Static and Dynamic Light Scattering for biological macromolecules in solution

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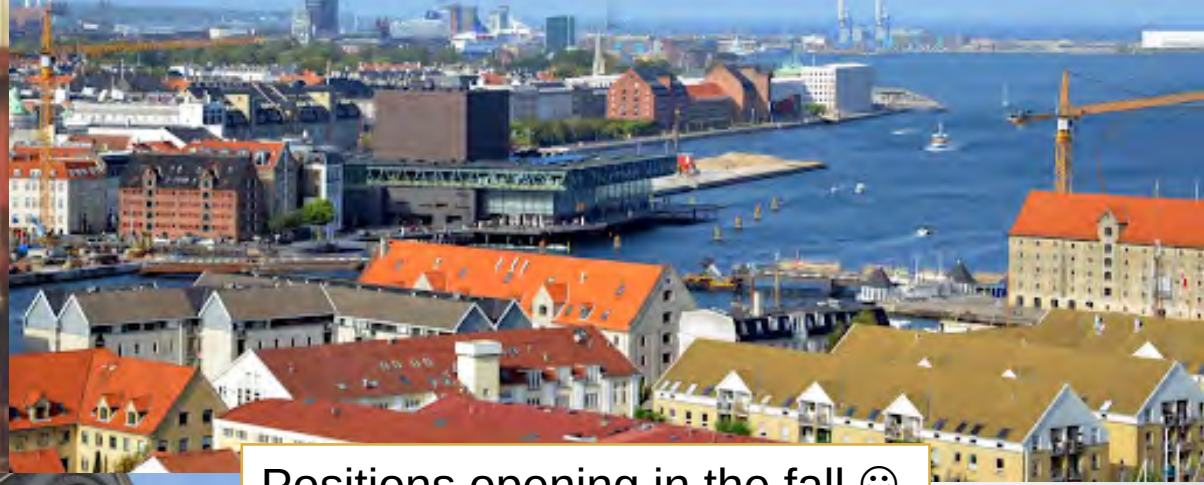
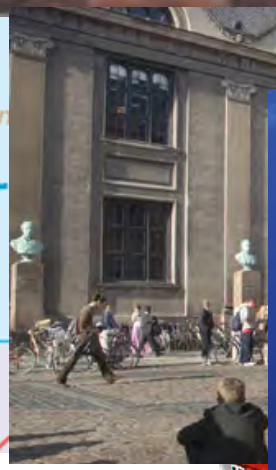
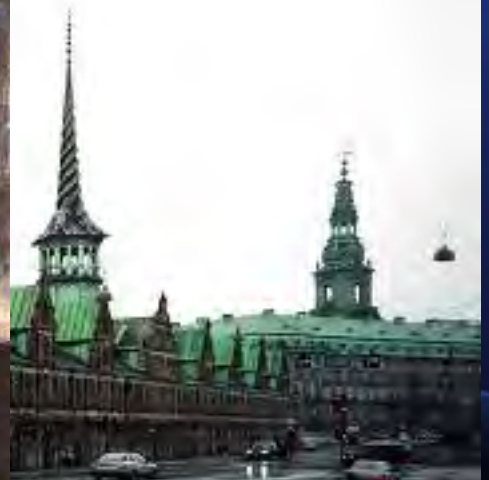
EMBO Global Exchange Lecture Course

1479

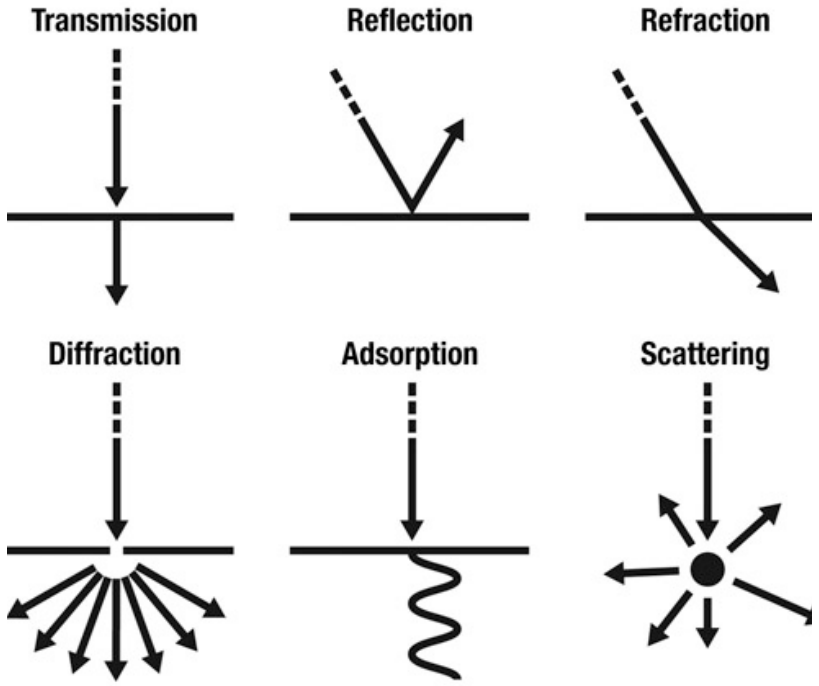


Positions opening in the fall ☺





When light interacts with matter, it can….



- Be absorbed
  - and re-emitted at modified λ (fluorescence)
- Change polarisation
- Be scattered
  - Reflected/refracted/diffracted from ordered matter
  - Inelastically (change of λ) e.g. Raman
- - all disregarded here

Rayleigh scattering: λ of light is significantly larger than the dimensions of the scattering particles (point scatterers)

- Be scattered
  - Elastic (same λ)

    SLS
  - Quasi-elastic (nearly same λ)

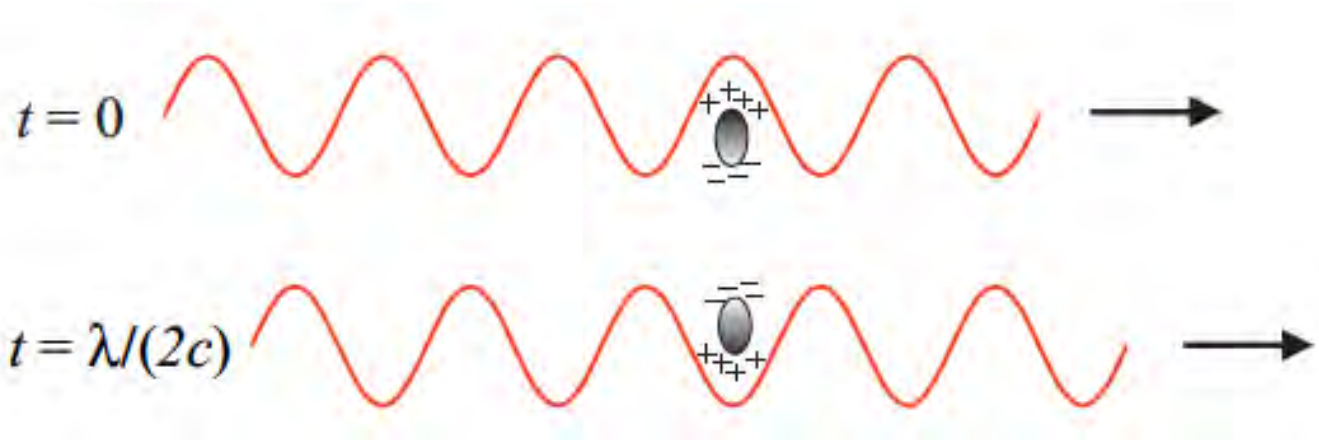
    DLS or QELS
    - movement of particles modifies l (Doppler effect)

Why is light scattered?

Electrodynamics:
An oscillating dipole emits electromagnetic radiation in all directions
Induced dipole momentum (oscillating)

$$\mu = \alpha \bullet m \bullet E_{0,laser}$$

Polarizability Mass of dipole

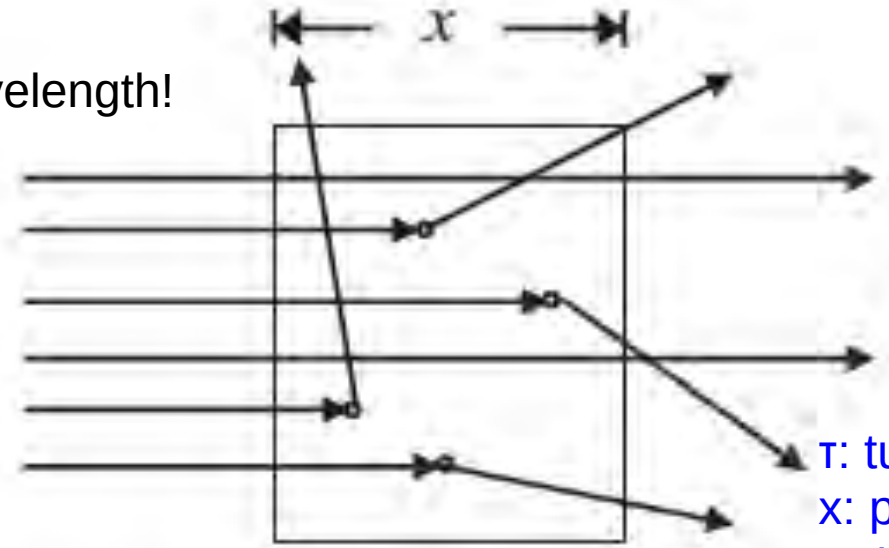


$$I_{s,1}(r) = I_0 \cdot \frac{\pi^2 \alpha^2}{r^2 \lambda^4}$$

Choose right wavelength!

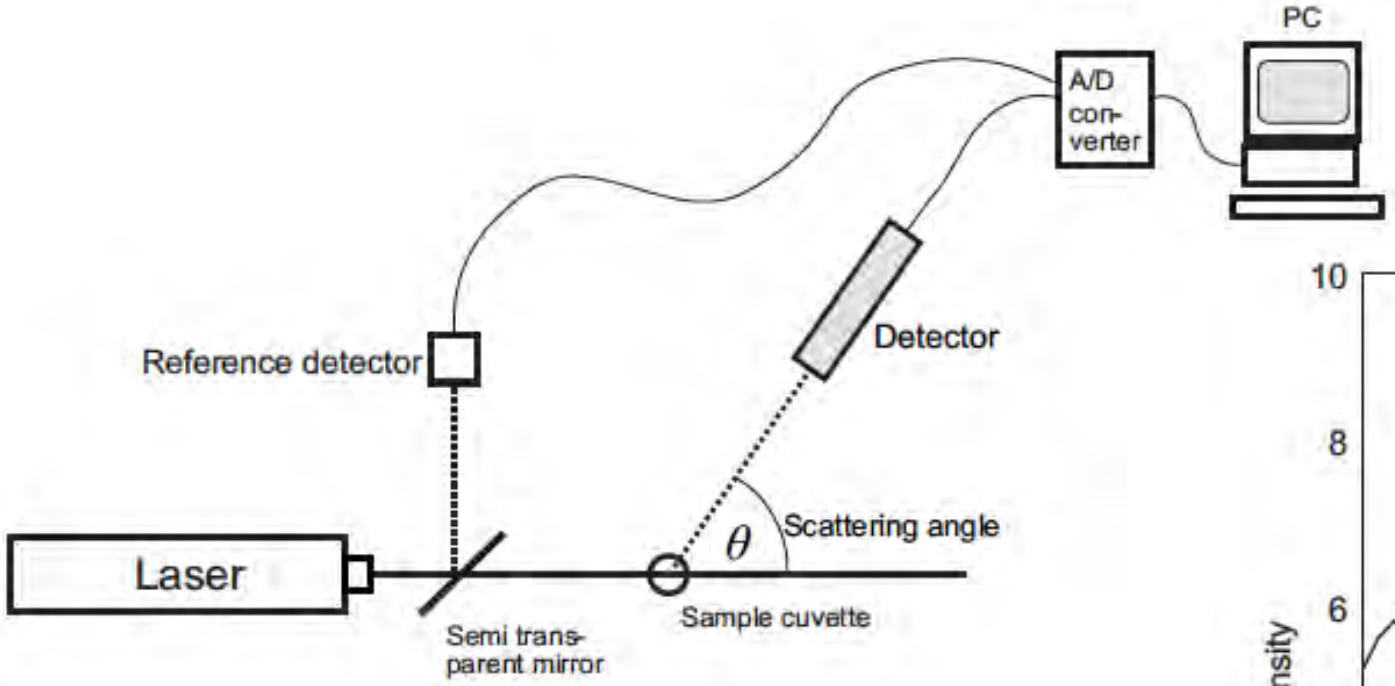
The scattering intensity is proportional to the square of the particle molecular weight.

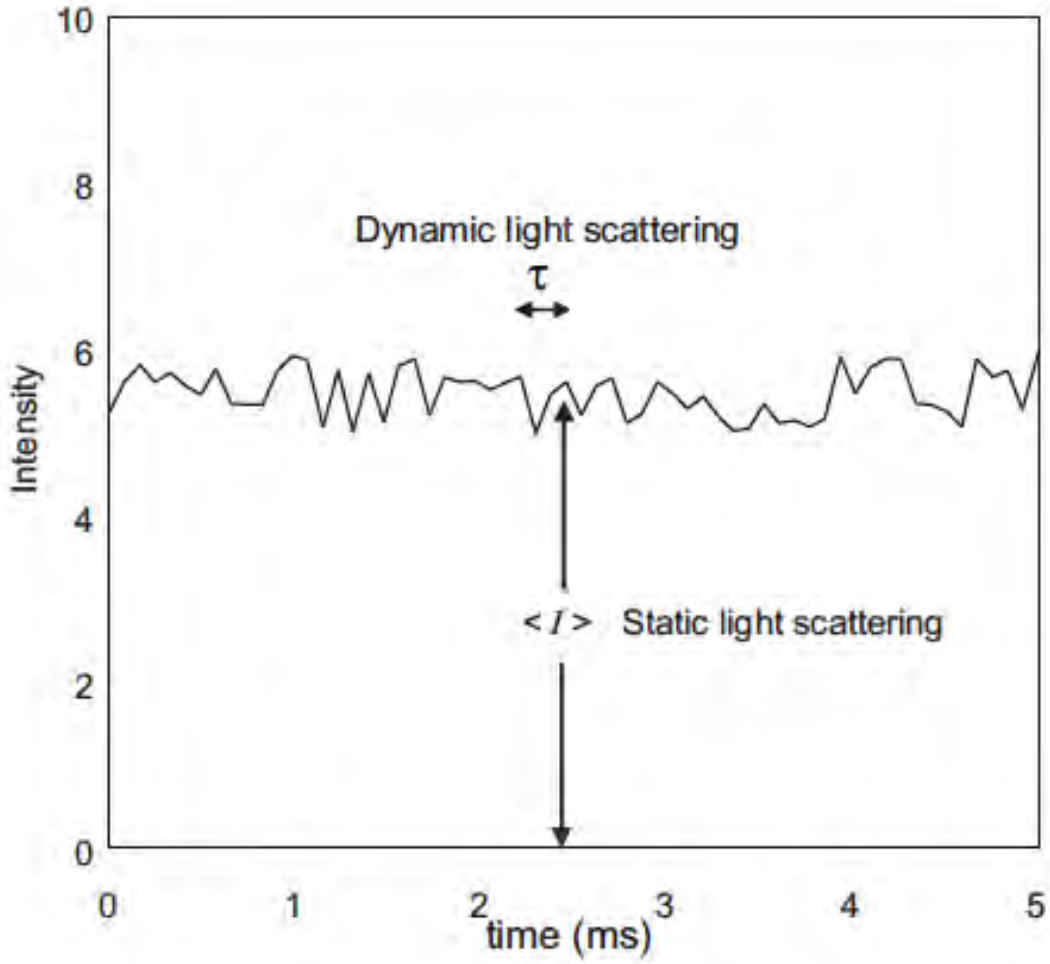
The scattered light is proportional to the concentration of the particle.



