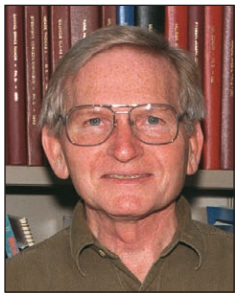
Emeritus Profile: Professor Perry Frey

Recollections of a life in chemical mechanistic enzymology and 27 years in Madison



In response to a request for a profile of my development as a chemical mechanistic enzymologist, I offer the following. My parents were John and Inez Frey, who were happily married for 68 years in Plain City, Ohio. This was a small, rural community of ~1500 at the time I was born in 1935. My twin sister and I started school in first grade at the local public school in September of 1941, and three months later we learned about the attack at Pearl Harbor and the start for America in World War II. About two years later, I began carrying and delivering a newspaper to neighbors, and I read about the war. It was very hard to learn about local soldiers who lost their lives or returned with life-long injuries.

Early Education

By the time I reached high school I had learned how fortunate I was. My parents were intelligent people who had been prevented by cultural pressures from obtaining higher education. My father grew up in an Amish family and was prevented by that culture at the time from attending high school. He was allowed an eighth-grade education, after which he worked on the family farm until he turned 21 in 1930, the depth of the Great Depression. He could have been any kind of professional man if he had been able to obtain a higher education. GEDs did not exist then, but he read widely and became socially and politically mature; and he taught himself music on the violin, guitar, and organ. He worked as a farm laborer for a couple of years and then acquired skills in seasonally complementary trades as a sheep shearer and painting contractor. My mother grew up a Mennonite, which did not restrict her education. However, she had to leave high school in the 10th grade to help support her younger siblings after the departure of their father. She worked at her own job after we children entered grade school, and she became manager of the local office of the General Telephone Company. My parents impressed on me the importance of a high school diploma, which they regarded as a key to success. My father employed me in his contracting business through junior and senior high school, and later in college. I graduated from high school in 1953.

College at Ohio State

In high school I became interested in science, especially chemistry. I have often been asked by students and colleagues if I intended to have an academic research career. My answer was always that I had no specific intention other than to be a chemist. I was fortunate to live within 19 miles of The Ohio State University, where tuition was $50.00 per quarter at the time, and the Chemistry Department was excellent. I spent the year after high school commuting daily to and from Ohio State. The commute was inconvenient, but I could not afford to live on campus, and there were other pressures. At the time, federal law required all healthy young men to complete 8 years of military service. Three main paths were available. One could enlist in the Army, Navy or Air Force and serve for eight years or as a career. Alternatively, one could join an Active Reserve or National Guard unit for eight years. This involved limited training on active duty, followed by regular training as a "weekend warrior" for the balance of the eight years. A third option was to volunteer for military conscription and serve on active duty for two years. For the balance of the eight years you were in the Ready Reserve, which did not require meetings or other training. College deferments were possible to avoid active duty, but I did not know anyone who had one. Religious objectors were given alternative options. I chose the third option because it would satisfy my military requirement in essentially two years and qualify me for college support under the GI Bill of Rights. I entered the U. S. Army in 1954. After training in basics and tank weapons mechanics, I was transferred to Germany for the balance of my active duty.

I have been told that my military service was a waste. I do not agree. I met people from the streets of New York, the mountains of Appalachia, the forests and cotton fields of the deep South, the deserts of the Southwest, and the mountains and forests of the Northwest. And I spent more than a year in Germany. It was a social education.

I returned to Ohio State in 1956 and completed the curriculum for a Bachelor of Science degree in chemistry. The chemistry courses were generally excellent. As I made progress I became increasingly comfortable with my choice of major, while placing a high and appropriate value on all of my other courses, including philosophy, English composition, German, comparative literature, economics, political science, and of course physics and mathematics.

