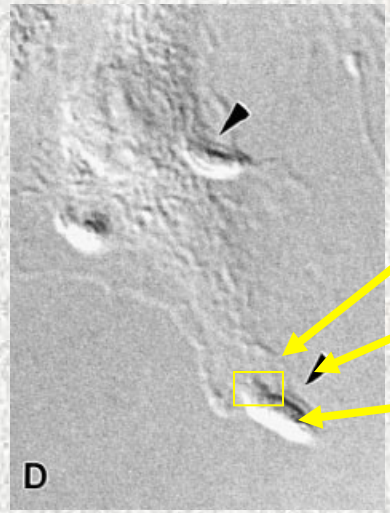
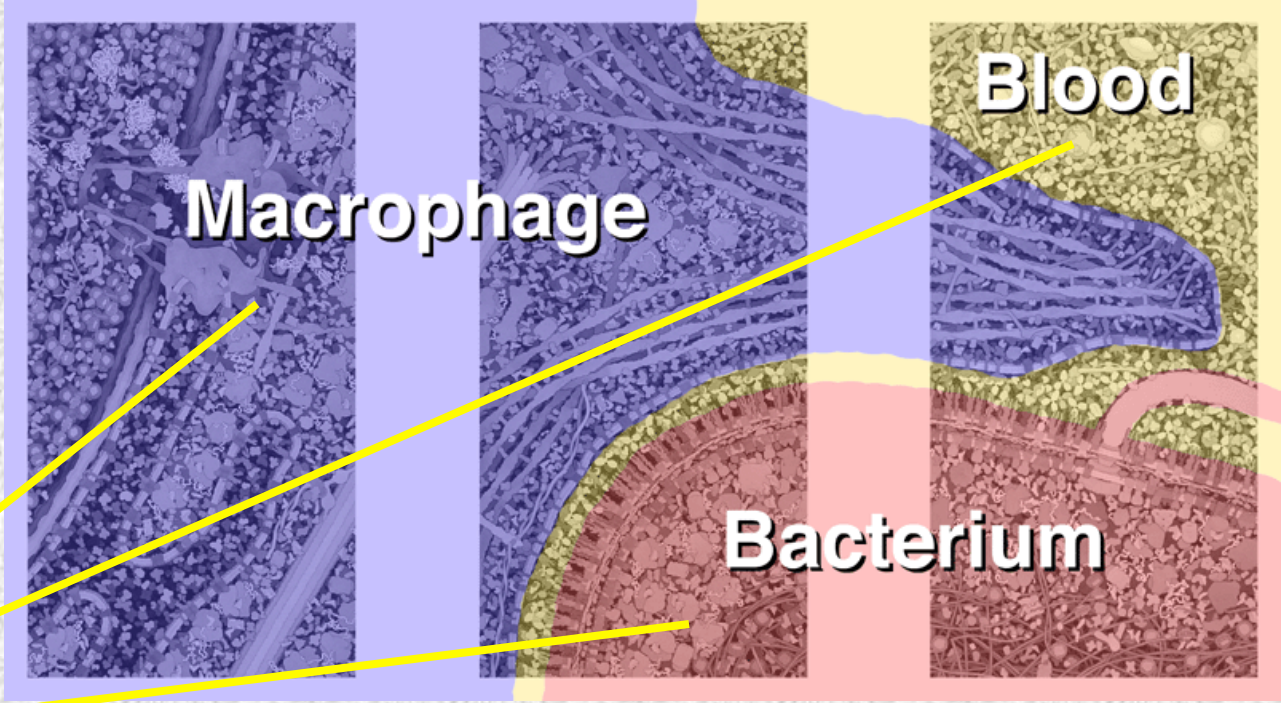
Crowding !!!!





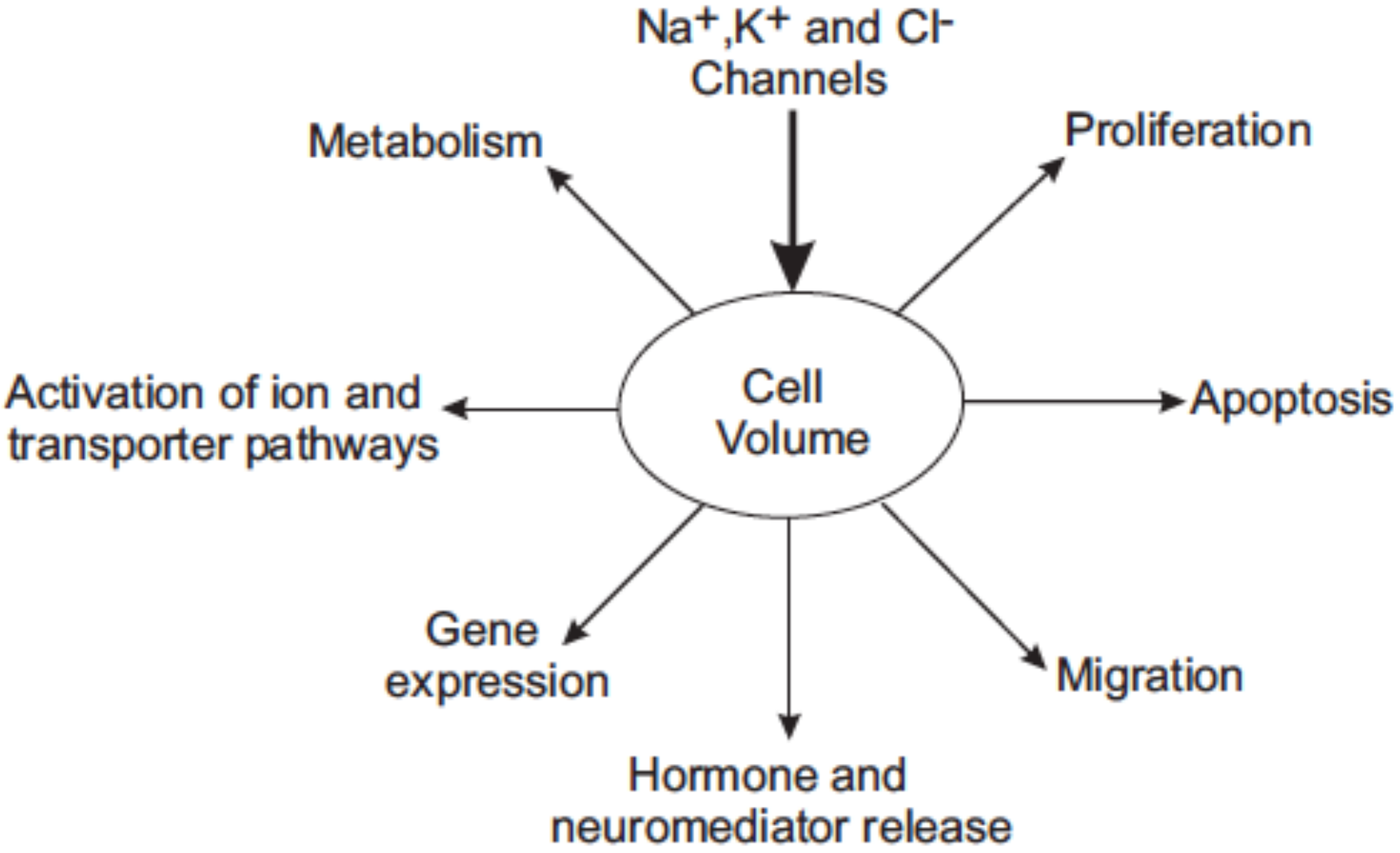
Biochemical reactions in living systems take place in media containing 50–400 mg/ml of macromolecules.

Diffusion of large and small molecules inside cells.

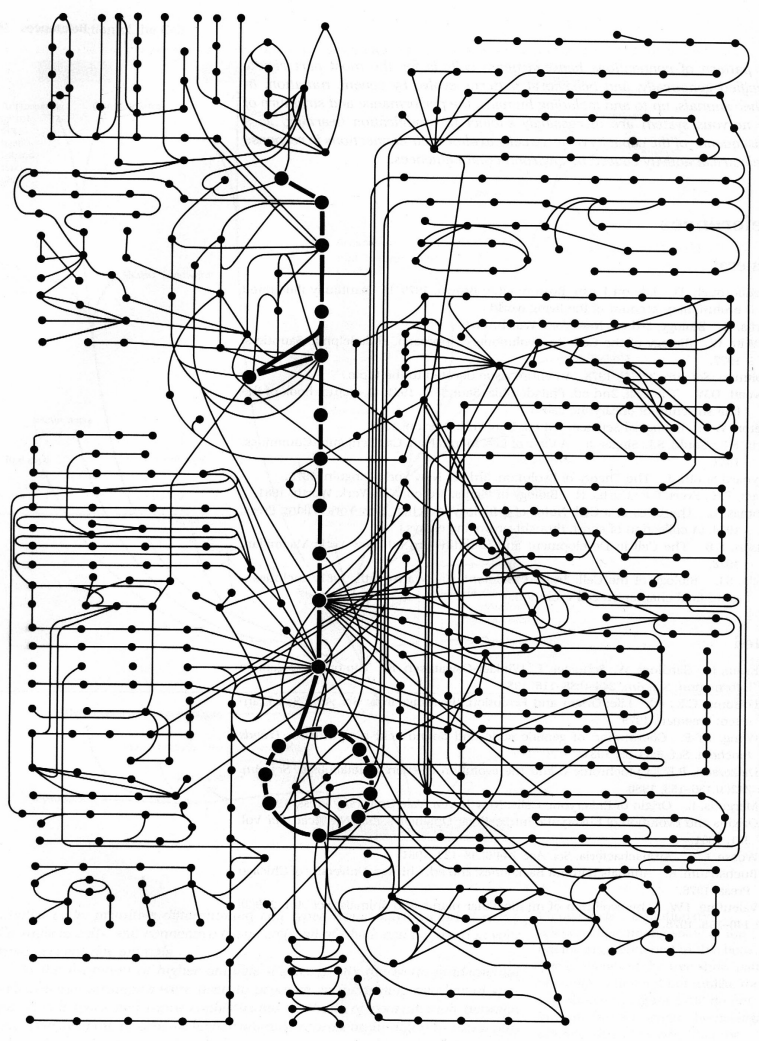
System	Molecular species	D/D_0*
Water	GFP	1
CHO cell cytoplasm	GFP	0.31
CHO cell mitochondria	GFP	0.23–0.34
E. coli cytoplasm	GFP	0.088
BSA (200 g/l)	FITC BSA	0.25
3T3 fibroblast cytoplasm	Carboxyfluorescein	0.27
3T3 fibroblast cytoplasm	FITC dextrans	0.27
Erythrocyte cytoplasm	Lactate	0.32

*Ratio of translational diffusion coefficient to that in water.

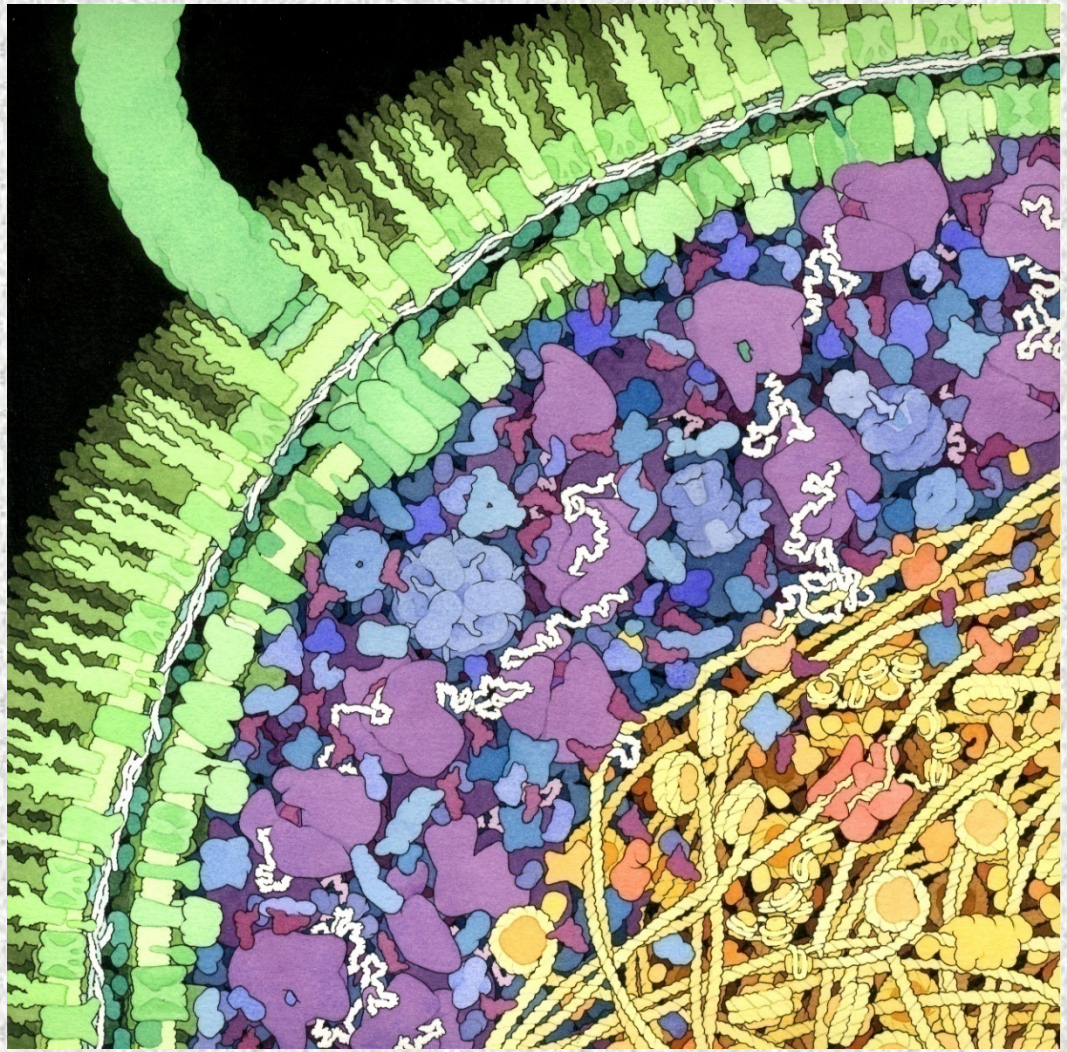
The volume factor



Two different views of a Cell



Molecular Bilogy of the Cell -Alberts et Al., 1983.



Escherichia coli - © David S. Goodsell 1999.

Composition of cell

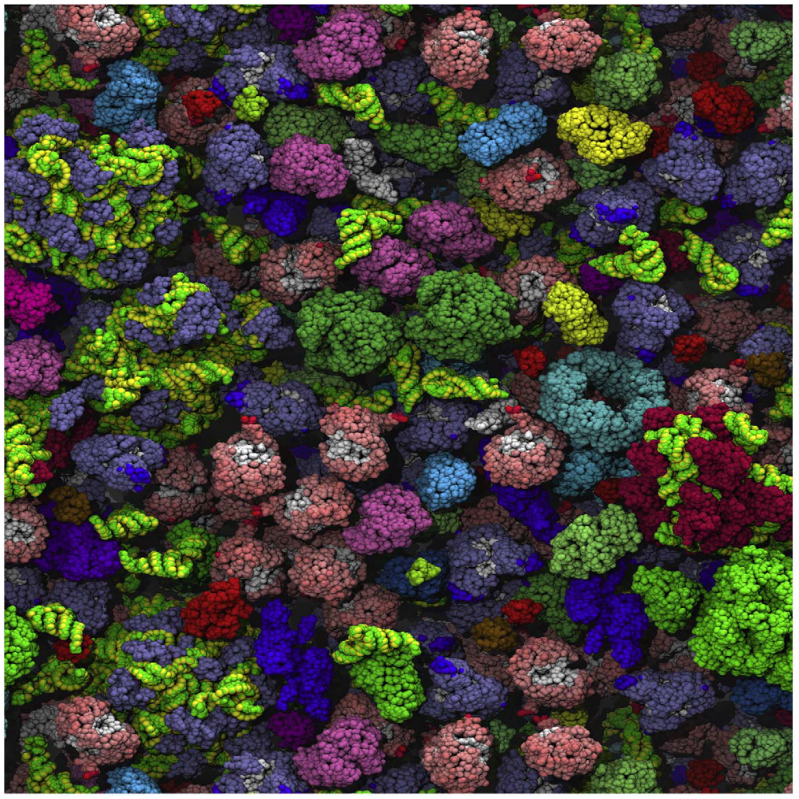
20 x 10^{12} water molecules per cell !

Human cell contains 2 copies of the genome (3.2 x 10^9 bases) = 2 x 3.2x10^9 bases/cell ~ 6.4x10^9 bases/cell.

