

Supplemental Information:

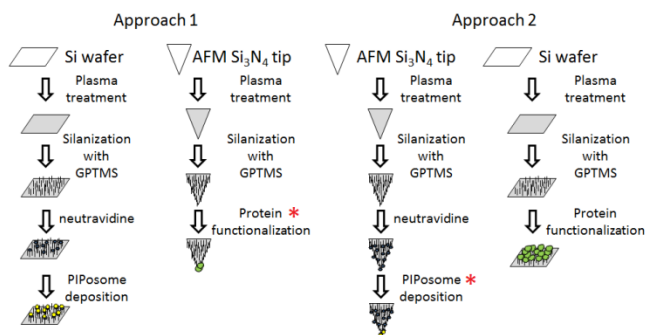


Figure S1. Schematics of the wafer and tip functionalization steps for FDS experiments.

Details of AFM tip and wafer functionalization:

All of the issues outlined in the introduction should be addressed in order to standardize an FDS measurement. The substrate should be well-characterized and exhibit almost 100% coverage of the species of interest to maximize the probability of binding. The tip coverage with the molecules that would interact with the substrate should be brought to an absolute minimum. At the same time, the possibility of non-specific binding events should be very low, in particular, events with a higher binding force than that of interest should not be observed at all.

Effective substrate functionalization with almost 100% coverage is a fairly straightforward task for small molecules. GPTMS has exposed epoxy groups which can directly bind carboxylic, amino and, with a much lower efficiency, hydroxyl groups of other molecules.

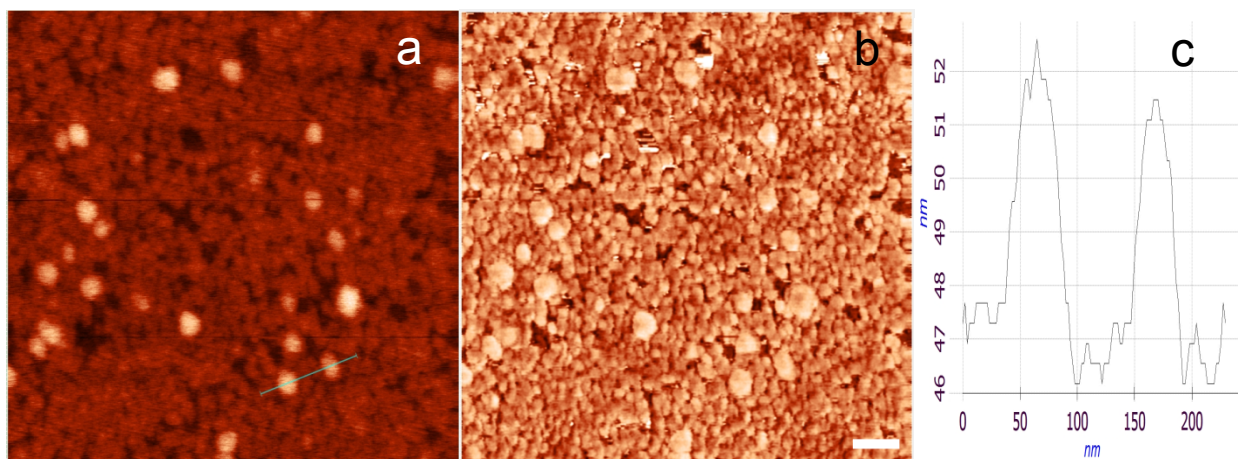


Figure S2. AFM topography (a) and phase (b) images of a Si wafer, after functionalization by GPTMS in a vacuum chamber and a subsequent functionalization by neutravidin molecules. Scale bar – 100 nm. The profile of line section marked in (a) is shown in (c).

Below, we will describe the functionalization procedures we used for the preparation of the substrates for the AFM FDS measurements. In the first approach, we decided to keep all of the larger particles (the large PIPosomes) confined to the substrate, leaving the AFM tip with a fairly thin functionalization layer.

First, we demonstrate the high uniformity of the silane layer, produced by vacuum deposition of GPTMS onto a silicon wafer and not by the much more commonly used conventional solution casting [1, 2]. Fig. S2a shows an AFM topography image of a GPTMS-functionalized silicon wafer. One can see that the surface is not smooth on the nanometer level and is covered by fairly well-defined globular-shaped particles of about 2 nm height on average. The phase image (Fig. S2(b)) shows that the underlying material is the bare wafer, which is significantly harder than the organic molecules, and shows that this is indeed a monolayer silane

film. The monolayer nature of the silane film agrees with ellipsometry results in the literature [3]. Assuming the length of the fully-extended GPTMS chain to be about 1 nm [4; 5], it can be said that such structures could not have formed by simple “grafting to” approach of individual chains in a “mushroom” regime with average chain-to-chain distance of $s > 2R_g$. While the distances between particles is indeed quite significant, it was concluded that each “mushroom” is not a single chain, but a complex aggregate, with some molecules bound not to the hydroxyl groups on the surface, but to silanol groups of neighboring GPTMS molecules, which have been hydrolyzed by traces of water present in the air. Given that silanol groups are located only at one end of the molecule which is facing the wafer, the particles can only grow to a finite size due to steric hindrances, since a longer silane functionalization did not result in a thicker film. This contributes to very good macroscopic homogeneity of the resulting silane functionalization of the wafer.

