4D structure

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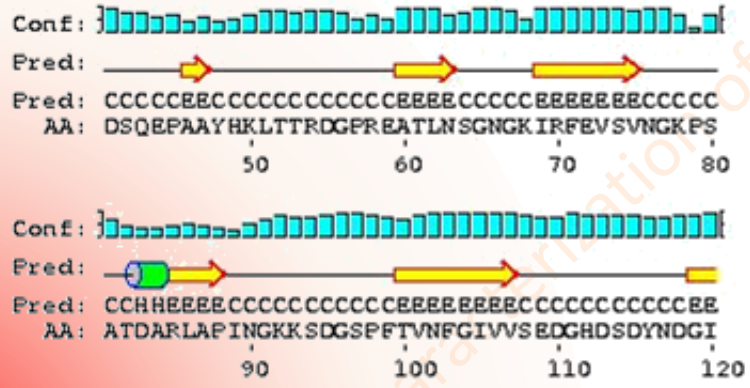
S1004 Methods for structural characterization of biomolecules

Structural hierarchy

1D

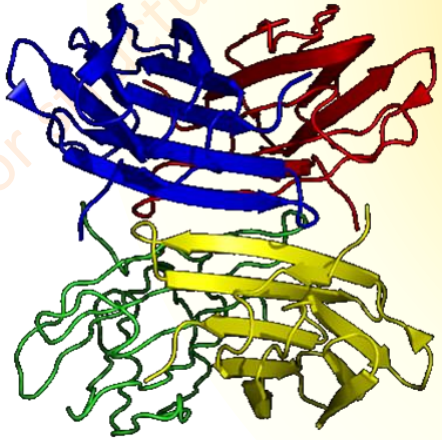
ADSQTSSNRAGEFSIPPNTDFRAIFFANAAE
QQHIKLFIGDSQEPAAYHKLTTRDGPREATL
NSGNGKIRFEVSVNGKPSATDARLAPINGK
KSDGSPFTVNFGIVVSEDGHDSDYNDGIVV
LQWPIG

primary (sequence)

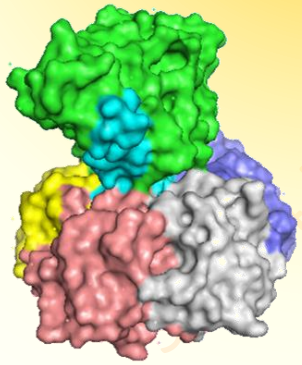


secondary

2D

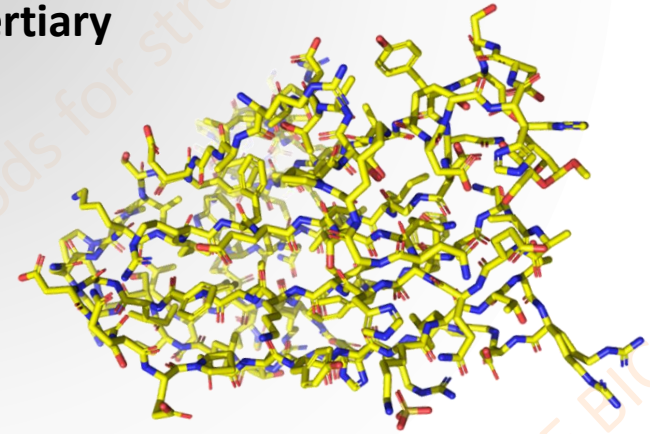


quaternary



4D

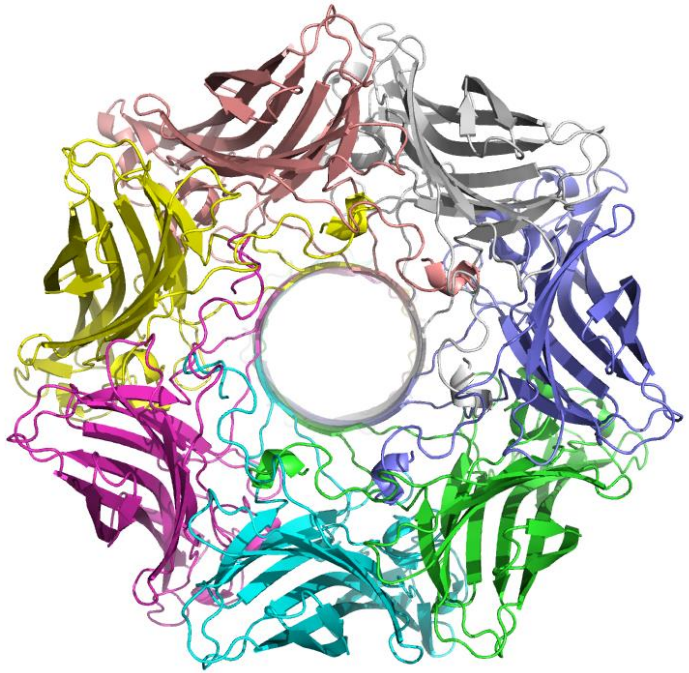
tertiary

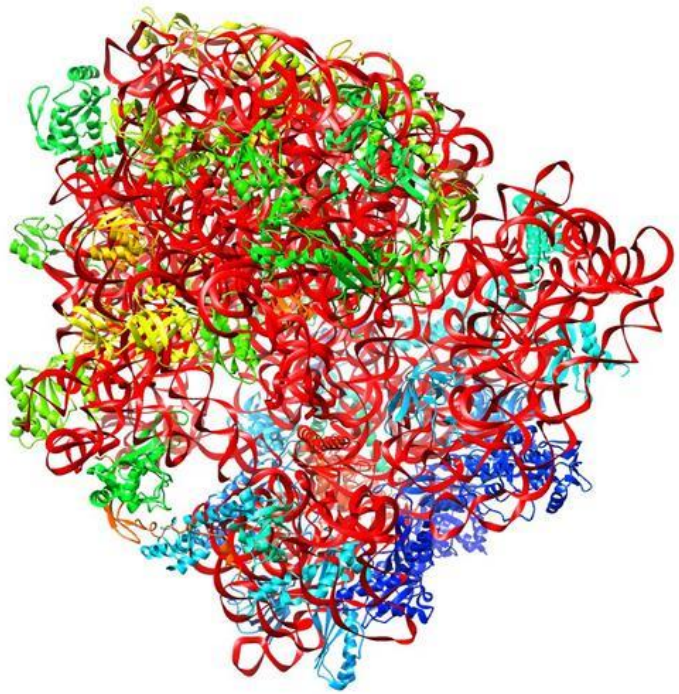


3D

Quaternary structure

- **Association of individual** (protein) **chains**
- Consisting of identical chains (**homooligomers**) or different chains (**heterooligomers**), including non-protein molecules, e.g. nucleic acids





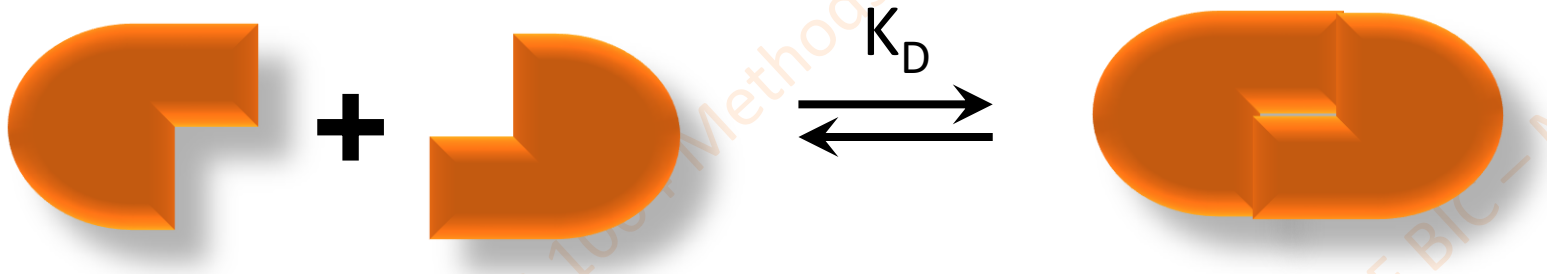
Quaternary structure

- **Composition** of the complex molecule may be obtained:
  - Via **dedicated experiment** (MS, SEC-MALS, ...)
  - From **3D structure**
  - **Combination** of both

