Structure of soybean oleosomes studied by small angle neutron scattering

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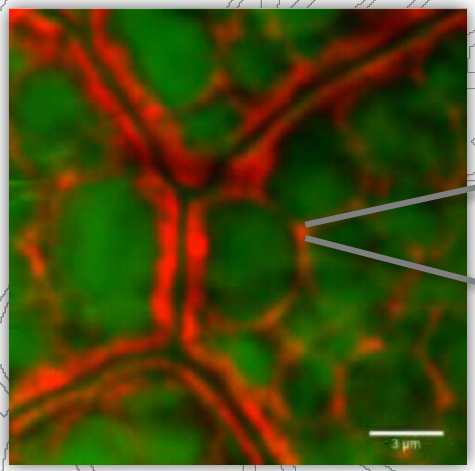
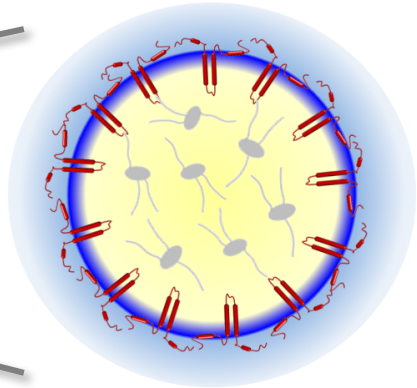
Oleosomes

Oleosomes: Intracellular oil storage particles in plants



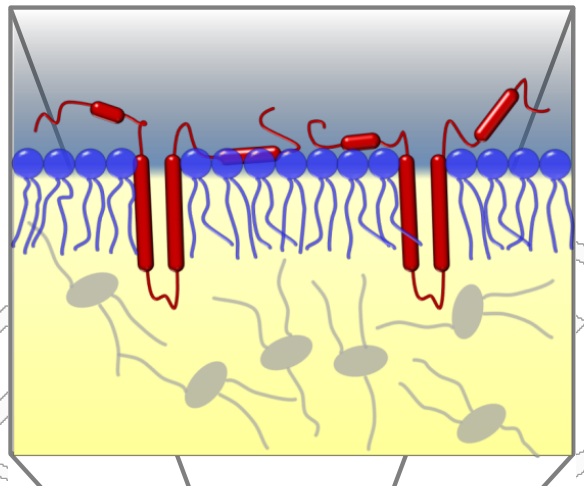



aqueous extraction



Natural emulsion of highly stabilised oil droplets

Oleosomes



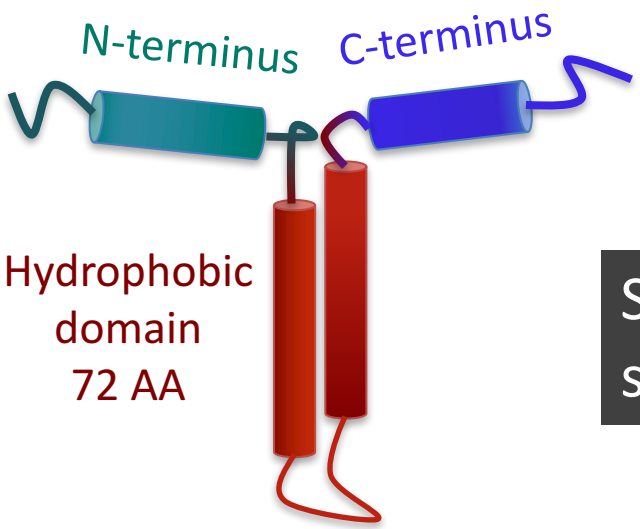
Oleosins

Phospholipids

Triacylgycerides

ca. 350 nm

Oleosin



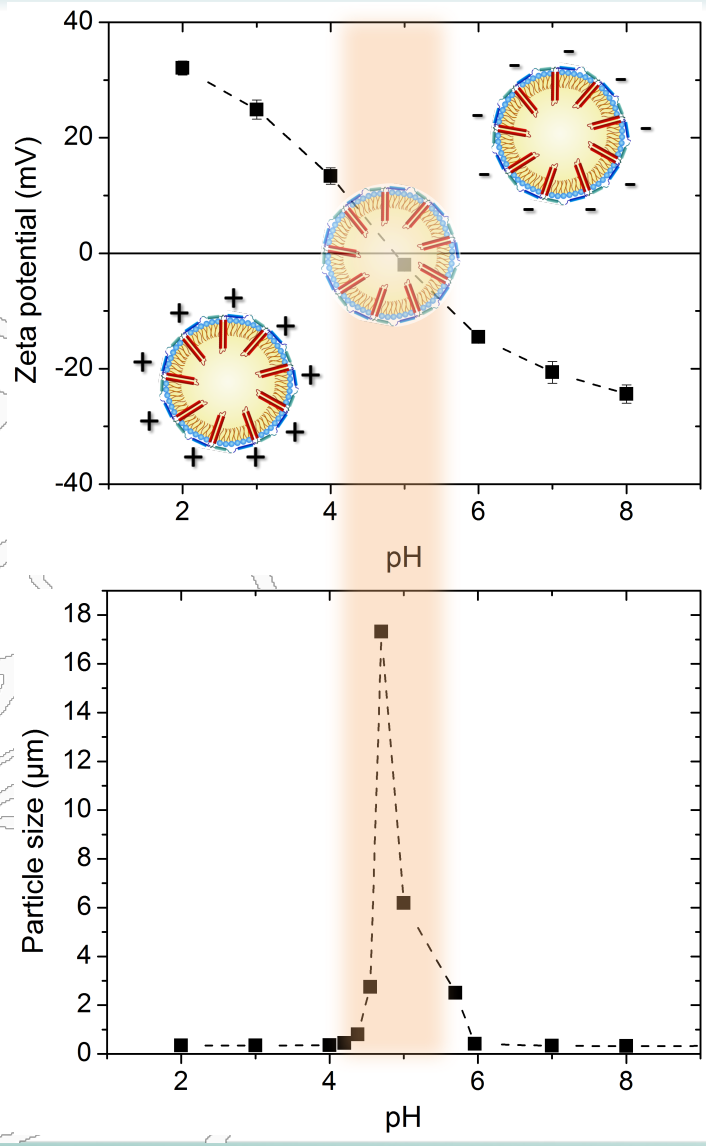
Secondary structure?

