Analytical Ultracentrifugation

Biophysical techniques for measuring mass and protein-protein interactions.

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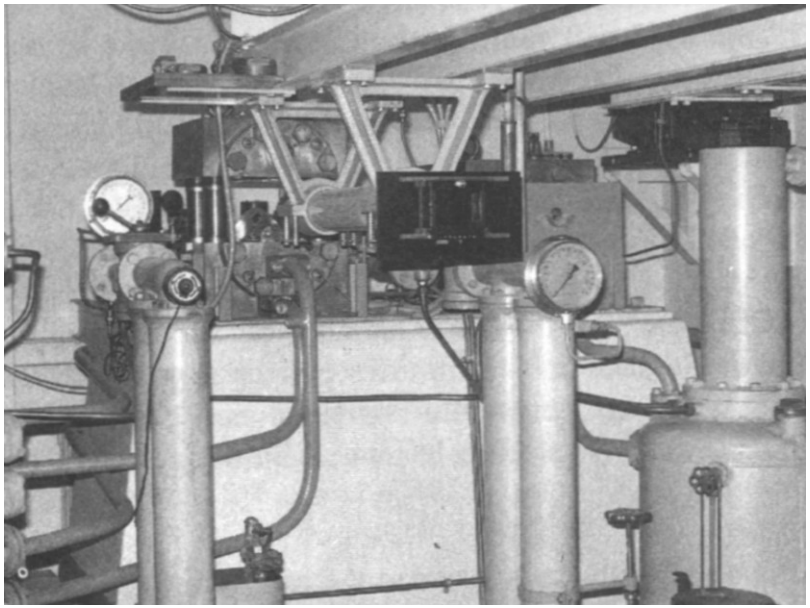
1686
Newtons Second Law of Motion

F = MA

- So if we can apply a Force and measure acceleration, then we can obtain Mass for a given particle

High speed analytical ultracentrifugation was first developed in 1910-20's, Upsalla University, Sweden.

Oil turbine analytical ultracentrifuge



Theodore Svedberg pioneered their use for study of gold nanoparticles.

Awarded Nobel Prize in Chemistry in 1926 for work "for his work on disperse systems."

Todays Ultracentrifugation

Features

Analytical Ultracentrifugation (AUC)

- AUC remains the most versatile, rigorous and accurate means for determining the molecular weight and hydrodynamic/thermodynamic properties of a protein or other macromolecule.
- No other technique is capable of providing the same range of information with a comparable level of precision and accuracy.

Provides data which can answer more critical questions than any other comparable technique, including:

- Shape
- Mass
- Diameter
- Stoichiometry
- Heterogeneity
- Association
- Aggregation
- Purity
- Formulation

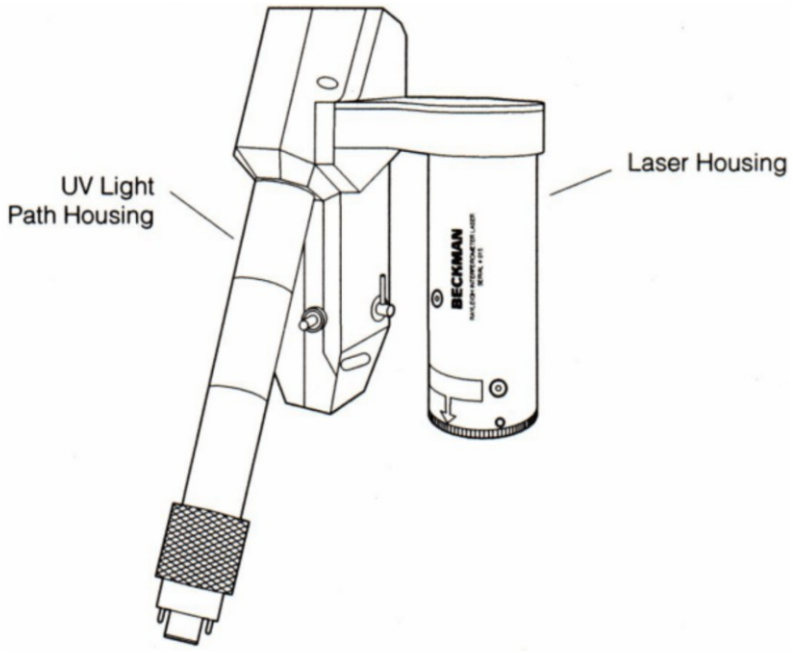
Analyzes a wide array of particles in native, matrix-free conditions, including:

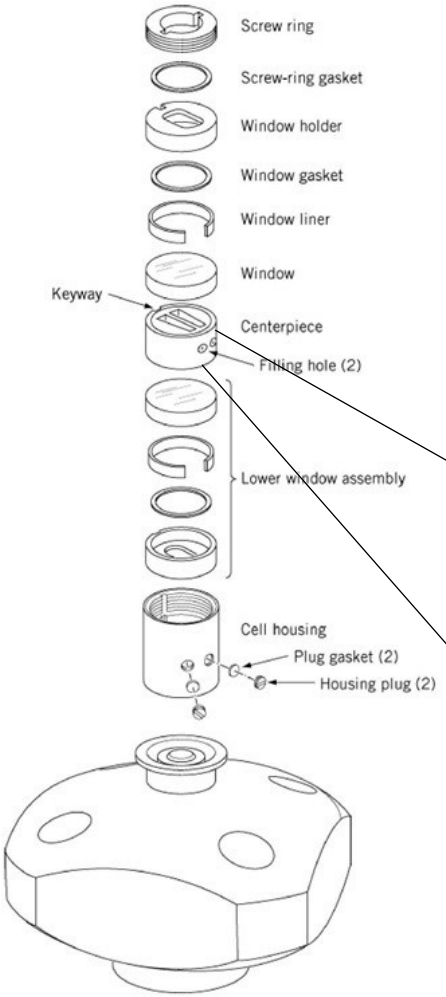
- Proteins
- Nanoparticles
- Peptides
- Polymers
- Micelles
- Liposomes
- Extracellular vesicles
- Drug conjugates
- Viral payload

The analytical ultracentrifuge is unsurpassed for directly measuring molecular weights of solutes in their native state and as they exist in solution without relying on calibration or making assumptions concerning shape

