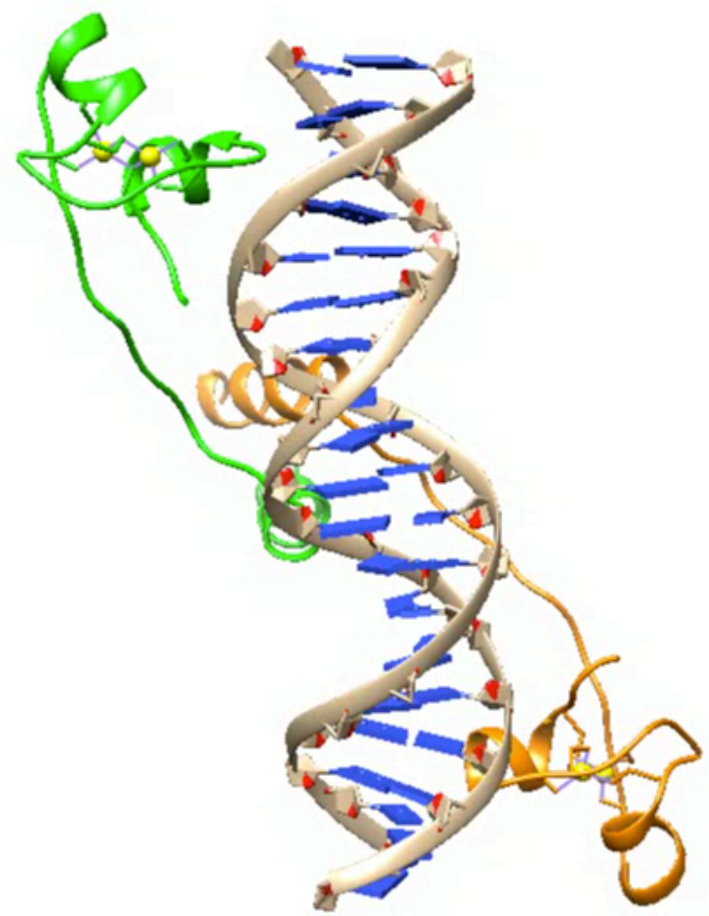
Understanding Protein Transport on DNA Track

Arnab Bhattacherjee

Associate Professor

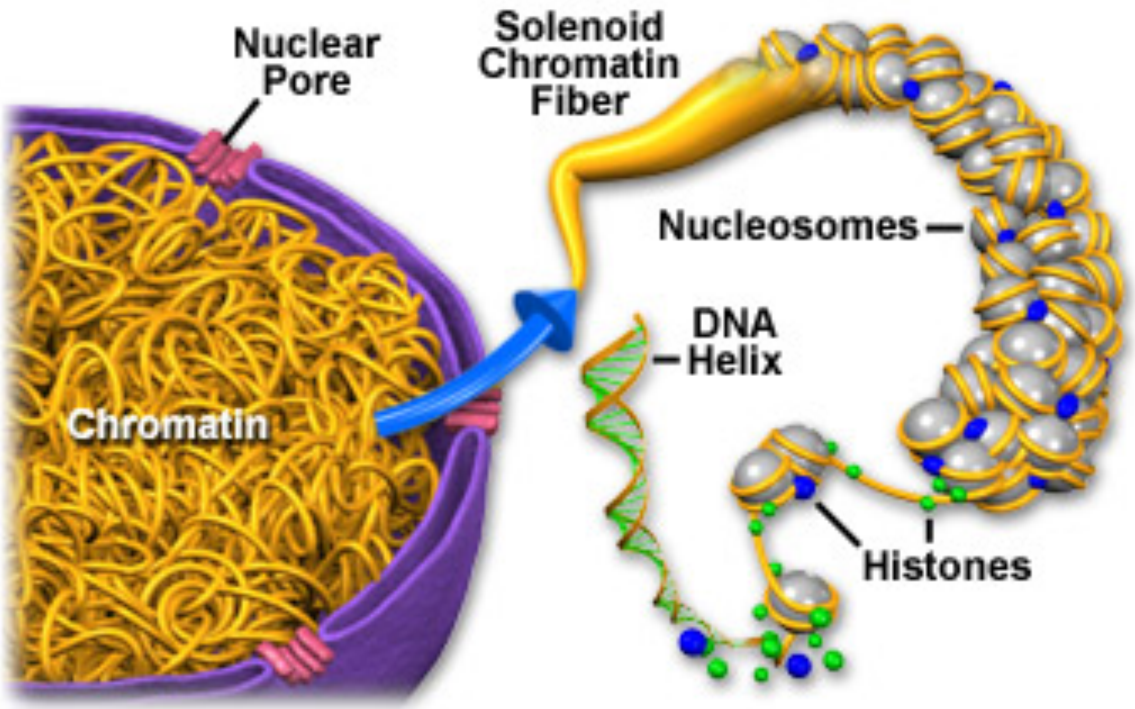
SCIS, JNU

Specific Protein-DNA Complex



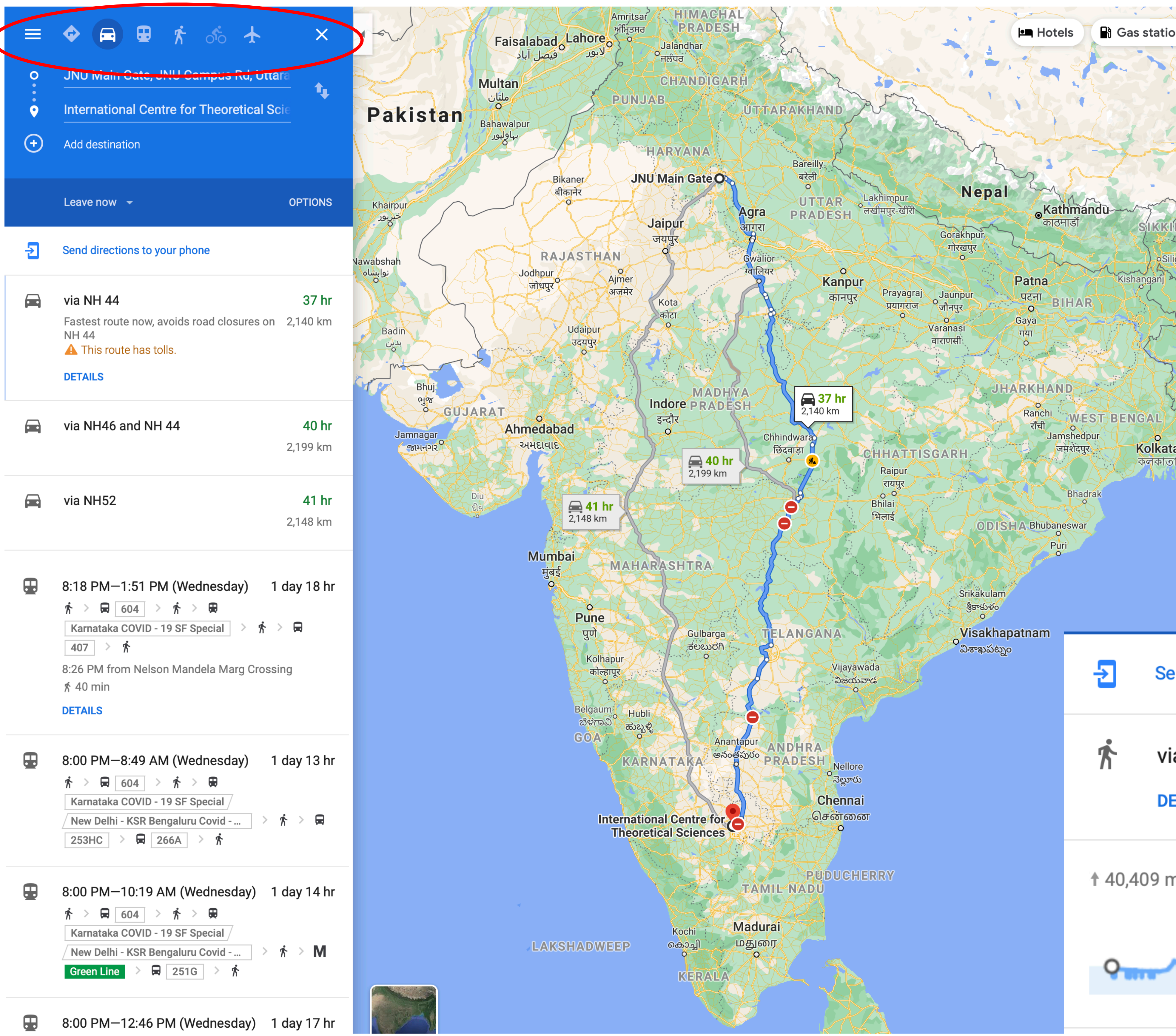
Journey is often more beautiful than the destination….

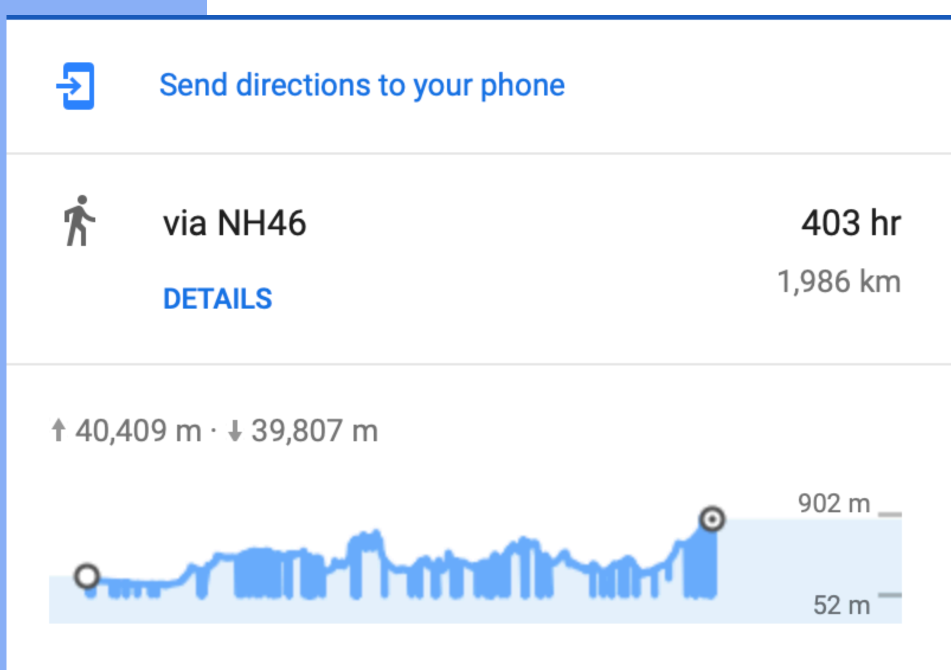
TRANSPORT and TRAFFIC



How Do Proteins Manage to move ?

How Do We Manage It ?





Modes of Protein Transport on DNA

Sliding Dynamics

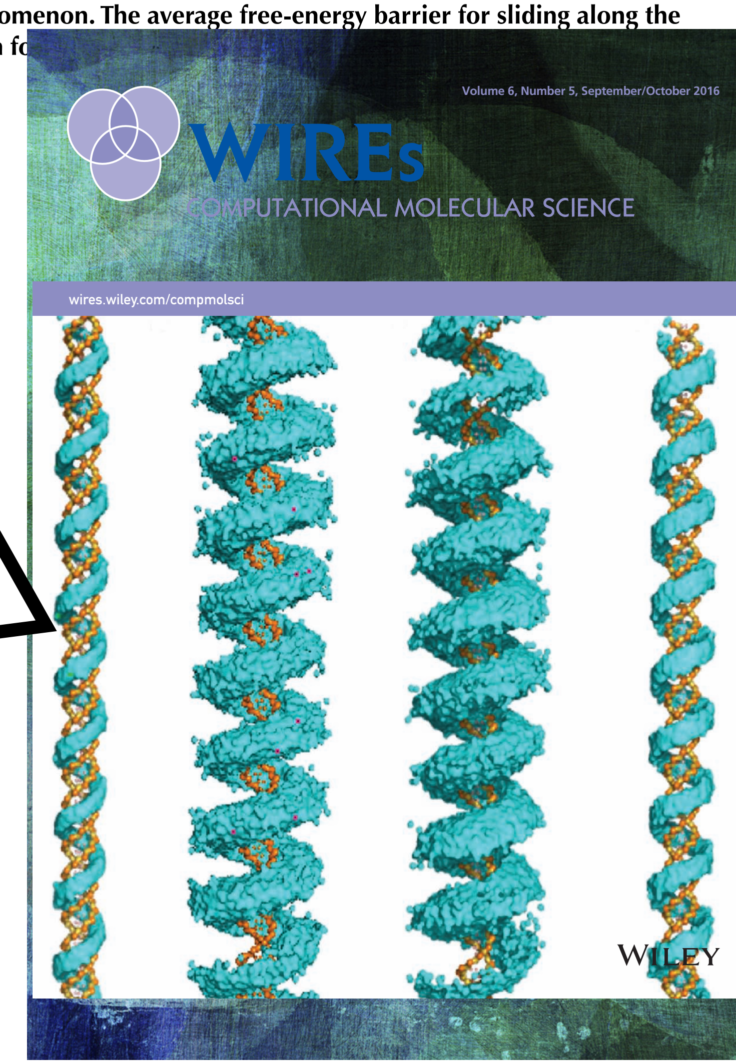
ARTICLES

nature structural & molecular biology

Nonspecifically bound proteins spin while diffusing along DNA

Paul C Blainey[1], Guobin Luo[1], S C Kou[5], Walter F Mangel[3], Gregory L Verdine[1,4], Biman Bagchi[2] & X Sunney Xie[1]

It is known that DNA-binding proteins can slide along the DNA helix while searching for specific binding sites, but their path of motion remains obscure. Do these proteins undergo simple one-dimensional (1D) translational diffusion, or do they rotate to maintain a specific orientation with respect to the DNA helix? We measured 1D diffusion constants as a function of protein size while maintaining the DNA-protein interface. Using bootstrap analysis of single-molecule diffusion data, we compared the results to theoretical predictions for pure translational motion and rotation-coupled sliding along the DNA. The data indicate that DNA-binding proteins undergo rotation-coupled sliding along the DNA helix and can be described by a model of diffusion along the DNA helix on a rugged free-energy landscape. A similar analysis including the 1D diffusion constants of eight proteins of varying size shows that rotation-coupled sliding is a general phenomenon. The average free-energy barrier for sliding along the DNA was 1.1 ± 0.2 k_BT. Such small barriers facilitate rapid search fo



