Protein adsorption at the air/water interface

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- β-casein and β-lactoglobulin in solution
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- .. at air-water interface, kinetics
- Conclusions

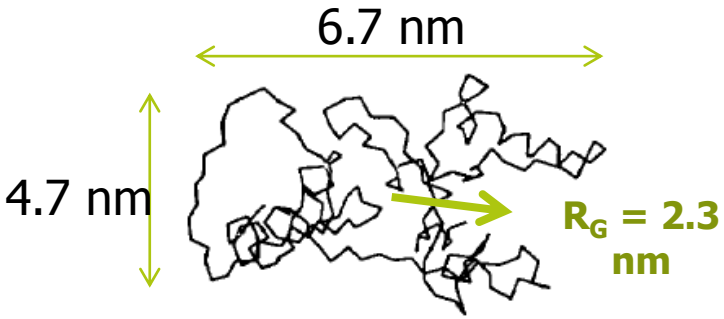
Introduction

- Foam is stabilized by surface-active molecules (by lowering the surface tension)
- In food industry: proteins
- What is the structure of the protein film?
- What is the kinetics of protein film formation?

The proteins

β-casein

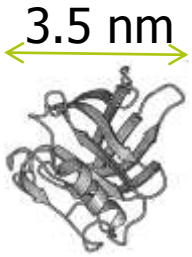
- 21% of milk proteins
- 209 amino-acid residues
- M = 24 kDa
- little secondary structure
- random-coil-like polymer?



Kumosinski 1993

β-lactoglobulin

- whey protein (10%)
- 162 amino-acid residues
- M = 18.3 kDa
- more 'rigid' globular molecule



Brownlow 1997
Verheul 1999

The proteins in solution

β-casein

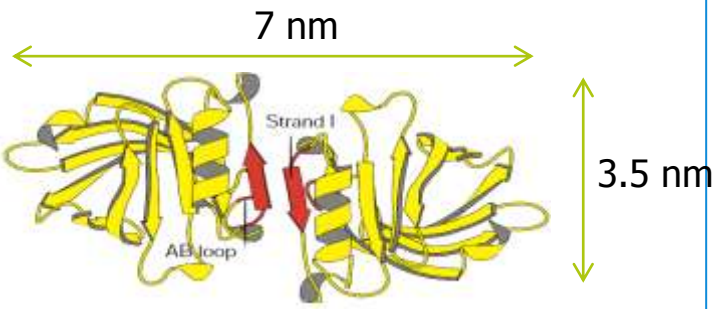
- amphiphilic
- 'block-copolymer'
- RT, pH7: forms micelles

~ 15mer
$R_G \approx 8$ nm

SAXS *Kajiwara 1988*

β-lactoglobulin

- RT, pH7: forms dimers

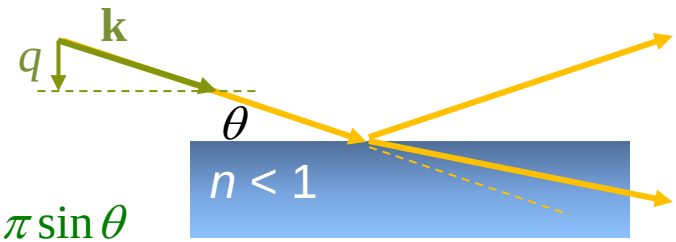


Brownlow 1997
Verheul 1999

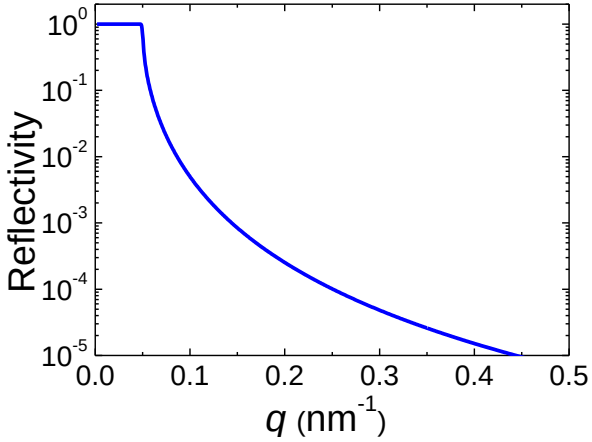
Neutron Reflectometry

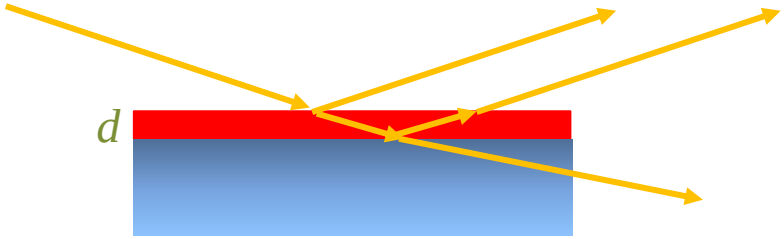
Principle

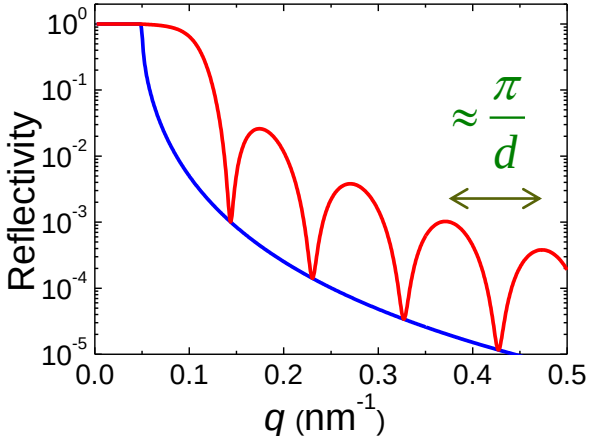
Specular reflection → perpendicular profile $z \sim 1 - 100$ nm



