Supporting Information

Single Molecule Sensing of Amyloid-β Aggregation by Confined Glass Nanopore

Ru-Jia Yu[1,2†], Si-Min Lu[1,2†], Su-Wen Xu[1], Yuan-Jie Li[1], Qun Xu[3*], Yi-Lun Ying[2*], Yi-Tao Long[2*]

[1]School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, 200237, P. R. China

[2]State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, P. R. China

[3]Department of Neurology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200127, China

Experimental Section

1. Chemicals

Tris(hydroxymethyl)aminomethane (Tris) and KCl was purchased from Sigma-Aldrich (St. Louis, MO, USA). Aβ1-42 monomer was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All the chemicals are in analytical grade and used without further purification and the aqueous solutions were prepared using ultrapure water (>18 MΩ cm). Quartz capillary (inner diameter 0.5 mm, outer diameter 1.0 mm) was purchased from Sutter Instrument.

2. Preparation and characterization of the glass nanopore

Single conical shaped glass nanopore was fabricated by pulling quartz capillary with 0.5 mm inner diameter and 1.0 mm outer diameter. Capillaries were firstly repeatedly washed by separately sonicating in acetone, ethanol and distilled water for 30 min, respectively. After drying with nitrogen gas, each capillary was fixed on the middle of laser pipette puller (P 2000, Sutter Instrument Co., Novato, CA), whose center area was heated and pulled into two identical conical nanopores. The parameter for nanopore pulling was reported in our previous studies and adjusted accordingly due to the loss in laser power. The glass nanopore was pretreated by sputtering with gold for 20 s, and subsequently characterized with scanning electron microscopy (SEM) imaging. The tip diameter was about ~30 nm for peptide sensing.

3. Peptide preparation and characterizations

The peptide powder was first dissolved in distilled water and incubated for different times (0 h, 6 h and 20 h) to prepare the three types of peptide, monomers, oligomers and fibers respectively. First, the Aβ1-42 monomers in powder form were obtained from Sangon Biotech Co., Ltd. (Shanghai, China) with specific hexafluoro-2-propanol (HFIP) purification. The monomers were directly dissolved into 10 mM KCl buffer solution (pH 8 with Tris) with a final concentration of monomer 10 μg/mL. As previous reported, Aβ1-42 peptide with low concentration could be stabilized in alkaline solution and thus the peptide could stay monomeric and used for nanopore sensing. To prepare peptide aggregates, Aβ1-42 monomer powder was first dissolved into distilled water with a concentration of 1 mg/mL. The solution samples were incubated at 25 ℃ for 6 h and 20 h respectively to form oligomers and fibers respectively. For nanopore sensing experiment, the peptide was diluted 100 times with 10 mM KCl buffer solution (pH 8 with Tris). Such prepared Aβ1-42 peptide both monomers and aggregates were characterized with transmission electron microscopy (TEM) images. Aβ 42 monomers could hardly be recognized clearly due to the present resolution of transmission electron microscopy.

4. Peptide sensing using glass nanopore sensors

The prepared glass nanopore was anchored in a suited holder, backfilled and tip immersed in 10 mM KCl buffer solution (pH 8 with Tris). A pair of Ag/AgCl electrodes were placed inside the nanopore (as working electrode) and in the external solution (as reference electrode, grounded) respectively to apply a set of biased voltages. The external buffer solution was replaced with peptide solution for peptide sensing. The final concentration of Aβ1-42 monomers, oligomers and fibers were 10 μg/mL. The experiment was conducted at room temperature. The ionic current was recorded and filter at 5 kHz by Axon 200B low-noise, with a sampling rate of 100 kHz by using a DigiData 1550A converter. The raw current data was further analyzed by using our self-developed integrated software system called “NANOPORE ANALYSIS” on the MATLAB platform and the data plots

were drawn by OriginLab 9.0. The errors in this paper were obtained from corresponding fitting.

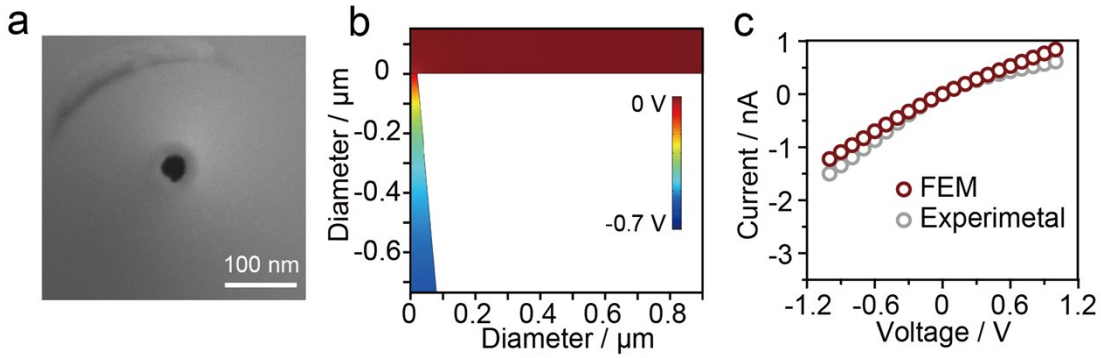

Figure S1. (a) Scanning electron microscope (SEM) image of the prepared glass nanopore. (b) The voltage distribution nearside the nanopore calculated by finite element method. (c) The I-V curves obtained from FEM simulation and the nanopore experiment.

