

Effect of lipid composition on peptide-induced coalescence in bicellar mixtures

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Protein-Lipid Interactions

Perturbation of bilayers by peptides can be important in many biological contexts. For example:

Lung surfactant
Antimicrobial activity

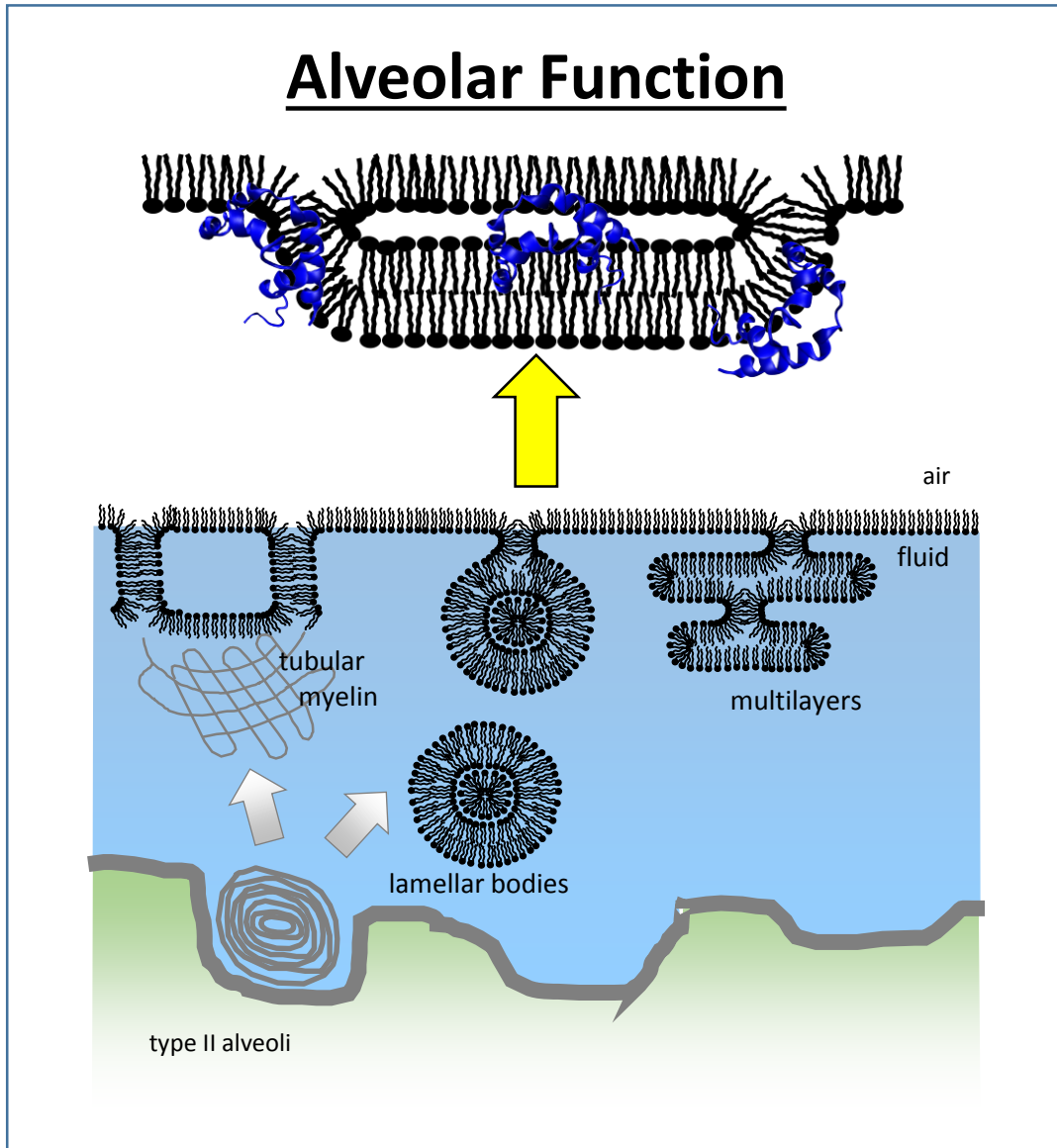
This Study:

- Compare effects of a lung surfactant peptide fragment and an antimicrobial peptide on a membrane model system (bicellar dispersions) whose organization is very sensitive to perturbing interactions

Ask:

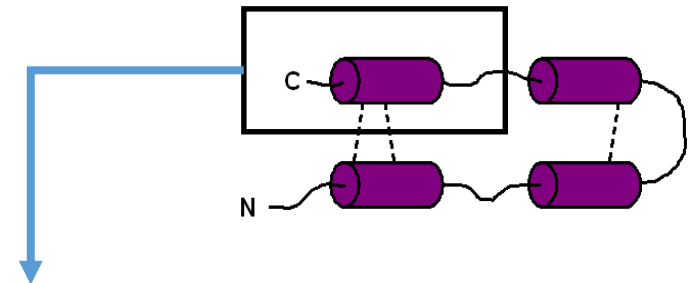
- Do two peptides (both cationic and amphipathic but apparently different functionally) perturb the model system differently?
- Is the observed perturbation sensitive to bilayer composition (i.e. anionic lipid content)?

