SUPPORTING INFORMATION

Protein Binding Kinetics in Multimodal Systems: Implications for Protein Separations

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This SI document lists:

Surface characterization

To evaluate the chemistry of the functionalized quartz crystal surfaces, XPS was carried out to provide an elemental analysis for the MM SAM surfaces at different ligand densities (**Table S1**). The data show that for the "Capto ligand" and the "Nuvia ligand" surfaces functionalized at 100% ligand density, the nitrogen content was roughly two times that of the sulphur which was expected based on the ligand chemistry (**Figure 1A** and **1B**). Further, for both ligand surfaces, a drop in the nitrogen content was observed at the lower ligand density of 40%. The data also indicate similar sulphur content across all the surfaces, which was expected due to the presence of the gold reactive thiols that terminate all of the linkers used in the functionalization, including the PEG linkers used for passivation. The slight differences in the carbon and oxygen content observed across the surfaces may be due to the presence of waters of hydration on these SAMs in varying concentrations.

Quartz crystal microbalance with dissipation

Figure S1 shows a representative QCM-D sensogram resulting from a typical experiment where Δf upon adsorption of proteins to the MM surfaces is presented at different overtones ($n = 3$ (15 MHz), $n = 5$ (25 MHz) and $n = 7$ (35 MHz)). As described in the experimental section, in these isocratic experiments the protein is first bound to the MM surface, then washed with the same running buffer and finally the MM surface is regenerated with a high salt wash. As can be seen in **Figure S1**, protein binding results in a large negative Δf and the regeneration step brings the value of Δf back to zero, signifying complete removal of bound protein from the surface.

The red curve in **Figure S1** represents the ΔD obtained at $n = 3$ (15 MHz) during the experiment and the negligible value of dissipation (i.e. $< 0.2 \cdot 10^{-6}$) upon adsorption signifies the formation of a rigid layer (generally $\Delta D/\Delta F < 1 \cdot 10^{-8}$ Hz^{-1}) of protein on the MM surface. In the presence of a rigid layer, as in the present case, the Sauerbrey equation holds, which provides a linear correlation between the change in mass per unit area adsorbed on the surface ($\Delta m''$) and Δf.[1] The Sauerbrey eq. is given by

$$\Delta m'' = -C\frac{\Delta f_n}{n} \quad (1)$$

where C is the mass sensitivity constant (C =-17.7 $ng.cm^{-2}.Hz^{-1}$ at 5 MHz) and $\Delta f_n/n$ is the normalized frequency of the n^{th} harmonic (n =1, 3,...), which is independent of the overtone number n if the adsorbed layer obeys the Sauerbrey equation. The use of the model is further validated by the close overlap of the different frequency overtones in **Figure S1**.[2,3]

Kinetic parameter estimation

Figure S2A shows representative real time protein binding curves under increasing concentrations of the protein in solution. A modified form of the Langmuir isotherm that incorporates a contribution from protein-protein self interactions was employed in this work to facilitate a more precise fitting of the protein binding curves under a wide range of conditions. A kinetic form of the isotherm is presented in eqs. 2-4. The assumptions of the model are as follows: (i) protein adsorption initially occurs according to the Langmuir adsorption isotherm (eq. 2) to give rise to a monolayer of protein on the MM SAM surface; (ii) subsequent protein adsorption can occur onto this previously adsorbed monolayer of protein (eq. 3). Hence, total protein adsorption (Δf_{model}) onto the surface is given by eq. 4 which includes both contributions. Since there is a linear correlation between the mass of protein adsorbed and the frequency change (eq. 1), eqs. 2-4 are written in terms of Δf. **Figure S2B** shows schematics of the first and second steps of the adsorption process as represented by eqs. 2 and 3, respectively. As shown in the figure, k_{ads} and k_{des} signify the adsorption and desorption kinetic rates of the protein binding to the functionalized quartz crystal surface and k_{ads}^{I} and k_{des}^{I} denote the kinetic rates of binding and unbinding between the protein in solution and previously adsorbed proteins, respectively. Δf_{max} in eq. 2 denotes the maximum frequency change attained by the protein under the specific set of fluid phase conditions. The parameters of eqs. 2-3 were estimated by using a global optimization curve-fitting algorithm written in MATLAB where the Δf_{model} was fit to the experimental values of Δf simultaneously across five different protein concentrations (C_{in}). This method provided a significantly greater accuracy in estimation of the fitted parameters (k_{ads}, k_{des}, k_{ads}^{I}, k_{des}^{I} and Δf_{max}). The optimized fits to this kinetic form of the modified Langmuir isotherm is presented by the dotted lines in **Figure S2A**.

