

ORAL PRESENTATIONS

FUNCTIONALIZED SYNTHETIC LIPIDS AS MOLECULAR TOOLS FOR CELL SURFACE ENGINEERING

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Natural glycolipids can insert from the extracellular environment into cell membranes, providing an opportunity for modifying the cell surface with various molecular fragments. However, natural glycolipids have several limitations: they are difficult to isolate from natural sources or obtain by chemical or chemoenzymatic synthesis, and their physical properties such as poor solubility in aqueous media are incompatible with experiments involving live cells. To overcome these limitations, a technology based on functionalized synthetic lipids, also known as FSL constructs (FSL: Function-Spacer-Lipid), was developed. FSLs are built from several structural blocks: a functional part, i.e., a glycan or another molecular fragment to be introduced onto the membrane surface, a spacer-arm, and a lipid anchor. FSLs insert into cell membranes without adversely affecting cell viability or functioning, and the membrane density of inserted FSLs can be varied making the process of modification controllable. The functional part of an FSL is actually any molecular fragment, which is required to be introduced onto the surface of the cell. The range of functional parts includes glycans, peptides, proteins, oligonucleotides, fluorescent labels, chelating groups, biotin, and reactive groups for click conjugation. Along with linear FSLs, various types of multiantennary constructs with complex architecture bearing several functional parts (same or different) have been synthesized. Short adipic or aminovaleric fragments were initially used as spacer-arms in FSLs. Later spacers of the carboxymethylglycine (CMG) family were introduced. CMG spacer-arms maintain an extended linear conformation thus distancing the functional part from the membrane and making it more accessible for receptor binding. The negative charge of CMG spacers also improves solubility in aqueous media expanding the range of functional parts to hydrophobic moieties such as biotin or fluorescent dyes. The most commonly used lipid anchor of FSLs is dioleoylphosphatidylethanolamine (DOPE). However, FSLs based on DOPE analogs containing saturated fatty acids, as well as constructs based on cholesterol derivatives, have also been synthesized. The use of various lipid parts enables targeted FSL insertion into different (raft or non-raft) membrane domains. FSLs are synthesized via modular assembly from their components. The most commonly used conjugation method is acylation of an amino group with activated ester. Nevertheless, other conjugation procedures such as thiol-maleimide or azide-alkyne click reactions were also employed. Recently, an enzymatic approach allowing conjugation of proteins or their fragments as functional parts of FSLs was developed. The possibility to vary the architecture of FSL constructs allows to tailor presentation of functional part to a specific experimental objective. In our study of antibody recognition of the tumor-associated ganglioside GD2 (Neu5Ac α 2-8Neu5Ac α 2-3(GalNAc β 1-4) Gal β 1-4Glc β 1-Cer), a panel of FSLs bearing the same GD2 glycan but differing in architecture was used to model various presentation of antigen on the cell surface. Antibody binding studies revealed that GD2 clustering plays a significant role in its recognition. Thus, FSL technology provides a simple and practical approach to engineering the molecular landscape of the cell membrane by introducing virtually any molecular moiety onto its surface. Moreover, the modular architecture of FSL constructs enables the generation of molecules with wide range of desired functions or properties. Together, these features open broad opportunities for applications in cell biology.

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