Molecular search in gene regulation

— Houston, 5th December 2019 —

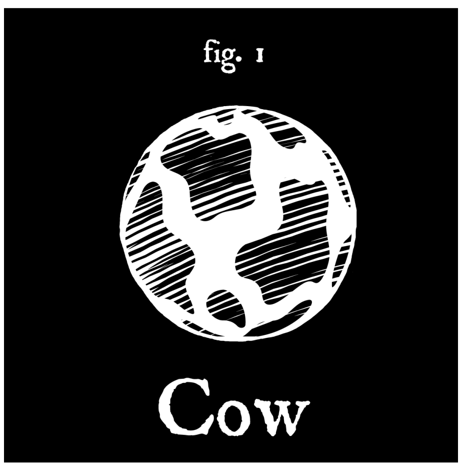
Main protagonist: bacteria cells such as E.coli

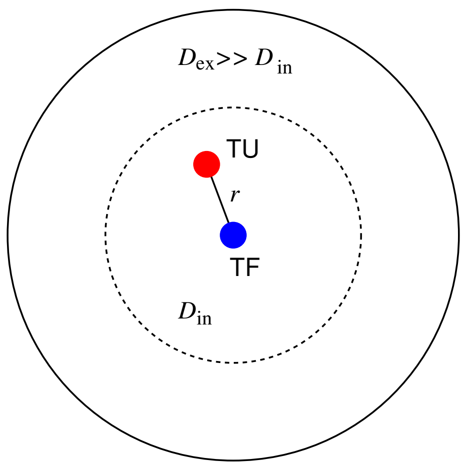
Cell size: roughly $2\mu\text{m} \times 1/2\mu\text{m}$
Cell volume: $\approx 1\mu\text{m}^3$

DNA length: 4.7×10^6 base pairs or $\approx 1.6\text{mm}$

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

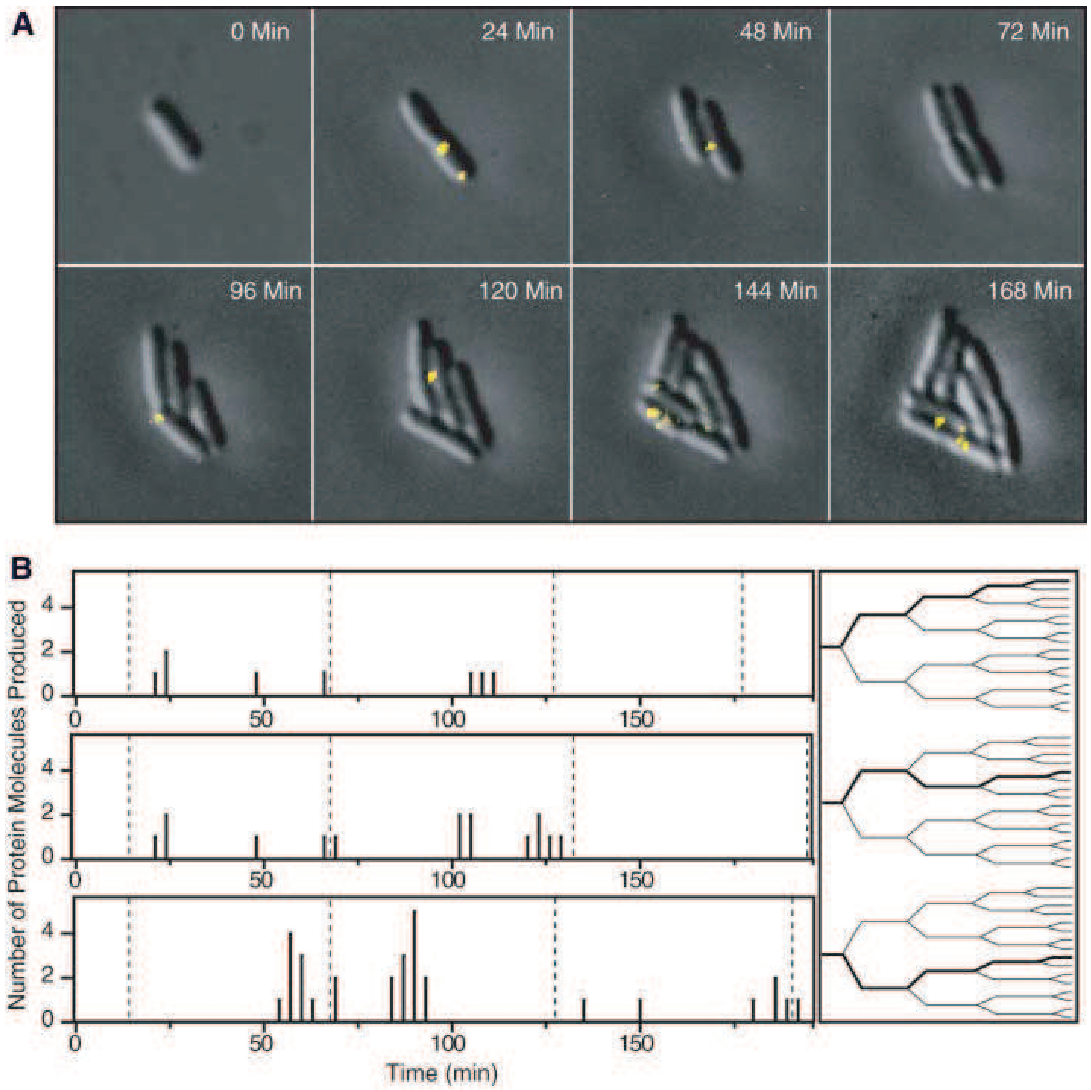
Some proteins occur only as few or few tens of copies/cell (nM concentrations)





RW Corbin et al, PNAS (2003)

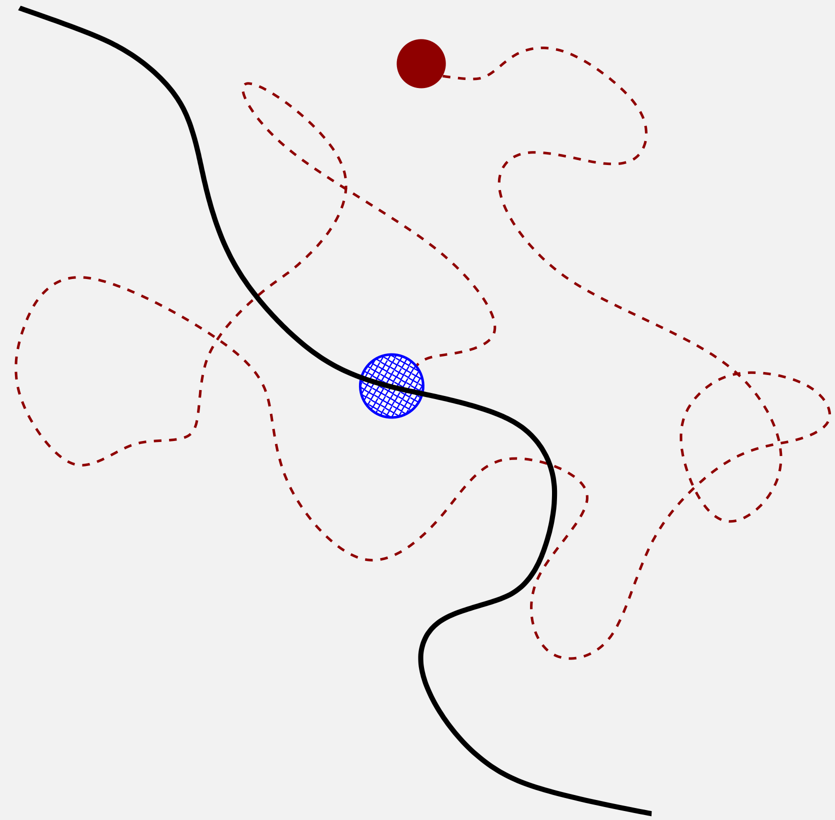
Gene expression one molecule at a time



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

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

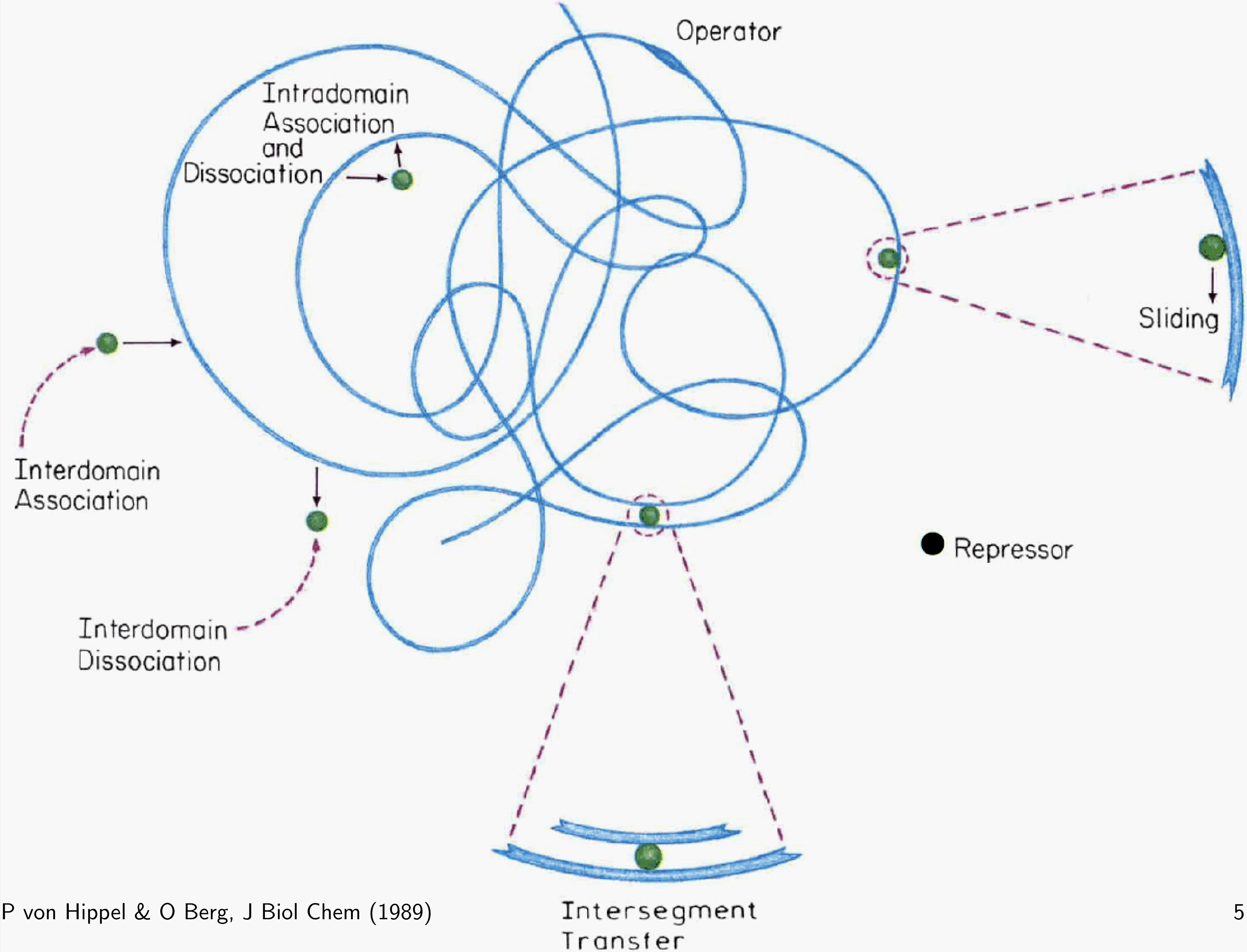
Lac repressor [AD Riggs, S Bourgeois, M Cohn, J Mol Biol 53, 401 (1970)]:

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

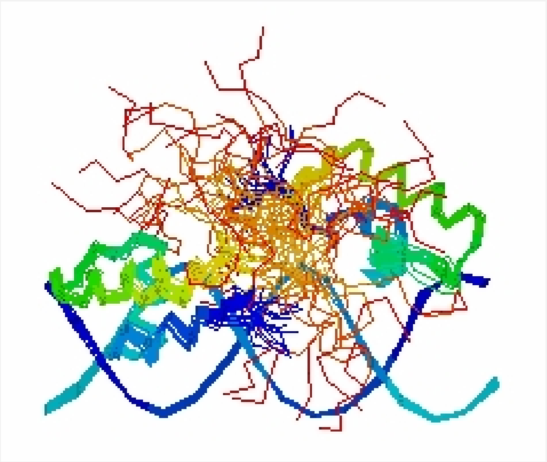
→ Facilitated diffusion picture

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

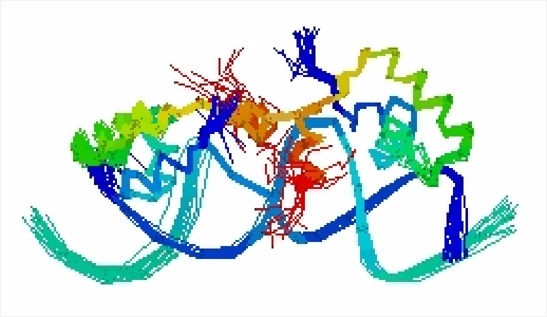
Facilitated diffusion: the Berg-von Hippel model



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



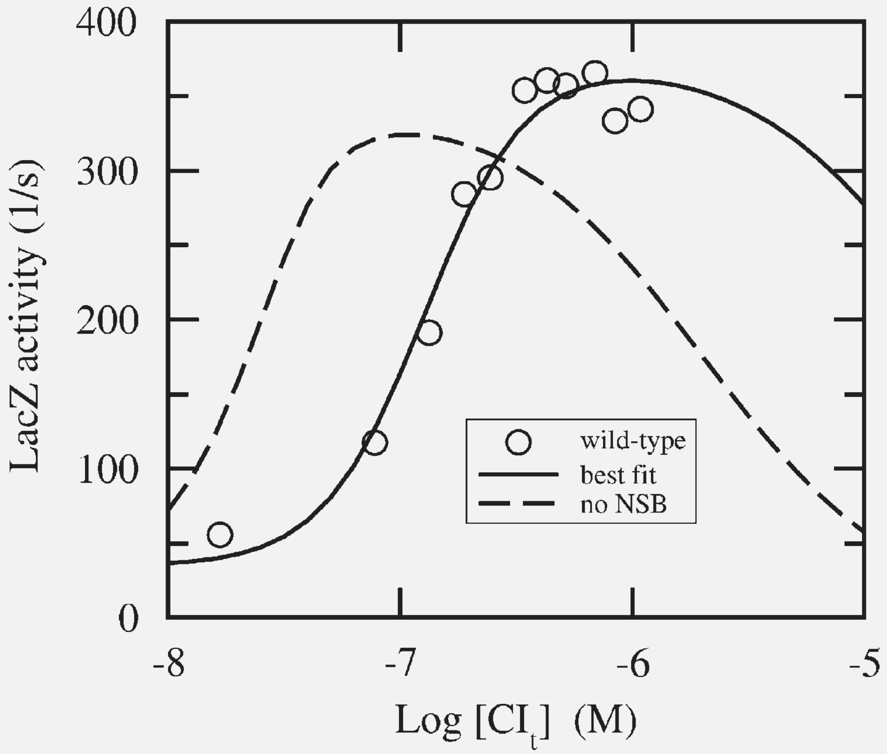
Lac repressor, nonspecific binding



Lac repressor, specific binding

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

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

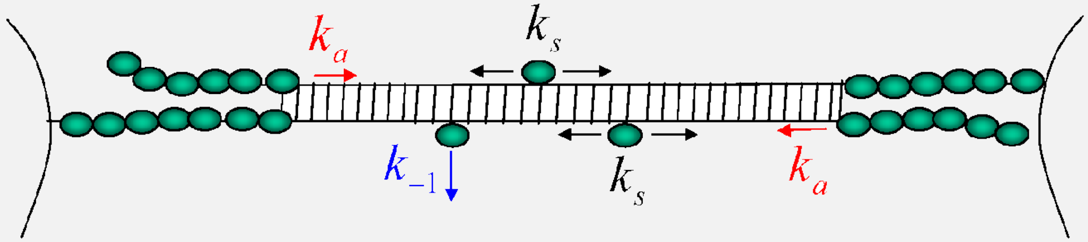


A Bakk & RM, FEBS Lett (2004); J Theoret Biol (2004)