- Composition depends on **conditions**

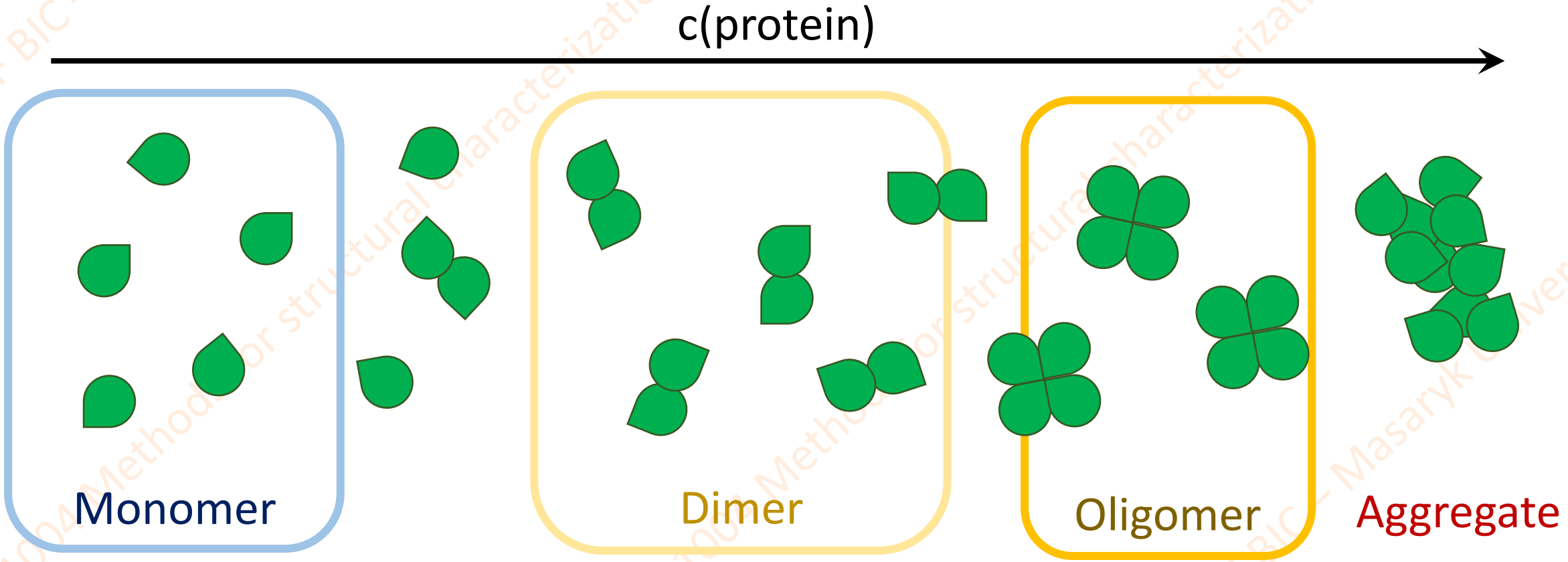
Concentration-dependent

- Dimerization – special type of protein interaction
- K_D(dimerization) can be determined – interaction techniques
- At $c < K_D \rightarrow$ dimer not stable !!!



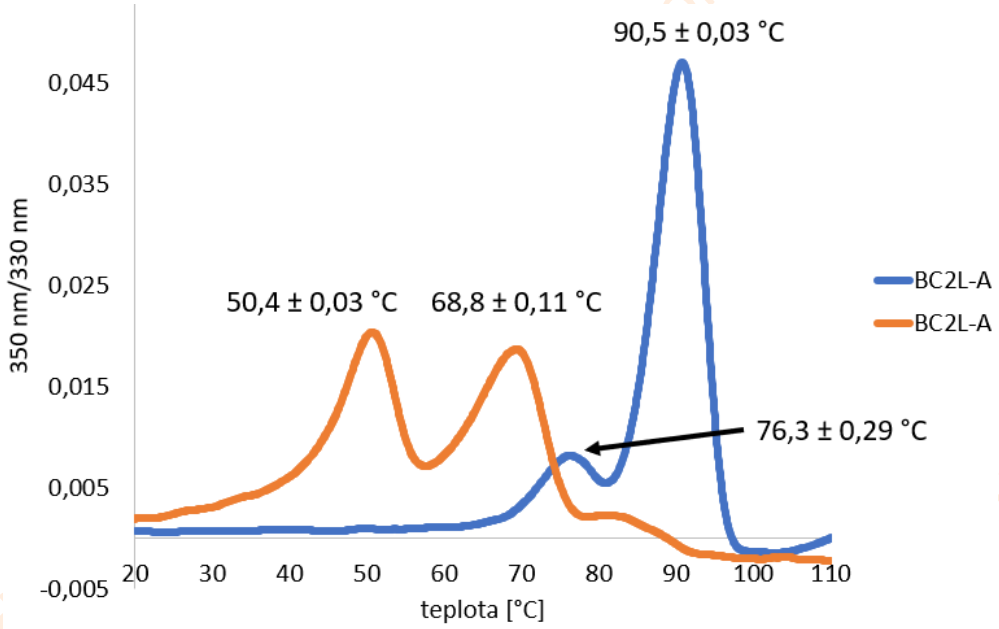
Sample homogeneity vs. oligomerization

- Working concentration determines protein state



Stability methods

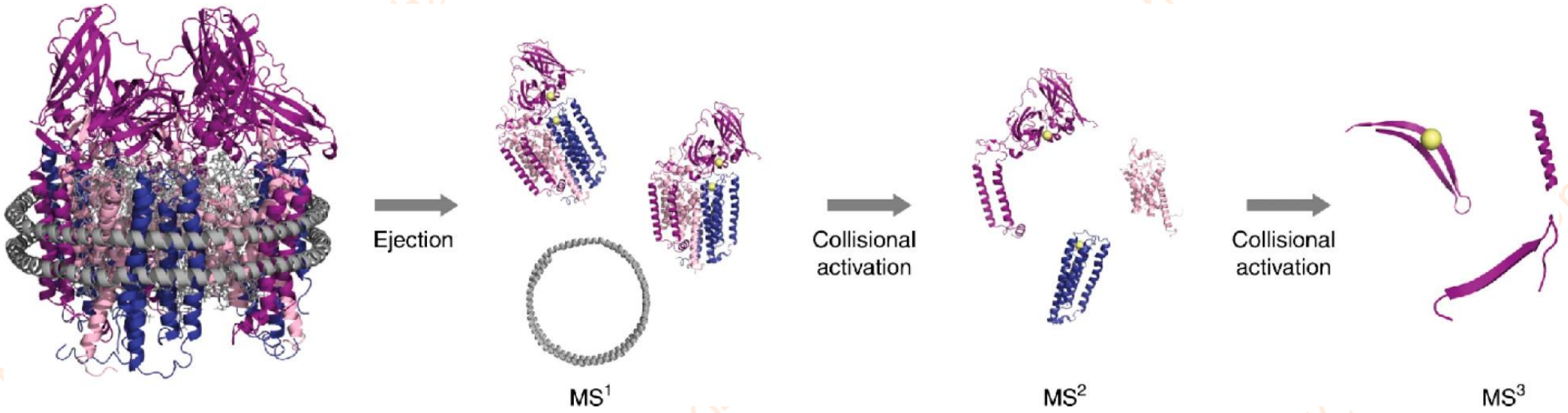
- Indirect evidence – double (multiple) transitions upon heating
- Thermal-shift assay
  Differential scanning fluorimetry



