Sample Preparation for Proteomics

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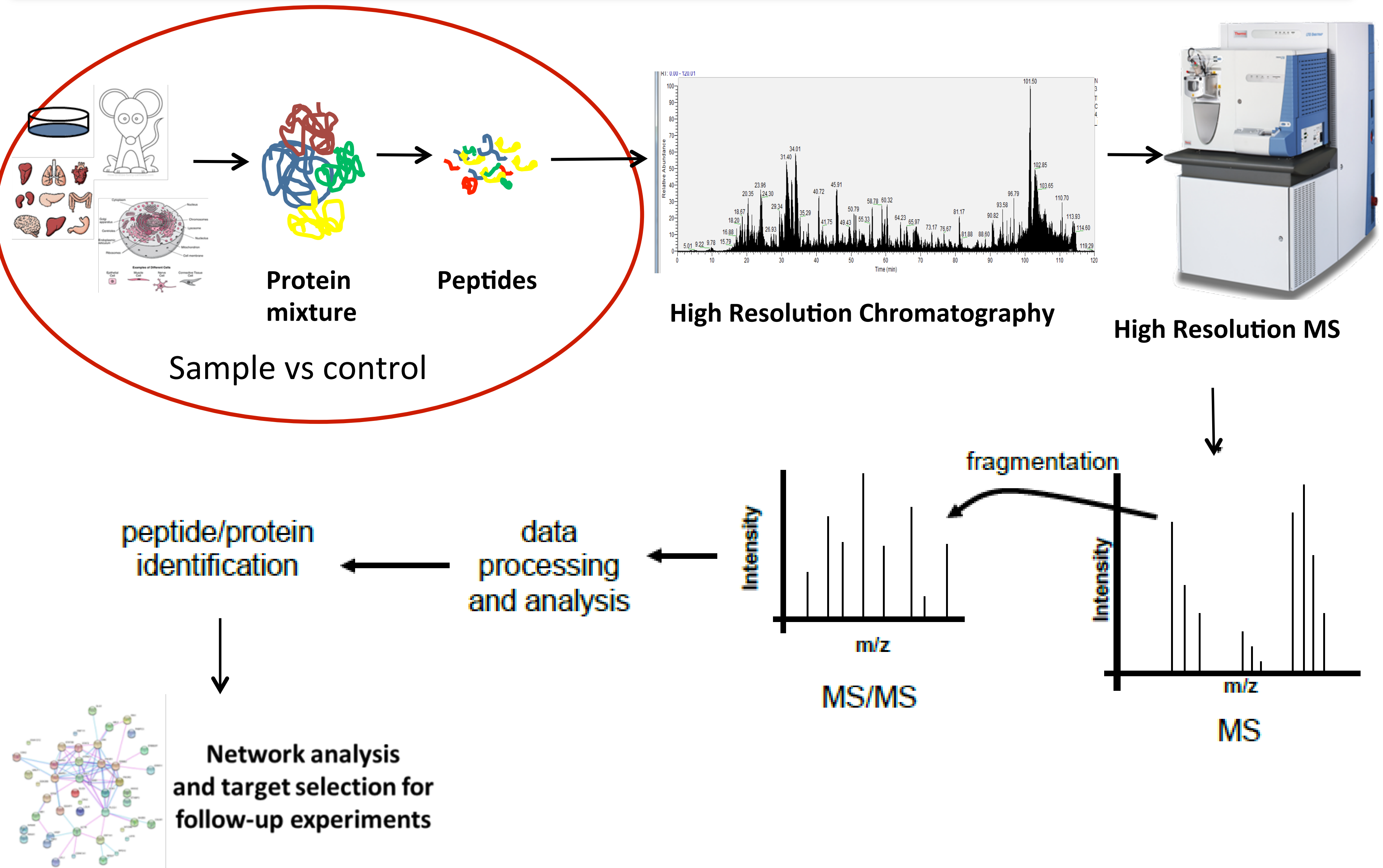
Broad Institute Proteomics Platform

