

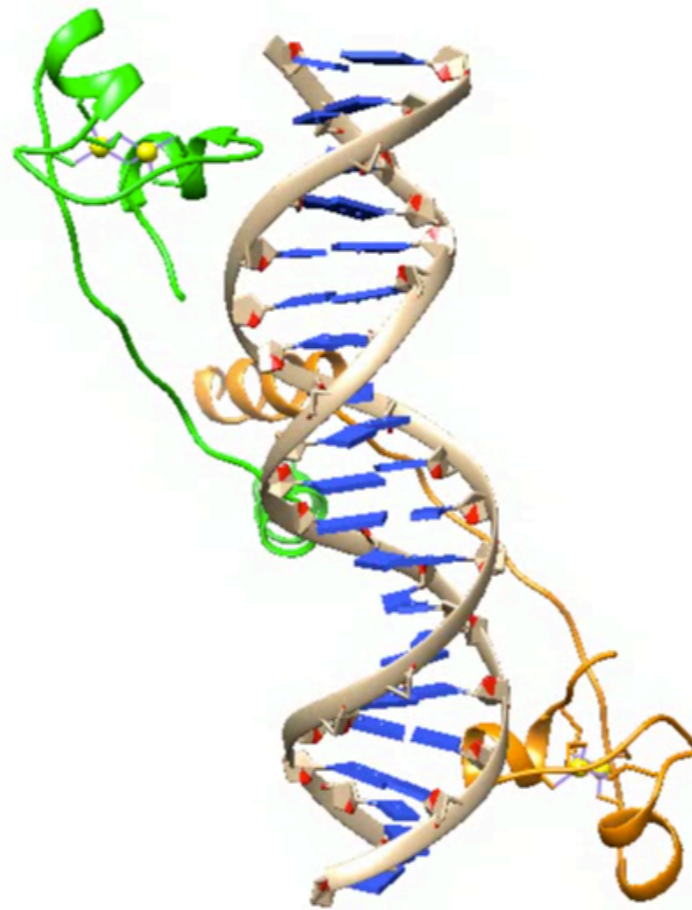


# Understanding Protein Transport on DNA Track



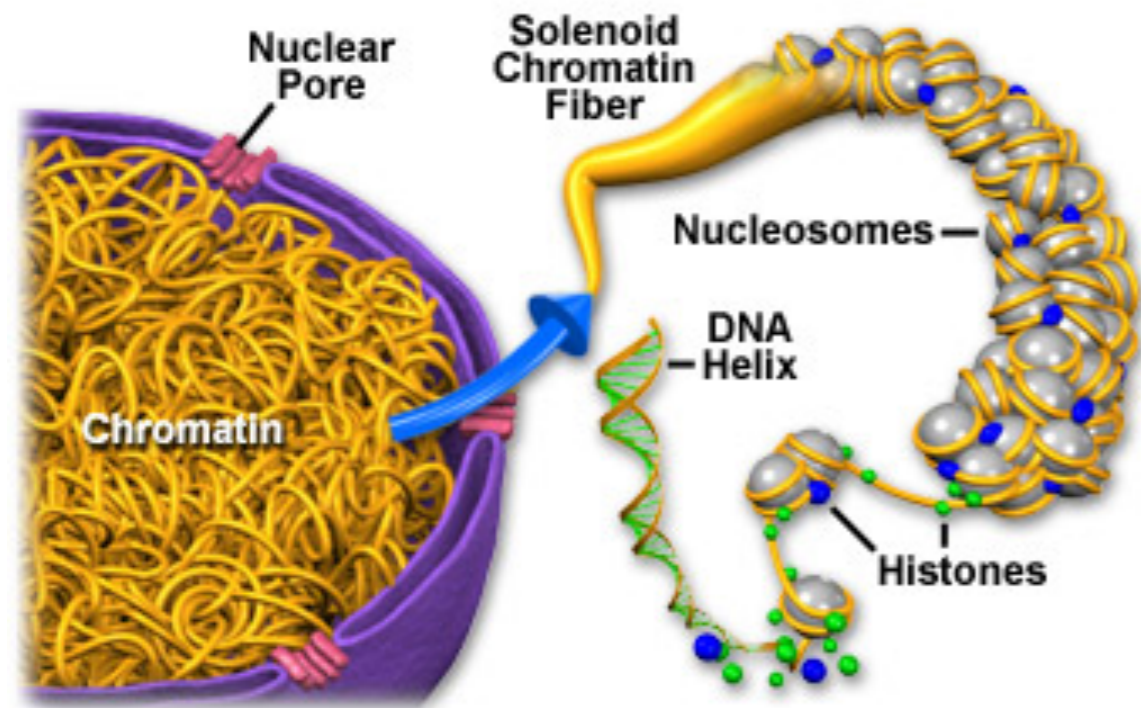
**Arnab Bhattacharjee**  
Associate Professor  
SCIS, JNU

# *Specific Protein-DNA Complex*



*Journey is often more beautiful than the destination....*

# TRANSPORT and TRAFFIC

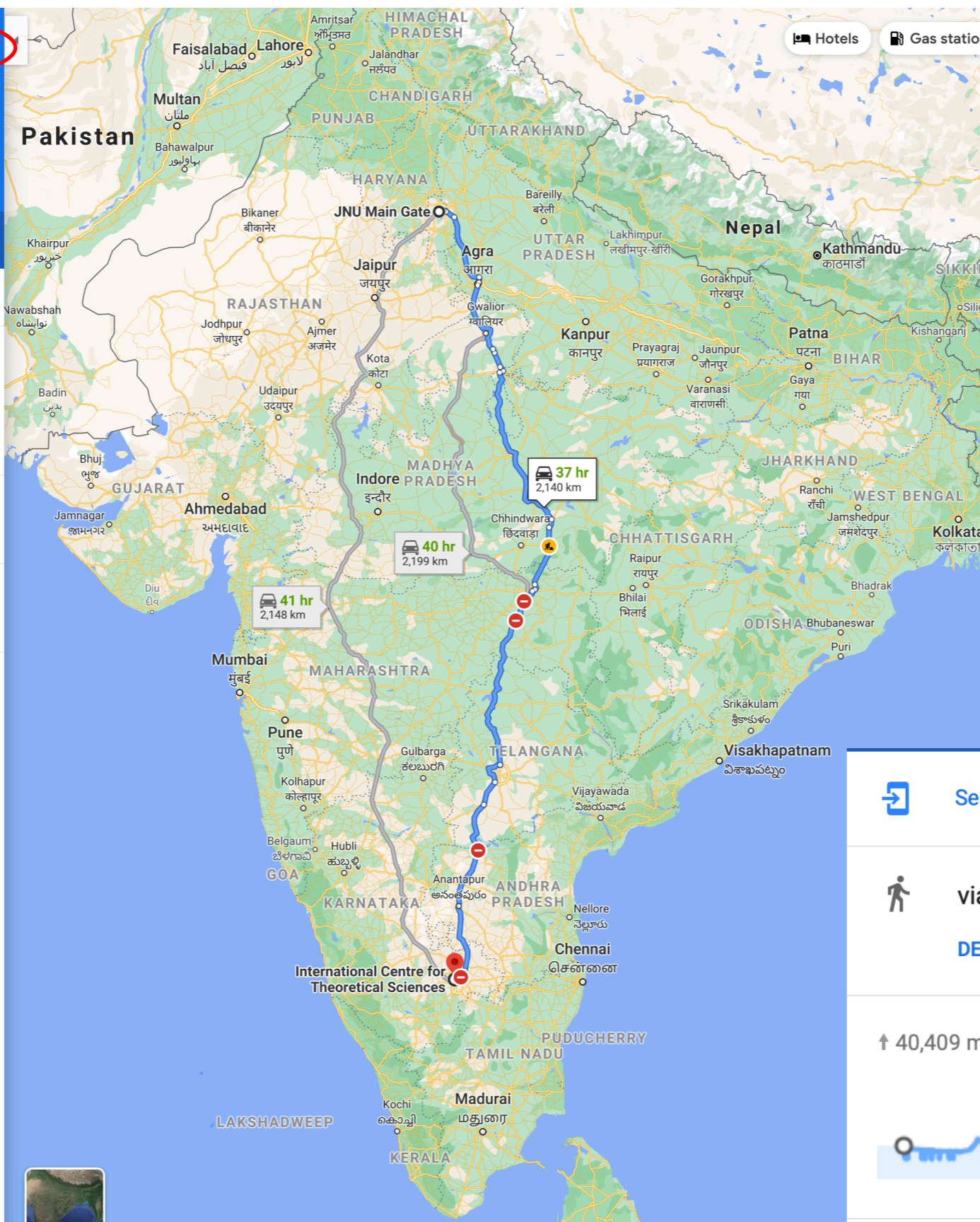


How Do Proteins  
Manage to move ?



# How Do We Manage It ?

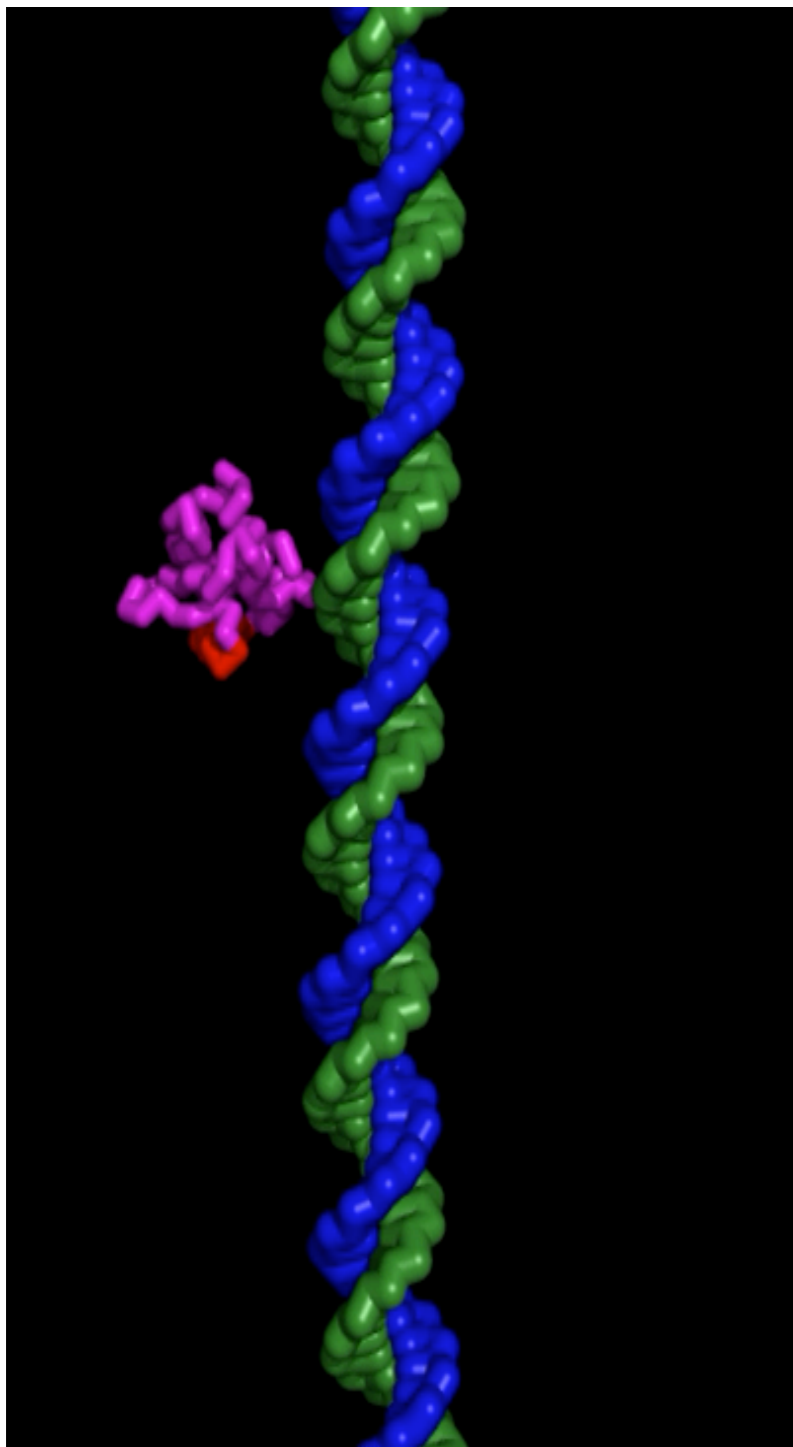
Navigation app interface showing transport mode icons (car, train, walking, bicycle, airplane) and search results for routes from JNU Main Gate to International Centre for Theoretical Sciences.



Summary panel for the route via NH46, showing travel time of 403 hours and distance of 1,986 km. It also includes a profile view of the route showing elevation changes, with a maximum elevation of 902 m and a minimum elevation of 52 m.

- via NH 44** 37 hr  
Fastest route now, avoids road closures on NH 44  
2,140 km  
⚠️ This route has tolls.  
[DETAILS](#)
- via NH46 and NH 44** 40 hr  
2,199 km
- via NH52** 41 hr  
2,148 km
- 8:18 PM—1:51 PM (Wednesday)** 1 day 18 hr  
Karnataka COVID - 19 SF Special  
8:26 PM from Nelson Mandela Marg Crossing  
40 min  
[DETAILS](#)
- 8:00 PM—8:49 AM (Wednesday)** 1 day 13 hr  
Karnataka COVID - 19 SF Special  
New Delhi - KSR Bengaluru Covid - ...  
[DETAILS](#)
- 8:00 PM—10:19 AM (Wednesday)** 1 day 14 hr  
Karnataka COVID - 19 SF Special  
New Delhi - KSR Bengaluru Covid - ...  
[DETAILS](#)
- 8:00 PM—12:46 PM (Wednesday)** 1 day 17 hr  
[DETAILS](#)

Sliding Dynamics

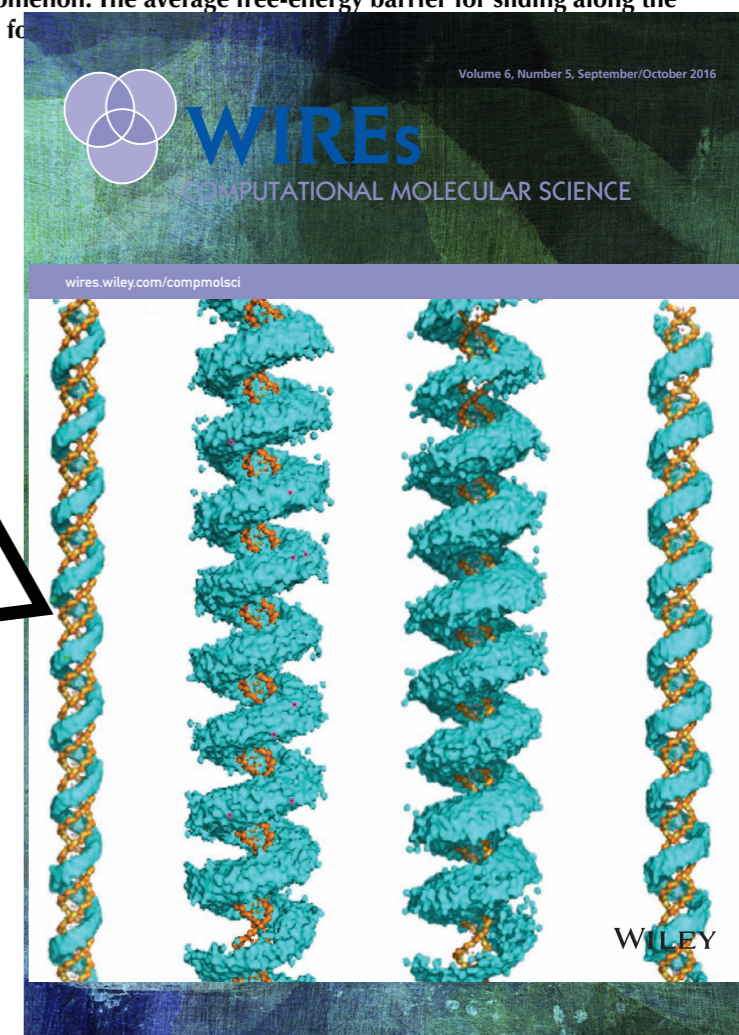


Nonspecifically bound proteins spin while diffusing along DNA

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

All rights reserved.

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  $1.1 \pm 0.2 k_B T$ . Such small barriers facilitate rapid search for



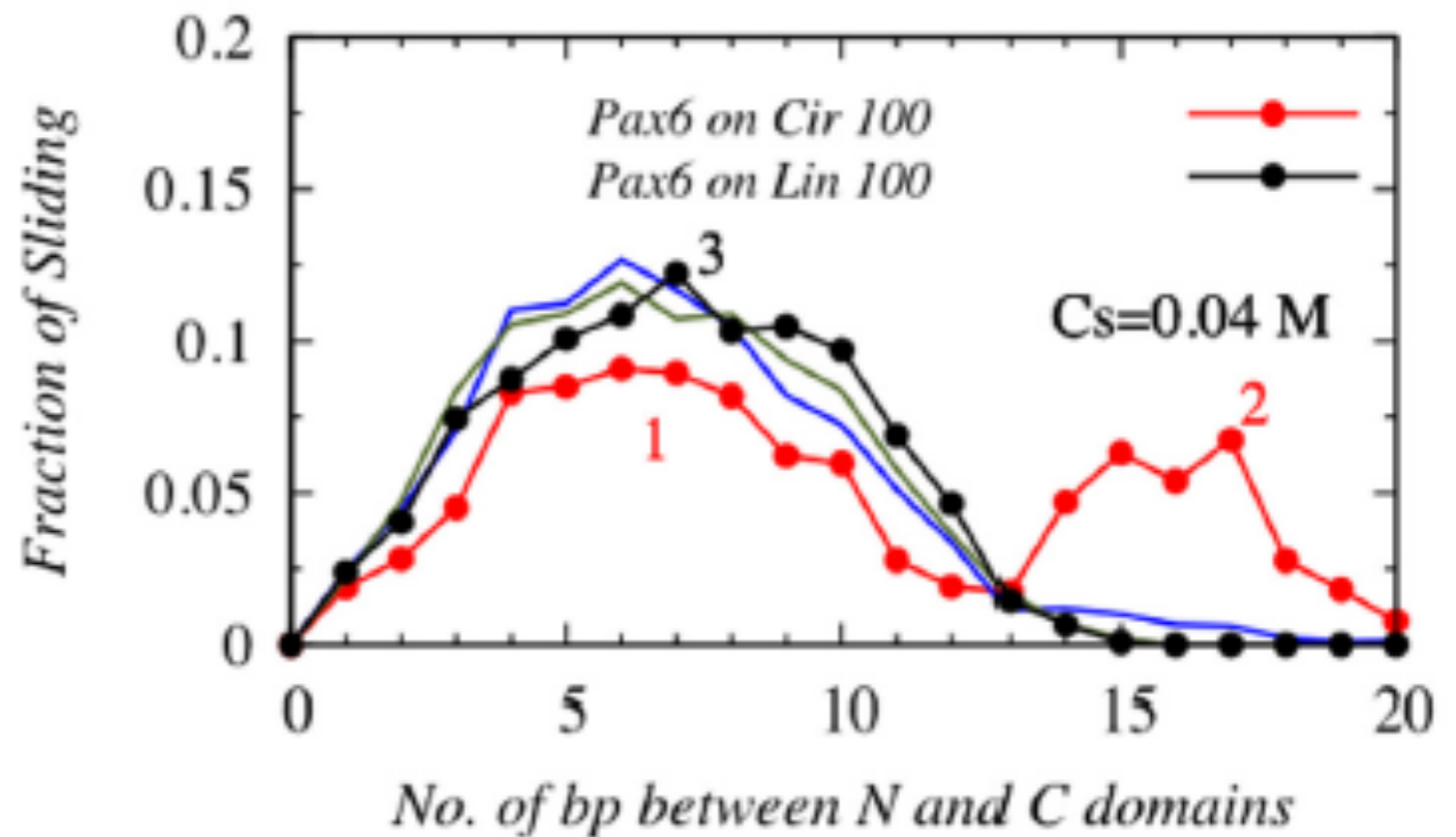
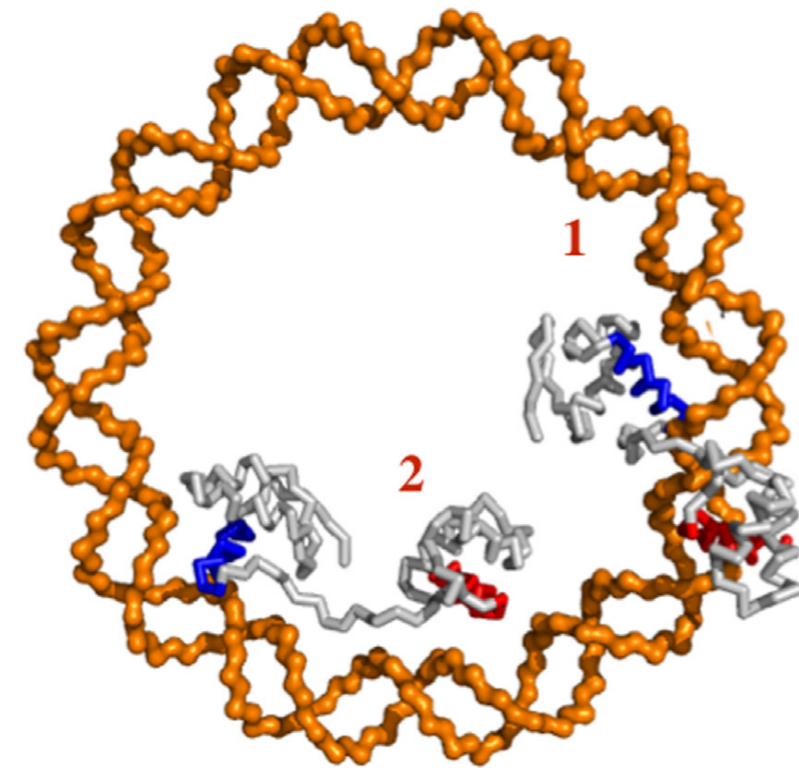
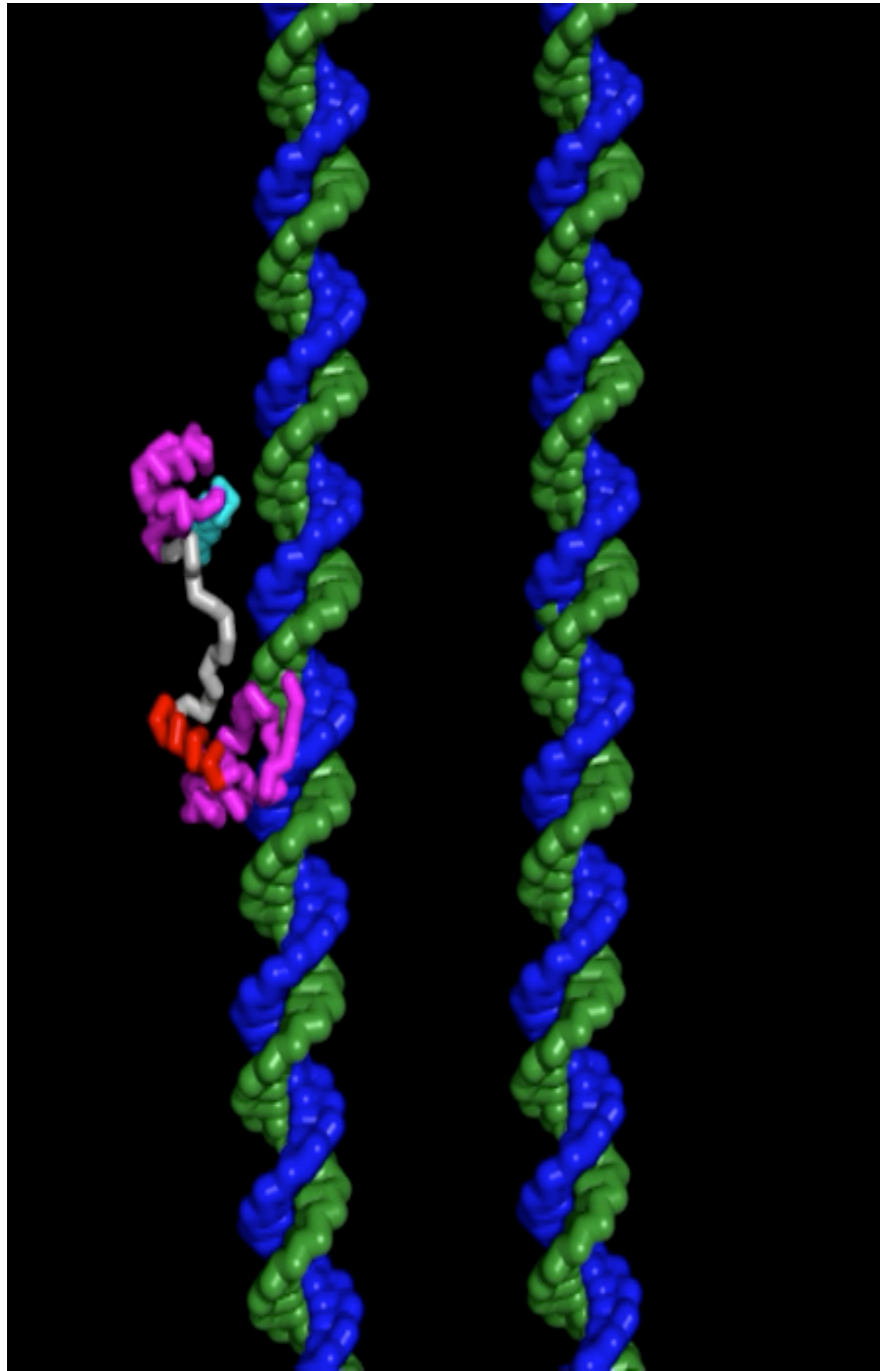
*WIREs Comp Mol Sci.* 6, 515, 2016