- **Advantage**: easy-to-obtain data
- **Disadvantage**: additional knowledge needed; risk of misinterpretation

Native Mass spectrometry (MS)

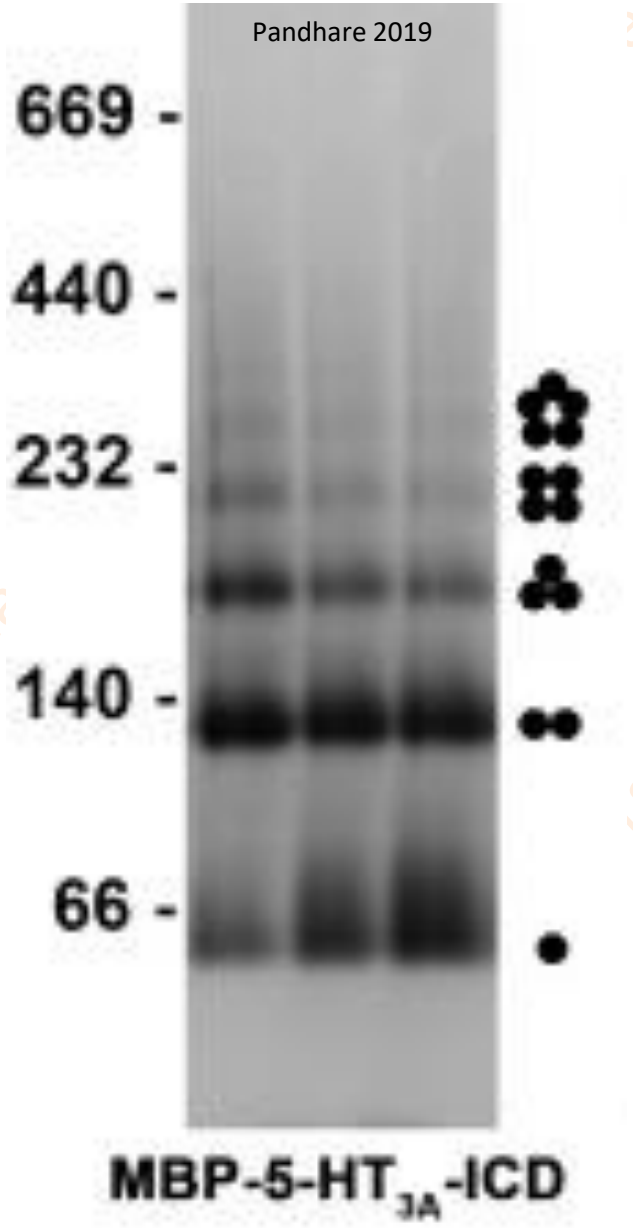
- High precision in MW determination
- Easier for stable oligomers – S-S bonds
- Mild ionization – ESI-MS



S.Tamara et al, Chem. Rev. 2022

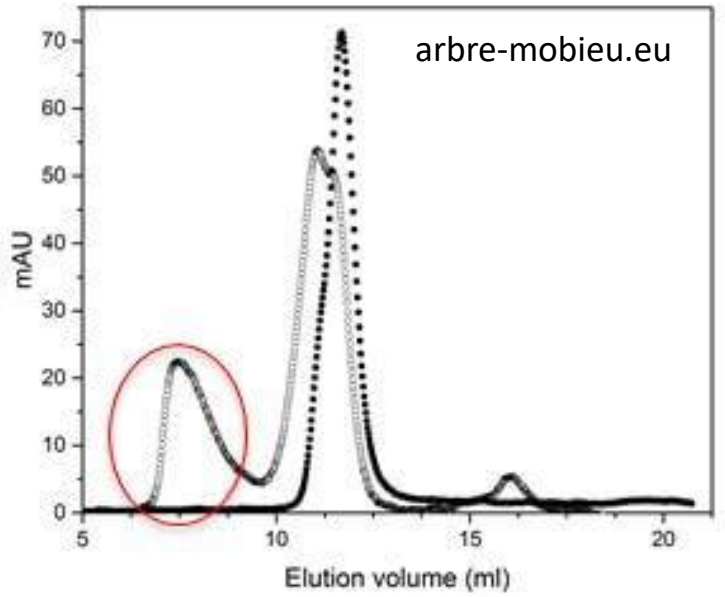
Native electrophoresis

- Possibility to observe various **oligomers**
- Relatively imprecise and unreliable
- Complicated in presence of protein **isoforms**



Size exclusion chromatography

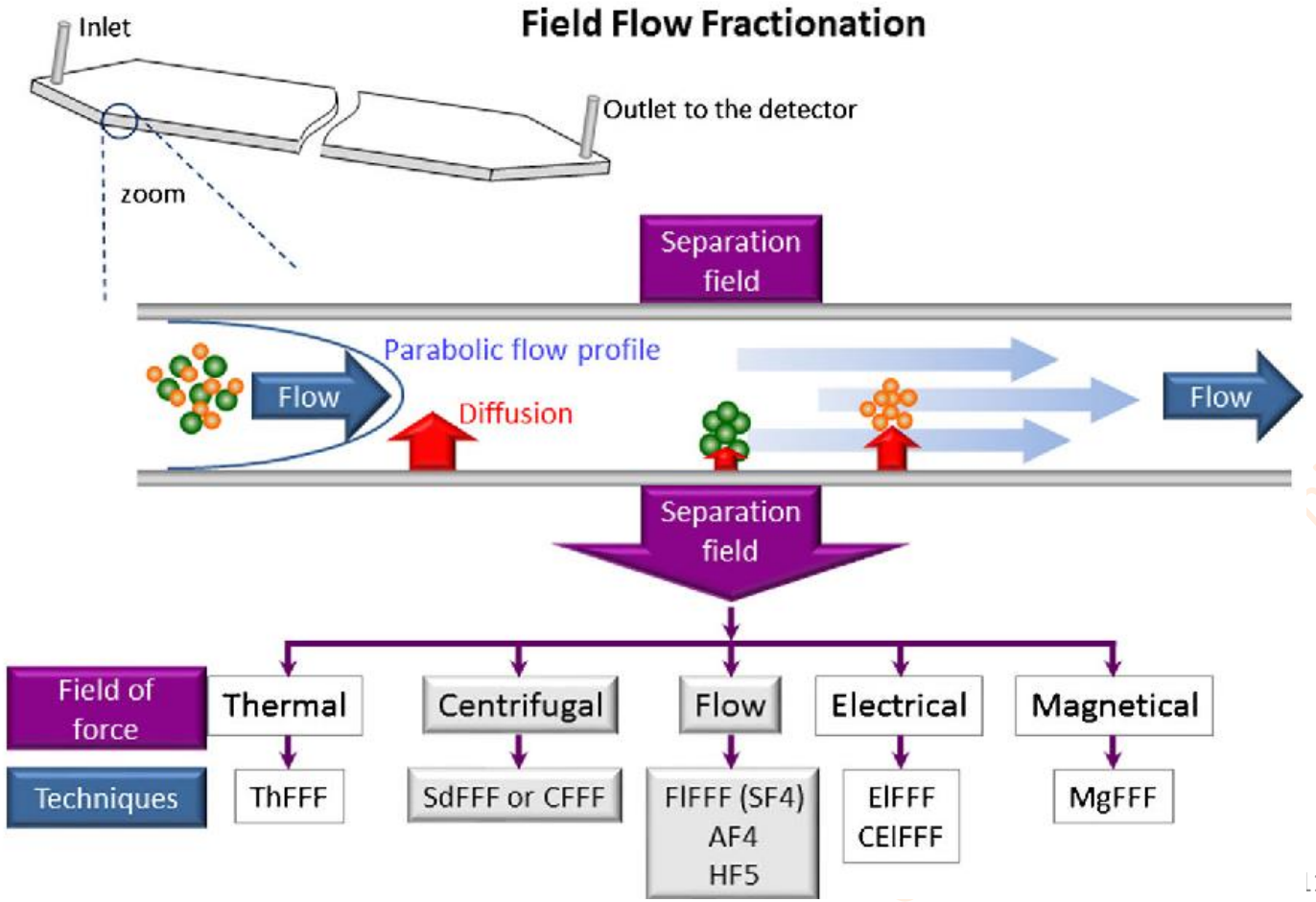
- Separation of particles based on “size”
- Interaction with matrix possible (!)