From biological samples to ‘protein lists’

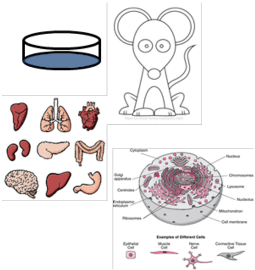


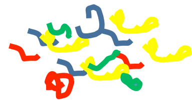
General sample preparation

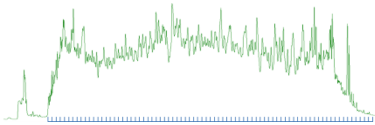
Cell/Tissue: Solubilize

Digestion

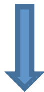
Fractionation (peptide)





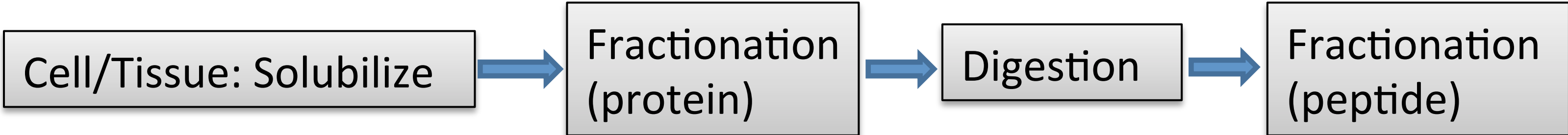


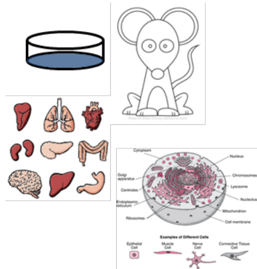
(sample vs control)

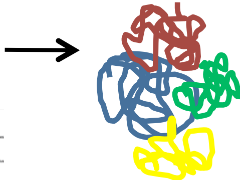


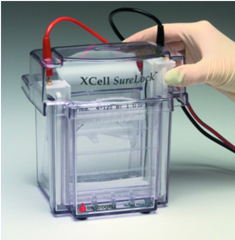
Desalting/Clean-up

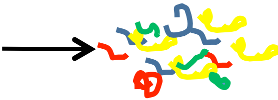
General sample preparation

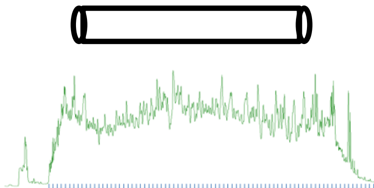












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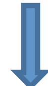


Desalting/Clean-up

Cell/Tissue/Subcellular fraction: Solubilization/Lysis

Denaturing Buffers
- SDS based
- Urea based (chaotropes in general)

Complete protein denaturation

Global proteome and PTM

Non-denaturing buffers
- Ripa(or Ripa-like) include
- Triton, NP40 or deoxycholate

Preserves protein complexes

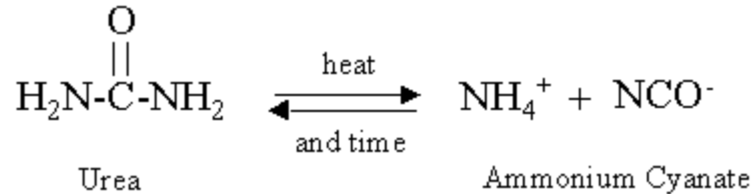
Affinity Proteomics

Physical disruption methods
- Sonication
- Grinding/homegenization
- Cryo-pulverization

Protease inhibitors are needed (phosphatase inhibitors optional except in phosphoPTM)

