Supporting Information

Single-molecule probing of amyloid nano-ensembles using the polymer nanoarray approach

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Synthesis of FNA-Aβ (14-23) dimer

FNA polymer was synthesized using phosphoramidite chemistry on the MerMade-12 oligonucleotide synthesizer (BioAutomation, TA, USA) with the standard 200 nmol DNA synthesis protocol (DMT removal-coupling-cap-oxidation-cap). Using appropriate phosphoramidite monomers two DBCO groups were placed at a distance of 12 nm in the middle and two ends were functionalized with a biotin and a thiol group (Figure S1). The thiol group was protected with suitable protecting group, which was activated when needed. Two copies of Aβ(14-23) peptides were covalently linked with the FNA tether using metal free 'click chemistry' (Figure S1), followed by purification by reverse phase HPLC. The product was characterized by mass spectroscopy.

TAPIN measurements

Labeling of Aβ(14-23) monomer and dimer with Cy3

0.1 mg of Cysteine-HQKLVFFAED was weighted with a high precision microbalance (Sartorius AG, Germany), and dissolved in 100 µL of 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP) and sonicated for 5 minute to break pre-aggregates. The solvent was evaporated in rotary vacuum (Eppendorf Vacufuge Concentrator, Fischer scientific, USA). The peptide was

then dissolved in 10 mM sodium carbonated buffer (pH 7.8) and freshly prepared NHS-Cy3 dye was added to a molar ratio of 1:1, and stirred for 2 h in dark place. The solvent was lyophilized and the product was purified by reverse phase-HPLC (Phenomenex C18 column, 250 mm x 4.6 mm, Solvent A: 0.1% TFA in water, Solvent B: 0.1% TFA in acetonitrile). Final product was conformed by MALDI-TOF mass spectroscopy (not shown).

For lifetime measurement purpose, thiol end of the FNA-Aβ(14-23) dimer was activated with TCEP and coupled with MAL-Cy3 in 10 mM sodium phosphate buffer. The molar ratio was 1:10 and the reaction was carried out at argon atmosphere for 5 h, at room temperature and in in dark place. The unreacted dye was filtered through Amicon spin filter (molecular cut off 3K) (Amicon Ultra, Merck Millipore, USA) at 14000 rpm for 20 minutes, followed by washing with fresh water for three times. The filtrate was discarded and retained product was collected by reverse spin at 1000 rpm for 5 min. The concentration was measured by absorbance spectrometer (Nanodrop 1000 Spectrometer, Thermo Scientific, USA) using extinction coefficient of Cy3 at 555 nm (ε_{Cy3}) = 150,000 $M^{-1}cm^{-1}$.

Control experiment: photobleaching and photoblinking tests

Experimental set up for photobleaching and blinking of fluorophore is shown in Figure S2A. The glass surface was cleaned with chromic acid for 30 min and washed thoroughly with fresh water. The coverslip was placed on sample holder (PicoQuant, Berlin, Germany), and covered by a 0.1-mm-thick teflon spacer and a 25-mm-diameter quartz disk at the top. The top quartz disk has two pinholes for injecting and draining the solutions. The chambers were, first, filled with 167 μM MAS for 30 min and then ringed multiple times with water. The Cy3 labeled Aβ(14-23) peptide was pretreated with TCEP and diluted to 50 pM in 10 mM sodium phosphate buffer (pH 7). This solution was injected to the chamber and kept in dark place for 1h. Unreacted maleimide groups were then quenched with 10 mM 2-mercapto ethanol,

followed by washing with water. The holder was set up into TIRF instrument, 30 µl of 10 mM sodium phosphate buffer (pH 7) was a added to chamber and TIRF videos were recorded for 2-3 min. The video file showed no photobleaching or blinking during the experiment, which can be further verified from a time trajectory in Figure S2B.

