Electronic Supplementary Material (ESI) for Journal of Materials Chemistry C.

Electronic Supplementary Information for

Amyloid fibrils as dispersing agents for oligothiophenes: control of photophysical properties through nanoscale templating and flow induced fibril alignment

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Importance of mixing 6Tand insulin in the solid state: to illustrate the importance of grinding 6T and insulin prior to addition of solvent we performed a simple control experiment following the standard procedure but omitting the grinding step. When adding 25 mM HCl to a mixture of insulin and 6T, the protein readily dissolves as expected. However, 6T does not dissolve and remains floating on the surface of the aqueous solvent, as shown in Figure S1a. Subsequent heating results in the formation of amyloid fibrils; however, 6T is not incorporated into the fibrils as seen in Figure S1b. A possible alternative method to functionalize amyloid fibrils is to dissolve the hydrophobic compound in a co-solvent that is itself miscible with water, such as THF or DMSO. Addition of the resulting solution to water may lead aqueous solutions of the hydrophobic compound. However, in the case of 6T the low solubility of 6T even in pure THF makes this approach difficult. 6T is only partially soluble in THF (Figure S1c) and even less so in DMSO (Figure S1d). A relative comparison of the absorption between 6T dissolved in pure THF and 6T ground with insulin is shown in Figures S1e-f. In Figure S1e is shown a spectrum of a solution made from insulin ground with 6T and dissolved in 25 mM HCl according to our standard procedure. The sample was diluted 40 times before measurement. In order to roughly compare the amount of 6T in solution

obtained by the grinding methodology with the amount of 6T in a THF solution we prepared a saturated THF solution that was diluted 40 times with THF before obtaining the spectra shown in Figure S1f. By comparing the spectra shown in Figures S1e-f it can readily be seen that the solution obtained from 6T ground with insulin has a higher content of 6T. The use of DMSO as co-solvent is not possible as 6T is insoluble in DMSO (Figure S1d). It will thus be impossible to obtain high concentrations of 6T in aqueous media by employing the co-solvent method. Using grinding to incorporate 6T in the protein structure prior to solvation is a far more efficient way of obtaining amyloid fibrils non-covalently functionalized with hydrophobic molecules.