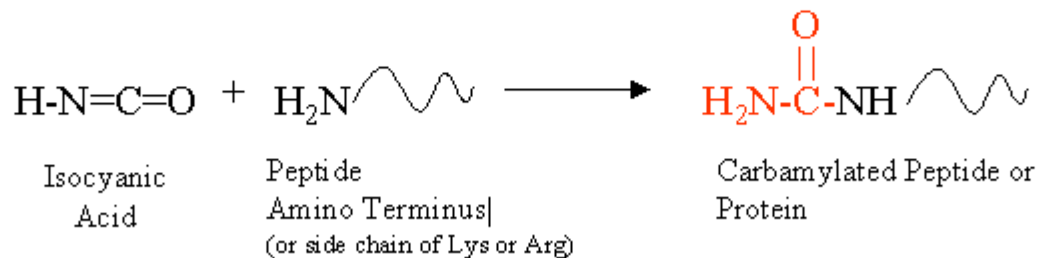
Cell/Tissue/Subcellular fraction: Solubilization/Lysis

Decomposition of Urea



Caution!!!
If in Urea!!!

Carbamylation of Proteins
(amino terminus of a peptide used as an example)



Do not heat urea lysis buffers!!!

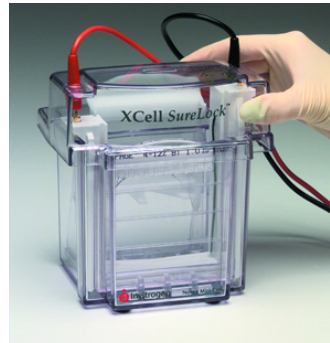
Fractionation (protein) prior to digestion

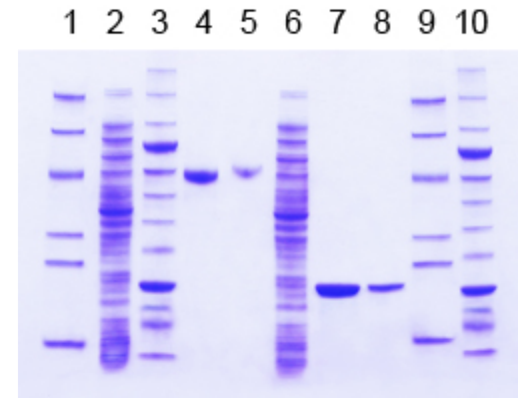
SDS-PAGE: Allows separation by molecular weight, ***and provides clean-up step*** to remove detergents (e.g. CHAPS, NP-40) that come from protein solubilization

