Electronic Supplementary Material (ESI) for Molecular BioSystems

Real-time probing of β-amyloid aggregation and inhibition using fluorescence self-quenching between neighbouring dyes

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SUPPLEMENTARY FIGURES

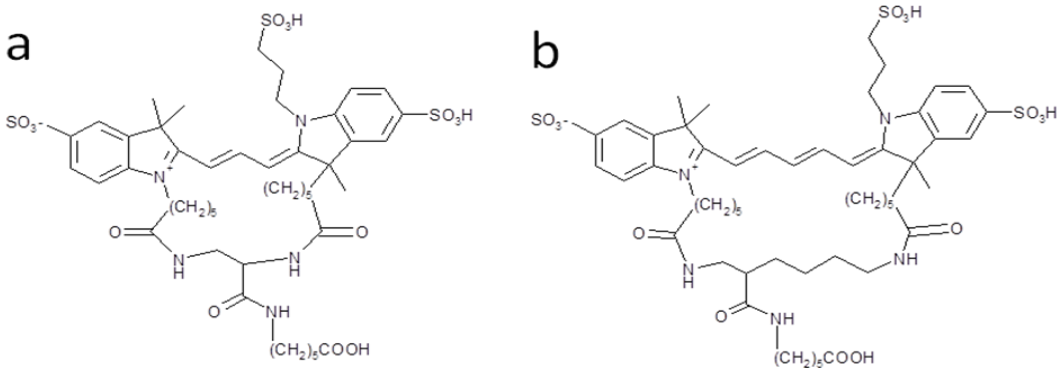

Figure S1 Chemical structures of the cyanine derivatives HiLyte Fluor 555 (a) and HiLyte Fluor 647 (b) used in this study.

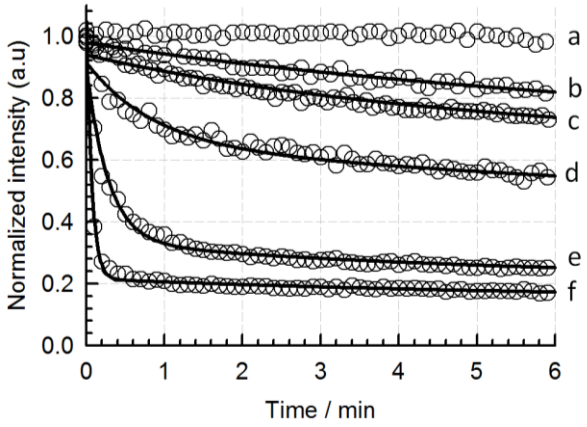

Figure S2 Normalized variation in the fluorescence intensity of $A\beta_{555}$ as a function of time with no HFIP added (a), and following the real-time injection of (b) 0.5% (v/v) HFIP, (c) 1% (v/v) HFIP, (d) 1.5 % (v/v) HFIP, (e) 2% HFIP and (f) 4 % (v/v) HFIP. [$A\beta_{555}$] = 0.1 μM, pH 7.9, T=4°C.

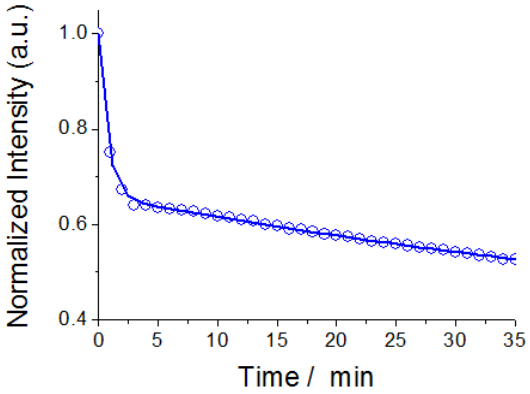

Figure S3 Aggregation time course and relative fluorescence quenching for HFIP-induced aggregates obtained using a 300 nM concentration of $A\beta_{555}$ peptides. after injection of 1.5% (v/v) HFIP (pH 7.9, 4°C).) The blue line is a non-linear squares fitting of the fluorescence trajectory to a bi-exponential decay function.

