*Review*

# Steps Along the Path From Microsomal Fex to 8500 Cytochromes P450

**Michael R. Waterman**

*Department of Biochemistry Vanderbilt University School of Medicine Nashville, Tennessee 37232-0146; Tel.: 615 322-3318*

** Corresponding author: E-mail: michael.waterman@vanderbilt.edu*

*Received: 30-08-2007*

## Abstract

This article arises from the closing lecture presented at the 15th International Conference on Cytochrome P450 held in Bled, Slovenia in June, 2007. It provides me an opportunity to highlight what I feel to be the most important elements of my scientific career while at the same time acknowledging key events in P450 research which have had particular impact on my research. On this latter point, there are far too many such discoveries to cover here, and I have selected for mention only a few. I apologize at the outset for not being able to highlight all the wonderful work carried by many investigators over the 46 years of my career. Finally, let me emphasize that this is not a retirement presentation, simply one of a 67 year old investigator who hopes to keep on working for years to come. The support of two friends and colleagues in making this public reflection possible, Fred Guengerich and Damjana Rozman, is gratefully acknowledged.

**Keywords:** Cytochrome P450. sterodogenic P450s, bacterial P450s, sterol 14α-demethylase

## 1. Path to the Fex

In the fall of 1961, I entered Dental School at the University of Oregon where I took my first course in Biochemistry. This was a mind-bending experience and the decision was quickly made to leave Dental School and move up the hill in Portland to the University of Oregon Medical School to study for a Ph. D. degree in Biochemistry. At this time the first of several unplanned and fortunate events throughout my career occurred. I was assigned as a student in the laboratory of Professor Howard S. Mason. Mason and Osamu Haiyashi in Kyoto had recently classified the enzymes involved in metabolism using molecular oxygen.[1,2] Mason was an outstanding scientist and was visited regularly by colleagues from around the world who were interested in oxidases. In this environment a very naive and not very good chemist (the author) found an opportunity to establish a foundation as a research scientist, a process that continues to this day. In addition to my good fortune of being assigned to Howard Mason's laboratory, he assigned me in 1962 to work on microsomal Fex, the beginning of a lifetime of study of hemoproteins, particularly P450s. Mason was a pioneer in the study of biological systems by electron spin resonance (ESR) and had identified a hemoprotein in endoplasmic reticulum from rabbit liver whose concentration was increased by treatment of the animals with phenobarbital. He named the protein generating this ESR signal, microsomal Fex,[3] and my job was to determine its oxidation-reduction potential using ESR and to begin investigation of its biophysical properties using optical rotatory dispersion.[4,5] Quite early during this work Mason realized that microsomal Fex was the same as the hemoprotein cytochrome P450, named by Omura and Sato and the name microsomal Fex was dropped.[6]

As I was approaching completion of my Ph.D. studies, plans were made for me to continue my research on P450 in Osaka, in the laboratory of Toshio Yamano, a leading investigator in the field. However, temporary disruption in the Japanese University system prevented me from taking advantage of this opportunity. This led to a second fortunate and unexpected turn in my career (as will be seen later) because Mason then decided that I should do my postdoctoral training with Ron Estabrook at the Johnson Foundation (JF) of the University of Pennsylvania. Just as I was completing my Ph. D., Ron accepted the position as Chairman of Biochemistry at the University of Texas Southwestern Medical Center in Dallas. He sugge-

sted that I take my postdoctoral training at the JF in the laboratory of Takashi Yonetani, a leader in the study of biophysical properties of hemoproteins. This was particularly attractive because my wife, Mimi, had been raised in the Philadelphia area. So I joined the Yonetani lab and worked on hemoglobin for two years. Nearing the end of those two years, I realized it was about time to set up my own academic laboratory and one day in the spring of 1970 when Ron was visiting the JF, I asked him if I could take a third year of postdoctoral training in his laboratory in Dallas so that he could help me find a position. He immediately asked if I would like an Assistant Professor position in Dallas and I replied "YES!" without hesitation. The unfortunate disruption of the Japanese University system and Howard Mason's direction to Ron Estabrook and the JF, were essential to my funding my first faculty position.

