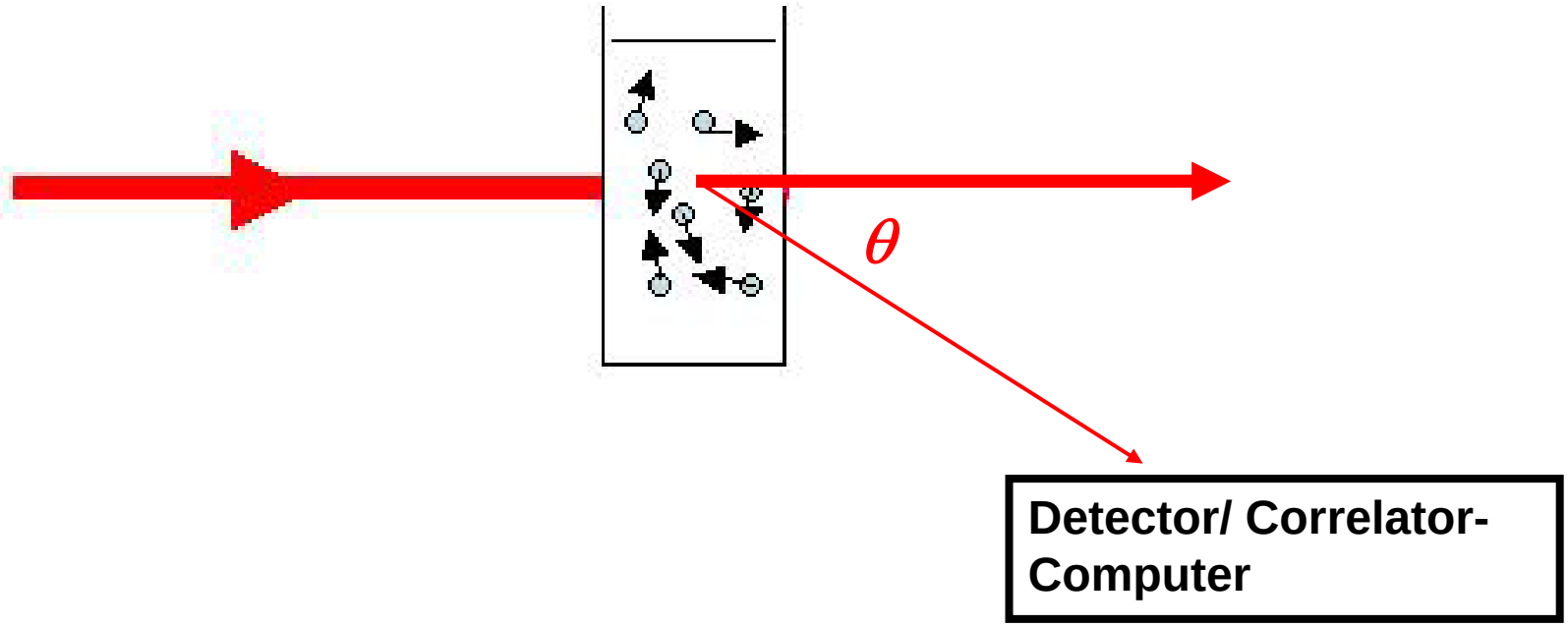
Datum/Zeit	Veranstaltungsort	Thema
Mo, 08.02.2010 10.00-11.30	Hörsaal Institut für Glaschemie Fraunhoferstrasse 6	***Albert Einstein and the Viscosity of Macromolecules***
Mo, 08.02.2010 12.15-13.45	Hörsaal Haus 1,IAAC, August-Bebel-Str. 2	***Light Scattering and SEC-MALLs***
Di, 09.02.2010 12.15-13.45	Institut für Materialwissenschaft und Werkstofftechnologie, HS 124 Löbdergraben 32	***Dynamic Light Scattering***
Mi, 10.02.2010 16.15-17.45	Hörsaal 3 Carl-Zeiss-Str. 3	***Analytical Ultracentrifugation I***
Do, 11.02.2010 14.15-15.45	Döbereiner Hörsaal	***Analytical Ultracentrifugation II: Interactions***

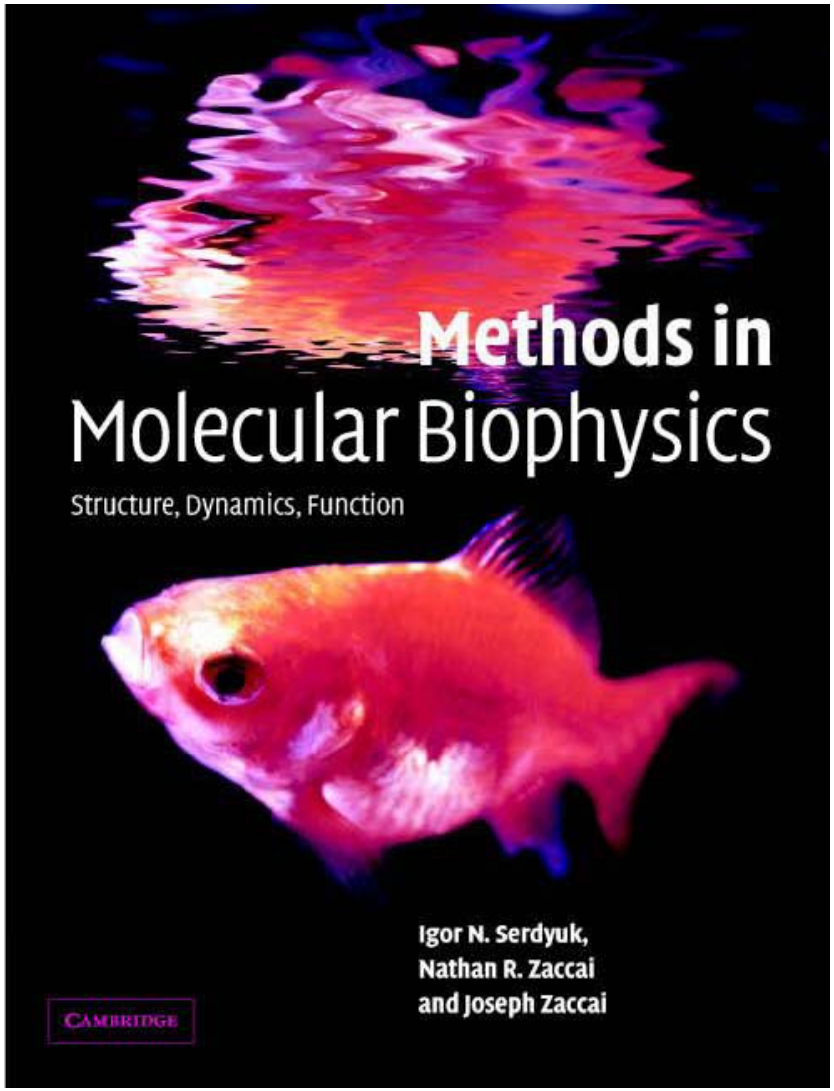
Lecture 3:

Dynamic Light Scattering

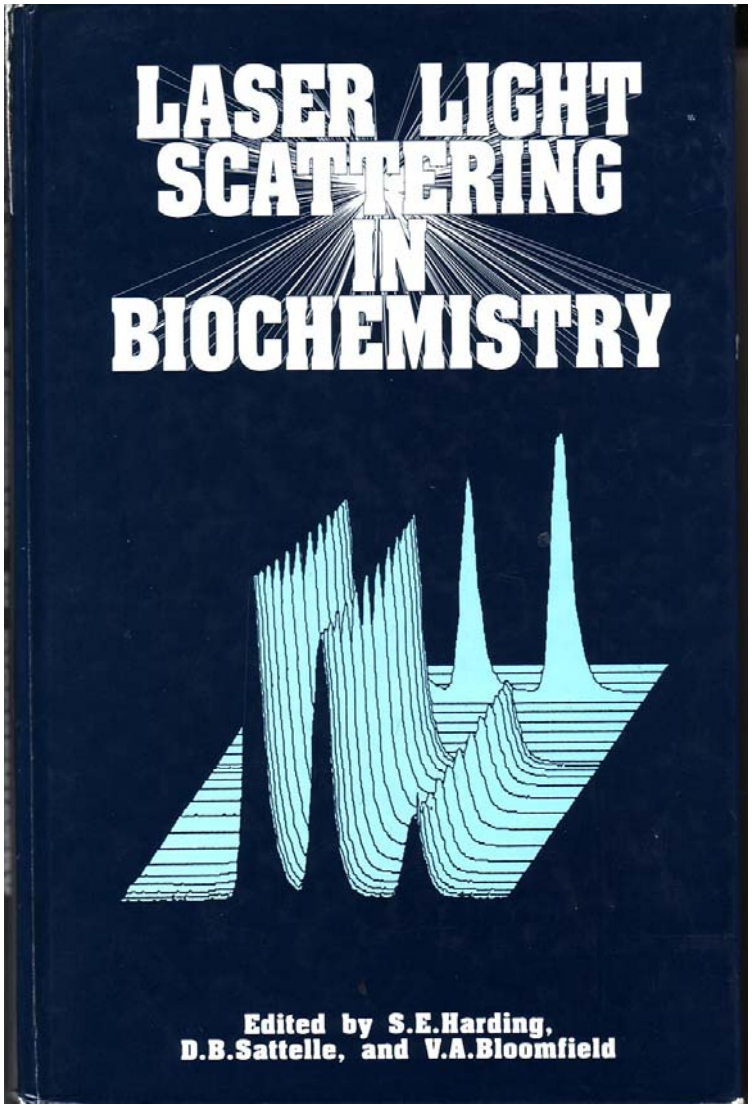


Part 1: DLS basic theory (overhead transparencies)

Part 2: Instrumentation – multi angle and fixed angle



Chapters D3 & D10



RSC Cambridge, 1992

Transport Probes :

DYNAMIC LIGHT SCATTERING

.... for measurement of diffusion coefficients

Why diffusion coefficients ?

(1) DIFFUSION COEFFICIENT → Structure & molecular weight of a biomolecule in solution

(2) POLYDISPERSITY / DISTRIBUTION OF DIFFUSION COEFFICIENTS → Size distribution (polysaccharides etc.)

(3) CHANGES IN DIFFUSION COEFFICIENT → DYNAMICS of a process

Strategy

PART I :

1. WHAT IS A DIFFUSION COEFFICIENT?
2. TECHNIQUES FOR OBTAINING IT
3. TYPES OF LIGHT SCATTERING ANALYSIS
4. DYNAMIC LIGHT SCATTERING – extraction of diffusion coefficients
5. EXPERIMENTAL POINTS

PART II : USE OF DIFFUSION COEFFICIENTS

PART I

1. DIFFUSION COEFFICIENTS

The diffusion coefficient, D is a manifestation of the ability of a particle to move under (i) the influence of a concentration gradient or (ii) under the influence of Brownian motion

(i) Concentration gradient

If J = mass of particles crossing 1 cm^2 cross section per sec.

$\partial c / \partial x$ = concentration gradient

then $J = -D \frac{\partial c}{\partial x}$ ("Fick's 1st law")

(ii) Brownian diffusion

$\overline{x^2}$ = average square displacement of a particle

$\overline{x^2} = 2Dt$

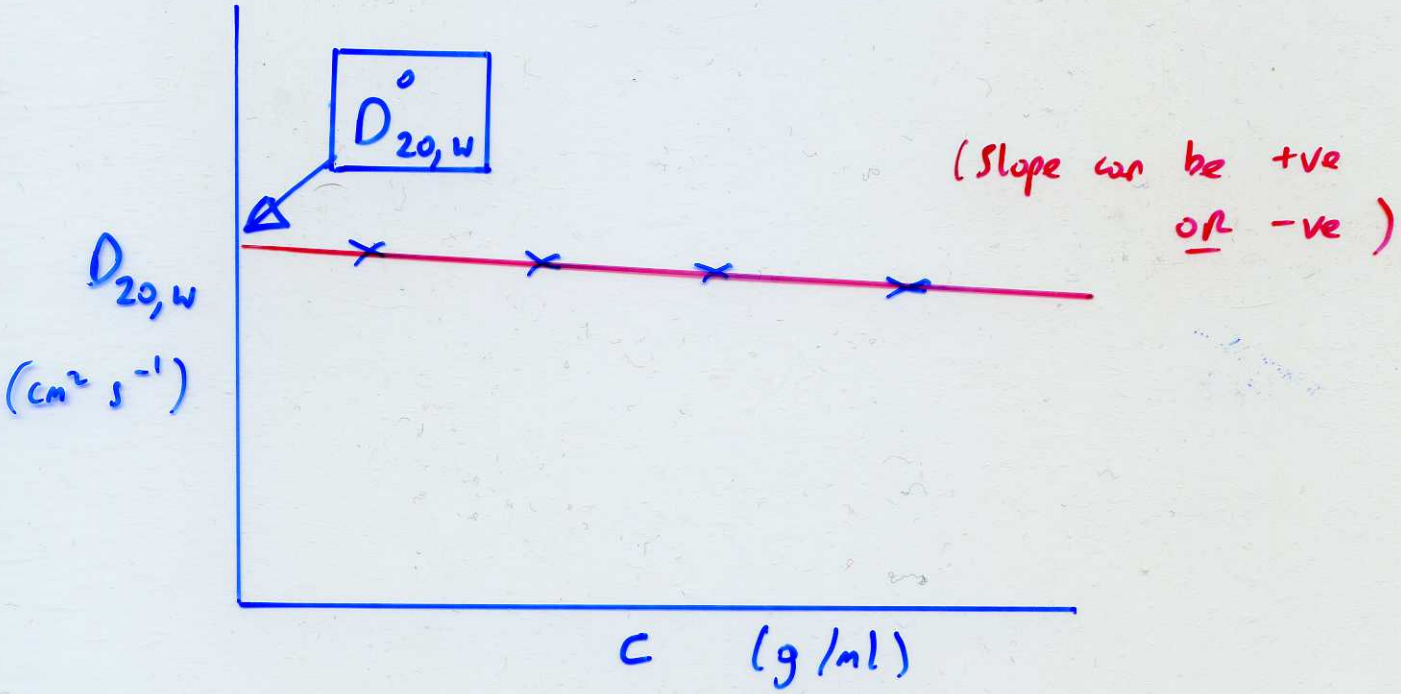
Units of D: $cm^2\ sec^{-1}$

(ii) More relevant to dynamic light scattering

In order to relate D to macromolecular properties we have to ① "standardize"/"normalize" to standard solvent conditions

$$D_{20,w} = \frac{293.1}{T} \frac{\eta_{T,b}}{\eta_{20,w}} \cdot D_{T,b}$$

② Extrapolate to zero concentration



$D^0_{20,w}$ so obtained can tell us many interesting things about biomolecules!

2. TECHNIQUES FOR OBTAINING $D^{\circ}_{20,w}$

DYNAMIC LIGHT SCATTERING

ANALYTICAL ULTRACENTRIFUGATION

("boundary spreading")

NUCLEAR MAGNETIC RESONANCE

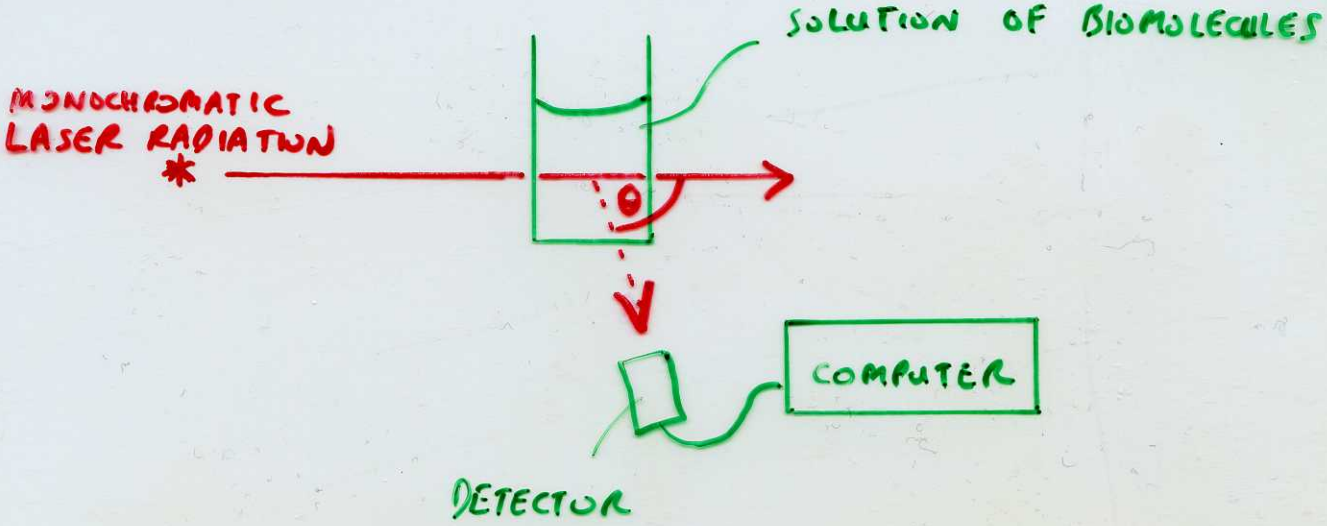
3. TYPES OF LIGHT SCATTERING

TURBIDITY (Use simple spectrophotometer)

