Deconstructing Receptor Signaling with Nanopatterned Supported Membranes

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Cells respond to external cues using signaling pathways that are typically initiated by membrane-bound receptors. These pathways do not simply transmit signals; instead signaling consists of cascades of chemical reactions with tightly controlled chemical kinetics to generate stimulus-specific responses. Importantly, the role of *spatial organization* of activated signal transducers is emerging as a novel concept in understanding cellular response. This has generated a need for developing biologically-relevant patterning approaches directed at understanding the role of micro- or nanoscale organization in signaling outputs. In this talk, we will examine the role of *spatial organization* in two classes of signaling pathways using hybrid live cell-supported membrane junctions.

First, we will highlight our efforts in understanding the immunological synapse, which is a specialized cell-cell junction that is defined by large-scale spatial patterns of receptors and signaling molecules. Supported membranes and nanometer-scale structures fabricated onto the underlying substrate are used to impose geometric constraints on immunological synapse formation. Analysis of the resulting alternatively patterned synapses reveals a causal relation between the radial position of T cell receptors (TCRs) and signaling activity, with prolonged signaling from TCR microclusters that are mechanically trapped in the peripheral regions of the synapse. These results are consistent with a model of the synapse in which spatial translocation of TCRs represents a direct mechanism of signal regulation.

The second system that will be discussed is the activation of the EphA2 receptor tyrosine kinase with its natively membrane-bound ligand, ephrin-A1, which plays an important role in breast cancer biology. We have found that ephrin-A1 displayed in supported lipid membranes activates EphA2 and triggers its signaling pathway. Realtime fluorescence imaging, immunostaining, and western blots all show that ephrin-A1 is active when tethered to a fluid supported lipid membrane (SLB), and biological function is altered when presented in solution, tethered to a non-fluid membrane, or covalently immobilized onto a surface. Importantly, the supported membrane platform enables visualization of mechanistic details easily lost in the irregularities of cell-cell contacts. In particular, we resolve a lateral transport step, in which EphA2-ephrinA1 complexes are actively driven across the membrane surface. The active transport step is revealed by receptor-ligand complex accumulation on substrate-imposed nanoscale corrals, and can be attenuated with cytoskeletal drug inhibitors.