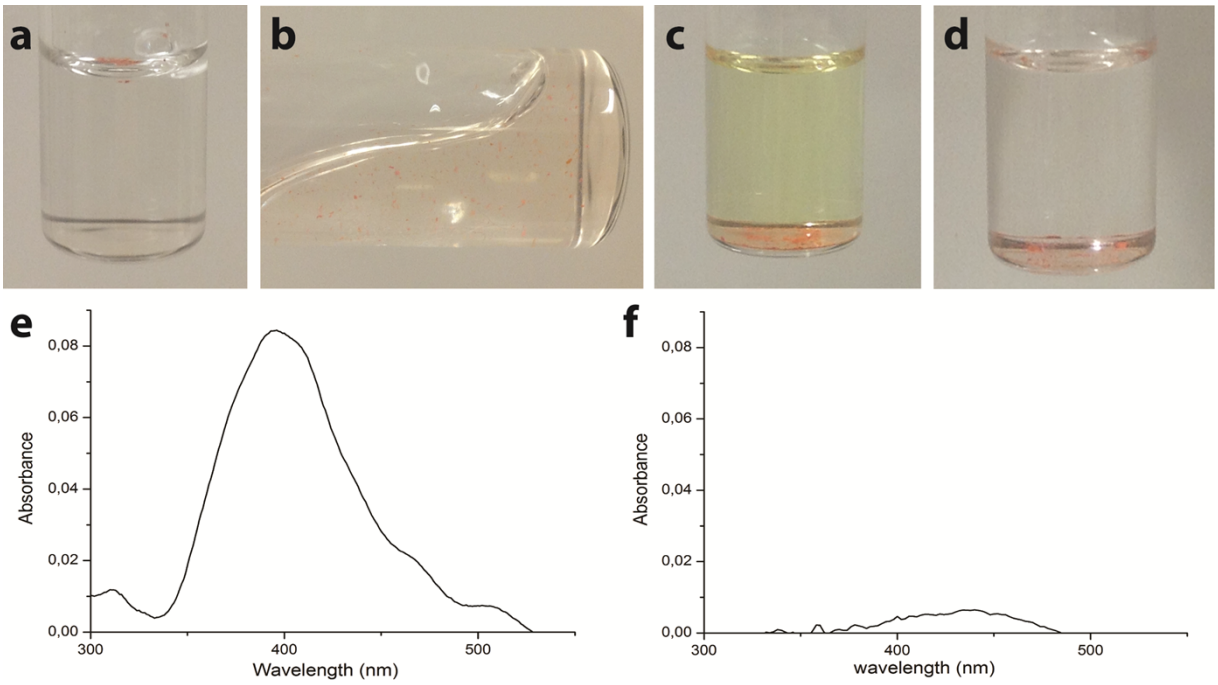

Figure S1. (a) Photograph of insulin and 6T in 25 mM HCl. (b) The mixture in figure S1a after heating. Fibrils have formed causing an increase in solution viscosity. (c) A saturated solution of 6T in THF. (d) An attempt to dissolve 6T in DMSO. (e) Absorbance spectrum of a ground 6T-insulin solution diluted 40 times. (f) Absorbance spectrum of a saturated solution of 6T in THF diluted 40 times.

Spectra of 6T dissolved in $CHCl_3$

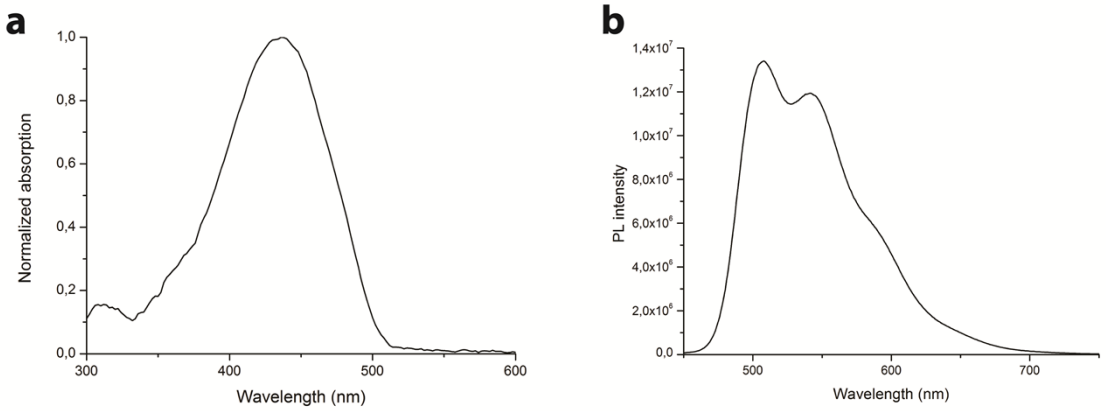

Figure S2. (a) Absorption spectrum of 6T dissolved in $CHCl_3$. Maximum at 436 nm. (b) Photoluminescence spectrum of 6T dissolved in $CHCl_3$. Sample excited at 436 nm. Maxima can be found at 508 nm and 542 nm.

