

Proteomics

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What is Proteomics?

Studies with focus on proteins (not necessarily MS based)

What tools do we have?

How do we study specific proteins that are known, or even unknown?

We generally want to obtain a measurement of an exact or relative change in the quantity of a protein (or protein modification) as they are effected by the introduction of a specific treatment or exposure.

This may be to an isolated protein in a test tube, in a cell culture setting, or a live animal.

Experimental side of things.....

Before we go forward, what are we measuring when we study a compound in a specific setting:

- 1) Test tube (purified protein + compound = modification to that protein? what modification at what concentration?)
- 2) Cell culture (biological mechanism can be studied; direct change to protein, indirect changes i.e. receptor mediated, parent or daughter molecule)
- 3) Animal model (translatable; system wide multi-organ effects, and discovery of biological markers to mark those effects)

Also, what problems and advantages do we have in the way of proteomics studies when we go from one setting to the next?

We go from a purified known protein in high quantity to increasingly complex systems, with the ability to measure indirect system wide effects.

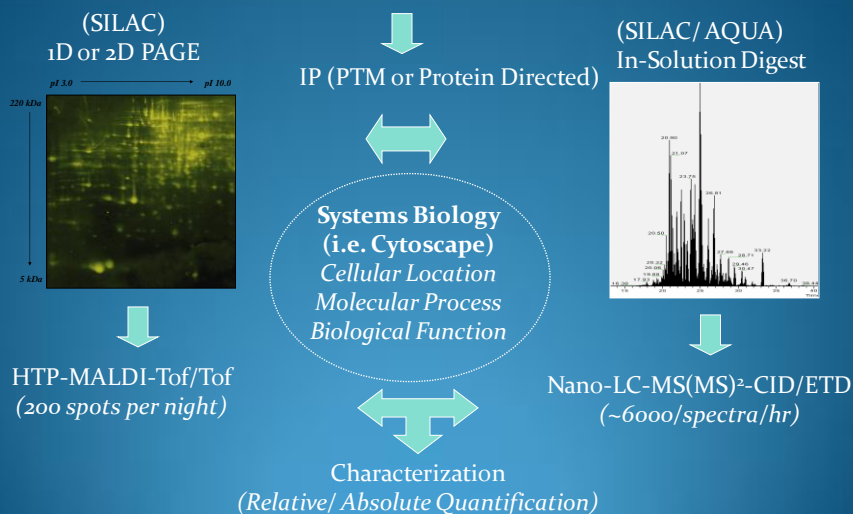
What Tools Encompass Proteomics

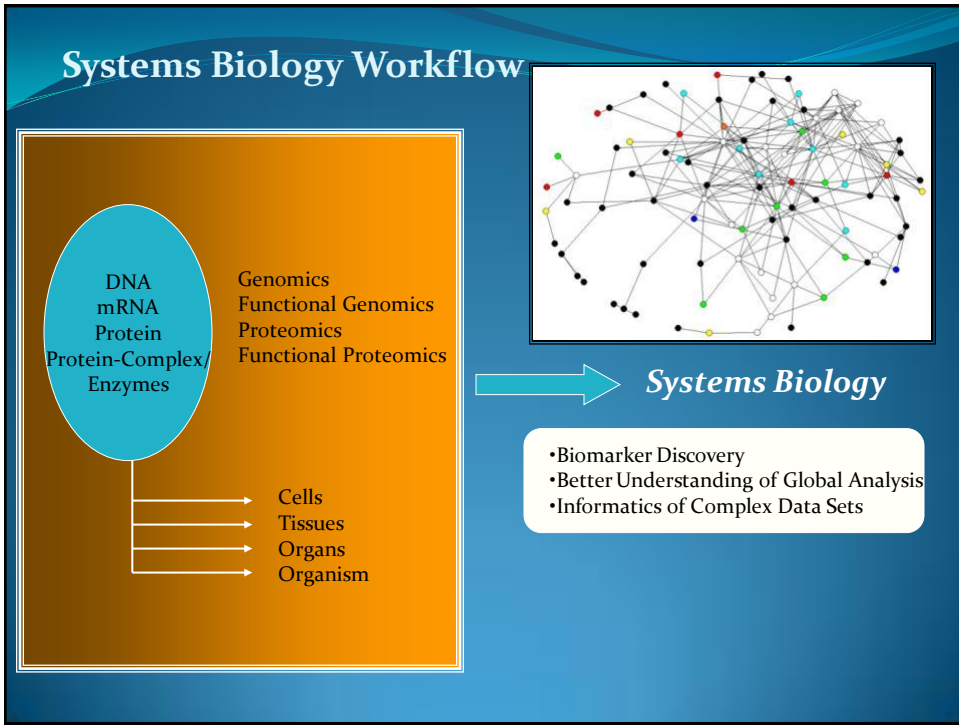
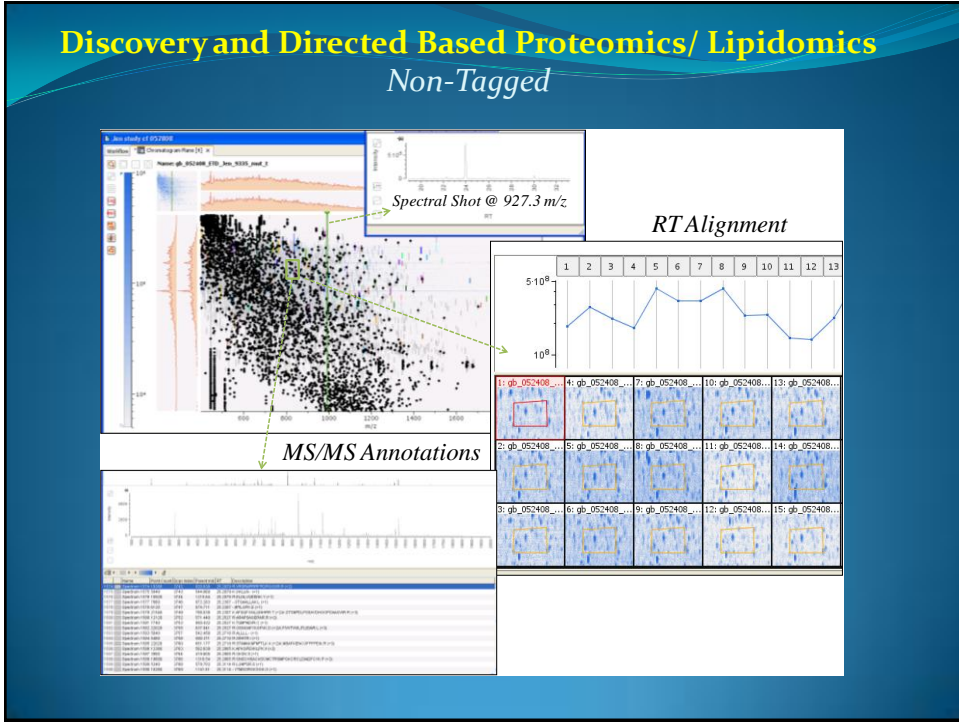
- **Relative or Absolute Quantification of Known Proteins.....**
 - Mass Spectrometry (Mass Tags/ AQUA)
 - Immuno-Directed (examples)
 - 1) Protein Arrays, 2) Western Blot, 3) ELISA, 4) Bioplex/ Luminex
- **Characterization of Unknown Proteins/ Mapping Post Translational Modifications (PTM's).....**
 - Sequencing of Unknowns, Mass Spectrometry alone!

Comparative proteomics often from a clinical perspective.....

- To find associations between biological components (i.e. SM, FA's, Proteins) and any clinical endpoint quickly, non-invasively, affordably.
- To non-invasively determine.....
 - Pathologic Changes (i.e. early detection of cancer)
 - Aggressiveness/ Stage of Disease
 - Predicting Rx Response
 - Drug Target Discovery
 - Mechanistic Studies (Systems Biology)
- **The Potential Clinical Impact is Tremendous!!**

General Theme: Mass Spectrometry Driven Discovery & Directed Quantitative Proteomics (non-Tagged/ SILAC/ AQUA)





Overview of Systems Biology Approaches

Now you have a long list of “interesting proteins”.....

How do you sidestep the “So What” factor..... Systems Biology!

Great Overview at.....

