Supporting Information

# ANALYSIS OF LIPID PHASE BEHAVIOR AND PROTEIN CONFORMATIONAL CHANGES IN NANOLIPOPROTEIN PARTICLES UPON ENTRAPMENT IN SOL-GEL DERIVED SILICA

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**Anisotropy Regressions.** The cooperativity and phase transition temperature were determined as described in the main article. The values of A and B determined from averaging all of the individually regressed samples were 4.37 X $10^{-4}$ ($°C^{-1}$) and -2.00 X $10^{-5}$ ($°C^{-2}$), respectively. These values were used to re-regress all of the anisotropy plots and to obtain the new values of the other four parameters ($r_{max}$, $r_{min}$, $T_m$, and n). These regressed parameters are shown for NLPs and liposomes in Table S1 and Table S2, respectively.

**Choice of Lipid (Di15:0PC).** Di15:0PC was chosen due to having a phase transition temperature in liposome form of approximately 35°C. Below 20°C, the Peltier element on the temperature control device for the fluorimeter was not able to change temperature at sufficiently fast rates. Using a lipid such as DMPC with a phase transition temperature of approximately 23°C would require anisotropy readings to start at 10°C and gradually increase to accurately capture the whole phase transition region. The speed at which the Peltier element heated/cooled samples in the 10-20°C region would only allow for one anisotropy measurement to be taken daily (~0.1°C/minute). Therefore, choosing Di15:0PC allowed for use of a PC lipid that conveniently shifted the phase transition region roughly 10°C higher. This allowed scan speeds of 0.4°C/minute to be obtained and 3-4 anisotropy readings to be performed per day, rather than

one. Use of DPPC was not considered due to its higher phase transition temperature of approximately 42°C. As shown in the results, incorporation of scaffold proteins and silica gel entrapment elevates the phase transition temperature of lipids. We wanted to minimize the necessity of any additional temperature elevation for examining phase behavior.

**Particle Size Analysis.** The sizes of particles in NLP and liposome samples were determined as described in the Materials and Methods section of the main article. The resulting sizes are shown in Table S3 as a Stokes diameter ($d_S$). This represents the diameter of an equivalent spherical particle, which is an accurate description for liposomes. However, NLPs are not spherical in shape. In order to find the discoidal diameter ($d_D$) of the NLPs Equation S1 was derived, where the area of a sphere was set equal to the area of a cylinder. This is a common approach used when techniques for imaging NLPs such as AFM are not available or overly cumbersome.[1]

$$d_D = \left(\frac{2d_S^3}{3h}\right)^{1/2} \qquad \text{(S1)}$$

The height "h" was estimated to be 5 nm, which is the average height of typical lipid bilayers and average height of various NLPs that was previously determined in other studies.[1-2] The discoidal diameter of 14.8 $\pm$ 4.4 nm for NLPs is slightly higher than that which was observed in other studies utilizing MSP1E3D1.[3] This is perhaps due to the Stokes diameter consisting of not only the protein-lipid moiety, but also water molecules surrounding the particle that cause it to seem larger (i.e. a hydrodynamic radius). Discoidal diameters could not be reasonably estimated or applied towards NLP samples in the presence of methanol as the effect of methanol on the morphology of NLPs is not known.

**Anisotropy Deconvolution.** As seen in Fig. 1 from the main article, there is a reduction in anisotropy range once NLPs and liposomes are entrapped in the silica gel. It was hypothesized

that this could be the result of a superposition of the probe diphenylhexatriene (DPH) being positioned in both lipid tails and pores of the silica gel. The small concentration of methanol in the pores of the silica gel (~5 v/v%) is high enough such that DPH can fluoresce in the pores. A horizontal line with an anisotropy value of roughly 0.150 was observed over the temperature range 20-60°C. To show that this superposition has minimal effect on the main parameters of this study (cooperativity "n" and phase transition temperature "$T_m$"), the raw anisotropy data points were deconvolved using Equation S2, where a liberal value of half DPH partitioning was assumed.