Surfactant Protein B



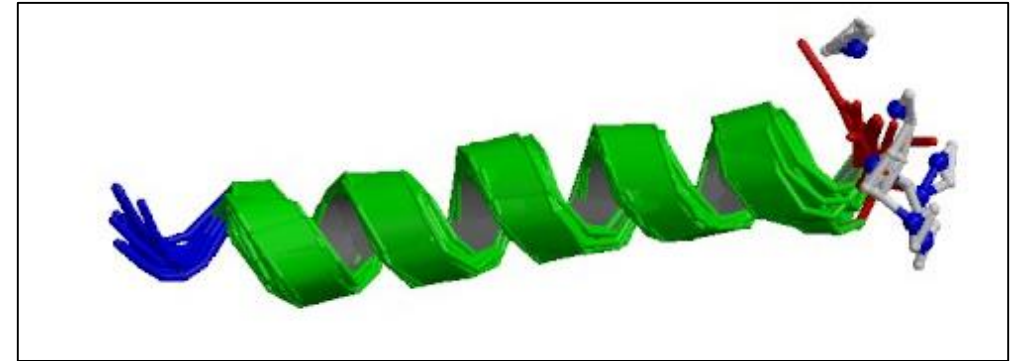
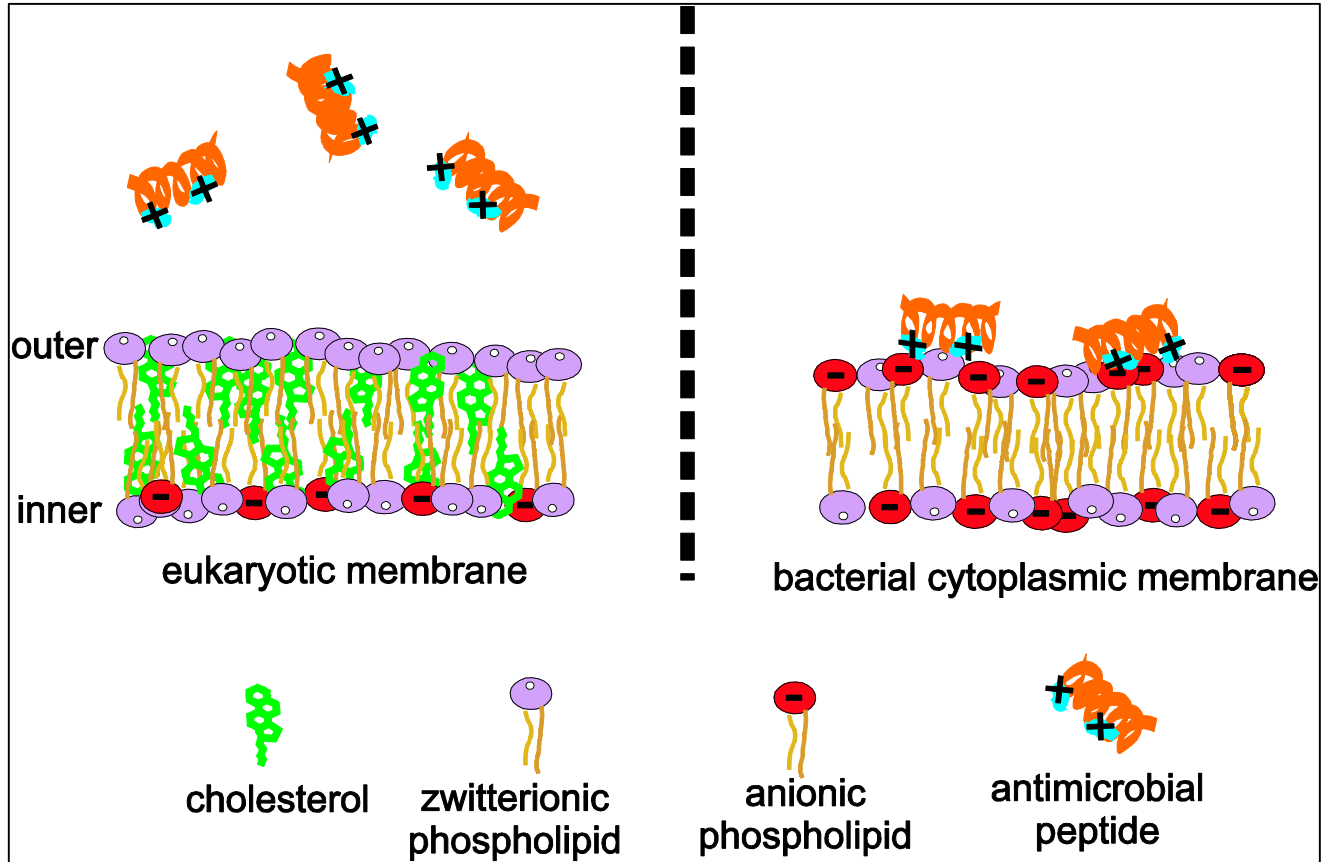
Surfactant Protein B

- SP-B:
 - 79 residue monomer
 - Cationic (+7), amphipathic, mainly hydrophobic
- SP-B_{C-TERM}
 - Amphipathic α -helix
 - Cationic (+3), mainly hydrophobic
 - Retains some functionality of full length SP-B



$\text{H}_3\text{N}^+ - \text{GR}^+ \text{MLPQLVCR}^+ \text{LVLR}^+ \text{CS} - \text{COO}^-$

Magainin 2



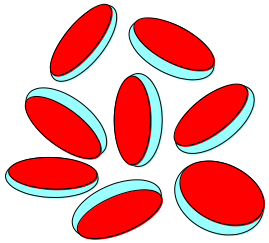
- Amphipathic α -helix
 - Cationic (+4) and hydrophobic
- Suggested to disrupt membrane via pore formation

PDBID: 2MAG

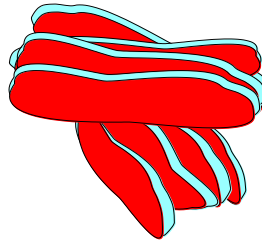
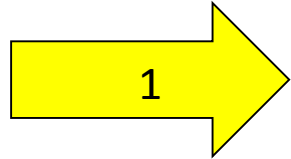
Gesell, J. . Z. M. . O. S. J. (1997). Two-dimensional 1H NMR experiments show that the 23-residue magainin antibiotic peptide is an alpha-helix in dodecylphosphocholine micelles, sodium dodecylsulfate micelles, and trifluoroethanol/water solution. *J.Biomol.NMR*, 9, 127–135.