Additional fluorescence data of as prepared and heated 6T-insulin samples

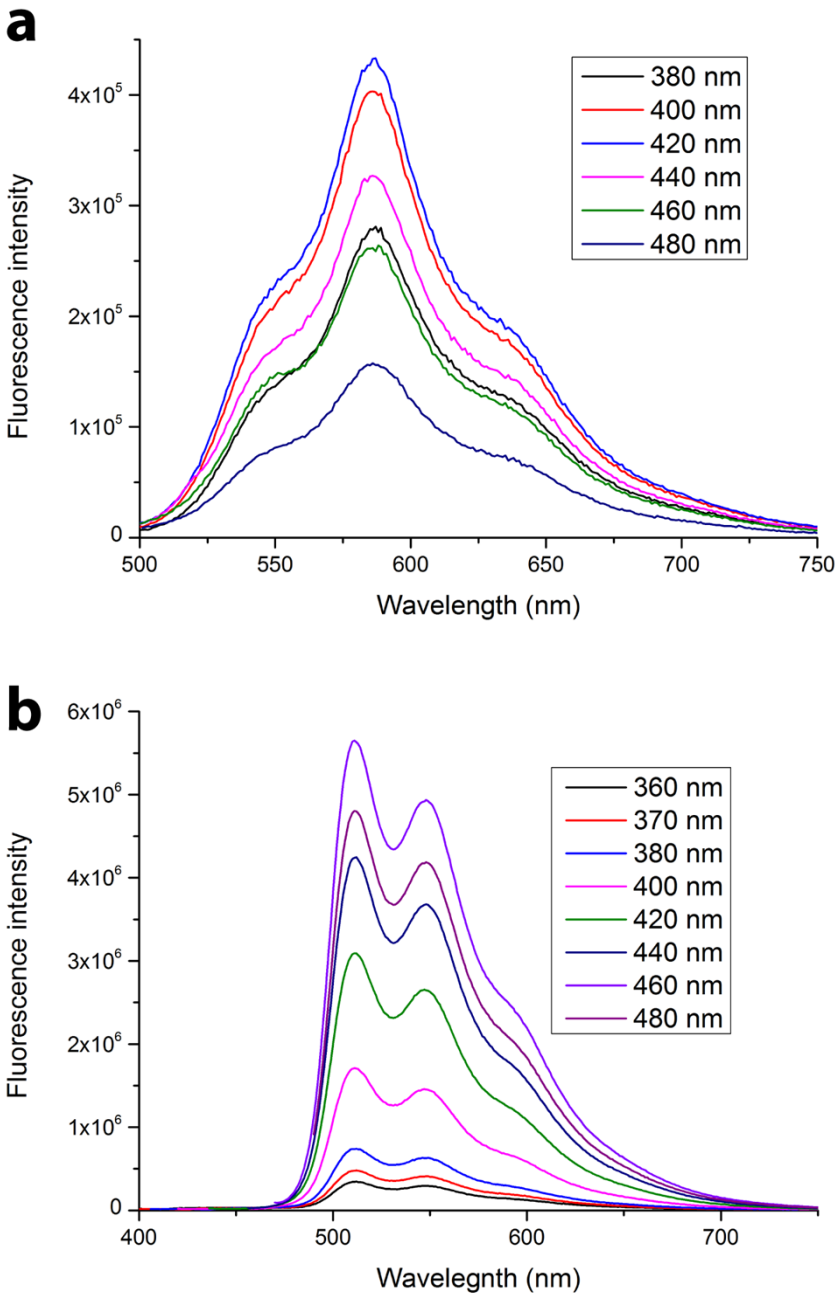

Figure S3. (a) Photoluminescence spectra of an as prepared 6T-insulin reaction mixture (0 h 6T-insulin) excited at selected wavelengths. (b) Photoluminescence spectra of a fibrillated 6T-insulin reaction mixture (24 h 6T-insulin) excited at selected wavelengths.

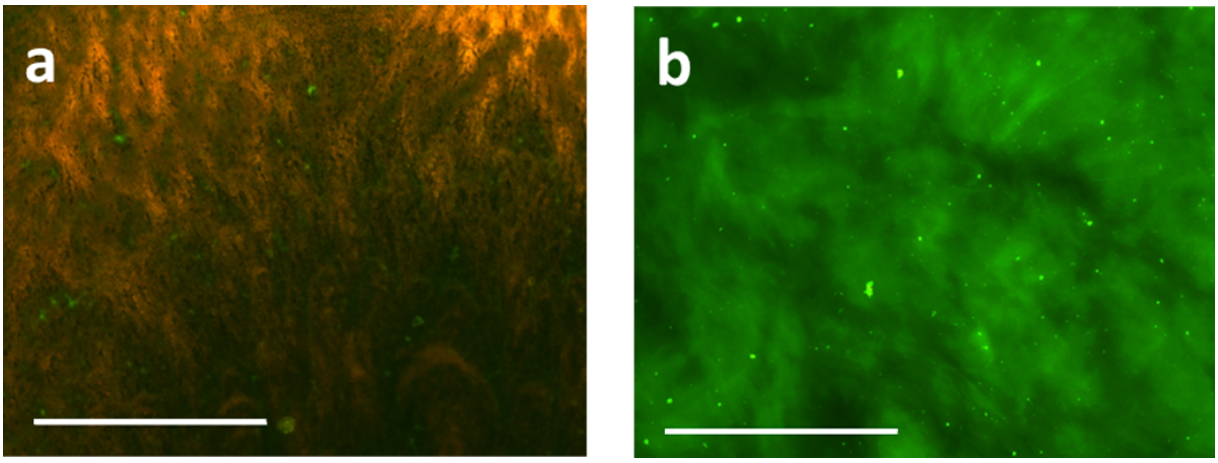

Figure S4. Epifluorescence microscope images of aliquots taken from a 6T- insulin reaction mixture drop casted on glass followed by drying of the sample. Samples were excited at 405 nm. Scale bars represent 20 µm. (a) An as prepared 6T-insulin reaction mixture prior to heating. (b) 6T-insulin reaction mixture after 24 h of heating.