Preparative	Analytical
Bigger volume	“Minimal” volume
Long runs (hrs)	Short runs (minutes)
Peak separation required	Peak separation advantageous

Field flow fractionation

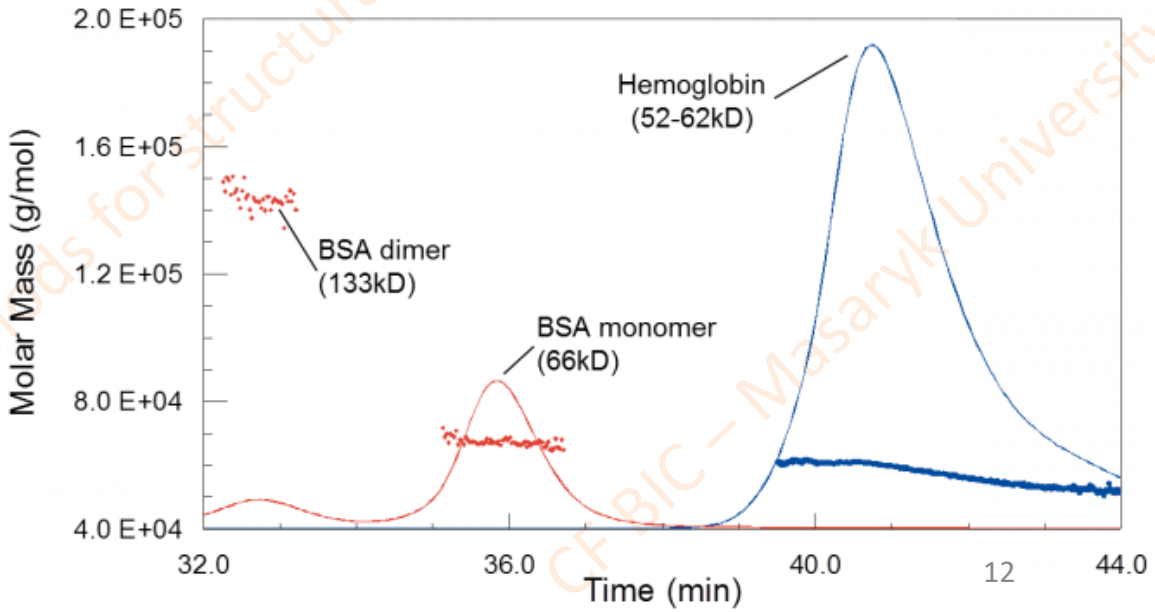
- Separation of particles in solution by external force
- Alternative to SEC



Size exclusion chromatography

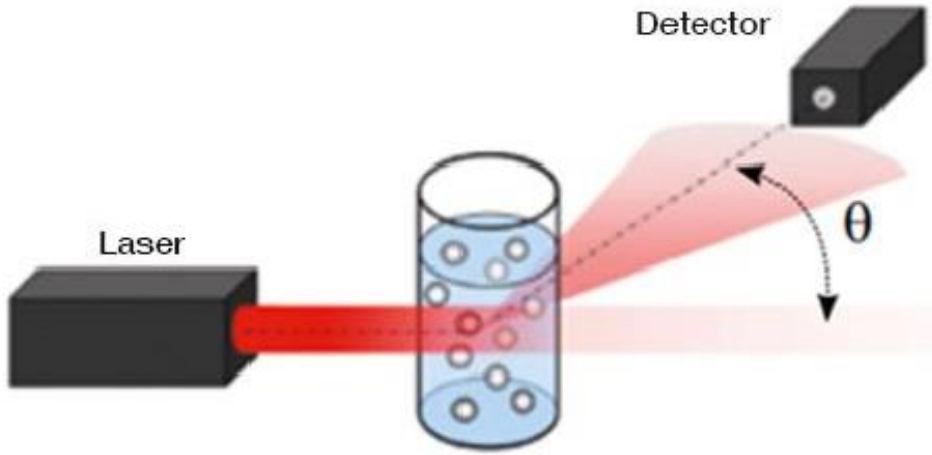
- Single detector (UV)
  - MW determination depends on standard elution volume
  - Non-standard behavior = MW error

- Multiple detectors (detector array)
  - Precise concentration (RI, ev. UV)
  - Accurate MW (MALS, RALS/LALS)



Light scattering

- Interaction of incident light with particles in solution
- Intensity of light at given angle
- Typically red/infrared light

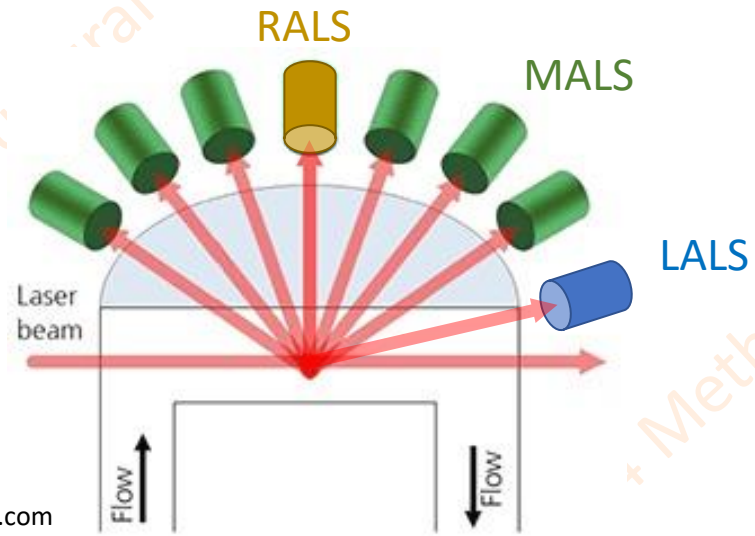


- **Dynamic light scattering**
  – size of particles
  – sensitive to aggregation
- **Static light scattering**
  – **mass of particles**

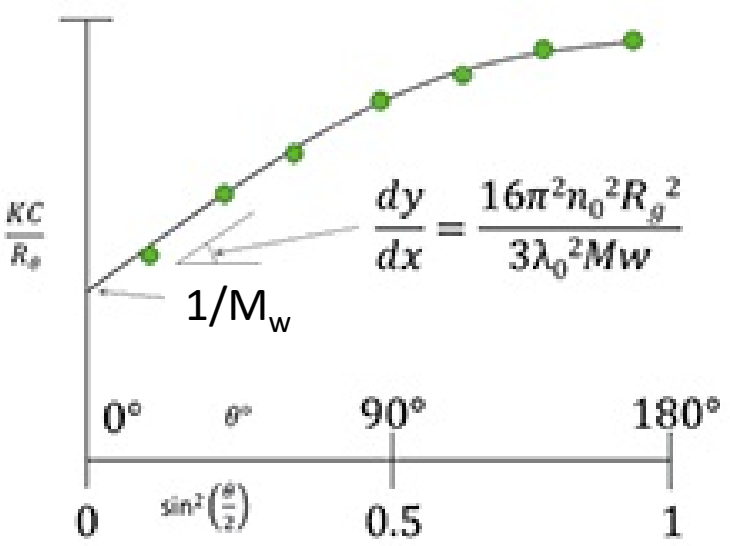
Static light scattering (SLS)