Control experiment: Specific and nonspecific adsorption test

Experimental set up for nonspecific and specific interactions of cy3 labeled dimers are shown in Figure S3A and C respectively. The glass-coverslips were cleaned and treated with 167 µM MAS solution for 30 min, followed by ringing with water. The surfaces were then treated with 10 mM 2-mercapto ethanol in sodium phosphate buffer (pH 7) for 1h to quench the maleimide groups on the surface, followed by multiple ringing with water. The surface was placed into holder and adjusted into TIRF instrument. The nonspecific adsorption was examined at pH 7 and 3.7. The chamber was filled with 1 nM Cy3 labeled Aβ(14-23) dimer in either 10 mM sodium phosphate buffer (pH 7) or 10 mM sodium acetate buffer (pH 3.7) and TIRF images were recorded. The surface showed a few (2-4) bright spots that correspond to nonspecific of binding Cy3 labeled Aβ(14-23) dimer with the surfaces in both the pHs (Figure 3B). But when Aβ(14-23) monomer or Aβ(14-23) dimer was tethered with surfaces, around 40-50 bright spots were observed, which corresponds to specific interactions (Figure S3D**)**.

Supporting Figures

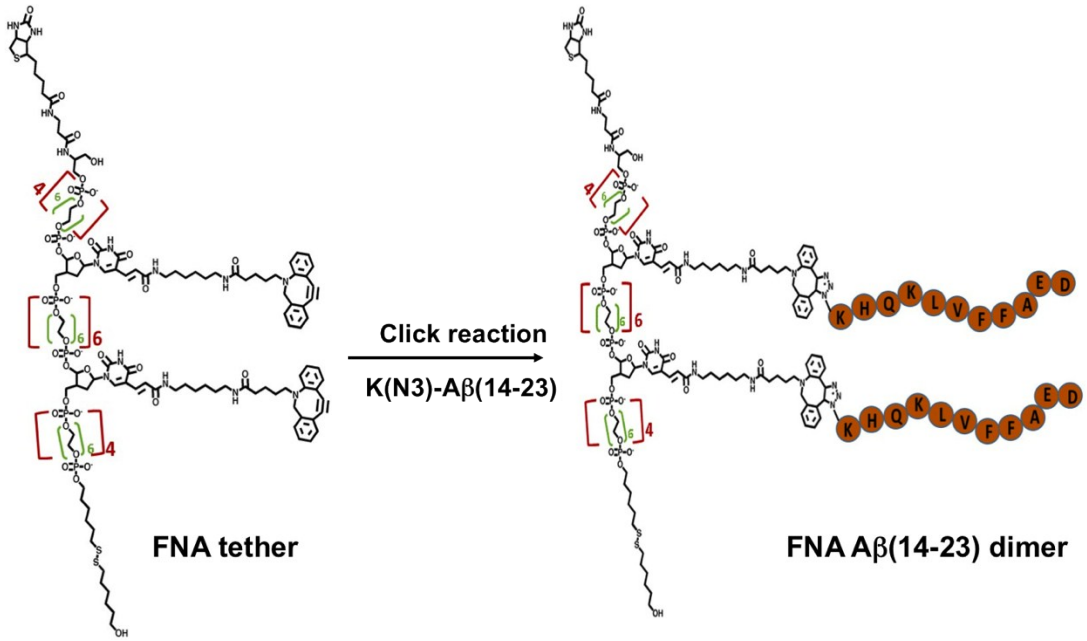

Figure S1. The scheme shows chemical structure of FNA tether and synthesis of FNA-Aβ(14-23) dimer. Two molecules of Aβ(14-23) peptide were covalently linked with two DBCO units of FNA tether via metal free 'click chemistry'.

