



UNIVERSITY OF SOUTHERN MAINE

Development and Screening of a Diagnostic Anti-Mercury

Using Novel Organomercury Bioconjugates

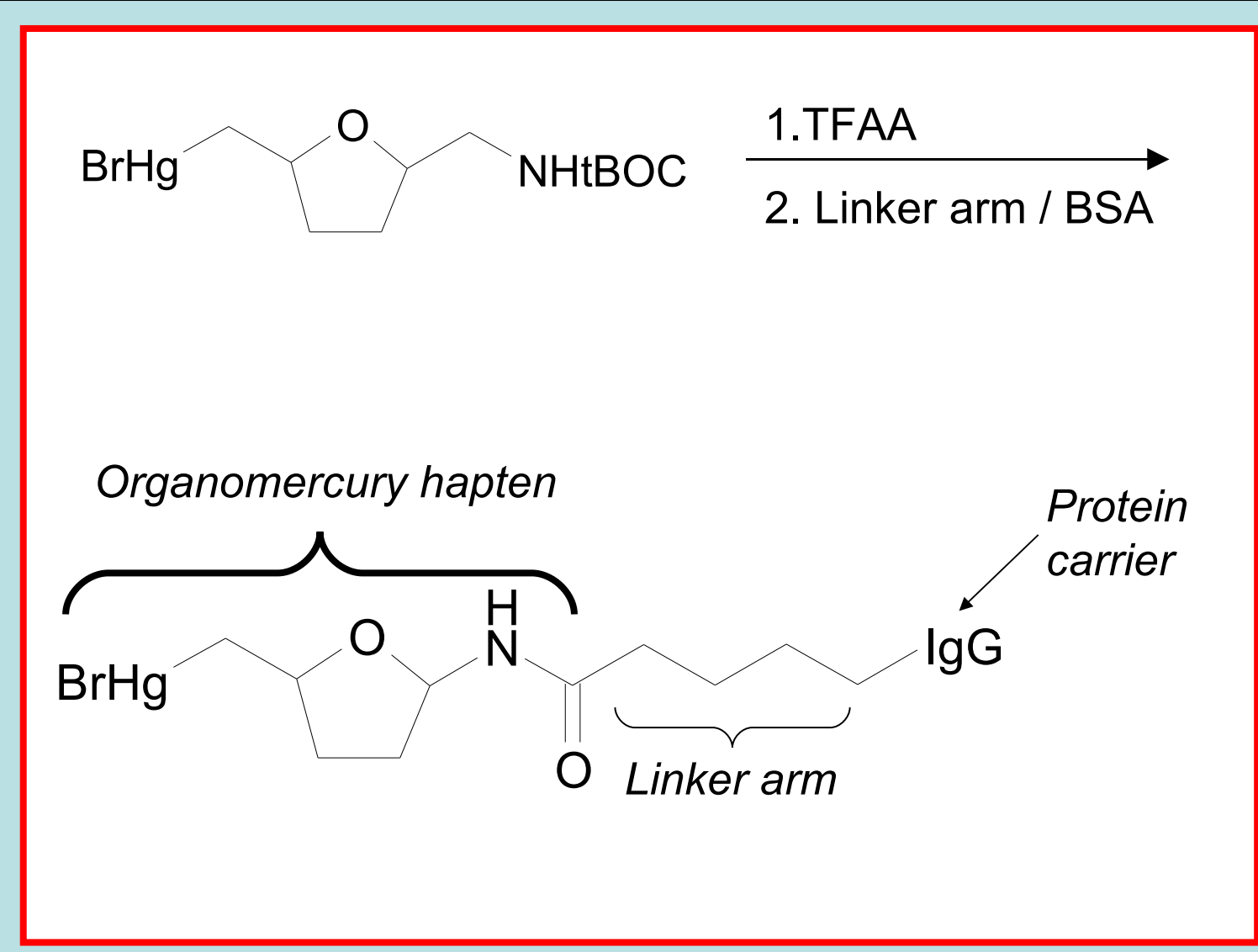
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Mercury: An Environmental Hazard