$$q = \frac{2\pi \sin\theta}{\lambda}$$

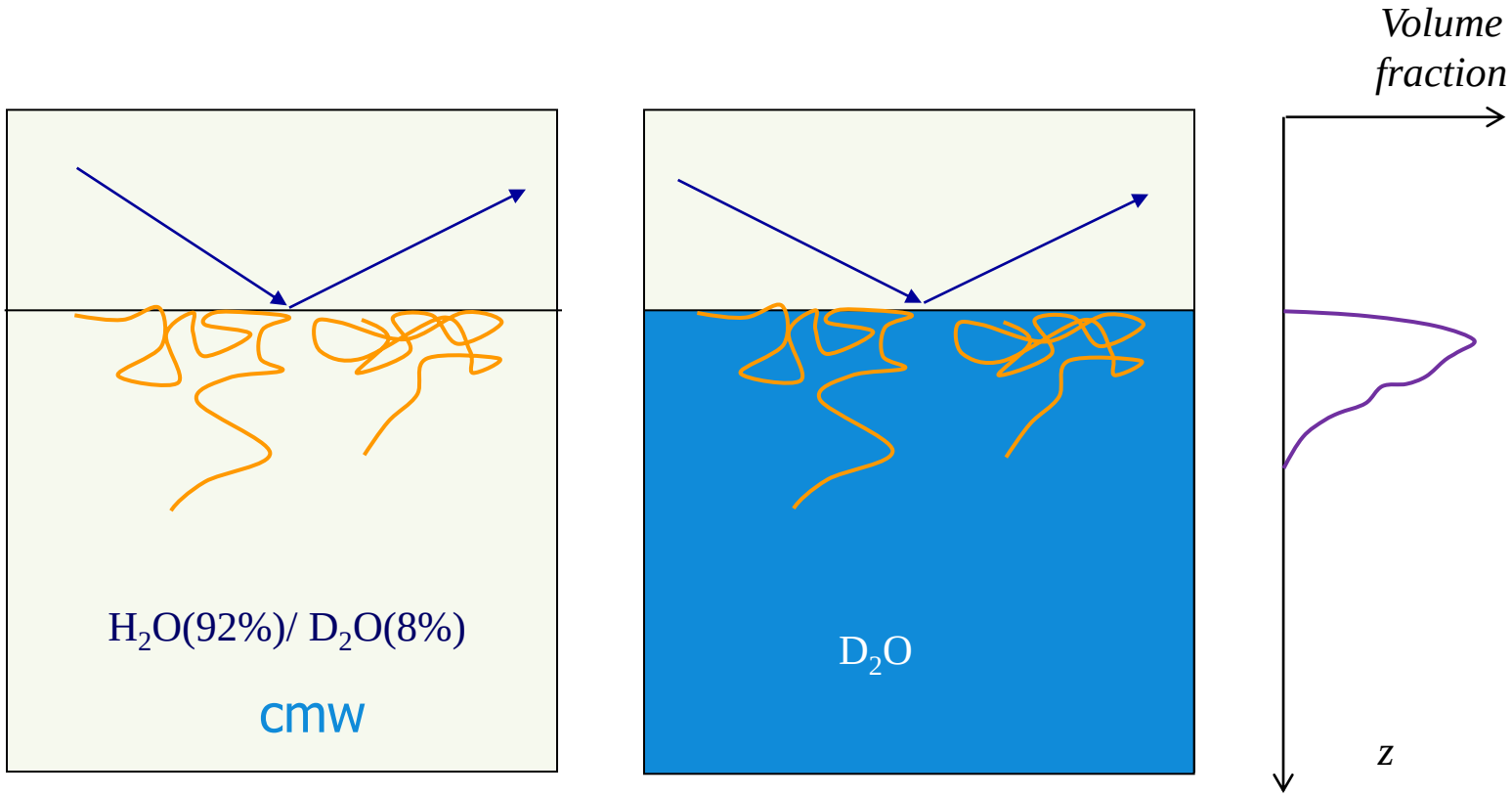






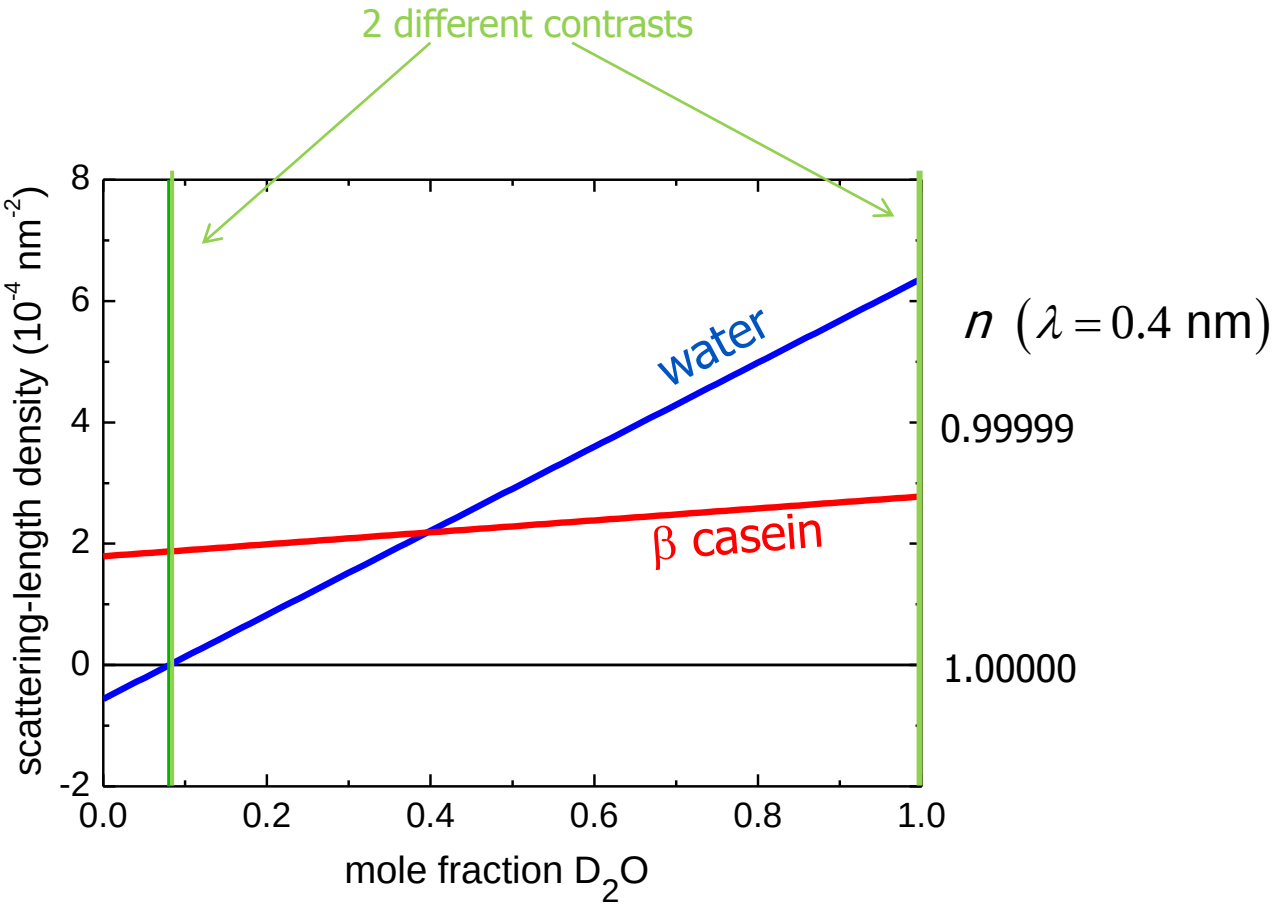
Neutron Reflectometry

Contrast variation / protein adsorption



Neutron Reflectometry

Contrast variation

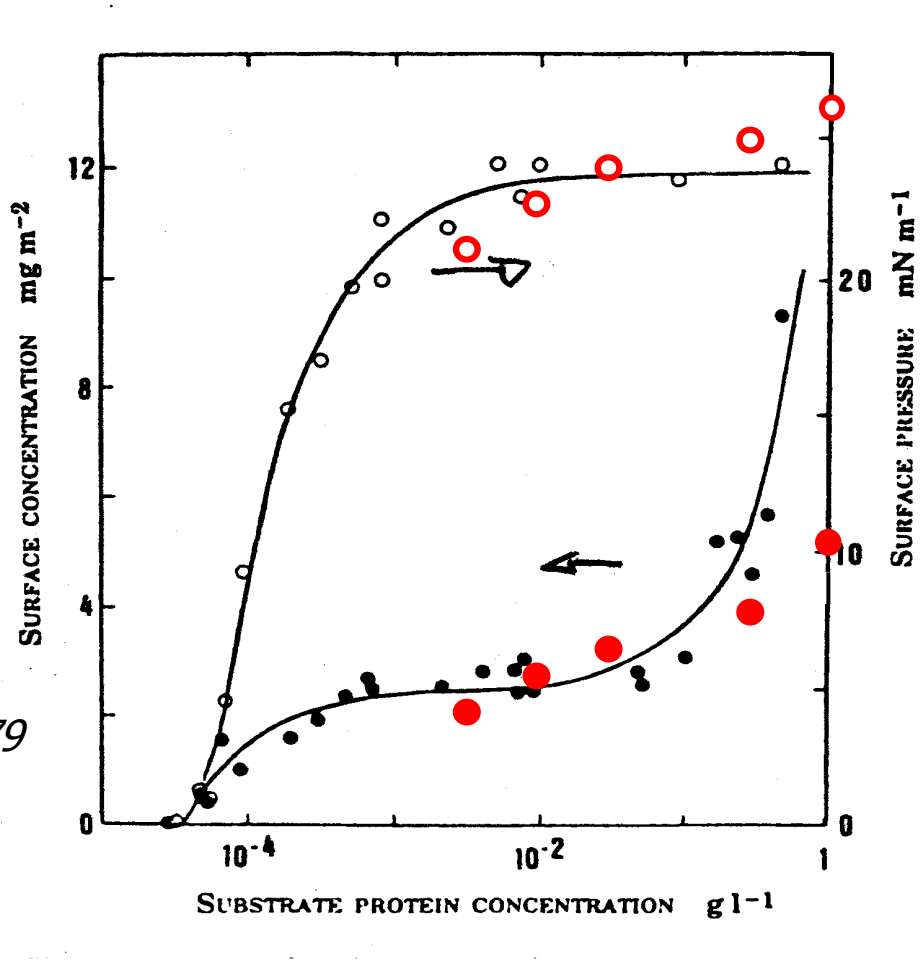


