Electronic supplementary information

Functionalization of insulin nanofibrils with fluorophores involved in cascade Förster resonance energy transfer

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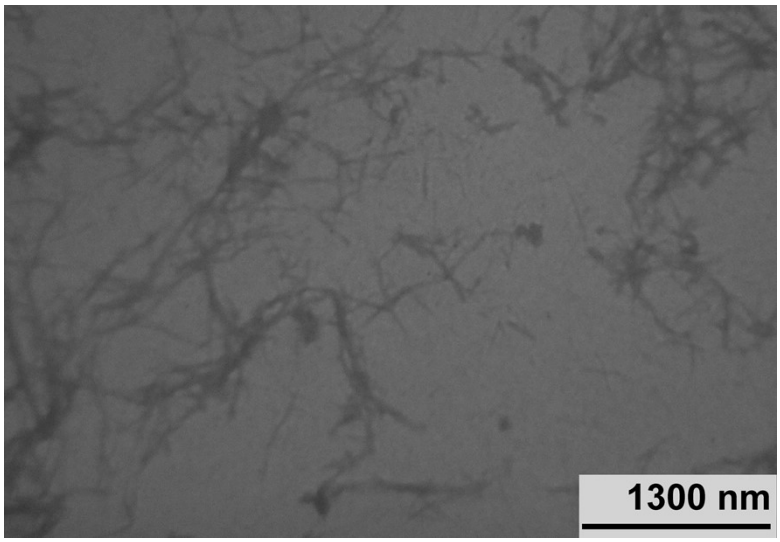


Fig. S1. Transmission electron microscopy image of the insulin amyloid fibrils.

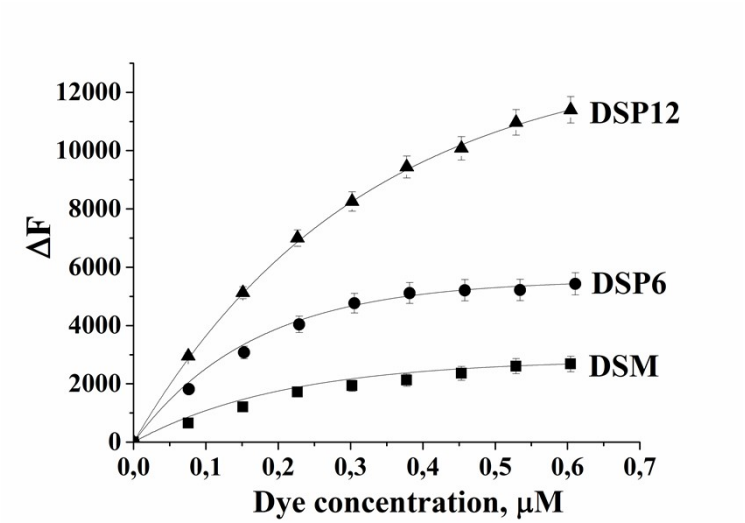


Fig. S2. The isotherms of the dye binding to the insulin fibrils obtained from the fluorimetric titration of the protein by the styryl pyridinium dyes. The changes in fluorescence intensity (ΔF) were measured at the emission wavelength 600 nm. The protein concentration was 2.4 μM.

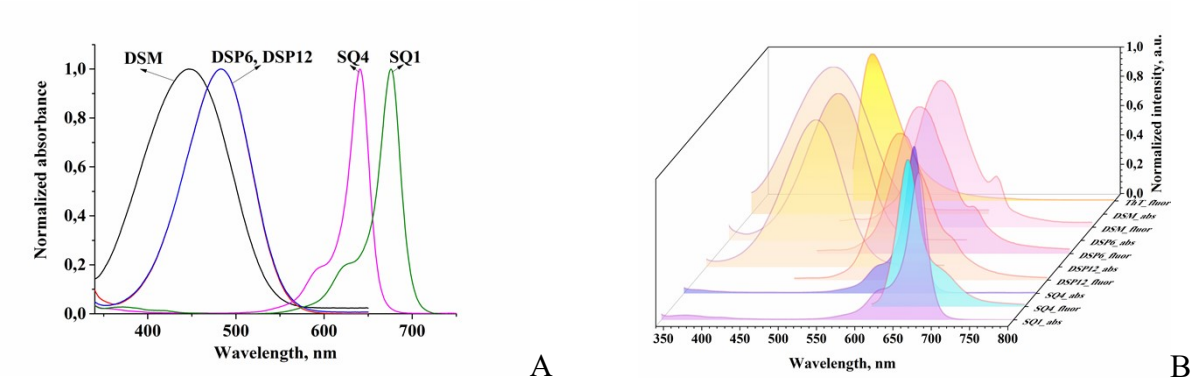


Fig. S3. (A) The normalized absorption spectra of the dyes acting as acceptors in the three-step FRET chain. The dyes were dissolved in ethanol (DSP6, DSP12), dimethyl sulfoxide (SQ1, SQ4) and 10 mM Tris-HCl buffer, pH 7.4 (DSM). (B) The overlap between the donor emission and acceptor absorption spectra illustrated for the donor-acceptor pairs ThT-SPD, SPD-SQ4 and SQ4-SQ1.