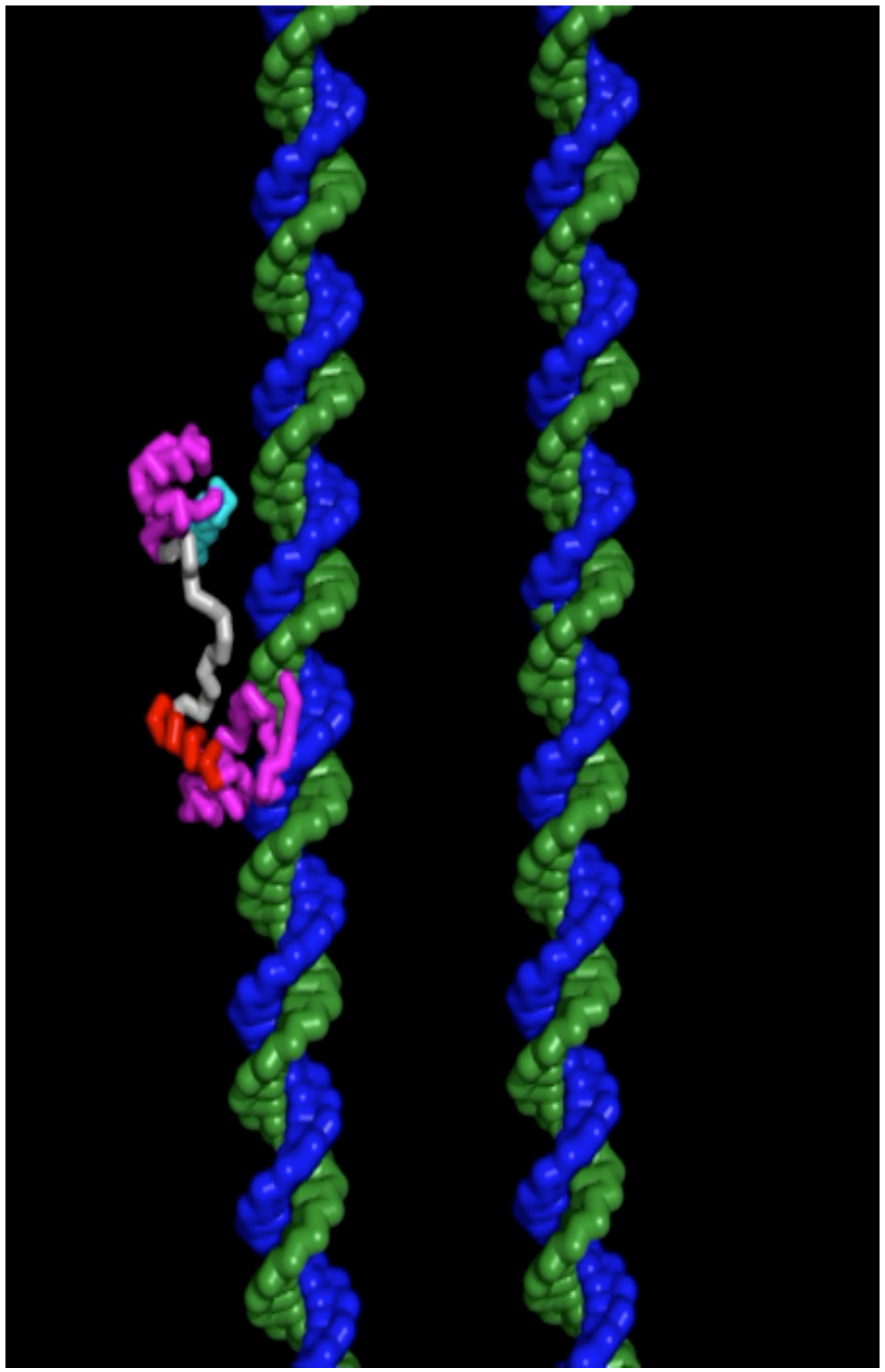
WIREs Comp Mol Sci. 6, 515, 2016

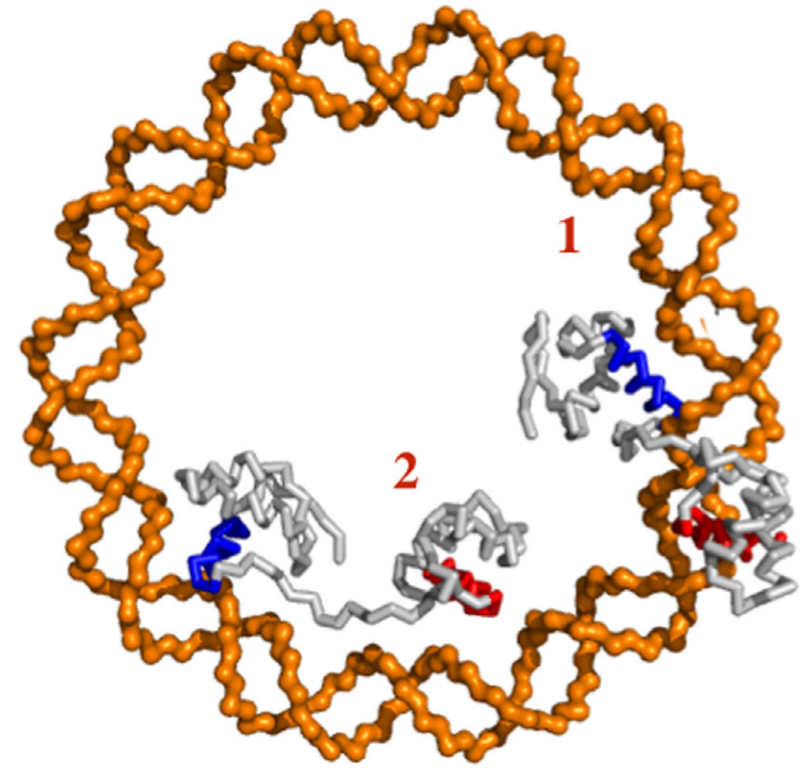
Nucleic Acids Res 43, 9176–9186, 2015

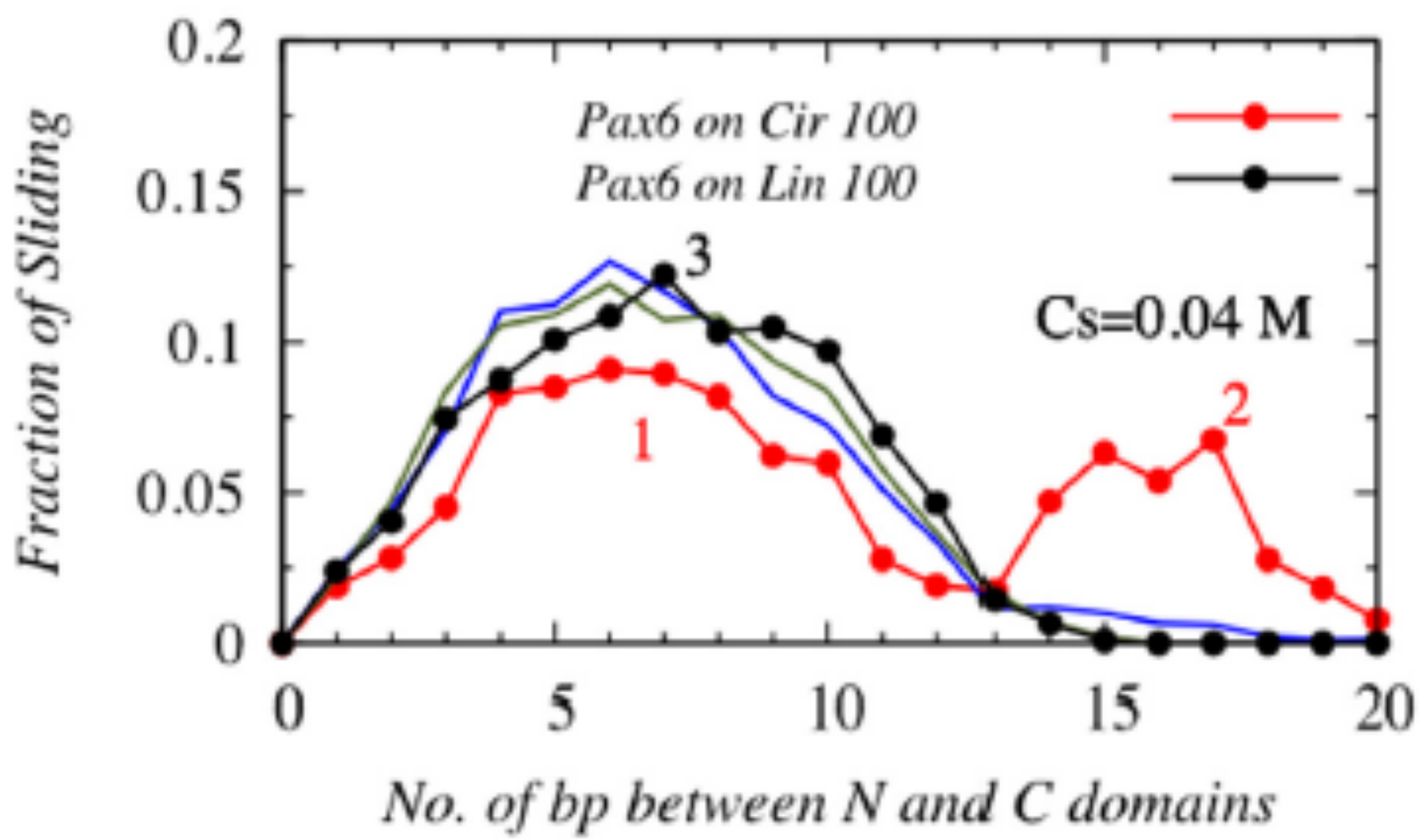
Nucleic Acids Res 42(20), 12404, 2014

Protein also takes Shortcuts

Intersegmental Transfer

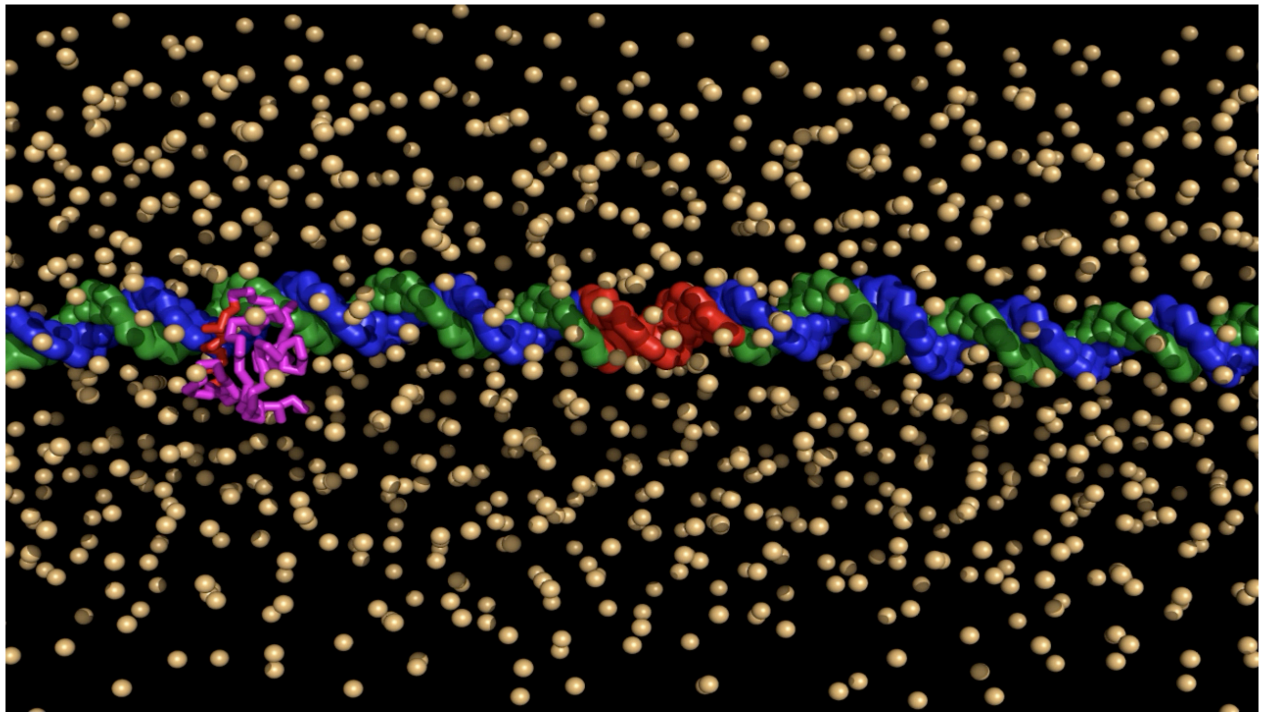


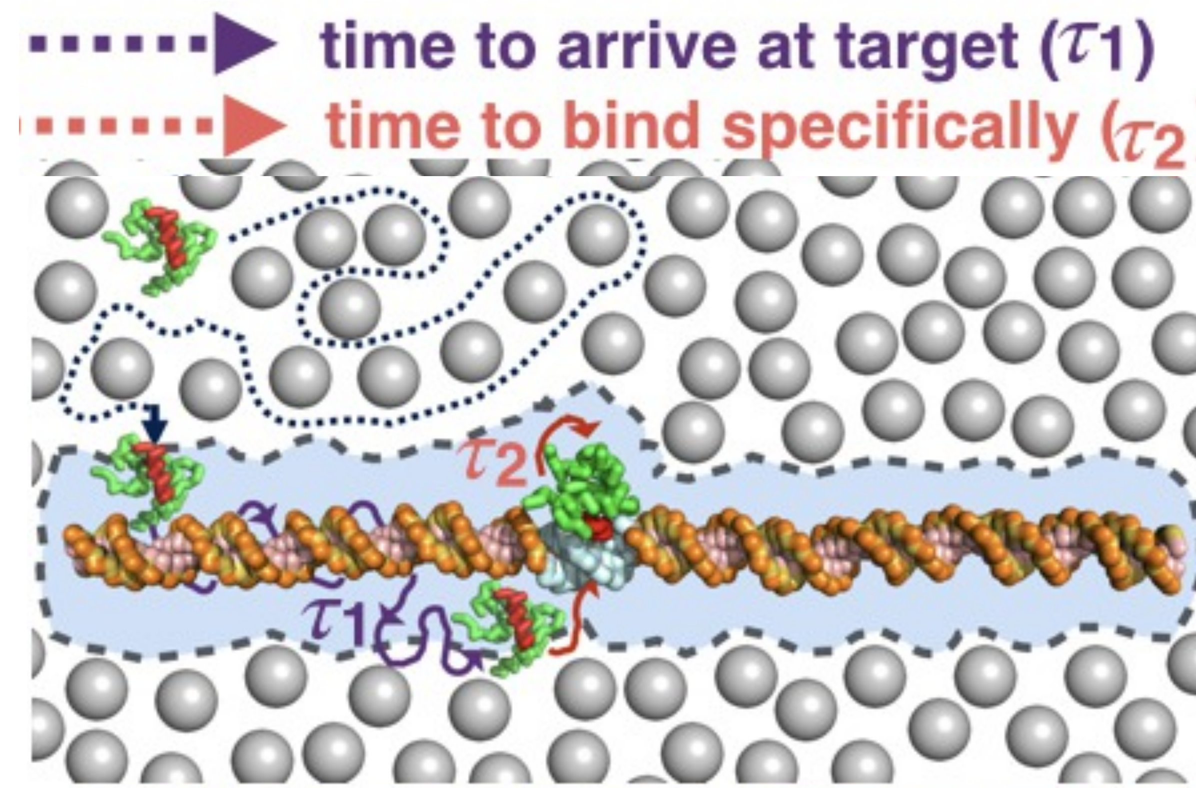


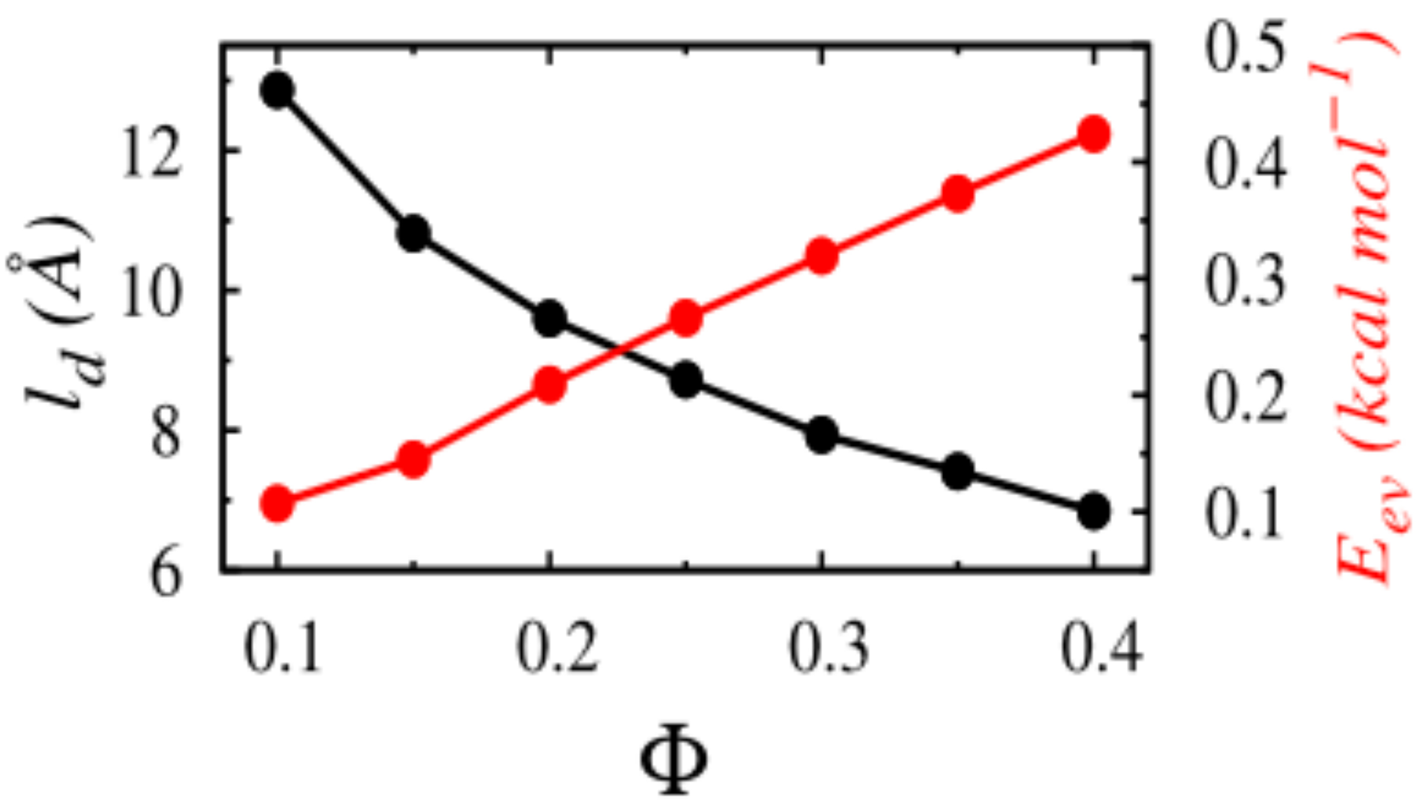


Nucleic Acid Research, 42(20), 12415, 2014

Making Passage Through Crowd

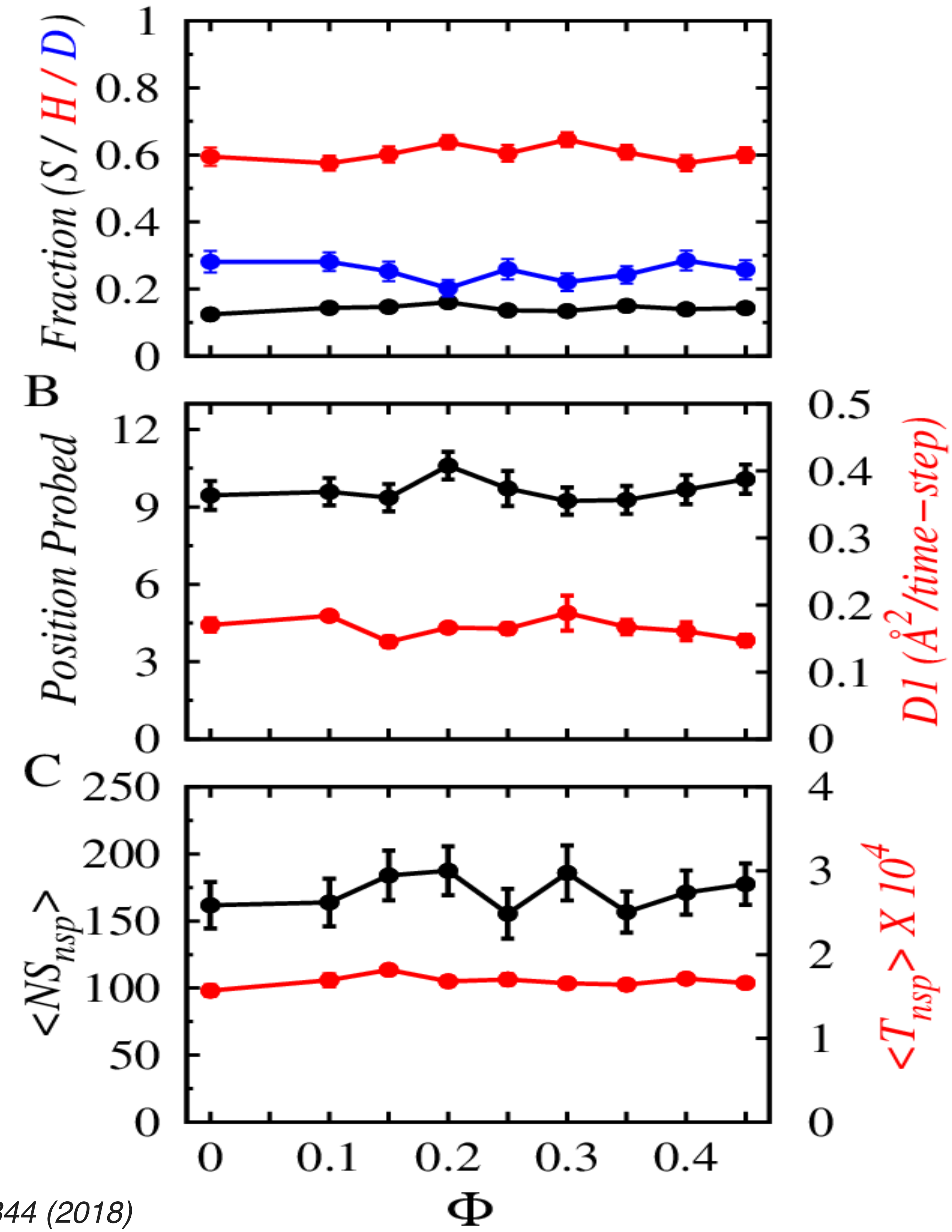






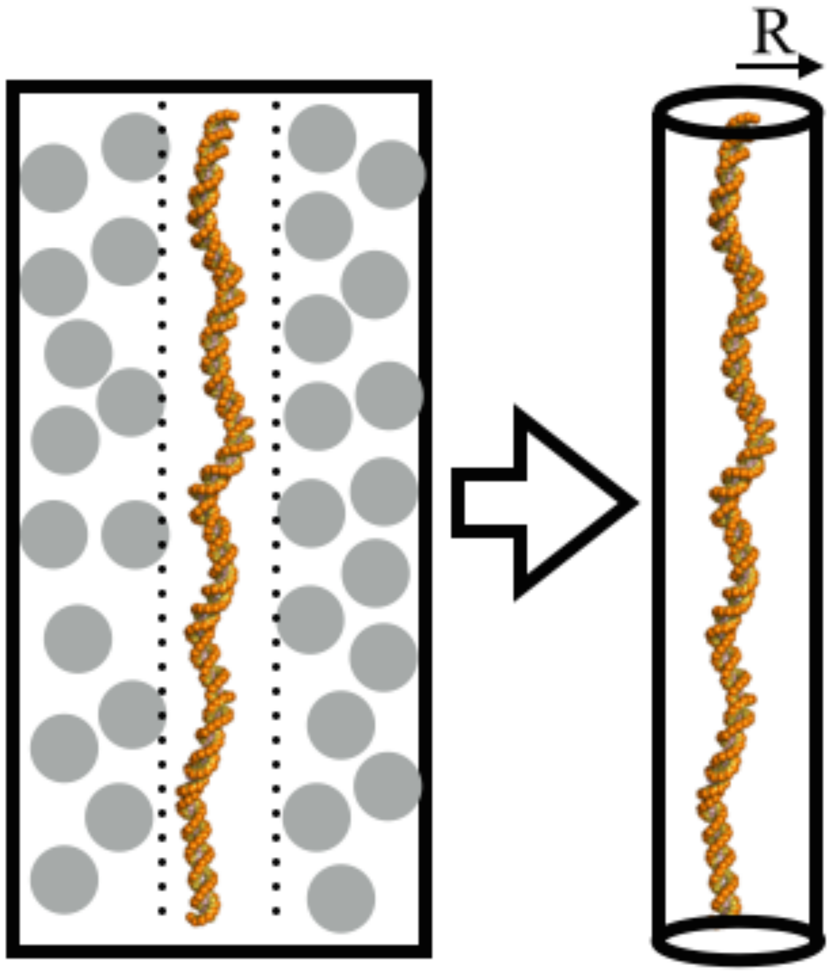
Scientific Reports volume 8, page 844 (2018)