refractive index:

$$n = 1 - \frac{SLD}{2\pi}\lambda^2$$

β-casein, static results

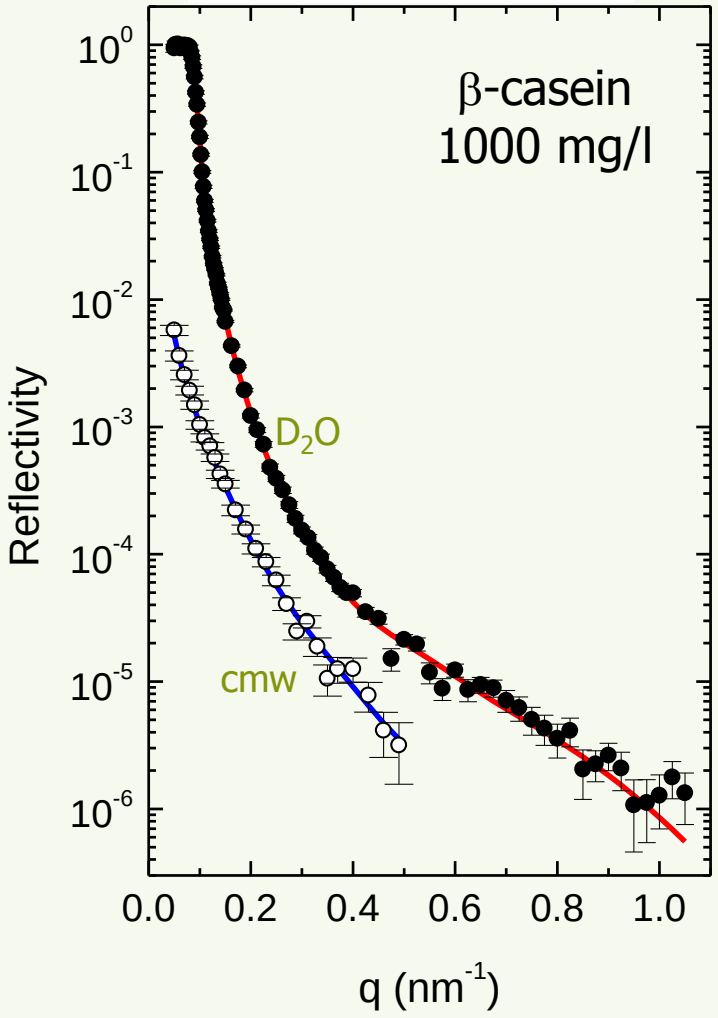
- 5 bulk concentration
- room temperature
- pH 6.7
  (Imidazol buffer)
- 0.2 g/l NaN_3
  (prevent bacteria growth)



