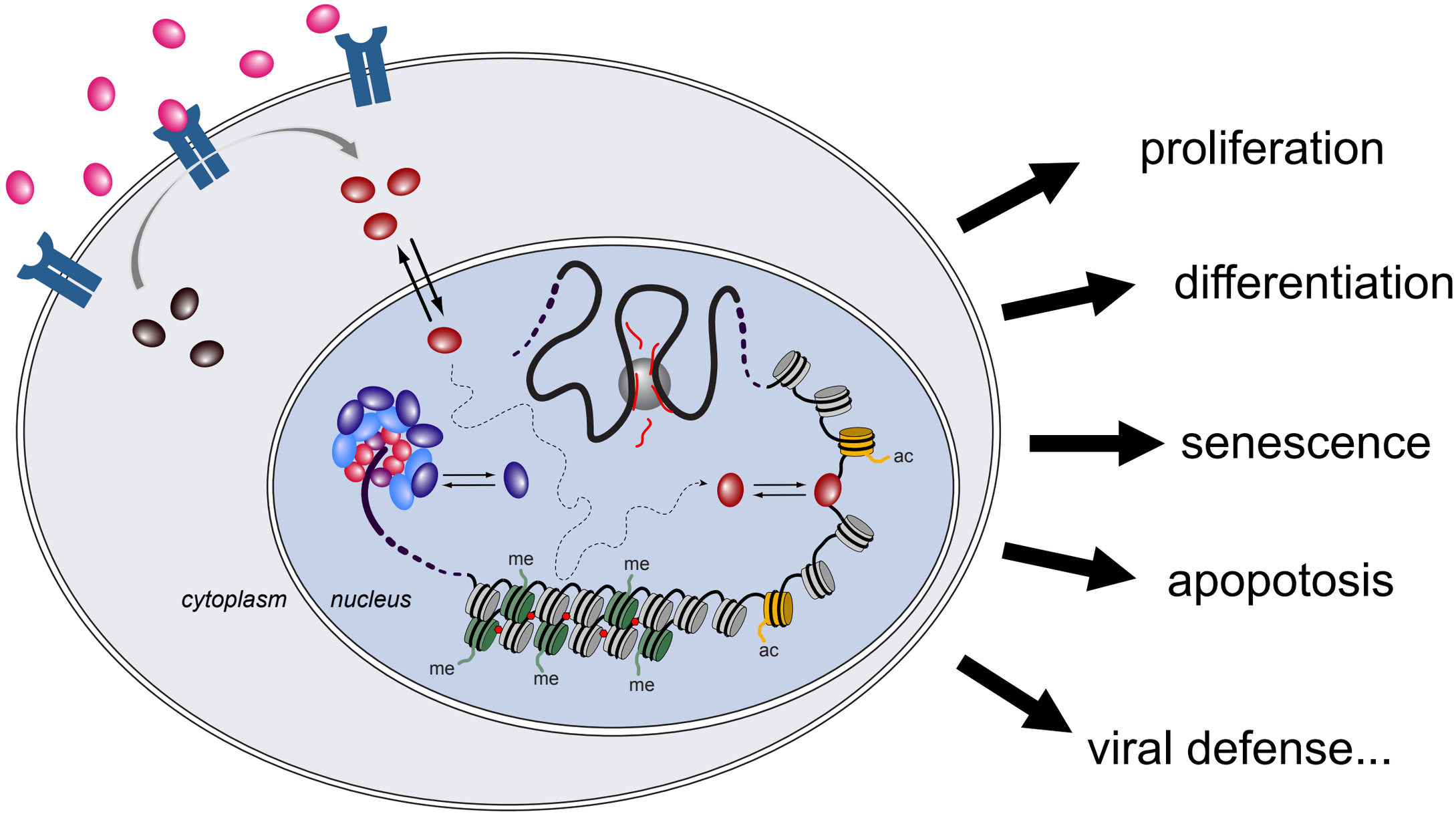
Protein mobility and genome interactions in the cell nucleus

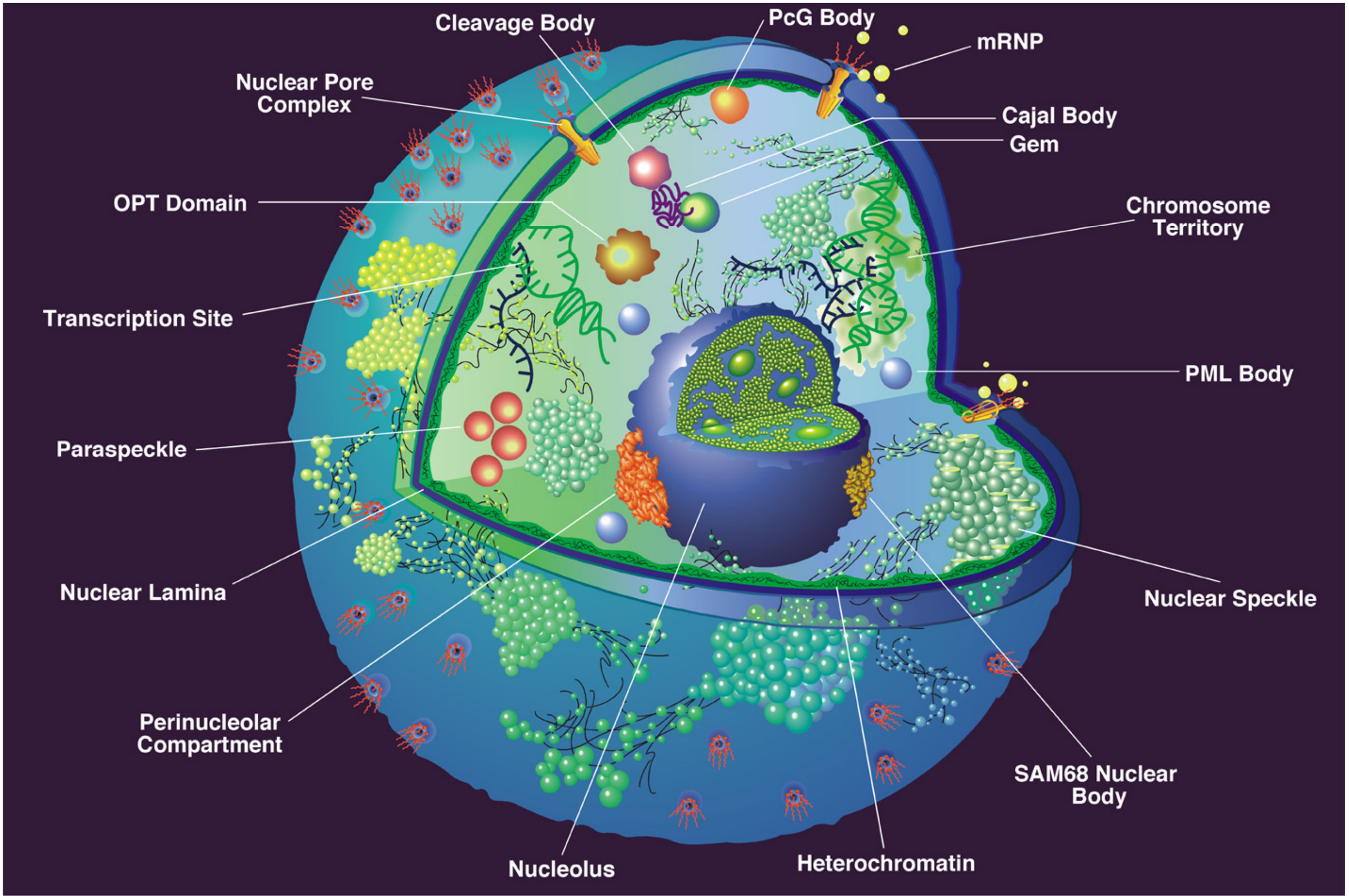
Important points for analyzing protein-DNA/RNA interactions in living cells

- Organization of the nucleus
- In vivo solution conditions
- Self-organization in the nucleus
- Chromatin dynamics and genome access

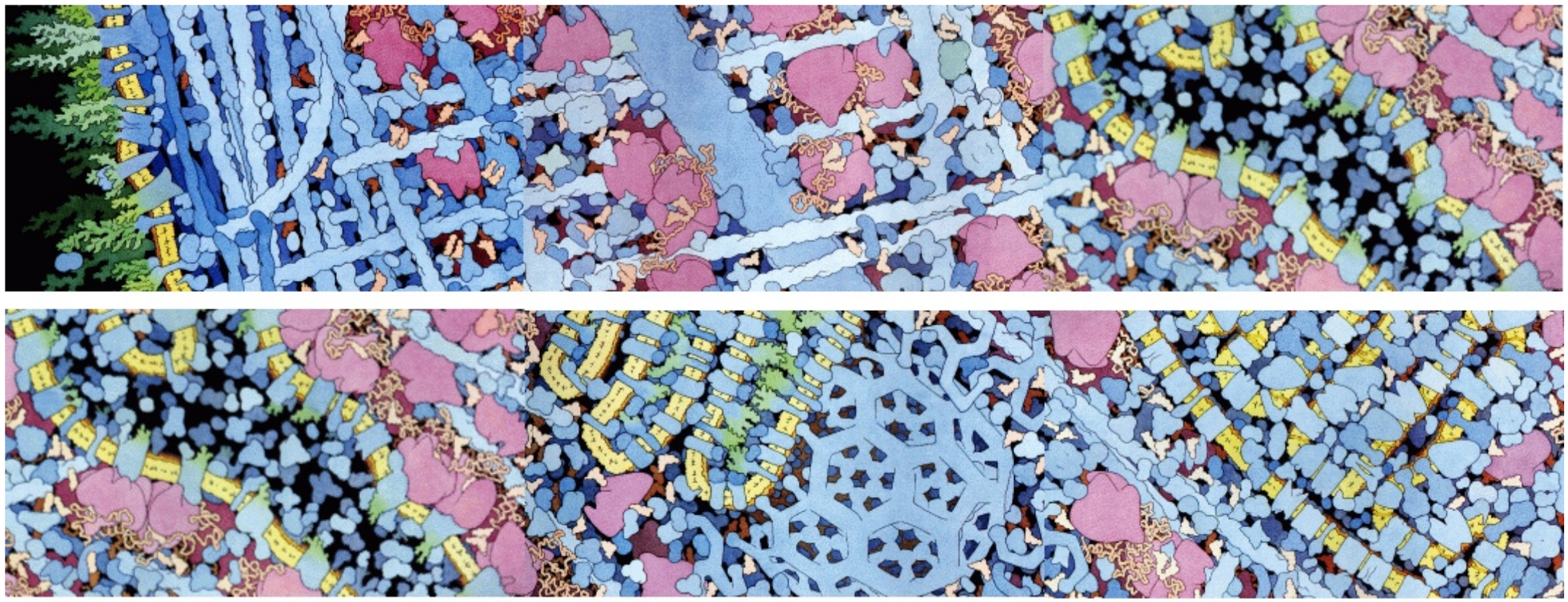
Decisions on the cell's fate made in the nucleus



The mammalian cell nucleus



The cell is a very crowded place (David Goodsell)



from left to right:

cell surface

cytoplasm

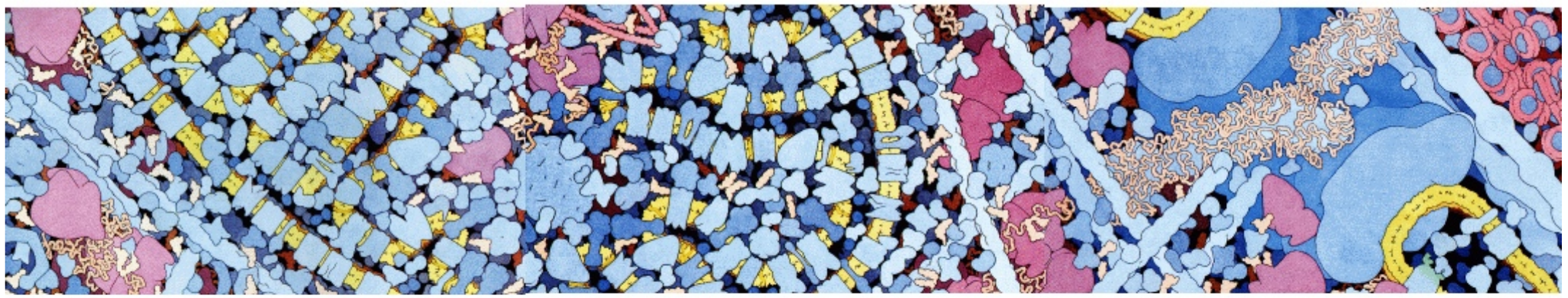
synthesis of proteins from the endoplasmic reticulum

Golgi apparatus,

coated vesicle

mitochondrion

nucleus



proteins: blue

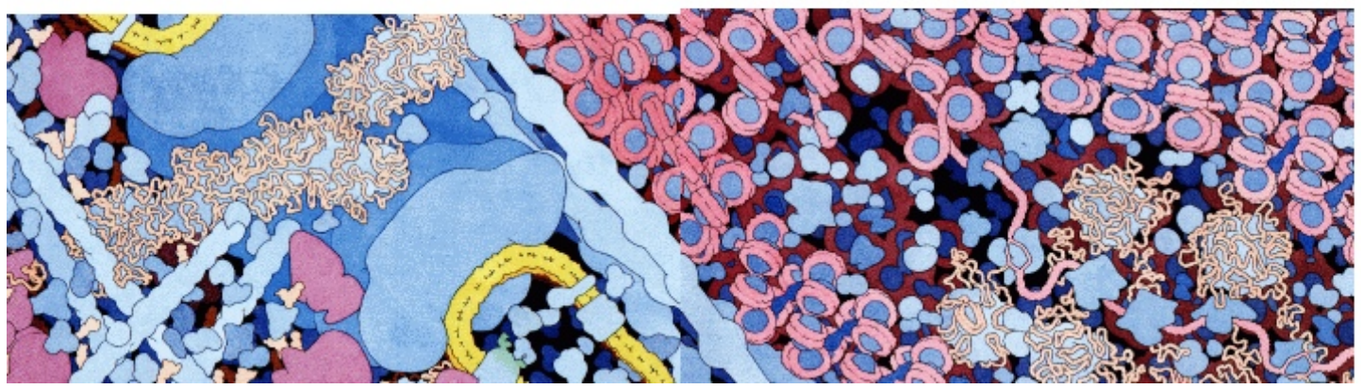
DNA and RNA: red and orange

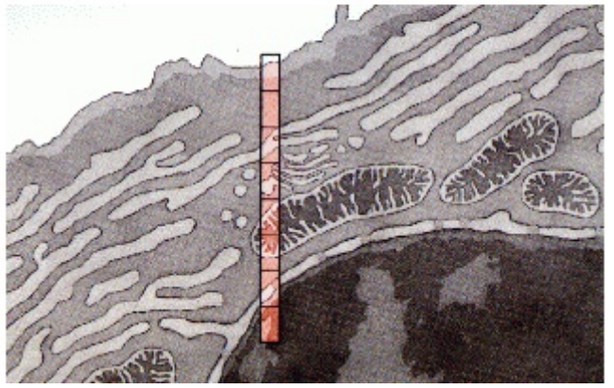
lipids: yellow

carbohydrates:

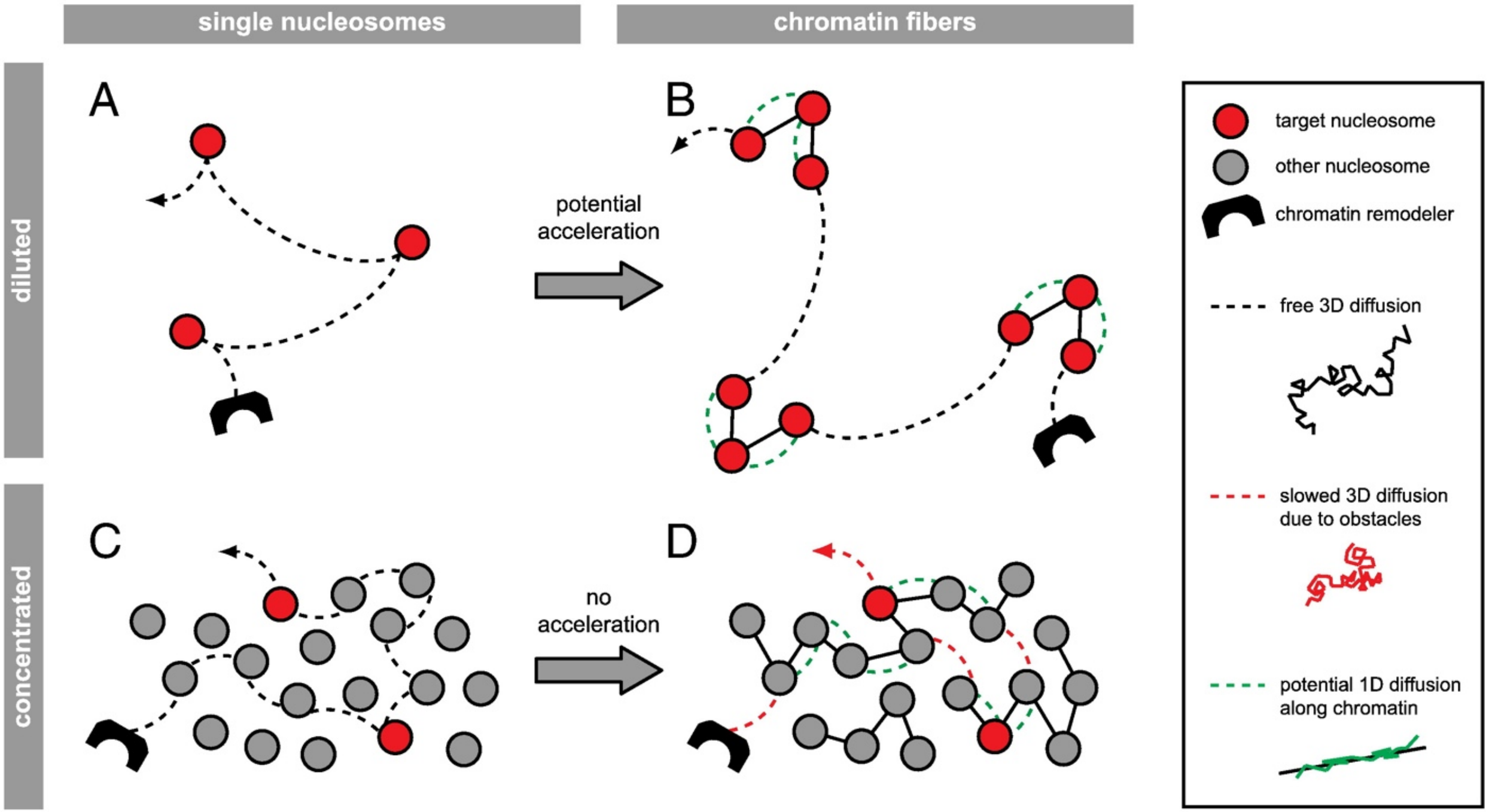
green

Ribosomes: magenta





How crowded is it?
target search, access, interactions..



