INDUSTRIAL DEVLOPMENT PROJECTS

SAXS and AF4 for Early-Stage Evaluation of Protein Therapeutics

THE INDUSTRIAL CHALLENGE

Strike Pharma is developing bispecific antibody formats for the next generation targeted delivery therapeutics. A major challenge in drug development is that promising candidates are sometimes rejected during early screening based on size or aggregation, without understanding the mechanisms behind those signals. These structural transitions are often short-lived and difficult to isolate, are hard to detect under realistic (in vivo-like) conditions with standard methods.

WHY USING A LARGE-SCALE FACILITY

To study how antibody–ligand conjugates behave under in vivo-like conditions, we need techniques that can detect the mechanism behind aggregation and conformational shifts, including at low concentrations and in complex media. Small-Angle X-ray Scattering (SAXS) enables us to observe phenomena such as conformational flexibility, partial unfolding, and early-stage aggregation. However, the high brilliance and coherence of synchrotron light is necessary to see weak signals and flexible molecules that are invisible with standard lab equipment.

HOW THE WORK WAS DONE

SAXS studies were performed at the CoSAXS beamline of the MAX IV Laboratory. To enable analysis in such a complex media as serum, SAXS was combined with Asymmetric Flow Field-Flow Fractionation (AF4), which separates molecules based on diffusion without a stationary phase. This AF4–SAXS setup enables real-time observation of structural changes of monomers, oligomers and aggregates under different peptide-to-antibody ratios and buffer compositions (e.g., histidine, arginine) and with and without serum. The collected SAXS data were modelled to assess conformational flexibility and aggregation behaviour.

THE RESULTS AND EXPECTED IMPACT

The SAXS experiments have given new insights into how target engagement and formulation choices of a bispecific antibody can lead to structural changes and how this can and should be analysed and considered when developing multi-specific antibodies.

With the support from SAXS data we could identify that binding to one of two ligand targets is in line with an induced fit model, where a certain antibody conformation state is favoured over others and that this stabilises the ligand binding. We also identified evidence of that the formulation can favour a certain confirmational state without ligand addition.

To be able to fully describe the mechanism of action and safety profile of antibody therapeutics ahead, bispecific antibodies should be investigated for how formulation and binding relate to antibody activity,

"SAXS data has helped us to understand how bispecific antibodies behave in different environments and in the absence or presence of a ligand interaction, this is valuable information that can and should be used to improve the mechanistic insights of bispecific antibodies in general" /Sara Mangsbo, co-founder Strike Pharma AB

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