Proteomic Studies of Radiation Response

Daniela Stricklin, Ph.D., MPH

Combined Injury Workshop

March 27-67, 2007

Washington, D.C.

FOI

Introduction to FOI

- **FOI, CBRN Defence and Security**
  - **Assignments ranging from threat/ risk assessment to basic research for all CBRN(E) areas**
- **Radiation Research**
  - **Field detection, forensics, biodosimetry**
  - **“Radiobiology” research focused on investigation of exposure assessment methods**
- **Overlapping Interests**
  - **Biomarkers of human exposure**
  - **Combined exposures**

FOI

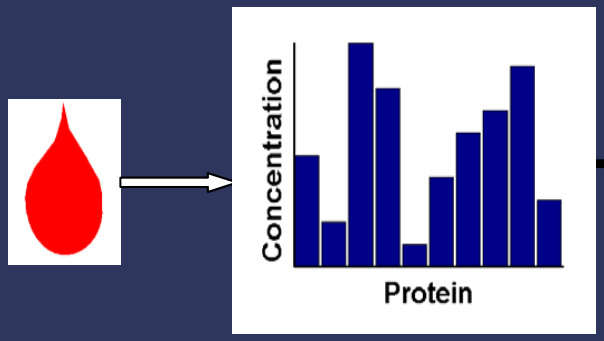
Why Proteomic Studies?

- Protein Expression
  - Proteins offer a large number of potential biomarkers with physiological relevance
  - Specificity may be found in individual proteins or in protein patterns (profiles)
- Protein measurement platforms
  - Protein chip/ array facilitates biomarker discovery
  - Chip or micro-bead for tailored assays
- Greater tool in the future
  - Potential to ID effects from any exposure (inc. combined exposures)

FOI

General Markers of Exposure

- Many different potentially harmful agents exists, ranging from actual NBC agents to industrial pollutants
- Exposure possibilities are variable and often unknown
- For biological monitoring, a sensitive but general biomarker to indicate exposure before toxicological effects would be advantageous



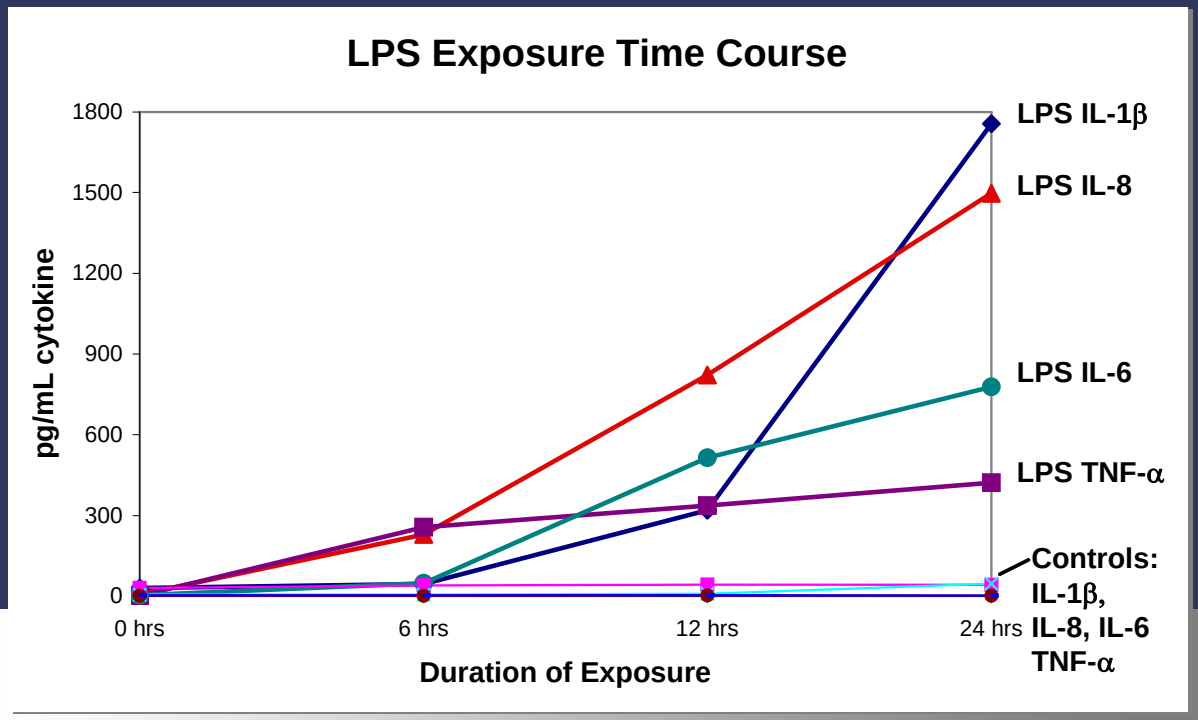
Exposed? Yes / No
What? NB or C
How much? High / Low

