Supporting Information

Effect of DNP-X-PE concentration on vesicle tethering

In preliminary experiments, we found that there was a slight dependence of the amount of vesicles tethered to the bilayer on the amount of DNP-X-PE they contained despite constant Biotin-X-PE concentration in both the bilayer (2 mol %) and the tethered vesicles (0.0125 mol %). This was evidenced as a slight decrease in the magnitude of the QCM-D frequency shifts at all of the overtones, especially at the higher DNP-X-PE concentrations (< 15% difference in the frequency shift at the third overtone over the range of DNP-X-PE concentrations investigated). We hypothesized that this was due to the slightly different surface charge of the vesicles because phosphatidylcholine lipids are zwitterionic with a net neutral charge, while both Biotin-X-PE and DNP-X-PE have a net negative charge. Thus, to hold the surface charge of the tethered vesicles constant, we adopted the practice of adding complementary amounts of POPS (up to 5 mol %), which also has a net negative charge, when varying DNP-X-PE concentrations in all subsequent experiments.

Calibrating the ELISA responses

As an independent measure of the amount of antibody bound to the surface of the tethered vesicles, a flow-based ELISA was developed so that the assay could be performed simultaneously with QCM-D measurements *in situ*. In typical ELISA binding assays, the response from an unknown sample is quantified by comparison to responses produced by samples of known surface density. For this work, known surface densities of antigens were generated by using a supported planar lipid bilayer. By controlling the lipid composition of the lipid bilayer, it was possible to accurately control the surface density of DNP-groups available for antibody binding and thus the antibody surface density. In this manner, the ELISA results of antibody binding to supported lipid bilayers composed of known amounts of DNP-X-PE served to calibrate the ELISA results of antibody binding to the tethered vesicle system. Additionally, we were also able to collect the QCM-D responses simultaneously, and the next sections describe the results of antibody binding to the supported lipid bilayer. These results guided the analysis of the of the tethered vesicle results.

Antibody binding to a supported lipid bilayer

Supported lipid bilayers composed of varying amounts of DNP-X-PE were prepared in a similar fashion as described for the tethered vesicle assembly. In Figure S1, both the average measured absorbance of the effluent stream and the average normalized net frequency shift upon antibody binding to DNP-X-PE containing bilayers at the third overtone are plotted against bilayer DNP-X-PE concentration. The absorbance measurements were conducted on a 100-μL sample collected from the effluent stream of the flow module that houses the QCM-D substrate upon which the supported lipid bilayer was constructed. Enough time was allowed to establish steady-state conditions for the hrp-catalyzed reaction before collection began. Each data point in the figure represents the average of three or four runs, and the error bars represent one standard deviation around the mean. The increase in absorbance was linear with increasing DNP surface density until surface saturation (~ 2 mol %). The frequency shift was also linear until the

antibody approaches surface saturation, although some nonlinearity was evident at higher surface densities, suggesting a deviation from Sauerbrey film properties at high antibody surface densities.