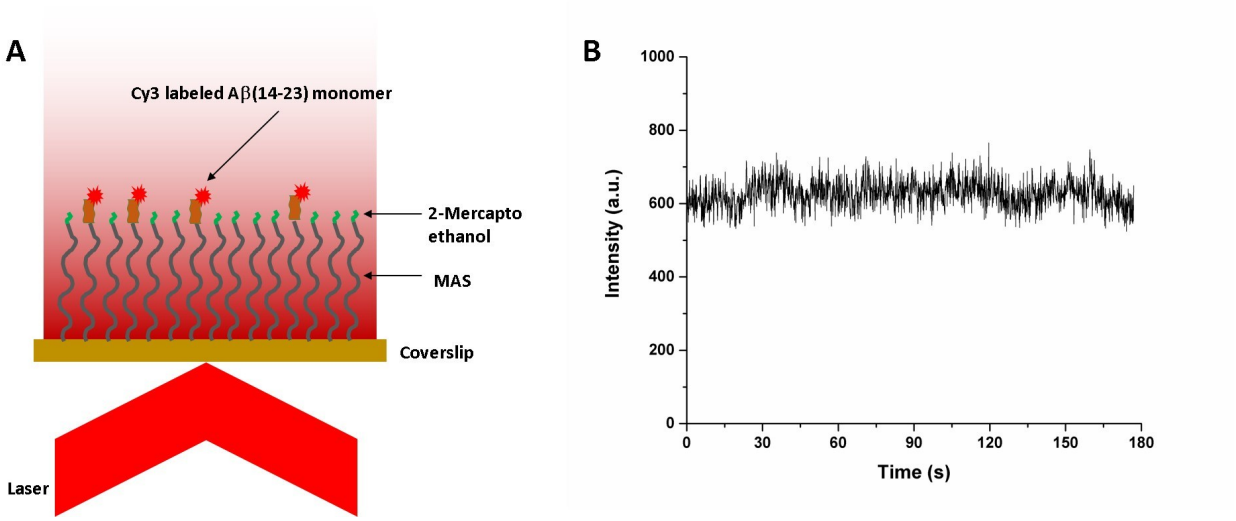

Figure S2. **Photobleaching and blinking test**; (A) Scheme shows experimental set up. (B) A fluorescence time trajectories after background subtraction, showing no bleaching or blinking under experimental condition up to 3 min.