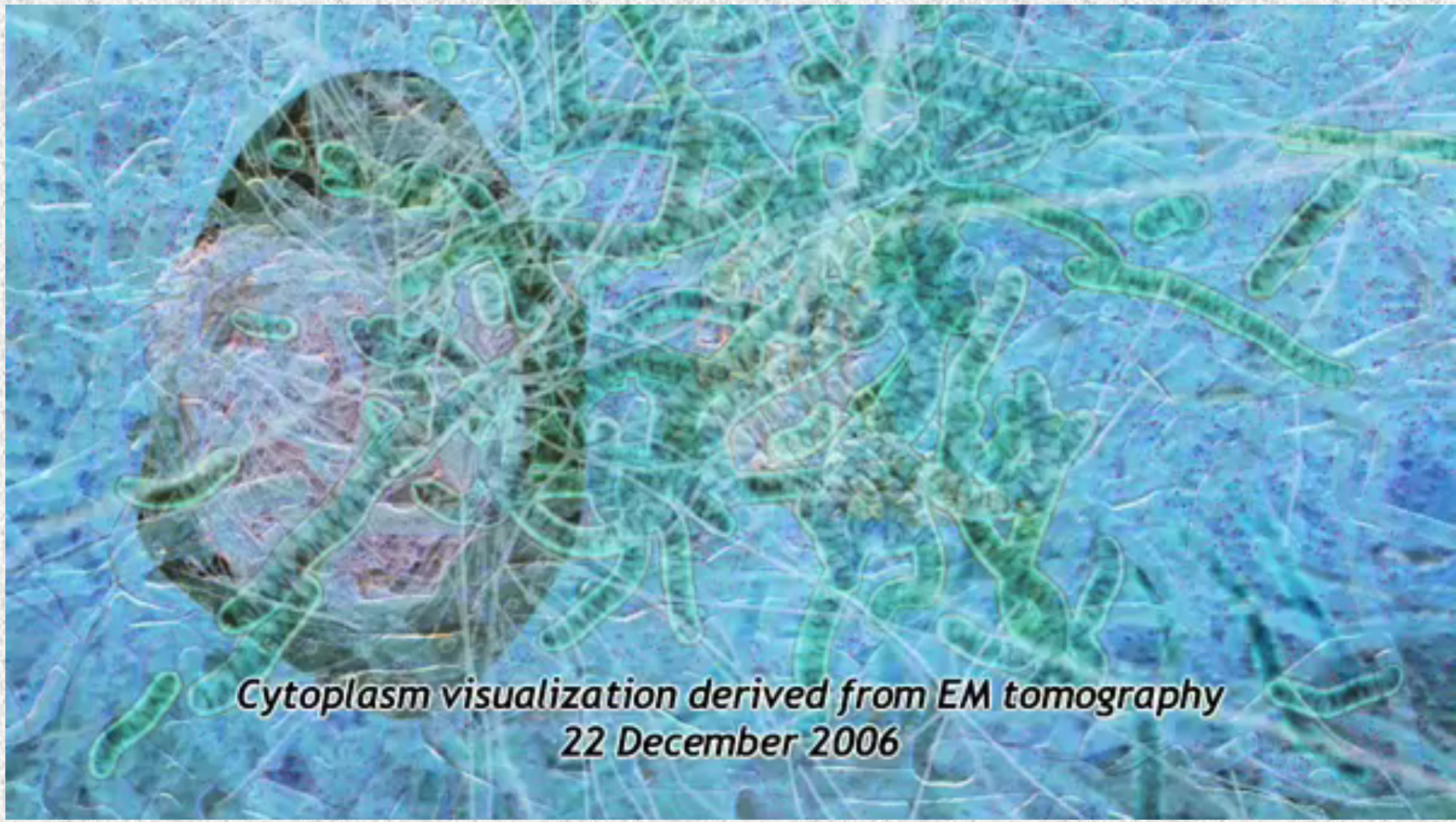
Cell contains ~ 10^9 proteins.

Cell plasma membrane is made of ~ 200 10^6 lipid molecules.

The total number of lipids is ~10^9 per cell.



Current Opinion in Biotechnology


Cytoplasm visualization derived from EM tomography
22 December 2006

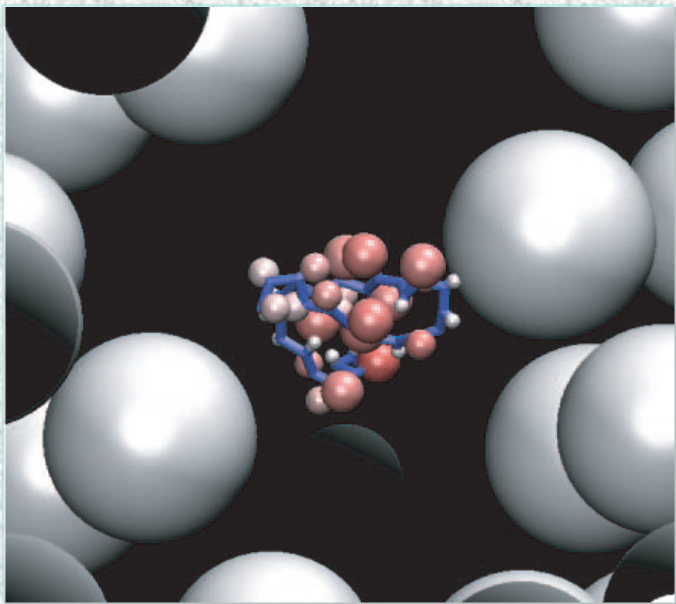
The volume of biological reaction in a cell

- the volume is between $10^{-16} – 10^{-20}$ L

- in bacteria a single copy of e molecule per bacterium will have a nominal concentration of 10^{-8} M

- a large surface with even low affinity for that species could adsorb the entire population.

- pH of the endosome is around 6 for a tipical spherical endosome 250 nm in diameter – this amount to one free proton.



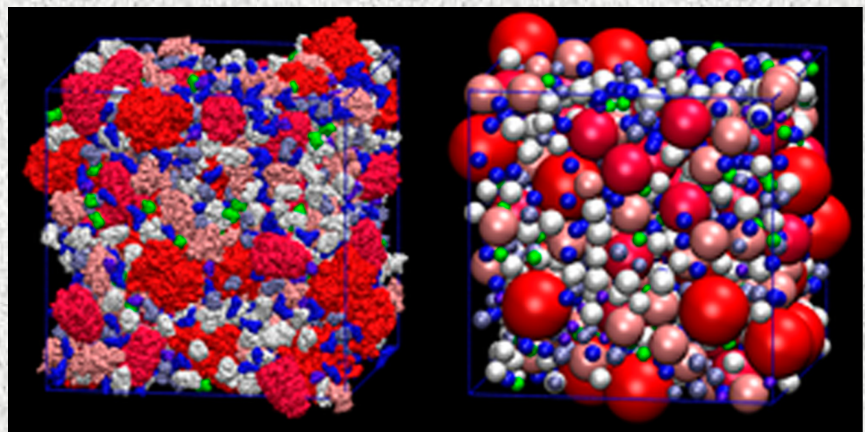
The effect of crowded space

- the protein content of a cell is 17% - 35 % by weight, 20-40% by volume,

- for a typical 50 kDa protein, the theoretical „overlap concentration" is 13g/100mL (the protein concentration inside the cell is 20-30g/100 mL,),

- the entropy benefit of crowded system arises from a reduction in particle number, this may drive association of components that associate weakly or not at all in dilute solution,

- the threshold for crowding effects is steep, therefore small changes in cellular volume may lead to large changes in the chemical activities of the solutes.



The issue of space organization

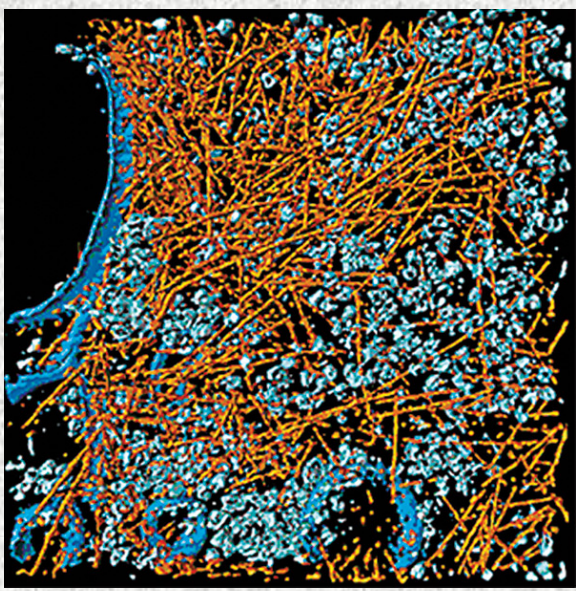
In cell there are

- 3 x 10^5 µm of actin filament per cell,

- 150 microtubules per cell,

- 10 000 µm stable intermediate filament per cell.

The biological solution is a well-structured space.



Medalia et al.

The Cryo-EM Image Reconstruction

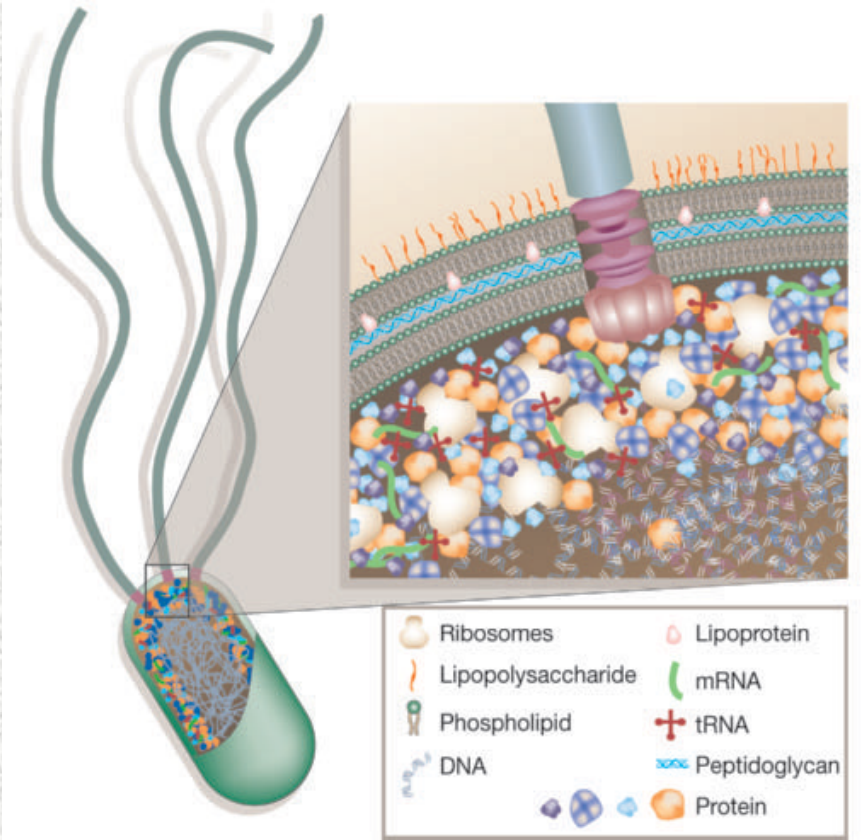
The limited accesibility of substrates

- if the K_d in vivo are the same as measured in vitro, a substantial proportion of substrates may be bound to enzymes, making the concentration of available substrate rate limiting,

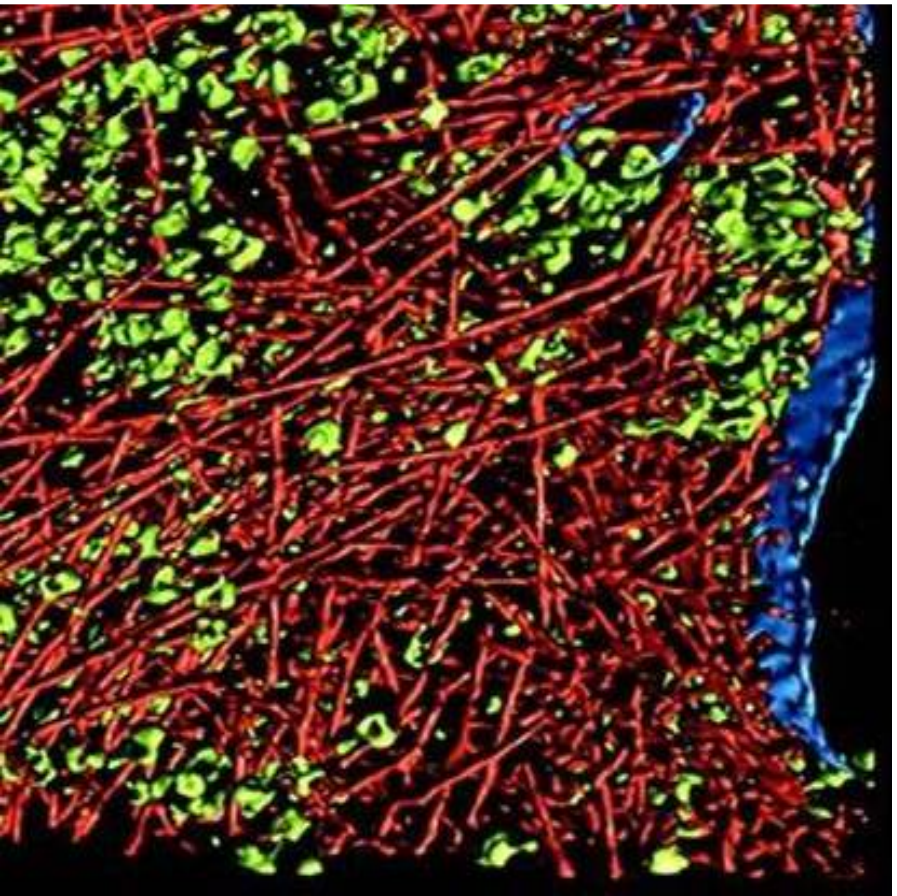
- in order to proceed efficiently under these conditions, channeling of substrates from enzyme to enzyme in a particular metabolic pathway may be necessary.

The concentrations of small molecules in cell, which act as osmolytes, can reach the molar range – the intracellular molarity can be rapidly altered

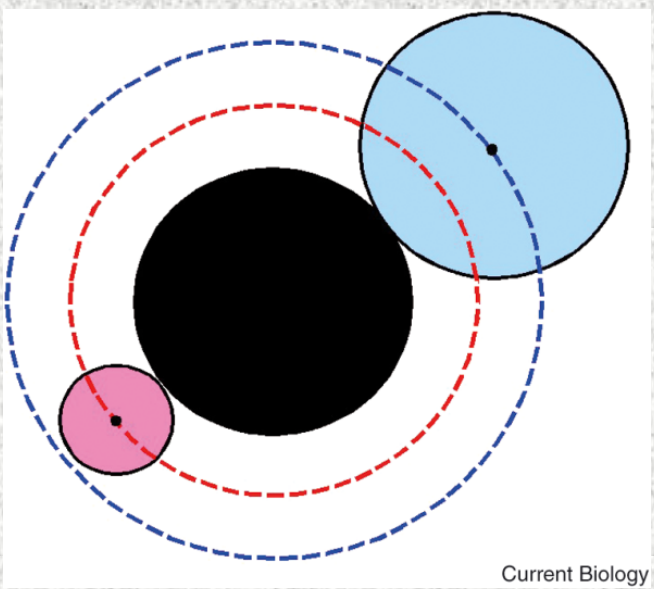
The most extreme example in mammals are kidney medulla cells that contain urea concentrations of up to 5.4 M, corresponding to 30% by mass.



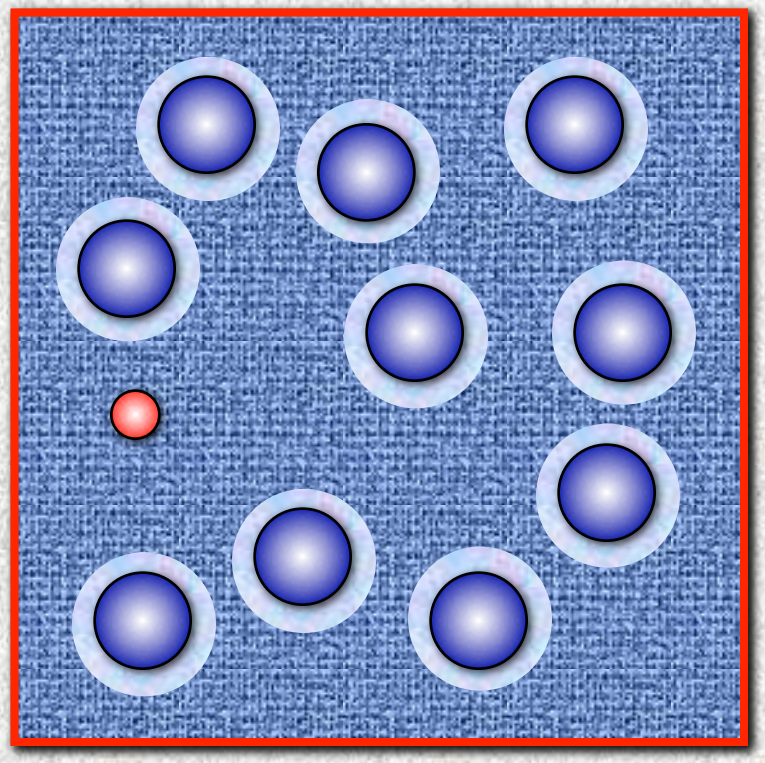
The term 'macromolecular crowding' was coined to connote the influence of mutual volume exclusion upon the energetics and transport properties of macromolecules within a crowded, or highly volume-occupied, medium.



