Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting information

Three-step Förster resonance energy transfer on amyloid fibril scaffold

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Supporting figures

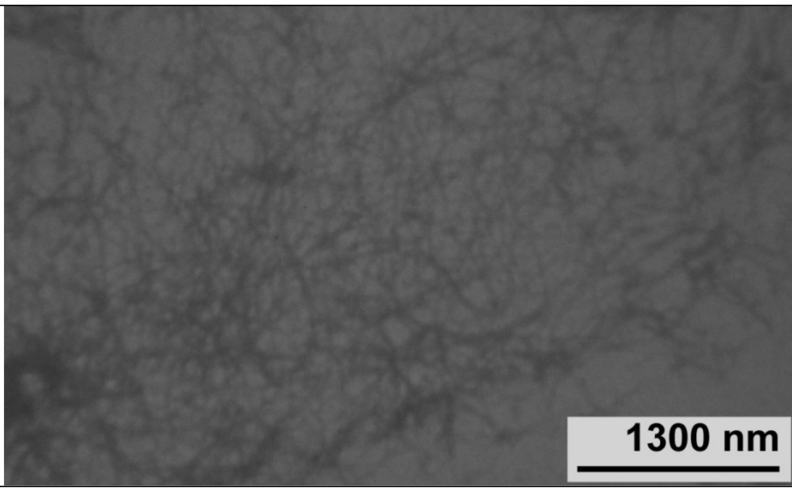


Figure S1. Transmission electron microscopy image of the insulin amyloid fibrils.

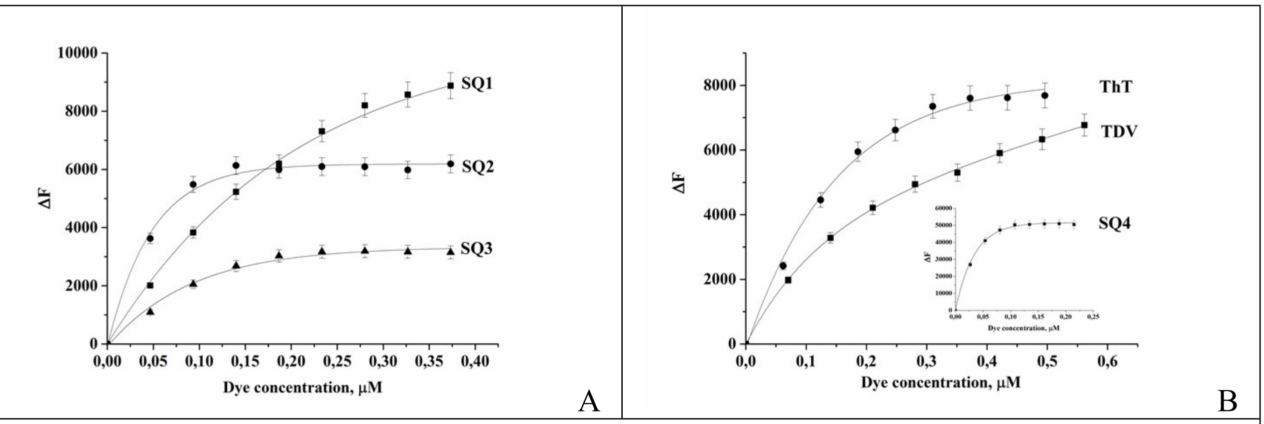


Figure S2. The isotherms of the dye binding to insulin fibrils obtained from the fluorimetric titration of the protein by the dye. The changes in fluorescence intensity (ΔF) were measured at the emission wavelengths 484 nm (ThT), 610 nm (TDV), 686 nm (SQ1), 692 nm (SQ2), 692 nm (SQ3), 648 nm (SQ4). The protein concentration was 2.4 μM.

