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The tubulin structure, a quarter of a century later

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ABSTRACT **This retrospective on the 25th anniversary of the publication of the first structure of tubulin is shaped by my own personal experiences rather than being a strict and complete historical account of the event. It is a reflection on how working in science felt many years ago, on the struggles and the joys of reaching for the high-hanging fruit, and, ultimately, on how relevant or not our personal scientific contributions are to the broader scientific community. Writing it brought back memories of my unique and sadly lost postdoctoral advisor Ken Downing, who dreamt of this structure and brought it to fruition against all odds.**

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The "almost" random winds of fortune had taken me to England following my undergraduate physics studies in Madrid. After playing with the idea of working on clathrin, I settled on tubulin for my graduate studies at the Daresbury synchrotron with Joan Bordas (another physicist) and used time-resolved small-angle X-ray scattering to study its assembly in the presence of anti-mitotic agents such as vinblastine or Taxol. In 1989 I was learning cryo-EM from visiting scientist Dick Wade (also a physicist), who happened to work on microtubule structure and during his time at Daresbury developed his lattice accommodation theory for different microtubule protofilament numbers (Wade *et al.*, 1990). I will not forget the year that followed, because it was 1990 when Richard Henderson (a physicist too) published the structure of bacteriorhodopsin using electron crystallography (Henderson *et al.*, 1990), demonstrating that two-dimensional protein crystals could lead to atomic models using electron microscopes.

The electron crystallography niche, which ultimately proved really narrow, centered on integral membrane proteins, which under the right conditions can be persuaded to form ordered arrays within the constraint of a lipid bilayer (thus, two-dimensional crystals). The bacteriorhodopsin structure, which built on the natural tendency of this protein to form ordered arrays in the membrane, was very exciting. Not only did it open a new path for structure determination, especially of a particularly challenging type of proteins, but also for years it served as the starting point for homology modeling of the pharmacologically very relevant G-protein coupled receptors to which bacteriorhodopsin is a distant cousin. It was not clear, however, that electron crystallography would provide any advantage in the study of soluble proteins. As it turns out, there was at least one obvious case for which it could.

Given the centrality of microtubules in cell biology, and the centrality of cell biology to life as we know it, tubulin had been a structural target for years. Major laboratories around the world had tried or were trying to crystalize tubulin to determine its structure by X-ray crystallography, already an almighty structural method that would dominate the field for years to come. One major problem for tubulin was the difficulty in generating a monodisperse sample, given its tendency to self-associate (after all, self-assembly is what tubulin does for a living!). If αβ tubulin dimers associated into a variety of oligomeric states (2,3,4 of them, all the way to a very large *N*), the mixture would not be conducive to crystallization. But what if tubulin's amazing capacity to polymerize could be used to our advantage?

Following the discovery and purification of tubulin, many biochemists had played with different buffer conditions to study different polymorphic forms of tubulin self-assembly in the test tube. Among the soup ingredients that they tried was zinc, which somehow made tubulin polymerize into open sheets (Larsson *et al.*, 1976). Linda Amos, who like Henderson was at the LMB (Laboratory of Molecular Biology, Cambridge) and thus at the epicenter of the emergent electron crystallography methodology, carried out early studies of these zinc-induced tubulin sheets using negative stained samples. Together with Tim Baker, she was able to determine that protofilaments, the head-to-tail arrangements of αβ tubulin dimers that associate in parallel in the microtubule making their cylindrical wall, associate in an antiparallel manner in the zinc-induced sheets, and as a result make a flat, two-dimensional polymer (Baker and Amos, 1978). I visited the LMB at the end of my PhD work and talked to both Richard and Linda. I could not have guessed then that I would end up using electron crystallography to pursue the

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Abbreviations used: EM, electron microscopy; PDB, Protein Data Bank.
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high-resolution structure of such tubulin sheets an ocean and a continent away from Cambridge.

When my then boyfriend, now husband, was offered a position at the Advanced Light Source in Berkeley, one of his friends arranged an encounter for me with Robert Glaeser (would you believe that he is also a physicist?). I had read Bob's critical papers in the 1970s, in which he demonstrated the power of cryofixation of biological samples for electron microscopy studies, not only allowing the use of a hydrated ("frozen" hydrated) protein sample inside the vacuum of the electron microscope, but also reducing radiation damage enough to allow high-resolution data collection (Taylor and Glaeser, 1974). In summary, Bob and his then student Ken Taylor had proven, for the first time, the huge potential of cryo-electron microscopy (cryo-EM). As you can imagine, I was quite intimidated! But Bob was incredibly kind, as he has been for many years as my colleague at Berkeley. When I mentioned that I had worked on tubulin, he walked me right to the office of Ken Downing (yes, you guessed correctly, another physicist).

