The mass equation law for binding of a protein P to its DNA

$$D_{\text{free}} + P_{\text{free}} \rightleftarrows DP \qquad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP}$$

binding of the first proteins with the dissociation constant K_1

D_{free}, concentration free DNA; P_{free}, concentration free protein

$$\text{binding constant } K_{\text{B}} = \frac{1}{\text{dissociation constant } K_{\text{D}}}$$

How fast is binding or dissociation

$$\mathrm{AB} \underset{k_{\mathrm{on}}}{\overset{k_{\mathrm{off}}}{\rightleftharpoons}} \mathrm{A+B}$$

k_{off} in s^{-1} is the reaction rate constant for dissociation

k_{on} in $M^{-1}\ s^{-1}$ is the reaction rate constant for binding

$$\frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} = K_{\mathrm{d}}$$

relation to the equilibrium dissociation constant

$$\frac{1}{k_{\mathrm{off}}} = \tau$$

life time of the complex

$$\frac{d[\mathrm{AB}]}{dt} = \mathrm{k_{on}} \cdot [\mathrm{A}] \cdot [\mathrm{B}] - \mathrm{k_{off}} \cdot [\mathrm{AB}]$$

rate equation for complex formation,

can be solved but it is already difficult

k_{on} cannot be higher than 10^8 - 10^9 $M^{-1}\ s^{-1}$ for a diffusion controlled reaction

What is the meaning of the dissociation constant for binding of a single ligand to its site?

1. K_d is a concentration and has units of mol per liter

2. K_d gives the concentration of ligand that saturates 50% of the sites (when the total sit concentration is much lower than K_D)

3. Almost all binding sites are saturated if the ligand concentration is 10 x K_d

4. The dissociation constant K_d is related to Gibbs free energy ΔG by the relation $\Delta G = - R\,T \ln(K_d)$

Our energy and time coordinate system

K (M)	concentration scale	ΔG (kcal/mol)		k_{off} (s-1)	complex life time	Binding interaction
			for k			
10	1 mM	-4.1		10	10 ms	ion-DNA ion-protein
10	0.1 mM	-5.5		10	0.1 sec	
10	10 µM	-6.8		1	1 sec	enzyme-ligand (weak)
10	1 uM	-8.2		10	10 sec	protein-DNA, unspecific
10	0.1 µM	-9.5		10	100 sec	enzyme-ligand (strong)
10	10 nM	-10.9		10	16.7 min	
10	1 nM	-12.3		10	2.8 hours	protein-DNA specific
10	0.1 nM	-13.6		10	28 hours	
10	10 pM	-15		10	11.6 days	antibody-antigen
10	1 pM	-16.4		10	116 days	

Biophysical Concepts and Theoretical Descriptions

Oct 16: Intro protein-DNA/RNA interactions

Oct 23: The energy, length and time coordinate system

Oct 30: Diffusion (Fabian Erdel)