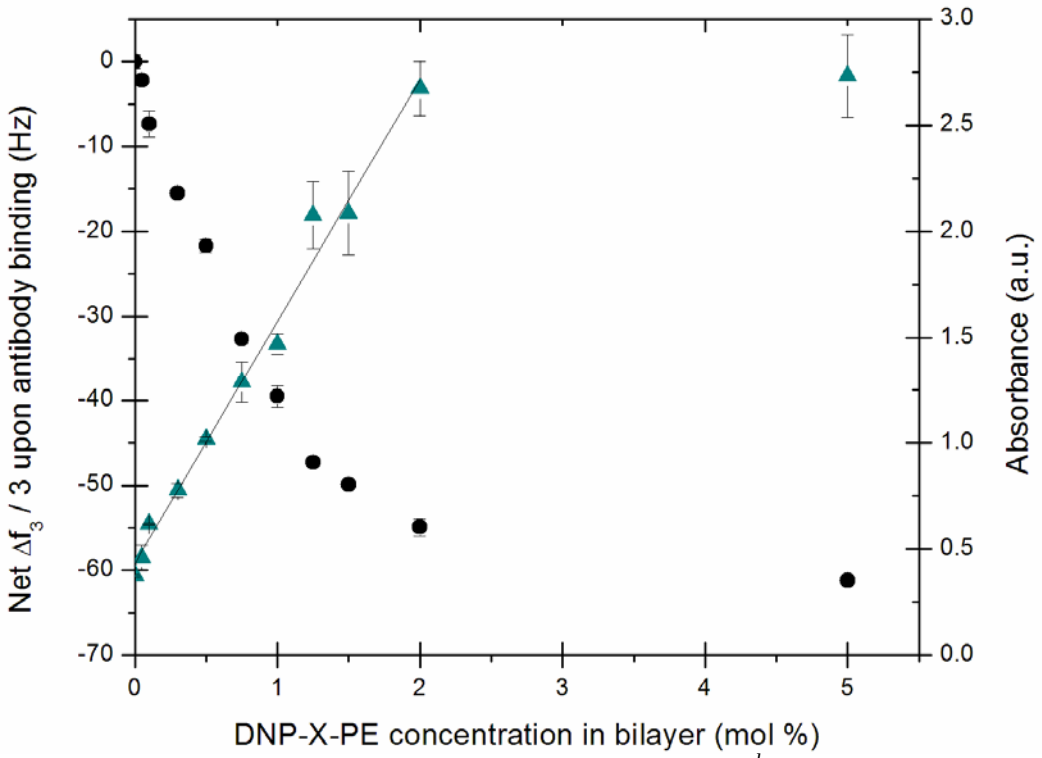

Figure S1: *The net changes in resonant frequency at the 3rd harmonic upon antibody binding to a supported lipid bilayer (black circles) and the corresponding ELISA absorbance measurements (green triangles). The error bars represent the standard deviations of at least three measurements.*

The absorbance data prior to surface saturation for the experiments focusing on the binding of antibody to a supported bilayer was empirically fit to a line. The slope and intercept of the line allowed the establishment of a relationship between the measured absorbance of the effluent stream and the antibody surface density. The bilayer DNP-X-PE concentrations were converted to antibody surface density assuming that all DNP hapten groups are each bound by a single anti-DNP IgG1 molecule. In this manner, the calibration obtained was applied to convert the absorbance measurements for the tethered vesicle system into antibody surface densities.

Examining the QCM-D results for antibody binding to a supported lipid bilayer, we find a significant departure from Sauerbrey behavior as evidenced by the nonlinearity of frequency responses with increasing bilayer DNP-X-PE concentration in Figure S1. As mentioned in the introduction to the paper, these findings are not necessarily unexpected and are in line with those of Svedhem, et al.,[1] who noted that a Sauerbrey treatment of antibody mass alone could not account for the frequency shift they observed for antibody binding to a supported lipid bilayer composed of lipids displaying an antigenic peptide sequence. With the analytical models available, it is not possible to precisely determine the amount of water above the bilayer that is

hydrodynamically coupled with the antibody layer because the extent to which the entrained water behaves in an inertial manner is difficult to estimate.

The situation of antibody binding to a lipid bilayer is distinct from that of streptavidin binding[2] in which we were able to show that the water entrained in a layer of bound streptavidin behaved in a purely inertial manner at high streptavidin surface densities. Streptavidin forms a regular, close-packed two-dimensional crystal on the surface of a biotinylated lipid bilayer[3, 4], while the bound antibody on the surface of the bilayer is not expected to do so. As a result, the net frequency shifts upon antibody binding to the hapten-displaying lipid bilayers do not exhibit an abrupt transition signifying surface saturation with increasing bilayer DNP-X-PE concentration, as was observed in our previous work with streptavidin[2]. Measured dissipation changes for antibody binding at saturation were found to be approximately 2×10^{-6}, compared to almost no measurable change for the case of streptavidin binding. Thus, while the dissipative losses are small, especially when compared to a film of tethered vesicles, the bound antibody film does exhibit a small amount of viscous losses, and the entrained water cannot be treated in a strictly inertial manner. Nevertheless, if the dissipative losses are assumed to be negligible, we estimate that roughly 25% of the antibody film mass could be accounted for by water.