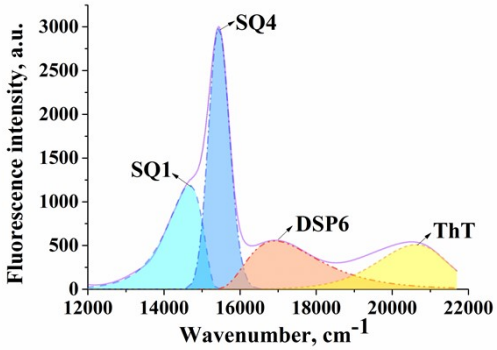
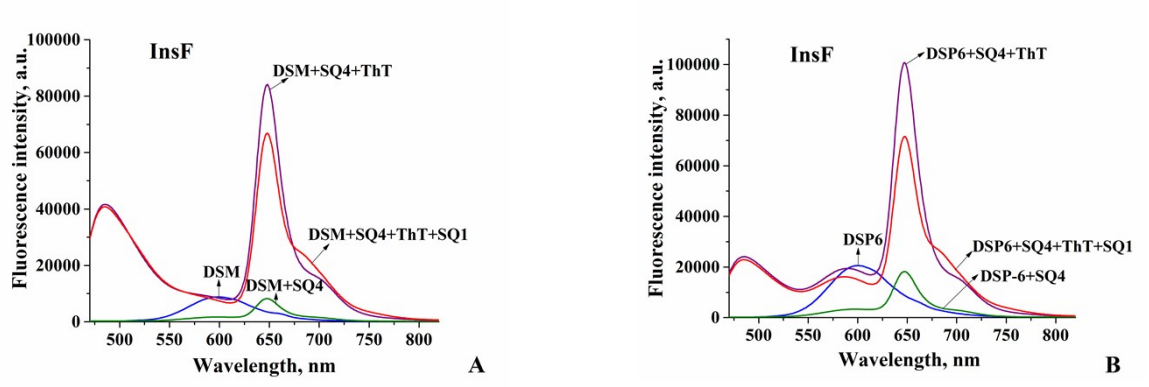


Fig. S4. Deconvolution of the fluorescence emission spectra in the four-chromophore system ThT-DSP6-SQ4-SQ1.



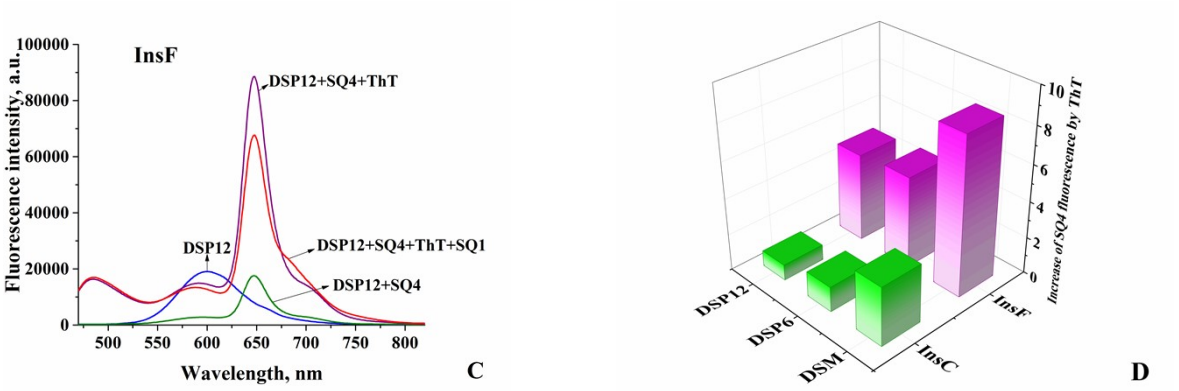


Fig. S5. The fluorescence emission spectra recorded upon sequential addition of SQ4, ThT and SQ1 to the mixtures of insulin fibrils with DSM (A), DSP6 (B) and DSP12 (C). The increase of SQ4 fluorescence upon the addition of ThT to the mixture InsF + SPD + SQ4 (D).The concentrations of the dyes were 11.2 μM (ThT), 0.45 μM (DSM, DSP6, DSP12), 0.3 μM (SQ4), 2.6 μM (SQ1). The protein concentration was 11.9 μM.

