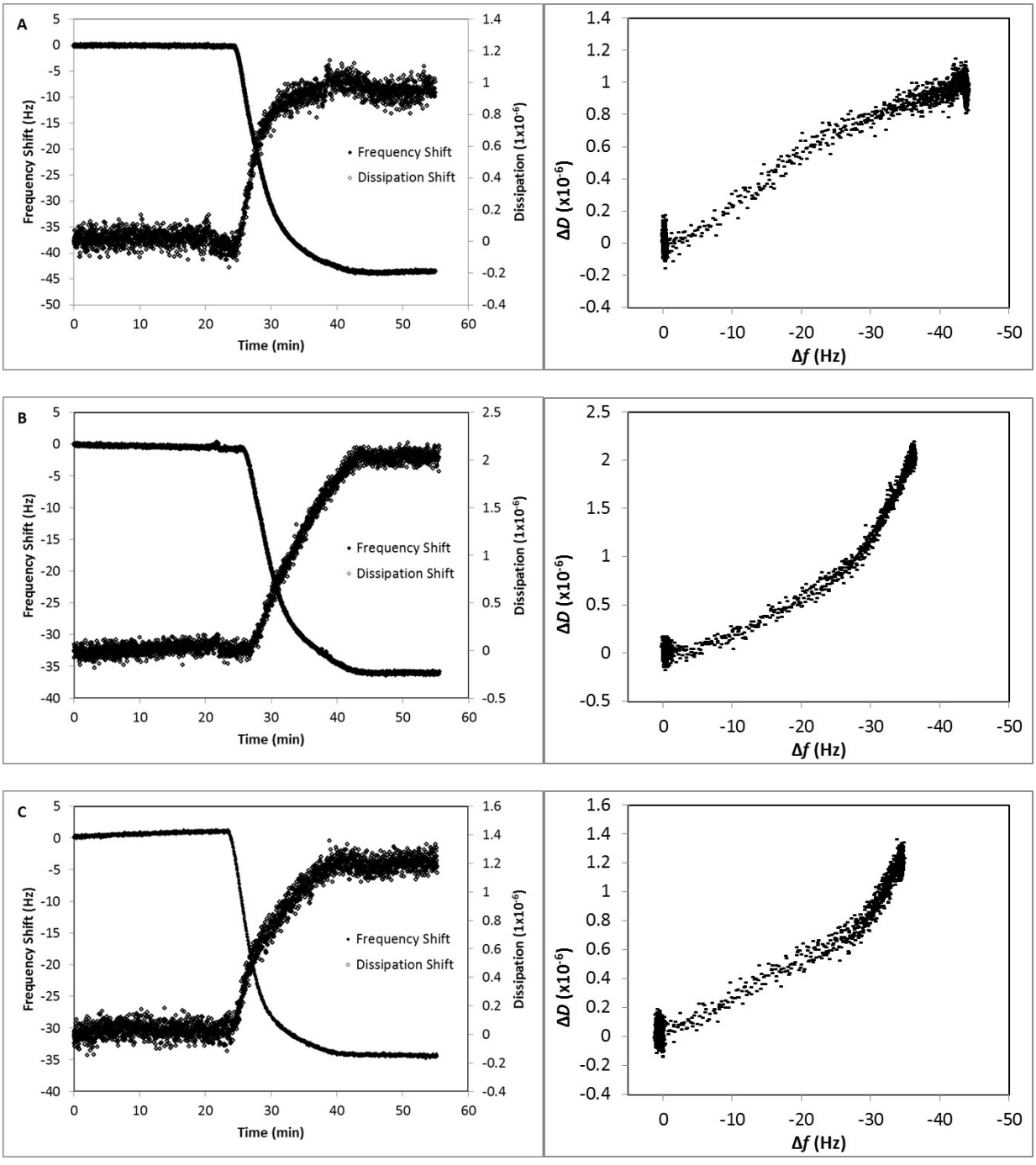
Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information: Discussion of Δ*D*-Δ*f* of the Affimer protein functionalized surface

The Δ*D*-Δ*f* plots can give an indication of the relative viscoelasticity of the protein layer as increasing number of molecules bind to the sensor surface. Examples of both the time dependent data and plots showing the change in dissipation versus the change in frequency (Δ*D*-Δ*f*) for Affimer B12 (A), Affimer B9 (B), and Affimer G12 (C) can be seen in the figure below. The 3rd overtone for the first 55 min of the experiment is plotted, encompassing the baseline in PBS, the adsorption of the Affimer protein to the surface, and the rinsing in PBS.



