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Conformational changes of surface immobilized proteins studied by combined Atomic Force Microscopy and Fluorescence Spectroscopy
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Atomic Force Microscopy (AFM) and Fluorescence Spectroscopy techniques have provided unique methods for characterizing conformational changes in proteins. Here we are using a technique called nanografting to immobilize proteins at well defined locations on atomically flat surfaces. In nanografting the AFM tip is used to shave alkanethiol molecules from a prescribed patch on a surface coated with an alkanethiol monolayer. Thiol-linked proteins in the surrounding solution are then able to self assemble on the newly exposed surface patch in a highly ordered array of the order of 100nm. Stable and meta-stable conformations of fluorescently tagged proteins and other molecules assembled in this manner can then be characterized using a combination of AFM and Fluorescent Resonance Energy Transfer (FRET). Due to the high spatial, temporal and force resolution provided by both AFM and FRET, a free energy landscape of the protein may be determined using this technique.

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