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Force Clamp Approach for Characterization of Nano-assembly in Amyloid beta 42 Dimer

Sibaprasad Maity and Yuri L. Lyubchenko*

Department of Pharmaceutical Sciences, University of Nebraska Medical Center,

986025 Nebraska Medical Center,

Omaha, NE 68198-6025, United States.

To whom correspondence should be addressed

Email: ylyubchenko@unmc.edu

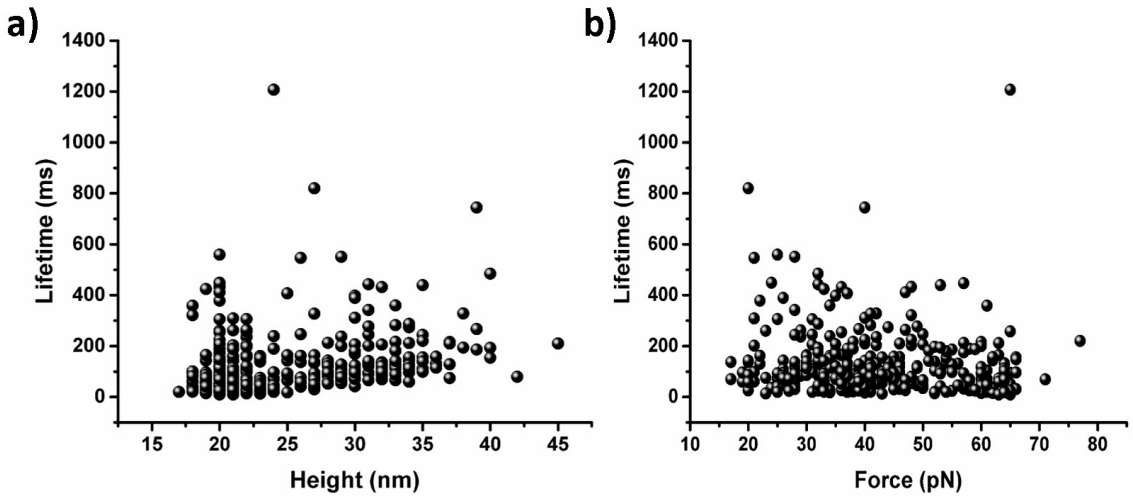

Fig. S1. Force clamp data were acquired in force 30, 40, 50 and 60 pN and data were combined and presented here as (a) height vs. lifetime and (b) force vs. lifetime.

