Supporting Information for:

Alternative Pathways of Human Islet Amyloid Polypeptide Aggregation Distinguished by ^{19}F NMR-Detected Kinetics of Monomer Consumption

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Running title: ^{19}F NMR measurements of amyloid fibrillogenesis

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Complete reference 78:

78. Miyata, M.; Sato, T.; Kugimiya, M.; Sho, M.; Nakamura, T.; Ikemizu, S.; Chirifu, M.; Mizuguchi, M.; Nabeshima, Y.; Suwa, Y.; Morioka, H.; Arimori, T.; Suico, M. A.; Shuto, T.; Sako, Y.; Momohara, M.; Koga, T.; Morino-Koga, S.; Yamagata, Y.; Kai, H. (2010) The Crystal Structure of the Green Tea Polyphenol (-)-Epigallocatechin Gallate-Transthyretin Complex Reveals a Novel Binding Site Distinct from the Thyroxine Binding Site. *Biochemistry 49*, 6104-6114.

Additional supporting figures referred to in the main text

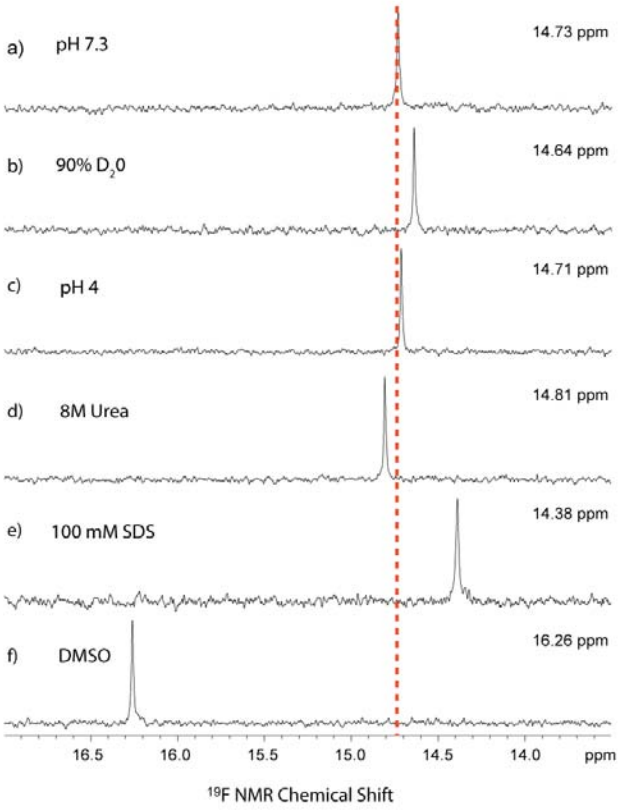

Figure S1. Environmental sensitivity of the $tfmF_{23}$ group. ^{19}F NMR spectra of IAPP-$tfmF_{23}$ were acquired under various conditions after 5 minutes of incubation at 25 °C: **a)** 20 mM sodium phosphate, 20 mM NaCl, 10% D_2O, pH 7.3 **b)** 90% D_2O, 20 mM sodium phosphate, 20 mM NaCl, pH 7.3 **c)** 100 μM HCl, 10% D_2O, pH 4 **d)** 8M Urea, 10% D_2O **e)** 100 mM SDS, 20 mM sodium phosphate, 20 mM NaCl, 10% D_2O, pH 7.3, **f)** 90% DMSO, 10% D_2O. Spectra were referenced to an external TFE standard (in solution of 20 mM Pi, 50 mM NaCl, pH 7.3) at 0 ppm.

