Effects of peptides on lipid phase behavior

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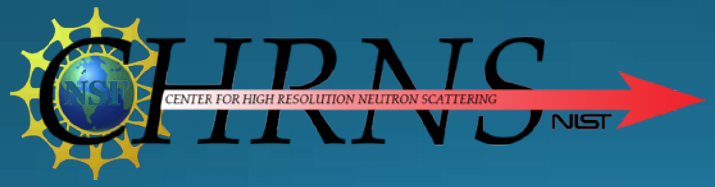
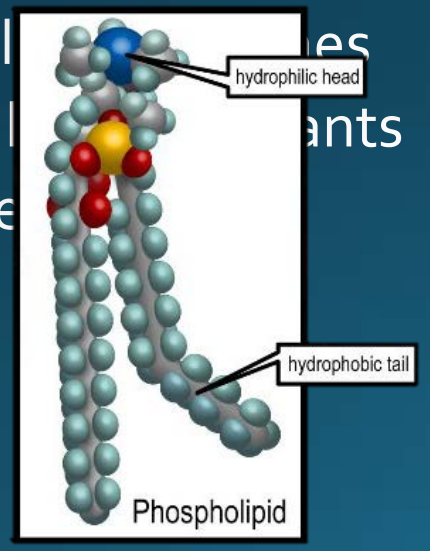
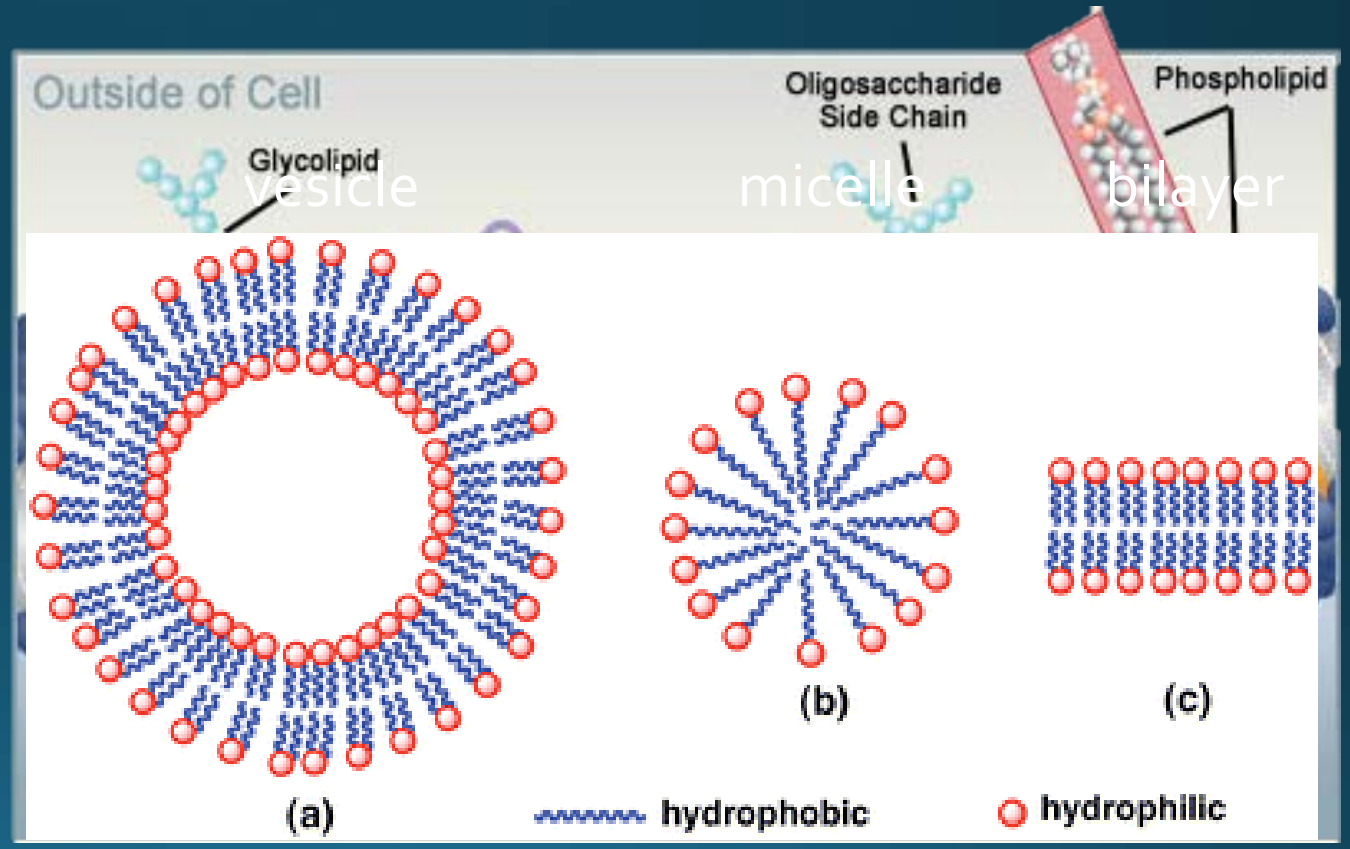


Background

- Fluid-mosaic model
  - Dynamic membrane
  - Amphipathic phospholipids
  - Proteins and sterols
- Amphipathic nanoassemblies
  - Vesicl…es
  - Micell…ants
  - Bilaye…





http://www.biology.arizona.edu/cell_bio/problem_sets/membranes/fluid_mosaic_model.html

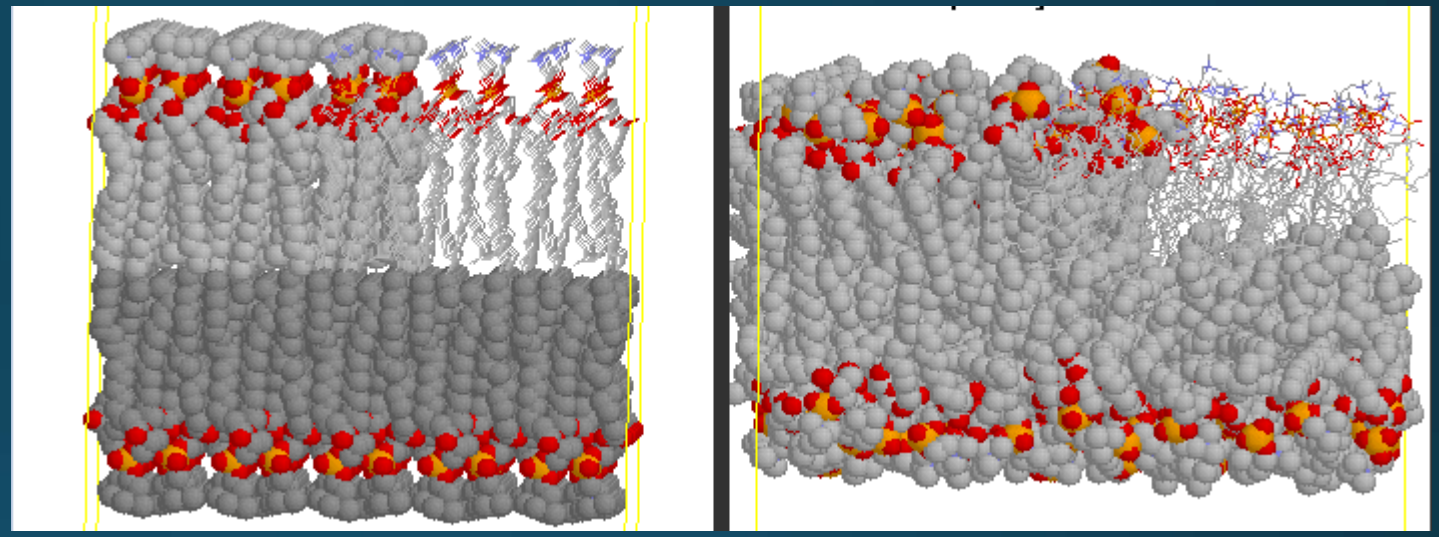
http://pubs.rsc.org/en/content/articlehtml/2009/an/b818181d