Chemistry at the USPHS

After graduating in 1959, I took additional higher level courses and worked as a teaching assistant. I was at loose ends, was not doing well, and was uncertain about my future. I was certain about one thing. I had met Carolyn Mae Scott on a date to see Madam Butterfly at the Cincinnati Summer Opera. Carolyn lived and worked in the Cincinnati area, and I thought it was important to see her every day. Therefore, I moved to Cincinnati and found a position as a food chemist at the Sanitary Engineering Center in Cincinnati. This center was operated by the United States Public Health Service (USPHS), and it was concerned with air and water pollution and food safety. The Center later became part of the Environmental Protection Agency. Carolyn and I were married in February 1961, and our first daughter Suzanne was born in February 1962.

As a food chemist in the USPHS, I worked under contract with the U. S. Army to neutralize the paralytic toxin from the red tide organism *Gonyaulax catenella*. This became known as saxitoxin. I purified it from Alaskan mussels and studied methods to chemically bind it to bovine serum albumin. I succeeded in chemically linking it to the protein, and in collaboration with microbiologists tested the conjugate for immunogenicity in rabbits. The conjugate elicited antibodies in rabbits, and some of the antibodies neutralized the paralytic effects of saxitoxin. Thus, saxitoxin linked to serum albumin served as a hapten in immunized rabbits. This work introduced me to immunology and toxicology.

Pursuit of the Ph.D.

During 1960-63 I furthered my chemical education by taking graduate courses in analytical, inorganic, organic, and physical chemistry in the Evening School at the University of Cincinnati. I decided to pursue a Ph.D. degree and applied to the National Institute of General Medical Sciences for a Predoctoral Fellowship. Based on my academic record the Institute awarded me a fellowship. I was drawn to the research of Professor Robert H. Abeles, then at the University of Michigan, whom I had met when he was an Assistant Professor of Chemistry at Ohio State. I called him, told him about my fellowship, and asked if he would accept me as a graduate student. He agreed. Our family moved to Ann Arbor in January 1964.

Upon arrival in Ann Arbor I learned that Professor Abeles had accepted a position in the Department of Biochemistry at Brandeis University in Massachusetts. He asked if I would be willing to move there the following August. I agreed and spent January to August at Michigan taking courses in biological chemistry, the chemical synthesis of natural products and starting my Ph.D. research. Abeles introduced me to the biochemistry of the Vitamin B_{12} coenzyme adenosylcobalamin, which he had found to be required in the bacterial dehydration of propane-1,2-diol to propionaldehyde. Abeles and his associates had purified dioldehdrase, the enzymatic catalyst. I synthesized 2*R*- and 2*S*-[1-^{2}H]propane-1,2-diols with identical configuration at C1 by chemical and enzymatic methods and showed that dioldehydrase transferred the 1-pro-*R* hydrogen from C1 of one stereoisomer and the 1-pro-*S* hydrogen from C1 of the other stereoisomer to C2 of propionaldehyde. We published this unusual stereochemistry and moved to Brandeis University.

At Brandeis I continued classroom studies in biochemistry, enzymology, and physical organic chemistry and moved forward with research on the role of adenosylcobalamin in the action of dioldehydrase. Abeles and associates had discovered that glycolaldehyde inactivated dioldehdrase in the presence of adenosylcobalamin, producing cob(II) alamin. This form of vitamin B_{12} lacked the adenosyl-moiety, and the cobalt was Co^{2+}, the one-electron reduced form the more common cob(III)alamin. I was charged with finding out what had happened to the adenosyl-moiety. I synthesized 5'-deoxyadenosine and identified it as the product derived from adenosylcobalamin in the inactivation. I also showed that inactivation by [2-^{3}H]glycolaldehyde led to the incorporation of tritium into 5'-deoxyadenosine. This keyed me to the idea that adenosyl-C5' might participate in hydrogen transfer in adenosylcobalamin-dependent reactions. In February 1965, Carolyn and I welcomed our younger daughter Cynthia into the world.

