

SUPPLEMENTARY INFORMATION

Supplementary Video 1: Brightfield Microscopy Formation of Myelin Figures

Myelin figure formation using 100 mol% POPC lipid cake hydrated with 30 μL of deionized water. Images were captured using brightfield microscopy.

Supplementary Video 2: Confocal Fluorescence Microscopy Formation of Myelin Figures

Myelin figure formation from a lipid cake (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) upon hydration with 30 μL of deionized water. Images were captured using confocal fluorescence microscopy.

Supplementary Video 3: Brightfield Microscopy Dehydration of Myelin Figures

Dehydration of stable myelin figures (100 mol% POPC) captured via brightfield microscopy.

Supplementary Video 4: Confocal Dehydration of Myelin Figures

Dehydration of stable myelin figures (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) captured via confocal fluorescence microscopy.

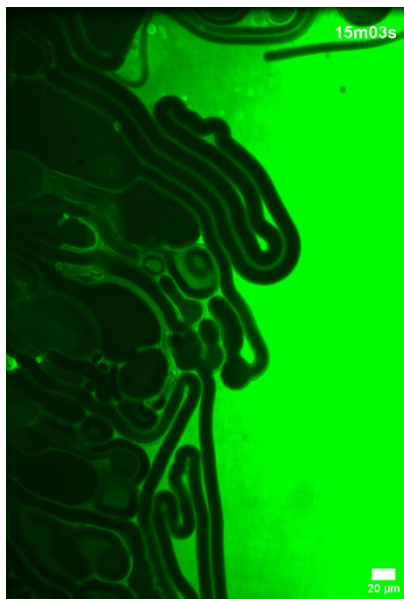
Supplementary Video 5: Secondary Hydration using Sucrose.

Secondary hydration of myelin figures (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) with 30 μL of 1000 mM sucrose. Images were captured via confocal fluorescence microscopy.

Supplementary Video 6: Secondary Hydration using Glycerol.

Secondary hydration of myelin figures (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) with 30 μL of 30 w/w% glycerol. Images were captured via confocal fluorescence microscopy.

Supplementary Files 7a: Confocal Snapshot of Secondary Hydration using Labeled



Sucrose.

Confocal fluorescence microscopy snapshot of myelin figures during secondary hydration with 30 μL of 773 mM sucrose solution containing 1 mol% NBD-glucose as a fluorescent label

Supplementary Files 7b: Secondary Hydration using Labeled Sucrose Solution.

Secondary hydration of myelin figures (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) with 30 μL of 773 mM sucrose containing 1 mol% NBD-glucose. Images were captured via confocal fluorescence microscopy.

Supplementary Video 8: Corrugation of Myelin Figures using PEG8K 12.7 mM.

Corrugation (wrinkling) seen in myelin figures 100 mol% POPC) subjected to secondary hydration via 30 μL PEG8K 12.7 mM ($\Pi = 0.266 \text{ MPa}$). Myelin figures were initially formed with 30 μL deionized water. Images were captured via brightfield microscopy.

Supplementary Video 9: Corrugation of Myelin Figures using PEG8K 37.5 mM.

Corrugation (wrinkling) seen in myelin figures (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) subjected to secondary hydration via 30 μL PEG8K 37.5 mM ($\Pi = 2.15 \text{ MPa}$). Myelin figures were initially formed with 30 μL deionized water. Images were captured via confocal fluorescence microscopy.

Supplementary Video 10: Corrugation of Myelin Figures using DEX10K 55.6 mM.

Corrugation (wrinkling) seen in myelin figures (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) subjected to secondary hydration via 30 μL DEX10K 55.6 mM ($\Pi = 2.15 \text{ MPa}$). Myelin figures were initially formed with 30 μL deionized water. Images were captured via confocal fluorescence microscopy.

Supplementary Video 11: Formation of 90/10 POPC/Cholesterol Myelin Figures using DI Water.

Myelin figure formation from a lipid cake (99.9 mol% lipid doped with 0.1 mol% lissamine-rhodamine-PE) upon hydration with 30 μ L of deionized water. These images represent the baseline morphology prior to the addition of PEG-laden solution and were captured using confocal fluorescence microscopy.

Supplementary Video 12: Corrugation of 90/10 POPC/Cholesterol Myelin Figures Induced by PEG8K 37.5 mM.

Corrugation (wrinkling) seen in myelin figures from Supplemental Video 11 subjected to secondary hydration via 30 μ L PEG8K 37.5 mM. Images were captured via confocal fluorescence microscopy.

