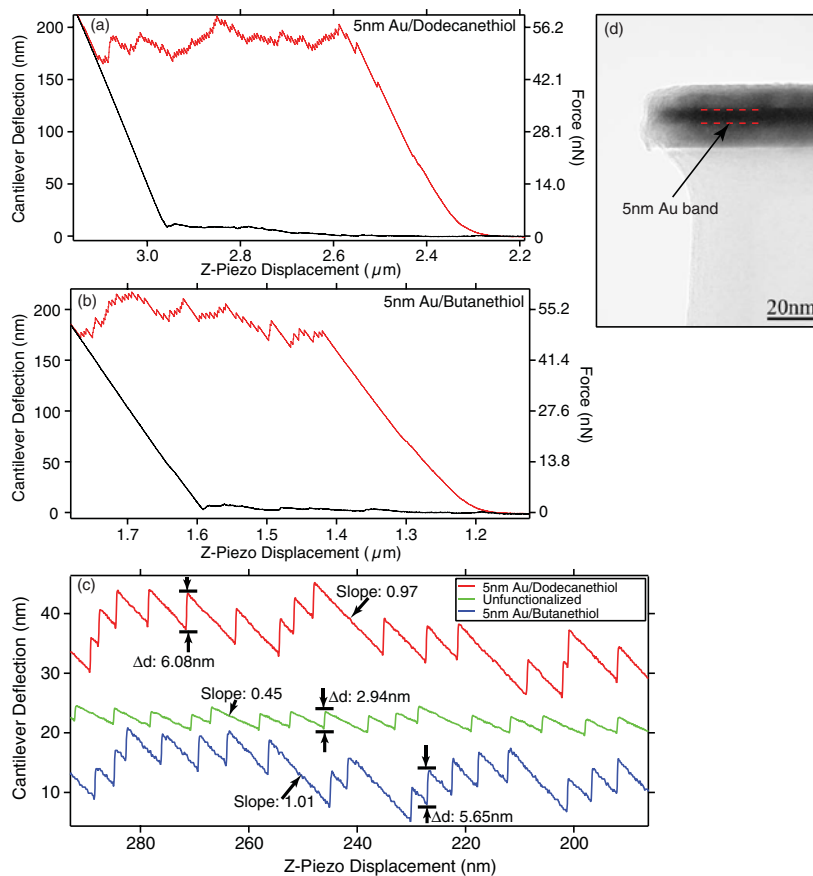
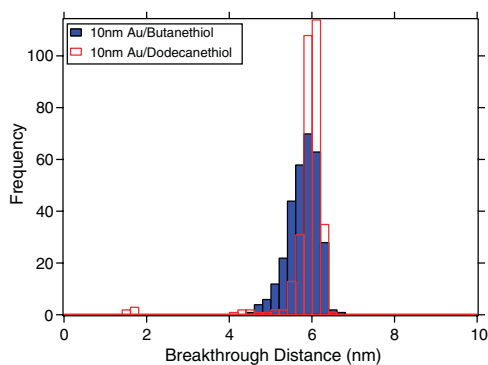


