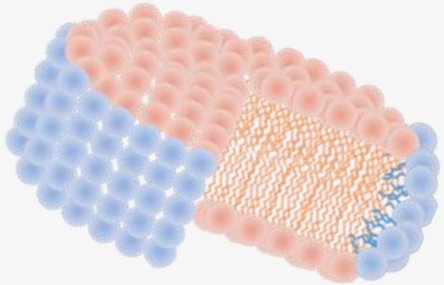
Analyzing the Structure of Bicelles with Small Angle Neutron Scattering



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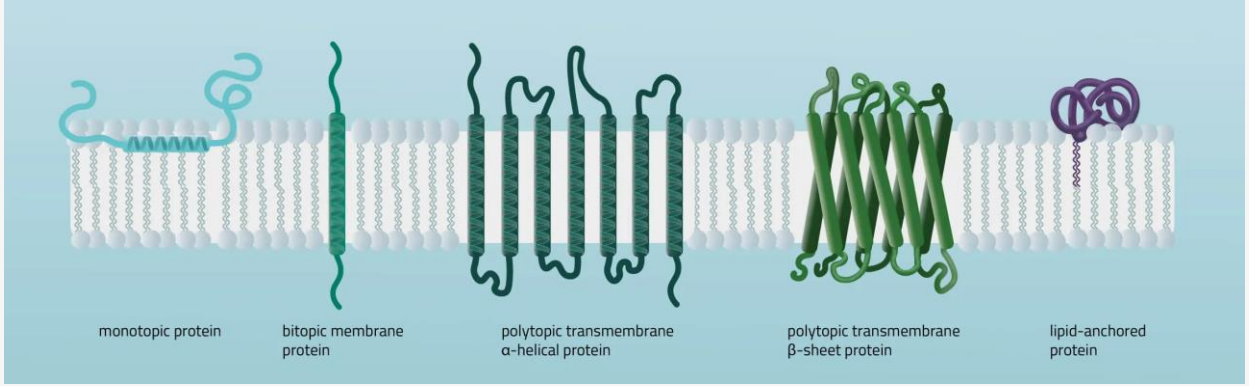
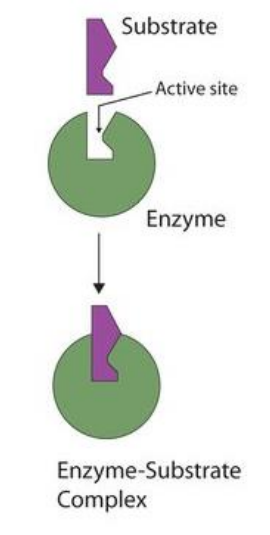
U.S. DEPARTMENT OF COMMERCE

Why do we care?

Membrane Proteins very important to study, but difficult!

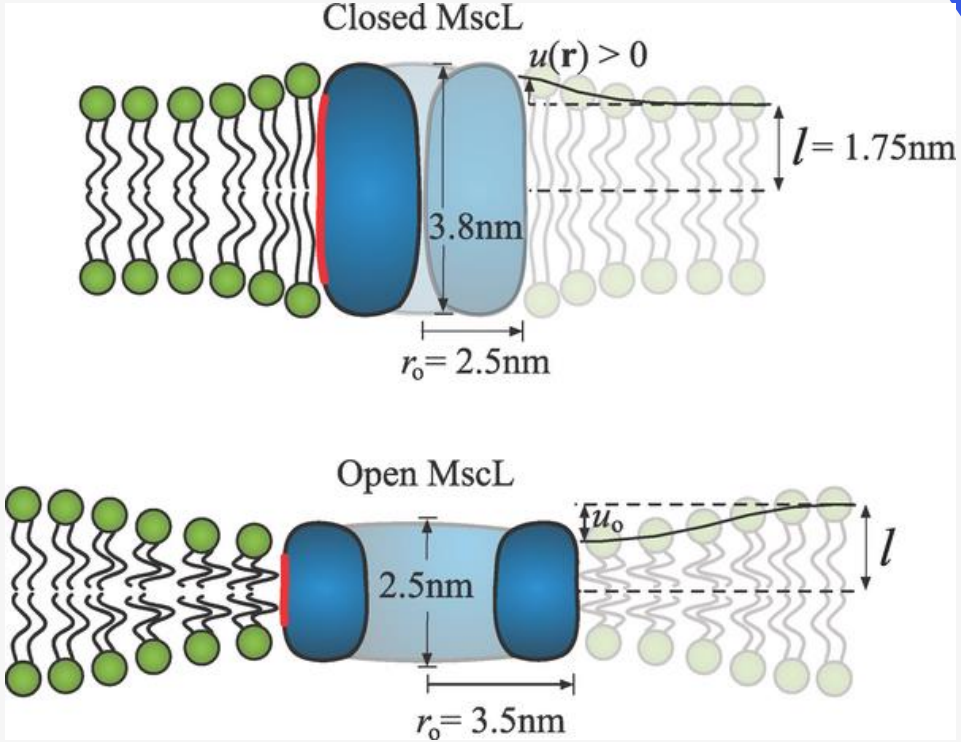




(Membrane Proteins - Definition, Types & Functions. (n.d.). Cube Biotech. https://cube-biotech.com/knowledge/membrane-protein-stabilization/membrane-proteins/)

But why study the membrane?

Studying the environment allows us to consider how it impacts the sample



(Ursell, T., Huang, K. C., Peterson, E., & Phillips, R. (2007). Cooperative Gating and Spatial Organization of Membrane Proteins through Elastic Interactions. *PLoS Computational Biology*, *3*(5), e81. https://doi.org/10.1371/journal.pcbi.0030081)

But why study the membrane?

