

BIOGRAPHICAL SKETCH

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NAME: Paul Alan Lindahl

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POSITION TITLE: Professor of Chemistry and of Biochemistry and Biophysics

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
North Park College, Chicago IL	B.A.	1975-1979	Chemistry, Mathematics
Illinois Institute of Technology, Chicago, IL	---	1978	Chemistry
University of California at Berkeley, CA	---	1979-1980	Biochemistry
Massachusetts Institute of Technology Cambridge MA.	Ph.D.	1980-1985	Chemistry
University of Minnesota, Minneapolis MN	---	1985-1988	Biophysics

A. Personal Statement

I have a unique combination of expertise and background experiences that allow me to direct research on ***Iron Trafficking and Regulation in Biological Systems***. I have over 40 years of experience doing research in the fields of bioinorganic and biophysical chemistry, including 32 years on the faculty at Texas A&M University (TAMU), 3 years as a post-doc in Eckard Münck's lab at the University of Minnesota, 5 years as a graduate student at the Massachusetts Institute of Technology, 1 year as a graduate student in the Biochemistry department at the University of California Berkeley, and 3 months as an undergraduate researcher at the Illinois Institute of Technology. I have published 138 research articles and have given invited lectures on my research at about the same number of conferences and universities. I have served as research advisor to 25 graduate students (22 obtaining Ph.D.'s), 9 post-docs, and over a dozen undergraduates. All but 1 of my PhD students have gone on to have successful careers in chemistry- or biochemistry-related fields. My research program has been funded continuously by the NIH since 1993 (8 R01's and 1 year of bridge funding). In the past 30 years, I have served on ca. 16 study sections at the National Institutes of Health, National Science Foundation and Department of Energy for which I have reviewed approximately 100 research proposals. I have reviewed hundreds of primary research articles and ca. 7 tenure cases. Other community service includes running Mössbauer and EPR spectra for numerous other scientists; occasionally a collaboration develops and papers are published, but more often spectra are collected and analyzed with no tangible benefit to me or my group. I was trained at the intersection of biochemistry, inorganic chemistry, and biophysical spectroscopy. For my first 20 years of research, I probed the catalytic mechanisms of metalloenzymes using various spectroscopic, kinetic, genetic, bioinorganic, and biochemical methods. I have studied nitrogenases, hydrogenases, acetyl-coenzyme A synthases, the associated corrinoid Fe/S protein, and carbon monoxide dehydrogenases. See my contributions I and II below. Starting about 17 years ago, I became fascinated with systems biology and mathematical modeling (Contribution III), and used these approaches to probe the mechanisms of cellular self-replication. Five years later, I began to apply these methods to iron trafficking in mitochondria, whole yeast cells, and human cells (Contribution IV). Towards this end I have outfitted my laboratory with novel instruments, including 4 Mössbauer spectrometers, an Electron Paramagnetic Resonance spectrometer (now a Departmental instrument), and a liquid chromatography system installed in a refrigerated anaerobic glove box and interfaced to an on-line inductively-coupled plasma mass spectrometer (LC-ICP-MS). These cutting-edge capabilities have allowed my lab to make significant and innovative advances in new directions – e.g. in understanding the

trafficking and regulation of iron in cells. In the past decade, numerous “labile metal pools” have been discovered in cells, using custom-designed chelator probes or sensors. The problem is that the small metal complexes that compose these pools cannot be well characterized by these methods, because the complexes are destroyed during detection. Using our LC-ICP-MS system, we have detected and can isolate numerous low-molecular-mass metal complexes that are involved in these processes. We are now actively identifying and characterizing some of these complexes. Five years ago, I began to apply these biophysical and bioanalytical techniques to the study of iron in ^{57}Fe -enriched mouse organs. In the past 4 years, we have begun to examine blood plasma from pigs and humans in efforts to characterize non-transferrin-bound iron (NTBI). We have made many exciting discoveries along the way – so many that I cannot possibly describe them all here. In summary, I have an unusually diverse background with decades of experience in biochemistry, metalloenzymology, cell biology, transition metal chemistry, chromatography, spectroscopy, kinetics, mathematical modeling, and with more recent experience in mammalian systems and bioanalytical chemistry. This diverse background and broad expertise, when combined with my enthusiasm, creativity, and courage to explore new avenues of research, ensure the continued success of my research program.

