Analytical Ultracentrifugation of Nanoparticles

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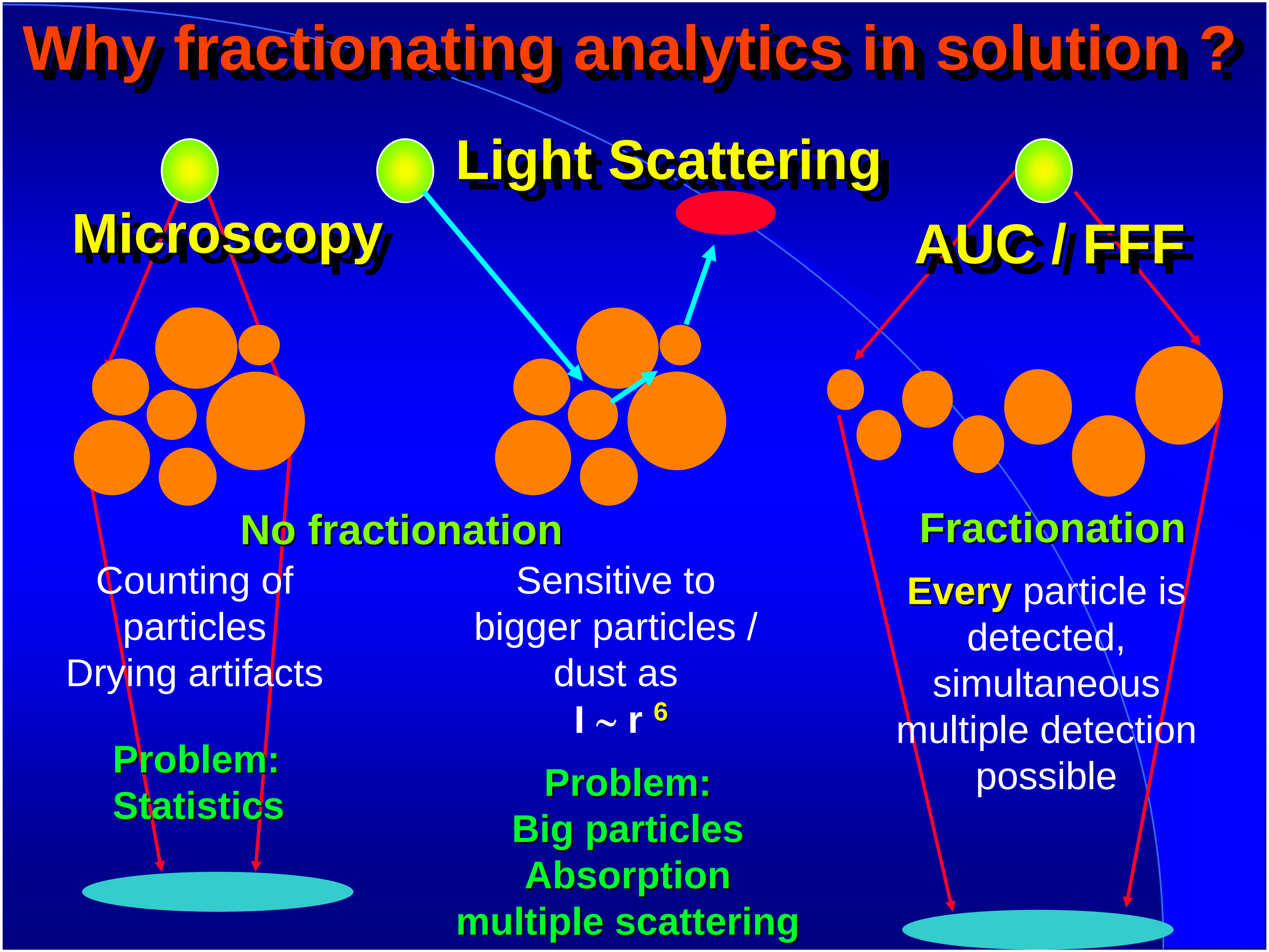

Why fractionating analytics in solution ?
Microscopy
Light Scattering
AUC / FFF
No fractionation
Fractionation
Counting of particles
Drying artifacts
Problem:
Statistics
Sensitive to bigger particles / dust as
I ~ r 6
Problem:
Big particles
Absorption
multiple scattering
Every particle is detected,
simultaneous multiple detection possible

Svedbergs Colloid Research

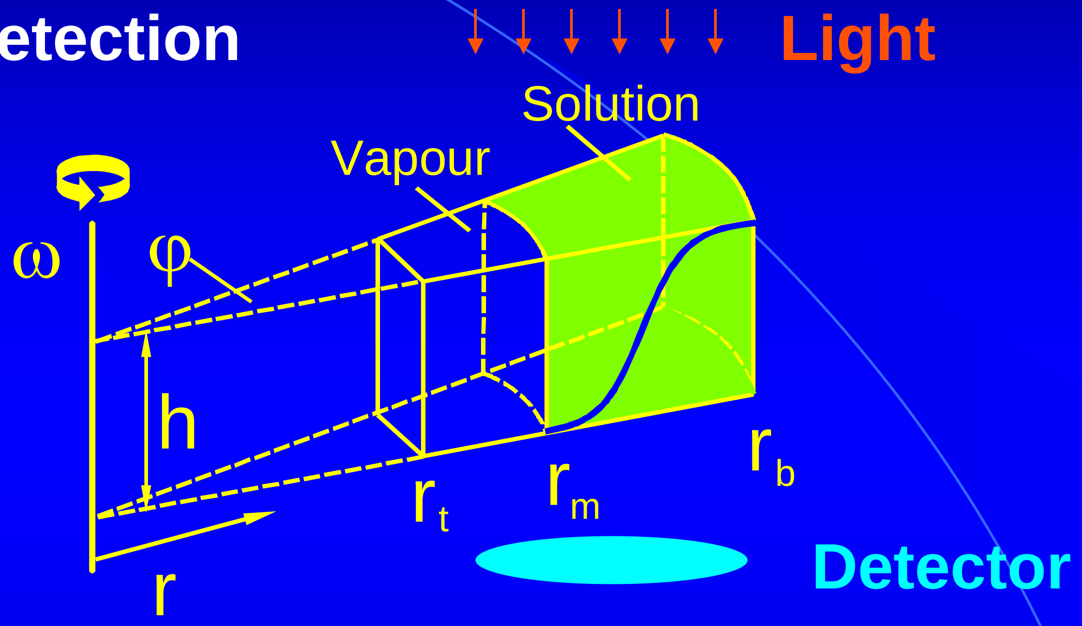
- T. Svedberg, J.B. Nichols, *J. Amer. Chem. Soc.* 45 (1923) 2910
- T. Svedberg, H. Rinde, *J. Amer. Chem. Soc.* 45 (1923) 943
- T. Svedberg, H. Rinde, *J. Amer. Chem. Soc.* 46 (1924) 2677
- T. Svedberg, Kolloid-Z. Zsigmondy Festschrift, Erg.-Bd. Zu 36 (1925) 53

1925: Nobel prize for colloid work

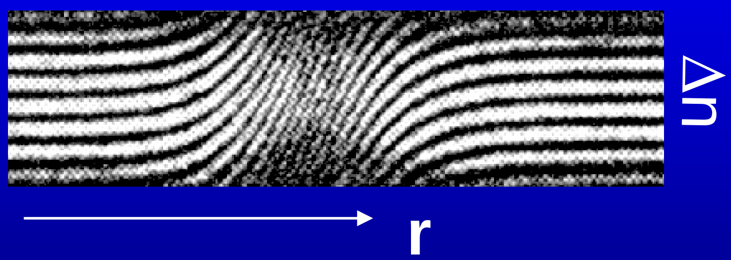
1926: First protein work

Principle of AUC

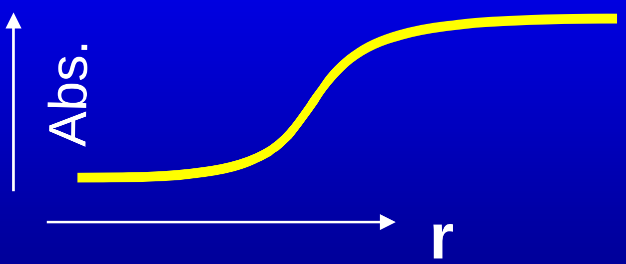
Radial Detection



Rayleigh Interference optics



UV/VIS Absorption optics

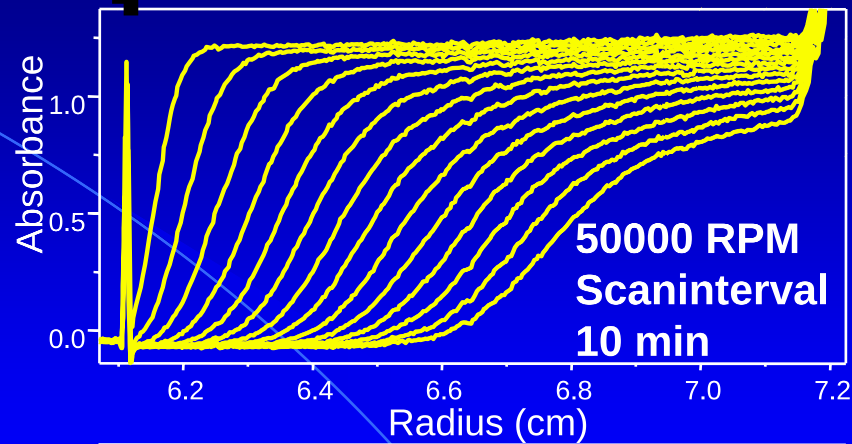


Experiments very simple, evaluation not always

Different AUC experiments

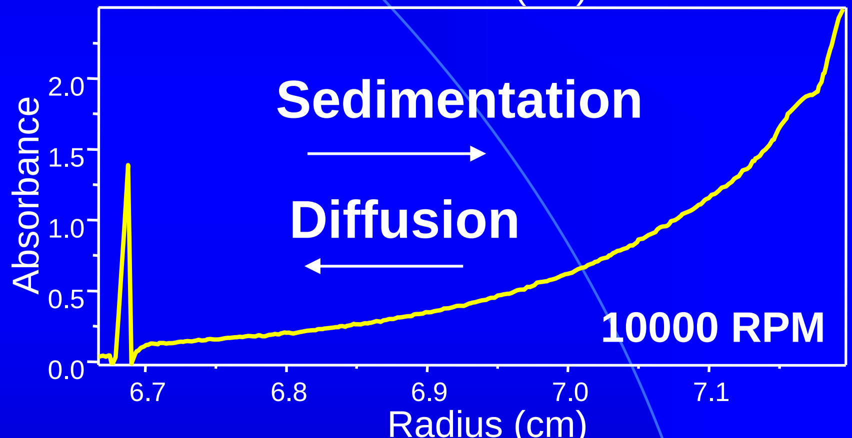
Sedimentation velocity

High centrifugal force
Sedimentation stronger than back diffusion



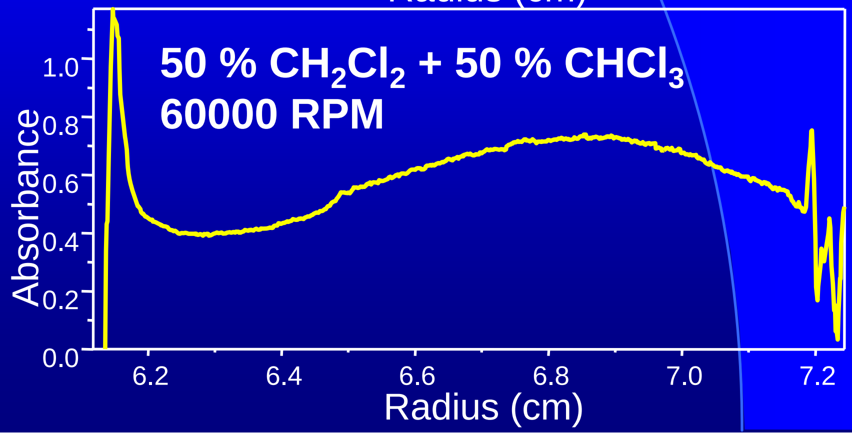
Sedimentation equilibrium

Moderate centrifugal force
Sedimentation in the order of back diffusion



Density gradient

High centrifugal force for distribution of a low molecular salt or a second solvent

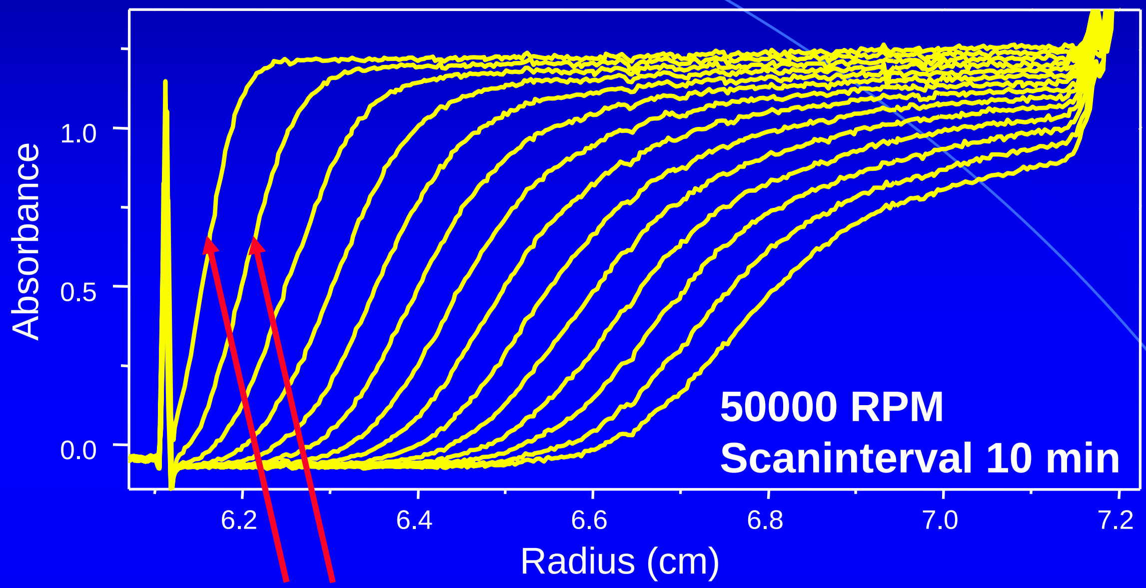


Lamm equation

$$\frac{\partial c}{\partial t} = \frac{1}{r}\frac{d}{dr}\left(\underbrace{r D \frac{dc}{dr}}_{\text{Diffusion term}} - \underbrace{s \omega^2 r^2 c}_{\text{Sedimentation term}} \right)$$

Experiment	Effective term in the Lamm equation	Characteristics
Sedimentation velocity	Sedimentation term much bigger than diffusion term	High rotational speed
Synthetic boundary experiment for the determination of D	Only diffusion term effective	Synthetic boundary cell, low rotational speed
Sedimentation velocity	Sedimentation and diffusion term effective / equilibrium between sedimentation and diffusion	Moderate / low rotational speed
Density gradient (special case of sedimentation equilibrium)	Sedimentation and diffusion term effective / equilibrium between sedimentation and diffusion	Moderate / high rotational speed, locally dependent solution density

Basic evaluation important for nanoparticles



1 step means 1 component

Flat baseline indicates purity

a) **Determine distance travelled in given time interval**

b) **Calculate sedimentation coefficient s or its distribution**

c) **Calculate particle size or distribution**

$$s = \frac{u}{\omega^2 r} \qquad s = \frac{\ln(r/r_m)}{\omega^2 t} \qquad d_i = \sqrt{\frac{18\eta s_i}{\rho_2 - \rho}}$$

Sedimentation velocity

$$M = \frac{sRT}{D\left(1 - \bar{v}\rho\right)} \qquad f = \frac{RT}{N_A D} \qquad d_i = \sqrt{\frac{18\eta s_i}{\rho_2 - \rho}}$$

Sedimentation velocity depends on:

Molar mass / particle size
Density
Shape / Friction
Charge

for given exptl. parameters (temperature, solvent density & viscosity etc.)