$$r_{read} = \frac{r_{lipids} + r_{pores}}{2} \qquad \text{(S2)}$$

In this equation, "$r_{lipids}$" represents the anisotropy of DPH in the lipid tails, "$r_{pores}$" represents the anisotropy of free DPH in the pores, and "$r_{read}$" represents the measured anisotropy value. The raw, unaveraged anisotropy values and regression is shown in Fig. S1A for an NLP sample aged 2 days in silica gel. The same sample was deconvolved by applying Equation S2 to each data point and re-regressing the overall data to obtain new parameters. The result of this is depicted in Fig. S1B. It can be seen that to the extent to which the significant figures were carried, there was no difference between the values obtained for cooperativity and phase transition temperature. The narrowing/expansion of the anisotropy range resulted in changes in $r_{max}$ and $r_{min}$, as well as the baseline parameters A and B. This effect was observed for several other samples when this method was applied. This result is mathematically intuitive, as a horizontal line intersecting a sigmoidal curve would not shift the inflection point of the sigmoidal curve, nor change the region through which there is a significant increase in slope. A horizontal line could only significantly alter the shape of the curve if its magnitude of contribution to the superposition approached

infinity, or its ordinate value was sufficiently higher/lower than the maximum/minimum ordinate values of the sigmoidal curve.

**Methanol Content Determination.** The content of methanol in the silica gel was determined by measuring the density of the liquid excreted from the gel once it hardened. The density was measured by simply measuring the mass and volume of the liquid. To correct for any error in measurement, the density of pure water was also measured in the same manner as the silica gel liquid. Afterward, the ratio of the measured density of the silica gel liquid to the measured density of pure water was used. A correlation between v/v% methanol and density of water/methanol mixtures is shown below in Fig. S2. This correlation was obtained from conversion of literature values for methanol/water mixture density as a function of methanol mass percentage.[4] Samples utilizing rotary evaporation were found to have a density of 0.990 g/mL and samples without had a density of 0.965 g/mL. These correspond to methanol concentrations of roughly 5 v/v% and 24 v/v%, respectively.

**Radius of Gyration Estimation.** The radius of gyration ($R_g$) was estimated using Equation S3.[5]

$$R_g = \frac{l\sqrt{n}}{\sqrt{6}} \qquad \text{(S3)}$$

This equation is derived for an entropically govered polymer chain where “l” corresponds to the length of a monomer and “n” corresponds to the number of monomers. For MSP, “l” would correspond to the average length of an amino acid residue backbone. This was estimated using the known bond lengths[6] of two C-N bonds (1.45 Å each) and one C-C bond (1.52 Å) for a total of 4.4 Å. For MSP, “n” is the total number if residues which is 277.

## Supporting Tables and Figures

Table S1: Regressed parameters for NLPs from Equation 1 after parameters A and B were determined.

