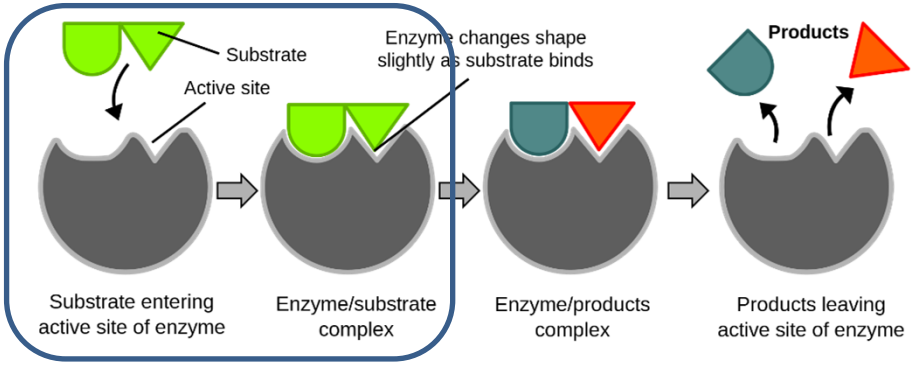
Bimolecular reactions in biology

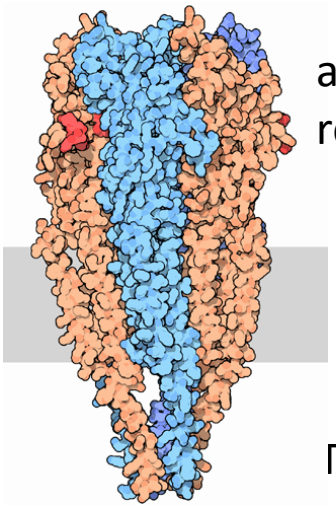
Enzymatic reactions
- first steps: formation of enzyme-substrate complex

Ligand binding
Receptor – ligand interactions, sigaling

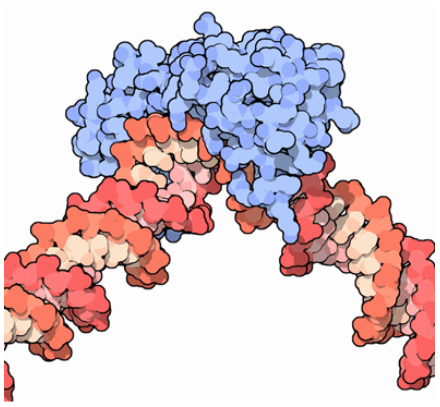
Complex formation
assembly of macromolecular complexes, aggregates

