ADVANCED MATERIALS

Supporting Information

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Supporting Information for:

Biospecific Recognition of Tethered Small Molecules Diluted in Self-Assembled Monolayers**

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Higher Surface Coverage of Small Molecule Probes Results in Nonselective Recognition

In addition to investigating insertion self-assembly to place carboxyl-terminated alkanethiol tethers into pre-existing monolayers at dilute coverage, we also performed experiments using self-assembly of mixed SAMs deposited directly from solution to produce higher probe molecule surface coverages. Here, the synthesis/assembly chemistry was similar to that described in the main text with the exception that Au surfaces were immersed in an equimolar mixture of oligoethylene glycol alkanethiols, 0.5 mM 1-(9-mercaptononyl)-3,6,9-trioxaundecan-11-ol **(1)** and 0.5 mM 23-(9-mercaptononyl)-3,6,9,12,15,18,21-heptaoxatricosanoic acid **(2)** for 24 h. The resulting self-assembled monolayers (SAMs) were washed with ethanol and dried under argon. Activated esters of the carboxyl-termini on **(2)** were formed by adding 150 mM N-ethyl-N-(dimethylaminopropyl)-carbodiimide (EDC)/30 mM N-hydroxysuccinimide (NHS) in deionized (DI) H_2O to the SAMs for 1 h, followed by a wash with DI H_2O. A 50 mM solution of serotonin in PBS, pH 7.4 was then added with gentle shaking at 4 ºC for 24 h, forming an amide bond between the primary amine on serotonin and the carboxyl moieties on the monolayer surfaces. Surfaces were washed with 0.1 M NaOH to inactivate unreacted NHS esters and to remove excess serotonin.

Ellipsometry was used to detect antibody binding to monolayer surfaces at higher probe coverages. Changes in surface film thicknesses were performed using an LSE Stokes Ellipsometer (Gaertner Scientific Co., Skokie, IL). The light source was a He-Ne laser (632.8 nm) aligned at an incidence angle of 70º with respect to the surface normal. Film thicknesses were calculated assuming a refractive index value of n=1.5 and an extinction coefficient of k=0. Recognition of higher coverage serotonin-derivatized monolayers by different antibodies is shown in **Figure SM-1**. Time point 1 shows the thicknesses of the initial monolayers that were

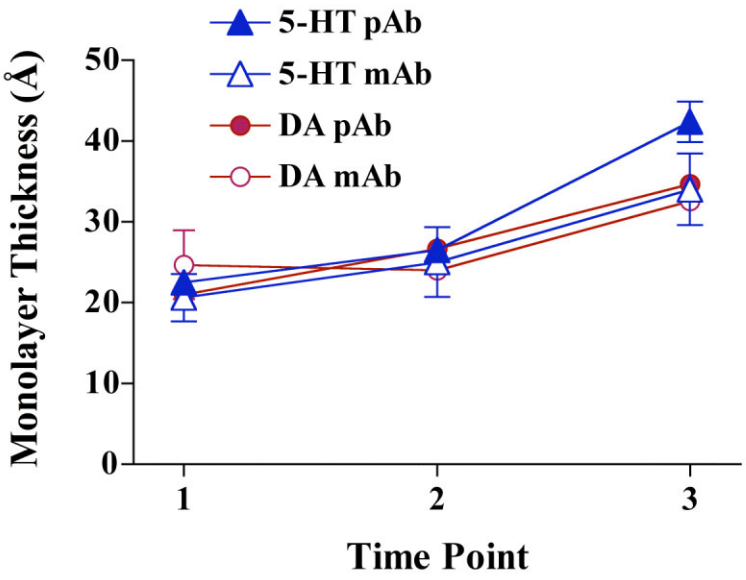

Figure SM-1: **Exposure of serotonin-functionalized monolayers with higher surface coverages (ca. 50%) to antibody targets.** Antibody capture was detected by increases in film thicknesses as measured by ellipsometry (in Å). However, in contrast to the dilute functionalization described in the main text, exposure of higher coverage serotonin-functionalized surfaces to monoclonal or polyclonal dopamine antibodies (DA mAb and DA pAb, respectively) also resulted in antibody binding, as evidenced by similar increases in film thicknesses to those detected for 5-HT antibodies. Time point 1: mixed SAMs prior to serotonin functionalization. Time point 2: following serotonin functionalization. Time point 3: following exposure of serotonin-functionalized SAMs to antibodies with different affinities for serotonin. Error bars are standard errors of the means. Measurements were taken for n=13 samples for 5-HT pAb, n=3 samples for 5-HT mAb and DA mAb, and n=1 sample for DA pAb. One-way analysis of variance indicated no statistically significant differences between mean film thicknesses for the different antibodies at time point 3 [$F(3,11)=0.87$; $p=0.48$].

