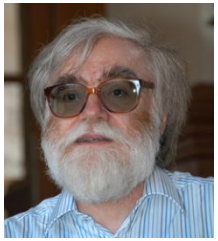
MODEL AND PARAMETER DETERMINATION FOR **MOLECULAR MOTORS** FROM SINGLE MOLECULE EXPERIMENTS

Francisco J. Cao
Universidad Complutense de Madrid (Spain)

Dedicated to the memory of my PhD. Advisor

Hector J. de Vega (CNRS, France)



Introduction

- Molecular motors = proteins able to do work
  perform different task in the cell
  (replication of DNA, transport of compounds, or of the whole cell, …)
- Their binding and conformational changes energies are of the order of $k_B T$ or one order of magnitude greater

  ⇒ thermal fluctuations are very present
- Single molecule experiments allow only to monitor one or a few distances of system.
- Thermal noise partially mask the signal of the system
- From this limited and noisy information one has to infer which is the system dynamics: determining the correct model and the values of its parameters.

We will show examples from our recent works

1. DNA replication speed masked by pauses

We measure distance between beads

- during s.d. (unwinding + replication) distance increases
- during p.e. (only replication) distance decreases

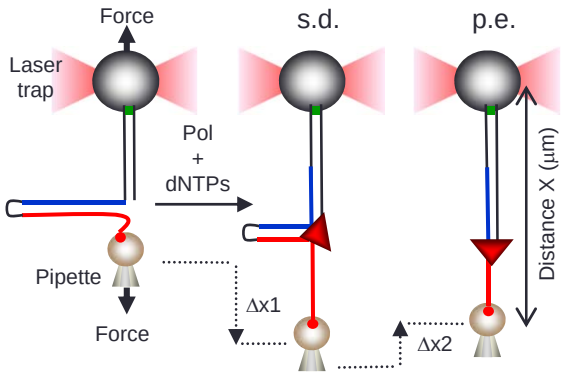
Distance between beads → polymerase trajectory

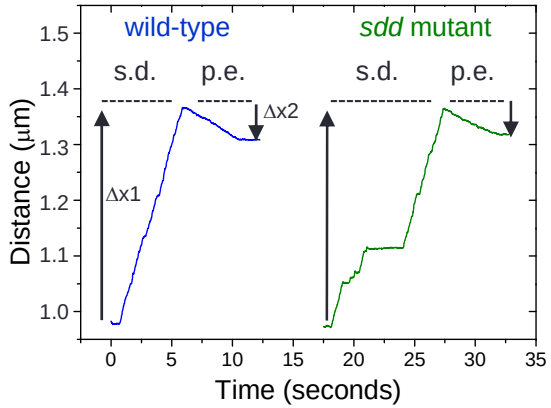
(after using the force extension curves for single and for double stranded DNA)

- Mutant Phi29 DNA polymerase showed slower s.d. replication speed than wild type.

  Trajectories suggest difference is due to the appearance of long pauses.

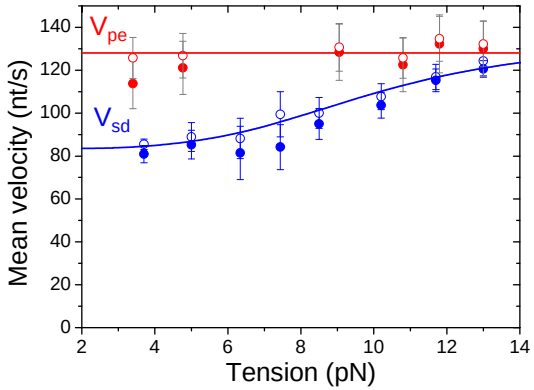
  We have to substract pauses to compare