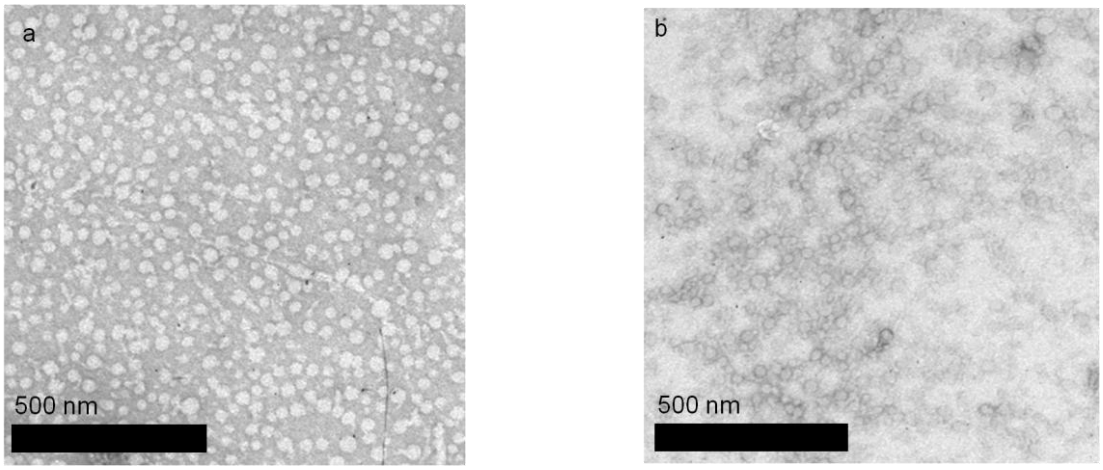

Figure S4 TEM images of HFIP-induced aggregates obtained using (a) 7 μM unlabelled $A\beta_{1-42}$ and (b) 7 μM $A\beta_{555}$. Aggregation conditions: 1.5 % (v/v) HFIP, pH 7.9, 4°C.

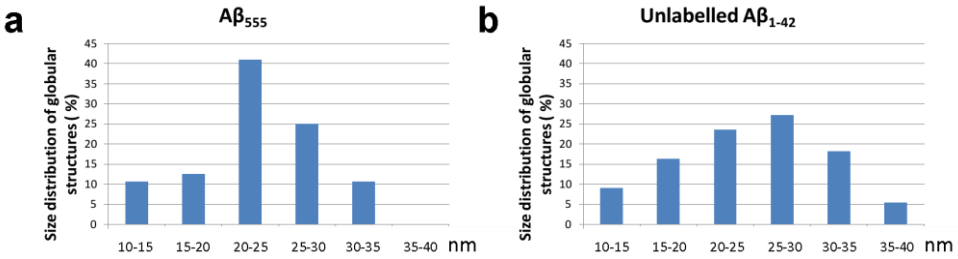

Figure S5 Quantitative comparison of diameter distribution of HFIP-induced globular aggregates composed of (a) 7 μM $A\beta_{555}$ and (b) 7 μM $A\beta_{1-42}$ obtained from negative stain TEM images.

