Thomas A. Steitz (1940–2018)

The world lost one of its best structural biologists when Thomas A. Steitz died at his home in Stony Creek, Connecticut on October 9, 2018. He was a Professor of Molecular Biophysics and Biochemistry at Yale University since 1970 and an HHMI investigator since 1986. A member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the Royal Society, his many honors and awards include the Keio Prize, the Gairdner Award, and the Nobel Prize in Chemistry. He continued to do research at his customary high level until shortly before his death at age 78. By focusing on the structures and mechanisms of many of the enzymes involved in the replication and expression of genes, he provided the framework for understanding how these critical pathways function at the atomic level. A crystallographer of such extraordinary breadth and depth and such exceptional accomplishment may never be seen again.

By far the best accounts of Tom Steitz's life and scientific career are his Nobel Prize autobiography (www.nobelprize.org/prizes/chemistry/2009/steitz/auto-biography/) and lecture (Steitz 2010). Since they also give one a sense of his personality, I strongly recommend them.

The arc of Steitz's astonishing career precisely matches the rise to prominence of X-ray crystallography in biochemistry and molecular biology. He was inspired to enter the field in 1963 by a lecture by Max Perutz describing the very first atomic structure of a protein, myoglobin. By 1967, Steitz was part of the team in the laboratory of William Lipscomb at Harvard that determined one of the first structures of an enzyme, carboxypeptidase A. In these early days of X-ray crystallography, progress was incremental and agonizingly slow. Obtaining diffraction quality crystals was so erratic it was considered an "art." Diffraction data

were measured manually, entered into cards or tapes, and then processed using computers far less powerful than current cell phones. Physical "ball and stick" molecular models were manually fit to the calculated electron density, one residue at a time, to obtain a final structure. To be a successful crystallographer, one had to be extraordinarily patient and overcome many roadblocks idiosyncratic to each protein. Not only did Steitz excel in this type of science, but, starting as a graduate student, he was a dedicated contributor to the many improvements that slowly made X-ray crystallography the powerful method that it is today. It is clear that growing up with the field was crucial for his later success.

Steitz was one of the early group of American postdocs who worked at the Laboratory of Molecular Biology in Cambridge, England during the heady years when molecular biology was born. He recalled frequent informal talks with John Kendrew, Francis Crick, Sydney Brenner, Fred Sanger, and Max Perutz in the LMB canteen (www.nobelprize.org/prizes/chemistry/2009/steitz/auto-biography/). It was during this time that Steitz set his sights on solving structures of the enzymes associated with the central dogma of molecular biology. Thus, like many of his peers, his postdoctoral experience not only set his scientific course for the remainder of his life, but also defined his view of how science should be performed.

In 1970, as a young expert in the emerging powerful tool of X-ray crystallography, Steitz began his job as an Assistant Professor at Yale. He recognized that, although clearly important, determining the structures of enzymes associated with the central dogma would be a daunting problem. While DNA replication, transcription, and translation could be assayed in extracts, the pathways were just beginning to be dissected into individual enzymes and only a handful of these had been purified. Furthermore, it was already clear that many of these enzymes were enormous, well beyond the capabilities of X-ray crystallography at the time. He therefore made the tactical decision to first focus on determining the structure of the more tractable (but still difficult) yeast hexokinase. In the meantime, he closely followed the progress of enzymologists working on the central dogma machinery and slowly began to purify and try to crystallize some of the smaller proteins in these pathways in his own laboratory. In other words, when taking on an impossible problem, start by doing what you can.

Virtually all of the publications from his first decade as an independent investigator documented steady progress on the structure and mechanism of yeast hexokinase. As was typical for crystallography laboratories at the time, nearly 20 papers reported incremental improvements in resolution, examined different crystal forms, and

documented the substrate binding pockets. An important conclusion was that the large conformational change that occurred upon hexose binding ensured that water molecules would not compete with the sugar and cause “wasteful” ATP hydrolysis. This was beautiful work that made Steitz well known among enzymologists and got him tenure, but was only a prelude to what would come.

