LSI SUPPORT FOR PhD-STUDENTS IN APPLIED AREAS

3D determination of synthetic peptide constructs in cancer vaccine development

THE PURPOSE OF THE PHD PROJECT

Oligonucleotides (ON:s), peptides, and proteins as modality for therapeutic intervention have been growing over the last years, first as single modalities and later also as constructs based on conjugating two or more of same and/or different modalities. The main purpose for conjugation is to enhance efficiency by e.g., conjugating targeting ligands to direct delivery to desired cell/tissue, but it also have a positive impact on stability, as well as cell penetration and the overall mode of action. In addition, there is an interest in increased knowledge of the 3D conformation of single constructs with and without interacting targets.

USING A LARGE SCALE INFRASTRUCTURE

Initial characterization using LC-MS to determine purity and identity was followed by small-angle X-ray scattering (CoSAXS MAX IV due to readily available within the project) to give initial information of overall size (radius of gyration) of the peptide/ON based constructs to be followed by higher resolution measurements. The possibilities that the synchrotron measurement techniques (SAXS) entails in the form of structural-related information in complex constructions consisting of several different biological active oligonucleotides and peptides units conjugated together is of outmost importance to obtain required detailed information regarding various interactions. The study aimed to use different longitudinal scales and highest available resolution to generate X-ray based data in atomic scale, surface properties, size, and format. These parameters are all important from a knowledge, functional and design perspective in further development and understanding of, in this case, as vaccine candidates. Developed designs of conjugated ON:s and peptides, which originate from research by Immuneed AB, Ultimovacs ASA and Uppsala University were measured at the CoSAXS beamline of MAX IV where the doctoral student also participated at site. She also produced additional conjugates with more favourable solubility properties compared to the samples measured at MAX IV (which showed aggregated constructs if/when dissolved and measurable). These were analysed through mail-in at the equivalent beamline P12 of Petra III, Hamburg.

RESULTS AND IMPACT

Finding combinations of water-based solvent systems including additives to generate a stable and measurable solution of constructs consisting of oligonucleotides and peptides proved to be more problematic than expected. The identified aqueous solution systems should also be relevant for the subsequent biological evaluations (in vitro/in vivo). The measurement methods and measurement possibilities to obtain data from size/shape down to atomic resolution highlights the importance and possibilities of synchrotron techniques. The method is clearly a very important component for continued understanding of the interaction between different conjugates and their targets in continued drug development in the therapeutic field. The project has generated important information and guidance on upcoming challenges for this type of conjugates which will be useful in coming evaluations to establish an easy soluble and non-aggregating conditions. The doctoral student has gained experience and insight into both the use of synchrotron measurement techniques and the proved difficulties in generating measurable ON-peptide conjugates.

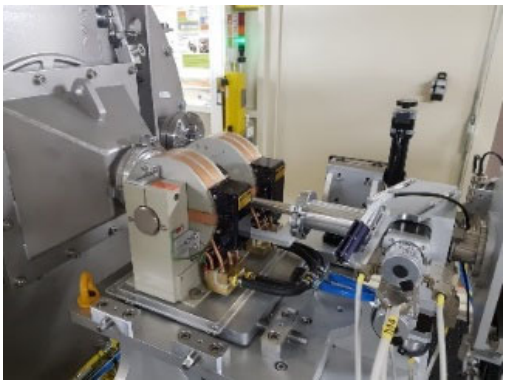

Figure 1. CoSAXS beamline at MAX IV.

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