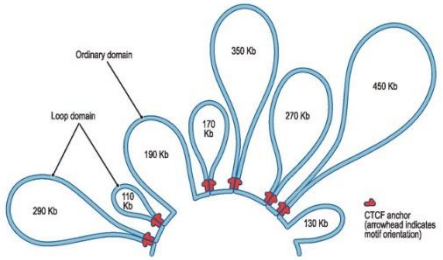
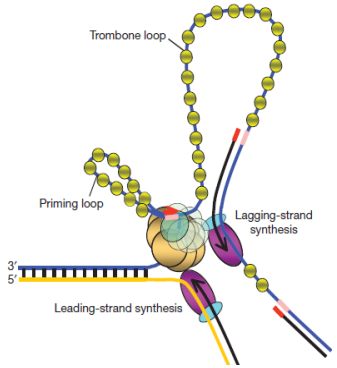
Protein-mediated DNA loops and supercoiling are ubiquitous

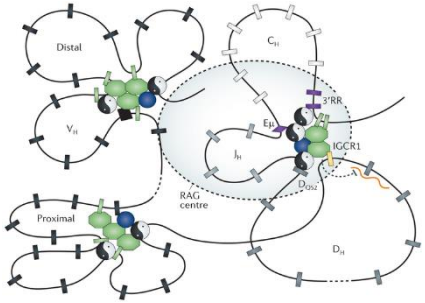
genomic structure



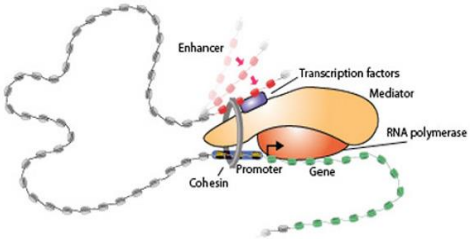
DNA replication

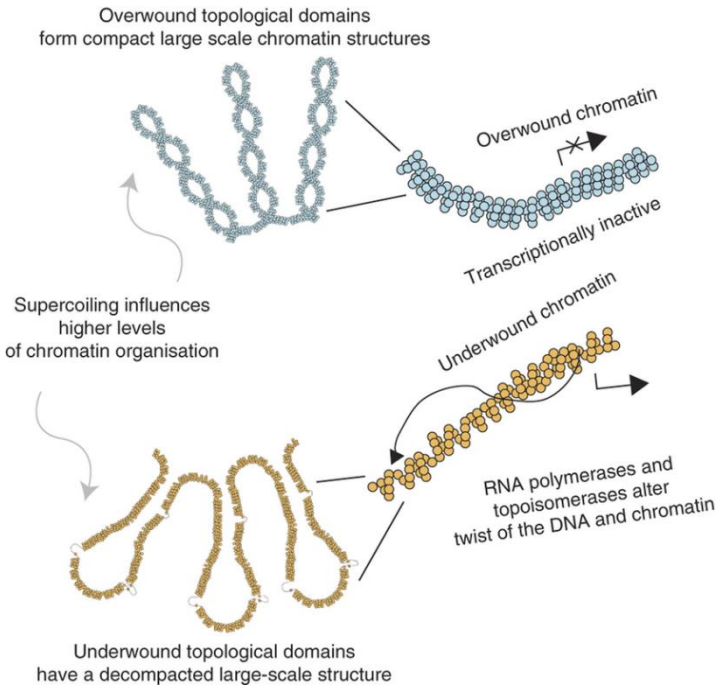


DNA recombination



DNA transcription





https://www.seas.harvard.edu/news/2014/12/scientists-map-human-loop-ome; Pandey, M., Syed, S., Donmez, I., Patel, G., Ha, T., & Patel, S. S. (2009). *Nature*, *462*(7275), 940-943; Ong, Chin-Tong, and Victor G. Corces. *Nature Reviews. Genetics* 15.4 (2014): 234; http://wi.mit.edu/news/archive/2010/surprise-genome-structure-linked-developmental-diseases

Naughton et al., Nat Struct & Mol Bio, 2013

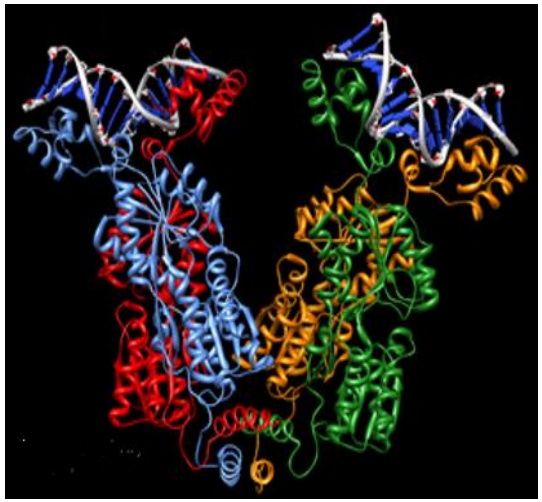
Questions

1. How does DNA supercoiling affect protein-mediated looping?

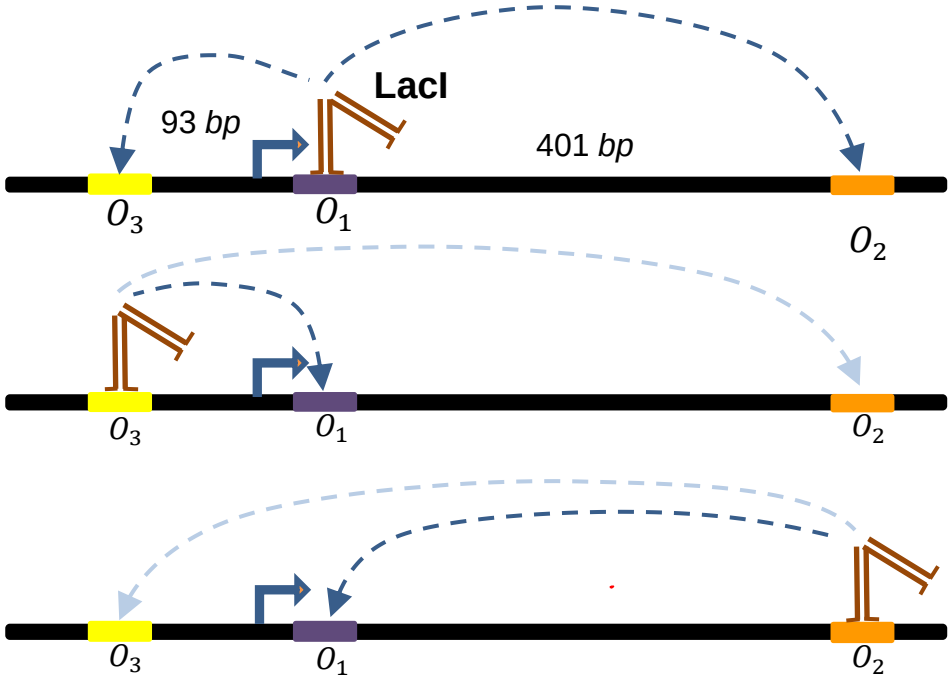
2. What effect do loops have on a transcribing RNA polymerase?

Lac repressor (LacI) mediates DNA looping

LacI Structure



Loop Enhances Repression



Binding Affinity of Lac Operators:

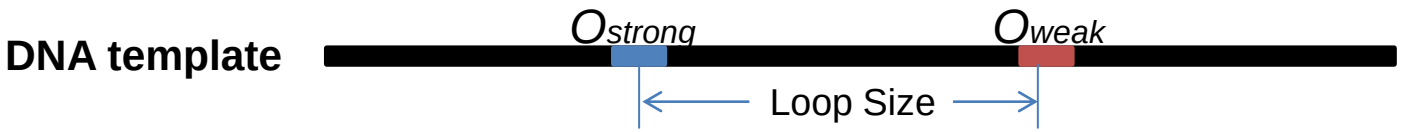
$$O_S > O_1 > O_2 > O_3$$

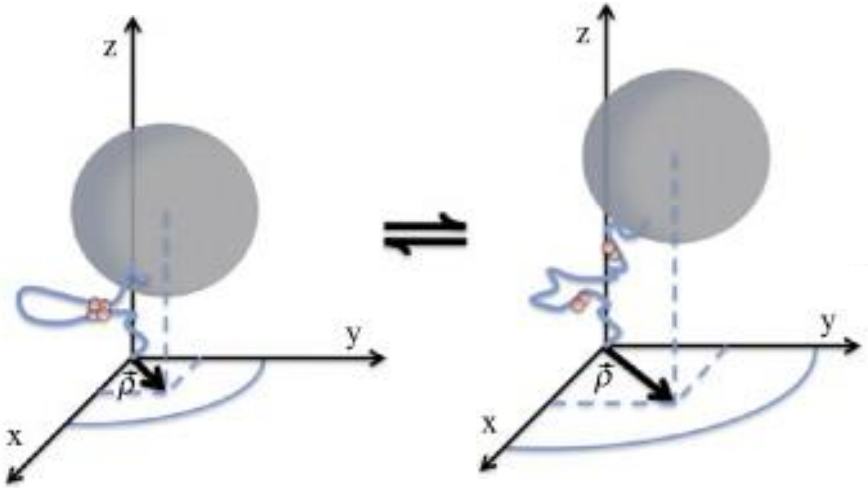
Engineered Operator

Wildtype Operators

Stefan Oehler J. Bacteriol. (2009);191:5301-5303; Vilar, Jose MG, and Leonor Saiz. 15.2 (2005): 136-144.

