Supporting Information

The Influence of Metal Ions on the Dynamics of Supported Phospholipid

Langmuir Films

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1. XPS Analysis

To determine the expected ratio between metal and phosphorus mediated by the surface or lipid, we first estimate the total number of phosphorus present:

$$P_{\textit{Total}} = P_{\textit{Surface}} + P_{\textit{Lipid}}$$

The content of surface phosphorus is estimated assuming complete phosphorylation of all present surface hydroxides (5-8 OH/nm²).¹⁻³ The phosphorus content provided from the lipids is estimated using the known mean molecular area from the Langmuir isotherms. At 25mN/m the average molecular area is ca. 69 Å²/molecule (0.69 nm²/molecule), at 1 P/Lipid this represents 1.4 P/nm².

The ratio of metal to phosphorus is given by:

$$M^{x+}: P = \frac{M^{x+}}{P_{Total}}$$

Assuming a surface mediated transfer and a 1:1 ratio of metal ion to surface phosphorus:

$$M^{x+} = P_{Surface}$$

Assuming a lipid mediated transfer and a 1:1 ratio of metal ion to lipid phosphorus:

$$M^{x+} = P_{Lipid}$$

Given these set of assumptions, the expected M^{x+} : P ratio for a surface mediated transfer process is 0.78 for 5 P/nm² and 0.85 for 8 P/nm² yielding an estimated ratio 0.82 +/- 0.04.



Figure S1. XPS survey spectra of DMPC (red), Mg^{2+} (blue), and Ba^{2+} (green) are shown.



Figure S2 (left). Fit of the XPS spectral data for a DMPC/NBD-LPC film containing Ba^{2+} is shown. The $Ba3d_{5/2}$ peak is fit to a Gaussian-Lorentzian curve after Shirley background subtraction. Figure S3 (right). Representative convoluted fits for the overlapping Sn4s and P2p peaks on the $Ba^{2+}/DMPC/NBD-LPC$ film.

2. Analysis of FRAP curves by the Full Reaction-Diffusion Model

The FRAP curves were fit using the full reaction-diffusion model described by Sprague et. Al.⁴ We briefly summarize the model system described by Sprague and relate the assumptions and equations to our lipid film system below:

The analysis starts with a simple single binding site model eq. 1:

$$F + M \underset{k_{off}}{\rightleftharpoons} B$$

$$(1)$$

where F = [f] represents the concentration of free diffusing molecule (NBD-LPC), M = [m] represents the concentration of available binding site (adsorbed metal ions), and B = [b] represents the concentration of bound complex between NBD-LPC and the adsorbed metal.

Assuming the system has reached equilibrium prior to the bleaching event, and that the binding sites are immobile, the recovery can be described by a set of coupled reaction-diffusion equations eq. 2:

$$\frac{\partial f}{\partial t} = D_t \nabla^2 f - k_{on}^* f + k_{off} b$$

$$\frac{\partial b}{\partial t} = k_{on}^* f + k_{off} b$$
(2)

where D_t is the diffusion constant of the NBD-LPC, $k_{on}^* = k_{on}^*[m]$ is the pseudo first-order rate constant derived by assuming that the M = [m] is constant, and ∇^2 is the Laplacian operator. In the case presented here, both assumptions are assumed valid. The surfaces were allowed to equilibrate ~10min prior to imaging and a period of 10 sec was collected prior to the bleaching event to assure equilibrium was reached. The binding sites in this case are considered to any binding interaction with the surface and the surface is assumed to be immobile on the timescale of the measurement.

Because the system is at equilibrium at the start of the FRAP experiment both *F* and *B* are at equilibrium:

$$\frac{df}{dt} = \frac{db}{dt} = 0$$

$$k_{on}^* F_{eq} = k_{off} B_{eq}$$
(3)

Assuming normalization of the FRAP curves, the final recovery of the free and bound species is equal to:

$$F_{eq} + B_{eq} = 1$$

$$F_{eq} = \frac{k_{off}}{k_{on}^* + k_{off}}, B_{eq} = \frac{k_{on}^*}{k_{on}^* + k_{off}}$$
(4)

One way to analytically solve the reaction-diffusion differential equations is to take the Laplace transform of the coupled equations. The inverse Laplace transform was performed using the matlab function *invlap.m* on the analytical equation:

$$frap(t) = \mathcal{L}^{-1}\left\{\frac{1}{p} - \frac{F_{eq}}{p} \cdot \left[1 - 2K_1(q\omega)I_1(q\omega)\right] \cdot \left(1 + \frac{k_{on}^*}{p + k_{off}}\right) - \left(\frac{B_{eq}}{p + k_{off}}\right)\right\}$$
(5)

where frap(t) is recovered by taking the inverse Laplace transform, F_{eq} and B_{eq} are related to k_{on}^* and k_{off} by eq. 4, K₁ and I₁ are the modified bessel functions, ω is the radius of the bleaching spot and q is described by eq. 6:

$$q = \sqrt{\left(\frac{p}{D_f}\right) \left(1 + \frac{k_{on}^*}{p + k_{off}}\right)}$$
(6)

3. FRAP Recovery Curves and Fitting

The FRAP recoveries were fit using the matlab routine *lsqcurvefit.m* with eqns. S5&S6 for the full reaction-diffusion model. After fitting, the FRAP recoveries were averaged for each surface and are shown in Table S1 for the dissociation constant k_{off} , and Table S2 for the diffusion coefficient D_T .

Table S1: Dissociation constant (k_{off}) data acquired by fitting FRAP curves with the reaction-dominant model.