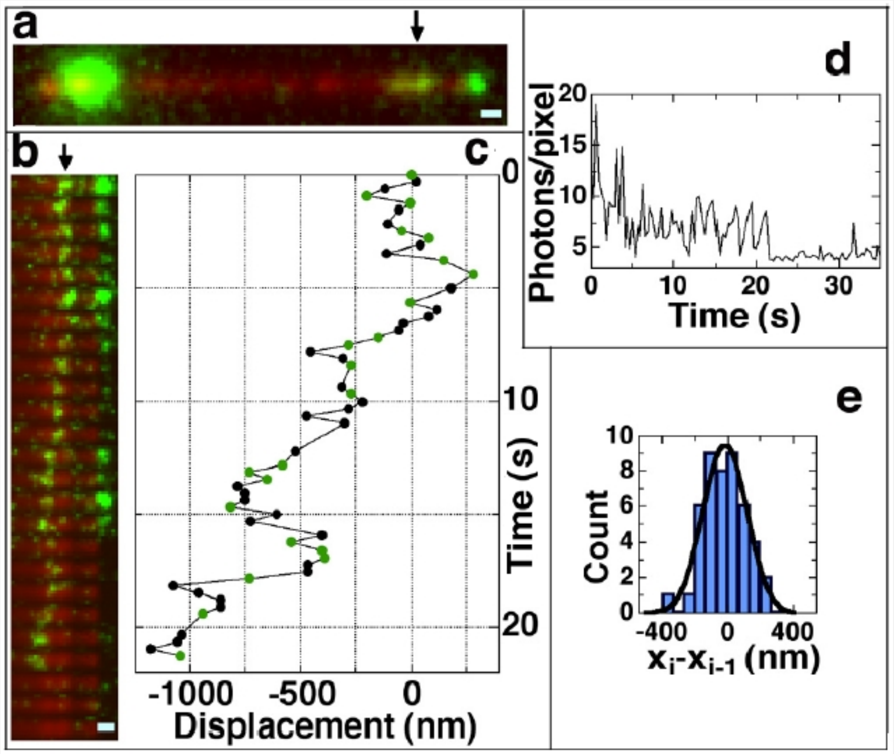
Proof of 1D search mode

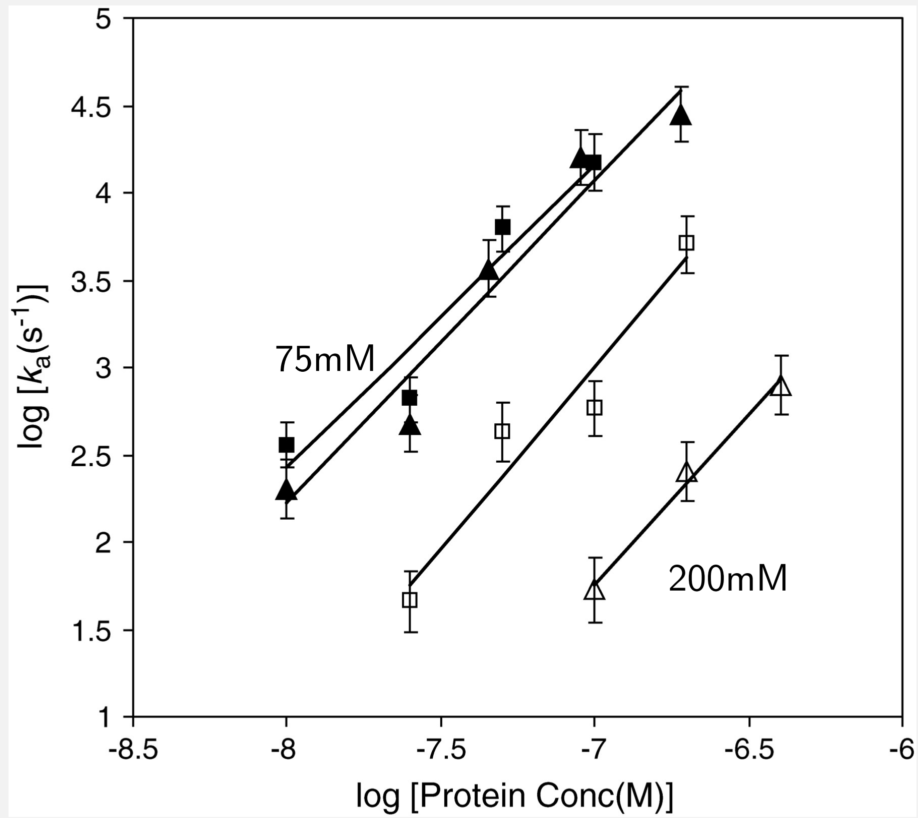
McGhee & von Hippel isotherm

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



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





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

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

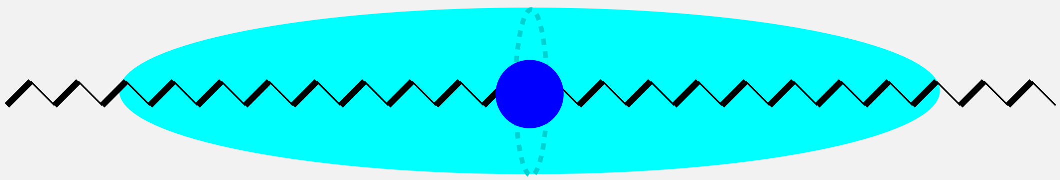
The antenna effect

Target search rate for cylindrical DNA model:

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

Sliding length:

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



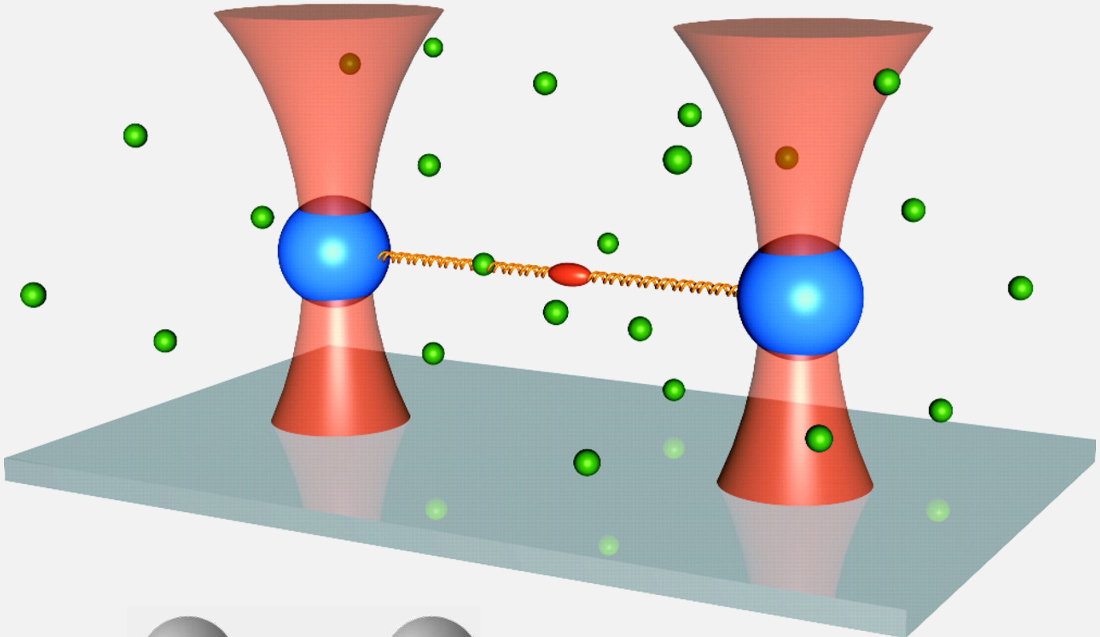
Effective sliding length:

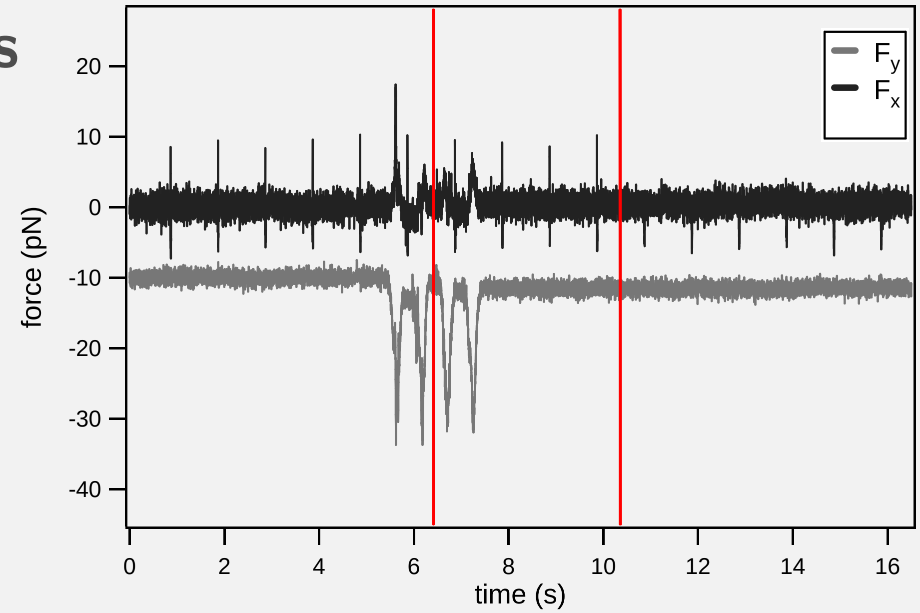
$$\ell_{\text{sl}}^{\text{eff}} = \sqrt{\frac{k_{\text{on}}}{2\pi D_{3d}}} \times \ell_{\text{sl}}$$

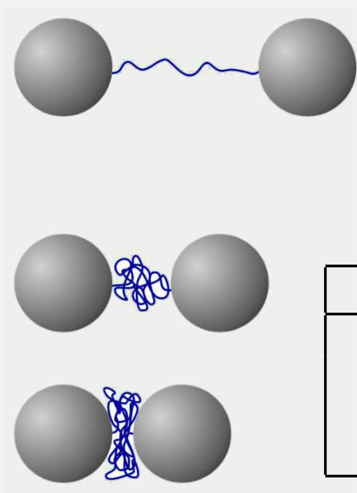
microhop correction: $\sqrt{\frac{k_{\text{on}}}{2\pi D_{3d}}}$

