Supporting Information to

Thioflavin-T binding to amyloid fibrils leads to fluorescence self-quenching and fibril compaction

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1. Materials and Methods

1.1 Materials

Bovine insulin (Sigma-Aldrich Catalog No I5500), thioflavin-T (ThT), and buffer chemicals were purchased from Sigma-Aldrich. Triple distilled water from a Milli-Q system was used in all experiments. The ThT powder was dissolved in methyltetrahydrofuran and recrystallized prior to use to remove impurities. The purified powder was dissolved in Milli-Q water. The buffer was 50 mM glycine-HCl, pH 2.2. All buffers were filtered prior to use.

1.2 Preparation of Insulin Amyloid Fibrils

Insulin fibrils were prepared by dissolving insulin to a concentration of 5 mg/ml in the glycine-HCl buffer followed by incubation at 60°C over night and without shaking. The resulting fibril solution was centrifuged for a few seconds in a table top centrifuge to remove gel-like particulates. The supernatant contained fibrils that were used for the experiments. The concentration of fibrils is reported as monomer mass concentration and was determined prior to each experiment by UV-Vis absorption spectroscopy using an extinction coefficient of 5600 $M^{-1}cm^{-1}$ at 280 nm for insulin.

1.3 Linear Dichroism Spectroscopy

Linear Dichroism was measured on a Chirascan Spectropolarimeter equipped with an LD.3 linear dichroism detector (Applied Photophysics Ltd, Leatherhead, UK). The insulin fibrils were shear aligned in the 0.5 mm gap between two concentric quartz cylinders in a custom made outer-rotating Couette cell under a shear gradient of 3100 s^{-1}. The total pathlength of the Couette cell was 1 mm. All spectra were corrected for background contributions by subtracting corresponding spectra recorded without shear/rotation.

1.4 Atomic Force Microscopy

Insulin amyloid fibrils were deposited on freshly cleaved mica. After 5 min, the mica was rinsed with filtered milli-Q water and dried under a gentle nitrogen stream. Images were recorded on an NTEGRA Prima setup (NT-MDT Spectrum Instruments, Moscow, Russia) using a gold-coated single crystal silicon cantilever (NT-MDT, NSG01, spring constant of ~5.1 N/m) and a

resonance frequency of ~150 kHz. Images were acquired at 512 pixel resolution with 0.5 Hz scan rate.

1.5 Steady-State Fluorescence Spectroscopy

Fluorescence spectra were recorded on a Varian Eclipse fluorimeter (Agilent Technologies, Santa Clara, California, US) in a reduced volume (60 µl) quartz cell with a pathlength of 3*3 mm (Hellma GmbH, Müllheim, Germany). Spectra were recorded from 450-600 nm upon excitation at 440 nm using a scan speed of 100 nm/min and an integration time of 0.6 s. The bandpass of the excitation and emission monochromators were respectively 5 and 10 nm. The recorded intensities were corrected to account for the primary inner filter effect according to the method by Lakowicz (Lakowicz, J., *Principles of Fluorescence Spectroscopy*, 3rd Ed. 2006. Springer Science & Business Media. ISBN: 9780387312781), where the corrected emission is calculated as:

$$F_{corr} = F_{obs} antilog(\frac{A_{ex} + A_{em}}{2})$$

With this correction, for the highest absorbing sample (20 µM ThT, A_{440} = 0.07), the primary inner filter attenuation was ~8%. The difference between corrected and non-corrected data for the entire data set is shown in Fig. S3.

1.6 Steady-state Fluorescence Anisotropy

Excitation spectra with were recorded at ambient temperature on a SPEX Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba) equipped with Glan polarizers and in a 3*3 mm quartz cell. The emission monochromator was set to 485 nm with a bandpass of 5 nm. The excitation monochromator was scanned from 380-470 nm in 5 nm increments and with a response time of 1 s. The excitation bandpass was 1 nm. The fluorescence anisotropy was calculated from the spectral data according to

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

I_{VV} is the fluorescence intensity measured with vertically polarized excitation and emission, and I_{VH} is the fluorescence intensity measured with vertically polarized excitation and horizontally polarized emission. G is defined as I_{HV}/I_{HH} and is an instrument correction factor giving the ratio of the sensitivity of the detection system for vertically and horizontally polarized light. In our case G was calculated to 0.66, a value that was constant across the entire scanned wavelength range. The anisotropy curve, overlaid onto the wavelength and lamp-intensity corrected isotropic excitation spectrum for ThT bound to insulin fibrils is shown in Fig. S5. The insulin fibril concentration was 20 µM and the ThT concentration was 10 µM.

