Mapping the internal structure of a living cell from a protein's point of view

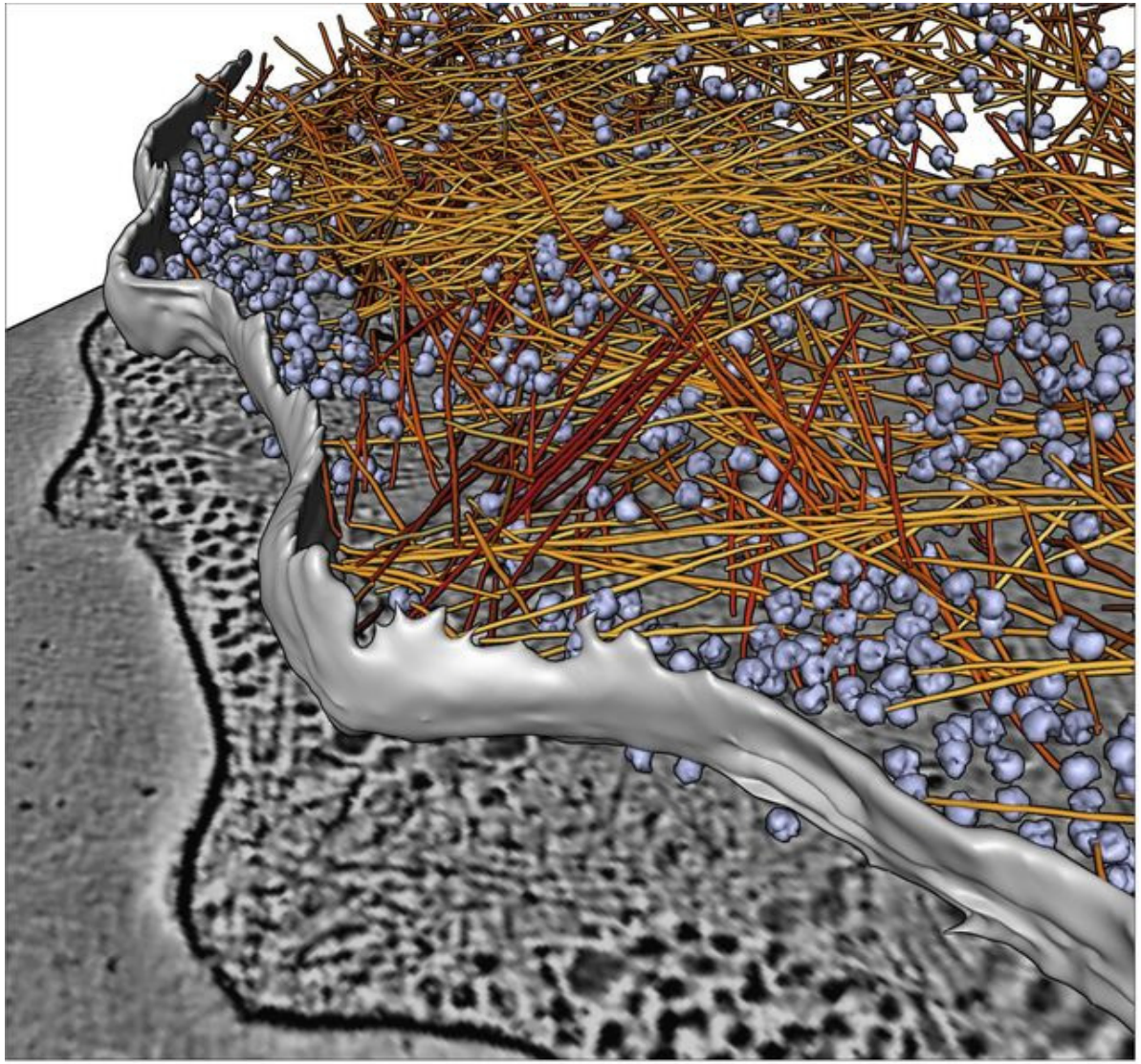
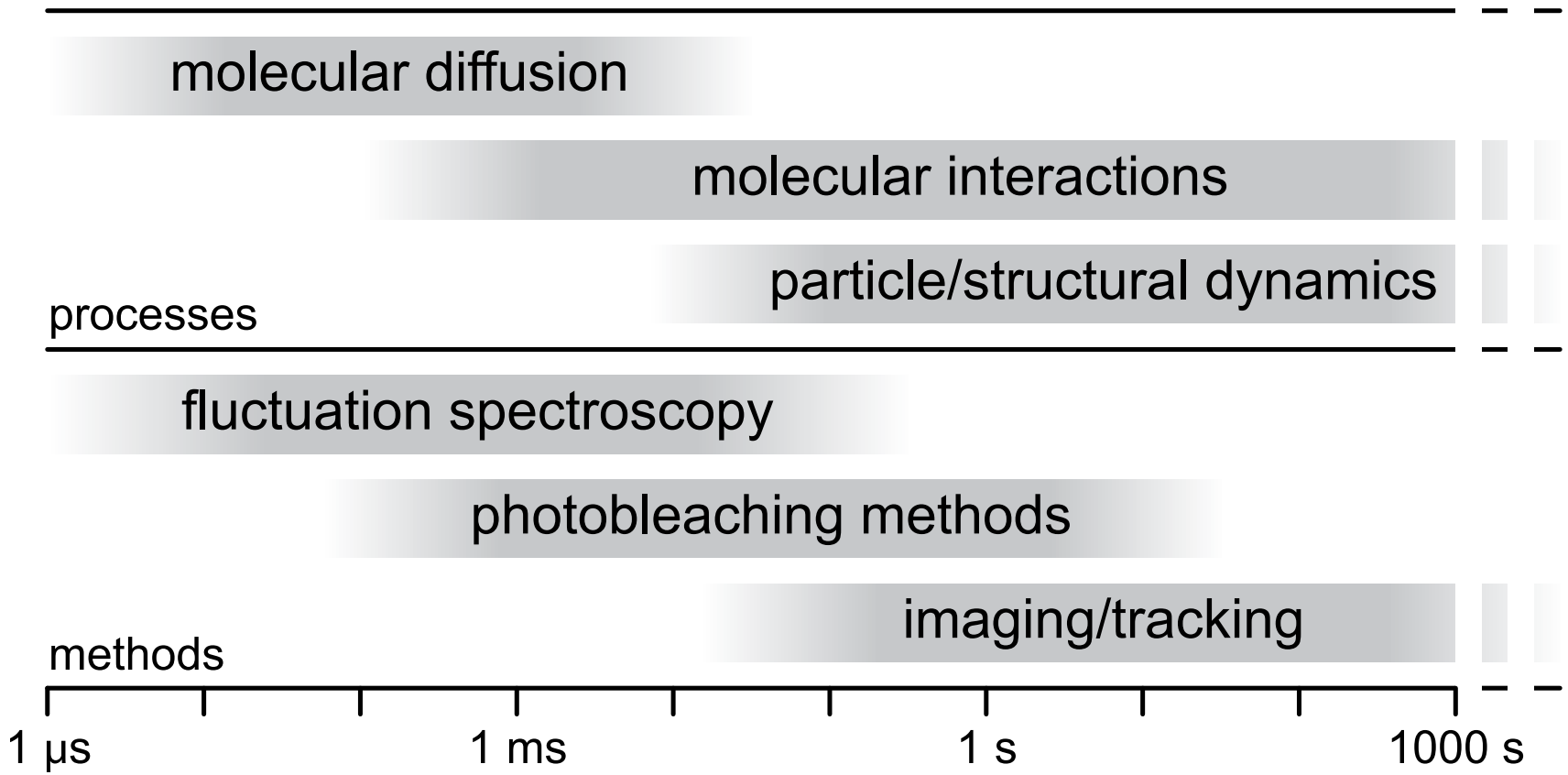


image from Lucic et al., JCB, 2012

Dynamic processes in the cell nucleus take place on the microsecond to hour time scale



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

	Typical resolution	Acquisition rate (frames/sec)	Light exposure
Wide field	250 nm (x,y) > 2 µm (z)	200	low
Confocal	250 nm (x,y) 600 nm (z)	1-10	high
Line scanner confocal	250 nm (x) 380 nm (y) 700 nm (z)	30	low

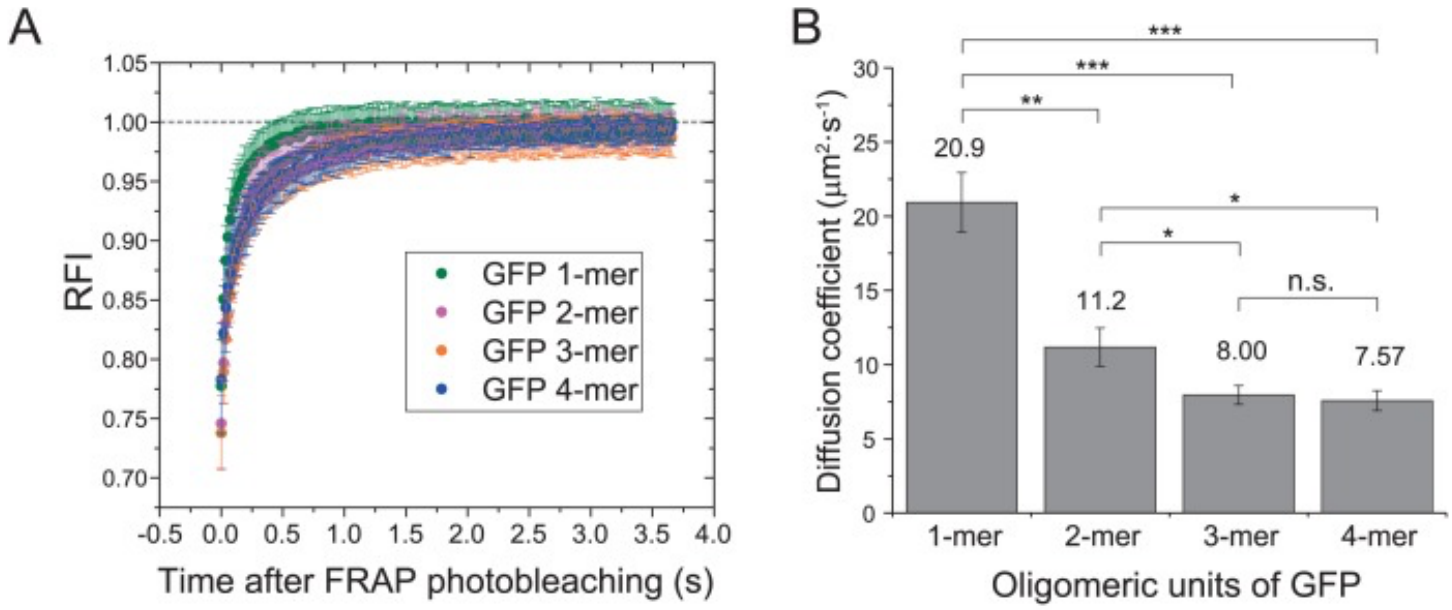
Typical values for diffusion coefficients in the nucleus

	D_{min} ($\mu m^2 s^{-1}$)	Accesible corral radius	Methods
Chromatin/ telomeres	$2 \cdot 10^{-3}$ $4 \cdot 10^{-4}$ $2 \cdot 10^{-5}$	0.08 µm 0.2 µm 0.3-0.8 µm	CLSM, single particle tracking
Transcription factor	10-15 (free) 0 - 0.01	up to 10 µm (nucleus)	FRAP (bound) FCS (free, transiently bound)
Membrane proteins	2-20 (2-D)	10 µm (nuclear membrane)	FRAP FCS