For all the Affimer proteins measured, the Δ*D*/Δ*f* values at equilibrium, lay between about 0.02 and 0.06 $\times 10^{-6}$ Hz^{-1}, less than the typical limit of 4×10^{-7} Hz^{-1}, allowing for Sauerbrey analysis of the data. All three Affimer proteins show a consistent linear increase in dissipation with a decrease in frequency for the first 20-30Hz. However, after this point, there is a change in slope in the Δ*D*-Δ*f* plots that varies somewhat from experiment to experiment. In the plots shown above, for Affimer B12 the rate of increase of dissipation slows for the same decrease in frequency. However, in many experiments including the ones portrayed in the plots above, for Affimer G12 and Affimer B9 the rate of dissipation change increases during the final rinse stage. While there are several possible interpretations of this multi stage behaviour, often distinct slopes in the Δ*D*-Δ*f* plot are associated with a rearrangement of the molecules on the surface. This rearrangement may lead to an increase in availability of the Affimer binding sites, allowing the specific binding of IgG2b seen in the following stages of the experiment. While there is a change in slope in the Δ*D*-Δ*f* plots of Affimer B12, the slope increase during the final rinsing stage is absent, indicating that the molecules may not be rearranging into the proper conformation to bind IgG2b whereas Affimers G12 and B9 do bind IgG2b in subsequent steps of the experiment.

Supplementary Information: Isotherm of mIgG2b binding to the Affimer G12 functionalized surface

As described in the paper, the Affimer-functionalized surfaces can be exposed to increasing concentrations of mIgG2b in solution in order to build an isotherm relating the initial concentration in solution to the equilibrium surface concentration of bound molecules. The adsorption of mIgG2b to the surface functionalized with the Affimer G12 displays the characteristic Langmuir adsorption behavior and can be well represented by the linearized Langmuir isotherm as seen in the figure below:

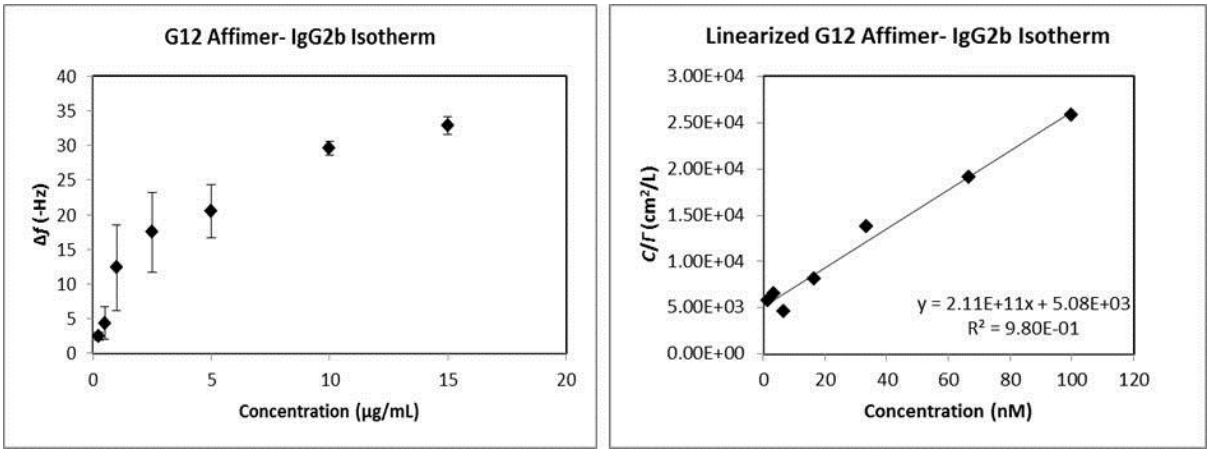


Figure S1: Isotherm of mIgG2b binding to the Affimer G12 surface showing the frequency shift at equilibrium surface concentration of mIgG2b versus the initial concentration of mIgG2b in solution. The isotherm on the left displays typical Langmuir adsorption behaviour and can be linearized as shown on the right. All experiments have been performed in triplicate.

The plot on the left exhibits typical Langmuir type behaviour with the equilibrium amount of mIgG2b binding to the surface increasing with the initial concentration in solution until the surface becomes saturated and plateaus at the maximum surface concentration. The isotherm can be linearized as seen

on the right which allows the extraction of the binding parameters of Affimer-mIgG2b from the slope and intercept of the line fit to the isotherm. The extracted binding data from this process is listed in Table 1 for both the Affimer B9 and Affimer G12.