Background

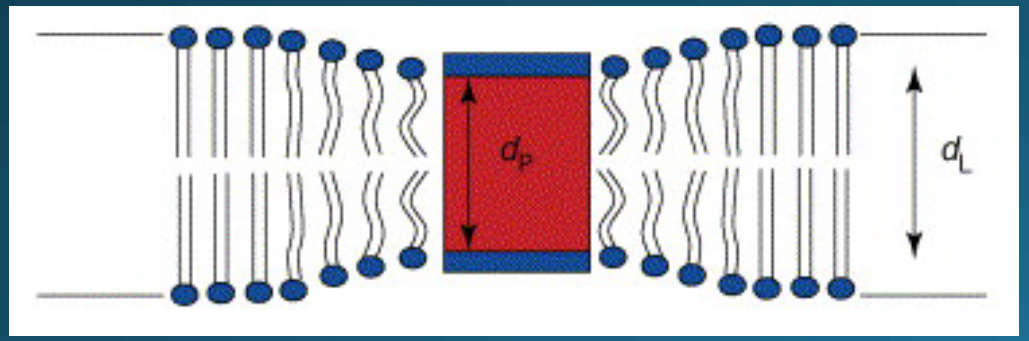
- Gel or liquid phase
  - Temperature
  - Peptide concentration
- Hydrophobic matching
  - Tail length
  - Peptide properties

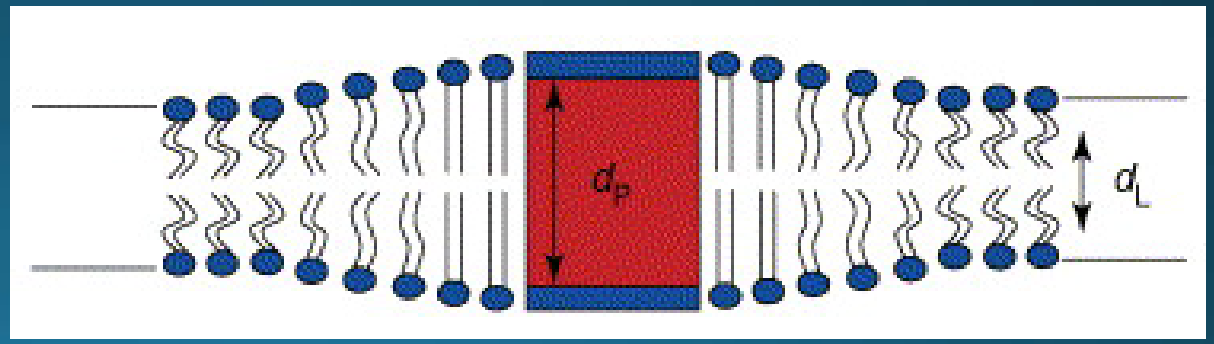
gel

fluid



http://biowiki.ucdavis.edu/Biochemistry/Lipids/Dynamics_of_Membrane_Lipids/Lipid_Conformational_Transitions

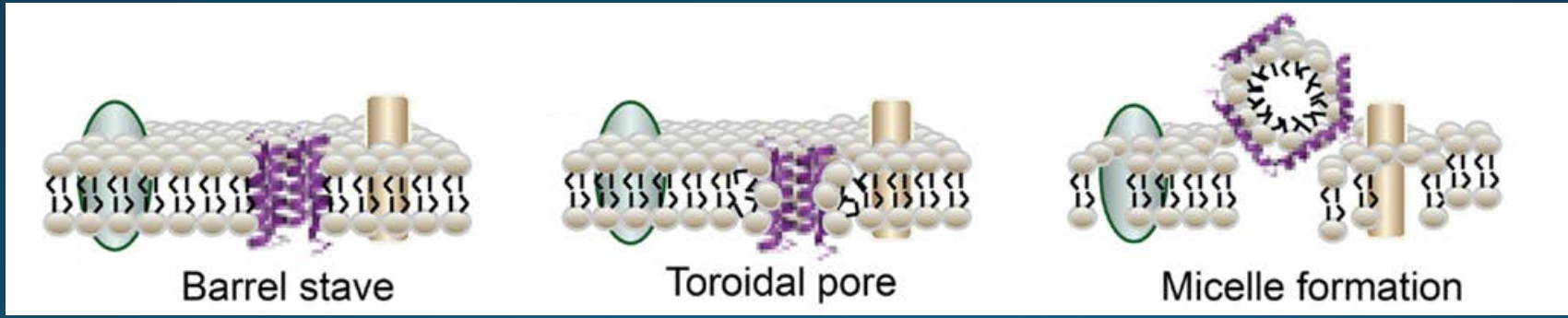




http://www.cell.com/cms/attachment/581128/4373622/gr1b3.jpg

Background

- Antimicrobial peptides
  - Kill pathogenic microorganisms by interacting with membrane
  - Key component of natural immune defense system



http://journal.frontiersin.org/article/10.3389/fimmu.2013.00143/full

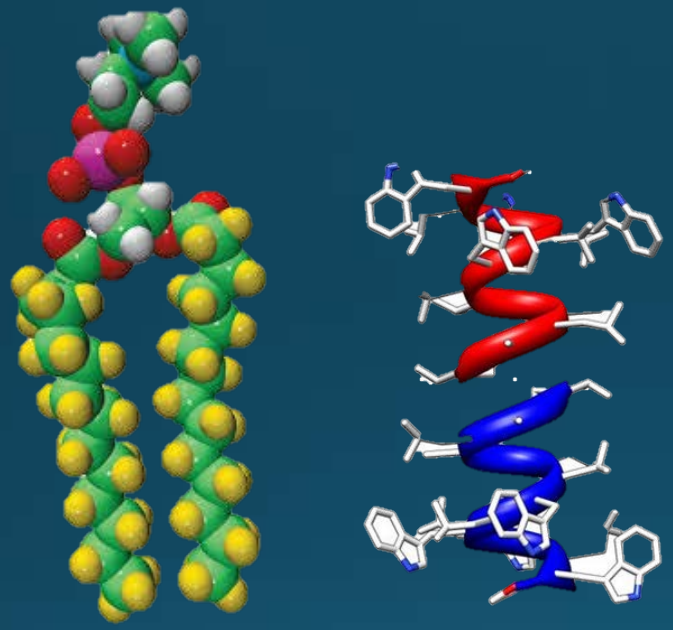
Purpose

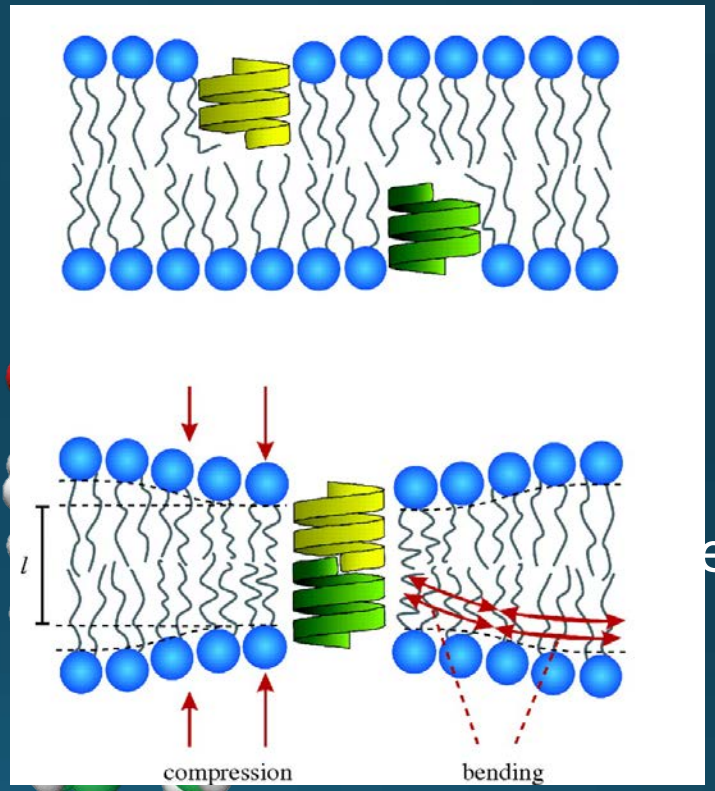
- Gain clearer understanding of interactions between lipids and peptides
- Future purposes of designing peptides for antibiotics
  - Tackling antibiotic resistance

