Supplementary Information (SI)

Synthesis of cysteine modified Aβ-40

The cysteine modified N-terminus Aβ-40 (Cys-Aβ-40) was synthesized at 0.1 mmole scale on a CEM Liberty microwave peptide synthesizer using a Val-HMPB Chemmatrix resin with a capacity of 0.58 mmol/g. A five-fold excess of HATU activated *N*-Fmoc amino acids were used in each coupling step. Deprotections using 20% (v/v) piperidine and 0.1 M HOBt in DMF were performed at 75 °C and 35 W irradiation energy for 3 minutes for the first 18 couplings and for 5 minutes for all subsequent couplings. The first 18 residues were coupled for 5 minutes at 75 °C and 25 W irradiation energy and all subsequent couplings for 10 minutes. His and Cys residues were coupled at 50 °C instead of 75 °C. The sequences Gly-Val, Ile-Ile, Phe-Ala, Phe-Phe, Arg-His, Cys-Asp were double coupled. Cys-Aβ-40 was cleaved from the resin by stirring the peptide resin with a mixture of 80/5/5/2.5/2.5/2.5/2.5 (v/v/v/v/v/v/v) TFA/Thioanisole/Phenol/H_2O/Dimethylsulfide/Ethanedi-thiol/Triisopropylsilane for 15 minutes at 0 °C and then at room temperature for 135 minutes. The peptide was precipitated with ice cold ether. Next, the peptide was dissolved in 100 uL TFA which was then diluted with 100 mL H_2O and injected into a Vydac C4 semi-preparative RP-HPLC column for purification. The purification gradient was from 3% to 40% over 60 minutes using a 0.1% TFA in H2O aqueous phase and a 0.09% TFA in acetonitrile organic phase. The purified peptide was characterized by MALDI mass spectrometry and SDS-gel electrophoresis (Fig. S1).

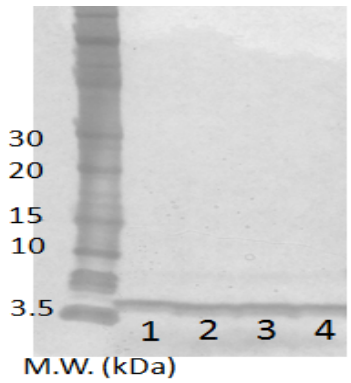

Fig. S1 SDS-PAGE gel image of Cysteine modified N-terminus of Aβ-40 (MW.~4.4 kDa) before and after addition of TCEP. Lane 1-4 are the Cys-Aβ-40 after incubation with TCEP at pH 10 (lane 1, 2) and at pH 7 (lane 3, 4). The molar ratio of Cys-Aβ-40 to TCEP is 1 to 1 (lane 1, 3) and 1 to 50 (lane 2, 4), respectively.

Nanoscale tetrahedral shaped tripodal silatrane terminating with a reactive maleimide moiety for AFM force spectroscopy

Synthesis and characterization of the nanoscale tetrahedral shaped tripodal silatrane **1** (T-silatrane; Fig. S2) incorporating a chemically reactive terminal maleimide group has been accomplished. Maleimide **1** consists of a rigid adamantane core that is substituted at the 1-, 3- and 5-positions with a semirigid 4-substituted phenylalkynylphenyl moiety. The substituent at

the 4-position of the phenyl group terminates with a silatrane unit designed to anchor the nanoscale molecule to the mica or silica surface. The 7-position of the adamantane core is substituted with a PEG-extended moiety that terminates with a reactive maleimide moiety for immobilization of thiol-terminated biological samples.

