Dear microtubule, I see you

Eva Nogales*
Molecular and Cell Biology Department and Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA 94720; Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

ABSTRACT This essay summarizes my personal journey toward the atomic visualization of microtubules and a mechanistic understanding of how these amazing polymers work. During this journey, I have been witness and partaker in the blooming of a technique I love—cryo-electron microscopy.

FROM PHYSICS TO BIOLOGY AND FROM X-RAYS TO ELECTRONS

I was trained in physics (solid state) in Madrid after I decided that medicine was not for me. I had amazing science and math teachers in high school, all very powerful and inspiring women! When I was getting ready to graduate from college and thinking about my PhD, the hot topic in Spain was synchrotron radiation. With third-generation synchrotron sources sprouting all over the globe, it was the time of x-rays! The European Synchrotron Radiation Source was being built in Grenoble, and the Spanish government wanted to train Spanish scientists in this area, so it was relatively easy to get a fellowship to carry out my thesis work at the British synchrotron. Quite by accident, I ended up deciding to study biological macromolecules and tried a number of interesting self-assembly systems (the acrosomal bundle of the horseshoe crab sperm; clathrin cages and coated vesicles) before settling on tubulin.

Eva Nogales

My studies used time-resolved small-angle x-ray scattering (SAXS) to follow the assembly of tubulin in the presence of vinblastine, an antimitotic, anticancer agent, and the effect that temperature had on the aberrant, spiral-like polymers that vinblastine induced (remember that, in the absence of drugs, the in vitro assembly of tubulin into microtubules can be controlled by temperature). At the time, computational analysis of SAXS scattering curves was limited, and part of our interpretation of the temperature changes needed help from a more direct visualization of the structures. This was why I first used cryo-electron microscopy (cryo-EM), helped by Dick Wade, who was visiting Daresbury to work with the computational group on his model of microtubule lattice accommodation (of all things!) (Wade *et al.*, 1990). At the time (ca. 1990), cryo-EM was in its infancy. A practical method for vitrification of a protein solution had just been developed a few years before by Jacques Dubochet (Lepault *et al.*, 1983). And 1990 was the year that Richard Henderson published the atomic model of bacteriorhodopsin using electron crystallography (Henderson *et al.*, 1990). But the idea that atomic structures would one day be obtained by cryo-EM analysis of frozen-hydrated samples in solution (i.e., single-particle studies) was then remote.

FROM AN ABERRANT POLYMER TO THE STRUCTURE OF TUBULIN

The biggest stroke of luck in my scientific career was to meet Ken Downing at the Lawrence Berkeley National Laboratory and to join his lab for my postdoctoral studies. Ken had been a player in the electron crystallographic studies of bacteriorhodopsin and was starting to use this methodology to study yet another aberrant polymer of tubulin. In the presence of zinc, tubulin assembles into straight protofilaments resembling those in microtubules. But while the natural polymer is formed by the parallel association of ~13 protofilaments

DOI:10.1091/mbc.E16-06-0372
Eva Nogales is the 2016 Porter Lecturer for the American Society for Cell Biology.
*Address correspondence to: Eva Nogales (enogales@lbl.gov).
Abbreviations used: cryo-EM, cryo-electron microscopy; SAXS, small-angle x-ray scattering; MAP, microtubule-associated protein.

to form a hollow tube, zinc makes protofilaments associate in an antiparallel way to form sheets. For all extents and purposes, these sheets can be considered small two-dimensional crystals, and therefore perfect samples for electron crystallography. Having worked with Taxol as a microtubule stabilizer during my PhD, I added this drug to the zinc-induced sheets and found that it also had a stabilizing effect. After about four years of data collection and analysis, my postdoc colleague Sharon Wolff and I obtained the first structure of tubulin in an assembled form and bound to one of the most broadly used anticancer agents (Nogales *et al.*, 1998b).

The structure showed that tubulin is not a classical GTPase (Nogales *et al.*, 1998a), provided the Taxol-binding site, explained the different nucleotide exchange properties of unassembled αβ-tubulin dimers and microtubules, described the longitudinal interaction between tubulin subunits along a protofilament, and explained the coupling of assembly and GTP hydrolysis central to the dynamic behavior of tubulin (discussed later) (Nogales *et al.*, 1998b; Lowe *et al.*, 2001). However, it told us nothing about how protofilaments came together to form the cylindrical microtubule. For that, we needed the structure of the bona fide microtubule.

