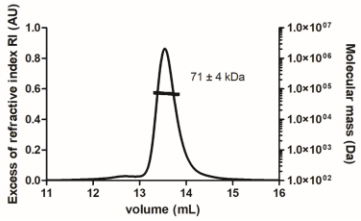
Solution studies : SEC-MALS, Analytical ultracentrifugation, SAXS and mass photometry

caroline.mas@ibs.fr

Size Exclusion Chromatography (SEC) coupled with Multi Angle Laser Light Scattering (MALLS)



What is SEC-MALLS?

Multi-Angle Laser Light Scattering (MALLS), UV Absorbance and Refractive Index (RI) coupled to a size-exclusion chromatography (SEC) system allows the **simultaneous determination of the molecular weight of each component of a sample.**

MALLS measurements work by calculating the amount of scattered light (LS) by a sample at various angles. The intensity of the light scattered of a solution is directly proportional to

- the concentration of its components (RI or UV)
- the average molecular weight (LS)

$$I_s(\theta) \propto c \times M \times \left(\frac{dn}{dc}\right)^2$$

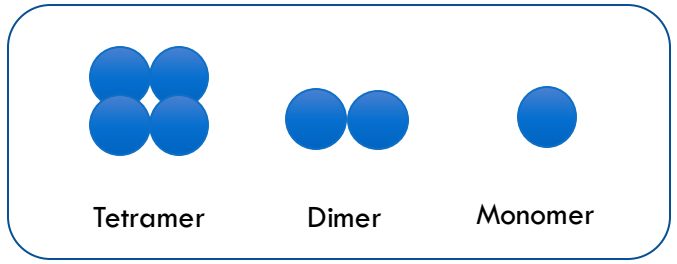
Sample requirement:

- 90% pure
- 2mg/mL
- 50 µL

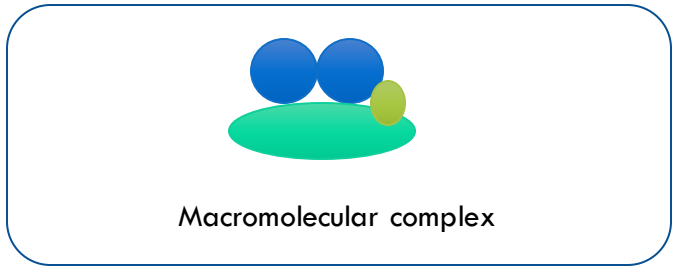
SEC-MALLS Applications

Weight-averaged molar mass (Mw) of particles separable by SEC

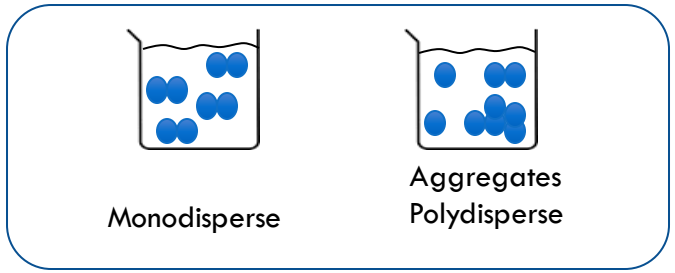
- Molecular weight in solution
- Oligomeric state
- Stoichiometry of my multimolecular complex
- **Mass contribution from two components**
  - **Protein and detergent micelle**
  - **Protein and glycan**
- Sample homogeneity, aggregation



Oligomeric state

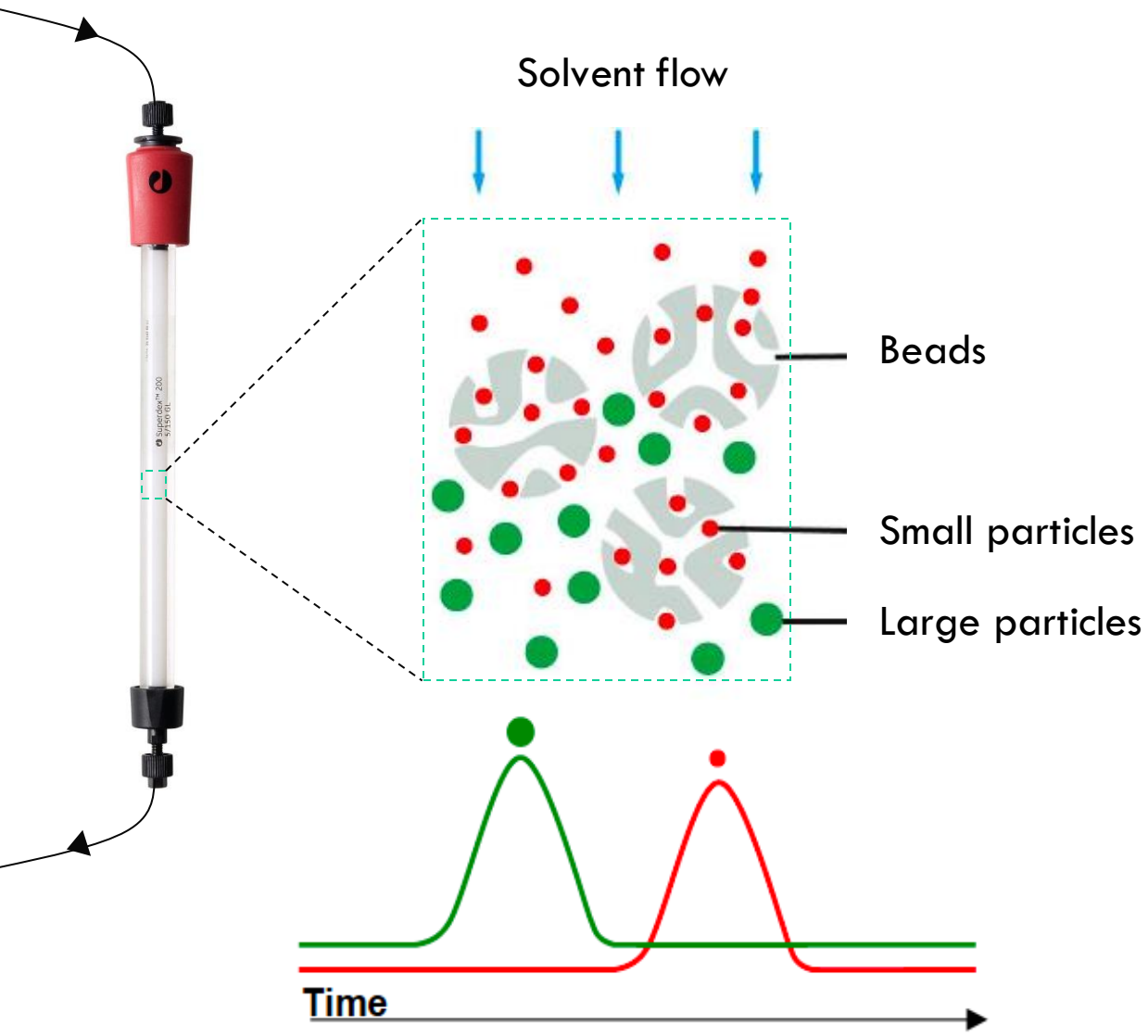


Stoichiometry of multimolecular complex



Homogenity

Size Exclusion Chromatography - SEC



Size Exclusion Chromatography (SEC) is a chromatographic method in which molecules are separated based on their size.

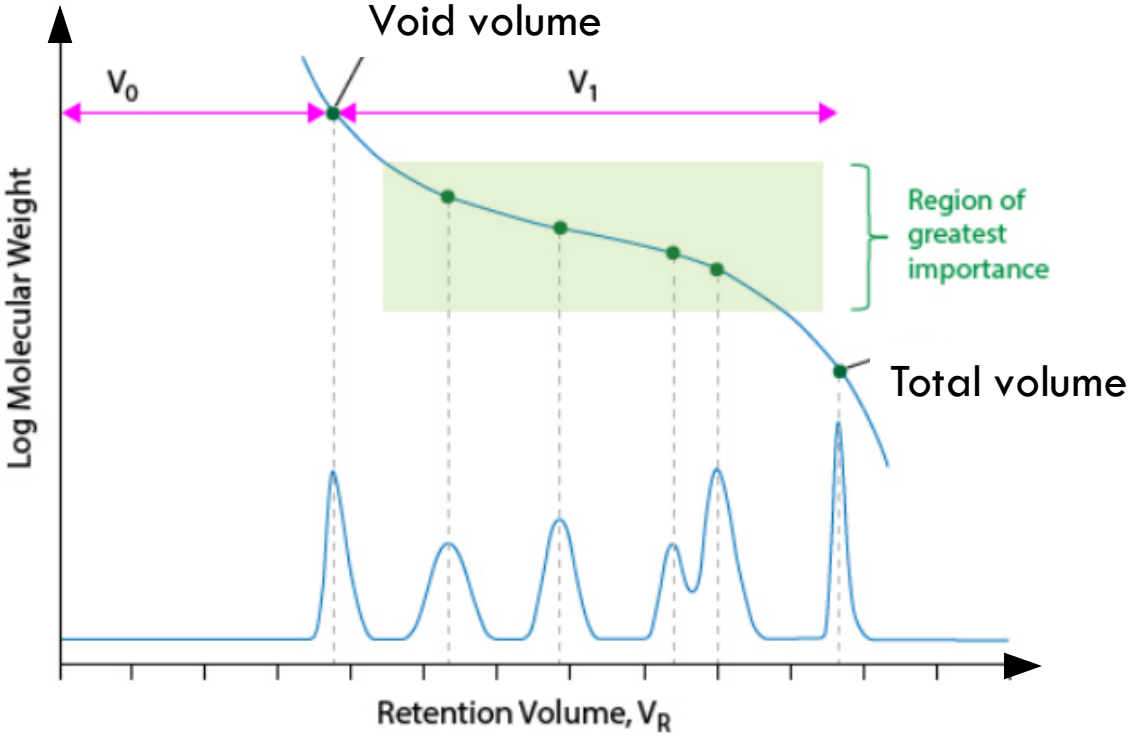
It means that the elution volume is related to their hydrodynamic volume / radius (Rh) and not to their molecular weight.

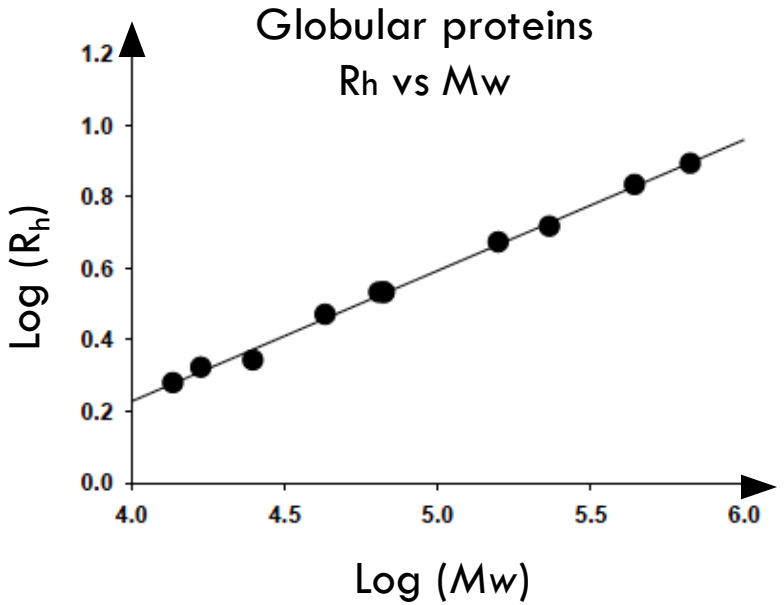
How does SEC works?

To connect the size (R_h) to the molecular weight, it is necessary to make a calibration with known globular molecular weight standards.

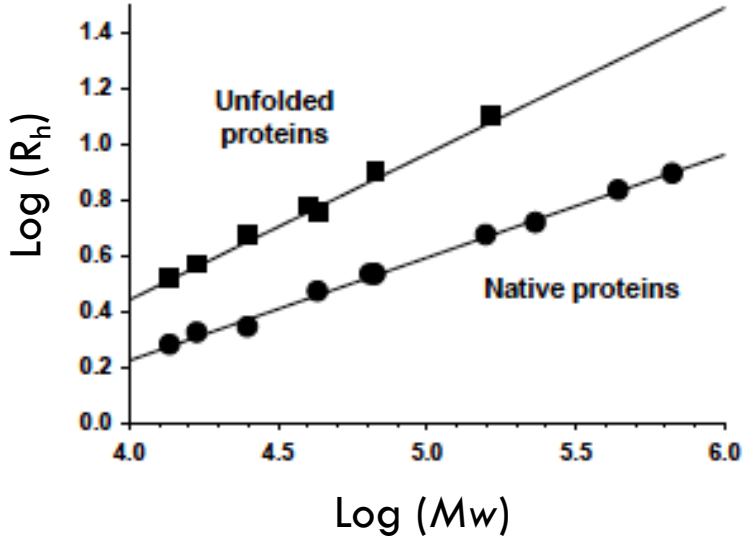
Traditional SEC assumes that the sample of interest:

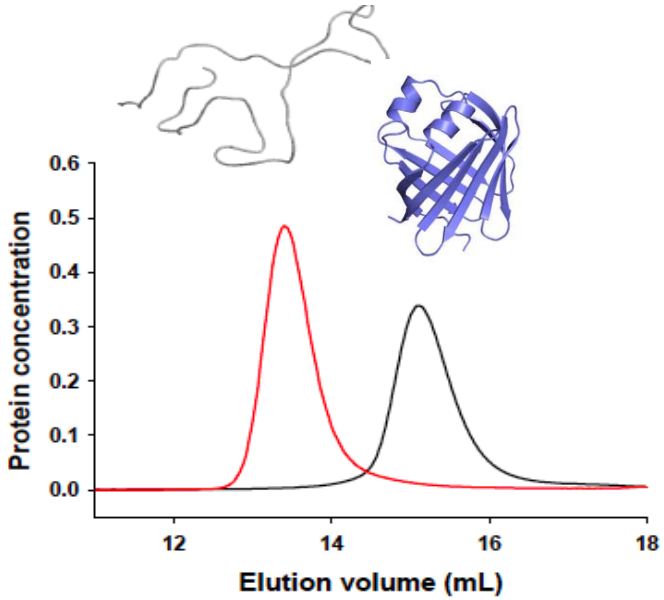
- has the same molecular conformation as the calibration standards
- does not interact with the stationary phase of the column





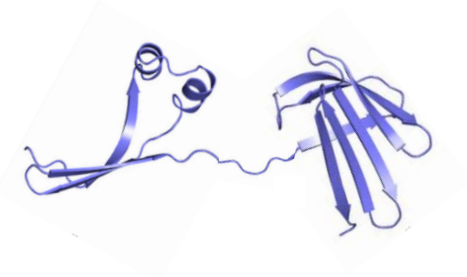
What about non-globular proteins?