It is important to note that since antibody-antigen binding is an equilibrium process and not irreversible, the amount of antibody bound to the lipid depends on the solution concentration of the antibody at any given bilayer DNP-X-PE concentration. Since the working antibody concentration was approximately 700 times higher than the apparent K_d[5], it was assumed that all available DNP groups were bound by anti-DNP for the purposes of converting bilayer hapten surface density to antibody surface density. This assumption allows calibration of the tethered vesicle ELISA results to those obtained for the supported bilayer. While this is not strictly correct, this assumption is reasonable given our operating conditions.

Dissipation kinetics of antibody binding to a bilayer and bivalent binding

As in the case for the tethered vesicles system, the changes in dissipation factor upon antibody binding to the lipid bilayer showed interesting kinetics. However, as illustrated in Figure S2, antibody binding to the supported bilayer showed distinct behavior and does not show strong overtone-dependent dissipation kinetics even under antibody surface saturation conditions. Instead, there is a small, long-term decrease observed at all of the overtones starting approximately 10 minutes after antibody exposure. This effect is only observed for bilayer DNP-X-PE concentrations of 1.25 mol % and higher. Before continuing with the discussion, we note that except for hydrodynamic coupling of water, all of the overtones are expected to behave in a similar manner and will be equally sensitive to antibody binding to the surface of the bilayer. The lipid bilayer acts in a Sauerbrey manner and the height of the antibody is expected to be well within the shear wave decay lengths at all of the overtones. Thus, whereas the sensitivity of QCM-D measurements at different overtones may be noticeably different for antibody binding to the tethered vesicle system, the overtones can be treated as if they are equally sensitive to antibody binding to the surface of the bilayer.