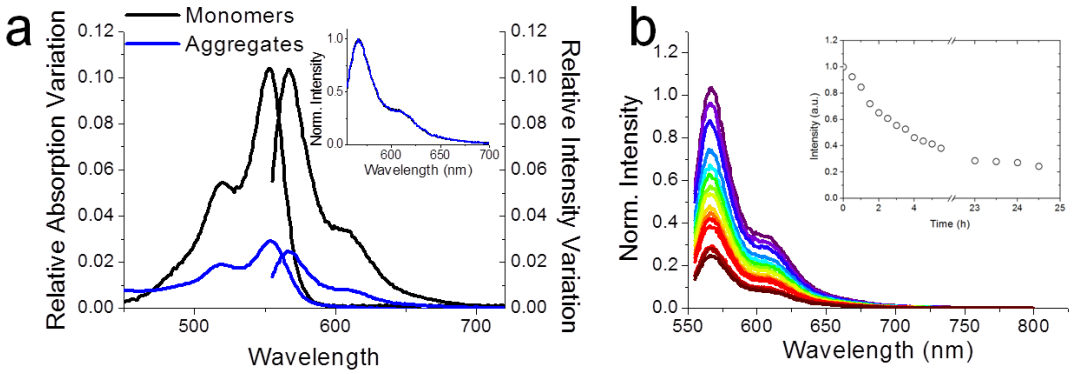

Figure S6 Representative aggregation time course and relative fluorescence quenching during the aggregation of $A\beta_{1-42}$ under ADDL-forming conditions. (a) Relative variation in the absorption and fluorescence spectra (λ_{exc} = 547 nm) between 0.5 μM $A\beta_{555}$ monomers (black lines) and $A\beta_{555}$ aggregates (blue lines) grown at 4°C, pH 7.3, phenol red free F-12 HAM's media. *Inset:* Normalized fluorescence spectra obtained at the initial (black) and final (blue) stages of aggregation. (b) Normalized variation in steady-state fluorescence emission spectra (λ_{exc} = 547 nm) as a function of time obtained during the aggregation process. Inset: normalized variation in the steady-state fluorescence emission intensity as a function of time under the same conditions.

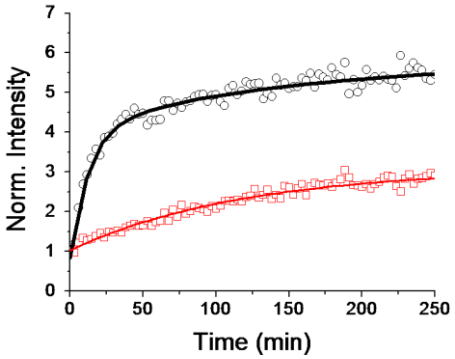

Figure S7 Disaggregation time courses of freshly prepared HFIP (1.5% v/v) aggregates (black circles) and aggregates allowed to mature for 18 hours after preparation (red squares). Each sample was composed of 7 μM $A\beta_{555}$ and disaggregation was induced by 10X dilution of the sample in buffer containing 50mM Tris-HCl (pH 7.9, 4°C).

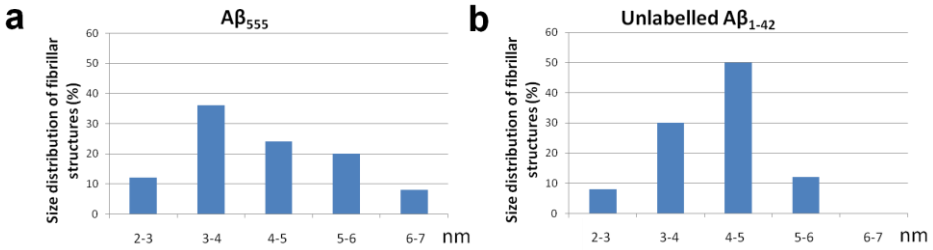

Figure S8 Quantitative comparison of the width distribution of (a) 7 μM $A\beta_{555}$ and (b) 7 μM $A\beta_{1-42}$ fibrillar aggregates obtained from negative stain TEM images.