Methods

<u>DMPC</u>

- 14 C tail
- Transition temp: 24˚C





emp: 55 ˚C

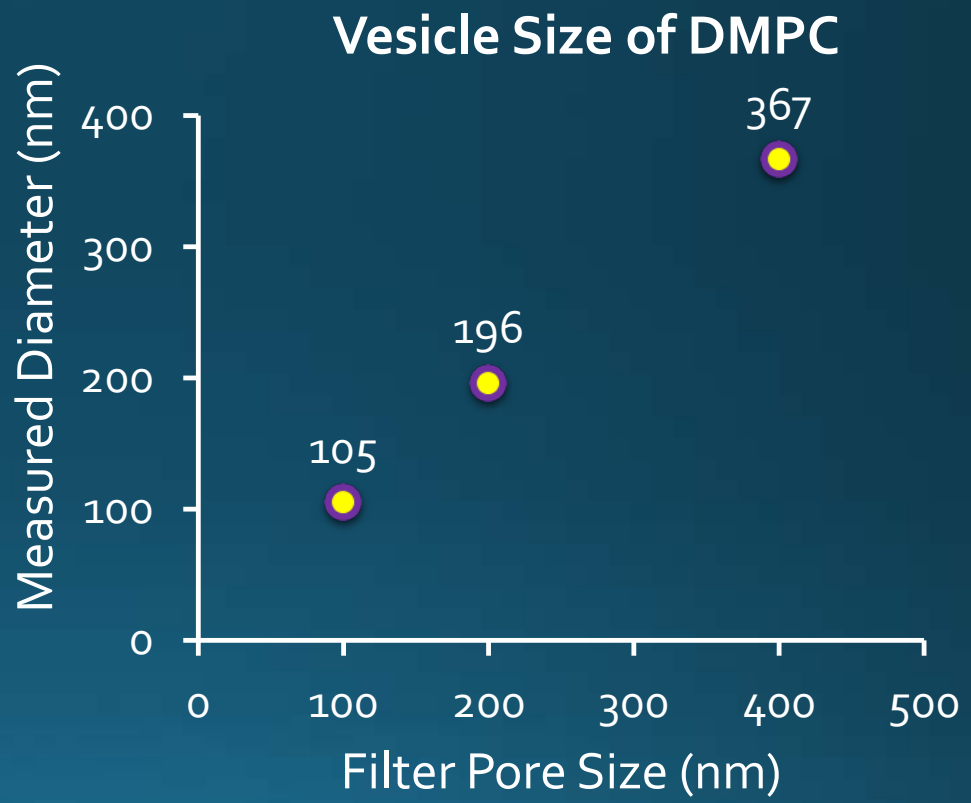
DOI: 10.1098/rsif.2009.0443 Lundbaek et al. J. R. Soc. Interface, 2010

<u>Gramicidin A from Bacillus brevis</u>

- Antimicrobial peptide that forms dimeric pores and leaks ions
- Extremely hydrophobic, helical structure
- Around 2.2 nm long

Methods

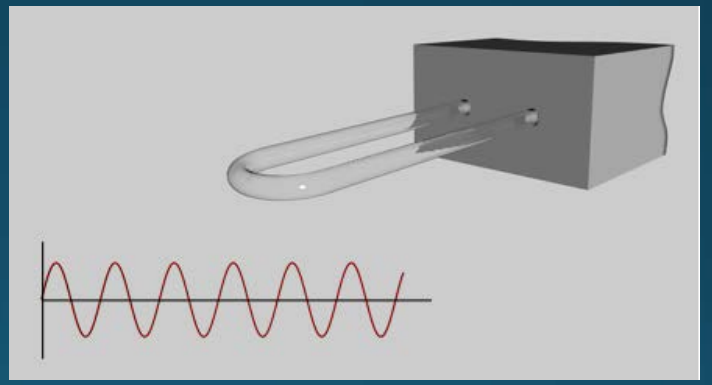
- Control vesicle size through extrusions
- Measure vesicle size using dynamic light scattering (DLS)



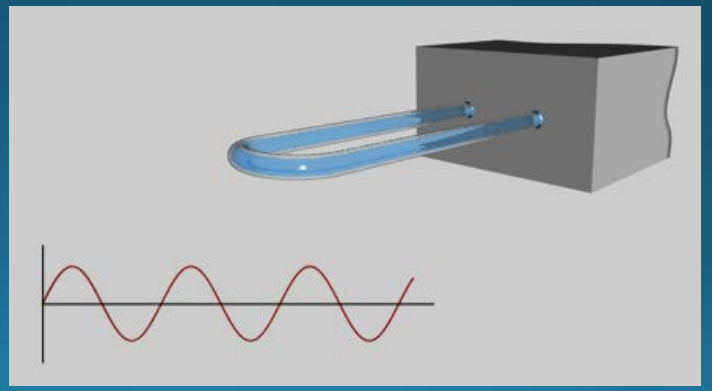
Methods

- Characterize phase transitions by measuring density at different temperatures

http://www.antonpaar.com/fileadmin/images/products/benchtop/density_and_sound_velocity_meter_dsa_5000_m/dsa_5000_m.jpg

