

# Structure and thermal stability of therapeutic proteins in solid state formulations: Analysis of the SAXS/WAXS data

## THE INDUSTRIAL CHALLENGE

The stability of biologically produced pharmaceuticals is the limiting factor to various applications. This can be improved by production in solid forms, mostly via lyophilization. However, drying is never absolute and the physical and chemical stability of biological drugs in the presence of residual moisture is complex. The processes occurring within semi-humid solid state protein formulations during storage affects long term stability. Therefore, increased understanding the mechanisms of destabilization of proteins in the dry state is essential to the development of more stable drugs.

## WHY USING A LARGE SCALE FACILITY

The proposed experiment using Small and Wide-Angle X-ray Scattering (SAXS/WAXS) requires synchrotron instrumentation to fully capture protein structures in complex formulations. Firstly, the scattering of proteins in a solid matrix of excipients is characterised by low X-ray contrast, thus high flux is required to achieve good statistics. Secondly, the sample environment required for the experiment, the Linkam heating stage, is not widely available outside synchrotron facilities. Finally, the high flux of the synchrotron source is needed to investigate these systems at realistic heating rates.

## NEED TO IMPROVE ANALYTICAL TOOL

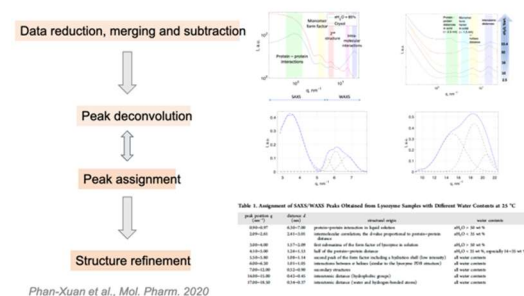
However, since the performed experiments generate large data sets we aimed to develop a data processing tool analysis of SAXS/WAXS data. The structural analysis of proteins in the solid state is novel and challenging since most of the available tools/fitting models only are valid for dilute aqueous systems. To fully understand the structure of the proteins in solid state it was therefore necessary with new strategies in data analysis. The team consists of different partners from industry (Swedish Orphan

Biovitrum) and public sector (Malmö University, MAX IV and RISE), providing expertise in scattering and diffraction of radiation but also in various areas such as mathematical modelling, thermodynamics and dynamics of solid state formulation and freeze drying of formulation.

## THE RESULTS AND EXPECTED IMPACT

We have successfully created and implemented a toolbox consisting of a complete set of Matlab scripts to perform different steps in the data processing in order to quantitatively identify changes of protein structure during dehydration and rehydration processes. This provides a better understanding of the relation between material structure and stability, thus contribute in the development of improved and novel formulation platforms.

The project results will be further developed together with other results collected in the collaborative project NextBioForm to provide guides for formulation of solid forms of proteins. Moreover, we also plan to incorporate the software in the data analysis pipeline of the CoSAXS beamline at MAX IV to make it available for all users.



**Figure.** Strategy for the analysis of the SAXS/WAXS data of protein structure in the solid state

**“Having a toolbox for processing of large synchrotron data is an important step towards understanding performance of biological products”**

/Jonas Fransson, Swedish Orphan Biovitrum



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