<http://www.cytoscape.org/>

<http://cytoscape.org/cgi-bin/moin.cgi/Presentations>

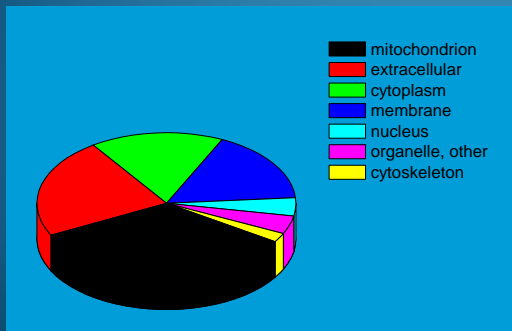
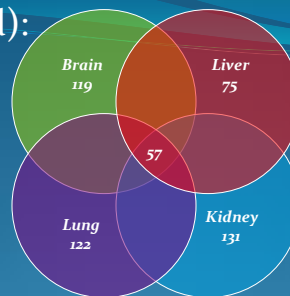
Example (Secreted):

Total Unique Proteins = 277

Brain (B)	119
Liver (Li)	75
Lung (Lu)	122
Kidney (K)	131

Common (>2) 57

*Common (all 4) 33

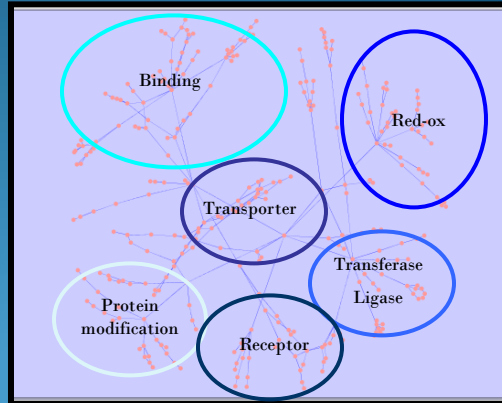
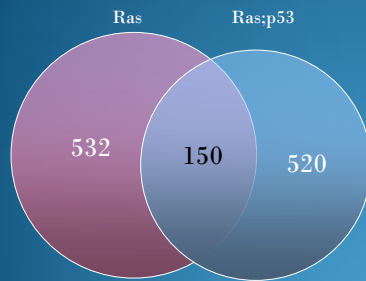


Example of Common Proteins

- *Acyl-CoA-binding protein
- Aldose reductase
- *Alpha-enolase
- *Apolipoprotein D precursor
- *ATP synthase-coupling factor
- Bisphosphoglycerate mutase
- *Copper transport protein ATOX1
- *Cytochrome c oxidase
- *D-dopachrome decarboxylase
- Dynein light chain roadblock-type 1
- *Glutathione S-transferase P 1
- Isopentenyl-diphosphate isomerase 1
- L-xylulose reductase
- *Peptidyl-prolyl cis-trans isomerase A
- *Phosphatidylethanolamine-binding protein
- Polyadenylate-binding protein 1
- Protein S100-A6
- *Ptms protein
- *Selenium-binding protein 1
- *Superoxide dismutase [Cu-Zn]
- Thioredoxin
- *Thymosin beta-4
- *TSC22 domain family protein 1
- *Albumin
- *Apolipoprotein A-II
- Calmodulin 1
- *Carbonic anhydrases (2)
- *Hb-alpha
- *Hb-beta
- *Keratin Subtypes (10)
- *MIF
- Mitochondrial Membrane Tim8 A
- Mitochondrial Membrane Tim8 B
- *Niemann Pick type C2
- *Prosaposins (3)
- Ubiquitin C, full insert sequence
- *Unknowns or Not Annotated (8)

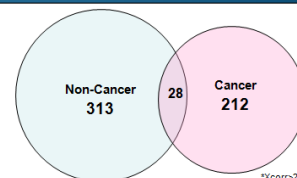
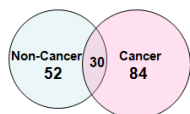
Example (Mouse Model – Pancreatic Cancer):

Protein bands were excised from the 1D gel for Ras and Ras:p53-63, digested with trypsin and run with LTQ-XL/CID mode. The results from LTQ-XL were run through SEQUEST to identify proteins. The proteins unique to Ras:p53-63 was run through *Cytoscape to see how those proteins relate to one another based on their molecular functions.



*Cytoscape is an open source bioinformatics software platform for **visualizing** molecular interaction networks and **integrating** these interactions with gene expression profiles and other state data.

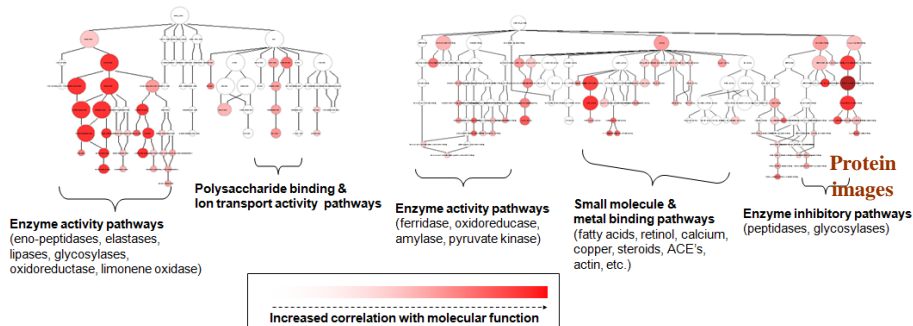
Non-Tagged Analysis of Pancreatic Juice (Cancer Vs. Non-Cancer)

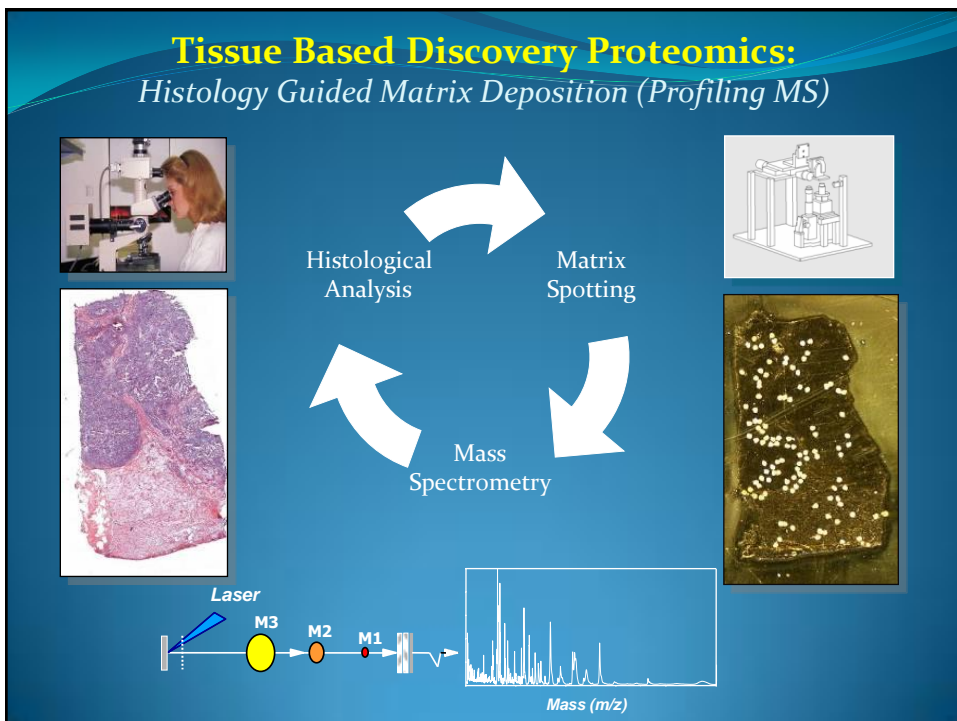
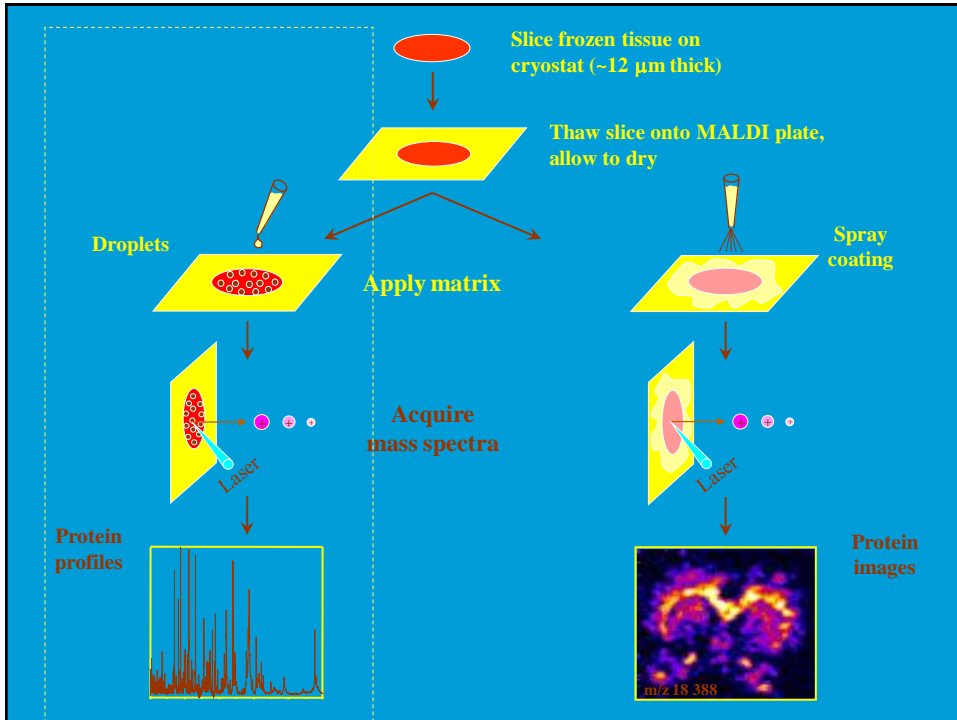


