Supporting Information

Differential roles of plasma protein corona on immune cell association and cytokine secretion of oligomeric and fibrillar beta-amyloid

Ava Faridi,[1] Wen Yang,[2] Hannah Gabrielle Kelly,[3,4] Chuanyu Wang,[2] Pouya Faridi,[5] Anthony W. Purcell,[5] Thomas P. Davis,[1,6*] Pengyu Chen,[2*] Stephen J. Kent[3,4,7*] and Pu Chun Ke[1*]

[1]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia

[2]Materials Research and Education Center, Auburn University, Auburn, AL 36849, United States

[3]Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

[4]ARC Centre for Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Melbourne, Australia

[5] Infection and Immunity Program & Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria 3800, Australia

[6]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane Qld 4072, Australia

[7]Melbourne Sexual Health Clinic and Infectious Diseases Department, Alfred Hospital, Monash University Central Clinical School, Carlton, Victoria, Australia

Corresponding Authors

Pengyu Chen: pengyuc@auburn.edu; Thomas P. Davis: thomas.p.davis@monash.edu;

Stephen Kent: skent@unimelb.edu.au; Pu Chun Ke: pu-chun.ke@monash.edu.

Localized surface plasmon resonance (LSPR) immunoassay chip fabrication

PDMS microfluidic-pattering layer preparation

The mold wafer for parallel microfluidic channels (200 μm (W) × 2.5 cm (L) × 50 μm) was fabricated on a silicon substrate by photolithography. A liquid PDMS (polydimethylsiloxane, Sylgard-184, Dow Corning) pre-polymer and cross linker were then mixed (10:1) and degassed twice, and poured onto the silicon mode wafer. The device was incubated over 6 h at 70 °C. After that, the PDMS microfluidic-pattering layer was peeled off from the mold wafer. Inlets and outlets of the follow channels were created by a hole puncher with a diameter of 1 mm.

Au nanorod barcode patterning

Glass slides were first washed by deionized (DI) water and immersed in Piranha solution (H_2SO_4:H_2O_2 = 3:1 v/v) for 10 min. The cleaned slides were rinsed with DI water and kept in an ultrasonic bath for 15 min, and then thoroughly washed with DI water. The prepared glass slides were fully dried at 80 °C. To create a negatively charged surface, oxygen plasma at 60 W (PE-50, Plasma Etch Inc.) was applied onto a glass substrate for 4 min. The PDMS microfluidic-pattering layer was quickly attached onto the surface of plasma-treated glass for Au nanorod (AuNR) barcode patterning. A suspension of CTAB-coated AuNRs (Nanoseedz, NR-40-650-10) was loaded into the channels and incubated overnight. The positively charged AuNRs were immobilized onto the glass surface through electrostatic interaction to form the barcode pattern on the glass substrate. Unbound AuNRs were washed away by DI water. The PDMS patterning layer was then carefully removed and a free PDMS flow channel layer (from the same mask) was attached perpendicularly to the AuNRs barcode patterns. Approximately 1 mM of 11-mercaptoundecanoic acid (Sigma-Aldrich, USA) was loaded into the channels to replace the CTAB layer on the AuNRs surface and incubated overnight. 0.1 M NHS (*N*-hydroxysuccinimide, Thermo Scientific) and 0.4 M EDC (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride, Thermo Scientific) prepared in 0.1 M MES (1-ethyl-3-[3-

(dimethylamino)propyl] carbodiimide hydrochloride Thermo Scientific) were mixed in equal volume. The mixture was then loaded into microfluidics channels, and incubated for 40 min. Probe antibody solutions (anti-human IL-6 or anti-human TNF-α, eBioscience, USA) in 50 μg/mL were injected into the channels and incubated for 1 h. All the excessive chemicals and molecules in each step were washed away by 1× PBS at 1.5 μL/min for 6 min (**Fig. S1**).

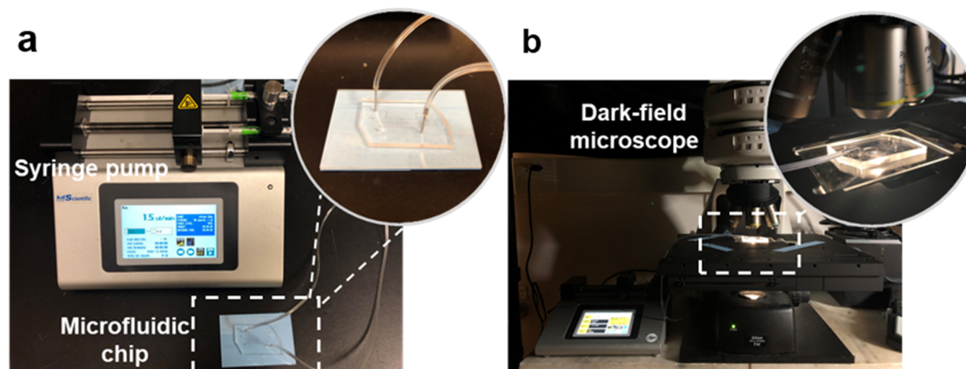

Figure S1. (a) Au nanorod (AuNR) barcode patterning. AuNRs and functional chemical solution were loaded into a microfluidic chip using a syringe. The liquid speed was precisely controlled by a syringe pump. (b) LSPR immunoassay under a dark-field microscope. A prepared LSPR chip was mounted on the sample stage. Scattering light was collected by a 10× objective lens. Dark-field images were captured by an EMCCD camera and analyzed by a customized Matlab code.

