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Single molecule FRET to resolve conformational fluctuations in proteins KEITH WENINGER, North Carolina State University, BRANDON CHOI, DAVID MURRAY, STEVE STORY — Proteins fold into complex shapes that are intimately linked to their function. High-resolution techniques are capable of determining static images of these structures with atomic detail, but biological function and regulation is achieved through dynamic changes in protein conformation. Single molecule fluorescence techniques have a unique capability to detect transient molecular conformations. The power of the single molecule approach arises because it avoids the averaging over molecules and over time that are inherent in ensemble measurements. We report application of single molecule fluorescence resonance energy transfer (smFRET) to tSNARE to directly observe a conformational transition that is postulated to have an auto-regulatory function. We present a series of measurements using mutants of the proteins as well as homologues of different species in order to gain a molecular level understanding of these transitions. The techniques demonstrated here are directly applicable to investigations of conformational dynamics in other protein based macro-molecular machines.

Keith Weninger
North Carolina State University

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