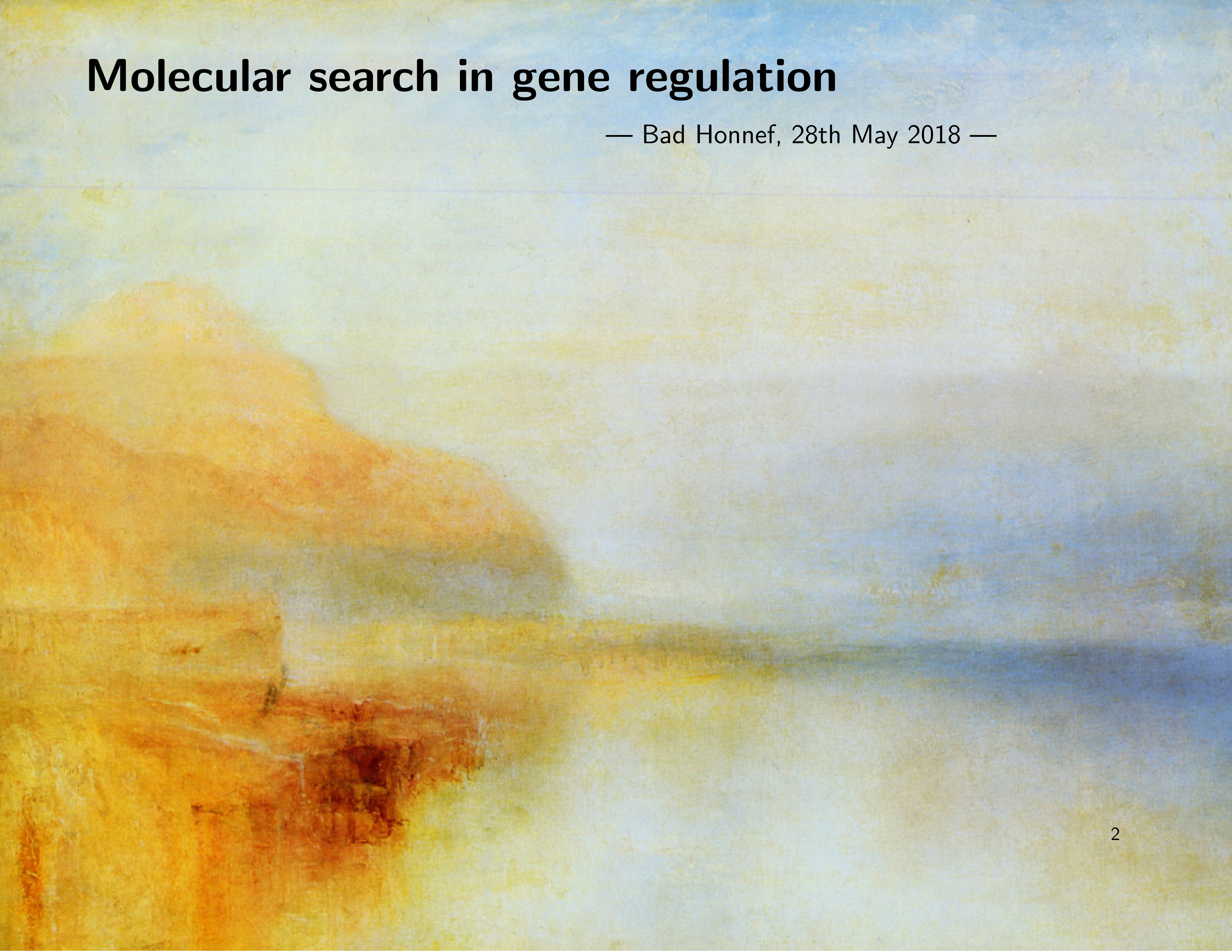
& now it's time for something completely different

Molecular search in gene regulation

— Bad Honnef, 28th May 2018 —



Main protagonist: bacteria cells such as E.coli

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)

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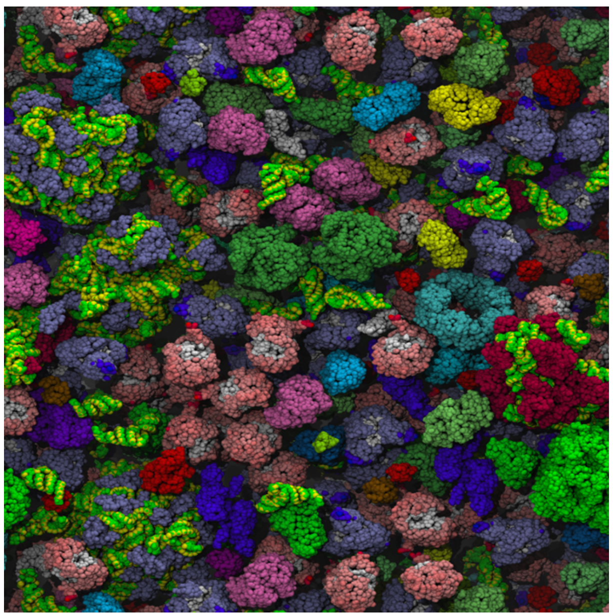
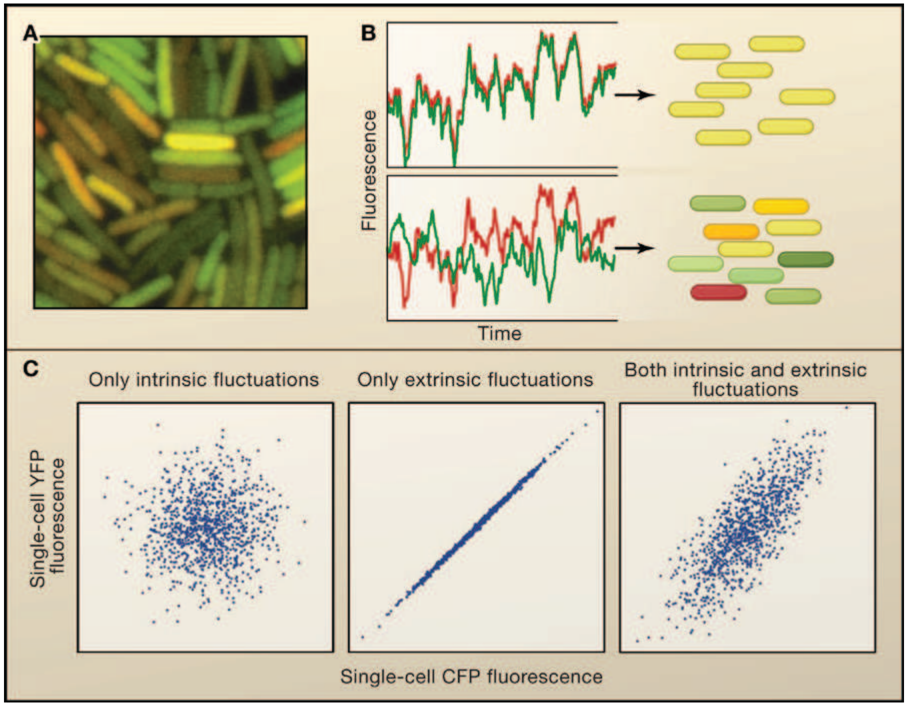
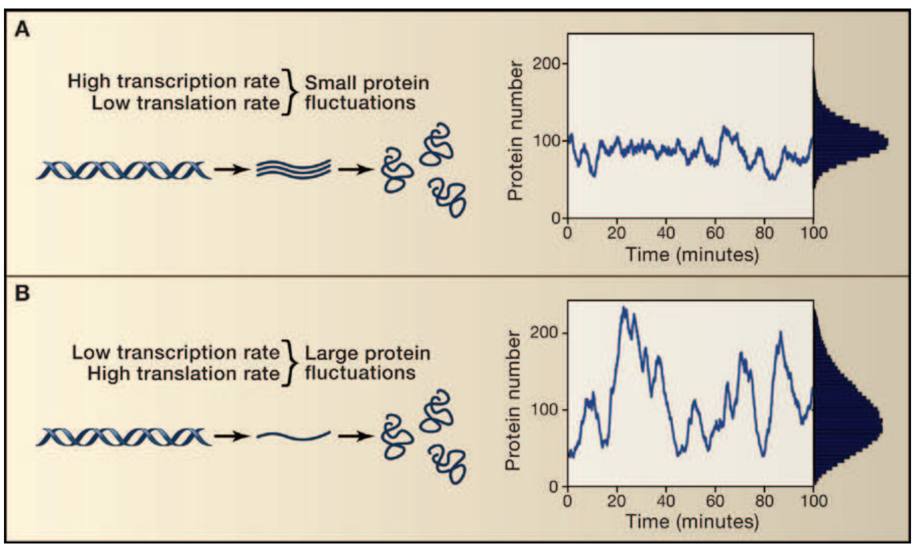
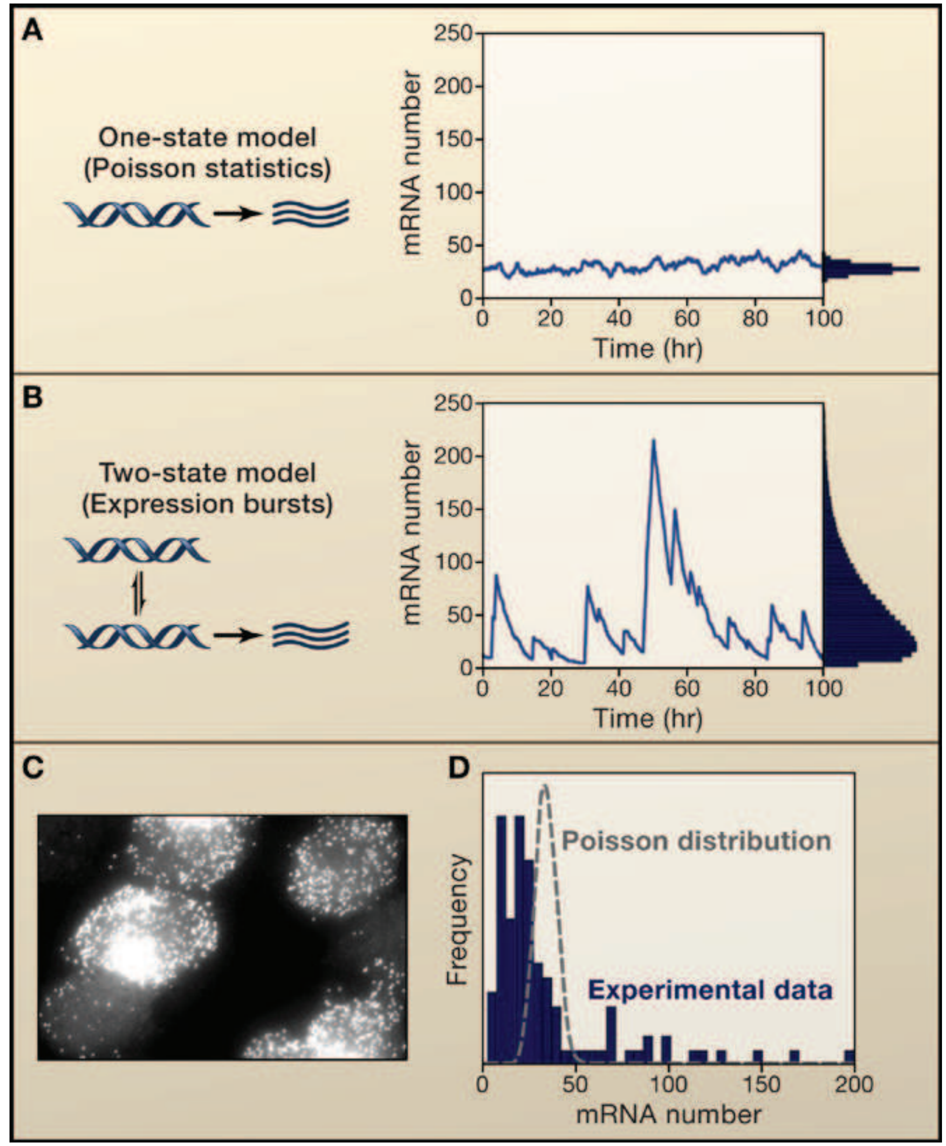


RW Corbin et al, PNAS (2003); McGuffee & Elcock, PLoS Comp Biol (2010)

Sources of cellular noisiness: chemical vs physical



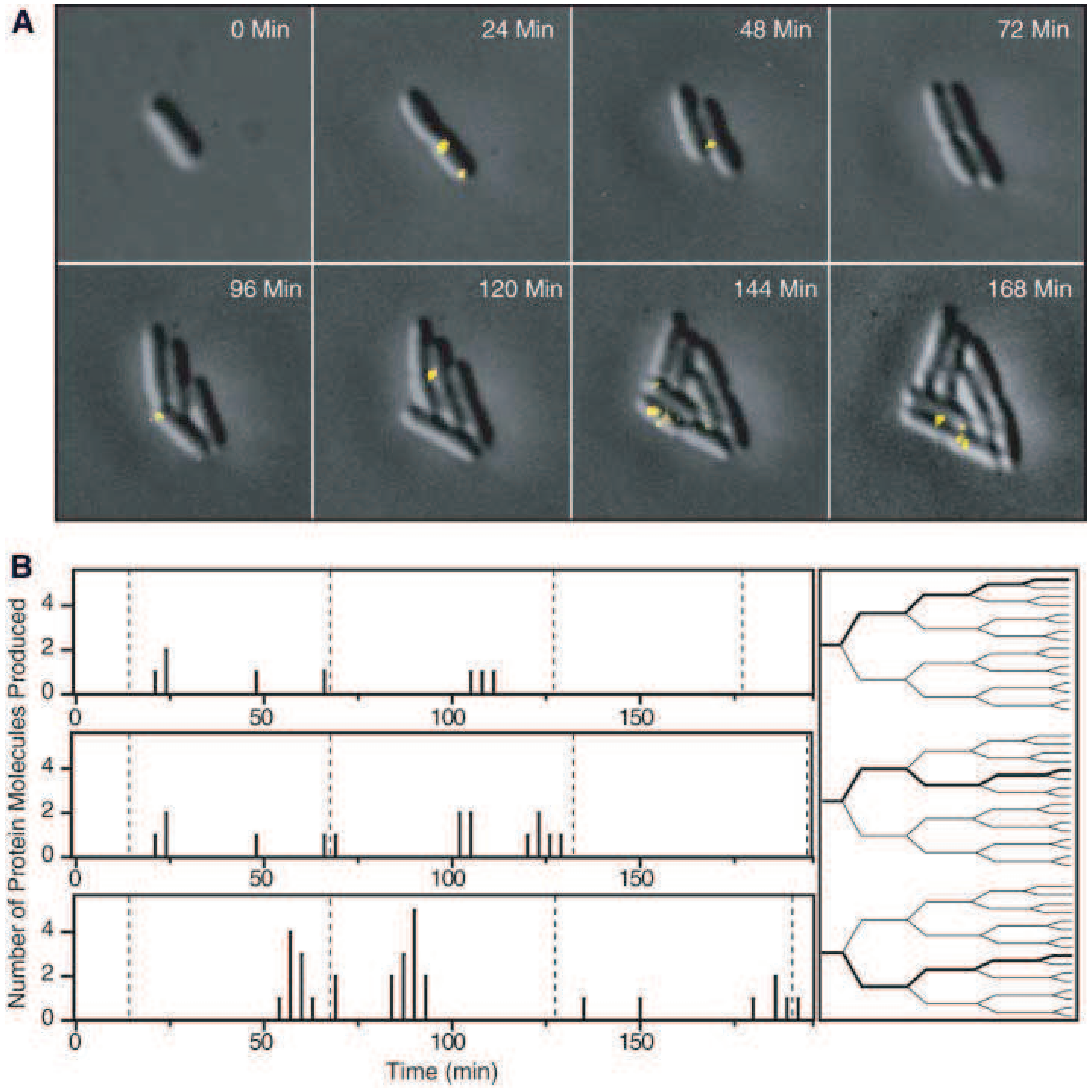




Plus noise due to spatial spreading of TFs!