Protein-DNA interactions
Transcription factor binding of DNA segments





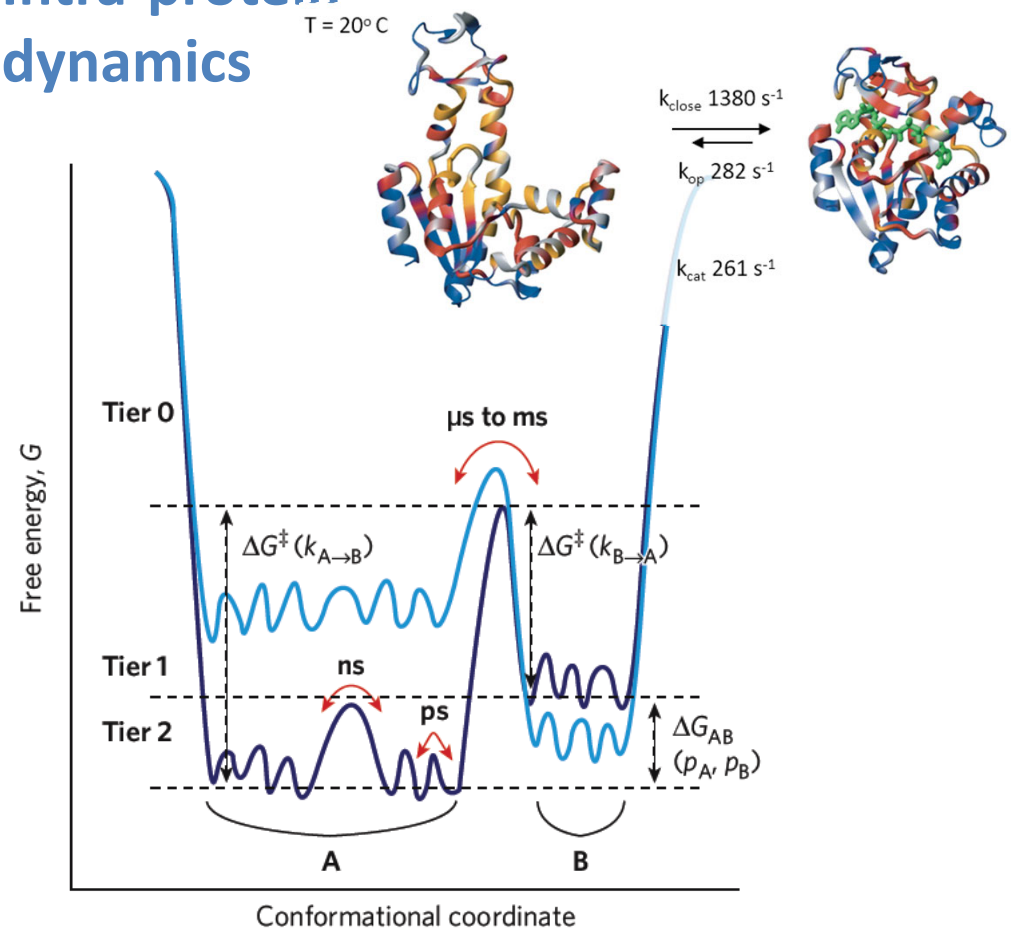
acetylcholine receptor

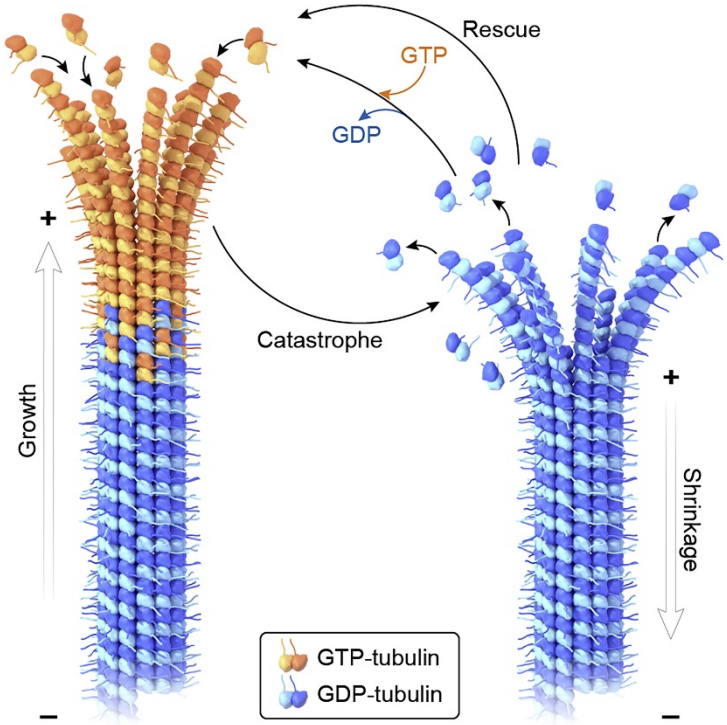


ГATA-binding protein

Protein dynamics

Intra-protein dynamics





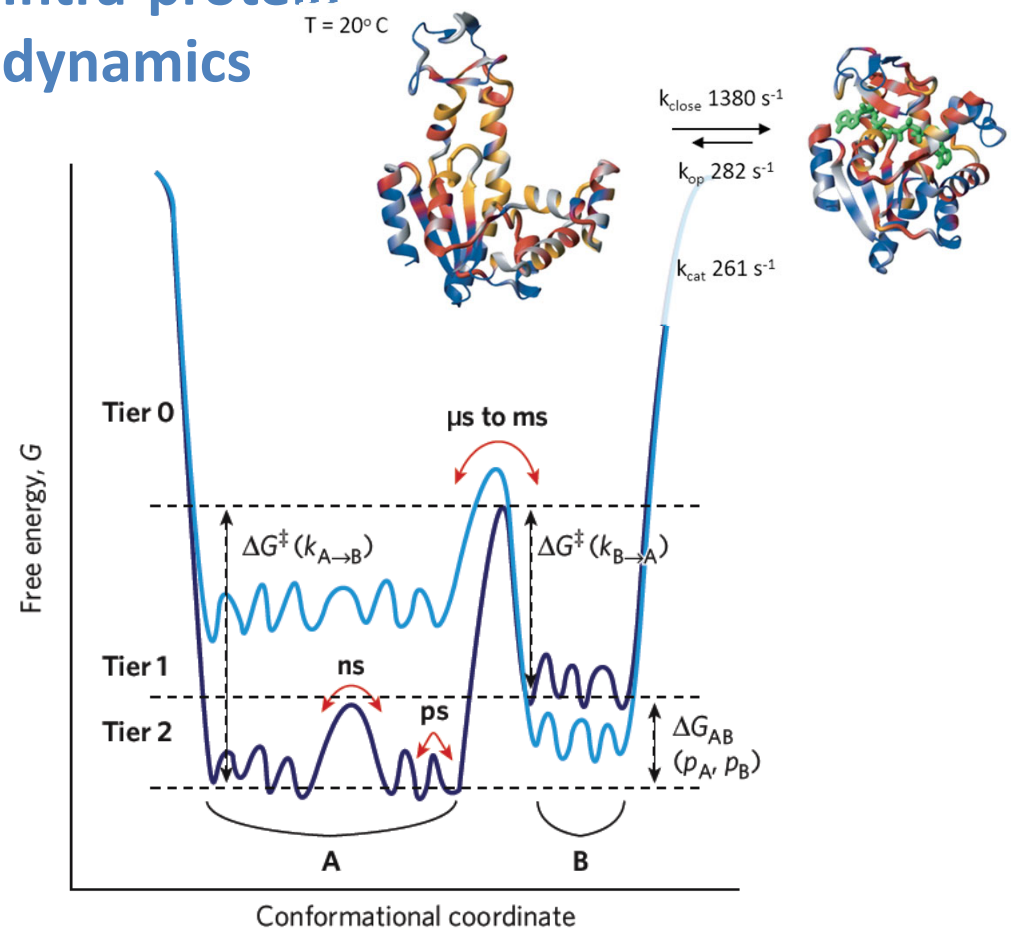
Inter-protein dynamics

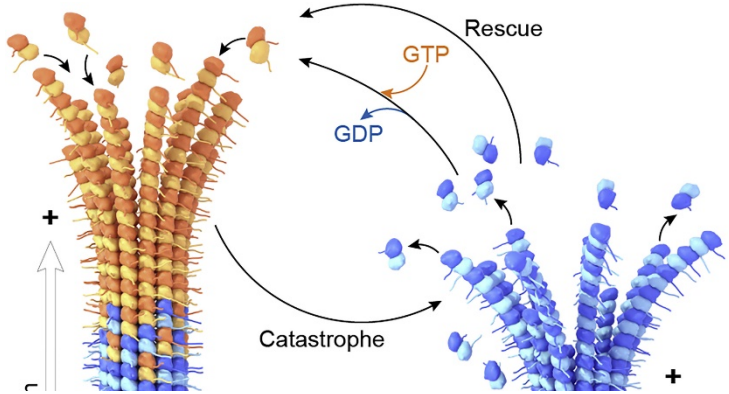
Nat Struct Mol Biol. 2004 Oct;11(10):945-9

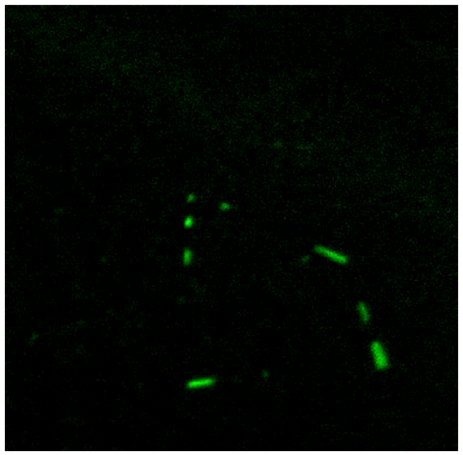
Dev Cell Volume 54, Issue 1, 6 July 2020, Pages 7-20

Protein dynamics

Intra-protein dynamics







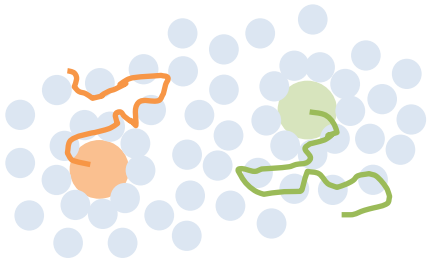
Nat Struct Mol Biol. 2004 Oct;11(10):945-9

Dev Cell Volume 54, Issue 1, 6 July 2020, Pages 7-20

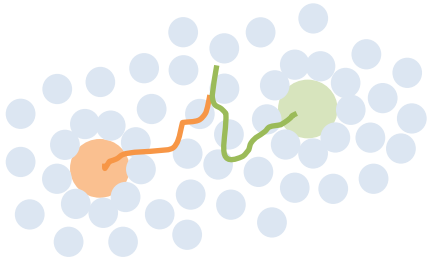
Bimolecular Reactions in solution

Steps for a reaction to occur:

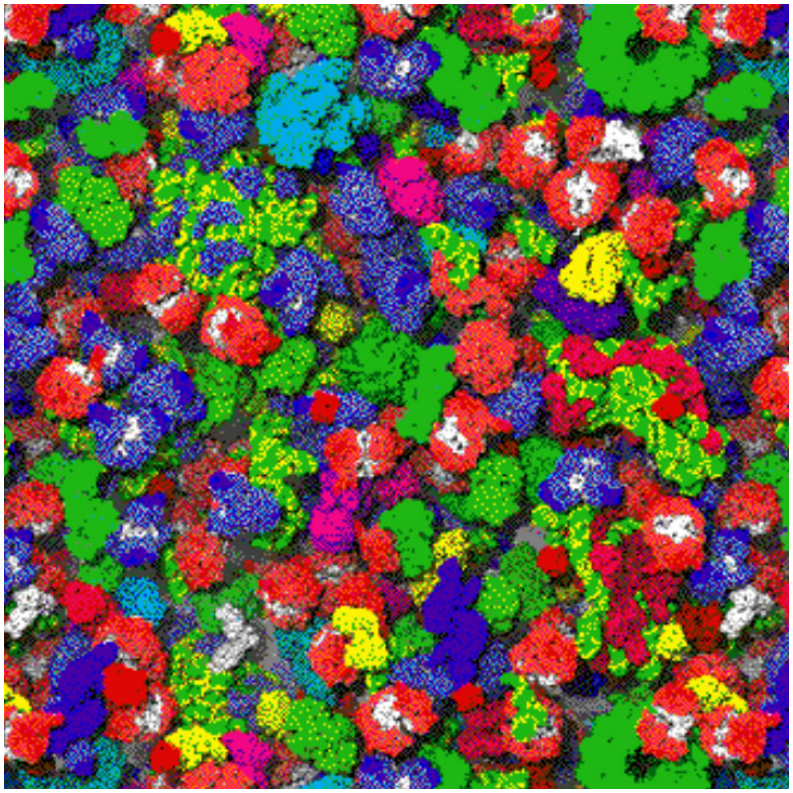
- molecular diffusion
- results in random collisions
- every molecule is surrounded by a solvation shell
- solvation shell is shared → encounter complex, repeated collisions
- **reaction?** not every encounter complex is productive → depends on the reaction
- dissociation of the complex



10^{-10} – 10^{-8} s for small molecules



Diffusion in biological systems



McGuffee & Elcock,
PLOS Comp Biol 2010

- Examples of diffusion processes:
  - enzymes and metabolites
  - receptors
  - protein protein interactions
  - transcription factor binding

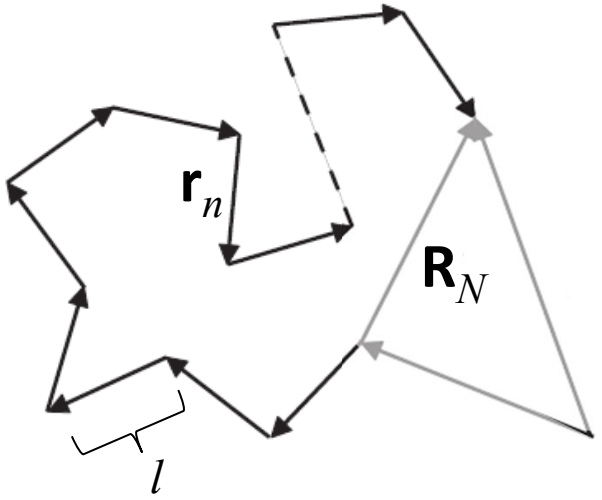
Average distance travelled in a random walk

Assuming, per time unit t, N' steps occur, then the number of steps is given by

$$N(t) = N' \cdot t$$

and the travelled distance can be expressed as:

$$\left\langle \mathbf{r}^2 \right\rangle = N' l^2 t \qquad \left\langle \mathbf{r} \right\rangle \propto \sqrt{t}$$

