HOKKAIDO UNIVERSITY

Title	A Single Spherical Assembly of Protein Amyloid Fibrils Formed by Laser Trapping
Author(s)	Yuyama, Ken-ichi; Ueda, Mariko; Nagao, Satoshi et al.
Citation	Angewandte chemie-international edition, 56(24), 6739-6743 https://doi.org/10.1002/anie.201702352
Issue Date	2017-06-06
Doc URL	https://hdl.handle.net/2115/70685
Rights	This is the peer reviewed version of the following article: K. Yuyama, M. Ueda, S. Nagao, S. Hirota, T. Sugiyama, H. Masuhara, Angew. Chem. Int. Ed. 2017, 56, 6739, which has been published in final form at http://dx.doi.org/10.1002/anie.201702352. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Type	journal article
File Information	A Single Spherical Assembly of Protein Amyloid Fibrils- Formed by Laser Trapping_SI.pdf, Supporting Information

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Supporting Information

Table of Contents

·Sample preparation……1
·Experimental procedure for laser trapping and fluorescence assay……1
·Electron microscopy analysis……2
·Time evolution of the size of the assembly formed by laser trapping of dimeric cyt *c*……2

Sample preparation

We prepared the sample solutions of monomeric cyt *c* and domain-swapped cyt *c* by the procedure similar to that in the reference [1]. After horse heart cyt *c* (Wako, Japan) (100 mg) was dissolved in 10 mL of 20 mM potassium phosphate buffer, pH 7.0, ethanol (final concentration, 60% (v/v)) was added to the cyt *c* solution. The cyt *c* solution was incubated at room temperature for 2 min, and the precipitate was collected by centrifugation. The precipitate was lyophilized to remove residual ethanol and subsequently dissolved in 10 mL of 50 mM potassium phosphate buffer, pH 7.0, at room temperature. Oxidized dimeric cyt *c* in the obtained solution was purified by gel chromatography (Superdex 75 30/100 GL, GE Healthcare, Buckinghamshire) using a fast protein liquid chromatography system (BioLogic DuoFlow 10, Bio-Rad, CA) (flow rate, 0.8 mL/min; monitoring wavelength, 415 nm; solvent, 50 mM potassium phosphate buffer; pH 7.0, temperature, 4ºC). The buffer of the purified oxidized dimeric cyt *c* solution was exchanged with D_2O using an Amicon ultra centrifugal filter (Millipore Corporation, Billerica) to prevent the absorption of the 1064-nm trapping laser by H_2O.[2] The concentration of monomeric and dimeric oxidized horse cyt *c* was adjusted to 0.5 mM (heme unit) by the intensity of the Soret band.

Experimental procedure for laser trapping and fluorescence assay

The D_2O solution of oxidized monomeric and dimeric horse cyt *c* was placed on a glass substrate that had been previously rinsed quickly with pure water. The volume of the solution placed on the glass substrate was 5–40 μL. Figure S1 shows a schematic illustration of the present optical system constructed based on an inverted microscope (Olympus, IX71). The sample was set on the computer-controllable motorized stage (Ludl Electronic Products, Ltd., LEP Motorized XY Stage) of the inverted microscope and subsequently covered with a glass bottle to suppress D_2O evaporation. A 1064-nm continuous-wave laser beam (Coherent. Inc., MATRIX 1064-10-CW) was used as the trapping light source and was focused to a position approximately 50 μm above the glass through an objective lens (100 magnification, NA = 1.4, oil immersion). The laser power was fixed to 200 mW after the objective lens by adjusting a half-wave plate placed in front of a polarizing beam splitter. The trapping behavior was observed with a charge-coupled device (CCD) camera (WATEC, WAT-231S2) under halogen-lamp illumination. A transmission image was sequentially captured at a video rate with a computer. We analyzed the images with the open-source software (Image J) and measured the diameter of a formed assembly manually at every 0.5 min. After the assembly was deposited on the glass substrate, an appropriate amount of a D_2O solution of ThT (50 μM) was added to the solution containing the assembly. The solution containing the assembly and 8.0

μM ThT was homogeneously illuminated with a UV laser at 405 nm (Coherent. Inc., CUBE), and the fluorescence of the solution was observed with the CCD camera. The excitation light was blocked by a long-pass edge filter (Semrock, 405 nm EdgeBasic) placed between the objective lens and the CCD camera. The fluorescence spectrum was measured with a CCD detector (Andor, Newton) coupled with a spectrograph (Andor, SHAMROCK-SR-303I-A).

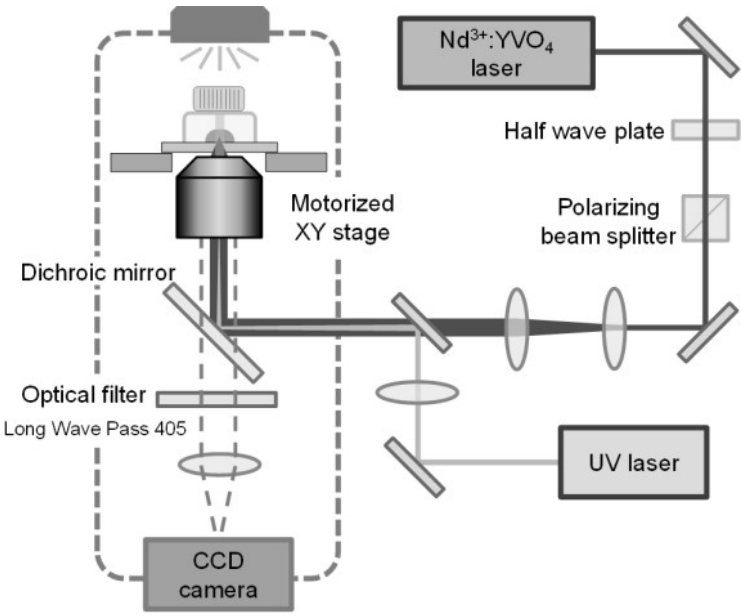


Figure S1. Schematic illustration of the present optical system for laser trapping and fluorescence assay.