Concept for using Protein Expression for Exposure Assessment

FOI

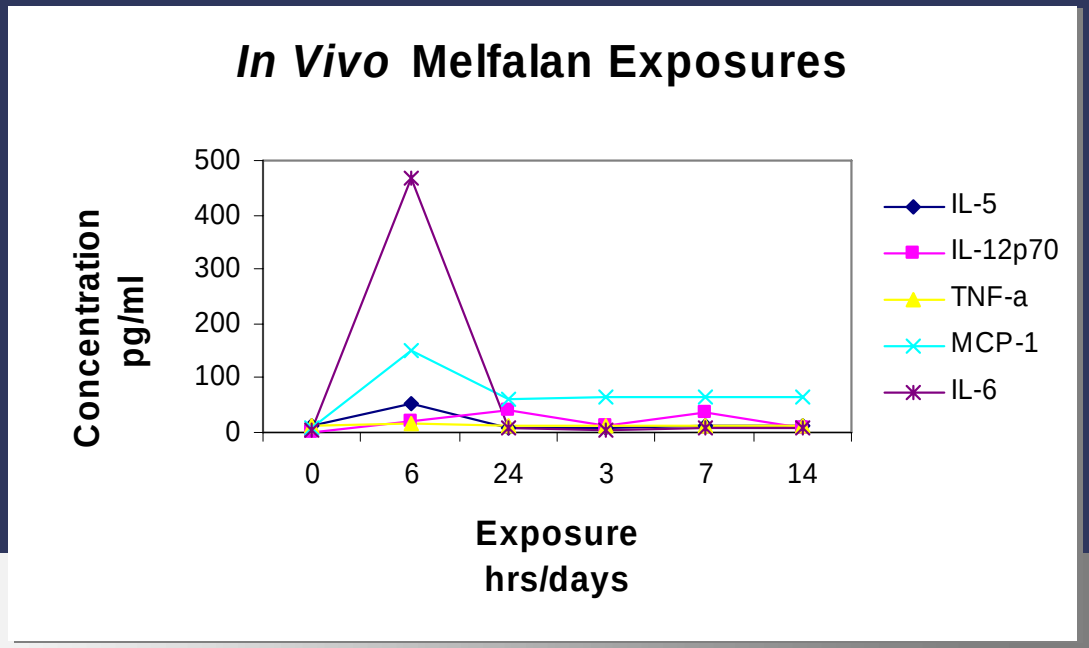
In Vitro Studies

- Increased cytokine levels of IL-1β, IL-8, IL-6, and TNF-α
  - 0, 6, 12, and 24 hours of exposure
  - 50 ng LPS / ml whole blood
  - Differential expression in high and low doses (IFN-γ)



In Vivo Studies: Chemical

- Cytokine levels in mouse serum
  - 6 and 24hrs, 3, 7, and 14 days after exposure
  - 1 mg melfalan / kg body weight
  - Significant increase in IL-5, IL-6, and MCP-1, confirmation in BAL fluid
  - Large variability