Accessible range of diffusion coefficients for CLSM, FRAP and FCS measurments

	t_{min} / t_{max}	Typical analysis volume	D_{min} / D_{max}
Confocal (single particle tracking)	0.4-2 sec / infinite	10 x 10 µm (x,y) 600 nm (z)	0 $\mu m^2 s^{-1}$ / 10 $\mu m^2 s^{-1}$
FRAP	0.4-2 sec / infinite	2 x 2 µm (x,y) 0.6 - 5 µm (z)	0 $\mu m^2 s^{-1}$ / 10 $\mu m^2 s^{-1}$
FCS	1 µsec / 1 sec	250 nm (x,y) 600 nm (z)	0.05 $\mu m^2 s^{-1}$ / 200 $\mu m^2 s^{-1}$

Diffusion coefficients of GFP multimere in the nucleus measured by FRAP (widefield microscope)



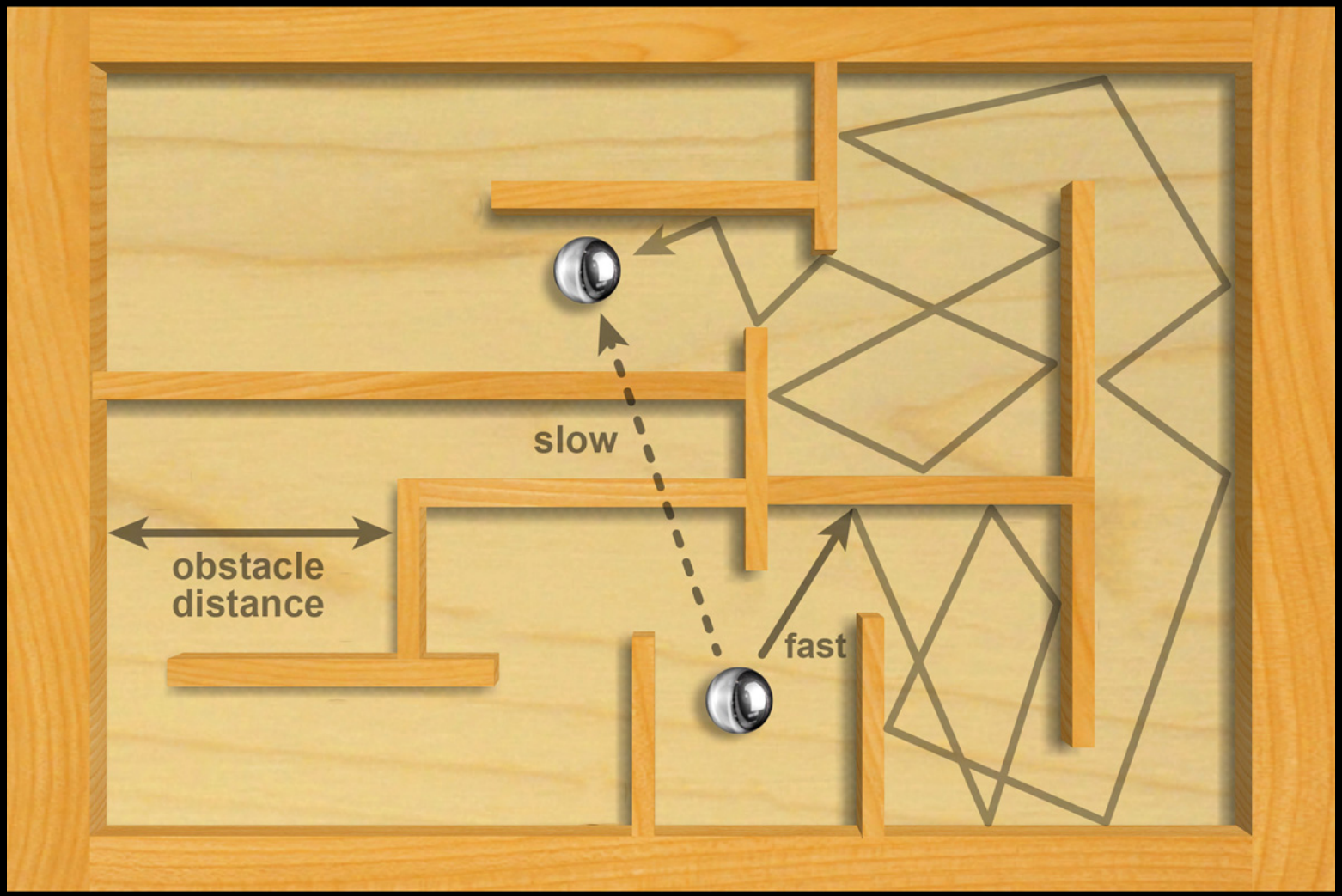
Kitamura & Kinja 2018, doi: 10.2142/biophysico.15.0_1

Diffusion coefficients of GFP multimeres in the nucleus measured by FCS

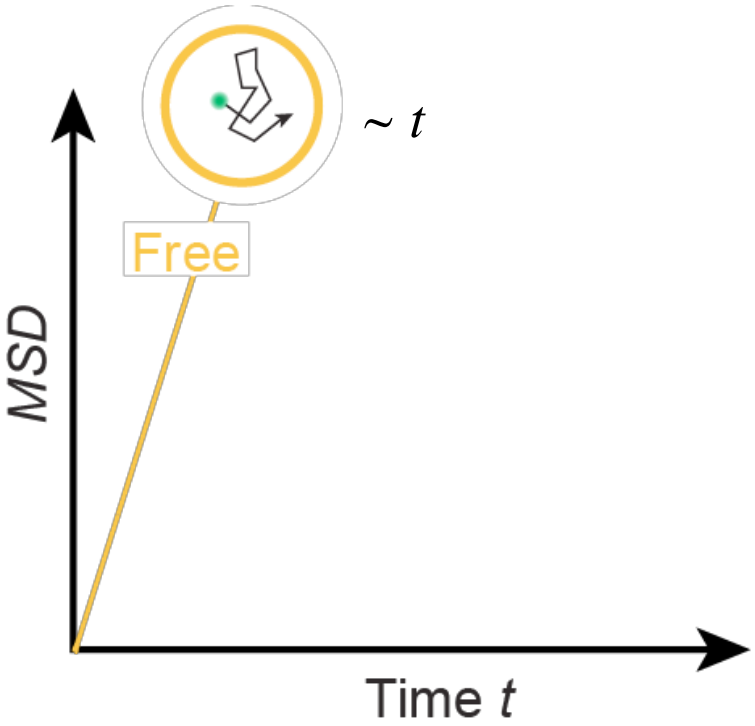
		NL (fast/first components)		FRAP
Cell line	Type of GFP	$D^{\ddagger}$ ($\mu m^2 s^{-1}$)	Fraction (%)	
HeLa	GFP_1	24.2 ± 1.3	–	20.9
	GFP_2	17.6 ± 1.2	–	11.2
	GFP_3	13.0 ± 1.0	–	8.00
	GFP_4	11.0 ± 0.7	–	7.57
	GFP_5	8.9 ± 0.4	–	

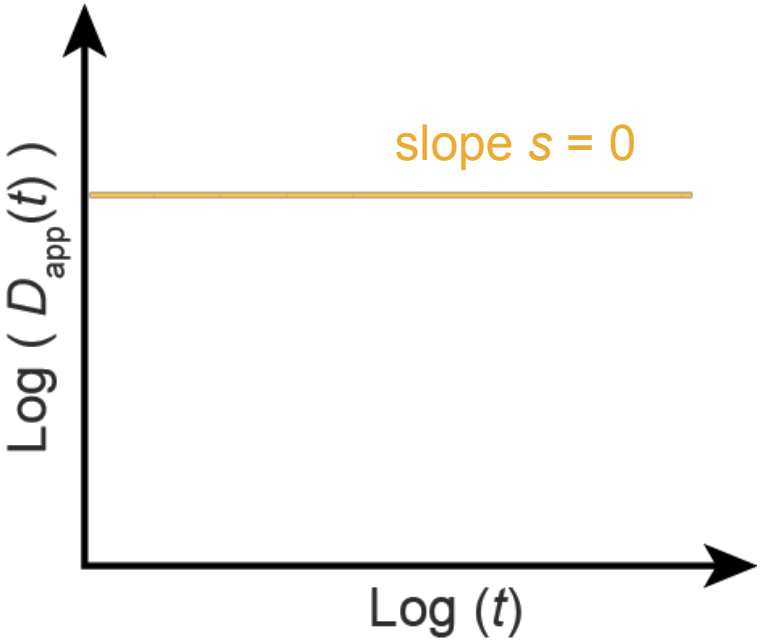
Pack et al. Biophysical journal 91, 3921–3936

Retrieving the intracellular topology from multi-scale protein mobility mapping in living cells