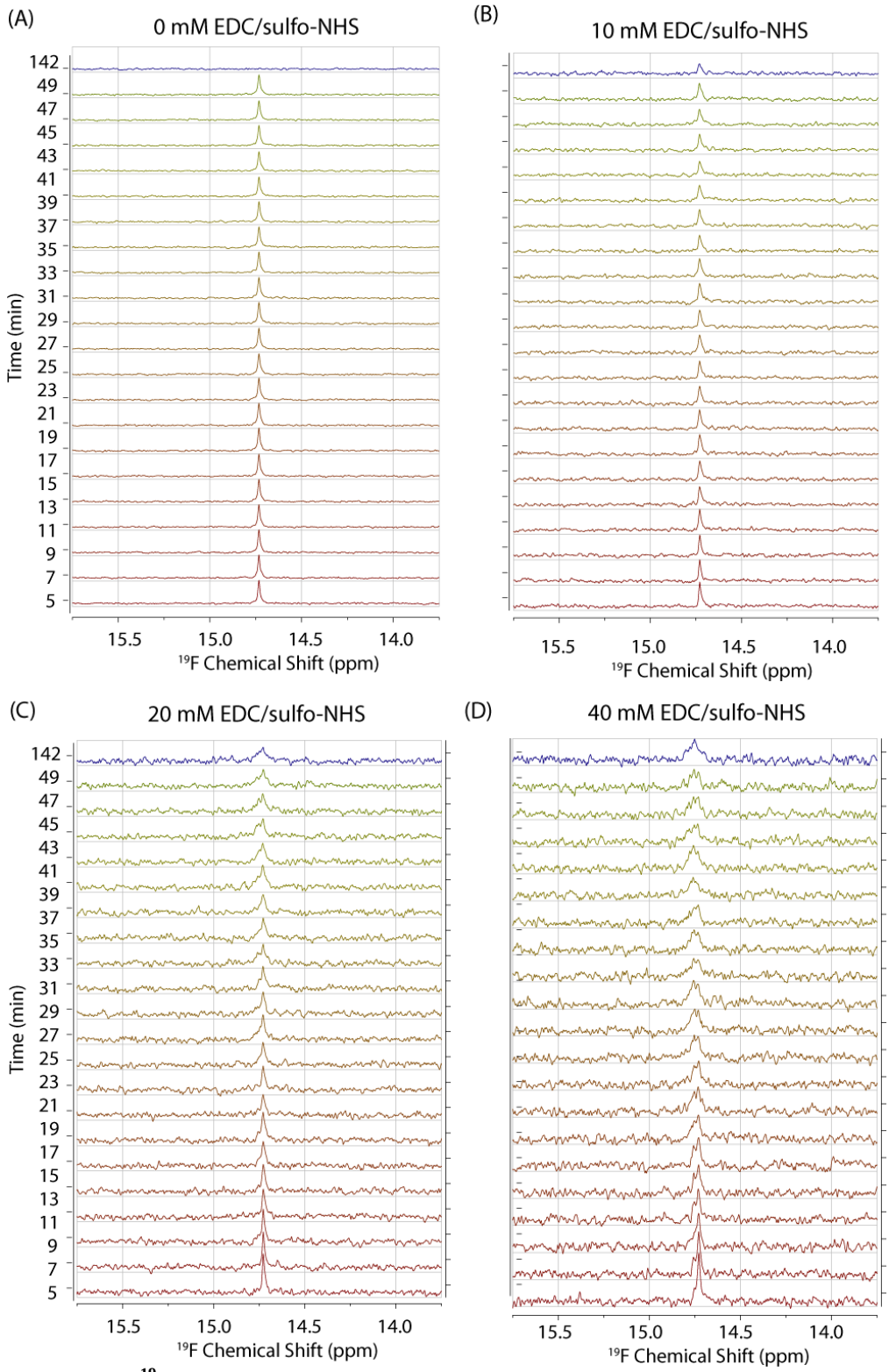

Figure S2. ^{19}F NMR detection of small oligomer formation during cross-linking by EDC/Sulfo-NHS. Intensity change with time of IAPP-tfmF23 when incubated with 0 (**A**), 10 **(B)**, 20 **(C)** and 40 mM EDC/Sulfo-NHS **(D)**. A second peak at 14.76 ppm next to the main peak at 14.73 ppm can be seen developing in the 40 mM EDC/Sulfo-NHS sample with time.

