

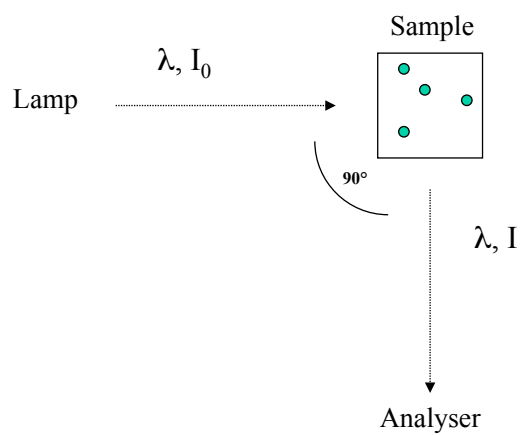
Dynamic Light Scattering

Effetto Tyndall: diffusione della luce da parte delle **particelle** in sospensione



1

Dynamic Light Scattering



autocorrelation function of the diffused intensity

$$G(\tau) = \langle I(0)I(\tau) \rangle \equiv \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T dt I(t)I(t+\tau)$$

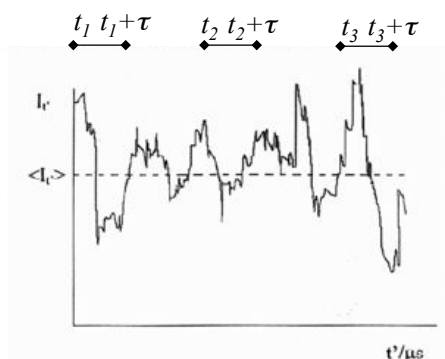
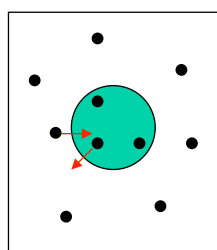
↑
tempo

2

the light intensity scattered by the sample fluctuates with the time around its mean value

$$G(\tau) = \langle I(0)I(\tau) \rangle \equiv \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T dt I(t)I(t+\tau) \approx \lim_{N \rightarrow \infty} \frac{1}{N} \sum_{n=0}^N I(t_n)I(t_n + \tau) =$$

$$\approx \frac{1}{N} [I(t_1)I(t_1 + \tau) + I(t_2)I(t_2 + \tau) + \dots]$$



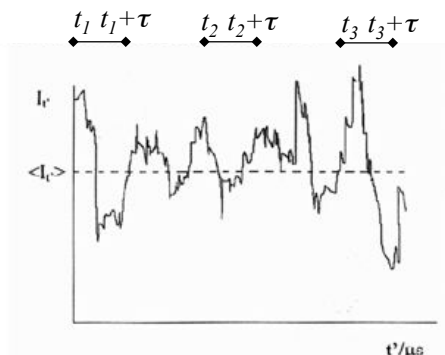
3

the autocorrelation function decreases with τ :

$$G(0) = \langle I(0)I(0) \rangle = \langle I(0)^2 \rangle = \lim_{N \rightarrow \infty} \frac{1}{N} \sum_{n=0}^N I(t_n)^2 \geq$$

$$\geq \lim_{N \rightarrow \infty} \frac{1}{N} \sum_{n=0}^N I(t_n)I(t_n + \tau) = \langle I(0)I(\tau) \rangle = G(\tau)$$

$$\forall \tau \quad G(0) = \langle I^2 \rangle \geq G(\tau)$$



the autocorrelation function has a limit value = uncorrelated intensities:

$$G(\tau) = \langle I(0)I(\tau) \rangle \xrightarrow{\tau \rightarrow \infty} \langle I \rangle \langle I \rangle = \langle I \rangle^2$$

4

The autocorrelation function is limited

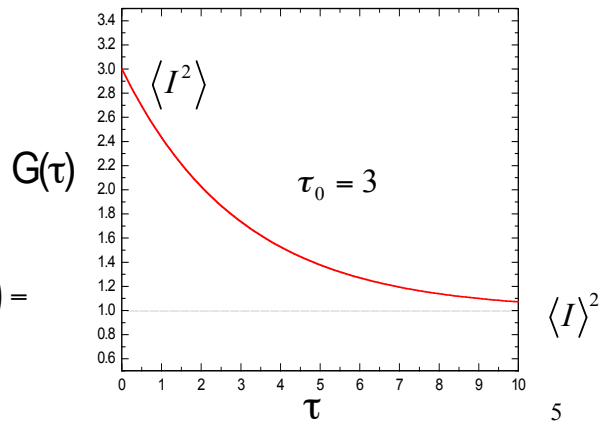
$$\langle I^2 \rangle \geq G(\tau) \geq \langle I \rangle^2$$

