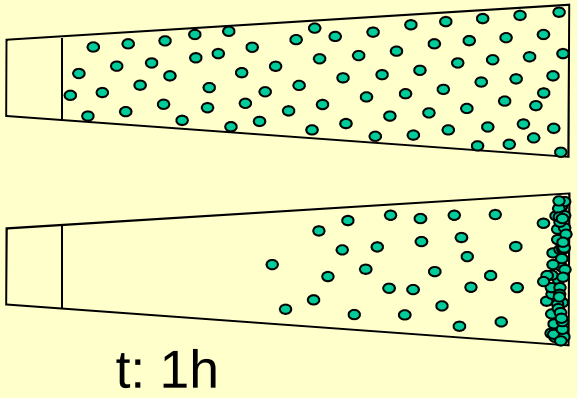
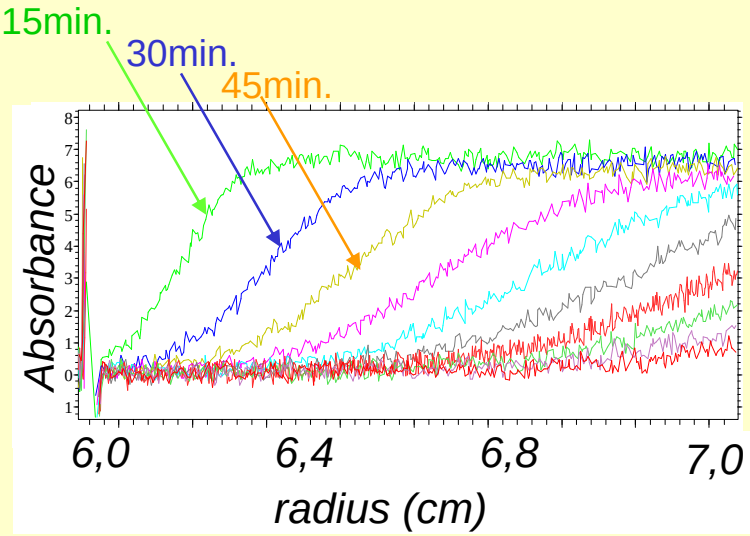
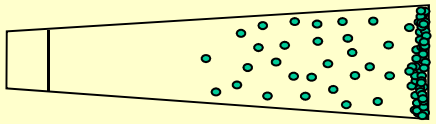
Sedimentation velocity SV



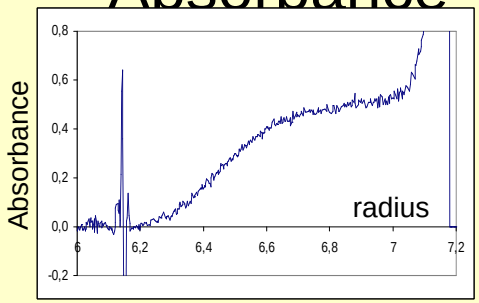


<u>Angular velocity</u>:	*Large compared to the ability of the particle to sediment (typically 42000 revs. per min.)*
<u>Duration:</u>	*Some hours*
<u>Analysis</u>:	*As a function of time* *Formation of a boundary*
<u>Sample:</u>	*420, 110 or 55 µl (l =12, 3 or 1.5 mm)*

Optical Systems



Absorbance

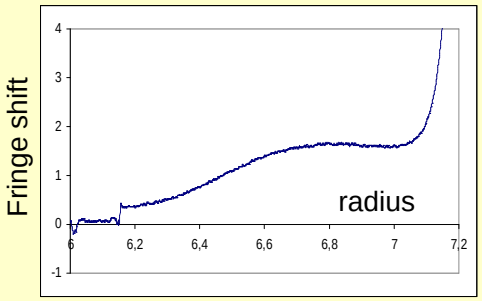


$A=E_{0.1\%}\,lc$

Selectivity depending on
presence of chromophore
220nm < λ < 600nm
Scan of 3 λ possible

0.1-2 mg/mL typically
(60-500 µL)

Interference



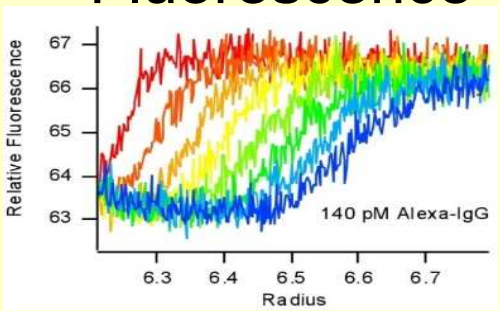
$\Delta J \propto (\partial n/\partial c)\, l\, c$

Not selective
Measures everything
(detergent, glycerol…)
SE difficult

0.1- >10 mg/mL
(60-500 µL)

