Proteomics and Mass Spectrometry 2011

The team

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Course plan

- **Meet Mondays/Wednesdays/Fridays in MCLM 401 from 9-10:30 am (Jan 5-Mar 18)**
- **Graduate Students taking this course are required to attend each session (unless there is advance communication with instructor)**
- **Evaluations will be made from exams and in-class presentations**
- **Where possible, class notes will be available on the UAB proteomics website (go to http://www.uab.edu/proteomics/index2.php - click on Class)**

Recommended general texts

- **Suggested text - "*Introduction to Proteomics*" by Daniel C. Liebler, 2002**
- **Also see "*The Expanding Role of Mass Spectrometry in Biotechnology*" by Gary Siuzdak (a 2003 edition of the 1996 first edition)**
- **"*Mass spectrometry data analysis in proteomics*", (ed., Mathiesson, R) in Methods in Molecular Biology, vol 367.**

Suggested readings

- **Kenyon G, et al. *Defining the mandate of proteomics in the post-genomics era: workshop report*. Mol Cell Proteomics, 1: 763-780 (2002)**
- **Kim H et al. *Proteomics and mass spectrometry in nutrition research*. Nutrition, 20: 155-165 (2004)**
- **Righetti P. et al. *Prefractionation techniques in proteome analysis: the mining tools of the third millennium*. Electrophoresis, 26: 297-319 (2005)**
- **Anderson NL. *The roles of multiple proteomic platforms in a pipeline for new diagnostics*. Mol Cell Proteomics, 4:1441-1444 (2005)**
- **Venkatesan et al. *An empirical framework for binary interactome mapping*. Nat Methods, 6:83-90 (2009) PMID: 19060904**
- **Yan W et al. *Evolution of organelle-associated protein profiling*. J Proteomics, 72:4-11 (2009) PMID: 19110081**
- **Pan S, et al. *Mass Spectrometry Based Targeted Protein Quantification: Methods and Applications*. J Proteome Res, 8:787-797 (2009) PMID: 19105742**

BMG/PHR 744 - section 1

Jan 5, Wed	**S Barnes/H Kim *The world of biomolecules. The proteome, proteomics and other –omics and where to start***
Jan 7, Fri	**M Renfrow *Mass spectrometry – gas phase transfer and instrumentation – including ETD***
Jan 10, Mon	**H. Kim Simplifying the proteome - techniques of protein purification**
Jan 12, Wed	**M. Renfrow *Methods for the identification of proteins: MALDI-TOF of proteins and peptide mass fingerprinting; LC analysis and peptide sequencing***
Jan 14, Fri	**M Renfrow *Ion fragmentation in mass spectrometry; application to proteomics***
Jan 17, Mon	**S Barnes *Isotopes in mass mass spectrometry***
Jan 19, Wed	**J Prasain *Ion Fragmentation of small molecules; Lipidomics***
Jan 21, Fri	**S. Asmellash *Sample preparation for proteomics and mass spectrometry***
Jan 24, Mon	**S. Barnes *Mass spectrometry in qualitative and quantitative burrowing of the proteome***
Jan 26, Wed	**J. Mobley *Connecting proteomics into bioinformatics; MUDPIT and SEQUEST; false discovery rates in complex systems***
Jan 28, Fri	**C. Crasto *The bioinformatics of the proteome; web tools; MRMPath***
Jan 31, Fri	***Mid-term take-home exam due***

BMG/PHR 744 - section 2

Feb 2, Wed	**S. Barnes *Course introduction***
Feb 4, Fri	**J. Prasain *Designing the metabolomics experiment***
Feb 7, Mon	**J. Prasain *Qualitative and quantitative analysis/method validation in metabolomics***
Feb 9, Wed	**H. Kim *Protein separation by electrophoresis and other 2D-methods***
Feb 11, Fri	**S. Barnes *Enzymology and mass spectrometry***
Feb 14, Mon	**Student presentations**
Feb 16, Wed	**M. Renfrow *Analysis of protein-protein interactions by affinity purification and mass spectrometry***
Feb 18, Fri	**P. Prevelige *Mass Spectrometry as a Tool for Studying Protein Structure***
Feb 21, Mon	**P. Prevelige *Study of macromolecular structures – protein complexes***

