A report on the Ocean Life Institute support for:

"Biodiversity of Cytochrome P450 Genes; Biomarkers for Coastal Pollution in Mussels"
(or "How Contaminants Affect Blue Mussels")

Submitted by
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The support from the Ocean Life Institute has been important to the progress in our efforts to bring the study of possible biomarkers in the mussel *Mytilus edulis*, as an important bivalve mollusk, to a new level. Many studies in mussels going back over 30 years have done little to advance our understanding of how these organisms deal with organic chemical pollutants. The effort we have undertaken to build a molecular biological foundation for understanding bivalve responses to specific types of chemicals has focused on the cytochrome P450 family of genes and proteins. (These are referred to as CYP or P450s.) Specifically, we are addressing regulation of CYP genes by chemicals and how the CYP proteins may be involved in metabolism of those chemicals. The funding from OLI has been important in enhancing studies and speeding progress in several of these research directions.

CYP gene relationships: A broad phylogenic analysis of all of the known molluscan cytochromes P450 genes has been performed. This analysis was based on the sequences obtained from various databases and from collaborators in the UK. Analysis of as many sequences as could be found revealed a *Mytilus edulis* CYP1-like protein. Genes in the CYP1 family in vertebrates are induced by and metabolize many of the most common hydrocarbon pollutants in the sea, including polynuclear aromatic hydrocarbons, and polychlorinated biphenyls. The close phylogenetic relationship which we had inferred at the time of proposal submission has been substantiated (Figure 1).

Figure 1. Evolutionary relationships between cytochromes P450 found in humans (blue), giant owl limpet (purple), deep sea mussel (orange), and *Mytilus* (black and red). The red-labeled genes have been cloned in our lab, including the CYP1-like gene described in the text. The pink and green highlighted regions include mussel sequences and the related human pollutant-metabolizing enzymes.

Molecular modeling of CYP proteins and substrate binding: We have taken a new approach to identify possible substrates for mussel cytochrome P450 enzymes, using computational methods to generate 3-dimensional structures of the proteins (molecular modeling), and then using programs to determine whether specific chemicals might bind to the protein in a proper "fit" for possible metabolism. This can identify candidates for direct biochemical study, much more efficiently than 'hit and miss' enzyme assays with different structures. After obtaining amino acid sequences inferred from cloning and sequencing of selected *M. edulis* CYP genes, we did computational modeling of the CYP sequence that is the molluscan CYP so far most similar to vertebrate CYP in the CYP gene family 1. CYP1 enzymes are well known in fish and other vertebrates, where they metabolize particular chemicals. We are doing *in silico* docking studies and beginning to predict likely substrates, focusing especially on those well-known xenobiotic substrates. We used X-ray crystallographic data from a human drug-metabolizing enzyme, CYP1A2, as a template structure from which to predict the three dimensional structure of the mussel protein target. A library of potential ligands was then computationally 'docked' to evaluate the affinity and positioning of the chemicals relative to the active site of the protein. In prior studies we have established the use of 'ensemble' modeling and docking in which multiple different homology models are docked many times to evaluate a distribution of computed ligand positions and energies. This is the approach we are applying to the mussel proteins.

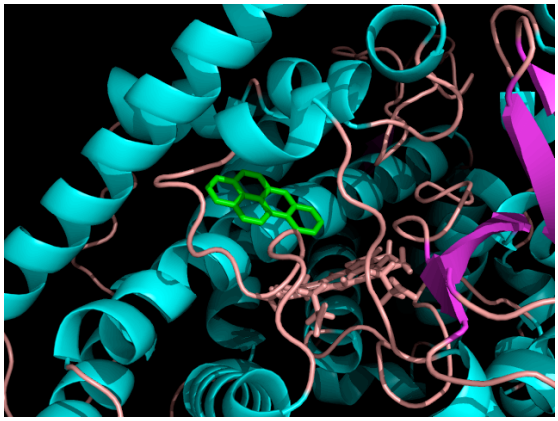

Figure 2. Representative image of benzo[a]pyrene (green) 'docked' into the model of the *Mytilus* CYP1-like protein, showing the close approach of susceptible carbons to the source of CYP oxidation, the iron-containing heme (tan).

Using this method we predict that the model polycyclic aromatic hydrocarbon carcinogenic contaminant benzo[a]pyrene (BaP) is a good ligand for this protein (Figures 2). Mussels have been reported to eliminate hydrocarbons including benzo[a]pyrene with half-lives of 8-14 days. By contrast, fish eliminate BaP with a half-life of 2-3 days. The clearance from mussels could involve metabolism of the hydrophobic contaminant, or indicate elimination by other pathways. In the computational docking, benzo[a]pyrene is tightly bound in a 'productive' position – that is, most dockings result in a computed position that is likely to result in benzo[a]pyrene oxidation by the CYP, based on the close approach of oxidizable BaP carbons in docked ligands to the computed position of the heme oxygen that would be transferred to the substrate.

Identifying reference populations for experimental exposures: Chemical analysis of offshore *Mytilus* samples is ongoing with our collaborators in Japan (Professor S. Tanabe). Samples were obtained from an experimental long-line aquaculture system off of Chilmark, Martha's Vineyard, funded in part by the NOAA Marine Aquaculture Program. This is an experimental aquaculture

system and it is not yet certain that it will be continued. Accordingly, samples for chemical analysis will be obtained from an alternative mussel farm, a commercial mussel aquaculture facility in the Gulf of Maine, located near Mount Desert Island (Pemaquid Mussel Company, courtesy of Dr. Carter Newell). Mussels from these populations will be used for gene expression studies, in animals exposed to selected chemicals. Chemicals selected for use in the defined exposure experiments include benzo[a]pyrene, PCB126, and a DDT metabolite, DDE. These chemicals are known to increase the expression of specific CYP genes in fish and mammals.

International collaborations*:* The funding from OLI also aided our efforts to develop an international working group of collaborators (in the UK, Italy, Sweden, Spain, Japan) who share the interest in understanding chemical effects in these organisms. At a meeting of the European Society of Comparative Biochemistry, Dr. Stegeman initiated group discussions that led to an "open web consortium" for mussel research (http://openmytilusconsortium.org/?q=node/1), engaging people from around the world in discussions and collaborations in the area of molecular responses. The discussions also led to proposal to the SciLifeLAb, Stockholm, for sequencing of the *Mytilus edulis* genome. That proposal, while not among those accepted for action this cycle, will be reconsidered in 2011.