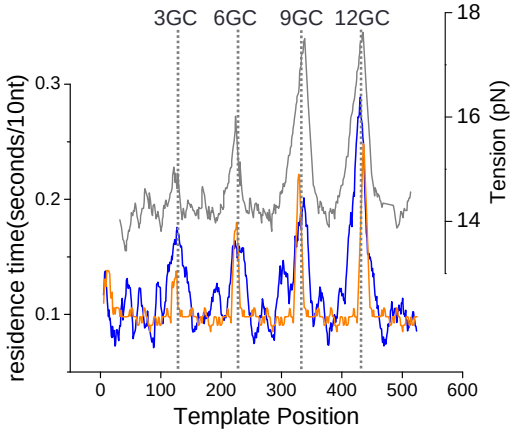




Wild type Phi29 polymerase

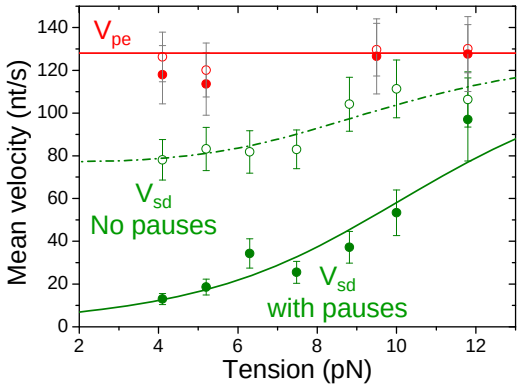
- Primer extension: Tension independent replication velocity. Replication rate k_0 ~ 128 nt/s.
- Strand displacement: Replication velocity is smaller and depends on tension. Tension helps DNA unwinding.
- During sd more time in GC positions (stronger binding than AT)

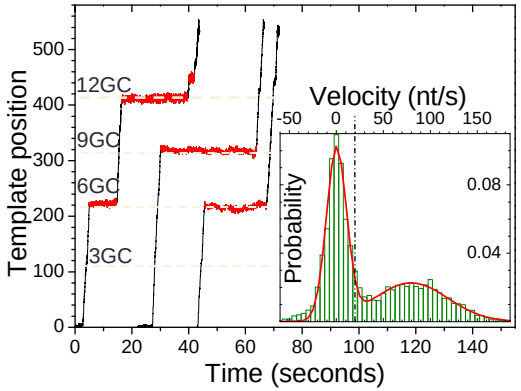




Mutant Phi29 polymerase

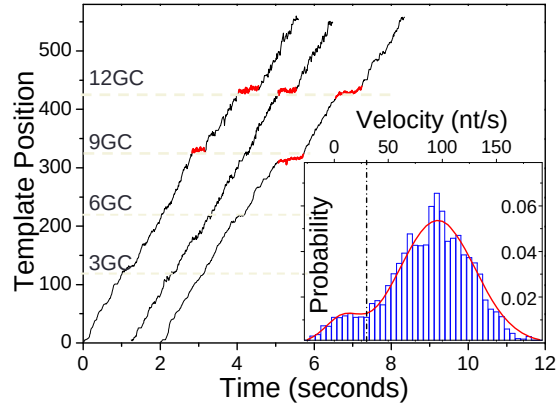
- Primer extension: same replication velocity as wild type. Replication rate k_0 ~ 128 nt/s.
- Strand displacement: smaller replication velocity
- Strand displacement deficiency is due to the appearance of long pauses in the dynamics

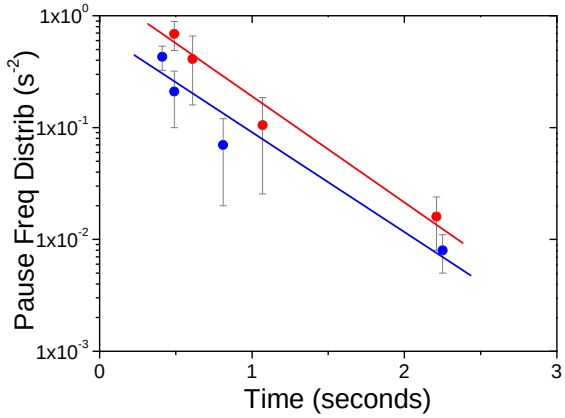


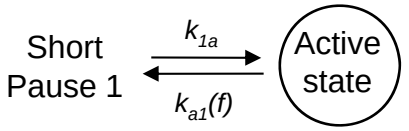


Wild type pauses

- Wild type also has pauses, but only shorter ones (with small influence in replication velocity).
- Short pauses only appear at GC locations, and pause frequency decreases with tension.