*xcarr>2.8, Sp>

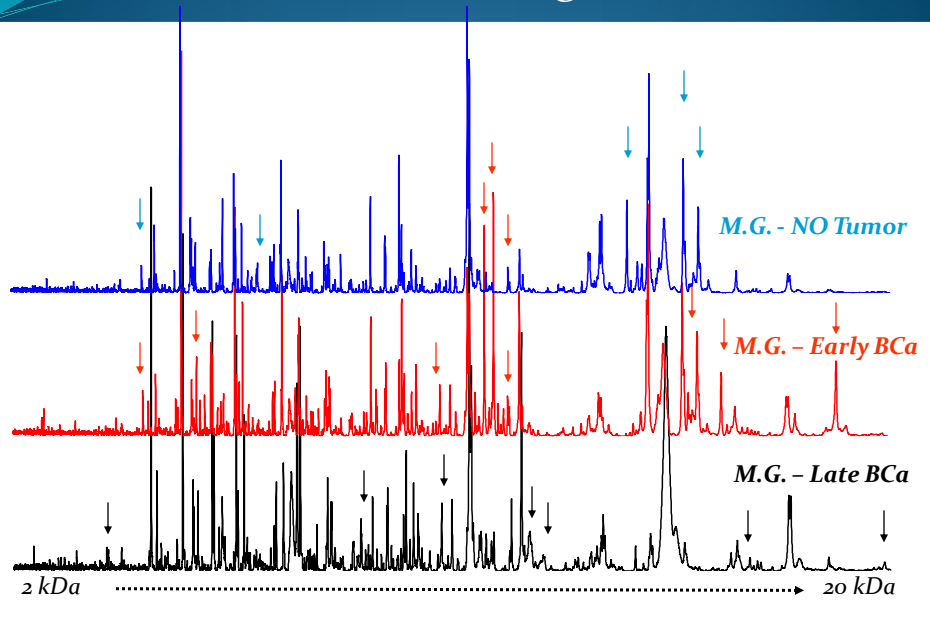
Non-Cancer Specific

Cancer Specific

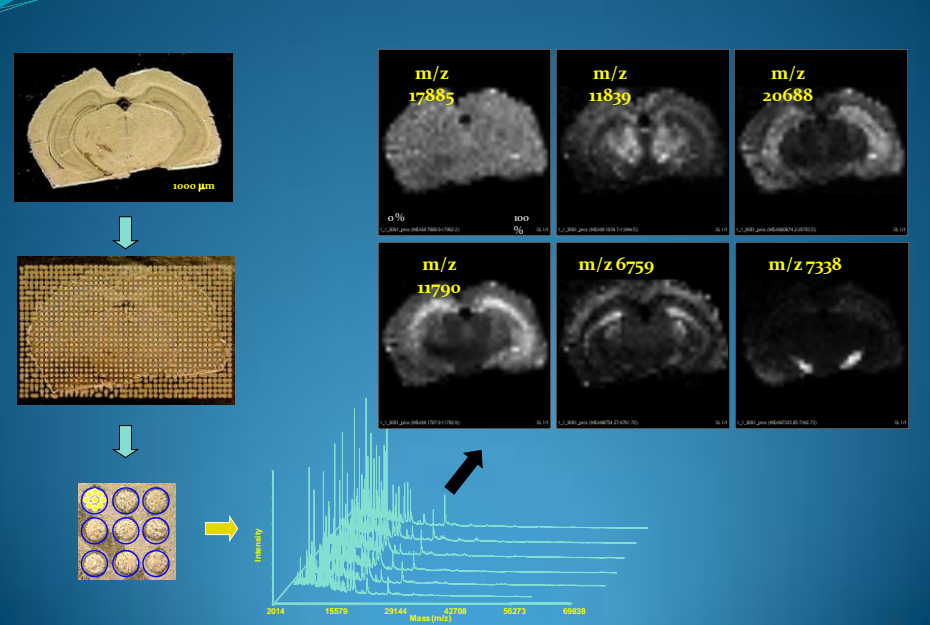




Results from Mass Profiling Tissue Sections



MS Imaging of a Mouse Brain Section



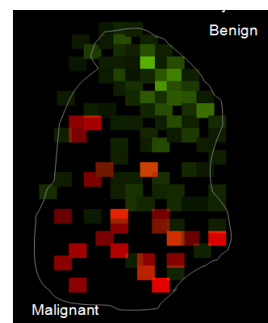
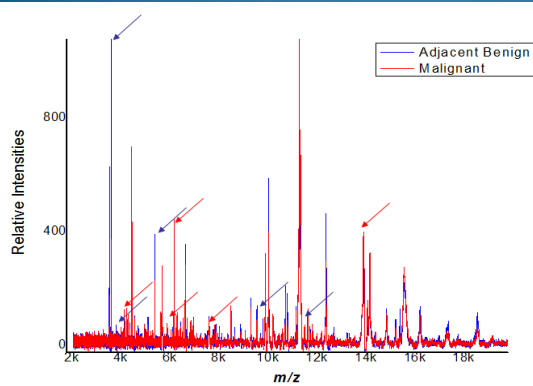
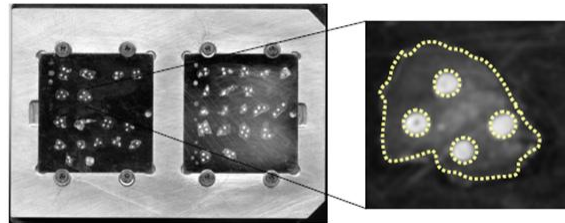
Focus Today:

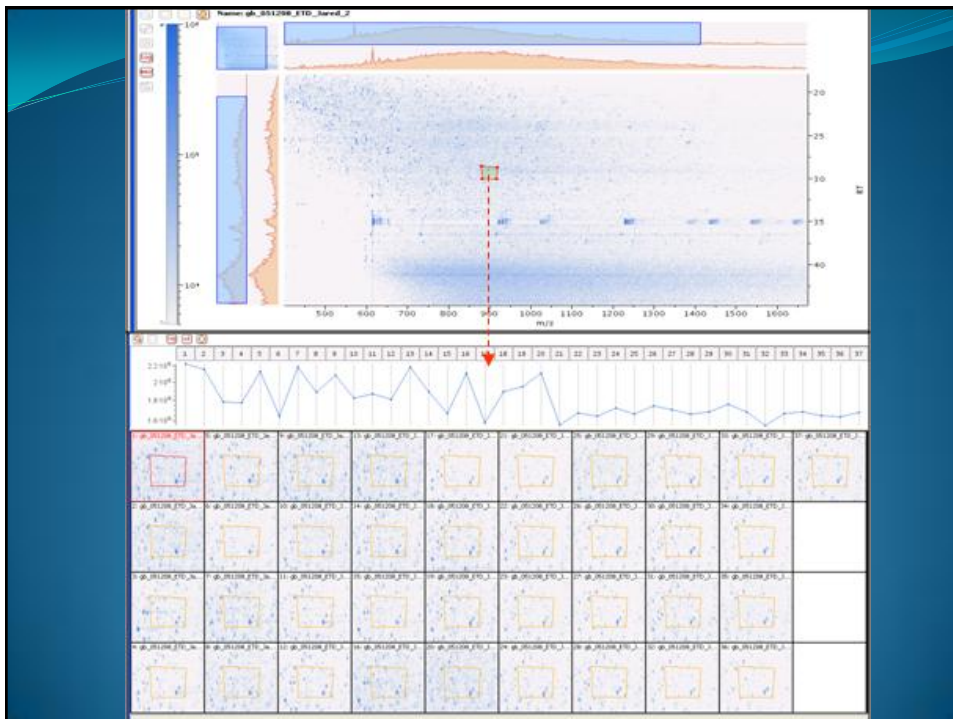
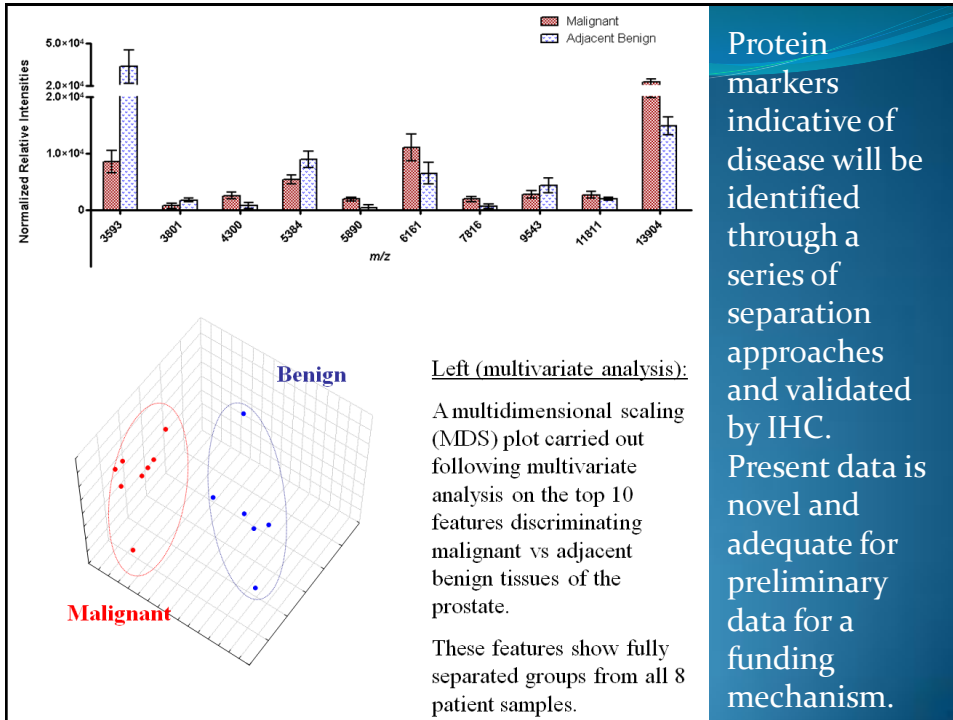
Tissue profiling ; LC/MS and directed MALDI