A Raj & A van Oudenaarden, Cell (2008)

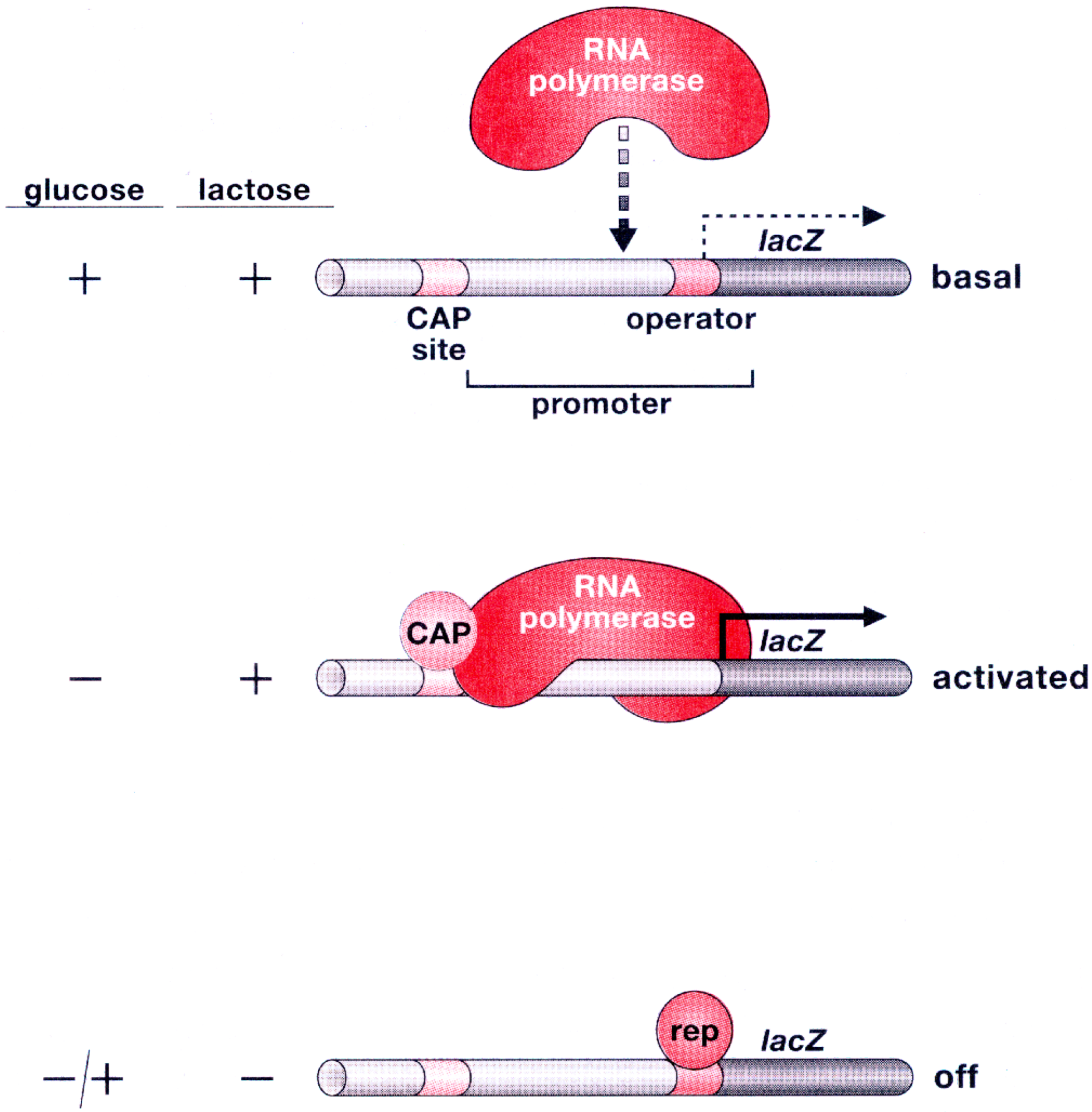
Gene expression one molecule at a time



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

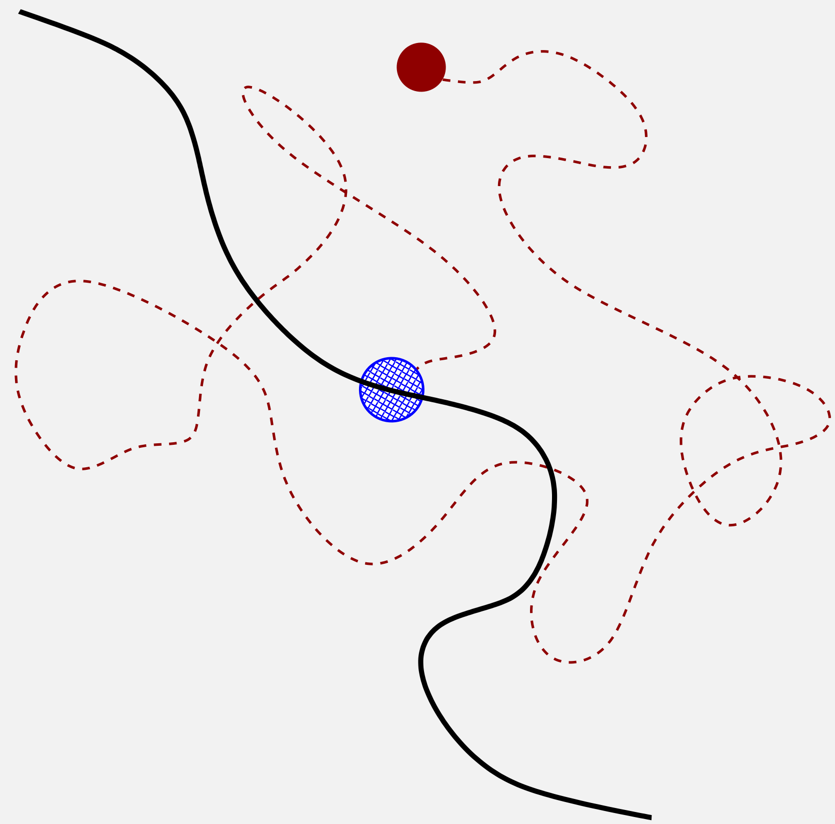
Yu et al, Science (2006); I Golding et al, Cell (2005)

Gene regulation by transcription factors: Lac repressor



M Ptashne, A genetic switch

Smoluchowski search picture



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

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

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

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

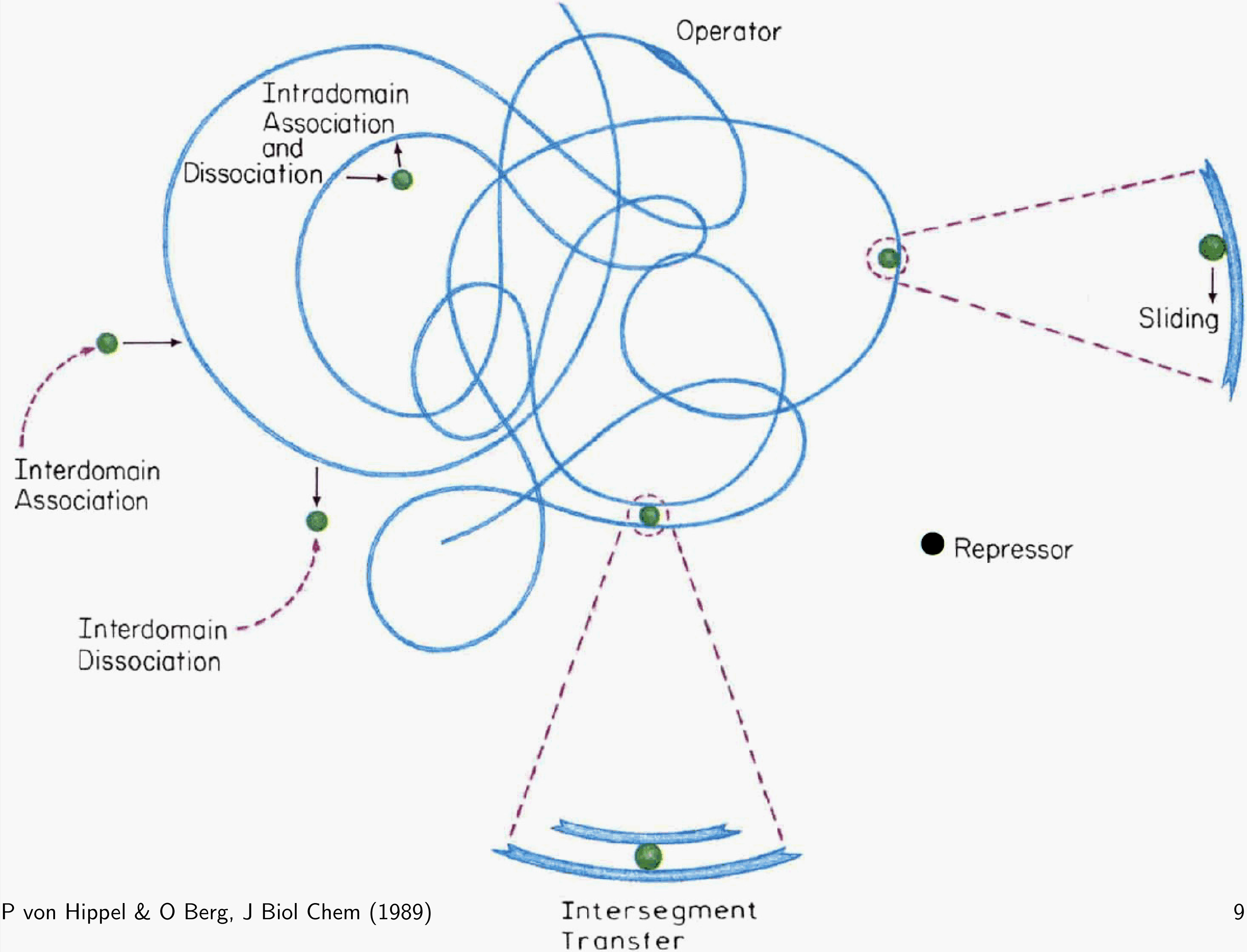
Lac repressor [AD Riggs, S Bourgeois, M Cohn, J Mol Biol 53, 401 (1970)]:

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

$\rightarrow$ Facilitated diffusion picture

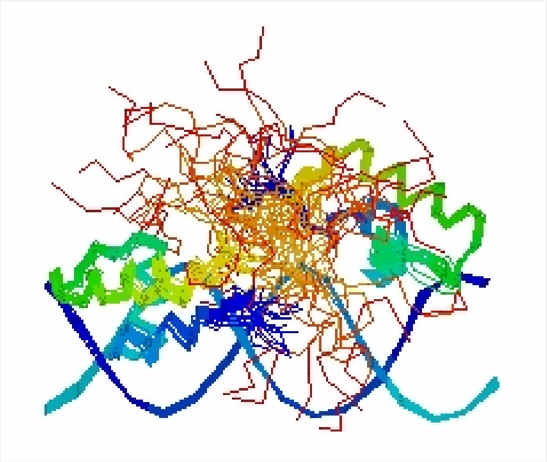
M v Smoluchowski, Physikal. Zeitschr. (1916); P von Hippel and O Berg, J Biol Chem (1989)

Facilitated diffusion: the Berg-von Hippel model

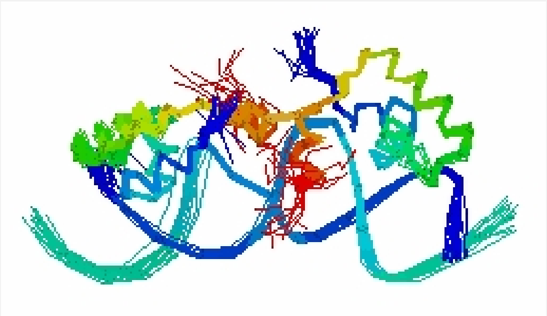


P von Hippel & O Berg, J Biol Chem (1989)

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



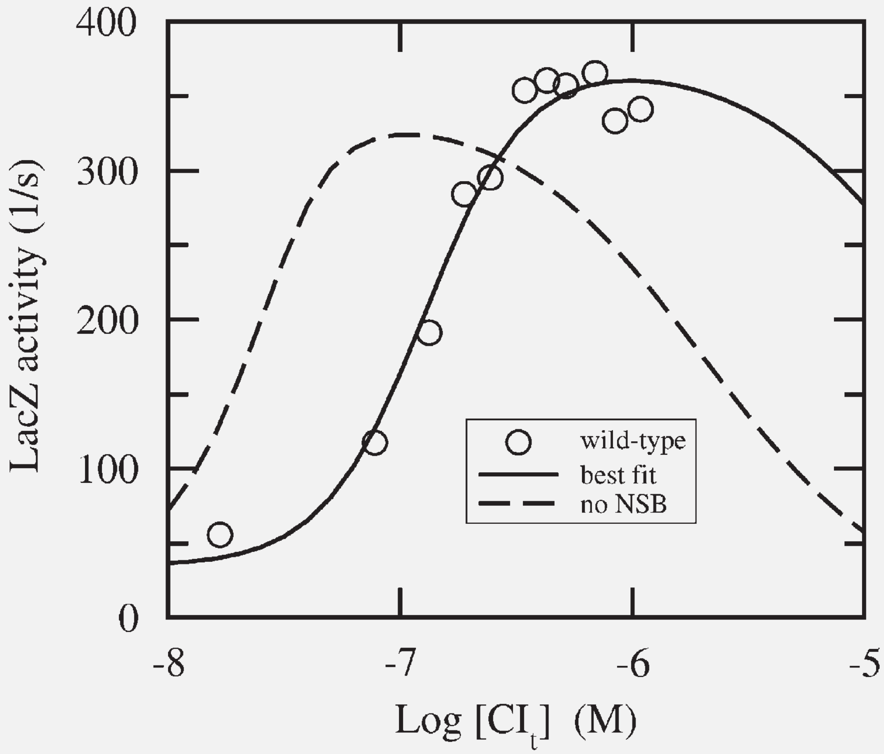
Lac repressor, nonspecific binding



Lac repressor, specific binding

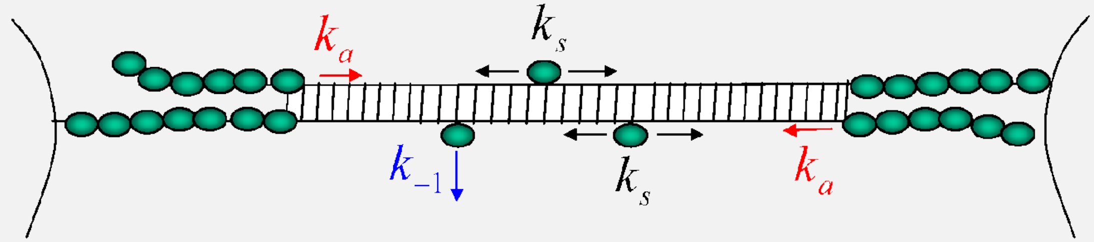
$$[X] = [X_{free}] + [X_{@O_P}] + [X_{NSB}]$$

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



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

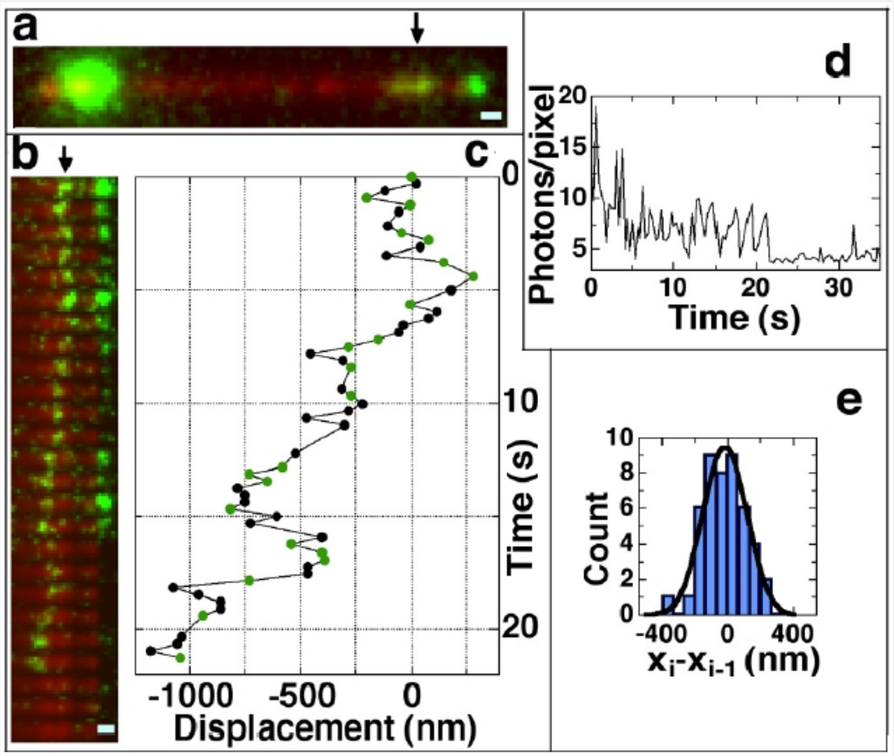
Proof of 1D search mode

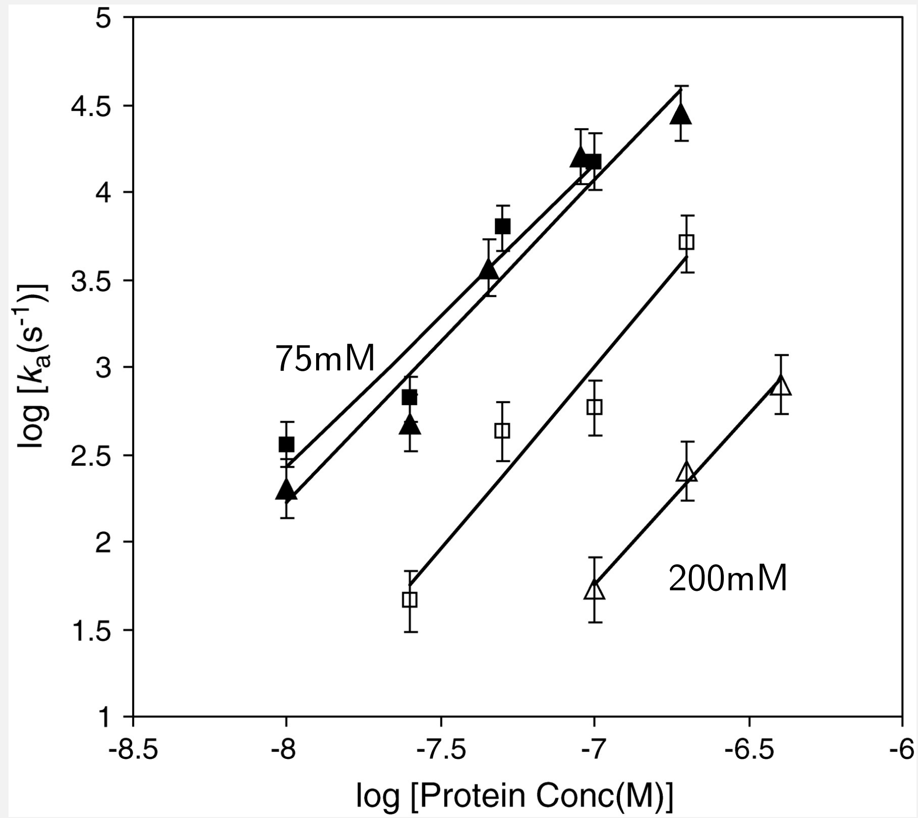


McGhee & von Hippel isotherm

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

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





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

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

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

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 & fractional derivatives

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

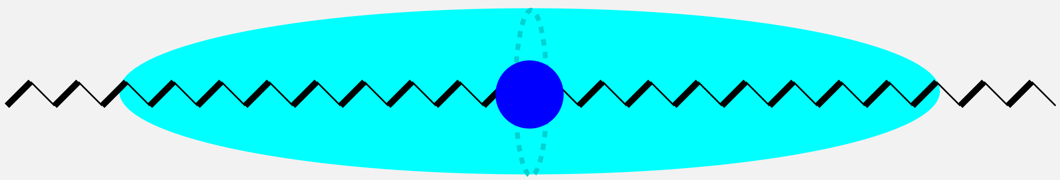
The antenna effect

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:

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

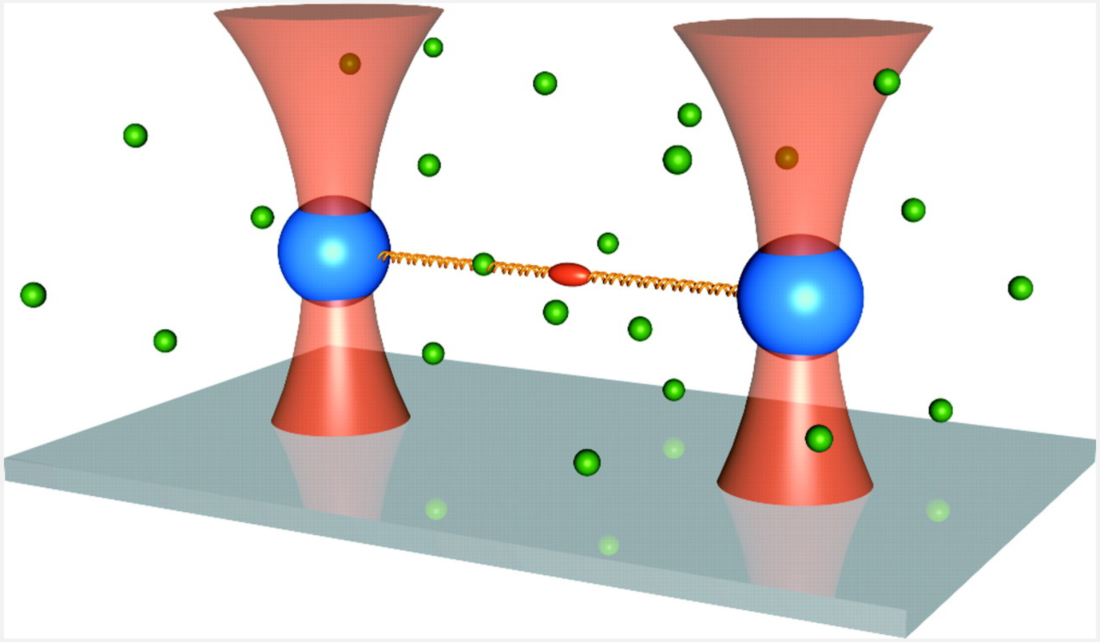


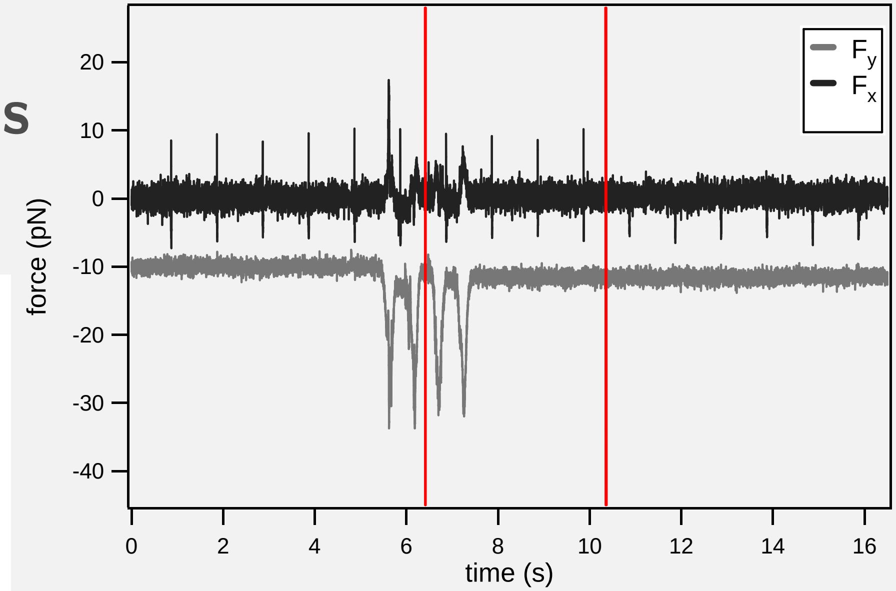
Effective sliding length:

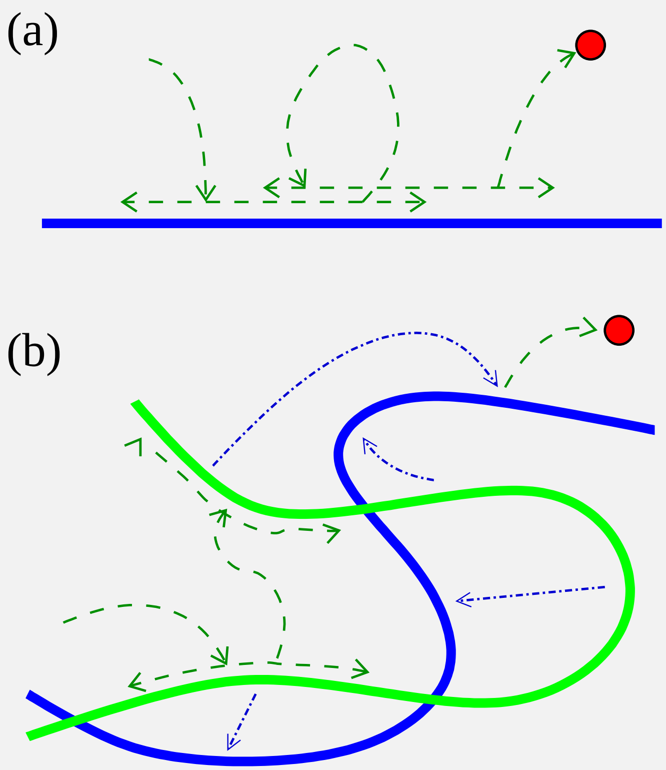
$$\ell_{\rm sl}^{\rm eff} = \sqrt{\frac{k_{\rm on}}{2\pi D_{3d}}} \times \ell_{\rm sl}$$

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

The rôle of DNA conformations







pCco5 plasmid DNA: 6538bp $\approx 2.2\mu$m $\approx 45\ell_p$

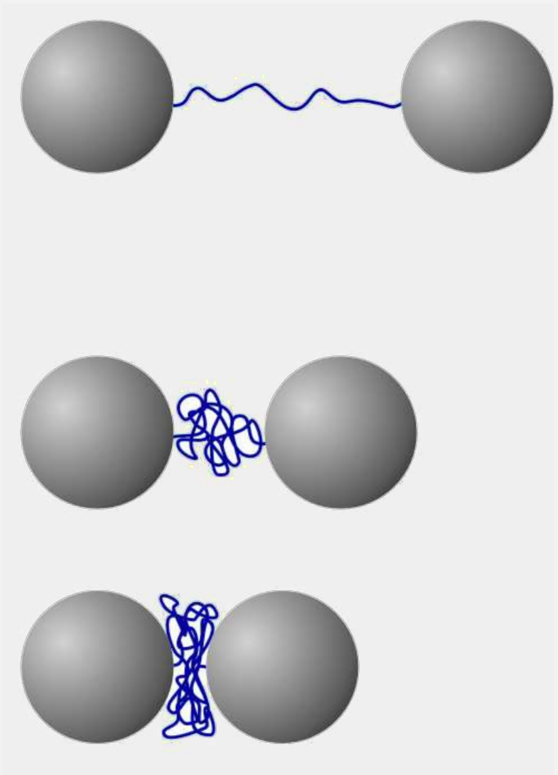
[comp λ DNA 48.5kbp]

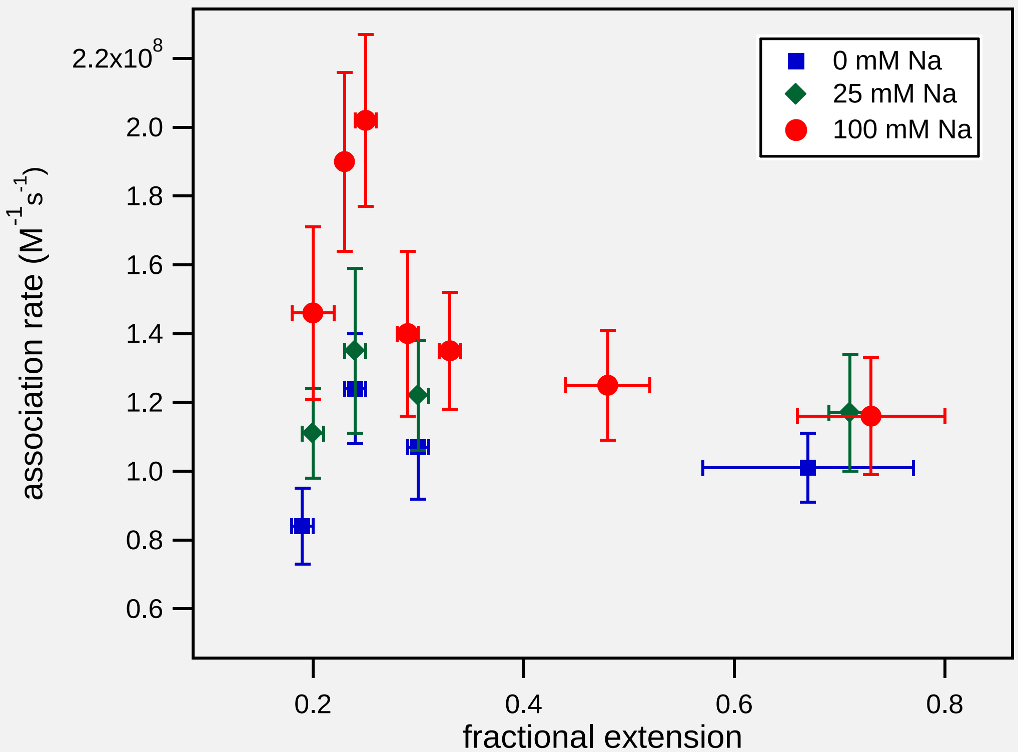
B van den Broek, MA Lomholt, S-M Kalisch, RM & GJL Wuite, PNAS (2008)

More compact DNA conformations speed up the search

[NaCl]	$k_{on}^{straight}$ [Ms]	l_{sl}^{eff} [bp]	$1/\sqrt{l_{DNA}}$ [bp]	ℓ_p [bp]	R_{theory}	$R_{measured}$
0 mM	0.8×10^8	195	518	188	1.18	1.3 ± 0.2
25 mM	1.0×10^8	250	485	175	1.23	1.1 ± 0.2
100 mM	1.0×10^8	250	150	159	1.67	1.7 ± 0.3
150 mM	0.9×10^9	15.5	120	153	1.15	1.3 ± 0.4

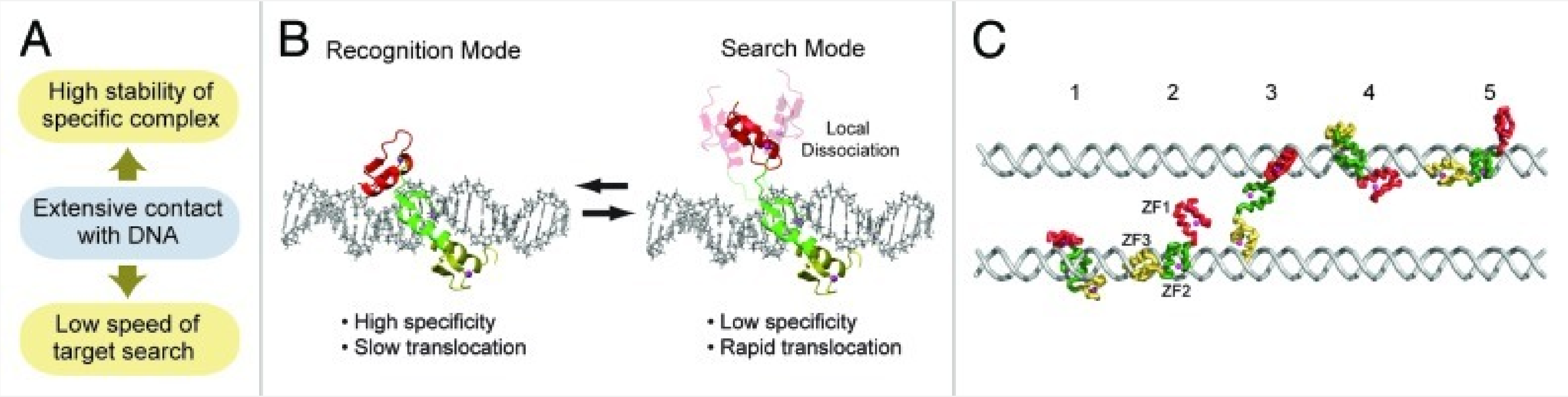
$R = k_{on}^{max}/k_{on}^{straight}$: enhancement ratio of attachment rates @ max and straight configuration)





MA Lomholt, B van den Broek, S-M Kalisch, GJL Wuite & RM, PNAS (2009)

Speed-stability paradox in TF search along DNA



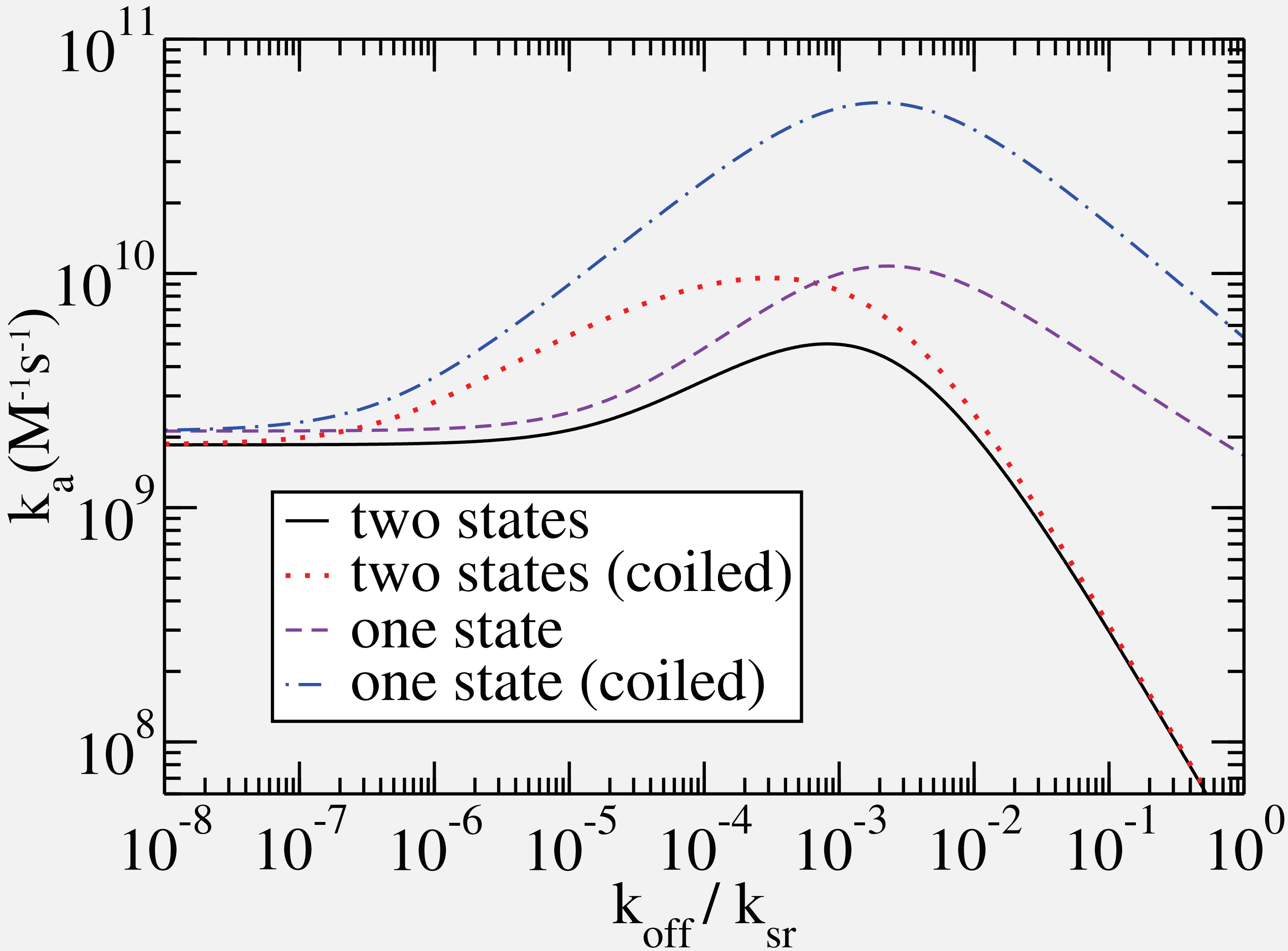
From simulations:

B: Search & recognition modes for a zinc finger protein

C: Intersegmental transfer of the protein

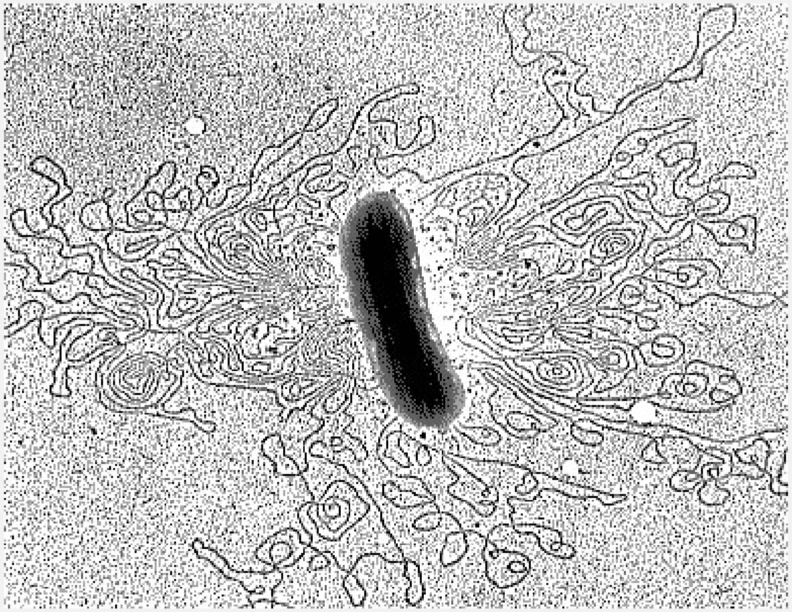
M Slutsky & L Mirny, Biophys J (2004); J Iwahara & Y Levy, Transcription (2013)

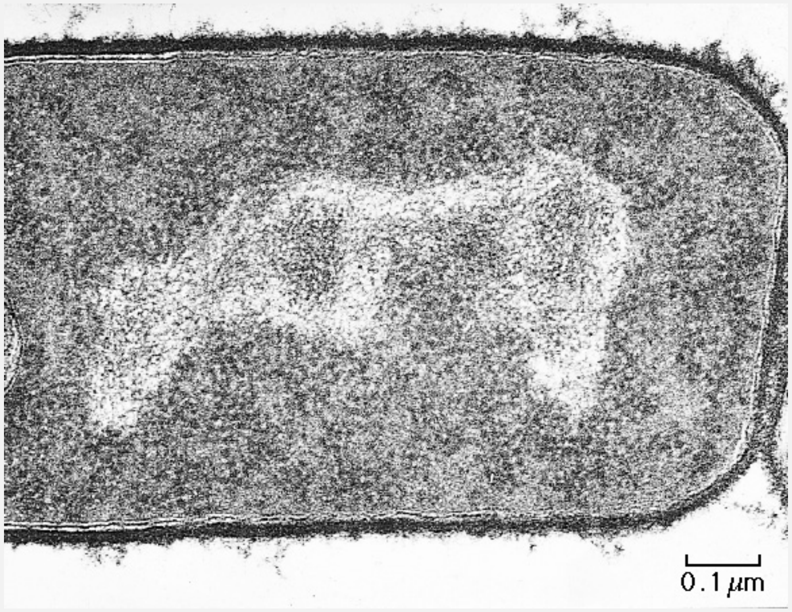
Facilitated diffusion: rate with search & recognition states