I do not play the lottery, as I do not believe in good luck, but that was the day I managed to be in the right place, at the right time, and I won the big prize. Ken was a hands-on expert in electron crystallography, who had been part of Henderson's team to solve the structure of bacteriorhodopsin. He had taken on the aim of improving the zinc-induced tubulin sheets and using the latest technical tricks to obtain the atomic model of tubulin that so far had proven unattainable by X-ray crystallography. Ken had already published a high-resolution two-dimensional projection map (Downing and Jontes, 1992) and had been awarded an National Institutes of Health (NIH) grant for this pursuit. And so it was that he invited me to join a small team consisting of Ken himself and my postdoctoral colleague Sharon Wolf (now at the Weizmann Institute). The three of us worked toward improving the size of the zinc-induced sheets and their stability and order, and took turns at the microscope collecting both images and electron diffraction patterns. While in many ways our resources were limited, we had an excellent electron microscope, and Ken had outfitted it to correct for drift, an important advantage in collecting high-resolution images when holders and stages were not as stable as they are today, and data had to be collected on film using long exposures. Within little more than a year, we were able to publish an initial structure at medium resolution (6.5 Å), as well as a two-dimensional projection map pointing to the binding site of the antimitotic drug Taxol, which we used to stabilize the sheets (Nogales *et al.*, 1995). But moving from there to better than 4 Å, a minimal requirement to trace a protein structure from scratch, proved very difficult.

The way meaningful data are collected in electron crystallography involves tilting the sample to get different views of the molecule. Unfortunately, as you tilt, the lack of planarity of the crystals (which are a few micrometers in diameter and just a few nanometers thick—the size of the tubulin protein) results in a dramatic loss of information. In fact, the daunting task of obtaining atomically flat crystals on the EM grid was likely the cause leading to the downfall of electron crystallography. After two years of struggle, of collecting micrographs that went in most cases directly into the dustbin, a breakthrough in sample preparation opened a window that allowed collecting all the data needed in less than a month. Just writing this now, when thousands of images are collected in a single day (enough for atomic resolution structure determination in modern cryo-EM), makes me smile. Science had a different pace back then. A typical data set from a day at the scope consisted of 50 micrographs, the number of films the cassette held. That was also about the number of cryo-EM practitioners at the time (well, really not much larger), when we all knew each other, knew what each of us was working on (for years and years). After each session was over, we would extract the cassette with the films from the scope and go to the darkroom, where we developed them in the mist of red light while listening to the radio to scare the night spirits away. The following day the films would be dried, and we could check them at the optical bench for diffraction spots that hopefully would extend isotropically in many rows of perfectly aligned starlight. Once the best images and diffraction data from the new preparation method were put together to generate a high-resolution density map of the tubulin sheets, I was left with the task of building the atomic model.

If Ken had been a different kind of mentor, after the years that it took to get there, he would have found an expert to build the structure fast. But he did not. He allowed me the time to learn to do it and finish the work myself. Ken was a true gentleman scientist (in so many ways). All of us who worked under Ken's mentorship know that Ken loved science for science's sake. He loved the day-to-day work, laboring around the struggles, solving problems, and he allowed his lab members to enjoy the same calm pace of things. Ken Downing passed away in the summer of 2018. We had the good fortune to celebrate his career with a symposium in his honor a few years before, and then to celebrate his memory in 2019 with a special session at the annual meeting of the Microscopy Society of America (MSA), which he presided over in 2000. His loving wife Linda instituted a special postdoctoral award in his honor awarded by the MSA every year (https://www.microscopy.org/awards/downing.cfm).

Fortune had it that the structure of the tubulin prokaryotic homolog, FtsZ, was obtained by Jan Löwe in the lab of Linda Amos using X-ray crystallography at about the same time that we obtained the tubulin structure. The publication of both structures was coordinated (Löwe and Amos, 1998; Nogales *et al.*, 1998), and the two sister structures made the cover of the January 8, 1998 issue of *Nature*. A few years later I spent some time at Cambridge working with Jan on the refinement of that original tubulin structure (Lowe *et al.*, 2001), in the process changing its Protein Data Bank (PDB) code from the beautiful 1TUB to 1JFF (uglier, but the correct one!!)

The first structure of tubulin 25 years ago, obtained from a polymer that preserved the protofilament structure and was bound to Taxol, did more than define the fold of the protein (a Rossmann fold, as I told and showed Michael Rossmann himself when he visited Berkeley in 1997 to give a seminar). The structure (Figure 1) positioned the nucleotide binding sites in α-tubulin and β-tubulin at the intra- and interdimer longitudinal interfaces along the protofilament, respectively, thus explaining their different exchangeability in solution and in the polymer, and also it provided the binding site of a major anticancer therapeutic. Just a year later, combined with the low-resolution cryo-EM structure of a microtubule (in this case Ron Milligan's), it led to a model of how protofilaments are oriented in microtubules (Nogales *et al.*, 1999), which was confirmed when higher-resolution structures of microtubules became available, thus defining which structural elements in tubulin face the lumen or the outside of the microtubule. And so we saw that Taxol binds on the luminal surface, while the variable, negatively charged, and highly modified C-terminal tails of tubulin extend from the surface of the microtubule. This even pointed to which regions were likely to be involved in lateral contacts, which would be defined in more detail years later (for a study out of my own lab see Alushin *et al.*, 2014).

