Supplementary Information for Interfacial Rheology of Lanthanide Binding Peptide Surfactants at the Air-Water Interface

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I. K_D MEASUREMENT FOR RR-LBT³⁻

To find the K_D of the RR-LBT³⁻ mutant, a Tecan Spark plate reader assay was performed as described in prior work[1]. In short, the Trp in the peptide is excited at 280 nm. Energy transfer between the Trp residue and bound Tb³⁺ results in Tb³⁺ emission that is measured at 545 nm. By holding the RR-LBT³⁻ concentration constant, and increasing the concentration of Tb³⁺ in solution, the luminescence intensity increases then plateaus as the peptide becomes saturated S1. The collected luminescence data were fitted to find the free energy of binding for Tb³⁺ to RR-LBT³⁻.



Figure S1. Tb³⁺ luminescence intensity, as sensitized by 50 nM RR-LBT³⁻, used to calculate K_D .

II. RESOLUTION OF MEAN SQUARED DISPLACEMENT

To find the minimum MSD our microscope can measure, dried polystyrene particles $(2a = 1\mu m)$ were placed directly on glass coverslip. The apparent motion of these fixed particles was tracked. The resulting MSD of the particles is shown in Fig. S2. This nearly flat MSD vs lag-time shows the minimum MSD we can resolve.

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Figure S2. The MSD resolution of the microscope used in this work is based on the perceived motion of static particles is about $0.003 \ \mu m^2$.

III. RELATIONSHIPS BETWEEN DIFFUSIVITY AND BQ

Three relationships between particle diffusivity, D, and surface viscosity, made non-dimensional in Bq, were considered. In each case the diffusivity is related to a drag coefficient through the Stokes-Einstein relation:

$$D = \frac{k_B T}{4\pi \eta a \Lambda} \tag{S1}$$

where η is the viscosity of the subphase, *a* is the particle radius, and Λ is the drag coefficient. The following expressions for Λ are taken from Hughes, et. al.[2], Saffman and Delbruck[3, 4], and Fischer et. al.[5]

$$\Lambda_{Hughes} = \left[\frac{1}{Bq} \left(\ln 2Bq - \gamma + \frac{4}{\pi Bq} - \frac{1}{2Bq^2} \ln 2Bq\right)\right]$$
(S2)

$$\Lambda_{Saffman} = \left[\frac{1}{Bq} \left(\ln 2Bq - \gamma\right)\right] \tag{S3}$$

$$\Lambda_{Fischer} = \left[\frac{1}{Bq} \left(\ln Bq \sin \theta - \gamma\right)\right] \tag{S4}$$

where γ is the Euler-Mascheroni constant and θ is the contact angle of the particle with the fluid interface. Fig. S3 shows Bq as calculated from D using these different expressions. For this work we chose to use the Hughes relationship since it covers the largest range of Bq.



Figure S3. Various expressions relating D to Bq all give similar results.

IV. INTERFACIAL RHEOLOGY OF CONTROL INTERFACES

To ensure the results we measure with PEPS and Tb^{3+} present are due to the adsorbed layer of PEPS, two controls are used. We measured the surface viscosity of buffer solution that contained no PEPS and a buffer solution that contained 260 μ M Tb³⁺ (Fig. S4). We find that the surface viscosity increases slightly when Tb³⁺ is present at 260 μ M, however, the measured Bq = 5.40 is significantly smaller than the Bq measured for solutions of PEPS and Tb³⁺ at high concentrations. Therefore, we are confident that the Bq we measure for PEPS containing solutions is largely attributable to the interfacial PEPS layer and not impurities in our buffer or Tb³⁺ solution.



Figure S4. Addition of Tb³⁺ to a buffer solution increases surface viscosity slightly. EMSD of probe particles $(2a = 1\mu m)$ at the air-water interface in the presence of bulk solution containing (a) 50 mM MES and 100 mM NaCl buffer solution; (b) buffer solution and 260 μ M Tb³⁺. Insets in (a) and (b) show the distribution of particle diffusivities. The black dashed lines are fits of the form MSD=4D τ . The solid black lines are guides to show a power law with an exponent of 1.

V. CORRELATED DISPLACEMENT VELOCIMETRY MEASUREMENT RESOLUTION

To ensure that the displacements calculated by correlated displacement velocimetry (CDV) are resolved above noise from Brownian motion the following inequality must be true:

$$U(|\mathbf{x}|) \gg \frac{\langle \Delta r^2 \rangle^{1/2}}{\sqrt{N}} \tag{S5}$$

where U is the displacement $(U = u_y \tau), \langle \Delta r^2 \rangle^{1/2}$ is the mean squared displacement at the lag time that the CDV is constructed, and N is the number of data points in a bin at the location $|\mathbf{x}|$. In words, the average Brownian noise is given by the MSD of the particles. We divide this average noise by the number of data points and take the square root to calculate a value akin to the standard error of the mean Brownian motion. If the displacement is larger than this value, then it can be resolved above the noise level. We define a significance criterion, S:

$$S \equiv \frac{U(|\mathbf{x}|)\sqrt{N}}{\langle\Delta r^2\rangle^{1/2}} \gg 1 \tag{S6}$$

and plot the left side of this expression versus $|\mathbf{x}|$ to determine whether sufficient data have been binned for displacements at given locations in the interface to be considered significant. Figure SS5 shows examples of these significance plots.