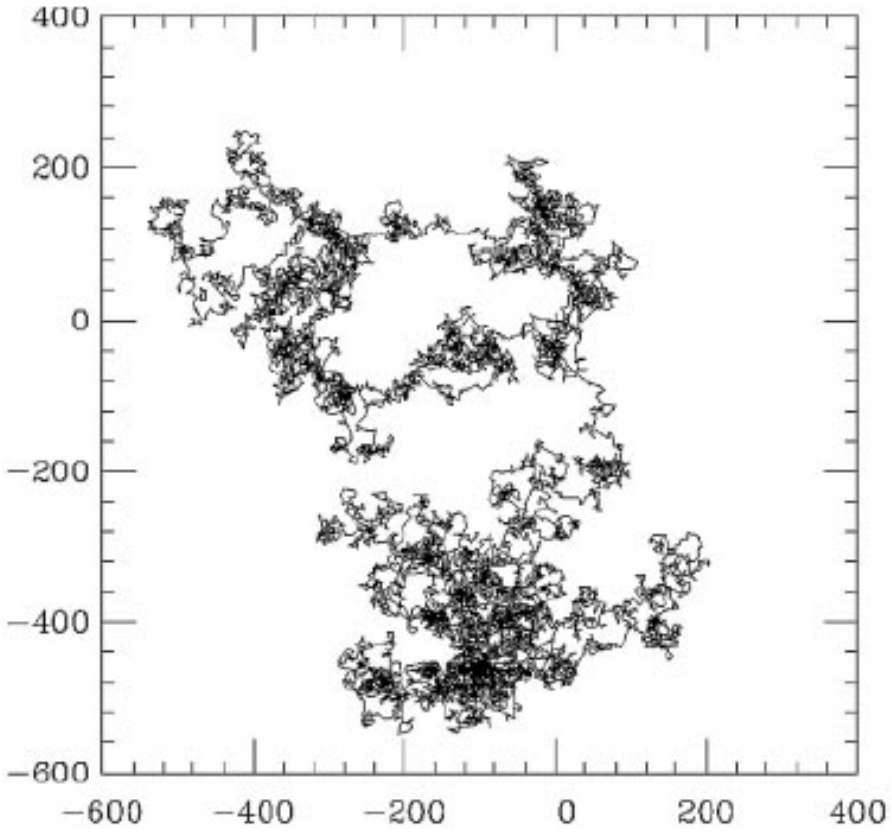


equivalence to freely jointed chain!

and the probabiliy of traveling a distance x

$$p(r)dr = \frac{4\pi x^2}{\left(2\pi N' l^2 t\right)^{3/2}} e^{-\frac{r^2}{2N' l^2 t}} dr$$

Molecules undergo a random walk in solution



Brownian motion

- steps in all directions are equally likely
- stepsize large in gaseous phase
- in solution, steps are infinitesimally short due to solvent interactions
- limited by mobility and size of molecules
- by solvent interactions / mobility → viscosity

Mean squared displacement with diffusion

Redefine the steplength and frequency as the **diffusion coefficient D**.

This yield the following equations, dependent on the dimensionality of the process:

1D – diffusion: $\left\langle x^2 \right\rangle = 2Dt$

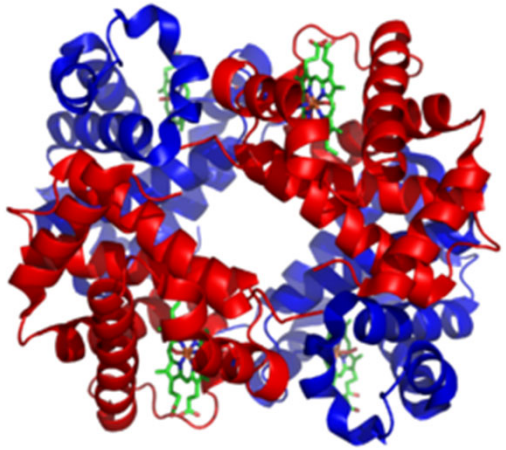
2D – diffusion: $\left\langle \rho^2 \right\rangle = 4Dt$

3D – diffusion: $\left\langle r^2 \right\rangle = 6Dt$

$$D = \frac{kT}{f} = \frac{kT}{6\pi\eta r}$$

Quiz...

- Estimate the diffusion coefficient of **hemoglobin**.
- **Estimate, how long** a hemoglobin molecule would need to diffuse the length of a red blood cell (7 μm)?



Hemoglobin (ca. 5 nm diameter)

Boltzmann constant =

$1.3806488 \times 10^{-23}\ m^2\ kg\ s^{-2}\ K^{-1}$

water viscosity

Temperature [°C]	Viscosity [mPa·s]
10	1.308
20	1.002
30	0.7978
40	0.6531
50	0.5471
60	0.4658
70	0.4044
80	0.3550
90	0.3150
100	0.2822

how long for 7 micrometers

D hemoglobin 69um^2 / s

viscosity 0.7mPa s

kb 1.38E-23m^2 kg s^-2 K^-1

equation $\left\langle r^2 \right\rangle = 6Dt$

0.016908s

16.90821ms

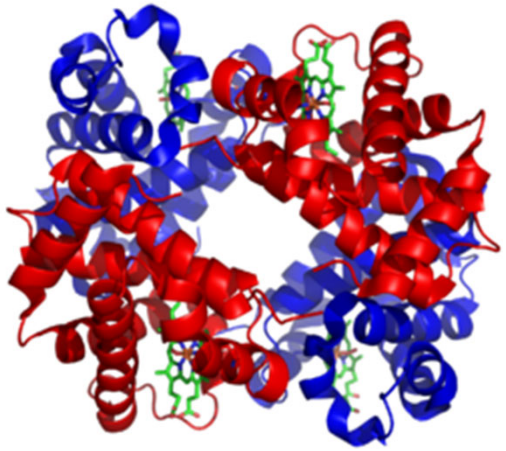
$$D = \frac{kT}{f} = \frac{kT}{6\pi\eta r}$$

D 1.29812E-10

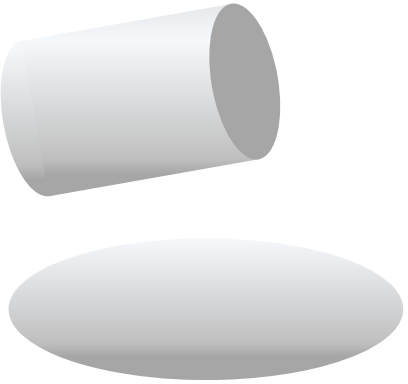
129.8122966um^2/s

Diffusion coefficients of proteins

- The radius of a spherical protein can be calculated from D (i.e. the Stokes radius)
- biological molecules are always **hydrated**
- solvation effectively increases the **hydrodynamic volume** of a molecule and therefore its frictional coefficient



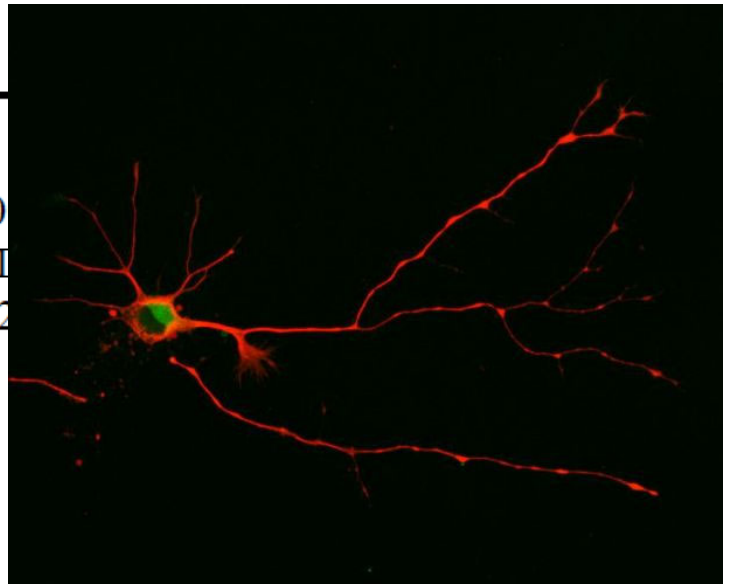
- Molecular shape will also influence the diffusion coefficient: deviation from spherical shape -> **larger frictional coefficient**



Diffusion is efficient for small particles over small distances

Object	Distance diffused			
	1 µm	100 µm	1 mm	1 m
K^+	0.25ms	2.5s	2.5×10^4s (7 hrs)	2.5×10^8s (8 yrs)
Protein	5ms	50s	5.0×10^5s (6 days)	5.0×10^9s (150 yrs)
Organelle	1s	10^4s (3 hrs)	10^8s (3 yrs)	

K+: Radius = 0.1nm, viscosity = 1mPa·s^{-1}; T = 25°C; D=20
Protein: Radius = 3nm, viscosity = 0.6915mPa·s^{-1}; T = 37; I
Organelle: Radis = 500nm, viscosity = 0.8904mPa·s^{-1}; T = 2



For long distances (axon) or large particles (vesicles), **active transport is required**

How to measure diffusion coefficients?