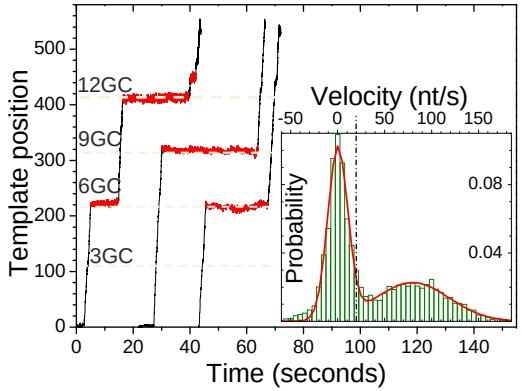


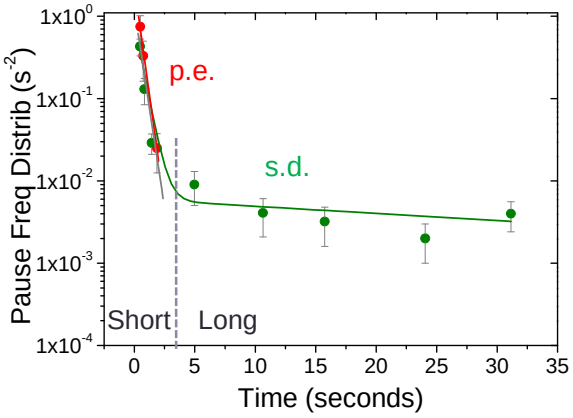




Mutant pauses

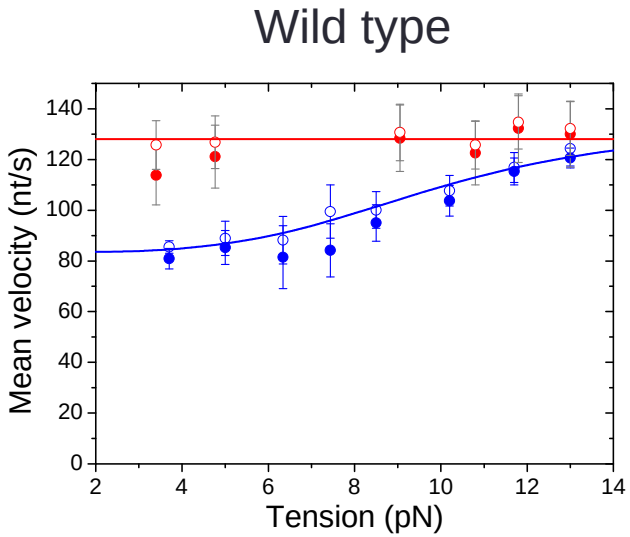
- Mutant polymerase shows long pauses during strand displacement.
- Pause length frequency distribution during s.d. shows two characteristic times indicating that there are at least two type of pauses (short and long).
- Short pauses are also present in p.e. and have a similar characteristic time as in wild type polymerase.

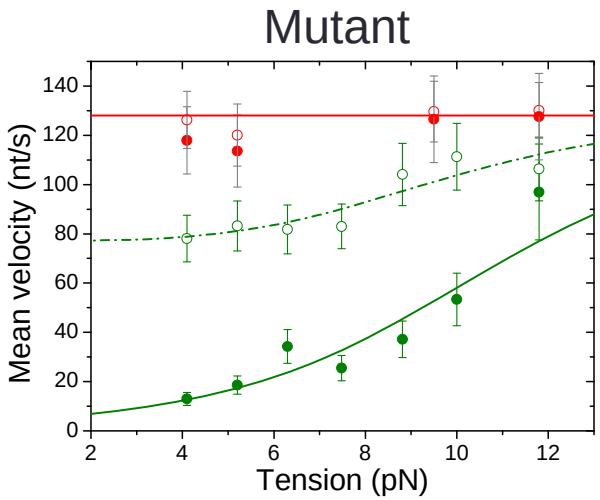




Replication speed without pauses

- After pause subtraction (empty circles), replication speeds are the same
- Analysis of trajectories showed that the mutation induced additional long pauses
- In addition we have been able to give an hypothesis for the origin of the pauses using its force dependence.