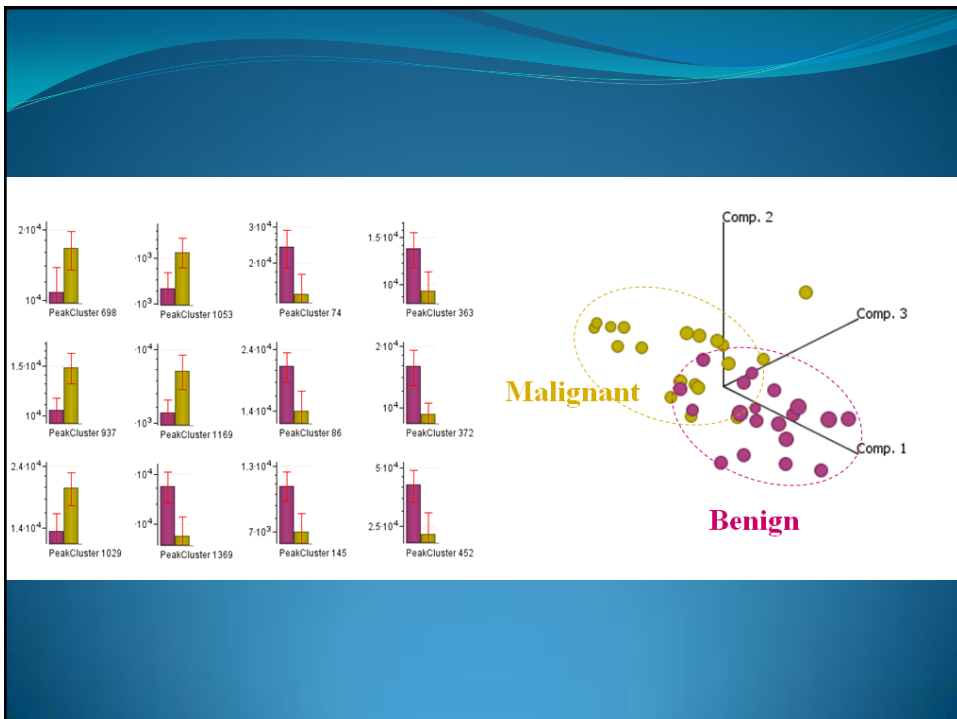
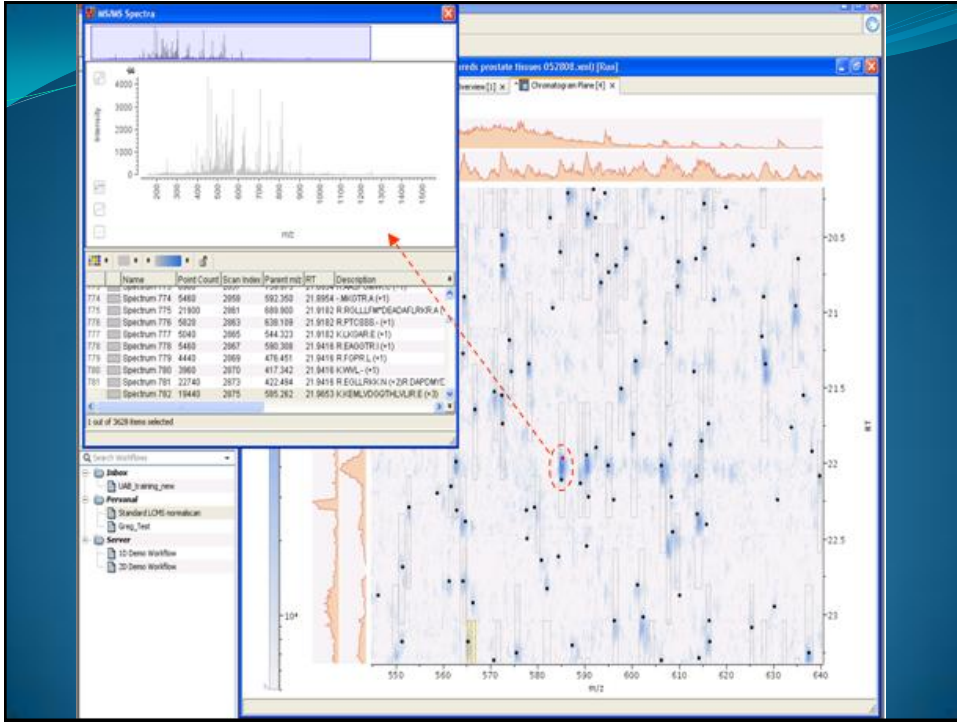
Pharmaco-Proteomics (pristanic acid & AMACR)

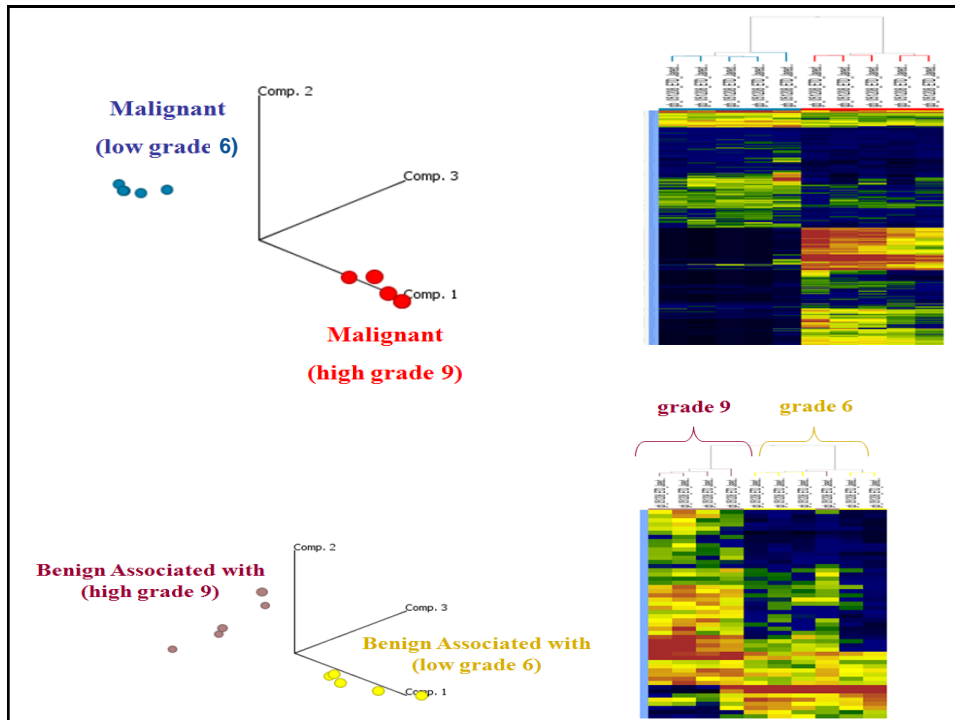
Identification of Markers from Gentamicin Induced
Kidney Damage in Monkey.....

