SUPPLEMENTAL INFORMATION

A FOLDING TRANSITION UNDERLIES THE EMERGENCE OF MEMBRANE AFFINITY IN AMYLOID-β

Suman Nag[†#@], *Bidyut Sarkar*[†#], *Muralidharan Chandrakesan*[‡], *Rajiv Abhyanakar*[†], *Debanjan Bhowmik*[†], *Mamata Kombrabail*[†], *Sucheta Dandekar*[‡], *Eitan Lerner*[§], *Elisha Haas*[§] *and Sudipta Maiti*[†*]

[†]Department of Chemical Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Mumbai 400005, India.

[‡]Department of Biochemistry, Seth G.S. Medical College and KEM Hospital, A.D. Marg, Parel, Mumbai 400012, India.

[§]The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel.

[@]Current address: B401, Beckman Center, Department of Biochemistry, Stanford School of Medicine, Stanford, CA-94025, USA.

[*]Email: maiti@tifr.res.in (S.M)

[#] S.N and B.S contributed equally to this work.

Materials, Reagent Preparation and Experimental Methodology

Materials

All the 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acids, Fmoc-Asp(EDANS)-OH, (2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU), O-benzotriazole-N,N,N',N'-tetramethyl uronium hexafluoro phosphate (HBTU), 1-hydroxybenzotriazole (HOBt), EDANS Novatag Resin, rink amide MBHA resin LL, triisopropylsilane (TIS) and N-(4-[4'-(Dimethylamino) phenylazo] benzoyloxy) succinimide (DABCYL-OSu) were purchased from Merck (Schuchardt, Germany). Trifluoroacetic acid (TFA), N-methyl morpholine (NMM), N,N-dimethylformamide (DMF), acetic anhydride, tert-butyl methyl ether, acetonitrile, chloroform, uranyl acetate, di sodium hydrogen orthophosphate dihydrate (Na_2HPO_4, $2H_2O$) and potassium dihydrogen orthophosphate (KH_2PO_4) were purchased from S.D. Fine Chem. Ltd. (Mumbai, India). Sodium chloride (NaCl) was obtained from Fisher Scientific (Mumbai, India). Potassium Chloride (KCl) and calcium chloride dihydrate ($CaCl_2$, $2H_2O$) were purchased from SRL (Mumbai, India). Magnesium sulphate ($MgSO_4$, $7H_2O$) was obtained from AnalaR, Glaxo laboratories (Mumbai, India). Hexafluoroisopropanol (HFIP), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU), dimethyl sulphoxide (DMSO), ploy-L-Lysine and dextrose were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). *N*,*N*-Diisopropylethylamine (DIPEA), phenol, ethane dithiol (EDT), thioanisole and trifluoroethanol (TFE) were obtained from from Fluka (St. Louis, MO, USA). 5-((((2-iodoacetyl) amino) ethyl) amino) naphthalene-1-sulfonic acid (IAEDANS) was purchased from Molecular Probes (Eugene, Oregon, USA). Rhodamine labeled amyloid beta 40 (R-Aβ_{40}), N-terminal fluorescein labeled amyloid beta 40 (FL-Aβ_{40}) and C-terminal fluorescein labeled amyloid beta 40 (Aβ_{40}-FL) were purchased from rPeptide (Bogart, GA, USA). Carbon-coated 100 mesh copper grids were purchased from Electron Microscopy Sciences (Hatfield,

PA, USA). PenStrep, Fetal Bovine Serum (FBS), DMEM-F12 media and Trypsin were purchased from Gibco (Grand Island, NY, USA).

Reagent Preparation

Buffer preparation

The artificial cerebrospinal fluid (ACSF) buffer used for all the experiments consisted of 20 mM Na_2HPO_4, 146 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.4 mM KH_2PO_4 and 5 mM dextrose (pH adjusted to ~7.4). Modified Thomson's buffer (TB) (consisting of 20mM sodium HEPES, 146 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.4 mM KH_2PO_4, 0.3 mM Na_2HPO_4 and 5 mM dextrose; pH adjusted to ~7.4) was used for a few of the measurements. Most experiments were repeated in both buffers and no substantial difference was found in the measurements between the two buffers (though the solubility of Aβ is lower in TB). All data reported here represent the average of all measurements, irrespective of the buffer used.

Peptide synthesis and purification

In brief, $A\beta_{40}$ was synthesized using Fmoc chemistry in an automated solid phase peptide synthesizer (PS3, Protein Technologies Inc. Tucson, AZ, USA). Use of rink amide MBHA resin for the synthesis yields peptide with carboxamide at the C-terminal. For each step of coupling, four-fold excess Fmoc amino acid was activated with equimolar HATU or HBTU and NMM (0.4M) in DMF. 4% DBU in DMF was used for deprotection of the Fmoc group. Finally, treatment with a mixture of 80% TFA + 2.5% TIS + 5% water + 2.5% EDT + 5% thioanisole + 5% phenol for 4 hours was used to cleave the acid labile side chain protecting groups, while at the same time producing the free peptide in solution. The peptide was then concentrated under nitrogen flow and precipitated from the solution using tert-butyl methyl ether. The precipitate was washed thrice using the same ether followed by evaporation *in vacuo* to obtain the peptide in dry powder form.

For the Aβ-EDANS (D-Aβ_{40}) peptide synthesis, instead of the rink amide resin, EDANS Novatag resin (resin loading 0.64mmol/g) was used, which had the fluorophore EDANS already attached to the resin. The synthesis proceeded from the C to the N-terminus with the first coupling between the carboxylic acid of C-terminal amino acid of Aβ and the free secondary amine group of resin bound EDANS. This coupling was effected using the amino acid (4-fold excess) and HATU (4-fold excess) and DIPEA (8-fold excess) in DMF. Since the first coupling was not very efficient this step was repeated to get maximum coupling. The coupling efficincy was found to be ~70% using Fmoc detection.[1] Rest of the unreacted secondary amine sites of the resin bound EDANS was bolcked by treatment with acetic anhydride. This step ensured that no unwanted chain of varying peptide length can grow. Rest of the coupling, peptide cleavage and recovery steps followed a method similar to that used for the unlabeled peptide. In this case the cleavage mixture cleaved the bond between EDANS and the resin yielding Aβ with EDANS at the C-terminal of the peptide.

To prepare the FRET pair DABCYL-Aβ-EDANS (DA-Aβ_{40}) peptide, DABCYL was attached to the N-terminus of the resin bound Aβ-EDANS peptide using DABCYL-OSu (4-fold excess) in the presence of HOBt (2-fold excess) and DIPEA(4-fold excess) in DMF medium with constant stirring for 24 hr. The cleavage and peptide recovery were performed as described earliar.