BMG/PHR 744 - section 3

Feb 23, Wed	**E. Shonsey**	***MS in Forensics***
Feb 25, Fri	**J. Mobley**	***Tissue and body fluid proteomics and mass spectrometry***
Feb 28, Mon	**Student presentations**	
Mar 2, Wed	**J. Mobley/D. Stella**	***Applications of MS to tissue imaging***
Mar 4, Fri	**M. Renfrow**	***Applications of FT-ICR-MS***
Mar 7, Mon	**J. Novak/M. Renfrow**	***Mass spectrometry in glycomics research - Application to IgA nephropathy***
Mar 9, Wed	**H. Kim**	***Use of proteomics and MS methods in the study of the brain proteome and neurodegenerative diseases***
Mar 11, Fri	**H. Kim/S. Barnes**	***Putting it all together – by-passing pyruvate kinase***
Mar 18	***Final report due***	

Course learning objectives

- **Introduction to the concepts and practice of systems biology**
- **Sample ionization and mass spectrometers**
- **Mass spectrometry and its principal methods**
  - **protein and peptide ID; peptide and metabolite ion fragmentation; stable isotope labeling; quantification**

Course learning objectives

- **Informatics, statistics and quality control in mass spectrometry**
- **Importance of prefractionation in proteomics - 2DE, LC and arrays**
- **Applying mass spectrometry to protein modifications, function, structure and biological location, and to other biological molecules**

Hopes and hazards of biomedical research

It boils down to whether having taken life apart into its distinct pieces, can we reassemble it in new ways? [*strong analogies to what have been the central quests of physics since the early 20th century*]

Can we create a form of life that might live in a very hostile extra-terrestrial environment and thereby save humanity?

Or will we (as well as our enemies) instead create life forms that can terrorize, or even eliminate us?
Will Einstein's and Oppenheimer's moral dilemmas surface in biomedical science?

History of proteomics

- **Essentially preceded genomics**
- **"Human protein index" conceived in the 1970s by Norman and Leigh Anderson**
- **The term "proteomics" coined by Marc Wilkins in 1994**
- **Human proteomics initiative (HPI) began in 2000 in Switzerland - http://www.hupo.org**
- **Human Proteome Organization (HUPO) had meetings in 2002 in Versailles, France; 2003 in Montreal, Canada; 2004 in Beijing, China; 2005 in Munich, Germany; 2006 in Long Beach, CA; 2007 in Seoul, Korea; 2008 in Amsterdam, The Netherlands; 2009 in Toronto, Canada; 2010 meeting in Sydney; 2011 meeting will be in Geneva**

What proteomics is, what it isn't

"Proteomics is not just a mass spectrum of a spot on a gel"

George Kenyon,
2002 National Academy of Sciences Symposium

Proteomics is the identities, quantities, structures, and biochemical and cellular functions of all proteins in an organism, organ or organelle, and how these vary in space, time and physiological state.

Collapse of the single target paradigm - the need for systems biology

Old paradigm

Diseases are due to single genes - by knocking out the gene, or designing specific inhibitors to its protein, disease can be cured

But the gene KO mouse didn't notice the loss of the gene

New paradigm

We have to understand gene and protein networks - proteins don't act alone - effective systems have built in redundancy

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Research styles

- **Classical NIH R01**
  - **A specific target and meaningful substrates**
  - **Emphasis on mechanism**
  - **Hypothesis-driven**
  - **Linearizes locally multi-dimensional space**
- **Example**
  - **Using an X-ray crystal structure of a protein to determine if a specific compound can fit into a binding pocket - from this "*a disease can be cured*" - this approach ignores whether the compound can get to the necessary biological site, whether it remains chemically intact, and where else it goes**

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From substrates to targets to systems - a changing paradigm

- **Classical approach - one substrate/one target**
- **Mid 1980s - use of a pure reagent to isolate DNAs from *cDNA libraries* (multiple targets)**
- **Early 1990s - use of a *reagent library* (multiple ligands) to perfect interaction with a specific target**
- **2000+ - effects of specific reagents on cell systems using *DNA microarrays* (500+ genes change, not just one)**
- **2008 - integration of transcriptomics, proteomics, peptidomics, metabolomics (everything changes, just like in ecology)**

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Exploring information space - the *Systems Biology* approach

- **Systems biology means measuring everything about a system at the same time**
- **For a long time, it was deemed as too complex for useful or purposeful investigation**
- **But are the tools available today?**

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Systems Biology

"To understand biology at the system level, we must examine the structure and dynamics of cellular and organismal function, rather than the characteristics of isolated parts of a cell or organism."