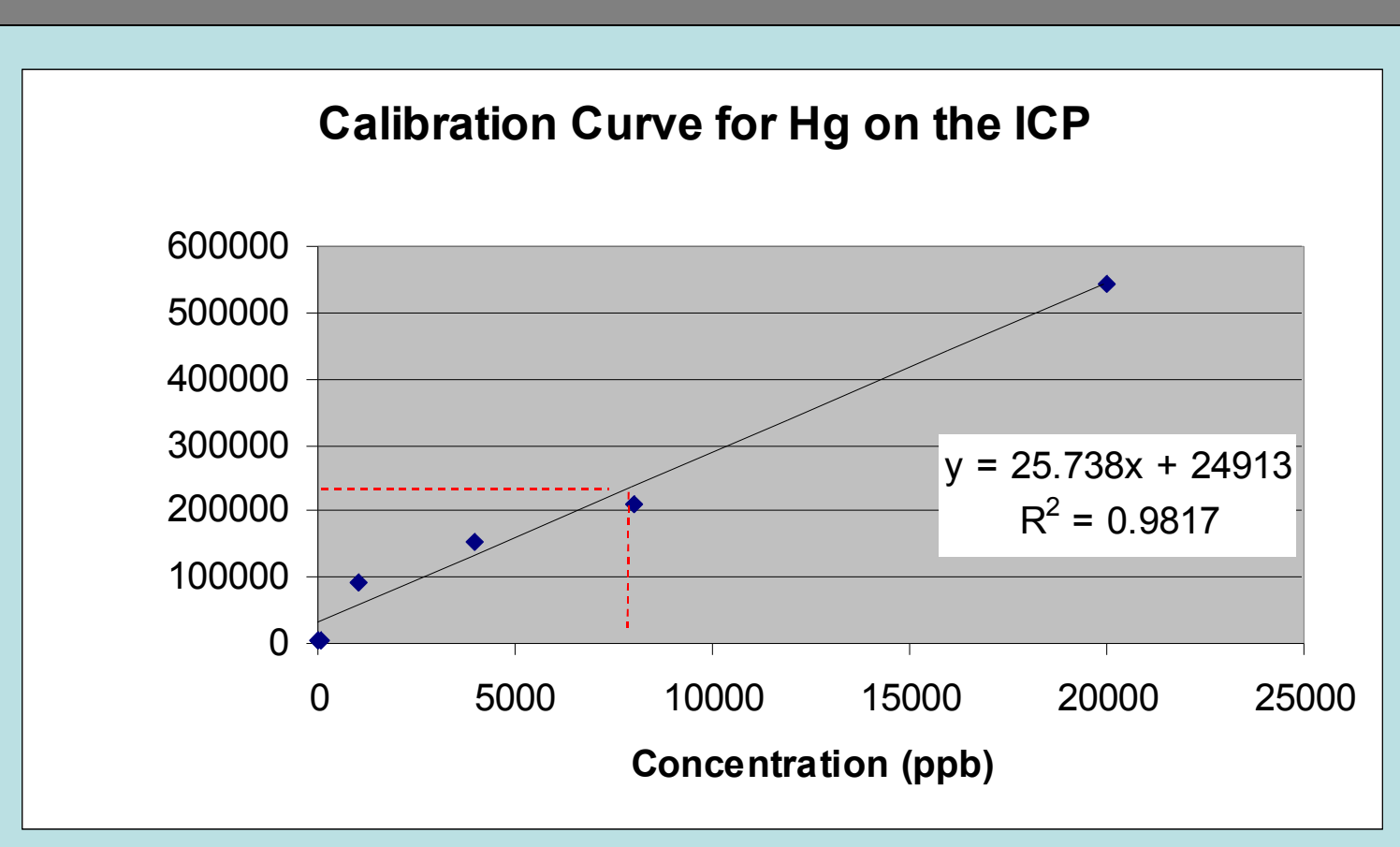
Manmade processes release mercury into the air. Mercury contaminates soil and water when it falls to the ground with rain or snow. Once in the environment, mercury can be converted to methylmercury, a highly toxic compound. Mercury levels in Maine wildlife are among the highest in North America [1].