$\exists \tau_0$ "characteristic" time $\begin{cases} \tau < \tau_0 \Rightarrow I(t_n) \approx I(t_n + \tau); \forall n \Rightarrow G(\tau) & \text{"big"} \\ \tau > \tau_0 \Rightarrow I(t_n) \neq I(t_n + \tau); \forall n \Rightarrow G(\tau) & \text{"small"} \end{cases}$

tale **tempo caratteristico**
e' legato alla **frequenza delle fluttuazioni** \Rightarrow **velocità di moto** delle particelle

In the simpler case the autocorrelation function decays like an exponential

$$G(\tau) = \langle I(0)I(\tau) \rangle = \langle I \rangle^2 - \exp\left(-\frac{\tau}{\tau_0}\right) (\langle I \rangle^2 - \langle I^2 \rangle) = A + B \exp(-\gamma\tau)$$



The autocorrelation function is normalized to vary between 2 and 1:

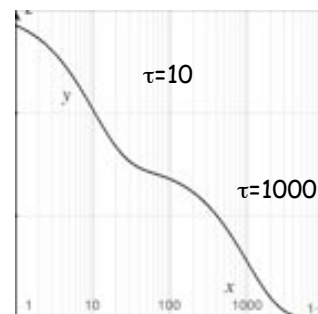
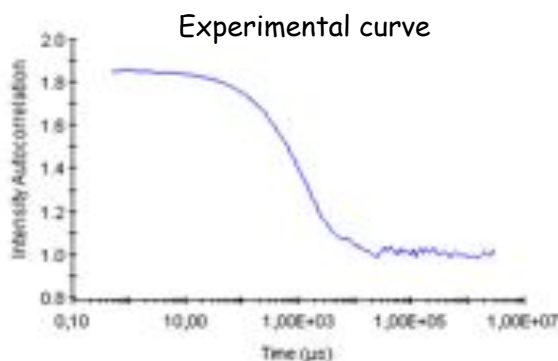
$$G(\tau) = \langle I(0)I(\tau) \rangle = \langle I \rangle^2 - \exp\left(-\frac{\tau}{\tau_0}\right) (\langle I \rangle^2 - \langle I^2 \rangle)$$

$$G(\tau) \Rightarrow \frac{G(\tau) - a}{b} = G'(\tau) = 1 + \exp(-\gamma_0 \tau) \quad \gamma_0 = \frac{1}{\tau_0} \quad \begin{cases} \langle I^2 \rangle = 2 \\ \langle I \rangle^2 = 1 \end{cases}$$

in general cases G depends on more than one exponential

$$G'(\tau) = 1 + \frac{1}{N} \sum_{n=1}^N \exp(-\gamma_n \tau)$$

Theoretical curve



In generale

se consideriamo la variazione istantanea di una grandezza A rispetto al suo valore medio (FLUTTUAZIONE):

$$\delta A(t) \equiv A(t) - \langle A \rangle$$

$$G(\tau) = \langle A(0)A(\tau) \rangle = \langle (\delta A(0) + \langle A \rangle)(\delta A(\tau) + \langle A \rangle) \rangle = \langle \delta A(0)\delta A(\tau) \rangle + \langle A \rangle^2$$

\downarrow
 $\langle \delta A(t) \rangle = 0$

$$\langle \delta A^2 \rangle = \langle \delta A(0)\delta A(0) \rangle = \langle (A(0) - \langle A \rangle)^2 \rangle = \langle A^2 \rangle - \langle A \rangle^2$$

nell'ipotesi che la funzione di autocorrelazione decada come un semplice esponenziale:

$$G(\tau) = \langle A(0)A(\tau) \rangle = \langle A \rangle^2 - \exp\left(-\frac{\tau}{\tau_0}\right) (\langle A \rangle^2 - \langle A^2 \rangle)$$

funzione di autocorrelazione della fluttuazione:

$$\langle \delta A(0)\delta A(\tau) \rangle = \exp\left(-\frac{\tau}{\tau_0}\right) (\langle \delta A^2 \rangle)$$

7

Se le fluttuazioni non decadono con un semplice esponenziale si può definire il tempo di correlazione come:

$$\langle \delta A(0)\delta A(\tau) \rangle = \exp\left(-\frac{\tau}{\tau_0}\right) (\langle \delta A^2 \rangle) \quad \longrightarrow \quad t_c \equiv \int_0^{\infty} dt \frac{\langle \delta A^2 \rangle}{\langle \delta A(0)\delta A(t) \rangle}$$

\downarrow

$$\int_0^{\infty} d\tau \left(\frac{\langle \delta A(0)\delta A(\tau) \rangle}{\langle \delta A^2 \rangle} \right) = \int_0^{\infty} d\tau \exp\left(-\frac{\tau}{\tau_0}\right) = -\tau_0(0-1) = \tau_0$$