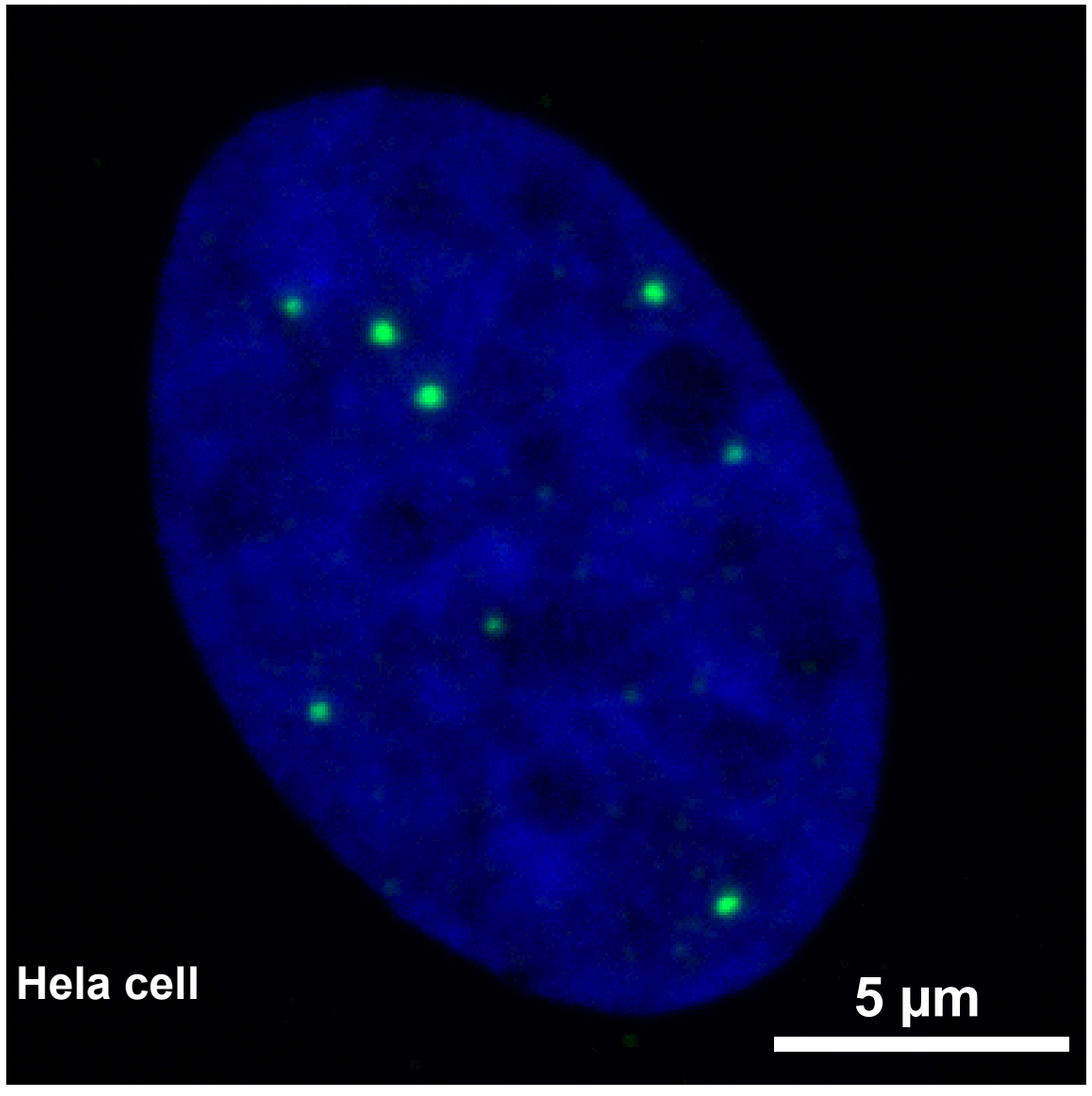
Concentration of proteins and DNA/RNA in the nucleus

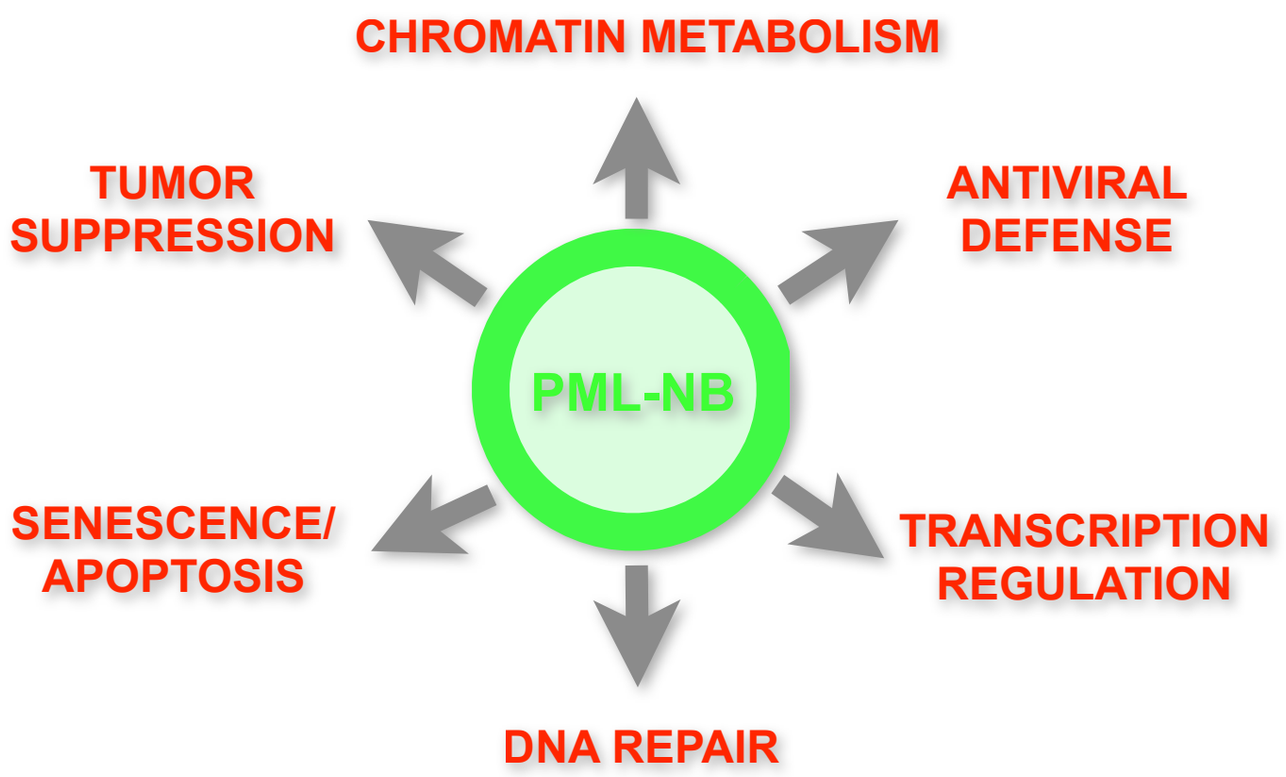
DNA	**RNA**	**Protein**
~ **15mg/ml** (6pg DNA per cell, [19] nucleus ~1/10 of cell volume $4x10^{-9}$ cm^3 typical) [20] **~18.5mg/ml** (56mM nucleosome concentration, [21] 200 bp/nucleosome, 2bases/bp,1Mbase/330g. [22] **~19 mg/ml** [23] ~**20-31 mg/ml** (8.1-12.5pg/cell, [24] nucleus ~1/10 of cell 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

The promyelocytic leukemia (PML) nuclear body

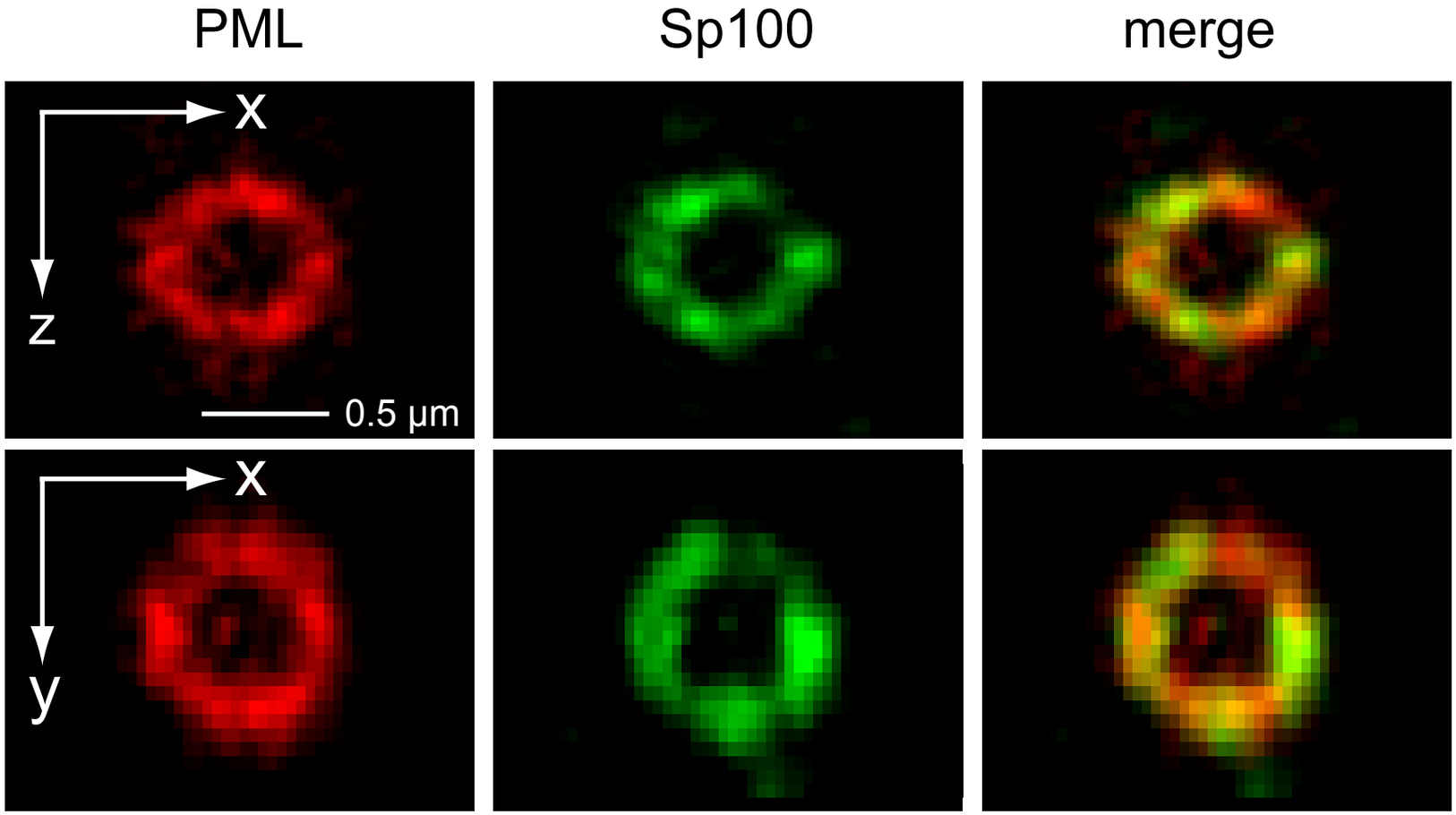


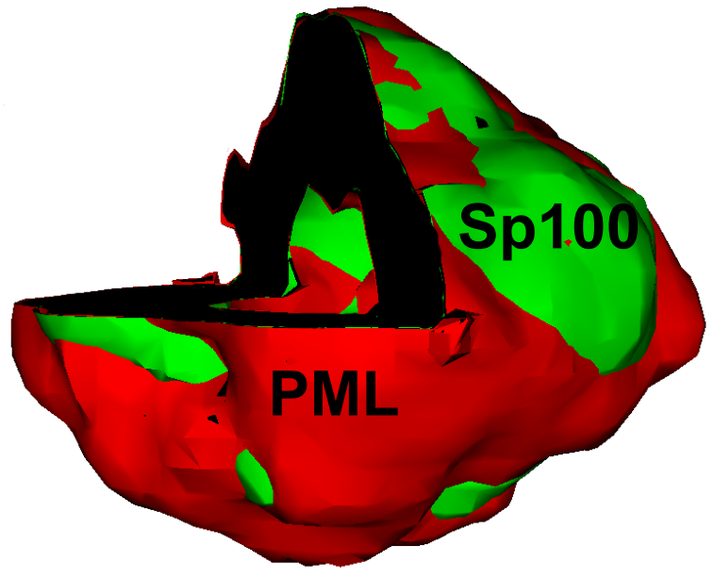


blue: DAPI; green: anti PML immunostaining

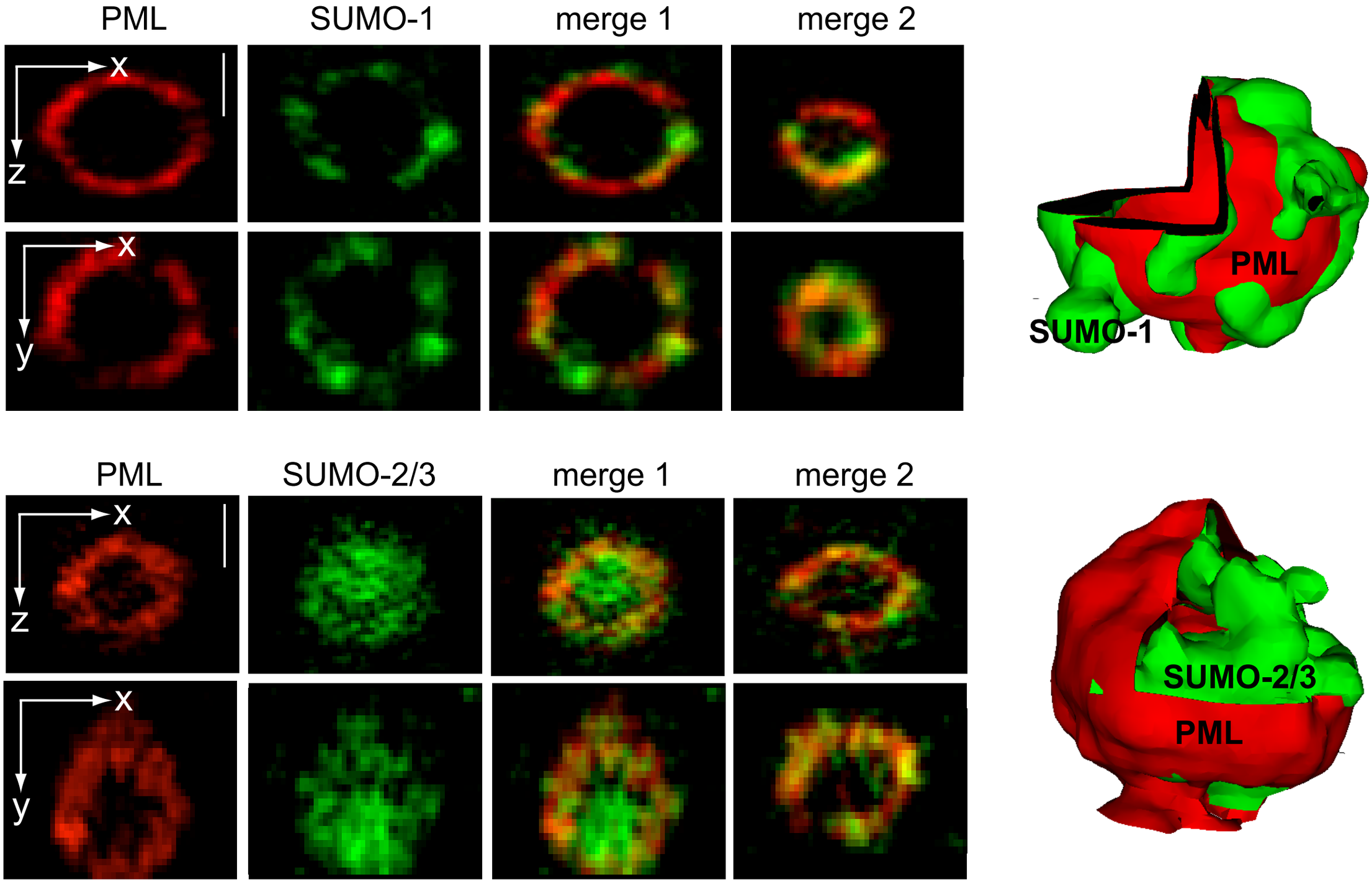
Görisch, Wachsmuth, Ittrich, Bacher, Rippe & Lichter (2004). Nuclear body movement is determined by chromatin accessibility and dynamics. *Proc Natl Acad Sci USA* **101**, 13221–13226 (2004).