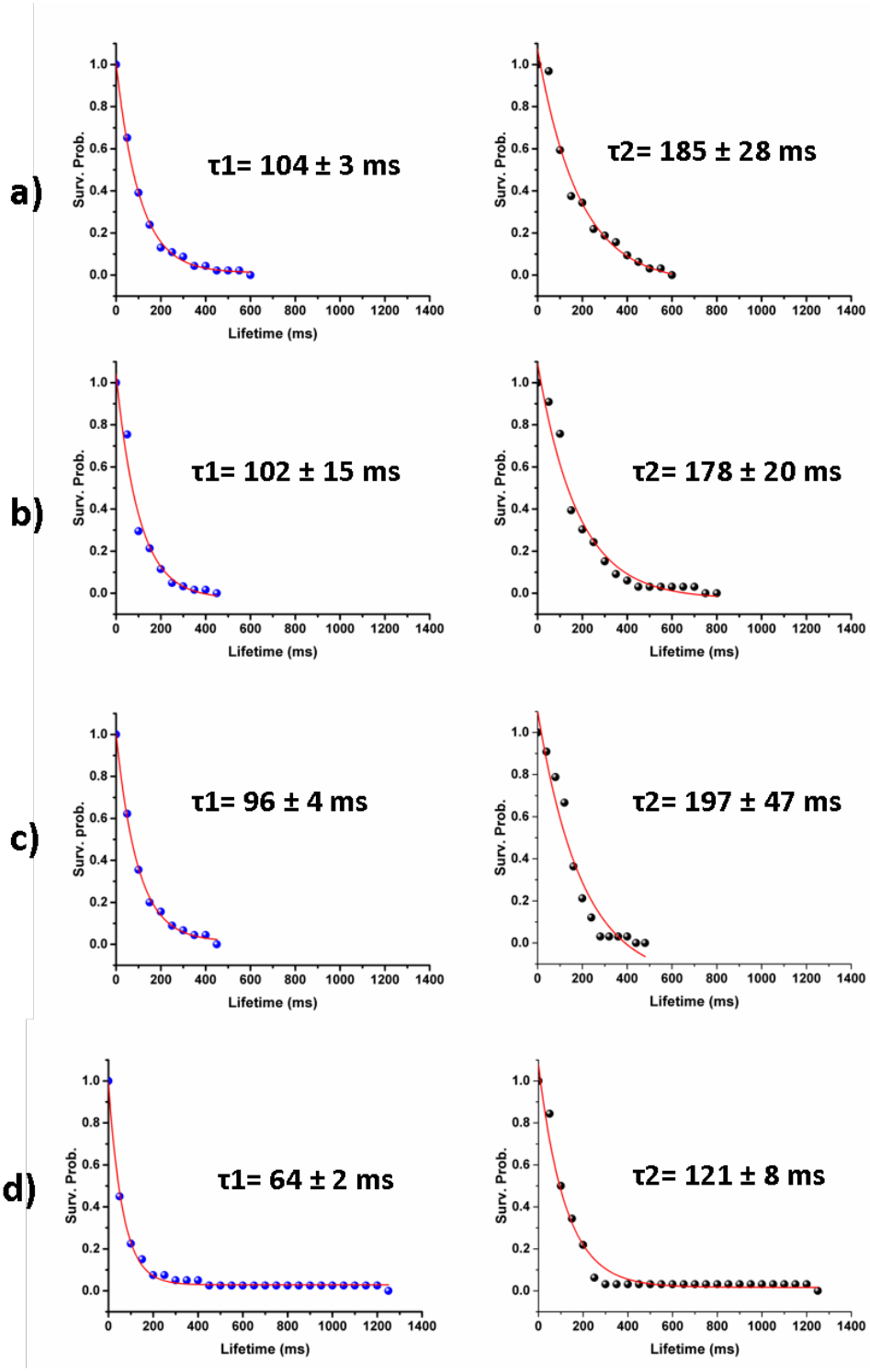

Fig. S2. Survival lifetime plot for two types of Aβ42 dimers in the force of (a) 29.9 ± 2.9 pN, (b) 39.4 ± 2.5 pN, (c) 49 ± 2.9 pN, and (d) 60.6 ± 2.9 pN. Blue (left) and black (right) data point corresponds to 1st and 2nd population respectively. The red line in each graph indicates single exponential fitting.

AFM tip functionalization and validation of method

Low density of probes on the AFM tip and targets (mica surface) is a critical factor for the single molecule probing experiments. We use very low concentrations of reagents (aminopropyl silatrane, APS or aminopropyltriethoxy silane, APTES) to achieve a low density of amines on the surface. According to the estimates made in (Lyubchenko and Shlyakhtenko 1997), the mean distance between the amino groups on the surface is between 10-30 nm. Importantly, the second step after APS functionalization surface functionalization, as described in the paper, involves the use of 1:10 mixture of reactive PEG with a non-reactive one. It was made intentionally to further decrease the density of proteins on the surface, which bind to reactive PEG residues only. As to the tip functionalization, we intentionally decrease the APS concentration for the tip treatment to make this distance between the amines even larger. The support for the low coverage density by the probe molecules on the AFM tip can be obtained by the analysis of the contour length values as the position of the rupture peak depends on the probe location on the tip. Given that the largest rupture length corresponds to the dimers assembled by the molecule located at the apex of the tip and the monomer on the surface, probes located non-apically produce rupture events with smaller rupture length. To test the contribution from the molecules located away from the apex of the AFM tip and simplify the analysis, we performed experiments with a short peptide Aβ(14-23), which according to our previous experimental studies and computational modeling the dimer of Aβ(14-23) ruptures by one-step mechanism (Zhang and Lyubchenko 2014). The data are shown below in Fig. S3.