*Nucleic Acids Res* 43, 9176–9186, 2015

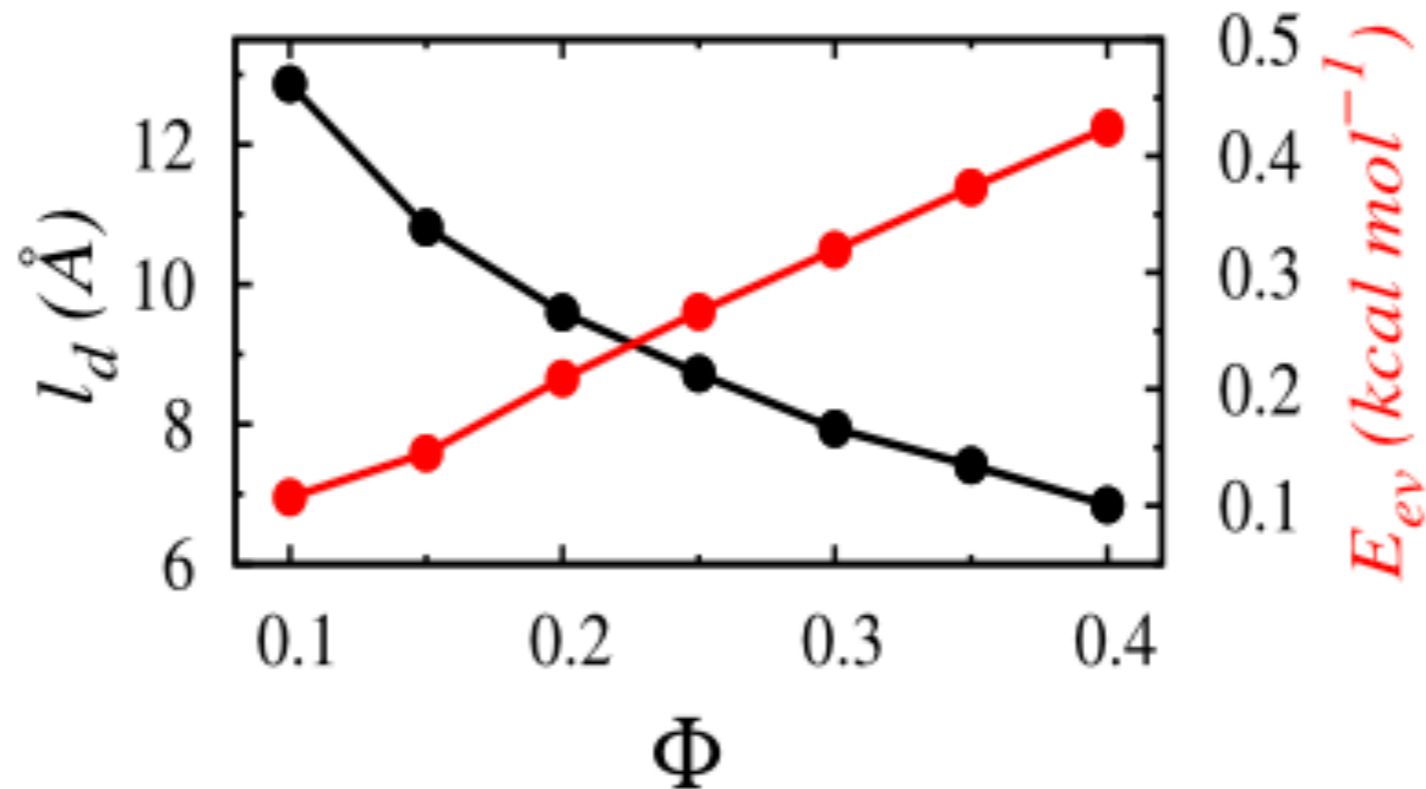
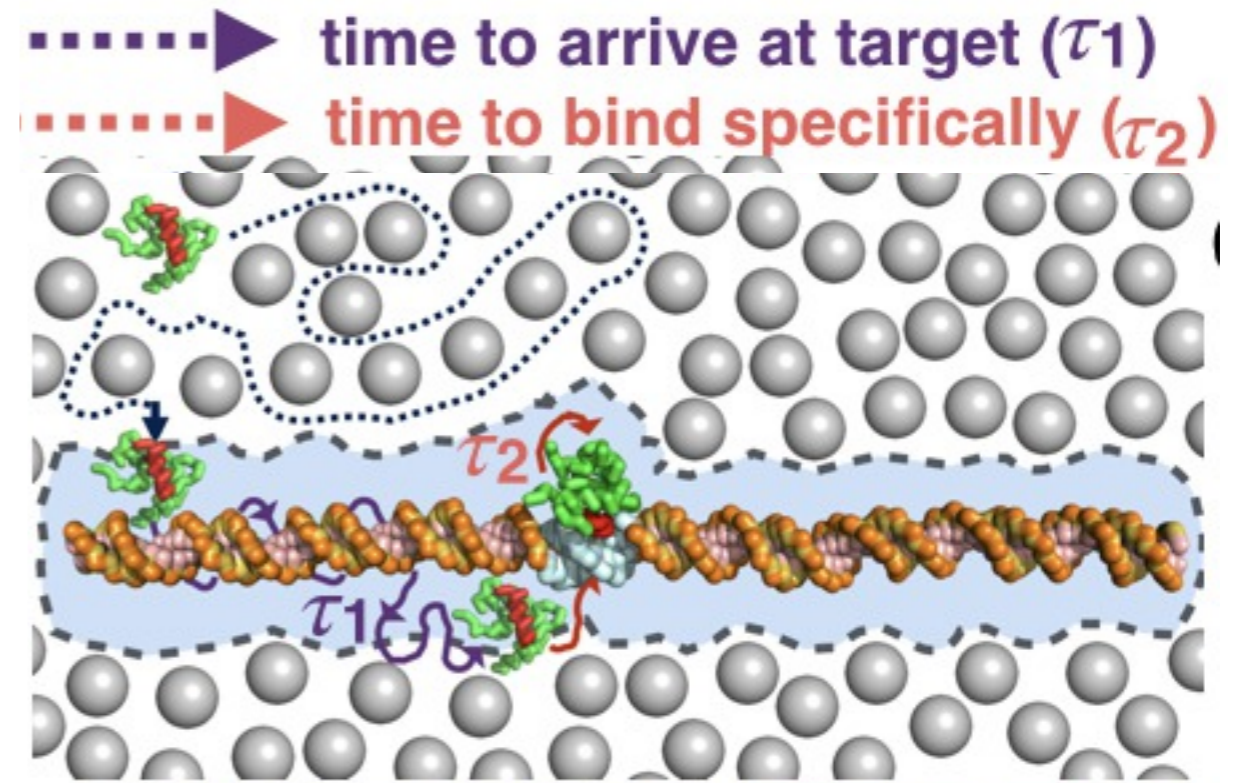
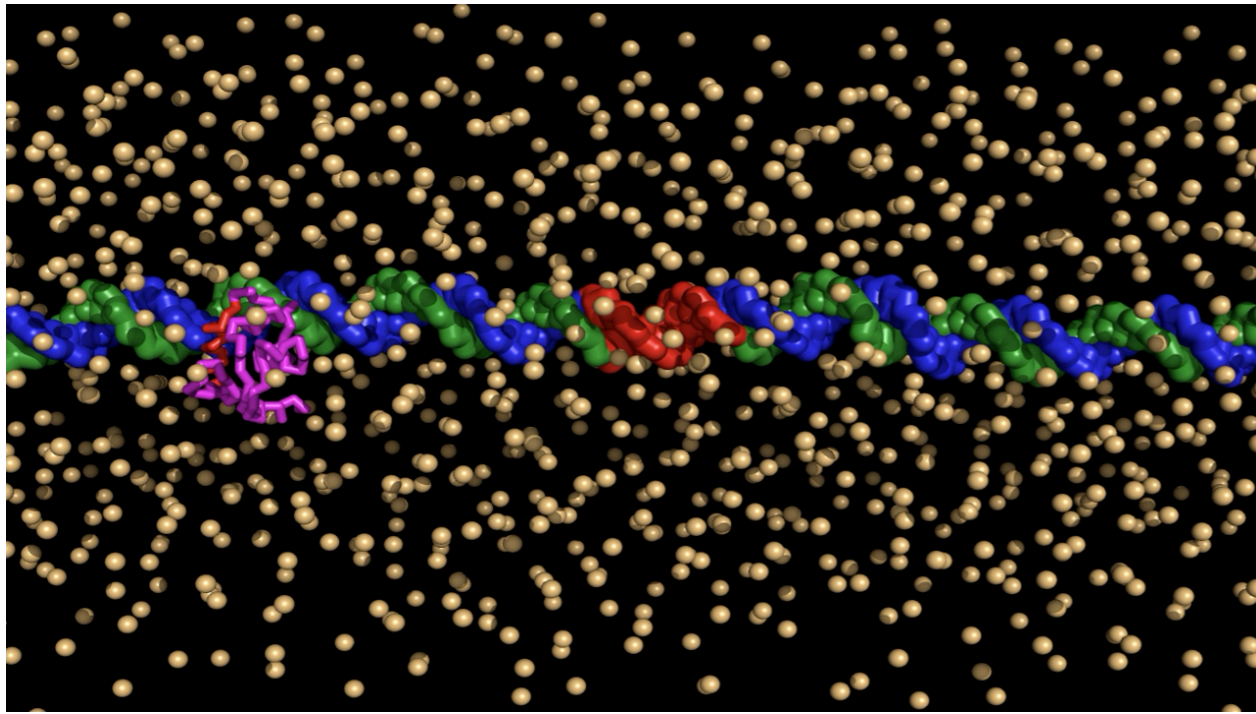
*Nucleic Acids Res* 42(20), 12404, 2014