Low-angle light scattering (LALS) – big molecules
Right-angle light scattering (RALS) – small molecules
Multi-angle light scattering (MALS) – M_w and R_g

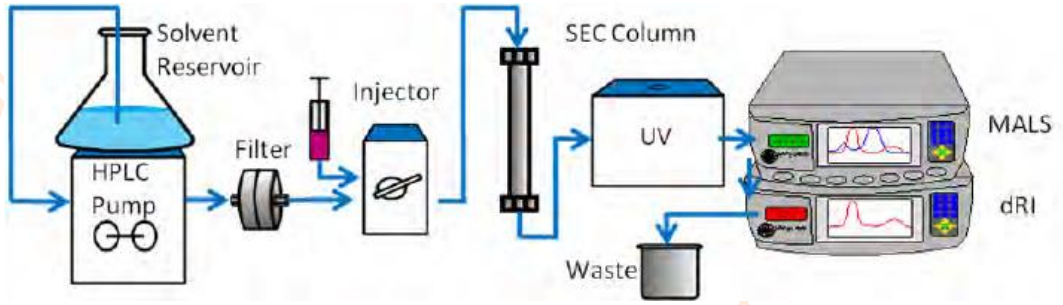
- Intensity of scattered light
- **Mass** of the particle (molecular weight)



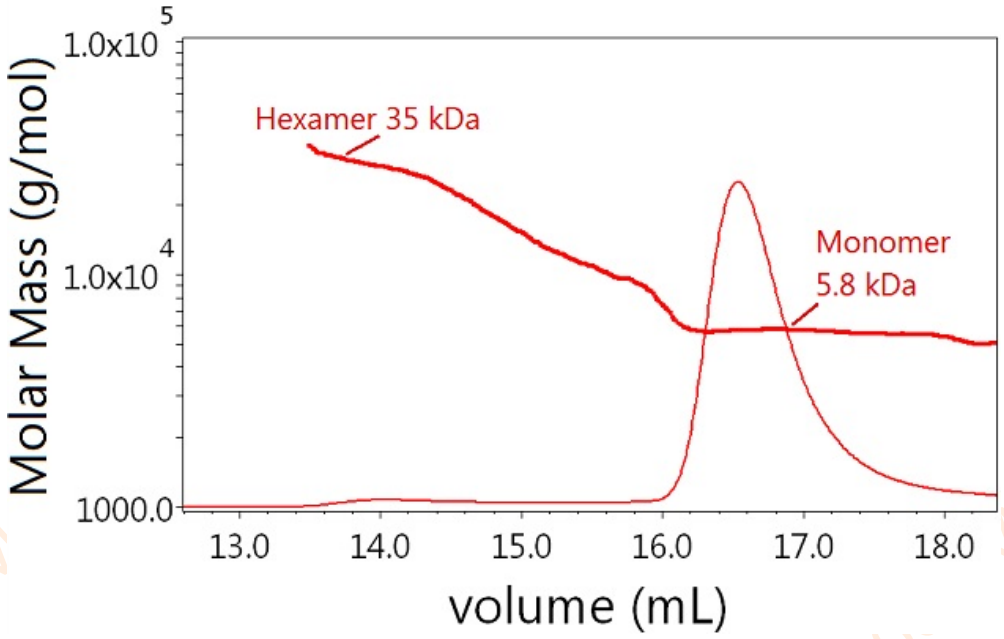
www.malvernpanalytical.com

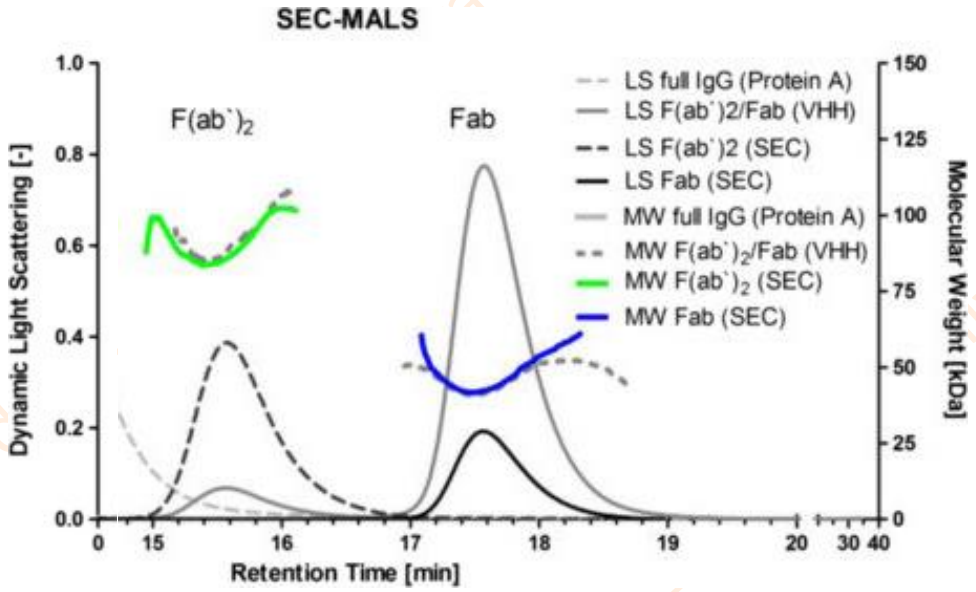


Static light scattering



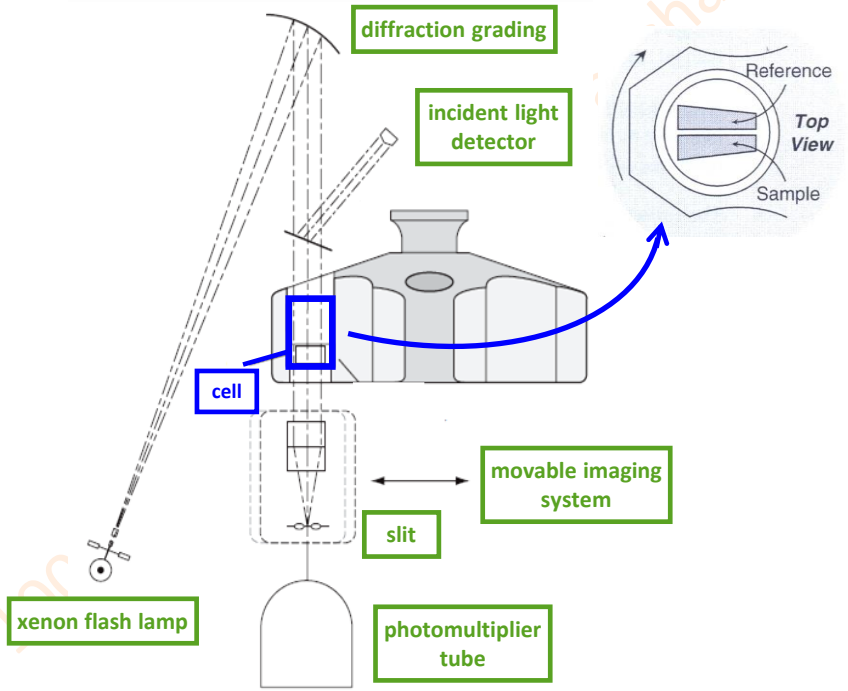
- Average of all sample particles !
- Typically coupled to separation (SEC, FFF)

