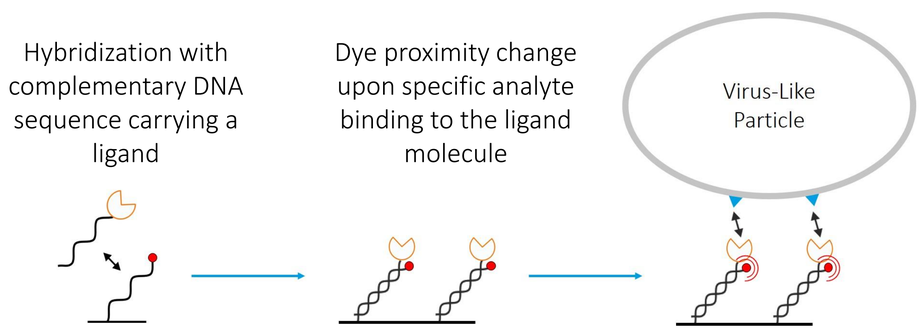
Biophysical Characterization of Liposomes and Antibody-Receptor Interactions on VLPs with the switchSENSE® Biosensor

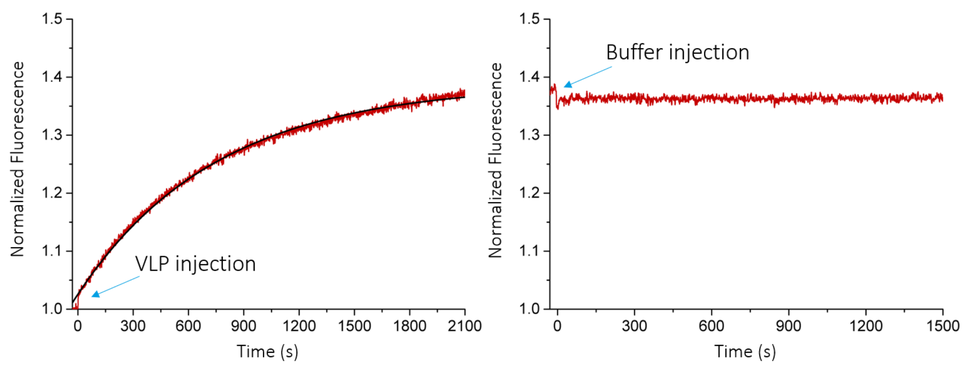
dynamic BIOSENSORS

Hanna Mueller-Landau, Ulrich Rant and Wolfgang Kaiser; Dynamic Biosensors GmbH, Martinsried, Germany

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. Temperature 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 thermosensitive drug-delivery systems.

Strategies for Surface Functionalization with Virus-Like Particles



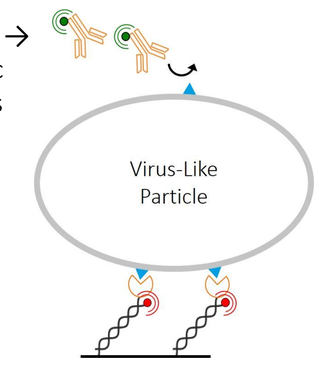


Red optical channel
Time-resolved monitoring of virus-like particle (VLP) immobilization via fluorescence proximity sensing

Anti-human CXCR4 modified DNA surface. CXCR4-VLPs are captured selectively on the sensor via specific antibody targeting. Antibody-receptor interaction in close proximity to the fluorophore results in an increase in light emission (red detection channel). One VLP can interlink multiple DNA conjugated anti-human CXCR4 antibodies leading to stable VLP functionalization (constant fluorescence signal during buffer injection).

