

Surface passivation with a perfluoroalkane brush improves the precision of single-molecule measurements

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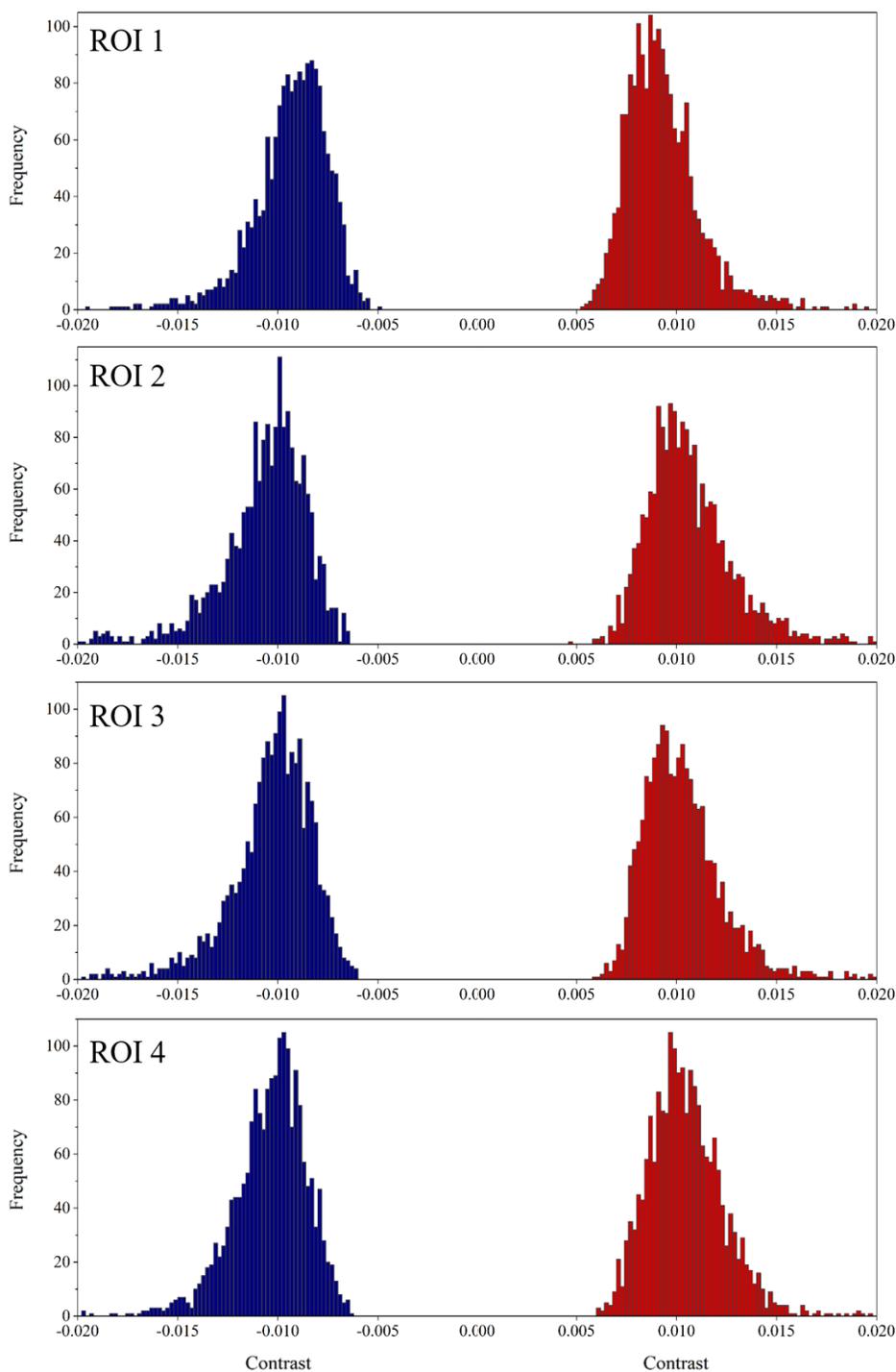


Figure S1. Histograms of the frequency for adsorption (blue) and desorption (red) of BSA as a function of the interferometric contrast of single-molecule events. A 20 μl volume of a 20 nM solution of BSA in T50 buffer was dispensed onto a fluoruous-coated cover glass. Data was obtained across 60 s from each of four different regions of interest (ROIs).