Il **tempo di decadimento** della funzione di autocorrelazione è legato alla **frequenza delle fluttuazioni di intensità** che è a sua volta legata alla **velocità** di spostamento delle particelle, legata alle **dimensioni** delle particelle stesse:

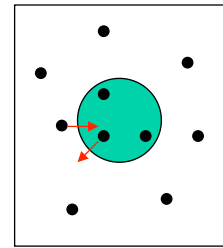
$$\gamma_n = \frac{1}{\tau_n} \rightarrow \langle v_n \rangle \rightarrow \langle R_n \rangle, T, \eta$$

$\langle v_n \rangle$ = Mean velocity of the particles of kind n

η = Viscosity of the solution

$\langle R_n \rangle$ = Mean radius of the particles of kind n

T = temperature



f_v = forza di frizione = forza richiesta per mantenere la distribuzione delle velocità perturbate delle molecole di solvente. Nel caso ideale di moto di una superficie di area A :

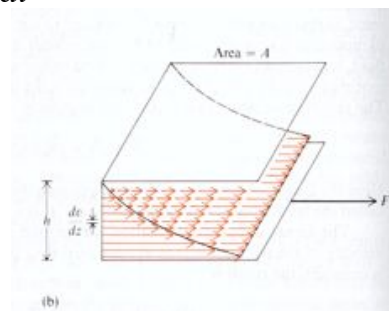
moto in presenza di attrito:
$$F - f_v = m \frac{dv}{dt}$$

\updownarrow
 η

attrito per una superficie di area A (moto laminare)

$$f_v \propto \frac{dv}{dz} A \Rightarrow f_v = \eta \frac{dv}{dz} A$$

$$[\eta] = \frac{gr}{\text{sec cm}}$$



9

in generale l'attrito sarà proporzionale alle dimensioni dell'oggetto e alla viscosità del mezzo: in termini puramente dimensionali:

$$f \propto \eta^x r^y$$

$$[f] = \frac{gr}{\text{sec}} \quad \left(\frac{gr}{\text{sec}} \right) = \left(\frac{gr}{\text{sec cm}} \right)^x \text{cm}^y \Rightarrow x = y = 1 \quad f \propto \eta r$$

$$[\eta] = \frac{gr}{\text{sec cm}}$$

Relazione di Stokes (per una **particella sferica**) $f = 6\pi\eta r$

Moto Browniano (spostamento quadratico medio)

$$\frac{Tt}{f} \leftarrow \langle (r(t) - r_0)^2 \rangle = \frac{6kT}{f} t$$

da dimostrare

Moto Browniano: particella sottoposta ad una forza casuale $F(t)$ in presenza di attrito (f):

$$m\ddot{x} = -f\dot{x} + F(t) \quad \longrightarrow \quad m\dot{x}\dot{x} = -f\dot{x}\dot{x} + xF(t) \quad \longrightarrow \quad m \frac{d}{dt}(\dot{x}\dot{x}) - m\dot{x}^2 = -f\dot{x}\dot{x} + xF(t)$$

moltiplico per \dot{x}

media nel tempo:

$$\langle xF(t) \rangle = \langle x \rangle \langle F(t) \rangle = 0$$

$$m \frac{d}{dt} \langle (\dot{x}\dot{x}) \rangle - m \langle \dot{x}^2 \rangle = -f \langle \dot{x}\dot{x} \rangle + \langle xF(t) \rangle$$

0
||
→

nel moto Browniano la forza è indipendente dalla posizione e la media della forza nel tempo è nulla!

$$\frac{1}{2} m \langle v^2 \rangle = \frac{1}{2} kT \Rightarrow \langle v^2 \rangle = \frac{kT}{m}$$

soluzione

$$\left(\frac{d}{dt} + \frac{f}{m} \right) \langle (\dot{x}\dot{x}) \rangle = \frac{kT}{m} \quad \longrightarrow \quad \left(\frac{d}{dt} + A \right) s(t) = B \quad \longrightarrow \quad s(t) = Ce^{-\gamma t} + D$$

$$-\gamma Ce^{-\gamma t} + ACe^{-\gamma t} + AD = B \quad \longrightarrow \quad \begin{cases} \gamma = A = \frac{f}{m} \\ D = \frac{B}{A} = \frac{kT}{f} \end{cases} \quad \longrightarrow \quad \langle \dot{x}\dot{x} \rangle = Ce^{-\gamma t} + \frac{kT}{f} = \frac{1}{2} \frac{d}{dt} \langle x^2 \rangle$$

