Resolved in Space and Time by N&B Analysis. This work was supported in part by a grant from Allergan, Inc.

In vitro lifetimes of non-interacting, monoexponential-decay fluorophores and intrinsic protein fluorescence using the phasor plot approach. Phasor points from binary mixtures of varying composition lie along the line connecting the individual component points on the universal circle, while tertiary mixture points fall in a triangle between the individual vectors. Molecular interactions such as protein dissociation, protein-ligand interaction, denaturation, and energy transfer resulted in changes in the position of the vector point allowing for a rapid, graphical representation of these complex reactions. Data at a single frequency may be recorded rapidly allowing resolution of kinetic processes that would be difficult to monitor using complete multifrequency approaches. The combined results demonstrate the value of the phasor plot method to in vitro lifetime analysis. This work was supported in part by a grant from Allergan, Inc.

Application of Phasor Plots to Analysis of Fluorophore Heterogeneity, Excited State Reactions and Protein Conformation

Christian Hellriegel1

resolved fluorescence data, obtained using either frequency or time domain methods, independently of model constraints. Using the phase and modulation approach, the phasor plot converts raw data at a single frequency to a vector. Single exponential decays appear on the universal circle (semicircle with radius 0.5 and center 0.5, 0) whereas decays due to multiple exponentials appear as points inside the universal circle. This method has been successfully applied to fluorescence lifetime imaging microscopy (FLIM) wherein the data are typically collected at only one frequency. Applications of phasor plots in FLIM studies have, to date, been largely limited to FRET studies in cells. We have extended the application of phasor plots to several in vitro systems. Specifically, we have analyzed frequency-domain data of binary and tertiary mixtures of non-interacting,

Anomalous Diffusion as a Readout for the Folding Status of Transmembrane Proteins

Nina Malchus1

In the ER, chaperones supervise their folding as unfolded polypeptide chains. In the ER, chaperones supervise their folding process and prevent, by still poorly understood mechanisms, a premature export from the ER. Here, we used Fluorescence Correlation Spectroscopy (FCS) to investigate the interaction of a prototypical transmembrane cargo protein, tsO-45-G, a temperature-sensitive mutant of VSV-G, with the ER quality control machinery in vivo by quantifying the proteins diffusion properties in the ER under various conditions. Our experimental data and accompanying simulations show that the diffusion of unfolded tsO-45-GFP in the ER is strongly anomalous, most likely due to a transient oligomerization with UDP-glucose:glycoprotein glucosyltransferase (UGT1). In contrast, folded tsO-45-G, calnexin-associated unfolded tsO-45-G, or a mutant tsO-45-G with only one glycan are significantly less obstructed in their diffusion behavior.

Counting Up the Molecules in Live Bacillus Subtilis by Fluctuation Imaging and Analysis: An in Vivo Study of Transcriptional Regulation

Matthew L. Ferguson1, Matthieu Jules2, Dominique Le Coq3, Stéphane Aymery2, Nathalie Declerck1, Catherine A. Royer1

We have developed an automated fluorescence cross-correlation screen to detect molecular interactions in yeast. Using fluorescence cross-correlation spectroscopy (FCCS) positive control strains containing linked eGFP and mCherry proteins could be reproducibly distinguished from negative controls with independently diffusing populations of the two fluorophores. Transmitted light images were acquired in parallel with FCCS measurements to determine protein localization and cell health. Data was taken in a 96-well format on a commercially available microscope with the addition of a ConfoCor3 (Carl Zeiss Jena GmbH, Germany). Custom software controlled navigation of the 96-well plates, detection of yeast cells, and selection of cellular regions for taking FCCS measurements, making the screen adaptable for larger scale experiments. Using this method, proteins of particular interest can be rapidly screened against large portions of the proteome to uncover their contribution to the function of a complex network of proteins.

Automated Screen of in Vivo Molecular Interactions using Fluorescence Correlation Spectroscopy (FCCS)


Stowers Institute for Medical Research, Kansas City, MO, USA.

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