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

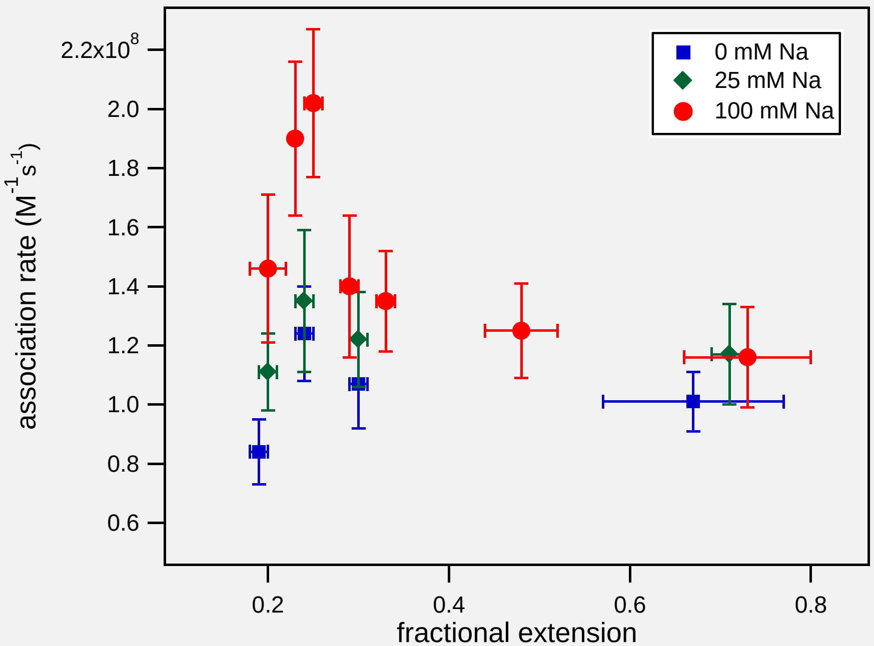
The rôle of DNA conformations





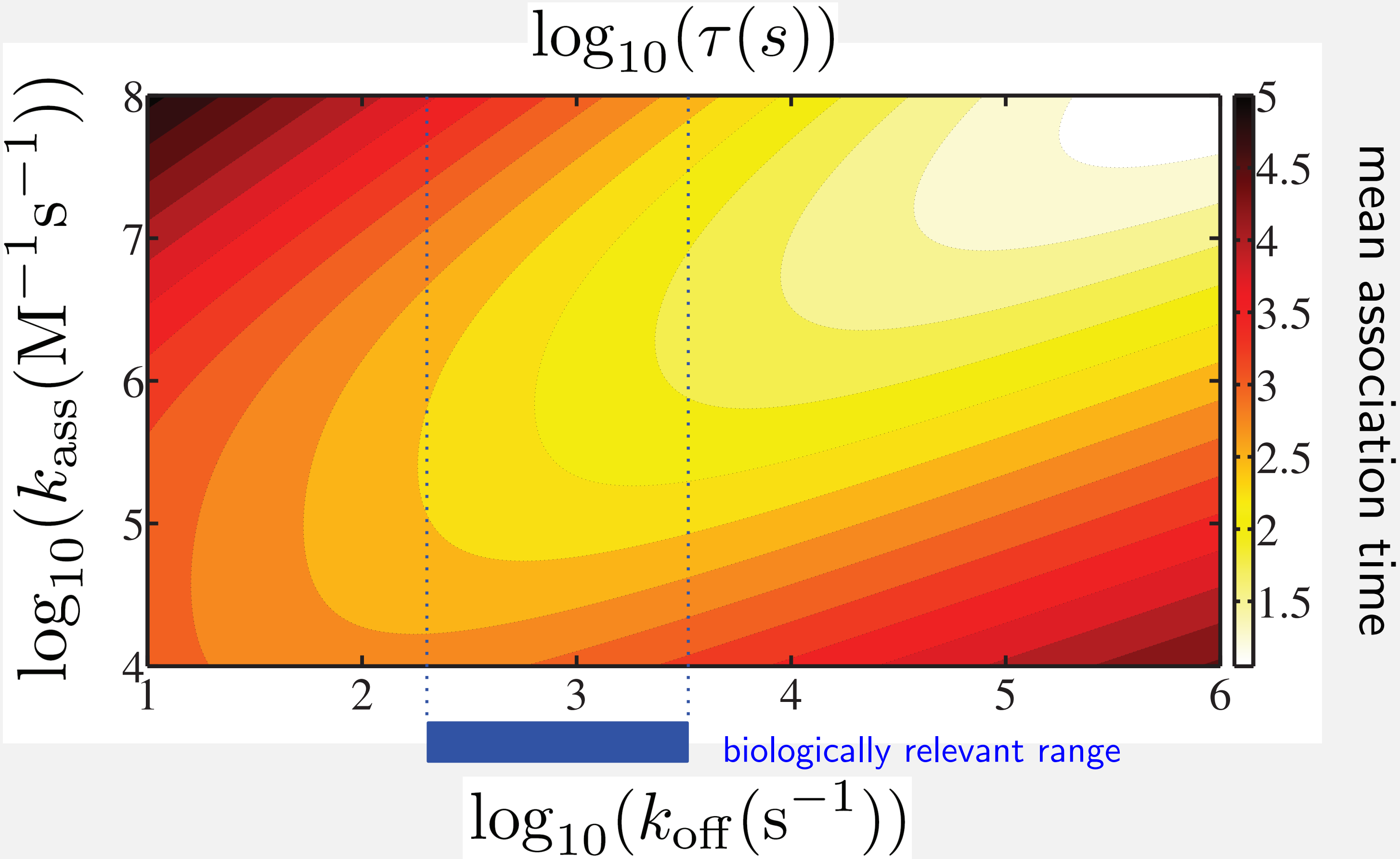


[NaCl]	R_{theory}	R_{measured}
0 mM	1.18	1.3 ± 0.2
25 mM	1.23	1.1 ± 0.2
100 mM	1.67	1.7 ± 0.3
150 mM	1.15	1.3 ± 0.4



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

In vivo gene regulation consistent with facilitated diffusion



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

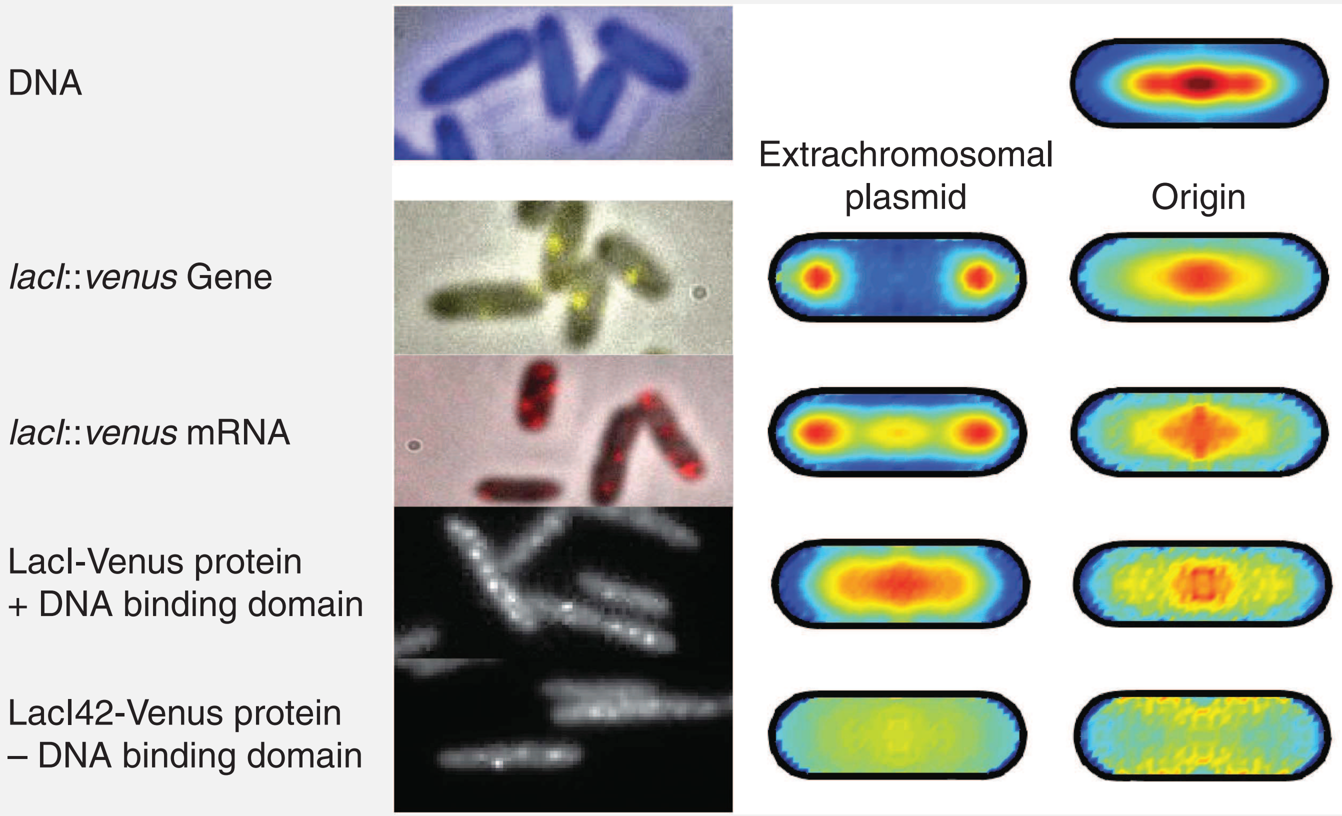
single molecule experiment: $\tau_{\text{exp}} = 354$sec [Elf et al, Science (2007)]

M Bauer & RM, PLoS ONE (2013)

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

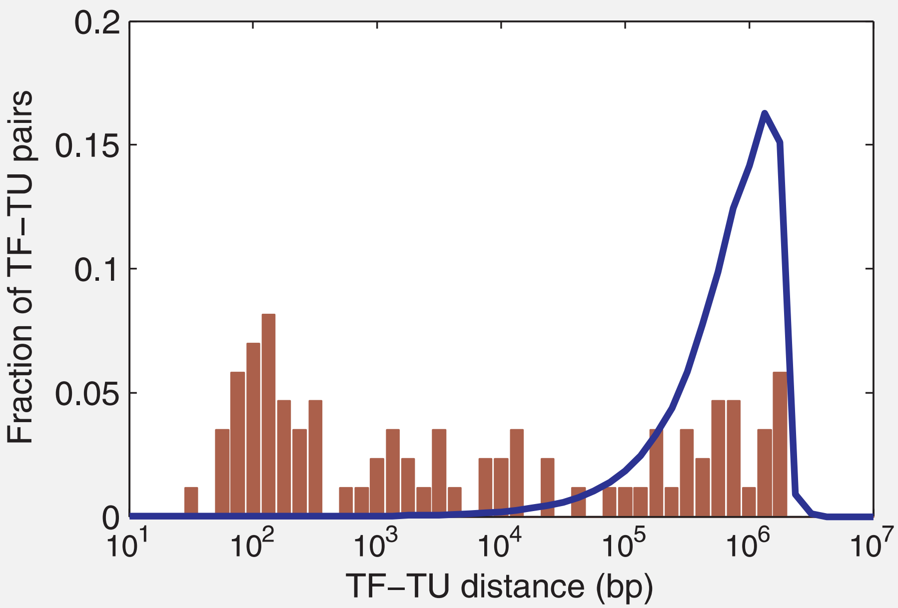
Kuhlman & Cox: • TF distribution highly heterogeneous • TF gene influences distribution: localisation of TF near TF gene