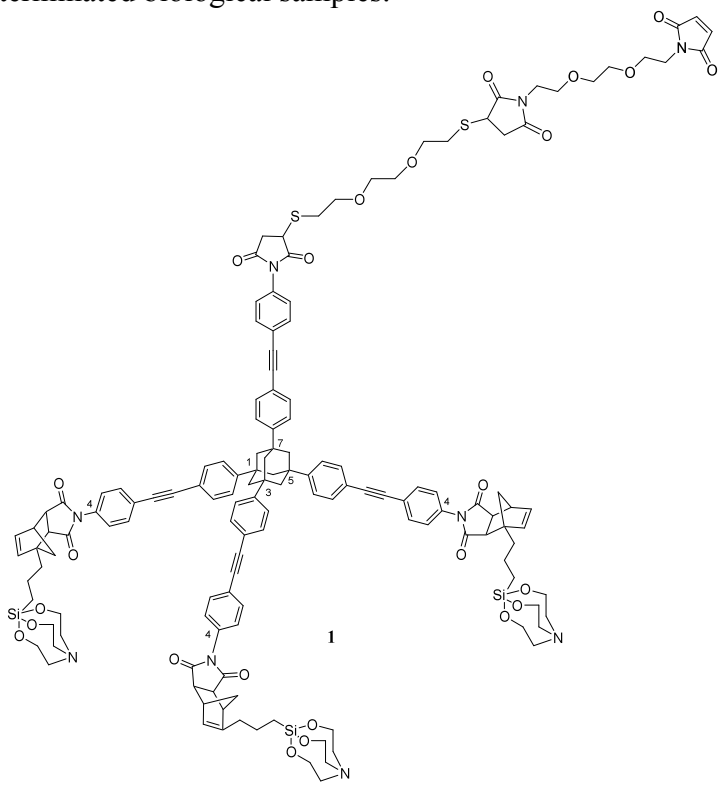

Fig. S2 Chemical formula of tetrahedral shaped tripodal silatrane.

The synthesis of cyclopentadiene-terminated silatranes **4a/4b** as a mixture is shown in Fig. S3. (3-Cyclopentadienylpropyl)trimethoxysilanes **3a/3b** were prepared as a mixture by reaction of one equiv of 3-iodopropyltrimethoxysilane **(2)** with one equiv of sodium cyclopentadienide in tetrahydrofuran at room temperature (*1*). Reaction of one equiv (cyclopentadienylpropyl) trimethoxysilanes **3a/3b** with one equiv of triethanolamine in methanol in the presence of sodium metal as a catalyst led to cyclopentadiene silatranes **4a/4b** as a mixture (*2*). That **4a/4b** is obtained as a mixture is of no consequence with regard to the AFM application of the derived nanoscale molecules.

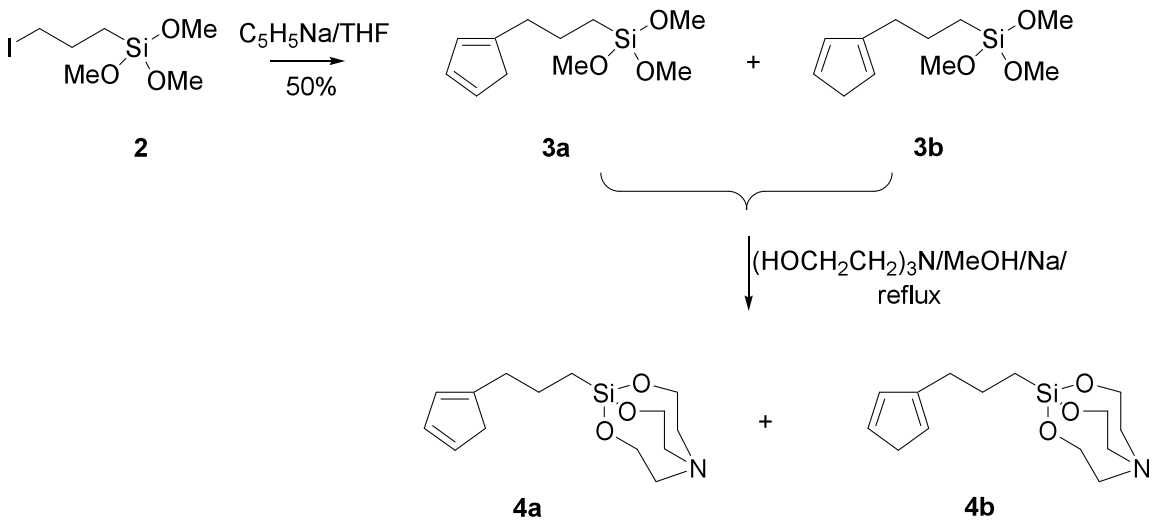

Fig. S3 Scheme of synthesis of cyclopentadiene-terminated silatranes 1.

