REAL-TIME INTERACTION STUDY OF COVALENTLY IMMOBILIZED SARS-COV-2 SPIKE PROTEIN AND SPECIFIC NANOBODIES

Smiltė Drobnytė[1], Mantvydas Usvaltas[2], Vincentas Maciulis[2], Asta Luciunaite[2], Ieva Plikusiene[2]

[1]Vilnius University, Faculty of Medicine, Pharmacy and Pharmacology Center, Vilnius, Lithuania
[2]State Research Institute Center for Physical and Technological Sciences, Sauletekio av. 3 Vilnius, Lithuania
Smilte.Drobnyte@gmail.com

The rapid evolution of SARS-CoV-2 and the emergence of variants with altered infectivity and immune escape properties continue to challenge existing therapeutic strategies. The viral spike (S) protein, which mediates host cell entry through interaction with the ACE2 receptor, remains a primary target for neutralizing monoclonal antibodies. Detailed characterization of spike–antibody interactions is therefore essential for the development and optimization of effective antibody-based therapies.

The aim of this study was to investigate and compare real time interactions between covalently immobilized SARS-CoV-2 spike protein with selected monoclonal antibodies and single-domain nanobodies. The SARS-CoV-2 spike protein was covalently immobilized on gold sensor surfaces using a self-assembled monolayer of 11-mercaptoundecanoic acid and EDC/NHS coupling surface chemistry. Real-time interaction analyses were performed using spectroscopic ellipsometry, quartz crystal microbalance with dissipation monitoring (QCM-D), and surface plasmon resonance. These complementary label-free techniques enabled monitoring of immobilization efficiency, antibody binding kinetics, and surface regeneration under controlled conditions.

During this study stable and reproducible conditions for immobilization of the spike protein were achieved. All investigated antibodies demonstrated specific binding to the immobilized antigen, with distinct interaction profiles. Quantitative analysis revealed differences in surface mass density and kinetic parameters among the antibodies, indicating variations in binding affinity and complex stability. Nanobody SB15 exhibited the strongest interaction with the spike protein, while CR3022 showed comparatively weaker binding .This study demonstrates the applicability of real-time, label-free biophysical methods for detailed evaluation of SARS-CoV-2 spike protein interactions with monoclonal antibodies. The obtained results provide valuable insight into antibody binding behavior and support the rational selection and design of antibody-based therapeutics against SARS-CoV-2 and its emerging variants.