Supplementary Video 13: Formation of 75/25 POPC/Cholesterol Myelin Figures using DI Water and PEG8K 37.5 mM.

Myelin figure formation from a lipid cake (99.9 mol% lipid doped with 0.1 mol% lissamine-rhodamine-PE) upon hydration with 30 μ L of deionized water. These images represent the baseline morphology prior to the addition of PEG-laden solution and were captured using confocal fluorescence microscopy.

Supplementary Video 14: Corrugation of 75/25 POPC/Cholesterol Myelin Figures using DI Water and PEG8K 37.5 mM.

Corrugation (wrinkling) seen in myelin figures from Supplemental Video 13 subjected to secondary hydration via 30 μ L PEG8K 37.5 mM. Images were captured via confocal fluorescence microscopy.

Supplementary Video 15: Formation of 60/40 POPC/Cholesterol Myelin Figures using DI Water and PEG8K 37.5 mM.

Myelin figure formation from a lipid cake (99.9 mol% lipid doped with 0.1 mol% lissamine-rhodamine-PE) upon hydration with 30 μ L of deionized water. These images represent the baseline morphology prior to the addition of PEG-laden solution and were captured using confocal fluorescence microscopy.

Supplementary Video 16: Corrugation of 60/40 POPC/Cholesterol Myelin Figures using DI Water and PEG8K 37.5 mM.

Corrugation (wrinkling) seen in myelin figures from Supplemental Video 15 subjected to secondary hydration via 30 μ L PEG8K 37.5 mM. Images were captured via confocal fluorescence microscopy.

Supplementary Video 17: Initial Formation of POPC Myelin Figures using DI Water before Adding DEX10K 55.6 mM (1000:1 unlabeled to labeled)

Myelin figure formation from a lipid cake (99.9 mol% POPC doped with 0.1 mol% lissamine-rhodamine-PE) upon hydration with 30 μ L of deionized water. These images represent the

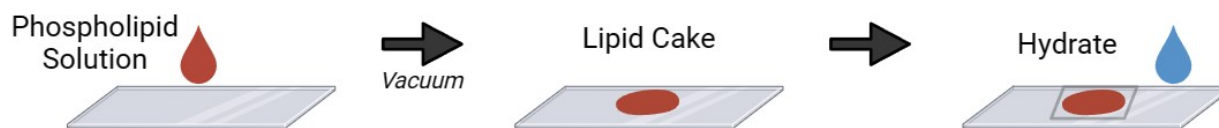
baseline morphology prior to the addition of labeled dextran solution and were captured using confocal fluorescence microscopy.

Supplementary Video 18: Corrugation of POPC Myelin Figures Induced by DEX10K 55.6 mM (1000:1 unlabeled to labeled)

Corrugation (wrinkling) seen in myelin figures from Supplemental Video 17 subjected to secondary hydration via 30 μ L DEX10K 55.6 mM (1000:1 unlabeled to labeled). Images were captured via confocal fluorescence microscopy.

Experimental Set-Up

The following illustration made on Biorender is a pictorial representation of how these myelin figures are formed.



Determining Osmotic Pressure via Modified van't Hoff's equation

The physical presence of macromolecules in solution gives rise to nonideal, colloidal osmotic pressure Π ¹ which is well appreciated in polymer physics. When dealing with ideal polymer

solutions, van't Hoff's law ($\Pi = RTc$) is valid, where $R = 8.314 \frac{J}{mol \cdot K}$ is the universal gas constant,

$T =$ temperature in *Kelvin*, and $c =$ concentration in $\frac{mol}{m^3}$. However, when dealing with non-ideal solutions as in this case, the expression must be extended to include non-colligative effects. A virial expansion of the osmotic pressure² accounts for these non-colligative effects and is written as:

$$\frac{\Pi}{RT} = \frac{c}{M_w} + A_2 c^2$$

In the above expression, $R = 8.314 \frac{J}{mol \cdot K}$, $T =$ temperature in *Kelvin*, $c =$ concentration in $\frac{g}{mL}$, M_w

is molecular weight in $\frac{g}{mol}$, and A_2 is the second virial coefficient with units of $\frac{mol \cdot mL}{g^2}$. The second virial coefficient accounts for the colligative non-ideality in the solution due to solute-solute and solute-solvent interactions. Higher order terms in the virial expansion, such as A_3, A_4, \dots can be neglected as we are primarily interested in the pairwise solute-solute interaction understanding and not three molecules at a time. It is also experimentally difficult to determine the higher order terms in the expansion.

