SANS in Soft Matter

Prof Karen Edler
Department of Chemistry
Centre for Analysis and Synthesis
Lund University

UNIVERSITY OF BATH

LUND UNIVERSITY

Soft Matter

- "molecular systems giving a strong response to very weak command signal" deGennes (1991)

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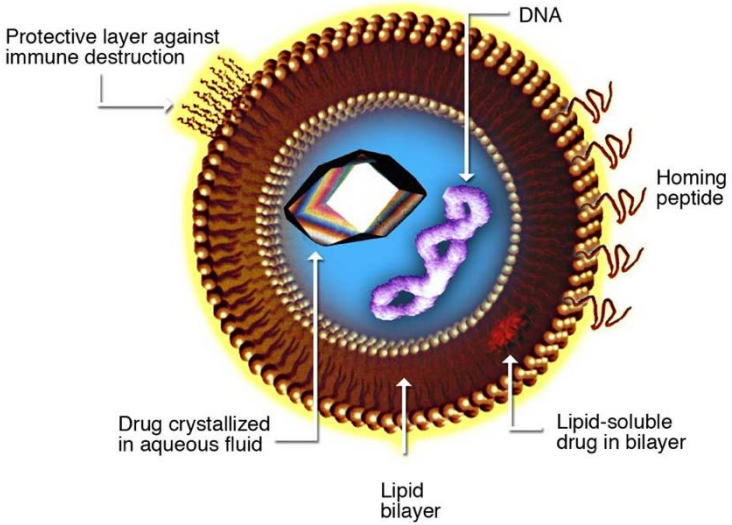
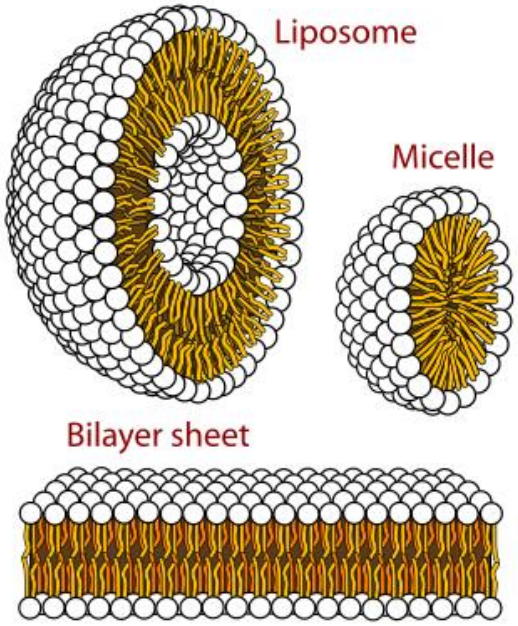
- Refers to condensed matter, but with states deformed by small external fields
  - thermal stress or fluctuations
  - mechanical stress; shear & flow
  - energy scales comparable with room temperature thermal energy

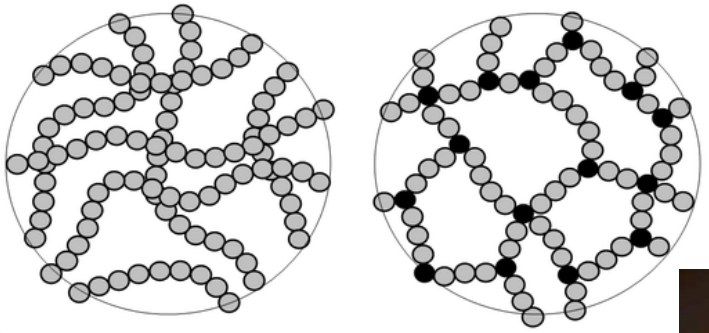


- Includes liquids, colloids, polymers, foams, gels, granular materials, liquid crystals, biological materials

Soft Matter

- Distinctive behaviour due to tendency to self-organize on mesoscale (1-50nm)
  - structures that are larger than atoms/molecules but smaller than macroscopic scale of the material





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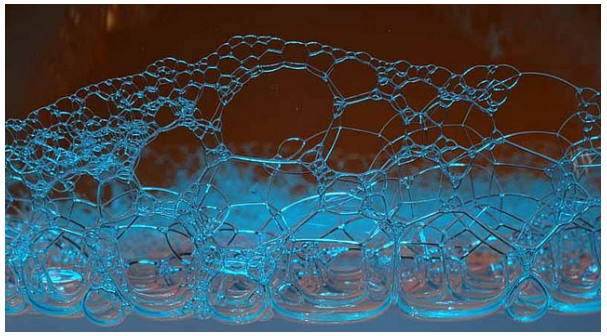
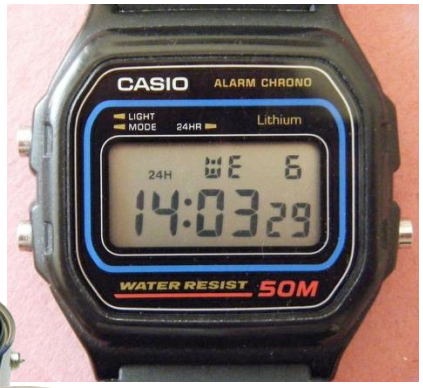
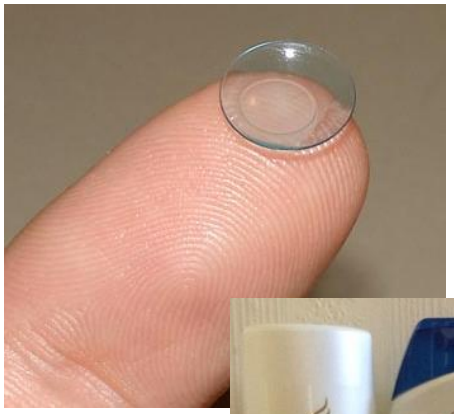


http://www.flickr.com/photos/ross_elliott/4568836584 Ross Elliot CC BY 2.0

Common Soft Matter Materials





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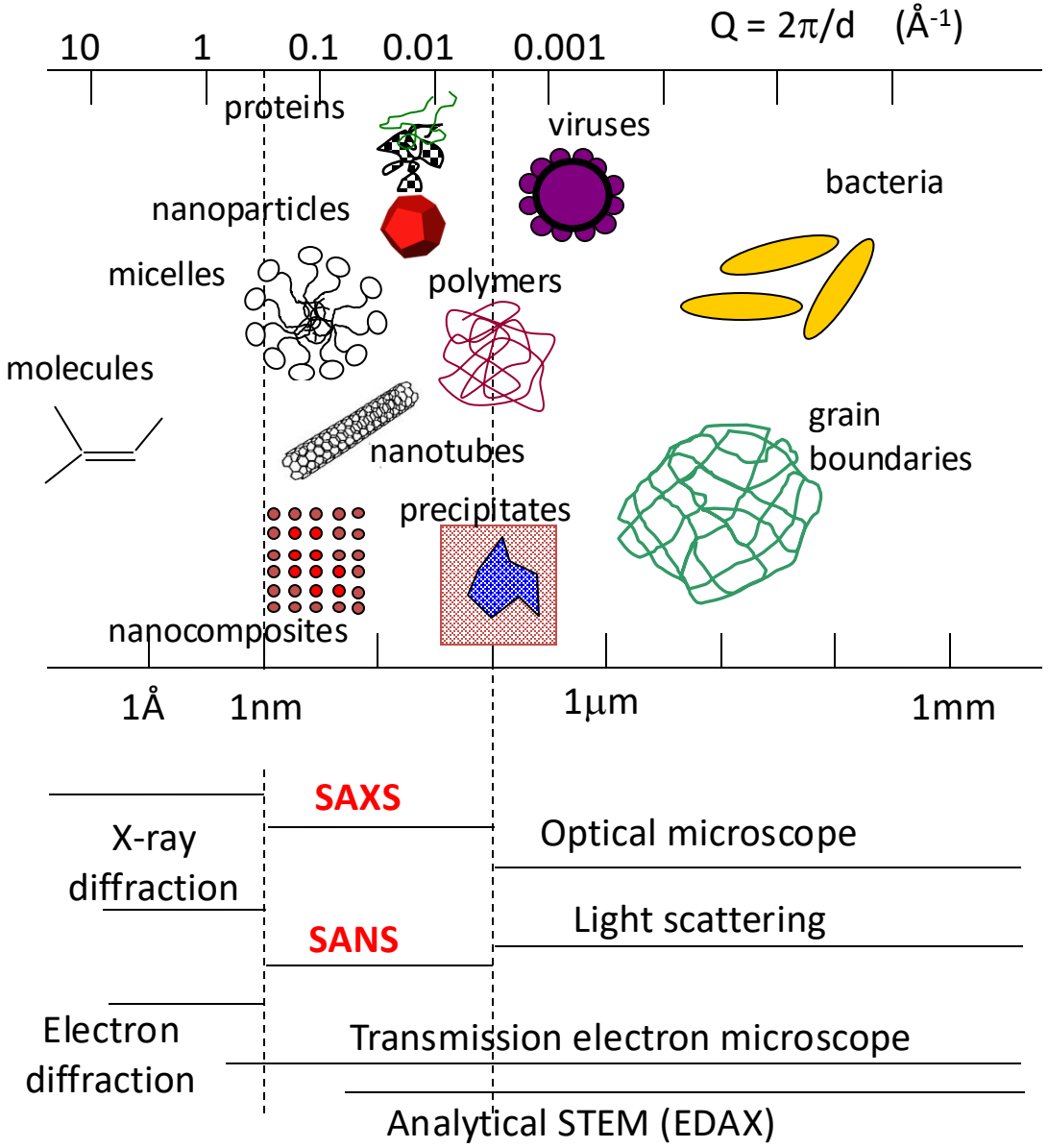
Size Range Comparisons



Neutron Scattering & Soft Condensed Matter

- Neutron techniques cover wide range of length and time-scales
- Key techniques for soft matter
  - Small angle scattering
  - Liquid scattering
  - Reflectivity
  - Quasielastic scattering
  - Neutron spin-echo

- Often rely on contrast variation since many soft matter systems contain hydrogen

http://www.isis.stfc.ac.uk/instruments/loq/

Scattered Intensity

- observed scattered intensity is Fourier Transform of real-space shapes

$$I(Q) = N_p V_p^2 (\rho_p - \rho_s)^2 F(Q) S(Q) + B$$

where: N_p = number of particles

V_p = volume of particle

ρ = scattering length density (of particle/solvent)

B = background

F(Q) = form factor

S(Q) = structure factor

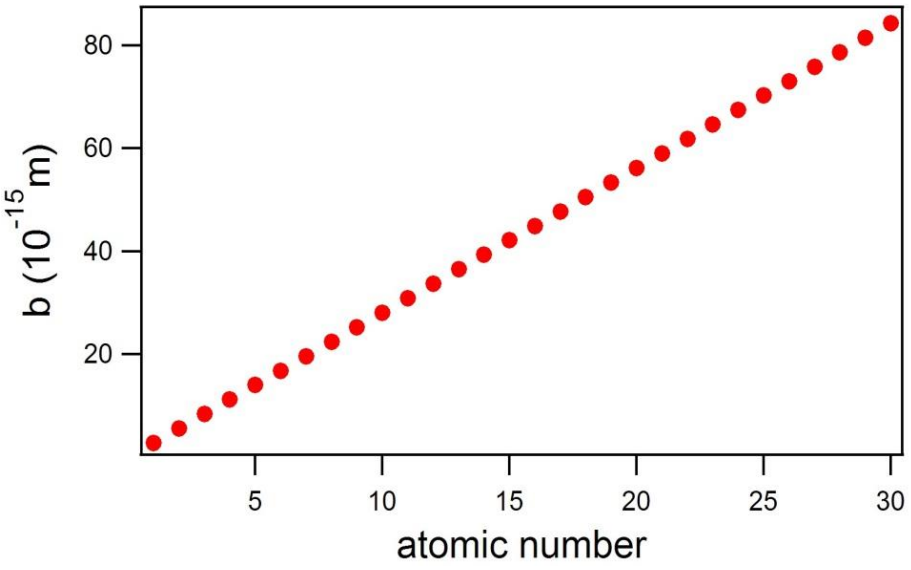
- Sample considerations…

Reminder: Scattering Length Density

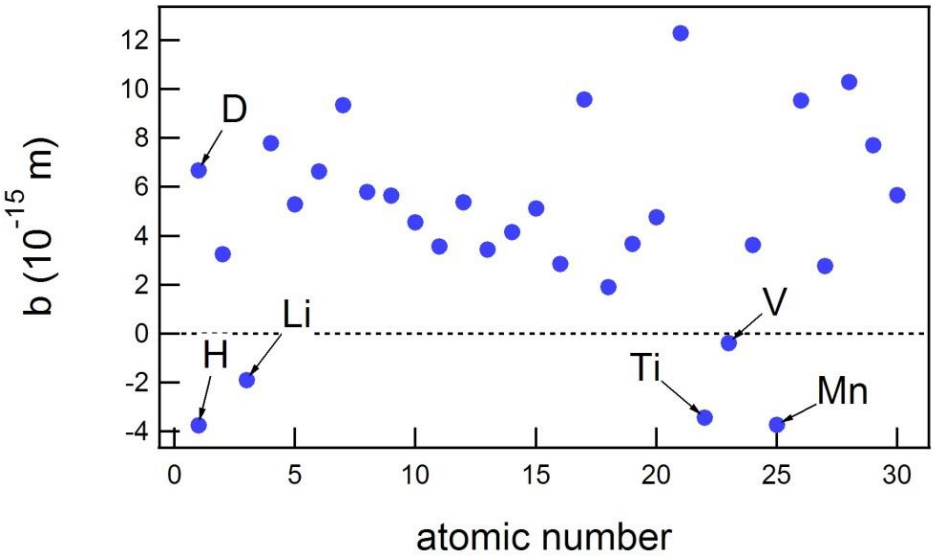
- Neutrons more penetrating than X-rays (interact less with matter)
- Interaction of neutrons with nuclei depends on isotope

$$I(Q)=N_p V_p^2(\rho_p-\rho_s)^2 F(Q)S(Q)+B$$

X-rays

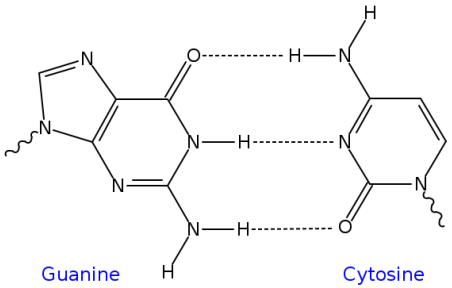


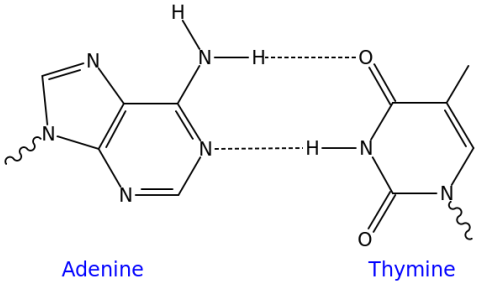
neutrons

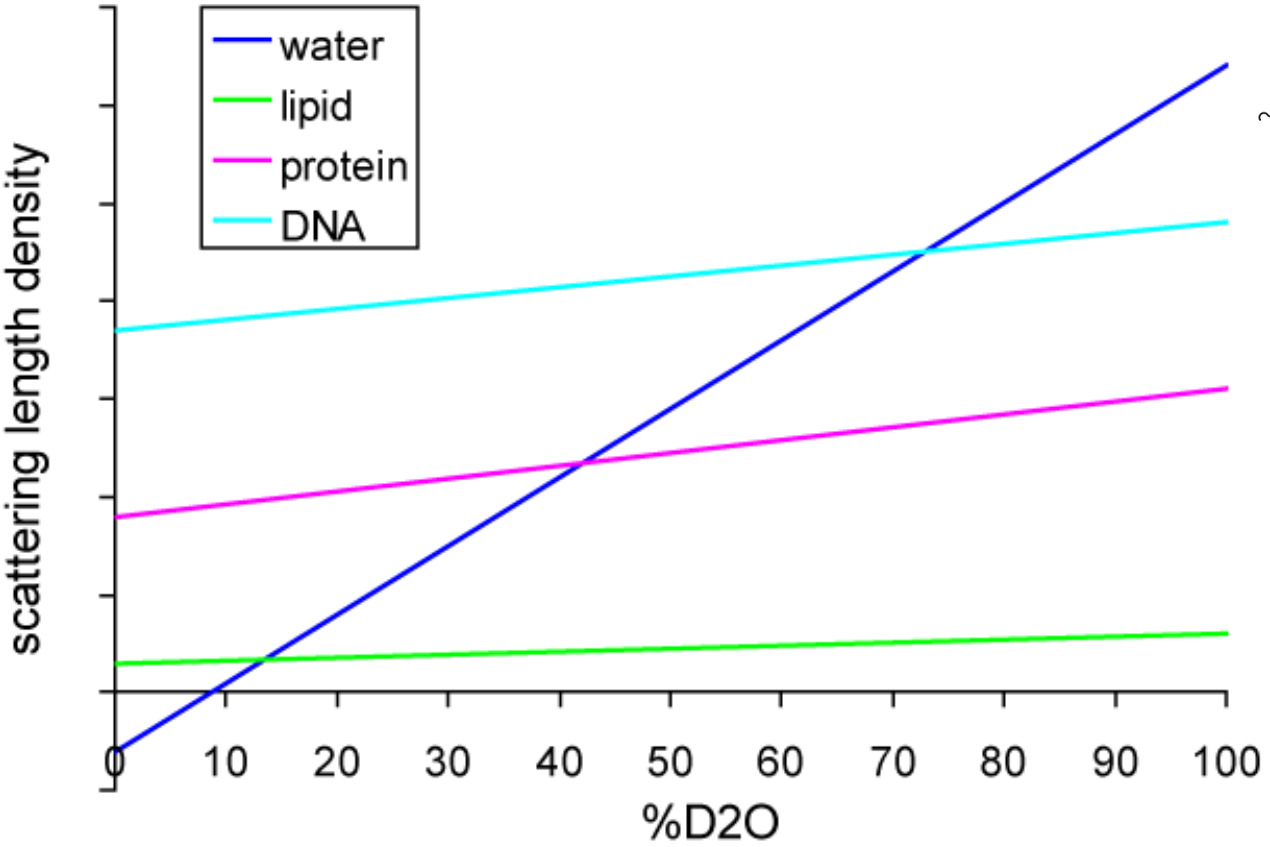


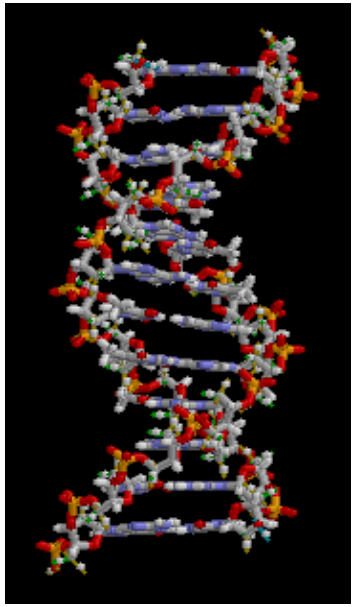
Predicting Contrast Match Point

- By calculating the SLD can predict %D_2O where the scattering signal will be zero
- BUT if have exchangeable hydrogens in the structure the SLD will vary with %D_2O