Nov 6: Kinetics

Nov 13: Essentials of protein-DNA/RNA interactions

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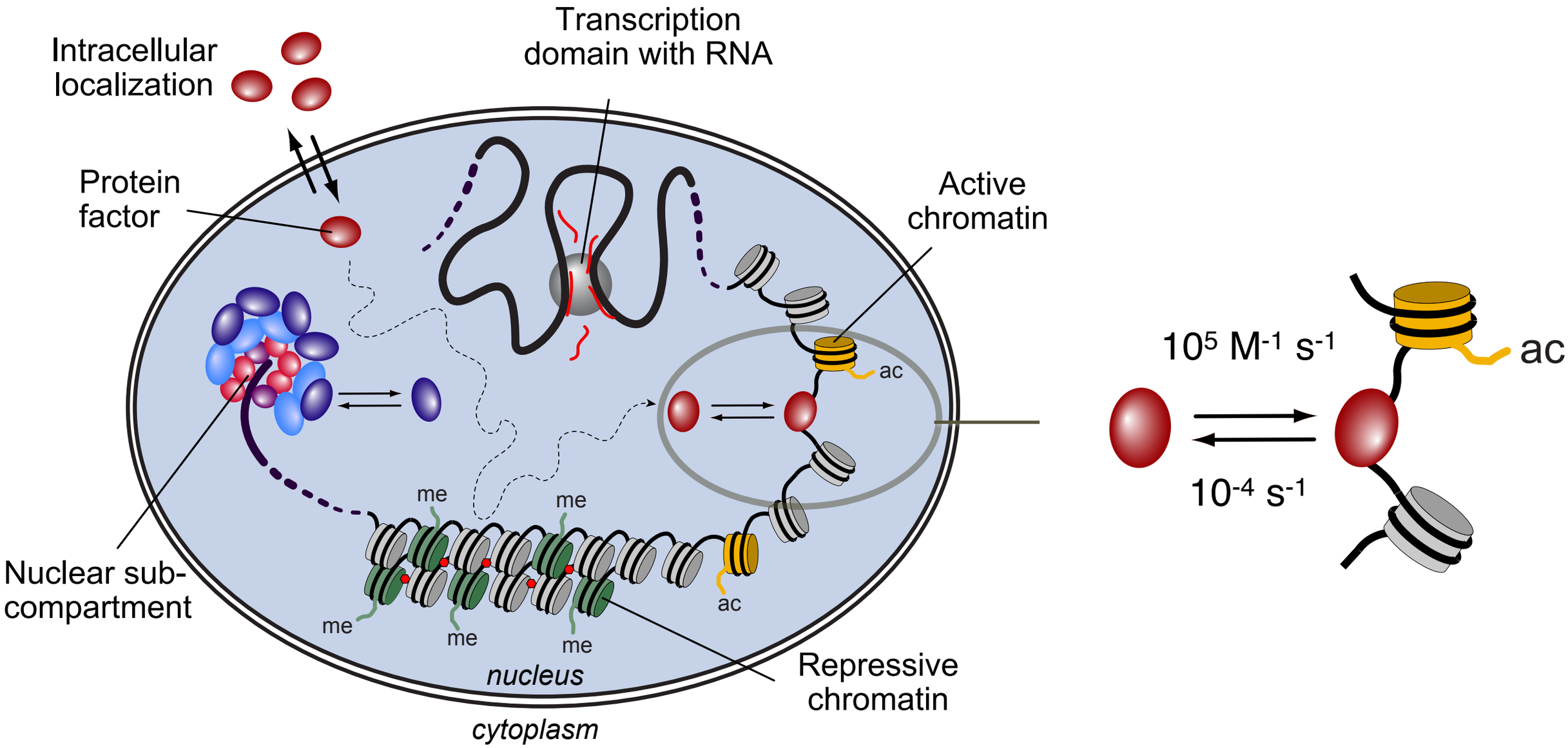
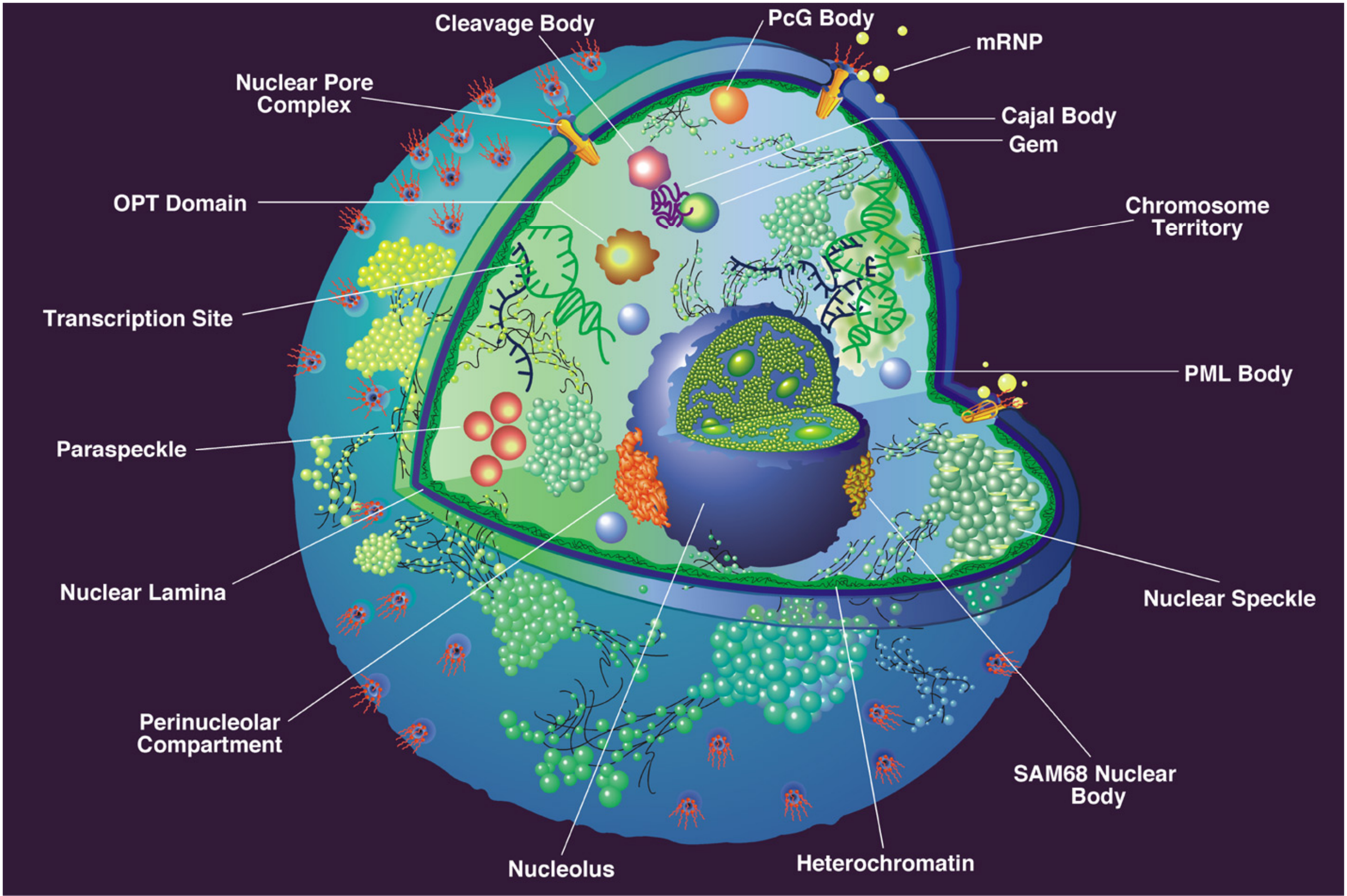
