Quantifying Binding Affinity of MLV Gag to Lipid Membranes

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March 31st, 2011

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Surface Plasmon Resonance (SPR) was used to quantify the binding affinity of MLV Gag to tBLMs composed of different ratios of DOPC:DOPS at different salt concentrations. The strong influence of electrostatics on binding is observed. We also see a minimum PS composition below which no significant amount of binding is detected.

I Introduction

Tethered Bilayer Lipid Membranes (tBLMs) were prepared using different ratios of DOPC:DOPS. By using vesicle fusion to prepare the bilayers and using the osmotic shock protocol, highly sealing bilayers were prepared on HC 30:70 without the use of cholesterol. MLV Gag was dialyzed into buffers of 2 different salt concentration in an attempt to quantify the role of electrostatics on membrane binding. SPR experiments were performed in a non-flow cell without the use of any stirring and the binding constant (k_d) and the amount of protein bound (b_{max}) were measured.

II Materials and Methods

1. Materials

HC18 and βME were obtained from David Vanderah. DOPC, DOPS and the extrusion kit were obtained from Avanti. MLV Gag was expressed and purified by Siddhartha Datta at the NIH. 3”x1”x1mm Fisherbrand microscope slides were obtained from Fisher Scientific.

2. SAM Preparation

Microscope slides were cleaned with Hellmanex solution, thoroughly rinsed with water and then incubated in Nochromix solution for 15 minutes. They were then rinsed thoroughly with water, followed

by Ethanol and then blown dry with N_2 gas. They were then coated with ~10Å Chromium and ~450Å Gold by a high-energy magnetron at CMU and immediately incubated with 0.2mM ethanolic solution of HC:βME = 30:70 for at least 12 hours.

3. Vesicle Preparation

5mg/ml solutions of DOPC and DOPS were prepared in Chlofororm. The DOPC:DOPS mixture was placed in a test tube under vacuum for ~3 hours to evaporate all the chloroform. Pentane was then added to the test tube and left to evaporate overnight under the hood. The dried lipid film was then resuspended in 500mM NaCl, 10mM HEPES, pH 7.0 buffer. The test tube was the sonicated for 1 hour and the lipid was then extruded 23 times through a membrane with 100nm pore size. Prepared vesicles were used within 24 hours of extrusion.

4. Tethered Bilayer Lipid Membrane Preparation

The glass slide coated with HC:βME = 30:70 was incorporated into the SPR sample holder. The SPR cell was then heated to 45C for one hour following which the extruded vesicles were added and incubated for a further hour. Finally the vesicles were gently rinsed off with 100mM NaCl, 10mM HEPES, pH 7.0 buffer which had already been heated to 45C. The cell was allowed to stay at 45C for a further 15 minutes (or till the SPR signal stabilized, whichever came second) and then cooled to 25C at which it was maintained for the rest of the experiment. Once the SPR signal stabilized the cell was rinsed with 50/100mM NaCl, 10mM Sodium Phosphate, 1mM βME buffer at pH 7.5.

5. Protein Dialysis

The stock protein was stored in a 500mM NaCl, 20mM HEPES, 1mM TCEP, 0.01mM $ZnCl_2$, 10% v/v glycerol buffer at pH 7.5. Prior to use, it was dialyzed overnight in 50/100mM NaCl, 10mM Sodium Phosphate, 1mM βME buffer at pH 7.5.

6. Surface Plasmon Resonance (SPR) Instrumentation

The SPR used was a custom-built device commissioned from SPR Biosystems. The instrument uses the Kretschmann configuration where a glass slide coated with gold is coupled to a prism. A Superluminescent Light Emitting Diode (SLED) (EXS7510 from Exalos AG, Switzerland) at 763.8nm is used to excite surface plasmons in the gold film. The illuminated sample area is 100mmx100µm with the former being along the direction perpendicular to propagation. The signal is detected using a Hamamatsu Digital CCD (C10990). The system has a temporal resolution of 0.1s with a sensitivity of $5x10^{-7}$ RU or better. A Wavelength Electronics temperature controller (LFI-3751) is used with a range from ambient to 50°C with 0.005°C resolution. The non-flow cell is composed of a Teflon cylinder of 6mm diameter with a volume of 1ml.

6. SPR Binding Measurement

The dialyzed protein was diluted to prepare 5-6 concentrations ranging from 0.01µM to 7µM. All the buffer from the SPR cell was removed, except for 200µl. To this, 200µl of the first concentration of

protein was added and the binding kinetics were recorded. Once equilibrium was achieved, 200µl was removed from the cell and 200µl of the next protein concentration was added. This was repeated till the last concentration had equilibrated.