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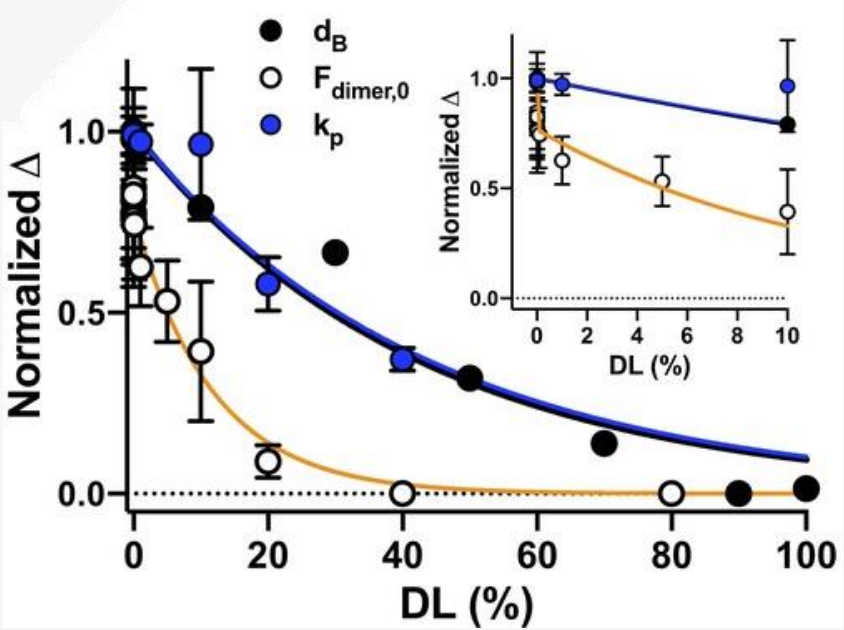
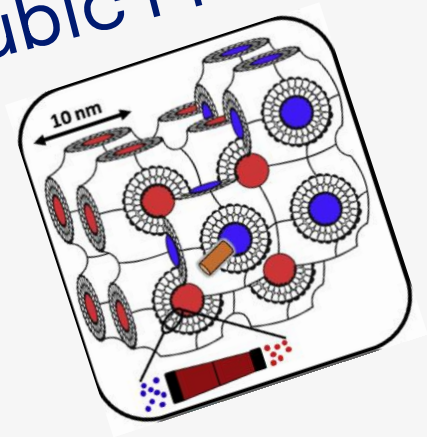


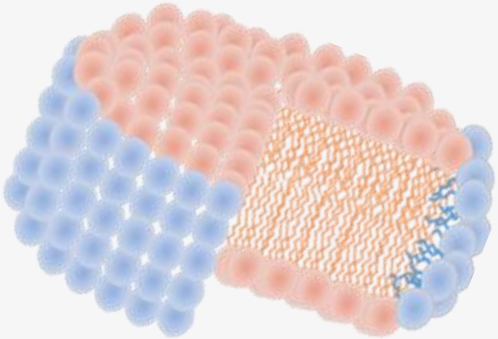
(Chadda, R., Bernhardt, N., Kelley, E. G., Teixeira, S. C., Griffith, K., Gil-Ley, A., Öztürk, T. N., Hughes, L. E., Forsythe, A., Krishnamani, V., Faraldo-Gómez, J. D., & Robertson, J. L. (2021). Membrane transporter dimerization driven by differential lipid solvation energetics of dissociated and associated states. *eLife*, *10*. https://doi.org/10.7554/elife.63288)

Model systems

Cubic Phase!



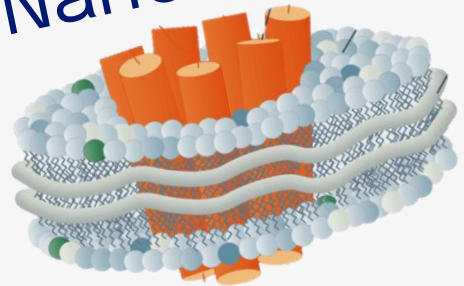
(Li, D., & Caffrey, M. (2020). Structure and functional characterization of membrane integral proteins in the lipid cubic phase. *Journal of Molecular Biology*, *432*(18), 5104–5123. https://doi.org/10.1016/j.jmb.2020.02.024)



Bicelle!

(Model Membranes | Columbus Lab. (n.d.). Columbus Lab. https://www.columbuslabs.org/model-membranes)

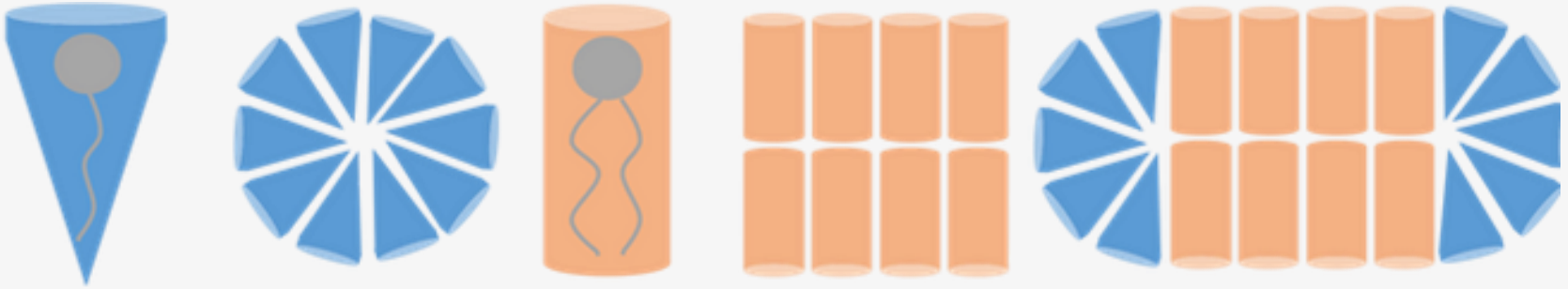
Nano Disk!



