New and Notable

Get Your kICS by Measuring Membrane Protein Dynamics

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Akin to the puck on an ice hockey rink, the lateral dynamics of a representative membrane protein exhibits rich behavior (1). Depending on the interaction with the hockey players and other obstacles (membrane constituents), the puck (protein) can dart around the ice (membrane surface) or be stalled momentarily (transient confinement zone). If we watched the motion of a single puck, we would need to watch many hockey games before we might eventually work out the rules of the game. But what if we could watch many games in parallel? This is where the suite of image correlation spectroscopy (ICS) techniques and its newest member, k-space image correlation spectroscopy (kICS) comes in Abu-Arish et al. (2).

ICS and kICS are members of a family of techniques which began with fluorescence correlation spectroscopy (3). Fluorescence fluctuation analysis provides the statistical mechanical foundation with correlations between fluorescence fluctuations measured relative to a given lag vector. Correlations can be made as a function of time (fixed space) called FCS (3), fluctuations in space (fixed time) called ICS (4), and space-time fluctuations called STICS (5). The ergodic principle ensures equivalence between occupancy fluctuations whether in time or space.

In temporal ICS or TICS, one essentially images in time (using a laser scanning fluorescence confocal microscope) many molecules in parallel as they change positions from one image to the next image. If the molecules do not move between the first image and the second image, the spatial correlation between image one and image two will be high. However, if movement does occur between image one and image two, there is a loss of spatial correlation. By analyzing the correlation between images collected at different times, information on protein motion could be obtained. This method provides information from many molecules without the need for tracking individual particles. A problem endemic to all these methods is that fluctuations in fluorescence are measured as opposed to particle fluctuations which are the desired quantities in evaluating transport properties.

In kICS (6), images collected from a time-series are first Fourier transformed into k-space before image cross-correlation is performed. This has two distinct advantages over (real space) ICS. First, fluctuations from photophysics (e.g., *cis-trans* isomerization, (de) protonation, reversible dark state quenching, reversible photobleaching) do not contribute to the determination of transport coefficients (6). Second, determination of the lateral point spread function dimension is not required, unlike conventional fluorescence fluctuation approaches (6).

In this issue of the *Biophysical Journal,* the pioneer of ICS and kICS, Paul Wiseman has teamed up with Asmahan Abu-Arish, Elvis Pandzic, Julie Goepp, Elizabeth Matthes, and John Hanrahan (2) to gain new insights into the dynamics of a biomedically important membrane protein, the cystic fibrosis transmembrane conductance regulator. Using the kICS technique, the authors have provided evidence for two populations of cystic fibrosis transmembrane conductance regulator (CFTR) molecules, which differed in degree of confinement and lateral motion on the cell surface. Impressively, they were able to extract information on the dynamics of CFTR inside domains, CFTR dynamics outside (and between) domains, fractional populations, and degree of confinement (within domains). ICS analysis delivered cluster densities and mean number of molecules per cluster.

The authors examined the effect of cholesterol on dynamics and clustering behavior of CFTR. Cholesterol has a remarkable effect on membrane protein assembly, dynamics and function (7–9). Depletion of cholesterol caused the confined fraction and average number of CFTR molecules per cluster to decrease, whereas increase in cholesterol were found to be associated with increase in clustering and increased confined fraction. Interestingly, viral infection was shown to increase clustering further into larger platforms with reduced CFTR mobility. These observations and analyses suggest that cholesterol-influenced membrane domains play an important role in the cell surface behavior and pathology of CFTR.

Aside from the important new insights into this anion channel, the results of this study revealed how complex cell surface dynamics and clustering can be measured using powerful fluorescence microscopy techniques. With these new methods in hand, biophysicists can sit back, relax and enjoy the hockey game.

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