7. SPR Data Evaluation

The SPR curve is fit to a 14th order polynomial in real-time to generate a plot of the SPR minimum as a function of time. Equilibrium analysis is performed where the difference between the initial and final position of the SPR minimum is plotted as a function of concentration. The data is then fit to the Langmuir isotherm equation:

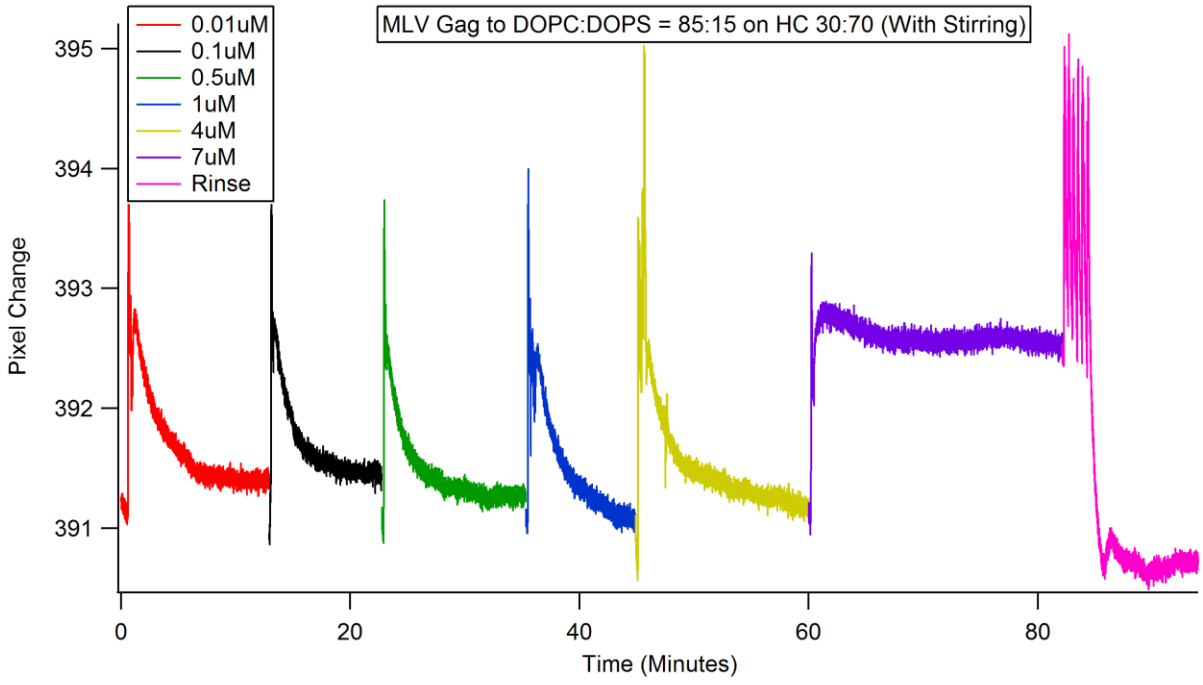
$$R_{eq} = \frac{c_0 B_{max}}{c_0 + k_d}$$

Where R_{eq} is the equilibrium response at a given protein concentration c_0, B_{max} is the saturation response and k_d is the dissociation constant.