- Crucial for stability
- Determines oleosome size
- Acts as lipase acceptor

Importance of oleosin

Zeta potential

Particle size



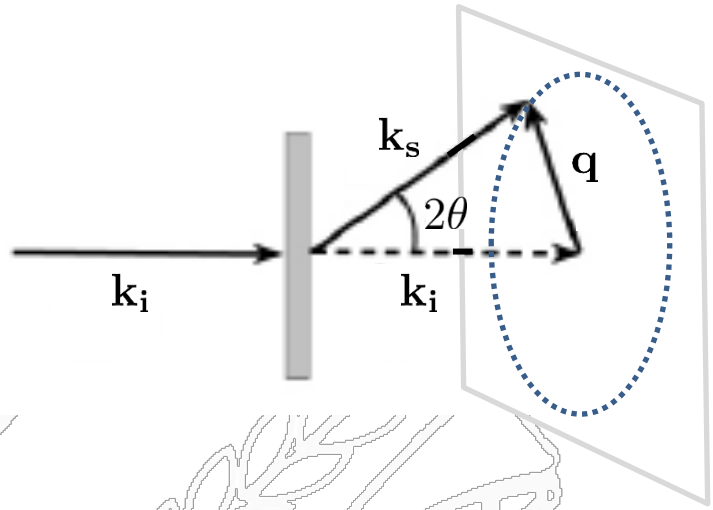
Maurer et al., *J. Phys. Chem. B* (2013) 117

SANS

- Alternative method for size determination
- Examine oleosin in its native environment – the oil/ water interface
- Detect early changes in interfacial layer upon environmental changes (e.g. temperature)

Scattering experiment

- Experiments performed at ISIS (Didcot, UK) and ILL (Grenoble, France)



$$\mathbf{q} = \mathbf{k_s} - \mathbf{k_i}$$

$$|\mathbf{q}| = \frac{4\pi}{\lambda}\sin\theta$$

Scattered intensity $I(q) = N(\Delta\bar{\rho}V)^2 P(q) S(q)$

molecules/volume

contrast

$$\Delta\bar{\rho} = \bar{\rho}_{particle} - \bar{\rho}_{solvent}$$

form factor: shape

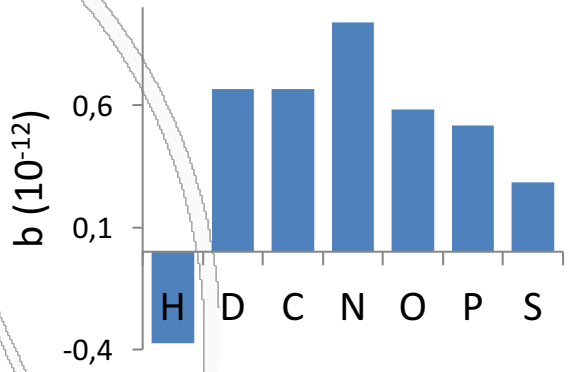
structure factor: interactions

Neutron scattering

Neutrons interact with nuclei → scattering depends on isotope

Scattering length *b* (10^{-12} cm):