## 2. Start with Cytochromes P450 – the Dallas Period

Arriving in Dallas in August of 1970 I began setting up my laboratory with emphasis on both P450 and hemoglobin, and obtained my first research funding to study hemoglobin. But the environment in the Estabrook Department, including colleagues like Bill Peterson, Bettie Sue Masters, Yuzuru Ishimura and subsequently Russ Prough, Jurgen Werringloer and Jorge Capdevila kept me up-to-date and involved from a distance on P450 research. In 1975 I was promoted to Associate Professor of Biochemistry with tenure based on my work studying hemoglobin, but quite honestly I was not headed toward a career with any distinction and probably I would not have remained funded for this research over the long term. At this time, I realized that I could not understand about 50% of the literature describing hemoglobin research because it was applying the new technologies of molecular biology to globin systems. This led to the third unexpected and fortunate turn in my career. I decided to take a sabbatical to learn this new technology. I contacted David Weatherall at Oxford about a position and he said he had nothing available but that I should contact Bob Williamson, Professor of Biochemistry, at St. Mary's Hospital Medical School in London. In February of 1977, Mimi and I and our children, Peter and Amanda, moved to London, and over a six month period I learned many of the techniques of molecular biology. St. Mary's was a particularly good place for me because Bob Smith and John Caldwell who were leaders in the study of the role of P450 in drug metabolism were key figures in the St. Mary's Pharmacology Department. So even though my training in molecular biology focused on hemoglobin, I was located in a hot bed of P450 research in the U.K. and came away with new ideas about P450s and molecular biology.

## 3. Key Moments of Cytochrome P450 Research in Seventies and Eighties

When I arrived back in Dallas, I realized that I could not compete with the many important laboratories applying molecular biology to hemoglobin research, and with support of Ron Estabrook, I began to apply what I had learned to the study of P450s. A particularly fortunate piece of this sabbatical experience was that Mimi and I rented our Dallas house to Evan and Norma Simpson while we were away, the beginning of my lifetime friendship with Evan. One day, shortly after I settled back into my laboratory in Dallas, Evan approached me about studying a remarkable primary cell culture system, bovine adrenocortical cells, which contained several different P450s. From this point forward I never looked away from P450 research and Evan and I shared a very productive collaboration of more than fifteen years.[7,8]

A number of key moments in P450 research occurred over this time period. Of those which had particular impact on my research, the earliest was that of Anthony Lu and Jud Coon who achieved a major accomplishment by partially purifying a phenobarbital inducible P450 from rat liver and reconstituting the activity with partially purified P450 reductase and phospholipids.[9] This was a particularly important step forward in our research field by showing others that purification of P450s was possible. Shortly after this, higher level purification of P450s was accomplished and the N-terminal amino acid sequence of three P450s from rat liver was reported at the 4$^{th}$ Annual Microsome and Drug Oxidation meeting in Ann Arbor, Michigan in 1979.[10] A lively discussion arose at this and subsequent meetings as to how many P450s there were.

We now know that there are 57 CYPs in humans.[11] At the next of this series of meetings in Tokyo in 1981 the first big payoff of the application of molecular biology to P450 research was presented. Yoshiaki Fijii-Kuriyama presented the first complete primary sequence of a cytochrome P450,[12] what we know to be a CYP2B. This was a moment of great accomplishment in our field. It is worth noting that there was considerable competition in the effort to report the first sequence since Dan Nebert, Masa Negishi, and colleagues were nearing completion of mouse CYP1A1[13] sequence at the time of the Fujii-Kuriyama report. Shortly after this major step forward in P450 research, a second one occurred. Tom Poulos, working in the laboratory of Joe Kraut and in collaboration with I. C. Gunsalus, determined the x-ray structure of P450cam, a soluble P450 from *Pseudomonas putida.*[14] Thus within the first half of the 1980s we learned how to clone and sequence P450s and what the three dimensional structure of these proteins looked like. Sequence alignments of P450s became common place including a major publication of alignment of thirty-four P450 sequences by David Nelson and Henry Strobel in 1988.[15] A few years later, in 1992, Osamu Gotoh used his insight from sequence alignments

to report the presence of six substrate recognition sequences in P450s which have been used extensively for site-directed mutagenesis studies of many different P450s.[16] During the 1980s a significant number of P450s were discovered in different organs of different animals, both by purification and sometimes by molecular cloning. As exciting as this was, it created a major problem for the field because every investigator gave their own name to the P450 they discovered. In some cases, the same P450 was given multiple names creating great confusion. Fortunately, Dan Nebert realized early on that a systematic nomenclature was essential in classifying P450s and took up the challenge of designing one. Together with David Nelson and a world wide advisory committee, Dan developed the CYP nomenclature as we know it today.[17] Without this nomenclature it is impossible to imagine how we could keep track of the more than 8,500 known CYP genes. In this context it is important to recognize that the extensive effort by David Nelson to catalog P450s (http://drnelson.utmem.edu/cytochromeP450.htm) has made many P450 discoveries possible.