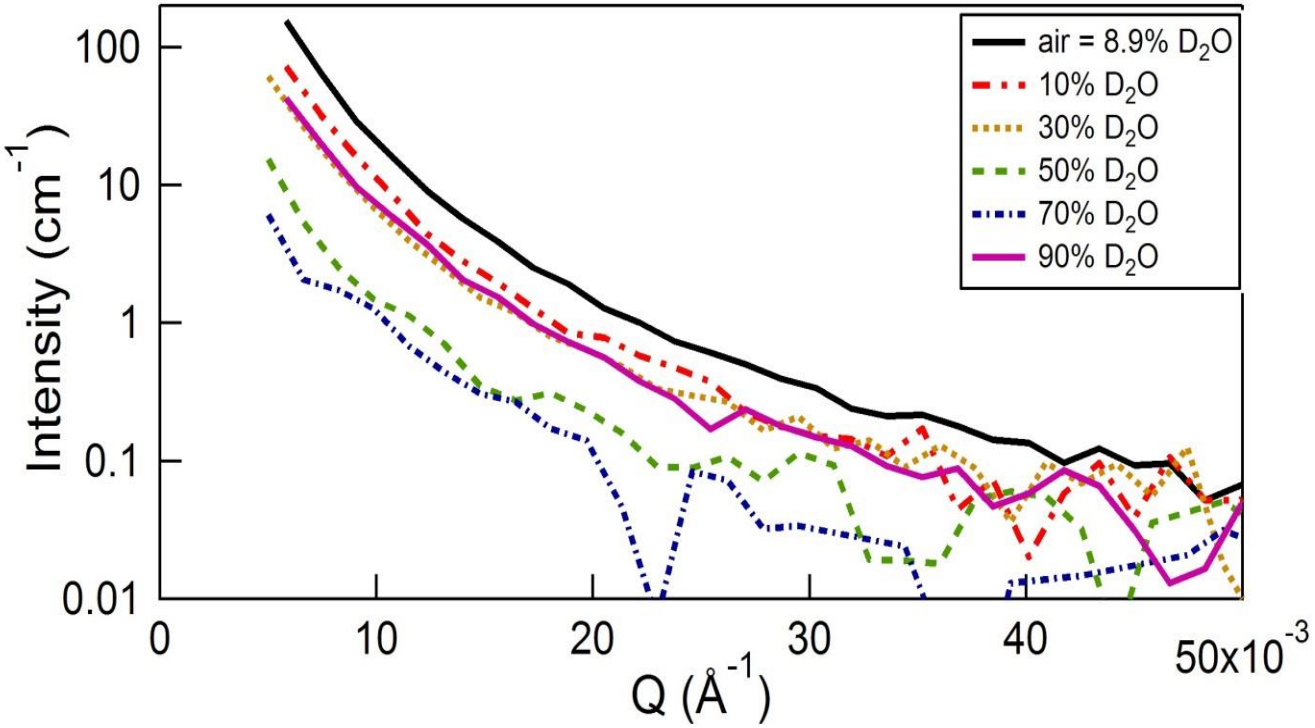


Neutron "Contrast" Series

- intensity of scattering depends on difference between particle and solution

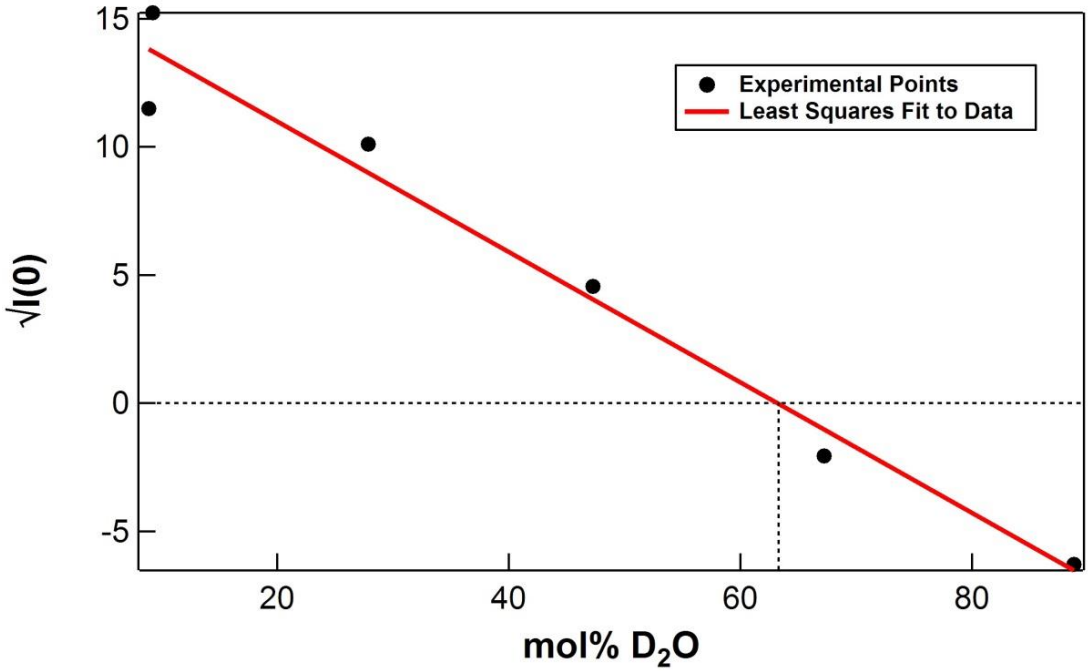
$$I \propto (\rho_{particle} - \rho_{solution})^2$$

- measure scattering at a series of solution contrasts
- extrapolate scattering to Q = 0 and measure I_0



Contrast Match Point

- Plot as $\sqrt{I_0}$ vs $[D_2O]$



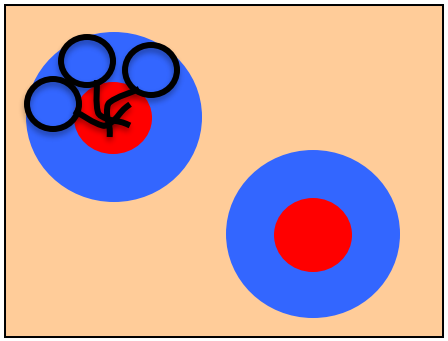
- Place where line cuts zero is where the solution has the same scattering length density as the particle
  $\Rightarrow$ *contrast matched*

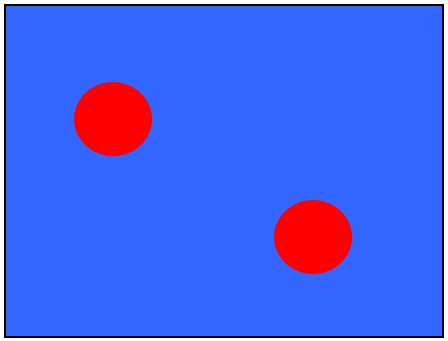
- Can use this to find the density of the particle

Neutron "Contrast" for Complex Objects

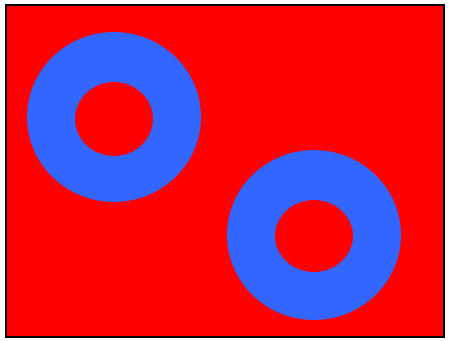
- At the nm scale, contrast matching allows us to "remove" scattering from parts of an object

	Scattering Length Density, /Å^{-2}
H_2O	-0.56×10^{-6}
D_2O	6.34×10^{-6}
D-C_{16}TAB	5.54×10^{-6}
H-C_{16}TAB	-0.24×10^{-6}





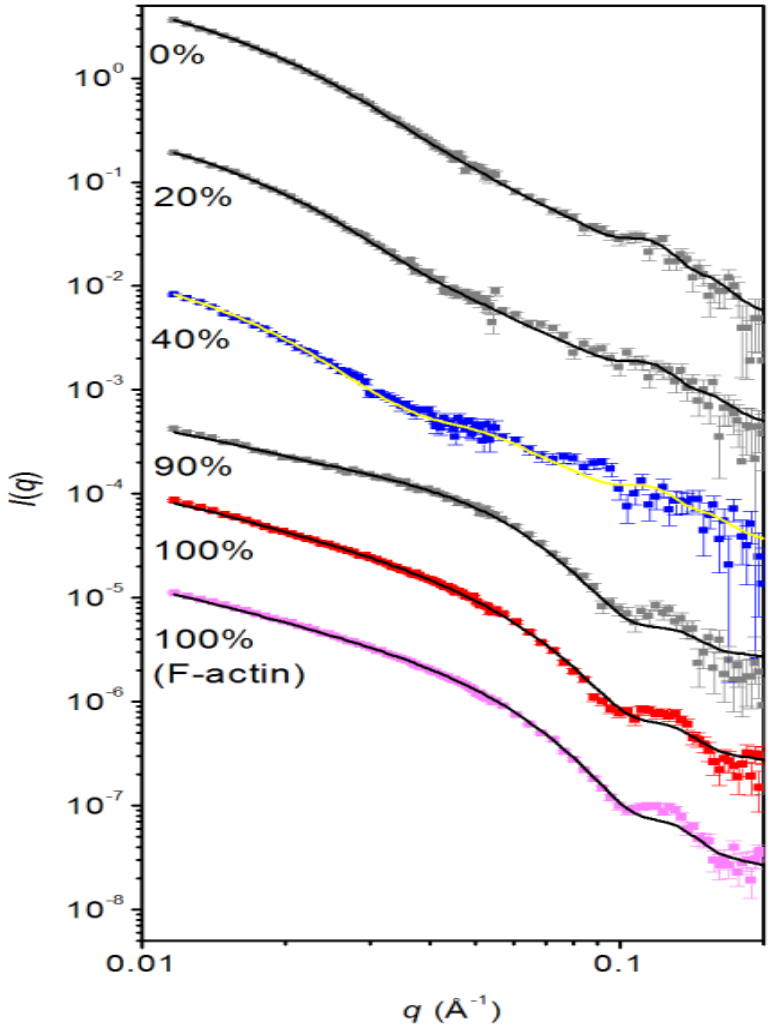
"shell-contrast"
⇒ see only core

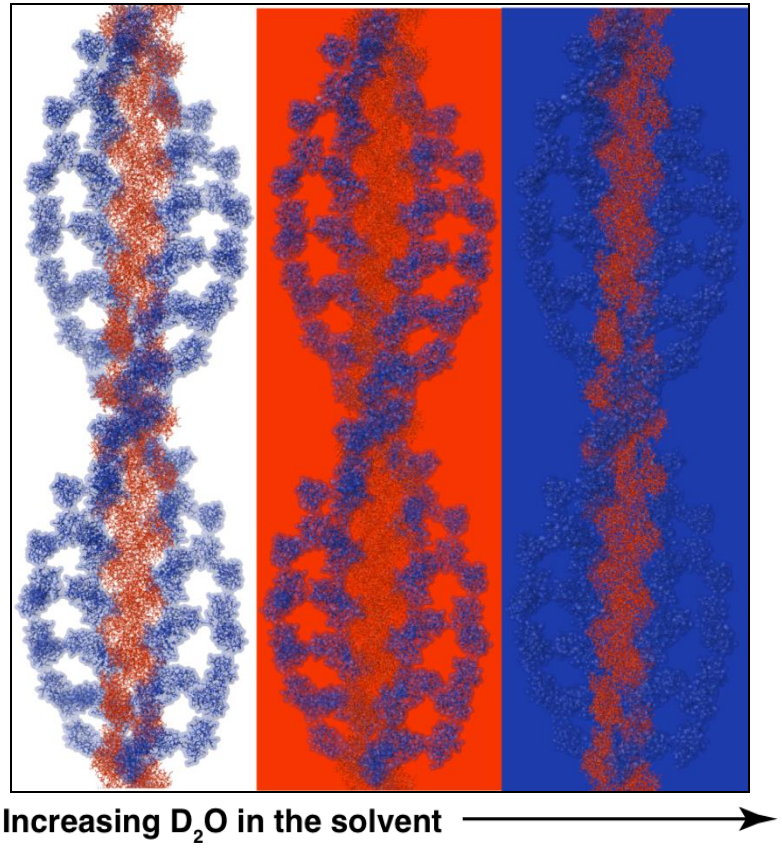


"core-contrast"
⇒ see only shell

Solvent matching for C0C2-actin assembly

- cardiac myosin binding protein C (C0C2) has extended modular structure
- Mixing C0C2 with G-actin solutions results in a dramatic increase in scattering signal due to formation of a large, rod-shaped assembly

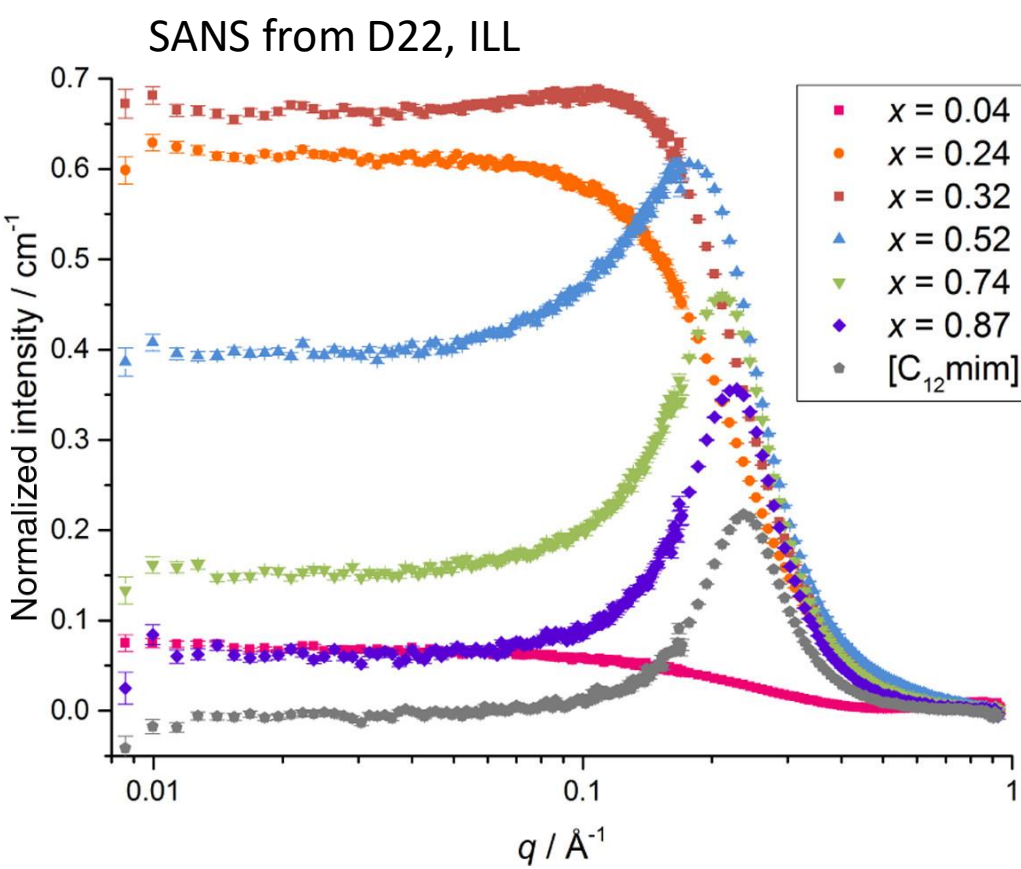


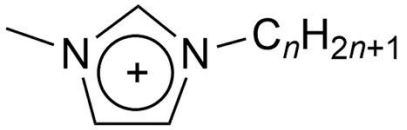


Whitten, Jeffries, Harris, Trewhella (2008)
Proc Natl Acad Sci USA 105, 18360-18365

Using Contrast in Soft Matter

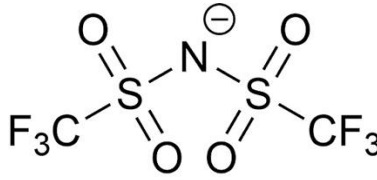
- Deuteration can highlight structure in low contrast systems for SANS measurements.





(n = 2) [C_2mim]$^+$

(n = 12) [C_{12}mim]$^+$



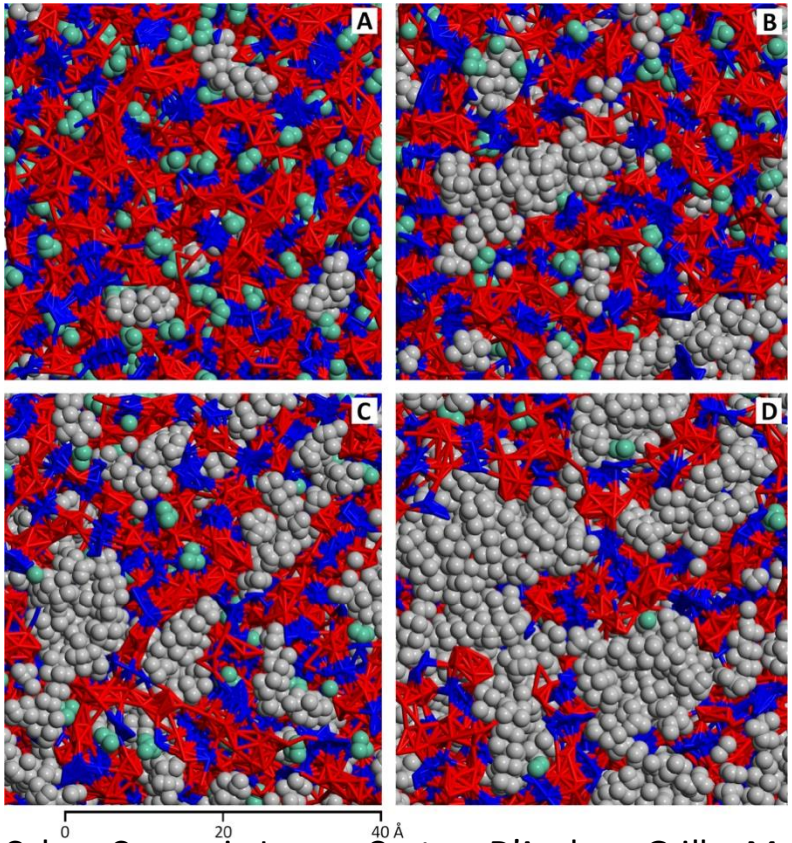
[Tf_2N]$^-$

- Solutions of [C_{12}mim]$^+$ in [C_2mim]$^+$[Tf_2N]$^-$

Bruce; Cabry; Canongia Lopes; Costen; D'Andrea; Grillo; Marshall; McKendrick; Minton; Purcell; Rogers; Slattery; Shimizu; Smoll; Tesa-Serrate; *J. Phys. Chem. B* **2017,** 121, 6002-6020. DOI: 10.1021/acs.jpcb.7b01654,

Segregation in Ionic Liquids

- Deuterated C_{12} chains on $[C_{12}mim]^+$ allowed mesostructure with changing $[C_{12}mim]^+$ concentration to be determined
  - Low concentrations, fitted to elliptical model,
  - High concentrations, fitted to bicontinuous network

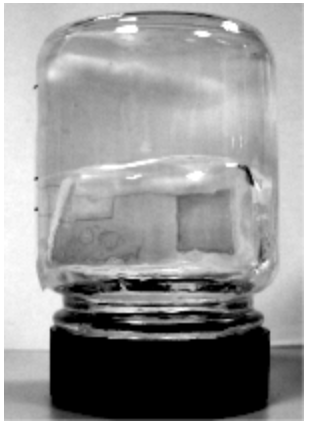


- SANS fitting compared to molecular dynamics simulations

Nanosegregation between the polar network (red/blue mesh) and nonpolar domains (grey and green beads) in $[C_2mim]_{1-x}[C_{12}mim]_x[Tf_2N]$ (a) $x = 0.04$, (b) $x = 0.24$, (c) $x = 0.52$, and (d) $x = 0.87$.