LacI-induced looping can be measured in torsionally relaxed DNA by TPM

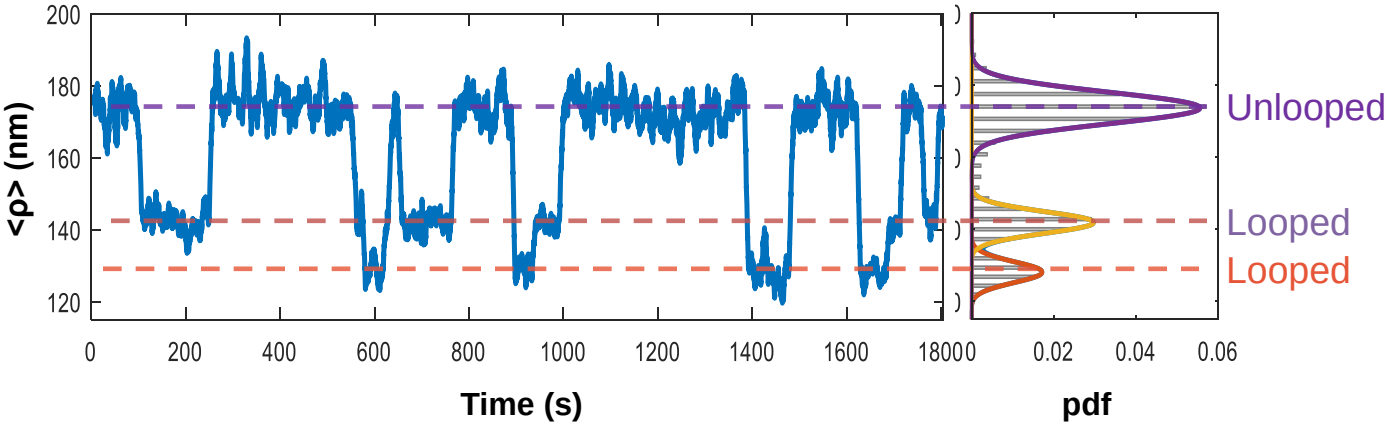




The motion of the bead projected in two dimensions (x-y) is used to calculate the time averaged excursion about the tether anchor:

$$< \rho >_t = < \sqrt{(x - \bar{x}_t)^2 + (y - \bar{y}_t)^2} >_t$$

The effective length of DNA molecule can then be calculated from a calibration curve.

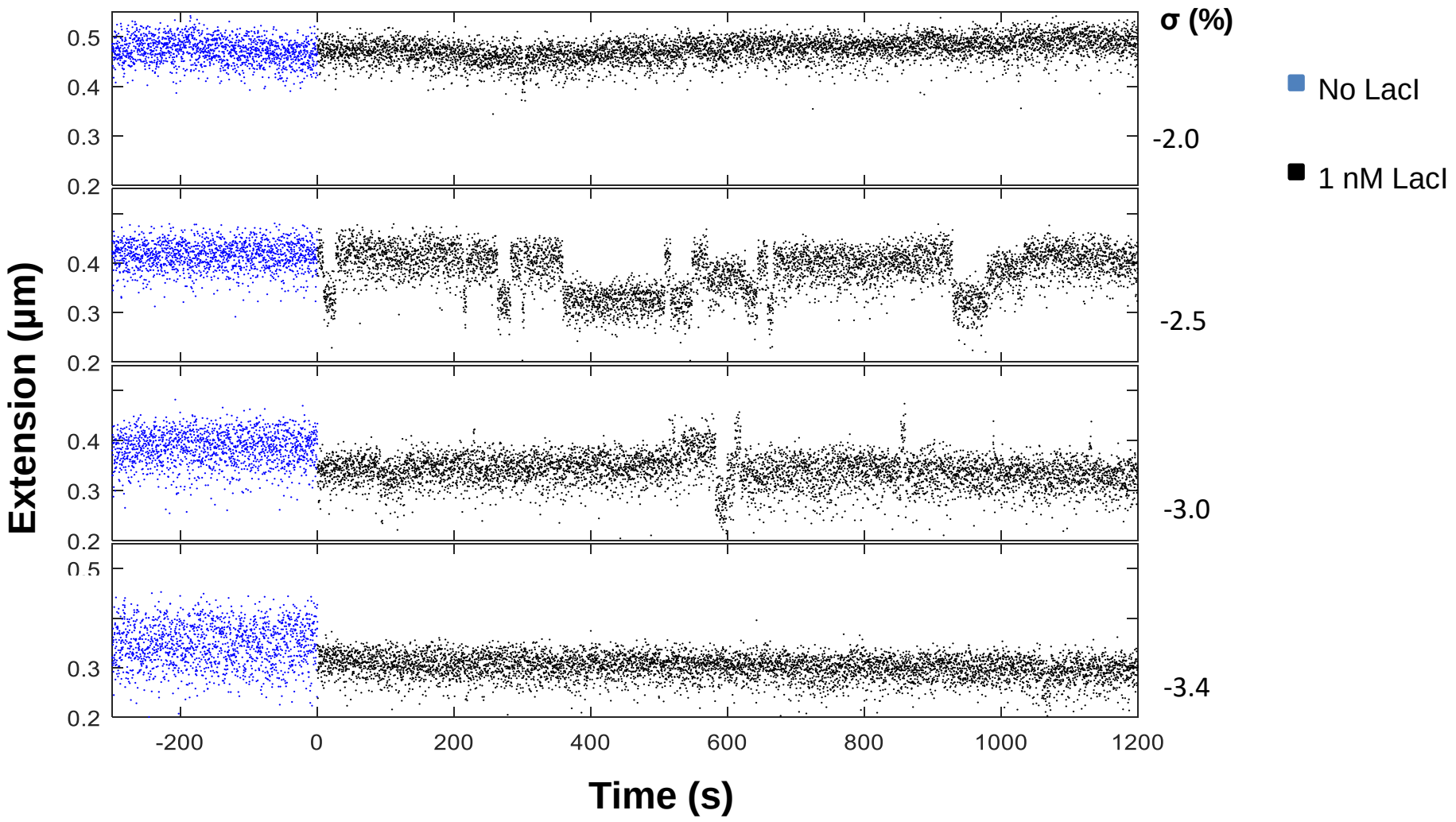


Looping Probability

$$P_{loop} = \frac{t_{loop}}{t_{total}}$$

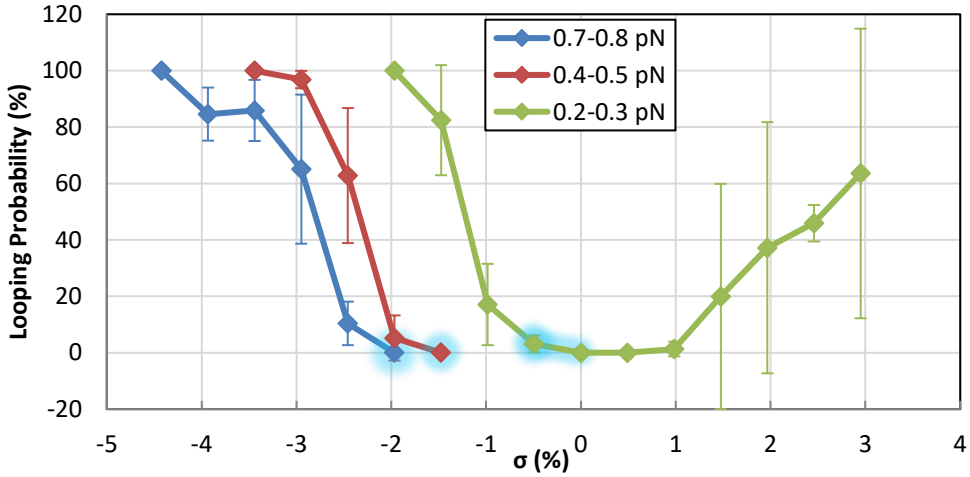
Yan Yan et al, "Protein-mediated looping of DNA under tension requires supercoiling", *Nucleic Acids Research (NAR)* 2018

