Exploring the molecular bases of cytoskeleton-cell membrane interactions, by live imaging approach

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Cellular architecture and compartmentalisation are organised as bi-dimensional fluid membranes, which are assembled by essential molecules for the life of a cell: the phospholipids. These are not just crucial for the cell membranes formations, since they also play a pivotal role in the formations of "raft" domains at the membrane level[1], together with the action of proteins. In addition, they participate in several other fundamental cell mechanisms including proteins regulations, vesicular trafficking, and cell motility[2].

Over the last years, many cellular processes have been deeply characterised, especially from a molecular biology and biophysics viewpoints. However, cell membrane dynamics still represent one of the most complex process yet to be fully elucidated. This is due mostly due to a general lack of technologies suitable in lipidomic investigations. In fact, bio-molecules such as phospholipids, ceramides, and sterols cannot undergo genetic modifications, and hence being then analysed in live cells using traditional fluorescence protein approaches.

In this work, we used polymersomes as synthetic self-assembled delivery system[3], to achieve a stable and optimal distribution, in live cell, of five different membrane and cytoskeleton probes: BODIPY®Phosphocholine (PC), NBD-Cholesterol (NBD-C), BODIPY®TB-Ceramide (BC), TopFluor®PI(4,5)P2 (PIP2) and Phalloidin-ATTO647 (PHAL). The effective delivery and the topological membrane-distributions of these probes were evaluated in mouse embryonic fibroblast (NIH-3T3 cell line). The results showed an efficient delivery for all the tested probes in live cell.

Furthermore, it was possible to characterize the specific interplay and overlap between the different lipid domains in diverse cell membrane areas, by means of confocal analyses. Moreover, the delivery of PHAL polymersomes enabled a clear staining of F-actin in live cell, without causing any toxicity.

Using such an approach, we have been able to elucidate the cooperation between PIP2 domains and cytoskeleton during sophisticated cellular mechanisms such as adhesion, polarization, and spreading process, showing a peculiar phosphatidylinositol active transportation to the membrane protrusion. In conclusion, this work enables to reach a better understanding of the complexity of lipid organization, and provides important explanation about the interplay that subsists between these bio-molecules and the F-actin network in live cell.

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