Project 1; Tissue
Profiling; mining
for markers of
diagnosis &
progression?









Protein Identified in this Study:

Diagnostic - Cancer Specific

Heat shock cognate 71 kDa protein, Prostate specific antigen, Alpha-2-HS-glycoprotein, IGHA2, Golgi phosphoprotein 2, APS protein, 60 kDa heat shock protein, RcTPM3, Macrophage migration inhibitory factor, Histone 1, Antithrombin-III, Peroxiredoxin-1, Alpha-enolase, Malate dehydrogenase, Fructose-bisphosphate aldolase, ATP synthase subunit alpha

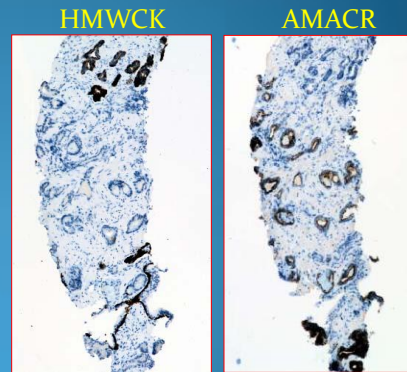
Prognostic - Grade Specific

Malate dehydrogenase, Alpha-enolase, Tropomyosin, Filamin-A, Apolipoprotein A-I precursor (Apo-AI), Zinc-alpha-2-glycoprotein, Serotransferrin, prostate specific antigen, Lipoma-preferred partner, Prostatic acid phosphatase, APS protein, Vimentin, Calponin-1, Cytochrome c oxidase, Alpha-1-antichymotrypsin, Smooth muscle myosin, Beta-microseminoprotein, Elongation factor 1-alpha 1

Project 2; AMACR in Prostate Cancer

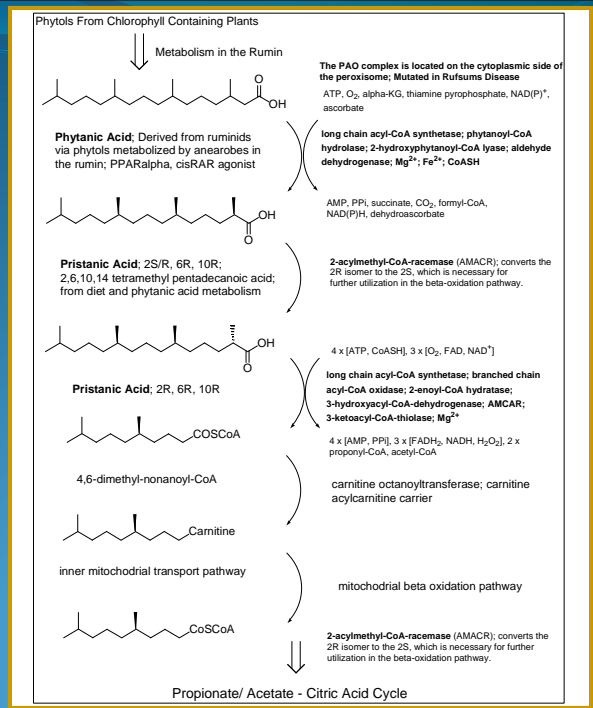
AMACR is overexpressed in the epithelial cells of high-grade prostatic intraepithelial neoplasia (PIN), and in a majority (60 –100 %) of prostate cancers (CaP) as compared to minimal expression in normal and benign hyperplastic lesions of the prostate

CaP Biopsy

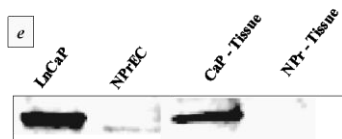
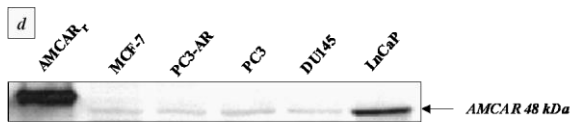
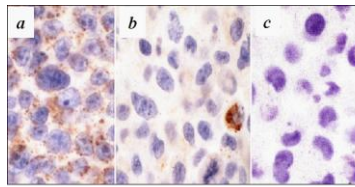


Metabolic Effects of AMACR

Production of:
 H_2O_2
 NADH
 FAS substrates
 Active - Intermediates



Optimizing With a Cellular Model



Transcript;
Real Time PCR:

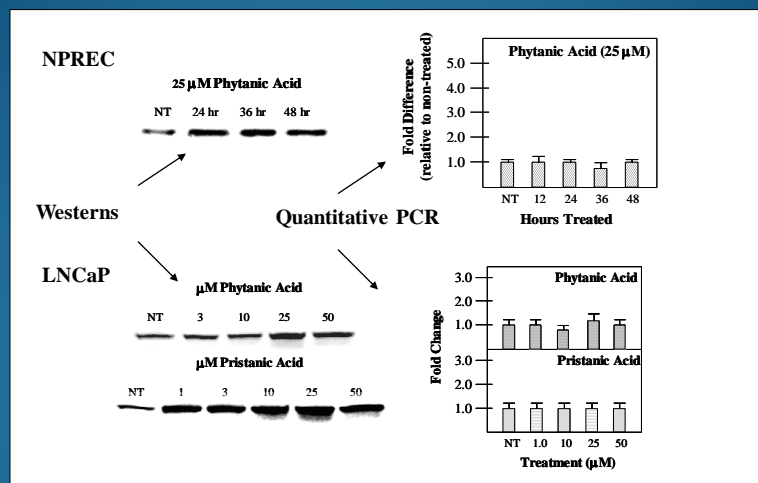
LNCaP Vs. NPrEC
12-16 Fold Increase

LNCaP Cells
2-4 cell sensitivity

Tested on 27 Patient
Samples;

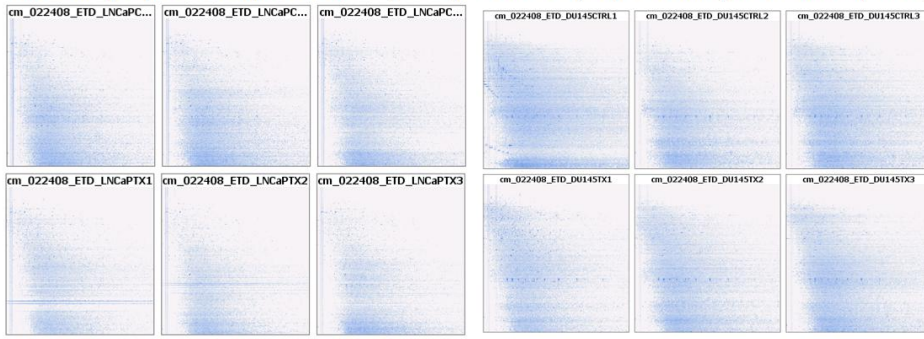
15 Healthy
12 CaP

AMACR is not Induced..... Rather Stabilized by Its Substrate



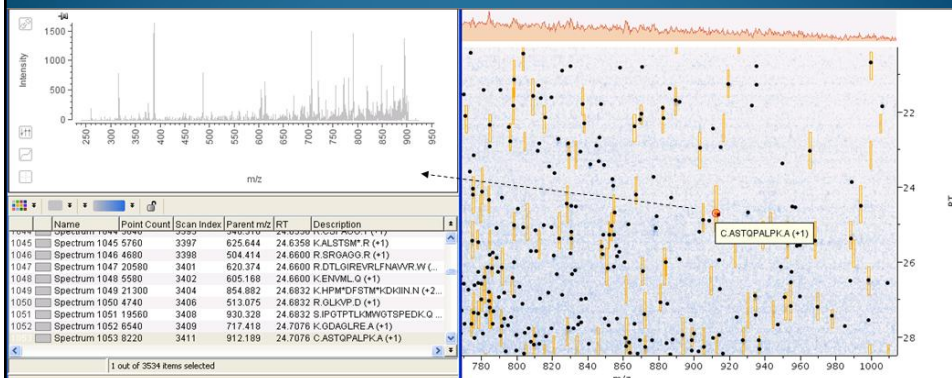
Treatment with Substrate.....2D Plots LC/MS Cells Expressing AMACR Vs. Cells that do not!