Aβ with EDANS at the N-terminus of the peptide was prepared by using Fmoc-Aspartate with EDANS attached to the carboxyl side chain instead of regular Fmoc-Aspartate (Aspartate is the N-terminal amino acid).

The powdered crude peptides were dissolved in a 1:1 (vol/vol) mixture of HFIP and TFE and purified by reverse phase HPLC (Model: Prominence 20A, Shimadzu, Columbia, MD, USA) using a Kromasil C4 column (Kromasil, Bohus, Sweden). Water-acetonitrile (both containing 0.1% TFA) solvent gradient was used as for the elution. The purity of the peptides was verified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Model:TOF SPEC 2E,

Micromass, Manchester, England) or electrospray ionization mass spectrometry (ESI-MS, Finnigan LCQ Deca electrospray quadrupole ion trap mass spectrometer, Thermo Electron Corporation, San Jose, CA, U.S.A).

Preparation of specimens with different sizes and their characterization

The methods for preparing monomers and oligomers have been described by us earlier.[2,3] Briefly, peptides were dissolved in physiological buffer solutions at pH 7.4 and at 25°C. We prepared a mixed solution of 100 μM of unlabeled $A\beta_{40}$ along with 10 μM of D-$A\beta_{40}$ or DA-$A\beta_{40}$ (as needed) and 150 nM R-$A\beta_{40}$, diluting from a stock solution prepared in water at pH 11. The time of this dilution (and consequent pH change to ~7.4) was taken as the initial time point of the measurement. $A\beta_{40}$ was supersaturated at these concentrations and started to aggregate, progressively producing large oligomers and insoluble precipitates. For preparing small oligomers, we centrifuged the solution at 2000×g for 20 minutes after ~ 1 day, and measured the size to ensure that the solution predominantly consisted of small oligomers. When this process was repeated for ~5 days, only monomers were obtained, as reported earlier.[2] The particle sizes were measured by FCS (which used the rhodamine fluorescence) (Fig. S1a-f). We tested its ability of the large precipitates to form classic amyloid fibrils using transmission electron microscopy (Fig. S1g-h). The measurements for small oligomers and monomers were repeated for sub-saturated solutions (1-2 μM) without centrifugation. This initially yielded small oligomers, and after ~5 days yielded monomers, also as reported earlier.[2] The small oligomeric and monomeric Aβ measurements were also separately repeated for fully labelled specimens at low concentrations (using pure D-$A\beta_{40}$ and pure R-$A\beta_{40}$). In each case, all sets of measurements gave very similar results (i.e. within the standard deviations of each other). Finally, the monomeric and the oligomeric solutions were tested for their affinity for RN46A cell membranes, by confocal microscopy (using a Zeiss LSM 710). We verified that the oligomers had strong affinity for the cell membranes at

250 nM concentration, while the monomers had very little affinity at the same concentration (Fig. S2), as found earlier.[3]

Preparation of specimens with DA-Aβ$_{40}$

It was difficult to obtain fully acceptor labeled DA-Aβ$_{40}$ specimen in large quantities, and data reported for larger aggregates (which requires higher sample concentration) in this manuscript are from specimens with 64% acceptor labelling (as estimated by absorbance measurements, see 'Estimation of DABCYL labelling efficiency' in SI). In other words, a DA-Aβ$_{40}$ molecule always had the donor (EDANS) at the C-terminal, but did not have the acceptor (DABCYL) in 36% of the cases. However we were successful in preparing a small amount of 100% acceptor labeled samples (see the mass spectrum in Fig. S3). We repeated the monomer and small oligomer FRET measurements with this sample (these measurements required small amounts of specimen). The data obtained using 100% and 64% DA-Aβ$_{40}$ samples (after due correction, see section 'Correction of FRET efficiency' in SI) for the monomers and the small oligomers agree within experimental errors. Section 'Estimation of FRET efficiency for 100% and 64% acceptor labelled DA-Aβ$_{40}$ peptides' in SI provides a full description of results comparing the 64% and 100% DABCYL labelled DA-Aβ$_{40}$ samples.

Experimental Methodology

Characterization of aggregates

Co-aggregation of DA-Aβ$_{40}$with unlabeled Aβ$_{40}$ was performed following a method described elsewhere.[2,4] FCS measurements were performed with an instrument constructed in-house.[5] Lifetime measurements of the solutions were performed by using the Time Correlated Single Photon Counting (TCSPC) method, with an instrument described elsewhere.[6] FCS data were fitted either with a discrete few-component diffusion model, and/or with a Maximum Entropy Method (MEM) based software written in house.[7] The FCS data were converted into hydrodynamic radii (R_h) using rhodamine B (R_h = 0.57 nm[8]) as a calibrant. Further details are provided in the 'FCS measurements' section in SI.

Estimation of DABCYL labelling efficiency

The solid state peptide synthesis method ensured that the donor only sample ($A\beta_{40}$-EDANS or D-$A\beta_{40}$) was completely labelled by EDANS (5-((2aminoethyl) amino) naphthalene-1-sulfonic acid) at the C-terminus. We calculated the percentage labelling of the acceptor DABCYL at the N-terminus of the donor-acceptor sample (DABCYL-$A\beta_{40}$-EDANS or DA-$A\beta_{40}$) by measuring the relative absorbance of the EDANS and DABCYL dyes attached to the peptide, and by using their molar extinction coefficients. The molar extinction coefficient for EDANS attached to the C-terminal of the peptide was measured to be 5300 M^{-1} cm^{-1} at 330 nm, which is close to values reported earlier (5700 M^{-1} cm^{-1} at 336 nm for IAEDANS, as reported by the manufacturer, Molecular Probes). To measure the molar extinction coefficient of the peptide-attached DABCYL, we subjected a small amount of DA-$A\beta_{40}$ specimen to multiple prolonged rounds of labelling with DABCYL-OSu under different solvent conditions (e.g in DMSO) for 10 days. This was continued until the purified DA-$A\beta_{40}$ specimen showed no detectable trace of unlabeled specimen in the mass spectra (Fig. S3). This was used as a standard for a fully (100%) labeled DA-$A\beta_{40}$ sample. Using this standard, we determined the extinction coefficient of the DABCYL attached to the N-terminus to be 6285 M^{-1} cm^{-1} at 450 nm. The ratio of the two absorbance peaks of the DA-$A\beta_{40}$ specimen was measured and was compared to the ratio of the molar extinction coefficients at 450 nm and 330 nm. This yielded a labelling efficiency of 64% for most the specimens used for the experiments (unless otherwise stated). This implied that the nominal DA-$A\beta_{40}$ sample had a mixture of D-$A\beta_{40}$ and actual DA-$A\beta_{40}$ in a ratio of ~ 1:2.