As a result of the prescient decision he made as a post-doc, Steitz became a founding crystallographer in five of the major subdisciplines of molecular biology: replication, recombination, transcription, reverse transcription, and translation. Over the succeeding years, his laboratory published structures in dozens of different macromolecular systems. His pivot from metabolic enzymes to the proteins associated with the central dogma occurred quite abruptly in the early 1980s. This critical period was ushered in by the structure of the bacterial cAMP activator protein (CAP) in 1981 and was soon followed by the very important structure of the Klenow fragment of DNA polymerase 1 (1985). During this same period, his reports of the crystallization of part of $\gamma\delta$ resolvase (1982), SSB (1983), and recA (1986) clearly established Steitz’s intent to focus on what then were called nucleic acid enzymes. There soon followed structures of several of the very first protein–nucleic acid complexes, including DNA bound to the Klenow fragment (1988) and the complex of tRNA^{Gln} bound to glutamyl tRNA synthetase (1989). Although each of these successes was the result of years of work, improvements in the production and structure determination of proteins during this period allowed his laboratory to simultaneously focus on multiple projects of ever increasing complexity. By the end of this critical second decade of his career, Steitz had not only established himself as one of the world’s best crystallographers, but also as a leader in the rapidly expanding field of molecular biology.

Tom Steitz was never a “one and done” crystallographer who simply solved structures. Instead, his commitment to molecular mechanism ensured that he continued working on each system, often for decades after the first structure was solved. Structures of enzyme–substrate and enzyme–inhibitor complexes, intermediates in the reaction mechanism and complexes with relevant accessory proteins would subsequently appear. One such example was his pursuit of how CAP activates transcription of nearby genes. The initial CAP–cAMP structure (1981) was followed by 15 more papers, including showing that DNA bends sharply upon protein binding (1991), explaining why CAP and lac repressor could not bind DNA simultaneously (1996), and documenting the different conformation of the apo-protein (2009). Finally, in 2017 his group determined a cryo-EM structure of an entire transcriptional activation complex containing a CAP-dependent promoter DNA bound to CAP, σ 70-RNA polymerase, and a de novo synthesized RNA chain. Thus, over 36 years, Steitz used the ever increasing power of structural biology to understand

different aspects of how this important process “worked.” This extraordinarily tenacious dedication to mechanism is seen in every system he studied.

Each one of the five subdisciplines that Steitz worked on evolved into a separate field, involving different groups of investigators, separate meetings, and even specialized journals. It is hard to comprehend how he managed to work at such a high level in so many different fields simultaneously. His Nobel Prize winning work, documenting the structure of the 50S ribosome, exploring the mechanism of the peptidyl transferase reaction, and determining the mode of action of numerous antibiotics, appeared in about a dozen papers between 1998 and 2003. Remarkably, during that same period he published more than two dozen additional papers in other areas, including structures of T7 RNA polymerase without and with promoter DNA, the structure of HIV reverse transcriptase bound to an inhibitor RNA, several structures of tRNA–synthetase complexes, and a structure of the CCA adding enzyme bound to substrates. This impressive breadth of Steitz’s work was matched by high productivity. From about 300 Steitz laboratory publications (67 in *Science/Nature/Cell*!), nearly 200 of them contained substantial new structural data. When I asked him how he managed all of this, he told me: “by not interfering with the excellent people in my lab.”

No single individual has sufficient scientific breadth to knowledgeably evaluate the impact of all of his accomplishments. Thus, here I select a few of my own favorite Steitz projects that involve RNA:

Synthetase “recognition” of tRNA. This area became hot in the early 1980s when molecular geneticists and biochemists (including myself) evaluated hundreds of tRNA mutations in an attempt to understand how the 20 tRNA synthetases could each accurately distinguish their “cognate” subset of tRNA substrates from the pool of about 100 structurally similar cloverleaves. Although these experiments led to the idea that there were certain “identity” residues that were critically important for each enzyme, it was unclear what made these residues important and how the overall specificity was achieved. Steitz’s first co-crystal structure of a tRNA synthetase bound to its cognate tRNA (Rould et al. 1989) was a game changer for the field. For the first time, tRNA specificity could be thought about in terms of hydrogen bonds in three dimensions rather than A, U, C, and G symbols on a cloverleaf. Subsequent co-crystal structures containing tRNAs with mutations in anticodon identity residues (Arnez and Steitz 1996) revealed that specificity actually involved a mutual adaptation of the protein and the tRNA molecules, often involving subtle long-range conformational changes. Thus, the structure showed that the digital “identity nucleotide” model of tRNA specificity was an oversimplification that obscured the subtlety of the macromolecular interaction.

tRNA nucleotidyl transferase. This beautiful story, somewhat obscured by the excitement over the con-

temporaneous appearance of the 50S ribosome structure, investigated how this enzyme was able to use a single active site to sequentially add two C residues followed by one A residue to the ends of cellular tRNAs with high specificity. By solving five different structures of an archaeal enzyme complexed to either CTP or ATP and several tRNA substrate intermediates, Steitz and colleagues found that subtle changes in the nucleoside triphosphate binding pocket and an alteration in the cleavage mechanism could account for change in specificity from CTP to ATP in the last step (Pan et al. 2010). This is a great example of how a thorough crystallographic study can explain a mysterious biochemical specificity.