M Bauer & RM, Biophys J (2012)

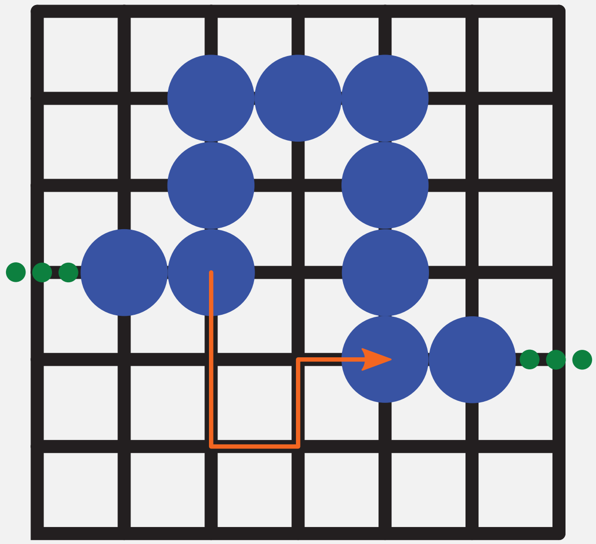
In vivo bacterial gene regulation: E.coli





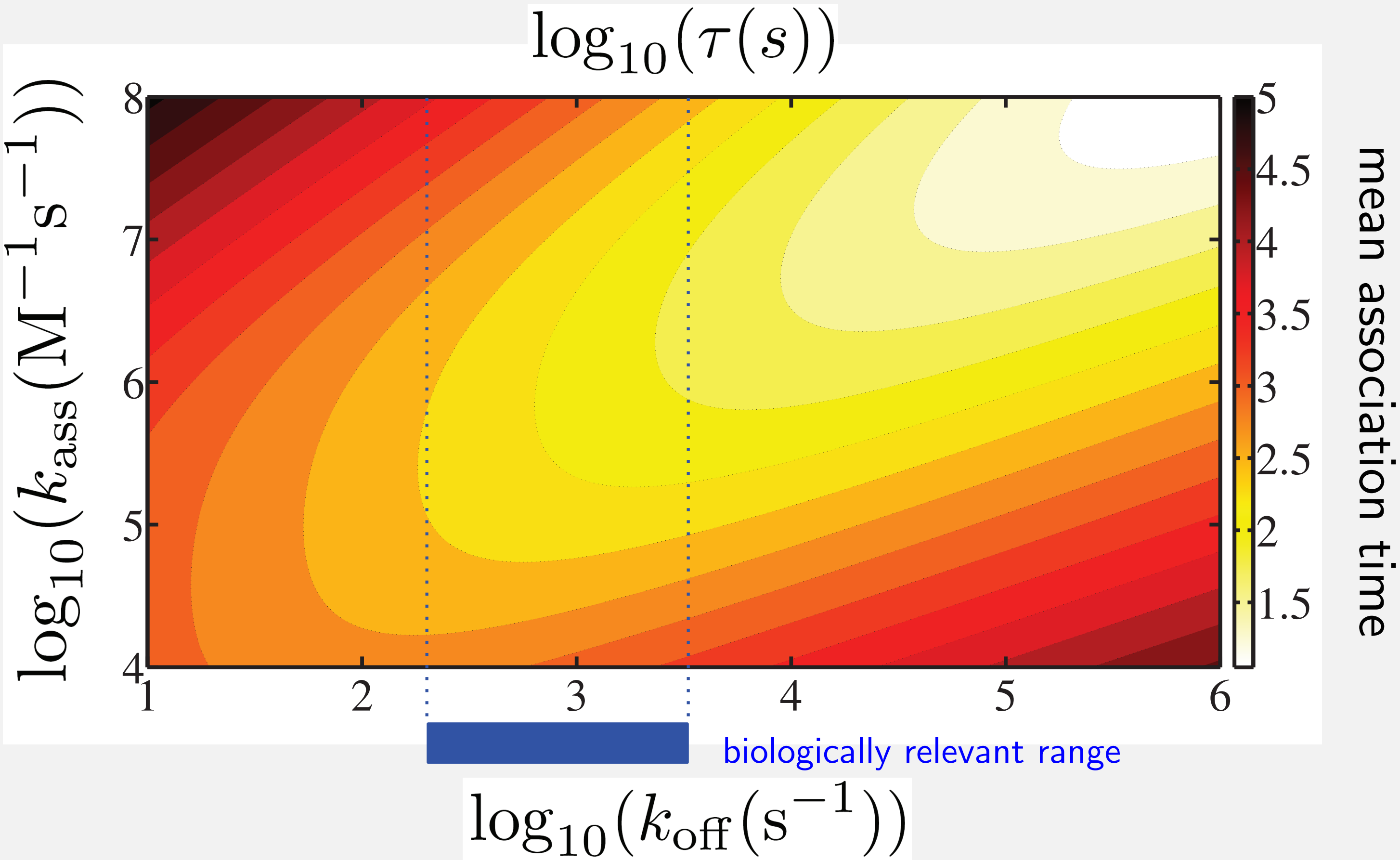
Chromosome is approx an SAW

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



M Bauer & RM, PLoS ONE (2013)

In vivo gene regulation consistent with facilitated diffusion



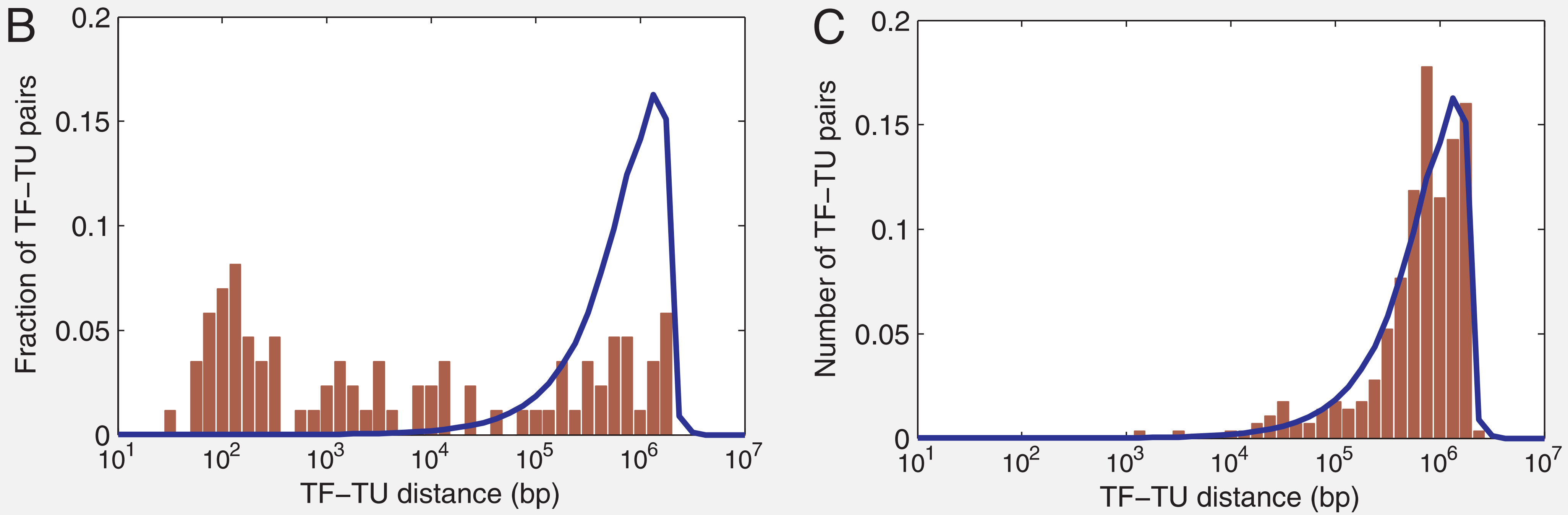
@ optimum the target association time is $\tau \approx 311$sec (no fit parameter)

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

TF regulation effects gene proximity

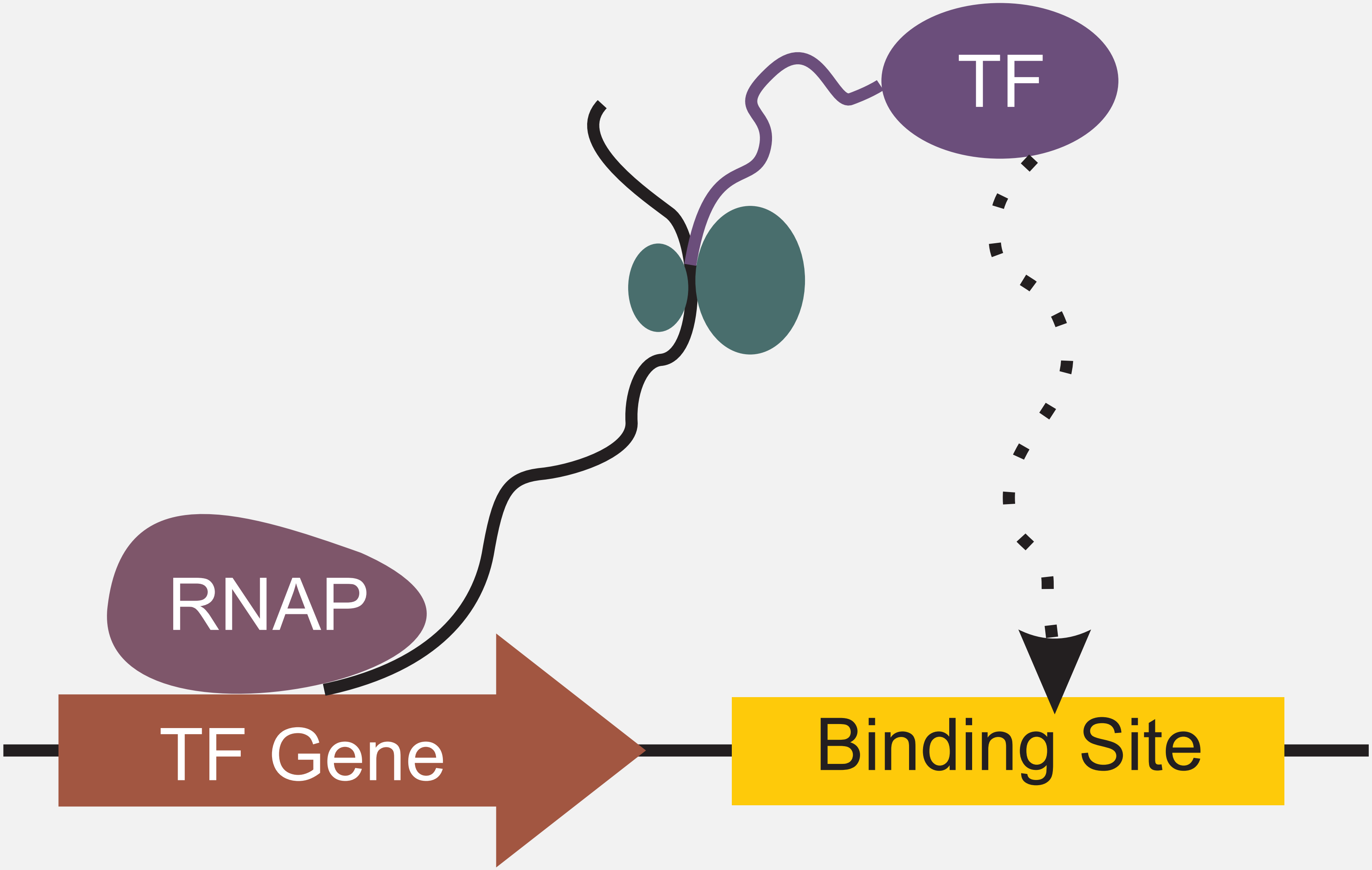
Does distance between genes interacting via TFs matter?

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)

Rapid search hypothesis

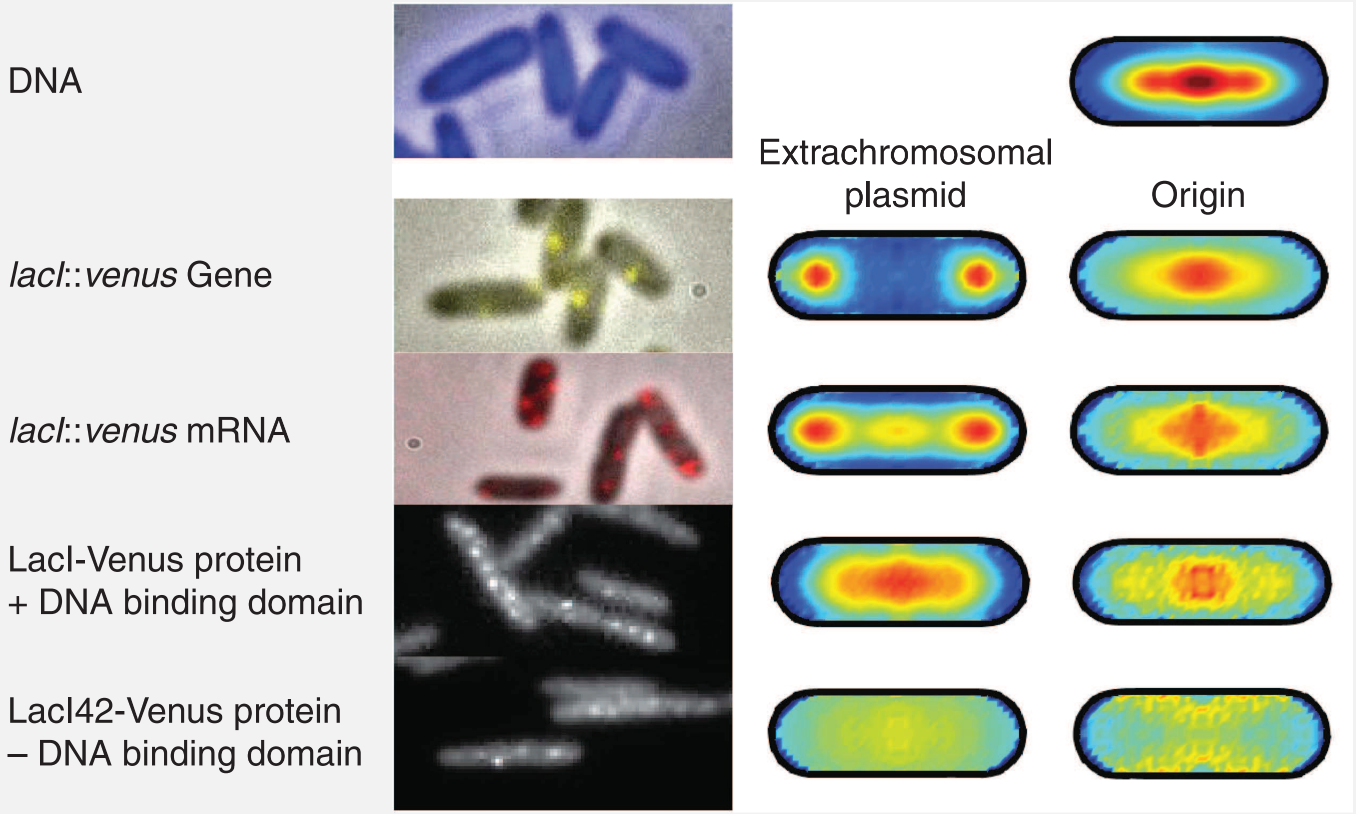


Kolesov et al, PNAS (2007)

Spatial aspects: do gene locations matter?

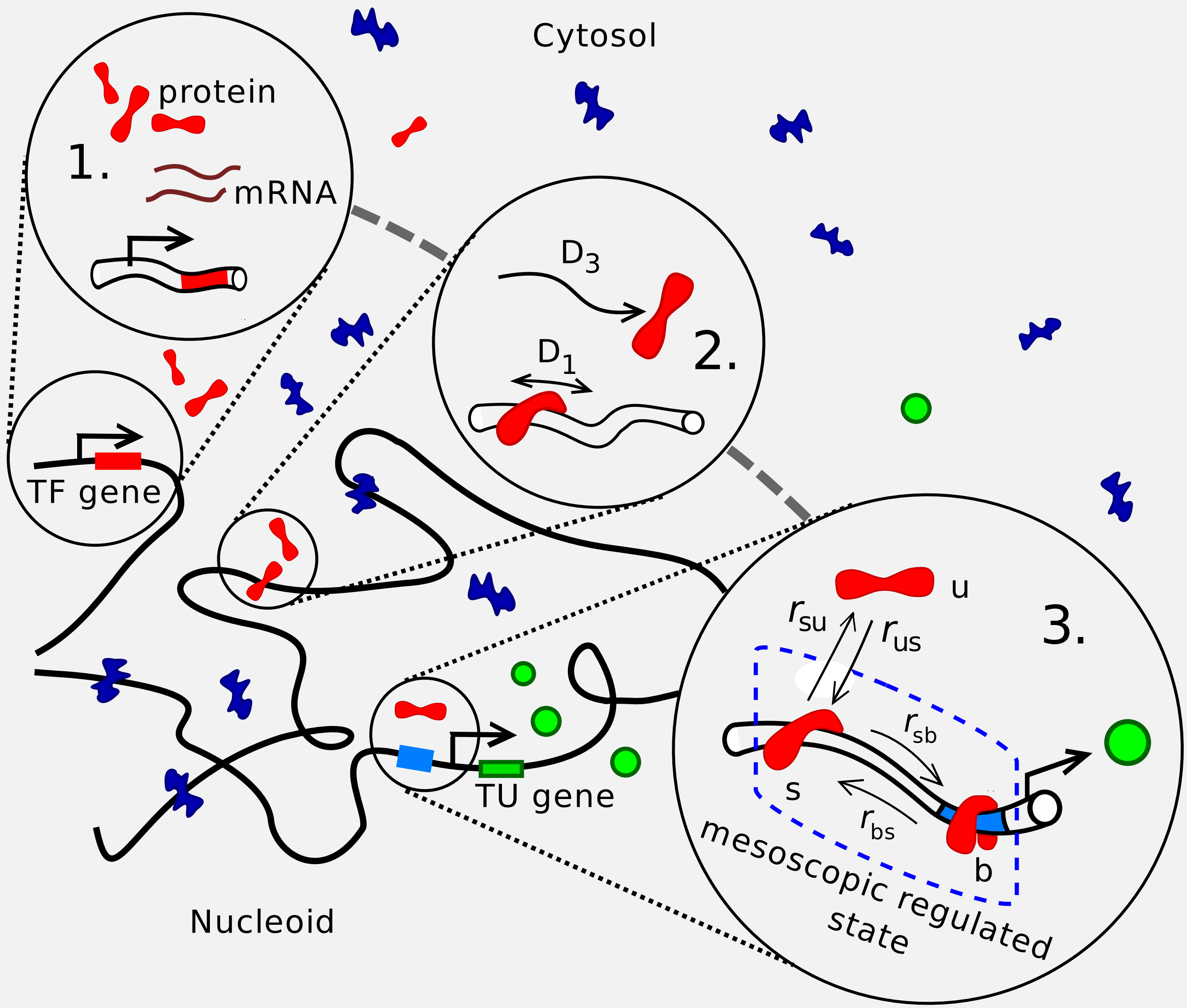
Képès: TF targets are typically located next to or at regular distances from the TF gene $\rightarrow$ TF gene-target pairs close in 3D