The analytical ultracentrifuge

Inside the analytical ultracentrifuge

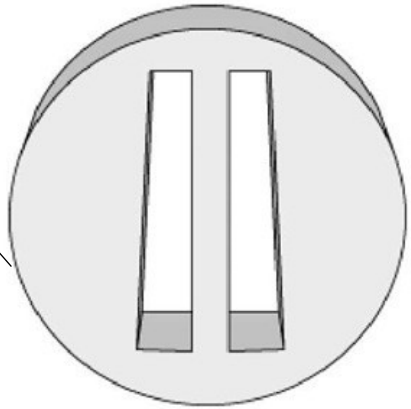




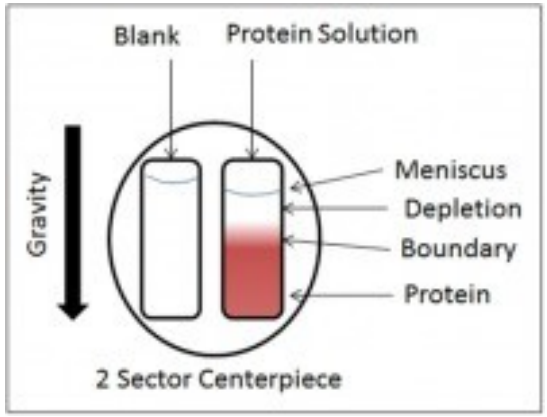
Sample volume
400 -500 uL

An absorbance of ~ 1 somewhere between 190 and 600 nm.

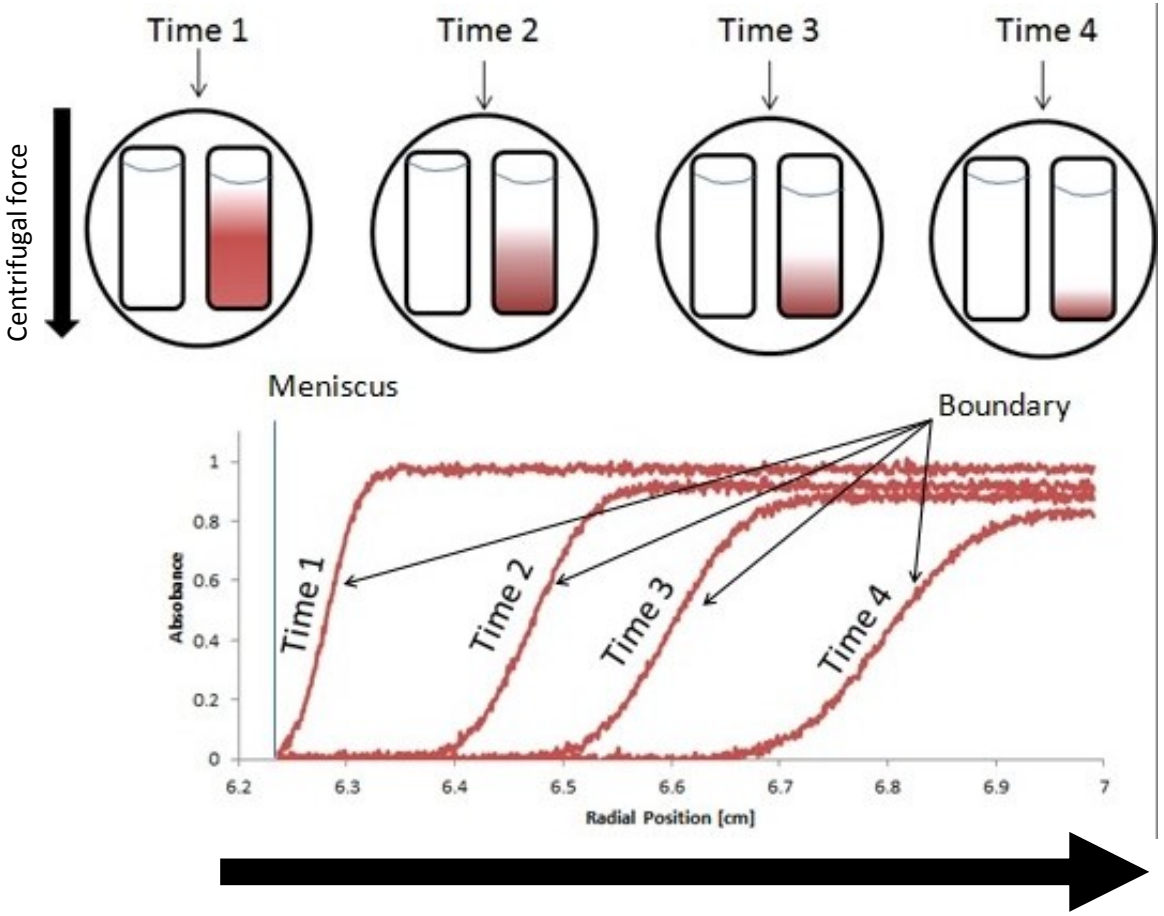
Salt (10 – 100 mM) added to remove non-specific charge interactions.



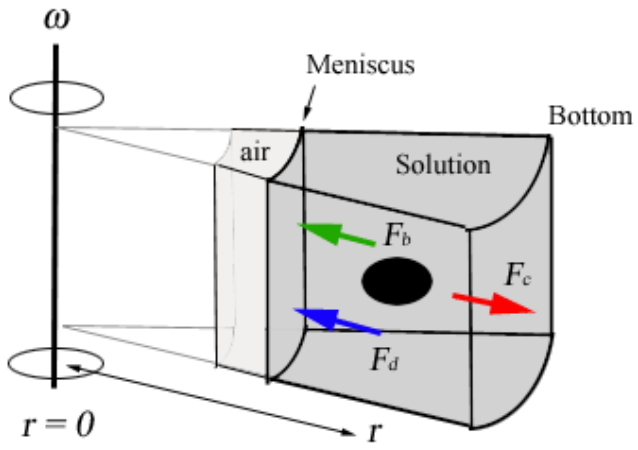
(a)



Sedimentation velocity applies a force and measures migration.



Forces Experienced by a Particle in an Ultracentrifuge.



Centrifugal force

$\boldsymbol{F}_c = \omega^2 rm$

ω: angular velocity of the rotor

r: distance form the center of the rotor

m: mass of the particle

Buoyancy force

$\boldsymbol{F}_b = -\omega^2 rm_0$

m_0: mass of the solution displaced by the particle

Frictional force

$\boldsymbol{F}_d = -fv$

f: frictional coefficient, the drag caused by the particle as it moves through solution. A function of particle size, shape, hydration and viscosity of solution.