11

$$\langle \dot{x}\dot{x} \rangle = Ce^{-\gamma t} + \frac{kT}{f} = \frac{1}{2} \frac{d}{dt} \langle x^2 \rangle$$

$$t=0, x=0 \quad \longrightarrow \quad C = -\frac{kT}{f} \quad \frac{1}{2} \frac{d}{dt} \langle x^2 \rangle = \frac{kT}{f} (1 - e^{-\gamma t})$$

integrando:

$$\int_0^t dt e^{-\gamma t} = -\frac{1}{\gamma} (e^{-\gamma t} - 1) \quad \longrightarrow \quad \langle x^2 \rangle = \frac{2kT}{f} \left(t + \frac{1}{\gamma} (e^{-\gamma t} - 1) \right) \xrightarrow{t \gg \gamma^{-1}} \frac{2kTt}{f}$$

$$\gamma = \frac{f}{m}$$

spostamento quadratico medio

$$\langle r^2 \rangle = 3 \langle x^2 \rangle = \frac{6kTt}{f} = 6Dt = \frac{kT}{\pi\eta r} t$$

coeff. di diffusione

Relazione di Stokes per una sfera: $f = 6\pi\eta r \quad \longrightarrow \quad D = \frac{kT}{6\pi\eta r}$

12

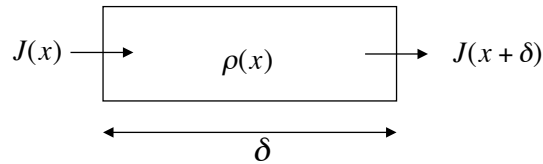
Si può scrivere l'eq. della diffusione (di Fourier):

$$J = -D \frac{\partial \rho}{\partial x}$$

I legge di Fick,
rapporto tra flusso e gradiente spaziale di densità

$J =$ flusso
 $D =$ coeff. di diff.
 $\rho =$ concentrazione

rapporto tra flusso e gradiente temporale di densità
in un elemento di volume



$$\delta \frac{\partial \rho}{\partial t} = J(x) - J(x + \delta) = J(x) - \left(J(x) + \frac{\partial J}{\partial x} \delta \right) = -\frac{\partial J}{\partial x} \delta$$

↑
velocità di variazione del numero di moli nell'elemento di volume

eq. della diffusione

$$\frac{\partial \rho}{\partial t} = -\frac{\partial J}{\partial x} = D \frac{\partial^2 \rho}{\partial x^2}$$

13

Si può definire la funzione G che rappresenta la
densità di probabilità della diffusione di una particella:

$$G(\vec{r}, t) \equiv \left\langle \delta(\vec{r} - [\vec{r}_j(t) - \vec{r}_j(0)]) \right\rangle$$

trasf. di Fourier

$$F(\vec{q}, t) = \int d^3 r \left\langle \delta(\vec{r} - [\vec{r}_j(t) - \vec{r}_j(0)]) \right\rangle \exp(i\vec{q} \cdot \vec{r}) = \left\langle \exp i\vec{q} \cdot [\vec{r}_j(t) - \vec{r}_j(0)] \right\rangle$$

$$\frac{\partial \rho}{\partial t} = D \frac{\partial^2 \rho}{\partial x^2}$$

l'eq. della diffusione (di Fourier):

$$\frac{\partial}{\partial t} G(\vec{r}, t) = DV^2 G(\vec{r}, t) \Rightarrow \int d^3 r \frac{\partial}{\partial t} G(\vec{r}, t) \exp(i\vec{q} \cdot \vec{r}) = \int d^3 r DV^2 G(\vec{r}, t) \exp(i\vec{q} \cdot \vec{r})$$

F.T.

$$\frac{\partial}{\partial t} F(\vec{q}, t) = -q^2 DF(\vec{q}, t)$$

l'eq. della diffusione (di Fourier):

$$\frac{\partial}{\partial t} F(\vec{q}, t) = -q^2 D F(\vec{q}, t)$$

soluzione



$$F(\vec{q}, t) \propto \exp(-q^2 D t)$$

dalla funzione di autocorrelazione dell'intensita

→ al coeff. di diff. →

alla forma/dimensione delle molecole (R)

$$F(\vec{q}, t) \rightarrow \exp(-q^2 D t) \rightarrow R$$

Per approfondire: Berne & Pecora; "Dynamic Light Scattering"; Dover

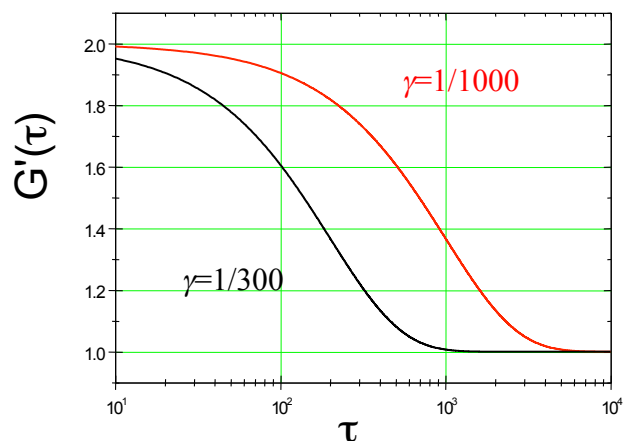
15

$$G(\tau) = A + B e^{-\gamma \tau} \longrightarrow \gamma \propto D \propto \frac{kT}{\eta R}$$