H	D	C	N	O	P	S
-0.3742	0.6671	0.6651	0.940	0.5804	0.517	0.2847

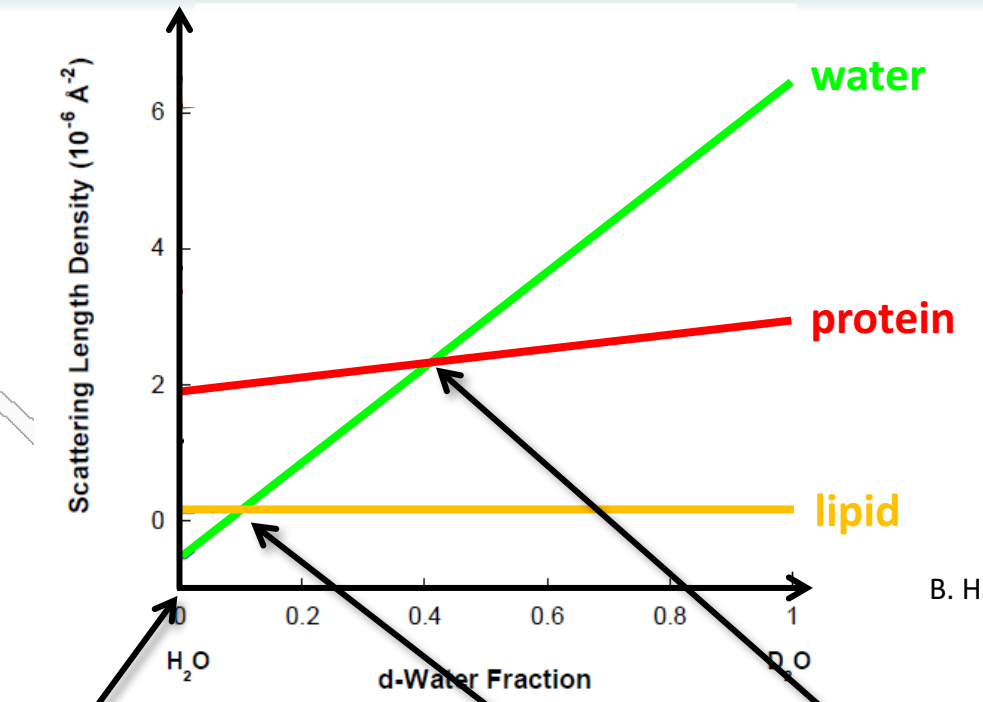


Scattering length density (SLD) ρ:

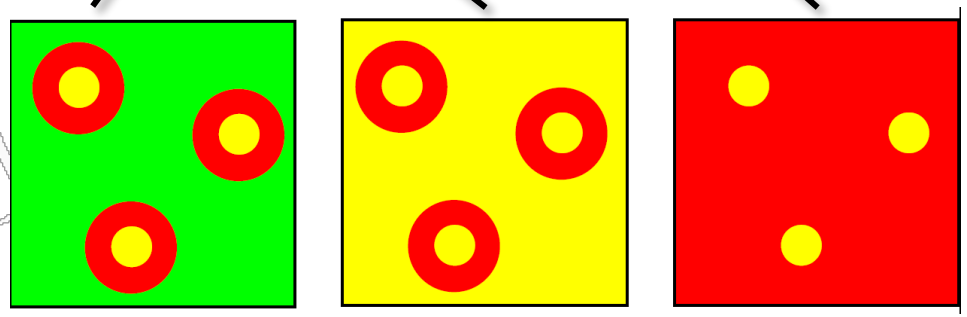
$$\rho = \frac{\sum_{i=1}^{N} b_i}{V}$$

Contrast variation

Biological systems



B. Hammouda (2008)

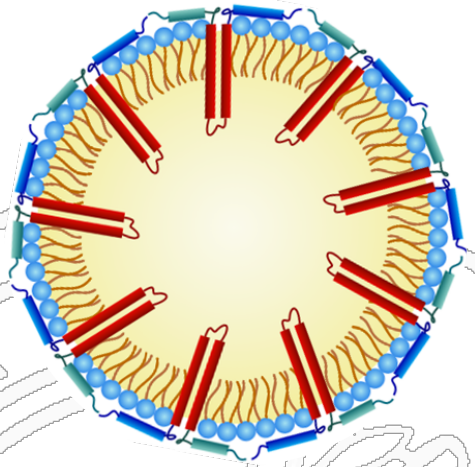


Natural contrast | ρ solv = ρ core (shell visible) | ρ solv = ρ shell (core visible)

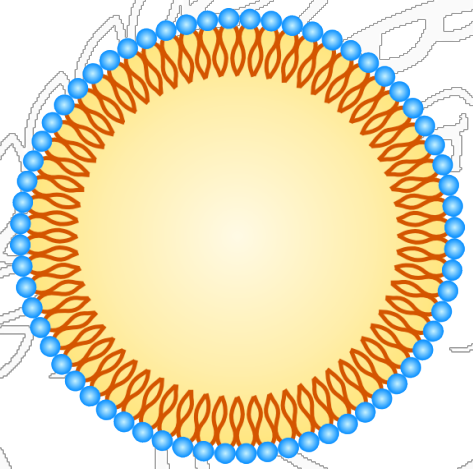
A. Jackson (2008)