At the time, the groups of Linda Amos, Ron Milligan, and Dick Wade were making significant progress in the cryo-EM study of different kinesins bound to microtubules (Arnal *et al.*, 1996; Hirose *et al.*, 1996; Sosa *et al.*, 1997). (The x-ray structure of the kinesin motor domain was then fresh from the oven [Kull *et al.*, 1996].) The resolution of those cryo-EM structures was typically 25 Å, but the use of hybrid methods to place crystal structures of components into the EM map was very powerful to define interfaces and interpret relative motions. We teamed up with Milligan and used his 20 Å map of the microtubule (Figure 1A) to "dock" the electron crystallographic structure of the protofilament into it. As a result, we were able to produce a model of the microtubule that positioned the different structural elements in the tubulin molecule with respect to the outside and the lumen of the microtubule, and we identified potential structural elements involved in lateral interfaces (Nogales *et al.*, 1999).

This work landed me a job as assistant professor at University of California, Berkeley (1998), so I did not have to move very far! As an independent investigator, I continued to use EM. By then, single-particle cryo-EM, pioneered by Joachim Frank, was gaining momentum, and I started using it to study GDP-bound tubulin rings, an assembly form that mimics the rams' horns of bent protofilaments seen at the end of depolymerizing microtubules. This methodology also allowed me to start the second line of research in my lab, the study of the eukaryotic transcription initiation machinery. But that is another story.

FROM BLOBS TO ATOMS IN THE VISUALIZATION OF MICROTUBULES

The regime of 20–30 Å resolution in cryo-EM is familiarly known as "blobology." Improving the resolution of cryo-EM maps of microtubules beyond blobs required the use of high-end instruments and the merging of large data sets. A major breakthrough came with Ken Downing and Huilin Li's visualization of the structure at ~10 Å resolution in 2002 (Li *et al.*, 2002). Ten years later, many cryo-EM studies of microtubules, alone or decorated with a number of motors or other associated factors, had provided a richness of biological information, but none of these microtubule structures had broken the 8 Å resolution barrier (Sindelar and Downing, 2010; Sui and Downing, 2010; Alushin *et al.*, 2012; Maurer *et al.*, 2012; Redwine *et al.*, 2012).

It was at this point that two brave souls in my lab, postdoc Gabe Lander and graduate student Greg Alushin, decided to go all out in a joint effort to break this resolution curse. They developed and implemented a reconstruction scheme that used helical/single-particle hybrid reconstruction methodology developed by Ed Egelman and that took advantage of the pseudohelical symmetry of the microtubule, while still accounting for the presence of the so-called seam (discussed later). As a result, we produced better than 5 Å resolution structures, a new record at the time (Alushin *et al.*, 2014). These studies used data collected on film, the best detection media for high-resolution cryo-EM studies available to us at the time. And then the revolution happened.

In cryo-EM images, resolution is limited by poor signal due to the intrinsic low contrast of proteins on a water background and the need to use low doses to minimize radiation damage. Historically, the problem was made significantly worse by charge-coupled device (CCD) detectors, which introduced noise and lowered the signal across the whole resolution spectrum. New direct electron-detection technology has recently dramatically improved the contrast

