

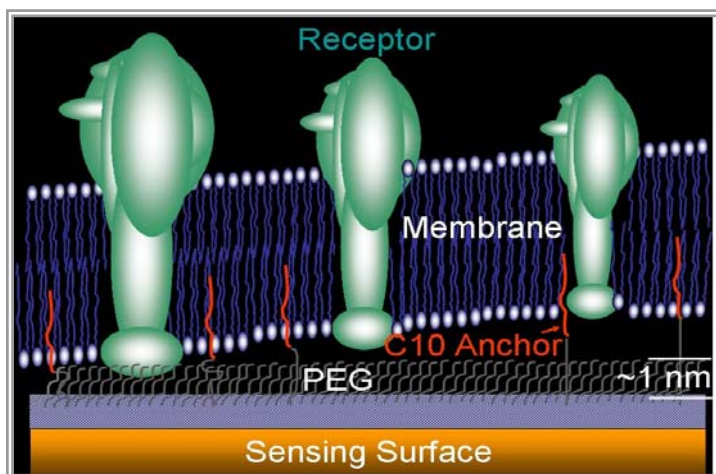
VESICLE CAPTURE FOR MEMBRANE BOUND RECEPTOR INTERACTIONS (VesCap CHIPS)

Informative real-time, label-free experiments on molecules that interact with cell membranes, or liposomes, are now possible using the ICx Nomadics Vesicle Capture chips.

VesCap

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On the VesCap chip, the lipid bilayer is maintained just as in the natural cellular environment, and cell membrane components in their native conformation can diffuse freely in this realistic model of the cell membrane. We have not confirmed that the vesicles fuse into a single membrane bilayer but this is very likely on such a two dimensional surface.



- An excellent model for experiments with drugs, toxins, and peripheral membrane-associated proteins involved in cell signaling.^{i,ii,iii}
- Membrane proteins and their partners interact just as in real cell membranes.¹
- Receptor/ligand embedded in liposomes may be immobilized.

VesCap CHIP PROPERTIES

- The lipid bilayer and the native structure of membrane proteins are maintained. Capture of vesicles onto the sensing surface is non-covalent, allowing free diffusion of membrane components in all directions.
- The PEG-decylamine layer presents a simple two dimensional interaction plane.
- Preparation of vesicles, or liposomes, for attachment to this surface is straightforward.
 - Purification steps can often be eliminated as samples can be relatively impure.
- VesCap chemistry is especially suitable for cell lines over-expressing a surface receptor.
- Regeneration of the VesCap chip surface is straightforward. A combination of surfactant, and solvent removes all vesicles from the surface allowing reuse of the VesCap chip

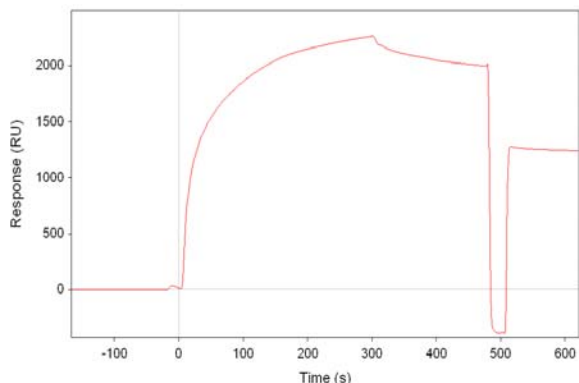
VesCap MECHANISM

The VesCap chemistry is prepared by covalently binding n-decylamine to ICx Nomadics' signature PEGylated sensing surface. The lipophilic hydrocarbon chains of the immobilized decylamine insert into the phospholipid bilayer. Membrane fragments, liposomes, micelles are thereby captured.

¹ Williams and Addona. (2000). The integration of SPR biosensors with mass spectrometry: possible applications for proteome analysis. Trends Biotechnol 18:45-8.

ATTACHMENT OF MEMBRANE TO VesCap SURFACE

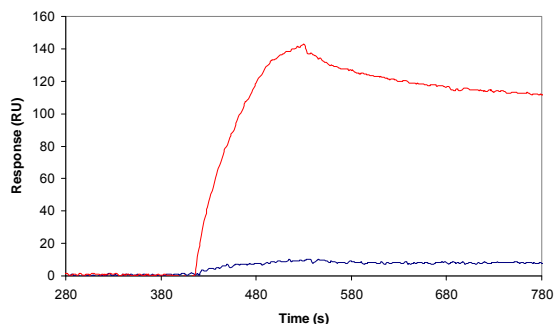
In the following example cell membrane fragments are immobilized. In brief, cells are lysed in hypotonic solution and the membrane recovered by centrifugation. The membrane pellet is washed to remove soluble protein and is then diluted in buffer. The membrane will spontaneously degrade producing small vesicles that have similar composition to the original parent cells. This preparation is sufficient for surface coating.



- Receptor-positive membrane solution was coated onto this surface yielding >2,000RU bound membrane vesicles as shown above.
- The surface was then exposed to HCL to stabilize the baseline by removing weakly attached material.
- Alternatively the HCl wash may be avoided if the surface is allowed to stabilize for 30 minutes prior to use.

In this example, a mass equivalent to 1000 RU of receptor membrane was immobilized. We recommend that a receptor negative membrane should be coated onto the reference sensing surface.

DEMONSTRATION OF VesCap CHIP BINDING OF ANTIBODY TO MEMBRANE ASSOCIATED RECEPTOR



- As a control non-specific antibody (blue curve) yielded a negligible binding response of 8 RU.
- Anti-receptor antibody yielded a binding response of 136 RU.

The data set shown above is the product of reference curve subtraction where the sample response curve from the reference surface (coated with receptor negative membrane) was subtracted from the sample response curve over the receptor positive surface. Note that this procedure eliminates baseline drift yielding flat baselines that are necessary for accurate data analysis.

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ⁱ Kim et al. (2004) Surface plasmon resonance studies of the direct interaction between a drug/intestinal brush border membrane. *Pharm Res* 21:1233-8.

ⁱⁱ Anderluh et al. (2005) Properties of nonfused liposomes immobilized on an L1 Biacore chip and their permeabilization by a eukaryotic pore-forming toxin. *Anal Biochem* 344:43-52.

ⁱⁱⁱ Gopinath et al. (2007) Snake-venom-derived Factor IX-binding protein specifically blocks the γ -carboxyglutamic acid-rich-domain-mediated membrane binding of human Factors IX and X. *Biochem J* 204:351-7.