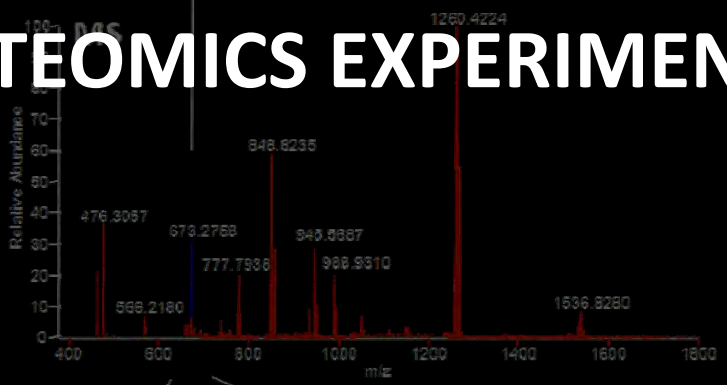
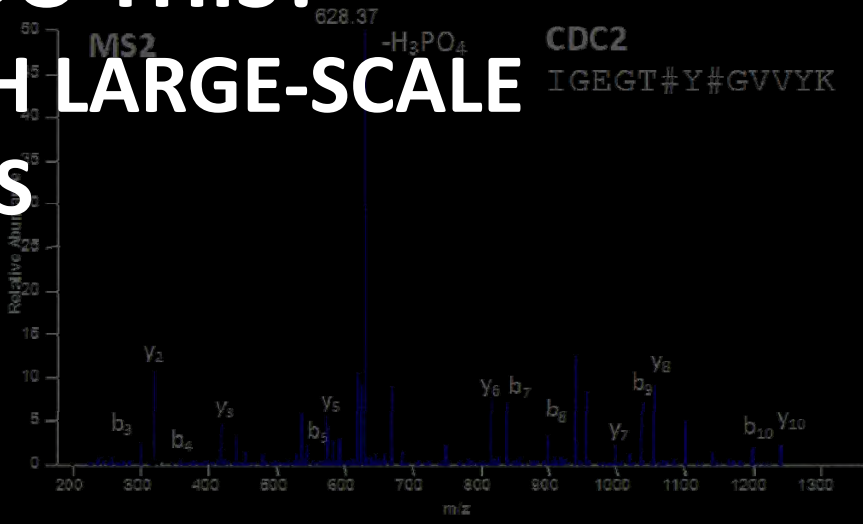
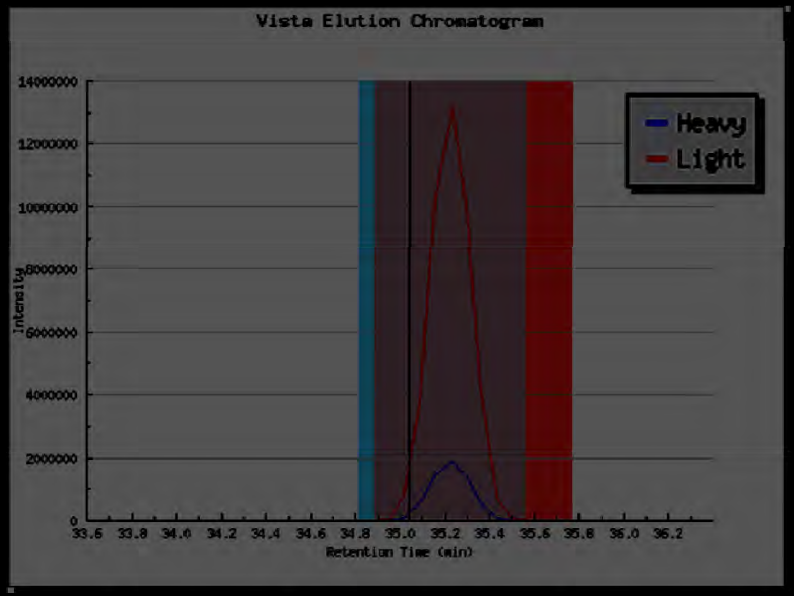
CAN YOUR MASS SPEC DO THIS?
COMMON CONCERNS WITH LARGE-SCALE PROTEOMICS EXPERIMENTS