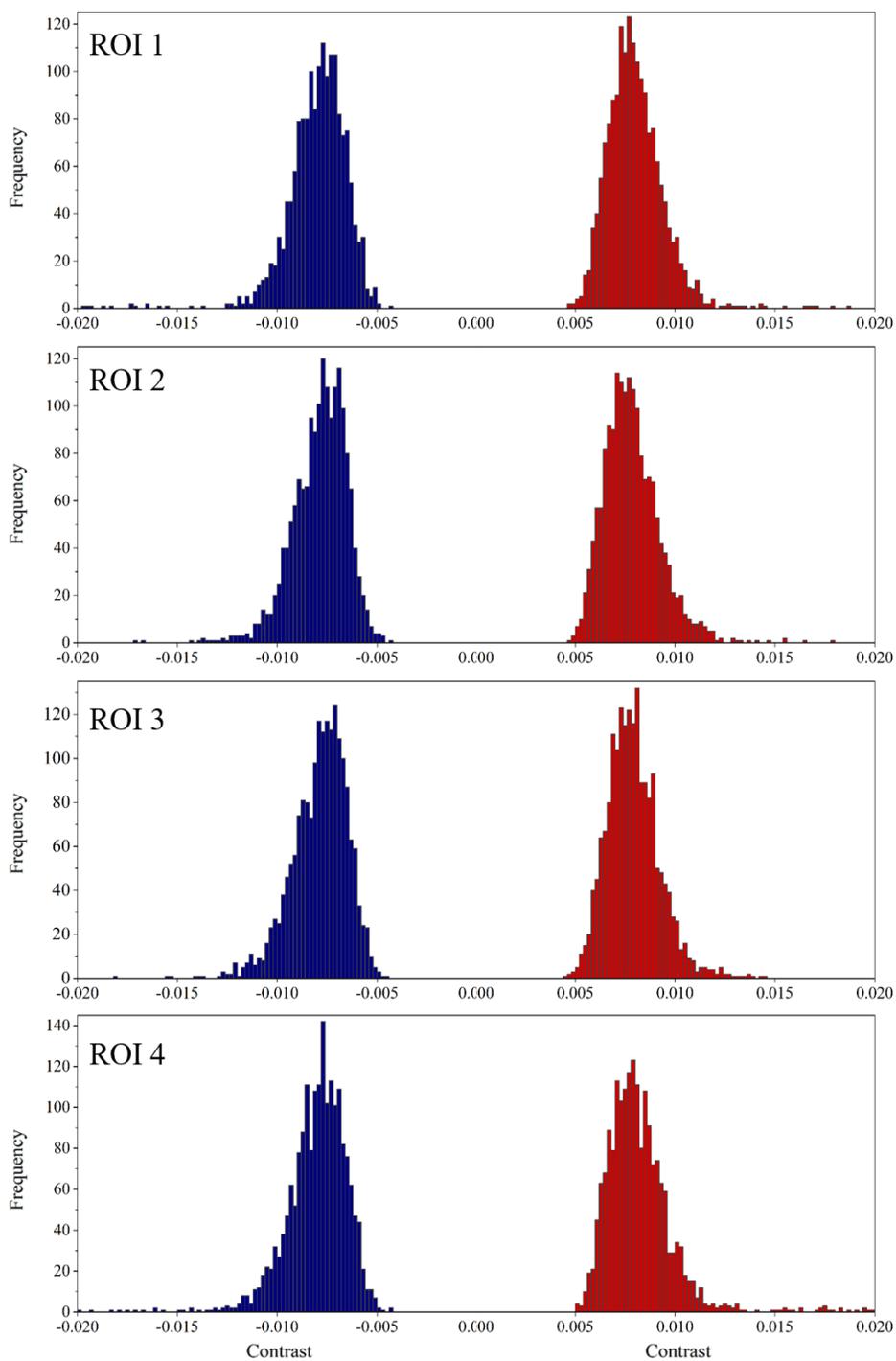


Figure S2. Histograms of the frequency for adsorption (blue) and desorption (red) of Streptavidin as a function of the interferometric contrast of single-molecule events. A 20 μl volume of a 20 nM solution of Streptavidin in T50 buffer was dispensed onto a fluoros-coated cover glass. Data was obtained across 60 s from each of four different regions of interest (ROIs).