$$I = I_0 \cdot e^{-\tau x}$$

τ: turbidity
x: pathlength
I_0: incoming intensity





Monochromatic
Collimated

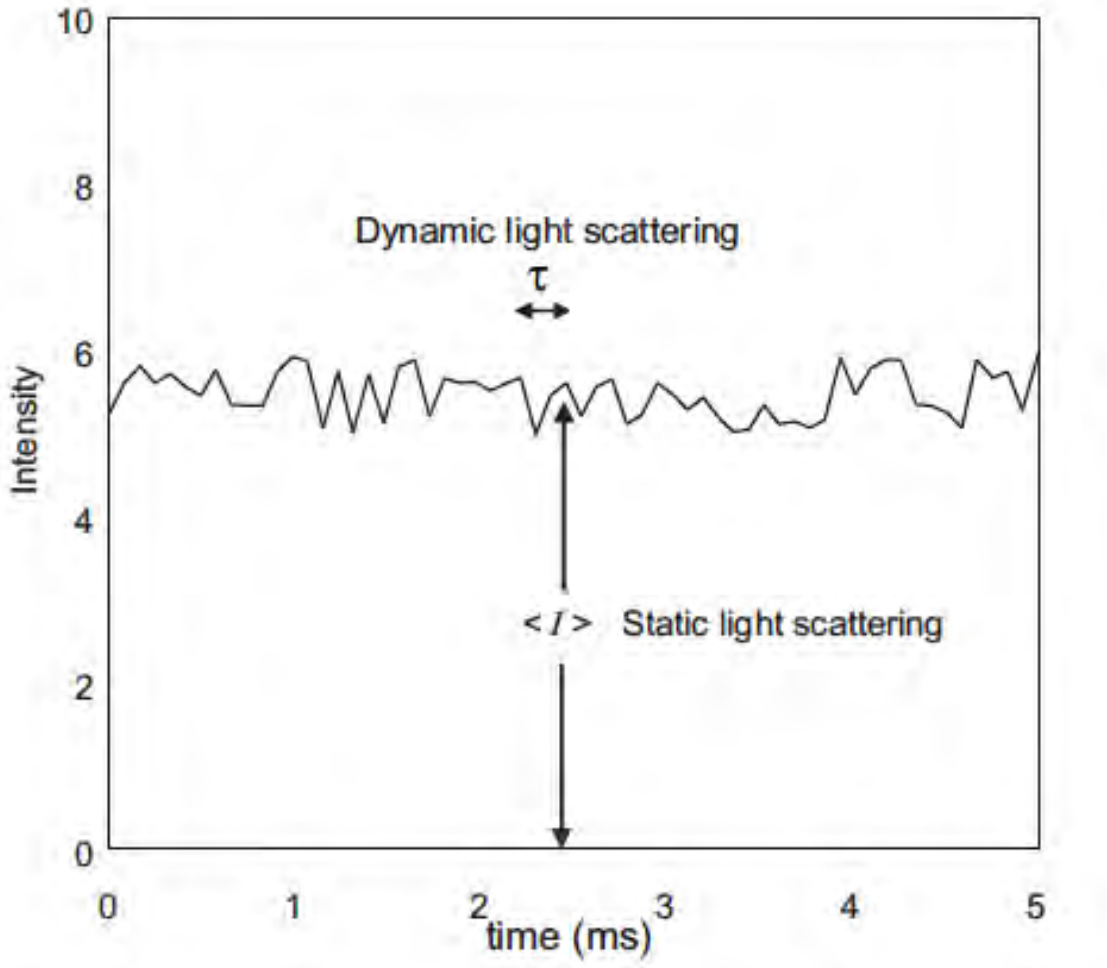
Intensity: Reflects the molecular weight of the particles

Fluctuations: Reflect the diffusion coefficient of the particles

Basics: SLS and DLS

Intensity: Reflects the molecular weight of the particles. SLS measures at many different angles (typically 10-100), intensity is averaged over time (1 sec or more)

Fluctuations: Reflect the diffusion coefficient of the particles. DLS employs measurements in a time series, averaging over very short time intervals (typically 100 nsec).



Basic comparison

- SAXS, SANS, SLS:
  - Same theory
  - Same epxerimental setup but different light sources
  - Measures the **structural characteristics** of the sample at different resolutions
  - Structure including both the form factor and structure factor
- DLS
  - Different theory
  - Different experimental setup
  - Measures the **diffusion** of the particles in the sample

Small Angle Scattering/Static light scattering

λ: Wavelength of X-ray, neutron or light

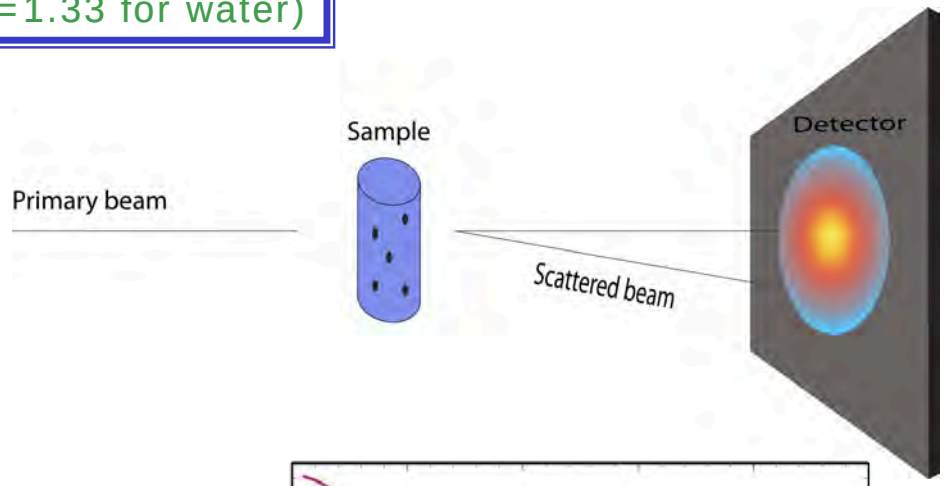
n_0: Refractive index of sample (=1.33 for water)

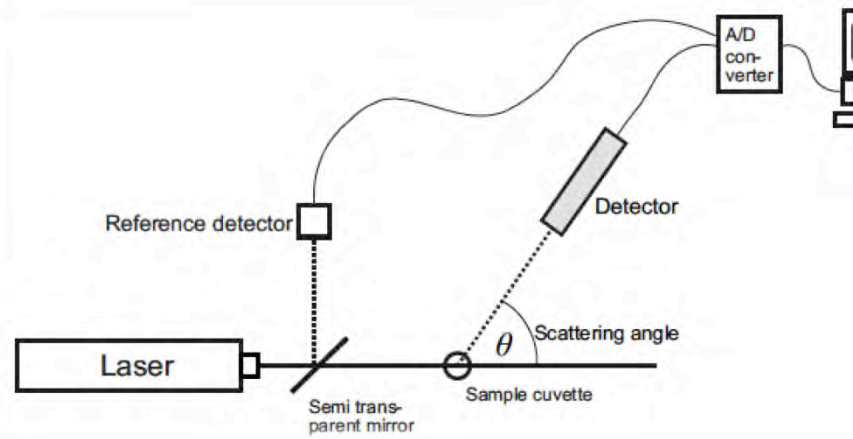
Beam:

Neutron (SANS)

X-ray (SAXS)

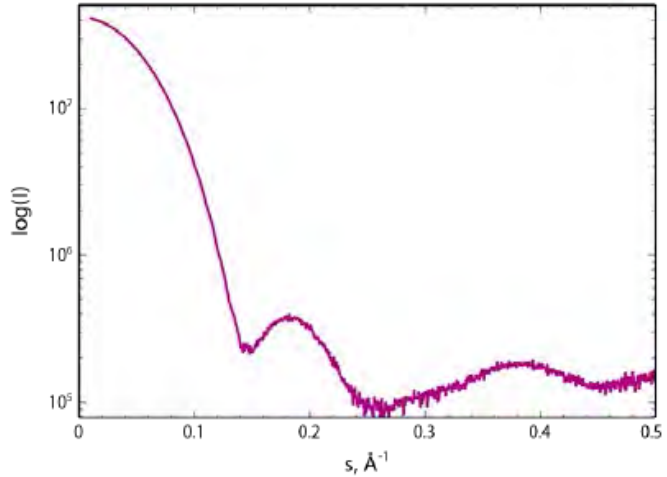
or light (SLS)

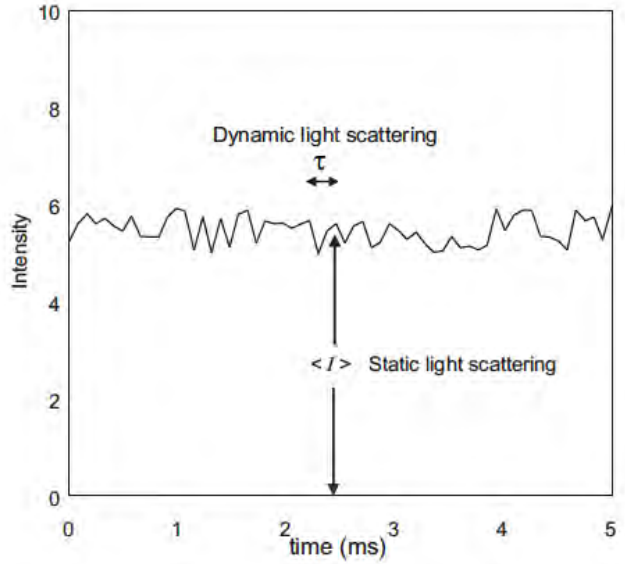




$$|Q_{SAS}| = \frac{4\pi \sin\theta}{\lambda}$$

$$|Q_{SLS}| = \frac{4\pi n_0 \sin\theta}{\lambda}$$



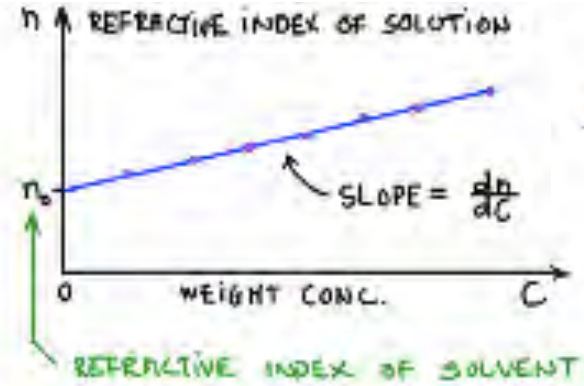


SAXS/SANS: θ_{min}≈0.03°, θ_{max}≈3°, Q=[0.001-0.5 1/Å], 1-200 nm

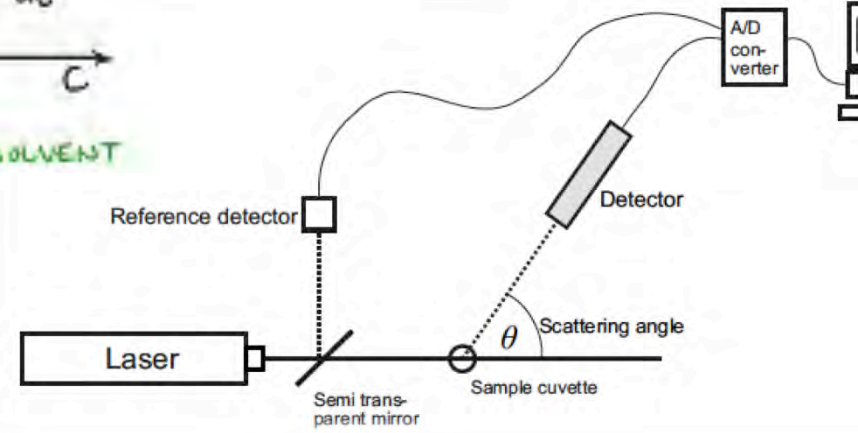
SLS: θ_{min}≈8°, θ_{max}≈160°, Q=[0.0004-0.001 1/Å], 200-2000 nm

Static light scattering

- Intensity depends on:
  - The molecular weight of the particles
  - The concentration of the particles
  - The size of the particles
  - The refractive index of the pure solvent
  - The refractive index of the suspended molecules
  - Interaction forces between particles

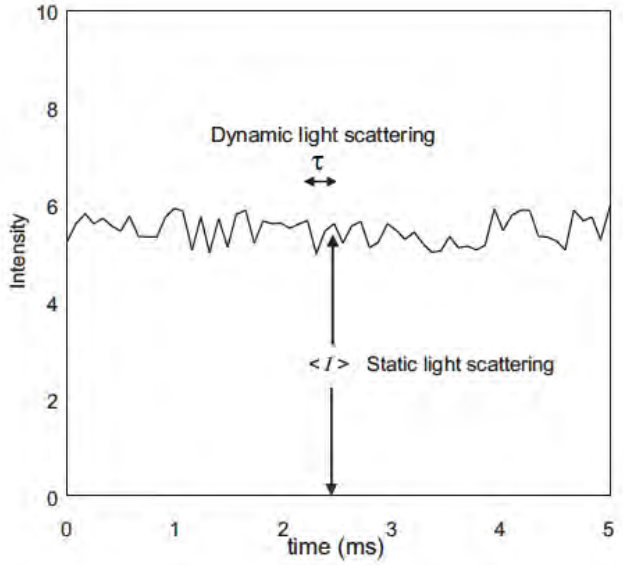


$$K = \frac{4\pi^2 n_0^2 (dn/dC)^2}{N_A \lambda_0^4}$$



$I_{total} = K I_0 V C M / r^2$

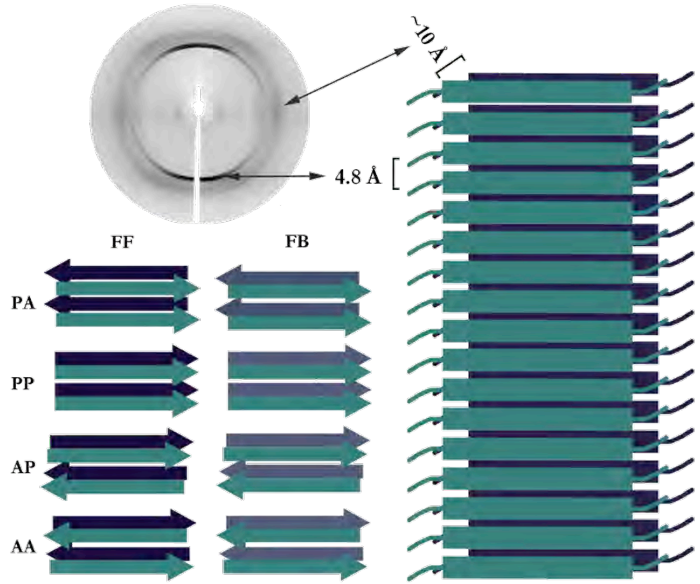
C: mg/ml
V: volume
M: mass
r: distance to detector
K: optical contrast constant

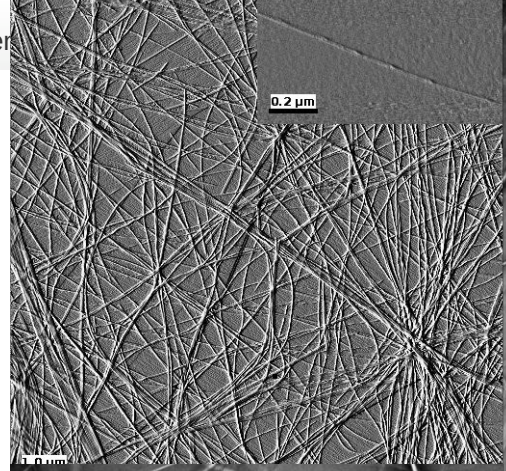


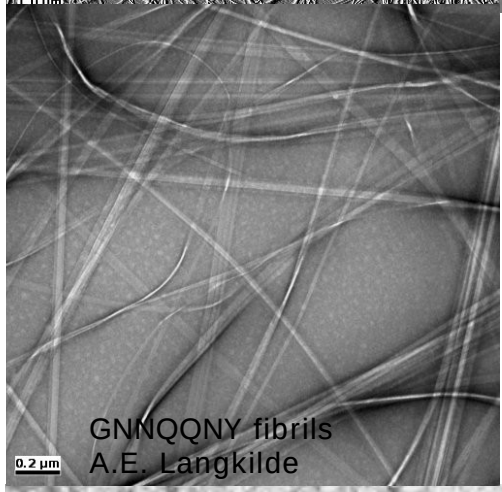
http://igm.fys.ku.dk/~lho/personal/lho/lightscattering_theory_and_practice.pdf