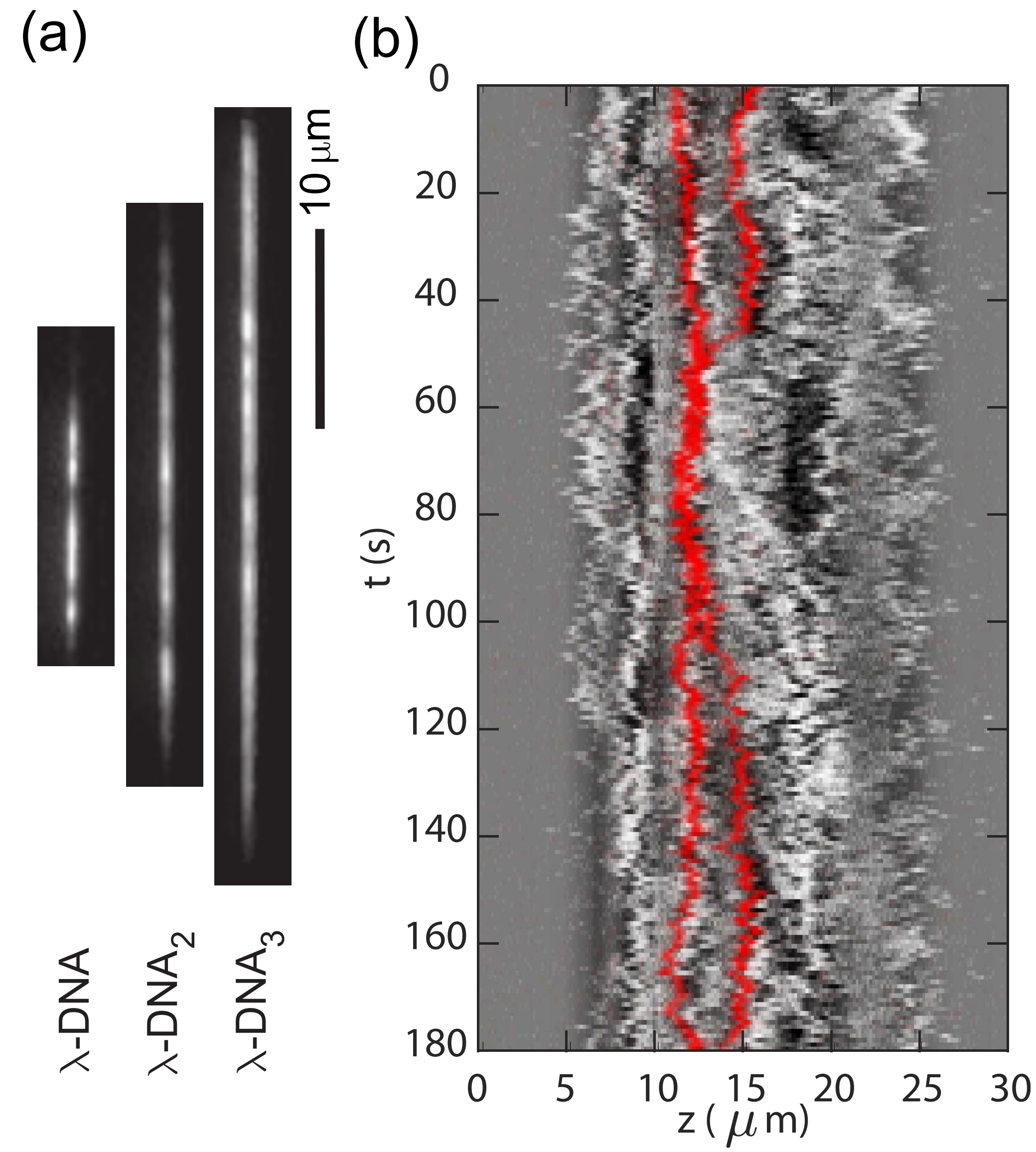
Non-specific search Regime

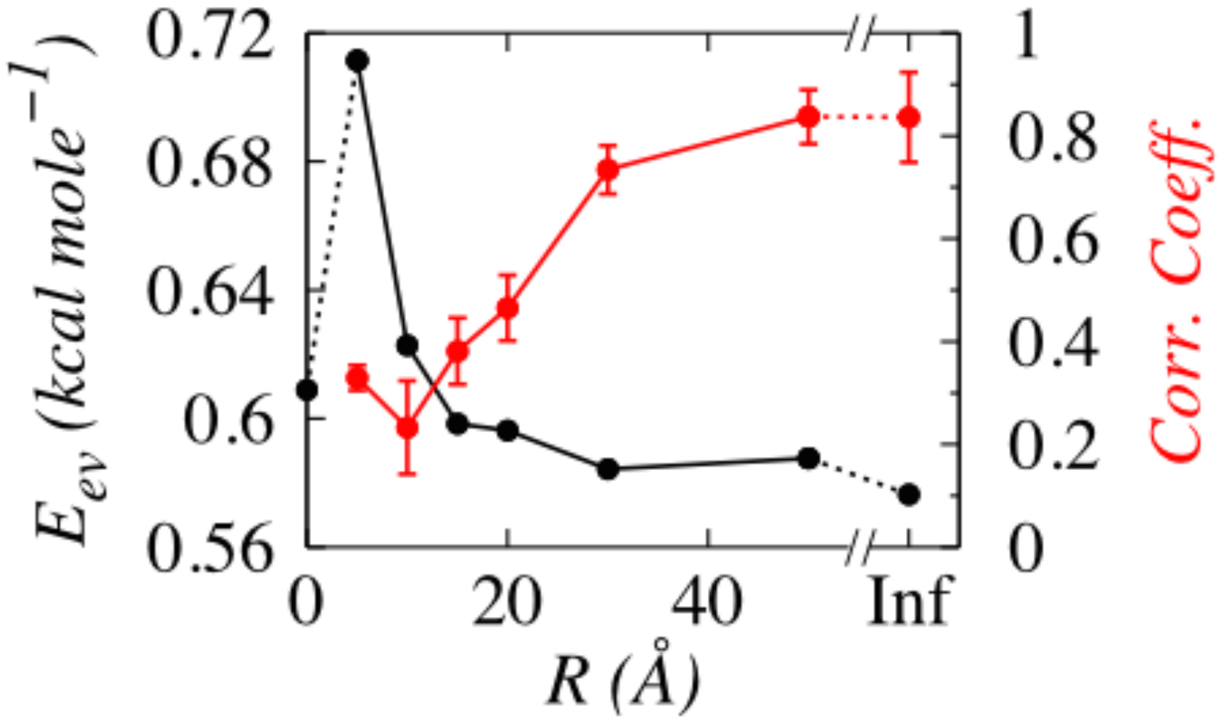


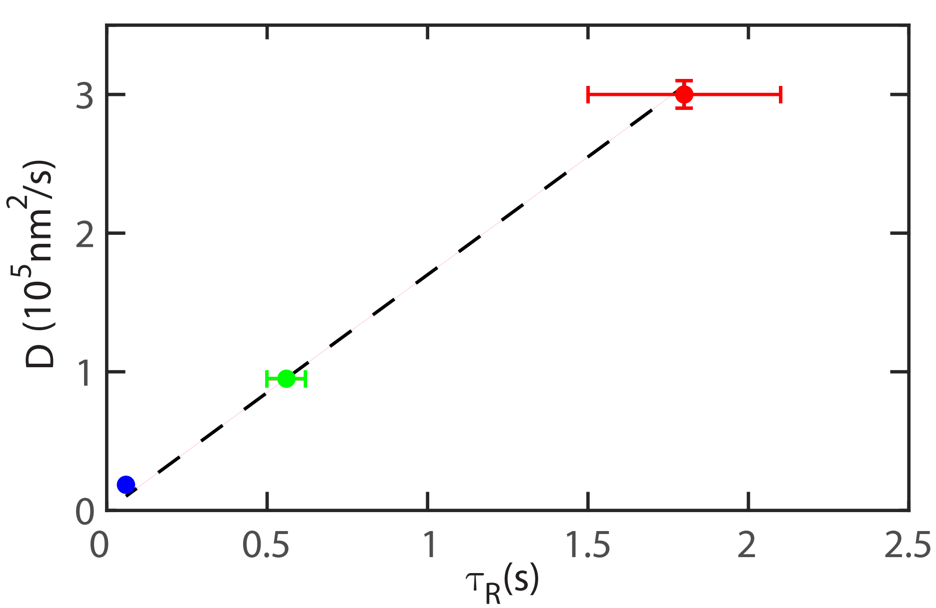
Scientific Reports volume 8, page 844 (2018)

JPC L 11, 8424, 2020



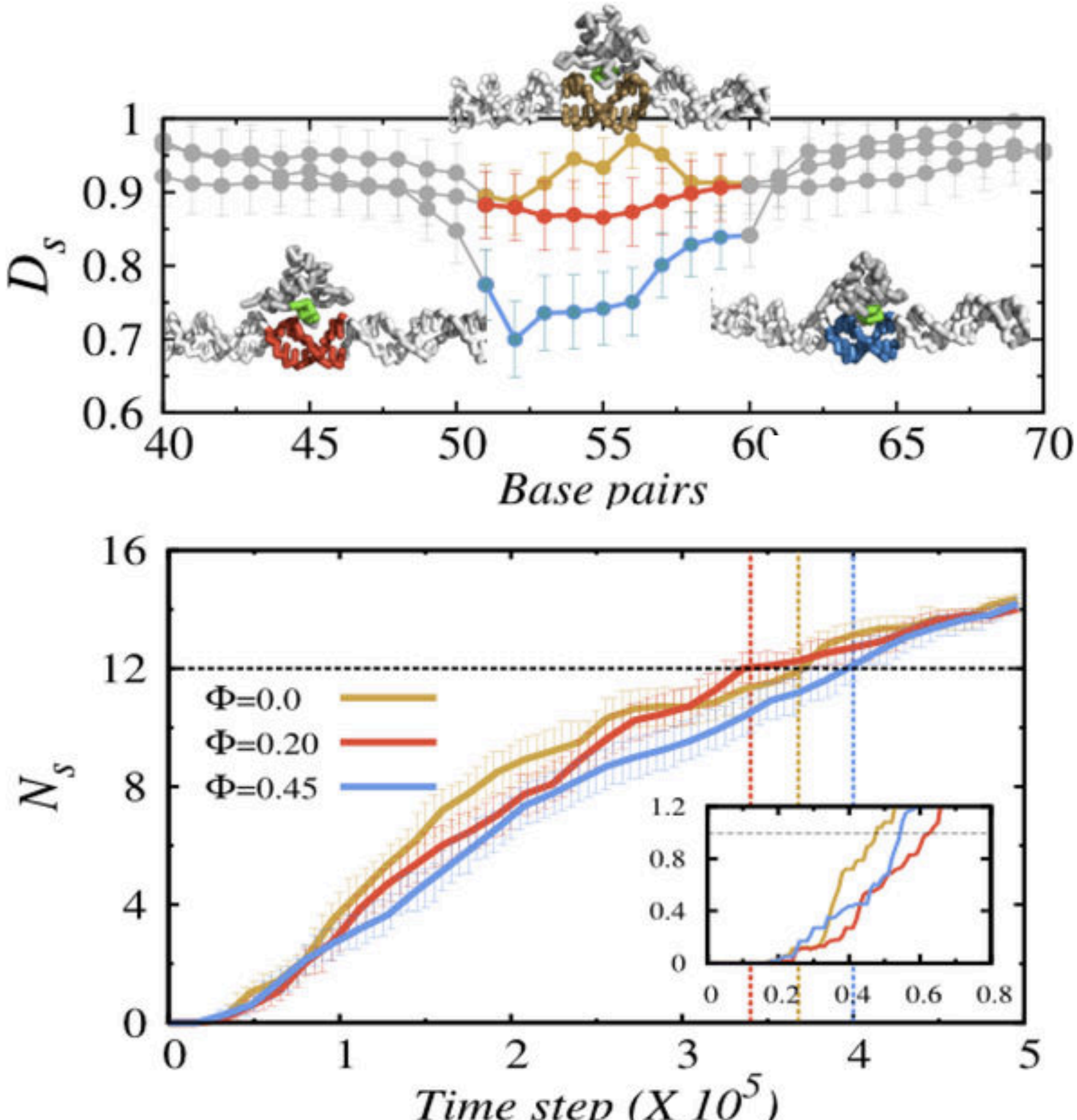






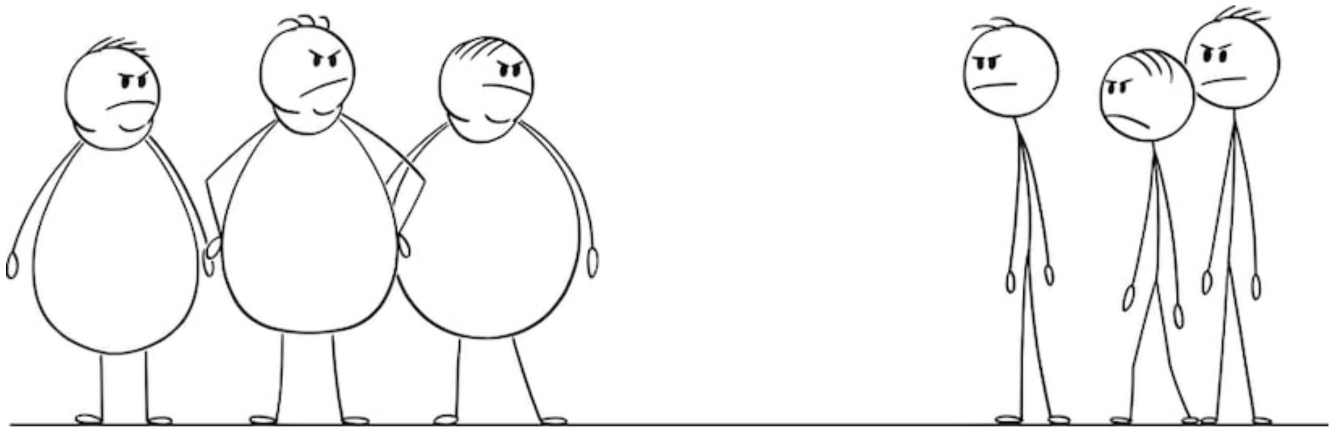
NAR 43, 9176–9186, 2015

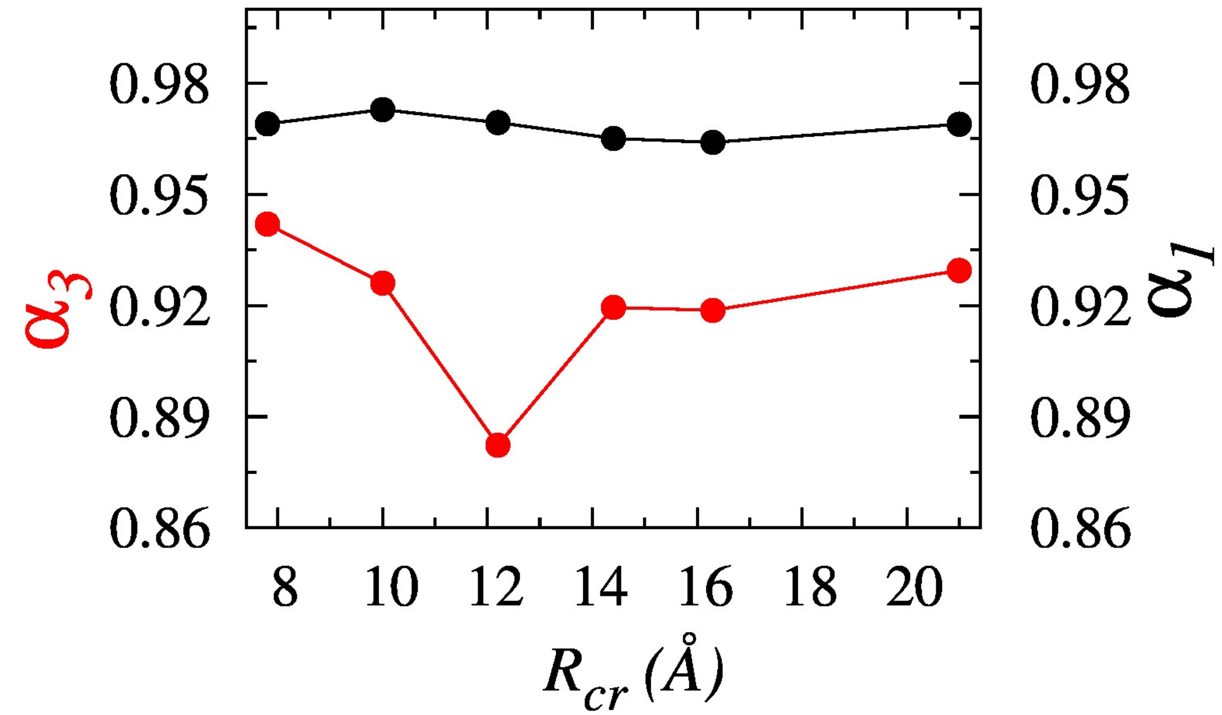
Impact on Specific Binding

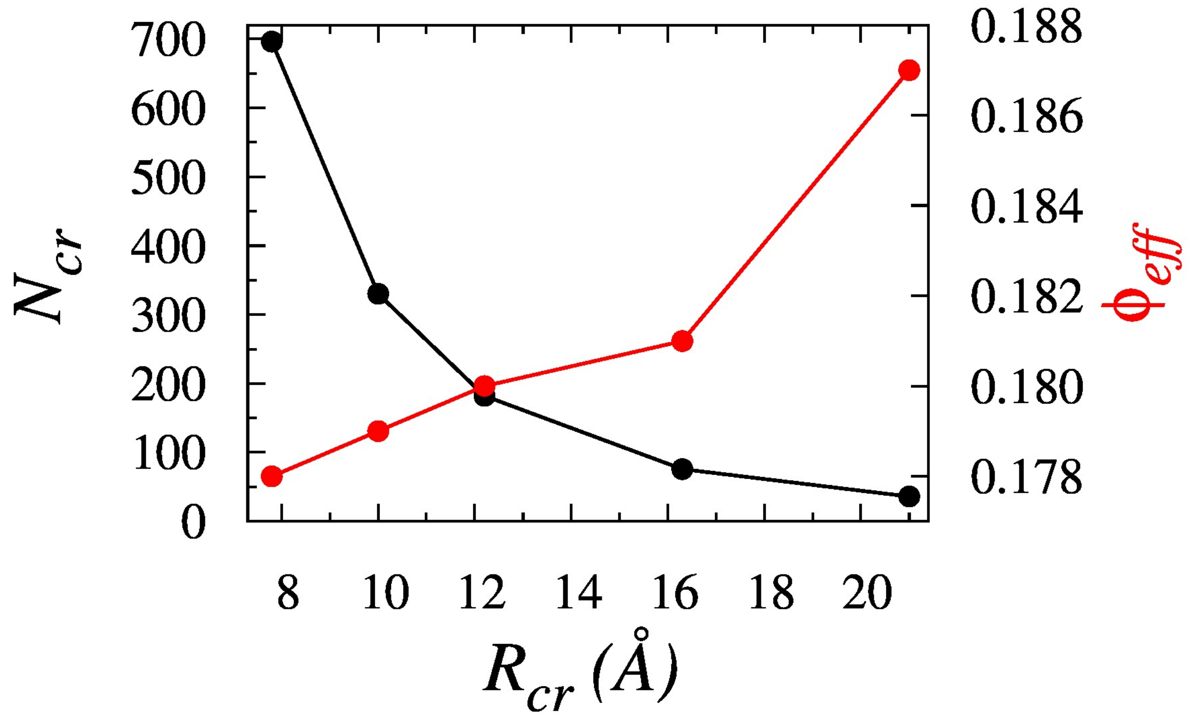


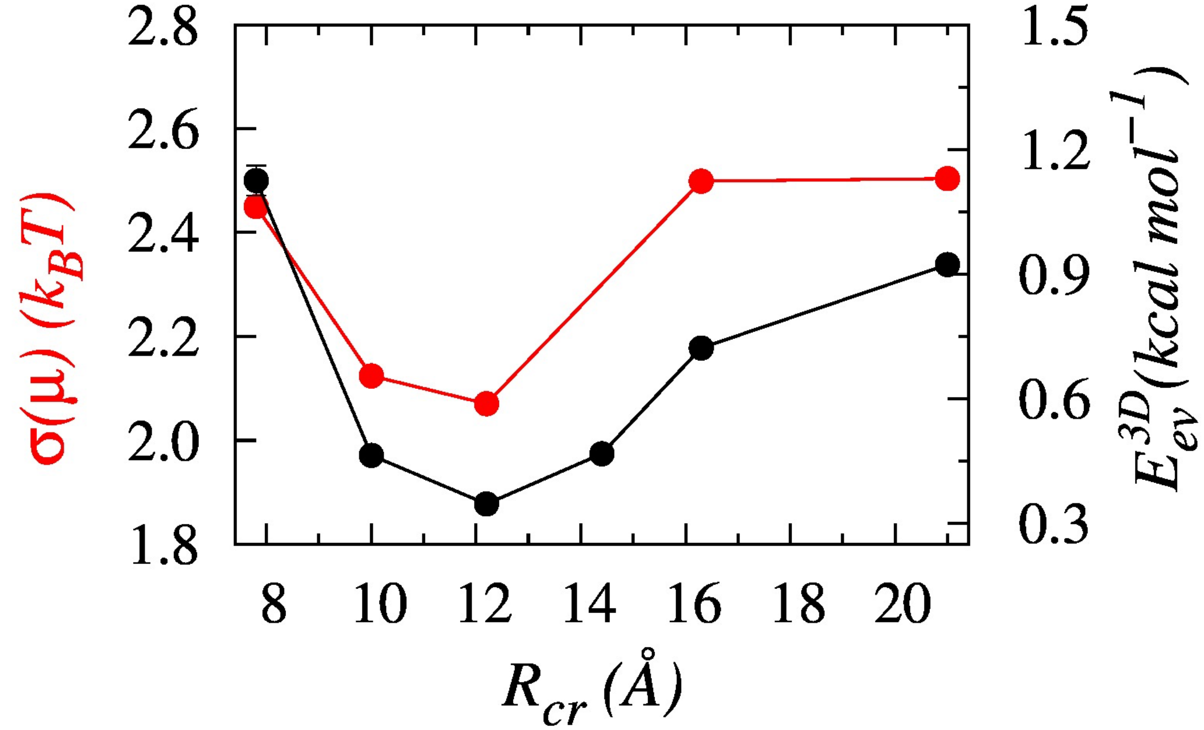
Scientific Reports volume 8, page 844 (2018)