Bicellar Mixtures

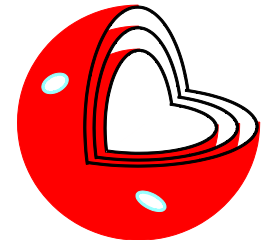
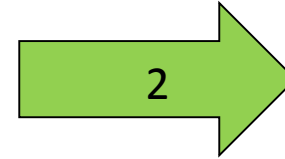
- Mixtures of long chain and short chain lipids
- Coalesce to larger structures upon warming



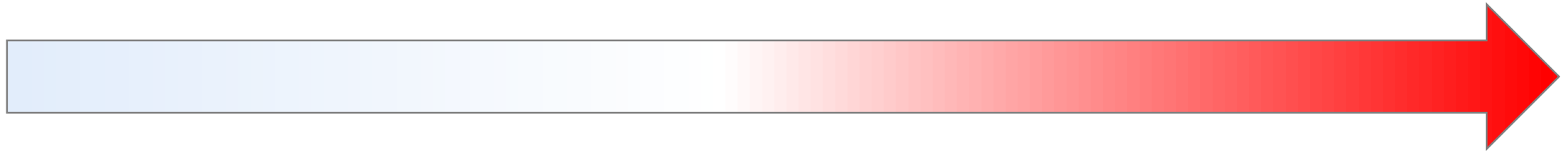
Isotropic Phase



Magnetically Oriented Phase



Extended Lamellar Phase



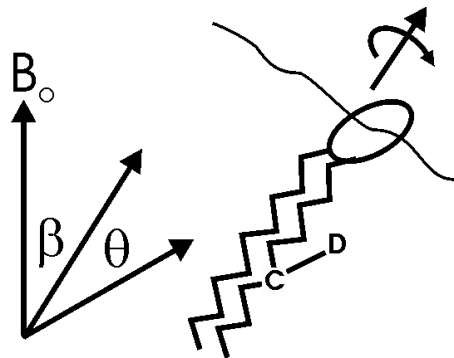
Low Temperature

High Temperature

^2H NMR of Bicelles

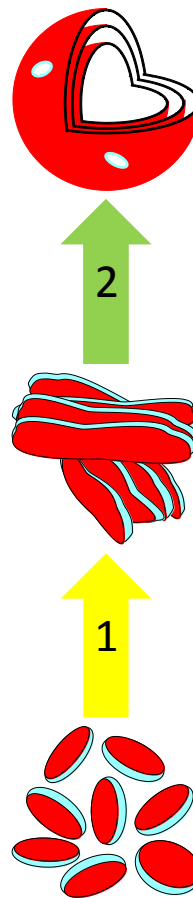
^2H NMR

Deuteron splittings reflect motional averaging of orientational dependent quadrupole interaction



$$\Delta\nu_q = \frac{3 e^2 q Q}{2 h} \left(\frac{3 \cos^2 \beta - 1}{2} \right) S_{CD}$$