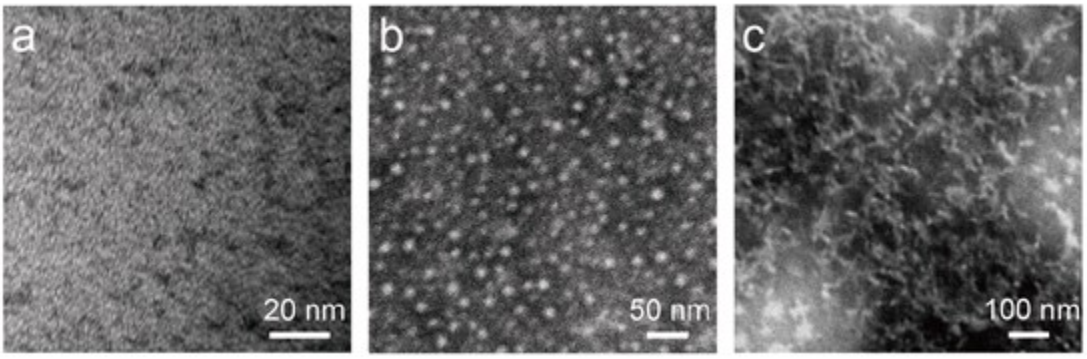

Figure S2. Transmission electron microscopy (TEM) images of prepared (a) Aβ 1-42 monomers, (b) oligomers and (c) fibers, Aβ1-42 protein was stained with 2% w/v phosphotungstic acid solution, pH 6.5.

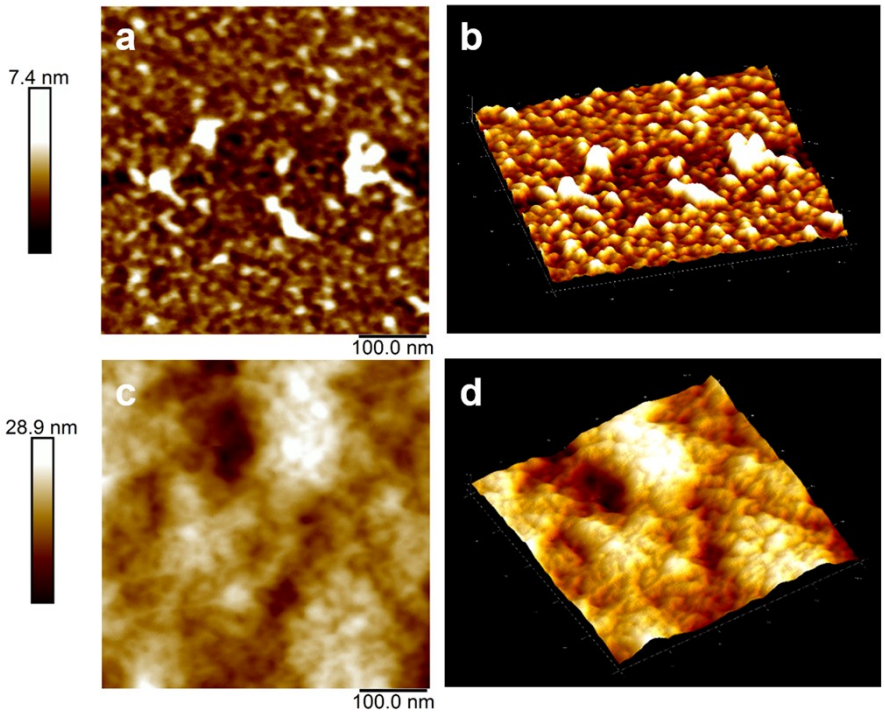

Figure S3. Atomic force microscope (AFM) Characterizations of the prepared Aβ1-42 (a, b) oligomers and (c, d) fibers.

