



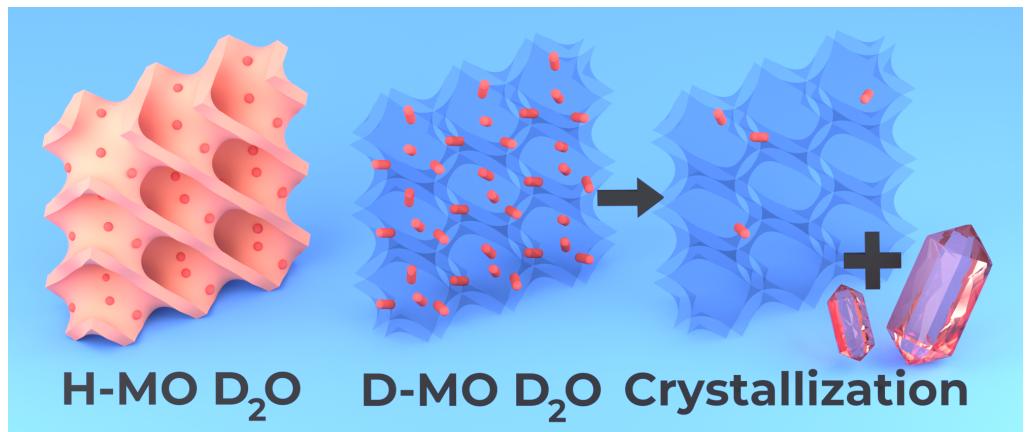
MONASH
University

Scattering studies on protein-lipid interactions for evolving biological, biomedical and food technology applications

Dr Leonie van 't Hag, Lecturer – Chemical & Biological Engineering

13 July 2022

SM^A2 Workshop

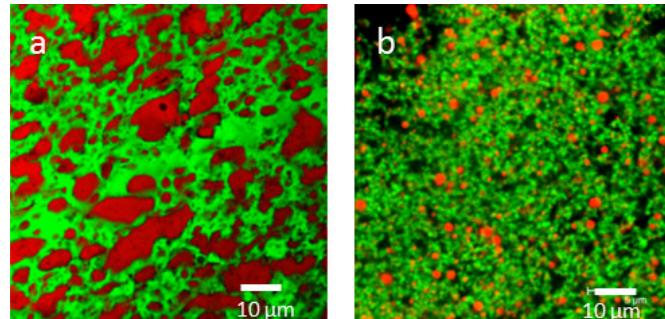
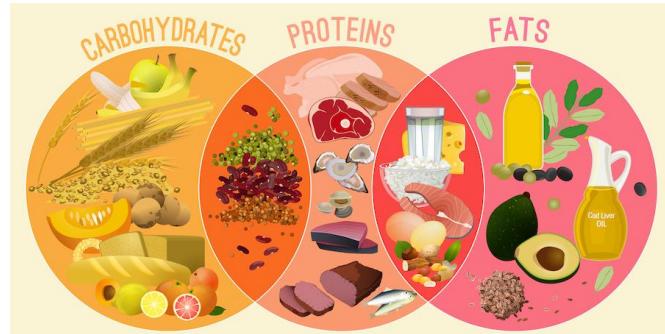


1. Peptide encapsulation: SAX/NS
2. Food Engineering
3. *In meso* crystallisation:
time-dependent measurements



Protein-lipid interactions important in 1) Biology,

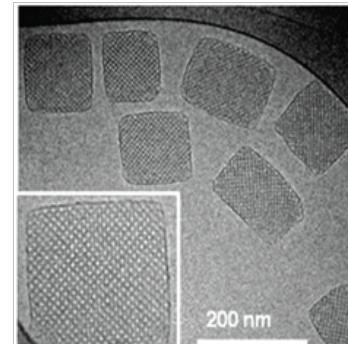
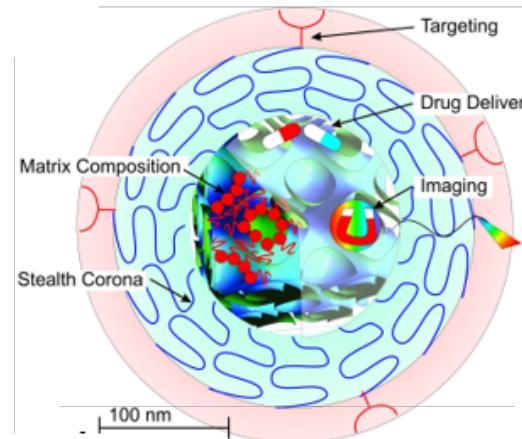
(2) Food technology



CLSM image (a) Cheddar cheese, (b) yoghurt. Fat (red), protein (green) and serum (black). Scale bars are 10 µm.

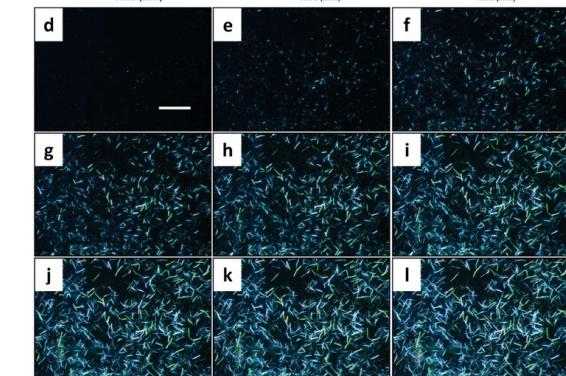
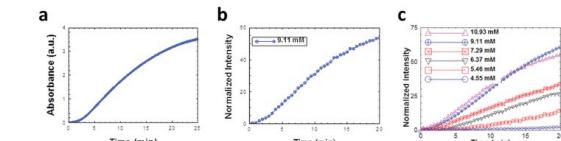
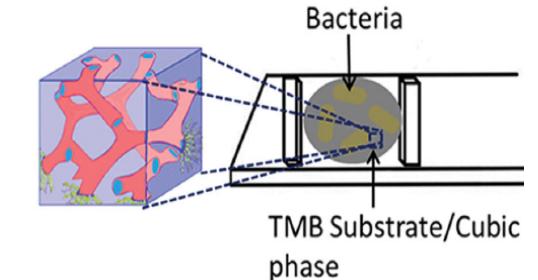
Soodam et al. *Food Structure* 2017, 14, 8–16.

3) Drug delivery



Mulet et al. *J. Colloid Interface Sci.* 2013, 393, 1–20.
Muir et al. *J. Phys. Chem. B* 2012, 116, 3551–3556.

4) Biosensors



Vallooran et al. *Adv. Funct. Mater.* 2016, 26, 181–190.

Membrane proteins are drug targets

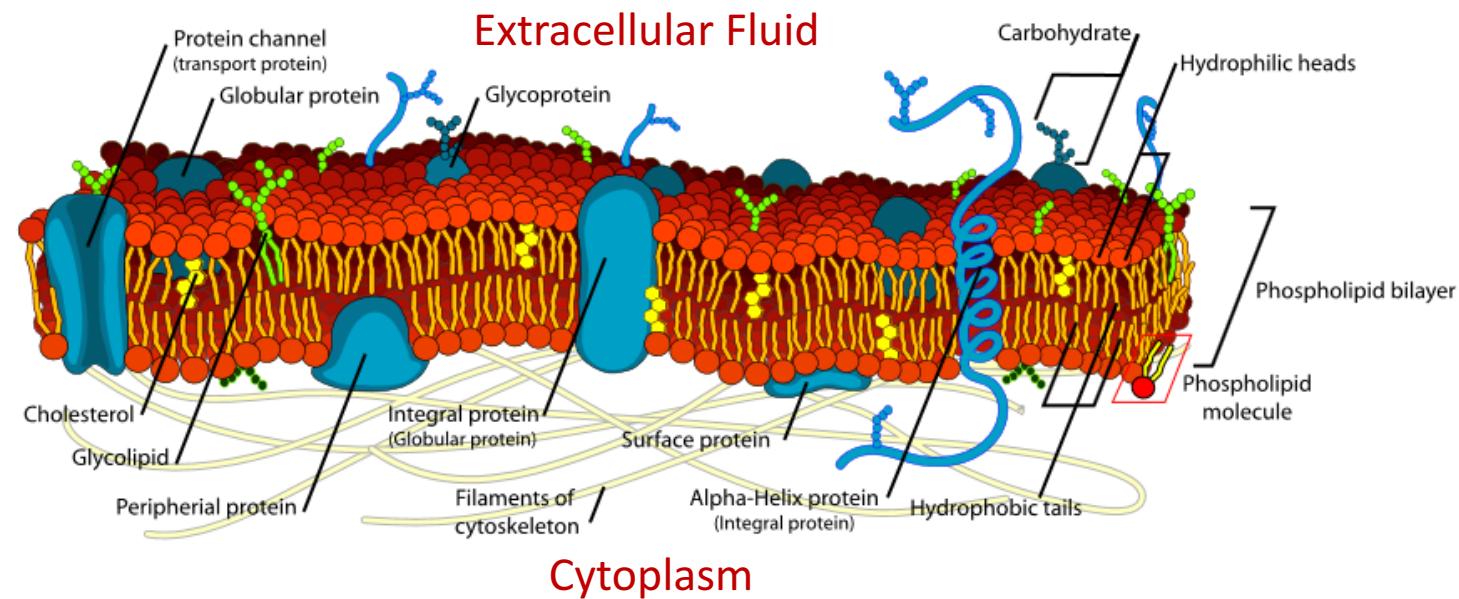
Membrane protein functions

- Signal transduction
- Solute transport
- Conversion of energy

Important drug targets

- Parkinson's disease
- Tourette's syndrome
- Sleep disorders

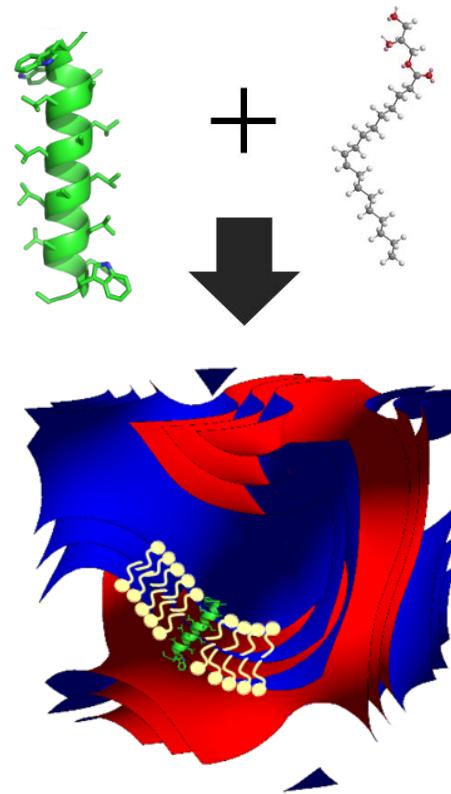
~60% of pharmaceutical compounds on the market target membrane proteins



[1] Landau and Rosenbusch, 1996. [2] Image credit: Mariana Ruiz Villarreal. [3] Service, 2014.

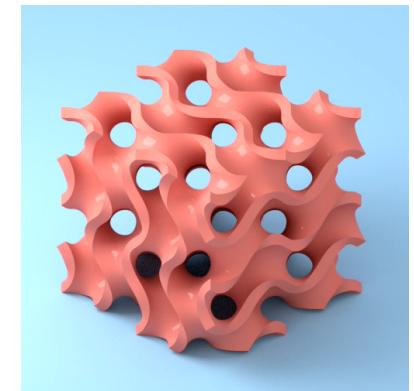
Membrane proteins in native state

Protein/peptide + *Lipid*



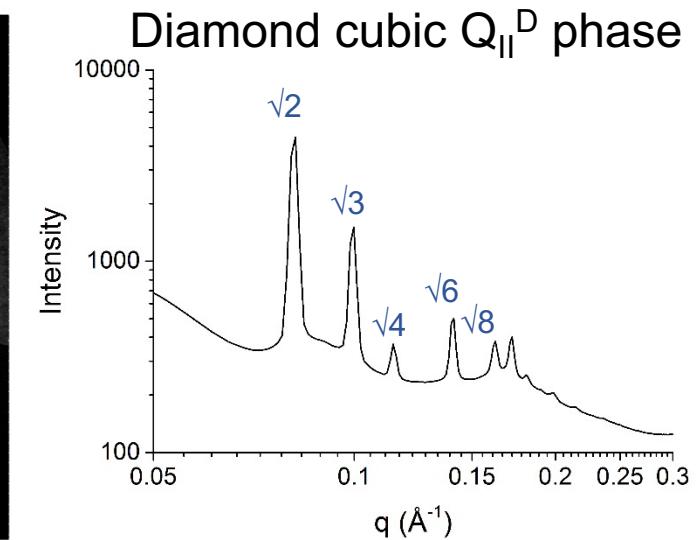
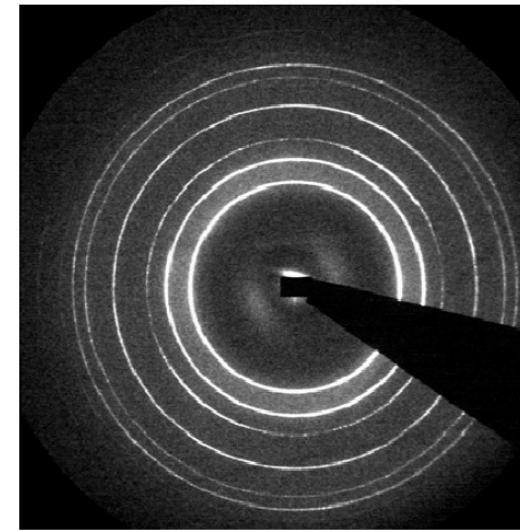
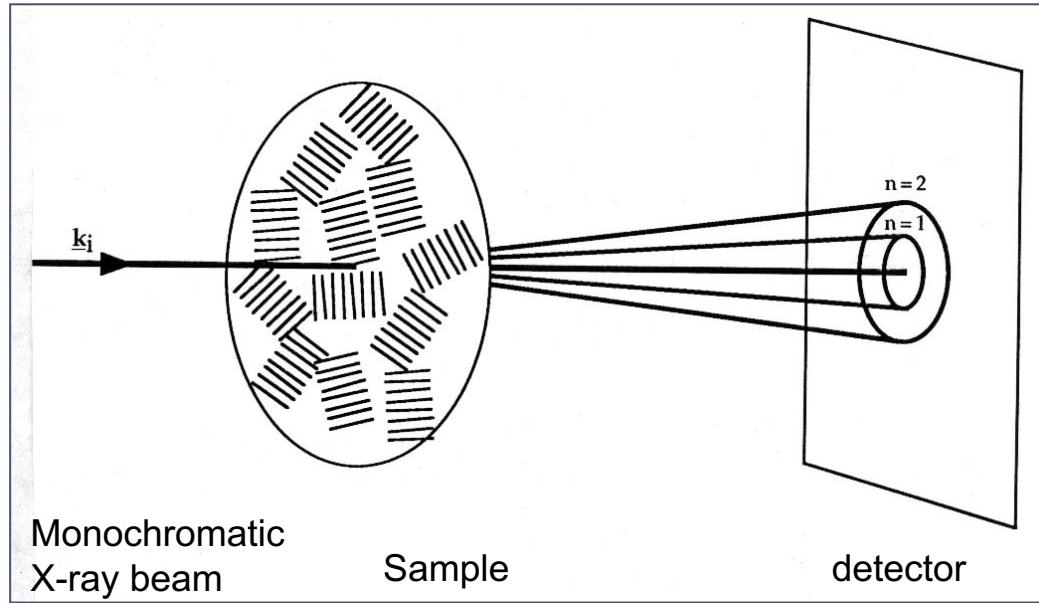
- Encapsulated in a membrane mimic
20 °C
>20% w/w H₂O

- Bicontinuous cubic phases
 - A single curved bilayer
 - Two separate networks of water channels
 - High surface area to volume ratios

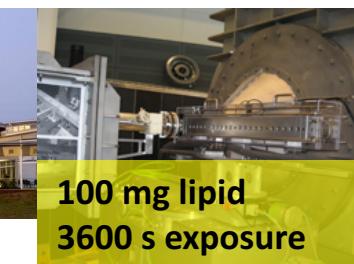


Luzzati et al. Nature 1968, 220, 485–488.

