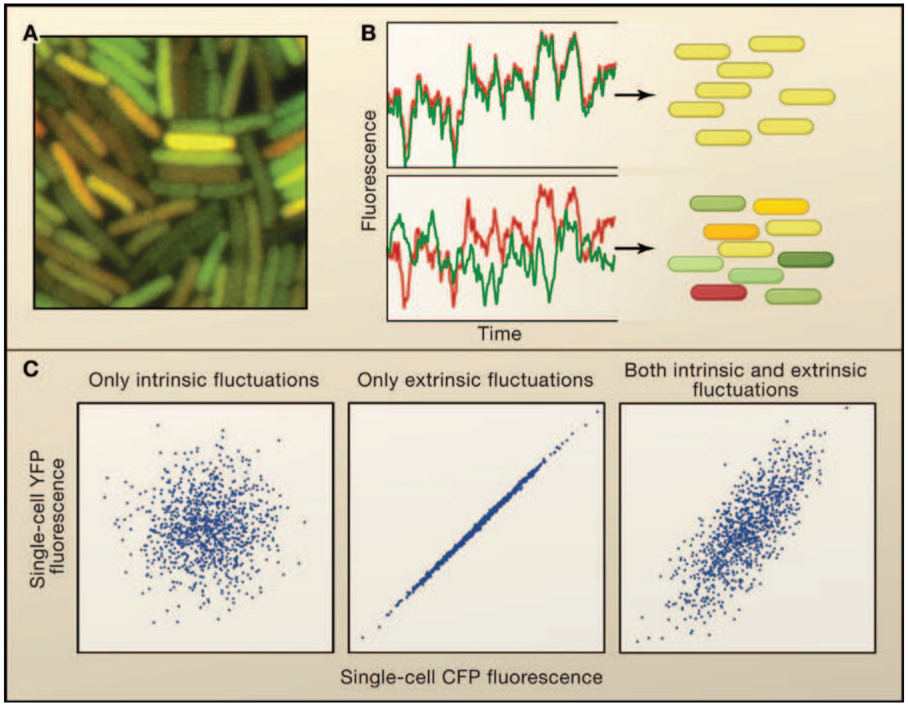
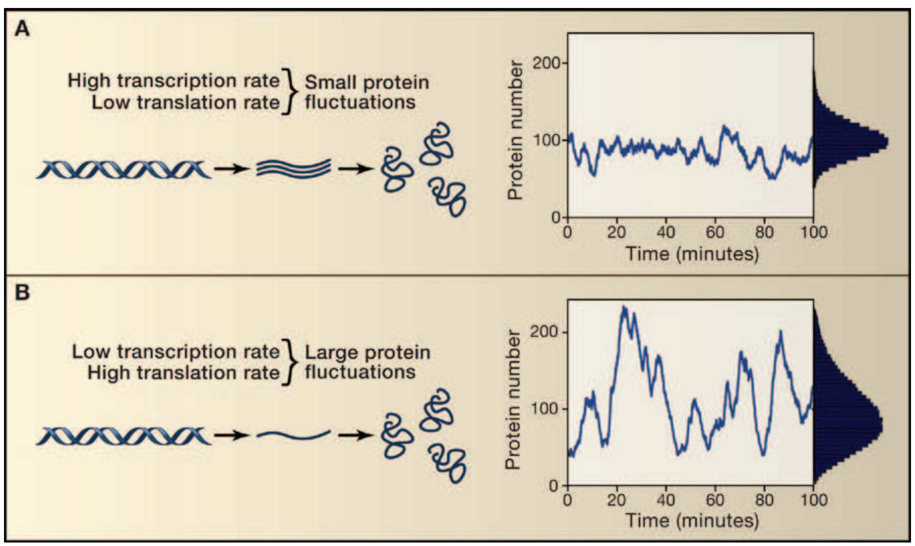
Molecular search in gene regulation

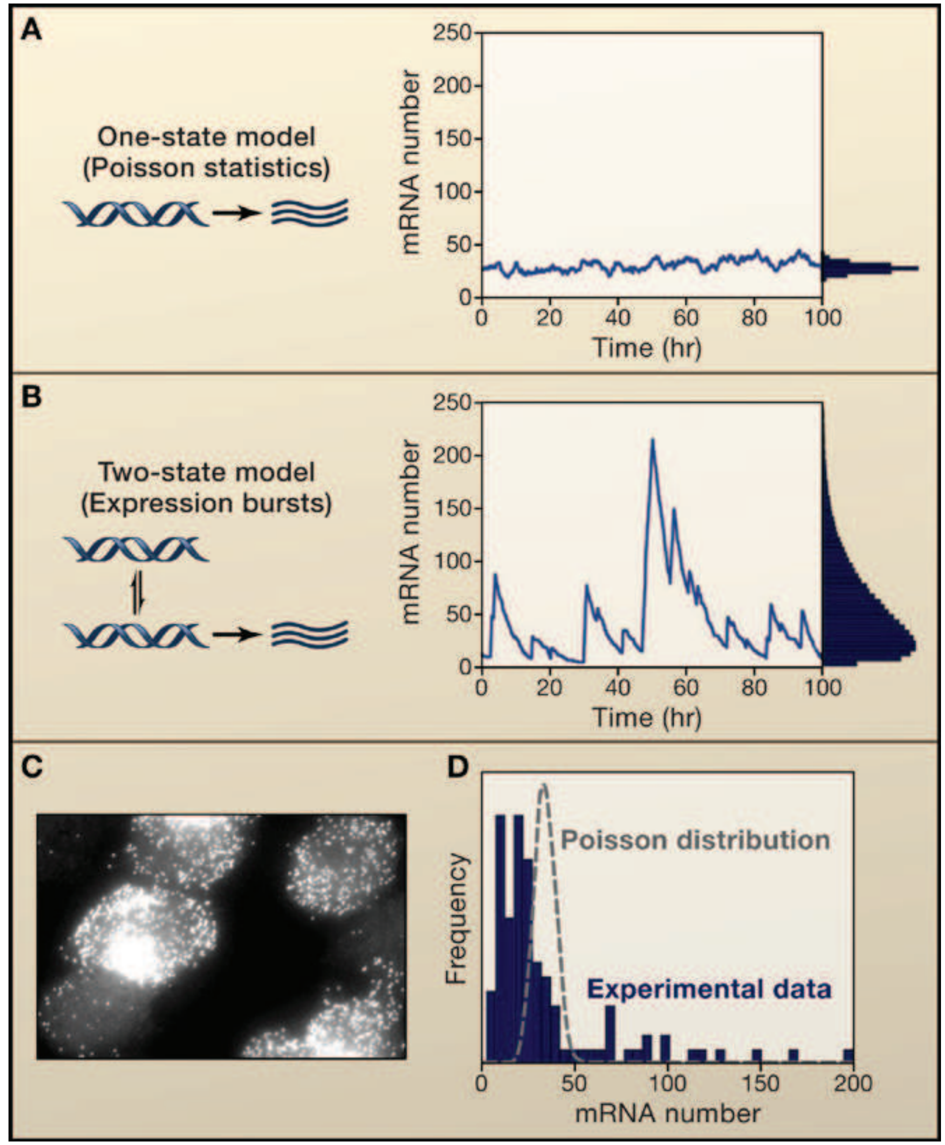
— POSTECH, Pohang 14th January 2020 —

Sources of cellular noisiness: chemical vs physical

Ellowitz et al, Science (2002)







Plus noise due to spatial spreading of TFs!

A Raj & A van Oudenaarden, Cell (2008)

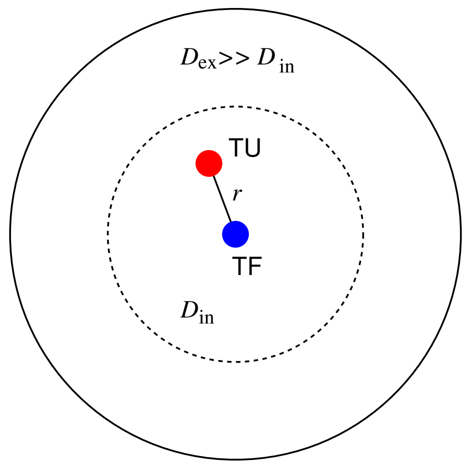
From bacteria cells (E.coli & Co.) to spherical cows

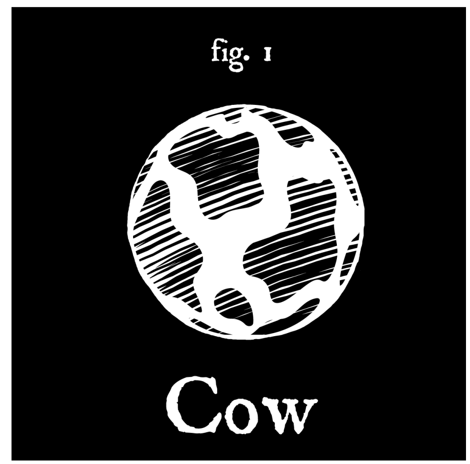
Cell size: roughly 2μm $\times$ $1/2\mu$m
Cell volume: $\approx 1\mu$m^3

DNA length: 4.7×10^6 base pairs or ≈ 1.6mm

Number of proteins in cell: $\approx 2.4 \times 10^6$
Different proteins (# genes): 4,300

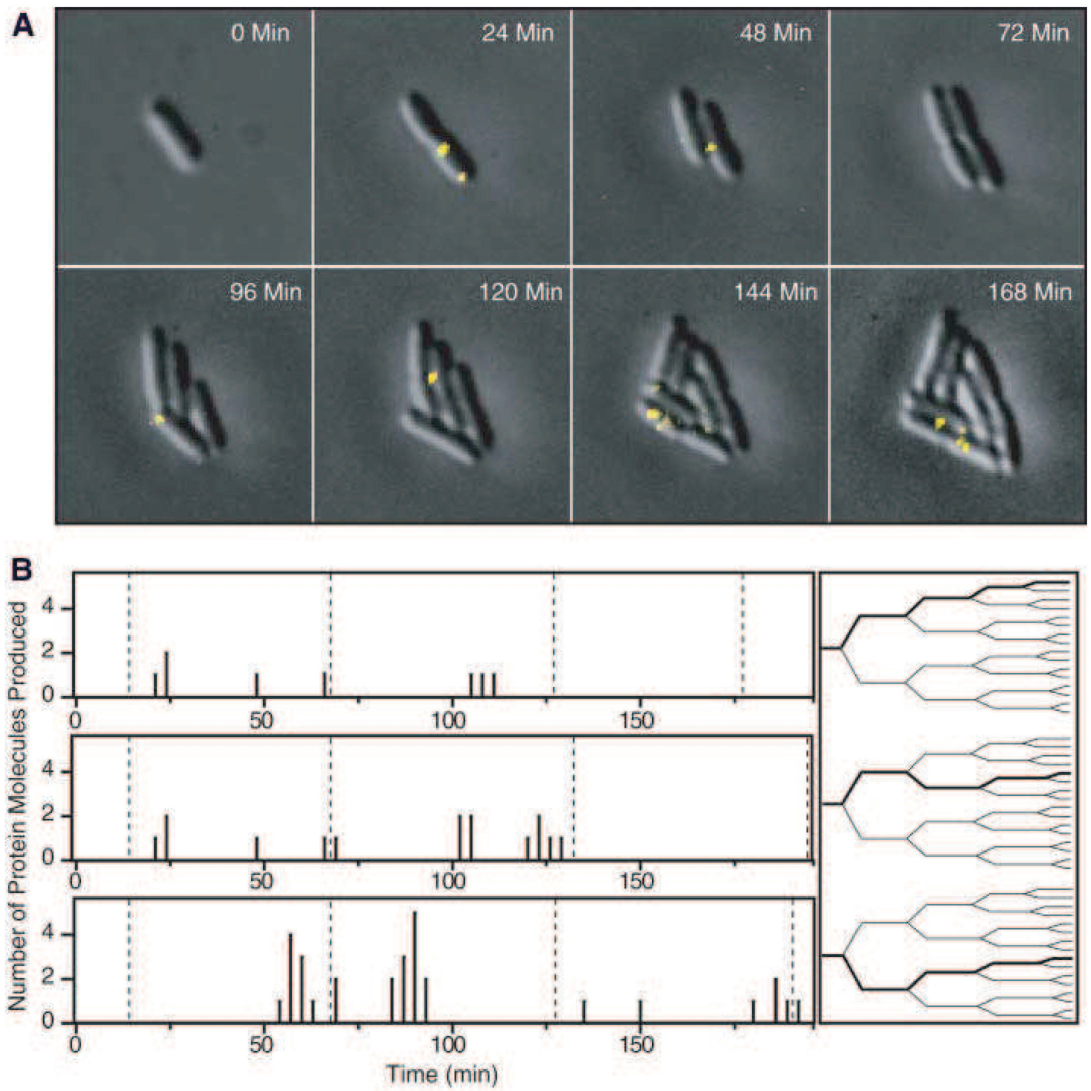
Some proteins occur only as few or few tens of copies/cell (nM concentrations)





RW Corbin et al, PNAS (2003)

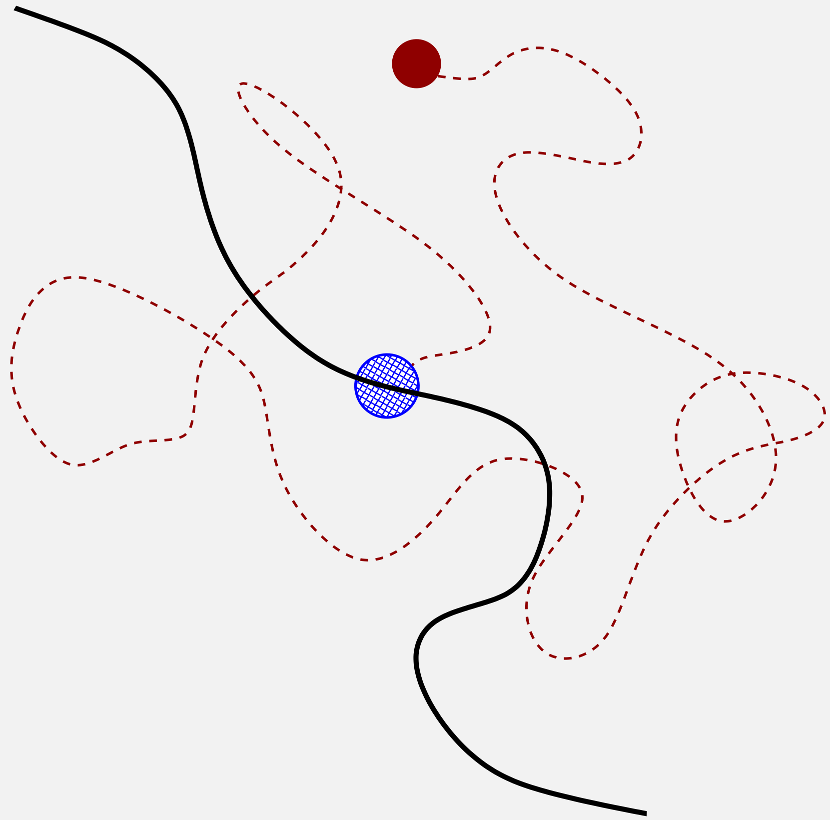
Gene expression one molecule at a time in E.coli



synthesised proteins (bursty) along three cell lineages, dashed lines marking cell divisions

Yu et al, Science (2006); I Golding et al, Cell (2005)

Smoluchowski search picture



Search rate for a particle with diffusivity D_{3d} to find an immobile target of radius a (assuming immediate binding):

$$k_{\mathrm{on}}^{S} = 4\pi D_{\mathrm{3d}} a$$

Protein-DNA interaction: $a \approx$ {few bp} $\approx$ 1nm
$D_{\mathrm{3d}} \approx 10\mu\mathrm{m}^2/\mathrm{sec}$ (typically $\varnothing_{\mathrm{TF}} \approx$ 5nm):

$$k_{\mathrm{on}}^{S} \approx \frac{10^8}{(\mathrm{mol/l}) \times \mathrm{sec}}$$

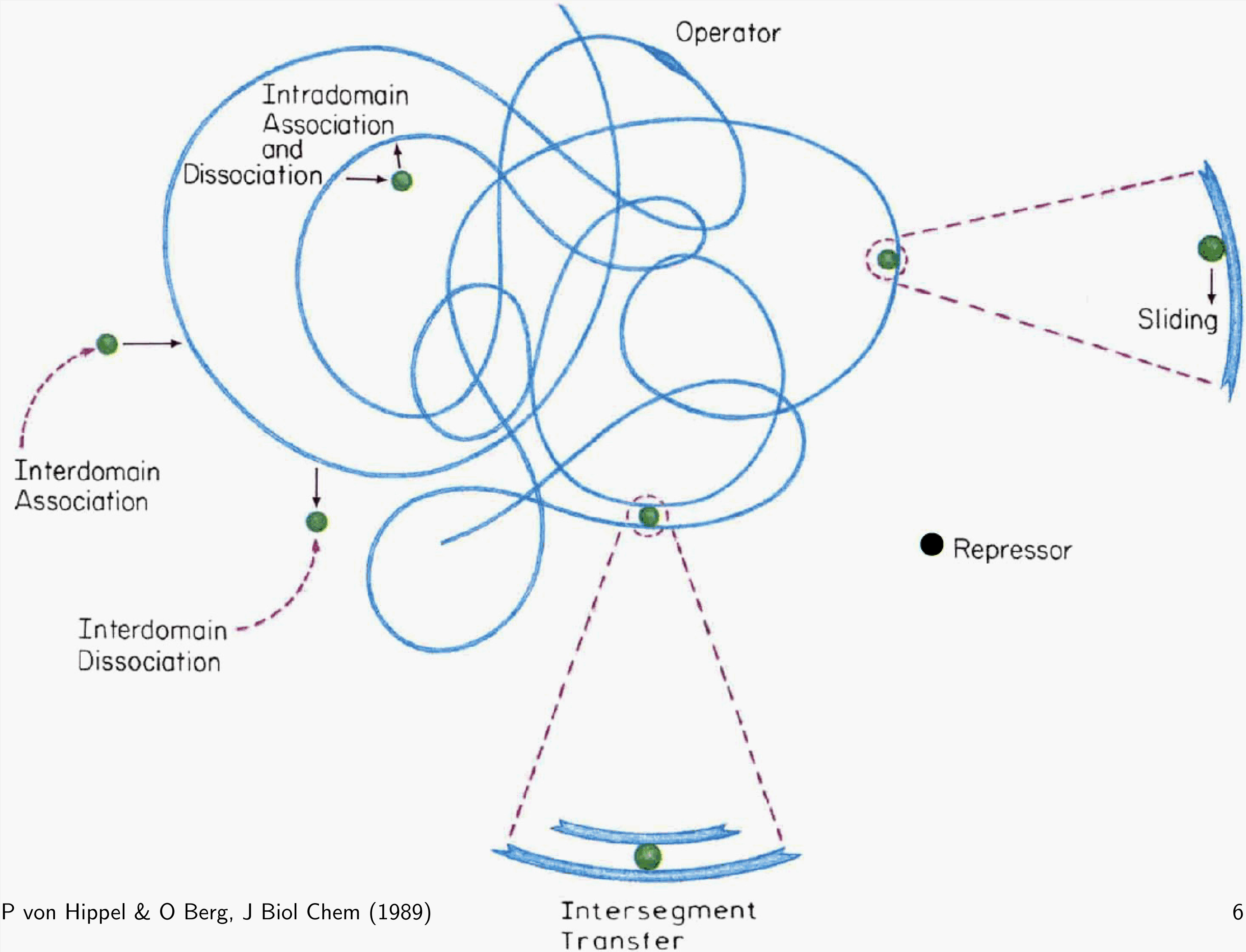
Lac repressor [AD Riggs, S Bourgeois, M Cohn, J Mol Biol 53, 401 (1970)]:

$$k_{\mathrm{on}} \approx \frac{10^{10}}{(\mathrm{mol}/l) \times \mathrm{sec}}$$

$\rightarrow$ Facilitated diffusion picture

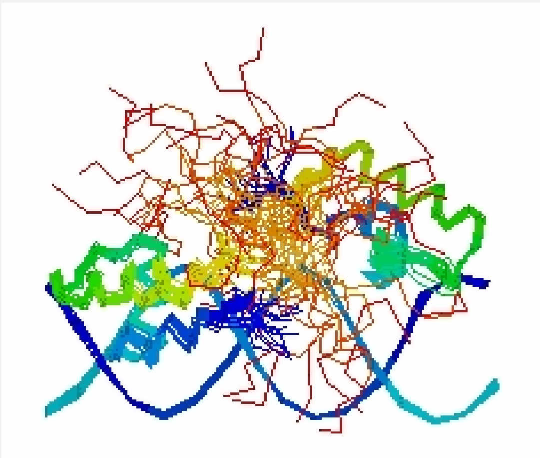
M v Smoluchowski, Physikal. Zeitschr. (1916); P von Hippel and O Berg, J Biol Chem (1989)