During the 1980s my laboratory in collaboration with Evan Simpson's carried out extended studies of steroid hydroxylases in the adrenal cortex. Using cloning technologies we discovered that mitochondrial P450s and their related reductases were synthesized as higher molecular weight precursors which were proteolytically processed upon uptake into mitochordia.[18,19] Similar results were obtained at the same time in Tsuneo Omura's laboratory.[20] In addition, we carried out many studies on transcriptional regulation of steroidogenic P450s in the adrenal cortex.[21] This work was supported by an NIH grant for 24 years and concluded with the most detailed analysis of the biochemistry of a P450 transcriptional regulatory system, that in CYP17.[22] With this success, I chose not to renew the grant as my interests moved in other directions and away from transcription.

An important spin-off of the cloning of P450s was the use of expression vectors for producing individual P450s in selected cell types for detailed study, eliminating the need to purify the monooxygenases from animal tissue. Initial studies focused on use of COS (monkey kidney) cells for P450 expression and study.[23,24] Then came the use of yeast cells[25] and insect cells[26] as systems for P450 expression. While the COS cell expression system provided us the opportunity to study P450 activities and the steroidogenic pathway, the need for a simple expression system that would produce large quantities of P450s which could be readily purified for detailed biophysical and biochemical studies was evident and multiple laboratories addressed the utility of bacteria for this purpose. We were successful in developing high level expression systems for both microsomal[27] and mitochondrial[28] P450s that have provided the base for expression of a large number of eukaryotic P450s in a large number of laboratories.

## 4. The Nashville Period and CYP51

Upon moving to Vanderbilt University in 1992 another unexpected and important event in my career took place. I was provided with adequate resources to begin a new project and we began our continuing studies of CYP51, the most widely distributed cytochrome P450 gene family which is found in all biological kingdoms.[29] Our early studies characterized the human CYP51 gene[30] and its regulation.[31] In 1998 the genome sequence of *Mycobacterium tuberculosis* (MT) was reported and it produced an unexpected result concerning cytochrome P450 enzymes.[32]Much to everyone's surprise there were 20 CYP genes in this genome. It had not been expected to find so many P450 genes in a single bacterium. Previously it was imagined that the three CYP genes in *Bacillus megatarium* might be the largest number of CYPs found in a bacterium, and it was known that many bacteria (such as *E. coli*) would be found to contain none. The biological function of none of the MT CYPs is clearly established and 19 of the 20 genes are members of novel gene families. However, one of the genes, according to amino acid sequence, was predicted to encode a member of the CYP51 family. Cloning and expression of this gene proved that it encoded a sterol 14α-demethylase,[33] although its function *in vivo* remains unknown. This unique finding provided us the opportunity to investigate the structure of a CYP51, since being a bacterial P450 it was soluble. At that time all other CYP51 family members were known to be membrane bound and therefore much more difficult to crystallize. In 2001 we reported the first high resolution x-ray structure of a CYP51,[34] and subsequent studies have identified 29 amino acid residues that are conserved among the more than 100 CYP51 sequences known, the CYP51 signature sequence.[35] CYP51 is a well known drug target for many pathogenic organisms.[29] One important part of our continuing research on CYP51 is study of the enzymes from trypanosomes, *T. brucei* and *T. cruzi.*[36,37] We believe that these forms of CYP51 represent excellent drug targets for these pathogenic protozoa.

In 2000 Eric Johnson reported a major accomplishment in the P450 field, the high resolution x-ray structure of a mammalian P450 from the endoplasmic reticulum.[38] Subsequently Johnson's laboratory and others have solved the structure of a number of P450s from mammalian gene families CYP1, CYP2 and CYP3. At the 15th International Conference on Cytochromes P450 held in Bled, Slovenia in June 2007, the structure of a microsomal P450 from another gene family, CYP46, was reported by Irina Pikuleva.[39] This is the first structure of a cholesterol metabolizing P450. Using strategies developed in the Johnson laboratory we have crystallized forms of CYP51 from both *T. brucei* and *T. cruzi* and studies are underway to improve the resolution of diffraction of these crystals in order to determine their structures.