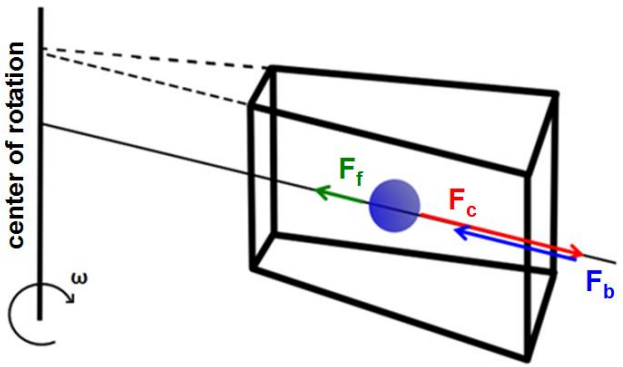


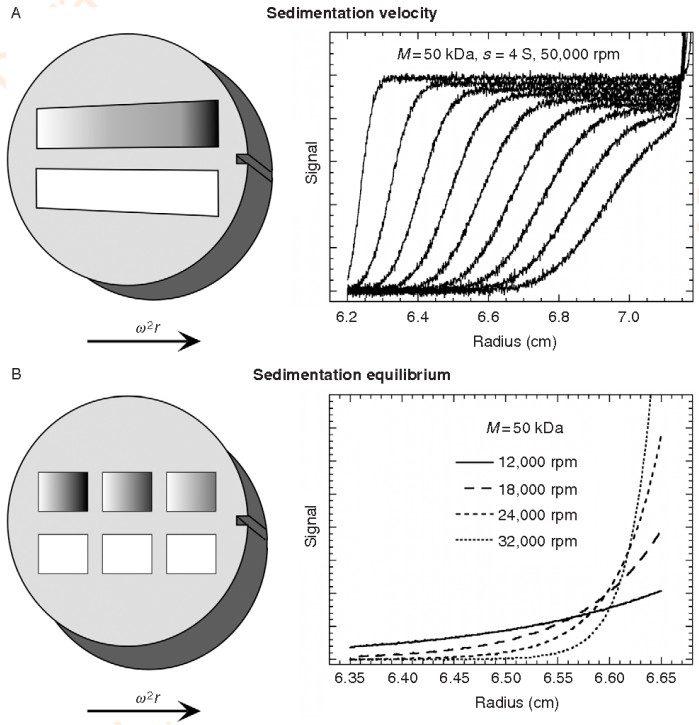


Analytical ultracentrifugation (AUC)

- Sedimentation of particles in centrifugal field by **hydrodynamic properties**
- Two modes:
  - Sedimentation equilibrium – mass determination
  - Sedimentation velocity – size distribution

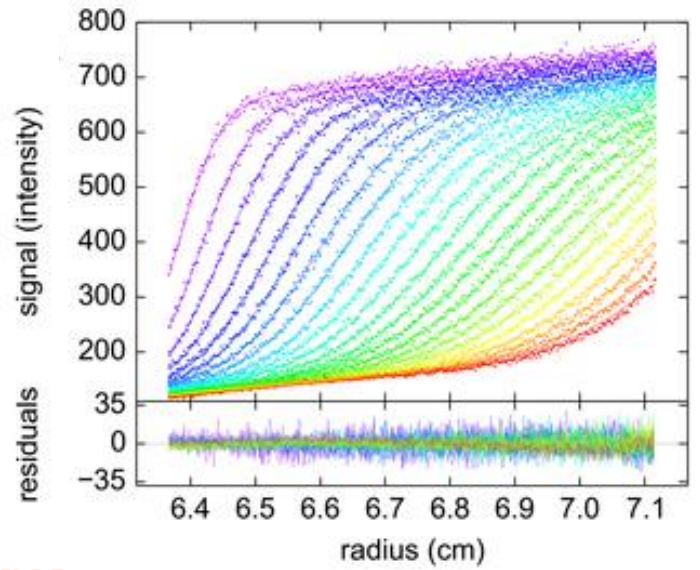


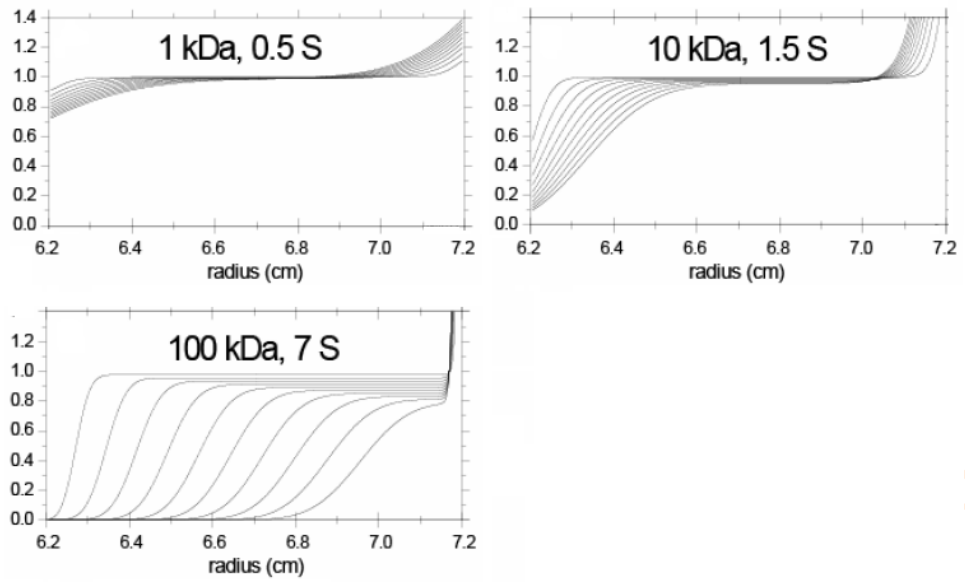




AUC – Sedimentation velocity

- Sedimentation of particles over time observed
- **Size** of the particle (hydrodynamic radius)





Equations for Rates of Settling in Centrifuges

- At the end of the residence time of the particle in the fluid, the particle is at a distance r_B m from the axis of rotation.
- If $r_B < r_2$, the particles leaves the fluid
- If $r_B = r_2$, it is deposited on the wall of the bowl and effectively removed from the liquid
- For settling in the Stokes' law range, the terminal settling velocity at a radius r is:

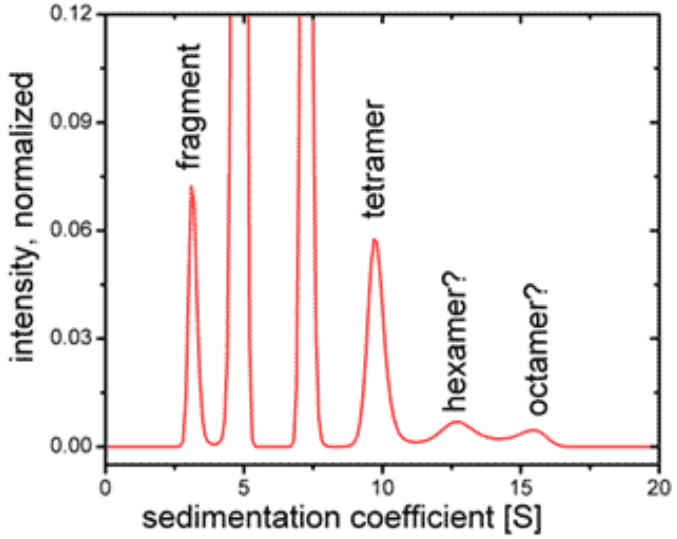
$$v_t = \frac{\omega^2 r D_p^2 (\rho_p - \rho)}{18\mu} \quad (8)$$