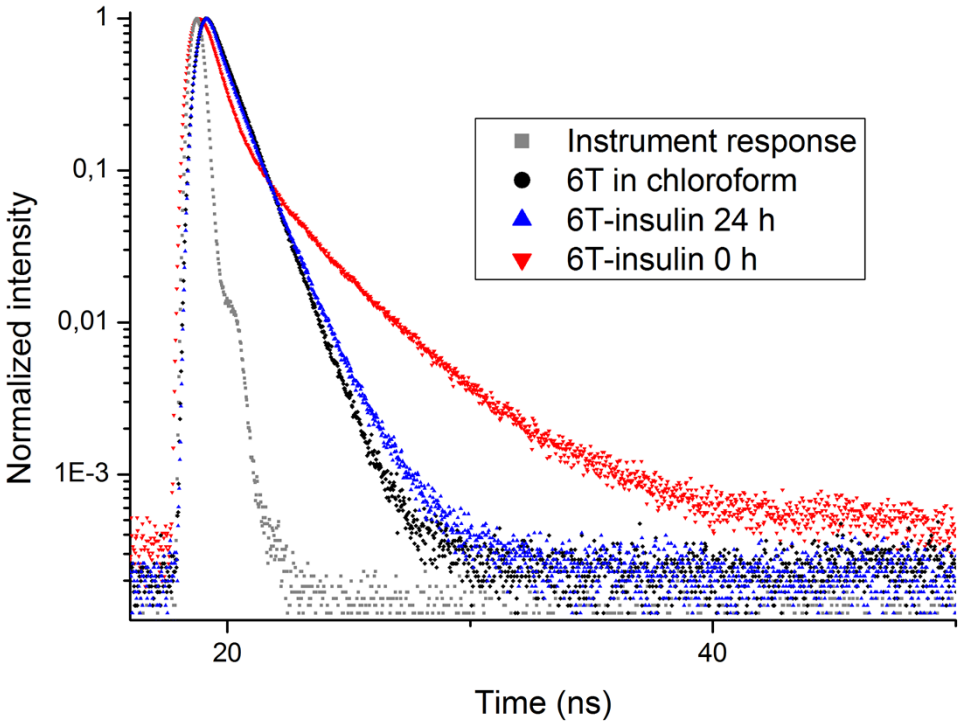

Figure S5. Fluorescence decay curves of samples excited at 460 nm. As prepared 6T-insulin reaction mixture (0 h of heating, in red), fibrillated 6T-insulin reaction mixture (24 h of heating, in blue), and 6T dissolved in chloroform (black).

