SYNOPSIS

Historically, the dynamic characteristics of molecules residing on the cell membrane has been assumed to be derivable from the classical equilibrium physics of artificial membranes. In a eukaryotic cell, while this works for many small lipid molecules, the cell membrane has far more proactive players, that associate with, and in turn modulate, cellular components other than the membrane itself, such as the highly dynamic actin cytoskeleton.

GPI Anchored Proteins are a class of molecules that associate with the actin cytoskeleton. These proteins are found anchored on to the outer leaflet of the plasma membrane via a glycolipid tail. They perform a variety of functions in the cell and are useful in signal transduction, cell adhesion etc. They are internalized into the cell via the so called ‘GEEC’ pathway that does not require the action of coat proteins. In contrast to trans-membrane proteins, they are distributed on the plasma membrane in a peculiar way: they are known to exist as nanoclusters (of 2-4 mers) or monomers on the cell surface; the cluster monomer ratio is found to be constant over a range of expression levels, indicating that they are organized in a way that violates the law of mass action. These results, known from earlier measurements of polarization anisotropy of the entire cell surface, form the basis of further experiments and analysis.

My thesis deals with the interpretation and modelling of experimental measurements of the spatial distribution and local dynamics of these proteins. A theoretical model that aims to make qualitative connections with these
observations is also proposed.

High resolution wide field and confocal polarization anisotropy measurements of fluorescently labeled GPI anchored proteins provides the required spatial and temporal information. Firstly, maps of the fluorescent anisotropy and intensity reveal gross features that change over a scale of about 450 nm. These include microvilli, lamellipodia, flat featureless regions etc, which are each characterized by their unique shape and nanocluster content. One can derive information about nanocluster arrangement from regions that are "typical": they do not contain any obvious membrane features. A large part of the membrane is made up of such regions and the information they contain lies in the single point nanocluster density distribution. Secondly, time resolved confocal imaging of a small membrane patch is adapted to provide nanocluster-monomer kinetics information. Cluster monomer interconversion rates, at different temperatures and different regions of the cell, are found. Both the spatial and temporal behaviour are probed when the cell is subjected to actin and cholesterol perturbations.

It is clear from the experimental observations that the architecture of GPI-APs on the plasma membrane is correlated with cortical actin. The cortical actin has distinct architectures - one predominantly tangential to the plasma membrane, and the other predominantly perpendicular to it. These two architectures modulate the composition and shape of the plasma membrane respectively. The two architectures of cortical actin are regulated by interdependent molecular players.

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A satisfactory theoretical model of particle motion in the plasma membrane
must include parts played by the cortical actin as well. Primarily, the model should provide a mechanism for the formation of nanoclusters, consistent with all other experimental observations. We propose that the GPI anchored protein is a 'passive' tracer particle, merely reflecting the architecture and dynamics of the underlying actin. The physics of actively crosslinked filaments then provides answers for the behaviour of these proteins.

The following section is a summary of the results of the thesis.