

We combined recognition imaging and force spectroscopy to study the interactions between receptors and ligands on the single molecule level. This method allowed the selection of a single receptor molecule reconstituted in a supported lipid membrane at low density, with the subsequent quantification of the receptor-ligand unbinding force. Based on atomic force microscopy (AFM) tapping mode, a cantilever tip carrying a ligand molecule was oscillated across a membrane. Topography and recognition images of reconstituted receptors were recorded simultaneously by analyzing the downward and upward parts of the oscillation, respectively. Functional receptor molecules were selected from the recognition image with nanometer resolution before the AFM was switched to the force spectroscopy mode, using positional feedback control. The combined mode allowed for dynamic force probing on different pre-selected molecules, resulting in higher throughput when compared with force mapping. We applied this method for a quantitative characterization of the binding mechanism between mitochondrial uncoupling protein 1 (UCP1) and its inhibitor adenosine triphosphate (ATP). Moreover the dynamics of force loading was varied to elucidate the binding dynamics and map the interaction energy landscape.