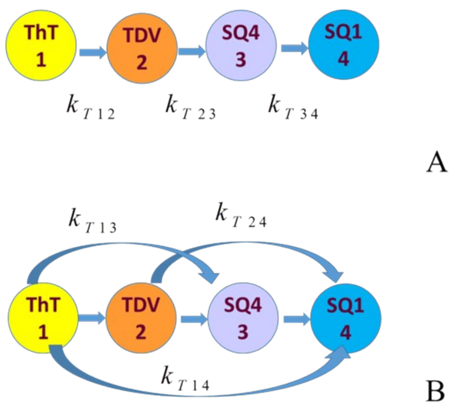


Figure S3. A four-chromophore system for energy transfer: (A) three-step FRET (energy is transferred from chromophore 1 to 4 via 2 and 3), and (B) all possible pathways of the energy transfer.

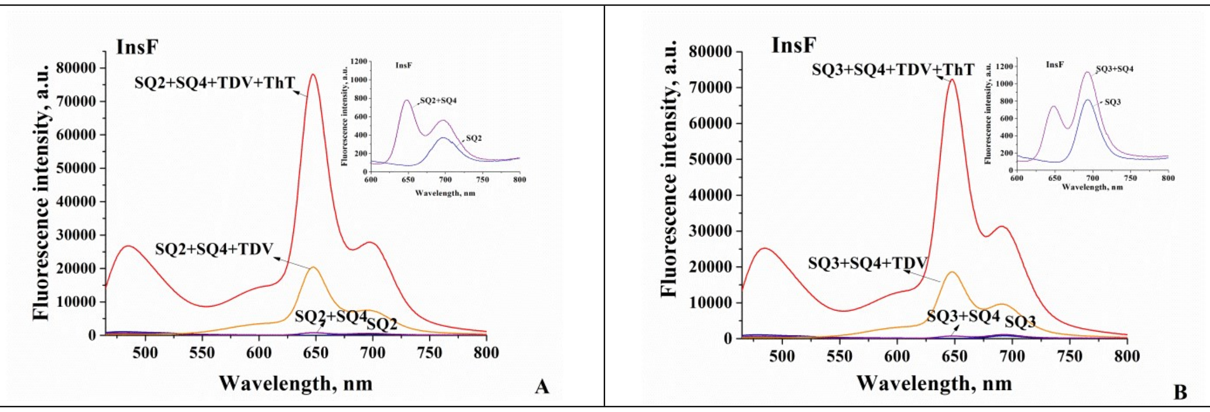


Figure S4. The fluorescence emission spectra recorded upon sequential addition of SQ4, TDV and ThT to the mixtures of insulin fibrils with SQ1 (A), SQ2 (B) and SQ3 (C). The concentrations of the dyes were 0.46 μM (SQ1), 0.46 μM (SQ2), 0.46 μM (SQ3), 0.26 μM (SQ4), 1 μM (TDV), 3.7 μM (ThT). The protein concentration was 14.5 μM.

