

CRYO-ELECTRON MICROSCOPY: A CLOSER LOOK AT CYTOSKELETAL INTERACTIONS

Interview with Professor Eva Nogales

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Dr. Eva Nogales is a Howard Hughes Medical Institute Investigator and Professor of Biochemistry, Biophysics, and Structural Biology at the University of California, Berkeley. Professor Nogales's research centers on using cryo-electron microscopy (cryo-EM) to investigate the mechanisms behind gene expression regulation and cytoskeletal dynamics in cell division. In this interview, we discuss the insights gleaned through the application of cryo-EM to study drug-stabilized microtubules.



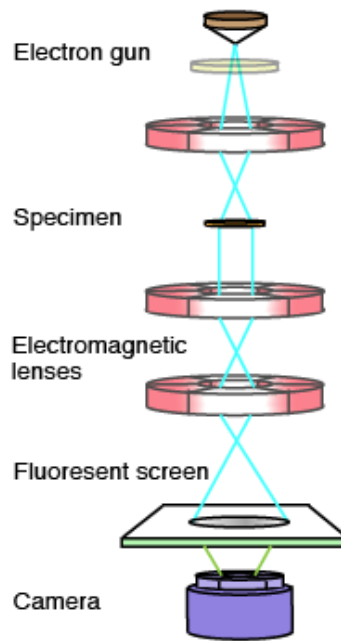
Professor Eva Nogales

BSJ: You originally studied solid-state physics. How did you get involved in the field of structural biology and cryo-EM?

EN: It was purely by chance. While I was an undergraduate in solid-state physics in Spain, I considered using synchrotron radiation techniques in the context of surface science. I met with the director of the British Synchrotron Radiation Source—he was a physicist and biologist, and a very charismatic individual, so I decided to join his research. The switch was just like that. It was very serendipitous, because the 21st century is the century of molecular biology, and I'm so glad to be in it. Once I made it to the synchrotron, I was using a technique called small-angle X-ray scattering to study tubulin self-assembly.

The technique was not that informative, so I started using cryo-EM to complement my studies with X-rays. It became obvious to me that cryo-EM is a very powerful technique. I came to Berkeley looking for postdoc positions and was referred to Kenneth Downing at Lawrence Berkeley National Laboratory. It was perfect—he was also a physicist, and because I had previous expertise with tubulin and he was very good at electron microscopy, we teamed up. But at the very beginning, it was purely accidental—moving from physics into biology and then getting exposed to cryo-EM at a time when the technique was just emerging.

Figure 1: Cryo-electron microscopy (cryo-EM) is a form of transmission electron microscopy (TEM) that allows the detailed observation of biological specimens at cryogenic temperatures, ranging from -180° to -269° C. In TEM, a thin layer of sample is hit with a high-energy beam of electrons. The electrons scatter onto a series of electromagnetic lenses, which produce a magnified 2D image of the specimen. The short wavelength of the electrons allows for sub-nanometer resolution in these images. [<http://cryoem.berkeley.edu/cryoem>]



BSJ: What are some advantages of cryo-EM over other conventional imaging technologies such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy?

EN: In cryo-EM, you don't need to crystallize your sample and you don't need a lot of it. You can also cope with conformational flexibility—in other words, you have a series of proteins that interact with one another in equilibrium, where they come together and go apart. This makes cryo-EM very generally applicable. That's the big benefit. In crystallography you need to be able to crystallize your sample, and in NMR you have to treat it with a special isotope. For determining protein structure *ab initio*, where you don't know what you're starting with, you're very limited in sample size for both techniques. Even for the average protein, the sample may be too small, while with cryo-EM you can study not just one protein, but an entire protein complex. Its advantage is its applicability, because it doesn't have all the requirements that other techniques have.

BSJ: A major factor in achieving near-atomic resolution was the advent of direct electron detectors in cryo-EM imaging. What is the advantage of direct electron detectors over charge-coupled detectors (CCDs)?