LNCaP (Top Control, Bottom Tx, n=3) Du145 (Top Control, Bottom Tx, n=3)

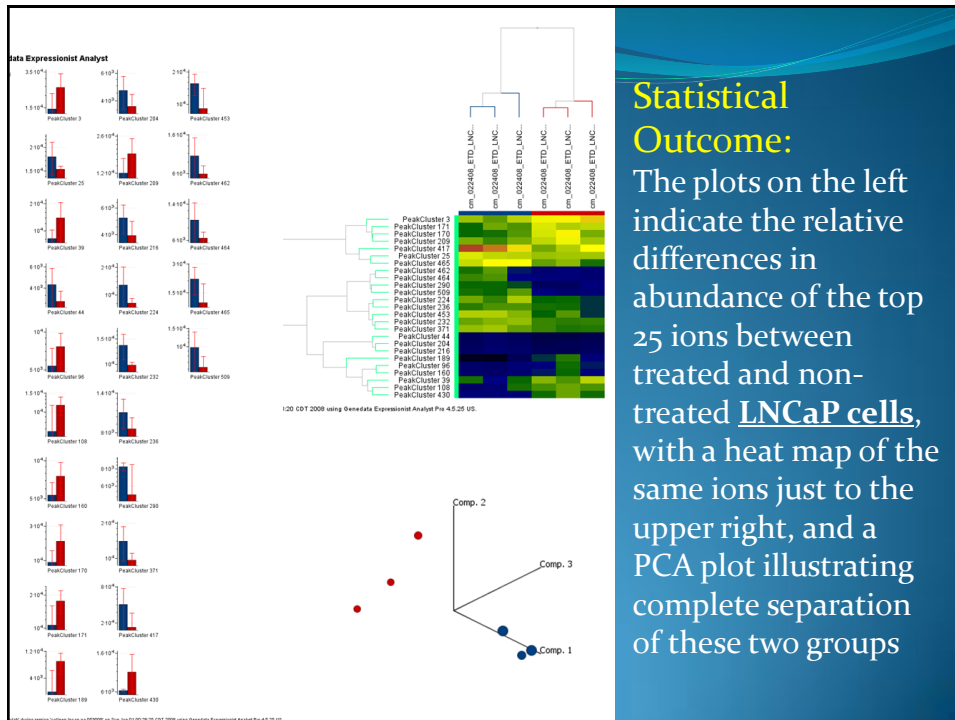


Above (LCMS ion plots from 3x3 treated vs non treated LNCaP and Du145 cell lines): Overall these ion plots illustrate the first step in overall consistency between runs, with minor adjustment to RT alignment required.

Treatment with Substrate.....2D Plots LC/MS Comparative Analysis and Protein ID's?

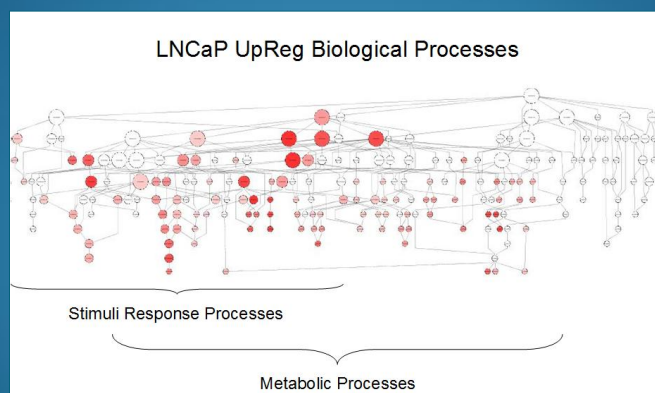


Above (LCMS/MS spectra from a single binned averaged representation of all runs with peptides sequence attached. Following RT alignment and binning (clustering all runs into single bins by time and mass) by Refiner MS by Genedata, statistical analysis can be carried out in Analyst by Genedata.



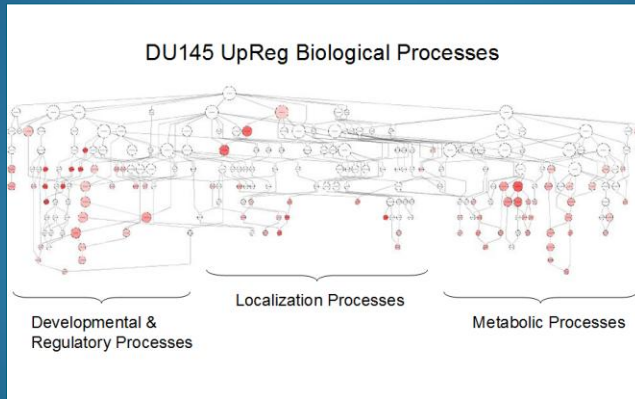
Proteins Specific to Treatment (LNCaP):

dihydrolipoamide dehydrogenase, H2B histone, RAS oncogene family, annexin A5, citrate synthase, karyopherin (importin), phosphatidylethanolamine binding protein 1, APEX nuclease (multifunctional DNA repair enzyme), lactate dehydrogenase B, non-metastatic cells (NM23A & NM23B), glycyl-tRNA synthetase, adenine phosphoribosyltransferase, Rho GDP dissociation inhibitor (GDI), aldehyde dehydrogenase, aldolase, fructose-bisphosphate, nucleolin, cathepsin B



Proteins Specific to Treatment Du145:

enolase alpha, non-metastatic cells (NM23B), eukaryotic translation elongation factor 1, ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1, SMT3 suppressor of mif, enoyl Coenzyme A hydratase, peptidylprolyl isomerase A, proteasome (macropain), tropomyosin 4, histone H2, integrin beta, thioredoxin reductase, vinculin, enolase (alpha), protein disulfide isomerase, nicotinamide N-methyltransferase, profilin 1, eukaryotic translation elongation factor, tyrosine 3/5-monoxygenase



Identification of Markers from Gentamicin Induced Kidney Damage in Monkey.....

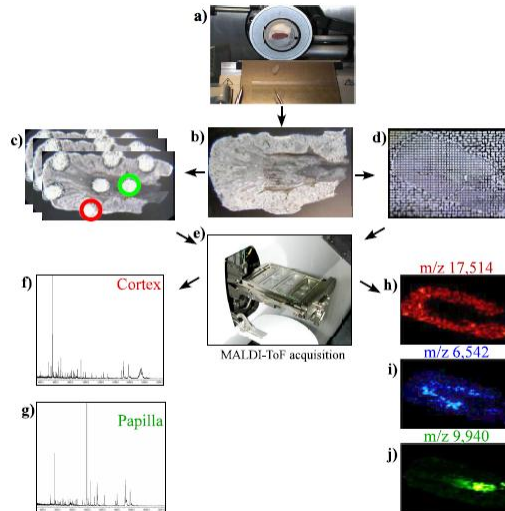
Gentamicin Information Center

Living with GENTAMICIN Poisoning

This website is **dedicated** to the many unfortunate persons who suffer a **balance or vision disorder** after receiving the antibiotic gentamicin.