# Protein also takes Shortcuts

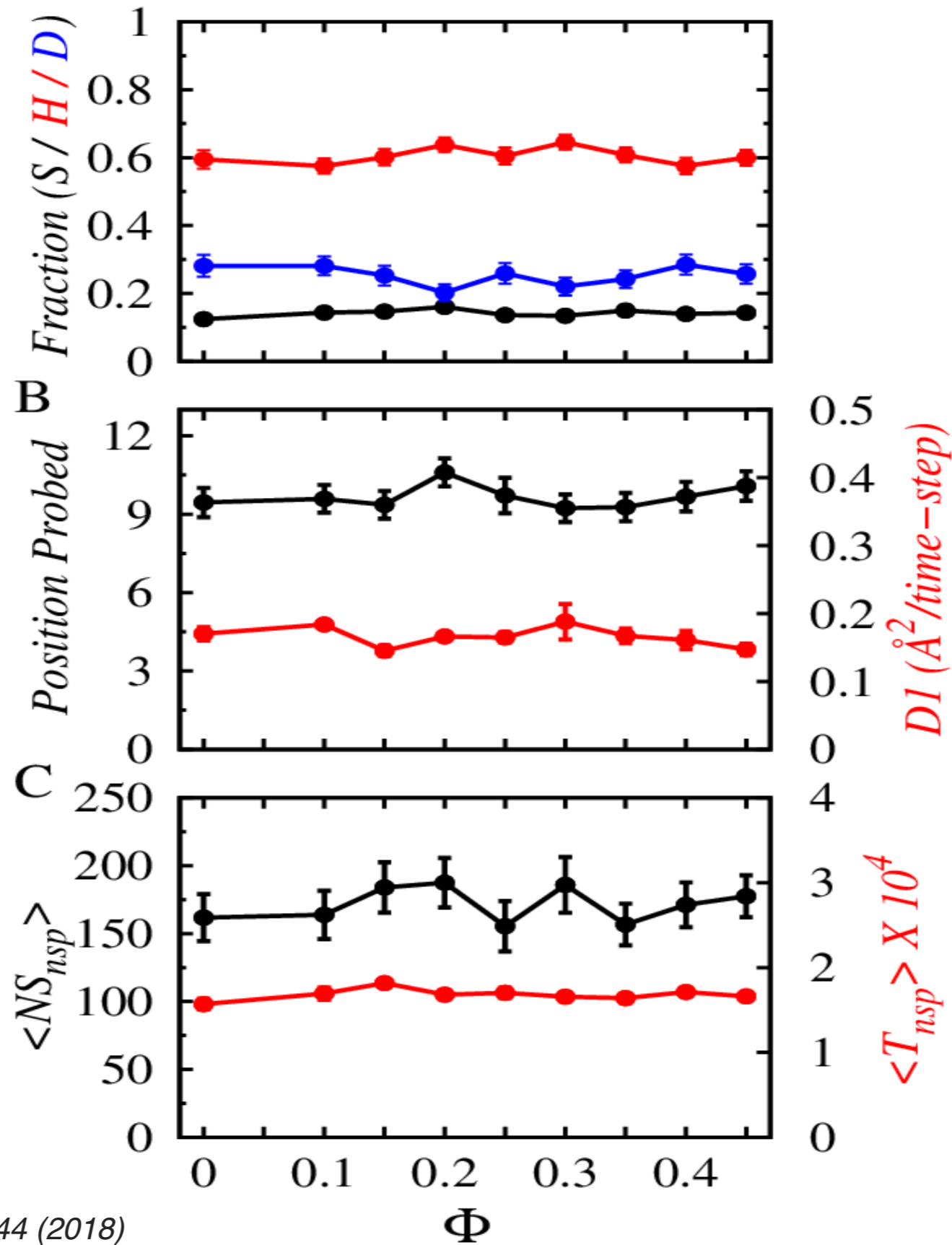
## Intersegmental Transfer



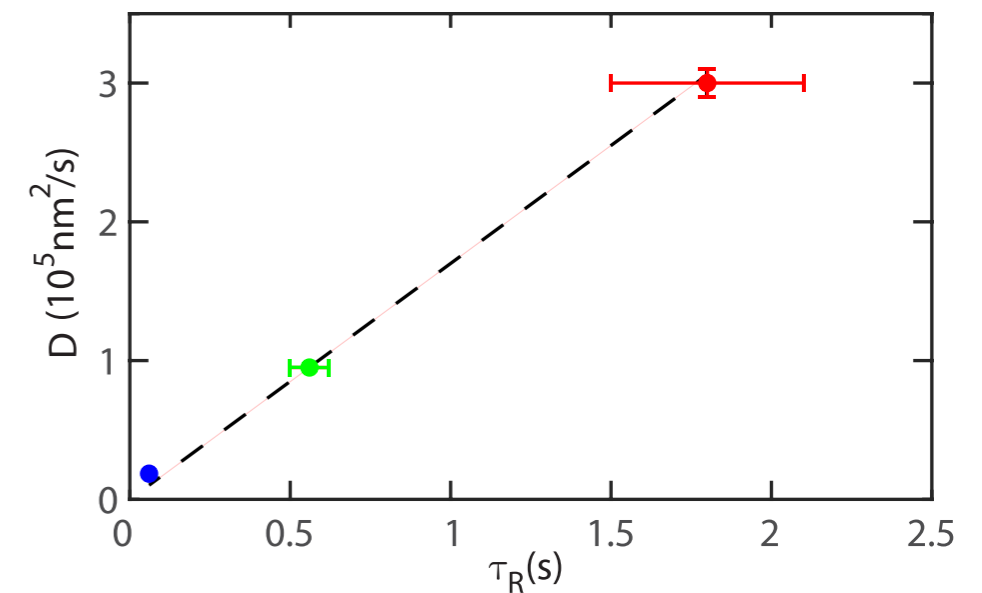
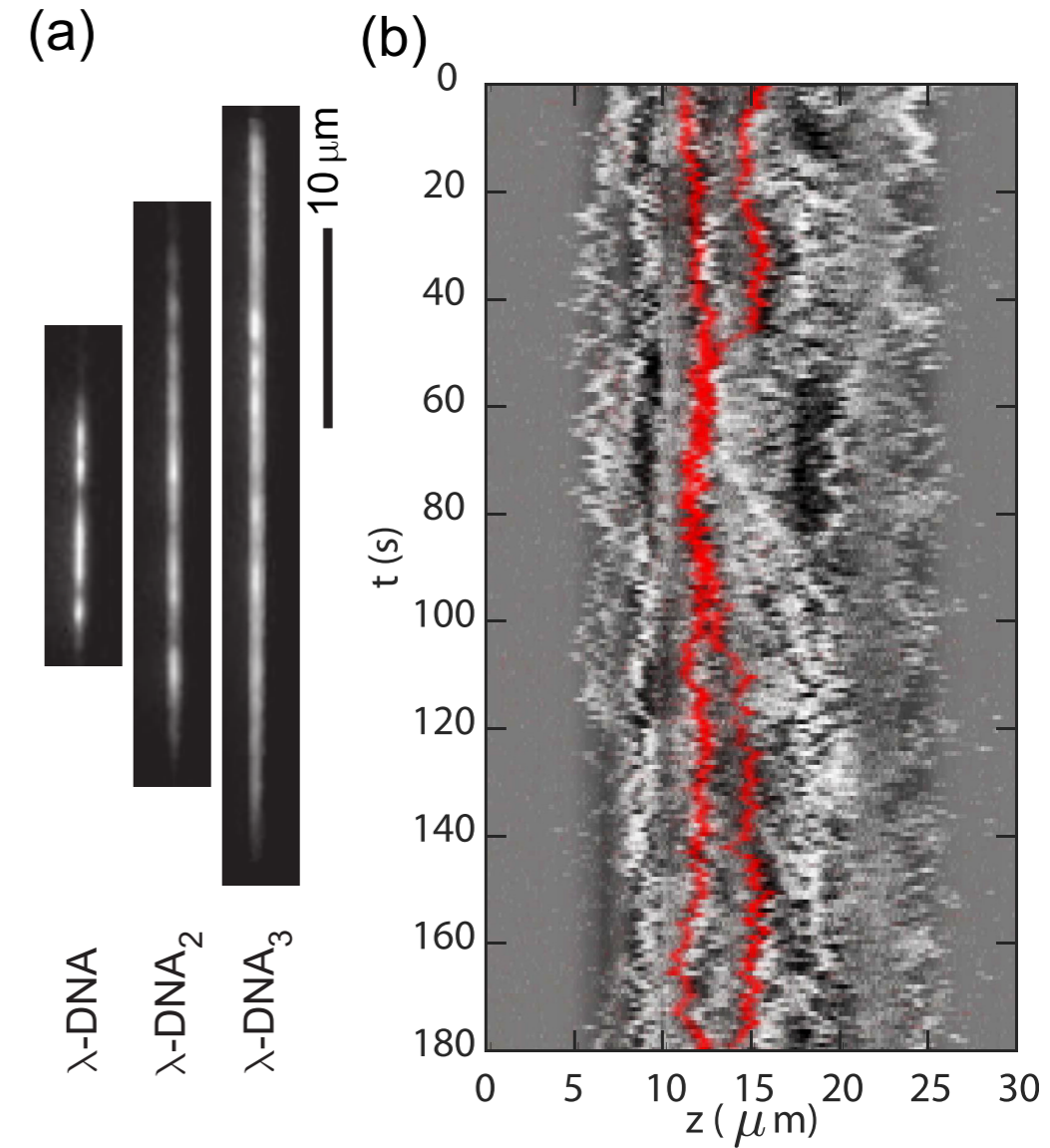
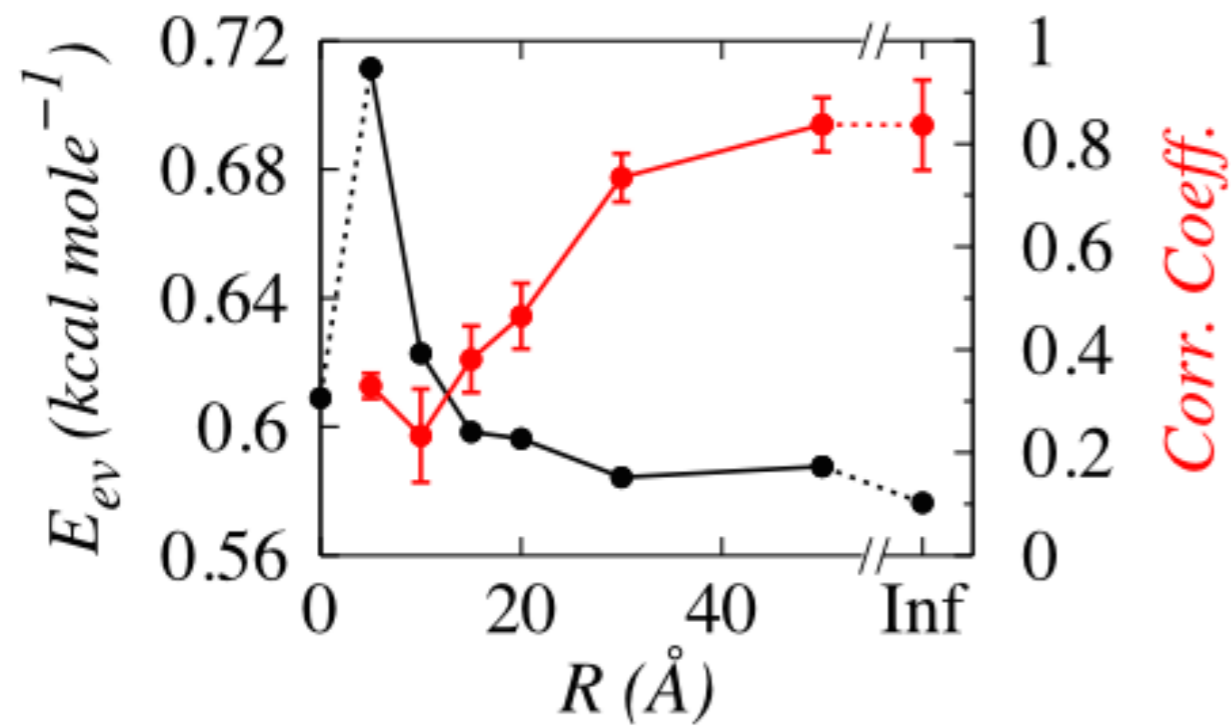
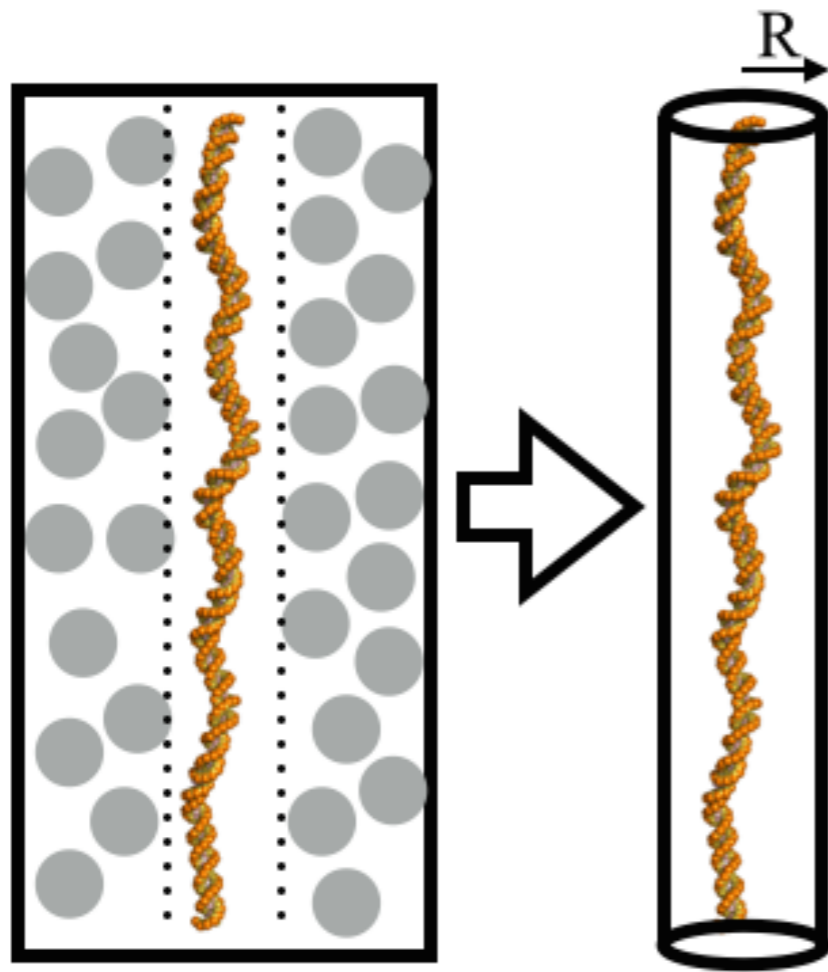
# Making Passage Through Crowd



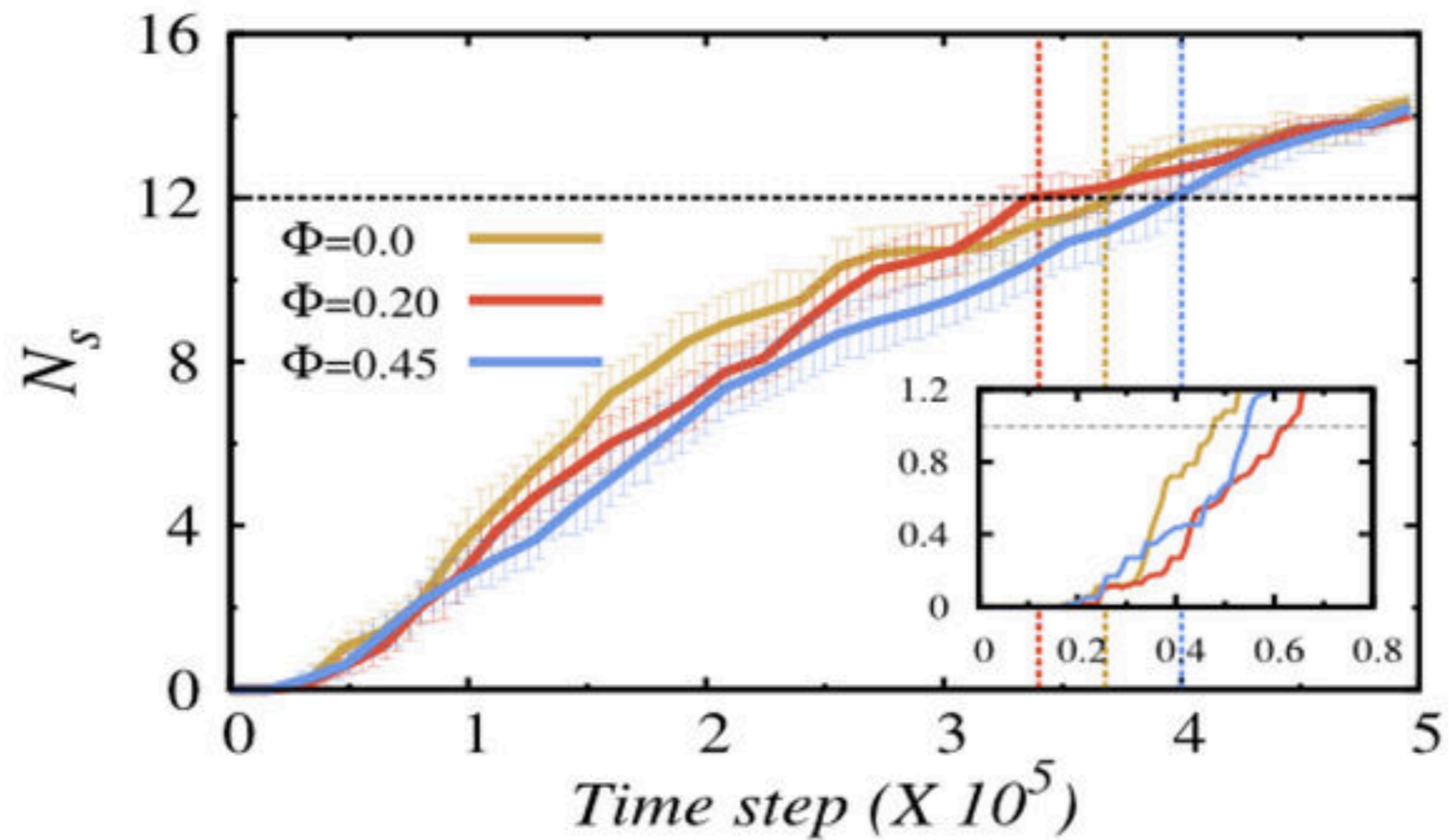
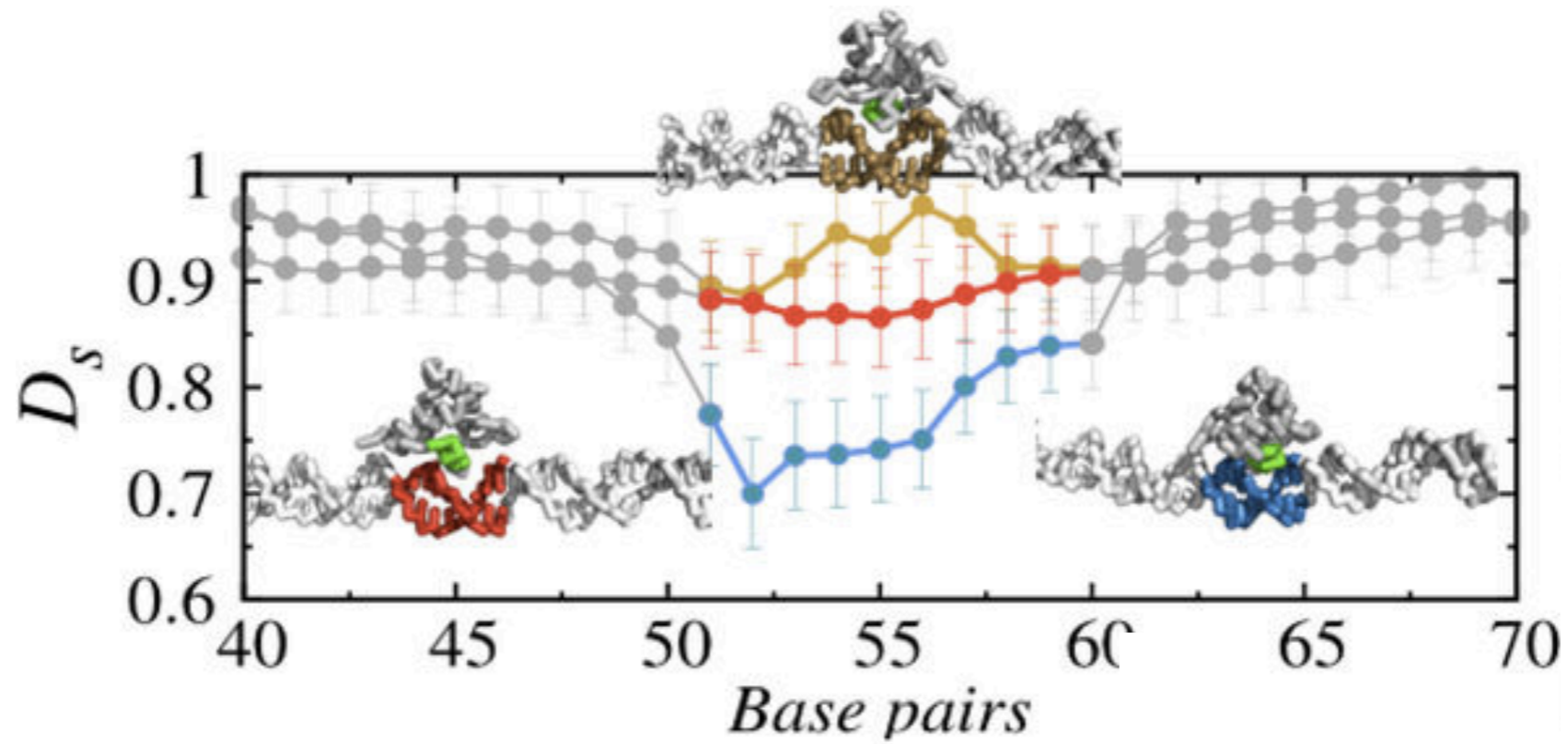
# Non-specific search Regime



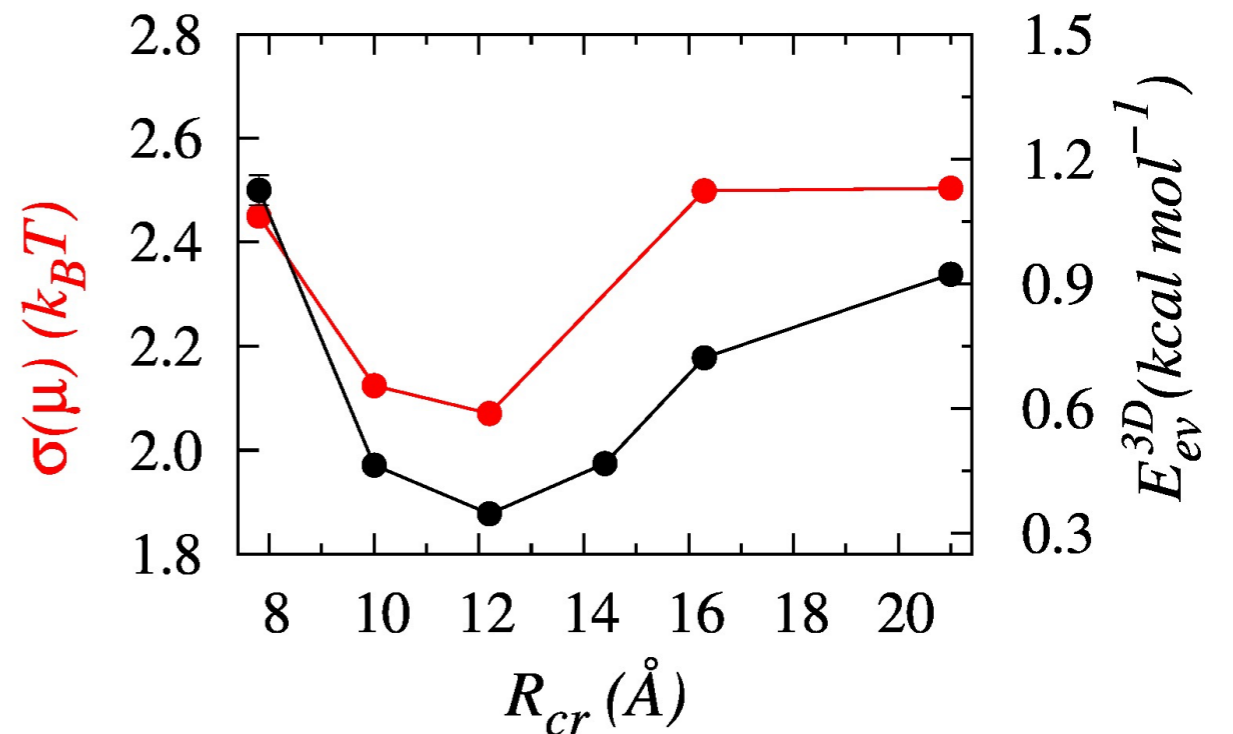
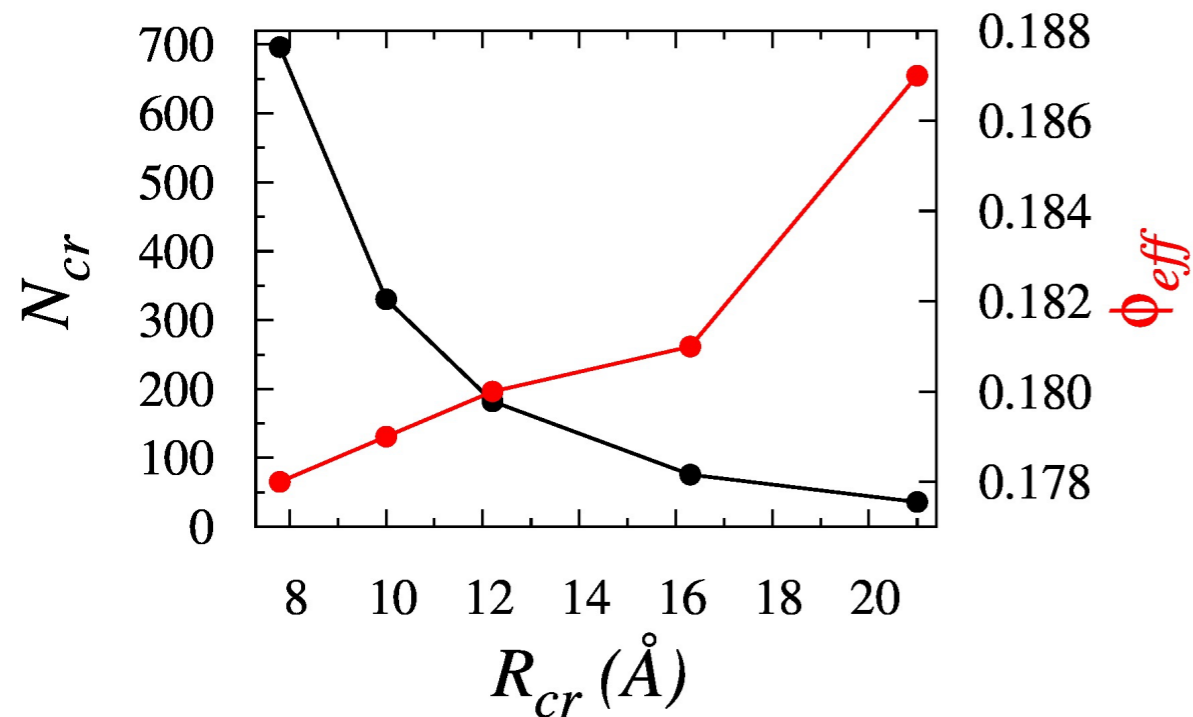
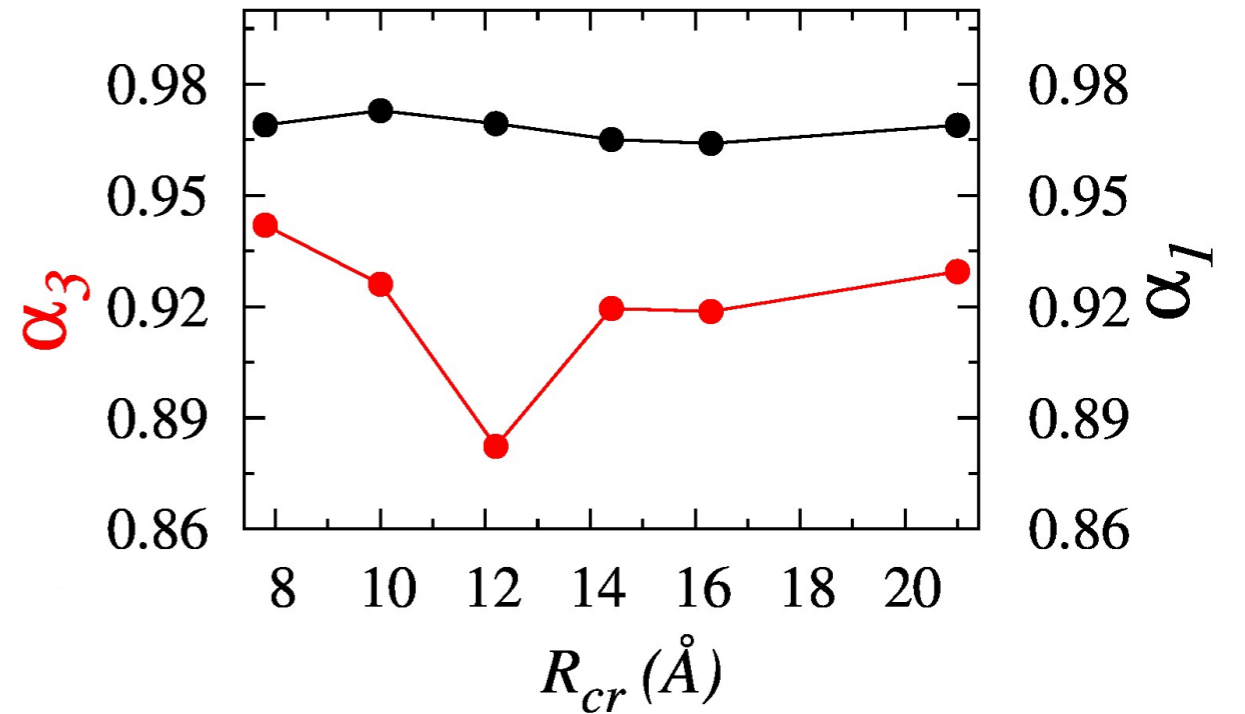
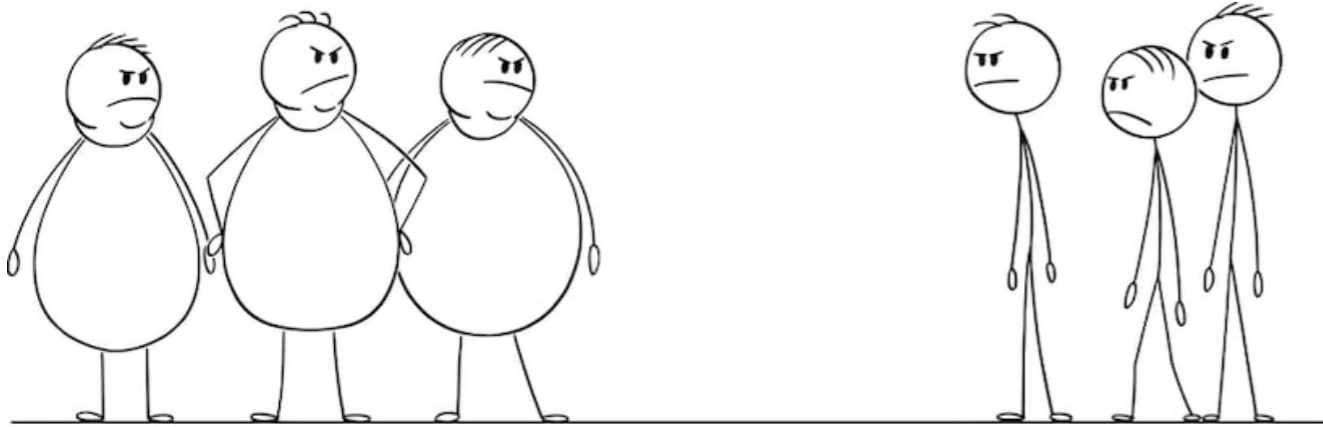