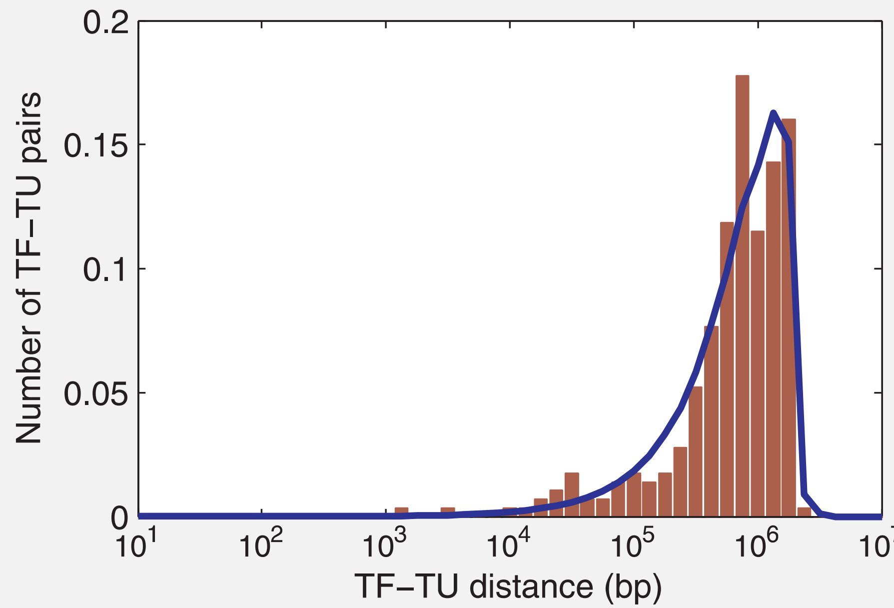


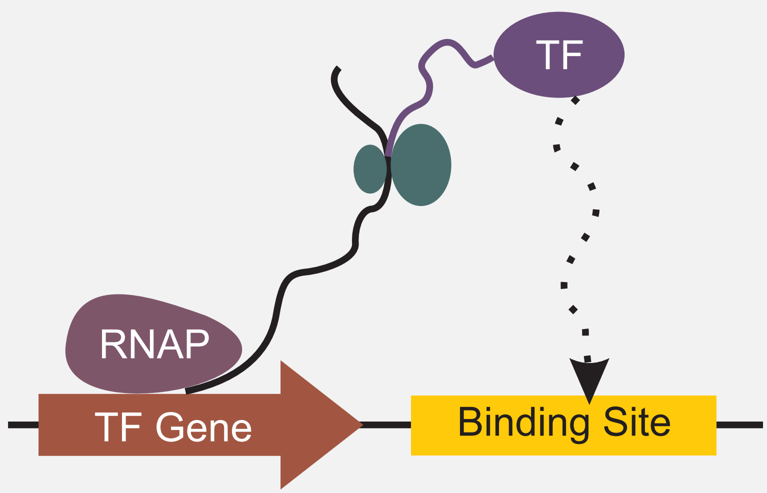
Kepes et al, J Mol Biol (2004); Kuhlman & Cox, Mol Syst Biol (2012)

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

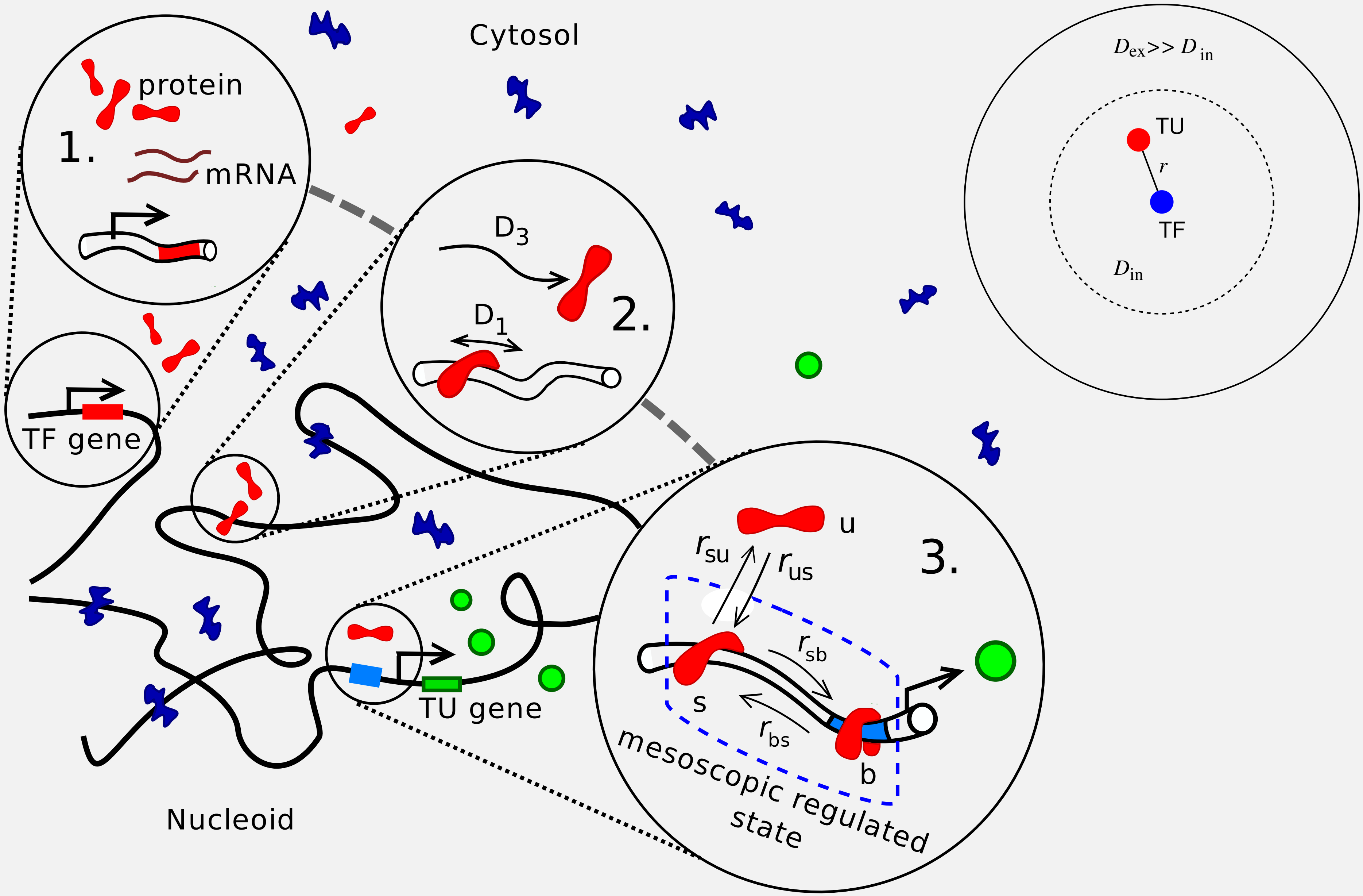






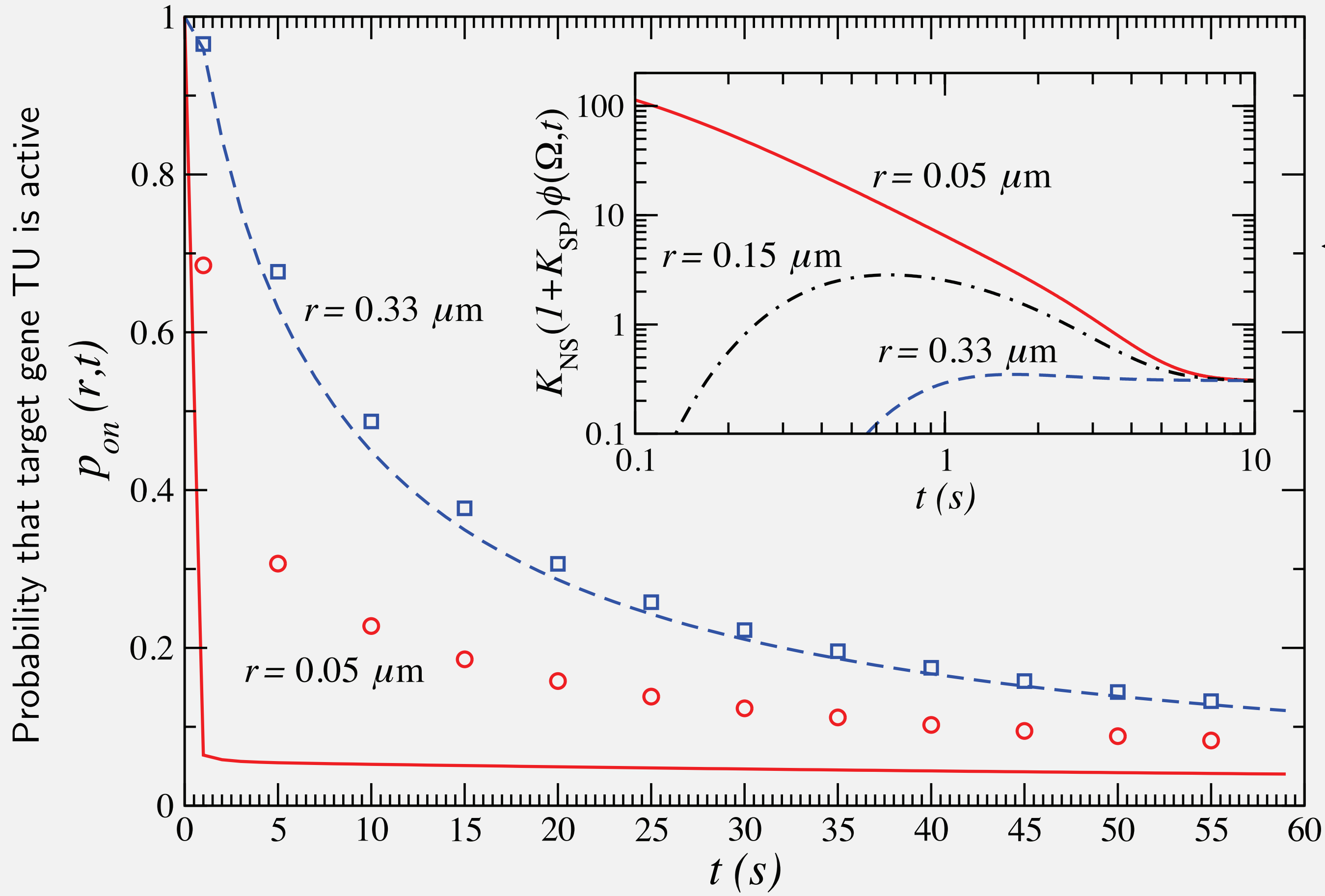
Kolesov et al, PNAS (2007)