"Properties of systems, such as robustness, emerge as central issues, and understanding these properties may have an impact on the future of medicine."

"However, many breakthroughs in experimental devices, advanced software, and analytical methods are required before the achievements of systems biology can live up to their much-touted potential."

Kitano, 2002

The Biological Data of the Future

Current nature of data	Elias Zerhouni, FASEB 2004
• Destructive • Qualitative • Uni-dimensional • Low temporal resolution • Low data density • Variable standards • Non cumulative	• Non-destructive • Quantitative • Multi-dimensional and spatially resolved • High Temporal resolution • High data density • Stricter standards • Cumulative

Techniques in Systems Biology

- **DNA microarrays to describe and *quantify* the transcriptosome**
- **Large scale and small scale proteomics**
- **Protein arrays**
- **Protein structure**
- **Metabolomics**
- **Integrated computational models**

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Papers on systems biology

http://www.nature.com/focus/systemsbiology userguide/index.html

This is a cluster of papers in Nature Cell Biology, Nature Reviews in Molecular and Cell Biology, and Molecular Systems Biology that was published in October, 2006

Also read the papers and reviews published in *Molecular Systems Biology* (electronically available from Lister Hill Library)

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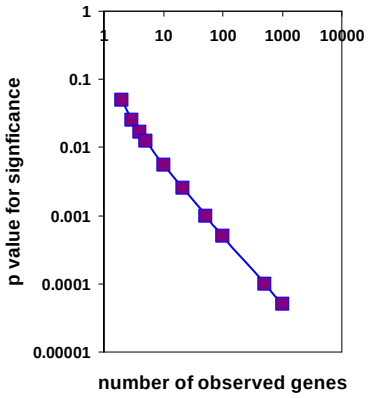
High dimensionality of microarray or proteomics data means you must understand statistics

While reproducible data can be obtained, the large numbers of parameters (individual genes or proteins) require large changes in expression before a change can be regarded as significant



Use of the Bonferroni correction:
A conservative correction

Properties of a system and fold-change

- **The primary assumption of most users of DNA microarrays (and proteomics) has been that the cut-off for assessing change is two-fold**
- **This is a very naïve view of properties of a system**
  - **Barnes' law "Fold-change is inversely related to biological importance"**

Properties of a system and fold-change

- For a system, items that are important are the least likely to change
  - when they do, catastrophic events may occur
  - Proliferation vs apoptosis (PTEN < 50% change)
- Items unimportant to the system can vary a lot (not a core value)
- How can we perceive "importance"?
  - Re-weight the data by dividing by the variance
  - Need to have enough information about each item to calculate its variance (n > 5)

Vulnerability of a system

- To really understand biological systems, you have to appreciate their dynamic state
  - Read about control theory
  - Realize that systems are subject to rhythms
  - Subject them to fourier transform analysis to detect their resonance (requires far more data than we can currently collect)
- A small signal at the right frequency can disrupt the system
  - Analogies "the small boy in the bath" and "the screech of chalk on a chalk board"

Hazards of interpreting microarray (proteomic) data

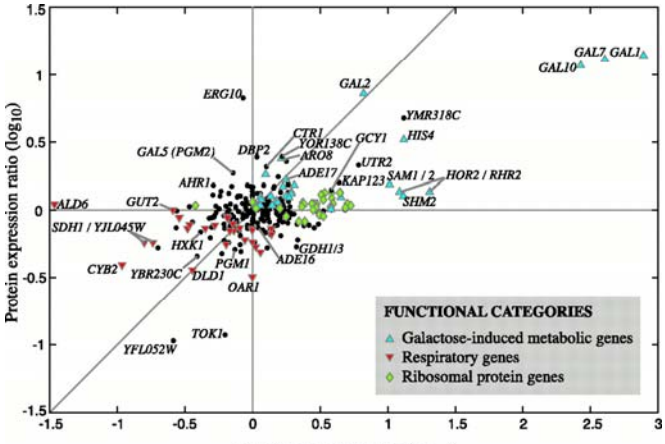
- "Expression patterns are the place where environmental variables and genetic variation come together. Environmental variables will affect gene expression levels."
- "Don't we need to be very careful to understand the environmental inputs that might have an impact on that expression? Perhaps an over-the-counter herbal supplement might cause an expression pattern that looks like that of a very aggressive tumor."

