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BIOPHYSICS

A toehold in cell surface dynamics

A combination of standard fluorescence microscopy and flow cytometry offers a practical new approach to study encounter rates and preferences during various live cell membrane signalling events.

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The plasma membrane is the cell's interface between its internal biochemistry and the outside environment that plays a fundamental role in cell communication with its surroundings. Being only 5 nm thick the membrane represents a complex and highly structured fluid mixture of lipids, proteins and sugars whose interplay and dynamics are instrumental in cell signalling and environmental responsiveness. Traditionally biophysicists have constructed physical membrane models composed of a minimal subset of biomembrane constituents to provide insight into their structure and properties. However to verify the validity of these predictions experimental studies on live cell membranes are required. Probing the interactions at the cell surface is technically challenging and many existing techniques still suffer from a number of practical shortcomings. In particular, transient yet important signalling encounters between membrane components are notoriously difficult to study due to their short lifetimes (usually on microsecond to millisecond timescales). Writing in Nature Nanotechnology, Mingxu You and colleagues at Hunan University, University of Florida, Zhejiang University, University of Massachusetts Amherst and Michigan State University present a potential solution to this challenge by applying a strategy commonly used in the design of DNA nanomachines[1].

Since the early 1970s, the traditional biochemical view of cellular membranes has been dominated by Nicolson and Singer's Fluid Mosaic Model that describes a liquid crystalline lipid bilayer with embedded membrane proteins free to diffuse within this matrix.[2] While this picture of biological membranes still largely holds, in the past couple of decades this model has been significantly

modified to account for membrane asymmetry and heterogeneity that remain topics of intense investigation and debate[3]. New advanced imaging modalities such as STED microscopy allow for such investigations but these techniques are not widely available and can be technically difficult to implement[4]. One important advantage of the approach developed by You and coworkers is that their method is compatible with readily accessible confocal microscopes and flow cytometers.

The use of DNA in controlling material assembly and interactions is now a well-established concept in nanotechnology[5]. DNA has previously been employed to synthetically functionalise lipid membranes to control their adhesion and fusion[6] and assemble artificial trans-membrane channels[7] and even used as artificial adhesion moieties on the membranes of cells[8]. Here, toehold strand displacement, a common strategy for integrating dynamic response within DNA nanomaterials, is employed to signal collisions between molecules on the cell surface[9] (Figure 1a). Toehold strand displacement of DNA requires a minimum of three strands: two anchor strands and one exchange strand. The exchange strand begins the experiment hybridized to its initial anchor strand with an overlapping single stranded segment. The target anchor strand has greater complementarity to the exchange strand offering a lower energy state for the hybridization interaction. The overlapping region of the exchange strand is complementary to the end of the target strand thus allowing nucleation of the binding interaction between the strands. The exchange strand rapidly unzips from its initial anchor while simultaneously binding to its new target. This strand exchange is coupled to an optical readout by attaching a fluorophore to the exchange strand and a quencher to the target strand therefore resulting in a decrease in fluorescence when the reaction is complete.

The authors first use their approach to investigate the relative encounter rates between different lipid types in the plasma membrane of Ramos cells. Lipid raft theories predict highly dynamic nanoscale compositional heterogeneities in the lipid distribution of biological membranes[3]. However the size and lifetime of lipid rafts make them challenging to directly image in live cells. Much of the evidence for their existence is inferred from the phase behaviour of experimental

model membranes composed of simplified lipid mixtures. You and coworkers use three different lipid anchors (diacyl lipid, tocopherol and cholesterol) covalently linked to DNA strands. All three molecules have different phase partitioning preferences in heterogeneous lipid membranes. Lipid encounter rates were shown to be strongly dependent on their chemical nature with like-species generally showing greater collision frequency while tocopherol and diacyl lipids, which strongly partition to the opposite liquid phases in model membranes, interact least frequently. This data provides strong further evidence for the existence of membrane heterogeneities such as lipid rafts that give rise to different free diffusion areas for these lipids on the cell surface (Figure 1b).

More sophisticated experiments are devised including a competition assay where the exchange strand can be transferred to one of the two competing target strands where only one of the targets contains the quencher. Therefore the relative drop in fluorescence is indicative of the relative encounter rates between the initial anchoring molecule and the two target species (Figure 1c).

The authors demonstrate that this method is not limited to the study of lipid interactions and can also be used to monitor collisions between membrane proteins (Figure 1d). Instead of covalent linking of the DNA strands to these proteins, DNA aptamer sequences are used. Aptamers are folded DNA strands that bind with high affinity and specificity to non-nucleic acid targets[10], in this case epitopes on the membrane proteins. The relative encounter rates of combinatorial pairs of four different membrane proteins are characterised in this work as proof of concept that the encounter rates of different types of membrane species can be characterised. Therefore DNA strand exchange is shown to be a potentially powerful toolbox for studying the structure and dynamics of the cell surface.

The long term impact of this work is yet to be seen but caution must be exercised when designing these experiments and interpreting their results. Even small molecules like lipids are sensitive to the symmetry of the interaction, i.e., encounter rates between two different membrane species were dependent on which species carried the initial anchor strand and which carried the target

strand. Therefore asymmetric interactions between different species need to be characterised by averaging the interactions between both allowed geometries. The interpretation of the encounters between membrane proteins will require the characterisation of their expression levels to give detailed insight into preferential interactions separate from mere random collisions due to the relative concentration effects. Strand exchange efficiencies in membrane protein encounters will also depend on the relative accessibility of DNA strands in collisions due to relative positioning of their anchoring epitopes. Furthermore, for any change in experimental conditions one needs to make sure that only encounter rates between membrane components are affected and not the DNA strand exchange efficiencies.

