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Observing the conformation of individual SNARE proteins inside live cells

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Protein conformational dynamics are directly linked to function in many instances. Within living cells, protein dynamics are rarely synchronized so observing ensemble-averaged behaviors can hide details of signaling pathways. Here we present an approach using single molecule fluorescence resonance energy transfer (FRET) to observe the conformation of individual SNARE proteins as they fold to enter the SNARE complex in living cells. Proteins were recombinantly expressed, labeled with small-molecule fluorescent dyes and microinjected for in vivo imaging and tracking using total internal reflection microscopy. Observing single molecules avoids the difficulties of averaging over unsynchronized ensembles. Our approach is easily generalized to a wide variety of proteins in many cellular signaling pathways.