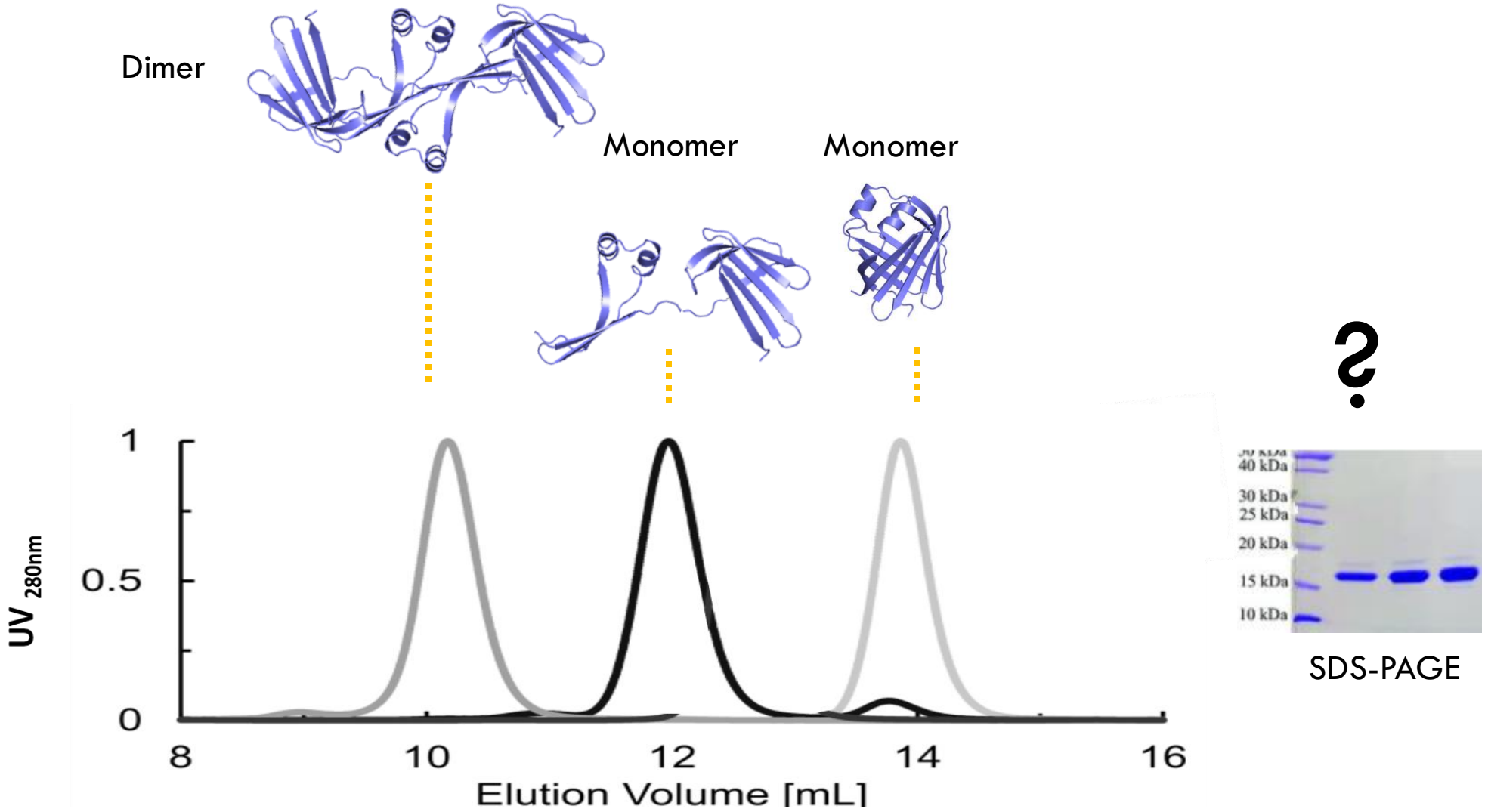


The hydrodynamic radius depends on the shape of the particle

Most proteins are not globular…



Interpretation of SEC data



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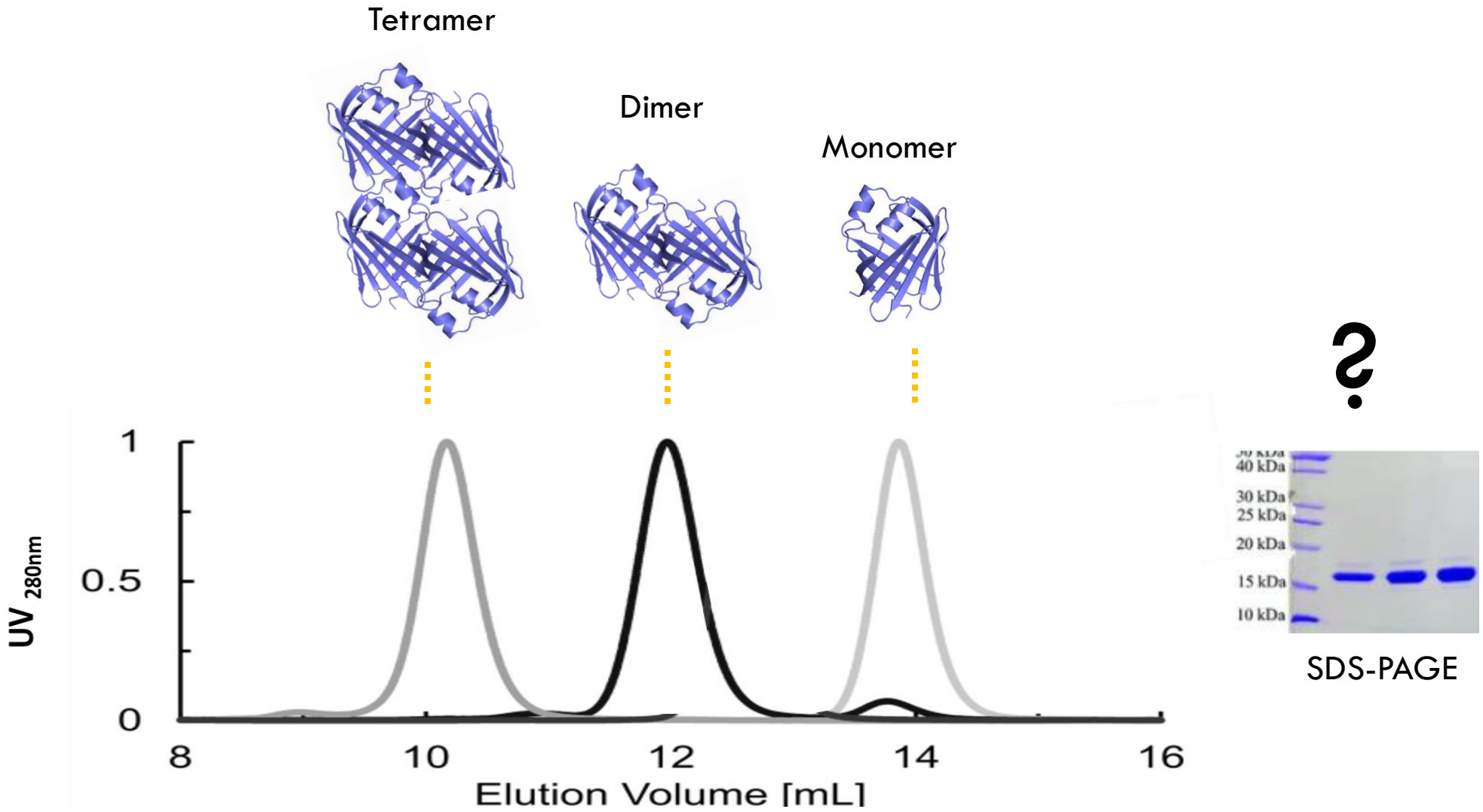
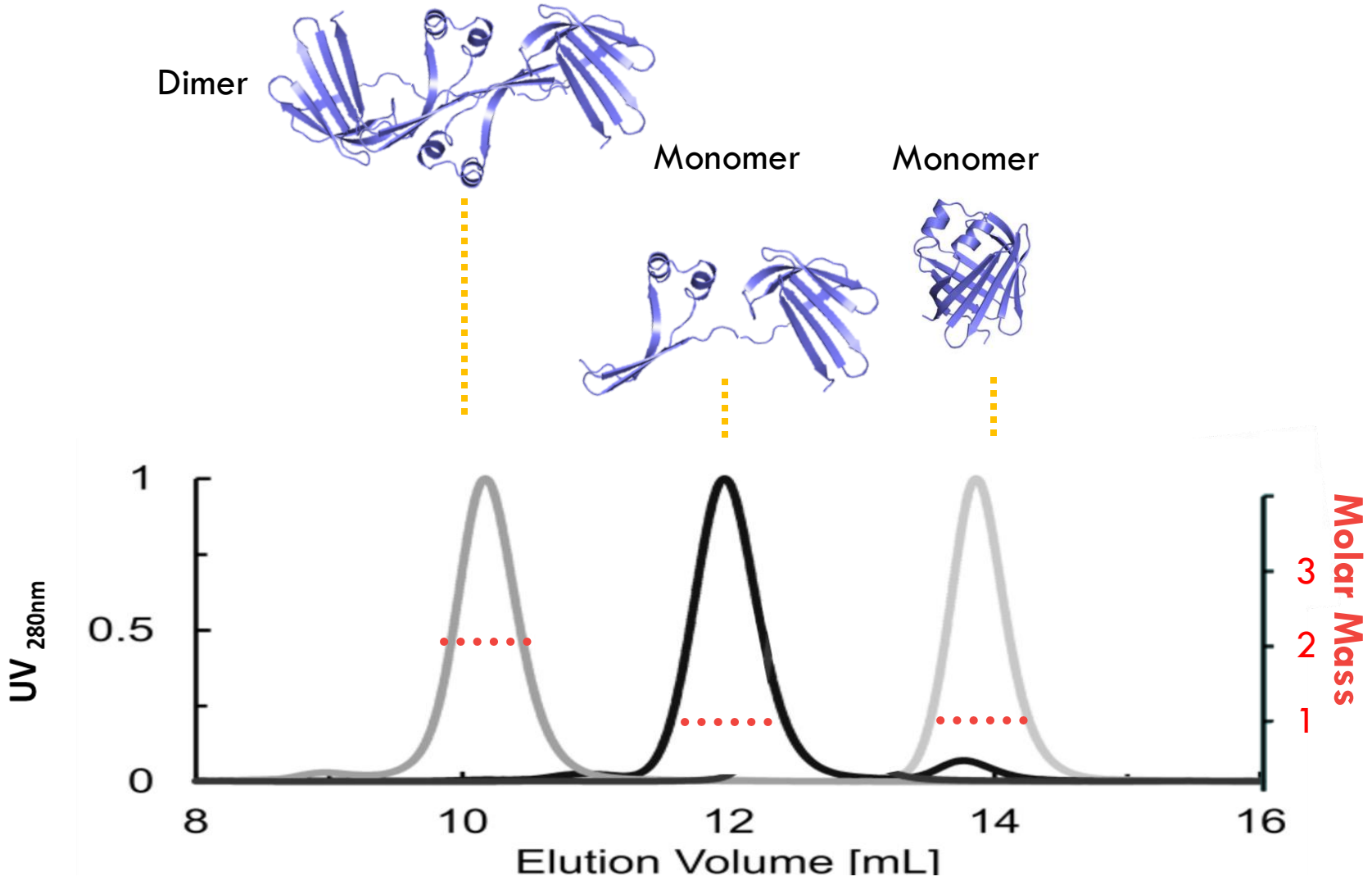
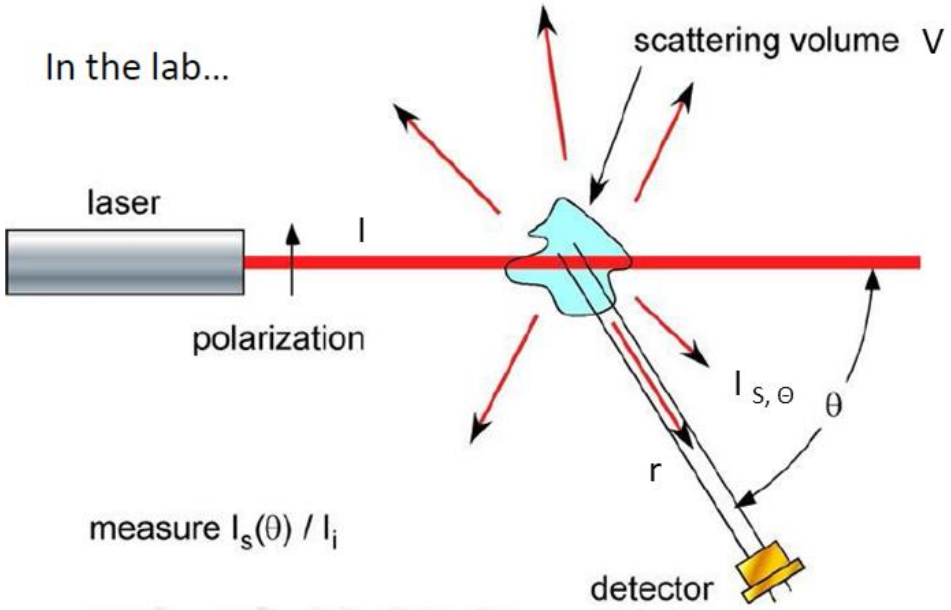


Interpretation of SEC data
Molar Mass information



Multi Angle Laser Light Scattering

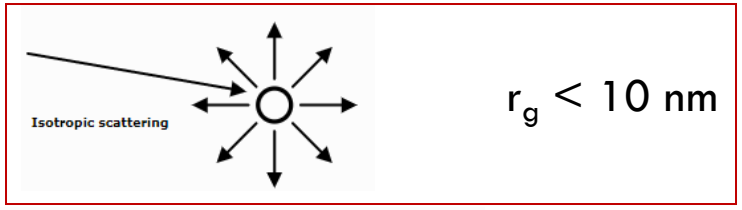
Static Light Scattering – SLS
MALLS measures the time-average intensity of scattered light

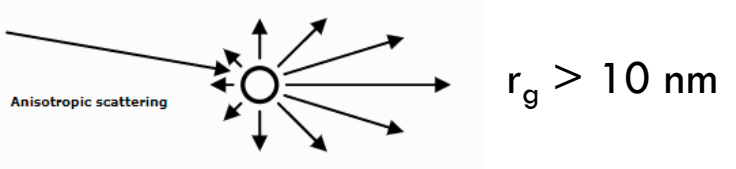


Rayleigh scattering

$$R(\theta) = \frac{I_{s,\theta}}{I_0}\frac{r^2}{V\ sin^2\theta}$$


Lord Rayleigh (John Strutt) (1842-1919)





Simple analytical description of Rayleigh scattering
The Rayleigh-Gans-Debye Equation

$$R(\theta) = \frac{4\pi^2 n_0^2}{N_A \lambda_0^4}\left(\frac{dn}{dc}\right)^2 McP(\theta)[1 - 2A_2McP(\theta)]$$

Excess Rayleigh scatter (above solvent) at angle θ

K : optical constant

M: molar mass
C: concentration

$P(\theta)$: Form factor
Angular term reflecting Rg
> Negligible (isotropic scattering)

Term accounting for the intermolecular interference terms in the second virial coefficient A_2
> Negligible

Multi Angle Laser Light Scattering

The intensity of light scattering of a solution is directly proportional to the average molecular weight and to the concentration of its components

$$I_s(\theta) \propto c \times M \times \left(\frac{dn}{dc}\right)^2$$

- M is the average molar mass of the scattering macromolecules, which is to be determined
- c is the concentration of the macromolecule(in mg/ml)
- dn/dc is a sample specific value, which relates changes of refractive index of the solution in relation to the change of concentration. Averaged value for proteins: 0.185 ml/g

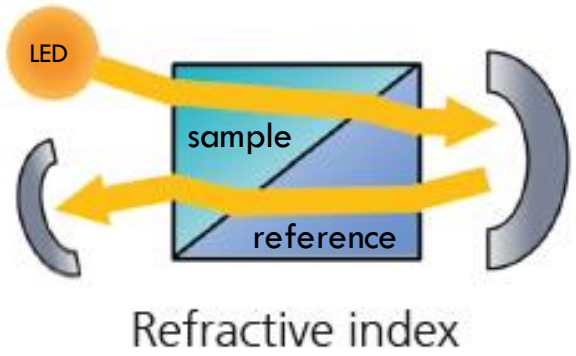
Refractive index

The Refractometer detector measures the difference of refractive index between sample and reference

Detection of all types of compounds even if they do not absorb.

> RI measurement is used to measure sample's concentration.

$$\Delta RI = n_s - n_r = c \frac{dn}{dc} \qquad c = \frac{\Delta RI}{\left(\frac{dn}{dc}\right)}$$



Refractive index

For **soluble proteins:** RI is used to measure the quantity of protein

For **membrane or glycosylated proteins:** RI and A_{280} are used for determination of detergent bound

$n = c/v$

c is the speed of light in vacuum

v is the speed of light in the in the medium

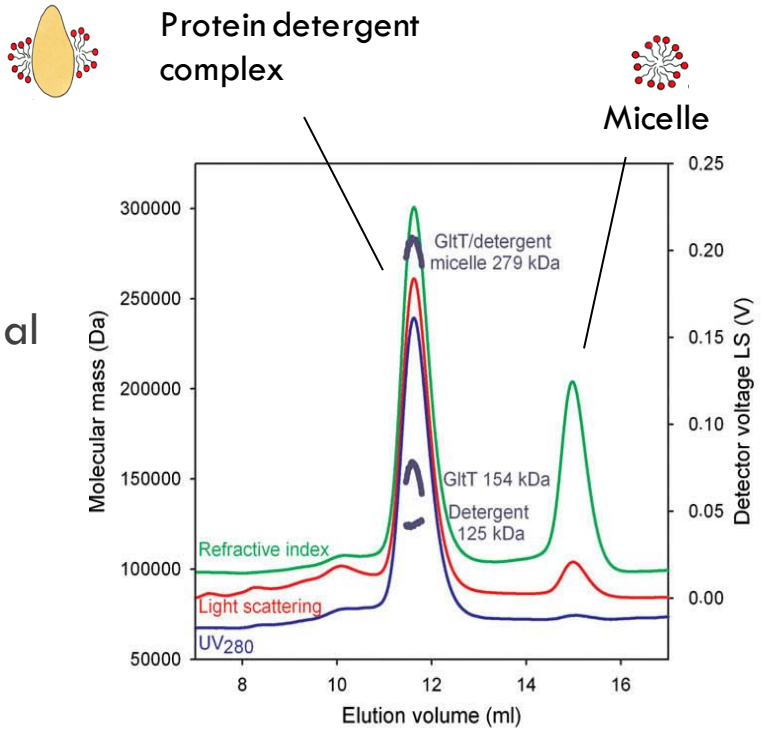
dn/dc is the specific refractive index increment = 0.185 ml/g for proteins

UV Absorbance

For **soluble proteins**
Absorbance is not used for the determination of the molar mass (RI is used)

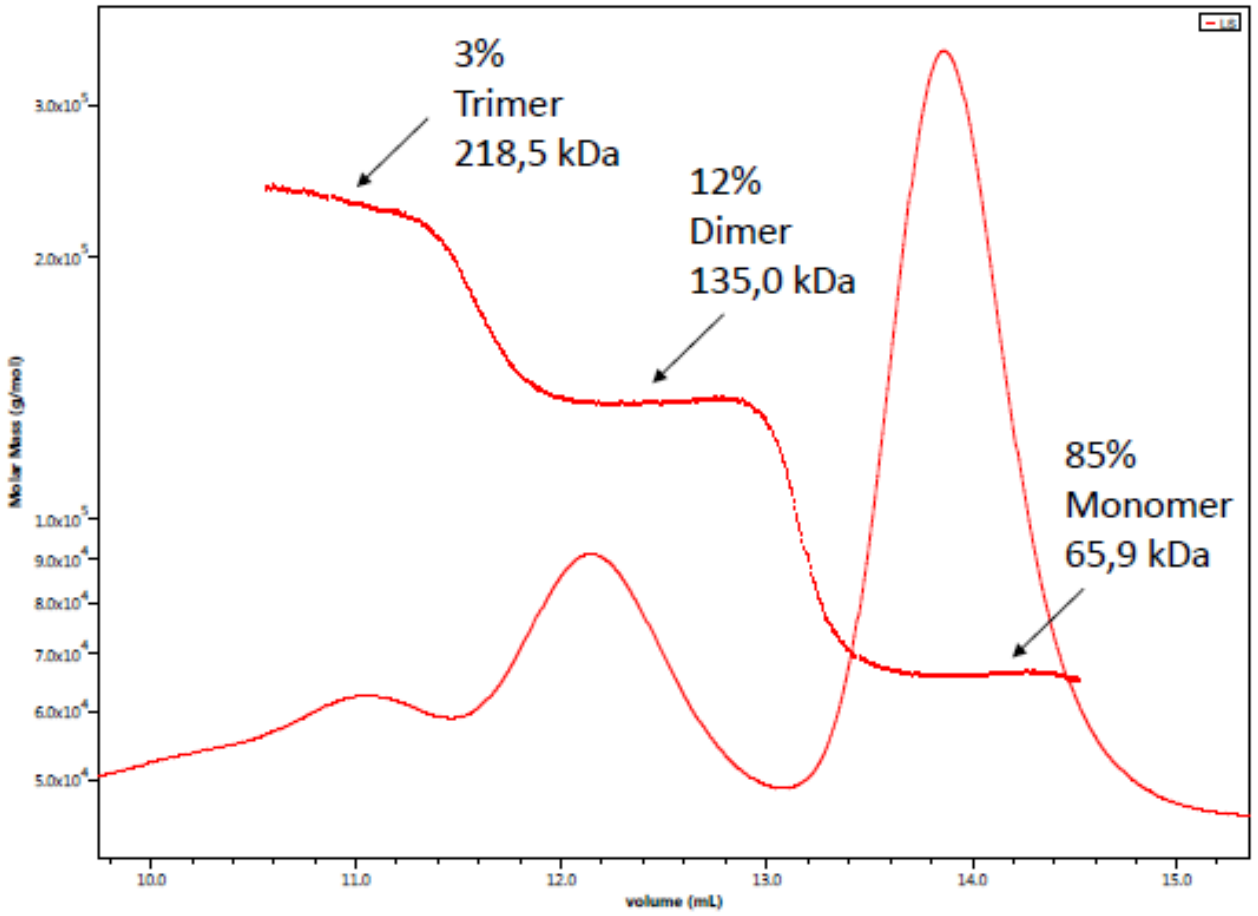
For **membrane proteins or Glycosylated proteins**
UV and RI detectors are used for concentration measurements in two orthogonal ways: Allows for deconvolution of mass contribution of two components (protein/detergent or protein/glycosylation)

Absorbance 280 nm → concentration of protein (ε_{prot})
Deconvolution of signal RI → used to calculate the amount and mass of detergent

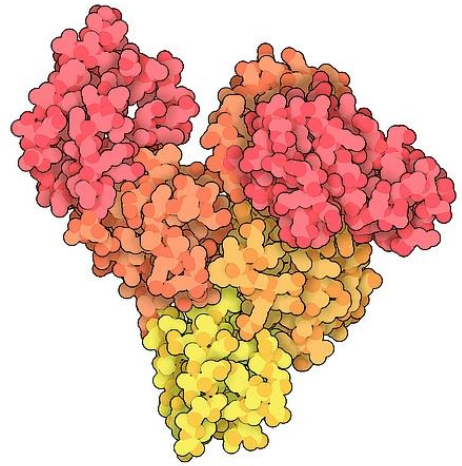


Analysis of Bovine serum albumin - BSA

- Example of the BSA (50uL at 2mg/mL injected on a KW 804 Shodex column)



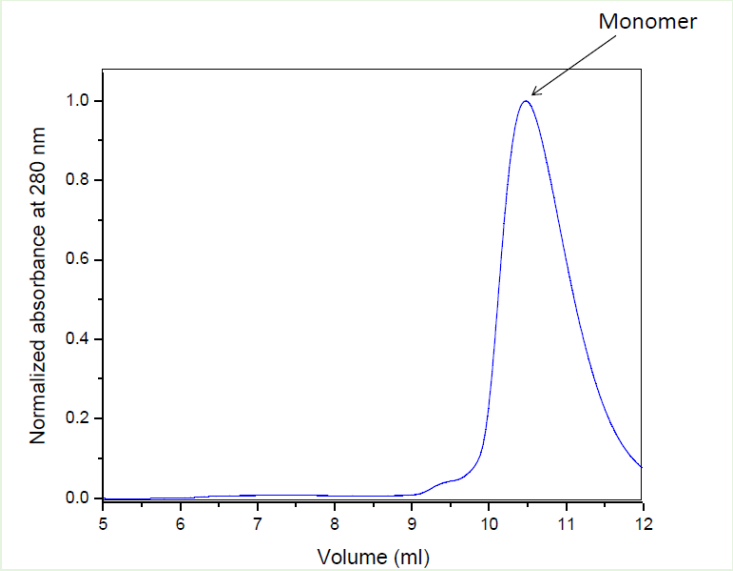
RI + LS => c + M
RI => *amount (µg) under each peak*
Input : $(\partial n/\partial c)$
UV *not used*



BSA molecular artwork, courtesy of Dr. David Goodsell.