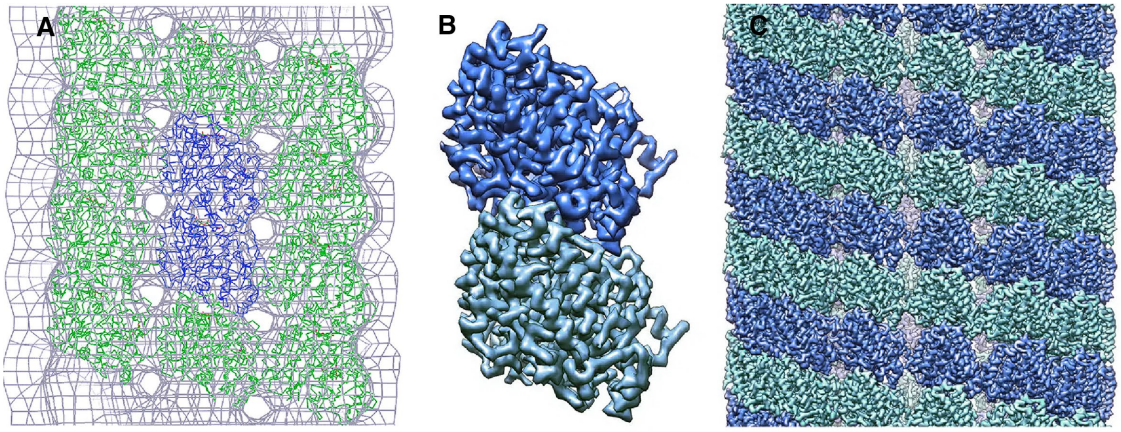

FIGURE 1: Microtubule and tubulin structures then and now. (A) A 20 Å cryo-EM density map (gray mesh) shown for three protofilaments, with the alpha trace of the docked electron crystallographic structure of the protofilament shown in green, except for one tubulin dimer highlighted in blue (modified from Nogales *et al.*, 1999). (B) A 3.5 Å cryo-EM map of one αβ-tubulin dimer segmented from the map of full microtubules shown in C. α- and β-tubulin are shown in lighter and darker blue, respectively (modified from Zhang *et al.*, 2015).

in cryo-EM data, resulting in an ever increasing number of atomic structures for molecules or assemblies that were considered structurally unreachable or highly challenging.

In our most recent studies, postdoc Rui Zhang has used a direct electron detector and improved data-processing strategies to obtain cryo-EM reconstructions of microtubules at 3.5 Å or better resolution (Figure 1B; Zhang *et al.*, 2015). This journey has taken us from blobs to atomic models for the full microtubule. Among other things, we can now see, for the first time, the details of the lateral contacts between protofilaments, the stitches that hold together the microtubule lattice (Figure 1C).

FROM THE BASIS OF DYNAMIC INSTABILITY TO UNDERSTANDING ITS REGULATION

Essential to most microtubule functions is the phenomenon of dynamic instability, a property first described by Mitchison and Kirschner in 1984. Microtubules switch stochastically between phases of slow growth and rapid shrinkage, a metastable behavior powered by the energy of GTP hydrolysis. A mechanistic understanding of this process requires a detailed description of the structural changes that accompany GTP hydrolysis in the microtubule. The present cryo-EM methodology has allowed us to obtain atomic models for microtubules bound to GMPCPP (a slowly hydrolyzable GTP analogue), to GDP (after GTP hydrolysis has taken place), and to GTPγS. Comparison of these states shows that hydrolysis results in a conformational change in α-tubulin and a compression of the dimer–dimer longitudinal interface along protofilaments that generates tension in the lattice (Zhang *et al.*, 2015). Interestingly, hydrolysis has a negligible effect on lateral interfaces, with the notable exception of the "seam." The seam is the special lateral contact between protofilaments in the microtubule that involves heterotypic contacts (α–β and β–α instead of α–α and β–β) and is thought to be involved in microtubule closure. By taking advantage of the higher contrast afforded by the direct electron detector, we have determined that the position of the two protofilaments involved in seam contacts deviates from the cylindrical shape of the rest of the tube (Zhang *et al.*, 2015) and that this deviation is larger after GTP hydrolysis.

Cryo-EM is also now providing us with the atomic details of the interactions microtubules establish with their associated cellular factors. Microtubule-associated proteins (MAPs) can regulate microtubule dynamics and organization and thus are critical to their essential cellular functions. We have visualized in atomic detail the interaction with microtubules of the +TIP protein EB3. EB proteins track growing microtubule ends by recognizing a particular microtubule structure and also directly regulate microtubule dynamics. Our structures provide a mechanistic understanding of how EB3 recognizes an intermediate state following hydrolysis, and how EB itself promotes GTP hydrolysis within the microtubule (Zhang *et al.*, 2015). With the myriad of MAPs that interact with and regulate microtubule function, our bucket list of studies is a long one.