Calculation of the FRET efficiency and inter-terminal distance distribution

We calculated the efficiency of energy transfer (E), using the equation:

$$E = 1 - \frac{\tau_{da}}{\tau_d} \quad \text{..... } \textit{Equation 1}$$

Where τ_{da} and τ_d are the mean lifetimes of the DA-$A\beta_{40}$ and D-$A\beta_{40}$ specimens respectively. Inter-terminal distance distributions and diffusion coefficients were calculated by global analysis of the

EDANS fluorescence decay curves using the model proposed by Beechem and Haas[9] (see 'Calculation of the inter-terminal distance distribution' in SI for details).

Correction of FRET efficiency

Most DA-Aβ$_{40}$ samples used in our experiments were only partially (64%) labelled with the acceptor (see 'Estimation of DABCYL labelling efficiency' in SI). Therefore any DA-Aβ$_{40}$ sample would have 36% peptide labelled with the donor only (D-Aβ$_{40}$). This means that the apparent lifetime decay, τ_{DA}^{app} from the DA-Aβ$_{40}$ sample would have contributions both from donor-only peptide (τ_D) and donor-acceptor labelled peptide (τ_{DA}^{real}), which would result in an incorrect estimate of FRET efficiency. This can be corrected if the fraction of labelling (x) of the DA-Aβ$_{40}$ sample is known. In our case x was 0.64. The apparent lifetime decay τ_{DA}^{app} from the DA sample is given by

$$\tau_{DA}^{app} = x\tau_{DA}^{real} + (1-x)\tau_D \quad \ldots\ldots\ldots \textit{Equation 2} \tag{169}$$

The apparent FRET efficiency is then calculated using Eq.3

$$E^{app} = 1 - \frac{\tau_{DA}^{app}}{\tau_D} \quad \ldots\ldots\ldots \textit{Equation 3} \tag{171}$$

However the real FRET efficiency is given by

$$E^{real} = 1 - \frac{\tau_{DA}^{real}}{\tau_D} \quad \ldots\ldots\ldots \textit{Equation 4} \tag{173}$$

Using equations 2, 3 and 4 it can be shown than

$$E^{real} = \frac{E^{app}}{x} \quad \ldots\ldots\ldots\ldots \textit{Equation 5} \tag{175}$$

Eq. 5 was used to correct for FRET efficiencies obtained using 64% labelled DA-Aβ$_{40}$ samples. We note that the accuracy of this correction does not affect the relative FRET efficiencies of the different specimens.

Calculation of the Förster distance for the EDANS-DABCYL pair

The dependence of the FRET efficiency E on the separation between the donor and acceptor chromophores (R) is given by

$$E = \frac{1}{1+\left(\frac{R}{R_0}\right)^6} \quad \ldots\ldots\ldots \textit{Equation 6}$$

where R_0 is the Förster distance at which energy transfer efficiency is 50%. R_0 can be calculated from the properties of the chromophores and the medium:

$$R_0^{\,6} = \frac{0.529 * \kappa^2 * \phi_D * J}{N * n^4} \quad \ldots\ldots\ldots \textit{Equation 7}$$

where R_0 and the wavelength λ are in centimetres, κ^2 describes the relative orientation of the fluorophores, ϕ_D is the quantum yield of the donor, N is the Avogadro number, and n is the index of refraction of the medium. J is the spectral overlap integral, given by:

$$J = \frac{\int_0^\infty \phi(\lambda) * \varepsilon(\lambda) * \lambda^4 * d\lambda}{\int_0^\infty \phi(\lambda) * d\lambda} \quad \ldots\ldots\ldots \textit{Equation 8}$$

Here $\phi(\lambda)$ is the area normalized donor emission spectrum and $\varepsilon(\lambda)$ is of the acceptor absorbance spectrum (M^{-1} cm^{-1}). We measured the value of J for the EDANS-DABCYL pair attached to $A\beta_{40}$ to be ~ $1*10^{14}$ M^{-1} cm^{-1} nm^4. The value of the orientation factor (κ^2) was taken to be 2/3 (for a freely rotating dye). This is a reasonable assumption, because the donor-acceptor pair is attached to the two termini of the peptide and should be relatively free to rotate. This was confirmed from the observed fast decay component (<0.2 ns) of the rotational anisotropy of EDANS attached to the peptide (data not shown). Moreover, for fluorophores which have multiple transition dipole moments, the effect of the orientation factor goes down drastically.[10] We note that EDANS has multiple lifetimes and is thus expected to have multiple transition dipoles. Using the values of 1.39 for n inside a protein and the value of 0.27

for ϕ_D (calibrated using standard fluorescein in pH 13), we obtained the Förster distance R_0 (using Eq.7) to be 2.7 nm.

Co-aggregation studies

We checked the effect of chromophore labeling on the aggregation properties of $A\beta_{40}$ by studying the co-aggregation of DA-$A\beta_{40}$ with unlabeled $A\beta_{40}$. A mixed solution of 100 μM of unlabeled $A\beta_{40}$ along with 10 μM of DA-$A\beta_{40}$ was prepared in buffer, by diluting a stock solution prepared in water at pH 11. The absorbance at 260 nm, 330 nm and 450 nm were monitored for tyrosine (in the peptide), EDANS and DABCYL respectively. The initial absorbance values (at 10min) were normalized at these three wavelengths. We obtained similar absorbance values for all three at the end of aggregation (within a factor of two). This suggests that the dye labeled and unlabeled peptides co-aggregate and their properties are grossly similar. Further, we have tested that the labeled peptide was able to form classic amyloid fibrils (Fig. S1h). These suggest that chromophore attachment does not have a major effect on the aggregation properties of the peptide.