(Membrane Proteins - Definition, Types & Functions. (n.d.). Cube Biotech. https://cube-biotech.com/knowledge/membrane-protein-stabilization/membrane-proteins/)

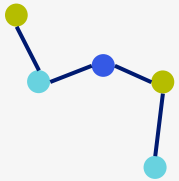
Bicelle + Lipid Model

x=0.5



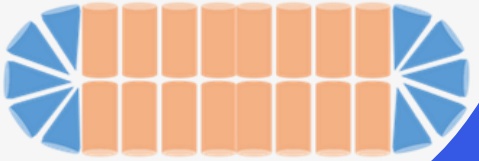
Detergent Micelle Lipid Lipid Bilayer Bicelle

x=1



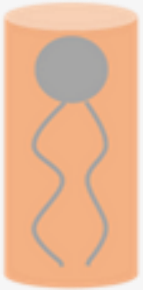
$$x_{eff} = \frac{[lipid_{\text{in bicelle}} - lipid_{\text{floating}}]}{[detergent_{\text{in bicelle}} - detergent_{\text{floating}}]}$$

x=2

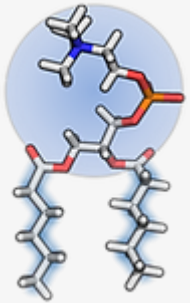


(*Model Membranes | Columbus Lab*. (n.d.). Columbus Lab. https://www.columbuslabs.org/model-membranes)

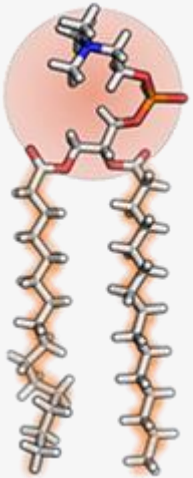
Bicelle + Lipid Model



Lipid



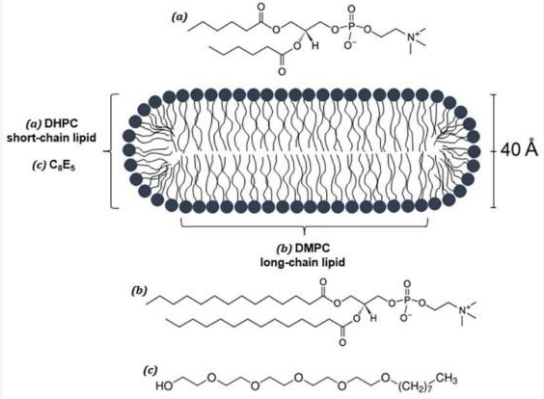
DHPC

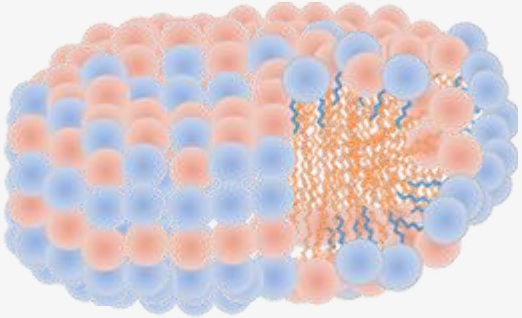


DMPC

(Cadwell et al, 2018)
(Katsaras et al , 2005)

(Rieth, M. D. (2018). The best of both worlds: A new lipid complex has micelle and bicelle-like properties. *bioRxiv (Cold Spring Harbor Laboratory)*. https://doi.org/10.1101/437327)





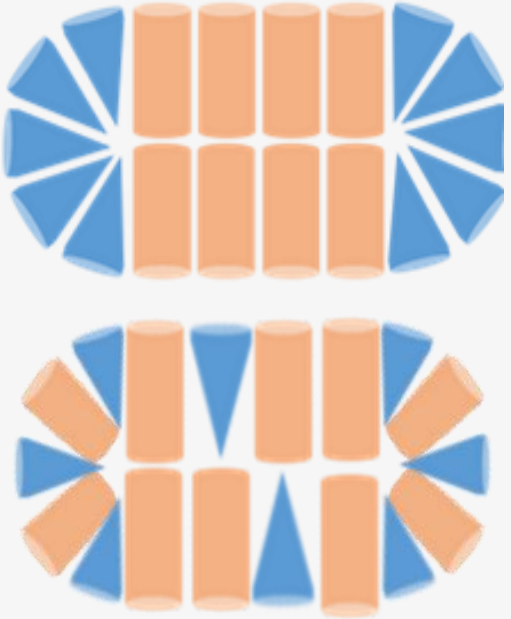
IDEAL MODEL!

(Model Membranes | Columbus Lab. (n.d.). Columbus Lab. https://www.columbuslabs.org/model-membranes)

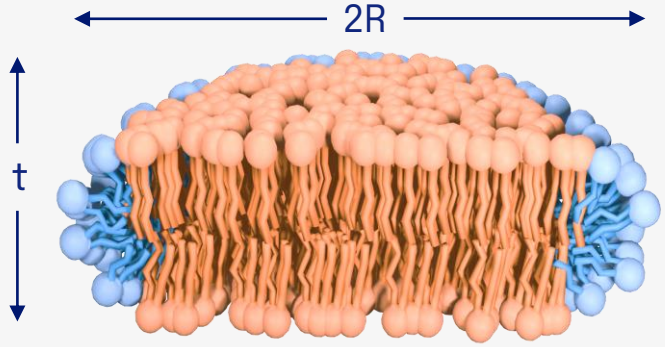
Research Question

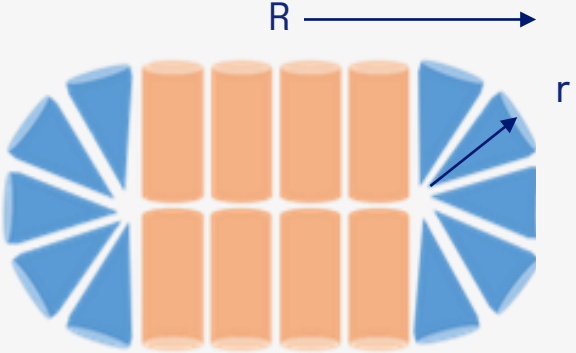
Is the ideal model correct? What is the true segregation of bicelles? Quantify?