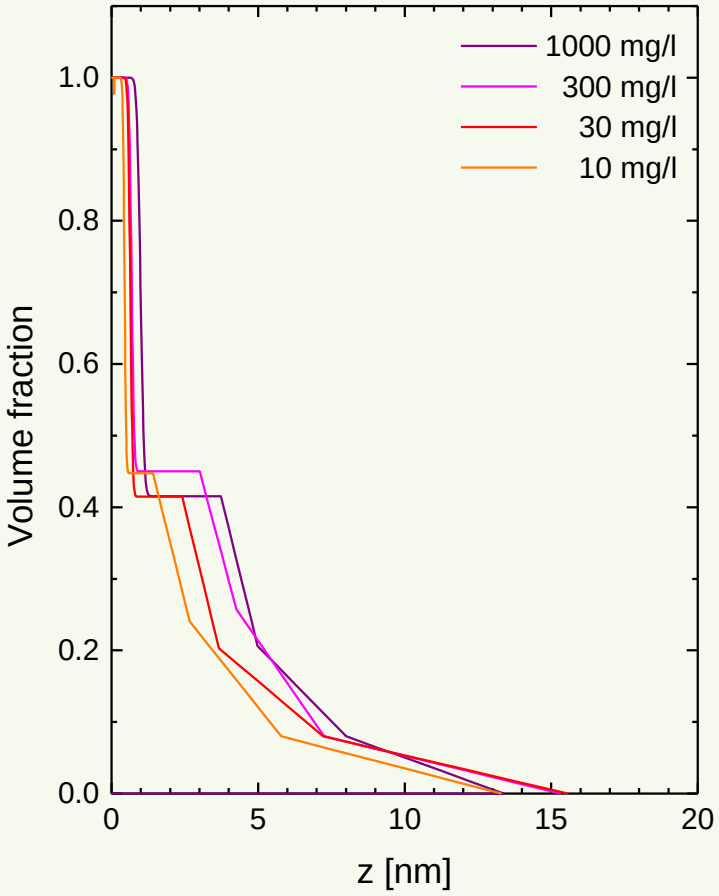
black: *Graham/Philips 1979*
red: our results