Bruce; Cabry; Canongia Lopes; Costen; D'Andrea; Grillo; Marshall; McKendrick; Minton; Purcell; Rogers; Slattery; Shimizu; Smoll; Tesa-Serrate; *J. Phys. Chem. B* **2017,** 121, 6002-6020. DOI: 10.1021/acs.jpcb.7b01654,

Cellulose based particulate rheology modifiers



Abundant, cheap material

From renewable resources

100 % non-petrochemical

Not food competitive

Clean derivatisation

Biodegradable

Functional

Gentle

structuring

foaming

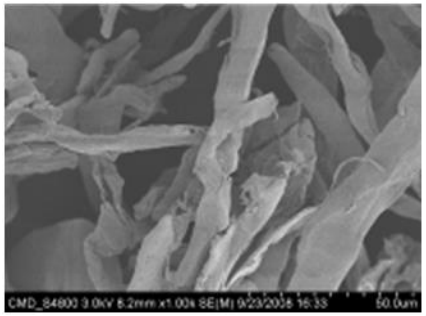
cleaning

Not dissolved! Well-dispersed fibrils with surface charge

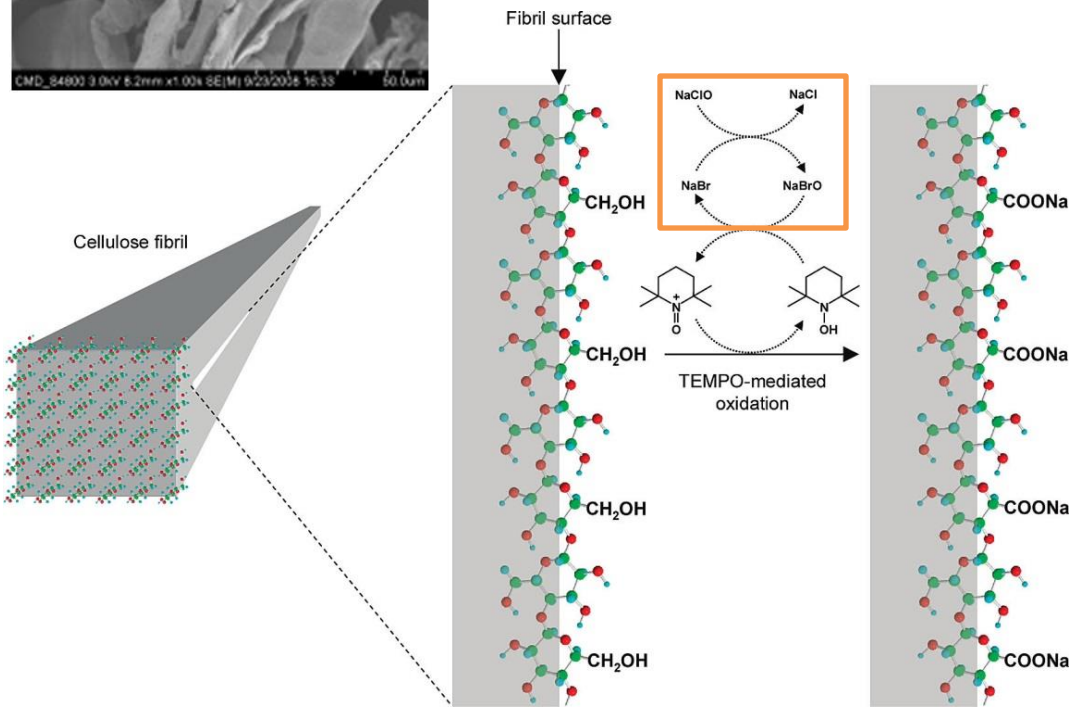
Partially Oxidised Cellulose

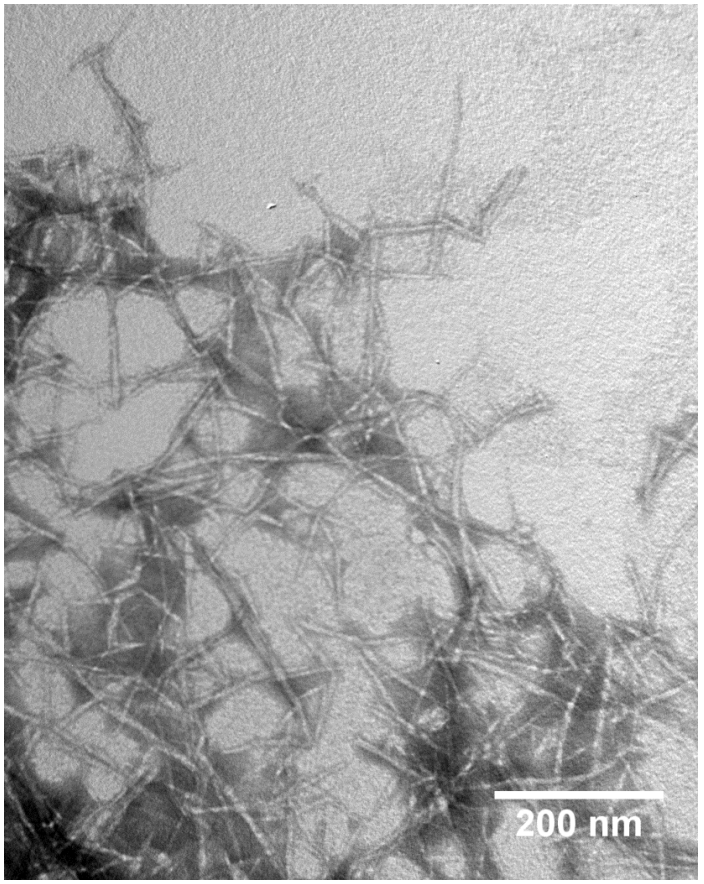
Cellulose: 40 000 000 000 *t* renewed annually,
not food competitive / petrochemical,
"waste" sources

BUT highly H-bonded ➔ *insoluble*



Partially oxidised fibrils
form stable dispersions





TEM

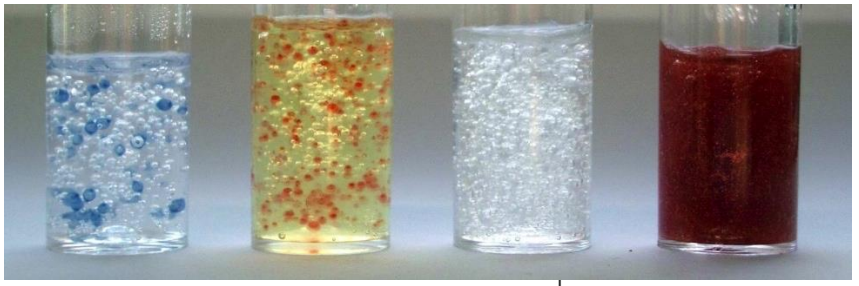
- Average width: 5.4 nm
- Standard deviation: 1.6 nm

ca 20 % of 1° alcohol oxidised

Y. Okita, T. Saito and A. Isogai, *Biomacromolecules*, 2010, **11**, 1696-1700.

Oxcell-Surfactant Gels

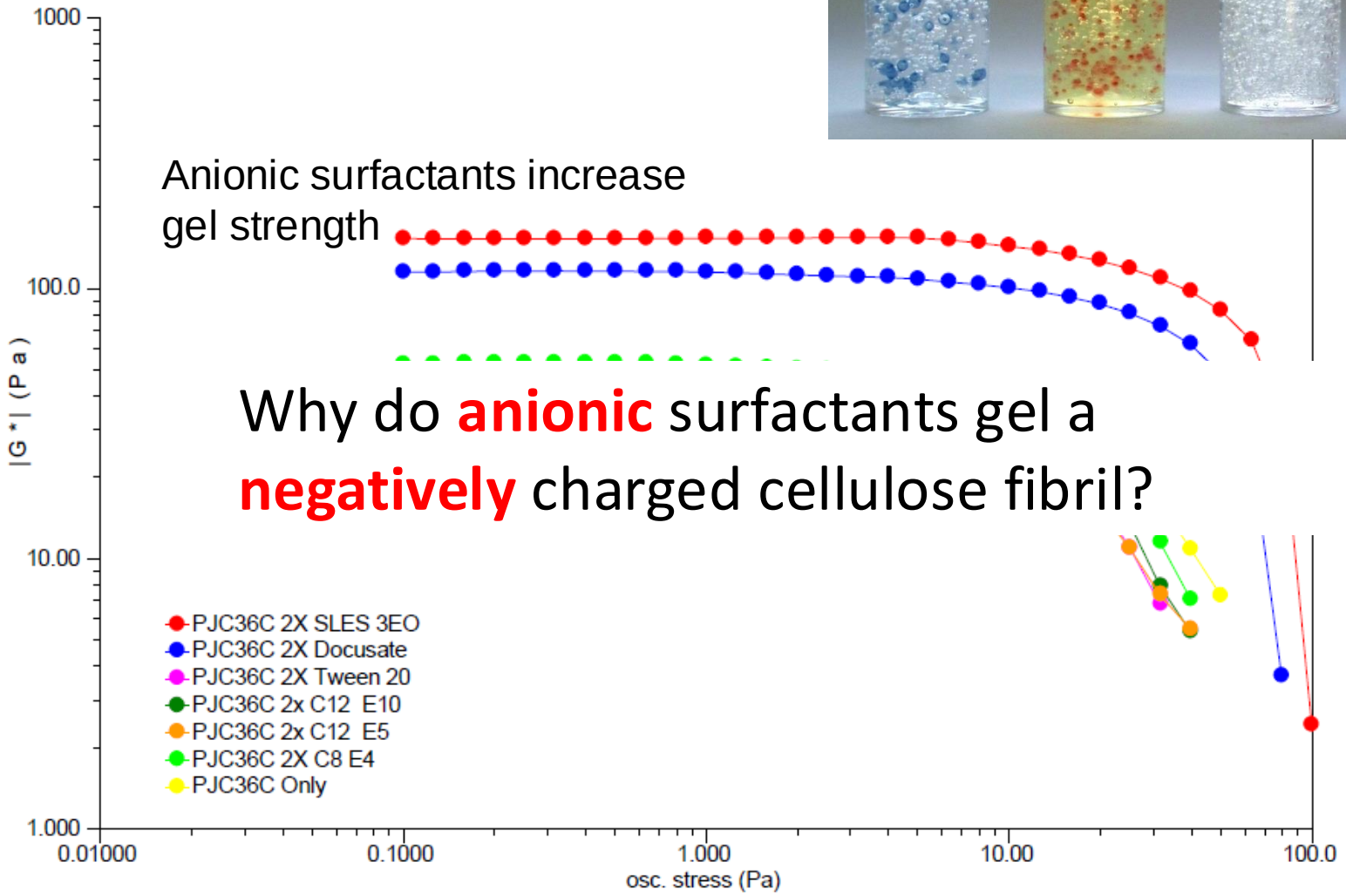
~5 wt% surfactant, ~1 wt% oxcel dispersion in water



Complex Modulus vs. Stress

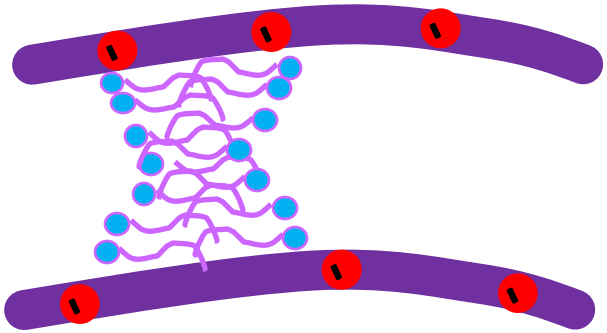
Anionic surfactants increase gel strength

Why do **anionic** surfactants gel a **negatively** charged cellulose fibril?

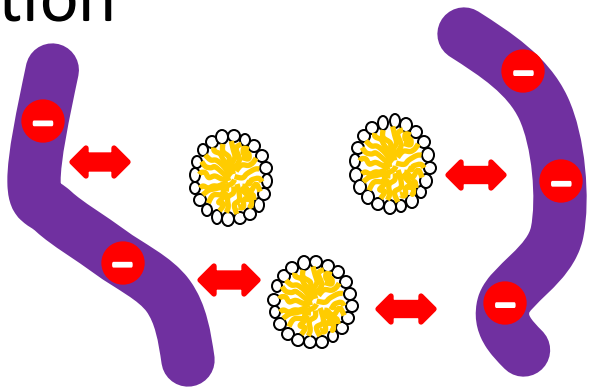


Possible SDS-Cellulose Gel Models

- Micelles as crosslinkers



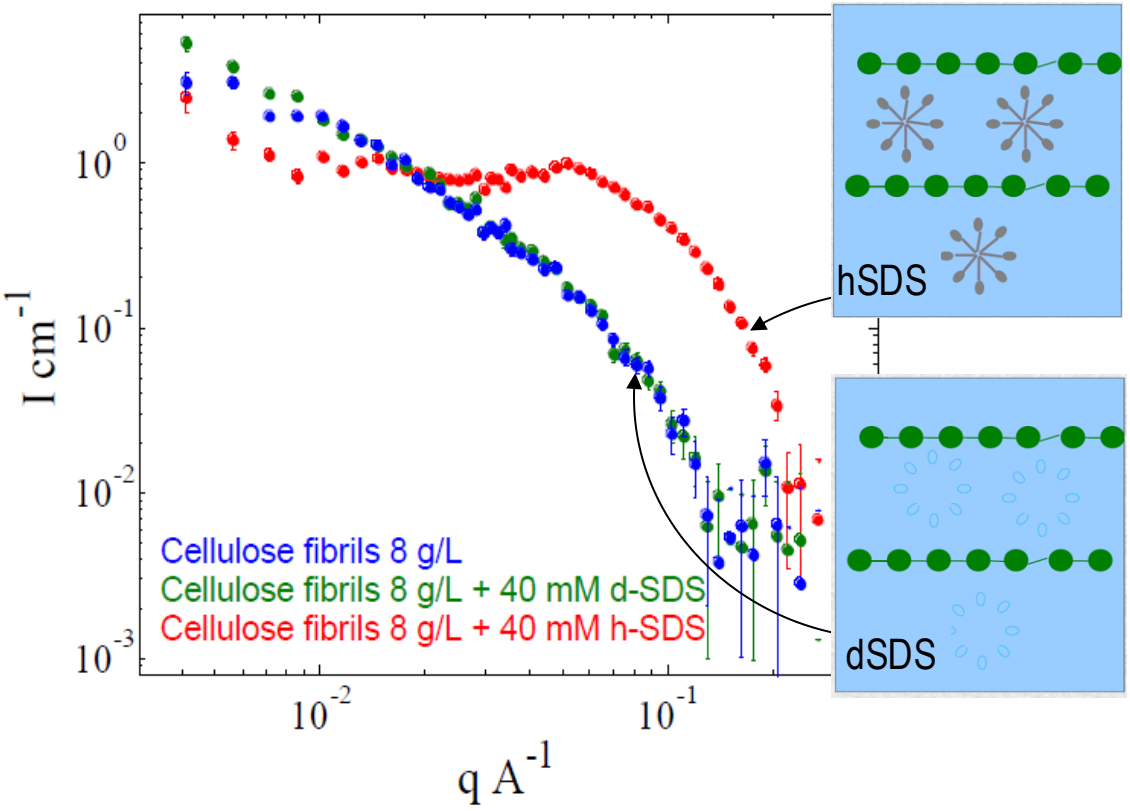
- Depletion flocculation



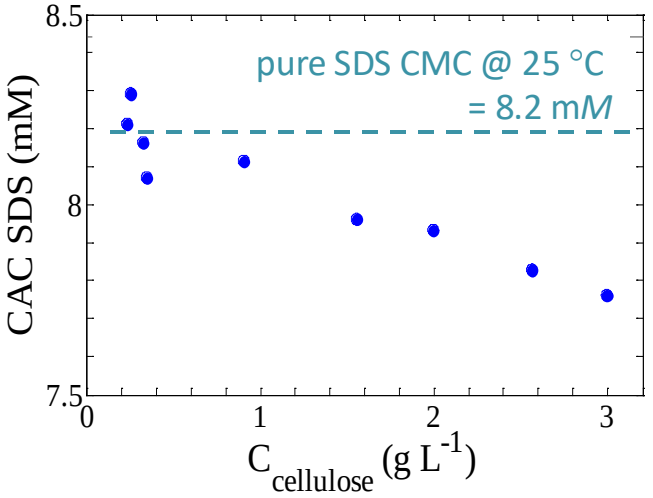
- Since cationic and anionic but not zwitterionic surfactants increase viscosity of cellulose suspensions are we just adding ions? ie salt

SANS: Oxcel and SDS

- Contrast matching SDS micelles in D_2O



- Surfactant CMC by conductivity

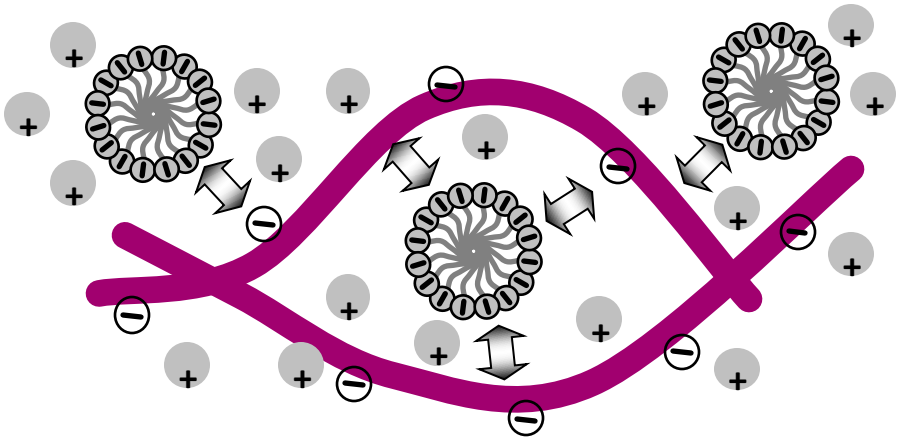


Micelles do not perturb Oxcel fibril structure or network

Edler, Lindhoud

Gelation of systems containing Oxcel fibrils and surfactants

- Increasing ionic strength (addition of electrolyte) is expected to cause collapse of the double layer on charged particles, allowing contact between fibres
- Anionic surfactant micelles result in depletion flocculation by effectively increasing the “concentration” of fibres and enhancing overlap



- Understanding the basis for gelation allows rational formulation (re)design

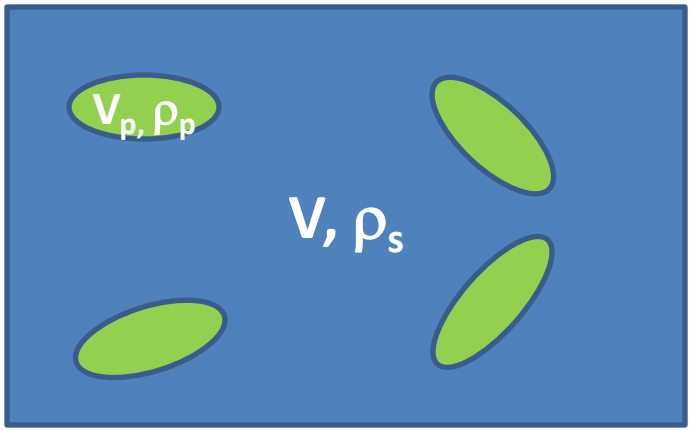
Crawford, Edler, Lindhoud, Scott, Unali, *Green Chem.*, 2012, **14**, 300-303

SAS Data Analysis

- Simple but not very accurate:
  - Porod slopes
  - Guinier analysis – see tutorial
- More helpful, but more complex:
  - fitting models to data
- Most complex (need more data):
  - fitting protein structures using crystal structures
  - monte carlo/simulated annealing methods

Scattering from Independent Particles

- Scattered intensity per unit volume of sample
  - arises from spatial distribution of regions with different scattering length density



$$I(q) = \frac{d\sigma}{d\Omega} = \frac{1}{V}\left|\int_V \rho(\boldsymbol{r})\, e^{i\boldsymbol{q}.\boldsymbol{r}}\, d\boldsymbol{r}\right|^2$$

- For identical particles:

$$I(q) = \frac{N}{V}(\rho_p - \rho_s)^2 V_p^2 \left\langle \frac{1}{V_p}\left|\int_{particle} e^{i\boldsymbol{q}.\boldsymbol{r}}\, d\boldsymbol{r}\right|^2 \right\rangle$$

Particle form factor, F(Q)

Dilute Randomly Ordered Uniform Particles

- scattering from independent particles:

$$I(q) = \frac{N}{V}(\rho_p - \rho_s)^2 V_p^2 \left\langle \frac{1}{V_p} \left| \int_{particle} e^{i\boldsymbol{q}.\boldsymbol{r}}\, d\boldsymbol{r} \right|^2 \right\rangle$$

- Assume: i) system is isotropic, then $\langle e^{-iqr} \rangle = \frac{\sin(qr)}{qr}$

  ii) no long range order, so no correlations between two widely separated particles

$$I(q) = I_e(q)(\rho_p - \rho_s)^2 V_p \int_0^\infty \gamma(r) \frac{\sin(qr)}{qr} 4\pi r^2 dr$$

$\gamma(r)$ = correlation function within particle

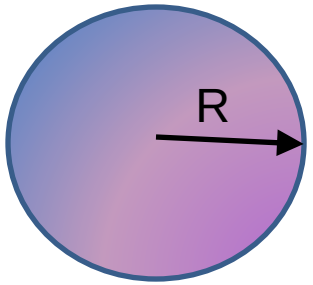
P(r)=$4\pi r^2\gamma(r)$ is the probability of finding two points in the particle separated by r

Porod's Law

- Start with form factor:

$$F(q) = \frac{1}{V_p}\int_0^\infty \gamma(r)\frac{\sin(qr)}{qr}4\pi r^2 dr$$

- Now consider radial pair correlation function for sphere, with sharp edges, radius R:



$$\gamma(r) = 1 - \frac{3}{4}\left(\frac{r}{R}\right) + \frac{1}{16}\left(\frac{r}{R}\right)^3$$

$$F(qR) = \frac{1}{V_p}\int_0^\infty \left[1 - \frac{3}{4}\left(\frac{r}{R}\right) + \frac{1}{16}\left(\frac{r}{R}\right)^3\right]\frac{\sin(qr)}{qr}4\pi r^2 dr$$

- Integrate by parts three times:

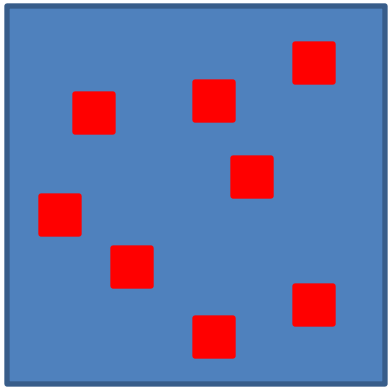
$$F(qR) \approx \frac{3}{2R^3}\frac{S_p}{V_p}\frac{1}{q^4}$$

At high scattering angles, for any system with sharp, smooth surfaces: $I(Q) \propto \frac{1}{q^4}$

Porod Scattering

- Slope at high q the same
- But point where slope changes depends on particle dimensions

A



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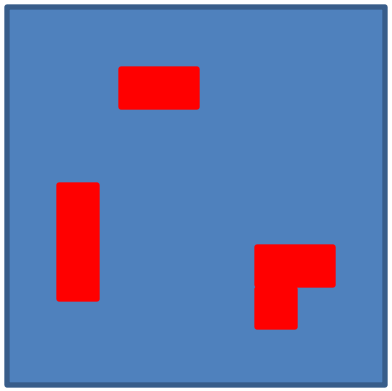
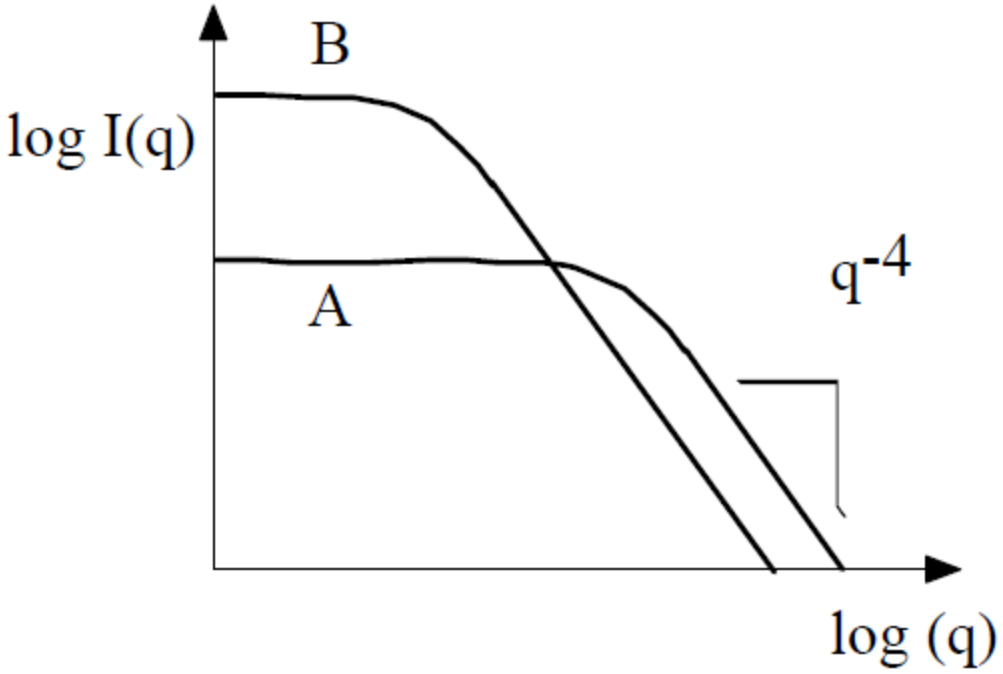


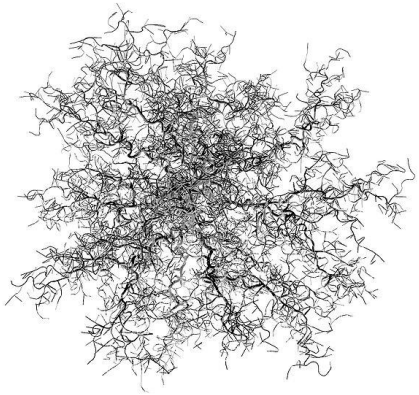
10% red / 90% blue in each square



*Glatter & Kratky pp. 30-1.