Test contrast variation

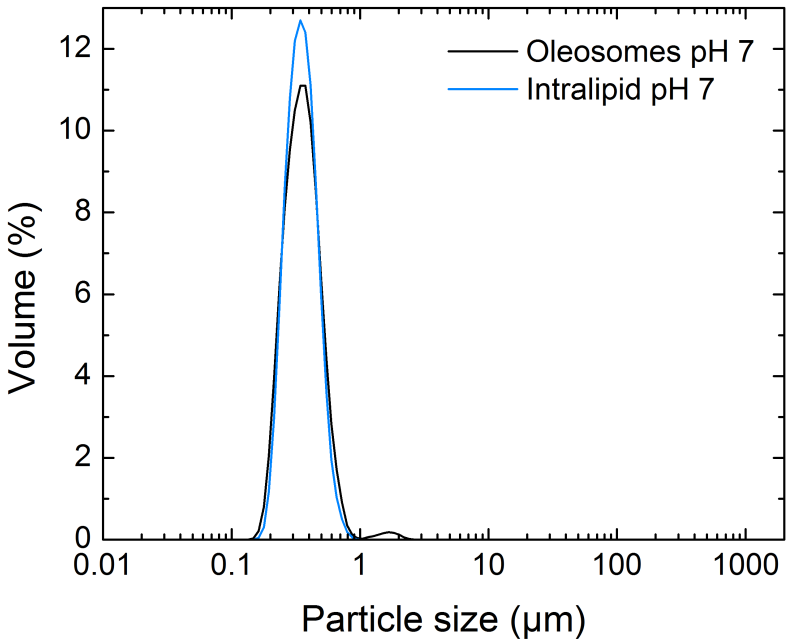
Oleosome



Intralipid



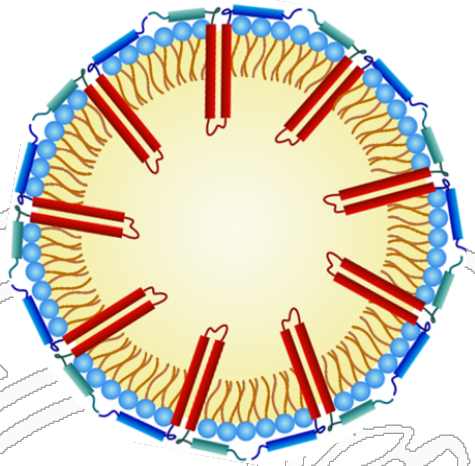
Size comparison



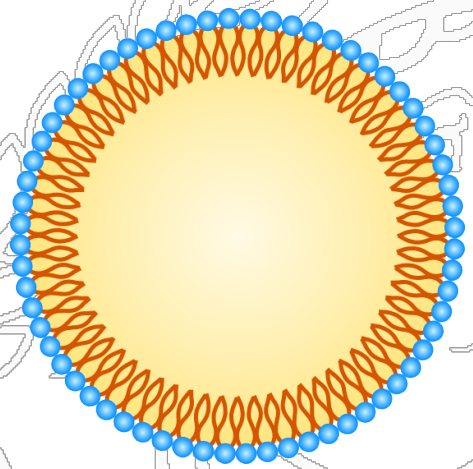
Intralipid:
comparable system without protein