Particle size distributions calculated on a hard sphere basis

Sedimentation velocity

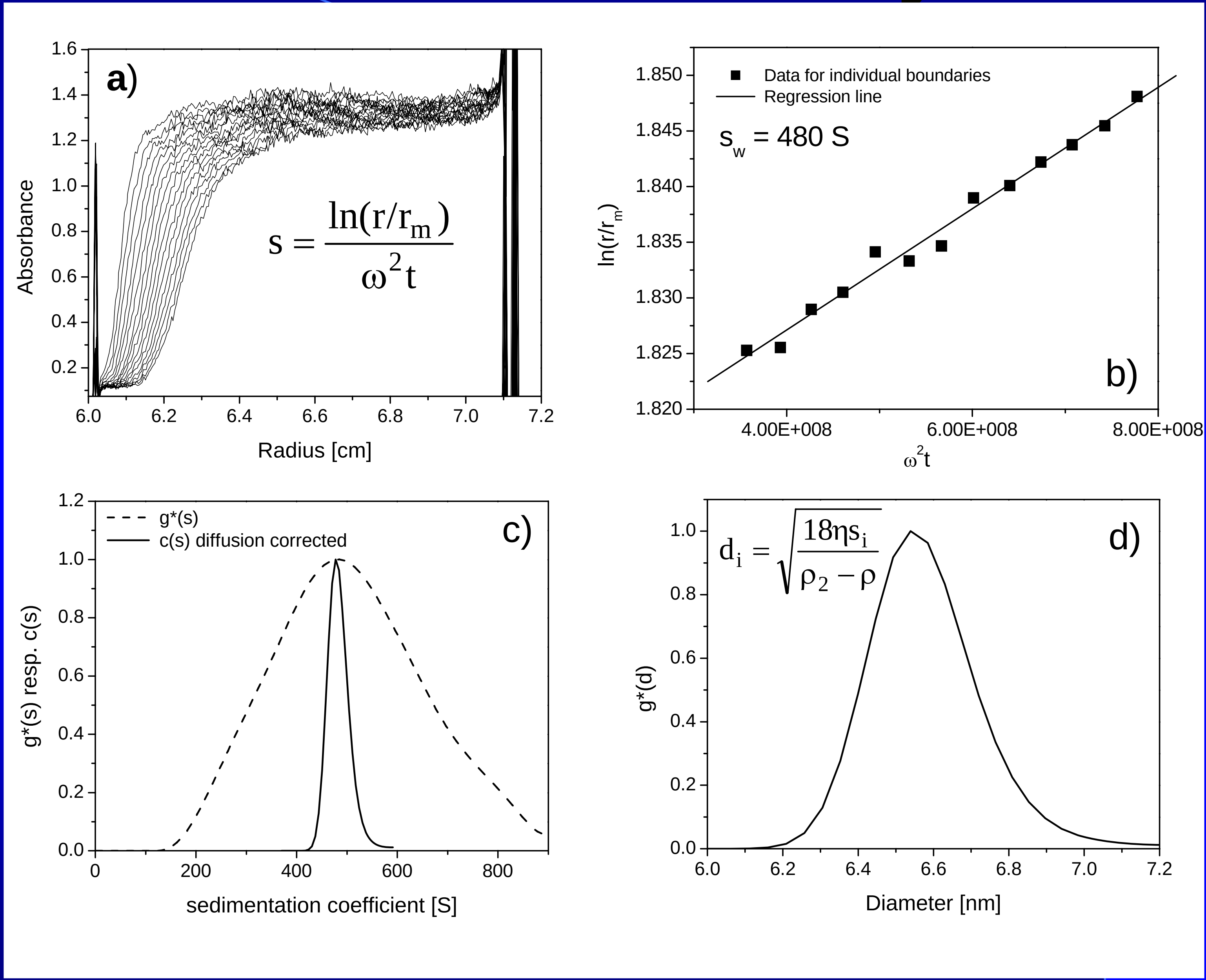
Gold in H_2O at 5000 RPM, 25 °C

a) Raw data

b) S-evaluation

c) s-distribution

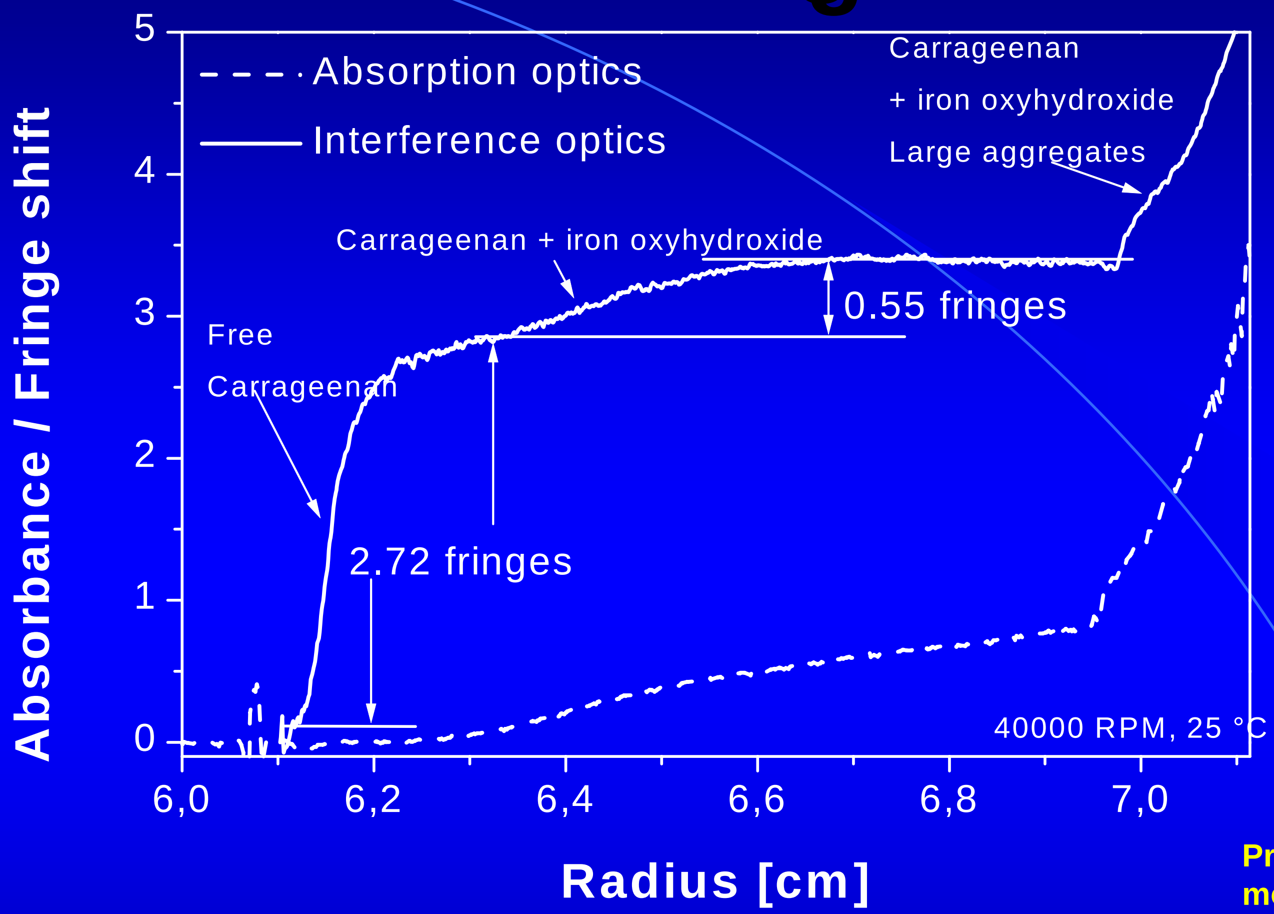
d) d-distribution

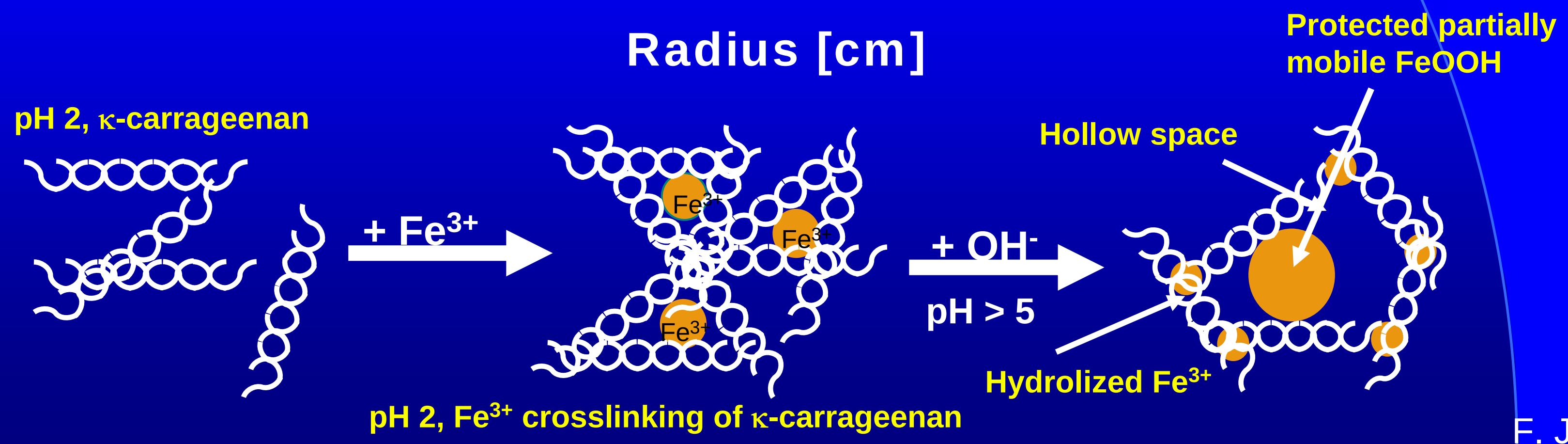


Common Problems

- Extremely broad s-distributions, Big aggregates or small impurities are not detected
- Colloids aggregate or grow during centrifugation (concentration dependent aggregation)
- Density of hybrid colloids is unknown to access particle size
- Electrostatic stabilization complicates analysis due to charge contributions
- Particle polydispersity in size, shape, density and hydration
- High particle density often makes density gradients or density variation methods impossible
- Often multicomponent mixtures

Fractionation of heterogeneous samples

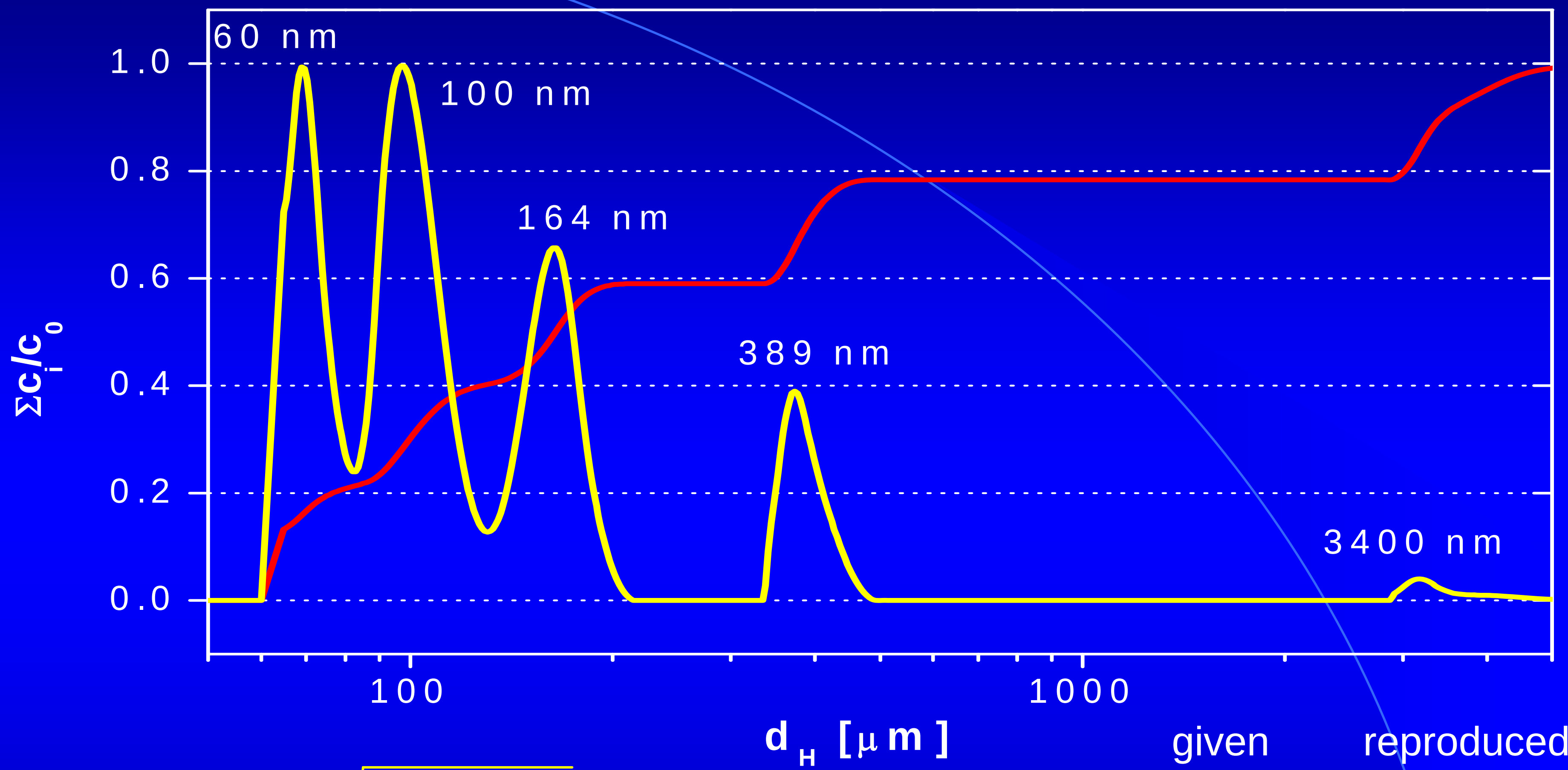




F. Jones

Latex mixture

A. Völkel



$$d = \sqrt{\frac{18\,\eta\, s}{\rho_P - \rho_{LM}}}$$

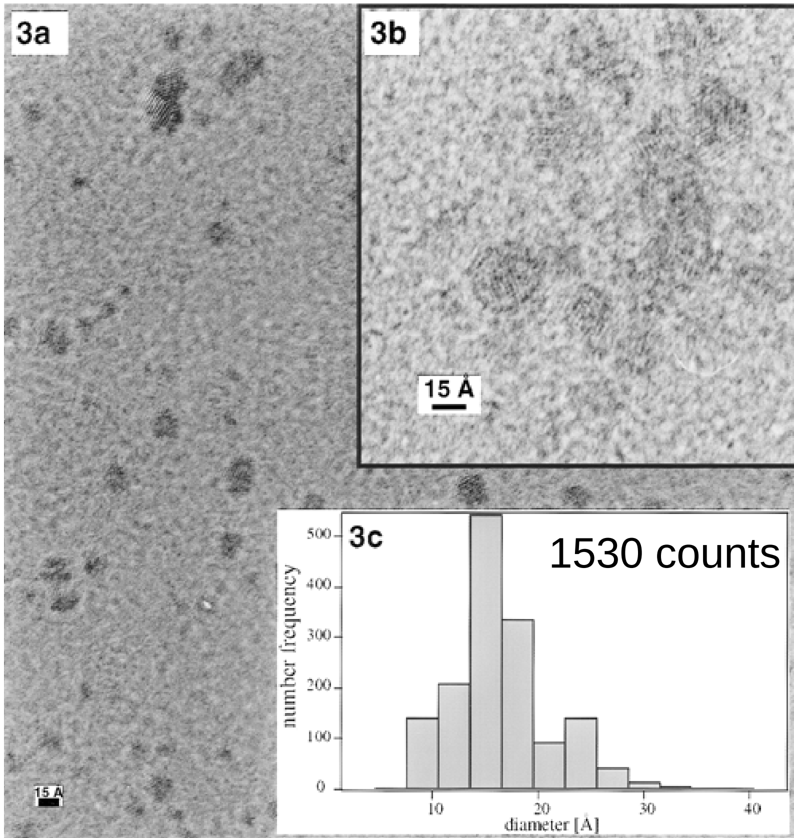
given		reproduced	
d [nm]	%	d [nm]	%
60	20	60	20
103	20	100	20
160	20	164	18
347	20	389	20
2800	20	3400	22

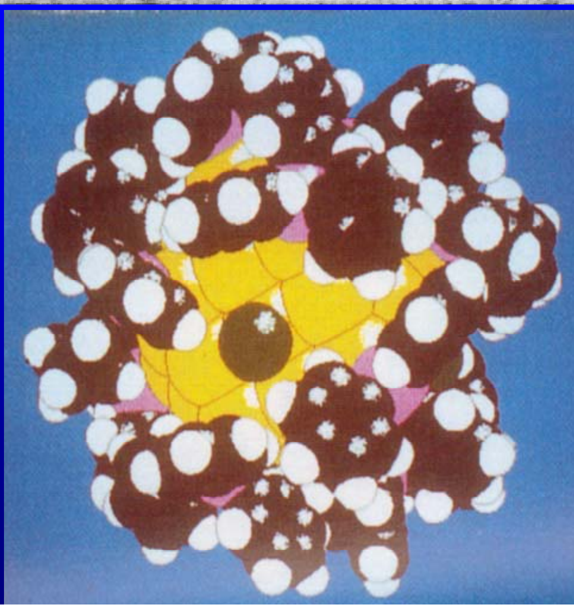
- **Particles analyzed over colloidal range in one experiment**

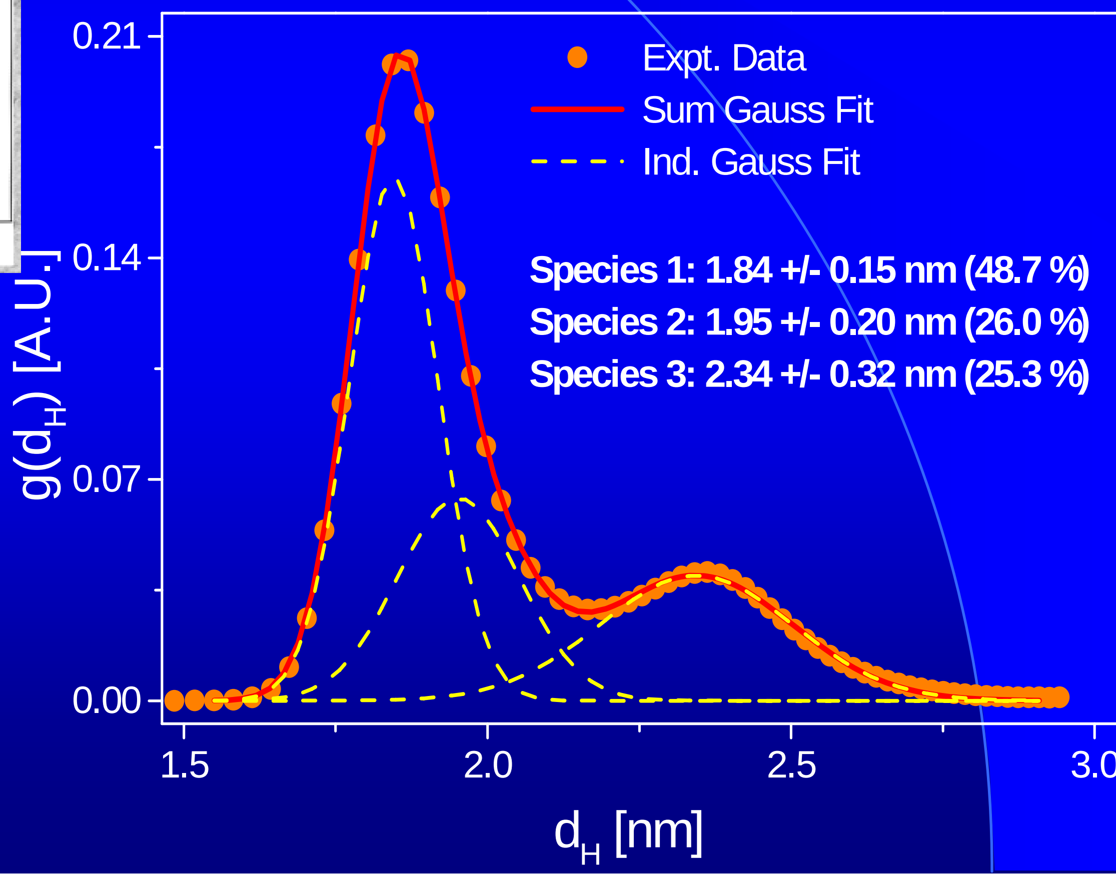
Very small colloids

$Au_{55}[P(Phe)_3]_{12}Cl_6$

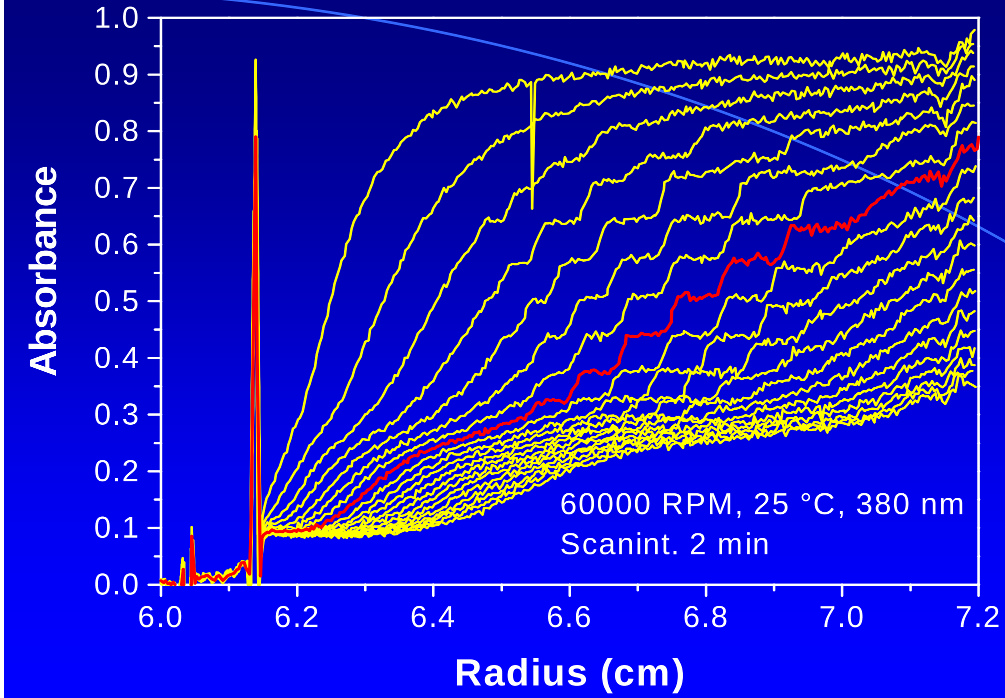
Reported to be definite cluster with 55 Au, Transition between metal and molecule



