Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Membrane affinity of individual toxic protein oligomers determined at the single-molecule level

Simli Dey[a], Anirban Das[a], Arpan Dey[a], and Sudipta Maiti*[a]

[a] Department of Chemical Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Mumbai 400005, India.

Supporting information:

SI_1: MALDI mass spectrum of lipid-tailed tris rhodamine labeled KSQKTTKI

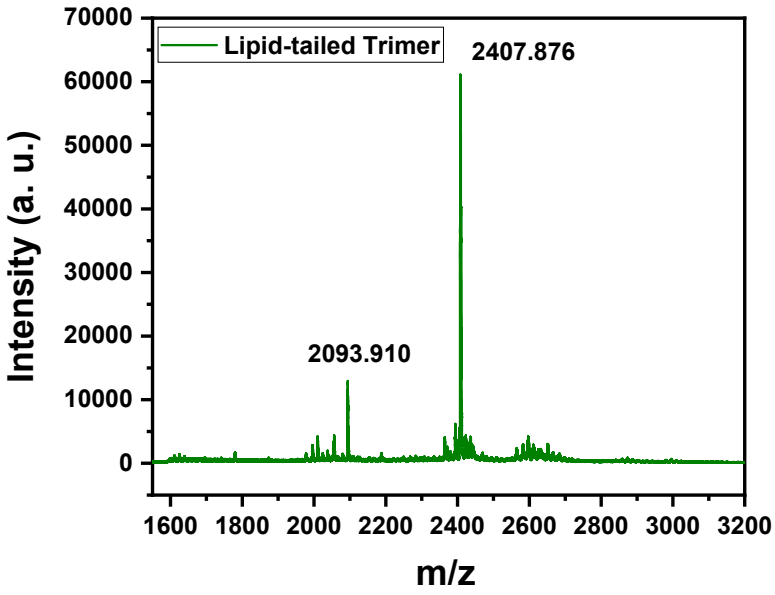

Fig. SI.1. MALDI spectrum of lipid-tailed tris-rhodamine labelled KSQKTTKI.

SI_2: Pooling of IAPP oligomers

The pooling of counts is done to improve the statistics. However, to provide a measure of the experimental variability, we have divided all the repeats into two datasets with an approximately similar number of counts. The distribution in Fig. SI.2 shows the degree of variability between the two halves is not large.

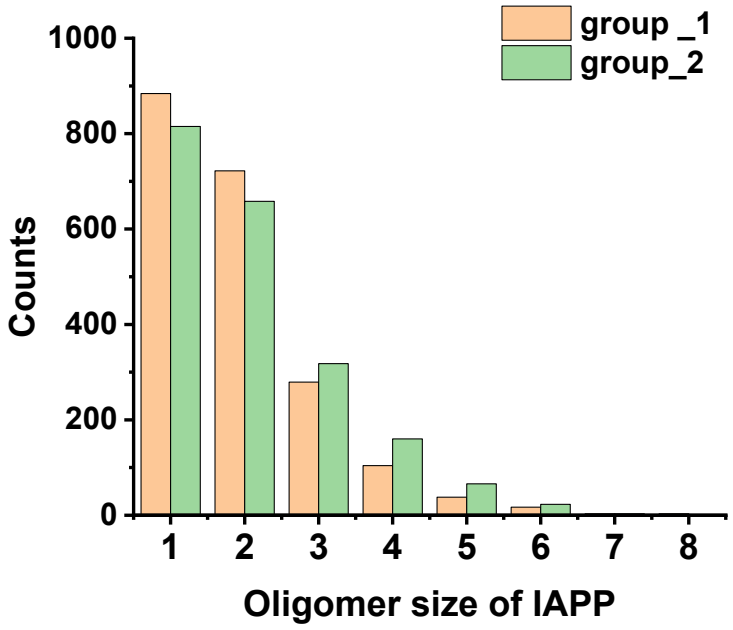

Fig. SI.2. Estimation of variability in the data. The orange and green bars show the distribution of IAPP oligomers in membrane, obtained from total data divided into two sub-groups.