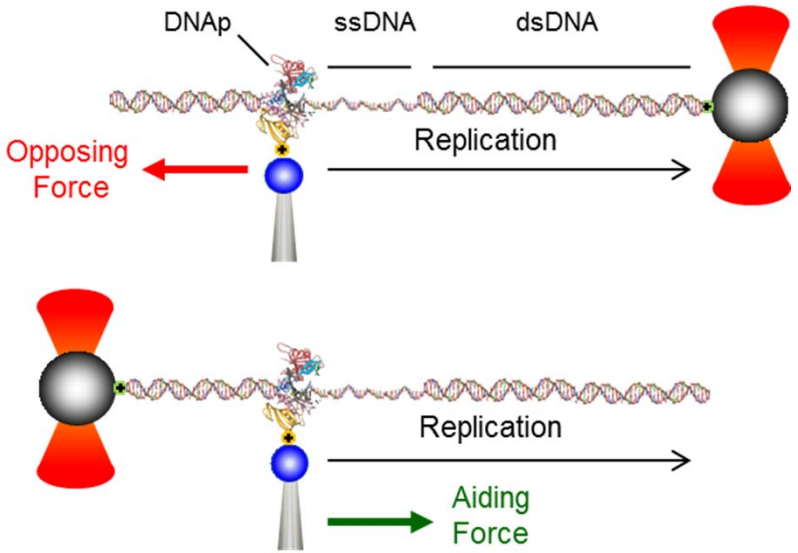




2. Stepping process in the DNA replication cycle

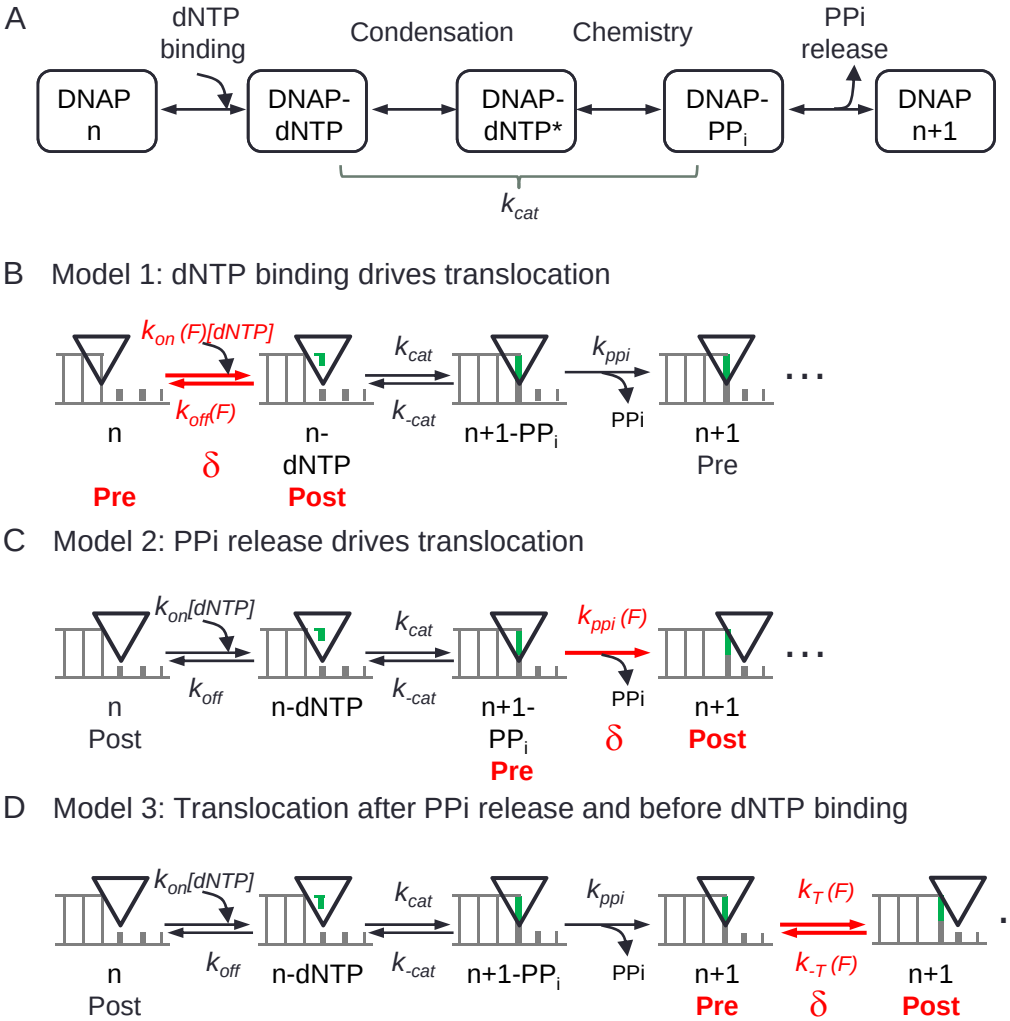
- Different force configuration
- Force pushes or pulls polymerase