Determining the Second Virial Coefficients for Osmolytes

To be able to calculate the osmotic pressure for the chosen osmolytes, the chosen osmolytes second virial coefficient needs to be determined. This was achieved by utilizing a technique known as

vapor pressure osmometry³. Vapor pressure osmometry measurements, using an osmometer, of these larger macromolecules can be determined by plotting mass concentration-normalized osmolality Osm/c_s against a mass concentration c_s of the solute⁴⁻⁶. As mentioned earlier, when the ideal expression for van't Hoff is expanded to include the virial coefficients, the expression becomes:

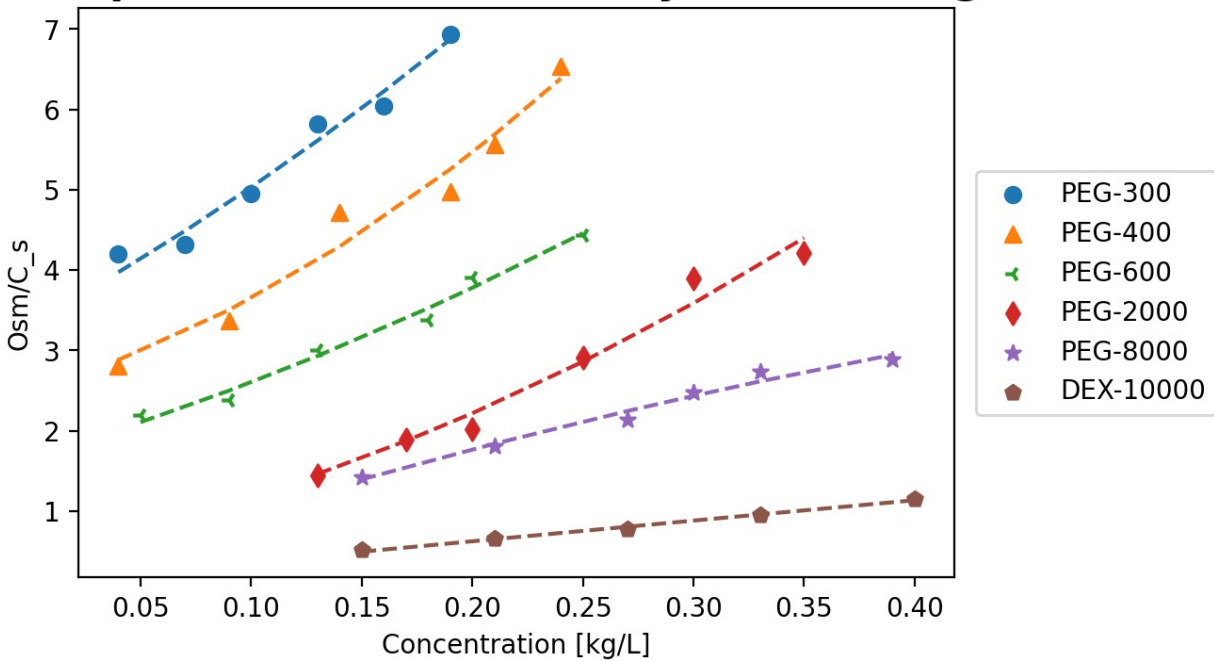
$$\frac{\Pi}{RT} = \frac{c}{M_w} + A_2c^2$$

Vapor pressure osmometry measures the osmolality of a solution as $Osm = \varphi m_s = -m_w^* \ln a_w$ where φ is the molal osmotic coefficient of the solute, m_s is the molality of solute, m_w^* is the molality of water ($55.51 \text{ mol} * \text{kg}^{-1}$), and a_w is water activity. Furthermore, the osmotic pressure is directly proportional to the water activity via $\Pi V_w = -RT \ln a_w$. If the three expressions from above are algebraically combined, it yields an expression which relates the output of a vapor pressure osmometer to the concentration, molar mass, and second virial coefficient:

$$\frac{Osm}{c_s} = V_w m_w^* \left(\frac{1}{M_s} + A_2 c_s + A_3 c_s^2 + \dots \right)$$

To determine the second virial coefficient of the depletant molecules, a plot of the concentration normalized osmolality (Osm/c_s) versus the mass concentration. The solution osmolality is measured directly from the vapor pressure osmometer. Given that these experiments are done in ambient conditions (25 °C, 1 atm), the molality of water $m_w^* = 55.51 \text{ mol} * \text{kg}^{-1}$, the molar volume of water $V_w = 18.01 \text{ mL} * \text{mol}^{-1}$, and the M_s is the molecular weight of each depletant measured. The results are shown in the figure and table below:

Vapor Pressure Osmolarity Data Fitting



Molecule	Molecular Weight	$A_2 \left[\frac{\text{mol} * \text{mL}}{\text{g}^2} \right]$	R^2
PEG	300	0.014960	0.971
PEG	400	0.007974	0.967
PEG	600	0.008088	0.982
PEG	2,000	0.005168	0.973
PEG	8,000	0.009215	0.979
DEX	10,000	0.002659	0.993

Sample Calculations for Osmotic Pressure Using Experimentally Determined Second Virial Coefficients

The following are calculations to show the determination of the osmotic pressure using the modified van't Hoff's law and the experimentally determined second virial coefficients.