1.6 Fluorescence Lifetime Measurements by Time-Correlated Single Photon Counting

Fluorescence lifetimes were measured by time-correlated single photon counting (TCSPC). A pulsed (10 MHz) laser diode emitting at 405 nm (PicoQuant GmbH, Berlin, Germany) was used for excitation. Emitted photons were collected at 485 ± 10 nm by an R3809U-50 microchannel-plate photomultiplier tube (Hamamatsu Photonics, Hamamatsu, Japan) and fed into a Lifespec II multi-channel analyser (Edinburgh Instruments Ltd, Kirkton Campus, UK) with its time window set to 50 ns divided into 2048 channels. 10,000 counts were recorded in the top channel. The instrument response function was recorded by placing a mirror in the sample holder. The

data were deconvoluted with the instrument response and fitted to a double exponential decay function by a Levenberg–Marquard non-linear least squares method using PicoQuant FluoFit software. The goodness of fit was assessed by the χ^2 value which was typically <1.2. A control measurement was made to assess the contribution from free ThT at 405 nm. Two samples were measured, one with 10 µM ThT, and one with 10 µM ThT and 10 µM insulin fibrils. Photons were collected for 5 min, and decays were compared to estimate the contribution from free ThT compared to bound ThT. The resulting decays are given in fig. S6, and show that the photon contribution from free ThT (red curve, Fig. S6A) is negligible in relation to fibril-bound ThT (blue curve, Fig. S6A), and that the acquisition time for 10,000 counts in the top channel is substantially longer for free ThT (Fig. S6B). This result is further discussed in the main text.

2. Supporting Results

2.1 Assessment of the degree of orientation of insulin during LD experiments.

In LD we aligned insulin fibrils by shear flow. We observed a reduction in LD signal from insulin (backbone transitions and tyrosine) with increasing ThT concentration. Here we show that this reduction is due to change in fibril alignment and not due to any structural perturbations to the fibril in response to ThT.

First, we recorded a set of LD spectra at a total insulin concentration of 10 µM and with ThT concentrations from 1-10 µM. This was necessary because higher insulin concentrations result in non-linear response of the LD detector at wavelengths below 200 nm. We first calculated the ratio of the 276nm (tyrosine) and 202 nm (β-sheet) LD signals (Fig. S2B). This ratio is near constant, strongly suggesting that the structure of the fibrils does not change. Still, to further analyse the non-systematic variation in the 276nm/202nm ratio we estimated the tilt angles of the tyrosine short axis relative to the fibril axis in these four spectra. The calculations are outlined below. First we used a literature value of the tyrosine tilt angle in insulin fibrils (α=42°, Kitts et al. Biochemistry, 2011, 50 (17), pp 3451–3461) and computed the orientation factor of the spectra with 1 µM ThT. This yielded S=0.13. We next assumed that in an unperturbed fibril the π-π* transitions in the fibril βsheet are parallel to the fibril axis (α=0°) (Bulheller et al. *J. Am. Chem. Soc.*, 2009, 131 (37), pp 13305–13314). Under the assumption that the β-sheet tilt relative to the fibril axis does not change we thereafter converted the LD ratio into angular information with help of Eq. 1 in the main text. The (irregular) variance in ratio that we observe would correspond to a change in tilt angle of the tyrosine of ≤ 2°. This is below the accuracy of a typical LD experiment. We also computed how much the β-sheet tilt relative the fibril axis must change in order to explain the drop in intensity in the 200 nm absorption band as entirely due to structural alterations to the fibril. The angle of the π-π* transition would change 40° from the lowest to highest ThT concentration in Fig. S2A. This corresponds to that the β-sheets would change their tilt from being perpendicular to the fibril axis to adopting a 50° angle. Such a major change is not possible within the cross-β geometry. Altogether it is therefore possible to conclude that ThT does not perturb the structure of insulin amyloid fibrils, nor does it affect the alignment of its tyrosine residues.