Scale independent free diffusion in solution

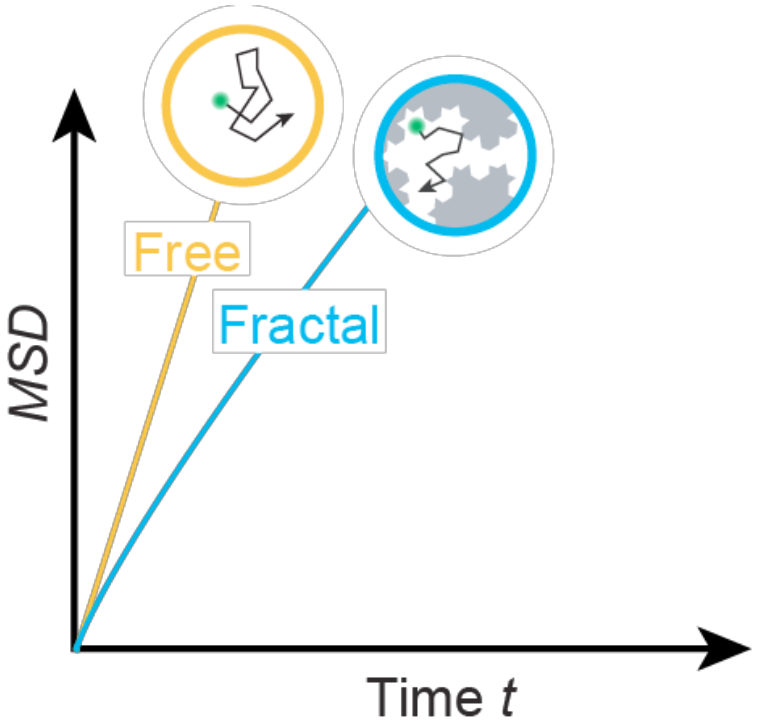


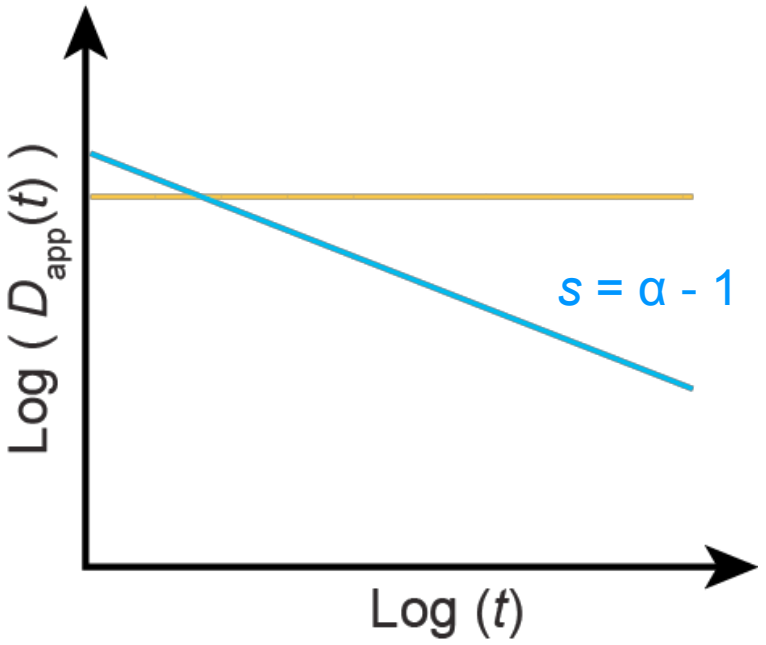


$$\left\langle d^2 \right\rangle \text{ or } MSD = 6 \cdot D \cdot t$$

$$D(t) = \text{const}$$

Anomalous diffusion in fractal environments



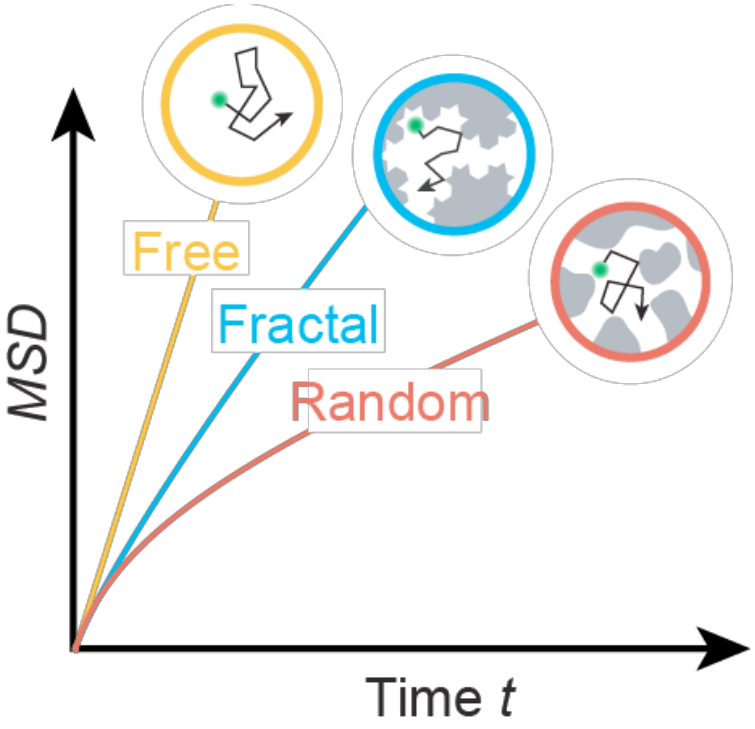


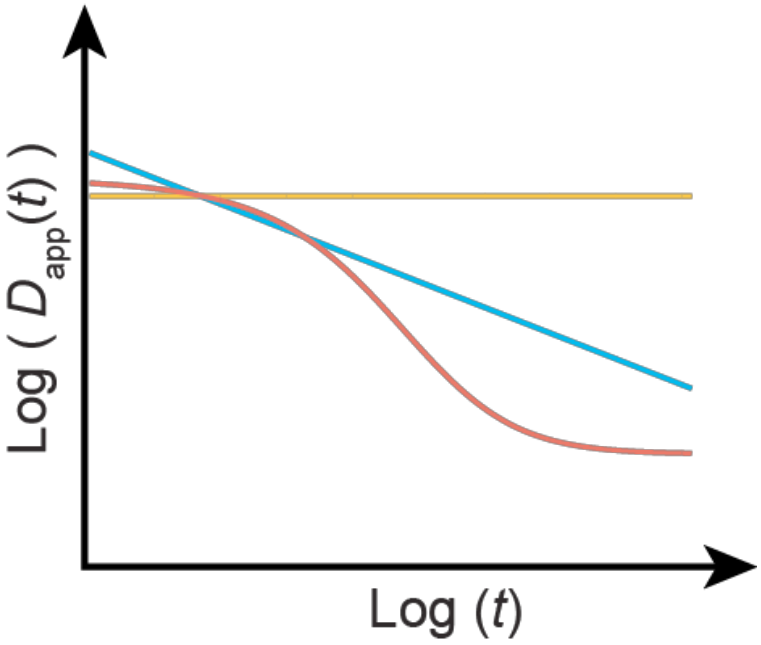
$$MSD = 6 \cdot \Gamma \cdot t^{\alpha}$$

$$MSD = 6 \cdot D_{\text{app}}(t) \cdot t$$

$$D_{\text{app}}(t) = \Gamma \cdot t^{\alpha-1}$$

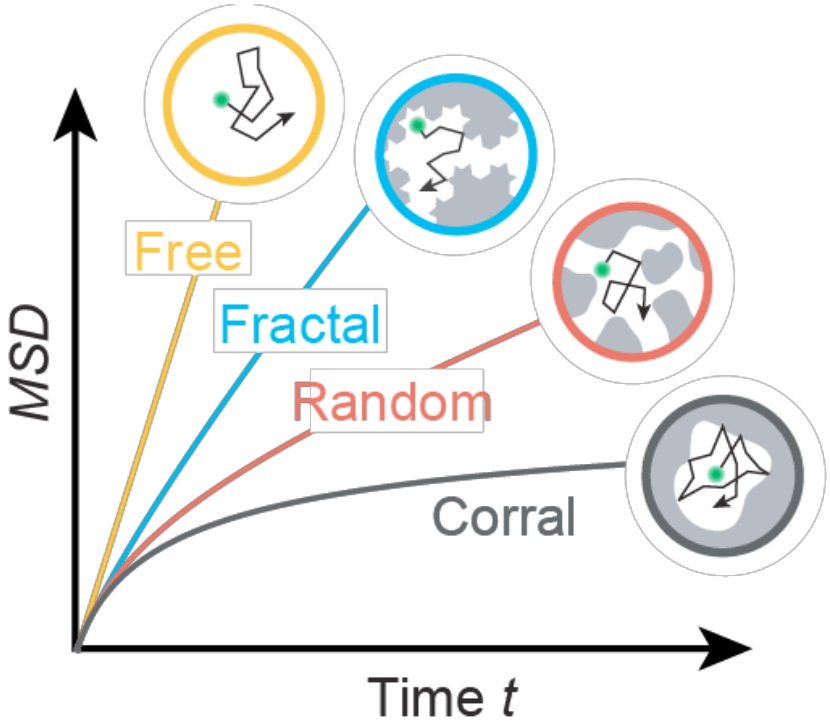
Anomalous diffusion in the presence of random obstacles/in porous media

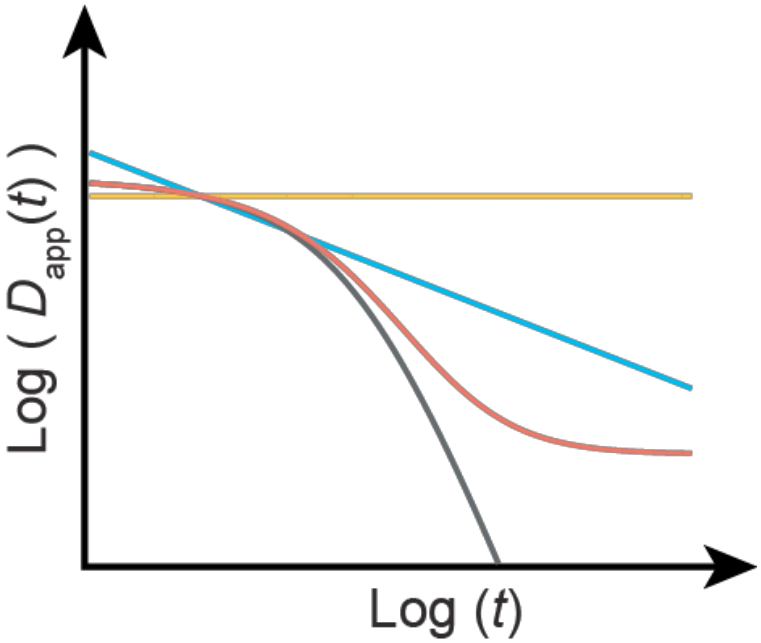




$$D(t) = (D_0 - D_\infty)\exp\left(-\frac{4\sqrt{D_0 t}}{\sqrt{\pi}\lambda}\right) + D_\infty$$

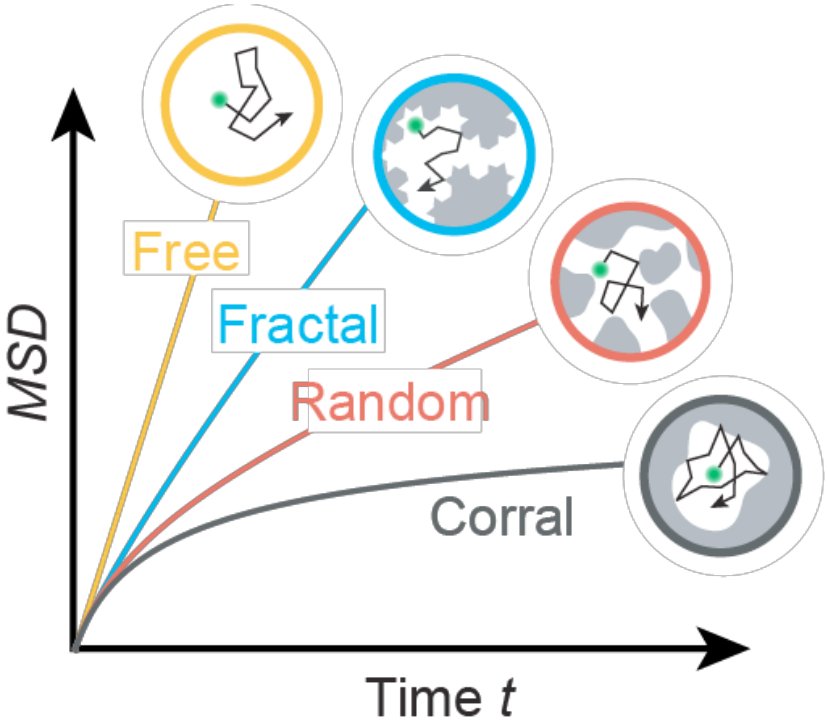
Trapping particles in corrals/confined diffusion