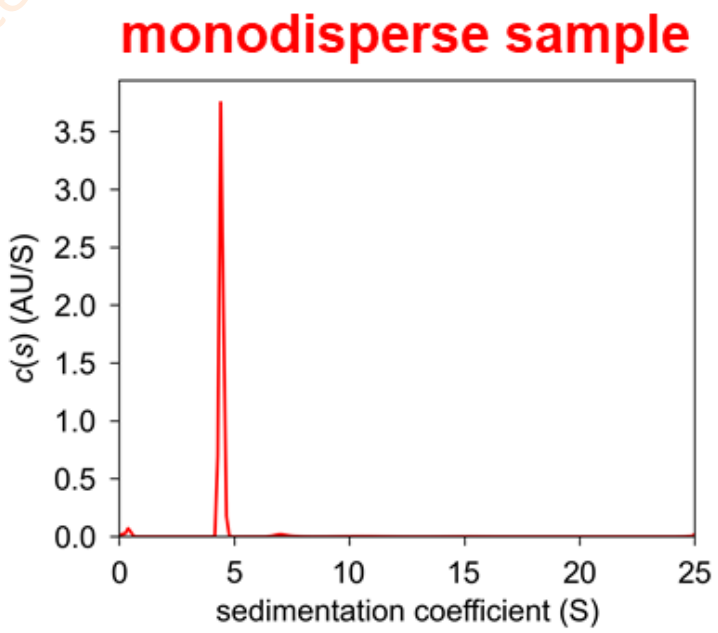
- Since $v_t = dr/dt$, and integrating between the limits $r = r_1$ at $t = 0$ and $r = r_2$ at $t = t_T$

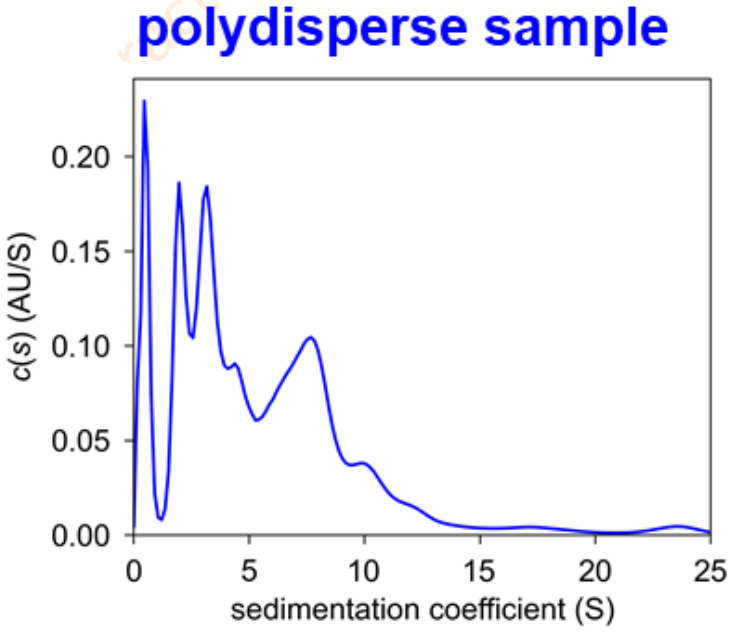
$$t_T = \frac{18\mu}{\omega^2 (\rho_p - \rho) D_p^2} \ln \frac{r_2}{r_1} \quad (10)$$

AUC – Sedimentation velocity

- Suiteable to detect and quantify **oligomers** and **aggregates**
- Sensitive to shape (and density)



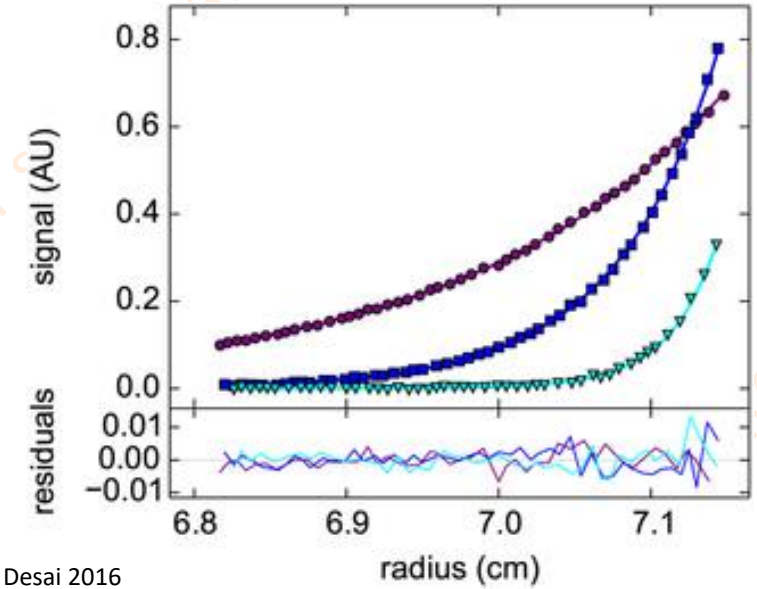




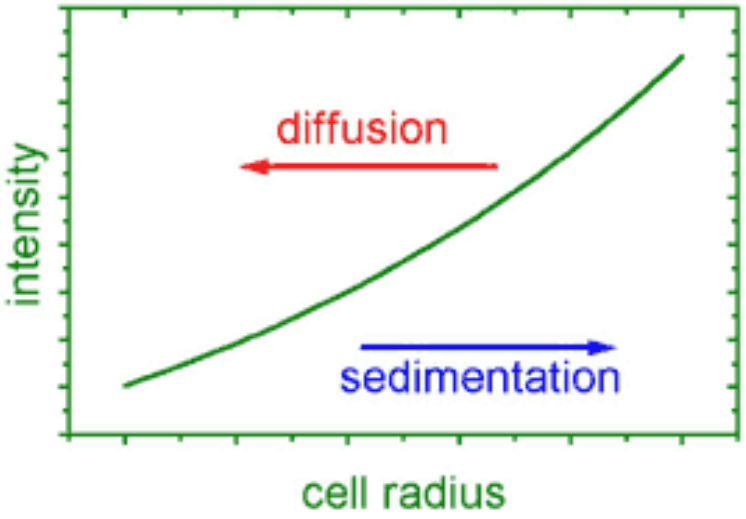
AUC – Sedimentation equilibrium

- Distribution of particles in cell

- **Molecular mass** of particle
- Problematic for mixtures – homogeneity !!!