PML and Sp100 proteins form distinct patches in the spherical shell of the PML nuclear body

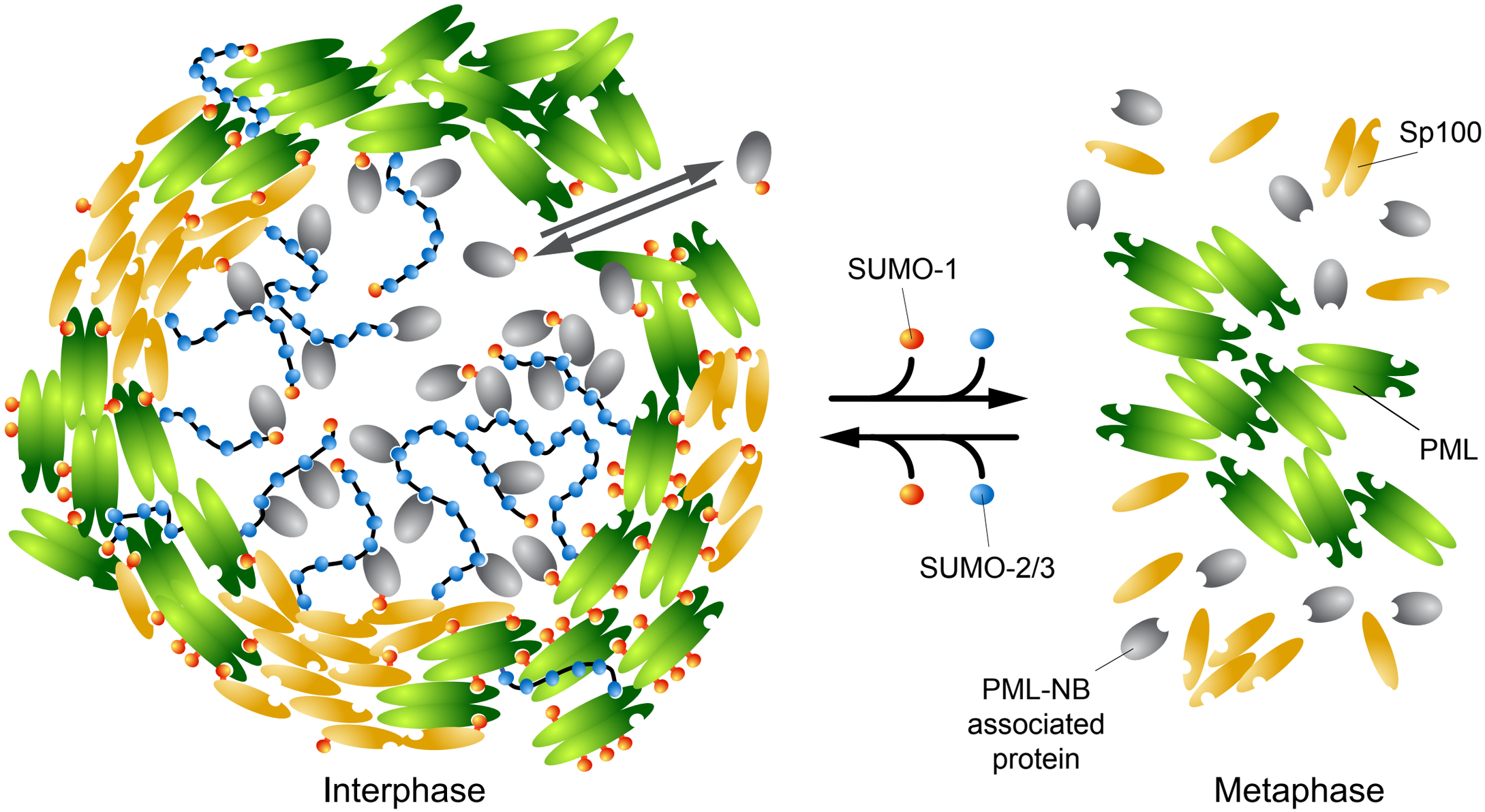




Localization of SUMO modification in PML-NBs



Model for the dynamic structure of a PML nuclear body



Self-assembly versus self-organization
(as defined by Tom Misteli)

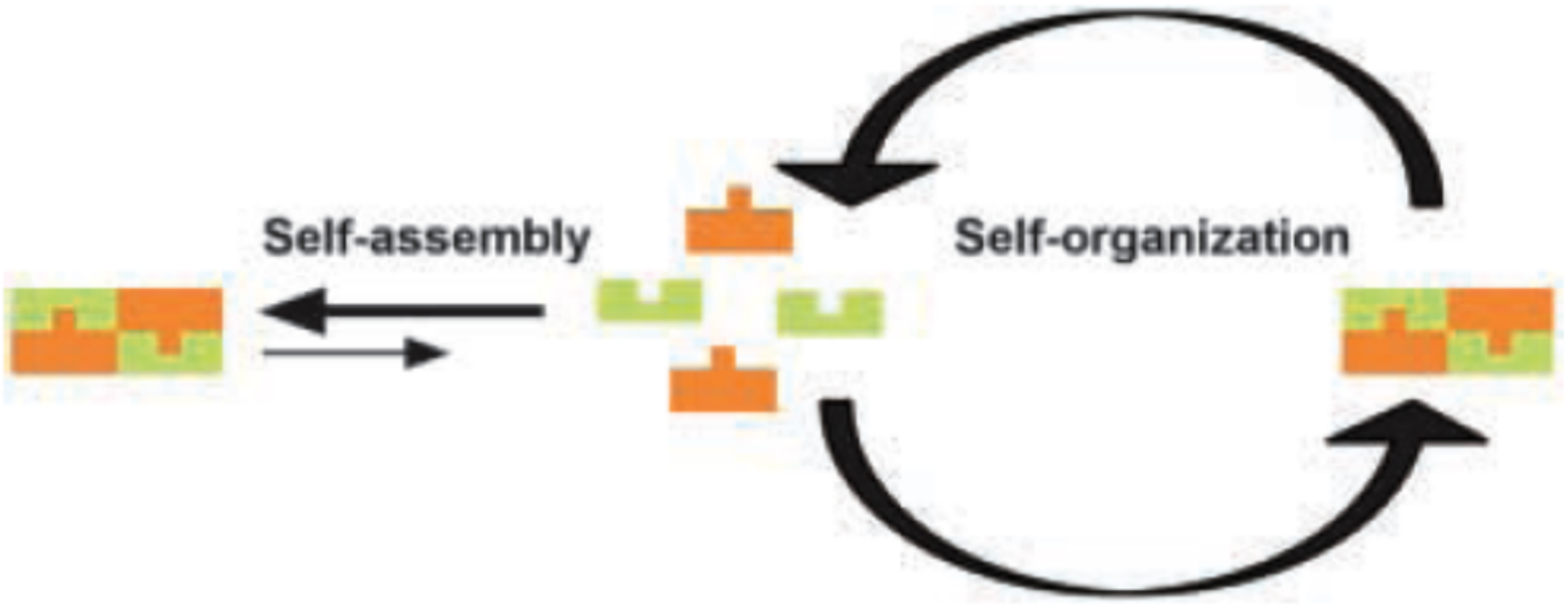
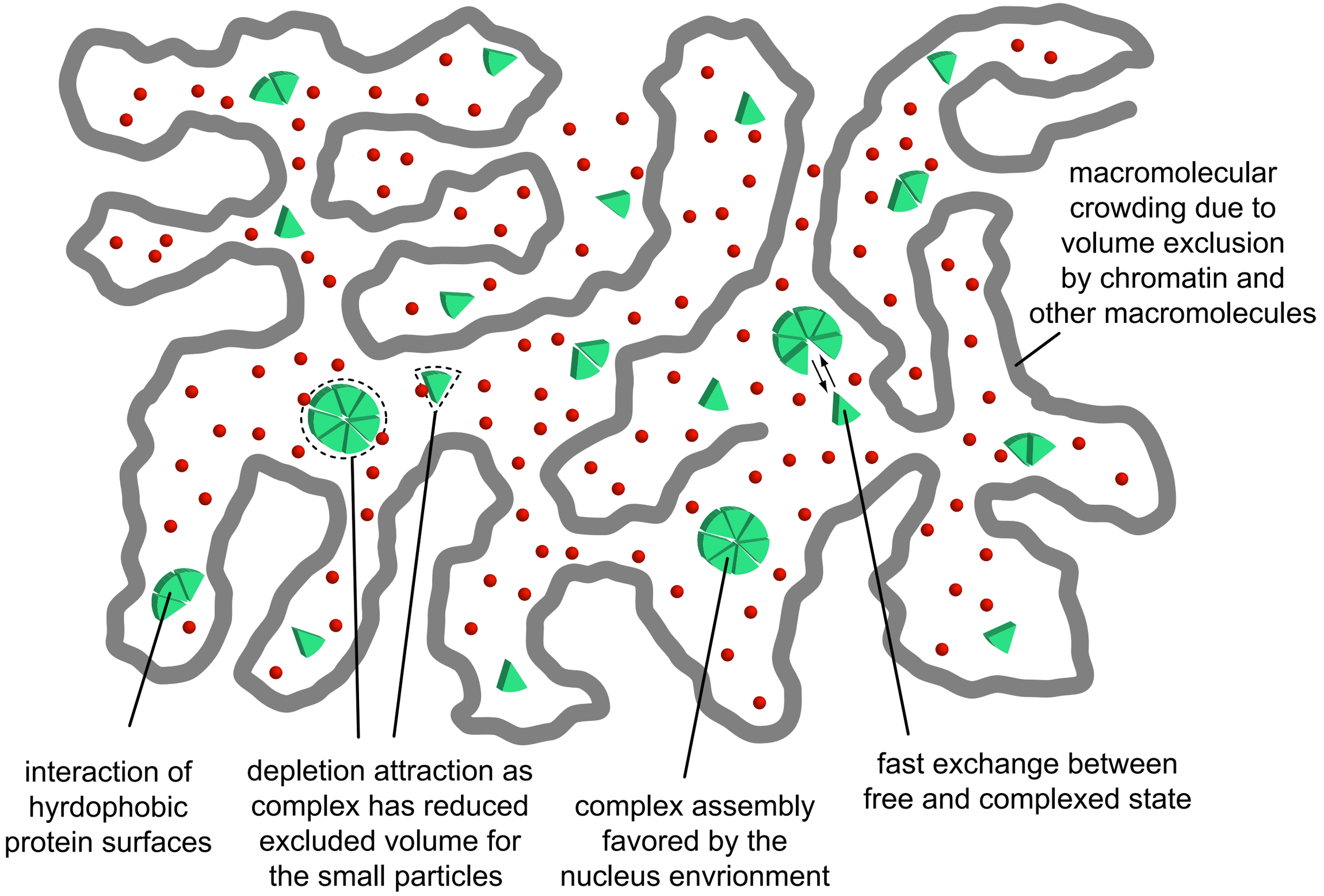
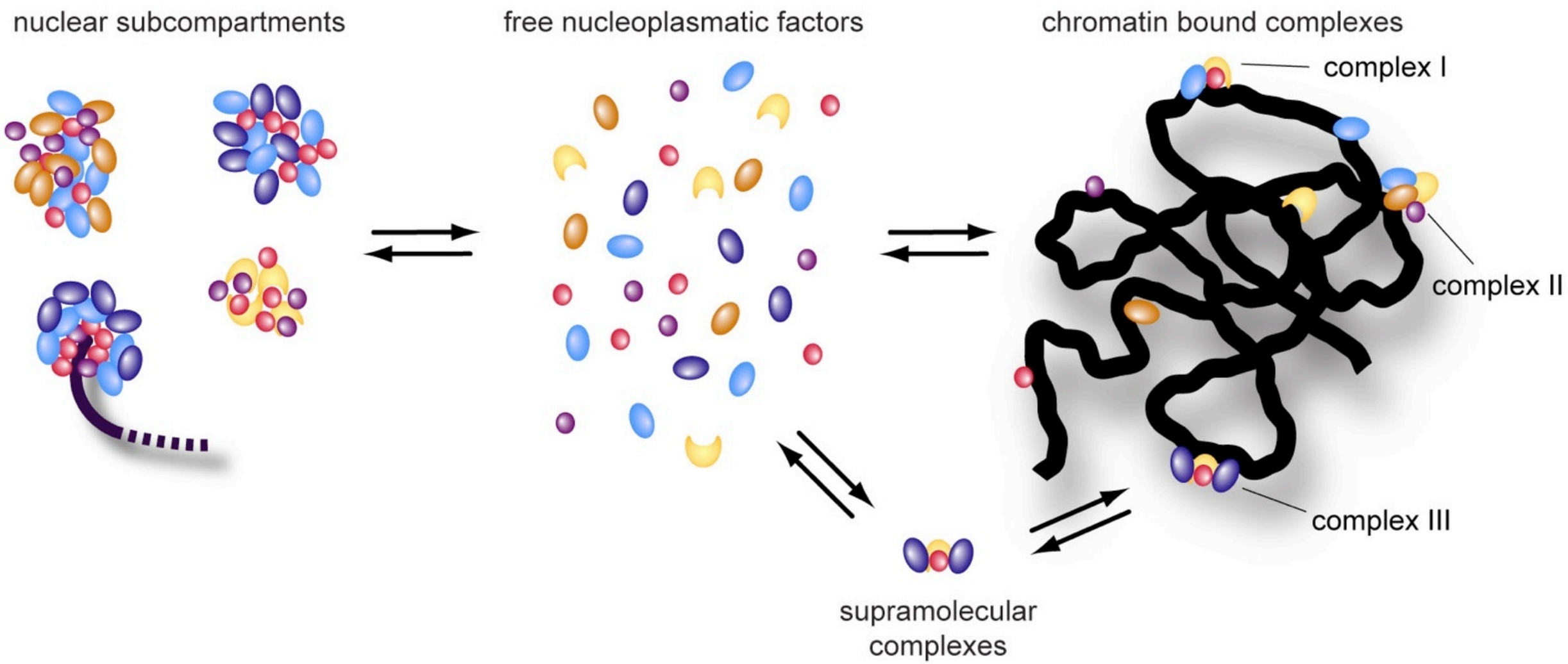


Figure 1. **Self-assembly versus self-organization.** In self-assembly, a set of components assembles into a stable, static structure that reaches a thermodynamic equilibrium. In self-organization, a set of components assembles into a steady-state, dynamic structure.

