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Fluorescence Correlation Spectroscopy of Biomolecules: Kinetic Studies Using Microfluidic Devices PETER GALAJDA, JASON PUCHALLA, ROBERT RIEHN, ROBERT AUSTIN, Princeton University — Fluorescence correlation spectroscopy (fcs) uses the autocorrelation of the fluctuation of the fluorescence intensity resulted by single fluorophores traversing a confined excitation volume. Information about motion and interactions can be gathered on the single molecule level. Usually either diffusion or convection is dominant on the timescales a molecule travels through the focal volume of a laser beam. In some conditions both can have a significant contribution to the fluorescence fluctuation, and the diffusion constant and the flow velocity can be determined simultaneously. In most cases an fcs measurement lasts for seconds, making it inadequate for kinetic studies of most biomolecular processes. However, when two or more reactants meet in a microfluidic diffusional mixer a steady-state flow establishes a direct mapping of the temporal evolution of the reaction to the spatial position along the channel. Probing the sample along the mixing channel gives information about the different stages of the reaction. This opens a way to perform kinetic fcs measurements with time resolutions of a fraction of a second. A variety of biological reactions might be studied by the above technique from protein folding to DNA-protein interactions. Examples will be given in the talk.

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