Supporting Information: Differential surface adsorption phenomena for conventional and novel surfactant correlates with changes in interfacial mAb stabilization

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Langmuir Adsorption Fit

The dynamic surface tension of surfactant alone in 20 mM histidine buffer, pH 6.0 was recorded for the concentration range 8.0×10^{-3} mg/mL – 3.0×10^{-2} mg/mL (or 0.6 – 24 µM for PS80, Fig. 2b) and 3.0×10^{-5} – 4.0×10^{-3} mg/mL (or 0.02 – 2.86 µM for FM1000, Fig. 2c over the time period of 1.0 – 1.0×10^{4} s. The change in surface tension value at longer time-scales for concentration > $PS80_{CMC}$ (1.6×10^{-2} mg/mL (12 µM)) and > $FM1000_{CMC}$ (3.0×10^{-3} mg/mL (2.14 µM)) was only ±0.2 mN/m. Based on this, the CMC for PS80 and FM1000 was found to be around 1.6×10^{-2} mg/mL and 3.0×10^{-3} mg/mL respectively, where no further change in equilibrium surface tension value was observed for long-time asymptotes.

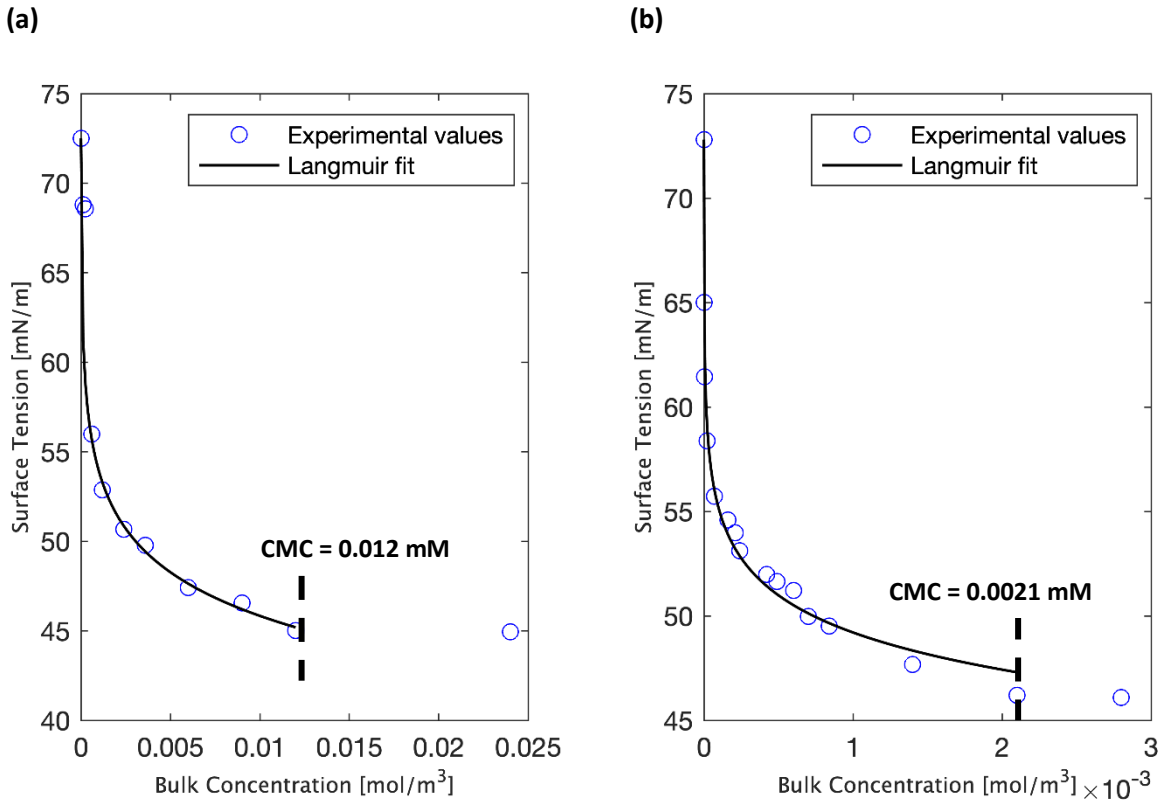

Figure S1: Langmuir adsorption isotherm fit (solid line) to the equilibrium surface tension data (blue markers) of (a) PS80 and (b) FM1000 obtained from pendant bubble tensiometry