Fractal Systems

- Fractals are systems that are self-similar as you change scale



Diffusion-limited aggregation in 3 dimensions
(Paul Bourke,
http://local.wasp.uwa.edu.au/~pbourke/fractals/dla3d/)

- For a Mass Fractal the number of particles within a sphere radius R is proportional to R^D where D = fractal dimension
- Thus:

$4\pi R^2 \gamma(R) dR$ = number of particles between distance R and R+dR

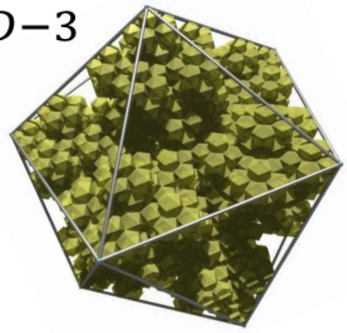
$= cR^{D-1} dR$

Fractal Systems Continued…

- So for a Mass Fractal:

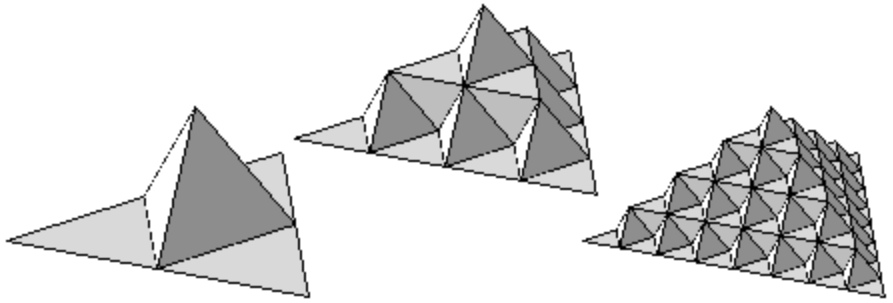
$$F(Q) = \int dR\, e^{iQR}\gamma(R) = \frac{2\pi}{Q}\int dR\,.\,Rsin(QR).\left(\frac{c}{4\pi}\right)R^{D-3}$$

$$= \frac{c}{2}\frac{1}{Q^D}\int dx.\,x^{D-2}.\,sinx = \frac{constant}{Q^D}$$

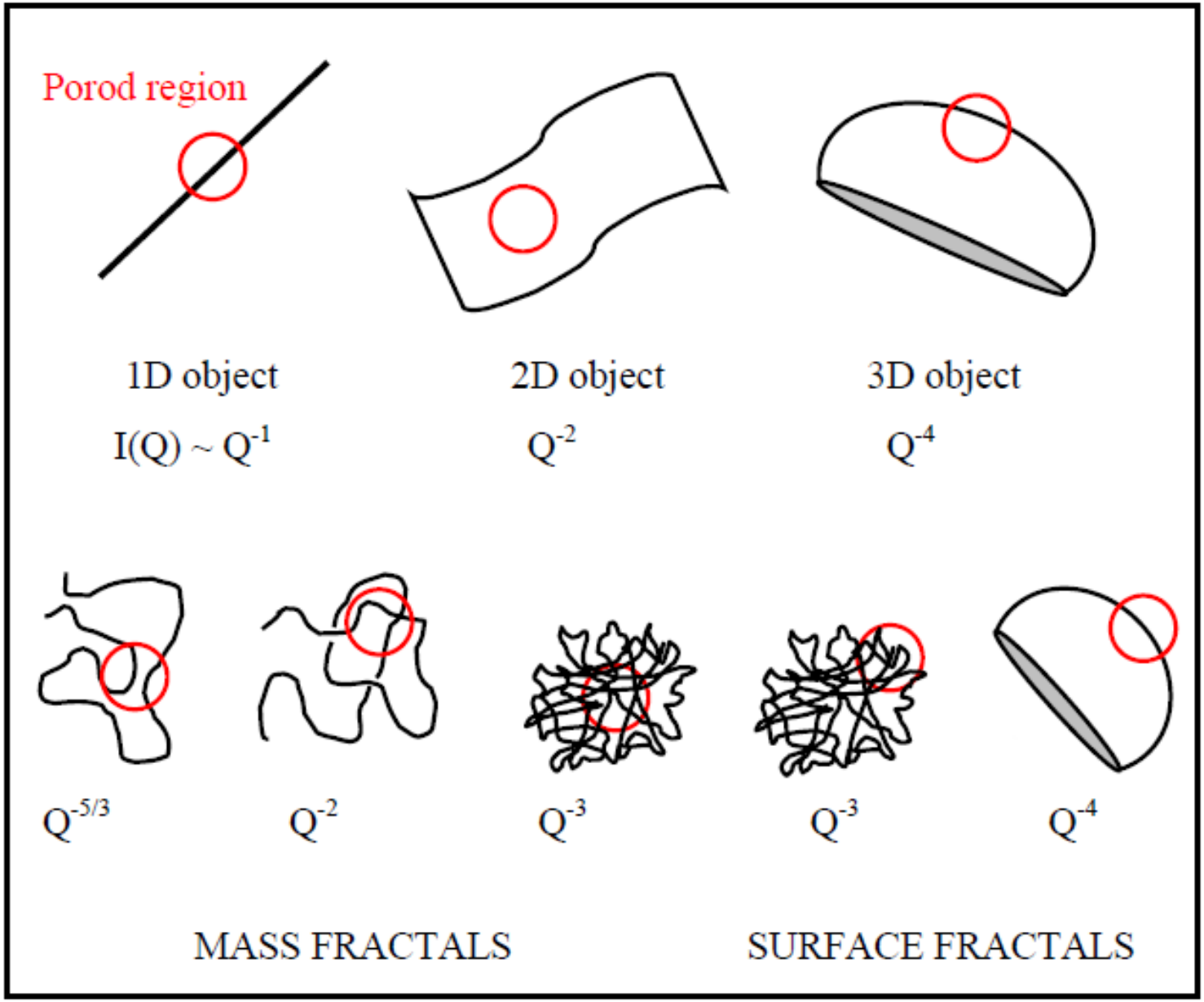


Paul Bourke

- For a fractal surface can show that $F(Q) = \frac{constant}{Q^{6-D}}$ (this reduces to the Porod Law for smooth surfaces of dimension 2)



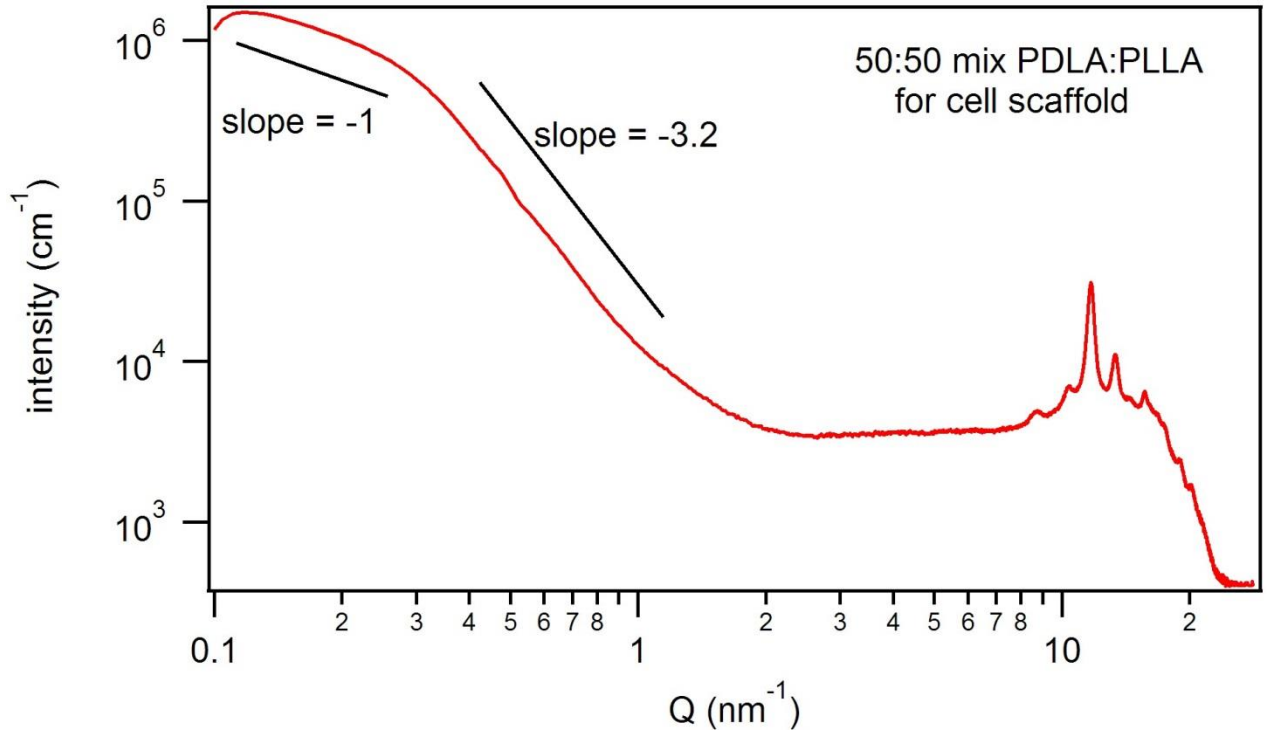
First stages of Koch (triangle) surface (Robert Dickau)



The SANS Toolbox. Boualem Hammouda, NIST

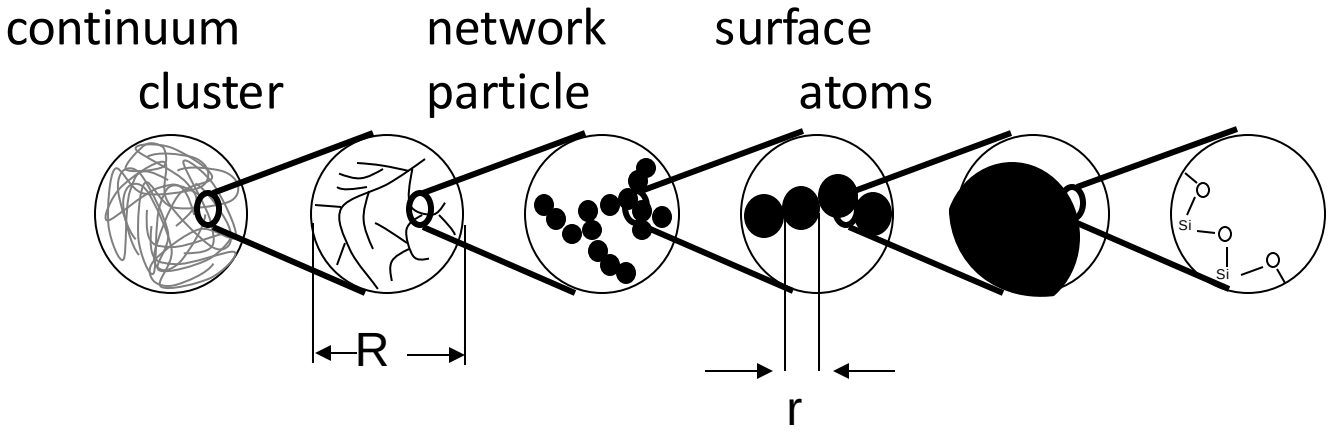
http://www.ncnr.nist.gov/staff/hammouda/the_SANS_toolbox.pdf

Porod Slopes & Structures



NB/ SAXS data, seldom measure such a wide Q range in SANS

eg



More Complex: Fitting Scattering

- observed scattered intensity is Fourier Transform of real-space shapes

$$I(Q)=N_p V_p^2(\rho_p-\rho_s)^2 F(Q)S(Q)+B$$

where: N_p = number of particles

V_p = volume of particle

ρ = scattering length density (of particle/solvent)

B = background

F(Q) = form factor

S(Q) = structure factor

Form Factor = scattering from within same particle

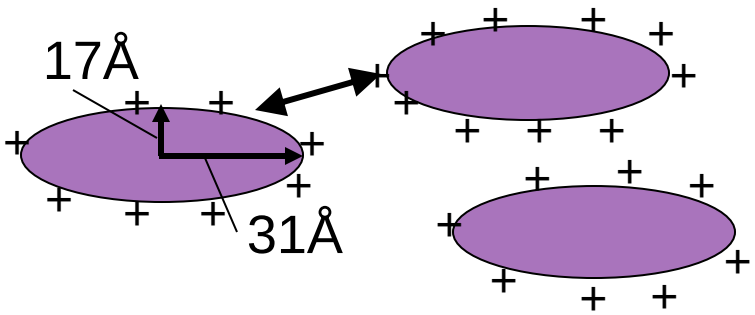
$\Rightarrow$ depends on particle shape

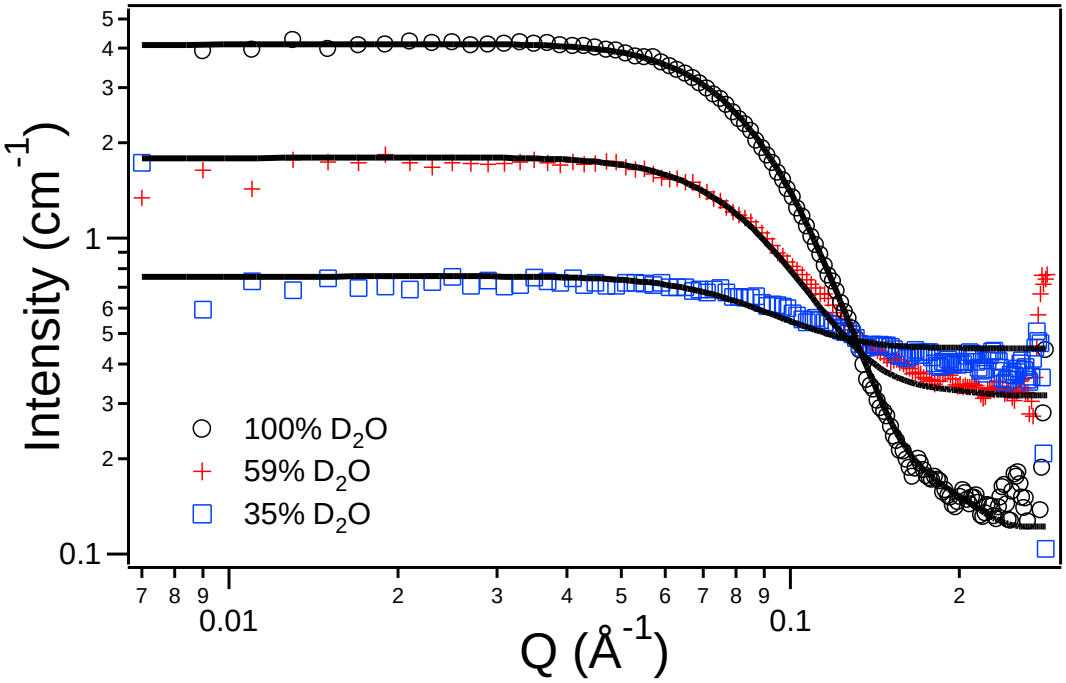
Structure Factor = scattering from different particles

$\Rightarrow$ depends on interactions between particles

Combining F(Q) & S(Q)

- Use computer programs to combine form factor and structure factor:



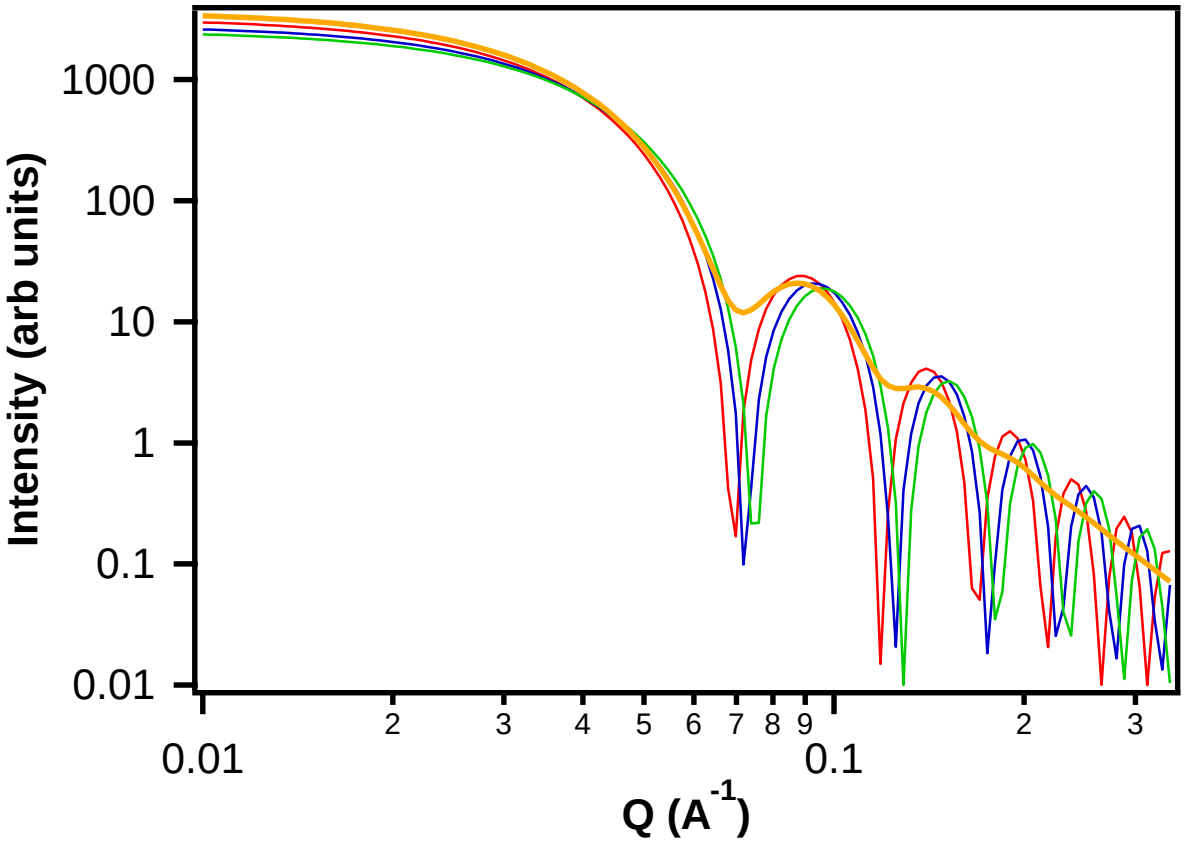


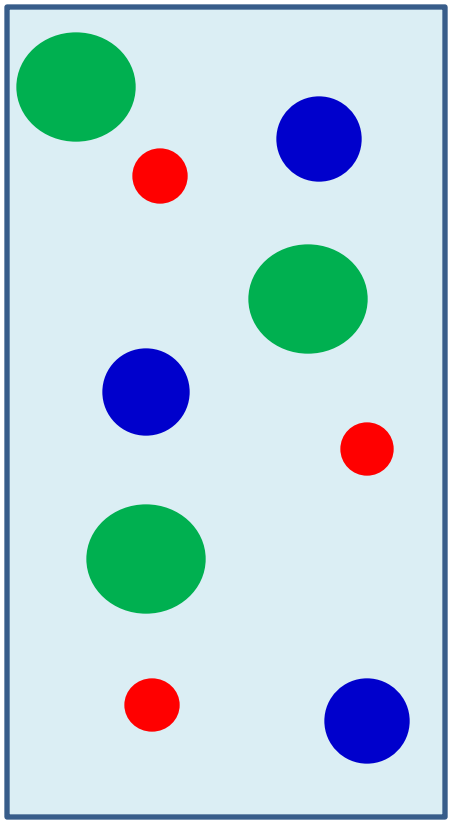
Brennan, Roser, Mann, Edler, *Chem. Mater.* **2002,** *14*, 4292