SUMS workshop

September 26, 2011

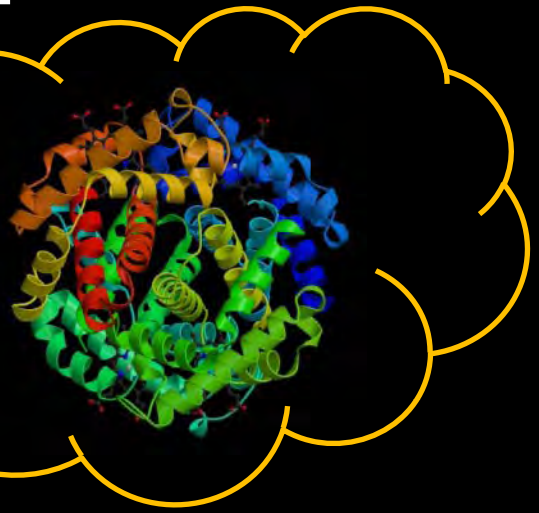
Josh Elias, Chemical & Systems Biology

josh.elias@stanford.edu

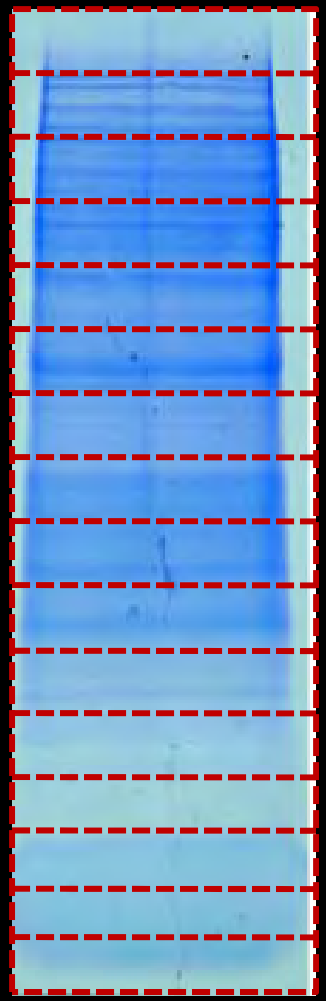
Presented at the 2011 Stanford Mass Spectrometry Users' Meeting

Aren’t there public facilities that do exactly what you do?

What do we do?

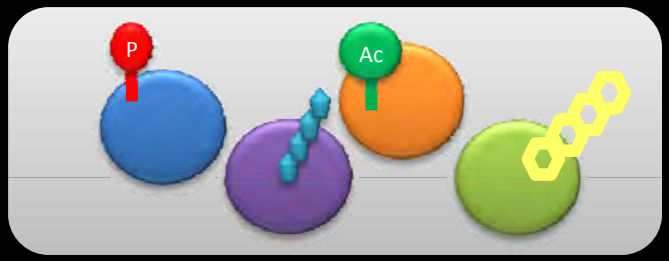


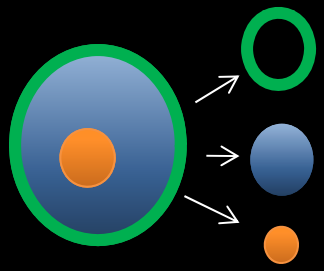
What do we do?



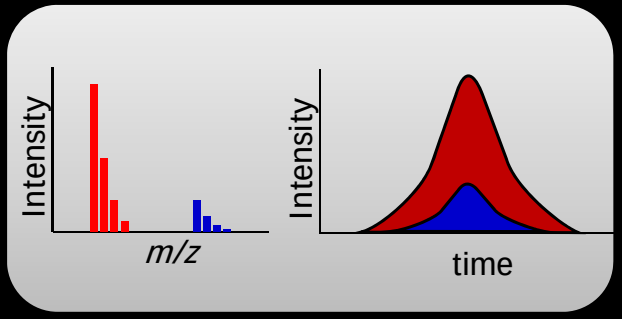
HUWE1	DHX30	USP7	SUPT16H
AICDA	LARP1	ACTN4	RUVBL1
TUBA4A	HSP90AB1	EIF4A1	AFF4
DNAJA1	TJP2	PABPC1	CSNK2A1
DYNC1H1	CTNNA1	CDK9	SUPT6H
MYH10	RIF1	ATP5B	MDN1
TUBB2C	DDB1	HNRNPM	GCN1L1
UBR5	NAP1L1	CCT7	SUPT6H
RUVBL2	ATP5A1	DHX9	UPF1
DNAJA2	USP9X	HNRNPF	CCT3
SYNCRIP	FSCN1	DDX3X	
PRPF4B	PPP1R12A	TCP1	LTV1
DDX17	MSH2	GTPBP4	AQR
CCT2	KPNB1	PA2G4	SMC1A
MLLT4	SSRP1	OGT	NOB1
HNRNPD	PTBP1	TUBB1	CCT8
SMC3	AHDC1	DDX1	RRP12
ACTN1	TUBB2A		
SET	HNRNPL		

What do we do?