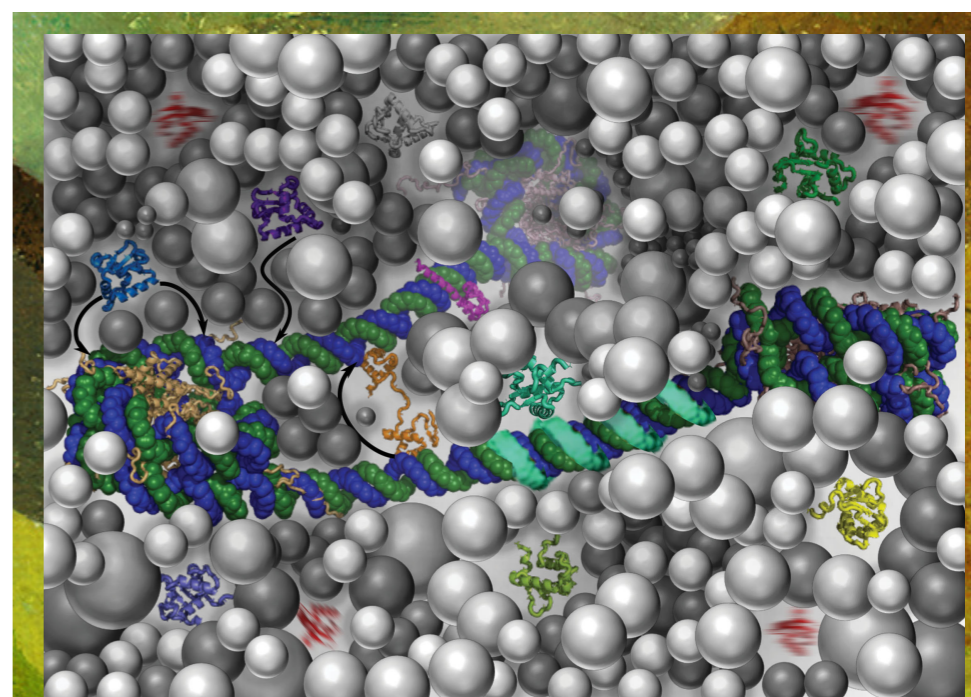
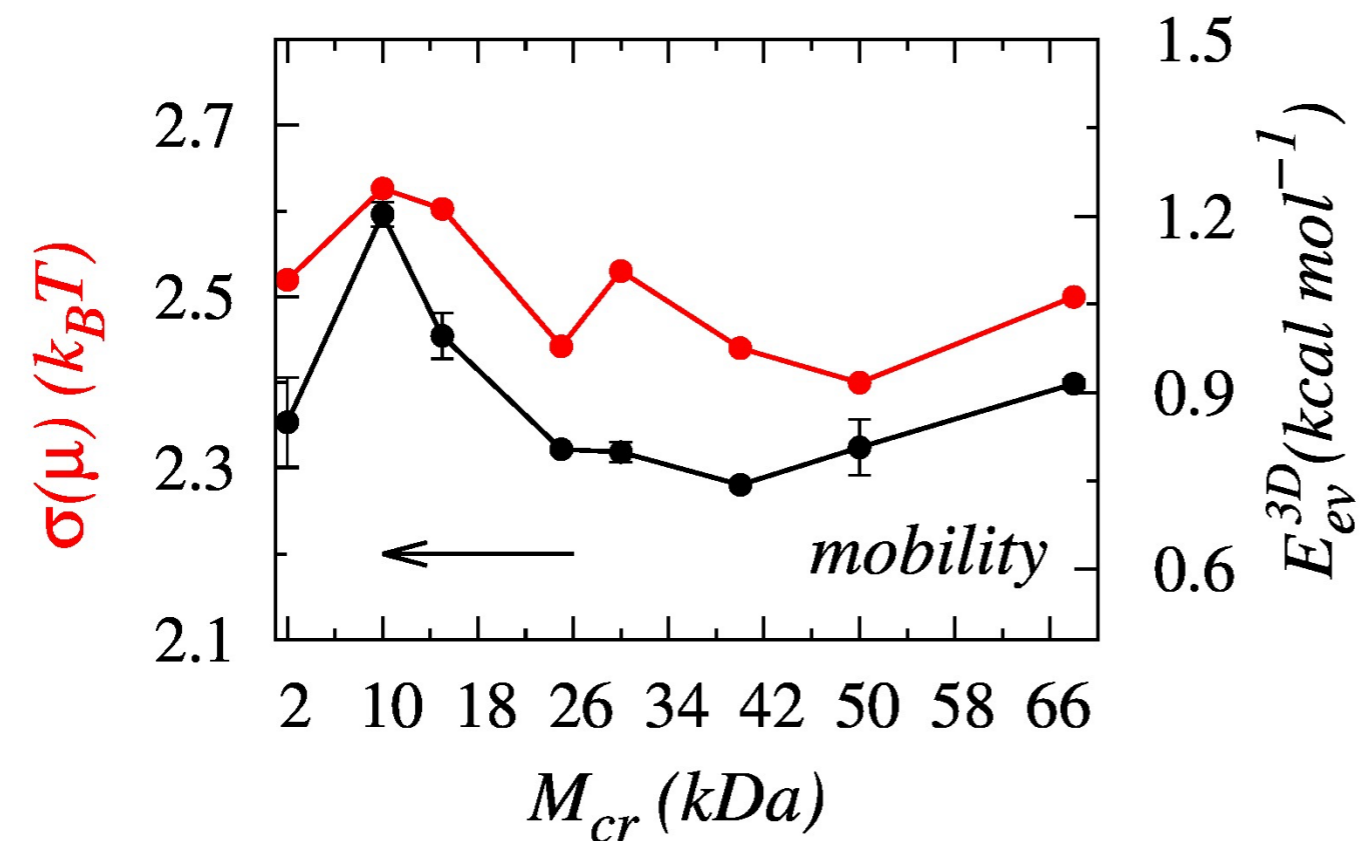
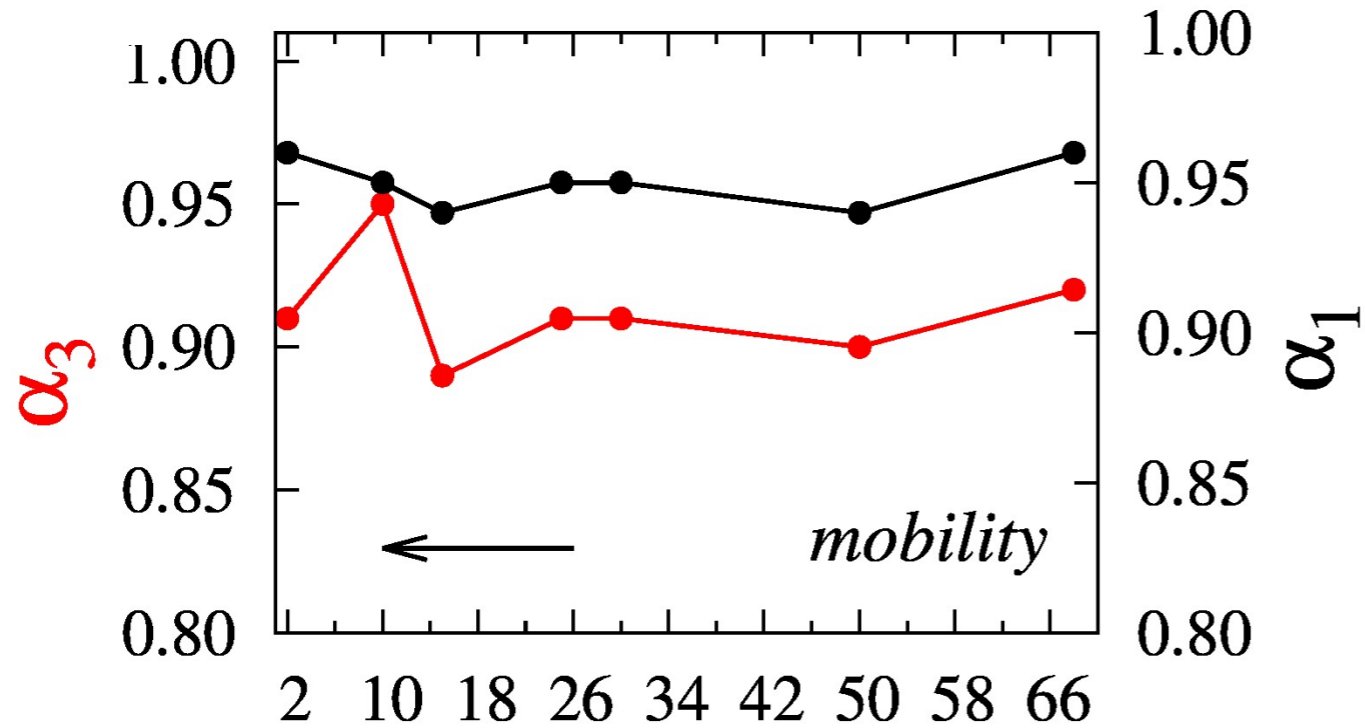


# Impact on Specific Binding



# Crowder Physiology - Size, Mobility, Shape





Highlighting research on multiscale simulations and theoretical biophysics of complex systems from the group of Dr Arnab Bhattacharjee.

Disparity in anomalous diffusion of proteins searching for their target DNA sites in a crowded medium is controlled by the size, shape and mobility of macromolecular crowders

Crucial biological processes take place when DNA Binding Proteins (DBPs) search and specifically bind to their respective target DNA sites by bypassing other macromolecules that act as crowding agents. Using extensive computer simulations, Dey et al. have analysed the role of the physical properties of these crowding agents, such as size, shape and mobility, on the target search dynamics of DNA binding proteins.

As featured in:

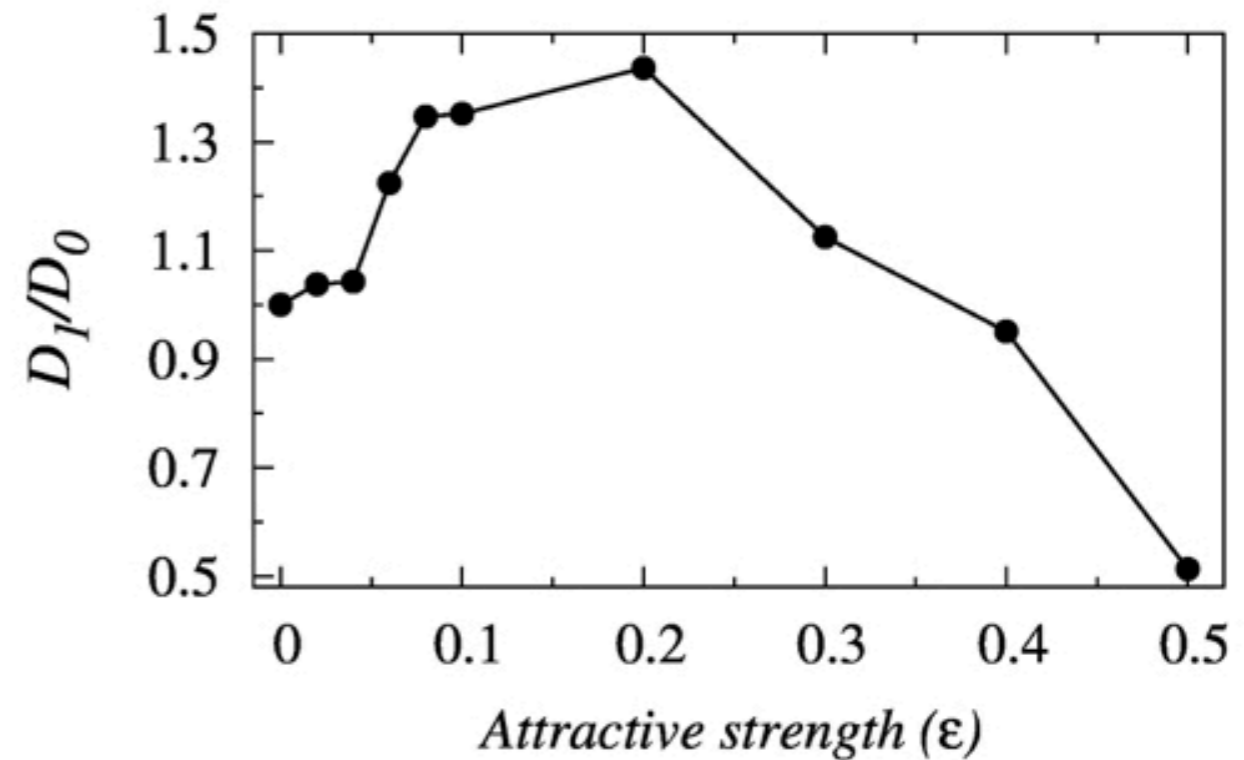
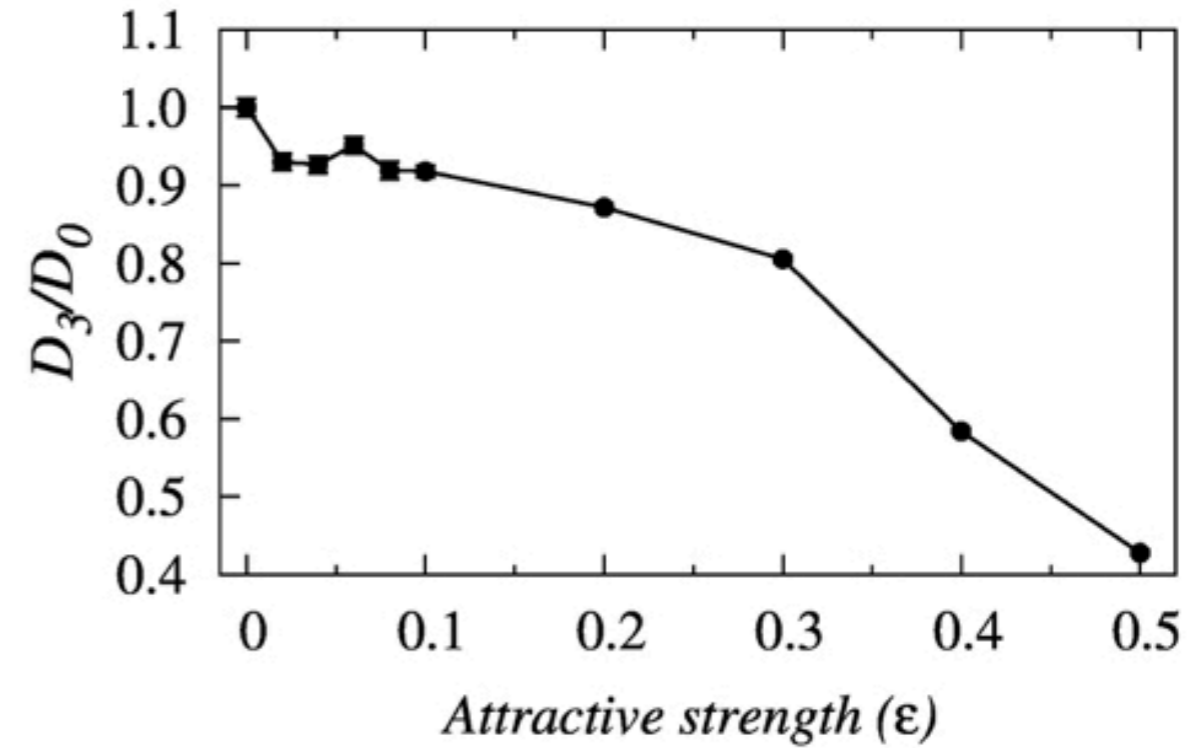
See Pinki Dey and Arnab Bhattacharjee, *Soft Matter*, 2019, 15, 1960.

