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Supplemental Information

How Fluorescent Tags Modify Oligomer Size Distributions of the Alzheimer Peptide

Jana Wägele, Silvia De Sio, Bruno Voigt, Jochen Balbach, and Maria Ott

S1 FCS of Aβ40 and Aβ42-solutions before fibrillation

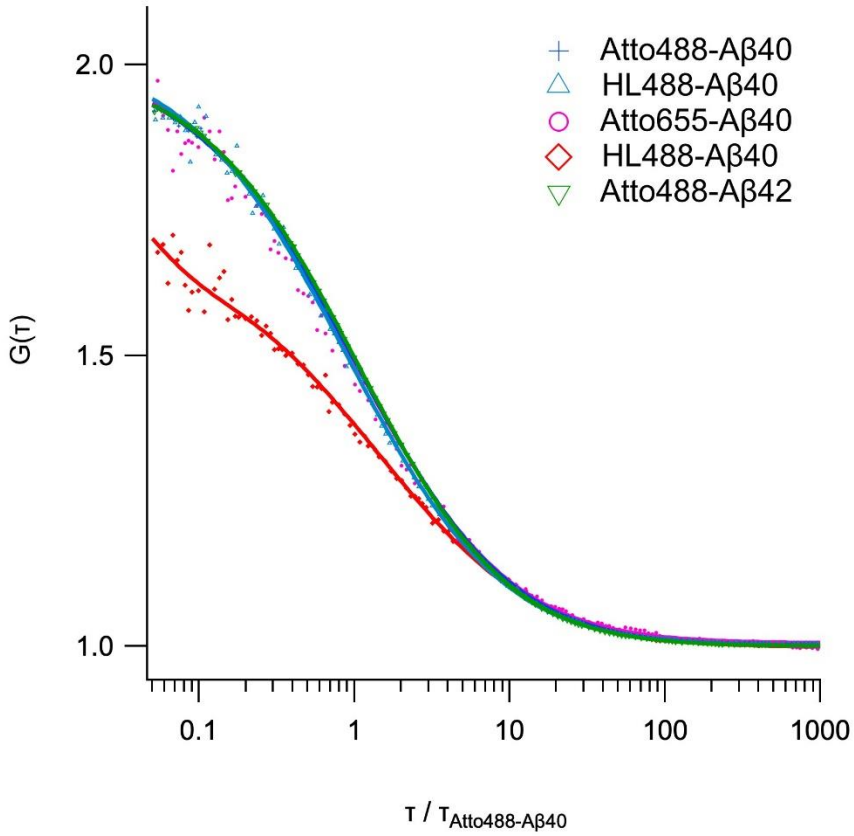

Fig. S1: Normalized time correlation function of labeled Aβ40-peptides before fibrillation (symbols) together with fitting curves (lines) in order to verify the absence of larger aggregates. The time axis was normalized to the diffusion time of Atto488- Aβ40 and Atto655-Aβ40 monomers with τ_d of (142±6) µs and (174±5) µs, respectively, to account for the different focus volumes for blue and red excitation. The peptide concentration was approx. 0.8 nM. The fast decay component of HL647 and Atto647N is due to triplet blinking kinetics.