# Supporting Information

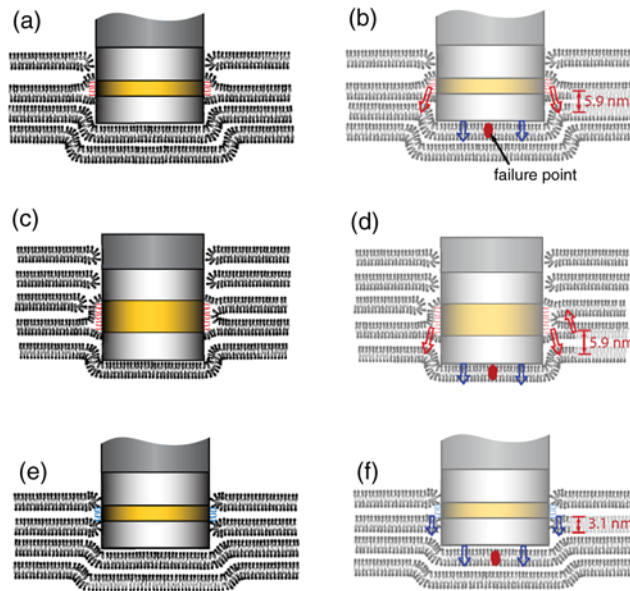
Almquist et al. 10.1073/pnas.0909250107



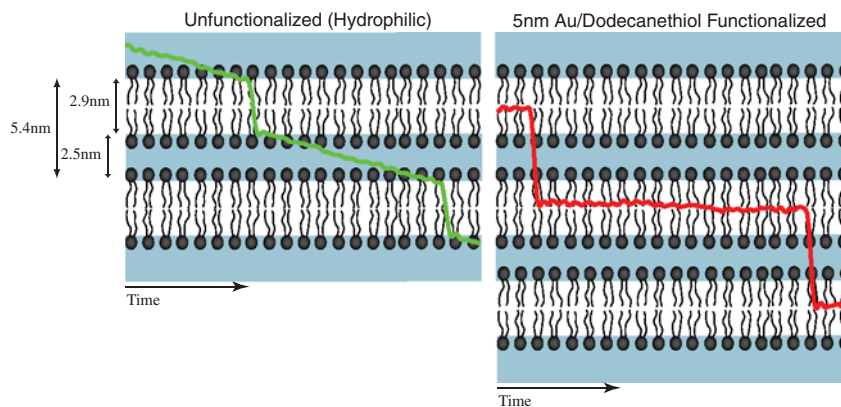
**Fig. S1.** (A and B) Force-displacement curves for 5 nm Au bands functionalized with dodecanethiol or butanethiol, respectively. (C) Comparison of cantilever deflection vs. displacement curves for curves shown in Fig. 3A and Fig. S1A and B. Functionalized probes remain fixed in the hydrophobic core of the membrane until breaking free and jumping to the subsequent bilayer core (see Fig. 3D and Fig. S3). This results in the slope of the sawtooth deflections being  $\approx 1$ , due to the cantilever deflection equaling the Z-piezo displacement. On the contrary, unfunctionalized hydrophilic probes are not held in a fixed location, instead traversing the hydrophilic region between bilayer cores. This results in a slope that is less than 1. (D) TEM image of a stealth probe with a 5 nm Au band.



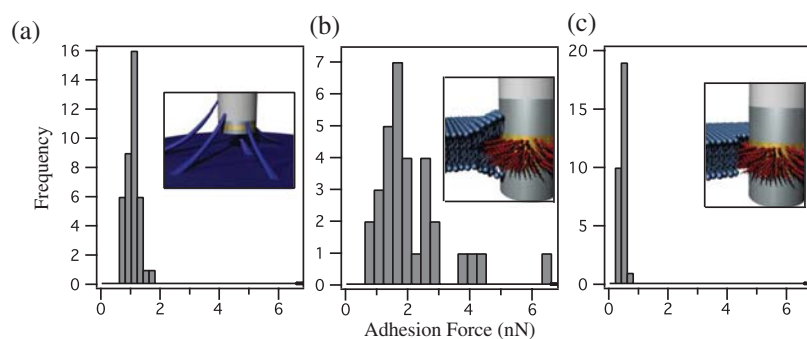
**Fig. S2.** (A) Histograms of stacked bilayer penetration distances for 10 nm Au stealth probes functionalized with either butanethiol or dodecanethiol.



**Fig. 53.** Schematic of proposed lipid structure during bilayer penetration. (A) A hydrophobic, 5 nm thick band inserts into a single bilayer. (B) During the failure event, the hydrophobic band jumps into the middle of the core of the next bilayer. Note that the distance required for the hydrophilic tip (Gray) to move is only the thickness of the bilayer. However, the hydrophobicity of the band drives the probe to move the extra distance so it may reside within the hydrophobic core, for a total displacement of 5.7 nm. (C) A hydrophobic, 10 nm band. In this case it is not clear whether one or two bilayers will interact with the band at one time (each case is shown on one side of the probe). The energy and breakthrough distances of these two cases are functionally equivalent, thus we are not able to distinguish between the two. However, the fact that only single pull-off events are observed during adhesion tests (Fig. 4B and C) suggests the single-bilayer interaction model. (D) Upon breakthrough, the hydrophobic band once again seeks to be in contact with the hydrophobic core of the next bilayer. The breakthrough distance is thus dictated by the center to center spacing of the bilayers, not upon the thickness of the band. (E) Structure of the hydrophilic band in contact with the bilayer. (F) During breakthrough of the tip the hydrophilic band traverses the thickness of the bilayer and enters the aqueous region. Since this is favorable, there is no driving force for the tip to immediate move farther, and the breakthrough distance is determined by the hydrophobic core thickness.



**Fig. 54.** Schematic showing how the penetration curves in Fig. 3D correspond to the various regions of the lipid bilayer stack. (Left) Unfunctionalized, hydrophilic probes penetrate through the hydrophobic core of the lipid bilayers, while hydrophobically functionalized probes (Right) jump from bilayer core to bilayer core.



**Fig. S5.** Histograms of maximum adhesion force for three types of membrane probes and a corresponding cartoon of the bilayer/probe interaction. (A) Unfunctionalized probes, which form lipid tethers (B) 10 nm Au/butanethiol functionalized probes, with strong adhesion with the bilayer. (C) 10 nm Au/dodecanethiol functionalized probes, which show very weak adhesion within the bilayer.