- Fit using ellipse + structure factor for charged objects which repel each other ⇒ many parameters!
- Use three contrasts to help pin down shape and size accurately

Polydispersity

- "smears out" sharp features in pattern
- "smearing" can also be due to poor Q resolution or beam shape (correct for this during data reduction)

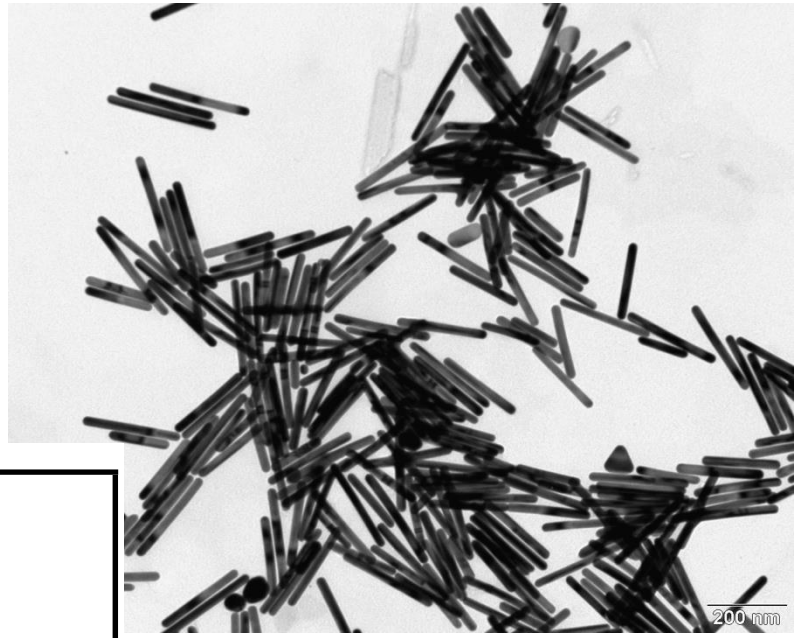


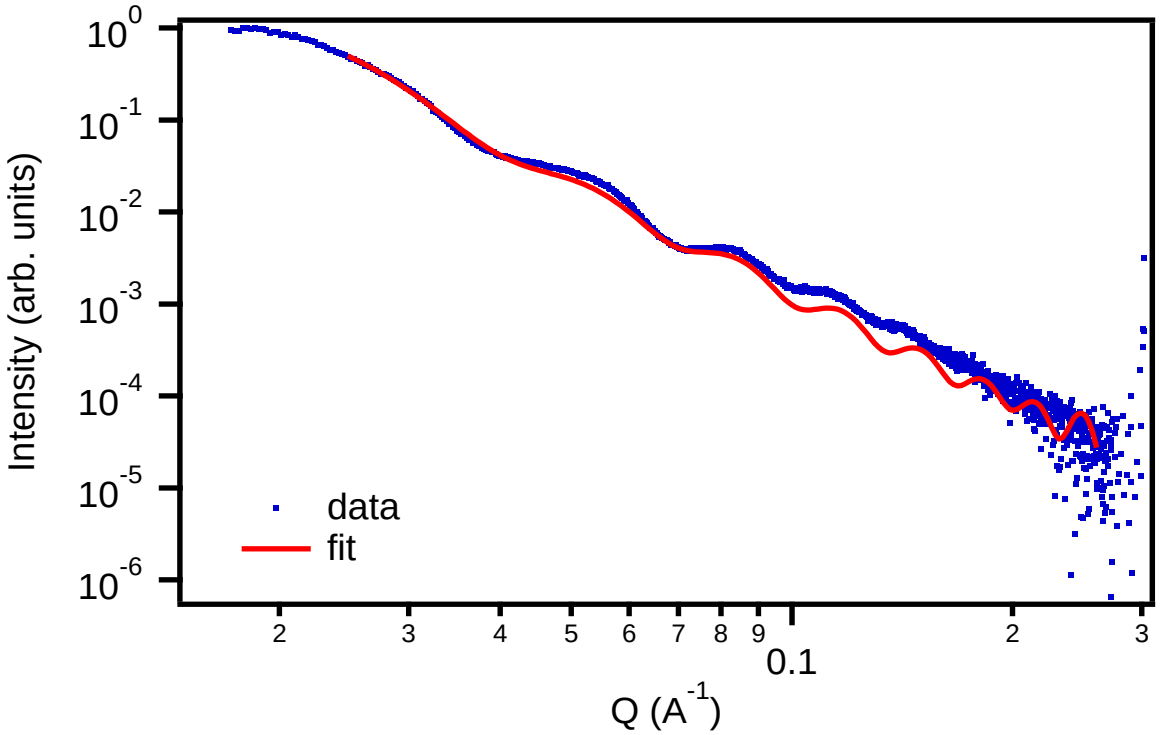


Au Nanorods

Fitted to charged cylinders

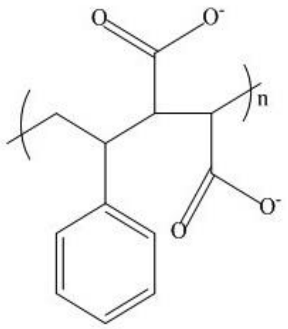
- Radius 80Å
- Length 190Å
- Polydispersity 0.29





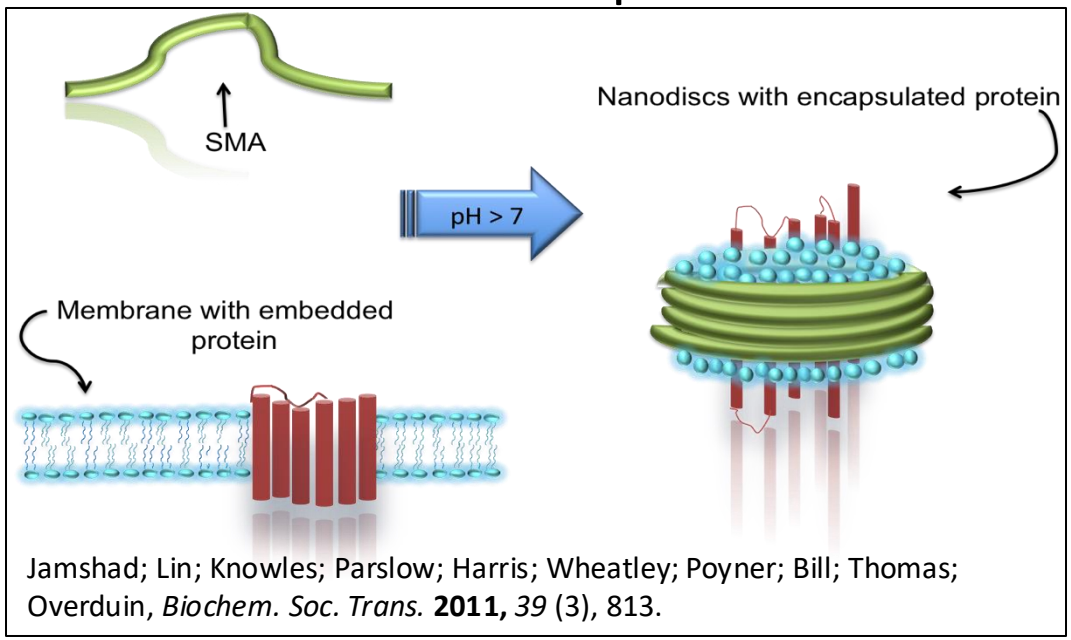
Polymer Cookie-Cutters: Nanodiscs

- A synthetic polymer belt can stabilise membrane proteins in discs



Poly(styrene-alt-maleic acid) (SMA)

Molecular weight	7kDa
Styrene:MA ratio	2:1
Polydispersity	1.6



Jamshad; Lin; Knowles; Parslow; Harris; Wheatley; Poyner; Bill; Thomas; Overduin, *Biochem. Soc. Trans.* **2011,** *39* (3), 813.

- Working to understand how discs form
- Improve the properties of the polymer belt

Lipid suspension

Add SMA → 1 min

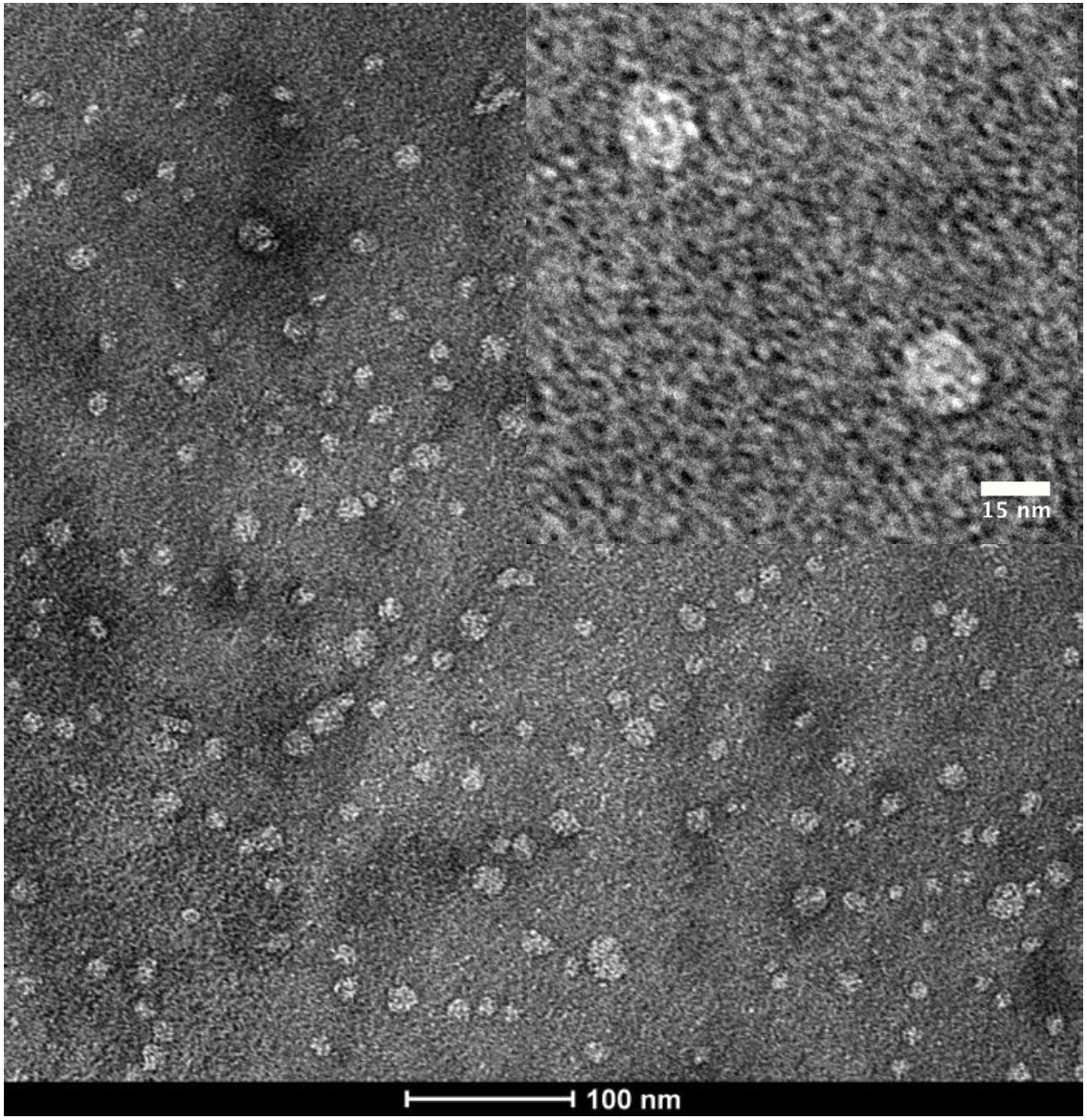
SMALP suspension

Knowles; Finka; Smith; Lin; Dafforn; Overduin, *JACS* **2009,** *131* (22), 7484

Transmission Electron Microscopy

- Empty SMALP stained with uranyl acetate

Maximum Feret Diameter/nm	15.3 ± 0.3
Minimum Feret diameter /nm	15.0 ± 0.3

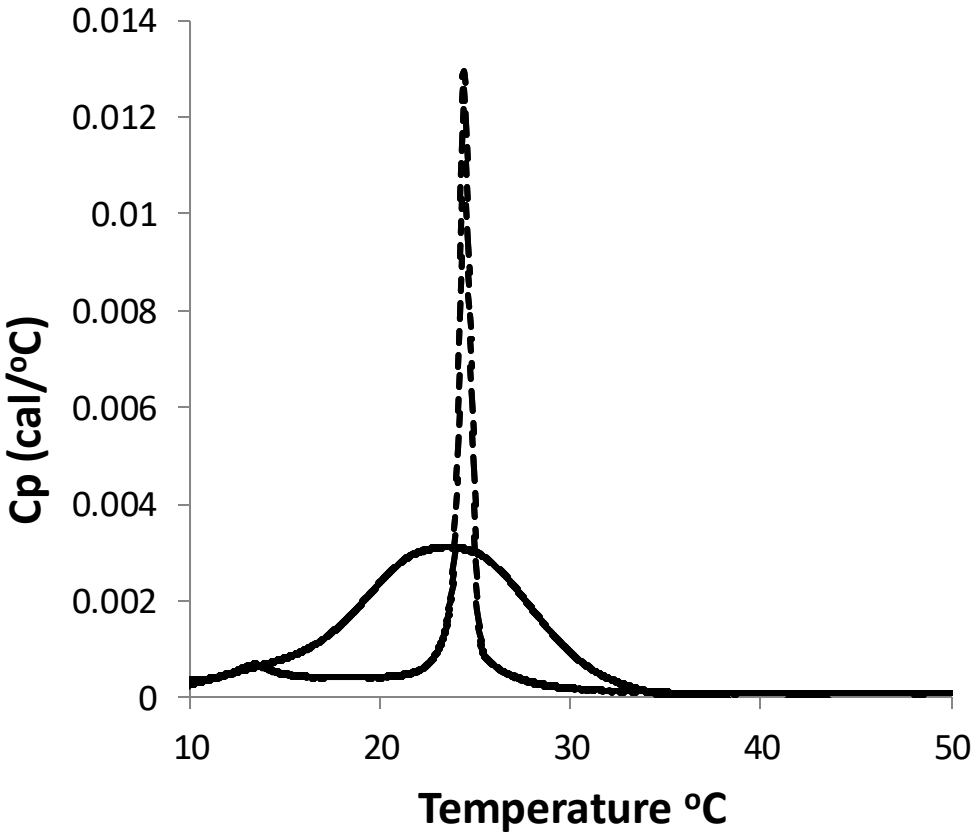


UNIVERSITY OF BIRMINGHAM

NPRL Nanoscale Physics Research Laboratory

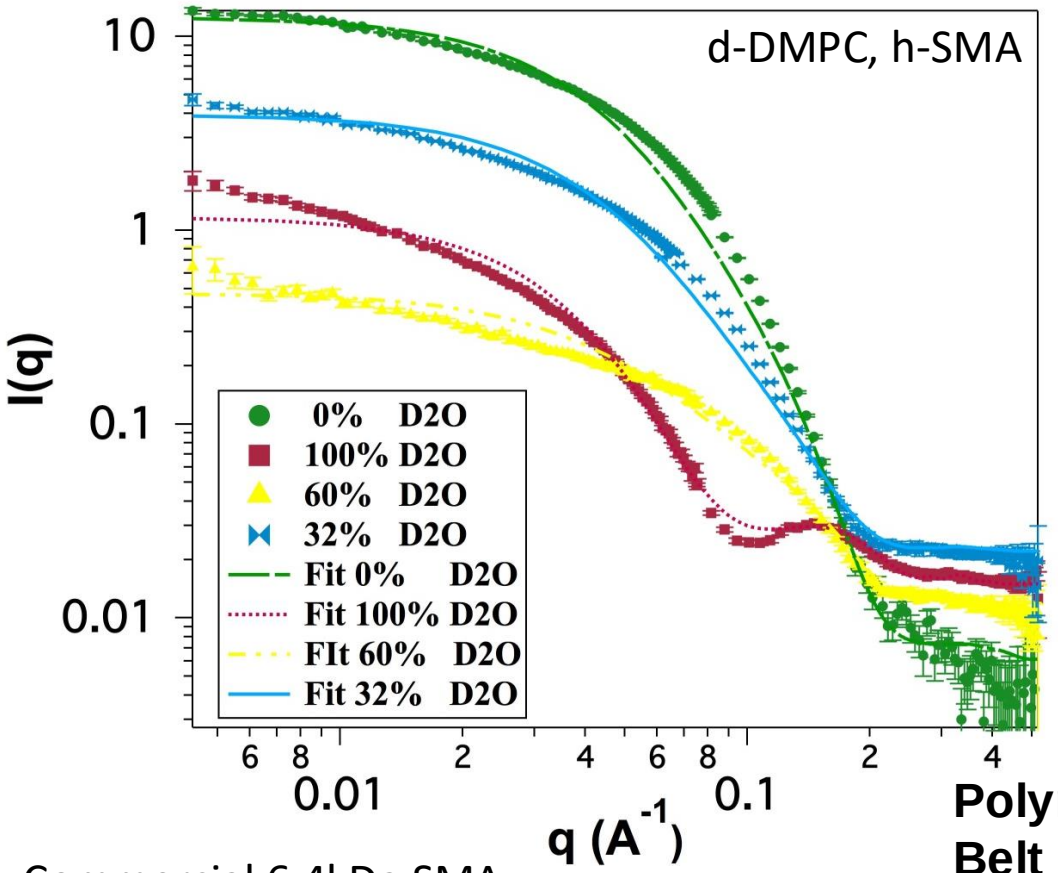
Differential Scanning Calorimetry

- DMPC phase transition in free lipid ~24°C
- When confined in disc transition broadens, shifts to ~ 23°C.
- (MSP stabilized discs ~28°C)



Jamshad; Grimard; Idini; Knowles; Dowle; Schofield; Lin; Finka; Palmer; Overduin; Govaerts; Ruysschaert; Edler; Dafforn, *Nano Res.* **2015,** *8* (3), 774.