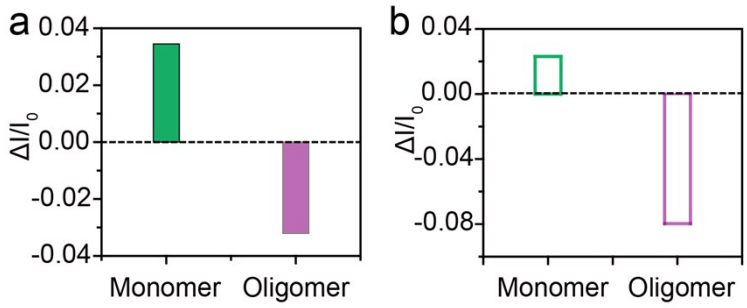

Figure S4. (a) The fitted $\Delta I/I_0$ values of the statistic analysis of Aβ1-42 monomers (green) and oligomers (purple) in figure 2. (b) The simulated $\Delta I/I_0$ values induced by the translocation of Aβ1-42 monomers (green) and oligomers (purple) in the prepared glass nanopore.

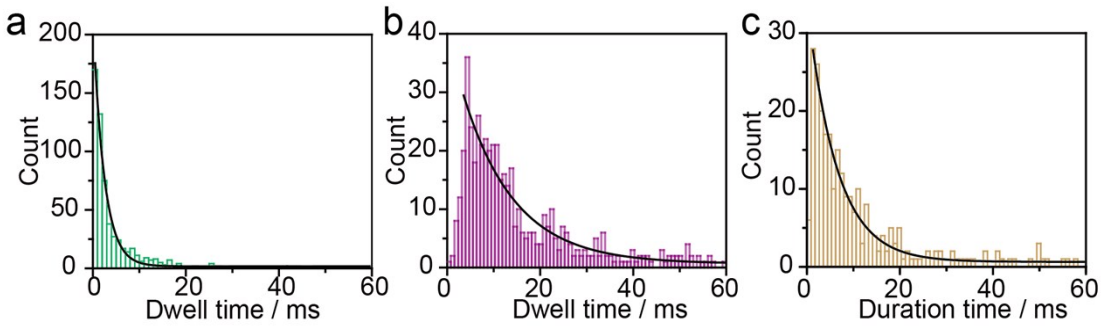

Figure S5. (a) The histograms of dwell time of Aβ1-42 monomers (a), oligomers (b) and fibers (c) under -700 mV and the normalized dwell time are 2.39 ± 0.08, 11.13 ± 1.11 and 6.32 ± 0.38 respectively.

5. Simulation of the current fluctuations in the nanopore sensing of Aβ1-42 peptide

The COMSOL Multiphysics software (COMSOL Inc., Burlington, MA, USA) is used to conduct the FEM simulation. The geometry of the model glass nanopore is set according to SEM result shown in Figure S1, which shows the diameter is 30 nm. According to the literature, the surface charge on the glass nanopore is set as -0.005 C/m^2.[1] The diffusion coefficient for the electrolyte are set as D_{K+}= 1.96e-9 m^2/s and D_{Cl^-}= 2.03e-9 m^2/s T = 293.15 K. The surface charge for monomer and oligomer are -0.03821 C/m^2 and 0.003821 C/m^2 respectively (calculated with the net charge of each peptide molecule and specific surface, defined as uniform distributed). Aβ1-42 monomer was regarded as solid sphere with diameter of 1 nm. Meanwhile, Aβ1-42 oligomer was regarded as disk with diameter of 15 nm, thickness of 4 nm. To simplify the simulation process, the system is regarded to be steady-state and all of processes happen in a 2D non-axisymmetric geometry. In addition, the monomer and oligomer locate at the tip of glass nanopore.

The Nernst-Planck (NP) equation is used to compute the ionic flow, which includes the diffusion, migration, and convection terms.

$$\boldsymbol{J}_i = -D_i \nabla c_i - (z_i F/RT) D_i c_i \nabla \boldsymbol{\Phi} + c_i \boldsymbol{u} \qquad (1)$$

As mentioned in equation (1), $\boldsymbol{J}_i$ represents ion flow vector, F is Faraday's constant, T is the absolute temperature, $\boldsymbol{\Phi}$ is the potential, $\boldsymbol{u}$ is the position-dependent fluid. Di, C_i, and Z_i represent the diffusion coefficient, the concentration, and the charge of species in the solution, respectively. As for the relationship between the ion concentration and electric potential, Poisson equation is utilized to illustrate it.

$$\nabla^2 \boldsymbol{\Phi} = -F/\epsilon \sum_i z_i c_i \qquad (2)$$

Here, ϵ represents the dielectric constant of the medium. In the stimulation, Navier-Sotkes equation is made use of to represent the flow distribution in the glass nanopore

$$\boldsymbol{u} \nabla \boldsymbol{u} = 1/\rho \left(-\nabla p + \eta \nabla^2 \boldsymbol{u} - F \left(\sum_i \sigma_i c_i\right) \nabla \boldsymbol{\Phi}\right) \qquad (3)$$

Therein, ρ and η are the density and viscosity of the fluid, respectively, and p is the pressure. p sets as 1× 10^{-3} kg/m^3 and η sets as 1× 10^{-3} Pa·s.

