Supplementary Material (ESI) for Chemical Communications

Light Harvesting Antenna on an Amyloid Scaffold

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Supporting Information

Materials and Methods

***Peptide synthesis*:** The amyloid β 16-22 peptide was synthesized using standard FMOC peptide synthesis protocols with an Applied Biosystems ABI431 peptide synthesizer. The peptide was cleaved from the resin using the solution of TFA/thioanisole /ehtanedithiol/anisole (90/5/3/2). The peptide was then precipitated from the cleavage solution and washed repeatedly using excess ice-cold diethyl ether. Reverse phase HPLC was used for the peptide purification. The solvents used for purification were acetonitrile and water, both of which contained 0.1% TFA. The molecular weights of the peptides were verified by MALDI mass spectroscopy.

***Fluorescent modifications*:** Rh16-22 was synthesized using standard FMOC peptide synthesis protocols with an Applied Biosystems ABI431 peptide synthesizer. Rh110 was attached to the peptide backbone at the N-terminus through standard FMOC peptide synthesis protocols. The peptide was cleaved from the resin using the solution of TFA/thioanisole/ehtanedithiol/anisole (90/5/3/2). The peptide was then precipitated from the cleavage solution and washed repeatedly using excess ice-cold diethyl ether. Reverse phase HPLC was used for the peptide purification. The solvents used for purification were acetonitrile and water, both of which contained 0.1% TFA. The molecular weights of the peptides were verified by MALDI mass spectroscopy. Peptides with F19 1-^{13}C labeling were synthesized as described using 1-^{13}C phenylalanine at the appropriate position.

Amyloid assembly Purified Aβ(16-22) was dissolved in 2:3 acetonitrile:water with 1%TFA to a final concentration of 1.0mM. Purified Rh16-22 was dissolved in 2:5 acetonitrile:water with 1%TFA to a final concentration of 1.0mM. The peptide solution was allowed to self-assemble and mature at room temperature for 2 weeks. The co-assembly system was prepared by dissolving purified Aβ(16-22) into Rh16-22 stock solution (4μM in 2:3 acetonitrile:water with 1%TFA). The samples of mature Aβ(16-22) nanotubes (1mM) mixed with Rh110 or Alexa555 were prepared by mixing mature Aβ(16-22) nanotubes (1mM) and the dye stock solution in 1:1 (v:v) ratio. Rh110 concentration was 4μM in 2:3 acetonitrile:water with 1%TFA, and Alexa555 concentration is 1μM in 2:3 acetonitrile:water with 1%TFA.

Transmission Electron Microscopy (TEM): Aliquots (20 μl) of an incubated Aβ(16-22) solution were applied to TEM grids (Formvar/carbon film coated 200 mesh, purchased from Electron microscopy sciences) and allowed to adsorb for 1 min after a certain fold dilution depending on the nanotube density. Excess peptide solution was wicked off with a filter paper. 10 μl of 5% uranyl acetate (purchased from Sigma-Aldrich) was then added to stain the sample for 3 min. Excess fluid was wicked off with a filter paper and grids were dried under house vacuum overnight before experiments. The micrographs were recorded on a Hitachi H -7500 transmission electron microscope instrument with a LaB6 emission filament at an accelerating voltage of 75 kV. Negatives were scanned at 600 dpi resolution on an Agfa DuoScan flatbed scanner (Agfa Corp., Ridgefield park, NJ).

Two photon fluorescence imaging: Two-photon fluorescence images were acquired on a home built laser scanning microscope[1]. Rh16-22 and Rh110 were excited at 780 nm using a mode-locked Tsunami Ti:Sapphire laser pumped with a 532-nm 5W Millenia solid-state Nd:YVO_4 laser (Spectra-Physics, Mountain View, CA). Alexa555 is not excited efficiently at 780nm, but can be excited at 980nm. The 4X-expanded laser beam was directed into a IX-71 Olympus microscope (Olympus America, Center Valley, PA) and focused into the sample with a 60× UPlanSApo 1.2 NA water-immersion objective lens (Olympus America, Center Valley, PA). Fluorescence collected through the objective lens passed through a dichroic mirror (675 DCSX) and a short pass filter (E680SP, Chroma Technology, Rockingham, VT), and was focused onto a photon-counting photomultiplier tube (Hamamatsu, H7421). Images were acquired with custom software.