Fully Segregated? Fully Mixed?



Radius versus q_{eff}



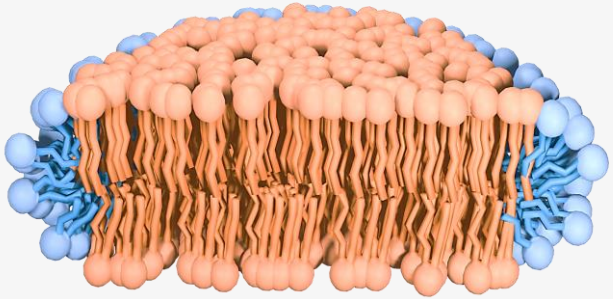


Only true for ideal model:

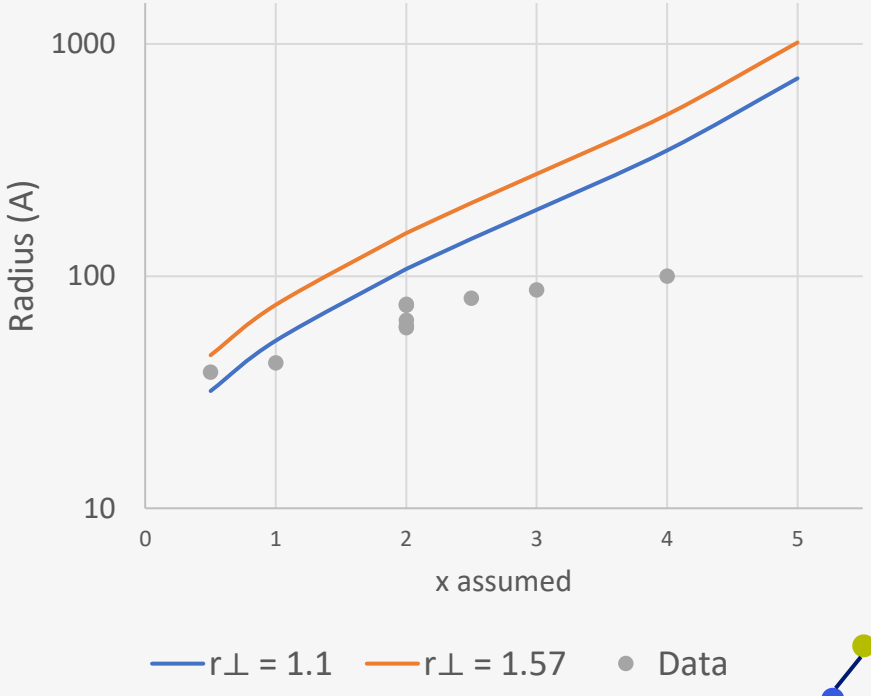
Predicts composition -> size

$$R_v = \frac{x_V r}{4}\left(\pi + \sqrt{\pi^2 + 32/3x_V}\right) + r$$

Radius versus Lipid Ratio



$$x_{eff} = \frac{[lipid_{in\ bicelle} - lipid_{floating}]}{[detergent_{in\ bicelle} - detergent_{floating}]}$$



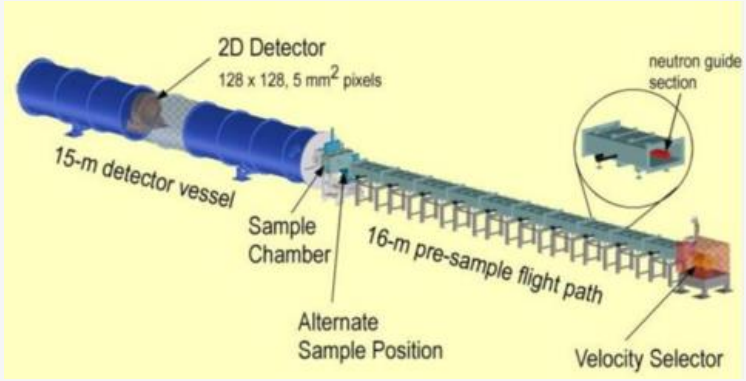
Ideal wrong!

Next steps:

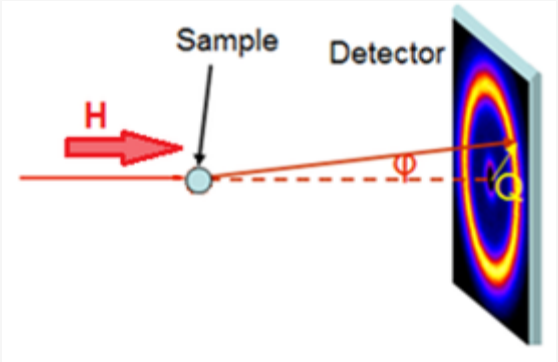
- Pick specific x value
- Continue doing analysis
- Quantify segregation

