One of the beauties of immobilizing intact cell membranes on a sensor chip is that trans-membrane proteins can be studied in their native configuration and in a natural microenvironment.

Biacore is set to play a lead role in the search for ligands to “orphan” G protein-coupled receptors, where the receptor, in order to bind and carry out its signal transducing function, requires the preservation of the structural integrity of the cell membrane.
The ability to reproducibly immobilize lipid-containing molecules on Biacore chips has been a major need for many years. The introduction of the HPA chip led to the development of useful protocols in many areas of lipid research. One area where there was still a need for alternate immobilization methodologies was with lipid membranes that contained integral membrane proteins. The introduction of the L1 chip has made it possible to immobilize many different types of lipid molecules for Biacore analysis. Two major areas of research that have been addressed by our lab are the ability to immobilize G-protein coupled receptor (GPCR)-containing membrane fragments and the stable immobilization of lipid vesicles for use in predictive adsorption studies of small molecules.

GPCRs have been described as the most clinically relevant class of receptors being researched today. Many of the most profitable therapeutic drugs on the market today target this class of receptor. GPCRs are membrane-bound receptors that have seven membrane-spanning domains with a ligand-binding extracellular domain and a signal-transducing intracellular domain. The known ligands for GPCRs are diverse in their make-up, ranging from gases and light, to peptides and proteins. The ability to discover novel GPCRs is being greatly enhanced by genomic-based technologies, which have increased the known number of receptors into the hundreds. Receptors with no known ligand are commonly called “orphans”, and orphan GPCRs, because of their documented therapeutic success, have the potential to be extremely valuable pharmaceutical targets in many clinical areas. De-orphaning GPCRs, or finding their natural ligand, has been a challenge for many years. The process is multi-stepped and usually takes two or more years. Using Biacore, our laboratory is trying to make this timeline much shorter (1).
Using the L1 chip, we are implementing a three-step process for identification of novel ligands for GPCRs. The first step involves the immobilization of membrane fragments that are prepared from mammalian cells that over-express certain GPCRs. We use one flow cell as a control surface that contains membranes from the parental (non-transfected) cell line and two flow cells for the membranes with receptor. We then inject our sample that possibly contains our new ligand over the surfaces and allow it to bind. Utilizing Biacore 3000’s micro-recovery feature, the bound material is collected from the individual flow cells and in the final step, the samples are analyzed by mass spectrometry for protein/peptide identification. Figure 1 shows a picture of L1 chip flow cells that have been immobilized with either a membrane preparation from cells that over-express a certain GPCR or the parental cell line. The known ligand for the receptor, which is fluorescently tagged, is injected and allowed to bind. The interaction is stopped and the chip is viewed under a fluorescent microscope. Only the flow cell that contains membranes with the over-expressed GPCR is seen, showing that there is very little non-specific binding to the control membrane. Figure 2 shows the results of a mass spectrometric analysis of angiotensin that had been eluted from a chip surface immobilized with membranes containing angiotensin receptors. The sequence was confirmed by tandem mass spectrometry (2). We have had very encouraging results using this methodology for ligand identification for GPCRs. The results mentioned here are early indications that the process can work. As we start to test more complex mixtures against orphan receptors, the process will become more complex, for example, issues concerning receptor number and the complexity of the ligand source must be addressed. Once all of these conditions are worked out, we believe that by using Biacore 3000, we can significantly decrease the time it takes to identify novel ligands for GPCRs when compared to other methods currently in use. Another area of research in which the L1 chip is having a significant impact is predictive pharmacology. Predictive pharmacology, or pre-ADMET studies (absorption, distribution, metabolism, excretion, and toxicology), deals with the in vivo testing and prediction of how small molecule drugs will act in the body before they are actually tested in vivo. There are active programs in many research laboratories that try and predict pharmacological properties of small molecules including (i) oral and tissue absorption, (ii) interaction with serum proteins (3), (iii) interaction with known enzymes of digestion and (iv) their tissue distribution properties. These types of experiments are valuable because ADMET studies are usually done when a compound is in pre-clinical trials or beyond. If the compound fails because of unfavorable ADMET properties, there is a tremendous loss of money and time to a company. By implementing ADMET studies earlier in the drug discovery process, unfavorable properties can be discovered and corrected before drugs enter clinical trials. The L1 chip, along with Biacore 3000, us has the potential to make a significant impact on predicting absorptive properties.

Figure 2 (a): Binding analysis of angiotensin binding to immobilized receptor membranes on an L1 chip surface. The bound ligand is eluted from the chip and analysed by MS. (b): Liquid chromatograph of eluted peptide from the chip. (c): Mass spectrum of the eluted peptide that yields molecular weight (1).
of small molecules by decreasing the time of analysis and enabling higher throughput.

Recent work by Biacore scientists (4) shows that by using liposomes of various fatty acid compositions, it is possible to reproducibly immobilize the liposomes and measure interactions with small molecules. These interactions and resulting analysis can be used to predict whether or not molecules are absorbed by the small intestine. This type of assay could prove to be very valuable as current methodologies used for absorption studies can be time consuming (1-3 weeks) and require extensive tissue culture and personnel resources. Using Biacore 3000 and the L1 chip, it could be possible to test hundreds of compounds a day, with relatively easy-to-prepare materials, and an automated system that requires little user intervention. Other areas of ADMET studies that involve the L1 chip are currently being developed that could address higher throughput methods for toxicology studies, including metabolism and tissue distribution.

The L1 chip is versatile and easy to use. It allows the stable immobilization of many lipid-containing reagents. It is essentially reusable, as regeneration with detergents allows for new surfaces to be created. Because of these properties, the L1 chip stands to play a major role in applications that require lipids and lipid-embedded proteins, including ligand identification from membrane receptors and in predictive ADMET studies.

References