Synthesis of Organomercury Immunogen



Details of organomercury hapten synthesis are described in [2]. Following synthesis the mercury hapten is connected to a carrier protein (IgG = chicken immunoglobulin) via a linker molecule. Connection to a protein carrier turns the hapten into an immunogen, or a molecule capable of causing an immune response.

Verification of Mercury in Biomolecule



An Inductively Coupled Plasma Emission Spectrometer (ICP) was used to analyze a sample of the bioconjugate for mercury content. Using the resulting calibration curve (shown above), the mercury concentration in the biomolecule sample was determined to be approximately 8000 ppb.

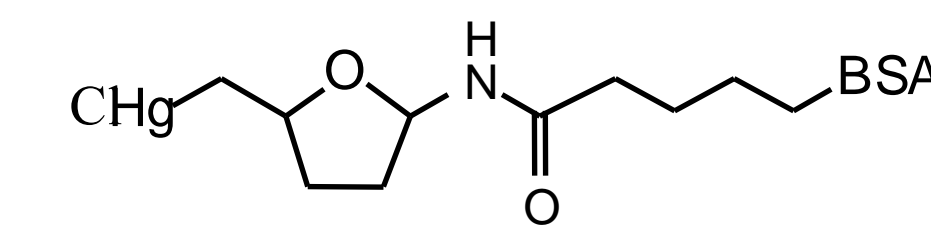
Abstract

Recent increases of mercury contamination in the environment necessitate the development of a sensitive and selective mercury assay for soil and water samples. This project focuses on developing such a method using a previously synthesized protein-mercury bioconjugate to stimulate an immune response to mercury in mice. Previous attempts to develop diagnostic metal antibodies have often employed chelating agents, such as EDTA, to link proteins with metals. Chelating agents are effective at binding metals, but this approach often produces antibodies with two undesirable characteristics: they are nonspecific for a particular type of metal and they produce a false positive response when the chelating cage is not bound to a metal at all. It is hoped that, due to the novel linkage connecting the protein and mercury entities in the bioconjugate to be employed in this study, this biomolecule will stimulate an antibody response that has higher sensitivity and specificity than previously developed anti-mercury antibodies. Mice will be injected with this new bioconjugate molecule and the antibodies they produce will be collected and isolated on a regular basis. We are currently screening serum samples using an Enzyme-Linked Immuno-Sorbent Assay (ELISA) to determine antibody specificity and sensitivity to mercury. Early screening assays demonstrate antibody binding to an organo-mercury based solid phase. Assay results have provided impetus to pursue monoclonal antibody production. Bioconjugate synthesis details, antibody-binding response to mercury over time and details of monoclonal antibody production will be discussed.

Project Goals

This project aims to develop an assay employing mercury antibodies as a probe to detect low-level mercury in a variety of sample media. Mercury antibodies will be developed using mice and screened using an ELISA assay.

ELISA Solid Phases



The solid phase used for ELISA plates (shown above) is very similar to the organomercury biomolecule used to immunize the mice. Because of this, antibody binding and positive signal response could be due to mouse antibodies responding to part of the solid phase other than the antigen. Varying the solid phase used to coat ELISA plates allows investigation into antibody-antigen binding, including specificity and selectivity of antibodies. To the right is a slide illustrating some of the solid phases investigated in this project.

