Supplementary Material (ESI) for Lab on a Chip

Electronic Supplementary Information for Lab on a chip

Size-selective concentration and label-free characterization of protein aggregates using a Raman active nanofluidic device

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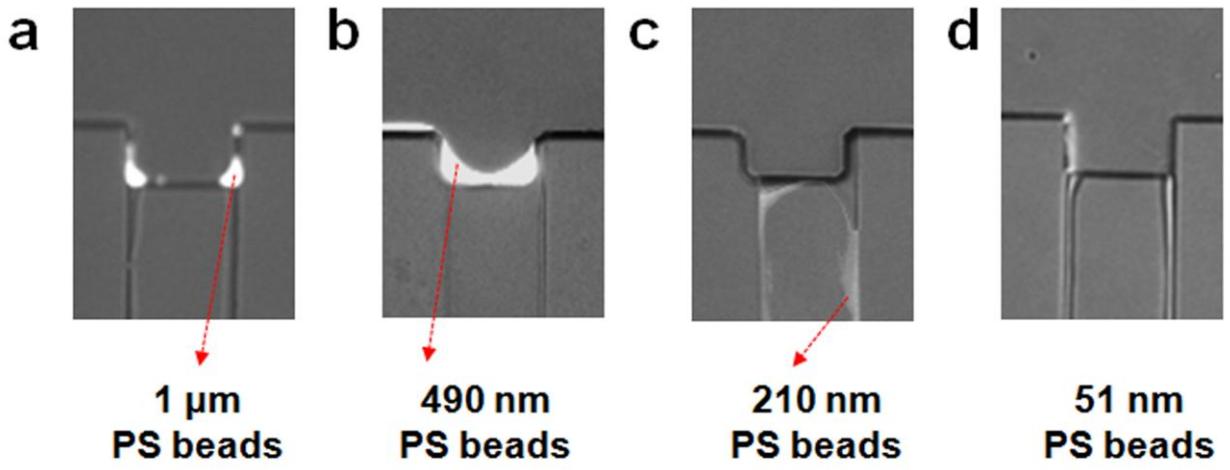
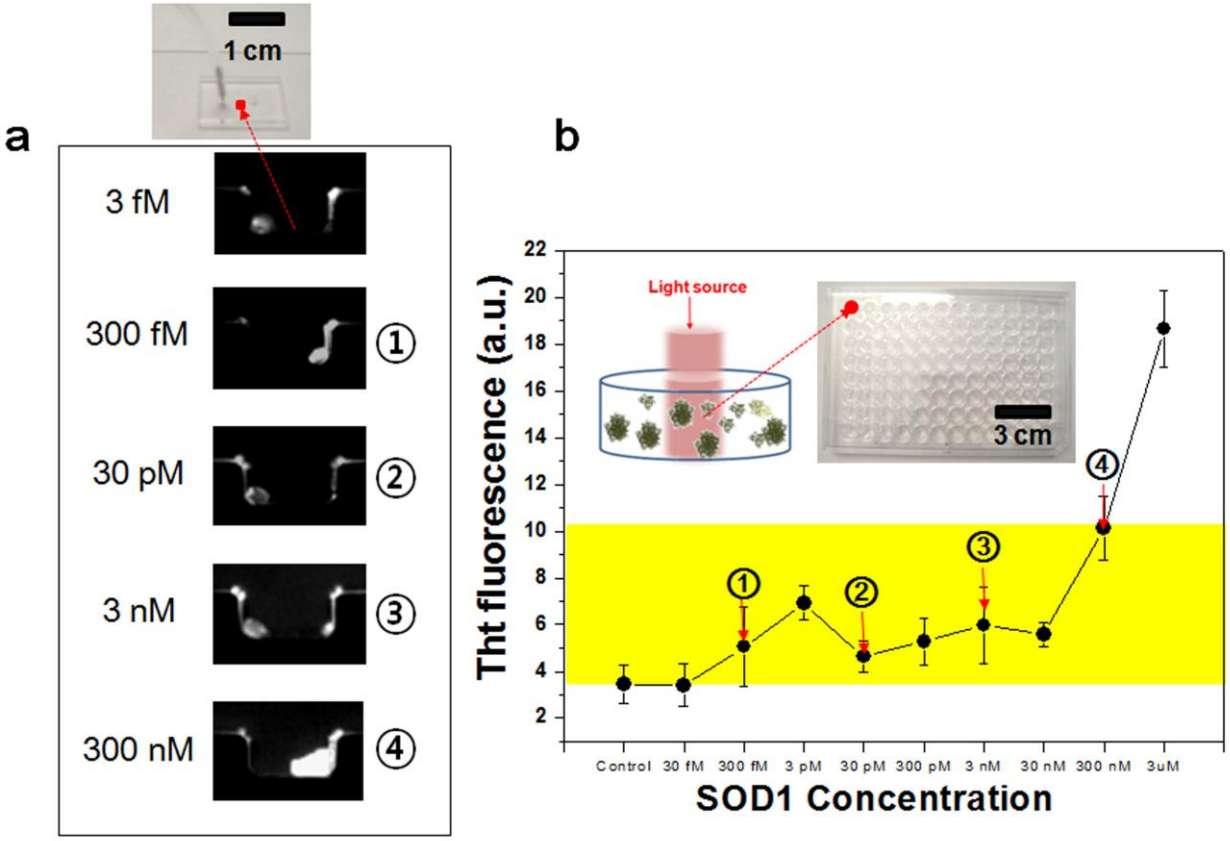
Supplementary figures