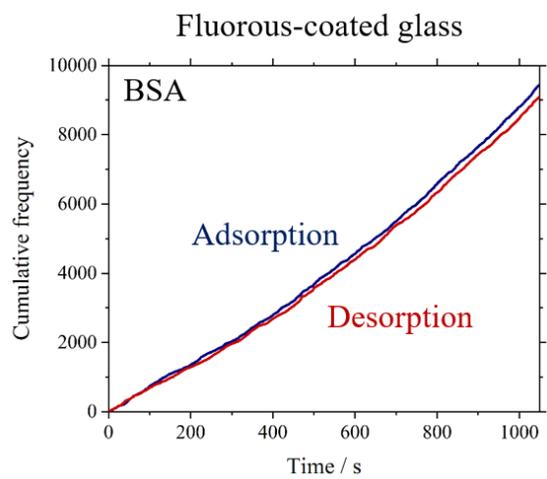


Figure S3. The cumulative frequency for adsorption (blue) and desorption (red) of BSA on fluorinated-coated glass.

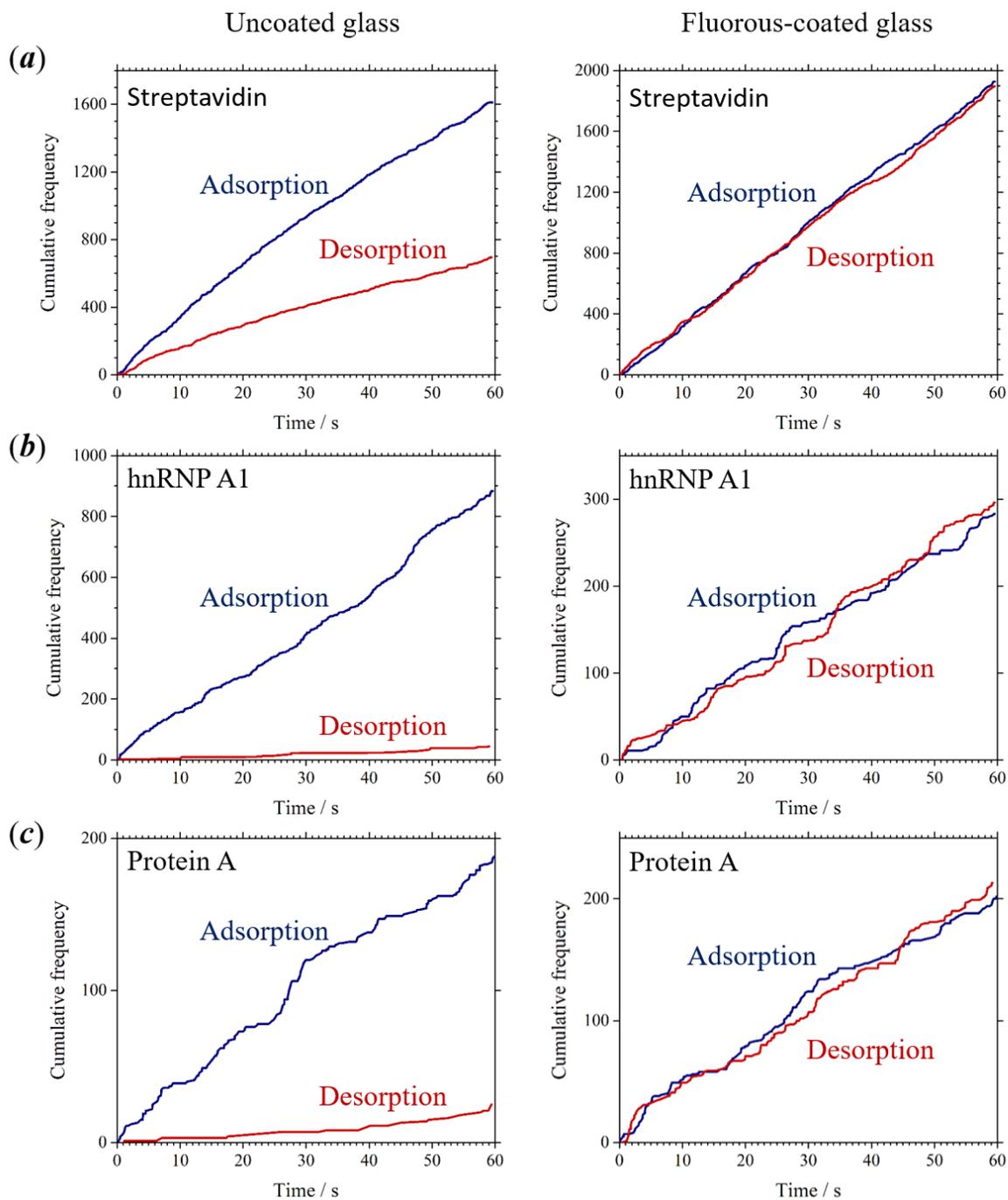


Figure S4. The cumulative frequency for adsorption (blue) and desorption (red) of (a) streptavidin, (b) hnRNP A1 and (c) protein A on fluorinated-coated glass over an interval of 60 seconds.

