The air-liquid interface to study drug interactions

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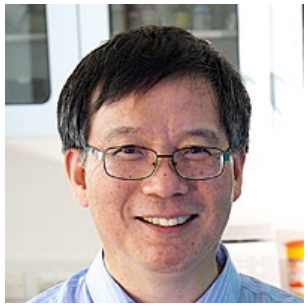


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Tutorial Outline

cutting-edge neutron reflectometer applied in the biosciences

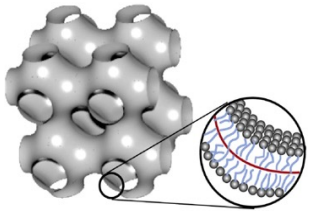
introduction to neutron reflectometry: 3 ways to exploit 1 technique

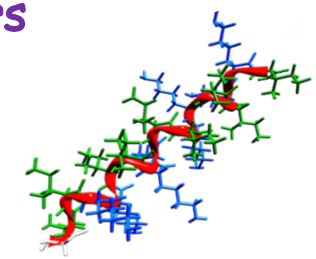
example 1 = structural disruption by antimicrobial peptides

example 2 = membrane binding of liquid crystalline nanocarriers

example 3 = interactions of anti-cancer drugs

summary & outlook





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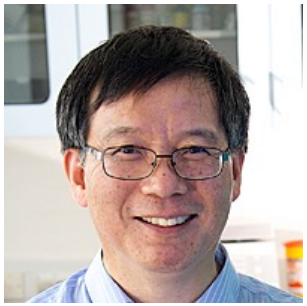

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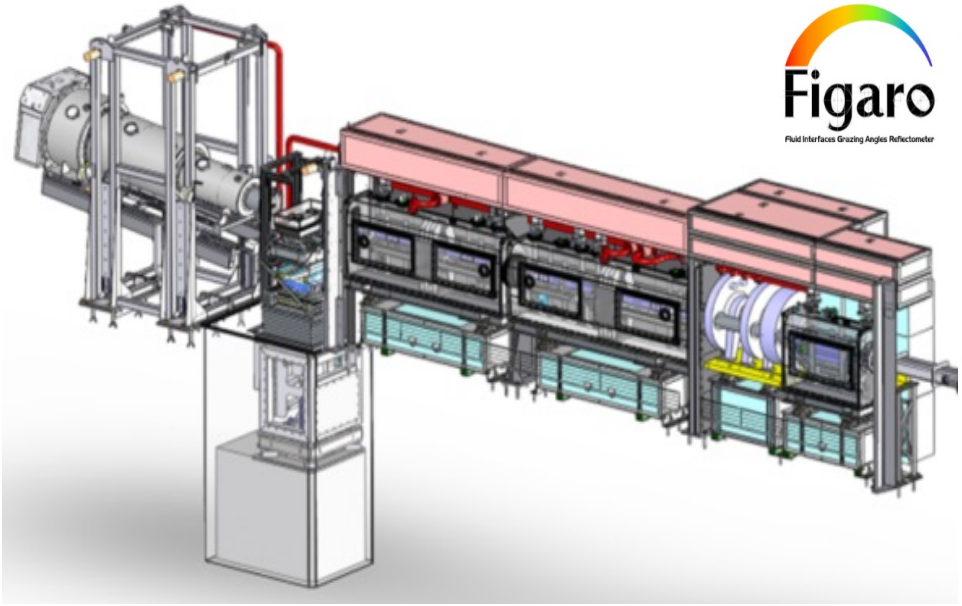
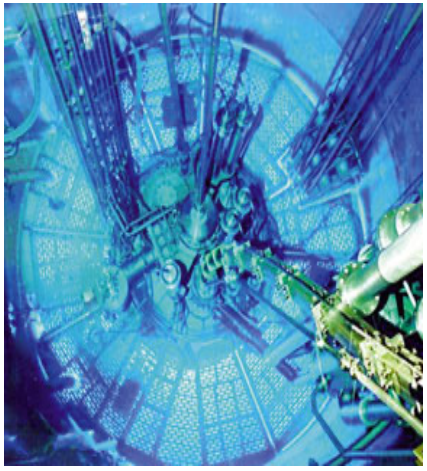
Fluid Interfaces Grazing Angles ReflectOmeter

cutting-edge neutron reflectometer



- FIGARO features:
  - powerful and versatile
  - reflection up or down modes
  - beam size of several tens of cm^2
  - data acquired in seconds to minutes



- FIGARO science:
  - peptide binding to model biomembranes
  - DNA interactions with lipid monolayers
  - reaction kinetics in health & environment
  - drug nanocarrier attachment to surfaces
  - interfacial mechanisms of formulations

Campbell et al. Eur. Phys. J Plus, 2011, 126, 107

Sample Environments & Applications in the Biosciences

adsorption troughs: self-assembly of formulations at the air/liquid interface

Langmuir trough: interactions involving lipid monolayers in health & environment

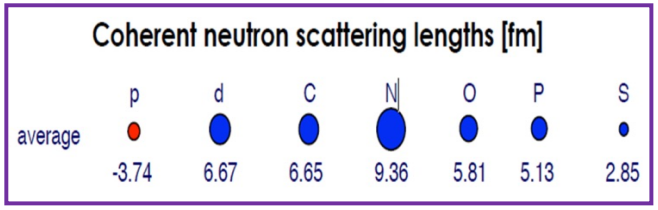
solid/liquid interface: binding of molecules and particles to model biomembranes

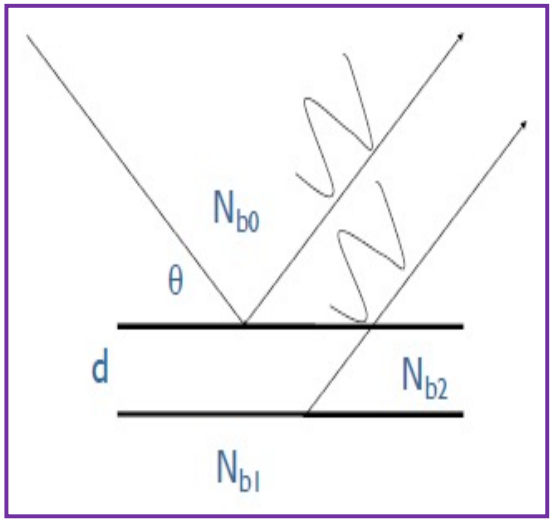
overflowing cylinder: adsorption of mixtures at a continually flowing liquid surface

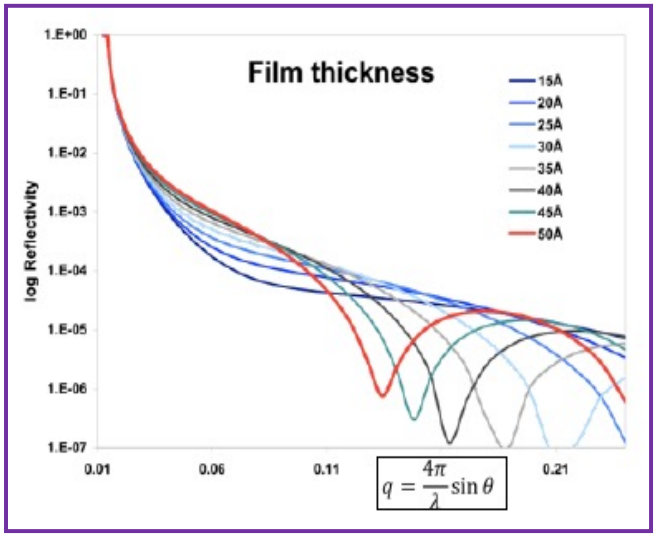
Campbell et al. Eur. Phys. J Plus, 2011, 126, 107

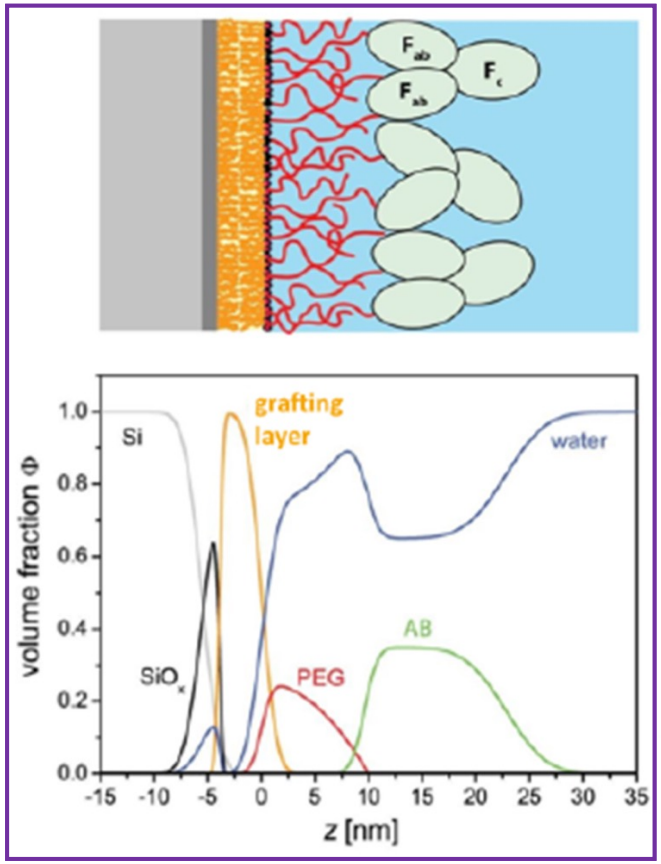
Neutron Reflectometry: 1. Interfacial Structure

- reflectivity of a collimated beam of neutrons at grazing incidence angles
- sensitivity to light elements on length scales in the nanometre range
- isotopic labelling & modelling gives density profiles normal to the interface
- non-invasive and surface-sensitive reflectometry technique
- resolution of the locations of different species in mixtures



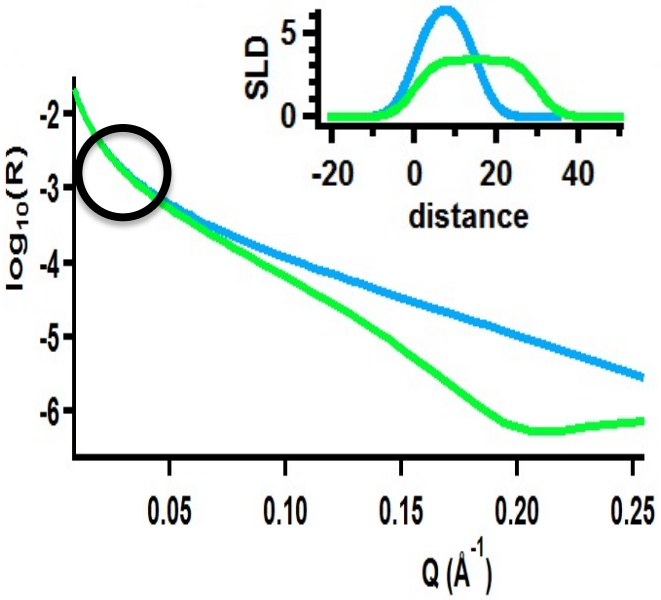


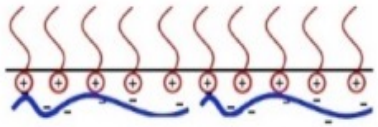




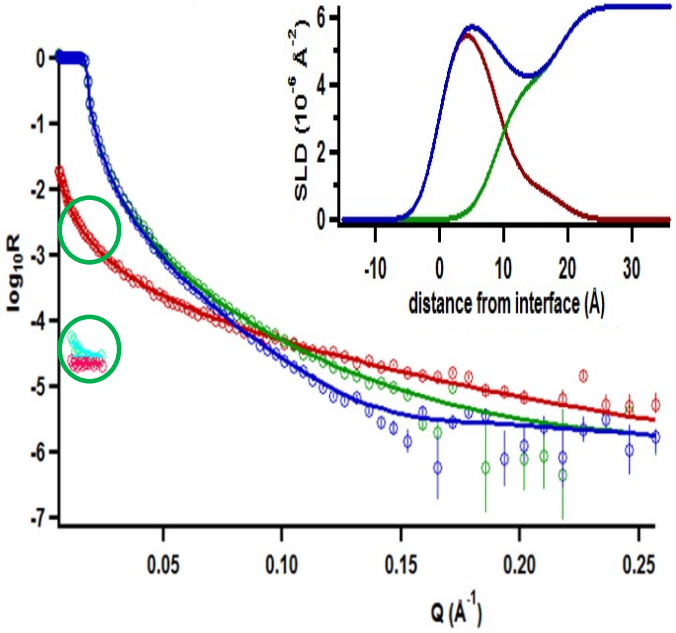
Skoda Curr. Opin. Colloid Interface Sci., 2019, 42, 41