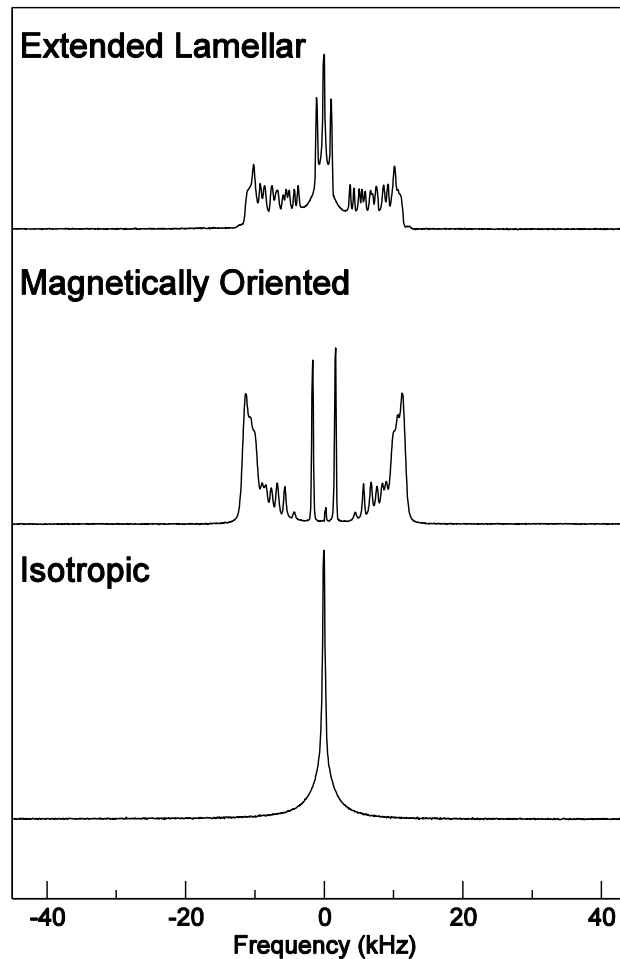
^2H NMR distinguishes bicelle phases



Extended Lamellar

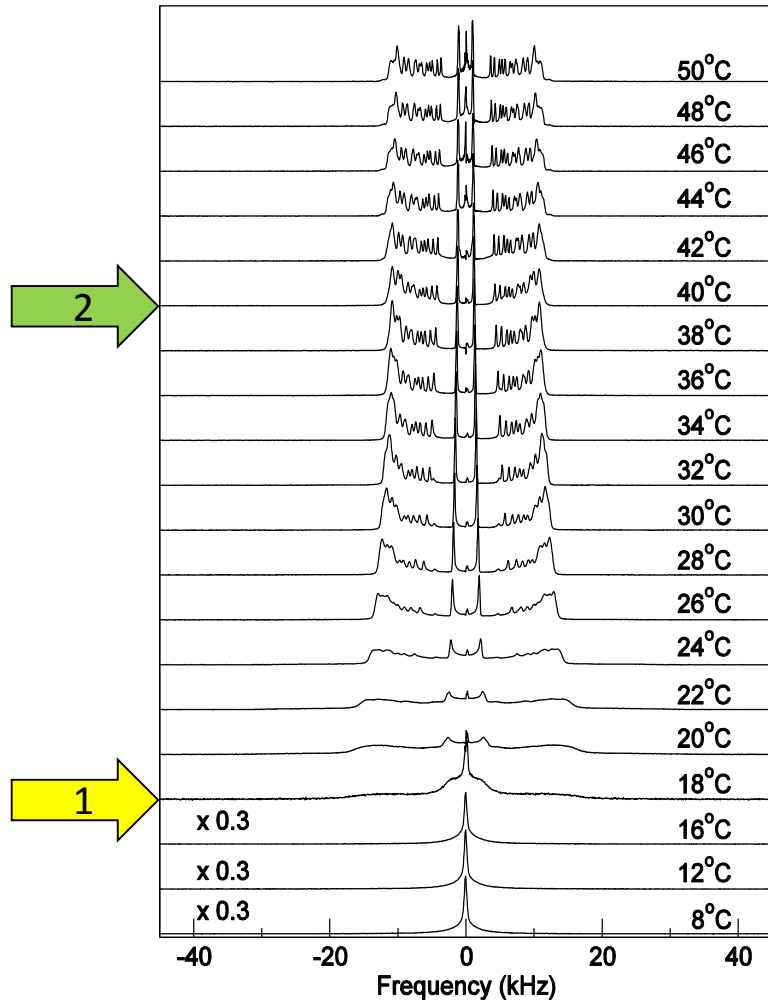
Magnetically Oriented

Isotropic

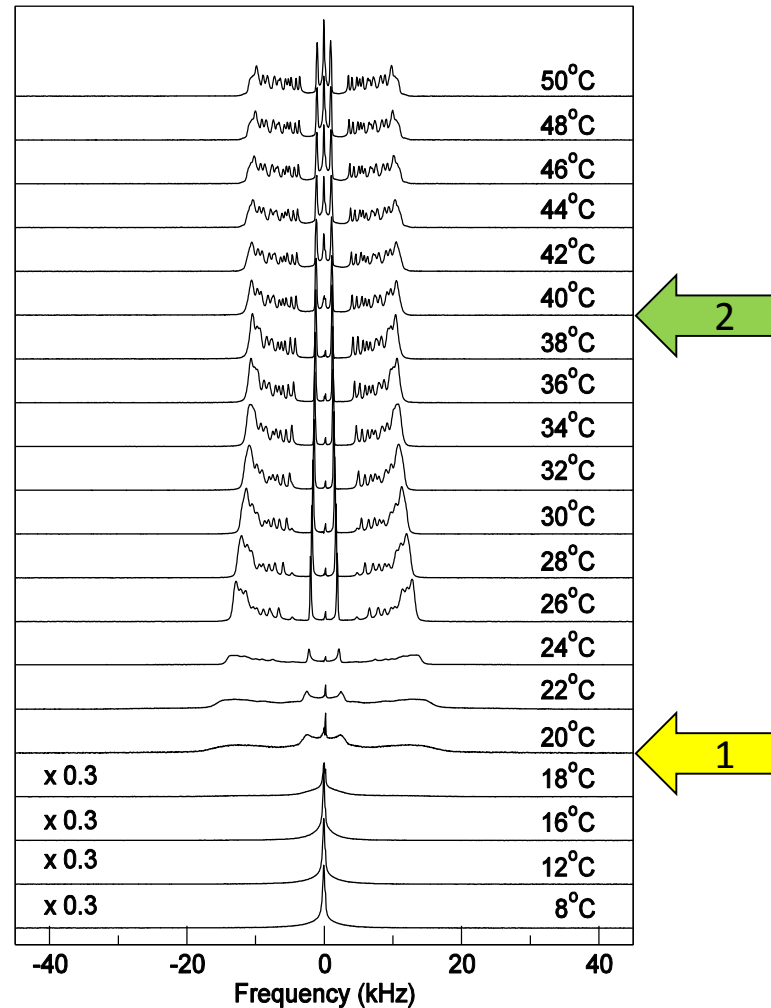


Zwitterionic Bicellar Mixtures with/without SP-B_{C-TERM}

DMPC-*d*₅₄/DMPC/DHPC (3:1:1)



DMPC-*d*₅₄/DMPC/DHPC (3:1:1)
With 10% SP-B_{C-TERM} (w/w)