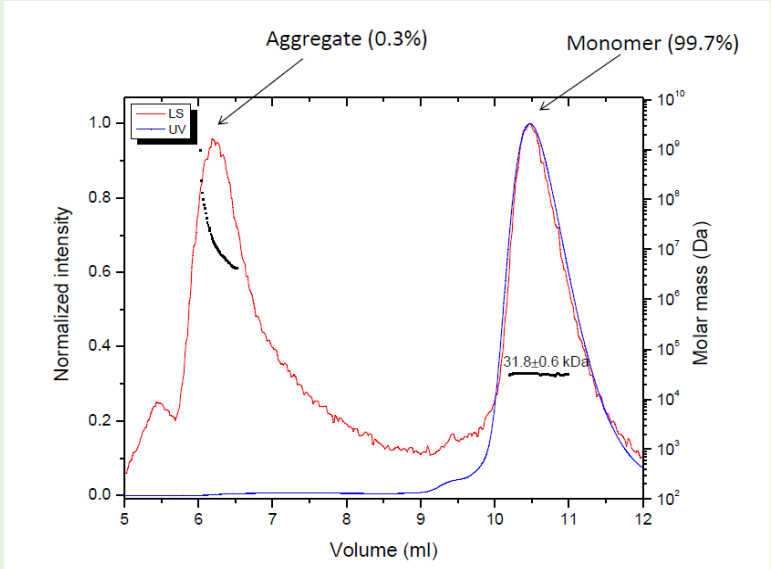
Detection of aggregates

SEC



- Separation by size
- Calculating *Mw* based on calibration curves of globular proteins
- Heterogeneity of a sample is undetectable

SEC- MALLS



- Separation by size
- Calculating *Mw* from the light scattering equations
- Calculate the *Mw* during the elution peaks, indicate homogeneity of a sample
- Detect low amount of aggregation : large molecules amplify the intensity of LS

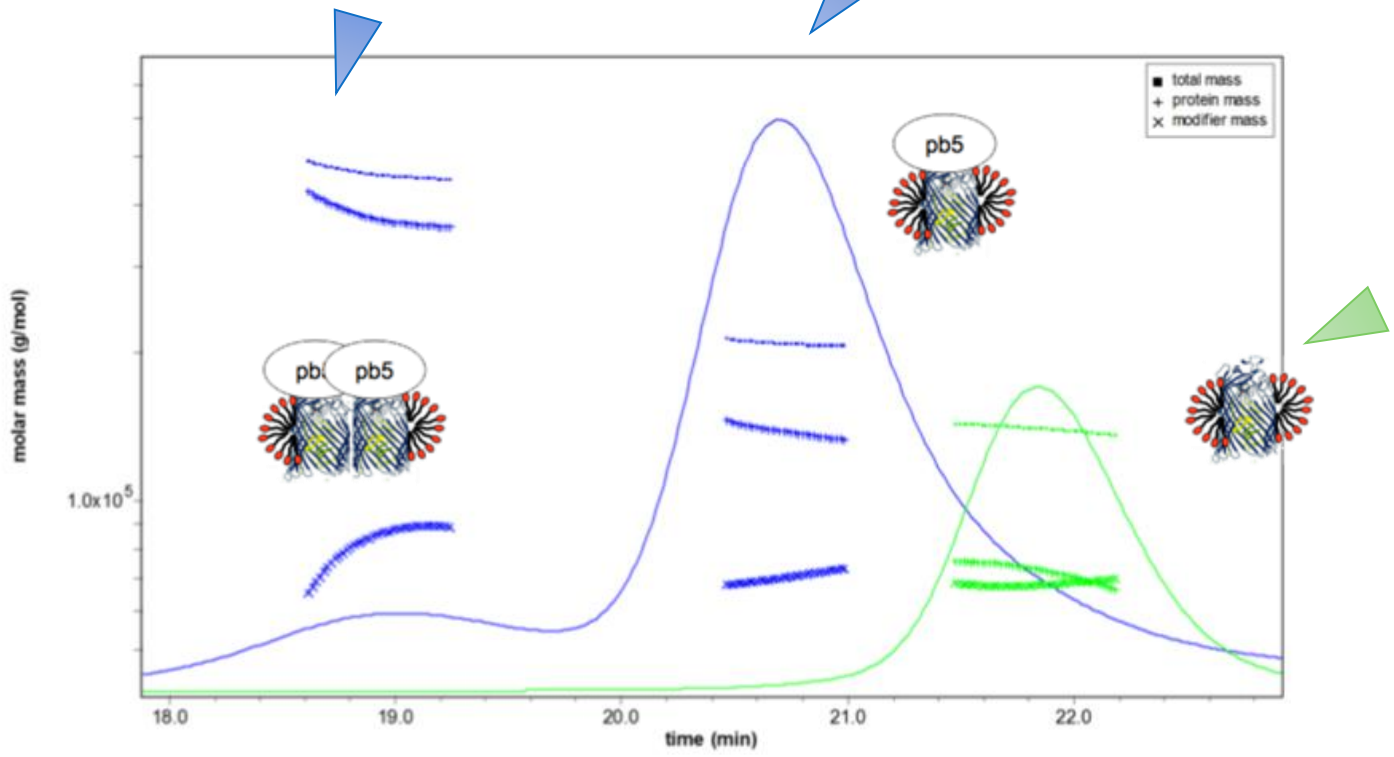
- Light scattering
- Absorbance 280 nm

Membrane protein

FhuA – pb5
M_p = 280 kDa
M_{det}= 85 kDa

FhuA – pb5 (main)
M_p = 140 kDa (theoretical 151kDa)
M_{det}= 70 kDa

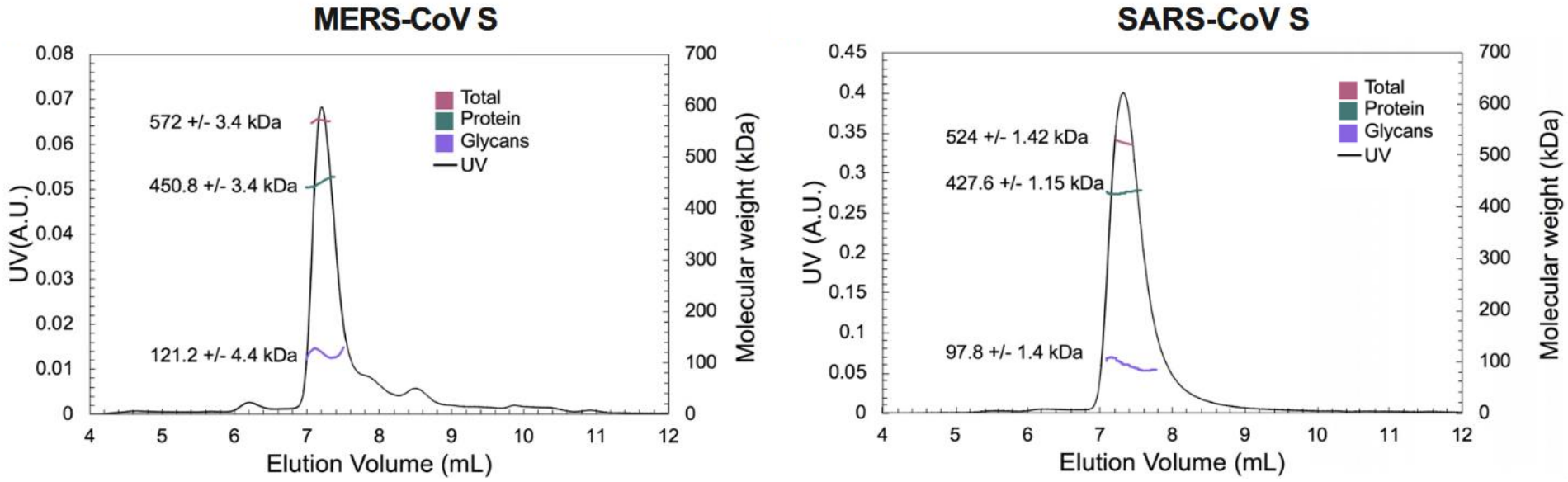
FhuA Ferrichrome transporter from *E. coli*. Pb5 receptor binding protein of the bacteriophage T5

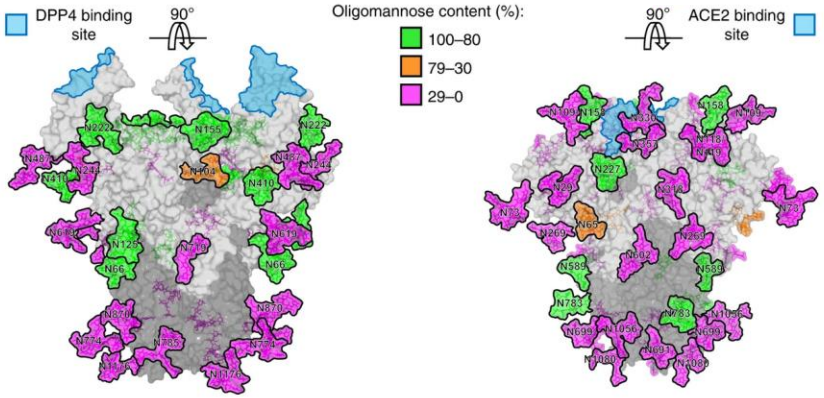


FhuA
M_p = 72 kDa (theoretical 80kDa)
M_{det}= 68 kDa

Christine Ebel

Glycosylated protein





Modelling of the experimentally observed glycosylation is illustrated on the prefusion structure of trimeric MERS S and SARS S glycoproteins.

Walls et al., Cell 2019
Watanabe et al., Nat Comm 2020

SEC-MALLS

- **LS + RI = > *M***
- For a two-component macromolecule (membrane protein, glycosylated protein)

Given extinction coefficients of the components are known:

Absorbance + LS + RI = > *M* of the partners within the complex

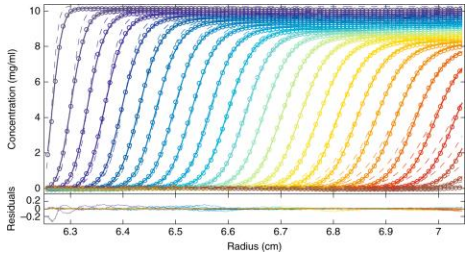
Advantages

- Simple and rapid (50 min for one sample)
- Absolute value of molecular mass
- Estimation of the polydispersity in a single chromatographic peak
- The column act as a filter and remove large aggregates

Limitations

- Uncontroled sample dilution upon elution
- Not adapted to weak complexes with fast dissociation
- Require the separation of the the various species upon elution

Analytical Ultracentrifugation - AUC



Analytical ultracentrifugation

Spinning and watching molecule transportation

Measures the rate of sedimentation of your molecule

Measures the concentration as a function of the radial position at various times of centrifugation

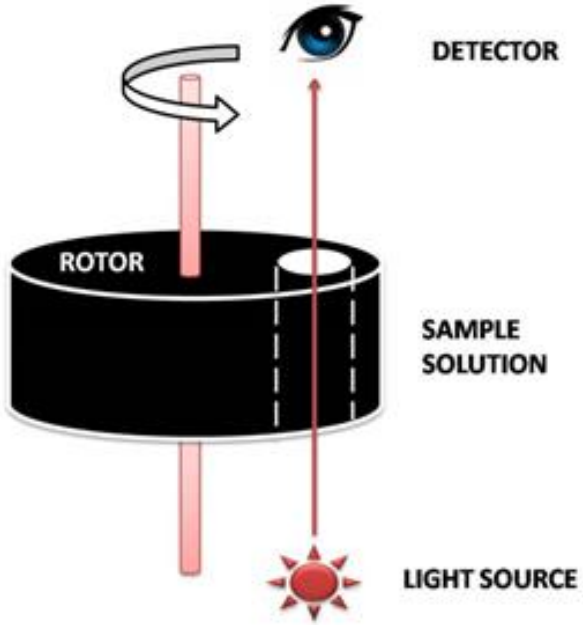
Centrifugal force: $F_c = m\omega^2 r$

m : *mass of the particule*

w : *60000 rpm*

r : *6-7cm*

➢ 300 000 g

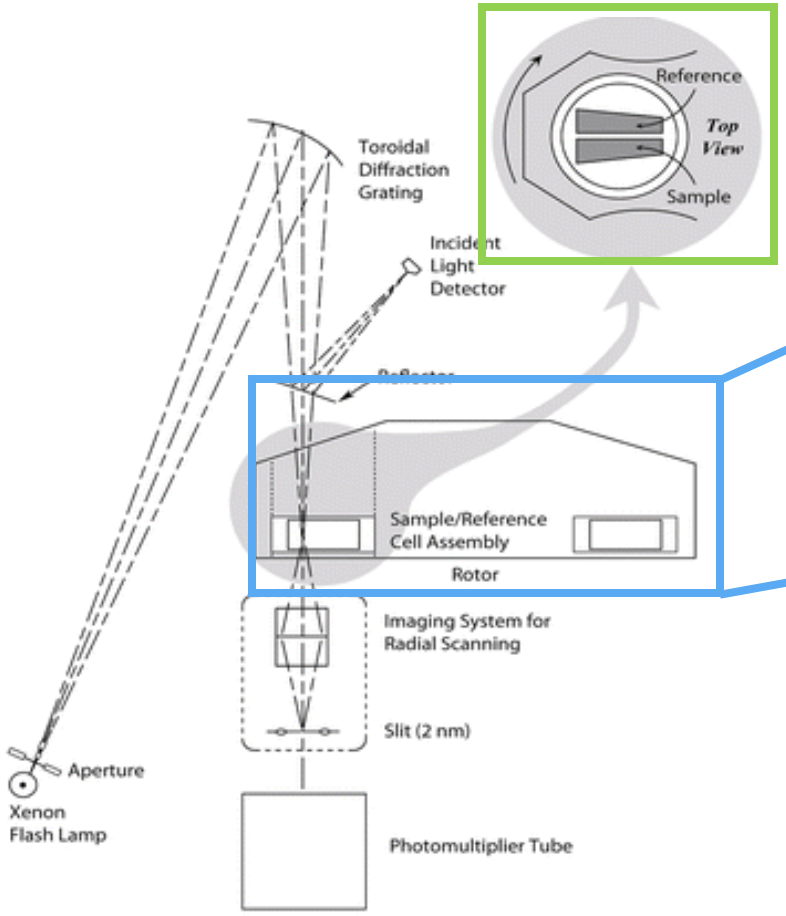


Applications

- Particle size distribution
- Particle composition
- Molecular weight distribution
- Shape factor
- Purity or heterogeneity
- Analysis of associating systems

Analytical ultracentrifugation

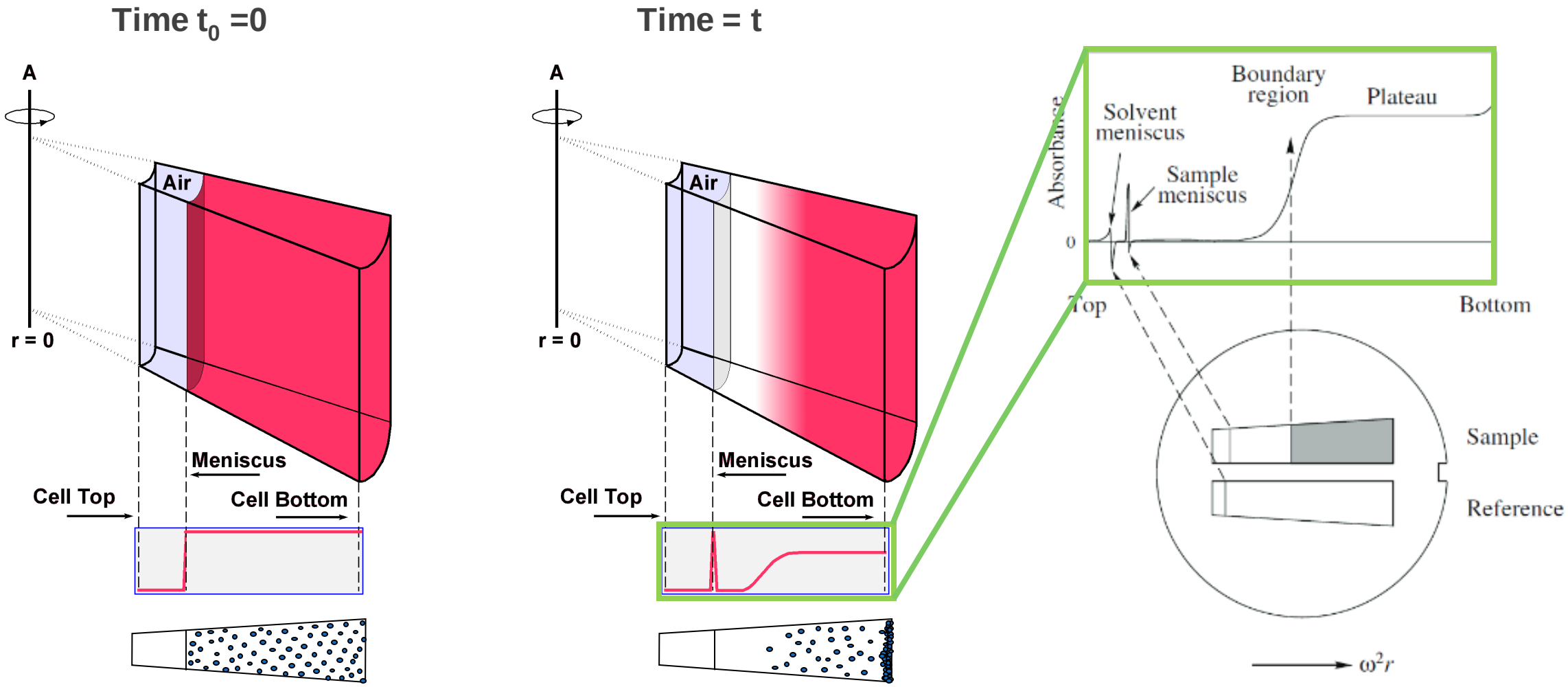
Spinning and watching molecule transportation



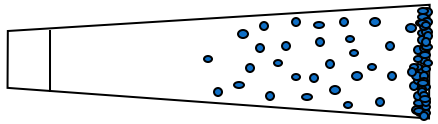
Path length (cm)	Volume (µL)	Required (µL)
1.2	400-420	450
0.3	100-110	150
0.15	50-55	75

Analytical ultracentrifugation

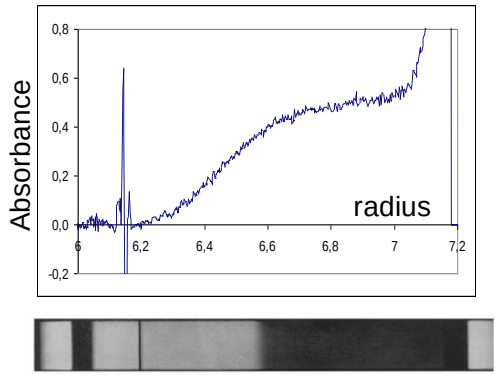
Spinning and watching molecule transportation



Optical system



Absorbance

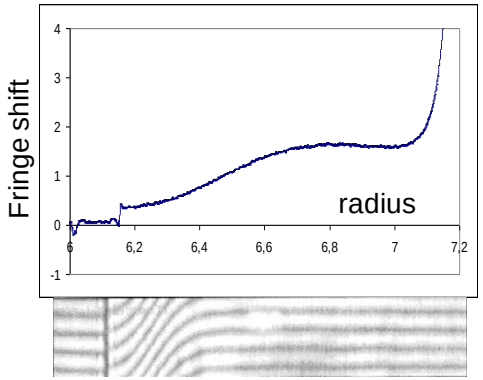


$A = E_{0.1\%}\, l\, c$

Selectivity depending on presence of chromophore

0.1-2 mg/mL typically
(60-500 µL)

Interference

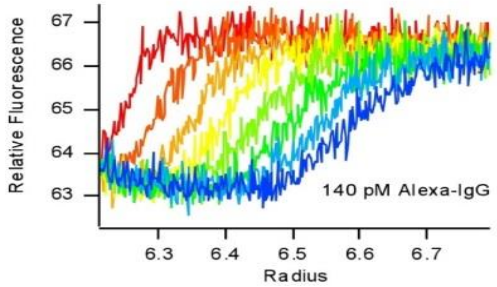


$\Delta J\ \alpha\ (\partial n/\partial c)\, l\, c$

Not selective
Measures everything
(detergent, glycerol…)

0.1- >10 mg/mL
(60-500 µL)

Fluorescence

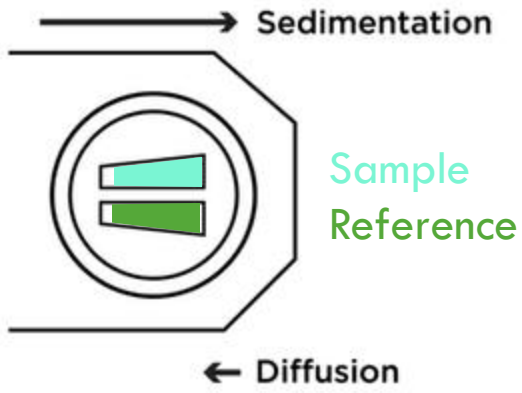


Signal in arbitrary units

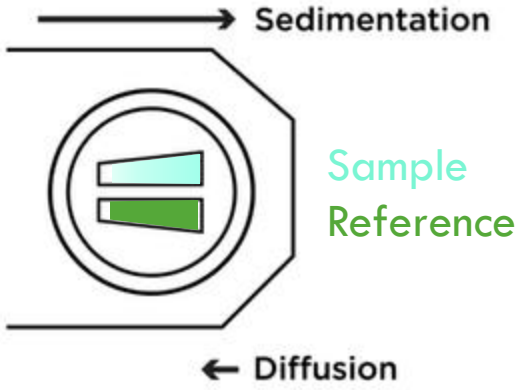
Highly selective
Requires fluorescent labeling

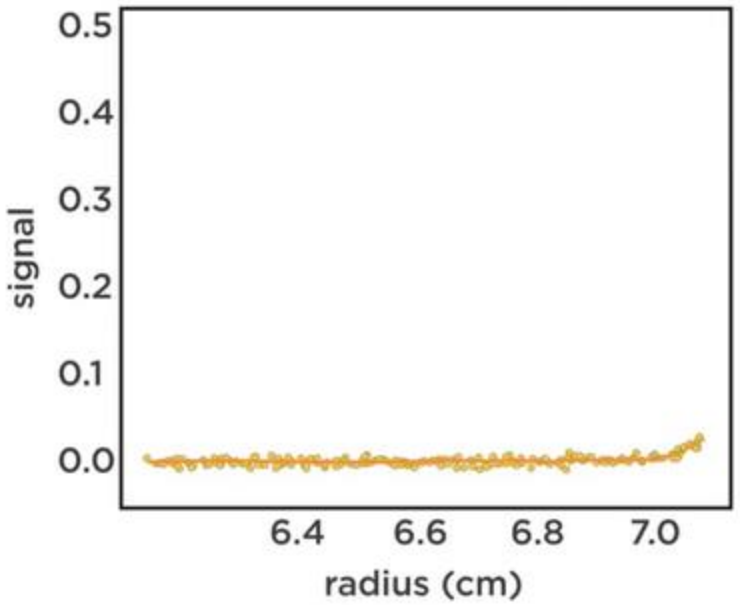
pM-µM
(500 µL)