FOI

In Vivo Studies: Radiation

- Study design
  - Mouse *in vivo* exposures
    - Control (6 animals)
    - Sham and 0.5Gy Exposed (5 animals/ group)
      - ^{137}Cs chamber, shutter closed or open for 47 sec
  - Wild-type only
  - Sacrifice at 6, 24, 48, and 96 hrs
  - Samples:
    - Whole-blood, serum (EDTA and no EDTA), blood cells, spleenocytes

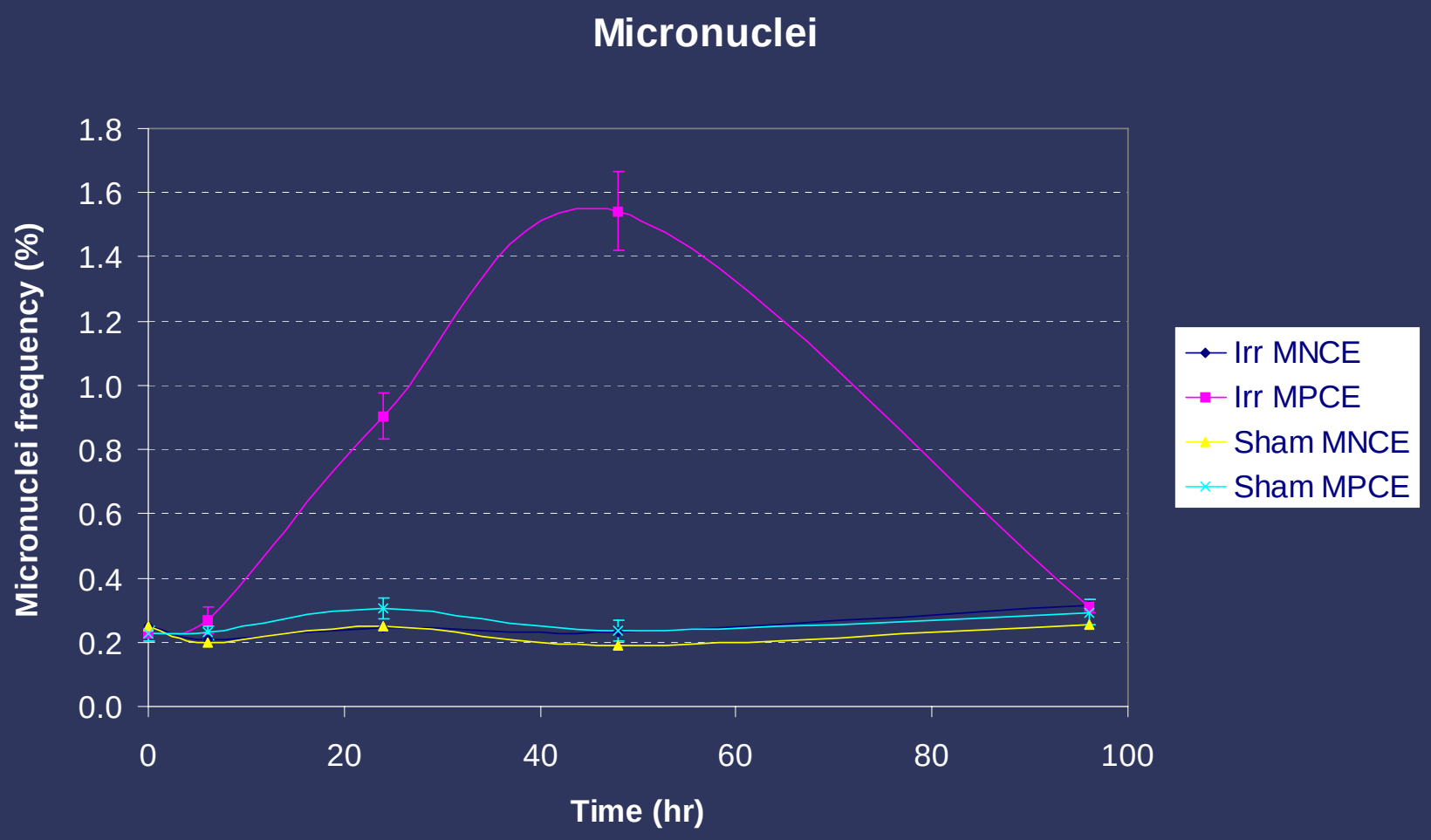
FOI

Measurements

- MN
  - Polychromatic and monochromatic rbc
  - Flow cytometry
- 23-plex cytokine
  - IL-α, IL-β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, Eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1, MIP-1a, MIP-1b, RANTES, TNF-α
- Protein expression, protein chip
  - Anionic, metal affinity, and hydrophobic surfaces
- Apoptosis
  - DNA fragmentation, future