prepared from solutions that were equimolar in **(1)** and **(2)** (monolayer composition is not expected to reflect solution compositions precisely[1]). Monolayer thicknesses after derivatization with serotonin changed very little due to the small size of serotonin (MW=176 amu; time point 2). High coverage serotonin-derivatized SAMs were then exposed to monoclonal and polyclonal antibodies raised against serotonin or dopamine. Both types of serotonin antibodies adhered to serotonin-derivatized surfaces, as indicated by the increases in monolayer thicknesses observed at time point 3. However, incubation of higher coverage serotonin-functionalized SAMs with

dopamine antibodies resulted in similar changes in monolayer thicknesses. These data indicate that at higher probe densities (*ca.* 50%), serotonin-derivatized SAMs are unable to discriminate between antibodies raised against serotonin versus antibodies raised against a different small molecule neurotransmitter. We infer that if small probe molecules are spaced too closely or irregularly under high density surface conditions, nonspecific binding increases and discrimination decreases.

"Like" molecules tend to aggregate or phase separate[1-4] in monolayers composed of two different types of molecules and this produces deleterious effects in two ways. First, since large molecule binding targets cover a surface area of approximately 5 nm × 5 nm, or *ca.* 100 matrix molecules, having more than one tethered probe molecule per 100-molecule area leads to no additional specific binding. Secondly, and perhaps more importantly, the functional groups on the probe molecules result in some degree of non-specific binding, so it is important to minimize any *excess* of probes. We have accomplished this by inserting tether molecules into preformed SAMs followed by functionalization with serotonin, thereby creating surfaces with highly dilute small molecule probes that are capable of specifically capturing antibody targets (see **Fig. 4** in main text). By advancing our investigation to very low surface coverages, however, we were not able to continue to use ellipsometry to investigate target binding. For later studies using insertion to place tether molecules into pre-existing SAMs, we utilized quartz crystal microbalance (QCM) gravimetry, a method that is capable of detecting small changes in the mass of surface bound species.

Surface Coverage Calculated from Changes in QCM Frequency

The Sauerbrey equation governs the relationship between the change in frequency of a QCM crystal and the corresponding mass uptake on that crystal. In its expanded form, it is written as:

$$\Delta F = \frac{-2F_0^2}{A\sqrt{\mu_q \rho_q}} \Delta m$$

where ΔF is the change in frequency of the crystal, F_0 is the resonant frequency of the fundamental mode of the crystal, A is the active measurement area, μ_q is the density of the crystal, ρ_q is the shear modulus, and Δm is the change in mass on the crystal. For our measurements, F_0 = 10 MHz, A = 0.2 cm^2, μ_q = 2.648 g/cm^3, and $\rho_q = 2.947 \times 10^{11}$. Because these values are constant throughout the experiment, the Sauerbrey equation can be simplified to:

$$\Delta F = -C \times \Delta m$$

where, for our system, $C = 1.1 \times 10^9$ Hz/g. This translates to 0.88 ng of mass uptake for every 1 Hz change in frequency.

The approximate mass of an IgG antibody, such as those used in this study, is 150 kDa or 2.4×10^{-19} g/molecule. Taking the frequency change as a result of the specific binding of serotonin antibodies to the surface to be ~100 Hz, there is a mass uptake of 88 ng, equivalent to 4×10^{11} molecules captured on the surface, or 2×10^{12} molecules/cm^2. The average spacing of alkanethiol molecules in a well-ordered SAM is ~5 Å, or four molecules per 100 Å^2. This implies a density of 4×10^{14} molecules/cm^2 or 8×10^{13} alkanethiol molecules on our crystal. Assuming a 1:1 binding of antibody to probe molecule and 100% functionalization, this implies 0.5% coverage of probe molecules on the surface.

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