- The force applied to the particle

$F = \omega^2 rm - \omega^2 rm_0 - fv$

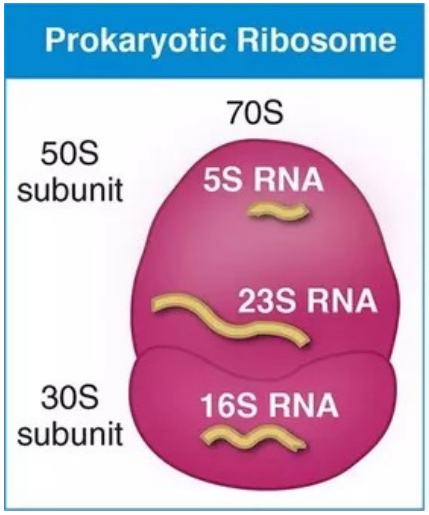
So the force a particle experiences is a combination of its buoyancy, shape and size, which can be summarised in a ***single coefficient.***

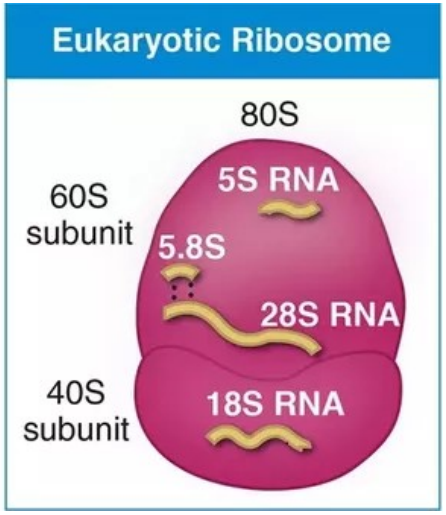
The Sedimentation Coefficient

- sedimentation coefficient = velocity / field strength
- The unit of sedimentation coefficient is defined as 1 Svedberg ($1x10^{-13}$ sec), denoted as **S**.

$$\frac{\quad}{\quad} \quad \frac{\quad}{\quad} = \frac{\quad}{\quad\quad\quad}$$

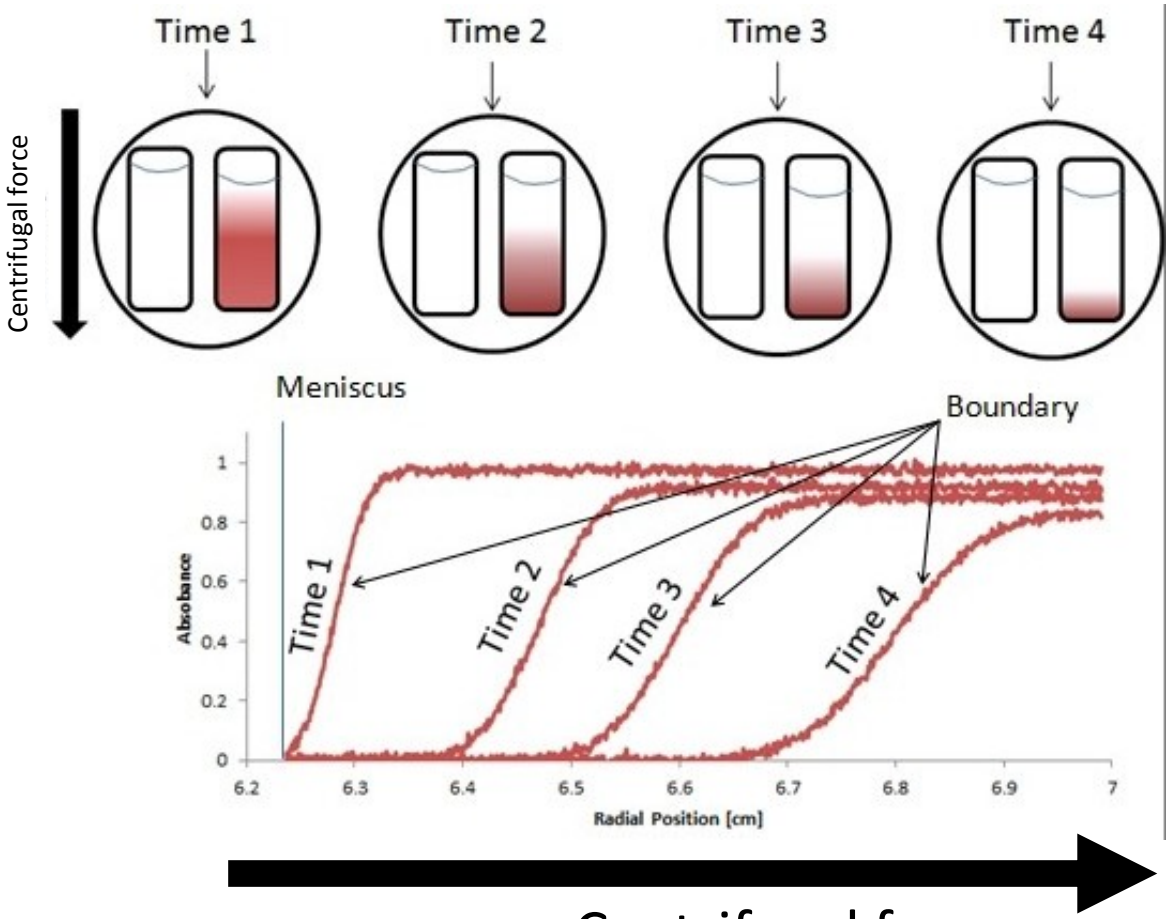
is partial specific volume
(volume displaced by mass)
is density of solvent.





Sedimentation coefficients defined early protein chararacterisation and understanding of protein complexes

The sedimenting boundary broadens with time due to **Diffusion**.



The opposing force - Diffusion

The Svedberg equation.