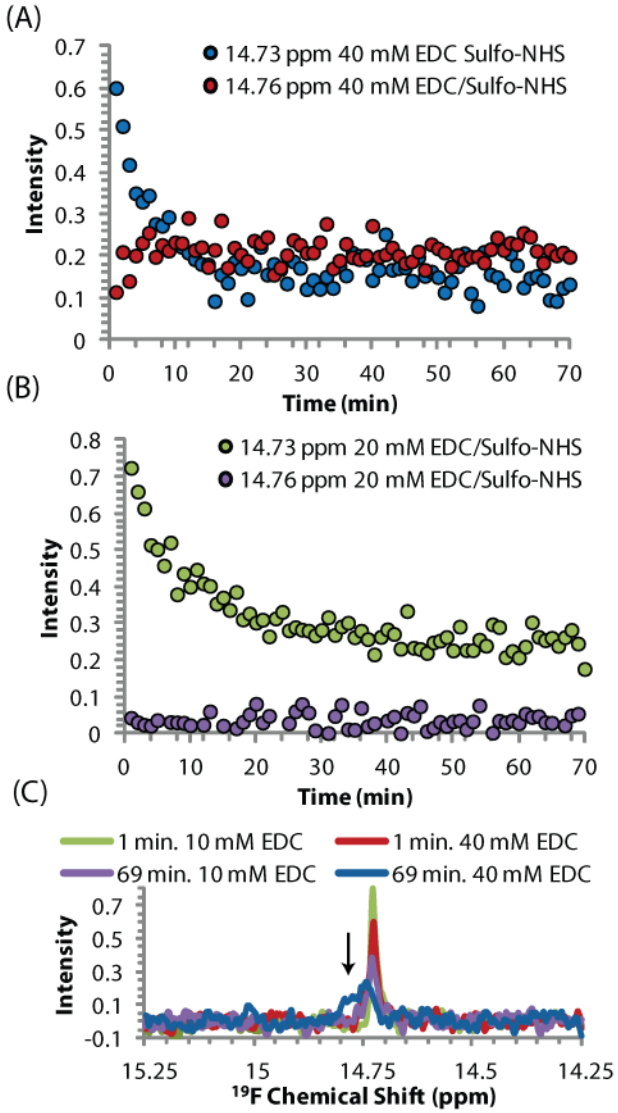

Figure S3. Changes in the ^{19}F spectra of IAPP-tfmF23 upon cross-linking. A and B: Intensity change with time of the main peak at 14.73 ppm and the second peak at 14.76 ppm with 40 mM **(A)** and 10 mM **(B)** EDC/Sulfo-NHS. **(C)** Superposition of the first and final spectra of IAPP-tfmF23 with 40 and 10 mM EDC/Sulfo-NHS. The position of the second peak at 14.76 ppm is indicated by an arrow.

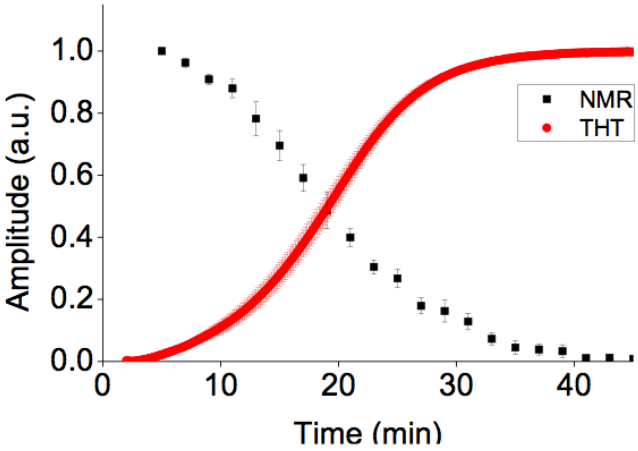

Figure S4. **Monomer consumption and fiber formation by IAPP-tfmF23 at 37 °C.** Overlay of kinetic traces from ^{19}F NMR (black), ThT fluorescence (red). Error bars indicate S.E.M. (n=4). The close correspondence between the curves suggests fiber formation closely follows monomer consumption at 37 °C, similar to the measurements at 37 °C shown in Figure 2.

