Atomic force microscopy of DNA-protein interactions

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Atomic force microscopy (AFM) can be used to distinguish proteins bound to nucleic acid templates. It has the capability of imaging under aqueous fluid conditions at nanometre resolution allowing the imaging of interactions and transactions between molecules in real-time. Current acquisition times for the raster-scanned images of the sharp tip across a surface are on the order of one minute for a micron squared area. This means that AFM is extremely adept at following slower processes, i.e. diffusion controlled events, such as facilitated searches for promoter sequences on DNA templates by transcription factors. Improvements in imaging times are on-going which will allow “movies” of biological processes to be acquired with image times close to video rate, a goal that has already been achieved by one or two research groups. As this technology becomes more widely available, AFM techniques will play a larger role in understanding of mechanisms of interaction between proteins and DNA.

Currently, we are studying two systems with AFM: DNA gyrase and a nested-gene transcription model.

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 been using AFM to study the length of DNA wrapped around the gyrase in the presence and absence of ADPStormp to understand in more detail how gyrase achieves the strand passage of one segment of DNA through another.

![Fig. 1 Tapping-mode AFM images of air-dried samples on mica. (A). Single DNA gyrase molecules in the presence of the drug ciprofloxacin bound on top of a layer of DNA molecules (428 x 428 nm). (B) Supercoiled pBR322 plasmid DNA molecules (681 x 681 nm)](image)

Nested-gene transcription model
“Nested gene” is a term coined for a gene that lies completely within the sequence of another gene. If the genes are arranged in a convergent orientation, the question of potential transcriptional collision arises. Nested genes therefore raise important implications for regulation of transcription and control of gene expression. Potential steric hindrance by RNA
polymerase transcribing the opposite strand and the possible generation of anti-sense RNAs have led to suggestions of co-regulation of expression. We are applying AFM to directly image two polymerases transcribing towards each other on opposite sides of a model DNA template. The template is based upon the highly conserved AMEL gene encoding for amelogenin (the principle component of the organic matrix of developing dental enamel), which is located entirely within (and in the opposite orientation to) the intron of a larger gene, ARHGAP6 (encoding a RhoGAP protein) on the human X chromosome. The initial AFM studies are concentrating on the study of the adsorption of DNA molecules to surfaces of varying chemistry to establish reliable protocols for imaging transcription processes in real-time.

![Tapping-mode AFM image of linear DNA molecules adsorbed to mica functionalised with aminosilane (1130 x 1130 nm)](image)

**Fig. 2** Tapping-mode AFM image of linear DNA molecules adsorbed to mica functionalised with aminosilane (1130 x 1130 nm)

**Collaborators**

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