$$M = \frac{S \cdot RT}{D(1 - \bar{\nu}\rho)}$$

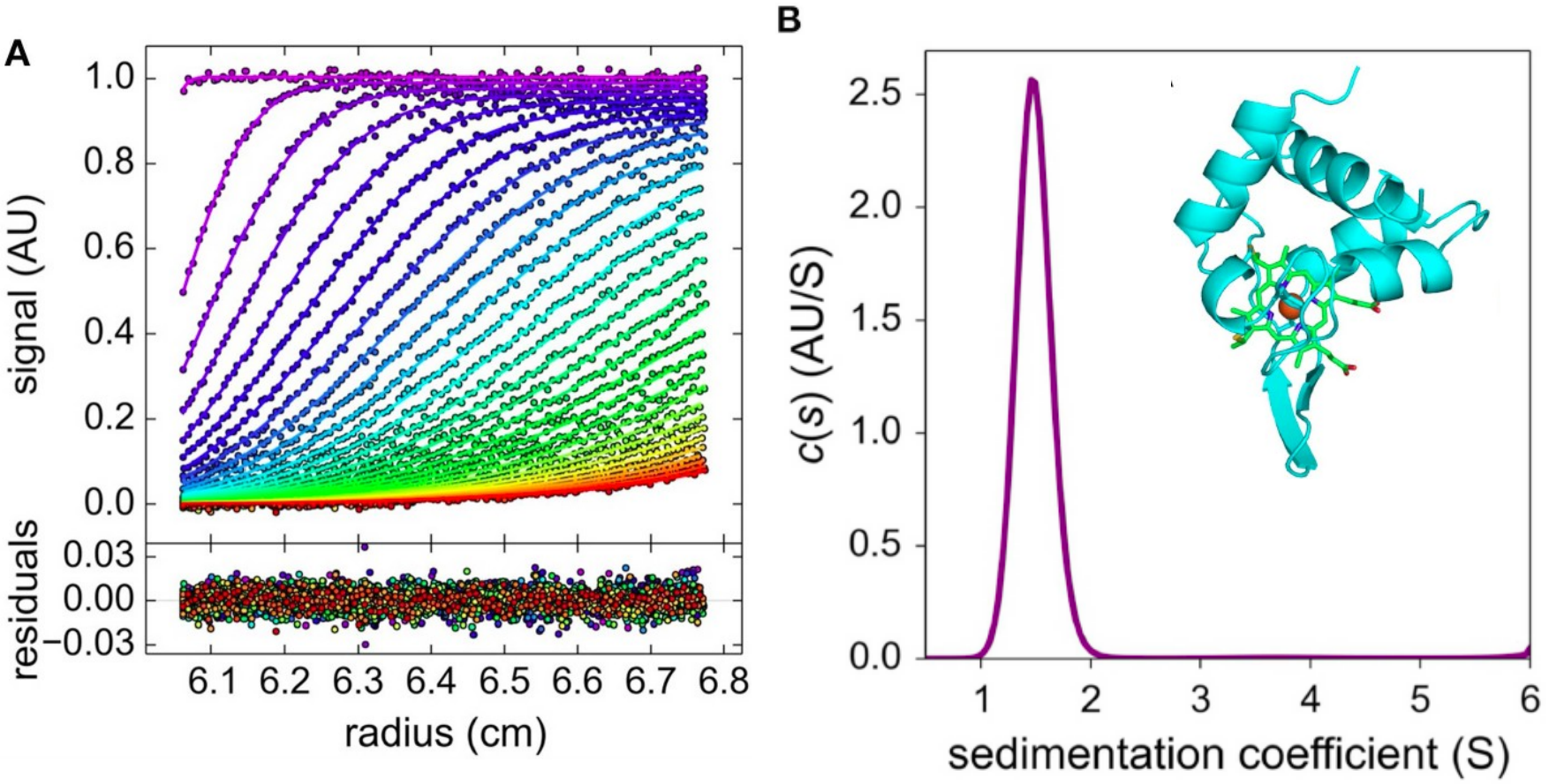
- By monitoring the motion and spread of a boundary it is possible to determine both the sedimentation coefficient and the translational diffusion coefficient.
- So if we can calculate S and D from the data, we can obtain the Mw of a particle.

The Lamm equation

$$\frac{\partial c}{\partial t} = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r}\right] - s\omega^2\left[r\frac{\partial c}{\partial r} + 2c\right]$$

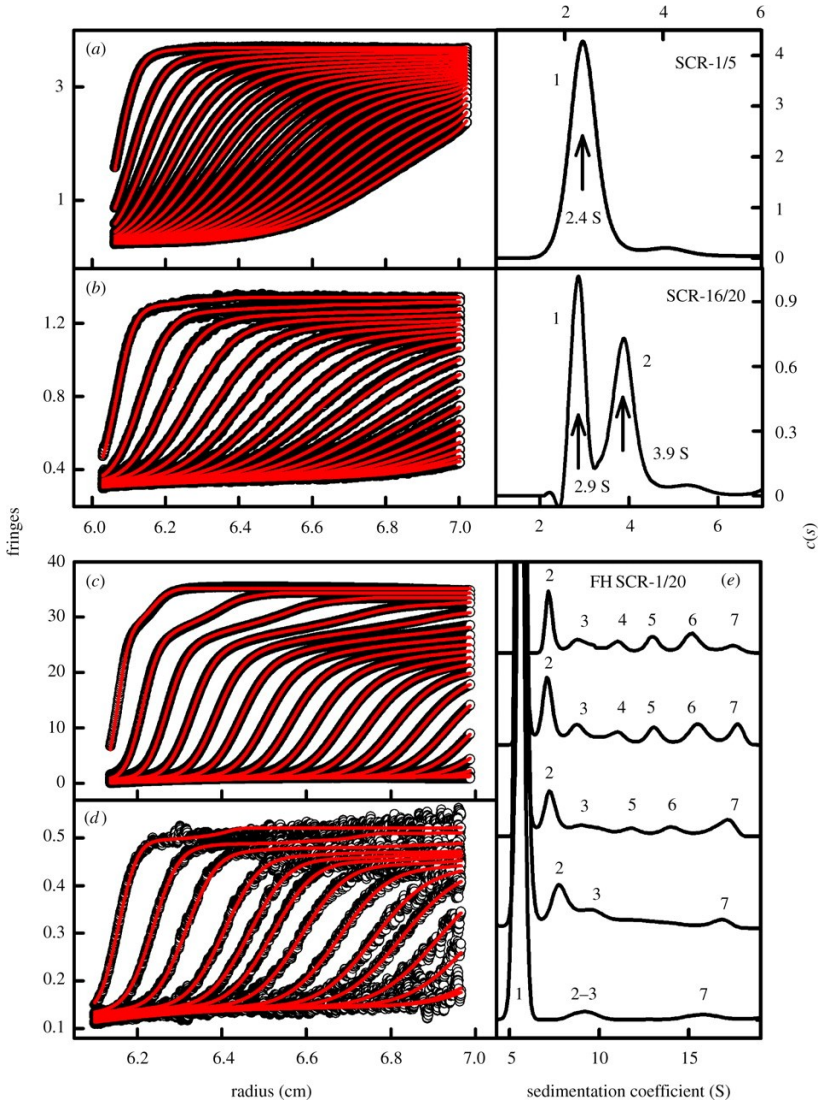
- A partial differential equation that describes the concentration distribution through a sedimenting cell.
- The sedimentation boundary of a particle in a 2 sector cell can be described by the Lamm equation.
- Various software programs can be used to fit the Lamm equation for sedimentation data and give values for S and D.

