X-rays Aid Production of Industrial Enzymes

Analysis of an enzyme produced by Novozymes in large-scale as a detergent ingredient, confirms that X-ray powder diffraction (XRPD) is highly relevant for protein-producing industries.

When your shirt has a sauce stain the enzyme Savinase in your washing powder chops up the proteins in the stain. This is an important first step, which allows the water and other detergent components to remove the tricky substance. By doing so Savinase – with the scientific name Bacillus lentus subtilisin – also helps the environment and protects the climate, as before the application of enzymes to washing powder, such stains could only be washed away at high temperatures, leading to shorter life of the textiles and to higher energy consumption. Therefore it is good news that a technique nursed by Associate Professor Pernille Harris and her group at DTU Chemistry is able to aid in the everyday production of Savinase.

The technique X-ray powder diffraction (XRPD) allows for fast characterization of the product. Just like all other proteins, Savinase can assume a variety of crystal structures, so called polymorphs. Depending on the actual production conditions, the distribution of polymorphs will vary.

"The collaboration has proven that the XRPD technology can provide Novozymes with a molecular insight into the complex biological process fluids," says Sune Jacobsen, Senior Manager at Novozymes – the world's leading producer of industrial enzymes.

Know your precipitation

It takes about an hour to set up the analytic procedures, and you obtain the results right away.

"This is fast enough for use in everyday production," says Industrial Postdoc Christian G. Frankær, who divides his time between DTU Chemistry and Novozymes.

Novozymes prefers to keep the details confidential on which exact distribution of Savinase polymorphs that would be ideal. But in general terms it can be stated that certain structures are better suited for the production flow than others. First of all the different structures correspond to different precipitation behavior.

"Controlling precipitation in the various steps in the production is key for Novozymes in order to run an efficient production. As precipitation can be beneficial at certain steps in the production the contrary is also true. In other words you want to control your precipitation, and getting the right distribution of polymorphs is a key feature," explains Associate Professor Pernille Harris, DTU Chemistry. "The point is that we have been able to show that XRPD is a good practical tool in this context".

XRPD is a standard method used in many types of industry. An example is characterization of the distribution of sand, clay and pebbles in soil for the building industry. However, characterization of protein structures is complicated as proteins are large molecules. Over the years, the group at DTU Chemistry has developed a number of software and analytical tools which has improved the accuracy of XRPD for protein characterization purposes greatly.

A highly representative method

Results from XRPD studies of Savinase have recently been published in the journal Acta Crystallographpica, the International Union of Crystallography. Christian Frankær, Pernille Harris, and Associate Professor Kenny Ståhl, all DTU Chemistry, co-authored with several Novozymes researchers and members of a group at University of York, UK.

"The article is a scientific landmark. We hope that it will spur further interest from industry," Pernille Harris says. "What we have done on Savinase is equally relevant to all other proteins. Many other proteins are manufactured both by Danish and foreign companies, and this is generally believed to be a rising trend."

For some years the prime technique for establishing the polymorphs of produced proteins has been single-crystal macromolecular crystallography (MX).

"MX will give you much more detailed information about the structure compared to XRPD. But MX will cost you a lot more time, it may also involve sending your samples to synchrotrons, and last but not least the results are probably not representative," says Christian Frankær.

Relevant in optimizing production

The problem with MX is that it requires large crystals – as indicated by the phrase macro-molecular. It is by no means given, that when you pick the largest crystals in your samples, you will get a representative picture.

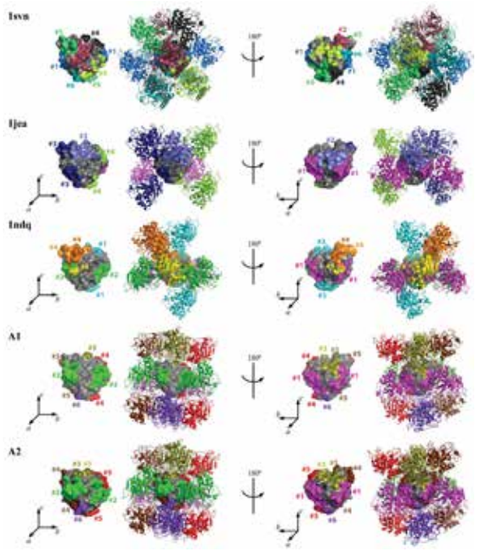
Pernille Harris points to the example of diamonds, which are a polymorph of carbon.

"Suppose you want to characterize the content of your coal mine, and you do that by looking at a few diamonds found in the mine. Similarly, you can get a false picture, if you try to fine-tune the production of an enzyme based on characterization of the largest crystals."

Pernille Harris and Christian G. Frankær admit that convincing industry about the attractive features of the method is not straight-forward.

"Industry is used to a development whereby science can help in looking at their products in ever finer detail. This is not the case here," says Pernille Harris, elaborating:

"On the contrary, MX and other techniques can give you much better detailing. The point is that XRPD is relevant not so much to the R&D people in industry, but rather to the process development and production optimization people. It will give you fast results, which are fully representative for your product. In other words, you get information, which you can actually use to optimize your processes. Once this is realized, we hope to see several new joint projects."



Crystal packing in five B. lentus subtilisin crystal forms.

Published article

The text is based on the scientific article published in the journal *Acta Crystallographica.*

You can download it as a pdf by scanning the QR-code below

Next step ...

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