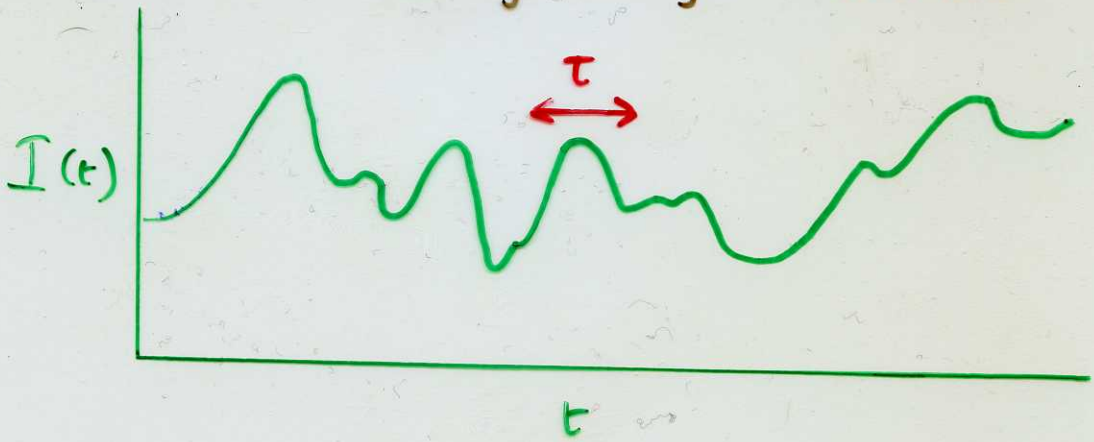
'CLASSICAL' LIGHT SCATTERING

DYNAMIC LIGHT SCATTERING.

4. DYNAMIC LIGHT SCATTERING



The moving macromolecules will "Doppler broaden" the otherwise monochromatic radiation. Intensity fluctuates because of different λ's beating amongst themselves



For a given angle θ:

An 'Autocorrelator' correlates intensities I(t) at time t with subsequent times $t + \delta\tau_s$ — sample time

CHANNEL NUMBER
1-64
or 1-128
or 1-256

$\delta\tau_s$ is referred to collectively as the "DELAY TIME" (τ)

Small delay times – GOOD CORRELATION

Long " " – POOR "

DEFINE a NORMALIZED CORRELATION FUNCTION

$$g^{(2)}(\tau) = \frac{\langle I(t) \cdot I(t+\tau) \rangle}{\langle I \rangle^2}$$

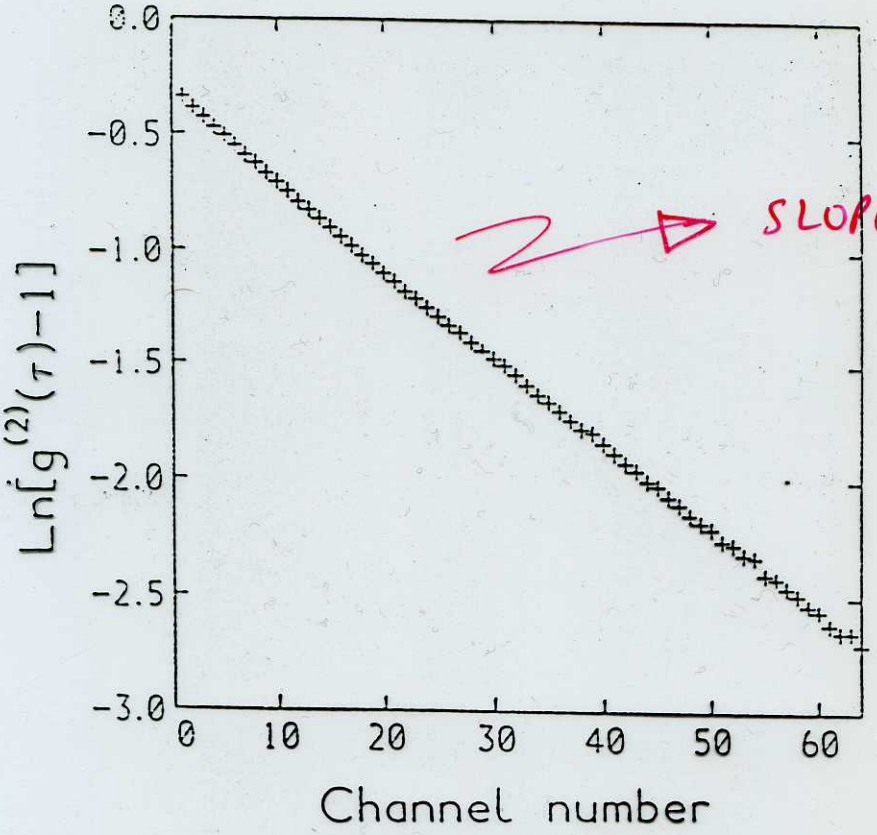
$\langle\ \rangle$ indicates product $I(t) \times I(t+\tau)$ averaged over long times compared with τ

For ≈ spherical Brownian systems

$$\ln [g^{(2)}(\tau) - 1] = -2Dk^2\tau$$

Bragg vector $= \frac{4\pi n}{\lambda \sin(\theta/2)}$

——— (1)



→ SLOPE → D.

(Dynein, $M \sim 2.5 \times 10^6$
$D_{20,w} = 1.1 \times 10^{-7}$ $cm^2 s^{-1}$)

N.B. For asymmetric scatterers eqn (1) becomes more complicated. These complications can be avoided if D measured according to (1) is extrapolated to zero angle

D values is obtained have to be

① corrected to $D_{20,w}$ values

② extrapolated to zero concentration to give

$D^{\circ}_{20,w.}$

9: Hydrodynamic properties of proteins

(a)

PART II : USE OF DIFFUSION COEFFICIENTS

1. MOLECULAR WEIGHT (M)

2. SIZE (STOKES RADIUS r_H)

3. FRICTIONAL COEFFICIENT - CONFORMATION

4. POLYDISPERSITY (Distribution of molecular weights)

5. DYNAMICS of processes.

1. MOLECULAR WEIGHT

$$M = \frac{s^{0}_{20,w}}{D^{0}_{20,w}} \times \frac{RT}{(1-\bar{v}\rho)}$$

1. MOLECULAR WEIGHT

$$M = \frac{s^{\circ}_{20,w}}{D^{\circ}_{20,w}} \times \frac{RT}{(1-\bar{v}\rho)}$$

Explanation :

$$D^{\circ}_{20,w} = \frac{RT}{N_A f} \qquad \text{(1)}$$

f : frictional coefficient

$$s^{\circ}_{20,w} = \frac{M(1-\bar{v}\rho)}{N_A f} \qquad \text{(2)}$$

(2) / (1) eliminates f.

2. SIZE

- in terms of the "STOKES RADIUS"

or radius of the equivalent sphere :

$$r_H = \frac{(k_B T)}{6\pi \eta_{20,w} D^{\circ}_{20,w}}$$

k_B : Boltzmann constant

$\eta_{20,w}$: viscosity of water at 20°C.