Real-Time Binding Analysis of Antibodies against Membrane Receptors

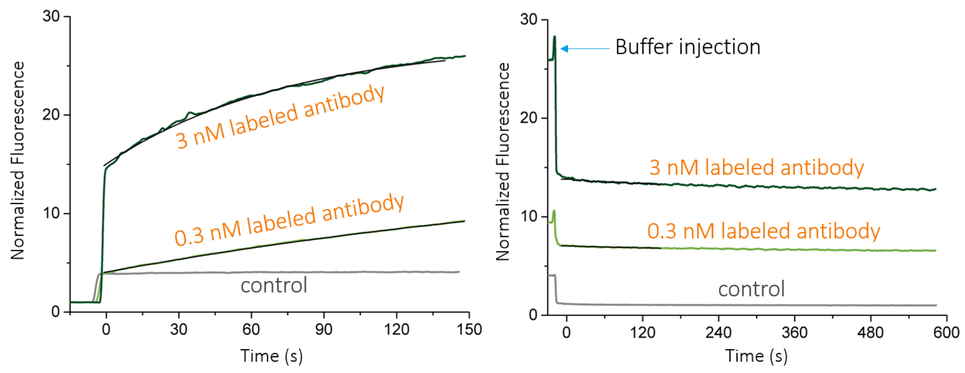
Binding of labeled antibodies to specific membrane receptors on immobilized VLPs →



← Independent monitoring of VLP stability on the surface in the red optical channel

Kinetic measurement of anti-human CXCR4 onto CXCR4-VLP functionalized surface yields specific antibody binding constants:

k_{ON} ≈ 3.3 x 10^6 $M^{-1}s^{-1}$
k_{OFF} < 3.2 x 10^{-3} s^{-1}
K_D < 1 x 10^{-9} M



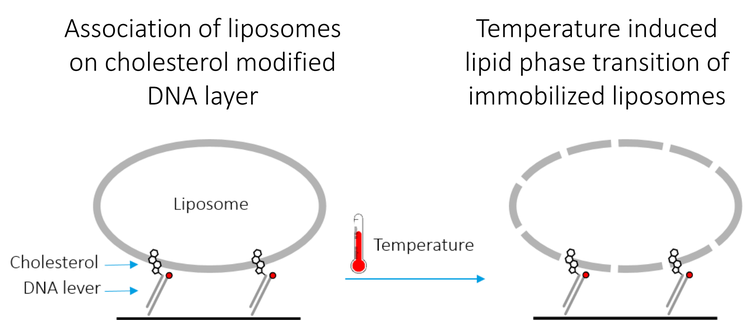
Green optical channel
Real-time binding analysis of antibodies towards membrane receptors via green fluorescence signal

Anti-human CXCR4 binding (green labeled) to CXCR4-VLP membrane receptors is detected in the green detection channel. Antibody binding is observed in real-time via concentration-dependent increase in fluorescence intensity after injection of green labeled antibody solution into the flow channel. No unspecific binding of antibodies onto the surface occurred (control: 0.3 nM antibody to unmodified surface). Unbound antibodies are removed and bound antibodies dissociate via buffer injection.

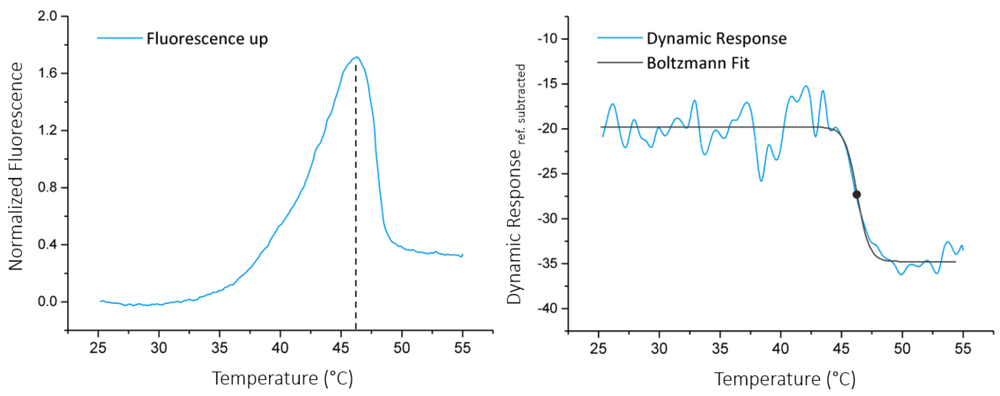
Detecting Antibody-Receptor Interactions with switchSENSE®