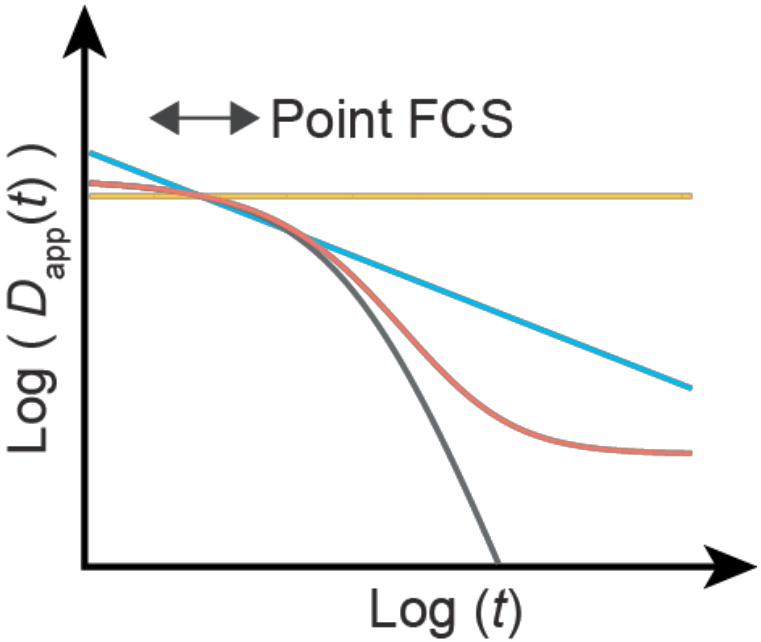


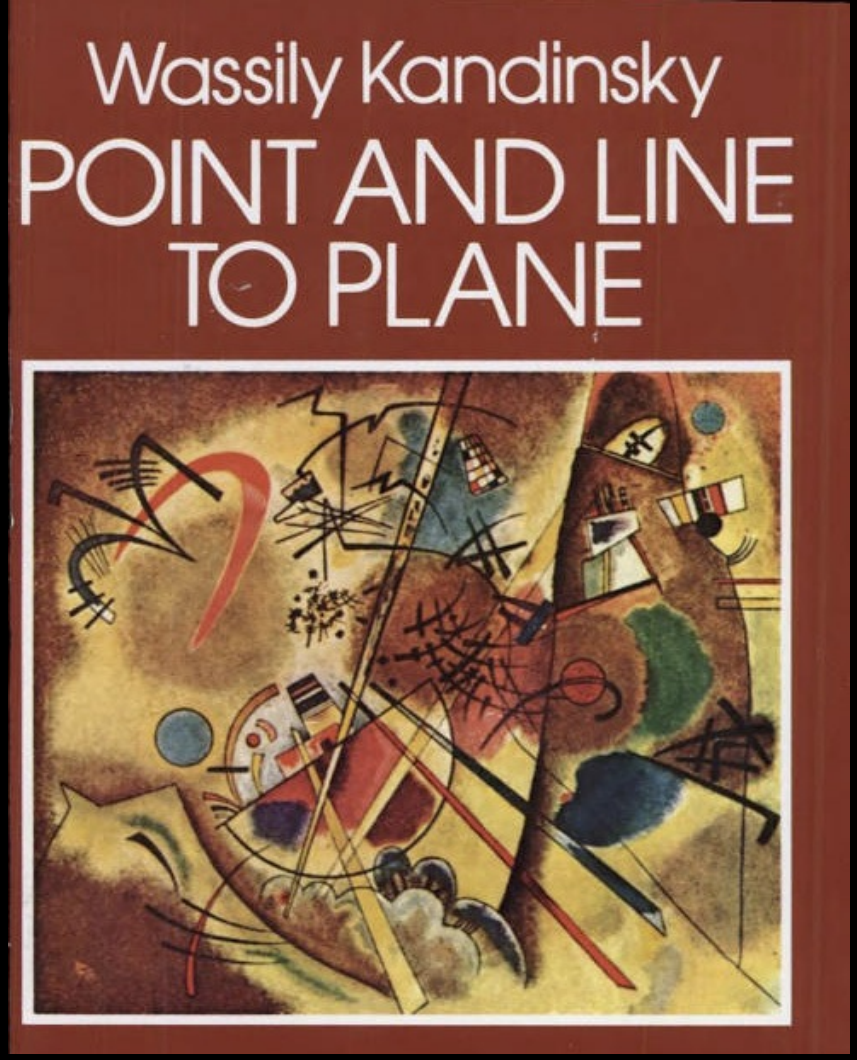


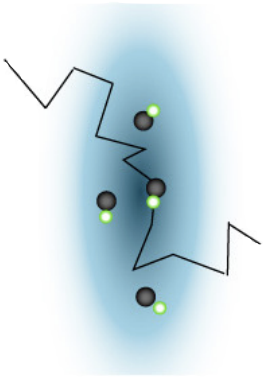
$$D(t) = D_0 \cdot \exp\left(-\frac{4\sqrt{D_0 t}}{\sqrt{\pi}\lambda} \right)$$

Small range of point FCS measurements

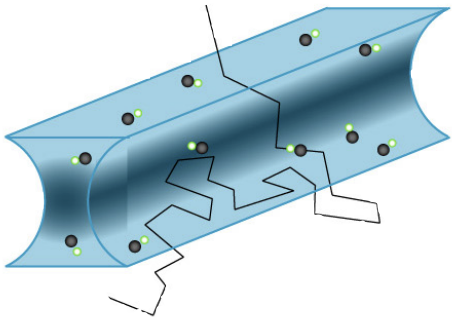




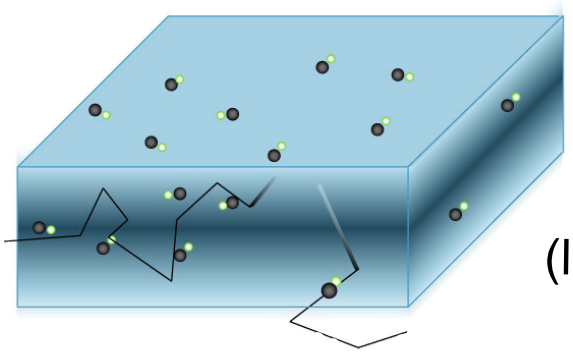




point (confocal)



line (cylindrical lens)



plane
(light sheet)

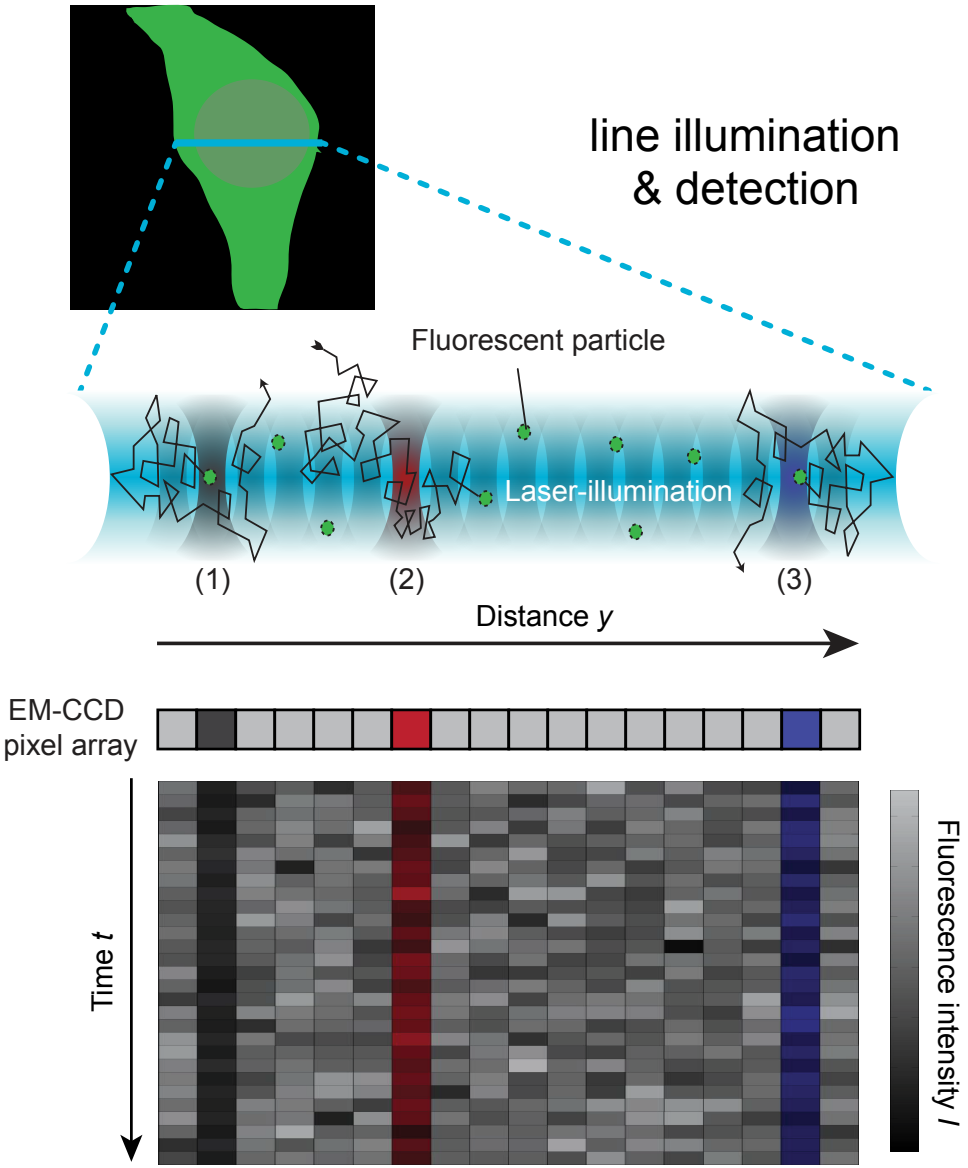
Gerrit Heuvelman

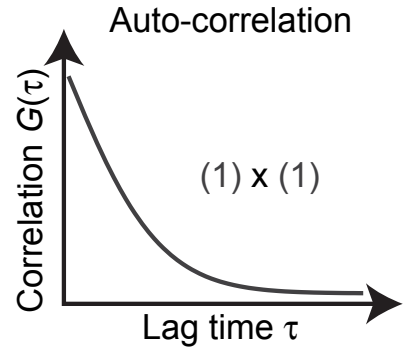
Malte Wachsmuth

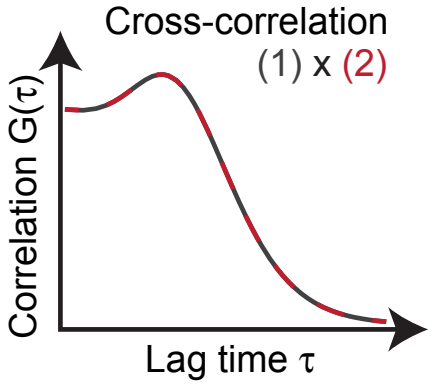
Michael Baum

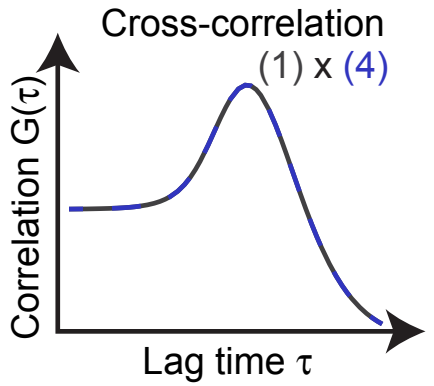
Fabian Erdel

Measuring time-dependence of protein mobility by multi-scale fluorescence cross-correlation spectroscopy (msFCCS)