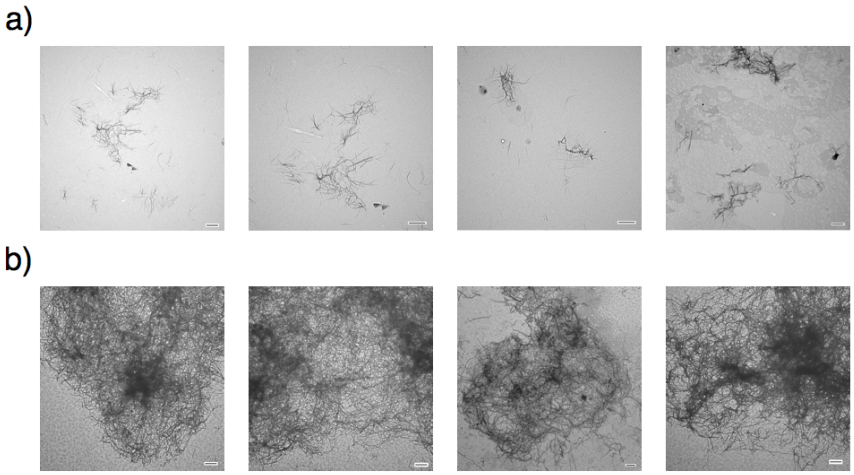

Figure S5. **Additional TEM images of IAPP-tfmF$_{23}$ fibers.** TEM images after ½ **(c)** and complete **(d)** depletion of the ^{19}F signal intensity at 25 °C. Scale bars represent 500 nm.