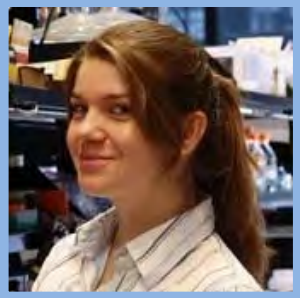


HUWE1 DHX30 USP7 SUPT16H
AICDA LARP1 ACTN4 RUVBL1
TUBA4A HSP90AB1 EIF4A1 AFF4
DNAJA1 TJP2 PABPC1 CSNK2A1
DYNC1H1 CTNNA1 CDK9 SUPT6H
MYH10 RIF1 ATP5B MDN1
TUBB2C DDB1 HNRNPM GCN1L1
UBR5 NAP1L1 CCT7 SUPT6H
RUVBL2 ATP5A1 DHX9 UPF1
DNAJA2 USP9X HNRNPF CCT3
SYNCRIP FSCN1 DDX3X
PRPF4B PPP1R12A TCP1 LTV1
DDX17 MSH2 GTPBP4 AQR
CCT2 KPNB1 PA2G4 SMC1A
MLLT4 SSRP1 OGT NOB1
HNRNPD PTBP1 TUBB1 CCT8
SMC3 AHDC1 DDX1 RRP12
ACTN1 TUBB2A
SET HNRNPL




Do you do have your own projects?
?