- ✓ Stable surface immobilization of VLPs via DNA encoded addressing
- ✓ Independent monitoring of multi-step assays on one detection spot using the DRX² dual color system
- ✓ Time-resolved observation of antibody binding towards membrane receptors

Characterization of Liposomes on Electro-Switchable Biosurfaces



Liposomes are captured by a cholesterol modified DNA layer. A temperature ramp crossing the critical lipid melting temperature results in a detectable change in structural properties of the lipid membrane.



Thermal melting curves of immobilized DPPC liposomes. A controlled temperature ramp is applied to the sensor surface (5°C/min) and the fluorescence signal (A) as well as the DNA switching speed (B) are recorded in real-time.

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 46°C. This indicates a temperature dependent change in the lipid bilayer around the T_m 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 inflection point at T = 46°C. This implicates a slowed DNA switching motion at T ≥ T_m caused by the change in liposome membrane properties.

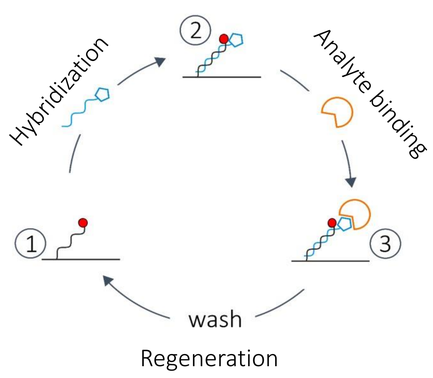
Characterizing Liposomes with switchSENSE®

- ✓ Stable surface immobilization of liposomes via DNA encoded addressing
- ✓ Time-resolved analysis of liposome binding and lipid phase transitions
- ✓ Characterization of specific melting temperatures of lipid formations

Electro-Switchable DNA Nanolevers

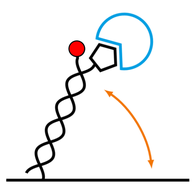
switchSENSE® | Measurement Cycle

Regeneration of sensor surface by DNA denaturation and repeated hybridization



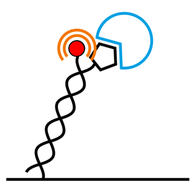
switchSENSE® | Measurement Modes

Two independent measurement modes for comprehensive signal acquisition



Molecular Dynamics

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



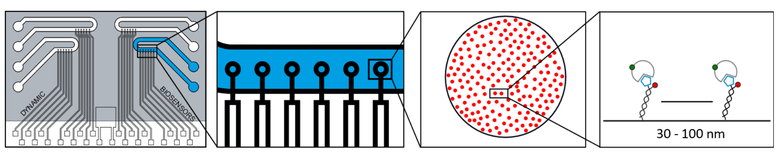
Proximity Sensing

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

switchSENSE® | DRX² Instrument

DRX² instrument featuring two light sources & two photon counters optimized for red & green fluorophores for dual binding analysis.

Multi-electrode biochip with 4 separate flow channels each comprising 6 detection spots.



High Sensitivity
Measurement of analyte concentrations from fM to mM.
From ultra-fast to ultra-slow kinetics.
LOD = 10 fM

Kinetics and Affinity
Determination of binding rate constants k_{ON}, k_{OFF} and dissociation constants K_D in real-time.

Size and Conformation
Analysis of protein diameters on chip with 0.1 nm accuracy, and monitoring of conformational changes.

Cooperativity and Avidity
Identification of multiple binding sites in a single measurement using variable capture molecule densities.

Thermodynamics
Characterization of melting transitions, thermal stability or thermodynamic analyses. 8° - 75°C

Nature Commun. 4:2099 (2013) | Bioanal. Rev. 4 (2) 97-114 (2012) | JACS 134, 15225 (2012) PNAS 107, 1397 (2010) | JACS 132, 7935 (2010)