CAN YOUR MASS SPEC DO THIS?
COMMON CONCERNS WITH LARGE-SCALE PROTEOMICS EXPERIMENTS

SUMS workshop
September 26, 2011
Josh Elias, Chemical & Systems Biology
josh.elias@stanford.edu
Aren’t there public facilities that do exactly what you do?
What do we do?
What do we do?

<table>
<thead>
<tr>
<th>Protein 1</th>
<th>Protein 2</th>
<th>Protein 3</th>
<th>Protein 4</th>
<th>Protein 5</th>
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- PA2G4
- OGT
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- TUBB2A
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- AFF4
- CSNK2A1
- SUPT6H
- MDN1
- GCN5L1
- SUPT6H
- UPF1
- CCT3
- LTV1
- AQR
- SMC1A
- NOB1
- CCT8
- RRP12

Intesity vs. time

Intensity vs. m/z
Do you do have your own projects?
Here’s the story of a lovely ORbl...
Large-Scale Protein Profiling

- Biological sample
- Sample fractionation
- Protein digestion
- Peptide separation
- Sample ionization
- Tandem mass spectrometry
- Data analysis
We learn through collaboration

- Working with small sample amounts
- Formaldehyde cross-linking
- Protein affinity purifications
- Unusual PTM identification
- Incompletely sequenced organisms
Large-Scale Protein Profiling

1. Biological sample
2. Sample fractionation
3. Protein digestion
4. Peptide separation
5. Sample ionization
6. Tandem mass spectrometry
7. Data analysis
FAQ for successful collaborations

Sample prep
IP’s
SILAC
Instrumentation capabilities
Collaboration interest
FAQ for successful collaborations

*Q: How much protein do I need?*

*A: It depends!*

q: How abundant is your protein(s) of interest?
q: How enriched are they in your sample

Lower detection limit ~ 0.5-1 fmol ≈ 5 billion molecules
Assume: 50 kDa protein -> 0.4 ng *of that protein*
Assume: 100% sample recovery!
Assume: 0.01% of sample: 4 ug
FAQ for successful collaborations

**Q: How much protein do I need?**

<table>
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<tr>
<th>Number of cells</th>
<th>Protein copies/cell</th>
<th>Total number of proteins</th>
<th>Moles of protein</th>
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FAQ for successful collaborations

Q: What form should sample be delivered?

• Beware of Keratins!
• Coomassie gels are good
  – do not reuse Coomassie!
• TCA, MeOH/CHCl₄ ppt (simple mixtures)
• Mass spec-friendly buffers
  – No detergents
  – No acids+plastic
  – No PEG
FAQ for successful collaborations

Q: Can you tell me what protein is in this gel band?

A: Probably, but:

- This is not the kind of collaboration we generally look for.
- It will probably take much longer to get around to this sample than you probably want. You’re better off using the SUMS facility.
FAQ for successful collaborations

Q: Can you tell me what proteins are cross-linked to my POI?

A: No. (for now, at least)
FAQ for successful collaborations

Q: Can you help me find biomarkers in serum?

A: This is not our forte. Perhaps check with Parag Mallick and Sharon Pitteri (Canary Center)?
FAQ for successful collaborations

Q: I know my POI is in my sample because I saw it with RTPCR/ microarray/ Western... why wasn’t it in your mass spec results?

A: Many possible reasons:

- Transcription ≠ protein expression
- False positive in other assay
- Unanticipated PTMs
- Incompatible with protease
  - Too many/ too few K’s, R’s
  - High homology with other proteins
- Low abundance
- Low S:N
- Insoluble
- Wrong sequence in database...
  (is it tagged?)
FAQ for successful collaborations

Q: I believe my POI is phosphorylated. Can you tell me where it’s phosphorylated?

A: Probably, but it’s not as straight-forward as you might think

q: What evidence do you already have that your POI is phosphorylated?
FAQ for successful collaborations

Q: I believe my POI is phosphorylated. Can you tell me how much it’s phosphorylated?

A: Can give a rough estimate, but a targeted strategy would be best.

2:05 PM

Using Selected Reaction Monitoring (SRM) Mass Spectrometry to Unmask Regulatory Feedback Loops Controlling Calcium Homeostasis and Adipogenesis

Mary Teruel, PhD, Dept. of Chemical & Systems Biology

Gerber S A et al. PNAS 2003;100:6940-6945
FAQ for successful collaborations

Q: I want to find all the proteins that interact with my POI. Can you do this?

A: Yes. We love this kind of experiment. But...

q: Have you already optimized binding and washing conditions?

Show me the gel and Western!
FAQ for successful collaborations

Q: I want to find all the proteins that interact with my POI. Can you do this?

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FAQ for successful collaborations

Q: I want to find all the proteins that interact with my POI. Can you do this?

A: Yes. We love this kind of experiment. But...

q: Have you already optimized binding and washing conditions?

q: How much of your input are you recovering?

Positive controls: Bait, known interactors
Negative controls: beads only, tag construct, (irrelevant protein, mutant POI)
FAQ for successful collaborations

Q: When I cut a protein out of a gel, could I be cutting it apart making it harder to identify??

A: No. A razor blade is much bigger than a protein.
FAQ for successful collaborations

Q: Can you send me your longer, unfiltered list of protein hits?

A: Yes, but you will probably get yourself into trouble.
FAQ for successful collaborations

Q: How can I sign up for time to use your mass spectrometer?

A: Step 1: Join my lab.
FAQ for successful collaborations

Q: I want to quantify differences in protein levels in my animal system. How can I use SILAC to do this?

A: Doing this with SILAC can be difficult and expensive. There are easier and cheaper alternatives.

• Label-free methods
• Chemical labeling (TMT/iTRAQ, others)
• SILAC-labeled cell culture lysate
FAQ for successful collaborations

Q: Can SILAC tell me the abundance difference between proteins X and Y?

A: Generally no. This is a job for targeted (AQUA/MRM) methods.
FAQ for successful collaborations

Q: Can you also tell me what metabolites are changing between my samples?

A: We don’t have experience with this. Check with the SUMS facility.
FAQ for successful collaborations

Q: I work with <weird organism>. Can we do proteomic analyses with it?

A: If it has a sequenced genome and annotated proteome, there shouldn’t be a problem.
FAQ for successful collaborations

Q: How long will the mass spec analysis take?

A: Probably longer than you expect.
FAQ for successful collaborations

Q: Are you looking for more collaborations?

A: Not actively, but always interested in cool projects that coincide with our lab’s ongoing research.
Have more questions?

- **CSB 230**: Current Methods in Proteomics
- **Terms**: Aut | **Units**: 3 | **Grading**: Medical Option (Med-Ltr-CR/NC)
- **Instructors**: Elias, J. (PI); Teruel, M. (PI)
- Josh.elias@stanford.edu