Supercoiling enhances loop formation



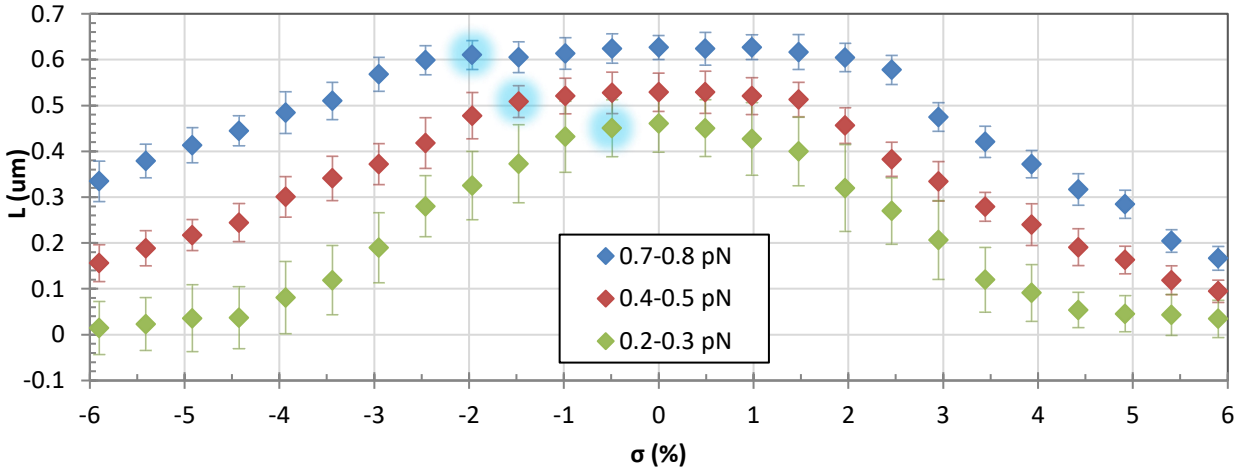
Yan Yan et al, “Protein-mediated looping of DNA under tension requires supercoiling”, *NAR*, 2018

Supercoiling enhances loop formation against tension



For each tension:

- Looping probability increased from 0 to 100% as negative supercoiling density increased.
- Negative supercoiling enhanced looping more than positive supercoiling.



Comparing looping probability with extension vs. twist curves:

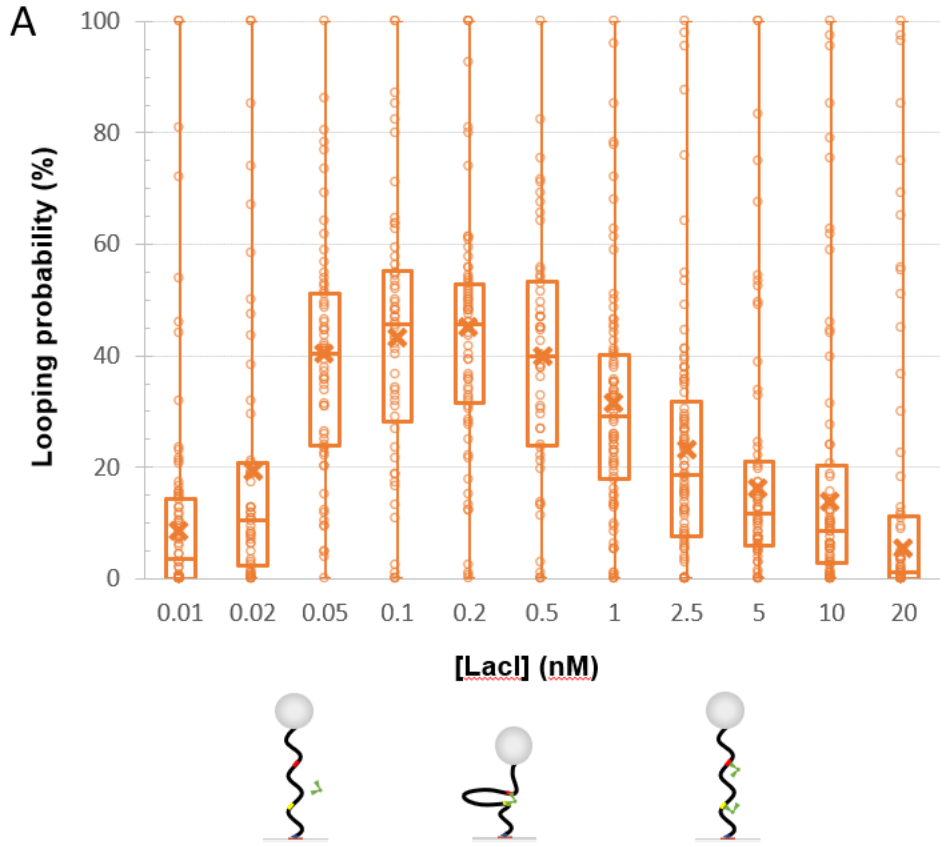
- The onset of loop formation catalyzed by negative supercoiling coincides with plectoneme formation.

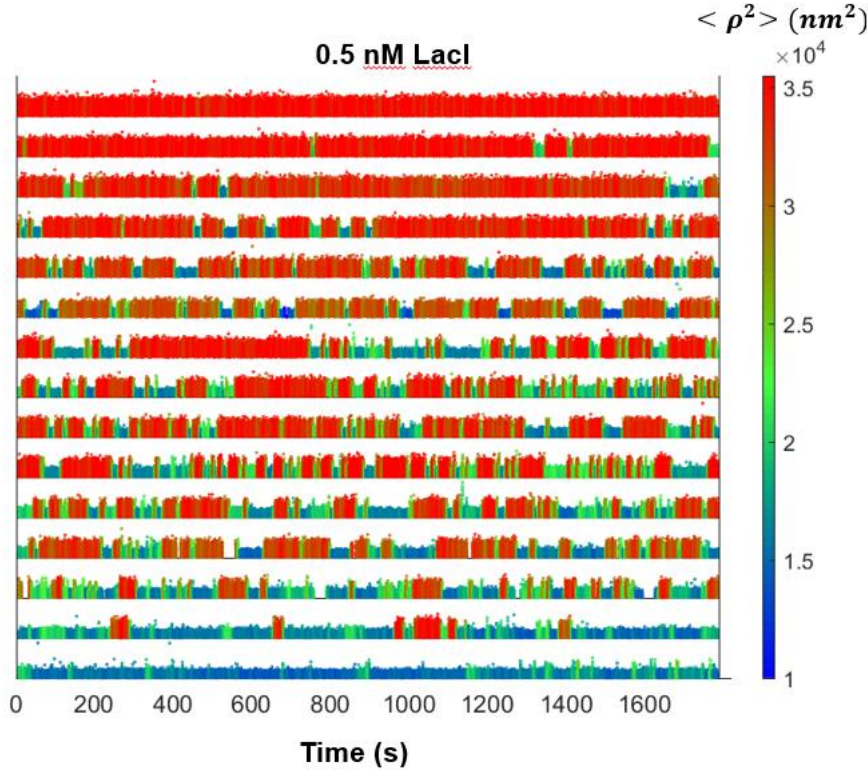
Comparing different tensions,

- At higher tension, more supercoiling is required to achieve same level of looping probability.
- Non-zero looping probabilities span a larger range of supercoiling at higher tension.

Yan Yan et al, “Protein-mediated looping of DNA under tension requires supercoiling”, *NAR*, 2018;

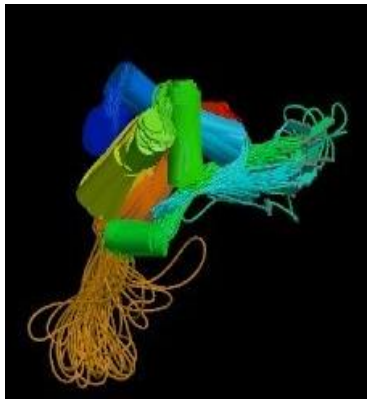
The looping probabilities of different DNA tethers vary widely in torsionally-relaxed DNA





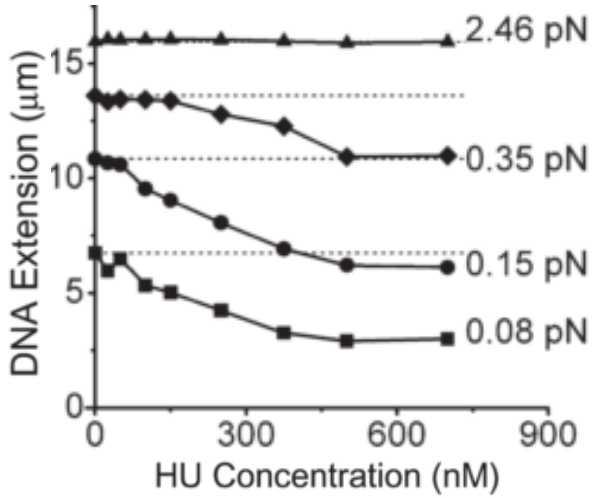
Yan et al, Nucleic Acids Research, 2021

The Heat Unstable (HU) protein is a NAP and compacts DNA



Structure of HU Protein
(From PDB)