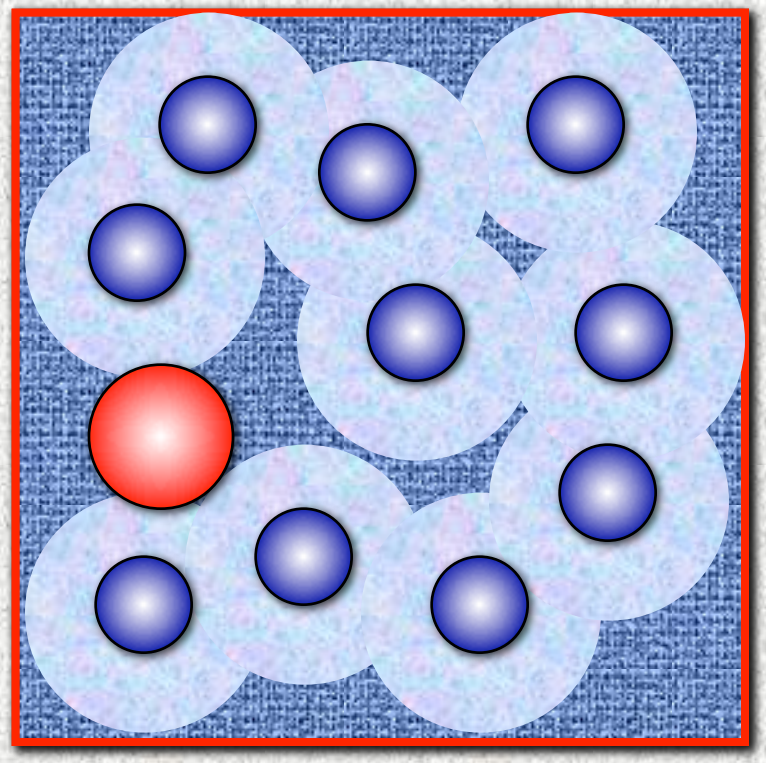
Volume exclusion



Effect of crowding on diffusion



diffusion of small solute unaffected



diffusion of large solute strongly slowed down

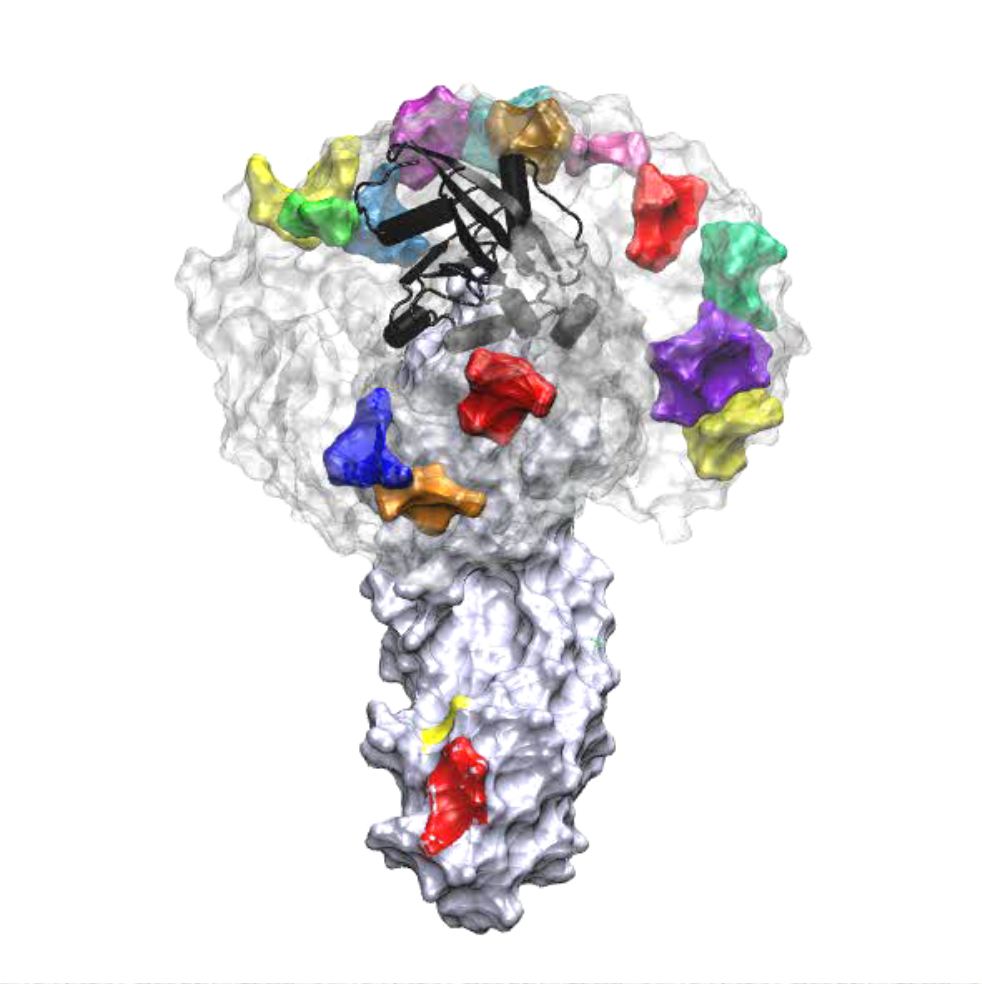
It has been reported that the diffusional mobility in the cytoplasm strongly decreases with an increasing radius of the tracked particle, leaving particles with a radius > 25–30 nm immobile.

Enzymes and crawding

- Metabolites are relatively small compared to enzymes and they can be neglected as a crawding agent.

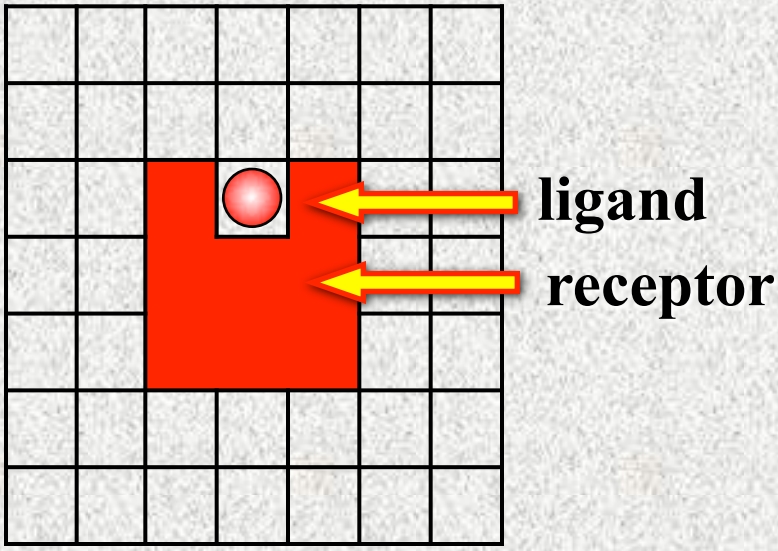
- The active site of most metabolic enzymes is relatively small compared to the whole molecule,

- For all practical purposes the inert enzyme region is equivalent to an inert crowding agent.

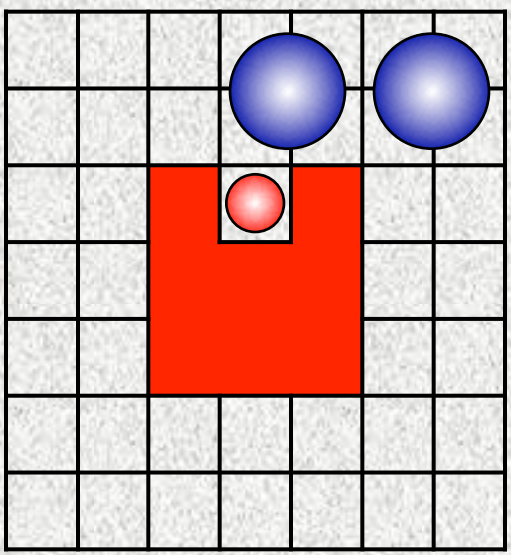


Effect of crowding on binding

- to fully understand a binding equilibrium we must consider two states:
  - bound state
  - unbound state
- imagine the following system:

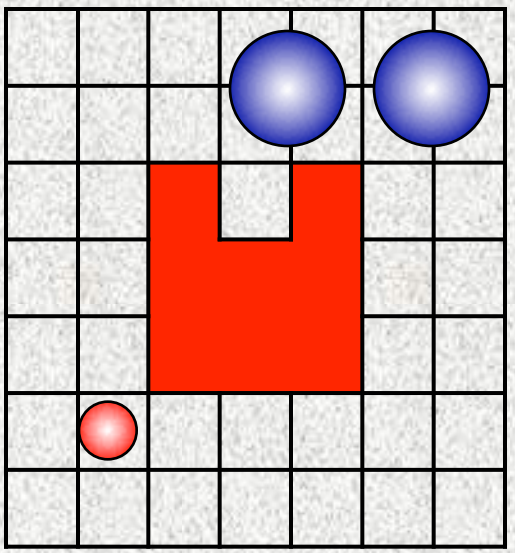


Effect of large crowding molecules:



ligand bound

many possible configurations for the crowder

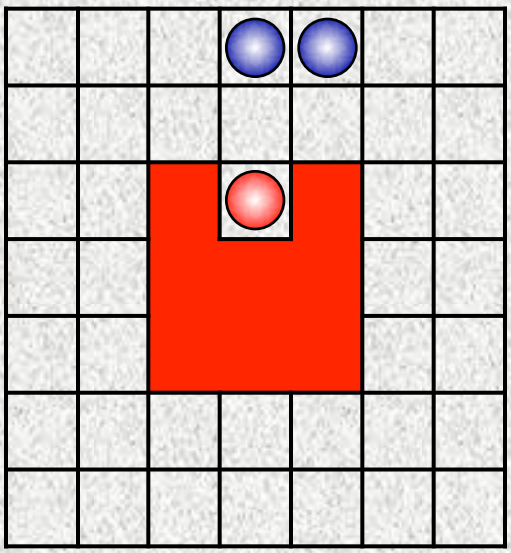


ligand unbound

fewer possible configurations for the crowder

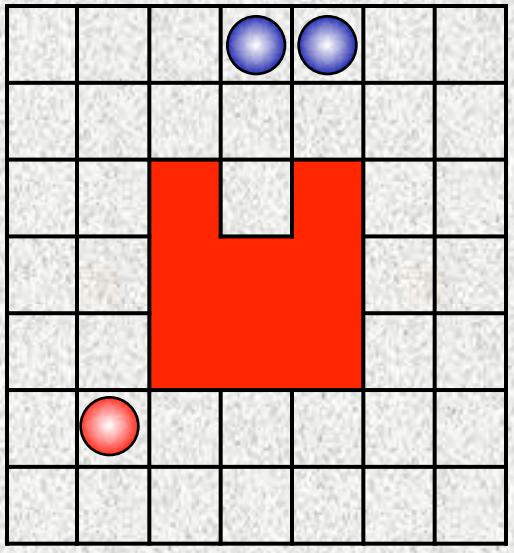
- on a purely statistical basis, large crowding molecules should cause an increase in ligand-receptor binding

Effect of small crowding molecules:



ligand bound

many possible configurations for the crowder



ligand unbound

many possible configurations for the crowder

- on a purely statistical basis, small crowding molecules should cause no change in ligand-receptor binding

Crowding and diffusion

1) The intracellular mobility of water is significantly reduced, leading to partitioning of metabolites between different water phases and to changes in binding constants.

2) Low-affinity adsorbtion of metabolites, especially if charged as ATP, to intracellular surfaces increases the viscosity.

3) The diffusion coefficient of metabolites is decreased by a factor of $(1+C/K_d)^{-1}$ where C is concentration of binding sites and K_d is dissociation constant of solute from these complexes.

4) Macromolecular crowding and cytoskeletal structures create barriers which increase the effective path-length of diffusion.

5) The movements of individual molecules become coordinated and vectorially directed due to organization of enzymes into multi-enzyme complexes, and the randomness of molecular events may be lost.

The ratio between the effective concentration C and the concentration in an ideal solution C_0 is denoted by the activity coefficient:

$$\gamma_C = C/C_0$$

It has been *even recommend not to use "diffusivities" or* "diffusion coefficients" for biological systems but to use some terms of the type of "empirical transport coefficient" when the Fick's equation is formally applied for intracellular processes.

The diffusion coefficient of a trace particle in a crowding media is affected by the concentration of crowding agents.

The diffusion coefficient is given by

$$D = \gamma_D D_0$$

where D_0 is the diffusion coefficient in aqueous solution and γ_D is a correction factor.

To quantify the impact of crowding on metabolites diffusion the empirical exponential law can be used:

$$\gamma_D = \exp(-\alpha v)$$

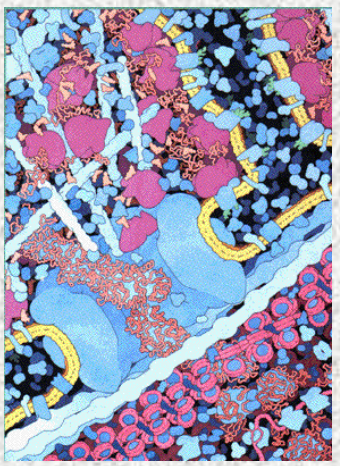
where the exponent α is an empirical parameter

The estimate for the cytoplasm equals $\alpha = 5.8$ for fibroblast cells.

- Crowding is a consequence of steric repulsion, a destabilizing interaction that increases the total free energy of the system.

- Crowding is expected to shift equilibria toward a state of the system in which excluded volume is minimized.

- Therefore crowding exerts a generalized pressure for the reduction of the surface to volume ratio.



This is accomplished in two ways;

- by favoring compact conformations over extended conformations of flexible macromolecules,
- by favoring both specific macromolecular associations leading to the formation of well-defined oligomeric species, and nonspecific macromolecular associations leading to the formation of large aggregates of native or nonnative species.