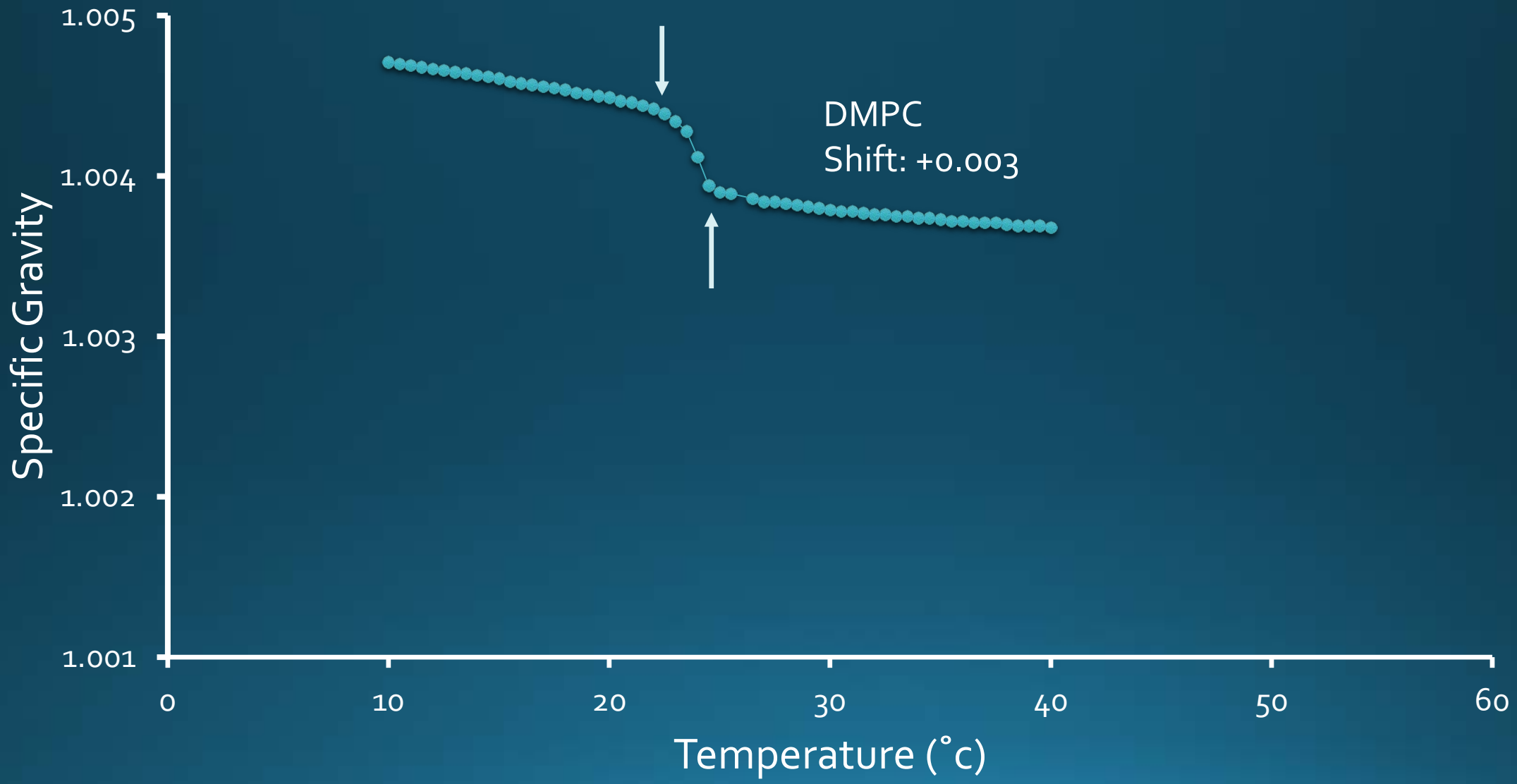


Characteristic frequency of air

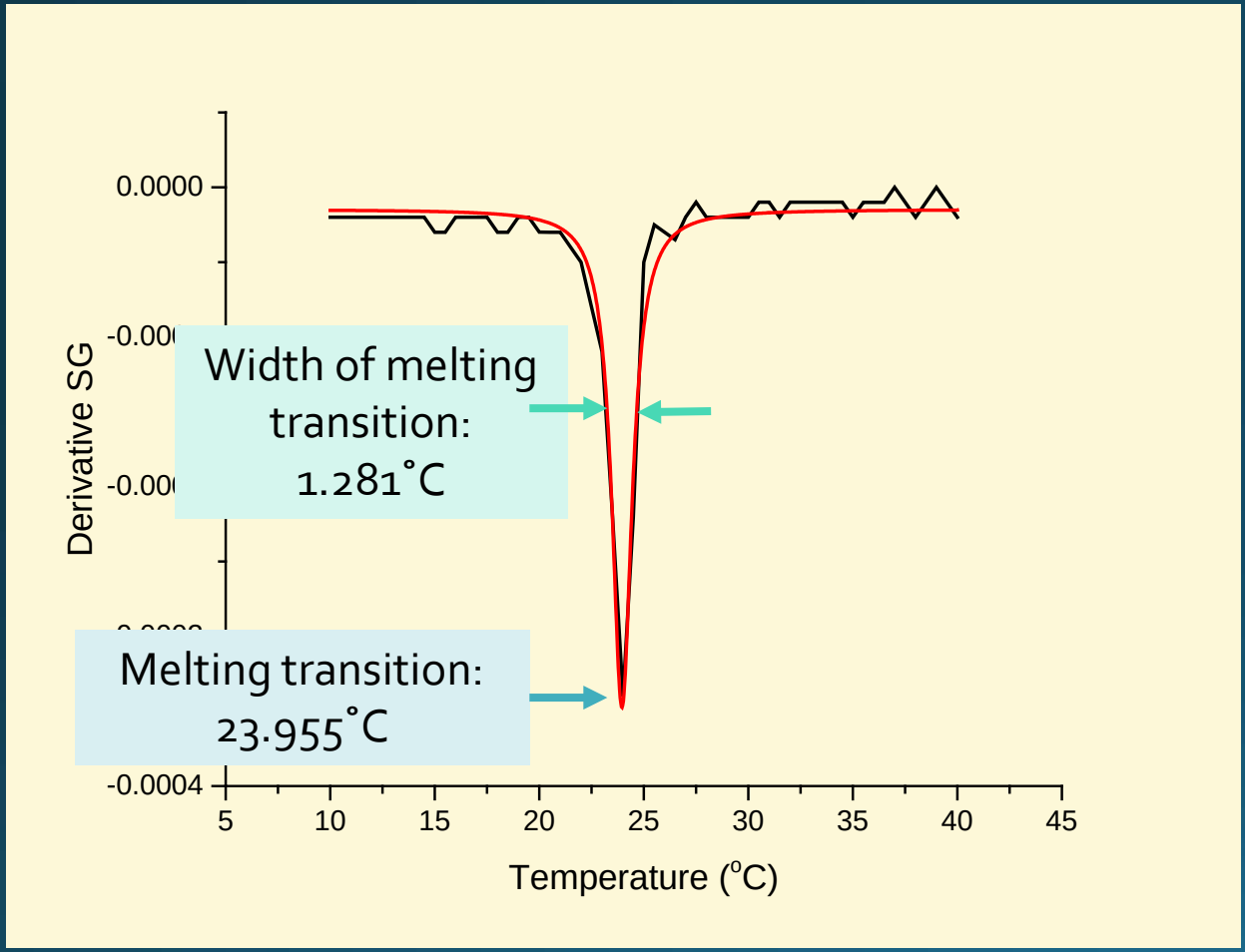


Characteristic frequency of water

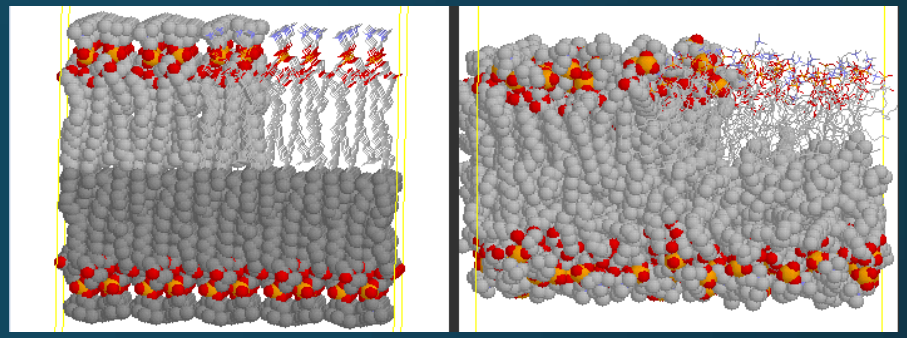
DMPC and Gramicidin Densities



Determining Melting Temperature



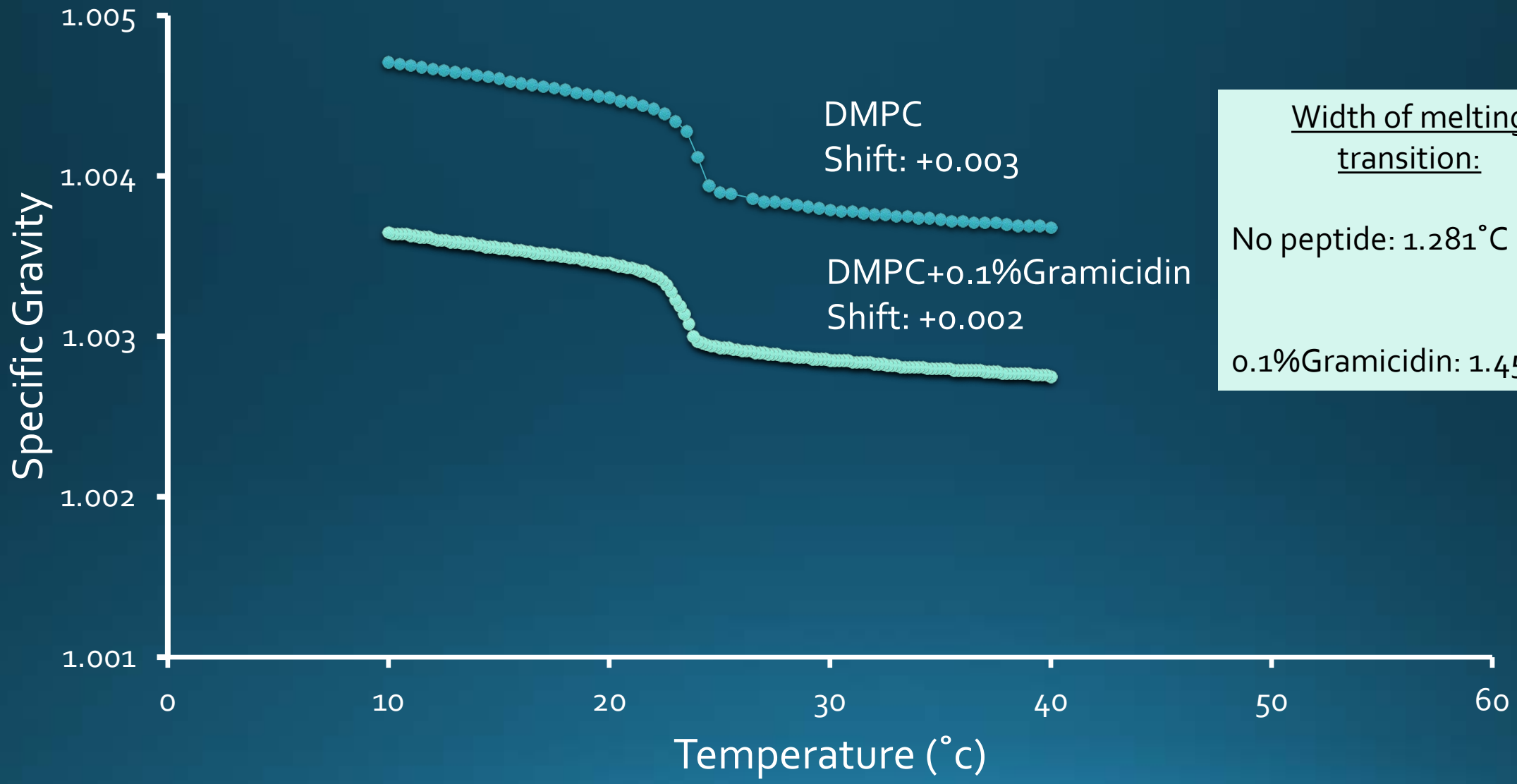
gel fluid



http://biowiki.ucdavis.edu/Biochemistry/Lipids/Dynamics_of_Membrane_Lipids/Lipid_Conformational_Transitions

