Le Interaction of charged Liposomes and antigen protein from AUC Analysis.

Materials and Methods:

A ThermoFisher Genesys 50 benchtop spectrophotometer was used to collect spectra for each liposome sample, scanning from 200-500 nm in a 1 cm quartz cuvette. Liposome control samples were prepared by diluting stocks, ~1:7 dilution, with the received DPBS buffer to obtain an optimal OD measurement of 0.6 OD for the 1.2 cm pathlength. The spike protein control was prepared with 12 uL of 2 mg/mL stock and 88 uL of DPBS. Interaction studies were prepared by combining 12 uL spike protein and 50 uL of liposome and incubated at 37 ºC for 10 minutes. 38 uL of DPBS buffer was then added, followed by gently tapping the tube. Samples were left to cool to room temperature and immediately ran in the AUC.

All sedimentation velocity experiments were performed using a Beckman-Coulter XLA AUC instrument at the Canadian Center for Hydrodynamics. Liposome controls were spun in cells assembled with 2-channel epon centerpieces and quartz windows, scanning at 280 nm with UV optics for 5.5 hours at 25 ºC. Spike protein control and all interaction studies were spun in 3 mm titanium centerpieces with sapphire windows, scanning at 488 nm with fluorescence optics for 6 hours at 25 ºC. Controls and interactions with liposomes 01 and 04 were spun at a speed of 14,500 rpm. Liposomes 03 and 06 were run at 40,000 rpm, and liposome 02 at 25,000 rpm. The spike protein control was spun at 30,000 rpm.

Intensity data was collected and converted to pseudo-absorbance for liposome controls using the UltraScan 4.0 R&D data acquisition module [1, 2]. UltraScan was used to further analyze all data. Models were fitted using the 2-dimensional spectrum analysis (2DSA) [3] in a multi-step refinement process to remove time- and radially-invariant noise, as well as to determine the boundary conditions. Refined data was then analyzed by the 2DSA iterative (IT) analysis [4]. Controls were compared to the fluorescence data acquired from the interaction runs using van Holde-Weischet plots, which generate a diffusion-corrected integral sedimentation coefficient distribution [5].

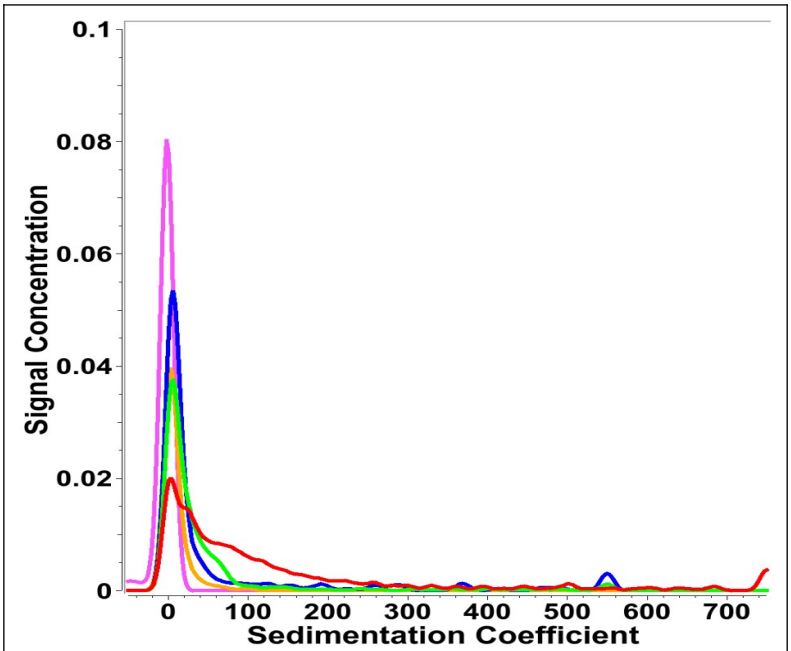

Figure 1: Sedimentation distributions for liposome controls. Liposome 01 (red), 02 (blue), 03 (magenta), 04 (green), and 06 (orange) are plotted.