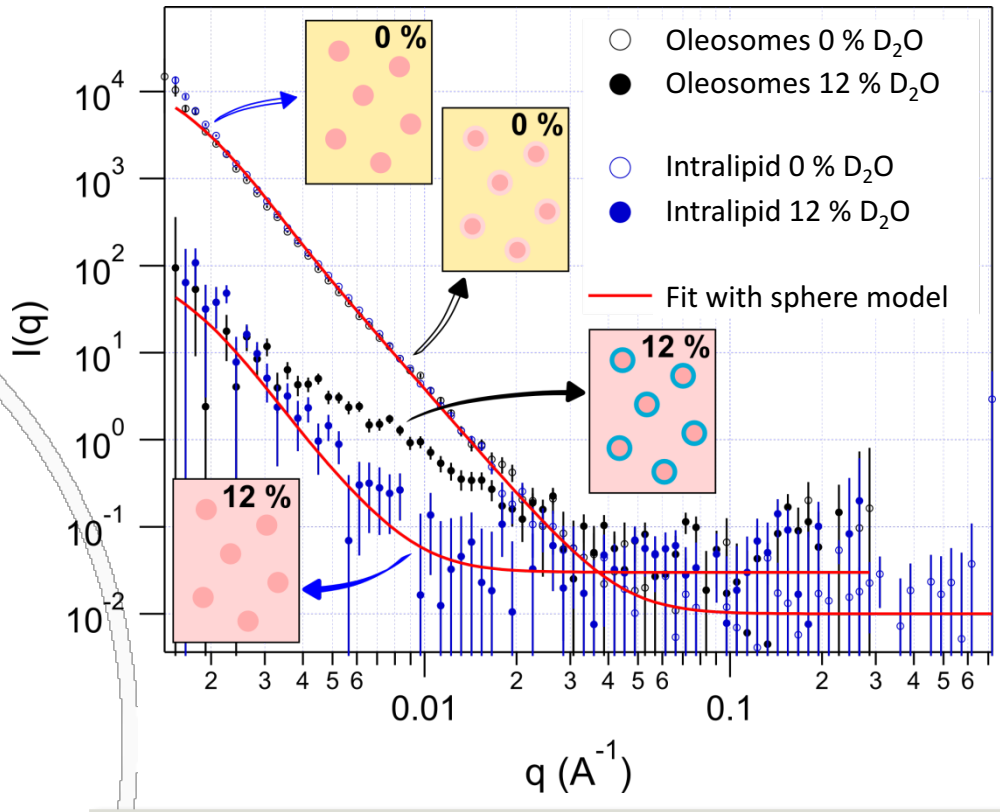
Test contrast variation

Oleosome



Intralipid



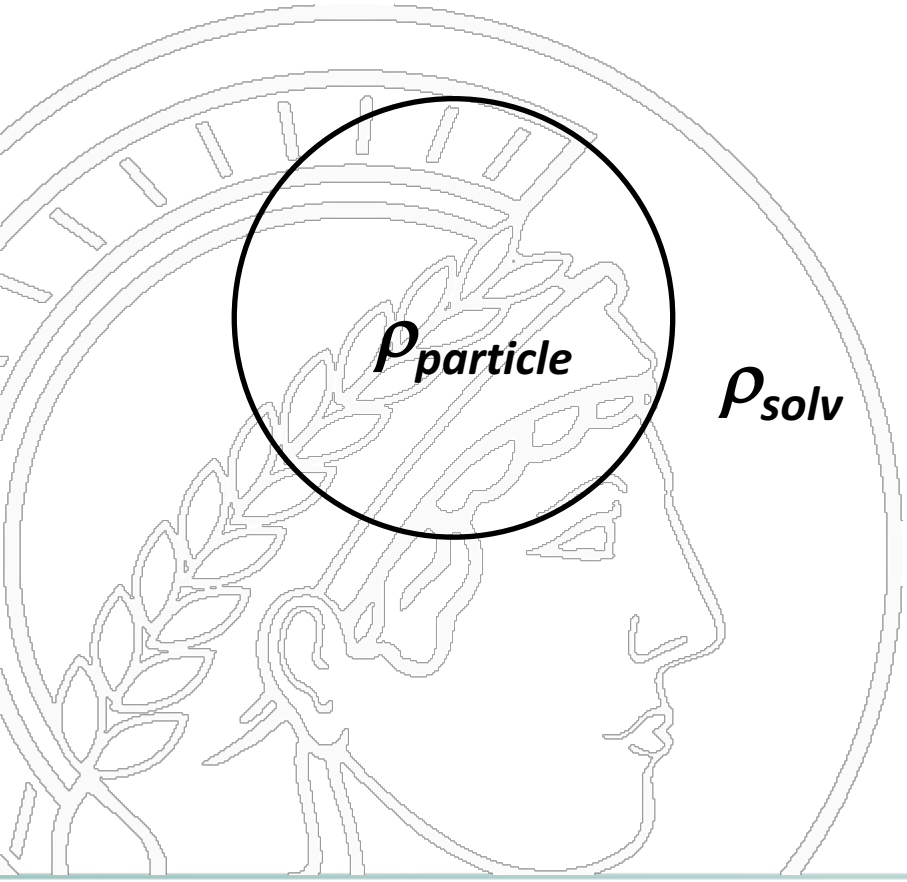


Oleosomes in 12% D_2O (oil core match) can not be fitted with sphere model

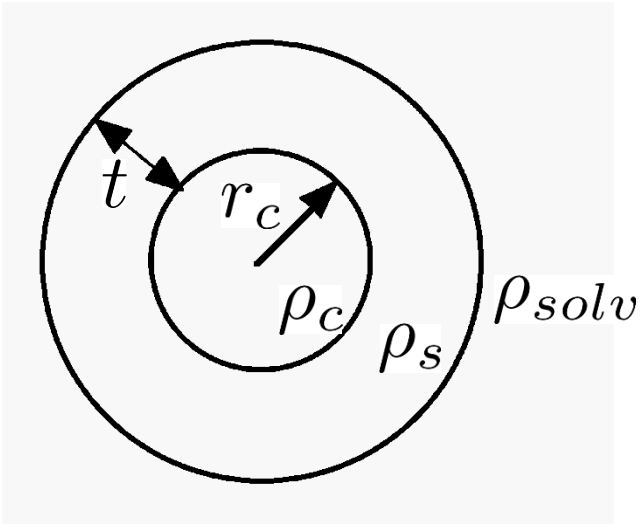
→ **signal of protein shell visible**

Geometry evaluation

sphere



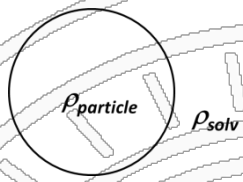
core-shell particle



Fitting

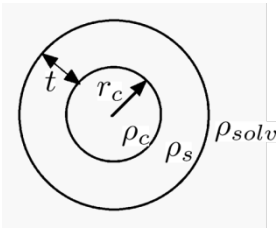
IGOR Pro + NIST SANS analysis macro (*"Reduction and Analysis of SANS and USANS Data Using IGOR Pro"* S. R. Kline, J. Appl. Cryst. 39 (2006) 895900)

Sphere model



- polydisperse spheres

Core-shell model

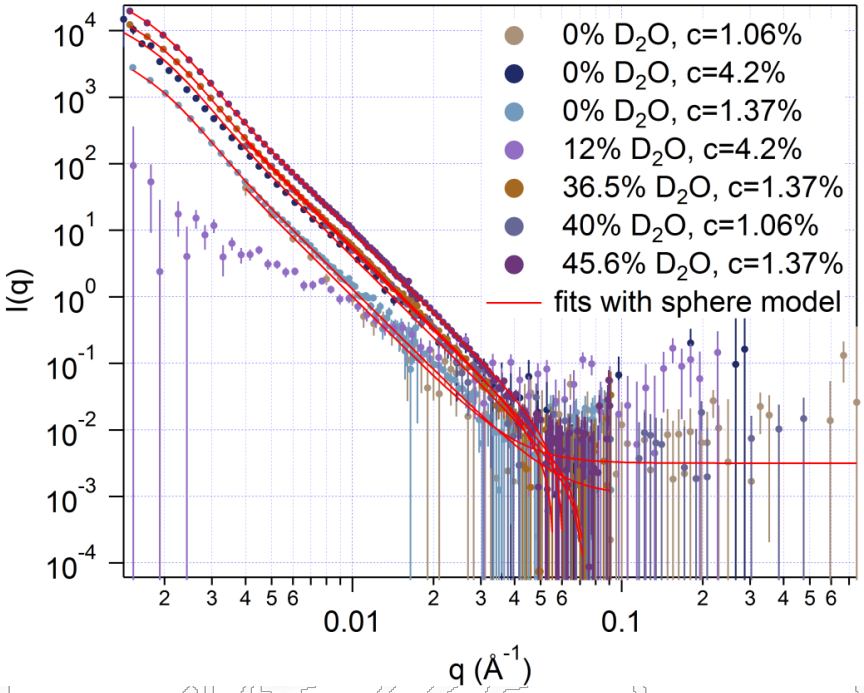


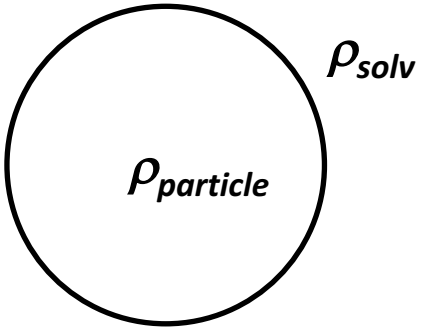
- polydisperse core
- constant shell thickness