Small-angle scattering (SAXS and SANS)



Lipid bilayer thickness 32 \AA

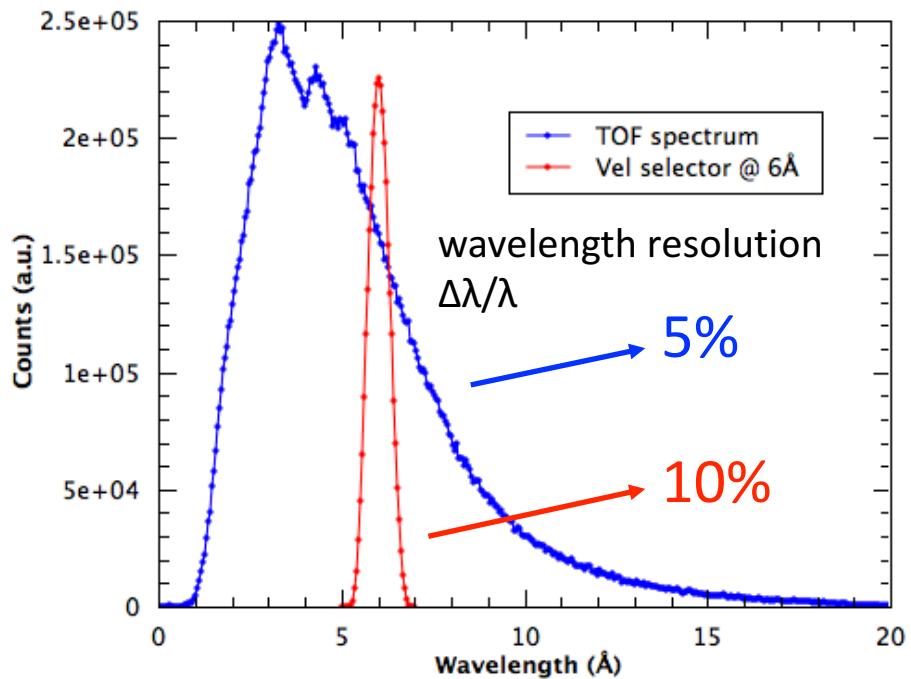


Ansto.gov.au

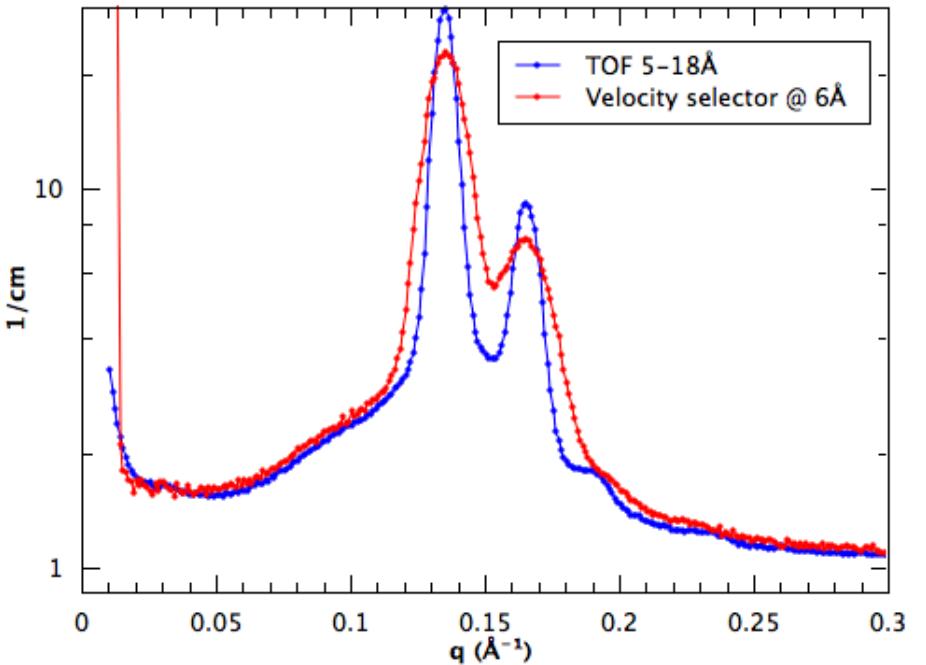
Time of Flight SANS (Bilby)



Bilby TOF Spectrum



Compare TOF - Velocity Selector



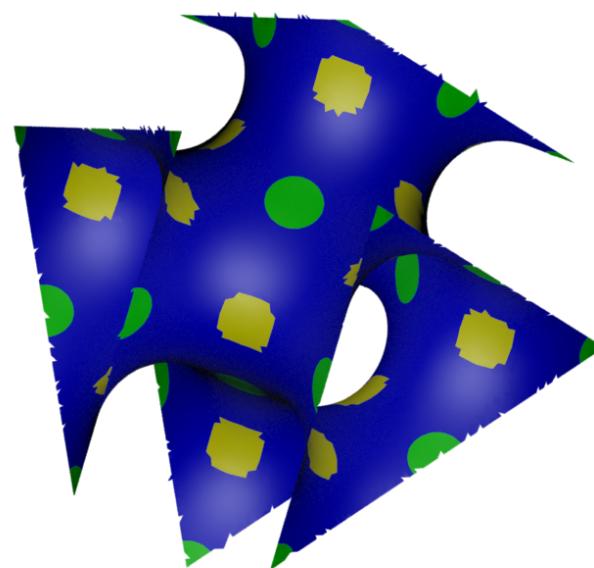
*flux is not displayed correctly

Lipid diamond cubic $Q_{||}^D$ phase

1. Varying Gaussian curvature: SANS

- Assembly of proteins or peptides at the **flat points** of the cubic phase shown to be energetically favorable, and **may drive crystallization**.
- The Gaussian curvature within the cubic phase varies:

Diamond ($Q_{||}^D$)



Points of highest Gaussian curvature

Mean curvature = 0

Gaussian curvature ≤ 0

$$I_2/I_1 Q_{||}^D \rightarrow 0$$

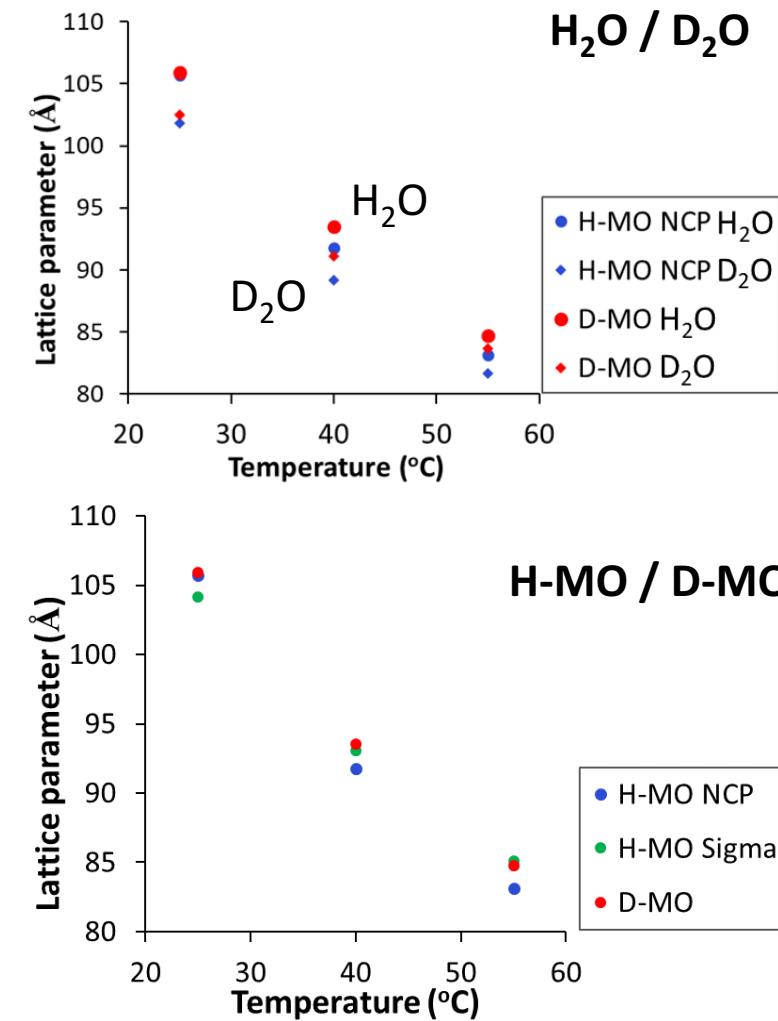
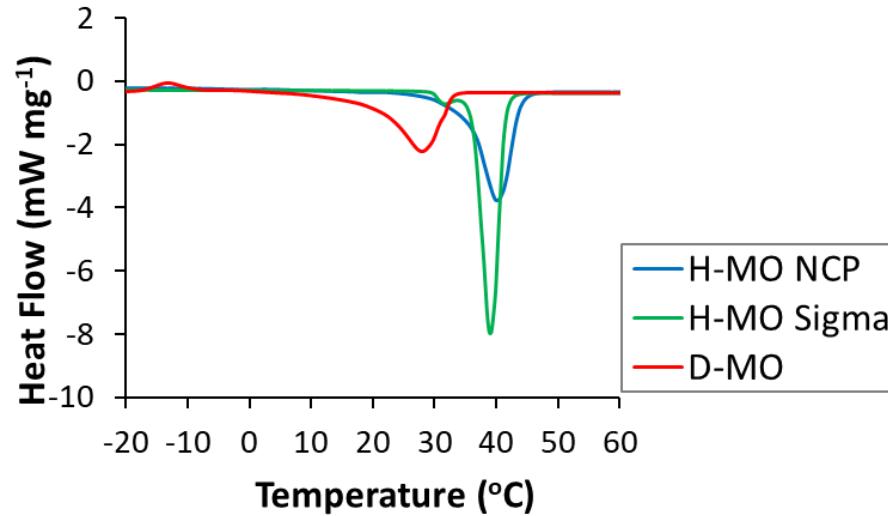
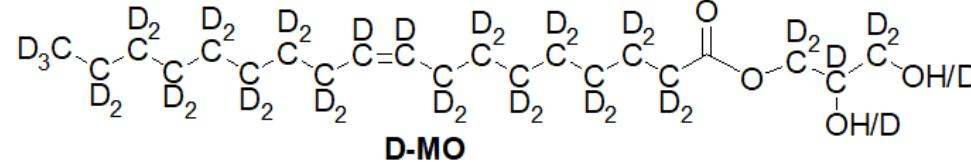
Flat points

Mean curvature = 0

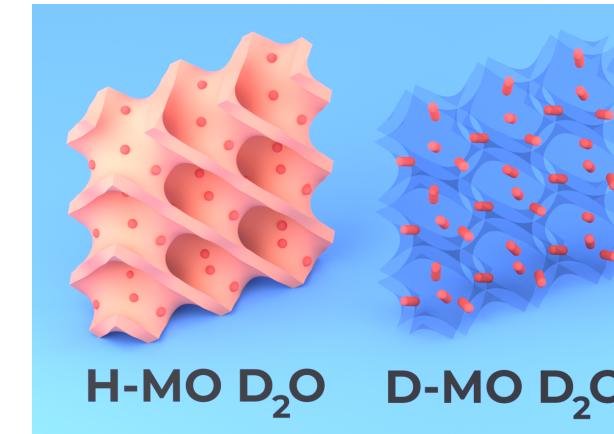
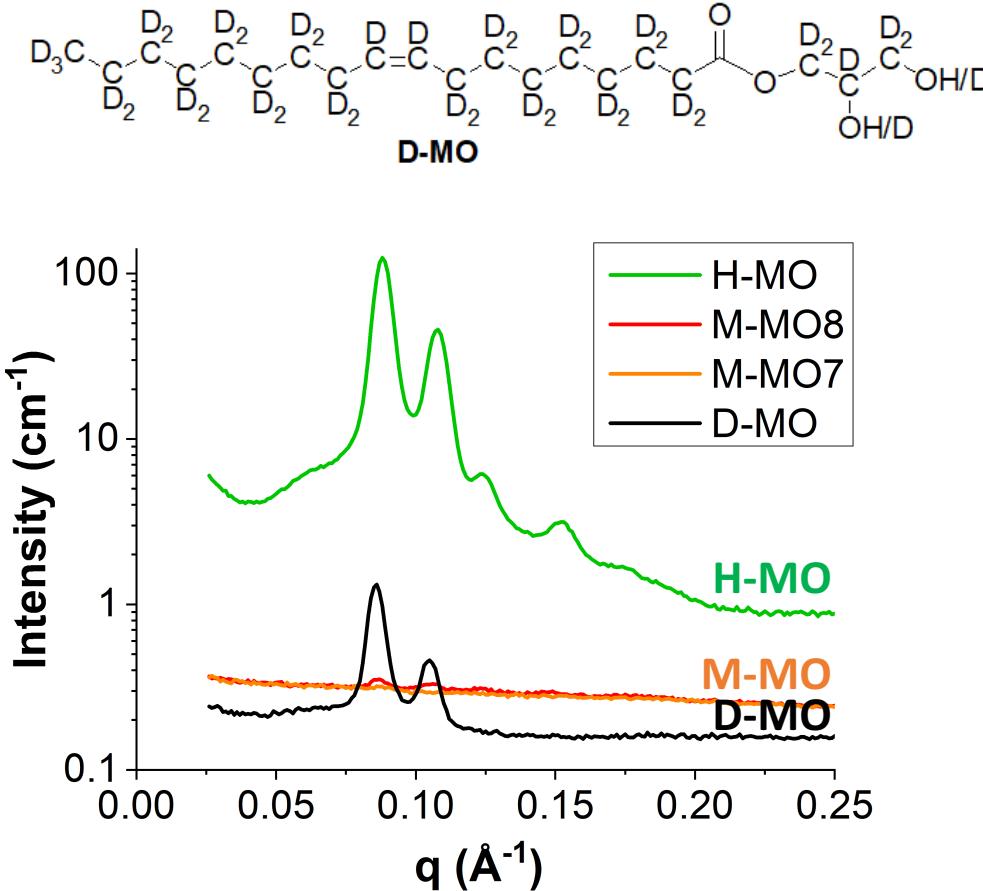
Gaussian curvature = 0

$$I_2/I_1 Q_{||}^D > 100\%$$

Minor effect of deuteration on phase behaviour MO



Contrast-matching of the cubic phase: M-MO in D₂O



$$SLD_{H/D-MO} = \frac{\sum_{i=1}^n b_{c_i}}{v_m}$$

Scattering Length Densities:

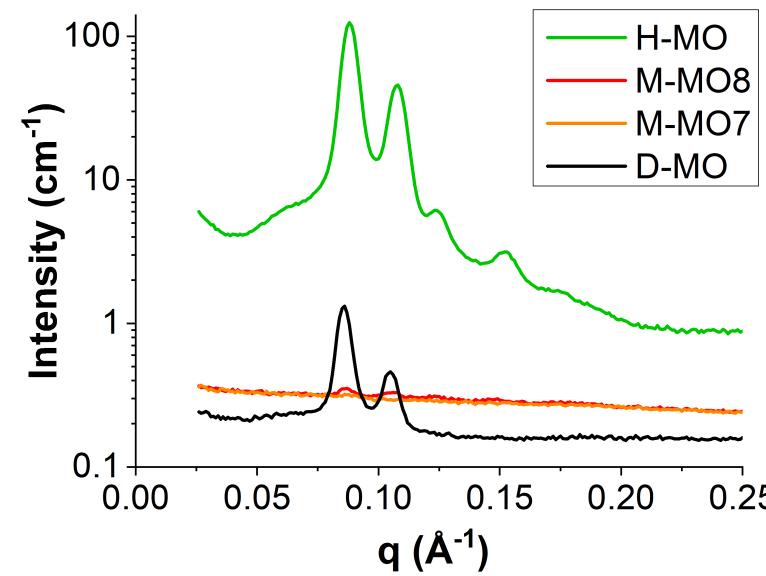
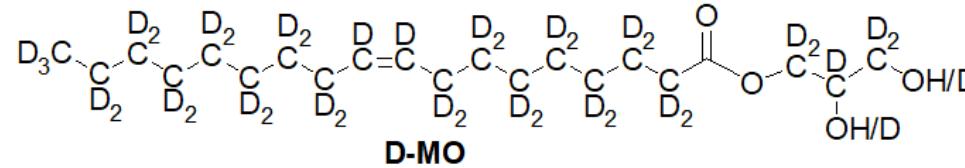
$$D\text{-MO} = 6.78 \times 10^{-6} \text{ \AA}^{-2}$$

$$D_{2,0} = 6.37 \times 10^{-6} \text{ \AA}^{-2}$$

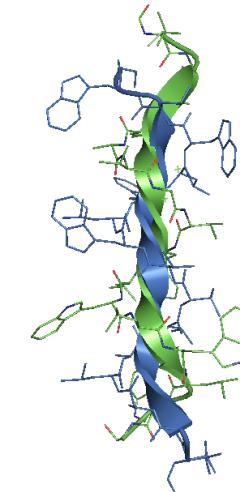
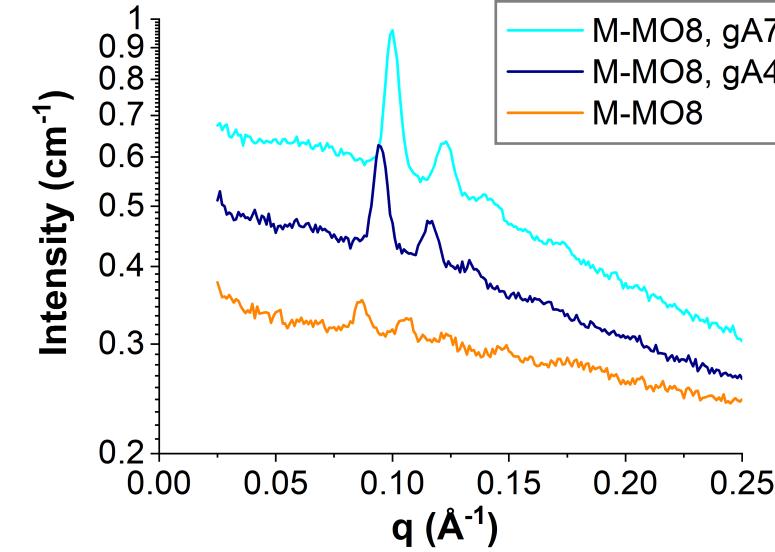
$$H-MO = 0.54 \times 10^{-6} \text{ \AA}^{-2}$$

94% v/v
M-MO
6% v/v

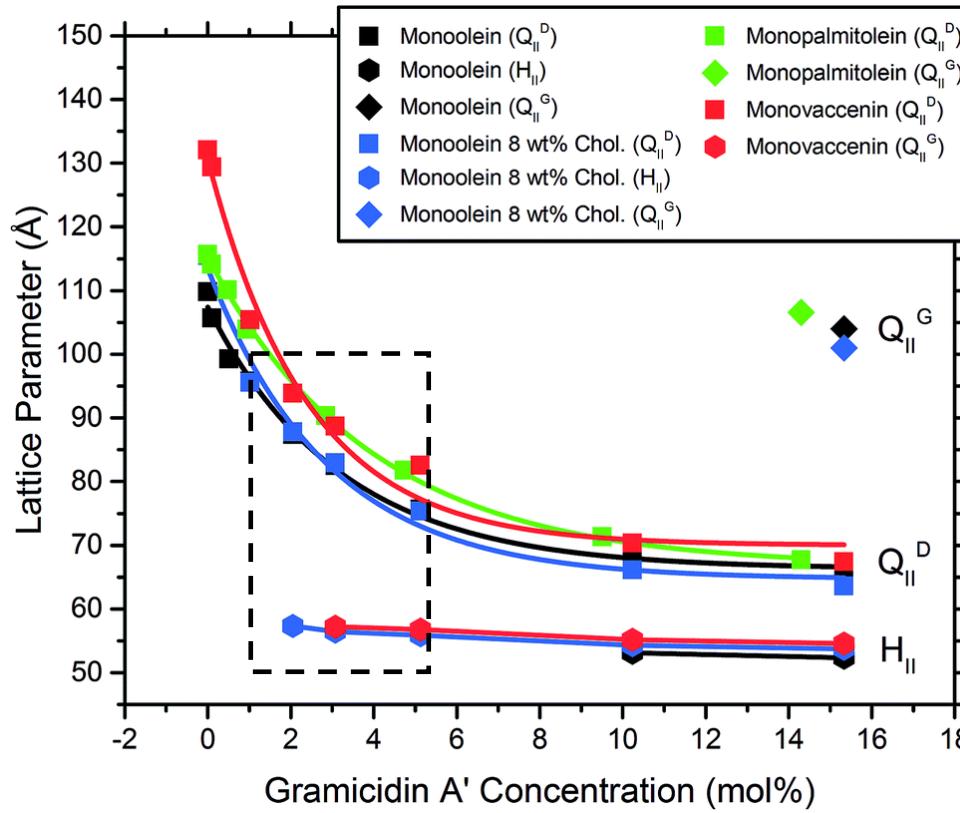
Isolate peptide scattering: gramicidin A encapsulation



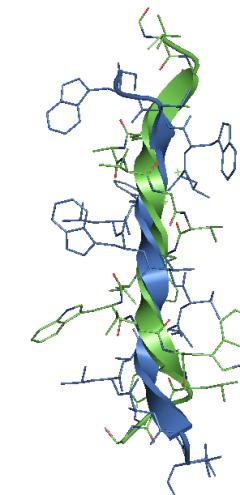
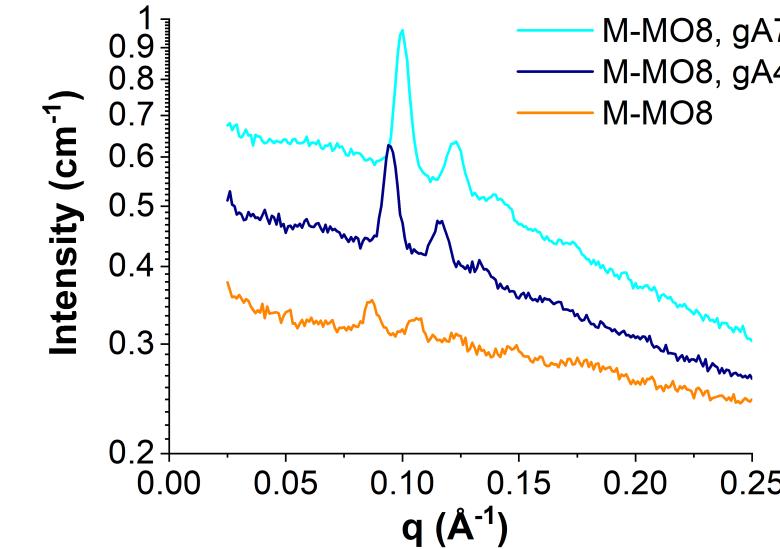
Gramicidin A encapsulation



High-throughput SAXS → Peptide Scattering with SANS



Gramicidin A encapsulation

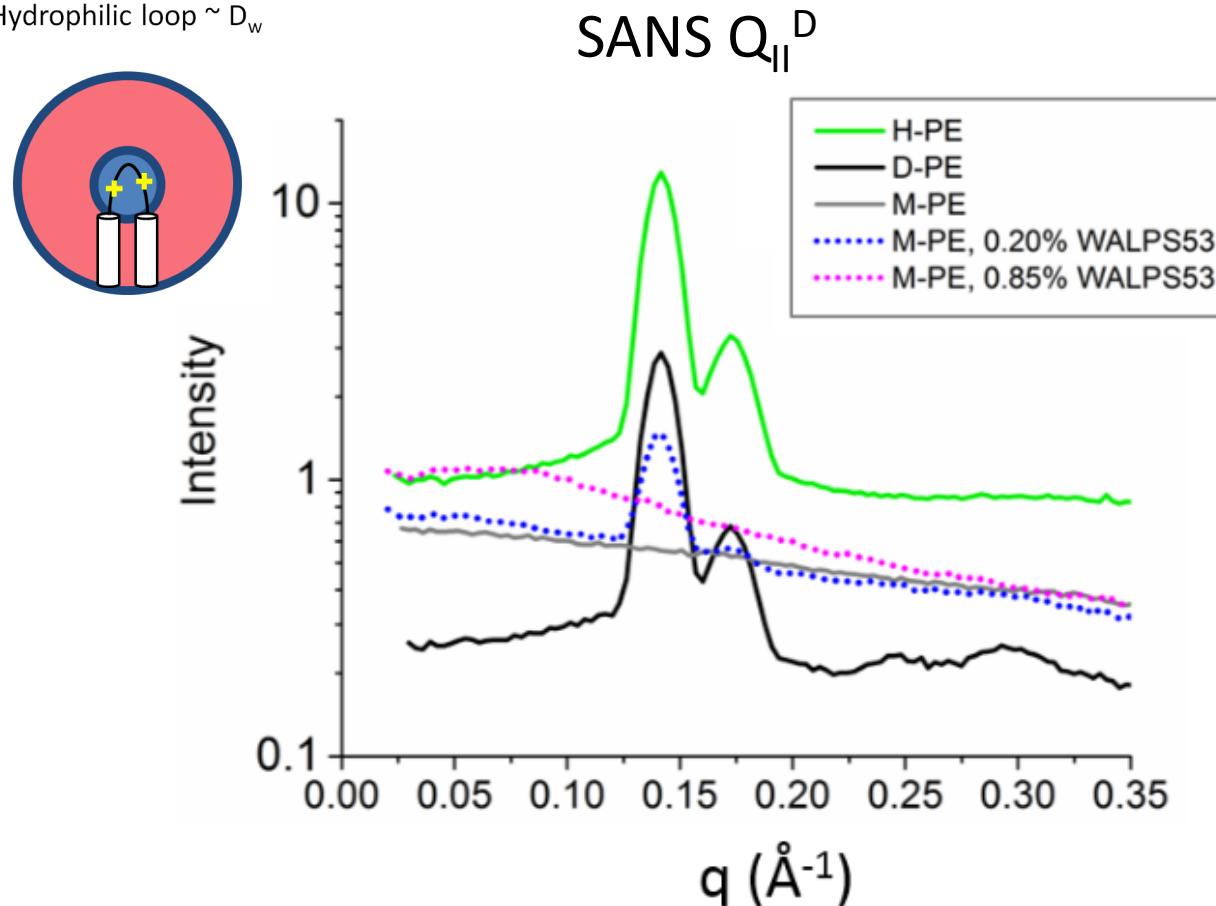


Meikle *et al*, RSC Adv. 2016, 6, 68685-68694.

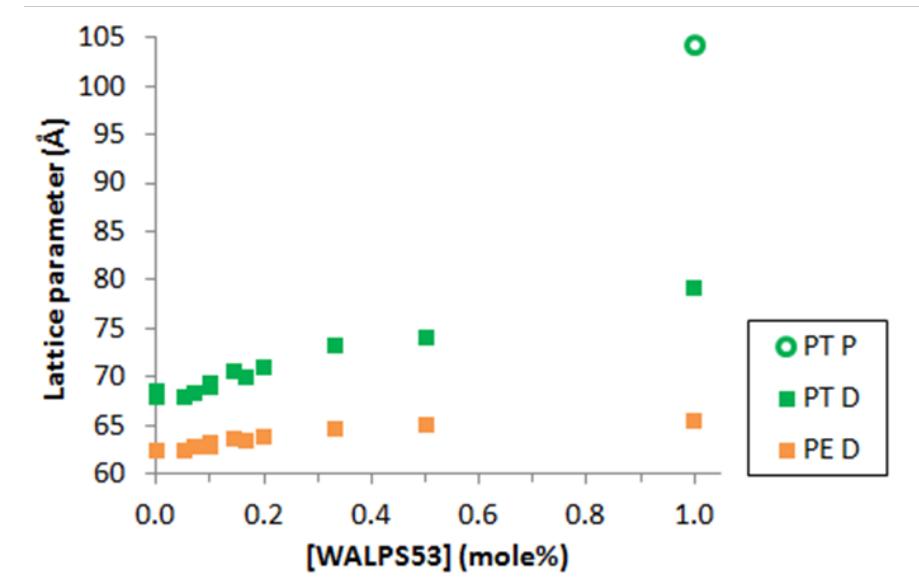
Frontiers in Chemistry 2021, , <https://doi.org/10.3389/fchem.2020.619470>.

