FLUORESCENCE CORRELATION SPECTROSCOPY TO PROBE MOLECULAR CONFINEMENT IN LIVE CELL MEMBRANES

Laure Wawrezinieck[1,2], Didier Marguet[2], Hervé Rigneault[1] and Pierre-François Lenne[1]

[1] Institut Fresnel
(CNRS - Univ. Paul Cézanne Aix Marseille III)
Domaine Universitaire de Saint Jerôme - 13397 Marseille cedex 20 - France
[2] Centre Immunologie Marseille Luminy
(CNRS - INSERM - Univ. de la Méditerranée Aix-Marseille II)
Parc scientifique de Luminy - 13009 Marseille cedex 9 - France
E-mail : lenne@fresnel.fr

KEY WORDS: Fluorescence Correlation Spectroscopy, hindered diffusion, cystoskeleton, lipid microdomains, live cell membranes

Fluorescence correlation spectroscopy (FCS) is a mature and powerful technique for measuring diffusion coefficients. In a standard experiment, it measures the spontaneous fluorescence fluctuations arising from a single observation volume defined by confocal optics. However, the study becomes uneasy when the diffusion is not free as it is the case for the cell membrane components in live cells. We have shown that doing FCS measurements at different sizes of observation volumes gives access to the diffusion laws without a priori knowledge of the landscape in which molecules are diffusing[1]. We investigate with this method the dynamics of cell membrane components.

Our present description of the plasma membrane includes heterogeneities or mechanisms that may hinder or even confine the diffusion of membrane particles. Indeed, the diffusion of proteins may be impeded by the presence of "corrals" formed by the actin-based membrane skeleton, whereas lipid microdomains are thought to transiently sequester molecules which are involved in signaling pathways and cell sorting.

We show that FCS observations enable to quantify an average confinement time within a microdomain and a related effective diffusion coefficient. Using this strategy on live cells, we report that microdomain-associated molecules exhibit different temporal confinements, which range from a few to tens of milliseconds. Variation of the cholesterol content results in drastic changes of confinement times. Hindrance by the cytoskeleton leads to diffusion laws having a different shape. Our method enables to distinguish different mechanisms responsible for the confinement of particles diffusing in the plasma membrane.

[1] L. Wawrezinieck, P.-F. Lenne, D. Marguet and H. Rigneault, "Fluorescence Correlation Spectroscopy to determine diffusion laws: application to live cell membranes", in *Proc. SPIE* **5462**, 92-102 (Biophotonics Micro- and Nano-Imaging, Dario Anselmetti, 2004)