Amyloid(-like) fibrils

- Amyloid diseases (Alzheimers, Parkinsons…)
- Functional Fibrils (Antimicrobial, Biofilm, Spider silk)
- Biopharmaceutical stability (insulin, glucagon, …)
- Self-assembly bio-systems
  - Drug delivery (Degarelix)
  - Nano-material: the strength of steel

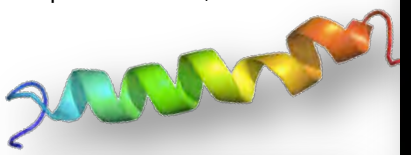


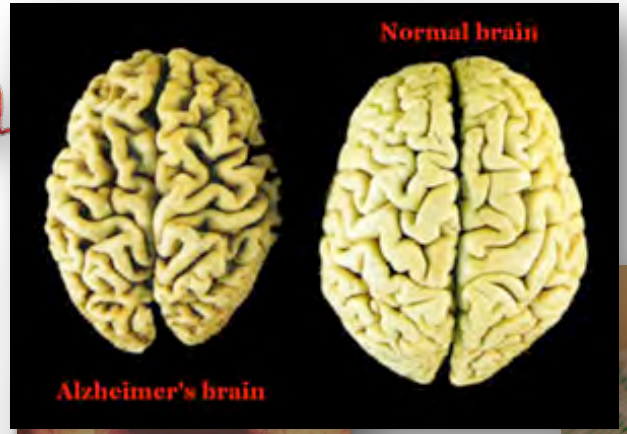




GNNQQNY fibrils
A.E. Langkilde

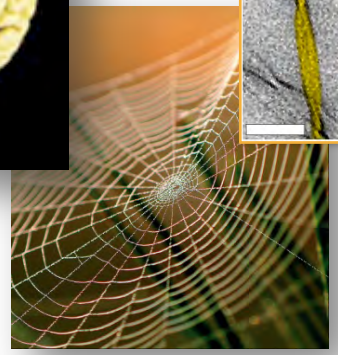
pdb-code 1d0r, GLP1

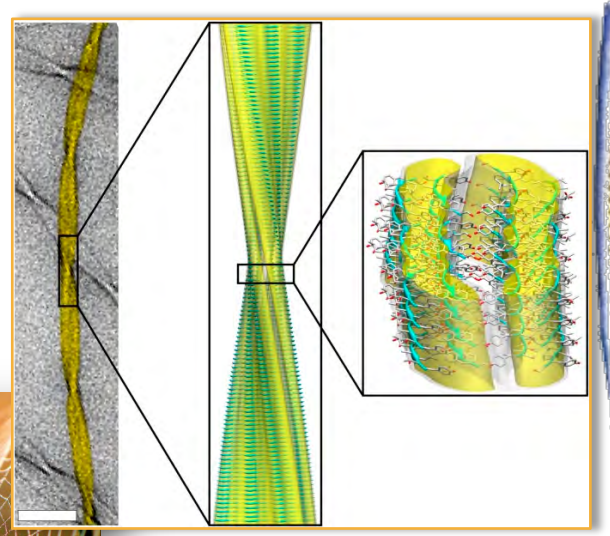


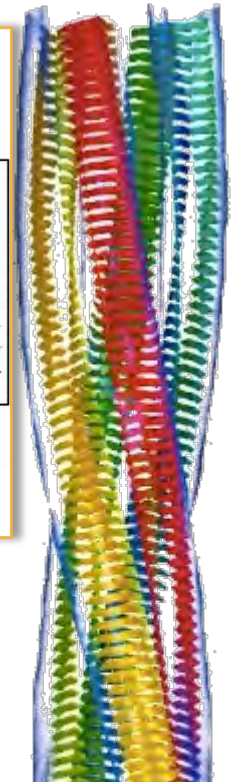


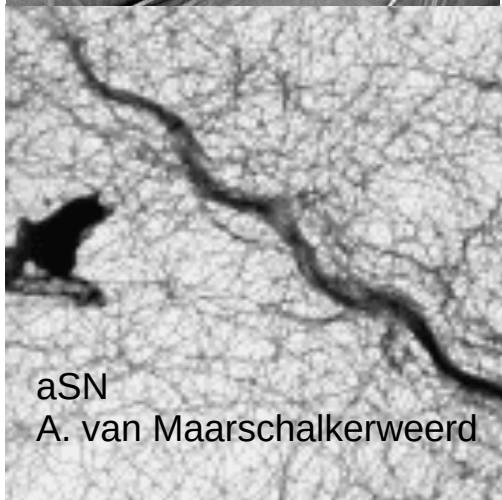
E. coli Biofilm AJC1/Flickr

Am. Soc.Hematology



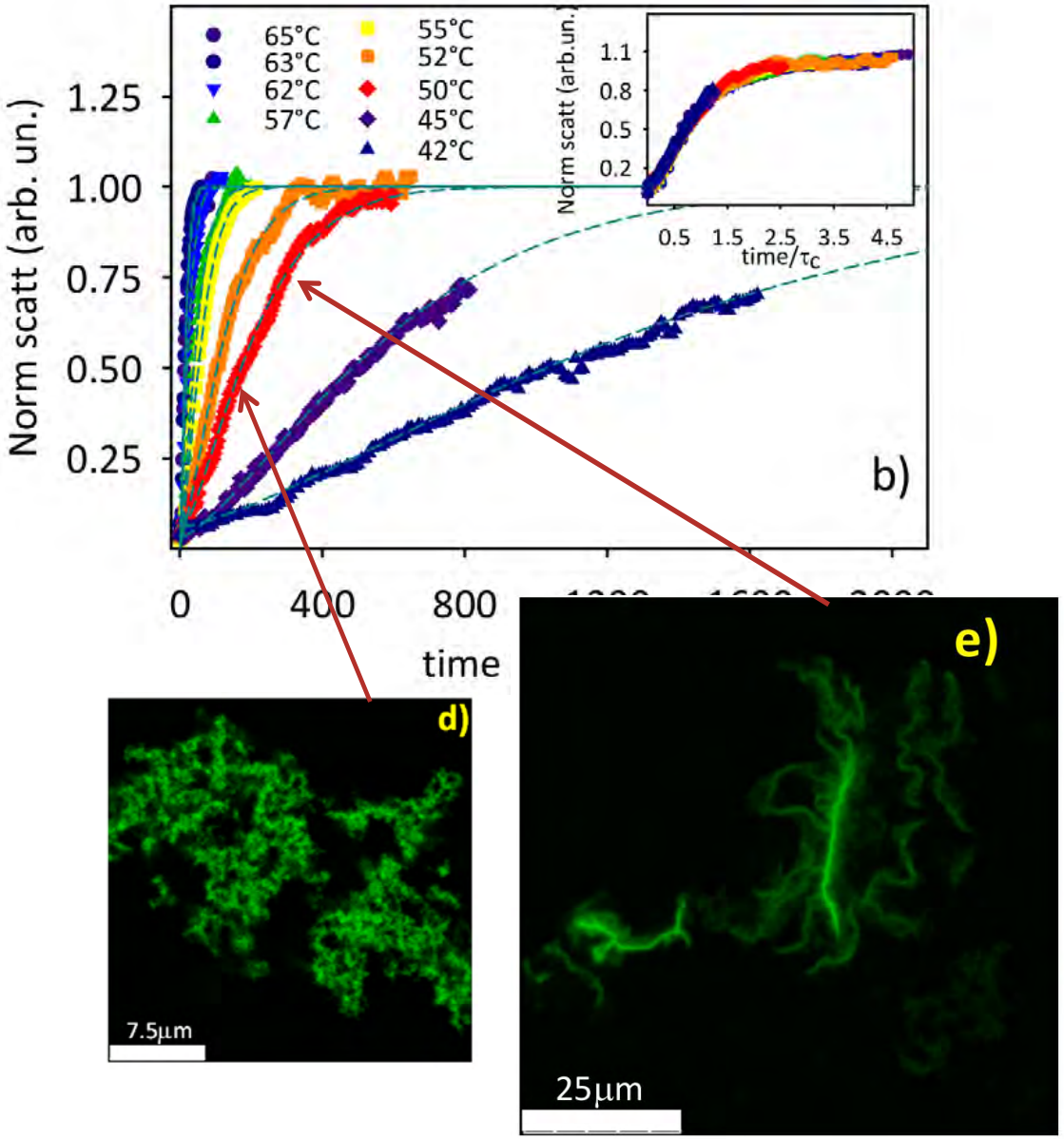




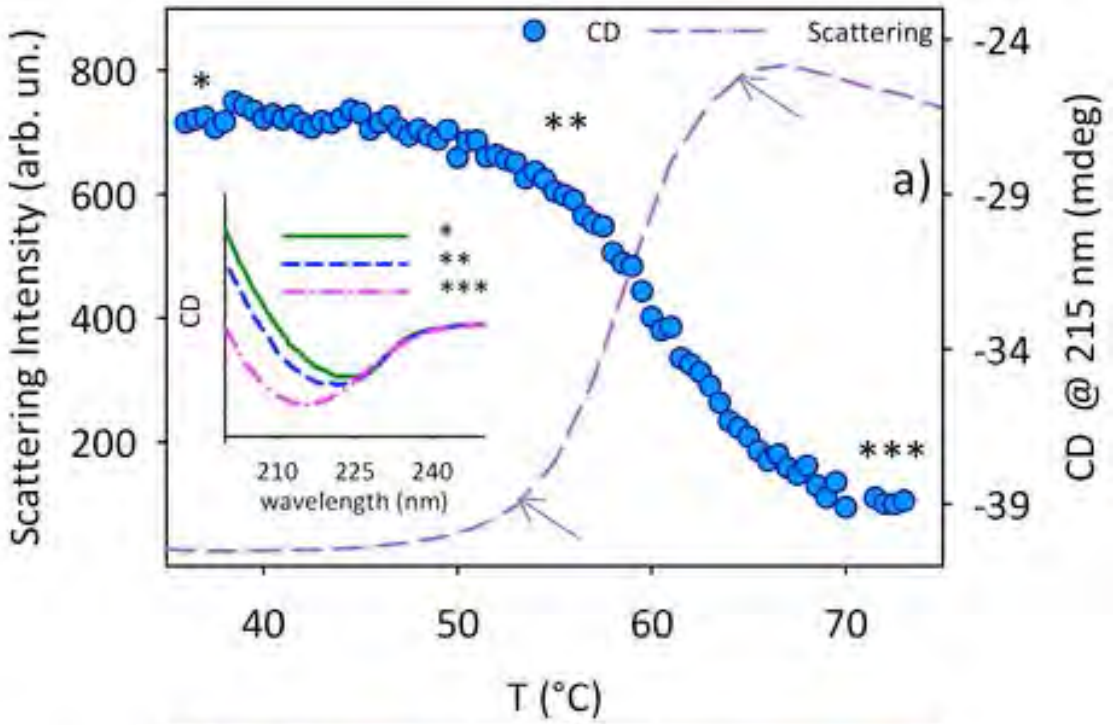


aSN
A. van Maarschalkerweerd

Applications: monitoring aggregate growth of ConA



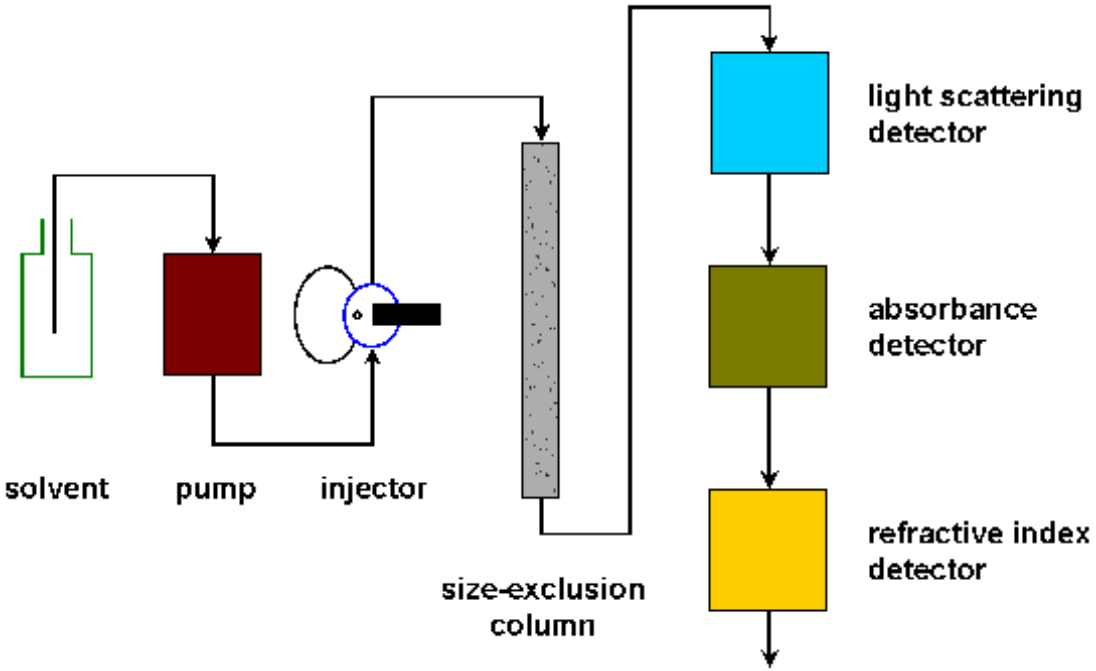
- Qualitative information
- Easy analysis
- Complemented with other techniques



