Supporting Information

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Plug-based microfluidics with defined surface chemistry to miniaturize and control aggregation of amyloidogenic peptides

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Supporting Information

Fabrication of the Microfluidic Device. Microchannels were fabricated as described before.[1] Briefly, microchannels with rectangular cross section were obtained by rapid prototyping in poly(dimethylsiloxane) (PDMS). The PDMS/PDMS devices were sealed using Plasma Prep II plasma cleaner. After sealing, we rendered the microchannels hydrophobic by baking the PDMS devices in a 120°C oven for over one hour.

ThT Aggregation Assay. $A\beta_{40}$ (NH_2-DAEFR^{5}HDSGY^{10}EVHHQ^{15}KLVFF^{20}AEDVG^{25} SNKGA^{30}IIGLM^{35}VGGVV^{40}-COOH) was obtained from rPeptide. HiLyte and fluorescein labeled $A\beta_{40}$ were obtained from Anaspec. To dissolve preformed peptide aggregates of $A\beta_{40}$, the lyophilized peptide was first dissolved in hexafluoroisopropanol (HFIP). The HFIP was evaporated from the samples of peptides, and the samples were then dried under a high vacuum overnight. The concentration of the solution of peptides was determined by absorbance at 280 nm using an extinction coefficient of $\varepsilon = 1360\ M^{-1}\ cm^{-1}$ for Tyr. $A\beta_{40}$ was dissolved in water shortly before use. Aggregation of $A\beta_{40}$ was detected by Thioflavin T (ThT) fluorescence.[2] Aggregation experiments in a well plate were done on a Polarstar Omega (BMG Labtech). For this we used 50 μM $A\beta_{40}$ and 50 μM ThT dissolved in 50 mM Tris (pH 7.3) at 37°C.

Microfluidic Experiment. Aqueous and fluorous solutions were filled into 10 μL gastight syringes (Hamilton Company), which were then connected to the microfluidic device by 30-gauge Teflon tubing (Weico-Wire & Cable). For $A\beta_{40}$ and CSF solutions, we filled the syringes with flourocarbon and aspirated 2 μL of either solution into Teflon tubing, which was then connected to a syringe and a PDMS device. Syringes were driven by PHD 2000 Infusion pumps (Harvard Apparatus). We used Fluorinert FC-40 or FC-70 (3M), purified by distillation before use, as a carrier fluid. The fluoro-surfactant 2-{2-[2-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-

Heptadecafluoro-nonyloxy)-ethoxy]-ethoxy}-ethanol (R_f-OEG_3) was synthesized as described elsewhere.[3]

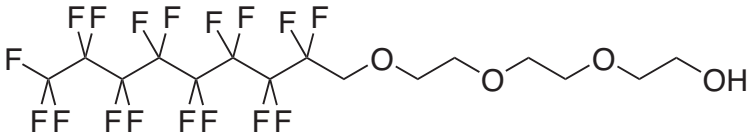

Figure SI-1. Chemical structure of 2-{2-[2-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluoro-nonyloxy)-ethoxy]-ethoxy}-ethanol (R_f-OEG_3).

1) Determination of the Concentration of Aβ40 in Plugs. To verify that $A\beta_{40}$ was encapsulated in plugs without nonspecific adsorption to PDMS or other interfaces, we encapsulated various concentration of fluorescein-labeled $A\beta_{40}$ in plugs. Additionally, we recorded a standard curve with fluorescein (dissolved in 0.05% NH_4OH) encapsulated in plugs. Figure SI-2 shows the fluorescence standard curve and the measured fluorescence intensities of labeled $A\beta_{40}$ at various concentrations. We observed a linear behavior of the fluorescence intensity of $A\beta_{40}$ in plugs with increasing $A\beta_{40}$ concentration (R^2 value 0.99). Furthermore, the fluorescence intensities of the peptide in the plugs were in agreement with the standard curve, indicating that $A\beta_{40}$ was not lost during the encapsulation process.