S2 TEM images of Aβ40wt oligomers and fibrils

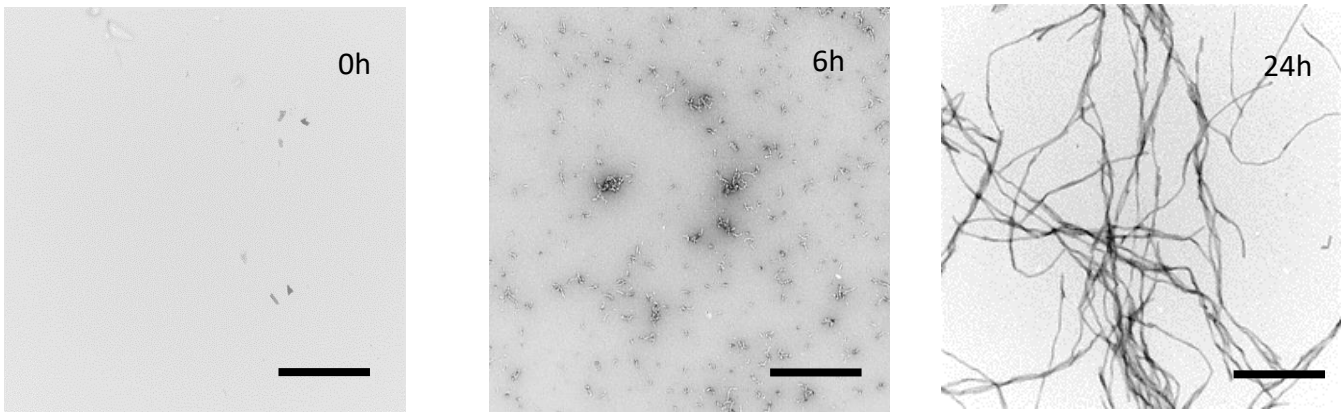

Fig. S2: Negative stained TEM images of Aβ40wt before and after 6 h and 24 h of fibrillation. The scale bar represents 500 nm.

S3 single-molecule fluorescence spectroscopy of labeled Aβ40-peptides

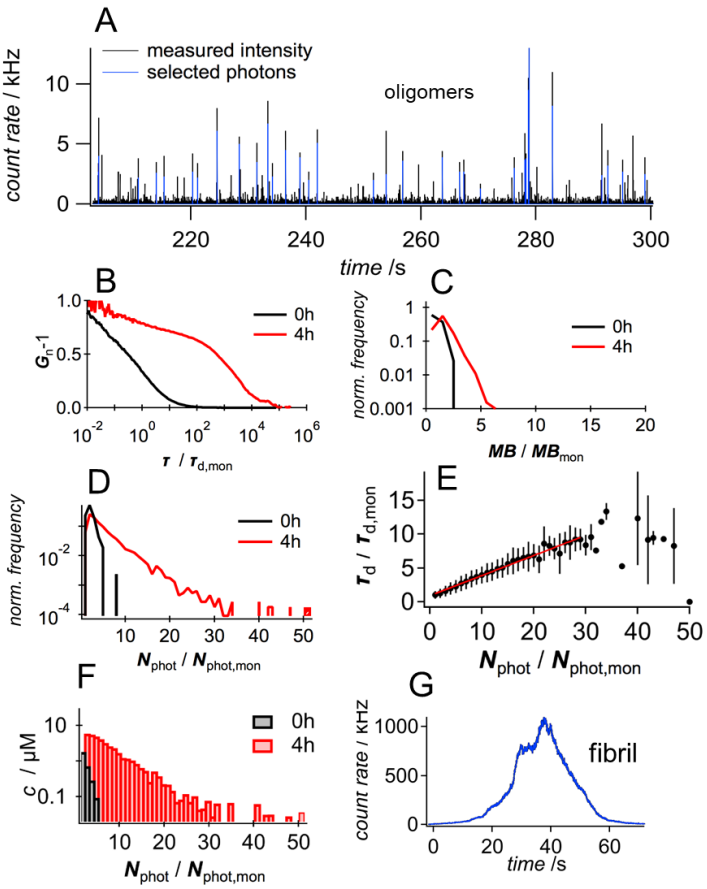

Fig. S3. A: Typical intensity trajectory of an 80 pM solution of HL647-Aβ40 (DOL 100%) after 4h of incubation at 37°C and 40 µM. B: Normalized time correlation function before (black) after (red) 4 h of incubation. D-F: Burst data analysis with normalized histograms of the relative molecular brightness, *MB* (C) and total photon number, N_{phot} (D), as well as the average dwell times (E) used to determine the concentration of the oligomeric species (F) before (black) after (red) 4 h of incubation. G: Fluorescence signal from a fibril (after 24h of incubation).

Data analysis of single-molecule trajectories:

Intensity time traces were analyzed via the calculation of a sliding density average of 10 adjacent photons detected by either of the two detection channels. All consecutive photons above a threshold density of 0.2 photons/ms were combined into one burst. Fig. S3A shows one of the fluorescence time-trajectories of HL647-Aβ40-peptides after 4 h of fibrillation and with a DOL of 100%.