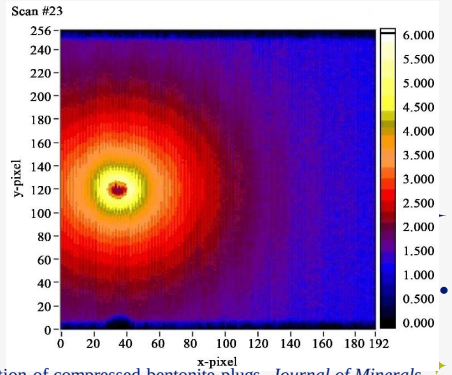
Small Angle Neutron Scattering (SANS)

- Two Instruments: NG3 and NG7
- Nuclear Generator -> Neutrons
- Long flight path = Thinner beam
- Hits Sample, Scatters
- Hits Detector



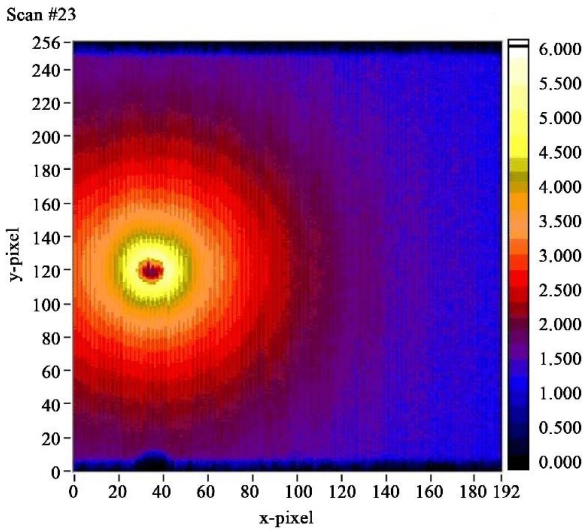
(Allen, A. J. (2005). Characterization of ceramics by X-Ray and neutron Small-Angle scattering. *Journal of the American Ceramic Society*, *88*(6), 1367–1381. https://doi.org/10.1111/j.1551-2916.2005.00463.x)





(Searching for Magnetic Skyrmions in Powdered B20 Materials with SANS | NIST. (2023, May 2). NIST. https://www.nist.gov/ncnr/2018-summer-school-fundamentals-neutron-scattering/course-materials/experiment-handouts-0)c

(Blach, T., & Holl, H. (2019). Small angle neutron scattering investigation of compressed bentonite plugs. *Journal of Minerals and Materials Characterization and Engineering*, *07*(05), 230–260. https://doi.org/10.4236/jmmce.2019.75018)


Scan #23
y-pixel
x-pixel
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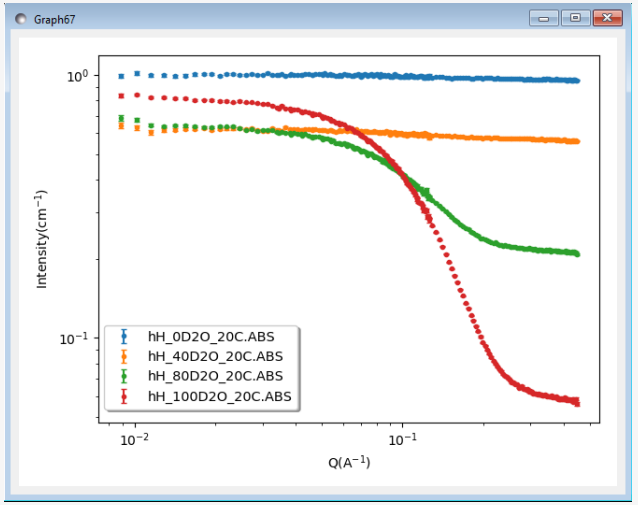
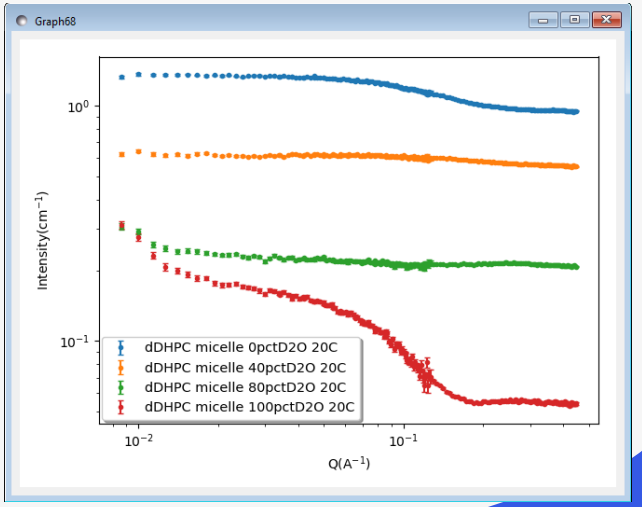

Graph67
Intensity(cm⁻¹)
Q(A⁻¹)
hH_0D2O_20C.ABS
hH_40D2O_20C.ABS
hH_80D2O_20C.ABS
hH_100D2O_20C.ABS

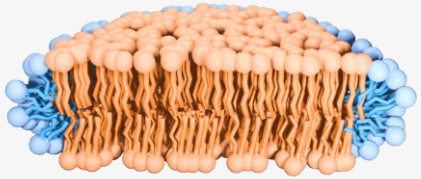

Graph68
Intensity(cm⁻¹)
Q(A⁻¹)
dDHPC micelle 0pctD2O 20C
dDHPC micelle 40pctD2O 20C
dDHPC micelle 80pctD2O 20C
dDHPC micelle 100pctD2O 20C

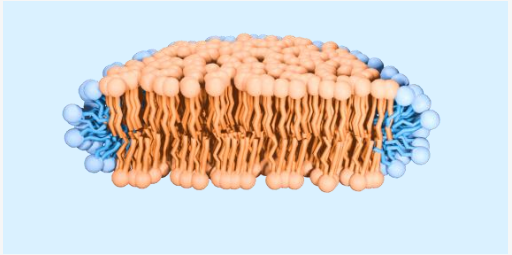
Why SANS?