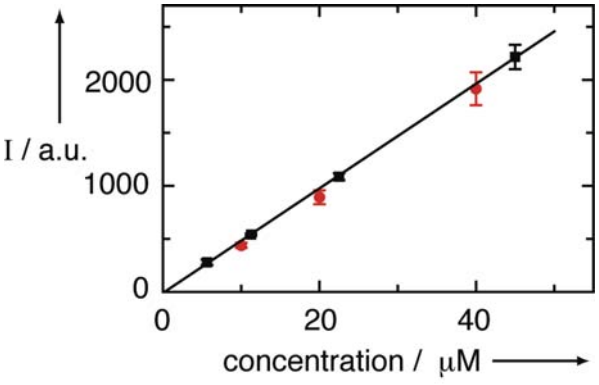

Figure SI-2. $A\beta_{40}$ concentration determination in plugs. Various concentrations of (■) fluorescein and (●) fluorescein labeled $A\beta_{40}$ encapsulated in plugs monitored by fluorescence microscopy. *I* is the absolute fluorescence intensity obtained using a Lecia DMI 6000B epi-fluorescence microscope with a 10x0.4 objective coupled to

Hammamatsu ORCA ERG 1394 CCD camera with an exposure time of 3ms. This procedure was used for both the Fluorescein standard curve and the labeled $A\beta_{40}$.

2) In vitro Aggregation Experiments in plugs (figure 1). Experiments were performed with a four-channel inlet device. Aqueous $A\beta_{40}$, Tris buffer, and a solution of ThT were loaded into syringes, and syringes were then connected to the convening channels (cross sectional diameters of 225 x 200 μm^2) of a microfluidic device. The final concentrations of $A\beta_{40}$, ThT, and Tris (pH 7.3 at 37°C) in plugs were always 50 μM, 50 μM and 50 mM, respectively. A syringe containing water-immiscible fluorocarbon FC-70, with or without 0.75 mg/ml R_f-OEG_3 was connected to a perpendicular channel. The flow of the aqueous solutions and fluorocarbon was established by driving syringes with syringes pumps. Typical flow rates for the total aqueous and fluorocarbon phase were 0.8 and 1.2 μL/min, respectively. Plugs were collected in Teflon tubing with an inner diameter of 200 μm. After the last plug was formed, the syringes were disconnected and the flow was stopped. To avoid evaporation of water from the plugs during the experiments, the plugs within the Teflon tubing were inserted into oil filled glass capillaries, and then the capillaries were sealed with sealing wax. Next, the capillaries were incubated at constant temperature (37°C), and the ThT fluorescence of the plugs was measured periodically.

3) Cerebrospinal Fluid Titration Experiments (figure 2). All animal experiments and protocols were approved by and conducted according to institutional and NIH guidelines. CSF was isolated from the cisterna magna compartment as previously described.[4] In brief, mice were anesthetized with a mixture of ketamine and xylene. An incision was made from the top of the skull to the dorsal thorax, and the musculature was removed from the base of the skull to the first vertebrae to expose the meninges overlying the cisterna magna. The tissue above the cisterna magna was excised. A microneedle was used to punctuate the arachnoid membrane covering the cistern. The CSF, which is under positive pressure as a result of blood pressure, respiration, and positioning of the animal, began to flow out of the needle entry site once the needle was removed. The fluid was then collected using a polypropylene narrow bore pipette as it exited the compartment.

Experiments with mouse CSF were done using a five-channel inlet device, where (1) $A\beta_{40}$, (2) buffer, (3) ThT in a salt solution, (4) CSF, and (5) FC-40 with 1.25 mg/ml R_f-OEG_3, were each allocated to one channel. The contact of CSF and $A\beta_{40}$ to PDMS is avoided in all titration experiments by inserting teflon tubing in the inlet channels of the solutions up the junction were plug formation takes place (see figure 2a). In order to screen the inhibitory potency of CSF on $A\beta_{40}$, we performed three experiments for each mouse CSF sample with three different pre-dilution factors of CSF (1:10, 1:5 and 1:1). Dilutions of CSF were done with a buffer that contained 150 mM NaCl and 10 mM PO_4^{2-} pH 7.3 at 37°C. Further dilutions (1:20, 1:10, 1:5, 1:4, and 1:2.5) of the pre-diluted samples were obtained by changing the flow rates of the aqueous streams. The flow rate ratio of the total aqueous phase and the FC-40 phase was kept constant (2μL min^{-1}/2μL min^{-1}). The flow rate of the $A\beta_{40}$ and ThT/salt solution were also kept constant. Only the flow rates of the buffer and CSF streams were changed to counteract each other. The length of the tubing used as a cartridge was matched with the total pumped volume in a titration experiment. The typical length of one condition filled approximately 6 cm of the cartridge. Plugs with the same condition in the cartridge were retrieved by calculating the position from the volume to length dependence. The cartridge tubing was placed in a PDMS mold (400 μm in width and 3 cm in length) and sealed to a glass slide (see figure 2a). Transitions between the two conditions were programmed to be located in the loop regions. Images of plugs were taken only in the region 1.5 cm from the starting and ending point of a single condition (i.e images only of plugs located in the PDMS mold). Although the position of the transition can vary between experiments (± 0.5 cm), 1.5 cm buffer volume on each side was sufficient to avoid shifts between the conditions (see SI-Fig.3). The pumps during a titration experiment were controlled with a LabView program.

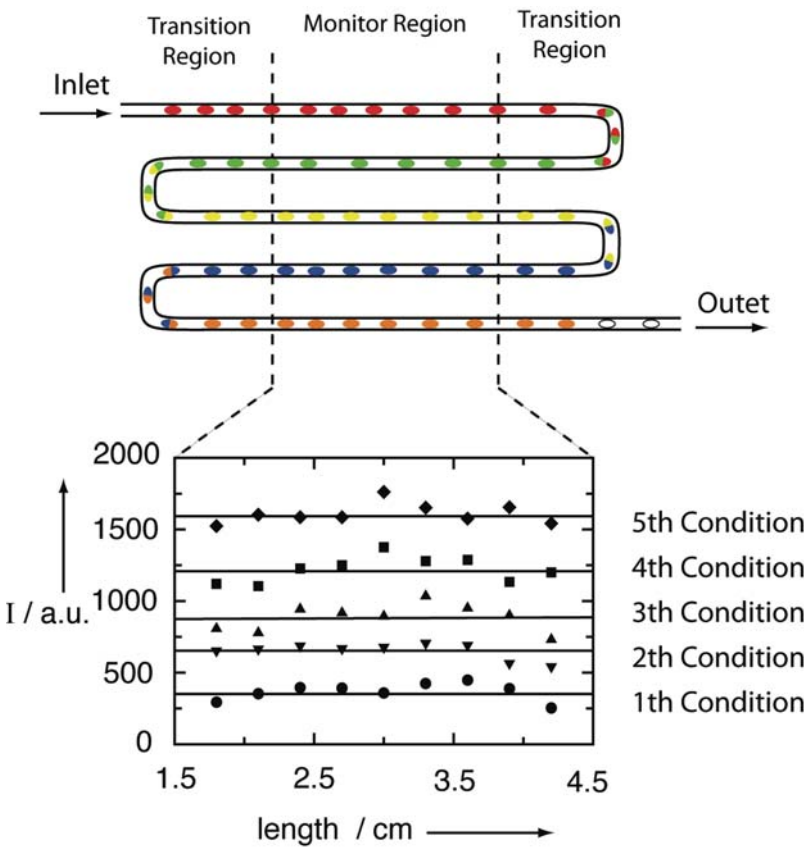

Figure SI-3. Plug position test. To test the position of the plugs in the cartridge we formed plugs with five different concentrations of fluorescein (◆) 8 μM, (■) 6 μM, (▲) 4 μM, (▼) 2 μM, and (●) 1 μM with the titration device. The total volume of each concentration series was calculated to occupy 6 cm in the storing cartridge. Fluorescence images of plugs were taken starting 1.5 cm from the calculated transition region and ending 1.5 cm for the next condition. The intensity of every 5th plug within this 3 cm region were measured and plotted against its position. Straight lines in the figure are the average fluorescence intensity of the plugs of each condition, respectively.