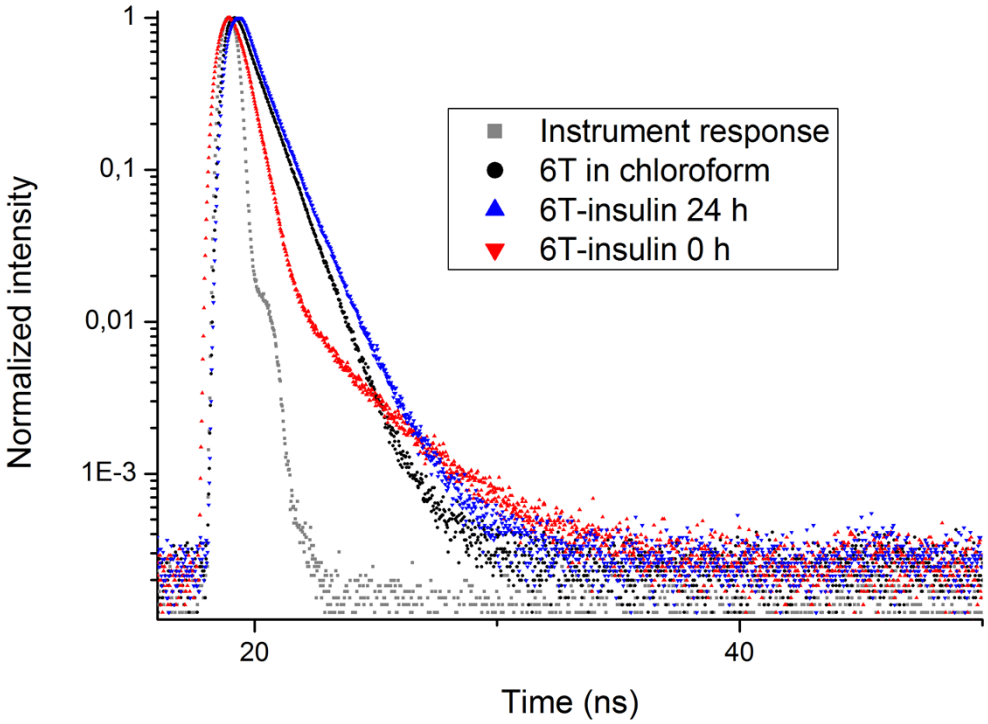

Figure S6. Fluorescence decay curves of samples excited at 500 nm. As prepared 6T-insulin reaction mixture (0 h of heating, in red), fibrillated 6T-insulin reaction mixture (after 24 h of heating, in blue), and 6T dissolved in chloroform (black).

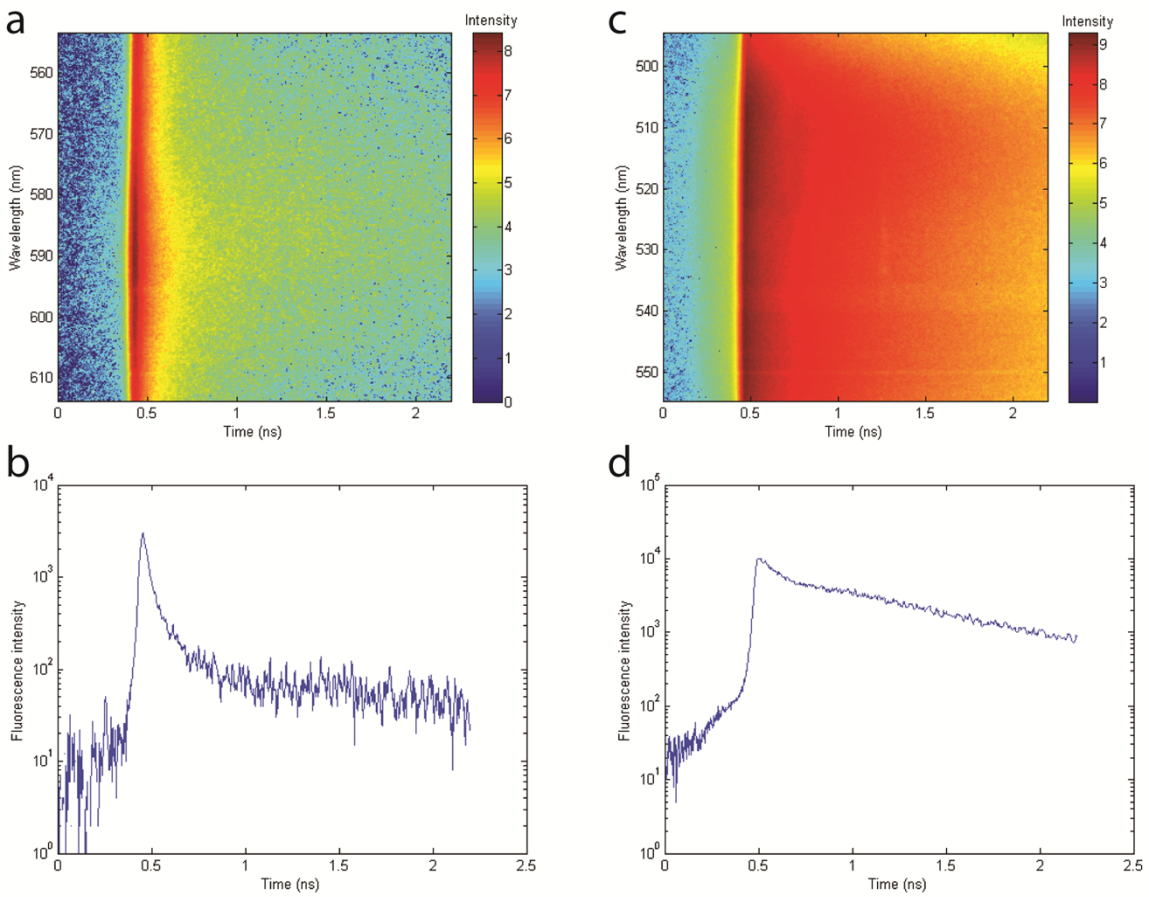

Figure S7. Streak camera measurements for samples excited at 377 nm. (a) Streak image of an as prepared 6T-insulin reaction mixture. (b) Fluorescence decay curve obtained at 587 nm from streak image data in (a). (c) Streak image of a fibrillated 6T-insulin reaction mixture (24 h of heating), (d) Fluorescence decay curve obtained at 508 nm from streak image data in (c).