THE JOURNEY SO FAR AND THE JOURNEY AHEAD

It is a rare thing in science to witness how your field of research undergoes a revolution of mind-blowing proportions. This is where I feel I am in my scientific career. I have been using cryo-EM since my PhD, and I have seen it grow and evolve at a consistent pace for more than two decades while I pursued the structural characterization of the microtubule. Cryo-EM practitioners steadily pushed the limits of resolution and applicability and along the way generated beautiful structures, landmarks of technical achievement, and new biological insights. But while all of us in this field knew that our technique was only going to get better, the explosion of results we have witnessed in the last few years and, even more, the potential that now has become all too apparent, has caught most of us by surprise. Our heads are spinning and the possibilities seem almost limitless. Keith Porter, an accomplished and pioneering electron microscopist, would have uniquely appreciated the exceptional moment we are experiencing in the visualization of macromolecular structures. He would be particularly excited about the potential to bring molecular resolution to the realm of cell biology, which we are expecting will be the next landmark of cryo-EM. It is a wondrous and exciting time, and there is little doubt in my mind that the cryo-EM revolution will lead to a bright new vision of how the cellular machinery works. You will SEE.

ACKNOWLEDGMENTS

Work on microtubules in the Nogales lab has been funded over the years by the National Institute of General Medical Sciences (GM051487). E.N. is a Howard Hughes Medical Institute Investigator.

REFERENCES

Alushin GM, Lander GC, Kellogg EH, Zhang R, Baker D, Nogales E (2014). High-resolution microtubule structures reveal the structural transitions in αβ-tubulin upon GTP hydrolysis. Cell 157, 1117–1129.

Alushin GM, Musinipally V, Matson D, Tooley J, Stukenberg PT, Nogales E (2012). Multimodal microtubule binding by the Ndc80 kinetochore complex. Nat Struct Mol Biol 19, 1161–1167.

Arnal I, Metoz F, DeBonis S, Wade RH (1996). Three-dimensional structure of functional motor proteins on microtubules. Curr Biol 6, 1265–1270.

Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckman E, Downing KH (1990). Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J Mol Biol 213, 899–929.

Hirose K, Lockhart A, Cross RA, Amos LA (1996). Three-dimensional cryoelectron microscopy of dimeric kinesin and ncd motor domains on microtubules. Proc Natl Acad Sci USA 93, 9539–9544.

Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD (1996). Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. Nature 380, 550–554.

Lepault J, Booy FP, Dubochet J (1983). Electron microscopy of frozen biological suspensions. J Microsc 129, 89–102.

Li H, DeRosier DJ, Nicholson WV, Nogales E, Downing KH (2002). Microtubule structure at 8 Å resolution. Structure 10, 1317–1328.

Lowe J, Li H, Downing KH, Nogales E (2001). Refined structure of αβ-tubulin at 3.5 Å resolution. J Mol Biol 313, 1045–1057.

Maurer SP, Fourniol FJ, Bohner G, Moores CA, Surrey T (2012). EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. Cell 149, 371–382.

Mitchison T, Kirschner M (1984). Dynamic instability of microtubule growth. Nature 312, 237–242.

Nogales E, Downing KH, Amos LA, Löwe J (1998a). Tubulin and FtsZ form a distinct family of GTPases. Nature Struc Biol 5, 451–458.

Nogales E, Whittaker M, Milligan RA, Downing KH (1999). High resolution structure of the microtubule. Cell 96, 79–88.

Nogales E, Wolf SG, Downing KH (1998b). Structure of the αβ tubulin dimer by electron crystallography. Nature 391, 199–203.

Redwine WB, Hernandez-Lopez R, Zou S, Huang J, Reck-Peterson SL, Leschziner AE (2012). Structural basis for microtubule binding and release by dynein. Science 337, 1532–1536.

Sindelar CV, Downing KH (2010). An atomic-level mechanism for activation of the kinesin molecular motors. Proc Natl Acad Sci USA 107, 4111–4116.

Sosa H, Dias DP, Hoenger A, Whittaker M, Wilson-Kubalek E, Sablin E, Fletterick RJ, Vale RD, Milligan RA (1997). A model for the microtubule-Ncd motor protein complex obtained by cryo-electron microscopy and image analysis. Cell 90, 217–224.

Sui H, Downing KH (2010). Structural basis of interprotofilament interaction and lateral deformation of microtubules. Structure 18, 1022–1031.

Wade RH, Chrétien D, Job D (1990). Characterization of microtubule protofilament numbers. How does the surface lattice accommodate? J Mol Biol 212, 775–786.

Zhang R, Alushin GM, Brown A, Nogales E (2015). Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins. Cell 162, 849–859.