*Proteins are generally **reduced** and **alkylated** prior to running the gel*

<u>Gel Systems</u>
BisTris with MOPS or MES buffers
Tris-Glycine

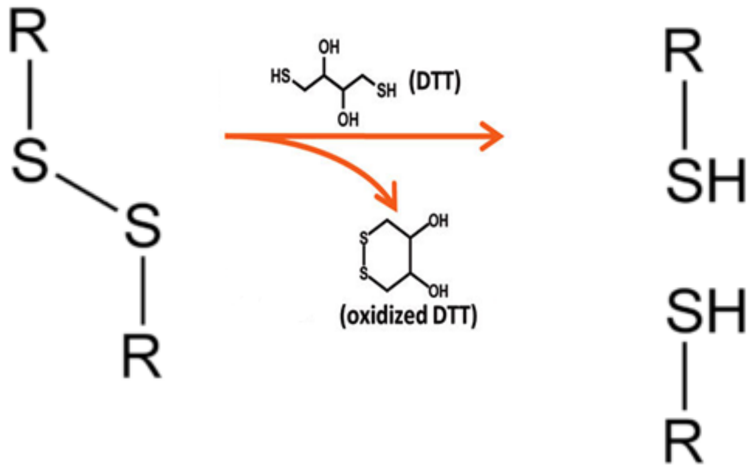
<u>Staining</u>
Coomassie

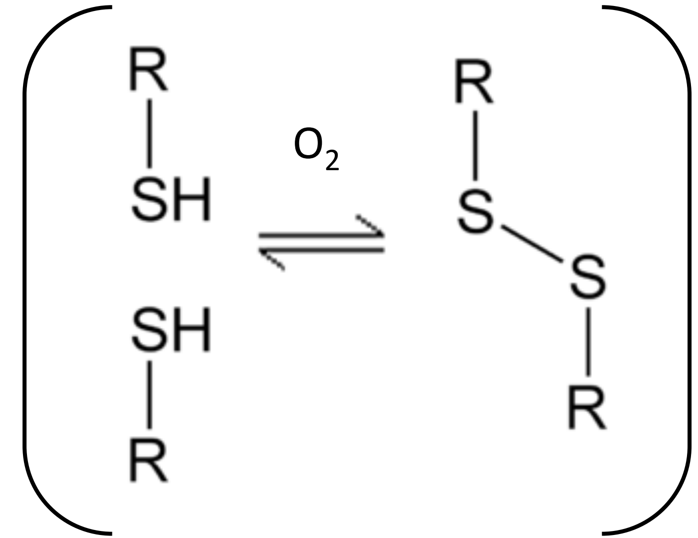




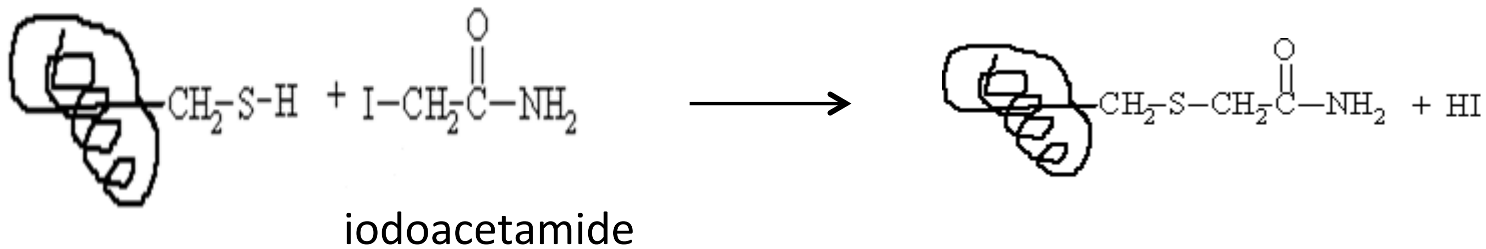
Digestion: *first* reduction and alkylation

1. Reduction: β-mercaptoethanol, **DTT**, TCEP





2. Alkylation: Iodoacetamide (IAA)



Digestion: *then* proteases

Proteases:

Trypsin (most commonly used)

Cleaves specifically *C-terminal of Arg and Lys* (if not followed by Pro)

Average size of peptides is 9 residues

Tryptic peptides are at least 2+ and easily ionized

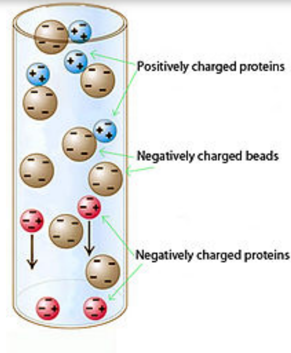
Autodigestion: Keep cool or acidic until contact with protein mixture

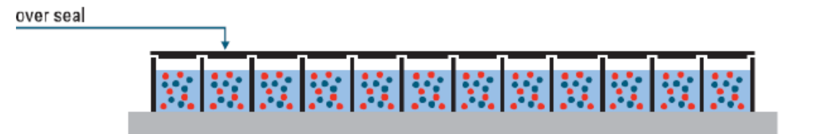
Other (common) proteases used:

LysC	K
ArgC	R \P (also K)
AspN	D
GluC	E (also D)
Chymotrypsin	W , Y , F \P

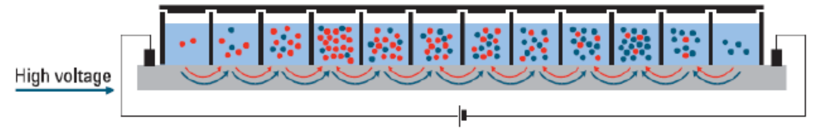
Fractionation post-digestion(peptide)

