Ferritin Research

Characteristics of Ferritins
- found in mammals, plants, invertebrates, and bacteria
- 4 major families of ferritins: classic (mammalian/vertebrate), bacterioferritins, DNA binding protein expressed during starvation (DPS), and plant
- shell forms a hollow spherical protein with a molecular weight of 450,000 daltons
- shell has an outer diameter of 120 Angstroms; an inner diameter of 80 Angstroms
- composed of 24 subunits, which assemble into a tetracosamer
- core is able to accommodate as many as 4500 iron atoms
- core contains ferrous iron which is reduced to ferrous iron when removed from the core.

Organisms and the Environment
Iron is an essential element to virtually all forms of life. Ferritin’s major role in the environment has always been assumed to be the storage of iron in its core. This function is important to the survival of specific organisms because the accumulation of excess iron can be very toxic to the cell. Once the apoferritin is formed, studies can be conducted to investigate the amount of iron that can be stored, what the molecules stress response is, and also whether or not the molecule can sequester elements aside from iron, to prevent the accumulation of other toxic elements.

Research Focus
The focus of my summer research was to test a new method of iron separation, and prove that the method works as well as the traditional method of iron separation. If proven to be successful, this new technique would greatly reduce the amount of preparation time involved when working with ferritins.

Dialysis, the old method of iron separation from the core of ferritins, involved the use of a semi-permeable membrane that allowed the passage of molecules (below a particular molecular weight cutoff) between the buffer and protein solution. Although this technique successfully removes the iron from the core, it requires at least 2 days to complete because the dialysis has to run for two 18-hour sessions.

Because the preparation of the apoferritin is essential to many studies of ferritin, a shorter separation technique would allow for more research to be completed. In the new method, membrane filtration by centrifugation, the iron, after reduction and chelation, is spun through the membrane of a centrifuge tube. The ferritin shell does not pass through the membrane because of the membrane’s molecular weight cutoff. This technique only takes approximately three spins at 20 minutes apiece, far less time than the dialysis method.

Below is some of the data that I accumulated in this study. The UV-Visible Spectrophotometer data shows that the technique works because of a decrease in the iron shoulder at approximately 295 nm. The iron shoulder at 295 nm is virtually nonexistent in the apoferritin. Figure 3 shows an iron assay of the filtrate. This shows the phenanthroline, the iron chelator used, chelated to the iron (II) and is present in the filtrate. An iron assay also showed that the amounts of ferric and ferrous iron in the apoferritin were significantly reduced from those of the holoferitin. Further quantization of this procedure is underway.