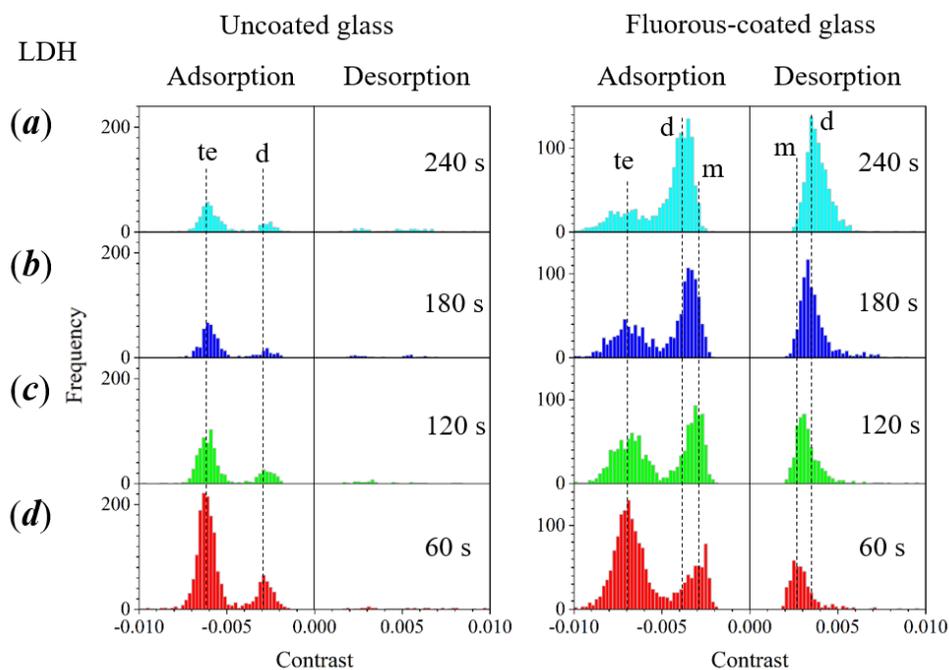


Figure S5. Sequential measurements of the frequency for adsorption of LDH onto uncoated cover glasses (left) and fluorinated-coated cover glasses (right). iSCAT measurements were made in the following time intervals: **(a)** 0 – 60 s; **(b)** 60 – 120 s; **(c)** 120 – 180 s; **(d)** 180 – 240 s. Each of the histograms represents an interval of 60 seconds (total experiment time shown in the figure is 5 minutes). Difference contrast values can be associated with the monomer (m), dimer (d) and tetramer (te). Data was obtained after a 10 μ l volume of a 20 nM solution of LDH in T50 buffer was dispensed (no further additions of protein were made after the start of the experiment).