*M. tuberculosis* is an actinomycete. During our study of MT we began to learn about other actinomycetes, parti-

cular streptomycetes which are known to produce a large number of secondary metabolites, many of which have important biomedical roles. Specifically, almost 70% of antibiotics used in human and animal medicine are produced by streptomycetes. In collaboration with Fred Guengerich here at Vanderbilt and Steve Kelly and David Lamb at University of Wales, Swansea, we have undertaken a project to characterize the 18 P450s in the prototypic streptomycete, *Streptomyces coelicolor.*[40] Our goal is to determine the function of these CYPs, then their structure and finally using site-directed mutagenesis generate novel secondary metabolites. By developing this paradigm it will be possible to produce novel biomedical molecules which might prove to be important drugs. We have identified the biochemical activity of P450s in *S. ceolicolor*[41,42] and in some cases their structure[43] and are just beginning site-directed mutagenesis studies aimed at developing a strategy for producing novel secondary metabolites with potential as important biomedical compounds.

## 5. Conclusion

P450 research has been underway for about 50 years. There probably are more than 10,000 scientists throughout the world who are currently actively involved in this research area, in industry, governmental laboratories and academic labs. While drug metabolism has led this research in the past, the topics of P450 research are much more widely distributed today. The discovery of the more than 8,500 CYP genes has arisen in a significant part because of the recently accelerated effort in genomic sequencing. While drug metabolism still provides the majority of efforts in P450 research, considerable effort is being invested in study of P40s which metabolize endogenous substrates, P450s in pathogens and other organisms which can serve as drug targets, and the many "orphan" (unknown function) P450s found in biology from humans to bacteria. Furthermore, considerable effort is still underway in trying to understand in detail the kinetic mechanisms of P450 function, the biophysical properties of P450s and structural changes which occur during function. Each researcher in this field could identify their top questions about P450s and while there would be considerable overlap, the number of novel questions would clearly demonstrate why this continues to be such an interesting field of research. My questions include 1) how does heme bind to both membrane bound and soluble forms of CYP and what roles does this binding play in the final protein conformation? 2) how does P450 interact with its reductase particularly in the endoplasmic reticulum where the amount of P450 is much greater than that of reductase and do P450s have to search for reductases after binding substrate? 3) how do very nonpolar substrates, such as lanosterol, find their way from a membrane environment into the P450 (CYP51) active site and are the enzymes of the postsqualene portion of the sterol biosynthetic pathway localized together in the membrane? 4) unlike sterol 14α-demethylases which contain a group of amino acids identified as a signature sequence, fatty acid hydroxylation is catalyzed by different gene families in different organisms: is there a small group of signature amino acids that would allow identification of fatty acid hydroxylases from different gene families? I present these questions to illustrate a point. While we know a great deal about the biochemical, biophysical and catalytic features of P450s, there is much to be done to understand the biological details of these features. Surely this field of research will continue at a rapid pace for many years to come. I am fortunate that my career has spanned virtually the complete lifetime of P450 research. I have had the opportunity to know a large number of investigators in this field over that time. But I see the first 50 years of P450 research as just the beginning. In the decades to come, we will learn many new and fascinating things about this superfamily. P450s indeed provide a rich and exciting area of research for the future.

## 6. Acknowledgments

In closing, I am grateful to all of my friends and colleagues from more than 46 years of biochemistry research. They are too numerous to mention here other than to highlight my mother and father (Storrs and Helen Waterman) who as part of their endless love supported my transition from dentistry to biochemistry at a time when most parents dreamed that their first-born would become a doctor or dentist. My wife, Mimi and children, Peter and Amanda, have graciously put up with my often selfish focus on research and I am forever grateful for their love and support. My two mentors, Howard Mason and Ron Estabrook taught me the lessons I needed to become a successful investigator even though I wasn't always the most willing student. Their support, friendship and commitment to my career can never be over stated. Finally, the many talented students and postdocs in the Waterman lab always made the boss look good. My deepest thanks to each of you. My work on CYP51 and *S. coelicolor* CYPs will continue as long as resources and energy permit.