higher is the temperature higher the particles velocity higher the γ value faster the decay of G

higher is the viscosity lower is the particles velocity lower the γ value slower is the decay of G

higher is the particles radius lower the particles velocity lower the γ value slower the decay of G



16

Summary

The scattered intensity oscillates randomly around its mean value with a typical frequency depending on the particles velocity

The autocorrelation function (G) is somehow a measure of this frequency and thus it gives information on the particles velocity and therefore on their radius

For a given viscosity and temperature, the particles velocity depends only on their dimension

DLS measures G \longrightarrow data analysis gives R_h

17

Hydrodynamic radius

Lysozyme:

Prolate ellipsoid of 26 and 45 Å

Axial ratio: 1.731 (=45/26)

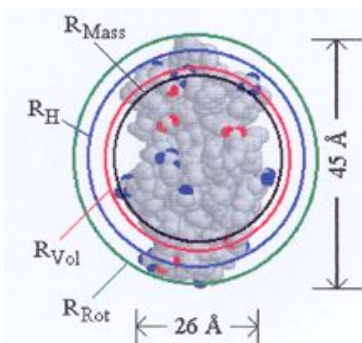
Mw: 14.7 kDa

Protein specific volume (protein volume/protein weight): 0.71-0.73 ml/gr = \bar{V}

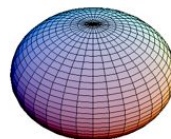
$$Mw \times gr \Leftrightarrow N_A \Rightarrow \frac{Mw}{N_A} = \text{protein weight in gr}$$

$$V = \frac{4\pi}{3} R^3 = \frac{Mw}{N_A} \bar{V} \Rightarrow R_{Mass} = \left(\frac{3}{4\pi} \frac{Mw}{N_A} \bar{V} \right)^{1/3} = 1.62nm$$

quantità teorica che si può sempre calcolare

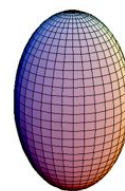


Oblate ellipsoid



having a polar axis shorter than the diameter of the equatorial circle

Prolate ellipsoid



the polar diameter is longer than the equatorial diameter.

18

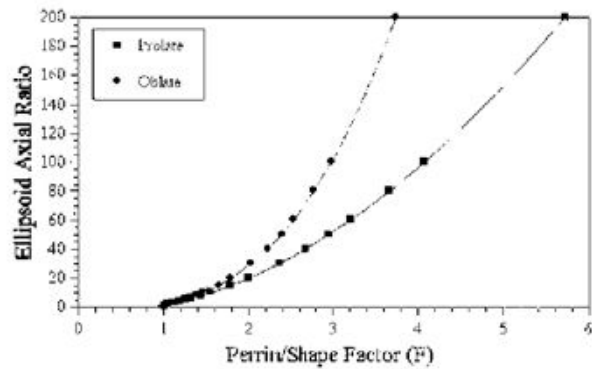
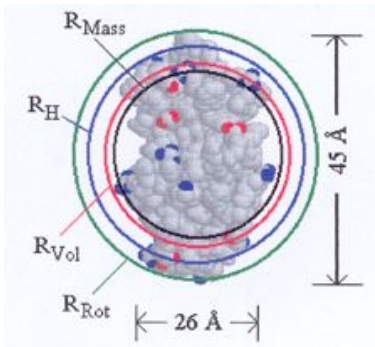
The **experimental hydrodynamic radius** is consistent by adding a single water shell around the protein (0.24-0.28 nm):

$$R_H = 1.90\text{nm} = R_{Vol} + r_{water} = (1.66 + 0.24)\text{nm}$$

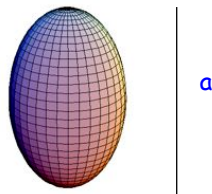
Form factor or Perrin factor (F) = a measure of the protein **non sphericity**.

$$F = \frac{R_{Vol}}{R_{Mass}} = \left(\frac{3Mw\bar{V}}{4\pi N_A} \right)^{-1/3} R_{Vol} = 1.02$$

For a prolate ellipsoid with $F = 1.02$, the **axial ratio** = 1.6, in agreement with the crystallographic axial ratio (1.7)