$$\frac{d\Delta f_1}{dt} = -k_{des}\Delta f_1 + k_{ads}C_{in}(\Delta f_{max} - \Delta f_1) \quad (2)$$

$$\frac{d\Delta f_2}{dt} = -k_{des}^{I}\Delta f_2 + k_{ads}^{I}C_{in}(\Delta f_1 - \Delta f_2) \quad (3)$$

$$\Delta f_{model} = \Delta f_1 + \Delta f_2 \quad (4)$$

References

(1) Sauerbrey, G. *Z. Physik* **1959**, *155*, 206-222.
(2) Höök, F.; Kasemo, B.; Nylander, T.; Fant, C.; Sott, K.; Elwing, H. *Anal. Chem.* **2001**, *73*, 5796-5804.
(3) Höök, F.; Rodahl, M.; Brzezinski, P.; Kasemo, B. *Langmuir* **1998**, *14*, 729-734.

Table S1. Elemental analysis by XPS of MM SAM surfaces prepared on gold coated quartz crystals.

		Atomic Composition (%)				
Surface	**Ligand density (%)**	**Au**	**C**	**O**	**N**	**S**
"Capto ligand"	**100**	37.9 ± 1.8	44.5 ± 1.4	13.2 ± 0.4	3.2 ± 0.2	1.5 ± 0.2
	40	42.1 ± 1.3	41.9 ± 0.9	12.6 ± 0.4	1.8 ± 0.6	1.6 ± 0.3
"Nuvia ligand"	**100**	39.6 ± 0.8	42.7 ± 0.2	13.9 ± 1.0	2.7 ± 0.3	1.3 ± 0.2
	40	43.6 ± 0.7	41.3 ± 0.2	12.5 ± 1.0	1.2 ± 0.3	1.5 ± 0.1