Ion Exchange:
Strong cation exchange
Strong anion exchange

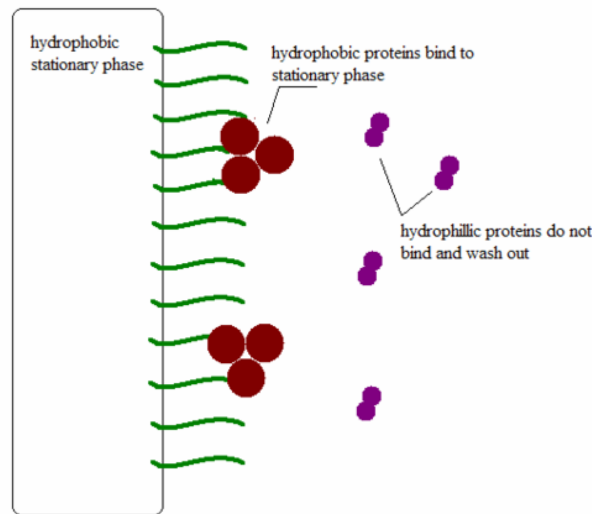




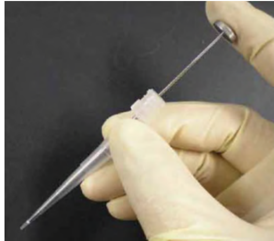
Isoelctric focusing (pI based)



Hydrophobicity:
Reversed phase
Basic Reversed phase



Desalting/Clean-up



Rappsilber J et al., Nat Protocols 2007

<u>Stop and Go Extraction (STaGE) tips</u>

Reverse Phase C-18 material loaded on a tip

<u>Commercially available desalting platforms</u>

Cartridges

Plates

What to avoid during sample preparation

Avoid….	Why we avoid it	How do we deal with it?
Salts	• Can cause adduct formation	• Yes! we de-salt by reversed phase • Use ammonium bicarbonate, it sublimates!
Detergents/Polymers	• Form m/z polymer series which competes with your sample, the spectrometer only spends time on them! • They can suppress ionization.	• We do not use them! (Especially Triton X-100) • …*unless* we go into a gel.. • or wash extensively (in the case of immobilized proteins)
Non-volatile organic solvents (like DMSO,DMF)	• Interfere with ion desolvation	• Avoid them!
Strong acids like TFA	• Can suppress signals from the analyte of interest	• Use Formic Acid
Keratins	• They can be so abundant the spectrometer will only spend time with them!	• Use common sense…