FCS measurements

The homebuilt FCS set up used here was similar to that described elsewhere.[5] A collimated laser beam was focused into the sample volume using an apochromatic 60× water immersion objective with NA of 1.2 (Olympus, America Inc, Center Valley, PA, USA). The emitted fluorescence was collected using the same objective and separated from the excitation beam by a dichroic mirror (Chroma, VT, USA). A 25 μm core-diameter optical fiber was used as a confocal pinhole to reject the out of focus fluorescence. The fluorescence was filtered by a suitable emission filter (Chroma, VT, USA) before being detected by a single photon avalanche photodiode (APD, PerkinElmer Inc, Waltham, MA, USA). The data were processed using a hardware correlator card (ALV 5000E, ALV Laser, VmbH, Langen, Germany) which yielded the autocorrelation curves. These curves were analyzed using

a discrete diffusing component model using non-linear curve fitting with the Origin 7.5 software (OriginLab, Northampton, MA, USA). Alternatively, we also used the MEMFCS fitting routine to obtain a size distribution from the FCS data in a model-free manner.[7] The peak hydrodynamic radius, as determined by MEMFCS analysis (Fig. S1b) of the FCS data (Fig. S1a; data: red circles, fit: black, residuals: blue, auto-correlation: green) obtained from the monomeric R-Aβ_{40} was 0.8±0.1 nm and it remained constant with time. Single component fit of the same data produced a size of 0.8±0.1 nm for the monomers (Fig. S1b, dotted line). This is close to the value expected for the monomer,[2] and established that the specimen was predominantly monomeric. MEMFCS fitting (Fig. S1d) of the FCS data (Fig. S1c) for small oligomers showed the presence of particles with size of 1.8± 0.1 nm, which would be characteristic of an octamer (assuming a compact spherical shape). Of course, oligomers are unlikely to be completely spherical or fully compact (indeed, lack of compactness has been suggested before[11]), and this would imply that the number of monomers constituting the oligomer species is substantially less than 8. A discrete component fit yielded a size of 1.9±.1 nm for the oligomers (Fig. S1d, dotted line). Size distribution of the large oligomers obtained from the FCS measurements (Fig. S1e) showed their hydrodynamic radii to be approximately 22 nm (Fig. S1f). We note that the size determination for monomers and small oligomers are essentially repeats of experiments reported by us previously.[2,12,13]

Lifetime measurements

Lifetime measurements of the solutions were performed in a 2-mm path-length cuvette. The set up was described elsewhere.[6] Briefly, the excitation wavelength of 370 nm was obtained by generating the second harmonic of the 740 nm fundamental wavelength produced by a Nd:YAG-pumped titanium: sapphire laser (Spectra Physics, model No. 375B, Mountain View, CA) pulse-picked at 20 MHz. The emission monochromator was set to 490 nm. The fluorescence was collected at magic angle (54.7°) with respect to the excitation polarization.The instrument response function (IRF) was obtained at 370 nm,

using a very dilute colloidal suspension of milk powder. The width (full width at half maximum) of the IRF was <80 ps. The decay was deconvoluted with respect to the IRF and analyzed using a sum of discrete exponentials.

Estimation of FRET efficiency for 100% and 64% labelled DA-Aβ_{40} peptides

It was difficult to achieve full DABCYL labelling of the DA-Aβ_{40} specimen, and all data (except as specifically mentioned) reported in the main manuscript is for specimens which are 64% DABCYL labeled (as estimated by absorbance measurements, see SI, 'Estimation of DABCYL labelling efficiency'). The FRET efficiency E reported in the manuscript was also obtained from the 64% DABCYL labeled DA-Aβ_{40} samples which were then corrected as described in the SI section 'Correction of FRET efficiency'. However, a 100% labeled DA-Aβ_{40} specimen was also prepared (see the mass spectrum in the Fig. S3), and the measurements with monomers and oligomers (which require small concentrations) were repeated with this specimen. This was to verify that the results obtained with the 64% DA-Aβ_{40} samples after correction agreed with 100% DA-Aβ_{40} sample. The FRET efficiency estimates were very similar between 100% and 64% DABCYL labeled DA-Aβ_{40} samples, as shown in Table S1.

For the monomers

The lifetime of D-Aβ_{40} monomers had a mean lifetime of 8.9± 0.6 ns, whereas the 64% DABCYL labelled DA-Aβ_{40} monomers had a mean lifetime of 8.0± 0.3 ns. This yields an average E of 14.5± 3.5%. Accounting for partial labelling (see SI section 'Correction of FRET efficiency'), this yields a corrected E value of 22.7±5.5%. On the other hand, 100% labelled DA-Aβ_{40} monomers yields a mean lifetime of 5.6± 0.3 ns. This implies an average E of 29.2± 3.6%, which is not significantly different.

For the Small Oligomers

The lifetime of D-Aβ_{40} small oligomer species had a mean value of 10.0± 0.7 ns. The 64% DABCYL labelled DA-Aβ_{40} for small oligomers had a mean lifetime of 5.4± 0.3 ns, yielding a

corrected E value of 71.1± 4.7%. On the other hand, the data obtained from 100% DABCYL labelled DA-Aβ_{40} yields a mean life-time of 3.5± 0.3 ns. This implies an E value of 67.4± 0.3%, which is similar to that obtained from the 64% DABCYL labelled DA-Aβ_{40} sample.

Together the monomer and the small oligomer data shows that the corrected FRET efficiency calculated from the 64% DABCYL labelled DA-Aβ_{40} samples and the FRET efficiency calculated from the 100% DABCYL labelled DA-Aβ_{40} samples match within experimental errors.

Calculation of the inter-terminal distance distribution

Inter-terminal distance distributions and self-diffusion coefficients were calculated by global analysis of the EDANS fluorescence decay curves of corresponding D-Aβ_{40} and the DA-Aβ_{40} specimens. All DA-Aβ_{40} donor fluorescence decay curves were globally fitted to the following model.[9] Equation 9 depicts the spatial and temporal survival probability of a donor electron in the electronic excited state in the presence of an acceptor which is at distances characterized by state j, which denotes one of the two states.

$$\frac{\partial \bar{N}_j^*(r,t)}{\partial t} = -\left\{\sum_{i=1}^{n}\frac{\alpha_i}{\tau_i}\left[1+\left(\frac{R_0}{r}\right)^6\right]\right\}\bar{N}_j^*(r,t) + \frac{1}{N_{0j}(r)}\frac{\partial}{\partial r}\left[N_{0j}(r)Diff_j(r)\frac{\partial \bar{N}_j^*(r,t)}{\partial r}\right] \quad \text{.....Equation 9}$$

$$\left[D^*\right]_j(t) = Norm\cdot\int_a^{r_{\max}} N_j^*(r,t)dr;\ \bar{N}_j^*(r,t) = \frac{N_j^*(r,t)}{N_{0j}(r)};\ N_{0j}(r) = N_j^*(r,t=0);\ j=1,2$$

Here r is the inter-fluorophore distance, t is the time after excitation moment, $Diff(r)$ is the inter-fluorophore self diffusion coefficient during excited state survival taken here as a constant over all inter-fluorophore distances, $N_0(r)$ represents the equilibrium distribution of distances, $N^*(r,t)$ represents the donor excited-state spatiotemporal survival probability and $\bar{N}^*(r,t)$ equals $N^*(r,t)$ normalized to the equilibrium distance distribution, R_0 is the Förster radius and τ_i is the ith lifetime component with

normalized amplitude of α_i.The integral over all available distances, $[D^*](t)$, represents the calculated donor fluorescence decay law for a given equilibrium distance distribution.