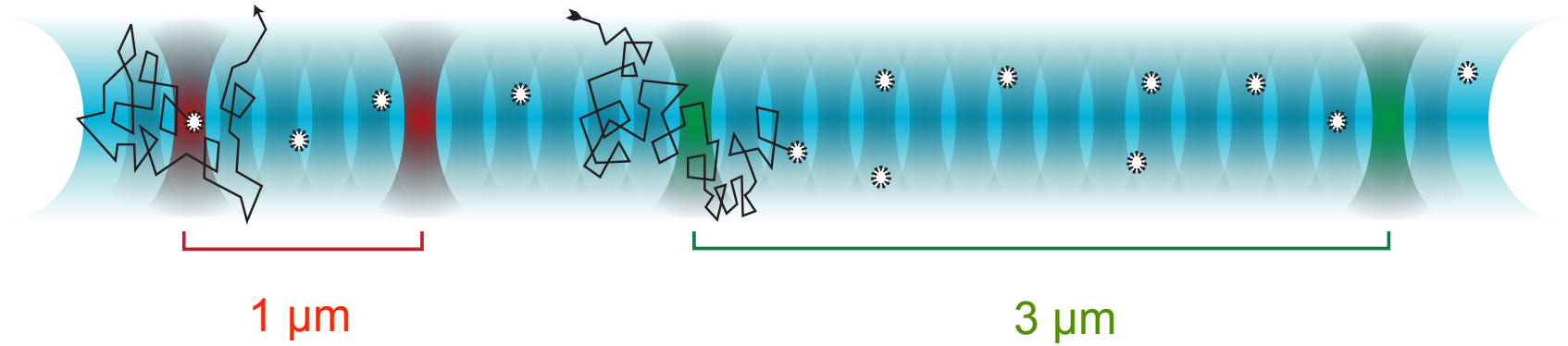


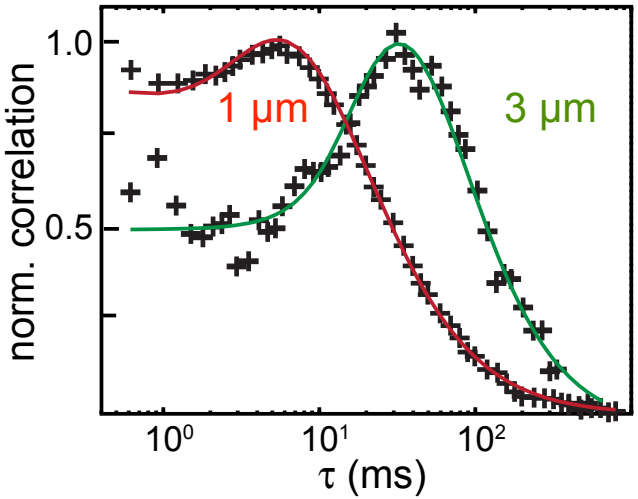


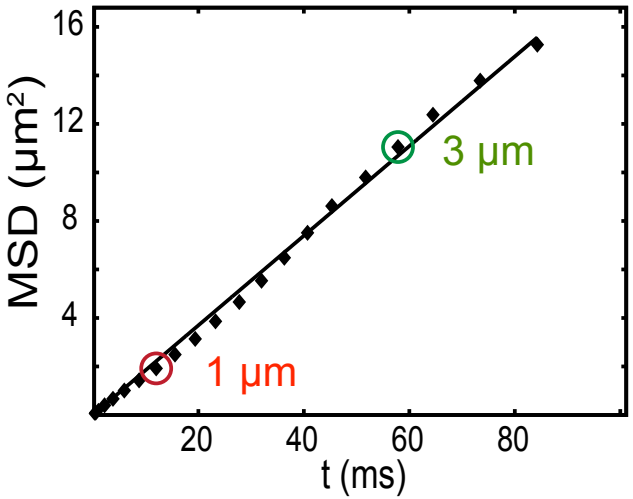


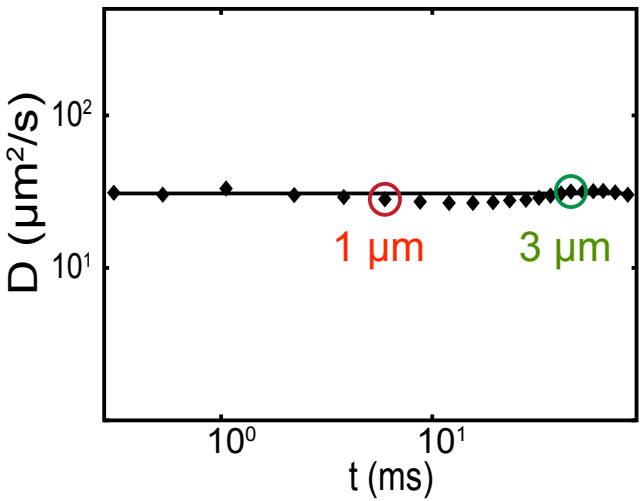
Heuvelman 2009, *Eur. Biophys. J.;* Baum 2014, *Nat. Commun.*

Validation: msFCCS of GFP *in vitro* in buffer solution







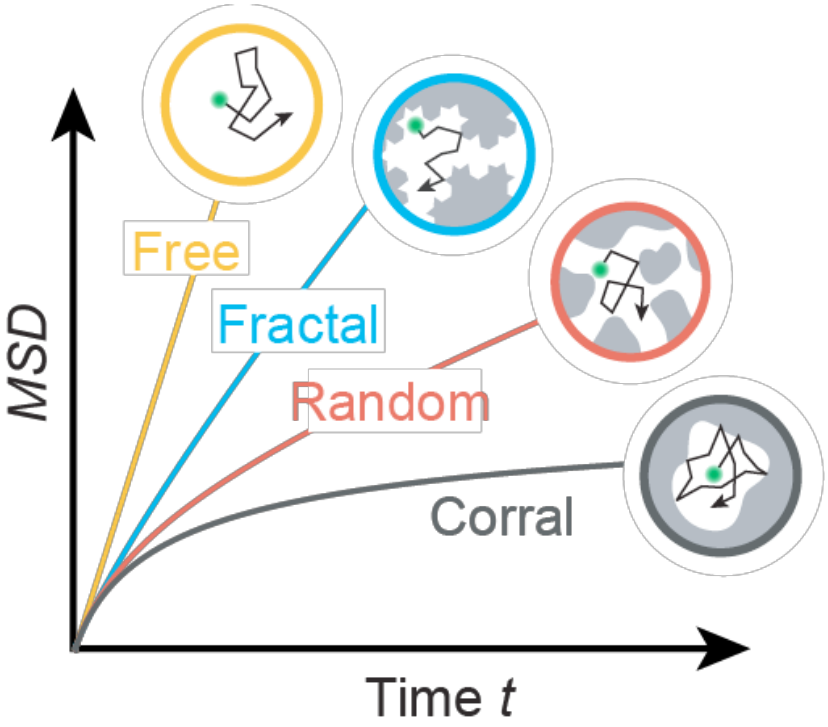


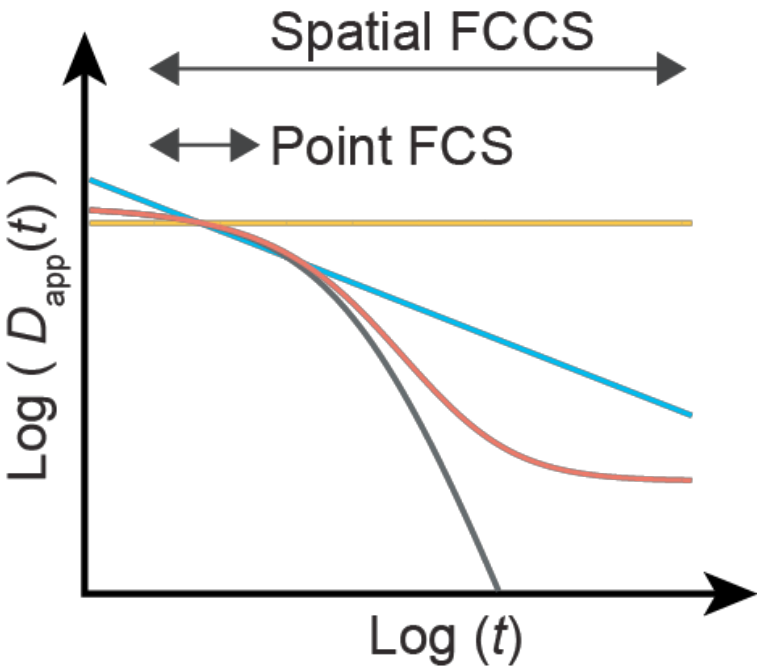
Quantitate random motion: Mean Squared Displacement (MSD) vs. time

$$MSD = 6 \cdot D \cdot t$$

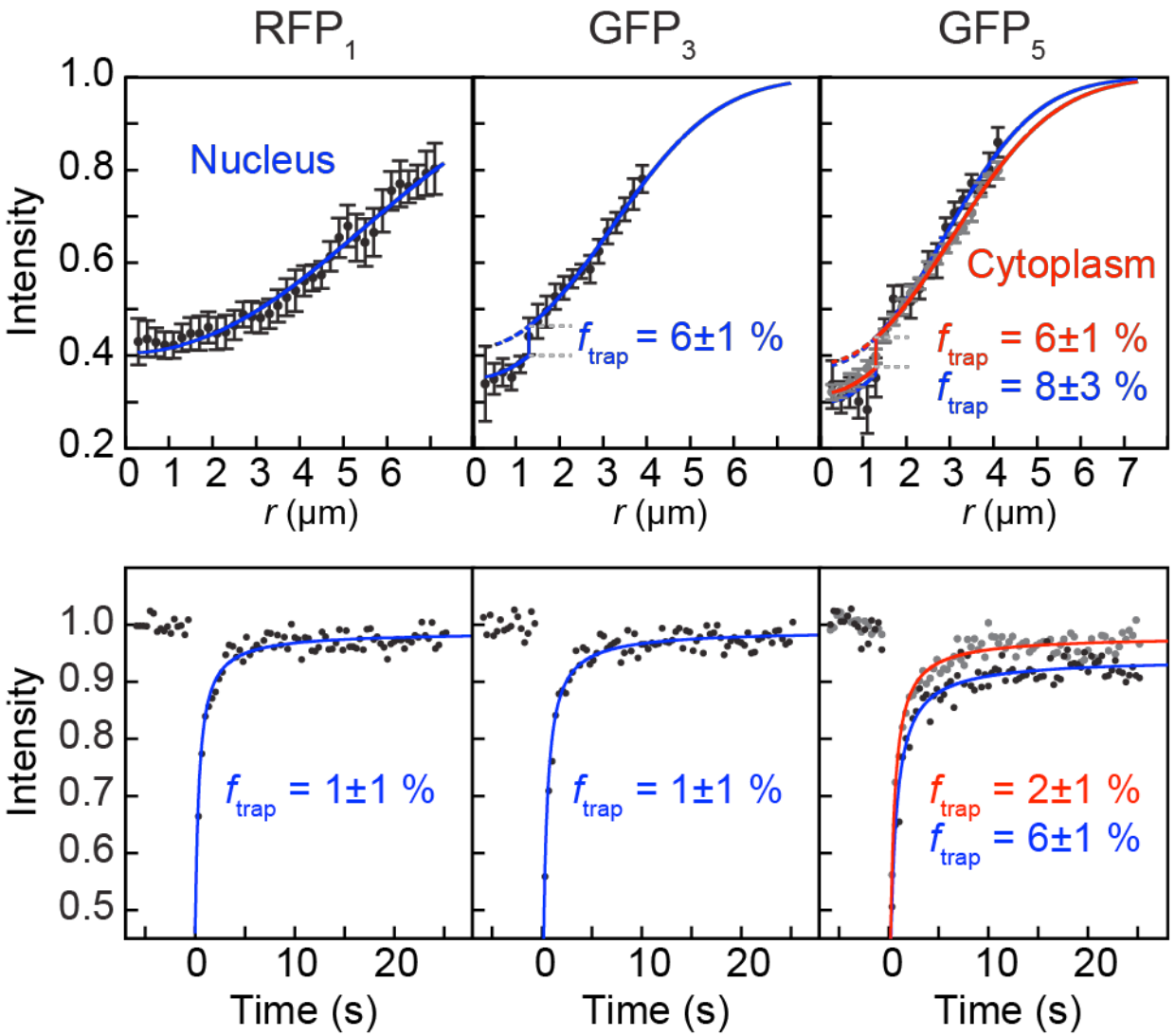
$$D_{cor} = \frac{d^2_{cor}}{6 \cdot \tau_{cor}}$$

Multi-scale mobility measurements reveal topology of the particle environment





Comparison of spatial msFCCS and FRAP results for measurements of a bleach spot of 1.3 µm diameter