Transient intracellular signalling is geometry-controlled



O Pulkkinen & RM, PRL (2013)

Result 1: transient response to repression



← TF concentration @ TU

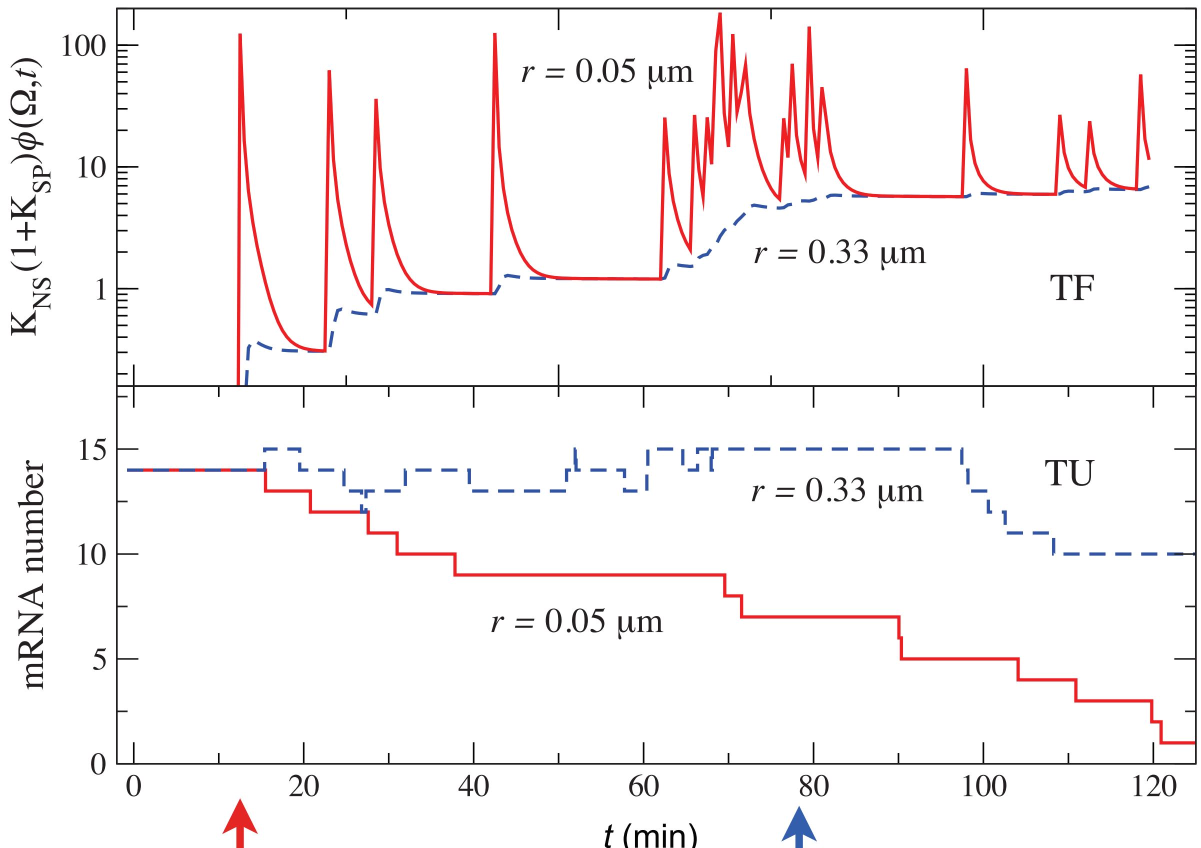
Mean field approximation (full & dashed lines):

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

O Pulkkinen & RM, PRL (2013)

Result 2: time dependence of gene response: bursts!

Sample paths from MC simulations:



First repressor binding at r = 0.05 μm

First repressor binding at r = 0.33 μm

TF density at the target operator

Target expression

O Pulkkinen & RM, PRL (2013)

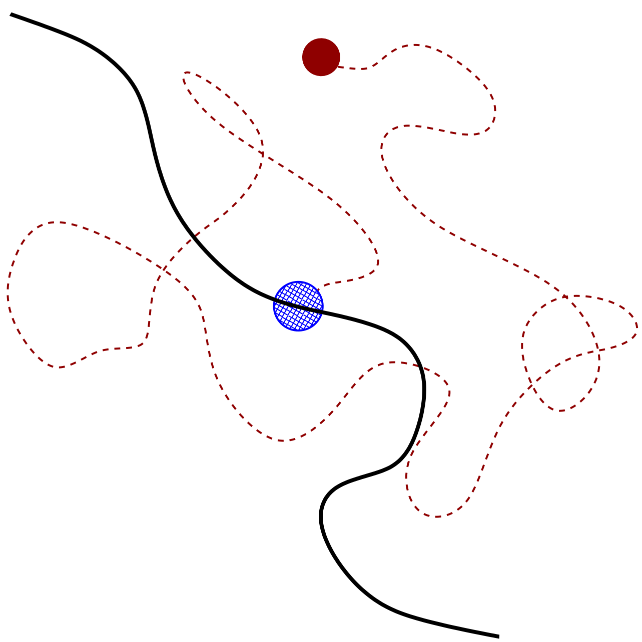
Numerical analysis confirms relevance of proximity effect

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

Brownian impromptu: from mere means to distributions

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

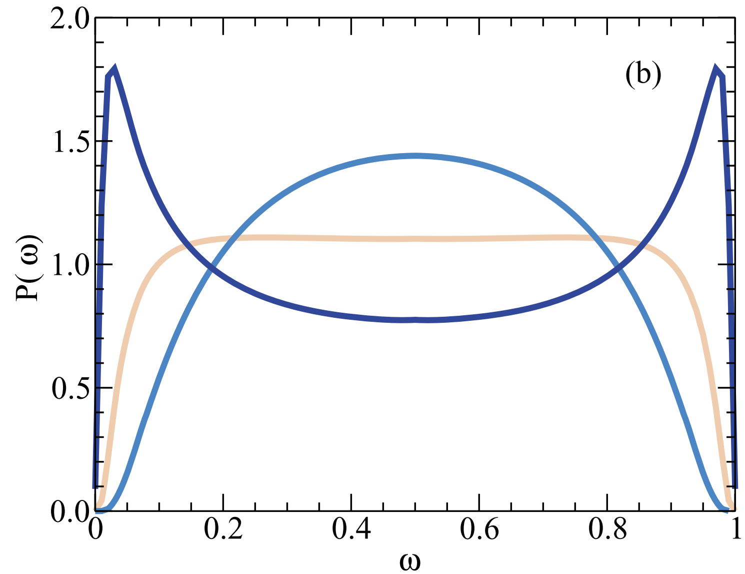
$$k_{\rm on} = 4\pi D_{3d} a$$



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)

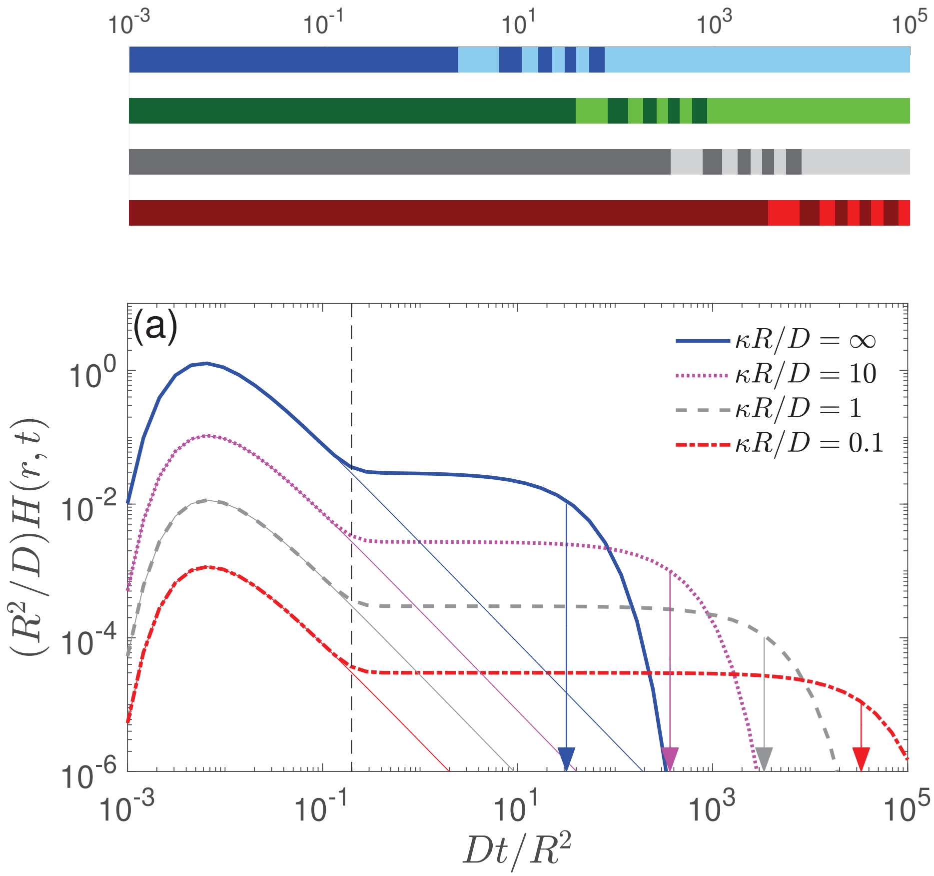
T Mattos, C Mejía-Monasterio, RM & G Oshanin, PRE (2012)