Neutron Reflectometry: 2. Interfacial Composition





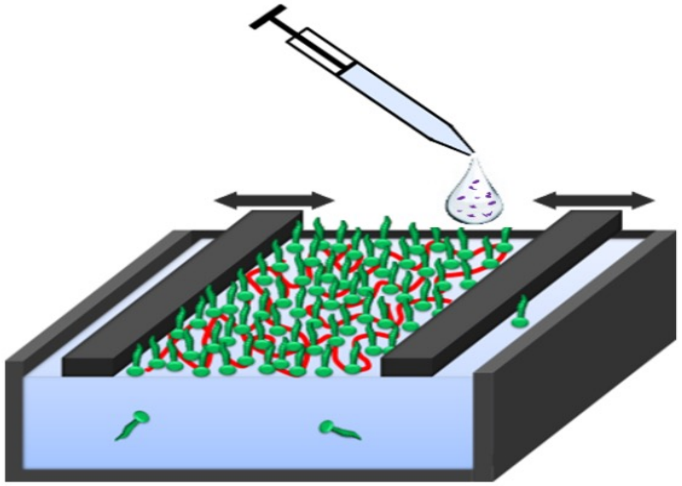
Nucleus	ρ_s / 10^{-5} Å
^{1}H	−3.7406
^{2}H	6.671



$$\sigma.d = \Gamma.b.\mathrm{N_A}$$

$$(\sigma\cdot d)_1 = N_A\cdot\Gamma_{\mathrm{poly}}\cdot b_{i,\mathrm{poly}}$$

$$(\sigma\cdot d)_2 = N_A\cdot(\Gamma_{\mathrm{surf}}\cdot b_{i,\mathrm{d\text{-}surf}} + \Gamma_{\mathrm{poly}}\cdot b_{i,\mathrm{poly}})$$



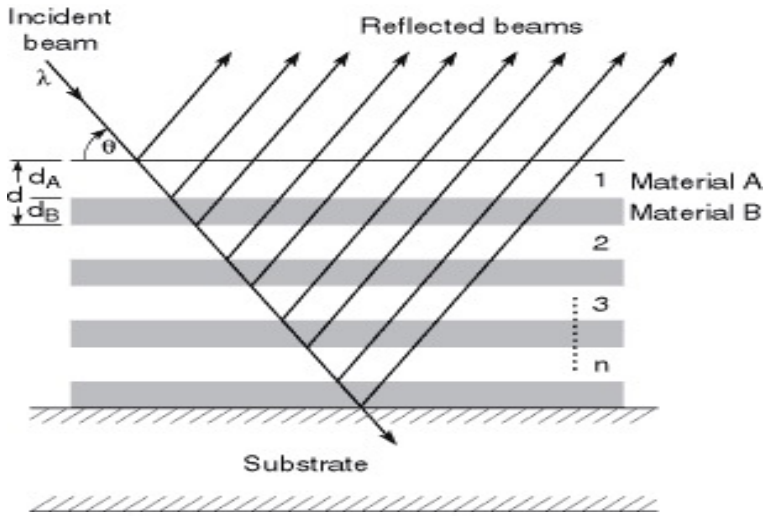
- interfacial composition can be resolved in situ
- measurements take 2 min *cf.* 2 hours
- has opened up research into dynamic systems

Campbell et al. Soft Matter, 2016, 12, 5304

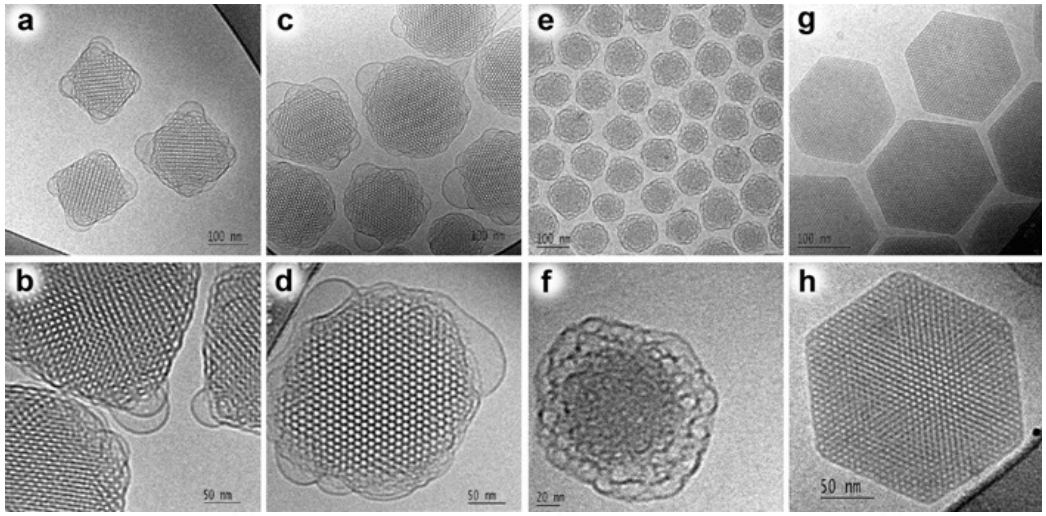
Campbell Curr. Opin. Collloid Interface Sci., 2018, 37, 49

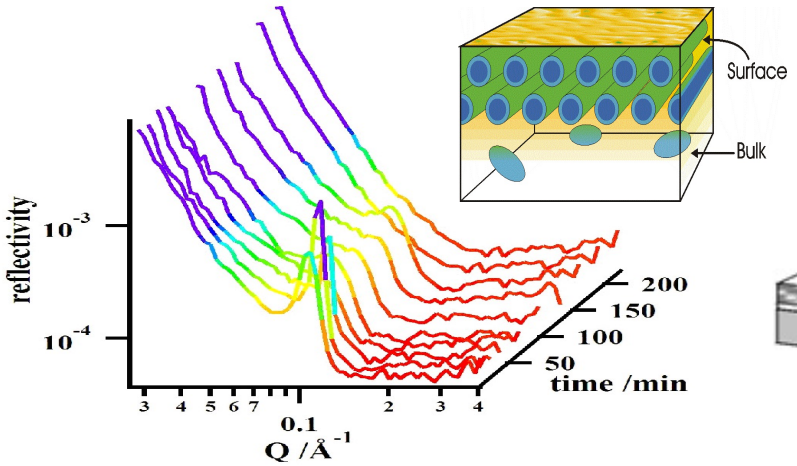
Neutron Reflectometry: 3. Particle Attachment

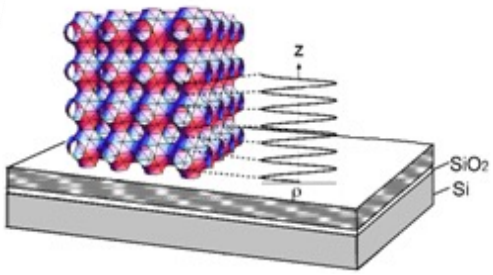
- special condition for multilayers:

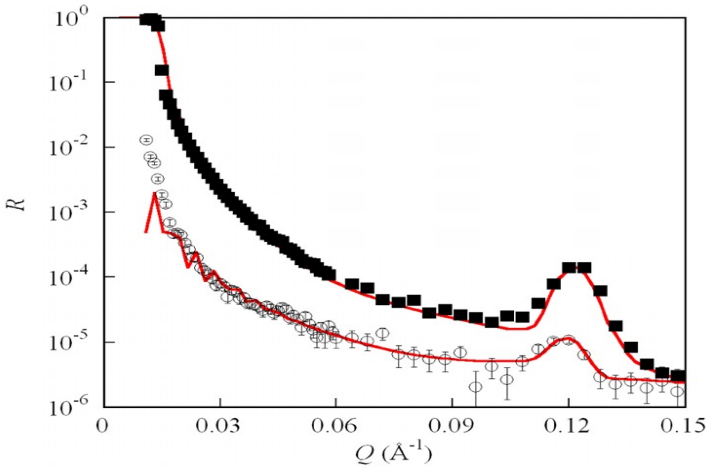


- same applies to liquid crystalline particles:







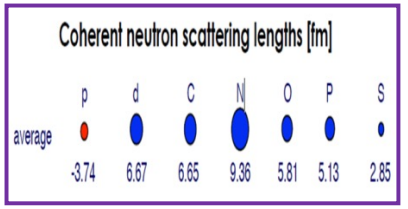


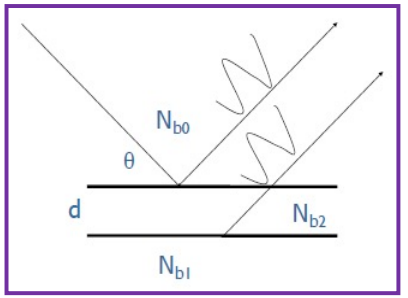
Campbell et al. Soft Matter, 2011, 7, 11125

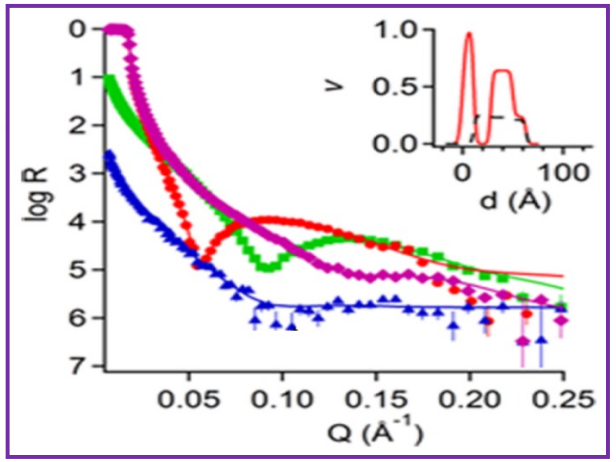
Vandoolaeghe et al. J. Phys. Chem. B, 2008, 113, 4483

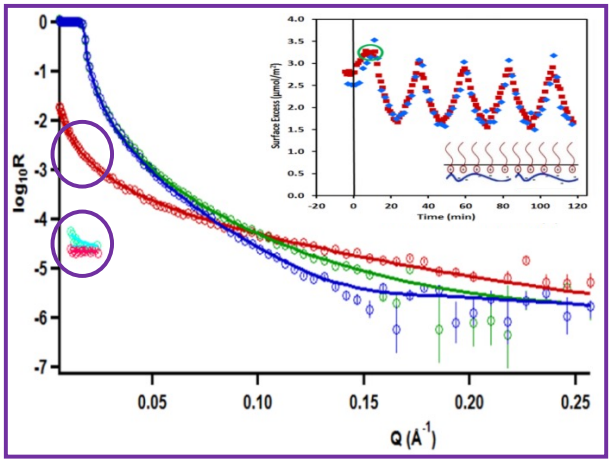
Neutron Reflectometry: Summary

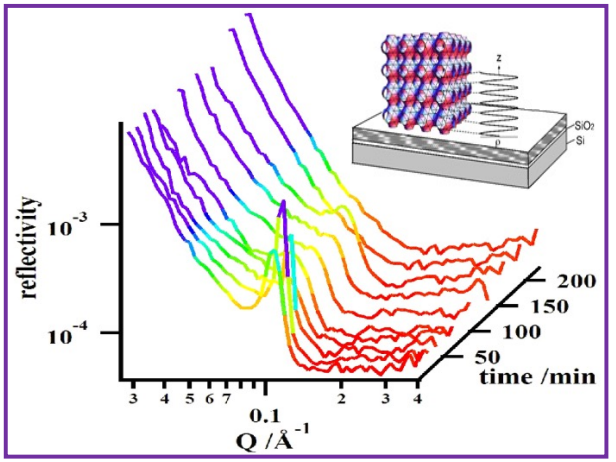
- interfacial structure:
  → full Q-range measurements in multiple isotopic contrasts
- interfacial composition:
  → low-Q measurements exploiting low contrast conditions
- particle attachment:
  → Bragg diffraction peak(s) in reflectivity profiles







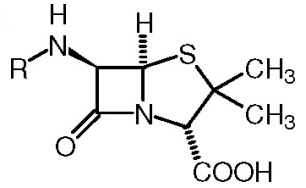


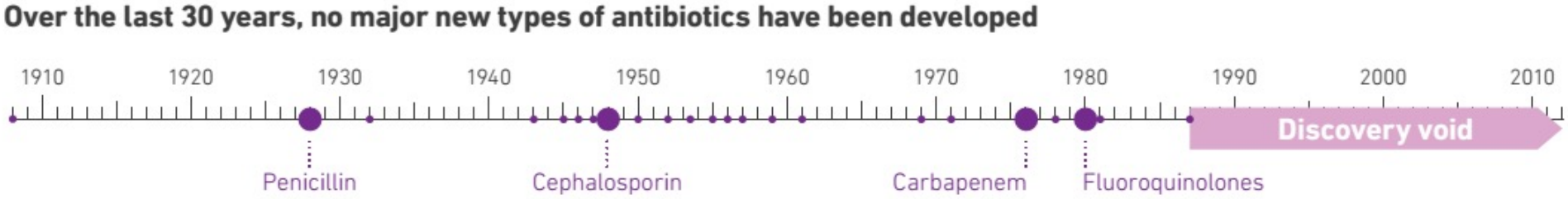


almost like three techniques in one!

