# Supplemental Information

## Interactions between a methoxy-terminated PEGolated bilayer and a smooth gold surface

Figure S1 shows the interaction measured between a smooth gold surface and a DSPE bilayer with 1% methoxy-terminated PEG lipids (all other experimental conditions are identical to those described the experimental section of the manuscript text). The adhesion measured is due to the PEG backbone in this case, and gives an adhesion energy of about 8 kT/molecule. We have subtracted this value from the measurement for smooth surfaces and amine-terminated PEG chains shown in Figure 3A of the main text, giving a value of about 32 kT for the specific interaction between the terminal amine group on the PEG molecule and the gold surface (see manuscript text for details).

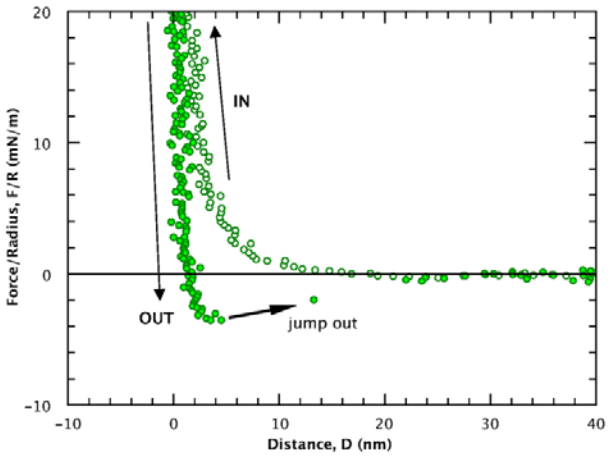


**Figure S1.** Interactions measured by SFA between a smooth gold surface and a PEGolated bilayer. In this case the PEG molecules are terminated with methoxy groups, rather than amino groups as shown in the manuscript text, and the adhesion from the PEG backbone is determined to be ~8 kT.

## Adhesion between a lipid monolayer and smooth gold

To demonstrate that a hydrocarbon lipid adheres strongly to a gold surface, we have measured the interaction between a lipid monolayer (prepared by Langmuir-Blodgett deposition) and a smooth gold surface. Figure S2 shows the interaction forces between a dioctadecyldimethylammonium (DODA) monolayer with a smooth gold surface (note: DSPE monolayers quickly overturn in water so we have chosen a more stable monolayer for this measurement). As Figure S2 shows, there is a long-range attraction, as well as very strong adhesion. The adhesion force of -300 mN/m is used to calculate the adhesion energy by the JKR theory, *i.e.*, $W_0 = 2F_{ad}/3\pi R = 63$ mJ/m$^2$. Two nominally hydrophobic surfaces have an adhesion energy of $W_0 = 100$ mJ/m$^2$. Thus, while gold is clearly not perfectly hydrophobic, the very large

adhesion energy of $W_0$ = 63 mJ/m$^2$ does indicate that the hydrophobic interaction plays a role in the adhesion between lipid monolayers and gold surfaces.

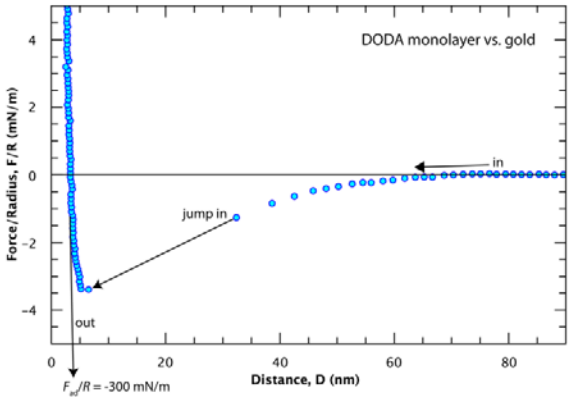


**Figure S2.** Interactions measured by SFA between a smooth gold surface and a DODA monolayer. The long-range attraction and strong adhesion indicate that there is hydrophobic attraction between the gold and DODA layer.

Distance determination between mica and gold surfaces

We have the case of a 2 layer interferometer, consisting of an ~5 μm thick piece of mica, and a < 50 nm thick layer of aqueous solution, between reflecting silver and gold layers, respectively. A lipid bilayer of thickness ~5-7 nm is adsorbed on the mica, which can be ignored at distances larger than the bilayer thickness. As detailed by Israelachvili[1], the exact solution for the 2-layer interferometer is identical to the equation for the even fringes in the 3 layer interferometer, thus, the distance $D$ and the refractive index of the medium $\mu_3$ cannot be determined separately, as shown in equation S1:

$$\tan(2k\mu_3 D) = \frac{(1-r^2)\sin(2n\pi\Delta\lambda/\lambda)}{-2r+(1+r^2)\cos(2n\pi\Delta\lambda/\lambda)} \quad , \qquad \text{(S1)}$$