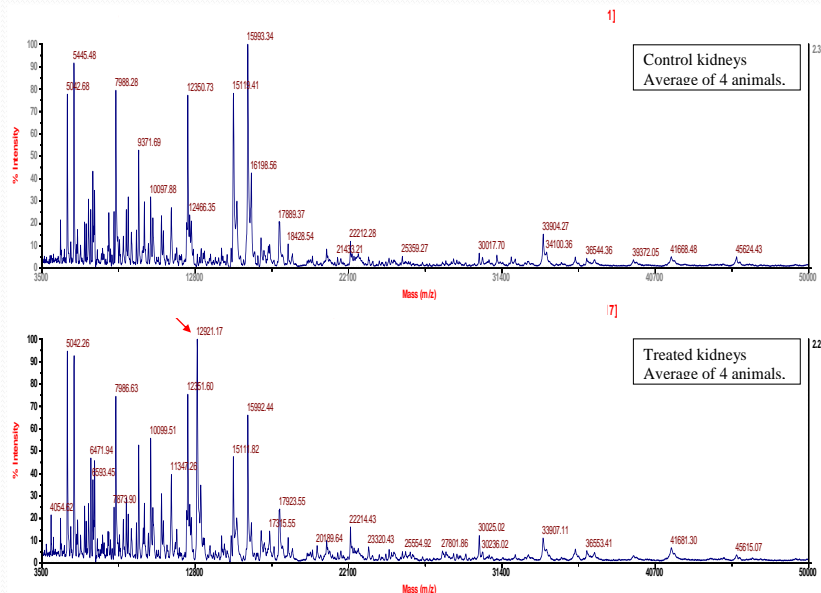


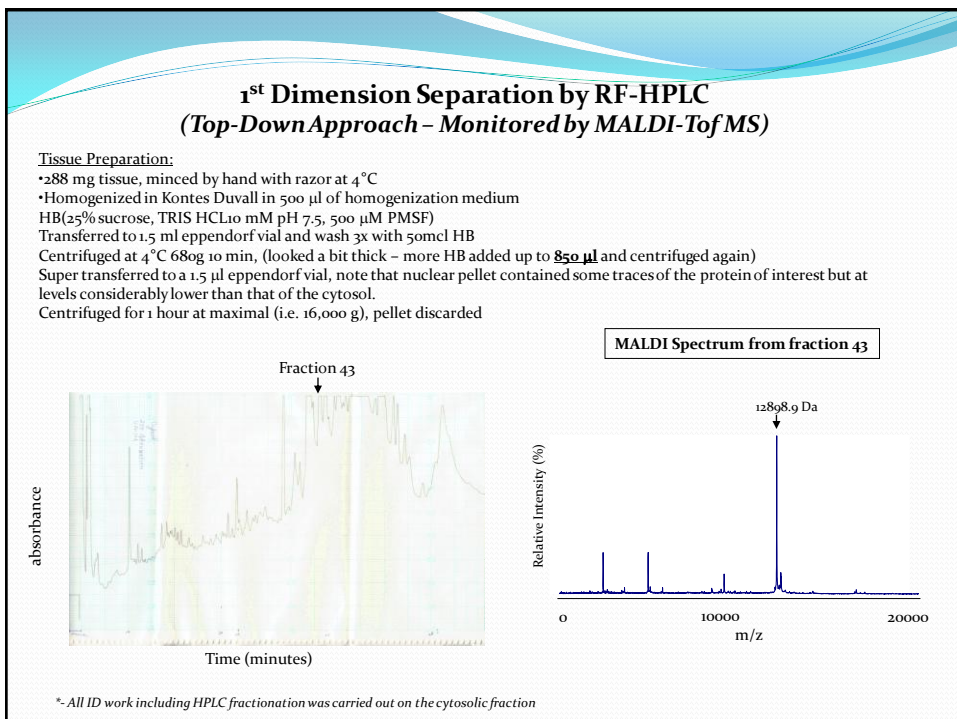
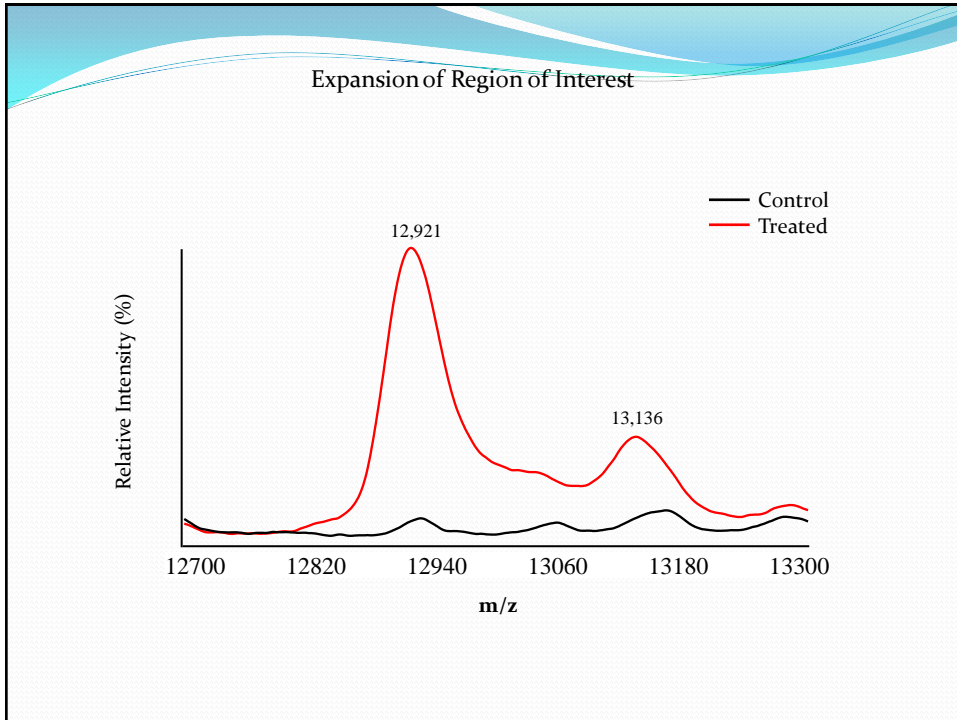
Overview of Approach From Similar Study on Rat

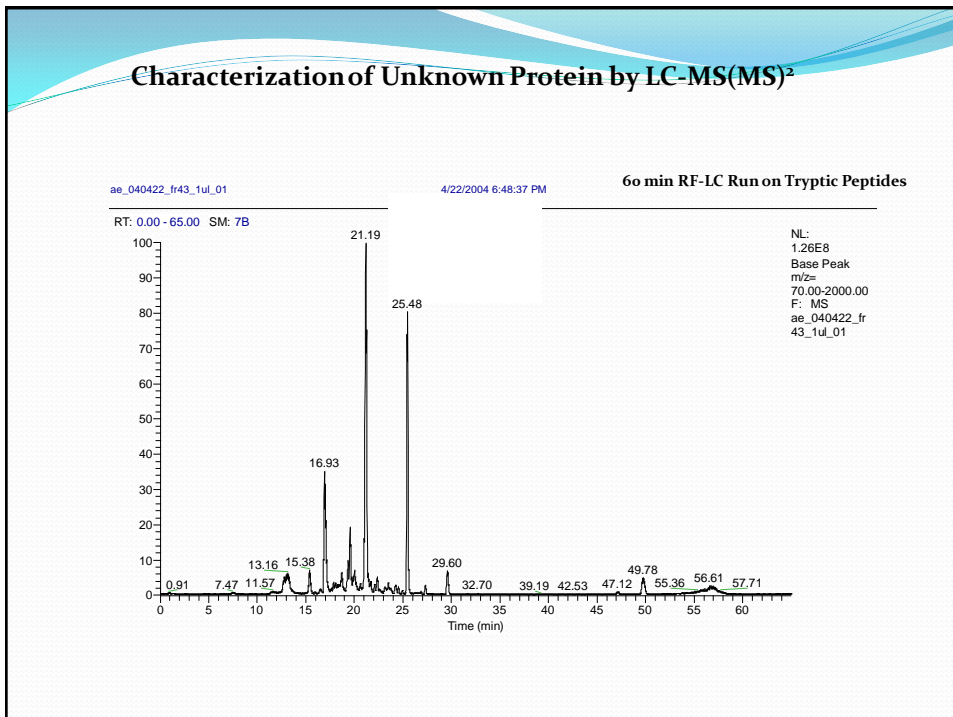
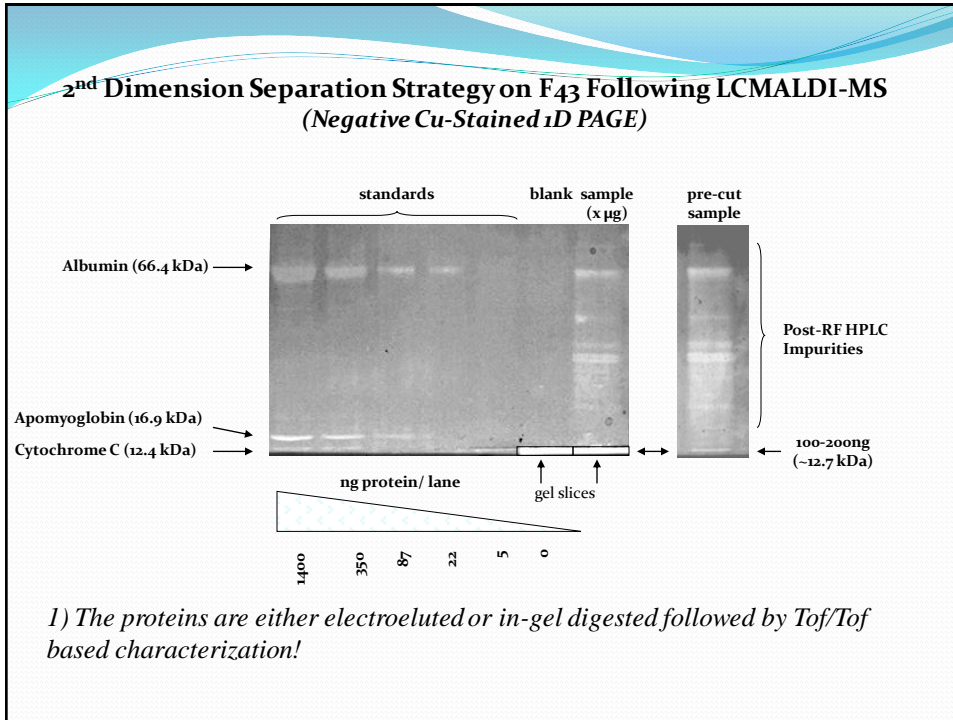