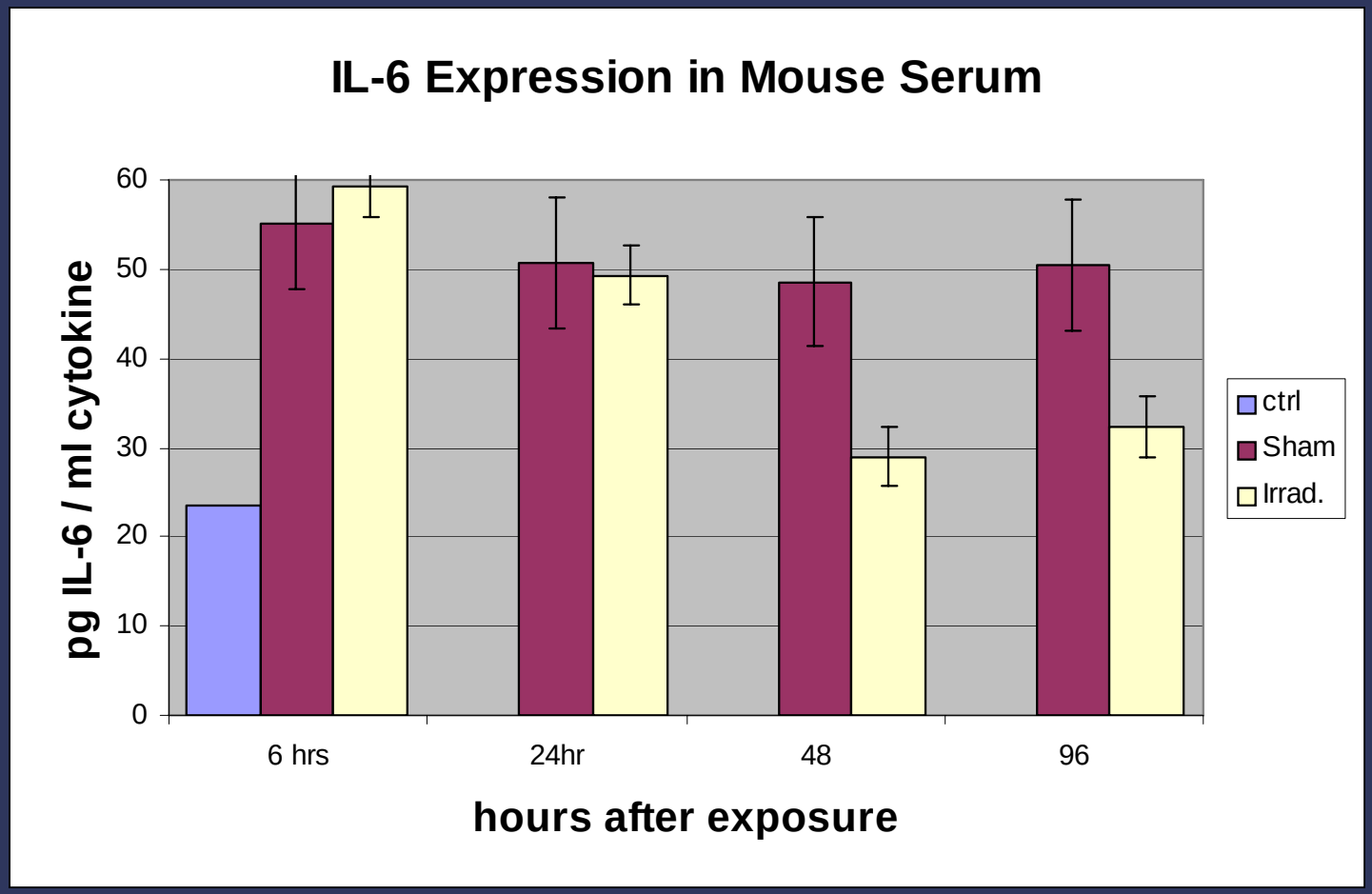
FOI

Summary of MN results



FOI

Example of Cytokine Profile



IL-6 is significantly lower in exp. as compared to sham at 48 and 96 hours.

FOI

Summary of cytokine results

- All cytokines were significantly affected by sham or irradiation as compared to control
- A significant trend was observed: irradiated samples returned to control levels at 96hrs, most sham irradiated samples did not.
- All increases or decreases in expression were subtle and of the same magnitude
- Different kinetics were observed for different cytokines and between sham and exposed
- Large variation was again observed, however, some significant differences were observed
- 12 cytokines were significantly different in exposed compared to sham
  - IL-a, IL-4, IL-6, IL-9, IL-12p40, IL-13, G-CSF, GM-CSF, KC, MCP-1, MIP-1b, TNF-a

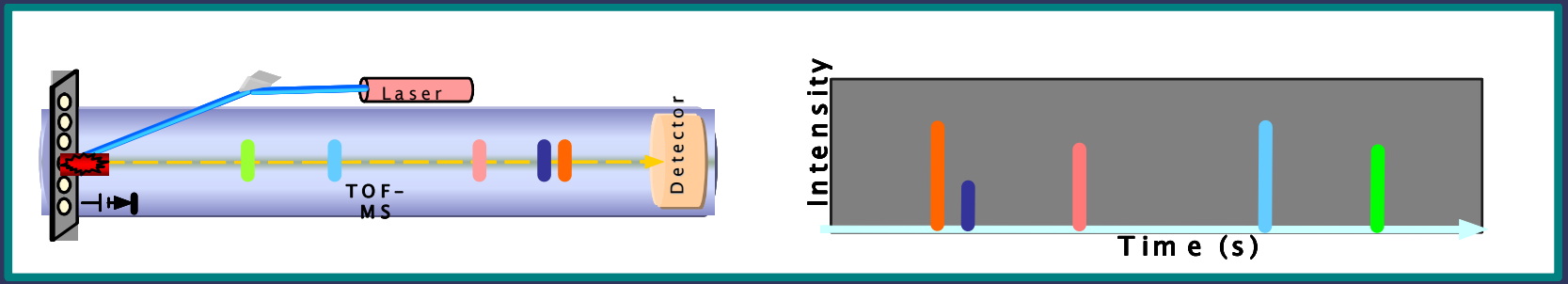
FOI

Overall Cytokine Summary

- Conclusions from cytokine experiments to date:
  - Distinct expression in exposures studied
