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FCS measurements of conformational fluctuations in proteins

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Don C Lamb, Yan Chen, Joachim D Müller, and Enrico Gratton.

**FCS measurements of conformational fluctuations in proteins.**

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**Abstract**

Fluctuation Correlation Spectroscopy (FCS) has proved to be a powerful tool in many areas of Biophysics. One of the features of FCS is the ability to accurately measure the molecule number fluctuations of different fluorescent species in the picomolar to micromolar range. We have applied this technique to the measurement of protein dynamics. FCS was used to measure the fluctuation of apomyoglobin between the folded and the denatured states. The binding of 1-anilino-8-naphthalene sulfonate (ANS) to apomyoglobin was monitored as the protein was denatured using Guanidine HCL. In the folded state, the ANS can bind in the hydrophobic pocket, and the fluorescence quantum yield is one. As the protein denatures, water can quench the fluorescence and the quantum yield drops dramatically. When the fluctuations between the folded and unfolded states occurs rapidly with respect to the diffusion time of the particle through the excitation volume, FCS will only observe the protein as one species. When the conformational fluctuations are slow relative to the time of diffusion through the excitation volume, FCS will measure two separate species. By varying the rate of conformational fluctuations with respect to the diffusion time of the particle through the excitation volume and monitoring the amplitude of the autocorrelation function, the rate of fluctuations between conformations can be measured.