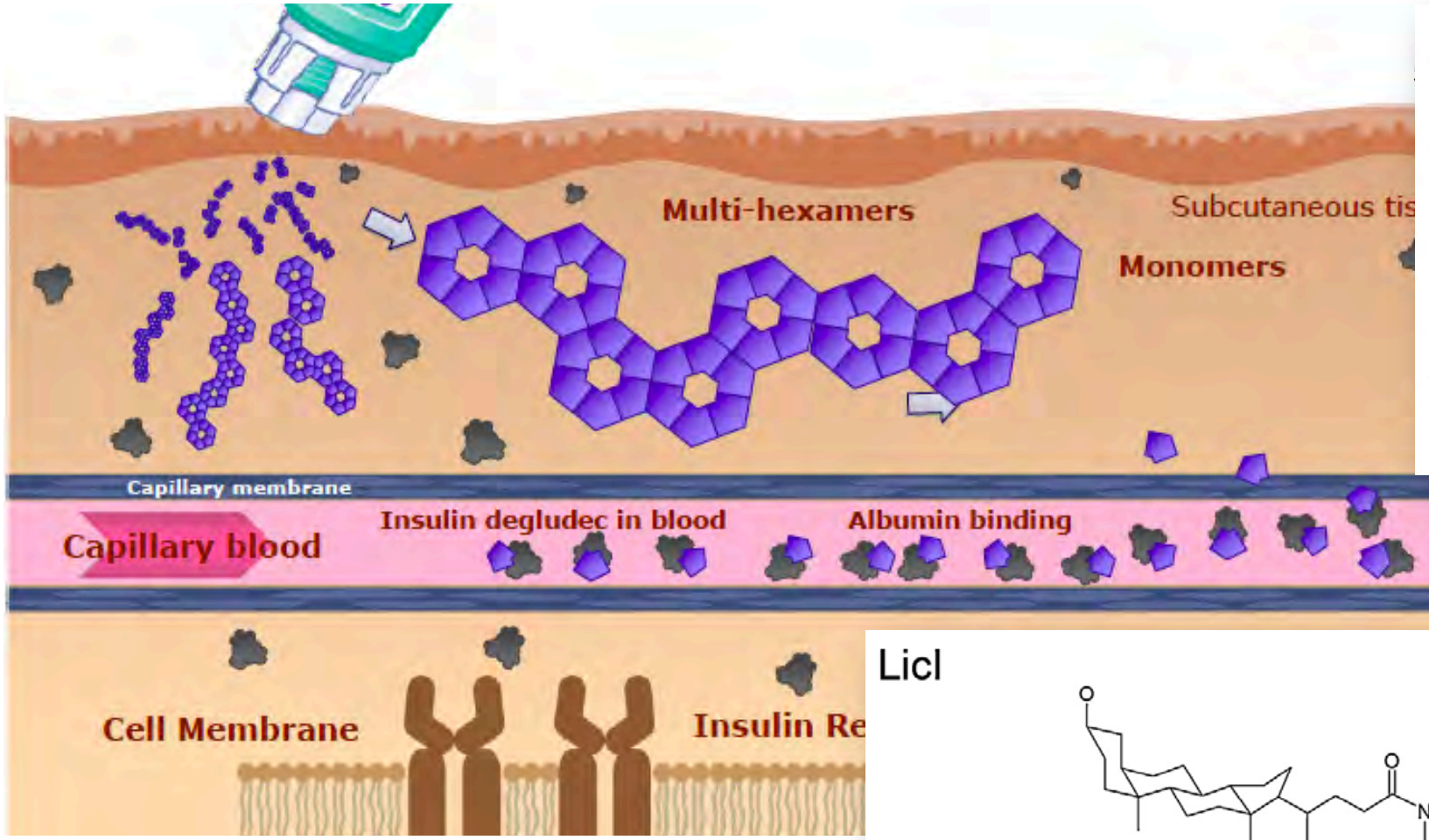
Vetri V.et al (2013) PloS One

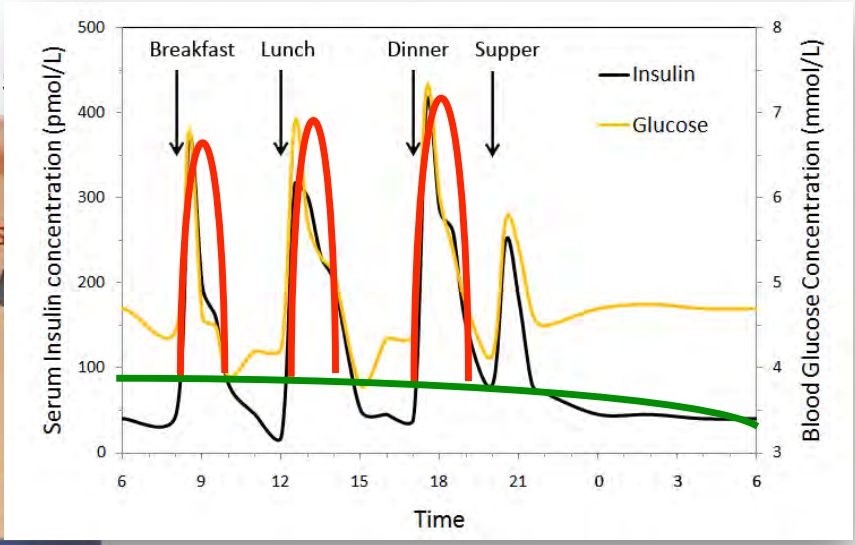
Coupling with Size Exclusion Chromatography

- Separate the molecular species according to size on a HPLC column
- Measure light scattering and derive molar mass on individual fractions
- Measure conc. of individual fractions via the refractive index



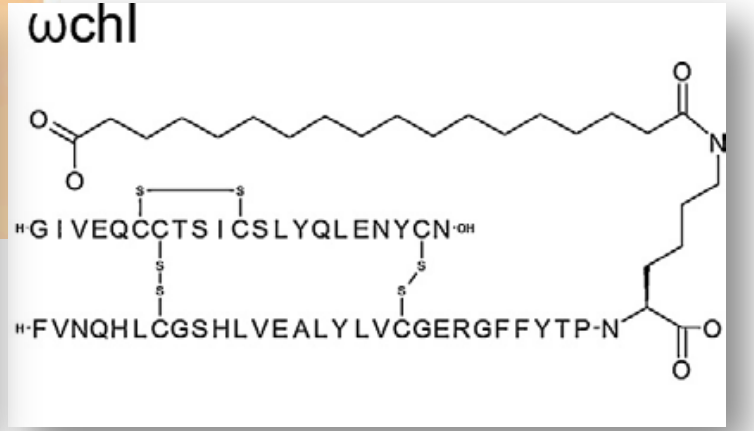
Therapeutically relevant insulin oligomerization





Fast acting insulin

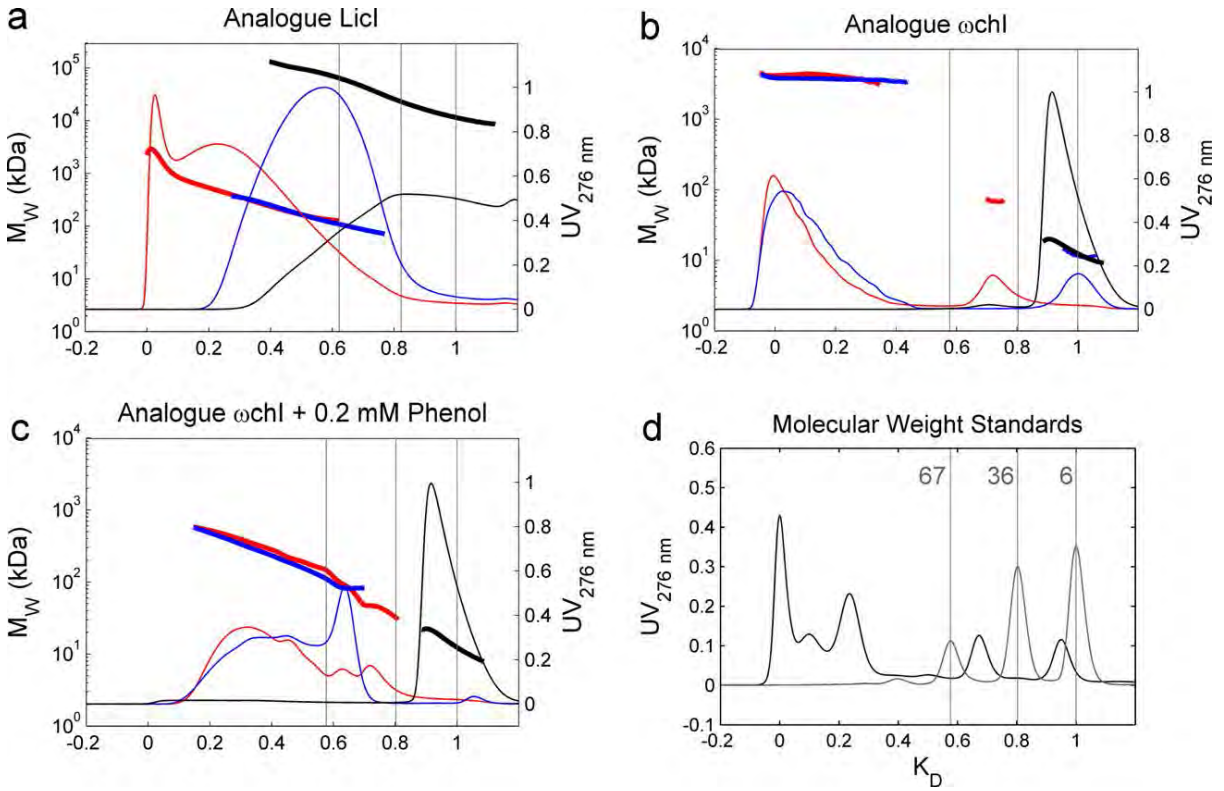
Long acting insulin



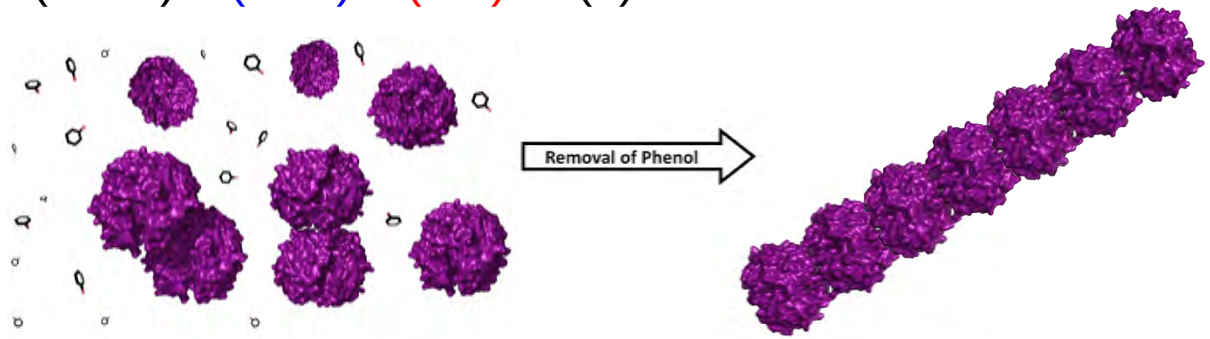
Protein based drugs:

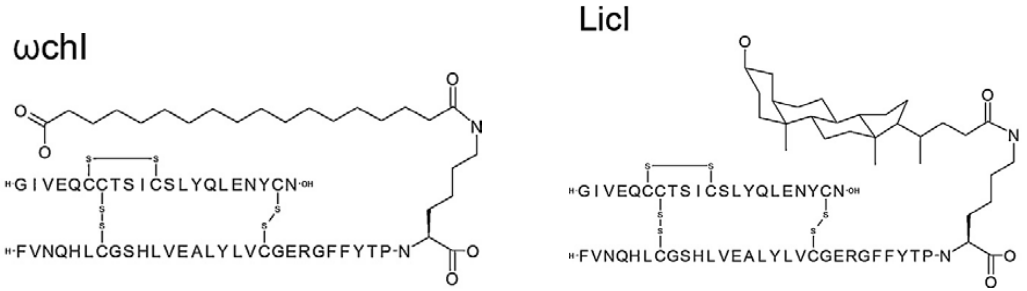
- Typically proteins in solution to be injected
- Control of release profile is desirable

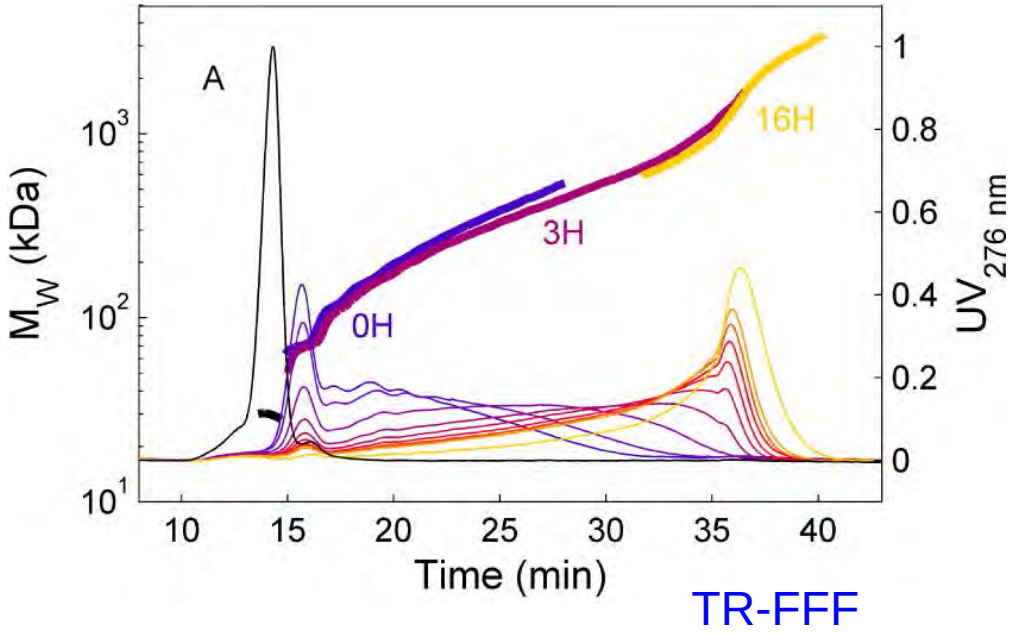
Tuning experimental conditions by SEC-MALS



0 (black) 3 (blue) 6 (red) Zn(II)/6 Ins.



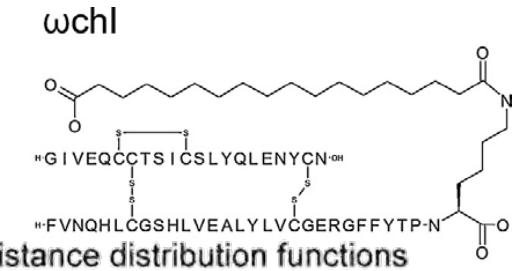


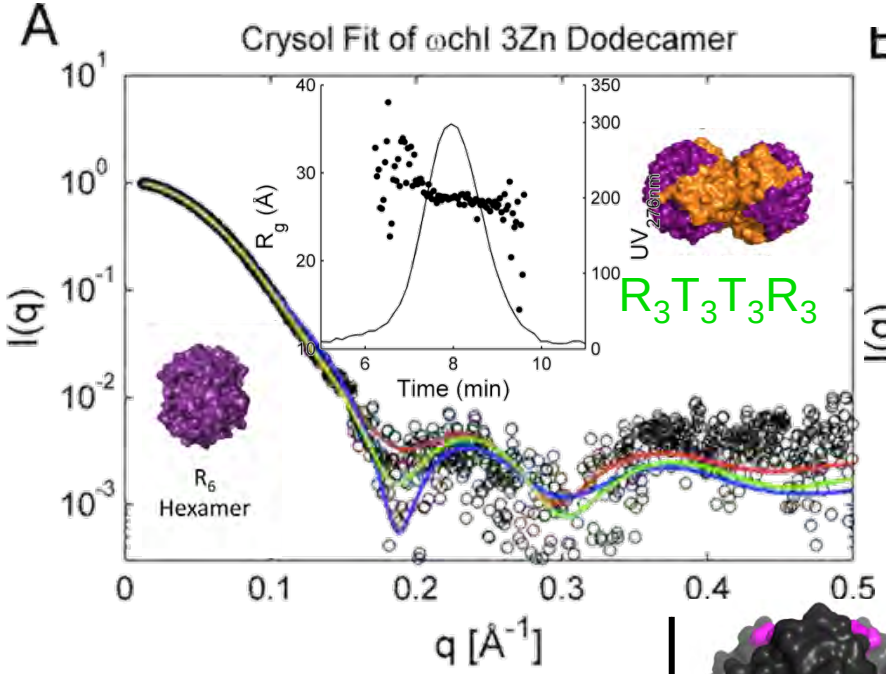


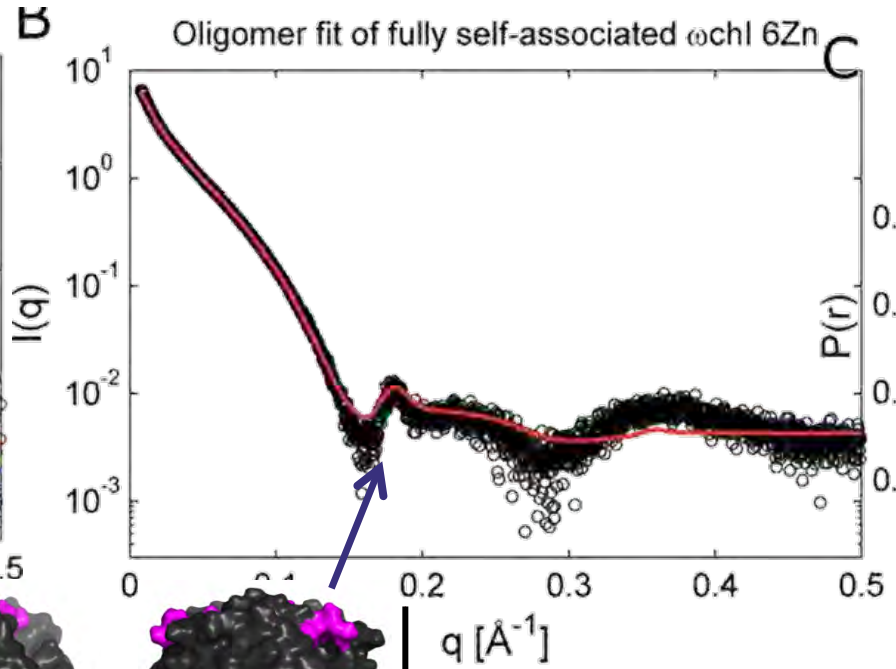
TR-FFF

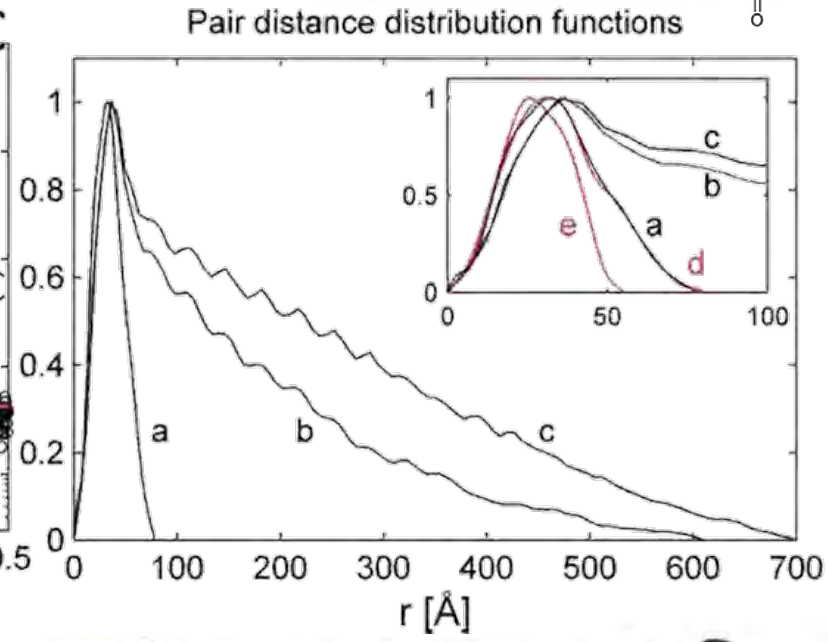
Jensen, M. H. et al (2011) J. Chrom. B;
Jensen, M.H. et al (2013) Biochemistry