Sedimentation velocity



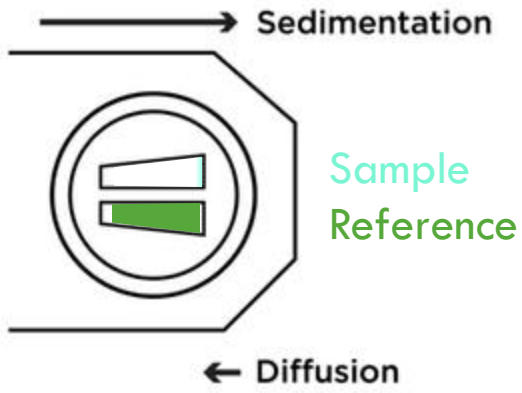
Sedimentation velocity

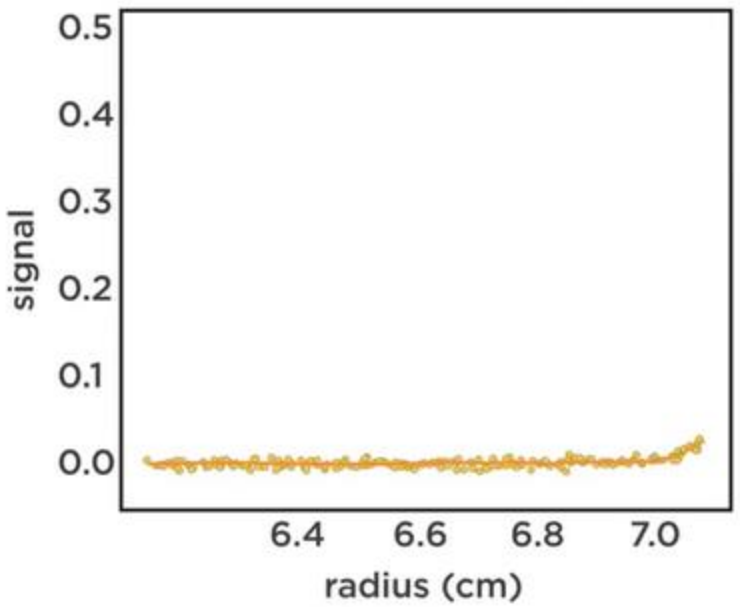




t= 0 min
t= 15 min
t= 30min
t= 1h
t= 3 h
t= 5 h
t= 6 h

Sedimentation velocity





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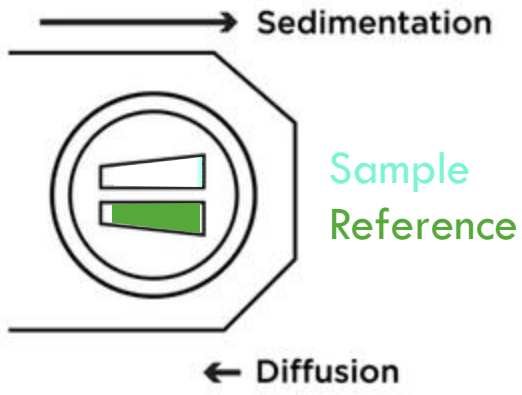
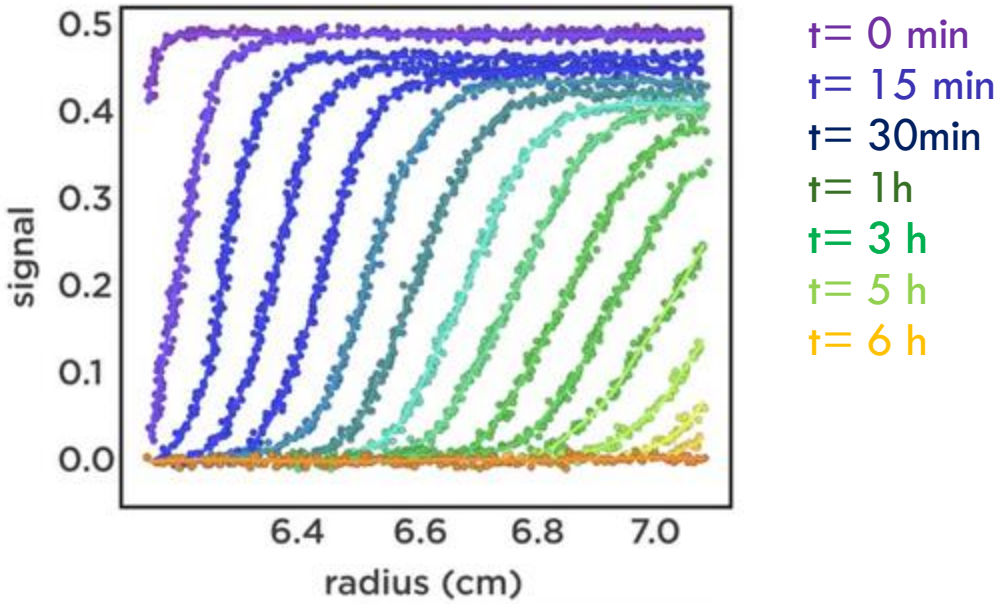
Sedimentation velocity





t= 0 min
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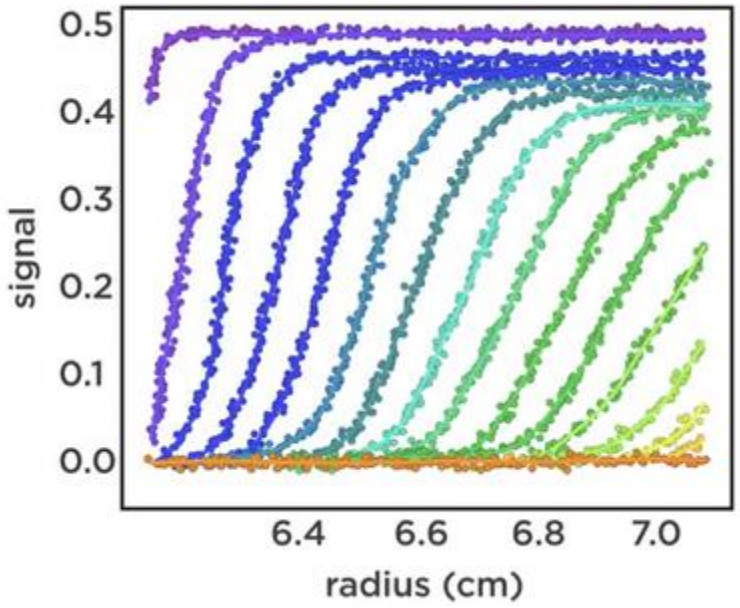
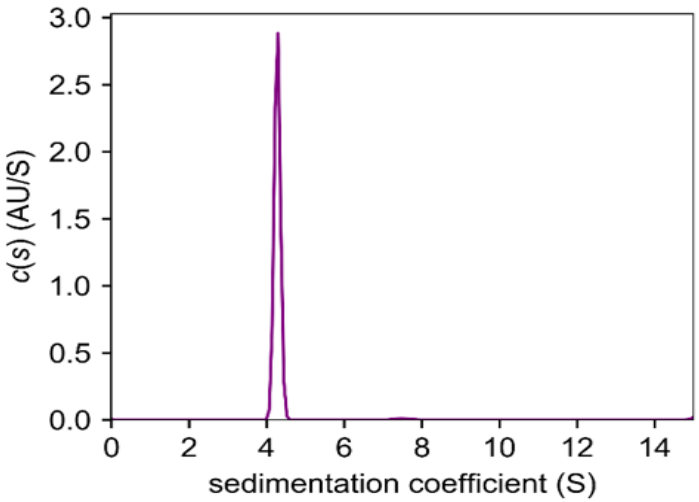
t= 6 h

Sedimentation velocity



Sedimentation profile

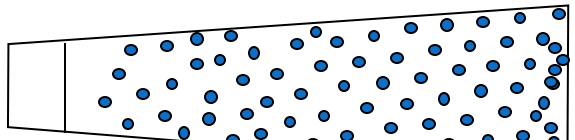
Data analysis

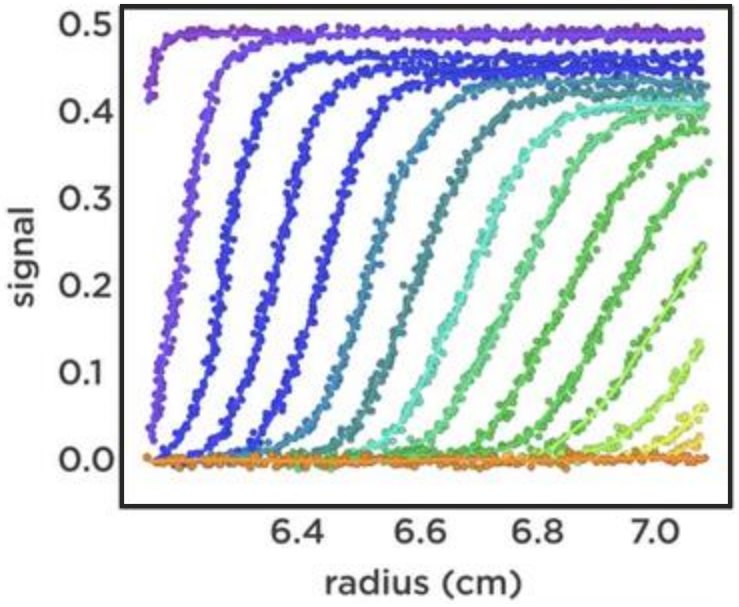


c(s) distribution

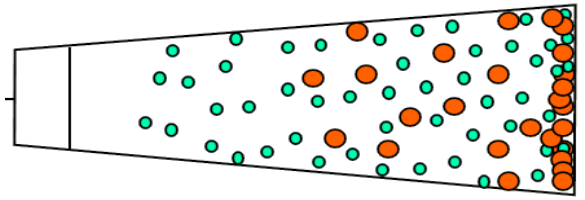
Sedimentation velocity

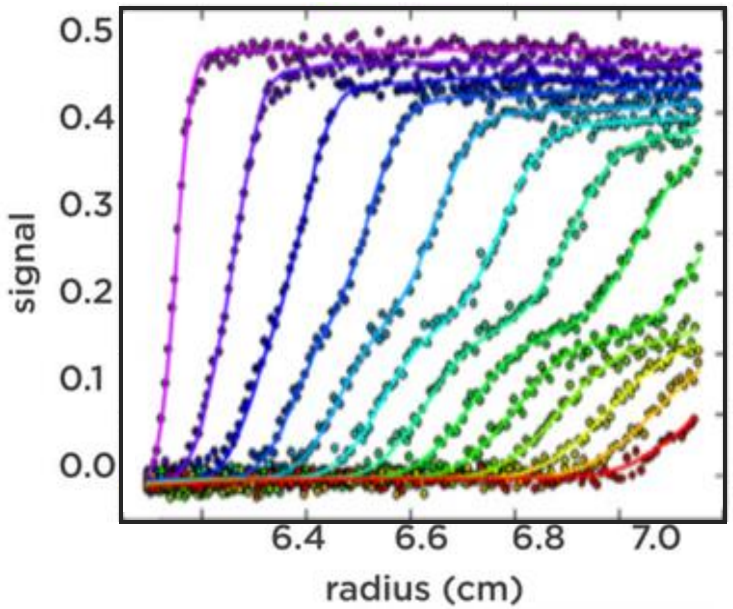
Single boundary



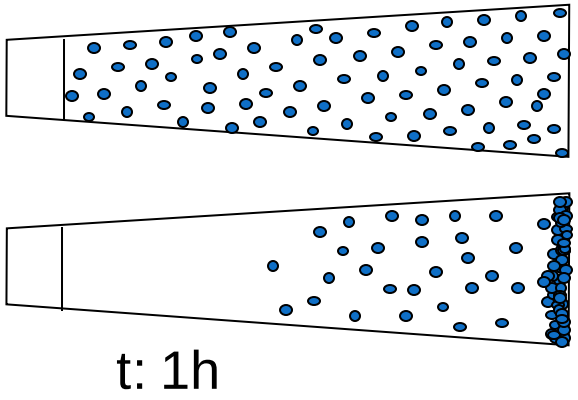


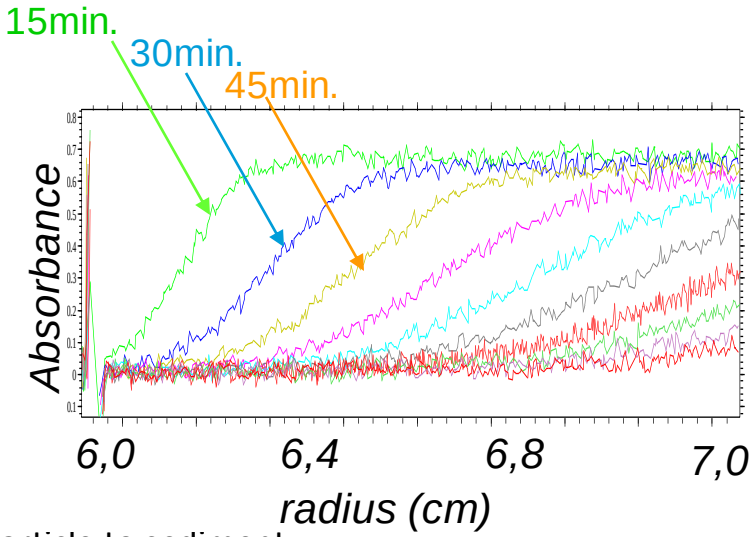
Multiple boundaries

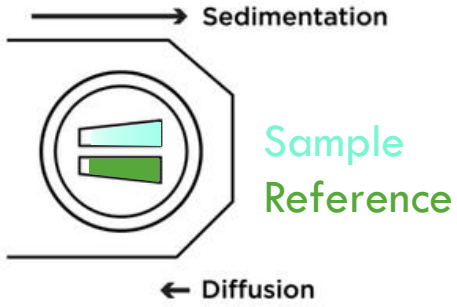




Sedimentation velocity







Angular velocity: Large compared to the ability of the particle to sediment (typically 42000 revs. per min)

Duration: Some hours

Analysis: Measures rate of sedimentation
As a function of time
Formation of a boundary

Sample: 420, 110 or 55 µl (l =12, 3 or 1.5 mm)

Information: Sedimentation coefficient (s), Frictional ratio f/fmin (shape), estimate Mw

AUC theoretical background

For a dilute homogeneous sample

Fc : centrifugal force

$$F_c = m\omega^2 r = \frac{M}{N_A}\omega^2 r$$

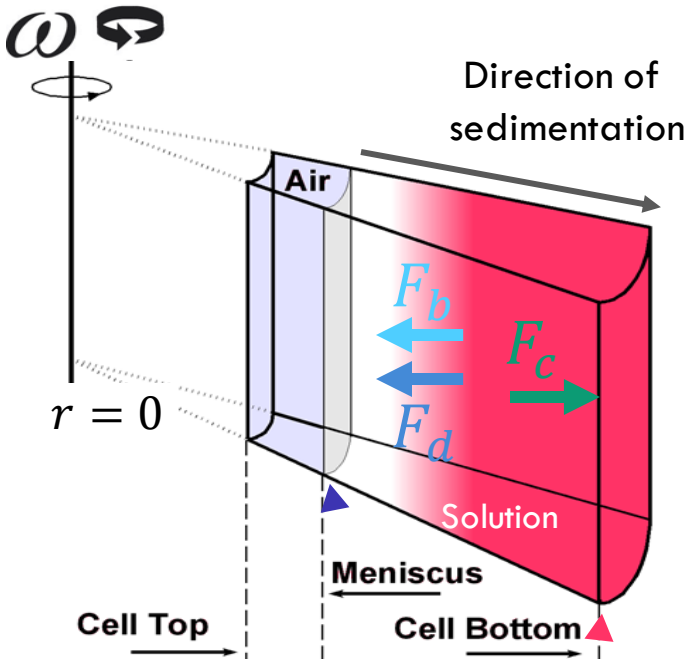
Fd : Viscous drag

$$F_d = -fv$$

Fb : buoyant force

from Archimedes' principle, is equal to the weight of fluid displaced

$$F_b = -m_0\omega^2 r = -(m\bar{v}\rho)\omega^2 r = -\left(\frac{M}{N_A}\bar{v}\rho\right)\omega^2 r$$



ω	angular velocity
r	distance to the axe of rotation
m	mass of the particle
M	molar mass
f	frictional coefficient, which depends on the shape and size of the particle
v	velocity

m_0	mass of fluid displaced by the particle $m_0 = m\bar{v}\rho = \frac{M}{N_A}\bar{v}\rho$
ρ	density of the solvent (g/mL)
$\bar{v}$	partial specific volume, is the volume that each gram of the solute occupies in solution (the inverse of its density)

AUC theoretical background

$$F_c + F_b + F_d = 0$$

$$\frac{M}{N_A}(1 - \bar{v}\rho)\omega^2 r - fv = 0$$

$$\frac{M(1 - \bar{v}\rho)}{N_A f} = \frac{f}{\omega^2 r} = s$$

Within a very short time
the 3 forces come into balance

Sedimentation coefficient s :
velocity of the particule per unit of gravitational acceleration

- measurement

AUC theoretical background

$$F_c + F_b + F_d = 0$$

The Svedberg equation

$$s = \frac{M(1 - \bar{v}\rho)}{N_A 6\pi\eta R_h}$$

sedimentation coefficient
s in Svedberg (S)
1 S = 10^{-13} s

R_h hydrodynamic radius
η solvent viscosity
f frictional coefficient of a molecule depends on the size of the particule, it is proportional to the hydrodynamic radius R_h

$$f = 6\pi\eta R_h$$

- Interpretation : macromolecular parameters

AUC theoretical background

mass relative density

$$s = \frac{M(1-\bar{v}\rho)}{N_A 6\pi\eta R_h}$$

M_b buoyant mass

Sedimentation coefficient velocity of the particles

shape, viscosity

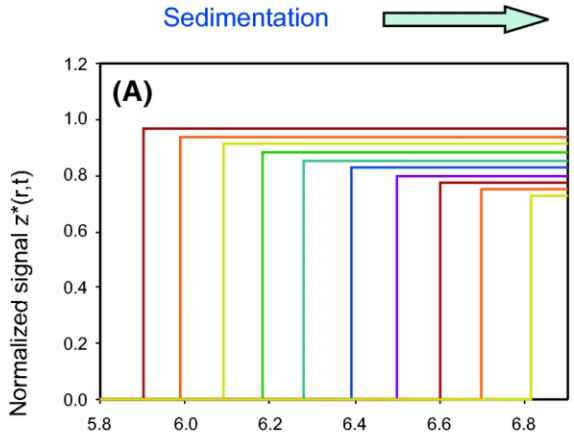
ρ	density of the solvent (g/mL)
$\bar{v}$	partial specific volume
D	translational diffusion coefficient
M_b	buoyant molar mass
f	frictional coefficient
f_{min}	frictional coefficient for a compact, spherical particle
f/f_{min}	frictional ratio
R_H	hydrodynamic radius
R_{min}	hydrodynamic radius for a compact, spherical particle

Spreading friction

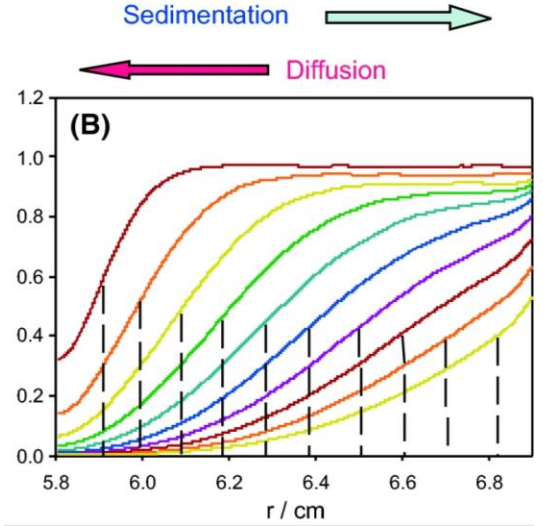
$$D = \frac{RT}{N_A f}$$ Stoke's Law

$$R_h = f/f_{min} R_{min}$$

frictional ratio *f/fmin*



Theoretical progress of the boundary if no diffusion had occurred



In real, we see diffusion against the centrifugal force, so the end results in a series a sigmoidal curve

- The steepness of the boundaries give information on the frictional ratio f/fmin
- Non-globular or elongated particles with higher frictional coefficients experience greater drag, which reduces the rate of diffusion and therefore reduces boundary spreading

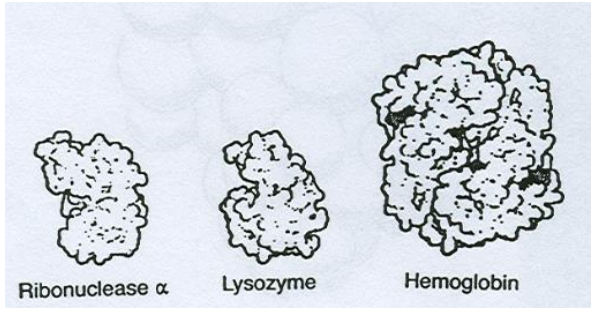
García de la Torre et al., 2018

Approximate Values of Partial Specific Volumes $\overline{v}$ and frictional ratios $f/fmin$

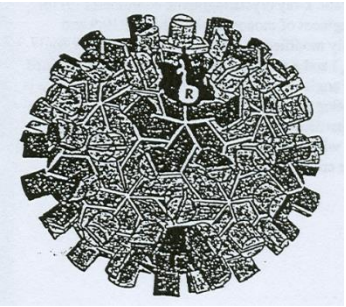
$\overline{v}$ (ml/g)