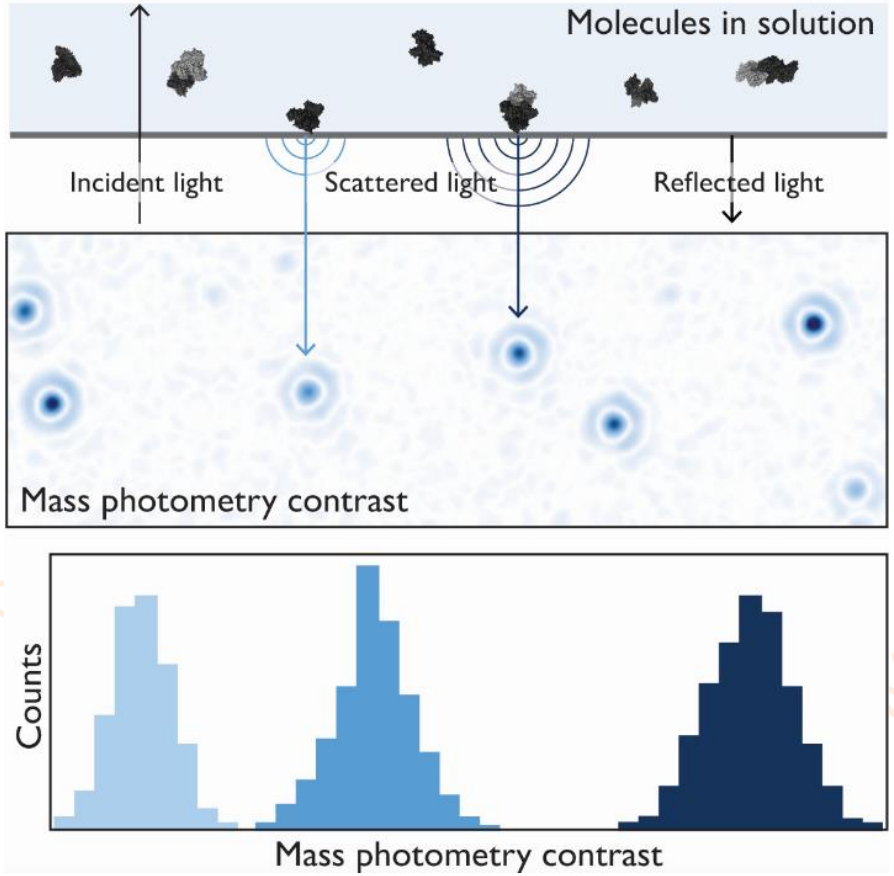


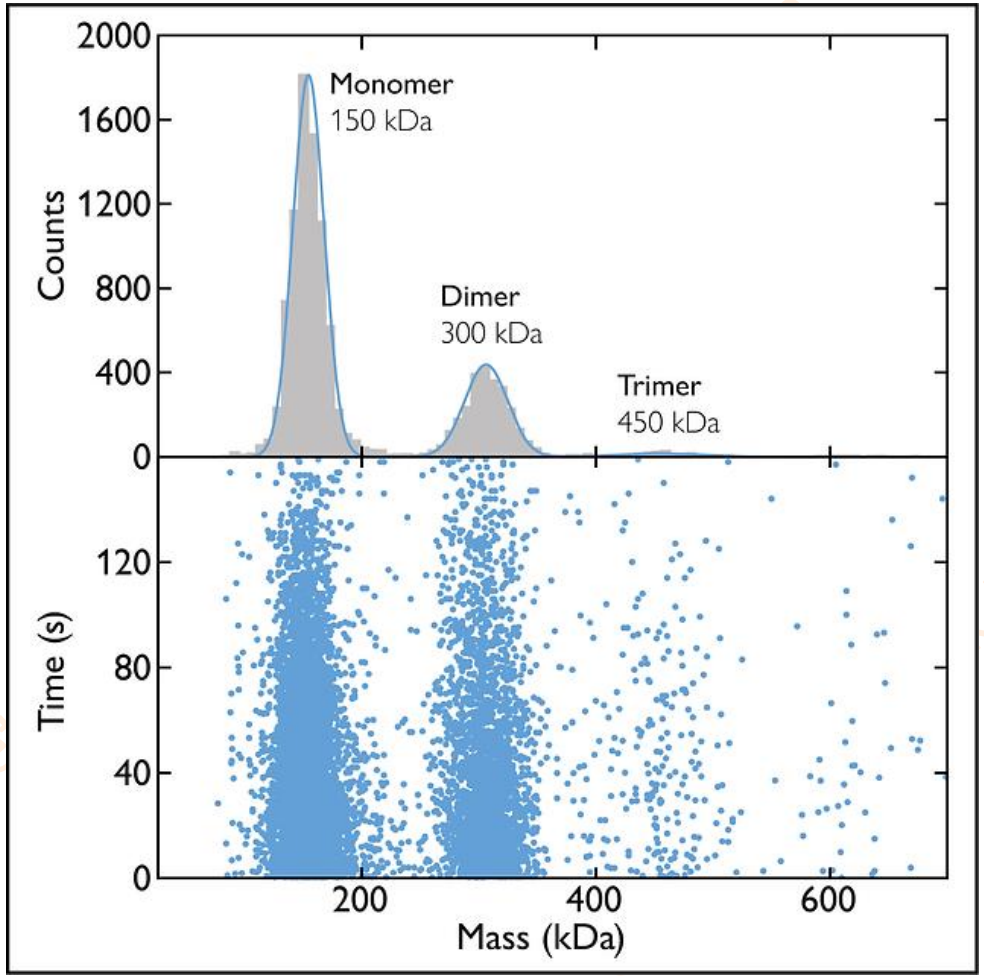
Desai 2016



Mass photometry (MS)

- Measurement of scattered light interference
- Mass of the particle





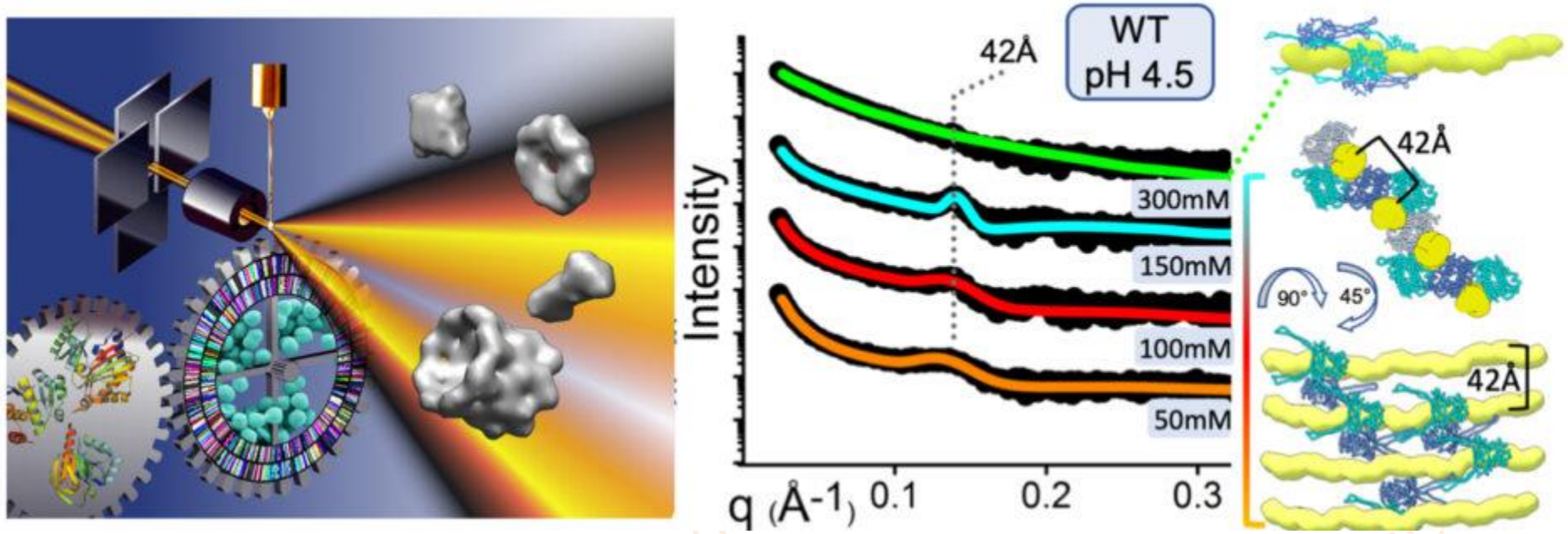
Mass photometry (MS) – instruments

- Single producer
- Manual and automated version

- Low volume at low sample concentration

Small-angle X-ray scattering (SAXS)

- Size and shape of molecules
- More in a separate lecture by Tomáš Klumpler



Summary

- Oligomeric state depends on **conditions (concentration)**
- Various techniques with **different requirements**
- Choose technique(s) **suitable** for your system
- Be aware of results **misinterpretation**

Questions?

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Interactions and
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