Langmuir adsorption framework was used to determine maximum surface coverage (Γ_∞) of surfactant molecules at the air/water interface based on the equilibrium surface tension (γ) as a function of bulk surfactant concentration (C_s). The corresponding adsorption isotherm and equation of sate are given by

$$\frac{\Gamma}{\Gamma_\infty} = \frac{1}{1+\frac{a}{C_s}}$$

$$\gamma = \gamma_c + RT\Gamma_\infty ln\left(1-\frac{\Gamma}{\Gamma_\infty}\right)$$

where, γ_c is the clean air/water surface tension (72.8 mN/m), R is the universal gas constant, T is the temperature, Γ is the surface concentration and a is the ratio of the desorption to adsorption rate constants. Figure S1 shows the equilibrium surface tension as a function of bulk concentration of (a)PS80 and (a) FM1000 on semi log axis along with the fit using Langmuir parameters (solid line). We note that the fit using Langmuir isotherm is good giving Γ_∞ = 1.88 mg/m^2 and a =5.09 × 10^{-9} mol/L for PS80 and Γ_∞ = 1.25 mg/m^2 and a = 9.45 × 10^{-11} mol/L for FM1000. Alternatively, the minimum area per molecule ($A_s = 1/\Gamma_\infty N_{Av}$) is around 116 Å^2/molecule for PS80 and 187 Å^2/molecule at the air/water interface.

X-ray reflectivity data analysis

The reflectivity, $R(Q_z)$ is measured as a function of the incident angle, α. The experimental reflectivity data in Figs. 3a, 4a and 5a is reported as the normalized reflectivity, $R(Q_z)/R_F(Q_z)$, where, $R_F(Q_z)$ is the Fresnel reflectivity from an ideally flat surface with a step-function based electron density profile (EDP). The normalization of the obtained reflectivity profile helps to calculate the thickness of the adsorbed layer and the structural features of the molecules in the form of oscillations at the interface. This serves as a qualitative distinguishing feature as proteins have a larger thickness (~200 Å) as compared to surfactants (~30-50 Å) and two maxima peaks in the normalized reflectivity are observed for proteins as compared to a broad maximum for the case of surfactants at $Q_z < 0.2$ Å^{-1}. The corresponding electron density profile to estimate thickness of the adsorbed layer and the adsorbed amount at the interface is obtained using the normalized reflectivity profile using the slab-model based on Parratt formalism. More details on this are provided elsewhere.[1,2]

Briefly, the normalized reflectivity data is fitted by assuming a constant electron density with a thickness value appropriate for the system (for example, 200 Å for mAb). This thickness layer is further sub-divided into additional sub-layers, each layer associated with an electron density, thickness and roughness. This initial EDP then undergoes an iterative procedure to minimize the mean square variation (χ^2). χ^2 represents the difference between the calculated and the experimental XR curves. To test the convergence of this fitting procedure and obtain a unique EDP, the above process is repeated using different initial guesses for electron density. The EDP from this model-independent approach is then used as an initial guess for the slab-model to get a smooth EDP.

Calculation of the Surface Adsorbed Amounts for Single Component System (mAb or PS80 or FM1000)

The surface adsorbed amount (surface concentration) for the single component system is calculated based on the volume balance at the interface and a total electron density balance:

$$d = \frac{N_{Av}}{MW_s}\Gamma_s v_s + \frac{N_{Av}}{MW_w}\Gamma_w v_w$$

$$e_T = \frac{N_{Av}}{MW_s}\Gamma_s e_s + \frac{N_{Av}}{MW_w}\Gamma_w e_w$$

where, the subscript, s and w denotes surfactant and water, N_{Av} is the Avogadro's number, MW is the molecular weight, v is the molecular volume and e is the number of electrons. d and e_T are obtained from the EDP profile (Figs. 3b of the main text, S2b and S3b) and represent the interfacial depth in the z-direction and the total number of electrons per unit area of adsorbed species respectively. d is set to an interfacial depth for which the electron density reaches the electron density of water (0.333 $electrons/$ Å^3) and e_T is integrated area under the EDP curve. The parameters required for solving the above two equations are given in Table S1 and are solved simultaneously to calculate the unknown surface concentration (Γ_s and Γ_w). The above equations are also used to calculate the adsorbed amounts of mAb Γ_p, with the molecular volume and number of electrons in the above equation replaced by the antibody values (Table S1). Alternatively, one can calculate the corresponding area per molecule for surfactant $(A_s = 1/\Gamma_s)$ and mAb $\left(A_p = 1/\Gamma_p\right)$ at the air/water interface. Using this methodology, Table 2 (PS80 and FM1000) and Table 3 (mAb) of the main text and Table S2 (PS80) of the Supporting Information gives the surface concentrations for pure component adsorption layers from solutions.