Protein: ≈ 0.74
Sugar: ≈ 0.62
DNA, Na^+: ≈ 0.54
DDM: 0.82
LAPAO: 1.067
lipid: ≈1
H_20: 1

$f/fmin$

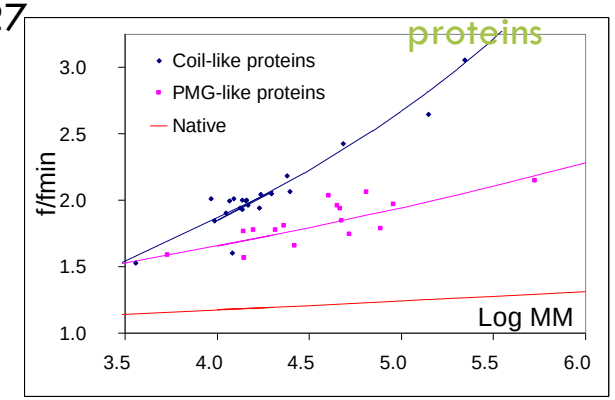


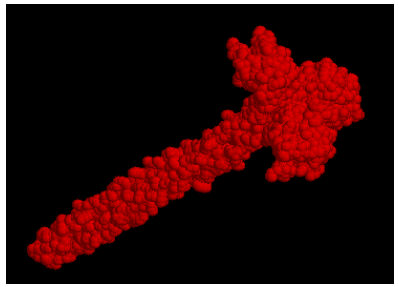
f/f_{min}:1.29 1.22 1.28



1.27

Unfolded proteins

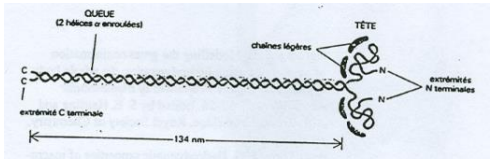




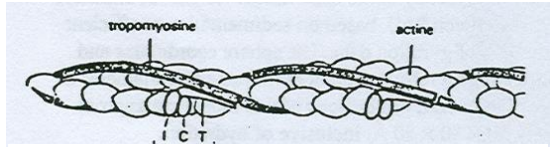
Langerin ECD f/f_{min}=1.8

Globular compact macromolecule
f/f_{min}≈1.25

Glycosylated protein:
typical $f/fmin$ = 1.5-1.8

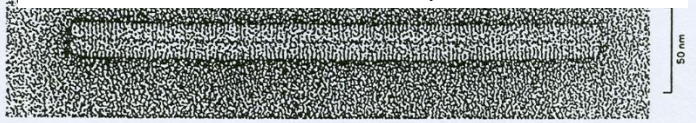


Myosin tail: f/f_{min}=3.63



tropomyosin: f/f_{min}=2.65

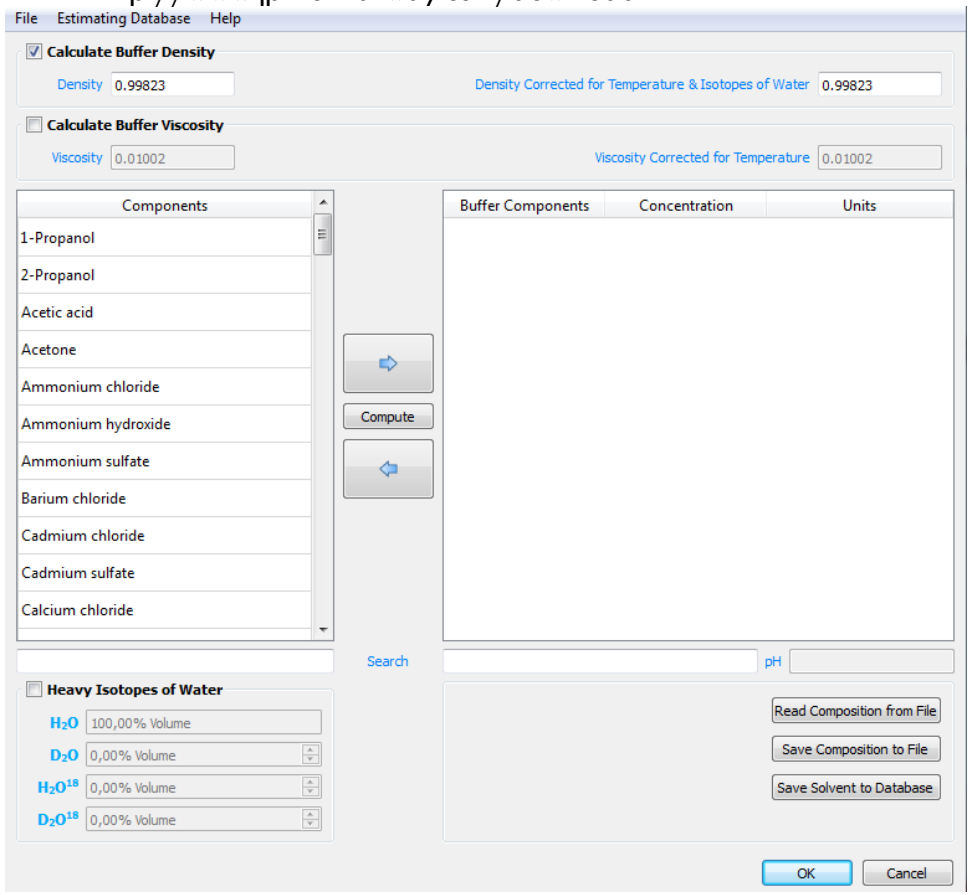
tobacco mosaic virus : f/f_{min}= 2.65



ρ, η : Density and viscosity of the solvents

calculated by SEDTERP / UltraScan

http://www.jphilo.mailway.com/download.htm



Or measured..

The Lamm equation

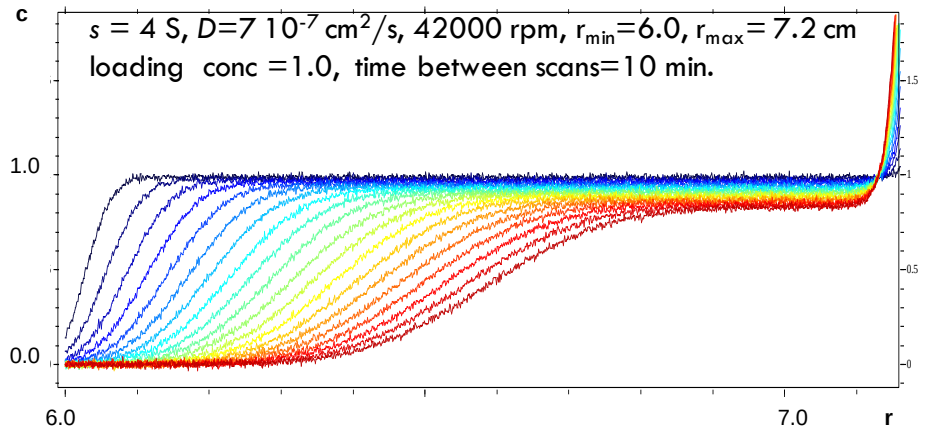
- Equation for the change in concentration over change in time
- Used to predict what the boundary shapes will look like

$$\frac{\delta C}{\delta t} = -\frac{1}{r}\left\{\frac{\delta}{\delta r}\left[\omega^2 r^2 s C - D r \frac{\delta C}{\delta r}\right]\right\}$$

Sedimentation flux Diffusion flux

The Lamm equation allows to **calculate the sedimentation concentration profile c(*r*,*t*)** for a **single component** with a sedimentation coefficient ***s*** and a diffusion coefficient ***D*** at a given angular velocity ω

s, *D* → c (*r*,*t*)



The Lamm equation

The Lamm equation can not be solved analytically, but it can be solved **numerically**. Computer programs like **SEDFIT** developed by P. Schuck fit the experimental data, by considering a finite number of solutions (50-250) and by applying a non linear least square analysis (or regularization process).

$$c(r,t) = \int c(s)\, f(s, D, r, t)\, ds \approx \sum c_n(s) f(s_n, D_n, r, t)$$

- $c(r,t)$: Experimental data for multiple components
- $c(s)$: Concentration of each specie
- $f(s, D, r, t)$: Lamm equation for one specie
- $c_n(s) f(s_n, D_n, r, t)$: Calculated data with *n* species defined by c, s and *D*

Different models are available, including:

- c(s) model: **diffusion + sedimentation** taken into account (proteins, small molecules)
- ls-g*(s): **Only sedimentation taken into account** (nanoparticles > 20 nm)

$s + D$ =>> M_b/R_H, R_H

www.analyticalultracentrifugation.com

The c(s) analysis

uses the simulation of the sedimentation for hundreds of particles

c(s) sedimentation coefficient distribution, fixes a reasonable relation between *s* and *D*.
Input : f/f_{min}, partial specific volume, i.e. same shape and density for all particles.

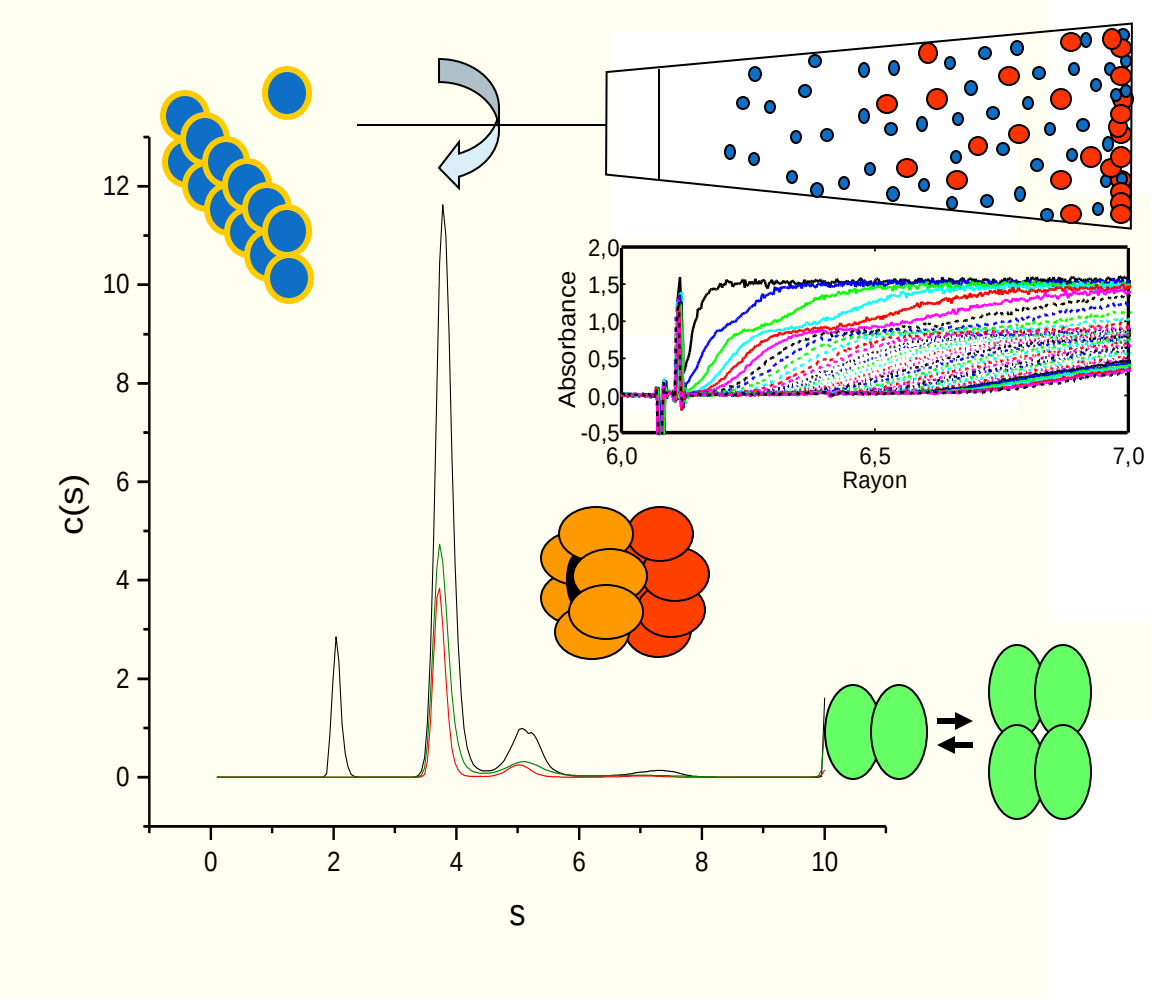
It allows deconvoluting boundary spreading for a high resolution distribution of *s*.

From peak integration

- *s* value
- signal (Absorbance, interference fringe shift…) related to concentration

•***M* can be determined** for samples with non-interacting species, from the analysis of the whole SV-profiles, where ***s* and *D*** are determined independently

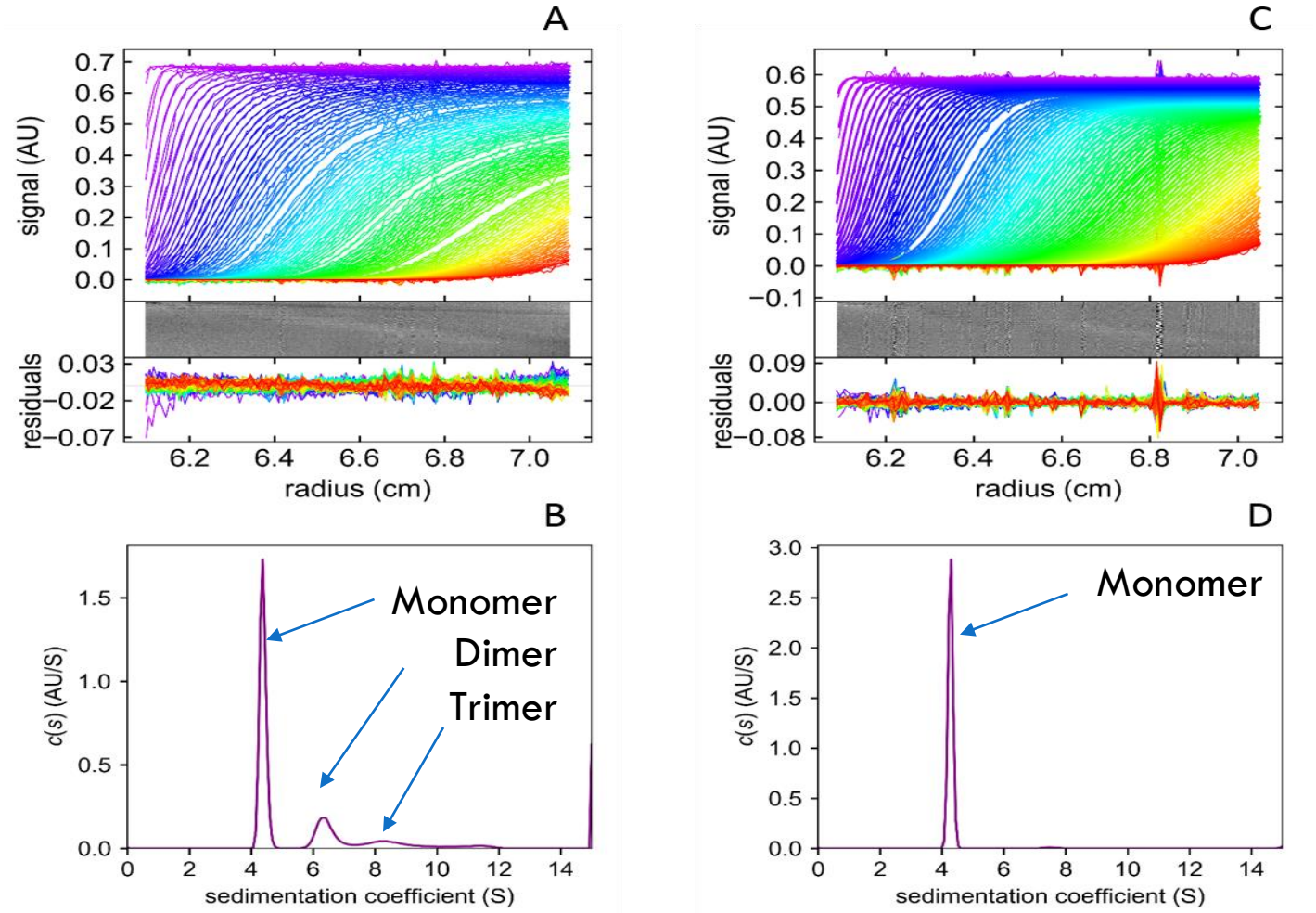
• **Numerical simulation** can describe complex interacting systems



BSA SV-AUC Sample homogeneity

Lyophilized BSA

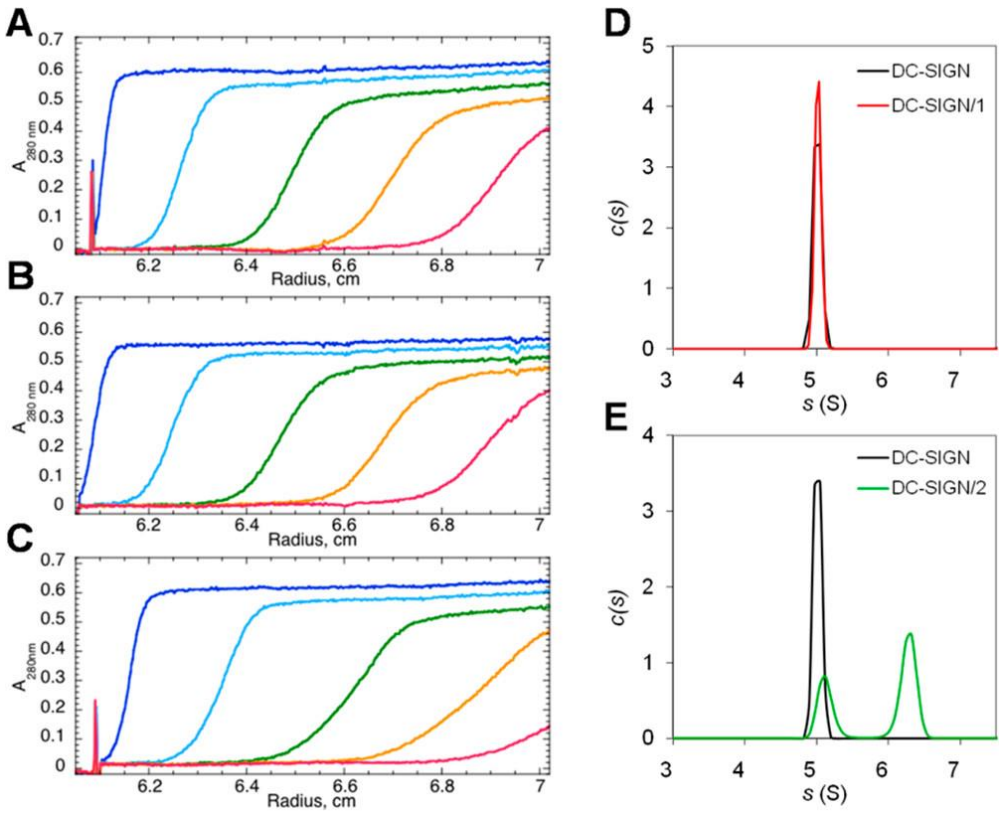
After purification of the monomer with size exclusion chromatography

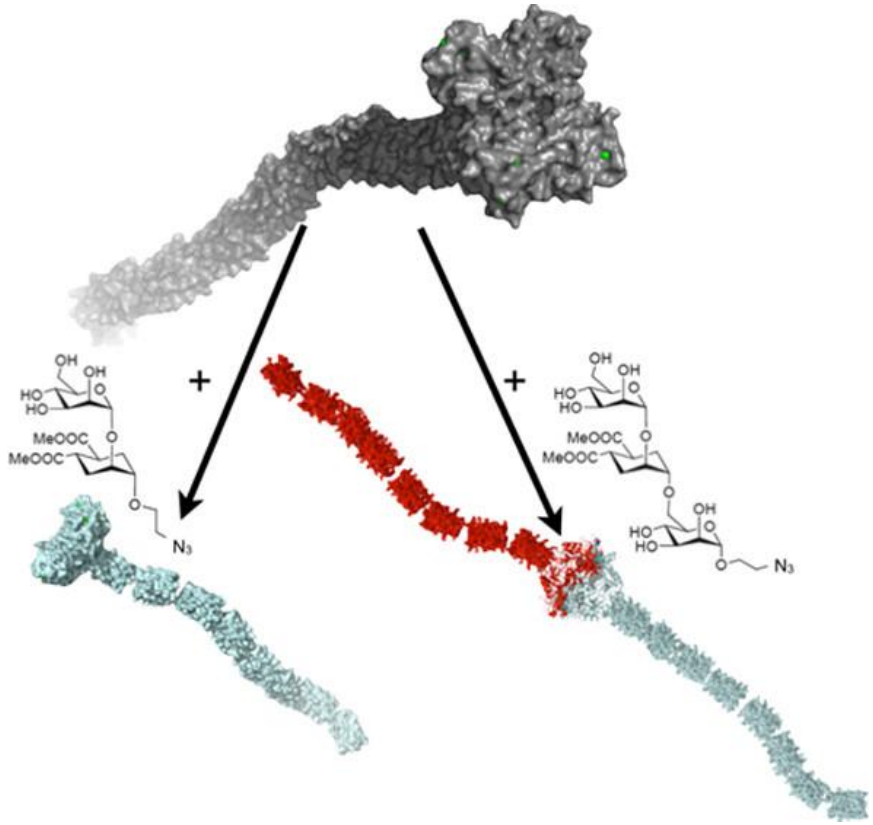


M = 65.9 kDa (theo: 65.5kDa)
s = 4.3 S
Rmsd = 0.009

Aline Le Roy & Christine Ebel

DC-SIGN -drug designed glycomimetic compound interactions





From ITC; AUC, DLS: molecule #2 is able, without any multivalent presentation, to cluster DCSIGN tetramers.