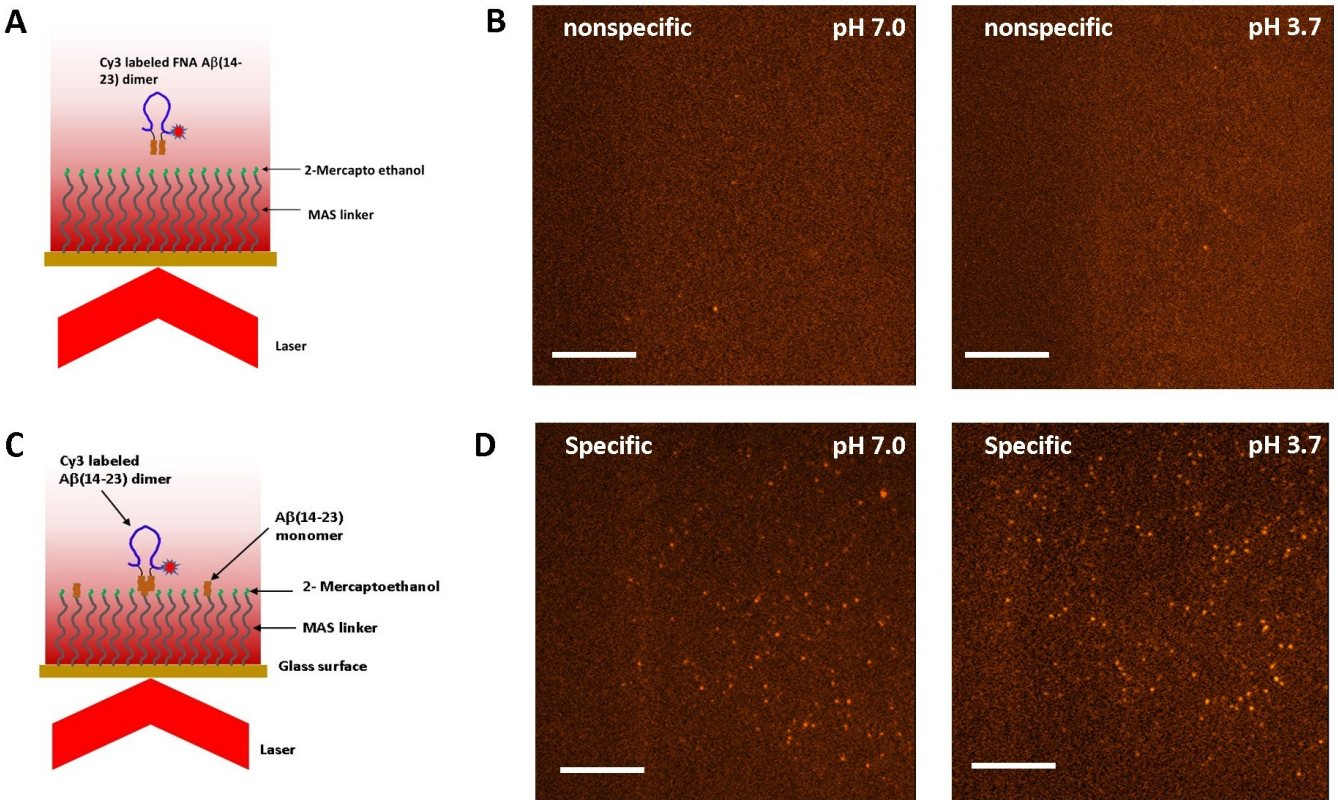

Figure S3. **Specific vs nonspecific interaction**; (A) Experimental set up for nonspecific interaction test; the surface without covalently attached Aβ(14-23) peptide (B) TIRF images from the surface show nonspecific binding of Cy3 labeled Aβ(14-23) dimer at pH 7 and at pH 3.7, which suggest a negligible amount of nonspecific binding; (C) experimental set up for specific interaction; surface contains covalently attached Aβ(14-23) monomers. (D) TIRF images of specific complexes at pH 7 and 3.7, indicating huge number of specific trimer complexes. Scale Bar 10 μM.

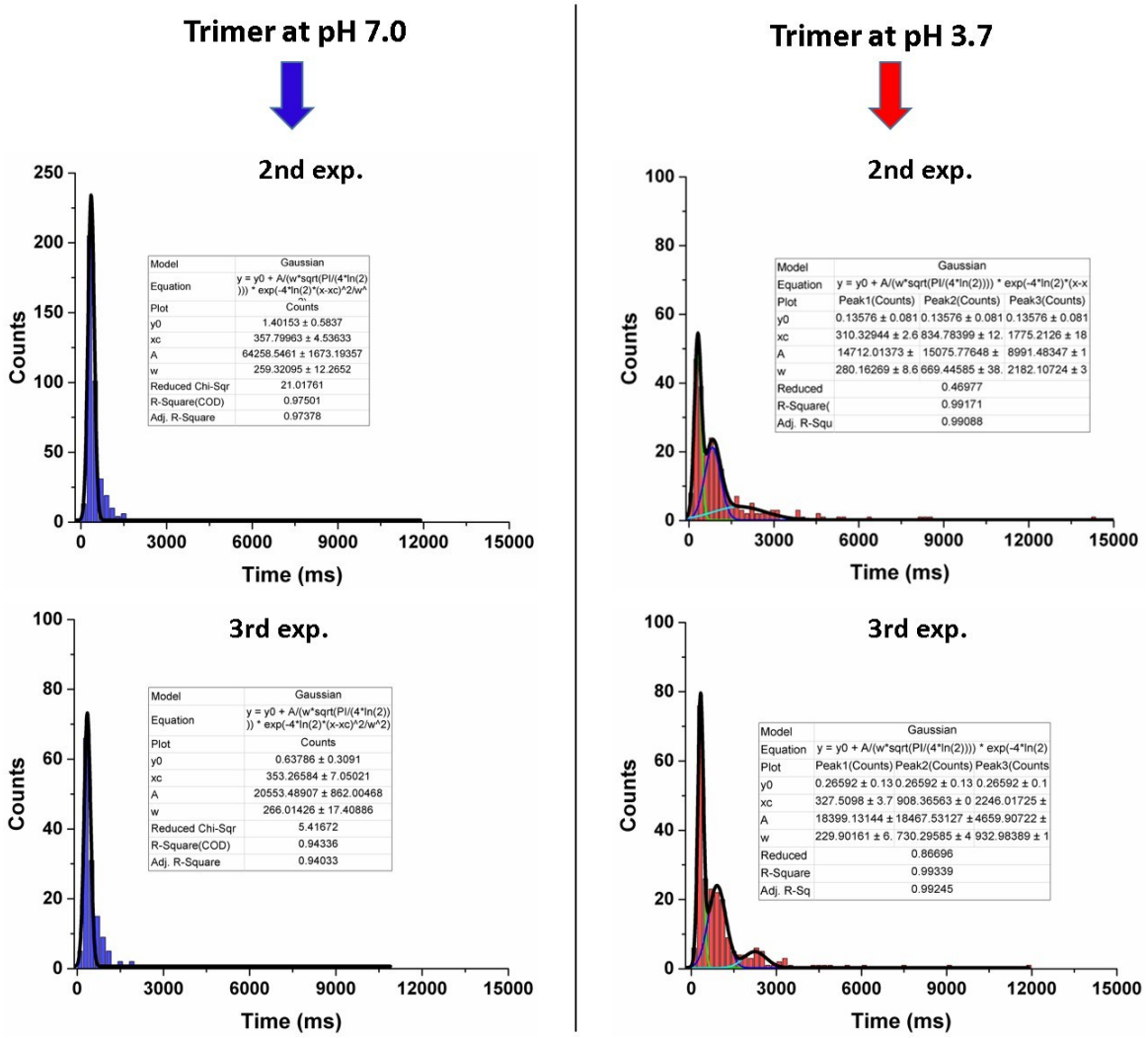

Figure S4. Additional TAPIN experiments for the Aβ(14-23) trimers at pH 7 (Left side, blue) and at acidic pH 3.7 (right side, red). The histograms were fitted with either single or three Gaussians.