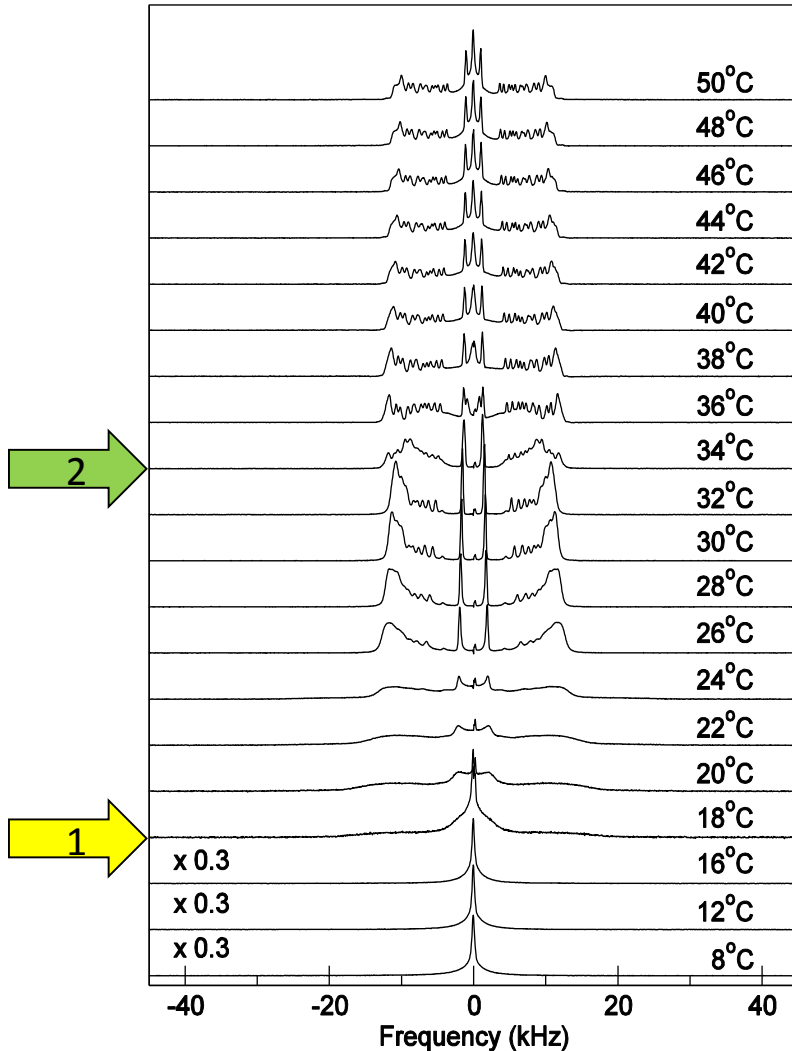


DMPC-*d*₅₄ /DHPC

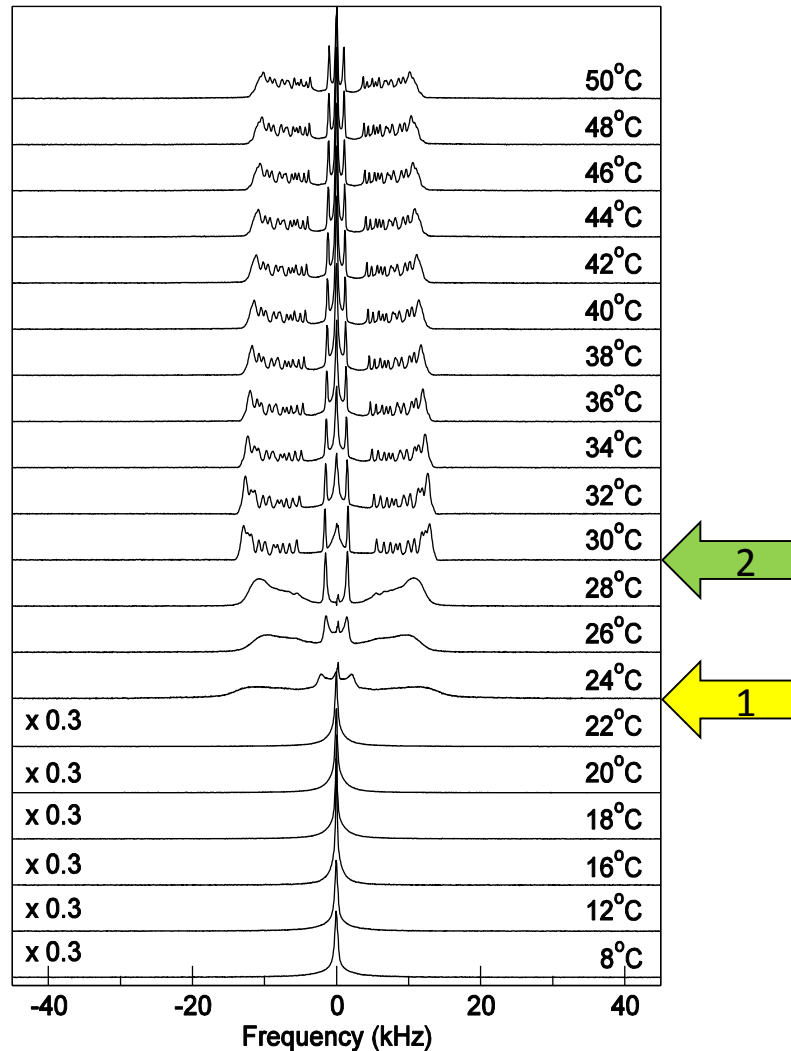
- SP-B_{C-TERM} has little effect on transitions in mixtures of DMPC/DHPC (both zwitterionic)

SP-B_{C-TERM} and Bicellar Mixtures with Anionic Lipids

DMPC-*d*₅₄/DMPG/DHPC (3:1:1)



DMPC-*d*₅₄/DMPG/DHPC (3:1:1)
With 10% SP-B_{C-TERM} (w/w)