2.2 Supporting Figures

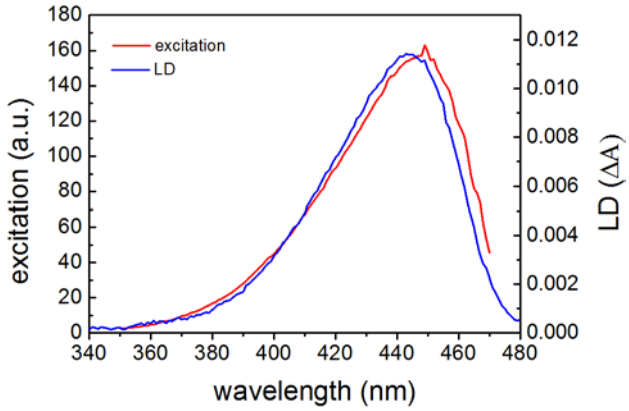

Fig. S1. Comparison between ThT absorption in LD and ThT excitation spectrum. (Blue): Absorption spectrum of bound ThT, as measured by LD. (Red): Excitation spectrum from ThT in presence of insulin fibrils. The two spectra are superimposed, indicating that the bound fraction is the same fraction that produces the excitation spectra, and thus is the emissive species.

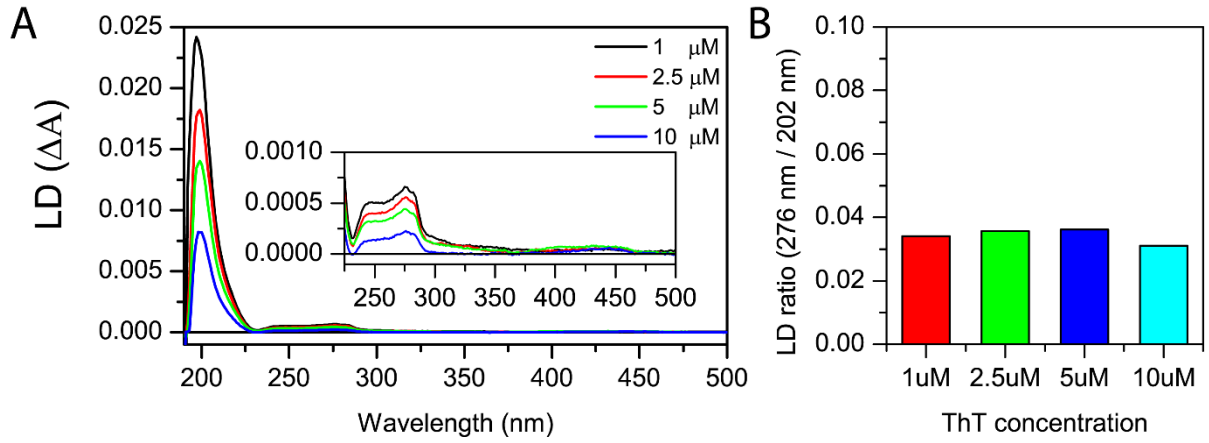

Figure S2. (A): LD spectrum of 10 μM insulin fibrils with increasing concentrations of ThT. (B): LD Ratio 276 nm / 202 nm as function of ThT concentration, data retrieved from the spectra in (A).

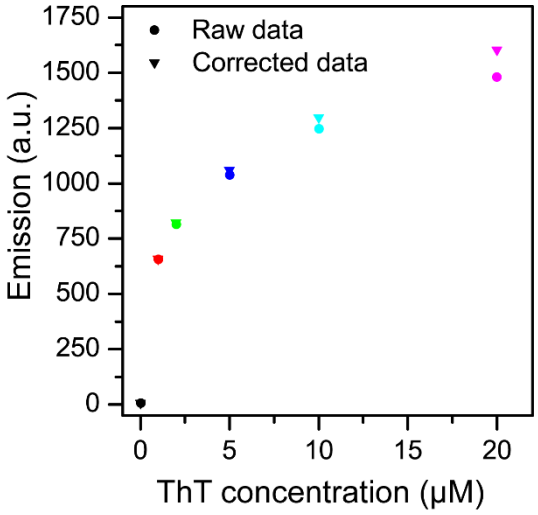

Fig. S3 Steady-state emission from 50 μM insulin fibrbils with increasing concentration of ThT. Circles denote raw data, down triangles denote data after correction for the primary inner filter effect according to Lakowicz (see materials and methods).

