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Abstract: 104O

Brightness cross correlation spectroscopy quantifies protein dynamics as a function of stoichiometry within live cells.

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The cellular environment is very crowded, and yet somehow, biological proteins efficiently navigate this framework to arrive at specific binding targets. Several proteins self-associate into homo or hetero oligomeric species, and the change in hydrodynamic radius associated with these events is thought to refine the cellular space accessible for protein diffusion, as well as ligand binding affinity. To what extent protein oligomerisation facilitates arrival at a specific target site is not entirely understood due to a lack of methods to dissect intracellular protein mobility as a function of homo versus hetero complex formation. To directly track this process in a living cell, here we present brightness cross correlation spectroscopy that is a new method for fluctuation analysis, which can extract protein mobility as a function stoichiometry throughout live cell confocal microscopy data [1]. From correlation of brightness fluctuations originating from one or two fluorescently tagged proteins within a single or dual channel frame acquisition, this approach has the capacity to extract and spatially map protein mobility as a function of homo and hetero oligomeric state with respect to intracellular architecture. Application of this technology to the signal transducer and activation of transcription (STAT) family of transcription factors reveals homo and hetero oligomer formation to differentially regulate chromatin accessibility and interaction with the DNA template, upon activation of distinct signalling pathways. Importantly, this mechanistic detail is only visible because of the unique capacity of brightness cross correlation spectroscopy to analyse fluorescent protein dynamics as a function of oligomeric state.

1. Solano, A., Lou, J., Scipioni, L., Gratton, E. and Hinde, E., 2022. Radial pair correlation of molecular brightness fluctuations maps protein diffusion as a function of oligomeric state within live-cell nuclear architecture. Biophysical Journal. 121(11), pp. 2152-2167.