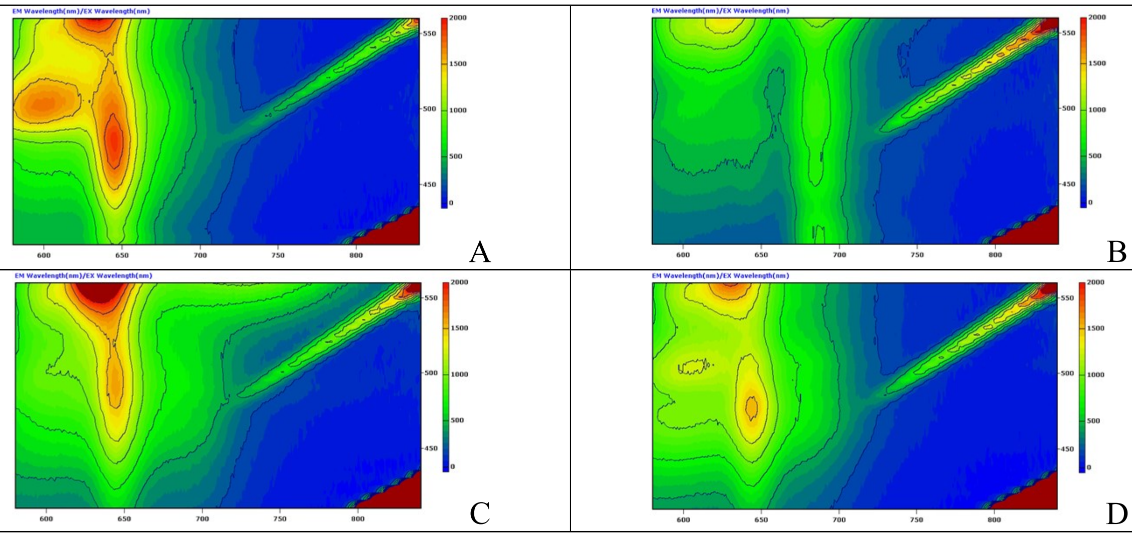


Figure S5. 3D fluorescence contour maps recorded in the presence of non-fibrillized insulin for the chromophore systems ThT-TDV-SQ4 (A) and ThT-TDV-SQ4-SQ1/2/3 (B)-(D). The dye concentrations were 3.7 μM (ThT), 0.7 μM (TDV), 0.13 μM (SQ4) and 1 μM (SQ1/2/3). The protein concentration was 14.7 μM.