Can be used in the same run - up to 3 or 7 samples
=>duplicate analysis
=>estimate of composite particle composition

Fluorescence



Signal in arbitrary units

Highly selective
Requires GFP fusion
or FITC labeling
SE difficult

pM-µM
(500 µL)

up to 6 or 14 samples

SE: sedimentation equilibrium

velocity of the particles

mass

relative density

particle distribution at equilibrium

buoyant mass

M_b

$$S = \frac{M(1-\rho\bar{v})}{N_A f}$$

Svedberg

shape, viscosity

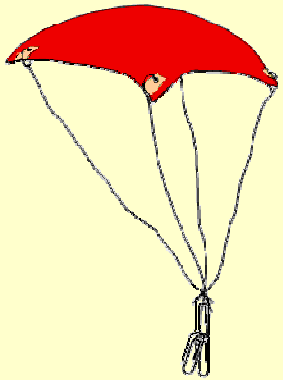
spreading friction

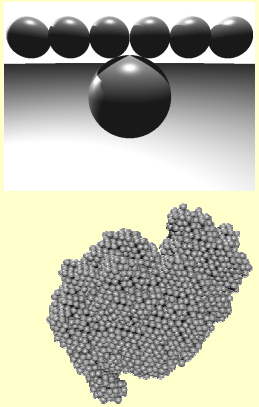
$D = RT / N_A.f$

$f = 6 \pi \eta R_H$

$R_H = f/f_{min} R°$

Stokes-Einstein



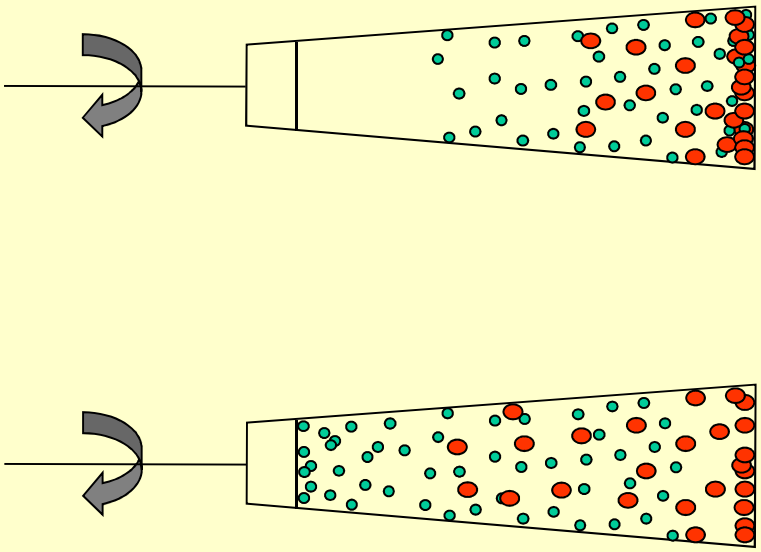


Parachutes : hppt://www.smm.org

partial specific volume

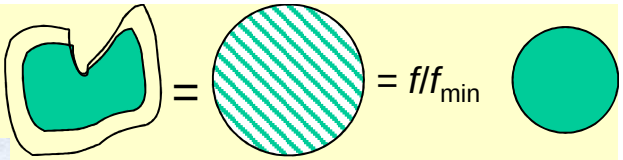
Measured or estimated from chemical structure
For protein: calculation from sequence: program SEDNTERP/SEDFIT
For chemicals, tabulated volumes (Durschschlag & Zipper)

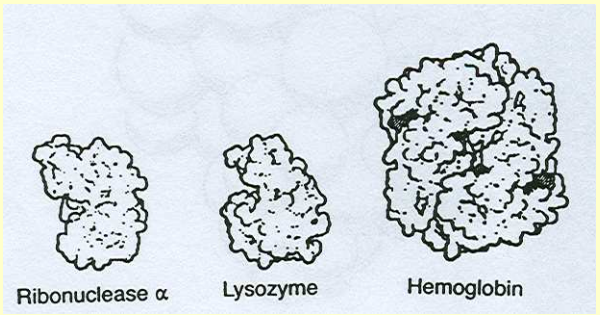
	$\bar{v}$ (mL/g)
NaCl	0.34
ADN, Na^+ salt	0.54
sucrose	0.62
protein	0.74
Detergent DDM	0.824
H_2O	1.00
Lipids:	≈1
Detergent LAPAO	1.067



$$\bar{v}_{complex} = \Sigma M_i \bar{v}_i \; / \; \Sigma M_i$$

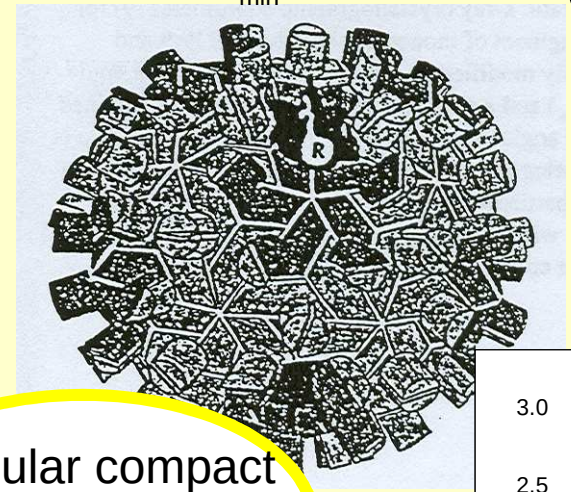
Frictional ratio





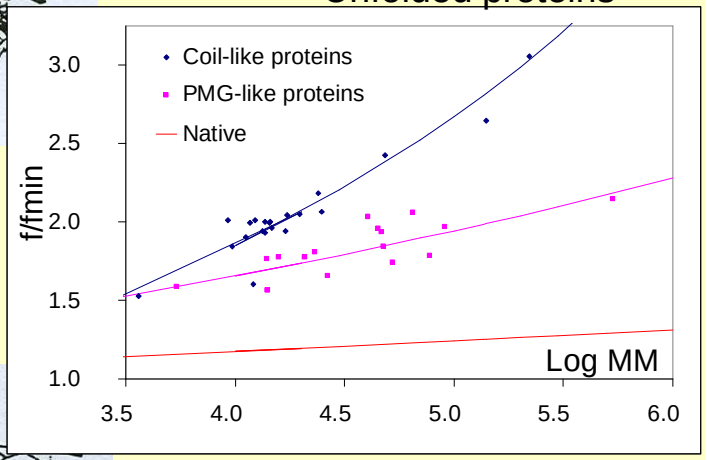
f/f_{min}:1.29 1.22 1.28

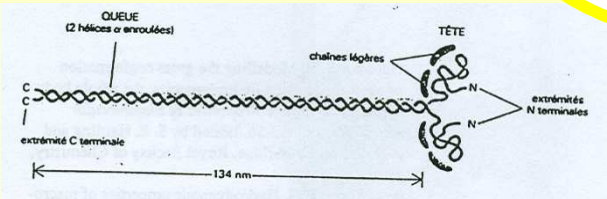
f/f_{min}=1.27



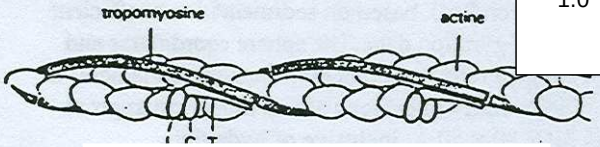
Globular compact macromolecule $f/f_{min}\approx1.25$