β-casein, static results

NR experimental results

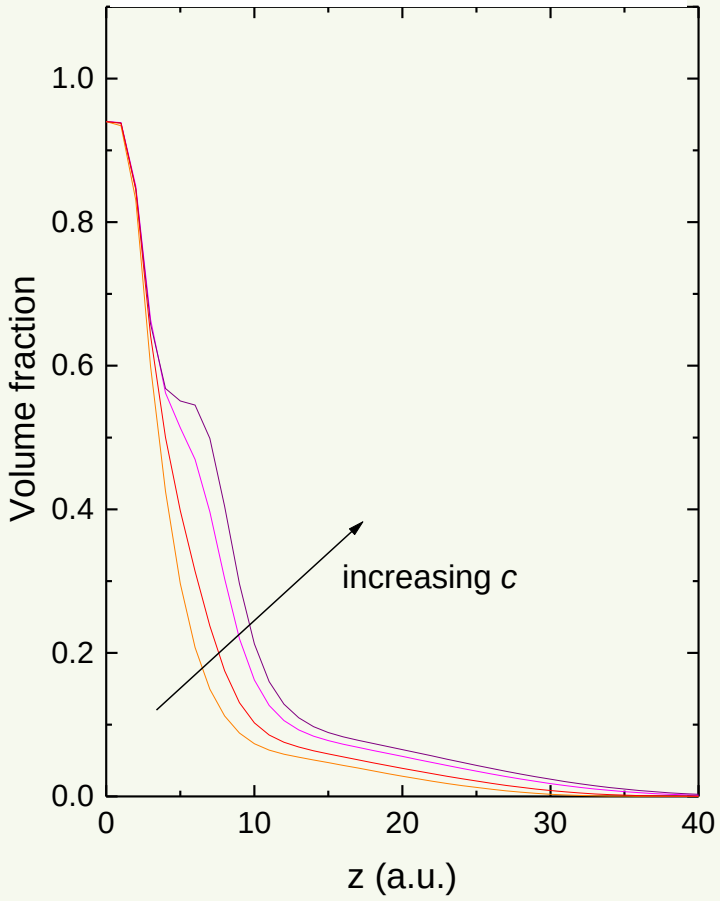


NR fit results



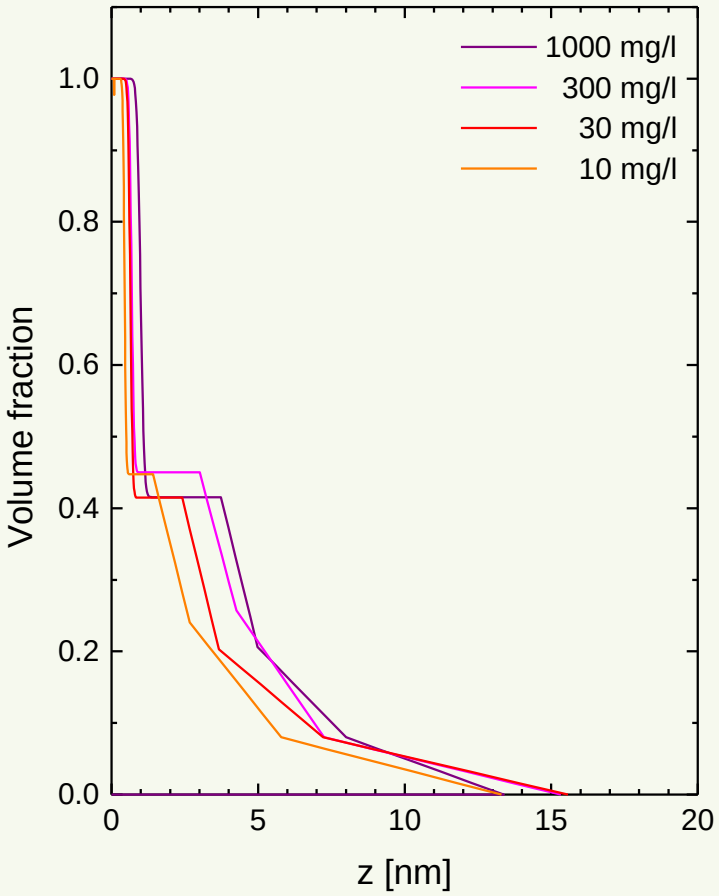
β-casein, static results

Self-consistent-field calculations



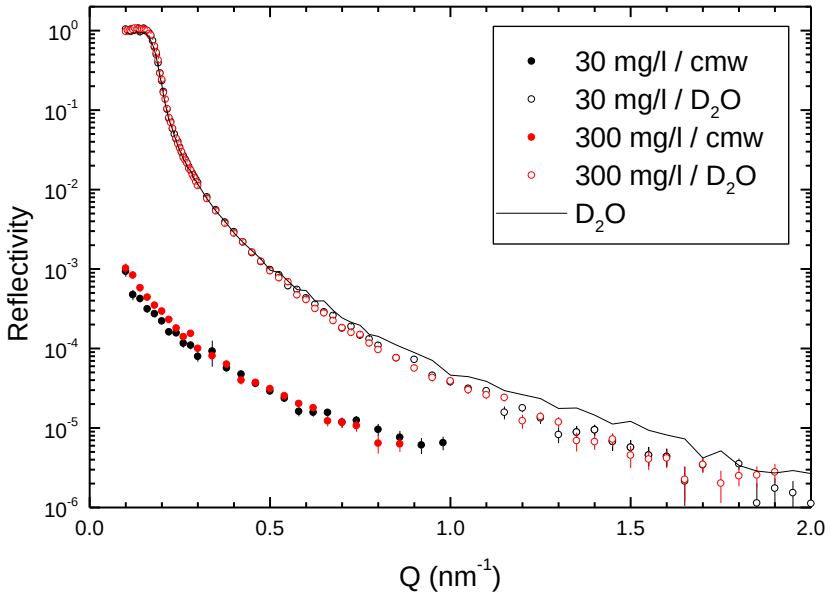
F. Leermakers 1996

NR fit results

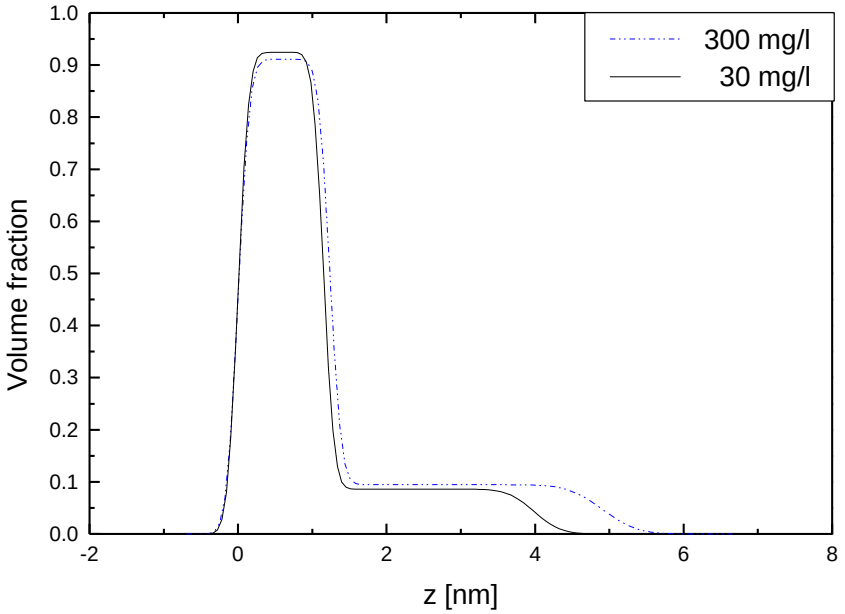


β-lactoglobulin, static results

NR experimental results



NR fit results

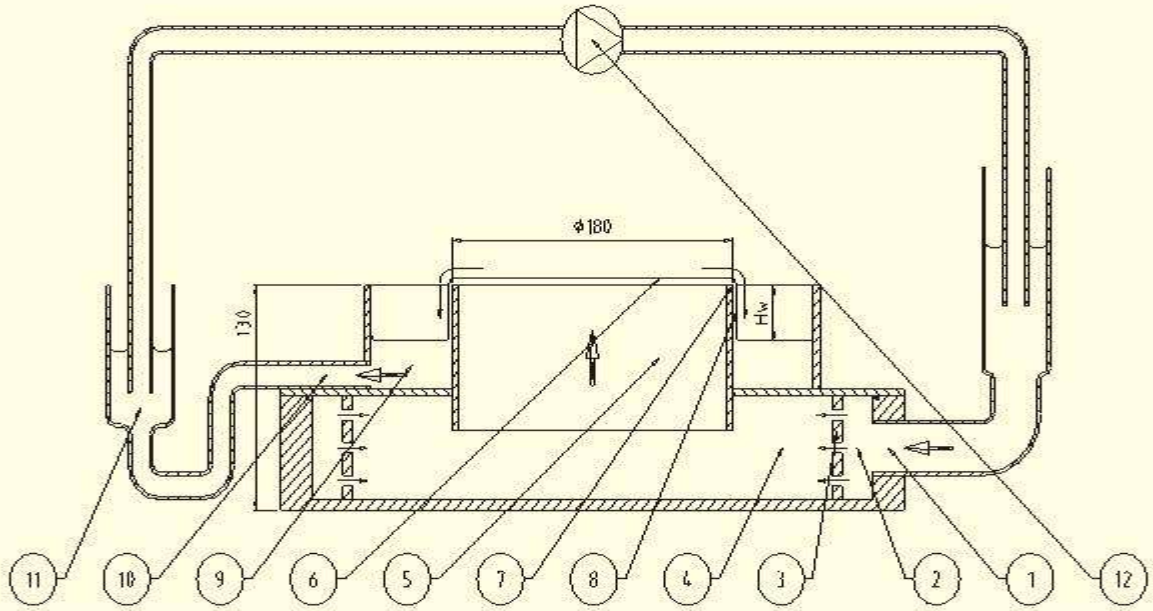


in solution

Kinetics of protein adsorption

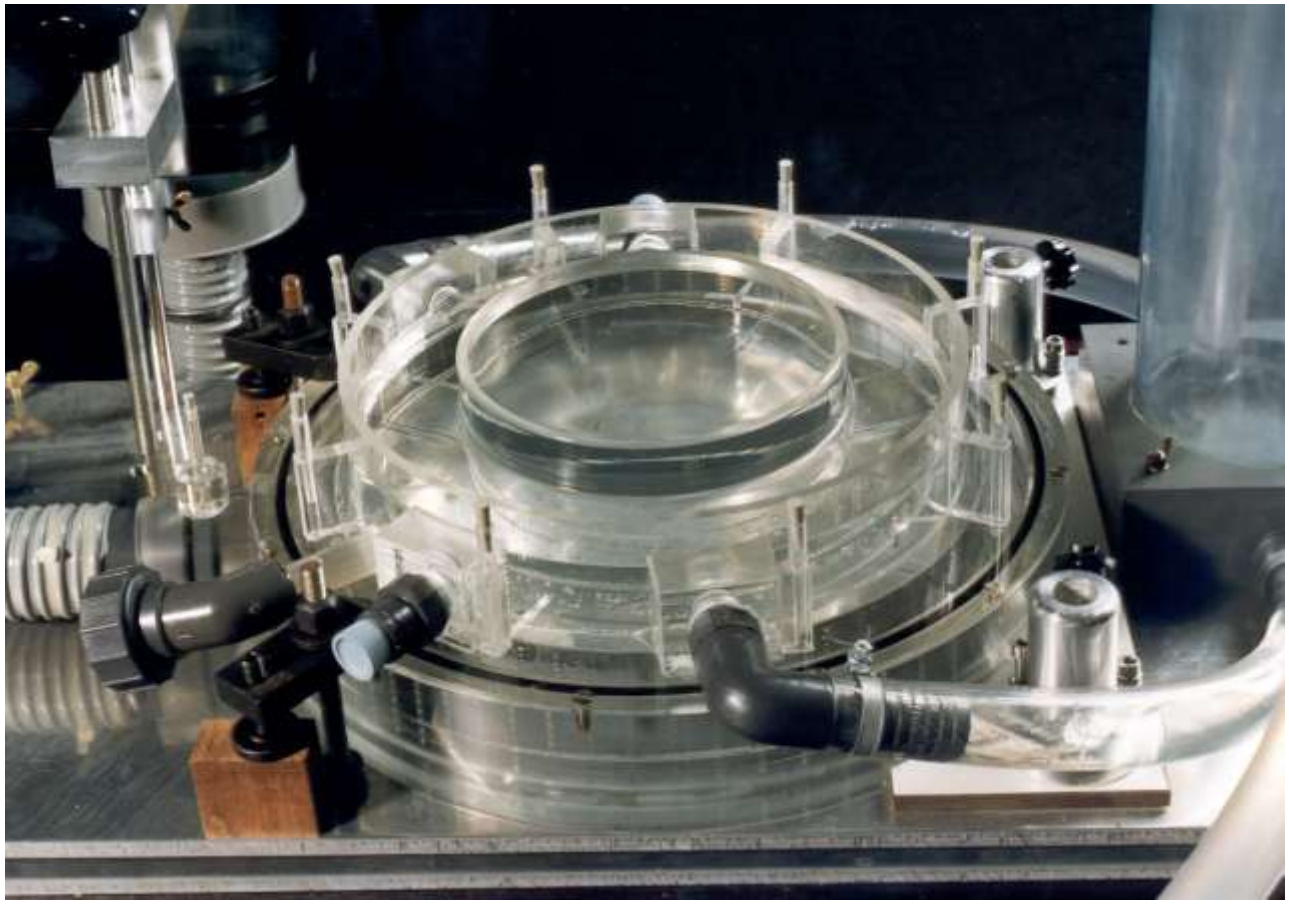
- Expanding interface