A single purified protein

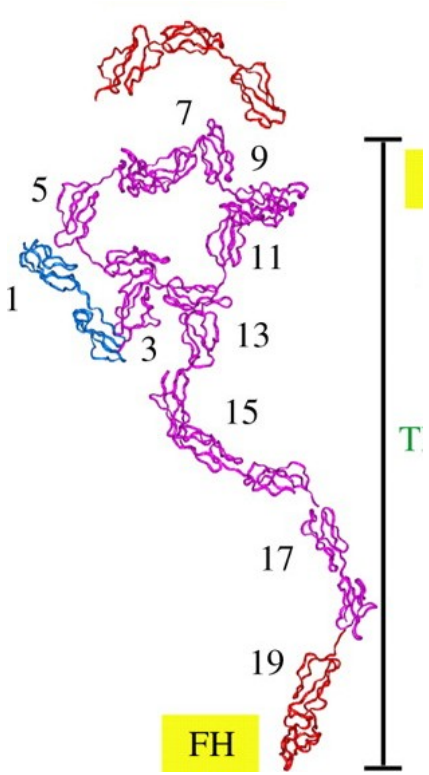


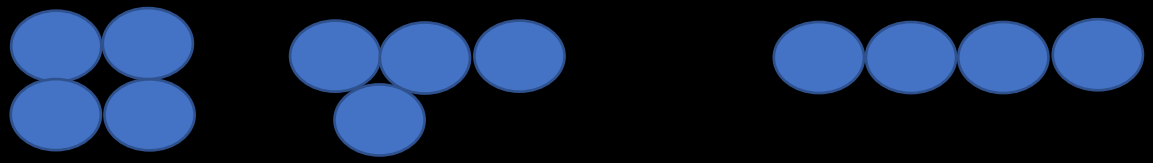
C(s) analysis shows a single sedimentation peak with a Mw of 11kA, the crystal structure confirms the sedimentation data.

More complex associations



- Increased oligomerization of different species as concentration increases.

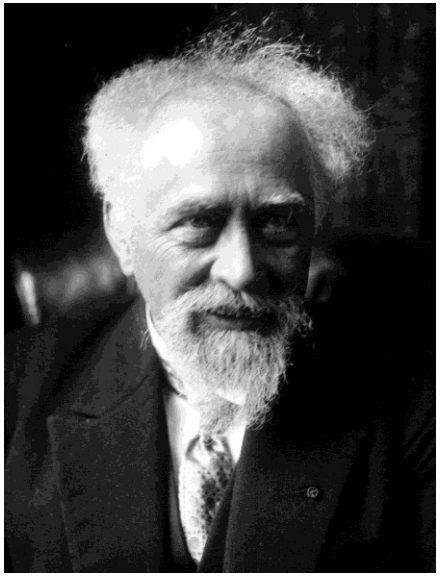




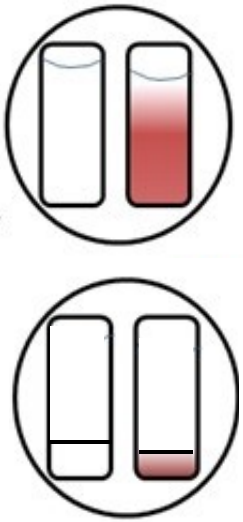
Sedimentation equilibrium

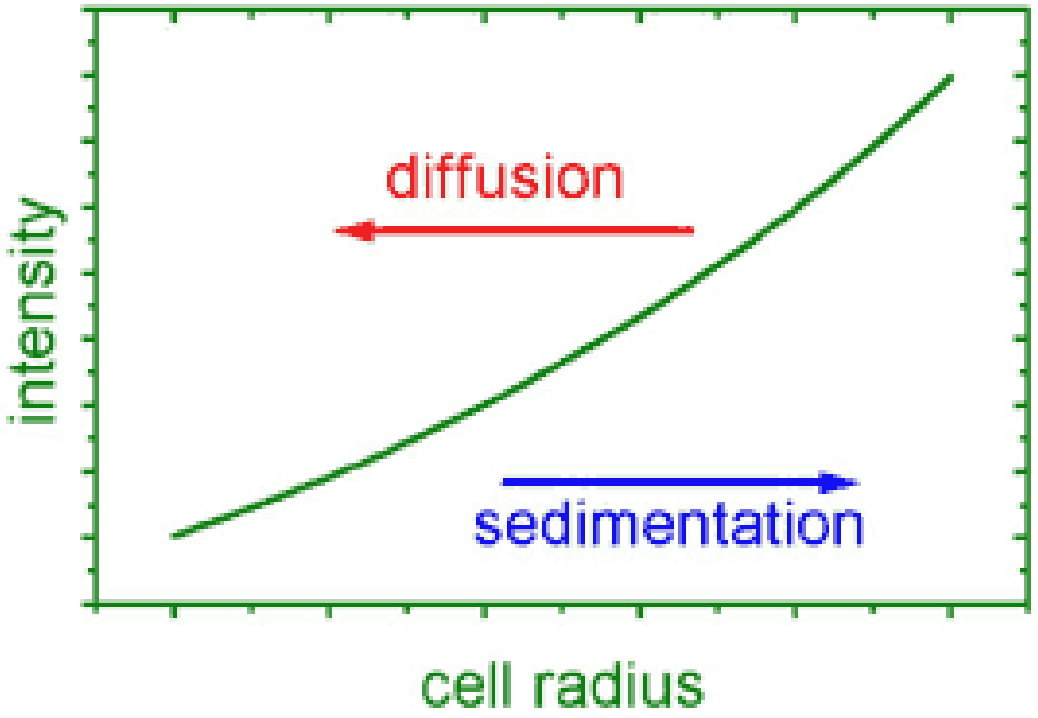
- When the centrifugal force is sufficiently small, an equilibrium concentration distribution of macromolecules is obtained throughout the cell where the flux due to sedimentation is exactly balanced by the flux due to diffusion.
- This equation describes the concentration gradient when sedimentation and diffusion forces are equal.

Jean Baptiste Perrin
1926 Nobel prize in physics
"for his work on the discontinuous structure of matter, and especially for his discovery of sedimentation equilibrium"