For this analysis, we used a skewed Gaussian model for the distribution of each population (Eq. 10),

$$N_0(r) = c \cdot 4\pi r^2 e^{-b(r-a)^2} \quad \ldots\ldots\ldots \textit{Equation 10}$$

Here a is a parameter representing the mean distance, b represents the reciprocal of the width and c is the normalization factor.

The analysis was performed for each population. The calculated donor fluorescence decay law for each population, $[D^*]_j(t)$ was weighted by their equilibrium fraction, x_j and summed. The convolution of the resultant fluorescence decay law with the instrument response function $IRF(t)$ is the calculated donor fluorescence decay (Eq. 11).

$$I_{DA}(t) = IRF(t) \otimes \left\{ \sum_{j=1}^{2} x_j [D^*]_j(t) \right\} \quad \ldots\ldots\ldots \textit{Equation 11}$$

For monomers only a single population was used. For small oligomers, two distance distributions were used: one for the monomer fraction and the other for the small oligomer fraction. The monomer distance distribution parameters were globally shared.

All D-Aβ_{40} donor fluorescence decay curves were also globally fitted to the convolution of the instrument response function with the weighted sum of exponentials used in Eq. 9 (see Eq. 12 below).

$$I_D(t) = IRF(t) \otimes \left\{ \sum_{i=1}^{n} \alpha_i e^{-\frac{t}{\tau_i}} \right\} \quad \ldots\ldots\ldots \textit{Equation 12}$$

The data from 64% labelled sample was also analysed for all the four species. This analysis is presented in Fig. 1f and it shows strong similarities with the analysis made from the 100% acceptor labelled samples for monomers and small oligomers (Fig. 1f, Inset). The data also shows that the large

oligomers and the fibrils have distance distributions which are very similar to those of the small oligomers. However, we note that the incomplete labelling makes the fitting less reliable.

Transmission electron microscopy

200 μM $A\beta_{40}$ (or DA-$A\beta_{40}$) solution was prepared in aqueous buffer and incubated for 7 days at room temperature. After 7 days, 10 μl of the solution was placed on a carbon-coated 100 mesh copper grid and allowed to be adsorbed for 2 minutes. The extra solution was removed with a filter paper, followed by three cycles of mild washing with double distilled water. For staining, a drop of 0.1 % uranyl acetate was added to the grid and left for 5 min. The grid was dried under an infrared lamp after removing the excess uranyl acetate solution using a filter paper. The samples were then examined with a transmission electron microscope (LIBRA 120, EFTEM, Carl Zeiss, Germany). The fibrils formed by DA-$A\beta_{40}$ (Fig. S1h) were mostly similar to that formed by unlabeled $A\beta_{40}$ (Fig. S1g).

Solvent accessibility of the termini

The solvent accessibility of fluorescein dye attached to the different termini of the Aβ peptide was assessed by collisional quenching of the fluorophore by KI in a home-built microscopic TCSPC setup. The setup was similar to the FCS set up described earlier. Briefly, we used a 490 nm pulsed diode laser (PicoQuant, Germany) for excitation and single photon avalanche photodiode (Micro Photon Devices, Italy) for detection, along with appropriate dichroic mirror and emission filters. This yielded simultaneous FCS and TCSPC measurements. The data were recorded with Picoharp 300 and analysed with Symphotime (Picoquant, Germany). We performed fluorescence lifetime measurements on 1 μM monomeric (or oligomeric) solution of $A\beta_{40}$ labelled fluorescein at the N-terminal (FL-$A\beta_{40}$) or at the C-terminal ($A\beta_{40}$-FL) in ACSF in a home-made glass bottomed Petri dish. The measurements were repeated following gradual addition of KI (from 1M stock solution in water) varying from 0 to 50 mM of KI. Fibrils were prepared by aggregating 100 μM $A\beta_{40}$ doped with 1μM FL-$A\beta_{40}$ (or $A\beta_{40}$-Fl) in

ACSF for 3 days. The fibrils were precipitated, and the precipitates were re-suspended in ACSF and mixed well in vortex shaker before measurements. The fibrils from the solution tend to settle at the bottom of Petri dish, so the measurements were performed near the surface with slow mixing of the solution away from the focus (without generation of bubbles which can scatter light). The fluorescence lifetime were analysed for 2-component exponential decay. The mean lifetime values were used to generate Stern-Volmer plots (Fig. S5), which yield the bi-molecular quenching rate constants (Fig. 2).

Membrane affinity studies

RN46A cells were cultured in Dulbecco's modified Eagle's medium (DMEM)-F12 (1:1) supplemented with 10% FBS, 50units/ml Penicillin and 50 µg/ml Streptomycin at 37ºC under humidified air containing 5% CO_2 in T-25 canted-neck flasks. For membrane binding studies the cells were cultured in home-made cover slip-bottomed Petri dish coated with poly-L-Lysine (0.1mg/ml). The initially imaged the cells for their autofluorescence (λ_{ex}= 543 nm, λ_{em}= 550-700 nm) in a confocal microscope (LSM-710, Carl Zeiss, Germany). Following 30 min of incubation with sham (TB), 250 nM monomeric or oligomeric R-Aβ_{40} solutions the dishes were washed with buffer and imaged again. We analyzed the brightness of the membrane region of the cells (at the brightest Z-position) after subtraction of the non-cell background, using ImageJ (open source software, available from the website http://rsbweb.nih.gov/ij/). Fig. S2a, S2b and S2c show three sets of cells at zero-time. Fig. S2d, S2e and S2f show the same cells after 30 min of treatment with sham, R-Aβ_{42} monomers (250 nM) and oligomers (250 nM) respectively. The membranes of the cells incubated with the oligomers brightened up considerably (~25×, Fig. S2c vs. Fig. S2f), while monomers hardly caused any change (Fig. S2b vs. Fig. S2e). We note that the membrane binding results shown here are repeats of experiments reported earlier by us.[2,3]

References

(1) M. Gude; J. Ryf and P. D. White, *Lett Pept Sci*, 2002, ***9***, 203-206.