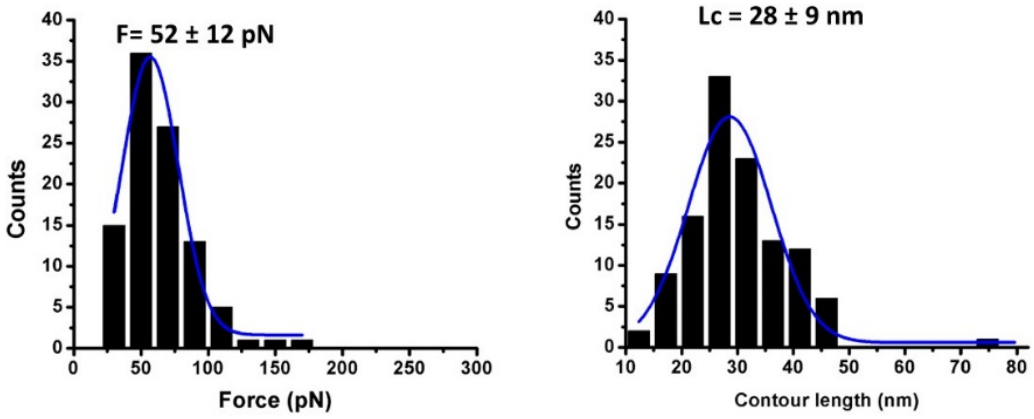

Fig. S3. AFM force spectroscopy for Aβ(14-23) monomer-monomer interaction; (a) force histogram (b) contour length histogram. Blue lines are Gaussian fittings.

AFM tip was functionalized with Aβ(14-23) monomer via a short maleimide silatrane (MAS) linker and the same monomer was immobilized on the surface with via PEG linker with MW 3400 g/mol. The force distribution is narrow as expected for the single-pair rupture events. The contour length distribution has only one peak with the maximum at 28 ± 9 nm. This value perfectly coincides with the contour lengths PEG tether (25 nm) and MAS linker (3-4 nm). There are a few factors contributing to the width of the

lengths distribution. First, according to the manufacturer, the molecular weight distribution for PEG corresponds to the contour length 25 ± 5 nm, which is a half of the experimentally determined distribution in Fig. S3. Second, we should take into account such factors as a thermal drift and inadvertently stretching molecules at an angle while measuring only the vertical component of the force evaluated in the recent paper (Walder, Van Patten et al. 2018), which according to the pulling experiments with the use of long DNA tethers can lead to the contour length distribution as broad as 50%. Thus we conclude that the data in Fig. S3 on the narrow distribution of the contour length support our conclusion on the low density of coverage of the tip surface with probes, so the closest probe to the mica surface produces the rupture events measured in the experiments. We came to a similar conclusion in paper (Kim and Lyubchenko 2014) in which rupture events of Aβ 42 dimers based on the contour length analysis was made.

Additional support to the conclusion on the selective interaction with the molecule on the surface the closest probe on the tip comes from the analysis of the contour length distribution profiles. As it is seen from Fig. 2 in the paper, the contour length distribution for the rupture Aβ 42 dimers is broad with the overall asymmetric pattern. Very similar asymmetric pattern was reported in our previous paper (Kim and Lyubchenko 2014) in which the rupture of Aβ 42 dimers was studied at different conditions including different pH values. We found that the rupture length pattern remains broad at acidic and neutral pH values, but the contour lengths patterns at these conditions were different. As one can see from Fig. 4 of paper (Kim and Lyubchenko 2014), the rupture length profile changes dramatically when the pH value changes from pH7 to pH2.7. This finding suggests that the pattern of the contour length distribution is defined by the interaction of specific segments of monomers within the dimers and this interaction pattern depends on pH. Importantly, the comparative experiments were performed with the same tip-surface pair, just the solution was replaced. Our computational modeling of the AFM rupture experiments with Aβ 42 dimers (Zhang, Hashemi et al. 2016) is fully in line with models proposed in (Lv, Condron et al. 2013, Kim and Lyubchenko 2014). Moreover, in paper (Lv, Roychaudhuri et al. 2013) we analyzed three mutants of Aβ42 along with the wild types. The rupture patterns for the wild type Aβ42 and for the mutants were very different from each other. Note also that rupture patterns for the wild type Aβ42 was very similar to the results in this paper.

Such a perfect correlation of the data obtained in the lab on the same system (Aβ42 dimers) at different times and different people points to a highly reproducible feature of the developed procedures for the functionalization of tips and surfaces, which we would like to emphasize as well.

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