Segment **7** **(**Fig. S4) was synthesized starting from commercially available 4-iodoaniline **(5)**. Iodide **5** was coupled with TMSA (trimethylsilylacetylene) in the presence of $Pd(PPh_3)_2Cl_2$ and CuI to afford the TMS derivative **6**, which was allowed to react with potassium carbonate in a dichloromethane/methanol to give the unprotected alkyne **7**. Tetrakis(4-iodophenyl)adamantane **(10)** was synthesized starting from 1-bromoadamantane **(8)** which was reacted with *t*-BuBr in hot benzene in the presence of $AlCl_3$ to give 1,3,5,7-tetraphenyl adamantane **(9)**. Treatment of **9** with I_2 and bis(trifluoroacetoxy)iodobenzene in $CHCl_3$ gave tetraiodide **10** (*3*). Alkynylaniline **7** then was allowed to react with a tetraiodide **10** under Sonogasira coupling conditions to give tetraamine **11**, which was treated with maleic anhydride at room temperature for 2 hours followed by cyclization in the presence of sodium acetate in acetic anhydride at 100°C for 3 hours to get tetramaleimide **12**.

Contour length distribution measured on the silatrane derivatized tetrahedral-shape molecule modified Si-wafer.

The 1 mg/ml stock solution of T-silatrane (see above) was obtained by dissolving a powder in DMSO and then diluted in DMSO/H_2O (80:20) before the use. The 30 μL of diluted T-silatrane (167 uM) was placed on the surface of cleaned wafer (1x1 cm^2) and left in a small and tightly closed chamber for overnight. Si-wafer was rinsed with DMSO and then plenty of DI water (>10 ml), dried under Ar flow and used within 1 day.

Figure S5 show the histogram of contour length distributions of Ab-40 peptides measured with the use of this substrate. The gray bars on the graph indicate the positions for the theoretical lengths of different conformational structures we discussed in the main text (Fig. 4).