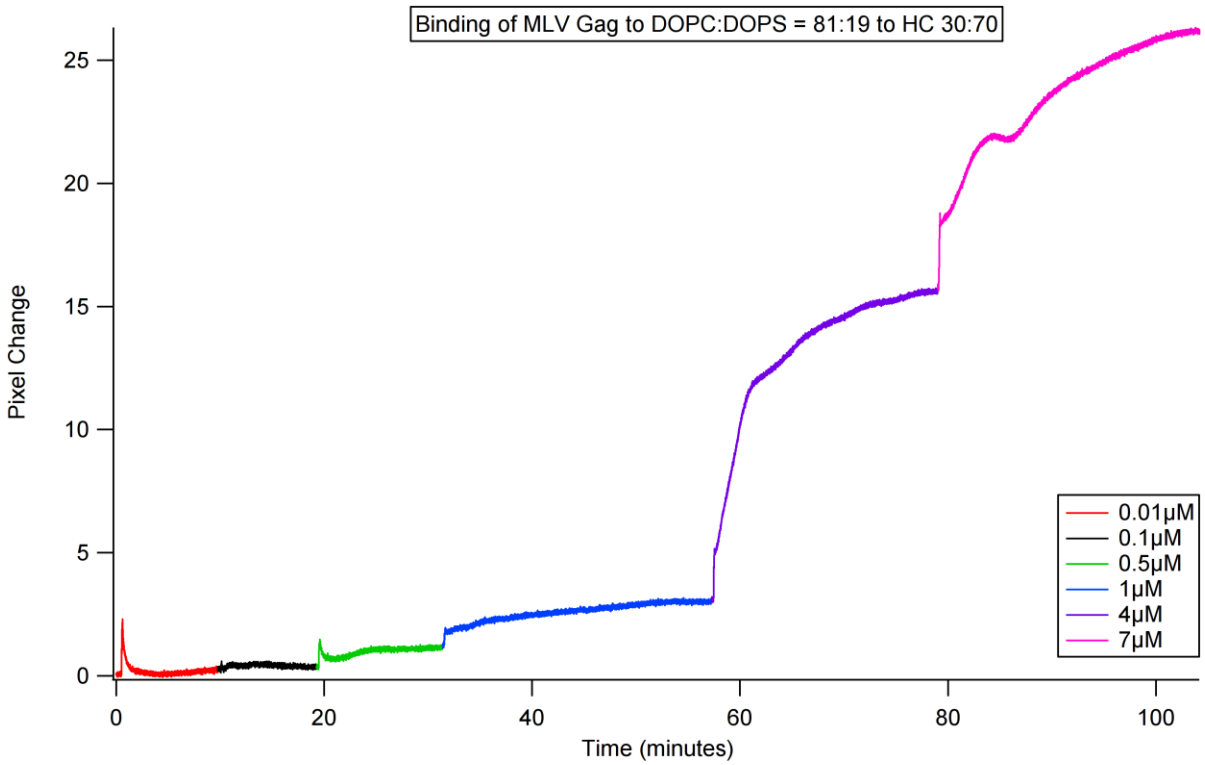
III Results

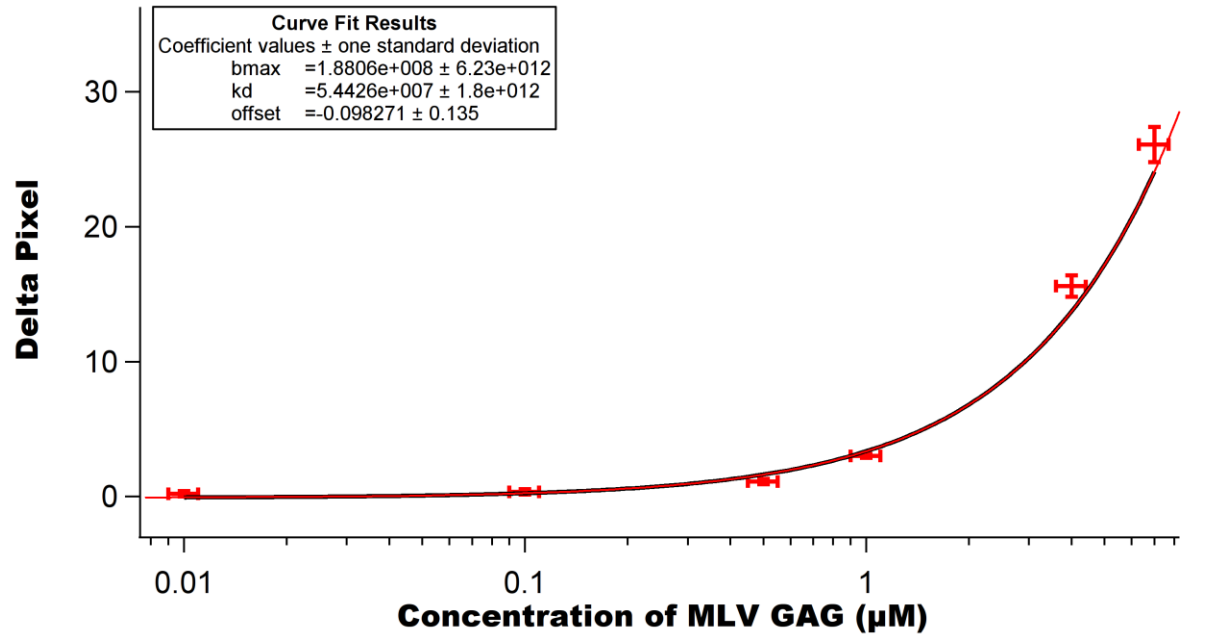
1. 50mM Salt Buffer

1.1 DOPC:DOPS = 85:15

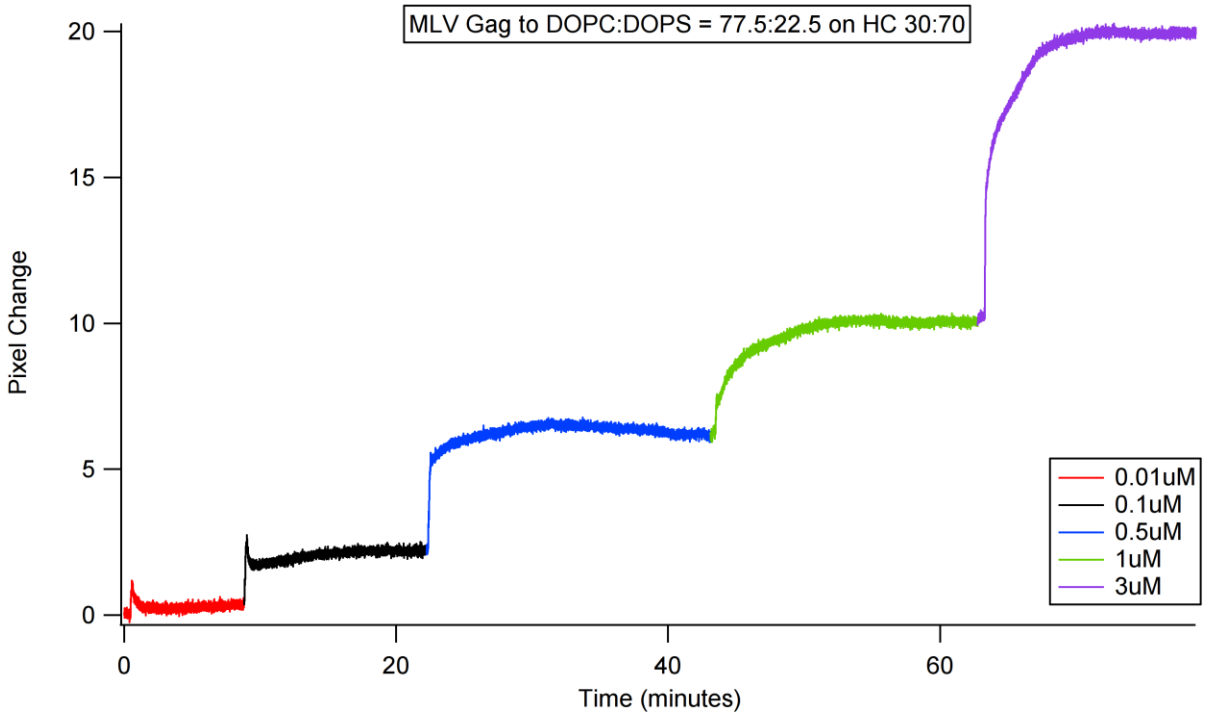


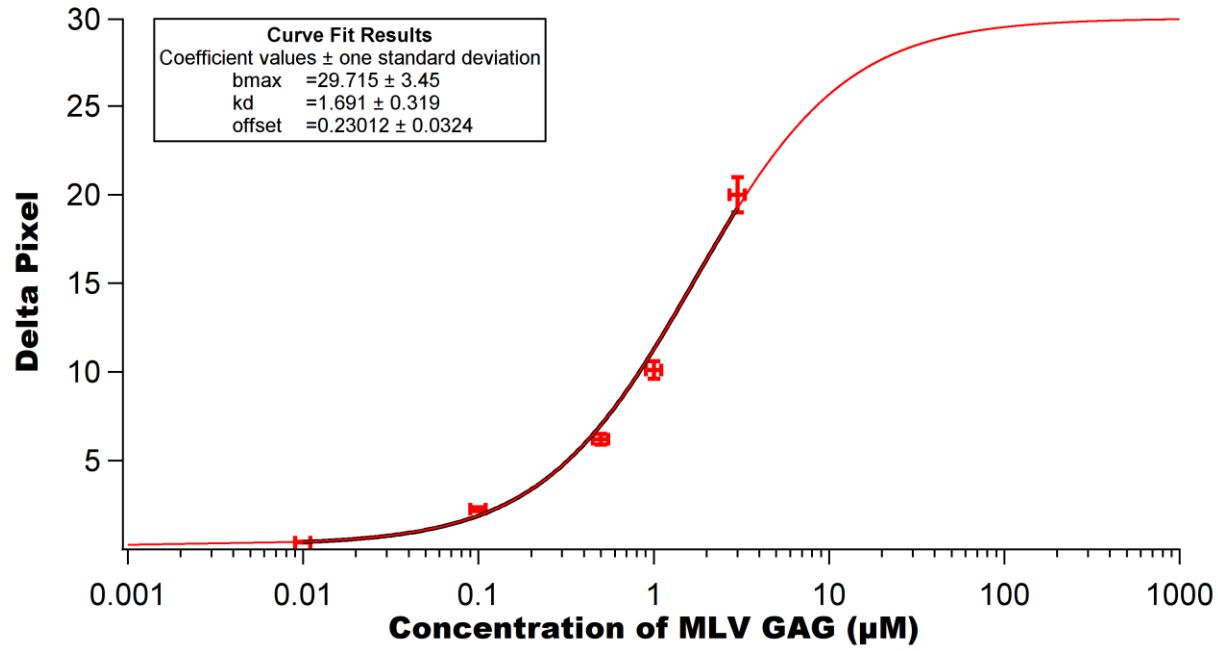
1.2 DOPC:DOPS = 81:19



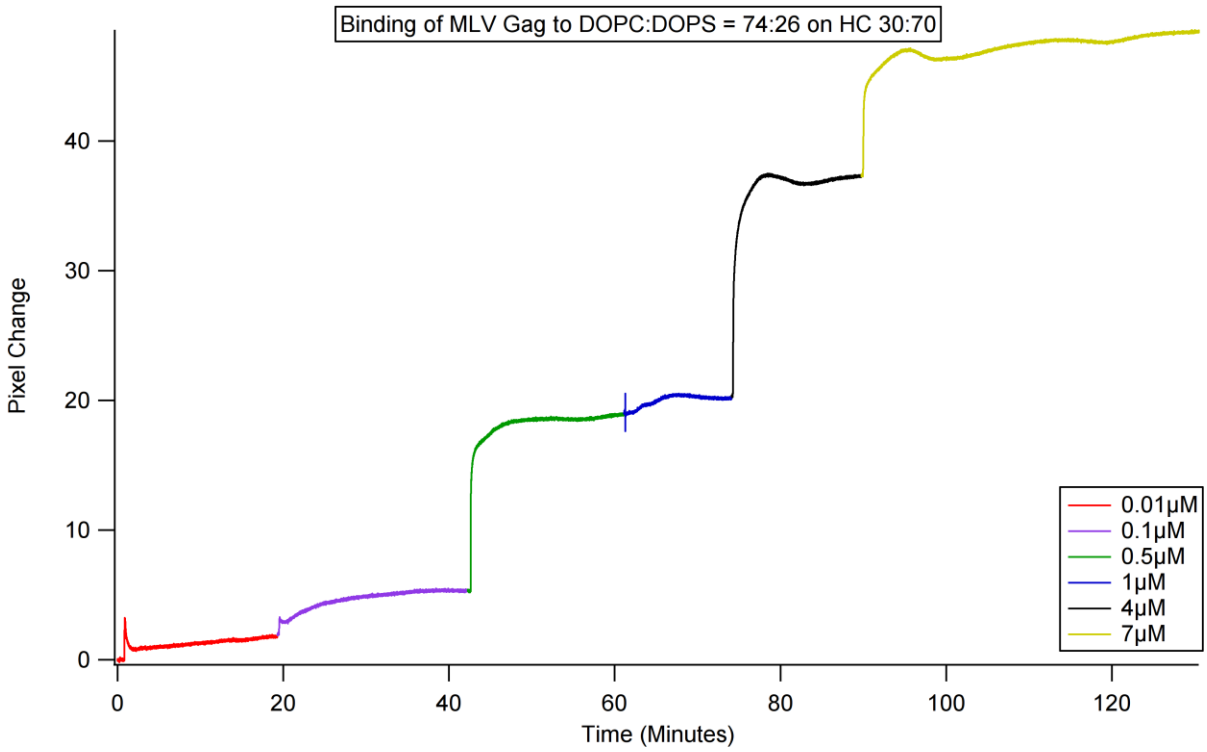


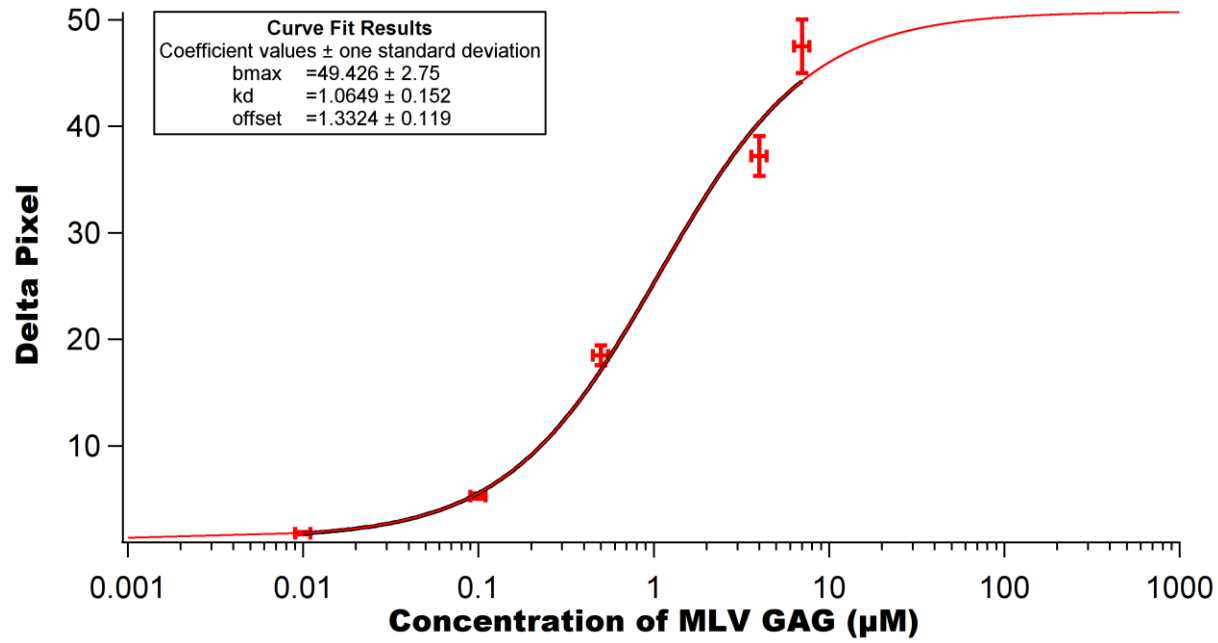
1.3 DOPC:DOPS = 77.5:22.5



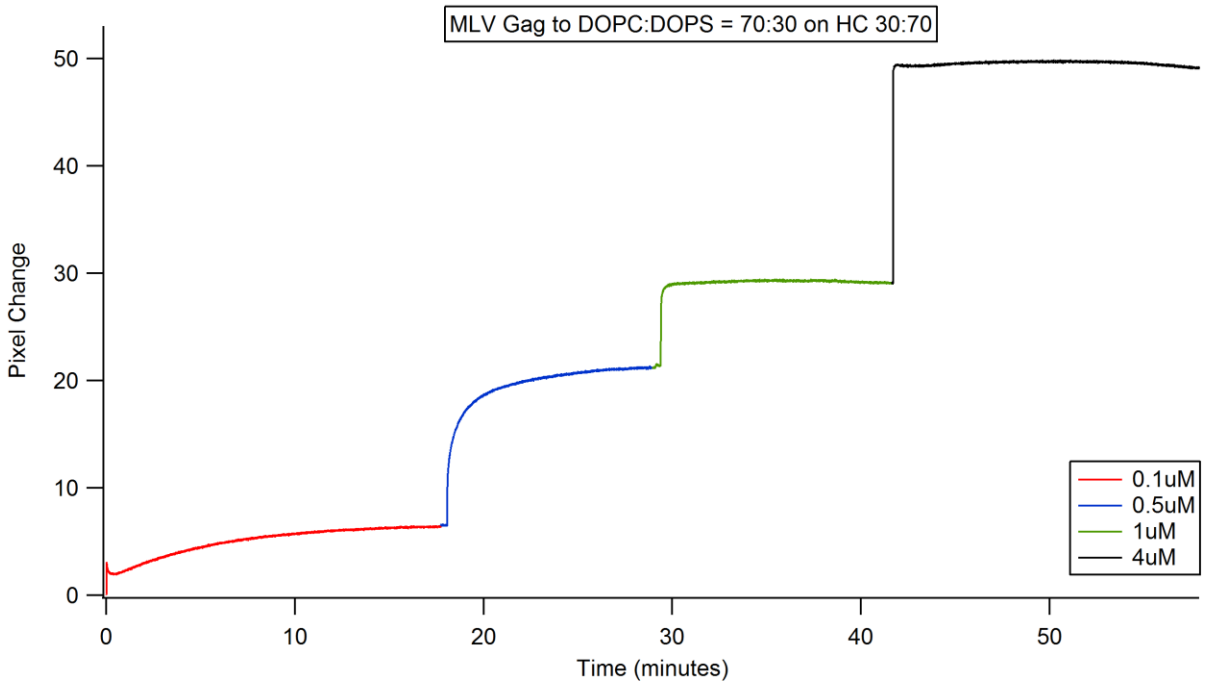


