Analysis of Protein Stoichiometry and Organelle Dynamics by Single-Molecule Fluorescence Imaging

Xiaohong Fang

Key Laboratory of Molecular Nanostructure and Nanotechnology, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China
E-mail: xfang@iccas.ac.cn

Single-molecule fluorescence imaging based stoichiometry analysis is becoming a powerful tool in cellular protein study since protein stoichiometry is fundamental to protein function. With the single-molecule imaging of the membrane receptors and quantification of receptor stoichiometry change, we have revealed the new activation models of transforming growth factor receptor and others. To achieve more accurate stoichiometry analysis by efficiently extract weak single-molecule signals from the high-level noises in cells, we developed a novel automated stoichiometry analysis of single-molecule fluorescence photobleaching traces via deep learning. We used convolutional layers to extract features of steplike photobleaching drops and long short term memory (LSTM) recurrent layers to distinguish between photobleaching and photoblinking events. Compared with traditional algorithms, our method shows higher accuracy with at least 2 orders of magnitude improvement of efficiency, and it does not require user-specified parameters. We further developed an unsupervised deep learning framework of discriminator-generator network (DGN) which consists of two bidirectional LSTM networks. The DGN can not only count the photobleaching steps but also extract dynamic information on stoichiometry change, such as durations of protein association, transition rates. The deep learning method is expected to provide a new strategy to stoichiometry study.

Photostable fluorescent probes are essential to long-time intracellular tracking. We developed a new type of photobleaching-resistant semiconducting polymer dots (Pdots) by nanoprecipitation of hydrophobic fluorescent polymer and amphiphilic polymer to form nanoparticles. The Pdots have large Stokes shifts, which make it possible to achieve dual-color STED imaging with a single pair of excitation and depletion laser beams. Tracking of cellular organelles by the Pdots has been realized in living cells, and the dynamic interaction of endosomes derived from clathrin-mediated and caveolae-mediated endocytic pathways has been monitored for the first time to propose their interaction models. The results demonstrated the promising potential of using Pdots as a new class of ultrastable fluorescent probe for cellular tracking.

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