| Condition | $r_{max}$ | $\delta r_{max}$ | $r_{min}$ | $\delta r_{min}$ | $T_m$ (°C) | $\delta T_m$ (°C) | n | δn |
|---|---|---|---|---|---|---|---|---|
| Gel (Day 1) | 0.216 | 0.001 | 0.167 | 0.001 | 46.0 | 0.3 | 0.32 | 0.03 |
| Gel (Day 1) | 0.222 | 0.001 | 0.174 | 0.001 | 47.2 | 0.4 | 0.34 | 0.04 |
| Gel (Day 1) | 0.213 | 0.001 | 0.158 | 0.001 | 45.7 | 0.3 | 0.33 | 0.03 |
| Gel (Day 2) | 0.202 | 0.001 | 0.156 | 0.000 | 46.2 | 0.2 | 0.31 | 0.02 |
| Gel (Day 2) | 0.187 | 0.001 | 0.145 | 0.001 | 47.3 | 0.3 | 0.41 | 0.04 |
| Gel (Day 2) | 0.216 | 0.001 | 0.166 | 0.001 | 47.4 | 0.4 | 0.28 | 0.03 |
| Gel (Day 3) | 0.211 | 0.001 | 0.170 | 0.001 | 46.3 | 0.5 | 0.36 | 0.05 |
| Gel (Day 4) | 0.223 | 0.001 | 0.186 | 0.001 | 45.8 | 0.5 | 0.38 | 0.07 |
| Gel (Day 4) | 0.188 | 0.001 | 0.150 | 0.001 | 48.1 | 0.4 | 0.33 | 0.04 |
| Gel (Day 4) | 0.215 | 0.001 | 0.162 | 0.001 | 45.5 | 0.3 | 0.30 | 0.03 |
| Gel (Day 5) | 0.198 | 0.001 | 0.143 | 0.001 | 46.3 | 0.3 | 0.31 | 0.03 |
| Gel (Day 6) | 0.236 | 0.001 | 0.178 | 0.001 | 47.4 | 0.4 | 0.30 | 0.03 |
| Gel (Day 7) | 0.220 | 0.001 | 0.177 | 0.002 | 49.9 | 0.6 | 0.29 | 0.04 |
| Gel (Day 7) | 0.202 | 0.001 | 0.144 | 0.001 | 45.8 | 0.4 | 0.30 | 0.03 |
| Gel (Day 9) | 0.198 | 0.001 | 0.148 | 0.001 | 47.3 | 0.3 | 0.27 | 0.02 |
| Gel (Day 11) | 0.172 | 0.000 | 0.135 | 0.001 | 48.0 | 0.3 | 0.31 | 0.02 |
| Gel (Day 13) | 0.230 | 0.001 | 0.185 | 0.002 | 51.1 | 0.6 | 0.24 | 0.03 |
| Gel (Day 14) | 0.175 | 0.001 | 0.125 | 0.001 | 48.2 | 0.4 | 0.25 | 0.02 |
| Gel (Day 24) | 0.207 | 0.001 | 0.158 | 0.001 | 50.0 | 0.4 | 0.24 | 0.02 |
| Gel (Day 36) | 0.182 | 0.001 | 0.154 | 0.001 | 49.5 | 0.8 | 0.25 | 0.05 |
| Gel (Day 1, no rotovap) | 0.186 | 0.002 | 0.125 | 0.002 | 40.0 | 0.5 | 0.35 | 0.05 |
| Solution (0% Methanol, Day 1) | 0.262 | 0.001 | 0.127 | 0.001 | 38.4 | 0.1 | 0.38 | 0.01 |
| Solution (0% Methanol, Day 1) | 0.244 | 0.001 | 0.099 | 0.002 | 37.5 | 0.1 | 0.35 | 0.02 |
| Solution (0% Methanol Day, 1) | 0.285 | 0.002 | 0.117 | 0.002 | 37.4 | 0.1 | 0.35 | 0.02 |
| Solution (0% Methanol, Day 21) | 0.300 | 0.001 | 0.127 | 0.002 | 37.6 | 0.1 | 0.35 | 0.01 |
| Solution (4% Methanol) | 0.240 | 0.002 | 0.090 | 0.002 | 36.2 | 0.1 | 0.36 | 0.02 |
| Solution (8% Methanol) | 0.234 | 0.003 | 0.076 | 0.001 | 35.3 | 0.2 | 0.39 | 0.03 |
| Solution (12% Methanol) | 0.219 | 0.002 | 0.066 | 0.001 | 33.8 | 0.2 | 0.39 | 0.03 |
| Solution (16% Methanol) | 0.208 | 0.001 | 0.069 | 0.001 | 32.6 | 0.2 | 0.53 | 0.04 |
| Solution (20% Methanol) | 0.204 | 0.001 | 0.054 | 0.001 | 31.7 | 0.1 | 0.53 | 0.03 |
| Solution (24% Methanol) | 0.197 | 0.002 | 0.064 | 0.002 | 29.9 | 0.1 | 0.82 | 0.09 |
| Solution (28% Methanol) | 0.197 | 0.002 | 0.063 | 0.001 | 29.1 | 0.1 | 0.81 | 0.06 |
| Solution (32% Methanol) | 0.193 | 0.004 | 0.061 | 0.001 | 31.3 | 0.2 | 0.90 | 0.11 |
| Solution (36% Methanol) | 0.191 | 0.008 | 0.084 | 0.002 | 33.5 | 0.2 | 2.14 | 0.83 |

