Model and parameter determination for molecular motors from single molecule experiments

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I. INTRODUCTION

In the last decades new experimental techniques have allowed the study of single molecule processes[1,2,3]. This allows in particular to explore in real time the operation of molecular motors. Molecular motors are proteins that are able to do work, and they operate to perform different task in the cell, which range from DNA replication to transport of compounds inside the cell, or even transport of the whole cell.

Stochastic processes are very present in single molecule experimentswith molecular motors. Molecular motors have stochastic dynamics, with binding energies, typically, of the order of the energy of thermal fluctuations, $k_B T$, or only an order of magnitude greater. In addition, thermal fluctuations also affect the measurement adding unwanted thermal noise, which partially mask the signal of the system dynamics.

In addition, single molecule experiments allow onlyto monitorone or a few distances of the system, and from the limited information contained in these distances and theirtime evolution one has to infer which the system dynamics was, determining the correct model and its parameter values. We will review all these questions with examples from our recent works in this field.

II. DETERMINATION OF DNA REPLICATION SPEED MASKED BY PAUSES

A mutated DNA polymerase (a molecular motor that replicates DNA) was observed to have a lower replication speed than its wild type counterpart (the one present in nature).

Our study of experimental polymerase trajectories for different forces applied to separate the two strands of DNA showed that the mutation induced additional pauses in the replication[4,5], see Fig. (1). The lower speed was due to the transition of the polymerase to a long pause state, while during the polymerization state the speed was the same. The force dependency of the entry and exit rates to this pause state, which we determined, were compatible with a transition to the long pause state induced by the interaction of the polymerase with the DNA fork, where the two strands of DNA merge.

This example shows how the detailed analysis of the single molecule trajectories can help to determine the mechanism underlying some effect, as in this case was the speed reduction.

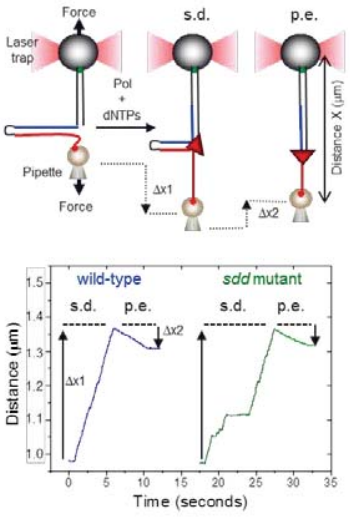

FIG. 1.**Top diagram** shows experimental configuration, in this configuration the force aids the separation of the two strands of DNA.At the left, previous to polymerase addition; at the center,on the strand displacement (s.d.) phase where the polymerase both unwinds the double strand of DNA and replicates one of the strands; and at the right, during the primer extension (p.e.) phase where the polymerase only replicates the remaining single strand of DNA. **Bottom plot** shows typical trajectories for the wild type and strand displacement deficient (ssd) mutant during both the s.d. and the p.e phases of the dynamics. Sdd mutant trajectories clearly show the appearance of long pauses during the s.d. phase.

III. DETERMINATION OF STEPPING PROCESS IN THE DNA REPLICATION CYCLE

We have studied the experimental trajectories of a DNA polymerase in the presence of aiding and opposing forces and for different abundances in the solution of the nucleotides needed for the replication[6], see Fig. (2). Our analysis shows that experimental results are incompatible with any of the two chemical steps that can lead to power stroke mechanisms leading the stepping process, while they are compatible with a Brownian ratchet mechanism for the polymerase advance.

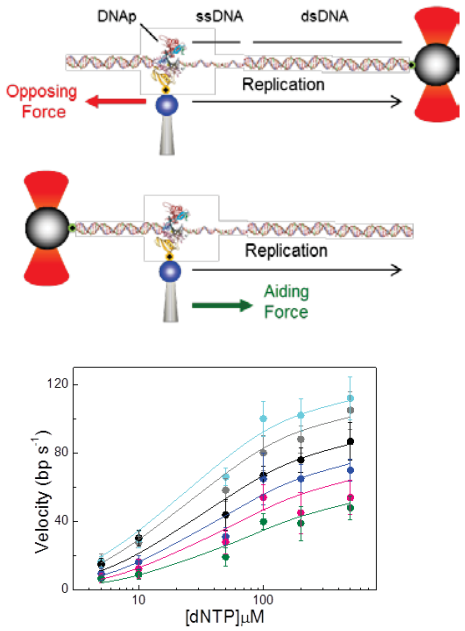

FIG. 2.**Top diagram** shows experimental configuration, in this configuration the force acts directly on the polymerase aiding or opposing the displacement associated with replication. **Bottom plot** shows replication velocity as a function of nucleotide concentration in the solution, for forces of 20, 5, -5, -10, -15 and -20 pN (from top to bottom curve, positive forces are aiding forces).

IV. OPEN PROBLEMS

We also want to point examples of openproblems that are expected to be solved through appropriate analysis of experimental trajectories combined with an appropriate experimental design:

- 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.

The two last points share in common that they imply the introduction of additional parameters in the model making more difficult to determine their values, and 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. Application of statistical inference methods can help to extract further information from the physical trajectories, and to combine the information of different experiments in a rigorous way. This combination of different experiments has already been done successfully in other fields of Science, as Cosmology, to successfully determine the values of models with a large number of parameters[7].

V. CONCLUSIONS

Single molecule experiments provide very detailed information of one or several of the distances involved in the system dynamics. From this partial information a lot of knowledge can be extracted mainly because it contains the temporal evolution of the distance which reflects transition processes.

Close and strong collaboration with biochemists and biologist is recommended to be able to do relevant contributions, structural details and bulk experiments give additional constraints to models, complementing the information from single molecule experiments. (Bulk experiments are traditional experiments performed in the test tube involving a macroscopic number of the molecules under study, in this case molecular motors.)

Molecular motors have a rich stochastic dynamics and its understanding challenge statistical physicist and stochastic dynamics mathematicians. 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. This provides abundant data to challenge our modelling ability and our statistical physics understanding.

We expect with this talk to attire more interest from the stochastic dynamics community to this area, where relevant and challenging problems are waiting for solutions, and will have a fast and strong impact in the development of Science.

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