Noninvasive label-free nanoplasmmonic optical imaging for real-time monitoring of in-vitro amyloid fibrogenesis

Sung Sik Lee$^{1,5}$, and Luke P. Lee$^{2,3,4,*}$

$^1$Department of Biosystems Science and Engineering, ETH Zurich, CH 4056, Basel, Switzerland
$^2$Department of Bioengineering, $^3$Biophysics Program,
$^3$Department of Electrical Engineering and Computer Science,
$^4$University of California Berkeley, CA 94720-1762, Berkeley, United States

$^5$Current Address: Institute of Biochemistry, ETH Zurich, CH 8093, Zurich, Switzerland

*to whom correspondence should be addressed:

Luke P. Lee
Department of Bioengineering, University of California at Berkeley, 408C Stanley Hall, Berkeley, CA 94720-1762, United States of America
Tel: (510) 642-5855
Fax: (510) 642-5835
E-mail: lplee@berkeley.edu
**Supporting Information Figure S1 (A)** Schematic illustration of dark-field imaging set up:

By using dark field condenser which numerical aperture (N.A) is higher than the one of objective, we collect only scattered light. The sample solution (Aβ42 and GNPs) was loaded in PDMS chamber and mounted on inverted microscope (B) Rayleigh scattering of nanoplasmonic particles: 80 nm Gold Nanoplasmonic Particles (GNPs) in water. (C) Example of trajectory of GNP' random movement: Yellow line starts from the center of GNP at initial time frame and it connects each center of GNP at the moment of imaging. In
this study, we took the image in every 60 ms and Fig.S1C shows the trajectory change in every 5 frames (D) Schematics for the calculation of mean square displacement (MSD)
Supplementary Table S1 Comparison of Dextran (M.W.=70,000) viscosity measured by nanoparticle tracking method (in this study) and conventional rheometer (Bohlin, C-VOR).

Viscosity unit: centi-poise (cp). The viscosities by nanoparticle tracking method could be calculated from MSD- τ plots (mean square displacement and observed lag time) and equations (MSD = 4Dτα, (D: Diffusivity, τ: observed lag time); D = kT / 6πηR (k: Boltzmann constant, T: Temperature, η: viscosity, R: radius of particle).

<table>
<thead>
<tr>
<th></th>
<th>Nanoparticle Tracking</th>
<th>Viscometer (rheometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Dextran 5%</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Dextran 10%</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Dextran 15%</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Dextran 20%</td>
<td>2.9</td>
<td>3.1</td>
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</tbody>
</table>
Supplementary Text

Methods

Aβ Sample preparation

Aβ peptides were prepared as described in previous studies \(^3\). The lyophilized peptide Aβ42 (Amyloid-β42 peptide, Sigma-Aldrich) was stored in sealed glass vials in desiccated containers at -80°C. Prior to re-suspension, the vial was allowed to equilibrate to room temperature for 30 min to avoid condensation upon opening the vial. The vial of lyophilized peptide was diluted in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma-Aldrich). The solution containing the dissolved peptide was then aliquoted in microcentrifuge tubes. Immediately prior to use, the HFIP-treated aliquots were carefully and completely re-suspended.

Nanoplasmonic Optics: Dark Field Imaging and Nanoplasmonic Particle Tracking

For dark field imaging, dark-field-occluded narrow beam of white light was delivered on top of sample through higher numerical aperture condenser (U-DCW, Olympus, N.A.=1.4), and lower numerical aperture of objective lens (UPlanFLN 100X, Olympus, N.A. = 0.6-1.3) was utilized to obtain the images of only scattered light (Fig. S1A)).

GNPs (80 nm, BBI international) were suspended in the Aβ42 solution which medium is either de-ionized water or DMSO with precursor concentration of Aβ42. The sample solution was prepared as 1:100 dilutions of GNP and Aβ42 solution. 5 µl from sample solution was loaded in PDMS chamber and sealed to prevent from drying, and immediately mounted on microscope (IX 81, Olympus).
We took sequential images of GNPs' Rayleigh scattering in sample solution every 60 ms for 200 frames. The trajectories of single GNPs in taken images were tracked in-house IDL (ITT Visual Information Solutions)–based software\textsuperscript{1,2}. We note that largely aggregated GNPs are neglected in the analysis. The single GNPs’ movements were analyzed by ensemble average of mean square displacement (MSD) from trajectories. MSD for every time interval \( \tau \) (observed lag time) was calculated as following equations.

\[
X (t) = (x_i, y_i) \quad (3)
\]

\[
MSD (t) = \langle \Delta X^2 \rangle \quad (4)
\]

\[
= \langle (X (t+ \tau) - X (t))^2 \rangle \quad (5)
\]

\[
= \langle \sum (x_{i+k} - x_{i+k-1})^2 + (y_{i+k} - y_{i+k-1})^2 \rangle \quad (6)
\]

It was calculated from where a particle at one position \((x_i, y_i)\) moved to other position \((x_{i+n}, y_{i+n})\) after a time interval given by \(n \times \) video frame time and we used the ensemble average of observed particles.

References