Calculation of the Surface Adsorbed Amounts for Mixed Component System (mAb + PS80 or mAb + FM1000)

The surface concentration of mAb and surfactant during co-adsorption is calculated by assuming that the surface structure of the both the mAb and surfactant in the mixed system is same as that in their pure (single component) system for a given bulk concentration. Therefore, we can say that the ratio of the water to mAb molecules (c_p) and the ratio of water to surfactant molecules (c_s) in the mixed system is similar to its pure mAb and pure surfactant system $\left(c_p = \frac{\Gamma_w^* MW_p}{\Gamma_p^* MW_w} \text{ and } c_s = \frac{\Gamma_w^* MW_s}{\Gamma_s^* MW_w}\right)$. The asterisks represent the surface concentration obtained from the pure component system (mAb alone or surfactant alone). This assumption can be justified because the number of water molecules per unit area was independent of the component (mAb or PS80) and its concentration in each of the pure systems. Thus, the surface concentrations can be found from:

$$e_T = a\frac{N_{Av}}{MW_p}\Gamma_p^* e_p + aN_{Av}\frac{MW_w}{MW_p}c_p\Gamma_p^* e_w + (1-a)\frac{N_{Av}}{MW_s}\Gamma_s^* e_s + (1-a)N_{Av}\frac{MW_w}{MW_s}c_s\Gamma_s^* e_w$$

where, a is the only unknown parameter to be solved for and represents the relative percent surface coverage of mAb. Therefore, the corresponding surface concentration of mAb and surfactant in the mixed system is given by $\Gamma_p = a\Gamma_p^*$ and $\Gamma_s = (1-a)\,\Gamma_s^*$. The value of e_T is obtained from the EDP (Figs. 4b, S4b for mAb+PS80 and 5b, S5b for mAb+FM1000). Using this methodology, Tables 4 and S3 (mAb+PS80) and Tables 5 and S4 (mAb+FM1000) gives the surface concentrations of mAb and surfactant for mixed adsorption layers from solutions. In each case where the fits resulted into negative numbers for surface adsorbed amount of protein or negative surface concentrations for proteins, the values are at the minimum resolvable concentration. This indicates protein adsorption is inhibited by the surfactant molecules, and a number of zero is reported. The values of a and $(1-a)$ are represented in Fig. 6 of

the main text to determine the minimum surfactant concentration for a given bulk mAb concentration to inhibit mAb adsorption onto the air/water interface.

Homology model

Based on the antibody sequence, the 3D structure of the mAb used in the study was built using the standard homology modeling protocol[3] in the MOE software.[4–6] The dimensions of the Fab and Fc domains of the mAb are shown in Figure S8. These were estimated based on the relevant inter-atomic distances from the homology model.