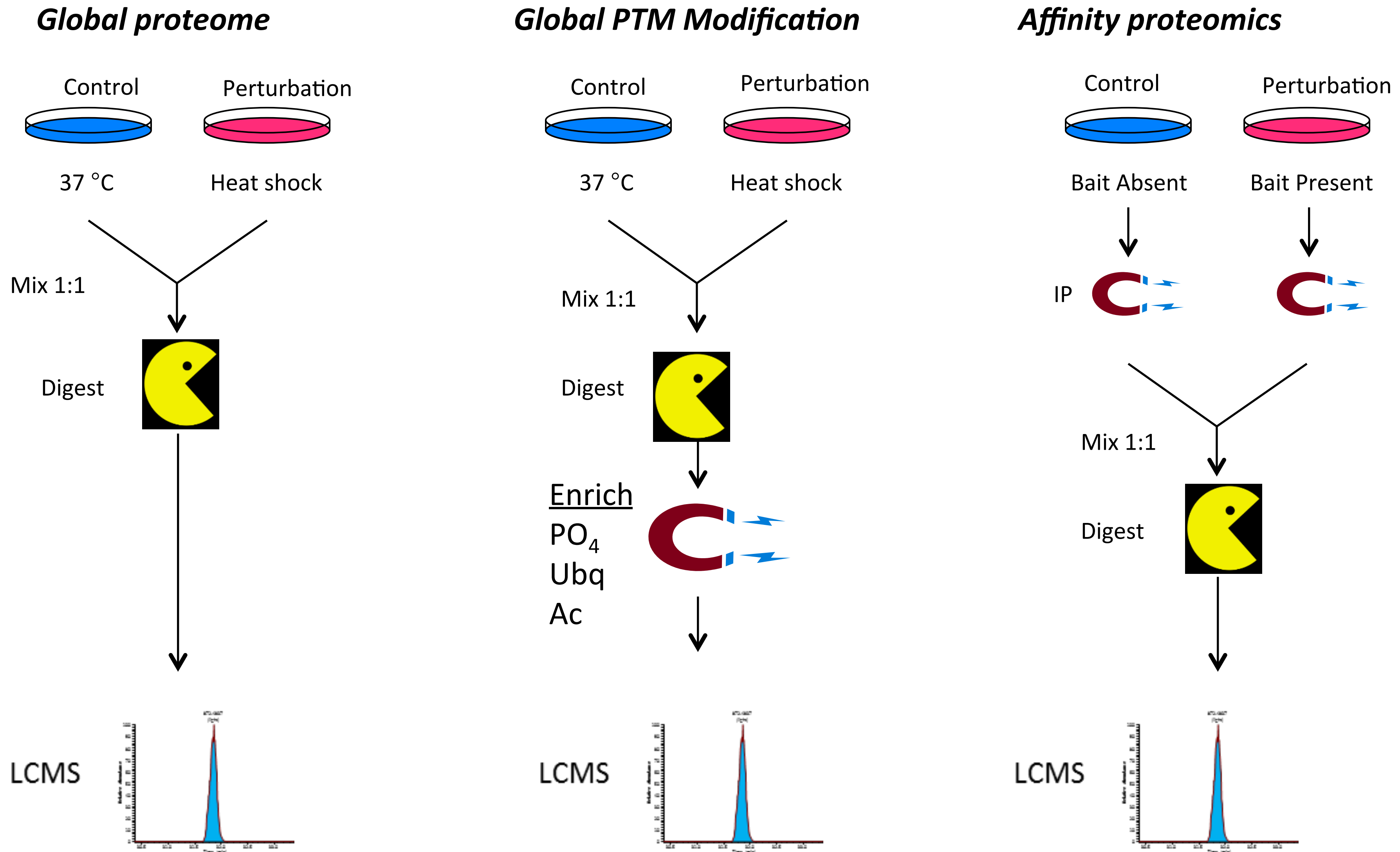
Defining the Most Important Classes of Proteomics Experiments; what do we want to know?

- **Global proteomics**: want to know what proteins are present and how they change as a result of a perturbation.

- **Global Post Translational Modifications** (PTM): want to know which proteins (and how) have been post-translationally modified.

- **Affinity Proteomics**: want to know the interactions of a protein with other proteins, small molecule or nucleic acid in a cellular context.

General workflows for the Most Important Classes of Proteomics Experiments



Global Proteome

Protein mixture

Comprehensive list of proteins and how their expression changed

In numbers: What do we mean by global proteomics?

Starting material 200 µg	Deep coverage
Cells	~ 8,000 proteins
Tissue	~ 10,000 proteins
Depleted Plasma	~ 3,000 proteins

Global Post Translational Modifications (PTM)

Protein mixture

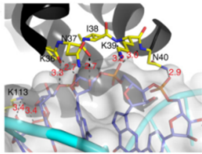
Comprehensive list of proteins with PTM

In numbers: How many sites do we mean by global post-translational modifications?

