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Force-Manipulation Single-Molecule Spectroscopy Studies of Enzymatic Dynamics H. PETER LU, YUFAN HE, MAOLIN LU, JIN CAO, QING GUO, Bowling Green State University, Department of Chemistry, Center for Photochemical Sciences, Bowling Green, Ohio 43403 — Subtle conformational changes play a crucial role in protein functions, especially in enzymatic reactions involving complex substrate-enzyme interactions and chemical reactions. We applied AFM-enhanced and magnetic tweezers-correlated single-molecule spectroscopy to study the mechanisms and dynamics of enzymatic reactions involved with kinase and lysozyme proteins. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules were observed simultaneously in real-time by single-molecule FRET detections. Our single-molecule spectroscopy measurements of enzymatic conformational dynamics have revealed time bunching effect and intermittent coherence in conformational state change dynamics involving in enzymatic reaction cycles. The coherent conformational state dynamics suggests that the enzymatic catalysis involves a multi-step conformational motion along the coordinates of substrate-enzyme complex formation and product releasing. Our results support a multiple-conformational state model, being consistent with a complementary conformation selection and induced-fit enzymatic loop-gated conformational change mechanism in substrate-enzyme active complex formation.

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