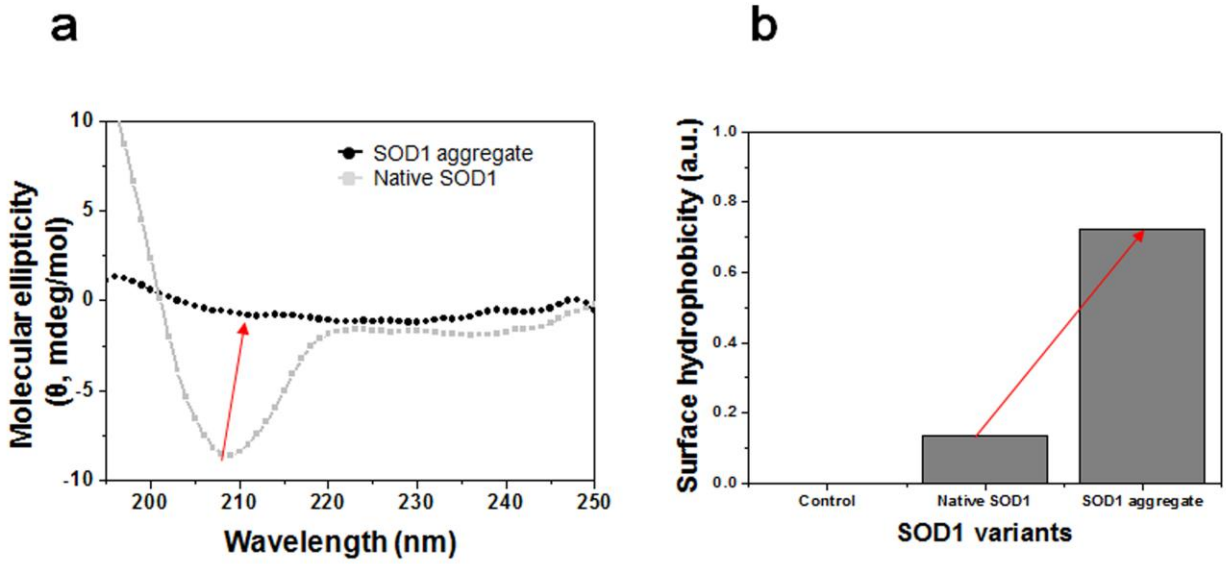
Supplementary Fig. S1. Demonstration of size-selective concentration of PS beads with different diameters. (a) and (b), 1μm and 490 nm PS beads, were accumulated at the micro/nanofluidic junction. (c) and (d), 210 nm and 51 nm PS beads, passed through the nanochannel as expected.



