High-throughput-, quantitative- single-molecule kinetic studies of the early steps of transcription

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In this project, we propose to use high-resolution, highly-parallel and quantitative bio-physical approaches to gain insight in the mechanisms that govern the initiation of transcription in bacteria.

Single-molecule techniques (force- or fluorescence-based) are appealing techniques that allow to probe reaction-intermediates, which are difficult, if not impossible, to observe when performing experiments on a large ensemble of molecules. Until recently, force-based techniques (Magnetic or Optical Tweezers) could only monitor one or a few molecules at a time and therefore prevented the acquisition of large datasets from experimental runs. Today, technical improvements (computing architectures such as CUDA, fast camera) allow Magnetic Tweezers (MT) to study hundred molecules in parallel (and in real time). As such, MT attracts a considerable attention in biophysics [1].

In this project, we plan to use MT to gain insight in a molecular mechanism (the initiation of the sigma-54 dependent transcription), which involves a complex kinetic pathway. Transcription is a conserved mechanism in which DNA is transcribed into RNA by a multi-subunit enzyme called RNA-polymerase (RNAp) [2]. As for other mechanisms involved in gene expression, transcription is a highly regulated process: sigma factors bind RNAp and modulate the association of RNAp with specific DNA sequences (known as promoter regions) located upstream a gene. For sigma-70 dependent promoters (those associated with house-keeping genes), additional proteins are dispensable for both the association of RNAp with the promoter and the initiation of transcription. The situation is however different for sigma-54 dependent promoters: there, ATP-dependent activators are required to initiate transcription and therefore involve a different control of gene regulation. Using MT data, we will be able to gain new results regarding the number of reaction intermediates as well as their characteristics (whether they involve the hydrolysis of nucleotides, changes in conformation of enzymes or the unwinding of DNA) [3]. Finally, we will develop a kinetic model that describes this relevant, yet not fully understood, process.

The research involves a good knowledge of experimental physics (optics, instrumentation, and programing). In particular, we plan to upgrade the current setup for kilo-molecule MT experiments. Knowledge of biology is not required but the successful candidate should show a strong interest for molecular biology and biochemistry (part of the biological samples will be obtained in collaboration).

References

[1] De Vlaminck, Iwijn and Dekker, Cees. Recent advances in Magnetic Tweezers. Annual Review of Biophysics (2012)
[2] Molecular Biology of the Cell. 6th Edition. Alberts B. *et al.* (2014)
[3] Reyvackin A. *et al.* Abortive initiation and productive initiation by RNA polymerase involve DNA scrunching. Science (2006)