WALPS53 peptide: excluded at high concentrations

Hydrophilic loop $\sim D_w$

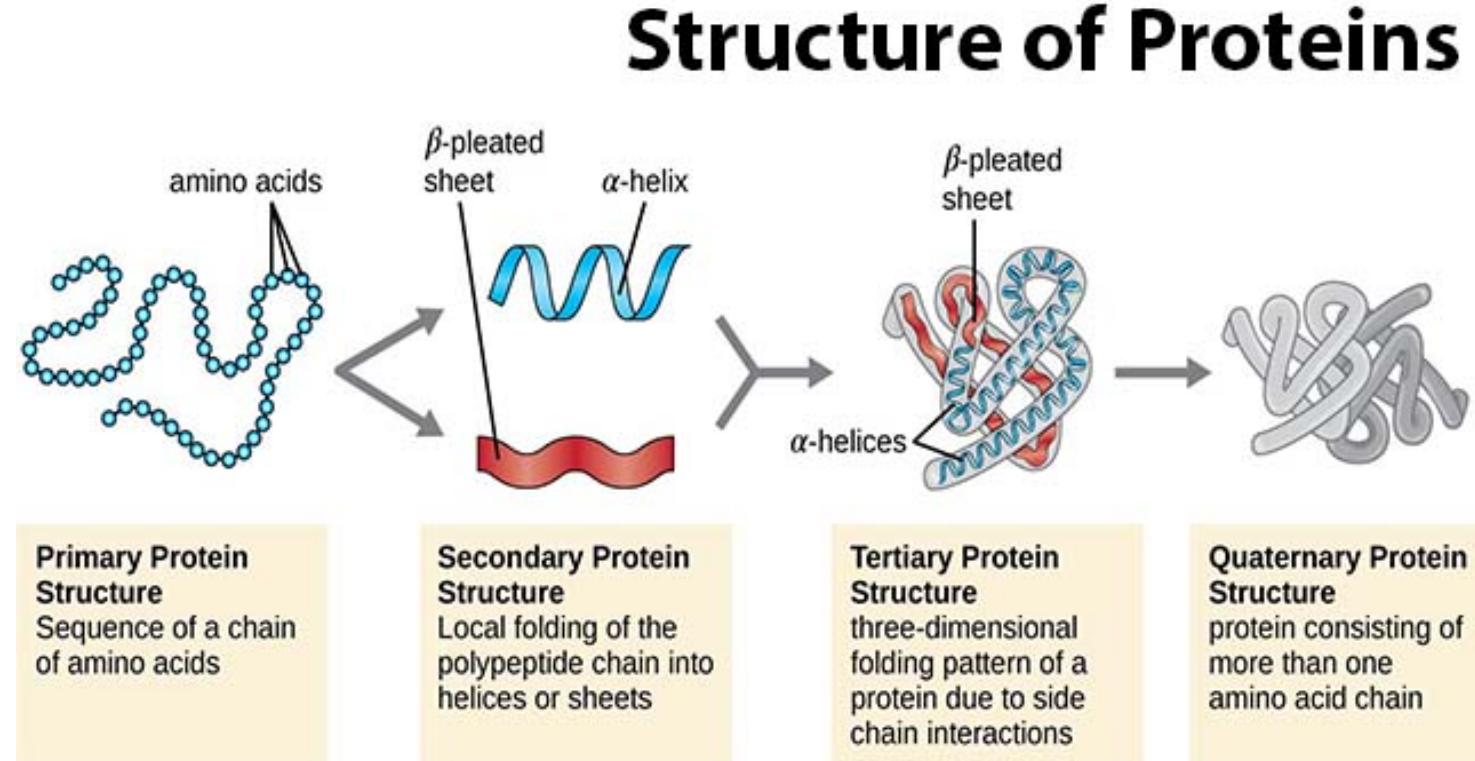
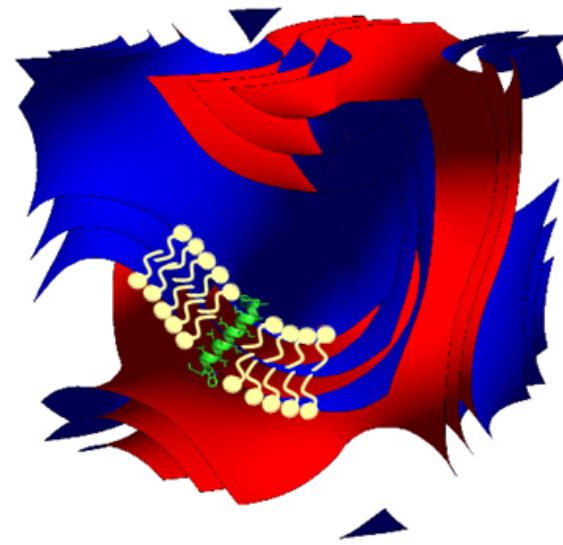


I_2/I_1 for WALPS53 similar: **no enrichment** at low concentration



→ Preferential hydrogel formation at high concentrations

Protein structures in lipid mesophases

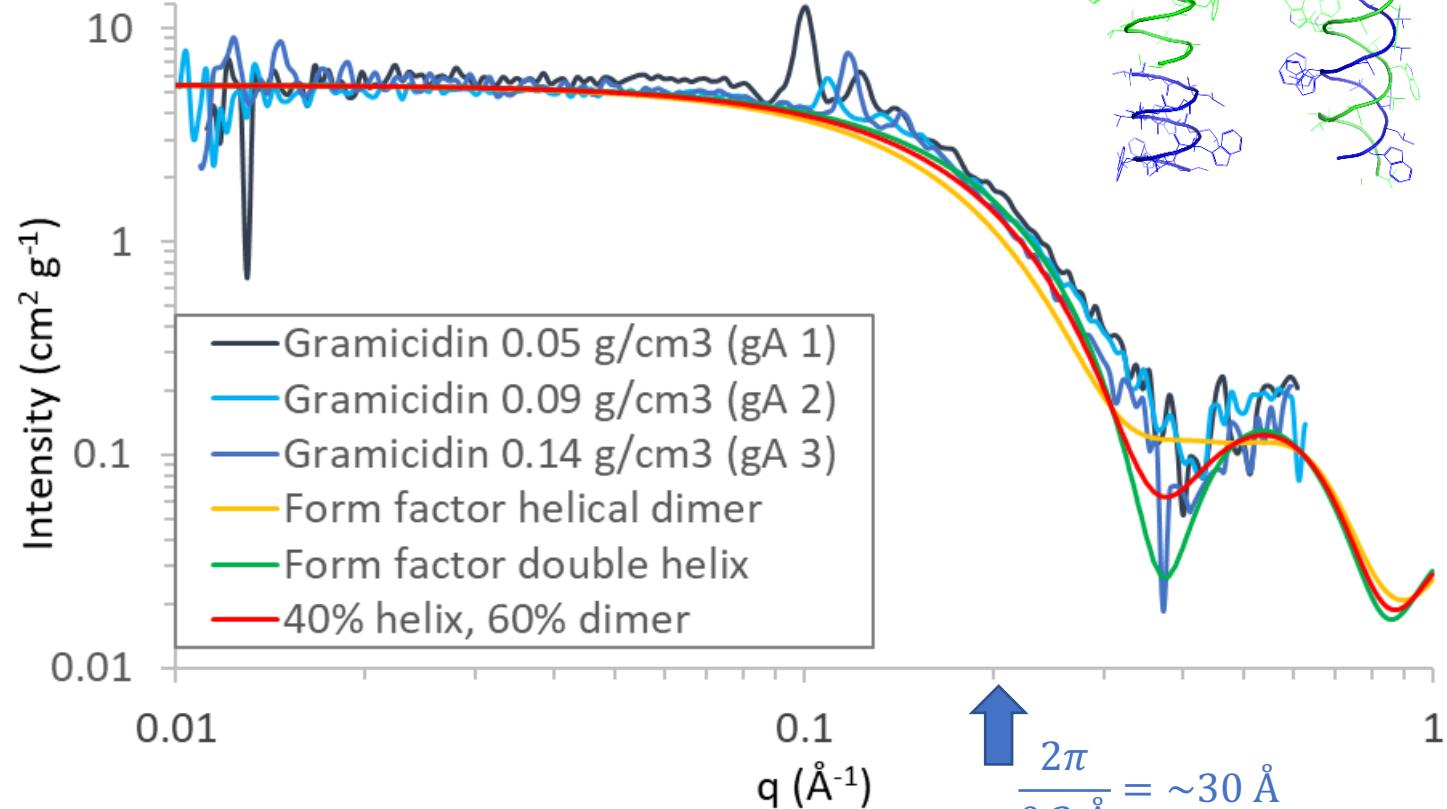
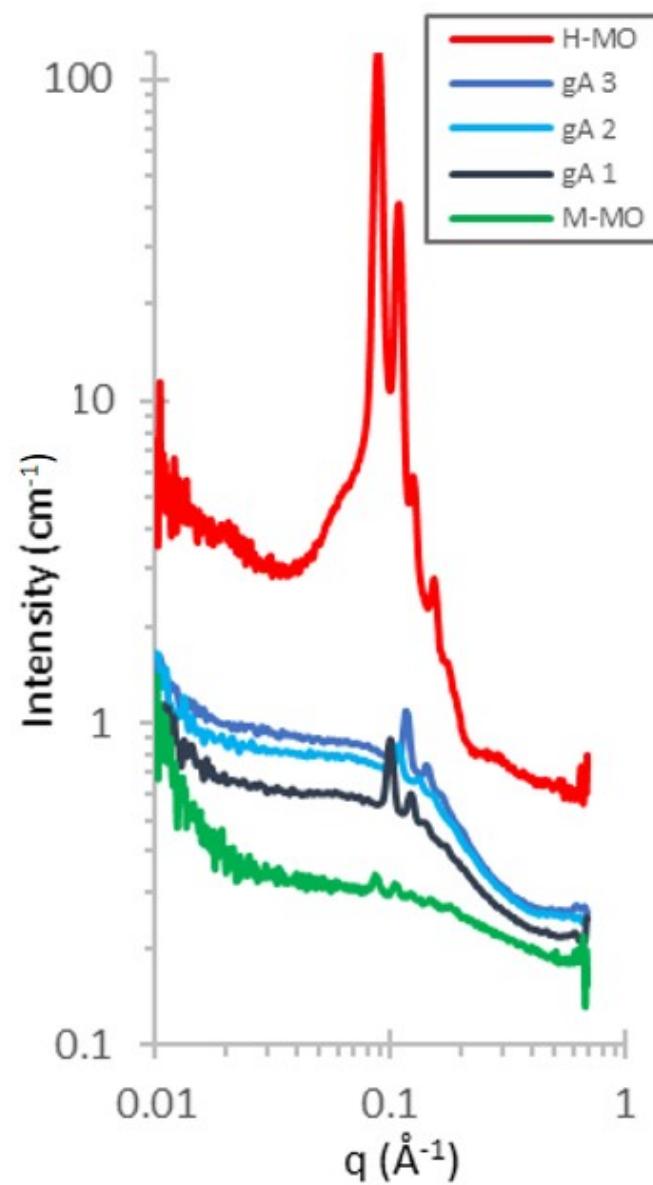


