

A theory for FRAP analysis at localized binding sites

Brian L. Sprague¹, Robert L. Pego², Peter M. Bungay³, Diana A. Stavreva¹, James G. McNally¹

¹Laboratory of Receptor Biology and Gene Expression, NCI, NIH, Bethesda, MD

²Department of Mathematical Sciences, Carnegie Mellon University, Pittsburgh, PA

³Division of Bioengineering & Physical Science, NIH, Bethesda, MD

Spatial heterogeneity is characteristic of cellular architecture. Numerous cellular structures, such as focal adhesions, centrosomes, nuclear bodies or transcription sites, are defined by the subset of specialized proteins that accumulate there. When a constituent protein from one of these structures is tagged with GFP, its dynamics at the localized binding site are often analyzed by fluorescence recovery after photobleaching or FRAP. Although a number of qualitative and a few quantitative analyses of such FRAP data have been performed, there is neither a general theory for understanding the range of possible FRAP behaviors at localized binding sites, nor a guide for how to extract binding parameters from such data. Here we develop and describe such a theory. We discuss the range of FRAP behaviors that arise under various limiting conditions, such as when binding is either fast or slow with respect to diffusion. For all of these scenarios, we obtain solutions for the FRAP recovery that can be used to estimate on and off rates of binding at the localized binding sites. We illustrate application of the method by analyzing binding of a transcription factor at its promoter target site, and obtain from these FRAP data an estimate for an *in vivo* binding constant. These FRAP analysis tools will be critical for measuring many of the cellular binding parameters necessary for a complete and accurate description of the protein networks that regulate cellular behavior.