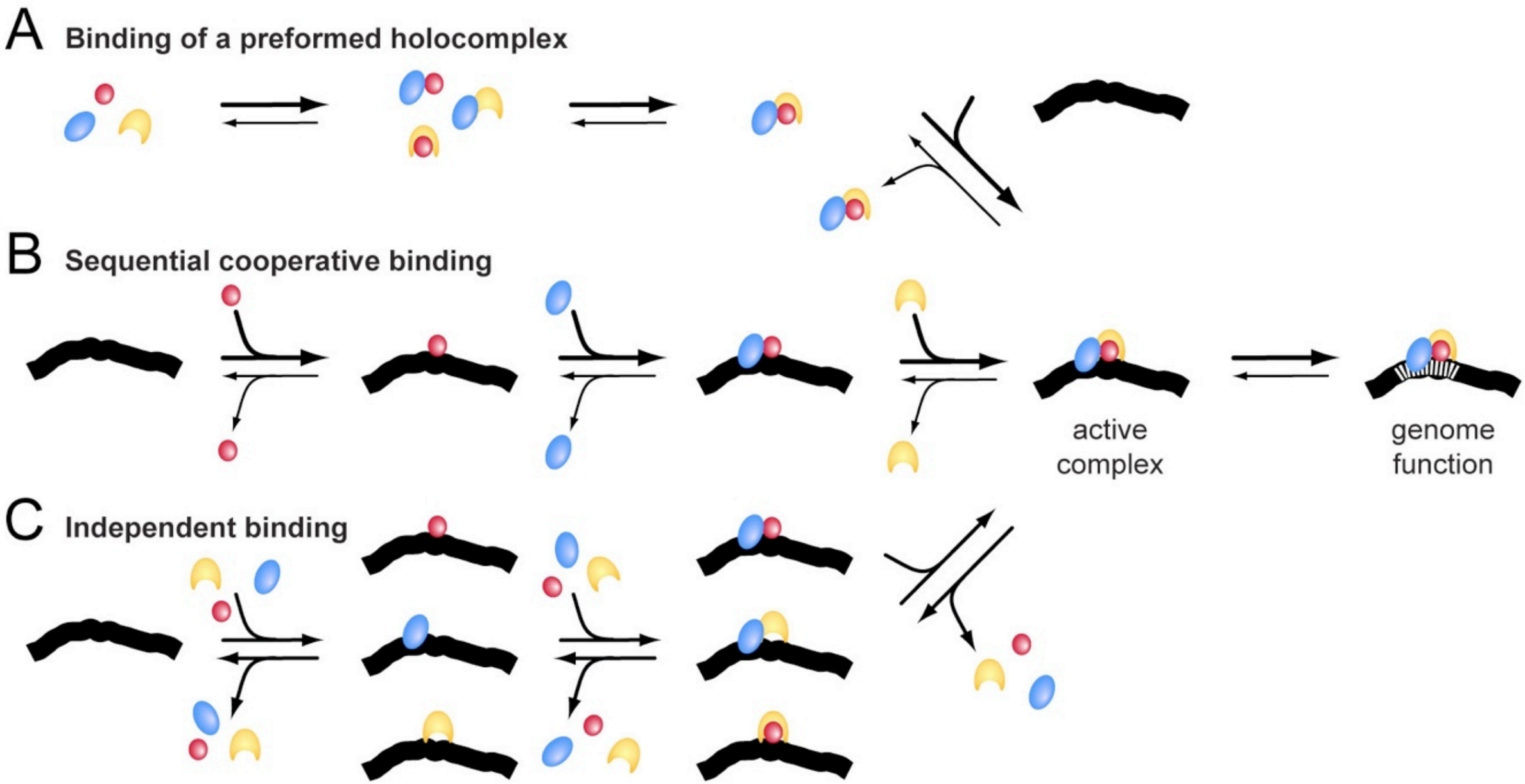
Self-organization in the nucleus



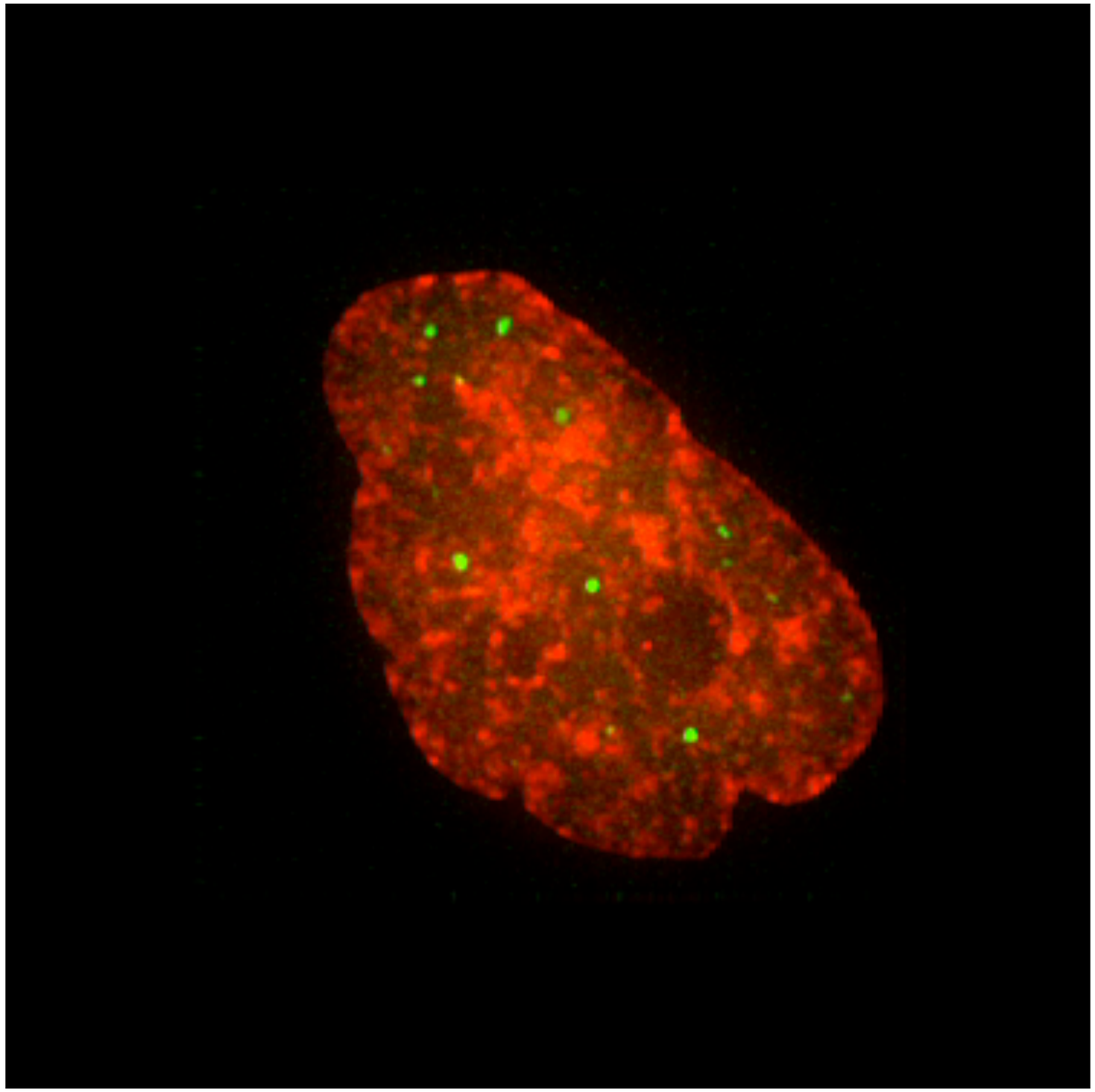
Dynamics of macromolecular interactions in the nucleus



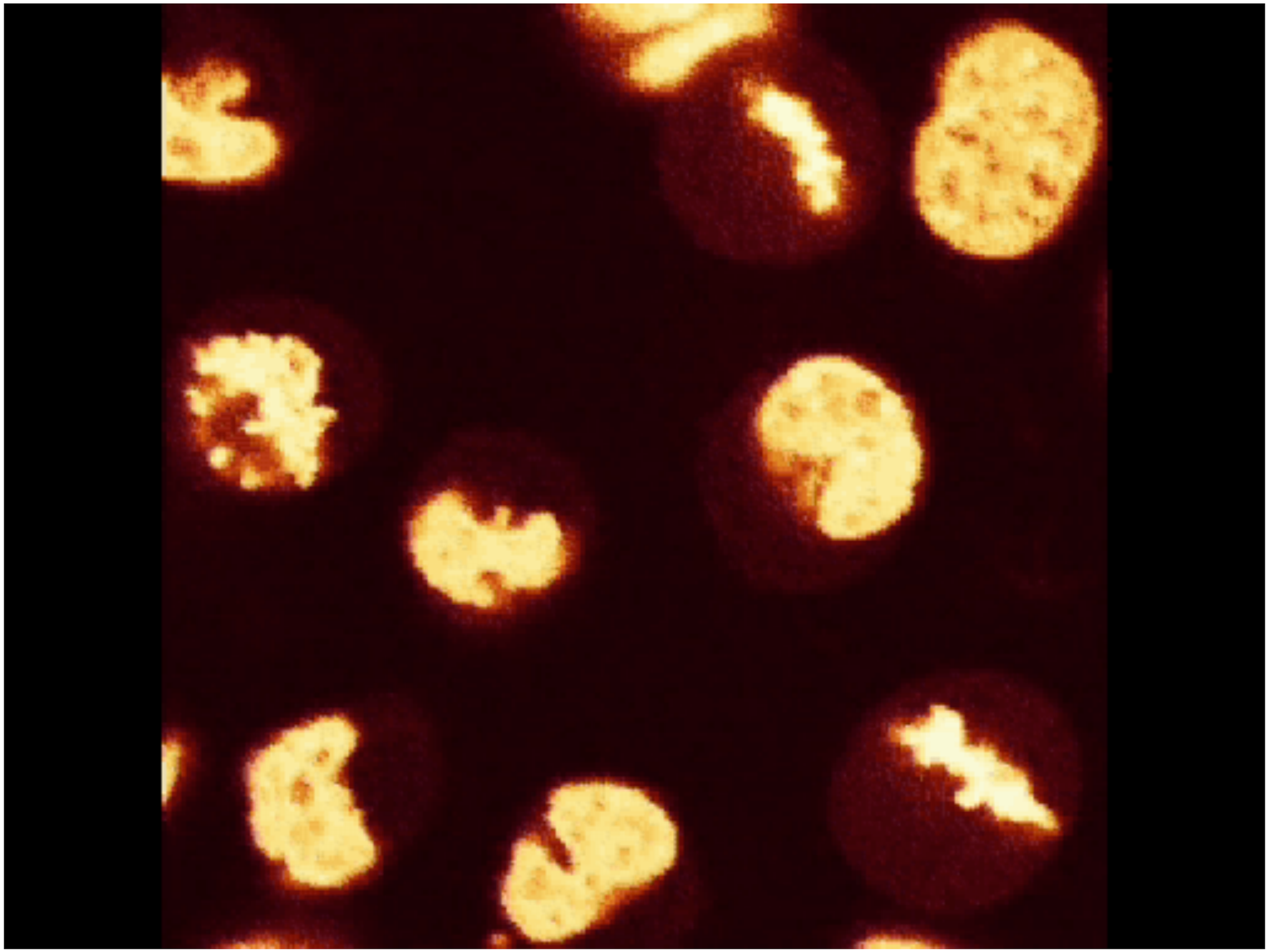
Different pathways for complex assembly



Movements of PML bodies (green) in the nucleus



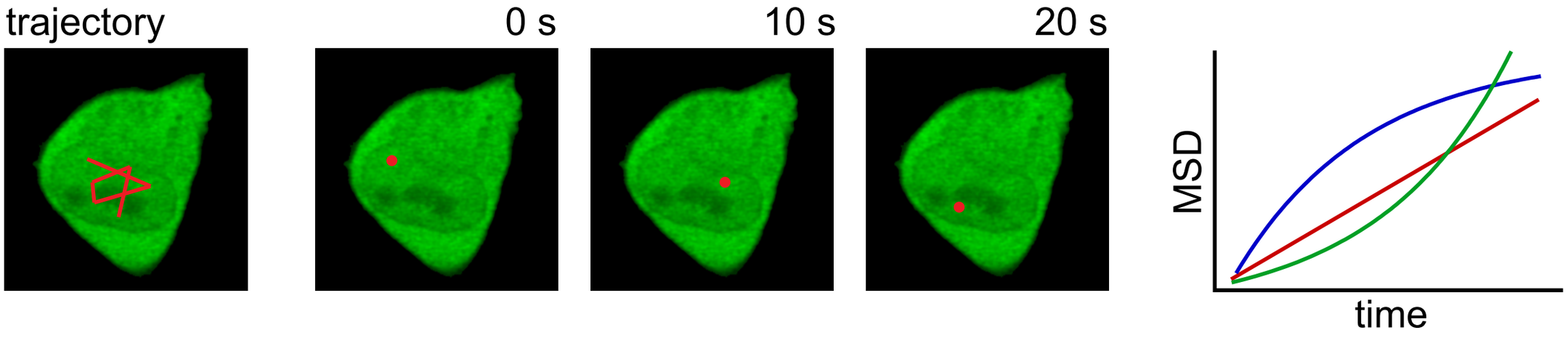
Chromatin dynamics of 24 h with a YFP-tagged histone



Single Particle tracking (SPT): nuclear bodies, chromatin loci, proteins, RNA

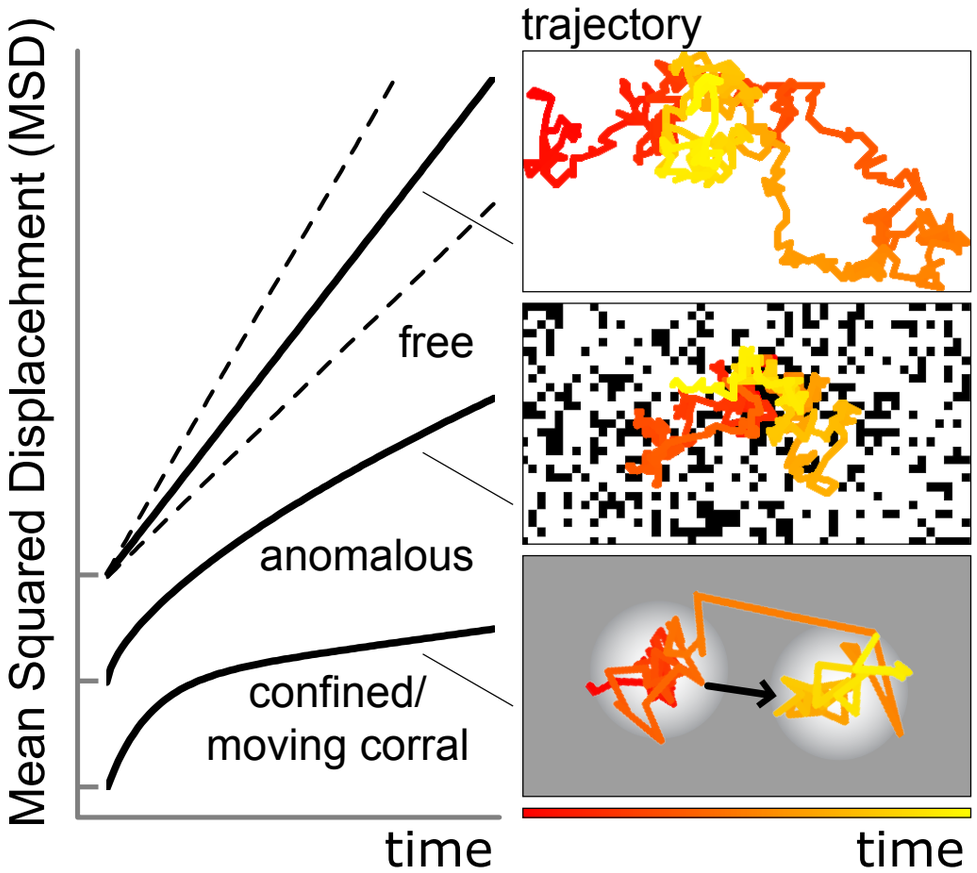
- Easiest approach to measure mobility:
  Directly watch single particles (over time)
- Prerequisites:
  Low concentration, bright & slow particles