Table S2: Regressed parameters for liposomes from Equation 1 after parameters A and B were determined.

| Condition | $r_{max}$ | $\delta r_{max}$ | $r_{min}$ | $\delta r_{min}$ | $T_m$ (°C) | $\delta T_m$ (°C) | n | δn |
|---|---|---|---|---|---|---|---|---|
| Gel (Day 1) | 0.209 | 0.000 | 0.113 | 0.002 | 40.9 | 0.3 | 0.25 | 0.02 |
| Gel (Day 1) | 0.206 | 0.002 | 0.109 | 0.006 | 41.7 | 0.7 | 0.21 | 0.02 |
| Gel (Day 20 | 0.198 | 0.001 | 0.111 | 0.002 | 41.6 | 0.3 | 0.28 | 0.02 |
| Gel (Day 4) | 0.183 | 0.002 | 0.095 | 0.002 | 40.0 | 0.4 | 0.22 | 0.02 |
| Gel (Day 5) | 0.152 | 0.001 | 0.084 | 0.001 | 40.4 | 0.3 | 0.29 | 0.02 |
| Gel (Day 7) | 0.179 | 0.001 | 0.108 | 0.004 | 42.1 | 0.6 | 0.26 | 0.03 |
| Gel (Day 13) | 0.169 | 0.002 | 0.100 | 0.003 | 41.8 | 0.5 | 0.28 | 0.04 |
| Gel (Day 22) | 0.199 | 0.002 | 0.110 | 0.003 | 39.3 | 0.5 | 0.23 | 0.03 |
| Gel (Day 39) | 0.168 | 0.002 | 0.109 | 0.004 | 40.4 | 0.9 | 0.23 | 0.04 |
| Solution (0% Methanol, Day 1) | 0.288 | 0.003 | 0.097 | 0.003 | 35.3 | 0.1 | 1.96 | 0.27 |
| Solution (0% Methanol, Day 1) | 0.290 | 0.002 | 0.100 | 0.002 | 35.1 | 0.1 | 1.63 | 0.14 |
| Solution (0% Methanol, Day 21) | 0.294 | 0.002 | 0.098 | 0.002 | 34.0 | 0.1 | 1.69 | 0.24 |
| Solution (4% Methanol) | 0.242 | 0.002 | 0.077 | 0.001 | 33.0 | 0.1 | 1.48 | 0.26 |
| Solution (8% Methanol) | 0.264 | 0.002 | 0.084 | 0.002 | 31.9 | 0.1 | 1.35 | 0.16 |
| Solution (12% Methanol) | 0.239 | 0.001 | 0.082 | 0.001 | 31.2 | 0.1 | 1.29 | 0.34 |
| Solution (16% Methanol) | 0.224 | 0.003 | 0.077 | 0.001 | 29.8 | 0.1 | 1.07 | 0.10 |
| Solution (20% Methanol) | 0.219 | 0.001 | 0.076 | 0.001 | 28.3 | 0.1 | 0.73 | 0.03 |
| Solution (24% Methanol) | 0.197 | 0.004 | 0.070 | 0.001 | 29.2 | 0.2 | 0.59 | 0.08 |
| Solution (28% Methanol) | 0.168 | 0.003 | 0.081 | 0.002 | 30.6 | 0.3 | 0.57 | 0.09 |

Table S3: Stokes diameters determined from dynamic light scattering for various samples and corresponding discoidal diameter of NLPs.

| Sample | Stokes Diameter (nm) | Discoidal Diameter (nm) |
|---|---|---|
| Liposomes (Solution) | 166.5 $\pm$ 1.8 | - |
| NLPs (Solution, 0% MeOH) | 11.7 $\pm$ 2.2 | 14.8 $\pm$ 4.4 |
| NLPs (Solution, 15% MeOH) | 17.3 $\pm$ 3.2 | - |
| NLPs (Solution, 30% MeOH) | 93.0 $\pm$ 3.5 | - |