E.g.'s : $r_H \sim 1.5$nm lysozyme

~ 3nm hemoglobin

30nm turnip yellow mosaic virus

~ 1000nm bacterial spore

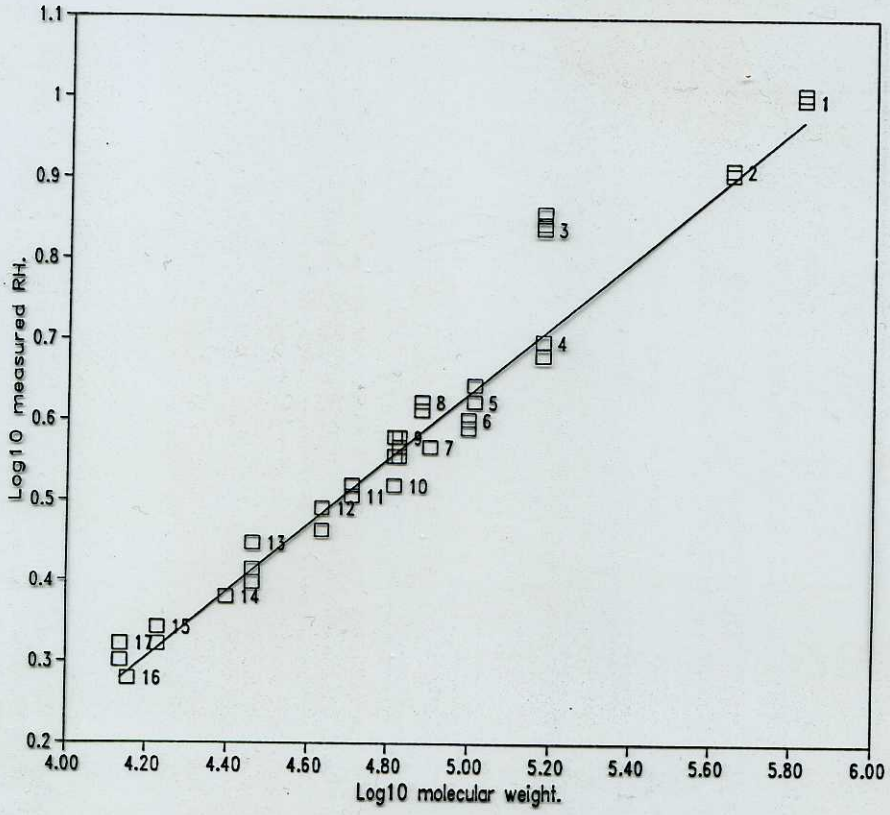
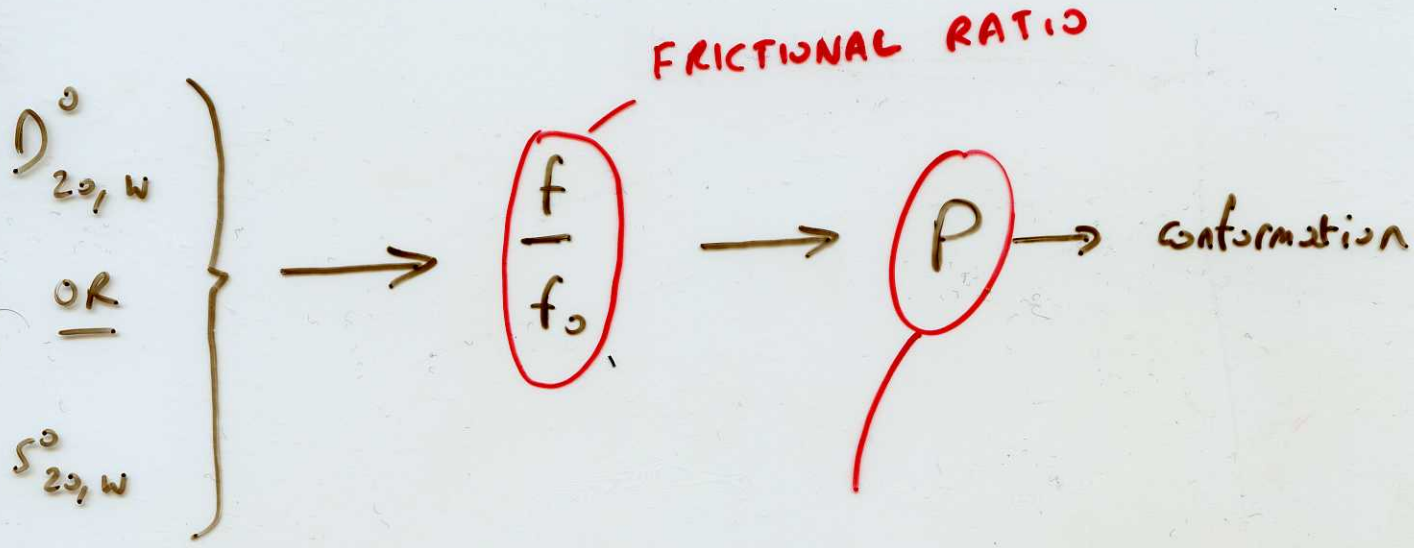


Figure 1. Plot of $\log_{10} R_H$ against $\log_{10}$ molecular weight. Proteins measured were: (1) thyroglobulin; (2) apoferritin; (3) IgG; (4) yeast alcohol dehydrogenase; (5) hexokinase; (6) amyloglucosidase; (7) horse alcohol dehydrogenase; (8) transferrin; (9) bovine serum albumin; (10) haemoglobin; (11) hexokinase sub-unit; (12) ovalbumin; (13) carbonic anhydrase; (14) chymotrypsinogen; (15) myoglobin; (16) lysozyme; (17) ribonuclease A. The relationship between $\log_{10} R_H$ and $\log_{10}$ molecular weight used in the estimation of molecular weight from measured R_H is also shown (——).

3. CONFORMATION



PERRIN FUNCTION

OR "FRICTIONAL RATIO due to SHAPE"

f: frictional coefficient of macromolecule

f_0: frictional coefficient of a sphere of the same mass and same dry (anhydrous) volume

$$\frac{f}{f_0} = \frac{k_B T}{6\pi\eta_0}\left(\frac{4\pi N_A}{3\bar{v}M}\right)^{1/3} \cdot \frac{1}{D^\circ_{20,w}}$$