Tuning experimental conditions by SEC-MALS



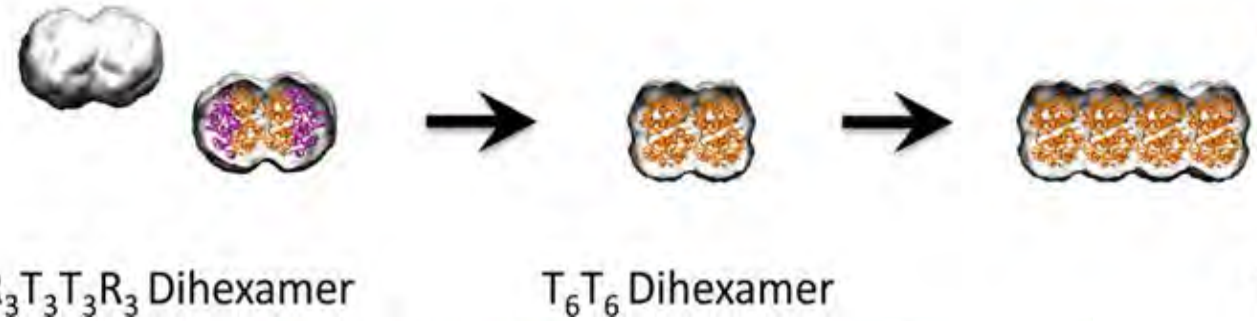


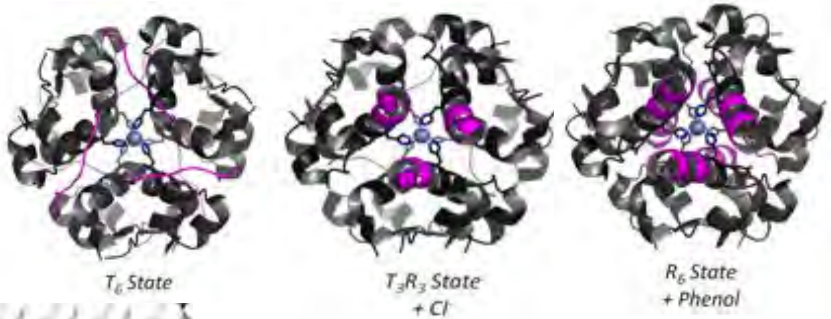




39.3±0.3 Å

33.9±0.2 Å





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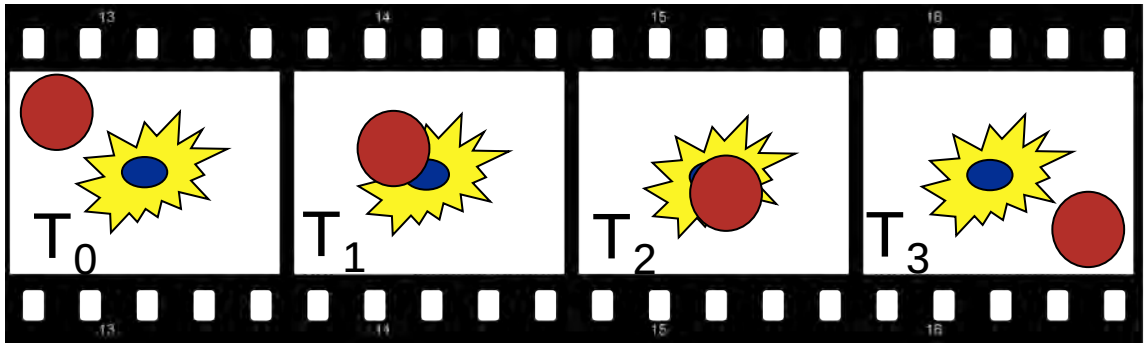
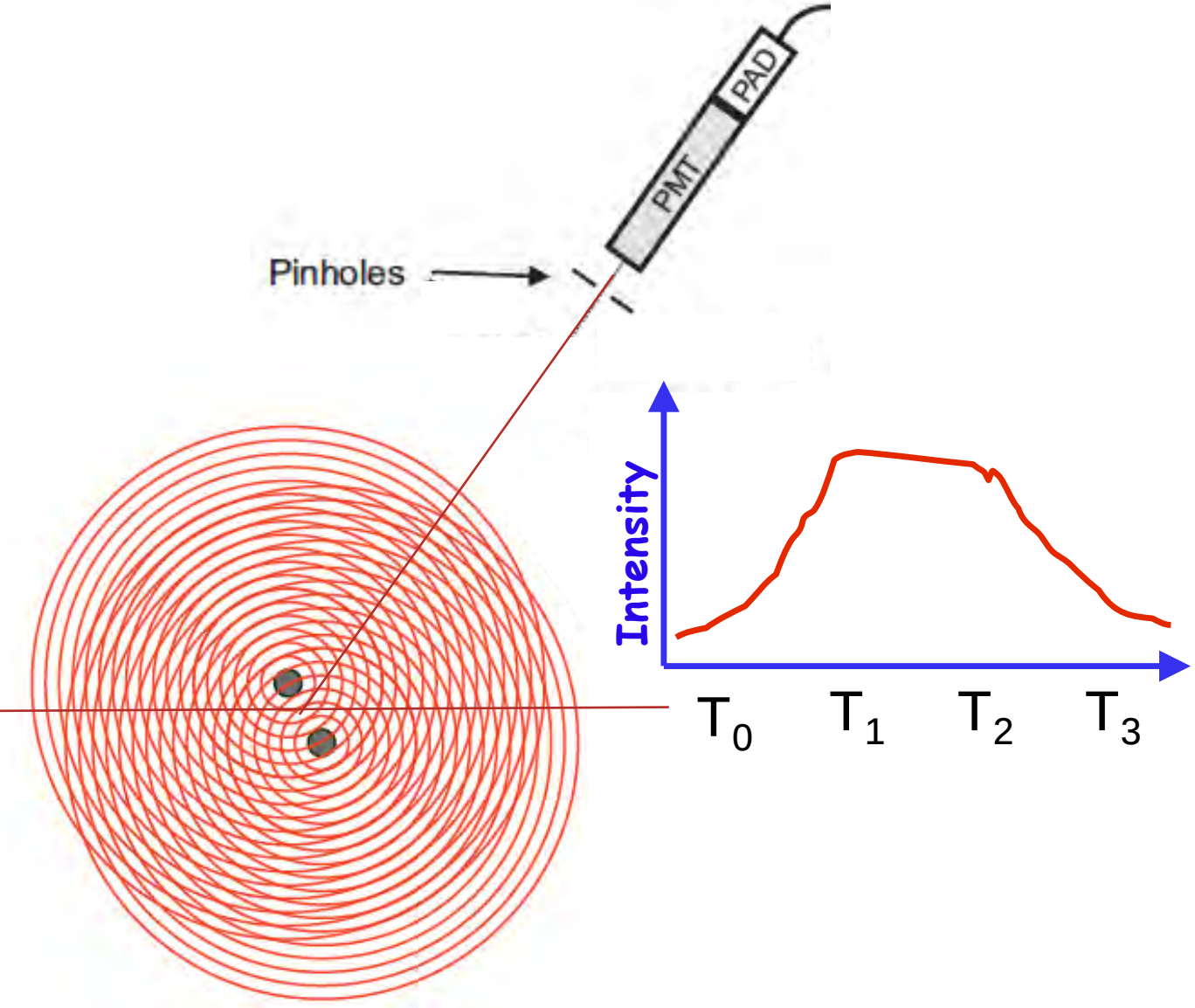
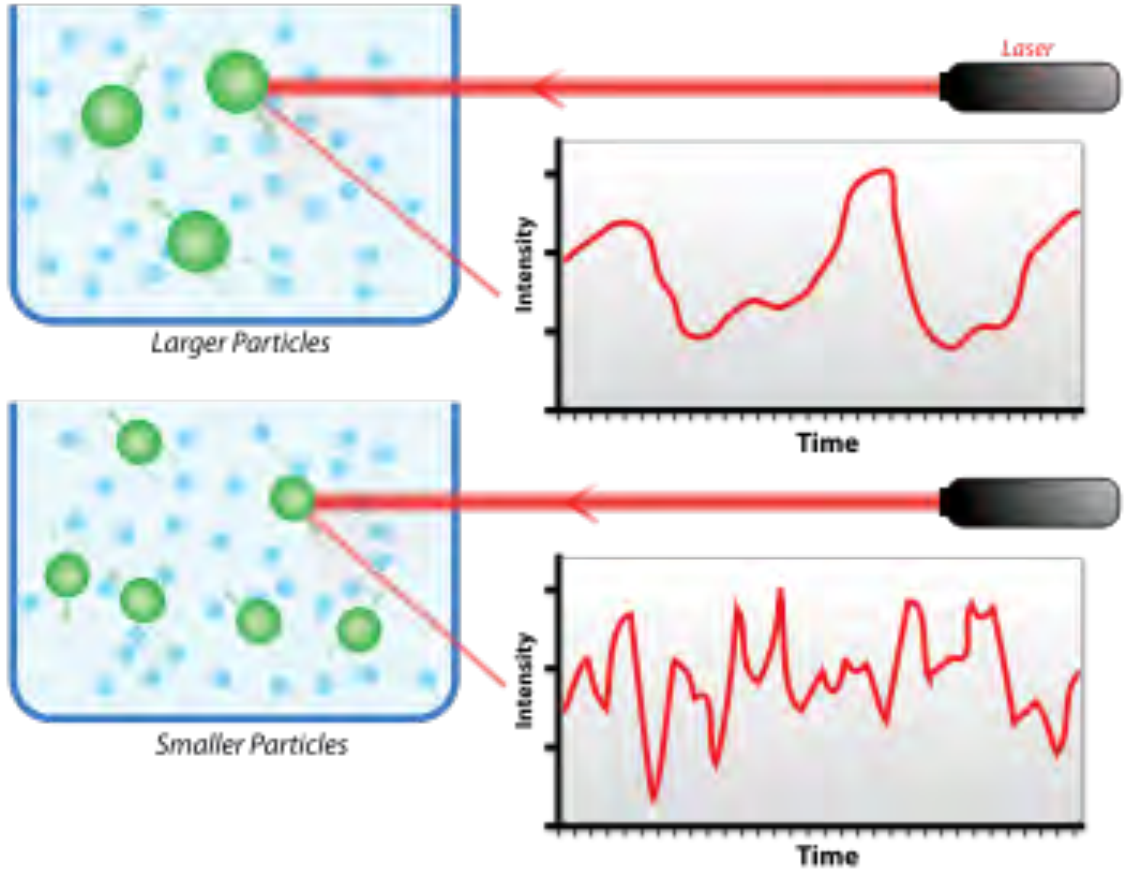
Jensen, M.H. et al (2013) Biochemistry

Dynamic Light Scattering

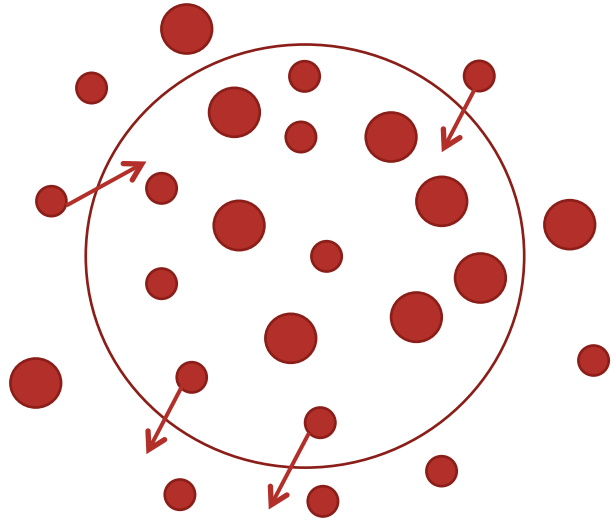


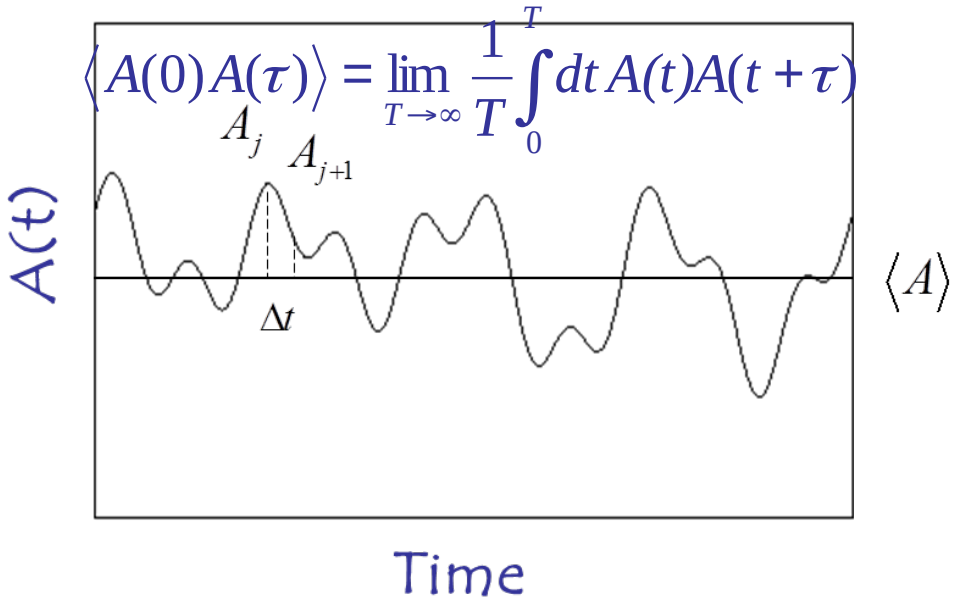




Dynamic light scattering – principle of measurement

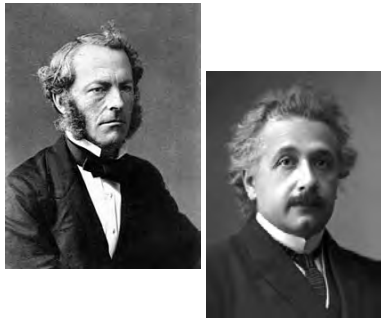
- Fluctuations: Reflect the diffusion coefficient of the particles. DLS employs measurements in a time series, averaging over very short time intervals (typically 100 nsec).
- Frequency of fluctuations depends on how fast the particles move (large particles move slowly – small particles move faster ...)
- Amplitude of fluctuations depends on particle size, contrast, and concentration (for a given fixed λ)