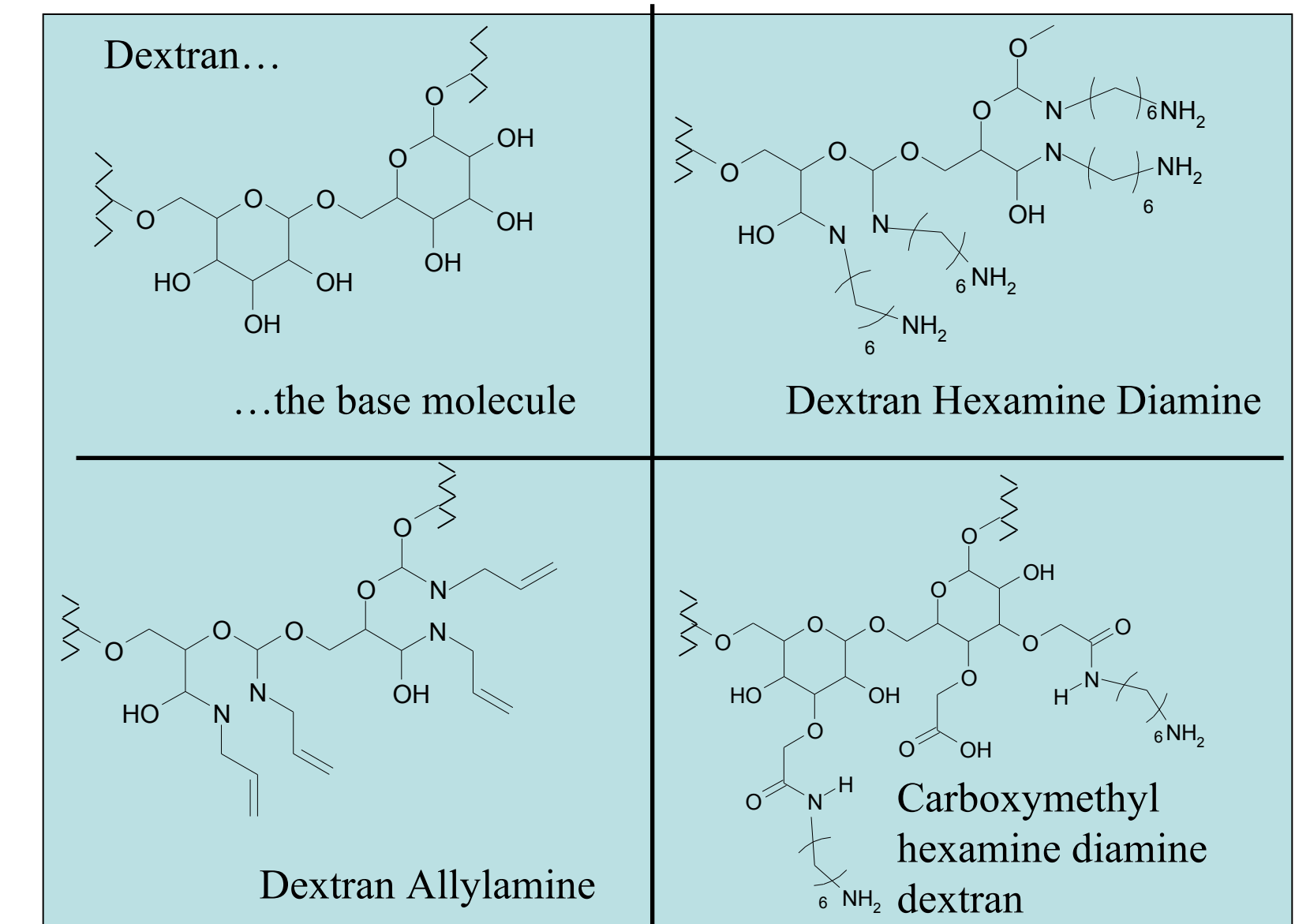
Future Work

Although monoclonal antibodies have not been successfully produced, efforts continue in this area. Work also continues on ELISA solid phase synthesis and characterization. As solid phases are produced, studies continue to classify the antibody binding and steps will be taken to increase selectivity and sensitivity of anti-mercury antibodies.