    - By exposure and dose
  - Potential utility has been demonstrated
  - Significant stress response has been observed
  - Large variability observed
    - Does cytokines expression correlate with other endpoints?

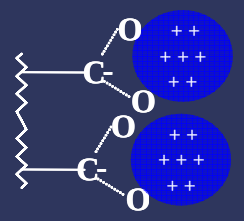
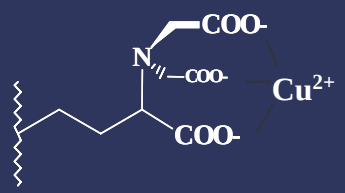
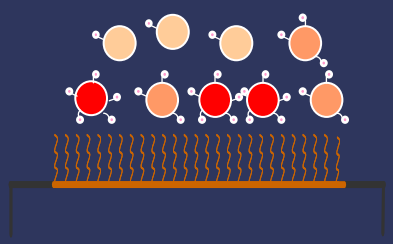
FOI

Protein chip array system



- Consists of a Protein Chip Array, a Protein Chip Reader, a Time of Flight Mass Spectrometer, and dedicated software for data handling
- Main component is the Protein Chip Array
  - Eight 2 mm spots, chemically or biochemically active.
- Biomarker identification
  - Chemically active surfaces (ionic, hydrophobic, chelating metal, etc.) captures a subset of proteins
  - Protein profiling
- Tailored application
  - Other highly reactive surfaces, ex. epoxy or carbonyldiimidazole groups, used to covalently bind a specific antibody or receptor.
  - Captures a specific protein or group of proteins.