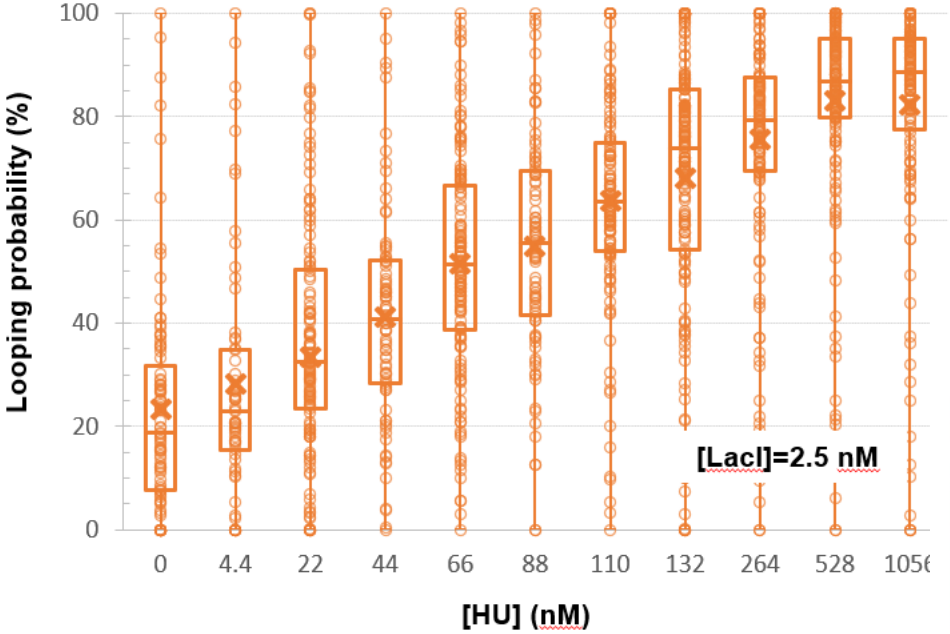
Structure	▪ Hetero-dimer (2 subunits HUα and HUβ)
Mass	▪ 18kDa (Mass of dimer)
Character	▪ Small basic protein
	▪Heat unstable
	▪ Non-specific DNA binding protein
	▪ Nucleoid associated protein
Function	▪ Introduces negative supercoiling
	▪ Condenses the bacterial chromosome
	▪ Influences DNA replication and transcription

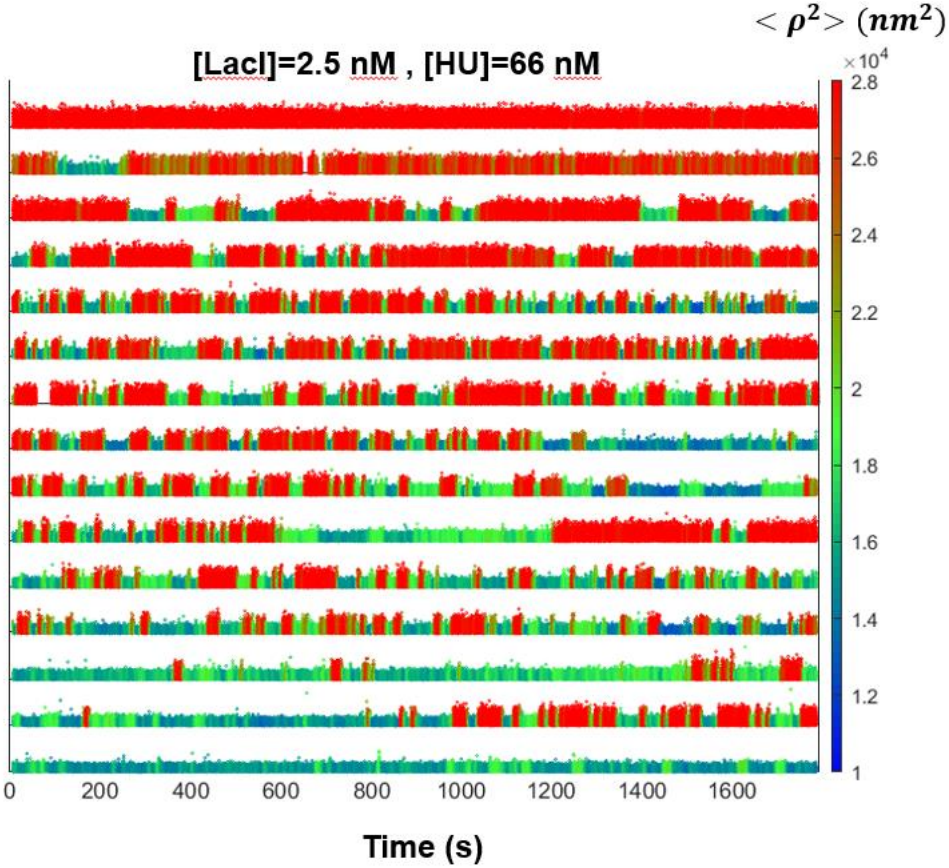


- At physiological monovalent salt concentration (150 mM NaCl) HU progressively compacts DNA and decreases its persistence length.

Xiao, B., Johnson, R. C. & Marko, J. F. (2010), *Nucleic Acids Res,* 38**,** 6176-85; Coste, F., Hervouet, N., Oberto, J., Zelwer, C., & Castaing, B. (1999). *55*(11), 1952-1954

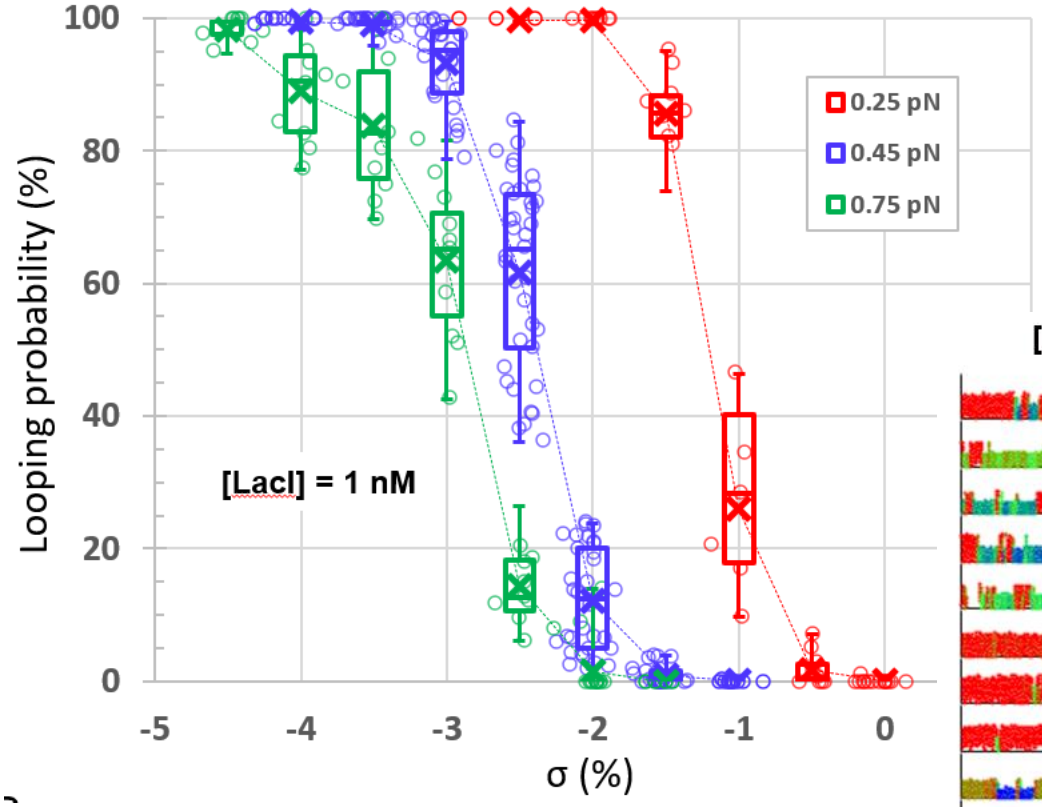
HU does not reduce the variation of looping probabilities among different tethers