Crowding affects proteins:

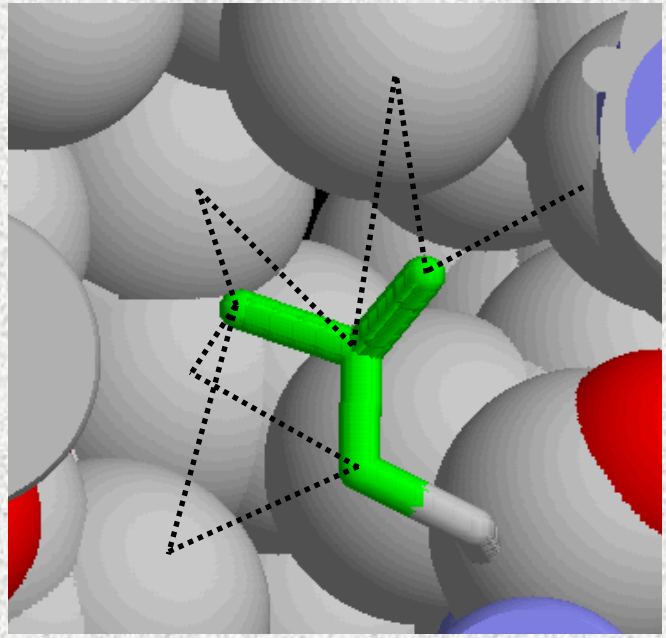
- thermodynamics of protein folding
- kinetics of protein folding
- extent of protein aggregation

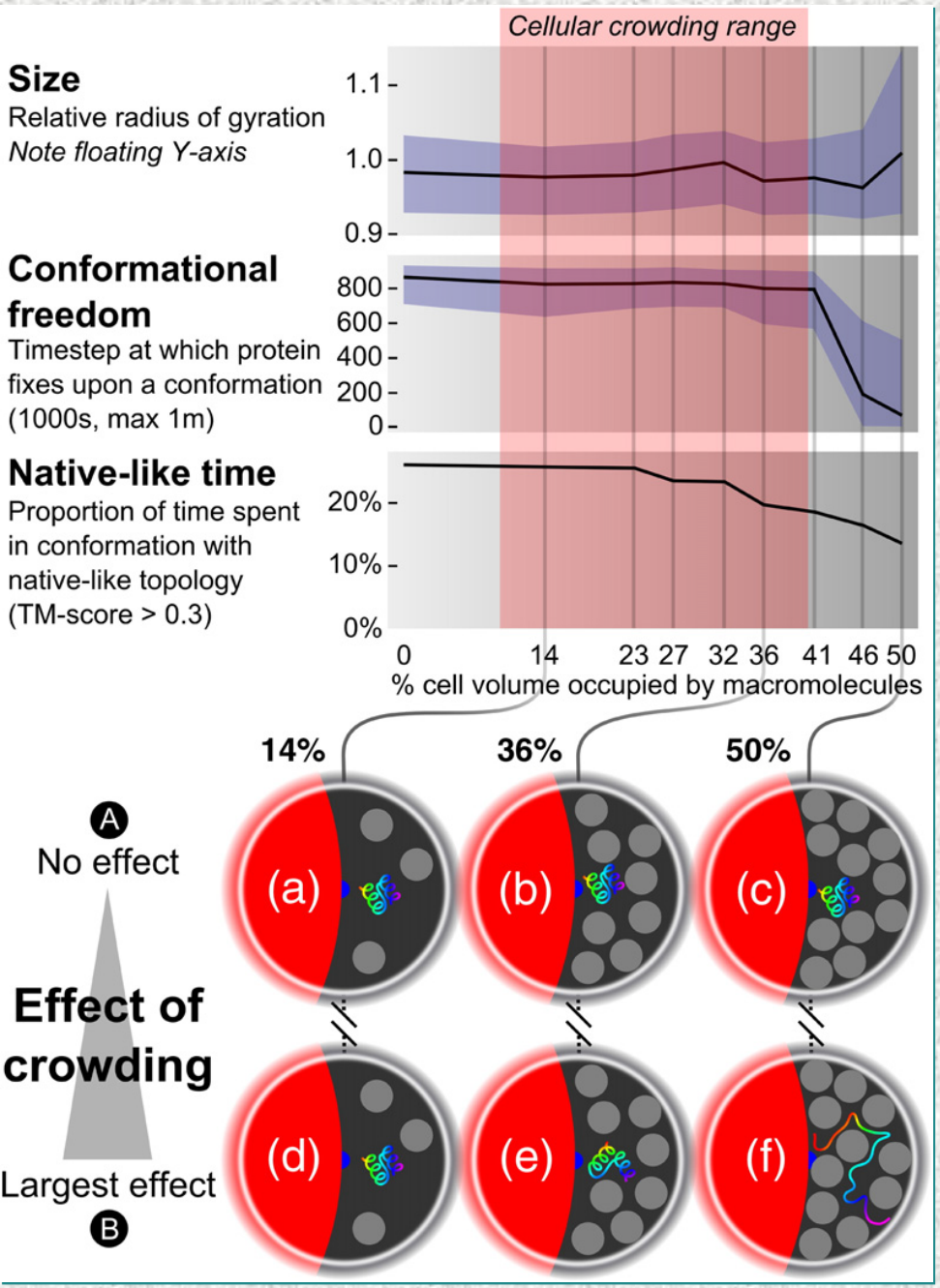
The density of atoms is higher in a protein's folded state than in water (proteins are very tightly packed) so dispersion interactions will stabilize proteins

favorable interactions with close-packed atoms in the folded state

are partly balanced by:

favorable interactions with water molecules in the unfolded state





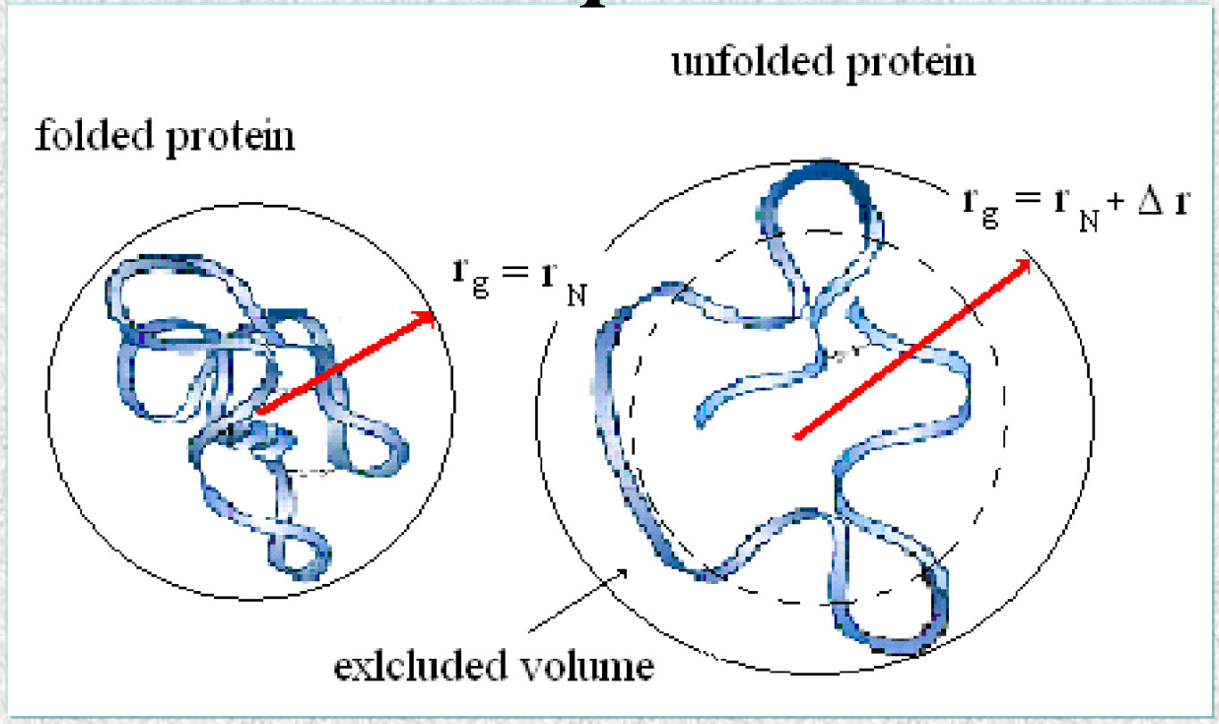
Effects of crowding on protein folding.

At 14% (d), there is no measurable effect on the folded protein.

At 36% (e), proteins are starting to get trapped in nonnative but compact conformations.

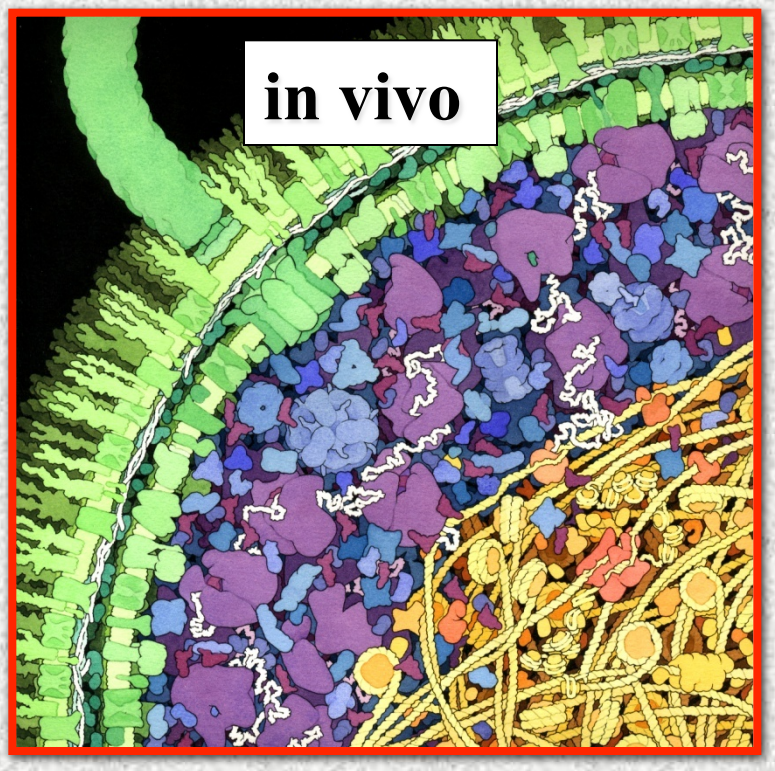
At 50% (f), proteins are becoming trapped in extended conformations.

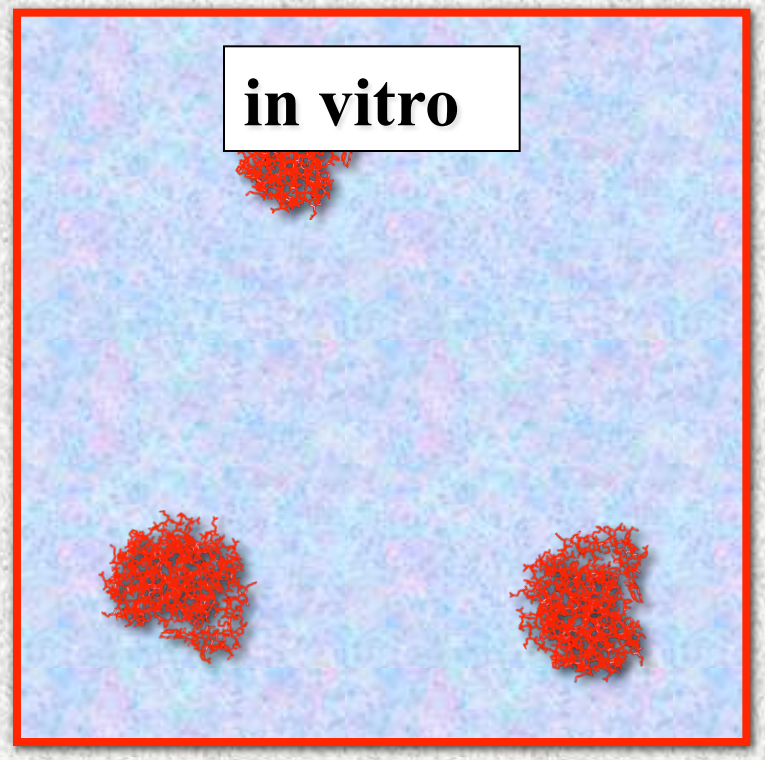
Unfoldng of the most thermolabile proteins in a cell increases the stability of the other cellular proteins.



The compactness of the protein structure in the folded state is different from that in the unfolded state. *rg expands during unfolding, excluding volume to other surrounding* proteins.

Macromolecular crowding - experimental approaches





David S. Goodsell

To mimic molecular crowding in vitro, various molecules can be utilized as crowding cosolutes.

The crowding cosolutes must meet the following criteria:

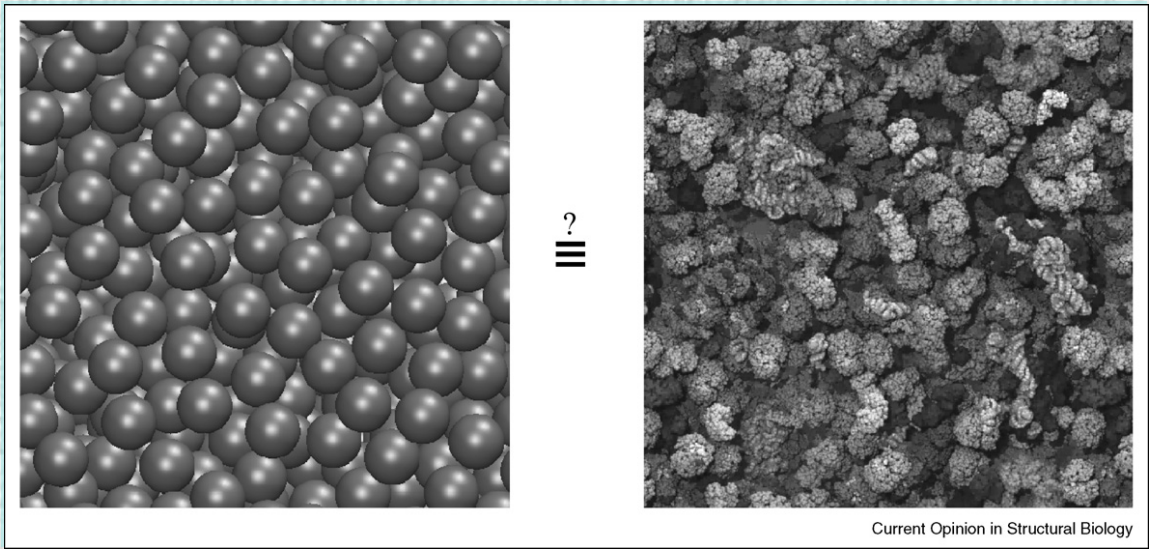
(1) they should be basically inert, resulting in no chemical interaction between a target molecule and a crowding cosolute;

(2) they should be highly dissolved in water to stimulate molecular crowding conditions;

(3) in the case of large cosolutes, different polymer sizes should be available;

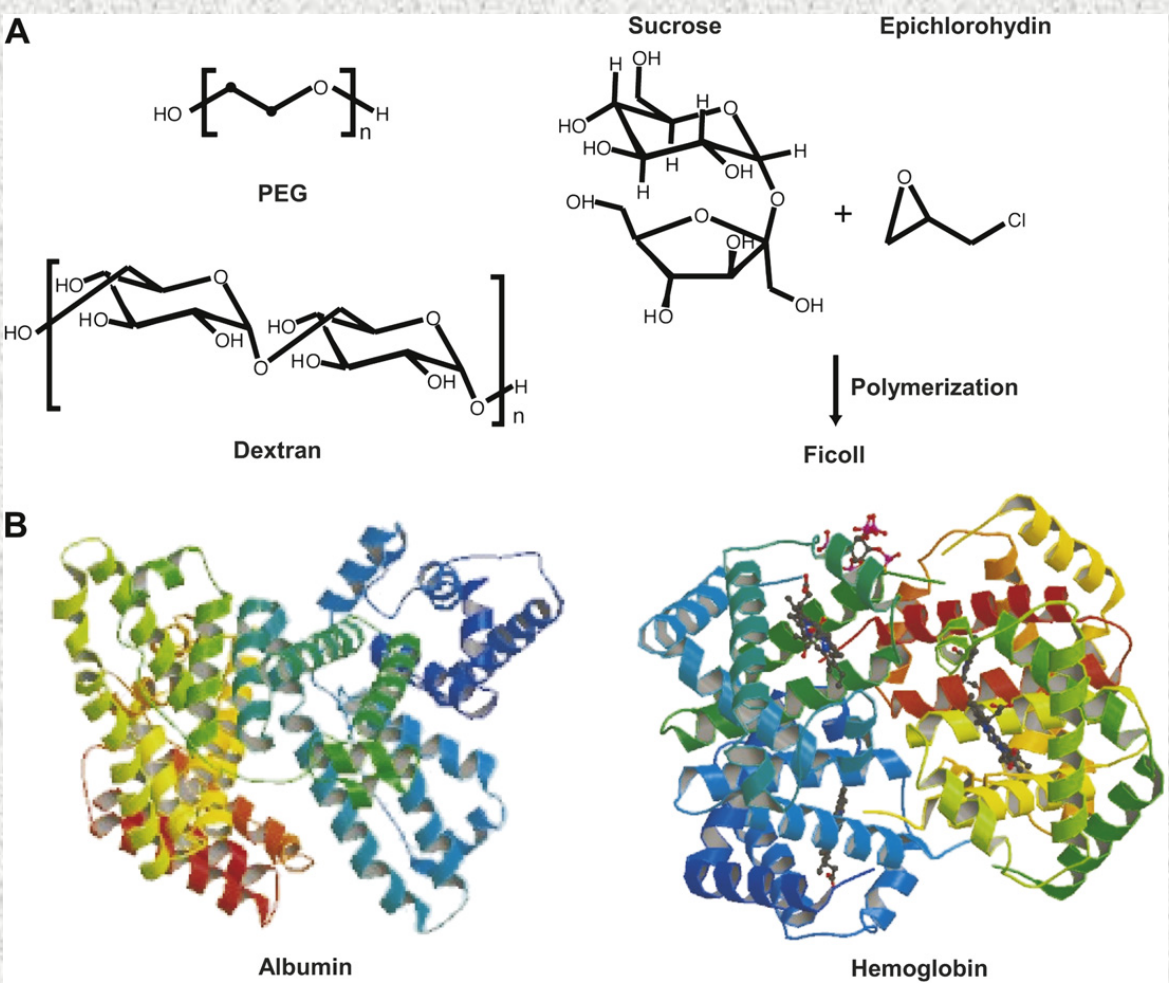
(4) in the case of small cosolutes, different chemical properties should be available.

Osmotic pressure is a critical factor that is perturbed by molecular crowding.



Model crowding cosolutes:

- large PEG, dextran, and Ficoll (a copolymer of sucrose and epichlorohydrin).
- proteins such as albumin, hemoglobin, and lysozyme
- small crowding cosolutes are alcohols, glycols, amino acids, and betaine.