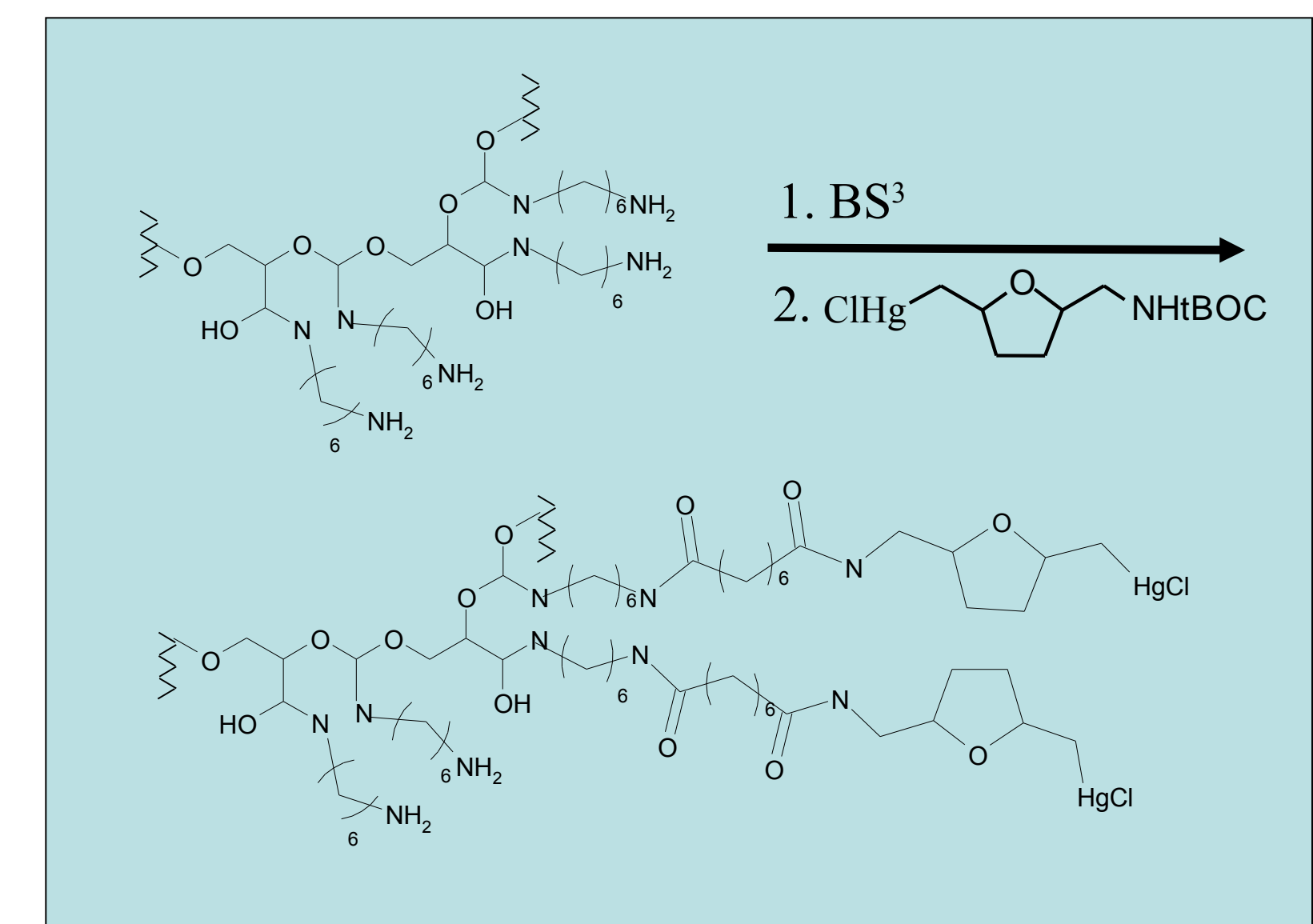
Acknowledgements

References

- [1] Maine Department of Environmental Protection. <http://www.maine.gov/dep/mercury/>
- [2] Caryn K. Prudenté and Margaret Hausman. "Organomercury Bioconjugate Synthesis and Characterization by Matrix Assisted Laser Desorption Ionization Mass Spectrometry," *Bioconjugate Chem.* **2003**, *14*, 1270-79.