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EN: First, let me state something very important. Biological materials are radiation-sensitive—we cannot pass a lot of electrons through them because the sample will get damaged. But when we take images with very few electrons, it's like taking a photograph with very little light. As a result, the image looks blurry. In a CCD, the electrons enter a scintillator, where they are converted to light, which is then converted back to an electron signal to produce a digital image. In the process of an electron scattering in the scintillator, it goes from what used to be a dot to a cloud that hits the detector. As a result, the images are very noisy. Some engineering was necessary to remove the scintillator and make these detectors resistant to high-energy electron damage. Once this solution was obtained, the detector basically became noise-free. You end up with an image that has much better contrast and more signal per image than anything we were getting before. A second gain is that the read-out of these detectors is very fast. Instead of producing a single exposure, we are able to obtain a little movie in the same amount of time. We then add all the frames into a single image. With this, we are able to correct for beam-induced motion, which is caused by the water in the sample buckling upon being hit by electrons. We can do this trick because the images have more contrast and are collected faster. We went from having images where maybe five percent had high resolution and those images still had poor contrast, to where 100% of images have good contrast and high resolution. It's completely like night and day.

BSJ: Image analysis methodologies also play a key role in improving the resolution of cryo-EM images. Can you explain the difference between geometry-based approaches, cross-correlation, and maximum-likelihood (ML) methods?

EN: When you take an image in a transmission electron microscope, your image is a projection—the addition of densities along the direction of the electron beam. Image reconstruction involves using the projection to get a full 3D view of the object. When you take an image of a molecule, it is randomly oriented in solution. If you take those projections and identify their relative angles, you can combine them and achieve a 3D image of the object. Geometric principles are the simplest way to do this. You put your sample in the electron microscope, take an image, and then rotate it. You know exactly how the two images are related, because you did the rotation. Now imagine that you've used geometric principles to achieve the structure of a molecule, and you want to study that molecule bound to a drug. You can use your starting molecule as a reference, and computationally generate all possible projections from all angles. Then you compare your experimental images to the computer-generated ones with a mathematical procedure called cross-correlation. At the end of the day, you get the relative orientations of all your images with respect to a common reference. Why doesn't this work very well? Sometimes it's very hard to tell which image corresponds to which view. So here come ML methods. In ML, you don't make any strong deterministic assumption to start. Instead of saying "this image corresponds to that view," you just say, "there is a 50% chance that it corresponds to this view, but there is a 25% percent chance that it corresponds to that view, a 10% chance that it corresponds to that view, and one percent chance to each of these 15 other views." You calculate your reconstruction using

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weights, so that you don't commit the image to one view or the other. It's a soft assumption that plays with probabilities. It takes more computer time, but in the end, it converges better to the right solution. Additionally, ML methods not only allow you to identify the different orientations, but also the different conformations of a molecule. This is incredibly powerful, because we cannot assume that a protein is in just one state. Many protein complexes actually work by having parts that move. ML methods are much better at identifying the presence of these states in a sample and describing them in parallel to each other.

BSJ: What are some challenges you continue to face with cryo-EM?

EN: The biggest challenge is sample preparation. We have a protein, an organic molecule that is made of carbon, nitrogen and oxygen. It is surrounded by water, which also has oxygen. The contrast in the image is given by different scattering of electrons, and if the background is as dense as your molecule, you don't see anything. You can minimize the amount of water in the sample, but you have an air-water interface, which is a large hydrophobic surface. If a protein has a very hydrophobic section, it is perfectly happy to unfold and interact with the air-water interface. Right now, the process of thinning the sample and keeping it happy is hit-or-miss. We use tricks, cross-linkers that keep the protein from unraveling. But sample preparation is our bottleneck. That, along with access to electron microscopes. I wish we had an electron microscope that anybody could use at any time. But we have to share it, for many projects and many labs.

BSJ: We read about some of your work imaging microtubules.¹ What is the seam and what role does it play in the structure of the microtubule?