Unfolded proteins

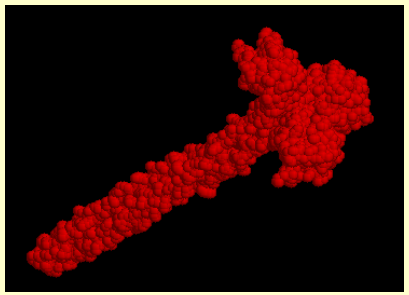




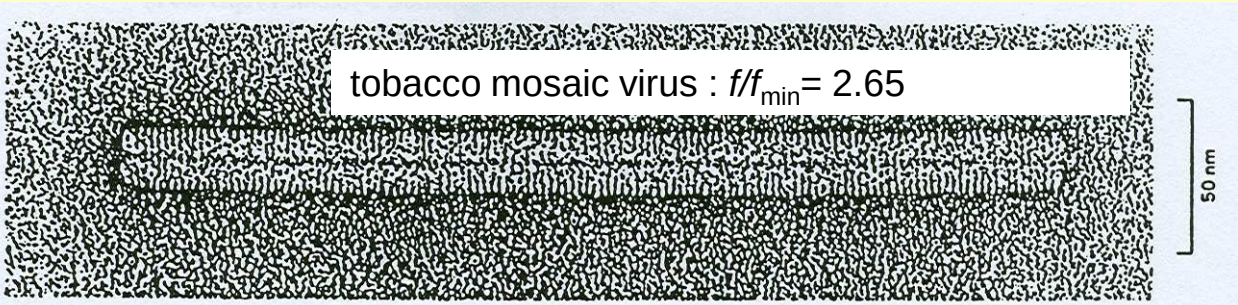
Myosin (queue): f/f_{min}=3.63



tropomyosin: f/f_{min}=2.65



Langerin ECD f/f_{min}=1.8



tobacco mosaic virus : f/f_{min}= 2.65

Transport equation: For each solute

s

Sedimentation coefficient

Movement of the particle
In response to
centrifugation forces

Force=$\omega^2 r$

s = velocity/centrifugation field

D

Diffusion coefficient

Movement of the particle
In response to
concentration gradients

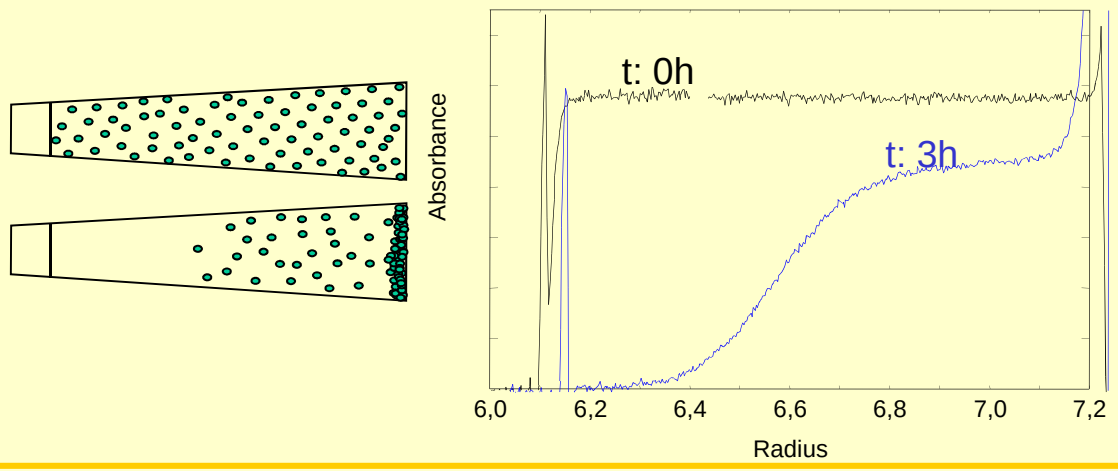
Force=$(\partial\mu/\partial r)$

Brownian motion

Q

Chemical reaction

Appearance or
Disappearance
of material



-position=>***s***
-dispersion=>***D***
-low concentration
=>dissociation

Lamm equation

$$(\partial c/\partial t) = -\,1/r\;\; \partial/\partial r\,[r(c\,s\omega^2 r - D\,\partial c/\partial r)]$$

Data treatments are based on numerical solutions of the Lamm equation
i.e. simulations

Input

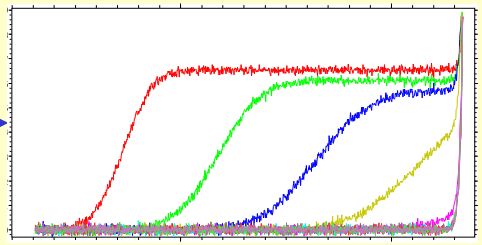
Simulation

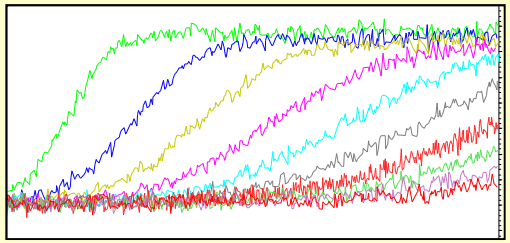
Comparison
with experiment

$s\ D\ c$

experimental
parameters

$r\ t\ \omega$





Problem: define the model for the analysisBoundary spreading= diffusion or heterogeneity ? How many species? Interactions?

•The *c*(*s*) analysis considers a distribution of particles, for which a plausible relationship between s and D is established (input : v, f/f_{min}, ρ and η): only concentrations and noises are determined.

•In the non-interacting species analysis, *s*, *D*, concentrations and noises are adjusted.

Experimental strategy

1: SV measured at different concentrations or other conditions

$c(s)$

- superposition of the *c*(*s*) for different samples/optics
  - ➔ Homogeneous?
  - ➔ Number of type of species?
  - ➔ Equilibrium of association?

- Examination of the signal intensity
  - ➔Concentration
  - ➔Bound detergent in membrane proteins

- analysis of the *s*-value
  - ➔compatibility with a given association state?
  - ➔ shape
  - ➔ equilibrium constant

NIS (non-interacting species analysis) if appropriate

2. SE if needed and appropriate (sedimentation equilibrium)

Programs:

- Sedfit :Analysis of one SV experiment: *c*(*s*); NIS…
- Gussi :figures and calculations after Sedfit
- Sedphat :Global analysis of ≠ experiments may adress more complex interacting systems