Facilitated diffusion: the Berg-von Hippel model

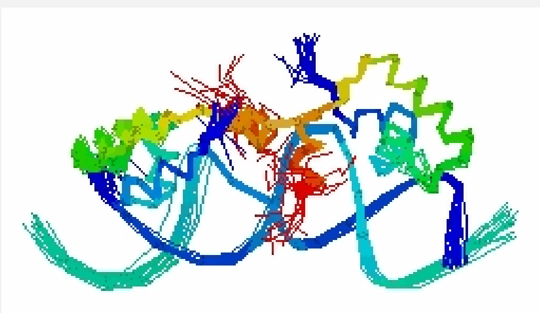


P von Hippel & O Berg, J Biol Chem (1989)

Non-specific binding energy based on *in vivo* data



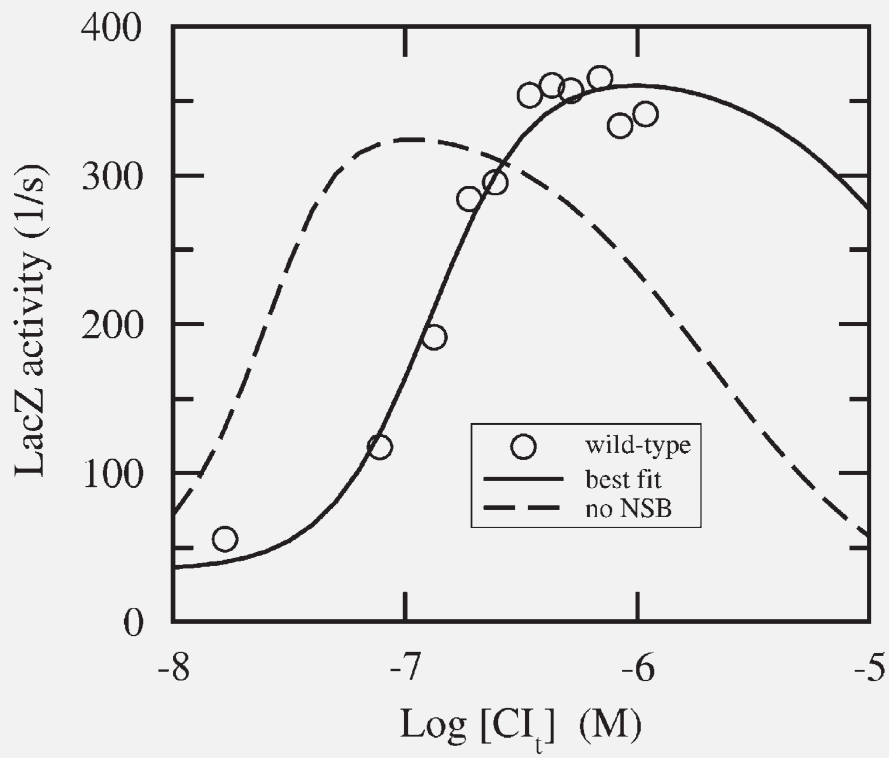
Lac repressor, nonspecific binding



Lac repressor, specific binding

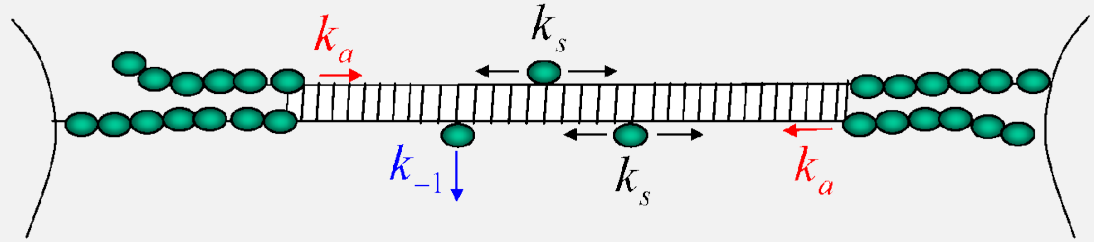
$$[\mathrm{X}] = [\mathrm{X}_{\mathrm{free}}] + \left[\mathrm{X}_{@\mathrm{O_P}}\right] + [\mathrm{X}_{\mathrm{NSB}}]$$

$$\Delta G_{\mathrm{NSB}}(\mathrm{CI}) = -4.1 \pm 0.9\,\mathrm{kcal/mol},$$
$$\Delta G_{\mathrm{NSB}}(\mathrm{Cro}) = -4.2 \pm 0.8\,\mathrm{kcal/mol}$$



A Bakk & RM, FEBS Lett (2004); J Theoret Biol (2004)

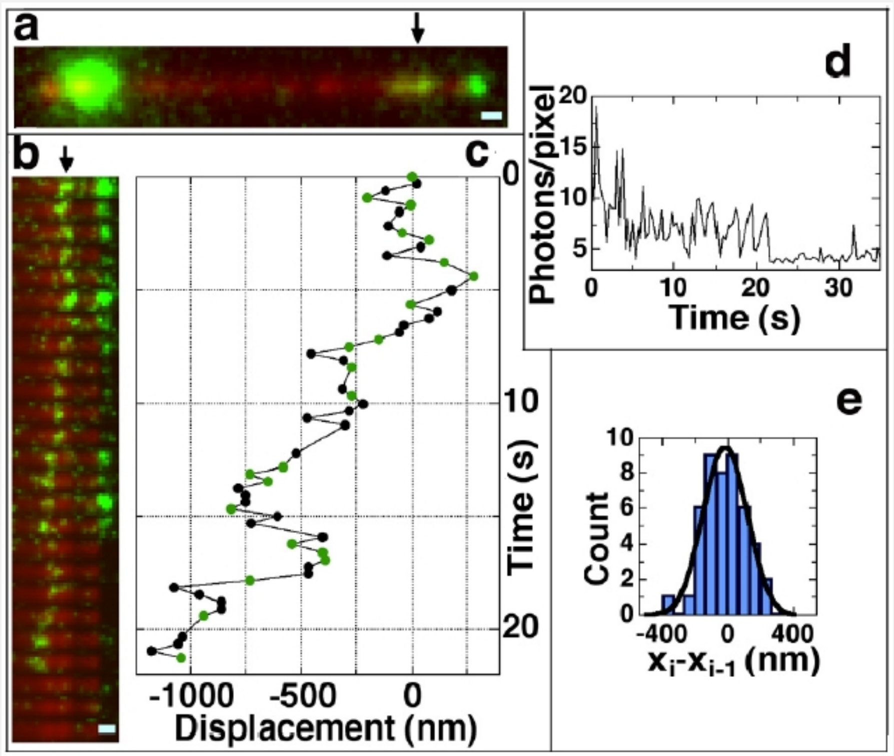
Proof of 1D search mode

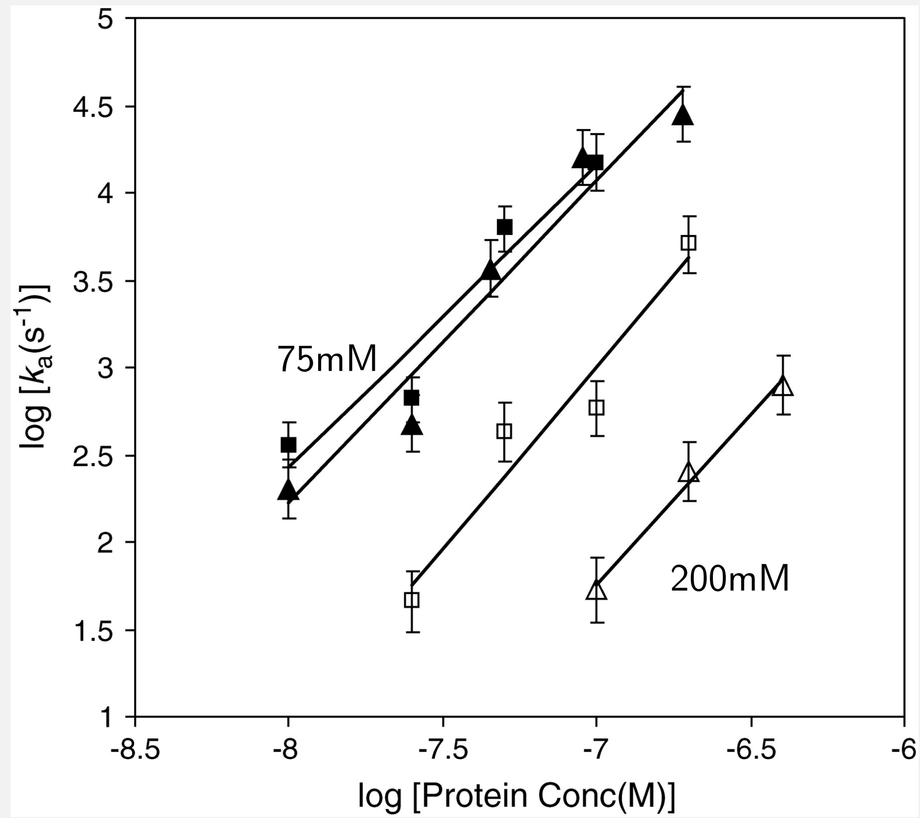


McGhee & von Hippel isotherm

$$f = \frac{N\lambda}{L} \simeq K_{\rm ns}\lambda C, \;\; f \ll 1$$

$$k_a \simeq \begin{cases} C, & \text{1D/3D Berg \& von Hippel} \\ C^2, & \text{Pure 1D search} \end{cases}$$





$$\Delta = 1.74 \pm 0.35,\ 1.85 \pm 0.24,\ 2.08 \pm 0.39,\ 1.95 \pm 0.17$$

YM Wang, RH Austin & EC Cox, PRL (2006); IM Sokolov, RM, K Pant & MC Williams, Biophys J (2005)

Calculating facilitated diffusion (our version): manifestation of intermittency

$$\boxed{\frac{\partial n(x,t)}{\partial t} = \left(D_{1d}\frac{\partial^2}{\partial x^2} - k_{\rm off}\right) n(x,t) - j(t)\delta(x) + G(x,t) + k_{\rm off}\int_{-\infty}^{\infty} dx' \int_0^t dt'\, W_{\rm bulk}(x-x', t-t')}$$

n: line density of TFs

x: chemical co-ordinate along DNA

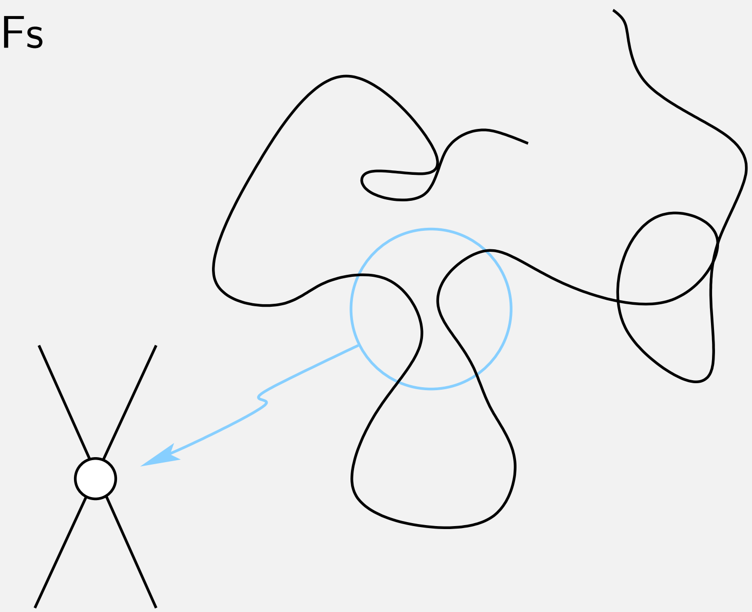
$k_{\rm off}$: unbinding rate of non-specifically bound TFs

D_{1d}: 1D diffusion constant ($\sim 10^{-2} D_{3d}$)

$j(t)$: flux into target (δ sink @ $x = 0$)

G: virgin flux of previously unbound TFs

$W_{\rm bulk}$: 3D diffusion propagator



Long chain, fast dynamics: Lévy flights

M Lomholt, T Ambjörnsson & RM, PRL (2005)

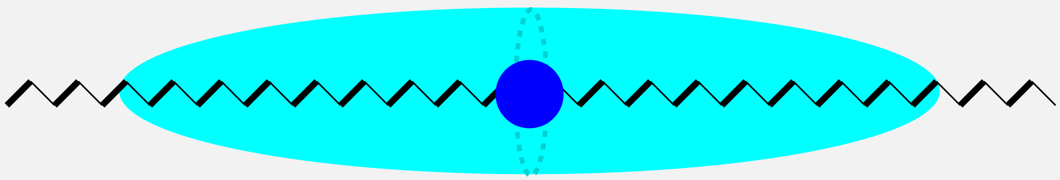
The antenna effect

Target search rate for cylindrical DNA model:

$$k_{\rm on} \sim 4\pi D_{3d}\ell_{\rm sl}^{\rm eff} \times \frac{1}{\sqrt{\ln(\ell_{\rm sl}^{\rm eff}/r_{\rm int})}}$$

Sliding length:

$$\ell_{\rm sl} = \sqrt{\frac{D_{1d}}{k_{\rm off}}}$$



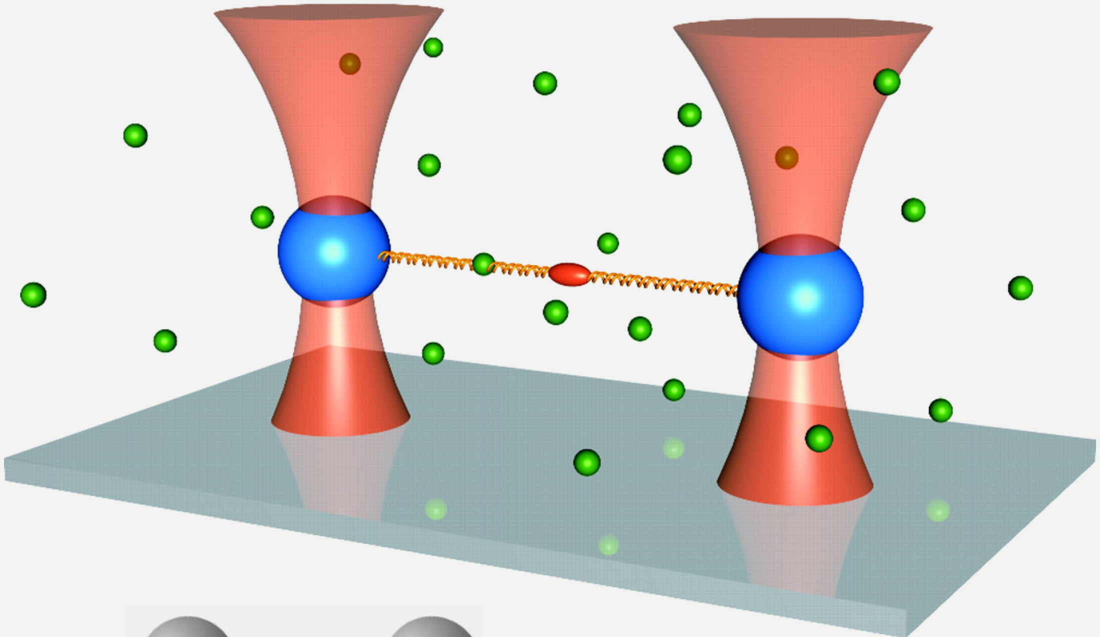
Effective sliding length:

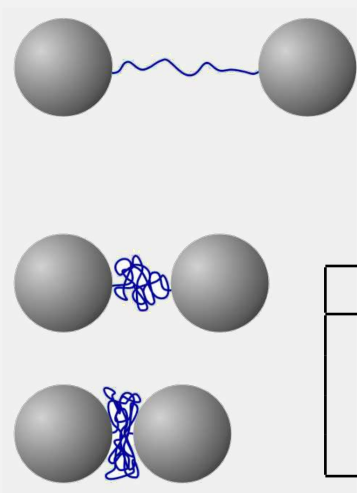
$$\ell_{\rm sl}^{\rm eff} = \sqrt{\frac{k_{\rm on}}{2\pi D_{3d}}} \times \ell_{\rm sl}$$