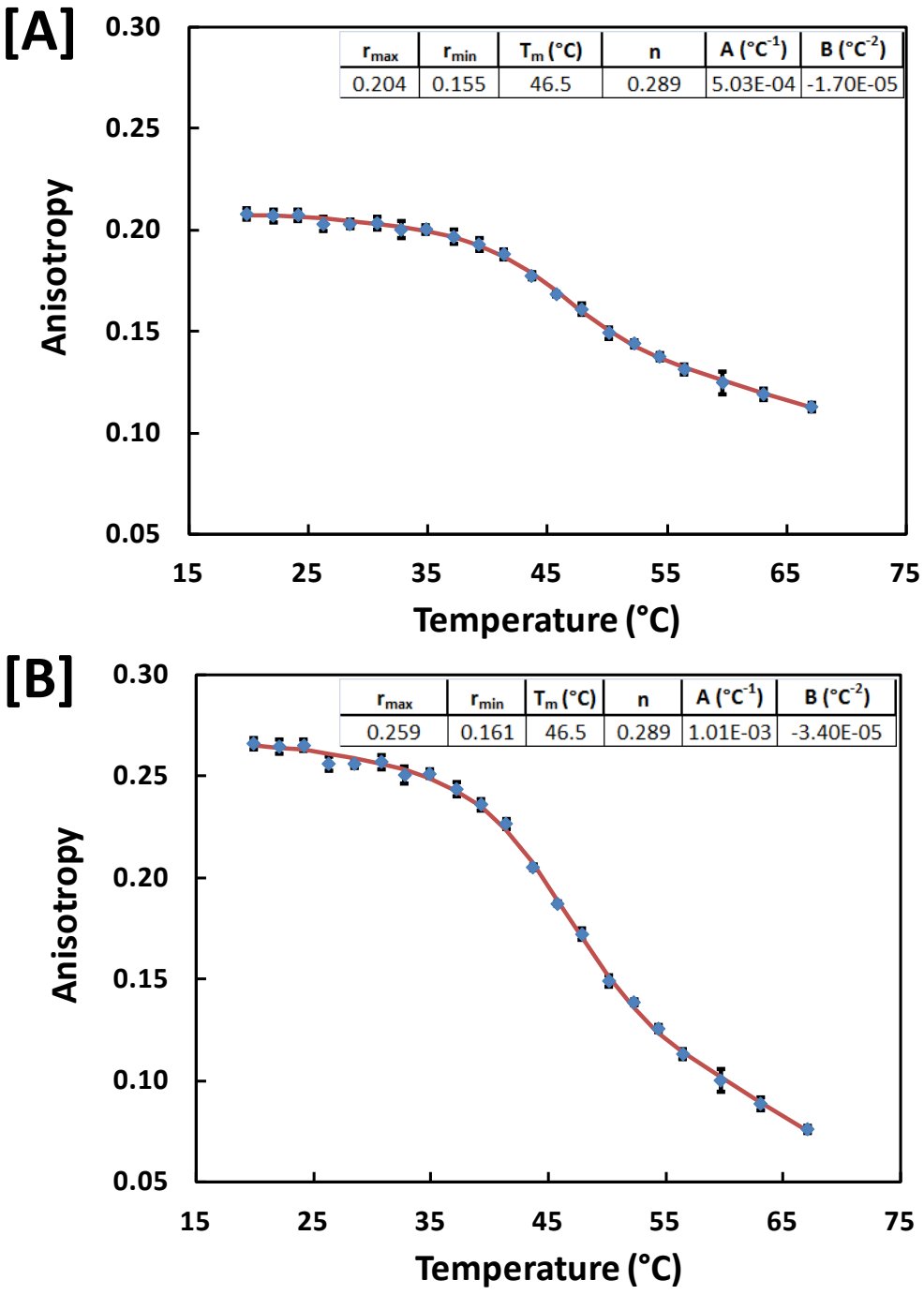


Figure S1: [A]Raw and [B] deconvolved anisotropy values and regression curve/parameters for a 2 day aged NLP sample in silica gel.

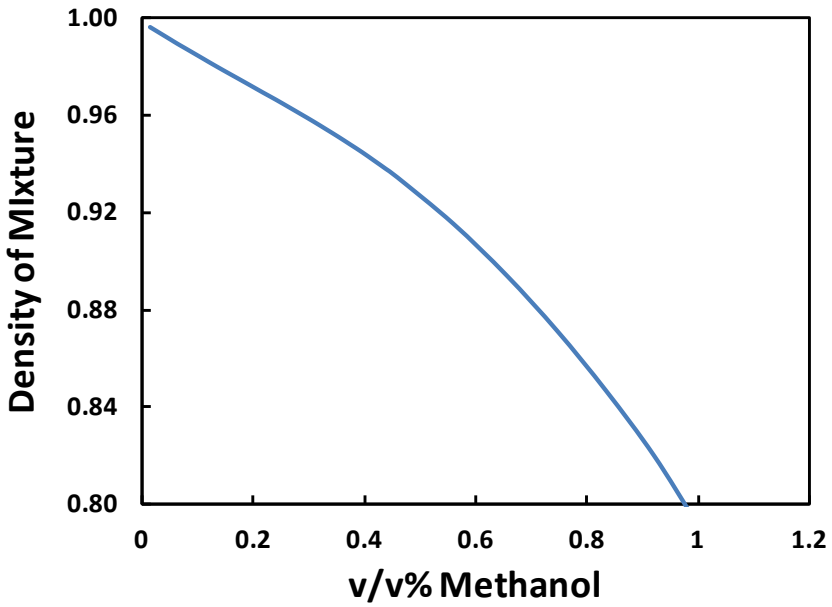


Figure S2: Plot of methanol and water mixture density versus v/v% methanol used for determining methanol content in silica gel.

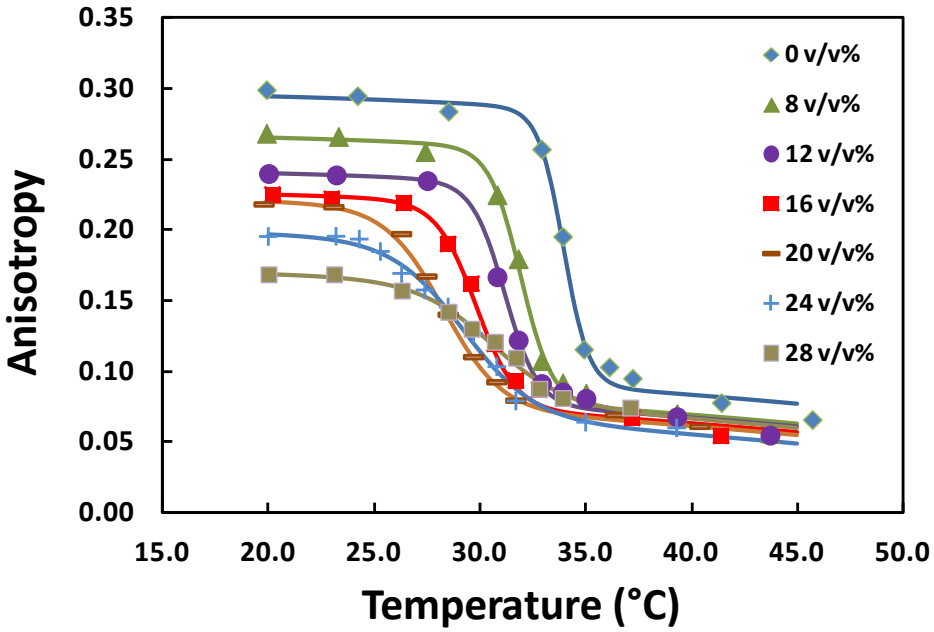


Figure S3: The measured anisotropy values and corresponding regression curves of different liposome samples in 20 mM Tris 100 mM NaCl at various concentrations of methanol (v/v%).

**References**


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