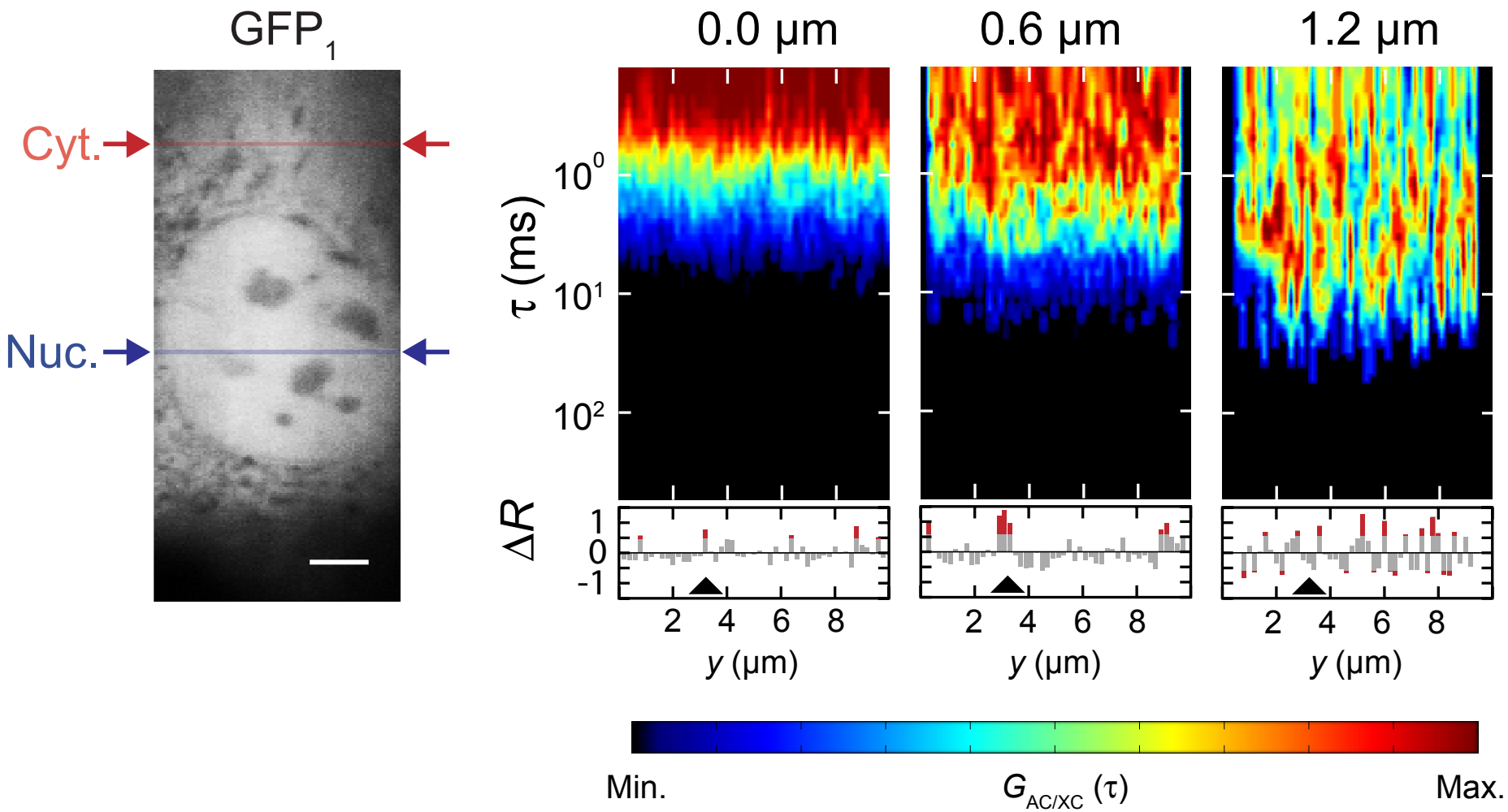
Spatial FCCS: D_{app} (µm^2·s^{-1})	
GFP_1	32 ± 3
GFP_3	14 ± 2
GFP_5	11 ± 1

d = 1.3 µm

FRAP: D_{app} (µm^2·s^{-1})	
RFP_1	31 ± 7
GFP_3	15 ± 4
GFP_5	10 ± 1

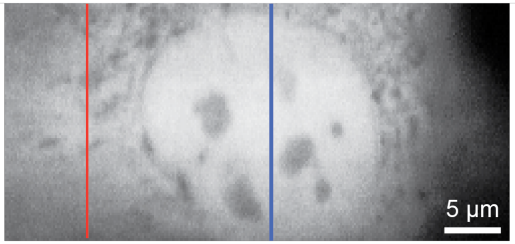
w_{bleach} = 1.3 µm

msFCCS protein mobility maps of GFP monomer in the nucleus



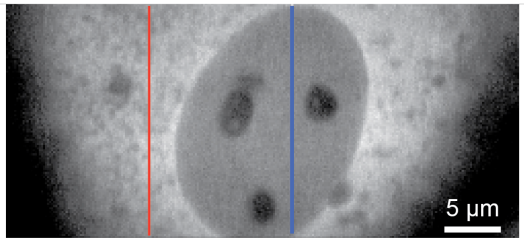
Spatiotemporal mobility analysis of GFP multimers in the nucleus and the cytoplasm of living cells