ROYAL SOCIETY OF CHEMISTRY | Celebrating IYPT 2019

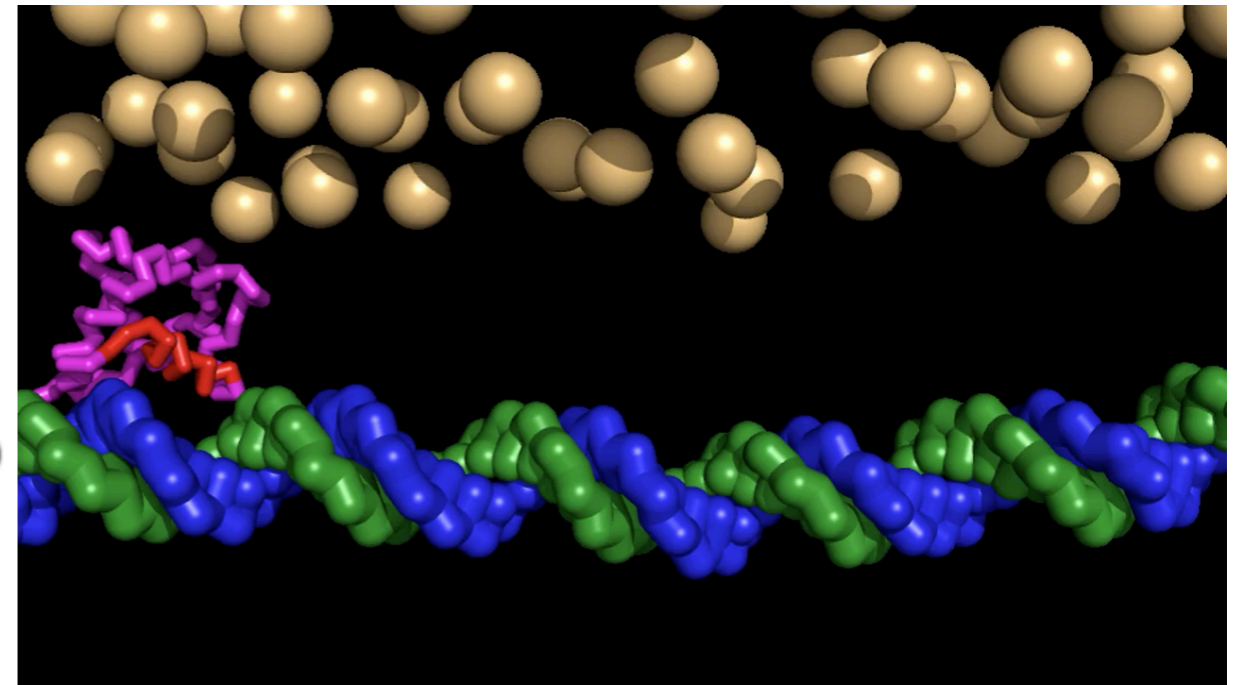
rsc.li/soft-matter-journal

Registered charity number: 207890

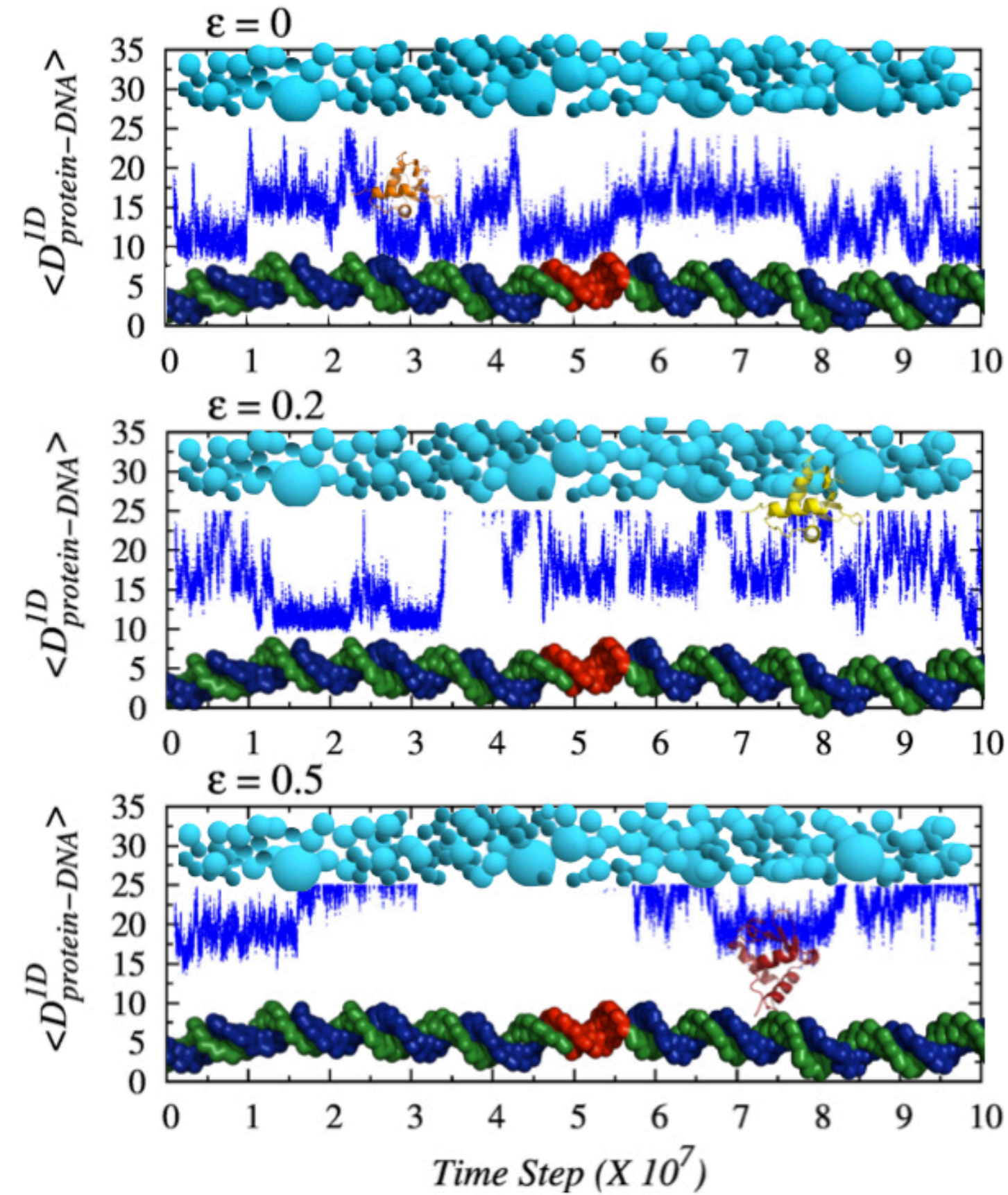
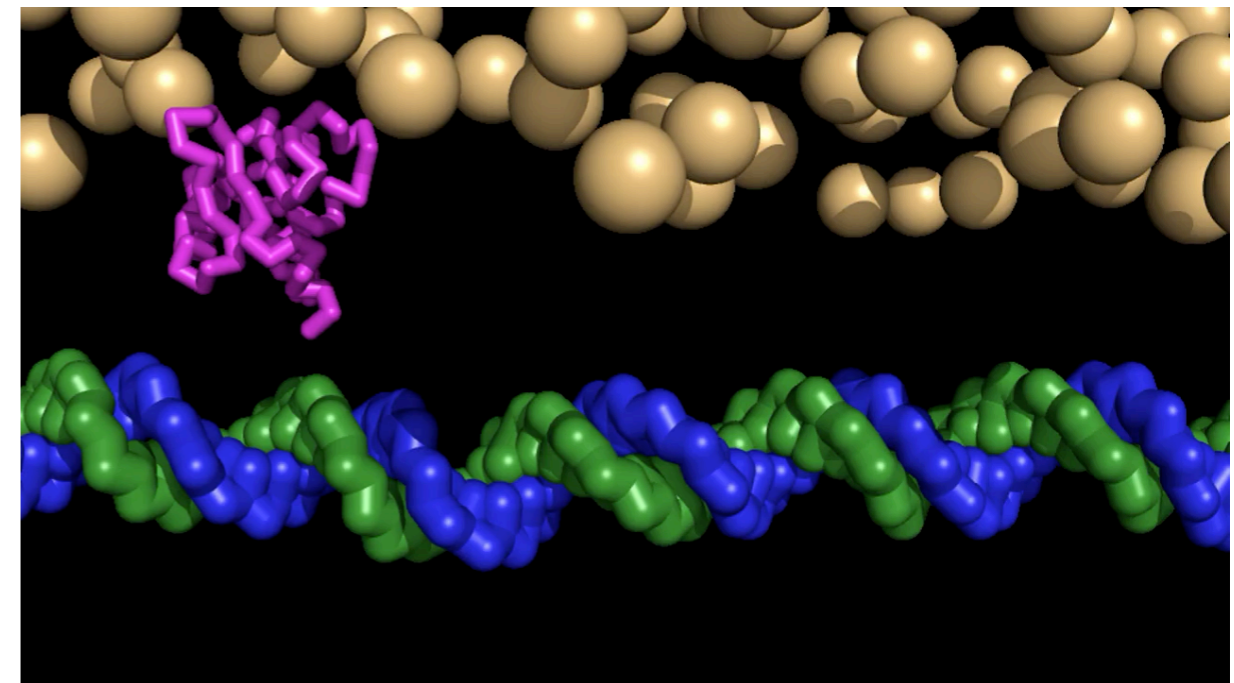
# Loving Crowd - Role of Protein-Crowder Interactions

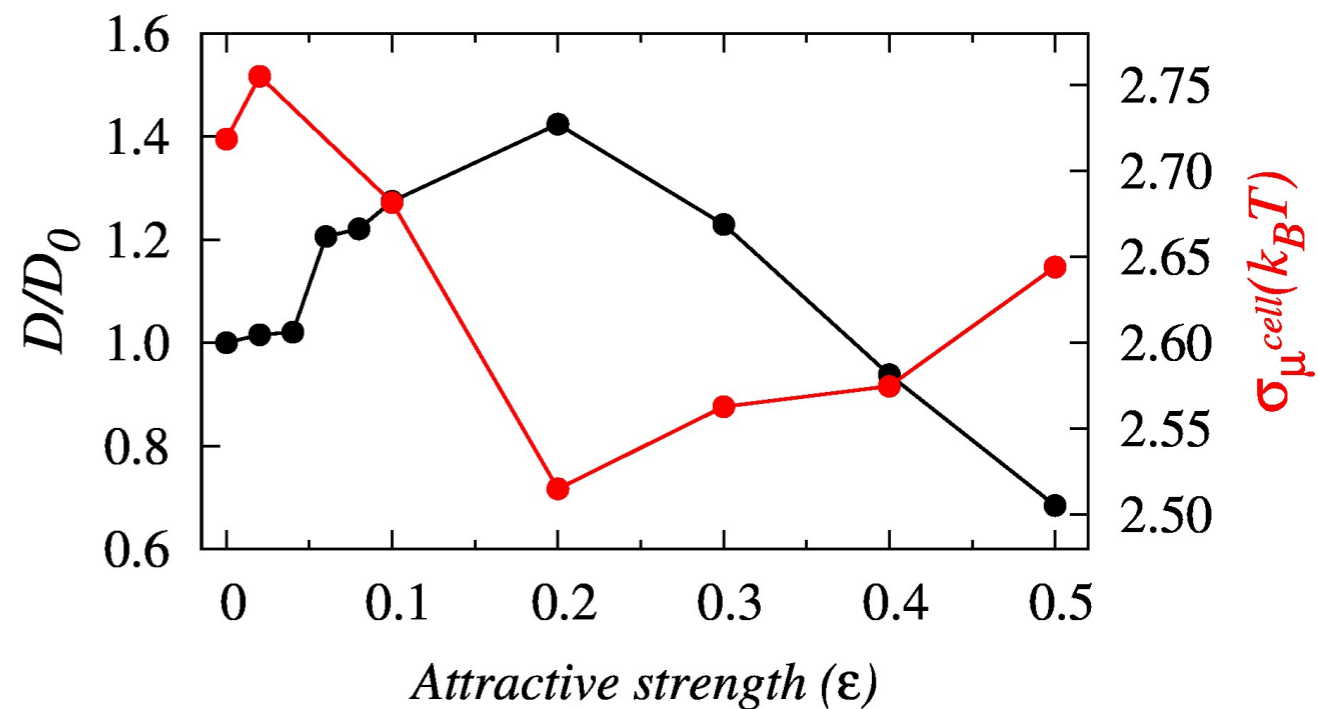
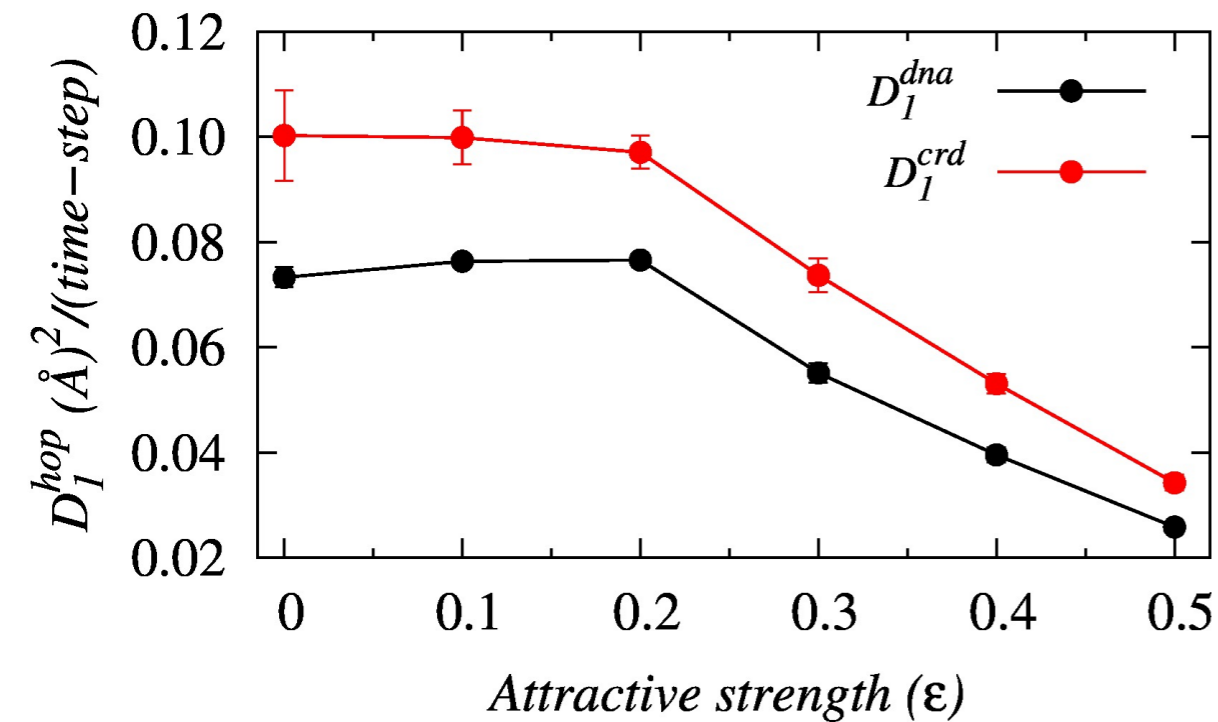


## Hopping on DNA



## Hopping away from DNA





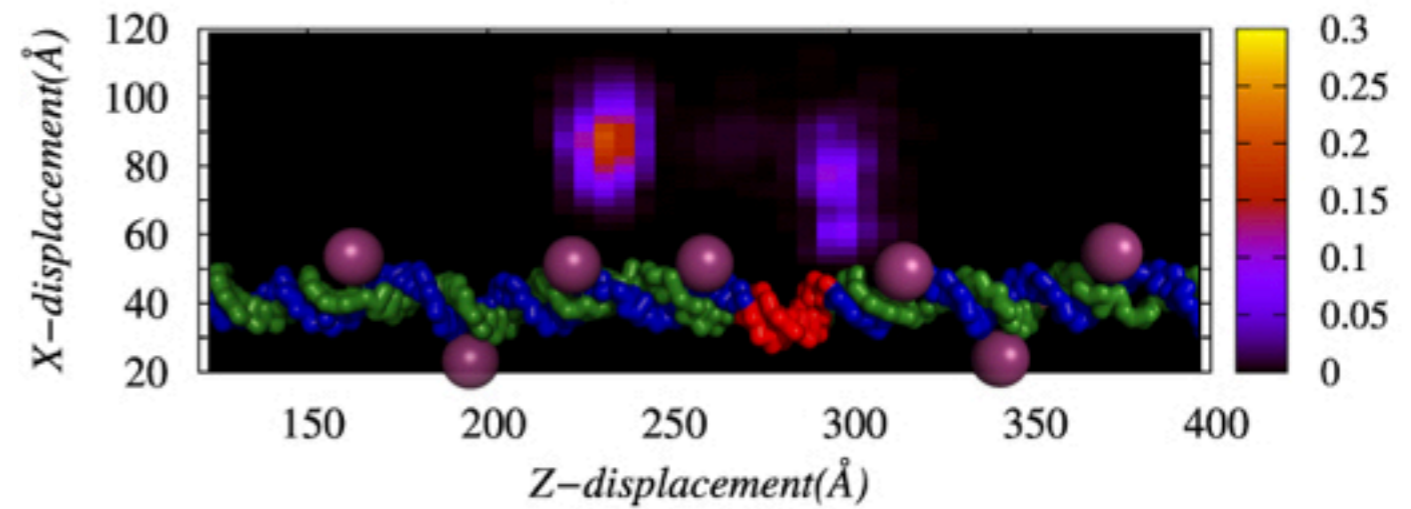
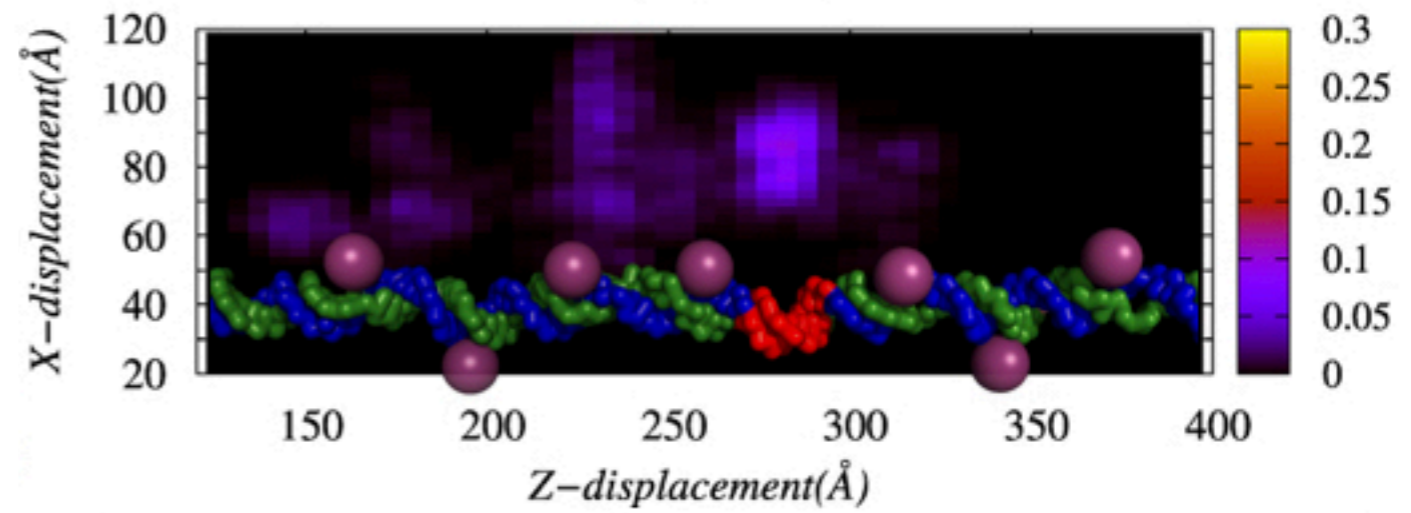
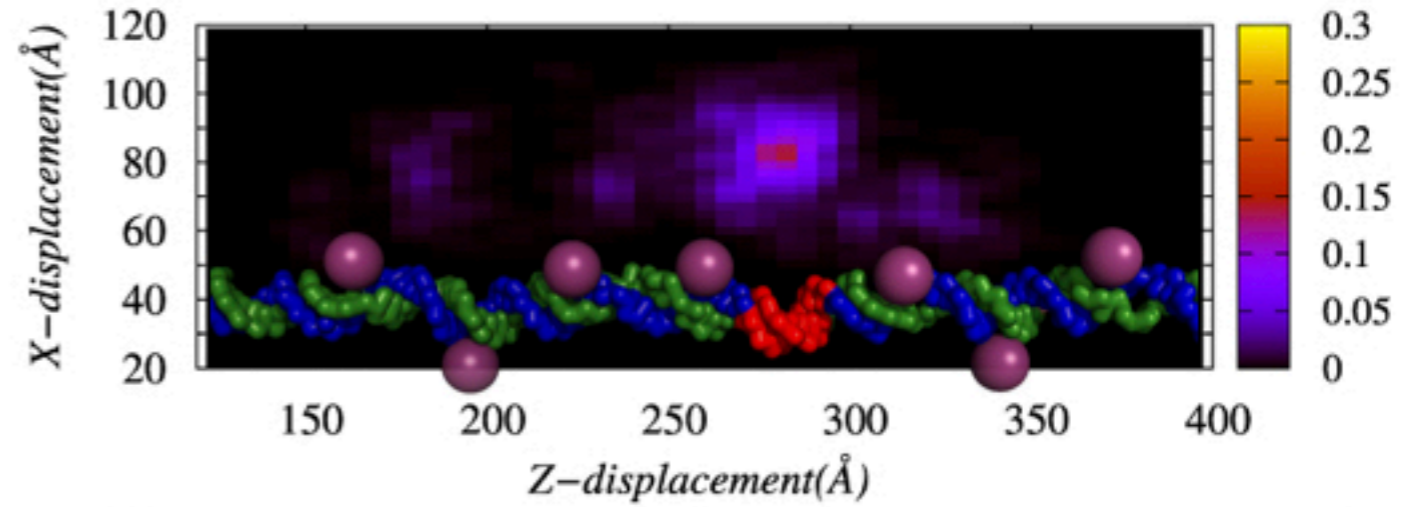
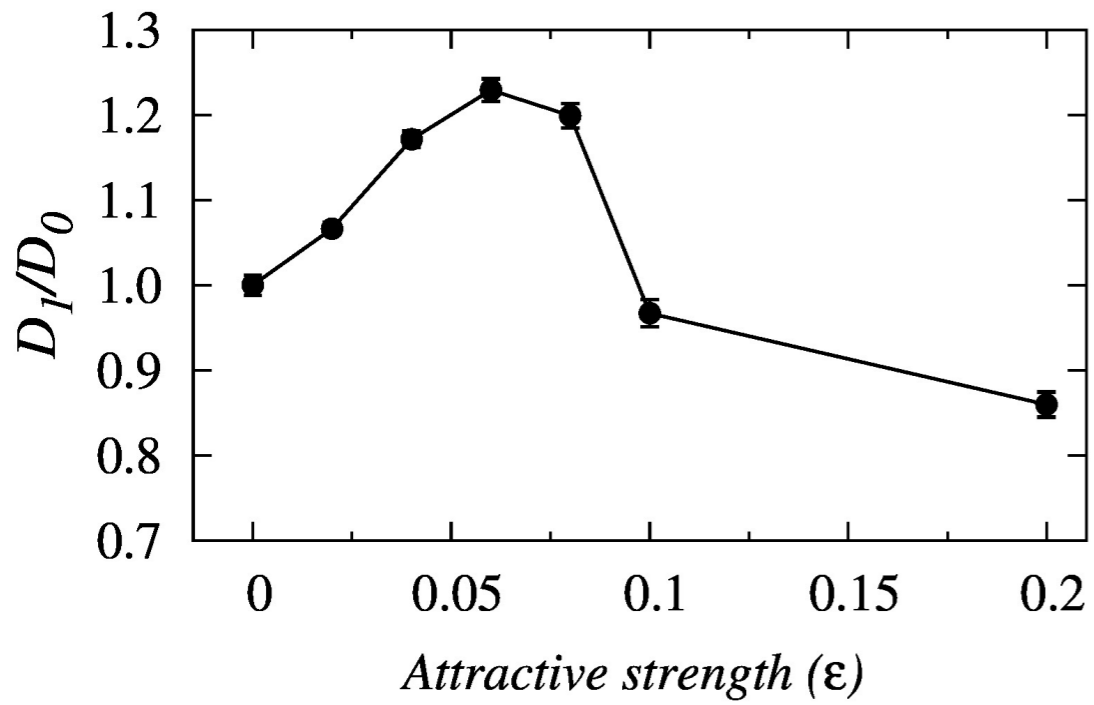
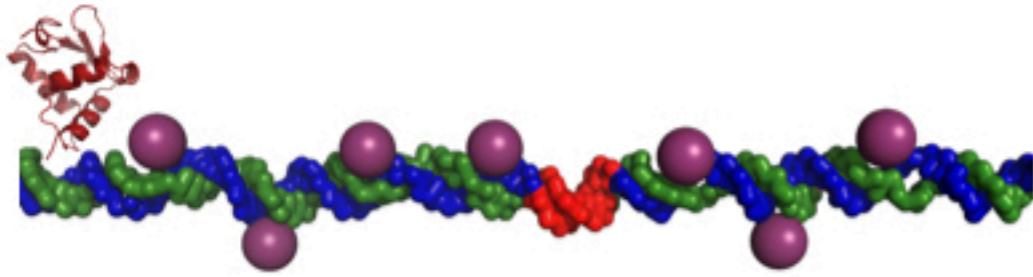
Published online 6 April 2015