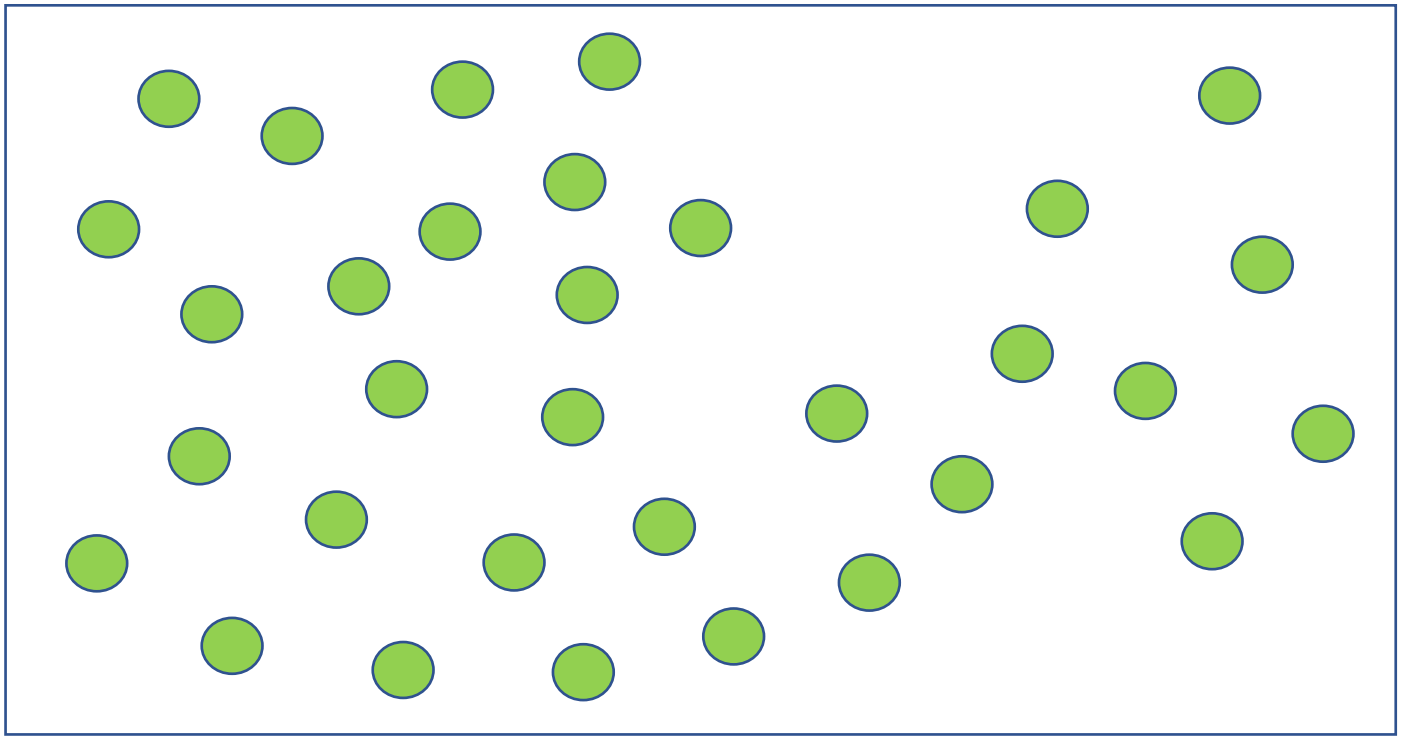
Application of sedimentation equilibrium





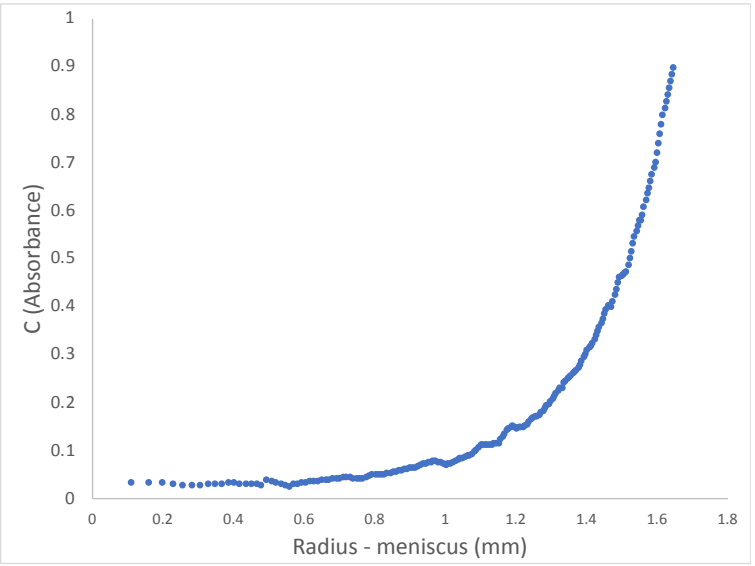
Can take 12 hours to more than a week to obtain equilibrium, depending on viscosity.