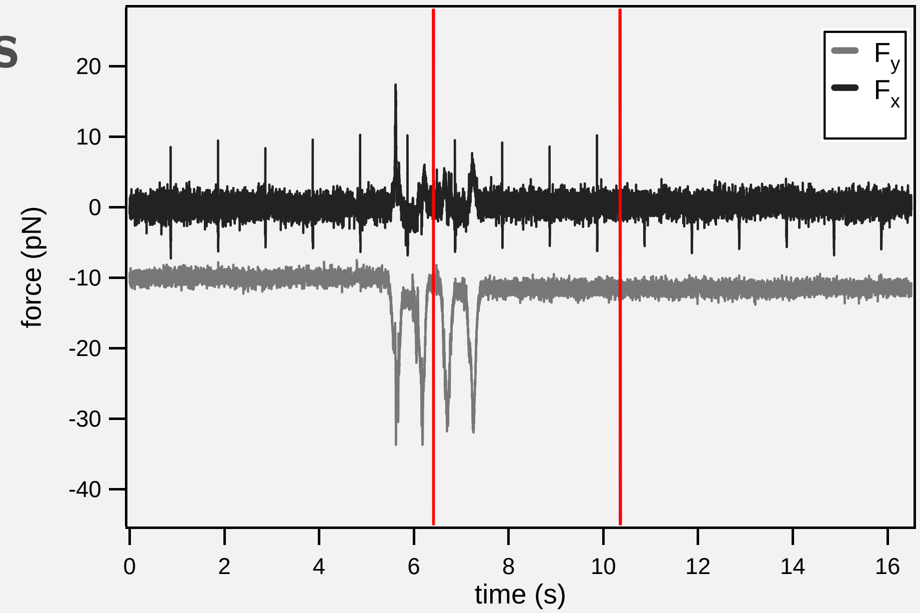
microhop correction: $\sqrt{\frac{k_{\rm on}}{2\pi D_{3d}}}$

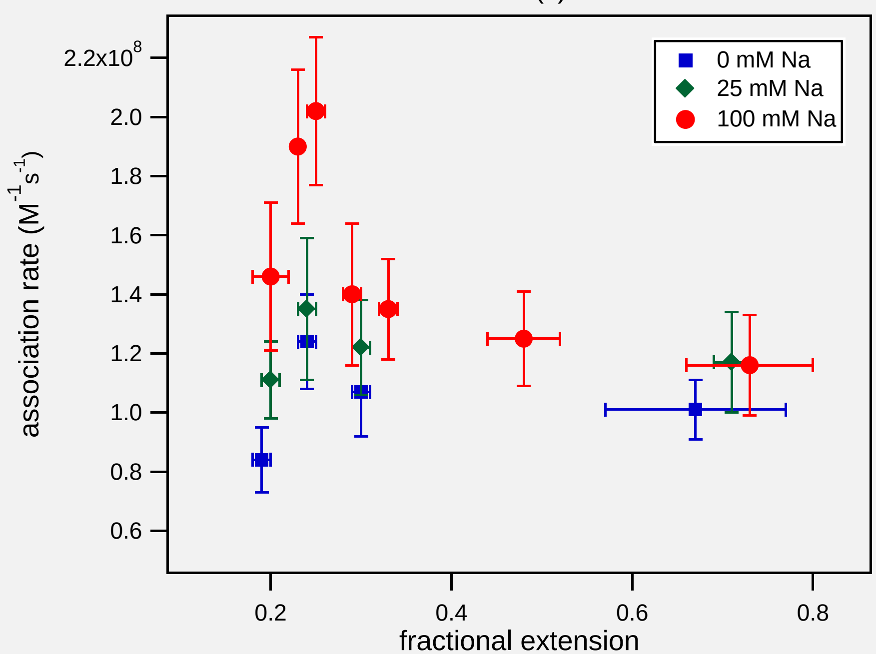
MA Lomholt, T Ambjörnsson & RM, PRL (2005)

The rôle of DNA conformations





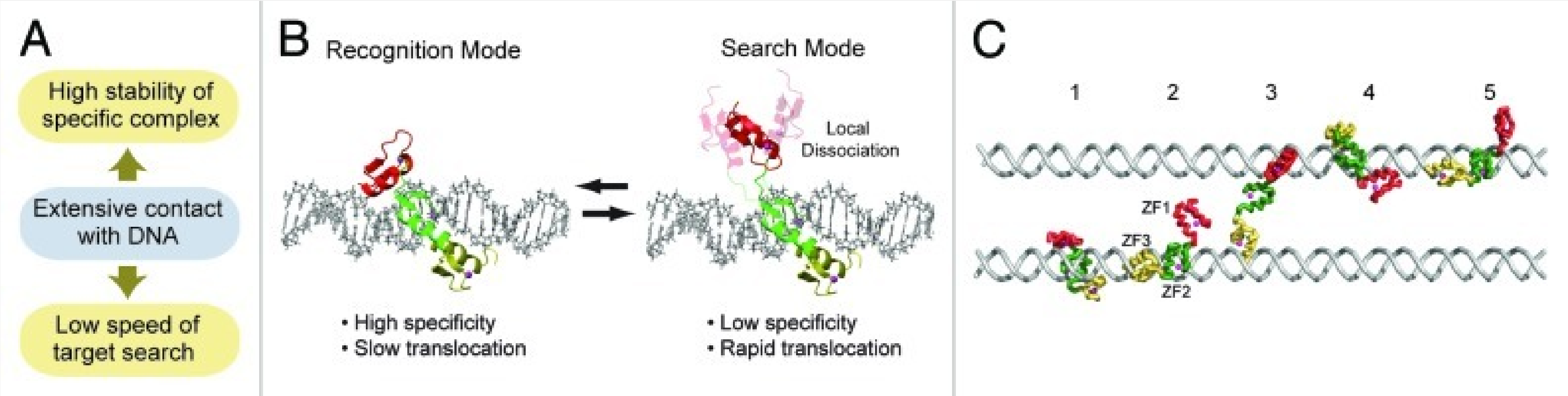




[NaCl]	R_{theory}	R_{measured}
0 mM	1.18	1.3 ± 0.2
25 mM	1.23	1.1 ± 0.2
100 mM	1.67	1.7 ± 0.3
150 mM	1.15	1.3 ± 0.4

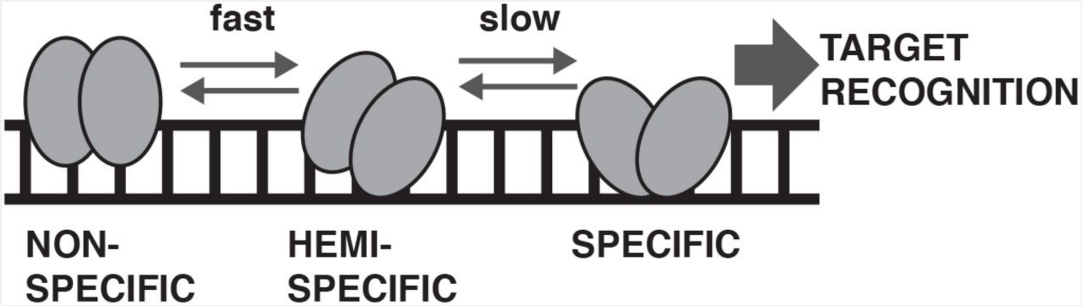
B vd Broek, SM Kalisch, MA Lomholt, RM & G Wuite, PNAS (2008); MA Lomholt, B vd Broek, SM Kalisch, G Wuite & RM, PNAS (2009)

Speed-stability paradox in TF search along DNA



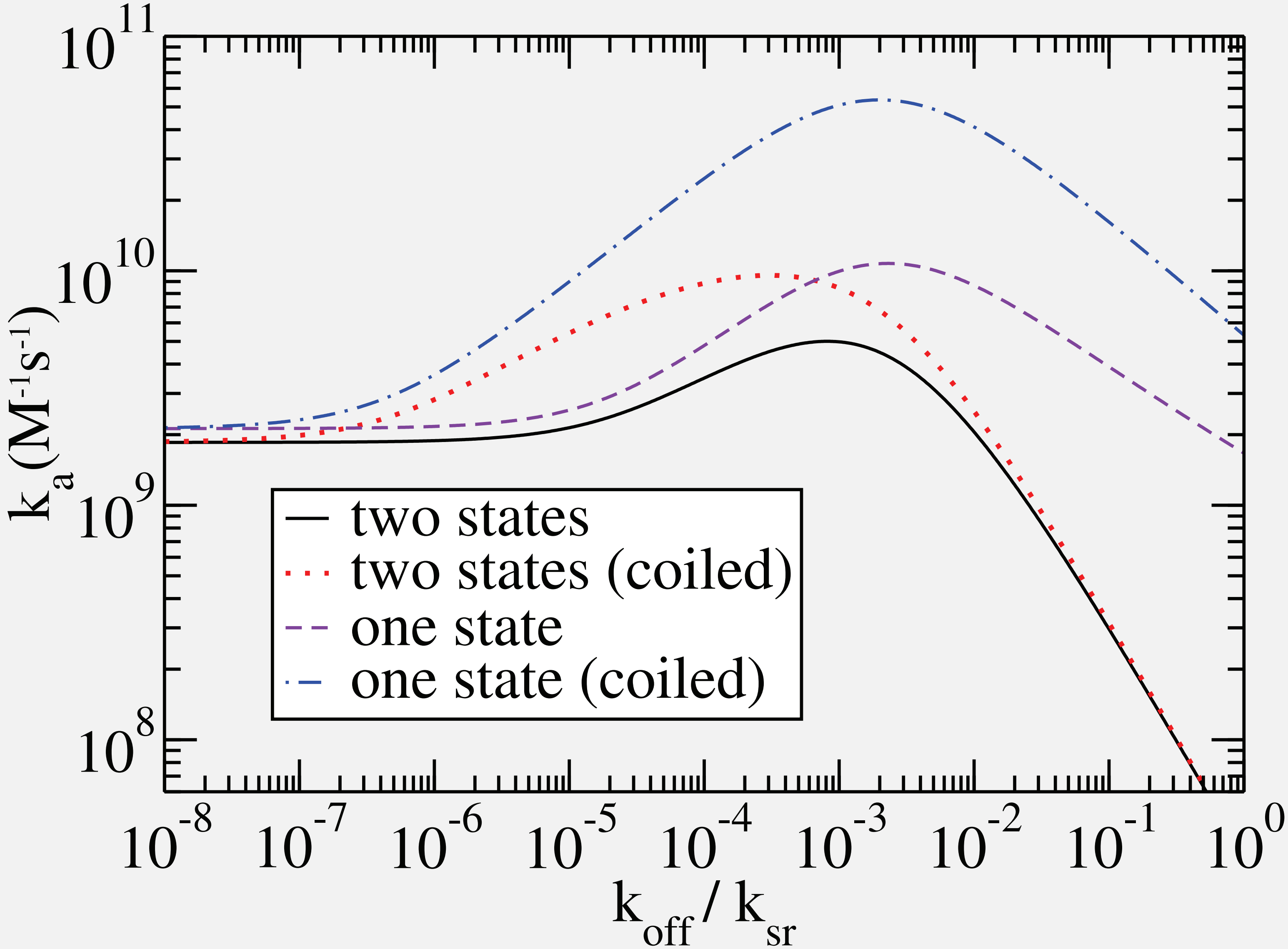
From simulations: B: Search & recognition modes for a zinc finger protein

C: Intersegmental transfer of the protein



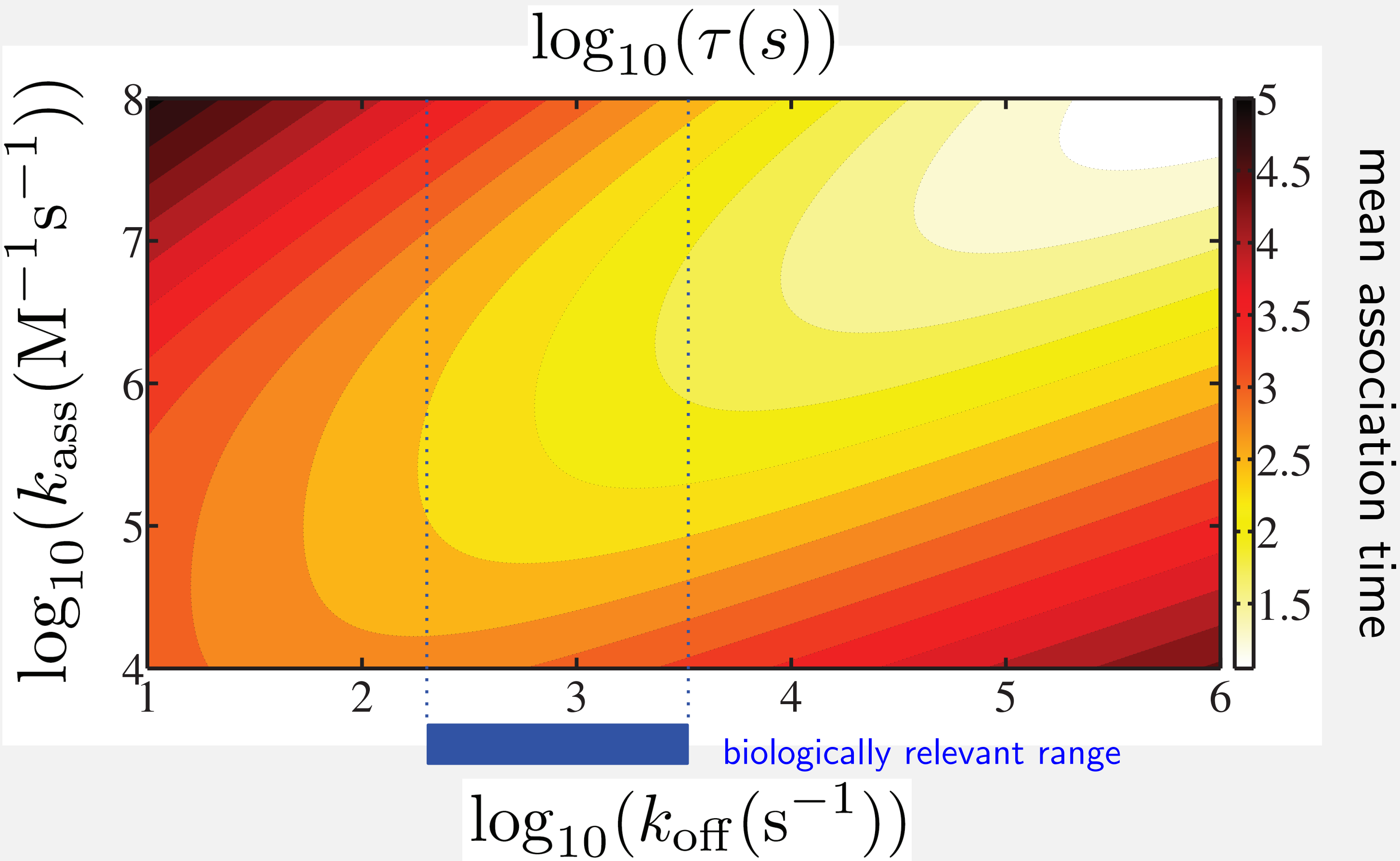
[SC Piatt, JJ Loparo & AC Price, bioRxiv (2018)]

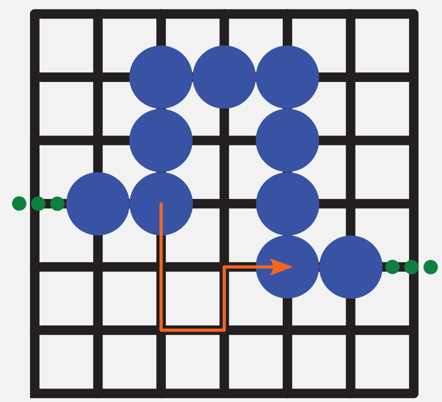
Facilitated diffusion: rate with search & recognition states



M Bauer & RM, Biophys J (2012)

In vivo gene regulation consistent with facilitated diffusion





[M Buenemann & P Lenz, PLoS ONE (2010)]

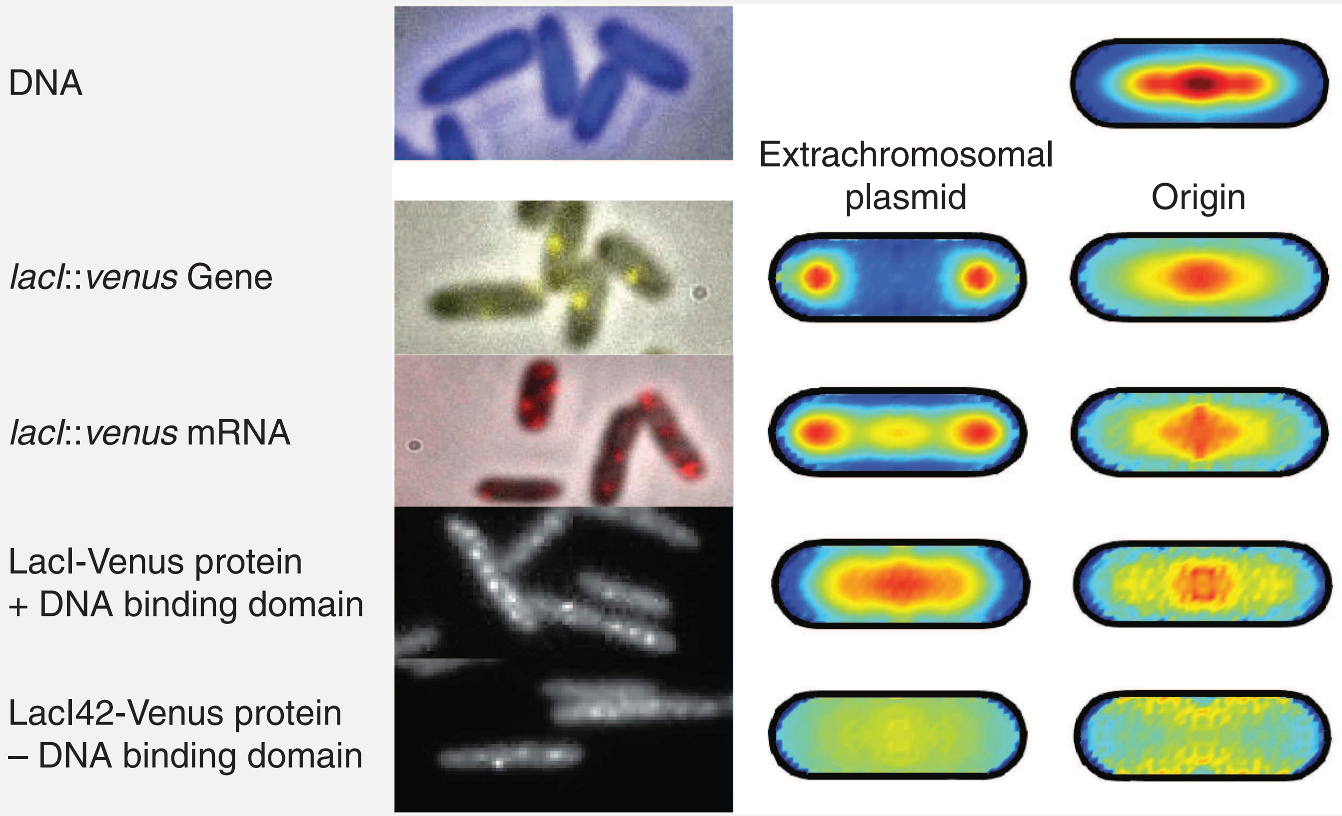
@ optimum the target association time is $\tau \approx 311$sec (no fit parameter)

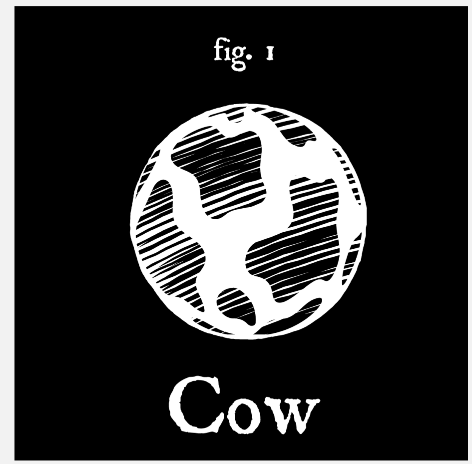
single molecule experiment: $\tau_{\rm exp} = 354$sec [Elf et al, Science (2007)]

Spatial aspects: do gene locations matter?