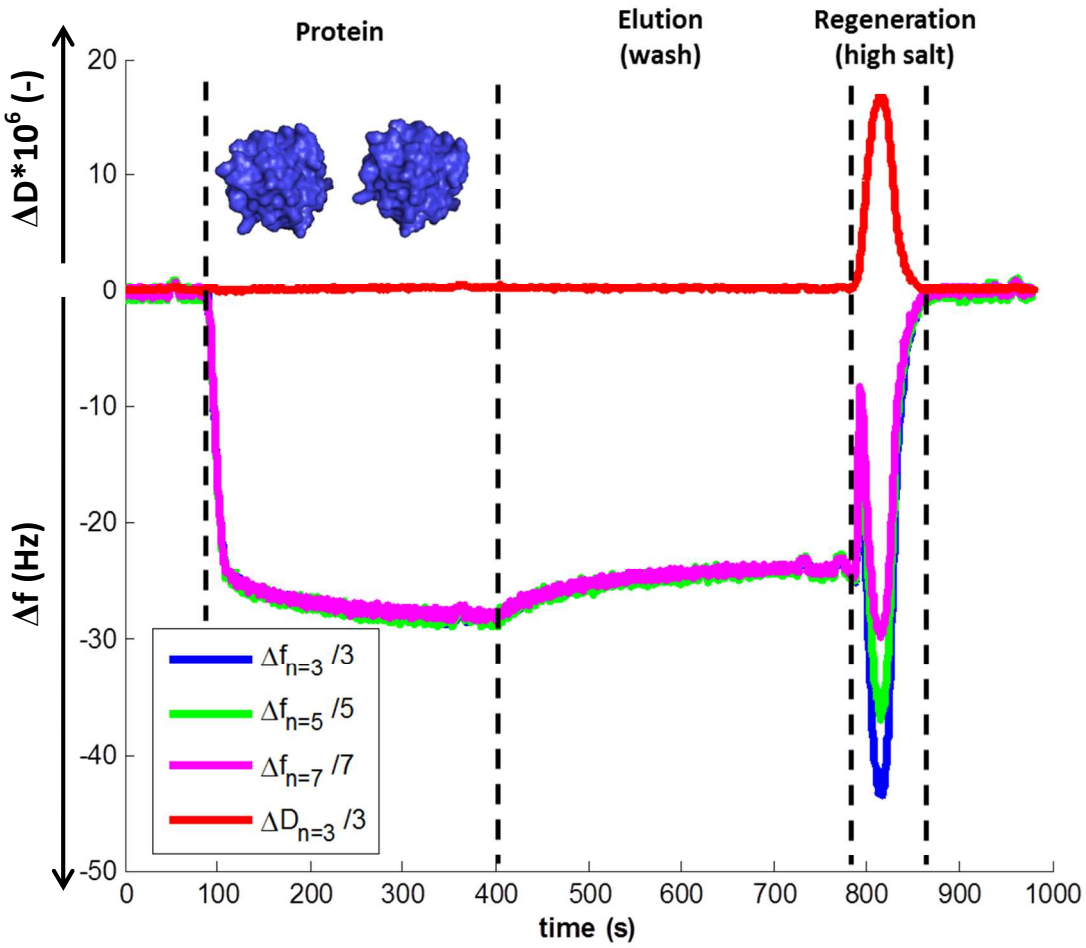

Figure S1. Representative QCM-D experiment showing binding of protein to the MM SAM functionalized gold-coated sensor followed by a wash and subsequent regeneration of the surface under high salt conditions. Blue, green and pink curves represent the different frequency overtones, 3rd, 5th and 7th, respectively, and the red curve represents the 3rd overtone for the dissipation change.

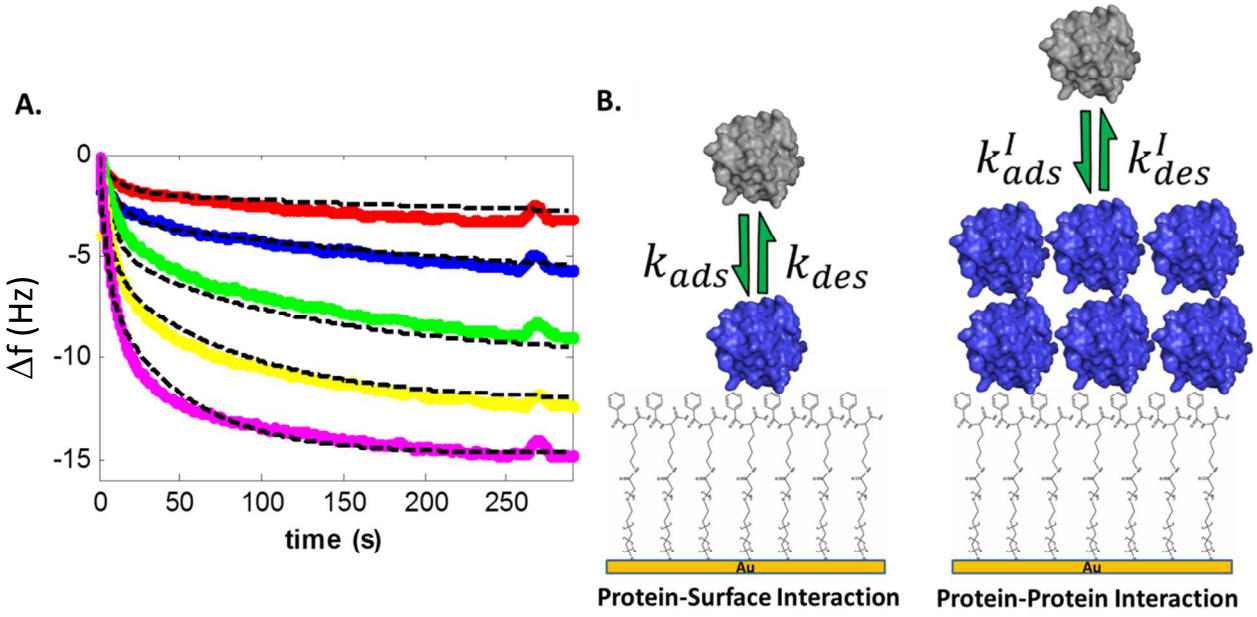

Figure S2. A. Representative binding curves under increasing concentrations of protein (lowest concentration displayed in red and highest in magenta) wherein the fits to the data are represented by dotted lines. **B.** Schematic of the sequential two step binding process represented by eqs. 2-4.