INDUSTRIAL PILOT PROJECT

Rational scaffold design of Affibody® molecules guided by atomic structure determination by X-ray crystallography

THE INDUSTRIAL CHALLENGE

Affibody AB is developing next generation biopharmaceuticals using a unique proprietary technology based on a minimized protein scaffold. Understanding the structure-function relationship is key towards optimized best-in class therapeutics. A time-efficient procedure for structure elucidation is crucial to enable its incorporation in the strained drug development process driven by fast-to-market needs.

WHY USING A LARGE SCALE FACILITY

Optimization of drug candidates by computer-aided rational protein design requires high-resolution structural information of the drug candidate and desirably also in complex with its antigen. Compared to laboratory-based X-ray sources synchrotron radiation provides higher intensity X-ray beams, resulting in better data quality. Furthermore, data acquisition at a synchrotron requires just a few seconds, compared to several hours at a laboratory source.

HOW THE WORK WAS DONE

Three Affibody® molecules and two antigens were produced. The stability of the Affibody® molecules were characterized by circular dichroism spectroscopy and antigen binding kinetic analyses were performed by surface plasmon resonance measurements. Antigen:Affibody® complexes were formed and purified by size-exclusion chromatography. Complex, antigen and Affibody® molecules, respectively, were further analysed by differential scanning fluorimetry. Crystallization experiments were setup using a nanoliter pipetting robot. Resulting crystals were analysed at the BioMAX beamline of MAX IV and at station I04 of Diamond Light Source (DLS) in UK. Crystal diffraction analyses and data collection were carried out remotely, which is the standard way of collecting crystallographic X-ray diffraction data nowadays.

THE RESULTS AND EXPECTED IMPACT

Three Affibody® molecules, two antigens and three antigen:Affibody® complexes were successfully generated using a combination of established platforms at Affibody AB and SARomics Biostructures AB. Well-diffracting crystals were obtained for one antigen and one Affibody® molecule that enabled structure determination of these molecules. The structure of the Affibody® molecule was solved and refined to 1.45 Å resolution with an R value of 0.177 and R_{free} of 0.208. As is often the case, the other samples only produced poorly diffracting crystals or did not crystallize.

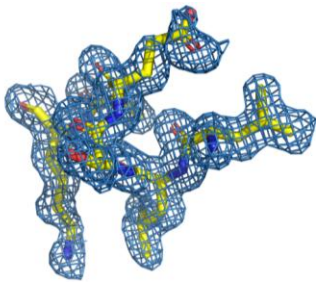

Figure 1. Selection of residues shown in stick representation and an example of the electron density contoured at the 1 σ level shown in blue of the 1.45 Å Affibody® crystal structure

The novel structure of the Affibody® molecule to atomic resolution is expected to enable further development of the Affibody® molecule scaffold, guiding the platform development process through rational protein design.

"We are excited about further enhancing our molecular platform by using rational design at atomic resolution with data from MAX IV and DLS in collaboration with SARomics" /Fredrik Frejd, CSO Affibody AB

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Vinnova's project No: 2019-02559 **Duration:** Aug 2019 -- Jan 2021

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.