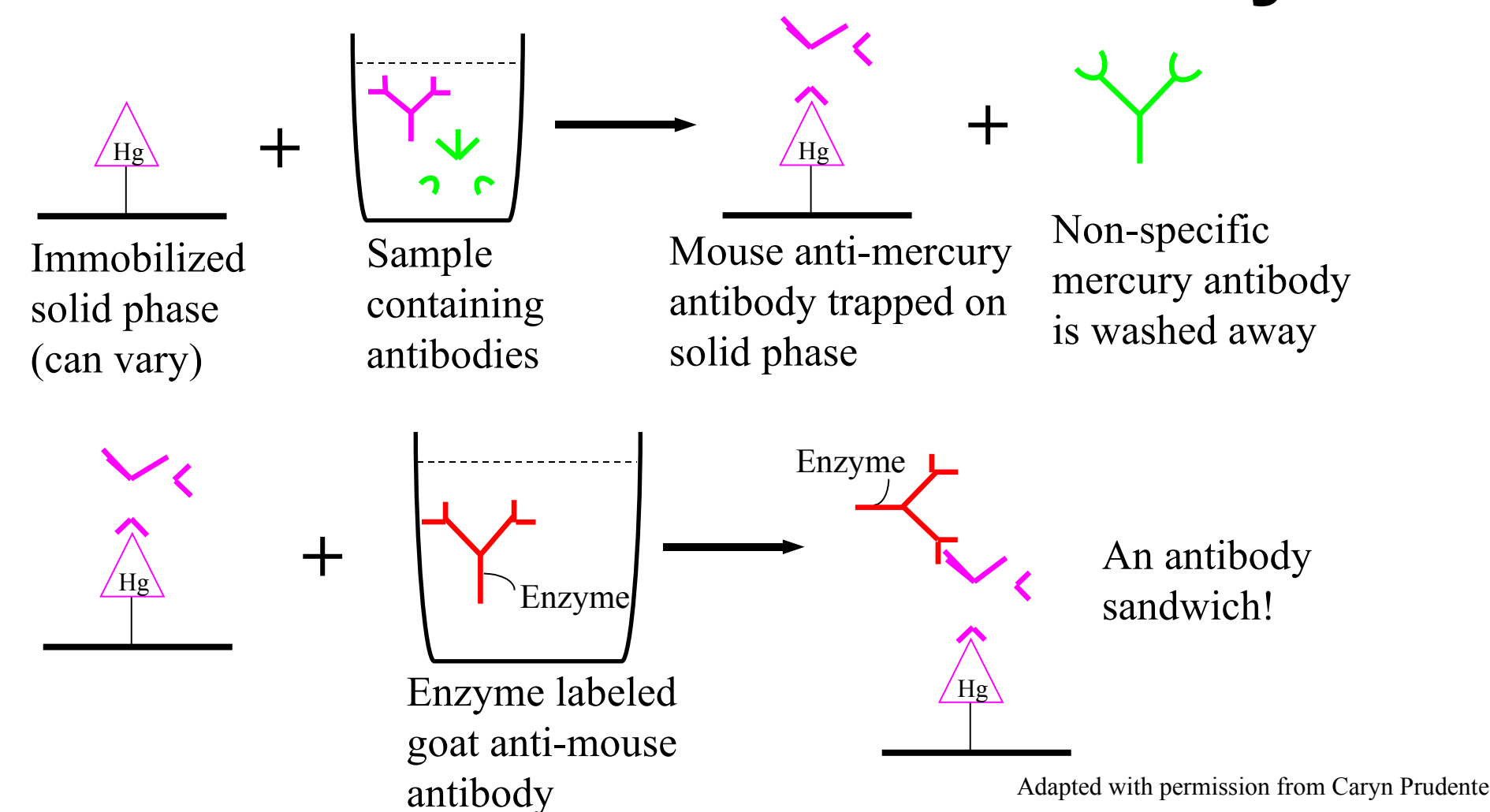


The above molecules are different solid phase precursors that were synthesized during this project. Dextran was the base molecule that was modified in a variety of ways to create new potential solid phases for ELISA assay. Once synthesized, each molecule can be coupled to mercury, as seen below with dextran hexamine diamine.