Képès: TF targets are typically located next to or at regular distances from the TF gene $\rightarrow$ TF gene-target pairs close in 3D

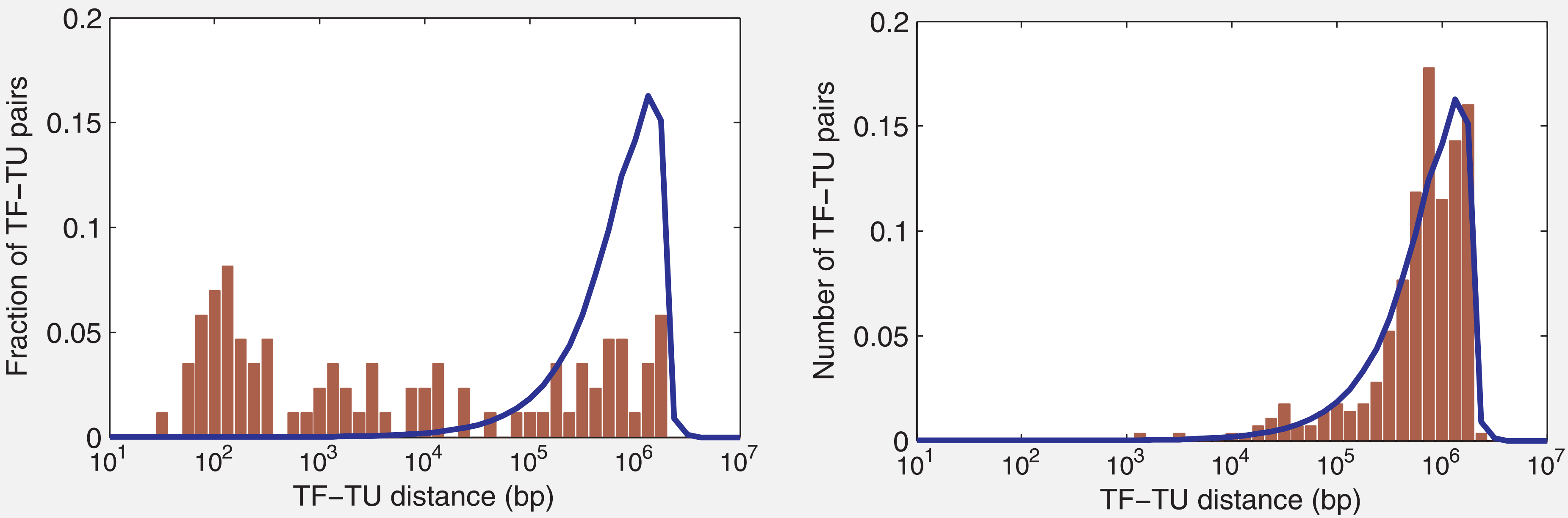
Kuhlman & Cox: • TF distribution highly heterogeneous • TF gene influences distribution: localisation of TF near TF gene

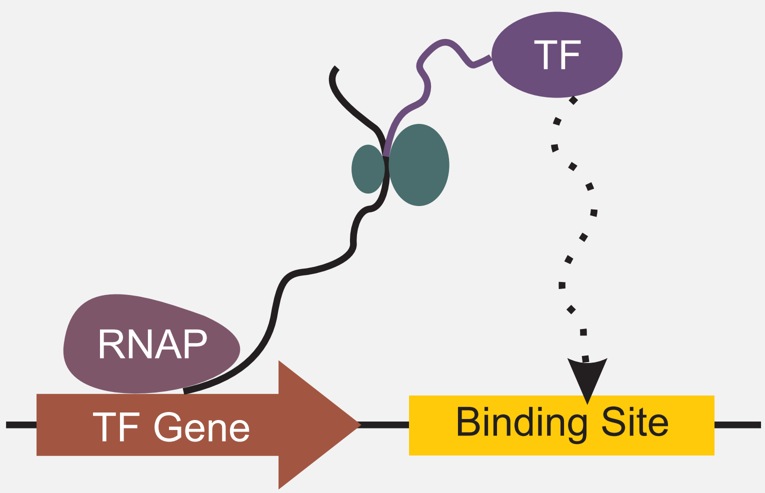




TF-regulation effects gene-proximity: rapid-search-hypothesis

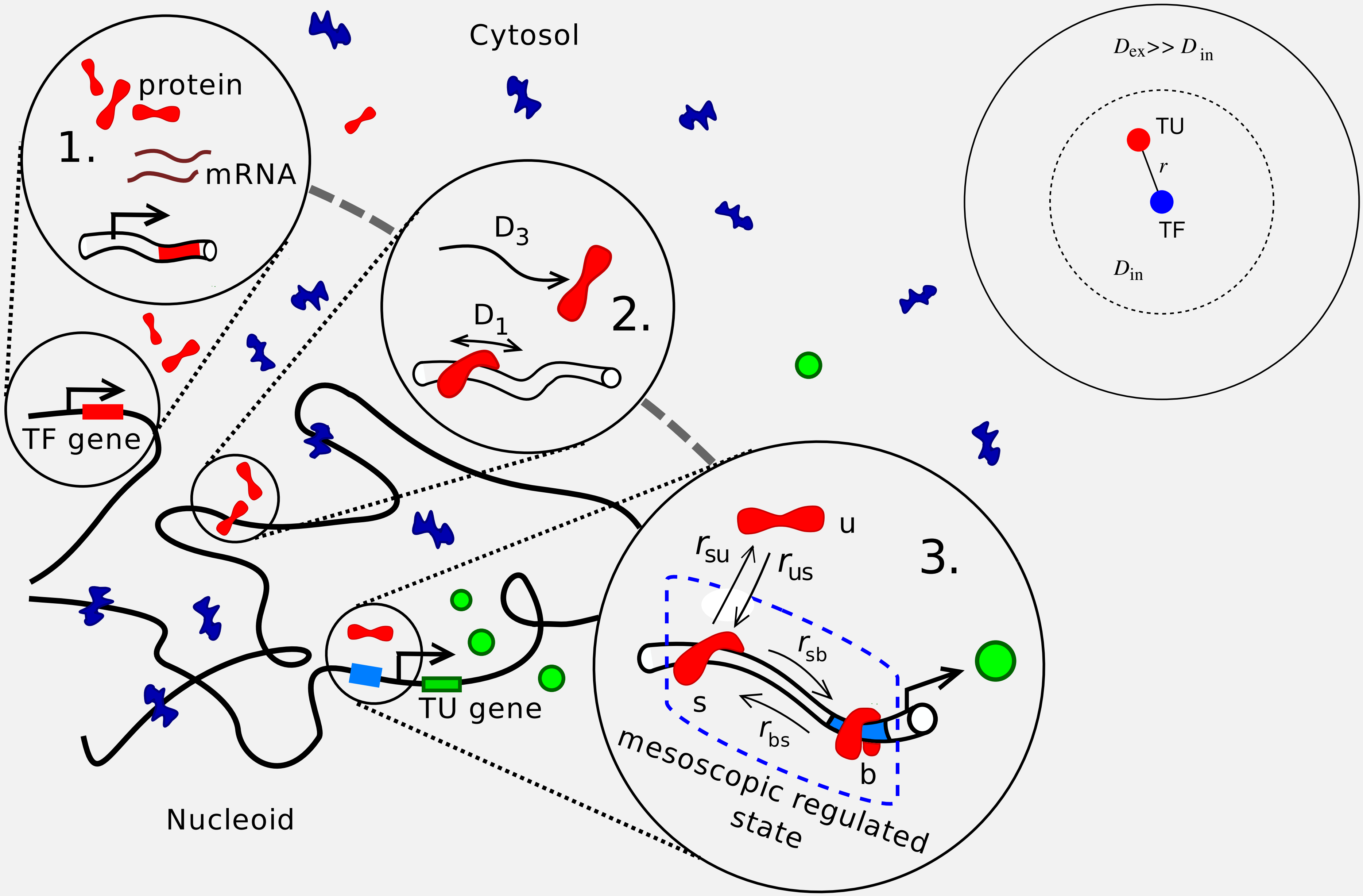
Gene-gene distance distribution for local TFs (regulate < 4 operons, left) and global (regulate ≥ 4 operons, right). Blue line: random location of genes





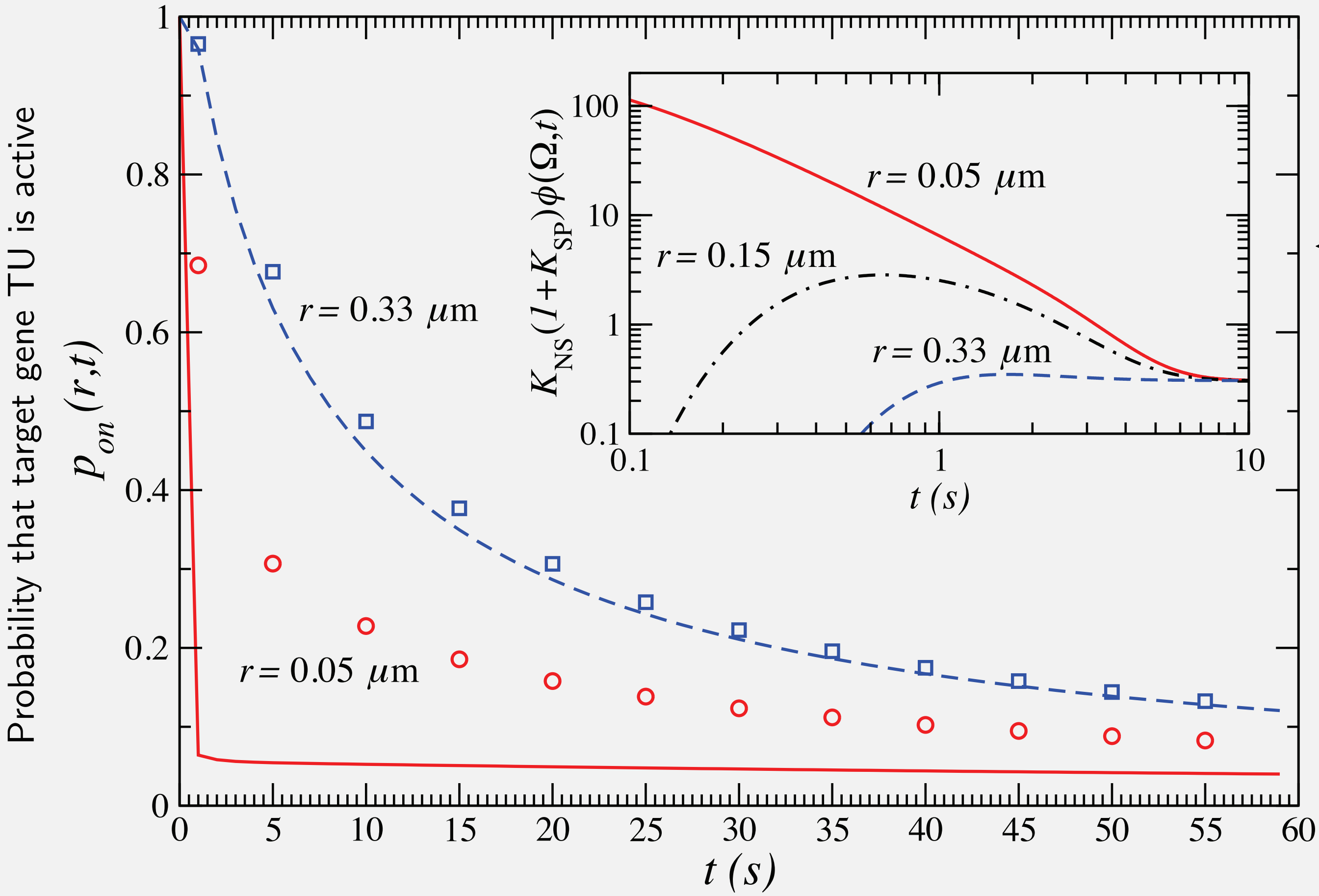
Kolesov et al, PNAS (2007)

Transient intracellular signalling is geometry-controlled



O Pulkkinen & RM, PRL (2013)

Result 1: transient response to repression



← TF concentration @ TU

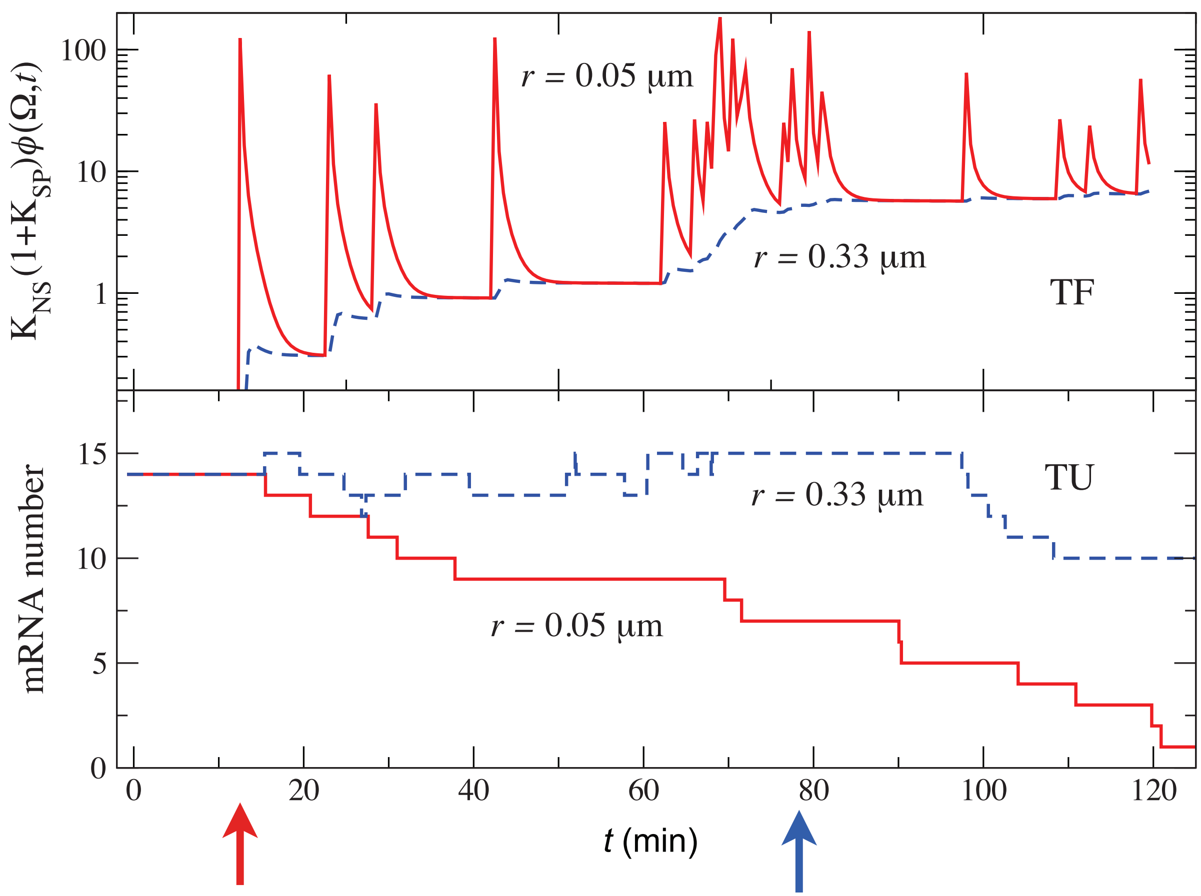
Mean field approximation (full & dashed lines):

$$p_{on}(r,t) = \left\langle \frac{1 + K_{\mathrm{NS}}\rho_{\mathrm{TF}}(r,t)}{1 + \tilde{K}\rho_{\mathrm{TF}}(r,t)} \right\rangle \approx \frac{1 + K_{\mathrm{NS}}\langle\rho_{\mathrm{TF}}(r,t)\rangle}{1 + \tilde{K}\langle\rho_{\mathrm{TF}}(r,t)\rangle}$$

O Pulkkinen & RM, PRL (2013)

Result 2: time dependence of gene response: bursts!