FOI

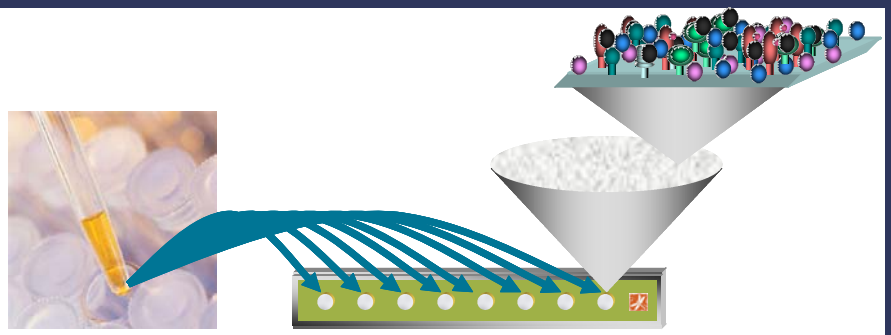
Surfaces	Binding buffer	Subset of proteins
 Anionic Surface	100mM NH_4HCO_3	Proteins that have positively charged groups e.g lysine, arginine or histidine.
 Immobilized Metal Affinity Capture	PBS pH 7,3	Proteins that bind divalent cationic metals such copper, i.e. with tryptophan, cysteine or histidine.
 Hydrophobic	PBS pH 7,3 + 10% Acetonitrile +0.25M NaCl	Peptides and proteins in lower mol. wt. through hydrophobic interactions, i.e. containing tryptophan, valine, alanine, leucine, phenylalanine or tryosine.

Surfaces and Binding Conditions

Sample Preparation on Pre-activated Surface

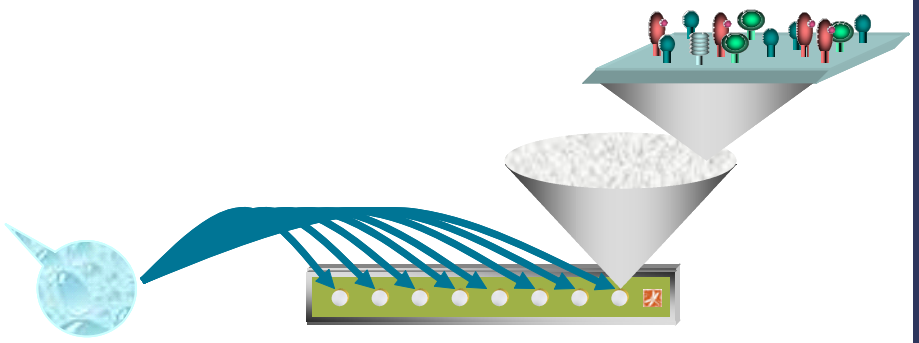
1. Apply Crude Sample

Proteins within the sample bind to chemical “docking sites” on the ProteinChip surface through an affinity interaction.