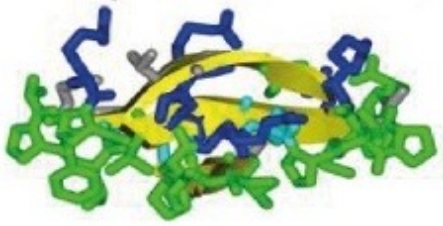
1. Structural Disruption by Antimicrobial Peptides

- antibiotic function mechanisms:
  - → interference with cell wall synthesis (penicillins, vanconmycin)
  - → protein synthesis inhibition (clindamycin, tetracyclines)
  - → interference with nucleic acid synthesis (fluoroquinolones, rifampin)
  - → inhibition of metabolic pathway (sulfonamides, folic acid)





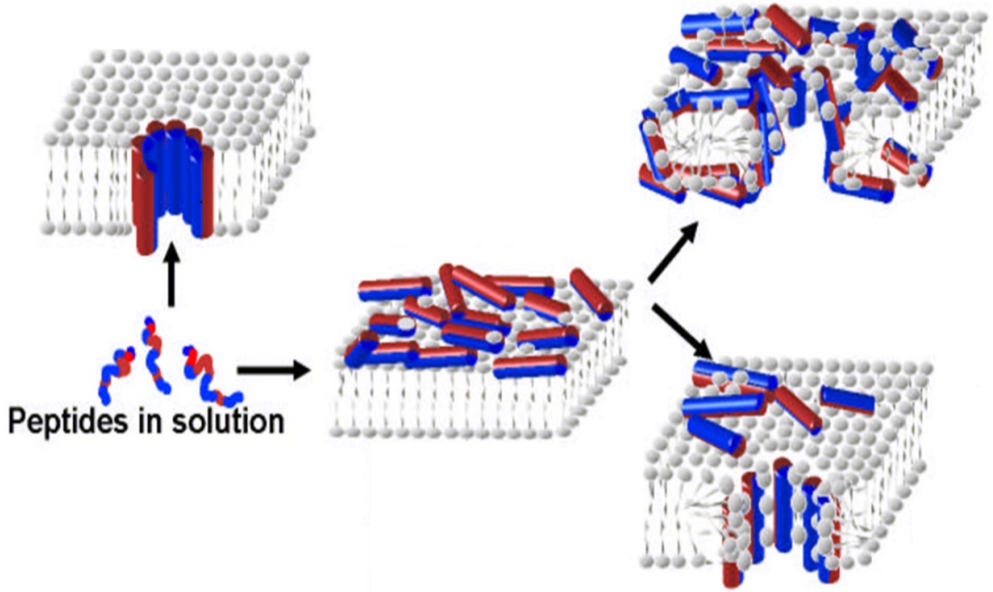
- disruption of bacterial membrane structure (polymyxins, daptomycin, AMPs)
- AMPs = antimicrobial peptides:
  - → at least 2 positively charged residues
  - → usually more than 50% hydrophobic residues

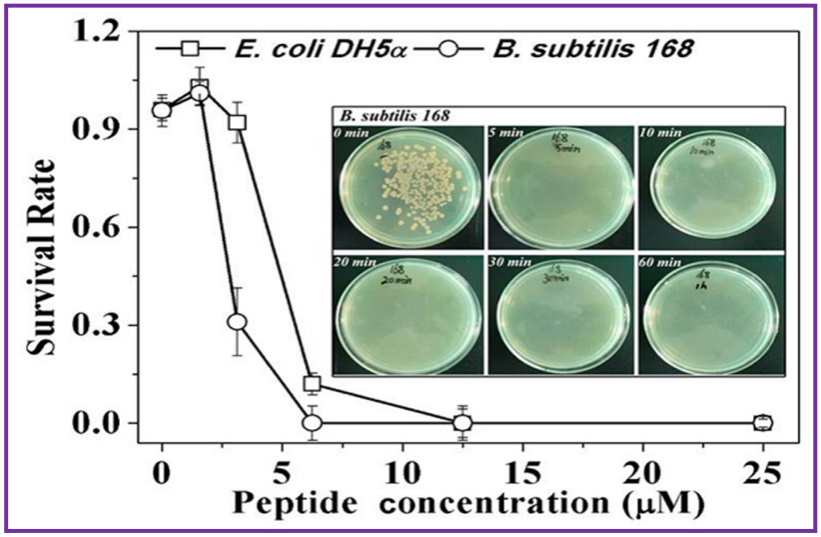


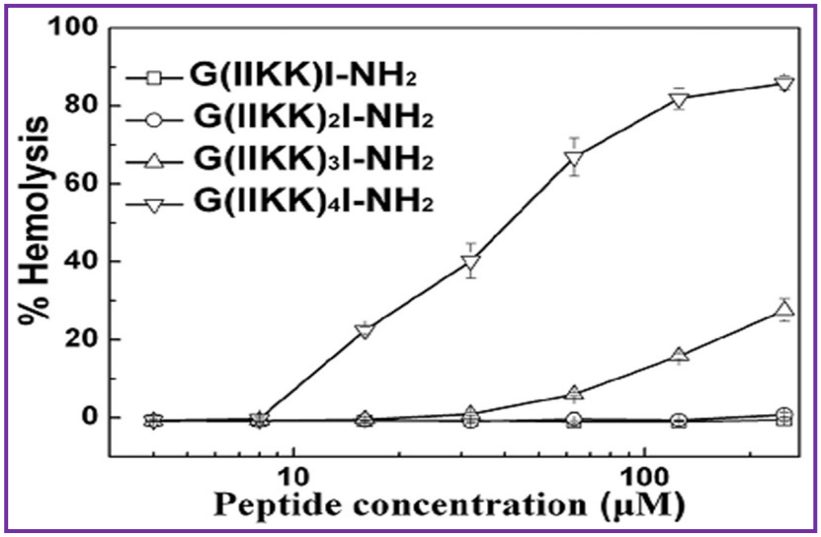
1. Structural Disruption by Antimicrobial Peptides

- work underway on designed AMP series:
  - → University of Manchester (UK)
  - → Petroleum University (China)
  - → Institut Laue-Langevin (France)

$$G(IIKK)_nI\text{-}NH_2$$



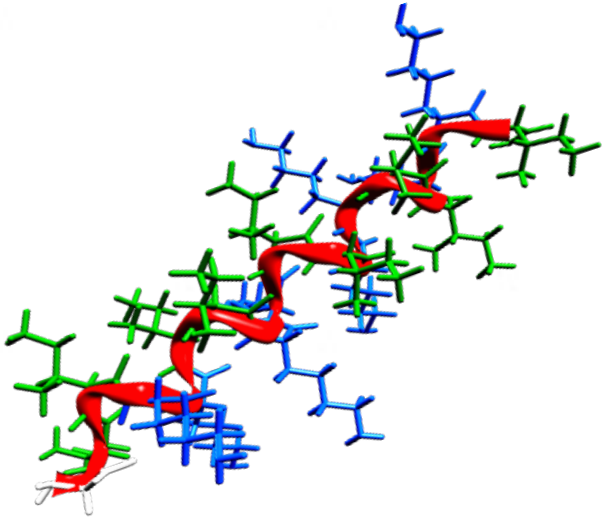




Chen et al. ACS Appl. Mat. Interfaces, 2014, 6, 16529

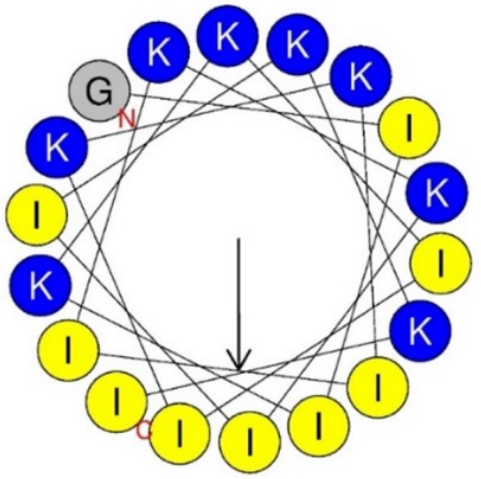
1. Structural Disruption by Antimicrobial Peptides

- minimum inhibitory concentration study conducted
- n = 4 peptide most promising
- wheel projection gives structural clues
- multi-technique interactions study initiated



$G(IIKK)_4I\text{-}NH_2$

peptides	MIC (μM) *E. coli*	MIC (μM) *B. subtilis*
G(IIKK)I		
$G(IIKK)_2I$	125 ± 5.6	30 ± 2.5
$G(IIKK)_3I$	8 ± 0.2	2 ± 0.5
$G(IIKK)_4I$	2 ± 0.5	0.5 ± 0.1
magainin-2[b]	38	>80
melittin[c]	3.9 ± 0.6	2.0 ± 0.2
ampicillin[d]	~11	5.5