- Identify melting transition temperature with 1st derivative
- Measure width of melting transition with full width half max

DMPC and Gramicidin Densities

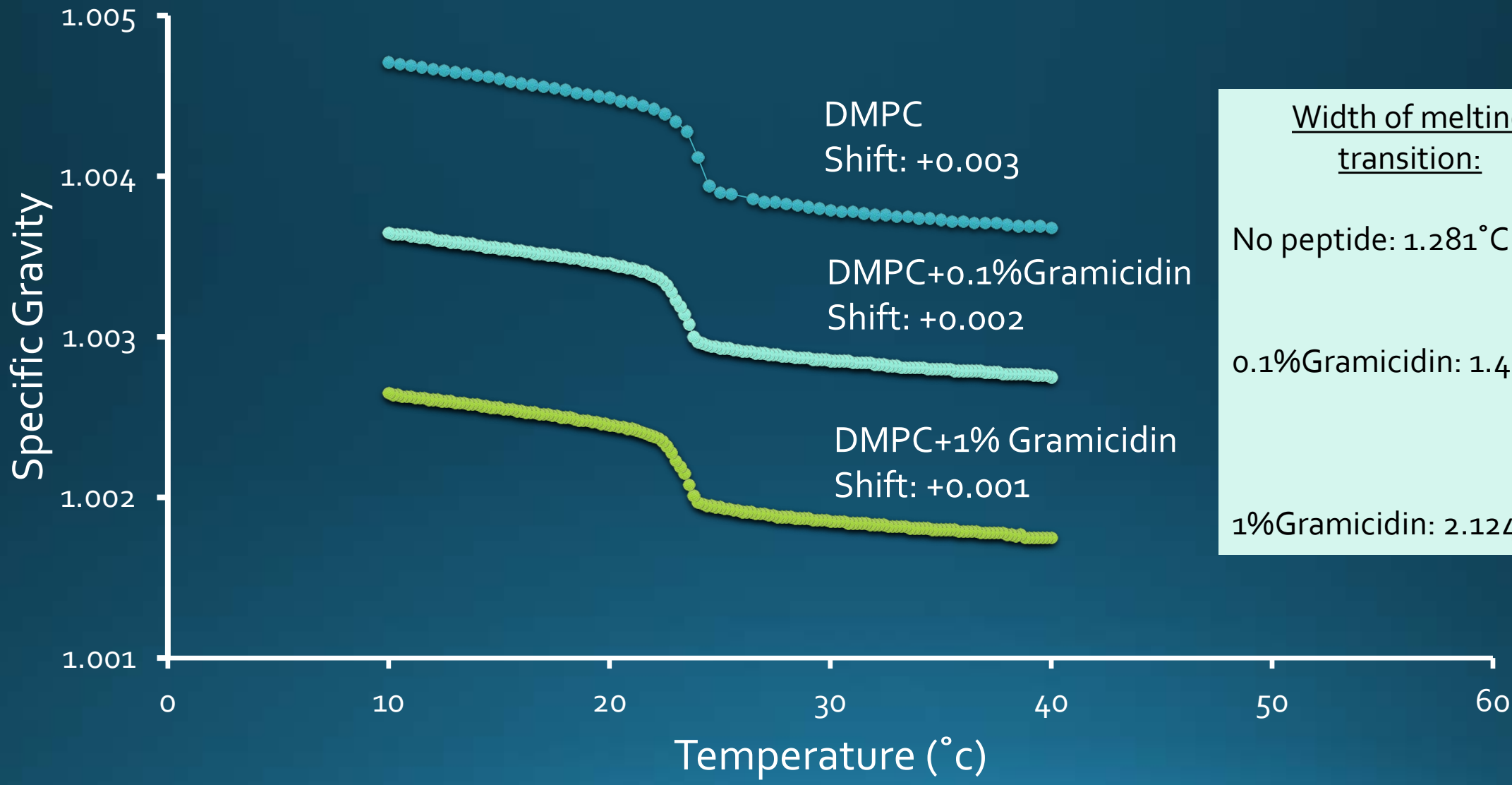


Width of melting transition:

No peptide: 1.281˚C

0.1%Gramicidin: 1.453 ˚C

DMPC and Gramicidin Densities



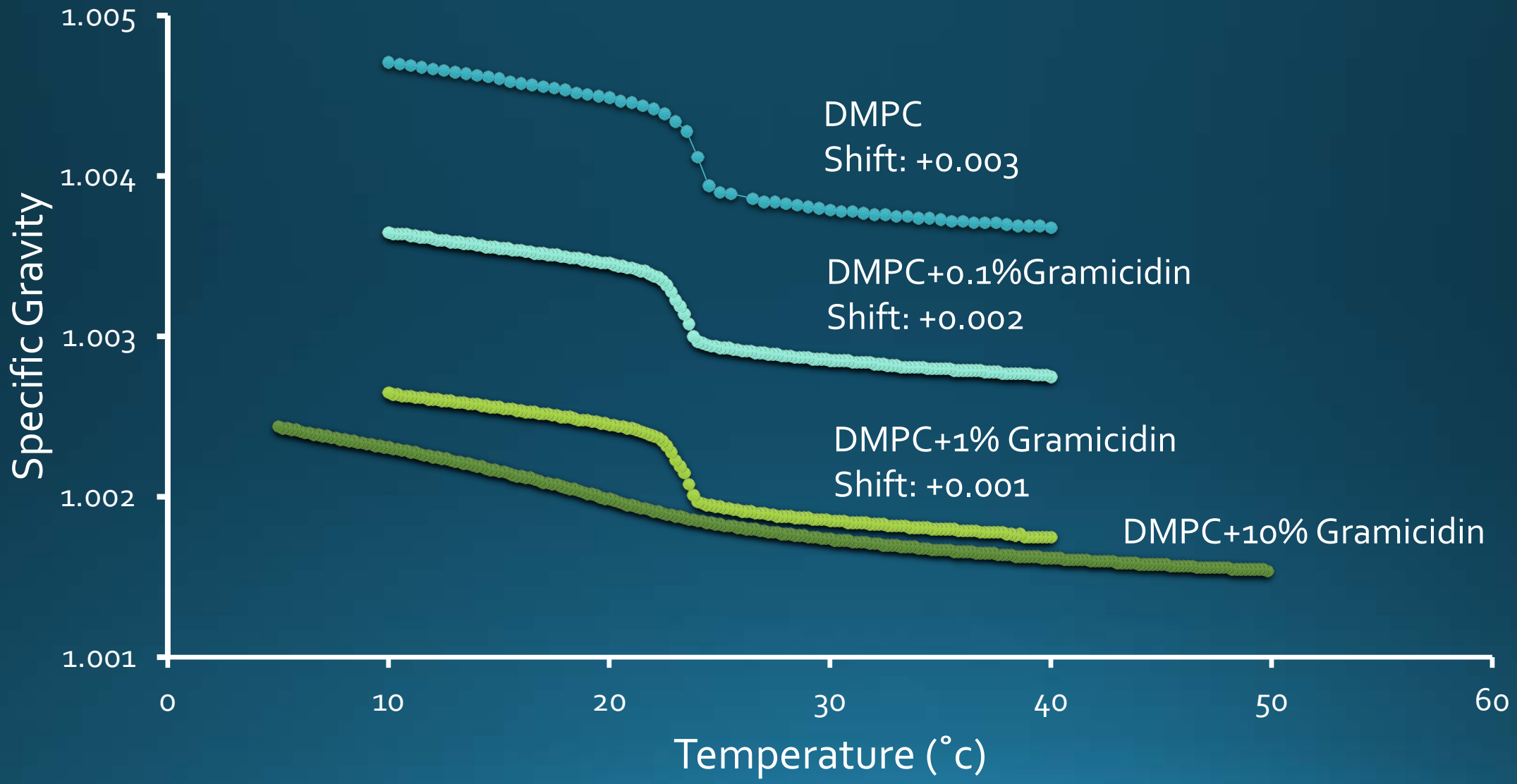
Width of melting transition:

No peptide: 1.281˚C

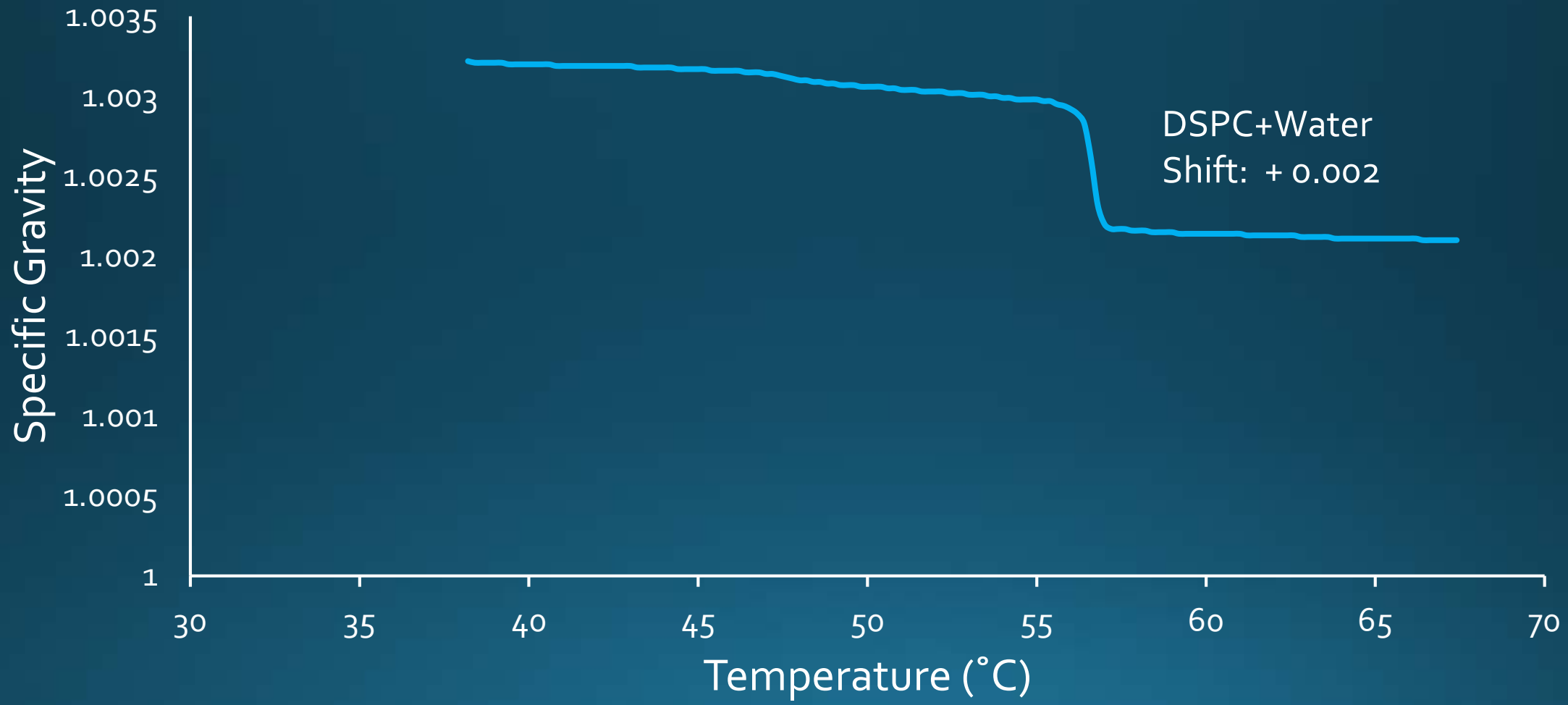
0.1%Gramicidin: 1.453 ˚C

1%Gramicidin: 2.124 ˚C

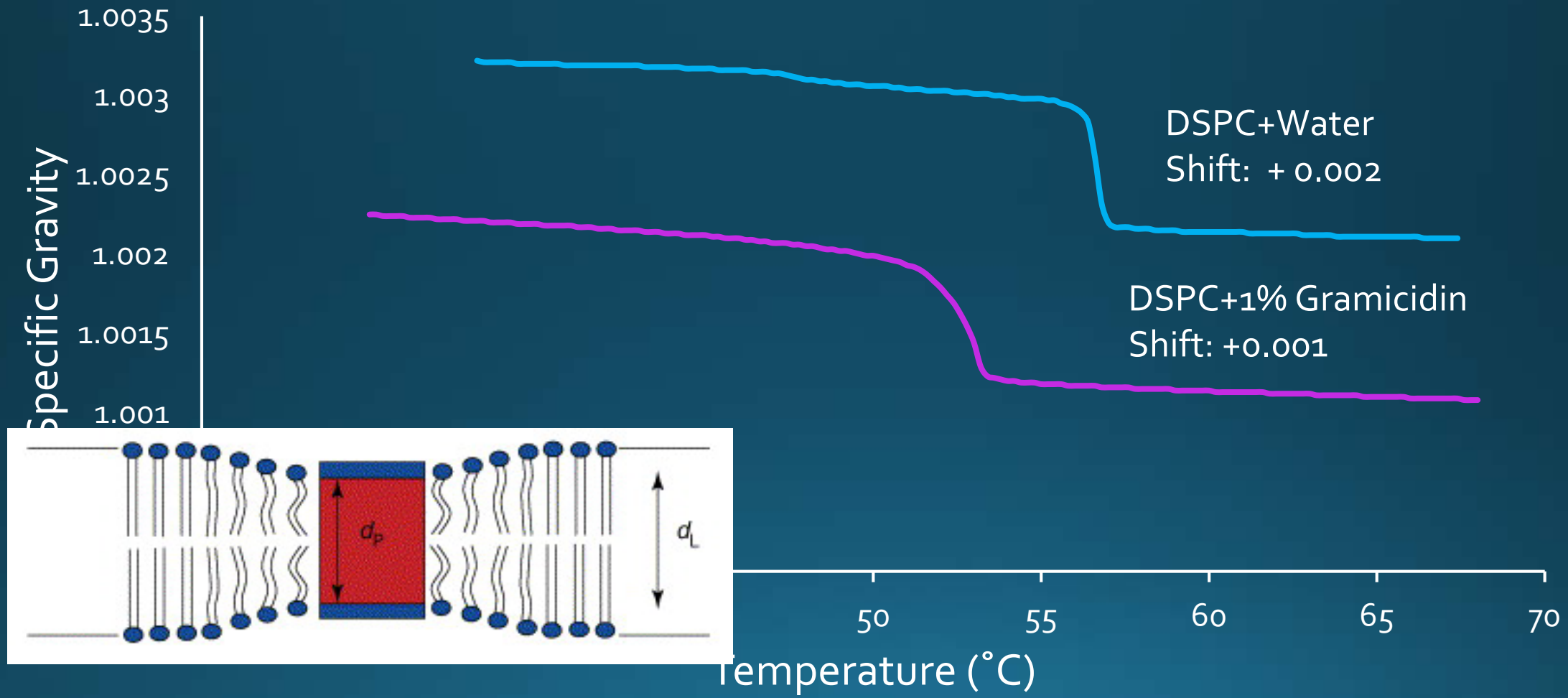
DMPC and Gramicidin Densities



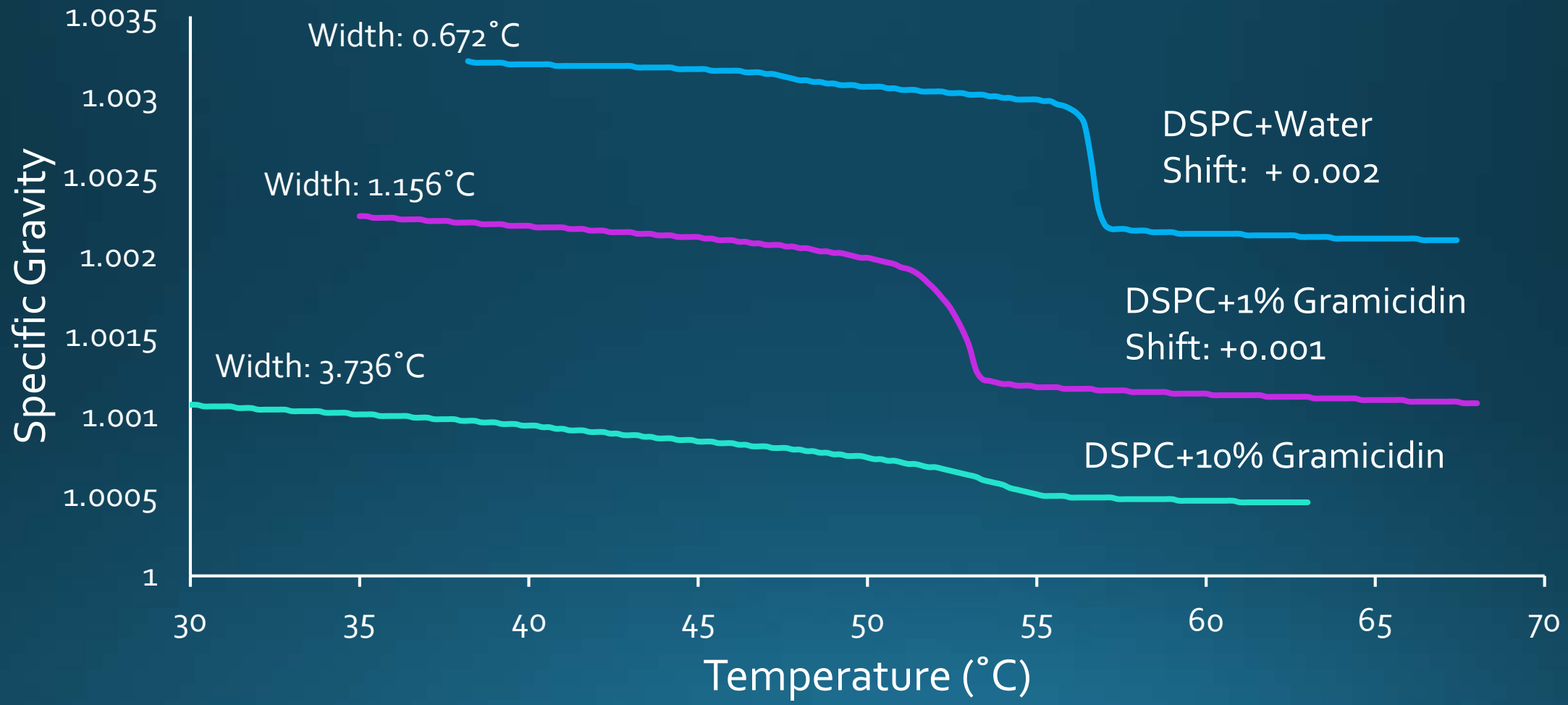
DSPC and Gramicidin Densities



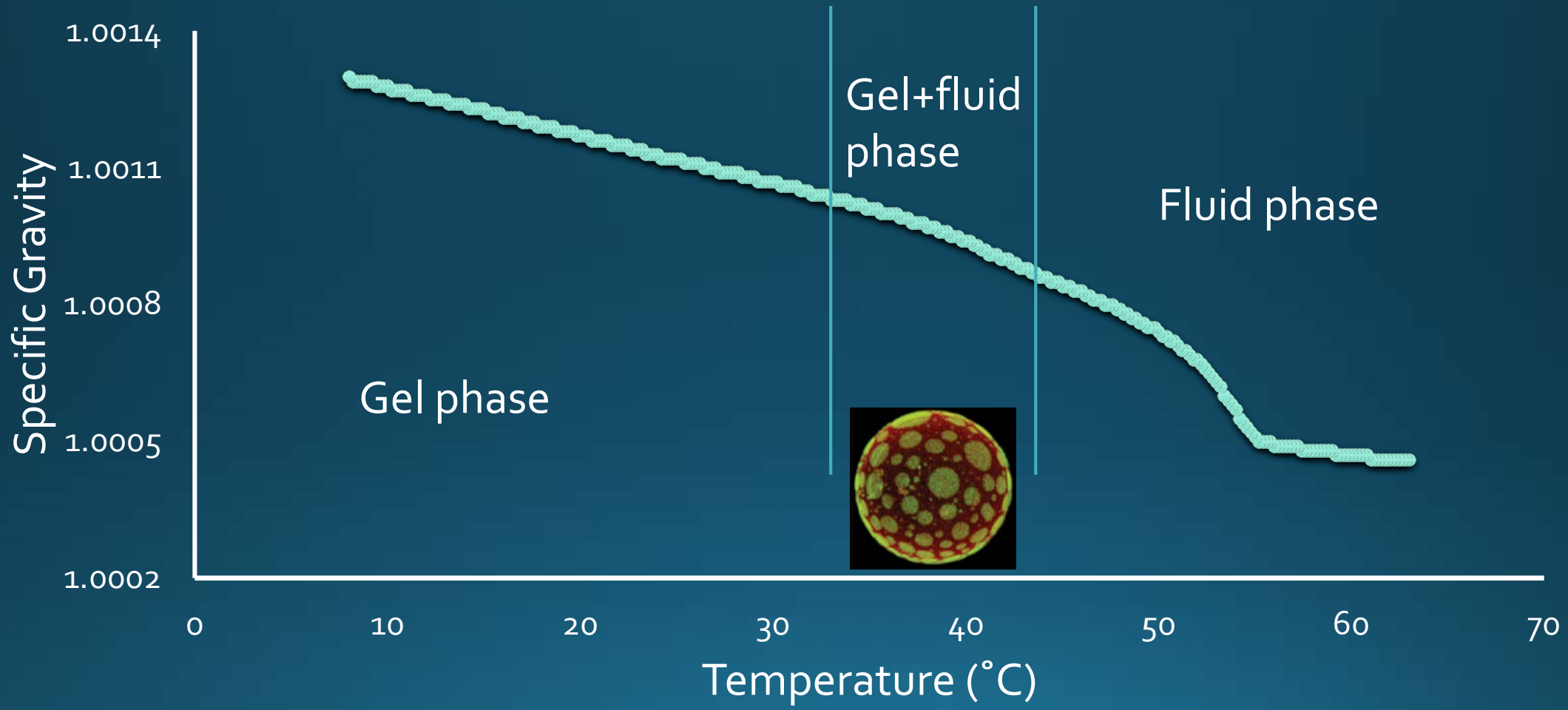
DSPC and Gramicidin Densities



DSPC and Gramicidin Densities

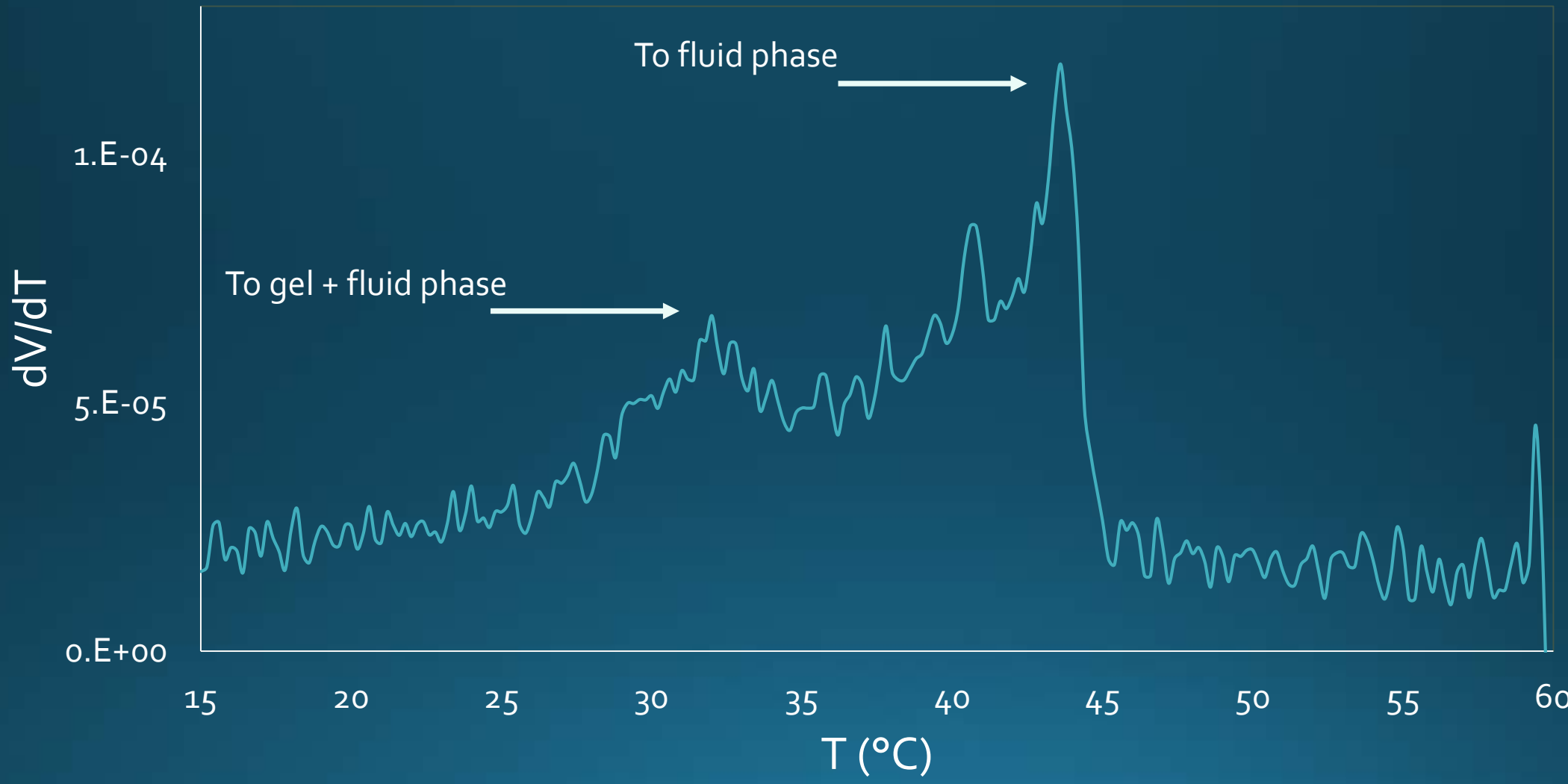


Density of DMPC+DSPC Mixture

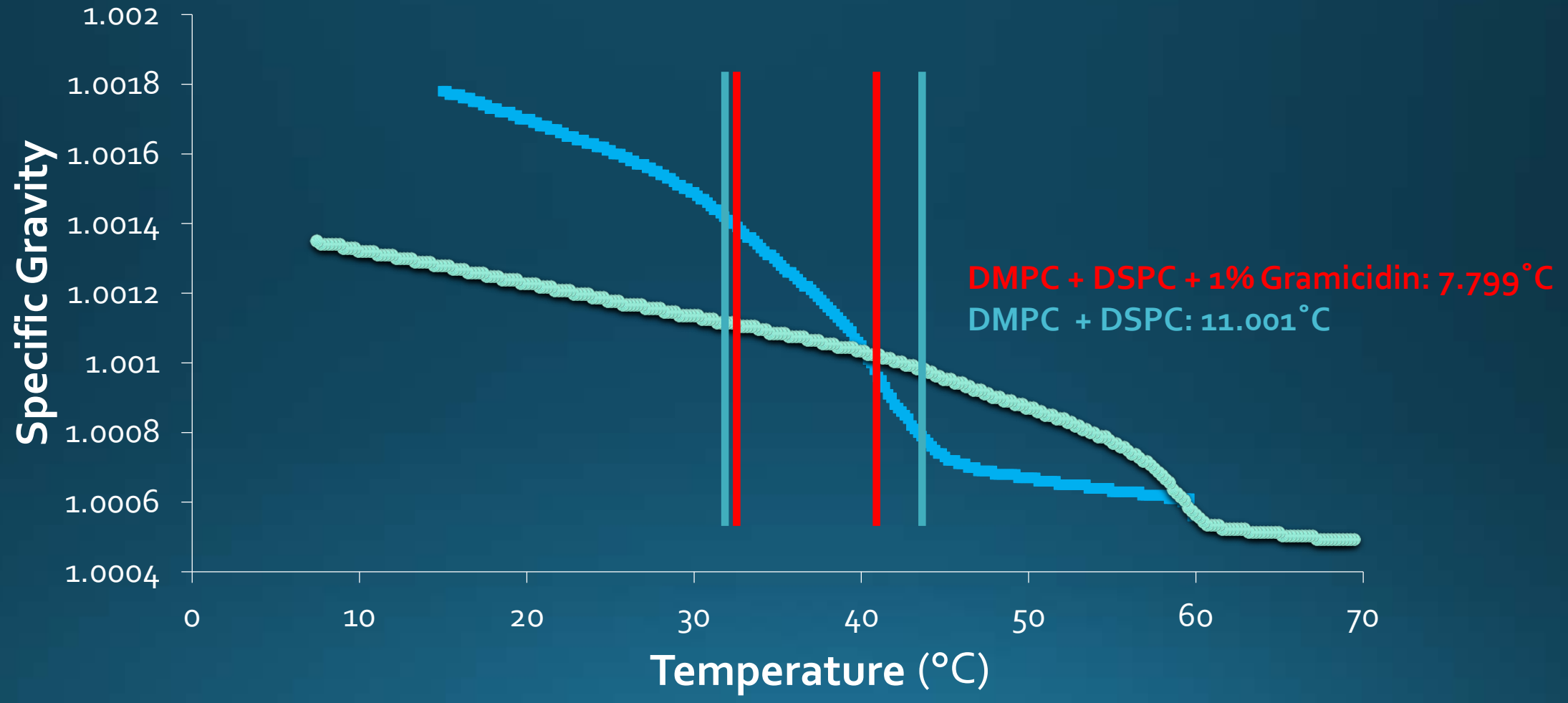