Recent ribosome stories. Steitz's famous structure of the *H. marismortui* 50S subunit helped to usher in the modern era of ribosome science that used structure to understand function. In the years that followed, I have admired how Steitz was able to learn the mature, complicated field of ribosome biochemistry and molecular biology and choose among the huge number of questions that could potentially be answered by high-resolution structures. Of his dozen "post-50S" papers, two of my favorites are (i) structures of complexes between three different bacterial hibernation factors and 70S ribosomes that explain how these proteins shut off translation during stationary phase (Polikanov et al. 2012) and (ii) structures of three different complexes of mammalian initiation factors bound to 40S ribosomes that begin to elucidate the scanning mechanism in eukaryotic translation initiation (Lomakin and Steitz 2013). Both are typical papers from the Steitz laboratory: a succinct, well-referenced introduction that leads to a clearly stated question, lots of data (multiple structures), a comprehensible description of the most important aspects of the structures using well-chosen figures, and an easy to understand conclusion. For non-crystallographers, these are a joy to read.

It could be argued that Steitz's overall influence on the field of crystallography exceeded his published work. As an indefatigable attendee of meetings and a willing and eloquent seminar speaker, he educated several generations of scientists on how much can be learned from an X-ray structure. Like Perutz did for him in 1963, he undoubtedly inspired many students to enter structural biology. In addition, many of the 50 graduate students and 87 postdocs who trained in his laboratory have gone on to use X-ray crystallography to study macromolecular structure and mechanism at universities and companies around the world. As a result, Steitz's standards and unique scientific style have been transferred to a large second generation of crystallographers and they are training the next generation. Thus, we can be assured that Steitz's legacy will continue.

Although the above paragraphs summarize the career of an exceptionally successful, gifted scientist, they do not really capture what he was like personally. Starting as a fellow graduate student at Harvard more than 50 years

ago, I have been fortunate to know Tom Steitz and his wife Joan as both professional colleagues and personal friends. Not only did we see one another several times a year at meetings, but, starting in the late 1980s, our families went skiing together every spring as part of a group that Tom named "Riboski" (www.nobelprize.org/prizes/chemistry/2009/steitz/auto-biography/). The group also sometimes trekked to more exotic locations (Galapagos 2000, New Zealand 2014). We also visited each other's houses, watched the kids grow up, cooked together, gossiped, drank wine, and marveled at the advances in RNA science. My wife Lori and I also went on many hiking trips with Tom and Joan, mostly in the mountains and deserts of the American west. Tom loved being outdoors. He was an enthusiastic, capable skier, a slow but powerful hiker, and a determined photographer of wildflowers.

Although Tom was not always outgoing, I found it easy to discuss virtually any topic with him, including cooking, wine, gardening, academic life, and (of course) science. So outside of meeting halls, along hiking trails, going up ski lifts, or eating lunch, we often would plunge randomly into conversation about something of mutual interest. A favorite topic was the relative advantages of X-ray crystallography versus solution measurements in determining how enzymes function. Although a confirmed crystallographer, Tom was well aware of the temptation of overinterpreting structures and was deeply appreciative of carefully thought out kinetic and thermodynamic experiments. Over the years, I learned a lot from him about the inner workings of crystallography, including the many experimental dilemmas encountered on the way to proposing a model. We discussed many of the big issues in determining macromolecular structures, including the structural genomics initiative (he was against it), the prospects of predicting structure from sequence (skeptical, but hopeful), and the recent rise of cryo-EM (loved it). Tom was great to argue with because he was always polite, listened carefully, and never took the conflict personally. He was also stubborn and clever at making you see the limitations of your position. Thus, Tom was a wonderful colleague—engaged, optimistic, modest, sympathetic, and easily amused.

Within our gregarious Riboski family, Tom was relatively quiet, amicable and, above all, kind. His reluctance to offend made him famously noncommittal and willing to defer to others. He was a specialist in terrible puns, often interjected in the middle of heated exchanges that stopped the conversation with universal groans. I will really miss him.

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