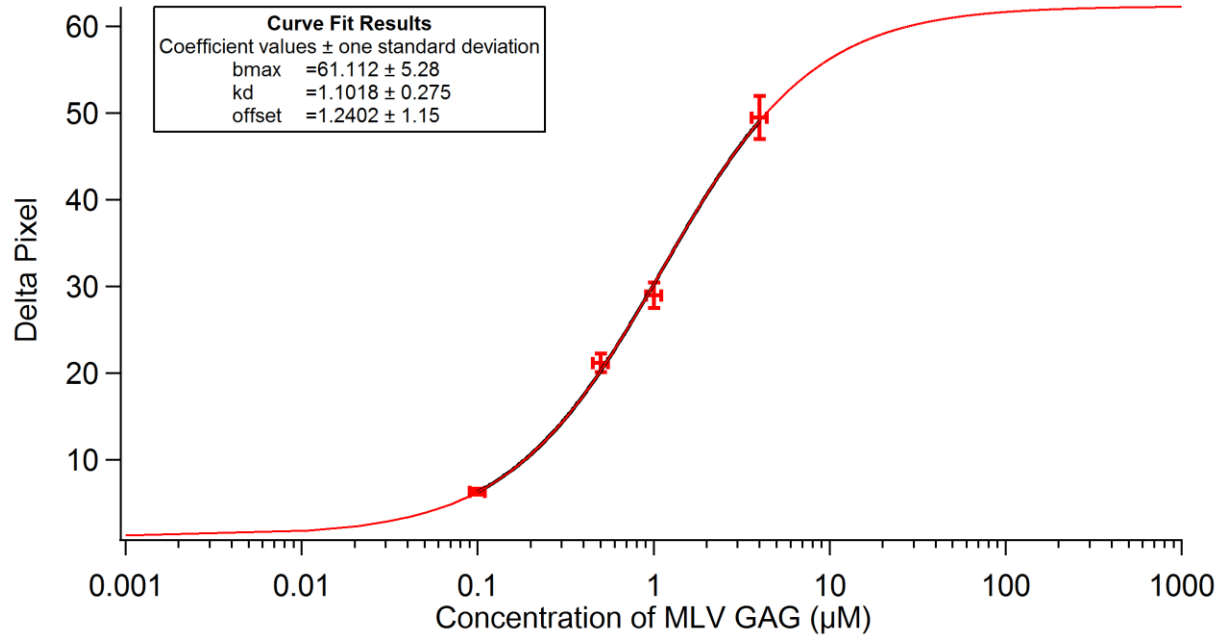
1.4 DOPC:DOPS = 74:26





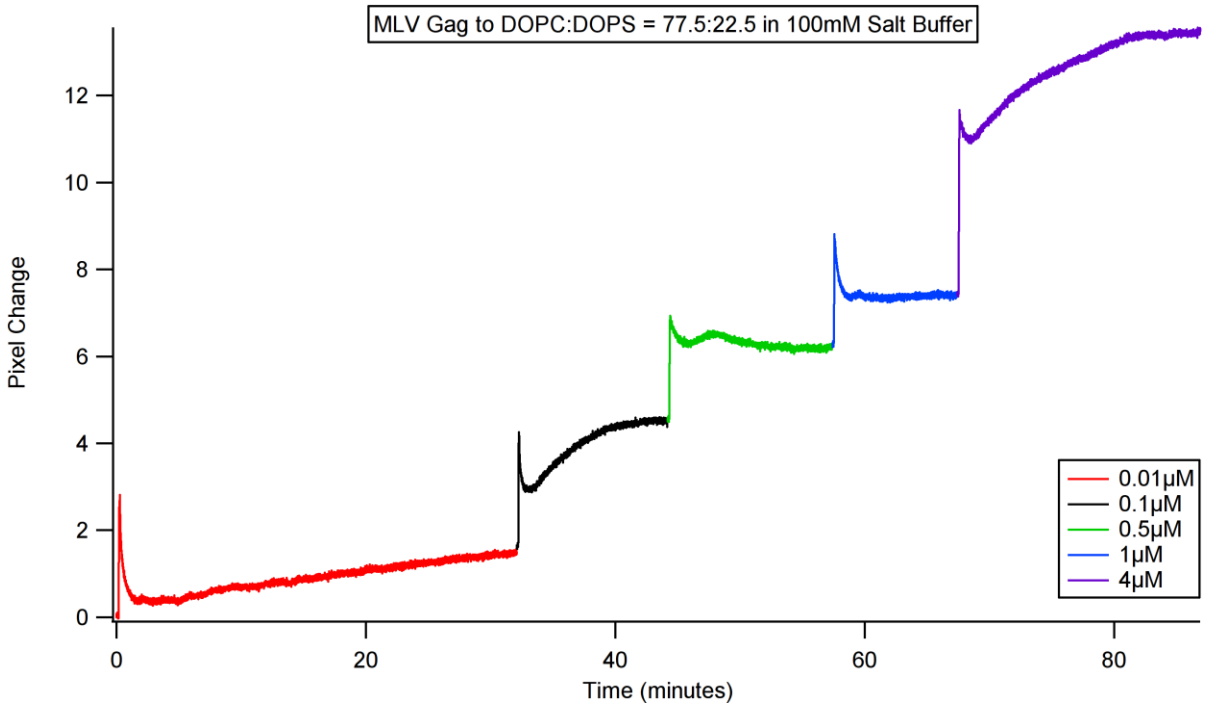
1.5 DOPC:DOPS = 70:30

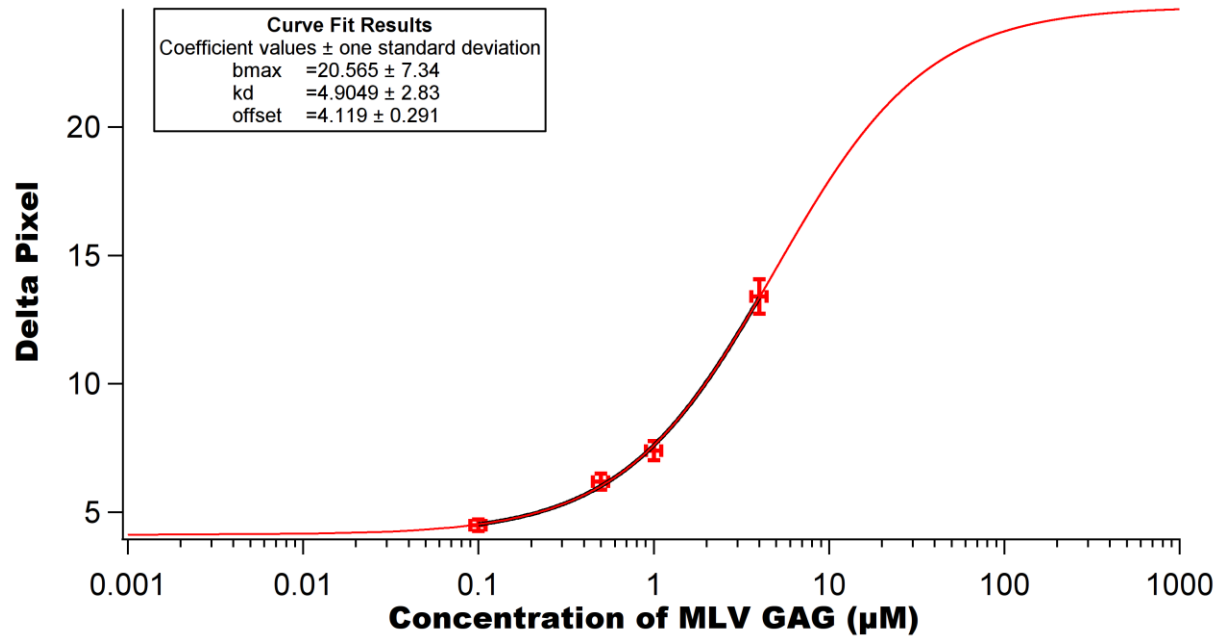




2. 100mM Salt Buffer

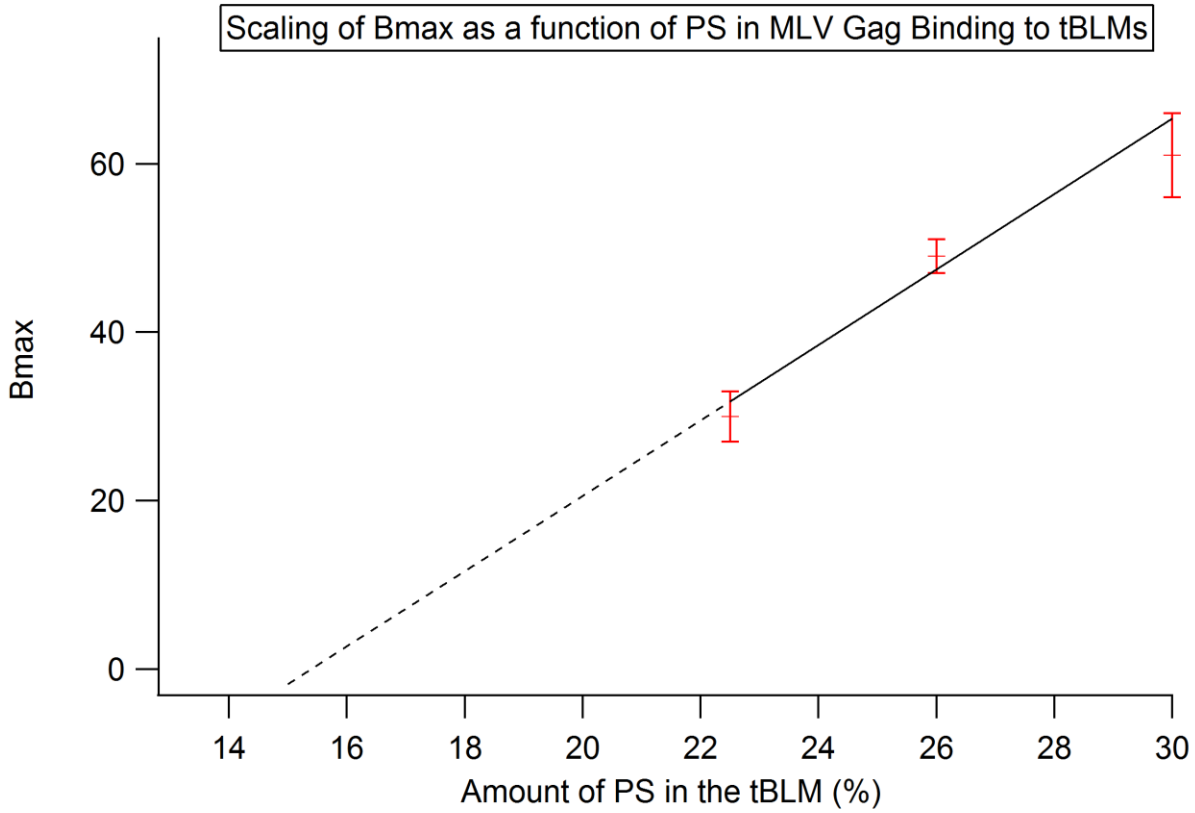
2.1 DOPC:DOPS = 77.5:22.5





IV Analysis

MLV Gag was bound to DOPC:DOPS tBLMS ranging in PS composition from 15% to 30%. At 15% PS, there is very little protein bound (2 pixel change for 7µM