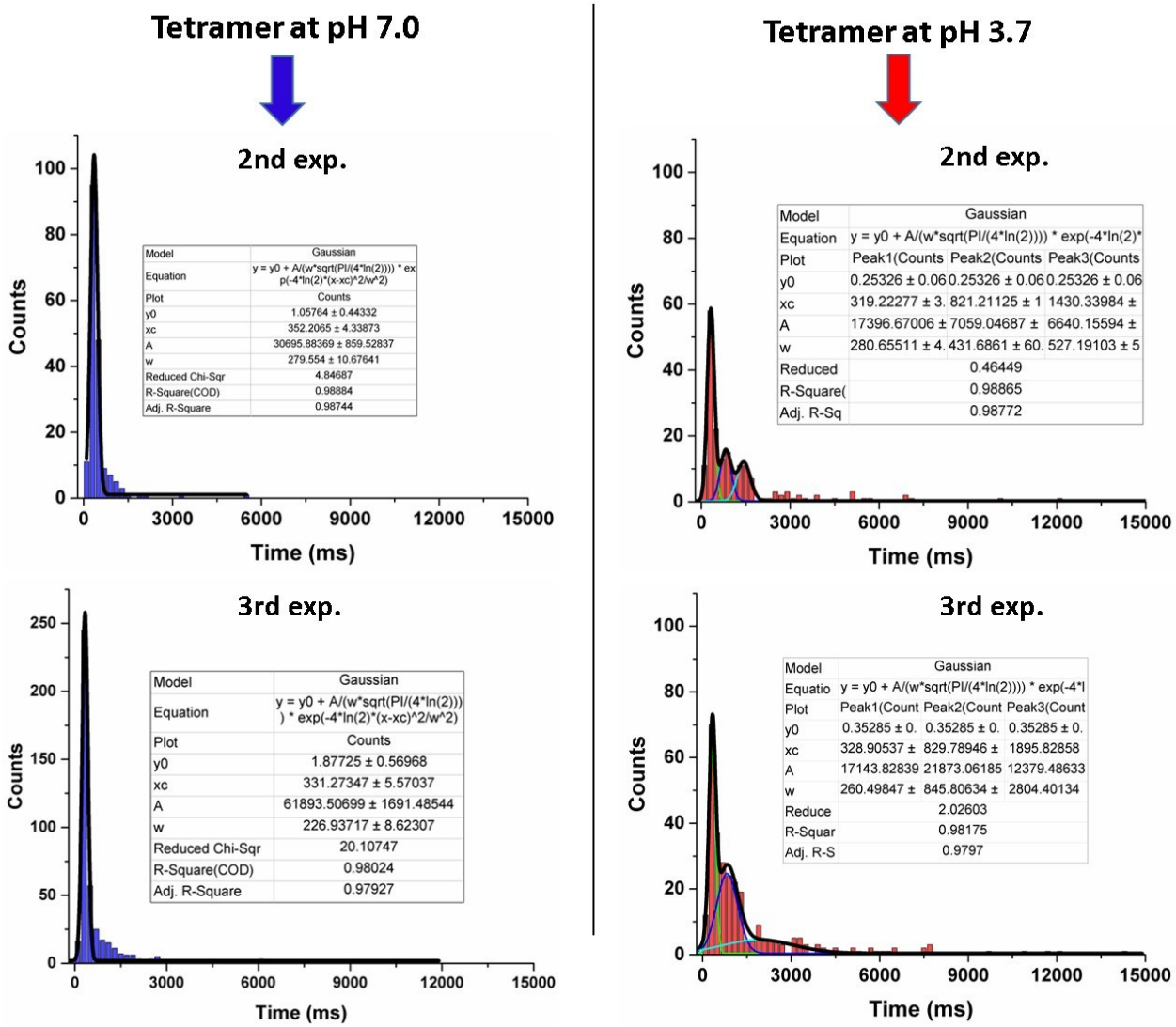

Figure S5. Additional TAPIN experiments for the Aβ(14-23) tetramers at pH 7 (Left side, blue) and at acidic pH 3.7 (right side, red). The histograms were fitted with either single or three Gaussian functions.