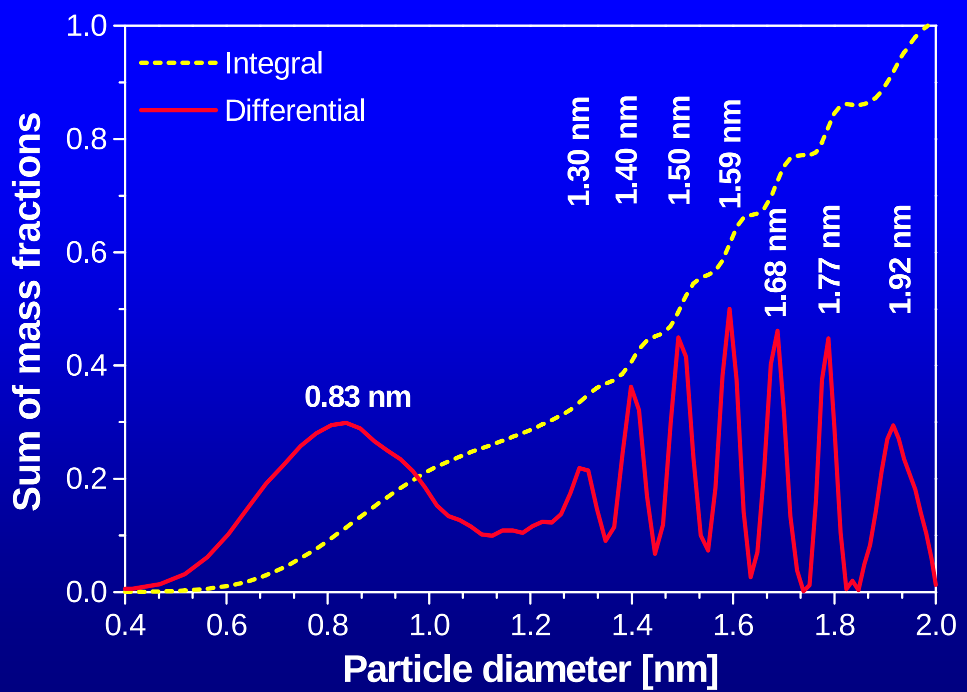




High resolution PSD



Pt colloid in MeOH / HAc

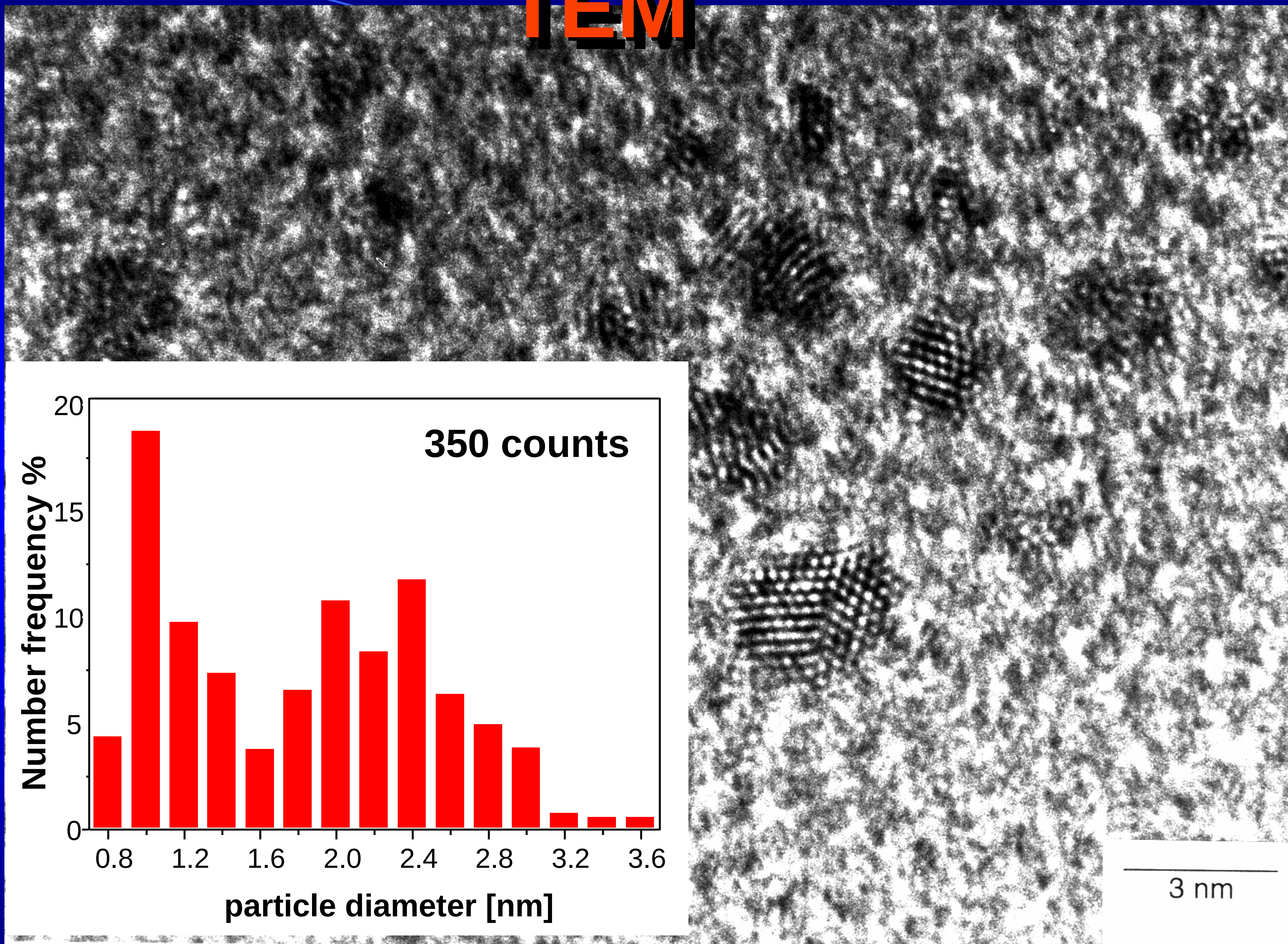


0.1 nm Baseline resolution

Problem:
Elimination of diffusion broadening by self-sharpening effects is not common

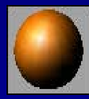
Baseline resolution > 1 Angström

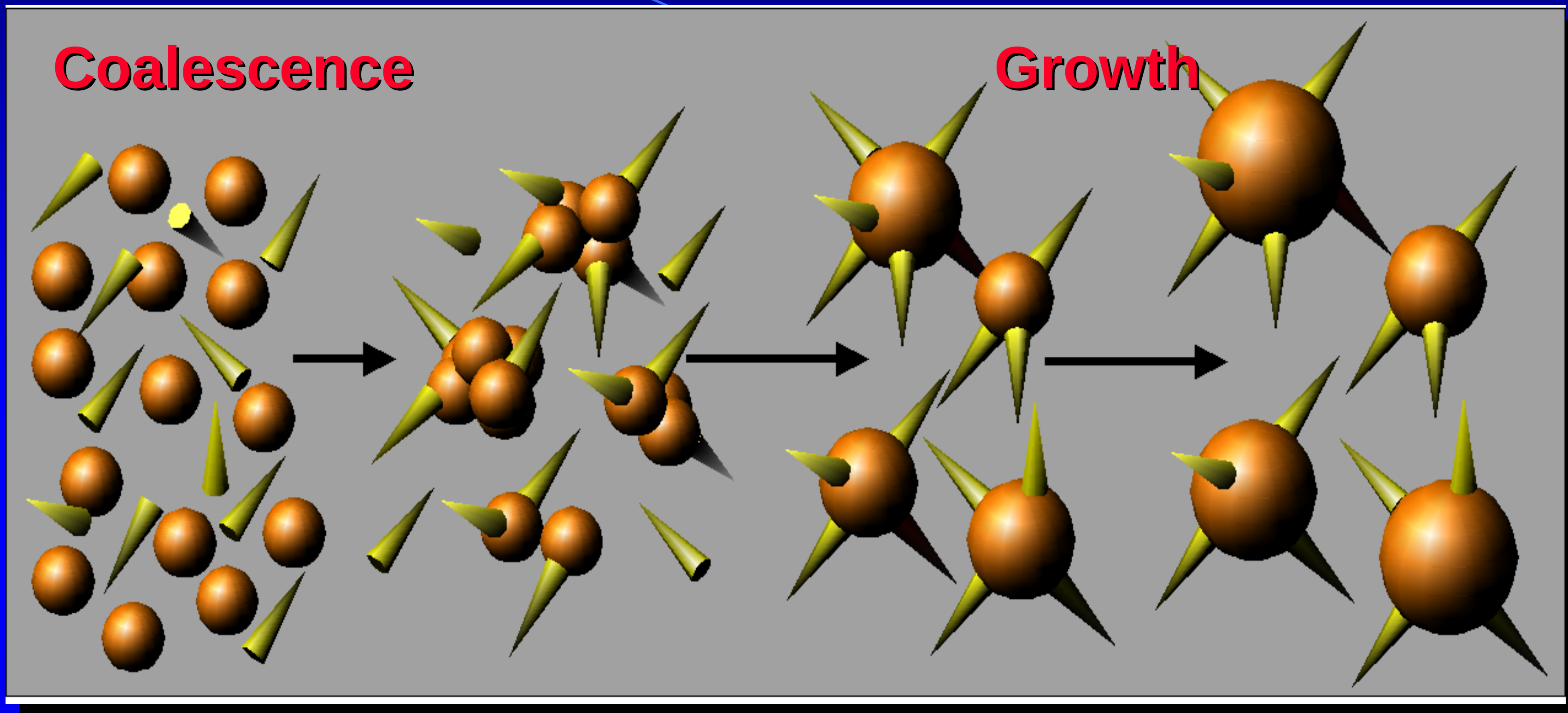
TEM



Particles slightly bigger than from AUC (Density)

Pt-Colloid growth

 = Stabilizer  = Pt-Particle



Critical crystal nucleus

Coalescence until particles are stabilized

Further growth through reduction of $PtCl_4$ at the crystal surface

$$\frac{dr}{dt} = \text{const.}$$

Differences in the particle size after coalescence are conserved

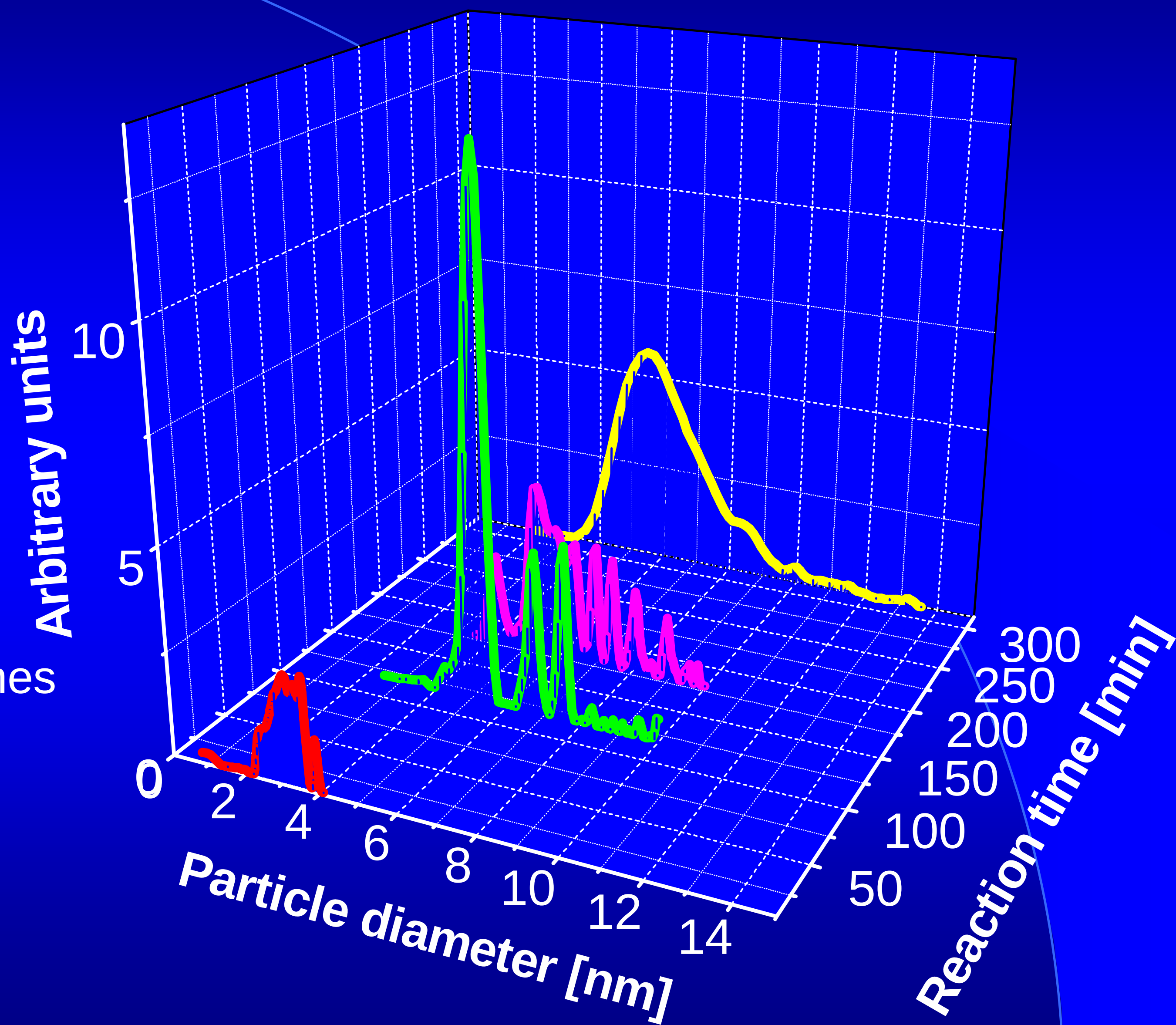
Mechanism in G.A. Braun, Ph-D dissertation, Aachen 1997

Growing ZnO Colloid

$Zn(Ac)_2$ (1 mmol/l)
+ 20 mmol/l NaOH
in water free
Isopropanol

Dilution 1 : 5 and
Heating to 65 °C

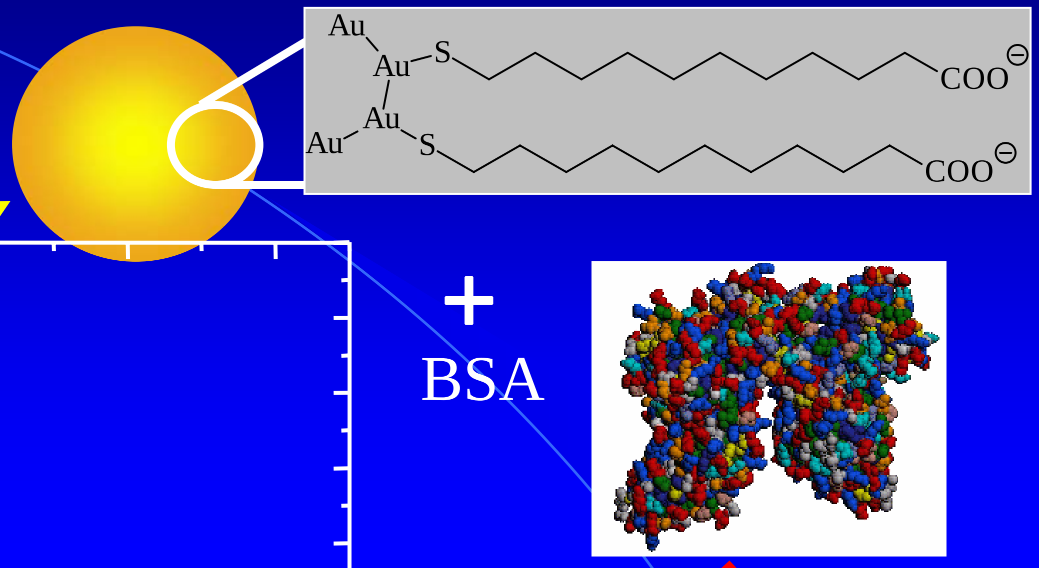
Start of heating defines
start of the reaction