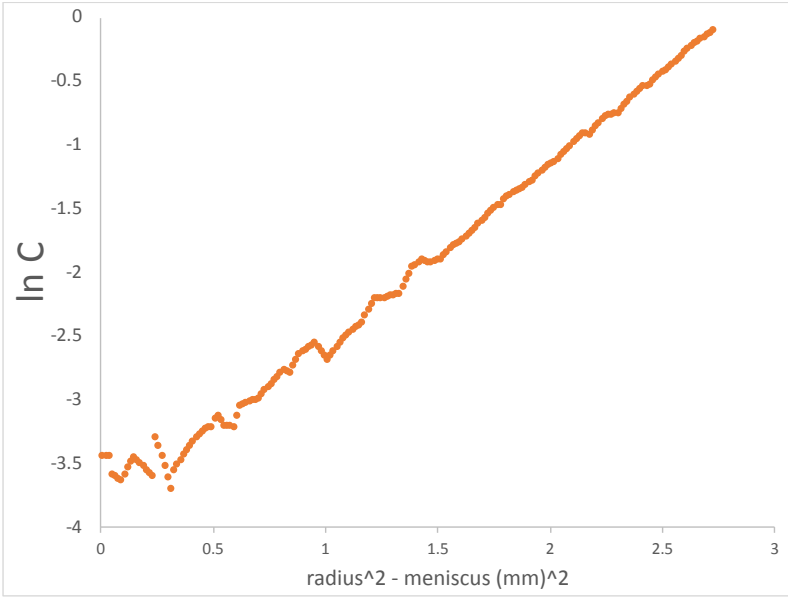
A monomeric ideal system



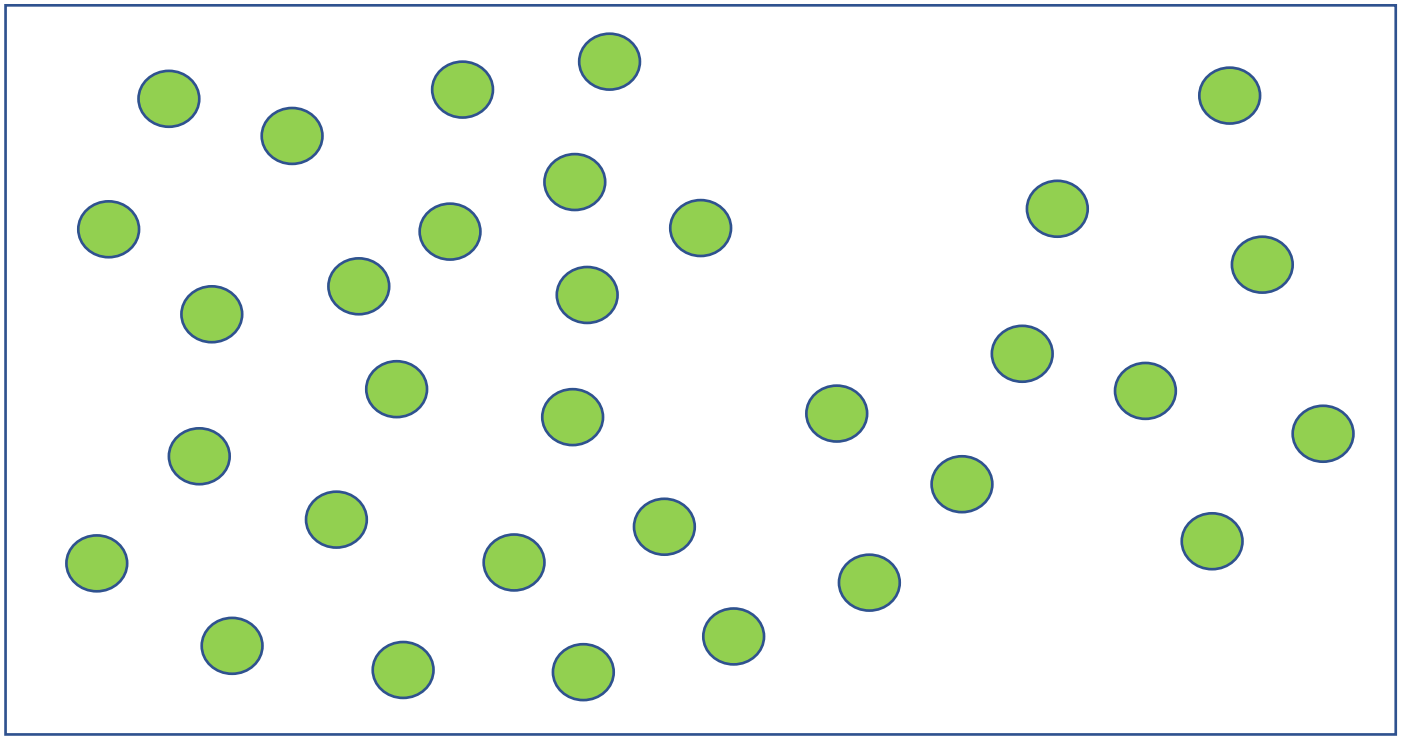
Each particle is subject to an identical sedimenting force
Monomer weight = 50 kDa
Measured weight = 50 kDa

A single particle can be determined easily





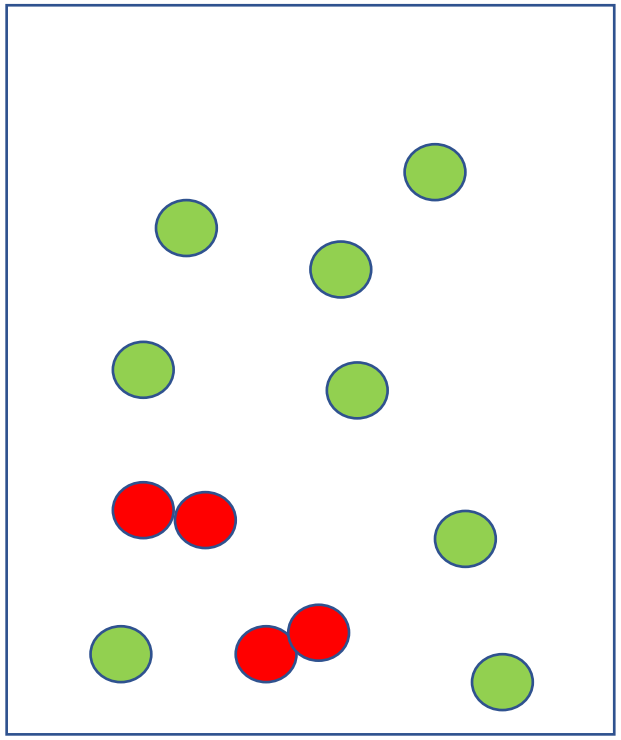
A monomeric system



Each particle is subject to an identical sedimenting force
Monomer weight = 50 kDa
Measured weight (Mw_{av}) = 50 kDa