Crowder Physiology - Size, Mobility, Shape

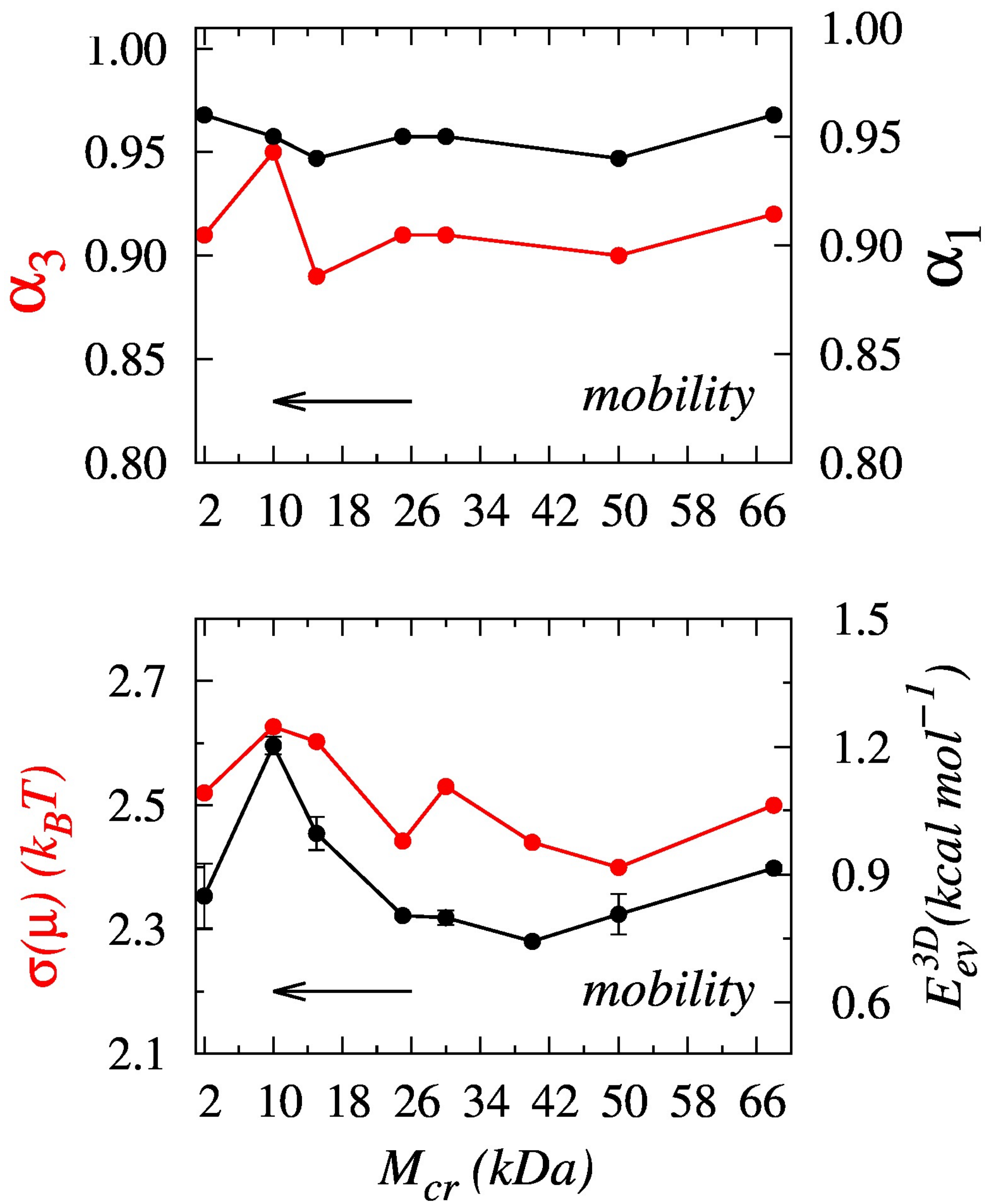


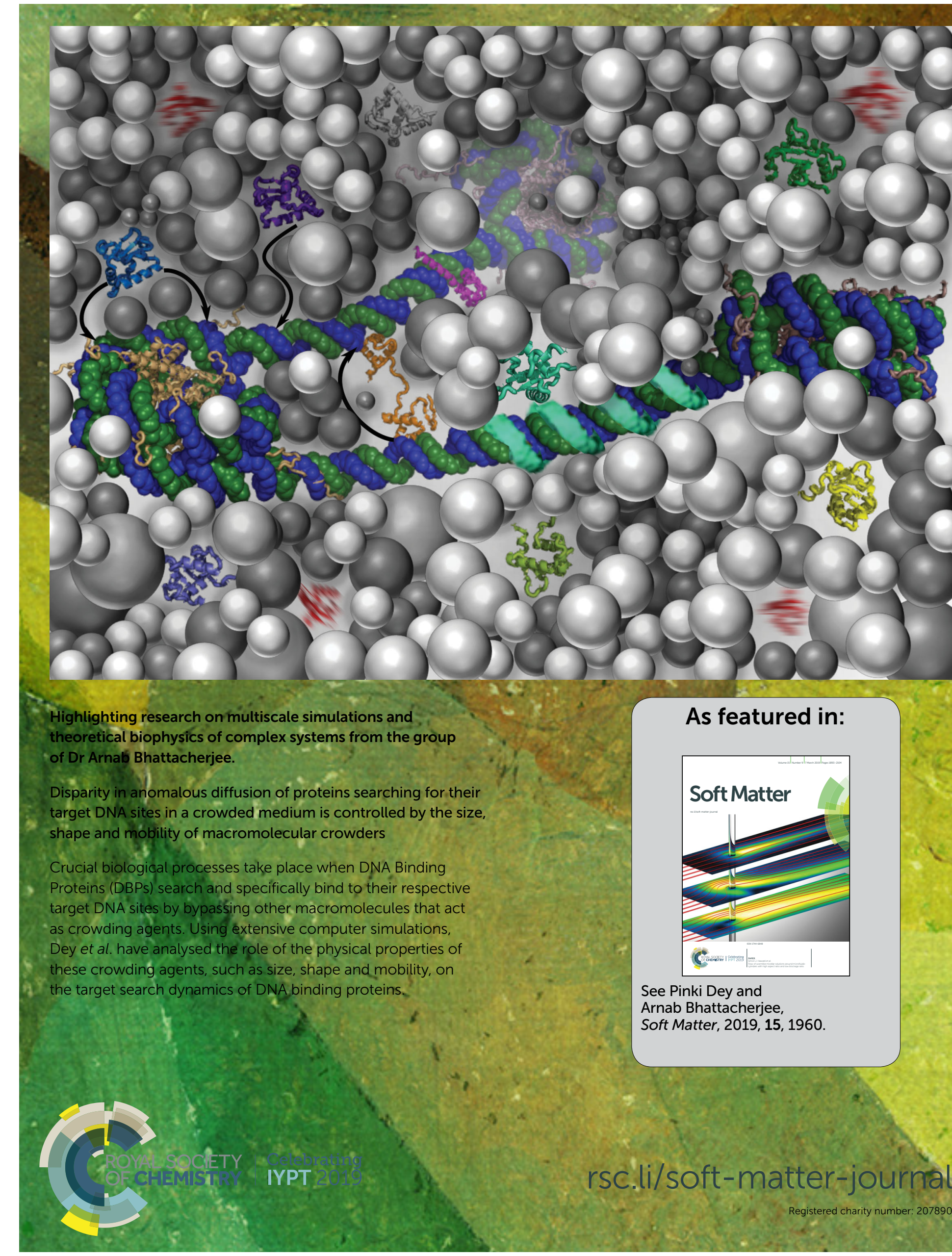






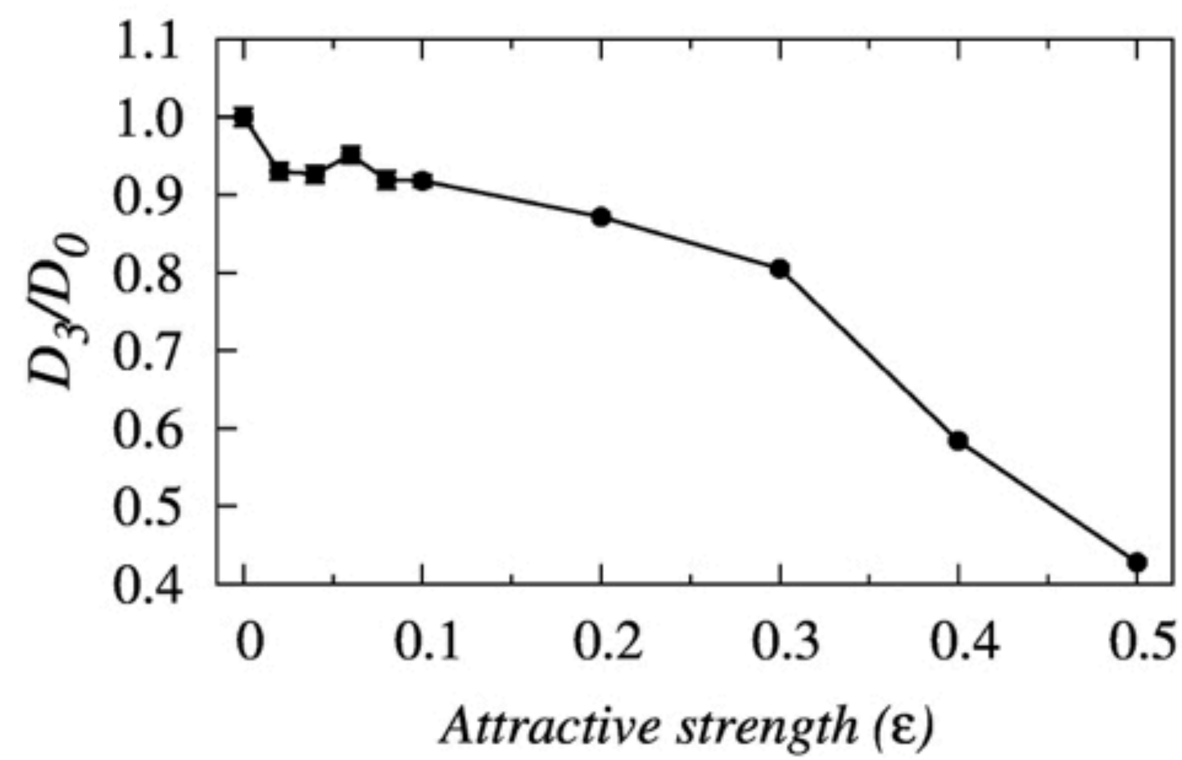
Soft Matter *volume* ***15****, page 1960-1969 (2019)*

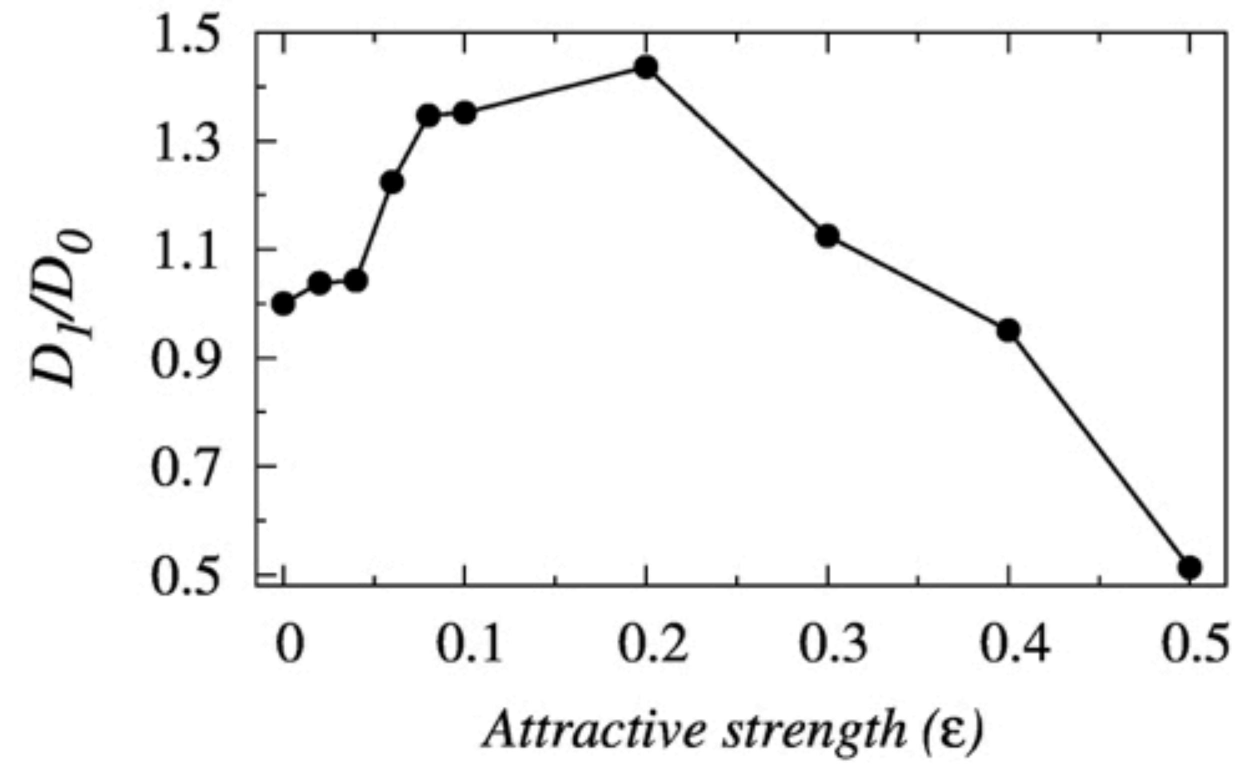




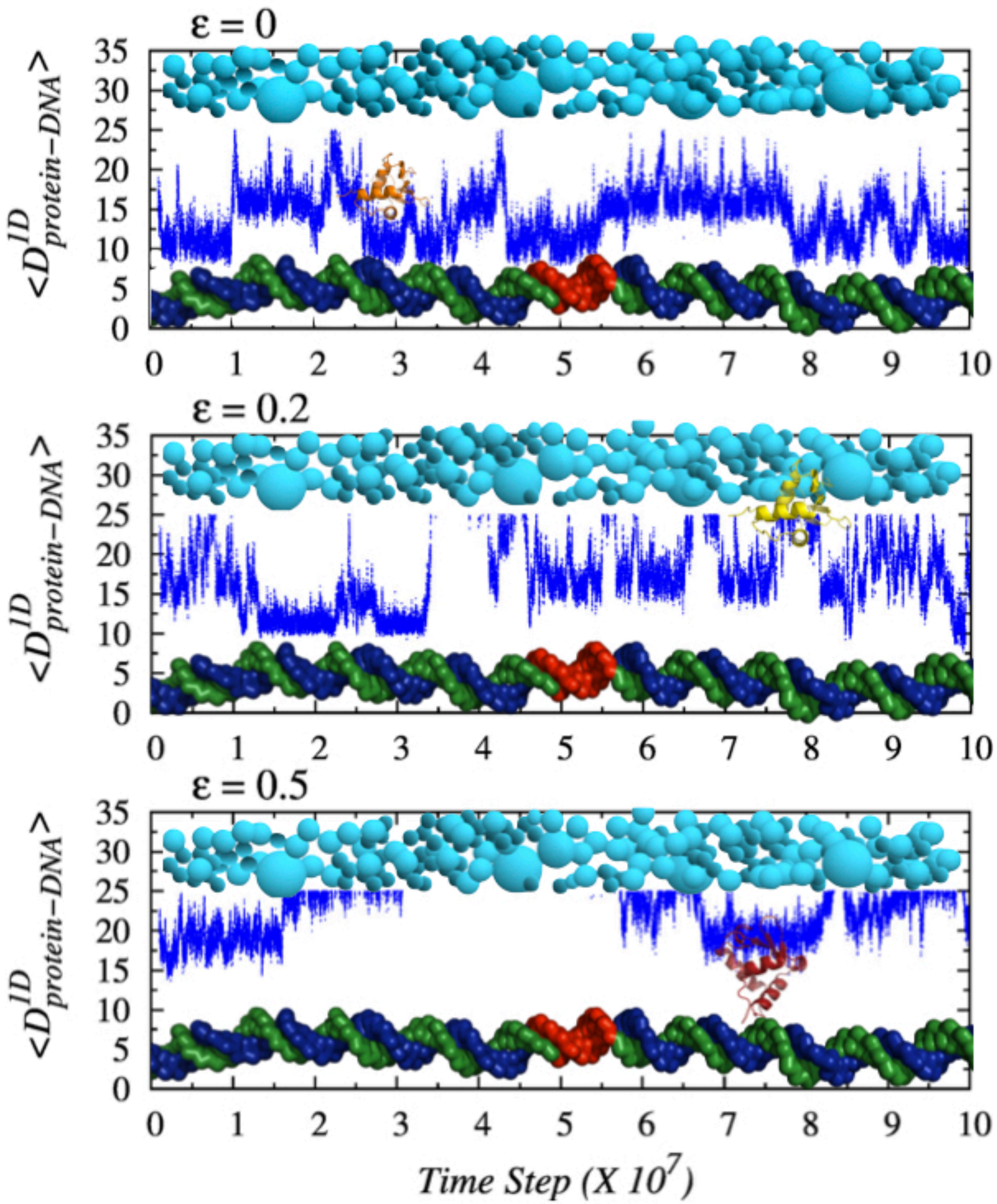
Soft Matter *volume* ***15****, page 1960-1969 (2019)*