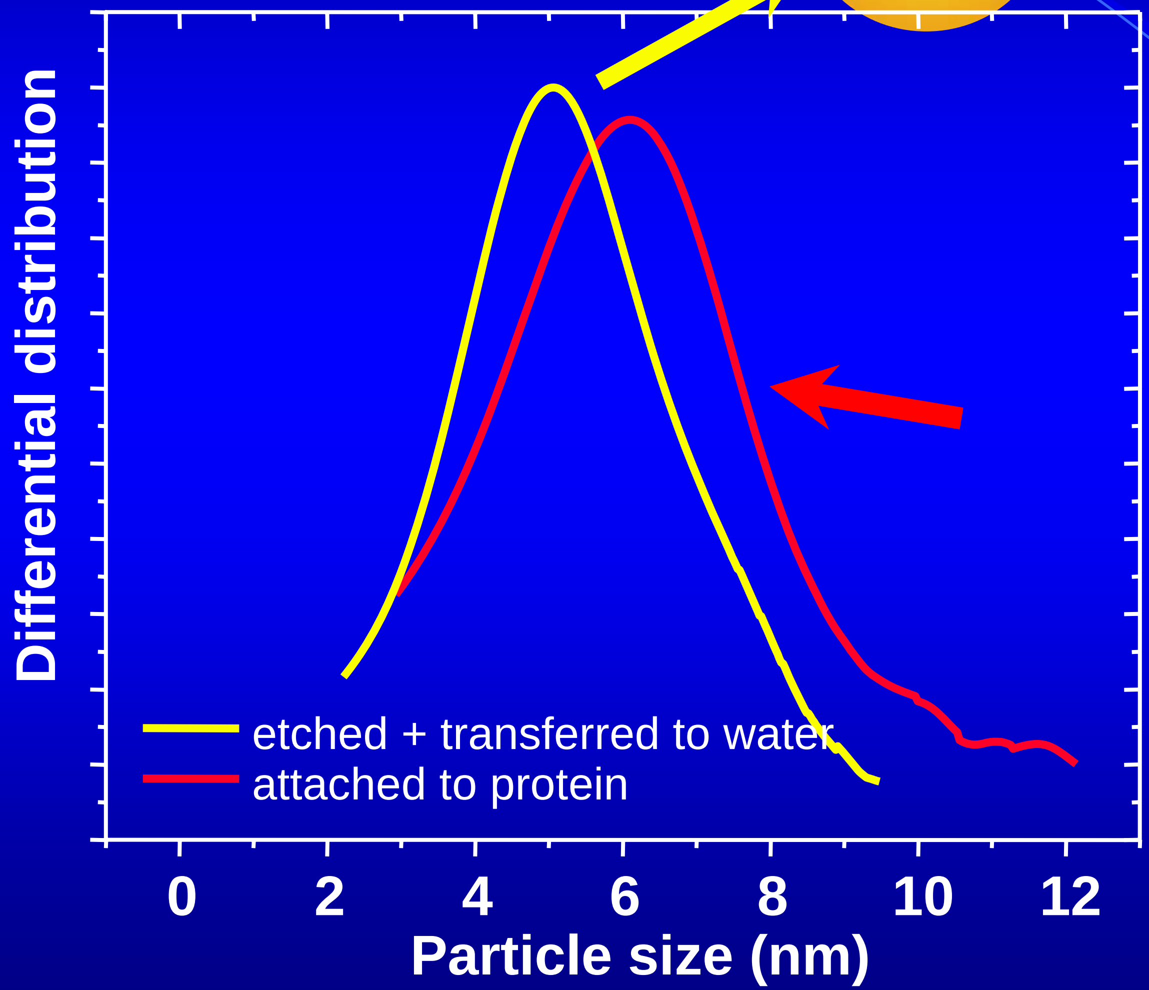


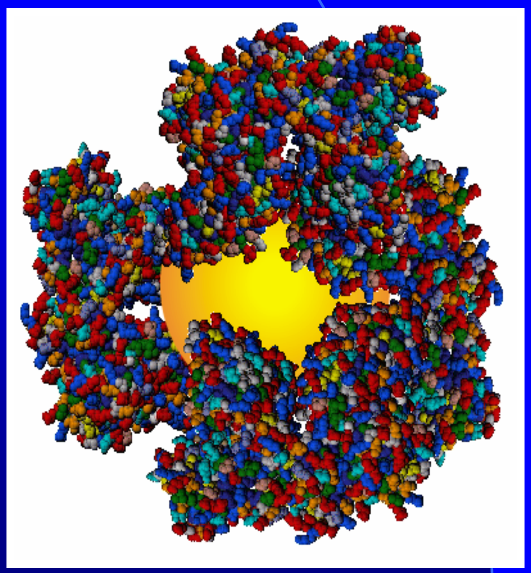
Problem: AUC has a low time resolution

T. Pauck

Bio-labeling

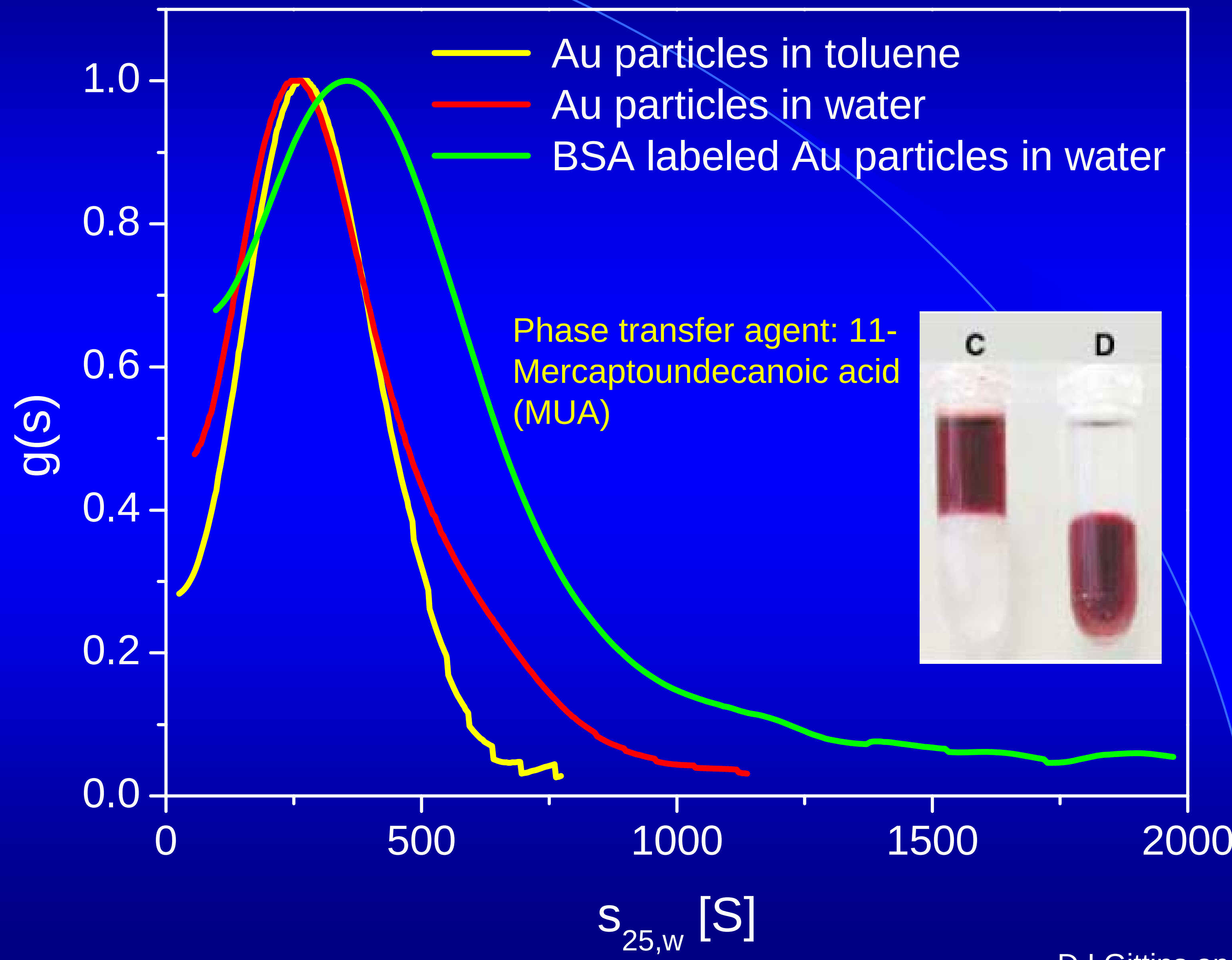






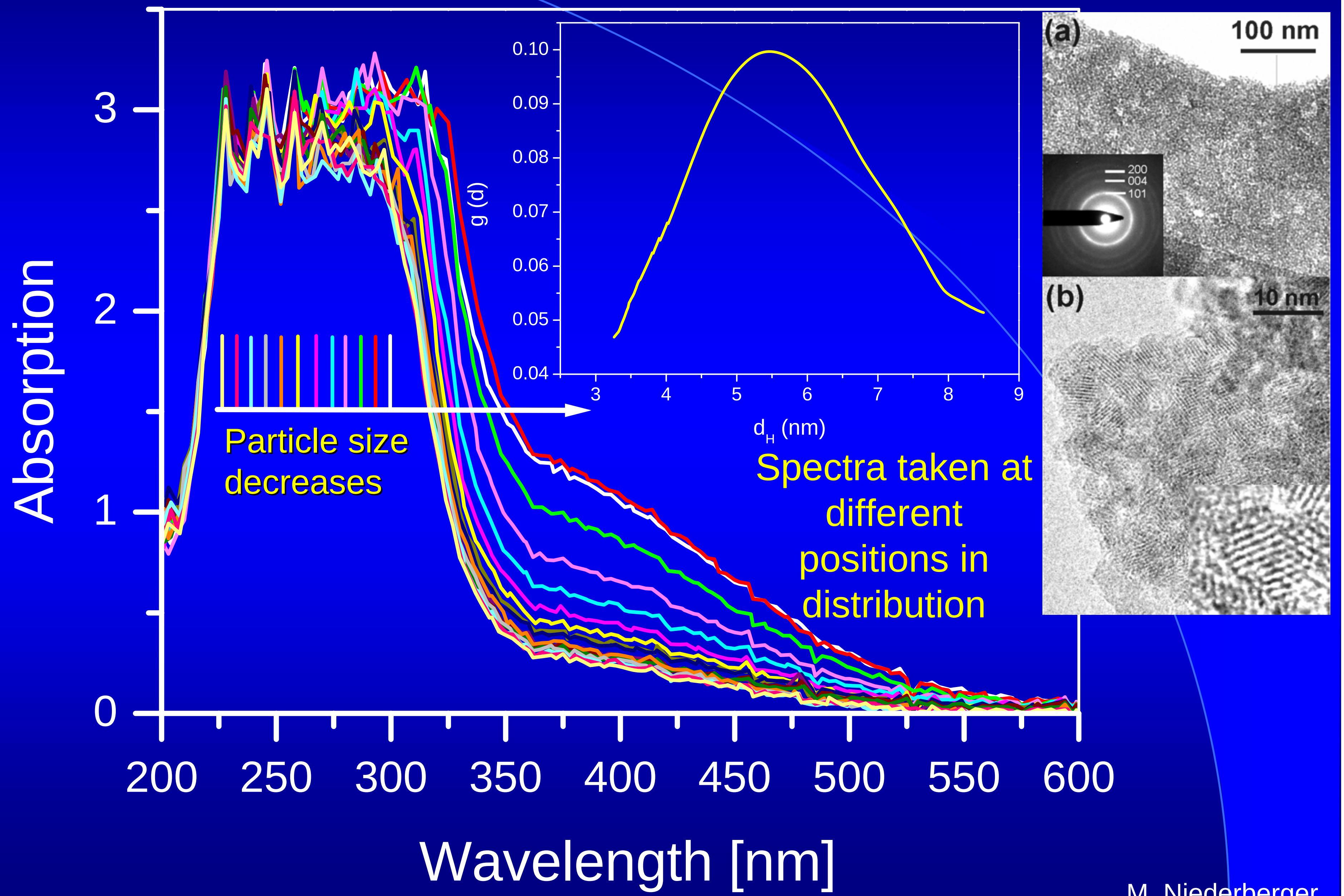
D.I.Gittins and F.Caruso

Phase transfer and bio-labeling



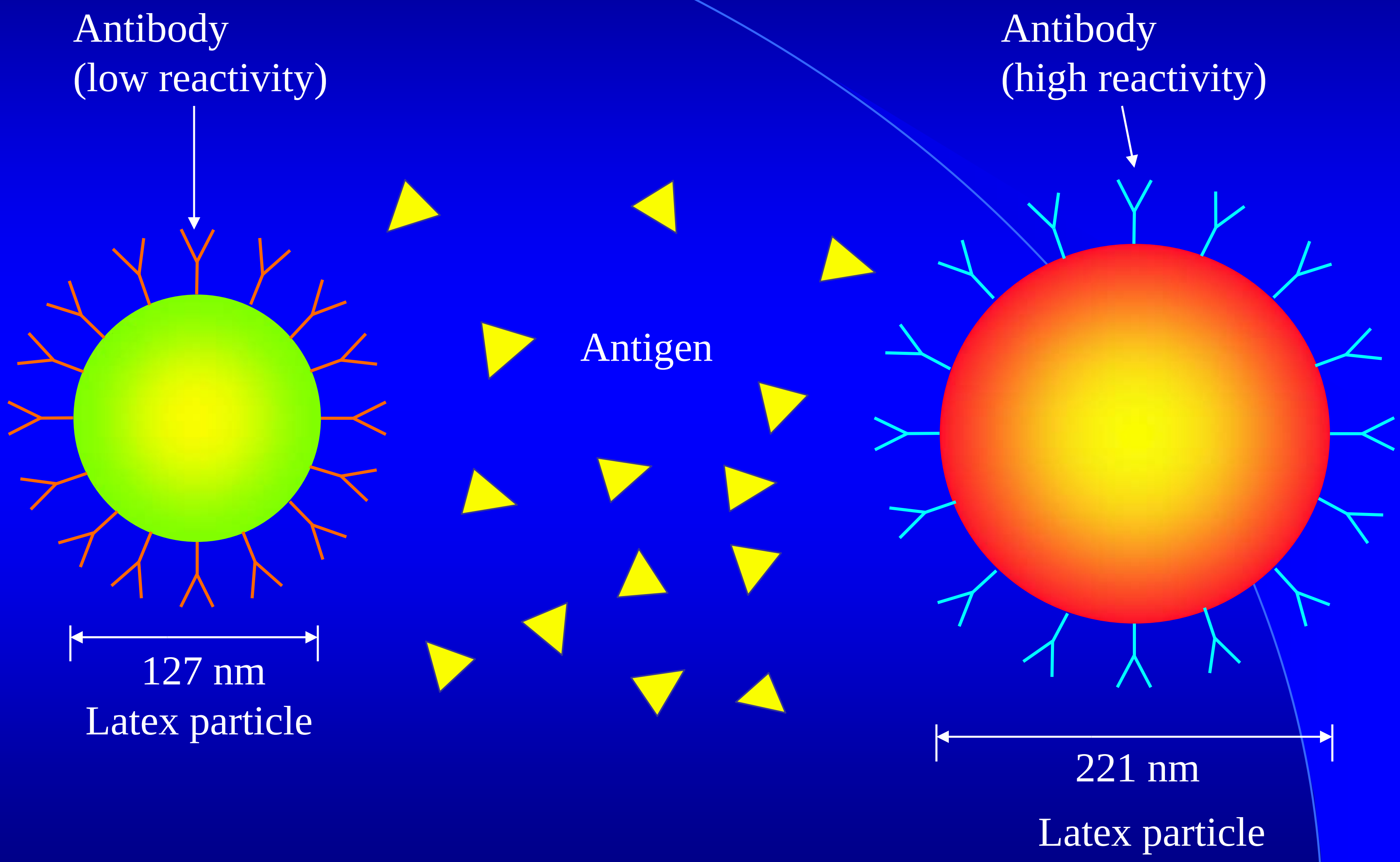
D.I.Gittins and F.Caruso

Dopamine functionalized TiO_2

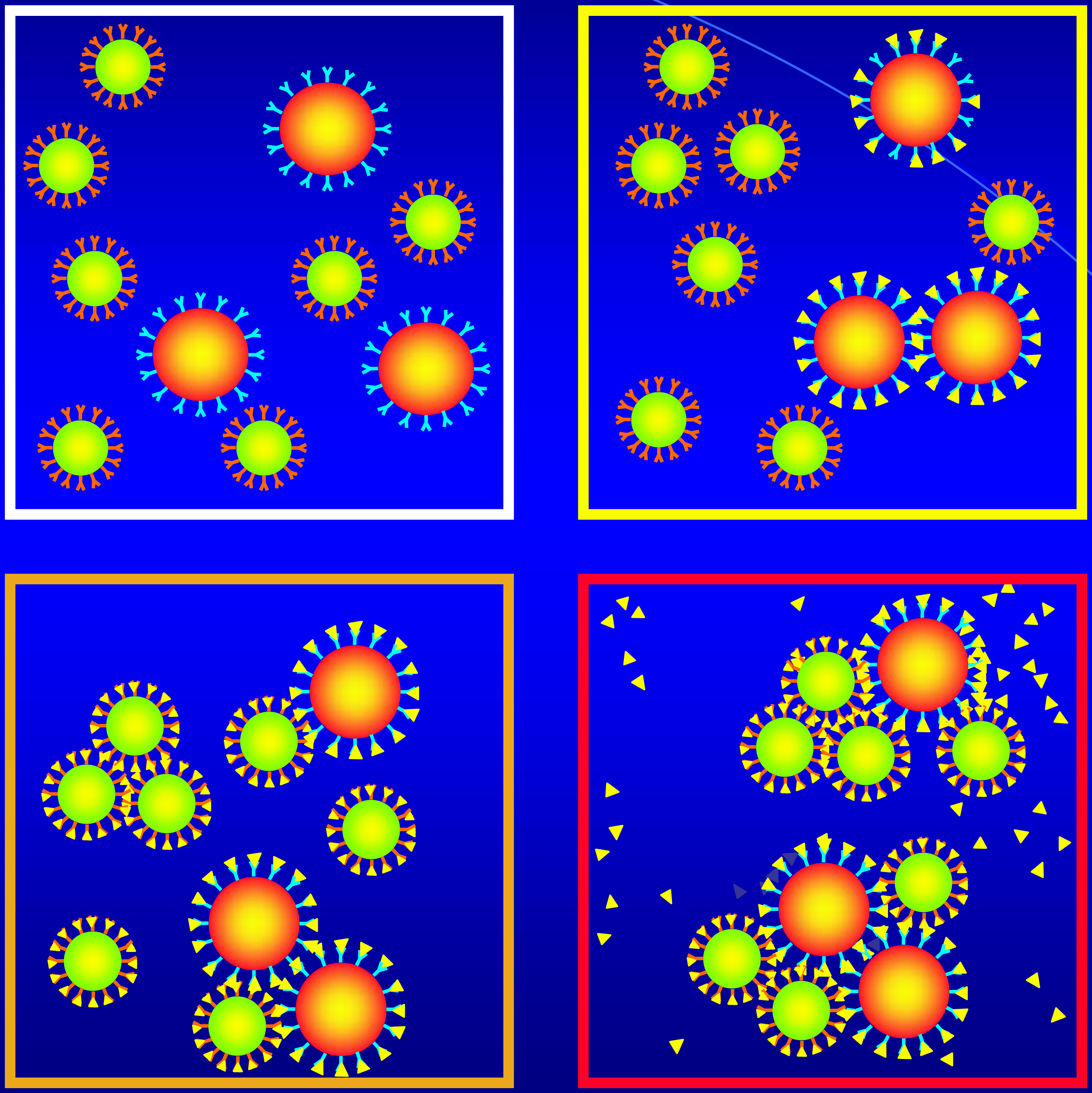


M. Niederberger

Concept of turbidimetric immunoassay



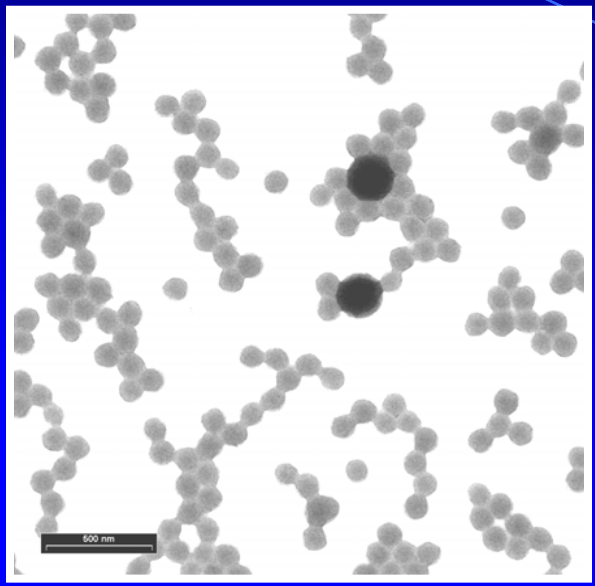
Concept of turbidimetric immunoassay



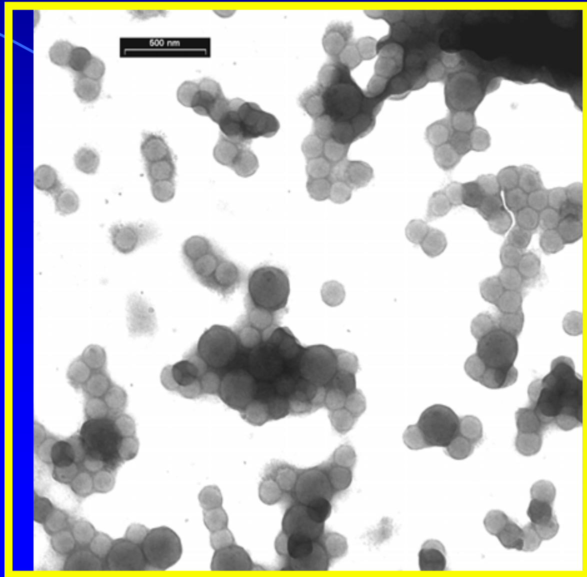
Big particles aggregate first, then the small ones

TEM of Immunoassay

0 mg/L CRP

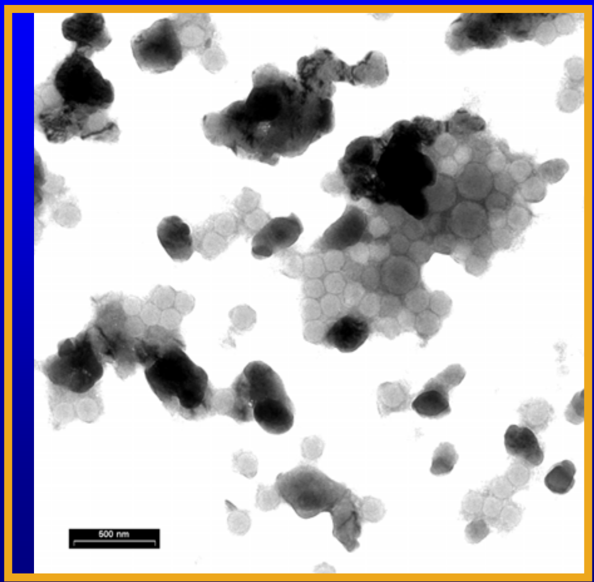


4.28 mg/L CRP

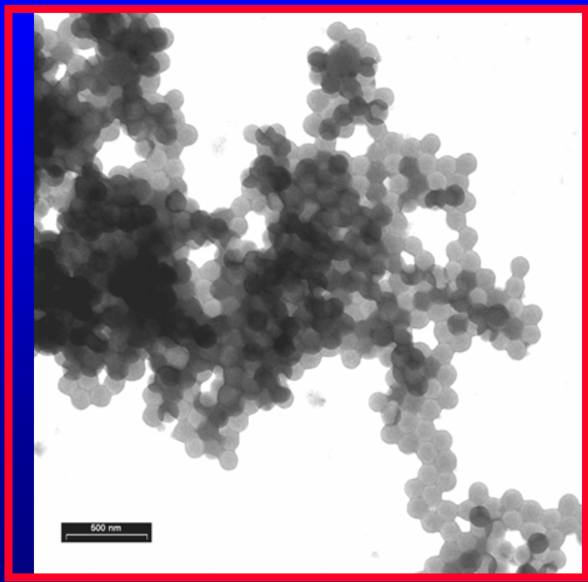


Statistics, drying artifacts ?