B. Positions and Honors

1978	National Science Foundation undergraduate fellowship
1979	Summa cum laude, North Park College
1985 – 1988	Post-doctoral fellow at the University of Minnesota
1986 – 1988	NIH Post-doctoral fellowship
1988 – 1994	Assistant Professor of Chemistry, Texas A&M University
1994 – 1999	Associate Professor of Chemistry, Texas A&M University
1996	Visiting Scholar, Department of Biochemistry, Rice University, Houston TX
1998	Associate Professor of Biochemistry and Biophysics, Texas A&M University
1999 – present	Full Professor of Chemistry and of Biochemistry and Biophysics, Texas A&M University

C. Contributions to Science

I. Nitrogenase: I have an extensive background in biophysical chemistry, especially with regard to the magnetic and electronic properties of metal-sulfur clusters. My Ph.D. research addressed a 15 year-old puzzle regarding an unusual magnetic property of an EPR signal from the nitrogenase iron protein. I used Mössbauer, EPR, X-ray absorption spectroscopies, and magnetic susceptibility to show that this protein contains a single Fe_4S_4 cluster in which the reduced 1+ core state is stabilized in a mixture of 2 spin states (30% $S = \frac{1}{2}$; 70% $S = \frac{3}{2}$). This was the first demonstration that $[\text{Fe}_4\text{S}_4]^{1+}$ clusters can be stabilized in a state other than $S = \frac{1}{2}$. This unusual spin-state mixture has now been observed in numerous $[\text{Fe}_4\text{S}_4]^{1+}$ proteins and is actually more common than proteins containing $[\text{Fe}_4\text{S}_4]^{1+}$ clusters that are exclusively $S = \frac{1}{2}$. $[\text{Fe}_4\text{S}_4]$ clusters are common in biology, for example in mitochondrial respiratory complexes, in hundreds of metalloenzymes, and in proteins involved in DNA replication and repair. In 2012, I published a paradigm-shifting hypothesis regarding the use of metal-metal bonds in nitrogenase, acetyl-coenzyme A synthase, carbon monoxide dehydrogenase and hydrogenase enzymes. This hypothesis, which has received additional support in the past few years, explains how very low redox states, e.g. Fe(I) and Ni(0), are stabilized in biology. This year, working with Mike Rose at UT Austin, we used Mössbauer and EPR spectroscopies to investigate the effect of a central carbon atom in a redox-active 6-Fe cluster on the redox properties of each Fe in the cluster. We discovered that differential bonding of the central carbon to the irons in the cluster is responsible for a redox disproportionation reaction in which some cluster irons are oxidized while others are reduced. A similar phenomenon might occur in the nitrogenase FeMoco cofactor, which also has a central carbon. Our study may help explain how the N_2 triple bond is reduced.

Highlighted Publications:

- "Mössbauer, EPR, and Magnetization Studies of the *Azotobacter vinelandii* Fe Protein"; **Paul A. Lindahl**, Edmund P. Day, Thomas A. Kent, William H. Orme-Johnson, and Eckard Münck. *J. Biol. Chem.* **1985**, 260(20), 11160 - 11173.
- "EPR and Mössbauer Studies of Nucleotide-Bound Nitrogenase Iron Protein from *Azotobacter vinelandii*"; Paul A. Lindahl, Nancy J. Gorelick, Eckard Münck, and William H. Orme-Johnson. *J. Biol. Chem.* **1987**, 262(31), 14945 - 14953.
- "Metal-Metal Bonds in Biology" **Paul A. Lindahl**. *J. Inorg. Biochem.* **2012**, 106, 172-178.
- "Structures, Interconversions and Spectroscopy of Iron Carbonyl Clusters with an Interstitial Carbide: Localized Metal Center Reduction by Overall Cluster Oxidation" Subramaniam Kuppaswamy, Joshua D.