⇒force will increase or decrease the rate of the stepping process



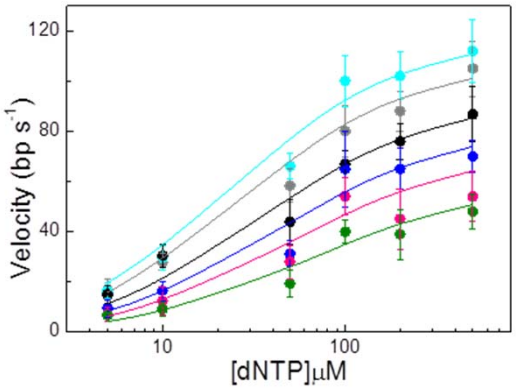
Different options for the stepping procces

- We aim to determine which is the stepping process (= the process where displacement occurs) within the polymerization cycle



Force and concentration dependence of speed gives the answer

- Increased nucleotide concentrations [dNTP] makes faster the nucleotide binding step

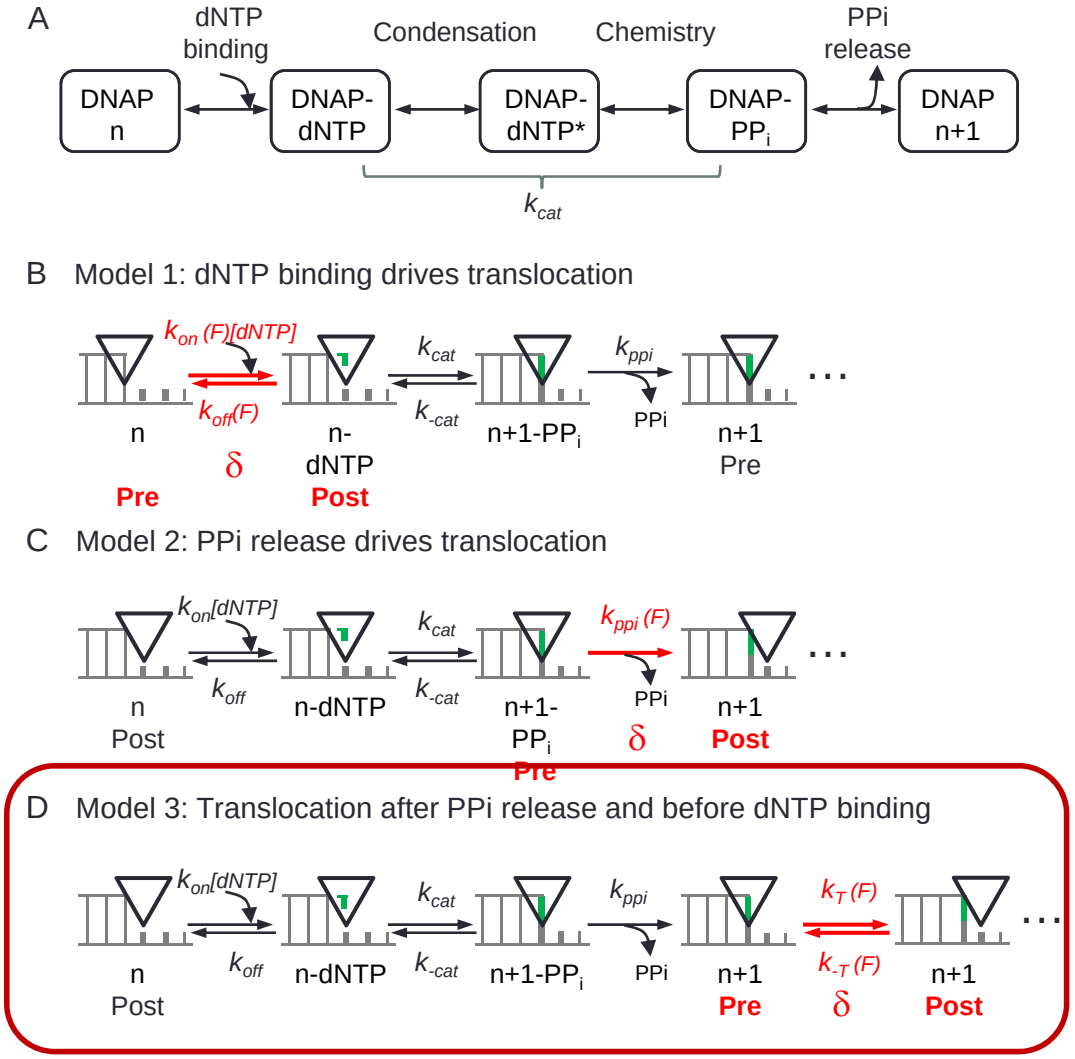


- Pushing force favors the stepping process, pulling force disfavors it.