The mesh format in the Finite Element Modeling, with the Aβ1-42 monomer and oligomers locating at the very tip of the nanopore.

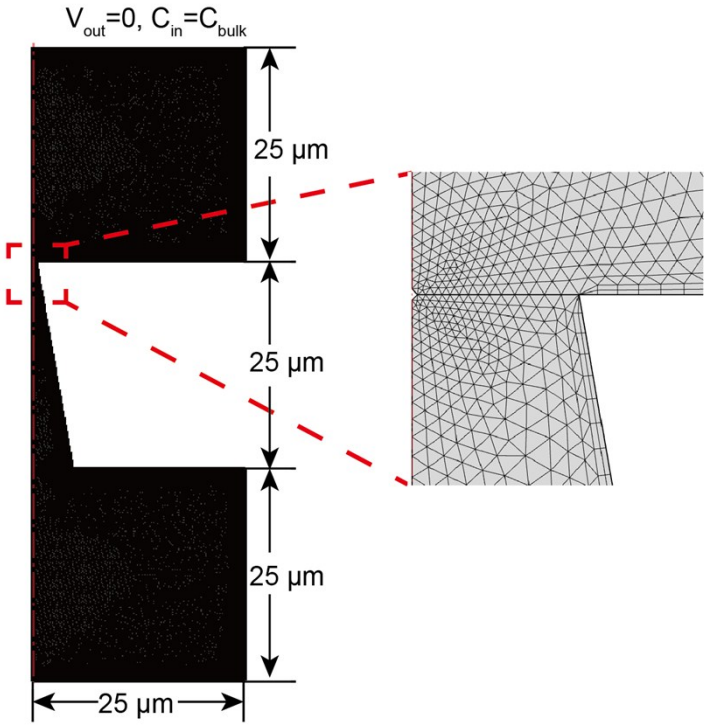

Figure S6. The geometry of nanopore, the boundary setting, and the mesh in the FEM simulation, the Aβ1-42 monomer locates at the tip of nanopore, the electrolyte parameters for the ionic species were as (D_{k^+} = 1.957 × 10^{-9} m^2 /s, c_{K^+} = 10 mM, D_{Cl^-} = 2.032 × 10-9 m^2 /s, c_{Cl^-} = 10 mM at T = 298 K).

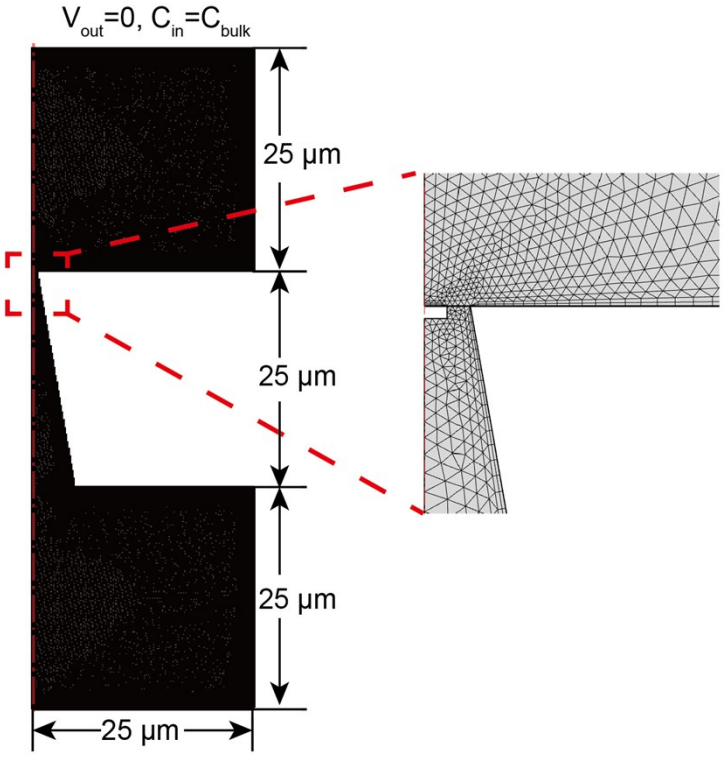

Figure S7. The geometry of nanopore, the boundary setting, and the mesh in the FEM simulation, the Aβ1-42 oligomer locates at the tip of nanopore, the electrolyte parameters for the ionic species were as (D_{k^+} = 1.957 × 10^{-9} m^2 /s, c_{K^+} = 10 mM, D_{Cl^-} = 2.032 × 10-9 m^2 /s, c_{Cl^-} = 10 mM at T = 298 K).

References

1. Yong-Xu Hu, Yi-Lun Ying, Rui Gao, Ru-Jia Yu, Yi-Tao Long. Characterization of the dynamic growth of the nanobubble within a confined glass nanopore. Analytical Chemistry, 2018, 90 (21), 12352–12355.