The final concentration of both $A\beta_{40}$ and ThT in the plugs was 50 μM. The salt concentration added to the ThT solution was adjusted to obtain a salt composition of 150 mM NaCl, 3 mM KCl, 0.8 mM $MgCl_2$, 1.4 mM $CaCl_2$, and 10 mM NaH_2PO_4/Na_2HPO_4 pH 7.3 at 37°C in a plug. Plugs were stored in Teflon tubing at 37°C, and fluorescence images of the plugs were taken periodically. For each CSF

concentration series, we formed 50 plugs, and of those 15 were monitored. We averaged the fluorescence intensity of the 15 plugs for the corresponding time points in figure 2 a and b.

We also confirmed that increasing the ionic strength of the buffer is responsible for nucleating $A\beta_{40}$ in plugs without a lag time. For this we measured the aggregation kinetics of $A\beta_{40}$ in dilutions of the above described buffer (1:1, 1:2, and 1:4). Figure SI-4 shows the effect of the salt concentration on $A\beta_{40}$ aggregation kinetics.

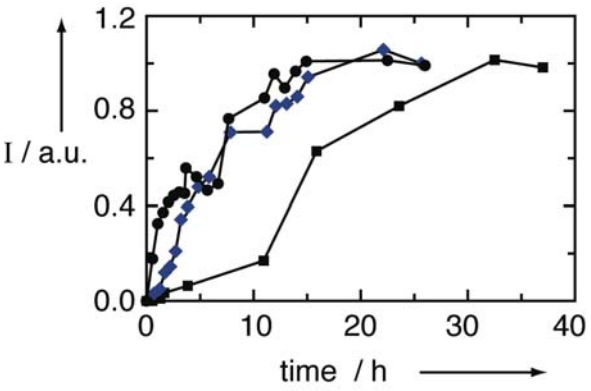

Figure SI-4. Aggregation kinetics of $A\beta_{40}$ in plugs with $R_f(OEG)_3$ measured by ThT under different ionic strength conditions: (●)150 mM NaCl, 3 mM KCl, 0.8 mM $MgCl_2$, 1.4 mM $CaCl_2$, and 10 mM NaH_2PO_4/Na_2HPO_4 , (◆) 1:2 dilution of (●), and (■) 1:4 dilution of (●). Each data point is averaged over 15 plugs.

4. TTR Titration Experiment

Titration experiments with TTR were performed under the same conditions as described for CSF experiment. Human TTR cDNA representing the mature protein without the endoplasmic reticulum-targeting signal sequence was inserted into the bacterial expression vector pProEx-HTa using standard molecular biology techniques. This construct was confirmed by sequencing and transformed into the Rosetta (DE3) bacterial strain. Protein expression was induced with 100mM isopropyl-beta-D thiogalactopyranoside for 20 hours at 25°C after which cells were pelleted and resuspended in lysis buffer (20mM Tris-Cl pH 8.0, 10mM 2-mercaptoethanol, 10ug/mL lysozyme, 1mM phenylmethylsulfonyl fluoride) and incubated on ice for

30’. Cell membranes were disrupted by sonication and lysates were spun at 100000 X g. From the supernatant, TTR was sequentially purified by Nickel-NTA, anion exchange and size exclusion chromatography. Samples were analyzed by SDS-PAGE and coomassie staining.

Data Evaluation. The fluorescence intensity in plugs was measured by taking fluorescence micrographs of single plugs using a Leica DMI 6000 microscope with a cooled CCD camera ORCA ERG 1394 (12-bit 1344 x 1024 resolution) (Hamamatsu Photonics). MetaMorph Imaging System version 6.1r3 (universal Imaging Corp) was used for imaging acquisition, and Matlab 7a (Mathworks) was used for image analysis. The fluorescence intensity was extracted from the images by calculating the average pixel intensity of the plugs at each time point. For comparison of the experiments in plugs and well plate, we normalized the ThT fluorescence intensity to the maximal intensity at the end point of the measurement: $I\text{-}I_0/I_{max}$, where I, I_0 and I_{max} are the fluorescence intensity, fluorescence intensity at time point 0, and the maximal fluorescence intensity at the end point of the measurement, respectively. In CSF experiments we normalized the ThT fluorescence signal to the starting value, $I\text{-}I_0$, were I_0 is the average fluorescence signal determined from 15 plugs.