Sample paths from MC simulations:



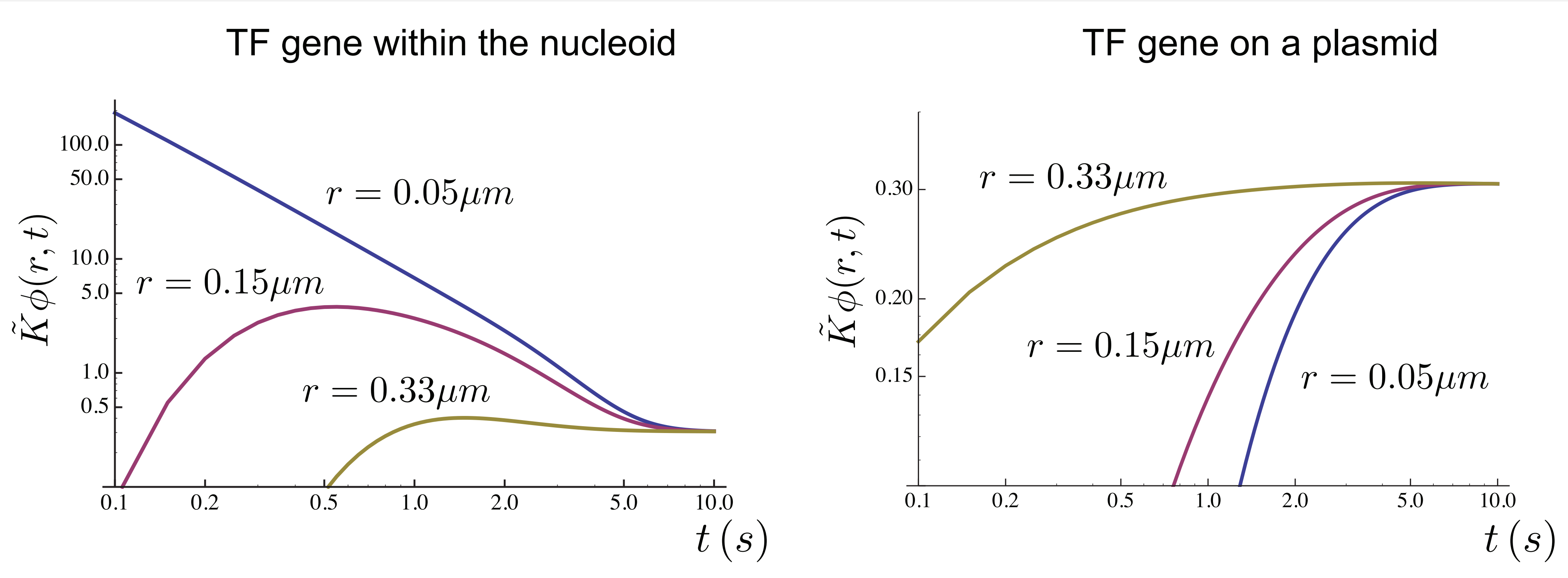
TF density at the target operator

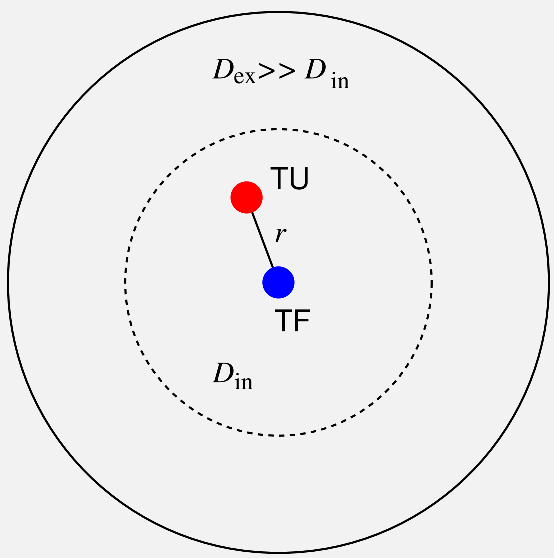
Target expression

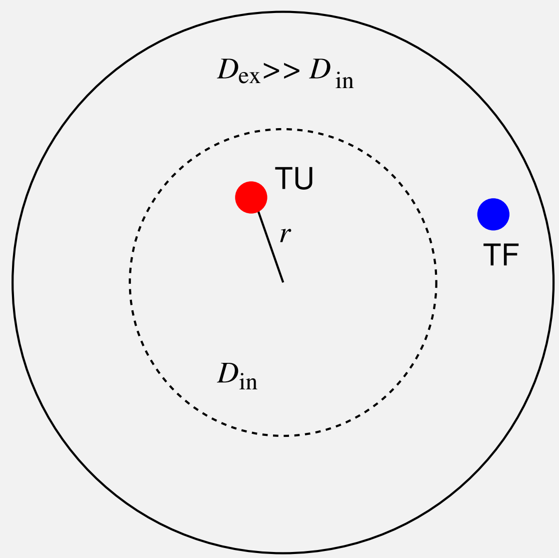
First repressor binding at $r = 0.05$ μm

First repressor binding at $r = 0.33$ μm

Result 3: gene location matters







O Pulkkinen & RM, PRL (2013)

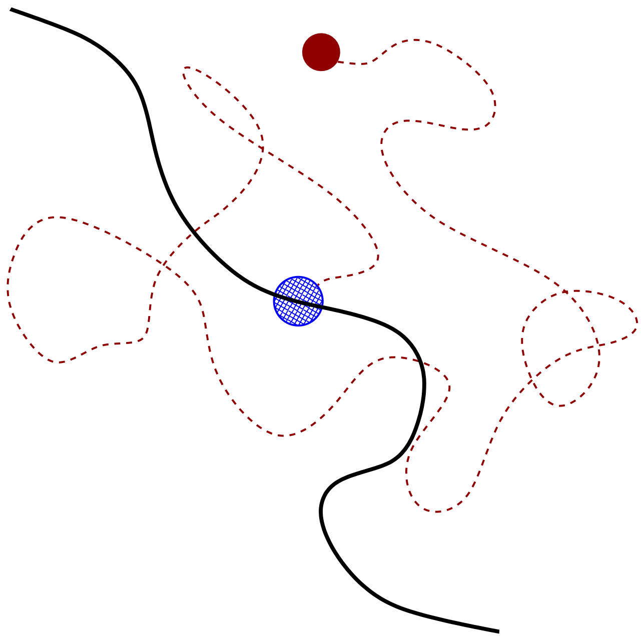
Numerical analysis confirms relevance of proximity effect

P Kar, AV Cherstvy & RM, PCCP (2018)

Brownian impromptu: first-passage time fluctuations

Search rate for particle with diffusivity $D_{3\text{d}}$ to find an immobile target of radius a (assuming immediate binding):

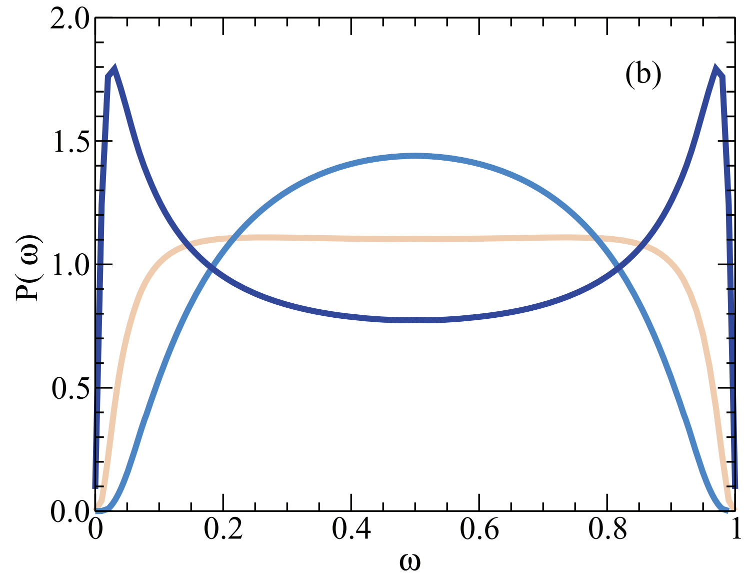
$$k_{\text{on}} = 4\pi D_{3\text{d}} a$$



Uniformity index for two independent first-passage times τ_1, τ_2:

$$\omega = \frac{\tau_1}{\tau_1 + \tau_2}$$

$\curvearrowright$ $\omega = 1/2$ means good reproducibility



M v Smoluchowski, Physikal Zeitschr (1916)

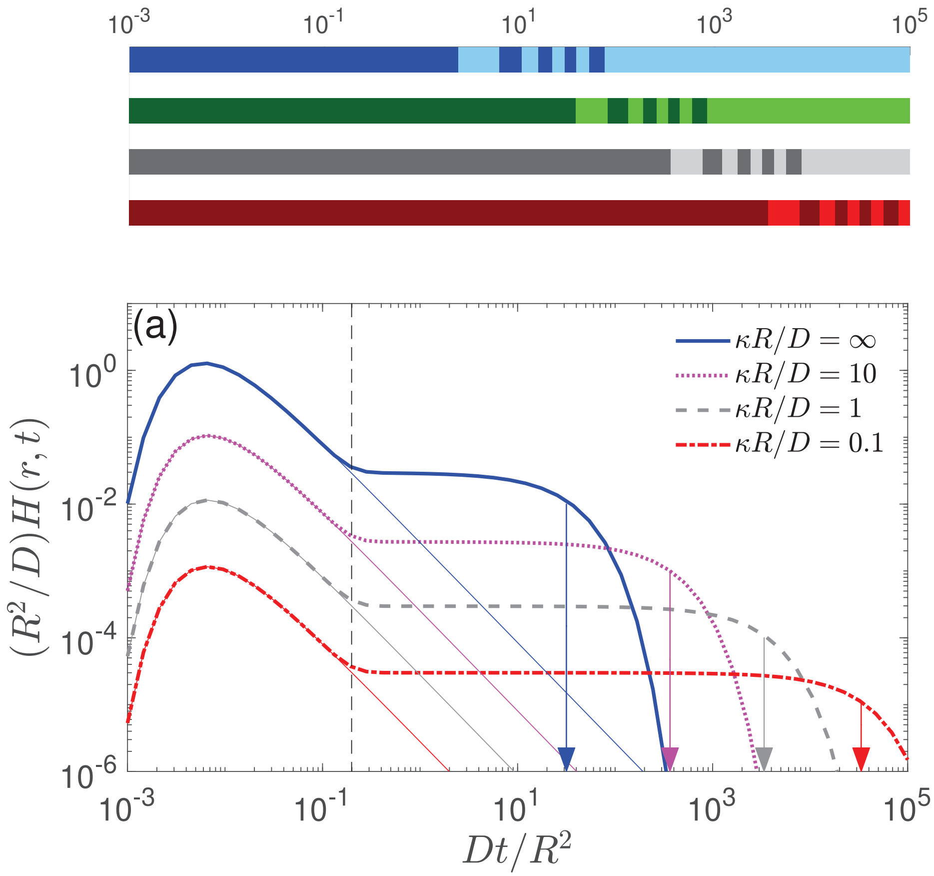
T Mattos, C Mejía-Monasterio, RM & G Oshanin, PRE (2012)

Beyond mere means: first-passage time distributions

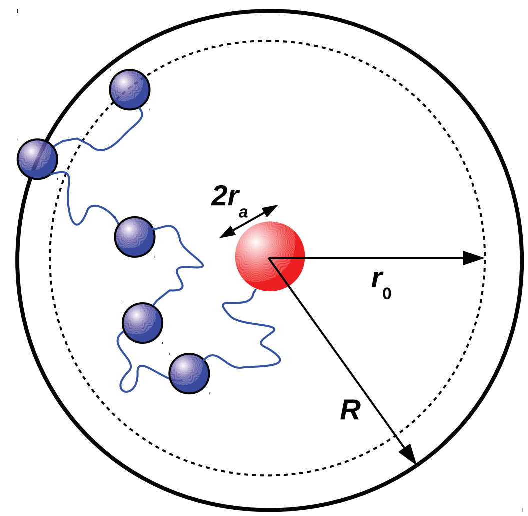
Even in μm-sized bacteria cells biochemical reactions are distance-dependent (geometry-controlled) [Kolesov et al, PNAS (2007); O Pulkkinen & RM, PRL (2013)]

Geometry- versus reaction-control in finite-reactivity scenario

Full first passage time density:



Direct vs indirect trajectories:

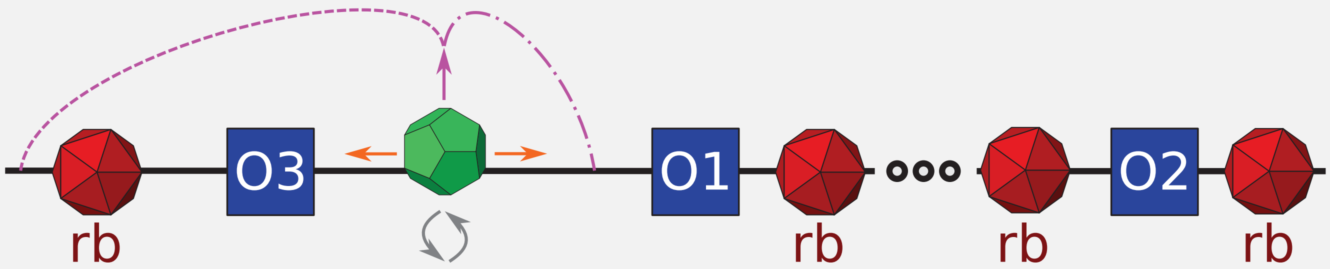


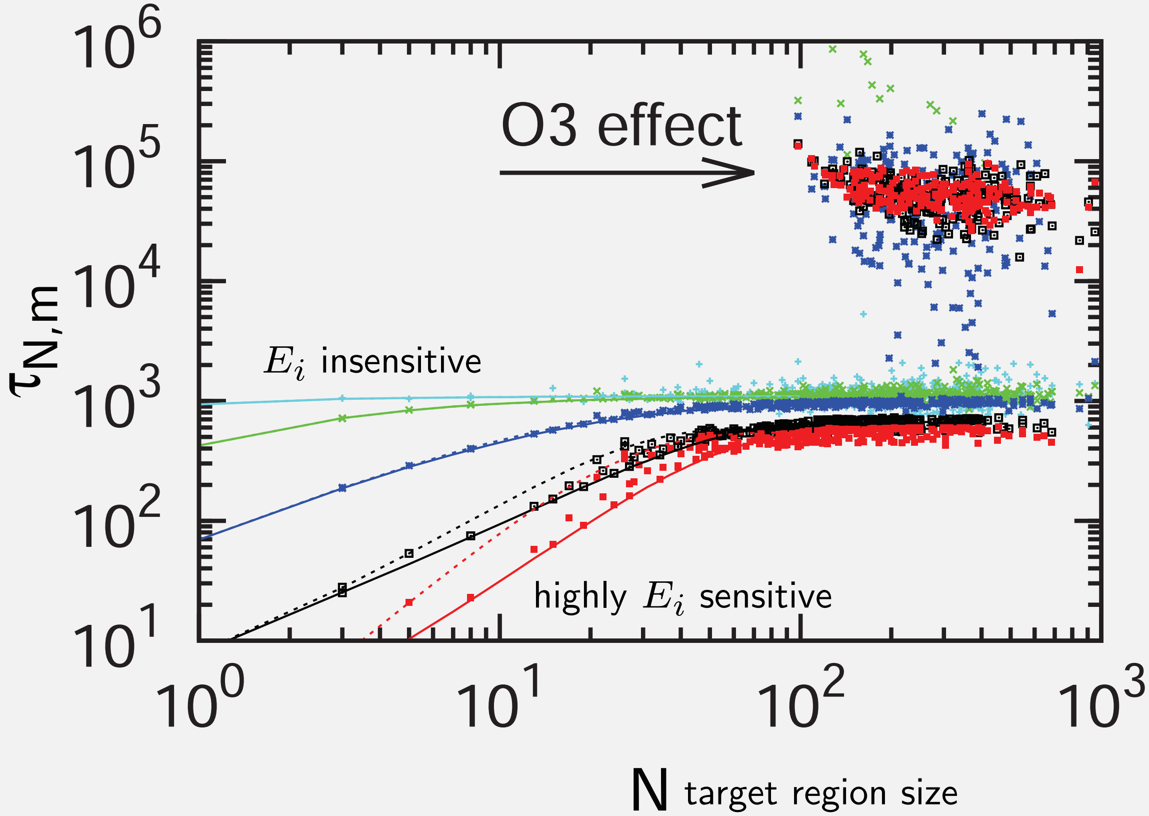
$$\langle t\rangle=\frac{(r_0-r_a)(2R^3-r_0r_a[r_0+r_a])}{6Dr_0r_a}$$

$$+\frac{R^3-r_a^3}{3\kappa r_a}$$

D Grebenkov, RM & G Oshanin, Comm Chem (2019), PCCP (2018); A Godec & RM, PRX (2016), Sci Rep (2016)

Sequence (binding energy) effects on target search time



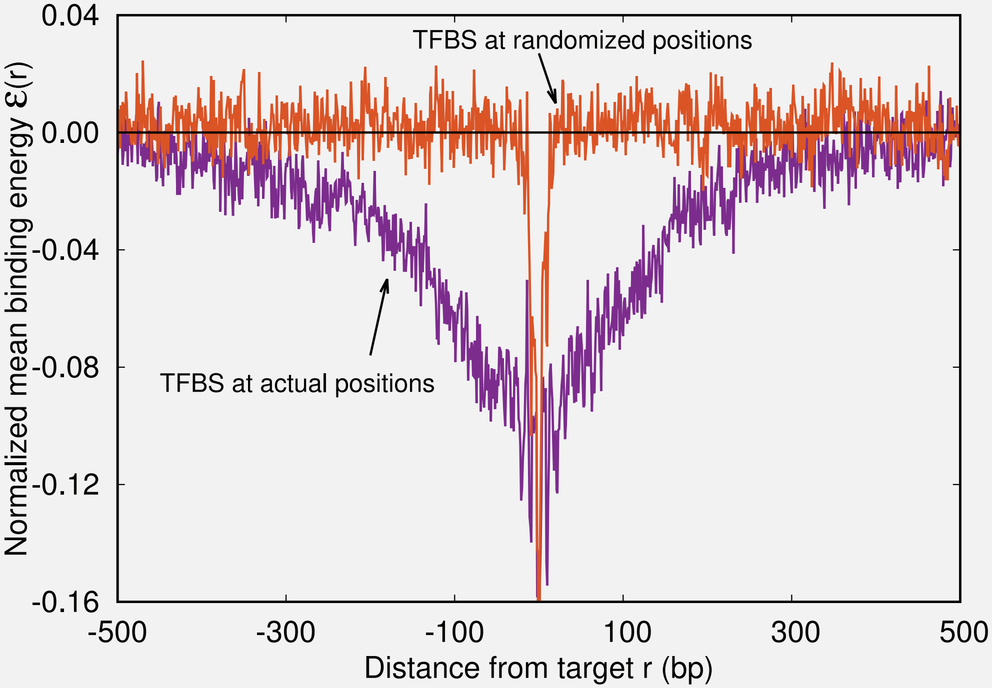


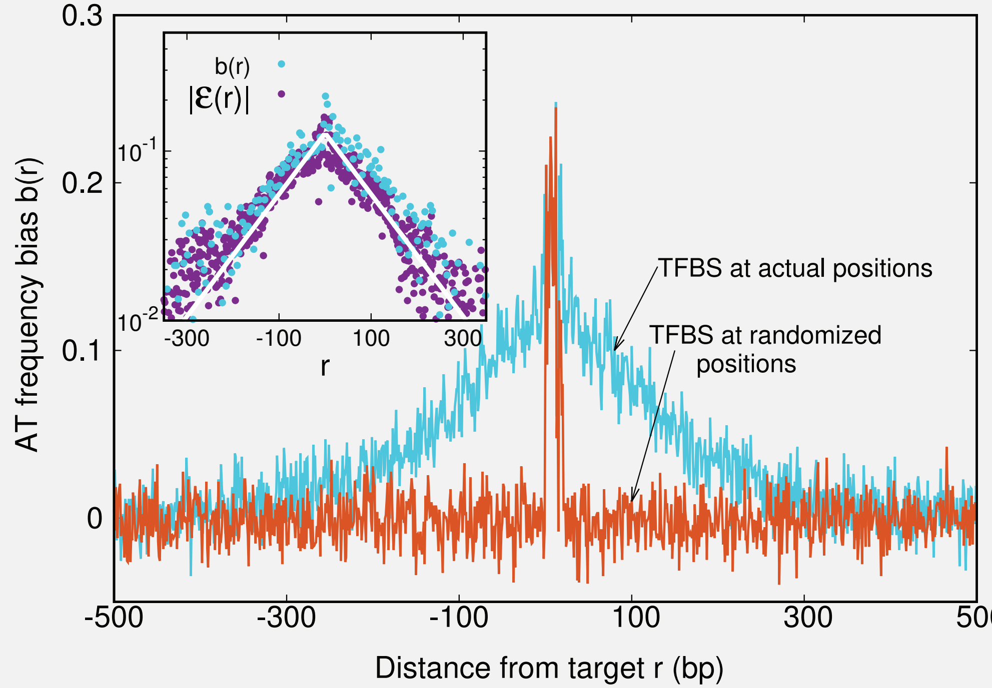
full line: centred target

dashed line: target @ boundary

M Bauer, E. S. Rasmussen, M. A. Lomholt, & RM, Sci Rep (2015)