Abridged from Karen Kline, 2002

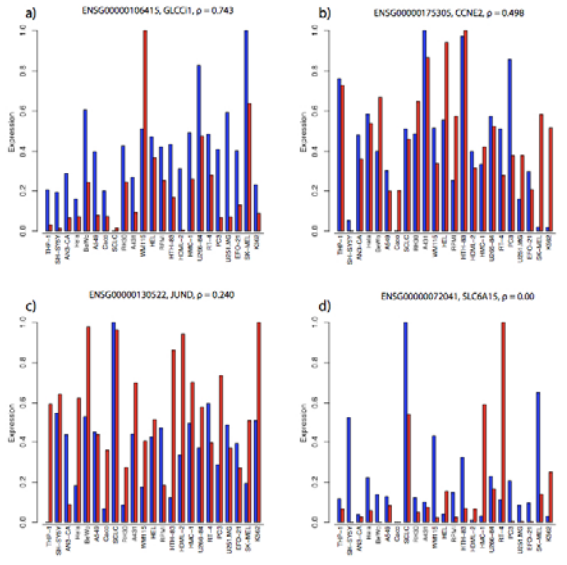
Why study the proteome when we can do DNA microarrays?

- DNA microarray analysis allows one to examine the mRNA levels of thousands and thousands of genes
- However, the correlation between gene expression and protein levels is often poor, although that may be an issue of the timing of sampling
- Is this a new finding? No, before the age of molecular biology, it was well known

Apparent structured relationship between gene expression and protein content



Ideker et al., Science 292: 929 (2001)



Differing correlations between RNA expression of a gene and its associated protein amount in 23 different cell lines

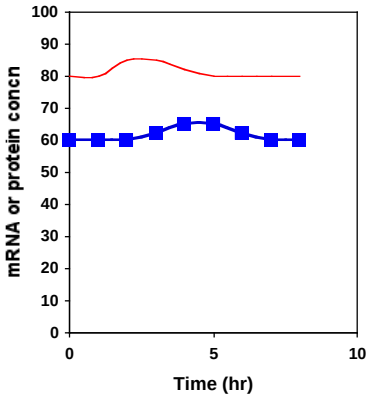
- a) glucocorticoid-induced transcript 1 protein
- b) G1/S-specific cyclin-E2
- c) jun-D, a transcription factor
- d) neurotransmitter transporter NTT73

From Gry et al. BMC Genomics 10:365 (2009)

Housekeeping genes and proteins are probably related

This is the relationship between mRNA (red) and protein (blue) levels expression of a house-keeping gene/protein, i.e., one that has to be expressed at all times

- Even with the small perturbation, the amounts of mRNA and protein are well correlated to each other

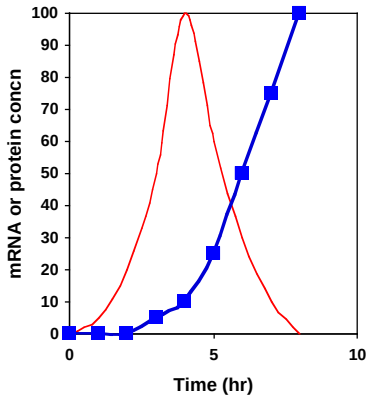


S Barnes/H Kim/M Renfrow BMG 744 1/05/11 (Barnes & Allison, 2004)

Sampling time affects interpretation of correlation between mRNA and protein expression for important proteins

Determining the relationship between mRNA (red) and protein (blue) levels depends totally on when you measure them - for the figure opposite, the ratio at 2.5 hr is 10:1, whereas at 7.5 hr it's 1:100

- better to measure the ratio over time and integrate the area under the curve



S Barnes/H Kim/M Renfrow BMG 744 1/05/11 (Barnes & Allison, 2004)

Experimental design and quality control issues

- How do we address quality control in a proteomics experiment?
  - Randomize sample analysis
  - Process samples blinded to identities
  - Standardize procedures and vendors of disposable plastics used in experiment;
    - minimize variation where possible;
    - eliminate variation where possible.
  - Consult with statistician before experiment; ensure enough "power" for the experiment so that statistical analysis yields significant data.