GFP_1
r_h = 2.8 nm



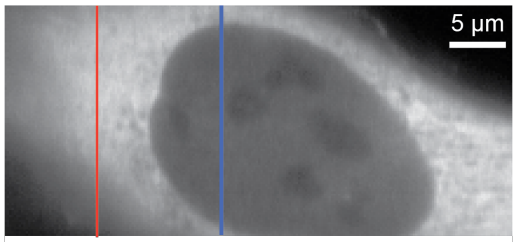
cytoplasm nucleus

GFP_3
r_h = 5.5 nm

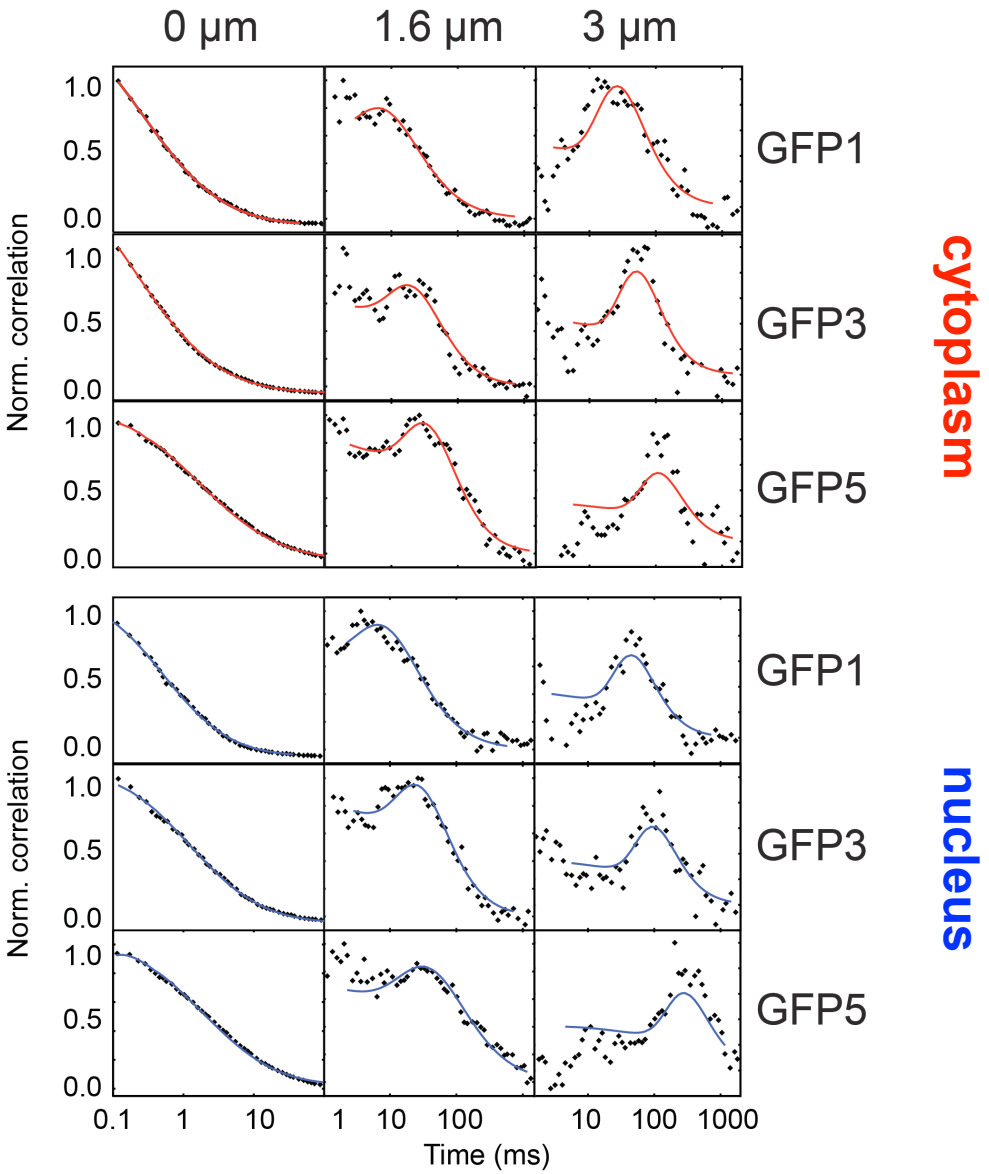


cytoplasm nucleus

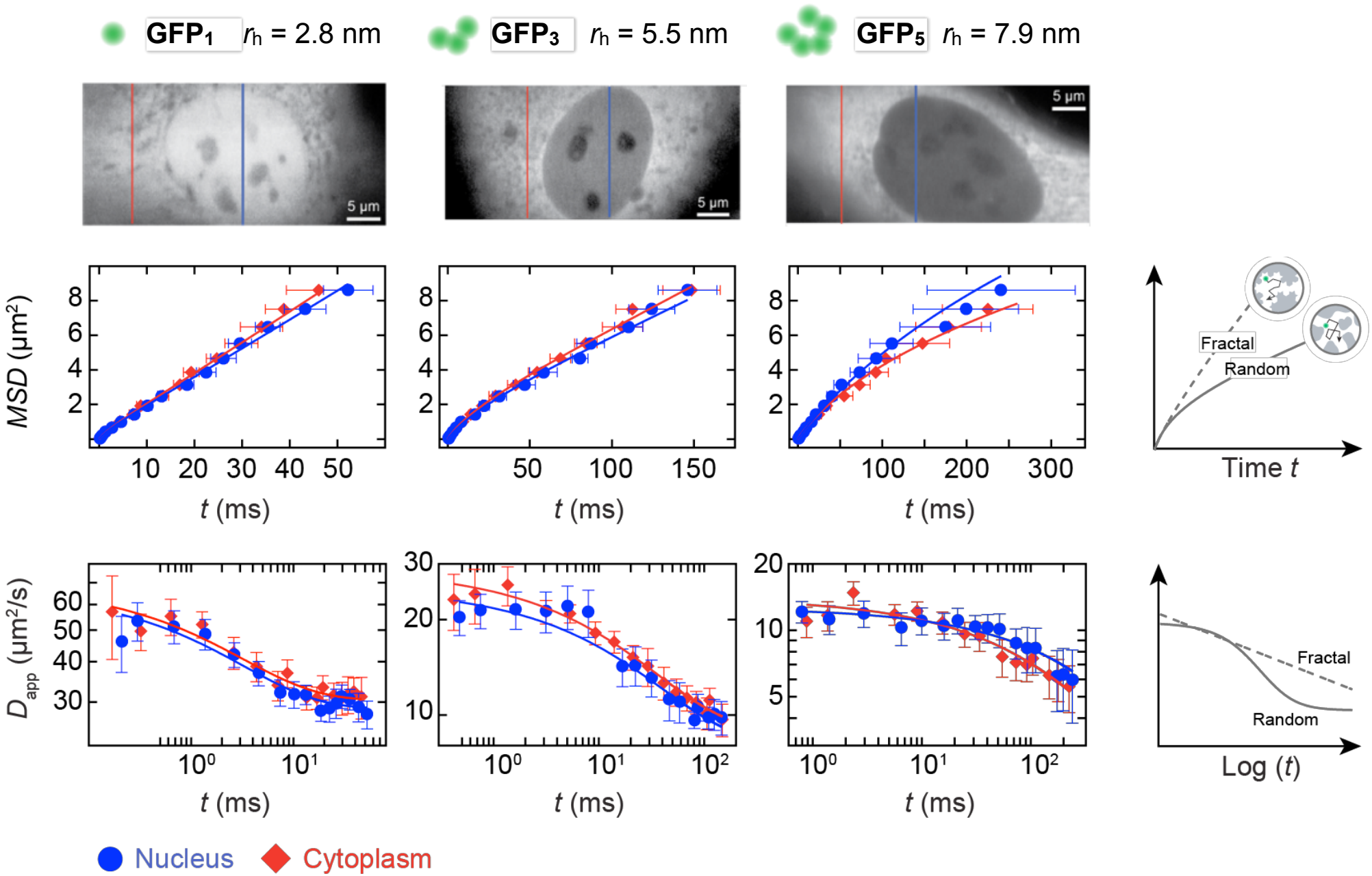
GFP_5
r_h = 7.9 nm



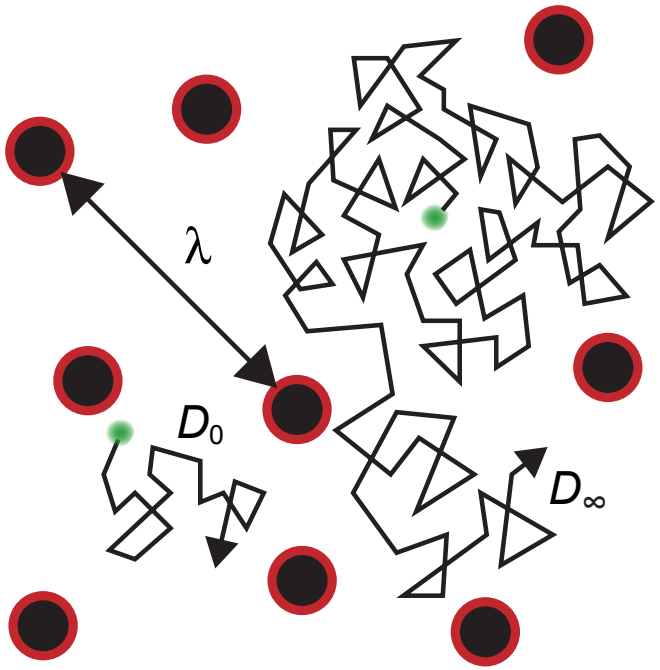
cytoplasm nucleus

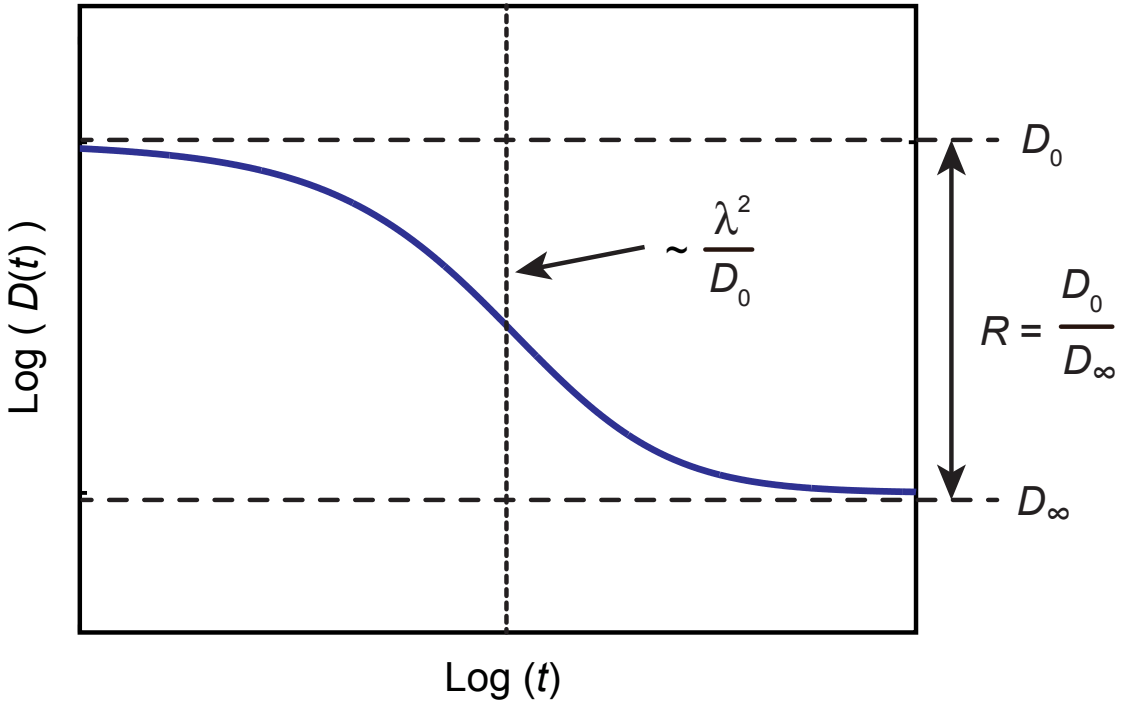


Time-dependent protein diffusion in living cells



Model for diffusion in porous media





$$D(t) = (D_0 - D_\infty)\exp\left(-\frac{4\sqrt{D_0 t}}{\sqrt{\pi}\lambda}\right) + D_\infty$$

Model parameters:

D_0 : Microscopic diffusion coefficient

D_∞ : Macroscopic diffusion coefficient

λ : Correlation length

Derived parameters:

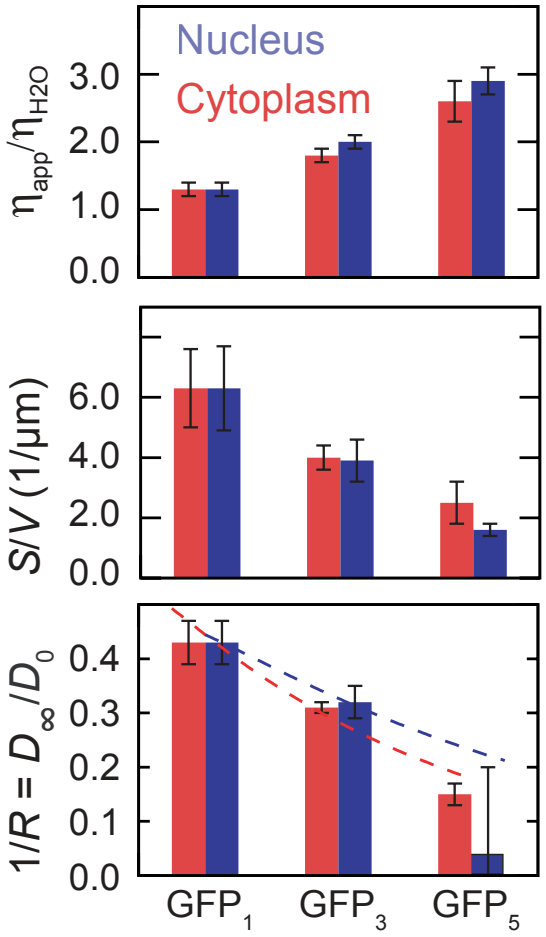
R : Retardation / tortuosity

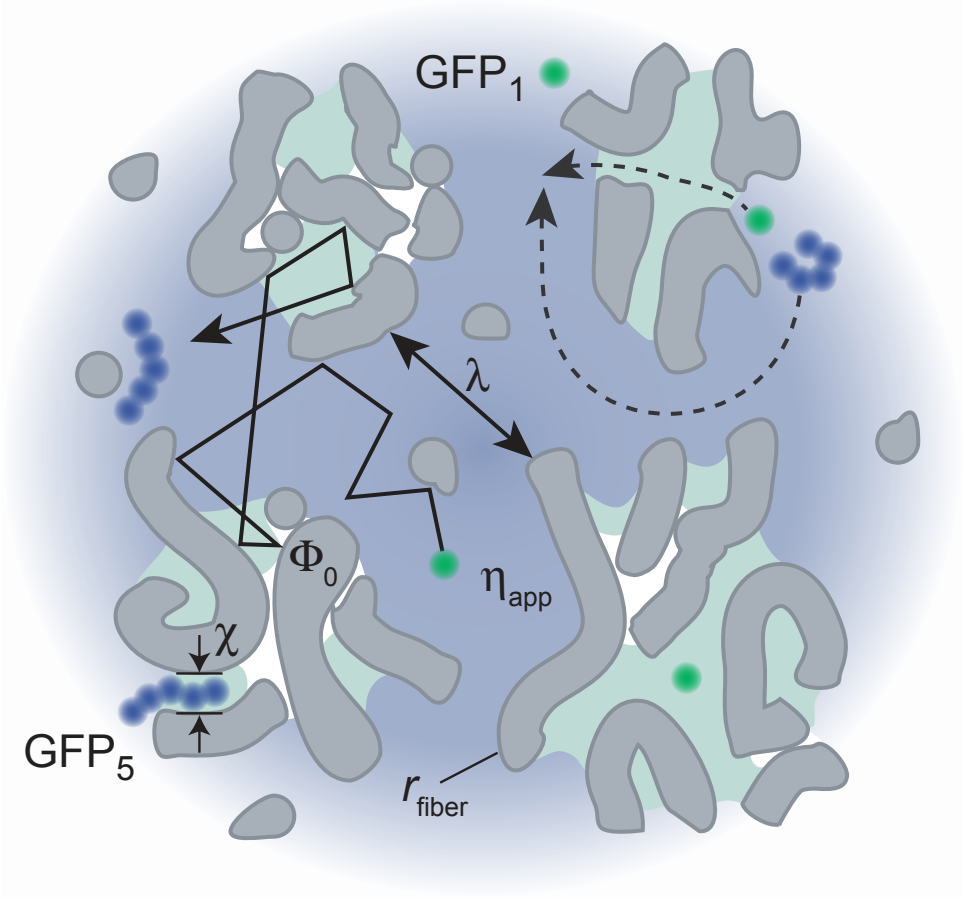
S/V : Specific surface

η_{app}/η_{H2O} : Viscosity ratio

Model from Loskutov et al., Journal of Magnetic Resonance, 2013

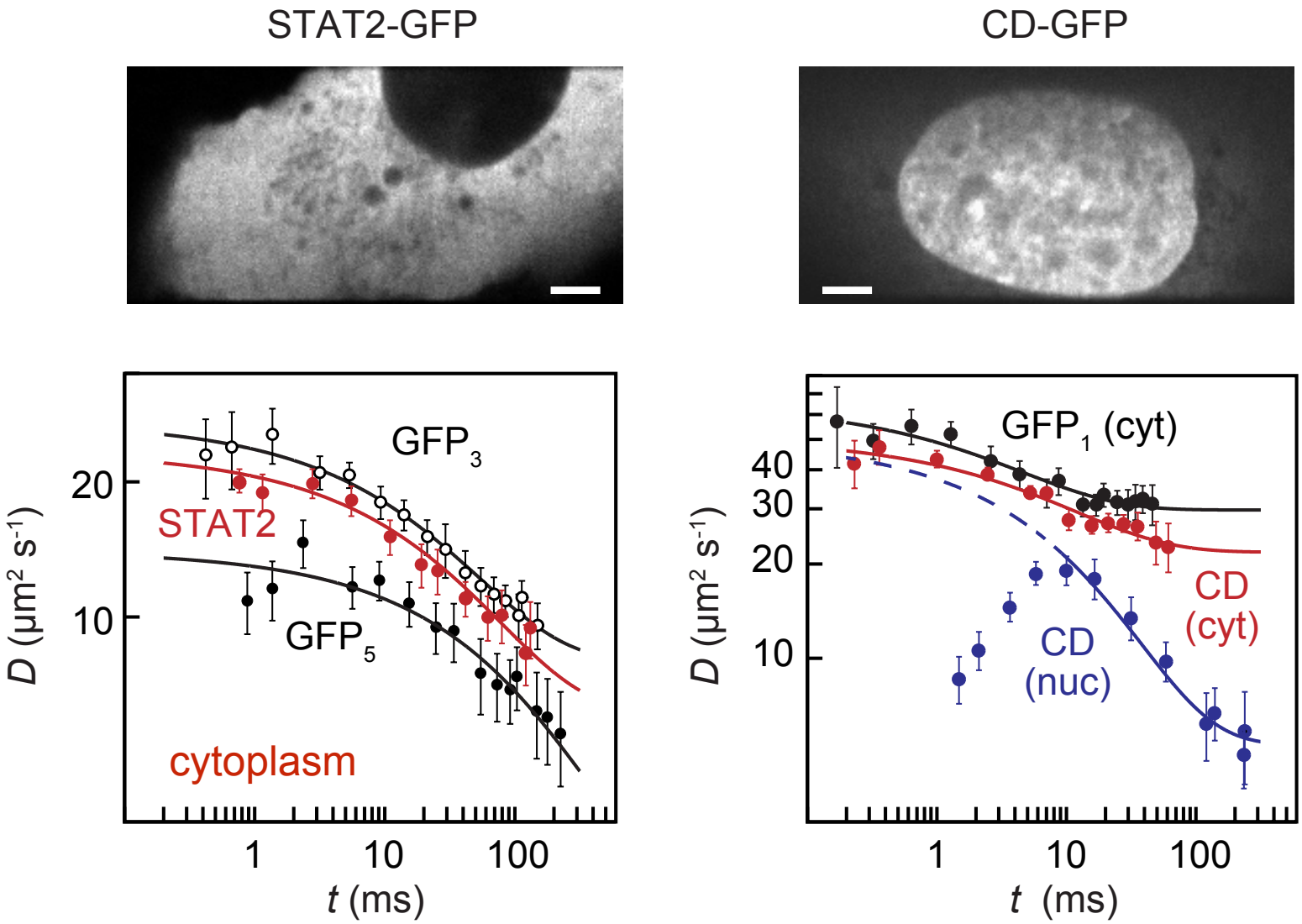
Parameters for intracellular obstacle structure derived from the fit to the porous medium model



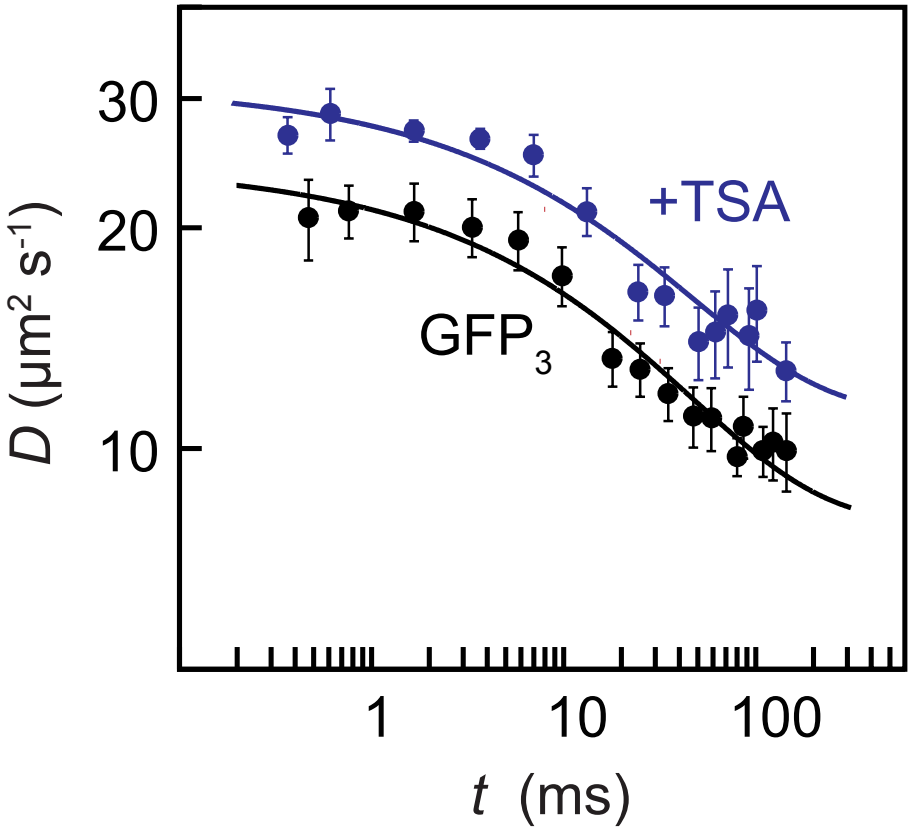


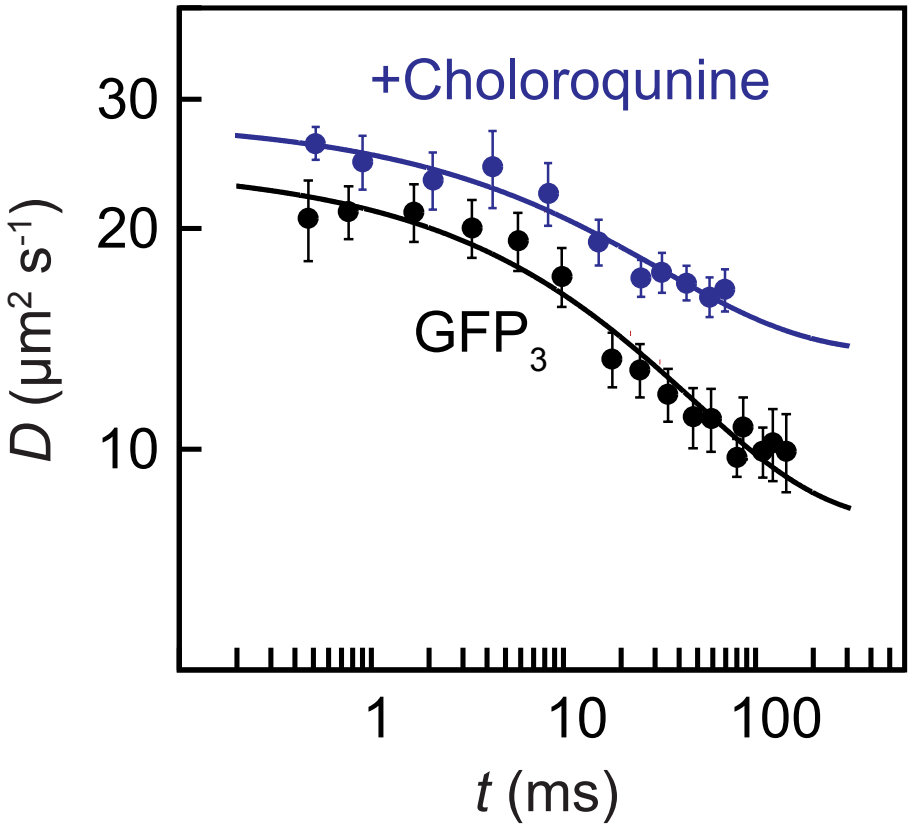
		Φ_0 (%)	r_{fiber} (nm)	λ_{GFP} (µm)	χ (nm)
Parameters for fiber shaped obstacle structure:	Nucleus	18 ± 5	6 ± 2	0.8 ± 0.2	~15
	Cytosol	12 ± 9	4 ± 2	0.8 ± 0.2	>15

msFCCS protein mobility maps of transcription factor STAT2 and a chromatin interacting chromodomain protein (CD)



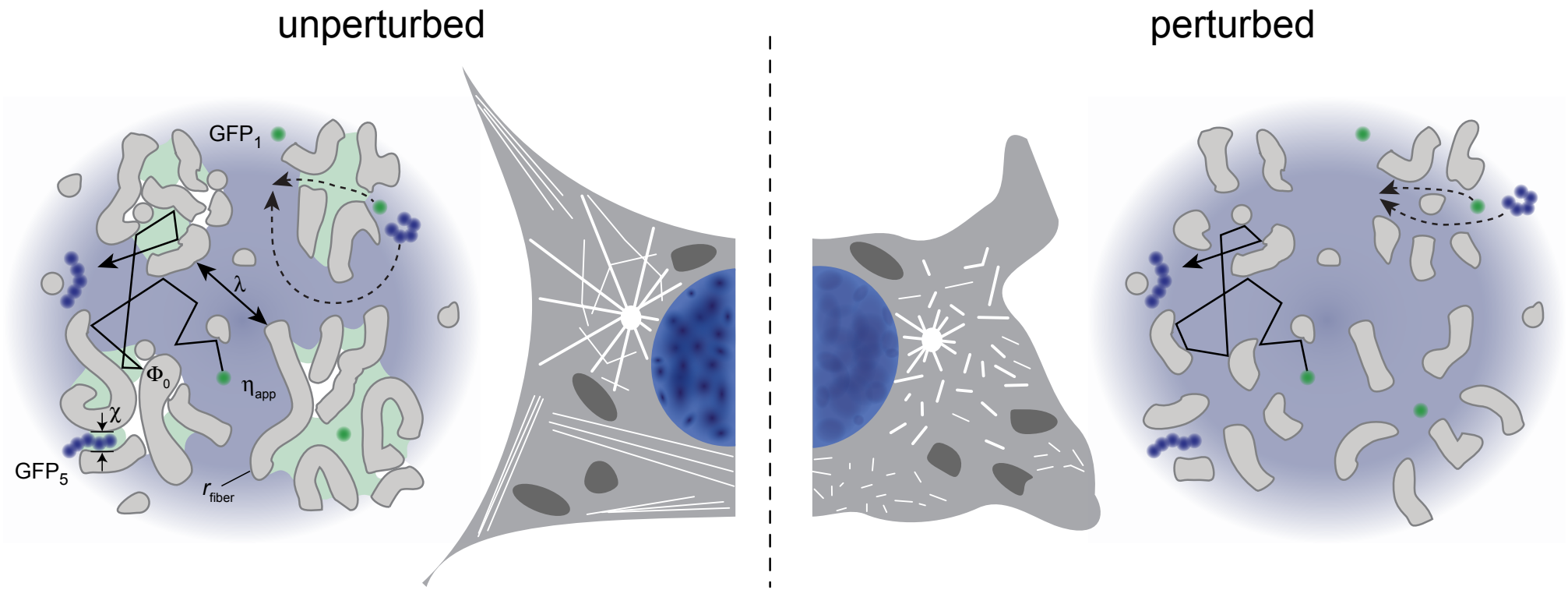
Drug induced chromatin decondensation increases overall nuclear protein mobility and reduces retardation R (= D_0/D_∞)





GFP_3 : R = 3.1 ± 0.3, λ = 1.6 ± 0.3
+ TSA: R = 2.8 ± 0.7, λ = 2.0 ± 0.8
+ CQ : R = 2.1 ± 0.3, λ = 1.7 ± 0.7

A quantitative model of the intracellular structure on the nanometer scale as it is “sensed” by a protein



- Cellular interior is not fractal but has random obstacle / porous medium topology
- Quantitative description with a three parameter model (D_0, D_∞, λ)
- Nucleoplasm and cytoplasm very similar except for some GFP_5 trapping in the nucleus
- Protein size dependent searched surface, larger particles partially excluded
- Determines target search processes in a size dependent manner
- Perturbances of cellular structure by drugs that target chromatin, cytoskeleton etc.

Baum 2014, *Nat. Commun.*; Erdel 2014, *J. Phys. Condens. Matter*, 2015