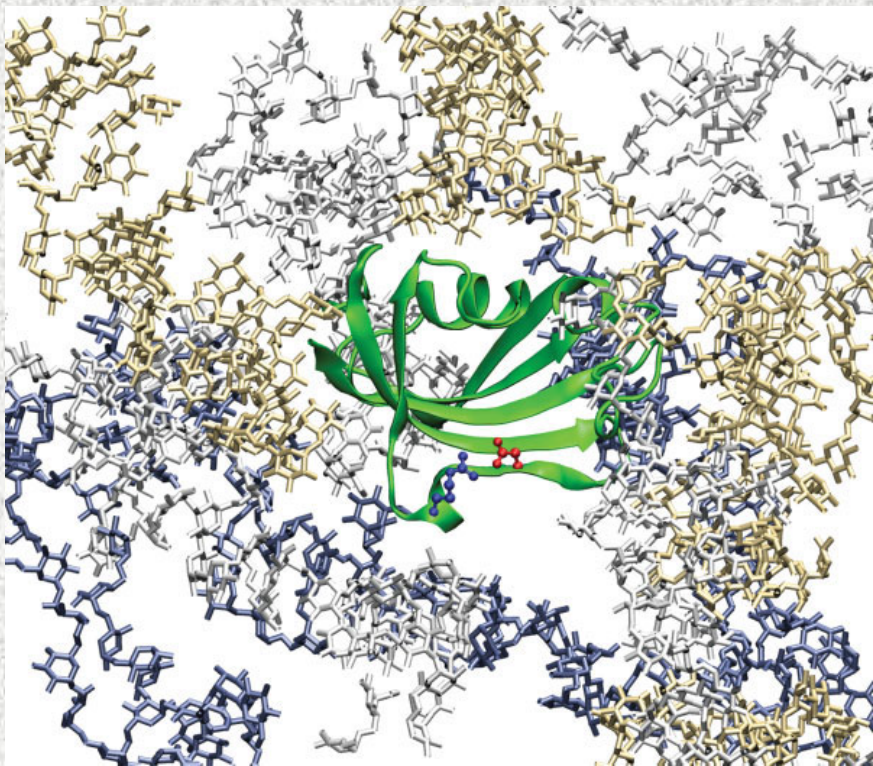


Overview of additives reviewed.

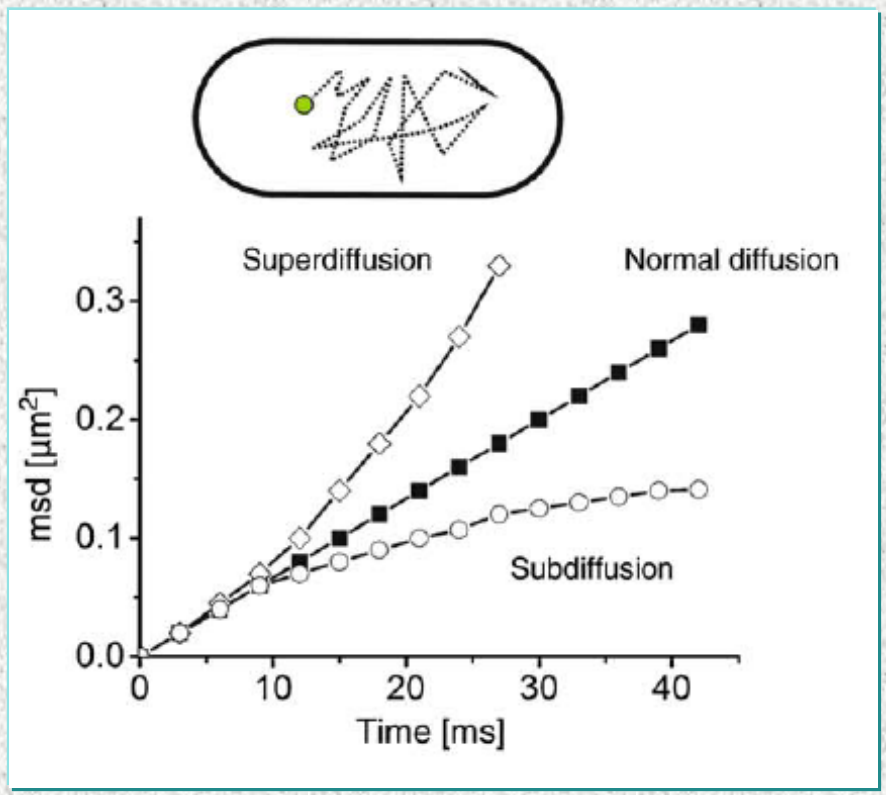
Class of additive	Examples reviewed	Action	Source of action
Polyols	Glycerol	Conformational stabilizer, association suppressor	Steric exclusion, repulsive electrostatic interactions, binding to hydrophobic patches
Sugars	Trehalose Sucrose	Conformational stabilizer	Steric exclusion, cohesive force, enhanced intra-protein interactions resulting from clustering
Denaturants	Urea Guanidinium chloride	Conformational destabilizer, solubilizer	Preferential binding from hydrogen bond and hydrophobic interactions
	2:1 Urea–TMAO mixture Guanidinium sulfate	No influence on conformation	Intra-solvent interactions inhibiting preferential binding
Amino acids	Proline	Refolding enhancer, solubilizer	Unclear, supramolecular assemblies disputed
	Arginine hydrochloride	Association suppressor	"Gap Effect", Arg–Arg attractive interactions
Arginine salts	Arginine sulfate	Conformational stabilizer, association suppressor	"Gap Effect", Arg–Arg and Arg–sulfate attractive interactions
	Arginine thiocyanate	Conformational destabilizer	Preferential binding of counterion
	1:1 Arg–Glu mixture	Association suppressor, solubilizer	"Gap Effect", Arg–Glu attractive interactions

Illustration of a FKBP molecule (green ribbon) surrounded by dextran molecules.

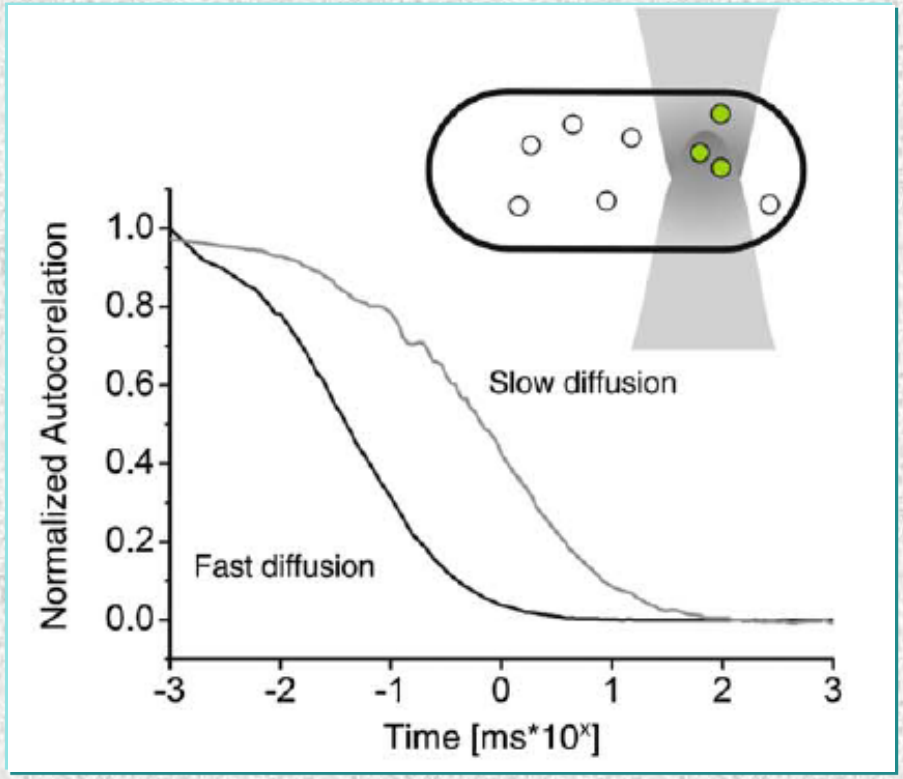
A salt bridge between Asp37 and Arg42 is shown as ball-and-stick.



Experimental techniqes for studies of processes in cell.

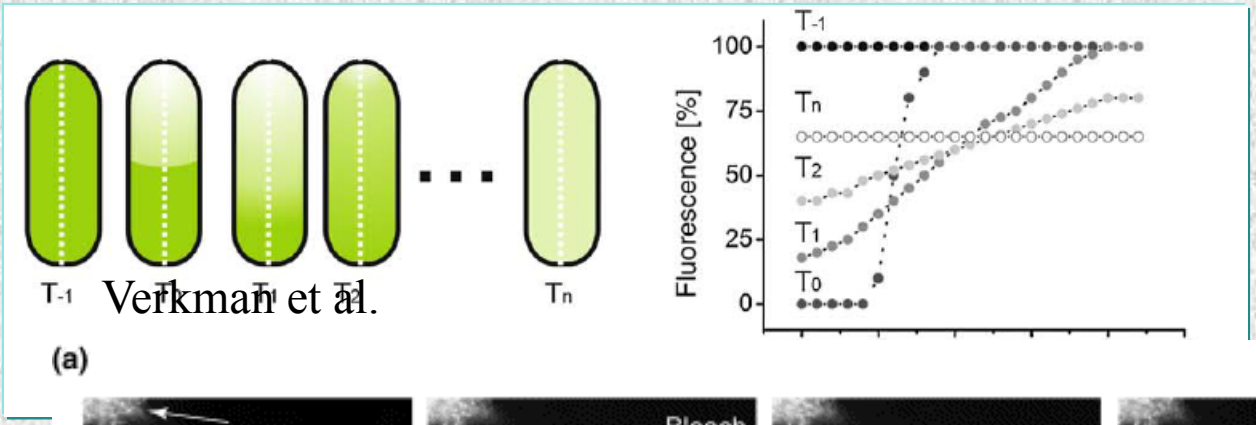


Single molecule tracking

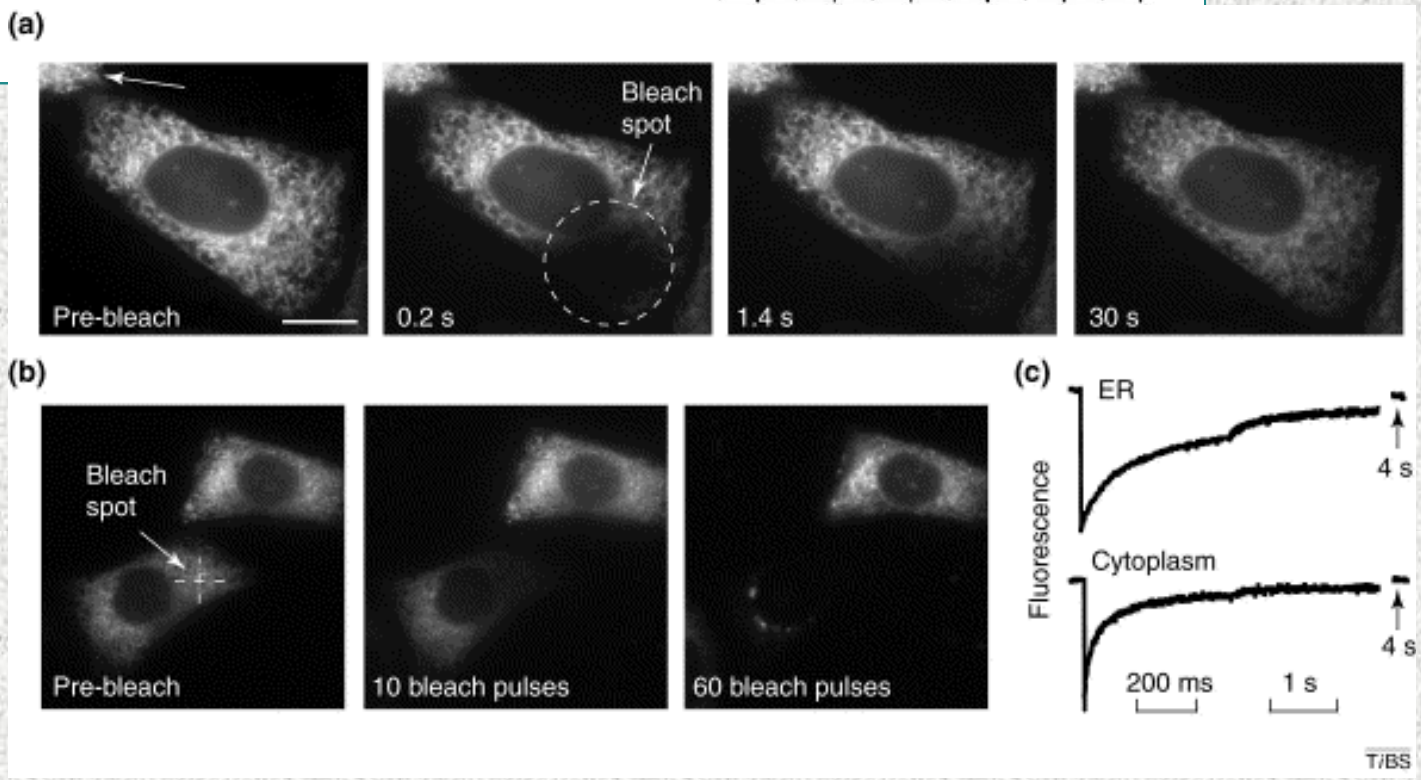


Fluorescence correlation spectroscopy

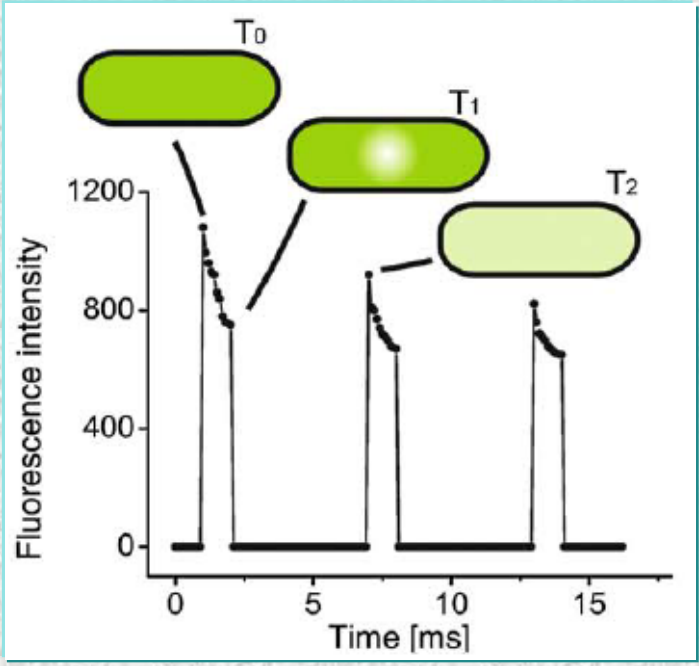
Conventional FRAP.



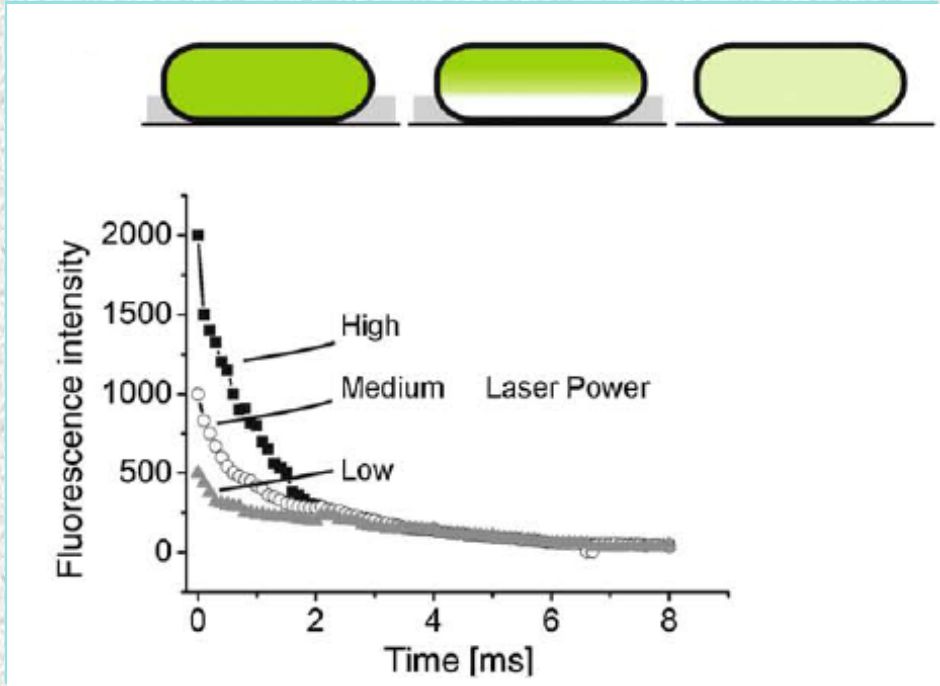
Verkman et al.