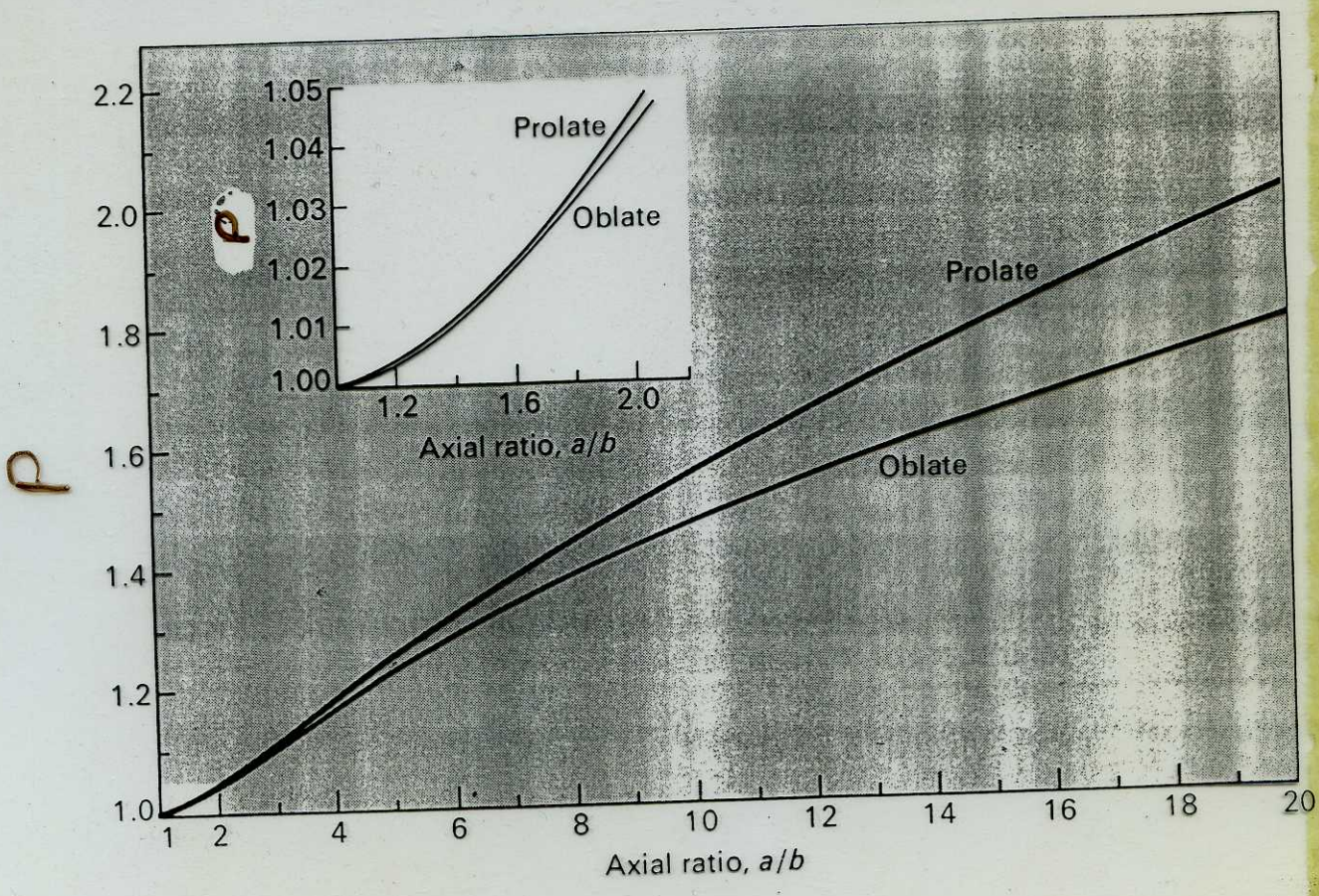
$$= \frac{M(1-\bar{v}\rho)}{N_A 6\pi\eta_0}\left(\frac{4\pi N_A}{3\bar{v}M}\right)^{1/3} \cdot \frac{1}{s^\circ_{20,w}}$$

$$\rho = \left(\frac{f}{f_0}\right) \cdot \left(\frac{\bar{v}}{v_s}\right)^{1/3}$$

hydration

v_s = swollen specific volume (ml/g) = $\bar{v} + \delta/\rho_0$

In terms of simple ellipsoids of revolution ...



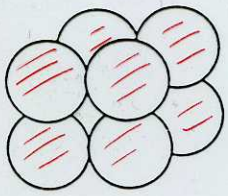
... or in terms of "BEAD MODELS"

Strategy: ① From a given model calculate P (& hence f/f_s, $D^\circ_{20,w}$ & $s^\circ_{20,w}$)

② Compare with experimental $D^\circ_{20,w}$, $s^\circ_{20,w}$

③ Chose appropriate model or refine model until agreement is reached

e.g. α-lactoglobulin {~~tetra~~ octameric protein}

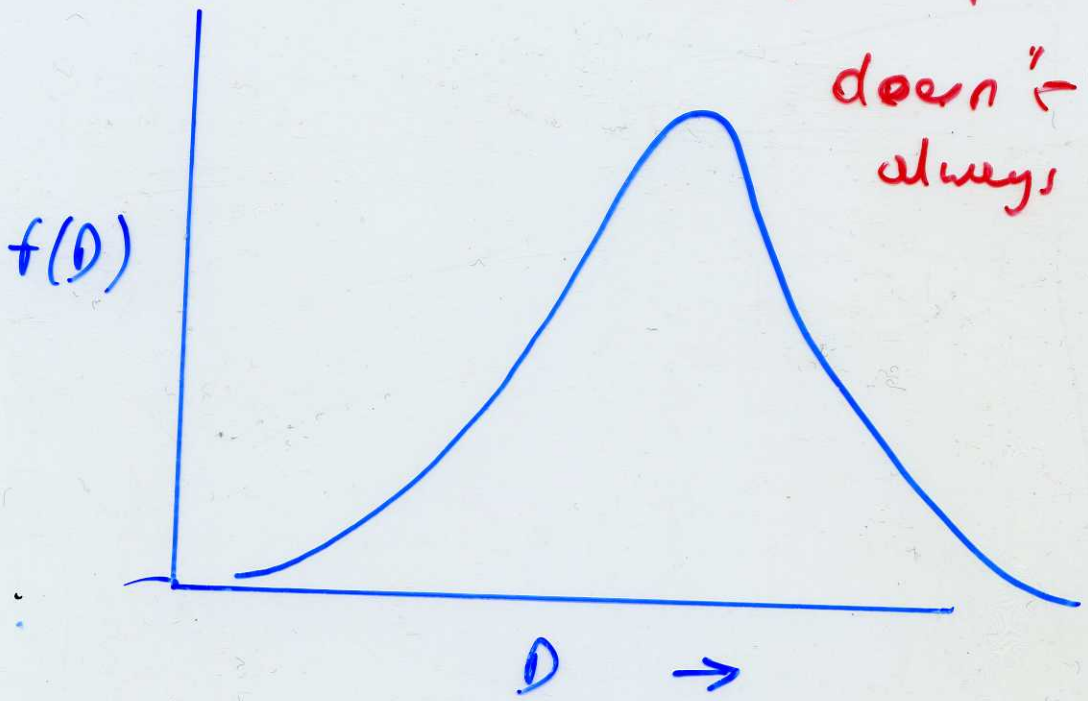


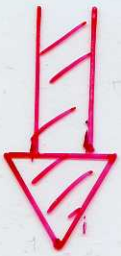
"Compact" form is the only form that agrees with the measured $s^\circ_{20,w}$ & $D^\circ_{20,w}$ data.

Another good example is the 11S soya bean globulin: also compact.

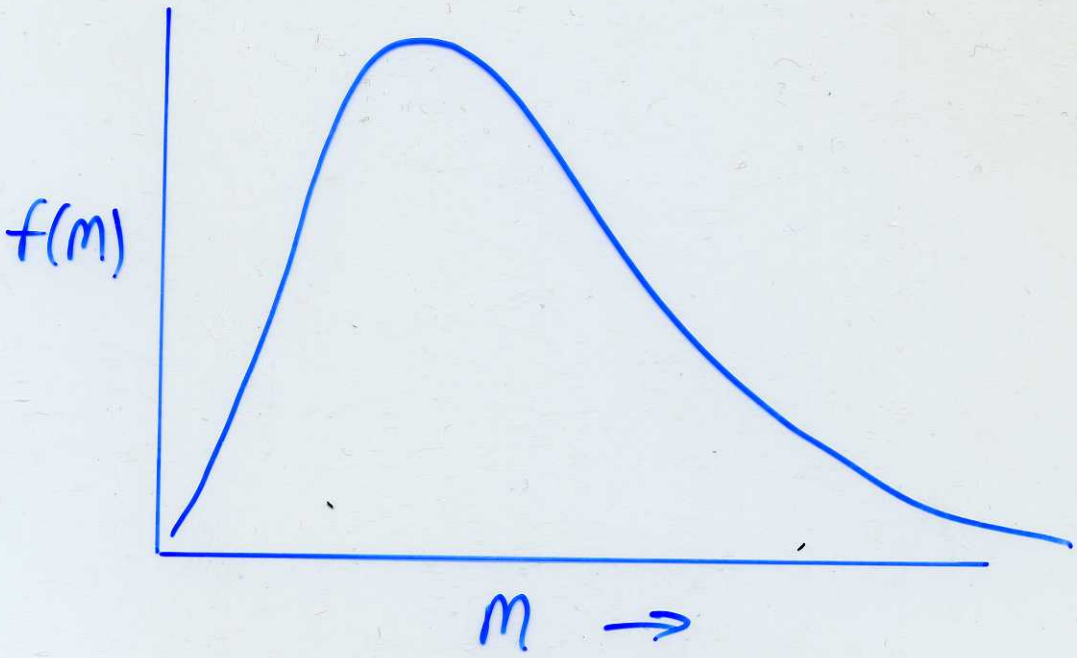
4. DISTRIBUTION OF SIZES

– in principle "doesn't" always work!