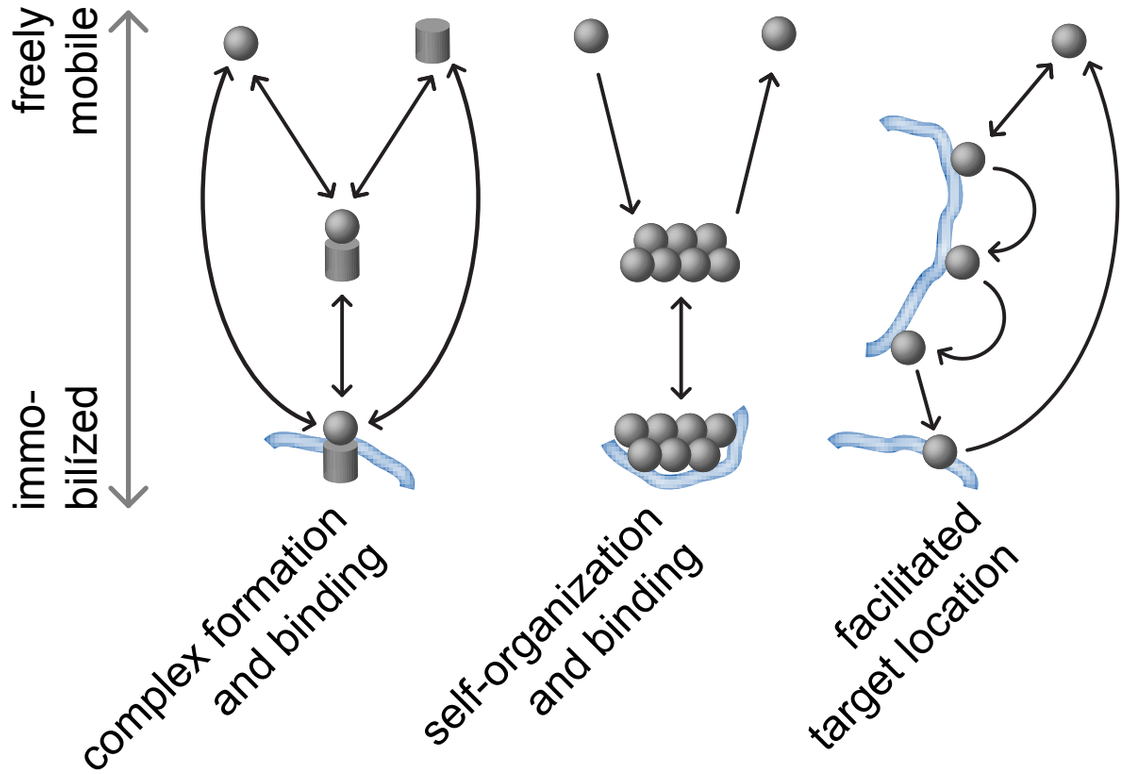
SPT



Protein mobility and interactions in the cell

$$MSD = 6\,D\,t^{\alpha}$$





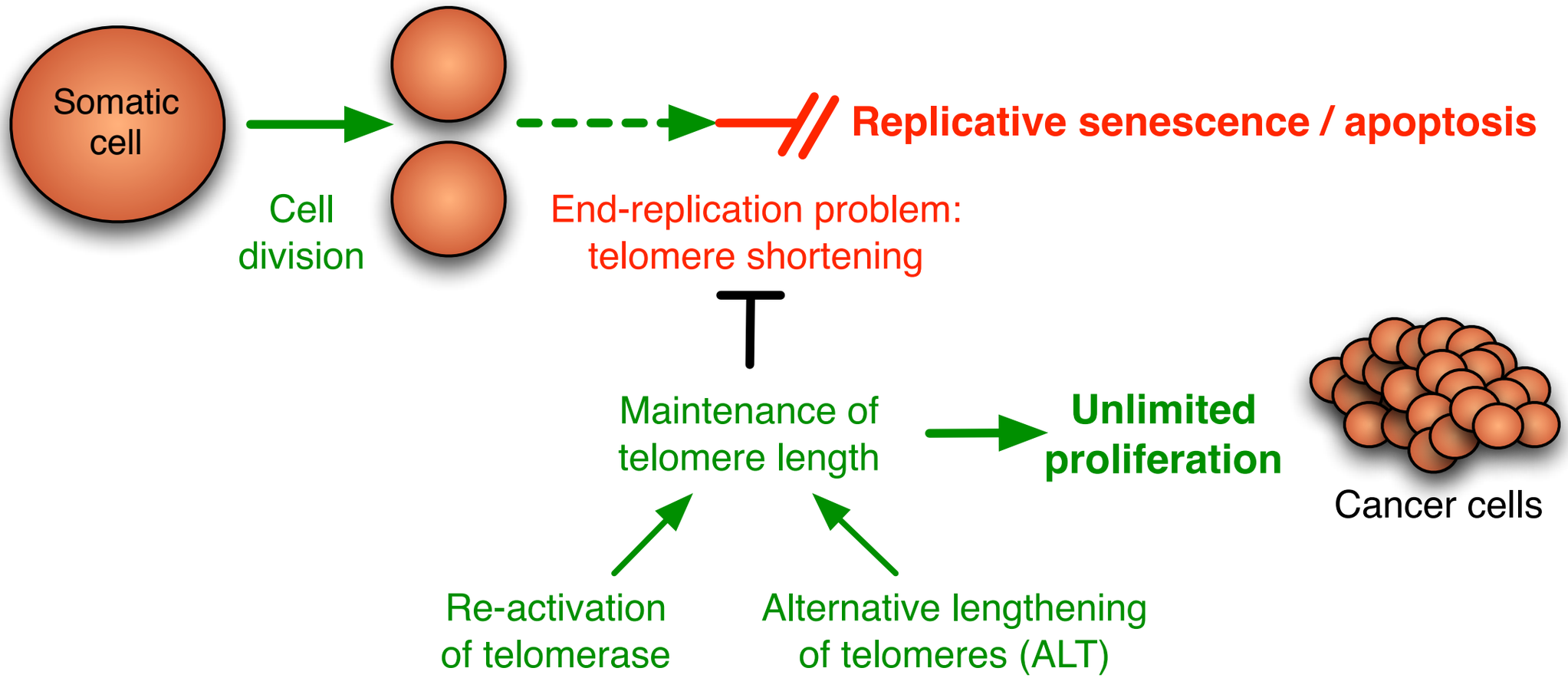
Dependence of diffusion coefficient D and molecular mass *M*

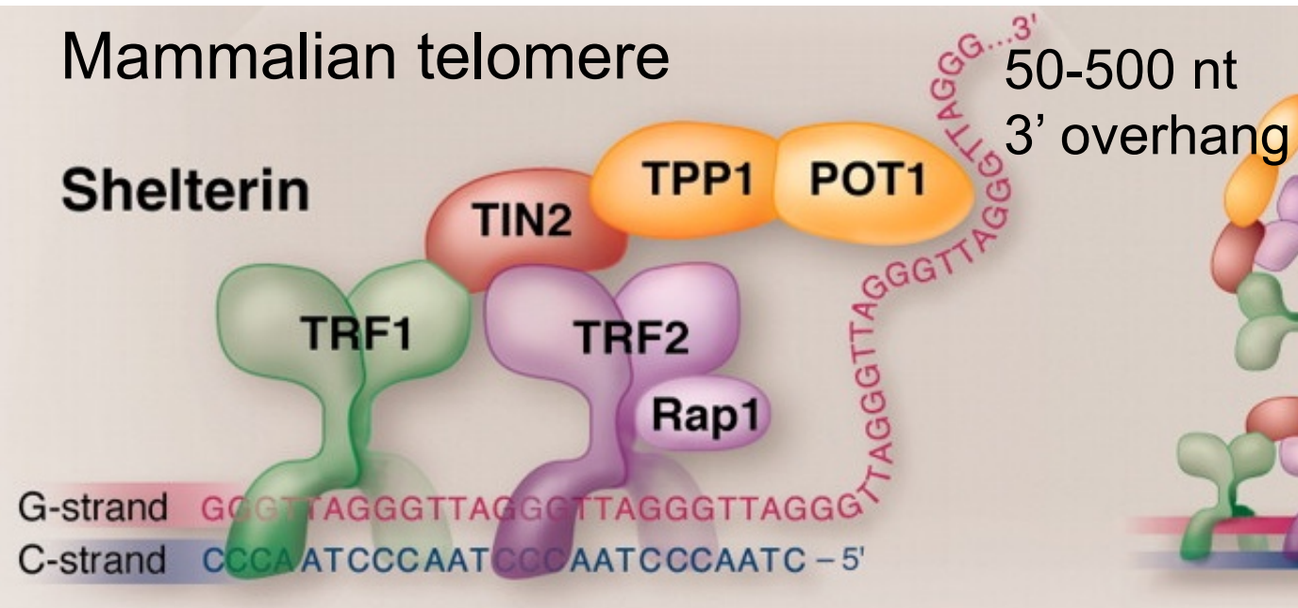
protein: $D \propto M^{-\frac{1}{3}}$ DNA: $D \propto M^{-\frac{1}{2}}$

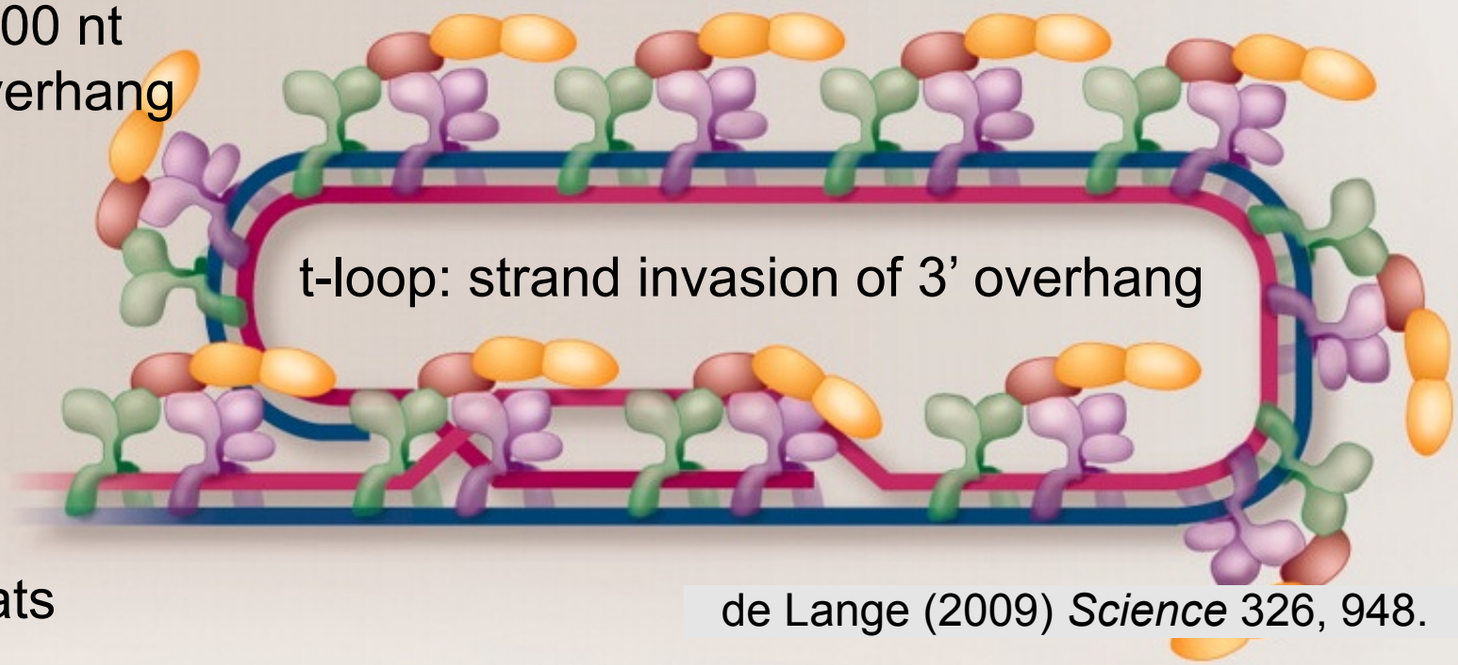
double mass *M* => 0.8 fold lower *D* double mass *M* => 0.7 fold lower *D*

Wachsmuth, M., Caudron-Herger, M. and Rippe, K. (2008). *Biochim. Biophys. Acta* **1783**, 2061-2079.

Tracing telomeres in living cells

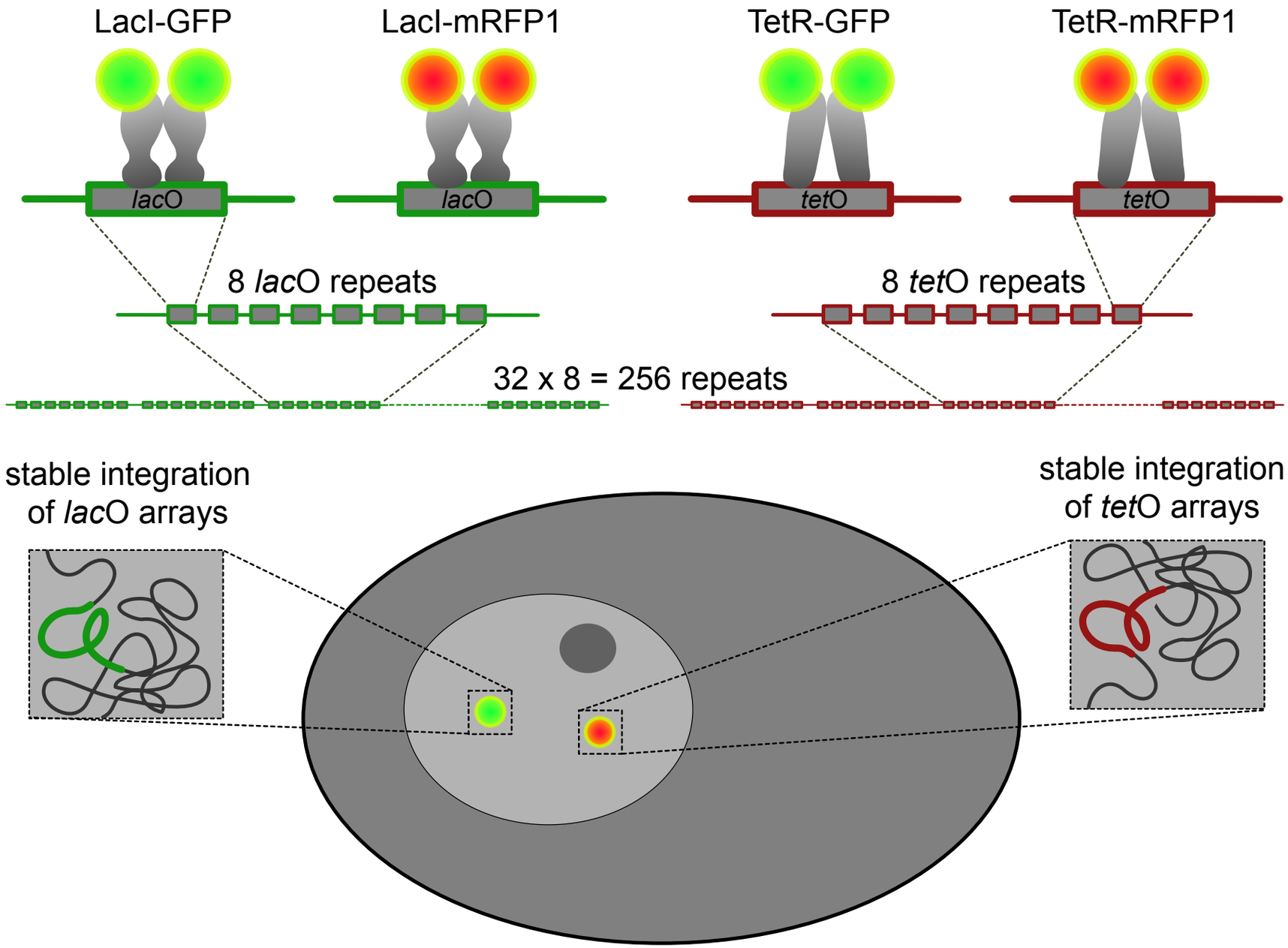






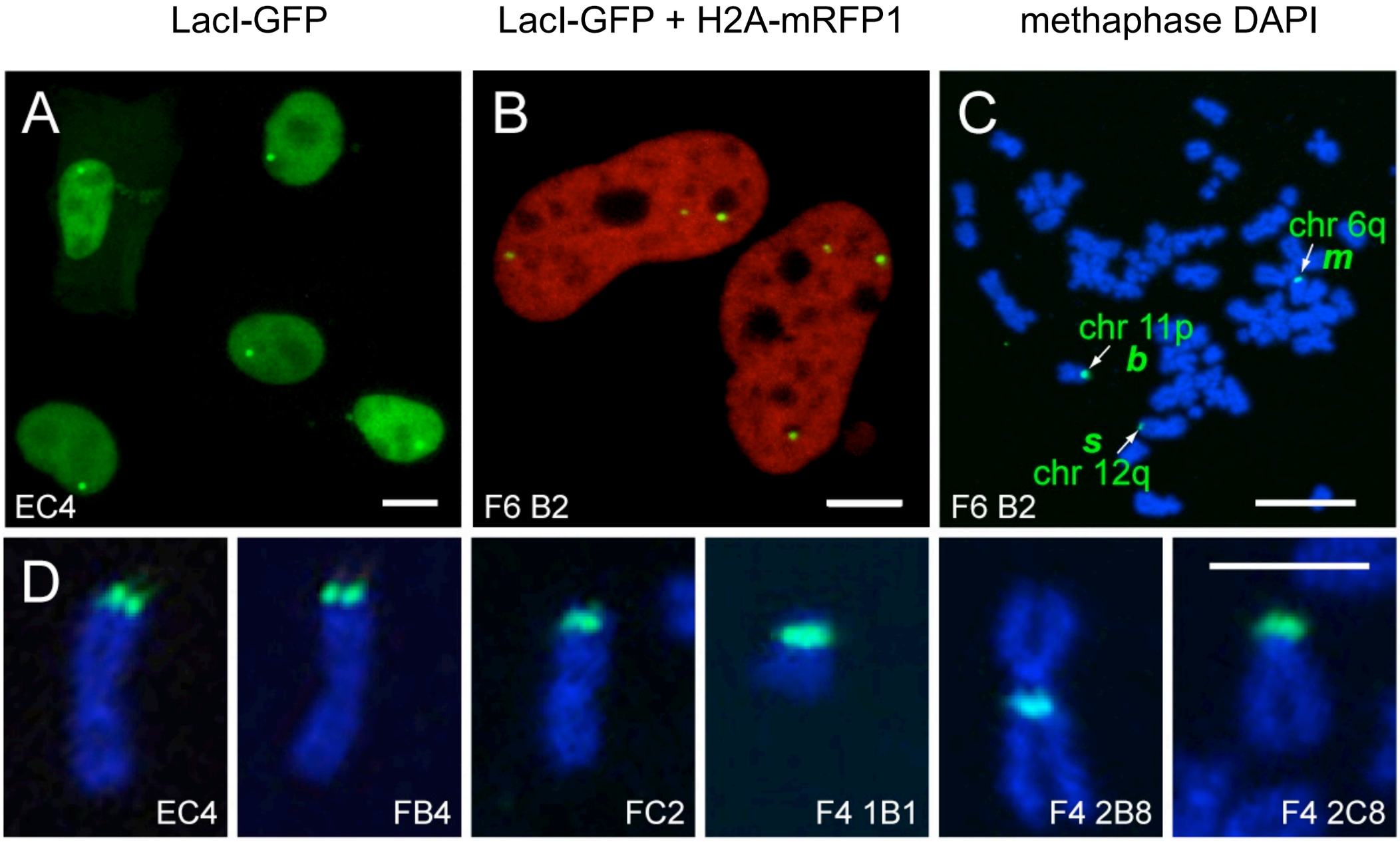
de Lange (2009) *Science* 326, 948.