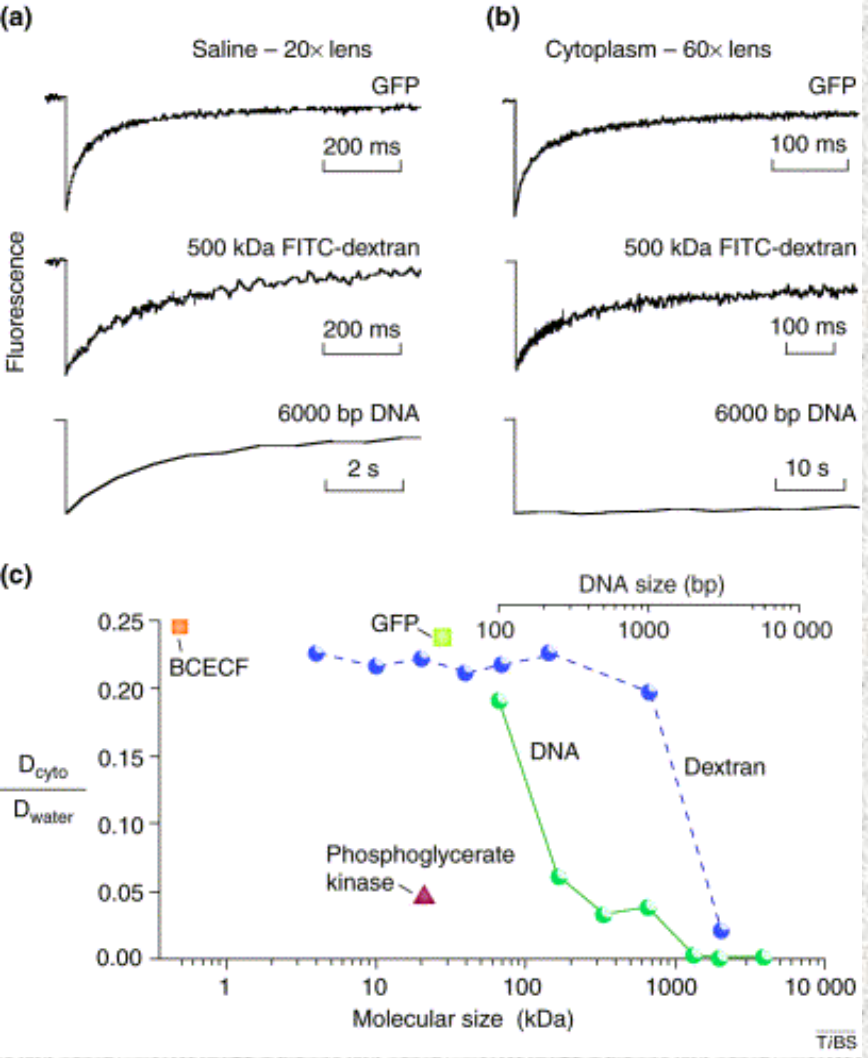
Pulsed-FRAP

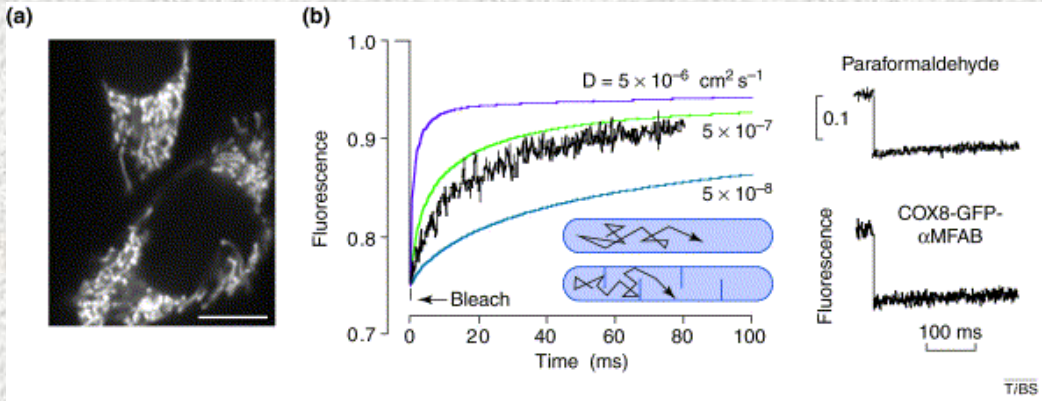


Continous photobleaching with total internal reflection microscopy.



FRAP Data and Macromolecular Diffusion





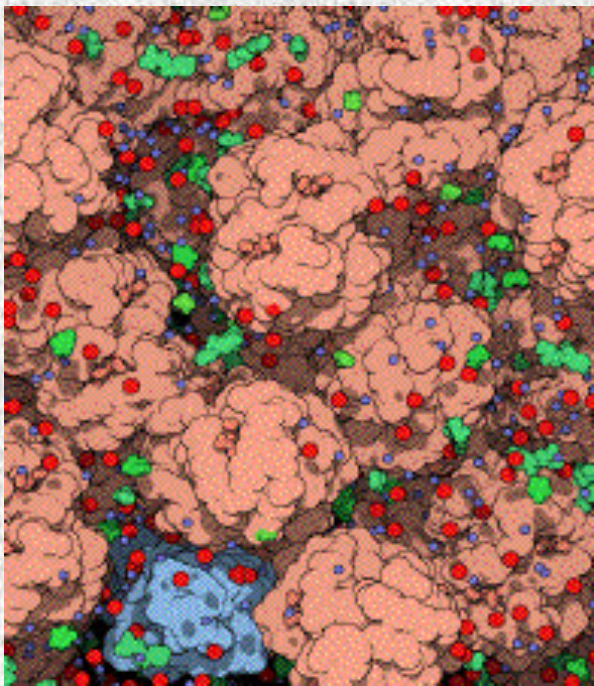
Verkman et al.

Crowding-induced order

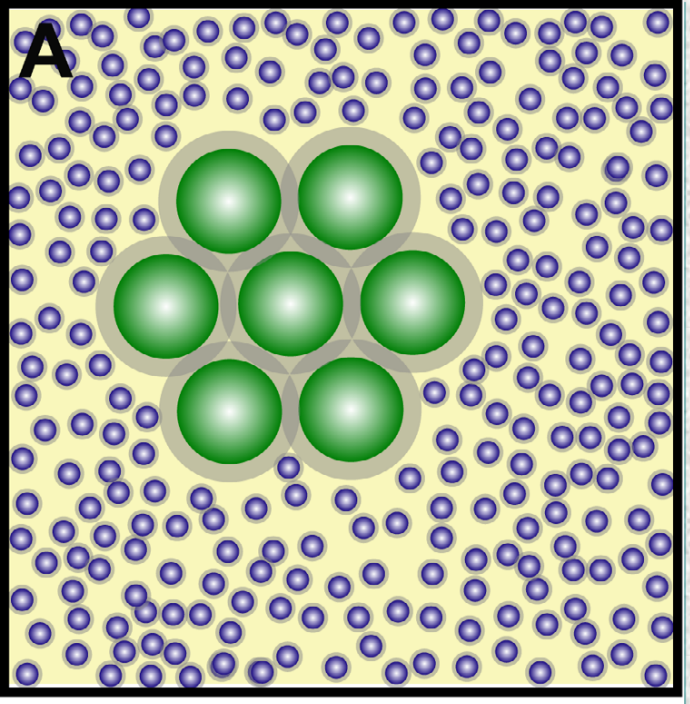
(1) ***orientational order*** – the spontaneous alignment of elongated particles along a common axis;

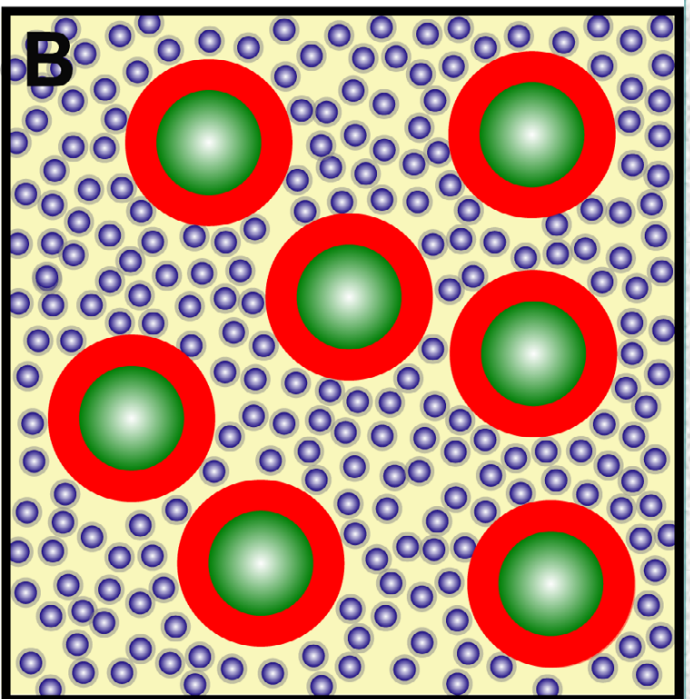
(2) ***positional order*** – the spontaneous arrangement of particles in layers, columns or lattices;

(3) ***demixing*** – the spontaneous segregation of different types of particles in different spatial domains.



An analogy between crowding and hydrophobic effects might be exploited in the form of an „agoraphobic effect" that drives the association of solute macromolecules through a desire to minimize disruption of favorable interactions between crowding molecules.

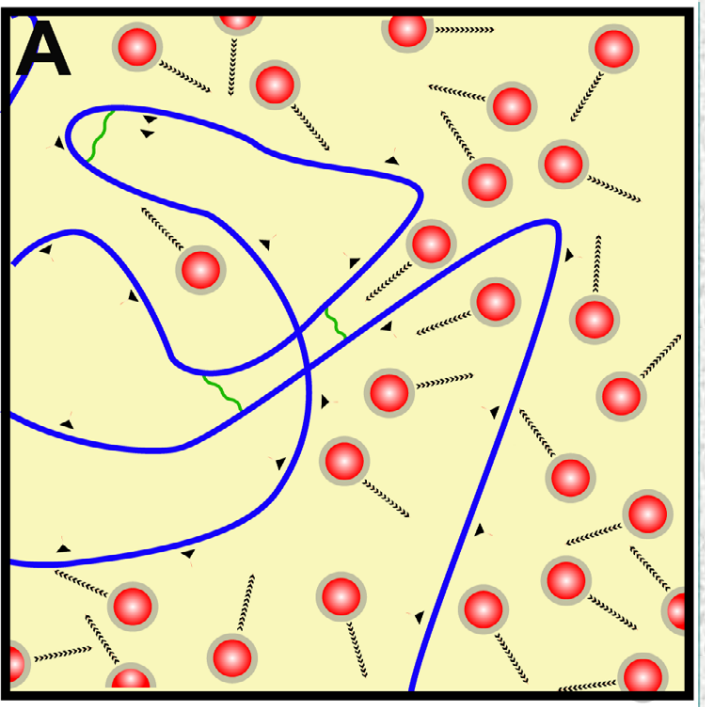


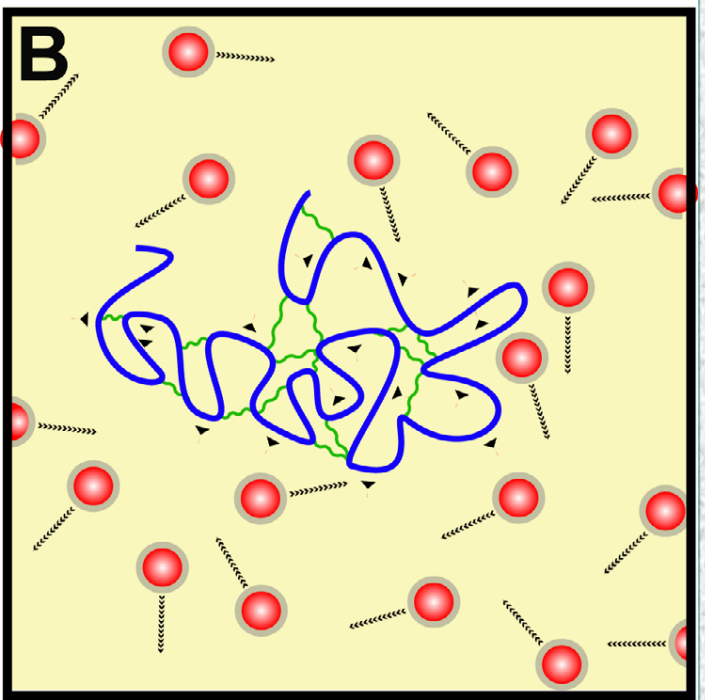


Colloidal particles aggregation

When the particles are not electrically charged (green circles with grey halo), excluding volume effects are the dominant force determining structure.

When particles become charged (phosphorylated, red halo around green circles), repulsive forces are stronger than excluding volume forces and the granule clusters become destabilized.





Viscoelastic phase separation

occurs in mixtures of components with very different dynamic regimes.

The mixture just after mixing, with large, slow polymers (blue lines) and smaller, very dynamic molecules (red balls). Long polymers show slow movement and tend to aggregate (wavy green line) and small molecules are very dynamic

.

Initially no phase separation can be observed.

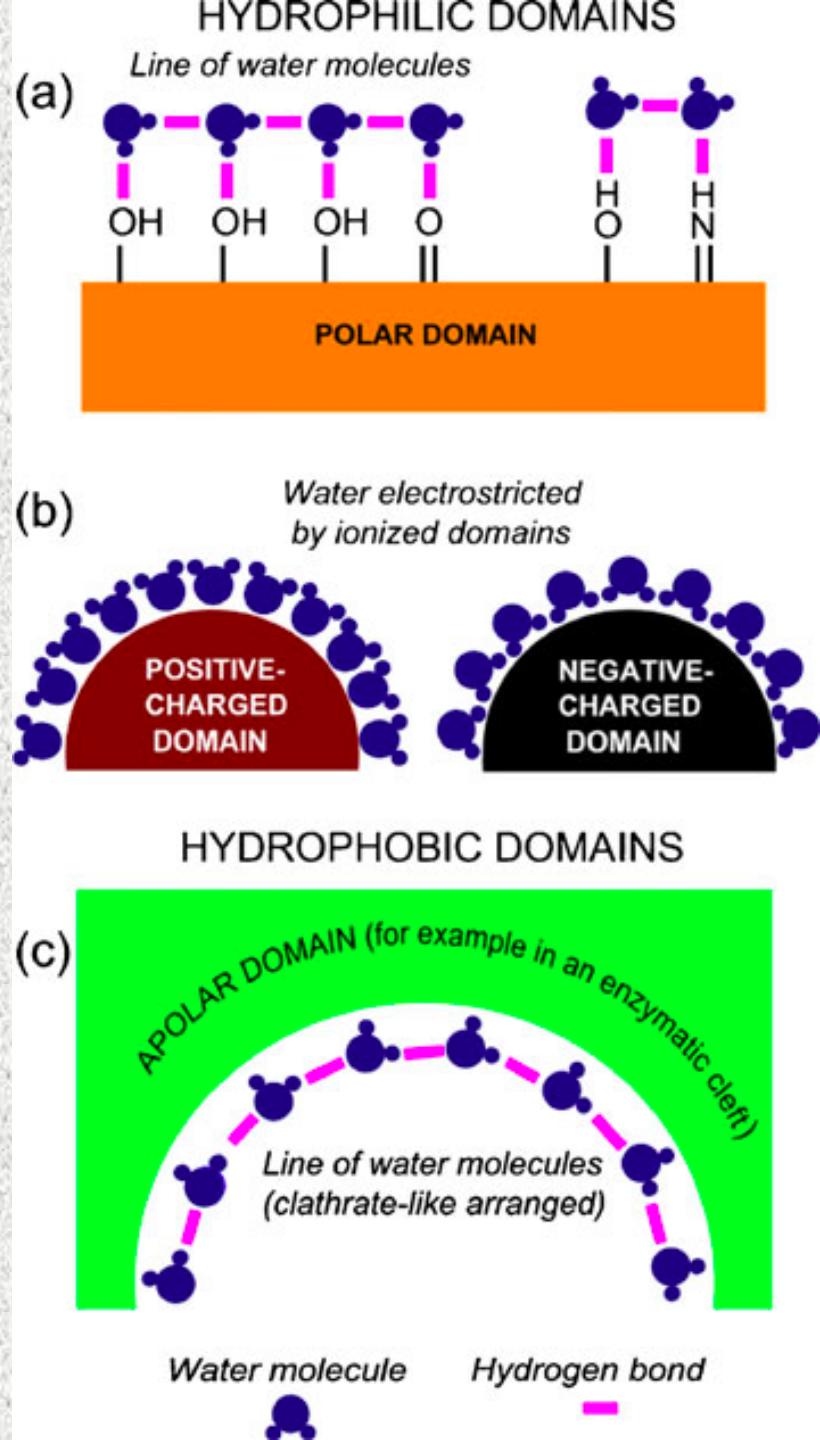
Later with more self-aggregation in the polymer, the polymer collapse in a separated phase.