SI_3: Final distribution is independent of laser power

To verify this, we have performed experiments with two different laser powers now (1.47 mW and 0.7 mW). We calculated the pre-bleaching probability at the different laser powers from tris-rhodamine labeled QKTTKI peptide by embedding it in polyvinylalcohol (PVA) matrix. We first imaged the standard sample at the two different laser powers. We then randomly divided the images into two groups, for each laser power. We calculated the B-value from one subgroup, and applied it to the other. The B-values obtained from two different laser powers are 50% (at high power) and 40% (at low power) respectively. After applying the correction, we obtained the trimeric population shown in Fig. SI.3 (black and red dashed line). We see that the percentage populations do not differ substantially between the two powers, so our results remain valid.

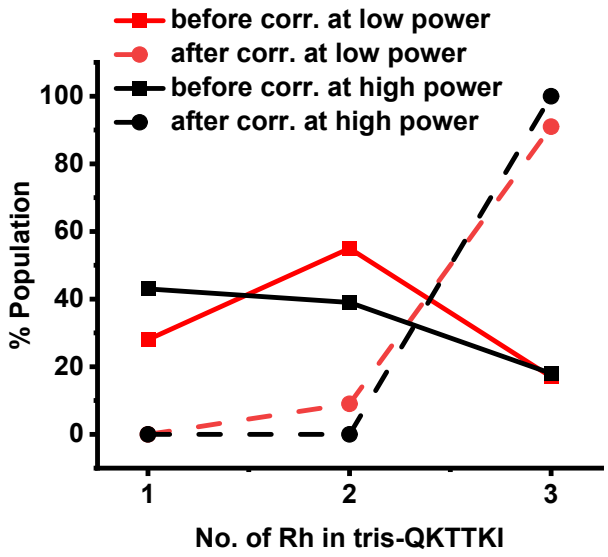

Fig. SI.3. Correction for prebleaching on the population obtained from standard tris-Rh-QKTTKI peptide as a function of laser power 0.7 mW (shown in red) and 1.47 mW (shown in black). Solid-squares and circles represent population before and after correction respectively at the two powers.

SI_4: Substantial bleaching observed in spite of using focus stabilization

In principle, pre-bleaching can be reduced if we are able to focus on the sample with minimal exposure. A focus stabilization system, which allows one to focus at one place of the object and move to another place while the focus is maintained, offers such an opportunity. We performed additional experiments with a "perfect focus system (PFS)" (Nikon) to test this possibility. We focused first at a region (power at back focal plane ~1.4 mW), and adjusted the PFS. For the actual measurements, we shuttered the excitation beam before moving to a different region, while the PFS took care of the focusing. We started recording the images before opening the shutter. We imaged the standard lipidated tris rhodamine-labeled sample on the membrane bilayer and estimated the B value. The observed histogram is shown in Fig. SI.4, which yielded a B value of 0.41, which is still substantial, and would therefore require pre-bleaching correction. So it appears that a substantial amount of pre-bleaching happens prior to the experiment. Therefore, the B value correction cannot be done away with.

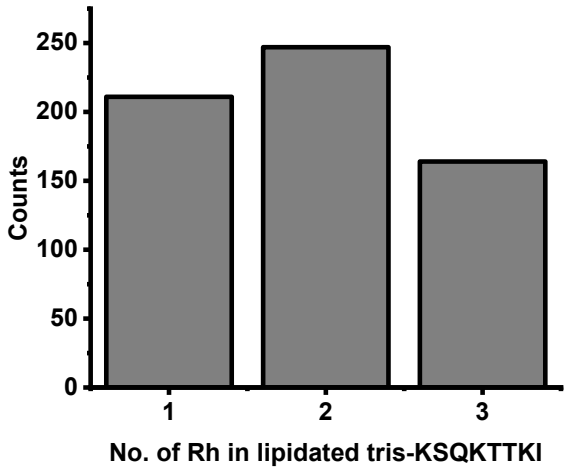

Fig. SI.4. Population histogram of prebleaching measurement of standard tris rhodamine labeled peptide using PFS and mechanical shutter

SI_5: IAPP stoichiometry is independent of time for incubation times up to ~1 hour

We have performed measurements as a function of incubation time. We observe that the data recorded with 30 minutes and 60 minutes of incubation are not substantially different at such low concentrations (shown in Fig. SI.5). All the data reported in the manuscript used 20 minutes of incubation time. So it is valid to presume that we are looking at direct oligomer attachments from the solution, and not post-attachment aggregation in the membrane.

