + B \cdot \exp(-kt)$ to the frequency versus time ($f-t$) curve of A β 25-35, giving the values of $k_{\beta\text{-sheet}} = 5.45 \times 10^{-5} \text{ s}^{-1}$ and $k_{\text{coil}} = 3.29 \times 10^{-5} \text{ s}^{-1}$. As the PI events of A β 25-35 caused by both random coil structure and bumping events, we think the aggregate rate extracted by fitting $f-t$ curve of PII is more appropriate to represent the aggregation rate of A β 25-35.

2. Nanopore analysis of A β 25-35 and A β 35-25

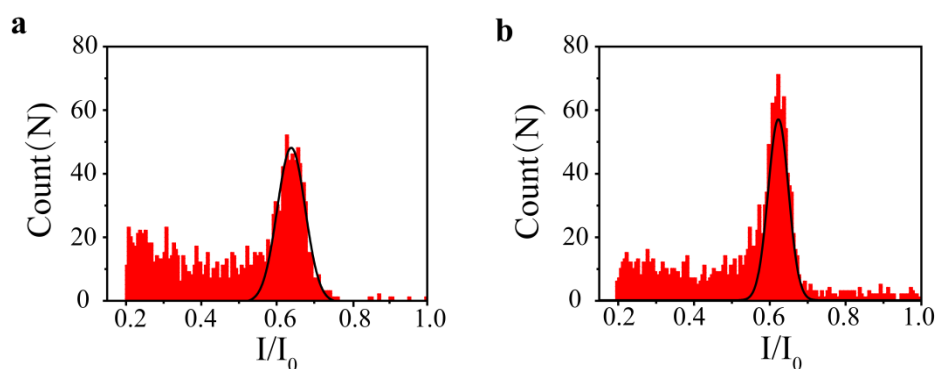


Fig. S1 Histograms of blockage currents for A β 25-35 at -60 mV (a) and -100 mV (b). The current distributions were fitted by Gaussian functions. The statistical analysis shows the $I/I_0 = 0.63$ and $I/I_0 = 0.62$ at -60 mV and -100 mV, respectively. The peak width of Gaussian fittings at -60 mV and -100 mV are 0.06 and 0.05 , respectively. The value of peak width are used to determine the distribution for PI and PII.

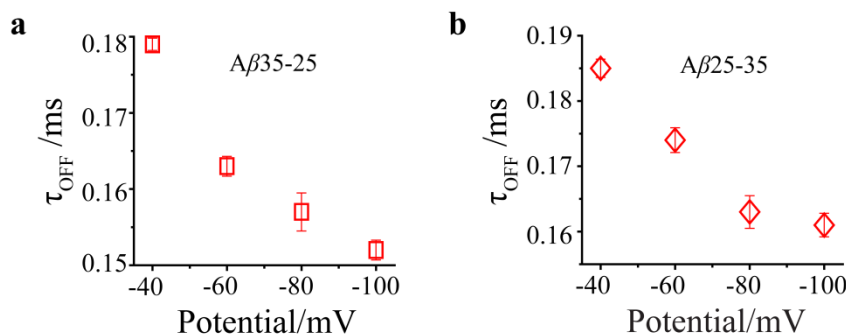


Fig S2. Voltage dependence of τ_{OFF} for A β 35-25(a) and coiled structure of A β 25-35(b).