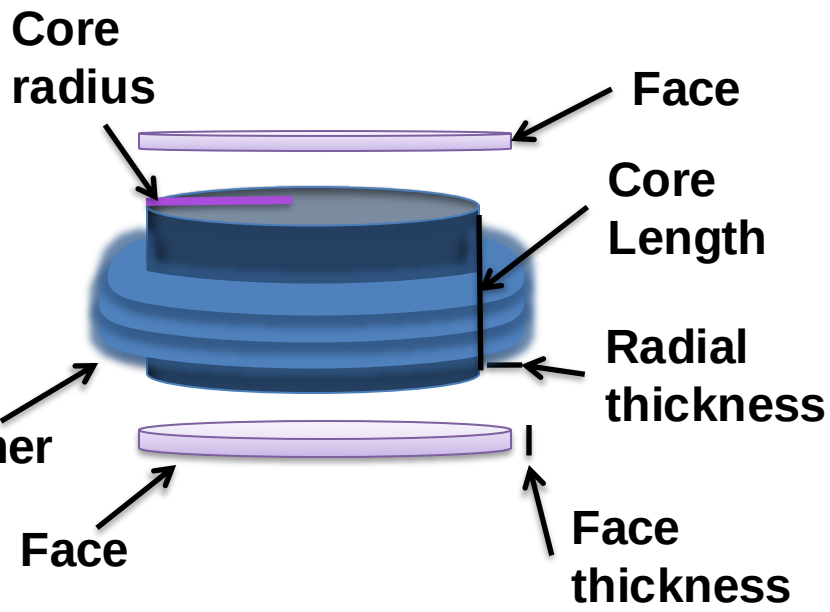
SMA-Lipid Disc SANS



Commercial 6.4kDa SMA-2000P, gel filtered

- diameter around 7.6 nm
- ~20 mol% copolymer in core
- Assuming lipid area ~0.59nm^2

$\Rightarrow$ 154 lipids (77 in each leaflet of bilayer)

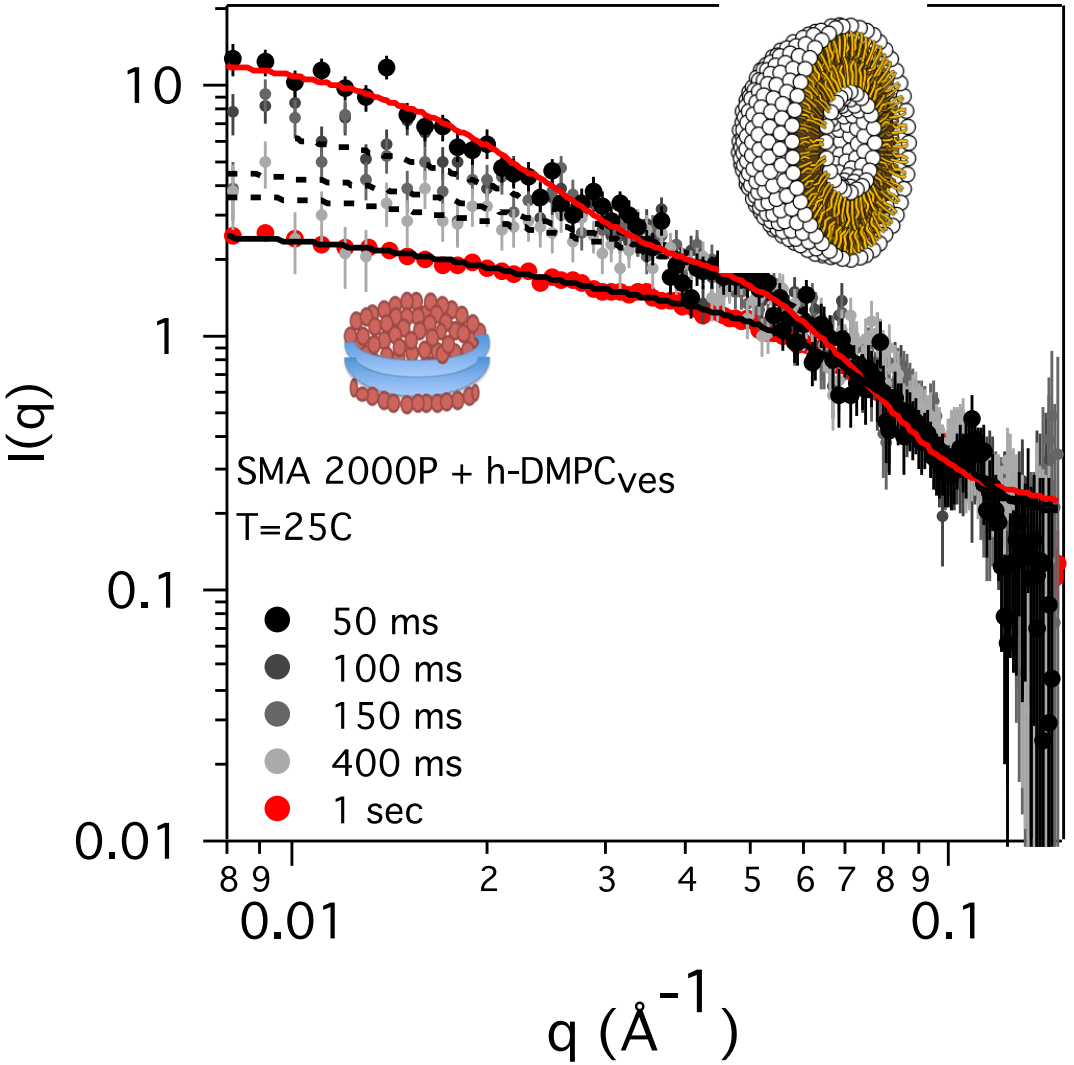


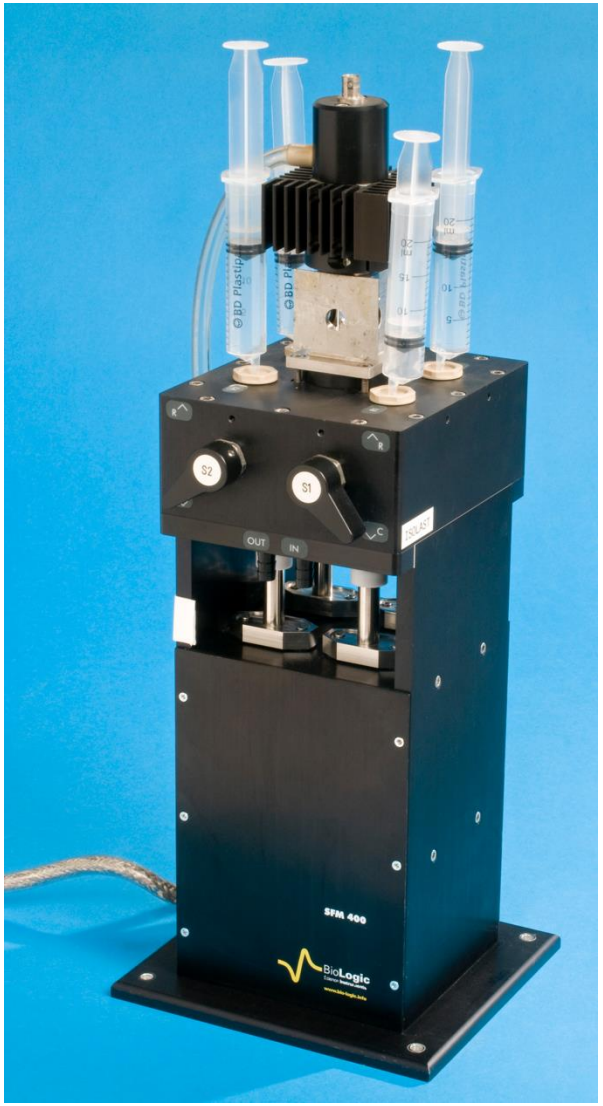
Poly-core bicelle model, NIST SANS Analysis Package

Jamshad; Grimard; Idini; Knowles; Dowle; Schofield; Lin; Finka; Palmer; Overduin; Govaerts; Ruysschaert; Edler; Dafforn, *Nano Res.* **2015,** *8* (3), 774.

How do Nanodiscs Form?

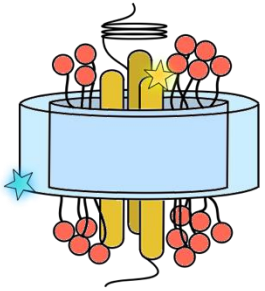
- Stopped flow SANS





Does SMA Affect Membrane Proteins?

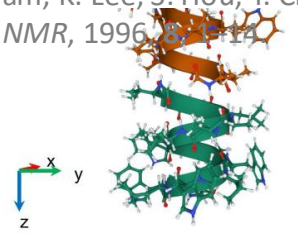
- Do the lipids prevent the polymer from interacting with the protein?
- Where are polymer and protein in a nanodisc?

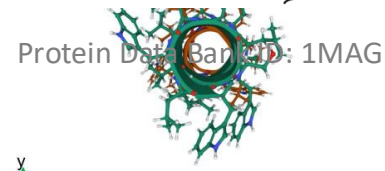


Model membrane proteins:

1. **Gramicidin A**
   - ➢ channel forming 15 amino acid peptide
   - ➢ forms membrane spanning α-helical ionophore as a dimer

R. Ketcham, K. Lee, S. Hou, T. Cross, *J. Biomol. NMR*, 1996, 8, 13-14

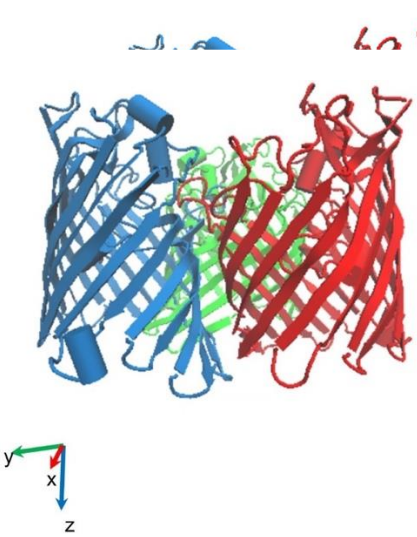


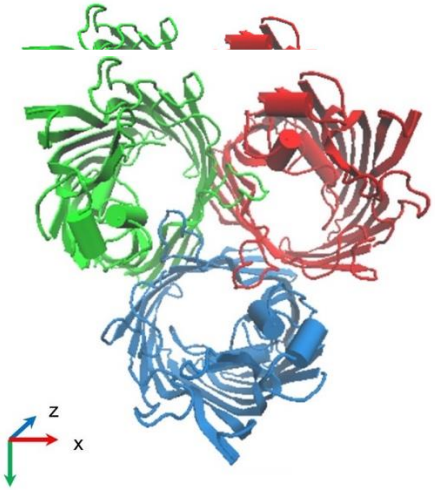


Protein Data Bank ID: 1MAG

2. **Outer membrane Protein F (OmpF)**
   - ➢ 16 β-stranded barrel
   - ➢ OmpF monomer ~ 37 kDa

➢ **OmpF is one of the earliest membrane protein porin crystal structures to be determined**



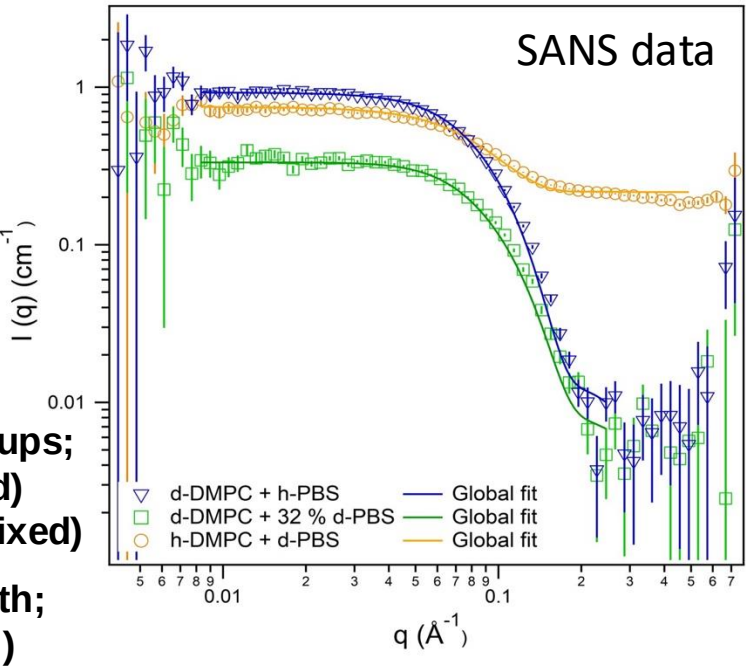


Protein Data Bank ID: 2OMF

J. M. David and A. K. Rajasekaran, J. Kidney Cancer VHL, 2015, 2, 15–24.

Gramicidin in SMALPs

- SMALPs prepared from DMPC-gramicidin vesicles at different DMPC contrasts in buffers:
  - d-DMPC + 100% H_2O PBS
  - d-DMPC + 32% D_2O in h-PBS
  - h-DMPC + 100% D_2O PBS
- gel filtered to remove excess polymer.

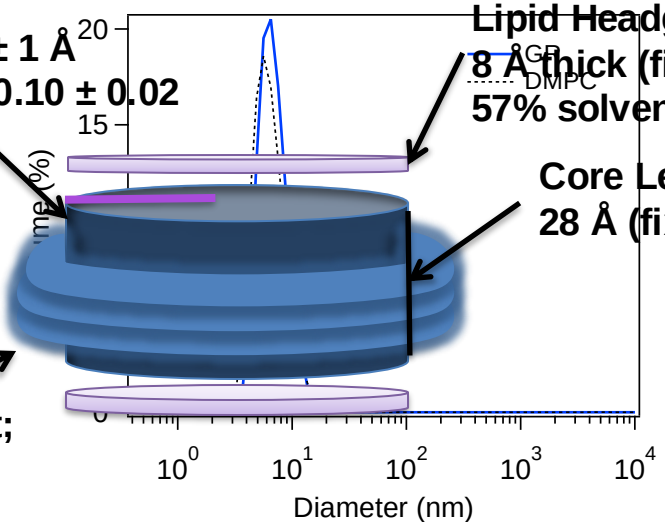


Lipid tails:
Core radius 23 ± 1 Å
polydispersity: 0.10 ± 0.02

DLS: 80 Å diameter

SANS: 62 Å diameter
44 Å tall

Polymer Belt;
8 ± 1 Å
75% solvent

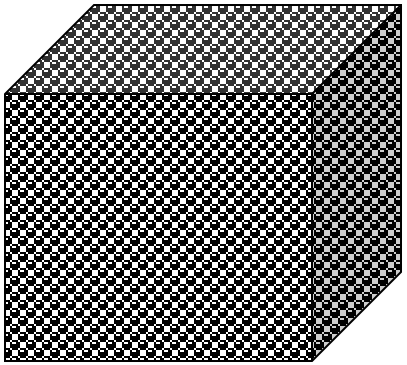


Lipid Headgroups;
8 Å thick (fixed)
57% solvent (fixed)

Core Length;
28 Å (fixed)

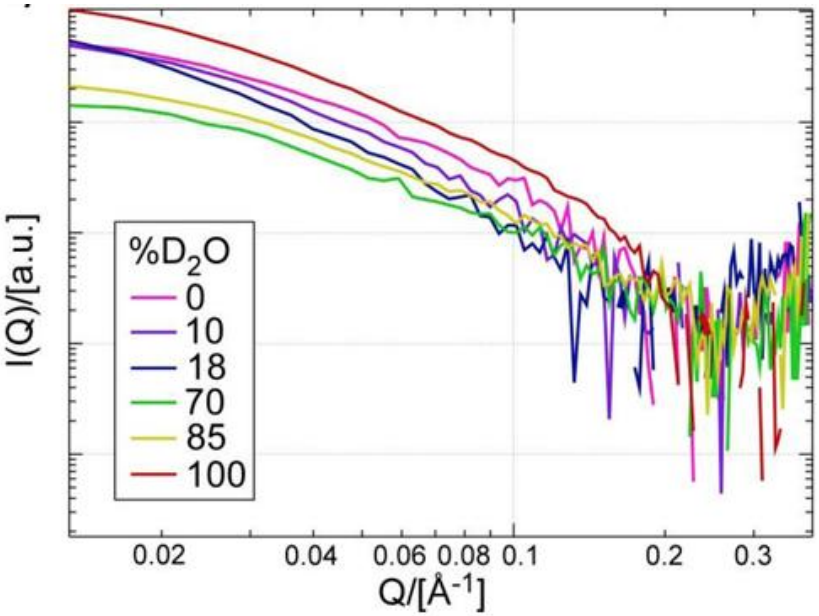
Morrison, Doekhie, Neville, Price, Whitley, Doutch, Edler, *BBA Advances* **2022**, *2*, 100033.

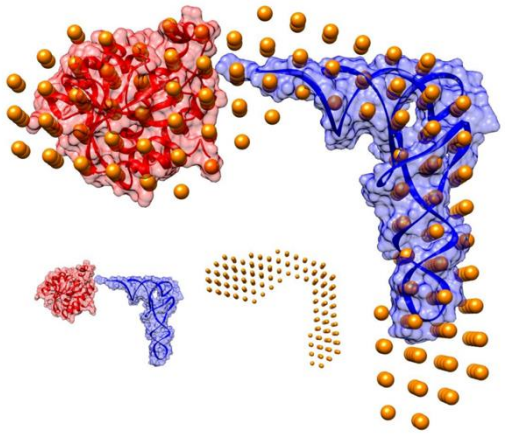
Modelling More Complex Shapes



Soluble proteins:

- simulated annealing
  - take box full of close packed spheres
  - allow spheres to change scattering length density
  - generate scattering pattern and compare to data
- Need to collect many different contrasts!

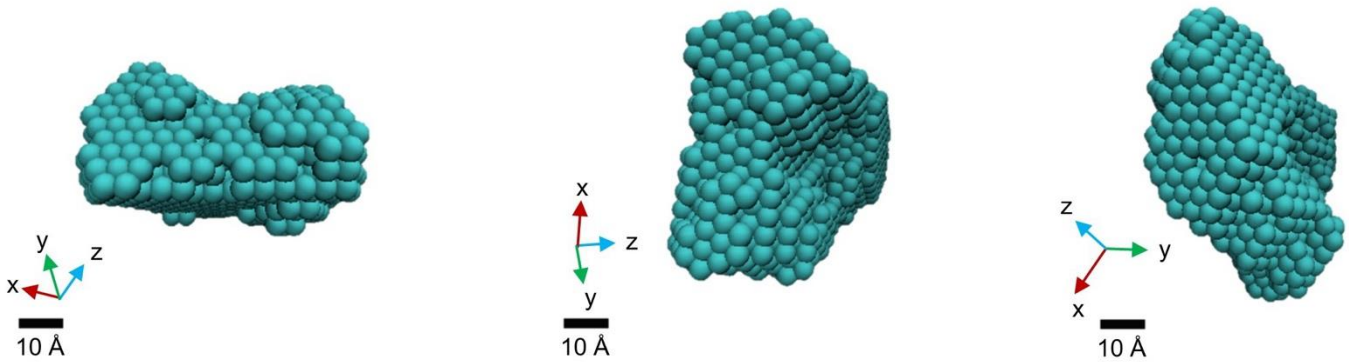




Gramicidin in SMALPs: MONSA

- MONSA: Part of the ATSAS data analysis software suite
- Single phase model; used only SANS data from d-DMPC with 32% D_2O PBS (highlights lipid core)
- In dimer form, gramicidin forms a channel 25 Å in length and 40 Å in diameter
- In bilayers > 25 Å thick, gramicidin causes compression of bilayer due to lipid length mismatch

model core: 70 by 30 Å

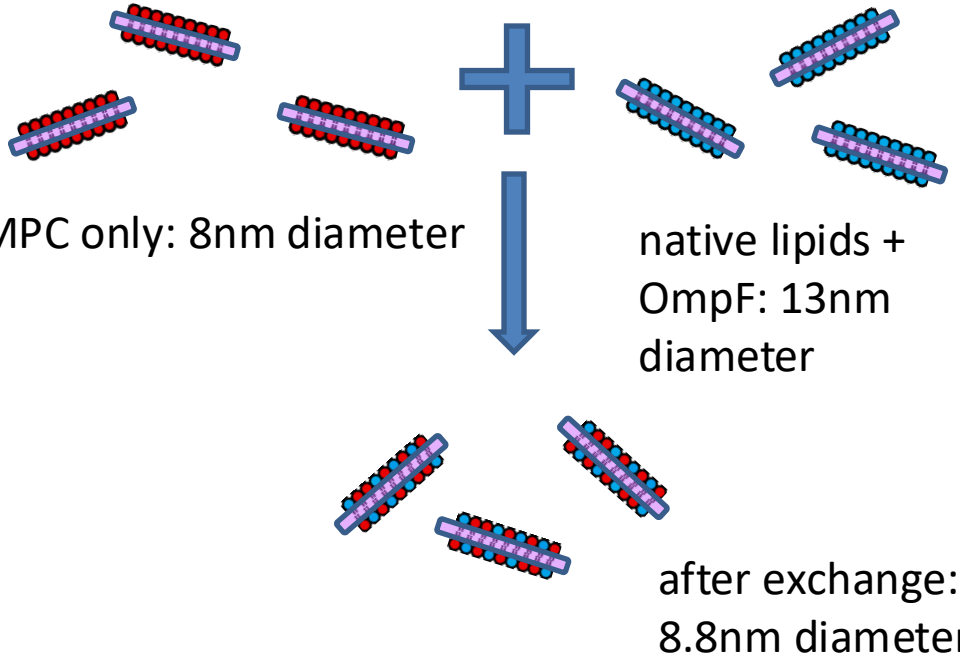


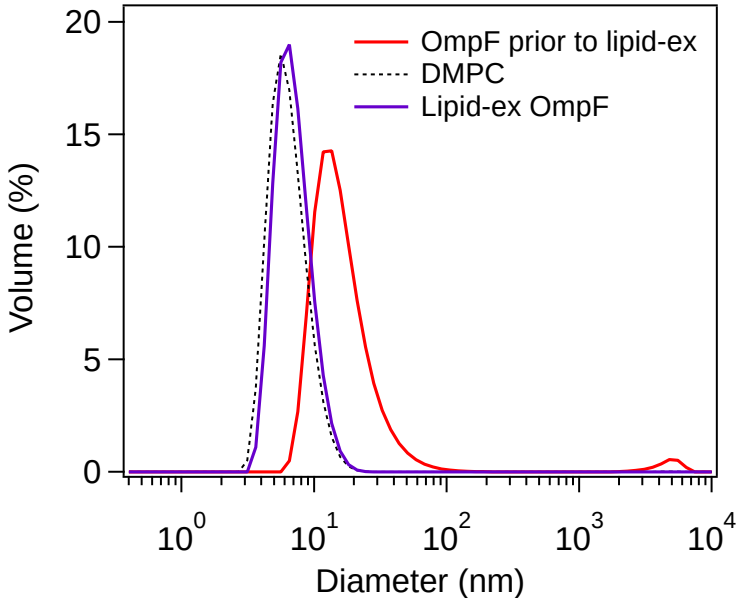
Morrison, Doekhie, Neville, Price, Whitley, Doutch, Edler, *BBA Advances* **2022**, *2*, 100033.

