Subunit Rotation and Twisting in FoF1-ATP Synthase by Single-Molecule Three-Color Förster Resonance Energy Transfer

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Abstract:

Catalytic activities of enzymes are associated with elastic conformational changes of the protein backbone. These conformational changes of single proteins can be monitored in real time by Förster resonance energy transfer, FRET. Two different fluorophores have to be attached to those protein domains, which move during function. Distance fluctuations between the fluorophores are measured by relative fluorescence intensity changes or fluorescence lifetime changes.
FoF1-ATP synthase is a rotary molecular machine which catalyzes the formation of adenosine triphosphate (ATP) known as the 'energy currency' of the living cell. The Escherichia coli enzyme consists of a membrane-bound Fo motor where proton translocation through Fo drives a 10-stepped rotary motion[1]. An internal central stalk transduces the energy of this rotation to the F1 motor, where ATP is synthesized in an 120° rotary stepping cycle[2, 3]. The exact mechanism of energy storage to bridge the symmetry mismatch of the two motors with different step sizes is not fully understood. The rotary mechanics of proton-driven FoF1-ATP synthase will be discussed and a single-molecule FRET approach to observe both rotations simultaneously in a triple-labeled single FoF1-ATP synthase at work will be presented[4].

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