References

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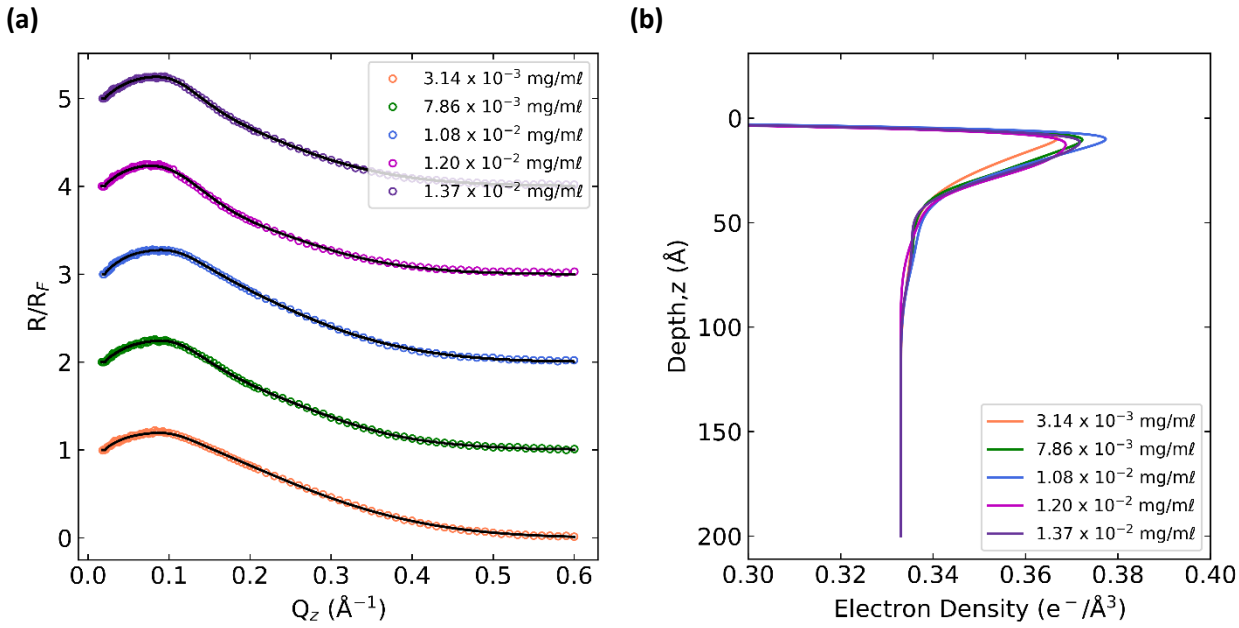

Figure S2: XRR measurements of the adsorbed layers of pure PS80 from bulk solution onto the air/water interface. (a) Representative normalized XRR profiles, R/R_F, as a function of wave vector transfer, Q_z, (symbols) and Parratt fits to the reflectivity profile (solid line) for PS80 in the concentration range 3.14 × 10^{-3} mg/mL to 1.37 × 10^{-2} mg/mL. XRR data for surfactants PS80 show a broad maximum for the studied concentration range. The upper four XRR curves are shifted for clarity; $R/R_F \rightarrow 1$ as $Q_z \rightarrow 0$ for all measurements. (b) Corresponding electron density profiles as a function of interfacial depth, z, obtained from the reflectivity fits for PS80.

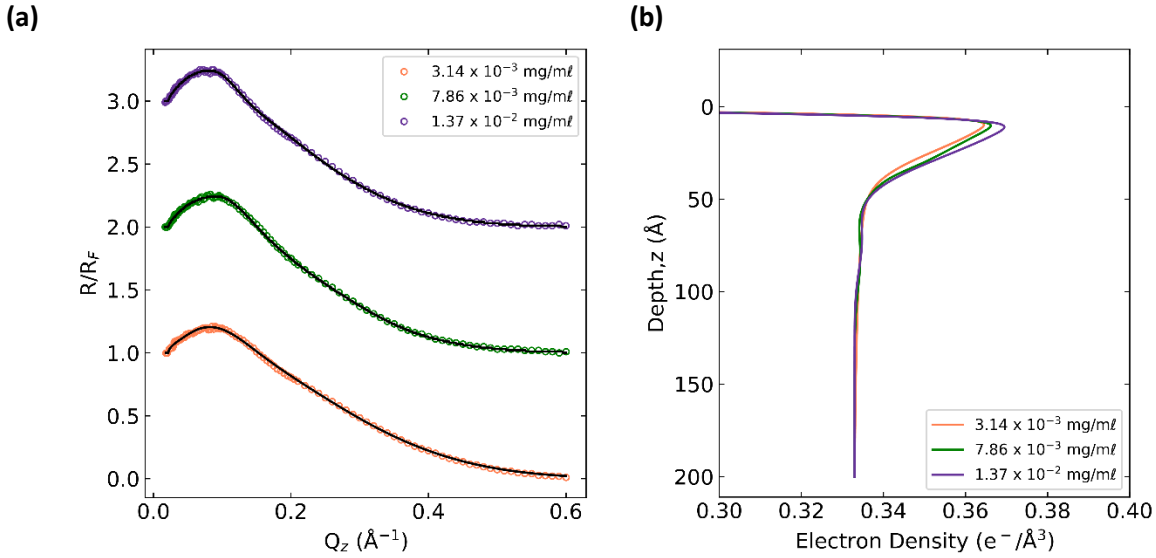

Figure S3: XRR measurements of the adsorbed layers of pure FM1000 from bulk solution onto the air/water interface. (a) Representative normalized XRR profiles, R/R_F, as a function of wave vector transfer, Q_z, (symbols) and Parratt fits to the reflectivity profile (solid line) for FM1000 in the concentration range 3.14 ×