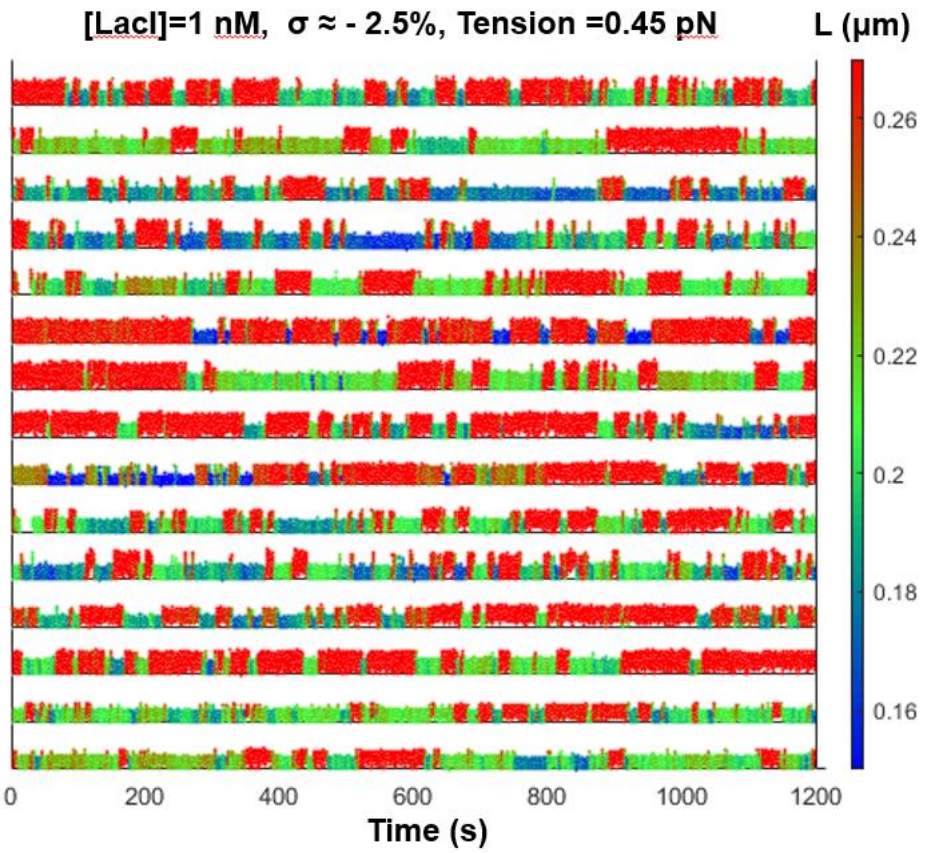




Yan et al, Nucleic Acids Research, 2021

Supercoiling dramatically reduces the variation of looping probabilities among DNA tethers





Yan et al, Nucleic Acids Research, 2021

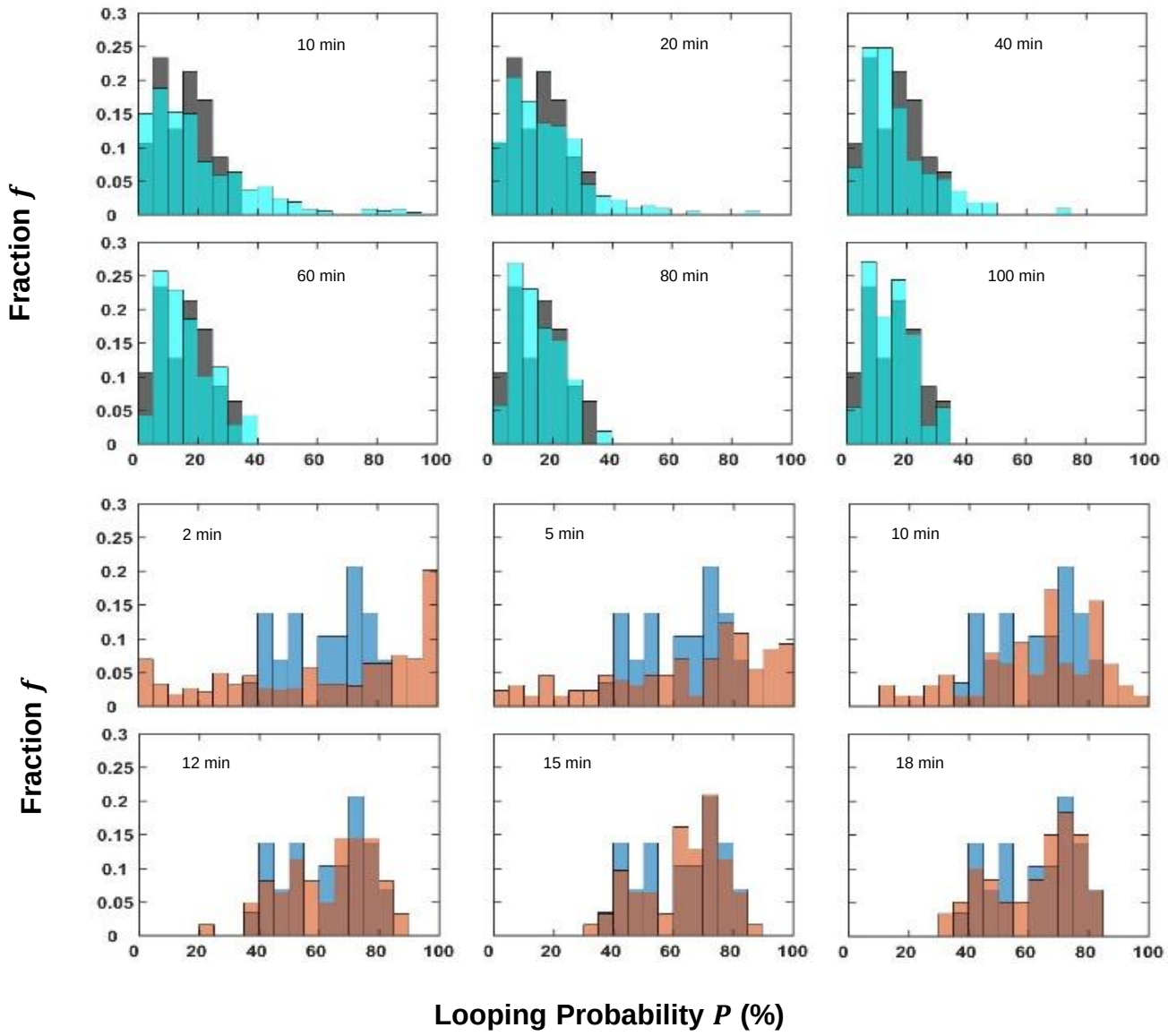
Sufficiently long observations are ergodic

TPM

MT

0.45 pN

-1.5% supercoiling



Yan et al, Nucleic Acids Research, 2021

Conclusions

1. DNA Supercoiling favors looping against tension.

2. When negatively supercoiled, each DNA molecule followed the average behavior.

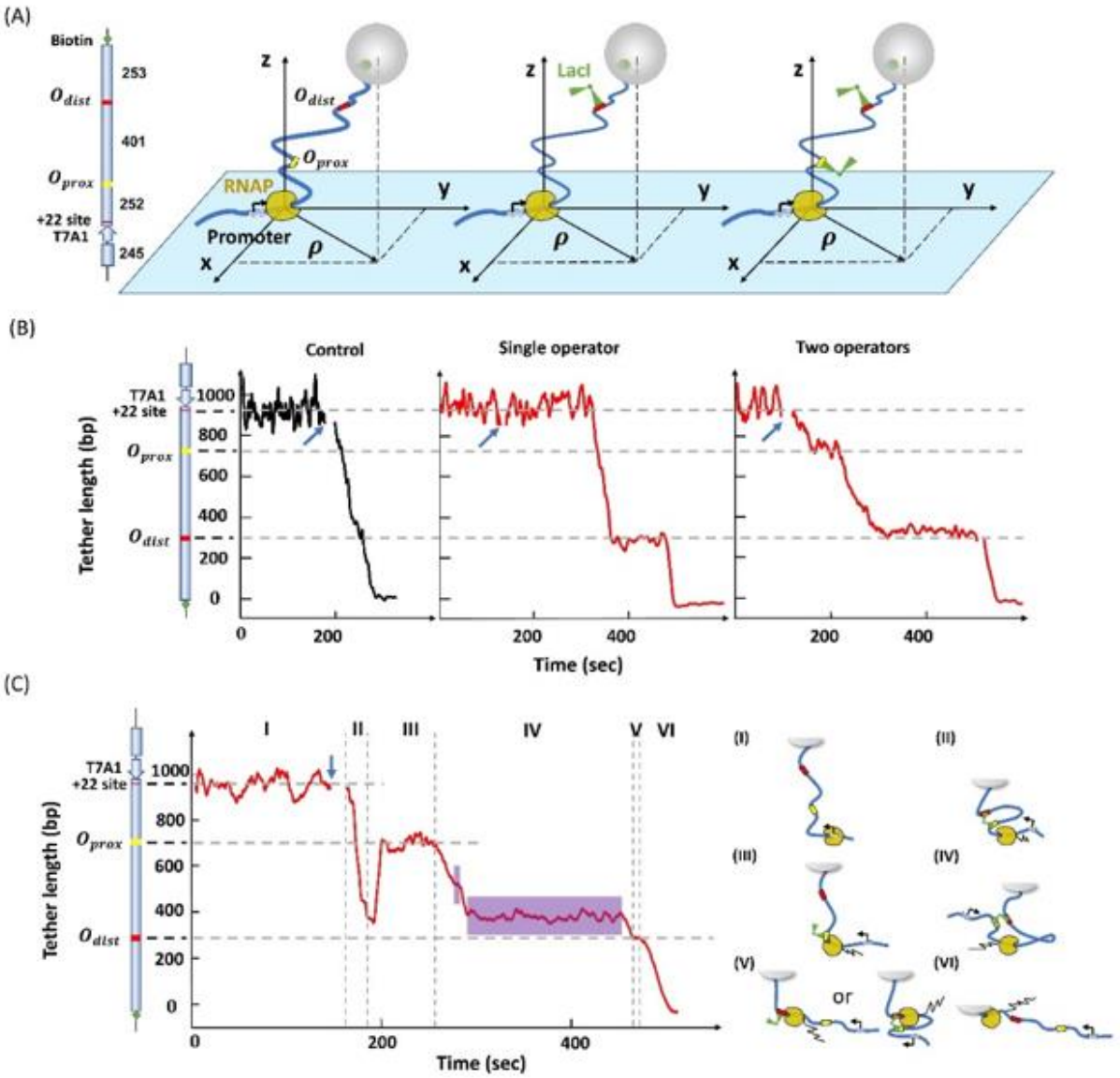
3. Furthermore, in only twelve minutes of observation, well within the doubling time of the bacterium, most molecules exhibited the looping probability of the ensemble.

