

Title	The application of mass spectrometry to identify proteins of the purple sea urchin <i>Strongylocentrotus purpuratus</i>
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Department	Biological Sciences
Description	<p>Here at Cal Poly, the Environmental Proteomics Laboratory ventures in to the relatively new scientific field of proteomics. Proteomics is the study of the overall protein expression of an organism. Through the introduction of various amounts of environmental stress, the scientists in the EPL can evaluate the physiological response by means of measuring the changing expression levels of proteins. By finding patterns in the levels of expression, related proteins can give light to the effects on the activities of physiological systems due to the external stress.</p> <p>At the Environmental Proteomics Laboratory, several undergraduates, graduate students as well laboratory assistants study environmental stresses on various species of marine animals. Species include mussels, sea urchins, sea snails, and arrow gobies. For example, heat, salinity, nitrate, and pollution stress are studied in these animals by means of protein expression. The varying protein expression levels are studied under many levels of stress. These studies can also put light to the issues of global warming and pollution.</p> <p>The animals of study are exposed to the stress, and then the tissue of interest is dissected and homogenized. The tissue is then centrifuged, and the soluble proteins are separated by isoelectric point and size on polyacrylamide gels. The gels are scanned into the computer, and the resulting gel images are used for computer analysis. A gel image analysis program, Delta2D, enables us to determine the expression levels of the proteins at the varying stress levels. This data is then put through various statistical models to determine which changes in protein expression levels are significant. The proteins that differ are then isolated from the gels and digested with an enzyme, trypsin, to analyze the peptide fragments of the protein. Analysis with our MALDI TOF/TOF, or matrix assisted laser desorption/ionization tandem time-of-flight mass spectrometer detects the mass of these peptides and compares the spectrum against a database in order find a protein that shows the same distribution of mass. With the proper identification of proteins that have significantly changed with some a specific environmental stress, hypotheses can be formulated on how certain stresses affect the physiology of an organism.</p> <p>This honors project would focus on the analysis portion of the proteome</p>

	<p>workflow. Delta2D is an extensive program, but a large portion of the efficiency of the program is left up to the user. For instance, when all of the proteins are properly detected and modified, the user has many statistical models to choose from in order to determine significance. Everything from simple t-tests and percentage increase of expression to two way ANOVA with a Pearson Correlation can be used. But what model is the most appropriate to use? This aspect, among many, of the Delta2D program will be studied.</p> <p>Secondly, the analysis power of the MALDI TOF/TOF mass spectrometer will also be a focus of this research project. The procedure for using the mass spectrometer is as followed: peptide mass fingerprinting, tandem mass spectrometry, database searching, and integration of results into a systems response model to environmental stress.</p> <p>The goal of this research project would be to apply the analysis techniques of the EPL to a set of gel images that are used to characterize the stress response to temperature changes in the purple sea urchin, <i>Strongylocentrotus purpuratus</i>. Delta2D, Trypsin digestion, and mass spectrometry will be applied to identify the proteins that changed expression levels in response to acute and chronic heat stress.</p> <p>3 honors students are required. Students majoring in Biology, Biochemistry and Computer Science are needed. Funds would be used for sample preparation including high quality reagents and sample vessel (mass spectrometry plates), mass spectrometry equipment and support for students.</p>
Interdisciplinary Description	<p>The project combines an ecological (biogeographical) question: "What determines the thermal limits of a key species in the marine environment, the purple sea urchin?" with a biochemical one: "Which proteins are changing expression levels in response to long- and short-term temperature stress?" We are now identifying the proteins that are changing by combining the biochemical results (2D gel electrophoresis to visualize protein expression patterns) with a physical methodology (mass spectrometry). The analysis and interpretation of the mass spectrometric data will involve bioinformatics: databases, specialized search engines for matching spectra with online databases etc. Thus this project looks at a range of disciplines: from biogeography and biochemical adaptations to the identification of proteins with mass spectrometry and bioinformatics. That's the reason why I am requesting a student each from biology, (bio-)chemistry and computer science.</p>
Links	http://bio.calpoly.edu/EPL/index.html
Students	3
Majors	BIO, CHEM, CSC
Desired Skills	A willingness to be intellectually adventurous! I will teach them the rest.

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