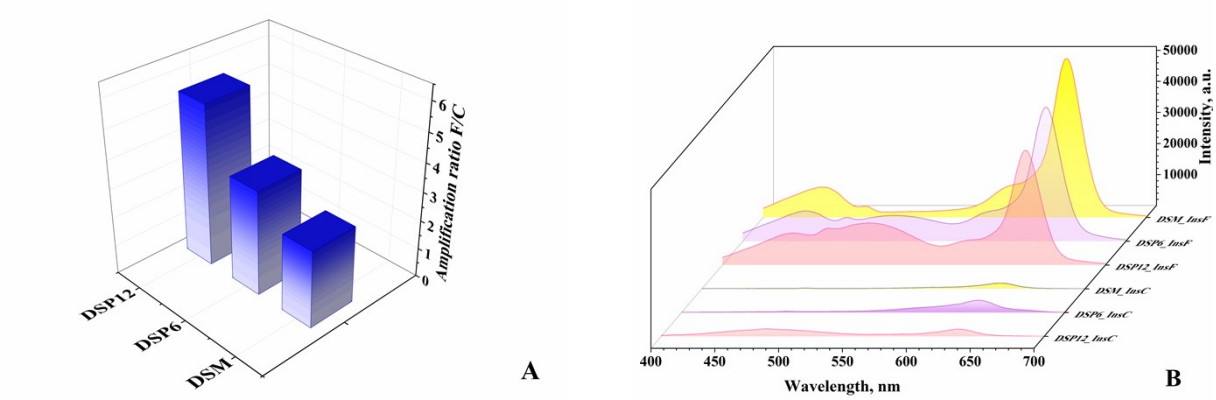
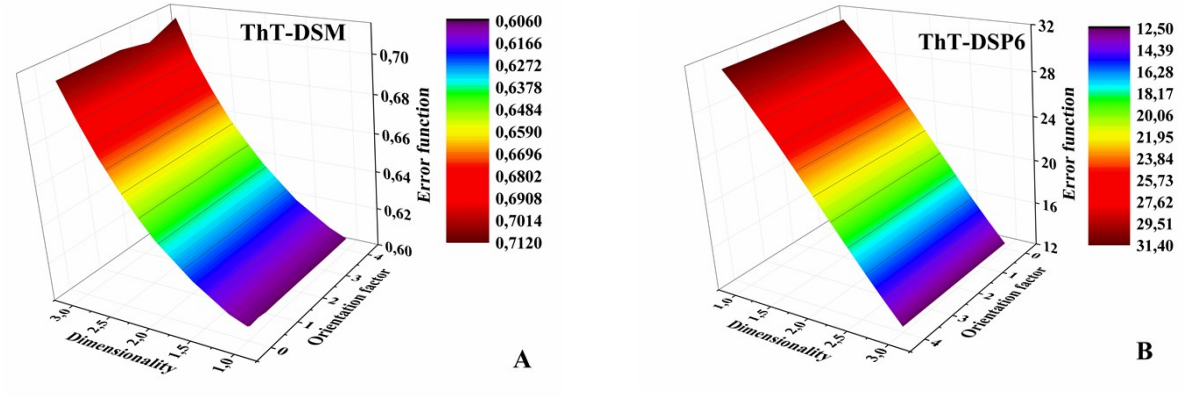


Fig. S6. The ratio of the ThT-induced increases of SQ4 fluorescence observed for the fibrillar and control insulin in the systems InsF/InsC + SPD + SQ4 + ThT (A). The fluorescence excitation spectra recorded in the systems InsF/InsC + SPD + SQ4 + ThT at the emission wavelength 720 nm (B). The concentrations of the dyes were 3.4 μM (ThT), 0.55 μM (DSM, DSP6, DSP12), 4 μM (SQ4). The protein concentration was 13 μM.



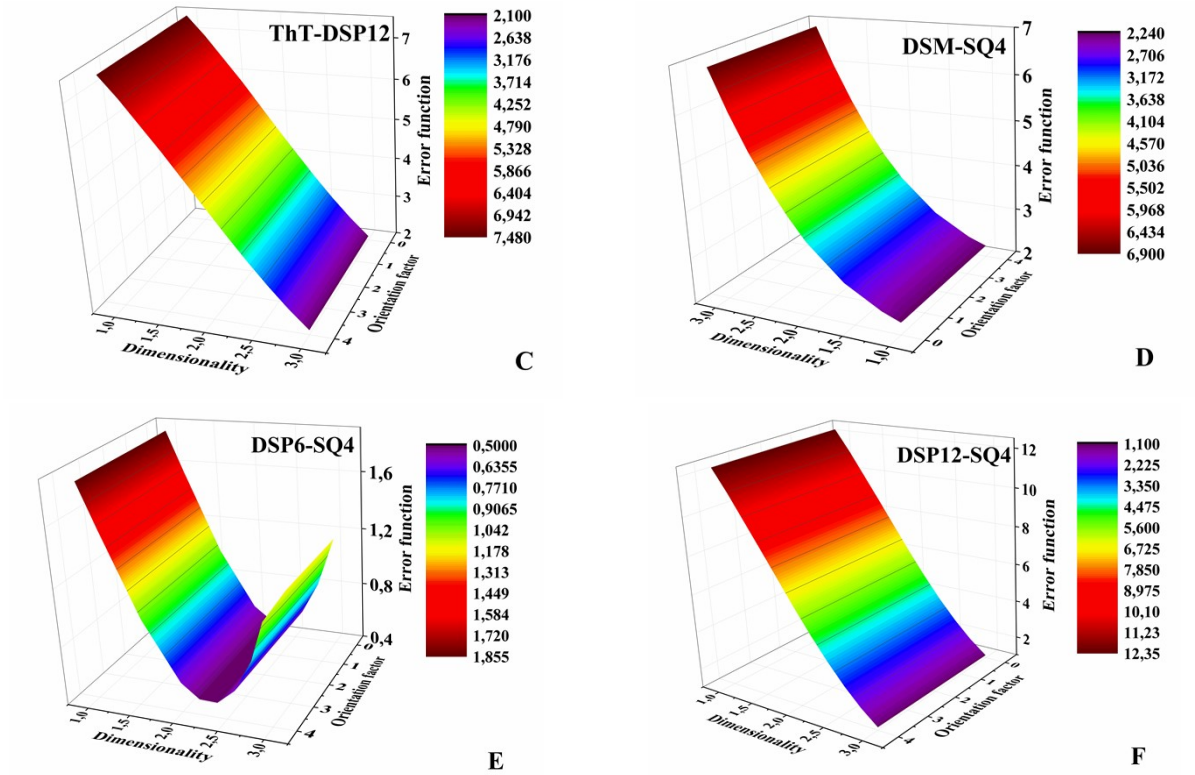


Fig. S7. The error function, characterizing the goodness of fit between the predictions of the stretched exponential model and the experimental FRET efficiencies, plotted vs the dimensionality of acceptor distribution and the orientation factor.