Bursts containing more than twice the average number of photons detected for monomeric solutions were analyzed with respect to its total number of photons, $\boldsymbol{N_{phot}}$, the molecular brightness, $\boldsymbol{\epsilon}$, and the average fluorescence lifetime, $\boldsymbol{\tau_F}$. The applied threshold criteria were optimized to reliably select not only bright molecules but also less intense events with long dwell times in the focus volume. These two characteristic types of smaller aggregates were usually found within 30 min after the initiation of fibrillation and were clearly different from the very large molecules, detected after 24 h of fibrillation. In order to remove quenching effects we used the fluorescence lifetime-corrected value , $\boldsymbol{N_{phot} = \left(N - N_{bg}\right) * \frac{\tau_{F,mon}}{\tau_{F,burst}}}$, where $\boldsymbol{\tau_{F,mon}}$, is the average fluorescence lifetime of the monomers and $\boldsymbol{N_{bg}}$ the fluorescent background of the buffer. The fluorescence lifetime of each burst was estimated by the average of the photon arrival times relative to the time of excitation.

In the same way a lifetime-corrected molecular brightness, $\boldsymbol{MB}$, was calculated for each burst by dividing $\boldsymbol{N_{phot}}$ by the individual dwell time, $\boldsymbol{t_d}$, of the molecule in the focus, which was estimated using the standard deviation of all photon arrival times grouped in one burst: $\boldsymbol{t_d = \sqrt{\frac{1}{N-1}\sum\left(t_i - t_{avg}\right)^2}}$. The intuitive choice of using the lifetime corrected values of $\boldsymbol{MB}$ to relate aggregate size to fluorescence properties was not applicable. $\boldsymbol{MB}$ is a measure for the average number of fluorescent labels attached to a single molecule and should linearly scale with the number of aggregated peptides. However, none of the investigated sampled displayed values of $\boldsymbol{MB}$, which could be meaningful related to aggregate sizes. Fig. S3F displays fluorescence time correlation functions of HL647-Aβ40-peptides before and after 4 h of fibrillation. The characteristic correlation time for measurements after 4 h is increased by three orders of magnitude in comparison to the measurement at 0 h. Since the decay time is related to translational diffusion and scales with the hydrodynamic radius, the increase indicates the presence of large aggregates. In contrast, the relative distribution of $\boldsymbol{MB}$ is hardly affected (Fig. S3C). However, the increased dwell times affect $\boldsymbol{N_{phot}}$ as shown in Fig. S3D, where the distributions for 0 and 4 h reveal the presence of large soluble aggregates after 4 h of fibrillation. Using a characteristic dwell time of all bursts belonging to one histogram bar of $\boldsymbol{N_{phot}}$, an average dwell time, $\boldsymbol{\tau_d}$, was determined by $\boldsymbol{\tau_d = \frac{1}{N_{burst}}\sum t_{d,i}}$, which was found to scale linearly with $\boldsymbol{N_{phot}}$ (Fig. S3E). Finally, $\boldsymbol{\tau_d}$ was used to determine the concentration of the oligomers characterized by $\boldsymbol{N_{phot}}$ (see Fig. S3F and Method section in the main text).

