

# A Proteomic Approach to Estrogen Agonist Screening

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TOXICOGENOMICS

## Background

As part of a tiered approach, new molecular techniques are being developed to assist the U.S. Environmental Protection Agency in evaluating the toxic potential of chemicals. Screening methods for use in chemical risk assessments should be high throughput and have low animal requirements. We describe a method for screening chemicals for estrogenic activity using a small fish assay and either surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS) or matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The techniques suggest protein expression profiling can be adapted for other chemical mode of actions and would provide a tool for screening and prioritization of existing chemical inventories.

## Methods

- Adult male sheepshead minnows (*Cyprinodon variegatus*) were exposed to the native ligand 17- $\beta$ -estradiol (E2) for 7-10 days, and blood plasma was analyzed for differential expression of proteins in control versus E2 treated fish using MALDI-TOF-MS and SELDI-TOF-MS.
- Samples were prepared for MALDI-TOF-MS by diluting plasma 1:100 in matrix buffer and applying to the array. SELDI-TOF-MS samples were diluted and bound to array surfaces through a series of binding and washing steps to remove unbound sample proteins and buffers. Proteins bound to the selective array were analyzed.



Exposure System



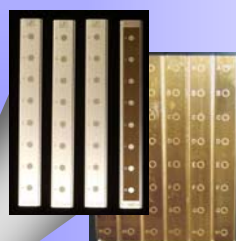
Tissue Collection



Sample Processing



Automated Workstation



Sample Surfaces



Mass Spectrometry Analysis

SELDI-TOF-MS

MALDI-TOF-MS

- Surface Enhanced Laser Desorption Ionization sample surface
- binds proteins to array surface according to physico-chemical properties of array
- can wash away unbound proteins to allow for capture of low abundance biomarkers
- adaptable to automated liquid-handling workstation format for high throughput processing
- arrays not reusable

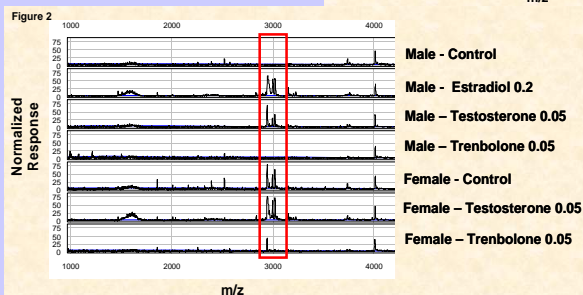
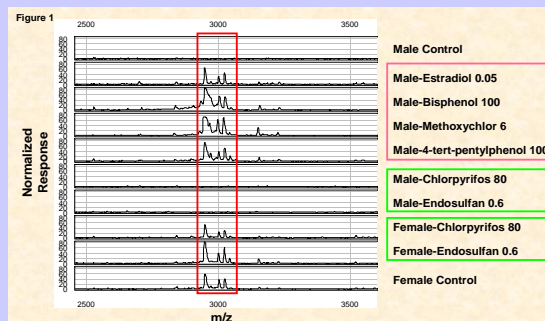
- Matrix Assisted Laser Desorption Ionization sample surface
- array does not selectively bind proteins
- plasma samples can be diluted and applied to surface with or without prior cleanup for high abundance proteins
- adaptable to automated liquid-handling workstation format for high throughput processing
- arrays are reusable

## Applications

### In vivo Diagnostics

The mass spectra shown here are representative of spectra generated using SELDI-TOF-MS and MALDI-TOF-MS methods.

- Protein biomarkers were found to be diagnostic of E2 exposure and demonstrated 100% sensitivity and specificity for fish exposed to **known estrogen agonists**, and to **non-estrogenic chemicals**. Chemical concentrations are in  $\mu\text{g/l}$  (Fig. 1).



- The estrogen-responsive protein biomarkers can be used to evaluate estrogenic and anti-estrogenic activity in fish exposed to androgenic chemicals. Chemical concentrations are in  $\mu\text{g/l}$  (Fig. 2).

- Expression of plasma protein biomarkers follows a clear dose response pattern for a 7 day exposure to E2. Error bars represent one standard deviation (Fig. 3).

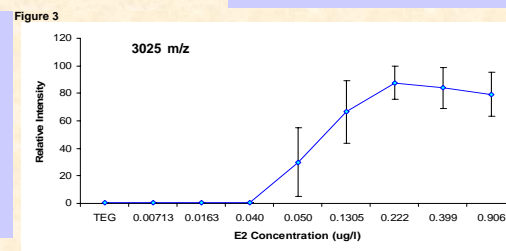
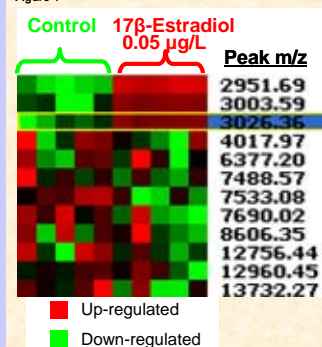
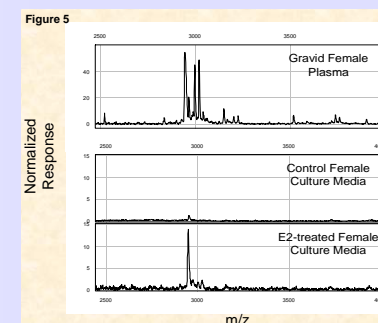


Figure 4



- A heat map allows for an alternate view of discriminatory vs. non-discriminatory peaks from 0.05  $\mu\text{g/l}$  E2 and control male fish; highlighted mass indicates identified protein fragment (Fig. 4).

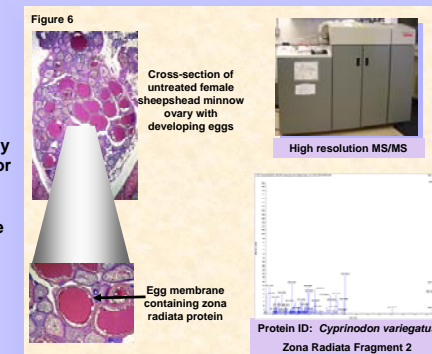
### In vitro Diagnostics



- The estrogen-responsive protein biomarkers were found in media of cultured sheepshead minnow liver cells exposed to E2, indicating that the biomarkers are hepatic in origin (Fig. 5).

### Protein Identification

- One of the estrogen-responsive biomarkers was identified by high resolution tandem mass spectrometry (MS/MS) as zona radiata fragment 1, an egg envelope protein that is naturally produced in gravid female fish or male fish which have been exposed to estrogenic chemicals. Biological relevance of the biomarker facilitates linkage of proteomics data to traditional reproductive endpoints (Fig. 6).



## Summary

- MALDI and SELDI differ in arrays, sample application, binding properties, preparation time and cost. MALDI arrays can be washed and reused, while SELDI arrays allow for a more in-depth look at the proteome by focusing on low abundance proteins that may otherwise go undetected.
- MALDI/SELDI-TOF coupled with a short term *in vivo* or *in vitro* assay provided a rapid means to identify estrogenic and anti-estrogenic activity while reducing our dependence on animal and time intensive methodologies.
- Protein biomarkers characteristic of a specific mode of action can be used as a chemical screening tool for chemicals with unknown properties.