Adhesion of giant unilamellar vesicles and living cells revealed by quantitative total internal reflection fluorescence microscopy

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Total Internal Reflection Fluorescence (TIRF) microscopy is becoming a widespread technique to study cellular processes occurring near the contact region with the glass substrate [1]. The characteristics of TIRF microscopy are directly related to the singular properties of evanescent waves, such as the exponential decay of the electric field along the z direction. This allows to provide a selective excitation of fluorescent molecules close to the interface. Determination of the accurate distance from the surface to the plasma membrane constitutes a crucial issue to investigate the physical basis of cellular adhesion process [2]. However, quantitative interpretation of TIRF pictures regarding the distance z between a labeled membrane and the substrate is not trivial. Indeed, the contrast of TIRF images depends on several parameters. The first one is obviously the distance z, which separates the dye molecules from the surface, as the emitted fluorescence signal is mainly governed by the exponential decay of the evanescent wave. But TIRF images contrast is also affected by unknown parameters such as the local concentration of dyes, their electric dipole moment orientation, their related angular emission pattern (which influences the detection efficiency), and also their fluorescence lifetime (which plays a role on the quantum yield). Moreover, these last two parameters can be strongly altered as a function of z near the surface [3].

To get around this problem, we propose two strategies allowing us to map the membrane-substrate separation distance with a nanometric resolution (typically 10-20 nm). The first one is dedicated to study the adhesion of Giant Unilamellar Vesicles (GUVs), which are often used as a biomimetic system to reproduce cells spreading. This approach, called nTIRF, is based on dual observation, which combine epi-fluorescence microscopy and TIRF microscopy: TIRF images are normalized by epi-fluorescence ones [4, 5]. Figure 1 shows an example of a negatively charged GUV in interaction with a thin layer of a positively charged polyelectrolyte recovering a coverslip. The second technique is devoted to explore the adhesion of living cells. This last is called variable-angle TIRF (vaTIRF) microscopy. vaTIRF was developed by Burmeister, Truskey and Reichert at the beginning of 1990s with a prism-based TIRF microscope for determine the membrane-surface separation distance on the so-called focal adhesion zones [6]. This first study and more recent other ones have shown that the plasma membrane was situated at typically 40-50 nm from the surface on the focal adhesion points, while the major part of the membrane was located beyond 100 nm. We propose a more convenient prismless setup, which uses only a rotatable mirror to adjust precisely the laser beam on the back focal plane of the oil immersion objective (no azimuthal scanning is needed with our experimental setup). Our technique consists to record a series of pictures at different incident angles in evanescent regime. This series of TIRF images allows us to calculate accurately membrane-surface distances in each pixel, as illustrated in figure 2 for a MDA MB 231 cell in adhesion on fibronectin coated coverslip [7].

Finally, we demonstrate that these two techniques (nTIRF and vaTIRF) are useful to

quantify the adhesion of vesicles and cells from weak to strong membrane-surface interactions, achieved on various functionalized substrates with polymers or proteins, such as collagen and fibronectin.

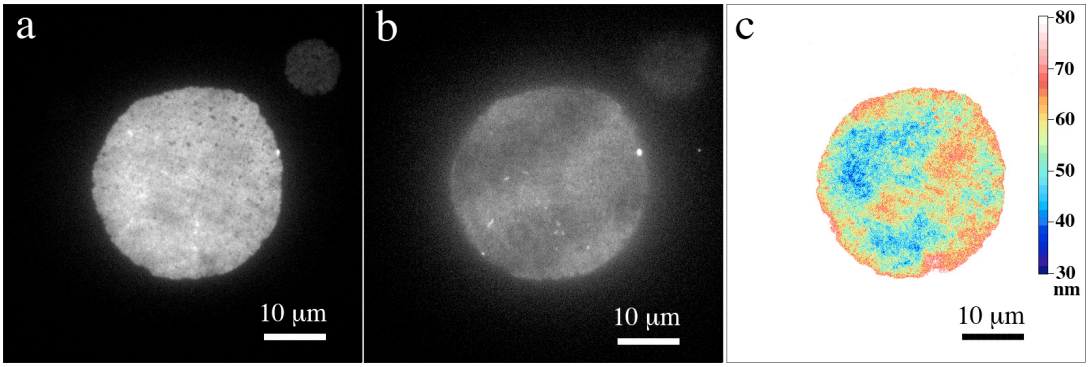

Figure 1: DOPC vesicle in adhesion on a coverslip coated with PDDA. The membrane was labeled with DOPE-ATTO488. (a): TIRF image at the incident angle θ=67°, (b): epi-fluorescence image at θ=0°, (c): corresponding membrane-surface distance image. Fluorescence images were recorded in 300 ms, with a laser excitation at 488 nm at 25 W/cm^2.

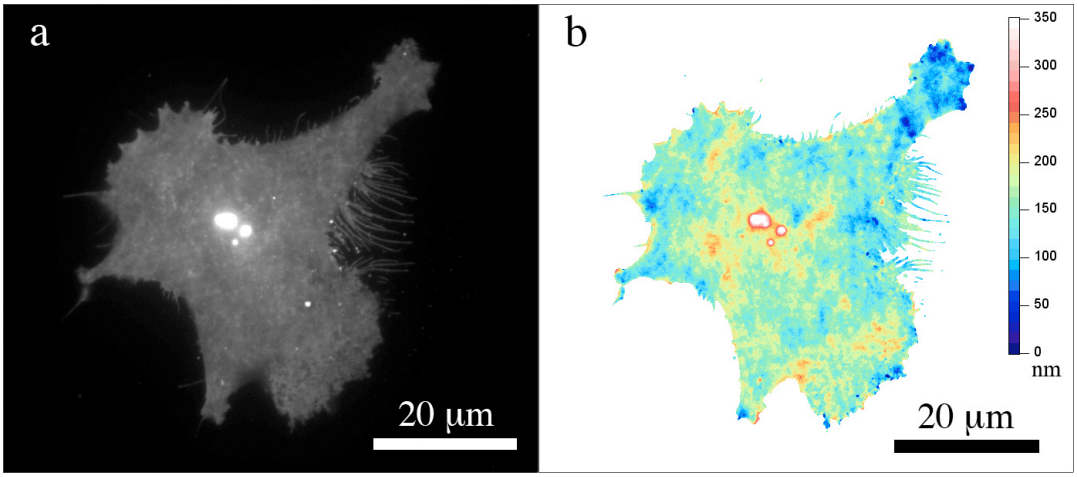

Figure 2: MDA MB 231 cell in adhesion on a thin layer of fibronectin (3-4 nm of thickness). The plasma membrane of cell was labeled with DiO. (a): TIRF image at the incident angle θ=64°. (b): corresponding membrane-surface distance image. TIRF images were recorded in 100 ms, at 37°C, with a laser excitation at 488 nm at 5 W/cm^2.

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