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

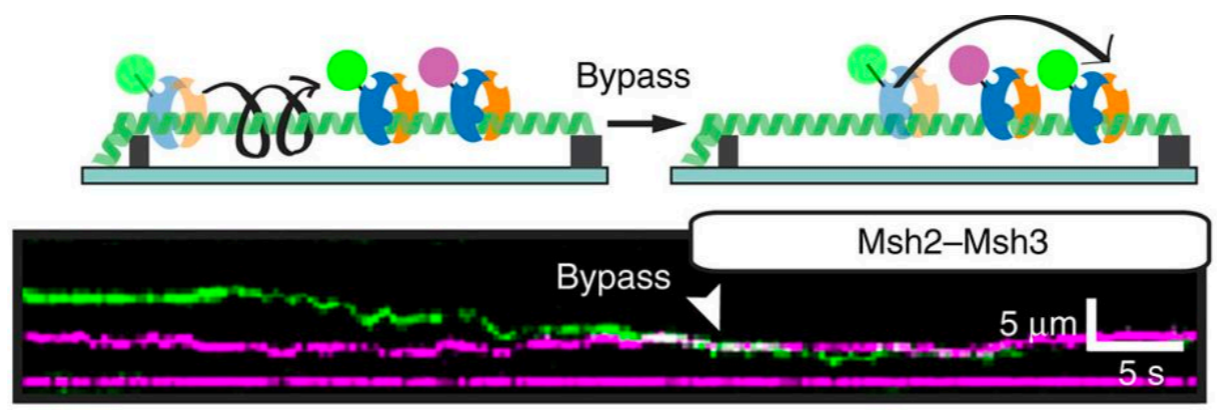
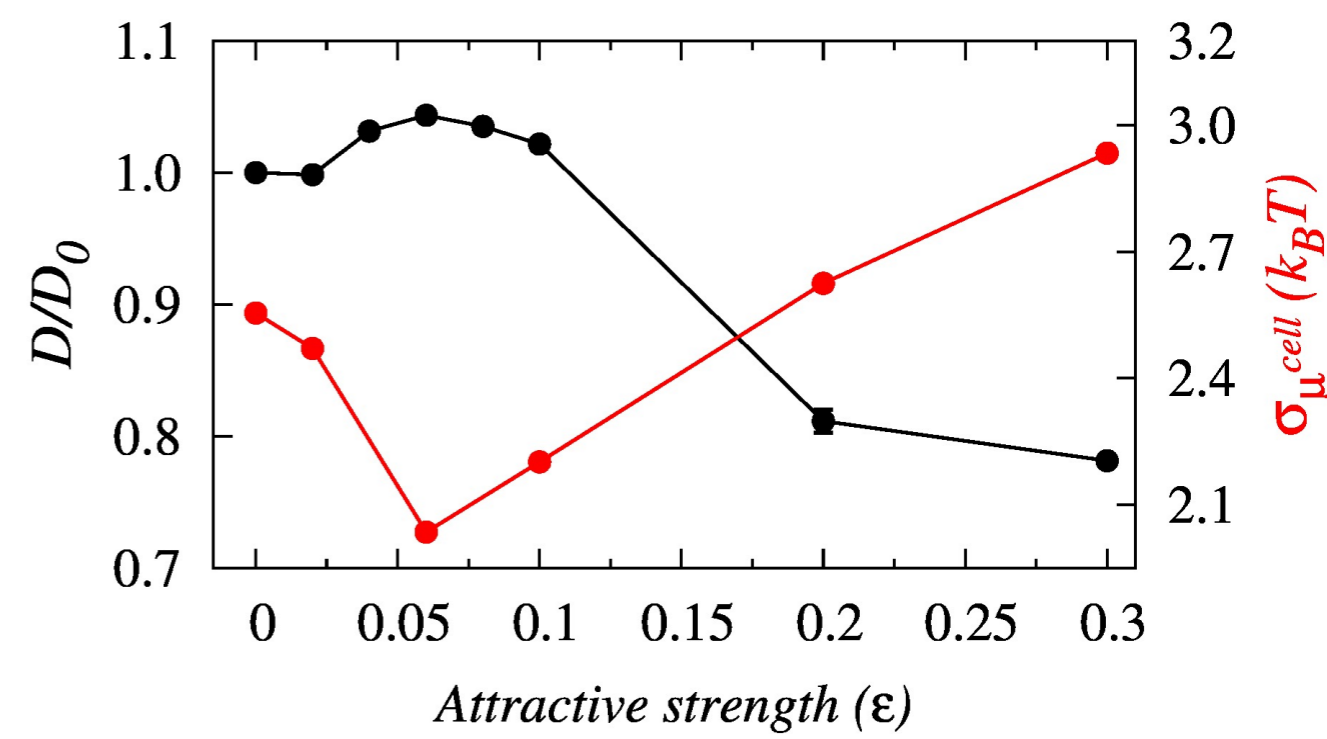
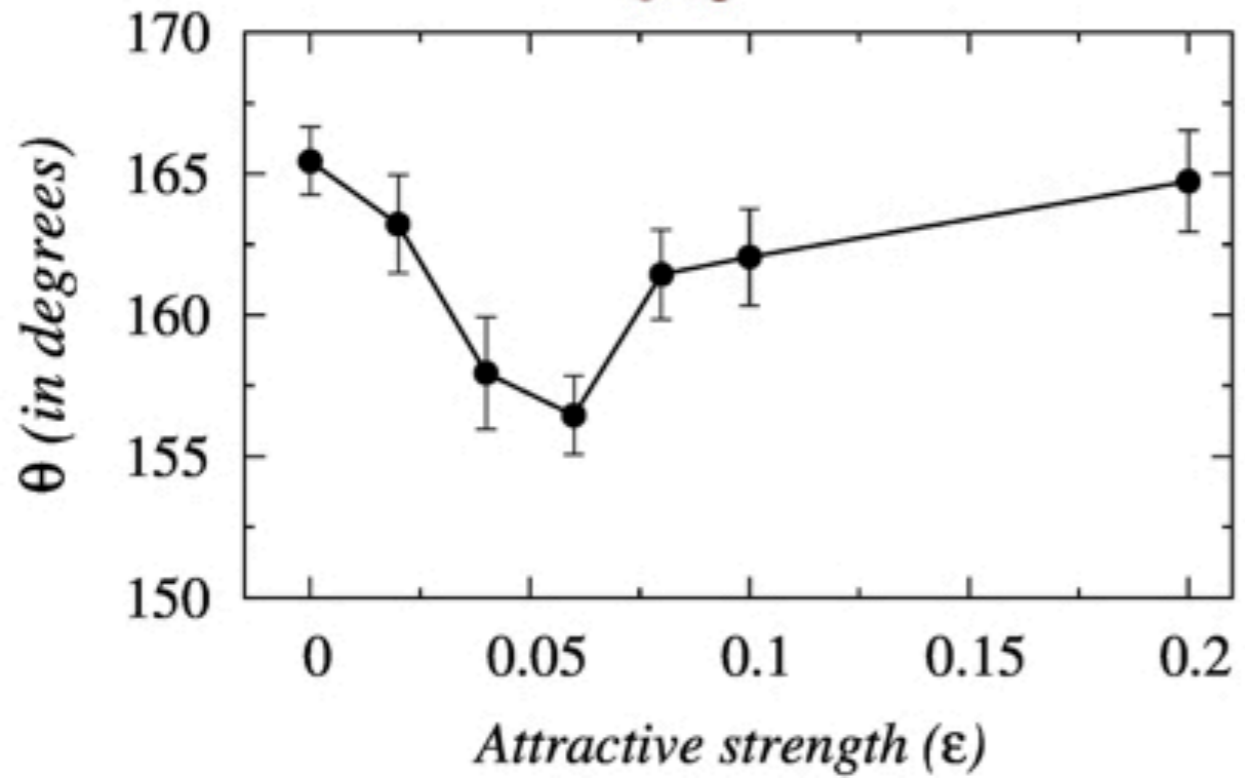
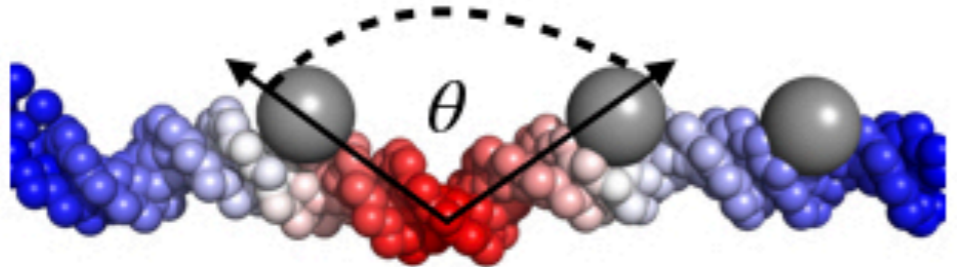
## Molecular crowding enhances facilitated diffusion of two human DNA glycosylases

Shannen L. Cravens<sup>1,†</sup>, Joseph D. Schonhoft<sup>1,†</sup>, Meng M. Rowland<sup>1</sup>, Alyssa A. Rodriguez<sup>2</sup>, Breeana G. Anderson<sup>1</sup> and James T. Stivers<sup>1,\*</sup>

# Genomic Crowders

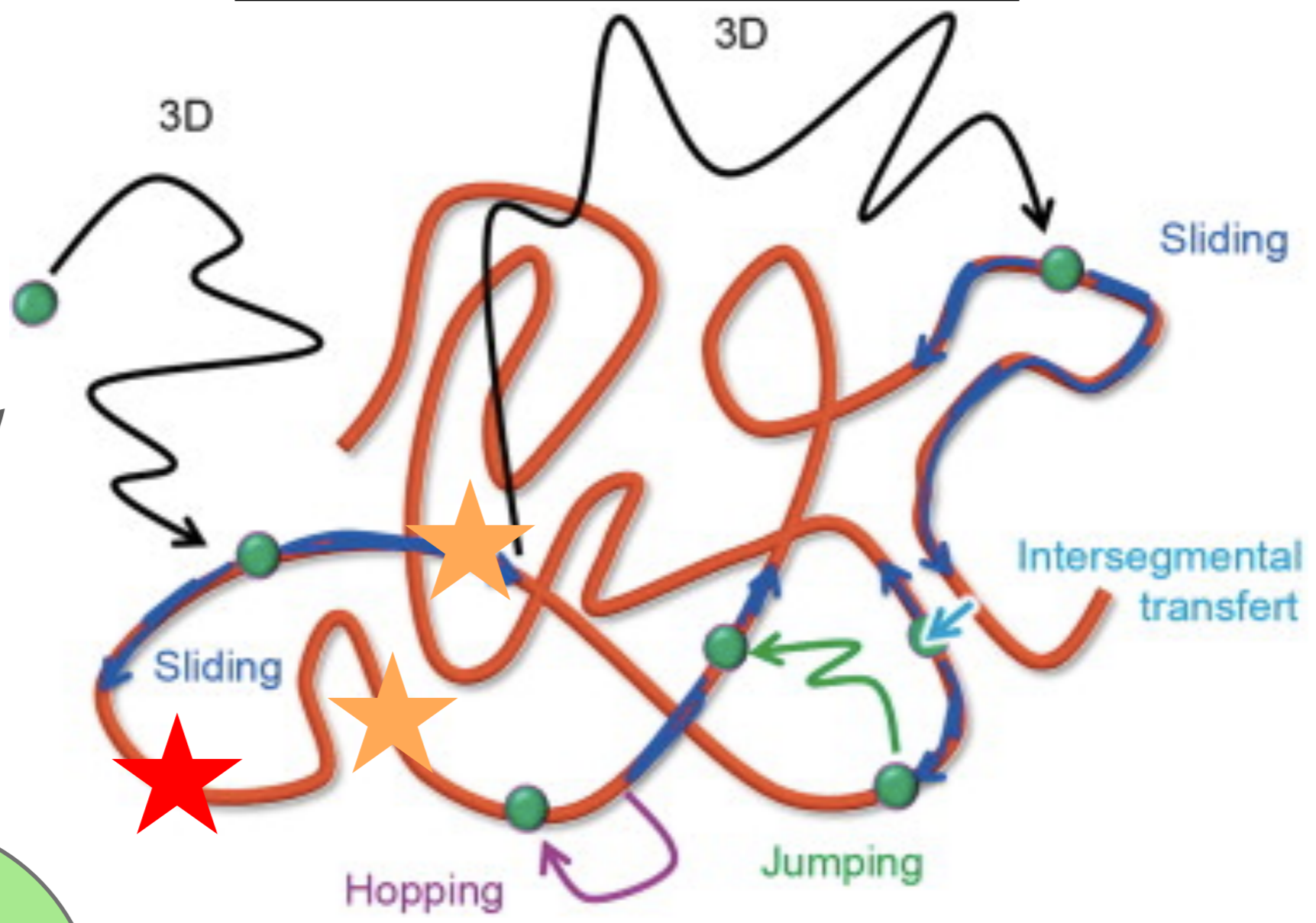




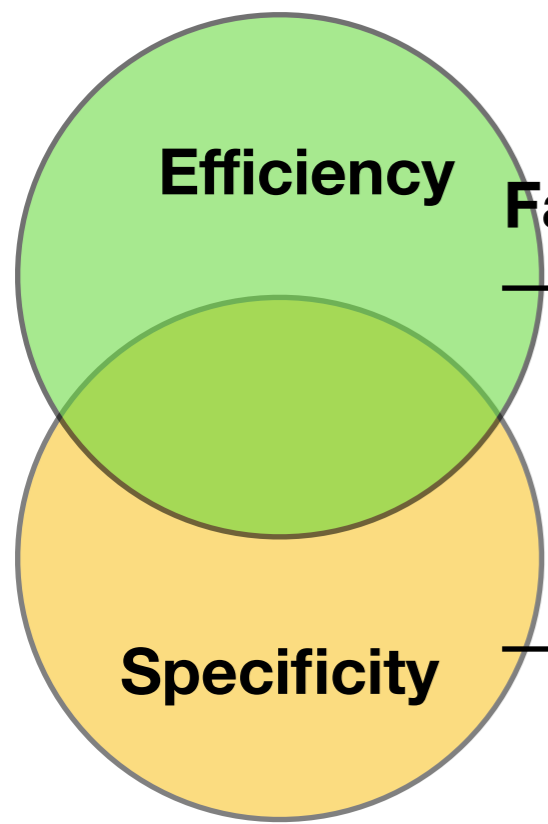


*Dynamic DNA binding licenses a repair factor to bypass roadblocks in search of DNA lesions, Finkelstein et. al. Nature Communications, 7: 10607, (2016)*

Slow down by Traffic Signal



*Antenna Effect !*



**Fast Kinetics**

**Stability**

Stability

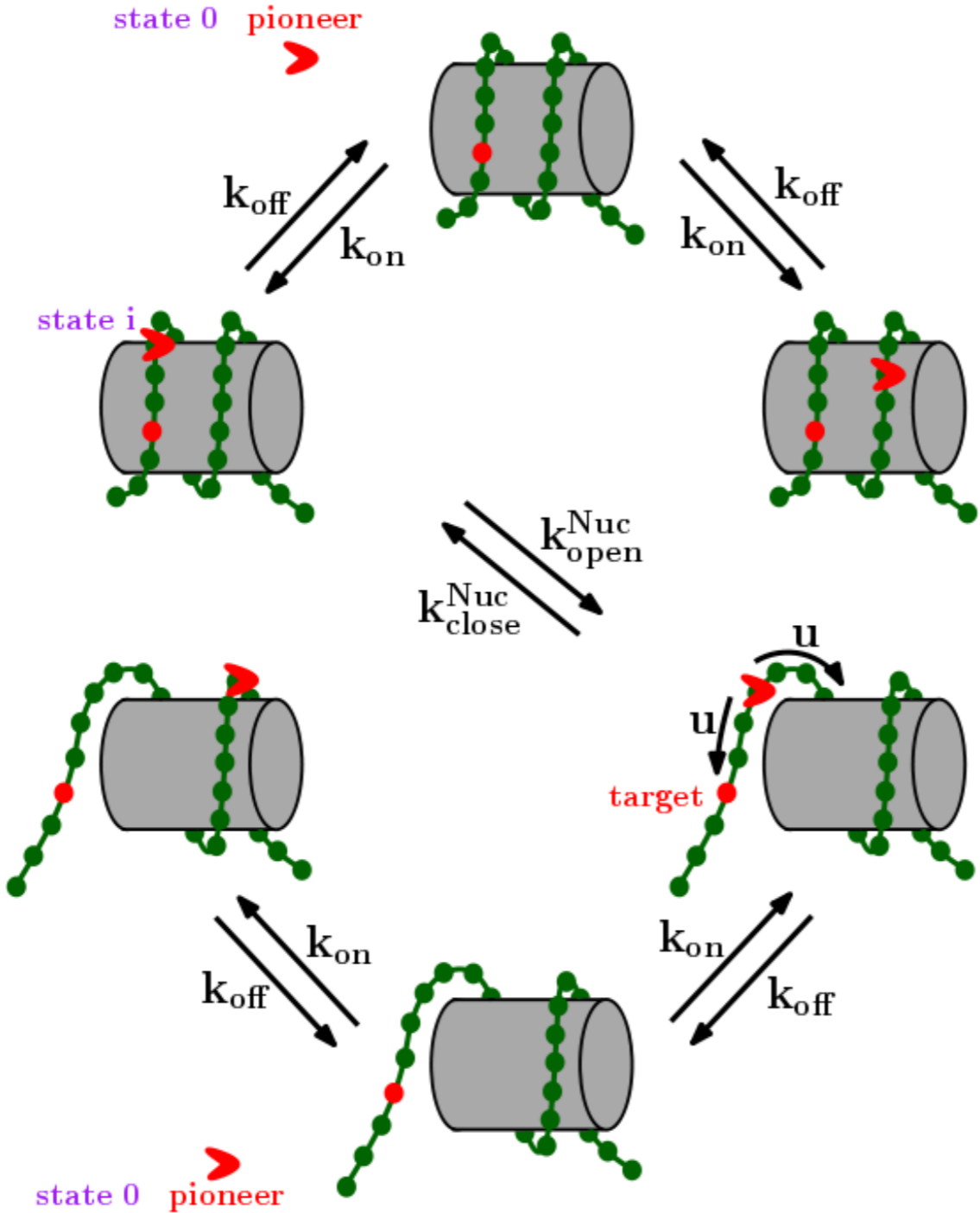
speed

**Speed-Stability Paradox**

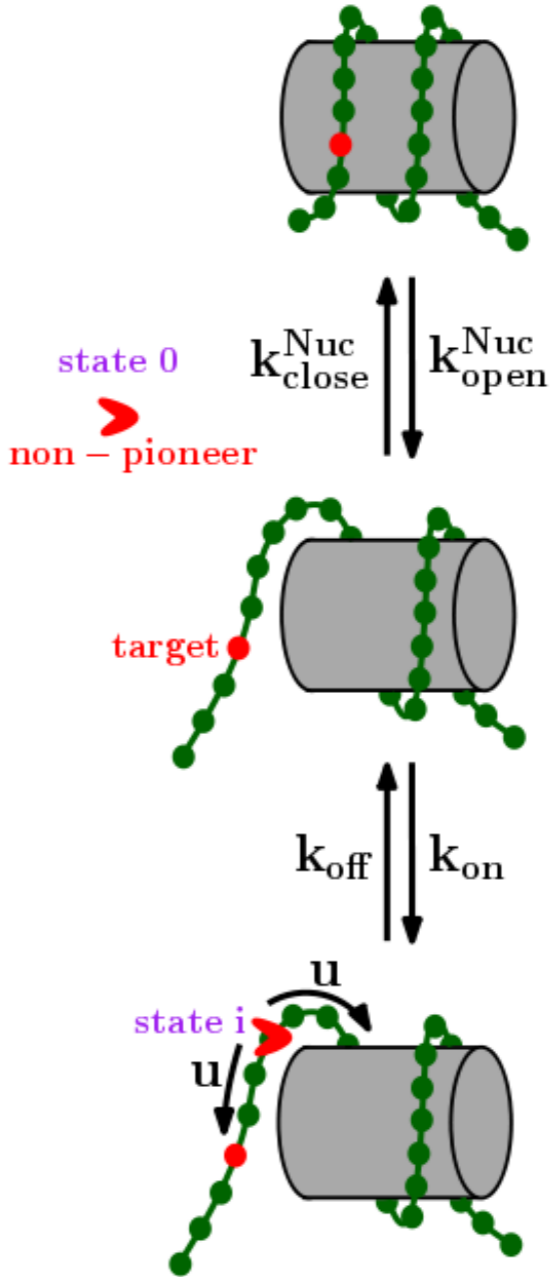


# Does Speed always come at the cost of Stability? How Protein searches on Nucleosomal DNA

Pioneer Search :



Non-Pioneer Search :



