Single molecule study of initial structural features on the amloidosis process

Yong-Xu Hu, a Yi-Lun Ying, a Zhen Gu, a Chan Cao, a Bing-Yong Yan, b Hui-Feng Wang, b and Yi-Tao Long a

a Key Laboratory for Advanced Materials & Department of Chemistry, East China University of Science and Technology, Shanghai 200237, P. R. China
b School of Information Science and Engineering, East China University of Science and Technology, Shanghai 200237, P. R. China

Email: ytlong@ecust.edu.cn; yilunying@ecust.edu.cn; Fax: +86-21-64250032 Tel: +86-21-64250032

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. Aβ35-25 and Aβ25-35 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 Aβ25-35 is NH2-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-COOH. Aβ35-25 is a mutant of Aβ25-35 with reversible amino acid sequence. Aβ25-35 and Aβ35-25 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 μm 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, 50μL Aβ25-35 or Aβ35-25 solution (Tris-HCl, pH = 8) were added in the trans chamber and the final concentration is 500 μM. 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](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 $f = A + B*exp(-kt)$ to the frequency versus time ($f$-$t$) curve of $\beta_25-35$, giving the values of $k_{\beta}$-sheet = $5.45 \times 10^{-5}$ s$^{-1}$ and $k_{\text{coil}} = 3.29 \times 10^{-5}$ s$^{-1}$. As the PI events of $\beta_25-35$ 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 $\beta_25-35$.

2. **Nanopore analysis of $\beta_25-35$ and $\beta_35-25$**

![Fig S1](image1.png) Histograms of blockage currents for $\beta_25-35$ 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](image2.png) Voltage dependence of $\tau_{OFF}$ for $\beta_35-25$(a) and coiled structure of $\beta_25-35$(b).
3. **Frequency of Aβ25-35 versus peptide concentration**

Fig. S4. The frequency of Aβ peptides versus different concentrations. (a) The frequency of all events versus Aβ25-35 concentration. (b) The frequency of PII events versus peptide concentration. Since the PII events is assigned to β-sheet structure of Aβ25-35, the proportion of β-sheet structure scales to the increasing concentration of fresh prepared Aβ25-35.

4. **Current traces of Aβ35-25**
Fig. S5 The raw current traces of Aβ35-25 with incubation time of 0 h, 24 h and 48 h.

Reference