(2) S. Nag; B. Sarkar; A. Bandyopadhyay; B. Sahoo; V. K. Sreenivasan; M. Kombrabail; C. Muralidharan and S. Maiti, *J Biol Chem*, 2011, ***286***, 13827-13833.

(3) B. Sarkar; A. K. Das and S. Maiti, *Frontiers in Physiology*, 2013, ***4***.

(4) P. Sengupta; K. Garai; B. Sahoo; Y. Shi; D. J. Callaway and S. Maiti, *Biochemistry*, 2003, ***42***, 10506-10513.

(5) P. Sengupta; J. Balaji and S. Maiti, *Methods*, 2002, ***27***, 374-387.

(6) A. Jha; J. B. Udgaonkar and G. Krishnamoorthy, *J Mol Biol*, 2009, ***393***, 735-752.

(7) P. Sengupta; K. Garai; J. Balaji; N. Periasamy and S. Maiti, *Biophys J*, 2003, ***84***, 1977-1984.

(8) C. T. Culbertson; S. C. Jacobson and J. Michael Ramsey, *Talanta*, 2002, ***56***, 365-373.

(9) J. M. Beechem and E. Haas, *Biophys J*, 1989, ***55***, 1225-1236.

(10) E. Haas; E. Katchalski-Katzir and I. Z. Steinberg, *Biochemistry*, 1978, ***17***, 5064-5070.

(11) K. Ono; M. M. Condron and D. B. Teplow, *Proc Natl Acad Sci U S A*, 2009, ***106***, 14745-14750.

(12) K. Garai; B. Sahoo; S. K. Kaushalya; R. Desai and S. Maiti, *Biochemistry*, 2007, ***46***, 10655-10663.

(13) B. Sahoo; J. Balaji; S. Nag; S. K. Kaushalya and S. Maiti, *J Chem Phys*, 2008, ***129***, 075103.

Figure legend

Figure S1. Size and morphology of Aβ monomers and aggregates: (a, c, e) Size of $A\beta_{40}$ monomers **(a)** small oligomers **(c)** and large oligomers **(e)** determined by MEMFCS fit (black) to autocorrelation curves (red circles) obtained for Fluorescence correlation spectroscopy of rhodamine labeled $A\beta_{40}$; residuals (Res): blue, 10 times magnified and autocorrelation of residuals (AC): green. **(b)**, **(d)** and **(f)** show the size distribution obtained from the MEMFCS fit (circles) and discrete component fit (dotted vertical lines) to the data in **(a)**, **(c)** and **(e)** respectively. **(g, h)** Negatively stained Transmission Electron Microscopy image of fibrils obtained by maturing 200 μM of $A\beta_{40}$ **(g)** and 200 μM of DA-$A\beta_{40}$ **(h)** in buffer for 7 days; Scale bar = 500 nm.

Figure S2. Binding of $A\beta_{40}$ monomers and oligomers to RN46A cell membranes: confocal images of RN46A cells at 0 min **(a, b, c)** and after 30 min (**d, e, f)** of incubation with buffer **(a, d)**, 250 nM of monomeric R-$A\beta_{40}$ **(b, e)**, and 250 nM of small oligomeric R-$A\beta_{40}$ **(c, f)**. Intensity is false color coded. Scale bar = 20μm. We note that the data shown here are repeats of experiments reported earlier by us.[2,3]

Figure S3. Mass spectrum of FRET-labeled $A\beta_{40}$: The multiply charged species of the 100% donor-acceptor labeled $A\beta_{40}$ (DA-$A\beta_{40}$) sample are marked in the spectrum. **Inset:** Data after deconvolution shows only 100% labeled DA-$A\beta_{40}$ (Expected mass: 4829). The donor only labeled $A\beta_{40}$ (D-$A\beta_{40}$) has a mass of 4575, which is absent.).

Figure S4. Stern-Volmer plots for the termini quenching of $A\beta_{40}$: for monomers **(a)**, oligomers **(b)** and fibrils **(c)** determined from fluorescence lifetime of fluorescein labeled at the N-terminus (black squares) and C-terminus (red circles) of $A\beta_{40}$ respectively.