S4 Additional TEM and AFM Images of fluorescently labelled Aβ40

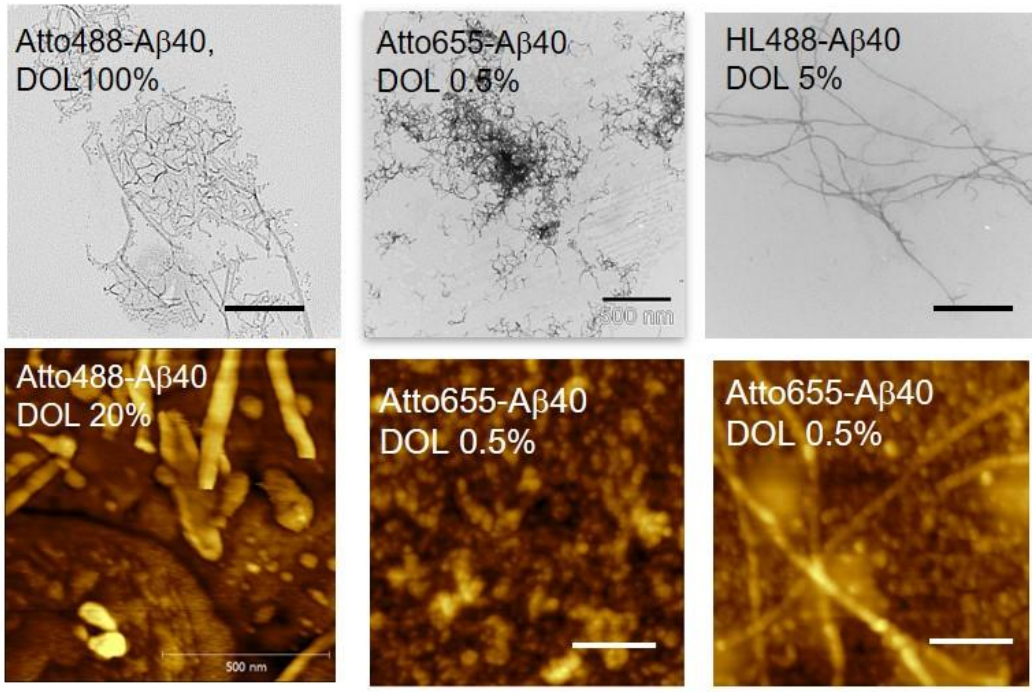

Fig. S4: TEM (up) and AFM (down) images of fibrils of fluorescently labelled Aβ40 after 24 h of incubation. The scale bars are 500 nm and 200 nm for TEM and AFM, respectively.