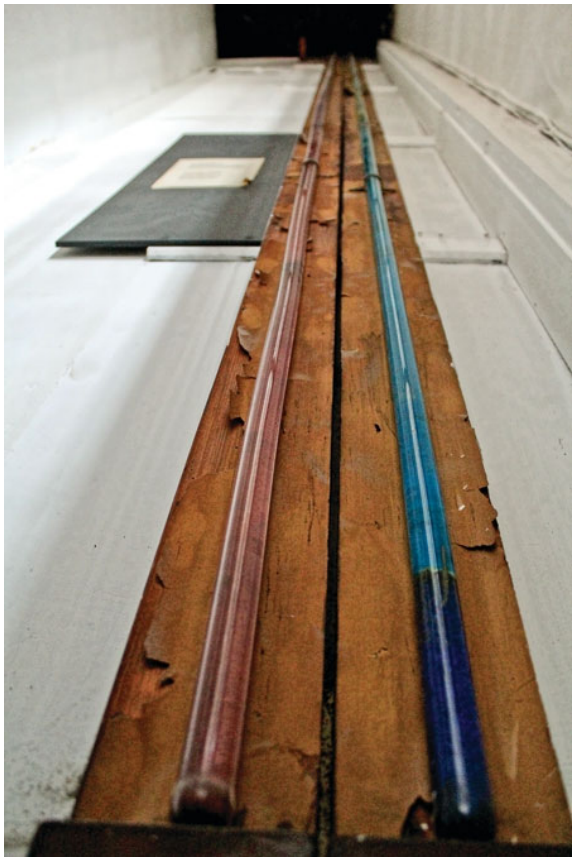
- **Macroscopic measurement (dye in a cuvette)**
- **Analytical ultracentrifugation**
- **Dynamic light scattering**
- Fluorescence correlation spectroscopy

} correlation spectroscopy

- single molecule experiments, e.g. single particle tracking

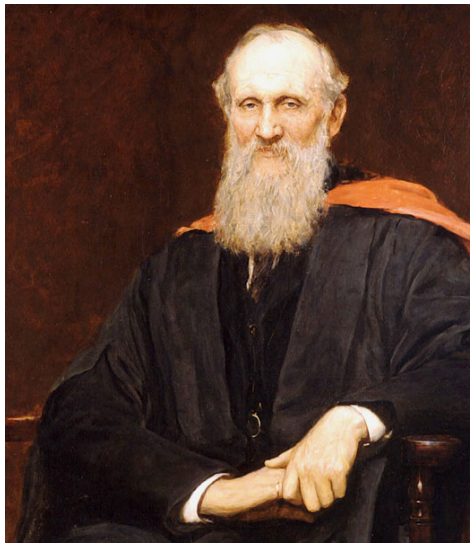
} direct observation

Macroscopic observations



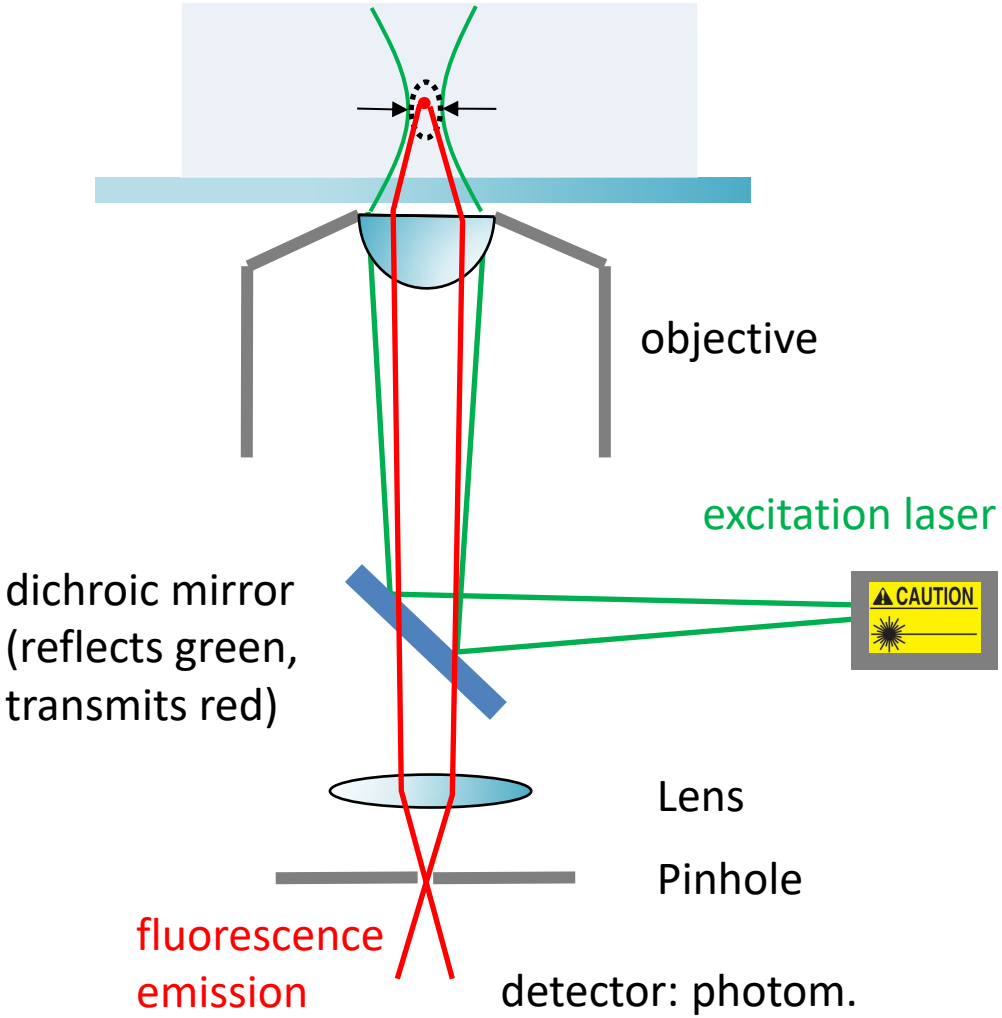
"In 1872 [Lord Kelvin] set up two vertical 17.5-foot glass tubes in a lecture theater [at Glasgow]. Kelvin partly filled one tube with a copper sulfate solution and then carefully topped it off with water. He partly filled the second tube with water and then topped it off with colored alcohol."

One of the longest running scientific experiments: it is undisturbed since 142 years



Lord Kelvin,
1824-1907

Measuring dynamics of fluorescent molecules



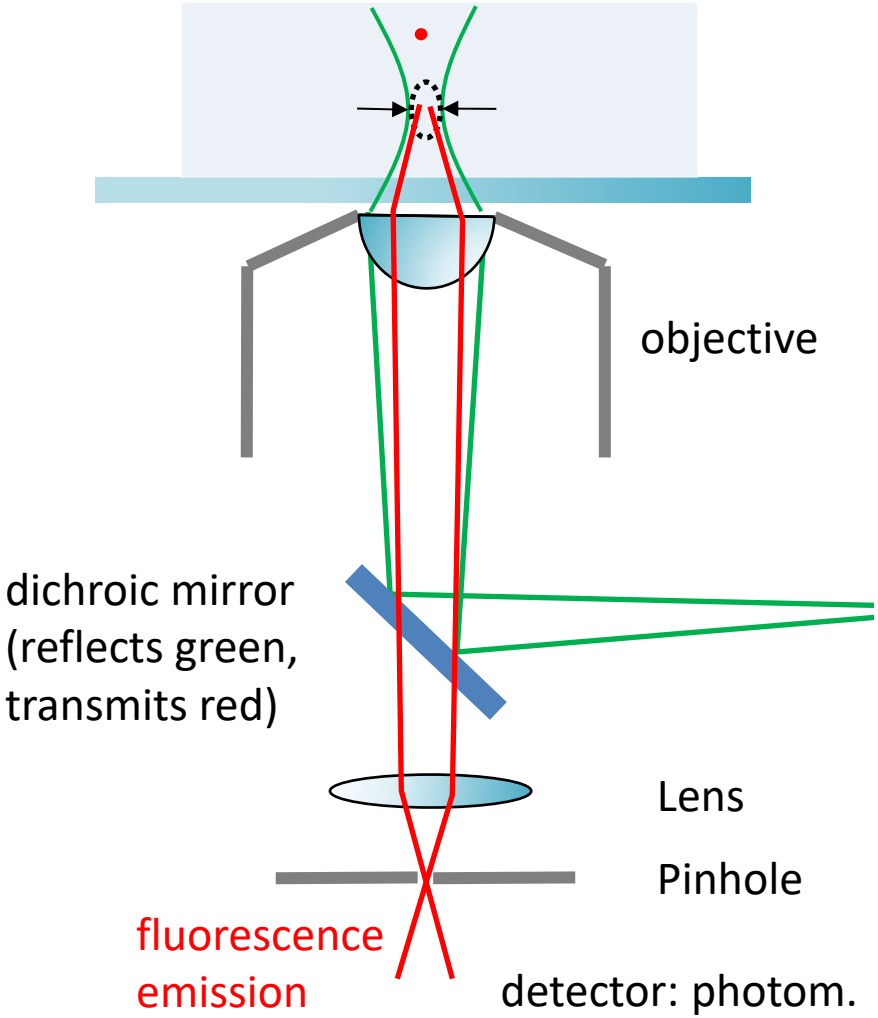
If the concentration of fluorescent molecules is low:

Only a low number of molecules occupy the confocal volume at any time

Fluorescence emission is no longer continuous, but fluctuates with the number of molecules

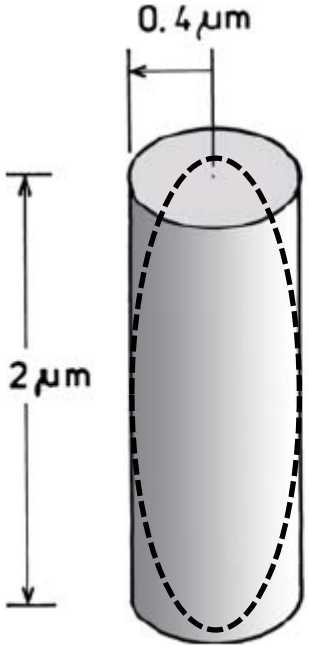
→ this can be exploited to study the motion of individual molecules in cells

Confocal Imaging



Confocal imaging of molecules:

Only light from a small volume is recorded, all other light from the sample is blocked by a **pinhole**.



Volume = 1 fl

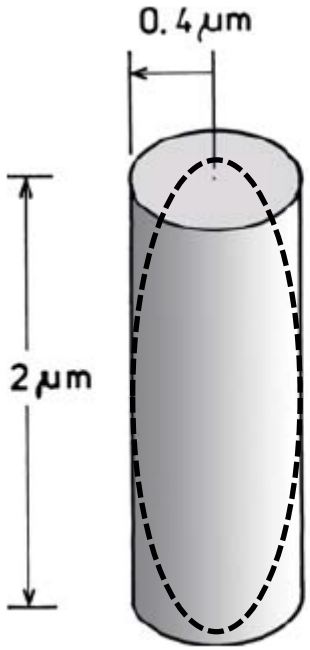
Lakowicz, Principles of fluorescence spectroscopy

Figure 24.1. Typical volume in an FCS experiment.

Fluorescence correlation spectroscopy (FCS)

FCS:
based on the analysis of time-dependent **fluorescence intensity fluctuations** resulting from dynamic processes

Confocal volume (CV)



Volume = 1 fl

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Lakowicz, Principles of fluorescence spectroscopy

Fluorescence correlation spectroscopy (FCS)

FCS:
based on the analysis of time-dependent **fluorescence intensity fluctuations** resulting from dynamic processes

Confocal volume (CV)

Diffusion of a fluorescent molecule into the CV

→ burst of fluorescence emission

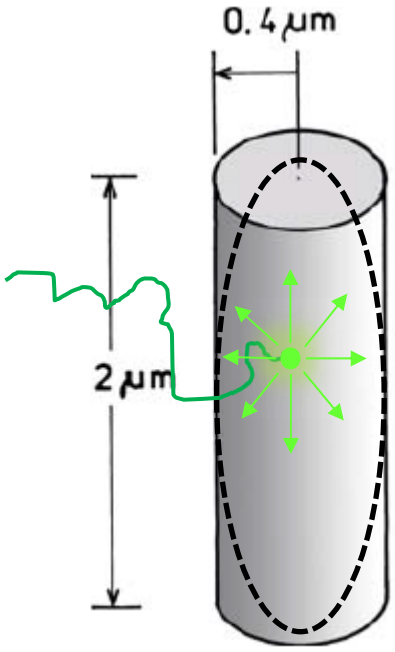

Figure 24.1. Typical volume in an FCS experiment.

Concentration

at low concentrations (nM), only few molecules simultaneously occupy CV

→ Diffuse in and out randomly

Volume = 1 fl

c = 1 nM

N = 0.6 molecules

Lakowicz, Principles of fluorescence spectroscopy

Origin of fluorescence fluctuations at low concentrations

FCS is governed by Poission statistics

Probability of having n mol. in the CV, when average number in CV is N

$$P(n,N) = \frac{N^n}{n!} e^{-N}$$

e.g. for N = 0.6

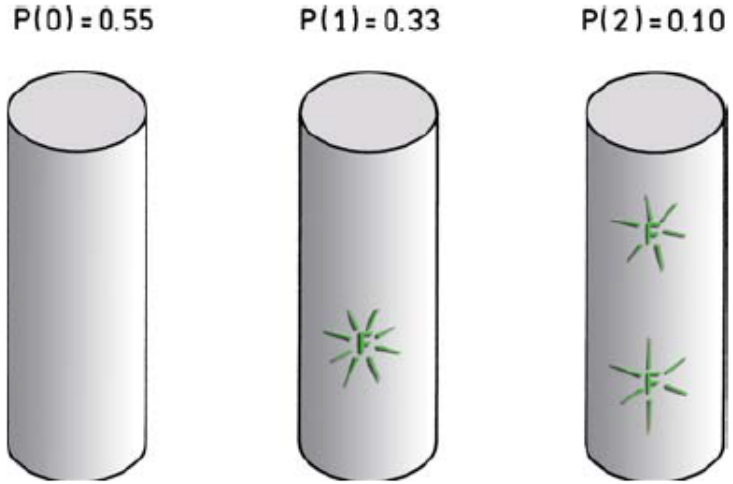

Figure 24.2. Poisson distribution of a 1-nM fluorophore solution in a 1-fl volume, $N = 0.6$.

Measured fluroescence intensities

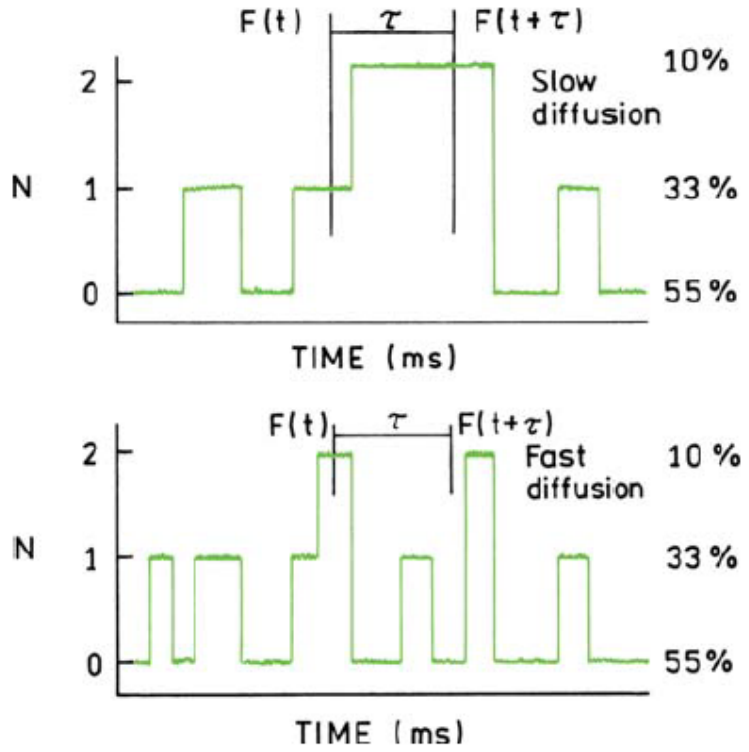

Figure 24.3. Fluctuations in the number of fluorophores (N) in the observed volume of 1 fl with c = 1 nM. The intensity axis is in units of the intensity from a single fluorophore.