405

406

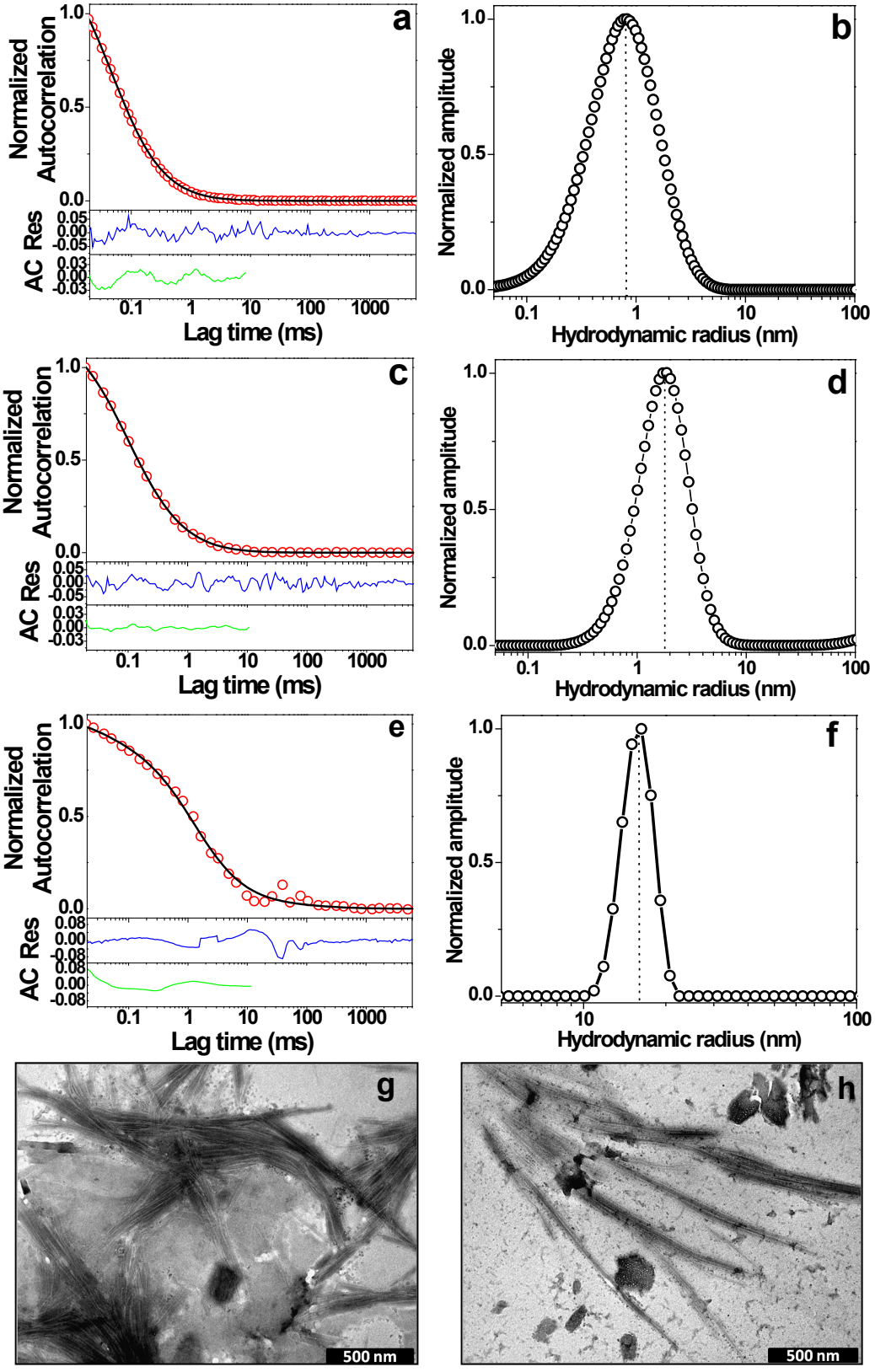

Figure S1

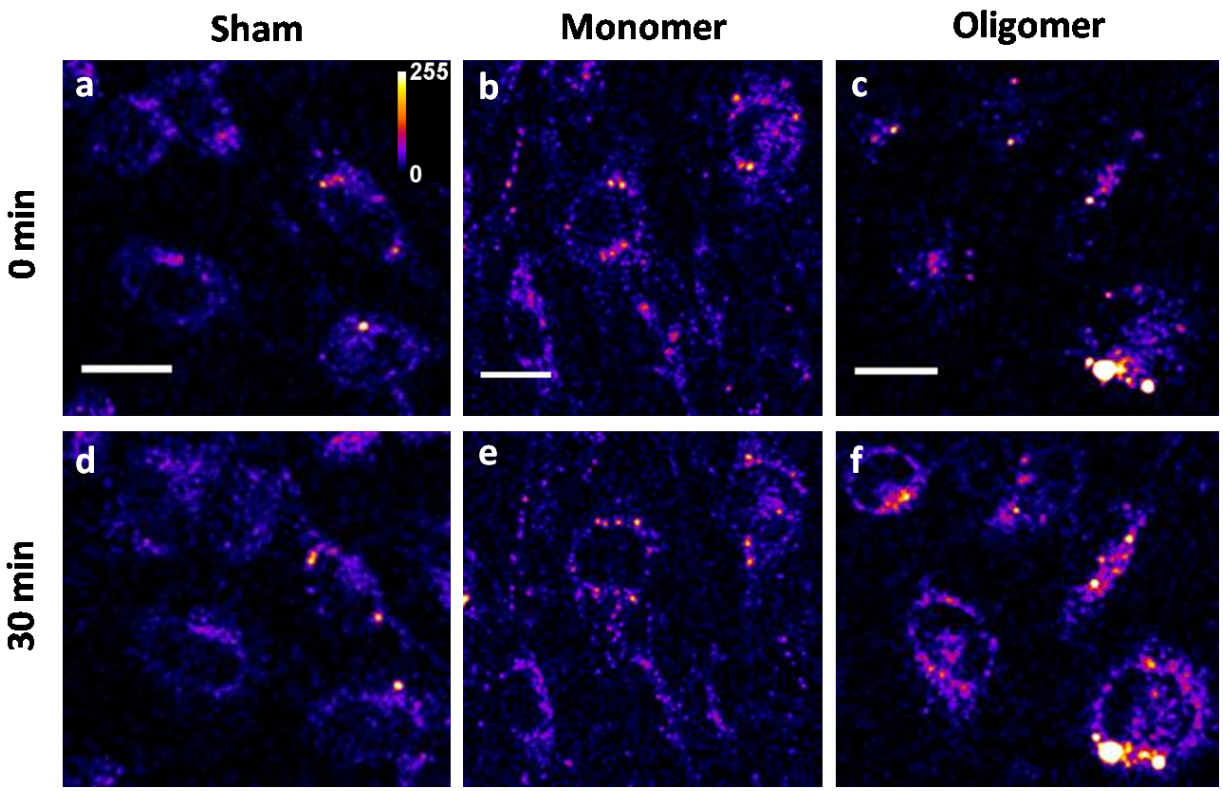

Figure S2

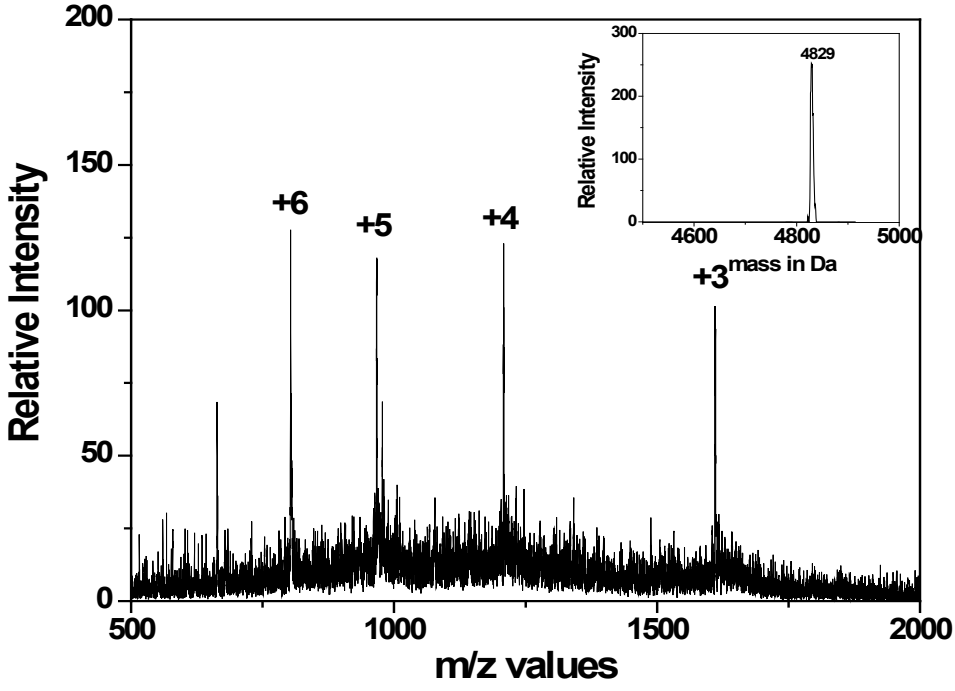

Figure S3

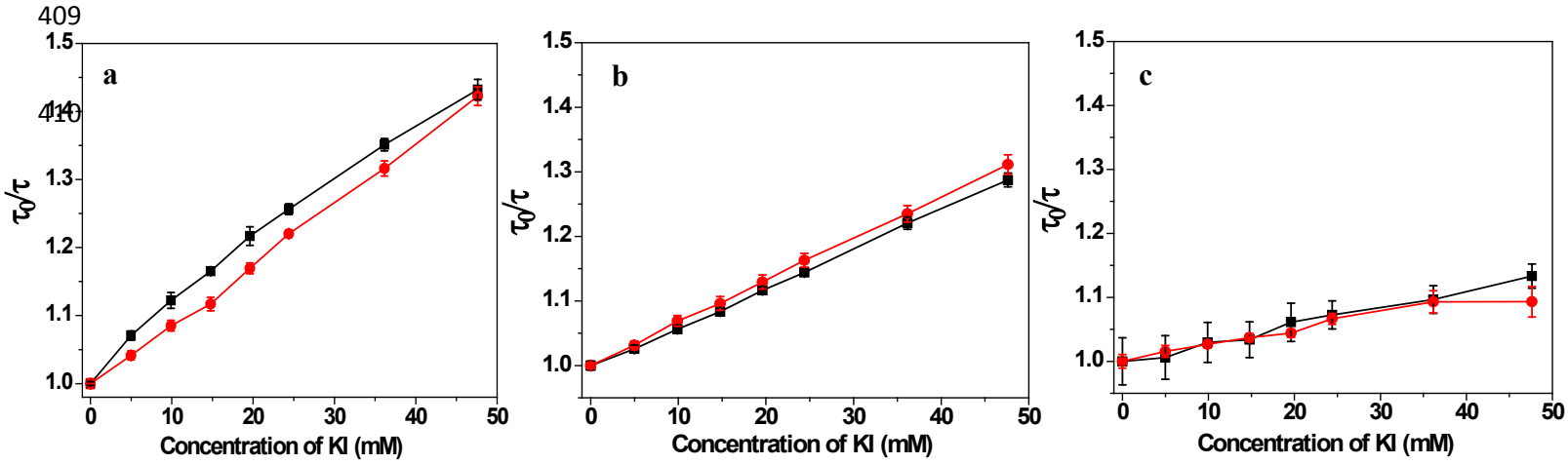
a
b
c
τ_0/τ
Concentration of KI (mM)
1.0
1.1
1.2
1.3
1.4
1.5
0
10
20
30
40
50

Figure S4

Table S1. Lifetime components for different species obtained from multi-exponential fits

Sample	Type	τ_1	a_1	τ_2	a_2	τ_3	a_3	τ_4	a_4	τ_m	E%
M	D-Aβ_{40}	0.42±0.09	0.19±0.05	3.2±0.3	0.13±0.05	12.5±0.8	0.7±0.1	-	-	8.9 ±0.6	-
M	DA-Aβ_{40}	0.41±0.12	0.22±0.03	2.8±0.3	0.11±0.01	11.3±0.1	0.67±0.02	-	-	8.0±0.3	22.7±5.5
M	DA-Aβ_{40} (100%)	0.29±0.06	0.40±0.09	2.4±1.2	0.18±0.03	12.0±0.3	0.42±0.06	-	-	5.6±0.3	29.2±3.6
SO	D-Aβ_{40}	0.62±0.12	0.17±0.04	3.9±0.4	0.23±0.03	14.7±1.0	0.60±0.04	-	-	10.0±0.7	-
SO	DA-Aβ_{40}	0.45±0.05	0.37±0.05	2.3±0.3	0.23±0.02	11.4±0.6	0.41±0.04	-	-	5.4±0.3	71.4±4.7
SO	DA-Aβ_{40} (100%)	0.25±0.03	0.43±0.06	1.5±0.3	0.26±0.03	9.15±0.03	0.33±0.03	-	-	3.5±0.3	67.4±0.3
LO	D-Aβ_{40}	10.50±0.26	0.08±0.01	3.4±0.7	0.19±0.02	15.2±0.5	0.74±0.02	-	-	12.1±0.5	-
LO	DA-Aβ_{40}	0.34±0.10	0.23±0.01	2.2±0.3	0.22±0.04	11.5±0.3	0.56±0.04	-	-	7.0±0.7	65.0±10.0
PPT	D-Aβ_{40}	1.20±0.34	0.22±0.13	11.0±1.0	0.46±0.07	22.1±1.7	0.30±0.07	-	-	12.1±1.0	-
PPT	DA-Aβ_{40}	0.30±0.27	0.20±0.07	1.5±0.3	0.35±0.07	8.0±0.7	0.25±0.07	19.0±1.3	0.25±0.07	7.2±0.5	63.0±7.8