Results from fits

	Sphere model	Core-shell model
sizes	sphere radius	core radius, shell thickness
polydispersity	$p = \sigma/R$	
SLDs	$\rho_{particle}$	$\rho_{core}, \rho_{shell}$

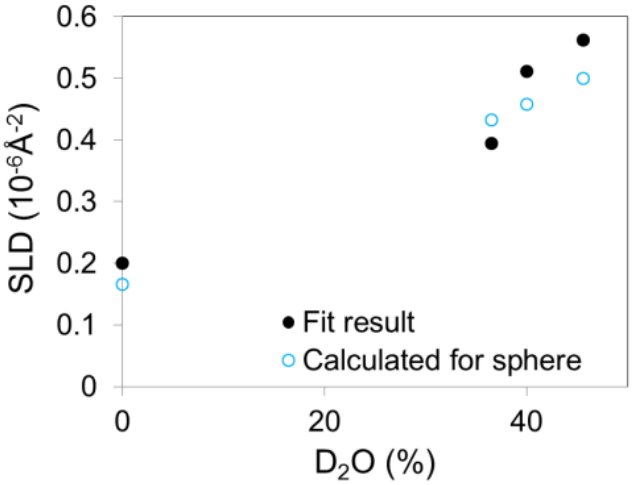
Sphere model





Component	% (w/w)
Protein	4.65
Phospholipid	2.95
Solvent	10.5

- **Average SLD** based on composition
- **contrast dependent SLD** (solvent and hydrophilic protein domains)

