Dynamic Modification of Lipid-DNA Probes on Live Cell Membranes: A Quantitative Evaluation

Yousef Bagheri and Mingxu You

EasyChair preprints are intended for rapid dissemination of research results and are integrated with the rest of EasyChair.

February 26, 2020
Dynamic Modification of Lipid-DNA Probes on Live Cell Membranes: A Quantitative Evaluation

Yousef Bagheri, Mingxu You*

Department of Chemistry, University of Massachusetts, Amherst, USA

Corresponding author: mingxuyou@chem.umass.edu

Cell membrane provides a physical barrier between the intracellular compartments and the extracellular environment. Moreover, cell membrane plays important roles in the signal transmission between cells and extracellular matrix or among neighboring cells. Better understanding and modulation of the compositions and functions of cell membranes is critical for regulating cell signaling and interactions. Among different methods developed for cell membrane study and regulation, lipid-DNA conjugates are powerful tools with great potentials. These conjugates have recently attracted much attention for cell membrane analysis, transmembrane signal transduction, and regulating intercellular networks. These lipid-DNA probes can be spontaneously inserted into the membrane simply by incubation. The highly precise and controllable DNA interactions have further allowed the programmable manipulation of these membrane-anchored functional probes. However, our understanding is still quite limited on how these lipid-DNA probes can interact with the cell membranes and what parameters determine this process.

Here, we aim to provide an in-depth understanding of how lipid-DNA conjugates interact with cell membranes. With quantitative assessment using several methods, we have determined the cell membrane insertion kinetics, magnitudes, and durations of different lipid-DNA probes. We have systematically studied the effect of lipid/DNA structure, hydrophobicity, concentration, temperature, and cell type on these membrane interactions. We studied the probe modification density on different cell membrane and realized that, modification is highly subjected the lipid linker (Figure 1a). By measuring the relative hydrophobicity of each probe, we found that there is an optimum probe hydrophobicity for maximum probe density on the cell membrane. (Figure 1b). We further discovered that probe detachment from the cell membrane usually happens through two main mechanisms which result in probe density decay (Figure 1 c). Probes either internalize into the cells through endocytosis or diffuse out into the solution. Understanding these pathways quantitively, helped us to develop a general mechanism of probe interaction with membrane and their removal from cell surface (Figure 1d).

First, there is equilibrium between the monomeric and aggregated forms of lipid–DNA probes in the solution. Monomeric form lipid-DNA probes insert into the cell membranes. Then, some of the cell membrane-anchored probes are internalized into the cells through clathrin- or caveolae-mediated endocytosis. Most of the internalized probes are then located inside the late endosomes or lysosomes where they are degraded or rejected out of the cells. Some probes are likely transferred to the Golgi apparatus or endoplasmic
reticulum as well. On the other hand, the membrane-anchored probes can also directly flow out into the extracellular solution, which is the reverse process of the initial probe membrane insertion. It is worth mentioning that direct probe exchange between neighboring cells can also occur at the cell–cell junctions.

![Diagram](image)

**Figure 1:** (a) Effect of different lipid linker on probe modification efficiency on MDCK cell membrane. (b) Membrane probe density on four different types of cell lines plotted against probe corresponding hydrophobicity. (c) Probe density decay from the MDCK plasma membrane. (d) Schematic of the dynamic process of lipid-DNA probe modification on cell membranes.

Detailed understanding of Lipid-DNA conjugates interaction with cell membrane enabled us to minimize probe removal from the cell membrane and extend their effective time window to over 24 hours. Therefore, these lipid-DNA conjugates now can be applied for various membrane studies that require long-term immobilization on the cell membrane. In conclusion, our quantitative data have dramatically improved our understanding on how lipid-DNA probes can dynamically interact with the cell membranes. These results can be further used to allow broad applications of lipid-DNA probes for cell membrane analysis and regulations.

**References:**