Prolate ellipsoid



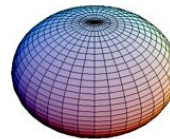
b

$$\rho = \frac{b}{a} \leq 1$$

$$\Gamma(\rho) = (1 - \rho^2)^{-1/2} \ln \left\{ \frac{1 + (1 - \rho^2)^{1/2}}{\rho} \right\}$$

axial ratio

Oblate ellipsoid



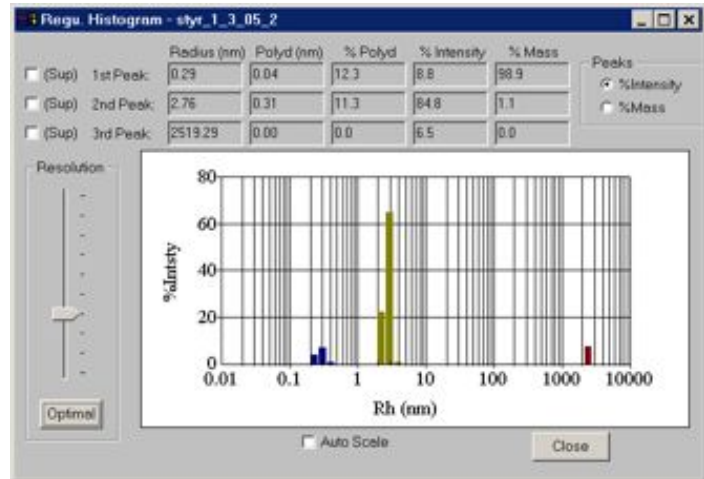
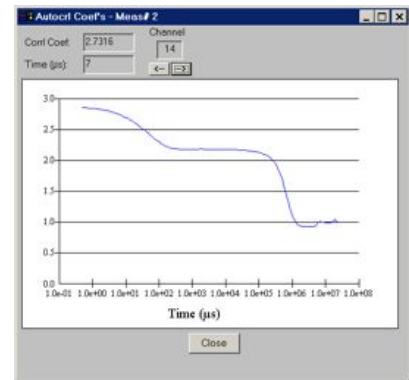
$$\rho = \frac{b}{a} \geq 1$$

$$\Gamma(\rho) = (\rho^2 - 1)^{1/2} \rho \tan^{-1} \left\{ (\rho^2 - 1)^{1/2} \right\}$$

$$D = \frac{kT}{6\pi\eta r} \Gamma(\rho)$$

Typical experiment

- The protein concentration (Mw); e.g. [lysozyme] > 0.1 mg/ml
- Filtrate or centrifuge the sample
- Buffer (η)
- Acquisition time = 30 sec (signal/noise)
- Kcounts/sec > 100
- Number of measures = 15-20
- Aggregation/dust
- Histogram