25 mg/L CRP

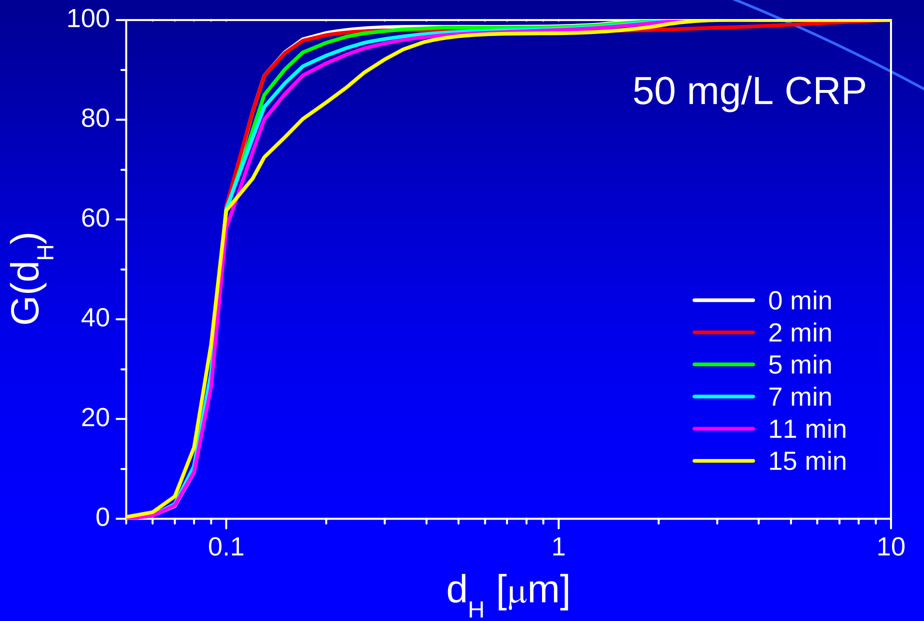


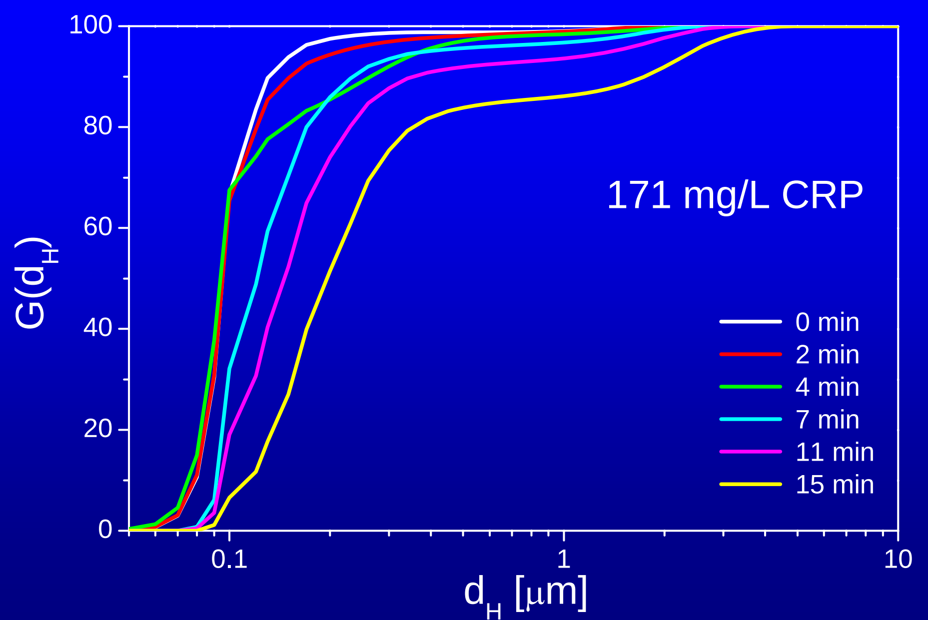
156 mg/L CRP



Scale bars 500 nm

SLS of Immunoassay

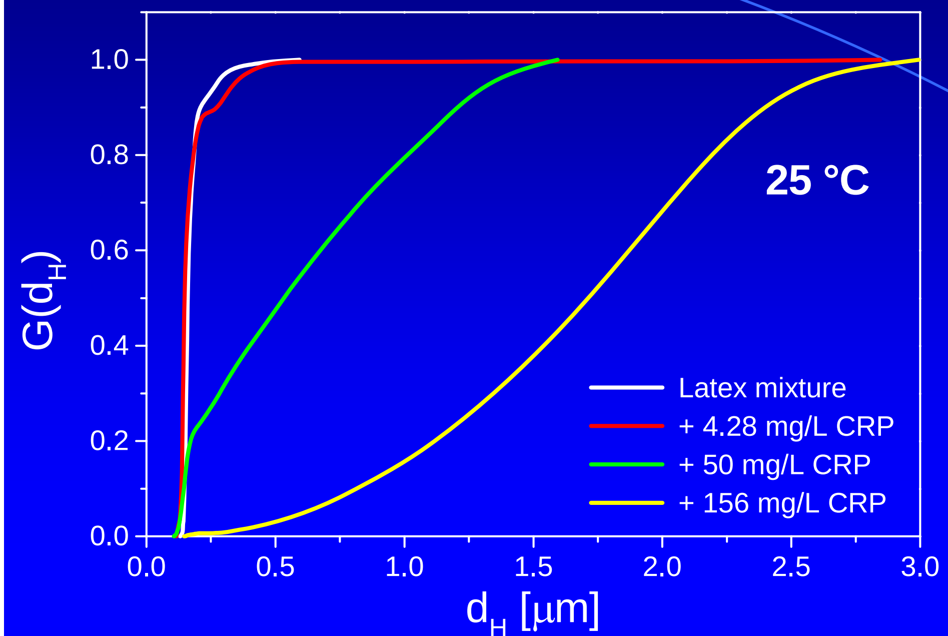


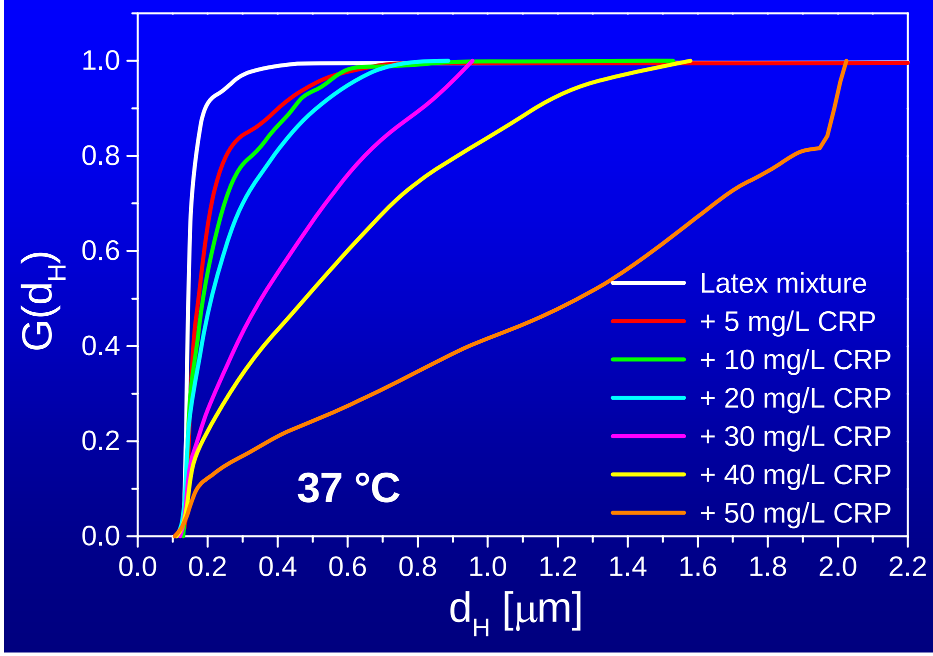


- Fast measurement without equilibration needs
- Kinetic measurements possible
- Ambient conditions without any pressure effects
- Low resolution in particle size
- Low statistical resolution (No fractionation)

92 wt-% small latices

AUC of Immunoassay





- AUC has equilibration time > 10 min before experiment
- Fractionation enables to determine correct particle quantities and sizes by turbidity detection (MIE correction) with speed profile even over decades of s-values
- 92 wt-% small latices in mixture correctly detected

Microgels

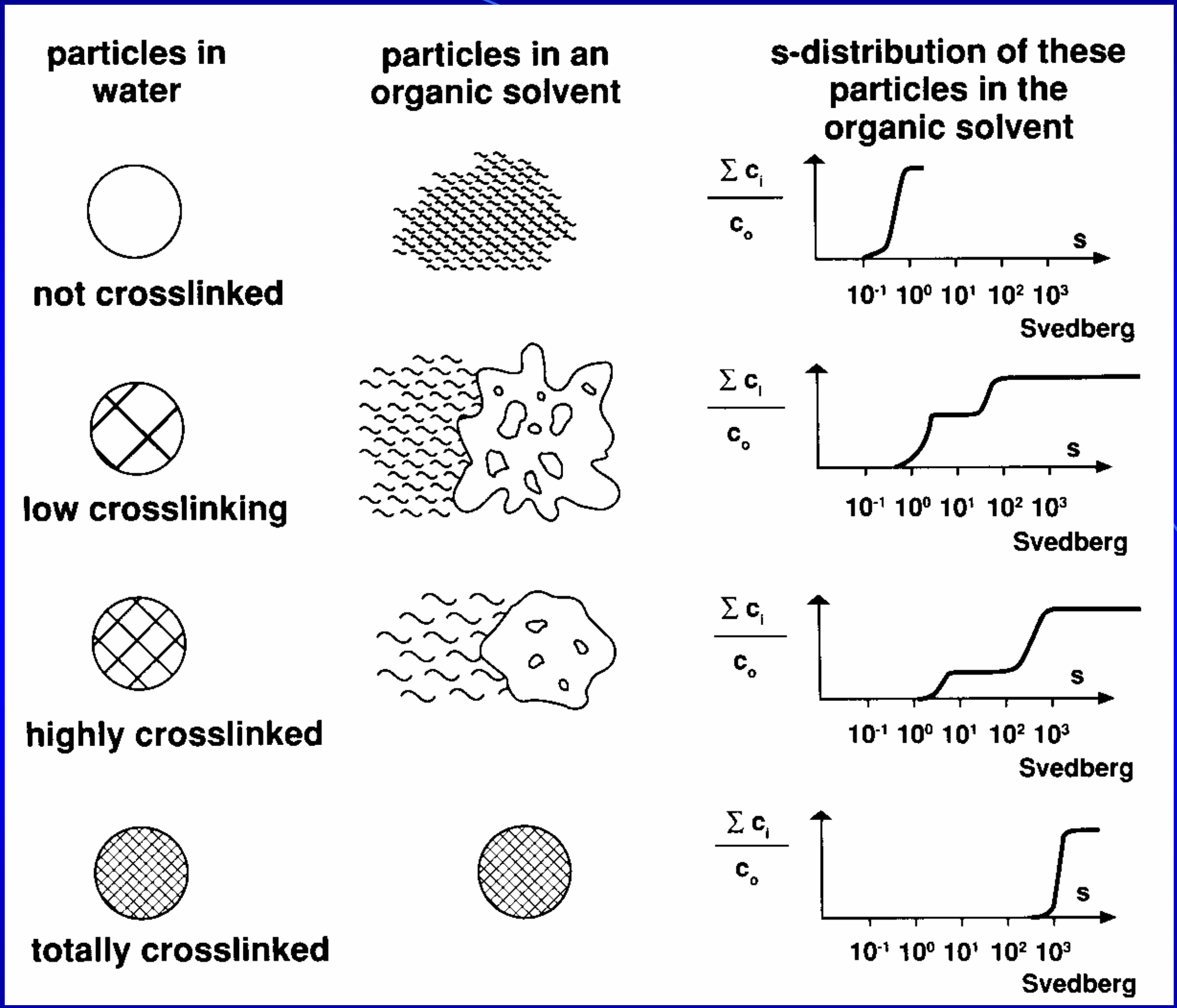


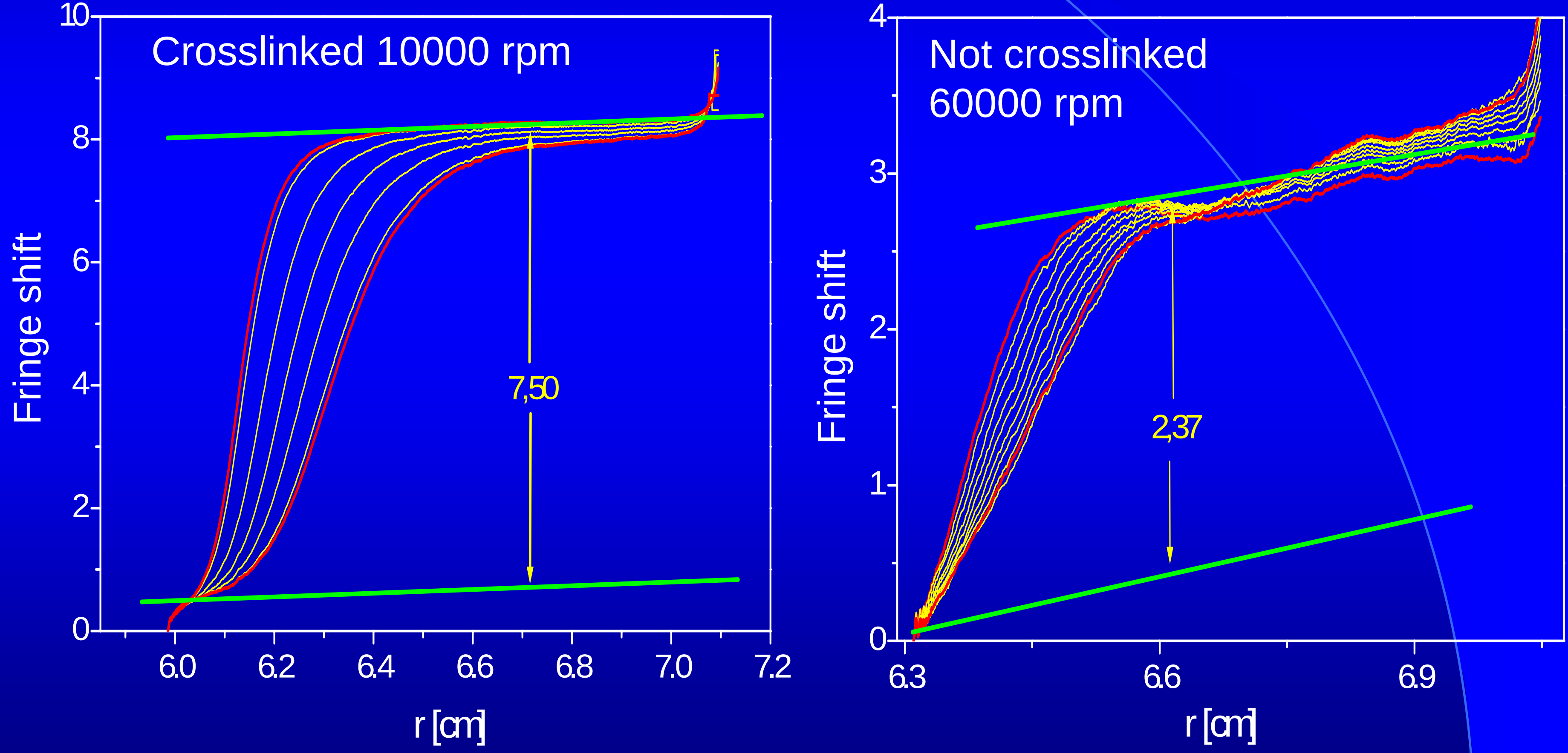
Figure from:
H.G. Müller, A. Schmidt, D. Kranz; Progr. Colloid Polym. Sci. **86, 70 (1991)**