Reversed-phase high performance liquid chromatography (RP-HPLC). Samples of Aβm, Aβo and $A\beta_f$ (300 μM) were dissolved in 10% acetic acid and ran on a 4.6-mm (internal diameter) × 100-mm (length) monolithic reversed-phase C18 high-performance liquid chromatography (HPLC) column of Chromolith SpeedROD; Merck Millipore, Darmstadt, Germany, using an ÄKTAmicro HPLC system (GE Healthcare, Little Chalfont, UK). Buffer A (0.1% trifluoroacetic acid; Thermo Fisher Scientific) and buffer B (80% acetonitrile, 0.1% trifluoroacetic acid; Thermo Fisher Scientific) were used in the mobile phase. After a blank

run, the Aβ peptide samples were injected into the column and separated using the following chromatographic conditions: 2-15% buffer B over 0.25 min (2 mL/min), 15-30% buffer B over 4 min (2 mL/min), 30-40% buffer B over 8 min (2 mL/min), 40-45% buffer B over 10 min (2 mL/min), 45-99% buffer B over 2 min (1 mL/min), 99-100% over 2 min (1mL/min), and re-equilibrated 6 min in 2% buffer B at 2 mL/min.

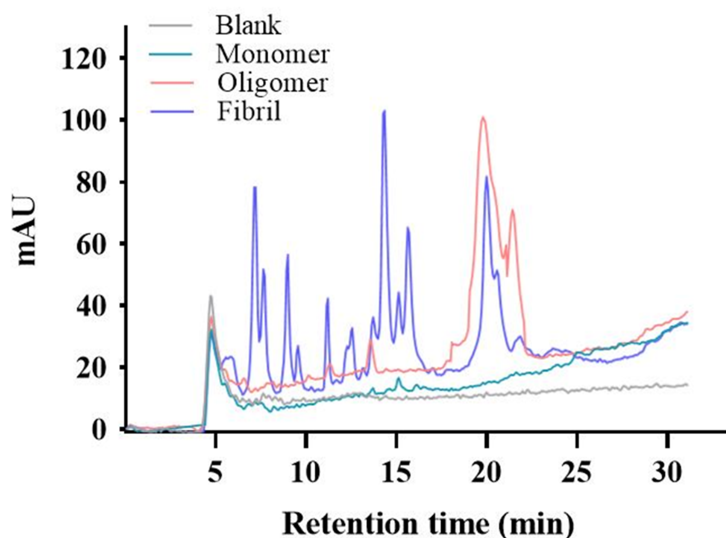

Fig. S2. RP-HPLC elution of Aβm, Aβo and Aβf structures. Longer retention time corresponded to increased hydrophobicity. Compared with Aβo, the oligomeric structures in the sample of Aβf were mostly depleted and converted into various heterogeneous and highly hydrophilic structures especially at earlier retention times. The overall data confirmed a conformational conversion of Aβm to Aβf over time, from disordered to β-sheet-dominant structures. mAU: milli-absorbance unit. Aβ concentration: 300 μM. For simplicity of comparison, this figure represents the first 30 min of HPLC runs (as there were no major differences between the curves after 30 min).

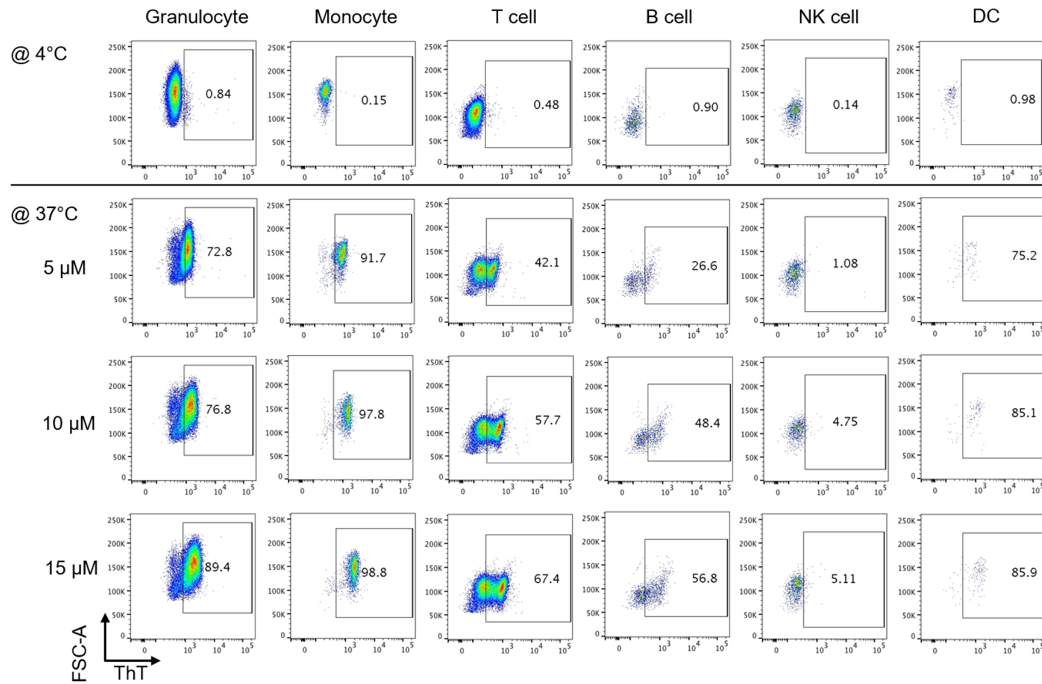

Figure S3. Gating was applied to identify the population of immune cells with ThT-labelled Aβo. Side and forward scatter were used to locate white blood cells before doublets were excluded. The following cell types were identified based on expression of surface markers or scatter: high side scatter granulocytes; CD3+ T cells; CD14+ monocytes; CD56+ NK cells; CD19+ B cells; and Lin1-HLA-DR+ dendritic cells. The percentage of each cell type positive for the ThT-labelled Aβ was recorded.