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Microfluidity mapping using fluorescence correlation spectroscopy: A new way to investigate plasma membrane microorganization of living cells

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ABSTRACT

Diffusion time distribution analysis has been employed to highlight the microfluidity fingerprint of plasma membrane of living cells. Diffusion time measurements were obtained through fluorescence correlation spectroscopy performed at the single cell level, over various eukaryotic cell lines (MCF7, LR73, KB3.1, MESSA and MDCKII). The nonsymmetric profile of the diffusion time distributions established experimentally, is discussed according to Monte Carlo simulations, which reproduce the diffusion of the fluorescent probe in heterogeneous membrane.

1. Introduction

The understanding of the plasma membrane organization requires powerful tools, for example to decipher and study submicronic domains. A typical membrane is a fluid assembly of hundreds of different lipids and proteins. All these molecules interact with each other through different physical interactions which take place at the nanoscale, such as Van der Waals forces, electrostatic forces, or hydrophobic forces. These fundamental molecular interactions give rise within the bilayer to laterally differentiated areas, characterized by a specific composition and packing, the so-called lipid rafts, corrals, and caveolae for example [1,2]. Moreover, proteins anchored to the cytoskeleton can provide effective fences or corrals, which lead to transient or permanent membrane domains [3]. This small lateral organization implies differentiation and compartmentalization of the lipid bilayer and has consequences onto the diffusional properties of membrane-bound molecules, such as enzymes and receptors. Its local organization may also influence the kinetics of chemical reactions and thus support membrane functions, such as signaling, protein and lipid trafficking, or cell growth [4,5]. The lateral bilayer structure is still not well established, especially regarding small heterogeneities called microdomains. The reason is that the spatial scale of these domains is significantly beyond the diffraction limit of optical microscope. It is thus experimentally difficult to directly visualize and investigate them in living cells.

Since membrane heterogeneities constrain molecular dynamic and thus locally alter plasma membrane fluidity [6], membrane fluidity can be measured and quantified as relevant biophysical parameter in the investigation of membrane microorganization of living cells. Currently, well-known methods such as fluorescence recovery after photobleaching (FRAP) or fluorescence anisotropy are widely used to study membrane fluidity. However these methods require a high concentration of dye molecules for labeling, which can locally perturb the membrane fluidity. Single molecule fluorescence spectroscopy techniques constitute an original experimental approach to overcome this invasive aspect [7]. Among the single molecule techniques, fluorescence correlation spectroscopy (FCS) is becoming a popular analytical method, well suited to investigate the fluidity of biological membranes through the lateral diffusion of a fluorescent membrane probe. The potential of FCS to relate heterogeneity in model and natural membranes was previously demonstrated [8,9]. FCS measurements at different spatial scales were also done to probe membrane domains organization with so-called FCS diffusion laws [10–12]. Based on FCS, diffusion-time distribution analysis (DDA) was recently applied to analyze heterogeneous samples such as plasma membrane of living cells [13,14]. In this paper, we propose to explore the plasma membrane fluidity of several cell lines (MCF7, LR73, KB3.1, MESSA,

Abbreviations: FCS, fluorescence correlation spectroscopy; GUV, giant unilamellar vesicle; FRAP, fluorescence recovery after photobleaching; ACF, autocorrelation function; DDA, diffusion-time distribution analysis

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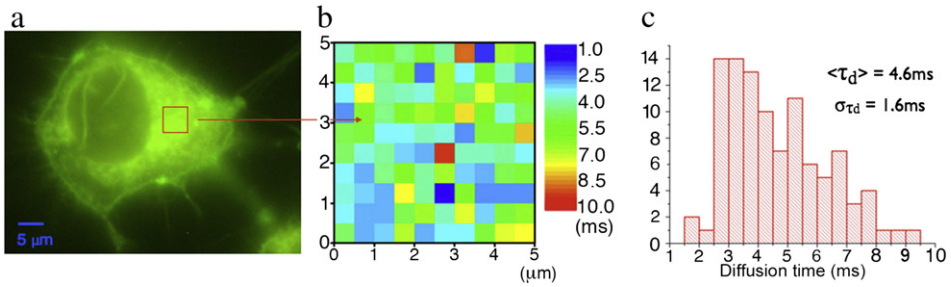

Fig. 1. Diffusion time mapping on a MDCKII cell. (a) Fluorescence image of a DiA labeled cell (bar = 5 µm). (b) Diffusion time mapping: 10×10 pixel image on a 5 µm square area of plasma membrane. (c) The corresponding diffusion time distribution. Autocorrelation functions were fitted by Eq. (1).

MDCKII) using DDA, in order to highlight the unexpected microfluidity fingerprint of living cells. For this, we implement at the single cell level a multi point FCS measurement. To obtain the microfluidity mapping of the plasma membrane we probe the diffusion of a small amphiphile dye (DiA), well known to label homogeneously the membrane of cells (no specific targeting). Additionally, we propose a simulation based on a Monte Carlo model to correlate the membrane microfluidity distribution with their organization in terms of viscous microdomains.