Tracing specific telomeres in living cells to study their dynamics



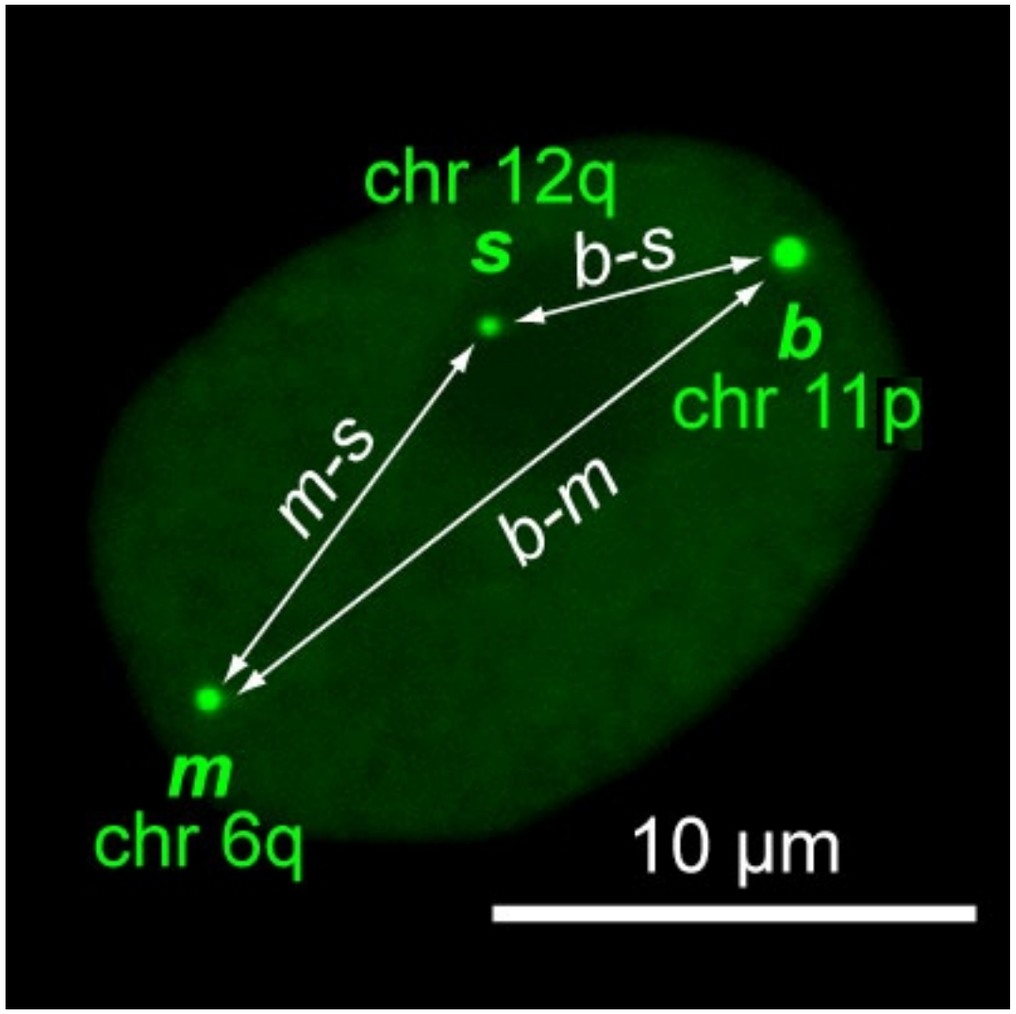
Jegou, Chung, Heuvelmann, Wachsmuth, Görisch, Greulich-Bode, Boukamp, Lichter & Rippe (2009). *Mol. Biol. Cell* **20**, 2070.

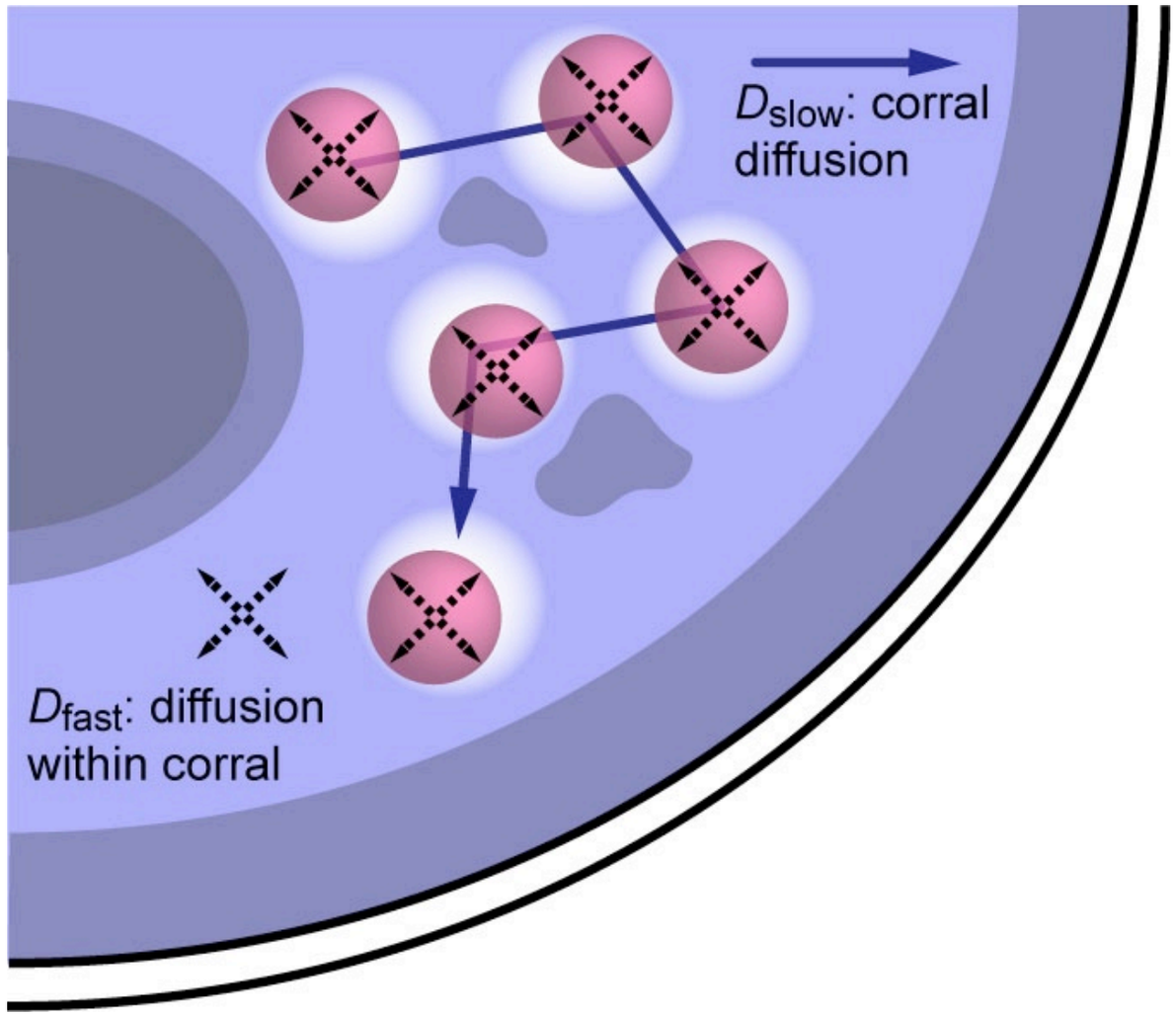
In vivo labeling of telomeres in human osteosarcoma U2OS cells with LacI-GFP via integrated *lac*O repeats



Metaphase FISH reveals preferred *lac*O integration into telomeres

The telomere mobility is derived from distance changes between two loci according to a “moving corral” model

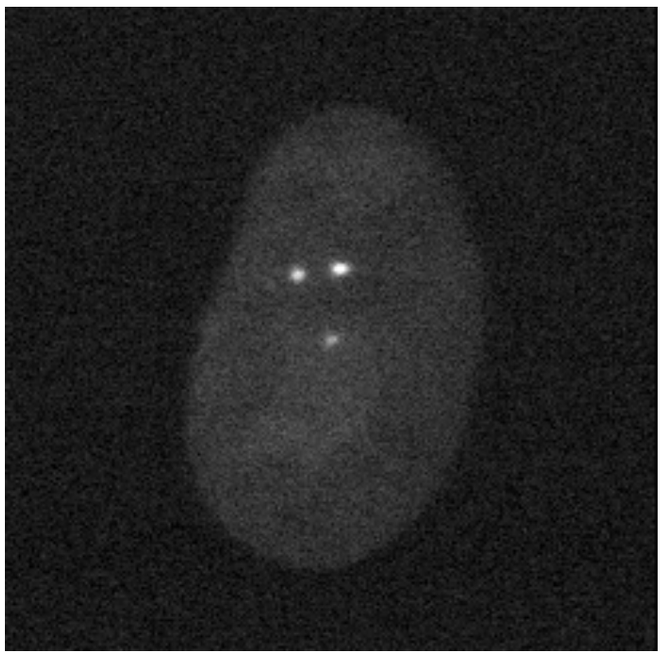




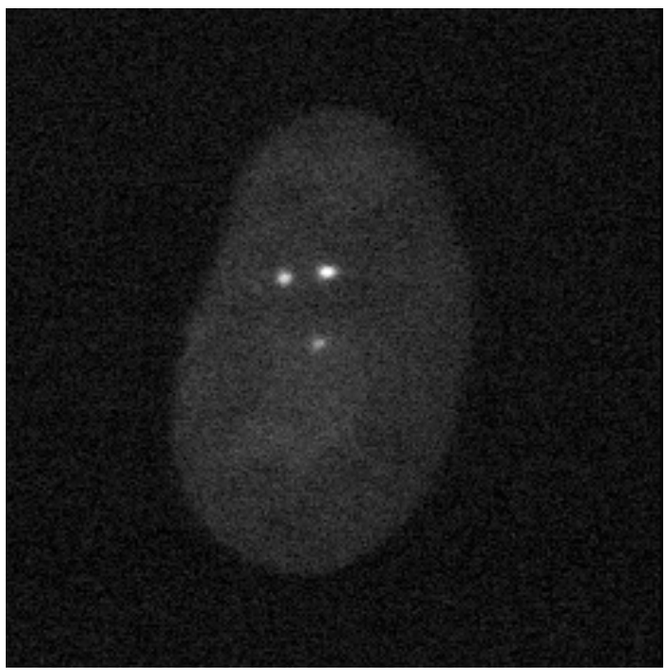
$$MSD = \left\langle r_c^{\,2} \right\rangle \cdot \left(1 + \frac{2n\,D_{slow}\,\Delta t}{\left\langle r_c^{\,2} \right\rangle} \right) \cdot \left[1 - \exp\left(- \frac{2n\,D_{fast}\,\Delta t}{\left\langle r_c^{\,2} \right\rangle} \right) \right]$$

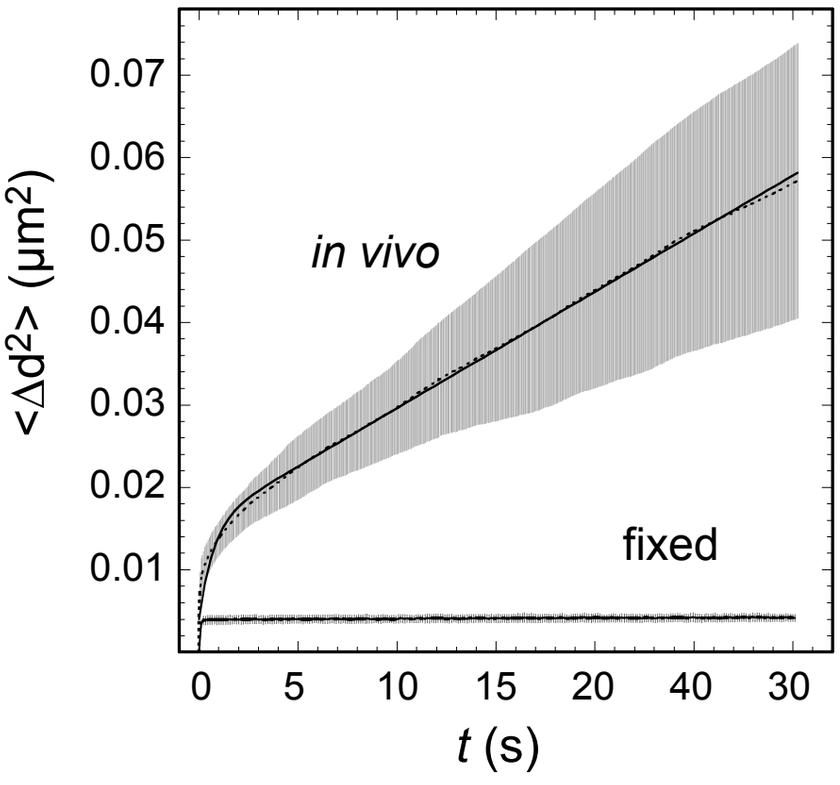
Mobility of telomeres at the second and the minute time scale

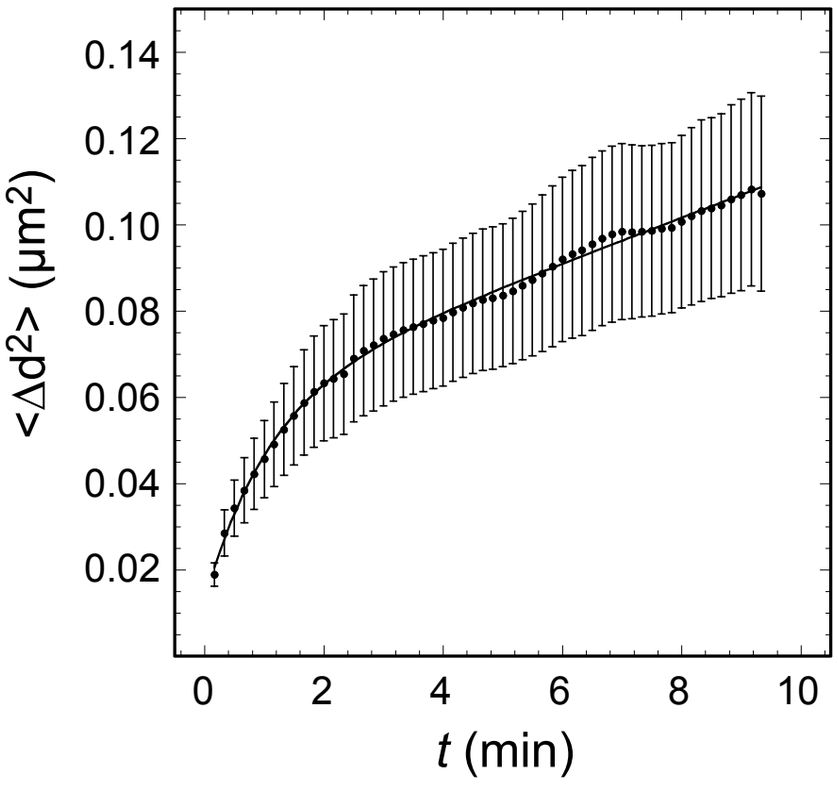
second scale
“real time” (Δt = 70 msec)