II. Acetyl-CoA synthase/Carbon Monoxide Dehydrogenase (ACS/CODH). This highly complex and fascinating metalloenzyme is found in anaerobic bacteria; it plays a major role in energy metabolism and the global carbon cycle. I studied this enzyme for 25 years and published over 60 research articles focused on it. When I began to work on ACS/CODH, little was known regarding the structure and function of its metal clusters, and little was known about the mechanism of catalysis. In 1992, my student Woonsup Shin and I discovered that the two catalytic activities of the enzyme originated from distinct active sites (the A cluster and the C cluster), and that the site used for acetyl-coenzyme A synthesis (the A-cluster) contains a labile Ni center. My student David Barondeau and I performed a remarkable series of experiments showing that the most popular mechanism of catalysis (involving Ni(II)) was incorrect, that the actual mechanism required a two-electron activation of the A-cluster and the probable formation of the highly unusual Ni(0) state. Two years later, my student Ernie Maynard and I discovered that there is a tunnel connecting the two active sites, through which CO is channeled. Working in collaboration with Juan Fonticella-Camps (Grenoble FR), we solved the x-ray structure of the enzyme. This was the first structure to show that the active A-cluster site contains a Ni site (rather than a Cu site which an MIT group erroneously thought was the active form of the enzyme). Virtually all of our earlier predictions (e.g. the existence of the tunnel and cluster heterogeneity) were confirmed by the structure. Using rapid-kinetic methods, post-docs Xiangshi Tan and Ivan Surovtsev and I developed the first experimentally-grounded mathematical model describing the catalytic mechanism of the enzyme. The model identifies the rate-limiting step and includes all known intermediates. My contribution to this field has been much more extensive than this, but it cannot be adequately described in a paragraph.

Highlighted Publications:

- “Methylation of Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum* and the Mechanism of Acetyl-CoA Synthesis” David P. Barondeau and **Paul A. Lindahl** *J. Am. Chem. Soc.* **1997**, 119, 3959-3970.
- “Active Acetyl-CoA Synthase from *Clostridium thermoaceticum* obtained by Cloning and Heterologous Expression of *acsAB* in *Escherichia coli*”, Huay Keng Loke, George Bennett, and **Paul A. Lindahl**, *Proc. Natl. Acad. Sci. USA* **2000**, 97, 12530-12535
- “Ni-Zn-[Fe₄S₄] and Ni-Ni-[Fe₄S₄] Clusters in Closed and Open Subunits of Acetyl-CoA Synthase/Carbon Monoxide Dehydrogenase” Claudine Darnault, Anne Volbeda, Eun Jin Kim, P. Legrand, Xavier Vernede, **Paul A. Lindahl**, and Juan C. Fontecilla-Camps, *Nature Structural Biology*, **2003**, 10, 271-279.
- “Acetyl-coenzyme A synthase: a beautiful metalloenzyme” **Paul A. Lindahl** in “Bioorganometallic Chemistry” (Ulf-Peter Apfel and Wolfgang Weigand, Editors). De Gruyter, Berlin, Germany. **2020**, Chapter 7, pp 279-312. <https://doi.org/10.1515/9783110496574-007>

III. Computational models of cellular self-replication. With the advent of the -omics era in the mid 90's and with new and exciting proposals coming from the origin-of-life field, I became fascinated with the possibility of describing the process of cellular self-replication from a chemical perspective. I contacted a mathematician (Jeff Morgan, then at TAMU) who has expertise in differential equations and dynamical systems, and we began to collaborate on a project focused on this grand goal. A post-doc in my lab (Ivan Surovtsev) with expertise in chemical physics soon joined the project, and the three of us developed a mathematical framework for modeling a whole growing and dividing *in silico* cell. A post-doc from Morgan's group (Zhigang Zhang) joined us a few years later. Together we modeled the shape of the synthetic cell and found that it was unable to divide. We realized the need for a model that describes the final steps of cell division in which a protein ring forms at midcell, and then constricts until two cells are generated. Thus, we modeled the assembly and contraction of the FtsZ ring in *E. coli*. We then incorporated this aspect into a whole cell math model possessing sophisticated growth and cell cycle division properties. In a recent study, we developed a mathematical model to describe the mechanism by which the ring is positioned at midcell. With all members of the modeling group now dispersed, this theoretical project is in hibernation. However, some of the concepts that we developed are being used in the field, and I have incorporated some of them in modeling iron trafficking and regulation in growing cells, a project which is ongoing in my lab.