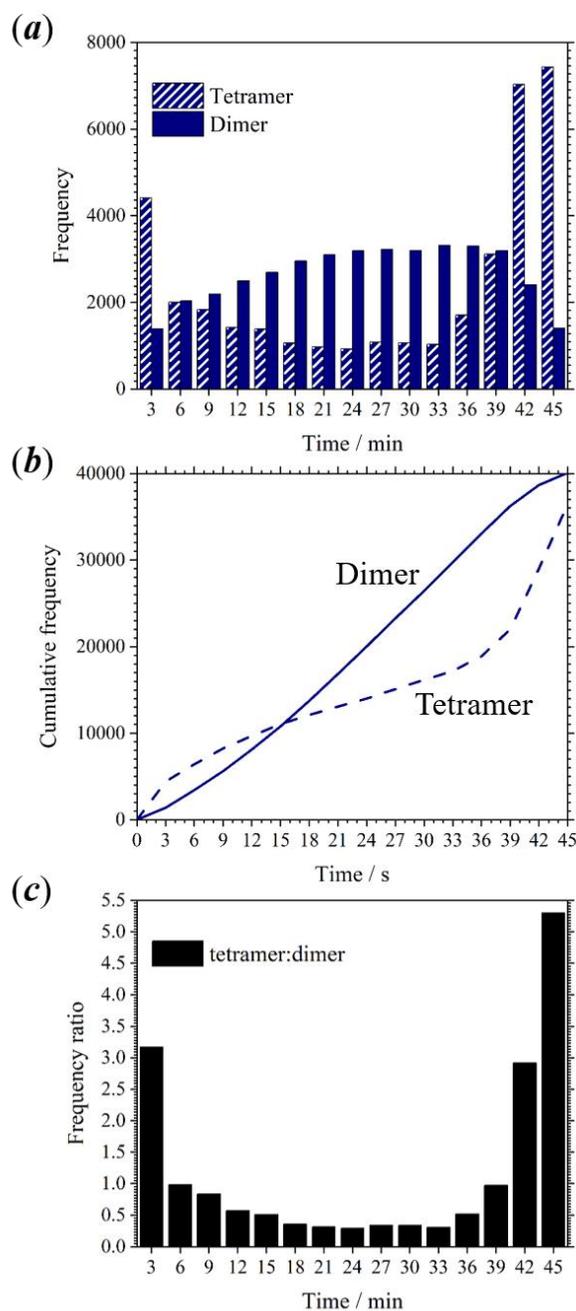


Figure S6. (a) Bar chart illustrating the frequency of adsorption of LDH dimer (solid) and LDH tetramer (diagonal line pattern) onto fluoruous-coated glass. The data was acquired for 45 minutes and partitioned into 3 minute intervals of time. A 20 μ l volume of a 20 nM solution of LDH in T50 buffer was dispensed onto the cover glass, and the droplet evaporated gradually during the experiment. (b) The cumulative frequency for adsorption of LDH dimer (solid) and LDH tetramers (dashed) onto fluoruous-coated glass. (c) The frequency ratio for adsorption of the tetramer to the dimer of LDH onto fluoruous-coated glass.

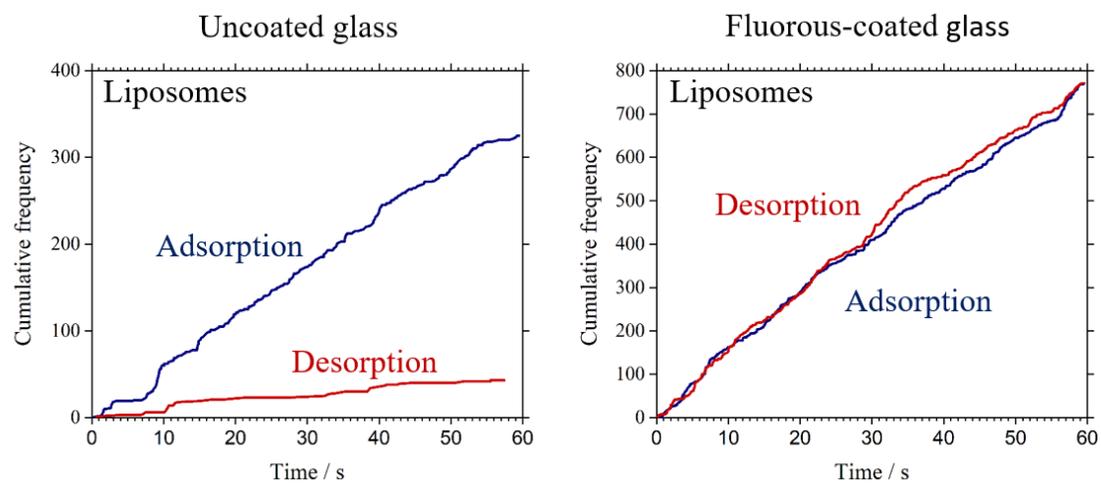


Figure S7. The cumulative frequency for adsorption and desorption of liposomes on uncoated and fluorinated-coated glass.

Appendix: Back-of-the-envelope calculation of the cumulative frequency of protein adsorption on measurement surfaces

A simplified model was adopted for a sessile drop corresponding to the protein sample dispensed onto the measurement surface: a cylindrical droplet shape. For the 20 μl sample volume, the cylinder was assigned a 4 mm diameter and a 1.6 mm height.

The protein sample of 20 nM concentration (*i.e.* 2.4×10^{11} molecules in 20 μl) was initially confined to this volume. We considered the axial diffusion of protein molecules only over a time interval, $t = 5$ minutes. The one-dimensional displacement of protein would follow a normal distribution with a standard deviation of $(2 D t)^{1/2}$; where the diffusion coefficient of protein, D is ca. $10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

In the model, the diffusion of protein was not confined to the volume of the cylindrical droplet shape on the measurement surface. A value was obtained for the number of protein molecules that would have a final location below the measurement surface. Note that this fraction would represent a lower limit to the collision frequency of protein with the cover glass, since many more protein trajectories will involve a collision with the cover glass but remain within the original droplet volume after 5 minutes.