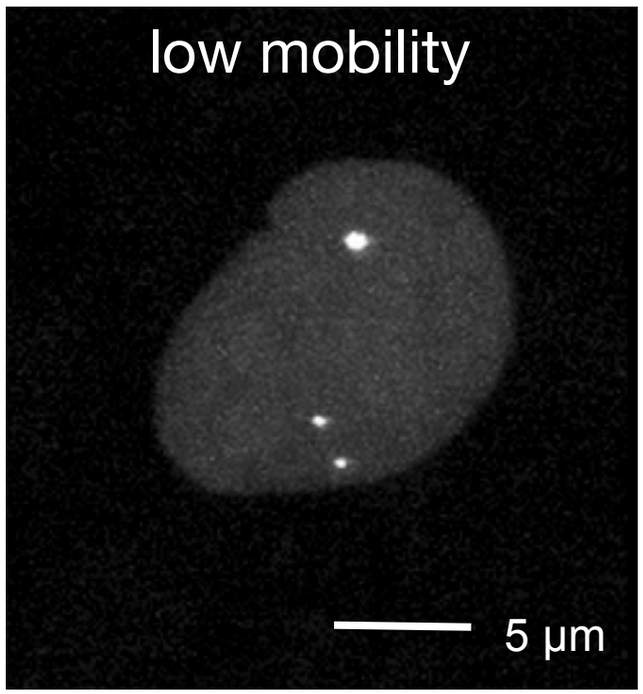
minute scale
25x higher speed

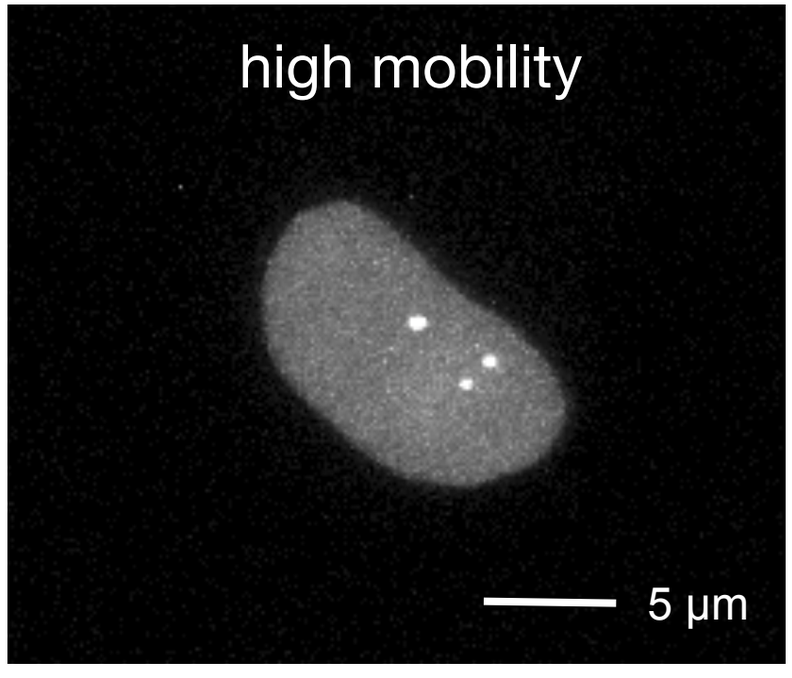


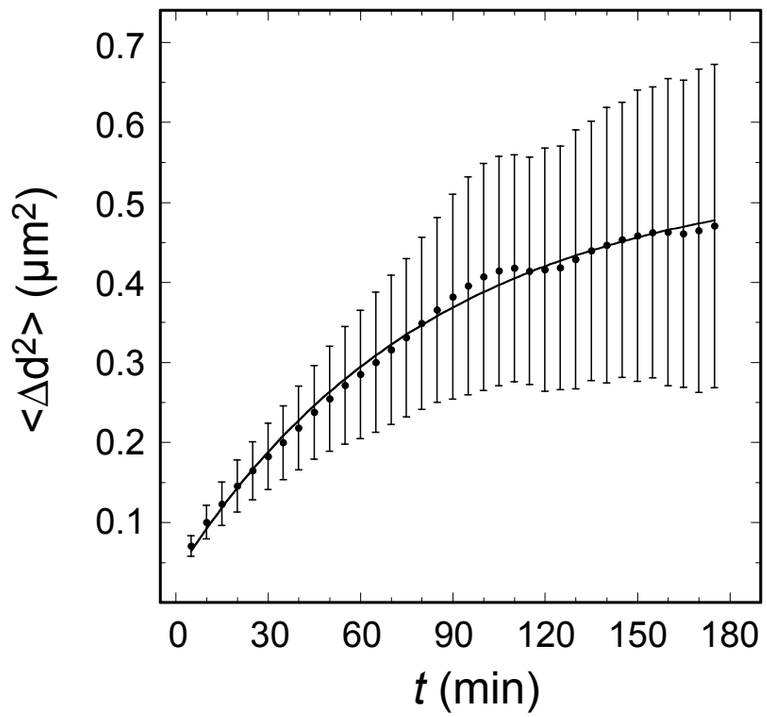


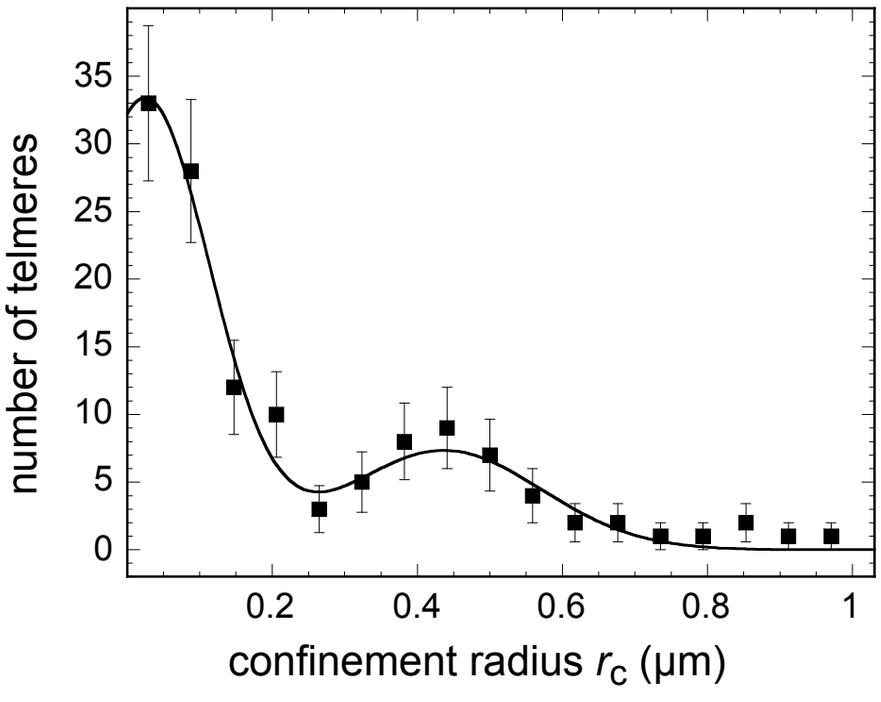


Mobility measurements over ~3 h (looped, 700x higher speed) show a state of extended mobility for some telomeres

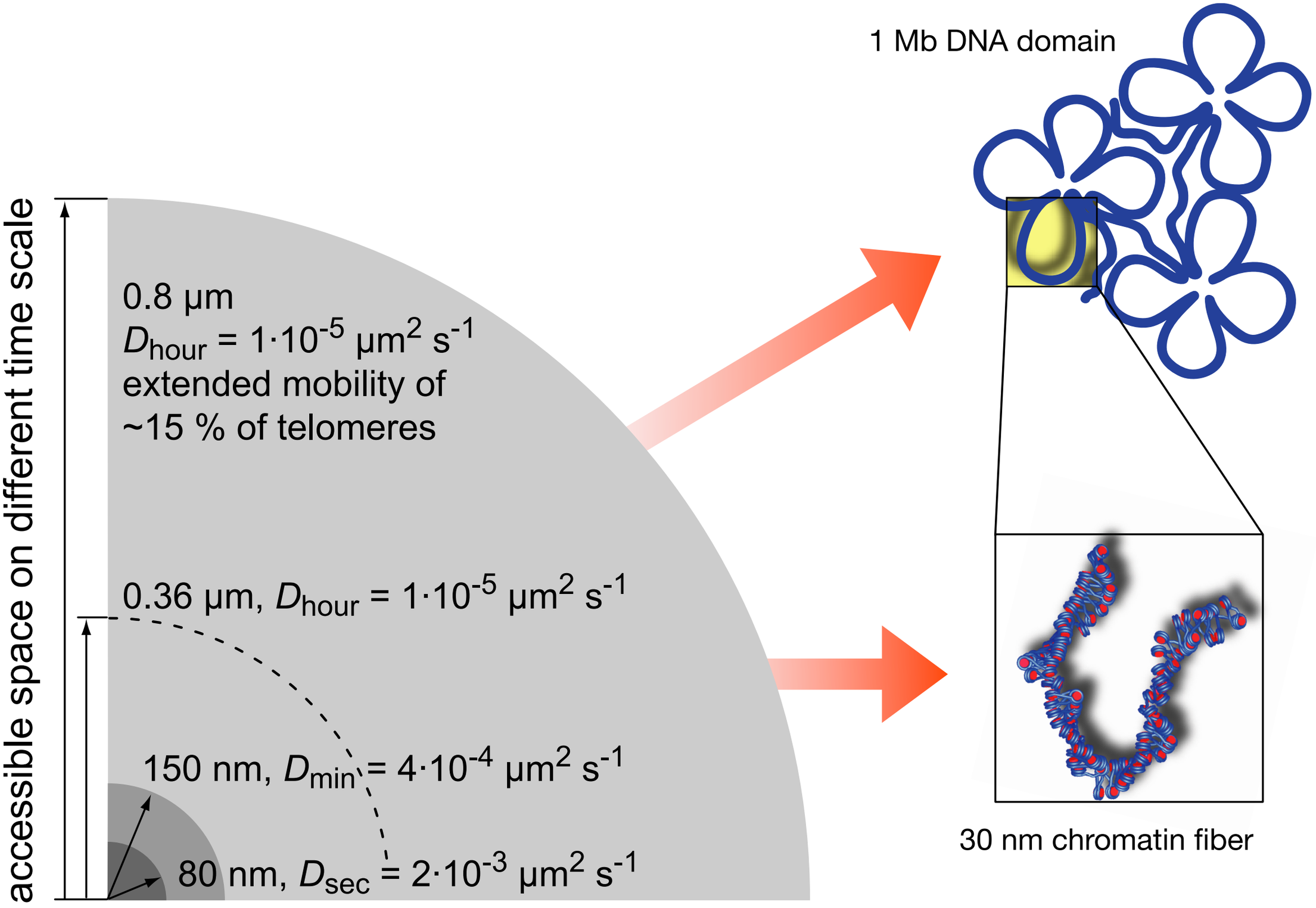




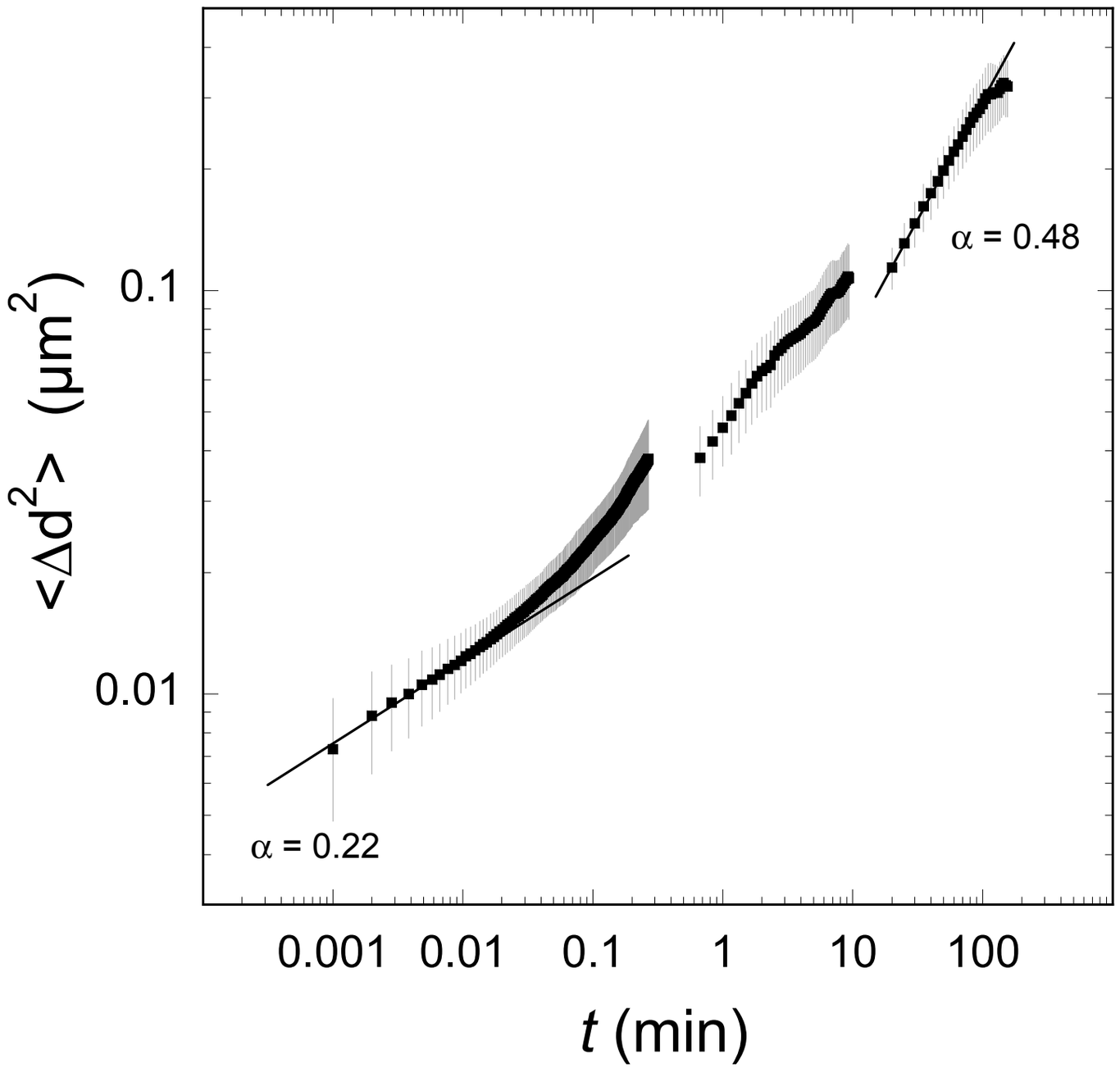




Telomere mobility over different time scales



Telomere mobilityis that of a polymer in a crowded environment according to the “reptation model”



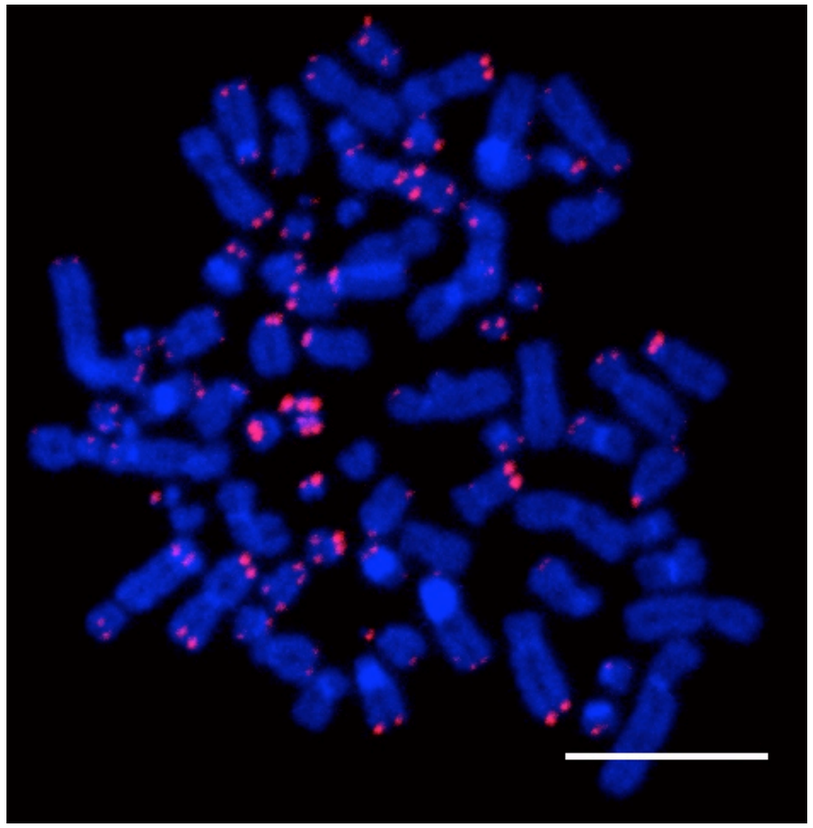
$$\mathrm{MSD} = \langle d^2(\Delta t) \rangle = 2n\Gamma\Delta t^{\alpha}$$