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



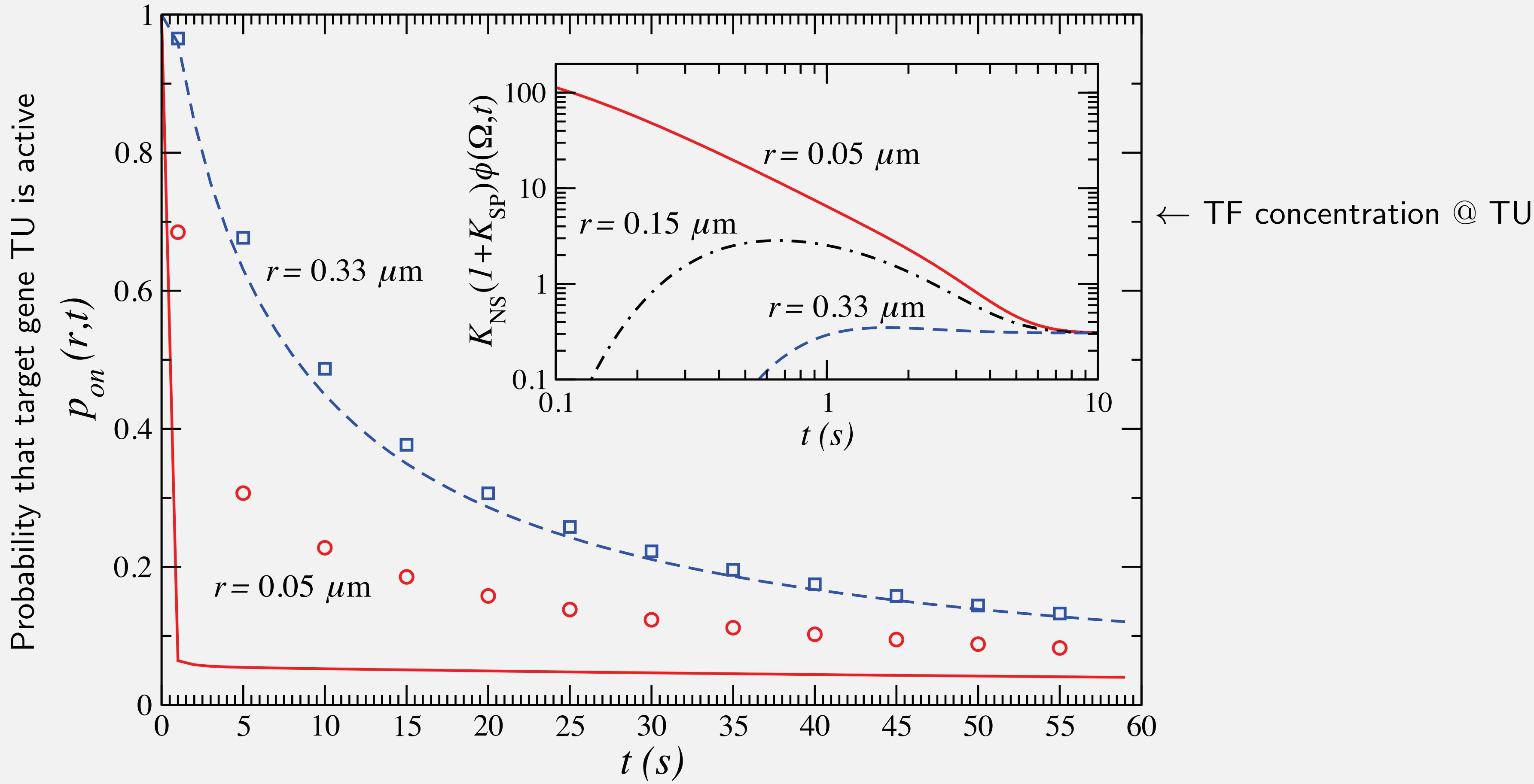
Kepes et al, J Mol Biol (2004); Kuhlman & Cox, Mol Syst Biol (2012)

Transient intracellular signalling is diffusion controlled



O Pulkkinen & RM, PRL (2013)

Result 1: transient response to repression



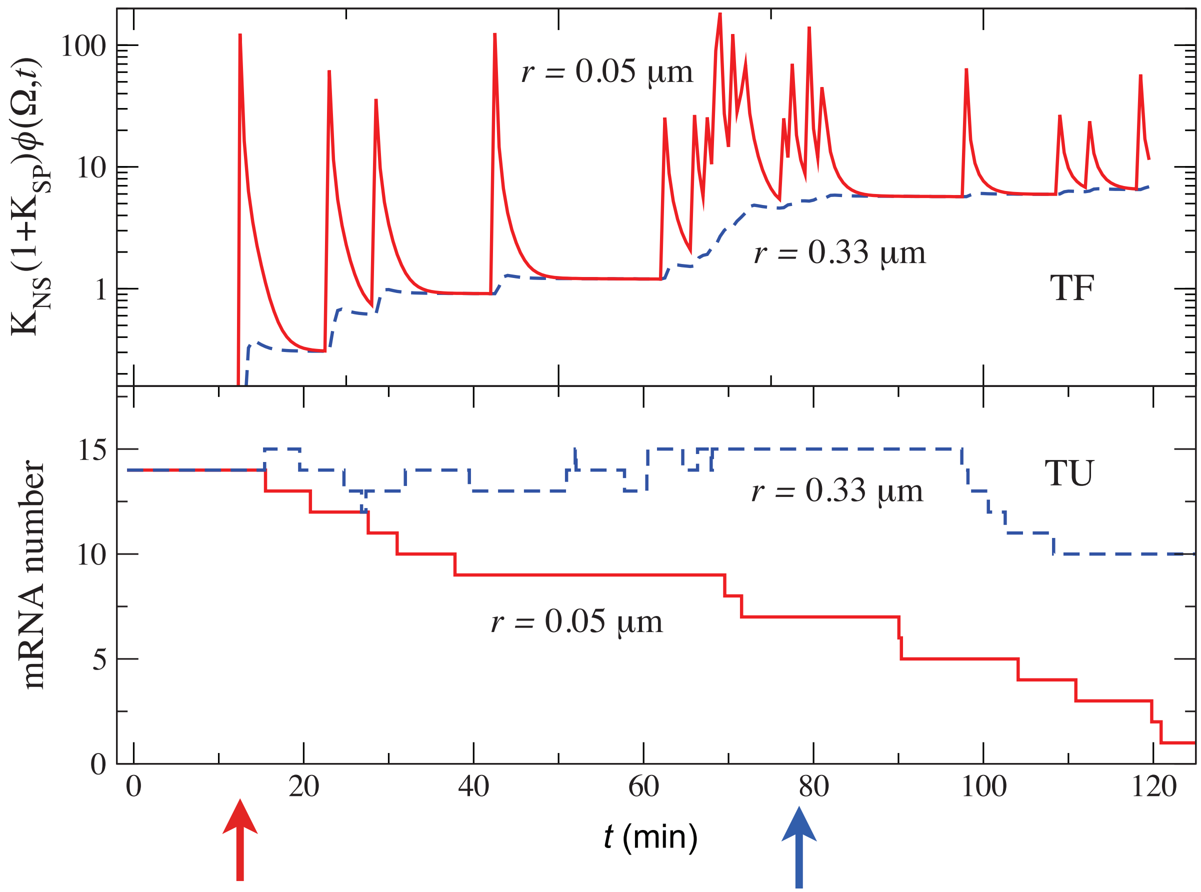
Mean field approximation (full & dashed lines):

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

O Pulkkinen & RM, PRL (2013)

Result 2: time dependence of gene response

Sample paths from MC simulations:



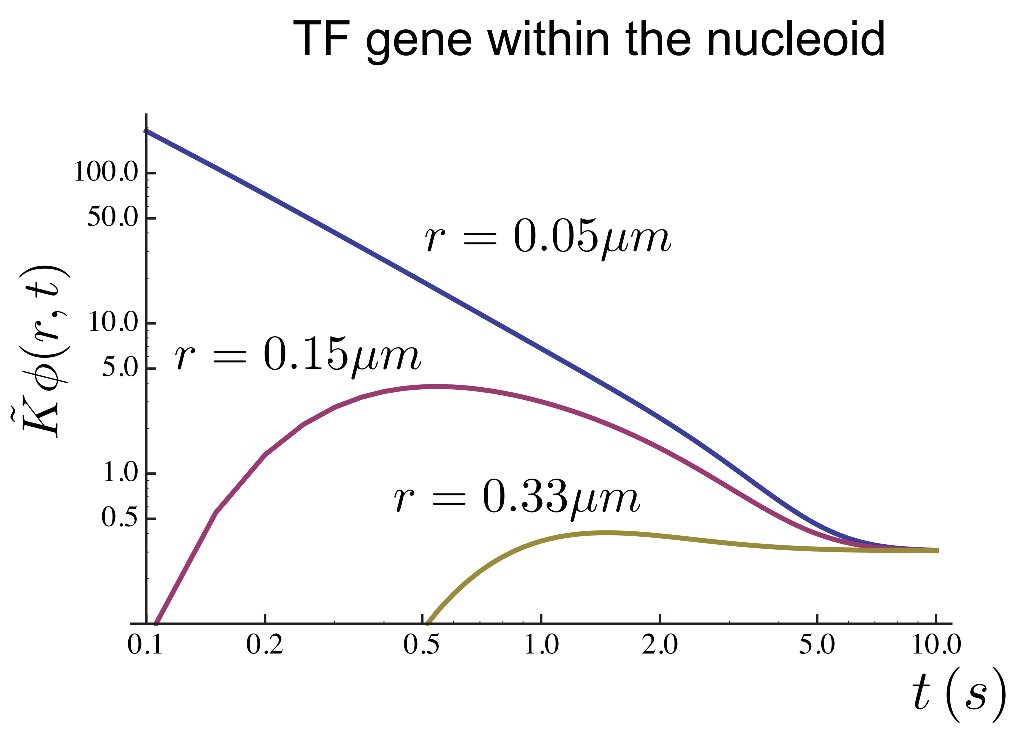
TF density at the target operator

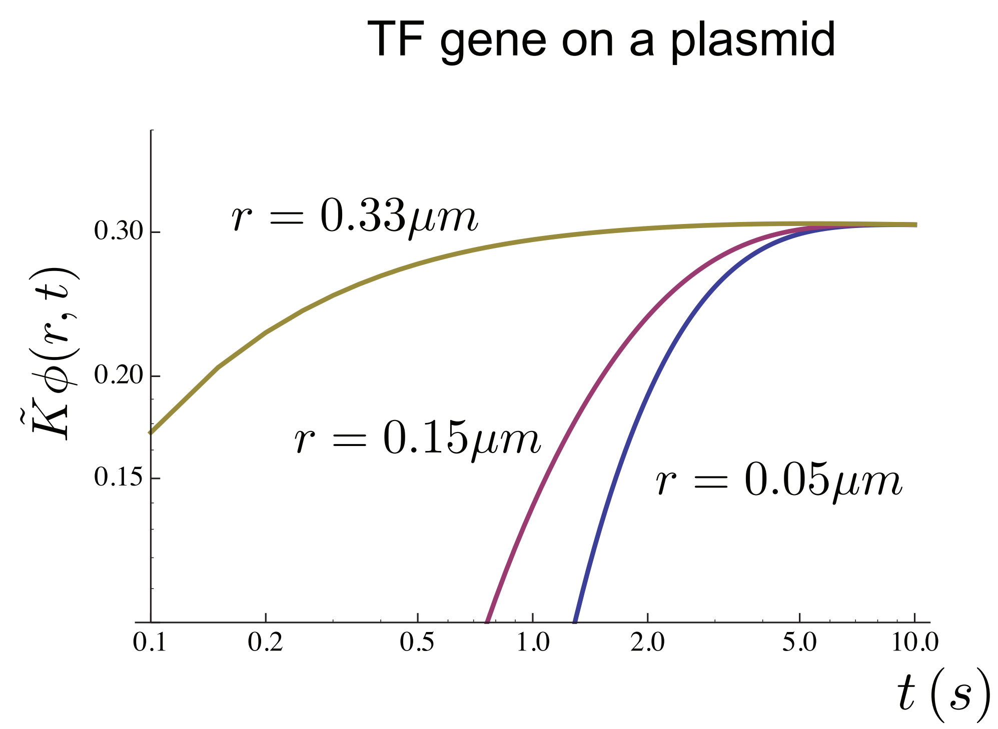
Target expression

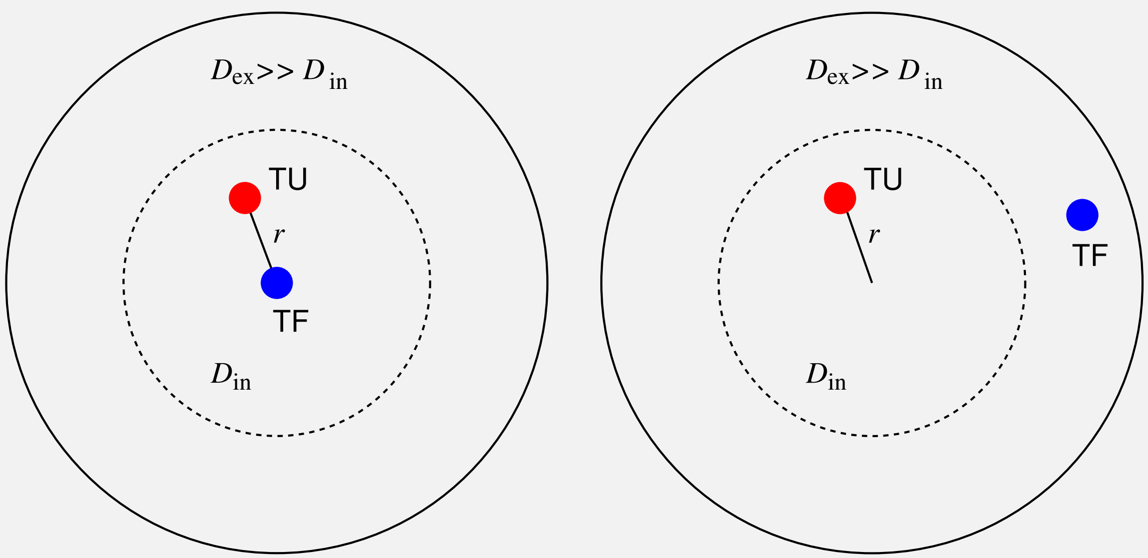
First repressor binding at r = 0.05 μm

First repressor binding at r = 0.33 μm

Result 3: gene location matters

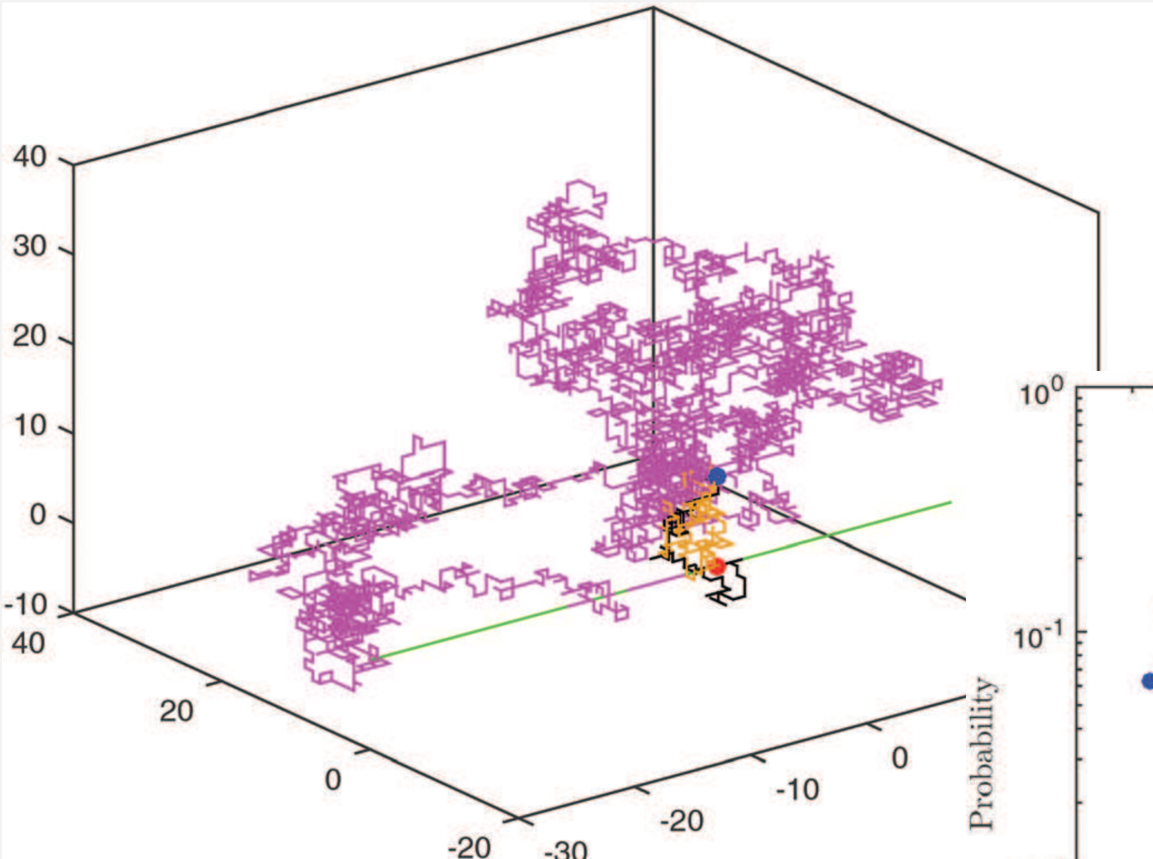


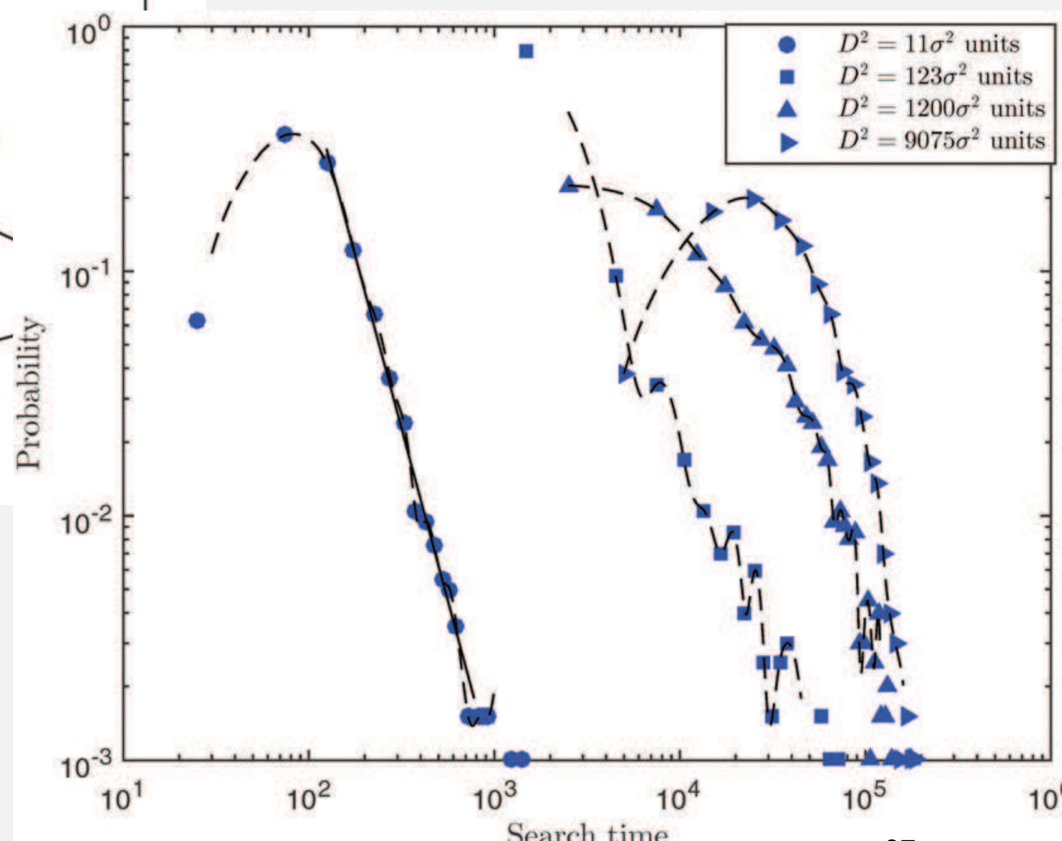




O Pulkkinen & RM, PRL (2013)