The Stokes-Einstein relation for spherical particles:

$$D = \frac{k_B T}{6\pi\eta r}$$



$$r_h = \frac{k_B T}{6\pi\eta D_{meas.}}$$

The hydrodynamic radius

T: absolute temperature
k_b: Boltzmann's constant
η: Viscosity of liquid

The characteristic decay time

Diffusion in one dimension:

$$\langle x^2 \rangle = 2D \cdot t$$

D: Diffusion coefficient
t: time
x: displacement

$$x = \frac{1}{q}$$

Characteristic diffusion distance for change in interference:

$$\left(\frac{1}{q}\right)^2 = 2D\tau_0 \Rightarrow \tau_0 = \frac{1}{2Dq^2}$$

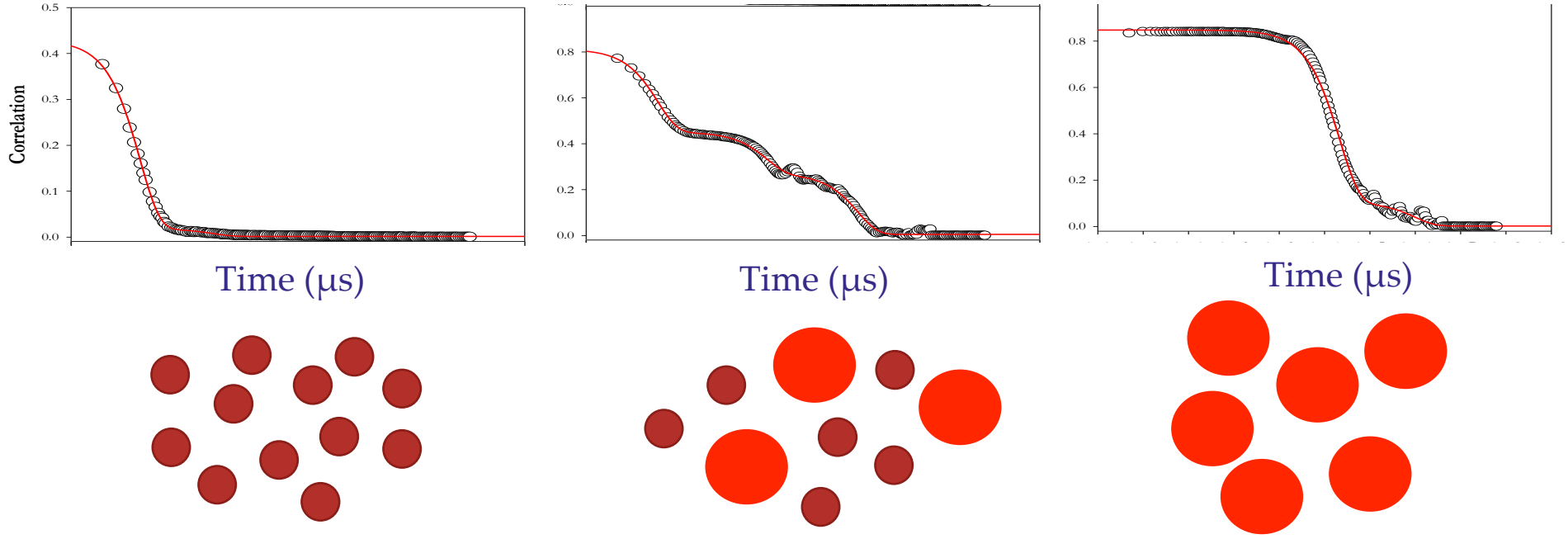
Measure of the diffusion coefficient *D*
Then calculate equivalent hydrodynamic radius:

Range: Down to $r_h \sim 1$ nm
Up to $r_h \sim 1000$ nm

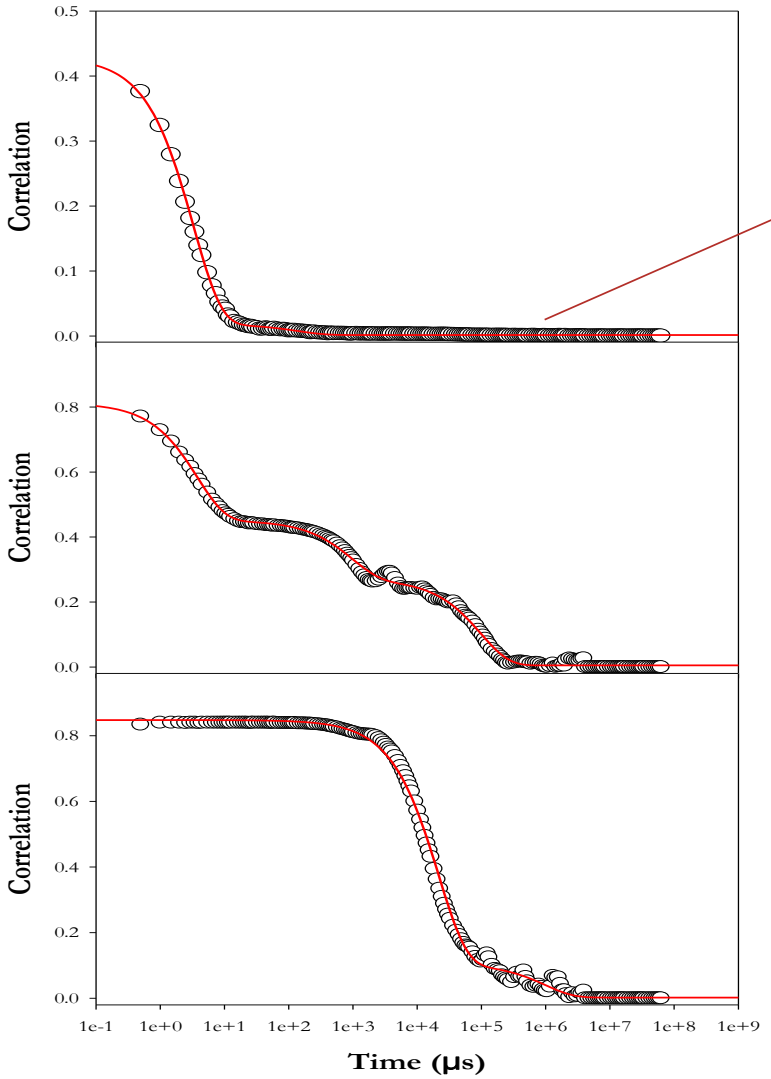
Dynamic light scattering – principle of measurement

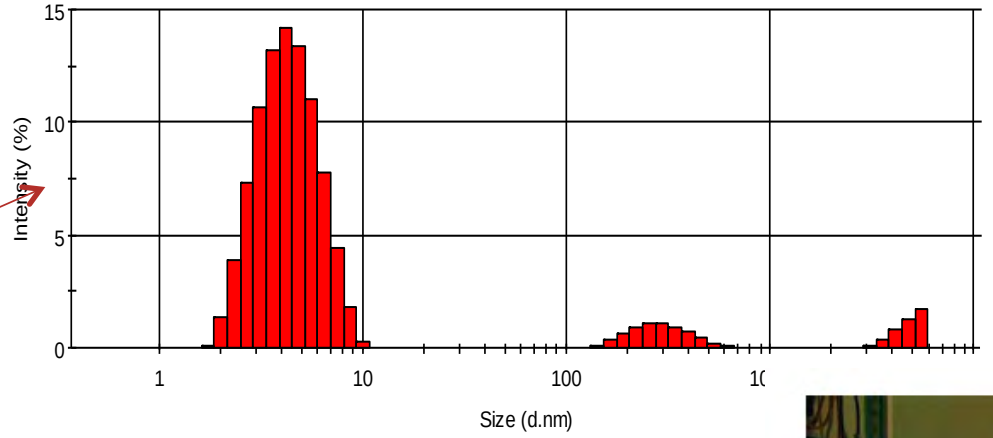
$$G_2(\vec{q},t) = \frac{< \left|A(\vec{q},t)\right| \ \left|A(\vec{q},t+\tau)\right| >}{< \left|A(\vec{q},t)\right| >^2} = < N >^2 + < N >^2 \ e^{-2Dq^2\tau}$$

The Auto-correlation funtion: Cross-correlation of a signal with itself over time (similarity as a function of the time-lag between signals)

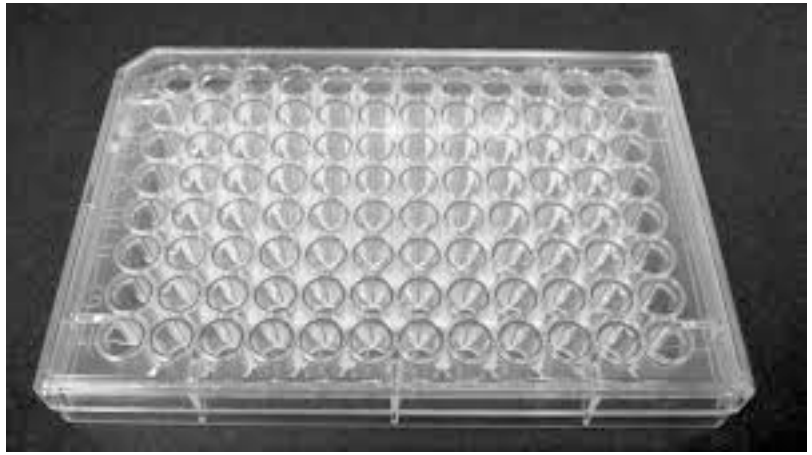


Size distribution for macromolecules in solution

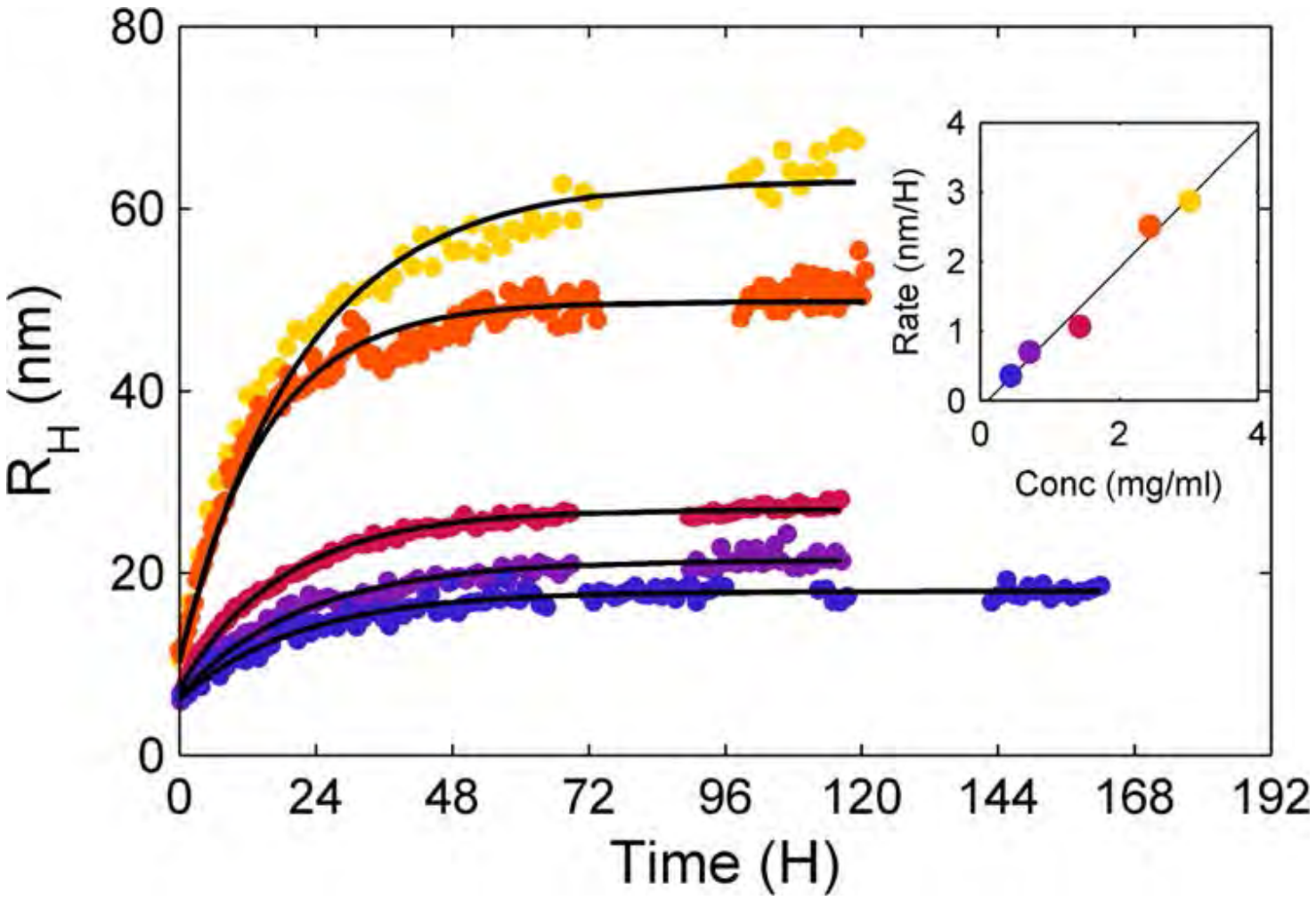


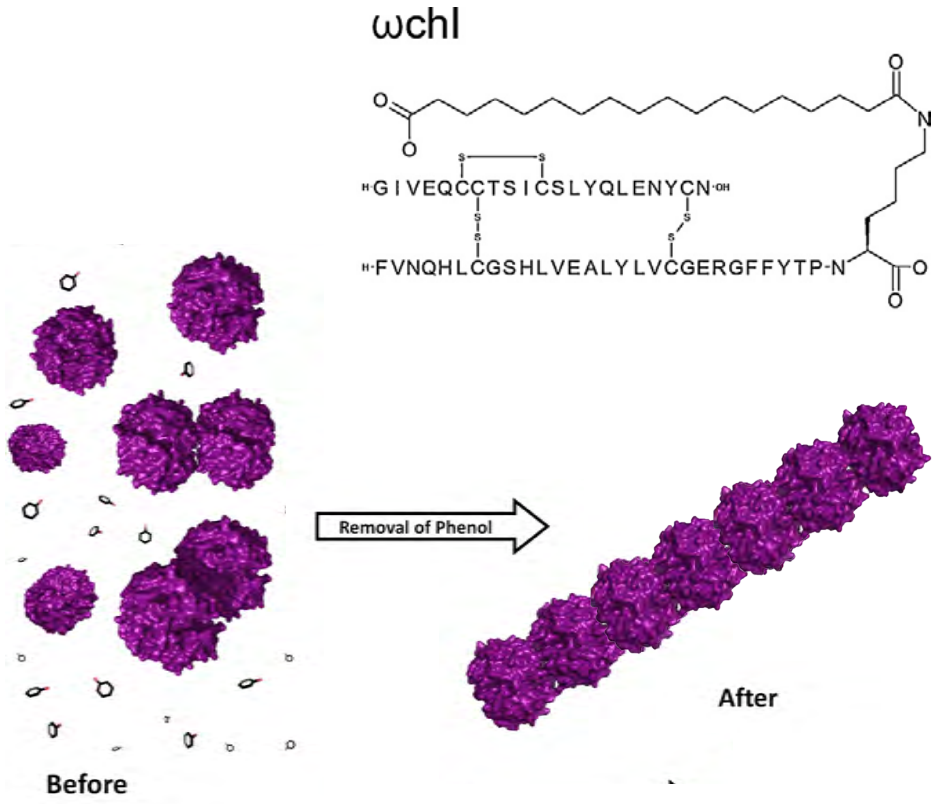


- Quantitative information
- Easy analysis (if the software automatically does it)



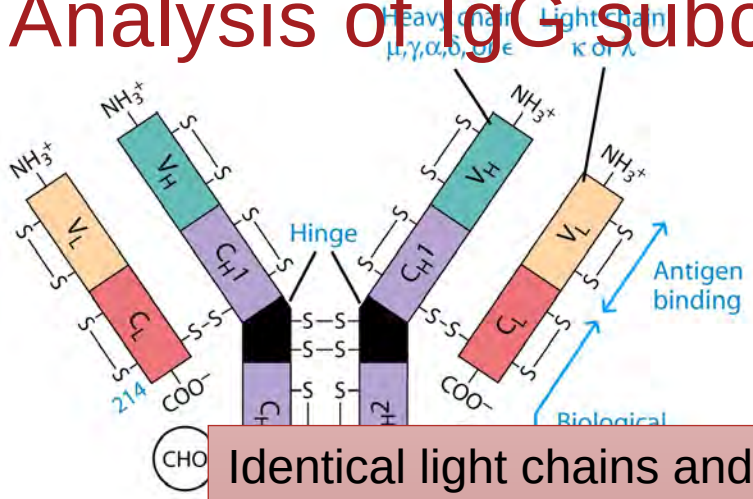
Back to the growing oligomers of insulin analogues



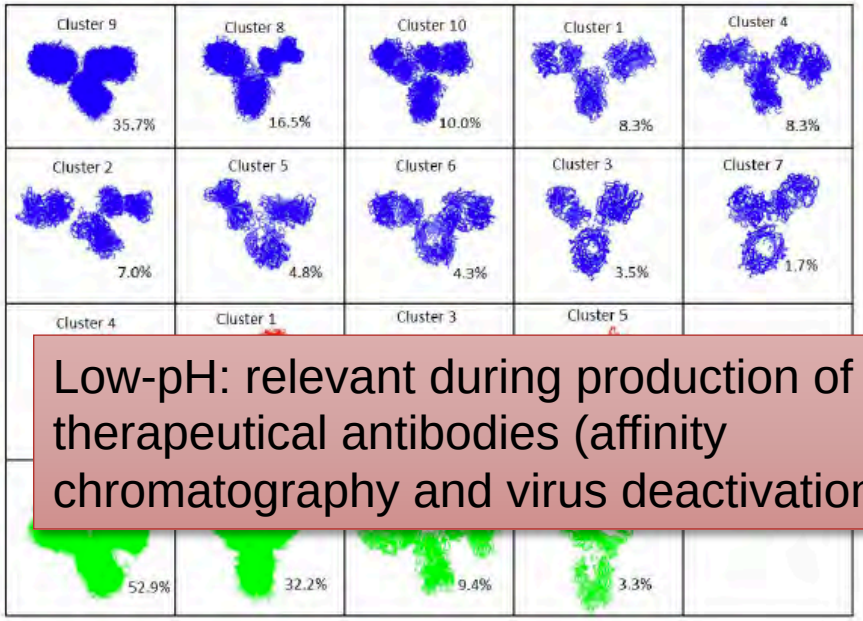


Note complementarity and advantages with DLS versus SLS?