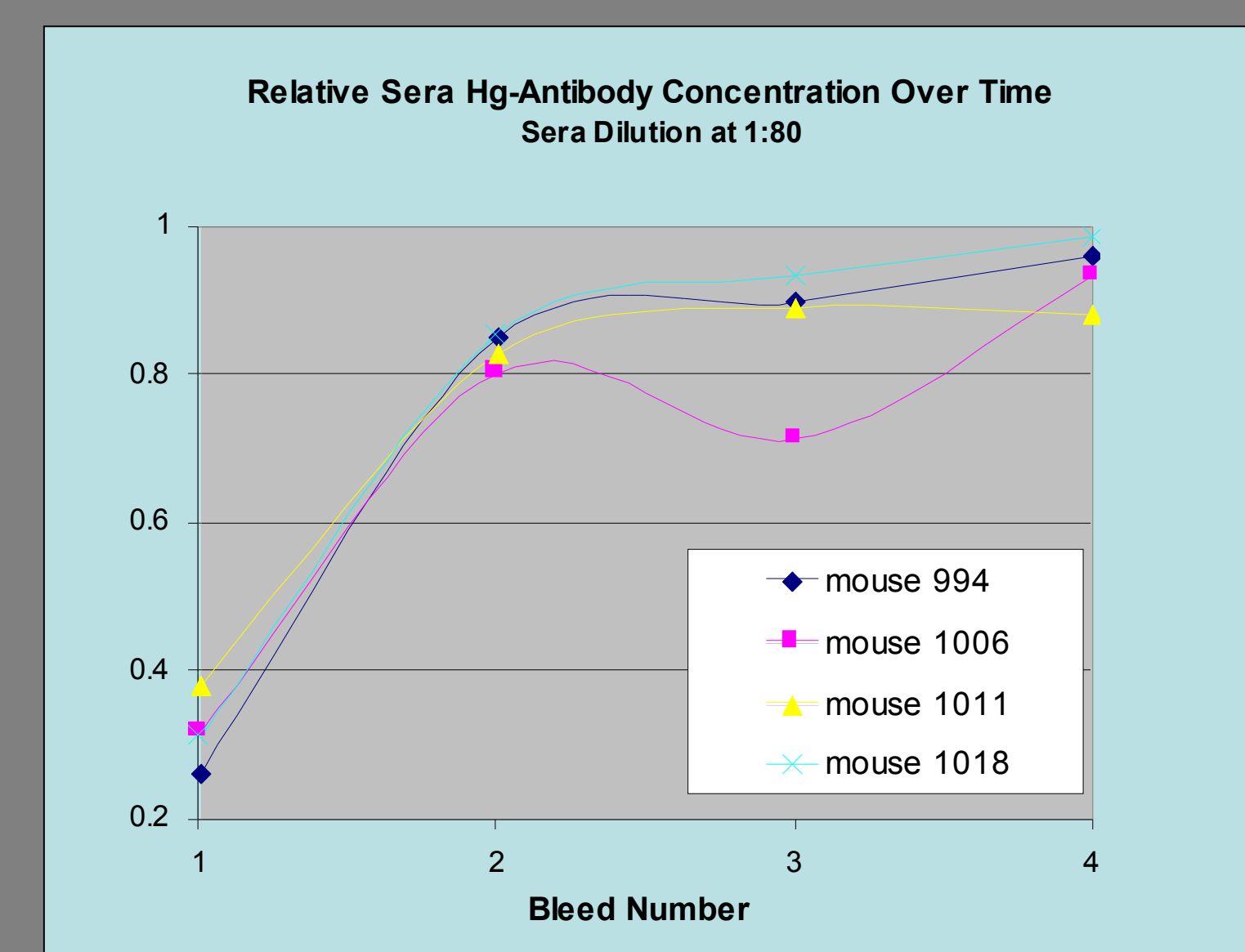


The above reaction couples dextran hexamine diamine to mercury using a linker arm (BS³) and the organomercury hapten. Although mercury is presented in the same way, the carrier molecule is dramatically different, which provides a solid phase to study antibody epitope sites and selectivity.