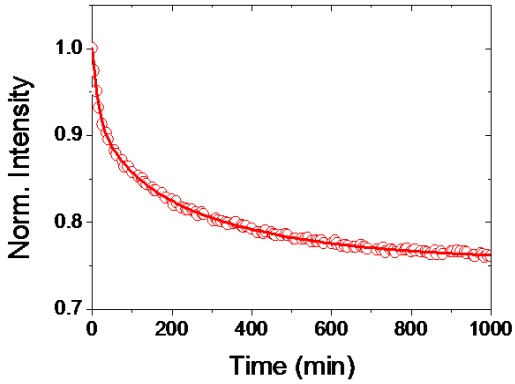

Figure S9 Normalized variation in the steady-state fluorescence intensity of a 1 μM freshly prepared non-aggregated sample of $A\beta_{555}$ as a function of time at 37°C in the presence of 150 mM NaCl. The red line is a non-linear squares fitting of the fluorescence trajectory to a bi-exponential decay function.

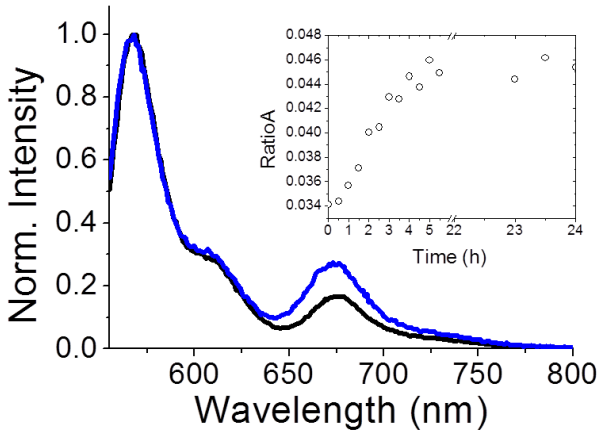

Figure S10 Normalized fluorescence spectra at the donor emission maximum corresponding to the initial (black line) and final stages (> 24 hours) of the aggregation process (blue line) of 0.5 μM $A\beta_{1-42}$ (molar ratios of $A\beta_{1-42}$: $A\beta_{555:}$ $A\beta_{647}$ as described in the main text) induced by phenol red free F-12 HAM's cell culture media (pH 7.3, 4°C), showing the relative increase in acceptor fluorescence. Inset: real-time variation in RatioA during the aggregation process.

Table S1 Comparison of the kinetic parameters corresponding to the aggregation of $A\beta_{1-42}$ induced by HFIP monitored using $A\beta_{555}$ or ThT fluorescence enhancement as shown in Figure 1a. Kinetic parameters were obtained from the individual and global non-linear squares fitting of the variation in fluorescence intensity to a biexponential function ($I(t)=I_0+a_1*\exp(-k_1*t)+a_2*\exp(-k_2*t)$). For the global fit, rate constants k_1 and k_2 were declared as global variables and optimized as similar values for both assays. Experimental conditions as described in the main text.

	Individual Fit		**Global Fit**	
	$A\beta_{555}$ (0.994)*	**ThT (0.996) ***	**$A\beta_{555}$ (0.99) ***	**ThT (0.99) ***
I_0	0.365 ± 0.002	2.81 ± 0.01	0.367 ± 0.002	2.861 ± 0.001
a_1	0.282 ± 0.001	-0.50 ± 0.01	0.297 ± 0.001	-0.393 ± 0.003
k_1 / min^{-1}	0.074 ± 0.004	0.152±0.007	0.079 ± 0.001	0.079 ± 0.001
a_2	0.268 ±0.002	-1.45±0.03	0.278 ± 0.003	-1.548 ± 0.009
k_2 / min^{-1}	1.367 ± 0.002	2.01±0.08	1.56 ± 0.02	1.56 ± 0.02

*Numbers in brackets represent the values obtained for the goodness of the fit expressed as reduced Chi-square (χ_r^2) calculated following the equation $\chi_r^2 = \frac{1}{N-p}\left(\sum_{i=1}^{N}\frac{(d_i - f_i)^2}{d_i}\right)$ where N represents the number of data points, p the number of fitting parameters, d_i the experimental data and f_i the fitting result

Table S2 Pre-exponential factors and fluorescence lifetimes obtained for freshly prepared non-aggregated $A\beta_{555}$ and at the final stage of the aggregation process induced by injection of 1.5% (v/v) HFIP in samples containing 100% $A\beta_{555}$ or a 1:1 ratio of $A\beta_{555}$ and unlabeled peptide. Aggregation conditions: total peptide concentration was 7 μM, pH 7.9, 4°C.