Figure S5. Resolution analysis for representative Bq interfaces. (a, c, e) Significance metric, S, for decreasing Bq. Inset is the number of datapoints at each location on the interface in 1000s of datapoints. The circles represent the y-directed displacements along the y axis, the black squares represent the y-directed displacements along the x axis. The red squares represent the total displacement. (b, d, f) Fits to the velocity decays for increasing Bq demonstrating how the data is cutoff when it is below resolution.

Our data shows for large Bq the resolution criterion is always met. This is because the Brownian noise is considerably smaller for such viscous interfaces. As Bq decreases, the Brownian noise increases and the velocity decays faster. As a result, the resolution criterion begins to fail. Still, the flow fields are largely well-resolved. The red squares, which represent the total displacement (Fig. S5c, e), typically meet the criterion despite the y-directed displacements not being strong enough. This implies that while the entire flow field may not be quantitatively accurate for fitting to find Bq, the streamlines and velocity magnitudes are accurate. Lastly, we highlight that the y-directed velocities along the y axis are always above the resolution criterion which allows Bq fitting to always take place. Generally, the y-directed velocities along the x axis are most sensitive to Bq, but when these velocities are not well resolved, the y axis decays are sufficient.

VI. CDV FLOW FIELD CORRECTIONS

In most cases, drift affects the data which causes the flow field to show a recirculatory flow. Since such flow is not expected, as shown in Fig. S6, we apply a correction to the flow field by subtracting the average y-directed velocity along the x axis far from the origin multiplied by a constant close to 1.0. The exact value of the constant is found by looking at the resulting velocity decays and picking a value where those decays are smoothest. In addition, there are sometimes significant asymmetries which we remove by averaging. This averaging is done by taking the average of the velocities at the same location relative to the x and y axes. We perform this correction on all flow fields regardless of whether they are asymmetric or not. Some examples of these corrections are shown in Fig. S6.



Figure S6. Flow field (a) before drift correction and (b) after drift correction. The average y-directed velocity is taken from the points highlighted by the cyan bar and the multiplier for this mean velocity is 1.15. Flow field (c) before symmetrization and (d) after symmetrization. Scale bars are 20 μ m.

VII. CORRELATED DISPLACEMENT VELOCIMETRY

The theoretical flow fields that were derived for a point force in the interface[6] are shown in the top row of Fig. S7. Bq increases from 3 (Fig. S7a) to 30 (Fig. S7b) to 300 (Fig. S7c). The streamlines show a waist which widens with increasing Bq. The colormap which depicts the velocity magnitude shows that there is slower decay for larger Bq. The decay of the y-directed velocities along the x and y axes as a function of the separation distance $|\mathbf{x}|$ divided by the Boussinesq length ($L_B = \eta_s/\eta$) is depicted in Fig. S7d. Once $|\mathbf{x}|/L_B$ becomes greater than ~5, the decay of the y-directed velocity along the y-axis becomes proportional to $|\mathbf{x}|^{-1}$ and the decay of the y-directed velocity along the x-axis is proportional to $|\mathbf{x}|^{-2}$. We extract y-directed velocities from measured flow fields[7] and fit their decays to these theoretical forms to find the Bq of the interface. The fitting is achieved by first finding the magnitude of the point force which shifts the entire decay curve up or down. Next, LB¬ is found using interval halving around an initial guess based on the single point value for Bq until a minimum sum of square error is found. L_B is multiplied by 2.0 to get Bq since Bq = L_B/a and a = 0.5 μ m.



Figure S7. Theoretical flow fields for increasing Bq show a widening waist; y-directed velocity along the x and y axes decays as $|\mathbf{x}|^{-2}$ and $|\mathbf{x}|^{-1}$, respectively, for separation distances larger than L_B . Flow fields with (a) Bq = 3; (b) Bq = 30; (c) Bq = 300. The black lines are guides to show $|\mathbf{x}|^{-2}$ and $|\mathbf{x}|^{-1}$ decays. (d) y-directed velocity along the x axis (orange) and y axis (blue) versus separation distance divided by the Boussinesq length L_B .

VIII. SIMULATION SNAPSHOTS

The simulation box, along with some snapshots are shown below. Note in Fig. S8b and d the difference in the location of most excess Tb^{3+} . For LBT⁵⁻ (Fig. S8b) the excess Tb^{3+} localize in the interfacial layer, whereas for LBT³⁻ (Fig. S8d) the excess Tb^{3+} are more distributed throughout the solution.

Table S1. MD Simulation Parameters. The first column is the system label, the second column is the peptide type, the third column is the Tb³⁺ to peptide concentration ratio, and the fourth column is the peptide surface area density in *molecules*/Å². N_P , N_w , N_{Tb} , N_{Na} , and N_{Cl} represent the number of peptide molecules, water molecules, Tb³⁺ ions, Na⁺ ions, and Cl⁻ ions, respectively.