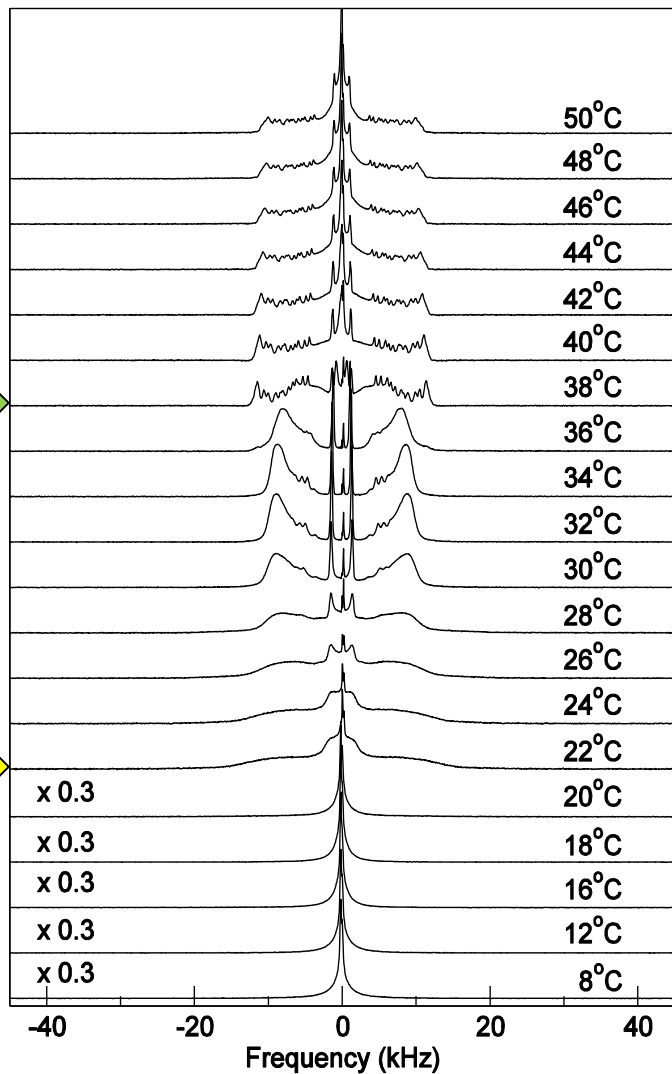


DMPC-*d*₅₄ /DMPG/DHPC

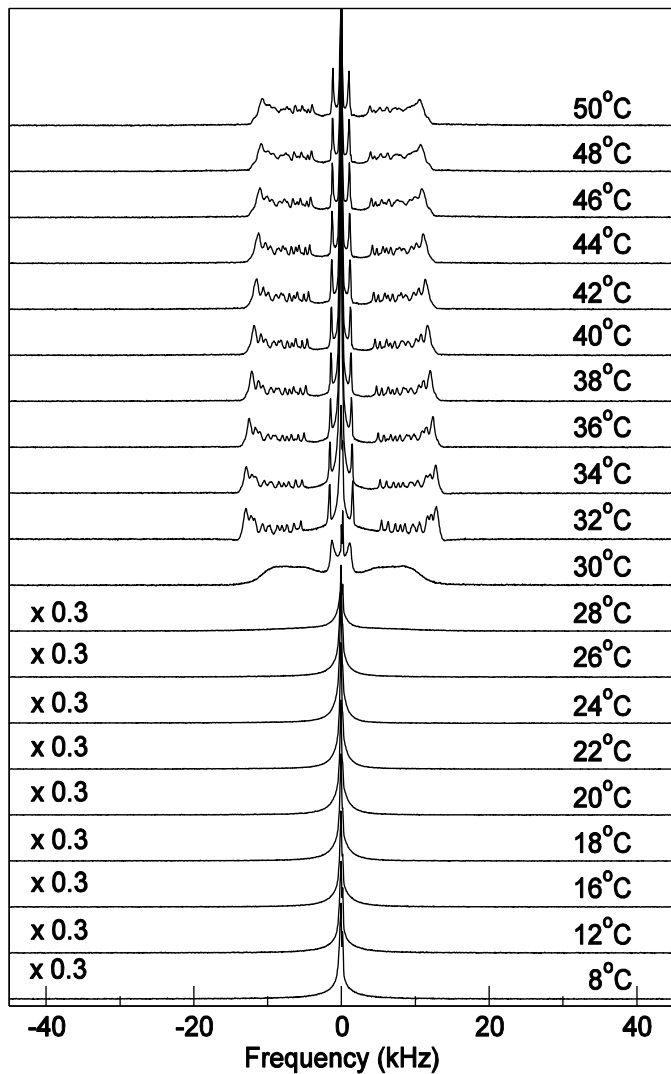
- In lipid-only bicellar mixtures, anionic lipid lowers and sharpens the ribbon-to-extended lamellar transition.
- SP-B_{C-TERM} further lowers the ribbon-to-extended lamellar transition
- Implies that SP-B_{C-TERM} depends on the presence of anionic lipids to promote ribbon-micelle coalescence

Does SP-B_{C-TERM} segregate DMPG?

DMPC/DMPG-*d*₅₄/DHPC (3:1:1)



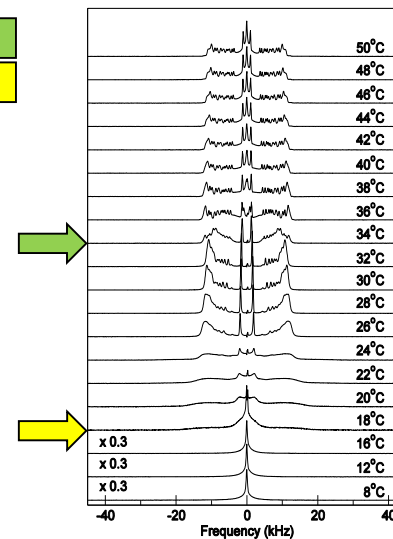
DMPC/DMPG-*d*₅₄/DHPC (3:1:1)
With 10% SP-B_{C-TERM} (w/w)



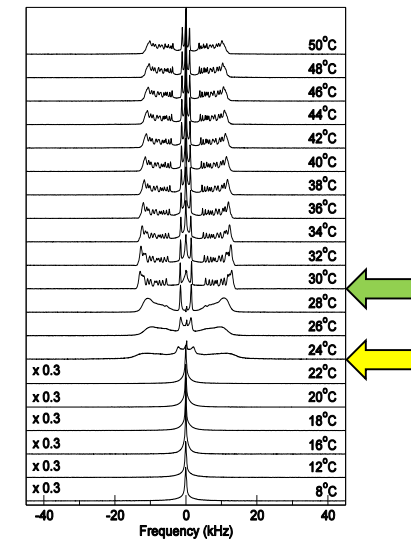
DMPC /DMPG-*d*₅₄/DHPC

- DMPG-*d*₅₄ and DMPC-*d*₅₄ report similar behaviours

DMPC-*d*₅₄/DMPG/DHPC (3:1:1)