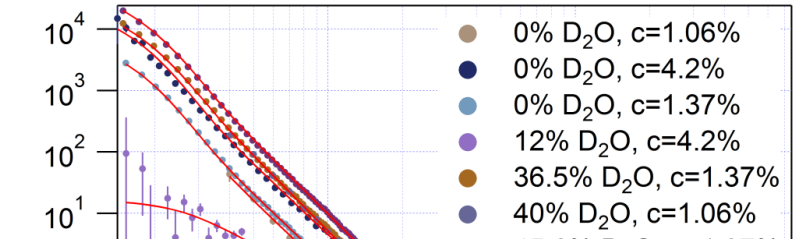


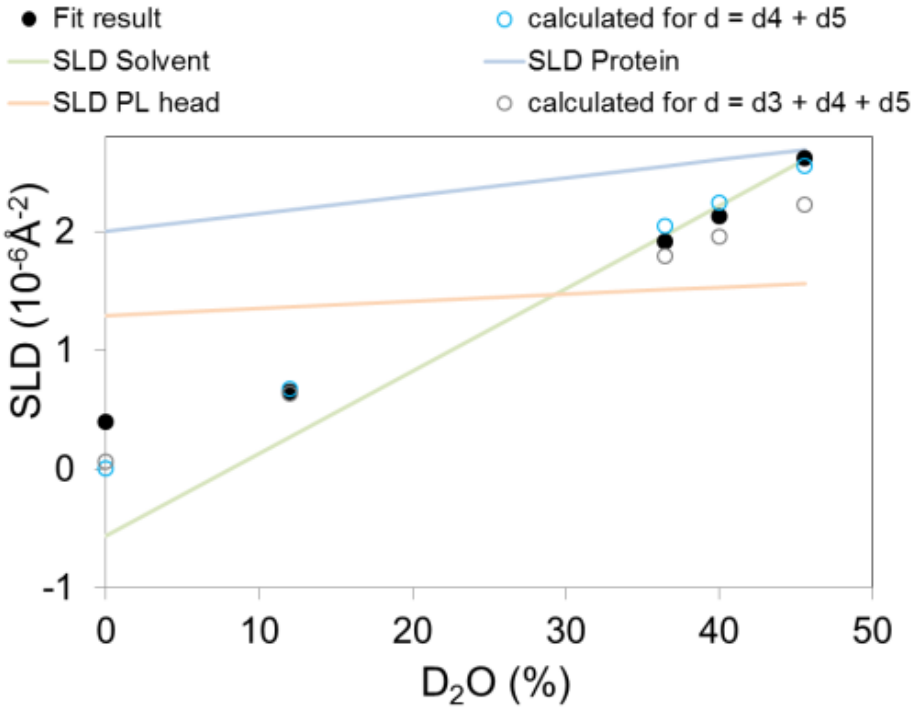
Fit results

- R = 1771 Å
- p = 0.395

→ **start values for core-shell fit**

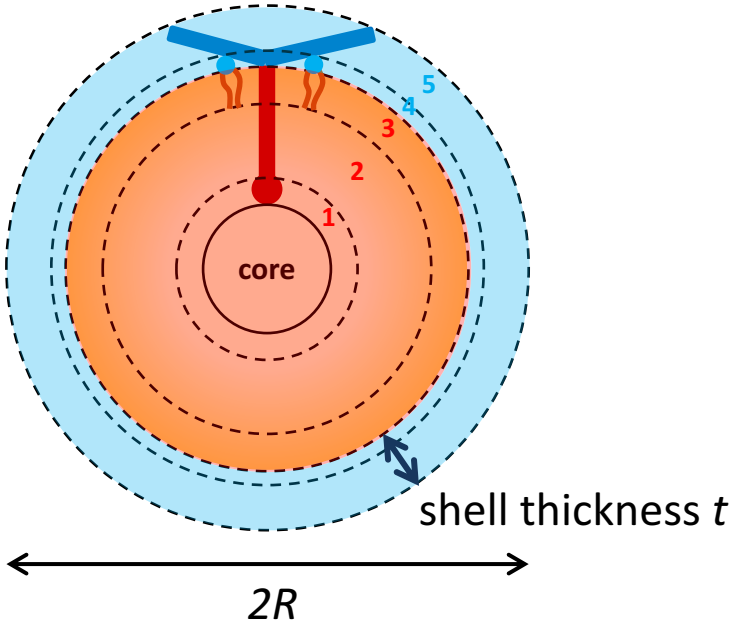
Core-shell model





Fit results

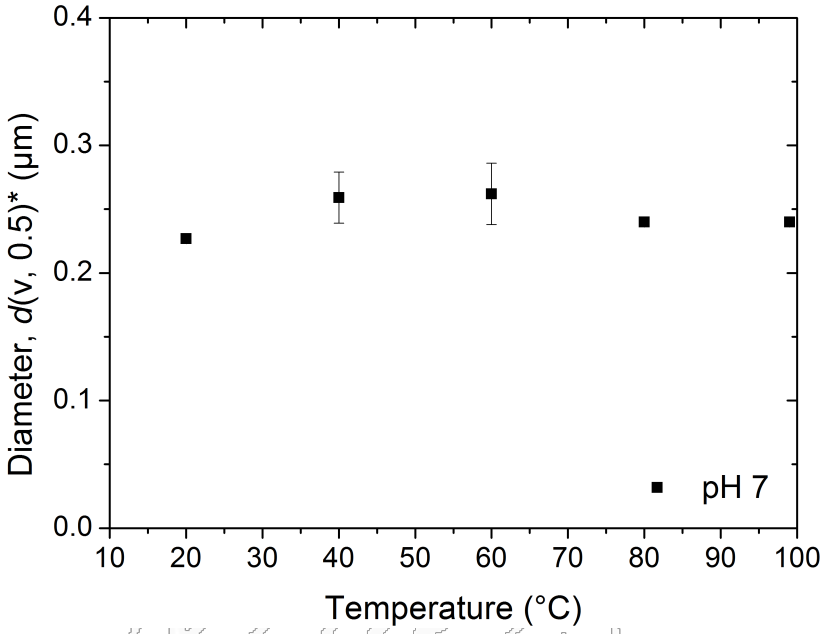
- R = 1848 Å
- p = 0.396
- t = 91 Å → **6.6 – 8.3 nm protein shell**



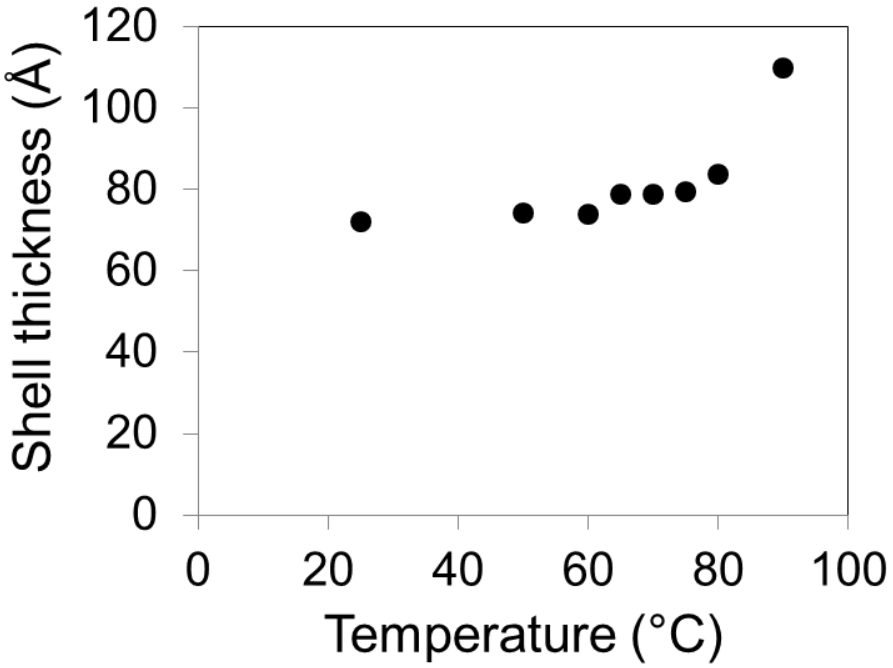
- theoretical shells
- calculate **SLDs for individual shells**