Next, I found that reaction of [1-^{3}H] propane-1,2-diol as substrate with the complex of adenosylcobalamin and dioldehydrase produced [^{3}H] adenosylcobalamin, with all of the tritium bonded to C5' of the adenosyl-moiety. Reaction of unlabeled propane-1,2-diol with [^{3}H]adenosylcobalamin and dioldehydrase produced [^{3}H] propionaldehyde. Therefore the adenosyl-moiety of adenosylcobalamin mediated hydrogen transfer. This function of adenosylcobalamin was subsequently found in all other adenosylcobalamin-dependent enzymatic reactions known at the time. I completed my Ph.D. research in 1967.

Postdoctoral Research

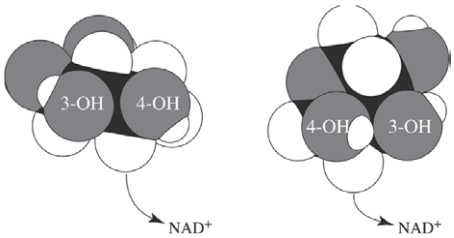
Near the end of my time at Brandeis, Professor Abeles suggested that I do postdoctoral work with F. H. Westheimer at Harvard. Knowing that Abeles had done postdoctoral work with Westheimer, a leading mechanistic enzymologist, I agreed. Abeles called Westheimer and informed me on the same day that I had a postdoctoral commitment. I interviewed with Westheimer, was awarded an NIH postdoctoral fellowship, and joined his group in November 1967. Westheimer had led research on bacterial acetoacetate decarboxylase and showed the role of the lysyl-6-amine in iminium-formation with the Beta-keto-group of acetoacetate, thereby promoting decarboxylation. Researchers preceding me had found that the pH-rate profile in acylation of this lysyl-amino group displayed a p*K*a of 6, 4.5-p*K*a units below that of lysine in water. Westheimer was concerned about kinetic ambiguities and asked me to look into this. I conducted reporter group spectrophotometric studies and found the active site microenvironment to lower the thermodynamically measured p*K*a-values of both neutral and positively charged acidic groups in the active site by 4 p*K*a-units. This could happen only in a positive electrostatic field.

University Faculty Career

Three weeks after starting my postdoctoral research, I received a letter from the Department of Chemistry at Ohio State requesting my application for an Assistant Professorship. I had been recommended by my mentors. I responded by submitting my *Curriculum Vitae* and application. I was interviewed and offered the position. I accepted the appointment and started my own laboratory in January 1969. In April 1969 I was awarded my first individual research grant from the NIH and accepted my first graduate students at Ohio State.

During my career at Ohio State from 1969-81 and at UW-Madison 1981-2008, more than 90 graduate and postdoctoral researchers collaborated with me in work on the chemical mechanisms of action of enzymes. These students were excellent in their own ways and produced research with me in the refereed literature. The students became friends and went on to their own careers in academia, research institutes, the biotechnology, pharmaceutical, and agricultural chemical industries in the U. S. and around the world. I cannot name them all in this space, but their names appear in our group website.

At Ohio State, our group pursued three main lines of research. We started with the Leloir Pathway, UDP-galactose 4-epimerase (GalE) and galactose-1-P uridylytransferase (GalT), which had been sparingly studied and presented significant mechanistic issues. We found that the interconversion of UDP-galactose and UDP-glucose by the GalE-NAD^+ complex involved the intermediate UDP-4-ketoglucose. Experiments on substrate selectivity and binding, kinetics, and isotope tracing suggested that nonstereospecific hydride transfer at glycosyl-C4 could be explained by substrates presenting different glycosyl rotamers to NAD^+, as illustrated (*next page*).



Hydride transfer rotamers of Gal E substrates UDP-xylose and UDP-arabinose.