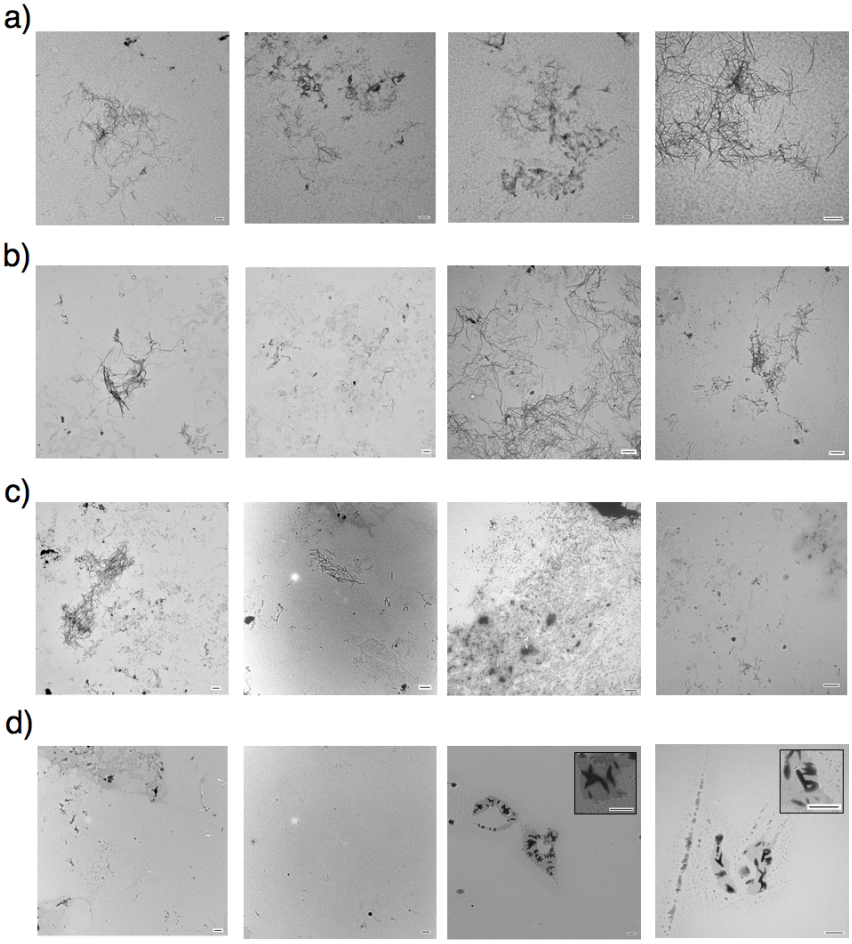

Figure S6. **Additional TEM images of IAPP-tfmF_{23} aggregation as a function of EGCG concentration.** TEM images after the complete depletion of the ^{19}F signal for IAPP-tfmF_{23} in the presence of (a) 0.2 eq, (b) 0.5 eq, (c) 1.0 eq, and (d) 5.0 eq of EGCG in the absence of ThT. Scale bars represent 500 nm.

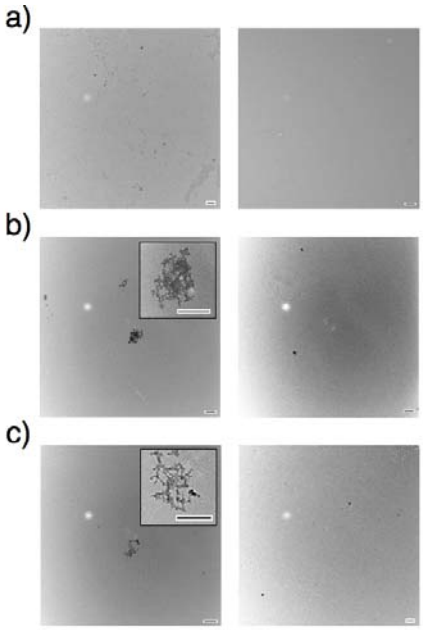

Figure S7. **Aggregate formation by EGCG in the absence of IAPP-tfmF$_{23}$.** TEM images for **(a)** phosphate buffer alone (20 mM Pi, 50 mM NaCl, pH 7.3), **(b)** 425 μM EGCG in phosphate buffer, **(c)** 425 μM EGCG with 100 μM THT in phosphate buffer. Samples were incubated for 3 hours at 37 °C before acquisition. Scale bars represent 500 nm.

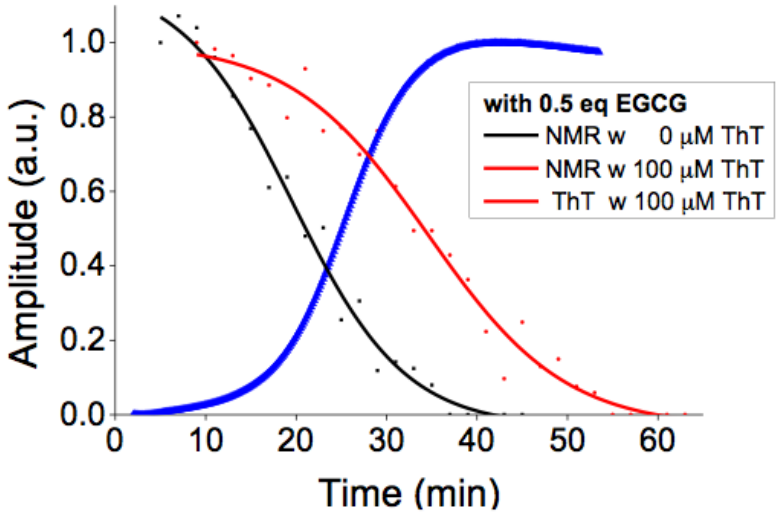

Figure S8. Competition for amyloid binding between ThT and EGCG. Overlay of kinetic traces from ^{19}F NMR (black: 0 μM ThT and red: 100 μM ThT) and ThT fluorescence (blue: 100 μM ThT) in the presence of 0.5 eq. of EGCG.