Despite all these challenges, the described strategy opens exciting new possibilities to study wide-ranging membrane phenomena in live cells that were previously inaccessible. Examples include oriented interactions between membrane proteins by varying the binding epitopes of aptamers on a specific species of interest and the effects of the cell cycle phase, drugs and disease pathologies on intramembrane interactions and dynamics. Combining this technique with compositional profiling of the membrane (proteome, lipidome and glycome) could offer a powerful tool to advance our understanding of the biological membranes and their role in health and disease.

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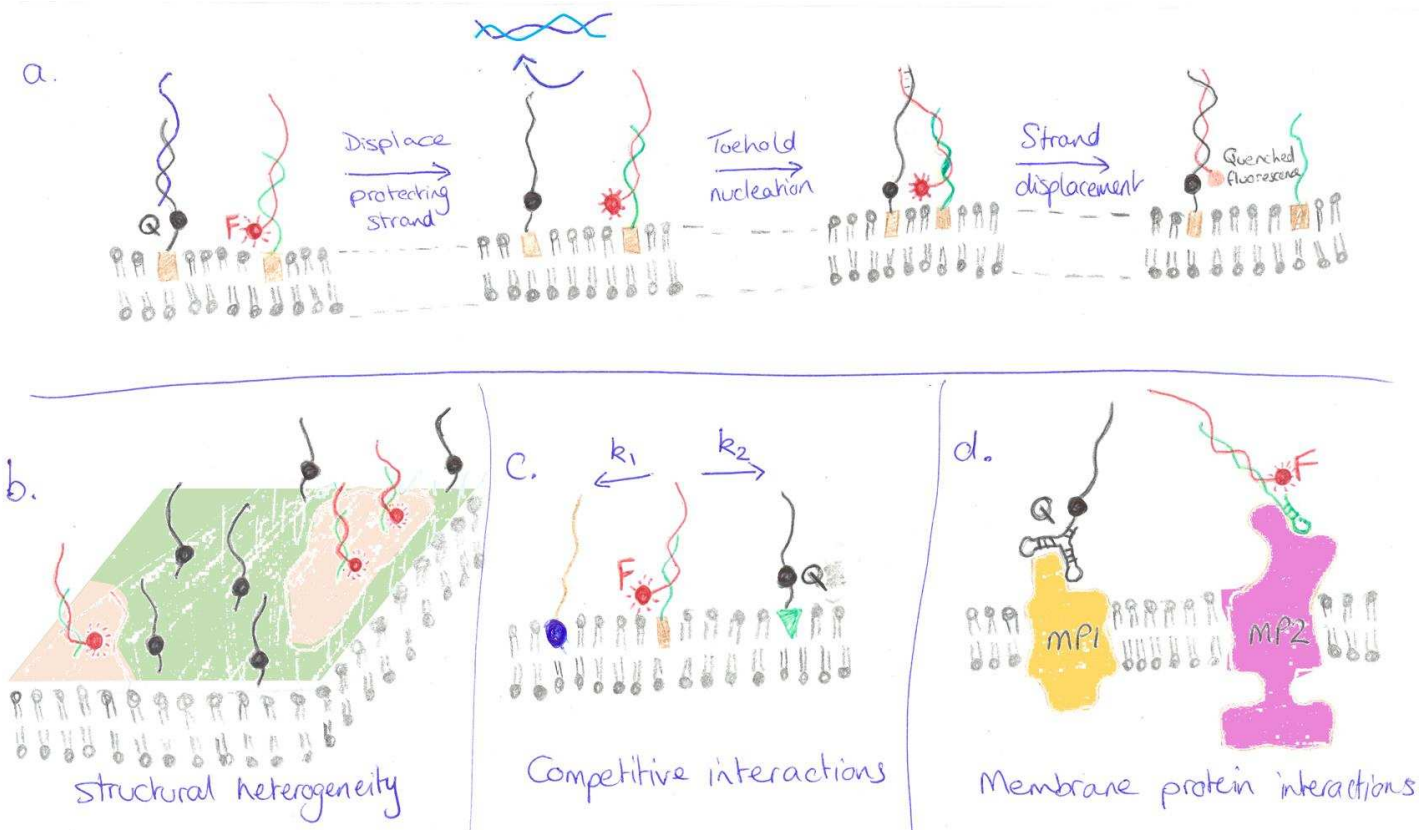

Figure 1: Membrane components exchange contact information via DNA toehold strand displacement. a, Lipid-DNA conjugates anchor designed DNA sequences to the membrane. A quencher (Q) on one lipid-DNA species is protected by a hybridized strand. A second lipid-DNA species is hybridized to the fluorescent probe strand (F). The experiment is started by displacement of the protecting strand from the quencher sequence. The lipid-DNA probes then interact through lateral diffusion on the membrane. Upon contact, the overlapping single-stranded region of the probe strand nucleates a toehold bond with the quencher strand. The probe then transfers to the quencher strand resulting in a quenching of its florescence that signals an interaction has taken place. (Adapted from Figure 1a of reference 1). **b,** Different lipid anchors preferably partition into different membrane domains. Therefore the structural heterogeneity of the membrane influences the collision rate between probes. The diagram shows probe and quencher strands that strongly partition into different domains from each other, which would result in a slow rate of probe transfer interaction and hence a slow rate of fluorescence quenching. **c,** Competition assays can be designed by having two target strands for the probe on different membrane components. Only one target strand has the quencher. Therefore the relative interaction rates (k_1,

k_2) determine the efficiency of fluorescence quenching in the strand exchange reactions. **d,** Membrane protein interactions between two membrane proteins (MP1 and MP2) can also be investigated by anchoring the quencher and probe sequences to epitopes on these proteins through aptamer-mediated interactions.