Sutkeviciute et al ACS Chem Biol 2014

Samll Angle X-ray Scattering - SAXS

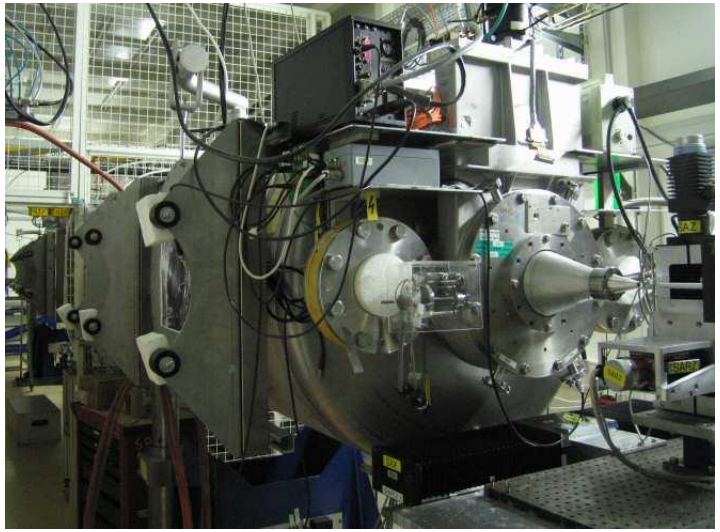
Small angle scattering

D22, ILL

Small angle neutron scattering

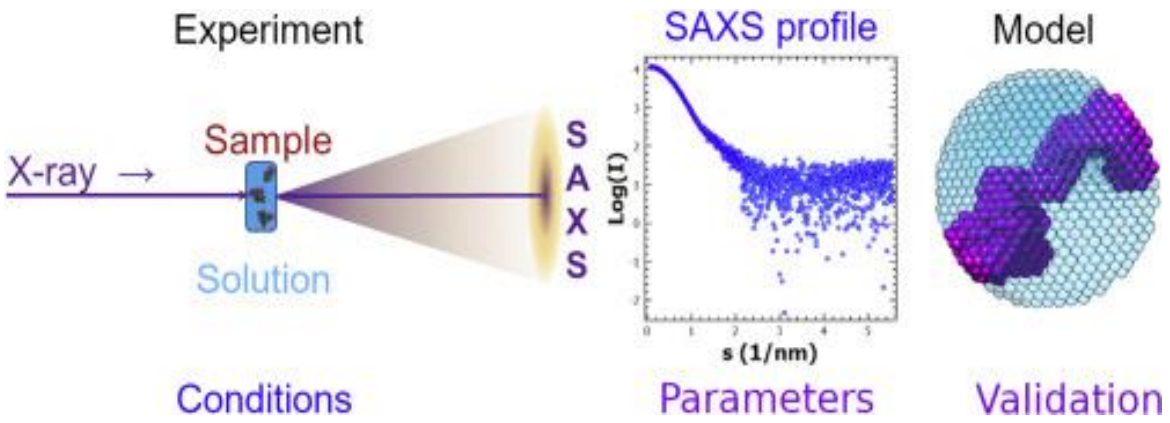
ID02, ESRF

BM29 BioSAXS



Small angle X-rays scattering

SAXS data for structural information



The scattering is typically isotropic and a radial averaging of the 2D scattering pattern yields a 1D intensity curve

The signal comes from macromolecules themselves but also from the buffer

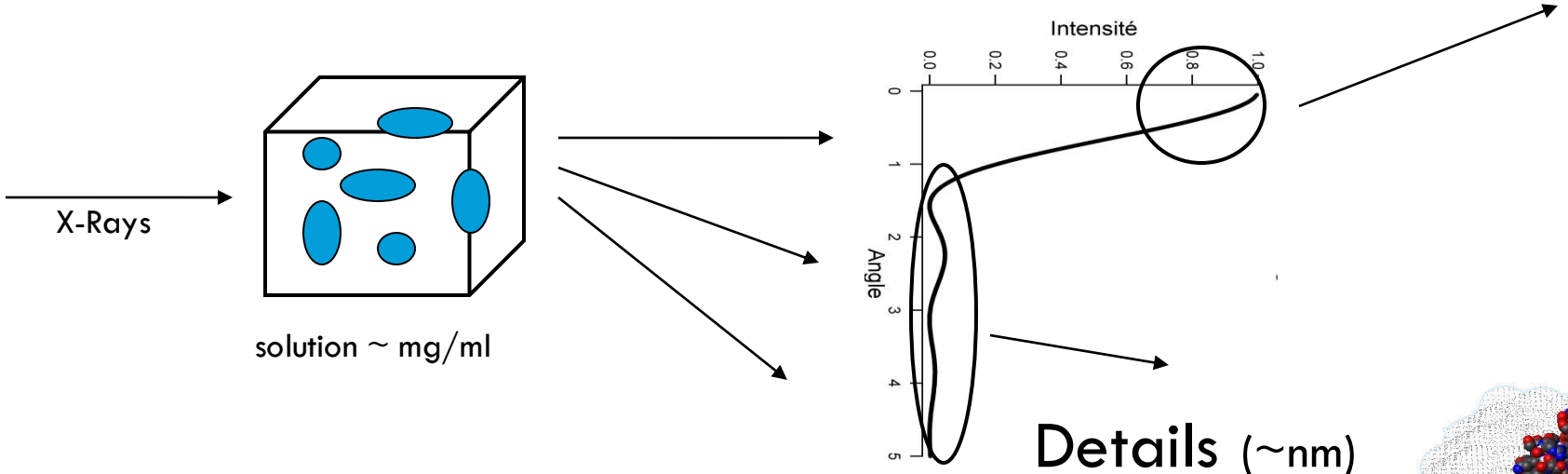
The subtracted SAXS profile yields the intensity from the macromolecules as a function of the scattering angle

The scattered intensity is a reflection of the structure of the macromolecule of interest for dilute solutions, if contributions from aggregates, self-association or positional correlations between different molecules are neglectable

Requirements

- monodisperse sample in solution
- There should be no correlation between particle positions and orientations
- c=1-10 mg/mL
- The scattered intensity depends on a contrast term between the particle and the solvant.
- Buffer signal will be substracted

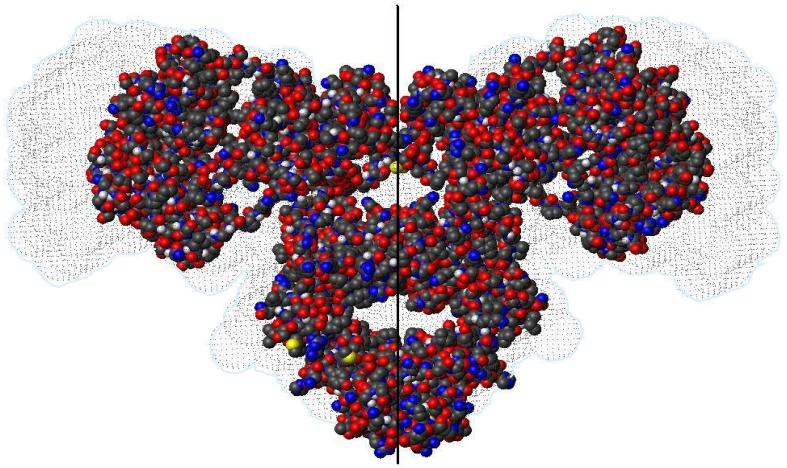
SAXS data for structural information



global properties

Radius of gyration,
Molecular weight
Particle composition..

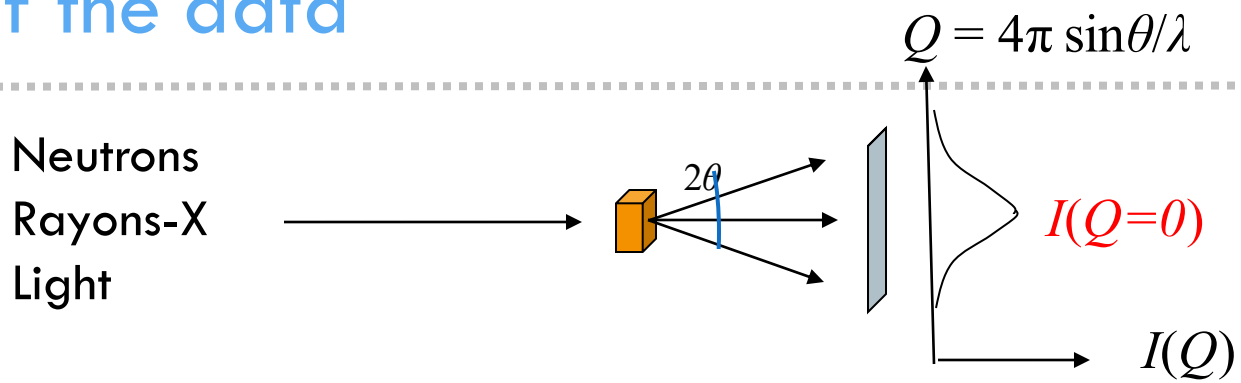
Details (~nm)



Low resolution structure

Antibody SAS vs. crystallography
Gabel et al., *J. Mol. Biol.*, 2006

Analysis of the data



For a dilute homogeneous solution

Forward intensity $I(0) = n \,.\, F(0)^2$

$$\underline{Neutrons} \quad I(0)/c = 1/N_A \,.\, M \,(\partial\rho_N/\partial c)^2$$

$$\underline{X\text{-}Rays} \quad I(0)/c = 1/N_A \,.\, M \,(\partial\rho_{el}/\partial c)^2$$

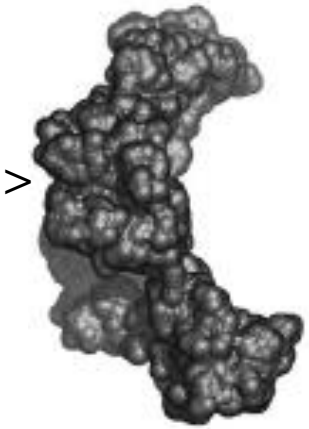
$$\underline{Light} \quad I(0)/c = 1/N_A \,.\, M \,(\partial n/\partial c)^2$$

Extrapolated *I*(0)
molar mass via a contrast term
that also determines the whole *I*(Q)

I(Q) changes at *small angle*
=> Radius of gyration

Whole I(q)
⇒Ensemble of the distances
within the particle P(r)

=>



$I(0)$: normalized forward intensity.
c: weigth concentration.
ρ_N and ρ_{el}°: neutron and electron scattering length density increments.
n, refractive index

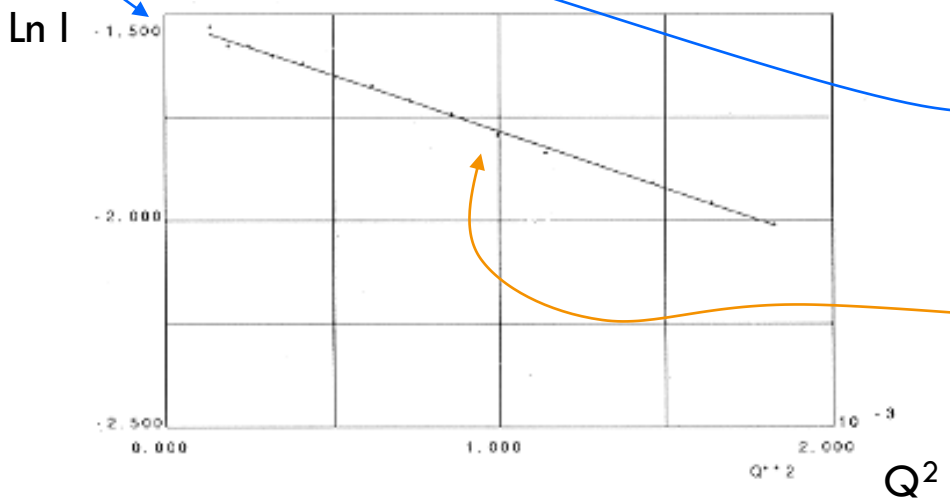
From the scattering curve to structural informations

Small* Q -range: the Guinier approximation

$$I(Q) = I(0)\exp -1/3\, R_G{}^2 Q^2$$

$$\ln I(Q) = \ln I(0) - 1/3\, R_G{}^2 Q^2$$

Intercept



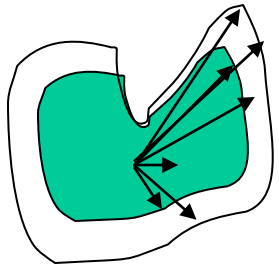
Slope

Q^2

*: small??? such as $R_G Q \ll 1$ in principle and for spheres;
$R_G Q < 1.3$ for globular non spherical particles.

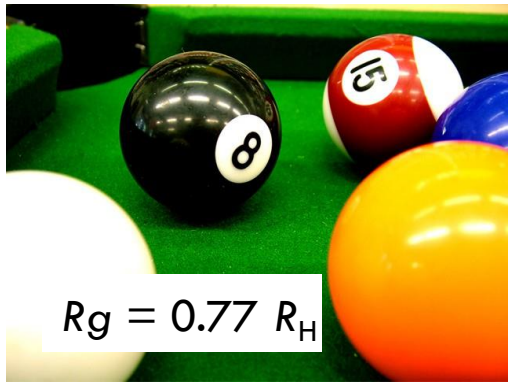
Rg from scattering

Rg tells about mass distribution around center-of-gravity (inertia)
Rg will tell about conformation changes
Rg is ponderated by scattering length density contrast



$$R_g^2 = \int r^2 g(r) dr$$

g(r): pair distance function

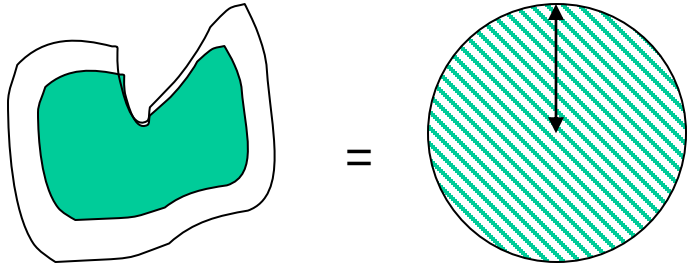


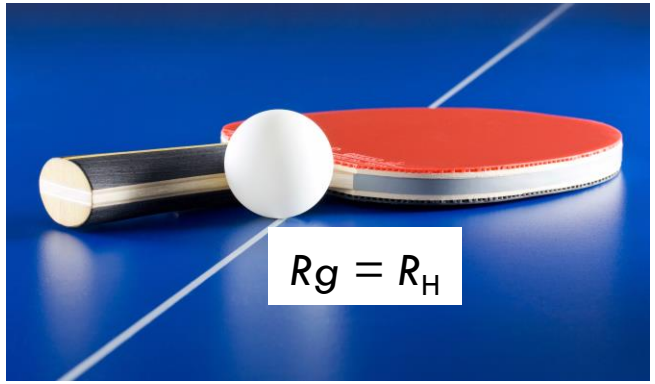
$Rg = 0.77\ R_H$

Rg differs slightly in SANS in D_2O and H_2O, and in SAXS, because it probes hydration

R_H from hydrodynamics

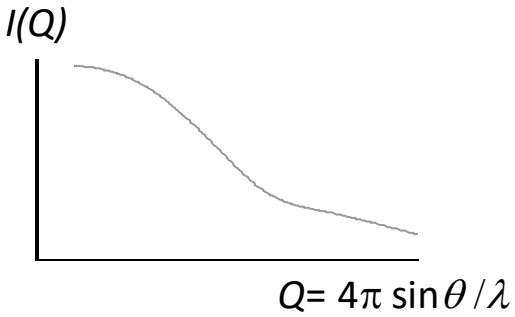
R_H probes distances to the surface (approximatively)





$Rg = R_H$

Pair distance distribution - p(r) from the whole scattering curve



Reciprocal space

F.T.$^{-1}$

Fourier Transform

p(r)

Pair distribution function

Real space

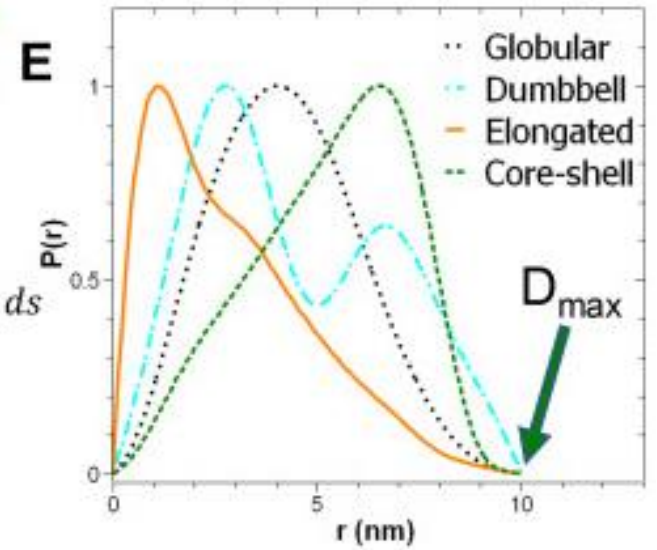
Pair distance distribution

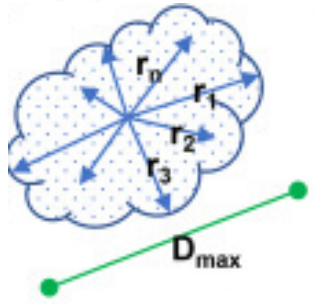
Real-space transformation of the data, conveys information on **shape** and **size** of the macromolecule

$$P(r) = \frac{1}{2\pi^2}\int_0^\infty I(s)\cdot sr\cdot \sin(sr)\,ds$$

P(r) related to the probability of finding two points at distance ***r*** within the macromolecule

P(r)



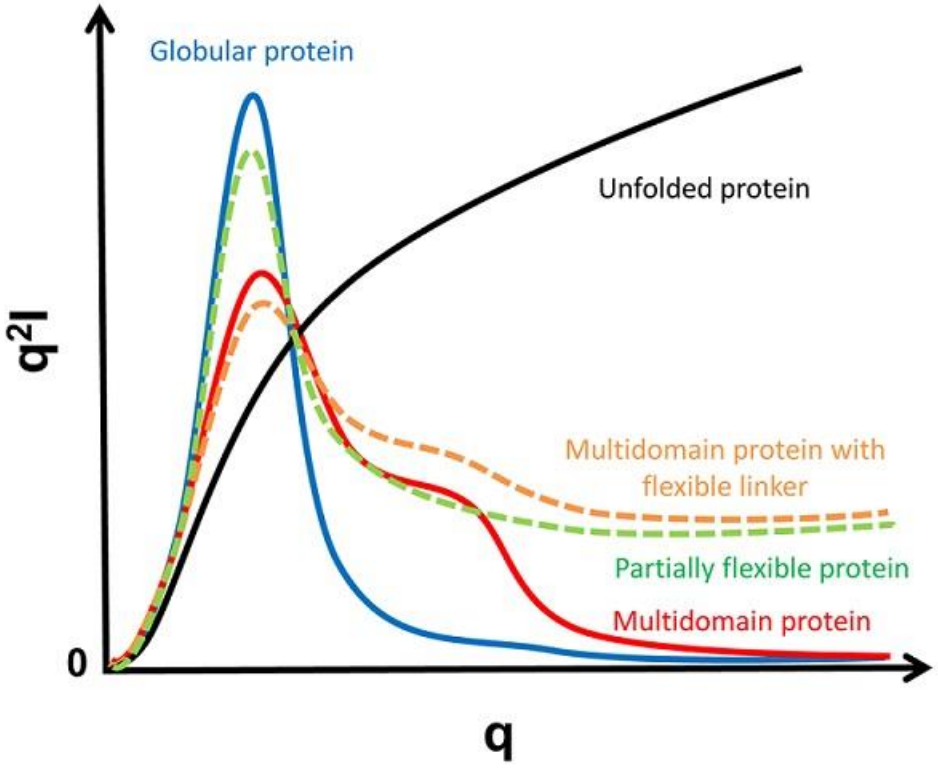


The Kratky plot

Considering larger angles

Kratky plot identifies unfolded samples:

- Globular macromolecules follow Porod's law have a bell shape curve
- Extended molecules, as such unfolded proteins, lack this peak and have a plateau or are slightly increasing in the larger *q* range

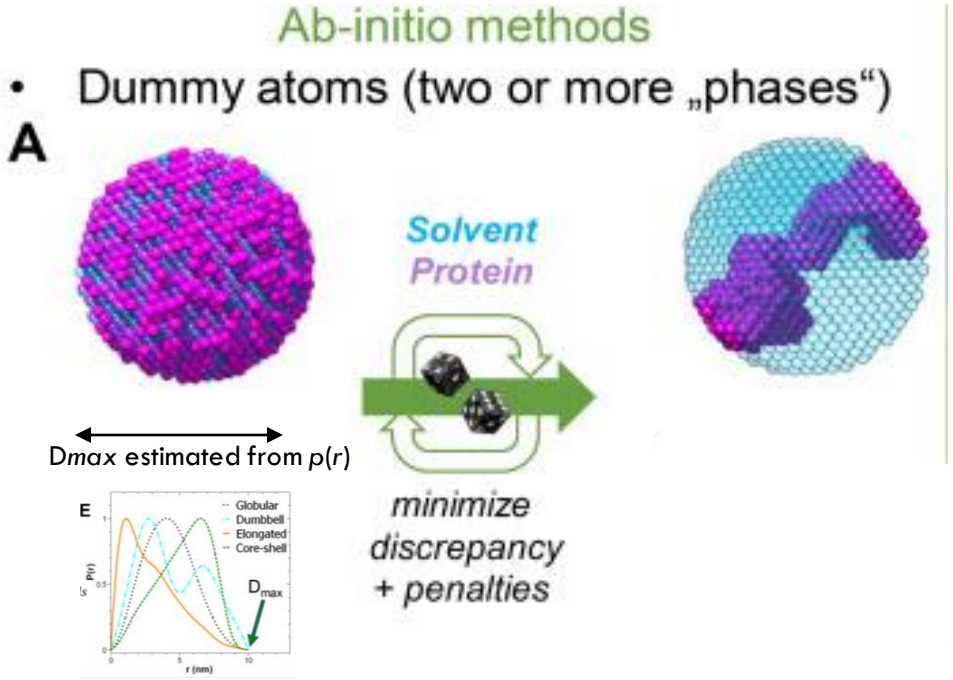


Schematic representation of typical Kratky plots. Curvature is depending on molecular shape, degree of flexibility, etc…

Courtesy of *Putnam, C.D., Hammel, M., Hura, G.L., and Tainer, J.A. Q Rev Biophys. 2007 40(3):191-285*

Modeling and fitting

Ab-initio Methods



The protein structure is represented beads

Each bead: particle or solvent.

