Atomic force microscopy of DNA-protein interactions

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Introduction

Atomic force microscopy (AFM) can be used to distinguish proteins bound to nucleic acid templates. Imaging can be conducted in air or under fluid, the only requirement being that the protein-DNA complexes must be bound to a flat substrate. By scanning across the support with a sharp AFM tip we are able to produce a topographic image of the support and the bound DNA-protein complexes. By measuring quantities such as the position of the protein on the template, its height, or its width, we are able to infer changes in the molecular conformation of the protein under different conditions. Also, the amount of template wrapping by the protein can be discerned, by measuring the protein induced bend angle, and the apparent shortening of the template. A central aim of our work is to study such systems under fluid in real time. In this setup, we can potentially study the transition of single molecules between different conformational states. Diffusion controlled processes such as a promoter search occur on time scales of seconds to minutes and are readily studied with current AFM technology. However enzymatically controlled processes such as transcription occur at much faster time scales, and in order to observe enzymatic processes, imaging times must be reduced. Fast scan technologies are being developed by a number of research groups worldwide. As these technologies become more widely available, AFM will play a larger role in understanding of mechanisms of interaction between proteins and DNA.

Nested-gene transcription model

“Nested gene” is a term coined for a gene that lies completely within the sequence of another gene. Such genes are known to exist in the human genome and eukaryotes such as Saccharomyces cerevisiae. Whether these genes come about as an unavoidable consequence of their compressed genetic arrangement, or whether such genes have an intrinsic effect on transcriptional regulation is unclear. We aim to study the effect two convergently aligned genes have on the expression of these gene’s mRNAs, either through collision of polymerases, RNA interference of sense and antisense transcripts, or topological constraints introduced by the template.

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Fig. 1: Gallery of representative images of a DNA template with T7 polymerases bound. Polymerases bound specifically, as judged by their position, are marked by +, and those non-specifically bound are marked *. The scale bar is 200nm.
Present work has focused on bacteriophage T3 and T7 polymerases due to their high specificities for their promoters. Using this system, we have been able to bind the polymerases to the template DNA, and, by measuring their position along the template, we are able to determine if they are bound at the promoter. We have studied the effect of omission of each nucleoside triphosphate (NTP) and their respective affinity to form stalled complexes downstream of the promoter. Future work will investigate different methods of polymerase stalling, in an attempt to decouple promoter binding and polymerase stalling. Different templates are currently being constructed with the aim to move to a true nested gene, when single molecule real time imaging will be undertaken.

**DNA gyrase**

DNA gyrase is a bacterial motor protein in a class known as topoisomerases, which are responsible for controlling the topological properties of DNA (i.e. amount of supercoiling or catenation). Most topoisomerases can relax supercoiled DNA, which is an energetically favourable process. DNA gyrase is unique amongst this class, because it can introduce supercoils as well as remove them. To wind or unwind DNA, it must break both strands of DNA, capture another segment of the same DNA molecule and pass this through the double-strand break before resealing. We have used AFM to study the length of linear DNA wrapped around the gyrase in the presence, and absence of 5'-adenylyl β,γ-imidodiphosphate (ADPNP). There is a shortening of the DNA when the gyrase binds consistent with a full wrap of DNA around the protein. On binding of ADPNP this wrap is completely lost. These AFM data have been compared with previous foot-printing data from the Maxwell group in the presence and absence of ADPNP. Both data sets are consistent and have allowed a new model for DNA wrapping in gyrase to be proposed.

![Fig. 2: DNA gyrase bound to a 1070bp linear DNA template. This DNA-protein complex is deposited on a mica surface and imaged using tapping-mode AFM in air.](image)

**Collaborators**

**Nested-gene Project:**
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**DNA gyrase Project:**
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**Publication**

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