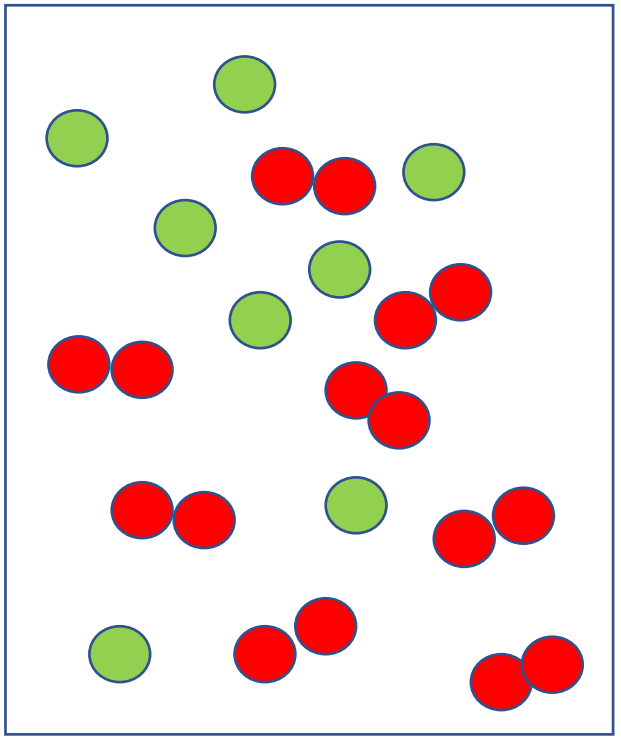
A dimeric system

x 1 concentration



Mwt_{av} = ~ 60 kDa

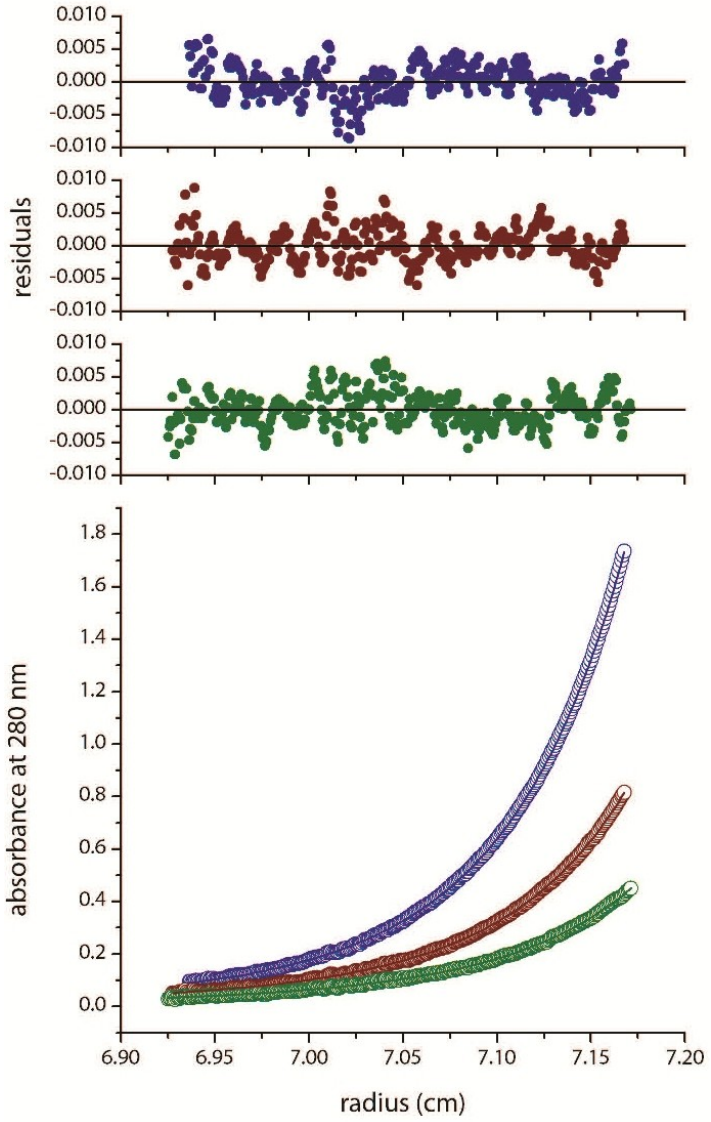
x 2 concentration



Mwt_{av} = ~ 80 kDa

Reversible association causes an increase in dimer concentration
Monomer Mwt = 50 kDa, Dimer Mwt = 100 kDa

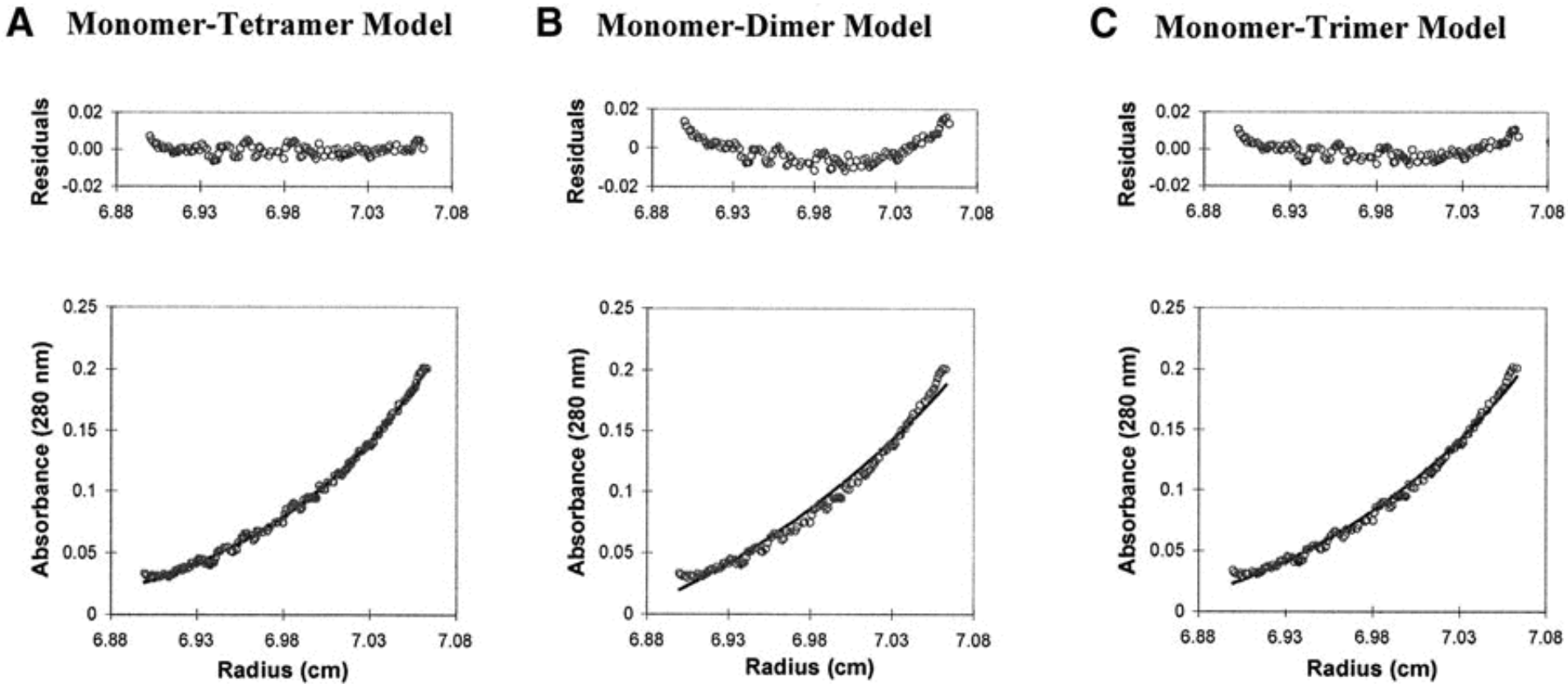
A tetrameric system



0.6.1.2,2.5 uM protein concentrations revealed a tetramer with a dissociation concentration of 0.5 uM

Blood 2010 115:4843-4852; doi: https://doi.org/10.1182/blood-2010-01-261396

A tetrameric example



Fitting data to different interacting models can reveal important interactive details

DOI 10.1093/emboj/19.23.6536 | Published online 01.12.2000 The EMBO Journal (2000) 19, 6536-6545

Analytical Ultracentrifuge Summary

- Sedimentation velocity
  - 500 uL sample
  - Measure S and D
  - Molecular weight of individual complexes
  - Measure f/fo ratio if weight known
  - Hard to measure transiently interacting systems
- Sedimentation equilibrium
  - 100 uL sample
  - Measure Mw_{av}
  - Can measure monomers and oligomers
  - Measure K_d

Values derived are obtained from first principles of laws - if the values are not or as expected interpretable then there are other parameters to have not been taking into account.

Sedfit: http://www.analyticalultracentrifugation.com
Sedanal: http://www.sedanal.org/
Ultrascan: http://ultrascan.aucsolutions.com/