Diameter = max. particle size

The macromolecule is an ensemble of packed beads (dummy atoms)

An iterative simulated annealing algorithm changes the configuration of the beads which includes the discrepancy to the SAXS data as well as structural constraints to ensure a physically plausible solution

Search of the best structure fitting I(Q)

Da Vela S, Svergun DI.
Methods, development and applications of small-angle X-ray scattering to characterize biological macromolecules in solution.
Curr Res Struct Biol. 2020 Aug

Combining structures of domains, with SAXS, SANS AUC & molecular modeling to propose full antibody structures

Structure determinations of human and chimaeric antibodies by solution scattering and constrained molecular modelling

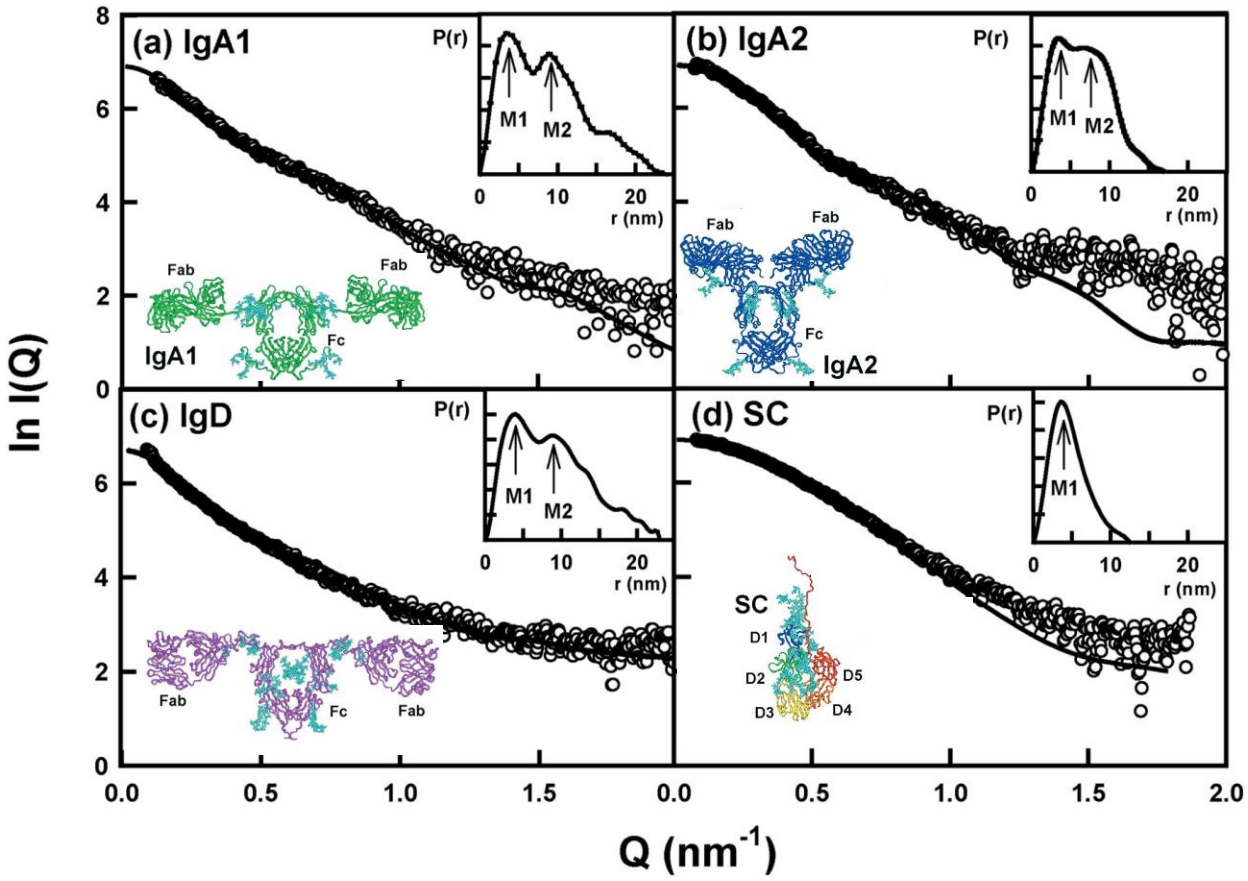
Stephen J. Perkins[1] and Alexandra Bonner
Department of Biochemistry and Molecular Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT, U.K.

Biochemical Society Transactions (2008)

"The prerequisite is a full starting co-ordinate model, including all carbohydrate chains if present.
The three major constraints are:

- the known sequence and composition to fix the macromolecular volume
- the use of relevant homologous crystal or NMR structures or good homology models to fix the domain shapes
- the known covalent peptide linkers between the subunits to limit the structures allowed"

- ➢ Different conformations of the proteins are derived from the linkers.
- ➢ Molecular Dynamics then randomizes this to generate libraries of 500–10000 conformers.
- ➢ Comparison with exp. data



MASS PHOTOMETRY

Mass Photometry - Principle

Mass Photometry is **a label free single-molecule** technique based on optical detection of the **scattering signal generated by a single particule at glass-water interface**

Measures the **mass of individual molecules** and thereby determines **mass distributions of biomolecule samples in solution**

Mass Photometry - Principle

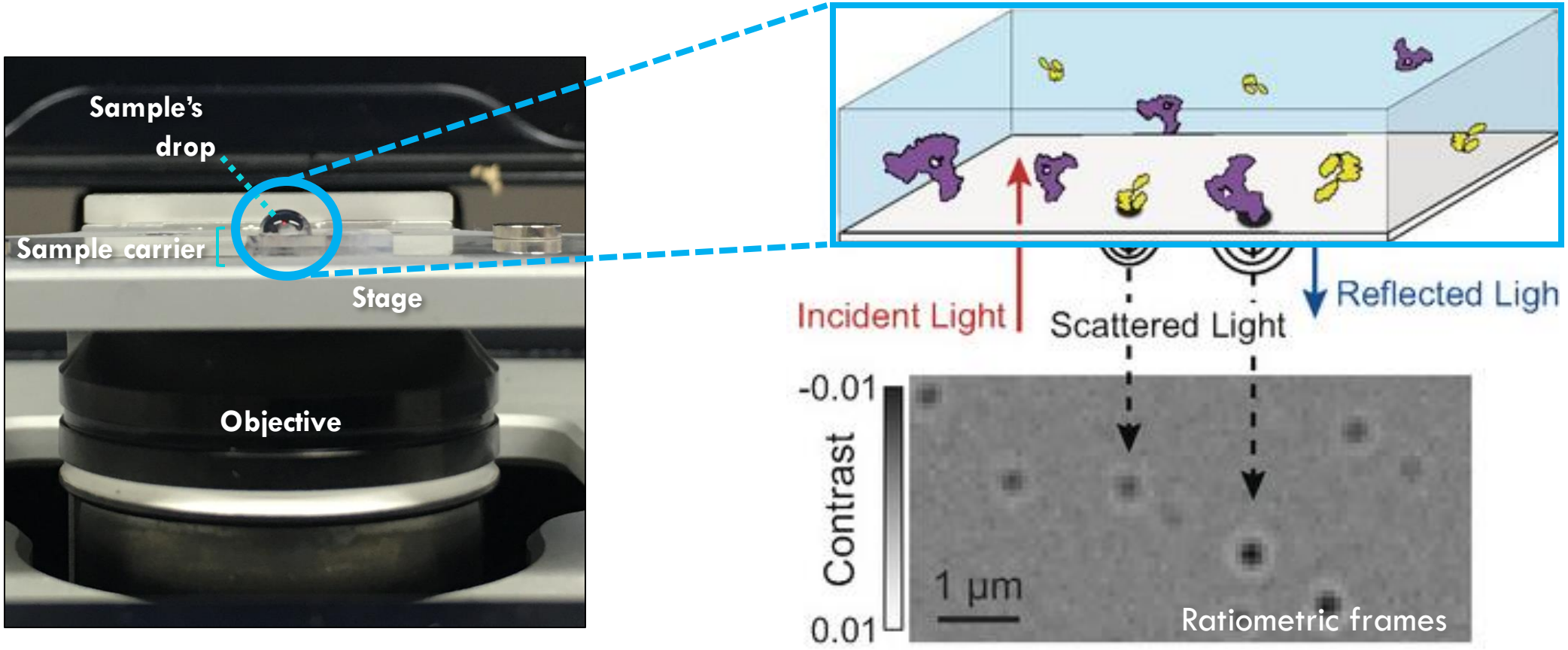


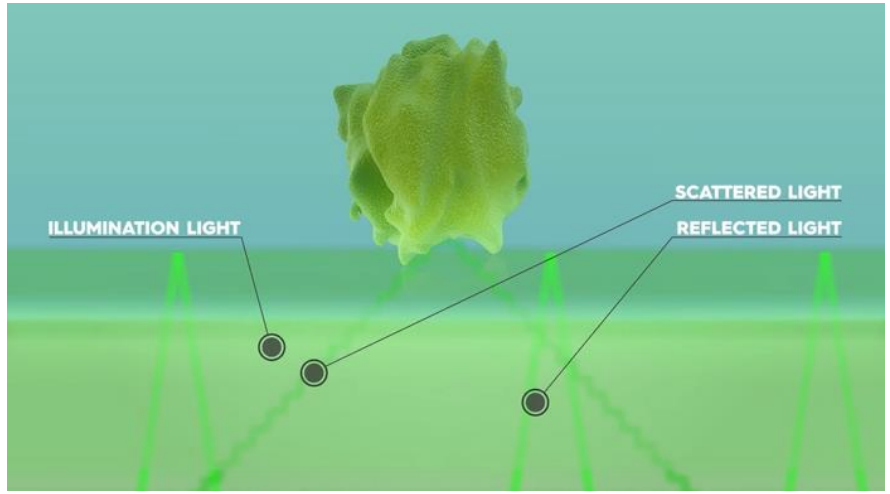
Image the coverslip to record a movie of the landings of macromolecules

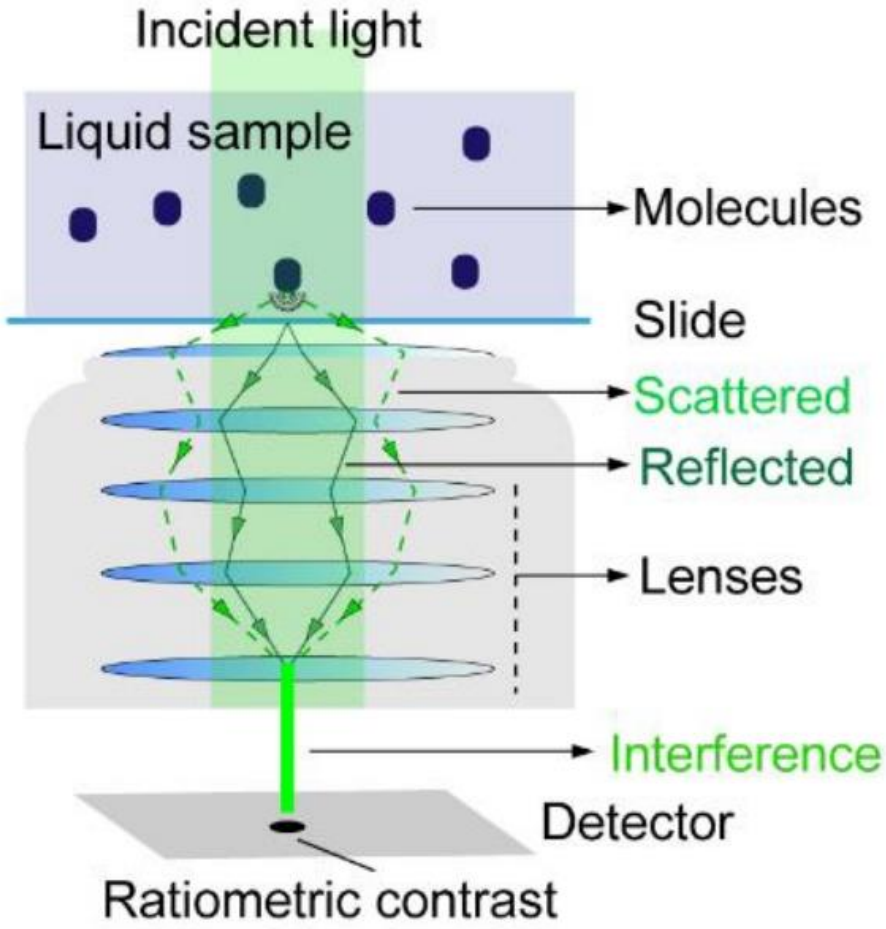
Angew. Chem. Int. Ed., Volume: 59, Issue: 27, Pages: 10774-10779 2020

Mass Photometry – Principle

Interferometric scattering iSCAT

coverslip





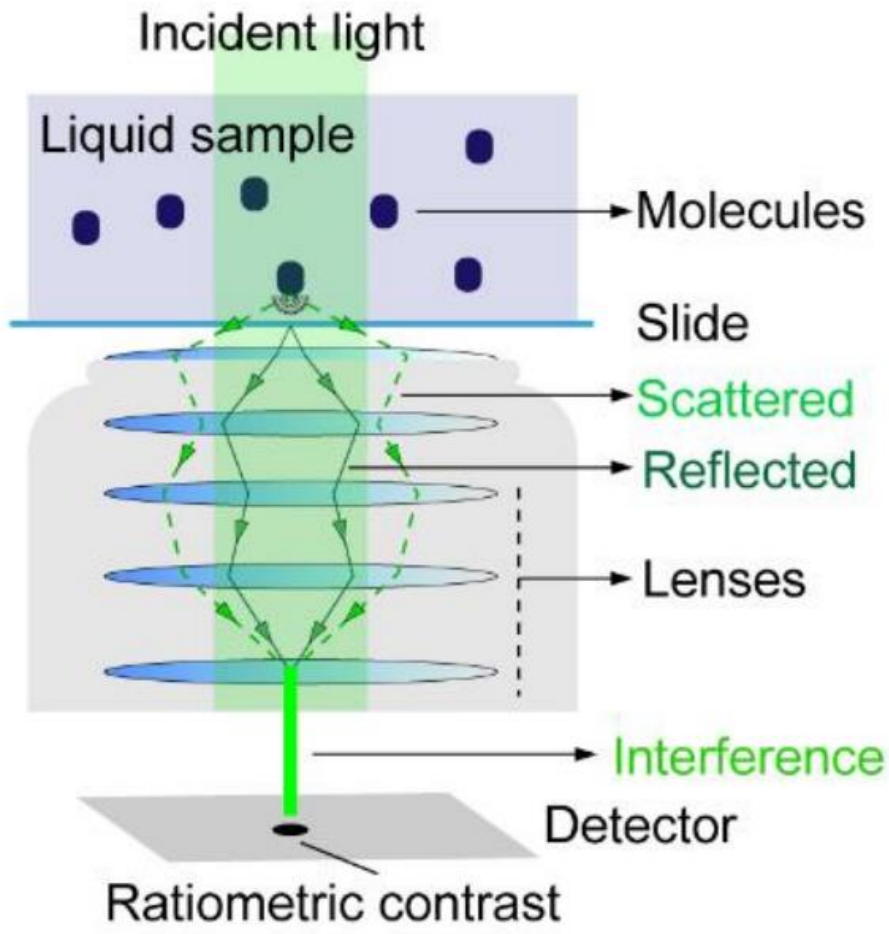
Mass Photometry – Principle
Interferometric scattering iSCAT

Interferometric scattering (iSCAT) microscope collects light that is reflected at the interface between a glass coverslip and an aqueous solution along with light scattered by objects at that interface

The detected light intensity is a combination of the two interfering electric fields of reflected and scattered light that can be described by

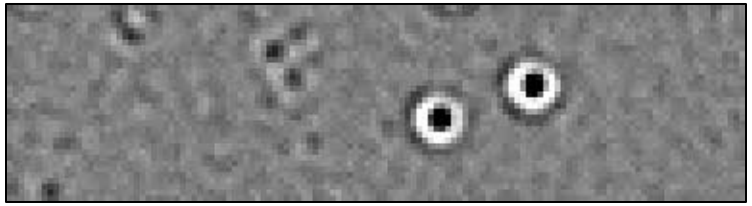
$$I_{\mathrm{det}} = I_{inc}(r^2 + |s|^2 + 2r\,|s|\cos\varphi)$$

I_{inc} is the incident light intensity used for illumination
r^2 is the reflectivity of the interface
$|s|^2$ is the scattering cross section
φ the phase difference between reflected and scattered light

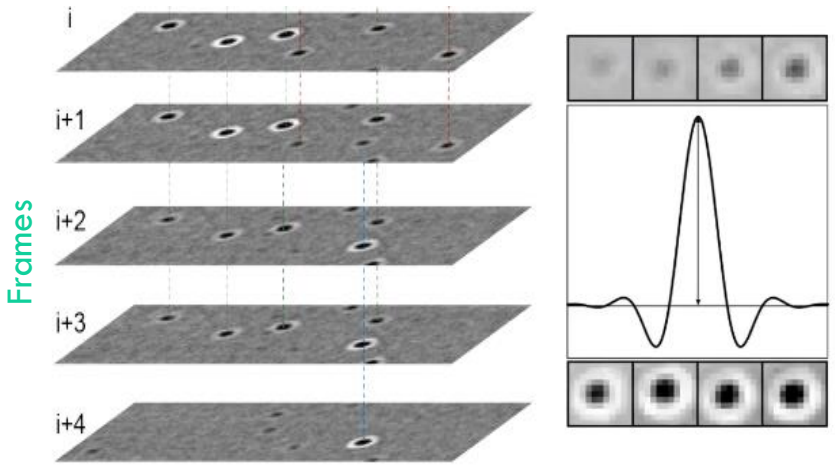


Mass Photometry - Principle

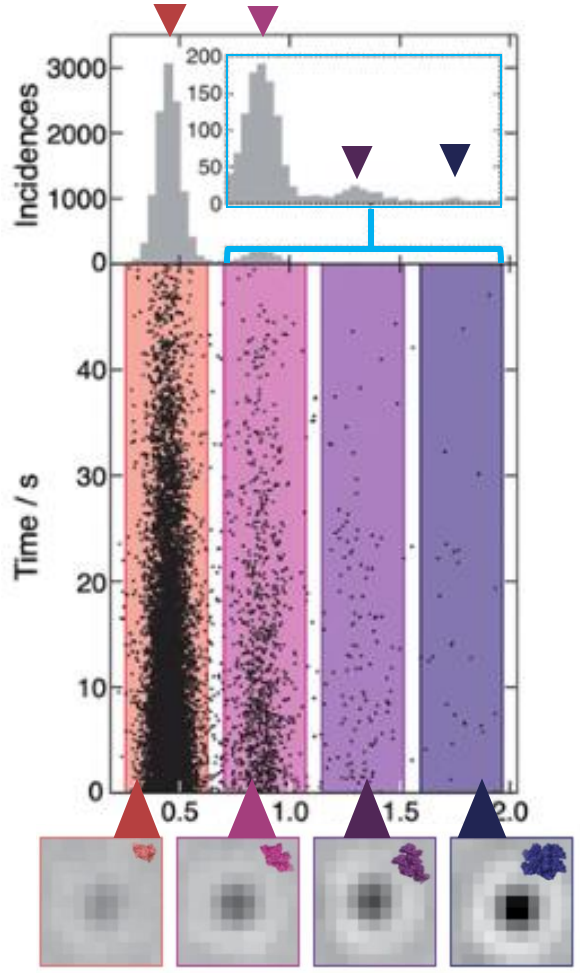
1 - Movie recording



Movie of particles landing on the coverslip



2 - Contrast / Mass



Contrast of various oligomeric states

3 - Sample's requirement

- Few µL at ~100-10 nM
- 40 kDa - 5 MDa

4 - Applications

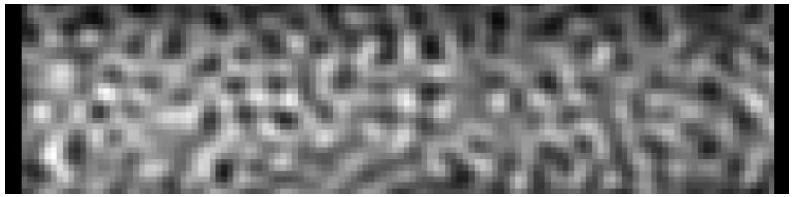
- Protein
- Unfolded protein
- RNA / DNA
- Membrane proteins
- Adeno-associated virus
- Interactions