Beyond mere means: first-passage time distributions

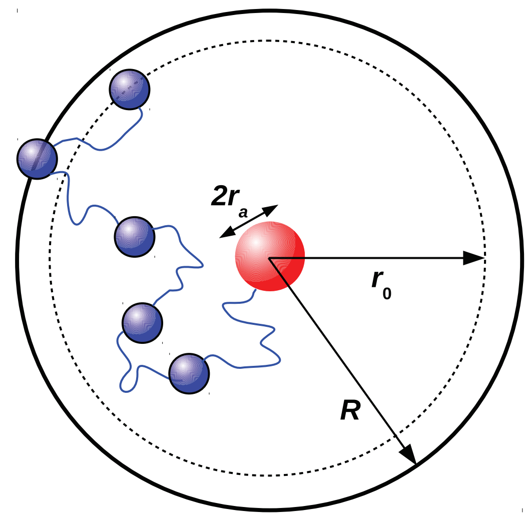
Even in μm-sized bacteria cells biochemical reactions are distance-dependent (geometry-controlled) [Kolesov et al, PNAS (2007); O Pulkkinen & RM, PRL (2013)]

Geometry- versus reaction-control in finite-reactivity scenario

Full first passage time density:



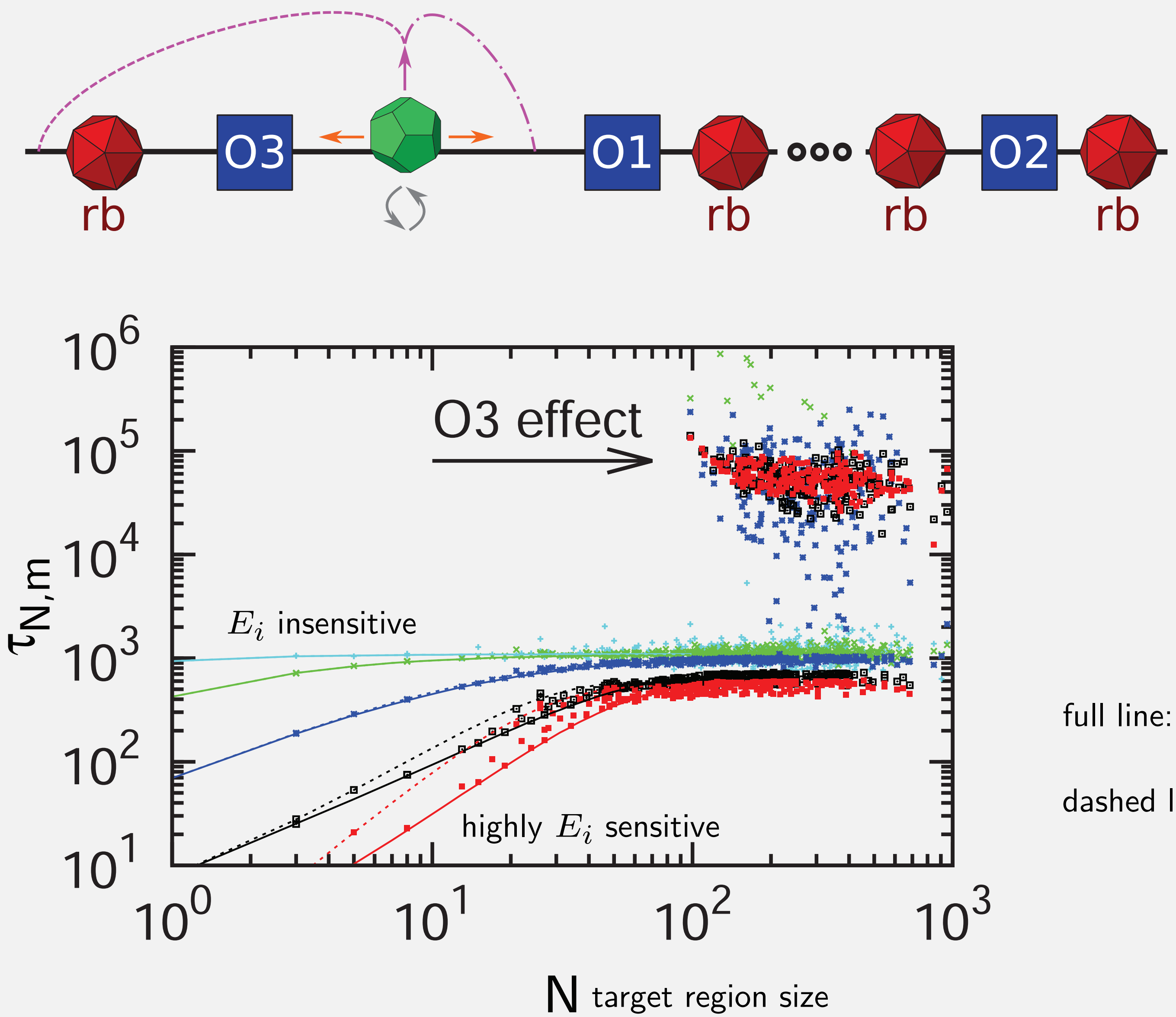
Direct vs indirect trajectories:



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

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

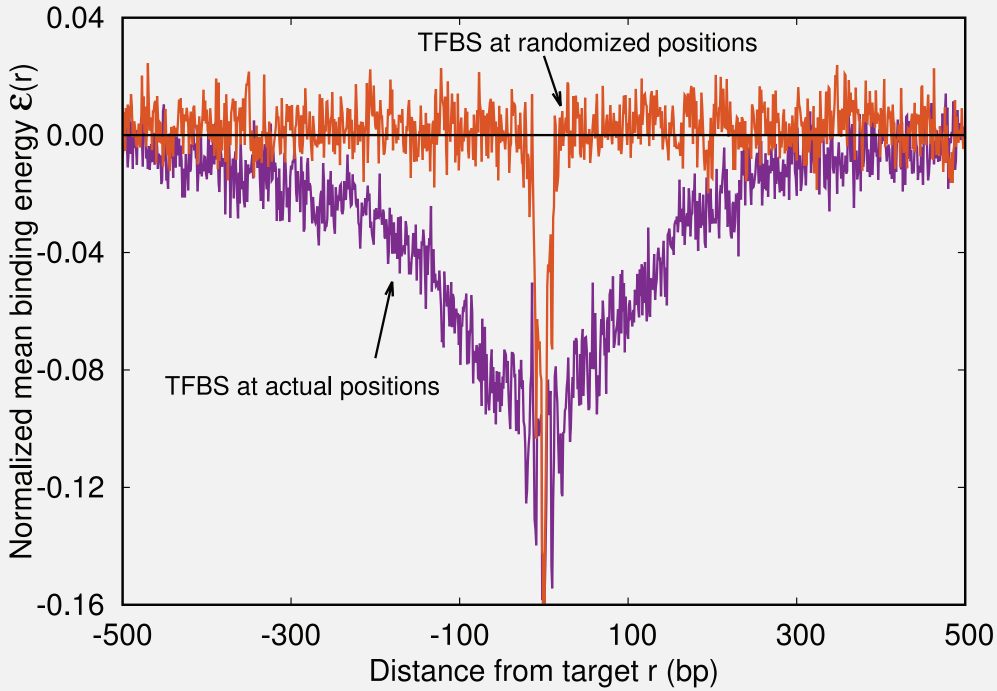


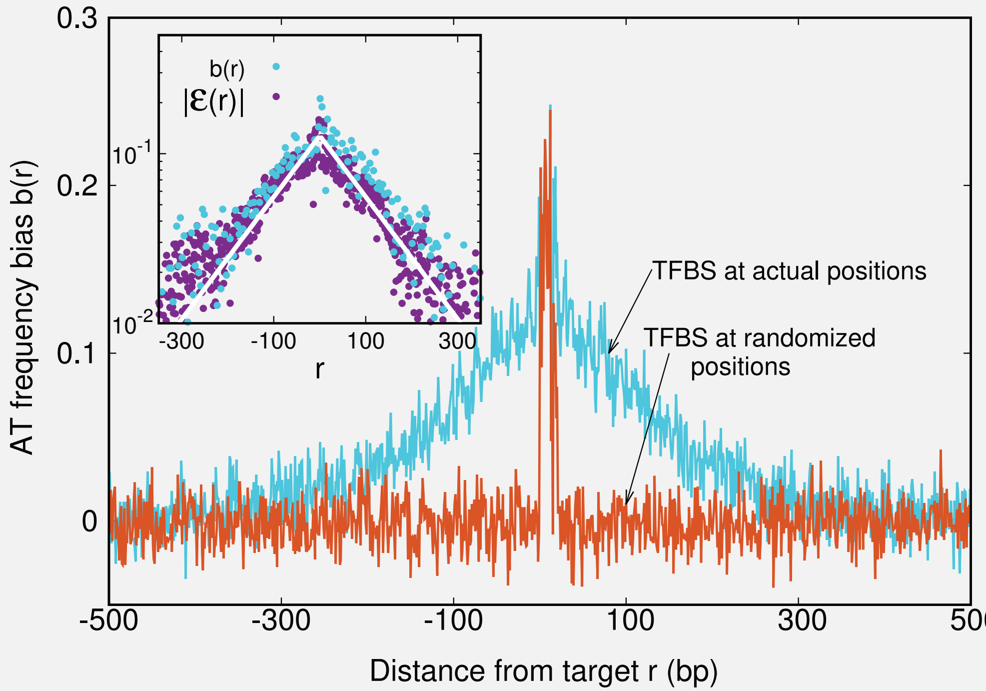
full line: centred target

dashed line: target @ boundary

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

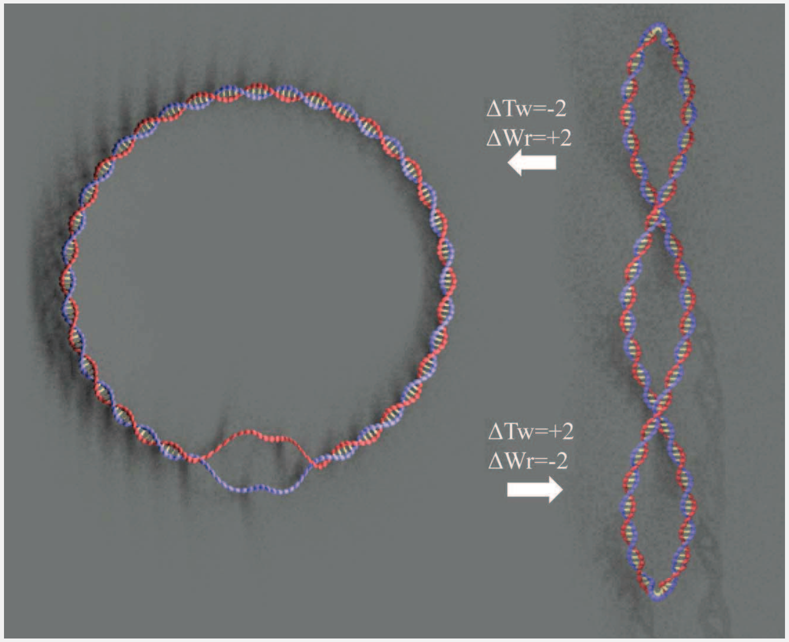
Energetic funnel facilitated diffusion

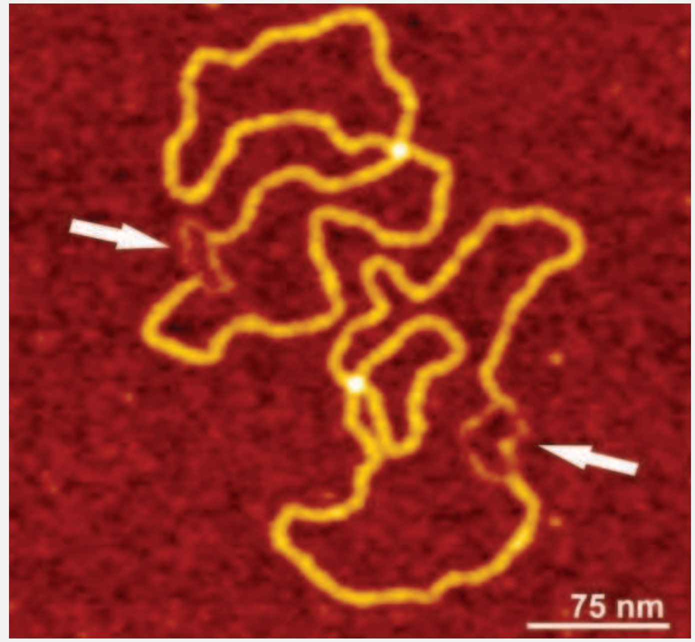




M Cencini & S Pigolotti, NAR (2017)

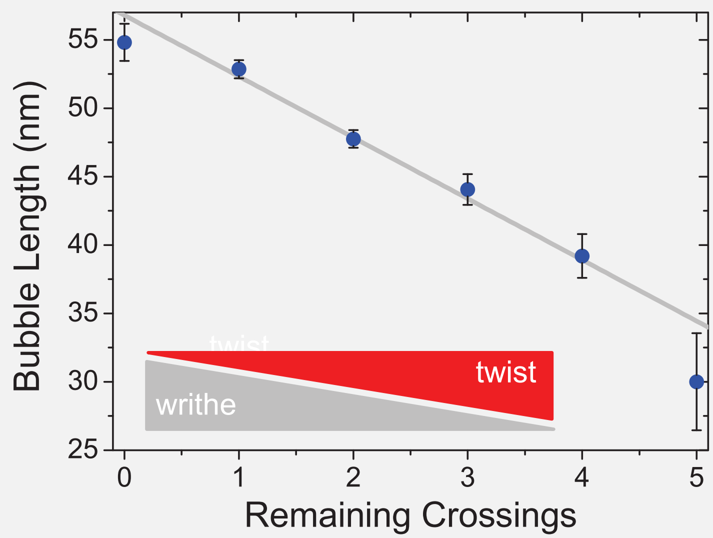
Weak regions at gene starts promote DNA denaturation





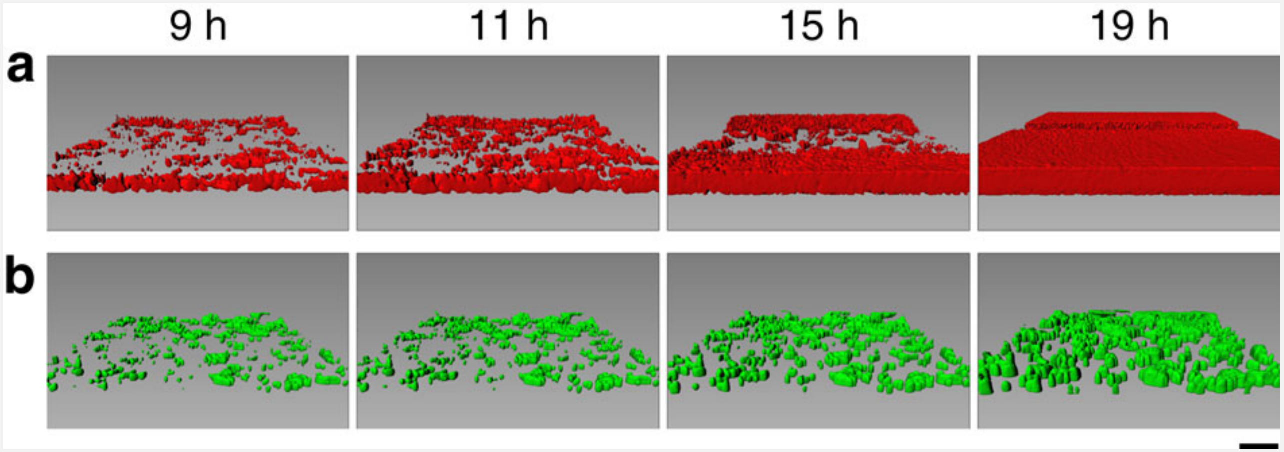
DNA superhelical density:

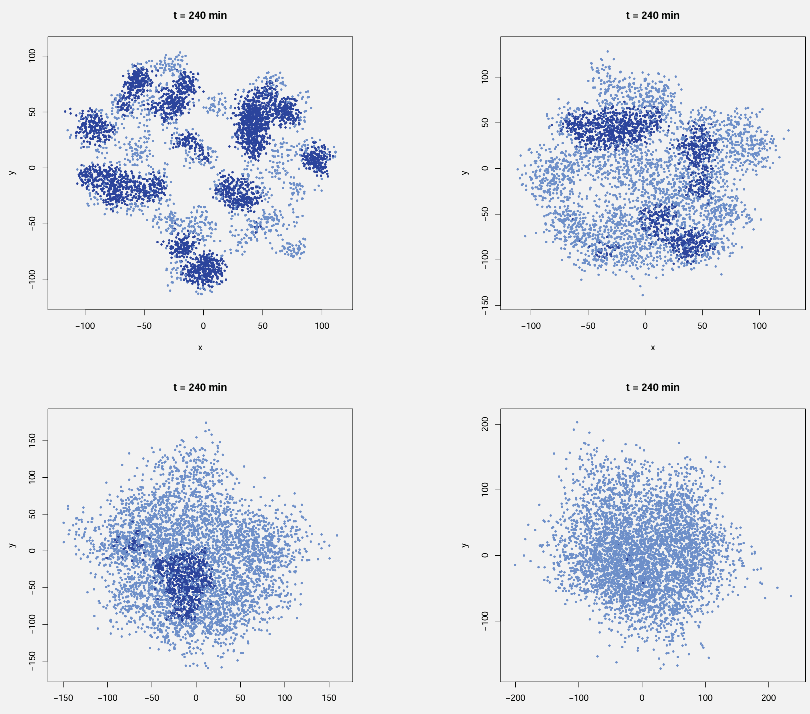
$$\sigma = \frac{\mathrm{Lk} - \mathrm{Lk}_0}{\mathrm{Lk}_0} \approx -0.06$$

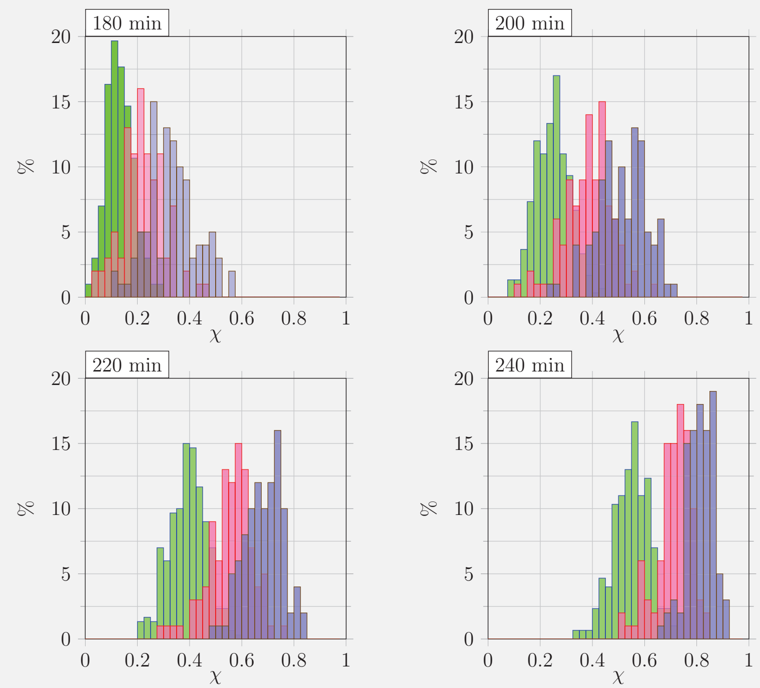


J-H Jeon, J Adamczik, G Dietler & RM, PRL (2010); J Adamczik, J-H Jeon, RM & G Dietler, Soft Matter (2012)

Quorum sensing dynamics in heterogeneous populations







SH Hong et al, Nat Comm **3**, 613 (2011); O Kindler, O Pulkkinen, AG Cherstvy & RM, Sci Rep (2019)

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DFG Deutsche Forschungsgemeinschaft

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