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

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

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

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

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

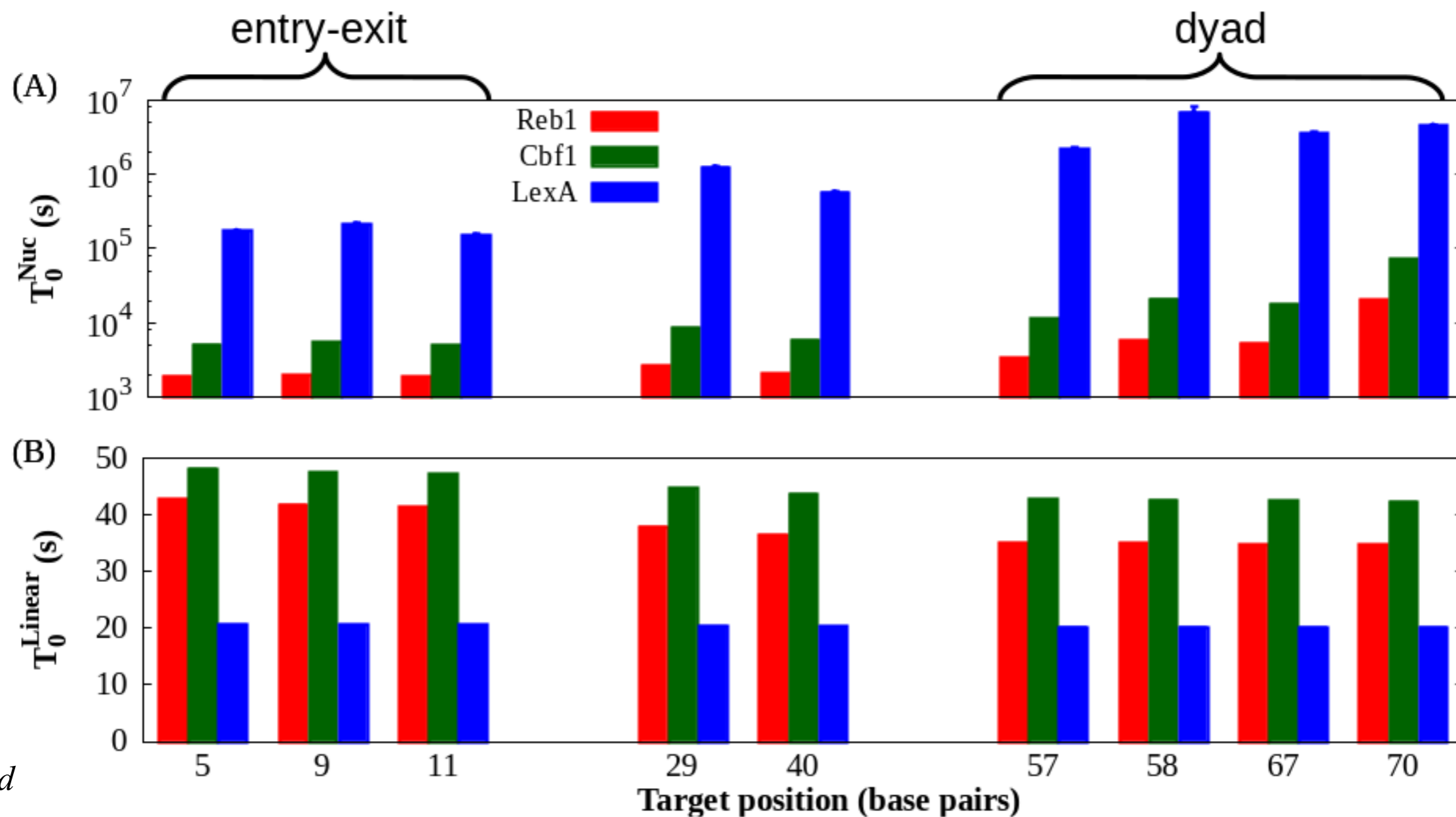
## Boundary Conditions

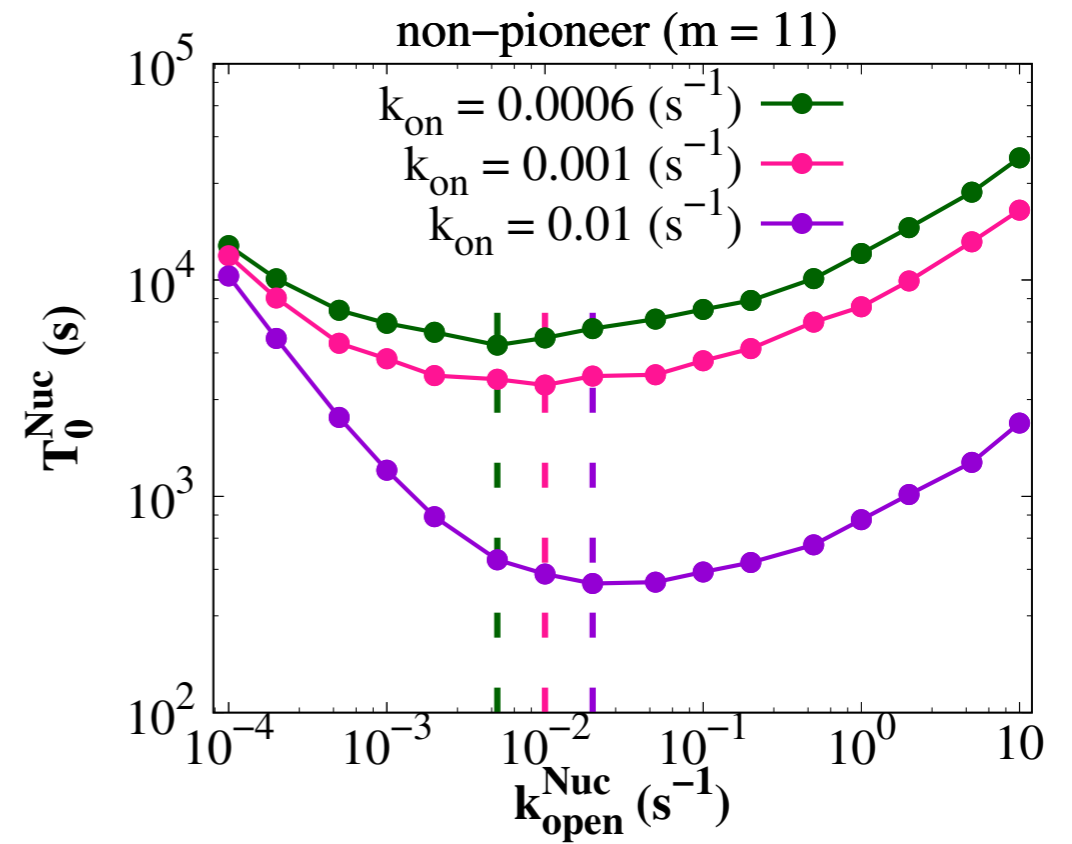
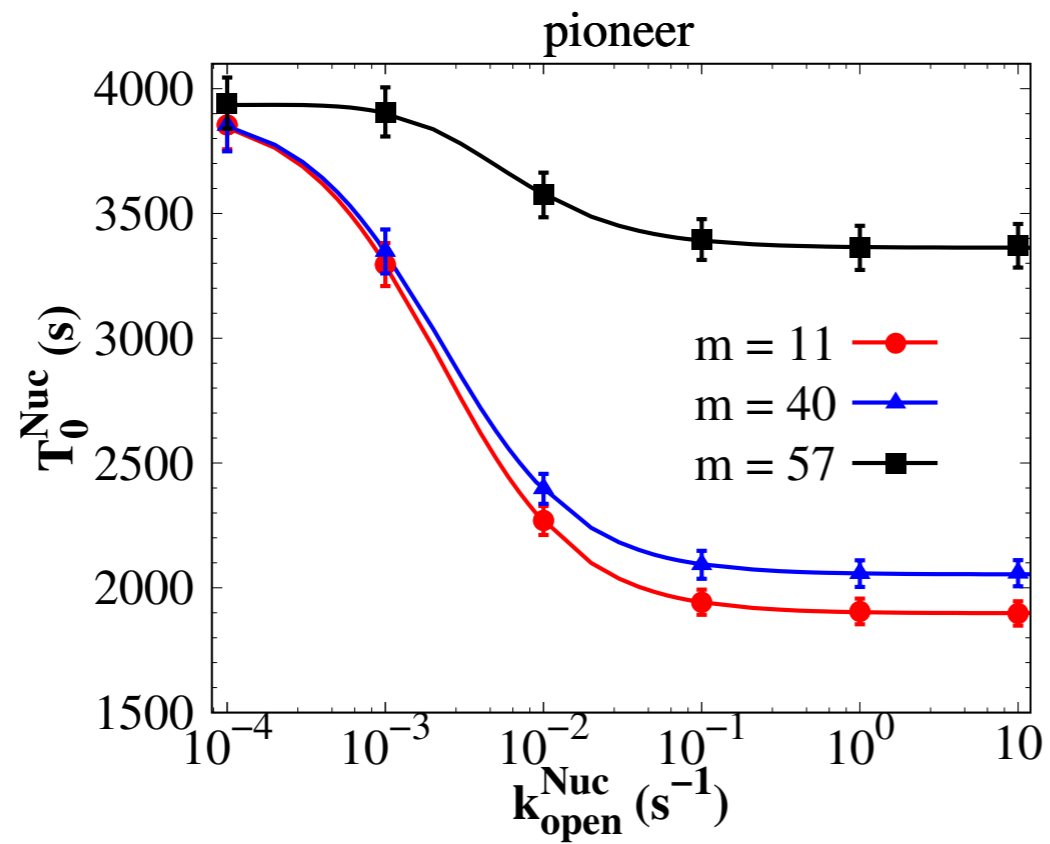
$$\frac{dP_1^{PUNuc}(t)}{dt} = uP_2^{PUNuc}(t) + k_{off}P_0(t) + k_{close}^{Nuc}P_1^{FWNuc}(t) - \left(u + k_{off} + k_{close}^{Nuc}\right)P_1^{PUNuc}(t)$$

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

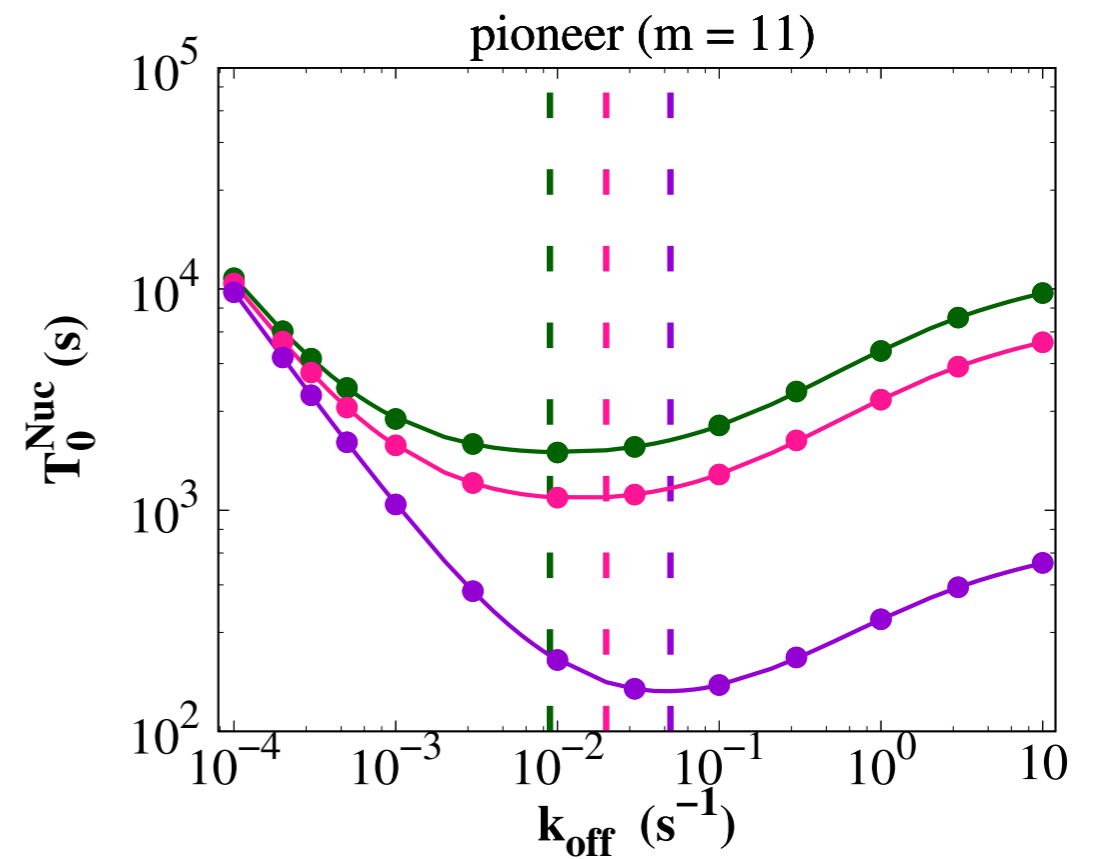
$$k_{D(nuc)}^{Reb1} < k_{D(nuc)}^{Cbf1} < k_{D(nuc)}^{LexA}$$

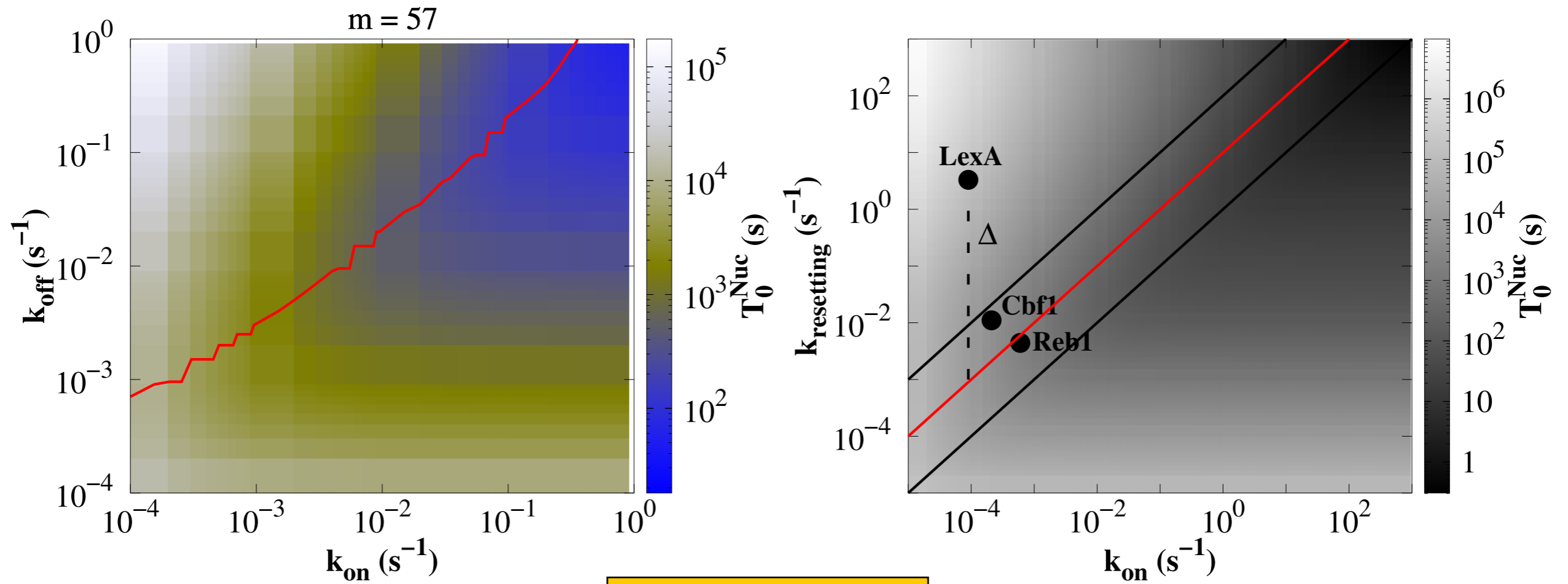
Transcription Factors	$k_{off}^{Nuc}$ ( $s^{-1}$ )	$k_{off}^{Linear}$ ( $s^{-1}$ )	$k_{on}^{Nuc}$ ( $s^{-1}nM^{-1}$ )	$k_{on}^{Linear}$ ( $s^{-1}nM^{-1}$ )
Reb1 (pioneer)	$(4.4 \pm 0.5) \times 10^{-3}$	$0.58 \pm 0.08$	$(6 \pm 1) \times 10^{-4}$	$(3.2 \pm 0.3) \times 10^{-2}$
Cbf1 (pioneer)	$(1.1 \pm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





*Optimal Error Correction !*

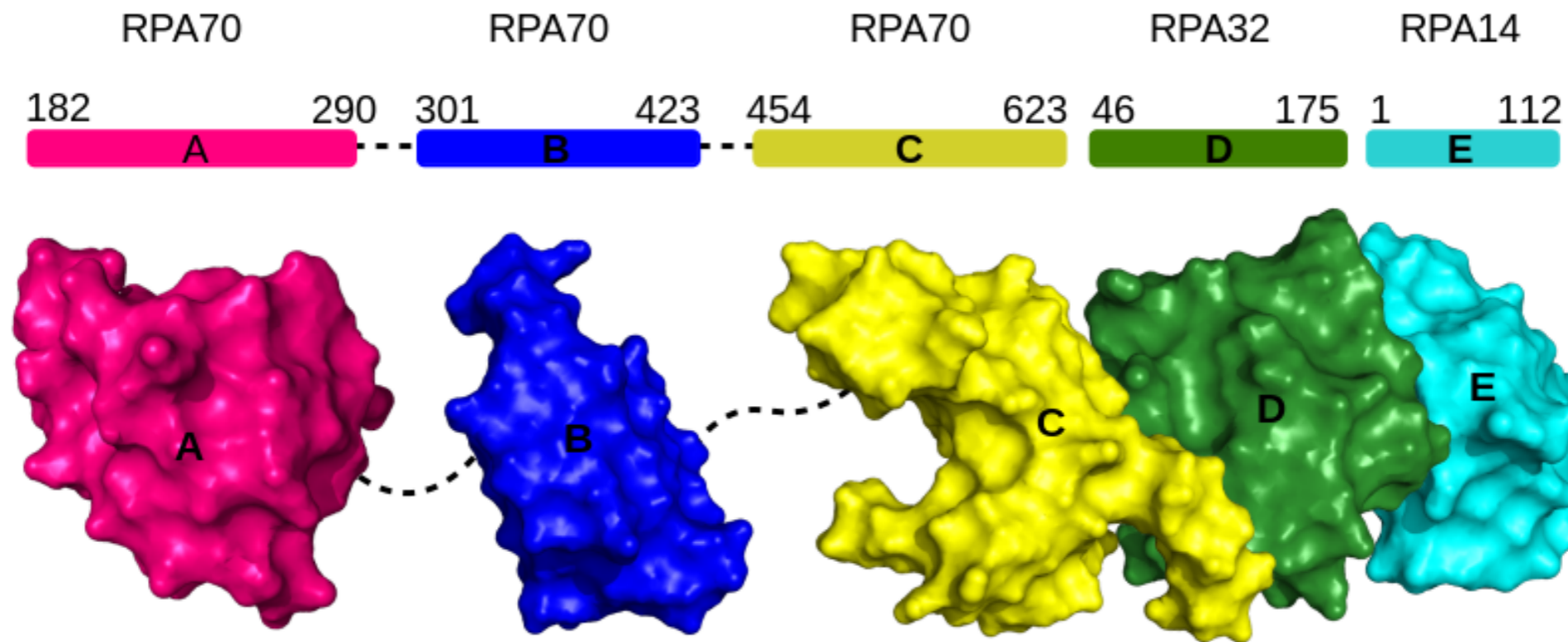




## Conclusions

- *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.*

## Changing the Path : TRANSPORT of Protein on ssDNA track



ssDNA length dependent RPA activity

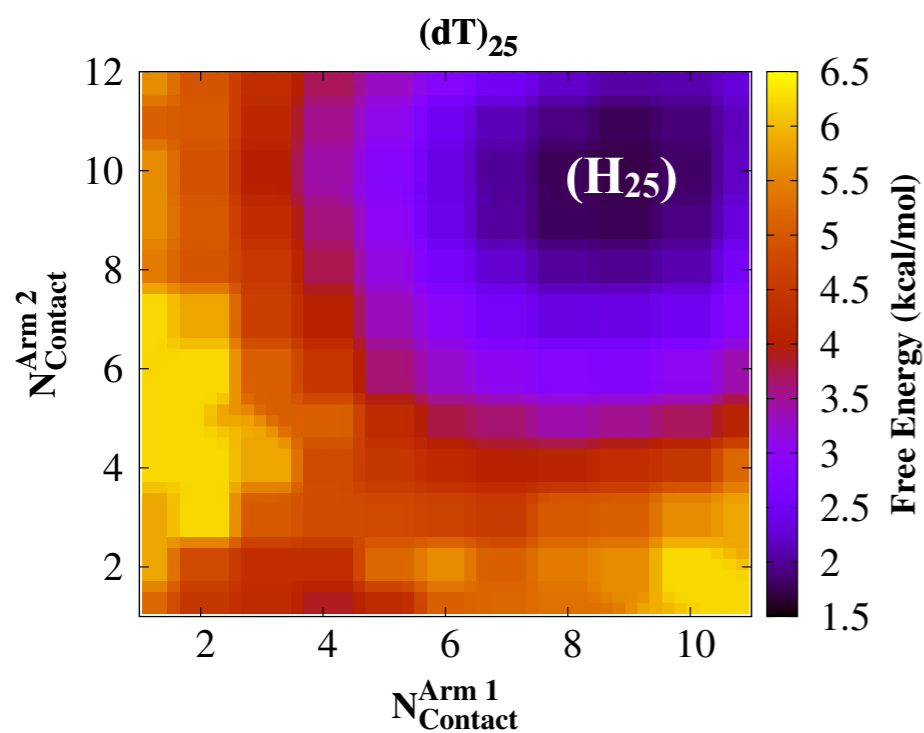
- ◆ Long but short-lived ssDNA intermediate binds to RPA during DNA replication.
- ◆ Shorter ssDNA intermediates (<30 nt) form a stable RPA-ssDNA complex for processing and repairing the DNA damage.