Temperature stability

Laser diffraction



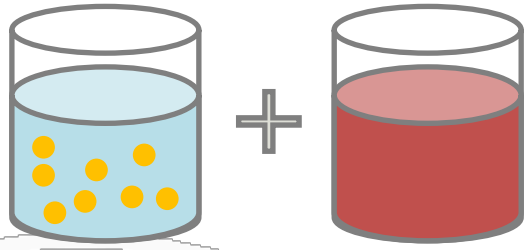
SANS



No significant change in shell thickness up to 80 °C

→ **high temperature stability**

Encapsulation

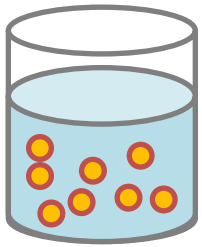


Oleosomes
7% w/w, pH 7

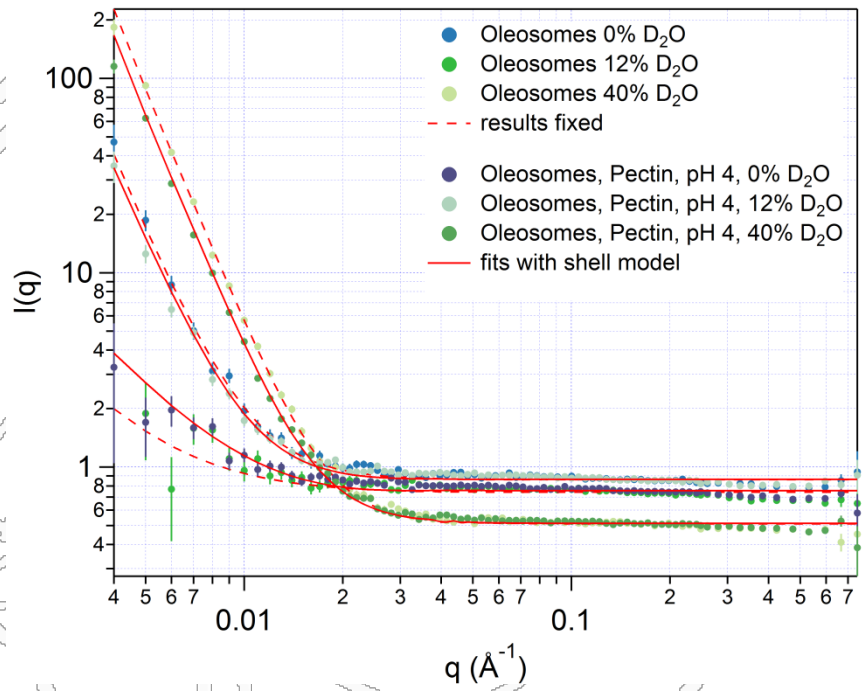
Pectin
0.5% w/w, pH 7

+ HCl

pH 4



Oleosomes encapsulated with pectin



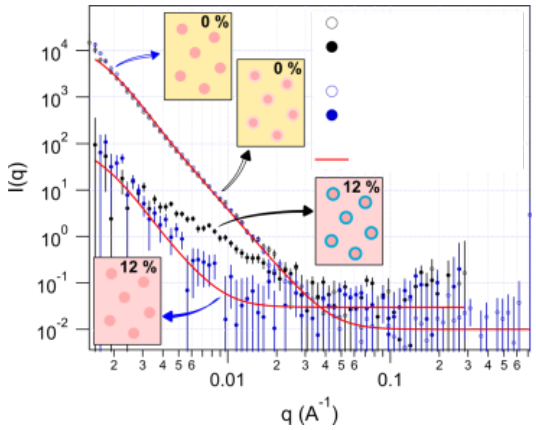
Shell thickness for encapsulated oleosomes 197 Å

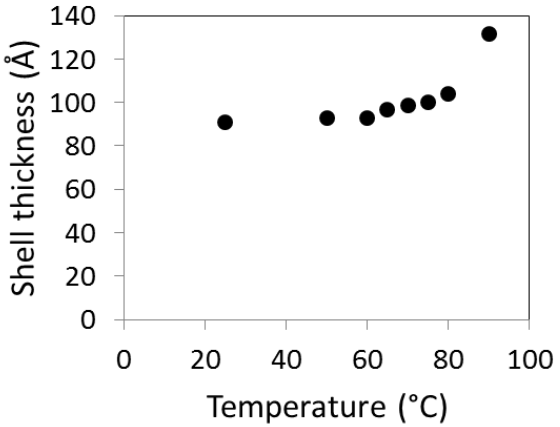
→ **106 Å pectin layer**

Conclusions

SANS can be used to study

- oleosomes‘ native interface
- temperature stability
- encapsulation





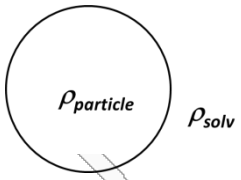
Thank you!

Fitting

IGOR Pro + NIST SANS analysis macro (*"Reduction and Analysis of SANS and USANS Data Using IGOR Pro"* S. R. Kline, J. Appl. Cryst. 39 (2006) 895900)

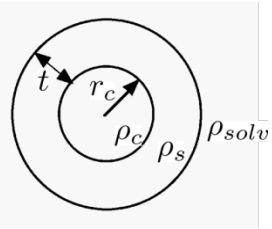
a) Sphere model

- polydisperse spheres



b) Core-shell model

- polydisperse core
- constant shell thickness



Schulz distribution

$$f(R) = (z+1)^{z+1} x^z \frac{exp[-(z+1)x]}{R_{avg}\Gamma(z+1)}$$

where $x = R/R_{avg}; z = 1/p^2 - 1$

with polydispersity $p = \sigma/R_{avg}$

and variance σ^2

Guinier radius: $$R_g = \sqrt{\frac{3\langle R^8\rangle}{5\langle R^6\rangle}} = R_{avg}\sqrt{\frac{3(z+8)(z+7)}{5(z+1)^2}}$$