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Watching Single Enzymes and Fluorescent Proteins in Action in Solution Using a Microfluidic Trap

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Observation of dynamics of single biomolecules over a prolonged time without altering the biomolecule via immobilization is achieved with a specialized microfluidic device. This device, the Anti-Brownian ELectrokinetic (ABEL) Trap, uses real-time electrokinetic feedback to cancel Brownian motion of single objects in solution. First, we use the ABEL Trap to study Allophycocyanin (APC), a photosynthetic antenna-protein and popular fluorescent probe. A complex relationship between fluorescence intensity and lifetime is observed, suggesting light-induced conformational changes and radiative and non-radiative rate fluctuations. Second, we apply the ABEL Trap to single molecules of the multi-copper enzyme blue Nitrite Reductase where a fluorescent label reports on the oxidation state of the Type I Copper. Redox cycling is observed and kinetic analysis allows extraction of the microscopic rate constants in the kinetic scheme. Evidence of a substrate-induced shift of the intramolecular electron transfer rate is seen. Taken together, these observations provide windows of unprecedented detail into the dynamics of solution-phase biomolecules.