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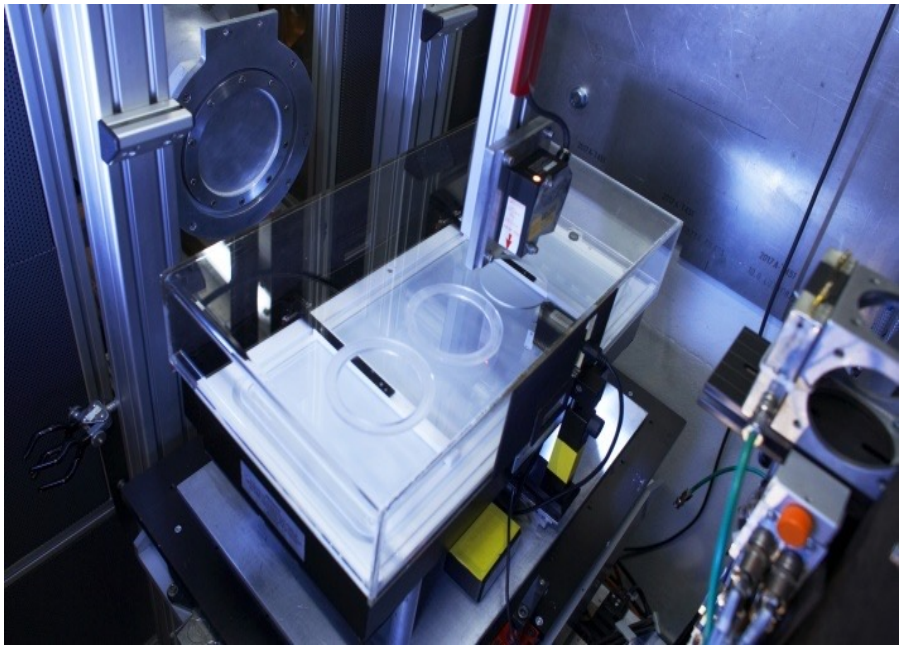
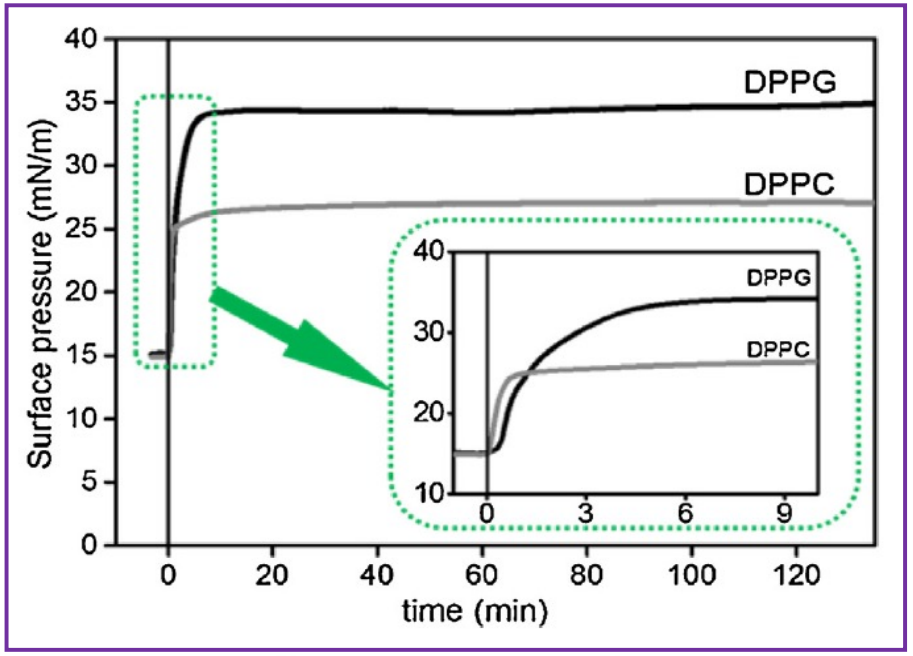
1. Structural Disruption by Antimicrobial Peptides

- charge on outer surfaces of cells: bacterial = negative *cf.* mammalian = lower
- first let's characterize the interactions of $G(IIKK)_4I\text{-}NH_2$ with 2 model saturated phospholipid membranes: DPPC (zwitterionic) and DPPG (anionic)
- 3 μM of $G(IIKK)_4I\text{-}NH_2$ was injected in the subphase at 15 mN/m





- but extent of peptide binding & interfacial structure were not resolved, so...

Ciumac et al. Colloids Surf. B, 2017, 150, 308

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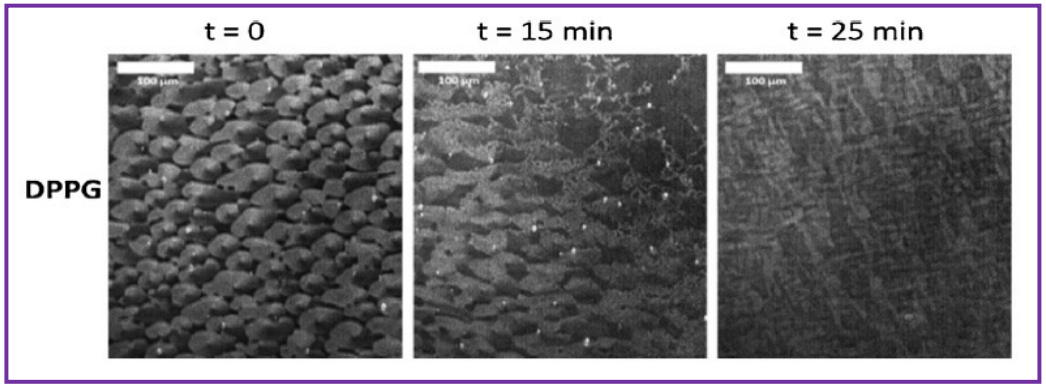
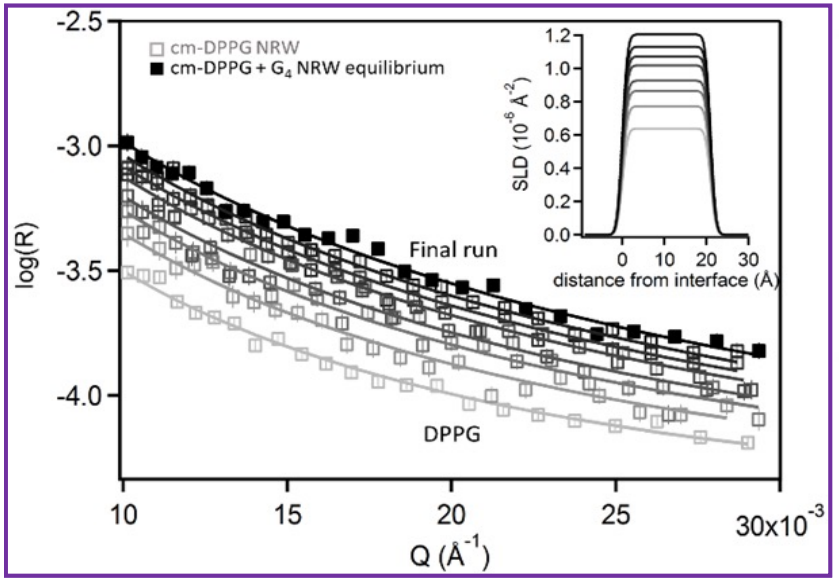
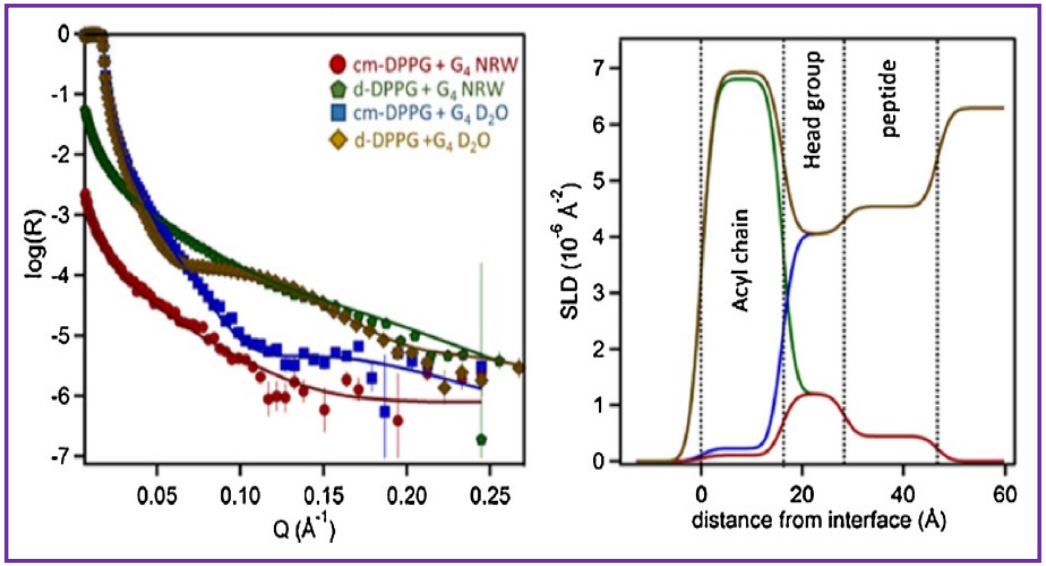
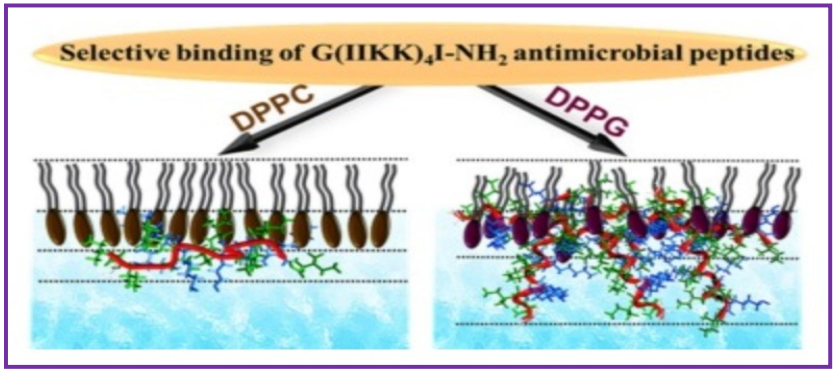
1. Structural Disruption by Antimicrobial Peptides







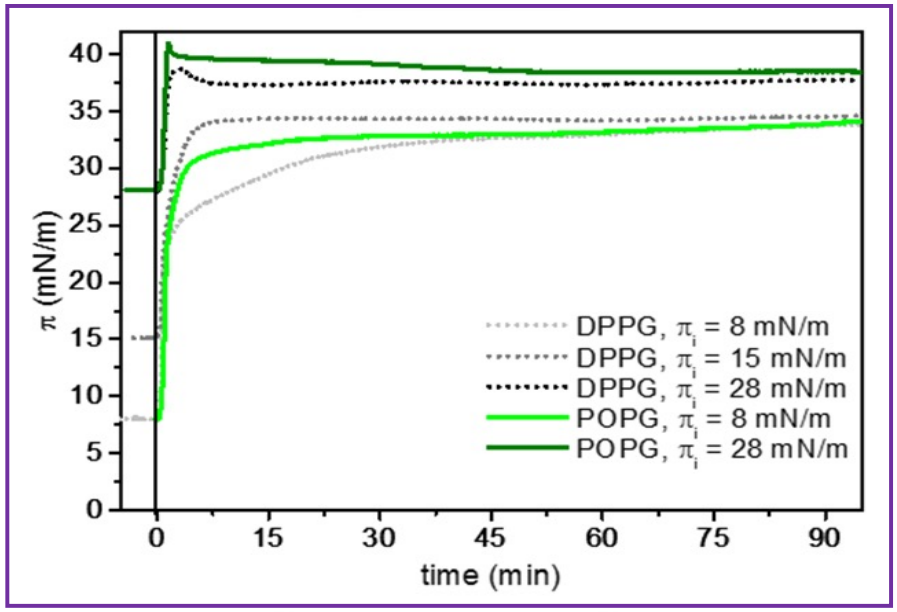


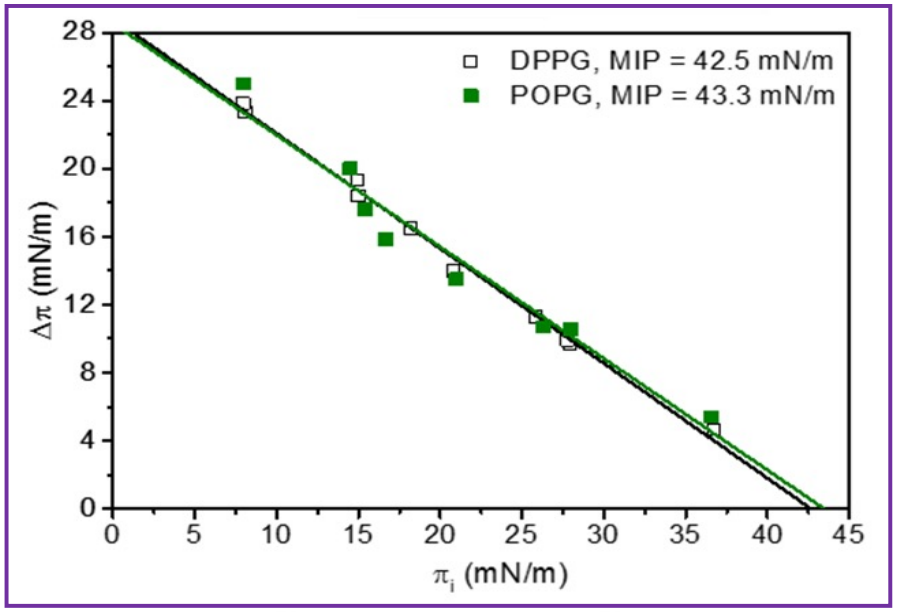
- G_4 binding is faster, more disruptive & more extensive to DPPG than DPPC