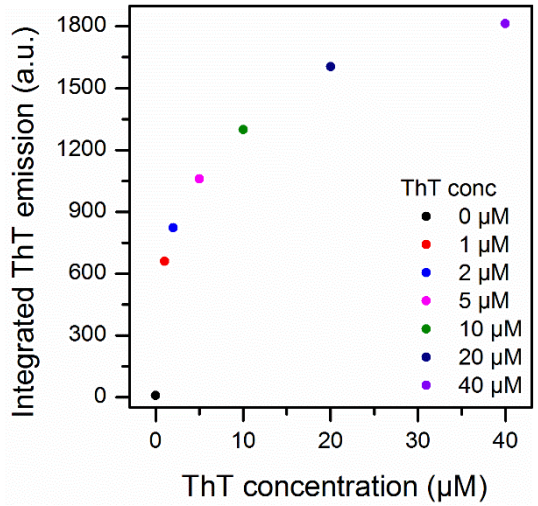

Fig. S4. ThT emission as function of total ThT concentration obtained upon titration of ThT to a 50 μM insulin fibril solution. Extended data set of the data shown in Fig. 2D in the main text.

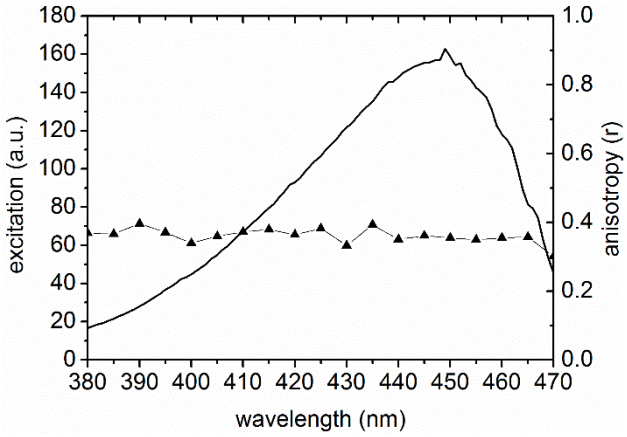

Figure S5. Steady state fluorescence anisotropy of ThT in presence of insulin fibrils (triangles). The insulin fibril concentration was 20 µM and the ThT concentration was 10 µM. The excitation spectrum of ThT in presence of insulin is shown as a solid line for comparison. The fluorescence anisotropy is constant across the entire excitation band.

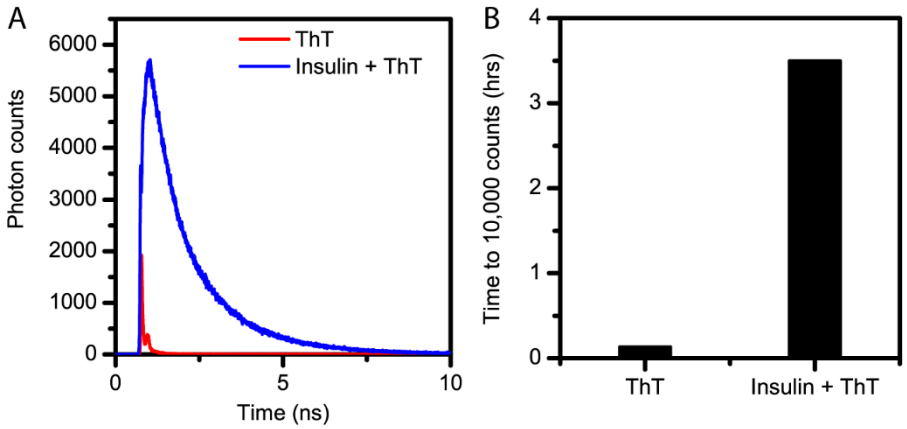

Fig. S6 (Right): (A): Fluorescence decays curves of 10 µM ThT in buffer (red) and in presence of 10 µM insulin fibrils (blue). Both data sets were recorded by collecting photons for 5 minutes, the count rate for collecting photons for the blue curve was ~50 times higher than for the red curve. (B): Time required to collect 10,000 photons in the top channel for ThT in buffer andr ThT in presence of insulin fibrils.