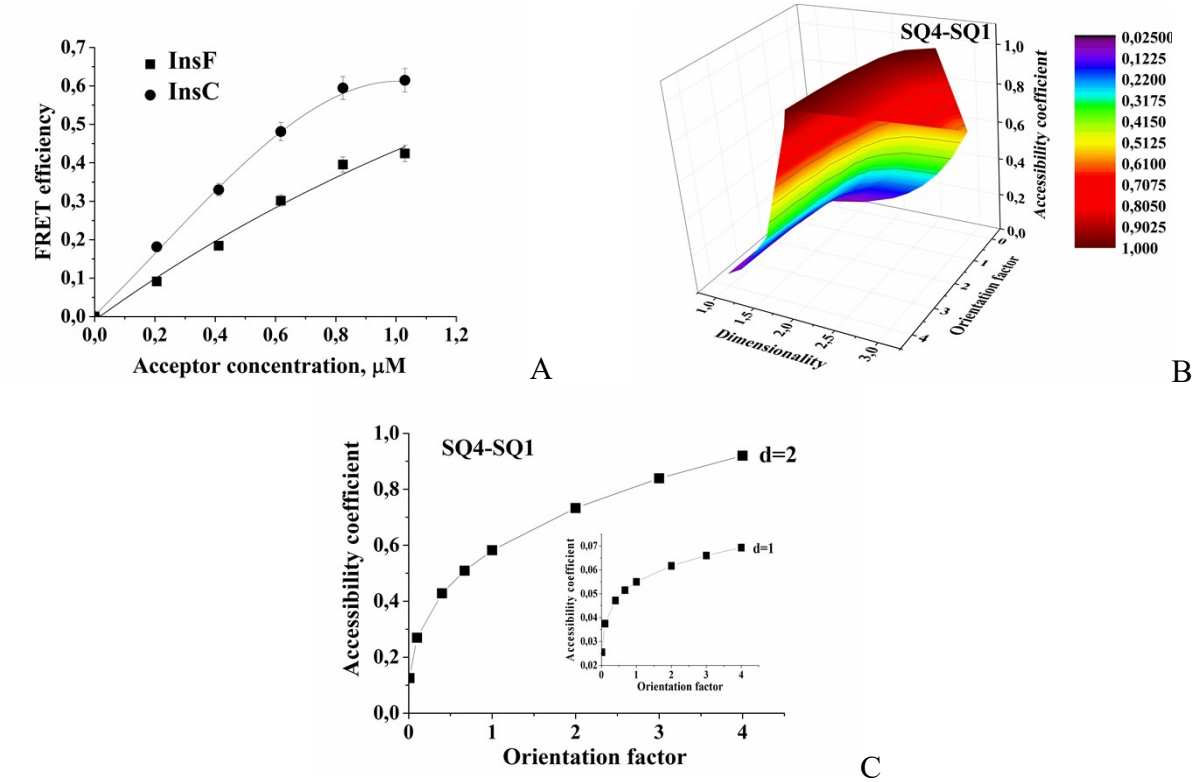
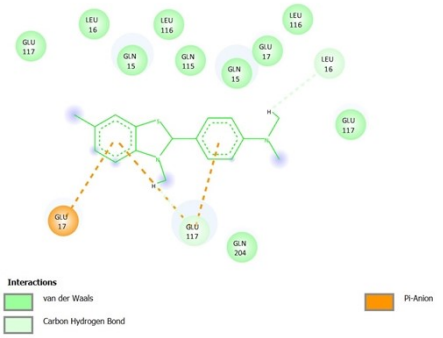
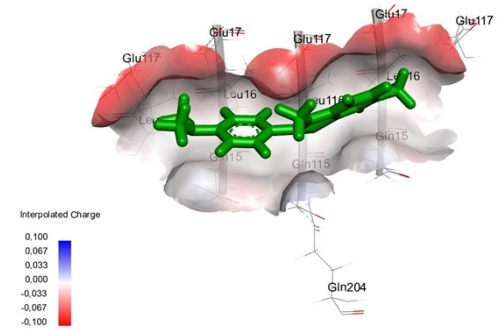
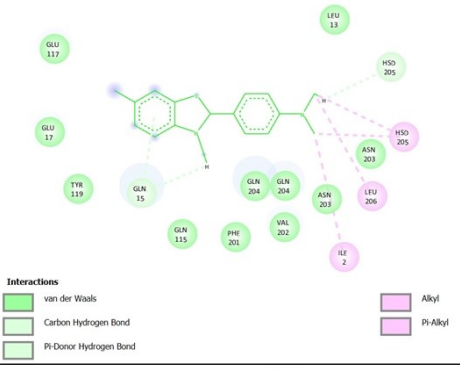
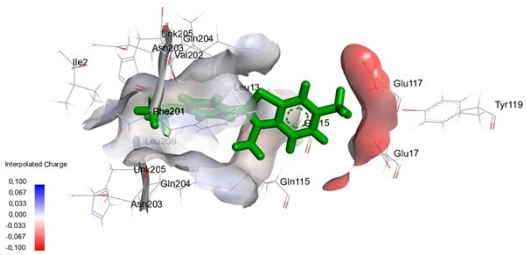
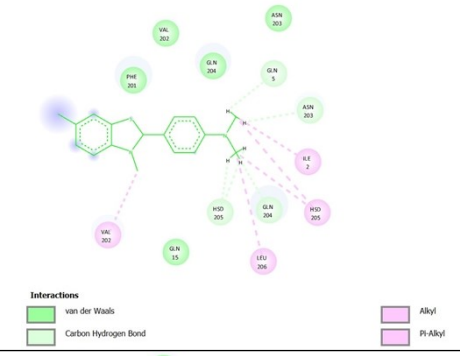
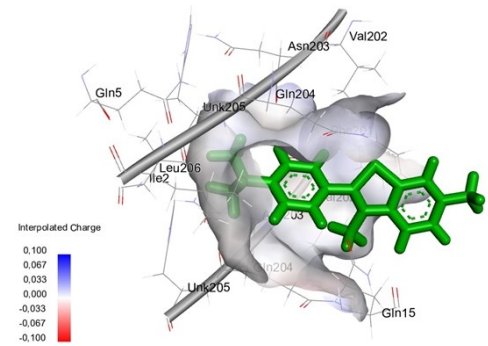
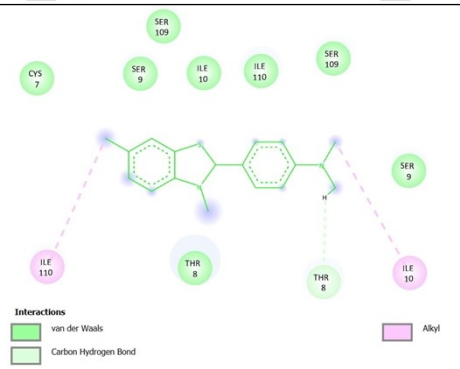