Ciumac et al. Colloids Surf. B, 2017, 150, 308

1. Structural Disruption by Antimicrobial Peptides

- further important variables are the degree of saturation of the phospholipids and the surface pressure in the model membrane
- next let's characterize the interactions of $G(IIKK)_4I\text{-}NH_2$ with respect to 2 model anionic membranes: DPPG (saturated) and POPG (unsaturated)
- work was carried out at surface pressures of 8, 15 and 28 mN/m

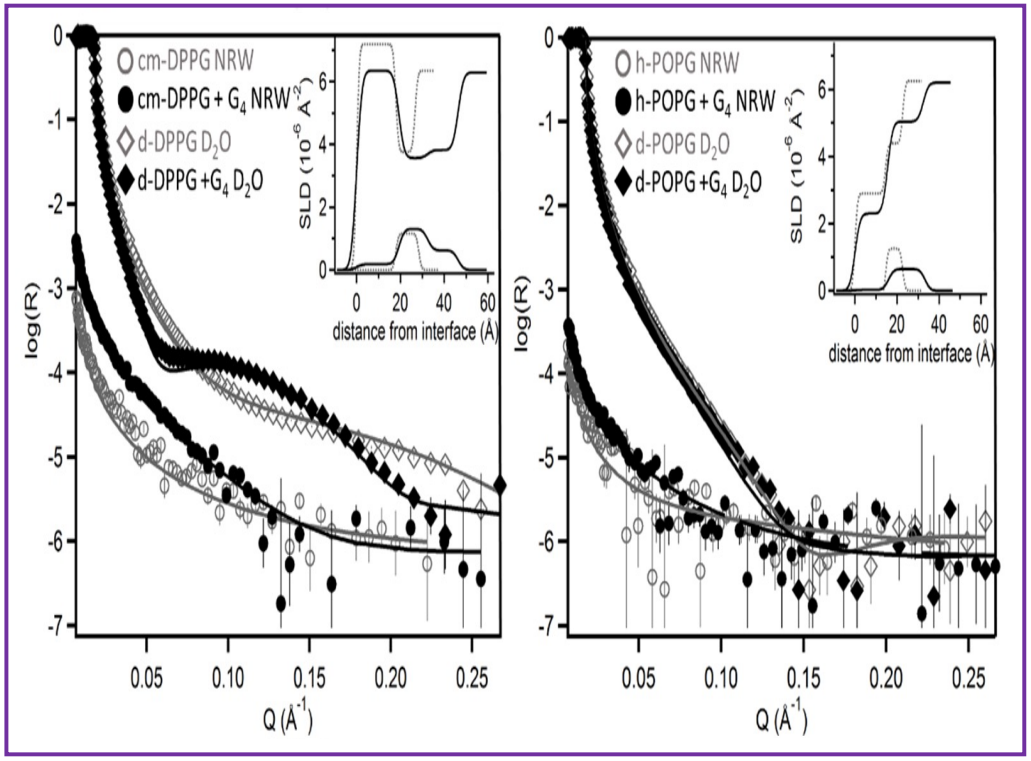


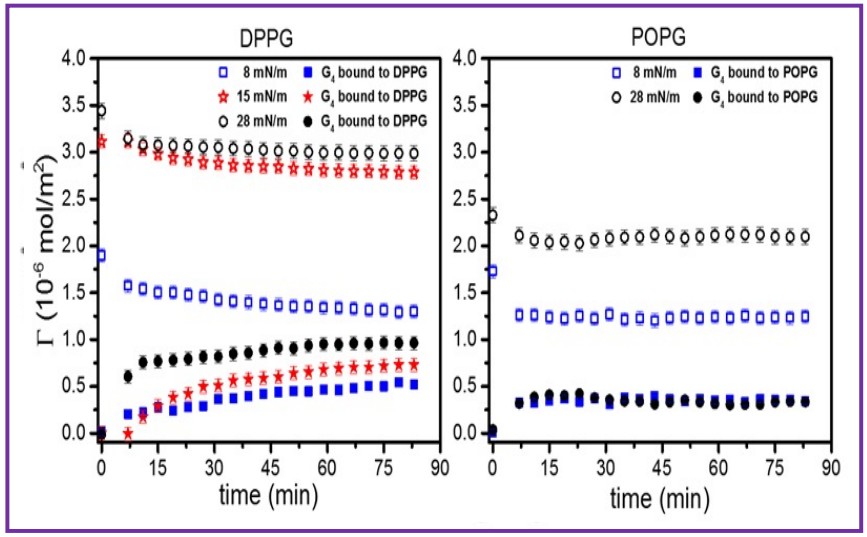


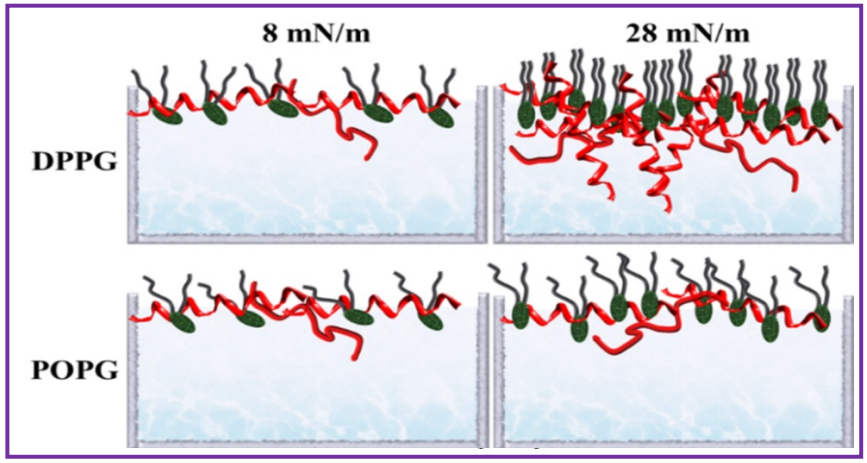
- maximum insertion pressure about the same (43 mN/m) for both lipids, so...

Ciumac et al. ACS Omega, 2017, 2, 7482

1. Structural Disruption by Antimicrobial Peptides







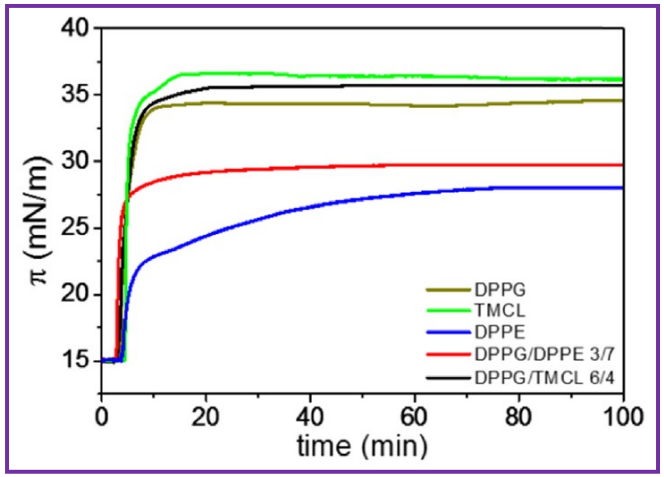
- acyl chain saturation deeply promotes binding of $G(IIKK)_4I\text{-}NH_2$
- binding is much more extensive at higher surface pressures of greater relevance to those found in cell membranes

- next: mixed monolayers...

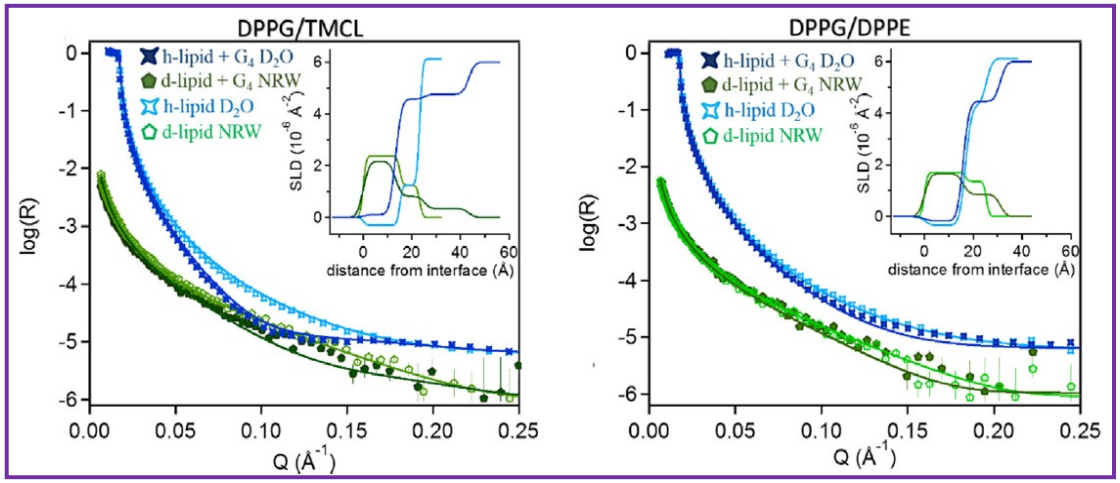
Ciumac et al. ACS Omega, 2017, 2, 7482

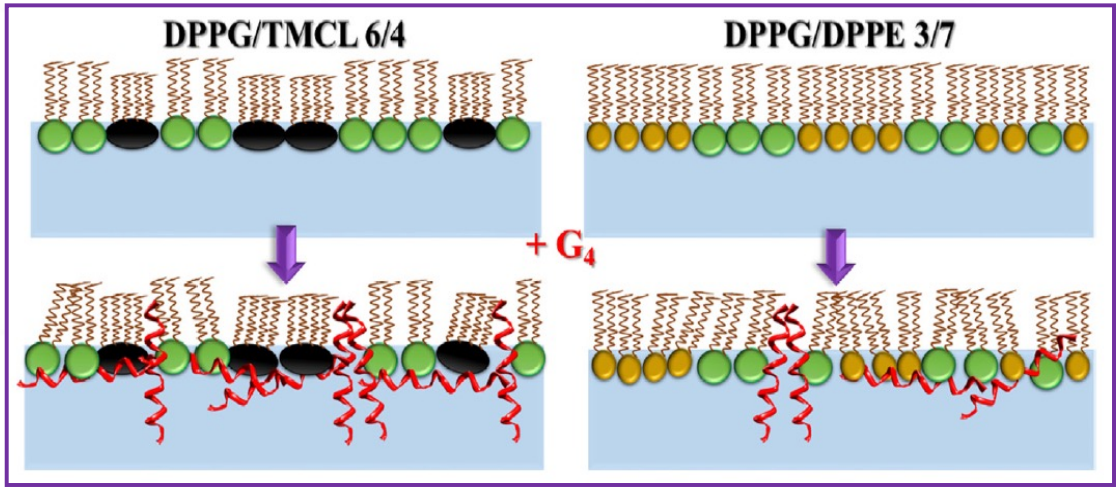
1. Structural Disruption by Antimicrobial Peptides

- initial studies were built on to study the peptide interactions with lipid mixtures
- DPPG/TMCL 6:4 mimiked gram positive bacterial membranes
- DPPG/DPPE 3:7 mimiked gram negative bacterial membranes



- work indicated greater efficacy of this peptide to gram positive bacterial membranes