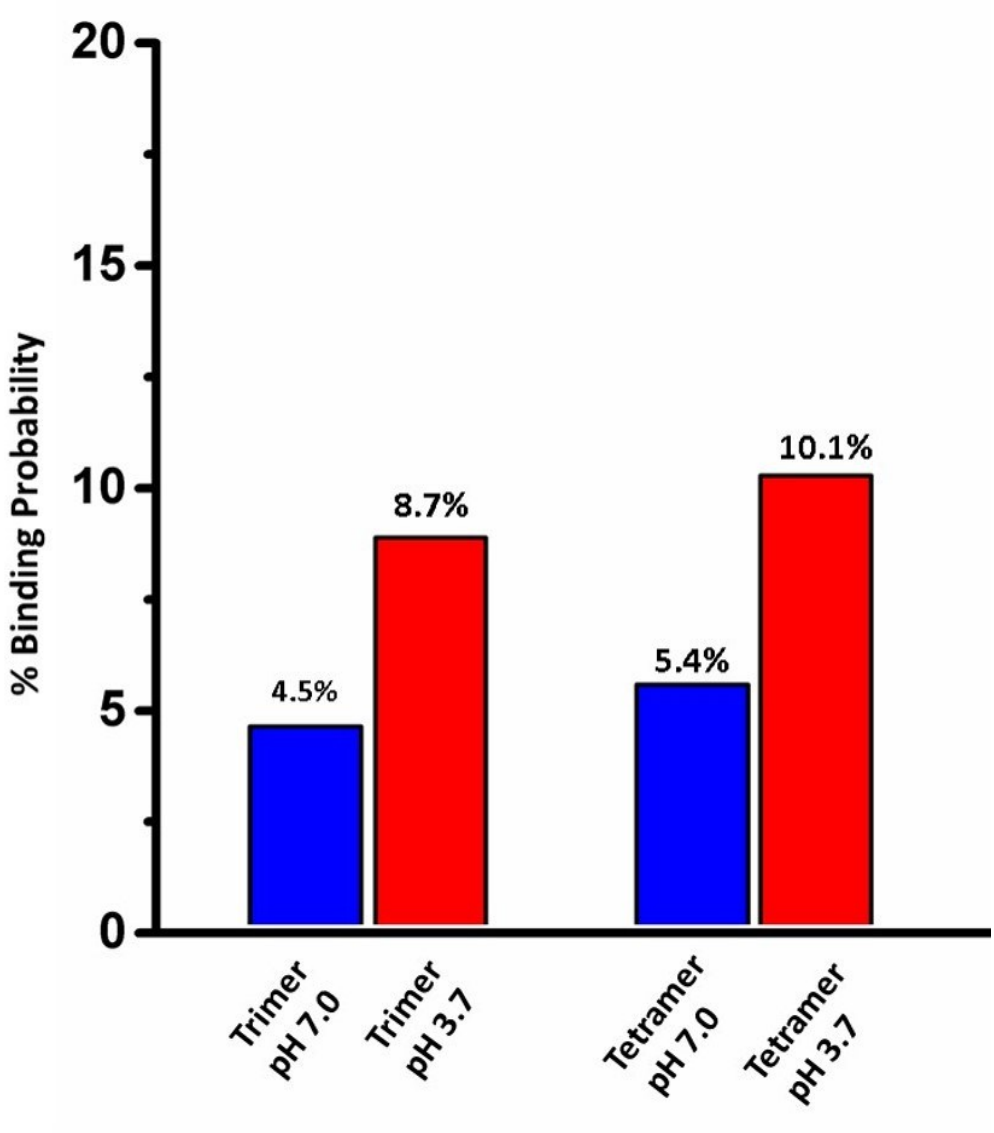

Figure S6. Binding probability (BP) of the Aβ(14-23) trimers and tetramers at pH 7 (blue bar) and 3.7 (red bar) respectively. BP was calculated from AFM single molecule force measurement by calculating percentage of F-D curve showing specific binding events over total number of F-D curve recorded.