As a second step of the functionalization procedure, neutravidine molecules have been deposited by simple drop-casting of a concentrated solution (20 ug/mL in 100 mM Sodium bicarbonate) of neutravidine in water onto the wafer. The wafer with the solution was then allowed to stay overnight at 4°C. The deposited molecules can be seen on top of the GPTMS film in Fig. S2(a). The size of the particles has been estimated to be about 5.5 nm in height (Fig. S2(c)) from their individual AFM topography profiles, which is in good agreement with literature results of 5.8 nm for the size of such molecules [6].

Finally, a third layer of polymerized liposomes, or “PIPosomes” [main text ref.21], were deposited onto the wafers by a simple drop-casting method. After the removal of the droplet and several steps of gentle rinsing of the wafer in DI water, the wafer has been imaged in SEM and AFM (Fig. S3). Only the largest particles of sizes averaging 50 nm in height being deposited

onto the wafer, which could be the result of Brownian motion overcoming the speed of sedimentation for smaller particles. Since the surface coverage was not sufficient, an alternative deposition method was used. The piposome solution was added to a narrow plastic vial with a confined Si wafer and consequently subjected to mild vortexing for 1 min. The size of the wafer was matched with the diameter of the vial to avoid its touching of the plastic walls. The wafer was washed the same way and imaged in SEM and AFM. Unlike with the drop deposition method, small particles had prevalence, as seen from Fig. S3(d). The particle size range was 5-15 nm (it is possible to distinguish between piposomes and neutravidine molecules of same height, since the former have an ellipsoid shape, while the latter are more spherical). The second approach resulted in a much higher surface coverage, as seen both from SEM and AFM images.

The tips were functionalized following a modified published procedure (main text ref.44). The novel method introduces minimal tip functionalization with the molecule of interest, where only the tip apex would be functionalized, leaving the rest of the tip and cantilever unaltered, which results in both better cantilever backside reflectivity and minimizes non-specific binding. In this paper, we show the advantage of this functionalization procedure and also visualize the extent of tip coverage by the functionalization molecules, which has not been done previously for this method. Briefly, the tip was functionalized with GPTMS and gently approached to a narrow paper strip, soaked in 10 μ L of functionalization solution, for the duration of one minute. Due to exposed epoxy groups of GPTMS, the attachment of molecules occurred spontaneously via their carboxyl- or amino- group termini. Unreacted epoxy groups were blocked by soaking tips in ethanolamine for 5 min.

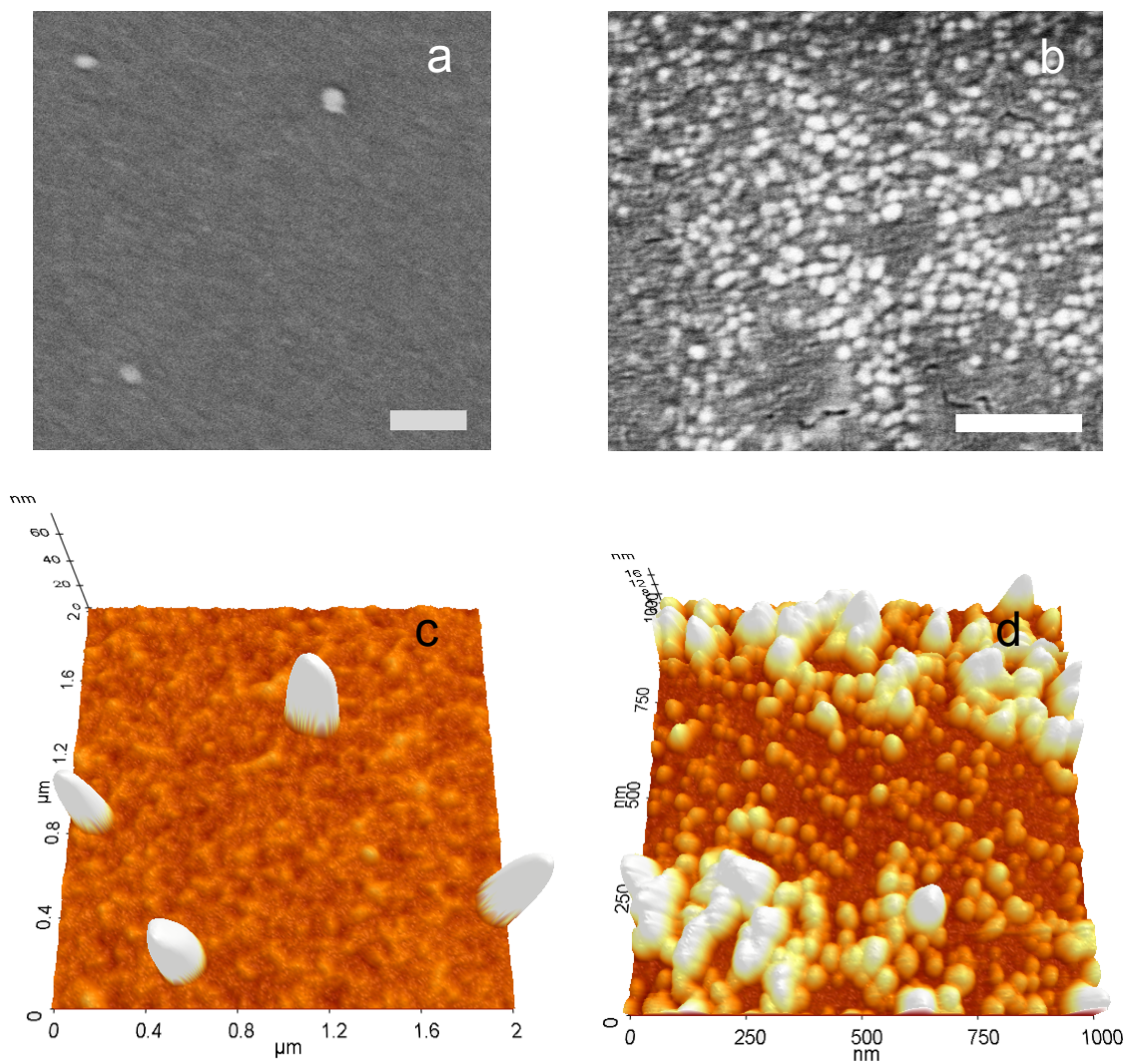
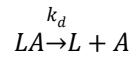


