Report experiment LS-2807

Investigating the heme extraction mechanism of hemophores with time-resolved WAXS

The aim of the experiment was to clarify the mechanism by which the IsdB hemophores (from *Staphylococcus aureus*) interacts with human hemoglobin (Hb), resulting in heme extraction and binding to IsdB, a necessary step for *Staphylococcus aureus* to acquire iron and hence proliferate in the host. To obtain structural insights on this protein-protein interaction, resulting in the heme transfer from one protein to the other, we exploit the possibility to carry out on ID09 beamline pioneering time-resolved wide-angle X-ray scattering measurements on proteins.

Instead of using laser to trigger photoactive compounds, we worked on the setup of a kinetic measurement by using a stopped flow apparatus online with ID09.

We initially performed static X-ray scattering measurements on single proteins (IsdB, oxidized and reduced human Hb) and stoichiometric complexes between IsdB and Hb. This allowed to set up proper experimental conditions and to have reference WAXS signals to be used in the future analysis of time resolved WAXS data.

Before installing stopped flow apparatus on ID09 beamline, we tried the instrument and set up mixing conditions by using a 532 nm laser and a diode, since the reaction can be also spectroscopically followed.

Then, after a proper installation and alignment of the stopped flow apparatus on the beamline, we worked on the definition of acquisition timing, based on previous results obtained with UV-visible absorption spectroscopy.

Time resolved WAXS experiments were preliminary carried out on buffer mixed with buffer, and oxidized hemoglobin mixed with buffer to check if solution mixing caused WAXS artifacts.

Time-resolved WAXS experiments on IsdB mixed with oxidized hemoglobin was properly carry out, and a clearly detectable change was observed. The use of molecular dynamic models of single proteins and complexes (work in progress) will allow to analyse time resolved WAXS traces and hypothesize a kinetic mechanism coupled with molecular events.

Future perspective is to investigate again this protein-protein interaction in the presence of inhibitors, which potentially contribute to the development of new antimicrobials, with significant potential impact on public health.