

SURFACE IMMOBILIZATION OF RECEPTORS FOR BIOASSAY DEVELOPMENT

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INTRODUCTION: Incorporation of membrane receptor proteins in supported membranes for the study of receptor-ligand interactions and signal transduction events by surface sensitive evanescent wave techniques necessitates a method to immobilise these receptors in a manner that preserves all their functional features. Here we present two complementary approaches for the functional surface immobilization of membrane receptor proteins.

Immobilization via affinity tags: A detergent solubilised, His-tagged 5-HT₃ serotonin receptor was fixed to quartz slides; the surface of the quartz slides was modified with NTA, a metal-ion chelating group, allowing the reversible attachment of the His-tagged receptor proteins (Fig.1, left panel). The specific binding of a fluorescently labelled antagonist to the receptor was detected by total internal reflection fluorescence (TIRF). The immobilized receptor exhibited identical ligand binding and pharmacology as the native receptor in brain tissues, indicating that surface immobilization did not effect these properties of the receptor¹. The TIRF assay is extremely sensitive; a few cells express sufficient receptor to perform ligand screening.

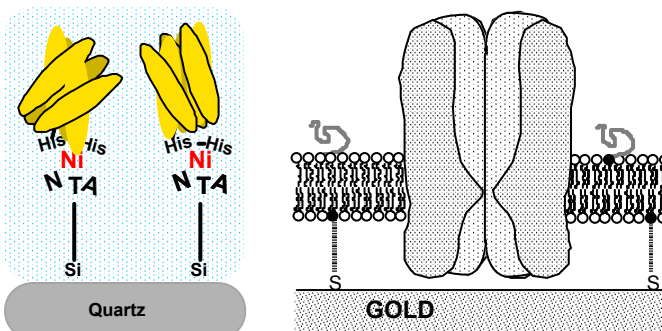
Immobilization in tethered membranes: The nicotinic acetylcholine receptor (nAChR) was reconstituted into vesicles containing a mixture of thiolipids and conventional lipids by detergent dialysis. The thiolipids, bearing in the headgroup a thiol group attached to the terminus of a long polyethyleneoxide spacer, bind covalently to the gold surface of surface plasmon resonance (SPR) sensor. The nAChR containing supported membrane is attached to the surface via the long spacer forming an aqueous compartment between the surface and the membrane, giving space to the extramembraneous parts of the nAChR (Fig.1, right panel).

The ligand binding site of the nAChR was predominantly native and facing the solution in the flow cell, as determined using epitope specific monoclonal antibodies. The pharmacological properties of the surface-immobilised receptor, tested by the competition of binding between certain ligands and a monoclonal antibody specific for the ligand binding site, were in good agreement

with the values found in radioligand binding assays².

These results demonstrate that the nAChR-containing supported membranes can be applied for ligand screening by SPR.

Fig. 1: Immobilisation of membrane protein receptors onto sensor surfaces. Detergent-



solubilised His-tagged 5HT₃ receptors were reversibly attached to NTA-modified quartz slides (left). The nicotinic acetylcholine receptor was reconstituted in tethered membranes onto a gold surface using so-called thiolipids, which serve both to covalently anchor the membrane to the surface and to decouple the membrane from the surface to accommodate the extramembraneous parts of the receptor protein (right).

DISCUSSION & CONCLUSIONS: We believe that the methods developed in this study are generally applicable for membrane receptor proteins and will be important for the discovery of new pharmacologically active compounds.

REFERENCES: ¹E. Schmid, et al., (1998) *Anal. Chem.*, **70**: 1331-1338. ²A. Sévin-Landais, et al., (2000) *Biophys. Chem.*, **85**, 141-152.

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