Loving Crowd - Role of Protein-Crowder Interactions

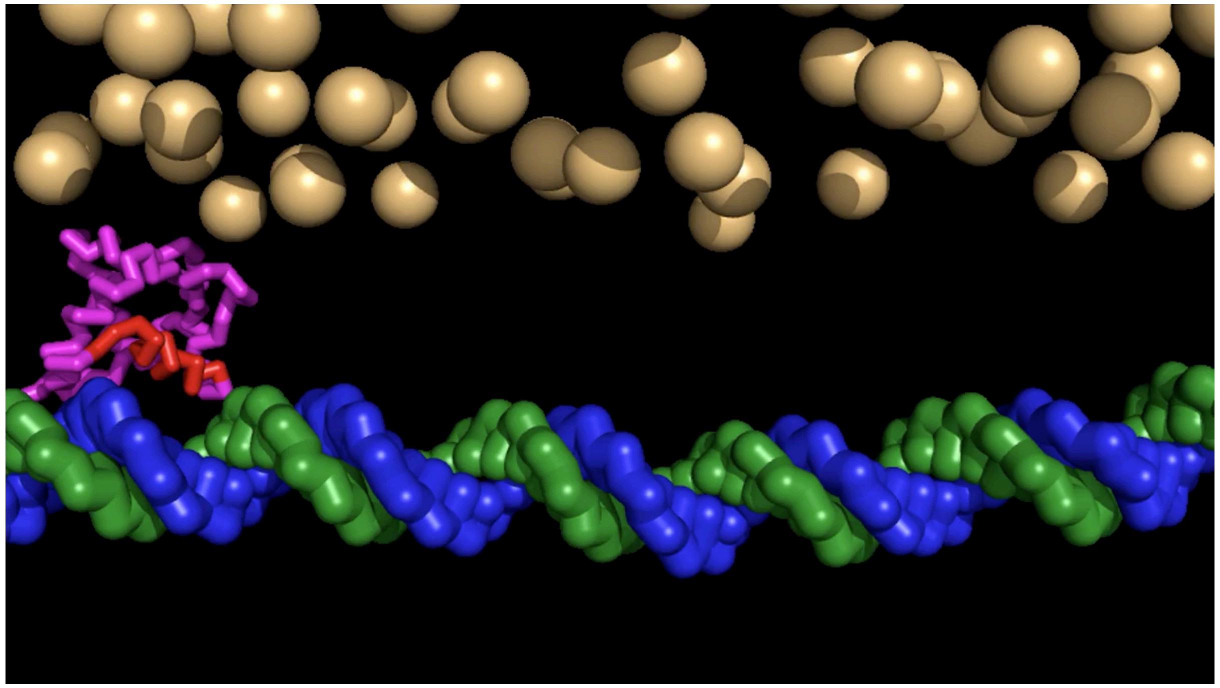




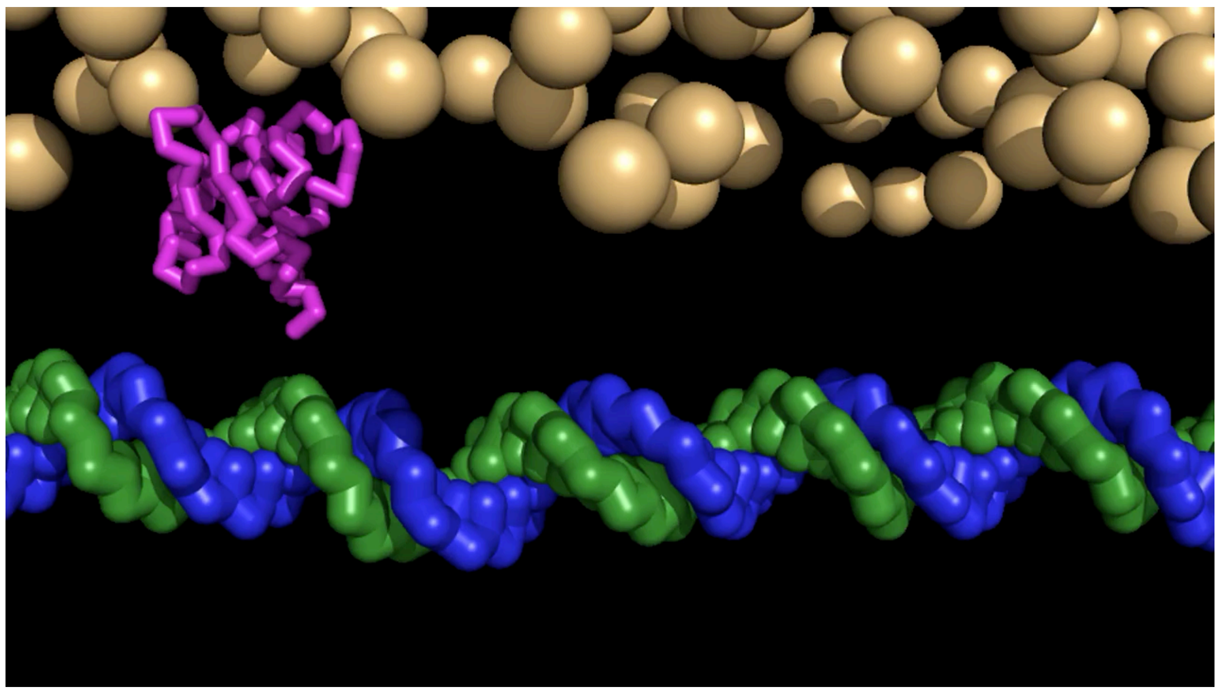
Biophys J *118(2) 1505-517 (2020)*



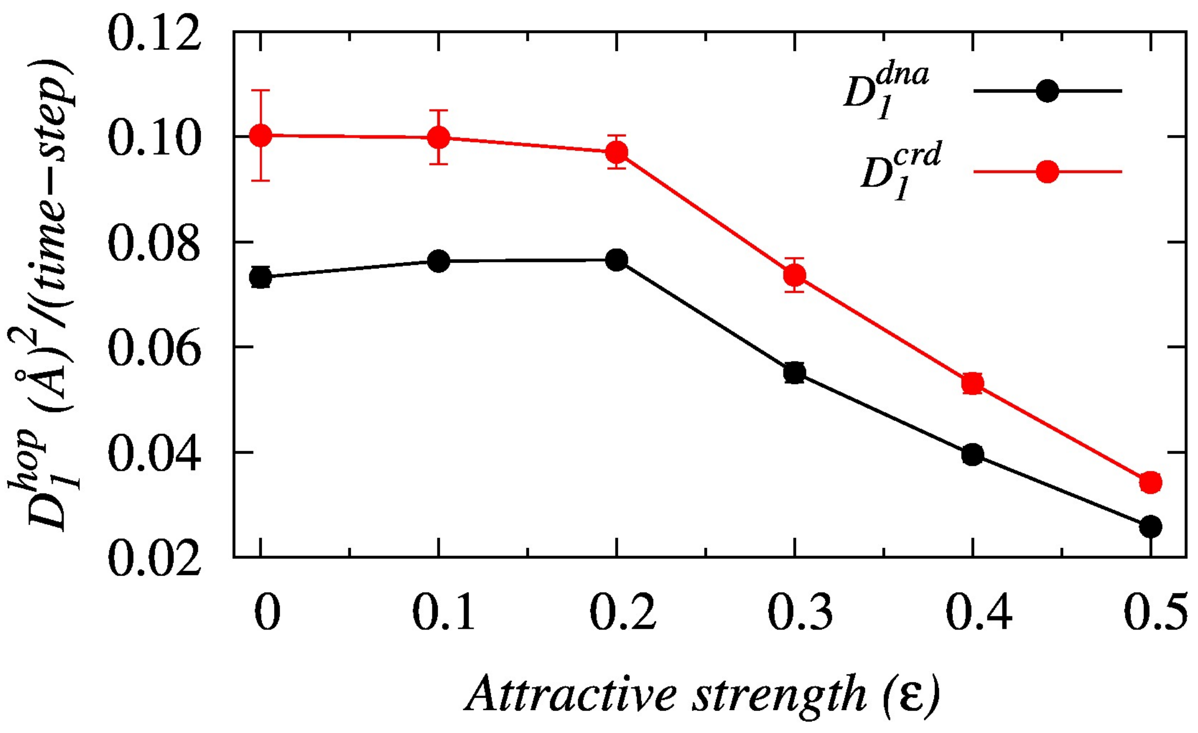
Hopping on DNA

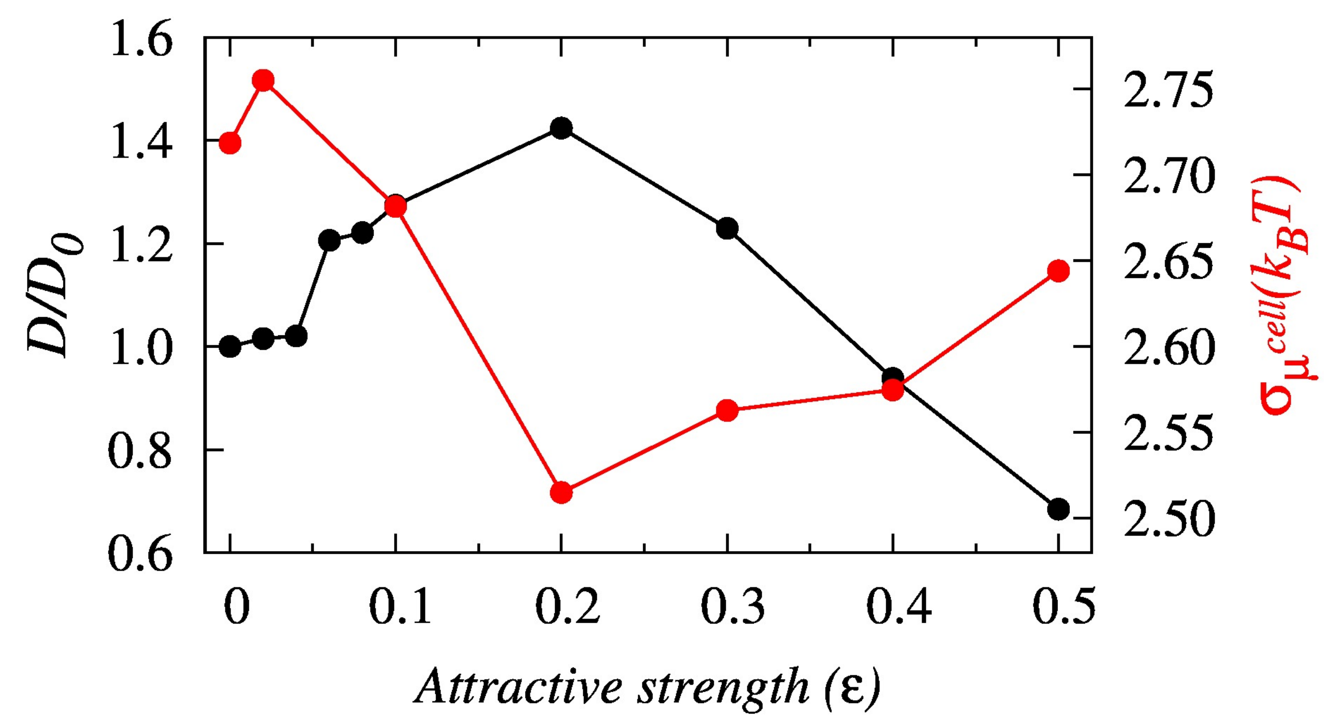


Hopping away from DNA



Biophys J *118(2) 1505-517 (2020)*





Published online 6 April 2015

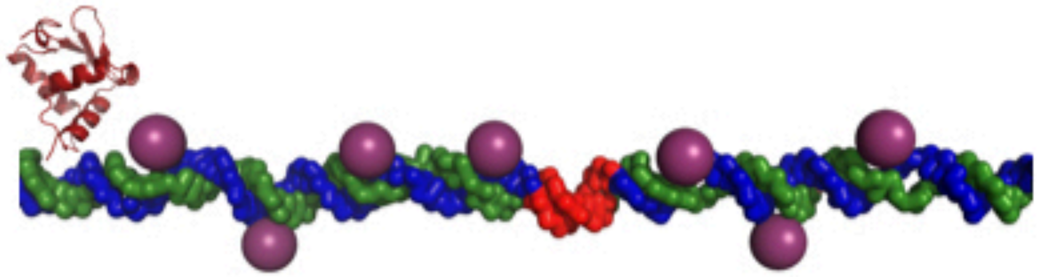
Nucleic Acids Research, 2015, Vol. 43, No. 8 ***4087–4097***
doi: 10.1093/nar/gkv301

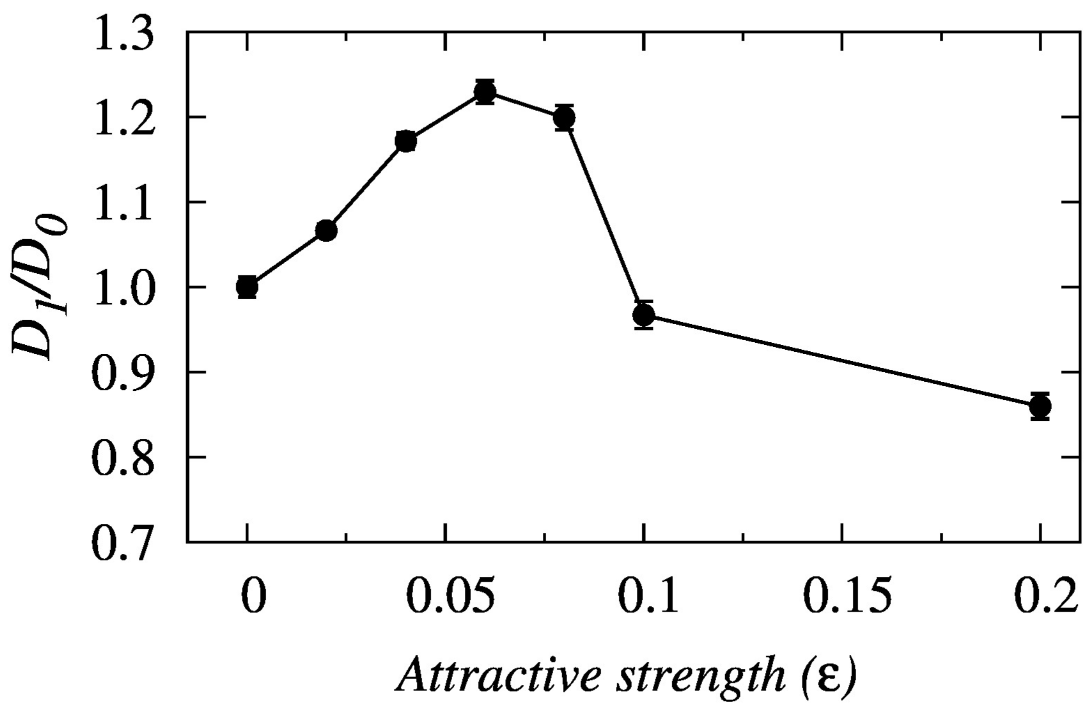
Molecular crowding enhances facilitated diffusion of two human DNA glycosylases

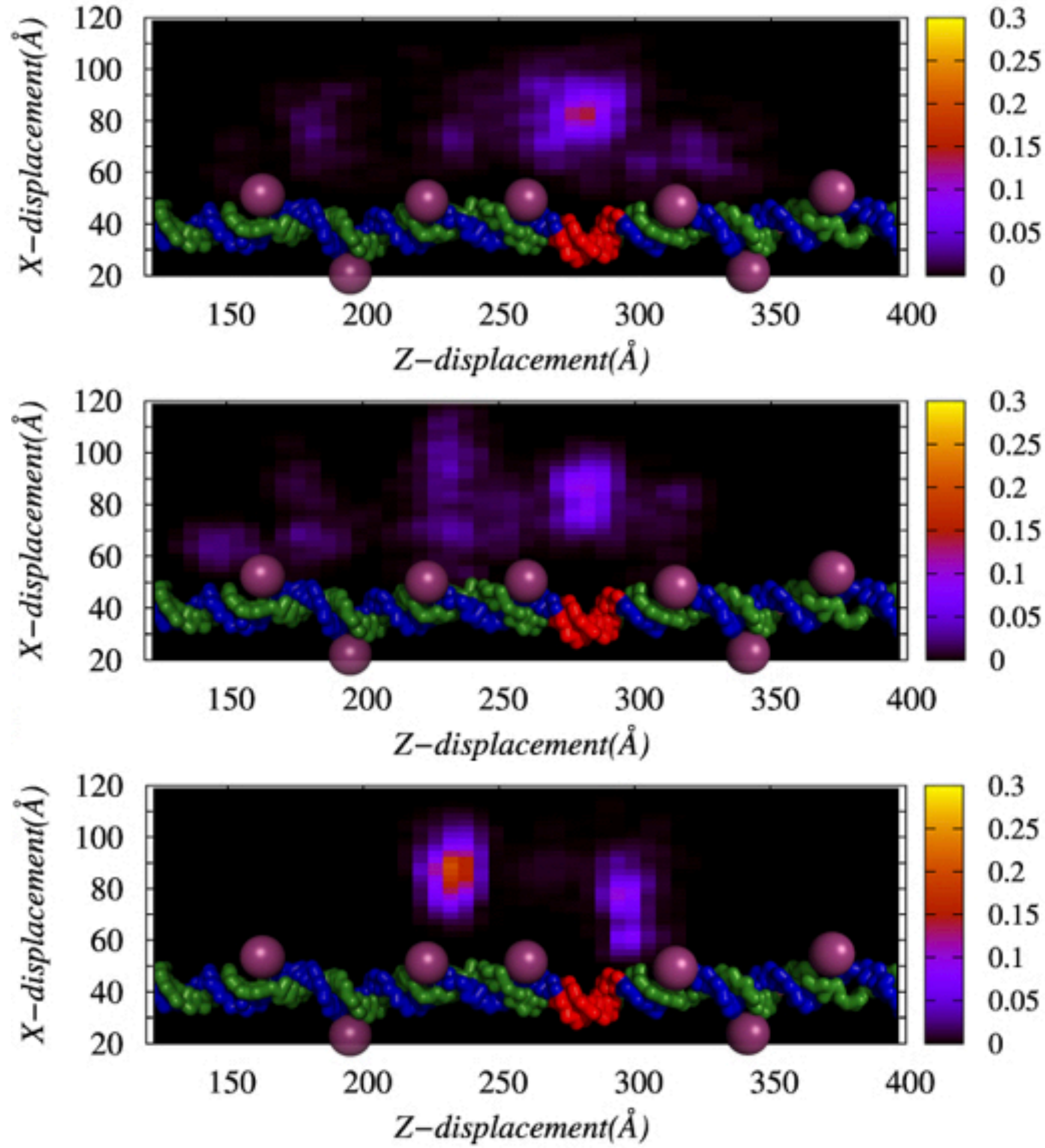
Shannen L. Cravens[1,†], Joseph D. Schonhoft[1,†], Meng M. Rowland[1], Alyssa A. Rodriguez[2], Breeana G. Anderson[1] and James T. Stivers[1,*]