MONSA: D. Svergun, Biophys. J., 1999, 76, 2879–2886.
D. Franke and D. I. Svergun, J. Appl. Crystallogr., 2009, 42, 342–346.

OmpF Samples

- endogenous OmpF extracted from E. coli cells following Efremov & Sazanov.
- outer membrane pellet solubilized with SMA2000P to make SMALPs, gel filtered.
- lipid exchange with deuterated DMPC-SMALPs to alter contrast.



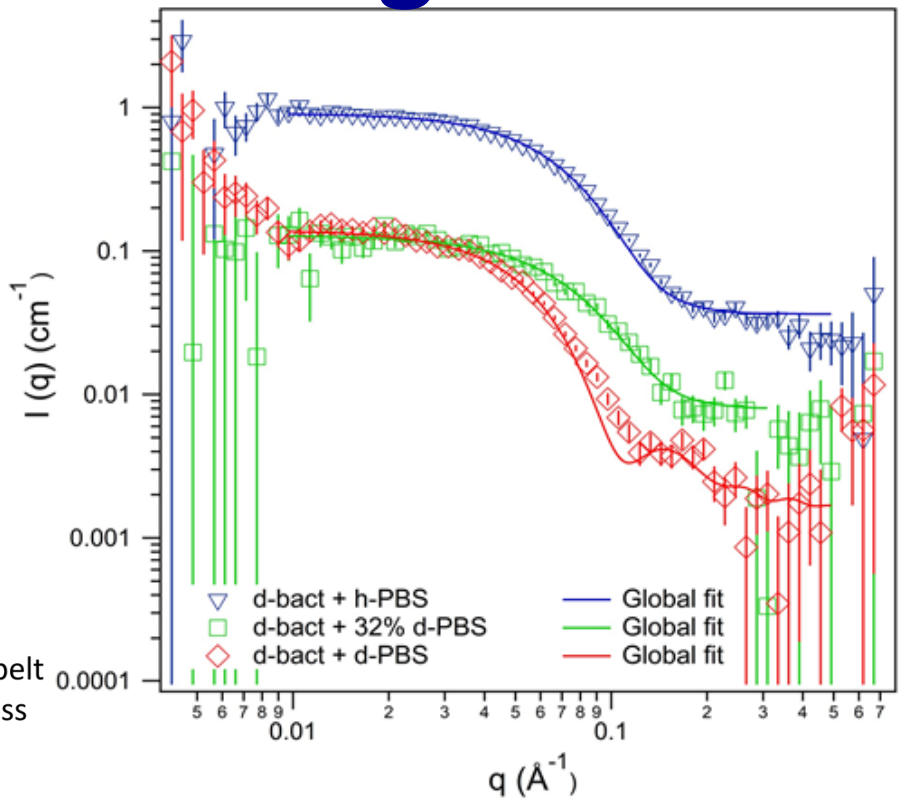


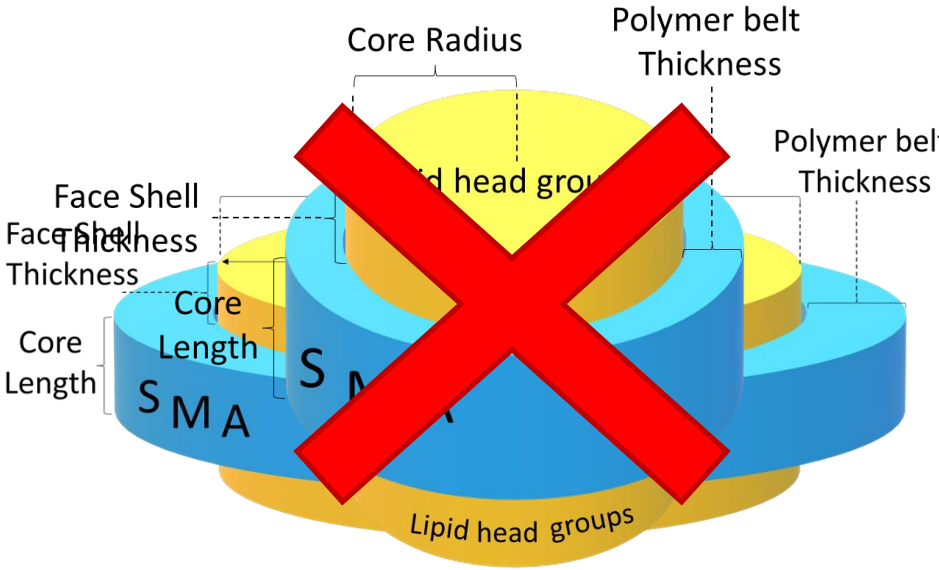
Efremov, Sazanov, J. Struct. Biol. 178 (2012) 311–318.

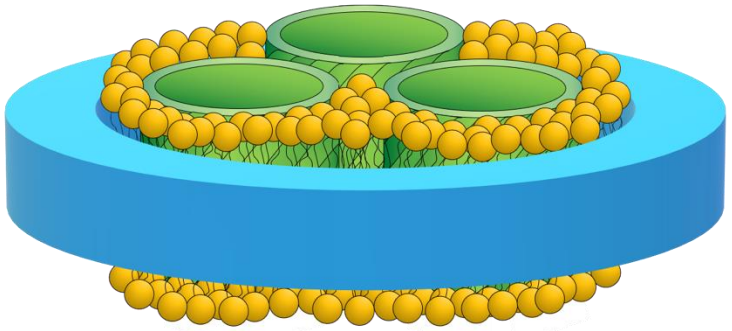
Morrison, Doekhie, Neville, Price, Whitley, Doutch, Edler, *BBA Advances* **2022**, *2*, 100033.

OmpF SANS Fitting

- OmpF in SMALPs
  - Core radii: 30 by 70 Å
  - Bilayer length increased to 60 Å
  - Polymer belt 9 Å, 30% hydrated



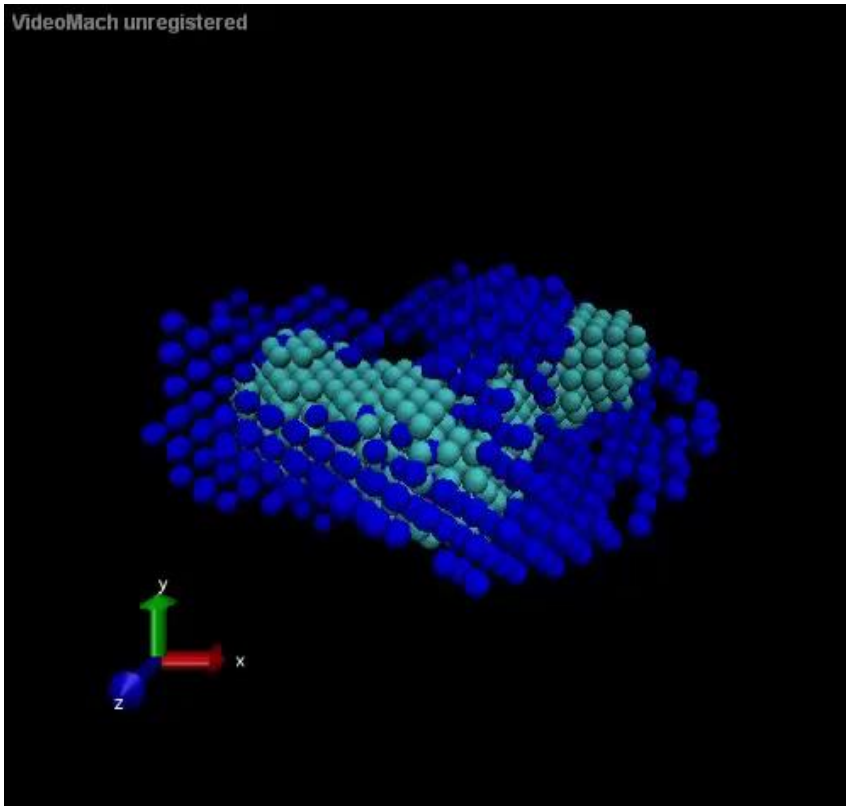




Morrison, Doekhie, Neville, Price, Whitley, Doutch, Edler, *BBA Advances* **2022**, *2*, 100033.

Ab initio Modeling: MONSA

- Simultaneous multi-phase "dummy atom" modelling
- Allows discrimination of two "phases" within model
  - polymer
  - lipid + proteins
- Assumed interconnected phases within particle
- Models visualised using Visual Molecular Dynamics (VMD)(3) software.

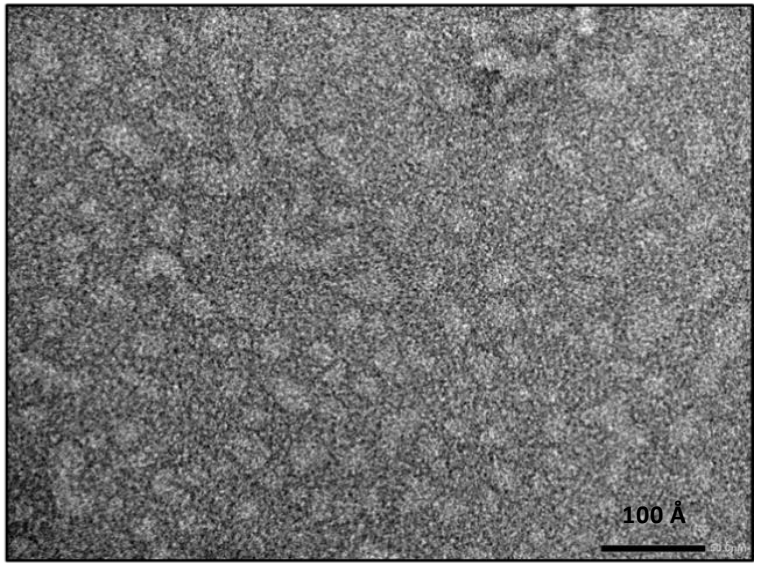


D. Svergun, Biophys. J., 1999, 76, 2879–2886.
D. Franke and D. I. Svergun, J. Appl. Crystallogr., 2009, 42, 342–346.

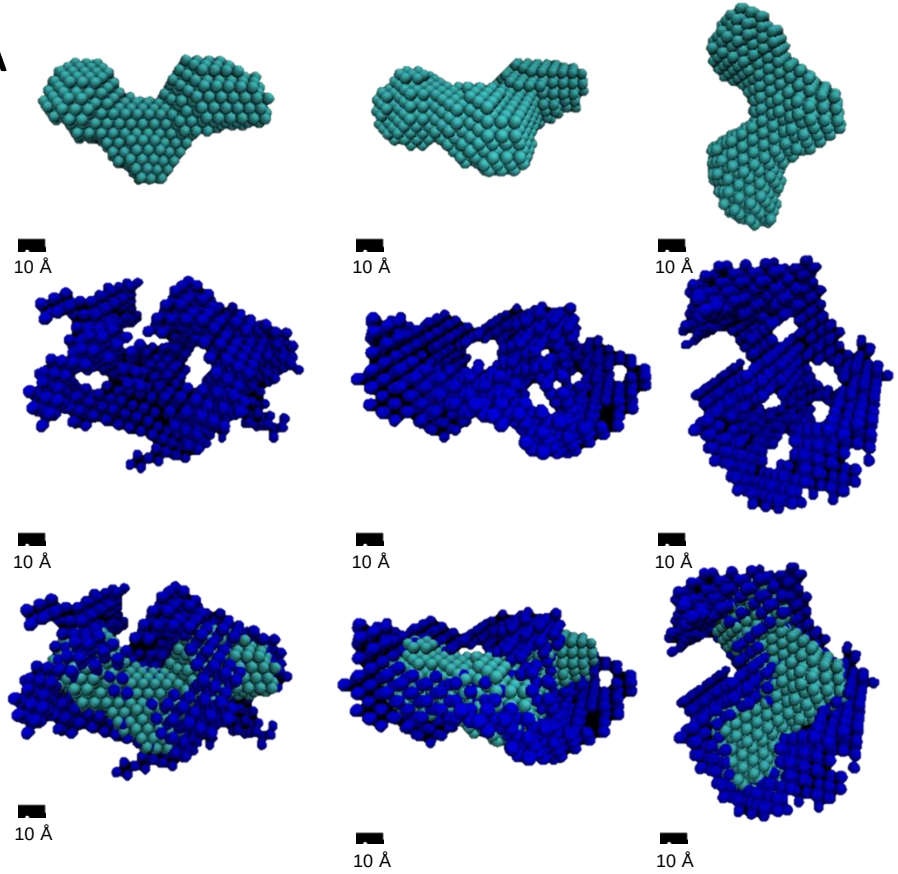
Morrison, Doekhie, Neville, Price, Whitley, Doutch, Edler, *BBA Advances* **2022**, *2*, 100033.

Modeling Outcome

- Approximately 80 by 30 Å.
  - ➢ cf bicelle model: 30 by 54 Å



TEM: uranyl acetate stained



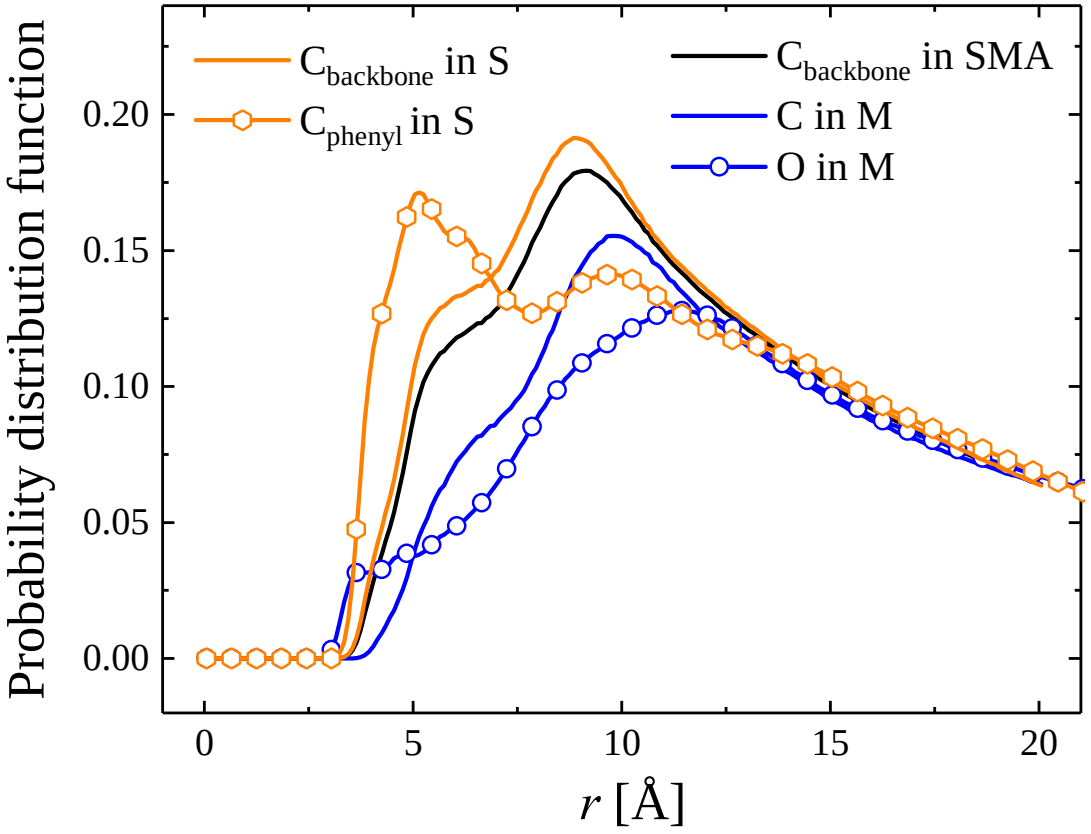
"Dummy atom" model for nanodiscs incorporating OmpF. [cyan] lipid/ protein phase; [blue] polymer phase.

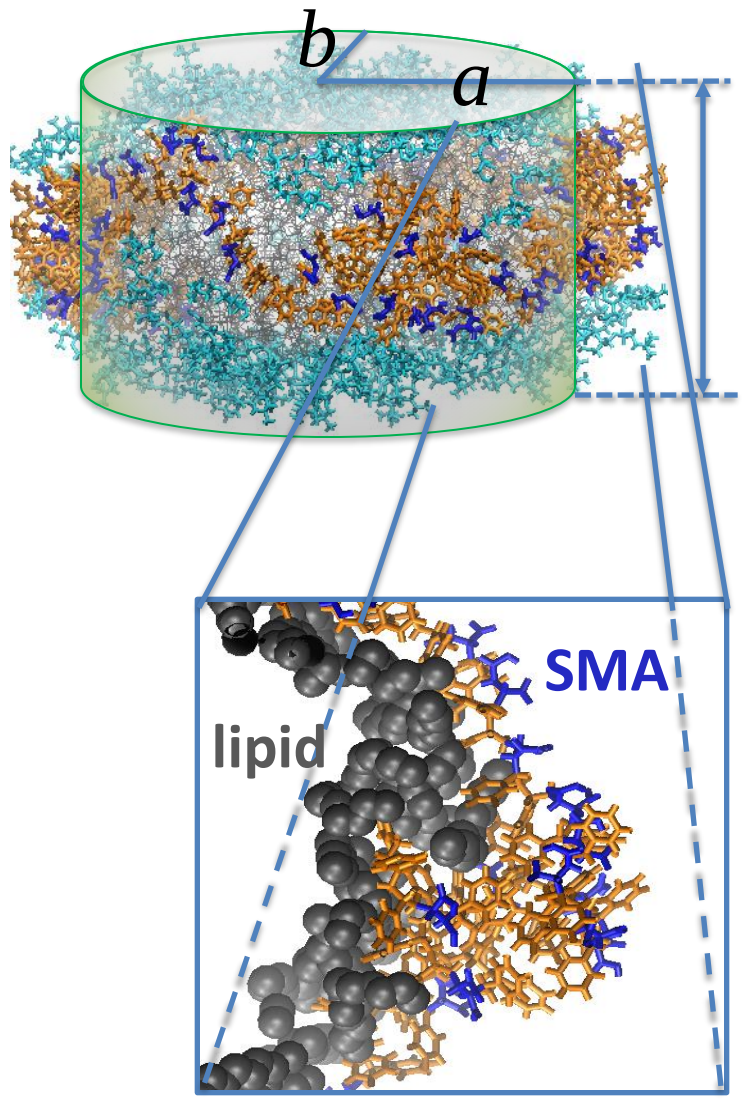
NEXT? Size Exclusion Chromatography (SEC)-SANS

Morrison, Doekhie, Neville, Price, Whitley, Doutch, Edler, *BBA Advances* **2022**, *2*, 100033.

Use Simulation in Scattering Analysis?