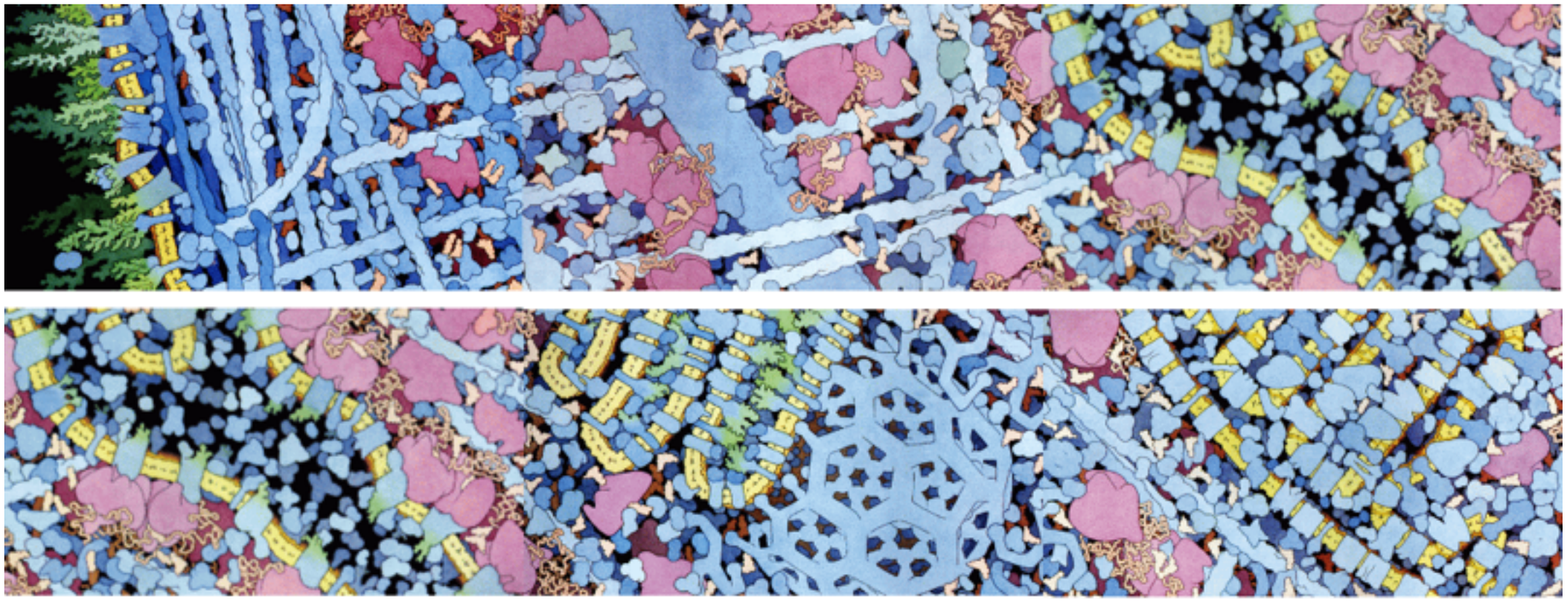
How to link in vitro measurements to interactions in the cell?