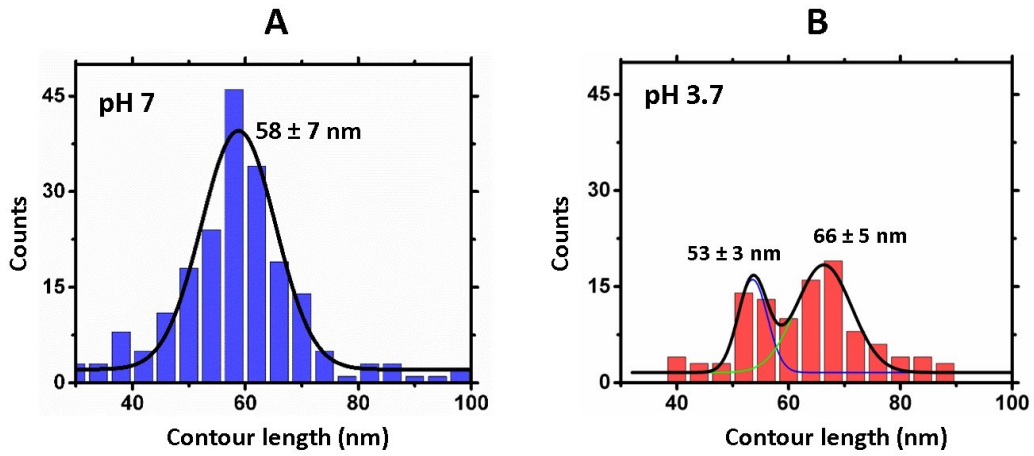

Figure S7. Contour length distribution for Aβ(14-24) tetramer; (A) at pH 7, narrow distribution fitted with single Gaussian; (B) at pH 3.7, broad distribution fitted with double Gaussian.