This proved to be consistent with the crystal structure subsequently determined at UW-Madison in collaboration with Professor Hazel M. Holden. The structure also keyed us to finer points of the hydride transfer mechanism.

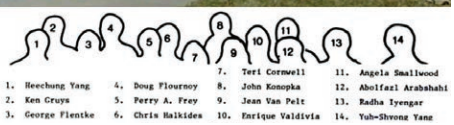
GalT catalyzes the reaction of galactose-1-P (Gal-1P) with UDP-glucose to produce UDP-galactose and glucose-1-P (Glc-1-P). I thought that Gal-1P and Glc-1-P should bind to the same site, so that the overall kinetics should be Ping Pong Bi Bi, in the Cleland nomenclature, and the intermediate should be a covalent UMP–X-GalT. Our group showed this to be correct kinetically and by isolation and characterization of the kinetically competent covalent intermediate. The gene and amino acid sequences, site-directed mutagenesis, and chemical rescue experiments pointed to His166 as the active site nucleophile X linked to UMP. The crystal structure later determined in collaboration with Professors Ivan Rayment and Hazel Holden at UW-Madison verified this assessment.

Our work on GalT inspired us to look more broadly into the mechanisms of phospho- and nucleotidyltransferases. Most were controversial regarding the involvement of covalent (E—P) intermediates. I thought that stereochemistry at the transferred phosphorus could resolve controversies. We devised syntheses of substrates with chiral P, using ^{18}O, ^{17}O, ^{16}O and/or S as substituents on P to generate chirality. And we developed spectroscopic and chemical methods to assign configurations.

We found the GalT reaction to proceed with overall retention at P, presumably by way of inversion in two transfer steps. We confirmed inversion in the first transfer step to form the E-His166—UMP. We subsequently completed stereochemical analyses on 13 other transferases. All studies indicated inversion at each transfer and retention whenever a covalent E—P was an intermediate. The results supported the rule that whenever both forward and reverse acceptors were similar structurally and electrostatically, the mechanism was two-step phosphotransfer with retention, like GalT. Whenever the two acceptors were unlike, the mechanism was a single phosphotransfer with inversion at P.

Beginning at Ohio State, we also undertook studies of a central enzyme in energy metabolism, the pyruvate dehydrogenase (PDH) complex. We simplified and improved the purification from E. coli. Other labs had shown it to consist of three enzymes – pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase – but its subunit composition was controversial. We managed to provide strong evidence for 24:24:12 as the subunit composition, using chemical and molecular mass analysis.

In 1981, we moved to UW-Madison and continued with research on GalE, GalT and other phosphotransferases, and we focused attention on the chemical mechanism of the PDH complex. The cofactors thiamine diphosphate (ThDP) and lipoamide were involved, as well as the substrates pyruvate, NAD^+/NADH, and CoA/acetyl-CoA. Various chemical mechanisms could be considered. We were able to prove the intermediacy of S^8-acetyldihydrolipoamide. We also generated indirect evidence for the participation of 2-acetyl-ThDP. Finally, we synthesized 2-acetyl-ThDP, determined its


1. Heechung Yang 2. Ken Gruys 3. George Flentke 4. Doug Flournoy 5. Perry A. Frey 6. Chris Halkides 7. Teri Cornwell 8. John Konopka 9. Jean Van Pelt 10. Enrique Valdivia 11. Angela Smallwood 12. Abolfazl Arabshahi 13. Radha Iyengar 14. Yuh-Shyong Yang

Frey Group December 1984

chemical properties, and proved it to be an intermediate in the reaction of PDH complex.

