INDUSTRIAL PILOT PROJECT

Small-angle X-ray scattering characterization of higher-order structures (HOS) of biosimilars as a business opportunity

THE INDUSTRIAL CHALLENGE

SARomics Biostructure is focused on providing services to small biotech and large pharmaceutical companies in the field of structure-based drug design. Our services include validation of higher-order structures (HOS, three-dimensional structure) of biosimilars. These are copies of therapeutic antibodies, usually produced by an industry actor after expiration of the original patent that belonged to a different company. Before entering the market, biosimilars need to be approved by regulatory agencies like EMA or FDA. The validation process demands assessment of the comparability of the new biosimilar's HOS with that of the originating biologics. Until now we offered to our clients a combination of X-ray crystallography and nuclear magnetic resonance (NMR) for this purpose. In this project we wanted to explore the use of synchrotron-based small-angle X-ray scattering (SAXS) in biosimilars comparison.

WHY USING A LARGE SCALE FACILITY

SAXS at synchrotrons provides higher intensity X-ray beams, resulting in better data quality than data obtained from laboratory equipment. And most importantly, exposure of each sample at a synchrotron requires just few seconds, compared to several hours at a laboratory source. For each project this provides considerable time and human resources saving, two essential factors in industry. In addition, taking into account the high costs, a laboratory SAXS equipment is simply not accessible for small companies.

HOW THE WORK WAS DONE

For the experiments, a number of buffer solutions were tested with dynamic light scattering (DLS). In addition, the samples were subjected to size-exclusion chromatography (SEC) and the promising solutions were selected for SAXS. Data were collected at beamline P12, at the Petra III synchrotron in Hamburg. Due to difficulties with sample instability and aggregation, two measurements were required. We also used two different antibodies with their biosimilars (Humira-Amgevita and Herceptin-Ontruzant) as a way of verification of the results. The experiments included testing of a total of 48 different conditions with different protein concentrations, different buffers as well as SEC-SAXS and took about 10 hours. Using a laboratory source this work would take at least 2-3 weeks! We received direct assistance from Dr Svergun's group at EMBL (Dr Cy M. Jeffries and Dr T. Gräwert) who run EMBL's industrial services through the company Biosax GmbH.

THE RESULTS AND EXPECTED IMPACT

The experiments allowed us to obtain the experience required for future use of synchrotrons for biosimilars characterization. The results based on the comparison of the scattering curves for the samples clearly confirm that our procedures were sufficient for the purpose (see Figure). However, a surprise in this context was that the scattering curves for the samples in the formulation solutions (the solutions used for injection in humans) showed clear differences between the antibodies and their biosimilars, as well as between the antibodies when compared to our optimized buffers. This suggests that antibody conformation depends on the buffer solution, suggesting in turn that some standard buffers may be required in future characterizations of HOS.

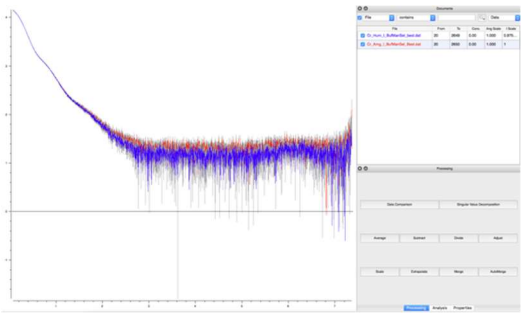

Figure. The scaling of the scattering curves of the antibody Humira and its biosimilar Amgevita. This shows that both curves are essentially identical.

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