10^{-3} mg/mL to 1.37 × 10^{-2} mg/mL. XRR data for surfactants FM1000 show a single peak. The upper two XRR curves are shifted for clarity; $R/R_F \rightarrow 1$ as $Q_z \rightarrow 0$ for all measurements. (b) Corresponding electron density profiles as a function of interfacial depth, z, obtained from the reflectivity fits for FM1000.

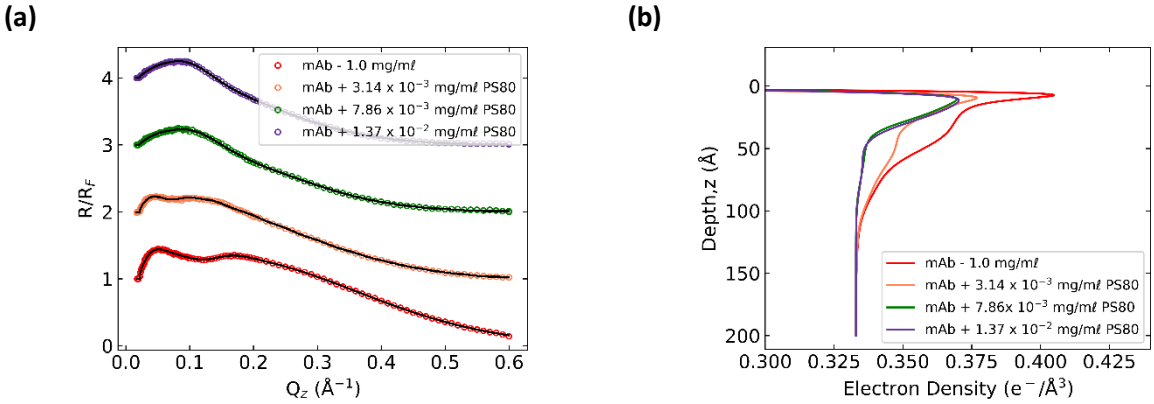

Figure S4: XRR measurements of the adsorbed layers from competitive adsorption of mAb and PS80 from bulk solution onto the air/water interface. (a) Normalized XRR profiles, R/R_F, as a function of wave vector transfer, Q_z, (symbols) and Parratt fits to the reflectivity profile (solid line) for mAb (1.0 mg/mL) and PS80 at increasing concentration (3.14 × 10^{-3} mg/mL, 7.86 × 10^{-3} mg/mL, and 1.37 × 10^{-2} mg/mL). The XRR curves are shifted for clarity; $R/R_F \rightarrow 1$ as $Q_z \rightarrow 0$ for all measurements. (b) Corresponding electron density profiles as a function of interfacial depth, z, obtained from the reflectivity fits for the mixed component system derived from (a).