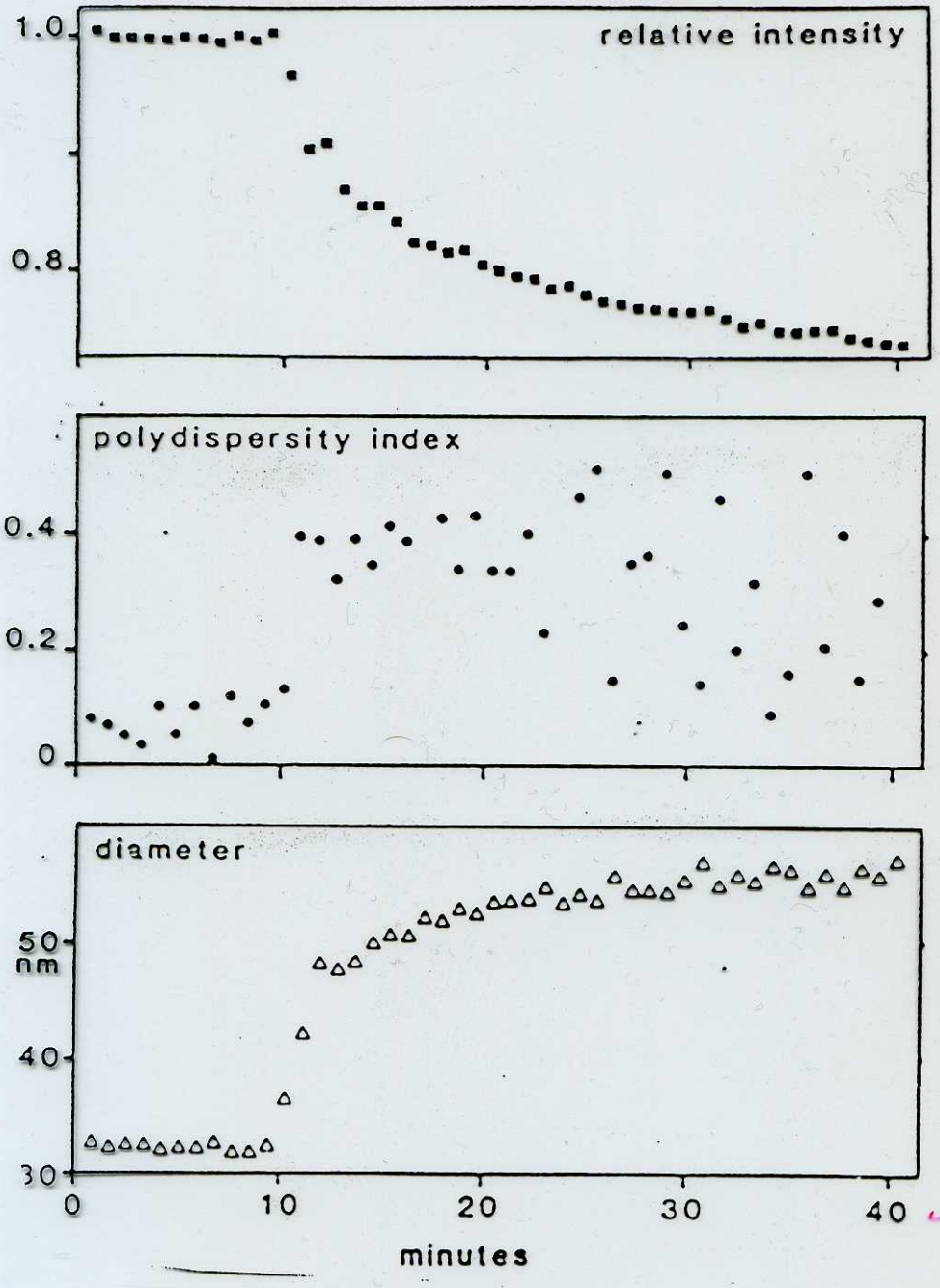
ASSUME A CONFORMATION



5. DYNAMIC LIGHT SCATTERING can be used to follow dynamic processes:

e.g. Swelling of VIRUS particles

[SOUTHERN BEAN MOSAIC VIRUS]

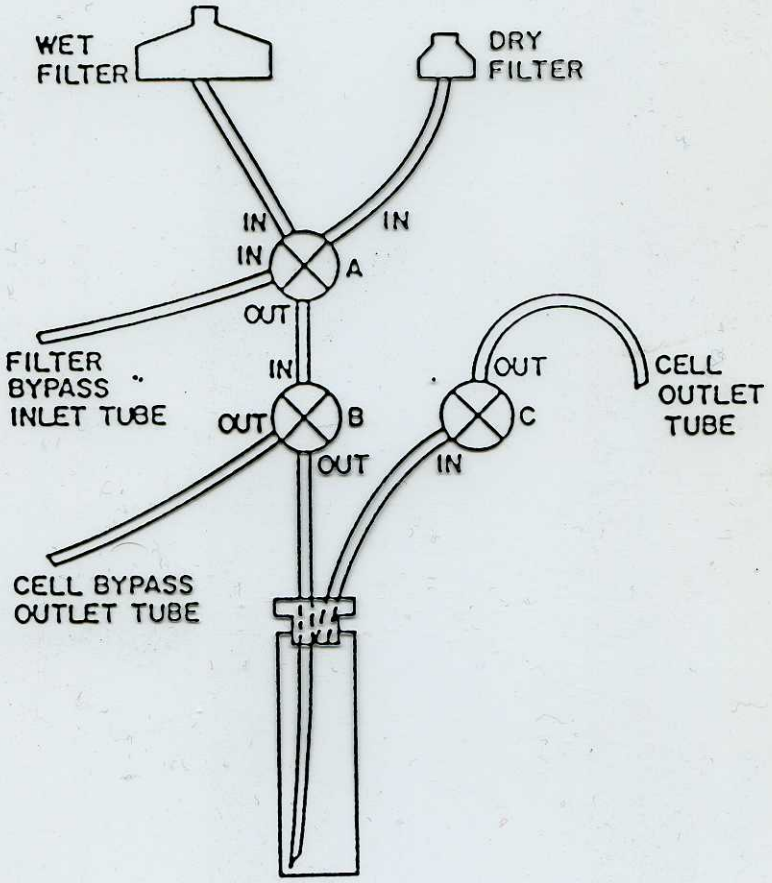


from spectrophotometer

from dynamic light scattering

... solutions & scattering cells / cuvettes

have to be scrupulously clean



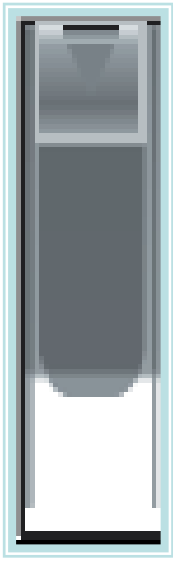
Multi-angle DLS – Malvern 4700 system

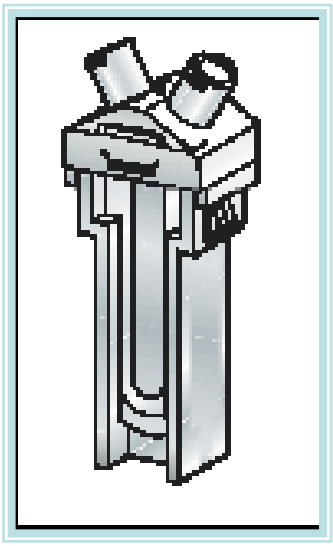
Fixed angle DLS – Protein Solutions System

