Single Molecule Recognition Forces and Recognition Imaging

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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, the ligand density on the AFM tip is sufficiently dilute for the allowence of single molecule studies. 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 reveals a logarithmic dependence of the unbinding force from the force velocity. These studies give insight into the molecular dynamics of the receptor-ligand recognition process and yield information about the binding pocket, binding energy barriers, and kinetic reaction rates. Applications on isolated proteins, native membranes, viruses, and cells will be presented.

We have also developed 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. 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 8 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. The recognition signals were well separated from the topographic signals arising from the surface, both in space (z ~ 5 nm) and time (half oscillation period ~ 0.1 ms). 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.