Here's the story of a lovely ORbI…

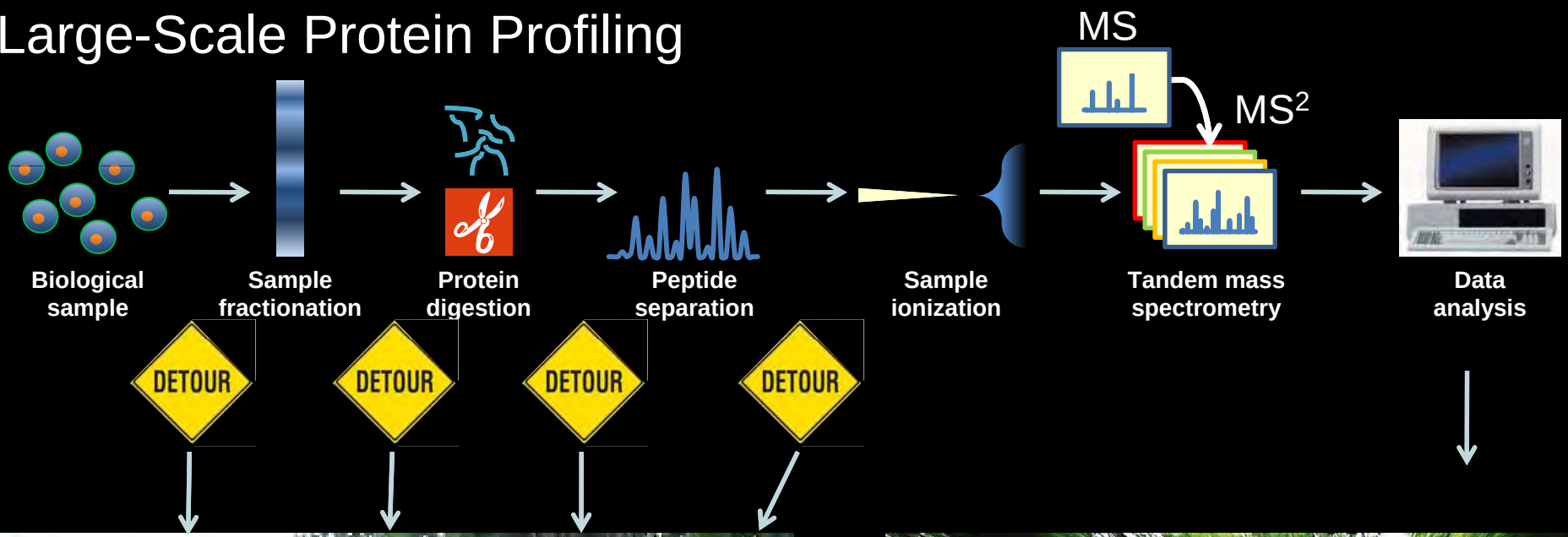








Large-Scale Protein Profiling

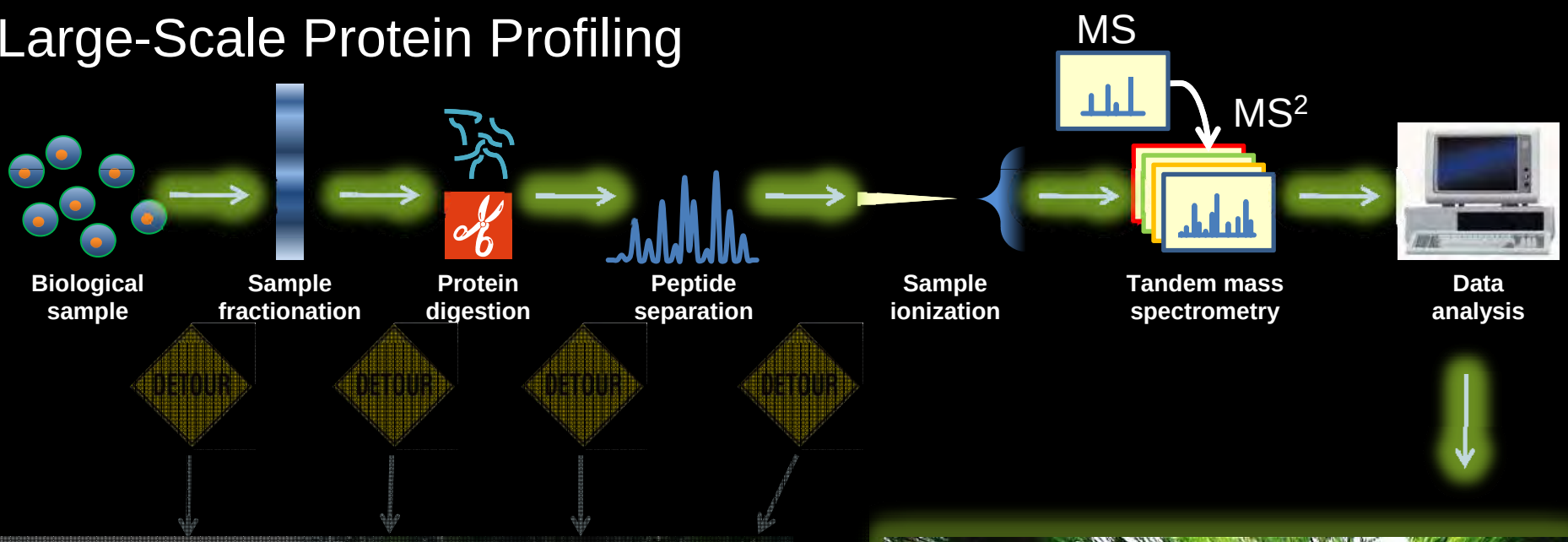


We learn through collaboration

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

Manish Butte
Ben Barres
Helen Blau
John Boothroyd
Pat Brown
James Chen
Karlene Cimprich
Martha Cyert
Crislyn D'Souza-Schorey
James Ferrell
Magali Fontaine
Judith Frydman
Chris Garcia
Jennie Lill (Genentech)
Betsy Mellins
Tobias Meyer
Daria Mochly-Rosen
Ashby Morrison
Max Nachury
Edward Rubenstein
Peter Sarnow
Justin Sonnenberg
Aaron Straight
Mary Teruel
Tom Wandless

Large-Scale Protein Profiling



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 1. Detection limits for proteins assuming 100% recovery