EN: Tubulin has this amazing property of self-association, meaning that it forms a polymer by itself. Tubulin dimers interact head-to-tail to form linear protofilaments, which then associate laterally to close into a tube. Most of the contacts are beta-to-beta and alpha-to-alpha, except at one point where beta interacts with alpha and alpha interacts with beta. This is the seam. What function does it have? We actually don't know, but we think it's involved in the process of microtubule closure, a zipping-up process in which many contacts are made simultaneously. It could also be a weak point where contacts are not very optimal, and that's what allows the microtubule to break down in dynamic instability.

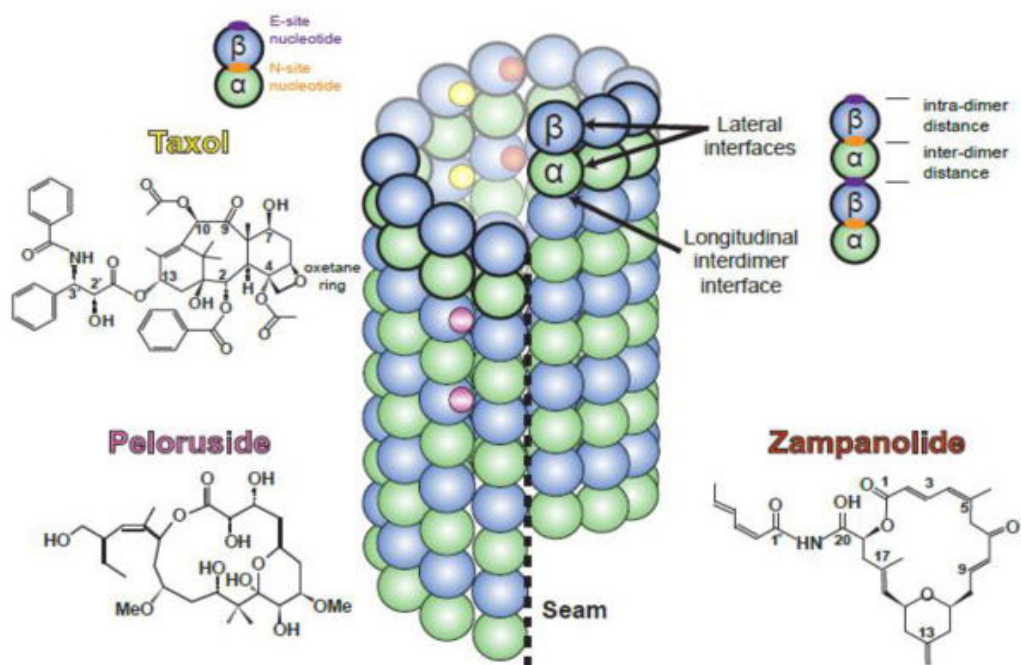


Figure 2: Schematic of a microtubule featuring the seam and the three binding sites for antimitotic drugs Taxol, peloruside, and zampanolide.²

BSJ: What is the property of dynamic instability in microtubules? What are microtubule-stabilizing agents and how do they relate to this phenomenon?

EN: Microtubules are not rigid. If you fluorescently label a microtubule and see how it behaves in real time, you see that it grows for a while and then disassembles—this phenomenon is called dynamic instability. It is very weird behavior for a polymer. In the process of cell division, microtubules grab onto chromosomes and pull and push on them until they are aligned. Then the two chromatids split apart and the microtubules pull them to each side of the cell. In order for microtubules to go through all of this growing and shrinking, they need to be dynamic. If that is stopped—if you give the cell a drug that makes the microtubule very stable, for example—it gets stuck in cell division and commits apoptosis, or cell death. That’s how these antimitotic drugs work in cancer cells.

BSJ: You have investigated a variety of antimitotic cancer drugs, including Taxol, peloruside, and zampanolide.² What motivated your selection of these drugs to investigate?

EN: Taxol is the most famous and more broadly-used of the antimitotic drugs. In fact, it’s used in the treatment of almost every single solid tumor. Therapeutically, it is the most important antimitotic

drug. Zampanolide is interesting because it binds to the same pocket as Taxol, but it binds covalently. We wanted to see whether these two drugs, which bind to the same place, have the same effect on the microtubule. Peloruside binds in a completely different place, but it also stabilizes the microtubule. The three drugs were chosen because we wanted to know whether they stabilize the microtubule in a similar or different way.