The ordered water

Types of organization of water at the contact of hydrophilic domains (a and b) and hydrophobic domains (c).

H-bonded water molecules are good conductors of protons.

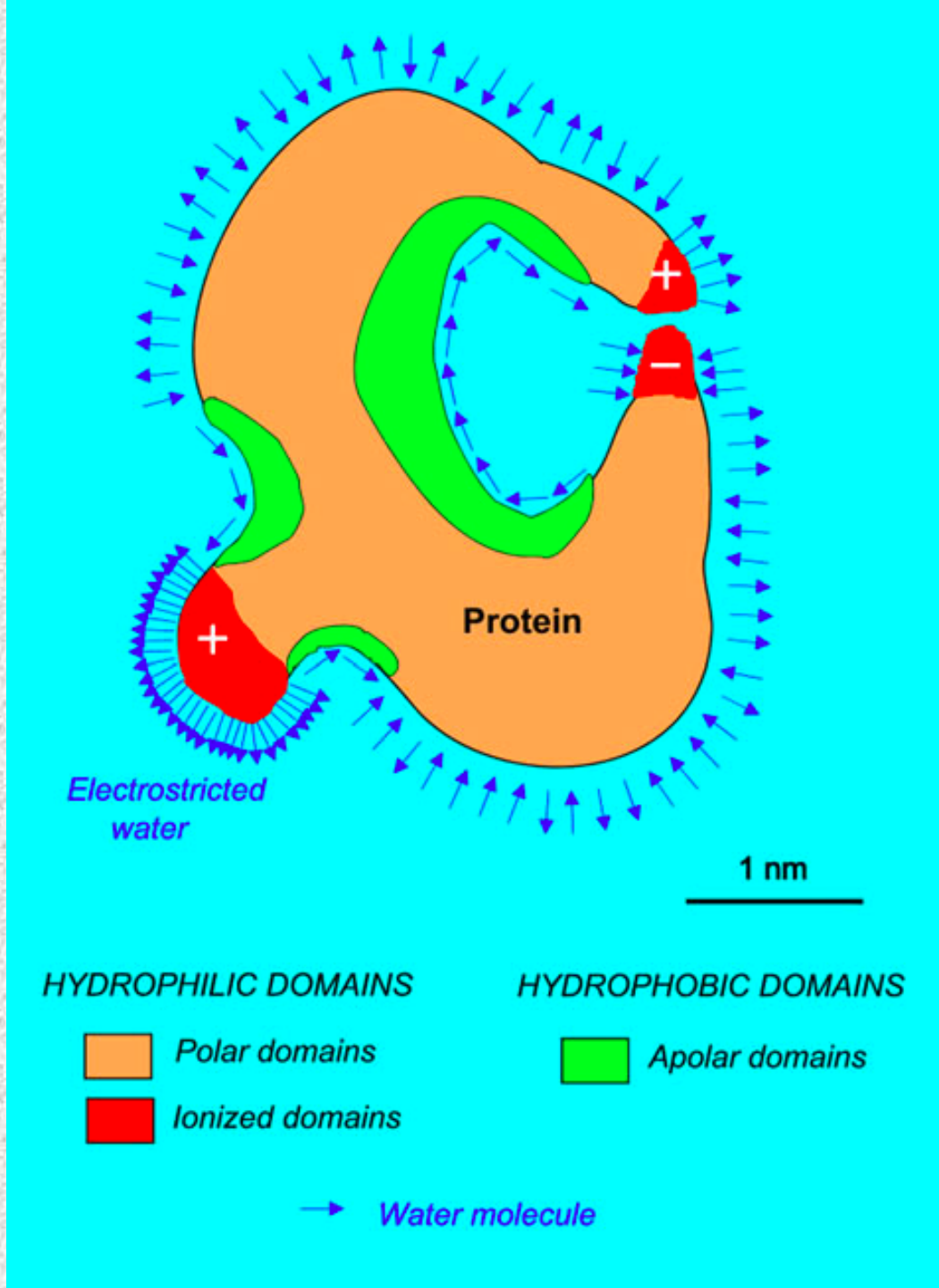
They can form along sequences of polar amino acids in polypeptide chains (a), or in clathrate-like structures (ibidem) covering apolar (hydrophobic) domains (c).



The first layer of interfacial water at the surface of a protein.

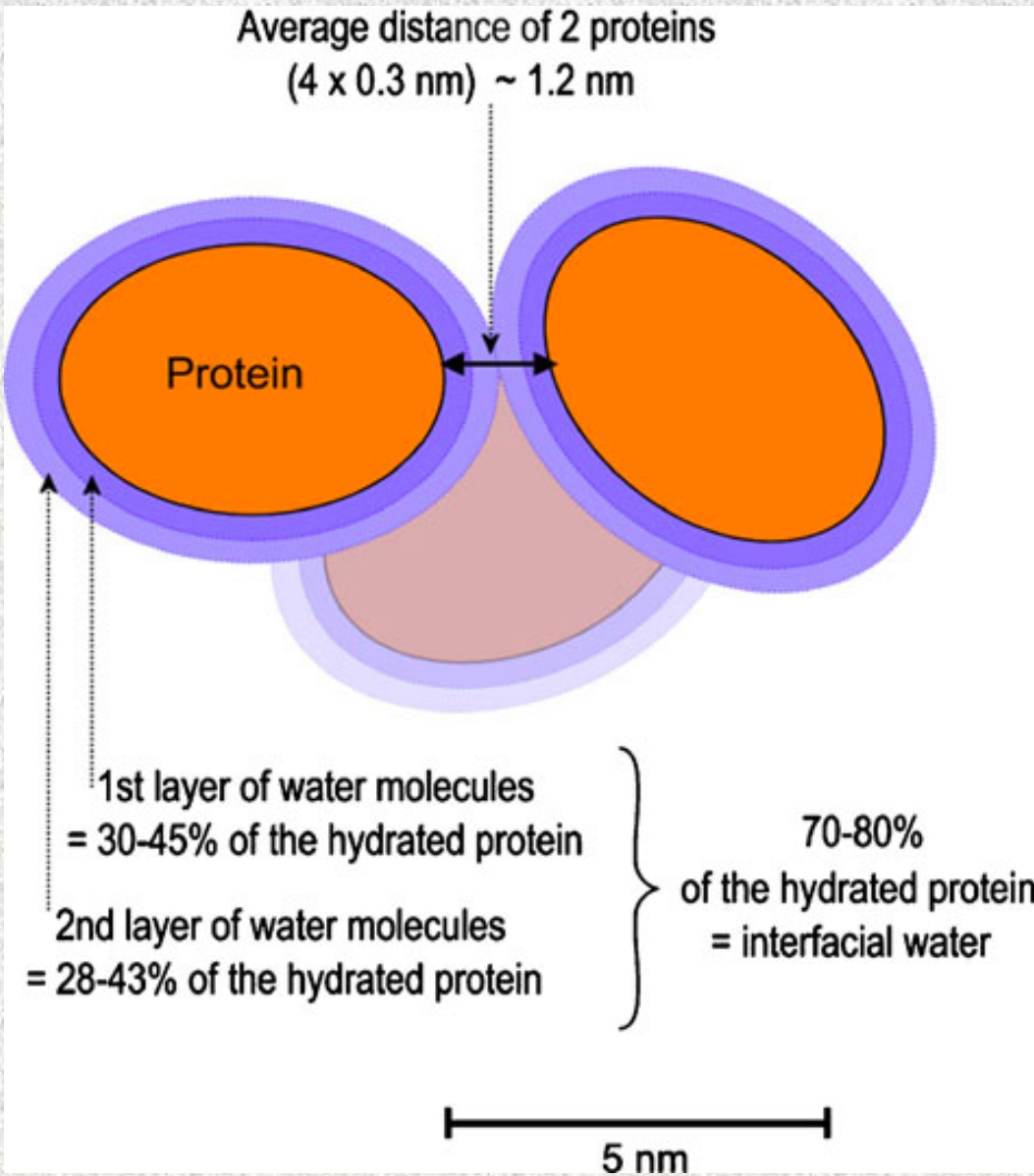
Its heterogeneous structure reflects the heterogeneity of the macromolecule surface.

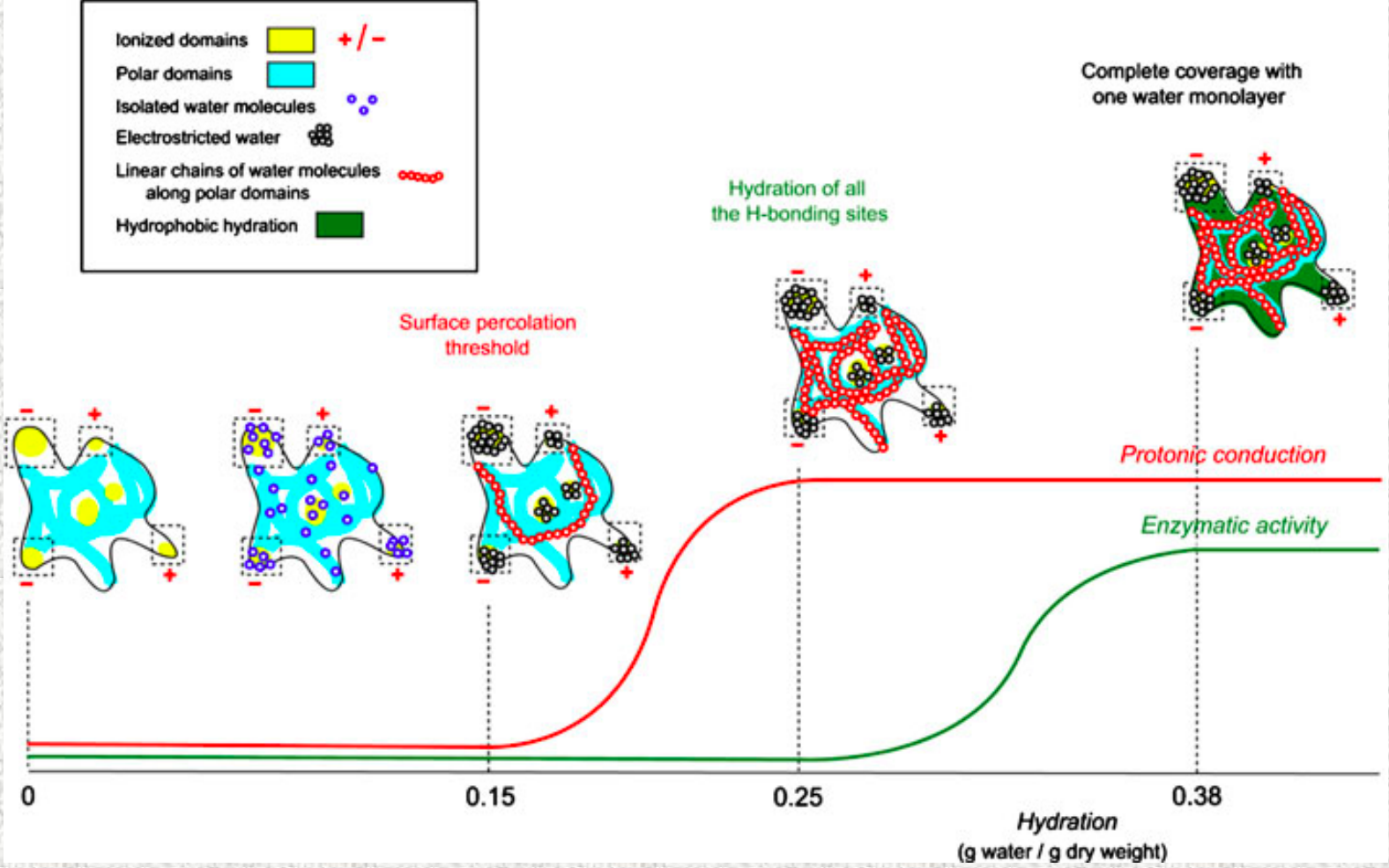
Changes in protein configuration must involve changes in volume, therefore mechanical effects.



The whole cell water (70–80% of the total mass), statistically, is distributed into only two to three hydration layers around macromolecules.

The size of a water molecule being 0.3 nm, the film of interfacial water between cell macromolecules is not very different from 1.2 nm.





Hydration enzymatic activity.

Water coverage exhibits a percolative transition at 0.15 water/g dry weight, detectable by the sudden appearance of proton conductivity, with emergence of protein mobility and catalytic activity at 0.25 g water/g dry weight.

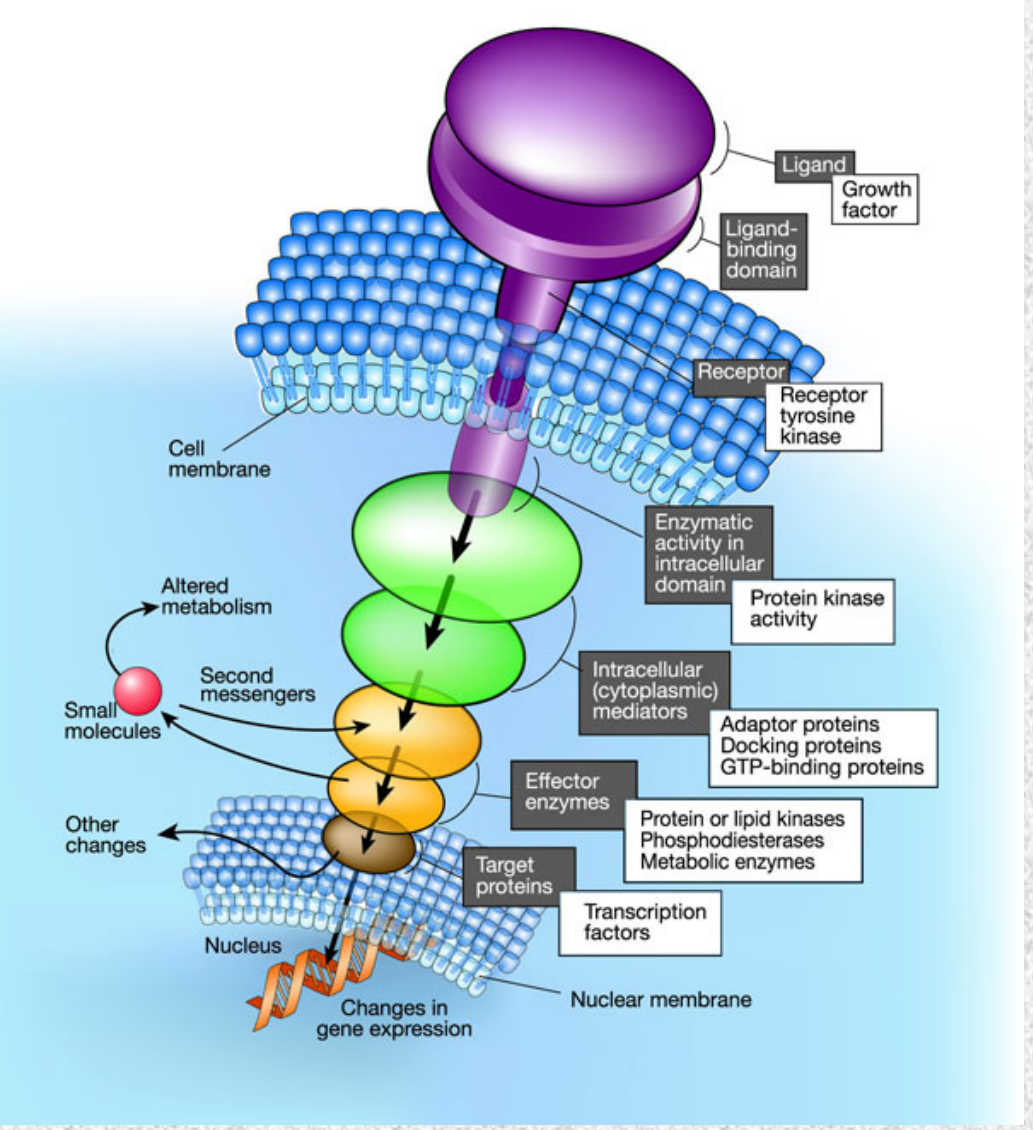
Biological pathways and networks

***Metabolic pathway*:** a series of enzymatic reactions that produce a specific product

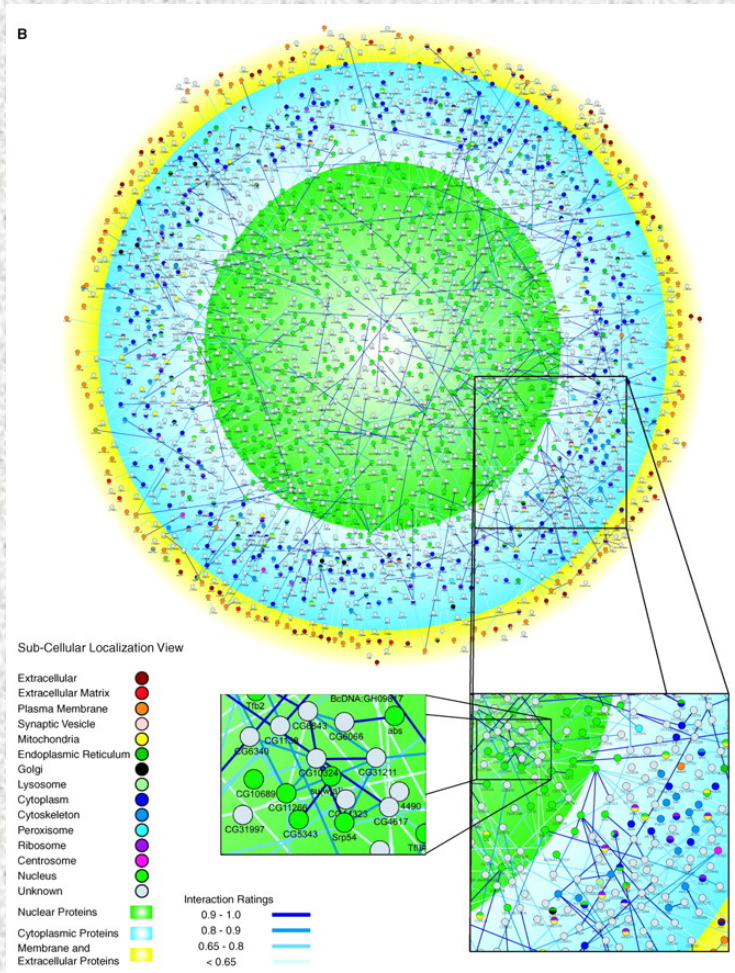
***Regulatory networks*:** pathways that regulate a cell's behaviors, including transcription, translation, degradation, motility, …..