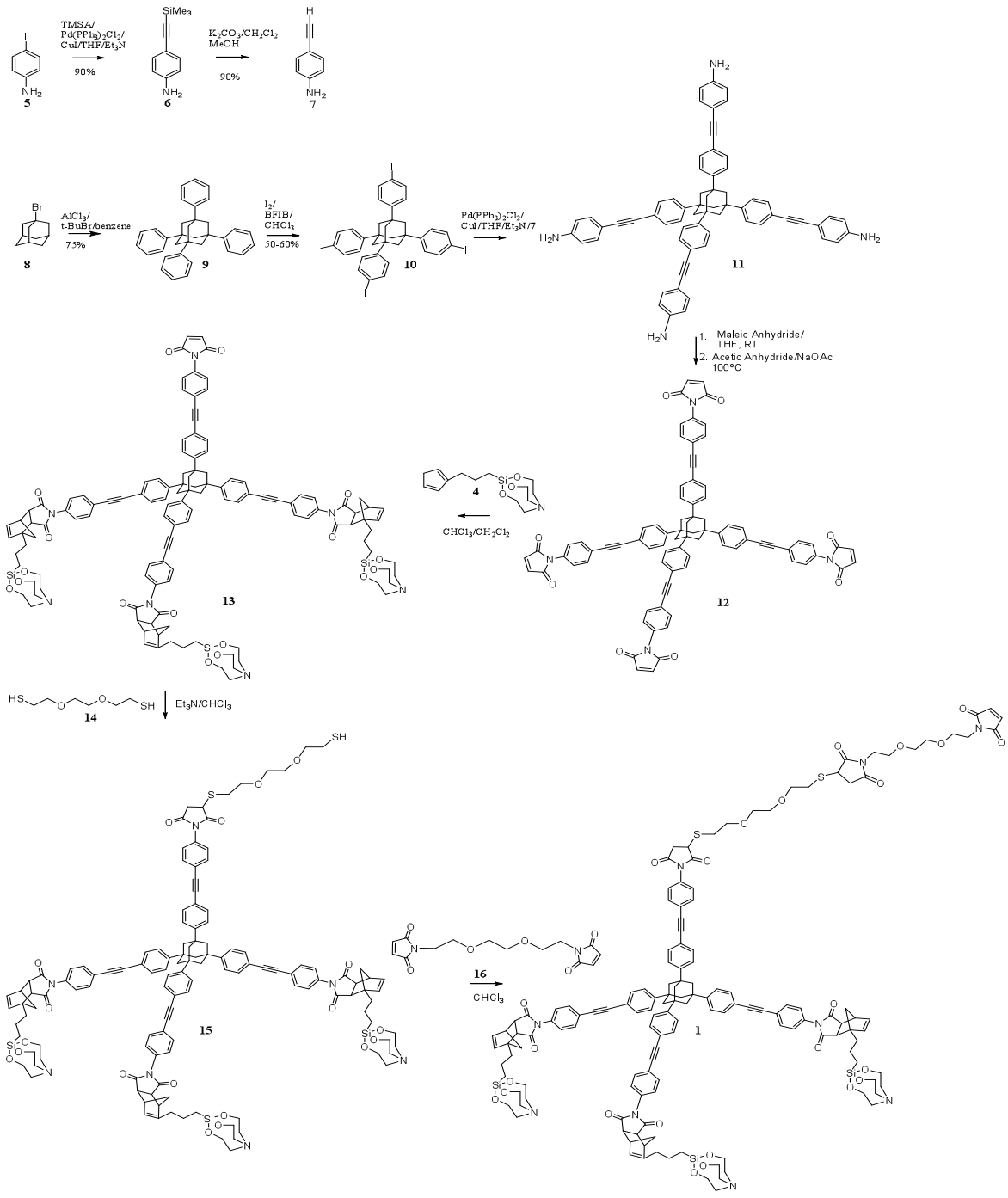

Fig. S4 Scheme of synthesis of cyclopentadiene-terminated silatranes 2

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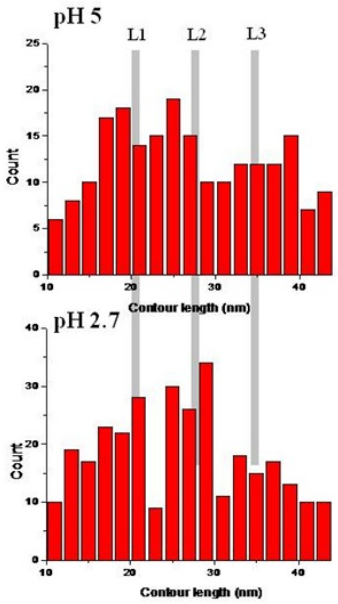

Fig. S5 Histogram of contour length measured on the substrate modified by silatrane derivatized tetrahedral-shape molecule at pH 5 (top) and 2.7 (bottom). L1, L2, and L3 are pointing the theoretical position of contour length for three different conformational structures of dimer.

Polydispersity of linker polyethylene glycol (PEG)

The L_C was calculated by WLC model and then classified with 1 nm of bin size as shown in Fig. 4. The histogram of contour length distribution was fitted by multi-Gaussian method with constant standard deviation. To calculate the total contour length including PEG, the extended Langevin function was used to estimate the length of PEG (*4*). In the Mass spectroscopy spectra of our PEG linker, the half maximum of Gaussian distribution of mass intensity was 3100 Da at lower range and 4100 Da. at higher range. The center of peak was corresponded to the evaluated value using the polydispersity measured by NMR (provided by the company). From the full width at half maximum of mass spectroscopy spectra, the polydispersity of PEG used was estimated to have 76±10 mer of ethylene glycol. The calculated PEG length including all linker molecules (APS, NHS-PEG-MAL, MAS) was 26±4 nm. This value was used to estimate the contour length of four different conformational structures we hypothesized in the text which are listed on table S1.