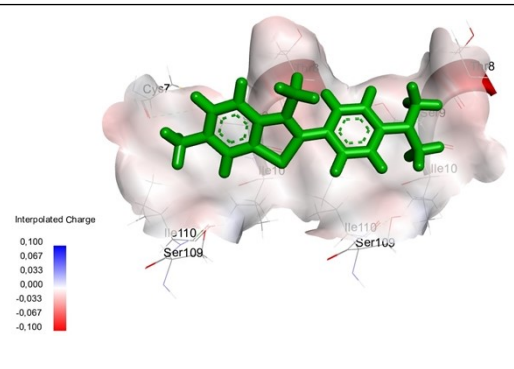
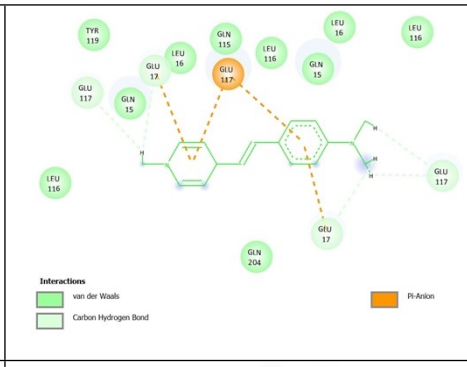
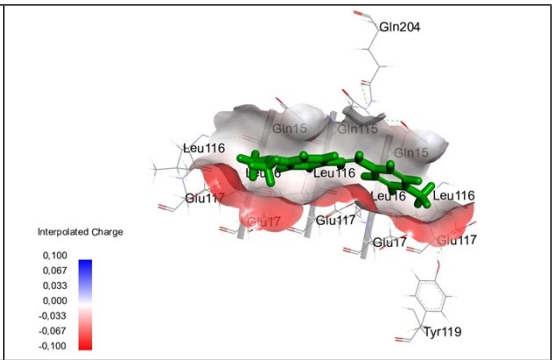
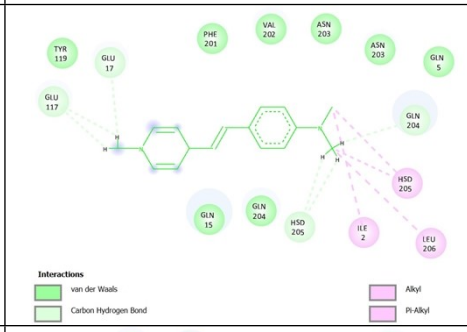
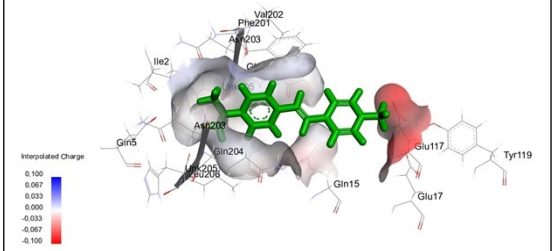
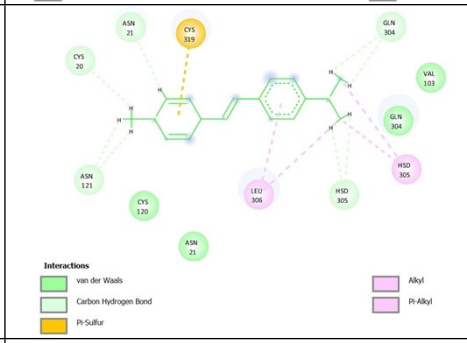
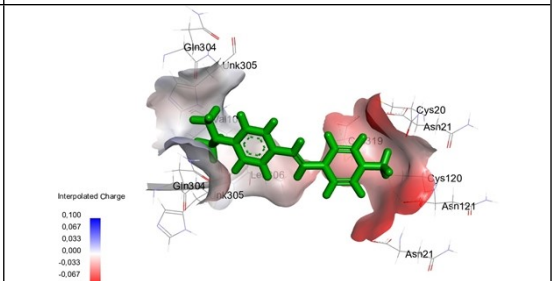
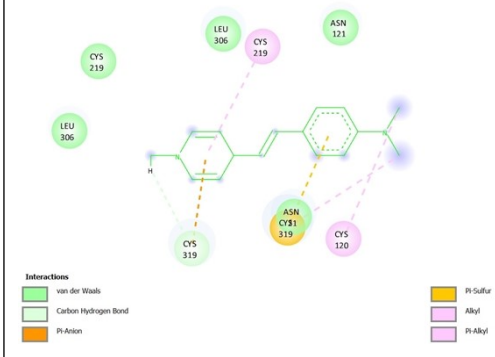