$$Q = \frac{b \cdot d_h^2}{s} \cdot \frac{\rho_P - \rho_s}{18 \cdot \eta}$$

b = mass ratio of soluble parts

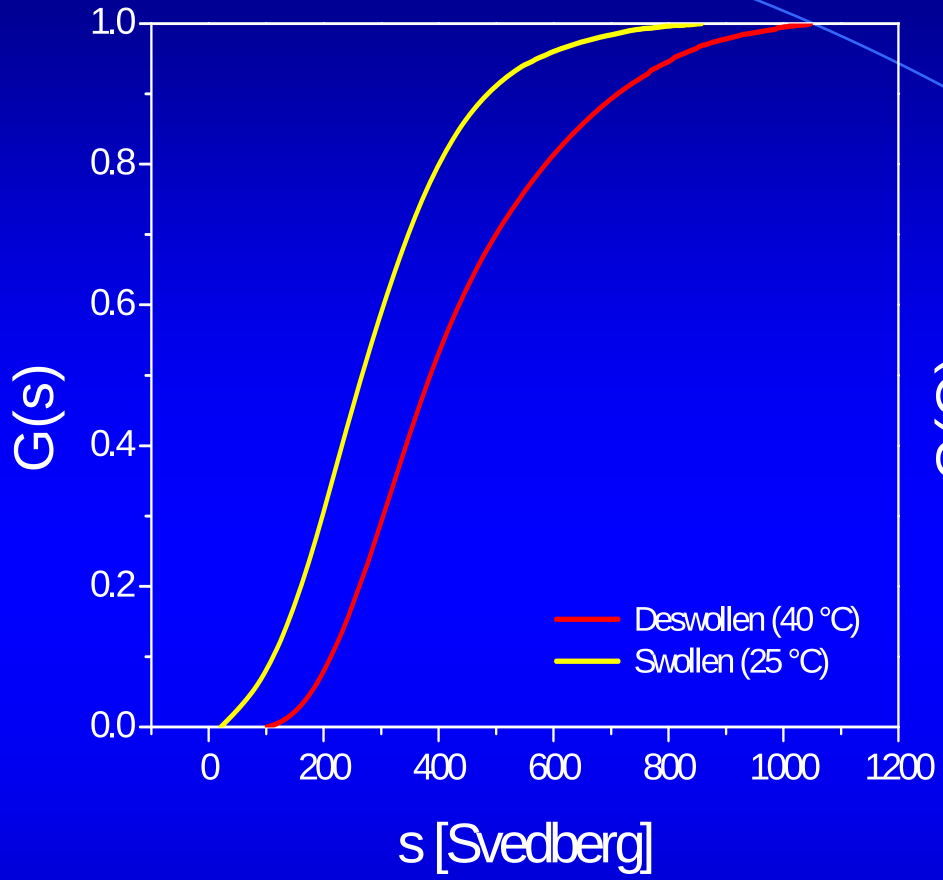
PNIPAM Microgels

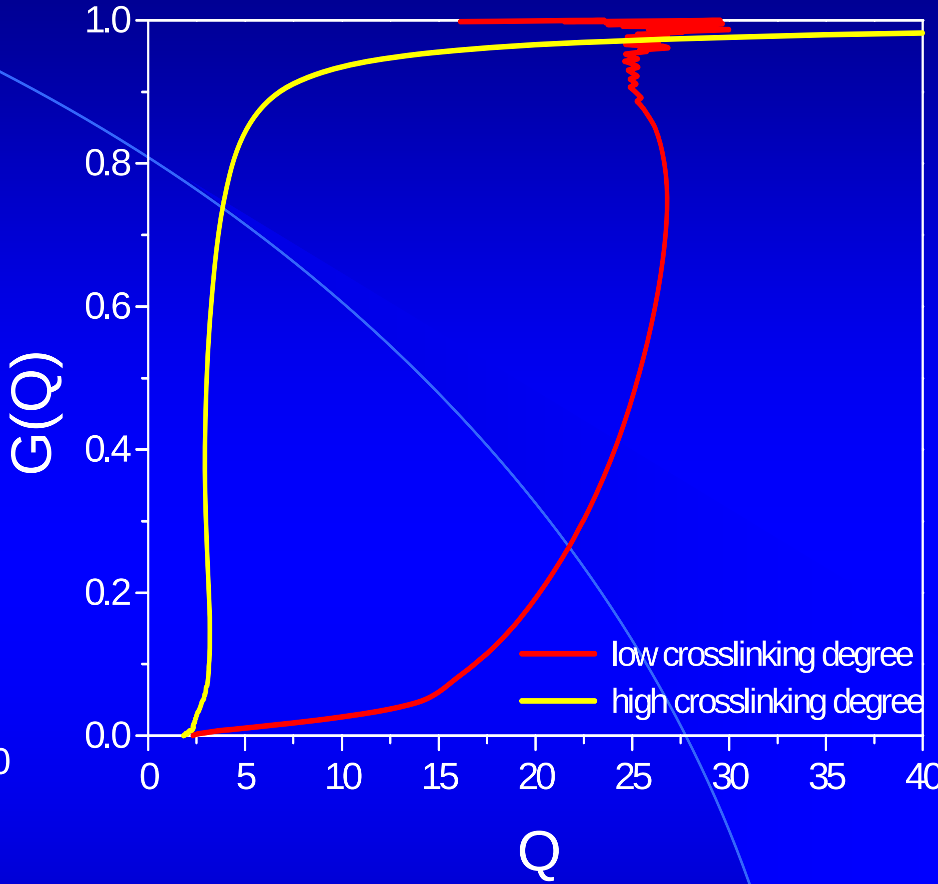
Fringe shift of crosslinked part: 7.90 **76%**
Fringe shift of free chains : 2.37 **24%**



D. Kuckling

Swelling degree distribution





$$Q = \left(\frac{s_{unswollen}}{s_{swollen}} \right)^3$$

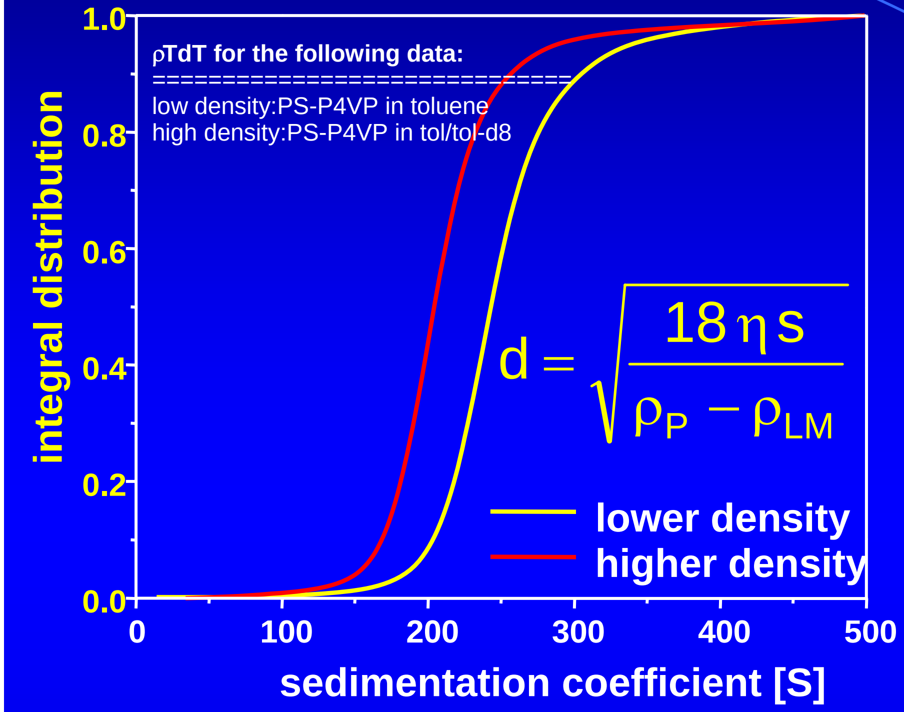
In principle also possible with soluble fraction b

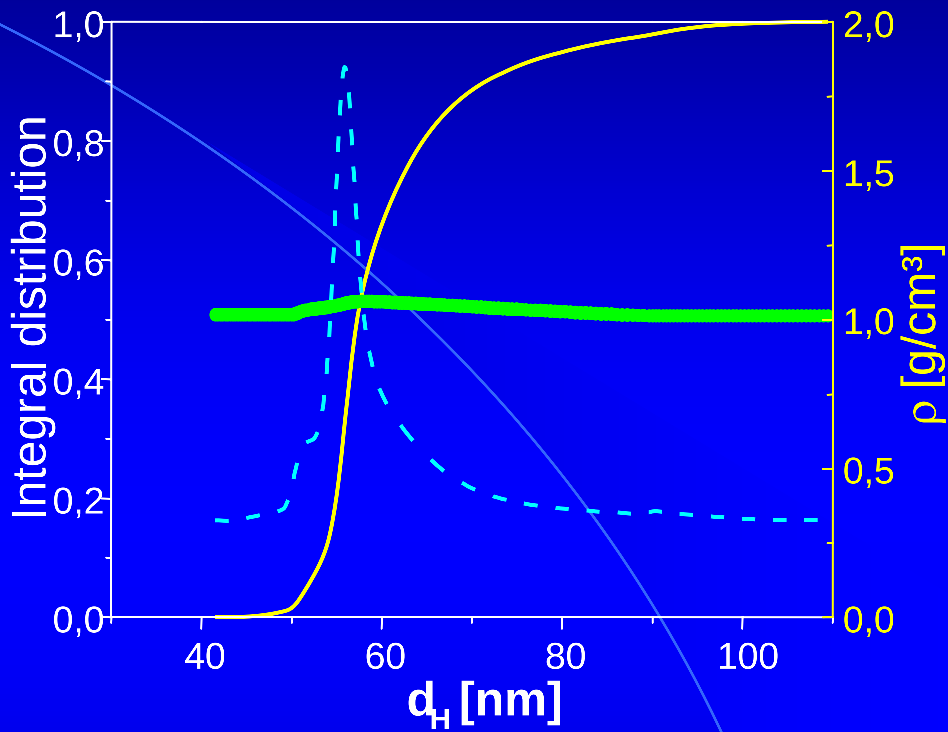
$$Q = \frac{b \cdot d_h^2}{s} \cdot \frac{\rho_P - \rho_s}{18 \cdot \eta}$$

D. Kuckling

Solvent density variation

PS_{123}-$P4VP_{145}$ in toluene resp. d-toluene



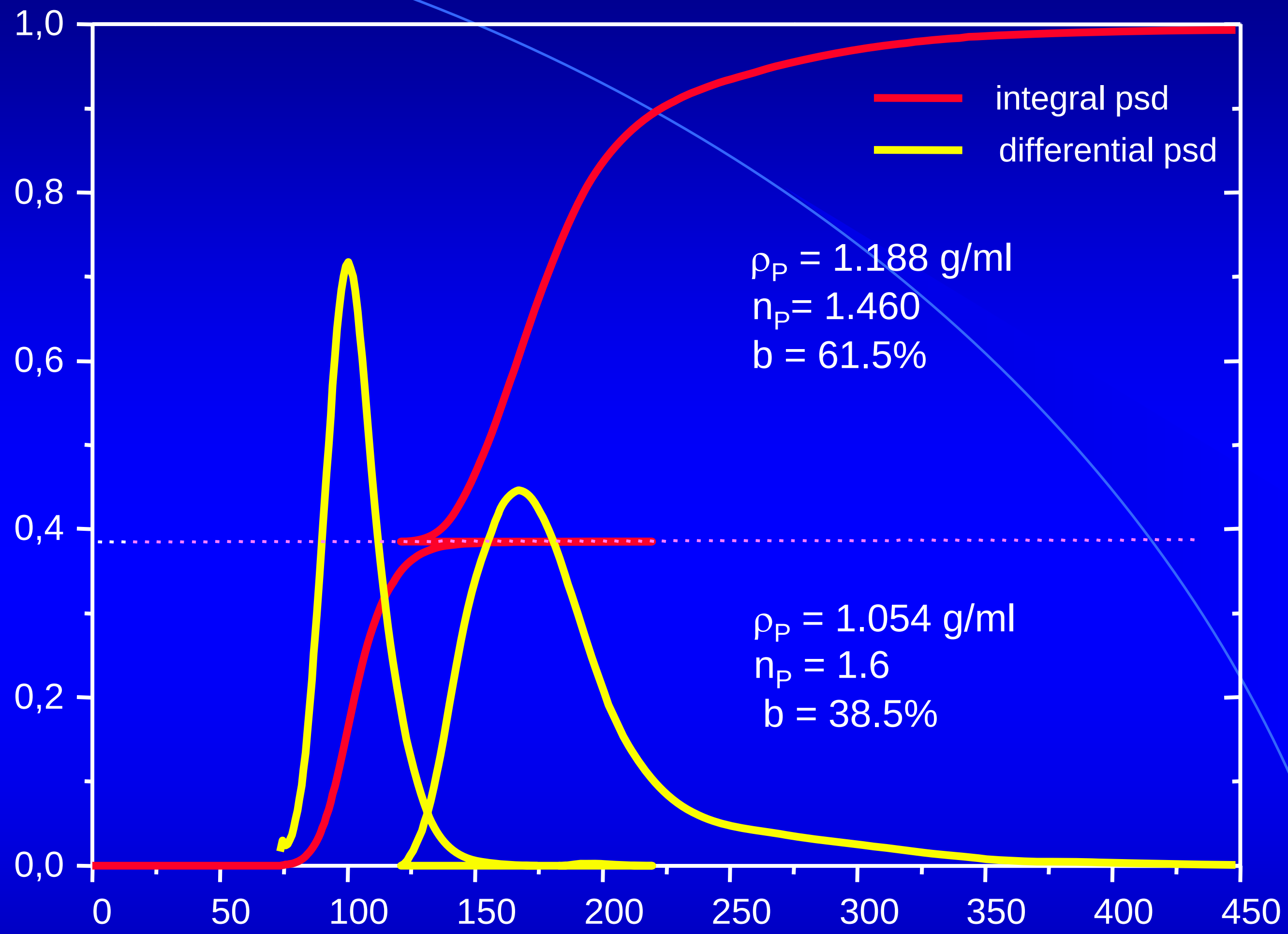


Combination of 2 s-distributions in 2 chemically identical solvents yields particle size and density distribution

- Density in good agreement with values from density meter
- Particle size in agreement with results from DLS or viscosity measurements
- Same result for core crosslinked micelles

K. Schilling

PS-PMAA Latex mixture 40 : 60



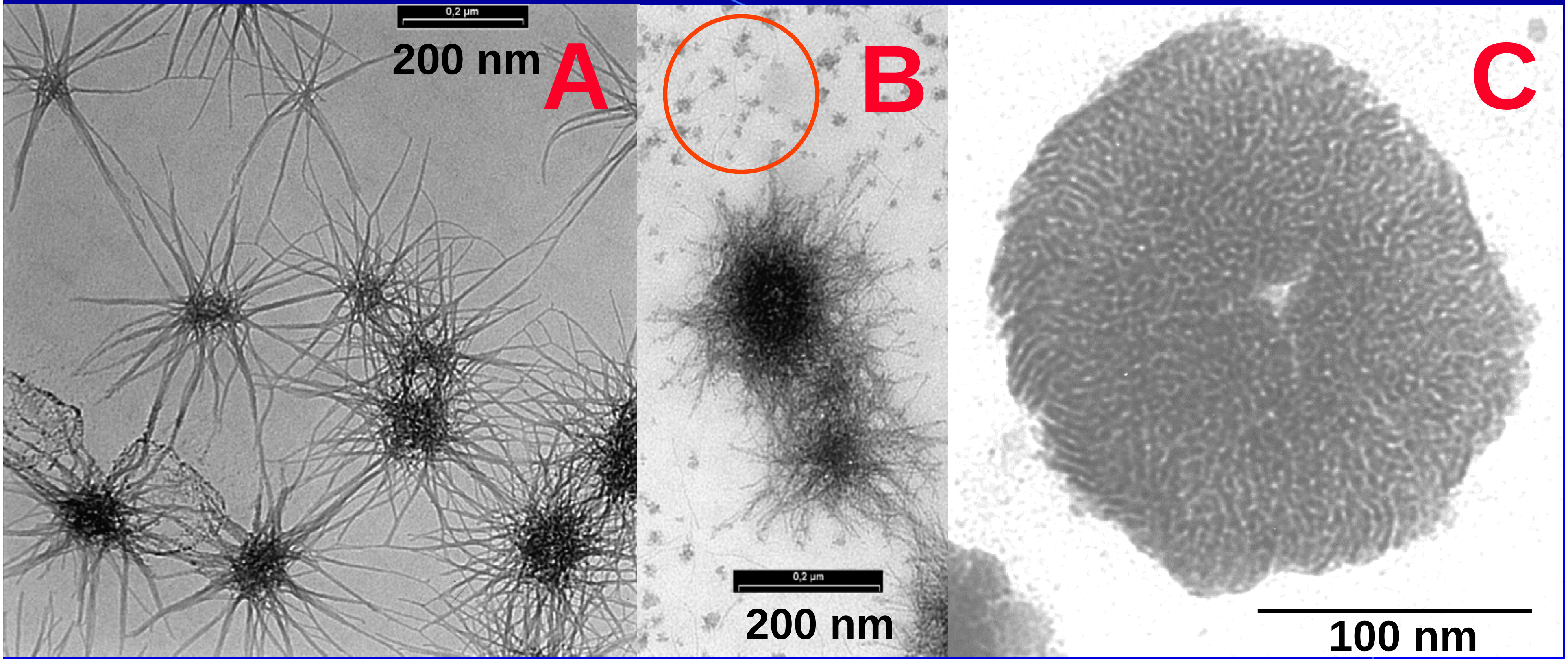
$$d = \sqrt{\frac{18(s_2\,\eta_{s2} - s_1\,\eta_{s1})}{\rho_{s1} - \rho_{s2}}}$$

$$\rho_p = \frac{s_1\,\eta_{s1}\,\rho_{s2} - s_2\,\eta_{s2}\,\rho_{s1}}{s_1\,\eta_{s1} - s_2\,\eta_{s2}}$$

A. Völkel

Synthetic hybrid colloids

Brushite $CaHPO_4 * 2H_2O$
TG 18% Mineral



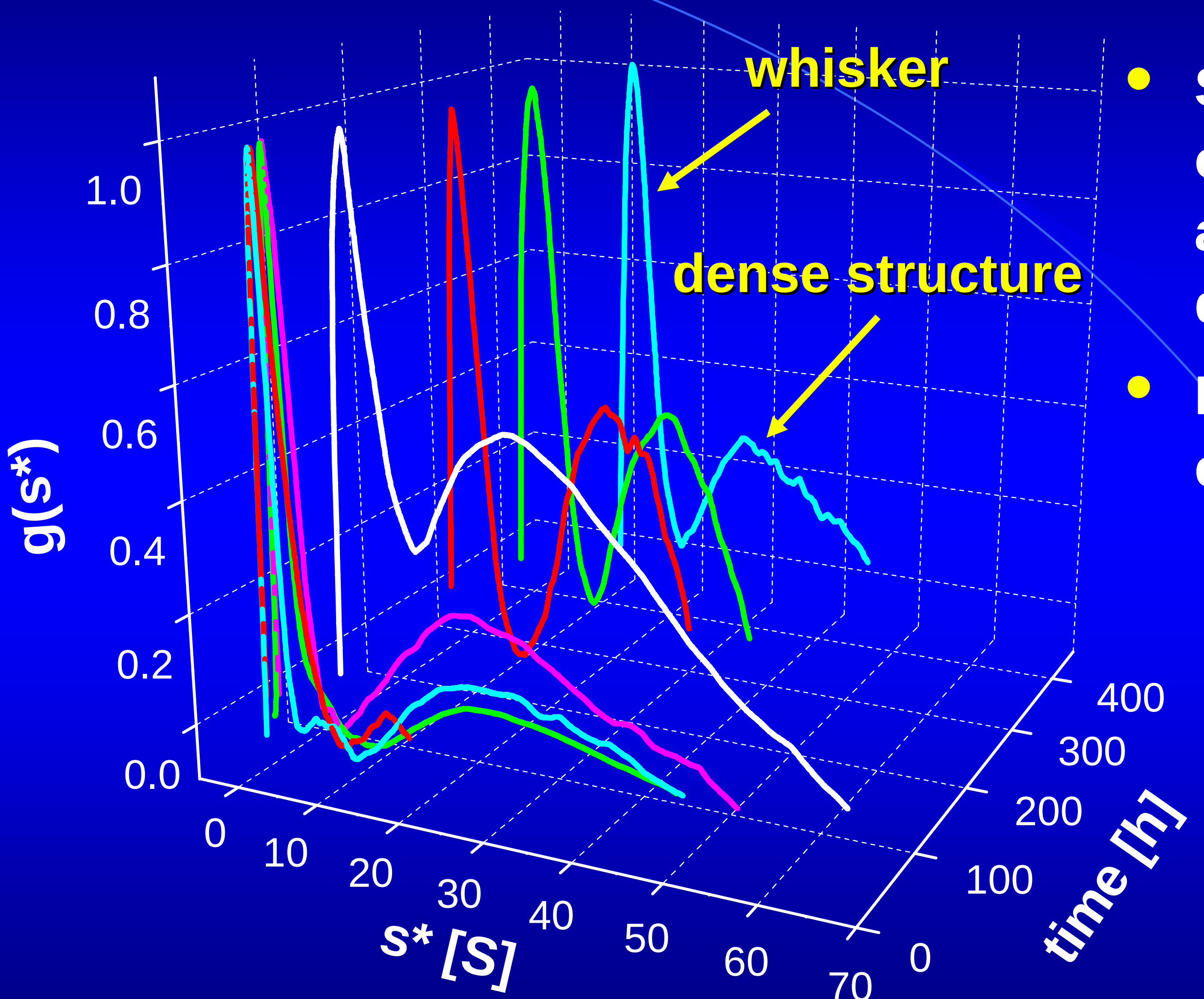
A: Star like crystals/whisker, d = 17 nm, l = 184 nm