Figure S3. SEM images (a, b) and 3D rendering of AFM images (c, d) of Piposomes, deposited on the surface of GTPMS-functionalized Si wafers: drop deposition for 1 hour (a, c) and deposition under agitation for 1 min (b, d). Scale bar for SEM images – 200 nm.

Details of SPR data analysis:

The fit for the SPR dissociation curves is derived as follows:



$$-\frac{d[LA]}{dt} = k_d \cdot [LA]$$

where L is the immobilized ligand, A is the analyte that is flown on the surface, LA is the bound compound, $[LA]$ is the concentration of LA and k_d is the rate constant of its dissociation. Separating the variable and integrating from time t_0 to t and from initial concentration of $[LA_0]$ to the concentration $[LA]$ at time t , we get

$$[LA] = B_0 + [LA_0] \cdot e^{-k_d(t-t_0)} \quad [1]$$

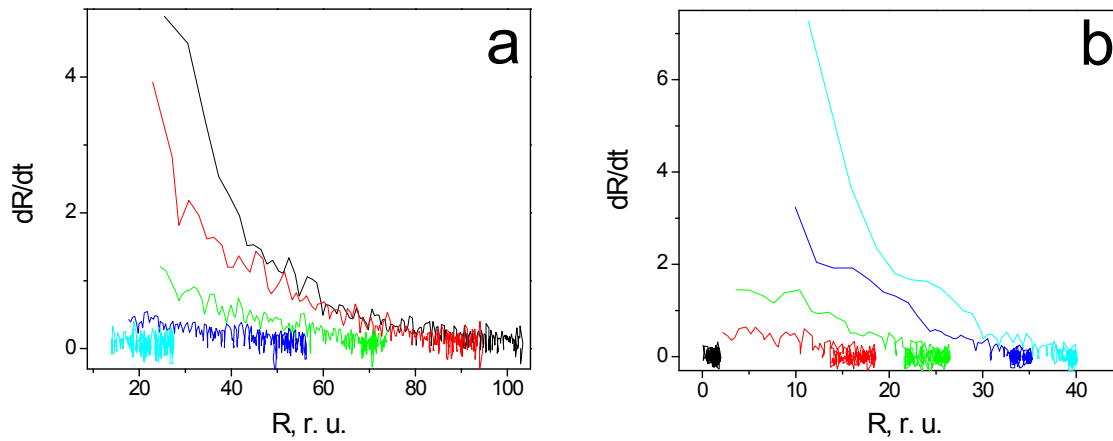


Figure S4. First derivatives of the response curves plotted versus response units for GRIP-PIP4,5 (a) and GRIP-PIPcontrol (b).

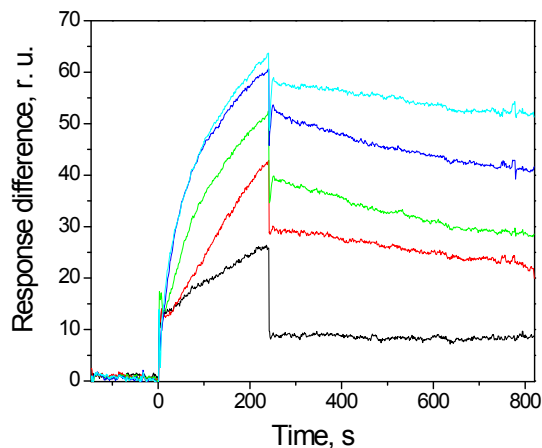


Figure S5. The result of subtraction of GRIP-PIPcontrol data from GRIP-PIP4,5 experiments.

Supplemental Information references:

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