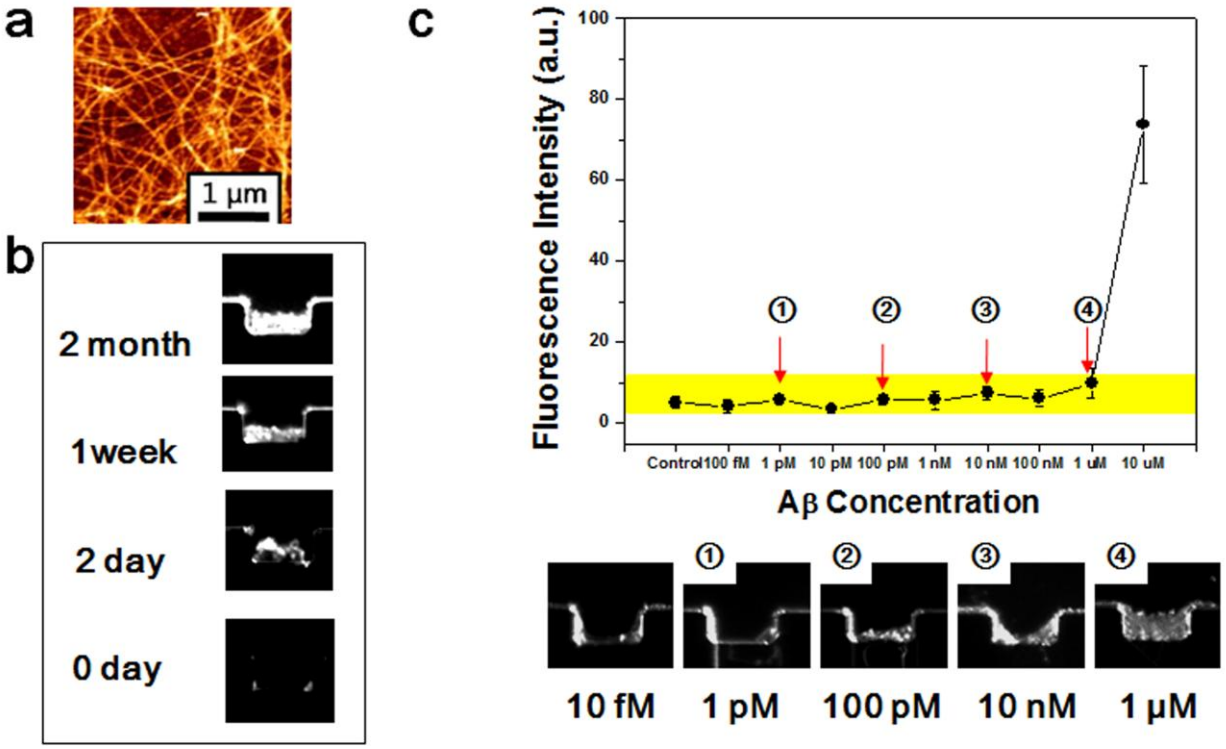
Supplementary Fig. S2. Comparison of fluorescence signals obtained from nanofluidic device with traditional fluorescence analysis. To identify the existence of protein aggregates, each protein solution was mixed with a 30 μM ThT solution with volume ratio of 1:10, and then it was characterized as below. Proteins were incubated for 47 days. (a) With the nanofluidic device: each sample solution with volume of 10 μl was injected into a nanofluidic device and then the concentrated protein aggregates were observed by imaging with fluorescence microscopy. (b) Traditional analysis: each sample solution with volume of 200 μl was added into a well in a 96-microwell plate, and then fluorescence intensities were measured by fluorescence spectroscopy. The yellow band of a graph indicates the range of intensities measured for the concentrations examined with our device. As can be seen for the traditional fluorescence method, it is hard to obtain reliable fluorescent intensities below the μM level.