## 7. References

1. H. S. Mason, *Adv. Enzymol.* **1957**, *19*, 179–233.
2. O. Hayaishi, *Ann. Rev. Biochem.* **1962**, *31,* 25–46.
3. Y. Hashimoto, T. Yamano, H. S. Mason, *J. Biol. Chem.* **1962**, *237*, PC3843–PC3844.
4. M. R. Waterman, H. S. Mason, *Biochem. Biopys. Res. Commun.* **1970**, *39*, 450–454.
5. F. C. Yong, T. E. King, S. Oldham, M. R. Waterman, H. S. Mason, *Arch. Biochem. Biophys.* **1970**, *13*, 96–100.

6. T. Omura, R. Sato, *J. Biol. Chem.* **1962**, *237*, PC1375–PC1376.
7. E. R. Simpson, M. R. Waterman, *Ann. Rev. Physiol.* **1988**, *50,* 427–440.
8. E. R. Simpson, M.R. Waterman in: J. J. DeGrott (Ed.): *Endocrinology* (3rd Edition) Grune and Staton, Inc. **1995**, 1630–1641.
9. A. Y. H. Lu, M. J. Coon, *J. Biol. Chem.* **1968**, *243*, 1331–1332.
10. L. H. Bothelo, D. E. Ryan, W. Levin, *J. Biol. Chem.* **1979,** *254,* 5635–5640.
11. F. P. Guengerich in: P. R. Ortiz de Montellano (Ed.): *Cytochrome P450: Structure, Mechanism and Biochemistry* **2005,** pp. 377–530.
12. Y. Fujii-Kuriyama, Y. Mizukami, T. Taniguchi, M. Muramatsu in: R. Sato, R. Kato, (Eds.); *Microsomes, Drug Oxidations and Drug Toxicity*, Japan Scientific Societies Press, Tokyo **1982,** pp. 589–596.
13. M. Nakamura, M. Negishi, M. Altieri, Y. J. Chen, T. Ikeda, D.W. Nebert, *Eur. J. Biochem* **1983,** *134,* 19–25.
14. T. L. Poulos, B. C. Finzel, I. C. Gunsalus, G. C. Wagner, J. Kraut, *J. Biol. Chem.* **1985**, *260*, 16122–16130.
15. D. R. Nelson, H. W. Strobel, *J. Biol. Chem.* **1988,** *263,* 6038–6050
16. O. Gotoh, *J. Biol. Chem.* **1992**, *267*, 83–90.
17. D. W. Nebert, M. Adesnik, M. J. Coon, R. W. Estabrook, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, B. Kemper, W. Levin, I. R. Phillips, R. Sato, M. R. Waterman, *DNA* **1987**, *6,* 1–11.
18. R. N. DuBois, E. R. Simpson, J. Tuckey, J. D. Lambeth, M. R. Waterman, *Proc. Natl. Acad. Sci. USA*, **1981**, *78*, 1028–1032.
19. M. F. Matocha, M. R. Waterman, *J. Biol. Chem.* **1985**, *260*, 12259–12265.
20. N. Nabi, T. Ishikawa, M. Ohashi, T. Omura, *J. Biolchem. (Tokyo)* **1983***,* 94 1595–1515,
21. R. N. DuBois, E.R. Simpson, R. E. Kramer, M. R. Waterman, *J. Biol. Chem.* **1981**, *256,* 700–7005.
22. M. B. Sewer, M. R. Waterman, *Endocrinol.* **2002**, *143*, 1769–1777.
23. M. X. Zuber, E. R. Simpson, M. R. Waterman, *Science* **1986**, *234,* 1258–1261.
24. M. X. Zuber, J. I. Mason, E. R. Simpson, M. R. Waterman, *Proc. Natl. Acad. Sci. USA* **1988**, *85,* 699–703.
25. T. Sakai, M. Shibata, Y. Yabusaki, H. Murikami, H. Ohkawa, *DNA* **1989**, *88,* 5597–5601.
26. A. Asseffa, S. J. Smith, K. Nagata, J. Gillette, H. V. Gelboin, F. J. Gonzalez, *Arch. Biochem.* **1989***, 274,* 481–490.
27. H. J. Barnes, M. P. Arlotto, M. R. Waterman, *Proc. Natl. Acad. Sci. USA* **1991**, *88,* 5597–5601.
28. A. Wada, P.A. Mathew, H. J. Barnes, D. Sanders, R.W. Estabrook, M.R. Waterman, *Arch. Biochem. Biophys.* **1991**, *290*, 376–380.