protein). At 19% PS, we see nearly 30 pixels bound for 7µM but the k_d is much greater than 7µM indicating very weak binding. For concentrations above 15% PS (i.e., 22.5%, 26% and 30% PS) we get a k_d of approximately 1µM with saturation responses (B_{max}) proportional to the amount of PS present in the bilayer. This is plotted in the figure below.



The linear fit to the 3 highest PS compositions gives a B_{max} of zero at ~15% PS, independently verifying the very small amount of bound protein seen for DOPC:DOPS = 85:15. The fact that B_{max} is directly proportional to the amount of PS present shows that the binding is electrostatically driven. It is interesting that there is a minimum threshold of PS present in the bilayer below which no significant binding is observed. The decrease in B_{max} and the increase in k_d for DOPC:DOPS = 77.5:22.5 upon the increase in salt concentration from 50mM to 100mM also supports the strong role of electrostatics in MLV Gag binding to tBLMs.

V Conclusions

SPR binding measurements of MLV Gag to tBLMs composed of DOPC:DOPS were performed. The strong role of electrostatics in binding is evident from the linear dependence of amount of protein bound to the amount of PS in the bilayer as well as the decrease in bound protein and

the increase in the dissociation constant upon increasing the amount of salt in the buffer. We find a minimum threshold of 15% PS below which there is no significant amount of protein bound to the bilayer. DOPC:DOPS = 81:19 constitutes a transition composition at which weak binding to the bilayer is observed. For all higher PS compositions, the binding constant is approximately 1μM.