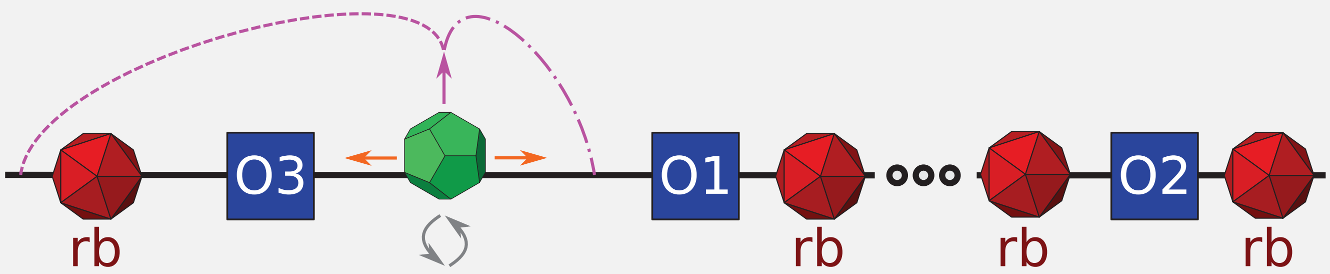
Numerical analysis confirms relevance of proximity effect

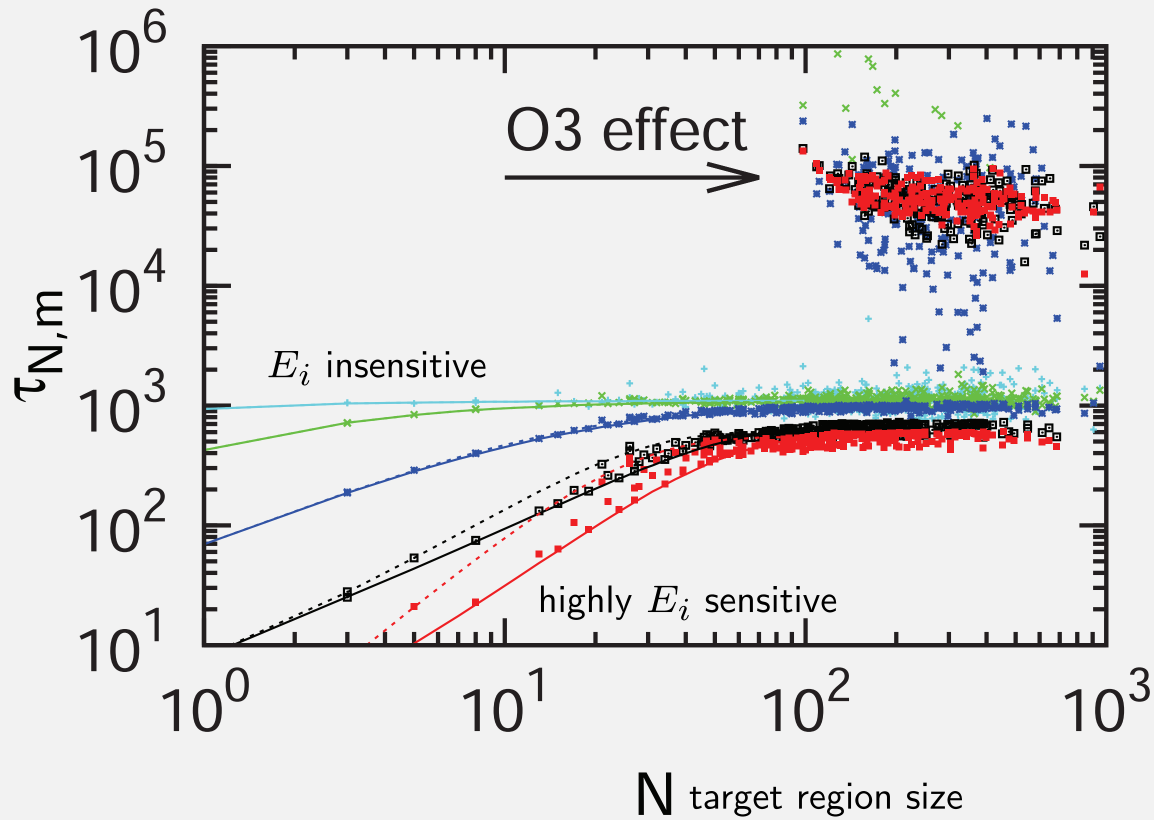




P Kar, AV Cherstvy & RM, PCCP (2018)

Sequence (binding energy) effects on target search time



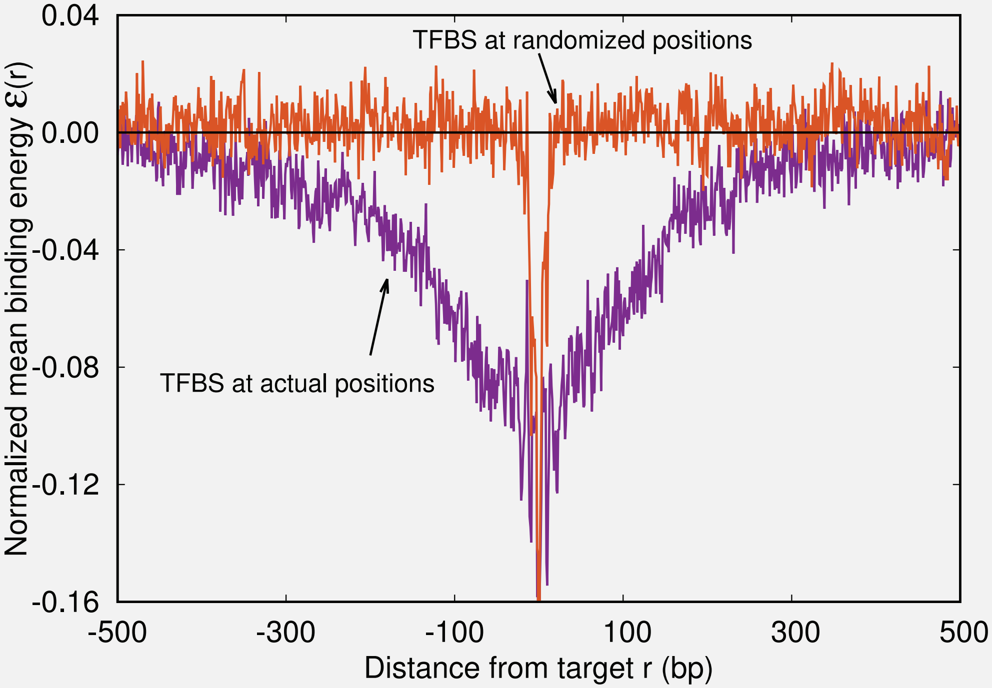


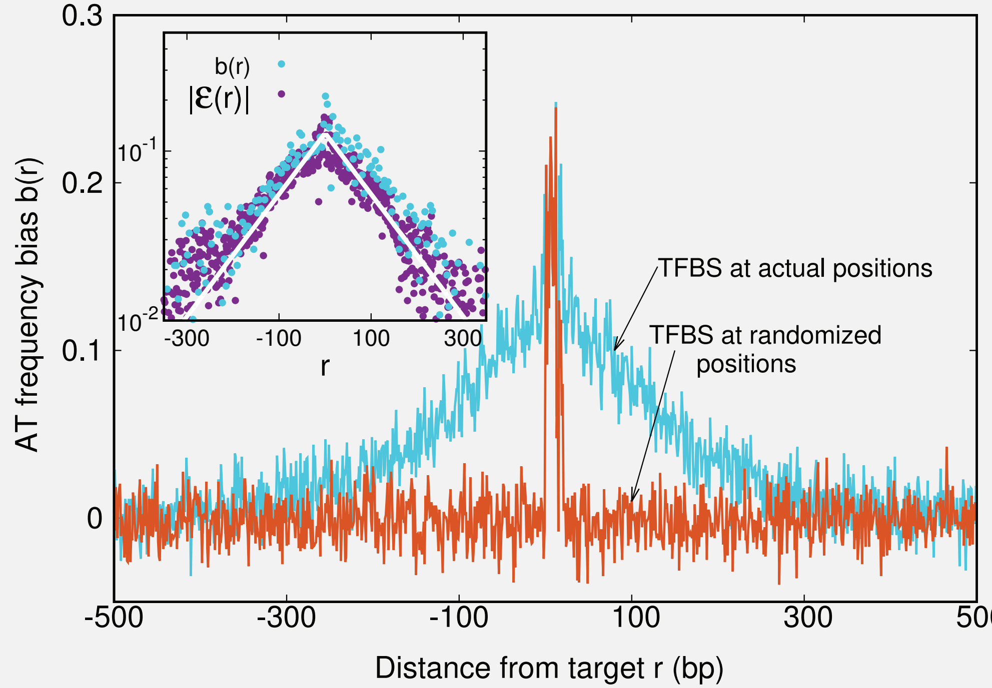
full line: centred target

dashed line: target @ boundary

M Bauer & RM, Sci Rep (2015)

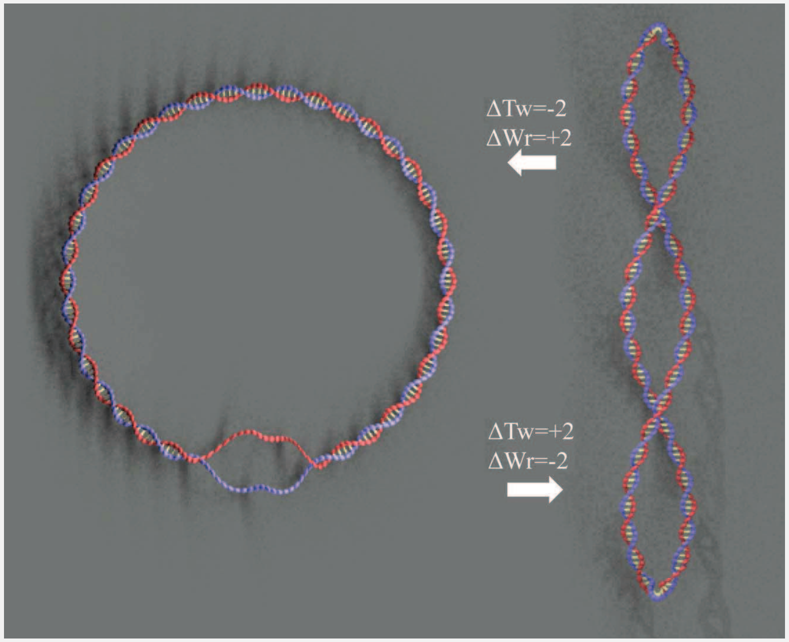
Energetic funnel facilitated diffusion

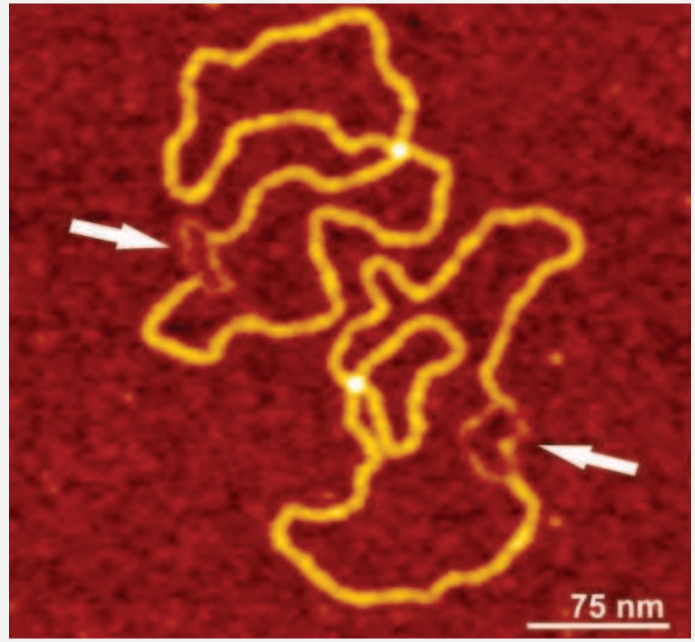




M Cencini & S Pigolotti, NAR (2017)

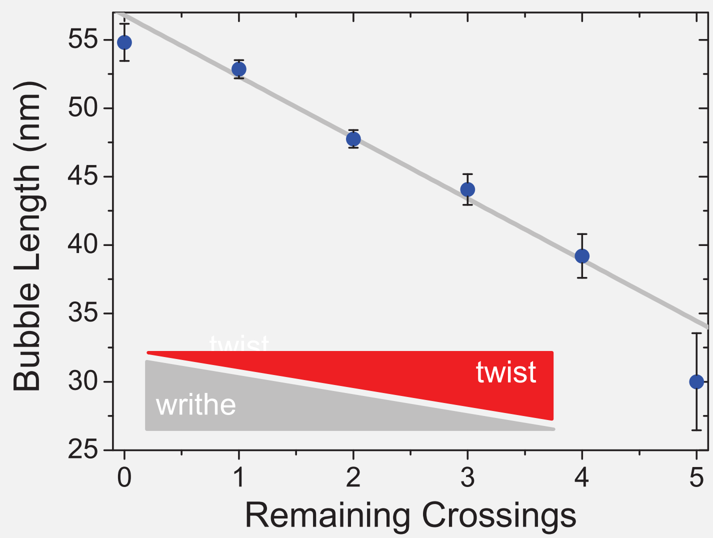
Weak regions at gene starts promote DNA denaturation





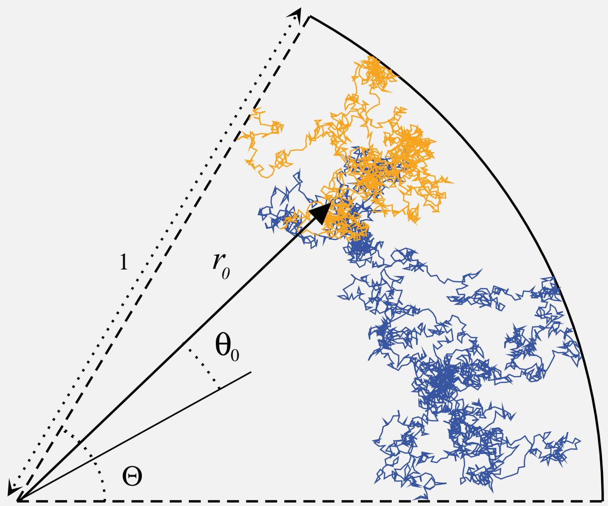
DNA superhelical density:

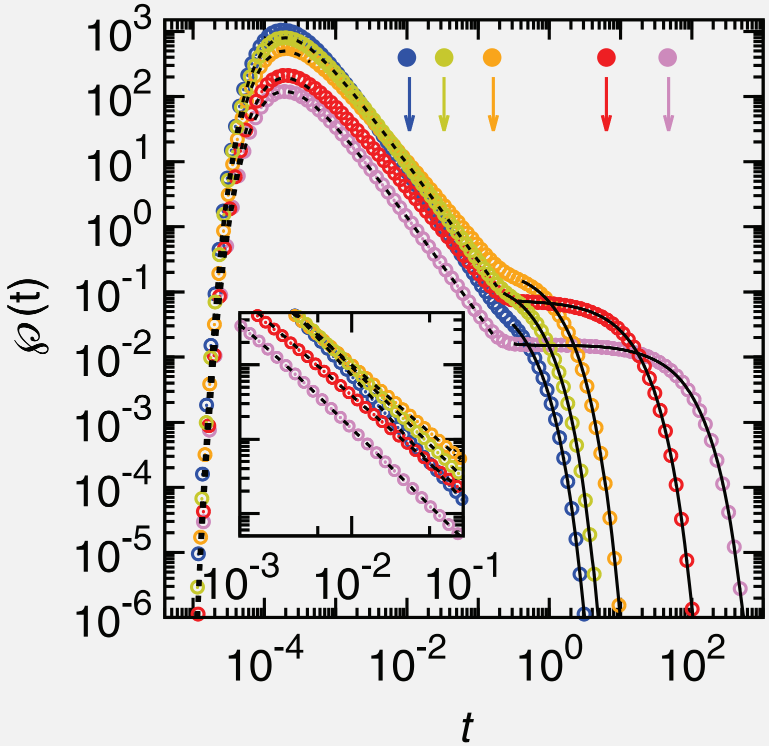
$$\sigma = \frac{\mathrm{Lk} - \mathrm{Lk}_0}{\mathrm{Lk}_0} \approx -0.06$$