To put the tubulin structure in a larger context, the 1990s were years of great excitement for the cytoskeletal community interested in structure/function. In 1990, Ken Holmes and Wolfgang Kabsch published the X-ray crystallography structure of actin, which they blocked from polymerization by binding it to DNAse1 (Kabsch *et al.*, 1990), as well as a model of F-actin using fiber diffraction of

FIGURE 1: Ribbon diagram of the structure of the $\alpha\beta$ tubulin dimer obtained by electron crystallography of zinc-induced tubulin sheets stabilized by Taxol (yellow). β-Tubulin is at the top, bound to GDP. α-Tubulin is at the bottom, bound to GTP. As shown, the view corresponds to that from the inside of the microtubule.

aligned filaments (Holmes *et al.*, 1990). Then, in 1993, Ivan Rayment produced the crystal structure of a myosin motor head (Rayment *et al.*, 1993b), and with Ron Milligan, in the first clear example of hybrid methodology, used the cryo-EM reconstruction of actomyosin filaments and the atomic models of actin and myosin to generate the first model of their interaction (Rayment *et al.*, 1993a). These were two back-to-back papers that I remember presenting in journal club! In 1996, Ron Vale and Robert Fletterick obtained the structure of the kinesin motor domain (Kull *et al.*, 1996). I remember Ron presenting the structure at an American Society for Cell Biology (ASCB) annual meeting session with thousands of people spellbound by this structure and its remarkable similarity to myosin, even though they translocate on different cytoskeletal filaments. I also remember a seminar in Berkeley in 1997 by Steve Block, who was studying the stepping of kinesin on microtubules. I was sitting in the first row of the small lecture hall. In his introduction he showed a cartoon representation of a microtubule with the crystal structure of kinesin on top of it. He said something like "We have kinesin, but we need the structure of tubulin, and whoever gets it will be famous overnight." I was at the time occupied tracing the tubulin chains on our density map and at that moment I feared the rest of the audience would be able to hear the loud, fast thumping of my heart. The cytoskeletal and structural biology communities did prove very appreciative. We enjoyed presenting our structure at meetings and seminars, and even more seeing how it was put to good use by other scientists interested in tubulin and microtubules.

In 2000, the first X-ray crystallography structure of tubulin, in a polymerization-inhibited state (using a principle similar to that used for actin, in this case binding tubulin to a stathmin-like domain), was obtained by molecular replacement by Marcel Knossow's team using the 1998 structure of the tubulin dimer as the model (Gigant *et al.*, 2000). Since then, many such structures bound to different drugs and tubulin-binding partners have been published, providing a richness of information. Microtubule structures, both alone and with proteins that uniquely bind to the polymerized form of tubulin, remained the realm of cryo-EM (too many to reference here). As this technique developed, so did our view of microtubules. Today, cryo-EM microtubule structures are obtained in a small fraction of the time that it took to get that of the zinc-induced tubulin sheets 25 years ago, the resolution is often better than it was then, the structures correspond to a physiological polymer, not an aberrant tubulin assembly, and often they include important microtubule interactors. Perhaps the most spectacular examples to date are those of microtubule arrangements with associated ciliary proteins (Ma *et al.*, 2019). How humble they make the tubulin structure of 25 years ago feel! I am sure Ken Downing would have been in awe at their beauty.

The almost perplexing complexity of the cell drives the scientific efforts of all of us. Our understanding of that unit of all life requires many approaches, at many scales, from the generation of a parts list to the unraveling of the circuitry of evolved networks that connects them in ways that are robust, always adjusting, and often puzzling. I feel very fortunate that "almost" random events led me from physics to cell biology through protein structure. And while it seems that laboring away to produce a simple gallery of images of protein shapes, motions, and interaction is a contribution as small as the molecules I study, I would like to think that it is a useful part of our collective effort to understand nature and marvel at it. Celebrating a structure may be meaningless or may be a way to reflect on the contributions of each research endeavor to the ones that follow, but also on how science and the life of scientists moves forward, always accelerating, always surprising us. Ken, Sharon, and I liked to think that our efforts of those years, culminating in a structure that many did not think we would ever get, would have some value for the research community, for our understanding of the cell. On a personal level, that tubulin structure made my career and allowed me to work with all those who have come through my laboratory and contributed their own structural discoveries. What a treat!

Today the final "structural frontier" for tubulin and microtubules, as for any other cellular components, is visualization in atomic detail within the cellular milieu. It is not the time to expand on the physical laws that make this goal impossible in a literal sense, or the ways in which we can successfully approximate it with human ingenuity. Whether we call it structural cell biology, cellular structural biology, or something else more creative, suffice it to say that the task is one worth working toward, and that the rewards could be many.

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