Biophysical Characterization of Liposomes and Antibody-Receptor Interactions on VLPs with the switchSENSE® Biosensor

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Virus-Like-Particles (VLPs) are membrane bound vesicles that are an attractive vaccine platform authentically resembling their host virus in structure and antigen expression. We report a novel automated methodology for characterizing VLPs with electro-switchable biosurfaces (switchSENSE®). Stable surface functionalization with VLPs (200 nm in diameter) using antibody modified DNA monolayers enables characterization of molecular interactions towards membrane proteins in physiological conditions. Additionally, a strategy to immobilize liposomes on the sensor surface using cholesterol modification is presented. Time-dependent phase transitions of immobilized liposomes was monitored in real-time. Information about specific lipid melting temperatures is crucial for the application of liposomes as thermostable drug delivery systems.

Strategies for Surface Functionalization with Virus-Like Particles

- Hybridization with complementary DNA sequence carrying a ligand
- Dye proximity change upon specific antibody binding to the ligand molecule
- Viruses

Real-Time Binding Analysis of Antibodies against Membrane Receptors

- Binding of labeled antibodies to specific membrane receptors on immobilized VLPs
- Independent monitoring of VLP stability on the surface in the red optical channel

Characterization of Liposomes on Electro-Switchable Biosurfaces

- Association of liposomes on cholesterol modified DNA layer
- Temperature induced lipid phase transition of immobilized liposomes

Electro-Switchable DNA Nanolevers

- switchSENSE® | Measurement Cycle: Regeneration of sensor surface by DNA denaturation and repeated hybridization
- switchSENSE® | Measurement Modes: Two independent measurement modes for comprehensive signal acquisition

Molecular Dynamics

- Detection of absolute size and shape of interacting molecules by electrically actuating DNA nanolevers

Proximity Sensing

- Real time size-dependent kinetics through changes in local environment of the fluorescent dye

Characterization of specific melting temperatures of lipid formations

- Stable surface immobilization of liposomes via DNA encoded addressing
- Time-resolved analysis of antibody binding and lipid phase transitions

Both independent signals (Fluorescence and Dynamic Response) are used to analyze specific phase transitions of lipid formations on the sensor surface.

- (A) Fluorescence proximity sensing: Fluorescence peaks at a surface temperature of 48°C. This indicates a temperature dependent change in the lipid bilayer around the Tc of DPPC lipids affecting the direct environment of the dye.
- (B) Molecular Dynamics: A decrease in Dynamic Response (DR) values is observed above a sensor temperature of 44°C with an infection point at T ≥ Tc caused by the change in liposome membrane properties.

High Sensitivity Measurement of analyte concentrations from fM to nM. From ultra-fast to ultra-slow kinetics. LOD > 10 fM
Detection of binding rate constants k_on, k_off and dissociation constants K_d in real-time. 8 fM
Analysis of protein diameters on chip with 0.3 nm accuracy and monitoring of conformational changes.
Characterization of melting transitions, thermal stability or thermodynamic analysis. 8°-75°C

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