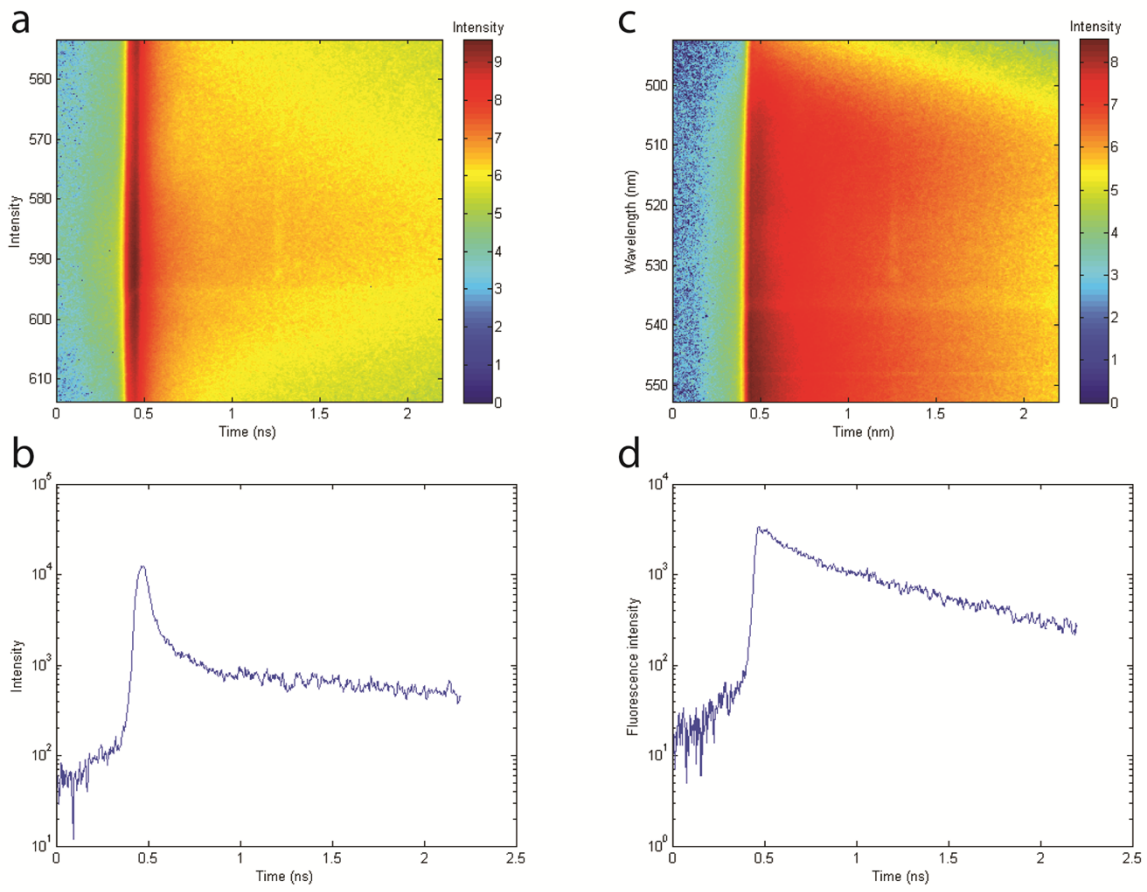

Figure S8. Streak camera measurements excited at 397nm. (a) Streak image of an as prepared 6T-insulin reaction mixture, (b) Fluorescence decay curve obtained at 587 nm from streak image data in (a). (c) Streak image of a fibrillated 6T-insulin reaction mixture (24 h of heating), (d) Fluorescence decay curve obtained at 508 nm from streak image data in (c).

