Title
Molecular aggregation and ligand-receptor interaction probed at the single molecule level using two-photon microscopy.

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Abstract

Two-photon excitation spectroscopy has inherent 3-D resolution with excitation volumes as small as 0.1 fL, which compared to conventional fluorometers constitutes a 10^10 times reduction of the excitation volume. Via fluctuation correlation spectroscopy (FCS), the fluorescence fluctuations within the small excitation volume provide a unique way to study interesting biological phenomena. Molecular aggregates are easily identified at the molecular level, both in terms of number fluctuation and changes in the translational diffusion coefficient. These molecular characteristics are obscured once they are averaged over an assembly of millions of molecules. We apply both Photon Counting Histogram (PCH) and autocorrelation analysis to study ligand binding to estrogen receptors. These two methods complement each other and accurately recover the number of molecules of free ligand and ligand bound to the receptor. In addition, kinetic information can be obtained using these two analysis methods. Supported by the National Institutes of Health, RR03155.