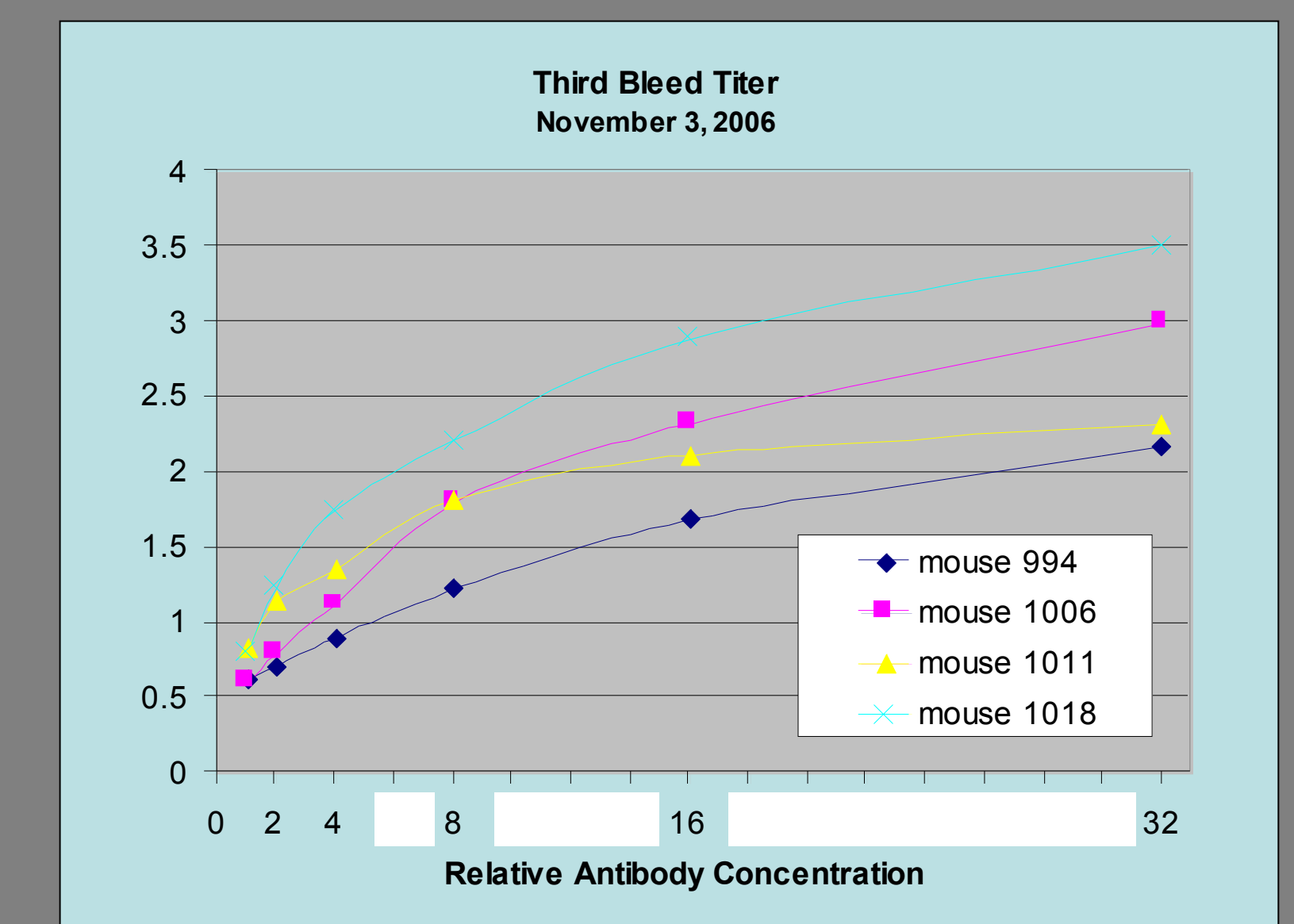
Sandwich ELISA Assay



Polystyrene ELISA plates are coated with a solid phase containing the antigen of interest coupled to a protein or sugar. The inherent "stickiness" of the solid phase protein adheres it to the plate. A serum sample from immunized mice is then added to the ELISA plate. Mouse anti-mercury antibodies from the sample are retained on the plate as they bind to the mercury immobilized in the solid phase. Any non-mercury-specific antibodies are rinsed away. In a second step an enzyme labeled anti-mouse antibody is added to the plate and binds to the mouse anti-mercury antibodies. The enzyme labeled antibody can be used for detection and quantification. Once the ELISA is optimized it will provide a tool to detect and quantify mercury in various samples



The above graph illustrates the change in the concentration of anti-mercury antibody in mouse sera through the course of the immunization process. Each bleed was taken at different time points throughout the immunization course. Bleed 1 was a pre-immunization sample, taken as a baseline reading. This initial data confirms that the organomercury hapten was effective at stimulating mice immune systems to make antibodies.



The above graph illustrates a titer. An increase in antibody concentration results in a corresponding increase in signal strength. This is good evidence that the signal results from antibodies and not from a contaminant or another source. Based on the ELISA results obtained in this study, the decision was made to produce monoclonal antibodies.