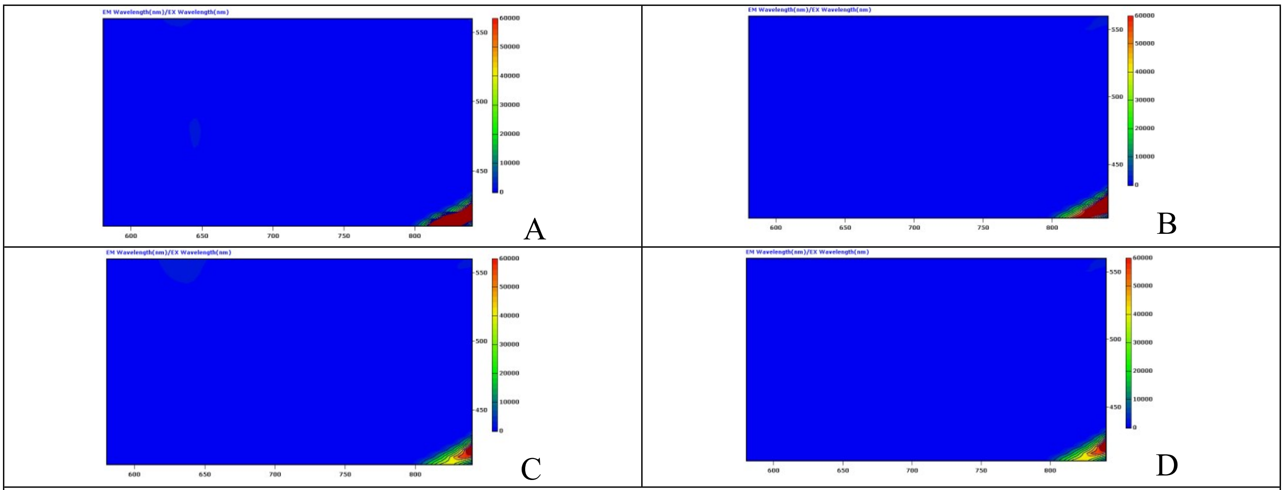
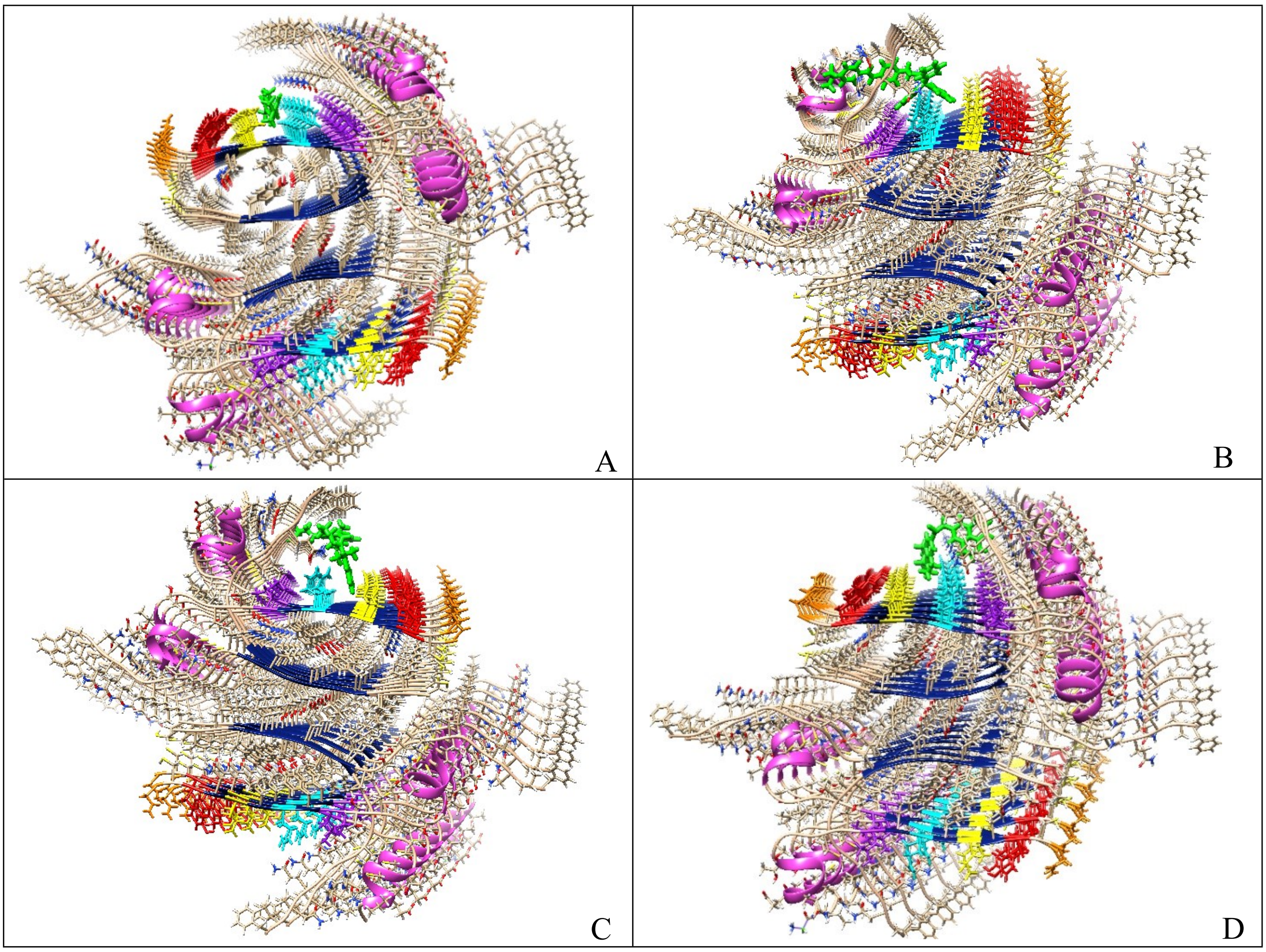
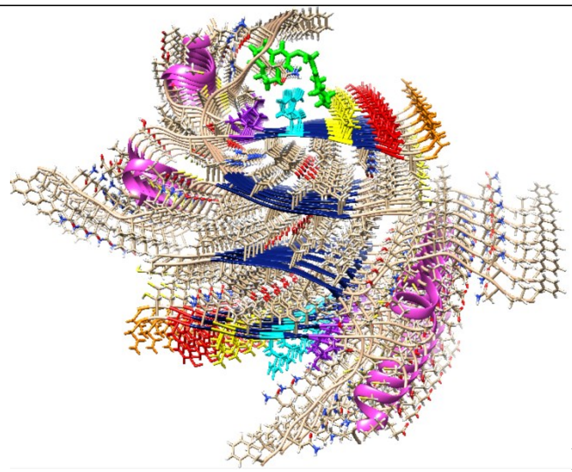


Figure S6. 3D fluorescence contour maps of non-fibrillized insulin plotted in the same scale as those obtained for fibrillar protein (Figure 3) with the chromophore systems ThT-TDV-SQ4 (A) and ThT-TDV-SQ4-SQ1/2/3 (B)-(D).