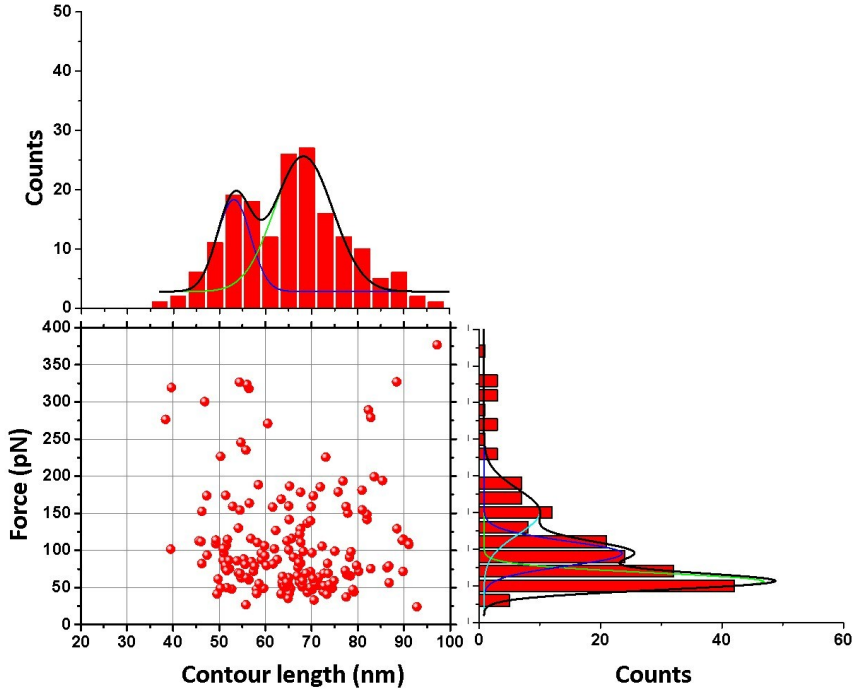

Figure S8. 2D plot Force Vs. Contour length for Aβ(14-23) trimer at pH 3.7

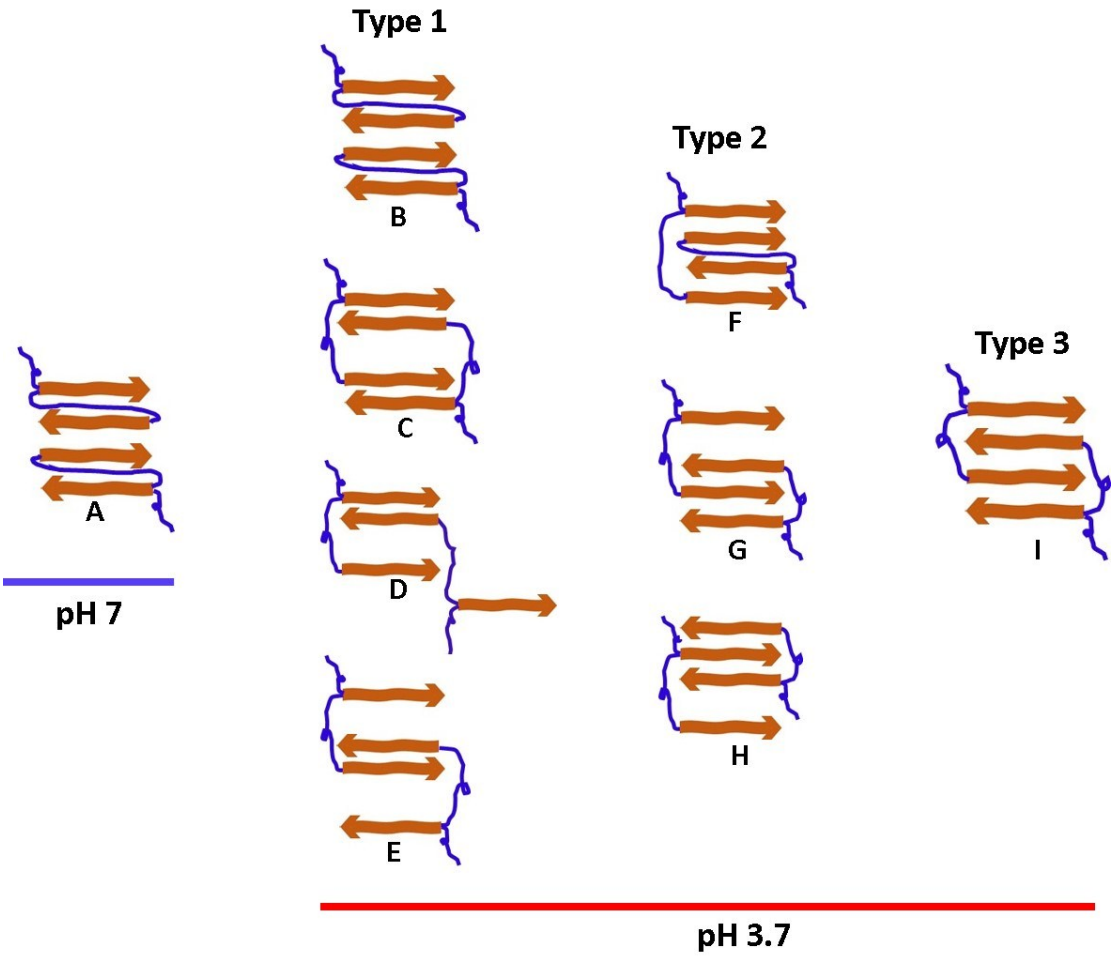

Figure S9. Schematic presentation of the hypothetical molecular arrangements for the Aβ(14-23) tetramer at pH 7 and 3.7. The blue tether and orange arrows indicate FNA and Aβ(14-23) monomers respectively.

Table S1: TAPIN lifetime measurements in three independent experiments at pH 7 and 3.7.

	Trimer, pH 7	Trimer, pH 3.7	Tetramer, pH 7	Tetramer, pH 7
Exp.1	τ=356 ± 147 ms	τ1=349 ± 99 ms τ2=902 ± 231 ms τ3=1704 ± 234 ms	τ=353 ± 167 ms	τ1=352 ± 135 ms τ2=946 ± 261 ms τ3=1702 ± 169 ms
Exp.2	τ=357 ± 129 ms	τ1=310 ± 140 ms τ2=835 ± 334 ms τ3=1775 ± 1091 ms	τ=352 ± 139 ms	τ1=319 ± 140 ms τ2=821 ± 215 ms τ3=1430 ± 263 ms
Exp.3	τ=353 ± 133 ms	τ1=327 ± 114 ms τ2=908 ± 365 ms τ3=2246 ± 466 ms	τ=331 ± 113 ms	τ1=328 ± 130 ms τ2=829 ± 422 ms τ3=1895 ± 1402 ms