- Static non-equilibrium system
- Overflowing cylinder technique
- Continuously fresh surface $\rightarrow$ probing first stages of adsorption
- Modelling complicated (hydrodynamics of system, mass transport)

Kinetics of protein adsorption



overflowing cylinder

Kinetics of protein adsorption



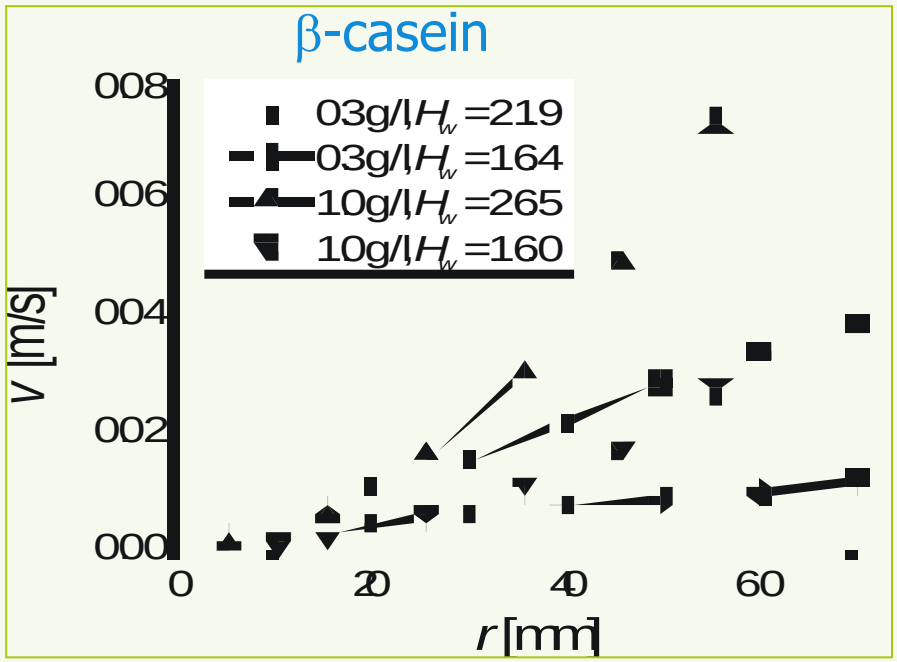
Kinetics of protein adsorption

surface expansion rate $$\theta = \frac{1}{A}\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{\mathrm{d}\ \ln A}{\mathrm{d}t} = \frac{v_r}{r} + \frac{\mathrm{d}v_r}{\mathrm{d}r}$$