http://dbpdra1nk4ncl.cloudfront.net/content/ppebio/57/1/F5.large.jpg

Derivative of DMPC + DSPC



Density of DMPC + DSPC + 1% Gramicidin

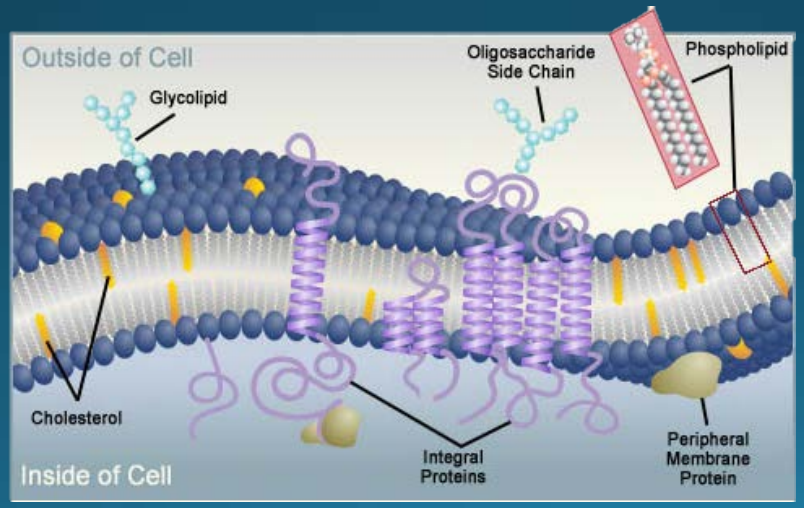


Conclusion

- The DMPC and DSPC solutions have one phase transition, from gel to fluid phase, that becomes broader as peptide concentration increases
- Mixtures of DMPC and DSPC exhibit two phase transitions: one from the gel to gel+fluid phase, where DSPC is still in gel phase, and from gel+fluid phase to fluid phase, where all components of the solution have melted
- The period of the gel+fluid phase is shorter for the solution with gramicidin, indicating the effect of peptide in solution

Future Work

- Observing the location of the peptides using small angle neutron scattering
- Studying different combinations of lipids and peptides and increasing the concentration of the peptide even more



Acknowledgments

- Elizabeth Kelley, NCNR
- NIST, NCNR, CHRNS
- Dr. Miller, Mr. Lee, Poolesville HS

Questions?