Title: Using the i-motif as a pH sensor in single molecule fluorescence microscopy imaging

Abstract

Förster resonance energy transfer (FRET) allows for observing behavior of single molecule processes. Two fluorescent molecules are attached to the ends of the biological structure. One molecule acts as the donor (Cy3), the other (Cy5) acts as an acceptor for the energy emitted from the donor molecule. The fraction of energy that is transferred depends on the distance between the molecules. Changes in the shape of the biological structure, which are accompanied by a change in the separation between donor and acceptor fluorophores, can be observed by FRET. The sub-nanometer sensitivity of FRET to such changes makes it an ideal tool to study conformational switches, structures which change their shape in response to an input signal. Conformational switches have applications in nanoengineering, biotechnology, and medicine. The i-motif structure is a conformational switch which responds to the pH of its surrounding and attains a compact structure at pH around 6.0. We used FRET techniques to study the folding and unfolding of the i-motif at different pH levels at the single molecule level. Using the i-motif as a sensor, we measured how the pH of a commonly used fluorescence imaging buffer changes in time.