Circular Dichroism spectroscopy

FTIR

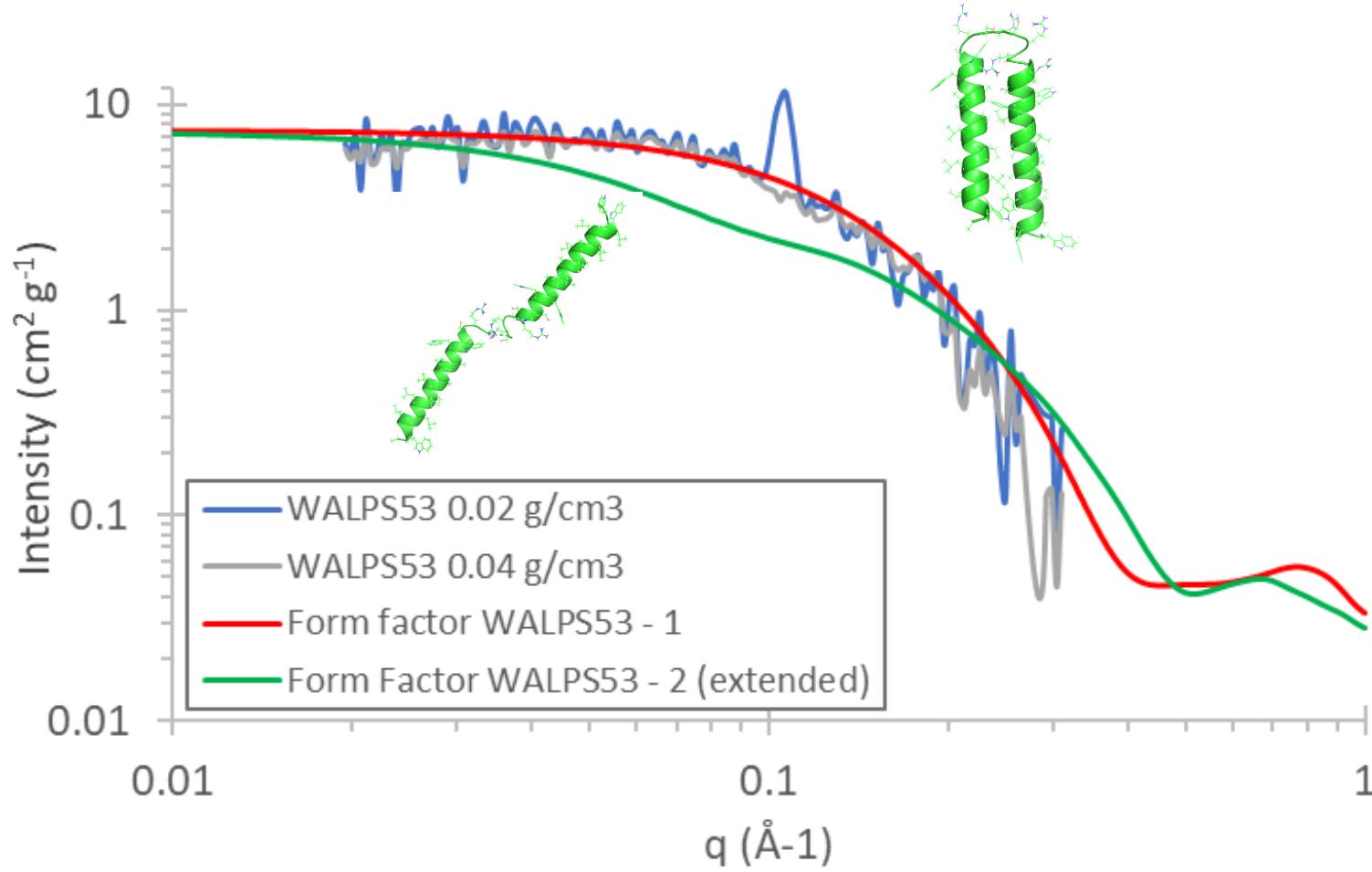
Solution structures SANS/SAXS

SANS peptide structure gA

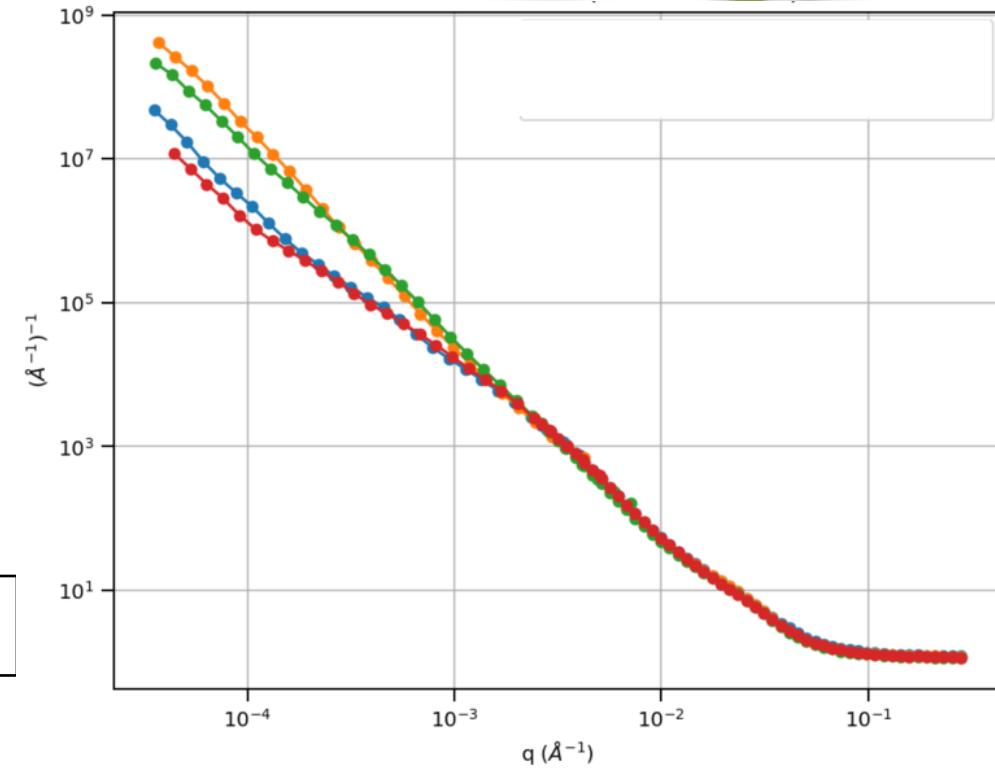
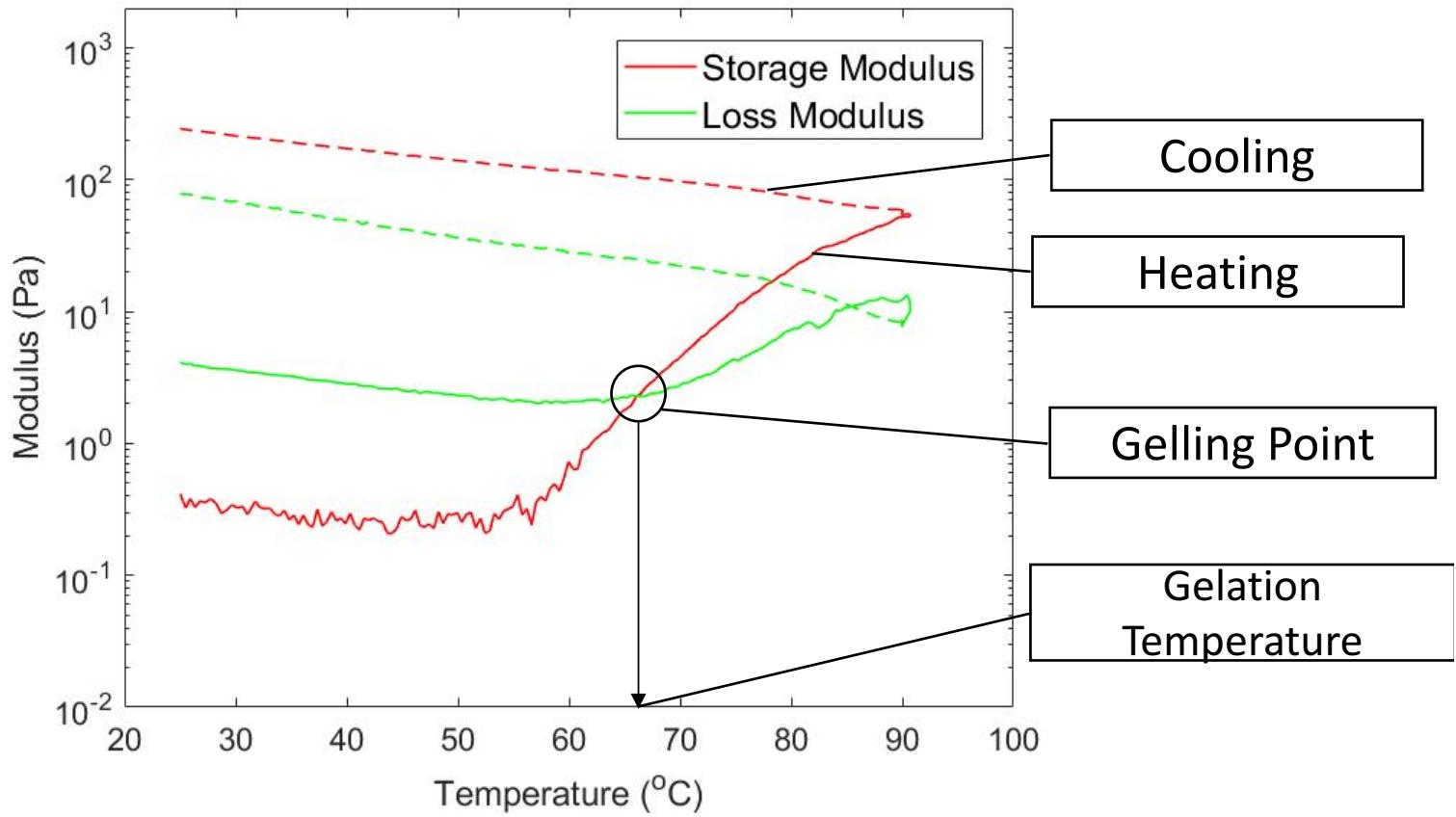


Frontiers in Chemistry 2021, , <https://doi.org/10.3389/fchem.2020.619470>.

SANS Peptide structure WALPS53



2. Plant Protein Assembly: (U)SANS



Protein aggregates
~ten μm

Soluble
~tens nm

Summary Hybrid Self-Assembly materials

- Effect of protein encapsulation on cubic phase nanostructure
 - Lipid system, buffer pH and salt concentration has to be considered: charge and hydrophilic domain size had the most significant effect.
 - Cubic phase was also shown to affect peptide and protein secondary structures.
 - Neutron scattering with contrast matched cubic phases opens up the possibility to study biomolecule location and conformation.
 - Deuteration effects were minor.
 - Explains changes in cubic phase nanostructure: optimize protein loading and develop new fusion inhibitors.
 - Supporting evidence for the *in meso* crystallization mechanism was obtained.
 - Technique with equipment available in standard crystallography laboratories enables testing with various proteins and peptides. No evidence had been reported since 2007.
- **Structure-property relationships:** evolve materials science, food and biomedical applications
- *More complex (mixed) membrane and protein systems*

Acknowledgements & Thank you!



Dr Nigel Kirby
Dr Susi Seibt
Dr Timothy Ryan



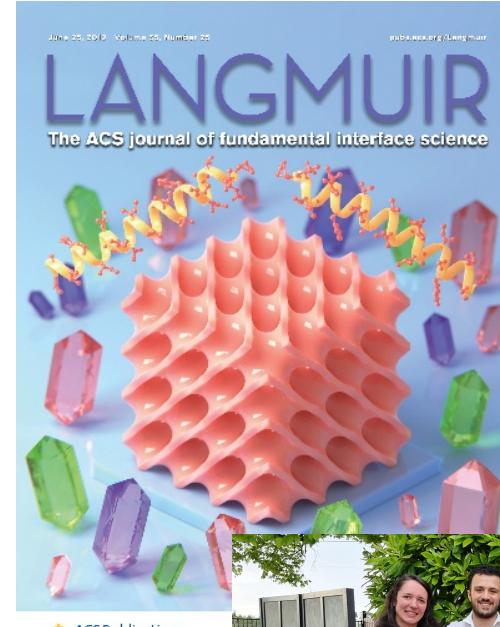
Prof. Calum Drummond
A/Prof. Charlotte Conn
Dr. Nhiem Tran
Dr. Thomas Meikle



Australian Government



Dr. Liliana de Campo
Dr. Anton LeBrun
Dr. Alice Klaproth
Dr. Christopher Garvey
Dr. Robert Knott
Dr. Tamim Darwish
Dr. Anna Leung
Dr. Anwen Krause-Heuer
Dr. Michael Moir
Dr. Karyn Wilde



Leonie van 't Hag

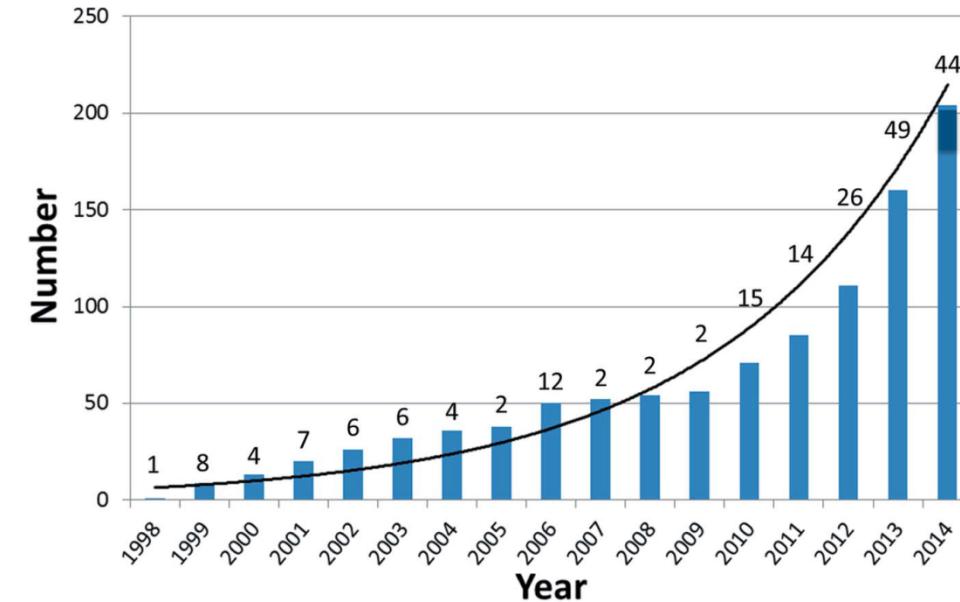
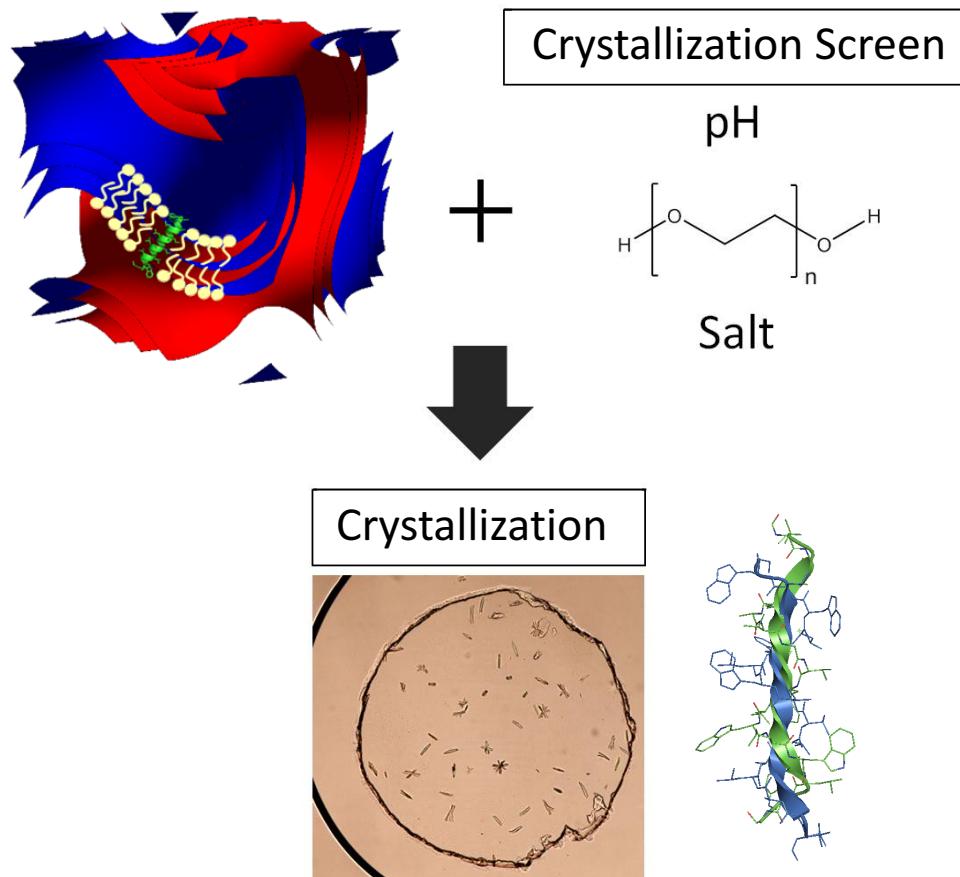
e-mail: leonie.vanthag@monash.edu
<https://www.monash.edu/engineering/hybrid-assembly>



- Processing-Structure-property relationships: evolve materials science, food and biomedical applications
- More complex (mixed) membrane and protein systems



2. In meso crystallization → structure XRD



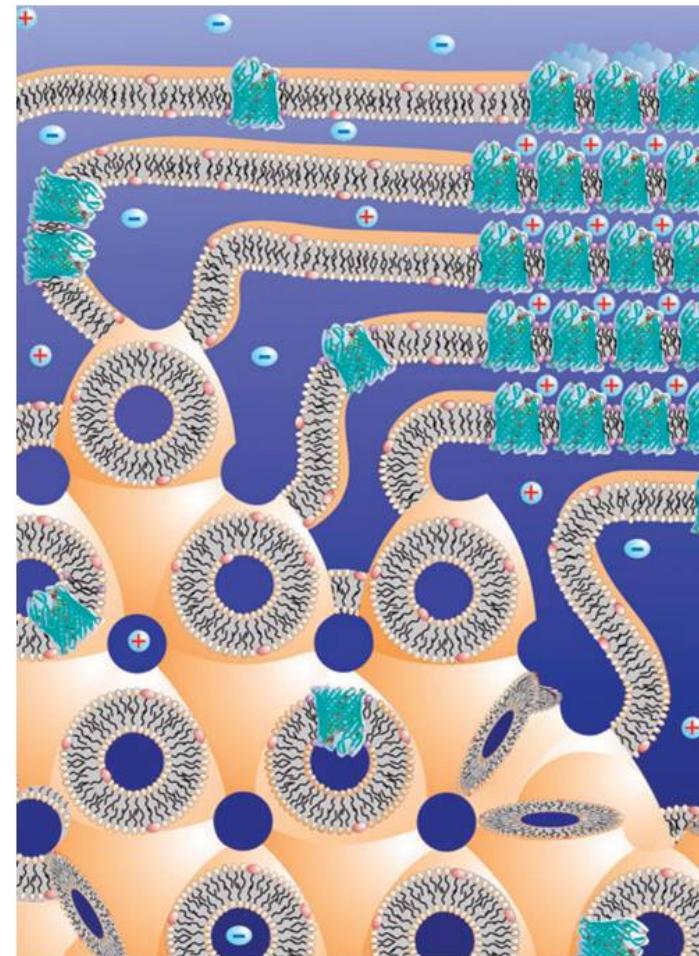
1 – 2% of structures in PDB are membrane proteins, while
 20 – 30% of structures in most organisms are membrane proteins

[1] Landau and Rosenbusch, 1996. [2] M. Caffrey, 2015. [3] Doerr, 2009. [4] Wallin *et al*, 1998.