4. DNA supercoiling, an inherent feature of all genomes, appears to impose time-constrained, emergent behavior on otherwise random molecular activity.

Yan et al, Nucleic Acids Research, 2021

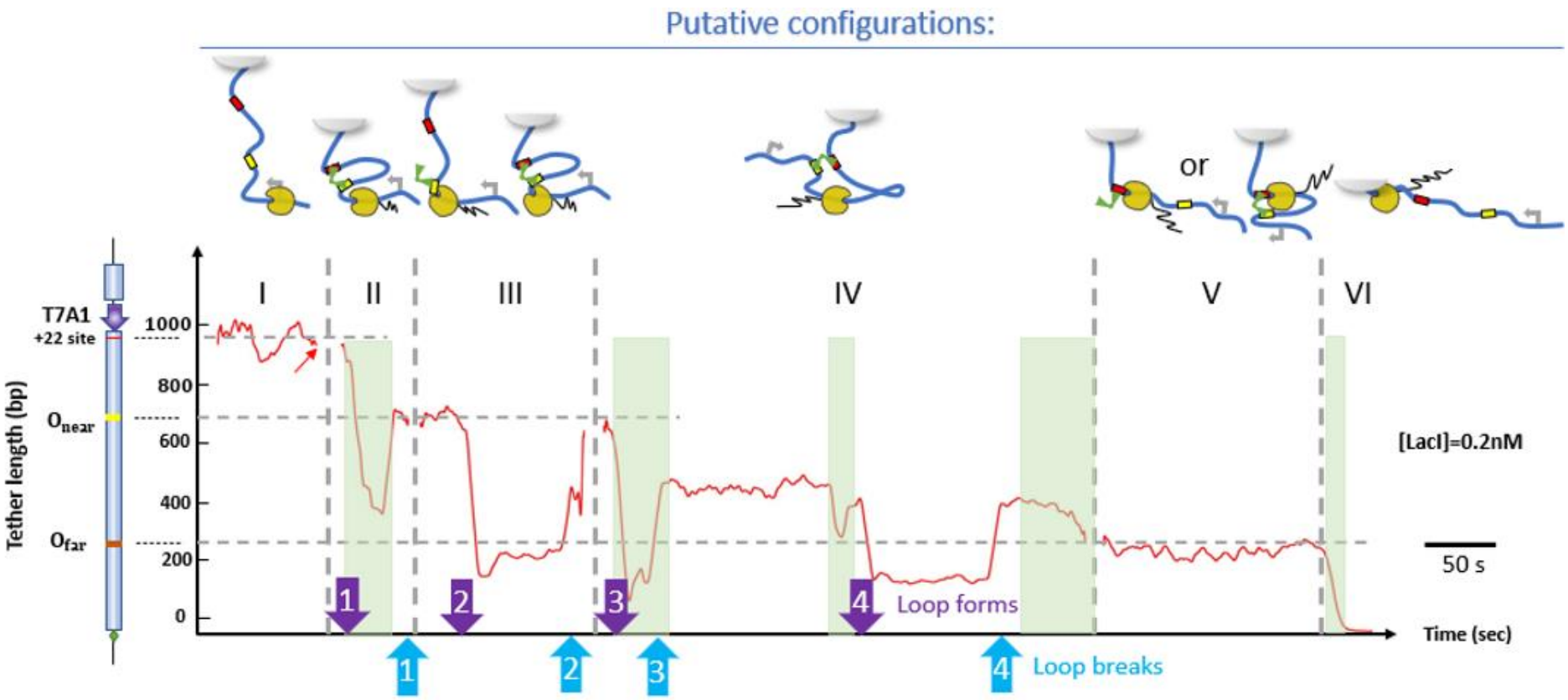
RNAP can transcribe through a LacI-mediated loop after a pause

TPM



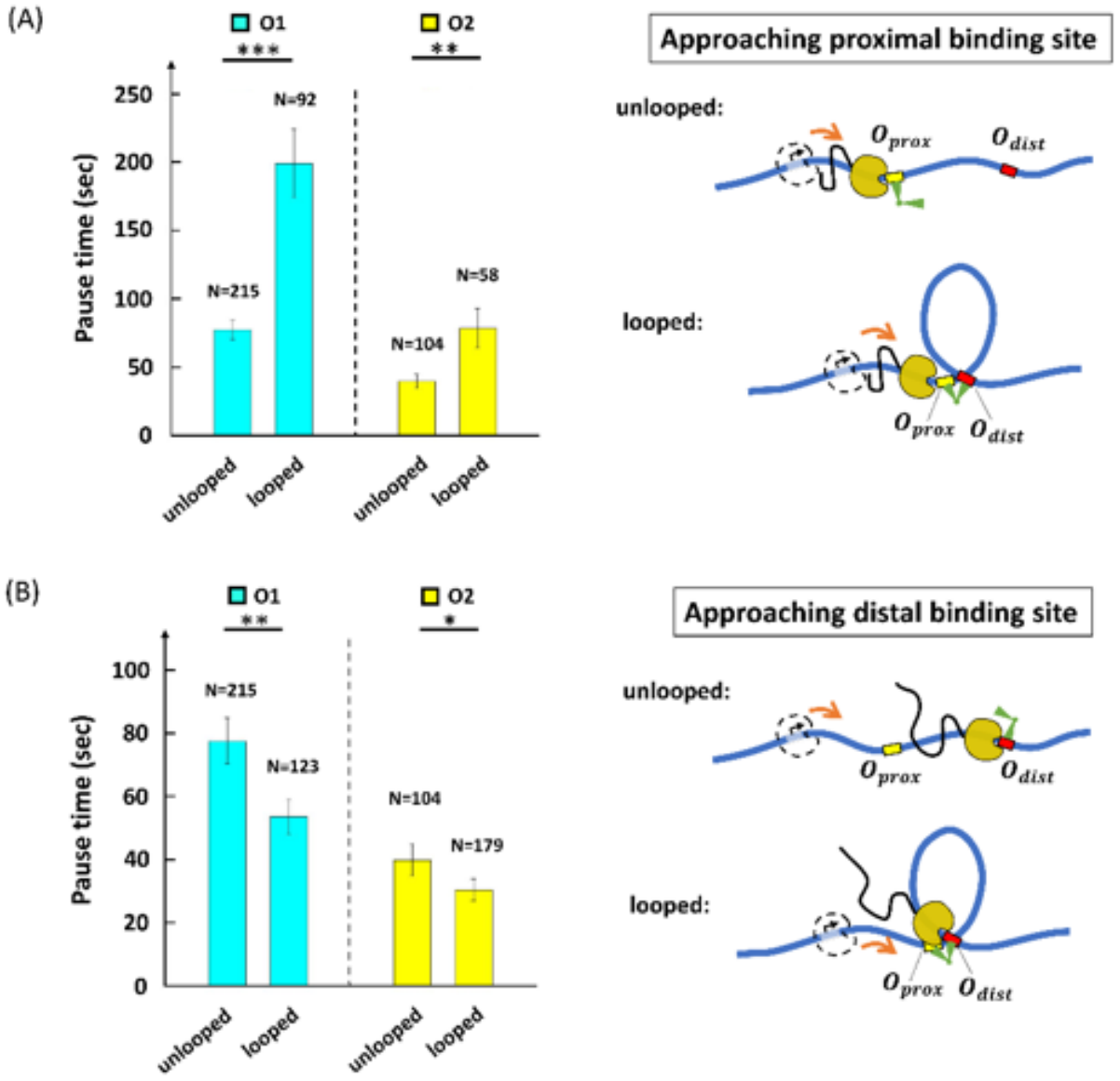
Xu et al, NAR, 2022

A LacI-mediated loop can produce discrete jumps or plateaus as transcription decreases the tether length