pure water: determined by hydrostatic forces

surfactants: determined by surface tension gradient

different for soap and protein, due to lateral interactions

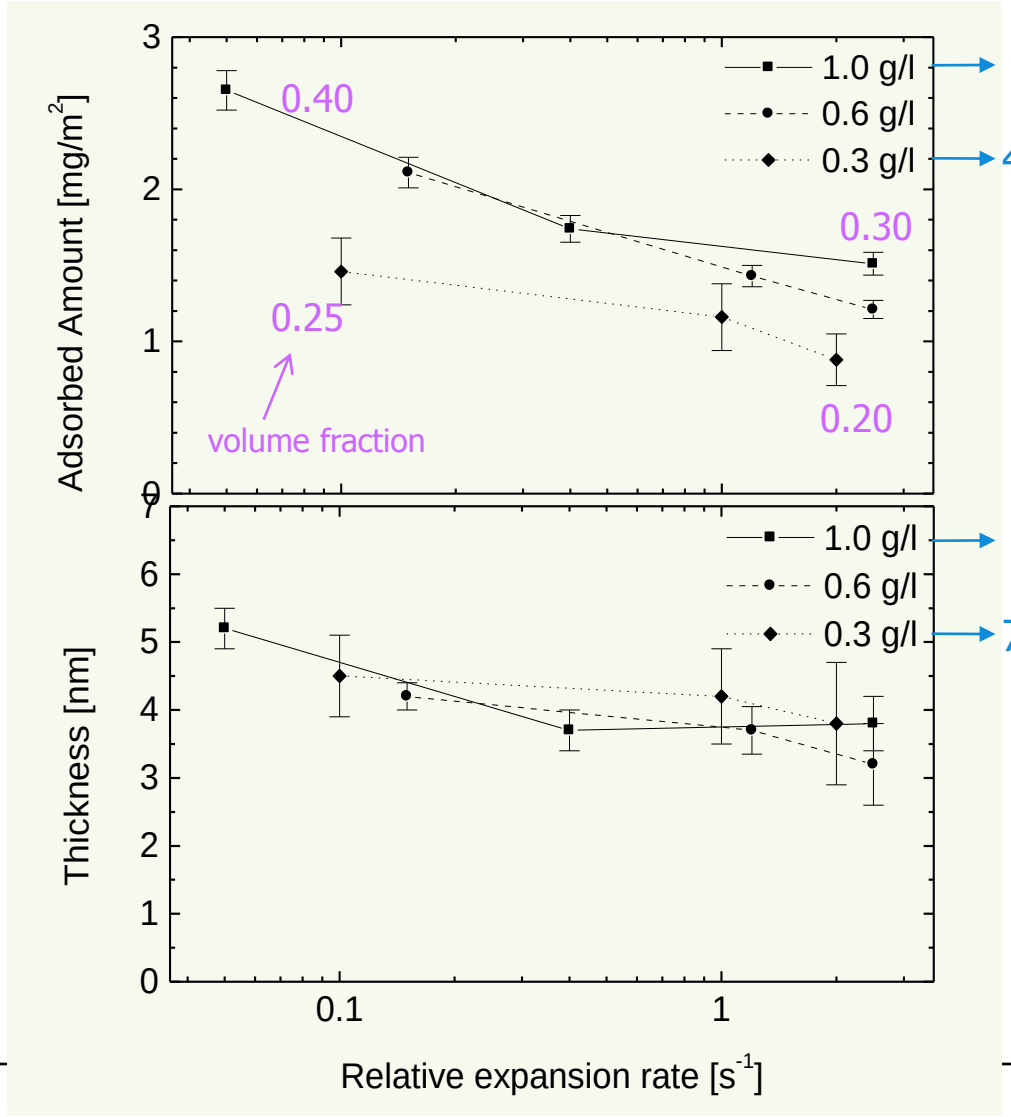


- expansion determined by film height
- if v_r proportional to r $\rightarrow$ constant expansion rate
- footprint exp: 30 x 60 mm

β-casein, kinetic results

results static measurements:

- Footprint: 30 x 60 mm^2
- only CMW (15 l)
- one-layer fit
- 1/θ : 0.3 – 30 s



Conclusions

- Neutron reflectometry is very well suited for studying the structure of proteins adsorbed at an interface
- β-casein can very well be described by polymer adsorption according to the SCF theory
- The overflowing cylinder (ofc) technique makes it possible to study the early stages of the adsorption process
- Full interpretation of ofc-results?
  Should include stagnant flow towards surface, unfolding of proteins, lateral interactions of proteins, ..

