INDUSTRIAL DEVELOPMENT PROJECTS

Verification of SAXS and SANS techniques for characterization of saponin-based vaccine adjuvant

THE INDUSTRIAL CHALLENGE

The saponin-based vaccine adjuvant enhances the immune response to vaccines. It is a lipid nanoparticle composed of phospholipid, cholesterol, and saponin with a self-assembled cage structure with an average diameter of around 40 nm. Detailed information such as component localization and distribution within the nanoparticle is key for product development, but a challenge to characterize due to small particle size, low concentration, and structural complexity.

WHY USE A LARGE-SCALE FACILITY?

Small angle x-ray scattering (SAXS) provides more detailed information on structural details such as shell thickness and morphology compared to standard bulk techniques, such as dynamic light scattering (DLS), and complement techniques, such as cryo-electron microscopy (CryoTEM) and asymmetrical flow field-flow fractionation (AF4). SAXS at a large-scale infrastructure, compared to a lab-based benchtop SAXS, is favored, since it has higher flux, resolution, and coherence and thus giving information on structural features at shorter length scales e.g. holes and bridges of the cage structure. Applying neutron scattering (SANS) on samples with deuterated lipid analogues is one of a few techniques that can provide molecular-level insight.

HOW THE WORK WAS DONE

To increase the dimensional resolution in the SAS-measurements, the formulations were optimized to produce monodisperse particles. For the SANS measurements, the saponin nanoparticles were formulated with deuterated cholesterol (Cholesterol-d45) and phosphatidylcholine (POPC-d64) and diluted in contrast matched buffers, e.g. by varying D_2O:H_2O ratios (10-90%). Neutrons interact differently with hydrogen and deuterium atoms (i.e., it is isotope sensitive), and through contrast matching series using different D_2O:H_2O ratios as solvent specific components were highlighted in the nanoparticle. The SANS measurements were performed at the Zoom beamline at the ISIS Neutron and Muon Source, England, in collaboration with beamline scientist Dr. James Doutch. The SAXS data was received at the CoSAXS beamline of MAX IV, Sweden, with support from beamline scientist Dr. Fátima Herranz. All data were analyzed using shape-dependent and shape-independent modelling and compared to data from DLS, AF4 and CryoTEM.

THE RESULTS AND EXPECTED IMPACT

The use of both SAXS and SANS allowed in-depth understanding of the morphology of nanoparticle cage structure i.e. membrane thickness, bridge dimensions and hole sizes. Additionally, SANS was able to reveal insights into the molecular distribution of the components. Converting the SANS data to pair-distribution functions P(r) by using inverse Fourier transformation shows that the apparent cage structure is changing in the contrast matching SANS experiment. This indicates an inhomogeneous distribution of the components and the exact component distribution within the nano particle is further pinpointed in ongoing advanced modelling work. The large-scale SAXS and SANS results have both direct and indirect implications for Novavax, with an increased molecular-level knowledge of the nanoparticle system important for product development, as well as improved analytical capabilities. The team has drafted a scientific manuscript and plans to continue the collaboration.

Contacts: Per Wessman – Novavax AB, pwessman@novavax.com
Margaret Holme – Chalmers University of Technology (Chalmers), holmema@chalmers.se
Christopher Söderberg – Research Institutes of Sweden (RISE), christopher.soderberg@ri.se

Vinnova's project No: 2023-02825 **Duration:** November 2023 – November 2025

Funded by Sweden's Innovation Agency, Vinnova, in order to build competence and capacity regarding industrial utilisation of large-scale research infrastructures such as MAX IV and ESS.