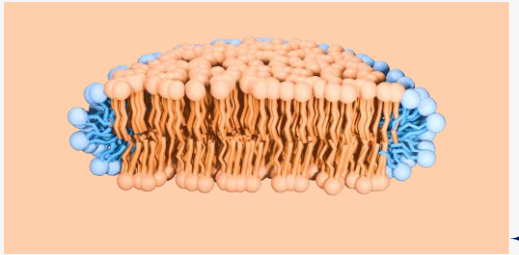
The special thing about SANS:

- Nanoscale info + molecular composition = **small** angle scattering
- Contrast!

ρ = Scattering Length Density
All Materials have scattering lengths!







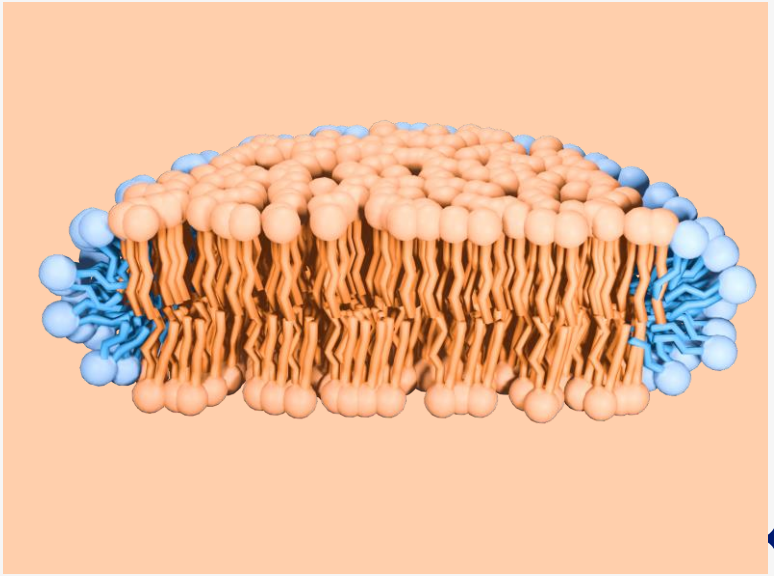
Make invisible

dDMPC contrast hDHPC

100D2O to match with dDMPC

Deuteration/Contrast!