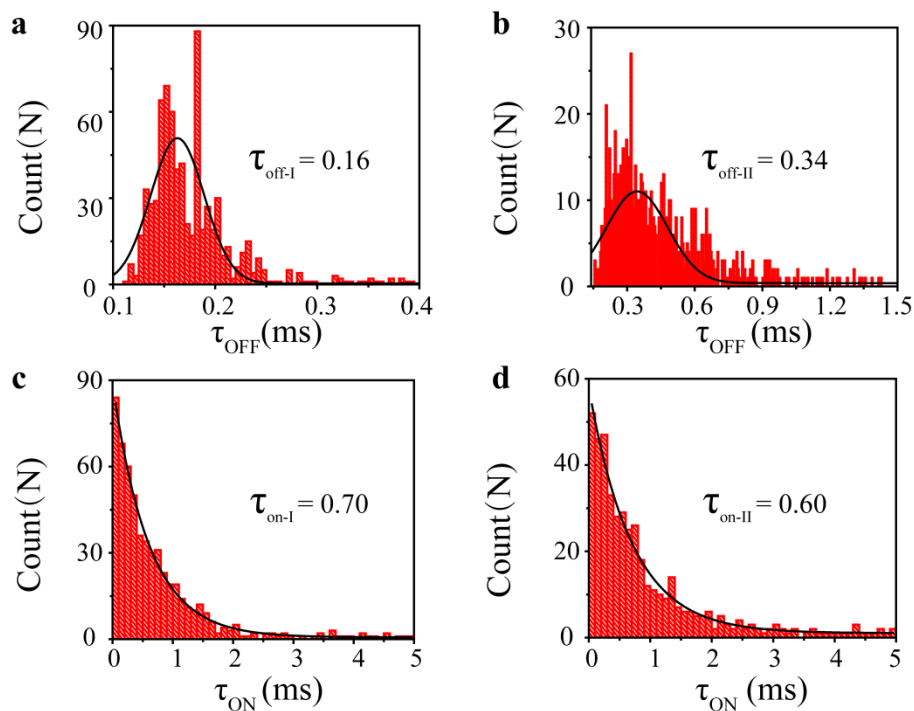


Fig. S3. Histograms of durations (τ_{off}) for PI (a) and PII (b). The histograms of events interval (τ_{ON}) for PI (c) and PII (d). Histograms of duration time were fitted by Gaussian functions whereas histograms of events interval were fitted by single exponential functions. The data was recorded at -100 mV.

3. Frequency of A β 25-35 versus peptide concentration

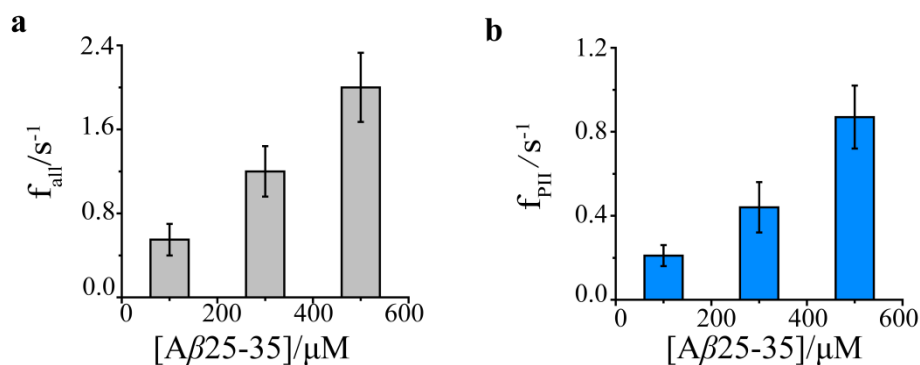


Fig. S4 The frequency of A β peptides versus different concentrations. (a) The frequency of all events versus A β 25-35 concentration. (b) The frequency of PII events versus peptide concentration. Since the PII events is assigned to β -sheet structure of A β 25-35, the proportion of β -sheet structure scales to the increasing concentration of fresh prepared A β 25-35.

4. Current traces of A β 35-25

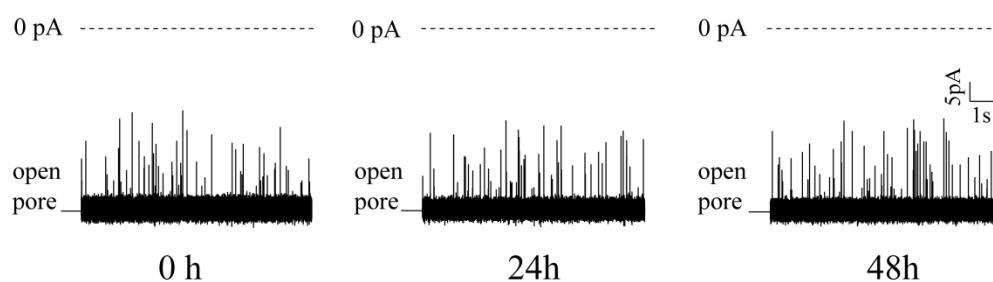


Fig. S5 The raw current traces of A β 35-25 with incubation time of 0 h, 24 h and 48 h.

Reference

1. F.-N. Meng, X. Yao, Y.-L. Ying, J. Zhang, H. Tian and Y.-T. Long, *Chem. Commun.*, 2015,**51**,1202-1205.
2. F. Chiti, N. Taddei, F. Baroni, C. Capanni, M. Stefani, G. Ramponi and C. M. Dobson, *Nat. Struct. Mol. Biol.*, 2002, **9**, 137-143.