**Introduction:** All eukaryotic cells, from yeast to humans, require transition metals such as iron, copper, manganese, and zinc. In fact, metals bind to nearly half of all proteins in cells. Transition metals have unique catalytic properties that make them essential for cellular survival. Ironically, these same properties also make them dangerous to cells; thus, the “trafficking” of metals within cells must be tightly regulated. Dysregulation of metal ion trafficking is associated with certain diseases, often involving oxidative damage and perhaps aging. Our lab is interested in the cell biology of such metal ions. Our primary focus is iron, but we are also interested in copper (and currently nickel) trafficking. Most of our studies involve budding yeast, *Saccharomyces cerevisiae*, as it is the “workhorse” of eukaryotic cells in which molecular-level mechanisms can be most readily probed. However, we also study or have recently studied bacteria (mainly *Escherichia coli*), archaea (*Pyrococcus furiosus*), human Jurkat cells, as well as blood and tissues from various genetic strains of mice – and even pigs!

**Labile Metal Pools:** Some metals are trafficked by binding to “chaperone” proteins in the cytosol which deliver them to various recipient proteins. However, “labile metal pools” (LMPs) are also involved in trafficking although their exact roles are less well understood. LMPs consist of small metal complexes with nonproteinaceous ligands. They were discovered 50 years ago, but little is known regarding their chemical compositions or structures. We are using liquid-chromatography interfaced with inductively-coupled plasma mass spectrometry (LC-ICP-MS) to detect, identify, and characterize these pools. This is challenging because of the inherent lability of the complexes – they tend to fall apart as they migrate through chromatography columns. Many of these complexes are redox-active, such that the LC must be located in an anaerobic glove box. We have invented various methods for dealing with this problem, and are making progress on identifying these pools, by interfacing with electrospray ionization mass spectrometry (ESI-MS). We also use various genetic strains of yeast to probe the cellular functions of these pools.

In the future, we want to examine labile metal pools in human cytosol and other organelles using our LC-ICP-MS system. We suspect that these pools will be similar to those in yeast, but there are indications that imply significant differences. These studies would have direct relevance to various metal-associated diseases in which trafficking has become dysregulated. On a more practical side, we will continue to explore the effect of non-aqueous solvents on these pools; our goal is to find conditions causing these complexes to remain intact thereby allowing unambiguous chemical identification using downstream powerful analytical tools such as ESI-MS and NMR spectroscopy. Most of our studies have used size-exclusion chromatography (SEC) columns, but in the future, hydrophilic interaction chromatography (HILIC) and other types of chromatography will be explored. We are considering purchasing a dedicated ESI-MS that would allow us to characterize the metal-related metabolome of the cell. This would be a huge advantage in our quest to characterize these mysterious labile metal pools.

**Ironomics of a yeast cell:** Rather than focus on a single iron-containing protein, we take a “systems’ level” approach to understanding iron trafficking. Iron is at the active site of nearly 100 proteins in a yeast cell. Iron plays important roles in cellular energetics, metabolism, and DNA replication and repair. When iron enters a cell, it is trafficked through the cytosol to various organelles, such as mitochondria, nuclei, vacuoles, endoplasmic reticula, and the Golgi. How can we hope to understand such a complicated process as a unified system? We use a few novel approaches to do this.

**Mössbauer Spectroscopy of whole cells and organelles:** We use many standard biochemical and molecular-biological methods for studying these processes, but Mössbauer spectroscopy stands out because it is ***the*** most powerful spectroscopic probe of iron. Like NMR, Mössbauer measures the energies of nuclear transitions involving 57Fe (I = ½); it can distinguish different oxidation states and spin states of iron, as well as identify various types of iron-sulfur clusters, heme centers, and nonheme iron centers. There are very few Mössbauer spectrometers world-wide, and we have 4 instruments in the lab. Using Mössbauer we analyze various genetic strains of yeast, grown under various conditions. We compare the iron content of such cells to that of wild-type cells. Typically, we isolate organelles and identify their iron contents. We have previously isolated cytosol, mitochondria, and vacuoles, and are currently focused on nuclei and endoplasmic reticula. We aim to assemble the “pieces of the puzzle” to characterize the entire iron-ome of the cell. No one does this.

**Computational kinetic Modeling:** A second approach that stands out is our use of computational methods to help analyze the kinetics of iron trafficking in yeast. Working in collaboration with Dr. Jay Walton (emeritus Professor of Mathematics at TAMU), we are developing ordinary-differential-equations-based models that describe the kinetics of iron trafficking and regulation in yeast cells. Students interested in this project should have some experience in computer programing and chemical kinetics. We are currently developing “toy” models (10-15 reactions) that describes the mechanism driving the transformation of a healthy cell to a state that characterizes the mitochondrial-based iron-related disease called *Friedreich’s Ataxia*. We are also developing a larger more comprehensive model (169 reactions and 79 components), which includes all iron-related processes that occur in a yeast cells. For both models, we use our experimental results to connect these models to what is observed experimentally.

Model simulations and predictions will be used to help direct the most informative experiments to pursue. New experimental results are used to modify and improve models and their predictive powers. The long-term goal is to understand – on the molecular level – the phenotypes that are observed when various iron-related genes are deleted or mutated in the cell. Currently, these phenotypes are interpreted superficially. Imagine that such a program, with real predictive power, would be available to medical doctors attempting to diagnose and treat a patient with an iron-related disease. With such a program, they could treat the disease at an unprecedented level of sophistication and effectiveness.

**Iron metabolism in mice:** We are just completing a study of mice that have the genetic disease *Hereditary Hemochromatosis*. We are characterizing an iron-complex in blood plasma called NTBI (nontransferrin-bound iron). We have also characterized the accumulation of iron in their tissues (liver, heart, spleen, etc) by enriching the mice with 57Fe and then collecting Mössbauer spectra of whole organs. The results of our current study are quite exciting, and they call for future follow-up studies in similar directions. Another animal study of interest would involve cancer. Iron plays an important role in cancer (it accumulates in cancerous tumors), and it would be exciting to examine this form of iron using our novel methods. We would use a genetic strain of mice that is unusually sensitive to developing cancer, raise them with 57Fe in their diet, then dissect the tumors and collecting Mössbauer spectra.

**Copper trafficking:** Although most of our studies involve iron, we are also probing the means by which copper is trafficked from the site at which it enters the cell to the mitochondria where it is installed in Respiratory Complex IV, cytochrome c oxidase. We are attempting to solve this 50-year-old puzzle using our LC-ICP-MS system in conjunction with ESI-MS, selected genetic strains of yeast, and various copper isotopes. We have identified a copper-containing species that is found in both cytosol and mitochondria and are probing whether it might be the sought-after trafficking species. We have also detected some low-intensity low-molecular-mass copper species in the cytosol that have not been observed previously and are investigating whether they may be involved in inter-organelle trafficking.