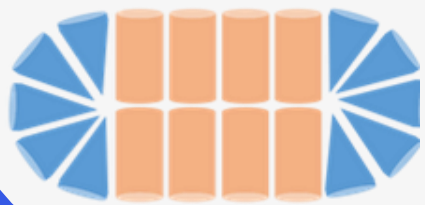
Deuterate the Head W/ 100D2O

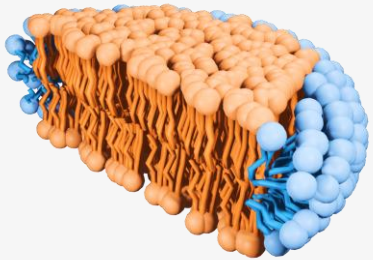


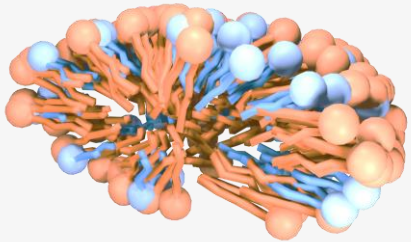
Segregated and Fully Mixed SLDs

Segregated	Value
SLD Core	7.4
SLD Face	6.2
SLD Rim	3.5

Fully Mixed	Value
SLD Core	5.5
SLD Face	5.7
SLD Rim	5.9

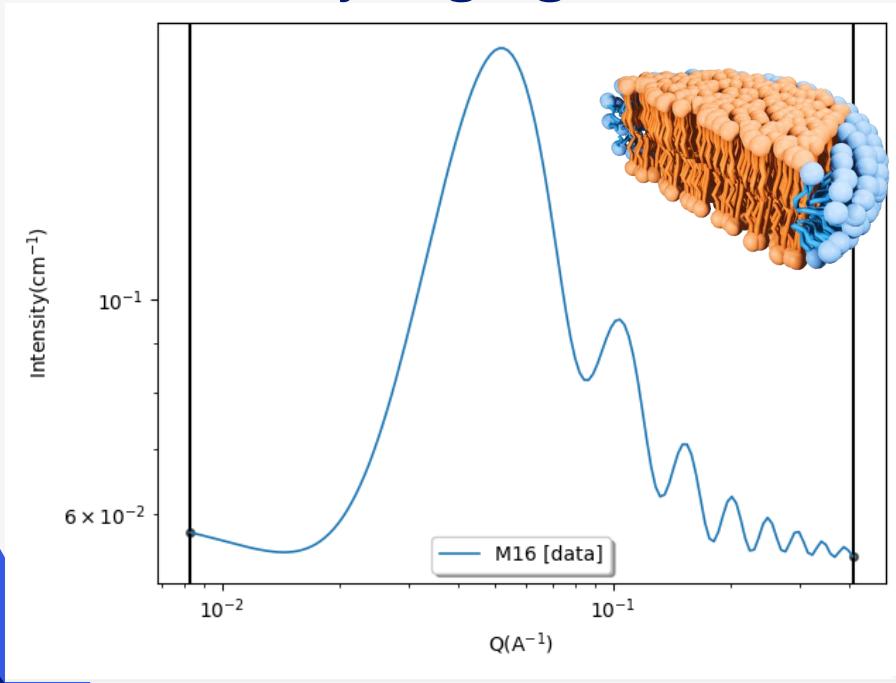




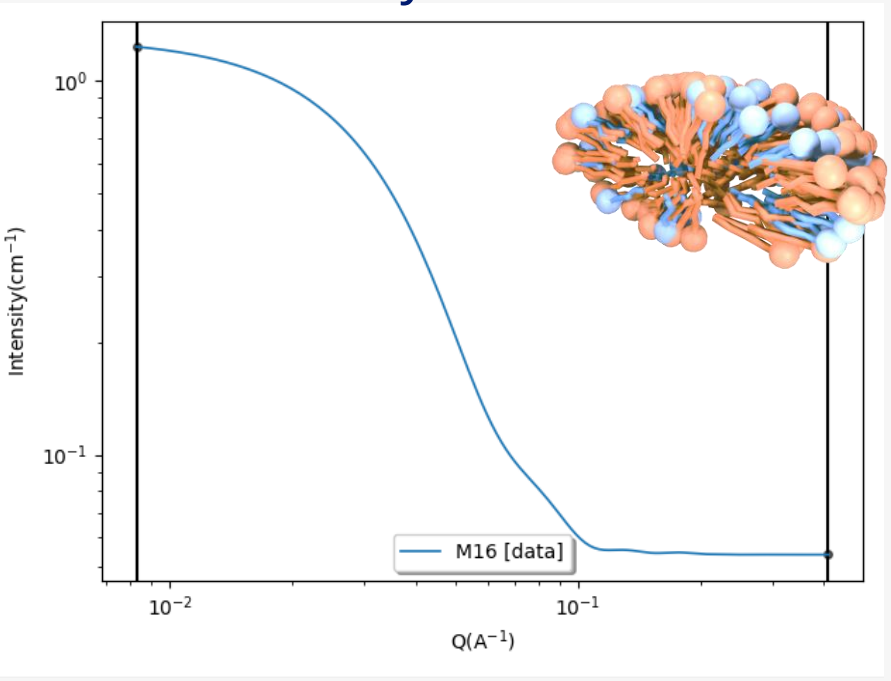


Model Curves

Fully Segregated

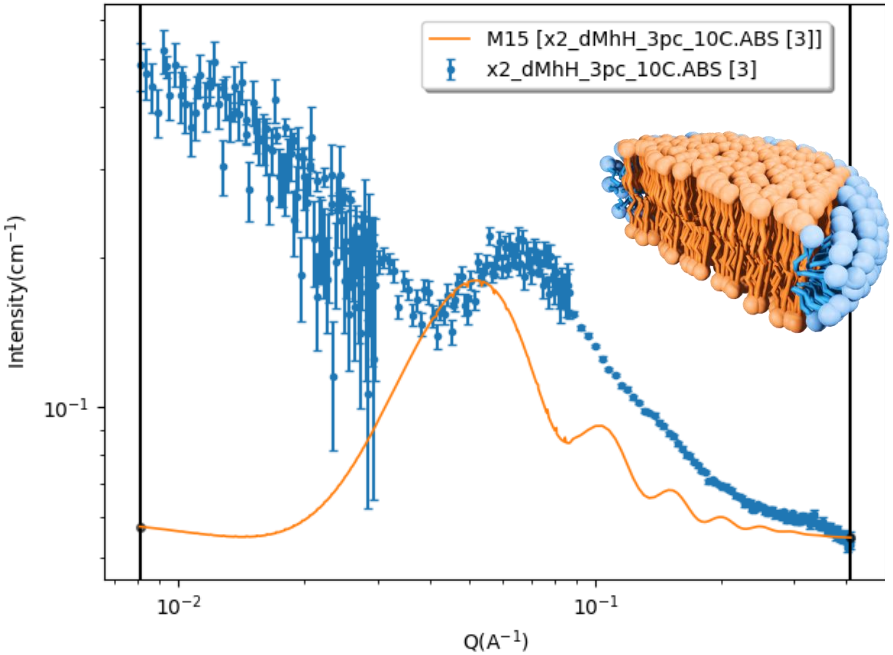


Fully Mixed

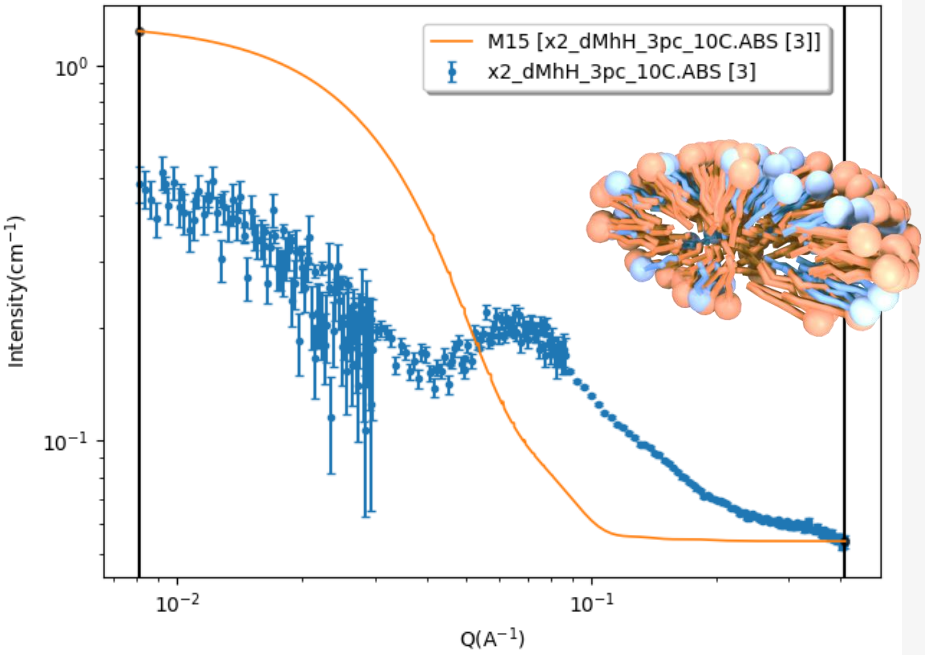


Plot Model w/ Data

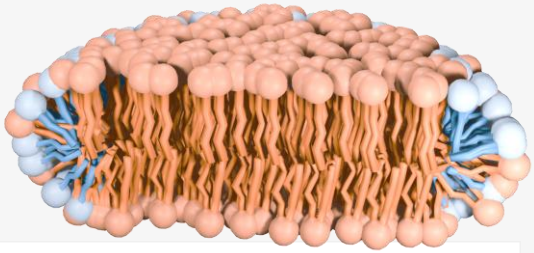
Fully Segregated



Fully Mixed

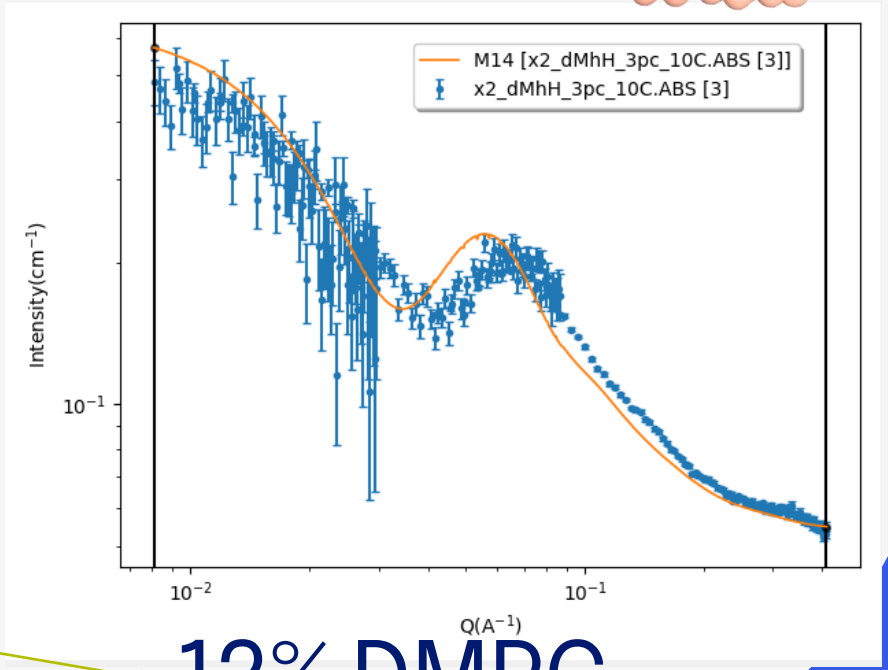


Final Fitting



dDMPC/hDHPC and dtail Lipids

