

Protein Measurements on Surfaces

G-protein-coupled receptors (also known as GPCRs), represent the largest protein family in the human genome, with about 900 members in total, of which about 420 appear of relevance to the pharmaceutical industry. These transmembrane protein receptors and their ligands play key roles in almost all aspects of physiology and hence have broad relevance to major disorders including heart disease, obesity, cancer, pain, and diabetes. Robust cell-based GPCR assays are impeded by difficulties with membrane-bound protein isolation and reconstitution and signal attenuation due to non-specific protein binding. Recently, we have demonstrated a new method for immobilization of GPCRs that appears to be promising for the development of robust cell-based GPCR assays.

D.J. Vanderah and K.D. Ridge (Div. 831)

The method developed by NIST researchers uses a sensitive surface plasmon resonance (SPR) method that shows selective immobilization of intact GPCR-containing vesicles to a surface highly resistant to non-specific protein adsorption. This approach avoids the problems associated with the isolation and reconstitution of low-abundance proteins. This is especially important to the pharmaceutical industry as they are studying these receptors in cell-based assays as potential drug delivery targets. The method of measurement was demonstrated with CCR5, a member of the GPCR family that specifically binds chemokine ligands and also acts as a secondary receptor for HIV-1. Intact CCR5-containing vesicles were immobilized on an activated initial template consisting of gold modified with oligo(ethylene oxide)s, which impart a high resistance to protein adsorption, and a low concentration of biotin-terminated compounds, which impart a specific recognition element for the subsequent surface activation steps. Surface activation of the initial template was accomplished by sequential capture of avidin, a protein with a high affinity for biotin; subsequent antibodies that ultimately present an epitope for specific recognition of CCR5; and, finally, the CCR5-containing vesicles. Each step of the process was accurately monitored in real time by an in-house-built, state-of-the-art SPR device.

This approach establishes a broad outline for the development and application of a variety of assays for CCR5 functions and (cell-based) GPCR assays. The generic nature of the initial surface and subsequent surface activation for specific immobilization can be applied to the development of assays for virtually all transmembrane receptors for which antibodies are available, or can be engineered to contain a particular antibody epitope, and can be expanded

to include the detection of specific proteins, cellular membranes, cells, bacteria, and viruses in complex biofluid mixtures.

This work establishes a sensitive, reproducible metrology for the detection and study of transmembrane proteins that are of significant importance to protein array technologies, drug discovery, disease-state diagnoses, and high-throughput sensor/biosensor development.

This approach is currently being applied for the immobilization of T cells via peptide-major histocompatibility complexes on microarrays for high-throughput analysis of antigen-specific, heterogeneous T cell populations.

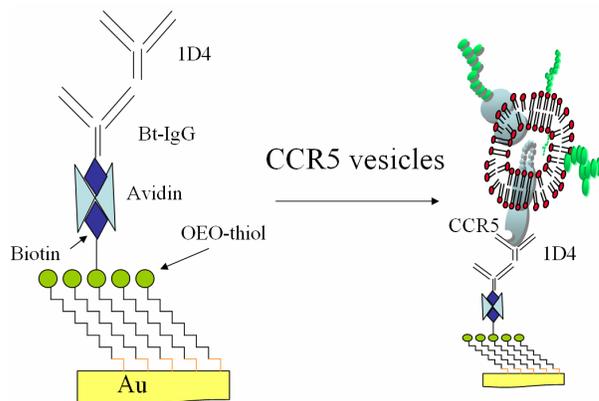


Illustration of immobilization on a protein resistant template (OEO-thiol) activated with (avidin → biotinylated immunoglobulin G (Bt-IgG) → rho 1D4) for selective capture (rho 1D4-CCR5) of CCR5-containing vesicles.

Publication:

Silin, V. I., Karlik, E. A., Ridge, K. D., Vanderah, D.J., "Development of Surface-Based Assays for Transmembrane Proteins: Selective Immobilization of Functional CCR5, a G-Protein-coupled Receptor," *Analytical Biochemistry, Anal Biochem.* 2006, 349, 247-253.