Polyethylene Glycol, 600 g/mol, 400 mM

$$\frac{\Pi}{RT} = \frac{c}{M_w} + A_2 c^2$$

$$\frac{\Pi}{RT} = \frac{(400 \text{ mM})}{600 \text{ g/mol}} + \left(0.008088 \frac{\text{mol} * \text{mL}}{\text{g}^2}\right) (400 \text{ mM})^2$$

$$\frac{\Pi}{RT} = \frac{(0.24 \text{ g/mL})}{600 \text{ g/mol}} + \left(0.008088 \frac{\text{mol} * \text{mL}}{\text{g}^2}\right) (0.24 \text{ g/mL})^2$$

$$\frac{\Pi}{RT} = 4e-4 \frac{\text{mol}}{\text{mL}} + 4.658e-4 \frac{\text{mol}}{\text{mL}}$$

$$\Pi = \left(8.658e-4 \frac{\text{mol}}{\text{mL}}\right) * RT$$

$$\Pi = \left(8.658e-4 \frac{\text{mol}}{\text{mL}}\right) \left(8.314 \frac{\text{J}}{\text{mol} * \text{K}}\right) (298 \text{ K})$$

$$\Pi = 2.146 \frac{\text{J}}{\text{mL}}$$

$$\Pi \cong 2.15 \text{ MPa}$$

Polyethylene Glycol, 2000 g/mol, 182 mM

$$\frac{\Pi}{RT} = \frac{c}{M_w} + A_2 c^2$$

$$\frac{\Pi}{RT} = \frac{(182 \text{ mM})}{2000 \text{ g/mol}} + \left(0.005168 \frac{\text{mol} * \text{mL}}{\text{g}^2}\right) (182 \text{ mM})^2$$

$$\frac{\Pi}{RT} = \frac{(0.364 \text{ g/mL})}{2000 \text{ g/mol}} + \left(0.005168 \frac{\text{mol} * \text{mL}}{\text{g}^2}\right) (0.364 \text{ g/mL})^2$$

$$\frac{\Pi}{RT} = 1.82e-4 \frac{\text{mol}}{\text{mL}} + 6.847e-4 \frac{\text{mol}}{\text{mL}}$$

$$\Pi = \left(8.667e-4 \frac{\text{mol}}{\text{mL}}\right) * RT$$

$$\Pi = \left(8.667e-4 \frac{\text{mol}}{\text{mL}}\right) \left(8.314 \frac{\text{J}}{\text{mol} * \text{K}}\right) (298 \text{ K})$$

$$\Pi = 2.147 \frac{\text{J}}{\text{mL}}$$

$$\Pi \cong 2.15 \text{ MPa}$$

Polyethylene Glycol, 8000 g/mol, 37.5 mM

$$\frac{\Pi}{RT} = \frac{c}{M_w} + A_2 c^2$$

$$\frac{\Pi}{RT} = \frac{(37.5 \text{ mM})}{8000 \text{ g/mol}} + \left(0.009215 \frac{\text{mol} * \text{mL}}{\text{g}^2}\right) (37.5 \text{ mM})^2$$

$$\frac{\Pi}{RT} = \frac{(0.3 \text{ g/mL})}{8000 \text{ g/mol}} + \left(0.009215 \frac{\text{mol} * \text{mL}}{\text{g}^2}\right) (0.3 \text{ g/mL})^2$$

$$\frac{\Pi}{RT} = 3.75e - 5 \frac{\text{mol}}{\text{mL}} + 8.293e - 4 \frac{\text{mol}}{\text{mL}}$$

$$\Pi = \left(8.668e - 4 \frac{\text{mol}}{\text{mL}}\right) * RT$$

$$\Pi = \left(8.668e - 4 \frac{\text{mol}}{\text{mL}}\right) \left(8.314 \frac{\text{J}}{\text{mol} * \text{K}}\right) (298 \text{ K})$$

$$\Pi = 2.147 \frac{\text{J}}{\text{mL}}$$

$$\Pi \cong 2.15 \text{ MPa}$$

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