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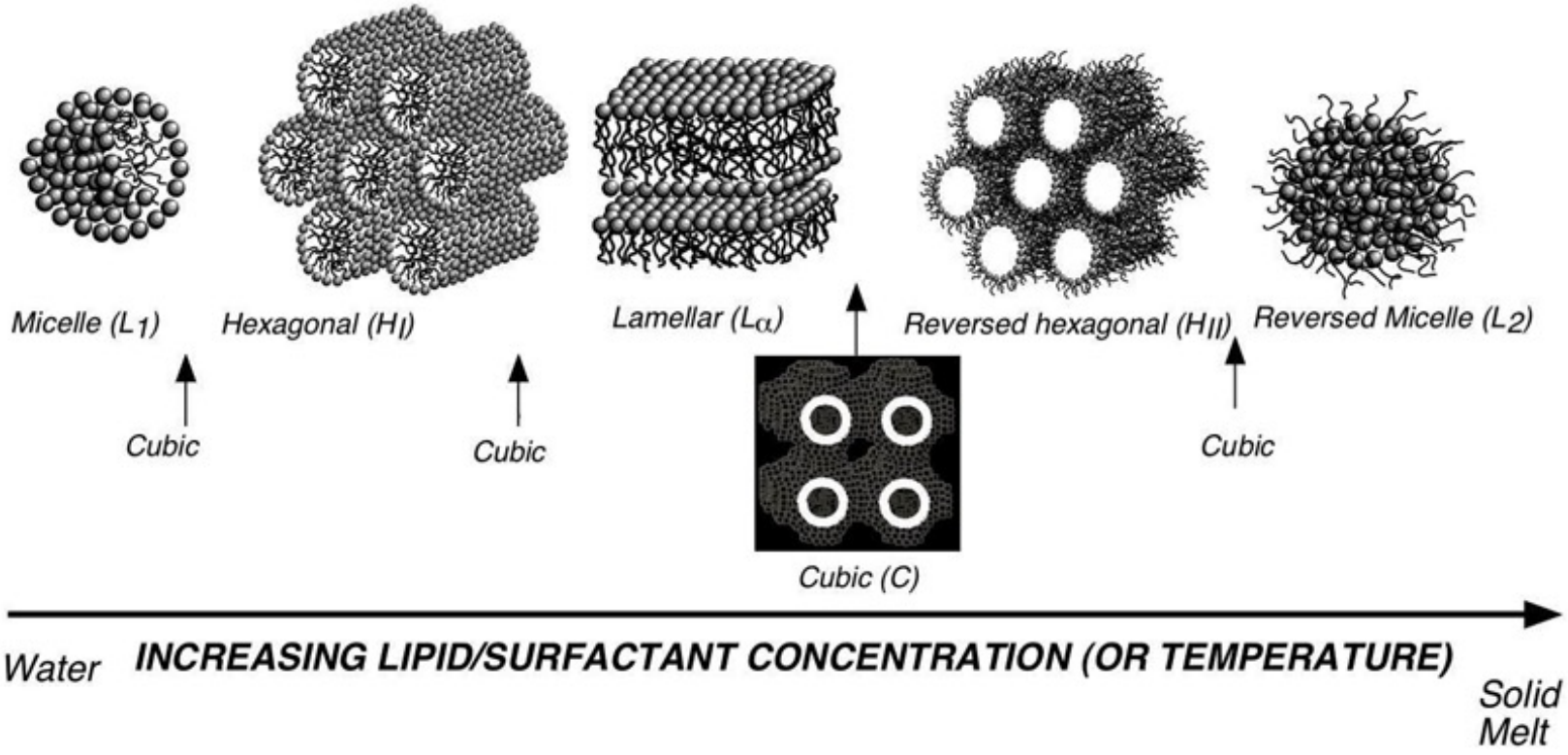
2. Membrane Binding of Liquid Crystalline Nanocarriers

- self-assembled lipid-based structures have potential as delivery vehicles
- lipids can be dispersed into liquid crystalline nanoparticles
- variety of 3D structures in which drugs can be solubilised
- resulting formulations can have improved drug uptake and storage time



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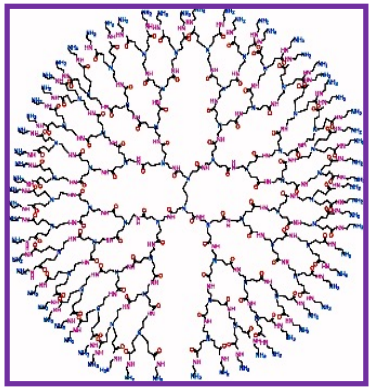
2. Membrane Binding of Liquid Crystalline Nanocarriers

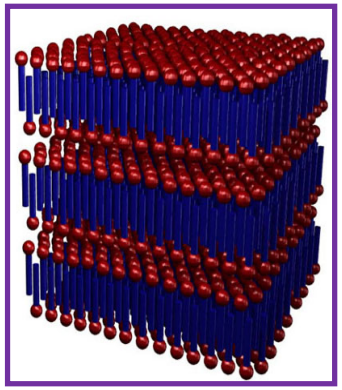
- dendrimer/phospholipid mixtures produce liquid crystalline nanoparticles
- poly(amido amine) dendrimers induce collapse of POPC/POPG lipid vesicles into lamellar multilayer stacks
- particles have potential to solubilise/encapsulate/deliver drug molecules
- work underway on the interactions of these particles with model biomembranes:

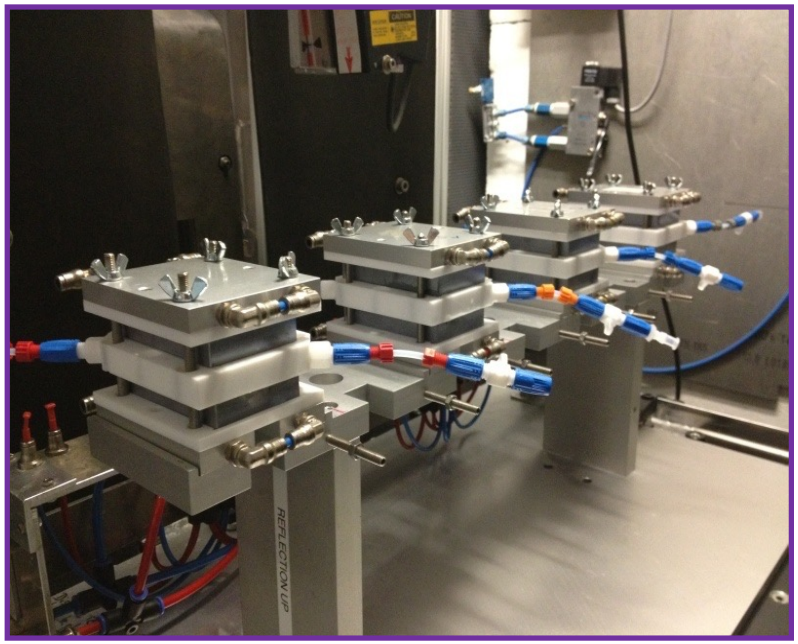
→ University of Copehagen (Denmark)

→ Los Alamos National Laboratory (USA)

→ Institut Laue-Langevin (France)



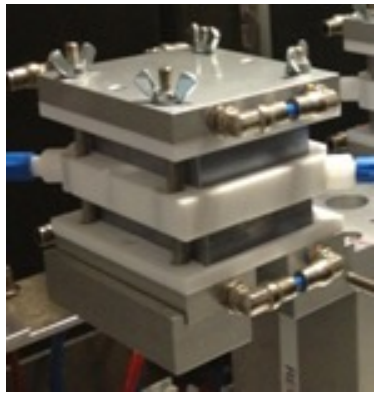


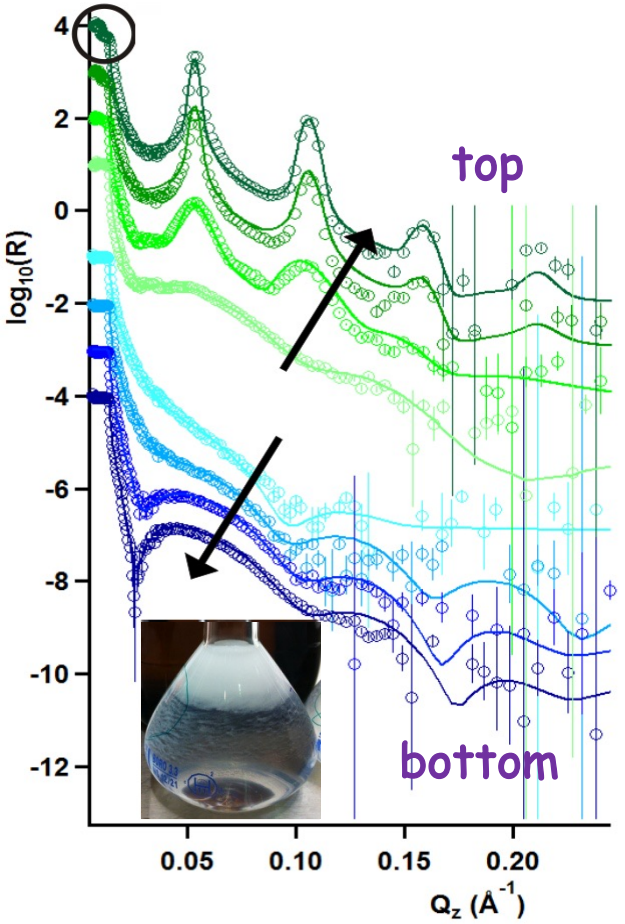


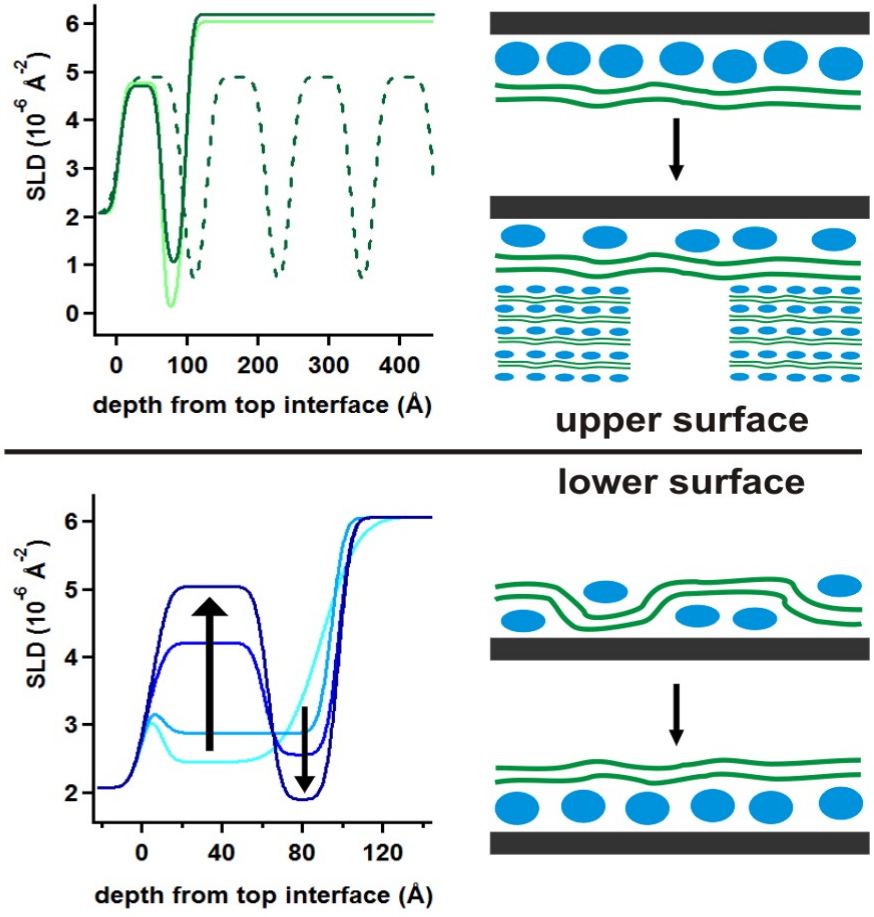
Campbell et al. J. Phys. Chem. B, 2012, 116, 7981

2. Membrane Binding of Liquid Crystalline Nanocarriers

- interactions with bare hydrophilic silica surfaes:





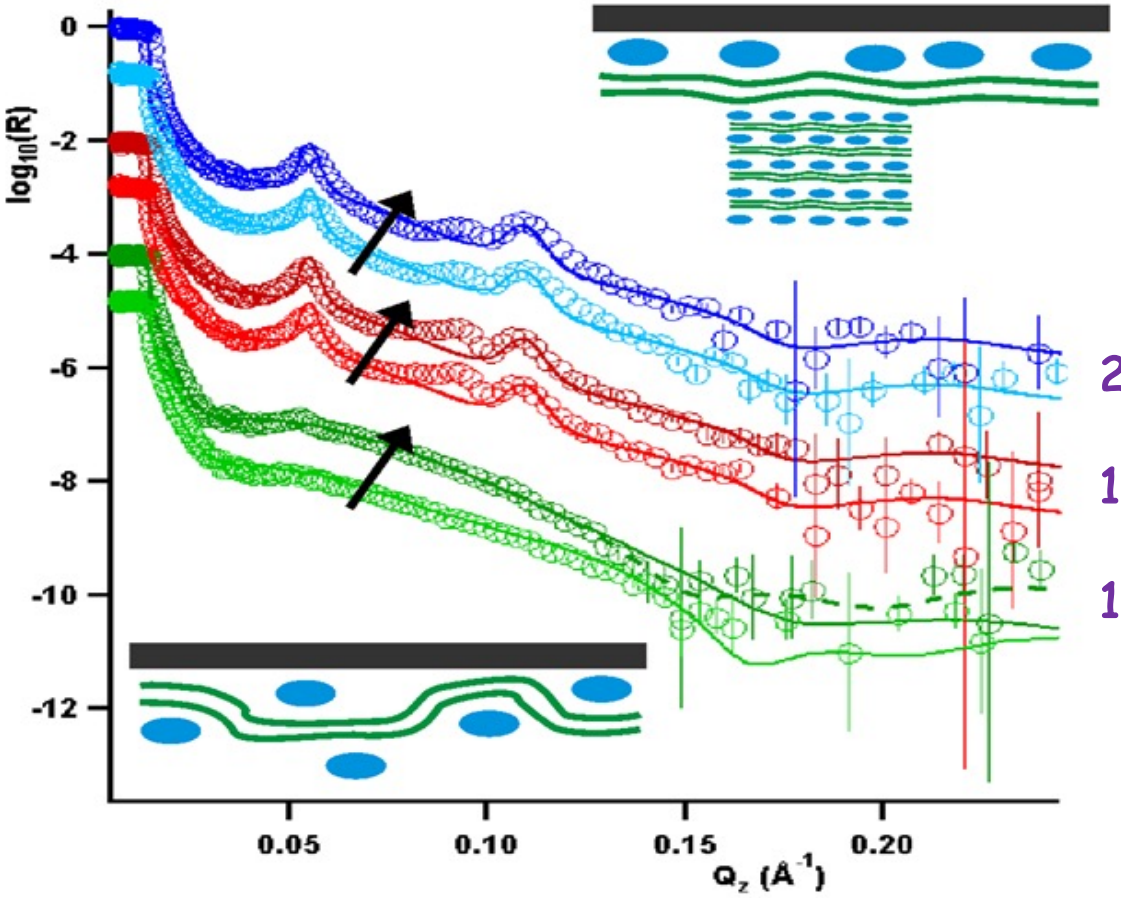


- gravity is an essential parameter to control in trials of such formulations

Campbell et al. ACS Macro Letters, 2014, 3, 121

2. Membrane Binding of Liquid Crystalline Nanocarriers

- interactions with pre-formed supported lipid POPC/POPG bilayers:



25% negative charge

17.5% negative charge

10% negative charge

- potential for exploiting interaction mechanism for targeting cancer cells

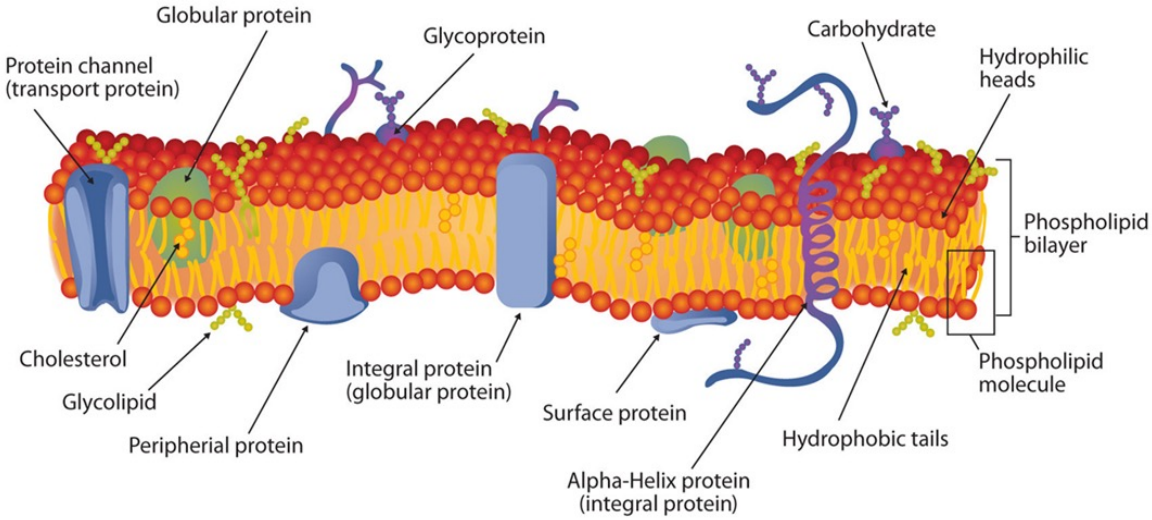
Campbell et al. ACS Macro Letters, 2014, 3, 121

3. Interactions of Anti-Cancer Drugs

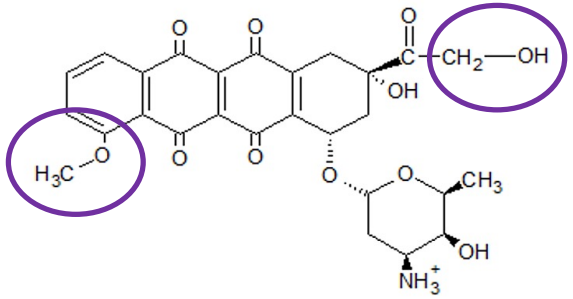
- anthracycline antibiotics including doxorubicin (DOx) and idarubicin are used as structurally similar anti-cancer drugs
- mode of action is through intercalation into DNA but the outer cell membrane is the first barrier to be crossed
- work underway on the interactions & delivery of DOx with model membranes:

→ University of Warsaw (Poland)

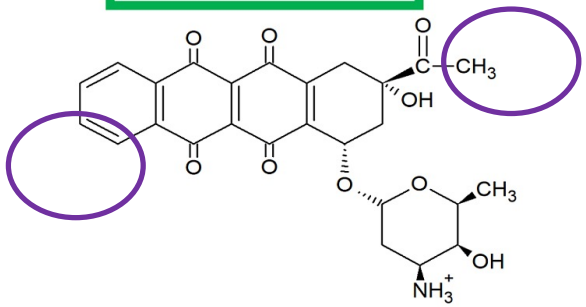
→ Institut Laue-Langevin (France)



DOXORUBICIN



IDARUBICIN



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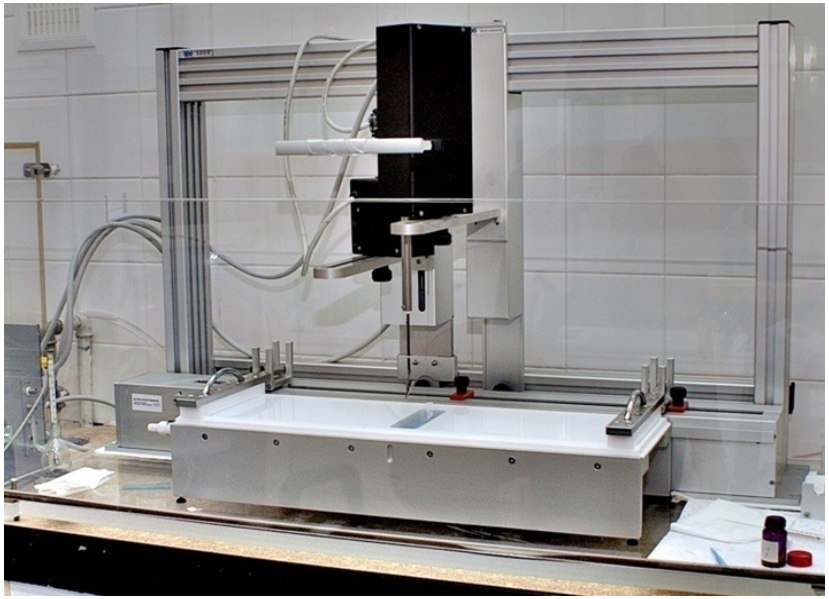
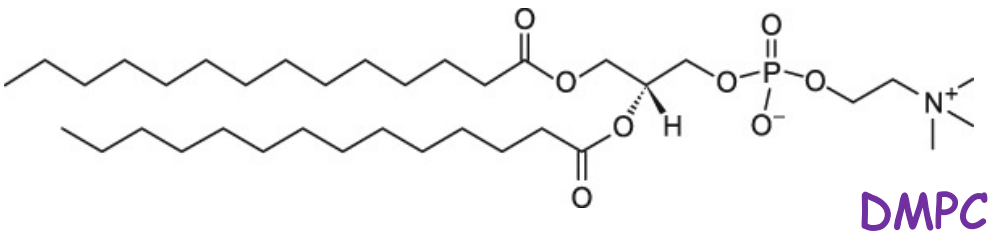
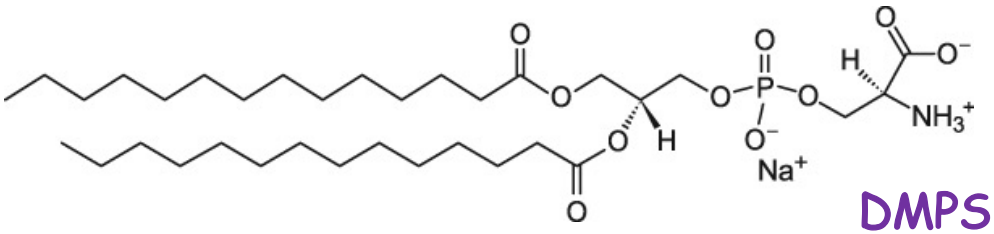
3. Interactions of Anti-Cancer Drugs

- healthy cell membranes are known to contain a high proportion of zwitterionic phosphatidylcholines such as DMPC
- cancer cell membranes have been shown to have elevated amounts of negatively charged phospatidylserines such as DMPS
- DOx has been shown to be more effective on breast cancer cells containing a higher proportion of negatively charged lipids
- Langmuir approach applied to DOx interaction with DMPC and DMPS monolayers