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Comparing fluorescence intensities in time

Calculating the auto correlation function

The autocorrelation function G(t) is given by the product of the intensity at time t, F(t) with the intensity at time t + τ , F(t + τ)

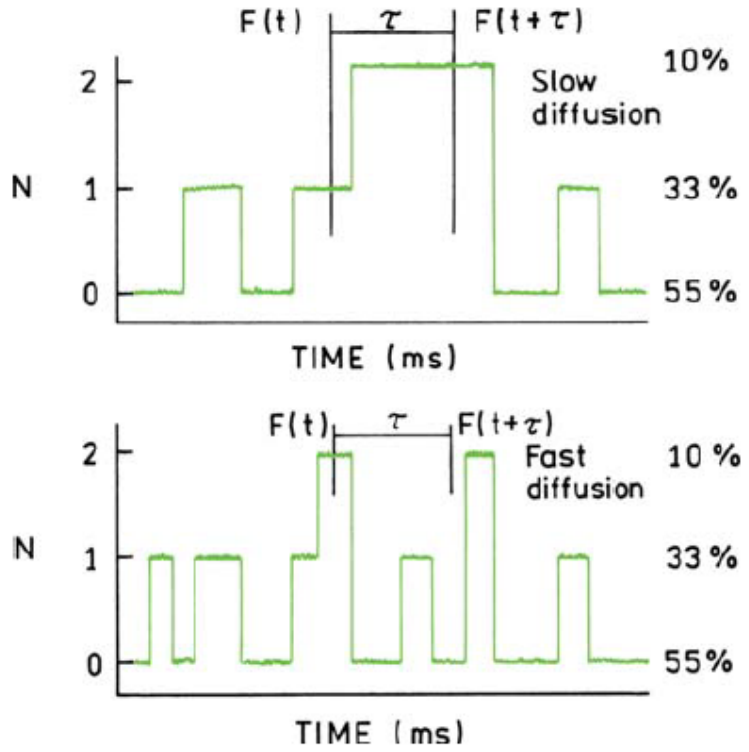
The autocorrelation is further normalized by the average intensity squared

$$G'(\tau) = \frac{\langle F(t)\,F(t+\tau)\rangle}{\langle F\rangle\langle F\rangle}$$

$$G(\tau) = \frac{\langle \delta F(0)\,\delta F(\tau)\rangle}{\langle F\rangle^2}$$

with δF as the variance of F

Measured fluroescence intensities



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Lakowicz, Principles of fluorescence spectroscopy

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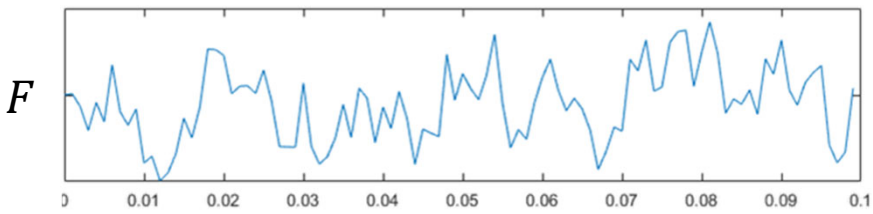
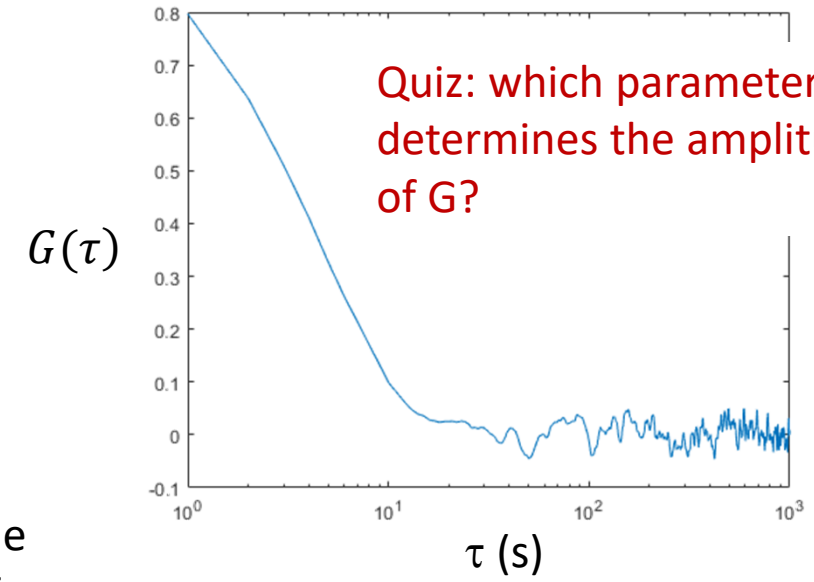




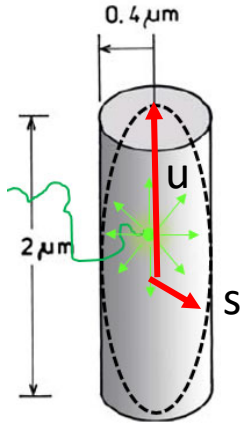
Quiz: which parameter determines the amplitude of G?

Diffusion and the autocorrelation function

The autocorrelation function of diffusing particles in 3D

$$\langle \delta C(r,0)\delta C(r',\tau)\rangle = \overline{C}(4\pi \mathrm{D}\,\tau)^{3/2}\exp\left[-|r-r'|2/4D\tau\right]$$

$C(r,t)$: no. fluorophores / molecules with position r in the confocal volume,
D: diffusion coefficient



Lakowicz, Principles of fluorescence spectroscopy

with this (and considering the differnet brightness of molecules in the confocal volume) the autocorrelation of the fluorescence becomes:

$$G(\tau) = G(0)\cdot\left(1+\frac{4D\tau}{s^2}\right)^{-1}\cdot\left(1+\frac{4D\tau}{u^2}\right)^{-1/2}$$

with u: long radius of the ellipsoid
s: short radius of the ellipsoid

$$G(\tau) = G(0)\cdot D(\tau)$$

Determining diffusion constant by FCS

$$G(\tau) = G(0) \cdot \left(1 + \frac{4D\tau}{s^2}\right)^{-1} \cdot \left(1 + \frac{4D\tau}{u^2}\right)^{-1/2}$$

expressed as a function of the diffusion time τ_D

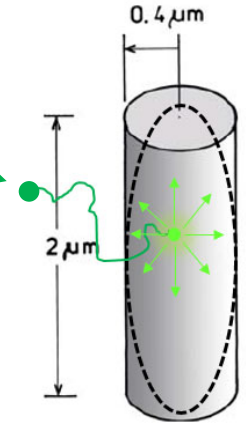
$$G(\tau) = G(0) \cdot \left(1 + \frac{\tau}{\tau_D}\right)^{-1} \cdot \left(1 + \left(\frac{s}{u}\right)^2 \frac{\tau}{\tau_D}\right)^{-1/2}$$

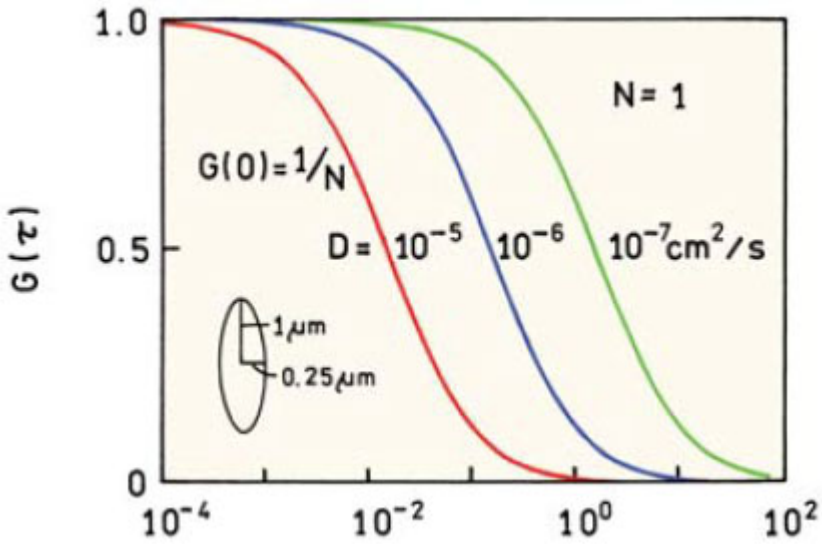
$$\tau_D = s^2 / 4D$$

the decay of G(τ) scales with the diffusion constant.