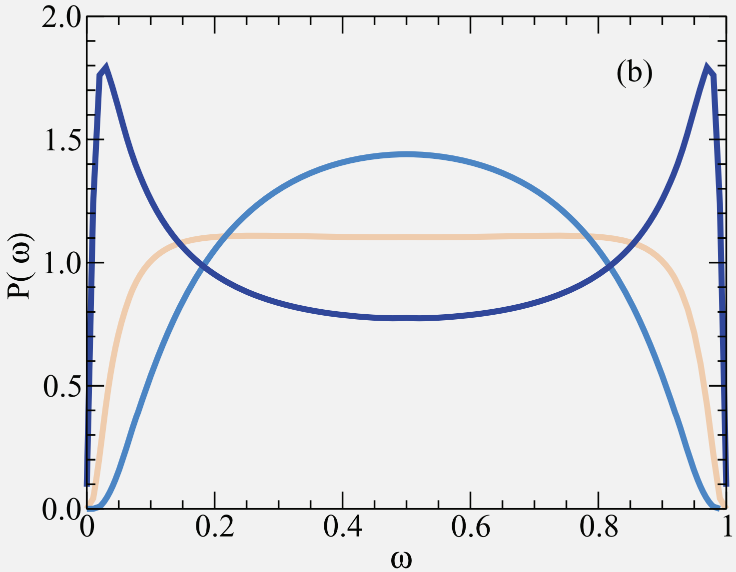


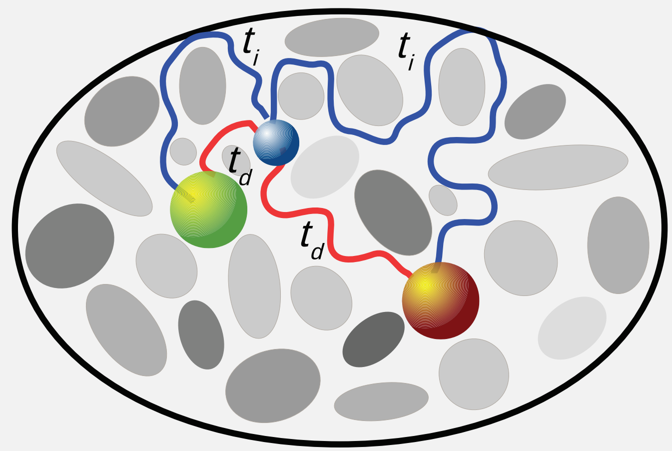
J-H Jeon, J Adamczik, G Dietler & RM, PRL (2010); J Adamczik, J-H Jeon, RM & G Dietler, Soft Matter (2012)

First-past-the-post: few-encounter limit & geometry control

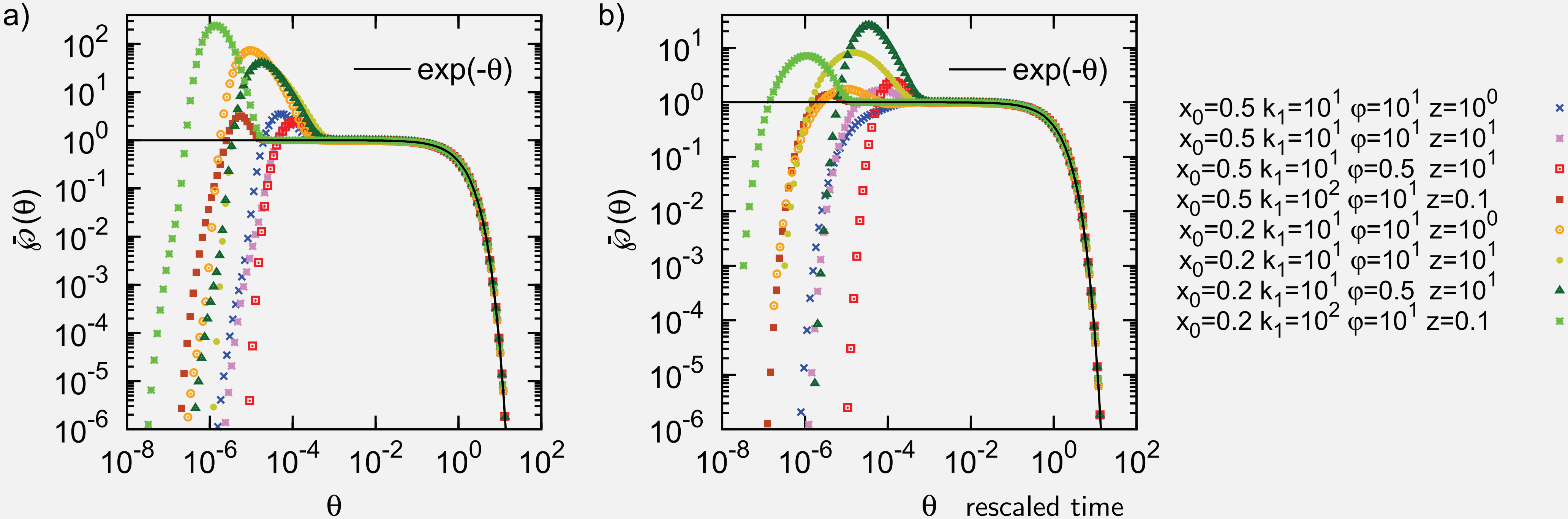


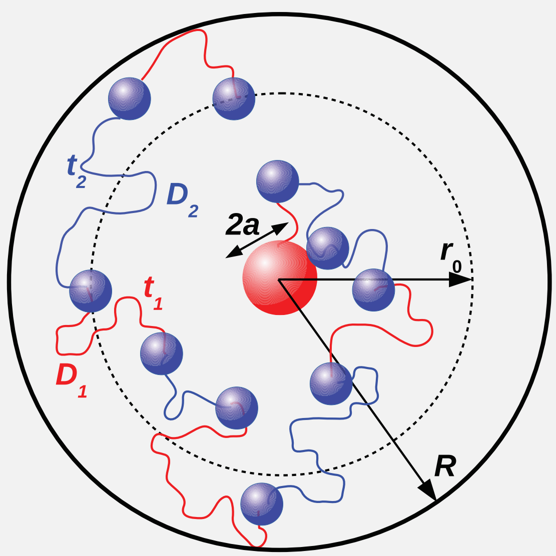






First-past-the-post for 2-channel diffusion



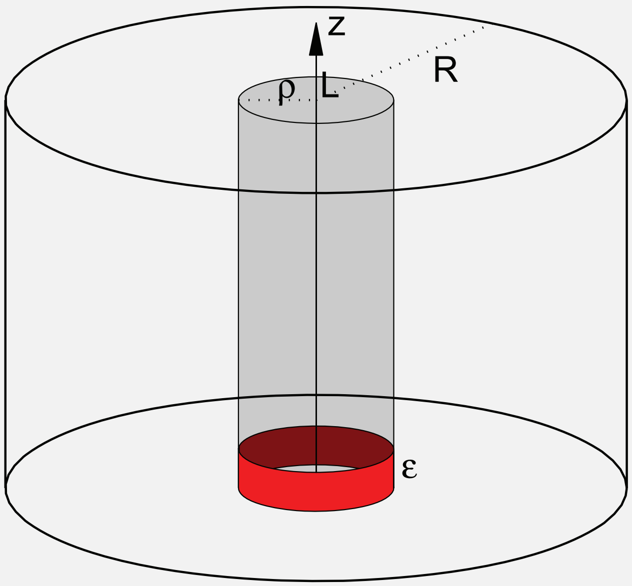


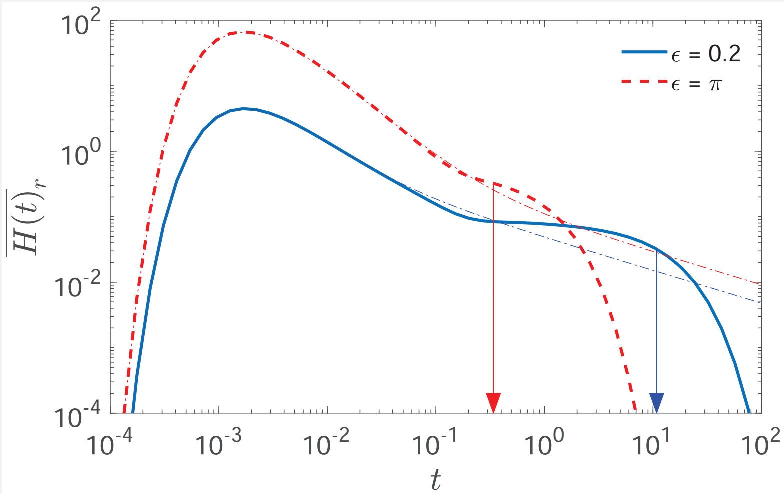
Search mode /w D_1

& recognition mode /w D_2

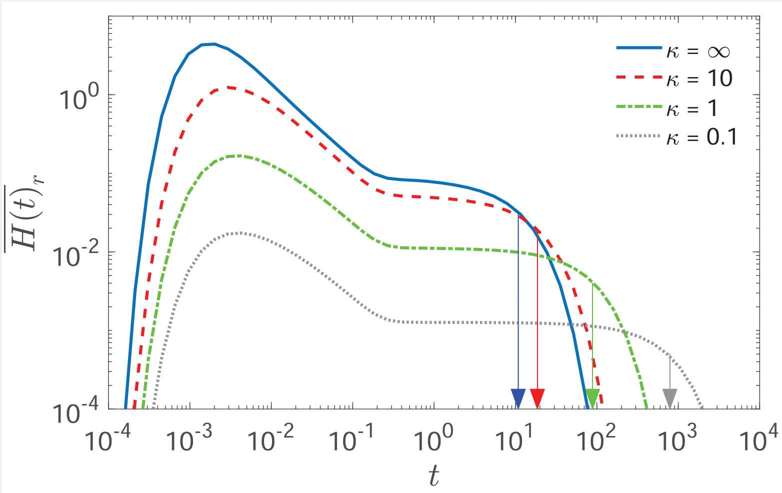
A Godec & RM, JPA (2017)

Few-encounter effect in cylindrical domain /w finite reactivity





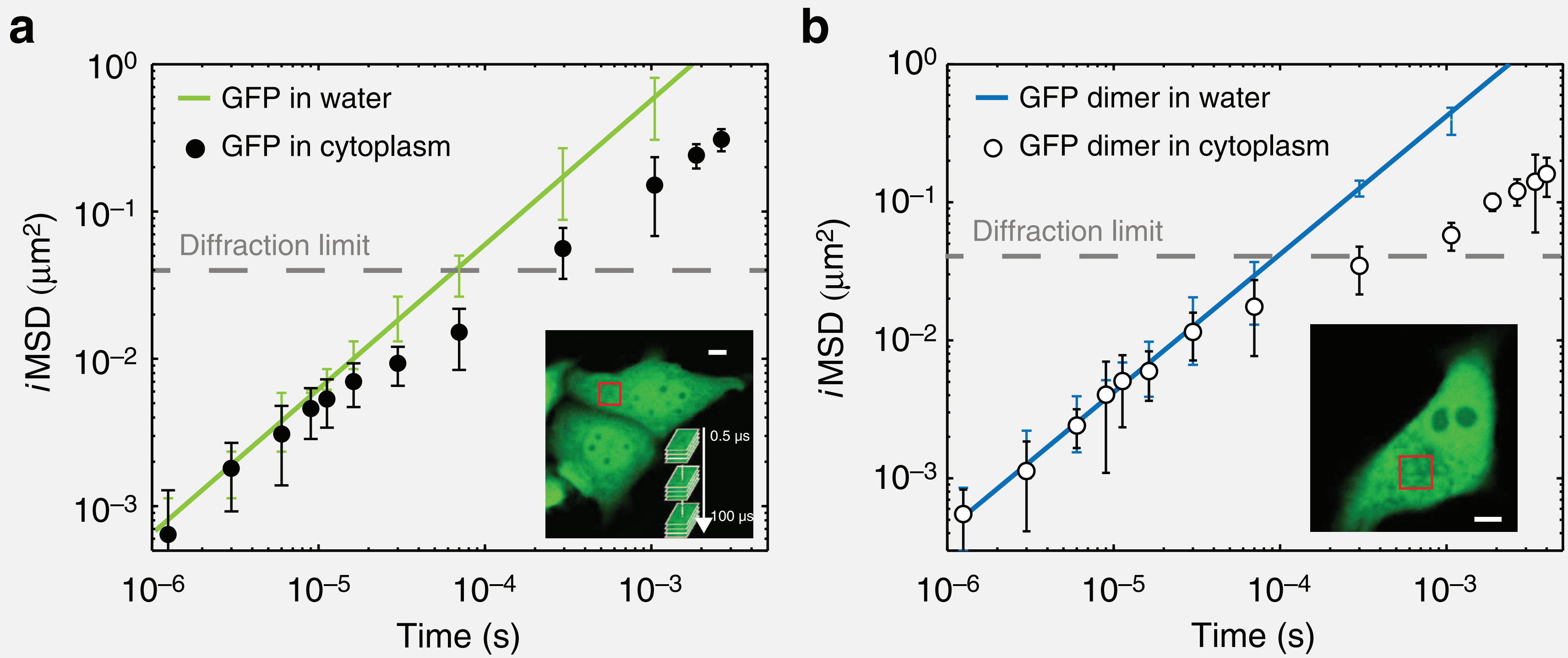
perfect reactivity $\kappa = \infty$



target size $\epsilon = 0.2$

D Grebenkov, RM & G Oshanin, NJP (2017); E-print (2018)

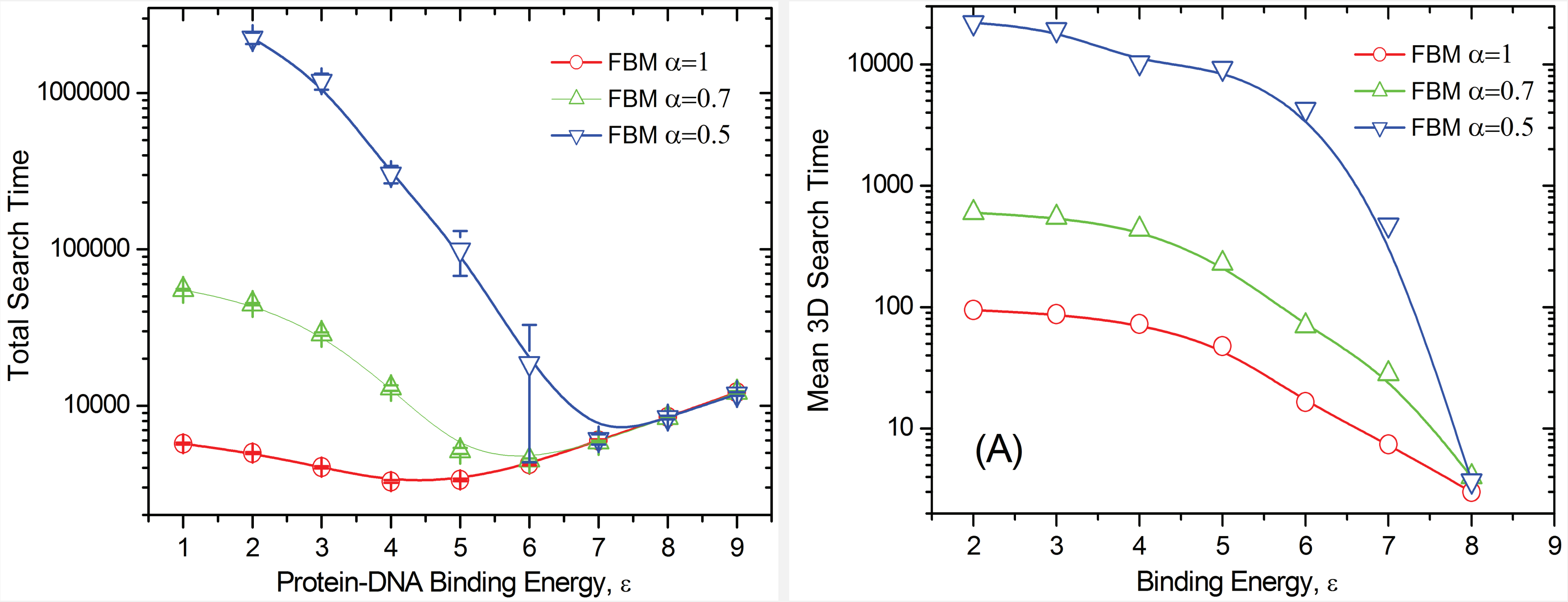
Anomalous diffusion of GFP in cell cytoplasm & nucleus



$\langle \mathbf{r}^2(t) \rangle \simeq K_\alpha t^\alpha$: 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:

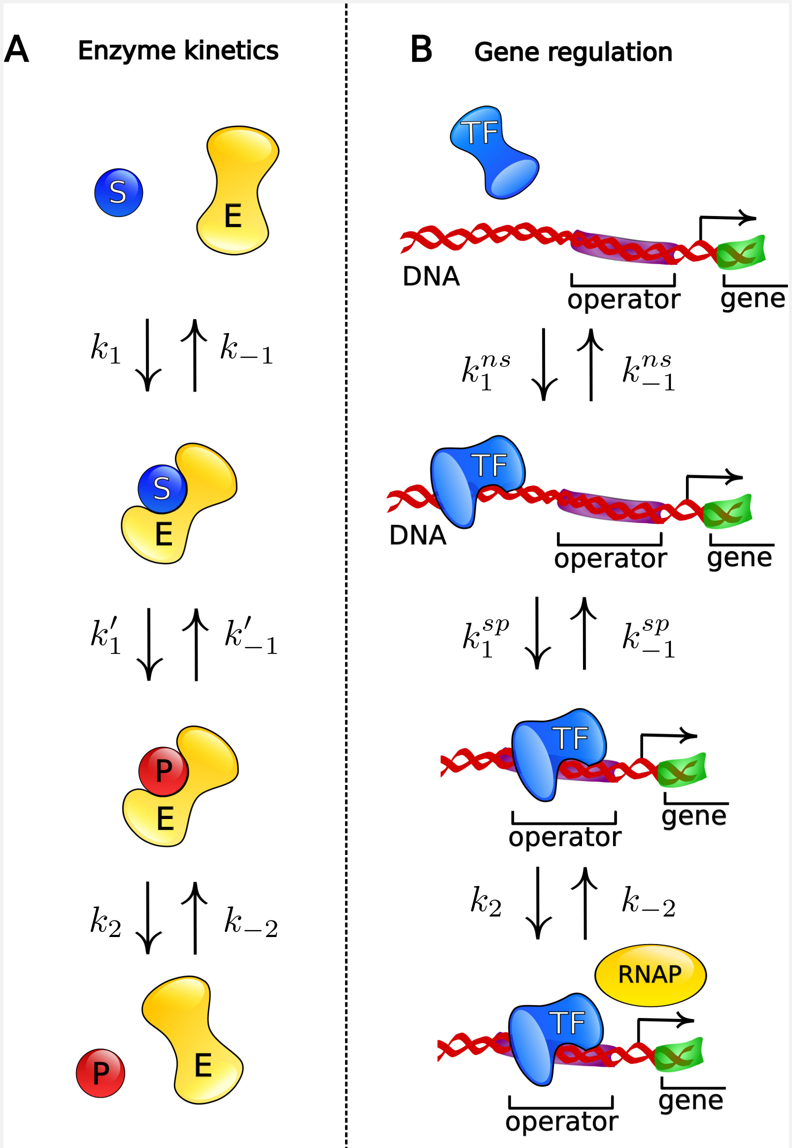
Physical mechanism of anomalous diffusion & cutoff time of anomalous motion?

