STUDY OF PROTEIN DYNAMICS AND INTERACTIONS AT THE SINGLE-MOLECULE LEVEL BY LIVE-CELL FLUORESCENCE MICROSCOPY

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

Live-cell fluorescence microscopy is powerful in directly characterizing the dynamics of cellular events. By single-molecule imaging of the membrane protein receptors, we realized the quantification of receptor stoichiometry and monitoring of receptor transportation dynamics, which led to the discovery of several new activation and internalization pathways.

To achieve more accurate stoichiometry analysis, the weak signal-molecule signals should be efficiently extracted from the high-level noises in cells. Thus we developed a novel automated stoichiometry analysis of single-molecule fluorescence photobleaching traces via deep learning, in which the convolutional layers are designed 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. This provided a new strategy to stoichiometry study and time series analysis in chemistry.

Photostable fluorescent probes are essential to long-time intracellular protein tracking. We developed a new type of photobleaching-resistant semiconducting polymer dots (Pdots) by nanoprecipitation of the mixture of hydrophobic fluorescent polymer poly and amphiphilic polymer to form monodispersed nanoparticles. The Pdots showed superior photostability, bright fluorescence, large Stokes shift, and easy surface functionalization. Long-term (2 h) continuous super-resolution STED imaging of cellular Pdots and live-cell dynamic tracking are achieved. The results demonstrated the promising potential of using Pdots as a new class of ultrastable fluorescent probe with super-resolution imaging capability.

REFERENCES