Results:

Heterogeneity in all liposome controls is seen by comparing the sedimentation distributions generated by 2DSA-IT models (Figure 3). Samples such as 01 and 02 have larger liposomes that sediment above 500 s, while liposome 03 floated, having negative sedimentation coefficients. The fluorescence data collected from the spike protein control showed free label and the labeled spike protein. The labeled spike protein sediments at 13 s, with any other fluorescent signal with a

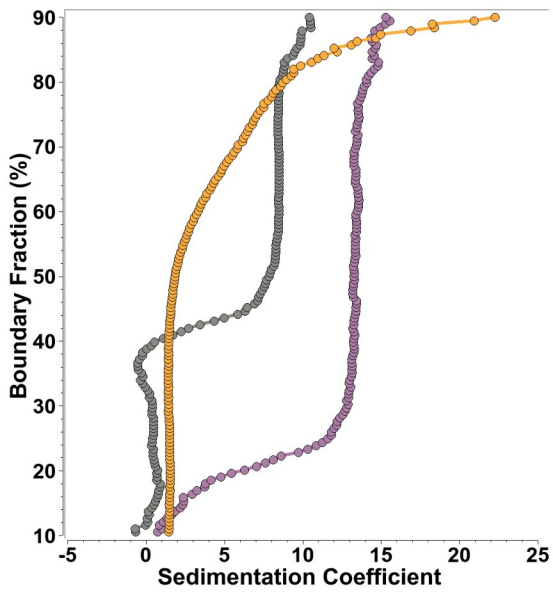

Figure 2: van Holde-Weischet plot of spike protein (purple), 06 liposome control (orange), and 06 liposome and spike protein combined (gray) integral sedimentation coefficients distribution.

lower sedimentation coefficient coming from the free label or degraded protein retaining the label.

A van Holde-Weischet plot of the interaction study with 06 liposomes (Figure 2) compares the sedimentation coefficients of the controls and the collected interaction data. The combined sample shows liposome sedimenting at two sedimentation values, the lower having integrated the free label and the higher having bound the spike protein. Figure 3 compares the spike protein control, the liposome control, and the 03 liposome and spike protein combined sample in a combined integral sedimentation distribution plot. This plot shows the stark difference between the behaviour of the liposome control and the combined sample. The combined sample shows that this cationic liposome did not absorb any free label, nor bind to the spike protein, as the combined sample resembles the sedimentation profile of the spike protein control.

Raw data comparing the spike protein control and all of the combined samples run is shown in Figure 4. The boundaries seen in combined samples identify any integration of free label as seen in liposome 06 by the slower sedimenting boundary within the once baseline signal. Liposome 01 and spike protein combination data was not modeled due to a leaking cell changing the boundary positions, but from the raw data, however, no free label is seen to sediment with the liposomes. Unfortunately, the sedimenting species visible in this sample cannot be identified as bound or unbound protein. Liposome 03 combined sample shows the baseline signal from the free label along with a homogeneous sedimenting species. This data along with the integral sedimentation plot identified unbound protein. Liposome 04 combined sample does not have any baseline signal and no distinct individual boundaries. This presumably resulted from the integration of the free label and spike protein into the liposomes, with a large distribution of sedimenting species. No quantitative sedimentation data could be modeled from this data set due to incompatibility with the modeling software, possibly due to changes in interactions throughout the run.

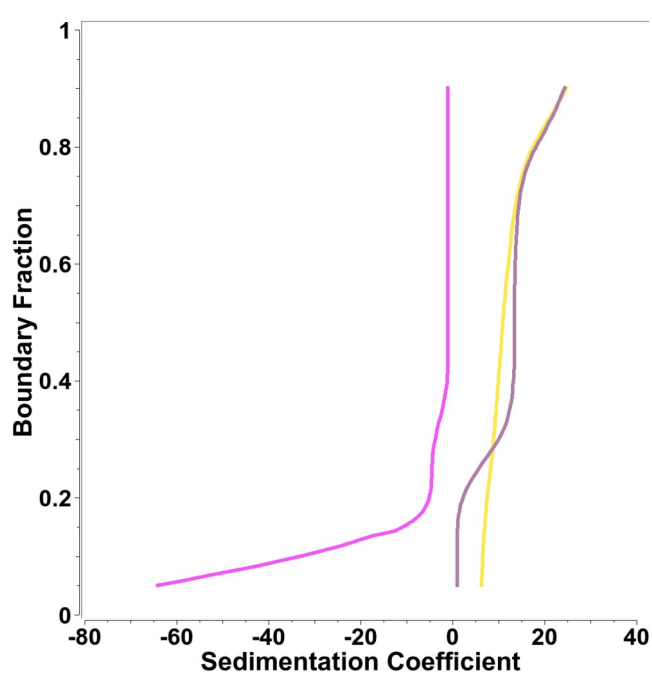

Figure 3: Combined integral sedimentation distribution plot of spike protein (purple) and 03 liposome and spike protein combined (yellow) integral sedimentation coefficient distribution.

