

NANOMETER LOCALIZATION AND IDENTIFICATION OF DNA REPAIR PROTEINS BY COMBINED AFM-FLUORESCENCE ANALYSIS

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Imaging of processes at the single-molecule level has revealed information otherwise inaccessible by “bulk” experiments. Atomic force microscopy is a powerful technique for studying biomolecular structures with nanometer resolution without necessity of external contrast agents. However, complex genetics transactions require the orchestrated action of different proteins, which would be undistinguishable by conventional AFM. On the other hand, fluorescence techniques can successfully localize proteins *in vivo* and *in vitro*, also for dynamic studies. However, optical resolution limits the details of structural studies in single molecule approaches. We show here the localization with nanometer resolution of recombination proteins using a combined atomic force and fluorescence microscope. Our experiments reveal the versatility of the system in the recognition and simultaneous localization of different fluorescent-tagged proteins on DNA. Moreover, we present the use of fluorescence polystyrene micro-spheres for a reliable alignment of the optical and the topographical images, which together with the DirectOverlay™ system (JPK instruments) have allowed us to precisely localized different proteins with nanometer resolution.