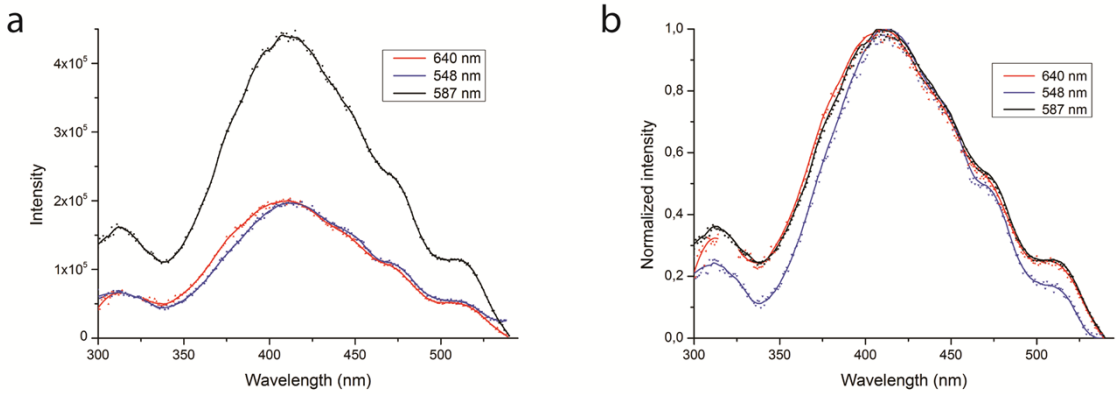

Figure S9. Excitation spectra of an as prepared 0 h 6T-insulin reaction mixture. Solid lines represent smoothed data. (a) Excitation spectra of an as prepared 6T-insulin reaction mixture monitored at 548 nm (blue), 587 nm (black) and 640 nm (red). (b) The same excitation spectra as in (a) but normalized.

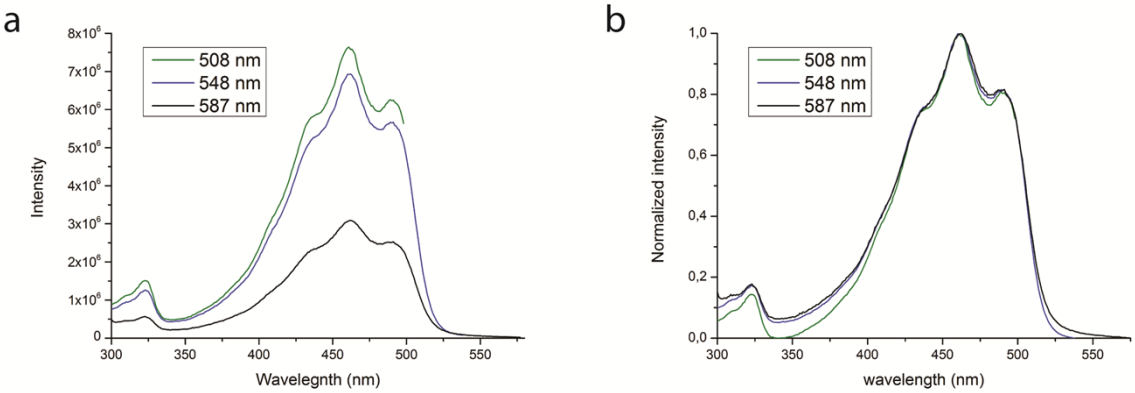

Figure S10. Excitation spectra of a fibrillated 6T-insulin reaction mixture (24 h of heating). (a) Excitation spectra of a 6T-insulin reaction mixture after 24 h, monitored at 508 nm (green), 548 nm (blue) and 587 nm (black). (b) The same excitation spectra as in (a) but normalized.

FTIR spectroscopy of 6T-insulin in D_20-DCl: To verify the results from the FTIR-ATR measurements we performed additional measurements on insulin and 6T-containing insulin reaction solutions following a previously reported procedure[1]. In order to reduce the influence of water on the signal to noise ratio for FTIR measurements, fibril-containing samples were

prepared using the standard procedure but with H_20-HCl replaced by D_20-DCl. 1 ml samples were removed after 24 h of heating and in order to concentrate the sample centrifuged at 20 000 G for 1 hour after which 900µl of the supernatant was removed. The remaining sample solution and aggregate was then resuspended and 15 µl of the resulting suspension was placed between two CaF_2 windows, separated by a 23 µm path length spacer, and fixed in a cell holder. 400 repeat measurements at a resolution of 2 cm^{-1} were recorded with the sample chamber continuously purged with nitrogen. Analysis of the amide-I region confirms that, after heating and fibril formation, the amide I peak for insulin ground with 6T as well as insulin alone is shifted to the same wavenumber position indicating the presence in both cases of insulin amyloid β-sheets.[2,3] With and without 6T the main peak position is located at 1621 cm^{-1}.

