Application Note # 167

In situ capture of T-cells and analysis of membrane receptor affinity with biofunctionalized lipid sensor

Assessment of T-cell specificity to tumor associated antigens is crucial for the development of personalized immunotherapies against cancer. Multi-Parametric Surface Plasmon Resonance (MP-SPR) was applied for characterization of interaction of T-cell receptors (TCR) from tumor-specific CD8+ T-cells. Intact live cells were captured onto biomimetic surfaces composed of artificial cell membranes functionalized with peptide-major histocompatibility complexes (pMHC). Real-time affinity analysis of immunological synapse formation between TCR and pMHC was completed from 4 different T-cell mutants.

Introduction

Surface Plasmon Resonance (SPR) is a well-established technique for measuring binding affinity and kinetics in real-time and label-free manner. Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments can perform measurements in a wide angular range (40-78 degrees) and at multiple wavelengths, enabling assessment of a wide range of interactants ranging from small molecules up to live cells. The technique can be used in a variety of innovative applications, such as characterization of interactions with live cells (both cultured on the sensor or captured in flow) as well as determination of thickness of the biological layers including conformation changes in lipid bilayers and biomaterials.

T-cells play a major role in the human immune defense system and their activation fires the adaptive immune responses. T-cells are activated upon the interaction of TCRs with antigenic peptides, presented by the major histocompatibility complex (MHC) in either tumor cells or antigen presenting cells. Immune cells specific to tumor-associated antigens with enhanced antitumor activity lay a foundation to development of personalized cancer immunotherapies. Such innovative therapy aims to activate tumor specific T-cells that help the patient's own immune system to attack the cancer cells. Successful immunotherapy requires reliable characterization of TCR-pMHC binding parameters to asses specificity and safety of the therapy.

Materials and methods

Measurements were conducted with an MP-SPR Navi™ 210A VASA instrument, equipped with 3 laser wavelengths (670, 785 and 980 nm in each flow channel). The flow rate of Krebbs-EDTA buffer was set to 10 µl/min and the measurement temperature to 16 °C.

Cell-based assays were carried out in 3 different sensor formats (Figure 1):
1. Au coated with PEGylated alkanethiols (SAM - Self-Assembled Monolayer),
2. SiO_2 surface functionalized with supported lipid bilayers (SLB) and
3. Au coated with hybrid bilayer membranes (HBM).

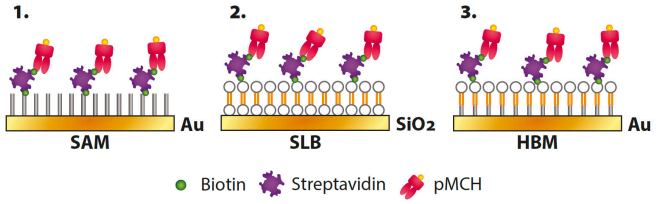
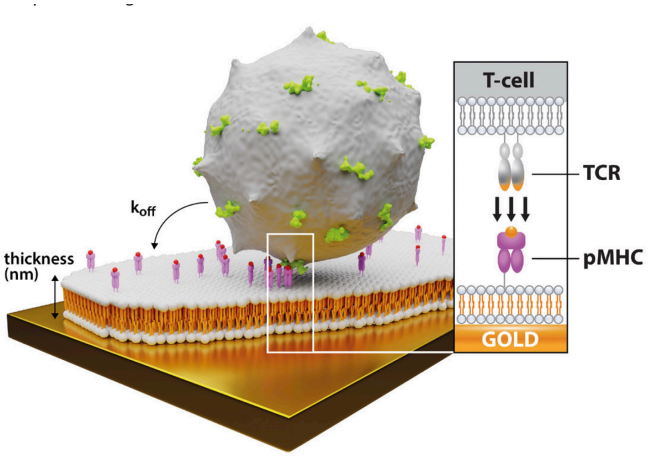


Figure 1. Different sensor formats: 1. Au coated with PEGylated SAM. 2. SiO_2 with SLB. 3. Au coated with HBM. All 3 surfaces were modified with biotin moieties, streptavidin and pMHC.

All 3 layers were modified with biotin moieties which enabled immobilization of biotin-pMHC complexes (HLA-A0201 monomers loaded with NY-ESO-I157–165 antigen) through streptavidin mediated binding. PEG-SAM surface was used as a reference while the functional SLB and HBM were considered as biomimetic planar matrices for determination of T-cell receptor binding.



Human tumor specific CD8+ T-cells were loaded onto the surface with 25 min injections with cell density from 10^2 to 10^5 cells/mL. The post-injection time was set to 10 minutes. Sequential injections of increasing cell densities were performed with 4 distinct cell populations where WT (wild type), DMβ and V49I were different TCR variants, while T1φ acted as a negative control.

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Results and discussion

Lipid-based sensor surfaces were generated *in situ* and layer formation was characterized by dedicated LayerSolver™ software. The thickness of the SLB film was 5.65 nm while HBM layer thickness was composed of 1.95 nm underlaying alkanethiol SAM and the actual lipid sublayer was 2.88 nm thick. MP-SPR curves and fittings of SLB deposition with three laser wavelengths are provided in Figure 3. SLB and HBM showed better sensitivity for cell detection and higher level of specific cell capture in comparison to the PEGylated SAM surface.

Real-time kinetic analysis of T-cell binding to pMHC functionalized surfaces was performed for all tested cell populations. Two-dimensional structural dissociation kinetics revealed different affinities of the three tumor-specific CD8+ T-cells (WT, DMβ, V49I). Table 1 presents the kinetic values of TCR binding to pMHC complexes as registered for different CD8+ T-cells variants on both biomimetic surfaces. The obtained data was in agreement with previously published *in silico* studies (Hebeisen *et al*., 2015, Irving *et al*., 2012). See Figure 4a for DMβ T-cells specific binding to pMHC. The negative control of T1φ T-cells showed no binding towards pMHC (Figure 4b). Here described approach demonstrated excellent specificity and selectivity for *in situ* detection of T-cell populations and characterization of binding affinities using MP-SPR technology.

Table 1. Kinetic parameters of TCR binding to pMHC and curve fitting (R^2) values.

TCR variant	SLB k_{off} ($x10^{-2}$ s^{-1})	HBM k_{off} ($x10^{-2}$ s^{-1})
DMß	**0.57** R^2 = 0.997	**0.63** R^2 = 0.999
Wild type	**3.65** R^2 = 0.993	**3.81** R^2 = 0.999
V491	**17.83** R^2 = 0.989	**19.54** R^2 = 0.999

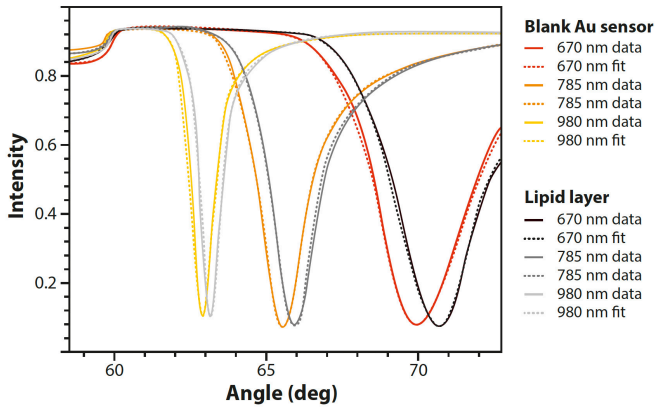

Figure 3. MP-SPR curves of suppoted lipid bilayer (SLB) deposition. The experimental data was fitted using LayerSolver™ software.

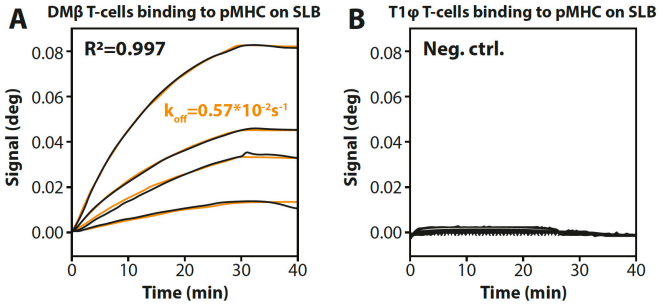

Figure 4. CD8+ T-cells with A. DMβ TCRs and B. T1φ TCRs binding to pMHC on SLB.

Conclusions

In this study, the biomimetic SLB and HBM layers were generated on sensor surfaces and the thickness of the layers was determined by MP-SPR and LayerSolver™ software. Different variants of tumor-specific CD8+ T-cells were successfully captured in flow onto pMHC-functionalized surfaces and the structural affinity of TCR to pMHC complexes was measured. MP-SPR based method has proven to be an efficient tool for determining anti-tumor activity of membrane receptors and might increase the pace of cancer immunotherapy development. BioNavis instruments enable cell-based measurements as it can be conducted in physiologically relevant conditions, at controlled temperature (15 to 45 °C) and under static or dynamic flow conditions. Therefore MP-SPR technology is a powerful tool for live-cell based assays which is essential for the development of novel cell therapies and biomaterials.

See how to measure layer thickness and refractive index simultaneously with MP-SPR in Application Note #128 and #139. See Application Notes #137 and #156 to see how live cells can be cultured over the sensor surface and applied in MP-SPR.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA OR 400 KONTIO

Sensors surfaces: Au or SiO_2

Software: MP-SPR Navi™ Control, DataViewer, LayerSolver™ TraceDrawer™ for MP-SPR Navi™

Original article:

Soler *et al.*, ACS Sensors Vol. 3 (11), 2018

References:

Hebeisen *et al*., Cancer Research Vol. 75 (10), 2015
Irving *et al*., Journal for Biological Chemistry Vol. 287 (27), 2012

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