B: Transversal growth, destabilization of filaments, further densification of core

C: Compact aggregates with periodicity („Chrysanthemun“ structure)

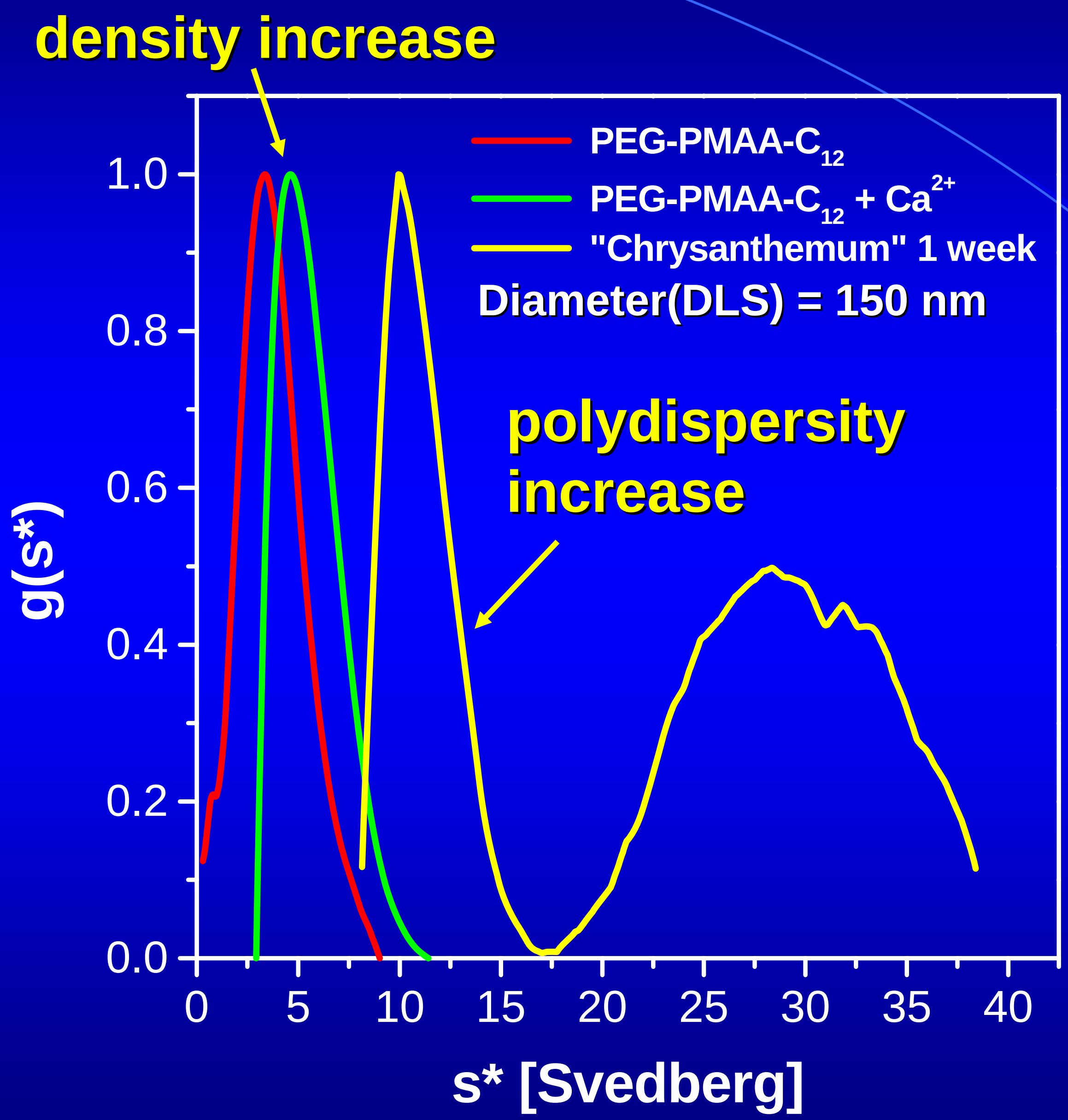
M. Breulmann

Synthetic hybrid colloids



- **Superposition of particle size and density distribution**
- **Densities can exceed 2 g/ml**

Synthetic hybrid colloids



- **Clear monitoring of density increase upon Ca^{2+} complexation**
- **Global Analysis could reveal density and molar mass**

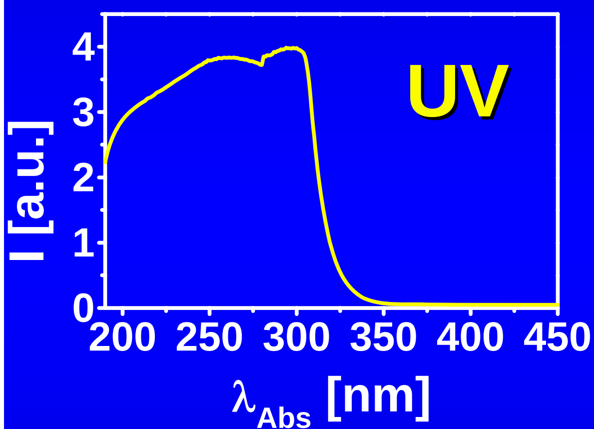
Combination of AUC with DLS or better Fl-FFF is highly desirable

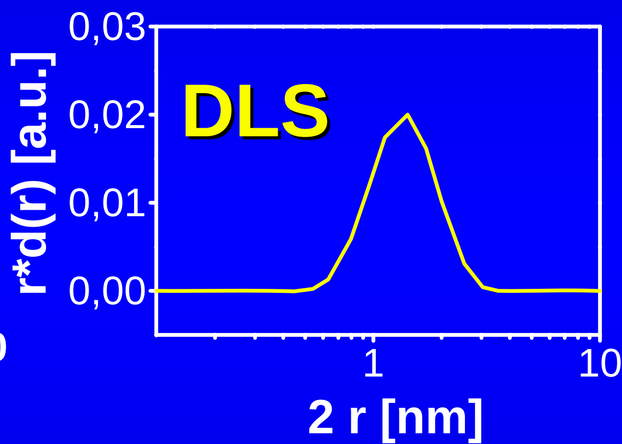
Particle size and density

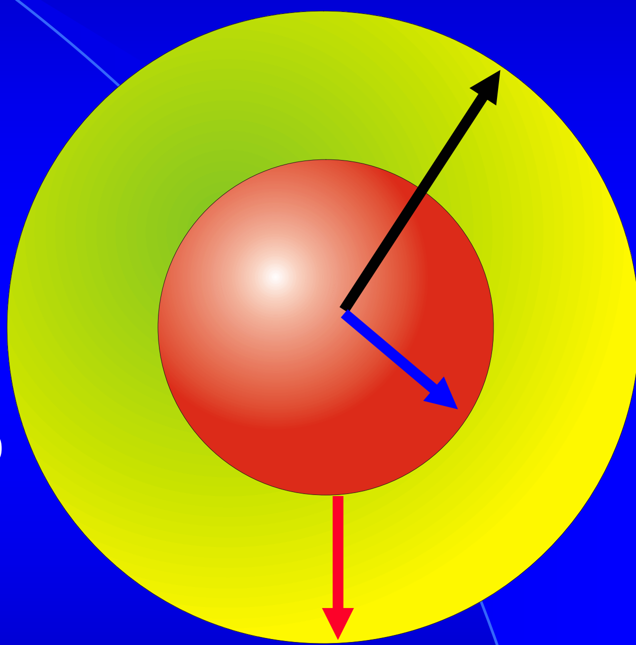
UV : d = 1.65 nm
DLS : d = 1.6 nm
AUC: ” d = 1.25 nm“ (s = 3.6 S)
calculated with ρ_{CdS} = 4.83 g/cm^3

CdS

$\rho_{particle}$ = 3.2 g/cm^3
$d_{particle}$ = 1.6 nm







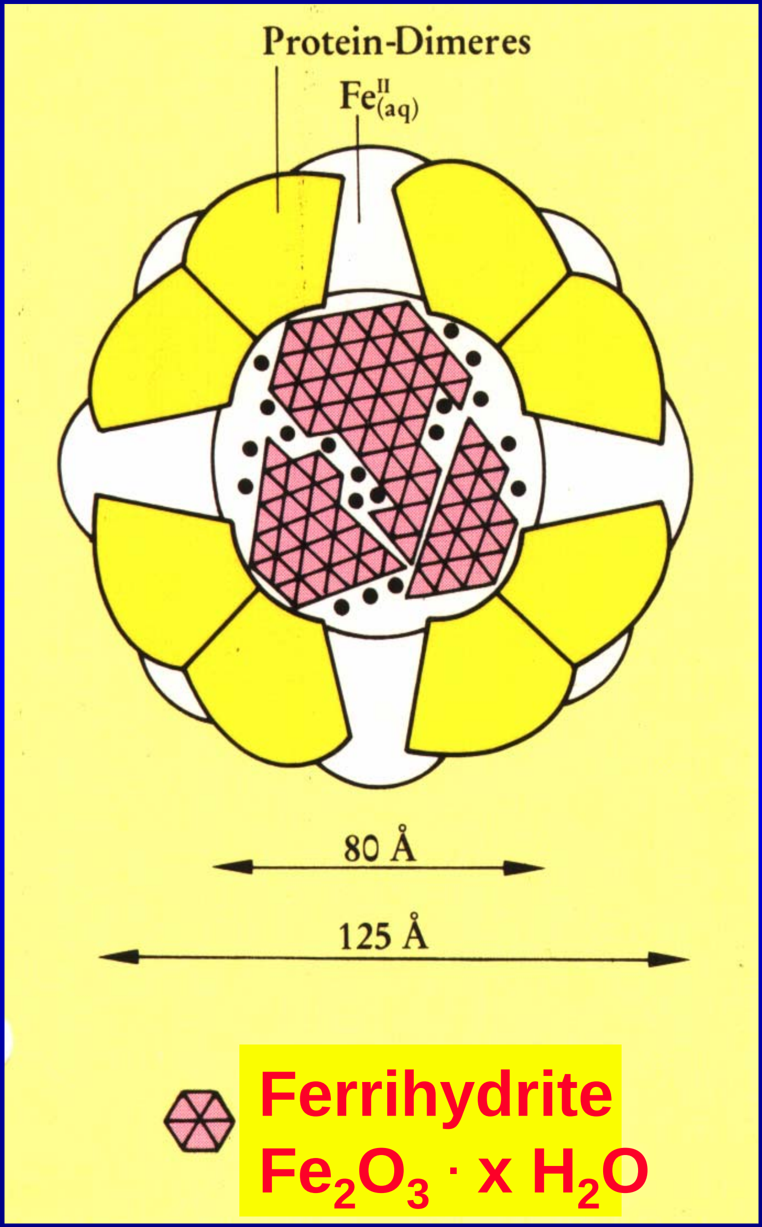
$$\rho_p = \frac{18\eta s}{d_p^2} + \rho_s$$

Density core and shell are known

d_{total} = 1.6 nm
d_{core} = 1.1 nm
d_{shell} = 0.5 nm
(Lit.: d_{shell} = 0.4 nm)

With independent size measurement, hybrid particle is fully characterized

Hybrid Colloids

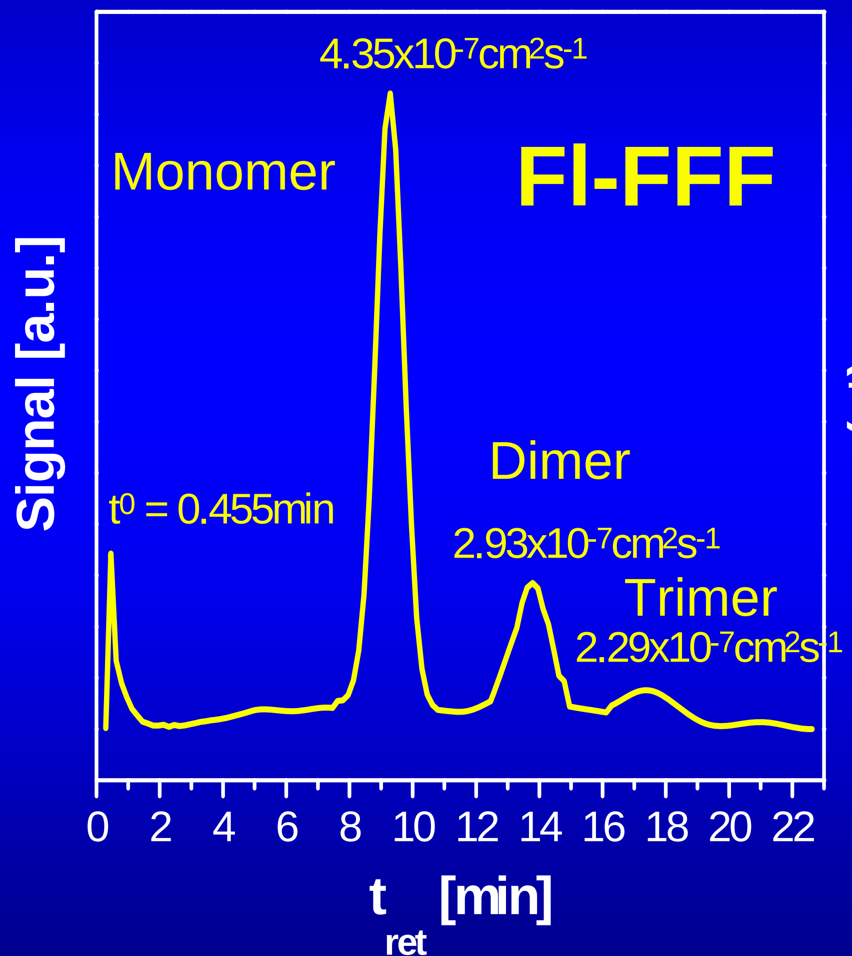


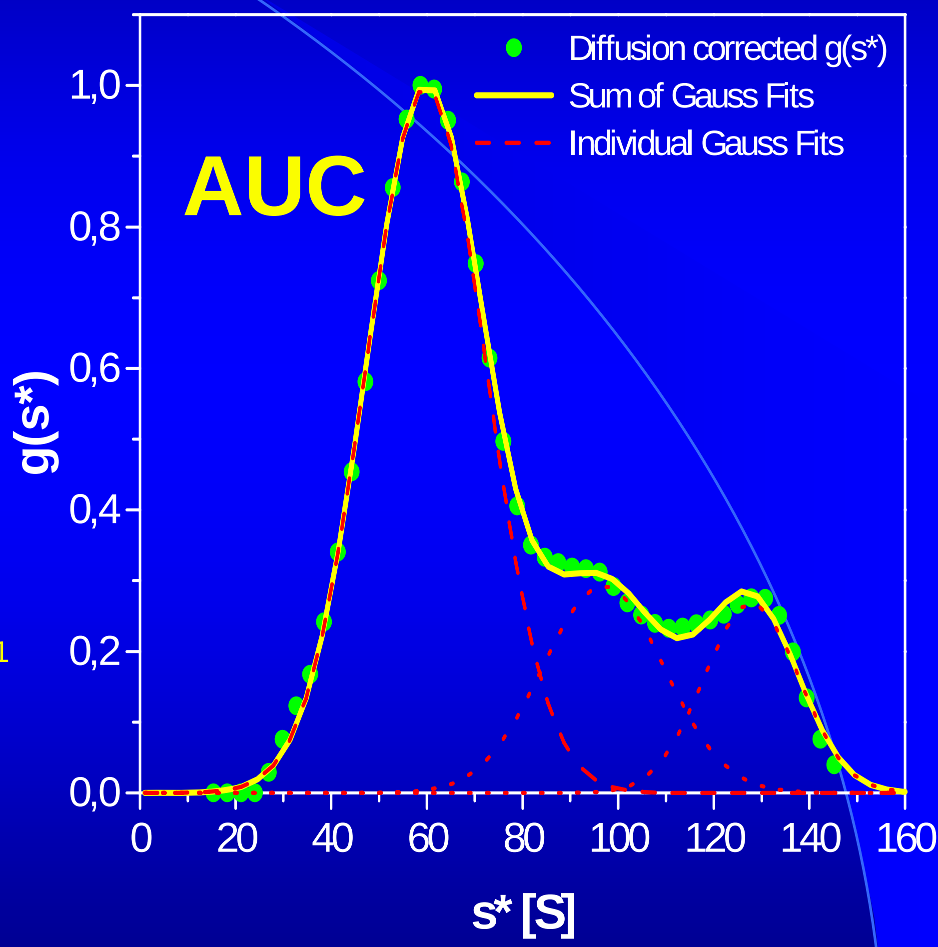
- Iron storage protein Ferritin forms robust hollow sphere from 24 dimeric subunits
- Ferritin oligomerizes
- Different amounts of ferrihydrite inside the core (up to 4500 Fe)
- Simultaneous particle size and density distribution
- Size and amount of oligomers ?
- Amount of ferrihydrite inside the core for the different oligomers ?