Nikolas Hundt, 2020

Native Image vs Ratiometric

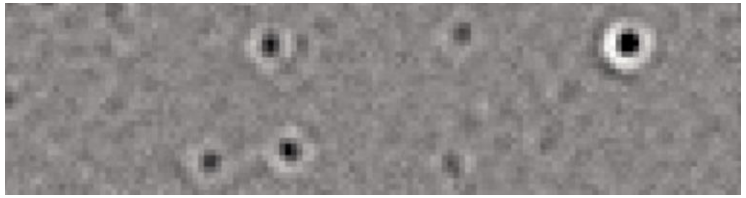
Native

Raw image of the coverslip

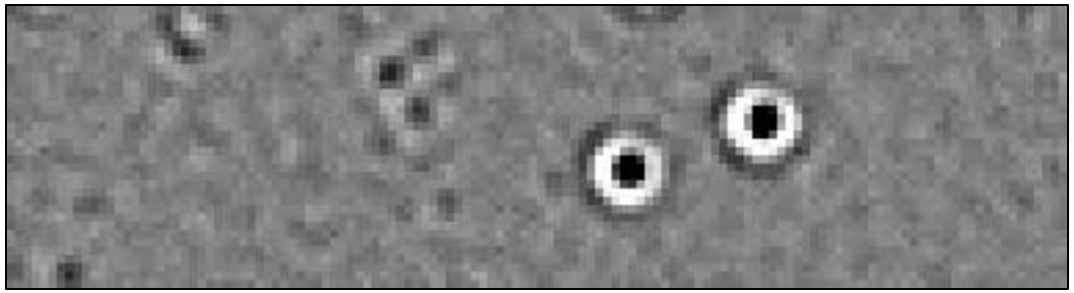


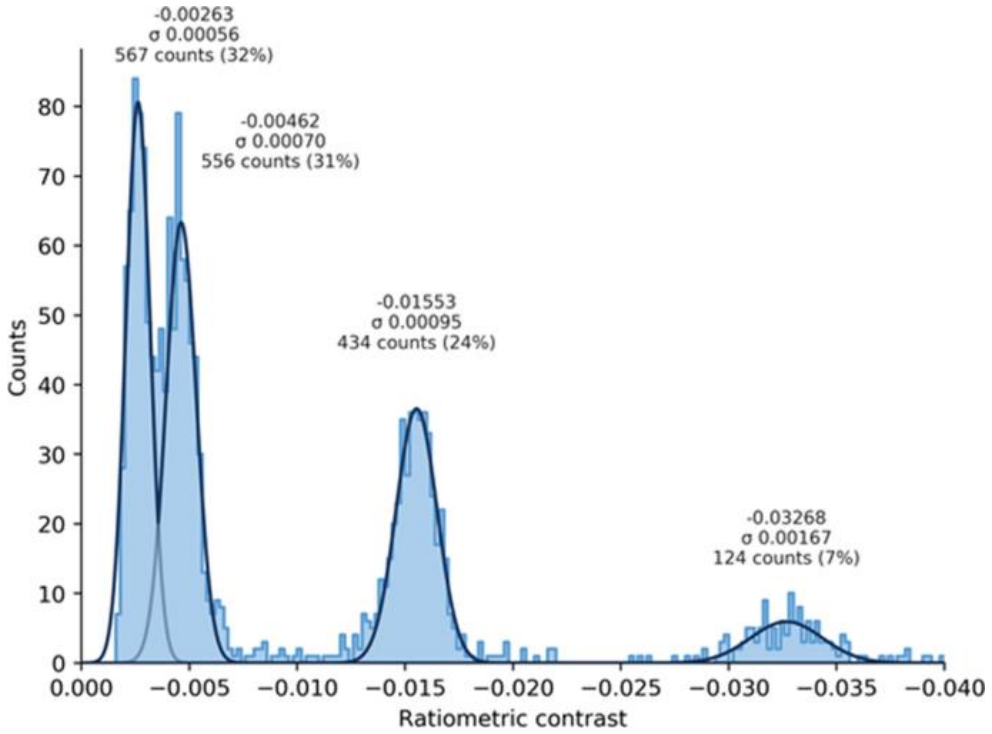
Ratiometric

Processed raw images

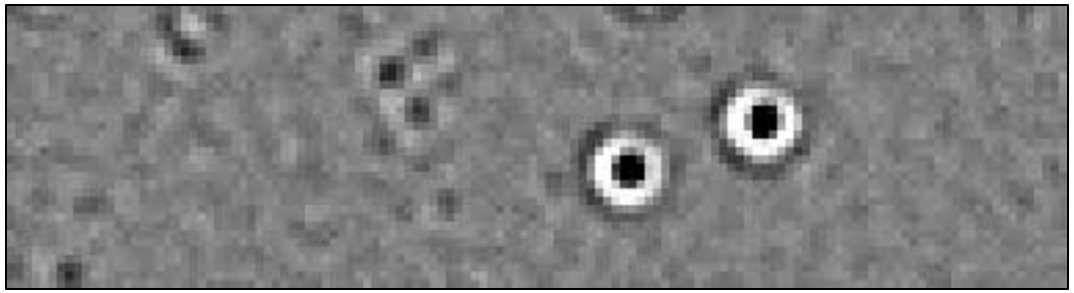


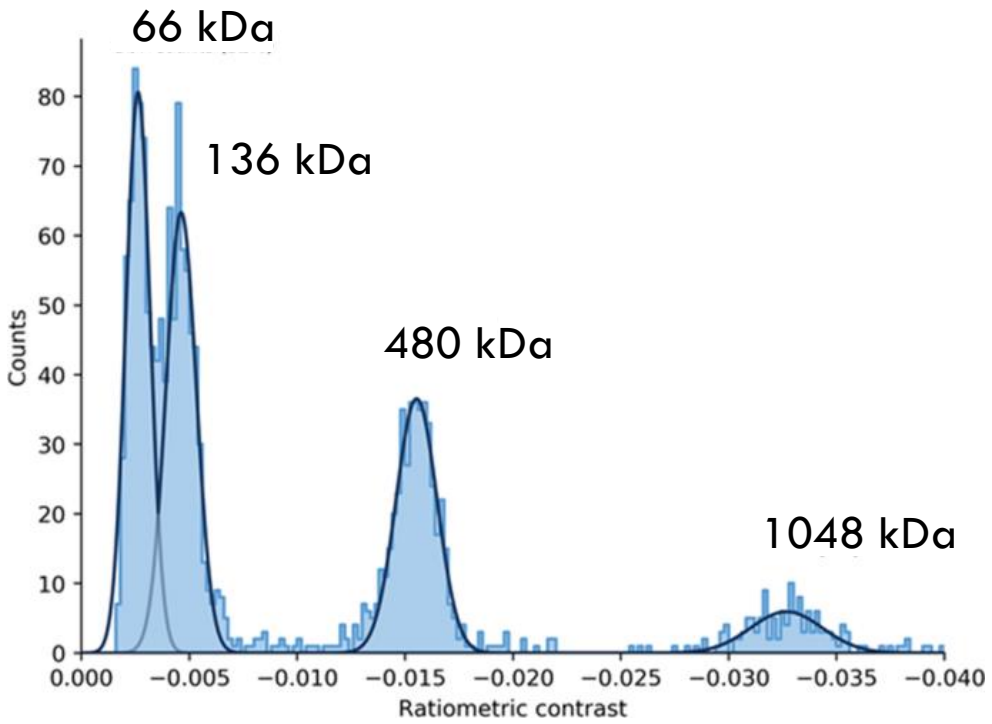
Mass Photometry Movie and Histogram



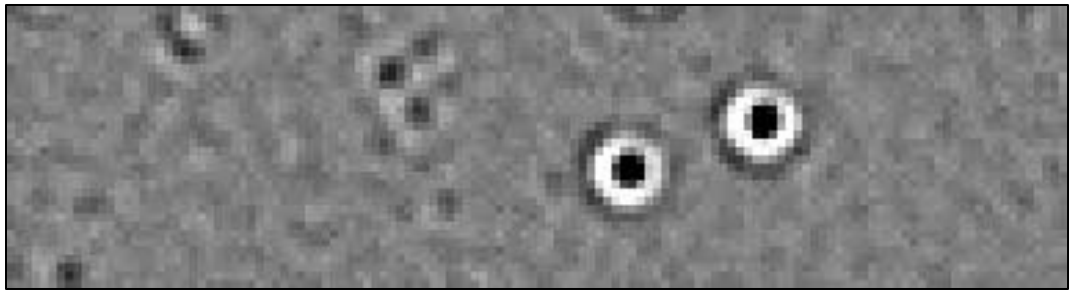


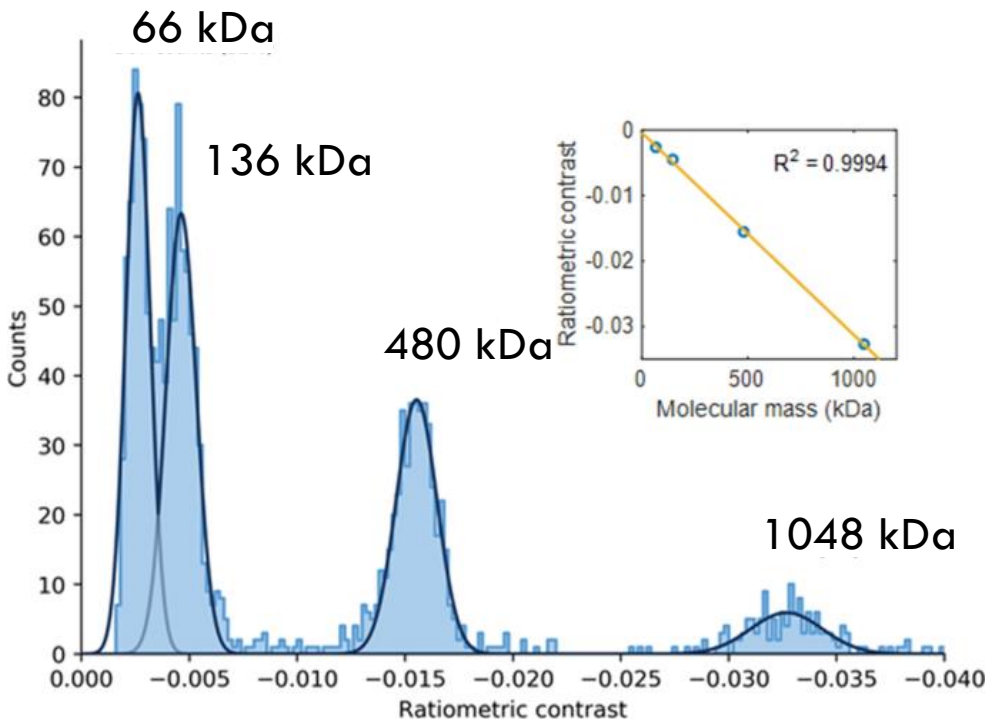
Mass Photometry Movie and Histogram





Mass Photometry Movie and Histogram





Example 1

communications biology

ARTICLE

https://doi.org/10.1038/s42003-022-03276-1 OPEN

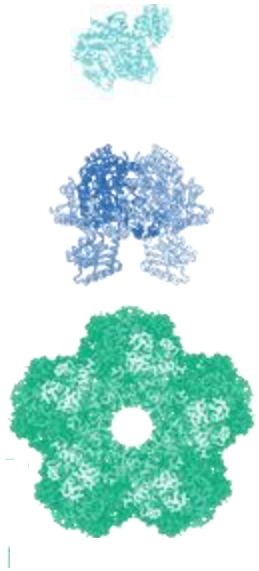
Structural and biochemical characterisation of the *Providencia stuartii* arginine decarboxylase shows distinct polymerisation and regulation

Matthew Jessop[1,2], Karine Huard[1], Ambroise Desfosses[1], Guillaume Tetreau[1], Diego Carriel[1], Maria Bacia-Verloop[1], Caroline Mas[1], Philippe Mas[1], Angélique Fraudeau[1], Jacques-Philippe Colletier[1] & Irina Gutsche[1]✉

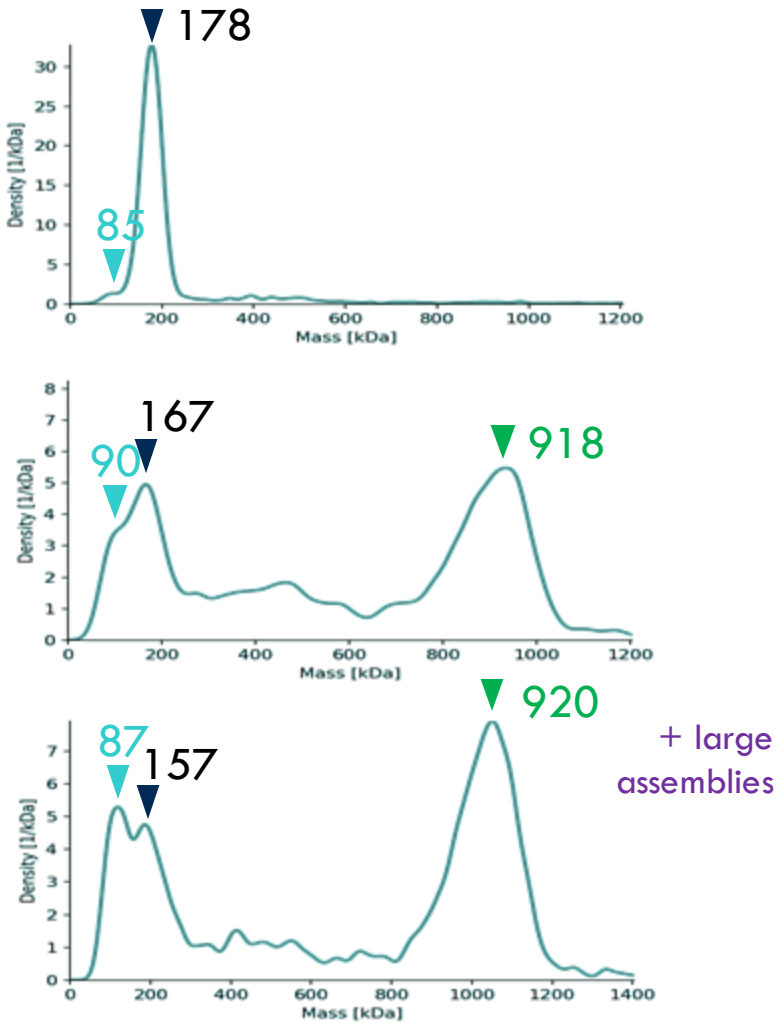
Monomer

Dimer

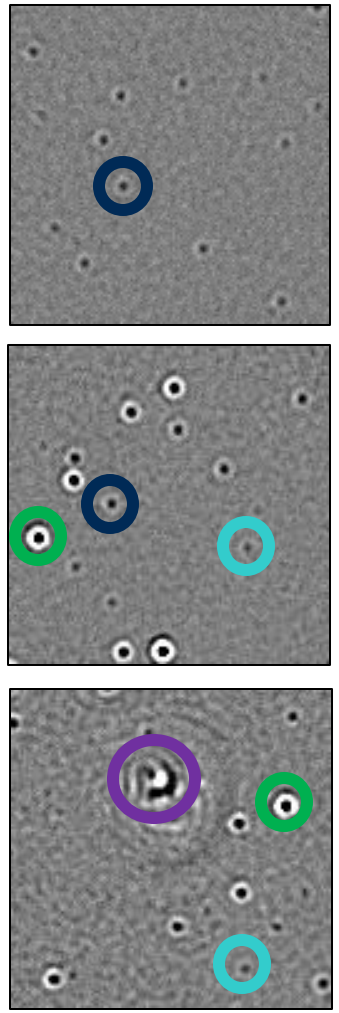
Decamer



Mass distribution



Ratiometric Frame



pH8

pH6.5

pH5

Jessop M et al, Comm Biol 2022

Example 2

Cell Reports

CellPress
OPEN ACCESS

Article

Extracellular endosulfatase Sulf-2 harbors a chondroitin/dermatan sulfate chain that modulates its enzyme activity

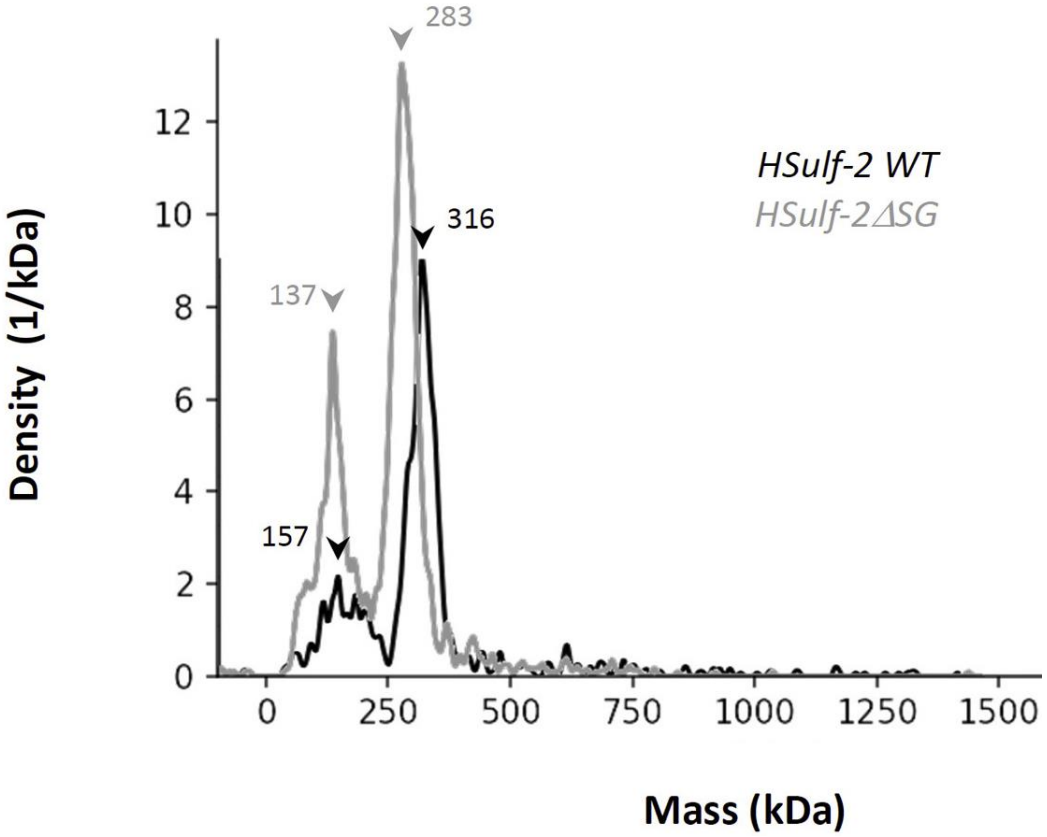
Rana El Masri,[1,8] Amal Seffouh,[1,8] Caroline Roelants,[2] Ilham Seffouh,[3] Evelyne Gout,[1] Julien Pérard,[4] Fabien Dalonneau,[1] Kazuchika Nishitsuji,[5] Fredrik Noborn,[6] Mahnaz Nikpour,[6] Göran Larson,[6] Yoann Crétinon,[1] Mélanie Friedel-Arboleas,[1] Kenji Uchimura,[7] Régis Daniel,[3] Hugues Lortat-Jacob,[1] Odile Filhol,[2,*] and Romain R. Vivès[1,9,*]

Human Sulfs are extracellular endosulfatases that catalyzed the specific 6-O-desulfation of cell-surface and extracellular matrix heparan sulfate (HS)

Mass Photometry analysis

- HSulf-2ΔSG : 137 kDa and 283 kDa
- HSulf-2 WT : 157 kDa and 316 kDa

> monomeric and dimeric forms for HSulf-2 harboring a ~20 kDa GAG chain.



Questions?

caroline.mas@ibs.fr