Supplementary Fig. S3. Characterization of changes in structural and surface properties by SOD1 aggregation. The conformational changes in proteins are generally accompanied by structural turnover and/or changes in surface residues. Characterization experiments were performed here using a 10 mg/ml (310 μM) SOD1, before and after the aggregation. (a) A change in secondary structures was measured by circular dichroism (CD) spectroscopy. The characteristic peak for secondary structures of SOD1 is shown to disappear following aggregation. (b) A change in surface hydrophobicity was measured by 8-anilino-1-naphthalene-sulfonic acid (ANS) fluorescence spectroscopy[1,2]. The enhancement of ANS fluorescence intensity reveals the increase of protein surface hydropbobicity by aggregation. Red arrows indicate the changes in protein properties induced by aggregation process.



Supplementary Fig. S4. Application of nanofluidic concentration device to amyloid beta (Aβ) protein aggregates. (a) A representative AFM image of Aβ fibrils. (b) Images showing the Aβ fibrils concentrated at a micro/nanofluidic junction. Samples were prepared with initial concentration of 1 μM, for different incubation times (2 month, 1 week, 2 day, and 0 day). (c) Comparison of fluorescence signals a obtained from the nanofluidic device with a traditional fluorescence analysis for different initial concentrations of Aβ (from 10 fM to 1 μM) incubated for 47 days. The yellow band in the graph indicates the range of intensities measured for the concentrations that were examined with our device. As can be seen for the traditional fluorescence method, it is hard to obtain reliable fluorescent intensities below the μM level.



Supplementary Table S1. Assignment of Bands in SERS Spectra of SOD1 aggregates[3-7]

Wavenumber (cm^{-1})	Assignment	Comment
823	Tyrosine doublet	Hydrophobic, aromatic residue
850	Tyrosine	Hydrophobic, aromatic residue
961	n(C-C)	Hydrophobicity
1000	Phenylalanine	Hydrophobic, aromatic residue
1030	Phenylalanine	Hydrophobic, aromatic residue
1087	Lysine, Arginine, Glutamine, Asparagine	Charged (positive)
1124	Valine and Isoleucine	Hydrophobic
1185	Phenylalanine and Tyrosine	Hydrophobic, aromatic residue
1208	Phenylalanine and Tyrosine	Hydrophobic, aromatic residue
1241	amide III (β-sheet)	β-sheet structure
1260	amide III (α-helix)	α-helix structure
1323	amide III and CH_2 twist/wag	amide III
1402	aspartic acid, glutamic acid, υ(COO-)	Metallation, charged (negative)
1488	Histidine	Charged (positive)
1547	Phenylalanine	Hydrophobic, aromatic residue
1589	Phenylalanine	Hydrophobic, aromatic residue

Supplementary methods

For Supplementary Fig. 3

a, **CD spectroscopy-** The secondary structure of proteins was determined by CD analysis (Jasco J-715 spectropolarimeter equipped with a Naslab temperature controller). A 0.1-cm quartz cell was used for the measurements and the CD spectra which was recorded from 190 to 250 nm. All CD measurements were carried out using the following parameters: 1 nm bandwidth, 50 nm/min run speed, 1 nm step size, 8.3 s response time, and an average of three runs.

b, **ANS fluorescence spectroscopy**[1,2]**-** For the measurement of surface hydrophobicity, the fluorescence property of the extrinsic fluorophore ANS (final concentration 20 μM, excitation at 360 nm, emission at 450 nm) was examined by 10 min of incubation with proteins in the PBS buffer via fluorescence spectroscopy.

Supplementary references

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