Highlighted Publications:

- “Whole-cell modeling framework in which biochemical dynamics impact aspects of cellular geometry” Ivan V Surovtsev, Jeffrey J Morgan and **Paul A Lindahl**, *Journal of Theoretical Biology*, **2007**, 244, 154-166.
- “Kinetic Modeling of the Assembly, Dynamic Steady State, and Contraction of the FtsZ Ring in Prokaryotic Cytokinesis” Ivan V. Surovtsev, Jeffrey J. Morgan, **Paul A. Lindahl**, **2008** *Plos Computational Biology*, 4, 1-19.
- “Mathematical modeling of a minimal protocell with coordinated growth and division” Ivan V. Surovtsev, Zhigang Zhang, **Paul A. Lindahl**, and Jeffrey J. Morgan **2009**, *Journal of Theoretical Biology* 260, 422-429.
- “Mathematical model for positioning the FtsZ contractile ring in *Escherichia coli*” Zhigang Zhang, Jeffrey J. Morgan, and **Paul A. Lindahl** *Journal of Mathematical Biology* **2014**, 68, 911-930

IV. Iron metabolism in mitochondria, yeast and human cells. Being an experimentalist at heart, I wanted an experimental program that would complement my interests in mathematical modeling and systems biology, and which would utilize my expertise in transition metal chemistry and spectroscopy. I became intrigued by mitochondria, as they are cell-derived organelles in which iron-sulfur clusters are assembled and Fe is inserted into porphyrins during heme biosynthesis. Most of the iron that enters the cell is trafficked to mitochondria for these purposes. Given my background in anaerobic biochemistry and Mössbauer spectroscopy (the most powerful tool to study Fe), this was an ideal focus. Our first experiment was to examine ⁵⁷Fe-enriched mitochondria using a suite of biophysical methods, to evaluate the types of Fe complexes present and determine their concentrations. Besides observing spectral features due to the respiratory complexes, we discovered a large pool of nonheme high-spin Fe^{II} which we eventually showed is used as feedstock for Fe/S cluster biosynthesis. We also characterized the “Fe accumulation” phenotype that is found with deletions in frataxin (giving rise to the disease Friedrich’s Ataxia) and other iron-sulfur-cluster-assembly mutants. The other major site of Fe in yeast cells is vacuoles; we discovered that these organelles store Fe as mononuclear nonheme high-spin Fe^{III} species. We have performed similar studies on human cells. Now we are using mathematics to model Fe trafficking and regulation in both yeast and human cells. We have also designed and built a novel liquid chromatography system linked to an on-line ICP-MS detector, and have used this hybrid LC-ICP-MS system to detect and isolate labile low-molecular-mass metal complexes that are involved in metal ion trafficking. We are the only group worldwide that has these combined bioanalytical and biophysical capabilities – capabilities that are stimulating major new insights into trafficking and regulation of iron in living systems.

Highlighted Publications:

- “Recovery of *mrs3Δmrs4Δ* *Saccharomyces cerevisiae* cells under iron-sufficient conditions and the role of Fe580” Michael J. Moore, Joshua D. Wofford, Andrew Dancis, and **Paul A. Lindahl** (2018) *Biochemistry* 57, 672-683.
- “Evidence that a respiratory shield in *Escherichia coli* protects a low-molecular-mass Fe^{II} pool from O₂-dependent oxidation” Joshua D. Wofford, Naimah Bolaji, Nathaniel Dziuba, F. Wayne Outten, and **Paul A. Lindahl** (2019) *Journal of Biological Chemistry*, 294, 50-62.
- “Chromatographic detection of low-molecular-mass metal complexes in the cytosol of *Saccharomyces cerevisiae*” Trang Q Nguyen, Joshua E Kim, Hayley N. Brawley, and **Paul A. Lindahl** (2020) *Metallomics*, 12, 1094-1105.
- “Mössbauer and LC-ICP-MS investigation of iron trafficking between vacuoles and mitochondria in *Vma2Δ* *Saccharomyces cerevisiae*. Joshua E. Kim, Shaik Waseem Vali, Trang Q. Nguyen, Andrew Dancis, and **Paul A. Lindahl** (2021) *Journal of Biological Chemistry*. <https://doi.org/10.1074/jbc.RA120.015907>

