

EMBL Podcast December 2010: Cryo-EM at EMBL

(lab sounds, fade into background when narration starts)

Sarah Stanley: The woods surrounding EMBL's main lab in Heidelberg, Germany, can get cold in the winter. But it's even colder inside some of the lab's most advanced microscopes.

(crescendo in lab sounds, then background again when narration starts)

Sarah: These are the sounds of the Polara, a powerful electron microscope. Unlike the commonly-used optical microscope, which uses a beam of light to magnify objects as small as bacteria, an electron microscope uses a beam of electrons, and can be powerful enough to detect individual atoms. John Briggs, a group leader at EMBL Heidelberg described the Polara to me:

John Briggs: At the top of the microscope you have an electron gun or a source of electrons, which is like the lamp in a light microscope. (Fade lab sounds to silent.) And you have a series of lenses, and at a certain point you put in your sample. And so in an electron microscope, or at least in a transmission electron microscope, which is the sort we use, the beam of electrons passes through the sample, through other lenses, and generates an image, which, these days, is most often collected on a CCD camera.

(lab sounds continue)

Sarah: Today, Alex De Marco, a PhD student in John's lab, is using the Polara to build a model of the structure of a protein complex. (meanwhile, fade lab sounds to silent) The Briggs group studies proteins associated with coated vesicles and membrane viruses. Coated vesicles are compartments used for things like storage and transportation in a cell. They're each surrounded by a membrane, a trait they share with membrane-bound viruses like influenza, ebola, and HIV. These viruses infect a cell, replicate inside it, and then use part of the cell's membrane to build themselves an ejection capsule: a bud that breaks off from the cell, taking the rest of the virus with it.

John: From a methodological point of view these are quite similar kinds of things; about the same size. And they both go through budding reactions so they both assemble proteins in order to manipulate a membrane to generate either a vesicle or a membrane-enveloped virus.

Sarah: John's group studies membrane manipulation using a variety of tools; things like fluorescence microscopy, which allows them to see if a protein is present, and where it is. But the lab's central focus is on the physical structures involved in membrane dynamics. And for that, the scientists often use cryo-electron microscopy, or cryo-EM.

Sarah: What sets cryo-EM apart from other types of electron microscopy is the way in which specimens are prepared. Instead of being hardened and sliced into sections, for instance, or stained with heavy metals, they're kept in or near biological conditions, and, as the name 'cryo' indicates, they're frozen.

(lab sounds)

John: We have the sample that we're interested in, so this might be a virus, in solution in a little tube. And we take some of the solution of virus and put it on a copper grid; a very small copper grid about two millimeters across. And on that grid there's a very thin layer of carbon film, which has holes in it. So you put a little droplet of your solution onto the grid.

You remove most of it with a bit of blotting paper, which just absorbs the moisture, and you're left with very thin layers of the solution stretched over the holes in the carbon film. Once you reach that stage you drop the grid into a little tub containing liquid ethane, and it cools extremely rapidly, and you end up with your sample in ice.

Sarah: But not normal ice, like you would find in a glass of cold water.

John: You have a kind of vitreous ice, so more like glass. And then you can put the sample in the electron microscope, and you pass your beam of electrons through the sample, and you end up on the other side with a projection image of whatever it is that you have in your solution. And if you collect enough projection images then we can use image-processing techniques on the computers to generate three-dimensional reconstructions of whatever it is we've imaged.

Sarah: Cryo-EM sample preparation was actually invented at EMBL by scientists Jaques Dubochet and Alasdair McDowall. They published their technique in a brief paper in 1981, which states, "We have devised a method for preparing vitrified ice or any frozen aqueous solution for direct observation in the electron microscope." Jaque and Alasdair's method is the foundation upon which today's cryo-EM procedures are still based. And cryo-EM at EMBL is about to enter a new phase with the recent arrival of a Titan Krios electron microscope.

Sarah: Carsten Sachse, also a group leader at EMBL in Heidelberg will be using the Titan Krios to continue his investigation into how cells deal with potentially harmful accumulation of large proteins.

Carsten Sachse: So the Titan has been used in the materials sciences, and now it has been adapted for cryo, so to hold biological samples, basically. And in terms of optics, it is quite a superior instrument. It produces very high resolution images, it has very good optics, and this will be quite beneficial, and there are quite a series of reports now that also it has been shown to be quite a boost for biology in terms of resolution.

Sarah: The new microscope will also improve sample stability, reducing tiny vibrations that can have a big impact on image quality. And it will allow for more efficient data collection.

John: Rather than what we currently do, which is to sit at the microscope and collect our data, one will actually sit most of the time in a separate room and control the microscope remotely. One can load multiple samples simultaneously into the microscope, and then over a period of days move through those samples and collect data on a lot of them, and a lot of that data collection should be automatable in a way which isn't particularly easy with current microscopes.

Sarah: So the Titan Krios in Heidelberg offers state-of-the-art technology and will be used by EMBL researchers and their collaborators. Christiane Schaffitzel, a group leader at EMBL Grenoble, is also a cryo-EM user. She studies the structures of biological molecules involved in targeting new proteins to different locations in a cell. I spoke with her on the phone about how cryo-EM fits in with other lab techniques, including biochemical methods and crystallography, rather like a piece in a puzzle.

Christiane Schaffitzel: Yes, it is like a puzzle. You take your electron microscopy data, you take your biochemical evidence, crosslinks, mutations, interactions, bits and pieces, and then, of course all the bits and pieces that have been crystallized, and put it together to a structure that makes sense.

(fade in lab sounds, fade into background when narration starts)

Sarah: This approach has led to a variety of findings by scientists at EMBL and beyond. It helped Christiane create a structural model of a translating ribosome, and John Briggs has used cryo-EM to investigate the lattice arrangement of a protein in the immature HIV virus.

Sarah: So next time you find yourself scraping ice off your car, or swirling the ice-cubes in your glass, remember that something as seemingly mundane as freezing water can help scientists to understand life at a microscopic level.

(crescendo lab sounds, play for a few seconds, then fade out)

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