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)

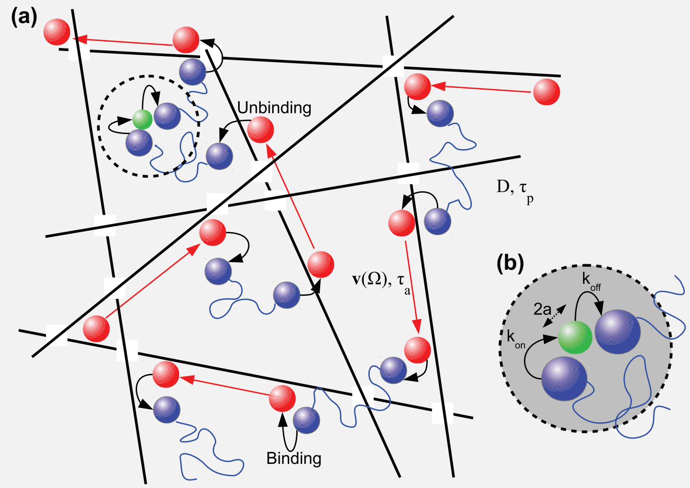
L Liu, AG Cherstvy & RM, JPC (2017)

Low-# Michaelis-Menten

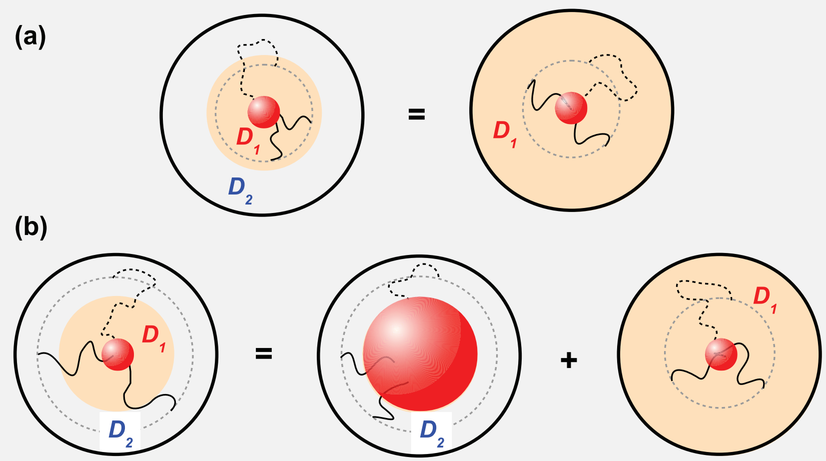


O Pulkkinen & RM, Sci Rep (2015)

Active sensing limit



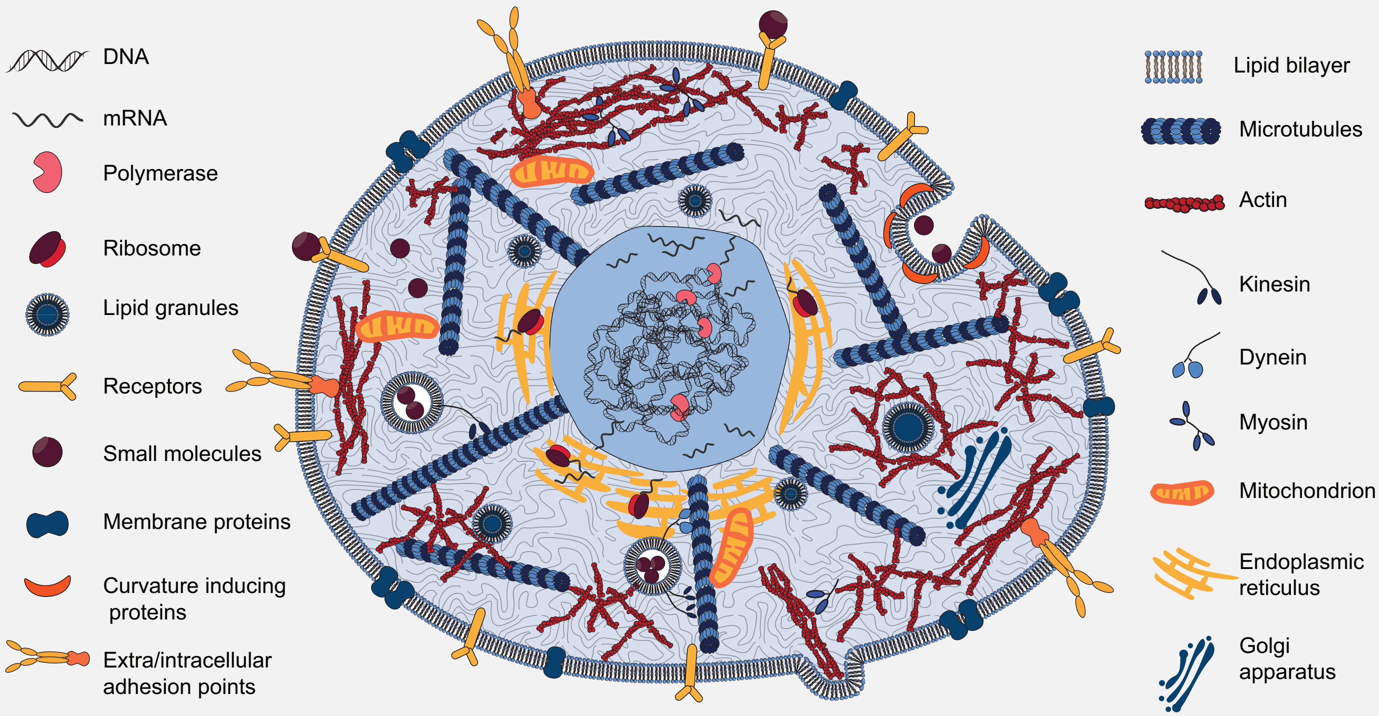
Heterogeneous FPT



New time scale in FP PDF!

A Godec & RM, PRE(R) & PRE (2015), Sci Rep (2016)

Gene regulation in eukaryotic cells

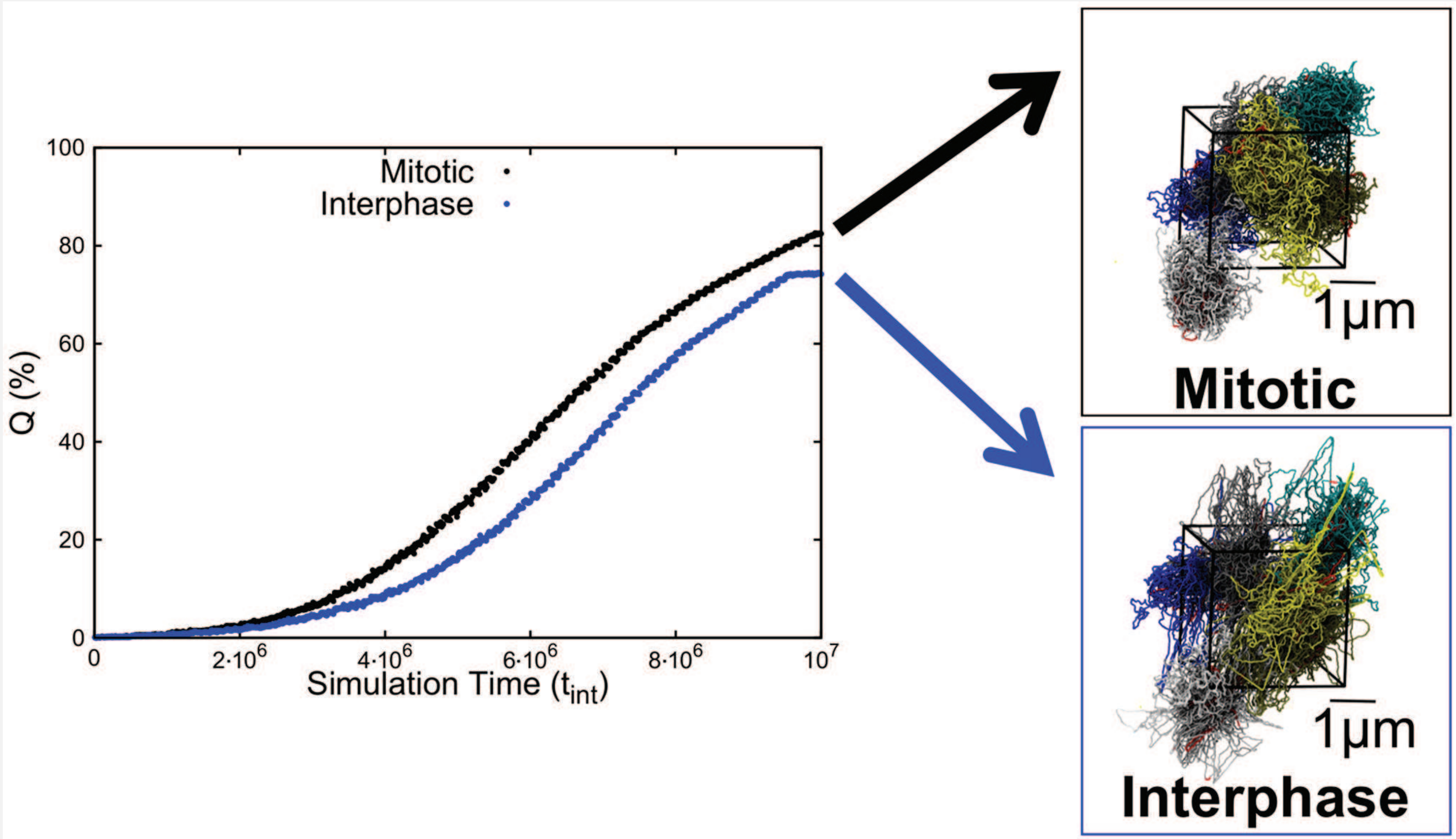


Exchange versus nucleic membrane, chromosomal dynamics & packaging

Active motion: motor transport, drag, or swirling (cytoplasmic streaming), see, e.g., Seisenberger et al, Science (2001) or Reverey et al, Sci Rep (2015)

K. Nørregaard, RM, C. Ritter, K. Berg-Sørensen & L. Oddershede, Chem Rev (2017)

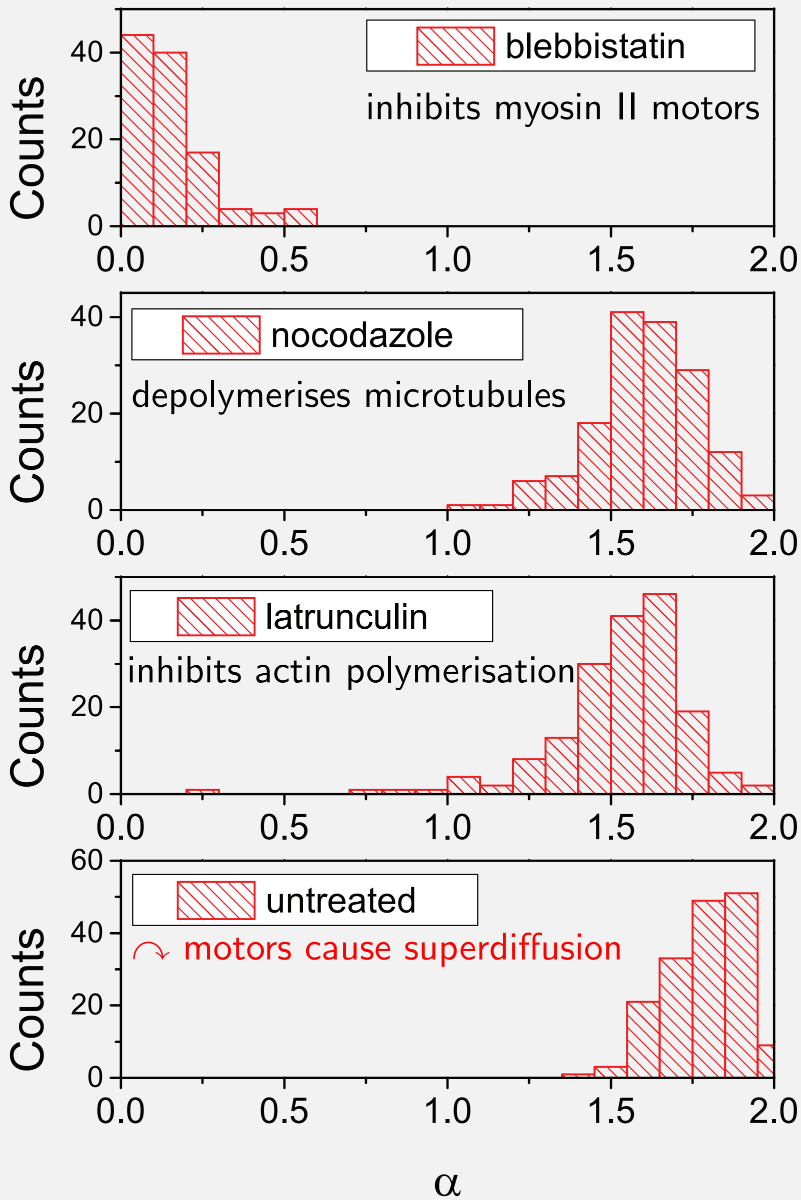
Colocalisation still exists in the nucleus

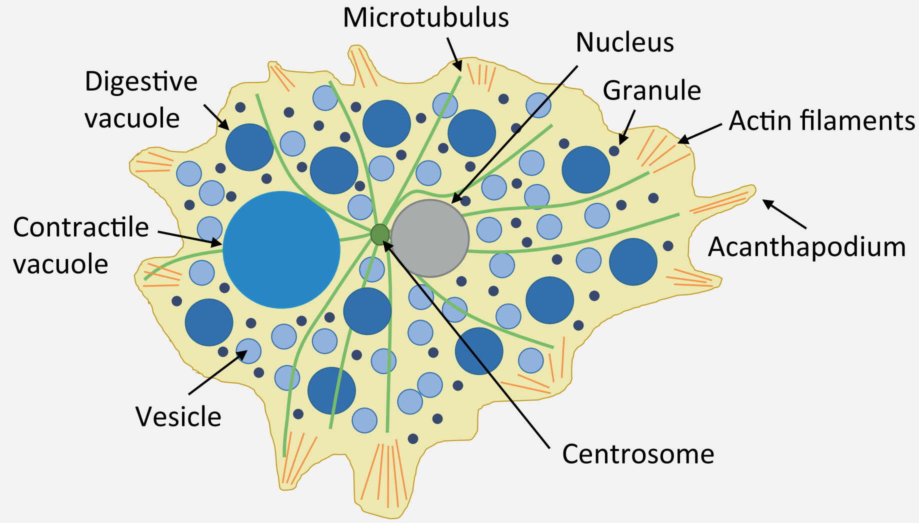


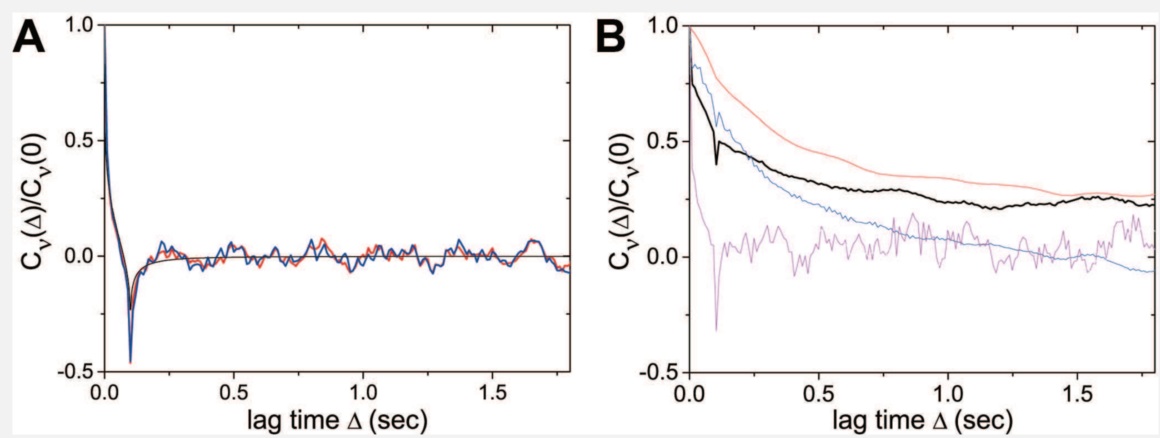
Increase of percentage Q of coregulated pairs of genes in chromosome 19 which colocalise during the MD protocol. Red (???) highlighted regions designate chromosome regions involved in the coregulatory network

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

Superdiffusion in living Acanthamoeba castellani







JF Reverey, J-H Jeon, H Bao, M Leippe, RM & C Selhuber-Unkel, Sci Rep (2015)

Journal of Physics A's new Biological Modelling section

Journal of Physics A

Mathematical and Theoretical

Biological Modelling

For anything interesting too mathematical for Biophys J, Phys Biol, or J Theoret Biol, or not general enough for PRL or NJP ...

Suggestions for topical reviews & special issues are welcome

∑ummary

I Gene expression based on stochastic binding of TFs; facilitated diffusion model verified in vitro for certain TFs. Speed-stability paradox

II Facilitated diffusion model also applies to in vivo gene regulation

III Distance matters: conformation of DNA in facilitated diffusion & gene-gene distance for TF-TU regulation—support for rapid search hypothesis

IIII (Transient) anomalous diffusion of TFs in vivo

卌 Anomalous diffusion models: RM & al, PCCP (2014)

Anomalous diffusion in membranes: RM & al, BBA Biomembranes (2016)

Single molecule manipulation & tracking: C Nørregaard et al, Chem Rev (2017)

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