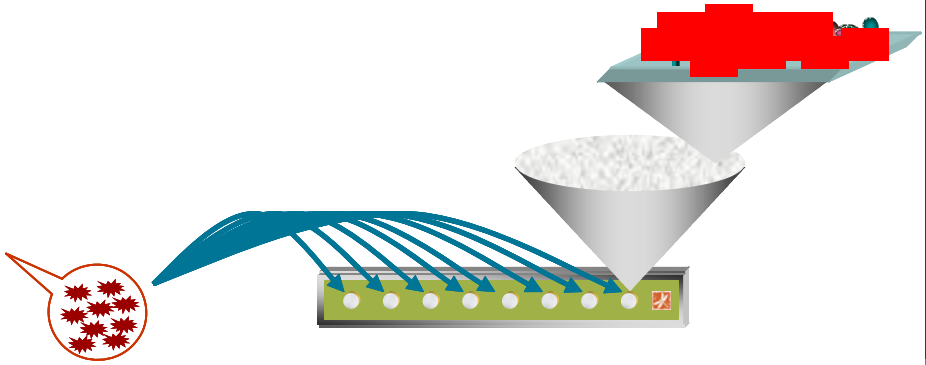
2. Wash ProteinChip Array

Proteins that bind non-specifically or buffer contaminants are washed away, eliminating sample “noise”.