Proposed *in meso* crystallization mechanism

Local L_α phase

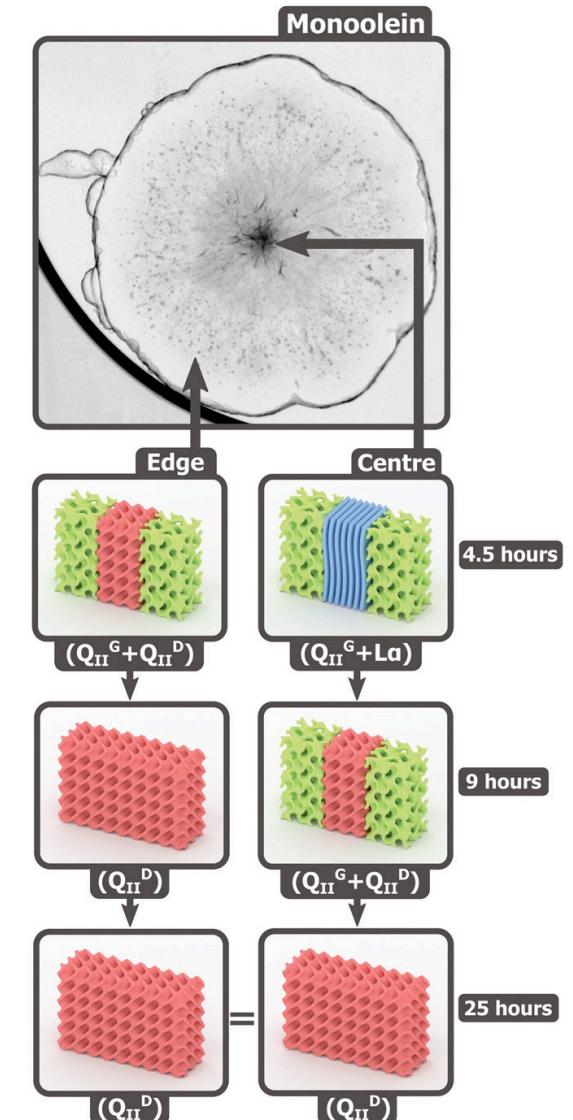
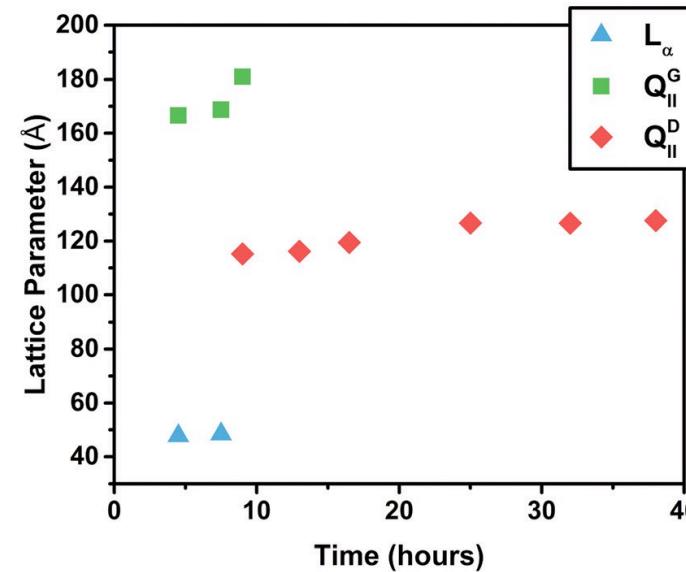
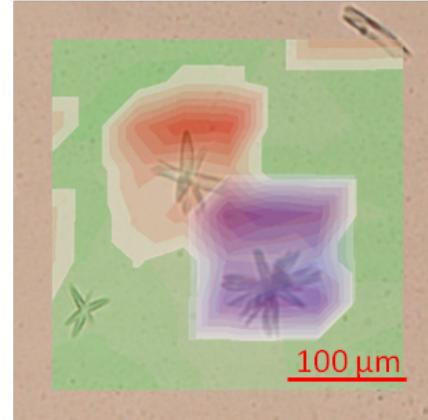
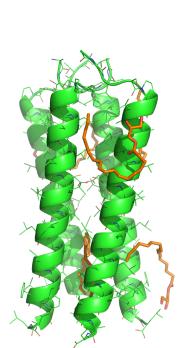
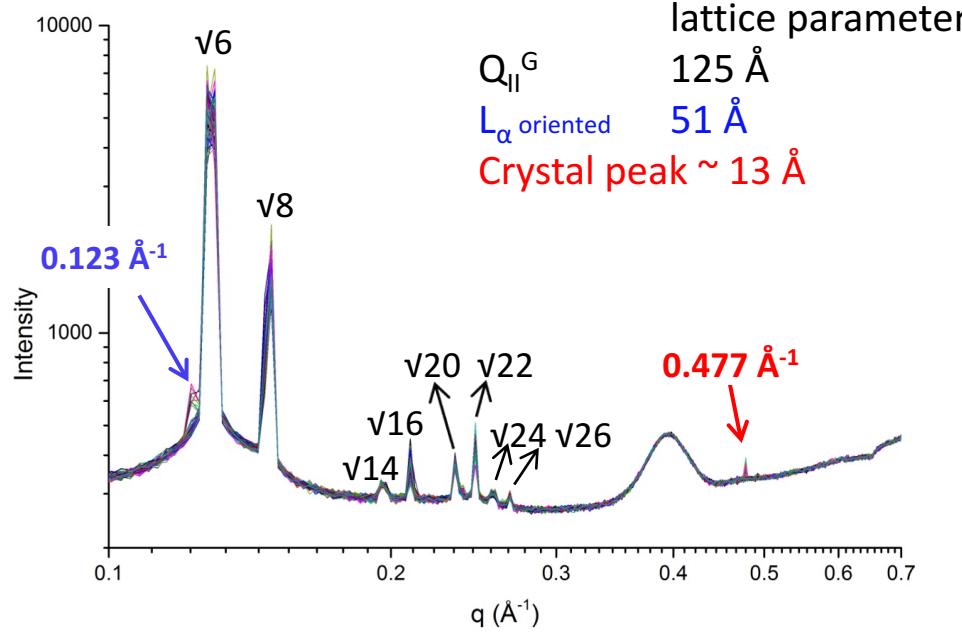
Cubic phase



Type I membrane
protein crystals

[1] M. Caffrey, 2003. [2] V. Cherezov and M. Caffrey, 2007.

SAXS analysis lipid micro-environment

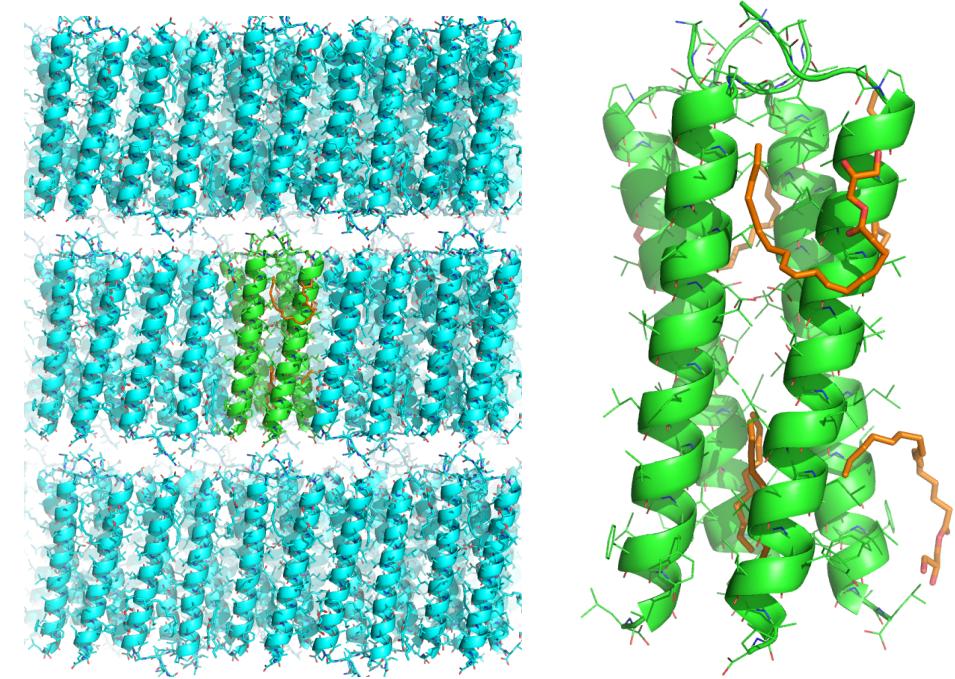


L. van 't Hag *et al*, Phil. Trans. R. Soc. A, 2017, 374(2072), 20150125.

A. Zabara *et al*, Nanoscale, 2017, 9, 754.

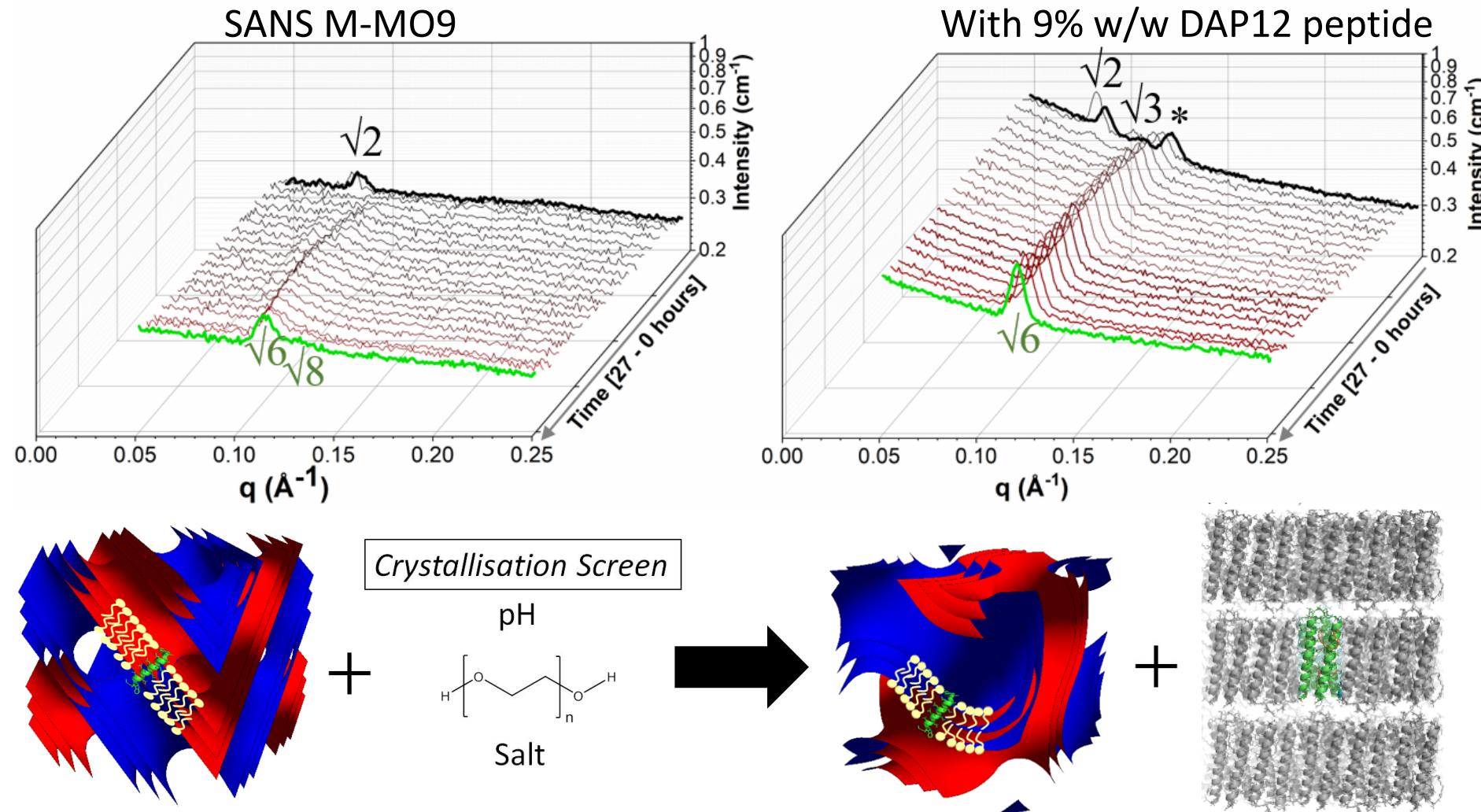
DAP12 in meso crystallization structure

- ▶ Human signalling module
- ▶ Forms transmembrane complexes with receptors that activate cells of the immune system
 - Understand interaction → manipulate immune response
 - Involved in chronic inflammatory diseases
- ▶ Crystallized with 0.1 M Bis-Tris propane (pH 6.4), 0.05 M CaCl₂, 20% w/v H-PEG 4000 g mol⁻¹
 - Lamellar spacing 50.6 Å

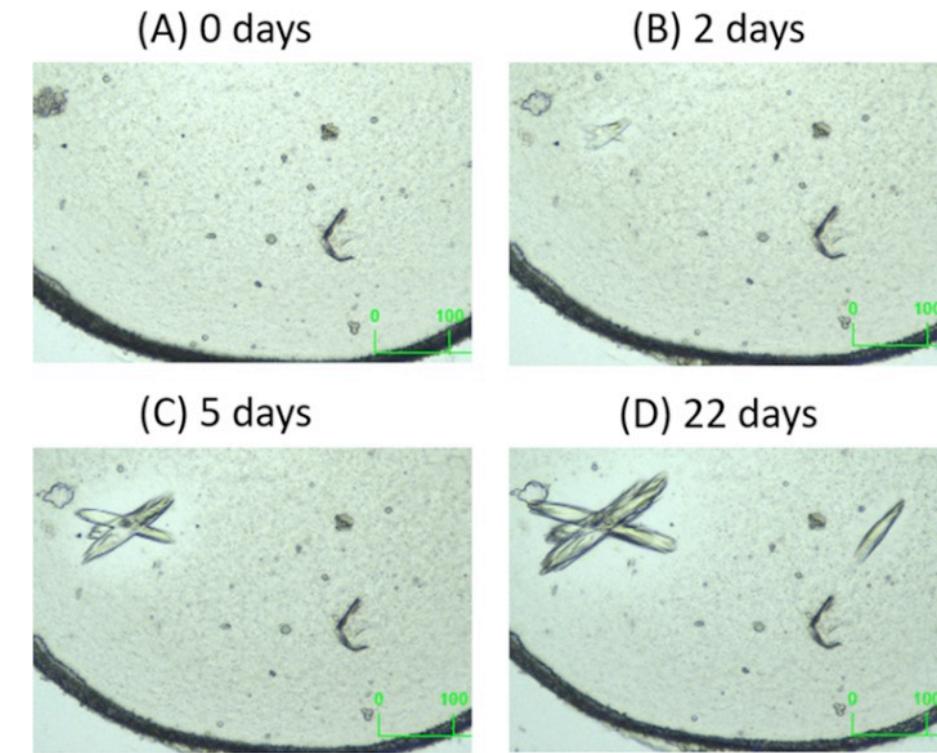
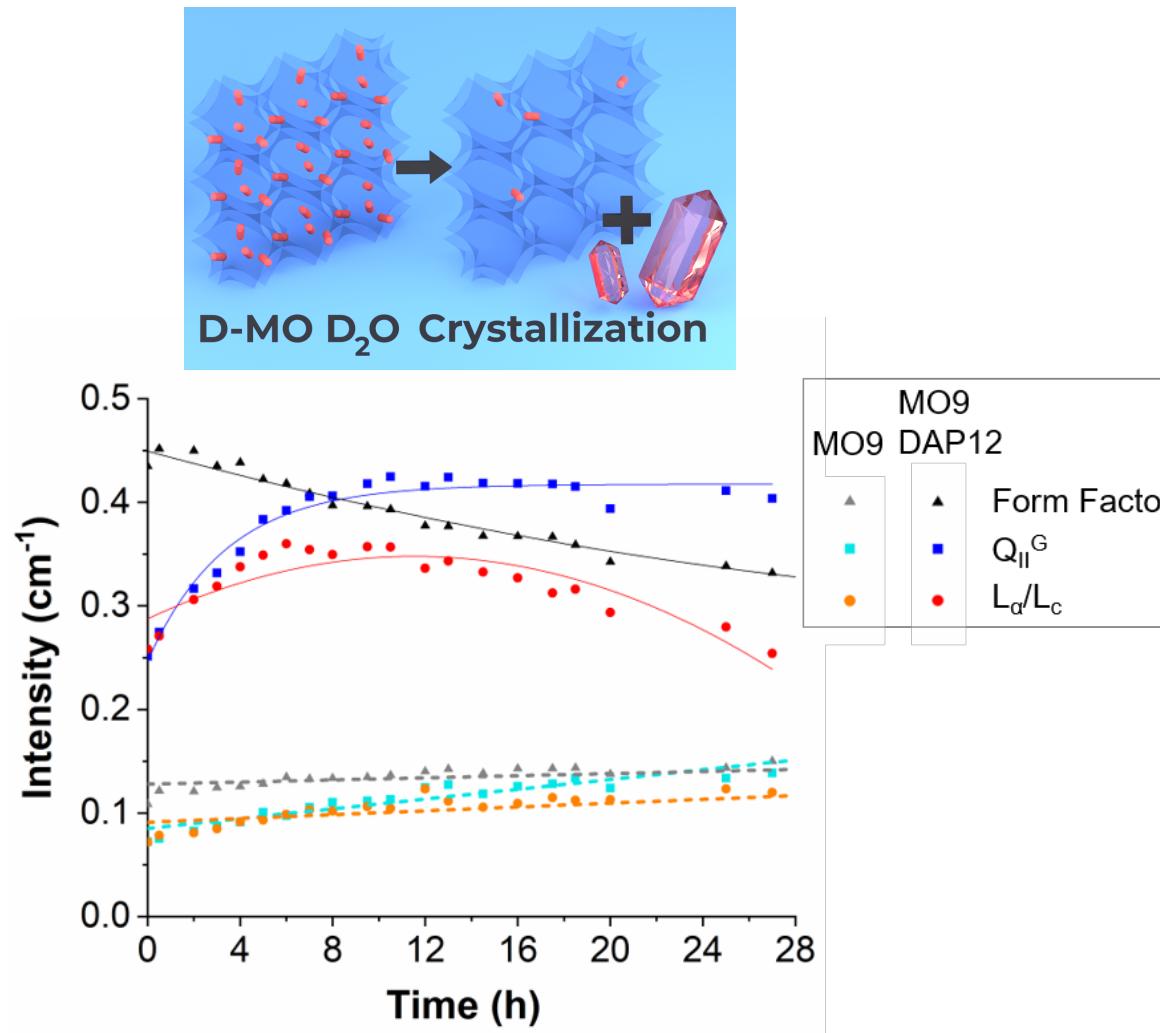