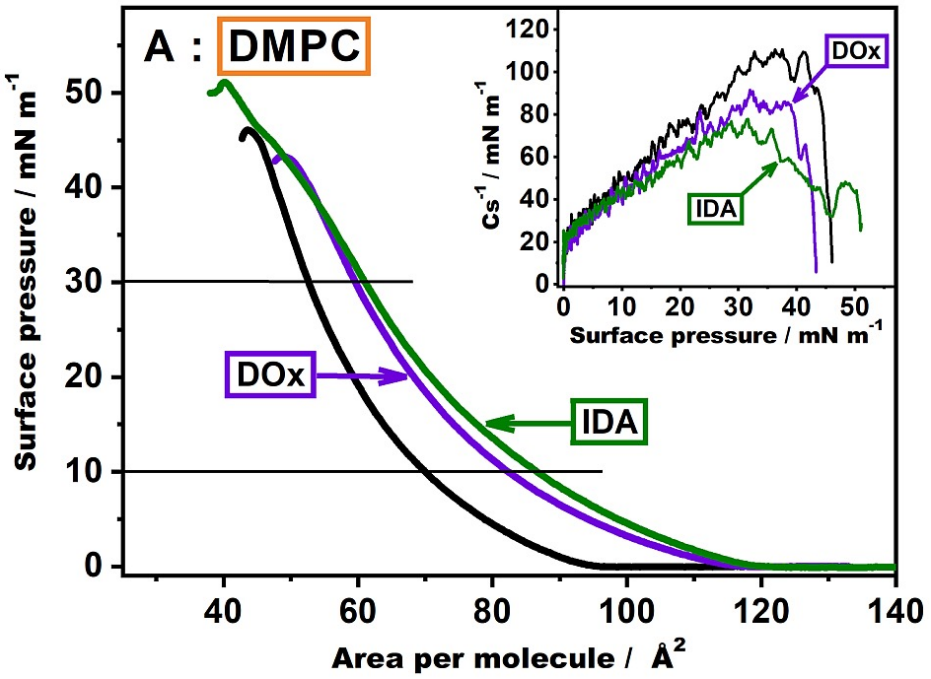
DMPC

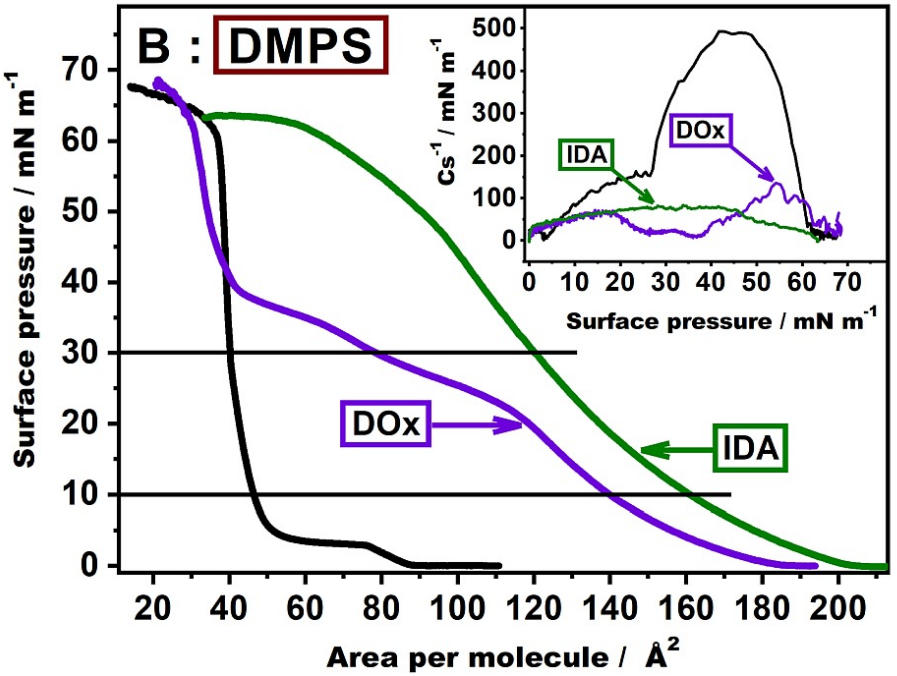


DMPS

3. Interactions of Anti-Cancer Drugs

- interactions of DOx with DMPC and DMPS monolayers were studied with surface pressure isotherms on a Langmuir trough
- stronger interaction of DOx with DMPS than with DMPC



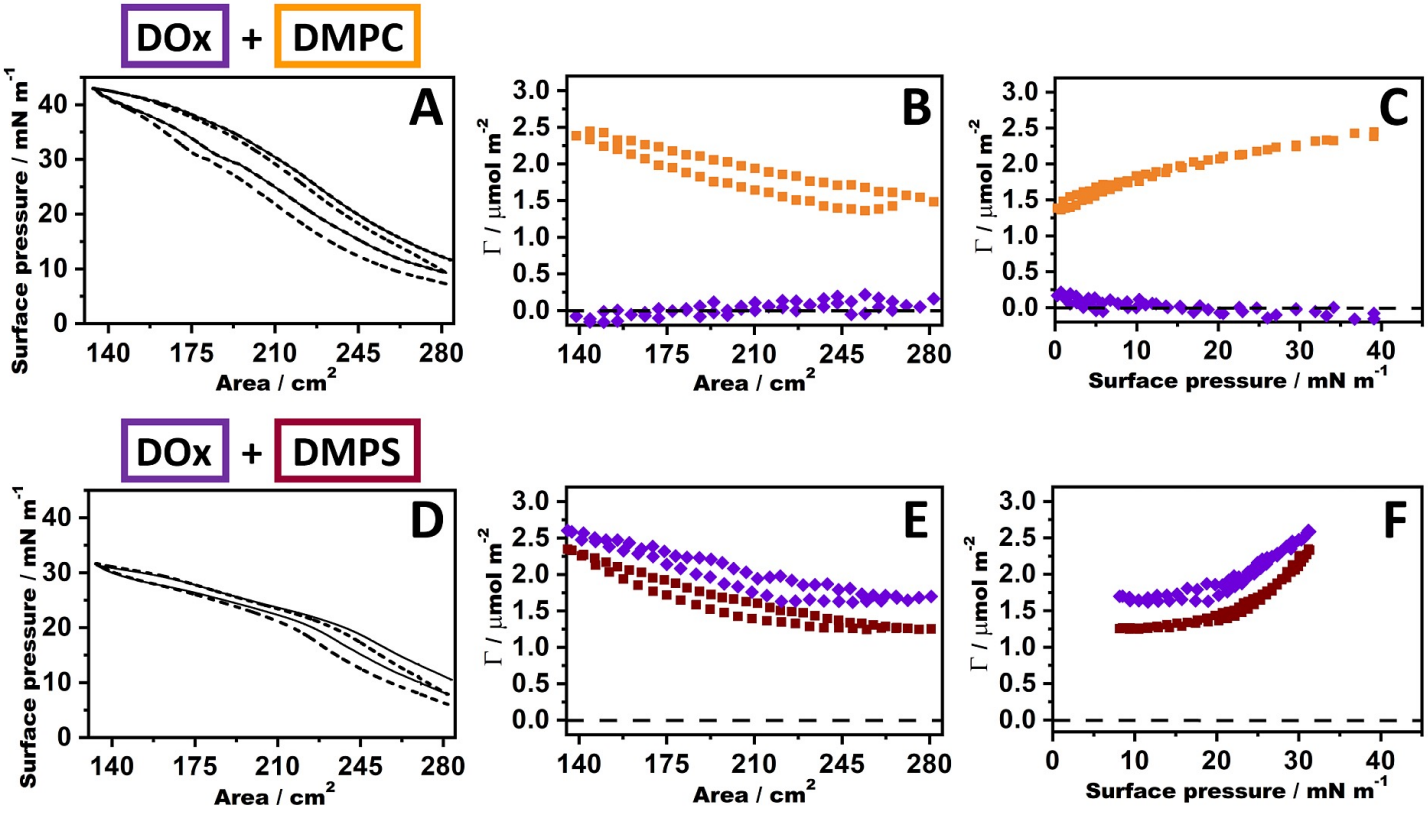


- but extent of interaction and interfacial structure were not resolved, so...

Matyszewska et al. J. Colloid Interface Sci., 2021, 581, 403

3. Interactions of Anti-Cancer Drugs

- studied interfacial composition using the low-Q analysis on FIGARO



- stronger DOx interaction with DMPS than DMPC quantified for the first time

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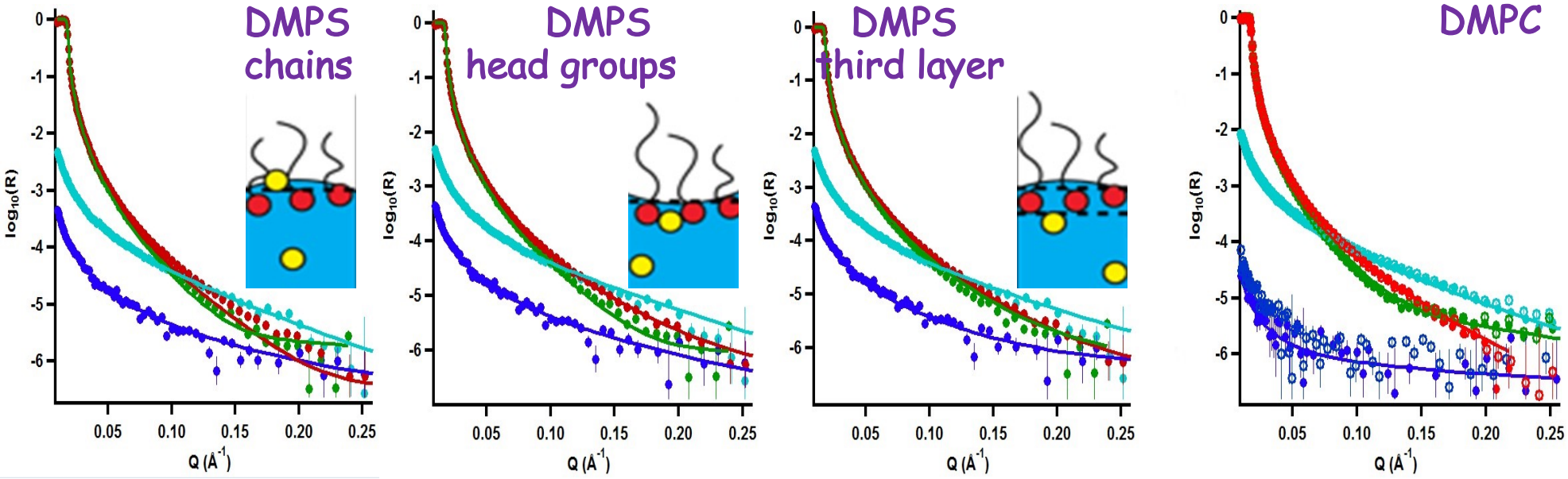
3. Interactions of Anti-Cancer Drugs

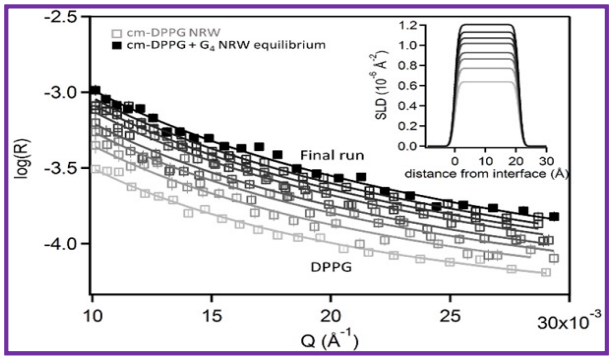
- different structural models were applied to data on the interaction of DOx with DMPS monolayers at a starting surface pressure of 10 mN/m

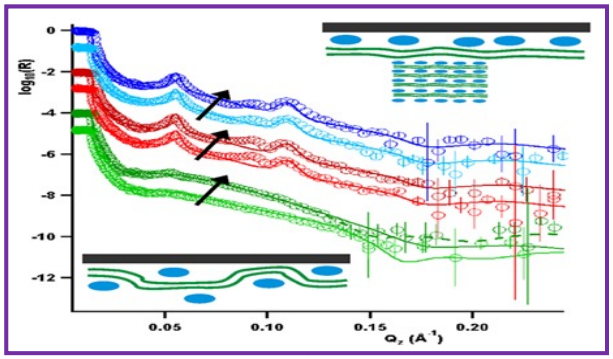


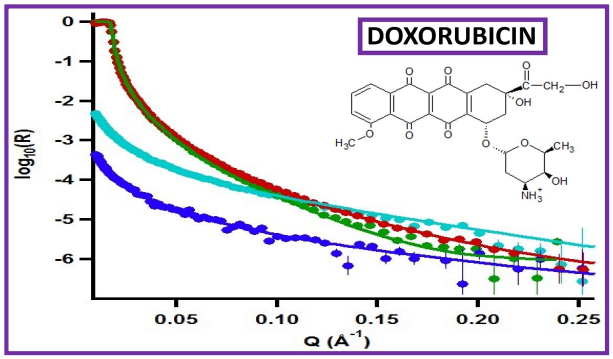
- model fits showed the drug binding preferentially in/around the head groups
- reduction of lipid surface excess by three quarters (4.4 to 1.1 μmol/m^2)
- equimolar replacement by drug (2.8 μmol/m^2)
- but binding of DOx to DMPC monolayers at 10 mN/m was minimal

Matyszewska et al. J. Colloid Interface Sci., 2021, 581, 403

Summary & Outlook







- structural disruption by antimicrobial peptides:
  - → effects of lipid charge, saturation and surface pressure investigated
  - → extension to more realistic model system will relate to results from trials
- membrane binding of liquid crystalline nanocarriers:
  - → effects of gravity and electrostatics on particle interactions resolved
  - → potential of using such systems for a range of novel biosensing devices
- interactions of anti-cancer drugs:
  - → initial studies conducted on interactions with model membranes
  - → extent of interaction and interfacial structure quantified for the first time