Electron microscopy analysis

Electron microscopy observations were carried out with an SEM (JEOL, JSM-7400F) for a linearly extended assembly and a TEM (JEOL, JEM-3100FEF) for the sonicated sample of the linearly extended assembly. After the deposition of the linearly extended assembly, the remaining cyt *c* solution was removed with a pipette, and the assembly was washed with pure water several times and dried spontaneously under ambient conditions. The assembly was coated with an osmium (VIII) oxide layer measuring a few nanometers in thickness to prevent surface charging and was observed by SEM. For TEM observation, the deposited assembly was scraped from the glass with a spatula and dispersed in pure water. The dispersion was sonicated for 60 min to reduce the size of the assembly by separating it into small pieces. After sonication, a small amount of the dispersion was placed on a copper grid with a support film. The grid was cleaned with a hydrophilic treatment device (JEOL, HDT-400) before placing the sample on the grid. Excess solution was removed with filter paper after waiting for 1 min. Small assemblies adsorbed on the grid were negatively stained with a 0.5–2% phosphotungstic acid (PTA) solution for 1 min before TEM observation.

Time evolution of the size of the assembly formed by laser trapping of dimeric cyt *c*

We examined the trapping behavior of dimeric cyt *c* for 5 samples in total. A representative result is shown in Fig. 1E, and the behaviors of other 4 samples are summarized in Fig. S2. In all samples, dimeric cyt *c* was initially not trapped, but a single assembly with a size of 1.5 μm was observed at the initial stage of trapping. The assembly grew and disappeared, which was often repeated as in Fig. S2 (a), (b), and (d). The incubation time before a single assembly became visible (I), the saturation tendency in the size increase (S), and the rapid growth of the assembly to 10 μm (R) varied from sample to sample. On the other hand, the sizes of the assemblies when they appeared and disappeared were always 1.5 μm and about 10 μm, respectively.

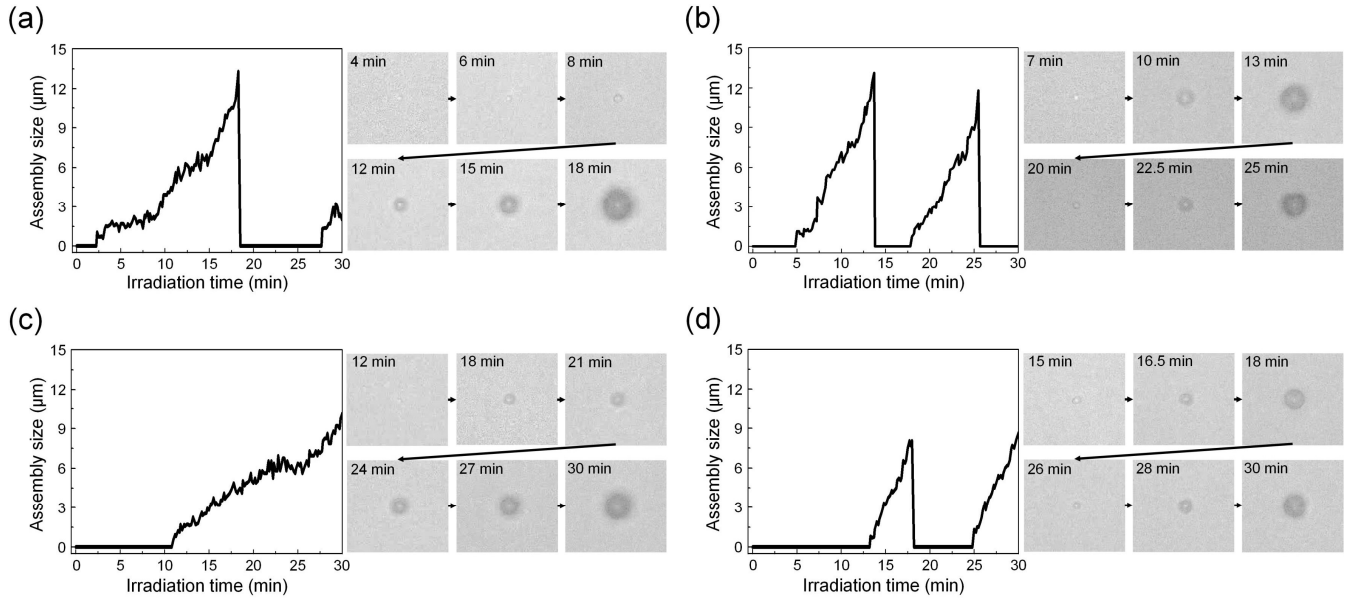


Figure S2. Time evolutions of the sizes of the assemblies formed by laser trapping of dimeric cyt *c*. For each sample, 6 captured images are shown. The size of the images is 40×35 μm^2.

References

[1] S. Hirota, Y. Hattori, S. Nagao, M. Taketa, H. Komori, H. Kamikubo, Z. Wang, I. Takahashi, S. Negi, Y. Sugiura, M. Kataoka, Y. Higuchi, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12854–12859.
[2] S. Ito, T. Sugiyama, N. Toitani, G. Katayama, H. Miyasaka, *J. Phys. Chem. B* **2007**, *111*, 2365–2371.