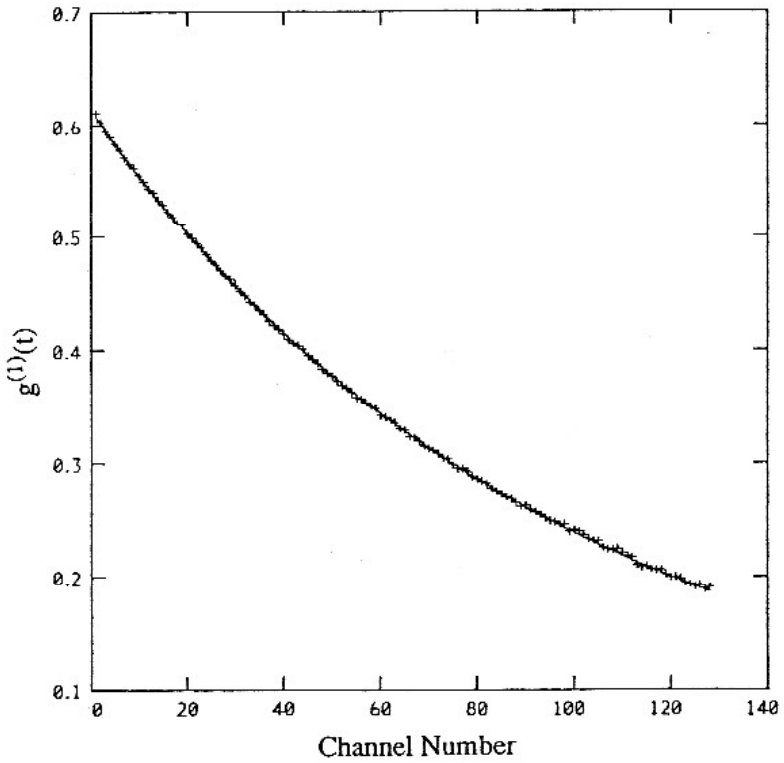
Fixed angle DLS – Malvern NanoS system

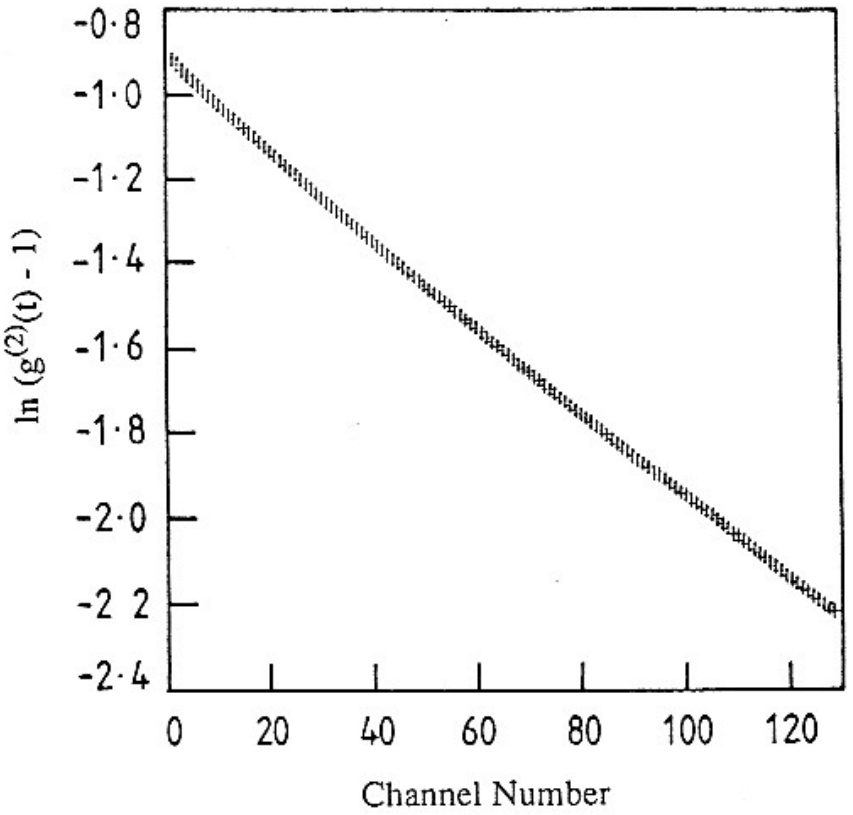
Malvern nanozetasizer 90

Cuvettes:



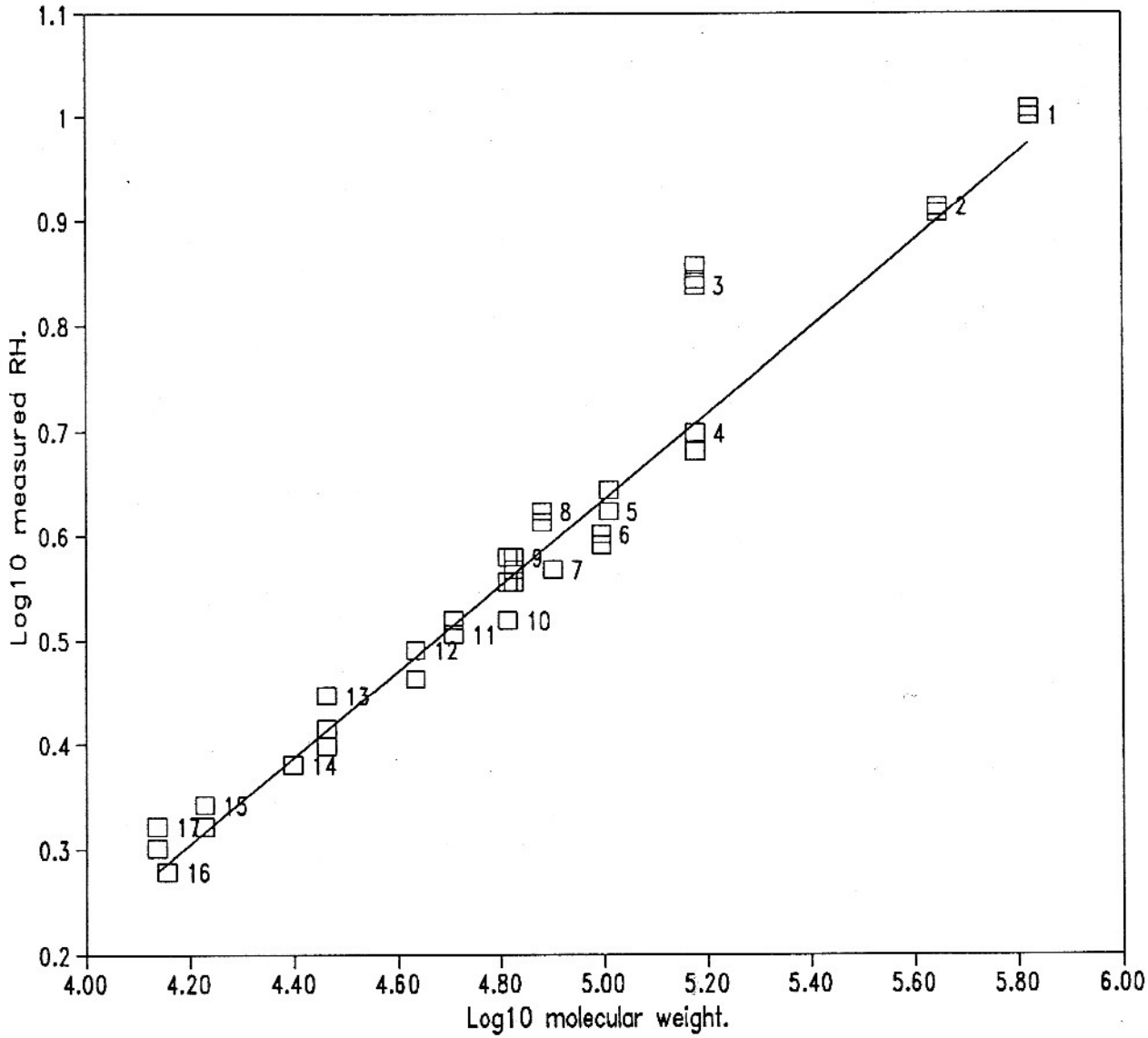


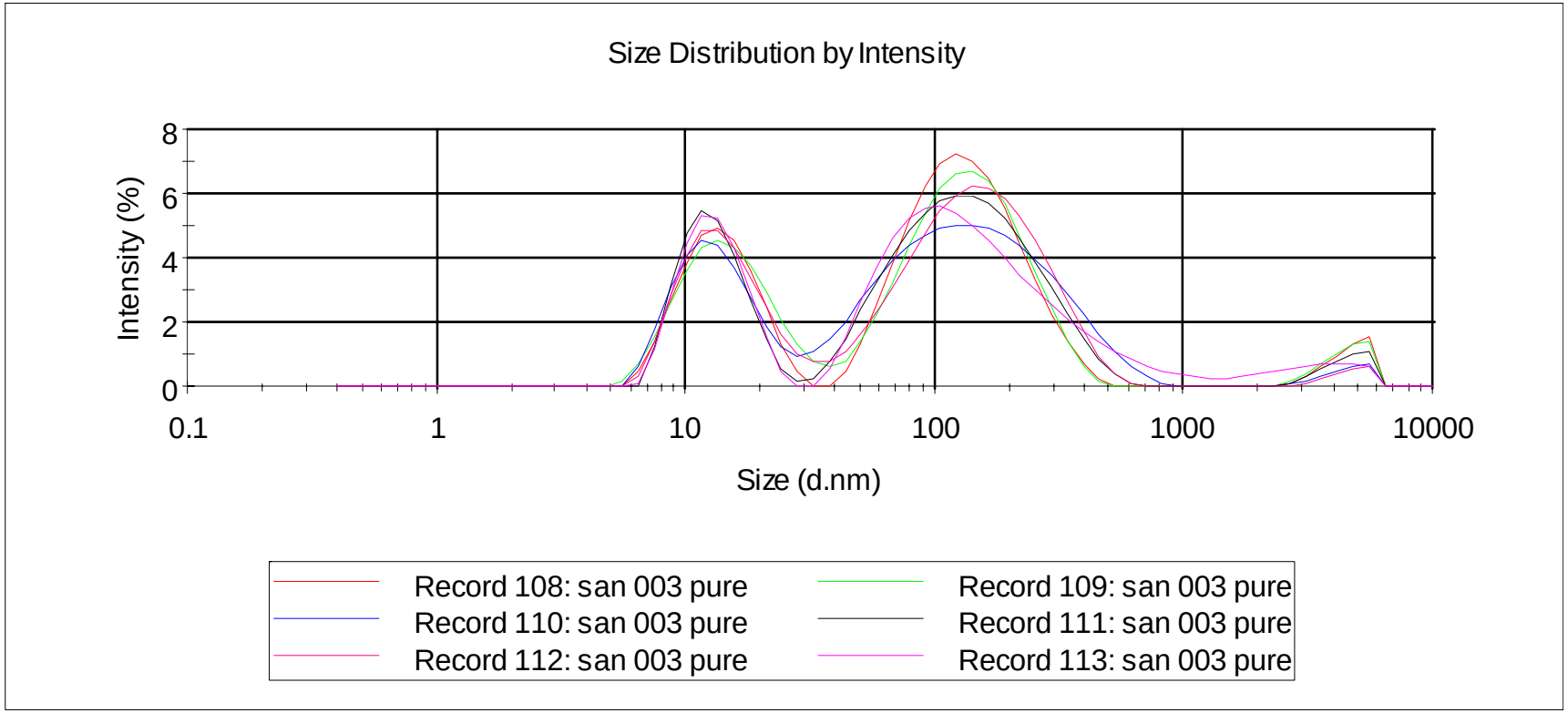


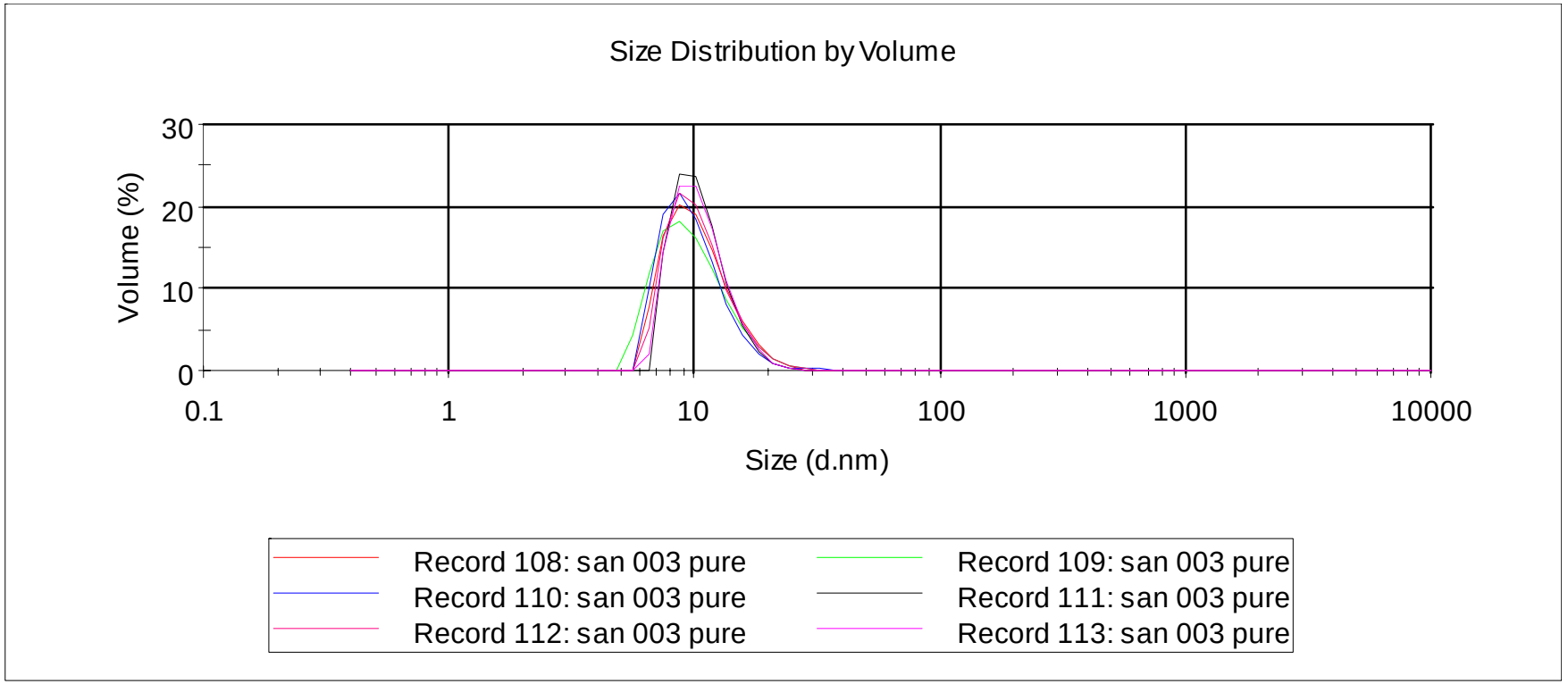


$$D = \frac{k_B T}{6\pi\eta R}$$

“size” d = 2*R*


Log10 measured RH.
Log10 molecular weight.
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
4.00
4.20
4.40
4.60
4.80
5.00
5.20
5.40
5.60
5.80
6.00
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17


Size Distribution by Intensity
Intensity (%)
8
6
4
2
0
0.1
1
10
100
1000
10000
Size (d.nm)
Record 108: san 003 pure
Record 109: san 003 pure
Record 110: san 003 pure
Record 111: san 003 pure
Record 112: san 003 pure
Record 113: san 003 pure


Size Distribution by Volume
Volume (%)
30
20
10
0
0.1
1
10
100
1000
10000
Size (d.nm)
Record 108: san 003 pure
Record 109: san 003 pure
Record 110: san 003 pure
Record 111: san 003 pure
Record 112: san 003 pure
Record 113: san 003 pure

Follow up bibliography:

1. On-line tutorials from: Malvern Instruments and Wyatt Technology (see their web sites)
2. Serydyuk, I.N., Zaccai, N.R. and Zaccai, J. (2006) *Methods in Molecular Biophysics*, Cambridge, Chapters D3 and D10
3. Harding, S.E., Sattelle, D.B. & Bloomfield, VA. Eds (1992) *Laser Light Scattering in Biochemistry* Royal Soc. Chem. Cambridge
4. Harding, S.E. & Johnson, P.J. (1985) The concentration dependence of macromolecular parameters, *Biochem. J.* 231, 543-547
5. Nobbmann U *et al* (2007) Dynamic light scattering as a relative tool for assessing the molecular integrity and stability of monoclonal antibodies. *Biotechnology and Genetic Engineering Reviews, 24,* 117-128
   Link: http://www.nottingham.ac.uk/ncmh/BGER/pdf/volume_24/04-Nobbmann-BGER.pdf