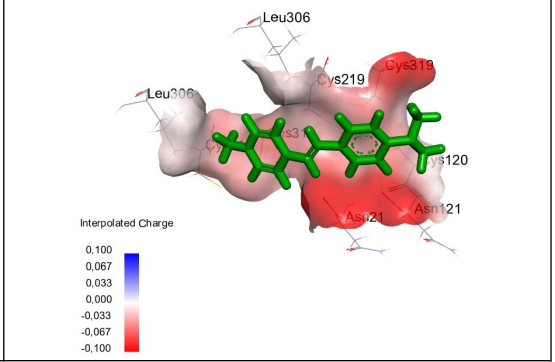
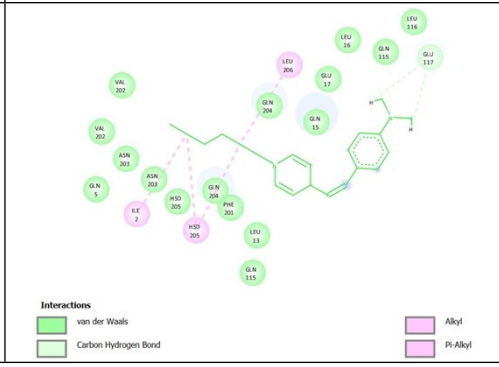
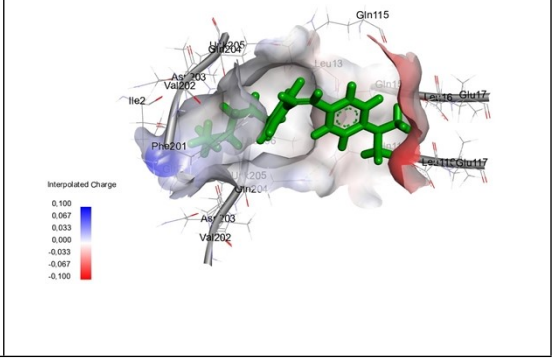
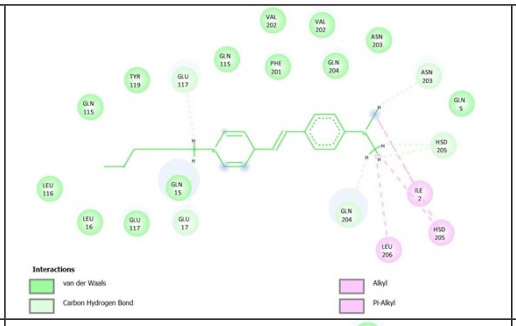
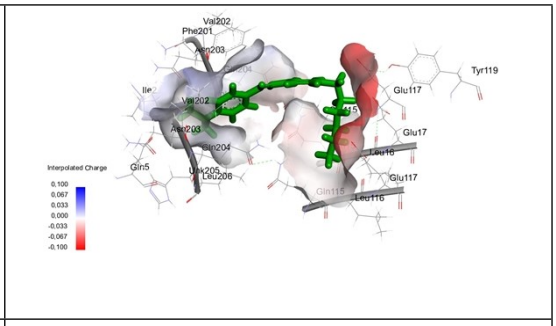
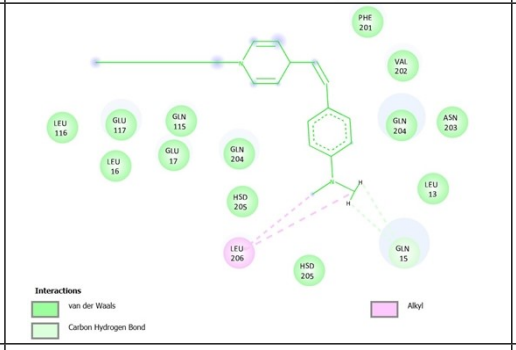
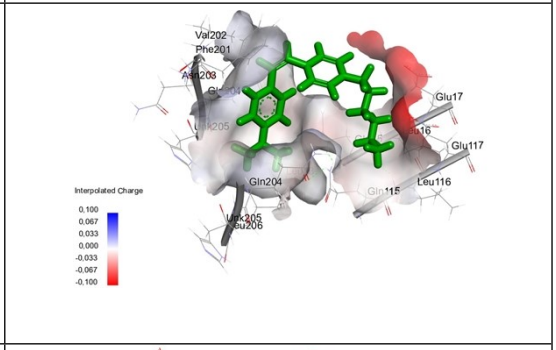