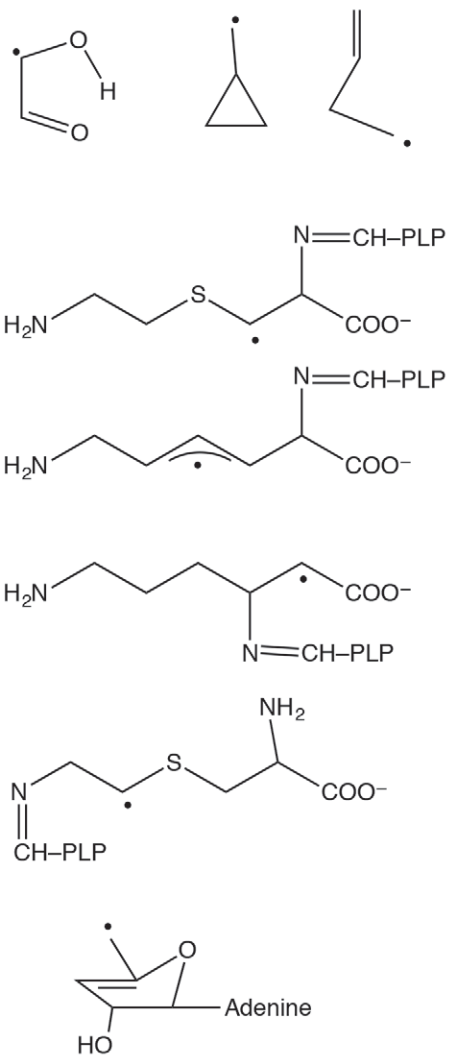
As a graduate student, I was taught that carbon-centered radicals could play no role in enzymatic reactions. They were too reactive and hard to control and would create havoc in an active site. By the 1970s, radicals began to appear in active sites, even in the active sites of dioldehydrase and other B_{12} enzymes.

By 1985 I began to take an interest in the role of S-adenosylmethionine (SAM) in reactions that, at the time, seemed unusual for this molecule. I was particularly interested in the role of SAM in the reaction of lysine 2,3-aminomutase (LAM), which had been discovered at UC-Berkeley by H. A. Barker and associates 15 years earlier. It catalyzed the interconversion of lysine and 3,6-diamininohexanoate, that is, migration of the alpha-amino group from C2 to C3. This reaction followed the pattern that had been established for adenosylcobalamin-dependent enzymes, but it did not require a vitamin B_{12} coenzyme. I wondered whether the adenosyl in SAM (Ado-CH_2—S^+-Met) could be made to function in the same way as the adenosyl group in adenosylcobalamin (Ado-CH_2—Co^{3+}-cobalamin). On its face, this did not seem reasonable. The Co—C bond in adenosylcobalamin was known to be weak (31 kcal/mol) and could undergo homolytic scission to the 5'-deoxyadenosyl radical and Co^{2+}, whereas the homolytic bond dissociation energy for a C—S^+ bond was >62 kcal/mol. However, the reaction of LAM was stimulated by Fe^{2+}, suggesting to me that complex chemistry might be at work.

Our group found that the adenosyl group in SAM mediated hydrogen transfer by LAM in exactly the same way as it did in adenosylcobalamin-dependent enzymes. We wrote a radical isomerization mechanism for the aminomutase reaction. We also discovered that the role of Fe^{2+} was in the assembly of a [4Fe-4S] cluster that ligated SAM through the carboxyl and amino groups. And we discovered that the iron-sulfur cluster mediated homolytic cleavage of the S+—CH_2 bond in SAM by inner sphere electron transfer.

Concurrently with the work on LAM, we undertook to examine the reaction of methane monooxygenase,

with support from the Amoco Research Foundation. I thought this reaction should proceed through a carbon-centered radical mechanism, and we succeeded in demonstrating this by chemical methods. We also extended the LAM research to an adenosylcobalamin-dependent lysine 5,6-aminomutase. We ultimately observed and characterized more than 15 carbon-centered radical intermediates by chemical and spectroscopic methods, mainly in collaboration with my colleague Professor George H. Reed and his associates, who specialized in characterizing carbon-centered radicals by isotope-edited electron paramagnetic resonance spectroscopy. Typical radicals found in active sites are shown below, where PLP is pyidoxal phosphate.