The mammalian cell nucleus



The cell is a very crowded place (David Goodsell)



from left to right:

cell surface

cytoplasm

synthesis of proteins from the endoplasmic reticulum

Golgi apparatus,

coated vesicle

mitochondrion

nucleus

proteins: blue

DNA and RNA: red and orange

lipids: yellow

carbohydrates:

green

Ribosomes: magenta

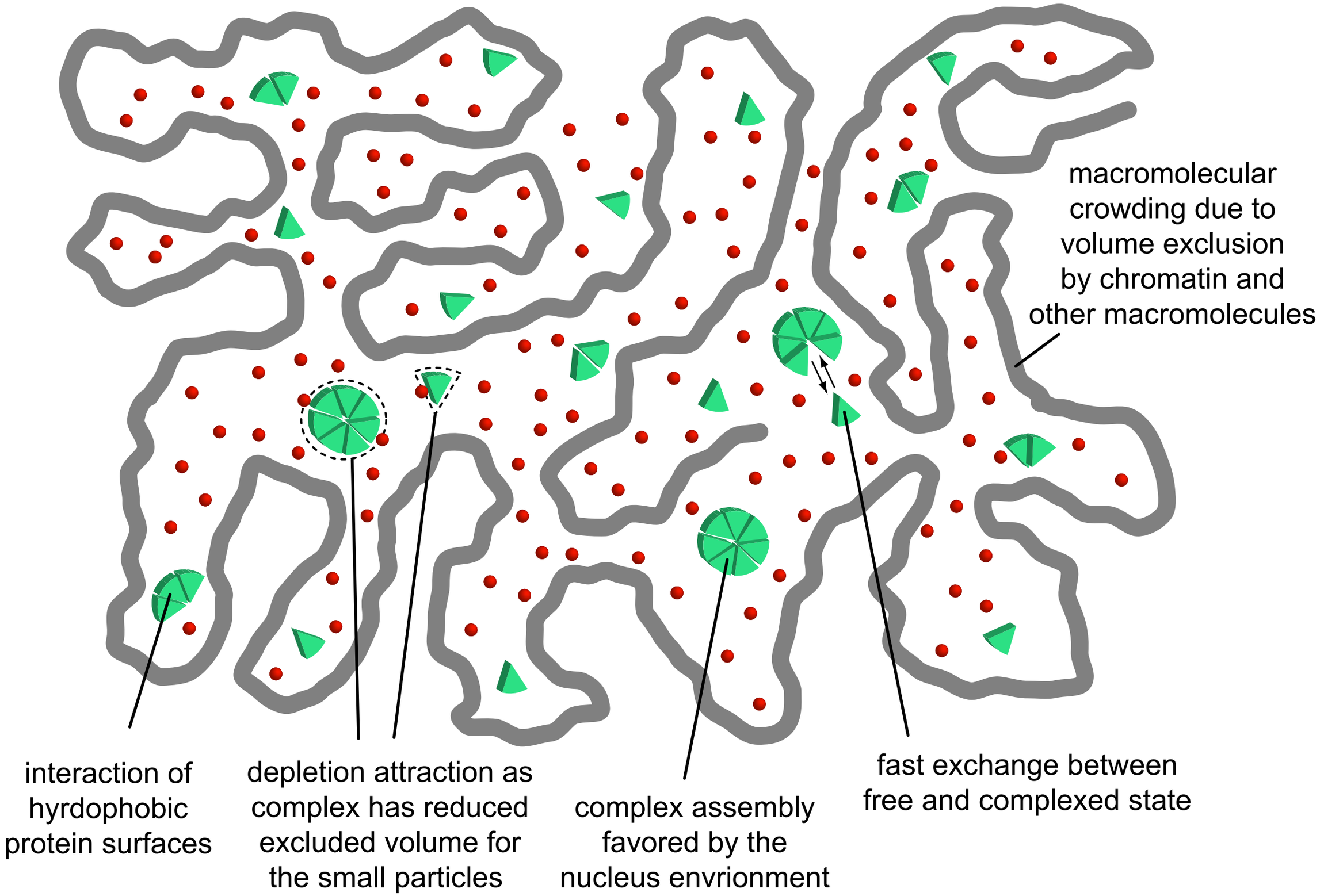
Concentration of proteins and DNA in the nucleus

DNA	**RNA**	**Protein**
~ **15mg/ml** (6pg DNA per cell, [19] nucleus ~1/10 of cell volume $4x10^{-9}$ cm^3 typical) [20] **~18.5mg/ml** (56mM nucleosome concentration, [21] 200 bp/nucleosome, 2bases/bp,1Mbase/330g. [22] **~19 mg/ml** [23] ~**20-31 mg/ml** (8.1-12.5pg/cell, [24] nucleus ~1/10 of cell volume $4x10^{-9}$ cm^3 typical) [20]	**~11 mg/ml** (5-25pg RNA per cell, [25] 18% in nucleus, [26] nucleus ~1/10 of cell volume $4x10^{-9}$ cm^3 typical). [20] **~12-15mg/ml** (27.1-33.1pg/cell, [24] 18% in nucleus, [26] nucleus ~1/10 of cell volume $4x10^{-9}$ cm^3 typical). [20]	**~106-215 mg/ml** in various regions of the nucleus. [27] **~108mg/ml** (6pg DNA per cell, [20] protein mass 72X DNA mass and cell volume $4x10^{-9}$ cm3 typical). [20] **~200-300mg/ml** in E.coli. [28]

Concentration of ions in the nucleus

- ~0.1 M K^+/Na^+ ($K^+ > Na^+$)
- 0.5-1 mM Mg^{2+}
- low µM values of Ca^{2+}
- 3.1 times higher apparent viscosity than water measured for the mobility of GFP (D = 25 $\mu m^2\ cm^{-1}$)
- inorganic cations are significantly more abundant than the corresponding mobile anions nucleic phosphate groups and negative protein charges are in excess of the positive protein charges
- high Cl^- concentration (in vitro!) can significantly disturb protein-protein or protein-DNA interactions

Macromolecule interactions in the nucleus are different from in vitro conditions



Measuring binding via protein mobility has a problem: many things affect the observed mobility