Using small-angle X-ray scattering to characterize higher-order structures of biosimilars

SARomics Biostructures AB focuses on providing services in the field of structure-based drug design to small biotech and large pharmaceutical companies. These services include the validation of higher-order structures (HOS, three-dimensional structure) of biosimilars. Biosimilars are copies of therapeutic antibodies, usually produced by an industry actor after the original patent belonging to a different company has expired.

CHALLENGE

Before entering the market, biosimilars need to be approved by regulatory agencies, such as the European Medicines Agency (EMA) or the Food and Drug Administration (FDA) in the United States of America. The validation process calls for the comparability of the new biosimilar's HOS with that of the originating biologics to be assessed.

Until now, SARomics Biostructures has offered its clients a combination of X-ray crystallography and nuclear magnetic resonance (NMR) for this purpose. In this project, SARomics Biostructures wanted to explore the use of synchrotron-based small-angle X-ray scattering (SAXS) for comparing biosimilars.

METHOD

For the experiments, a number of buffer solutions were tested using dynamic light scattering (DLS). In addition, the samples were subjected to size-exclusion chromatography (SEC) and promising solutions were selected for small-angle X-ray scattering (SAXS). Data were collected on the beamline P12, at the PETRA III synchrotron in Hamburg. Due to difficulties with sample instability and aggregation, two measurements were required. There were also used two different antibodies with their biosimilars (Humira Amgevita and Herceptin-Ontruzant) as a means of verifying the results. The experiments tested a total of 48 different conditions with different protein concentrations, different buffers as well as SEC-SAXS and took about 10 hours. SARomics Biostructures 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 BIOSAXS GmbH in Hamburg.

INSIGHTS & ANALYSIS

The experiments allowed SARomics Biostructures AB to gain the experience required in order to use synchrotrons for biosimilar characterization in the future. The results based on the comparison of the scattering curves for the samples clearly confirm that the procedures were adequate for the purpose. However, surprisingly the scattering curves for the samples in the formulation solutions (the solutions used for injection in humans) displayed clear differences between the antibodies and their biosimilars, as well as between the antibodies, when compared with the optimized buffers. This suggests that antibody conformation depends on the buffer solution, suggesting in turn that some standard buffers may be required in the future characterization of HOS.

BENEFITS

SAXS at synchrotrons provides higher intensity X-ray beams, resulting in better data quality than data obtained from laboratory equipment. And most importantly, exposing each sample takes just a few seconds in a synchrotron, compared with several hours using a laboratory source. Using a laboratory source this work would have taken at least 2-3 weeks.

This leads to considerable savings in terms of time and human resources for each project, two essential factors in industry. In addition, in view of the high costs, laboratory SAXS equipment is simply not accessible to small companies.

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For more information please contact:

Deutsches Elektronen-Synchrotron DESY
Ein Forschungszentrum der Helmholtz-Gemeinschaft
Notkestraße 85 I 22607 Hamburg

DESY Innovation & Technology Transfer:
Dr. Sabine Brock
E-mail: sabine.brock@desy.de
Phone: +49 40 8998-4579
www.innovation.desy.de