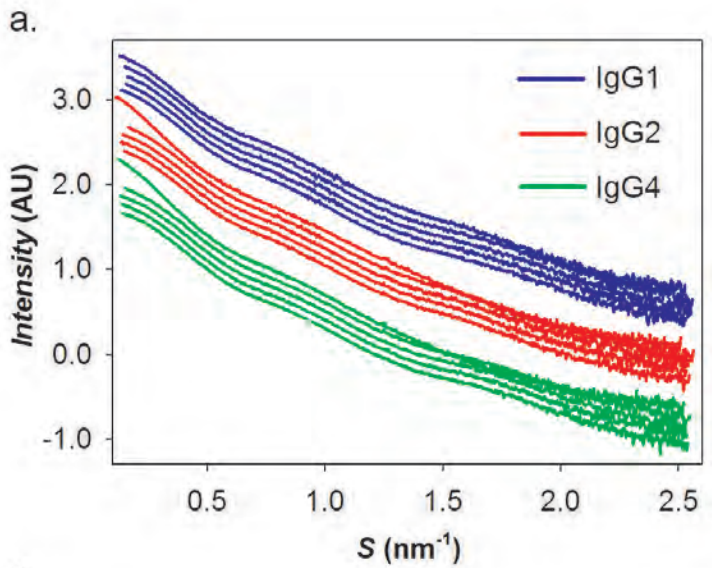
Analysis of IgG subclass structure and aggregation properties

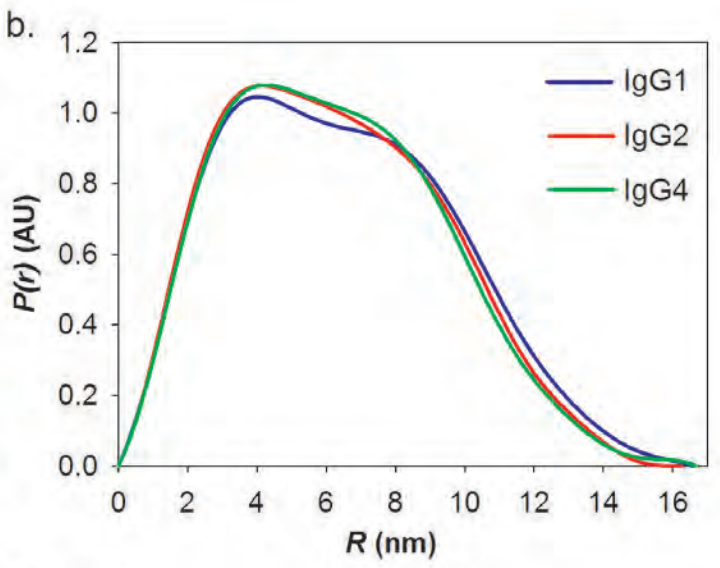


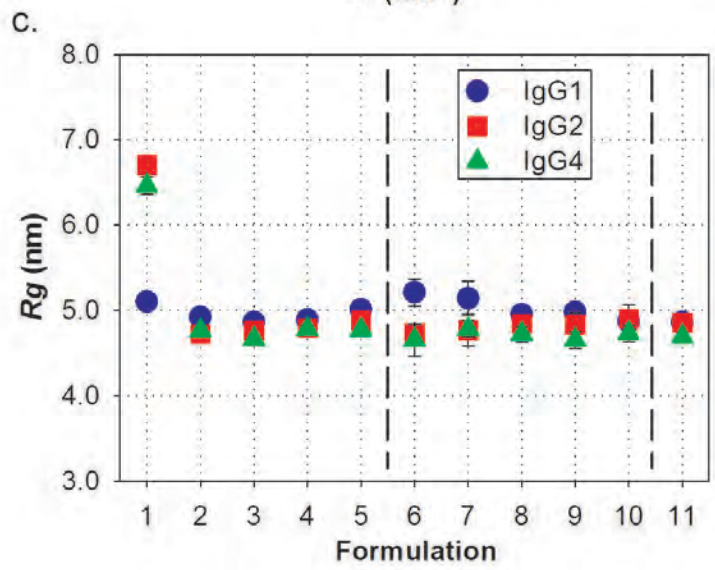
Identical light chains and identical variable regions in the heavy chain

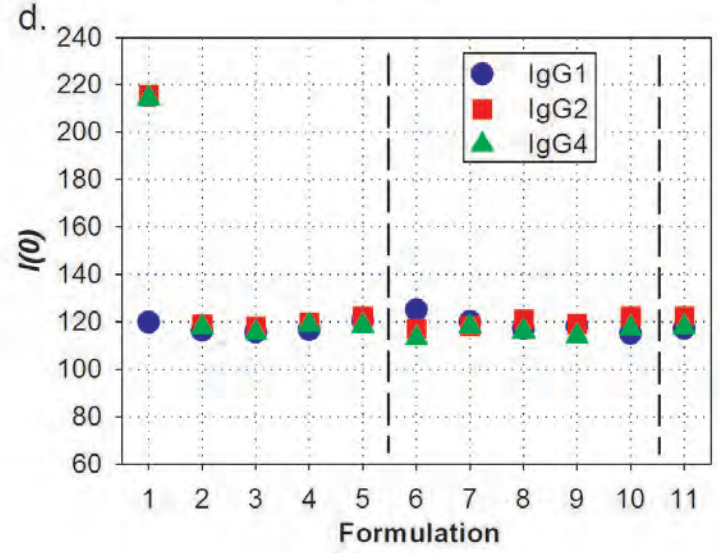


Low-pH: relevant during production of therapeutical antibodies (affinity chromatography and virus deactivation)



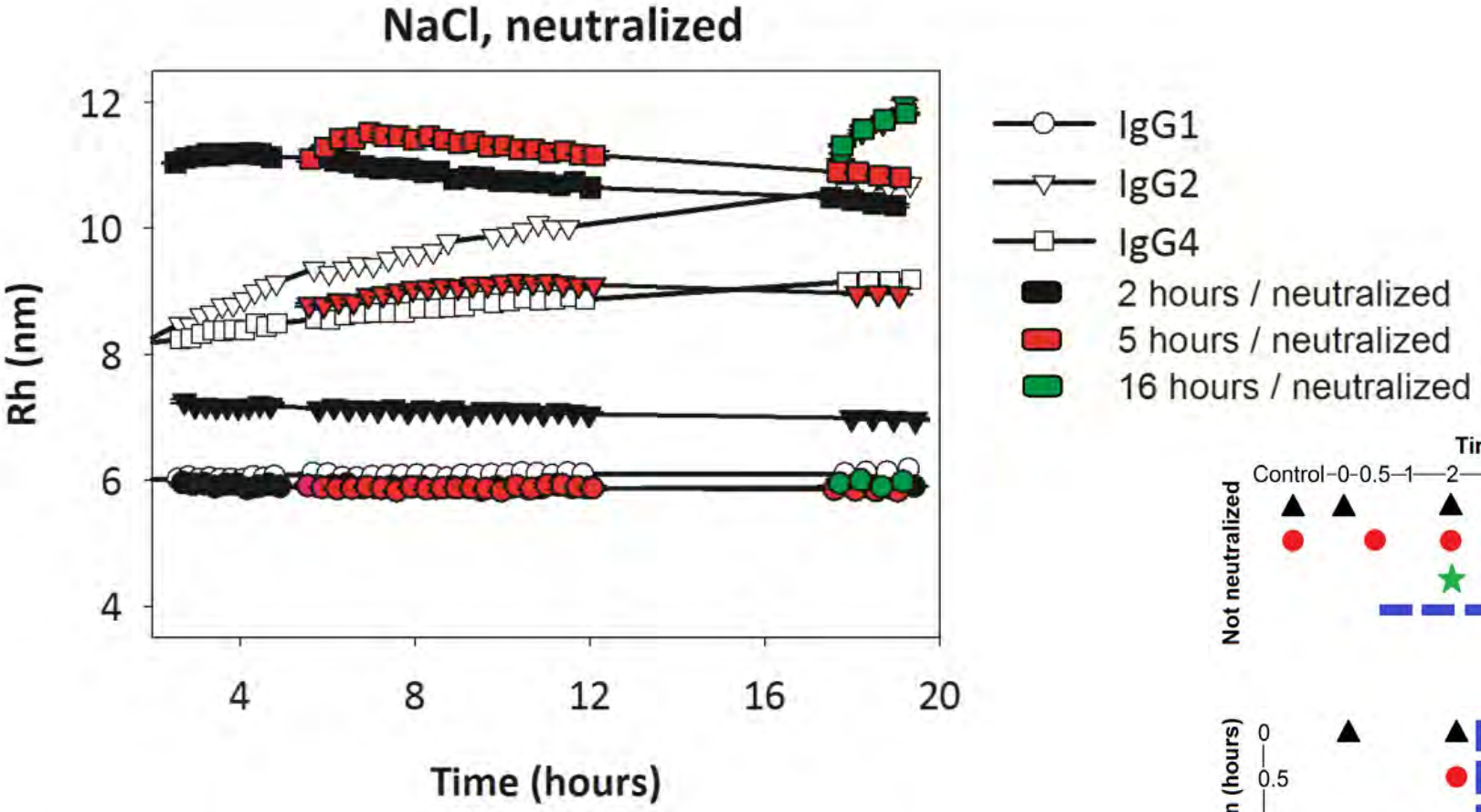


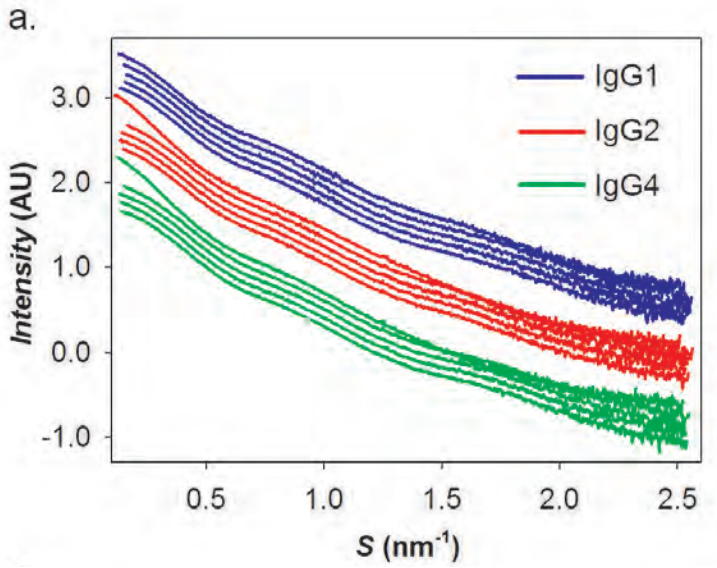


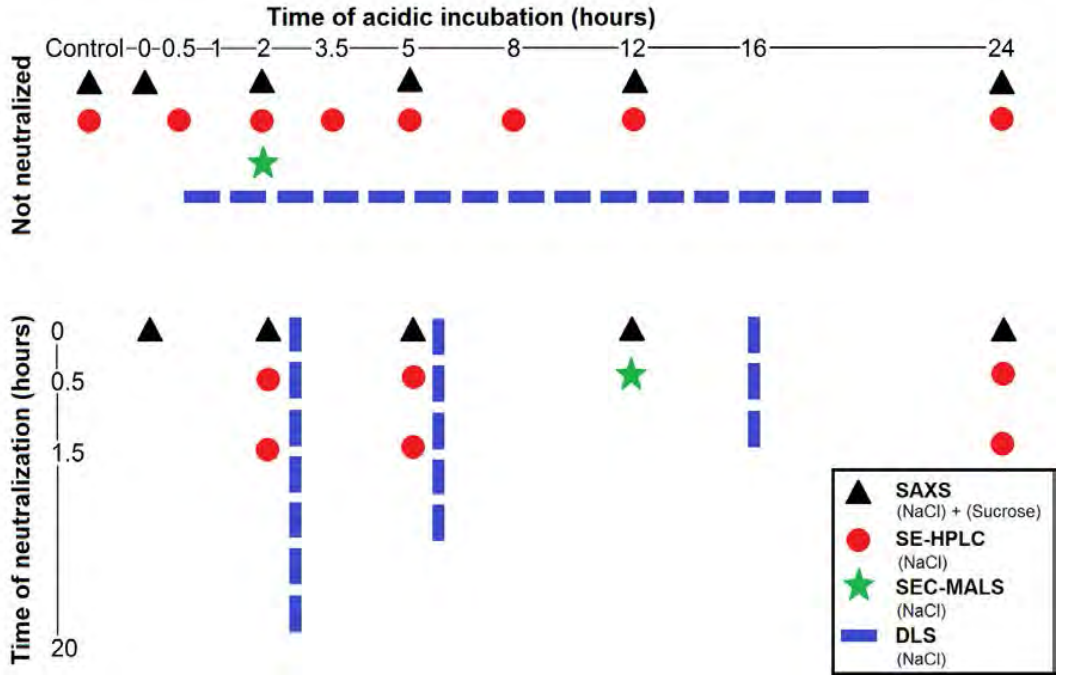


Tian et al. (2014) J. Pharm. Sci. Tian et al. (2015) IUCr J.