BSJ: How do Taxol-site binders such as Taxol and zampanolide affect microtubule structure? How does this compare with the effect of non-Taxol-site binders, such as peloruside?

EN: What we see with Taxol and zampanolide is that they cause the microtubule lattice to be flexible, so that there is variability in the lateral contacts between protofilaments. The microtubule is wiggling. It’s exactly the opposite for peloruside. Peloruside binds, bridging two protofilaments, and it makes the microtubule structure very ordered, so the opposite of wiggling—very well-defined lateral contacts between all the protofilaments.

BSJ: What is a doubly bound microtubule? What did you find in your investigation of the effect of a peloruside-Taxol doubly bound structure on microtubule structure?

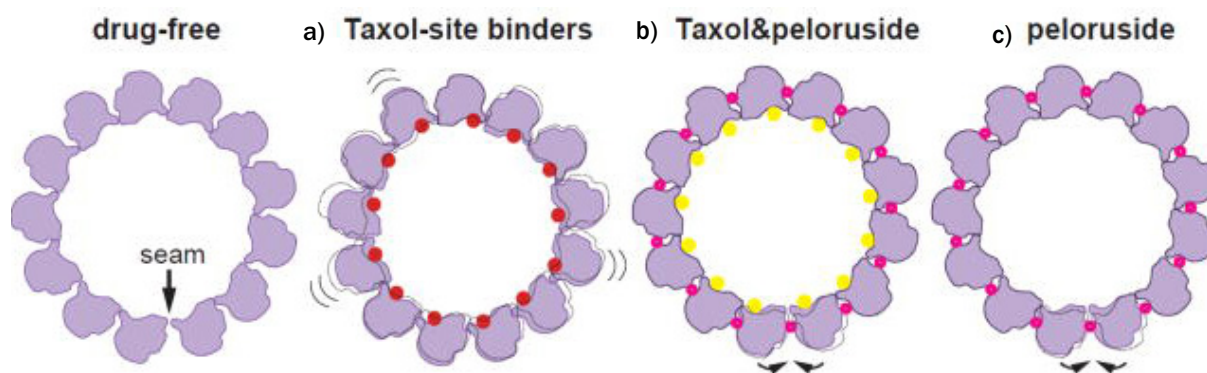


Figure 3. (a) Taxol-site binders Taxol and zampanolide causes the microtubule lattice to be flexible. (b) In a doubly-bound microtubule, peloruside overrides the effects of Taxol, making the microtubule lattice more ordered. (c) Peloruside promotes order in the microtubule lattice.²

EN: Since peloruside binds on one site, and Taxol binds on the other, we can add both agents to the microtubule because they don't compete with each other. This is interesting because you can conceive of the possibility of treating cancer by using a combination of both drugs. Because they have such different effects on the microtubule lattice, we decided to bind both of them and see their effects on microtubule structure. What we find is when both peloruside and Taxol are bound, the lattice becomes very well-ordered, so the effect of peloruside on lattice contacts wins over that of Taxol. We think that Taxol has more of an effect on longitudinal interactions (interactions between subunits along the length of the protofilament), while peloruside favors contacts between protofilaments. Both stabilization effects are additive, and, in terms of lattice order, peloruside makes even Taxol-bound microtubules well-ordered.

BSJ: What are some future directions of your research?

EN: Concerning microtubules, we are very interested in studying how different cellular factors affect microtubule behavior, especially dynamic instability. Many different factors can interact with microtubules at different states during the cell cycle or in different tissues. For example, we are now studying one microtubule-associated protein called tau that binds to axons. We are interested in investigating how tau stabilizes those microtubules and favors axonal regular function. We are also very interested in how microtubules interact with chromosomes via kinetochore complexes that can link together the microtubule and special nucleosomes in chromatin. These are both areas of research that are of great interest to us—cellular control of microtubule dynamics and the engagement of microtubules with chromosomes for the segregation of genetic material during cell division.

BSJ: Thank you very much for your time!

REFERENCES

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