

UC Irvine

UC Irvine Previously Published Works

Title

Heterogeneity on the single molecule level probed by fluorescence fluctuation spectroscopy

Permalink

<https://escholarship.org/uc/item/4h33r6xb>

Journal

BIOPHYSICAL JOURNAL, 76(1)

ISSN

0006-3495

Authors

Chen, Y
Muller, JD
Eid, J
[et al.](#)

Publication Date

1999

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Yan Chen, Joachim D Müller, John S Eid, Sergey Y Tetin, and Enrico Gratton.

Heterogeneity on the single molecule level probed by fluorescence fluctuation spectroscopy.

43rd Annual Meeting of the Biophysical Society, Baltimore, Maryland, 1999.

Biophys J. 1999; 76(1 Pt 2).

Abstract

Normalized variance of intensity fluctuations for a homogeneous species determined by Fluorescence Fluctuation Spectroscopy (FFS) is directly proportional to the number of molecules within the excitation volume. For a heterogeneous sample, however, the observed variance is determined by the number of molecules and the fractional intensity of each species in the mixture. Three different analysis methods, photon counting histogram, autocorrelation, and moment analysis have been employed to estimate the variance from experimental data of single and mixed species samples. As an illustration of such a system, we studied binding of fluorescein labeled digoxin to high-affinity ($K_d \sim 250$ pM) anti-digoxin antibody. The difference in variance between the completely bound and free ligand suggests heterogeneity in the bound species. In addition, two fluorescence lifetimes of 1.2 nsec and 4 nsec were determined for the bound species with fractional intensities of 20% and 80%, respectively. Combining this information with the results of the fluorescence fluctuation experiment led to a complete description of the FFS titration curve. Since the individual molecular species are stable during the diffusion time through the excitation volume, we conclude that the interconversion of individual species is slower than the transit time. Therefore, FFS presents a novel way to study molecular interactions of long lived states.