Xu et al, BioRxiv, 2021

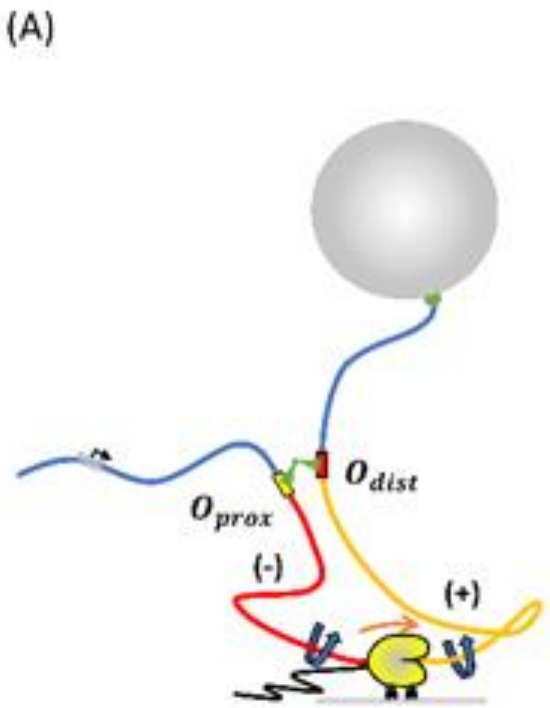
LacI-mediated looping influences pausing

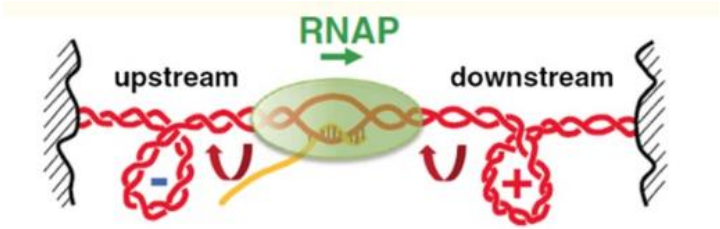


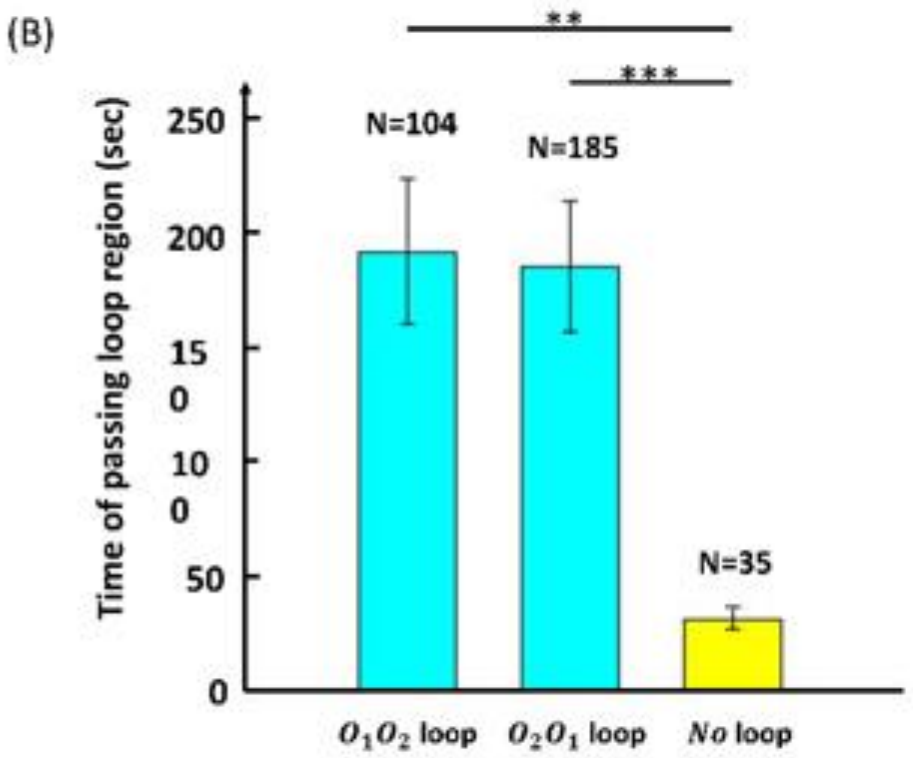
O1 > O2

Xu et al, BioRxiv, 2021

RNAP transcribes a loop more slowly

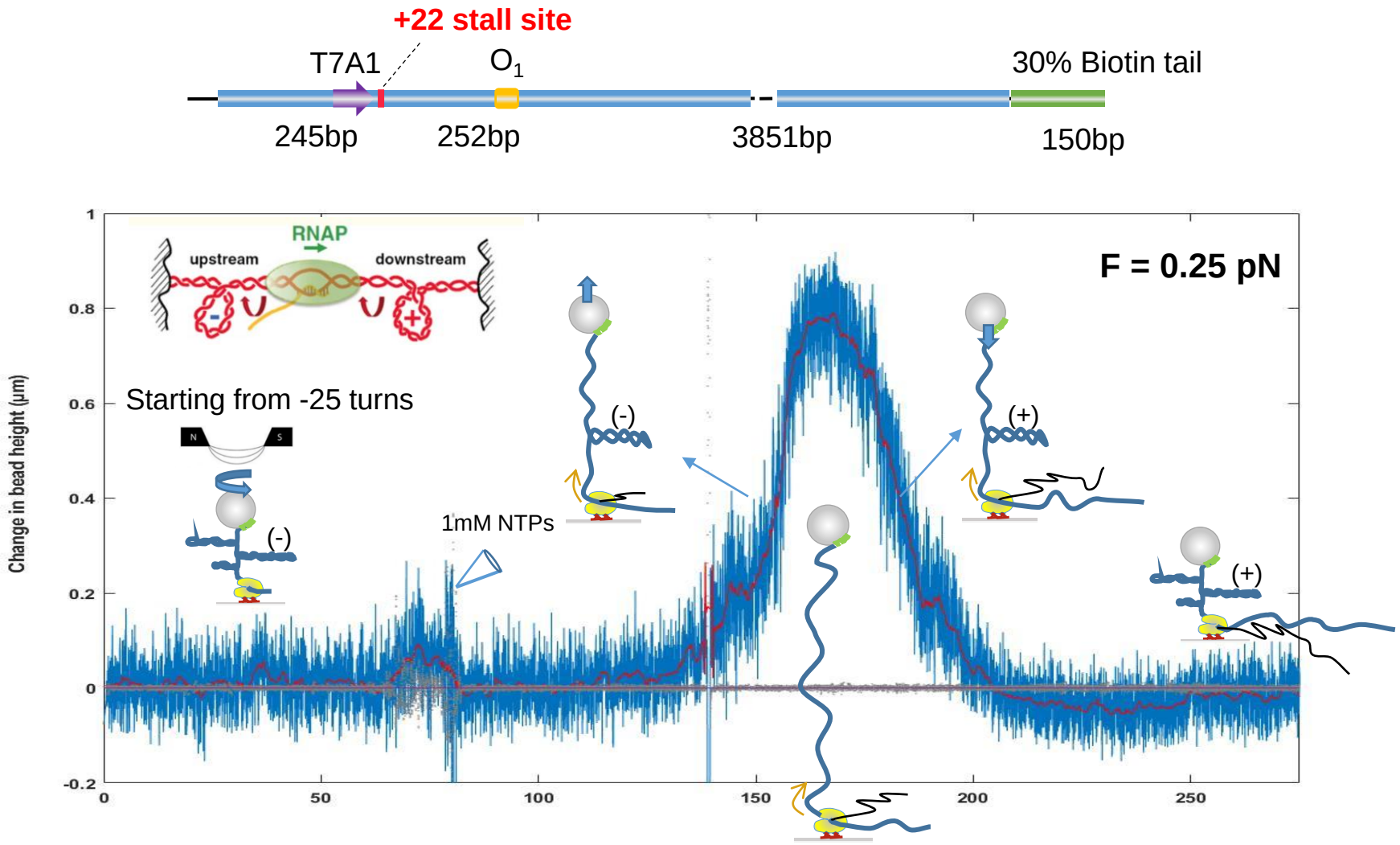






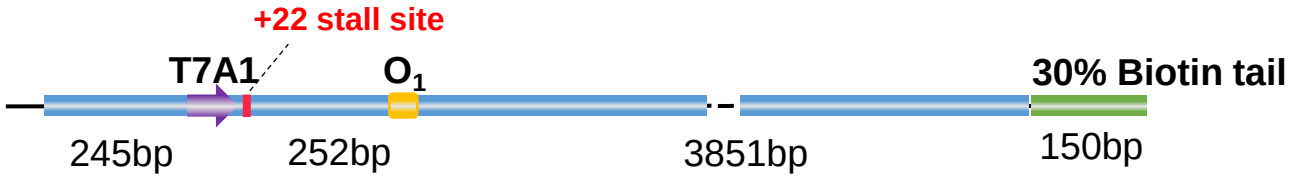
Xu et al, NAR, 2021

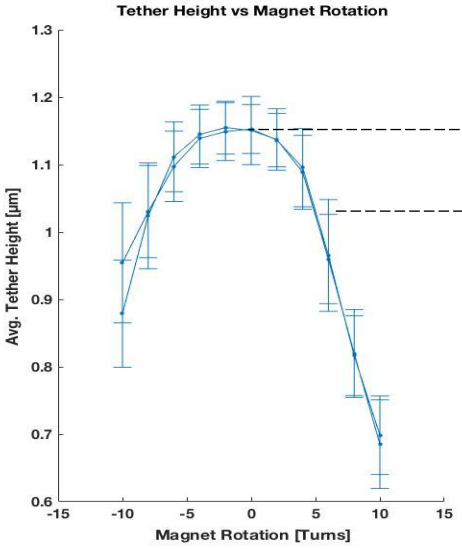
Transcription positively supercoils a DNA template