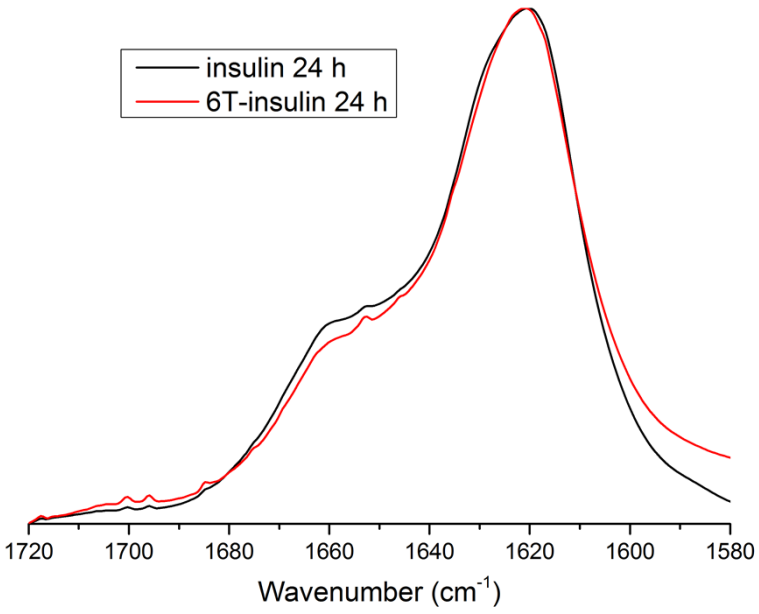

Figure S11. FTIR spectra of fibrillated insulin-only (black) and fibrillated 6T-insulin (red) reaction mixtures (24 h of heating). Spectra were normalized with respect to intensity in the region between 1720 cm^{-1} and 1580 cm^{-1}.

Additional polarized fluorescence microscope images

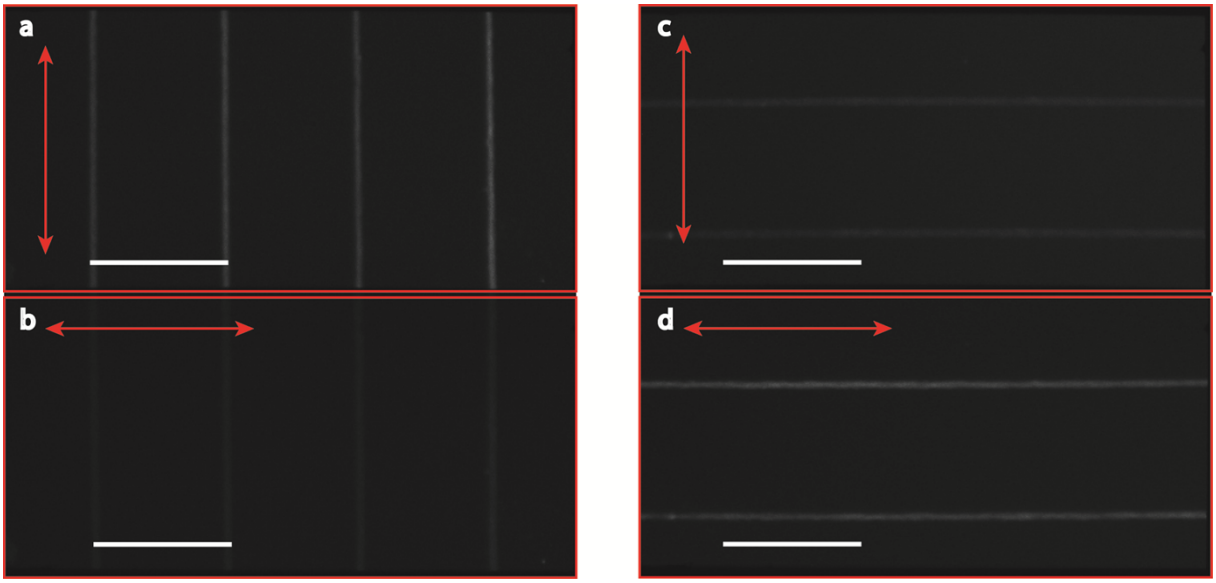

Figure S12. Fluorescence microscope images of two different areas (a-b, c-d) with 6T-containing protein insulin material aligned on a glass surface. Scale bars represent 100µm. The polarization direction is indicated by red arrows. Samples were excited at 475 nm.

1. R. Maeda, K. Ado, N. Takeda, Y. Taniguchi, *Biochimica et Biophysica Acta*, 2007, **1774**, 1619.

2. W. Dzwolak, A. Loksztejn, V. Smirnovas, *Biochemistry*, 2006, **45**, 8143.

3. M. R. Nilsson, *Methods*, 2004, **34**, 151.