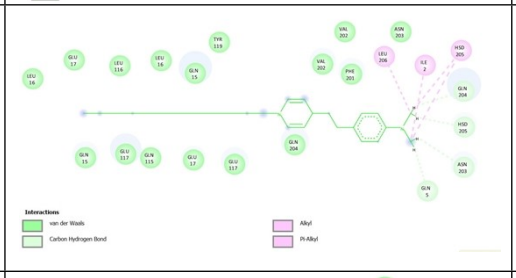
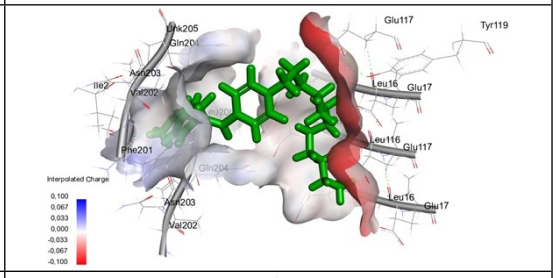
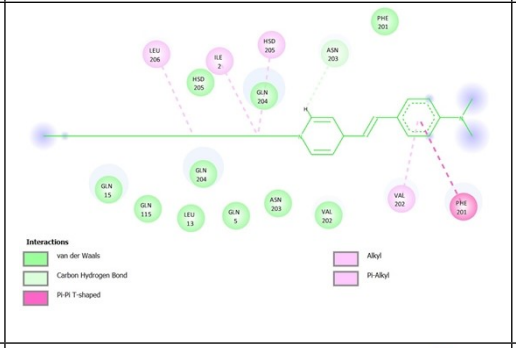
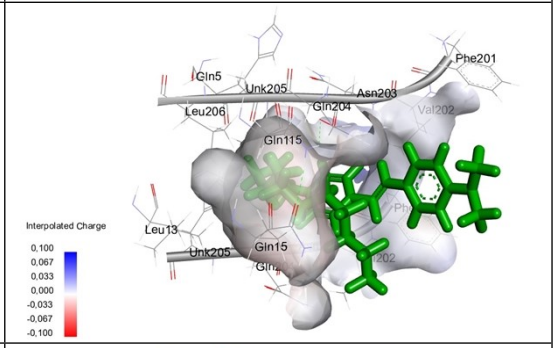
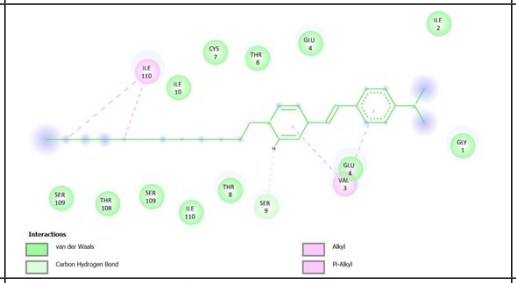
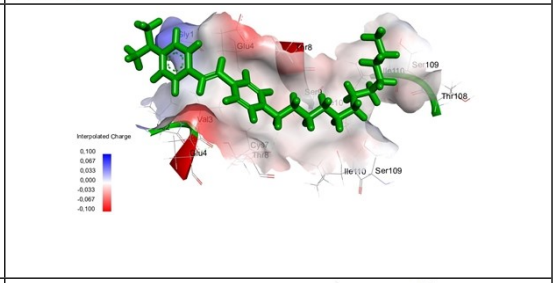
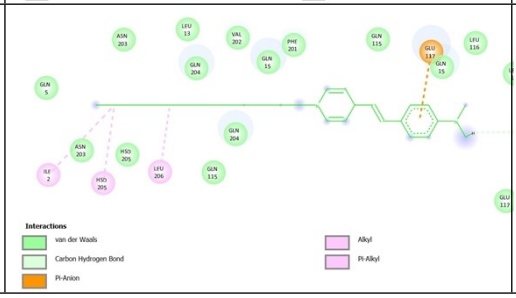
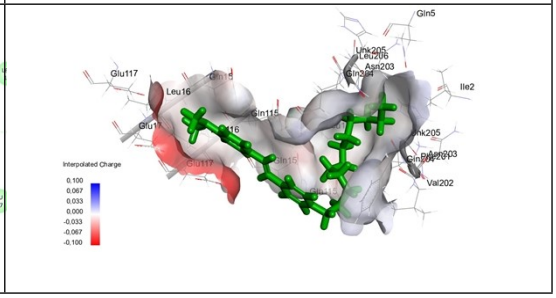