					95% Con Interval f	fidence for Mean	– Minimum	Maximum
Surface	Ν	Mean k _{off} (10 ⁻³ s ⁻¹)	Std. Deviation	Std. Error	Upper Bound	Lower Bound		
SiO _x	19	26.8	13.2	5.9	20.8	32.7	3.3	57.0
PO _x -SiO _x	20	27.2	18.3	8.0	19.1	35.2	0.1	55.4
Mg	19	34.3	22.3	10.0	24.2	44.3	1.0	68.6
Ca	22	32.9	20.2	8.4	24.5	41.3	0.0	75.3
Ba	18	26.7	18.7	8.6	18.0	35.3	2.5	77.0

Ni	21	39.1	20.6	8.8	30.2	47.9	2.4	75.7
Zn	25	28.9	15.4	6.0	22.8	34.9	2.8	78.0
Cd	17	29.9	24.9	11.9	18.0	41.7	1.5	84.4
Zr	9	27.1	15.7	10.2	16.9	37.3	7.7	52.1

 Table S2: Diffusion coefficient data acquired by fitting FRAP curves with the full reaction-diffusion model.

	N			Std. Error	95% Confidence Interval for Mean			
Surface		Mean (D _T)	Std. Deviation		Upper Bound	Lower Bound	Minimum	Maximum
SiO _x	19	4.50	0.72	0.32	4.18	4.82	3.53	6.17
PO _x -SiO _x	20	3.01	1.02	0.45	2.56	3.46	1.69	5.20
Mg	19	3.14	0.64	0.29	2.85	3.42	2.24	4.40
Ca	22	2.79	0.67	0.28	2.51	3.07	1.67	4.03
Ba	18	2.76	0.60	0.28	2.48	3.04	1.86	3.91
Ni	21	2.63	0.43	0.18	2.44	2.81	1.89	3.62
Zn	25	2.79	0.50	0.20	2.60	2.99	1.79	3.74
Cd	17	2.96	0.48	0.23	2.73	3.19	2.33	4.07
Zr	9	3.25	0.93	0.61	2.65	3.86	1.90	4.58

4. One-way ANOVA Testing of the Fitted Results

One-way ANOVA analysis was performed on the series of FRAP recoveries for each surface. The average results for each surface are shown as a box plot in figure S2. The central red mark represents the median and the edges of the box represent the 25^{th} and 75^{th} percentiles of the data. Whiskers are shown to extend to the most extreme data points, where outliers are represented individually. The notches in the plot correspond to the 95% confidence interval, where if the notches of one surface do not overlap another surface, the two medians are significantly different to 95% confidence.



Figure S2: Box plot for the fitted diffusion coefficients of all trials on each surface. The red line represents Median, whiskers represent maximum and minimum values, and red asterisks are considered outliers. Notches in the box plot represent 95% confidence intervals for each surface.

Using a Chi-Square goodness-of-fit test to the 5% significance level, trials for each surface were normally distributed and ANOVA analysis was performed. ANOVA analyses are shown for all surfaces (Table S2), among all metal surfaces (Table S3), among the alkaline metals only (Table S4), and among the heavy metals only (Table S5).

	Sum of Squares	df	Mean Square	F	Significance
Between Surfaces	49.0	8.0	6.1	13.5	6.9E-15
Within Surfaces	72.9	161.0	0.5		
Total	121.9	169.0			

Table S3: One-way ANOVA test among all surfaces

 Table S4: One-way ANOVA test among all metal surfaces

	Sum of Squares	df	Mean Square	F	Significance
Between Surfaces	4.6	6.0	0.8	2.2	5.0E-02
Within Surfaces	43.3	124.0	0.3		
Total	47.9	130.0			

Table S5: One-way ANOVA test among alkaline metal surfaces

-	Sum of Squares	df	Mean Square	F	Significance

Between Surfaces	1.7	2.0	0.8	2.0	0.14	
Within Surfaces	23.0	56.0	0.4			
Total	24.7	58.0				

Table S6: One-way ANOVA test among heavy metal surfaces								
	Sum of Squares	df	Mean Square	F	Significance			
Between Surfaces	2.82	3.00	0.94	3.2	0.03			
Within Surfaces	20.34	68.00	0.30					
Total	23.16	71.00						

5. Interaction Energy Calculations

The interaction energy between the DMPC molecules was estimated by a summation of the dispersive energies between two parallel (two-chained) 14-carbon alkyl chains.⁵⁻⁶ The dispersion attraction energy (W_{DMPC}) between two parallel saturated DMPC chains is given by:

$$W_{DMPC} = \alpha \frac{3\pi N}{8\lambda D^5}$$

where α is the interaction energy between two methylene units, *N* is the number of methylene units (14 for DMPC), λ is the length of the individual methylene units (1.26Å), and D is the separation distance between chains (calculated from the mean molecular area isotherms at the transfer pressure of 25mN/m).

The total interaction energy in the monolayer case assumes a hexagonal close packing arrangement and is a summation of attractive energies from the first closest interacting group at radius, r_1 , to the n^{th} group at a radius of r_n , where r is the interchain separation distance calculated from the radius of the mean molecular area isotherms at the transfer pressure.

$$r_{n} = 2nr$$
$$W_{M} = 2 \cdot \left(\sum_{1}^{n} N_{n} W_{DMPC}(r_{n}) - W_{DMPC}(r_{1}) \right)$$

where N_n is the number of nearest neighbors (i.e. $N_1 = 6$), and $W_{DMPC}(r_n)$ is the DMPC interaction energy at a separation distance of r_n and all summations have been made to n=12.

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