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Quantification of Membrane Protein Dynamics and Interactions in Plant Cells by Fluorescence Correlation Spectroscopy

Xiaojuan Li[1,6], Jingjing Xing[2,3,6], Zongbo Qiu[4], Qihua He[5] and Jinxing Lin[1,*]

[1]College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, China

[2]Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

[3]University of Chinese Academy of Sciences, Beijing 10049, China

[4]College of Life Sciences, Henan Normal University, Xinxiang 453007, China

[5]The Health Science Center, Peking University, Beijing 100191, China

[6]These authors contributed equally to this article.

***Correspondence: Jinxing Lin (linjx@ibcas.ac.cn)**

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ABSTRACT

Deciphering the dynamics of protein and lipid molecules on appropriate spatial and temporal scales may shed light on protein function and membrane organization. However, traditional bulk approaches cannot unambiguously quantify the extremely diverse mobility and interactions of proteins in living cells. Fluorescence correlation spectroscopy (FCS) is a powerful technique to describe events that occur at the single-molecule level and on the nanosecond to second timescales; therefore, FCS can provide data on the heterogeneous organization of membrane systems. FCS can also be combined with other microscopy techniques, such as super-resolution techniques. More importantly, FCS is minimally invasive, which makes it an ideal approach to detect the heterogeneous distribution and dynamics of key proteins during development. In this review, we give a brief introduction about the development of FCS and summarize the significant contributions of FCS in understanding the organization of plant cell membranes and the dynamics and interactions of membrane proteins .We also discuss the potential applications of this technique in plant biology.

Key words: fluorescence correlation spectroscopy, microdomain, membrane protein, dynamics, interaction

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INTRODUCTION

Our understanding of the cell membrane has evolved over the past decades, from the view that it plays a passive role as a barrier to the current view that the membrane plays a more active role in various cellular processes such as molecular transport, protein trafficking, and signaling pathways. Considering the fluid nature of the membrane as a key feature, molecular motion likely acts as an important determinant that can control the localization, abundance, and activity of proteins in the plasma membrane. Therefore, deciphering the motion of proteins and lipids in the membrane enables insights into protein function and organization of the cell membrane, particularly of high-viscosity membrane microdomains such as membrane rafts (Owen et al., 2009). Various *in vitro* tools have been developed to examine the mechanisms that regulate the function and localization of membrane proteins. However, the measured data do not necessarily reflect the complexity of membrane systems in a living cell and can sometimes produce inconsistent results. Even *in vivo* data from loss-of-function experiments or colocalization studies mainly provide indirect or qualitative information. Precisely deciphering the heterogeneous motions of proteins and organization of membranes with appropriate spatiotemporal resolution remains a challenge for physicists and biologists.

One of the techniques with great potential to address this challenge is fluorescence correlation spectroscopy (FCS). First developed in early 1970s, FCS involves analysis of fluctuations in the fluorescence emission of sample molecules, induced by spontaneous fluctuations of physical parameters. FCS has mainly been used in studies of translational diffusion of labeled macromolecules in solution (Magde et al., 1972, 1974) but has gained

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