Call et al. *J. Immunol.* 2010, 184, 138.16
Knoblich et al. *Cell Reports* 2015, 11, 1184-1192.

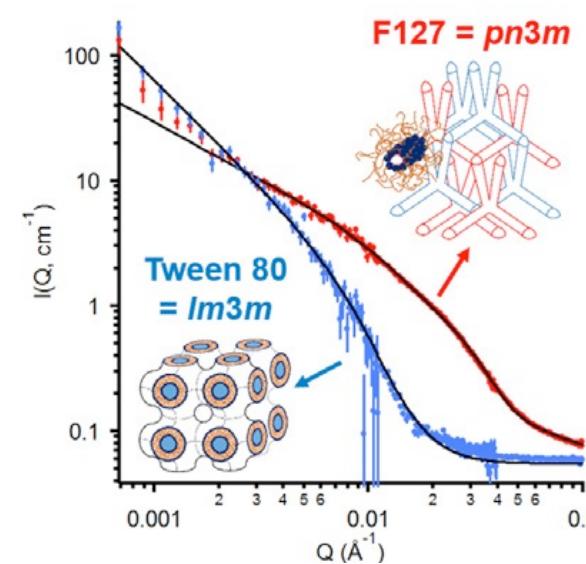
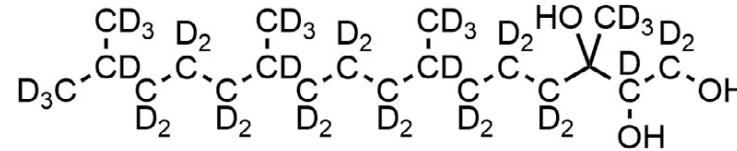
Crystallization DAP12 from cubic phase of M-MO



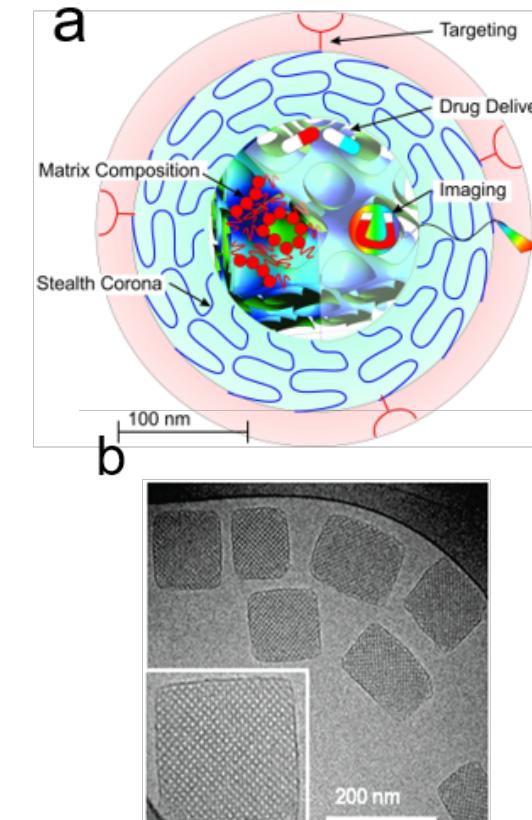
Crystallization over time: understand processes



Cubosomes D-phytantriol: stabilizer location

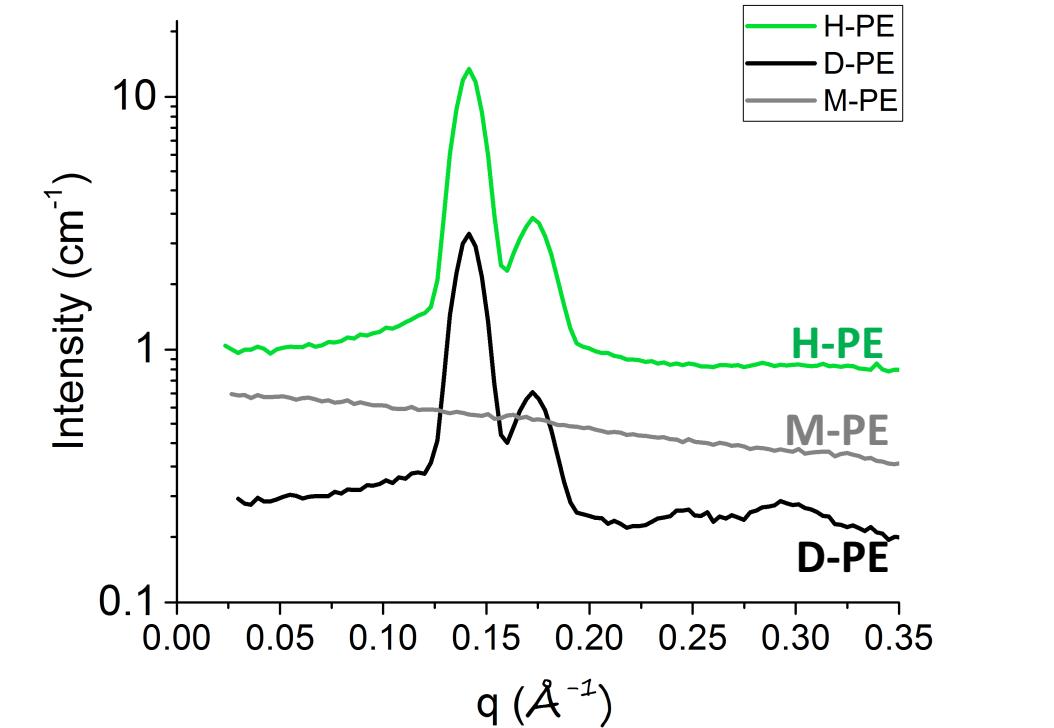
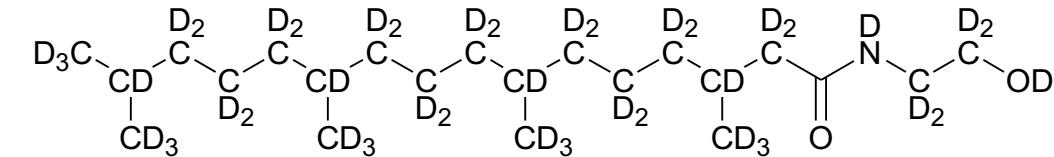
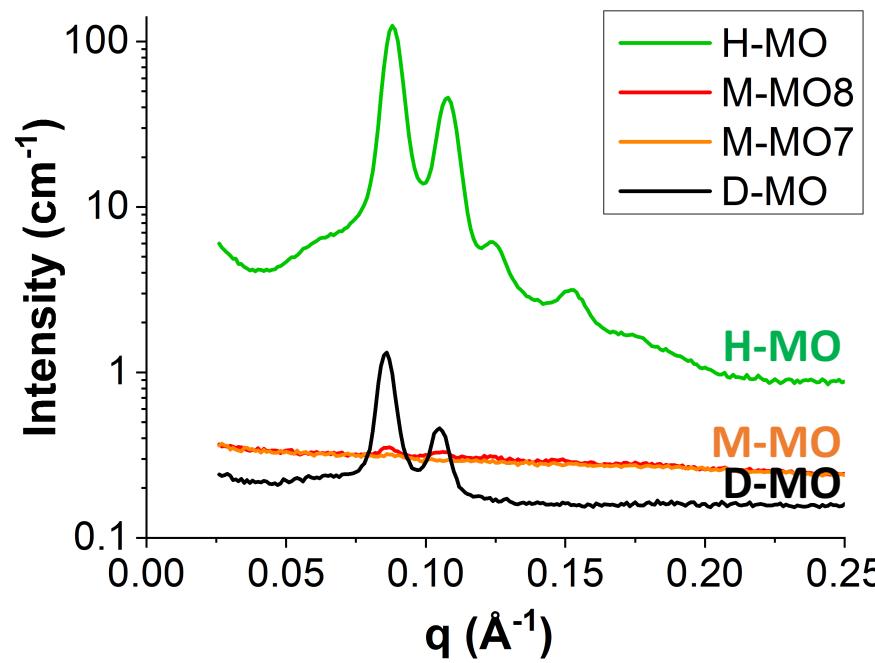
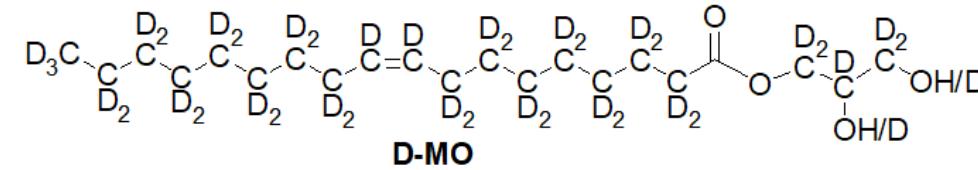


Yepuri, Clulow et al. *J. Coll. Int. Sci.* 2019, 534, 399–407.

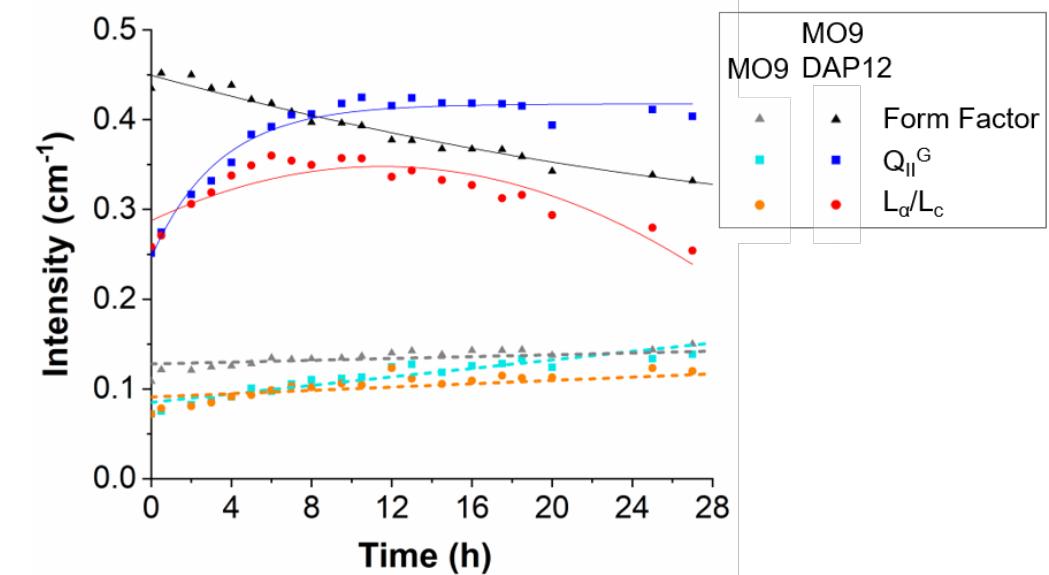
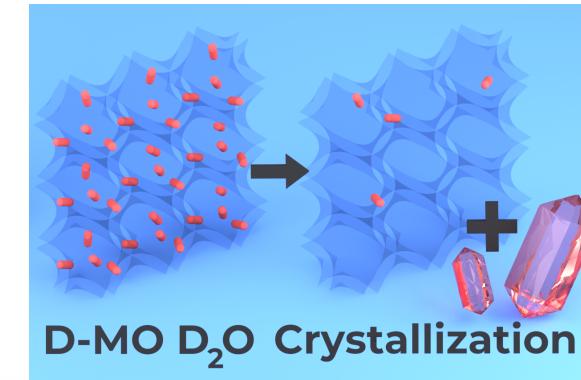
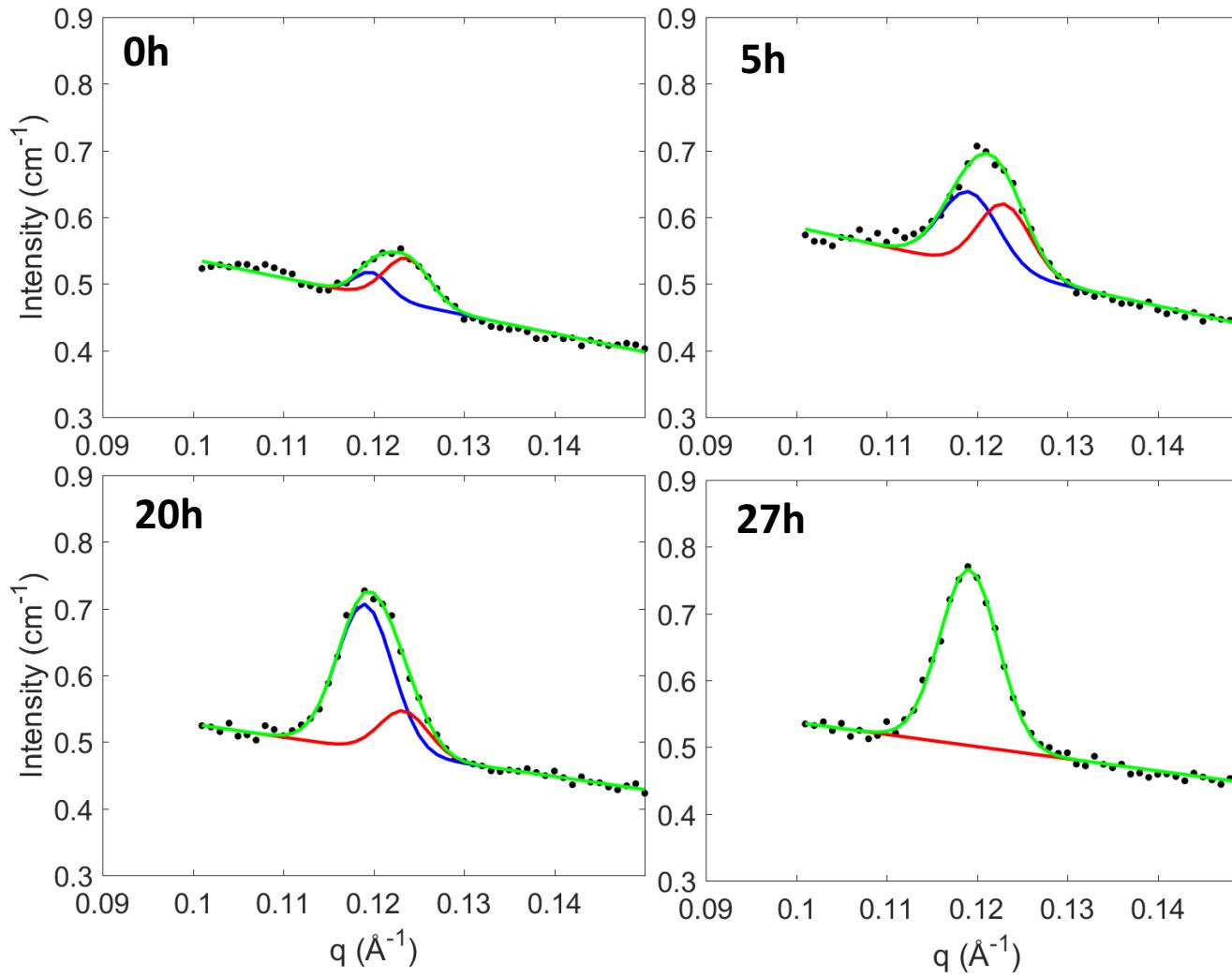