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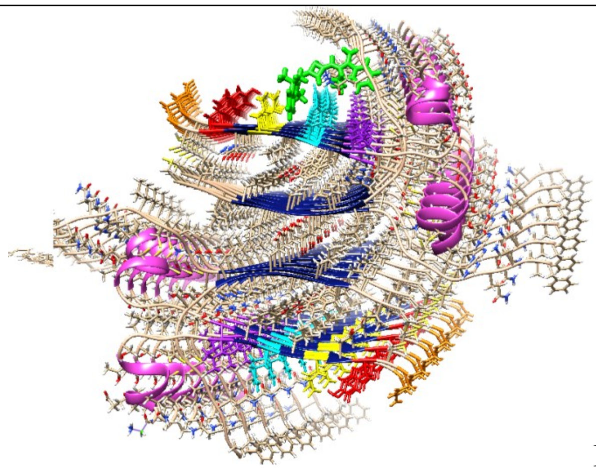

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Figure S7. The energetically most favorable complexes of ThT (A), TDV (B), SQ1 (C), SQ2 (D), SQ3 (E) and SQ4 (F) with the the insulin fibrils obtained with the SwissDock tool. The residues Leu13, Gln15, Glu17 and Tyr19 are colored in purple, cyan, yellow and red, respectively. The binding energies of the docked complexes were estimated to be -8.46 kcal/mol (ThT), -8.49 kcal/mol (TDV), -8.0 kcal/mol (SQ1), -8.12 kcal/mol (SQ2), -8.06 kcal/mol (SQ3), -9.26 kcal/mol (SQ4).

Supporting tables

Table S1. The Förster radii and overlap integrals for the donor-acceptor pairs involved in the cascade FRET

Donor-acceptor pair	Förster radius, nm	Overlap integral, $M^{-1}cm^{-1}nm^4$
ThT - TDV	2.41	8.14×10^{14}
TDV – SQ4	5.58	1.75×10^{16}
SQ4 – SQ1	4.96	1.65×10^{16}
SQ4 – SQ2	4.88	1.50×10^{16}
SQ4 – SQ3	5.18	2.14×10^{16}
ThT – SQ1	2.81	2.05×10^{15}
ThT – SQ2	2.71	1.67×10^{15}
ThT – SQ3	2.96	2.80×10^{15}
ThT – SQ4	2.95	2.78×10^{15}
TDV – SQ1	4.75	6.68×10^{15}
TDV – SQ2	4.61	5.55×10^{15}
TDV – SQ3	4.98	8.79×10^{15}

Table S2. Quantitative parameters of the dye binding to fibrillar and non-fibrillized insulin

Dye	Fibrillar insulin			Non-fibrillized insulin		
	F_{mol} , μM^{-1}	K_a , μM^{-1}	n	F_{mol} , μM^{-1}	K_a , μM^{-1}	n
SQ1	$9.9^{\pm1.9}\cdot10^4$	$23.6^{\pm5.1}$	$0.04^{\pm0.006}$	$5.3^{\pm1.1}\cdot10^3$	$25.1^{\pm4.9}$	$0.08^{\pm0.013}$
SQ2	$1.1^{\pm0.2}\cdot10^5$	$69.9^{\pm11}$	$0.03^{\pm0.005}$	$4.3^{\pm0.7}\cdot10^3$	$63.1^{\pm12}$	$0.01^{\pm0.002}$
SQ3	$2.2^{\pm0.3}\cdot10^4$	$71.4^{\pm13}$	$0.08^{\pm0.015}$	$1.1^{\pm0.2}\cdot10^3$	$63.2^{\pm11}$	$0.04^{\pm0.005}$
SQ4	$1.9^{\pm0.3}\cdot10^6$	$62.2^{\pm11}$	$0.01^{\pm0.002}$	$2.0^{\pm0.3}\cdot10^4$	$12.0^{\pm2.1}$	$0.05^{\pm0.009}$
TDV	$3.6^{\pm0.7}\cdot10^4$	$28.6^{\pm5.4}$	$0.07^{\pm0.013}$	$8.9^{\pm1.9}\cdot10^3$	$0.55^{\pm0.1}$	$0.25^{\pm0.04}$
ThT	$1.8^{\pm0.3}\cdot10^5$	$36.6^{\pm7.1}$	$0.02^{\pm0.005}$	*	*	*