DMPC-*d*₅₄/DMPG/DHPC (3:1:1)
With 10% SP-B_{C-TERM} (w/w)



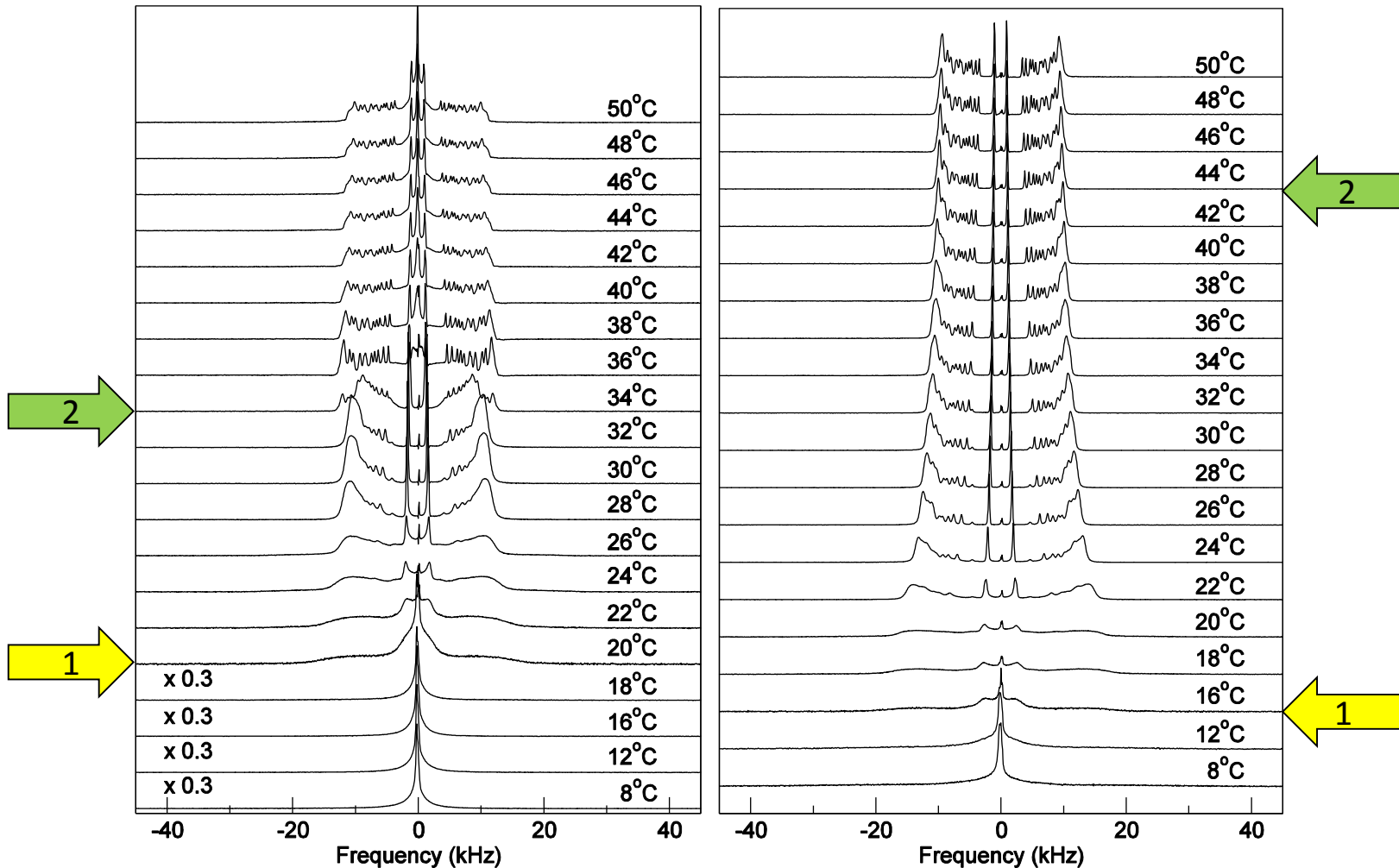
How does Magainin 2 affect bicellar phase behaviour?

DMPC-*d*₅₄/DMPG/DHPC (3:1:1)

DMPC-*d*₅₄/DMPG/DHPC (3:1:1)
With 10% Magainin 2 (w/w)

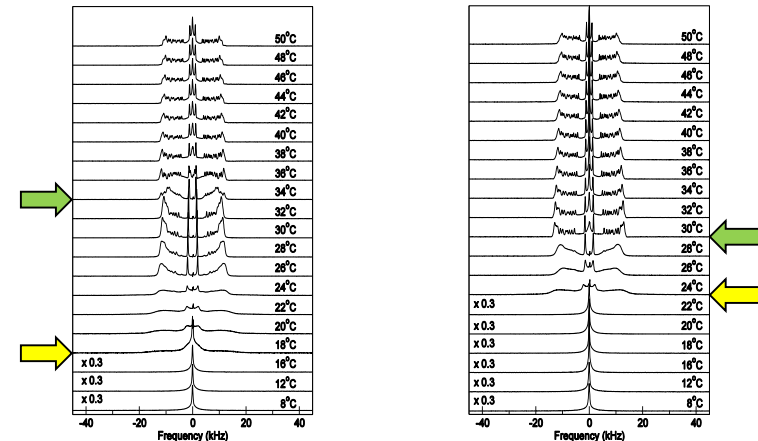
DMPC-*d*₅₄ /DMPG/DHPC

- Addition of Magainin 2 appeared to reverse the effect of DMPG on bicellar mixture phase behaviour
 - Spectra similar to DMPC-*d*₅₄/DHPC (4:1) bicelles
- Peptide-induced segregation of DMPG?