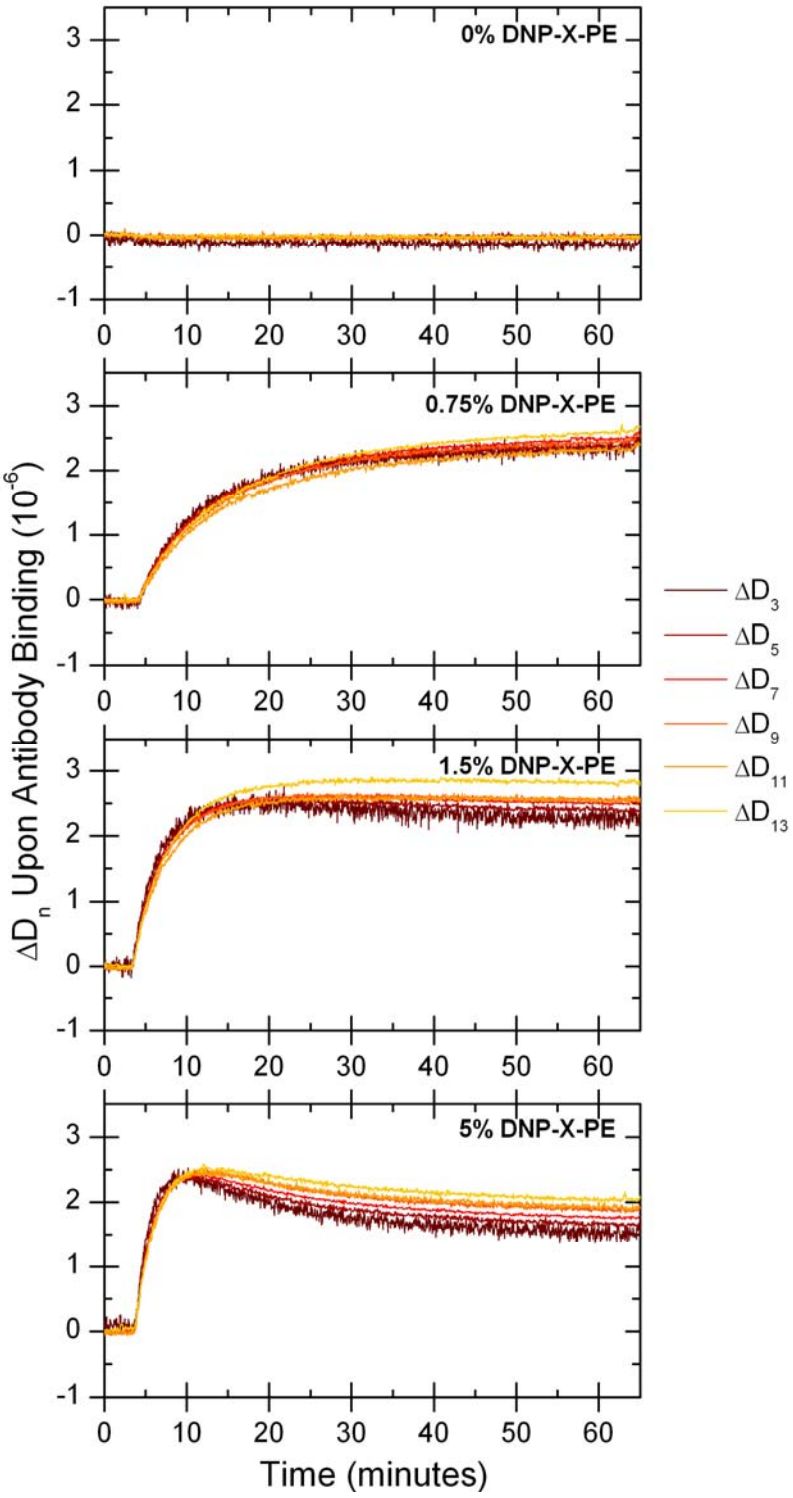

Figure S2: *Dissipation shifts upon antibody binding to supported lipid bilayers composed of varying amounts of DNP-X-PE groups. A long-term kinetic decrease in the dissipation value at all of the resonances becomes apparent for bilayer DNP-X-PE content of 1.25 mol % and higher. Note that the baseline for each resonance represents the respective measured value of the dissipation factor prior to the introduction of antibody, and time = 0 is taken to be 5 minutes prior to the introduction of antibody solution.*

We attribute the long-term, kinetic decrease to bivalent binding of the antibody to the hapten-displaying lipid bilayer, which is expected to result in stronger, less dissipative coupling. The decrease in dissipation factor increases in magnitude with increasing bilayer DNP-X-PE concentrations, as would be expected for an increasing amount of bivalent binding, but it remains less than 0.8×10^{-6}. Bivalent binding of antibodies to hapten-displaying lipid bilayers has been well characterized through fluorescence and radiolabeling methods[6-10]. These studies have characterized apparent binding affinities and report that at high bilayer DNP-X-PE concentrations, a larger proportion of antibodies are bound bivalently, resulting in higher apparent affinity coefficients. Using total internal reflection fluorescence microscopy, Yang, et al.,[9] have determined the fraction of bivalently bound antibody reaches approximately 0.8 at bilayer DNP-X-PE concentrations of 1.25 mol % and decreases quickly at DNP-X-PE concentrations below 1 mol %. They report a significant fraction of bivalently bound antibody at low DNP-X-PE concentrations (0.5 for bilayer DNP-X-PE concentration of 0.3 mol % in their results). Our dissipation results do not show the kinetic decrease in dissipation factor for low bilayer DNP-X-PE concentrations, suggesting that real-time dissipation shifts measured using QCM-D are not as sensitive to the valency of antibodies bound to hapten-containing bilayers as are equilibrium affinity measurements utilizing labeled antibodies. However, there is a measurable effect of bivalent binding at high antibody density. In addition, it is important to note that the percent of bivalently bound antibodies is based on estimates of the equilibrium binding affinities of the monoclonal antibody, which can vary from clone to clone. Therefore, the amount of bivalently bound antibody for a given hapten density can also vary between clones.

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