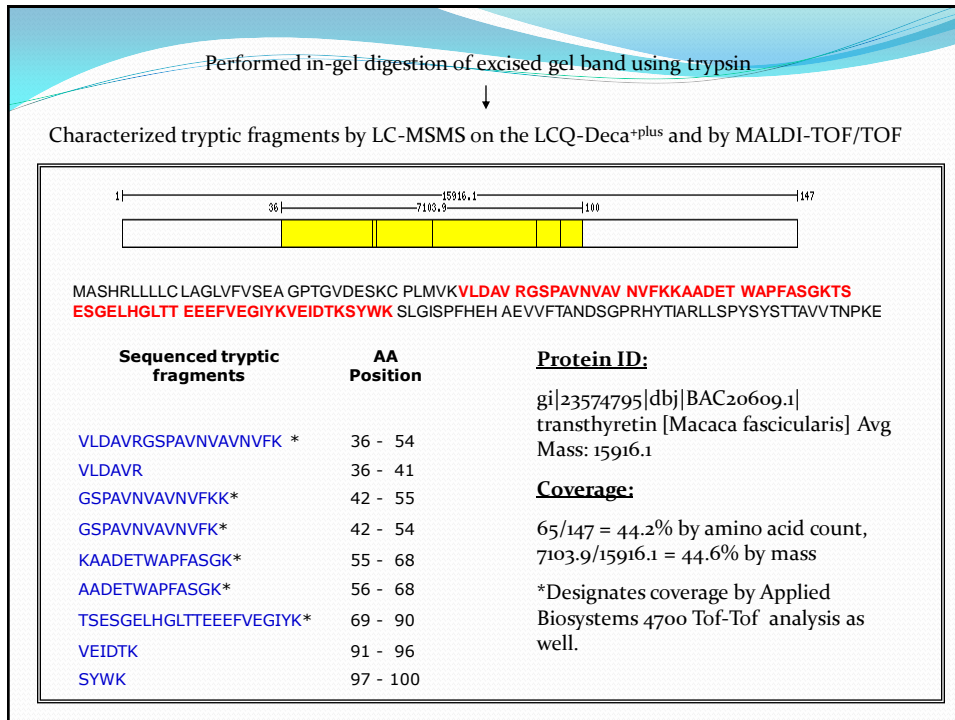
Meistermann, et. al. MCP 2006

Identification of Toxic Markers Via Mass Profiling (Monitored by MALDI-ToF MS)









Finding the Correct form of the Protein Can be a Challenge

Macaca TTR, 15918Da, 13792Da.

mashrllllc laglvfsea/gptgtgeskc/ (cleavage sites?)

1 plmvkvldav rgspainvav hvfrkaaddt wepfasgkts esgelhgltt eefvegiyk
61 veidtksywk algispfheh aevvftands gprrytiaallspysystta vvtnpke

127 AA, active form, 21-147, 13763 Da

gptgtgeskcplmvkvldavrgspainvavhvfrkaaddtwepfasgktsesgelhglteefvegiykveidtksy
wkalispfhehaevvftandsgprrytiaallspysysttavvtnpke

Extrahepatic sequence?, 31-147, 12845 Da

plmvkvldavrgspainvavhvfrkaaddtwepfasgktsesgelhglteefvegiykveidtksywkalispfh
ehaevvftandsgprrytiaallspysysttavvtnpke

Pro-form, 147 AA, 15887 Da

mashrllllclaglvfseagptgtgeskcplmvkvldavrgspainvavhvfrkaaddtwepfasgktsesgelhgltt
eefvegiykveidtksywkalispfhehaevvftandsgprrytiaallspysysttavvtnpke

11810.7 Da with potential cleavage at site aa:

gptgtgeskcplmvkvldavrgspainvavhvfrkaaddtwepfasgktsesgelhglteefvegiykveidtksy
wkalispfhehaevvftandsgprrytia

Initial Confirmation by Western Analysis (Rabbit anti-TTR 1°pAb (Novocastra Labs), Donkey anti-rabbit HRP 2°Ab)

Gel:

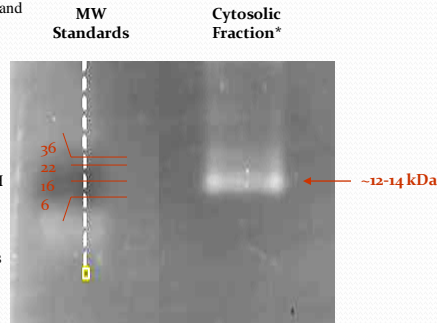
- Ran 4-12% Tris-Glycine gel at 150V with 50 μ l of cytosol sample (10 μ l cytosol in 20 μ l LDS buffer, 15 μ l H₂O, 5 μ l Reducing agent) approx. 1 hr. Sample was first heated on thermocycler for 10 min. at 70°C. Loaded 14 μ l (50 μ g) and 7 μ l (25 μ g) of sample in duplicate along w/ 10 μ l See Blue STD.
- Running Buffer (950 ml H₂O, 50 ml MOPS SDS 20X NuPage running buffer).

Electroblotting w/BioRad Trans-Blot SD cell:

- Transfer Buffer (5.82g Tris, 2.93 g Glycine, 0.0377g SDS, 200ml MeOH, 800 ml H₂O)
- Wetted Immobilon -psq Millipore membranes with MeOH then TB.
- Assembled Trans-blot apparatus and ran for 30 min. @ 15V.
- Placed gel in H₂O, then PBS/Tween (500 ml Dulbecco's PBS, 0.05 ml Tween 20).

Introduction of Antibodies:

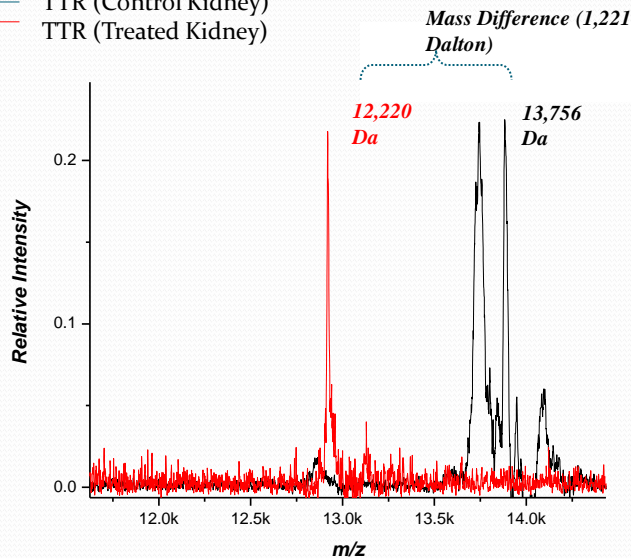
- Added Blocking Agent (2.5g BA from kit, 50 ml PBST), left on lab rotator for 1 hr @ RT, then washed 3X for 15min. w/PBST
- Diluted 1° Ab in 1 ml H₂O. Diluted further @ 1:1000(50 μ l Ab, 50 ml PBST)
- Added 1° Ab to membrane and rotated @ 4°C for 2 hrs., washed 2X quickly in PBST, 3X for 15 min.
- Added 2° Ab @ 1:5000 (10 μ l Ab, 50 ml PBST), rotated for 1 hr @ RT, washed 2X quickly, 3X for 15 min. in PBST.
- Using BCA reagents from Amersham ECL kit, combined 2 ml soln. A with 50 μ l of soln. B. Pipetted over membrane for approx. 5 min. in dark. Used Typhoon Imager with blue laser to image membrane and TTR band.



*. Single band shown in MW range of interest, non-specific bands present above 50kDa

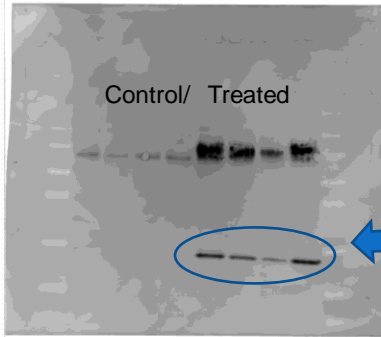
IP-MALDI-MS for TTR

— TTR (Control Kidney)
— TTR (Treated Kidney)



Initial Confirmation by Western Analysis
(Rabbit anti-TTR 1°pAb (Novocastra Labs), Donkey anti-rabbit HRP 2°Ab)

Urine Specimens



Truncated Protein
Specific to Disease

Questions?