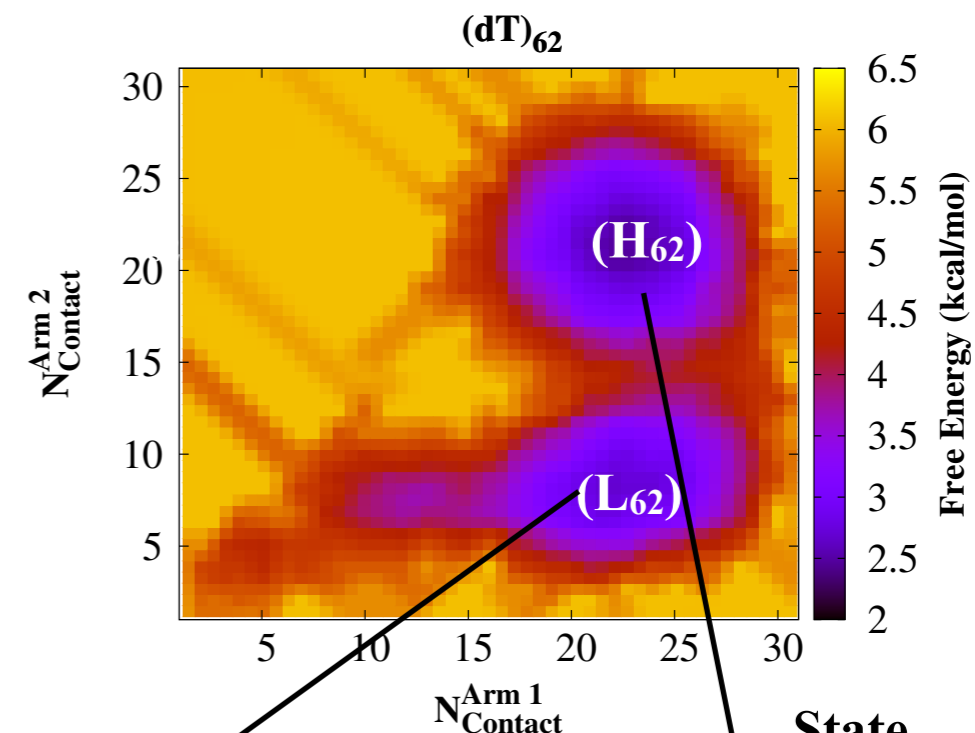
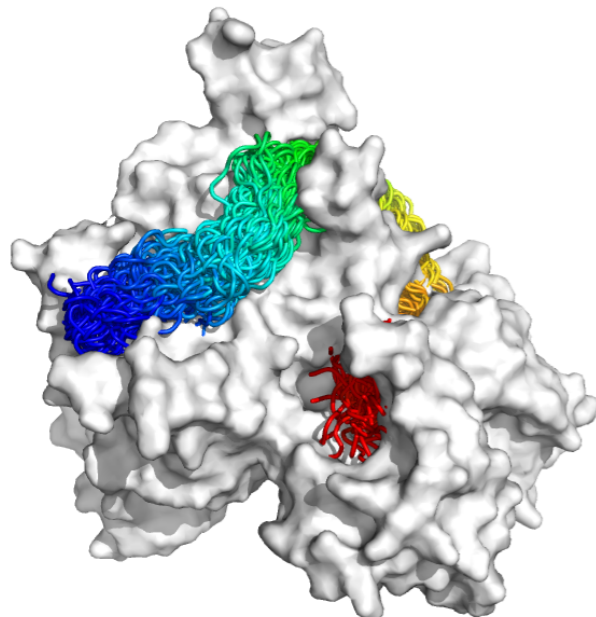
# TRANSPORT of RPA Protein on ssDNA

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

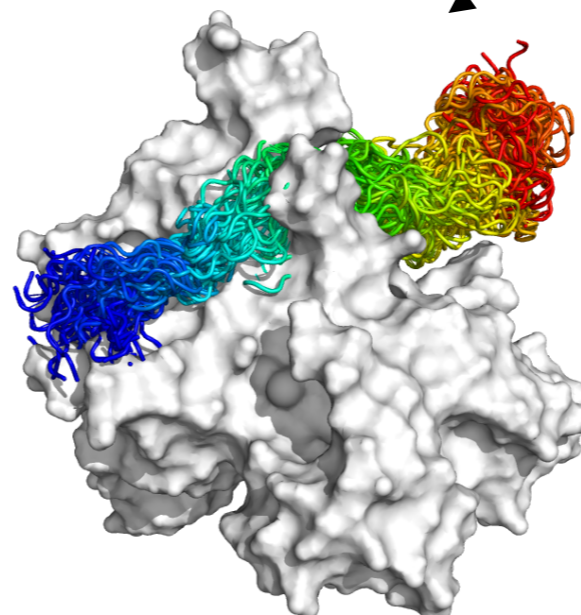
$$\Delta G_{H_{62}-L_{62}} = 0.7 \pm 0.001 \text{ kcal/mol}$$



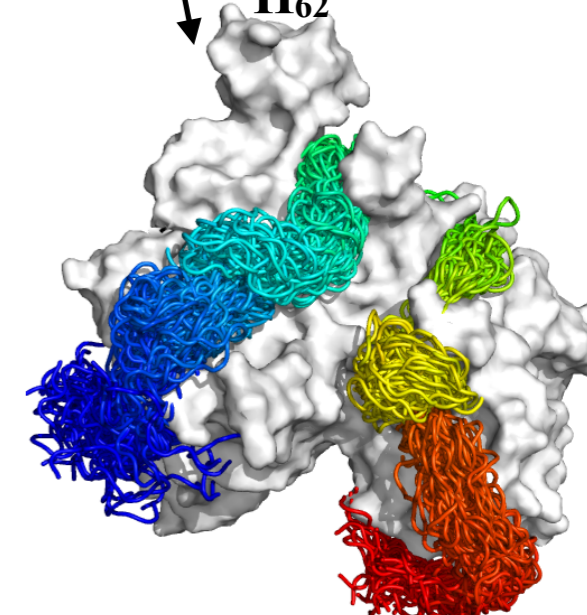
State H<sub>25</sub>



State L<sub>62</sub>

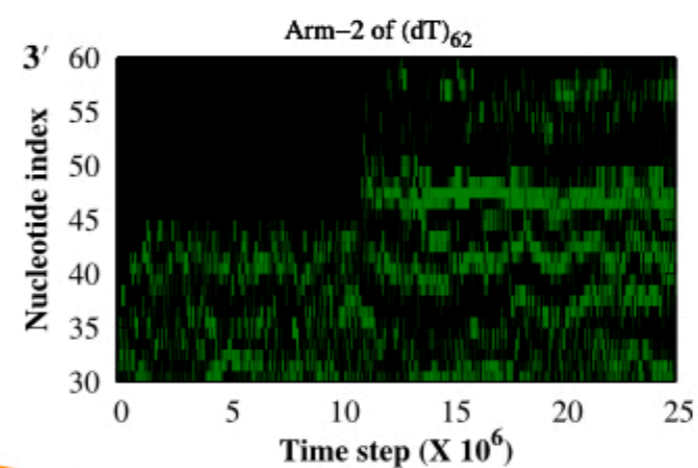
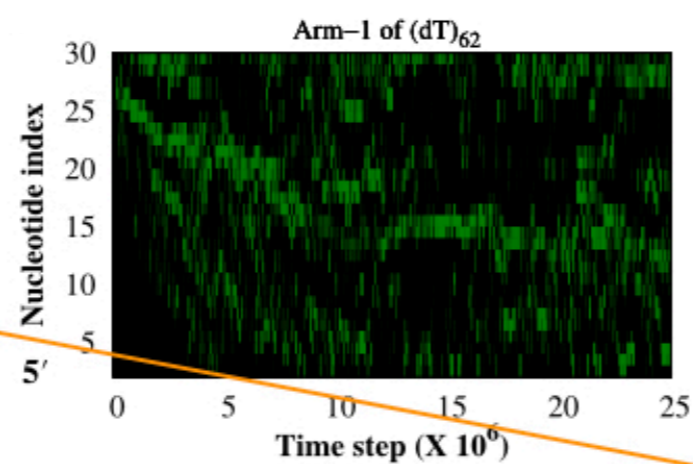
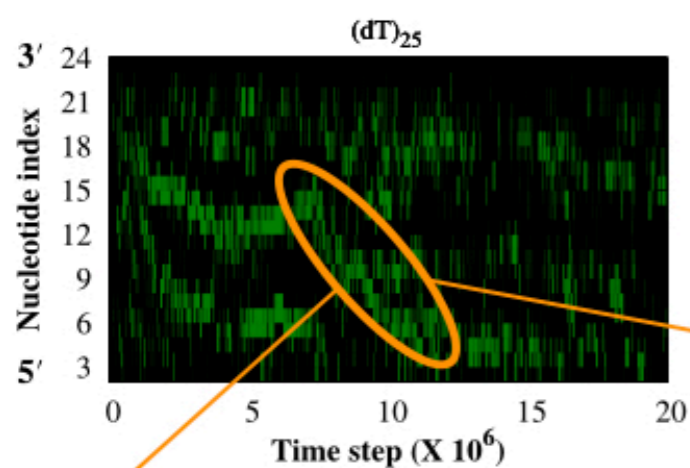
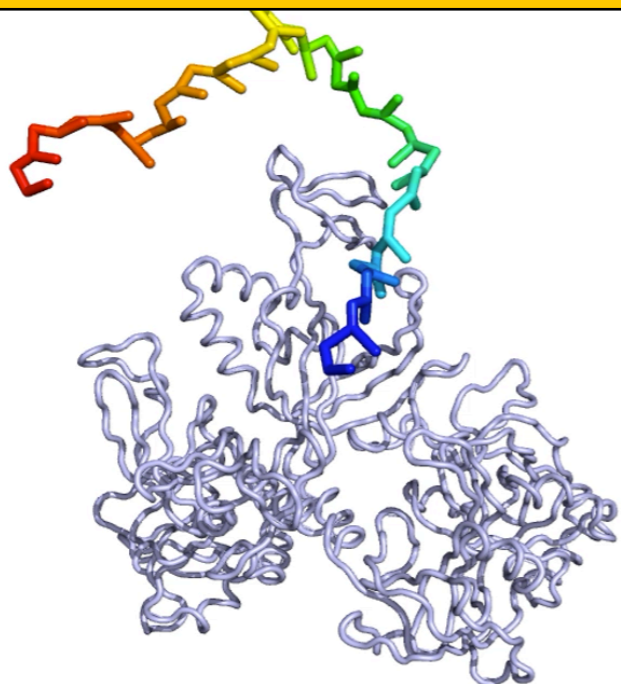


State H<sub>62</sub>

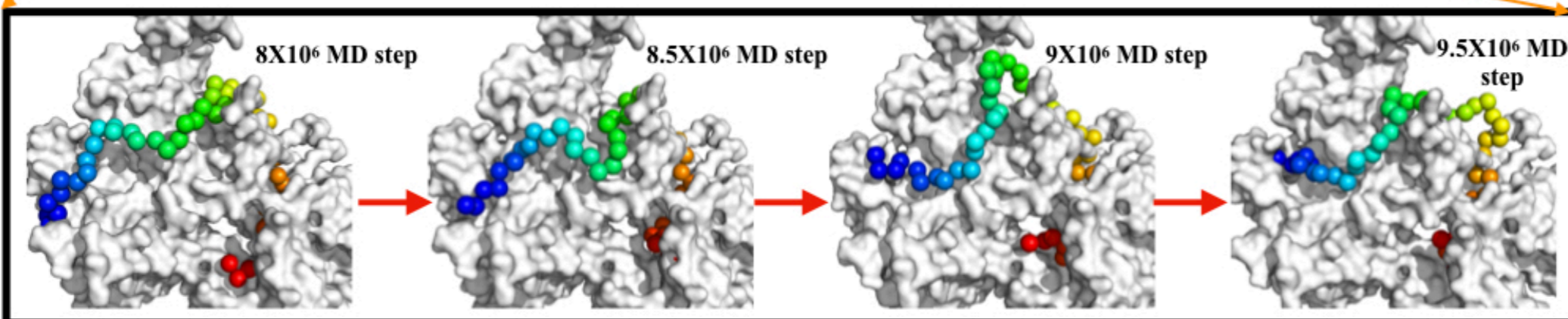


# TRANSPORT of RPA Protein on ssDNA

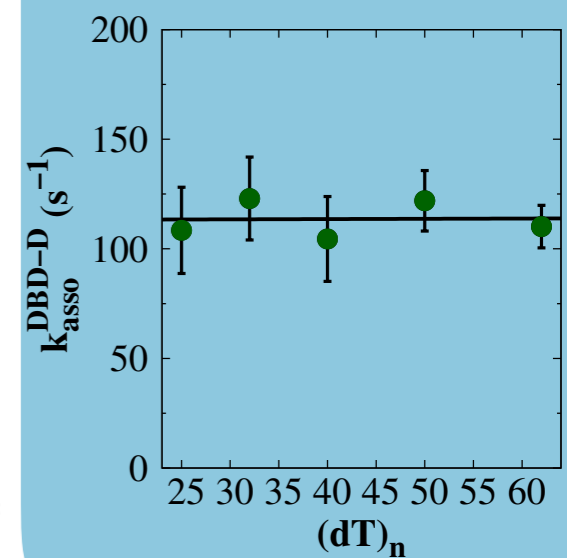
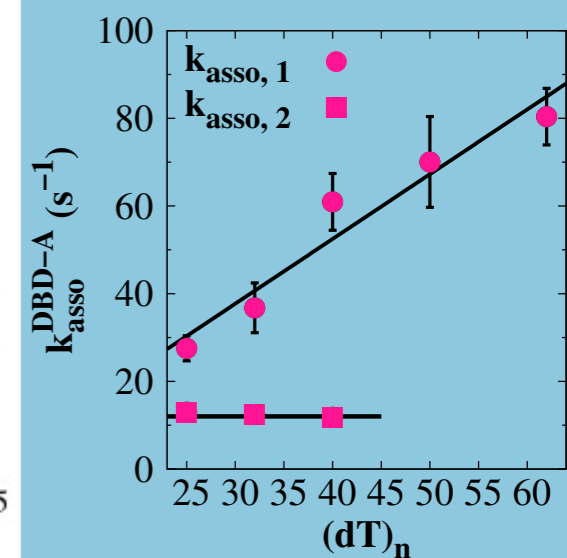
RPA-dT<sub>25</sub>



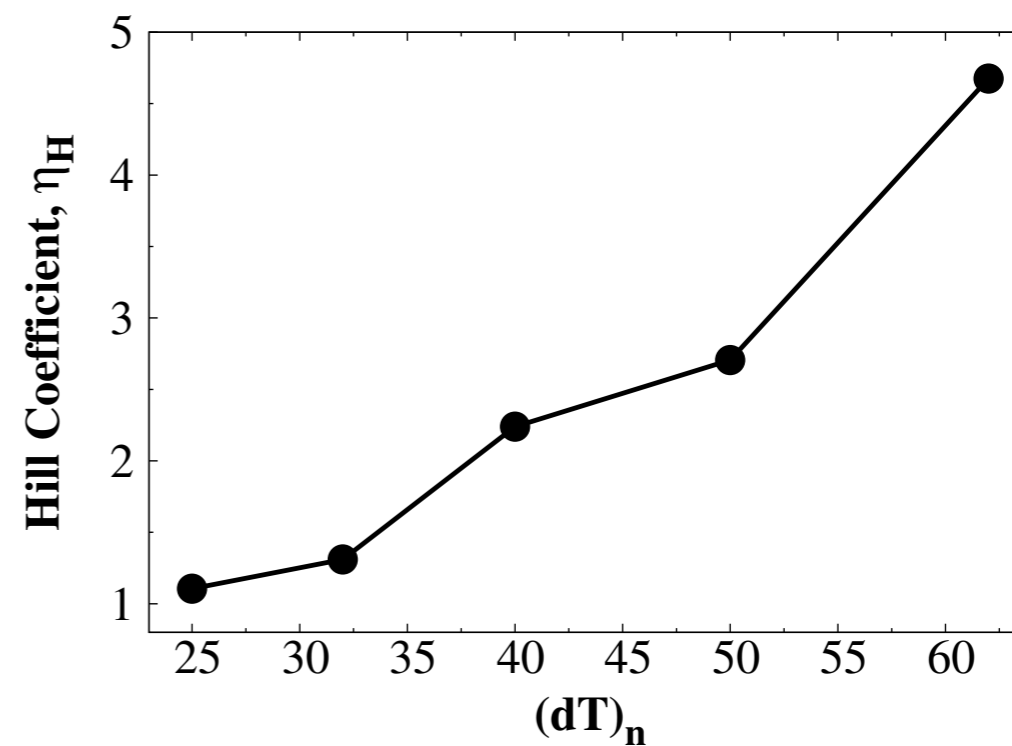
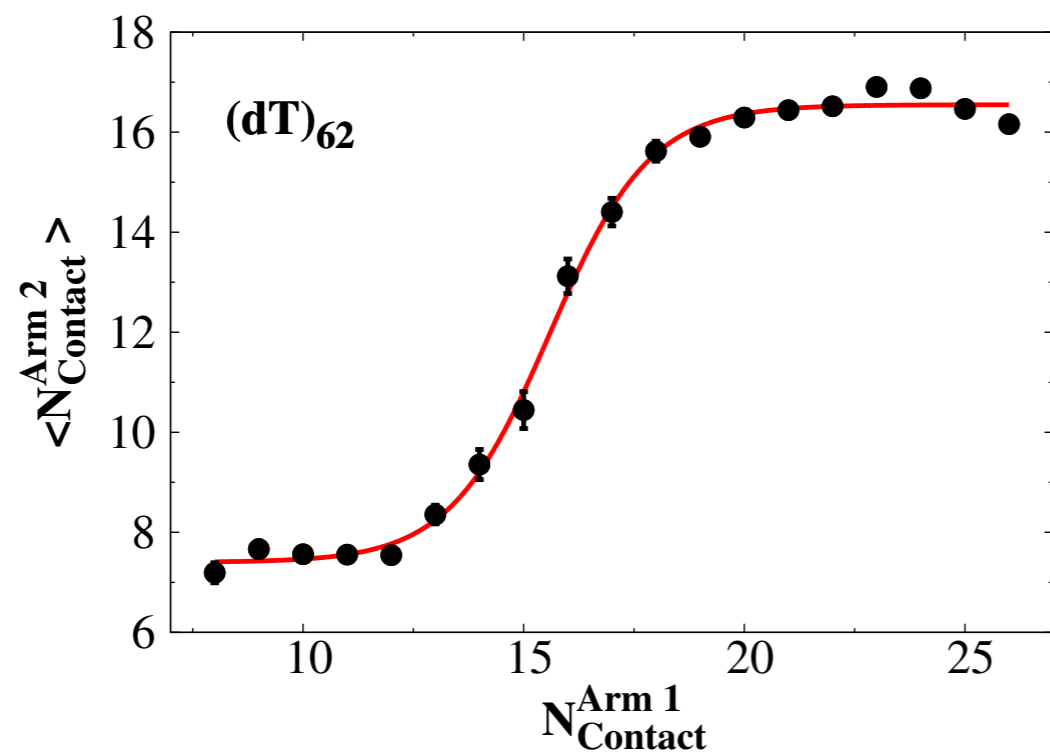
F)



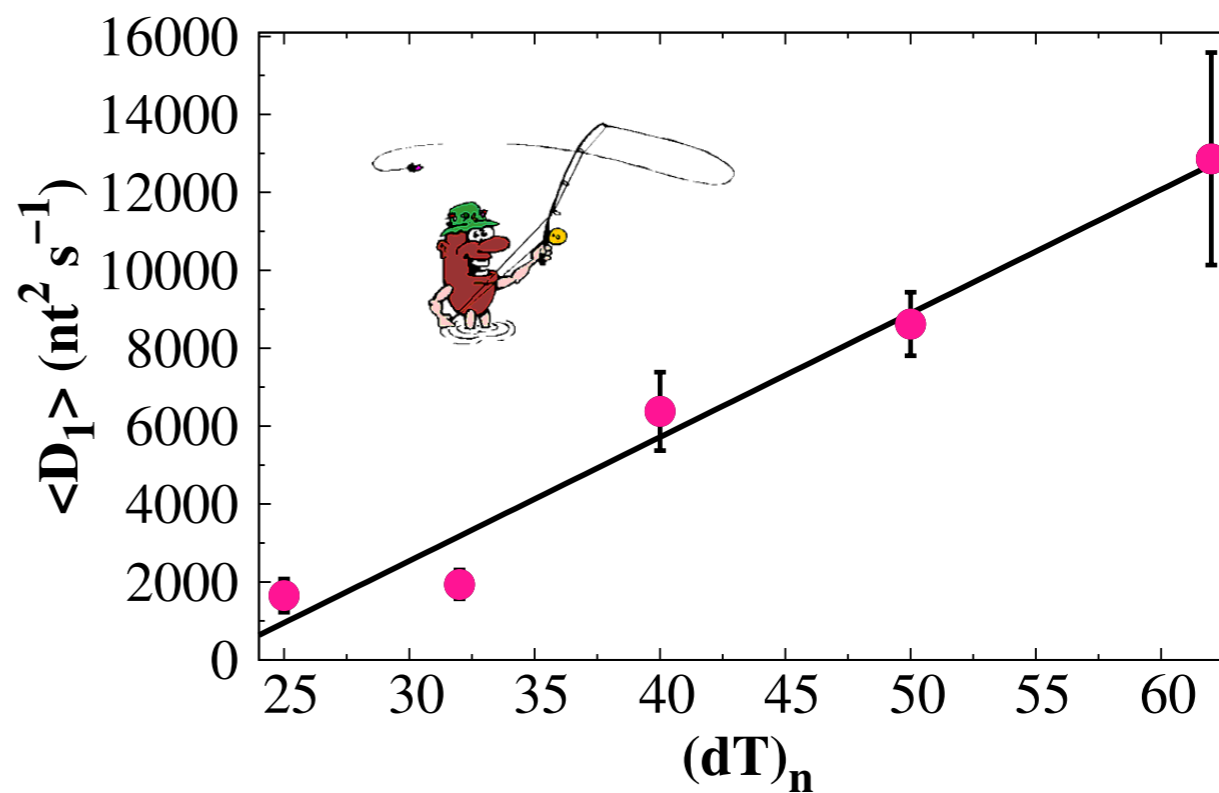
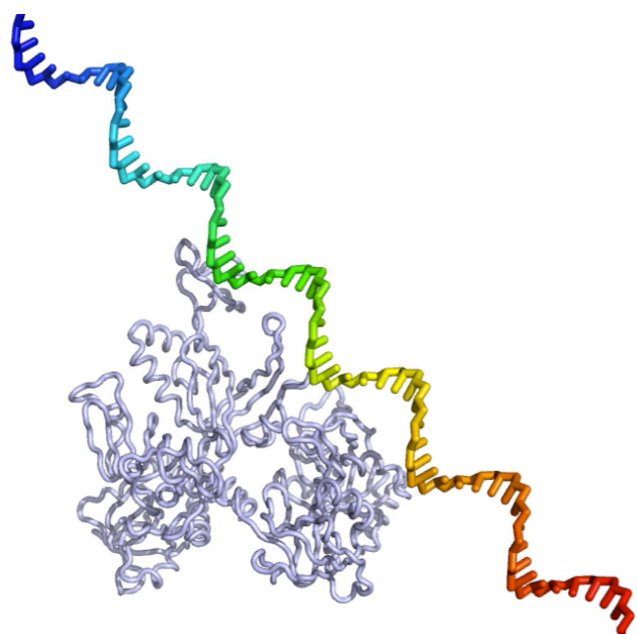
**BUT !**



# Cooperative Binding of ssDNA to RPA



RPA- $dT_{62}$



## 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 dissolve 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.

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*Funding:*

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*Enjoying our journey.....*



THANK YOU