

DIRECT MEASUREMENT OF PROTEIN DYNAMICS BY AFM

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Atomic force microscopy is a direct means to study the internal dynamics of proteins under physiological conditions. As was shown by Radmacher et.al., by measuring the fluctuation in apparent height of proteins adsorbed to a surface or fixed to a membrane, information can be gleaned about the internal dynamics of the molecule.

The protein of our particular interest is the 14-3-3 protein, which is a major participant in a range of signaling processes. Its main function is that of a carpenter's clamp by which other reaction partners are held in a mutually favourable conformation. 14-3-3 proteins are genetically modified such that they carry a N-terminal his₆-tag for immobilisation. Control of the surface coverage during the immobilisation procedure is obtained from simultaneous single-molecule fluorescence measurements of fluorescence labeled 14-3-3's. First results of imaging both lysozyme and 14-3-3 in physiological environments are presented. Initial measurements on the activity of lysozyme are used as an established test-bed for further system development.