MODEL-FREE ANALYSIS OF STEP-SIZE DISTRIBUTIONS TO DETECT AND QUANTIFY STOCHASTIC PROCESSES IN LIVE CELLS

Stefano Coppola[1], Veer I.P. Keizer[2], Marcel J.M. Schaaf[2], Thomas Schmidt[1]

[1]Physics of Life Processes, Huygens-Kamerlingh Onnes Laboratory; [2]Institute of Biology

Leiden University, Niels Bohrweg 2, 2333 CA Leiden, The Netherlands

Email: coppola@physics.leidenuniv.nl

KEY WORDS: fluorescence; protein dynamics; image correlation spectroscopy; single particle tracking; single molecule; phasors.

Microscopic information about the location and time of biological objects is at the heart of understanding of cellular processes. Nowadays this task is achieved at high spatial and temporal resolution by a plethora of available experimental techniques. Yet, analysis of such data is heavily affected by the models chosen a-priori in order to obtain quantitative information. Here we developed a novel model-free analysis tool to investigate the transport of biological objects. The methodology is based on the analysis of squared displacement distributions, generated by either single-molecule/particle tracking (SPT) [1] and image correlation spectroscopy (ICS) techniques [2, 3], by means of the model-free phasor approach known from fluorescence lifetime imaging [4]. Phasors exhibit unique fingerprint properties which allows one to determine, without a-priori knowledge on the motion, the appropriate mobility models which underlie the experimental data.

The strength of the method is exemplified on the DNA-binding dynamics of the glucocorticoid receptor (GR). The majority of activated GRs diffuses freely in the nucleus. Here we show that the remaining fraction could be split into an immobile fraction that is stably bound to DNA, and a less mobile fraction that exhibits frequent, short DNA-binding events. Disruption of the DNA-binding domain of GR altered the ratio between these fractions, resulting in a significant reduction in the fraction bound to DNA, paralleled by an increase of the free diffusive fraction. Our data supports models of search strategies for DNA-binding proteins, in which target search occurs through intermittent nonspecific binding to DNA, which is reflected in receptor slowdown.

[1] Schmidt T, Schütz G, Baumgartner W, Gruber H, & Schindler H (1996) Imaging of single molecule diffusion. *Proceedings of the National Academy of Sciences* **93**(7):2926-2929.

[2] Hebert B, Costantino S, & Wiseman PW (2005) Spatiotemporal image correlation spectroscopy (STICS) theory, verification, and application to protein velocity mapping in living CHO cells. *Biophysical journal* **88**(5):3601-3614.

[3] Semrau S & Schmidt T (2007) Particle image correlation spectroscopy (PICS): retrieving nanometer-scale correlations from high-density single-molecule position data. *Biophysical journal* **92**(2):613-621.

[4] Digman MA, Caiolfa VR, Zamai M, & Gratton E (2008) The phasor approach to fluorescence lifetime imaging analysis. *Biophysical journal* **94**(2):L14-L16.