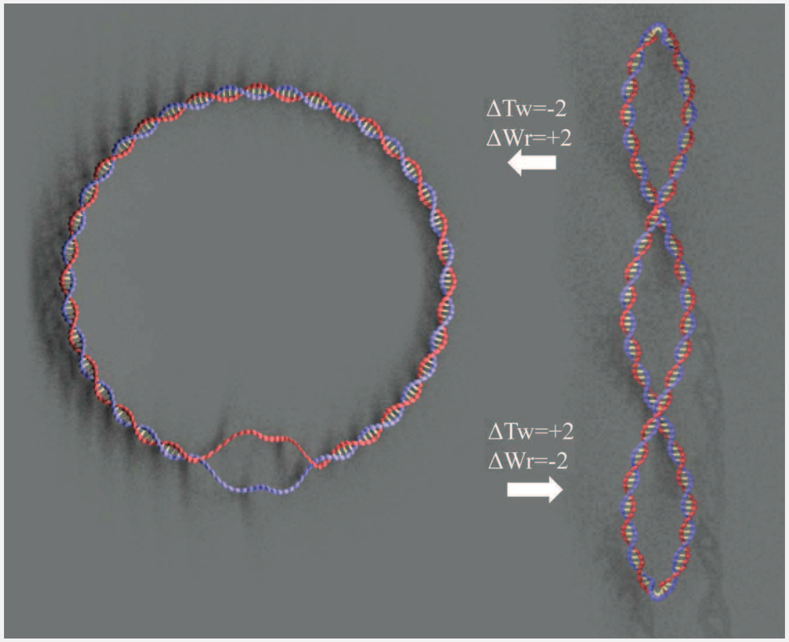
Energetic funnel facilitated diffusion

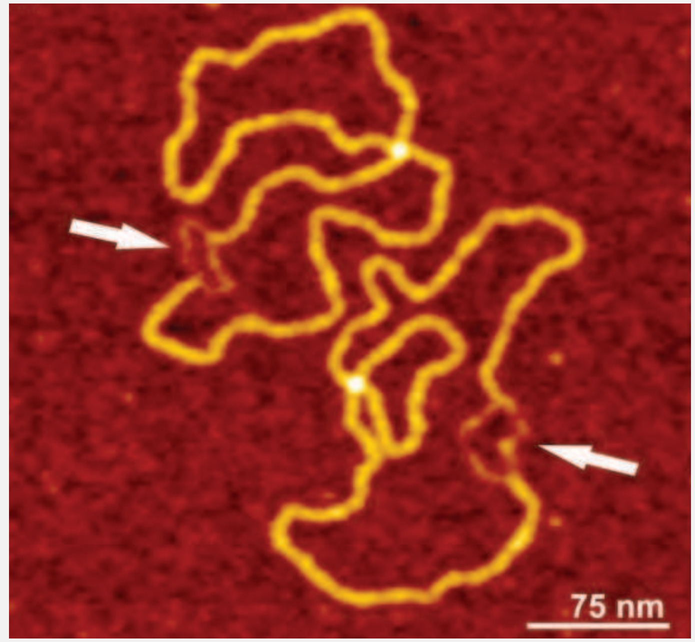




M Cencini & S Pigolotti, NAR (2017)

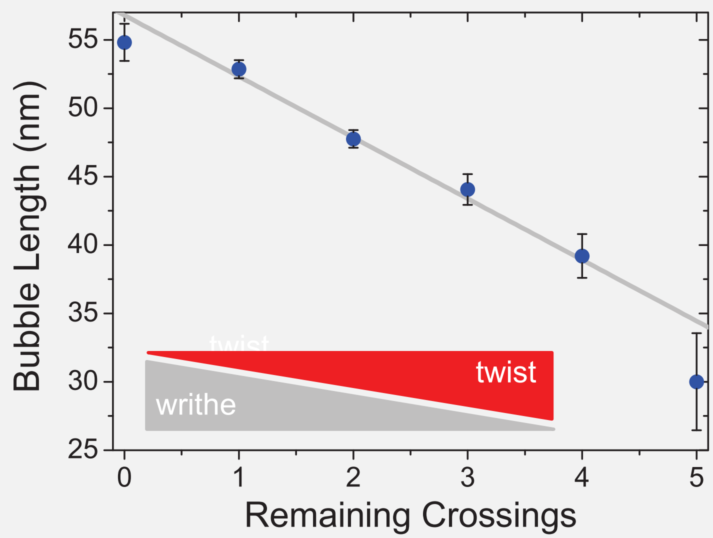
Weak regions at gene starts promote DNA denaturation





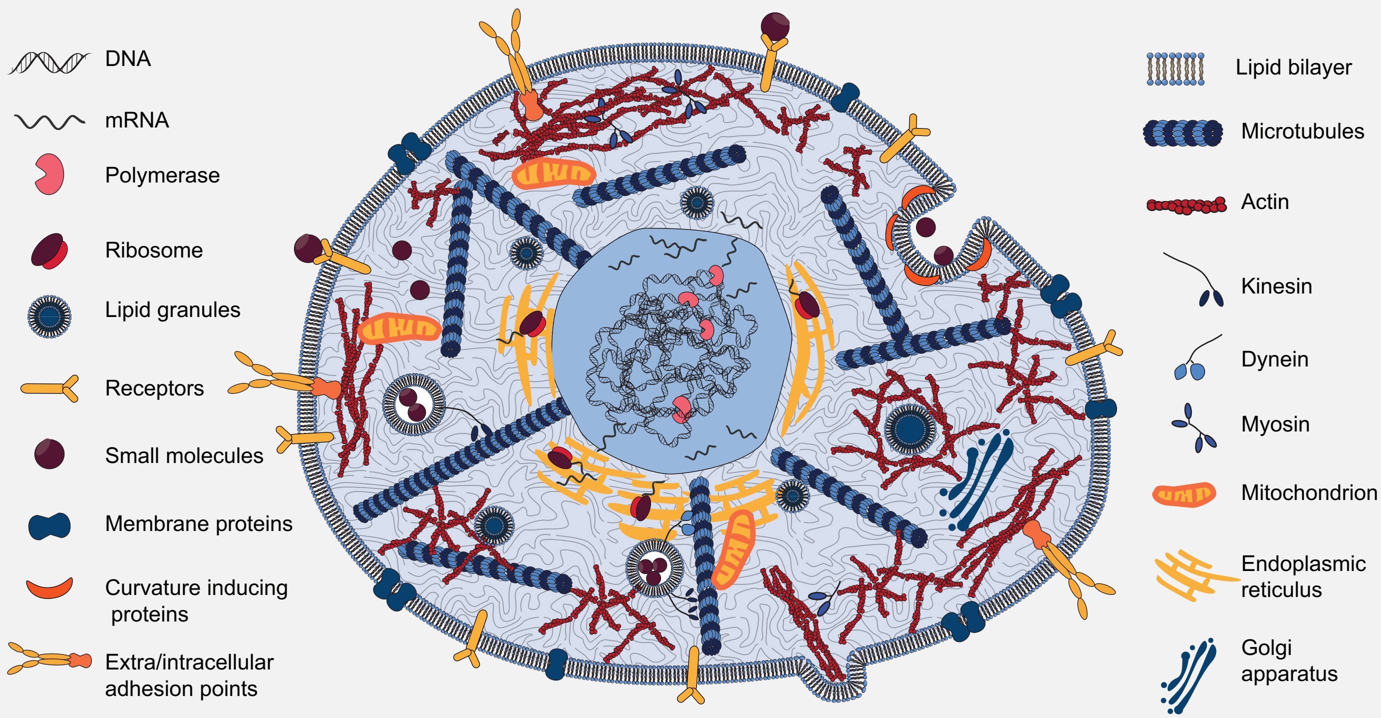
DNA superhelical density:

$$\sigma = \frac{\mathrm{Lk} - \mathrm{Lk}_0}{\mathrm{Lk}_0} \approx -0.06$$



J-H Jeon, J Adamczik, G Dietler & RM, PRL (2010); J Adamczik, J-H Jeon, RM & G Dietler, Soft Matter (2012)

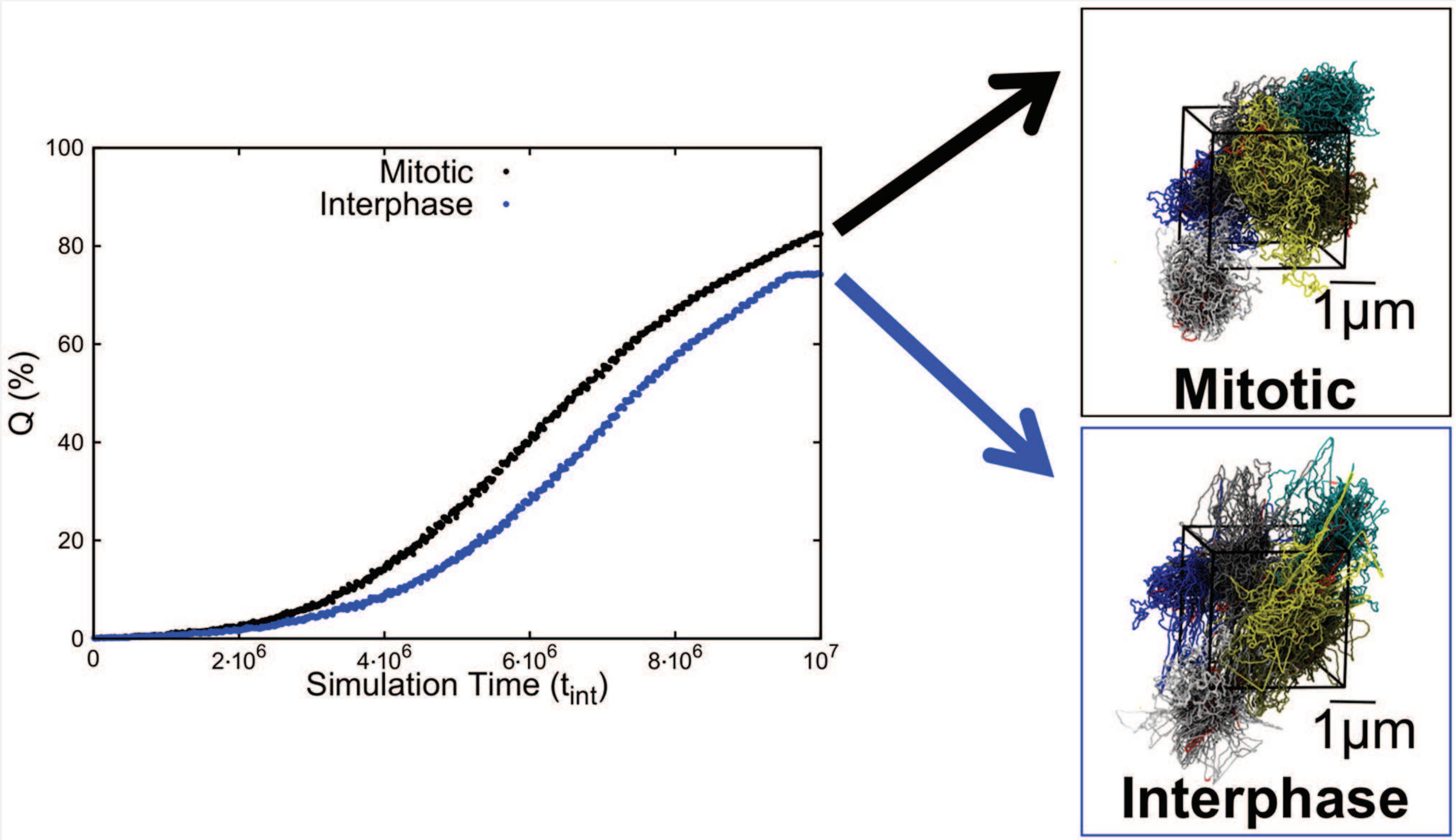
Gene regulation in eukaryotic cells



Exchange versus nucleic membrane, chromosomal dynamics & packaging

Active motion: motor transport, drag, or swirling (cytoplasmic streaming), see, e.g., Reverey et al, Sci Rep (2015) & Thapa et al, JCP (2019)

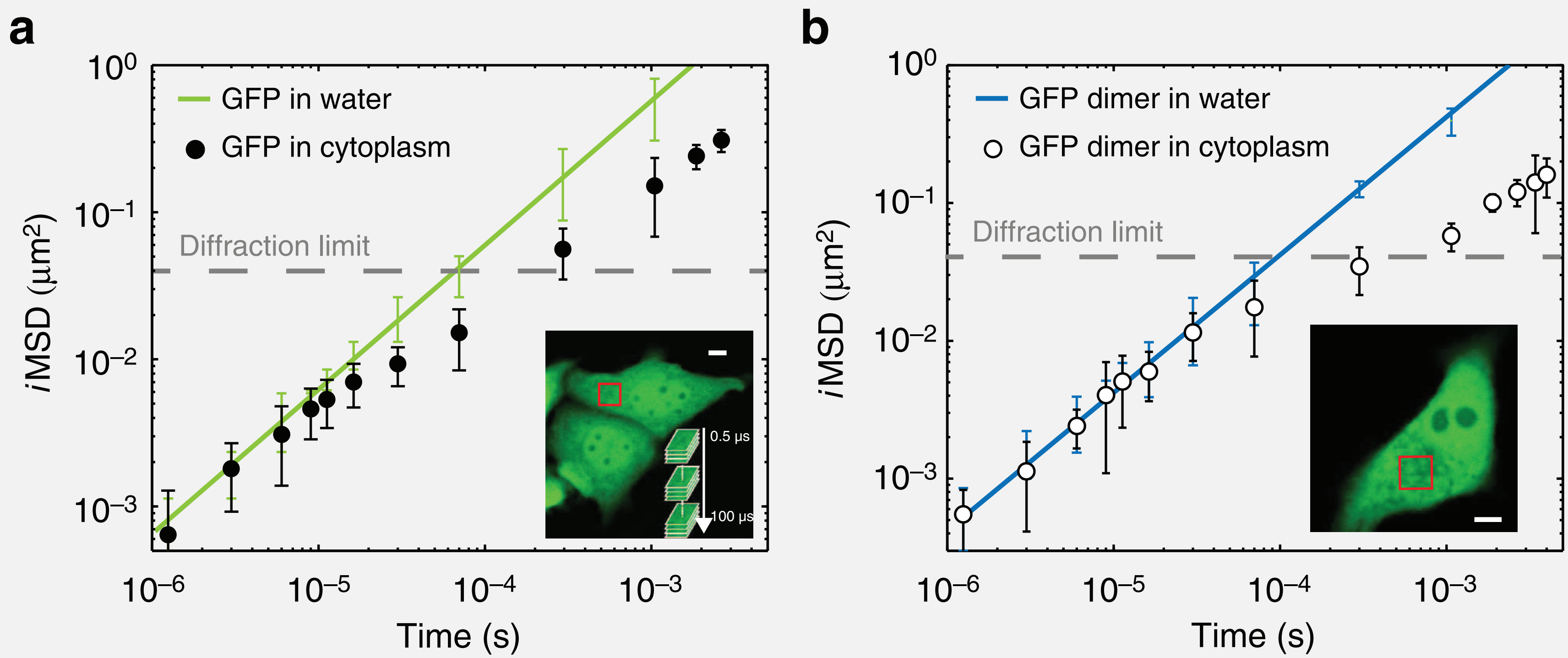
Colocalisation still exists in the nucleus



Increase of percentage Q of coregulated pairs of genes in chromosome 19 which colocalise during the MD protocol. Red (???) highlighted regions designate chromosome regions involved in the coregulatory network

M Di Stefano, A Rosa, V Belcastro, D di Bernardo, & C Micheletti, PLoS Comp Biol (2013)

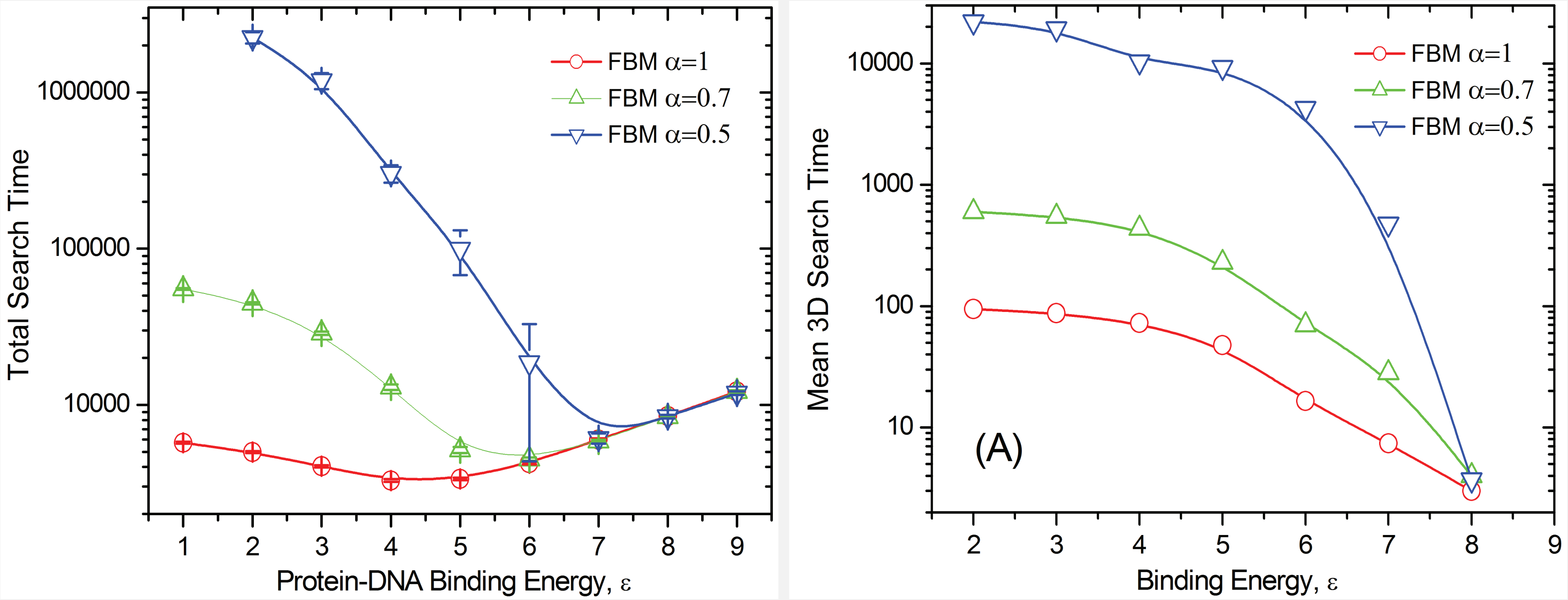
Anomalous diffusion of GFP in cell cytoplasm & nucleus



$$\langle \mathbf{r}^2(t) \rangle \simeq K_\alpha t^\alpha : \text{ Subdiffusion when } 0 < \alpha < 1$$