Biophys J *118(2) 1505-517 (2020)*

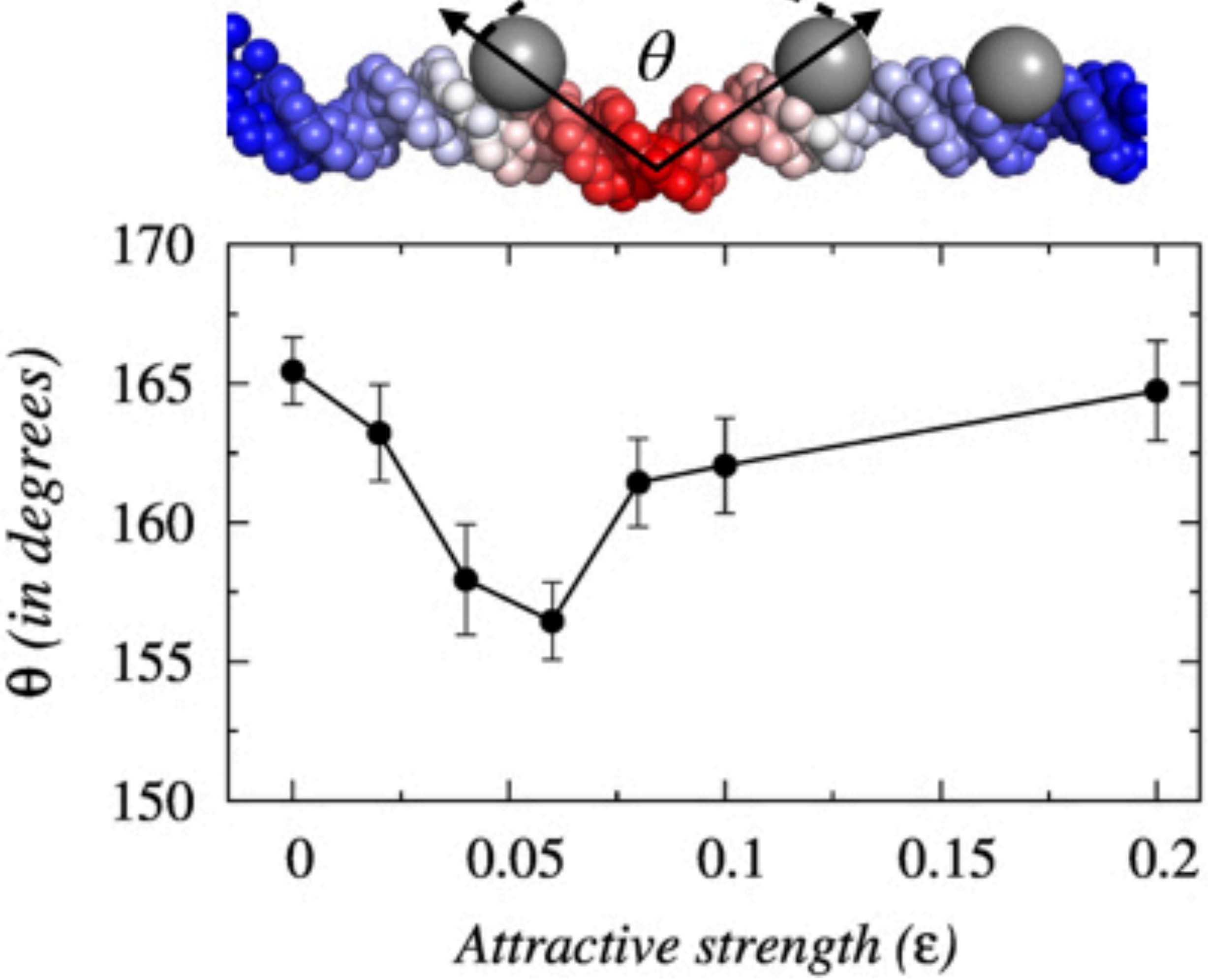
Genomic Crowders

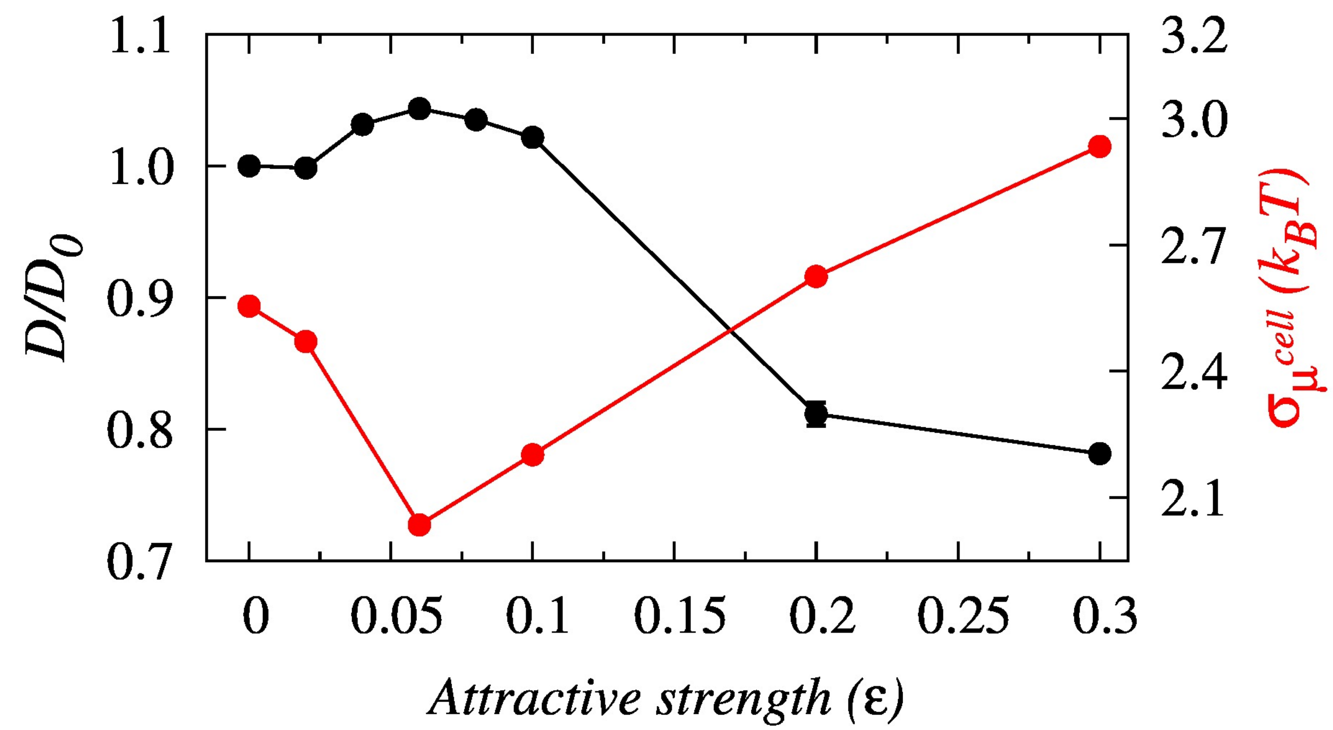


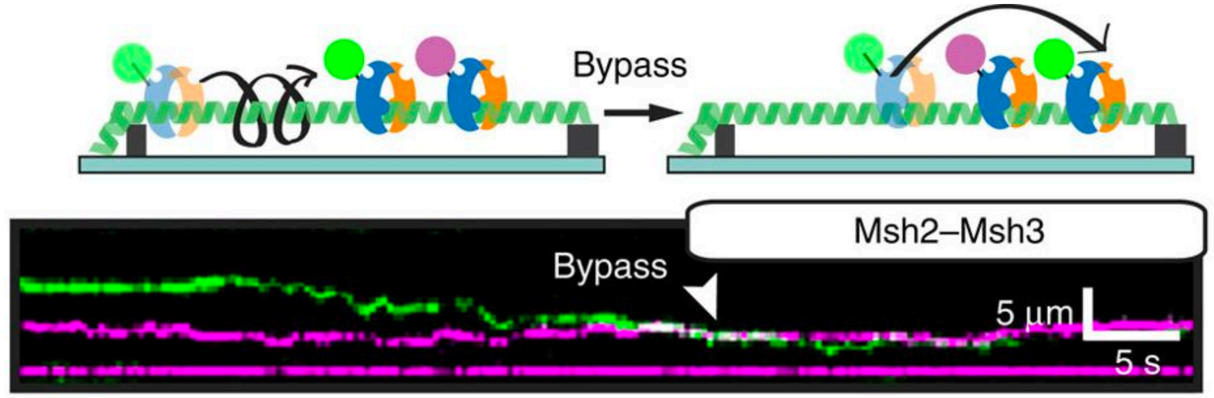




Biophys J *118(2) 1505-517 (2020)*





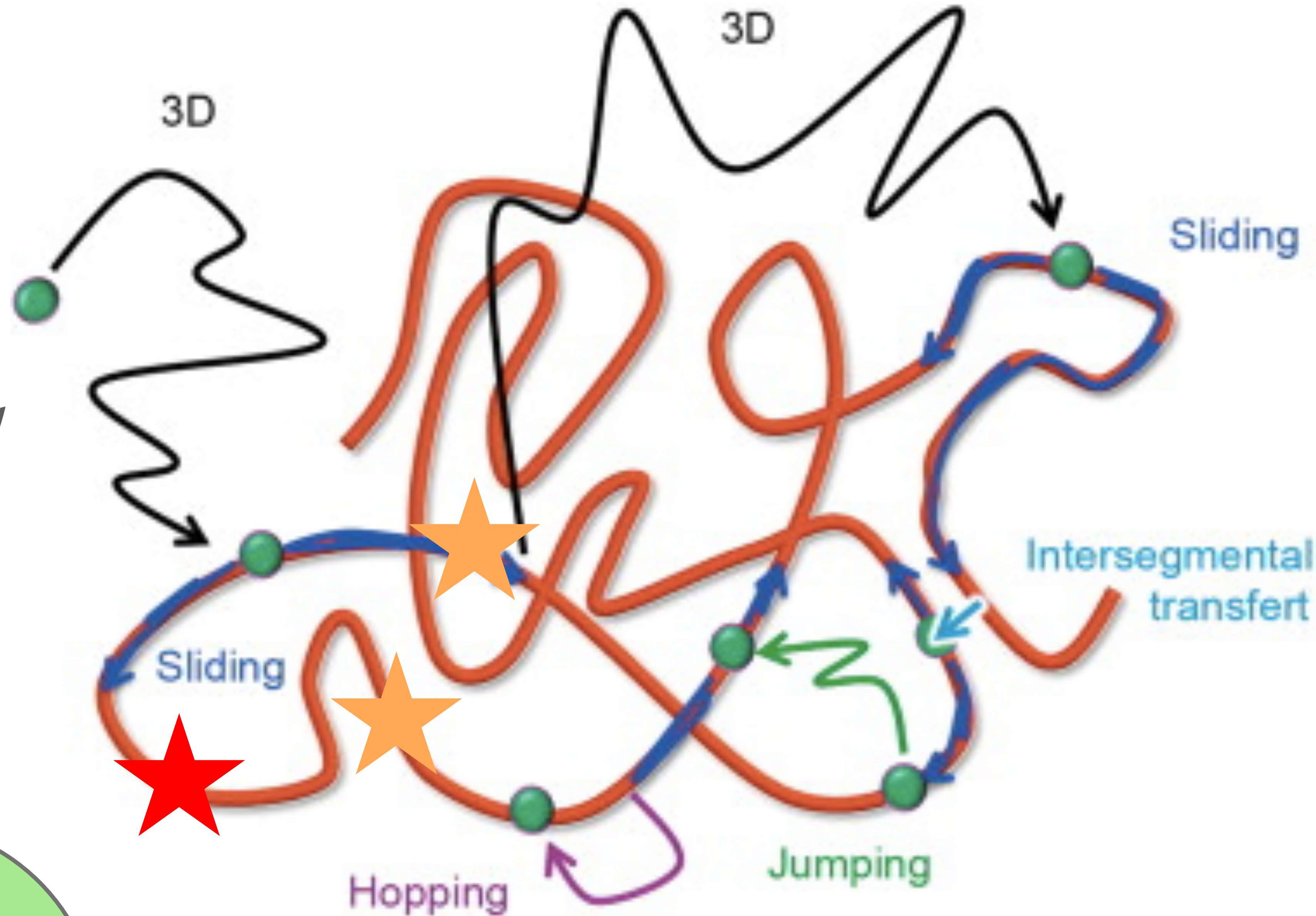


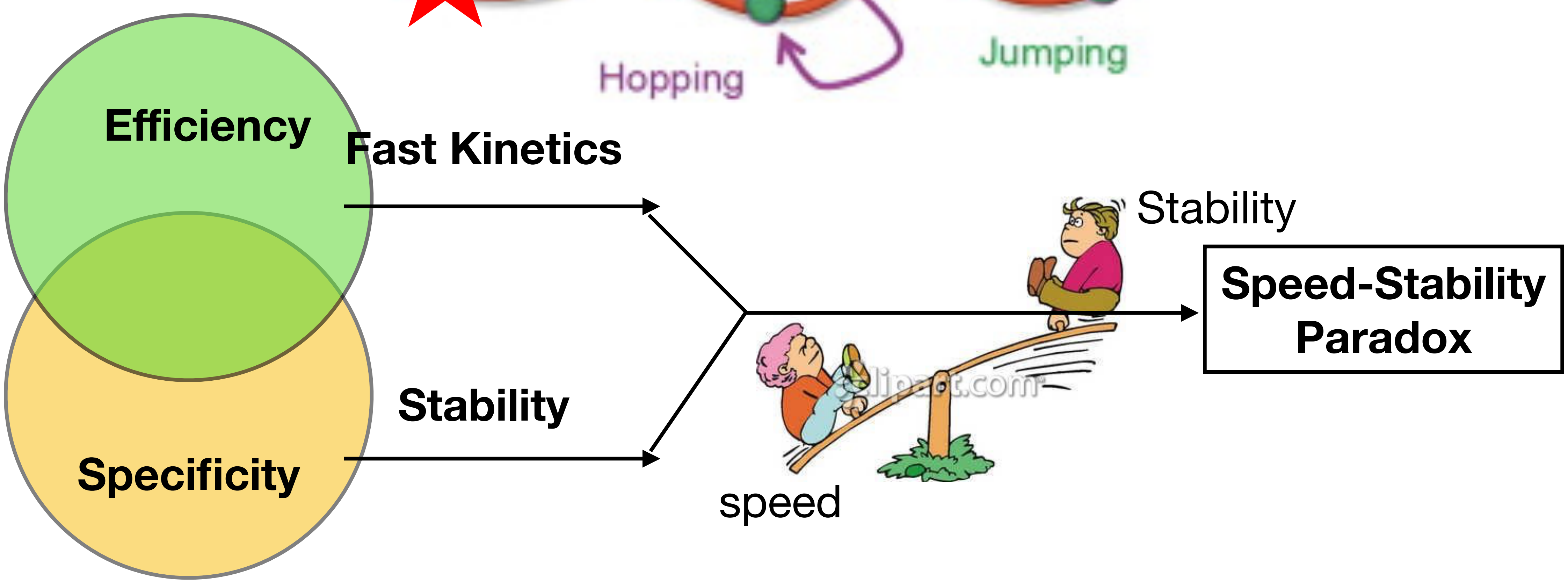
Dynamic DNA binding licenses a repair factor to bypass roadblocks in search of DNA lesions, Finkelstein et. al. **Nature Communications**, 7: 10607, (2016)

Biophys J *118(2) 1505-517 (2020)*

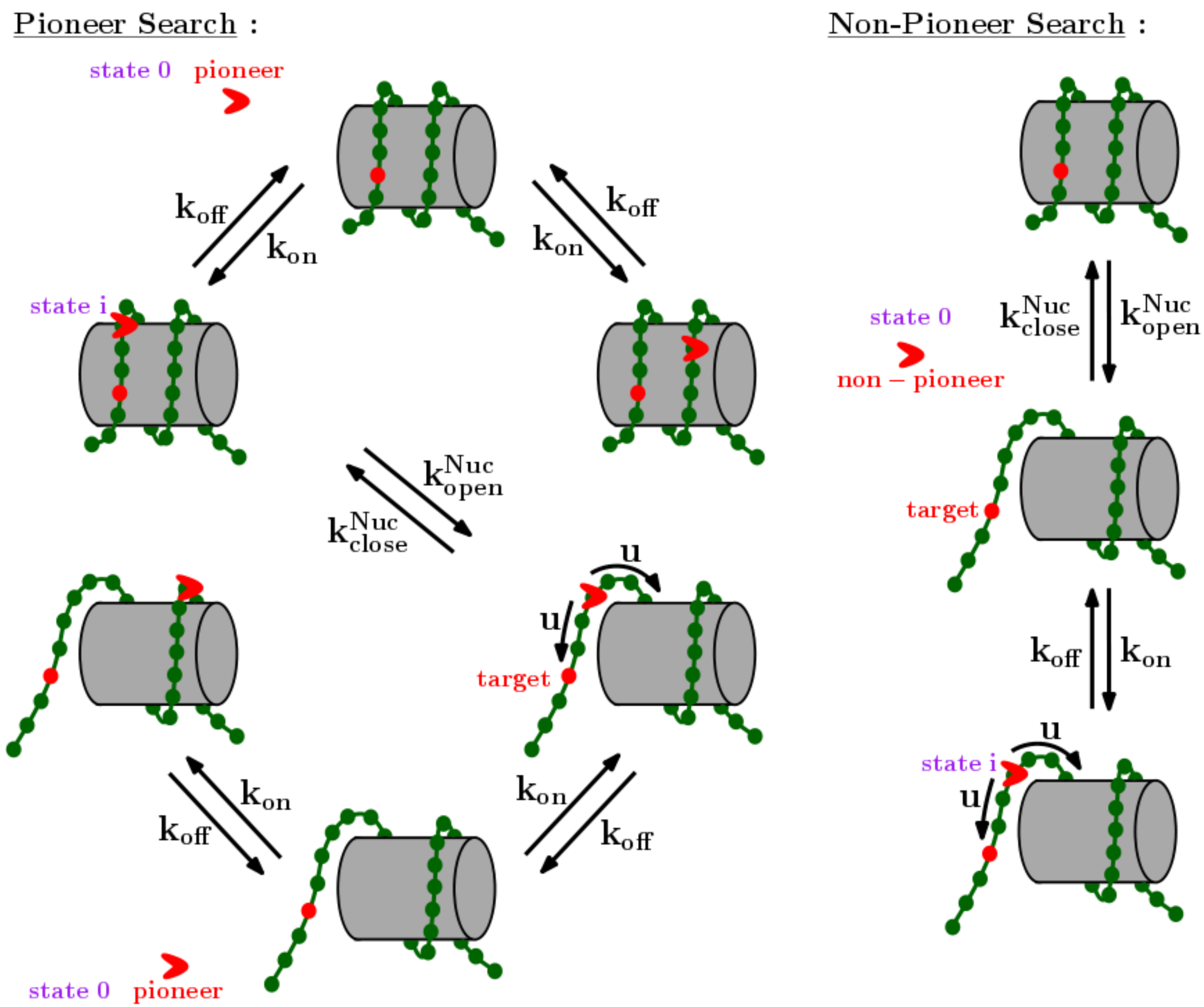
Slow down by Traffic Signal

Antenna Effect !





Does Speed always come at the cost of Stability?
How Protein searches on Nucleosomal DNA



$$\frac{dP_n^{FWNuc}(t)}{dt} = k_{off}P_0(t) + k_{open}^{Nuc}P_n^{PUNuc}(t) - \left(k_{off} + k_{open}^{Nuc}\right)P_n^{FWNuc}(t), \qquad 1 \leqslant n \leqslant l$$

$$\frac{dP_n^{FWNuc}(t)}{dt} = k_{off}P_0(t) - k_{off}P_n^{FWNuc}(t), \qquad l+1 \leqslant n \leqslant L$$

$$\frac{dP_n^{PUNuc}(t)}{dt} = u\left[P_{n-1}^{PUNuc}(t) + P_{n+1}^{PUNuc}(t)\right] + k_{off}P_0(t) + k_{close}^{Nuc}P_n^{FWNuc}(t) - \left(2u + k_{off} + k_{close}^{Nuc}\right)P_n^{PUNuc}(t), \quad 1 < n < l$$

$$\frac{dP_n^{PUNuc}(t)}{dt} = k_{off}P_0(t) - k_{off}P_n^{PUNuc}(t), \qquad l+1 \leqslant n \leqslant L$$

$$\frac{dP_0(t)}{dt} = \frac{k_{on}}{L}\left[\sum_{n=1}^{L} P_n^{FWNuc}(t) + \sum_{n=1}^{l} P_n^{PUNuc}(t) + \sum_{n=l+1}^{L} P_n^{PUNuc}(t)\right] - 2k_{on}P_0(t)$$