Mulet et al. *J. Colloid Interface Sci.* 2013, 393, 1–20.
Muir et al. *J. Phys. Chem. B* 2012, 116, 3551–3556.

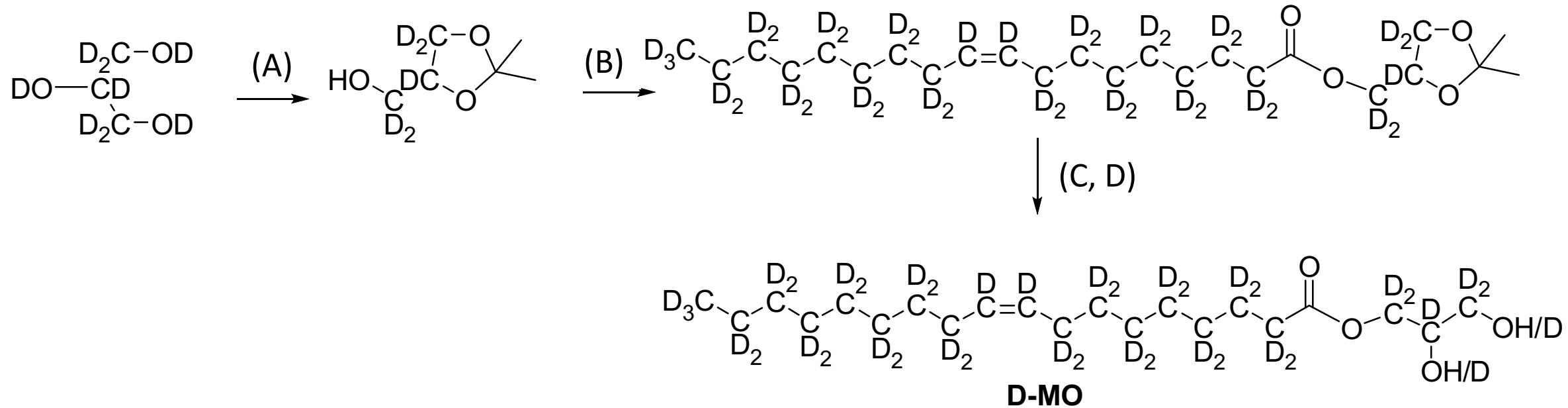
BILBY TOF-SANS vs QUOKKA data



Crystallization over time: understand processes

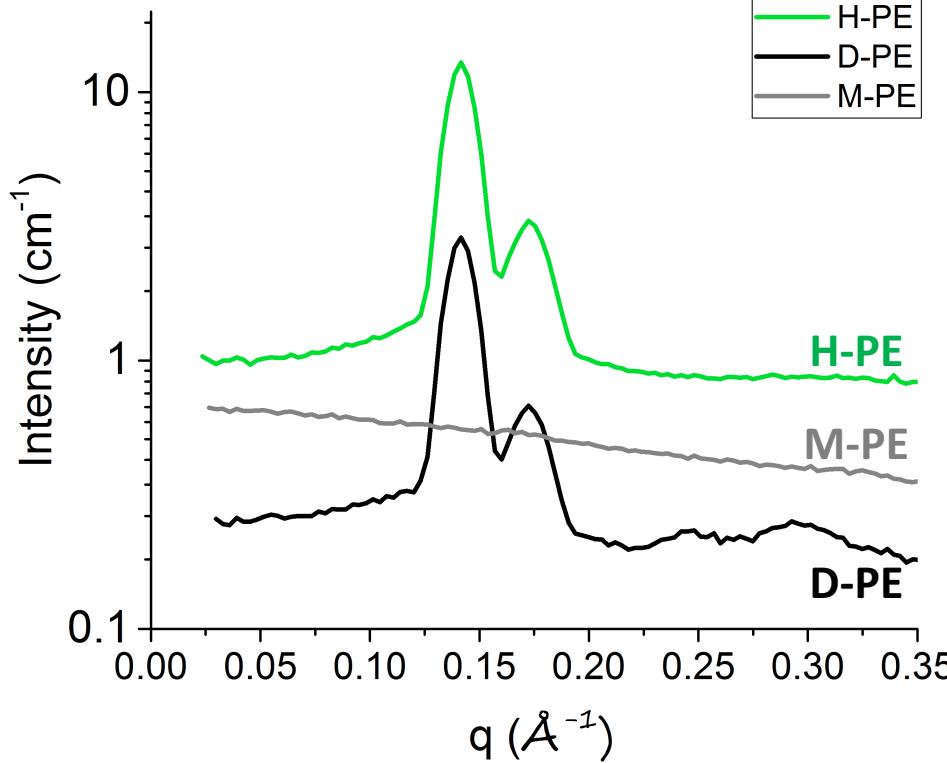
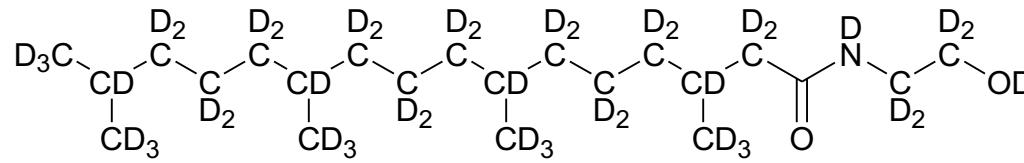


Synthesis of D-MO



Synthesis and chemical structure of deuterated monoolein (D-MO or MO-d₃₈). (A) Acetone, p-toluenesulfonic acid, reflux, 41 h. (B) Oleic acid-d₃₃ DCC/DMAP. (C) Amberlyst resin, 90 °C, 2 h, (D) MeOD.

Contrast-matching of the cubic phase: M-PE in D₂O



$$SLD_{H/D-PE} = \frac{\sum_{i=1}^n b_{c_i}}{v_m}$$

$$SLD_{M-PE} = \frac{\nu_{H-PE} * SLD_{H-PE} + \nu_{D-PE} * SLD_{D-PE}}{\nu_{H-PE} + \nu_{D-PE}}$$

Scattering Length Densities:

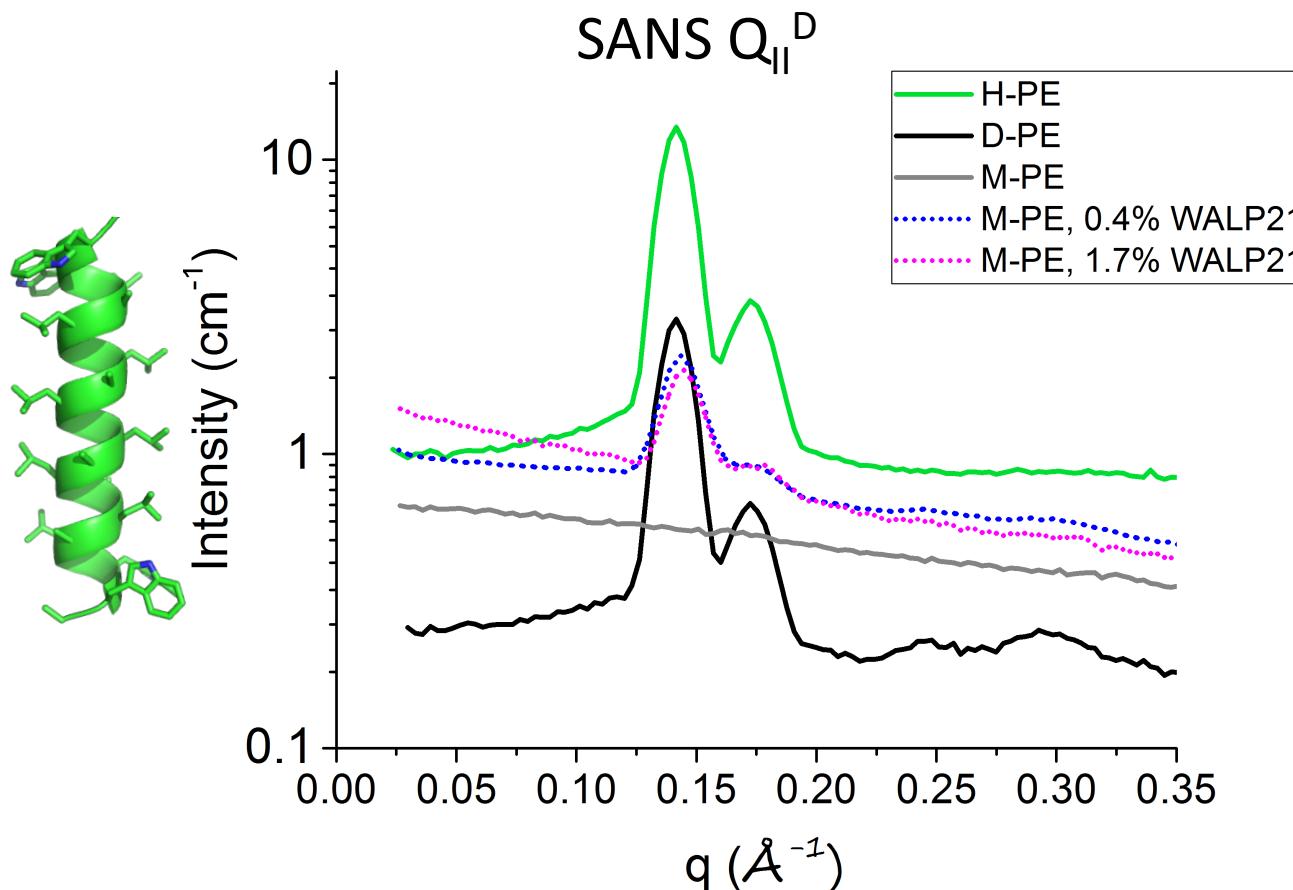
$$D\text{-PE} = 6.78 \times 10^{-6} \text{ \AA}^{-2}$$

$$D_{2\text{O}} = 6.37 \times 10^{-6} \text{ Å}^{-2}$$

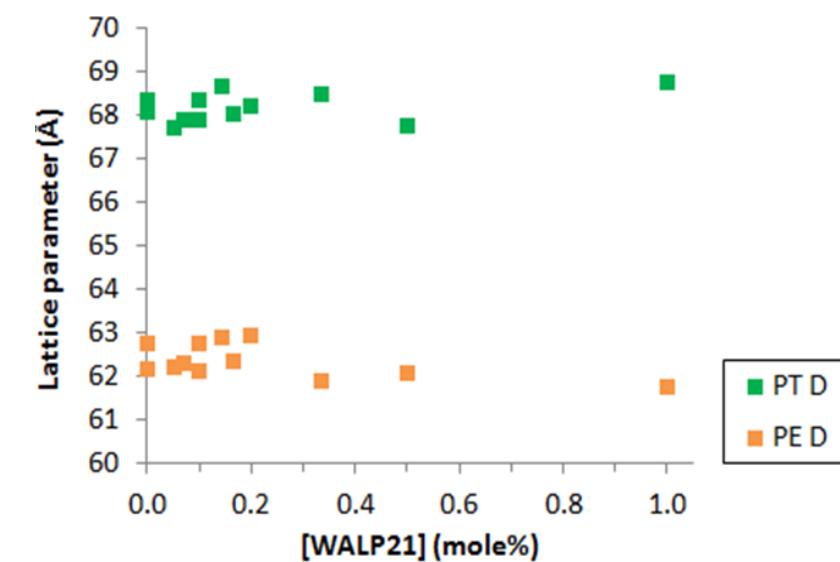
$$H-PE = -0.02 \times 10^{-6} \text{ \AA}^{-2}$$

↓ 86% v/v
↑ M-PE
14% v/v

WALP21 peptide scattering was obtained



I_2/I_1 for WALP21 similar: **no enrichment** flat or most curved points



→ Limited encapsulation due to high lateral bilayer pressure

One transmembrane domain per flat point

Molecular volume H/D-PE:

$$(S1.1) v_m = \frac{M_w (g mol^{-1})}{\rho (g nm^{-3})} = \frac{355.6}{0.94 \times 10^{21}} = 0.628 nm^3 molecule^{-1}$$

Unit cell volume Q_{II}^G (lp is lattice parameter):

$$(S1.2) v_{uc} = lp^3 = 8.8^3 = 681.5 nm^3$$

Lipid volume in Q_{II}^G at 15% w/w (14% v/v) water:

$$(S1.3) v_{ucl} = v_{uc} \times 86\% = 681.5 \times 0.86 = 584.3 nm^3$$

Number of PE molecules per unit cell Q_{II}^G :

$$(S1.4) n_{mol/uc} = \frac{v_{ucl} (nm^3)}{v_m (nm^3 molecule^{-1})} = \frac{584.3}{0.628} = 930.4$$

Number of PE molecules per flat point Q_{II}^G :

$$(S1.5) n_{mol/fp} = \frac{n_{mol/uc}}{16 flat points} = \frac{930.4}{16} = 58$$

WALP21 peptide concentration (mol %) with 1 transmembrane domain per flat point:

$$(S1.6) [WALP21] = \frac{1}{n_{mol/fp}} = \frac{1}{58} \times 100\% = 1.7 mol \%$$