Number of cells	Protein copies/cell	Total number of proteins	Moles of protein	Avogadro's challenge ng for 25 kDa protein	ng for 50 kDa protein	ng for 100 kDa protein	Visualization detection limits	Practical MS/MS detection limits
1.OE+09	1000000	1.OE+15	1600 pmole	41 528.00	83 056.00	166 112.00	Coomassie blue	LC-MS/MS MALDI-TOF
1.OE+09	100000	1.OE+14	160 pmole	4 152.00	8 304.00	16 608.00	Coomassie blue	LC-MS/MS MALDI-TOF
1.OE+09	10000	1.OE+13	16 pmole	415.00	830.00	1 660.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OE+09	1000	1.OE+12	1.6 pmole	41.00	82.00	164.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OE+09	100	1.OE+11	160 fmole	4.00	8.00	16.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OE+09	10	1.OE+10	16 fmole	0.40	0.80	1.60	Radio	–
1.OOE+08	1000000	1.OE+14	160 pmole	4 152.00	8 304.00	16 608.00	Coomassie blue	LC-MS/MS MALDI-TOF
1.OOE+08	100000	1.OE+13	16 pmole	415.00	830.00	1 660.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+08	10000	1.OE+12	1.6 pmole	41.00	82.00	164.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+08	1000	1.OE+11	160 fmole	4.00	8.00	16.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+08	100	1.OE+10	16 fmole	0.40	0.80	1.60	Radio	–
1.OOE+08	10	1.OE+09	1.6 fmole	0.04	0.08	0.16	Radio	–
1.OOE+07	1000000	1.OE+13	16 pmole	415.00	830.00	1.660.00	Silver staining	µC-MS/MS MALDI-TOF
1.OOE+07	100000	1.OE+12	1.6 pmole	41.00	82.00	164.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+07	10000	1.OE+11	160 fmole	4.00	8.00	16.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+07	1000	1.OE+10	16 fmole	0.40	0.80	1.60	Radio	–
1.OOE+07	100	1.OE+09	1.6 fmole	0.04	0.08	0.16	Radio	–
1.OOE+07	10	1.OE+08	0.2 fmole	0.004	0.008	0.016	Radio	–
1.OOE+06	1000000	1.OE+12	1.6 pmole	41.00	82.00	164.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+06	100000	1.OE+11	160 fmole	4.00	8.00	16.00	Silver staining	µLC-MS/MS MALDI-TOF
1.OOE+06		1.OE+10	16 fmole	0.40	0.80	1.60	Radio	–
1.OOE+06	1000	1.OE+09	1.6 fmole	0.04	0.08	0.16	Radio	–
1.OOE+06	100	1.OE+08	0.2 fmole	0.004	0.008	0.016	Radio	–

Corthals, G.L., Wasinger, V.C., Hochstrasser, D.F. & Sanchez, J.C. **The dynamic range of protein expression: a challenge for proteomic research**. *Electrophoresis* **21**, 1104-1115 (2000).

FAQ for successful collaborations

Q: What form should sample be delivered?

- Beware of Keratins!
- Coomassie gels are good
  - do not reuse Coomassie!
- TCA, MeOH/$CHCl_4$ 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

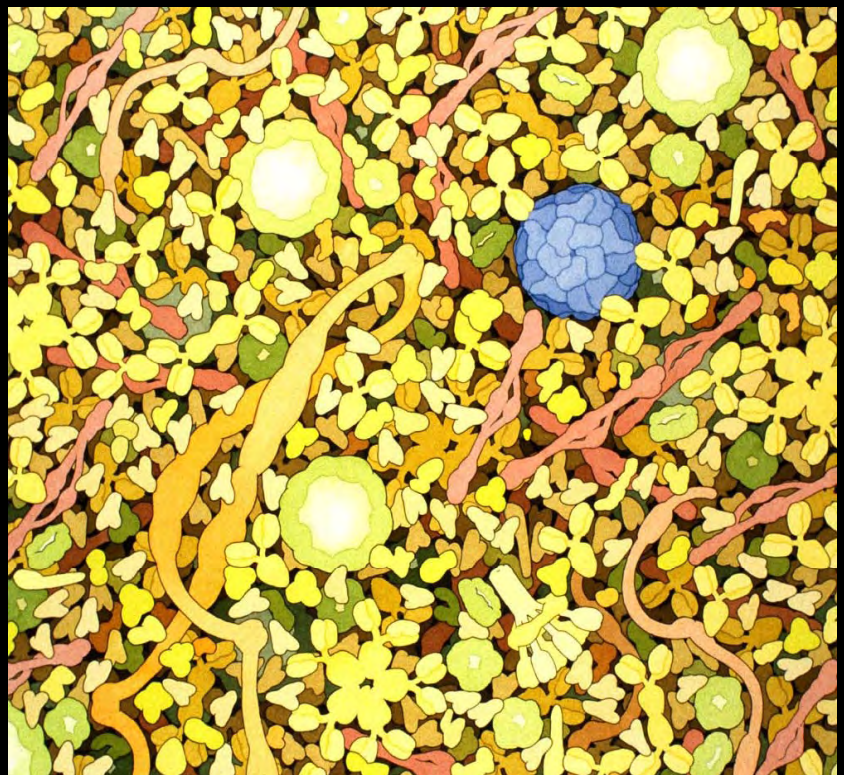
Q: Can you tell me what proteins are cross-linked to my POI?

