

Improving the shelf life of therapeutic proteins

Researchers at the University of Oxford have developed a new method for identifying changes to protein structure during a freeze-drying process used by the biopharmaceutical industry to improve the shelf-life of therapeutic proteins. The novel procedure shows what happens to the protein at each stage of the process, making it easier for companies to formulate drugs that can survive freeze-drying intact.

The researchers, led by Professor Zhanfeng Cui¹ and funded² through BBSRC's Bioprocessing Research Industry Club (BRIC – see box), have already used the process to characterise proteins from two BRIC industry members, Novozymes³ and Lonza plc⁴, and three German companies. The skills and training gained through the project have also enabled two of the researchers involved to move into new jobs with industry.

Preserving proteins

The UK pharmaceutical industry, worth more than £13.2Bbn (US\$21Bn) in 2011⁵ and employing around 25,000 people in research and development⁶, is developing many new therapeutic proteins to treat a range of illnesses. One of the challenges faced by the industry is to stop the proteins from denaturing – that is, suffering changes to their structure – while they are being stored or transported. “When the structure changes, they often lose their function,” explains Cui.

Storing and transporting the proteins at low temper-

atures, for instance using liquid nitrogen, would help solve the problem because it limits degradation, but it is difficult and expensive. Instead, companies freeze-dry proteins to remove any water (a process known as lyophilisation) that can lead to structural damage.

But freeze-drying raises several other problems. In particular, proteins naturally exist surrounded by water; without it their three dimensional structure will collapse. Pharmaceutical companies add chemicals called excipients to prevent such collapse, but it can be difficult to identify the right excipients because researchers cannot see what is happening to the protein during the freeze-drying process. There is also a risk of damage to the protein structure at each stage of freeze-drying.

According to Cui, “So far, it’s like a black box. They add in different salts, sugars, polymers and biopolymers. For this project we wanted to open that black box,” he adds.

Cui’s aim was to develop a method to observe the behaviour and structure of protein molecules as they were freeze-dried. The researchers first looked at a technique commonly used in chemistry laboratories called Fourier transform infrared spectroscopy (FTIR). In FTIR, infrared light is shone through a sample, and the resulting spectrum provides information about the sample’s molecular structure. Uniquely, the researchers were able to adapt the technique to produce a two-dimensional map of the protein in solution, show-

What is BRIC?

BRIC was established in 2005 by BBSRC, EPSRC and industry to support and develop the UK bioprocessing research community, and enable knowledge transfer between the science and engineering base and industry. BRIC includes 15 industry members, as of June 2012.

The first phase of BRIC awarded £13.7M of funding to 25 research projects. The second phase, which began in 2010 and will run for five years, will award a total of £10M.

For more information about BRIC please see: <http://www.bbsrc.ac.uk/business/collaborative-research/industry-clubs/bric/bric-index.aspx>



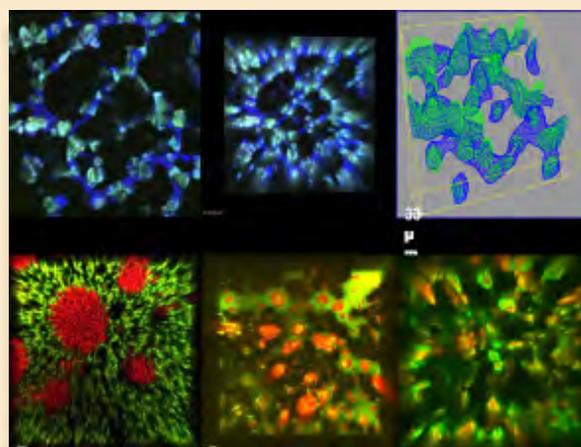
ing where and how much protein is denatured. “This is a useful tool, we can do it in liquid, partly-frozen, fully frozen, or dried states, so right through the process,” says Cui.

They then combined this with a modern three-dimensional microscopy method called multi-photon microscopy (MPM). During freezing, the protein molecules are pushed around as the ice forms; using MPM, the researchers could see how the protein and excipient molecules moved and interacted in three dimensions. They could also look at the structure of the dried powder at the end of the process, to see how it would respond when water was added to rehydrate the protein.

By using both imaging techniques, the researchers could build up a detailed picture of how process conditions and excipients affected protein structure during freeze-drying. “Through this process we don’t need the black box approach; we don’t need to do trial-and-error,” Cui says. “We can identify which excipients are better for a particular protein, and which is the key step for particular proteins as some are denatured more during the freezing step, and some during the drying,” he adds.

A service for industry

Cui’s new method has already been applied to industry products as part of the research project. Two BRIC members, Novozymes and Lonza, worked with Cui and had their own products characterised. In addition,



3D imaging of protein phase separation using multi-photon microscopy. *Credit: Professor Zhanfeng Cui/University of Oxford*

through Cui’s co-investigator Dr Heiko Schiffter, the team worked with three German companies who were not BRIC members. “They have their own therapeutic proteins, and we looked into their particular products, what is the best way to formulate their proteins. And this directly benefited the companies,” Cui explains.

According to Dr Phil Morton, Process Development Manager at Novozymes, “Through BRIC, industry has a unique opportunity to interact with academics and, via this collaboration with Oxford University and their novel

techniques, we gained an important insight into how our product behaved during lyophilisation.”

As well as providing a new method for the biopharmaceutical industry, the project also provided training for the researchers involved, two of whom have since moved into industry roles. Schiffter left the University of Oxford to work on drug formulation at chemicals company BASF, and post-doctoral researcher Dr Renchen Liu is now working with ISIS Innovation Ltd, Oxford’s technology transfer company, after he spent time working in pharmaceutical companies during the project.

The next stage for Cui is to commercialise the method and begin providing a service to a wider range of pharmaceutical companies. “We are looking to work with more pharmaceutical companies to meet their particular needs, because we feel this is protein-specific. We can’t have a rule of thumb for all proteins,” says Cui.

Notes and references

1. See: <http://www.eng.ox.ac.uk/chemeng/Cui/index.htm>
 2. See: <http://www.bbsrc.ac.uk/pa/grants/Award-Details.aspx?FundingReference=BB%2f-G010277%2f1>
 3. See: <http://www.novozymes.com/en/Pages/default.aspx>
 3. See: <http://www.lonza.com/>
 4. See: <http://www.abpi.org.uk/industry-info/knowledge-hub/global-industry/Pages/industry-market-.aspx>
 5. See: <http://www.abpi.org.uk/industry-info/knowledge-hub/uk-economy/Pages/uk-pharmaceutical-employment.aspx#3>
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