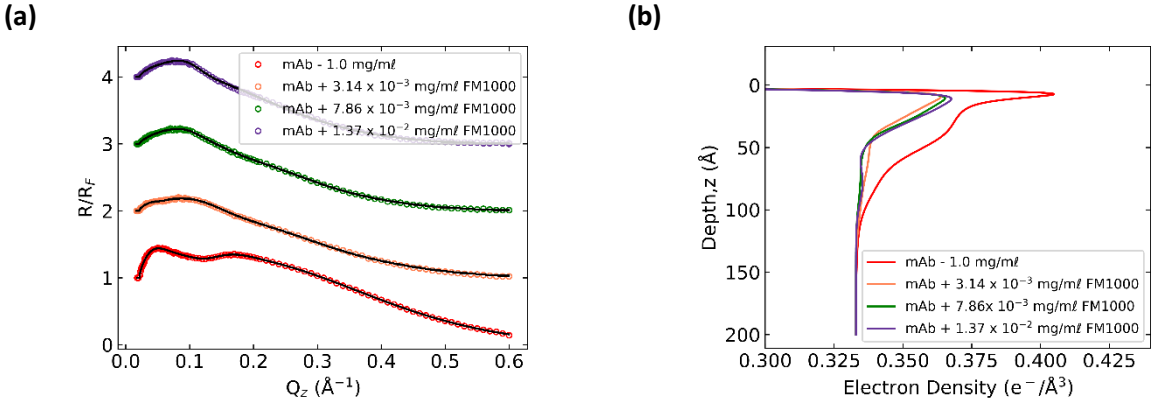

Figure S5: XRR measurements of the adsorbed layers from competitive adsorption of mAb and PS80 from bulk solution onto the air/water interface. (a) Normalized XRR profiles, R/R_F, as a function of wave vector transfer, Q_z, (symbols) and Parratt fits to the reflectivity profile (solid line) for mAb (1.0 mg/mL) and FM1000 at increasing concentration (3.14 × 10^{-3} mg/mL, 7.86 × 10^{-3} mg/mL, and 1.37 × 10^{-2} mg/mL). The XRR curves are shifted for clarity; $R/R_F \rightarrow 1$ as $Q_z \rightarrow 0$ for all measurements. (b) Corresponding electron density profiles as a function of interfacial depth, z, obtained from the reflectivity fits for the mixed component system derived from (a).

(a) (b)

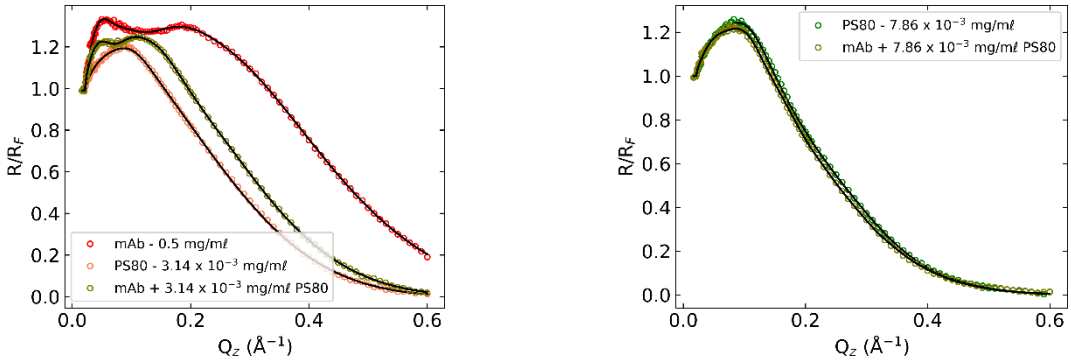

Figure S6: Overlap of the X-ray reflectivity measurements from adsorbed layers at the air/water interface for the mixed component system (a) X-ray reflectivity measurement of mAb (0.5 mg/mL) + 3.14 × 10^{-3} mg/mL PS80 concentration (olive circle marker) alongside with the pure mAb (0.5 mg/mL) (red circle marker) and pure PS80 (3.14 × 10^{-3} mg/mL) (orange circle marker) concentration. PS80 concentration of 3.14 × 10^{-3} mg/mL is not sufficient to prevent the adsorption of mAb to the surface as evident due to the presence of more than one peak as opposed to a single peak for PS80. The intensity of the peak is lower than that of pure mAb indicating co-adsorption of mAb + PS80. (b) X-ray reflectivity measurement of mAb (0.5 mg/mL) + 7.86 × 10^{-3} mg/mL PS80 concentration (olive circle marker) alongside with the pure PS80 (7.86 × 10^{-3} mg/mL) (green circle marker) concentration. Overlapping reflectivity profile of pure PS80 with the mixed system indicates PS80 dominating the surface and inhibiting the adsorption of mAb.

(a) (b)