Viral helicase

Mw: 48.9 kDa

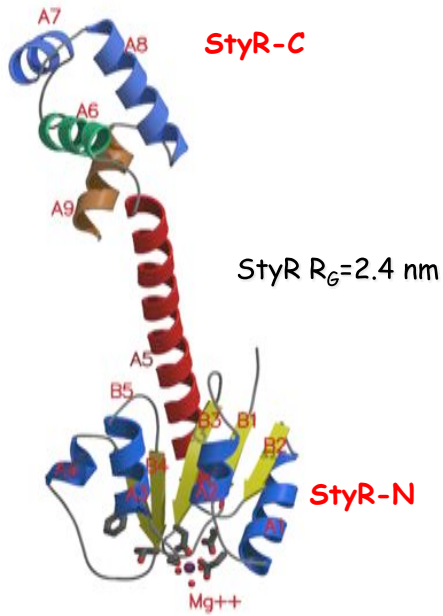
R_H (monomer) = 2.25 nm

R_H (dimer) = 3.27 nm

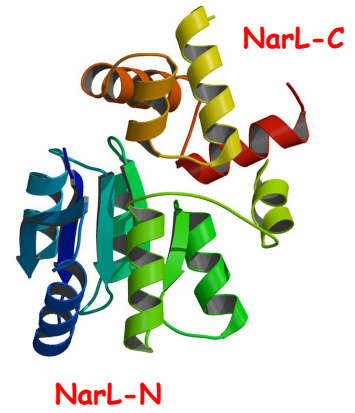
R_H (DLS) = 3.40 nm



Solution structure of StyR



NarL: $R_G=1.6$ nm

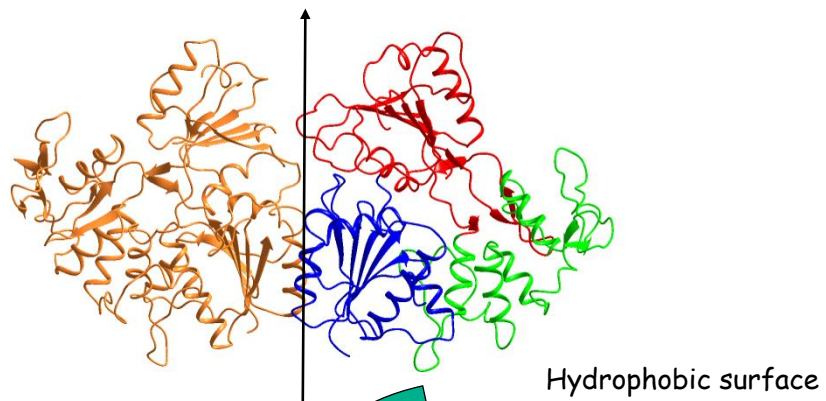


Dynamic Light Scattering: $R_H = (2.4 \pm 0.3)$ nm

prolate ellipsoid having an axial ratio of about 1/4.5

23

Quaternary assembly: dimer

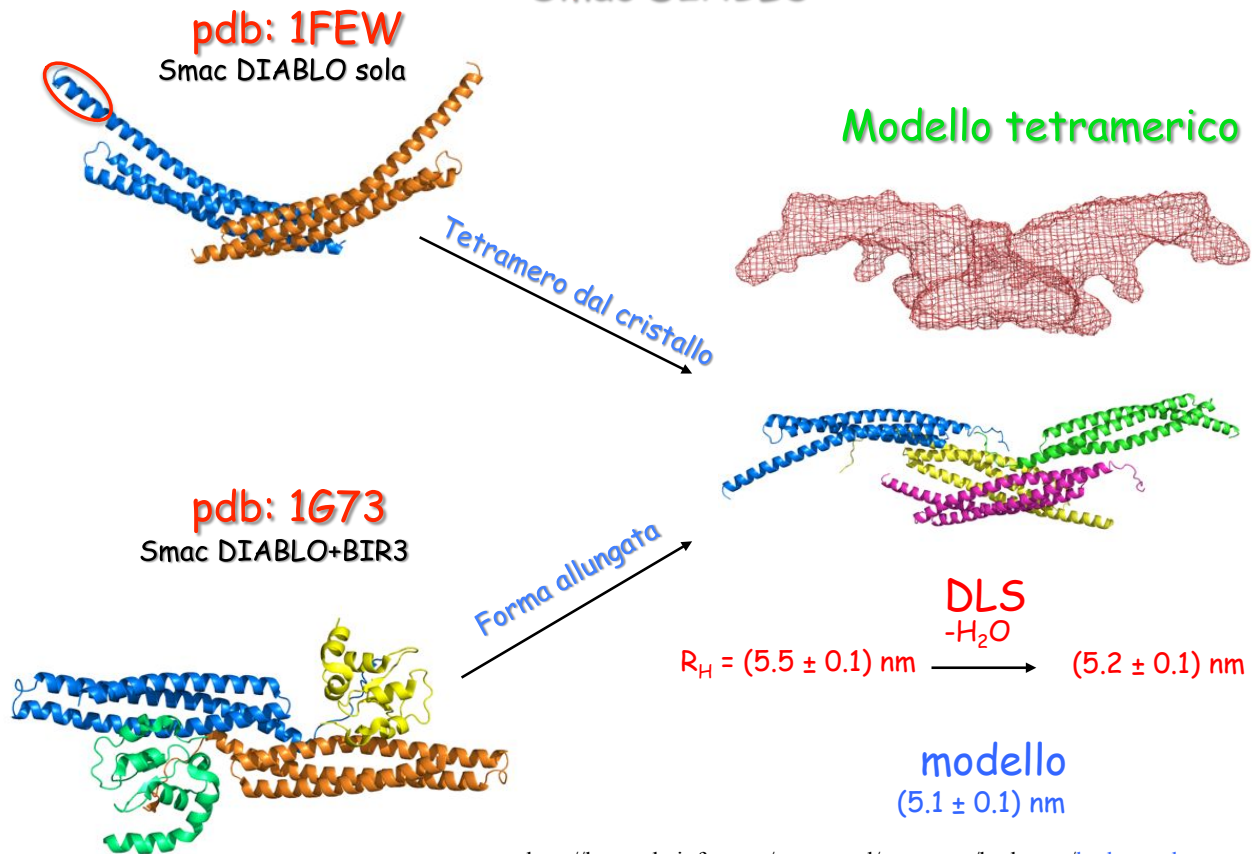


Kunjin 180	EP EM LR 185
Dengue	EDD I FR
Yellow	GSH ML K

R_H (D.L.S.) = 4.1 ± 0.3 nm
 R_H (dimer) = 4.1 ± 0.1 nm

24

Smac DIABLO



<http://leonardo.inf.um.es/macromol/programs/hydropro/hydropro.htm>

II virial coefficients B₂: interazioni di coppia

Pressione in funzione della densita'

$$\frac{P}{k_B T} = \rho + B_2(T)\rho^2 + B_3(T)\rho^3 + \dots$$



$$\frac{\beta p}{\rho} = 1 + \sum_{i=1}^{\infty} B_{i+1}(T)\rho^i$$

B₂ negativo = la pressione diminuisce quando aumenta la densita' interazioni attrattive tra le molecole