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)?



David Goodsell

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?

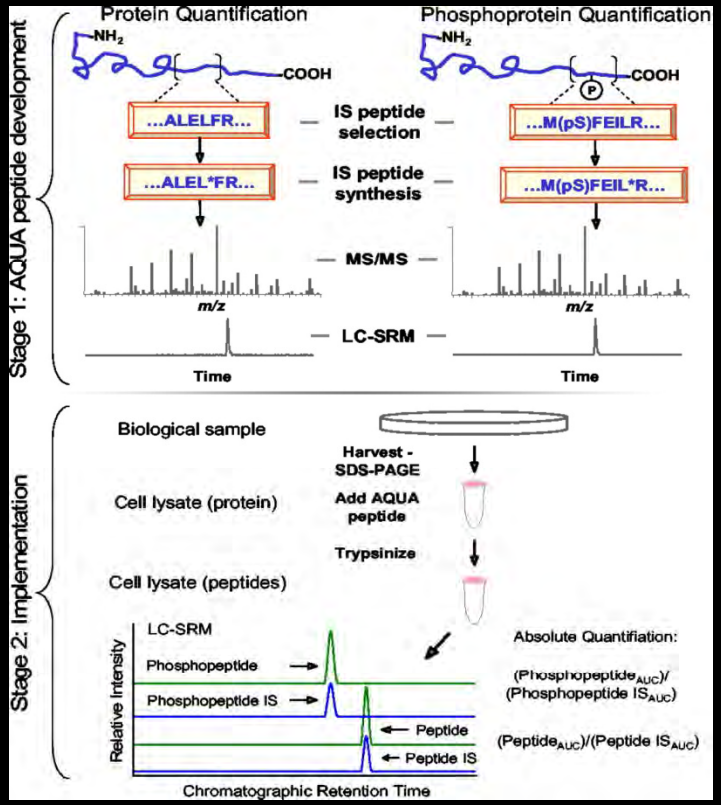
A: Probably, but it's not as straight-forward as you might think

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.



Gerber S A et al. PNAS 2003;100:6940-6945

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*

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?

FAQ for successful collaborations

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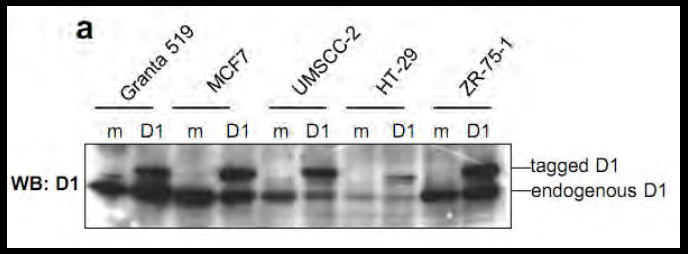

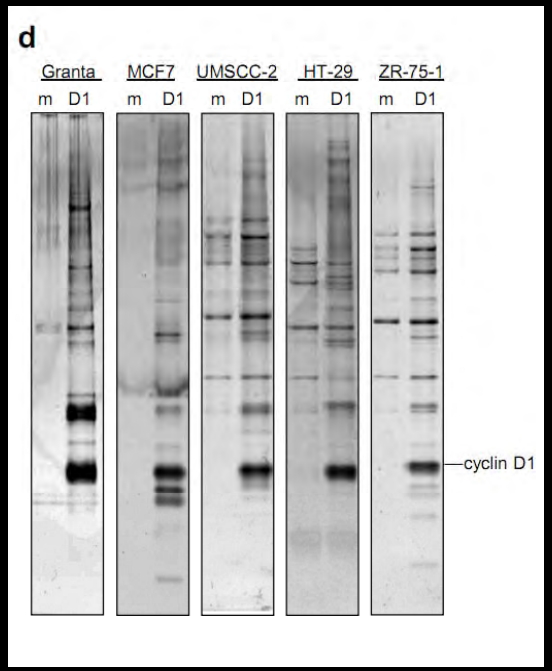

Jirawatnotai, S. et al, Nature 2011; 474, 230-234

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**