Faster diffusion → faster decay of the autocorrelation function

Molecule diffusing with D

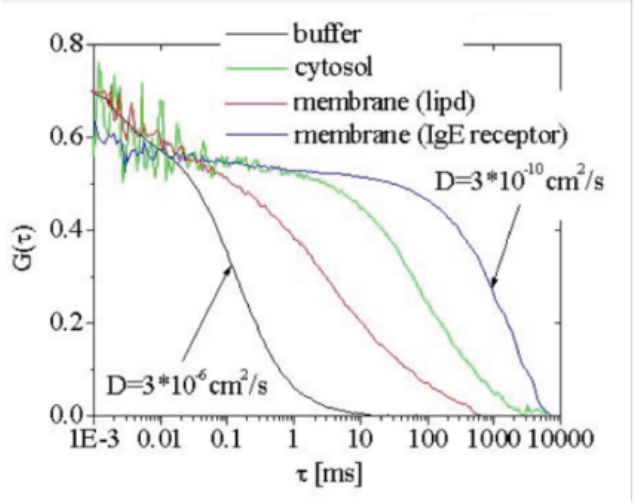




Lakowicz, Principles of fluorescence spectroscopy

Different environments and Binding reactions

Diffusion of dye:
÷ in buffer vs cytosol
÷ on a lipid or membrane protein



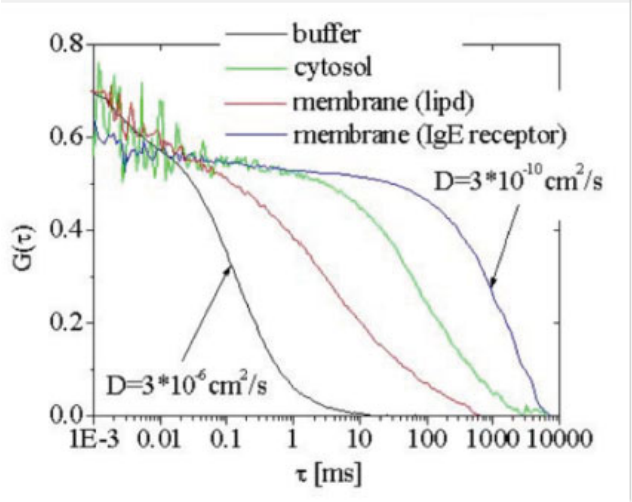
Quiz:

How can binding curves be determined?

Images : Petra Schwille

Different environments and Binding reactions

Diffusion of dye:
÷ in buffer vs cytosol
÷ on a lipid or membrane protein



Diffusion of ligand in the presence of increasing concentrations of receptor

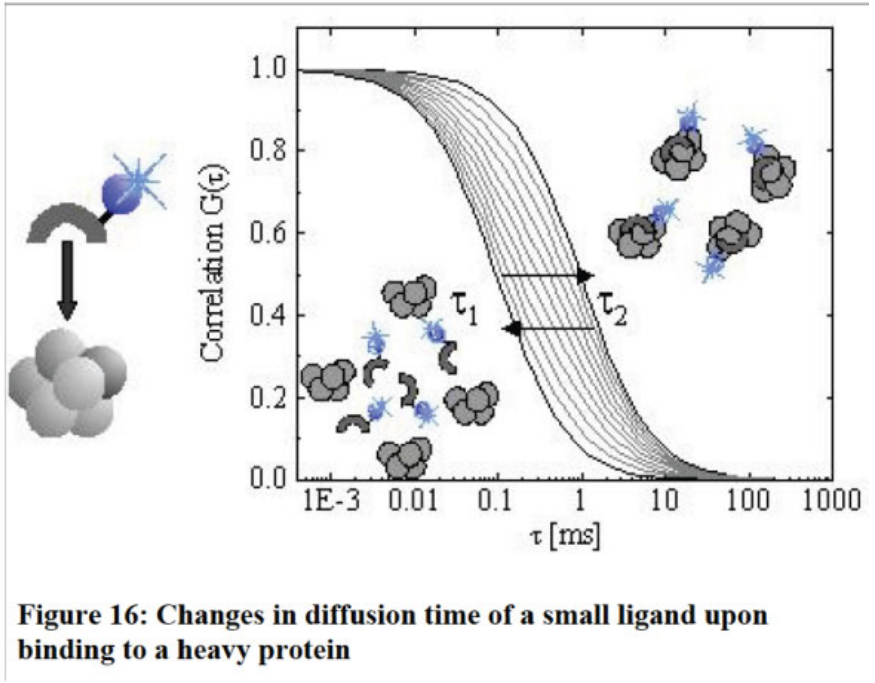
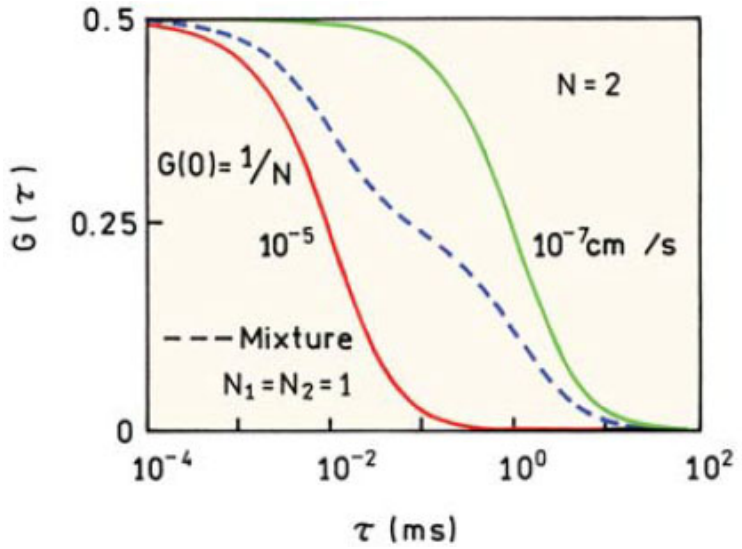


Figure 16: Changes in diffusion time of a small ligand upon binding to a heavy protein

Images : Petra Schwille

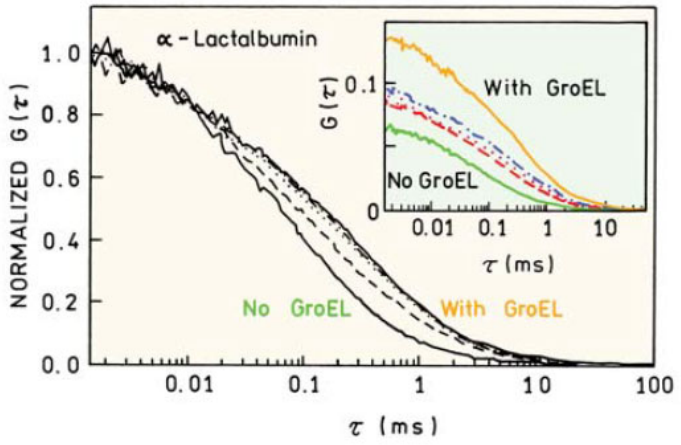
FCS with mixtures of species

Simulated FCS curve of mixture of two species with different diffusion coefficients.



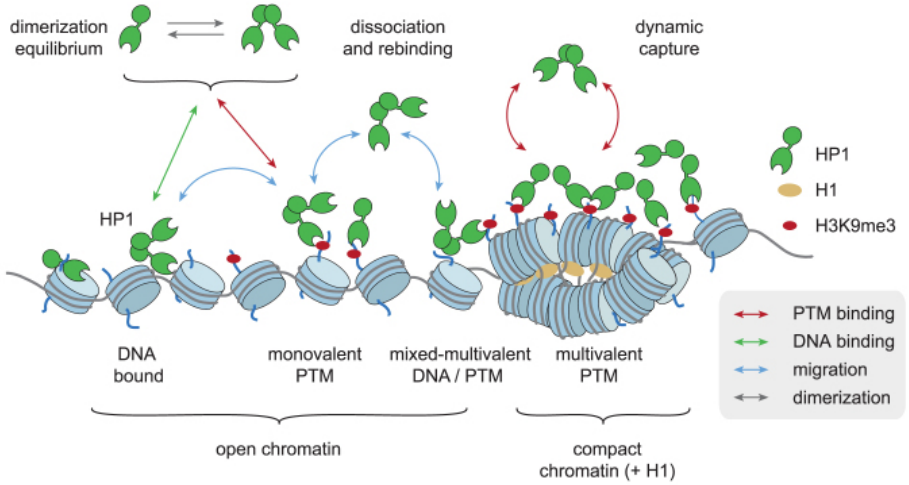
FCS can be used to determine binding reactions, by labeling one species, and titrating the unlabeled binding partner