29. G. I. Lepesheva, M. R. Waterman, *Mol. Cell. Endo.* **2004**, *215,* 165–170.
30. D. Rozman, M. Stromstedt, L-C. Tsui, S. W. Scherer, M. R. Waterman, *Genomics* **1996**, *38*, 371–381.
31. D. Rozman, M. Fink, G. M. Fimia, P. Sassone-Corsi, M. R. Waterman, *Mol. Endocrinol.* **1999**, *13,* 1951–1962.
32. S. T. Cole, R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Cas, C. E. Barry III, F. Tekaia, K. Babcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M.-A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead, B. G. Barrell, *Nature* **1998**, *393*, 537–544.
33. A. Bellamine, A. T. Magla, D. Nes, M. R. Waterman, *Proc. Natl. Acad. Sci. USA* **1999**, *96,* 8937–8942.
34. L. M. Podust, T. L. Poulos, M. R. Waterman, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3068–3073.
35. G. I. Lepesheva, C. Virus, M. R. Waterman, *Biochemistry* **2003**, *42*, 9091–9101.
36. G. I. Lepesheva, D. W. Nes, W. Zhou, G. C. Hill, M. R. Waterman, *Biochemistry* **2004**, *33,* 10789–10799.
37. G. I. Lepesheva, N. G. Zaitseva, D. W. Nes, W. Zhou, M. Arase, J. Liu, G. C. Hill, M. R. Waterman, *J. Biol. Chem.* **2006**, *281*, 3577–3585.
38. P. A. Williams, J. Cosme, V. Sridhar, E. F. Johnson, D. E. McRee, *Molec. Cell.* **2000**, *5*, 121–131.
39. I. A. Pikuleva, N. Mast, M. White, I. B. Jorkhem, E. F. Johnson, D. Stout, *15th International Conference on Cytochrome P450 Book of Abstracts* **2007**, pp. 34.
40. D. C. Lamb, F. P. Guengerich, S. L. Kelly, M. R. Waterman, *Expert Opinion on Drug Metabolism and Toxicology* **2006**, *2*, 27–40.
41. B. Zhao, F. P. Guengerich, A. Bellamine, D. C. Lamb, M. Fzunukawa, L. Lei, L.M. Podust, M. Sundarmoorthy, J. A. Kalatizis, L. M. Reddy, S. L. Kelly, B. S. Moore, D. Stec, M. Voekler, J. R. Falck, T. Shimada, M. R. Waterman, *J. Biol. Chem*. **2005**, *280*, 11599–11607.
42. Y-J. Chun, T. Shimada, R. Sanchez-Ponce, S. L. Kelly, M. R. Waterman, D. C. Lamb, F. P. Guengerich, *J. Biol. Chem.* **2007**, *282*, 17486–17500.
43. B. Zhao, F. P. Guengerich, M. Voehler, M. R. Waterman, *J. Biol. Chem.* **2005**, *280*, 42188–41297.

## Povzetek

Članek izhaja z zaključnega predavanja 15. mednarodne konference o citokromih P450, ki je potekala junija 2007 na Bledu. Avtorju je konferenca ponudila priložnost, da s svojega vidika osvetli najpomembnejše točke svoje znanstvene kariere in pri tem omeni tudi ključne dogodke v raziskavah citokromov P450, ki so vplivale na njegov raziskovalni razvoj. Nemogoče je omeniti vsa pomembna spoznanja. Avtor jih je izbral nekaj in se opravičuje, ker ni mogel povzeti vseh, ki so vplivala nanj v več kot 46 let dolgi raziskovalni poti. Poudarja, da nikakor ne gre za refleksijo upokojenega znanstvenika, ampak za 67 let starega raziskovalca, ki upa, da bo v znanstveno raziskovalnem delu ostal aktiven tudi v prihodnih letih. Za podporo, ki je omogočila nastanek tega pogleda v preteklost, se zahvaljuje dvema prijateljema in kolegoma, Fredu Guengerichu in Damjani Rozman.