Molecular Recognition Force Microscopy/Spectroscopy

Peter Hinterdorfer

Institute for Biophysics, Johannes Kepler University Linz, Altenbergerstr. 69, A-4040 Linz, Austria
E-mail: peter.hinterdorfer@jku.at

In molecular recognition force microscopy (MRFM), ligands are covalently attached to atomic force microscopy tips for the molecular recognition of their cognitive receptors on probe surfaces. Using an appropriate tip surface chemistry protocol with 6-nm long heterobifunctional crosslinkers as key elements, the ligand density on the AFM tip is sufficiently dilute for allowing single molecule studies. Our crosslinker library possesses many different chemical endgroups for various functional coupling strategies. Interaction forces between single receptor-ligand pairs are measured in force-distance cycles. A ligand-containing tip is approached towards the receptors on the probe surface, which possibly leads to formation of a receptor-ligand bond. The tip is subsequently retracted until the bond breaks at a certain force (unbinding force). In force spectroscopy (FS), the dynamics of the experiment is varied, which, in case of a single sharp activation barrier, reveals a logarithmic dependence of the unbinding force on the force velocity. From this curve the barrier height and width can be deduced, as shown on virus/cell receptor interactions. A more complex energy landscape dominates the interaction of the nuclear import receptor importin β with the small GTPase Ran. The complex switches between two distinct conformational states of different binding strength. Our results support a model whereby functional control of Ran-importin β is achieved by a population shift between pre-existing alternative conformation.

In another study it was shown that single molecule force measurements between single-stranded DNA containing multiple methylcytosines and an anti-methylcytosine antibody can survey the distances between methylcytosines with single nucleotide resolution. Two step unbinding events in force curves corresponded to sequential dissociation of two Fab-domains of one antibody from a single DNA molecule, with a distance in excellent agreement with the contour length of nucleotides in between two methylcytosines. Using different DNA sequences, the applicability for methylcytosine sequencing and the detection of single nucleotide polymorphism at the single molecule level was demonstrated.

Finally, a method for the localization specific binding sites and epitopes with nm positional accuracy by combining dynamic force microscopy with single molecule recognition force spectroscopy is presented. A magnetically driven AFM tip containing a ligand covalently bound via a tether molecule was oscillated at 5 nm amplitude while scanning along the surface. Since the tether had a length of 6 nm, the ligand on the tip was always kept in close proximity to the surface and showed a high probabilty of binding when a receptor site was passed. Topography and recognition images were obtained simultaneously using a specially designed electronic circuit. Maxima (U_{up}) and minima (U_{down}) of each sinusoidal cantilever deflection period were depicted, with U_{down} driving the feedback loop to record a height (topography) image and U_{up} providing the data for the recognition image. In this way, topography and recognition image were gained simultaneously and independently with nm lateral resolution. This method is capable of localizing distinct histones in chromation preparations and can visualize nm-sized receptor domains on cell surfaces.