APS+PEG+MAS (nm)	26±4			
	L_0	L_1	L_2	L_3
Expected contour length (nm)	33±4	38±4	45±4	53±4

Table S1. Theoretical contour length based on the hypothesized conformational structures of Aβ dimer. The expected contour length is included the deviation caused by the polydispersity of linker molecules and the unstructured part of Aβ-40.

Persistence length distribution

Figure S6 shows the typical force-distance curve (FDC) fitted by WLC model (A) and the histogram of persistence length distribution measured at pH 5 (B). (A) FDC was measured at pH 5 and calibrated the retracting trace as zero force line before. The contour length of this FDC was ~40 nm and the persistence length was 0.18 nm which was used as an adjustable parameter. (b) The histogram showed the distribution of the persistence lengths measured at pH 5, which hasa mean value of 0.16 ± 0.1 nm. (see the detail in text).

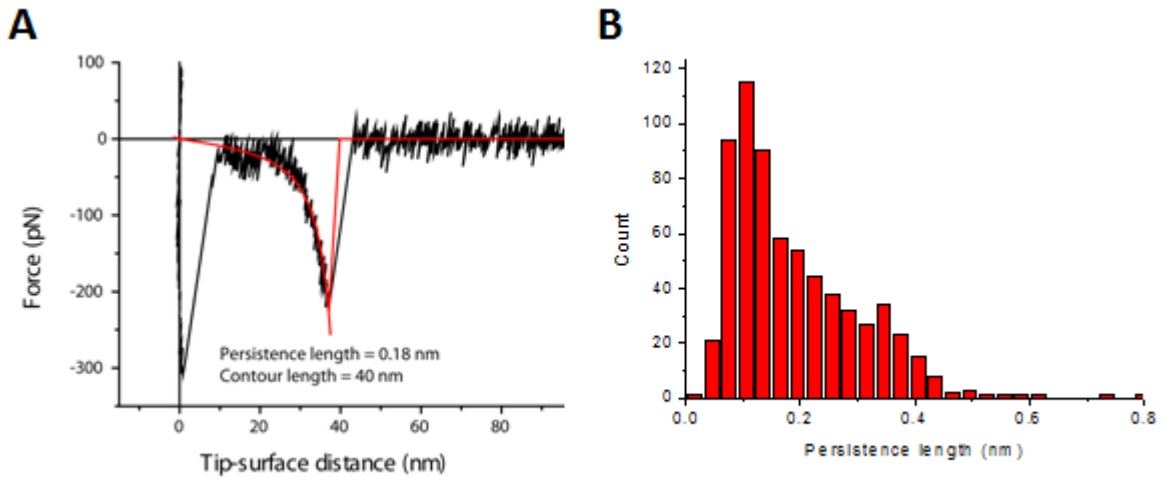

Figure S6 A) Specific force-distance curve. B) Histogram of persistence length distribution measured at pH 5

References

1. Booth, B. L. O., G. C.; Stacey, C.; Tai, P. J. T. (1986) Silica-supported cyclopentadienyl-Rodium(I),-Cobalt(I), and -Titanium(IV) complexes., *Journal of Organometallic Chemistry 315*, 143-156.
2. Shlyakhtenko, L. S., Gall, A. A., Filonov, A., Cerovac, Z., Lushnikov, A., and Lyubchenko, Y. L. (2003) Silatrane-based surface chemistry for immobilization of DNA, protein-DNA complexes and other biological materials, *Ultramicroscopy 97*, 279-287.
3. Li, Q., Rukavishnikov, A. V., Petukhov, P. A., Zaikova, T. O., and Keana, J. F. (2002) Nanoscale 1,3,5,7-tetrasubstituted adamantanes and p-substituted tetraphenyl-methanes for AFM applications, *Org Lett 4*, 3631-3634.
4. Oesterhelt, F., Rief, M., and Gaub, H. E. (1999) Single molecule force spectroscopy by AFM indicates helical structure of poly(ethylene-glycol) in water, *J New Journal of Physics 1*.