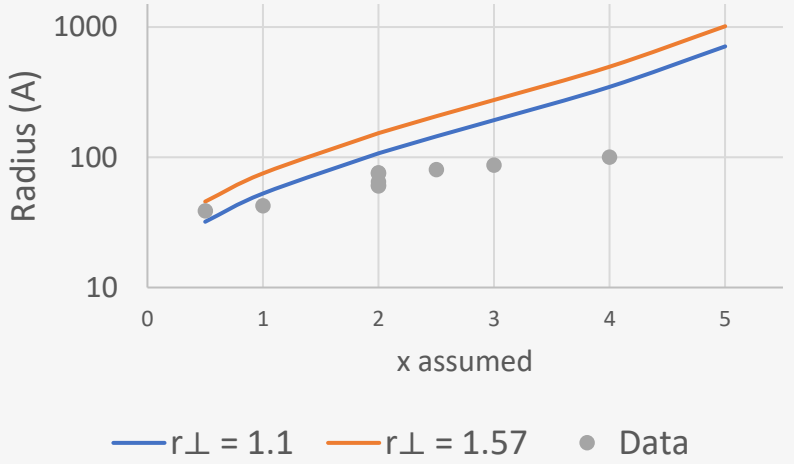
Scale	0.0273
Background	0.054
Radius	57.4
Thick Rim	8.3
Thick Face	11.8
Length	30.4
SLD_core	7.3
SLD_face	6.13
SLD_rim	1.44
SLD_solvent	6.4



12% DMPC

Conclusions

- Is a degree of mixing
- Around 12% mixing in rim
- Mixing increase as we increase x



Continued research needs to be made to get conclusive numbers:

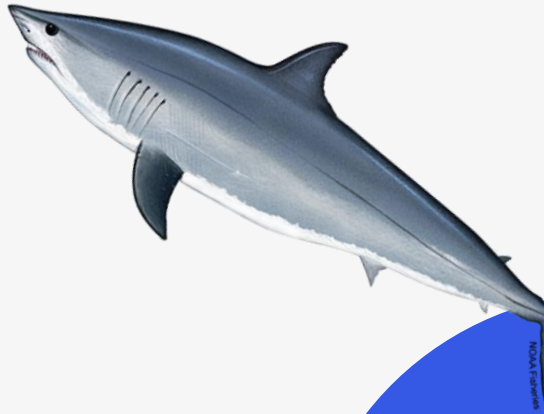
Range of x → h Lipids → dDMPC/dtail DHPC

SPECIAL THANKS

My Mentors: Elizabeth Kelley, Paul Butler
Coordinators of SURF: Julie Borchers, Leland Harringer, Susana Teixeira, Cara O'Malley

To the people at NCNR and sharktank room members...

And to YOU who sat through this!



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