Discussion:

Limitations in modeling combined samples resulted in a struggle for definitive results in this study. The van Holde-Weischet plot of combined samples with liposomes 06 did show that the

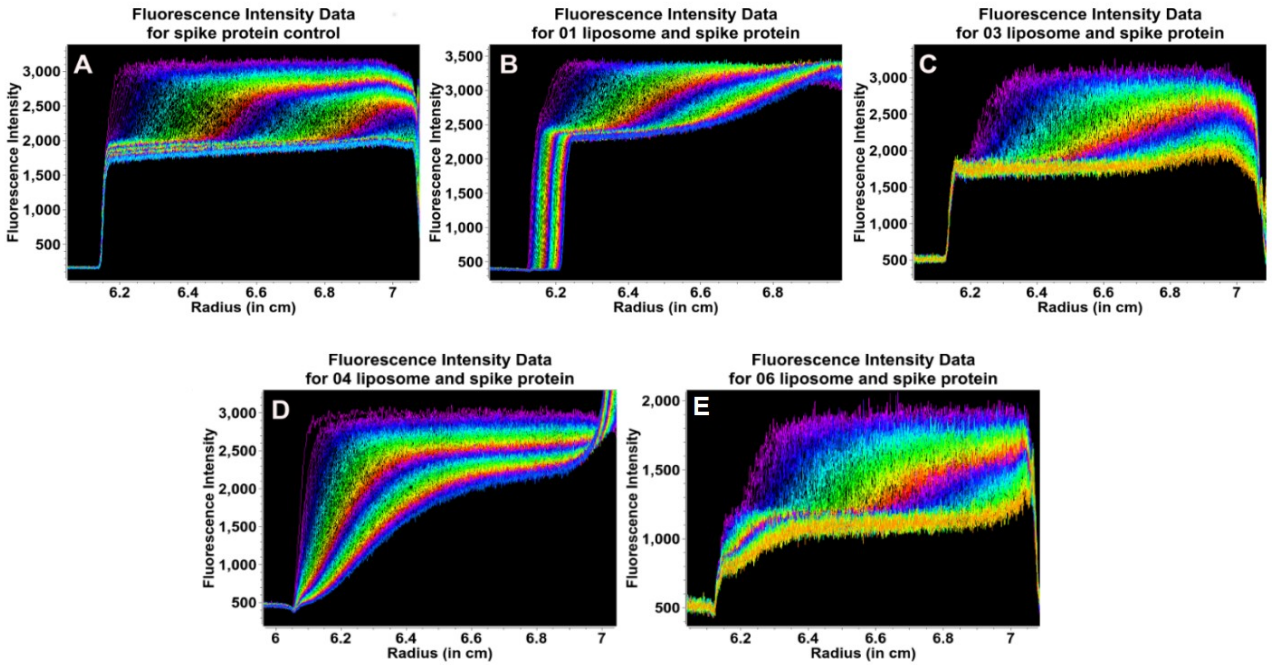

*Figure 4: Raw fluorescence intensity data collected from the spike protein control (**A**) and combined 01 (**B**), 03 (**C**), 04 (**D**), and 06 (**E**) liposomes with the spike protein. Combined samples with lipsomes 01 and 04 were ran at 14,5000 rpm, lipsomes 03 and 06 at 40,000 rpm, and the spike protein control at 30,000 rpm.*

mixed sample aligned with a sedimentation profile of bound liposome and protein and the free label. The homogeneity in the bound sample suggests that only one size of liposome interacts with the spike protein. Integrated label can be seen in both 06 and 04 liposome. The combined integral sedimentation distribution plot with the combined 03 liposomes showed no interaction with the liposome, with the detected signal overlaying the spike protein control, rather than the floating liposome.

Interaction studies with combined samples of 01 and 04 liposomes were also collected, but 01 liposome sample leaked during the run and 04 liposome sample unfortunately could not be modelled with acceptable fits. Raw data boundaries did provide some insight into any possible integration of free label, seen with 04 liposomes, but could not determine when was sedimenting.

References:

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