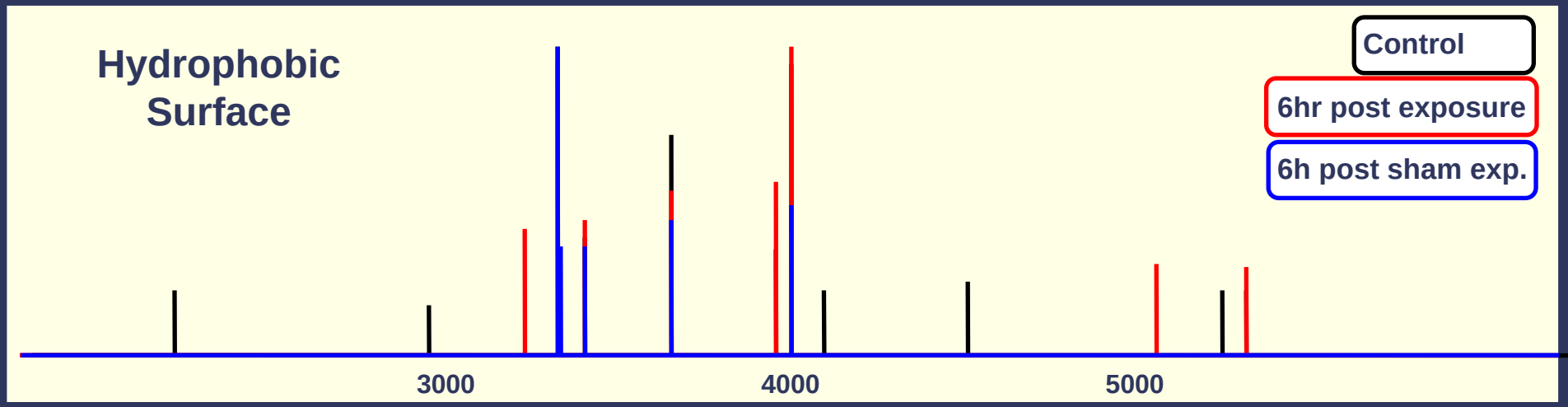
3. Add Energy Absorbing Molecules

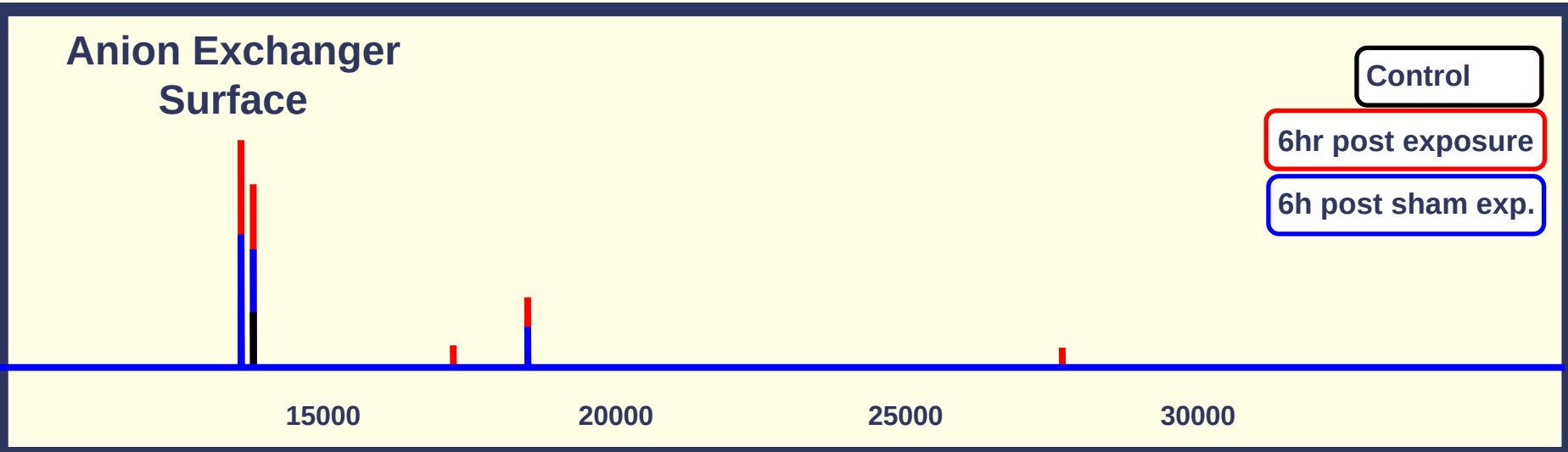
After sample processing the chip is dried and EAM is applied to each spot to facilitate desorption and ionization in the TOF-MS.



FOI

Example of profiles from serum





FOI

Summary of Proteomics Studies

- Several potential radiation specific markers identified
- Kinetics will play an important role in biomarker evaluation
  - Time for acquisition versus what marker to measure
- Complex dose response expected
  - Different proteins expressed at different dose severities
  - Potential for “categories” anticipated like in gene expression (Guel et al. 06)

FOI

Future Work

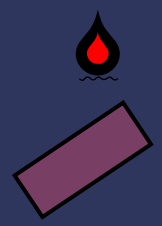
- Protein profiling
  - Analysis of individual variation
  - Further analysis, protein isolation and identification
  - Analysis from lymphocyte isolates or other subpopulations
- Protein work needs follow-up **Dose Response** studies
- Protein work needs follow-up **Comparison** studies
  - Other exposures, stress, individual variability, etc.
- Couple to gene expression studies

FOI

Potential for Early Diagnostics and Triage in Mass Casualty Accidents

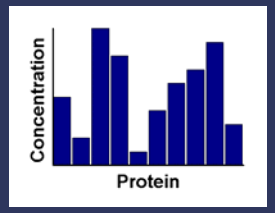
Incident involving 1000 persons

Single small volume sample



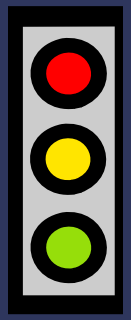
Multiplex Analysis

Fast Detection and Analysis



Automatic data manipulation

Group and prioritize individuals



10 Persons

100 Persons

890 Persons

Qualitative classification eliminating need for interpretation

Significant exposure indicated
Persons receive triage

Mild exposure indicated
Persons receive follow-up

No significant exposure indicated
Persons reassured

FOI

Inhalation Research

- Biomarkers of inhalation exposure
  - Systemic inflammatory response
    - Cytokines
  - Specific markers of lung injury
    - Leakage of lung specific proteins in the circulation
  - Proteomic assays for novel markers
    - 2-D gel from lung fluid
- Evaluation of countermeasures
  - Anti-oxidant vitamin E (vs. anti-inflammatory)

FOI

Combined Exposure Studies

- Scenario under consideration
  - Dirty bomb
- Expected consequences
  - External injuries from blast
  - Low level radiation exposures (potential internal contamination)
  - Inhalation exposure (particulate and possible chemical/ combined exposure)

FOI

Combined Exposure Studies

- Radiation exposure model
  - Rat and mouse, proteomics, biomarkers
- Chronic chemical exposure model
  - Auto-injection implant, rat model
- Inhalation model
  - Rat and mouse, acute lung injury
  - **Particulate** and chemical exposures
- Trauma injury model
  - Swine, physiological endpoints
  - Testing body armor

FOI