Atomistic SMALP model

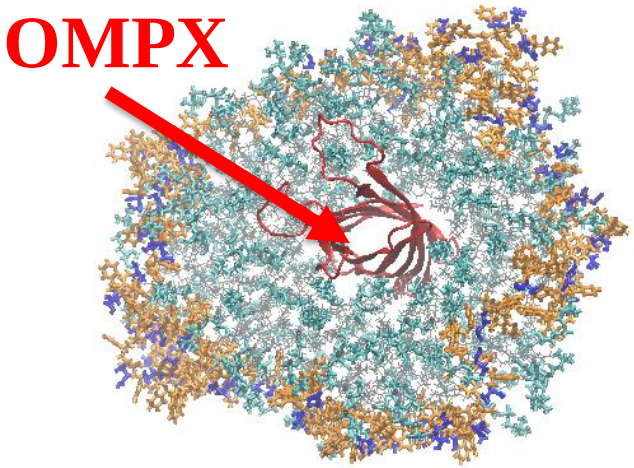


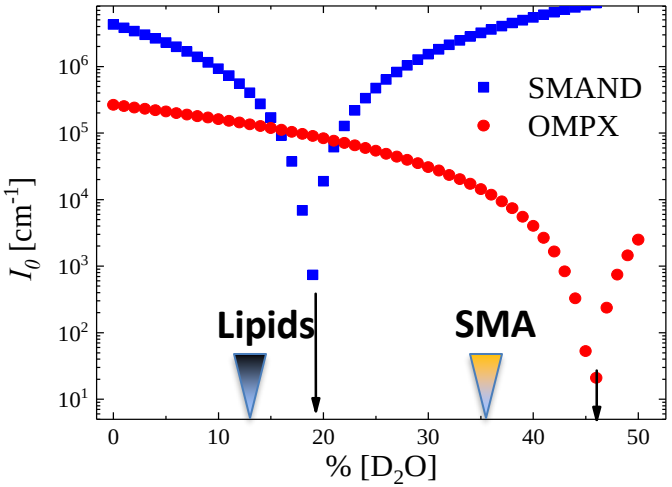


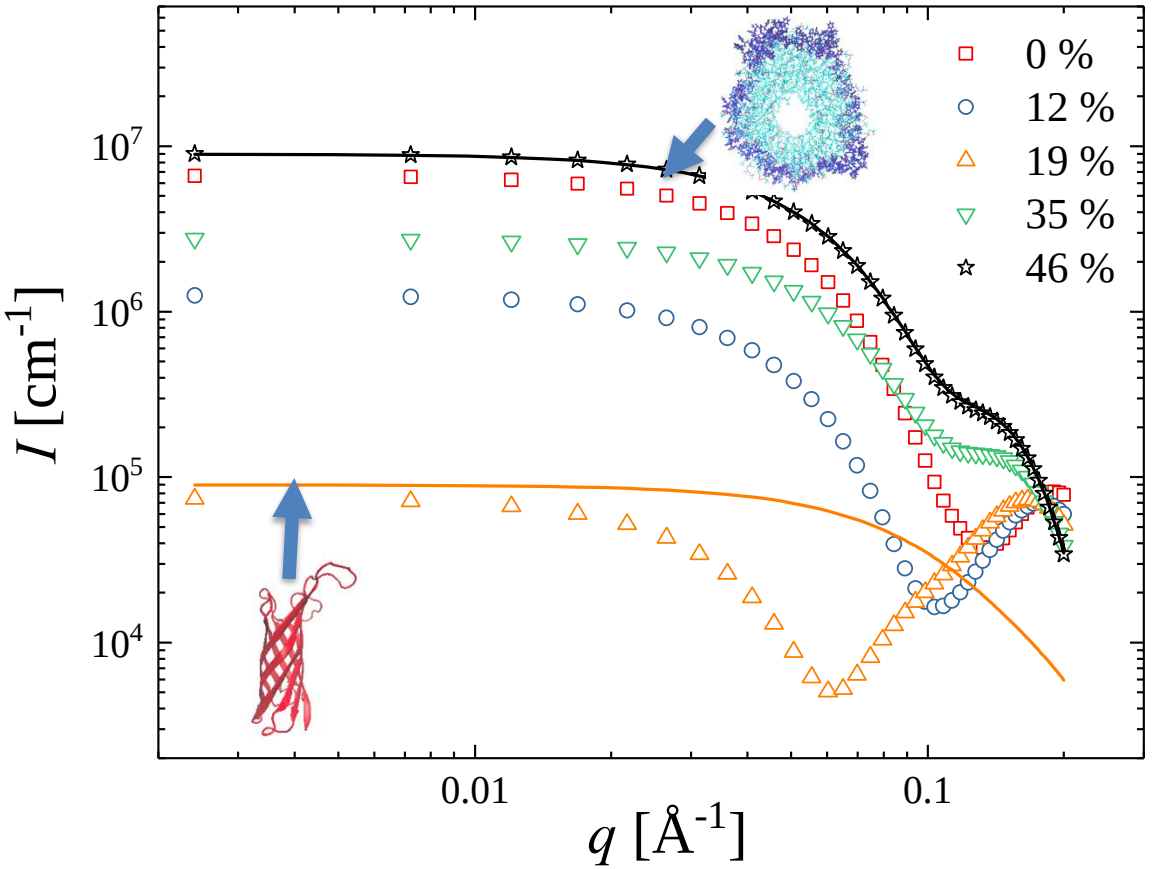
r = distance between outer boundary lipid tail carbons and SMA atoms

Jeong, C.; Franklin, R.; Edler, K. J.; Vanommeslaeghe, K.; Krueger, S.; Curtis, J. E., **J. Phys. Chem. B** 2022, DOI: 10.1021/acs.jpcb.1c05050.

Model: Integral MP in SMALP



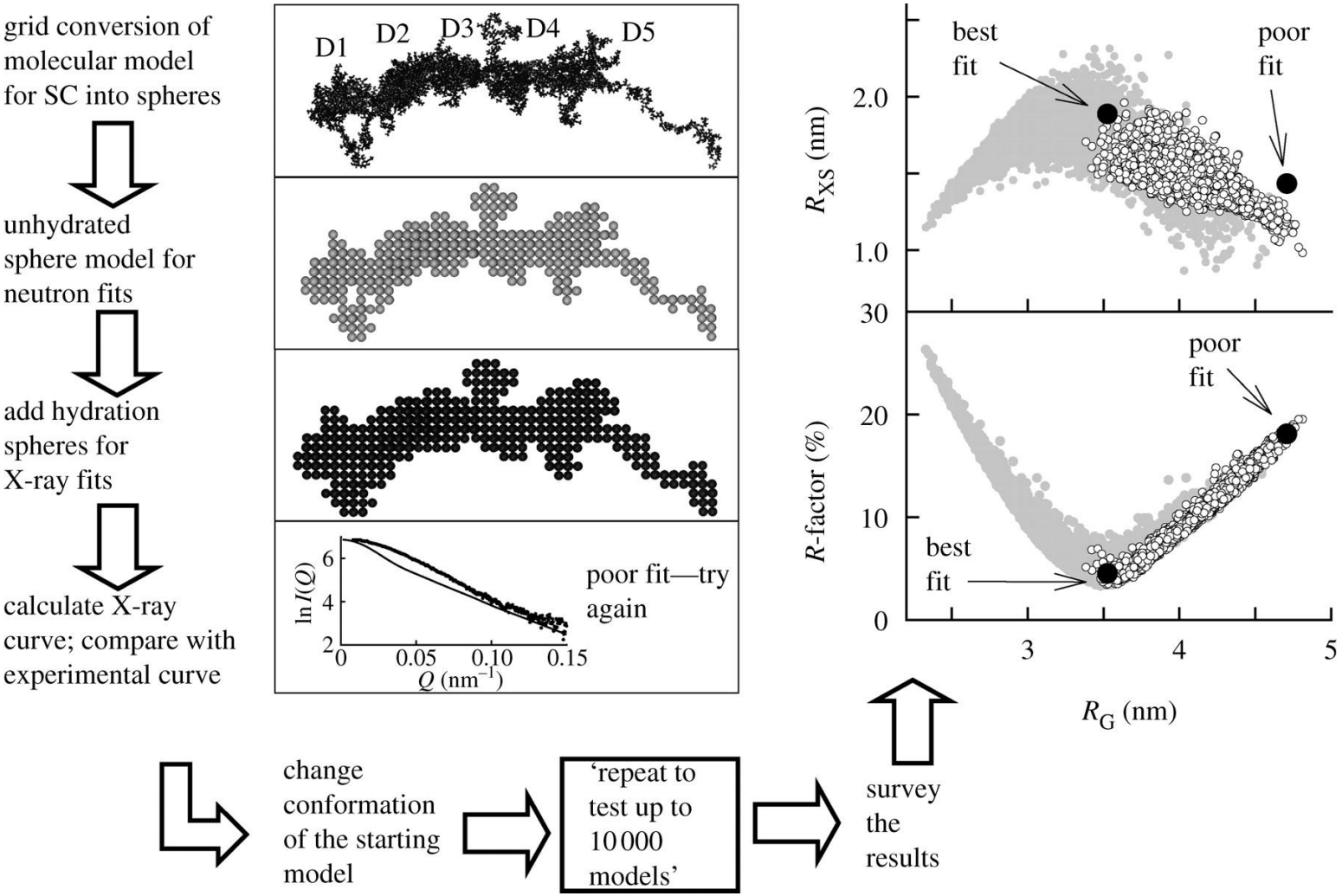




	OMPX	OMPX + SMALP
R_g [Å]	17.3	31.7

Jeong, C.; Franklin, R.; Edler, K. J.; Vanommeslaeghe, K.; Krueger, S.; Curtis, J. E.,
J. Phys. Chem. B 2022, DOI: 10.1021/acs.jpcb.1c05050.

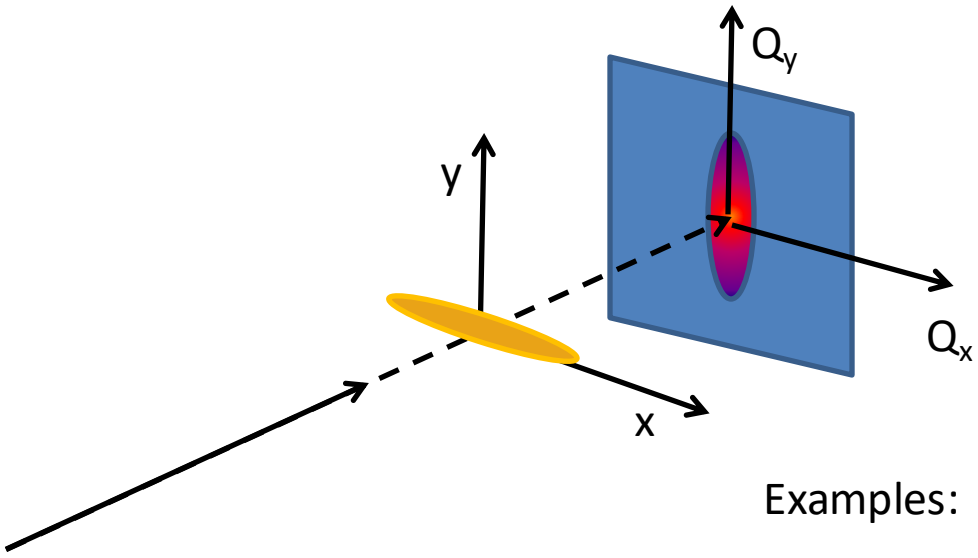
Constrained Modelling Algorithms & Output



S.J. Perkins et al. J. Royal Soc. Interface 2009;rsif.2009.0164.focus

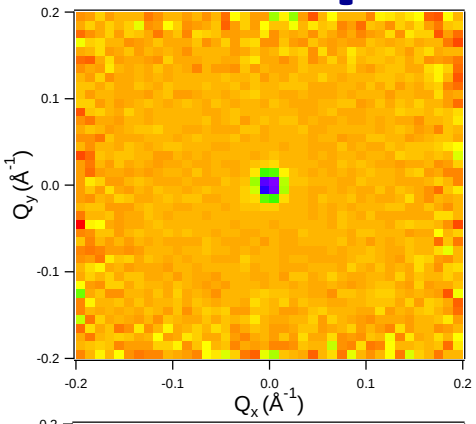
Effects of Sample Alignment

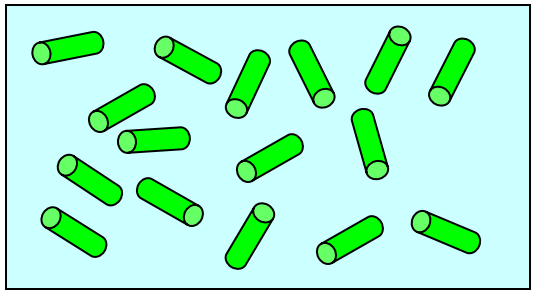
- Scattering no longer circular
- Form areas of high intensity perpendicular to direction of alignment



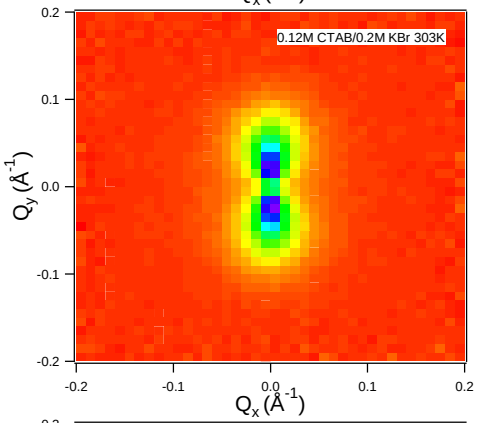
Examples: shear, flow
magnetic alignment

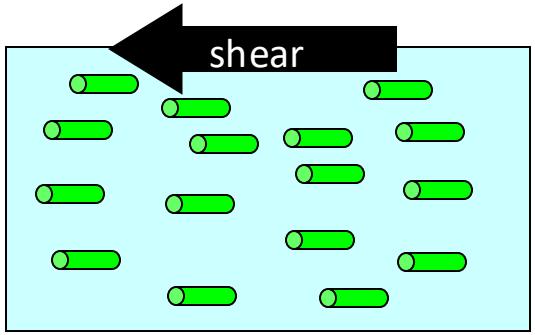
Isotropic vs Nonisotropic Structures



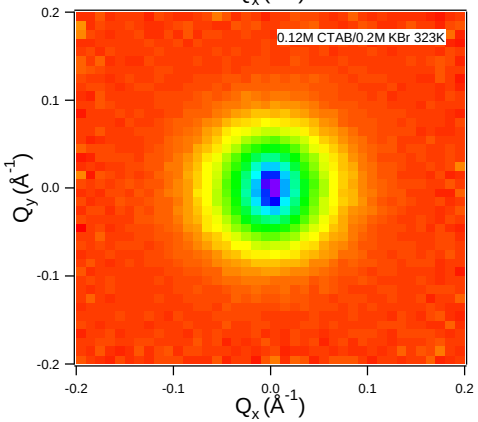


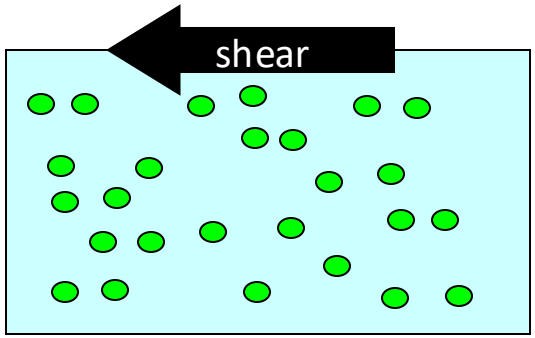
No shear
⇒Isotropic solution





Shear
⇒ aligned micelles



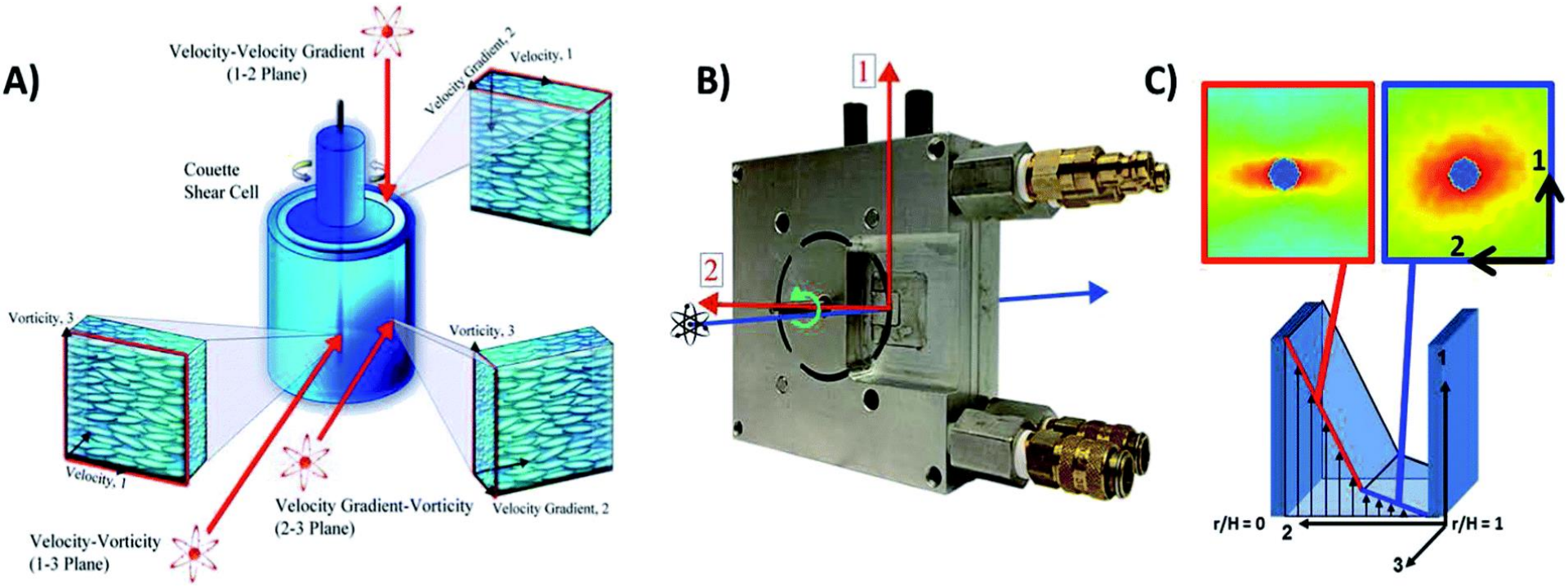


Shear + higher T
⇒ isotropic again

Edler, Reynolds, Brown, Slawecki, White, *J. Chem. Soc., Faraday Trans.* **1998,** *94*(9) 1287

Rheo-SANS

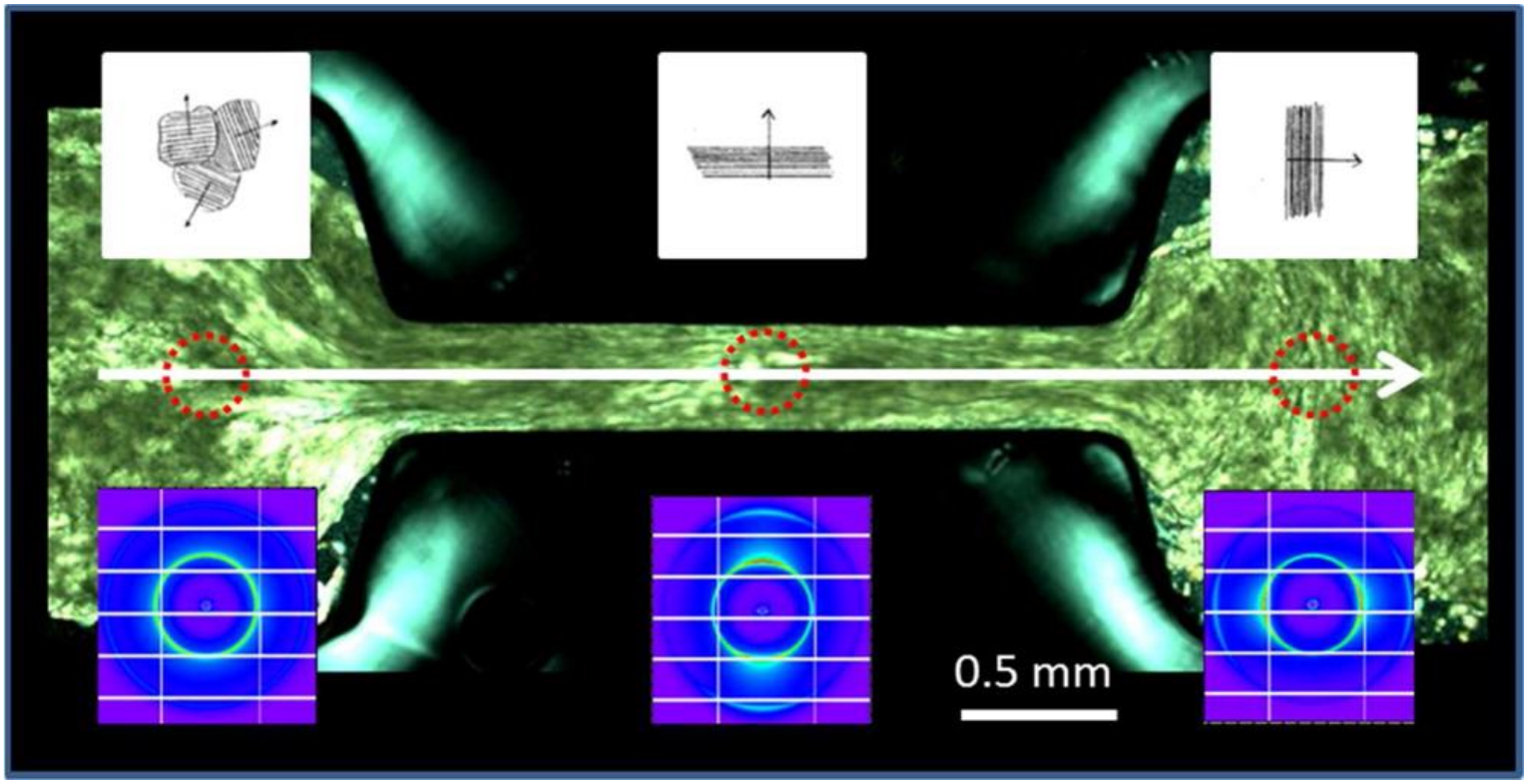
- Following material structure under shear
- 1–2 flow STR-SANS (spatiotemporally resolved small angle neutron scattering)



Gurnon; Lopez-Barron; Eberle; Porcar; Wagner, Spatiotemporal stress and structure evolution in dynamically sheared polymer-like micellar solutions. *Soft Matter* **2014,** *10* (16), 2889-2898. CC-BY 3.0

More Complex Shear Geometries

- Developments in sample environment & improved flux/reduced beam size mean new information becoming available in complex/realistic soft matter systems



Poulos; Nania; Lapham; Miller; Smith; Tantawy; Caragay; Gummel; Ces; Robles; Cabral; *Langmuir* **2016,** 32, 5852-5861. DOI: 10.1021/acs.langmuir.6b01240. CC-BY 3.0