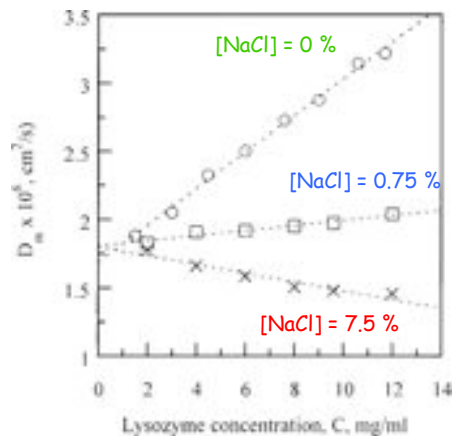
Coefficiente di diffusione

$$D_m(\rho) = D_0(1 + k_d \rho)$$

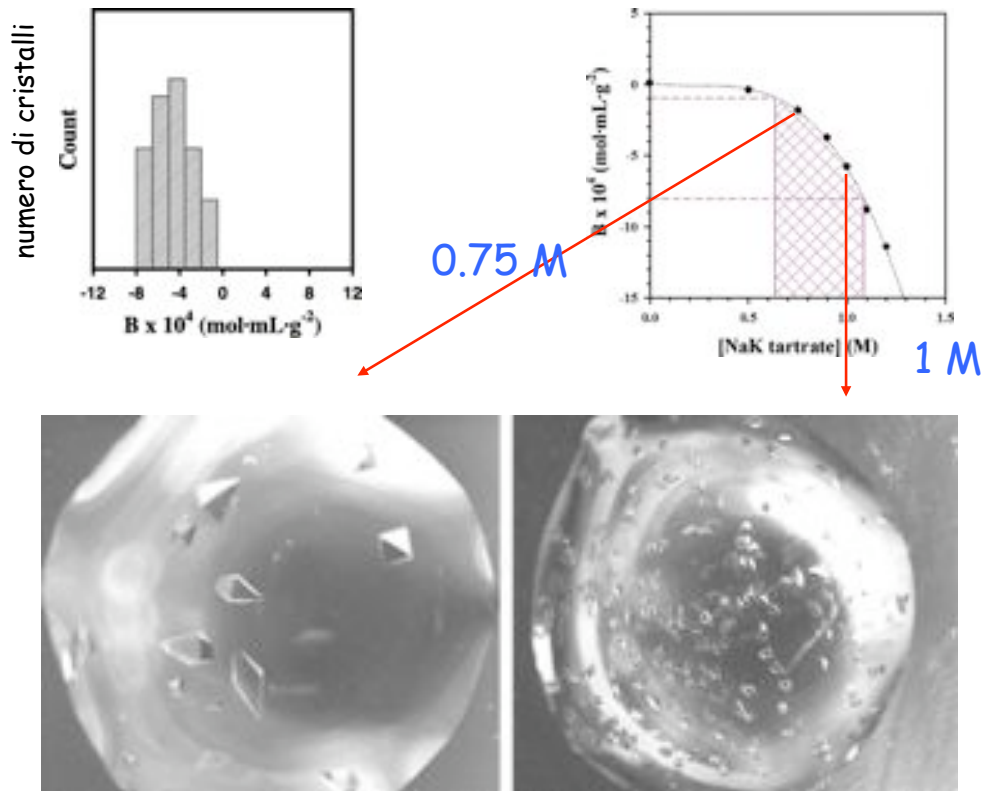
$$D_0 = \frac{k_B T}{6\pi\eta R_H}$$

$$k_d = k_d(B_2) = 2M_w B_2 - \dots$$

Valori negativi = interazione attrattiva



B: second virial coefficient



Wilson., J.Struct Biol, 142, 2003

Conclusions

DLS is a powerful tool to have information about:

1. Protein aggregation state: quaternary assembly
2. Protein low resolution shape: axial ratio
3. Protein polydispersity state: likelihood of crystallization

Programma per calcolare il raggio idrodinamico teorico da una struttura 3D

<http://leonardo.fcu.um.es/macromol/programs/hydropro/hydropro.htm>