C di Renzo, V Piazza, E Gratton, F Beltram & F Cardarelli, Nat Comm (2014)

Anomalous facilitated diffusion



Many unknowns in the modelling:

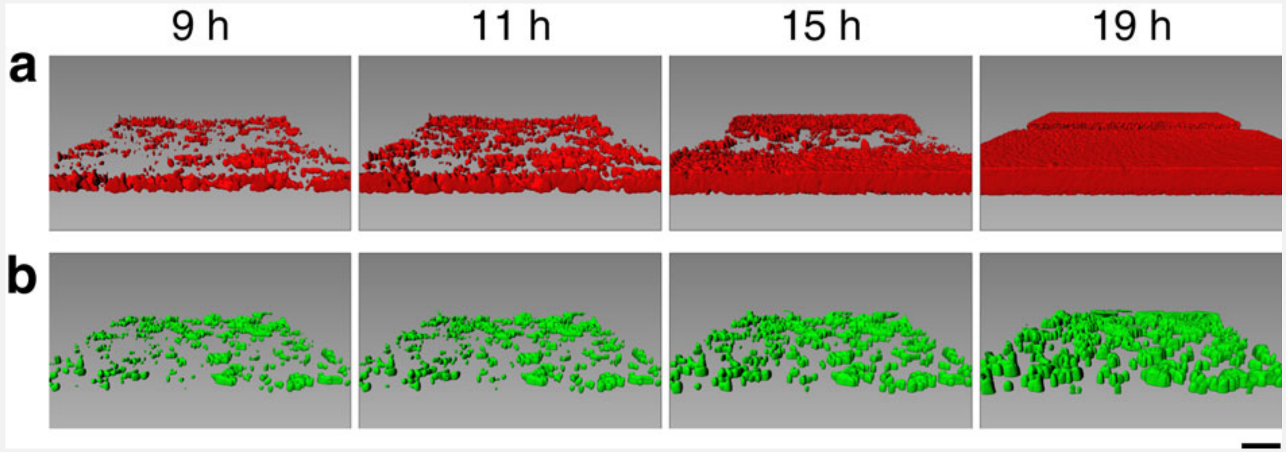
Physical mechanism of anomalous diffusion & cutoff time of anomalous motion?

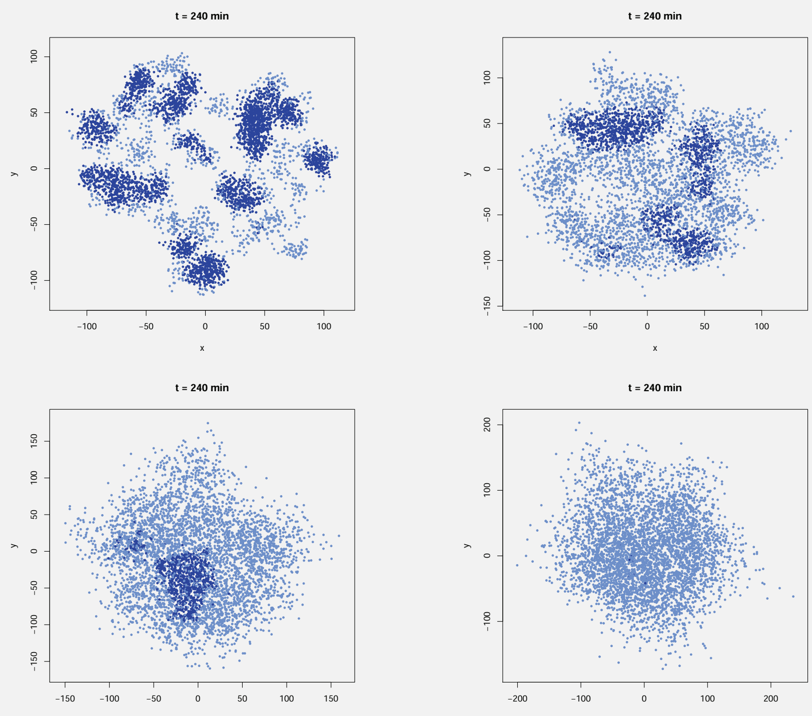
Effects of crowders with different sizes: see eg Shin et al, Soft Matter (2015) influencing immediate rebinding?

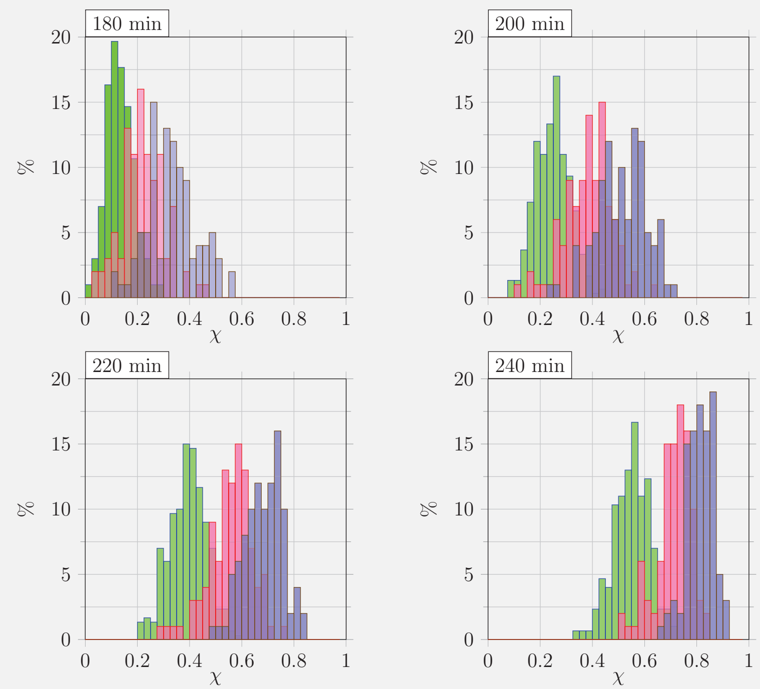
DNA conformations & dynamics due to crowding & active motion: Shin et al, NJP (2015), NJP (2016)

L Liu, AG Cherstvy & RM, JPC (2017)

Quorum sensing dynamics in heterogeneous populations



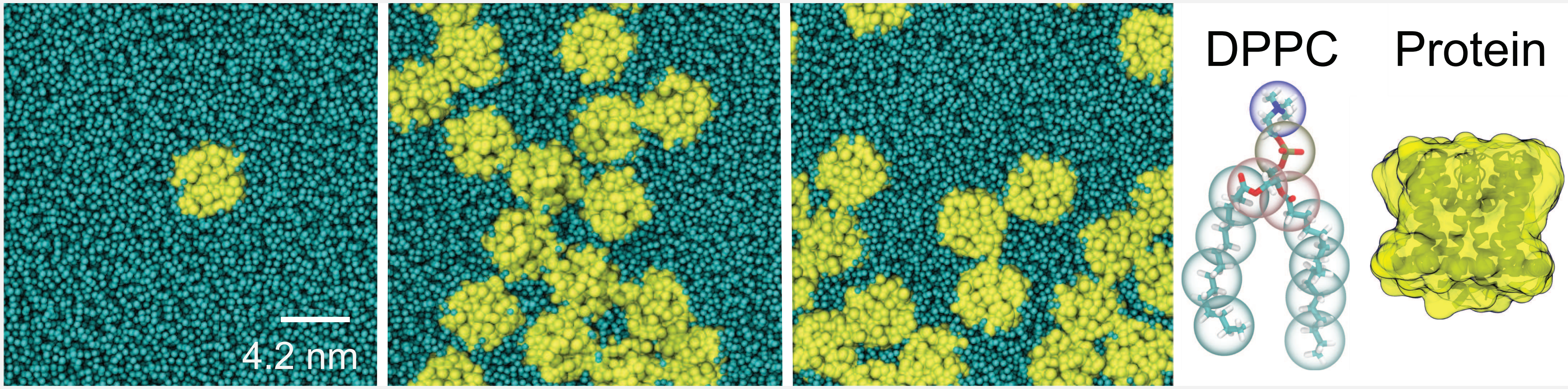


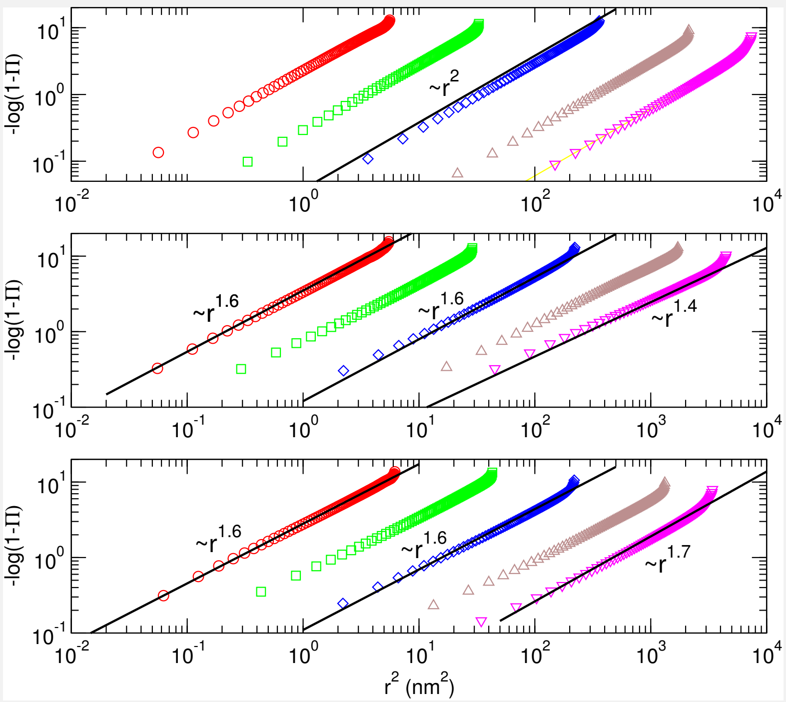


SH Hong et al, Nat Comm (2011); O Kindler, O Pulkkinen, AG Cherstvy & RM, Sci Rep (2019)

& now it's time for something completely different

Crowding in membranes: non-Gaussian lipid/protein diffusion

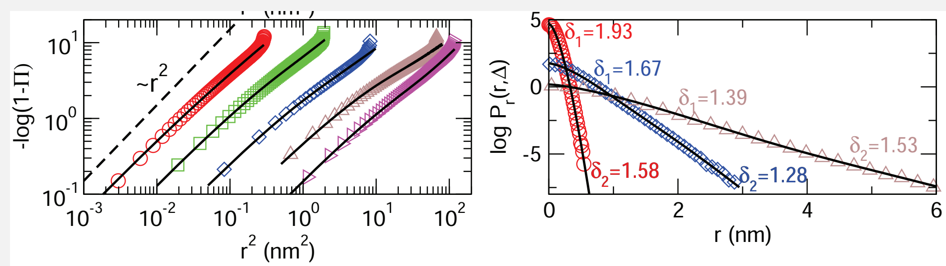




Dilute membrane: $P(r,t)$ Gauss

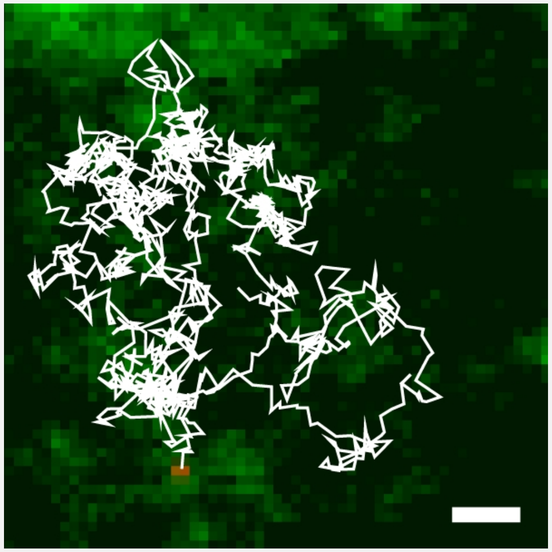
Crowded membrane ($\delta \approx 1.3 \ldots 1.7$):

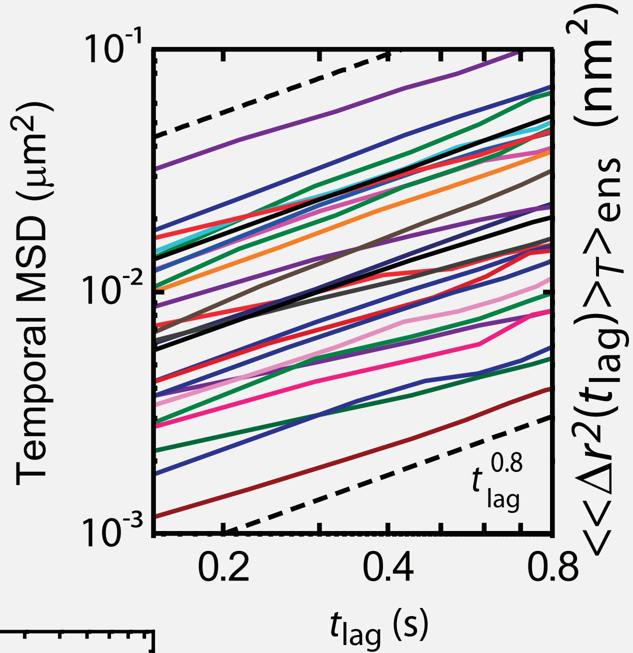
$$P(r,t) \propto \exp\left(-\left[\frac{r}{ct^{\alpha/2}}\right]^{\delta}\right)$$

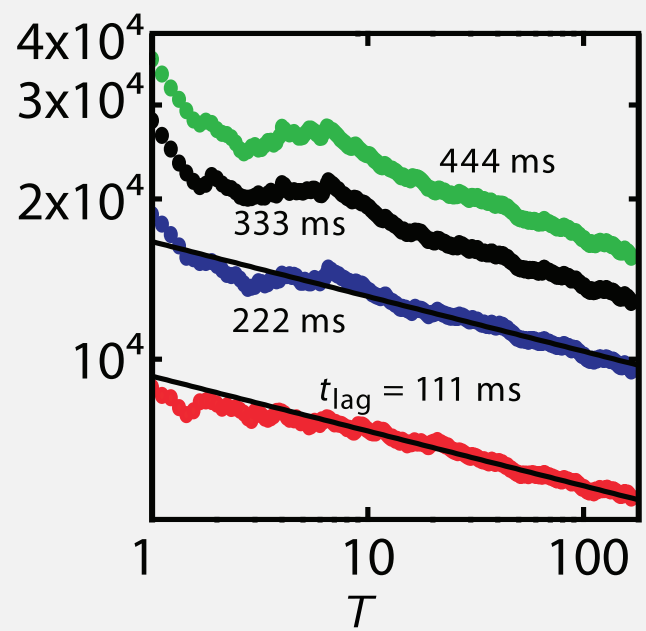


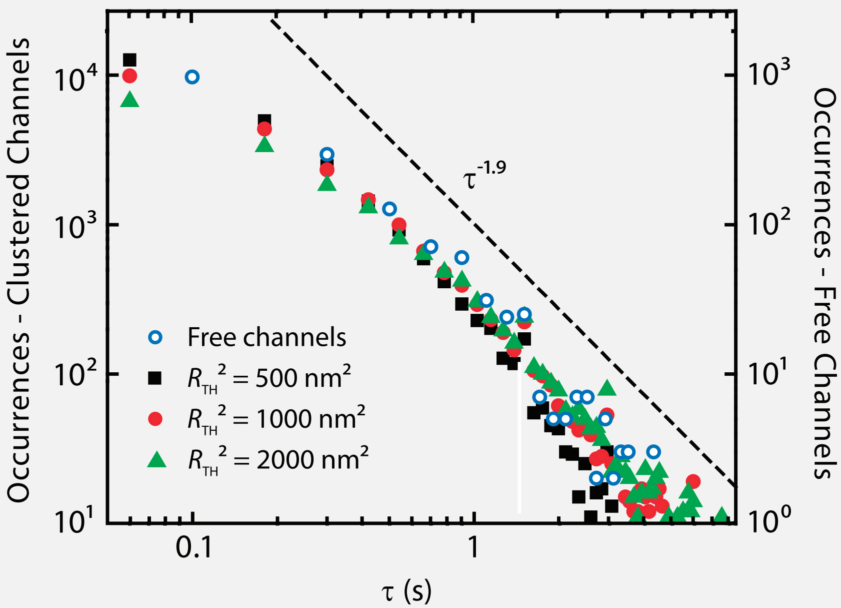
J-H Jeon, M Javanainen, H Martinez-Seara, RM & I Vattulainen, PRX (2016); see also Faraday Disc (2013)

