Hydrodynamic Trapping of Immune Synapse Proteins in Supported Lipid Bilayers

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Background: Much research has been devoted to the study of important proteins in the immune system and especially in the immunological synapse (IS). The IS functions as the contact area between immune cells and is crucial for initiating an immune response. However, static and kinetic information about intermolecular interactions in the IS are to a large extent lacking. We here use supported lipid bilayers (SLBs) to trap proteins on a planar SLB (1). This allows us to study intermolecular forces between different membrane proteins providing biophysical knowledge about their molecular behavior. The aim of this project is to investigate the intermolecular forces of characteristic molecules in the IS, including: CD45, CD4 and CD2, coupled to an SLB.

Methods: Hydrodynamic trapping of fluorescently-labelled human CD45 (hCD45), human CD4 (hCD4), rat CD2 (rCD2) and Streptavidin (SA) was done as previously described by Jönsson et al. (1). In brief, SLBs were prepared with vesicles containing 5 wt% 1,2-dioleoyl-sn-glycero-3-[N-(5-amino-1-carboxypentyl)[iminodiacetic acid]succinyl] (nickel salt) (DGS-NTA) mixed with 95 wt% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipids. Alternatively, vesicles containing 0.05 wt% 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt) (biotin-PE) mixed with 99.95 wt% POPC lipids was used for experiments on the protein streptavidin. A micropipette was mounted on a custom made micropipette holder that was placed above the SLB. Applying negative pressure over the micropipette allows for accumulation of the proteins below the pipette, which was captured using total internal reflection fluorescence microscopy (TIRFM).

Results and Discussion: We studied the trapping and accumulation of the T-cell proteins hCD45, hCD4 and rCD2, and the protein SA as a model protein. Accumulation of the proteins occurred in a circular area distinguishable from the surrounding bilayer by a higher fluorescence intensity, shown for hCD45 in Figure 1. The relative accumulation of proteins varies across the trap and is dependent on the applied hydrodynamic energy per μm², εhydro, at each position. The accumulation is also dependent on the dimensions of the protein as well as the intermolecular interactions between the proteins. The initial linear increase in Figure 1F is due to the hydrodynamic force from the trap being balanced by entropic forces, whereas at higher applied energies (protein densities) the concentration levels out at a value where repulsive intermolecular interactions between the proteins is high. We also saw that the taller molecules, hCD45 and hCD4, seem to bend at higher hydrodynamic forces.

Figure 1. Hydrodynamic trapping of hCD45.
Human CD45 accumulating under different pressures: (A) -2.6 kPa, (B) -9.4 kPa and (C) -2.6 kPa on the SLB. (D) Turning off the applied pressure leads to release of the trapped hCD45. (E) The intensity of trapped hCD45 (area indicated with the dashed circle) versus time at different applied pressures. (F) The relative accumulation of hCD45 as a function of εhydro from different experiments.

References: