Text transcript to video:

Laborversuch: Lysozym-Kristallisation
(Lab experiment: Lysozyme crystallization)

Prof. Dr. Oliver Daumke // MDC Berlin-Buch
Dr. Katja Fälber
Dr. Tobias Bock-Birnbaum

Research: Protein crystallization

Dear students, my name is Oliver Daumke and I lead a research group for structural biology here at the Max Delbrück Center.

Hello I am Tobi and I have been with Oli in the research group for 5 years ...
... and I am Katja and - like Tobi - a postdoc in Oli's group.

In order to understand how our human nervous system fulfills its different tasks, we structural biologists study the proteins of the nerve cells.
And we look very closely, right down to the atomic level.

The structural elucidation of complex proteins can take months, if not years. Today, we are therefore looking at the structural elucidation of a model protein in structural biology: that of the lysozyme.
Lysozyme is a protein that is found in our eye fluid and protects our eyes from bacterial infections.
And now Katja and I will show you the different working methods that structural biologists should know.

Question / Research

First of all, we think about the question we want to answer. Since we are structural biologists, we are of course interested in the 3-dimensional structure of proteins!
But it is also important where small fuel molecules bind to our protein. How does the spatial structure of our protein change when it operates? For each of these questions we can do a structure determination.

But first, we investigate what other researchers have already found out about this. To do this, we search in scientific databases or ask colleagues who have already done research on the protein.
And when we know which protein we want to take a closer look at, it's off to the lab.

Production

Unfortunately, we cannot buy our protein, but have to produce it ourselves. Fortunately, from our research, we know the DNA sequence, which contains the blueprint of our target protein, and introduce it into bacteria. These are actually quite easy to handle, they only need 37 degrees, a nutrient medium of amino acids and then a bit of shaking.

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And that's what they do here in this incubator, here the bacteria are shaken at a comfortable 37 degrees. Then after one night we can harvest them.

To do that, the nutrient solution has to be separated from the bacteria, so that we can get our protein. For this we have a centrifuge that rotates very fast, 4500 revolutions per minute for 20 min. This is faster than a washing machine in spin cycle. The heavy bacteria settle to the bottom and the nutrient solution can then simply be poured off.

Purification

However, the bacteria now contain their own proteins and also our target protein from the DNA we had introduced there. To access the proteins, we first have to break the bacteria and destroy the membranes. Unfortunately, we then have a mixture of the bacteria's own proteins and our target protein. However that's not a problem, because we can separate the proteins from each other. We do this using a chromatography system. We inject the protein cocktail and then it is pumped over so-called columns, which work like tiny sieves.

At the end, the proteins come off the column again, but this time sorted by size and collected in an automatic sample collector. So, in each tube there are only proteins of one size, with the smallest proteins in the last tube.

The computer shows us which tube contains our target protein by recording the UV light.

Crystallization

Now, finally, we have it: our target protein in pure form. To determine its structure, we need to measure it. However, the signal of a single protein would be much too small to measure. To amplify the signal, we use a trick: we let it crystallize, because we know that in a crystal quite a few molecules of our protein come together in an ordered lattice.

Unfortunately, it is often not clear under which conditions a crystal is formed. Therefore, we simply create as many condition scenarios as possible and test when it works. The preparation of the many different solutions is done fully automatically with the crystallization robot. For that, we use plates here containing 96 holes. We set up 10 - 15 plates per protein and can thus produce over 1000 different conditions in about 3 hours.

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And now the exciting phase begins!
But until a crystal really grows, it can take quite some time. In rare cases, this can happen in a few hours, but usually it takes days to weeks, and in rare cases even years.
Because it is so difficult to monitor these many preparations by hand with a microscope, we have so-called crystallization hotels. Here, the preparations are stored at 4 or 20°C and automatically taken out of their "room" at certain intervals and photographed by a camera. Yes, and sometimes they lie in there for years, and we monitor with a computer whether something is happening or not.
Sounds exciting, and it is.
And it is unfortunately the case that only very few of these many attempts lead to crystals. Many plates do not contain crystals even after a long time. In this overview we can see the 96 photos of a plate at a certain time.
But we can also look at the photos of a certain approach over a certain time: after one day, after 2 days after 1 week, after 2 weeks ....and then it is a nice feeling and a real sense of achievement when we really discover crystals and can virtually watch them grow.

And now you are asked:

Find out under which conditions your model protein lysozyme forms the largest crystals. The beauty of it is that lysozyme crystallizes within a few minutes. That means you can be there and watch it live.
Have fun!

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Part 2

After our target protein was produced by bacteria, we purified it with chromatography. Then we tried to crystallize the target protein by putting it in many different types of solutions. And after weeks or months, we managed to get crystals to form.
And we want to have those, because in crystals the proteins are all in an ordered lattice and act as signal amplifiers, so to speak.

X-ray structure analysis

Our protein crystals are small, similar to the diameter of a human hair. Nevertheless, we can manage to fish them out of the solution, so to speak, under the microscope with a tiny nylon loop. We then freeze our crystals in liquid nitrogen, at 100 Kelvin, which is - 197°C. And we do this because we now want to examine the crystal with the X-ray generator and we do not want the high-energy X-rays to destroy our crystal in the process.
The X-ray radiation hits our crystal in a single beam and is redirected in the process. Every single atom of the crystal deflects the beam a little bit differently and so we get these beautiful diffraction images.
In order for us to get a full three-dimensional image, we need to rotate the crystal in the X-ray beam and take many, many of these images. This measurement can take between 5 minutes and 2 hours.
And from this pattern, we can then calculate a so-called electron density cloud with the help of various computer programs.
This is like a shell that surrounds our protein.

It is rather rare that you can measure the one set of data with the first crystal to determine the structure. Normally, we test hundreds of crystals and measure them before we get to a data set that we can use to solve the structure.

Modeling

It depends somewhat on how good our crystal and our measurement was, and ultimately how well defined this electron density cloud is, to be able to see well where which amino acids fit in. If that's clear, computers can help us model it.

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But if it's not so clear, then the programs quickly reach their limits and we have to sit at the computer and fit amino acid by amino acid into the cloud and work our way through the protein.

Now here we come back to the beginning of the project, where we have inserted the DNA, which contains the blueprint for our protein. And this blueprint, the sequence of amino acids, we now see again here in the structure and in the electron density.

And we can use this information for modeling, because we know exactly in which sequence the amino acids occur one after the other. So, we can go through the sequence and pick out characteristic places in the electron density cloud where we can see exactly which amino acids need to be incorporated. Based on the amino acid sequence, we can finish building the model starting from this point.

And that is exactly what you will do now:
We have lost individual side chains in the lysozyme structure. And with the help of the sequence, you will find out which ones they are, reintegrate them and see how they fit into the electron density cloud.

Today you got the first insights into the work of structural biologists and carried out your own first steps to determine a protein structure.
We use these molecular structures to understand how molecular machines work. We also use them to understand the cause of genetic diseases and possibly develop drugs.

We hope that we were able to inspire you with our work today and look forward to seeing you here at MDC one day.

All the best, bye.

Project website: **bcp.fu-berlin.de/nos**