* - not determined

Table S3. The limits for donor-acceptor separation derived from Eq. (5) for given values of orientation factor

Donor-acceptor pair	R, nm*			
	$\kappa^2 = 0.01$	$\kappa^2 = 0.67$	$\kappa^2 = 1$	$\kappa^2 = 4$
ThT – TDV	1.22	2.47	2.64	3.32
TDV – SQ4	2.57	5.17	5.53	6.96
SQ4 – SQ1	2.59	5.22	5.58	7.03
SQ4 – SQ2	2.98	6.0	6.41	8.08
SQ4 – SQ3	3.02	6.08	6.49	8.18

The uncertainty in R value does not exceed 0.3 nm.

Table S4. The estimates of the Förster radius and orientation factor obtained from the approximation of experimental data by Eq. (7)

Donor-acceptor pair	Förster radius, nm	Orientation factor
ThT – TDV	2.27	0.47
TDV – SQ4	5.96	0.99
SQ4 – SQ1	2.30	6.7×10^{-3}
SQ4 – SQ2	3.13	4.7×10^{-2}
SQ4 – SQ3	1.56	5.0×10^{-4}

Theoretical description of FRET in four-chromophore system for the case when the energy is transferred sequentially from the initial donor to the terminal acceptor

$$\frac{d\left[C_1^*\right]}{dt} = -\left[C_1^*\right](k_{T12} + \tau_1^{-1}) \tag{S1}$$

$$\frac{d\left[C_2^*\right]}{dt} = -\left[C_2^*\right](k_{T23} + \tau_2^{-1}) + \left[C_1^*\right]k_{T12} \tag{S2}$$

$$\frac{d\left[C_3^*\right]}{dt} = -\left[C_3^*\right](k_{T34} + \tau_3^{-1}) + \left[C_2^*\right]k_{T23} \tag{S3}$$

$$\frac{d\left[C_4^*\right]}{dt} = -\left[C_4^*\right]\tau_4^{-1} + \left[C_3^*\right]k_{T34} \tag{S4}$$

where $\left[C_i^*\right]$ is the concentration of the *i-th* excited chromophore, τ_i is the fluorescence lifetime, k_{Tij} are the rate constant of energy transfer between the *i-th* and *j-th* chromophores.

Theoretical description of FRET in four-chromophore system for the case when the two- and three-step FRET take place in parallel with the one-step energy transfer events

$$\frac{d\left[C_1^*\right]}{dt} = -\left[C_1^*\right](k_{T12} + \tau_1^{-1}) \tag{S5}$$

$$\frac{d\left[C_2^*\right]}{dt} = -\left[C_2^*\right](k_{T23} + k_{T24} + \tau_2^{-1}) + \left[C_1^*\right]k_{T12} \tag{S6}$$

$$\frac{d\left[C_3^*\right]}{dt} = -\left[C_3^*\right](k_{T34} + \tau_3^{-1}) + \left[C_1^*\right]k_{T13} + \left[C_2^*\right]k_{T23} \tag{S7}$$

$$\frac{d\left[C_4^*\right]}{dt} = -\left[C_4^*\right]\tau_4^{-1} + \left[C_1^*\right]k_{T14} + \left[C_2^*\right]k_{T24} + \left[C_3^*\right]k_{T34} \tag{S8}$$