S5 SAXS and WAXS

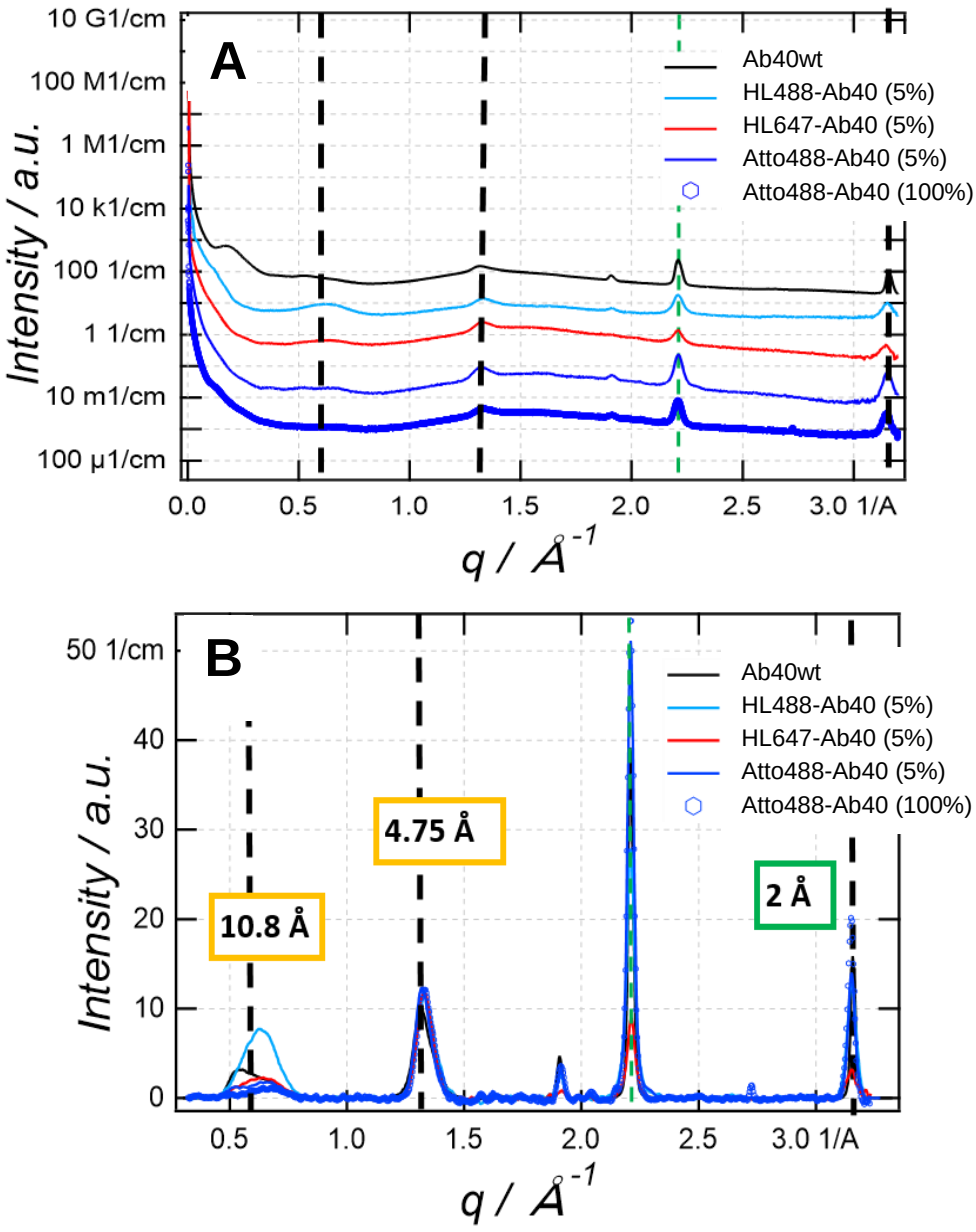

Fig. S5: 1D SAXS- and WAXS intensities of Aβ40wt and labelled peptides before (A) and after (B) the subtraction of the amorphous background. The numbers refer to peak positions of Aβ40 wildtype.

S6/S7 Atto488-Aβ42

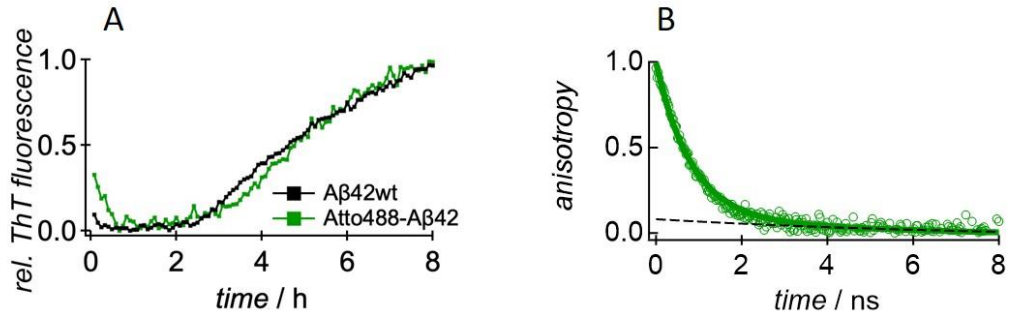

Fig. S6: A: The time dependent ThT fluorescence of Aβ42 without (black) and with 2% of Atto488-Aβ42 (green) at a monomer concentration of 10 µM. B: Time-dependent fluorescence anisotropy of labelled Atto488-Aβ42 before fibrillation.

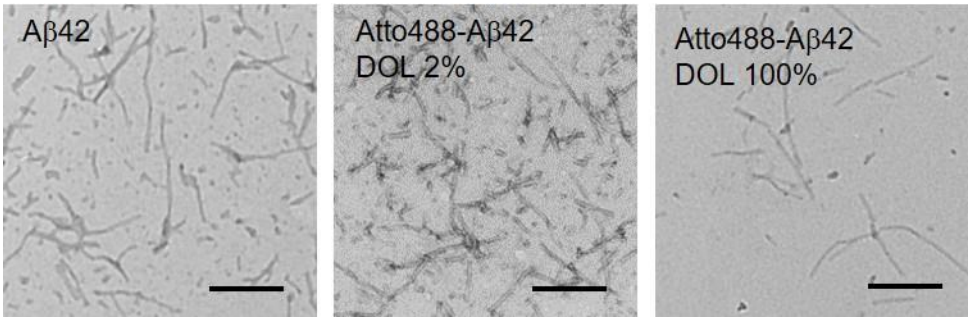

Fig. S7: TEM images of amyloid fibrils of wildtype and fluorescently labelled Aβ42 after 24 h of incubation. The scale bars represent 500 nm.