Jeremy R Knowles 1935–2008

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Perfection. It's one of the most tantalizing words in the English language, because it suggests something both desirable and unattainable. We apply it to food ("The steak was perfect."), weather ("the perfect storm") and work ("You're perfect for the job."), but we know that perfection can only be strived for, never achieved. So when Jeremy Knowles, the great Harvard chemist who died on April 3, 2008 from complications of prostate cancer, used the term "catalytic perfection" to describe the enzyme triosephosphate isomerase in 1976, he knew it would be controversial.

That enzymes demonstrate amazing catalytic efficiency was already appreciated—several were known whose kinetic parameters were similar to the time required for the catalyst and substrate to diffuse together. But there was no direct evidence that this was what determined their rate, and no theoretical framework for considering the major implication: that if such a "perfect" enzyme existed, it would have reached the end of its evolutionary development as a catalyst.

All that changed with the appearance of the December 14, 1976 issue of *Biochemistry*. It contained eight back-to-back papers with Jeremy Knowles as the corresponding author. Nothing like them had been seen in enzymology—or physical organic chemistry—before. In those papers Knowles and his Oxford collaborator W. John Albery first established both the theoretical and experimental foundation for using the effects of isotopic substitutions on reaction rates to dissect the free energies of each step in an enzyme-catalyzed reaction. They then applied that method to the enzyme triosephosphate isomerase (TIM), producing the first complete energy profile for an enzyme-catalyzed reaction. The profile was a descending staircase, in which each step was of lower energy than that preceding it. The highest energy step (and therefore the slowest, or rate-determining step) was a purely physical one—the diffusion-controlled association of enzyme with substrate. The internal steps, the chemical steps of catalysis, were all faster. TIM had evolved to optimize its chemistry to the point where no further improvement in catalytic efficiency was possible.

Knowles started the TIM project during his first academic appointment, as fellow and tutor of Wadham College, Oxford, attracted by the extreme simplicity of a reaction in which only two protons need be transferred in order to interconvert two phosphosugars. Another attraction was that David Phillips, who had made scientific history by determining the first crystal structure of an enzyme (lysozyme) only a few years earlier, had just joined the Oxford faculty and had decided that the structure of TIM would be his next major project. Finally, as Knowles first pointed out, because TIM simply catalyzes the equilibrium between a single substrate and a single product, it was possible in theory to observe a productive enzyme-substrate complex directly by X-ray crystallography so long as the concentration of substrate in the crystals was sufficiently high. That suggestion led eventually to an entire subdiscipline in which crystallography is used to look directly at enzyme-substrate and enzyme-intermediate complexes.

When the TIM papers were published, Knowles had already left Oxford for Harvard to become professor of chemistry—a position he would have had to wait years to attain in the hierarchical British university system. There he was to put his unique stamp on enzymology for the next 20 years. In a decade-long collaboration with the two of us, he combined site-directed mutagenesis, detailed kinetic and thermodynamic analysis, and X-ray crystallography to probe the role of every residue in the active site of TIM. When that project began it was difficult to publish structure-based mechanistic papers in biochemical journals. When it ended such papers had become the norm, largely thanks to the credibility his participation provided. We still remember with great fondness his patience, enthusiasm, good humor, attention to detail and generous support during this period.

Of his other achievements it is his work on phosphoryl transfer reactions that probably best demonstrates the elegance that characterized all his science. Starting with pioneering work by Westheimer, reactions in which a phosphate group is transferred from a donor molecule to an acceptor had long been central to mechanistic enzymology, but there was a huge unresolved question: in any given reaction, it was virtually impossible to determine whether the transfer occurred in a concerted fashion or by a stepwise mechanism with the formation of an intermediate. At an historic Gordon Research Conference on Enzymes in the summer of 1977, the doyens of enzymology discussed this problem and agreed that it could only be solved if it were possible to make a chiral phosphate group, one that could not be superimposed on its mirror image. A single-step reaction would by necessity invert the hand of such a phosphate, whereas a two-step process would invert it twice, resulting in retention of the original hand. Incredibly, by the following summer Knowles had synthesized a chiral phosphate using the stable isotopes of oxygen (^{16}O, ^{17}O, ^{18}O) to differentiate the stereochemical positions, and in a landmark paper in *Nature* on October 12, 1978, he used it to prove that the reaction catalyzed by alkaline phosphatase proceeds by a two-step mechanism.

In his final research Knowles returned to his favorite enzyme, this time using TIM as the basis for an experiment designed to test the question of whether there was more than one way to skin a cat, mechanistically speaking. He crippled a critical residue in the active site of TIM, replaced the wild-type enzyme with that mutant in a bacterial strain, randomly mutagenized the rest of the gene, and then looked for second-site mutations that could at least partially restore catalytic function. Remarkably, he found the same second-site suppressor could restore significant function to damaging mutations at two distinct sites. This study was one of the first demonstrations of the possibility of doing directed evolution, which is now a flourishing field of its own.

It is given to few scientists to quit while at the pinnacle of their profession. Jeremy Knowles did just that, and his departure from active research to become dean of the Faculty of Arts and Sciences at Harvard in 1991, exactly 30 years after receiving his DPhil at Oxford, left a hole in enzymology that has still not been filled. Every time he turned his hand to a new problem, he did so with such cleverness and clarity of thought and exposition that he not only opened up a new area, but also set its tone and style. He also produced a great many scientists who carry on his legacy of forefront research and attention to detail.

It may be that absolute perfection is only attainable by the most highly evolved enzymes—but as a scientist, collaborator and intellectual leader, Jeremy Knowles came close.

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