***Signal transduction pathway and networks*:** cellular processes that recognize extra- or intra-cellular signals and induce appropriate cellular responses

Cellular information processing and passing are carried out by networks of interacting molecules.



from Downward, Nature, August (2001)



From:
A Protein Interaction Map of Drosophila
Giot et al. Science **302**, *1727-1136 (2003)*

The phenomena ordeing the cytoplasmic macromolecules to perform their biochemical and physiological functions.

(i) Negatively charged macromolecules and their complexes mutually repel through the 'screened electrostatic forces' of classical physical chemistry to retain their individuality.

(ii) The high crowding of cytoplasmic macromolecules shapes the remaining cytosol into a system of electrolyte pools and pathways.

(iii) The charges on the surfaces of these pathways act as switches,which control the cytoplasmic transport of ions.

(iv) The pools have unequal 'bulk' concentrations of ionic metabolites, the gradient of which drives their electrochemical transport through the pathways.

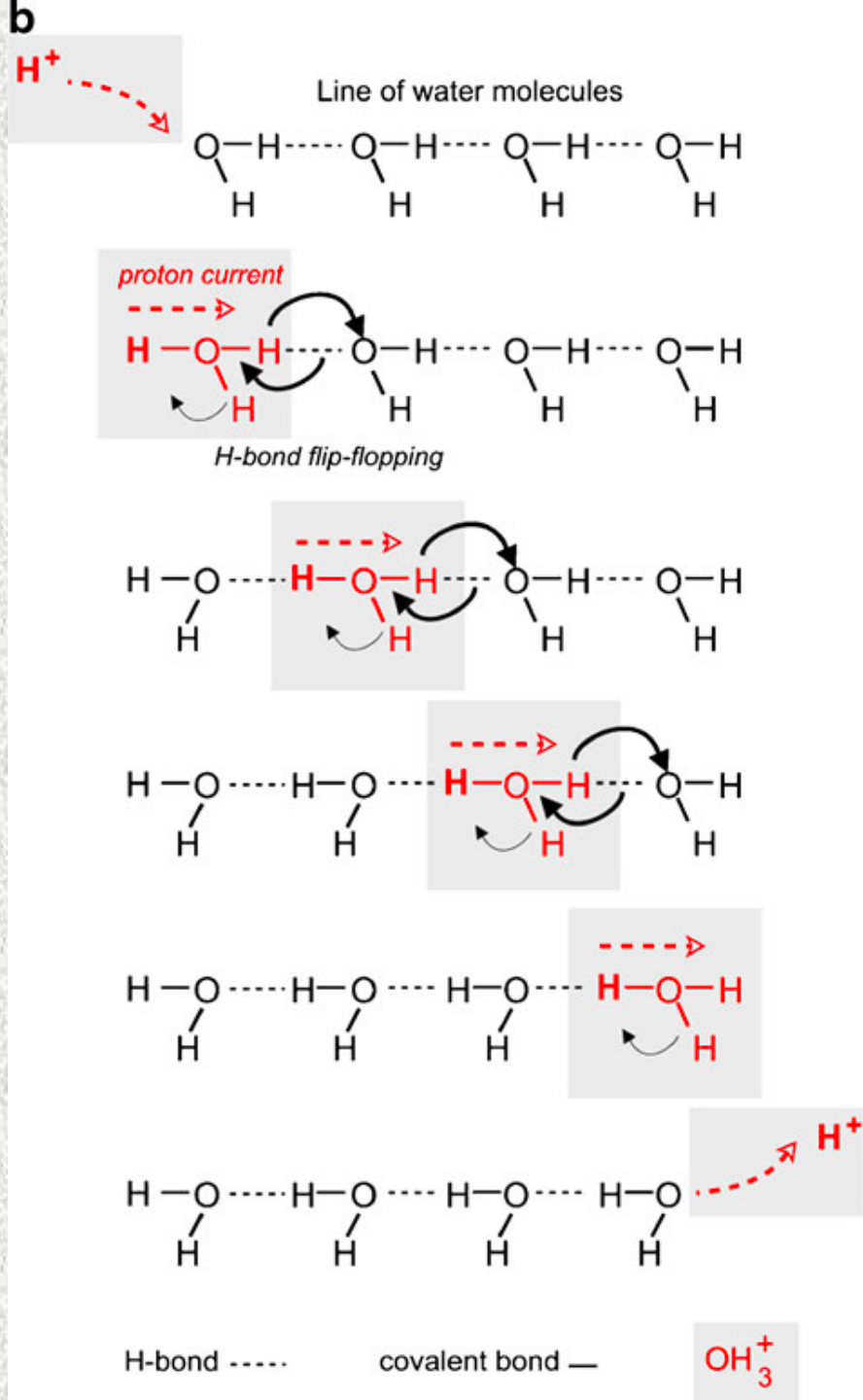
The Grotthus representation.

In the cell, protons are in countable quantity:

- just few tens in bacteria or in a mitochondrion

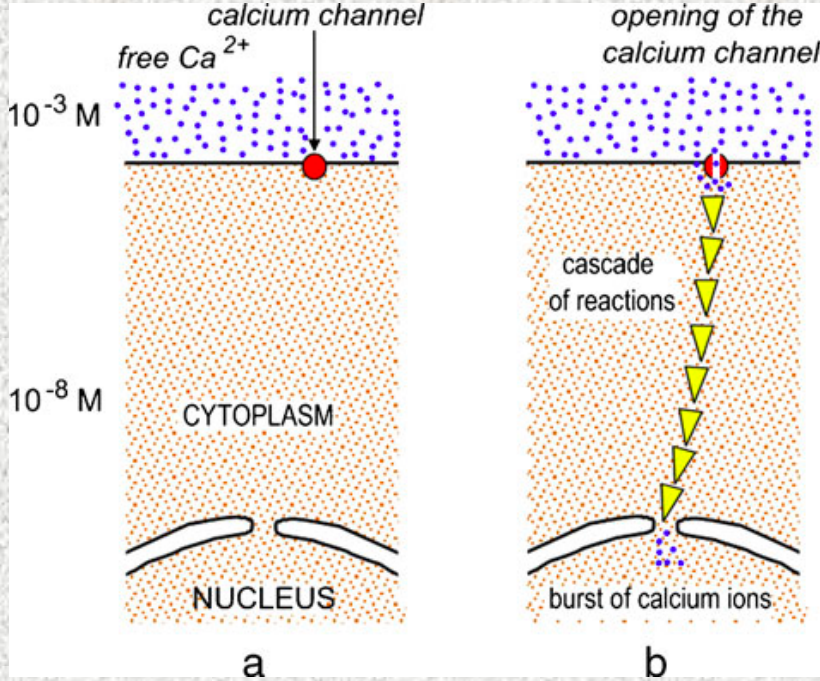
- at pH 7.0, the mean distance between two protons is 250 nm,

- a concentration gradient is not a force field. It is only a probability gradient.



A given ion (phosphate, Ca^{2+}, H^{+}) seems to be transported along a chain (cascade) of macromolecules containing this ion in a sequestered form.

This type of transport differs deeply from diffusion. It is not a transport of matter but a transfer of a level of energy.



The calcium signal would be transduced, without a real transport of calcium, along a cascade of phosphatase-kinase molecules, transmitting step by step an allosteric change triggered by the liberation of phosphate.

Signal transduction along a cascade of phosphatase-kinase molecules.

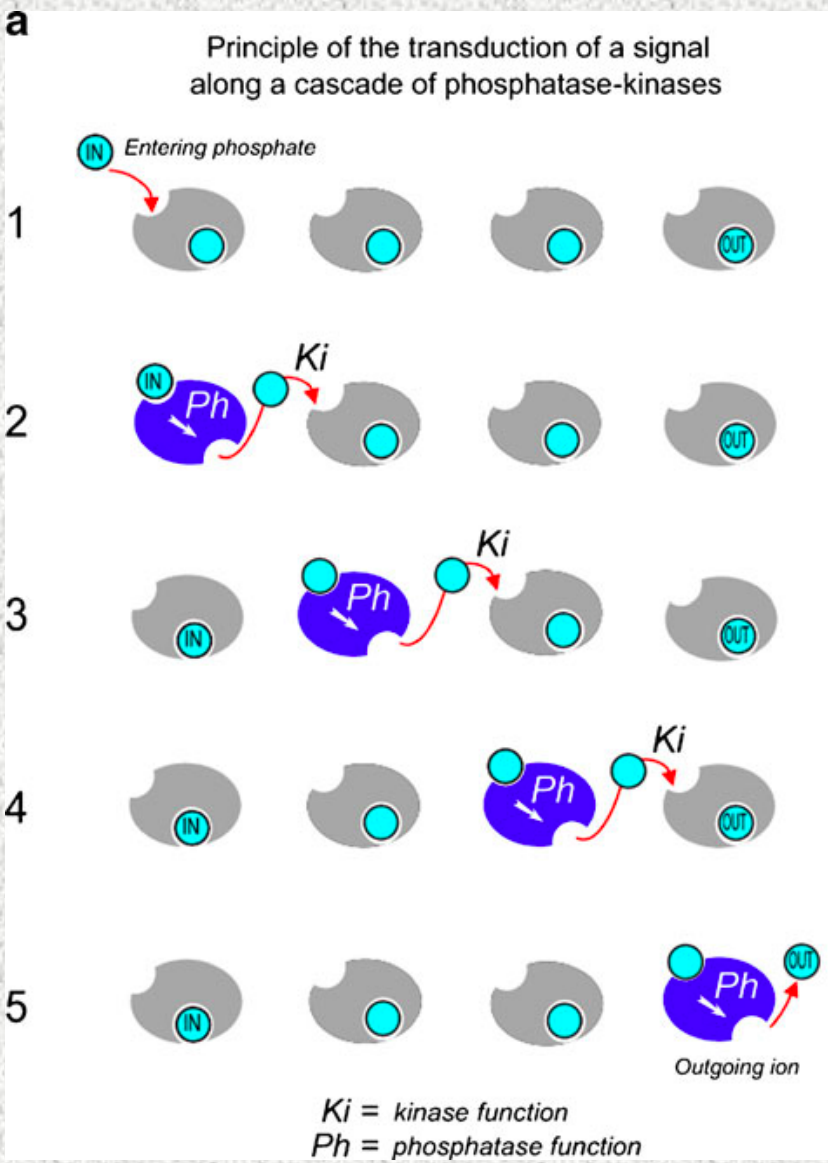
Each element of the chain has two functions: kinase (Ki), inhibited by phosphate ion, and phosphatase (Ph), activated by phosphate ion,.

(1) A phosphate ion "IN," liberated from one element of the chain, binds (by Ki action of this element) to the following one.

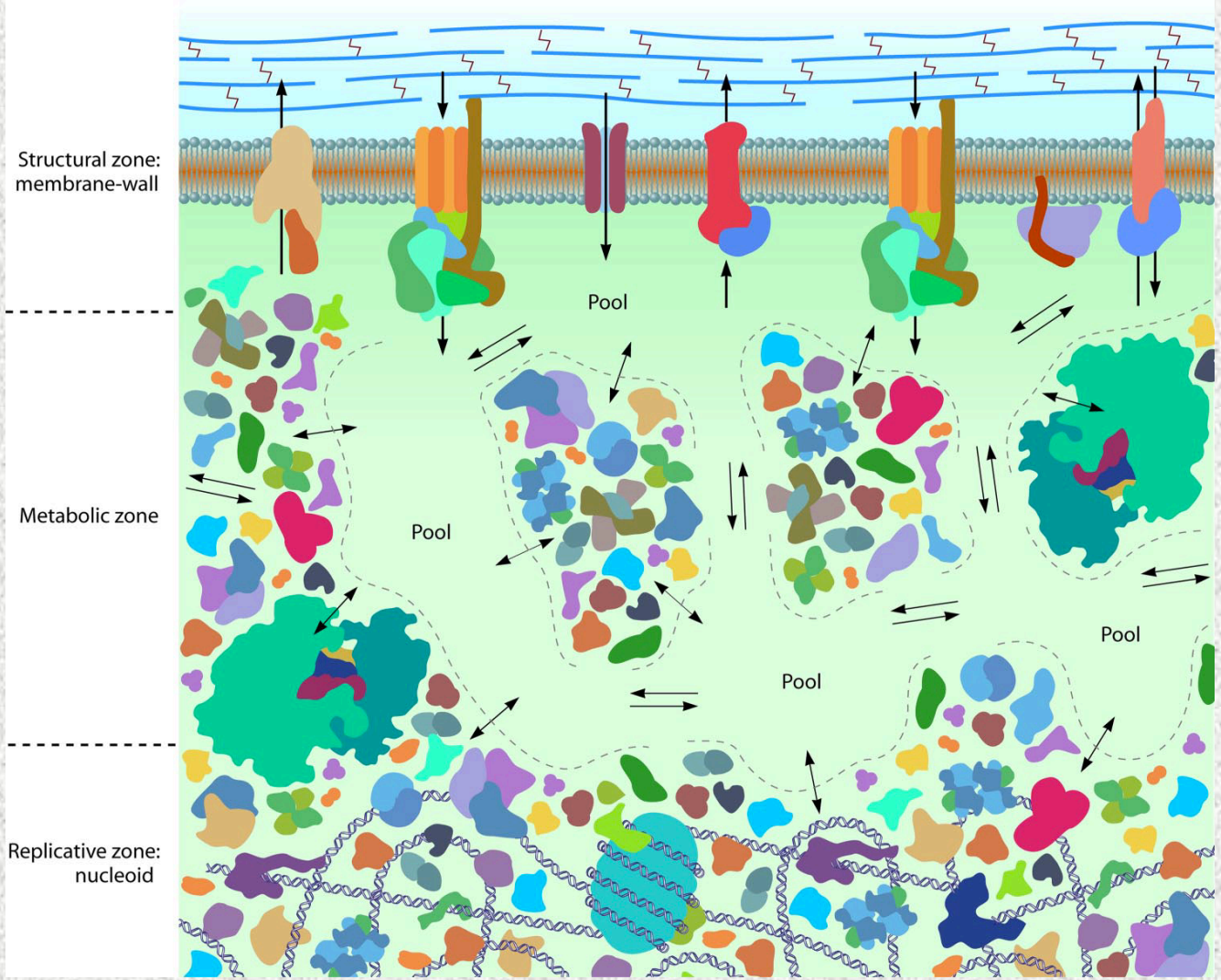
(2) This one undergoes an allostery transformation (in blue) which activates its Ph function. It exercises on itself its own phosphatase activity to liberate the phosphate ion which inhibits its Ki function (2).

This process is iterative (3), (4), (5).

At the end of the process, the entering ion has not left the first element of the cascade. The outgoing ion is the provided by the last element of the cascade.



The functionality of crowding



The model of complex vectorial biochemistry in a cell.

Spatial and temporal hierarchies.

The cytoplasmic phenomena can be divided into two spatial and temporal hierarchies:

- the large macromolecules moving slowly,
- the pseudo-equilibrated low molecular metabolites.

Spatial and temporal interactions of macromolecules will enable to construct an electrochemical wiring diagram of the cell.

Such a diagram will depend on the chemical composition of the extracellular environment and on other physicochemical variables that are external to the cell.