CTRW-like motion of Ka channels in plasma membrane









$\psi(\tau) \simeq \tau^{-1-\alpha}$ scale free

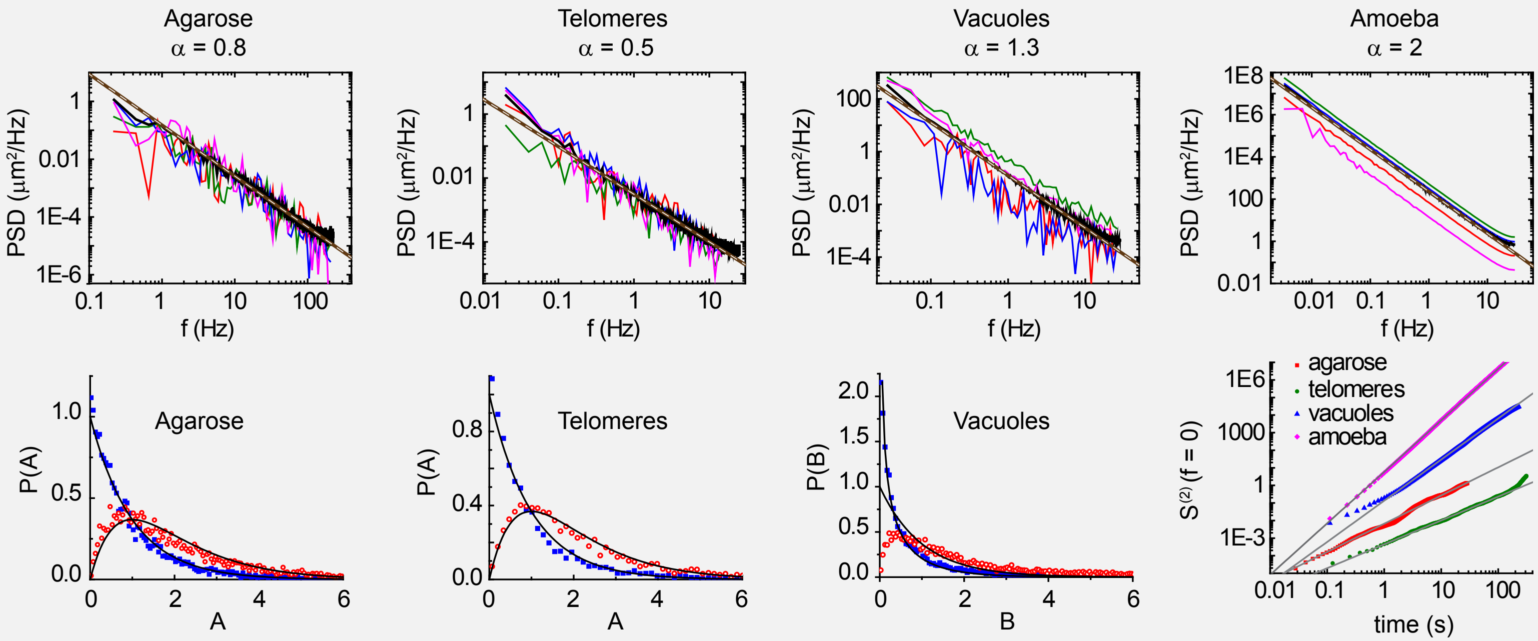
$\overline{\delta^2(\Delta)}$ apparently random

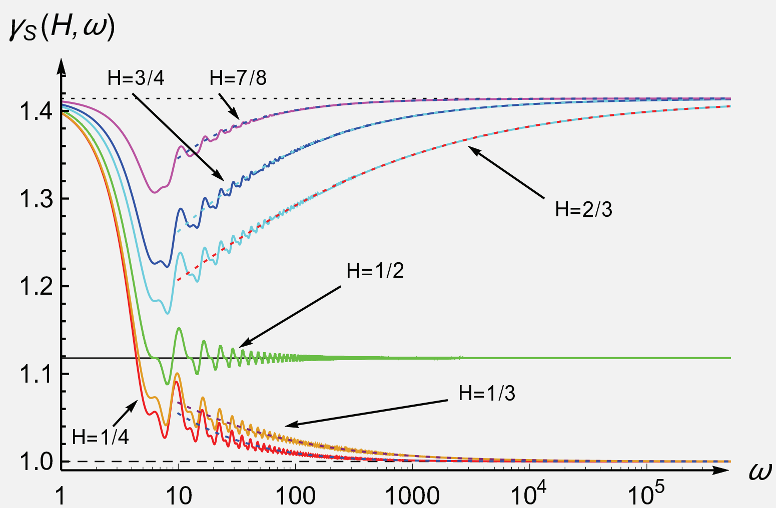
$\Delta/T^{1-\alpha} \simeq \overline{\delta^2(\Delta)} \neq \langle \mathbf{r}^2(\Delta)\rangle \simeq \Delta^\alpha$

$P(\mathbf{r},t) \simeq \exp\left(-\beta r^{1/[1-\alpha/2]}\right)$

AV Weigel, B Simon, MM Tamkun & D Krapf, PNAS (2011); theory: Y He, S Burov, RM & E Barkai, PRL (2008)

Power spectral density of a single FBM trajectory



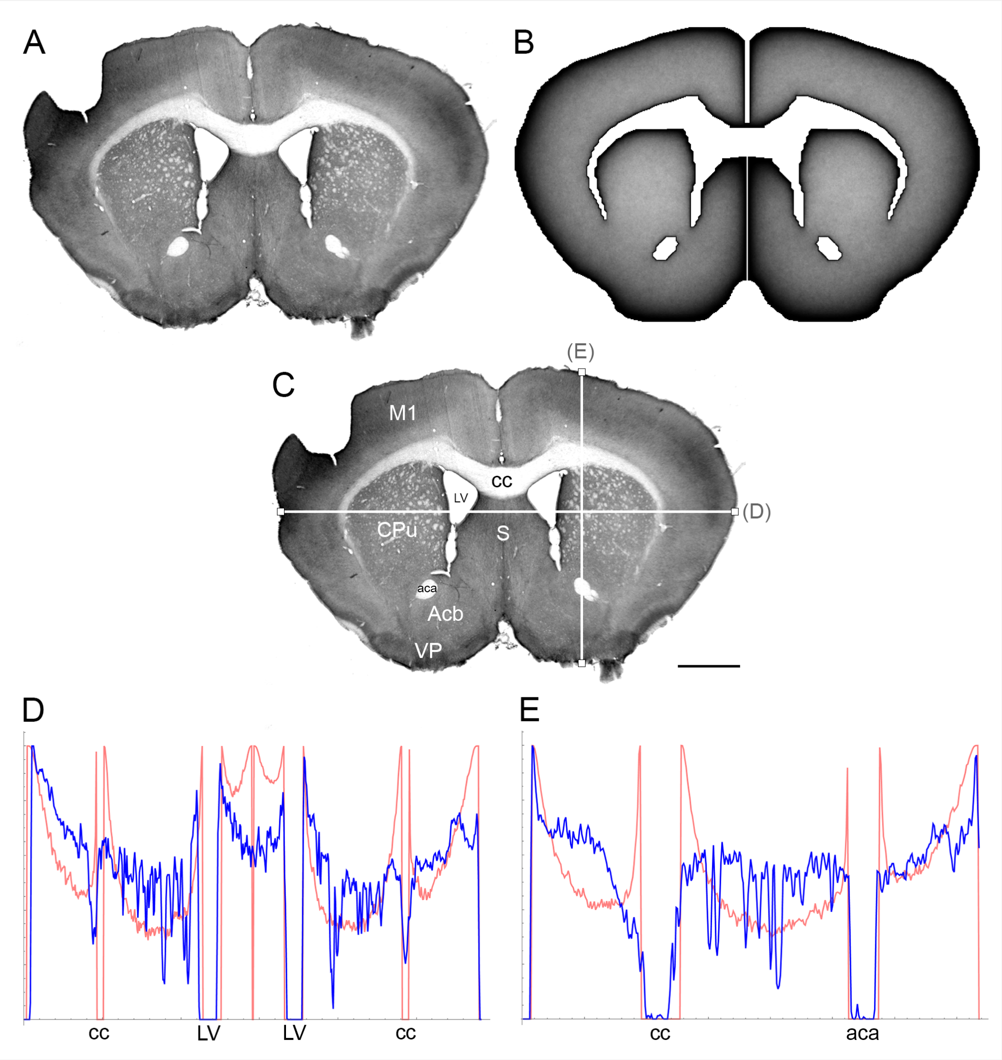


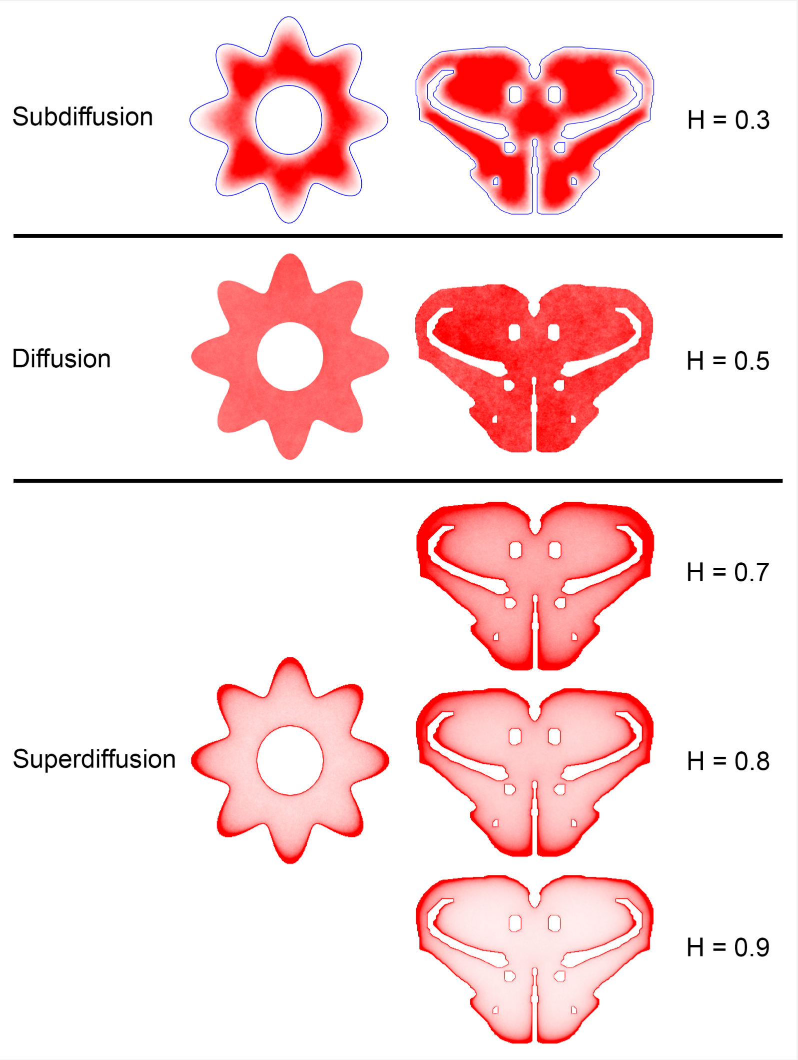
$$S(f,T) = \frac{1}{T}\left|\int_0^T \exp(ift)X_t dt\right|^2$$

$$\gamma = \frac{\left(\langle S_T^2(f)\rangle - \langle S_T(f)\rangle^2\right)^{1/2}}{\langle S_T(f)\rangle}$$

D Krapf, N Lukat, E Marinari, RM, G Oshanin, C Selhuber-Unkel, A Squarcini, L Stadler, M Weiss & X Xu, PRX (2019)

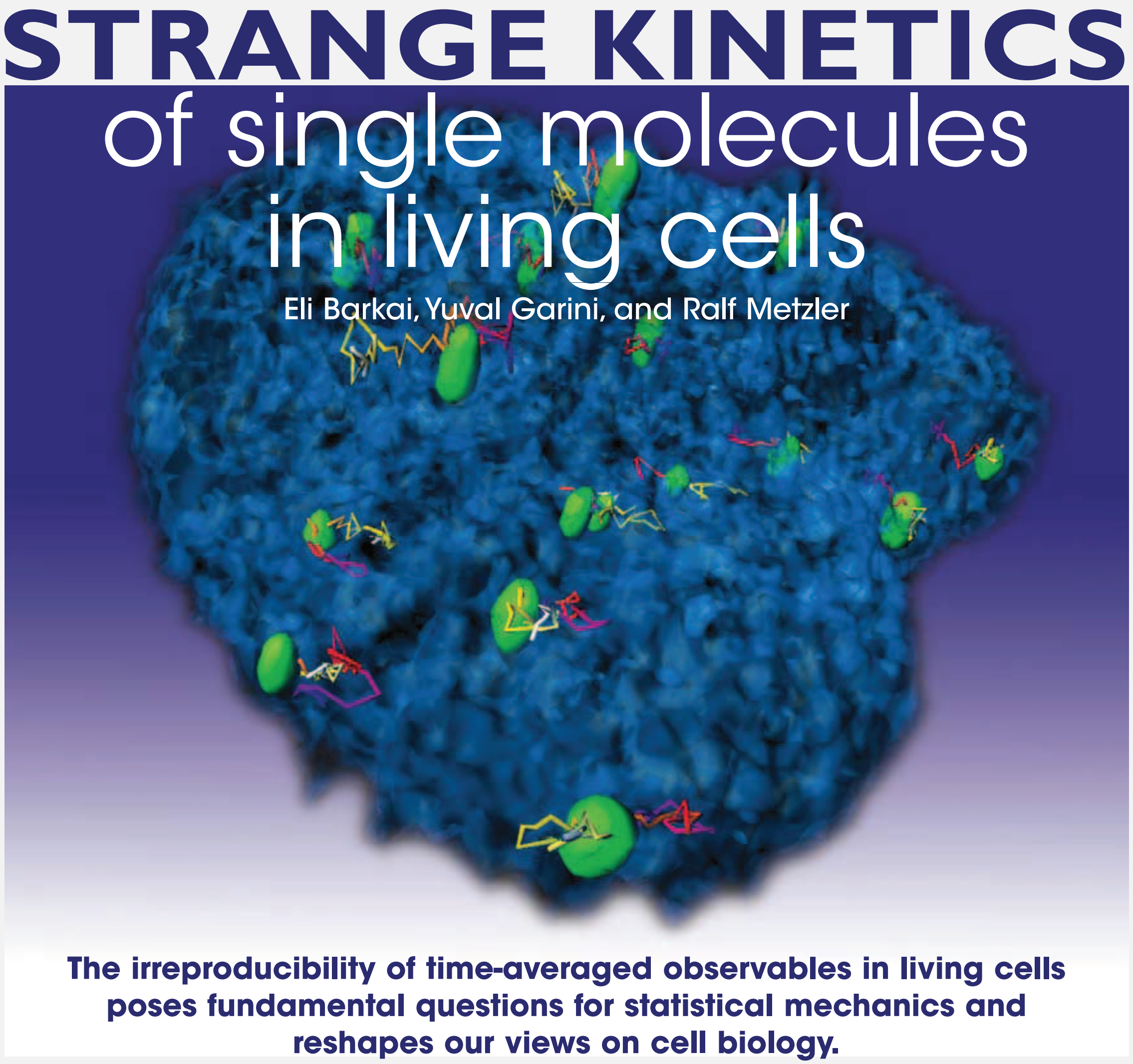
Brain serotonergic axons as FBM paths

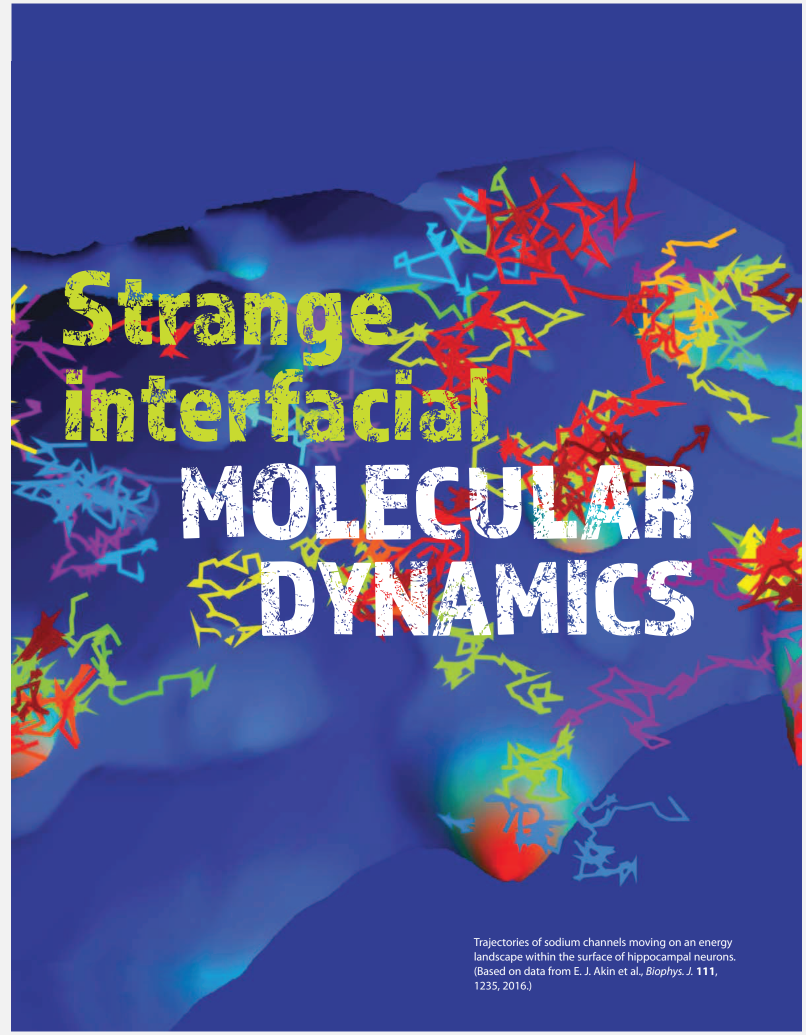




S Janušonis, N Detering, RM & T Vojta, bioRxiv (2020); T Guggenberger, T Vojta & RM, NJP (2019)

Popular articles





E Barkai, Y Garini & RM, Phys Today (2012); D Krapf & RM, Phys Today (2019)

Σummary

1. Facilitated diffusion picture well established, incl DNA conformations & in vivo formulation for bacteria
2. Heterogeneous DNA: speed-stability paradox $\rightsquigarrow$ two-state model;
3. Gene-gene proximity $\rightsquigarrow$ rapid search hypothesis, confirmed by explicit modelling: distance matters
4. Defocusing of reaction times in generic settings. Narrow escape: DS Grebenkov, RM & G Oshanin, NJP (2019)
5. Anomalous diffusion, (non-)ergodicity, ageing
6. Lévy flight search & optimisation
7. Dilute & protein-crowded lipid biliayer membranes

Acknowledgements

Eli Barkai (Bar-Ilan U Ramat Gan)
Carsten Beta (U Potsdam)
Andrey Cherstvy, Aleksei Chechkin (U Potsdam)
Aljaz Godec (MPIBC Göttingen)
Denis Grebenkov (École Polytechnique)
Jae-Hyung Jeon (POSTECH Pohang)
Michael Lomholt (Syddansk U Odense)
Marcin Magdziarz (Politechnika Wrocławska)
Lene Oddershede (NBI Københavns U)
Gleb Oshanin (Sorbonne)
Gianni Pagnini (BCAM Bilbao)
Christine Selhuber-Unkel (U Kiel)
Flavio Seno (Universitá di Padova)
Igor Sokolov (Humboldt U Berlin)
Ilpo Vattulainen (Helsingin Yliopisto)
Matthias Weiss (U Bayreuth)
Agnieszka Wyłomańska (Politechnika Wrocławska)

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DFG Deutsche Forschungsgemeinschaft

FIDIPRO Finland Distinguished Professor Programme