where $\lambda$ is the wavelength of the $n$-th fringe, $n$ is the fringe order number, $k = 2\pi/\lambda$, $\Delta\lambda = \lambda - \lambda_0$, $\lambda_0$ is the wavelength of the $n$-th fringe at contact, i.e., where $D$ = 0, and $r = (\mu_1-\mu_3)/(\mu_1+\mu_3)$, and $\mu_1$ and $\mu_3$ are the refractive indices of the mica and the medium respectively ($\mu_1$ = 1.4585 and $\mu_3$ = 1.333). The fringe order number $n$ is related to the fringe wavelengths by $n = \lambda_{n-1}/(\lambda_{n-1}-\lambda)$ where $\lambda_{n-1}$ is the wavelength of the $n$-1 fringe. For small values of $D$, *i.e.*, less than about 30 nm, the above equation reduces to the following much simpler form with less than 1% error:

$$D\mu_3^2 = n\Delta\lambda\mu_1/2 \qquad \text{(S2)}$$

To show that the distances measured in this study were not affected by using the short-range approximation in equation S2, we have directly calculated the distances from the measured

wavelengths for both the approximate and exact solutions. The comparison is shown in Figure S3. As shown, the agreement is great (to within 3% error) until about 50 nm, at which point the distances calculated by the approximate equation S2 begin to exhibit significantly larger values than the exact solution. We measure absolute separation $D < 30$ nm in this study, so the error involved in using the approximate equation is not significant. More error will come from the camera and optics, which we estimate limits the resolution in these experiments to about ±2.5 Å.

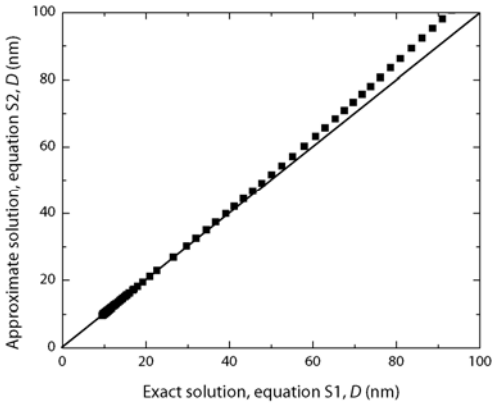


**Figure S3.** Comparison of approximate and exact solutions for the distance in a 2-layer interferometer. The y = x line represents perfect agreement between the exact and approximate solutions. The approximate solution significantly overestimates the distance for $D > 50$ nm.

## AFM imaging of PEGolated bilayers

Figure S4 displays an AFM image of a 0.05% $NH_2$-terminated PEGolated bilayer in water. Figure S4A shows the topography scan of a 300 nm x 300 nm area, while Figure S4B shows the corresponding phase signal for a section of the image shown in A. Figure S4C displays the height trace along the black line of Figure S4A. Analysis of this height trace indicates that the PEG molecules are likely being deformed by the AFM tip, i.e., they are flatter and more like "pancakes" than the expected, unperturbed "mushroom" configuration.

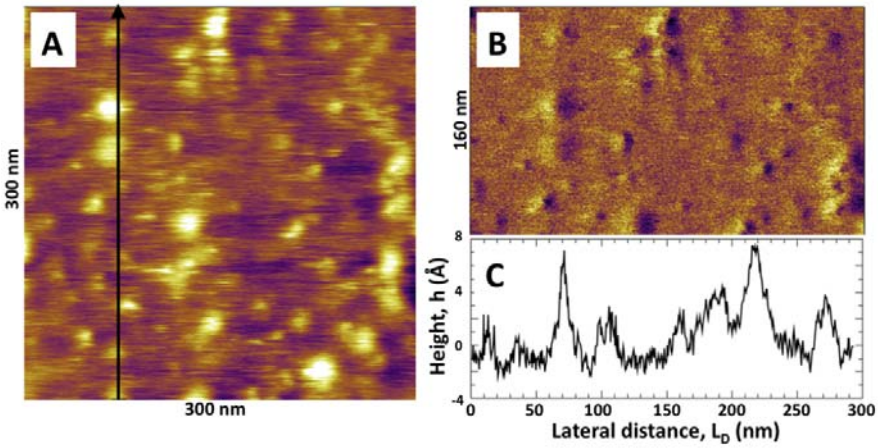


**Figure S4.** Single molecule AFM imaging of PEGolated bilayers: (A) AFM topography scan of a PEGolated lipid bilayer (0.05% PEGolation) recorded in tapping mode in pure water. This scan was recorded at the lowest possible amplitude damping in order to avoid puncturing the bilayer. The black arrow indicates the height trace shown in (C). The phase-signal (B) for a part of the scan shown in panel (A), shows a clear phase shift for the topographic features seen in (A), clearly distinguishing the mechanical properties of the topographic features (*i.e.*, the PEG mushrooms) and the matrix. Due to the tip-sample interaction during imaging, the height trace displayed in panel (C) shows that the imaged molecules are only 2 Å high, yet have a diameter of about 10 nm. The imaged volume, however, corresponds to the expected Flory volume of the PEG chains. It is observed that the molecules are dispersed randomly throughout the bilayer and the counted number corresponds with the expected area density.

## References

1. J. Israelachvili, *Journal of Colloid and Interface Science*, 1973, **44**, 259-272.