Acknowledgement

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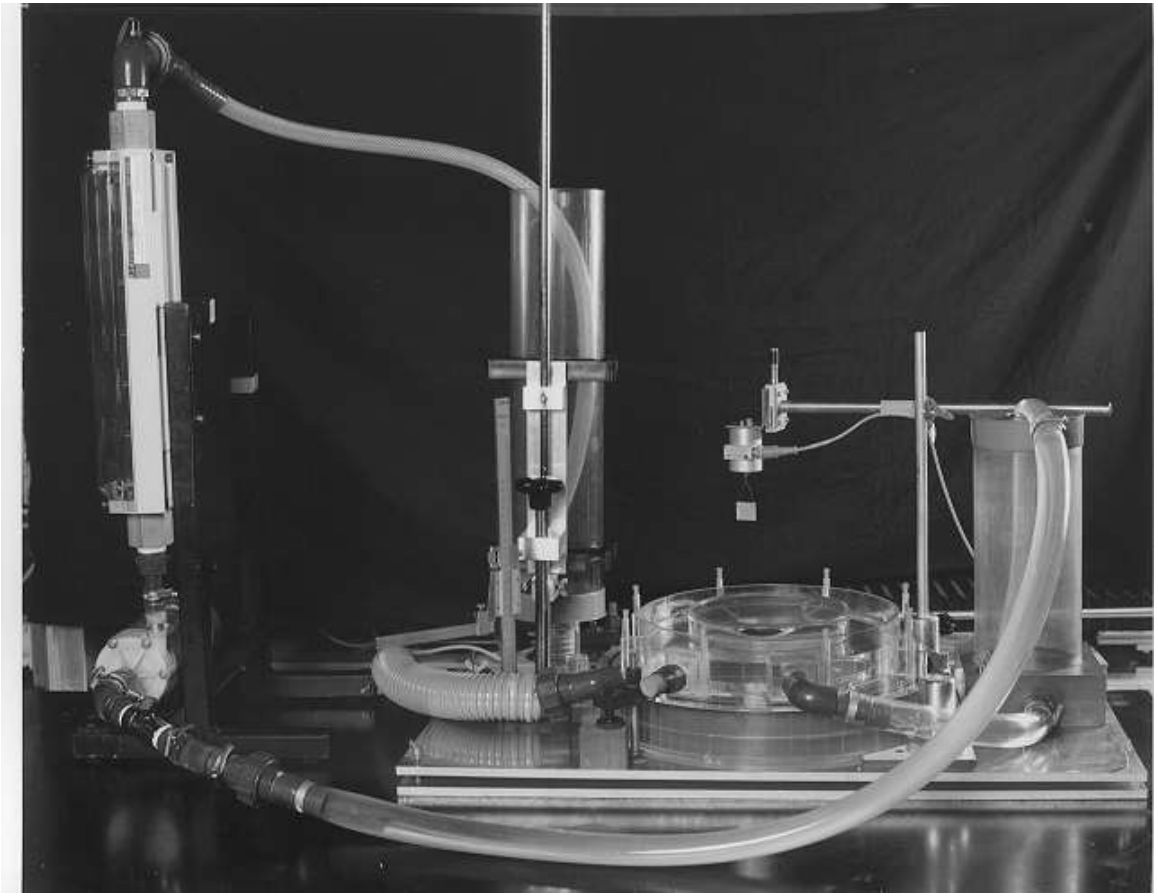
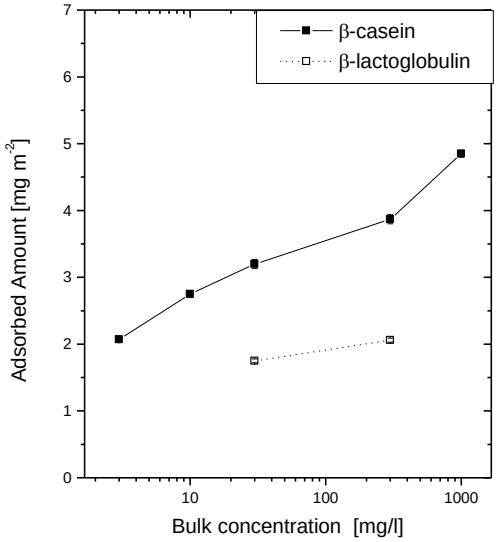
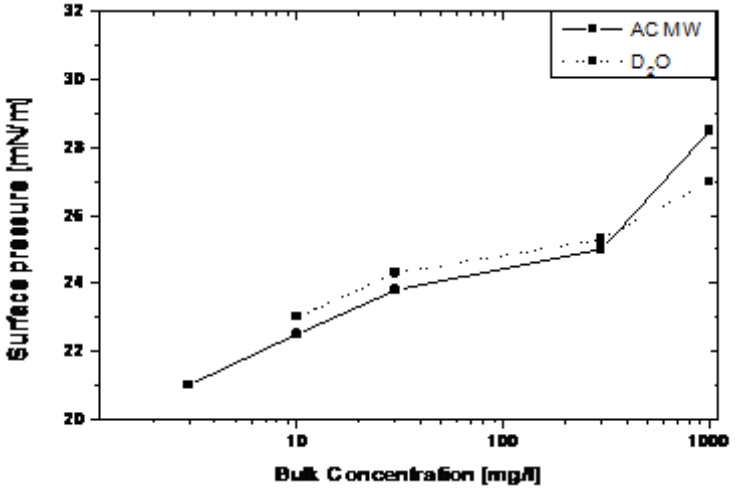
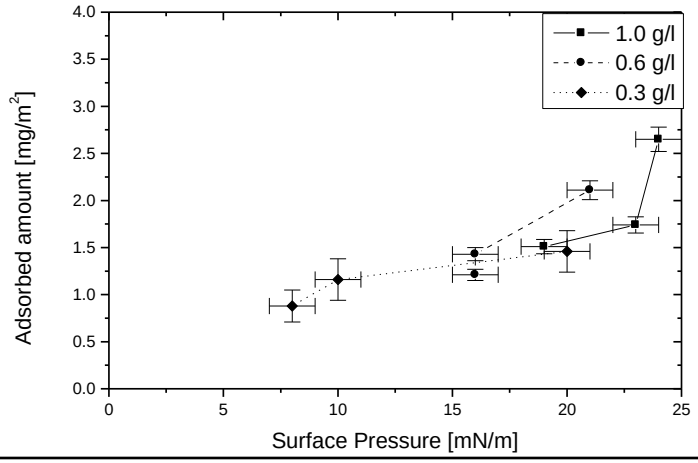
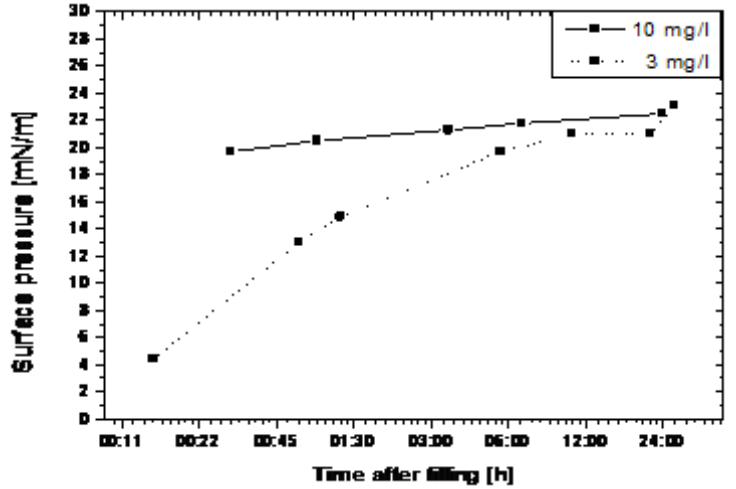
- Wageningen: A. Prins, F. Boerboom, A. van Kalsbeek
- Risø: Kristian Kjær
- ISIS: John Webster




β-casein
β-lactoglobulin
Adsorbed Amount [mg m-2]
Bulk concentration [mg/l]


AC MW
D2O
Surface pressure [mN/m]
Bulk Concentration [mg/l]


1.0 g/l
0.6 g/l
0.3 g/l
Adsorbed amount [mg/m2]
Surface Pressure [mN/m]


10 mg/l
3 mg/l
Surface pressure [mN/m]
Time after filling [h]