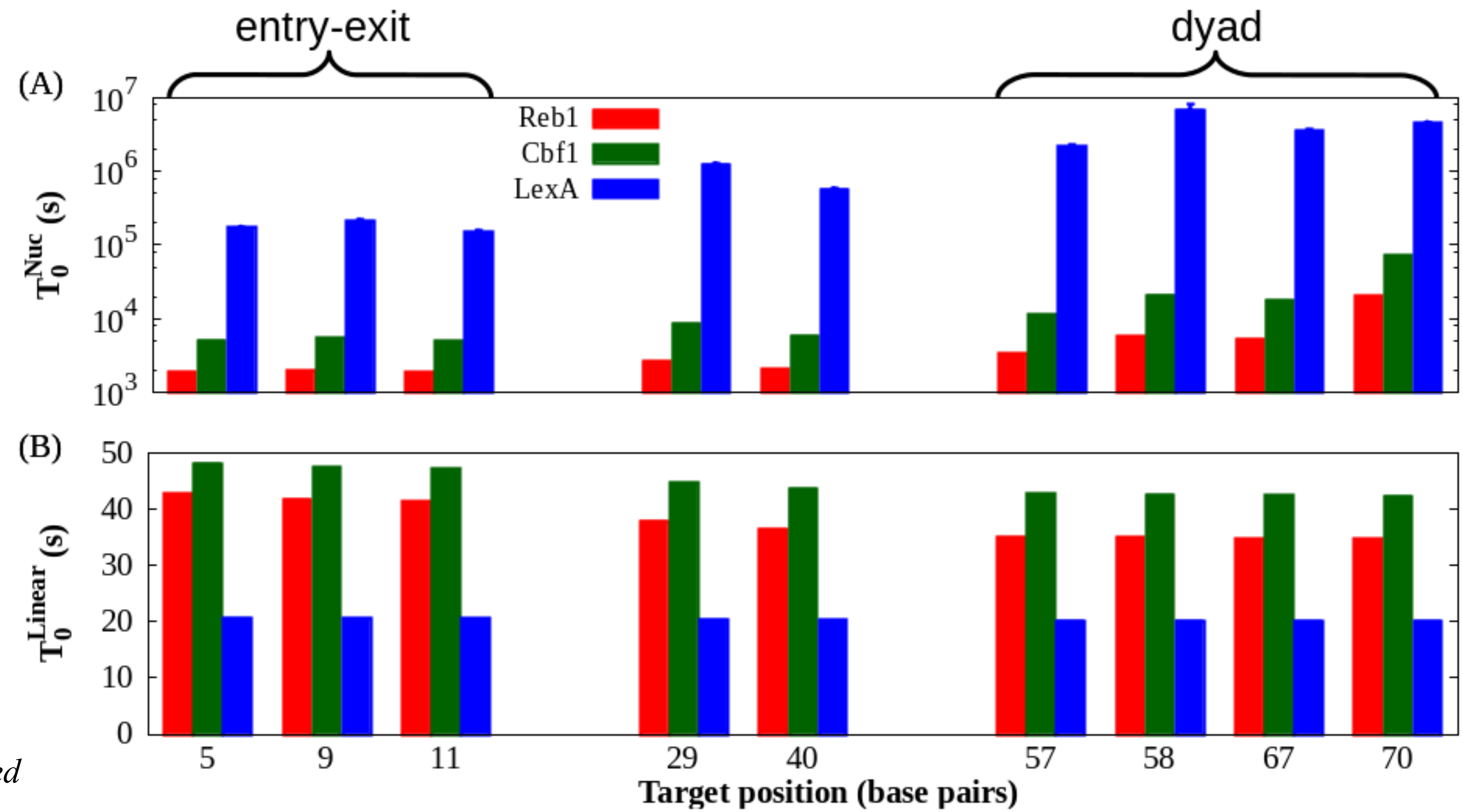
Boundary Conditions

$$\frac{dP_1^{PUNuc}(t)}{dt} = uP_2^{PUNuc}(t) + k_{off}P_0(t) + k_{close}^{Nuc}P_1^{FWNuc}(t) - \left(u + k_{off} + k_{close}^{Nuc}\right)P_1^{PUNuc}(t)$$

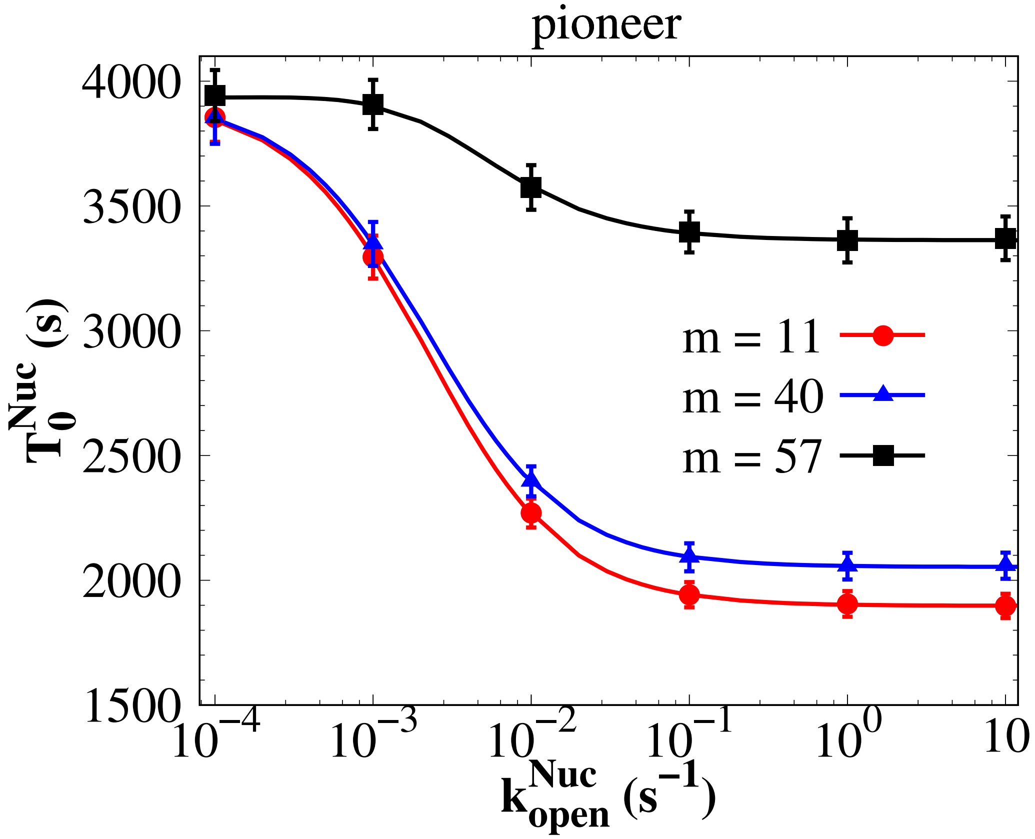
$$\frac{dP_l^{PUNuc}(t)}{dt} = uP_{l-1}^{PUNuc}(t) + k_{off}P_0(t) + k_{close}^{Nuc}P_l^{FWNuc}(t) - \left(u + k_{off} + k_{close}^{Nuc}\right)P_l^{PUNuc}(t)$$

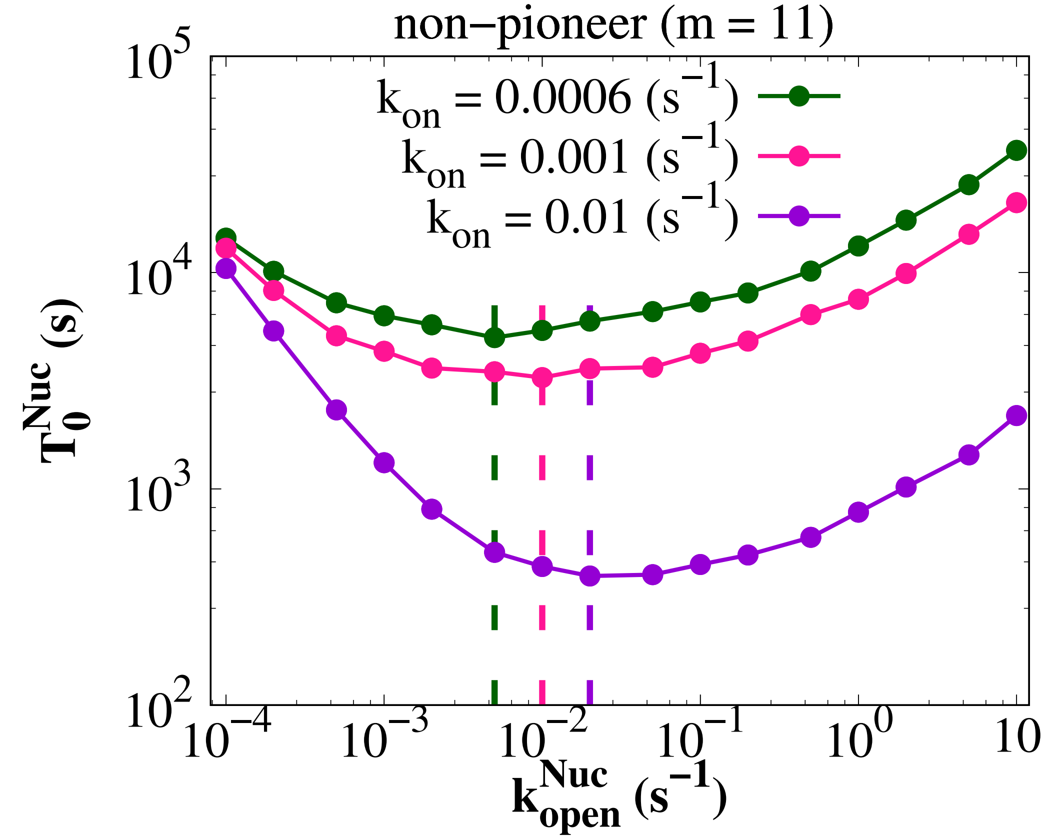
$$k_{D(nuc)}^{Reb1} < k_{D(nuc)}^{Cbf1} < k_{D(nuc)}^{LexA}$$

Transcription Factors	k_{off}^{Nuc} (s^{-1})	k_{off}^{Linear} (s^{-1})	k_{on}^{Nuc} ($s^{-1}nM^{-1}$)	k_{on}^{Linear} ($s^{-1}nM^{-1}$)
Reb1 (pioneer)	(4.4 ± 0.5) x 10^{-3}	0.58 ± 0.08	(6 ± 1) x 10^{-4}	(3.2 ± 0.3) x 10^{-2}
Cbf1 (pioneer)	(1.1 ± 0.1) x 10^{-2}	0.30 ± 0.05	(2.1 ± 0.2) x 10^{-4}	(2.5 ± 0.6) x 10^{-2}
LexA (non-pioneer)	3.3 ± 0.6	(3.4 ± 0.2) x 10^{-3}	(9 ± 2) x 10^{-5}	(5 ± 1) x 10^{-2}

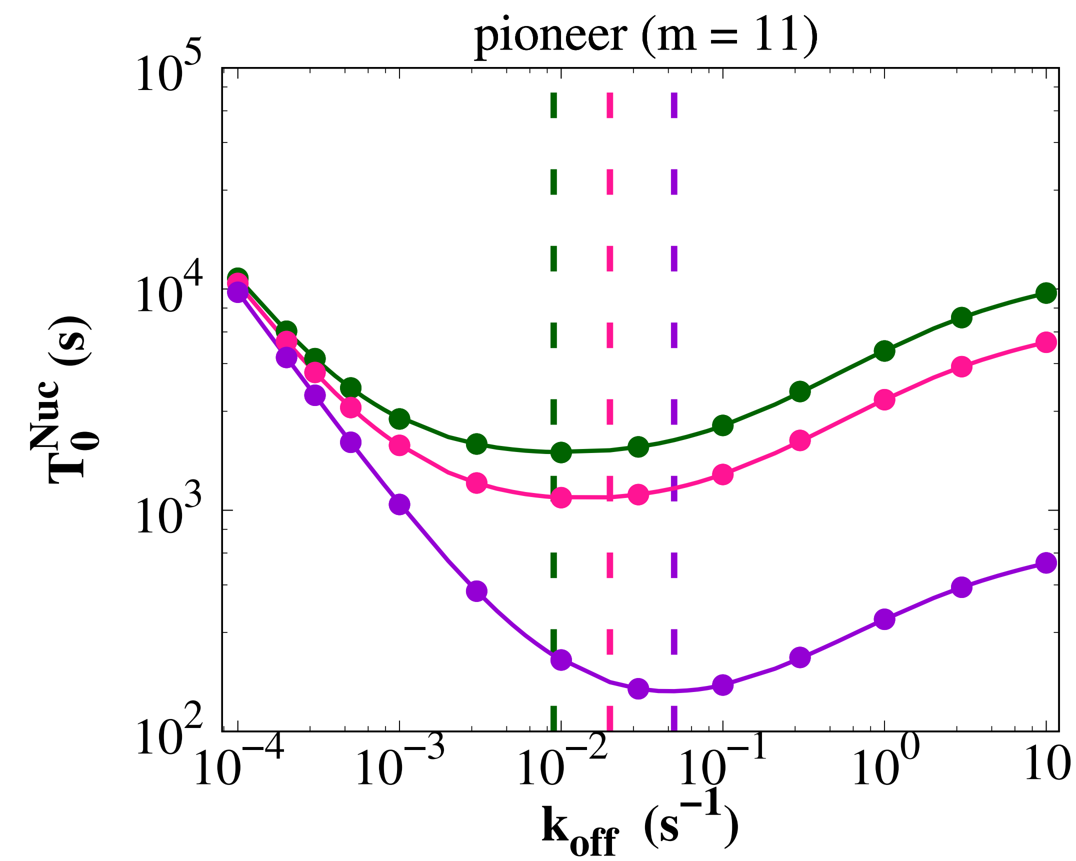


Submitted

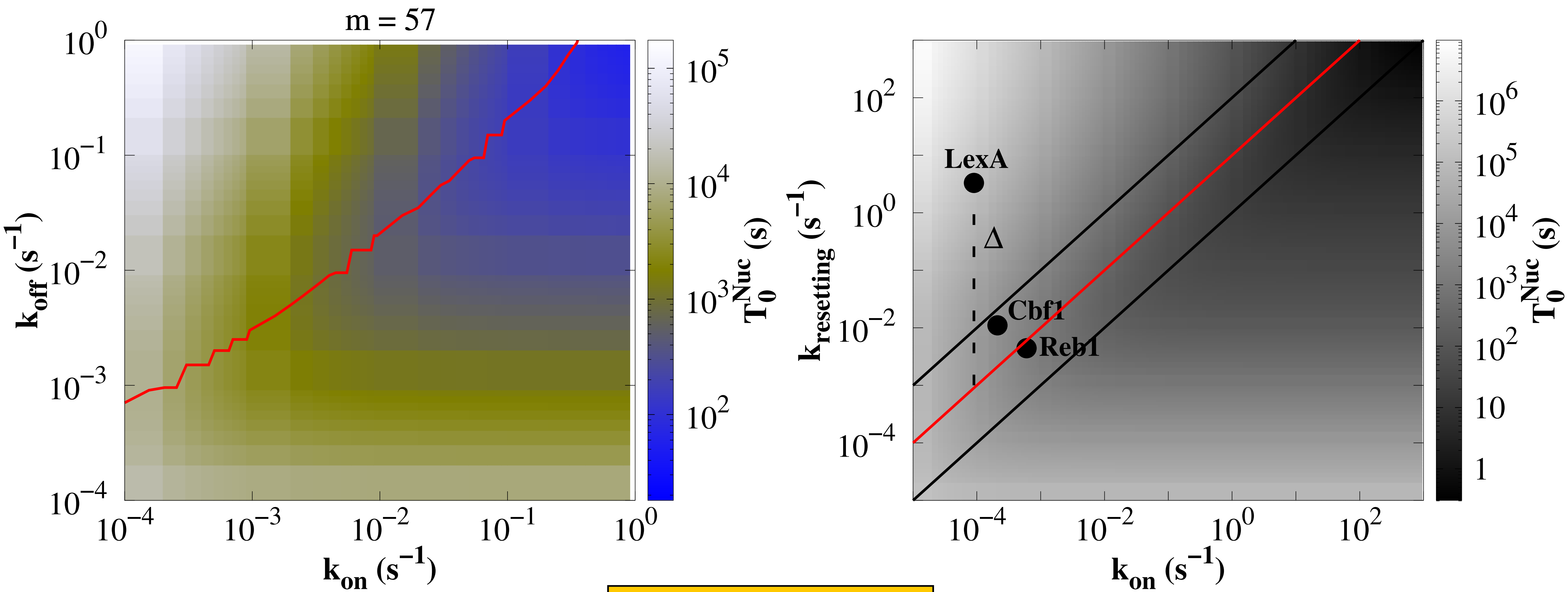




Optimal Error Correction !



Submitted



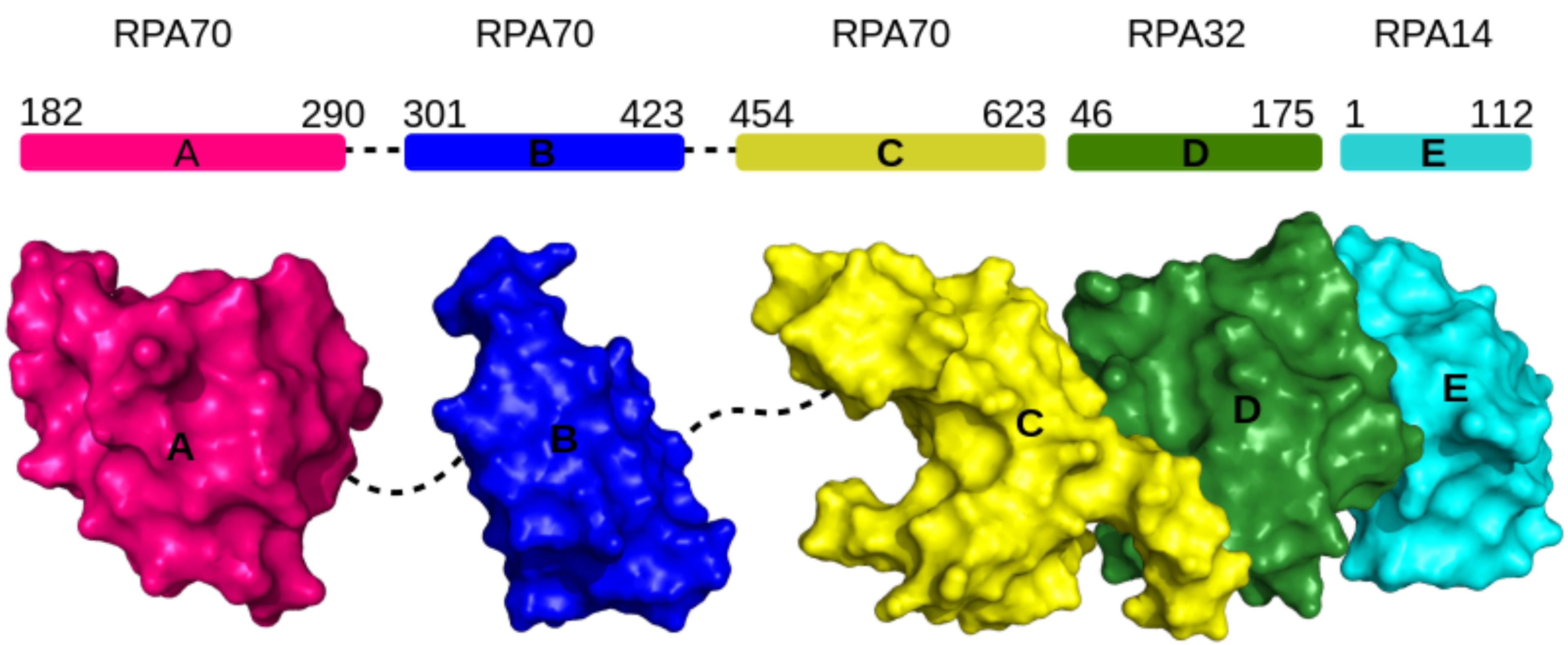
Conclusions

- *High binding affinity of proteins to DNA may not be a retarding factor, rather on nucleosomal DNA it helps pioneer factors to minimise the impact of nucleosome dynamics.*

- *Fastest transport to target DNA site is observed when nucleosome association and dissociation rates of a protein are comparable. The 'dissociation-compensated-association' ensures tradeoff between nuclear mobility and error in search process.*

Submitted

Changing the Path : TRANSPORT of Protein on ssDNA track



ssDNA length dependent RPA activity

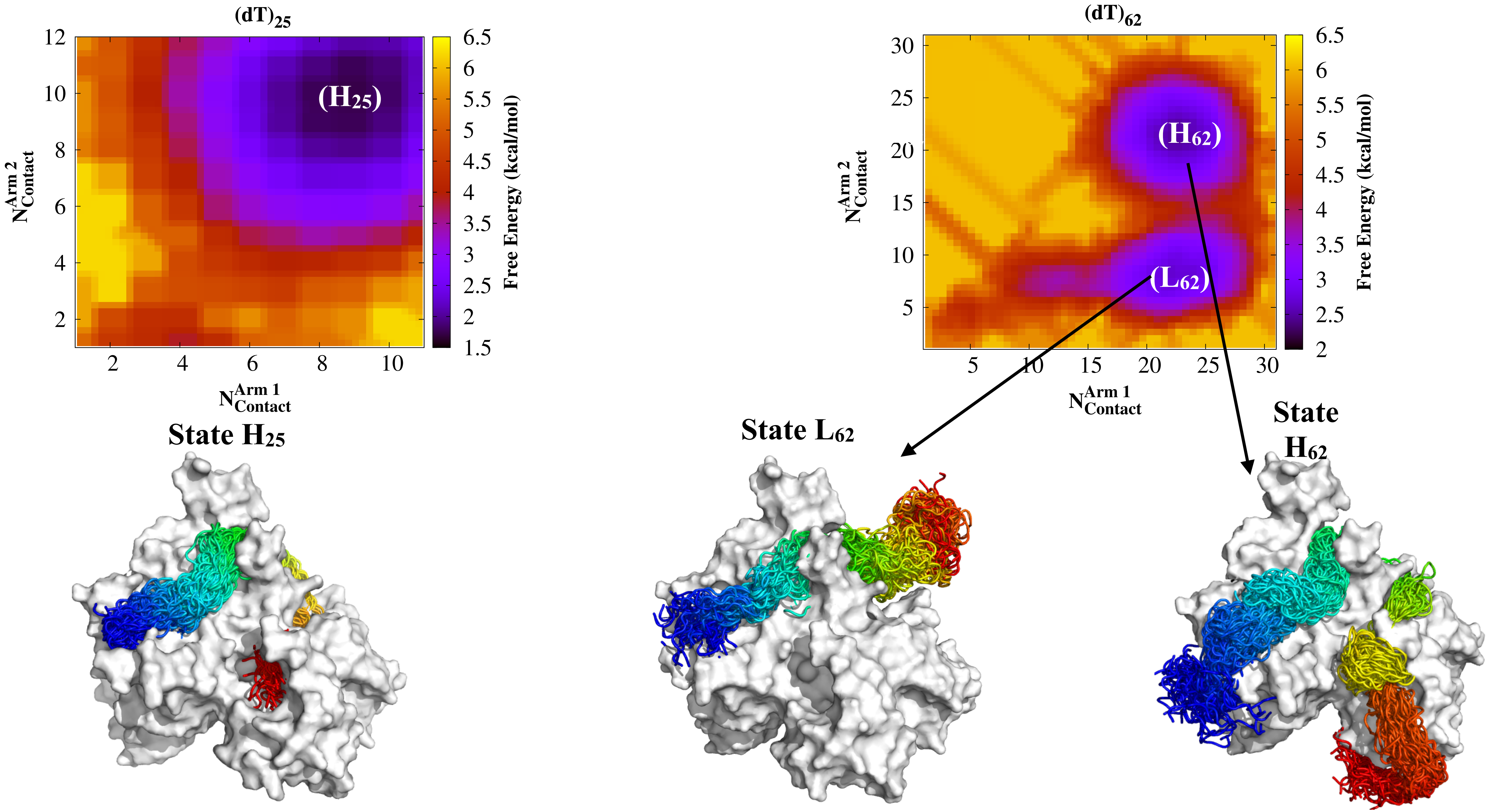
✦ **Long but short-lived ssDNA intermediate binds to RPA during DNA replication.**

✦ **Shorter ssDNA intermediates (<30 nt) form a stable RPA-ssDNA complex for processing and repairing the DNA damage.**

TRANSPORT of RPA Protein on ssDNA

$\Delta G_{H_{25}-H_{62}} = 0.46 \pm 0.001\ kcal/mol$

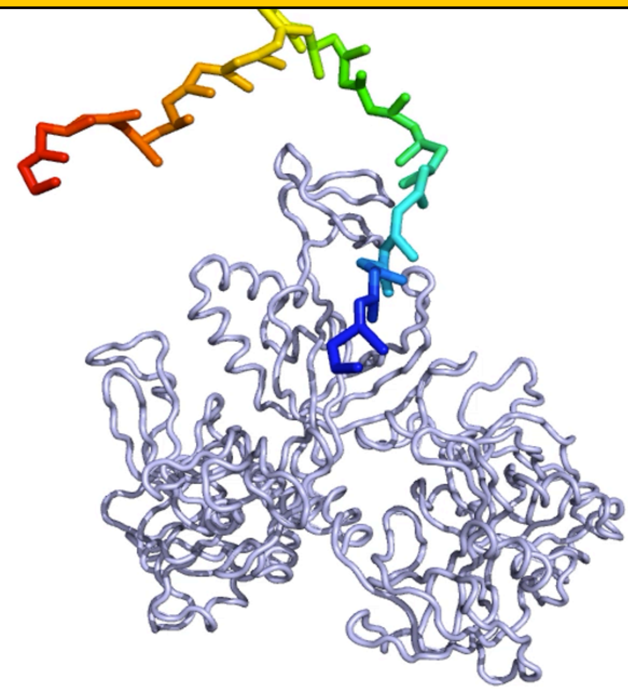
$\Delta G_{H_{62}-L_{62}} = 0.7 \pm 0.001\ kcal/mol$

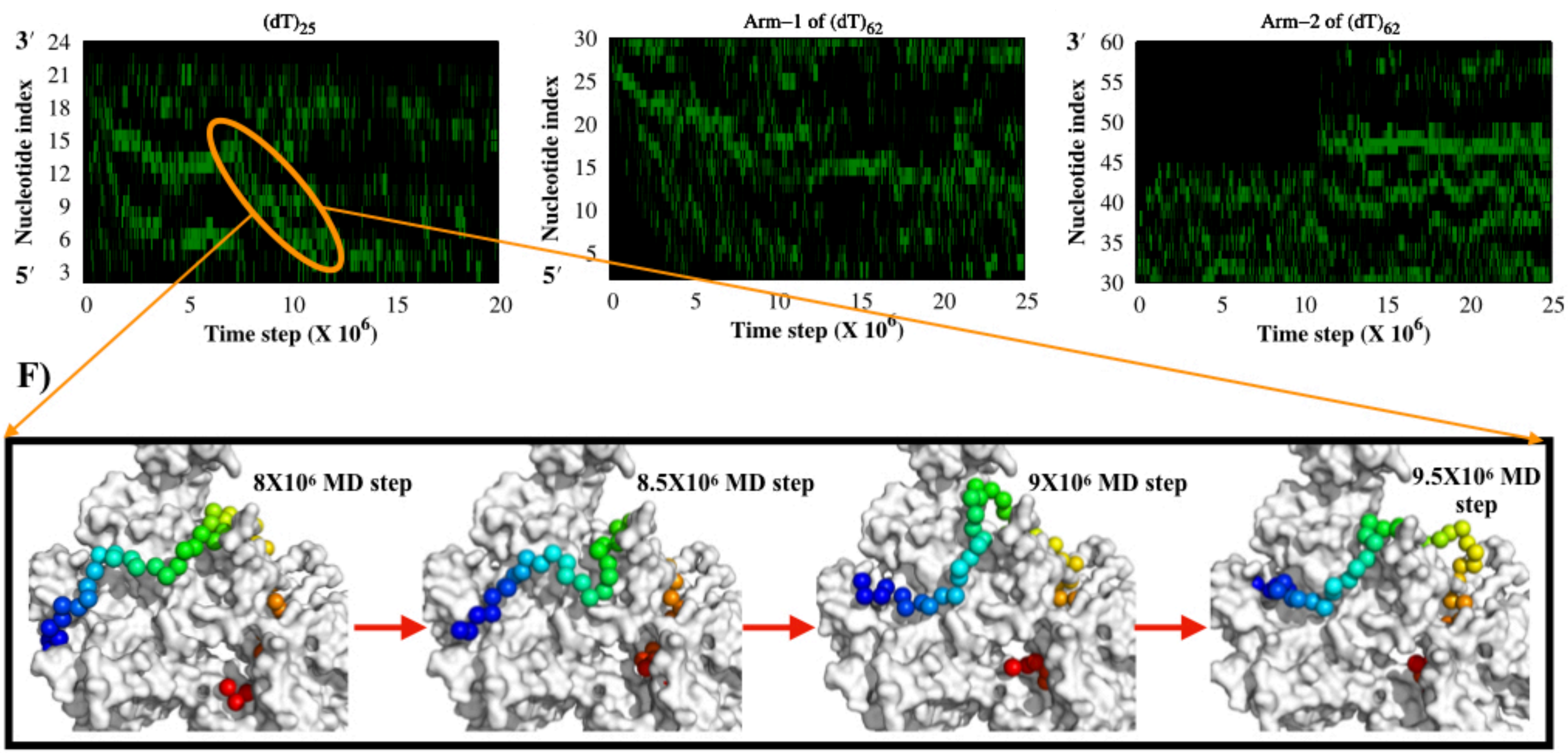


Journal Chemical Information and Modelling 60(20):5057-5069, 2020

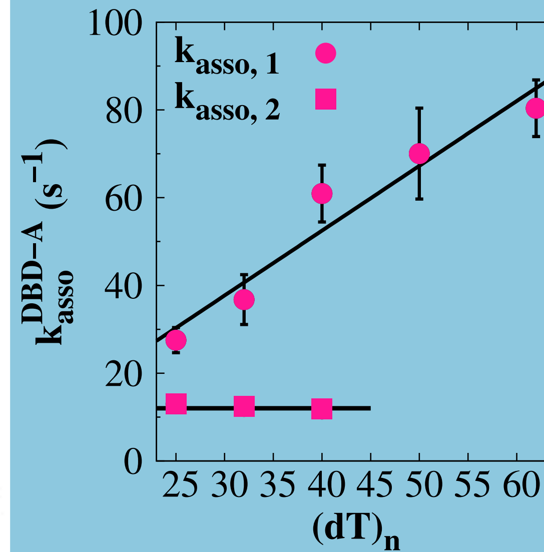
TRANSPORT of RPA Protein on ssDNA

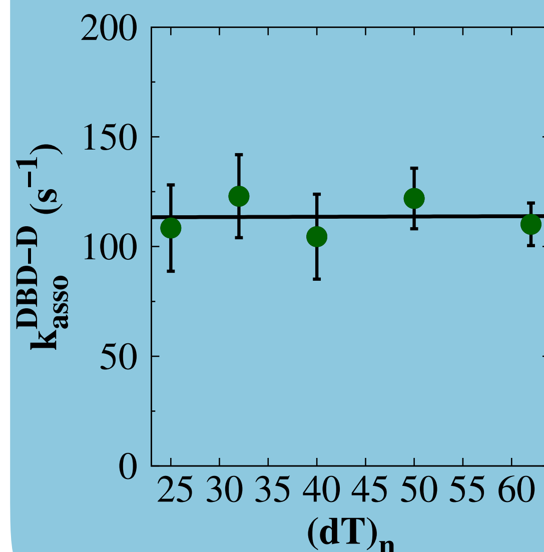
RPA-dT₂₅





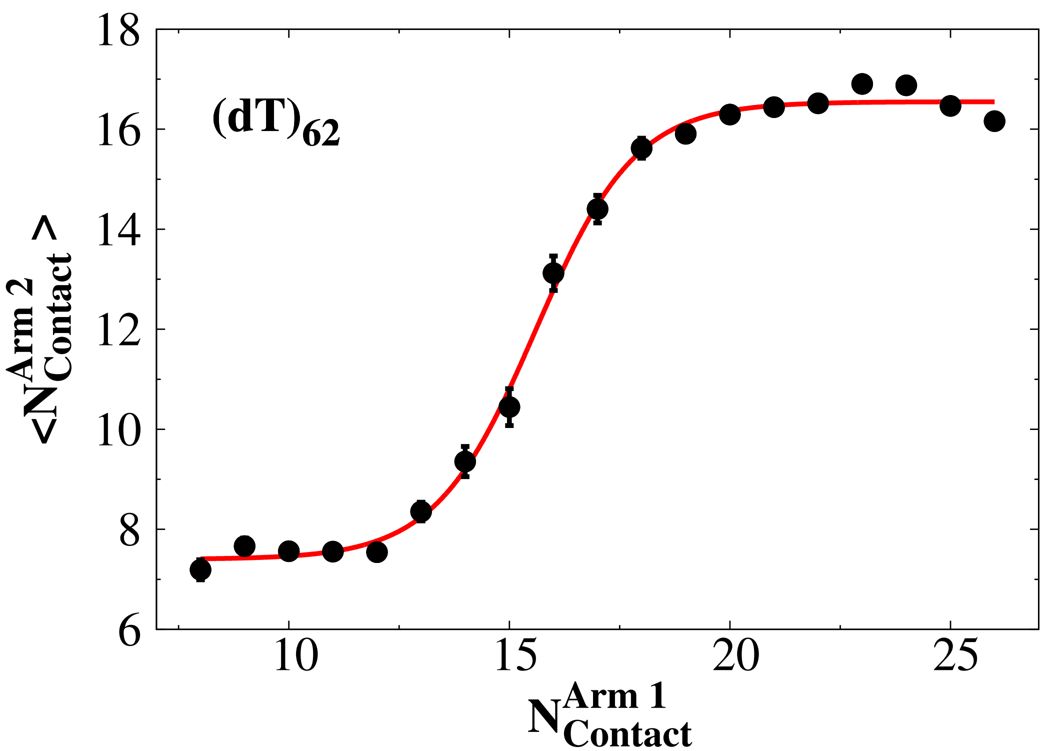
BUT !

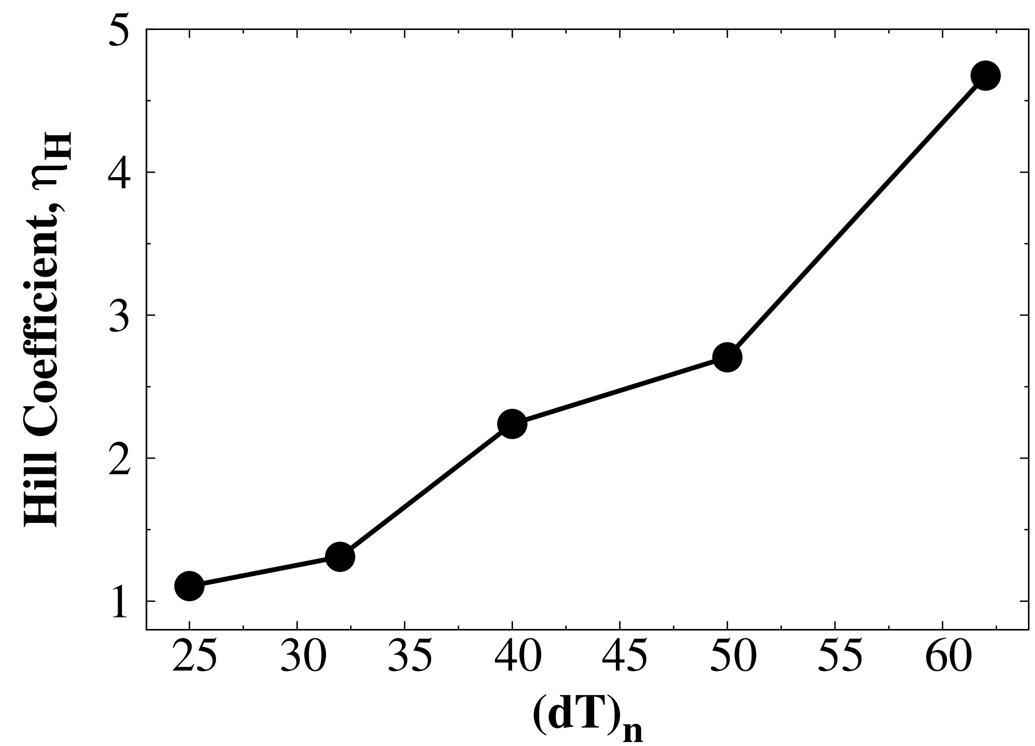




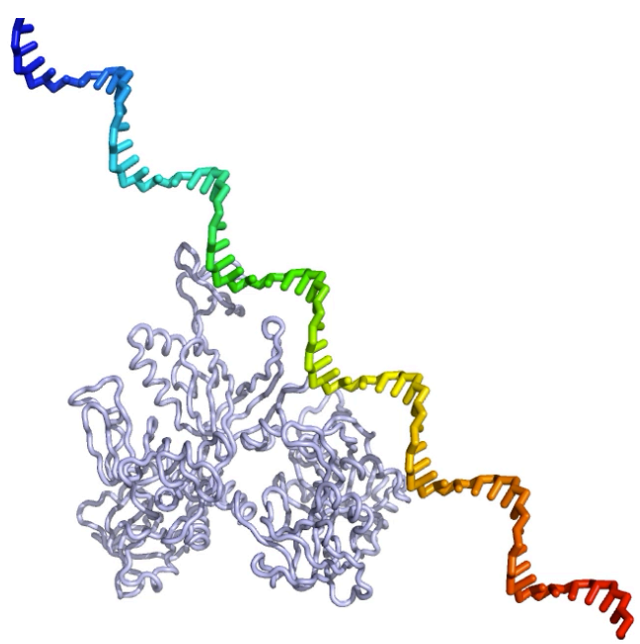
Journal Chemical Information and Modelling 60(20):5057-5069, 2020

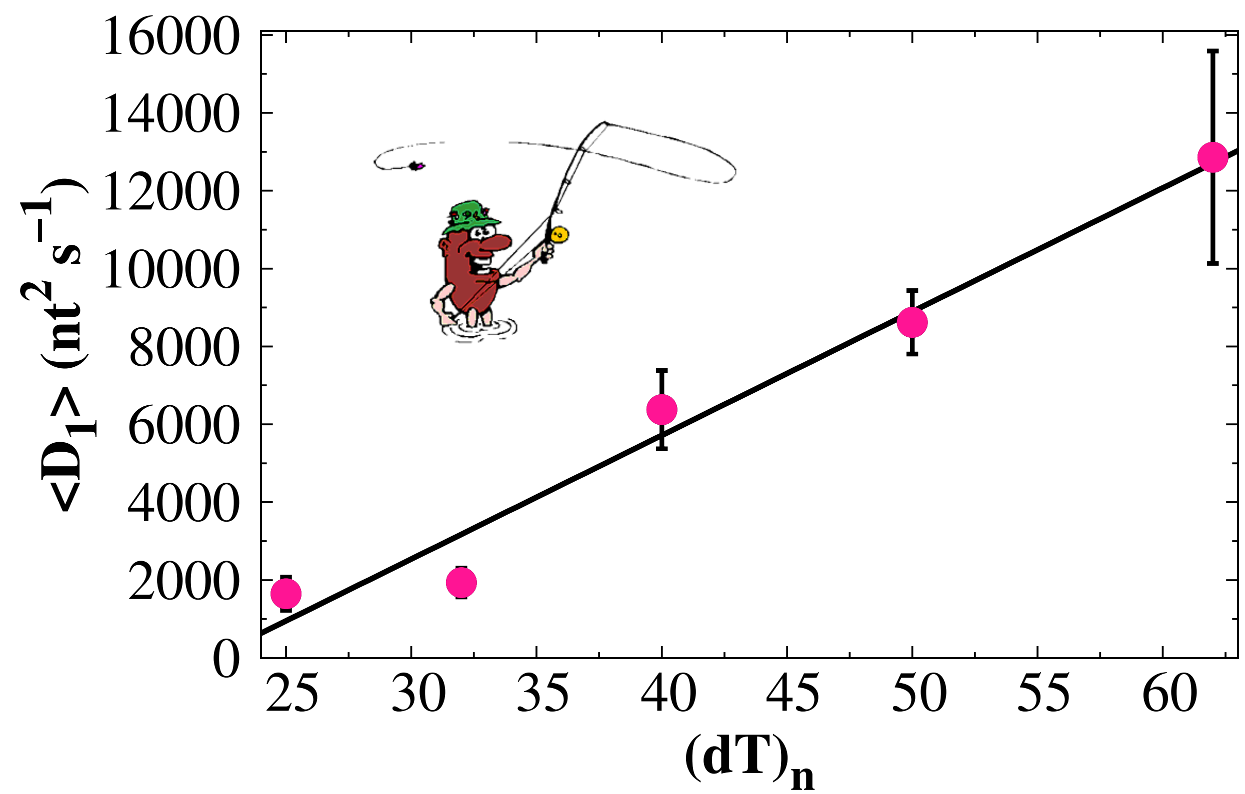
Cooperative Binding of ssDNA to RPA





RPA-dT62





Journal Chemical Information and Modelling 60(20):5057-5069, 2020

Conclusions

- The mechanism of binding of RPA to ssDNA differs with the length of ssDNA. The short length of ssDNA binds to RPA through 'reptation dynamics', where dynamic bulges on ssDNA form and dissolute continuously. In contrast, longer ssDNA binds to RPA in a cooperative fashion.

- The cooperative binding of ssDNA to RPA involves a conformational change from a stable ‘linear’ intermediate to a 'horse-shoe' shaped final state.

- The presence of these two distinct binding modes are connected via a dynamic equilibrium. The relative population of these states is a function of ssDNA length.

- RPA associates more strongly with short length of ssDNA compared to long ssDNA.

- The kinetic association for longer ssDNA is much faster compared to shorter one.

PhD Students:

Anupam Mondal

Pinky Dey

Sujeet Mishra

Sangeeta

Enjoying our journey…..

Funding:

DST SERB

THANK YOU