Replication velocity as a function of nucleotide concentration in the solution, for forces of 20, 5, -5, -10, -15 and -20 pN (from top to bottom curve, positive forces are aiding forces).

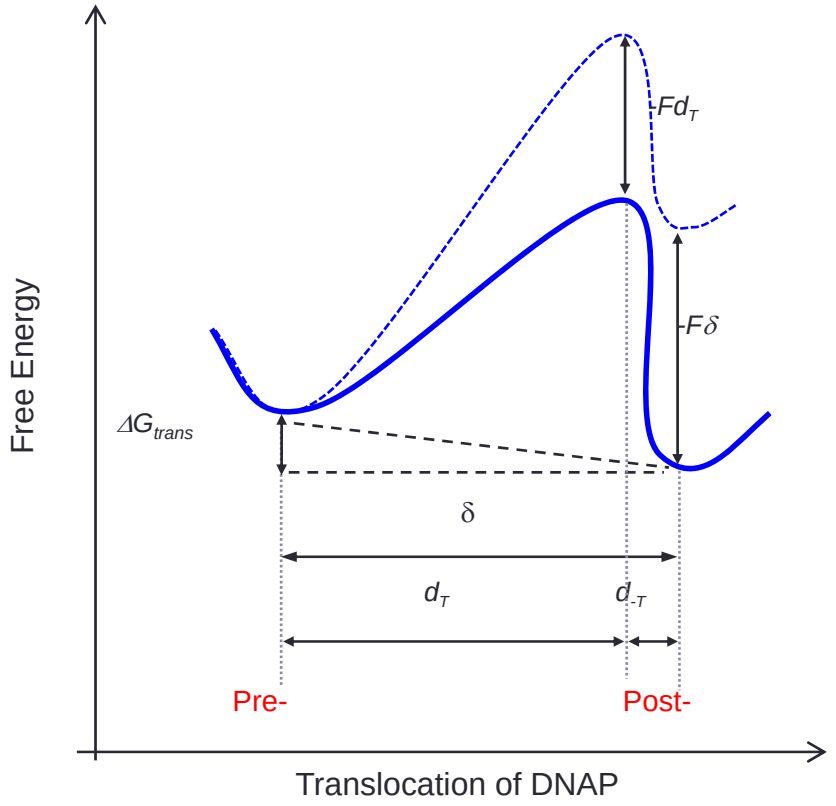
The stepping process

- Data favors the stepping process to be located after PPi release and before nucleotide binding.



Brownian ratchet mechanism

- Stepping occurs in a process that in the absence of force is energetically disfavored
- However the energetically favored and fast nucleotide incorporation, which follows, fix the slow events of going to the post-translocation state.



3. Open problems

- Determination of the step size when it is below the experimental resolution. We have a proposal to solve this problem which is expected to work for certain polymerases.
- Determination of possible transitions between fast and slow pause states, for the ssd mutant studied or for other molecular motors with two pause states.
- Detailed determination of whether stepping distributed among several of the processes in the chemical cycle can be excluded and in which cases, for the DNA polymerase studied or for other molecular motors.

Two last points imply the introduction of additional parameters, giving rise to degeneracies (i.e., several sets of values or even a region of the parameter space lead to good fits to the experimental data).

Statistical inference can help to extract further information from the physical trajectories, and to combine the information of different experiments in a rigorous way.

4. Conclusions

- The rich stochastic dynamics of molecular motors challenge statistical physicist and stochastic dynamics mathematicians
- Single molecule experiments provide very detailed information of one or several of the distances involved in the system dynamics.

  Biochemists provide their ability to completely inhibit certain processes or to block them with a certain probability, providing experimental data with more information in particular aspects of the involved dynamics.
- Close collaboration with biochemists and biologists is recommended to be able to do relevant contributions.

- References:

J. A. Morin, F. J. Cao, J. M. Lázaro, J. R. Arias-Gonzalez, J. M. Valpuesta, J. L. Carrascosa, M. Salas, B. Ibarra, *Active DNA unwinding dynamics during processive DNA replication*, **Proc. Natl. Acad. Sci. U. S. A.** 109, 8115 (2012) .

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- Future work: DNA replication in human mitochondria, effects of the SSB protein that protects one of the DNA strands during replication