V. Iron metabolism in Vertebrate Animals. The newest direction of my research program is to apply our novel bioanalytical and biophysical methods to biomedicine. In 2012 the NIH allowed me to alter an R01 grant such that we could perform studies on vertebrate animals. Mössbauer spectroscopy requires ⁵⁷Fe enrichment, and so we enriched a colony of mice with this isotope. I directed these studies with advice from Professor Louise Abbott in the School of Veterinary Medicine at TAMU. We investigated the brain, liver, heart, spleen and kidneys at different developmental stages - *no other lab worldwide had performed such studies*. We discovered that most of the Fe in the fetal brain is present as ferritin, but then, in the first few weeks of life, there is a burst of mitochondrialogenesis, such that the ferritin iron converts into mitochondrial Fe. Under Fe-deficient growth conditions, the brain depletes its stores of ferritin Fe, but the level of mitochondrial Fe remains no different than

in brains from Fe-sufficient mice. We performed similar studies on the liver. Here we see a major efflux of Fe during the first few weeks of life; this Fe is probably delivered to other organs for development. Under Fe-deficient conditions, the liver suffers a significant loss of mitochondria. We found that the heart contains more mitochondrial iron than the other organs, including the brain. Using our bioanalytical methods, we have discovered over a dozen low-molecular-mass metal complexes in the brain. Finally, we invented a new method of investigating the kinetics of nutrient Fe import into the blood and then into organs called *Pup Swapping*. In this method, mice that are enriched in one isotope of Fe (either ^{56}Fe or ^{57}Fe) are nursed by lactating females that are enriched in the other isotope. This has allowed us to follow the import kinetics with greater precision than in previous studies (where radioisotope tracers are injected). To our surprise, we found that non-transferrin-bound-iron rather than transferrin-bound-iron plays the major role in delivering Fe into developing healthy mouse organs. These intriguing results, which could have significance for treating Fe overload diseases, form the foundation for some current studies. In collaboration with Dr. Joanne Hardy (Vet school, TAMU), we are currently investigating NTBI in pig blood plasma. We have developed a novel experiment in which the kinetics of ^{57}Fe import from the stomach can be monitored as it passes through the intestines and into the blood where it ultimately binds transferrin. Using our LC-ICP-MS system, we have discovered numerous candidate NTBI species with masses < 2500 Da. We are now poised to examine blood from patients with hereditary hemochromatosis (our IRB was just approved). We hope to detect and identify NTBI species associated with iron-overload diseases.

Highlighted Publications:

- “Speciation of iron in mouse liver during development, iron deficiency, IRP2 deletion, and inflammatory hepatitis” Mrinmoy Chakrabarti, Allison L. Cockrell, Jinkyu Park, Sean P. McCormick, Lora S. Lindahl and **Paul A. Lindahl**, *Metallomics*, **2015**, 7, 93-101.
- “Mössbauer spectra of mouse hearts reveal age-dependent changes in mitochondrial and ferritin iron levels” Joshua D. Wofford, Mrinmoy Chakrabarti, and **Paul A. Lindahl**, *Journal of Biological Chemistry* **2017**, 292, 5546-5554.
- “Low-molecular-mass iron in healthy blood plasma is not predominately ferric citrate” Nathaniel Dziuba, Joanne Hardy, and **Paul A. Lindahl**, *Metallomics*, **2018**, 10, 802-817.
- “Low-molecular-mass iron complexes in blood plasma of iron-deficient pigs do not originate directly from nutrient iron” Nathaniel Dziuba, Joanne Hardy, and **Paul A. Lindahl**, *Metallomics*, **2019**, 11. DOI: 10.1039/c9mt00152b

The following is a URL to a full list of my published work as found SciENcv.

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1NQab5zxl0kj/bibliography/47398164/public/?sort=date&direction=ascending>

D. Additional Information: Research Support and/or Scholastic Performance

Organization: National Institutes of Health

Grant number: R35 GM127021

Title of Grant: "Iron Trafficking and Regulation in Biological Systems"

Dates: 2018-2023

Organization: Robert A. Welch Foundation

Grant number: A1170

Title: "Characterization of Low-Molecular-Mass Metal Complexes in Biological Systems"

Dates: 2020-2022

Organization: National Science Foundation

Grant number: MCB-1817389

Title of Grant: " Design and Testing of Math models of Iron Trafficking and Regulation in Eukaryotes"

Dates: 2018-2021