Alternative lengthening of telomeres (ALT)

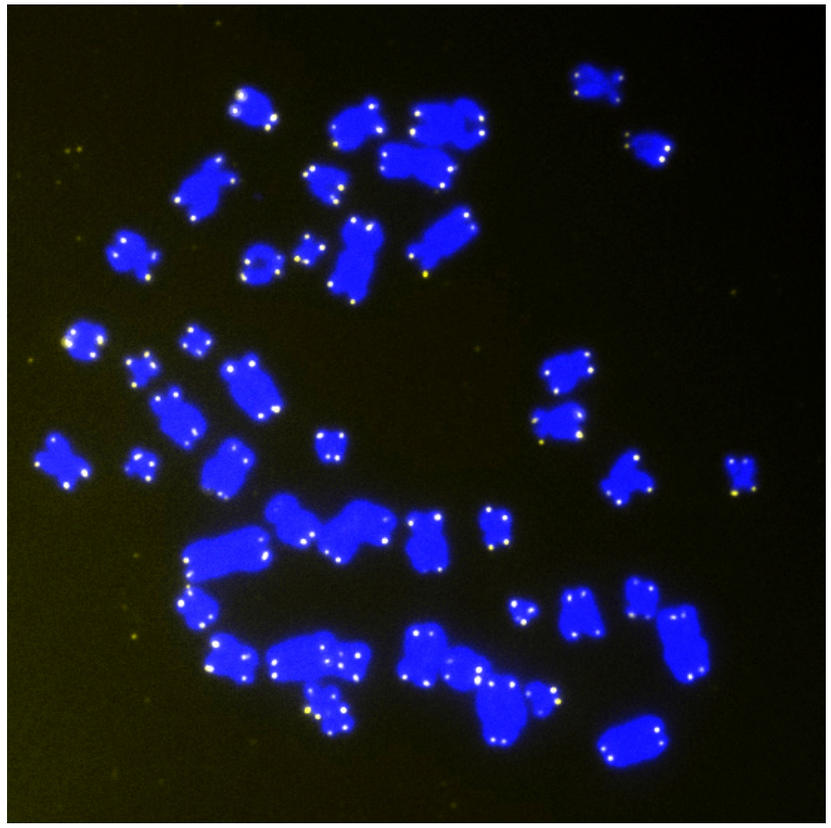
Maintaining telomere length without telomerase in ~30% of sarcomas and ~10% of carcinomas

- DNA repair/recombination based mechanism
- heterogenous telomere repeat length
- **A**LT-associated **P**ML **B**odies (APBs)
  = complexes of PML bodies at telomeres

Metaphase FISH of telomere repeats

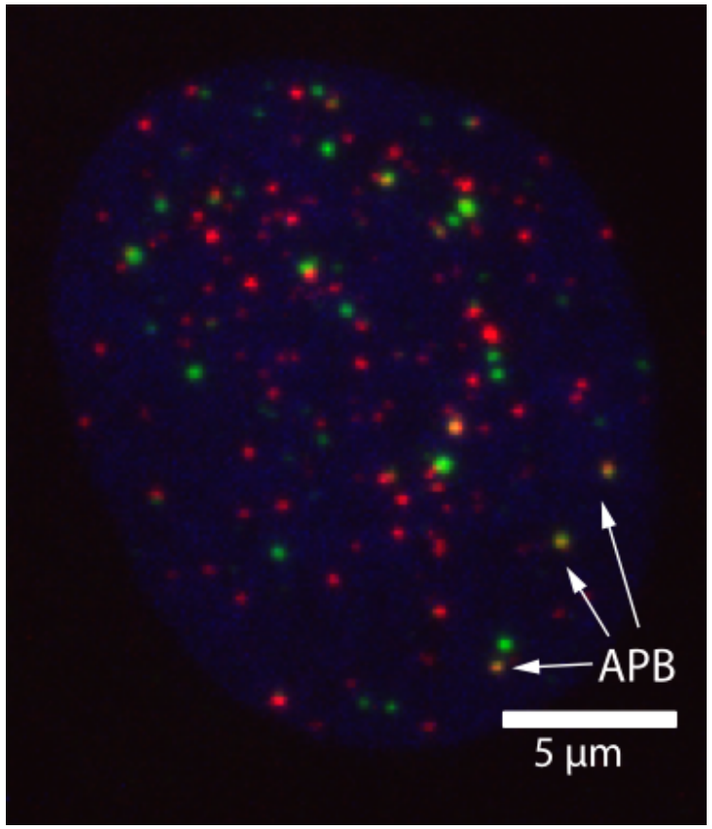


U2OS cells, ALT(+)



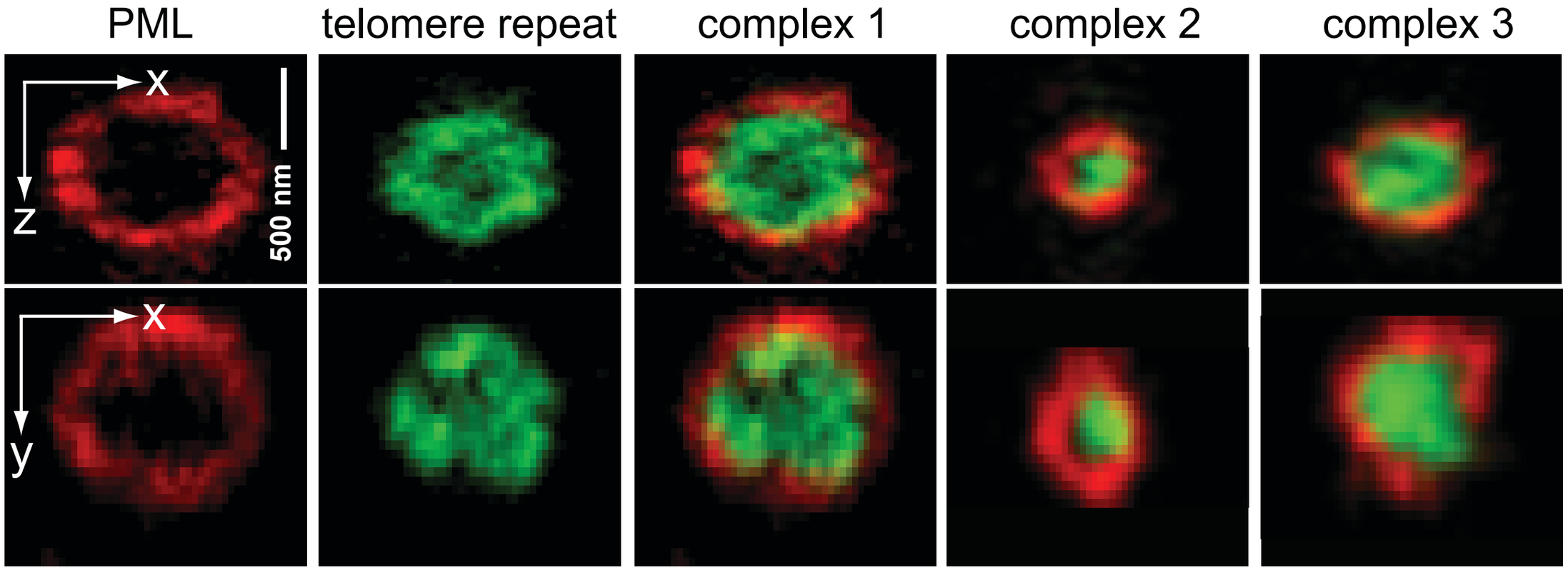
human lymphocytes, ALT(-)

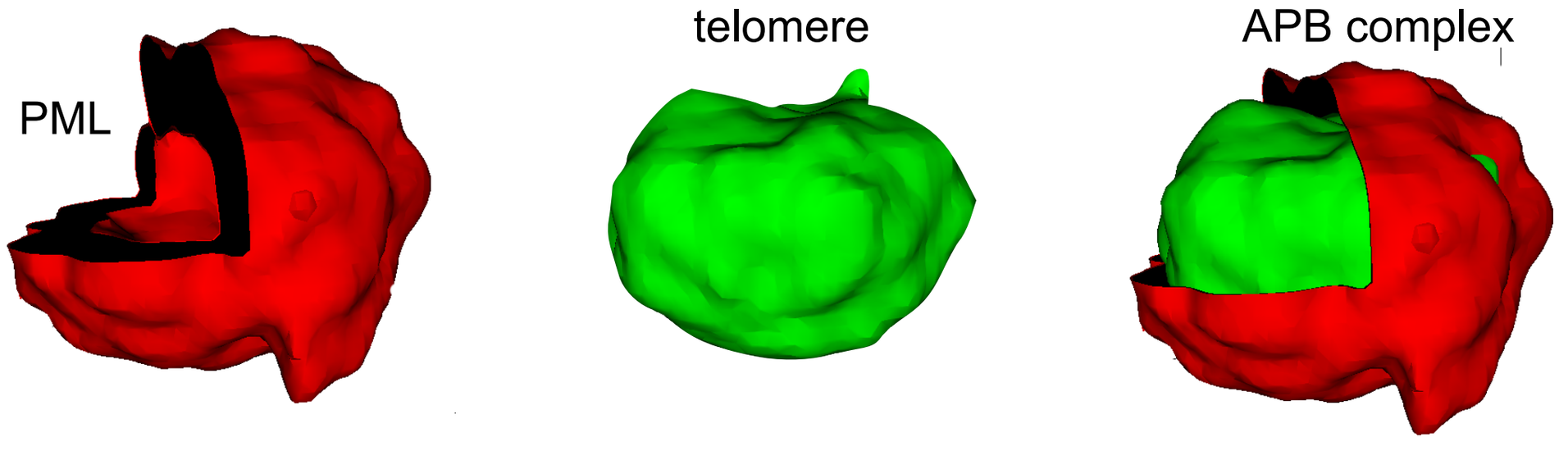
Immunostaining of telomeres and PML



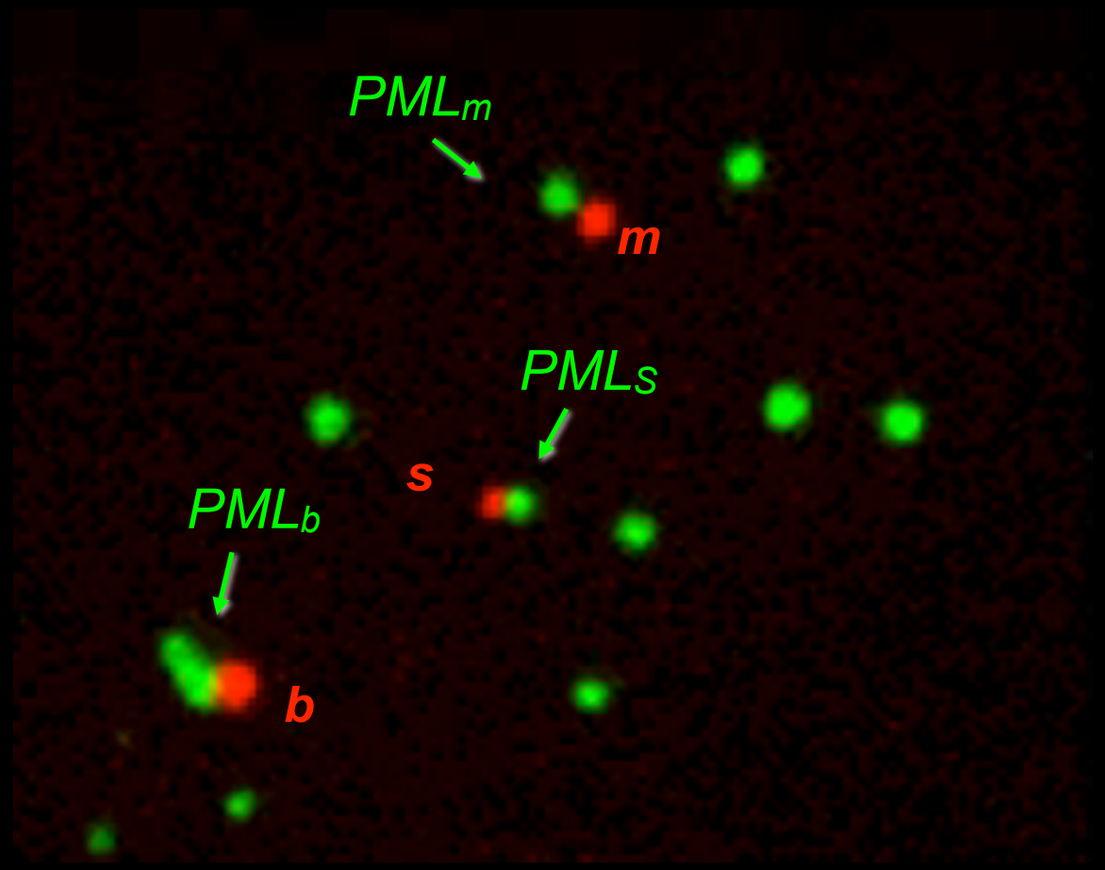
APBs in U2OS cells

High resolution imaging of APBs in U2OS cells



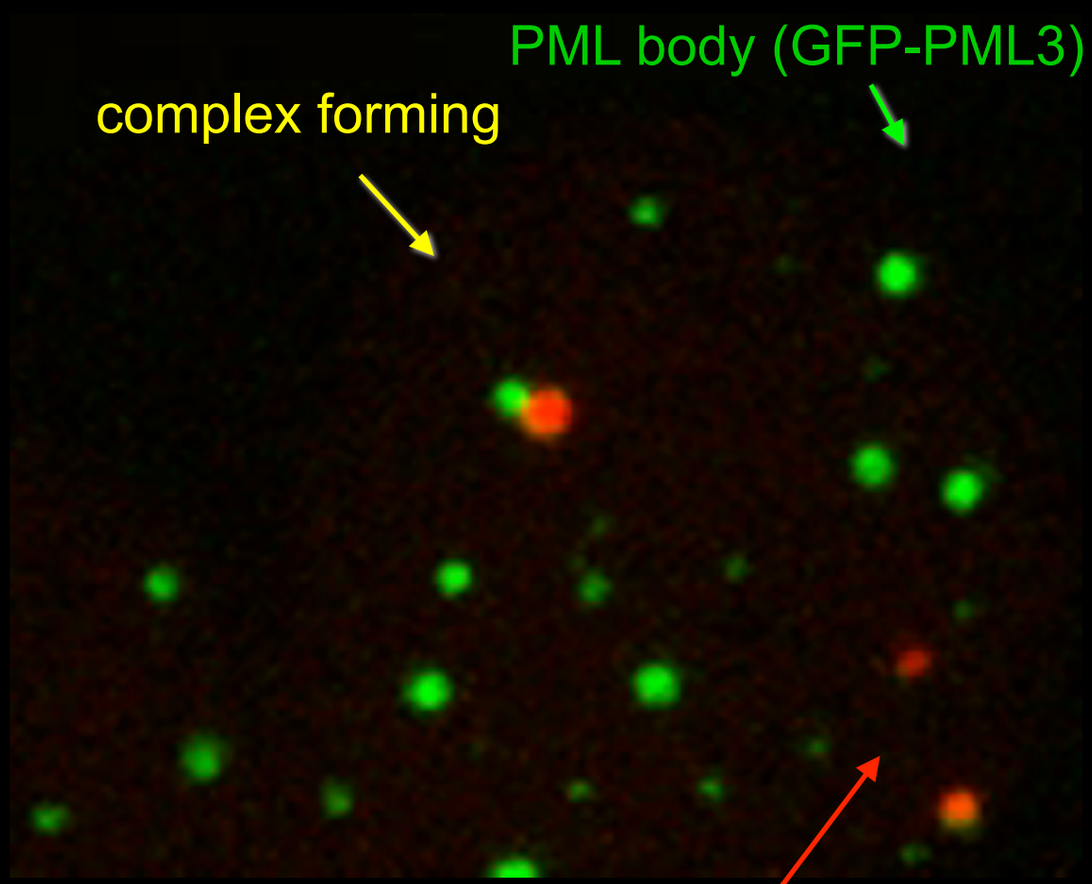


Mobility of PML bodies (green) close to telomeres (red)



70x higher speed

Complex formation between a PML body (green) and a telomere (red)



50x higher speed