A. Völkel

Ferritin

Fl-FFF separates only according to particle size, AUC according to particle size and density

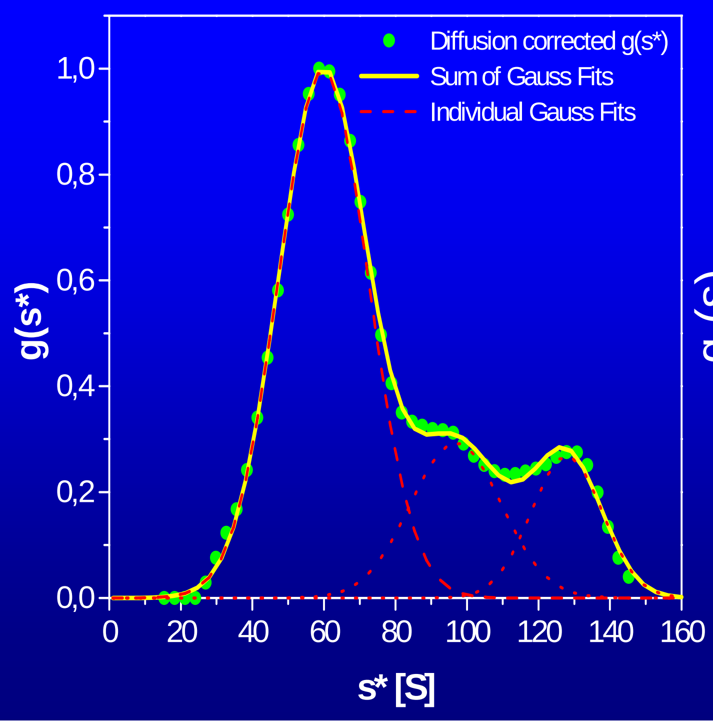


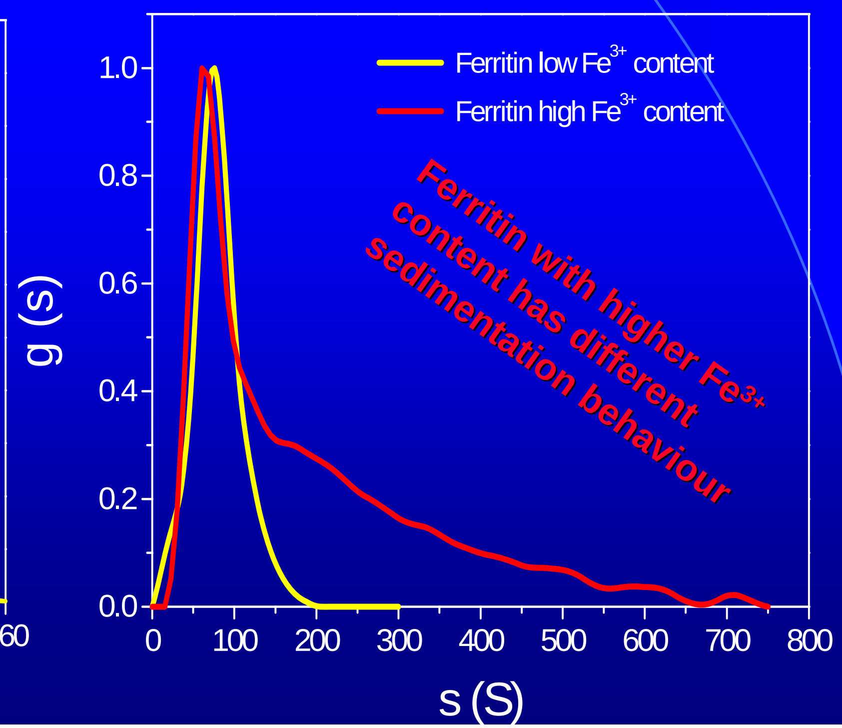


AUC no baseline separation of oligomers (density distribution)

Global analysis

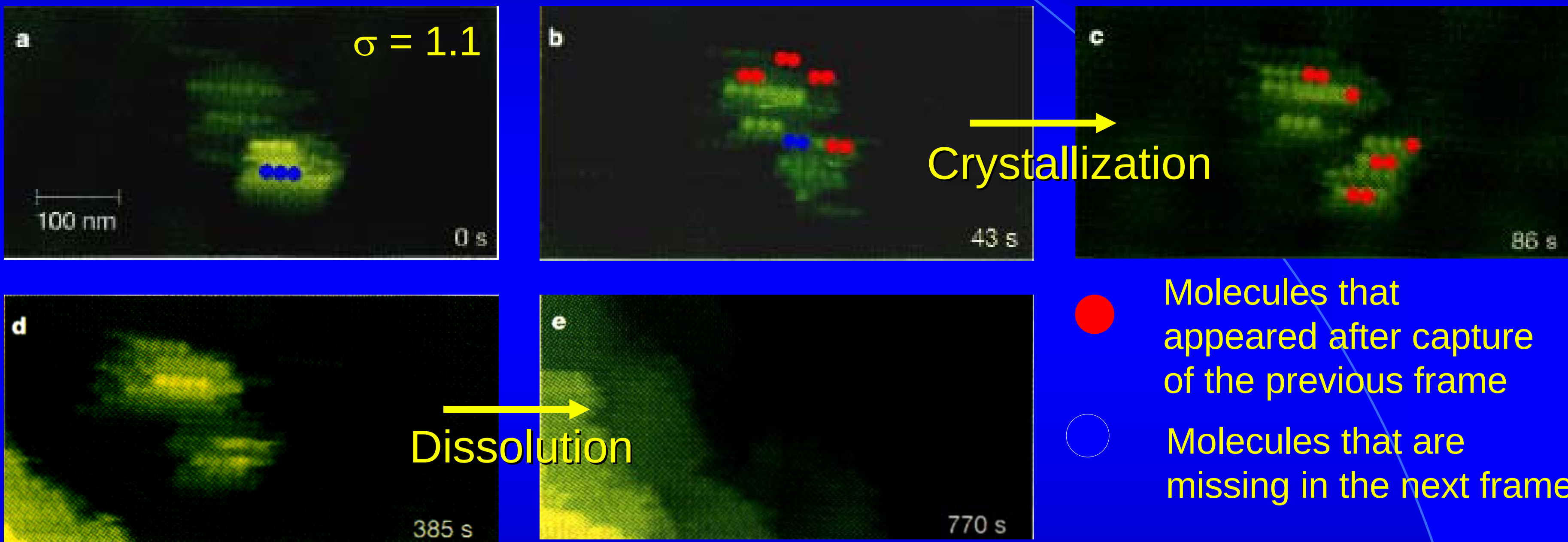
	Monomer	Dimer	Trimer
s* at 25 °C (AUC)	59.8 S	96.9 S	127.9 S
D* at 25 °C (Fl-FFF)	3.68 x 10^{-7} cm^2/s	2.41 x 10^{-7} cm^2/s	1.90 x 10^{-7} cm^2/s
d_H (Fl-FFF)	11.9 nm	18.1 nm	23.0 nm
ρ_P	**1.764 g/cm^3**	**1.531 g/cm^3**	**1.436 g/cm^3**
M_w	**586,700 g/mol**	**954,100 g/mol**	**1,367,400 g/mol**
Oligomer amount	**69.4 wt.-%**	**19.5 wt.-%**	**11.1 wt.-%**
n Fe^{3+}	**1476**	**667**	**34**





First direct observation of near critical size clusters by AFM

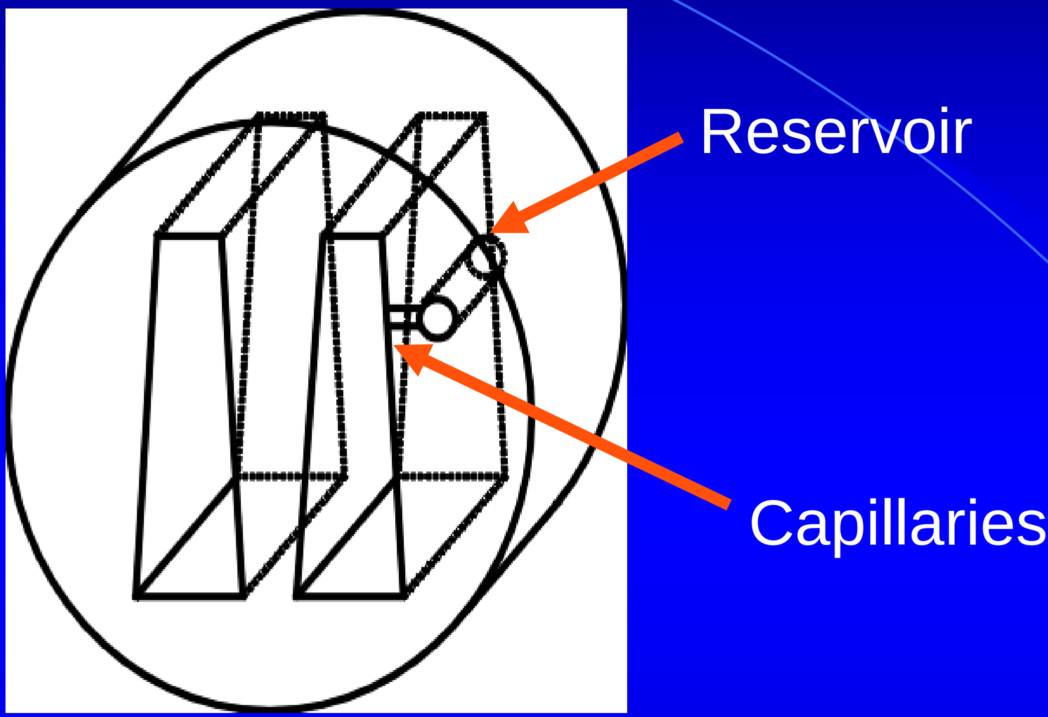
S.T. Yau, P.G. Vekilov „Quasi-planar nucleus structure in apoferritin crystallization“, Nature **406**, 494 - 497; 3 August 2000

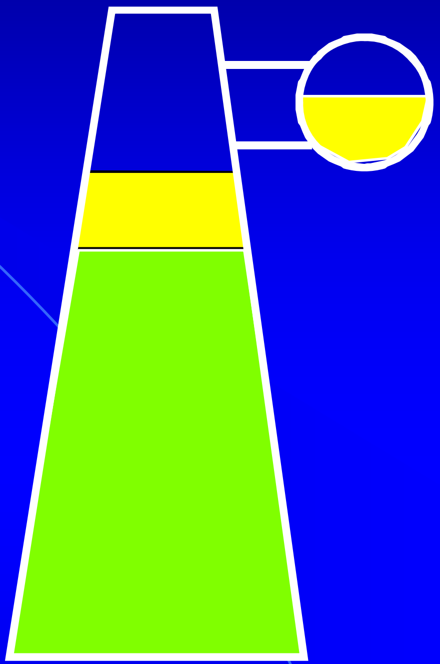


D.W. Oxtoby „Catching crystals at birth“, Nature **406**, 464 - 465; 3 August 2000:

„Although the first small crystallites involved in nucleation *form in the bulk of the solution*, Yau and Vekilov *can observe them only once they have fallen to the bottom of the vessel and attached themselves to the surface there*. How can the authors be sure that they are observing the critical early stages of crystallization described by nucleation ?“

Synthetic boundary cell of the Vinograd Type

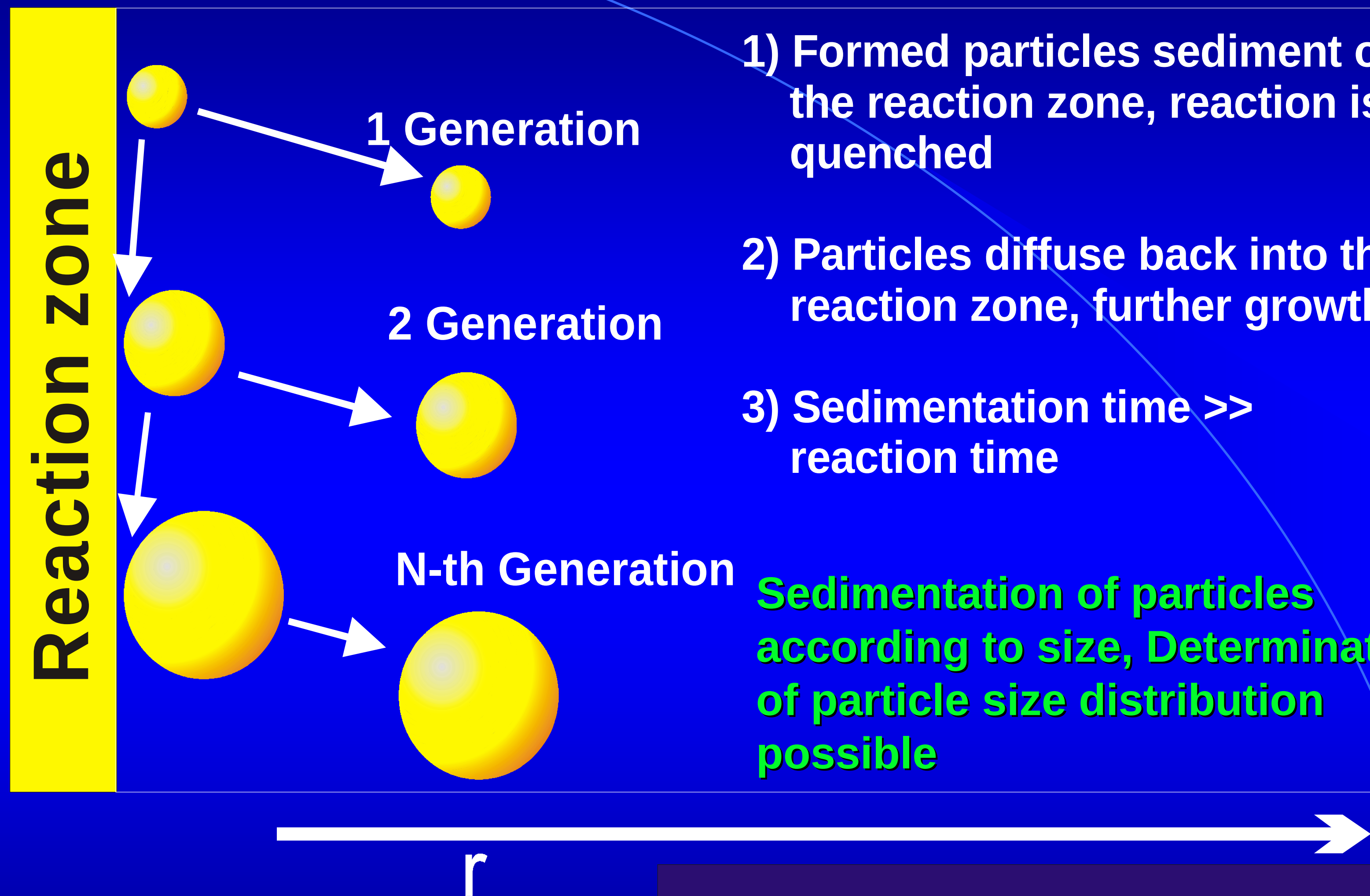




Reactant ( ) containing solution is layered onto the second reactant via the capillary ( )

Formation of a sharp reaction boundary

Particle formation in a synthetic boundary cell



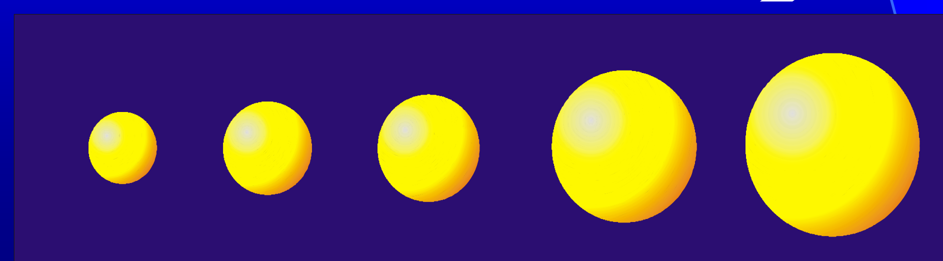
1) Formed particles sediment out of the reaction zone, reaction is quenched

2) Particles diffuse back into the reaction zone, further growth

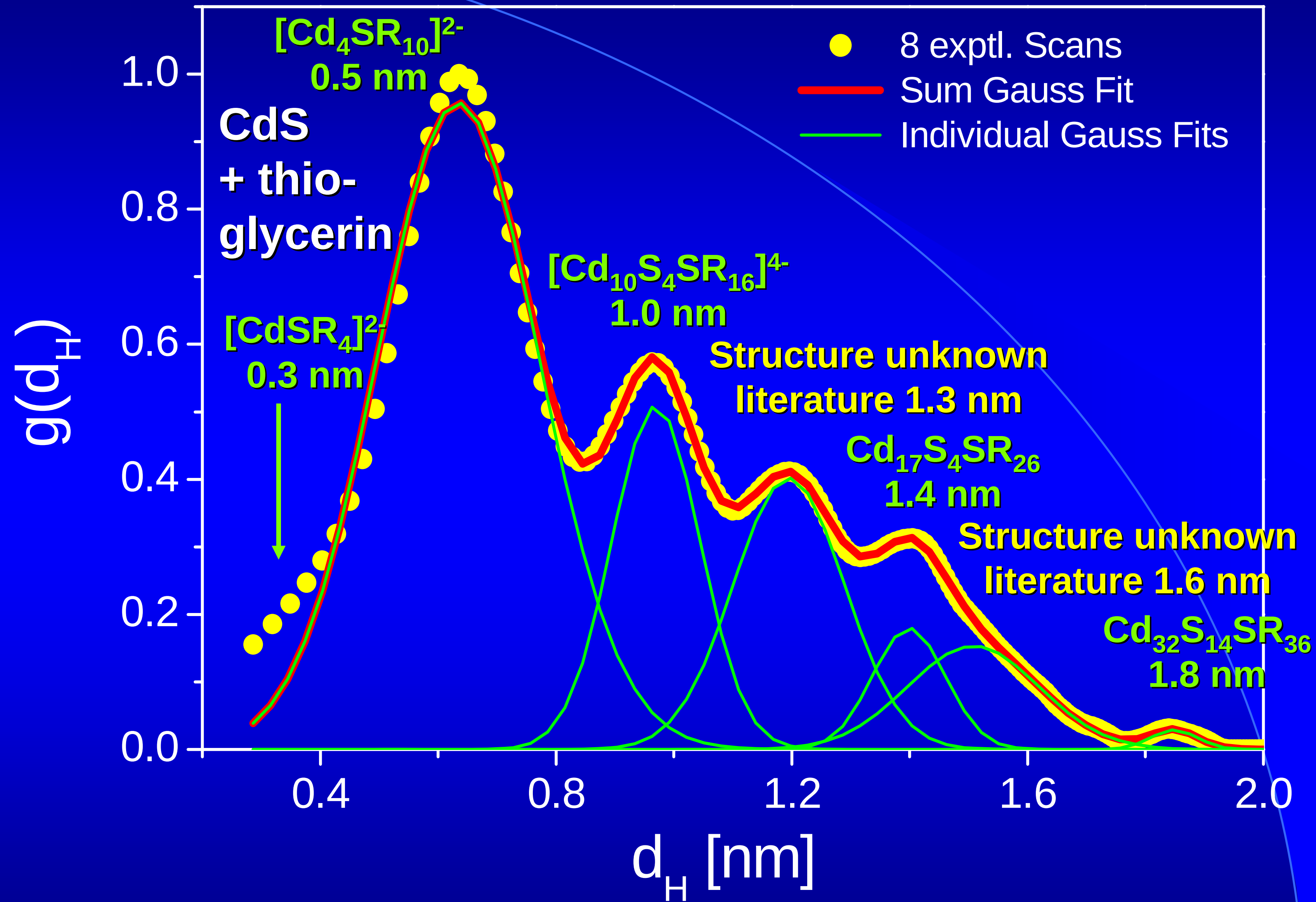
3) Sedimentation time >> reaction time

Sedimentation of particles according to size, Determination of particle size distribution possible

Transformation of a time distribution into a radial distribution



Synthetic boundary crystallization ultracentrifugation



All known stable CdS growth species simultaneously accessible in one experiment. Even subcritical clusters detectable in solution

Conclusion

- AUC is a very versatile instrument for colloid chemistry (Range of possible methods still not fully explored !!!)
- Fractionation allows the investigation of complex mixtures or very polydisperse samples with s ranging over decades (speed profile)
- High statistical accuracy
- Critical crystal nuclei and subcritical clusters can be resolved
- Crystal growth reactions can be investigated by transformation of time distribution into radial distribution

BUT:

- Colloids can express various unfavourable properties which makes them problematic to investigate
- Fast multidetection systems together with global analysis can potentially adress even most complex mixtures with particle size and VBAR distribution but role of charge still unadressed

Future Perspectives

- Fast speed profiles and detectors to suppress diffusion broadening
- Multi-detection systems (RI, UV-Vis, SLS and others)
- Global Analysis (promising with SEDVEL & SEDEQUIL + DLS & Fl-FFF) to obtain s, M, D & VBAR distributions
- New ultracentrifugation methods could allow to access new quantities

  Surface tension from phase transfer, particle charge determination via sedimentation in pH or charge gradient, Conformational changes in solvent quality gradients, new synthetic boundary techniques for membrane / crystal growth investigations or chemical reactions in the AUC, etc.

Recommended reading

. Svedberg, K.O. Pedersen, *The ultracentrifuge*, Clarendon Press, Oxford (1940)

he classical textbook about analytical ultracentrifugation still with much impact today

.K. Schachman, *Ultracentrifugation in biochemistry*, Academic Press, New York (1959)

compact and useful book covering experimental and theoretical aspects

.E. Harding, A.J. Rowe and J.C. Horton; *Analytical ultracentrifugation in biochemistry and polymer science*, The royal society of chemistry, Cambridge, ISBN 0-85186-345-0 (1992)

he most comprehensive modern book about analytical ultracentrifugation. A very good overview about methods & techniques of analytical ultracentrifugation and a valuable source of modern applications.