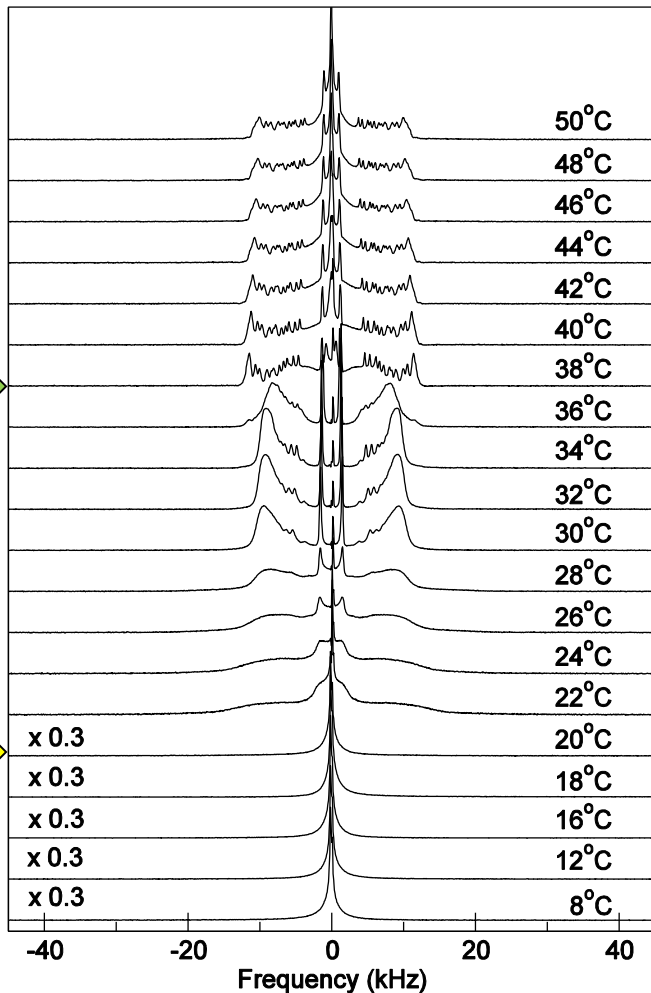
DMPC-*d*₅₄/DMPG/DHPC (3:1:1)

DMPC-*d*₅₄/DMPG/DHPC (3:1:1)
With 10% SP-B_{C-TERM} (w/w)

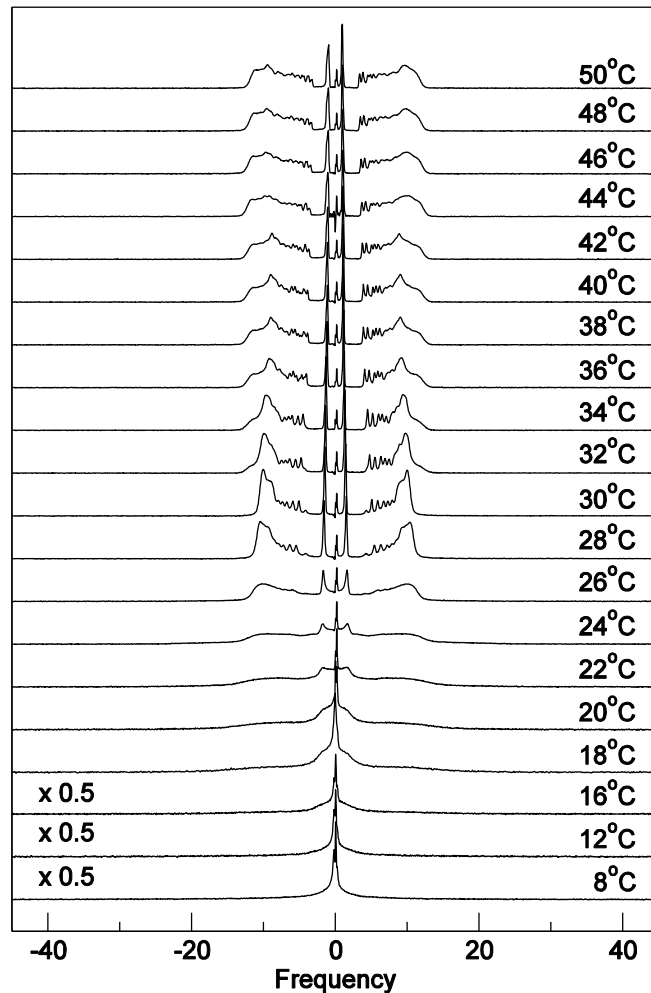


DMPC and DMPG appear to be in different environments in bicellar mixtures with Magainin 2

DMPC/DMPG- d_{54} /DHPC (3:1:1)



DMPC/DMPG- d_{54} /DHPC (3:1:1)
With 10% Magainin 2 (w/w)



DMPC /DMPG- d_{54} /DHPC

- Spectra suggest that Magainin 2 interaction promotes a distinct environment for DMPG (anionic lipid)
- Peptide-induced clustering anionic lipids?

Conclusions

- Peptide-induced reorganization of bicellar mixtures appears to be facilitated by the presence of anionic lipid
- SP-B_{C-TERM}
 - Promotes ribbon-to-extended lamellar coalescence at a lower temperature when anionic lipids are present
 - Does not appear to involve separation of DMPC and DMPG
- Magainin 2
 - Appears to preferentially interact with anionic lipids
 - Spectra suggest separation of anionic lipids, possibly clustering

Acknowledgements

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