Fig. S8. The efficiencies of energy transfer calculated from the donor fluorescence decrease (Eq. (2)) for the donor-acceptor pair SQ4-SQ1 (A).The dependence between the accessibility coefficient, the dimensionality of acceptor distribution and the orientation factor, calculated for the donor-acceptor pairs SQ4-SQ1 using the stretched exponential model (B). The dependencies between the accessibility coefficient and the orientation factor obtained for the donor-acceptor

pair SQ4-SQ1 (C).

Dye	2D diagram	Interpolated charge surface representation
Thioflavin T, pose 1		
Thioflavin T, pose 2		
Thioflavin T, pose 3		
Thioflavin T, pose 4		

DSM, pose 1		
DSM, pose 2		
DSM, pose 3		
DSM, pose 4		
DSP6, pose 1		

DSP6, pose 2		
DSP6, pose 3		
DSP12, pose 1		
DSP12, pose 2		
DSP12, pose 3		
DSP12, pose 4		

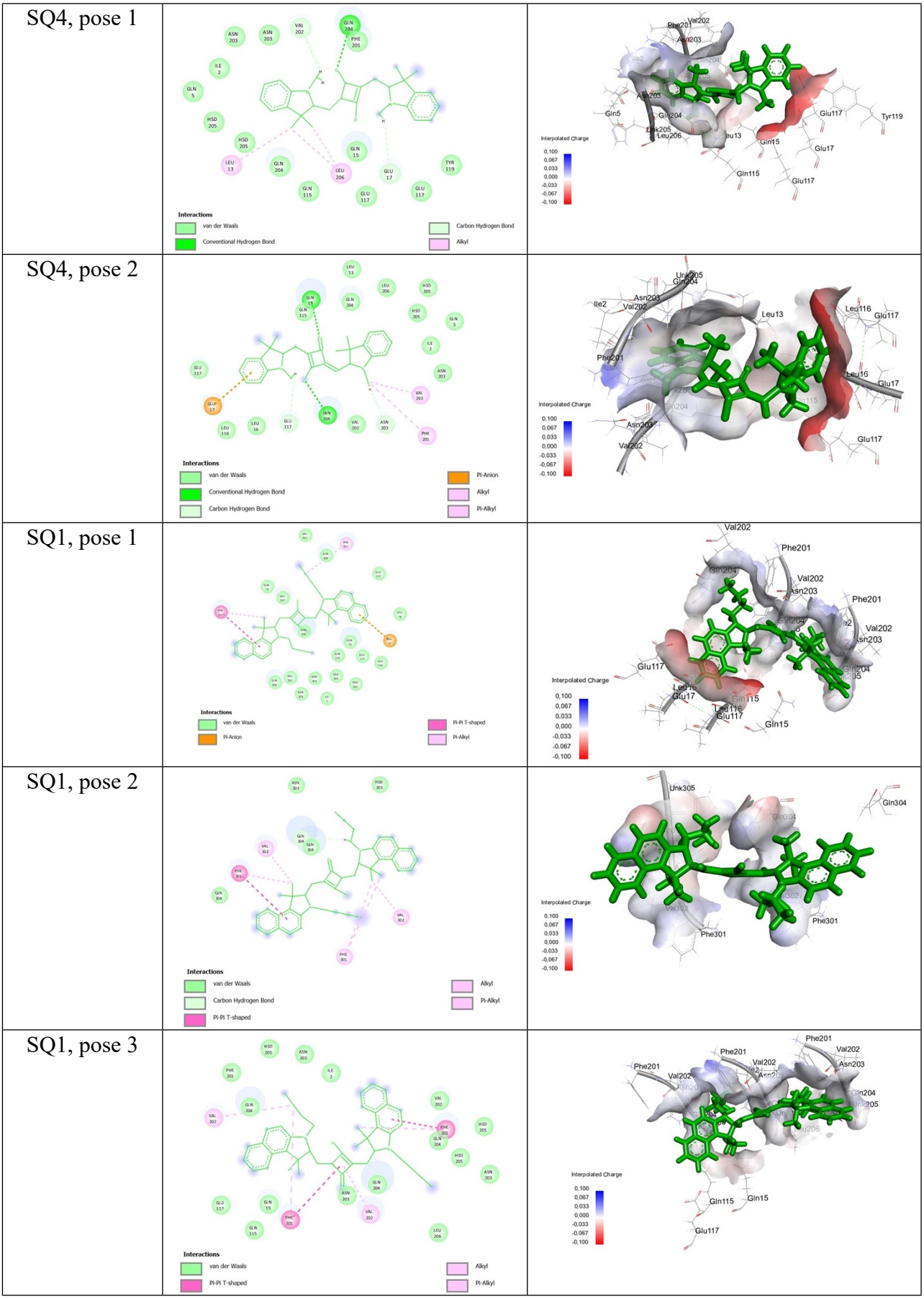


Fig. S9. Binding residues and types of interactions between the insulin amyloid fibrils and the examined dyes.

Table S1
Quantitative parameters of the binding of the styryl pyridinium dyes to the 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
DSM	$1.7^{\pm0.3}\cdot10^4$	$4.5^{\pm0.9}$	$0.09^{\pm0.016}$	$8.09^{\pm1.7}\cdot10^2$	$2.6^{\pm0.3}$	$0.10^{\pm0.015}$
DSP6	$3.5^{\pm0.6}\cdot10^4$	$15.1^{\pm2.9}$	$0.08^{\pm0.014}$	$1.2^{\pm0.2}\cdot10^3$	$5.6^{\pm1.2}$	$0.08^{\pm0.014}$
DSP12	$7.8^{\pm1.4}\cdot10^4$	$5.9^{\pm1.1}$	$0.08^{\pm0.016}$	$7.0^{\pm1.3}\cdot10^3$	$6.3^{\pm1.2}$	$0.09^{\pm0.014}$

Table S2
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 – DSM	2.39	7.99×10^{14}
ThT – DSP-6	2.79	2.01×10^{15}
ThT – DSP-12	2.79	2.02×10^{15}
DSM – SQ4	4.31	1.63×10^{16}
DSP6 – SQ4	6.10	1.71×10^{16}
DSP12 – SQ4	5.92	1.77×10^{16}
SQ4 – SQ1[*r]	4.96	1.65×10^{16}

[*]The Förster radius and overlap integral for the donor-acceptor pair SQ4-SQ1 were previously published in ref. [S1].

Table S3
Association constants calculated from the binding energies of the docked complexes SPD-InsF

Dye	K_a , μM^{-1}			
	Pose 1	Pose 2	Pose 3	Pose 4
DSM	0.97	1.59	26.21	2.19
DSP6	0.5	3.28	1.02	
DSP12	3.63	0.41	2.01	1.29

References

[S1] G. Gorbenko, O. Zhytniakivska, K. Vus, U. Tarabara and V. Trusova, Phys. Chem. Chem. Phys., 2021, **23**, 14746–14754.