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Unraveling novel features hidden in superresolution microscopy data

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Stochastic analysis of superresolution microscopy data obtained from receptor trafficking on neurons reveals novel organized molecular assembly generating long range forces. Would that have been possible with single particle tracking? How have we characterized these molecular assemblies?

High-density superresolution microscopy of live cells enables the simultaneous tracking of many thousands of individual trajectories.[1,2] Can we expect finding new biophysical features inside these new collections of data different from what has already been described by classical single particle tracking (SPT) data? In classical SPT, observed trajectories are of much longer time than in superresolution, but the number of them passing through any available point is drastically reduced. In the past 20 y, the classical framework[3-5] used to analyze these trajectories came from the theory of Brownian motion and the main tool employed was the mean square displacement (the sum of the local spatial increment squared). The nature of the motion (Brownian or any deviation of it), confined or free, diffusive, super or anomalous diffusion can be extracted from the MSD when it is plotted over various time intervals. This allows extracting mean velocities or the spread of the diffusion coefficient or other associated statistical quantities. For example, this analysis was applied successfully to characterize statistically diffusion of receptor trafficking on the neuronal dendrite, a field that helps clarifying the role of trafficking for synaptic transmission and plasticity.[6,7] However, the MSD and derived methods applied to SPT have several major limitations: indeed, the computations required pulling long trajectories together and the spatial organization underlying the nature of each trajectory is inevitably lost, preventing the access to the local geometrical structures underlying molecular organization. This obstacle is precisely overcome in the superresolution analysis. Indeed, contrary to SPT, by combining trajectories passing many times through plenty of points the superresolution analysis does not destroy the spatial nanometric structures. The analysis starts as follows: the physical description of the motion developed by Langevin and Smoluchowski[8,9] asserts that the dynamics of a stochastic particle is due to the sum of a local field of force plus a Brownian collision term. Using these two quantities, it is possible to simulate the motion of any stochastic trajectories. Interestingly, the superresolution data allows precisely solving the reverse engineering problem: given many trajectories, reconstruct locally the field and the local diffusion properties (diffusion tensor). Using these classical concepts derived from statistical physics, we thus extracted[10] the two terms (field of forces and Brownian forces) and the biophysical parameters responsible for AMPA receptor (AMPAR) trafficking in hippocampal dendrites. AMPAR are involved in mediating the glutamatergic synaptic transmission.

Using this analysis, we found[10] that regions of high density of receptors were described by potential wells, that are extensively used in chemistry to characterize molecule-molecule interaction. We found two categories of wells: the small ones associated with an energy less than 6 kT and large ones (> 8 kT). But what remains today unexplained is the large extension of the wells of the order of hundreds of

nanometers. It seems quite surprising to find field of forces with such extension in the plasma membrane of a cell.[11] Indeed the main molecular interactions are short range electrostatic forces, which extend few nanometers apart at most. The nature of these forces remains unexplained, how can they generate long range interaction organized in potential wells and in particular, what are the local molecular structures that can underlie these long range field of forces. In addition, these potential wells are not statics but dynamics in time and they can also regulate receptor trafficking in dendritic spines at the time scale of tens of minutes. Other features that we could observe are inward and outward flow fields inside dendritic spine neck. Finally, from the fluctuation of the diffusion coefficient and by using mathematical modeling of Brownian diffusion between generic round obstacles,[12] it is possible to get an idea of the density of obstacles that is generating the diffusion coefficient.

To conclude, the massive throughput superresolution data allows resolving reverse engineering problem to identify key biophysical features of receptor trafficking, which could not be previously addressed. This method is only at its infancy and novel concepts are now required to understand the long range interaction underlying potential wells. Receptors are not point-like particles and accounting for their complex transmembrane subunits will certainly play a fundamental role in determining their interaction with scaffolding molecules. A similar method can also be applied to analyze other superresolution data for viral capsid assembly.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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