$R_f(OEG)_3$-Concentration. To avoid $A\beta_{40}$ adsorption to the plug interface, correct concentration of $R_f(OEG)_3$ in carrier fluid was critical. Figure SI-3 shows aggregation experiments of $A\beta_{40}$, with various concentrations of $R_f(OEG)_3$ in FC-70. We found that a concentration lower than 0.5 mg/ml in FC-70 leads to $A\beta_{40}$ adsorption to the plug interface. For the carrier fluid FC-40, $A\beta_{40}$ adsorption could be avoided by using concentrations of $R_f(OEG)_3$ above 0.75 mg/ml. Therefore, saturation of the plug surface with surfactant molecules is dependent on the carrier fluid. $R_f(OEG)_3$ concentration above 1 in FC-70and above 1.5 mg/ml in FC-40, however, leads to the formation of aqueous micelles and plug coalescence.. ThT fluorescence in plugs formed in the stream of FC-70 with aggregated $A\beta_{40}$ after 250 hours is distributed over the whole plug volume, whereas in the case of a low $R_f\text{-}(OEG)_3$ concentration (Figure SI-5) or absence (Figure SI-6) the fluorescence signal is located at the plug interface. This finding supports the contention that aggregation of $A\beta_{40}$ is caused by adsorption to unfavorable interfaces.

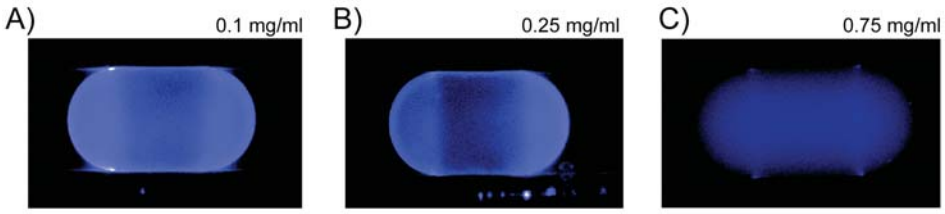

Figure SI-5. ThT fluorescence microscopy images of plugs formed with various concentrations of $R_f(OEG)_3$ containing 50 μM $A\beta_{40}$, 50 mM Tris pH 7.3. Adsorption of $A\beta_{40}$ to the FC-70/water interface of plugs is observed in plugs formed with a $R_f(OEG)_3$ concentration lower than 0.5 mg/ml.

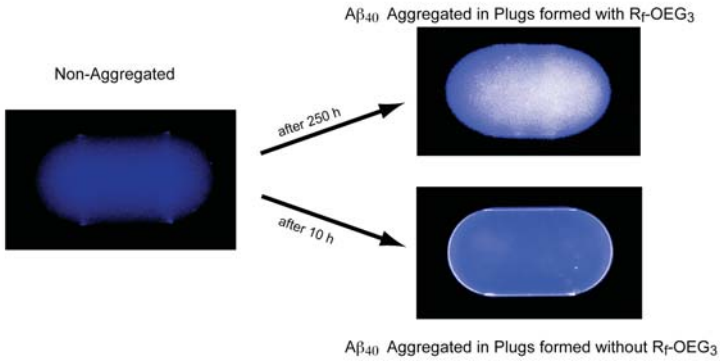

Figure SI-6. Representative ThT fluorescence microscopy images of plugs formed with and without R_f-OEG_3 containing non-aggregated and aggregated $A\beta_{40}$. The ThT fluorescence indicates that, in plugs formed without R_f-OEG_3, aggregates of $A\beta_{40}$ are located at the fluorocarbon interface; whereas in plugs formed with R_f-OEG_3, aggregates are more evenly distributed.

Supporting References

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[2] H. LeVine, *Meth Enzymol* **1999**, *309*, 274.
[3] L. S. Roach, H. Song, R. F. Ismagilov, *Anal Chem* **2005**, *77*, 785.
[4] R. B. DeMattos, K. R. Bales, M. Parsadanian, M. A. O'Dell, E. M. Foss, S. M. Paul, D. M. Holtzman, *J Neurochem* **2002**, *81*, 229.