Understanding disease using proteomics

- Disease/chronic condition may result from a single gene mutation, but ultimately involves changes in multiple proteins;
  - Aging
  - Cancer
  - Cardiovascular disease
  - Neurodegeneration
- Infectious disease and the microbial proteome

Why do we need to do broad scale "proteomics"?

- When you don't have a clue;
- When you have only a very small clue;
  i.e. you've done a microarray experiment, and you have a list of 3,284 genes that are differentially regulated in your system…which ones are "real"?
- When you knock out a gene (and hence a protein) that you're convinced is essential for life and health, and the animal pees as usual.

Challenges in proteomics

- So many combinations of sequences! How many 100-mers are possible using 20 different amino acids?
- Mature protein forms cannot be predicted entirely from genomic sequence data:
  - Posttranslational modifications
  - Differential splicing
  - Unknown transcriptional mechanisms
- The dynamic range (>10^9)
- No equivalent to the PCR reaction
- Avogadro's number

Avogadro's number comes back to haunt us: a reality check for proteomics

- One gram-mole of anything is 6.02×10^{23} molecules
- For mass spec detection, we need at least 100 femtomole (10^{-13} moles) or 6.02×10^{10} molecules

protein expression per cell	# cells needed for 100 fmoles
10	6.02×10^{9}
100	6.02×10^{8}
1,000	6.02×10^{7}
10,000	6.02×10^{6}
100,000	6.02×10^{5}

Central issue in proteome complexity: dynamic range

Cellular proteomes involve a very wide *dynamic range*: proteins can differ in their amounts by nine (9) orders of magnitude;

- In blood, albumin is 0.5×10^{-3} M, whereas cytokines are pM (10^{-12} M)
- The myocyte proteome is dominated by actin and myosin
- A 2D gel that is overloaded with respect to an abundant protein, may have *barely detectable* amounts of a low abundance protein…….And if you can't see it, you don't know it's changed, yet it may be the important protein change in the experiment.

Predicting the proteome

- ***Bioinformatics*** **is the basis of high throughput proteome analysis using mass spectrometry. Protein sequences can be computationally predicted from the genome sequence**
- **However, *bioinformatics* is not able to predict with accuracy the sites or chemistry of posttranslational modifications - these need to be defined chemically (using mass spectrometry)**
- **Proteins in individuals will have different sequences – there are 161 known natural mutations of the LDL receptor**

Predicting the proteome

- ***Predicting the proteome has elements of a circular argument***
  - **protein sequences were initially determined chemically and were correlated with the early gene sequences. It then became easier to sequence a protein from its mRNA (captured from a cDNA library). This could be checked (to a degree) by comparison to peptide sequences. Now we have the human genome. However, the ENCODE project is changing our view of what is a gene.**
- ***So, is it valid to predict the genes (and hence the proteome) from the sequence of the genome?***
  - **We're doing this in current research. But as we'll see, the mass spectrometer is the ultimate test of this hypothesis -**
  - **why? <u>because of its mass accuracy</u>**

Protein informatics

- The predicted sequences of the proteins encoded by genes in sequenced genomes are available in many publicly available databases (subject to the limitations mentioned earlier)
- The masses of tryptic peptides can be used to identify a protein in a matter of seconds

So, what do we do with all these data?

- Management of the data generated by DNA microarray and proteomics/protein arrays
  - High dimensional analysis
- Beyond the capabilities of individual investigators
- Urgent need for visualization tools
- The importance of new statistical methods for analysis of high dimensional systems

Protein structure

- Determined by folding - folding rules not yet defined - cannot predict structure *de novo*
- X-ray crystallography has been used to produce elegant structural information
- NMR and H/D exchange combined with mass spec enable the in-solution structure to be determined (see Peter Prevelige's lectures on February 18/21)

PROTIG and Videocast

- There is an NIH-based proteomics interest group (PROTIG)
  - Sign up at http://proteome.nih.gov
- Proteomics and mass spec talks are available for viewing
  - Log on at http://videocast.nih.gov
  - Jennifer van Eyck (December 2010 talk)
  - Podcasts are also available