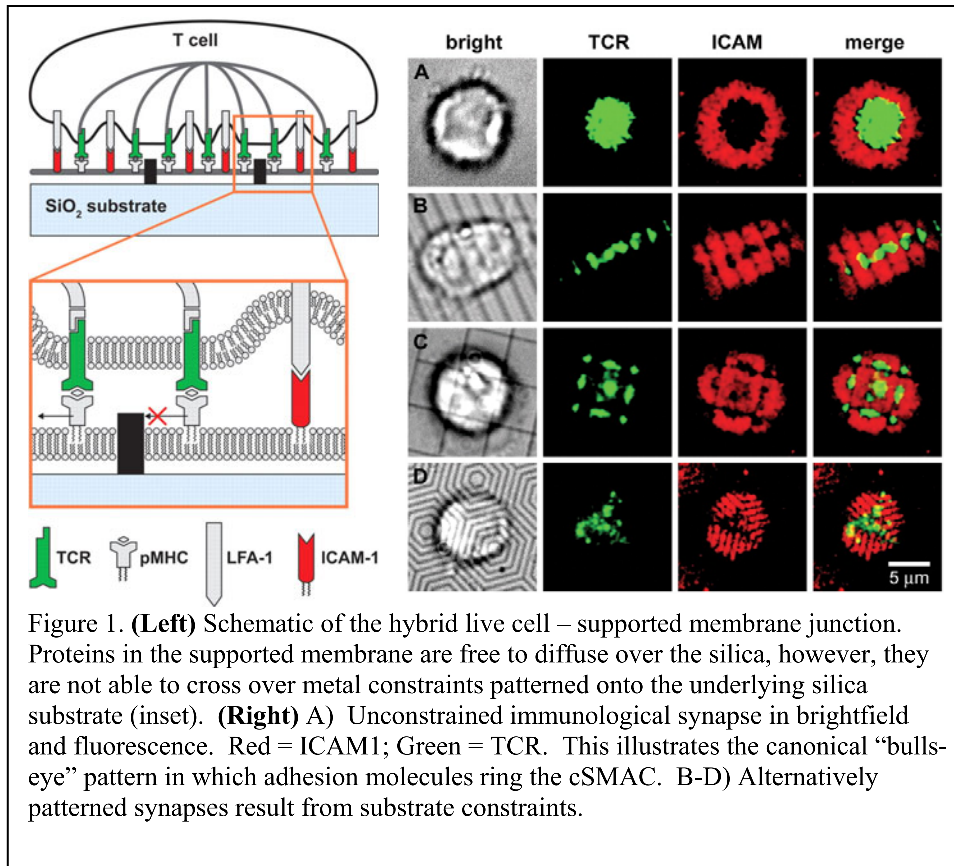

Figure 1. **(Left)** Schematic of the hybrid live cell – supported membrane junction. Proteins in the supported membrane are free to diffuse over the silica, however, they are not able to cross over metal constraints patterned onto the underlying silica substrate (inset). **(Right)** A) Unconstrained immunological synapse in brightfield and fluorescence. Red = ICAM1; Green = TCR. This illustrates the canonical "bulls-eye" pattern in which adhesion molecules ring the cSMAC. B-D) Alternatively patterned synapses result from substrate constraints.

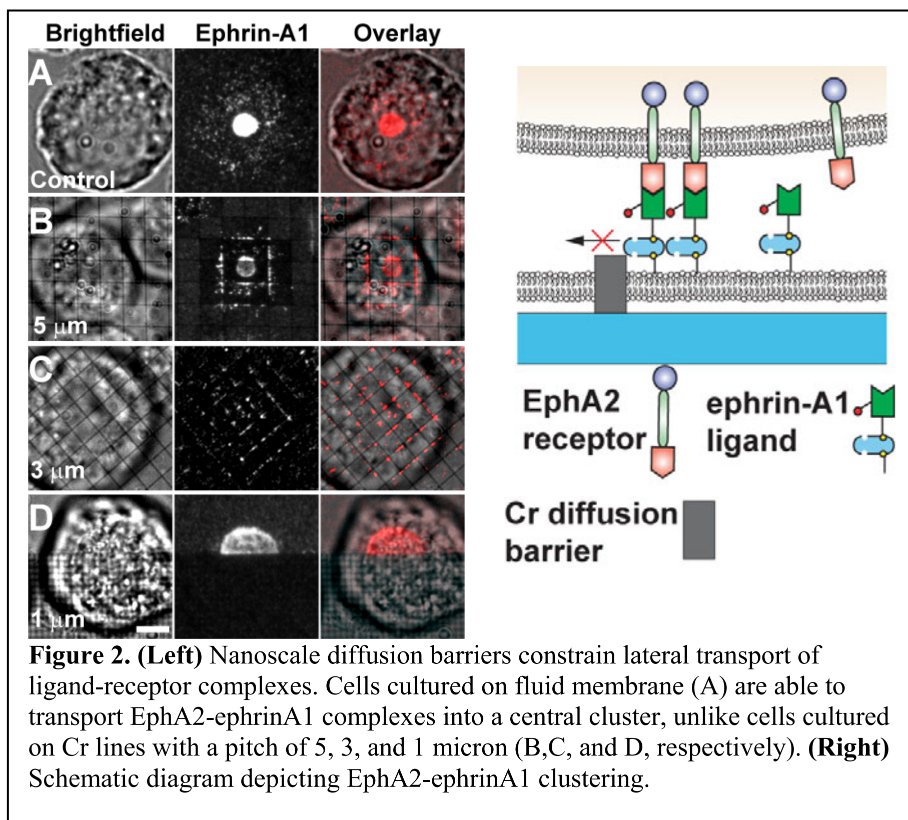

Figure 2. (Left) Nanoscale diffusion barriers constrain lateral transport of ligand-receptor complexes. Cells cultured on fluid membrane (A) are able to transport EphA2-ephrinA1 complexes into a central cluster, unlike cells cultured on Cr lines with a pitch of 5, 3, and 1 micron (B,C, and D, respectively). **(Right)** Schematic diagram depicting EphA2-ephrinA1 clustering.