	Monomeric	**50% $A\beta_{555}$**	**100% $A\beta_{555}$**
$\mathbf{a_o}$	0.347 ± 0 .005	0.250 ± 0 .004	0.204 ± 0.003
$\mathbf{a_1}$	0.629±0.004	0.707±0.004	0.451 ± 0.002
τ_1 **/ ns**	**1.24±0.04**	**0.71±0.01**	0.821 ± 0.003
$\mathbf{a_2}$	--	--	0.341 ± 0.004
τ_2 **/ ns**	--	--	0.104 ± 0.002
τ_{av}^{*} **/ ns**	N.A	N.A	**0.512 ± 0.002**
χ_r^2	0.991	0.992	0.998

* Intensity average lifetimes were calculated according to the equation: $\tau_{av} = \sum_{i=1}^{N} a_i \tau_i^2 / \sum_{i=1}^{N} a_i \tau_i$ where a_i represents the pre-exponential factor and τ_i the associated fluorescence lifetime.

Table S3 Comparison of the kinetic parameters corresponding to the aggregation of $A\beta_{1\text{-}42}$ at pH 7.9 (37°C and 150 mM NaCl) monitored using $A\beta_{555}$ or ThT fluorescence enhancement as shown in Figure 3b. Kinetic parameters were obtained from the individual and global non-linear squares fitting of the variation in fluorescence intensity to a biexponential function ($I(t)=I_0+a_1*\exp(-k_1*t)+a_2*\exp(-k_2*t)$). For the global fit, rate constants k_1 and k_2 were declared as global variables and optimized as similar values for both assays. Experimental conditions were as described in the main text.

	Individual Fit		**Global Fit**	
	$A\beta_{555}$ (0.999)*	**ThT (0.95)***	**$A\beta_{555}$ (0.98)***	**ThT (0.98)***
$\mathbf{I_0}$	0.701 ± 0.003	1.46 ± 0.03	0.66 1± 0.007	1.41 ± 0.07
$\mathbf{a_1}$	0.0652 ± 0.005	-0.18 ± 0.02	0.146 ± 0.005	-0.17 ± 0.01
$\mathbf{k_1}$ **/ min^{-1}**	0.041 ± 0.04	0.014 ± 0.003	0.011 ± 0.004	0.011 ± 0.004
$\mathbf{a_2}$	0.219 ±0.002	-0.24 ± 0.02	0.159 ± 0.007	-0.211 ± 0.004
$\mathbf{k_2}$ **/ min^{-1}**	0.0037 ± 0.0007	0.0011 ± 0.0004	0.0014 ± 0.0001	0.0014 ± 0.0001

* Numbers in brackets represent the values obtained for the goodness of the fit expressed as reduced Chi-square (χ_r^2) as described in Table 1

Table S4 Pre-exponential factors and fluorescence lifetimes obtained for freshly prepared non-aggregated $A\beta_{555}$ and at the final stage of the aggregation process induced by incubation at pH 7.9 (37°C and 150 mM NaCl). Decays were fitted to monoexponential decay functions of the form :$a_0+a_1*\exp(-t/\tau)$.