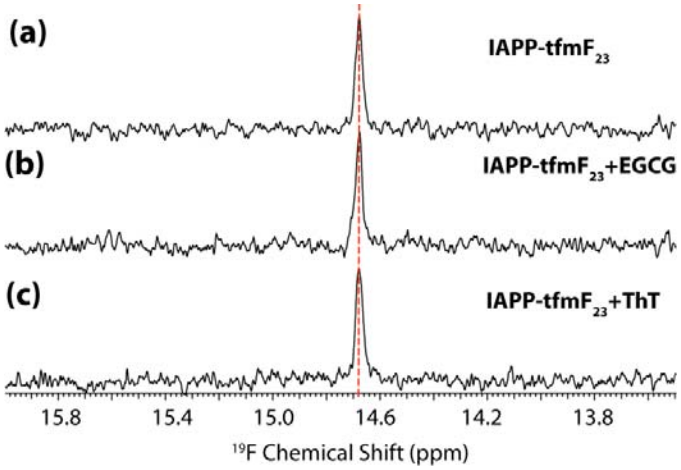

Figure S9.EGCG and ThT do not likely interact with monomeric IAPP-tfmF$_{23}$. ^{19}F NMR spectra of **(a)** IAPP-tfmF$_{23}$ alone**, (b)** IAPP-tfmF$_{23}$ with 85 μM EGCG, **(c)** with 100 μM THT **(c).** Spectra were acquired at pH 7.3, 37°C after 5 minutes of incubation.

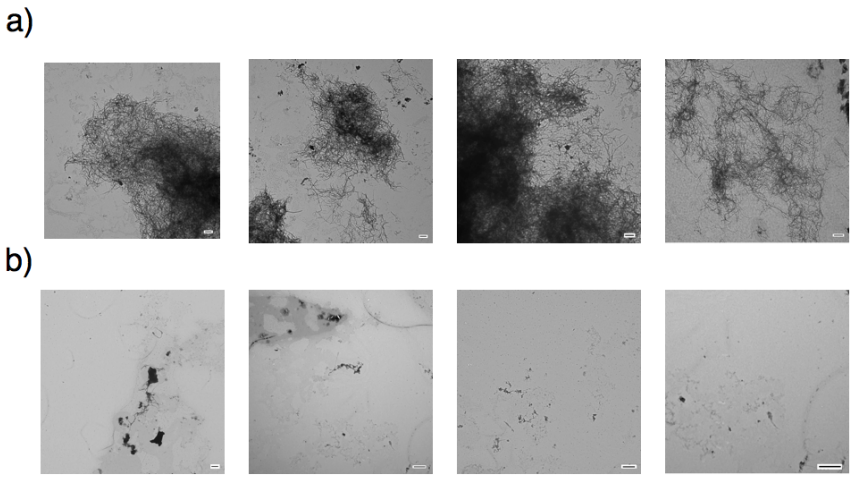

Figure S10. **EGCG disrupts preformed fibers of IAPP-tfmF$_{23}$.** Additional TEM images acquired just before the addition of 5 equivalents of EGCG to preformed fibers of IAPP-tfmF$_{23}$ (a) and after complete depletion of THT fluorescence intensity . Scale bar represents 500 nm.

Table S1

NMR/CD	ThT
$I = \frac{1}{1 + e^{\frac{x - t_{1/2}}{dx}}}$	$I = 1 - \frac{1}{1 + e^{\frac{x - t_{1/2}}{dx}}}$

25°C	**$t_{1/2}$**	**dx**
ThT	64.3±6.9	17.4±1.3
NMR	91.9±3.1	24.9±1.4
CD	91.8±6.3	22.8±1.4

37°C	**$t_{1/2}$**	**dx**
ThT	18.8±0.8	4.2±0.2
NMR	16.8±1.6	6.1±0.7