Flourier Transform Infrared Spectroscopy (FTIR) A 500 μL solution of mature samples formed by Aβ(16-22), fluorescence labeled peptides or the co-assembling system was spun down at 16,100 x g for 30 min and the pellet was frozen at -80^{o}C and lyophilized. The lyophilized pellet was mixed with dehydrated KBr crystals at a ratio of 1:10 (w/w), and the KBr/peptide pellet was analyzed on a MAGNA-IR 560 Spectrometer. E. S. P. instrument operated at 2cm^{-1} resolution.

Fluorescence lifetime imaging: The fluorescence lifetime imaging was measured using the time-correlated single-photon-counting (TCSPC) technique and Becker & Hickl electronics[2]. The detector was a Hamamatsu H7422-40 PMT (Hamamatsu Photonics, Japan) and a bandpass emission filter HQ530/50 (Chroma Technology) was used for lifetime measurement. The 256×256 pixel images were taken in a 100μs pixel-time scanning mode with a total collection time ranged from 120 to 180s. The fluorescence decay histogram for each pixel was well described by a single-exponential decay. A Levenberg–Marquardt routine for nonlinear fitting is applied to fit a decay curve to the data of this model function in a separate off-line software package SPCImage (Becker & Hickl Gmbh). The lifetime of rhodamine 6G in pure water (lifetime =4.08ns) was measured before each experiment.

Wide angle X-ray scattering (WAXS) Powder samples in 1.5mm diameter quartz capillary tubes were measured at the the 12-ID beamline at the APS with a shorter camera length and X-rays with E = 18 keV. The peaks in the plot $I(Q)$ versus Q are related to repeat distance d by the relation $d = (2\pi/Q)$. The intense, sharp and narrow peaks imply a high degree of order, and the weak and broad peaks relate to a low degree of repetition of the corresponding length scale.

Calculation of R_0: Föster radius is calculated through the overlap integral for the donor emission spectrum and the acceptor absorption spectrum. The definition is as below[3]:

$$R_0 = \sqrt[6]{\frac{8.8\times10^{-5}\kappa^2 Q_D J_{DA}}{n^4}}$$

$$J_{DA} = \int_0^\infty f_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

κ^2 is the orientation factor. In the calculation, the donor and acceptor are assumed to be randomly orientation dipoles, with the average value of κ^2, 2/3.
Q_D is the quantum yield of the donor, and the value is 0.6.
n is approximate index of refraction of the solution, the value is 1.343.
J_{DA} is the overlap integral of the donor emission spectrum and the acceptor absorption spectrum, and its definition as shown above.
ε_A is the extinction coefficient of the acceptor at each wavelength. The calculation uses a constant value of 150000.

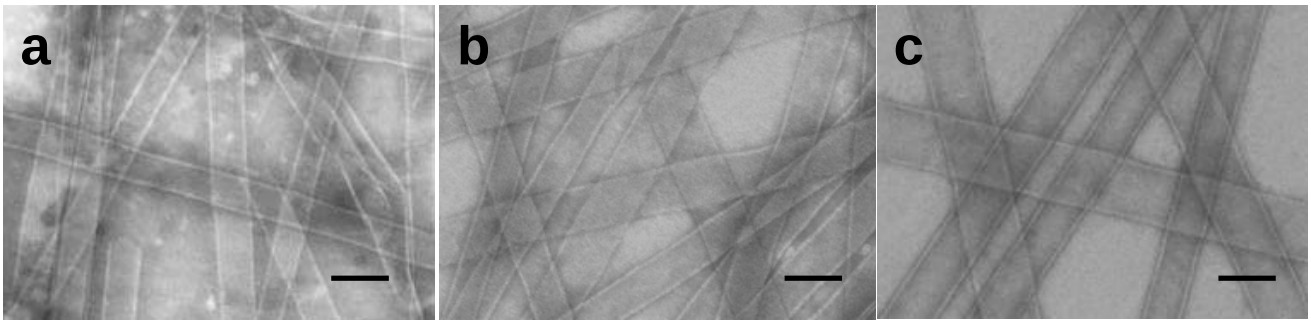

Figure S1 TEM of donor and acceptor with Aβ(16-22) nanotubes. (a) Aβ(16-22):Rh16-22 co-assembly (250:1 molar ratio); (b) Aβ(16-22) mature nanotubes with Rh110, (250:1 molar ratio); (c) Aβ(16-22) mature nanotubes with A555 (1000:1 molar ratio); Image scale=100nm.

Reference:

[1] A. Nagy, J. Wu, K. M. Berland, *Biophys. J.* **2005**, *89*, 2077.
[2] M. Peter, S. M. Ameer-Beg, *Biol. Cell* **2004**, *96*, 231
[3] B. W. v. d. Meer, Resonance energy transfer : theory and data, New York : VCH, **1994**.