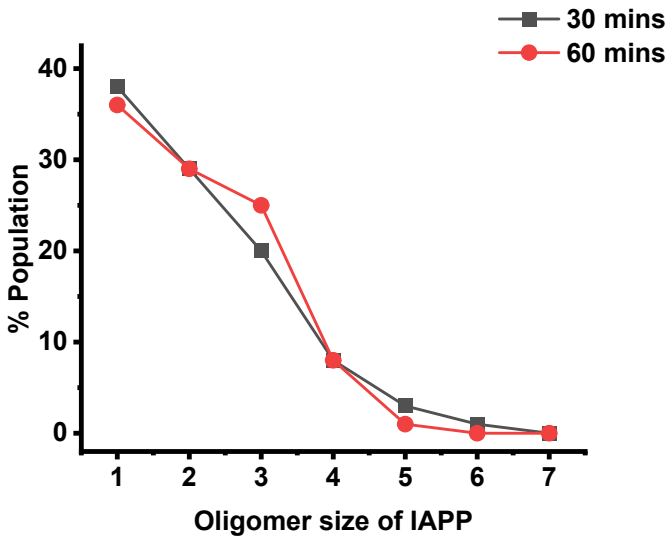

Fig. SI.5. Population histograms of IAPP oligomers on membranes at different incubation times.

SI_6: Monomerisation of IAPP

Freshly prepared rhodamine labeled IAPP oligomeric solution of ~40 nM concentration in PBS was taken to start the monomerization of IAPP. The size of IAPP oligomers was characterized using FCS to be ~1.7 nm. The IAPP oligomeric solution was kept in a centrifuge tube and gently rotated until nearly complete monomerization, which is characterized by the persistent size of the IAPP species (~0.8 nm) over ~90 days. After this the solution was subjected to smPB experiments. The solution which was taken as a monomer solution of IAPP contains ~35% of dimer, ~6 % of trimer and negligible quantity of higher oligomeric population.

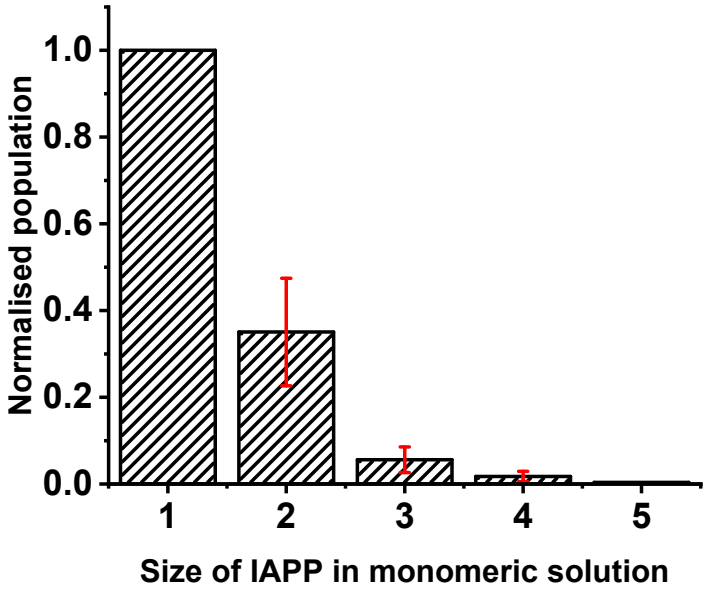

Fig. SI.6. Normalised population histogram of monomerized IAPP solution.