Agonist mobility in supported bilayers affects Fas mediated death response

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Abstract
Introduction
Extrinsic apoptosis is initiated by the heterologous binding and clustering of the single-pass transmembrane proteins, Fas ligand, expressed by natural killer lymphocytes, and its cognate receptor Fas (CD95) expressed at the surface of a target cell. While the Fas mediated death response was widely studied using soluble inducers, the mobility constraints of both receptor and ligand embedded in the plasma membrane of opposed cells are not yet fully determined. The reduced two-dimensional diffusion as well as the ligand induced oligomerization kinetics may affect the final apoptotic response.
Objectives
We explored the relevance of membrane confinement on apoptosis using model system employing a membrane-attached agonist ligand on supported lipid bilayers, where we assessed the diffusion coefficients with z-scan fluorescence correlation spectroscopy. We also used micro-contact printing technique to obtained surfaces with different topologies.
Materials and methods
We generate either homogeneous or micro patterned lipid bilayers of different compositions by the vesicle fusion method. For micro-contact printing, we use a stamp inked with fibronectin to passivate the surface before bilayer preparation. Biotinylated lipids in the bilayers allow us to bind streptavidin and biotinylated anti-Fas antibody to the surface. We used a stably transfected cell line which express Fas receptor-GFP.
Results and discussion
The mobility of the ligand is determined by the viscosity of the lipid phase. Despite both situations trigger apoptosis, in cells attached to bilayers Fas receptor becomes concentrated in regions in close contact with the bilayer, in comparison with cells over fibronectin in which clustering is all over the cell membrane.

Considering that lipidic composition is critical for protein diffusion, we compared bilayers with different lipid compositions and we suggest that the apoptotic response correlates positively with the mobility of the agonist ligand associated with the death inducing membrane.

Conclusion
Apoptosis triggered by Fas Receptor can be modulated by the mobility of the ligand or an agonist antibody.

**Key words:** fluorescence correlation spectroscopy; supported lipid bilayers; apoptosis; functionalized surfaces, Fas Receptor.