Monomer, M; small oligomers, SO; large oligomers, LO; and precipitate, PPT for donor only (D-Aβ_{40}) and donor–acceptor labeled specimens (DA-Aβ_{40}). τ_i (i= 1, 2, 3, 4) are the different lifetime components with corresponding amplitudes a_i, and τ_m is the mean lifetime. *E*% are the corrected percentage FRET efficiencies calculated from the mean lifetime and the estimated labeling efficiency values. For M and SO, DA-Aβ_{40} (100%) shows results from 100% acceptor labeled specimens. 64% acceptor labeled DA-Aβ_{40} specimens were used for the rest of the experiments.

Table S2. Biophysical parameters for different species obtained from fitting of the FRET data to a skewed Gaussian model:

Monomer inter-terminal Distance[a] (Å)	Monomer FWHM[b] (Å)	Small Oligomers inter-terminal Distance (Å)	Small Oligomers FWHM (Å)	Monomer Inter-terminal Diffusion Coefficient ($Å^2$/ns)	Small Oligomers Inter-terminal Diffusion Coefficient ($Å^2$/ns)	Fraction of Monomers in Small Oligomers Measurement
56.1 (56.1-61.4)	63.8 (63.0-69.9)	19.9 (19.4-21.0)	23.9 (19.7-23.9)	13.7 (13.0-39.1)	1.9 (1.0-4.9)	0.27 (0.18-0.33)

[a] – The most probable distance according to the distance distribution.

[b] - Full Width at Half of Maximum of the distance distribution.