Low-pH induced aggregation study



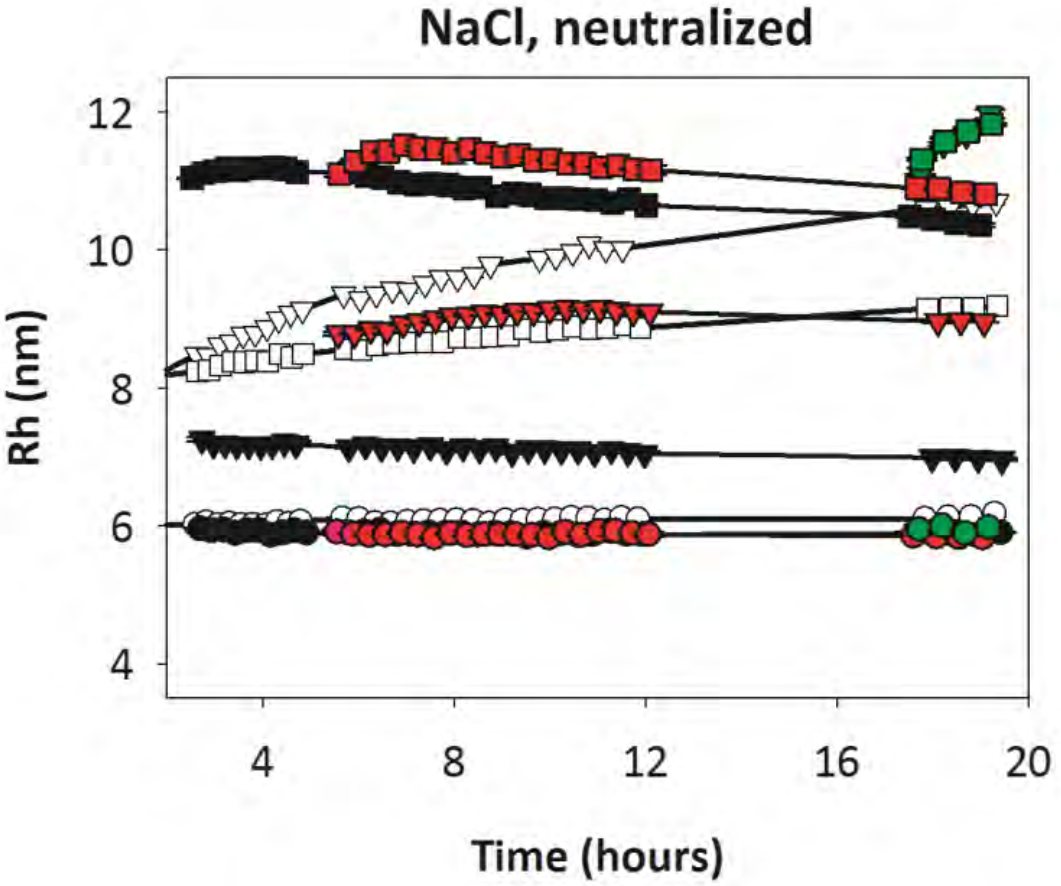


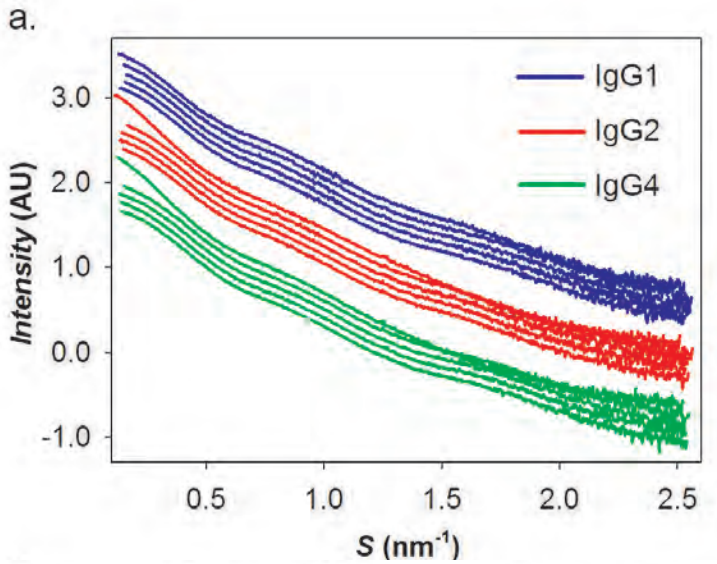


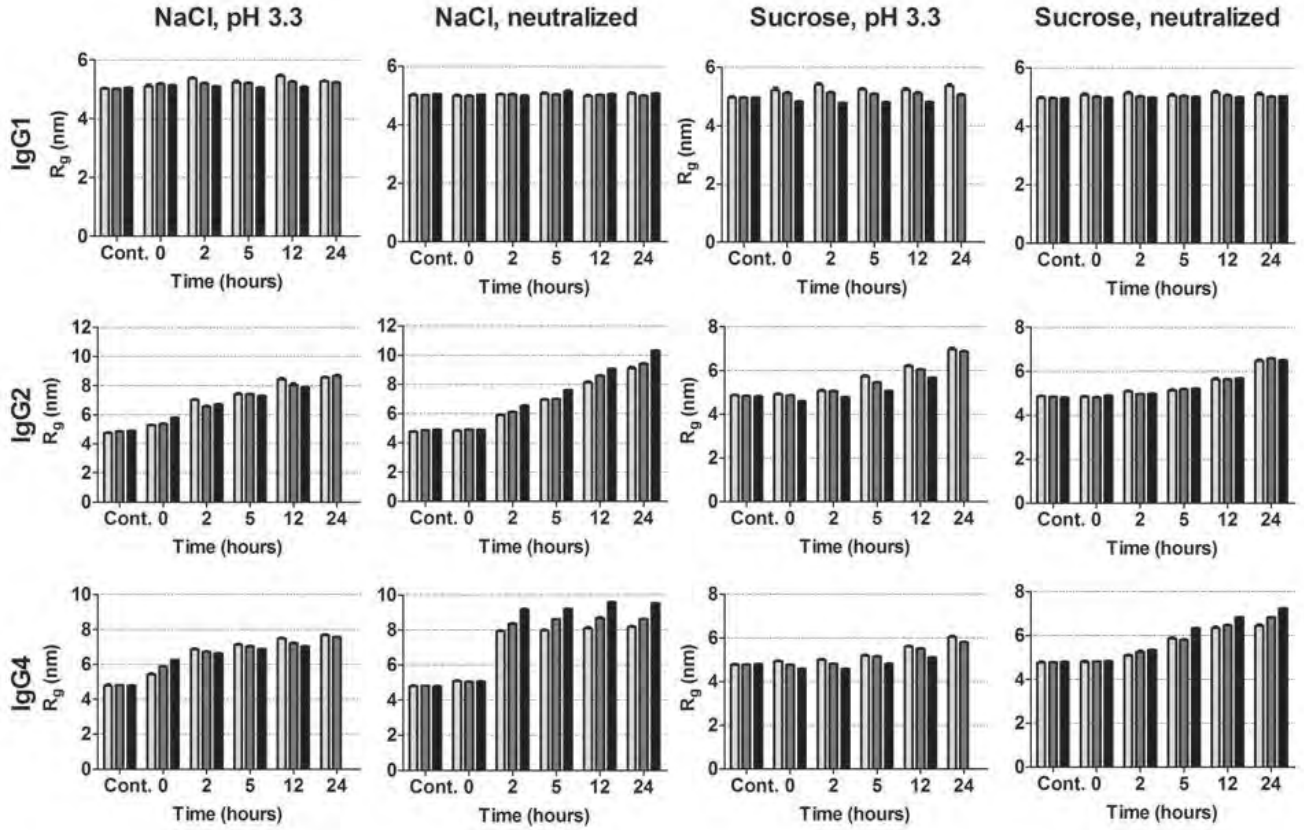
Low-pH: relevant during production of therapeutical antibodies (affinity chromatography and virus deactivation)

Skamris, Tian et al. (2016) Pharm Res

Low-pH induced aggregation study







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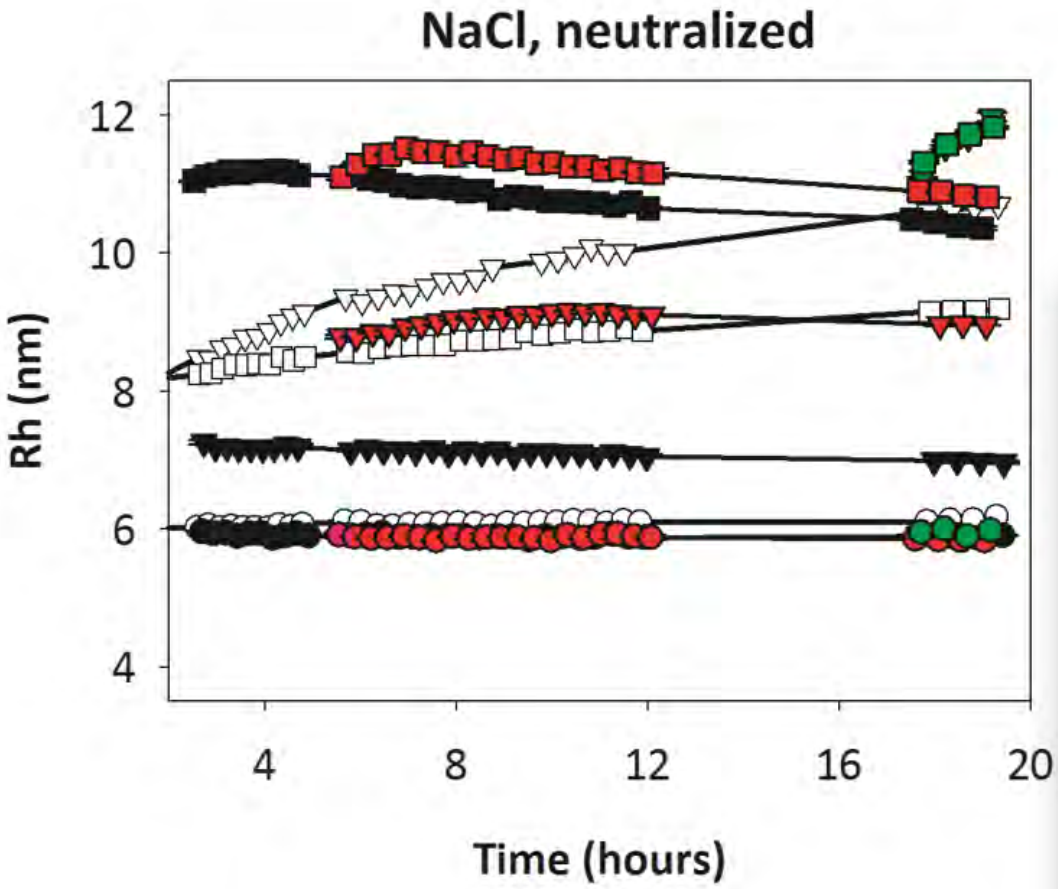
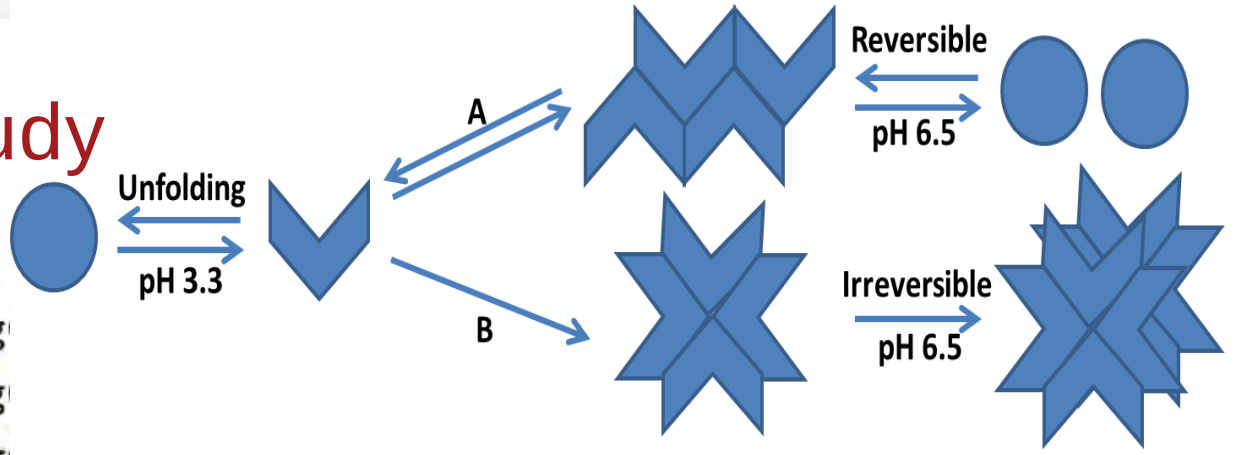
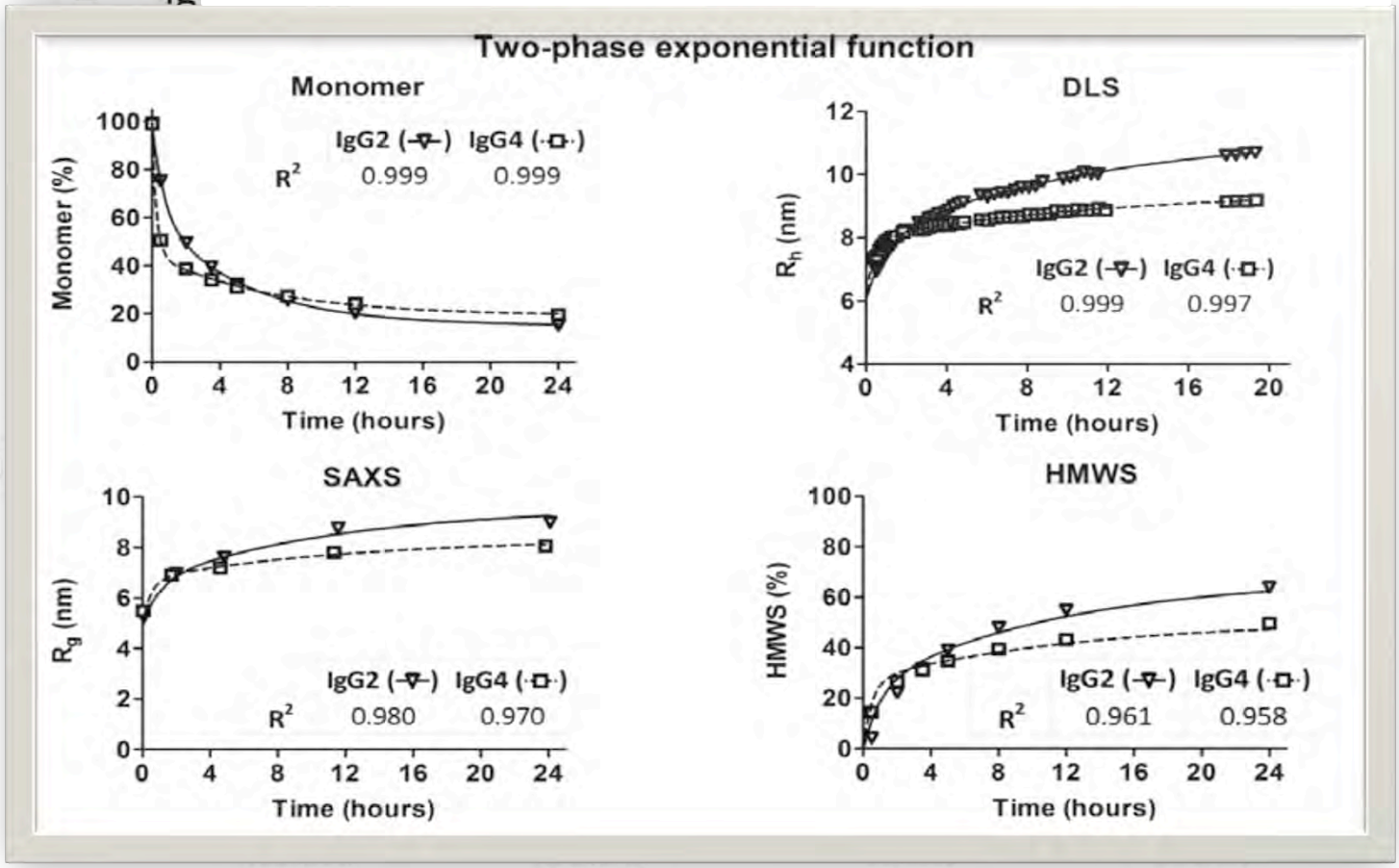
Low-pH induced aggregation study







Low-pH: relevant during production of therapeutical antibodies (affinity chromatography and virus deactivation)

Skamris, Tian et al. (2016) Pharm Res

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Thank you for your attention

Questions?

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ConA aggregation: Valeria Vetri & Maurizio Leone, University of Palermo; Vito Foderà, University of Copenhagen

Insulin analogue oligomerisation: Malene H. Jensen & Marco van de Weert, University of Copenhagen; Per-Olof Wahlund, Dorte B. Steensgaard, Jes K. Jacobsen & Svend Havelund, Novo Nordisk A/S

Antibody flexibility and oligomerisation: Xinsheng Tian, Thomas Skamris & Annette E. Langkilde, University of Copenhagen; Mattias Throlofsson, Hanne S. Karkov & Hanne Rasmussen, Novo Nordisk A/S

BioSAXS group @ University of Copenhagen

http://igm.fys.ku.dk/~lho/personal/lho/lightscattering_theory_and_practice.pdf