Typical carbon-centered radicals identified at enzymatic active sites.

Low-Barrier Hydrogen Bond (LBHB)

My colleague W. W. Cleland postulated in 1992 that certain enzymatic processes might be potentiated by the transient formation of LBHBs. This keyed me to recall a paper by G. Robillard and R. Shulman in 1972, in which they observed a downfield 1H-NMR signal at 18 ppm in chymotrypsin at low pH (pH < 7) and assigned it to the proton bridging His57 and Asp102 in the active site. It was unusual to observe such an exchangeable proton in aqueous solution by ^{1}H-NMR, and even more unusual to observe one so far downfield. I thought this should be a property of an LBHB.

Examination of the literature on such hydrogen bonds in small molecules supported my thoughts. The literature characterized three classes of hydrogen bonds according to their strengths, conventional, 2-10 kcal/mol; intermediate strong (LBHB), 10-20 kcal, mol; and very strong, >24 kcal/mol. The LBHBs were regarded as asymmetric, short hydrogen bonds intermediate in strength, displaying ^{1}H-NMR signals at 18-20 ppm, low deuterium fractionation factors, and deuterium and tritium isotope effects on chemical shifts. In very strong hydrogen bonds the proton was shared equally and centered between heteroatoms and displayed ^{1}H-NMR signals >20 ppm. I thought that the downfield proton in chymotrypsin fit the bill for an LBHB, and we assigned this. My colleague Professor John Markley and his associates concurred and published extensive properties, including deuterium fractionation and enthalpy of exchange with medium protons.

It was recognized that His57 would be protonated in the tetrahedral intermediate of the chemical mechanism at neutral pH. I thought that it should be engaged in an LBHB at that step. It was known that the peptidyl trifluoromethylketone (TFK) inhibitors, introduced by my former mentor R. H. Abeles, formed tetrahedral adducts with Ser195 analogous to tetrahedral intermediates. I called Abeles and asked if he could forward one of them to me. He agreed. Then I read his paper published in 1987, in which he and his associate observed the downfield signal at 18.7 ppm in one of these adducts, and the signal persisted at pH 9, exactly the behavior I expected. I called Abeles again and pointed out that he had already done the experiment that I envisioned. He agreed, although I had the impression that he might not have been convinced of the LBHB. He forwarded the inhibitor.

Our group synthesized the series of peptidyl TFKs that Abeles and associates had introduced, and we studied the properties of the corresponding chymotrypsin adducts. In collaborations with John Markley, we found downfield ^{1}H-NMR signals between 18.6 and 18.9 ppm, very low deuterium fractionation factors, very high enthalpies for exchange, and deuterium and tritium isotope effects on chemical shifts. We also constructed linear free energy correlations of the 1H-NMR chemical shifts with the published inhibition constants for the inhibitors, the p*K*a-values for His57 in the adducts, and the enzymatic reactivity of corresponding methyl ester substrates. In short, the evidence for LBHB was very strong, as was the correlation between strength of the LBHB with enzymatic reactivity.

Collaborators

Collaboration in science is very important to progress. I have enjoyed the pleasures and advantages of collaborating with more than 30 senior colleagues in Asia, Europe, and the Americas during my career. Ten of my collaborators have been colleagues in the Biochemistry Department of UW-Madison. I have mentioned my collaborations with graduate and postdoctoral students. They were no less valuable to and appreciated by me. My research was generously supported by the NIH for 44 consecutive years. This work could not have been done without the support of all my collaborators and financial supporters.

Conclusion

My professional life has been made possible by the support and understanding of my family. Carolyn and I have been joined by daughters Suzanne Frey and Cynthia Chapek; grandchildren Samantha and Ian Smith, Carrie and Bonnie Chapek; and great granddaughter Alice Smith. Their constant affection for us and for one another has been our sustaining force.