Figure: binding of α-LA to GroEL



Lakowicz, Principles of fluorescence spectroscopy

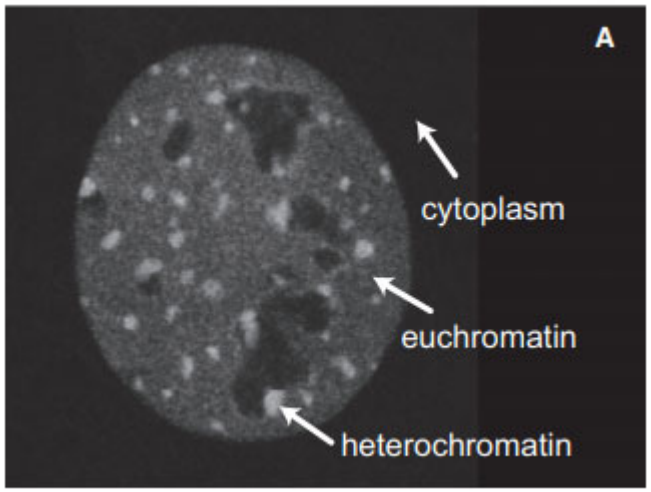
Measuring FCS in cells



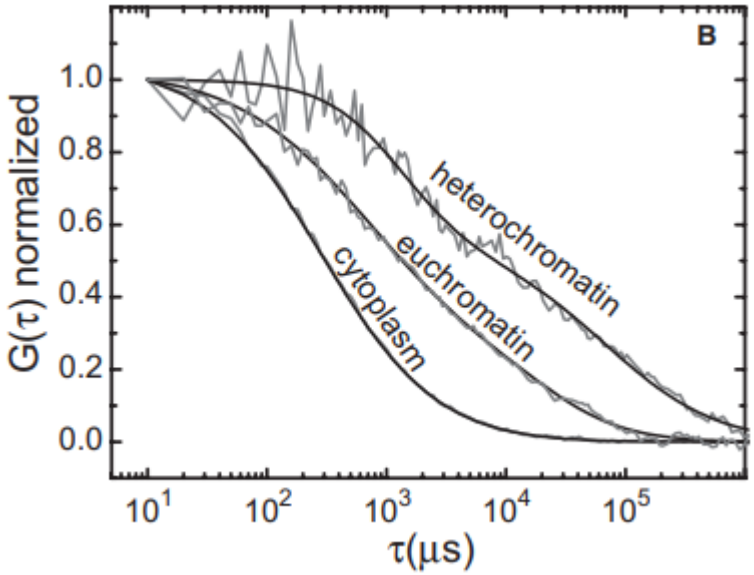
HP1 is a chromatin binding protein, organizing compact heterochromatin compartments.

euchromatin: loose, active chromatin

heterochromatin: compact, repressed chromatin



Müller et al. Biophysical journal 2009



Measuring FCS in cells

free GFP:

D = 23.7 5 4.5 $\mu m^2\ s^{-1}$

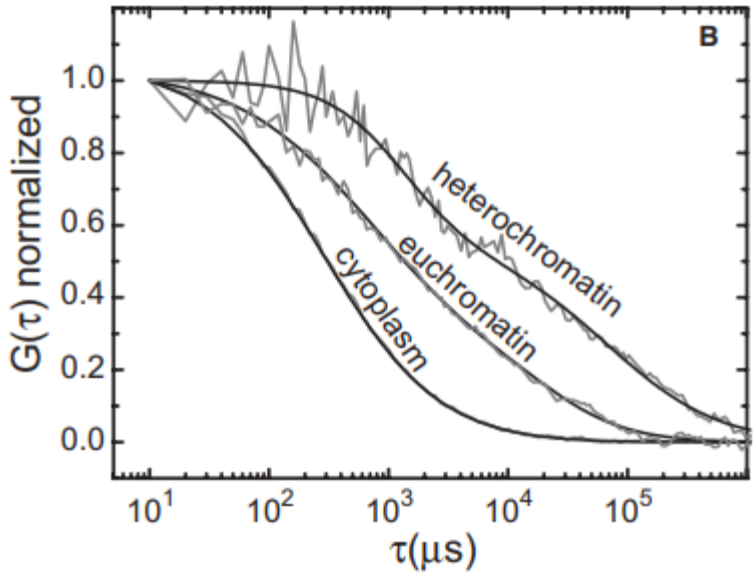


TABLE 2 FCS measurements of HP1α and HP1β in NIH 3T3 cells

	Cytoplasm*		Euchromatin†		Heterochromatin†	
	HP1α	HP1β	HP1α	HP1β	HP1α	HP1β
D_1 ($\mu m^2\ s^{-1}$)	23.4 ± 2.4	24.3 ± 5.6	7.7 ± 0.8	3.2 ± 0.8	3.9 ± 0.9	3.7 ± 0.6
α_1	0.83 ± 0.05	0.74 ± 0.05	0.81 ± 0.04	0.79 ± 0.06	0.88 ± 0.12	0.83 ± 0.08
D_2 ($\mu m^2\ s^{-1}$)	—	—	0.21 ± 0.04	0.07 ± 0.02	0.05 ± 0.02	0.04 ± 0.01
α_2	—	—	>1	>1	>1	>1

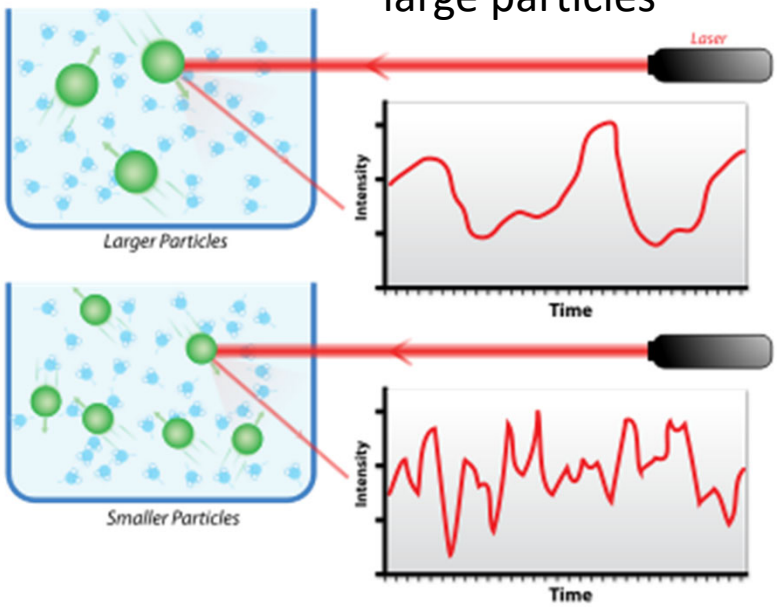
Data were analyzed with a one- or two-component anomalous diffusion model. As a reference, the diffusion constant of GFP was measured to be $D = 23.7 \pm 4.5\ \mu m^2\ s^{-1}$ in the cytoplasm ($\alpha = 0.97 \pm 0.04$) and $D = 21.5 \pm 4.8\ \mu m^2\ s^{-1}$ in the nucleus ($\alpha = 1.1 \pm 0.1$). Euchromatin and heterochromatin regions were not distinguishable in terms of the associated GFP mobility. See also the Supporting Material.

*The data for the HP1α and HP1β mobility in the cytoplasm were fit with a one-component anomalous diffusion model (D_1, α_1). (Error limits correspond to a 95% confidence interval.)

†For the nuclear fraction of HP1α and HP1β, a two-component model was required to describe the data. The faster-moving fraction with diffusion constant D_1 displayed a subdiffusion behavior ($\alpha < 1$), as expected for transient binding and/or diffusion in the presence of obstacles. For the second fraction, intensity fluctuations were very slow and displayed a value of $\alpha > 1$. A more detailed analysis of the associated intensity fluctuations revealed that they originate from chromatin-bound molecules and can be described by a confined diffusion model.

Correlation spectroscopy: Dynamic light scattering

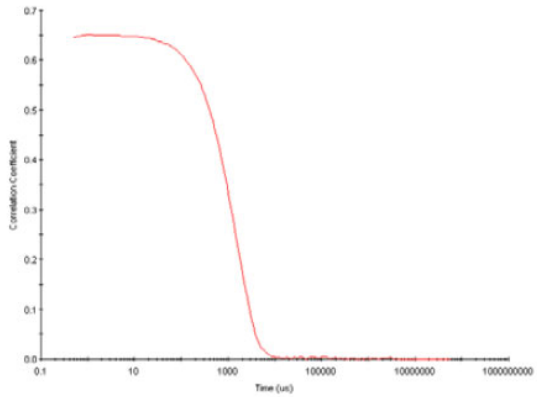
large particles



small particles

autocorrelation function

$$G(\tau) = \langle I(t) \cdot I(t+\tau) \rangle$$



laser with wavelength λ is passed through the solution with refractive index n.
Scattered light intensity is measured at angle θ in time, a correlation function is calculated

$$G(\tau) = A(1 + B\exp(-2Dq^2\tau)]$$

$$q = \frac{4\pi n}{\lambda_0}\sin(\theta/2)$$

Summary

- Diffusion of macromolecules often determines the kinetics of biological processes (see next week)
- The distance a particle diffuses depends on its friction (shape), the viscosity and scales with $(t)^{0.5}$
- The individual molecules undergo a random walk in 3 dimensions

Methods:

- Macroscopic methods, inclusing analytical ultracentrifugation, yield D
- Correlation spectroscopy, such as FCS or DLS allow determination of D and MW