Vesicle docking and delivery: Life in the TIRF zone

A.C.F. Coster, School of Mathematics & Statistics, University of New South Wales, NSW 2052, Australia and Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia.

Cells traffic membrane-embedded proteins to the plasma membrane *via* a variety of mechanisms. One of these is the docking and fusing of vesicles with the plasma membrane. Even this mechanism, however can happen *via* several modalities.

Given the live-cell imaging techniques using total internal reflection (TIRF) microscopy (in which the first ~200nm of the cell surface is observed using fluorescent markers attached to the molecules), it is now becoming possible to view the dynamics of these processes, albeit that in some systems the vesicles are below the resolution of the system.

In order to systematically extract these delivery events from other background traffic, we need robust, quantitative descriptions of the dynamics. These can then be employed in automated detection systems to analyse the system under a variety of perturbations.

Such an automated detection system has been developed within the Diabetes & Obesity Group at the Garvan Institute of Medical Research. The proteins of interest in this case are the glucose transporters GLUT4, which are highly insulin-responsive. These may be brought to the plasma membrane *via* methods including the full fusion of the vesicles, and also *via* a process termed “Kiss-and-Run”, whereby a pore connection is made between the vesicle and plasma membrane allowing diffusion of the GLUT4 proteins across the boundary. The vesicle does not, however, become fully integrated, but, after a period, detaches from the plasma membrane. Thus, the amount of GLUT4 delivered depends on the amount of time the “Kiss” persists, and also on the physical dimensions of the vesicle and pore connection.

The model for this process can also be used to explore the possibilities of differential diffusion rates of the molecules in the vesicle and the plasma membrane. In the case of GLUT4 vesicles in adipocytes, the analysis of TIRF imagery is further exacerbated by the fact that the ~80nm diameter vesicles are well below the microscope resolution. Advances in the understanding of these processes are still, however, being made, with the use of mathematical modelling and sophisticated image analysis techniques. Current work is exploring the modelling and imaging of the “Kiss-and-Run” delivery process, and the insights this may additionally give us into the diffusion characteristics of the membranes.