QCM-D/SE COMPARISON OF SB14 AND SB68 FC-FUSION NANOBODY IMMUNOCOMPLEX FORMATION WITH SARS-COV-2 VIRUS-LIKE PARTICLES

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Macrophages may induce a strong inflammatory response when substantial amounts of inflammatory cytokines are secreted, which can lead to hyperinflammation and tissue damage [1]. This inflammatory response can be mediated by antibody-opsonized antigens called immunocomplexes (ICs). It has been experimentally confirmed that SARS-CoV-2 can activate this response, and antibody-opsonized SARS-CoV-2 particles can further enhance inflammation by promoting inflammasome activation [2]. The structural properties of immunocomplexes formed between an antibody and an antigen may affect IC interactions with FcR. Therefore, comparing how different antibodies form ICs with virus-like particles can help understand how IC structure may influence macrophage FcR engagement and support strategies to modulate macrophage responses in viral infections or antibody therapies, thereby avoiding adverse reactions.

In this work we compared how llama nanobodies fused with human IgG1 Fc - SB14 and SB68, form ICs with SARS-CoV-2 VLPs, which are assembled from the viral structural proteins and mimic the native virion. A self-assembled monolayer of 11-mercaptoundecanoic acid was used to covalently immobilize Protein G, enabling oriented capture of the Fc-fusion nanobodies and subsequent IC formation upon exposure to VLPs. By combining the quartz crystal microbalance with dissipation monitoring (QCM-D) and spectroscopic ellipsometry (SE) methods, we calculated the binding kinetics using a two-step interaction model, determined the wet and dry surface mass densities, and evaluated the viscoelastic properties of the formed VLP layers. A substantial difference was determined while comparing the viscoelastic properties of the formed layers: VLPs bound to SB68 produced a more viscoelastic layer, with an approximately two-fold larger frequency shift (ΔF) and higher dissipation (ΔD) than VLPs bound to SB14.

In this work, QCM-D and SE were used to compare SARS-CoV-2 VLP immunocomplex formation on SB14 and SB68 Fc-fusion nanobody layers. Surface mass density of the formed layers and binding affinity of the antigen-antibody interaction were evaluated which showed similar results for both nanobodies. ΔF and ΔD shifts revealed that the VLPs bound to SB68 formed a more viscoelastic layer than VLPs bound to SB14.

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