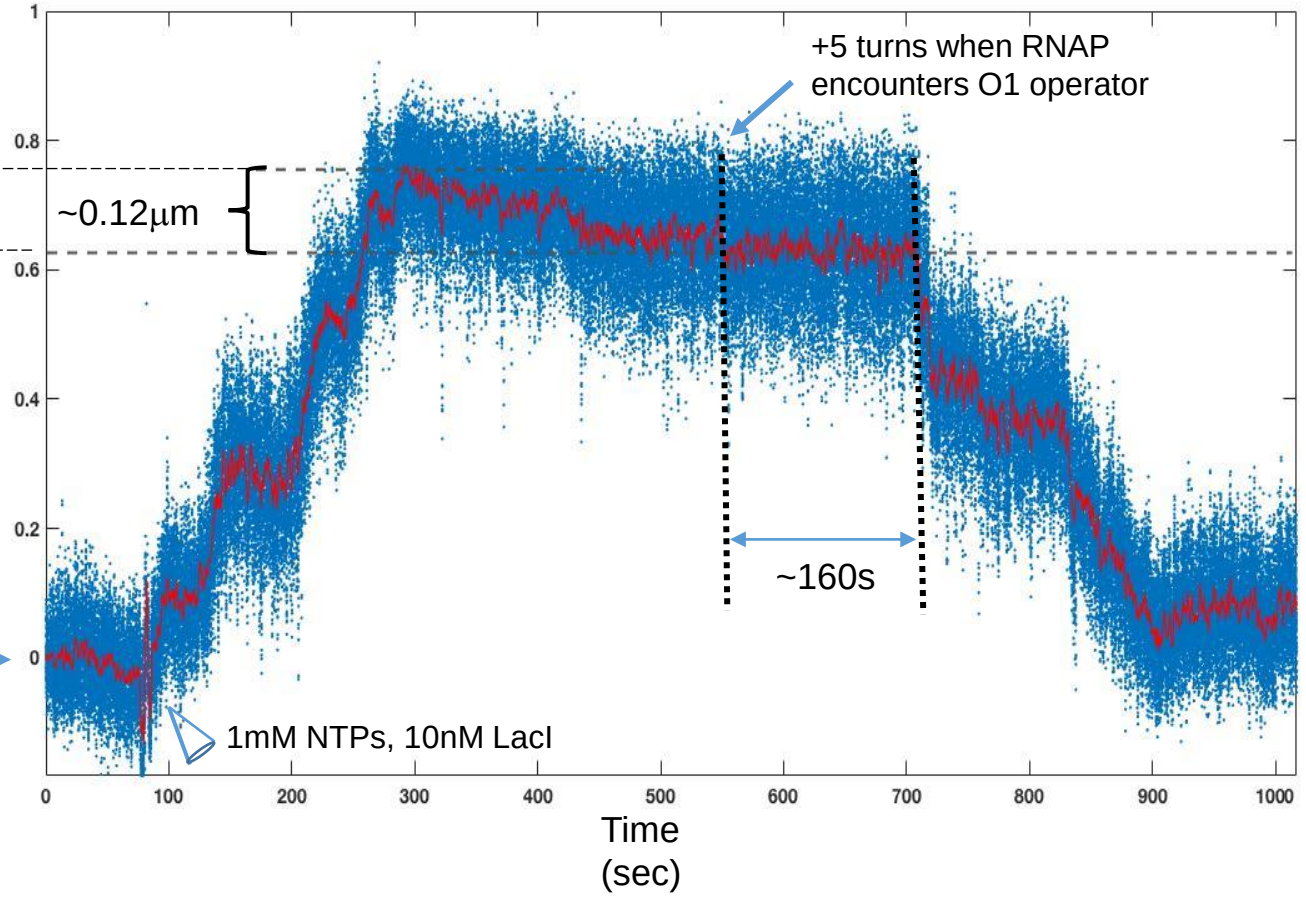
Xu et al, BioRxiv, 2021

Setting supercoiling of RNAP - LacI obstacle encounters



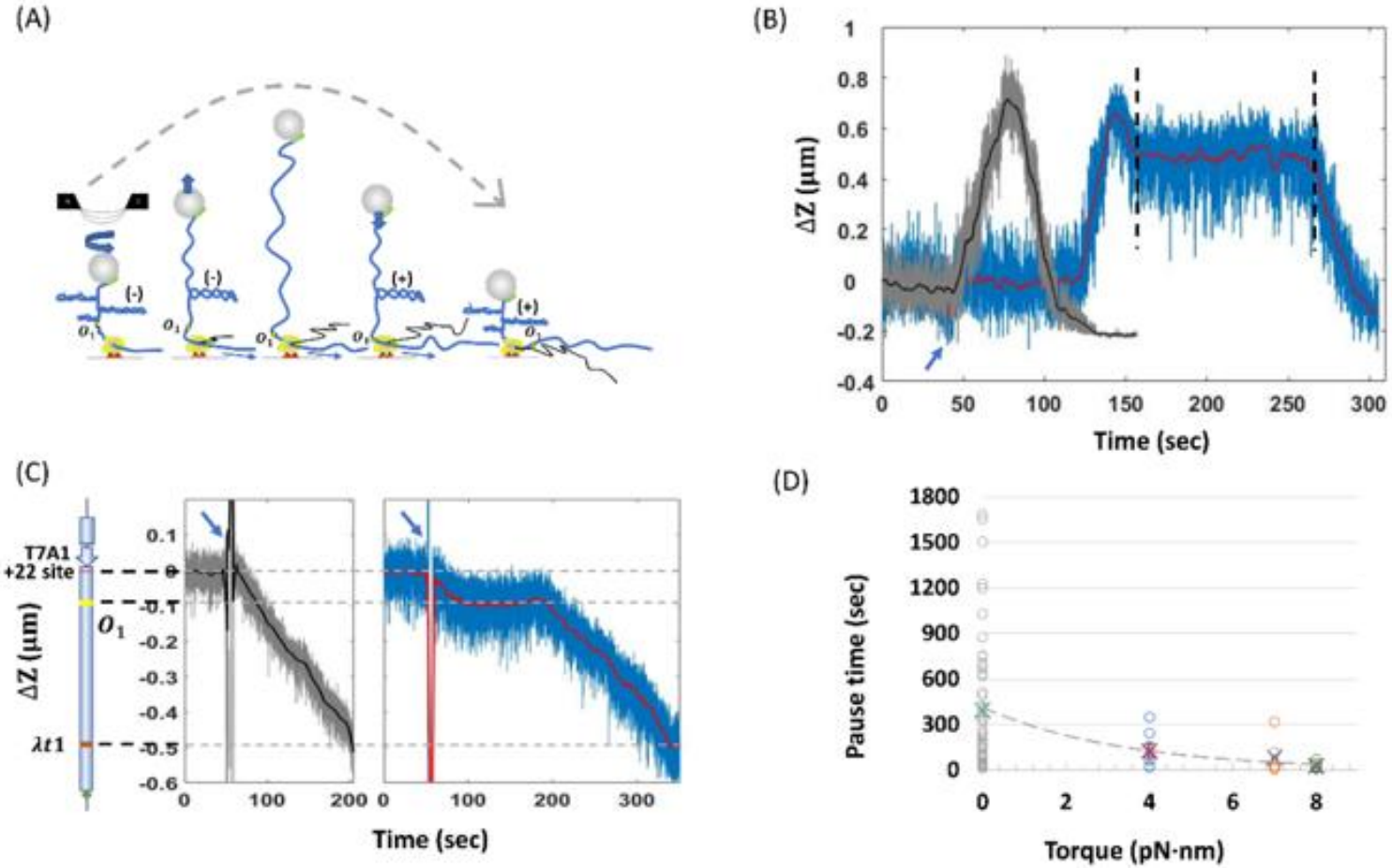


Starting from -19 turns



Xu et al, BioRxiv, 2021

Positive supercoiling may dislodge the obstacle



Xu et al, BioRxiv, 2021

Conclusions

1) LacI mediating a loop is a stronger roadblock than LacI bound at a single operator.

2) Positive supercoiling generated by RNAP helps it to surpass a roadblock and exit a loop.

Acknowledgments

and

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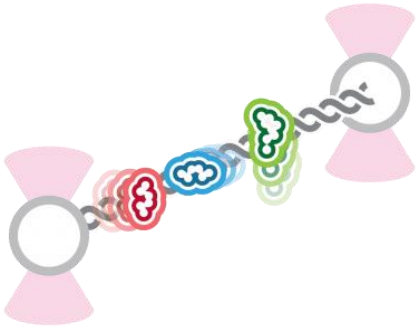
THE OHIO STATE UNIVERSITY

Postdoctoral positions available!



Lumicks Optical tweezers with s-m fluorescence detection