2. Materials and methods

2.1. FCS measurements

To know more about cell culture, see sample preparation and FCS measurements in the Supplementary material. In order to avoid perturbations due to the biological material inside the cell, measurements were performed on the plasma membrane in contact with the glass substrate (in practice at the vicinity of the glass–water interface). Plasma membrane is precisely localized by moving the observation volume, see Supplementary material, Fig. S2. Fig. 1 shows a diffusion time mapping on an MDCKII cell obtained with repeated measurements of 4 s, along with a 10×10 grid with 500 nm steps. This picture clearly confirms the heterogeneous nature of plasma membrane at the submicronic scale.

We need at least 20 to 30 s of time recording to obtain a satisfying ACF accuracy, see Supplementary material, Fig. S3. Unfortunately, cells can move after few tens of minutes and it is difficult to perform an important number of measurements on a single cell. Therefore we choose to target ~25 points of measurement on the plasma membrane of each cell, according to a 5×5 grid pattern of 4 µm step. Each FCS measurement was then recorded during 30 s. In these conditions, it was necessary to study several cells of a same line to obtain enough measurements to build an acceptable diffusion time distribution. Thereafter, all data were represented as probability density histograms. The column bin size was fixed at 0.33 ms. This value was determined by the measurement of the diffusion time relative uncertainty, estimated at 0.16 (or 16%) through 8 series of 5 consecutive measurements recorded on different areas of the plasma membrane.

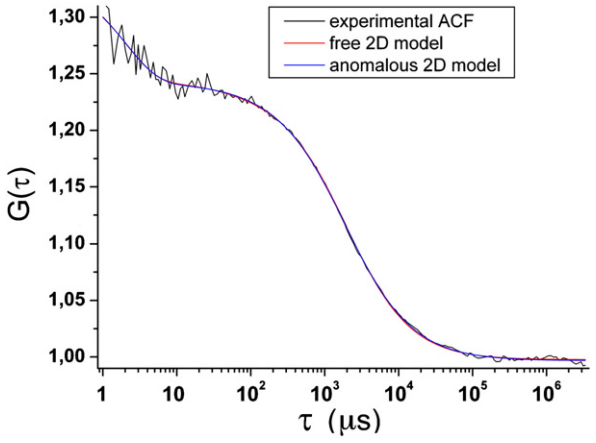

Fig. 2. Typical ACF on cells membrane fitted by the "Anomalous 2D" and "free 2D" models. Values from these fits are: "free 2D diffusion" (red curve): p = 0.28, $\tau_p = 2.0$ µs, $\tau_{2D}^{free} = 2.00$ ms, $F_{2D} = 0.94$, $N = 4.06$. "Anomalous 2D diffusion" (blue curve): p = 0.27, $\tau_p = 2.1$ µs, $\tau_{2D}^{ano} = 1.89$ ms, $\alpha = 0.95$, $F_{2D} = 0.98$, $N = 4.07$. The normalized residual sum of squares (see in the Supplementary material) of these fits is respectively: $\langle RSS\rangle_{free2D} = 1.66.10^{-5}$ and $\langle RSS\rangle_{Anomal2D} = 1.62.10^{-5}$.

2.2. Monte Carlo simulation

Monte Carlo simulation has been implemented to numerically reproduce FCS experiments on heterogeneous cells membrane and provide a numerical support to data analysis. To simulate diffusion in membrane, fluorescent molecules are randomly incorporated in a 2D square mesh, Supplementary material Fig. S5(a). The width of the elementary pattern, named box, is 5 nm. The excitation laser beam is supposed to be Gaussian and centered on the lattice. The radius of the illuminated observation area is given by the beam waist, $\omega_o = 226$ nm. The total size of the simulation area is 5 times larger than ω_o to avoid any side effects. The total number of molecules in the simulation window corresponds to a mean number N of one dye in the illuminated area. In order to simulate various proportions and viscosity of membrane heterogeneities, a relative viscosity value, between 0 and 1, is randomly attributed to each box of the simulation window (1 represents an impermeable viscous box and 0 is a standard fluid one). Each fluorescent molecule follows a random walk from a randomly selected starting position. The random walk was performed as following:

- at each time step, an adjacent box is randomly selected,
- the probability to effectively jump into this new box is given by the relative fluidity of the incoming box, defined as relative fluidity = 1 − relative viscosity, and
- if the molecule goes out of the simulation window, a new one is randomly inserted in the border.

10^8 steps are necessary to calculate each trajectory. At each time step, the detected intensity is computed assuming a fluorescence signal directly proportional to the Gaussian laser illumination profile, Supplementary material Fig. S5(a) and (b). The autocorrelation function of this simulated fluorescence signal was then calculated according to a logarithmic progression. A typical simulated ACF is shown in the Supplementary material Fig. S5(c).

2.3. Fitting function

The choice of an analytical fit function is a crucial point for data analysis in FCS. To fit ACF recorded on cells membrane, two standard models can be used, as seen in the literature. The first one is based on a free 2D diffusion model, and the other one on an anomalous 2D