System	Peptide	$\frac{[Tb^{3+}]}{[LBT]}$	$\Gamma_P(molecules/Å^2)$	N_P	N_w	N_{Tb}	N_{Na}	N_{Cl}
1	LBT^{5-}	2	0.0054	108	41980	216	96	204
2	LBT^{5-}	2	0.0040	80	44550	160	96	176
3	LBT^{5-}	1.75	0.0040	80	44550	140	96	116
4	LBT^{5-}	1.50	0.0040	80	44550	120	136	96
5	LBT^{5-}	1.25	0.0040	80	44550	100	196	96
6	LBT^{5-}	1	0.0040	80	44550	80	256	96
7	LBT^{3-}	2	0.0051	102	42436	204	96	402
8	LBT^{3-}	1	0.0051	102	42436	102	300	300



Figure S8. (a) The simulation box with PEPS in their starting position. Tb^{3+} ions are in purple, Cl^{-} ions in green, and Na^{+} ions in blue. Side views of the interface for (b) $Tb^{3+}:LBT^{5-}$ 2:1; (c) $Tb^{3+}:LBT^{5-}$ 1:1; (d) $Tb^{3+}:LBT^{3-}$ 2:1.



Figure S9. CDV for LBT³⁻. (Left column) EMSD of probe particles $(2a = 1\mu m)$ at the air-water interface. Insets show the distribution of particle diffusivities. The black dashed lines are fits of the form MSD=4D τ . The solid black lines are guides to show a power law with an exponent of 1. (Middle column) flow fields constructed from CDV. Scale bars are 20 μ m. (Right column) y-directed velocity decays along the y-axis (circles) and x-axis (squares) fits from the flow field functional form, used to find Bq, are shown in red dashed lines.

X. SIMULATION OF LBT³⁻ WITH CA²⁺



Figure S10. Snapshot of LBT^{3-} with Ca^{2+} bound after 1 μ s of simulation. Here the binding loop is formed around the Ca^{2+} , thereby making the anionic ligands not available for crosslinking.

XI. AGGREGATION AT THE AIR WATER INTERFACE



Figure S11. Aggregates present in the air-water interface upon addition of Al^{3+} . (Left) Micrograph of the air-water interface with 400 μ M Al^{3+} and 100 μ M LBT^{3-} containing solution. (Right) Micrograph of typical interface of solution containing Tb^{3+} and PEPS over the range of concentrations studied. The black dots are $2a = 1 \mu$ m polystyrene beads that are trapped in the interface which can be used to determine the scale.

XII. SIMULATION RESULTS COMPARING LBT⁵⁻, LBT³⁻, AND RR-LBT⁵⁻

The simulation snapshot below in Fig. S12d shows the interfacial layer of a solution containing RR-LBT³⁻ and excess Tb^{3+} cations. Inspection of this snapshot shows locations (highlighted) where the anionic ligands outside the binding loop are interacting with excess cations. In addition, we plot the number of excess Tb^{3+} that are free (n_0) , associated with one PEPS anionic ligand (n_1) , and associated with two PEPS anionic ligands (n_2) over the course of the simulation. For LBT⁵⁻ and RR-LBT³⁻ we see significant populations of Tb^{3+} associating with one and two anionic ligands in contrast to LBT³⁻, where no such population is found.



Figure S12. Additional simulation results for all PEPS studied for (a) LBT^{5-} , (b) $RR-LBT^{3-}$, and (c) LBT^{3-} . The number of each type of Tb^{3+} found in the simulation versus time where n_0 (blue) represents free Tb^{3+} , n_1 (red) represents Tb^{3+} associating with one PEPS anionic group, and n_2 (green) represents Tb^{3+} associating with two PEPS anionic groups. (d) Simulation snapshot of the interface laden with $RR-LBT^{3-}$ and excess Tb^{3+} . Two spots are highlighted showing crosslinking and an extra Tb^{3+} associating with a free anionic ligand.

XIII. CALCULATING BASIN FREE ENERGY

The free energy of each basin can be calculated according to:

$$\langle F_{basin} \rangle = -k_B T \log \left(\int_{s_1(b)}^{s_1(a)} \int_{s_2(d)}^{s_2(c)} e^{-F(s_1,s_2)/k_B T} ds_1 ds_2 \right)$$
(S7)

where $\langle F_{basin} \rangle$ is the free energy value integrated over a given two-dimensional area covered by collective variables s_1 and s_2 which corresponds to the coordination number (CN) of Tb³⁺ and the root mean square deviation of α carbon (C_{α} -RMSD) of the binding loop domain of LBT peptide respectively. k_B is the Boltzmann constant, T is the temperature.



Figure S13. Plot of free energies for different basins (CN=5-10) of (a) $LBT^{5-}:Tb^{3+}$ and (b) $LBT^{3-}:Tb^{3+}$ binding structures as a function of simulation time, for checking the convergence of the enhanced sampling.





Figure S14. Violin plots for (a) CN=8, $LBT^{5-}:Tb^{3+}$ and (b) CN=7 $LBT^{3-}:Tb^{3+}$.

XV. AGGREGATION VIDEOS

Supplemental videos can be found as separate files.

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