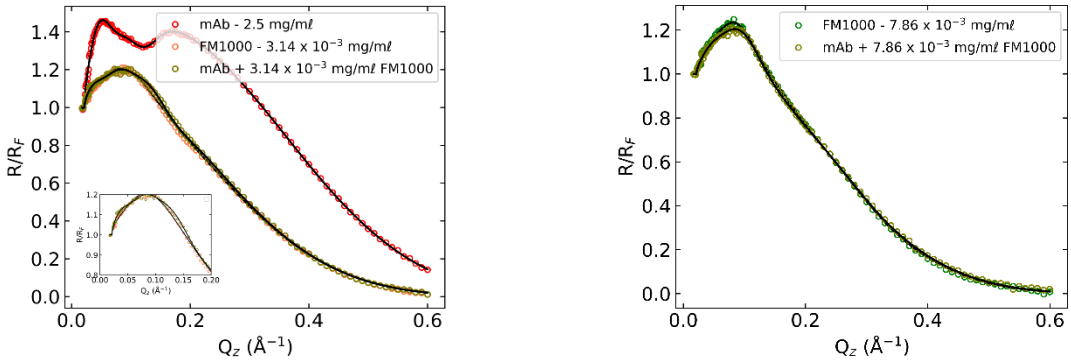

Figure S7: Overlap of the X-ray reflectivity measurements from adsorbed layers at the air/water interface for the mixed component system (a) X-ray reflectivity measurement of mAb (2.5 mg/mL) + 3.14 × 10^{-3} mg/mL FM1000 concentration (olive circle marker) alongside with the pure mAb (2.5 mg/mL) (red circle marker) and pure FM1000 (3.14 × 10^{-3} mg/mL) (orange circle marker) concentration. FM1000 concentration of 3.14 × 10^{-3} mg/mL is not sufficient to prevent the adsorption of mAb to the surface as evident due to the presence of more than one peak (subset plot) as opposed to a single peak for FM1000. (b) X-ray reflectivity measurement of mAb (2.5 mg/mL) + 7.86 × 10^{-3} mg/mL FM1000 concentration (olive circle marker) alongside with the pure FM1000 (7.86 × 10^{-3} mg/mL) (green circle marker) concentration. Overlapping reflectivity profile of pure PS80 with the mixed system indicates FM1000 dominating the surface and inhibiting the adsorption of mAb.

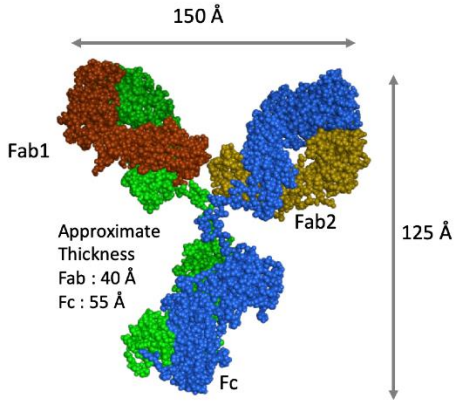

Figure S8: Homology model of the mAb where the variable domains are shown in brown and yellow and the constant domains are shown in green and blue for the light and heavy chains respectively. The dimensions of the Fab and Fc domains of the mAb that were estimated based on the relevant inter-atomic distances from the homology model are also shown.

Table S1: The molecular weight (MW), number of electrons (e) and molecular volume (υ) for the antibody (mAb), PS80, FM1000 and water used in the analysis of XR data.

Component	MW (g/mol)	e	υ (Å^3)
mAb	144481	77299	132565
PS80	1310	716	1803
FM1000	1400	766	1455
water	18	10	30

Table S2: Surface adsorbed amounts and area per molecule for pure layers of PS80

PS80 Conc. mg/mL	Γ_s mg/m^2	A_s Å^2
1.08×10^{-2}	1.88±0.21	116±14
1.20×10^{-2}	2.26±0.14	96±6

Table S3: Surface adsorbed amounts, area per molecule, and relative percent surface coverage of mAb in mixed layers of mAb and PS80

mAb Conc. mg/mL	PS80 Conc. mg/mL	Γ_p mg/m^2	A_p Å^2	Γ_s mg/m^2	A_s Å^2	$\%a$ obtained from fitting for protein coverage
1.0	3.14×10^{-3}	0.28±0.20	86047±36189	1.21±0.14	180±23	18
	7.86×10^{-3}	0	-	2.09±0.09	104±5	-
	1.37×10^{-2}	0	-	2.28±0.17	95±8	-

Table S4: Surface adsorbed amounts, area per molecule, and relative percent surface coverage of mAb in mixed layers of mAb and FM1000

mAb Conc.	FM1000 Conc.	Γ_p	A_p	Γ_s	A_s	$\%a$ obtained from fitting for protein coverage
mg/mL	mg/mL	mg/m^2	Å^2	mg/m^2	Å^2	
1.0	3.14×10^{-3}	0.29±0.06	82689±21267	0.43±0.17	543±156	19
	7.86×10^{-3}	0	-	0.80±0.02	292±9	-
	1.37×10^{-2}	0	-	1.00±0.08	232±21	-