	Monomeric	**Aggregated state**
$\mathbf{a_o}$	0.347 ± 0 .005	0.421± 0.006
$\mathbf{a_1}$	0.629±0.004	0.527 ± 0.004
$\mathbf{\tau_1}$ **/ ns**	**1.24±0.04**	**0.90 ± 0.02**
χ_r^2	0.991	0.985

Table S5 Pre-exponential factors and fluorescence lifetimes obtained for $A\beta_{555}$ aggregated at pH 6 and pH 4 (37°C and 150 mM NaCl).

	pH 6	**pH 4**
$\mathbf{a_o}$	0.056 ± 0 .002	0.031 ± 0.004
$\mathbf{a_1}$	0.531±0.006	0.491 ± 0.003
$\mathbf{\tau_1}$ **/ ns**	**0.060 ± 0.002**	**0.076 ± 0.001**
$\mathbf{a_2}$	0.447 ± 0.005	0.497 ± 0.005
$\mathbf{\tau_2}$ **/ ns**	**0.52 ± 0.01**	**0.417 ± 0.005**
τ_{av}^* **/ ns**	**0.47 ± 0.02**	**0.36 ± 0.01**
χ_r^2	0.992	0.998

* Intensity average lifetimes were calculated according to the equation: $\tau_{av} = \sum_{i=1}^{N} a_i\tau_i^2 / \sum_{i=1}^{N} a_i\tau_i$ where a_i represents the pre-exponential factor and τ_i the associated fluorescence lifetime.

Table S6 Kinetic parameters for the aggregation of $A\beta_{1\text{-}42}$ induced by HFIP and by incubation at pH 7.9 (37°C and 150 mM NaCl) as shown in Figure 7c and Figure 7d, respectively. Aggregation was monitored by following the time-dependent variation in FRET efficiency, quantified as RatioA, between HiLyte555 (donor) and HiLyte 647 (acceptor) N-terminally labelled $A\beta_{1\text{-}42}$ peptides. Kinetic parameters were obtained from the non-linear squares fitting of the variation in RatioA to a biexponential function ($I(t)=I_0+a_1*\exp(-k_1*t)+a_2*\exp(-k_2*t)$). Experimental conditions as described in main text.

Conditions	**1.5 % HFIP (0.95)***	**pH 7.9, 37°C, 150 mM NaCl (0.97)***
$\mathbf{I_0}$	0.091 ± 0.006	0.037 ± 0.006
$\mathbf{a_1}$	-0.022 ± 0.005	- 0.0016 ± 0.0007
$\mathbf{k_1}$ **/ min^{-1}**	0.037 ± 0.008	0.027 ± 0.004
$\mathbf{a_2}$	-0.041 ±0.005	-0.0014 ±0.0004
$\mathbf{k_2}$ **/ min^{-1}**	0.83 ± 0.05	0.0032 ± 0.0002

* Numbers in brackets represent the values obtained for the goodness of the fit expressed as reduced Chi-square (χ_r^2) as described in Table S1

Table S7 Pre-exponential factors, fluorescence lifetime components and intensity average lifetimes obtained for the FRET donor $A\beta_{555}$ in a mixture containing a 0.6:0.2:0.2 molar ratio of unlabelled $A\beta_{1\text{-}42}$, $A\beta_{555}$ and $A\beta_{647}$ acting as FRET acceptor at the final stage of the aggregation process induced by HFIP (left column) and by incubation at pH 7.9 (right column) with 150 mM NaCl at 37°C.

	HFIP	**pH 7.9**
$\mathbf{a_o}$	0.090 ± 0 .003	0.24 ± 0.03
$\mathbf{a_1}$	0.36±0.01	0.662 ± 0.004
$\boldsymbol{\tau_1}$ **/ ns**	**0.50 ± 0.01**	**0.48 ± 0.01**
$\mathbf{a_2}$	0.59 ± 0.02	
$\boldsymbol{\tau_2}$ **/ ns**	**0.07 ± 0.02**	**--**
$\boldsymbol{\tau_{av}}^{*}$ **/ ns**	**0.36 ± 0.02**	**--**
$\boldsymbol{\chi_r^2}$	0.990	0.987

* Intensity average lifetimes were calculated according to the equation: $\tau_{av} = \sum_{i=1}^{N} a_i \tau_i^2 / \sum_{i=1}^{N} a_i \tau_i$ where a_i represents the pre-exponential factor and τ_i the associated fluorescence lifetime.