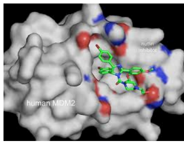
Modification/Starting material	Deep coverage
Phosphorylation/ 1-2 mg	~ 20,000 phosphopeptides
Acetylation/ 5-10 mg	~ 3,000 acetylated peptides
Ubiquitination/ 5-10 mg	~ 20,000 ubiquitinated peptides

Affinity Proteomics

- Protein-Protein (and disruption of these by small molecules)



- Protein-nucleic acid: DNA, RNA, lincRNA



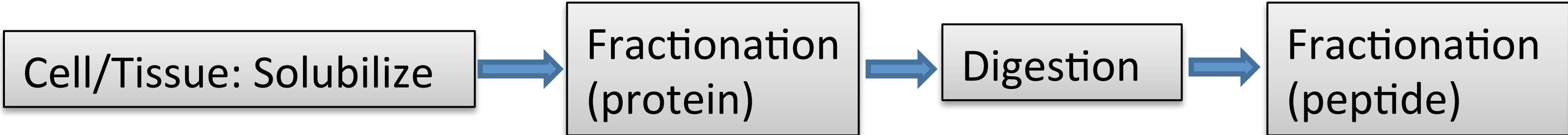
- Protein-small molecule

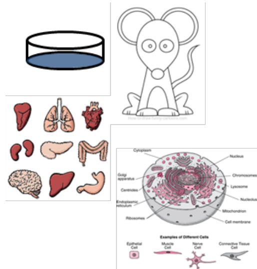
Conceptually these three are ***all the same:***
Affinity enrichment using a bait and solid support

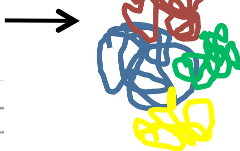
Protein mixture simplified
by ***enriched by the bait***

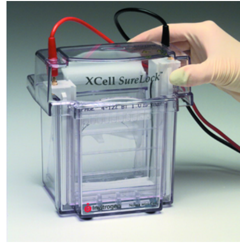
Rank-ordered list of
protein binders to the bait

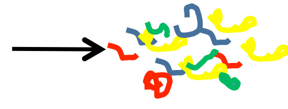
General sample preparation

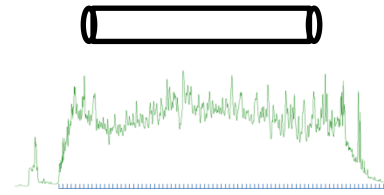






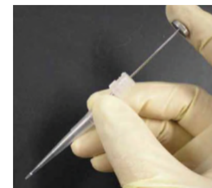






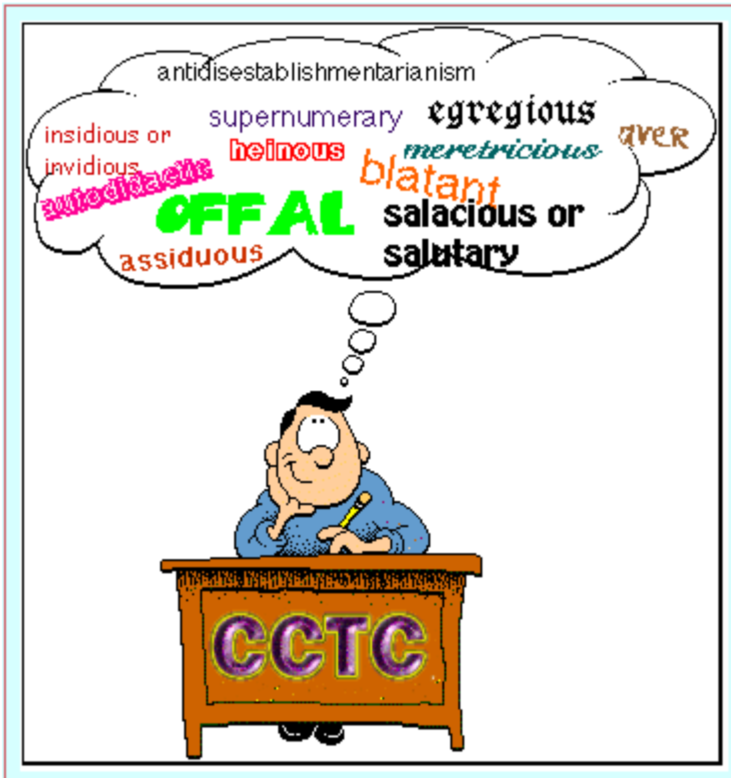
(sample vs control)

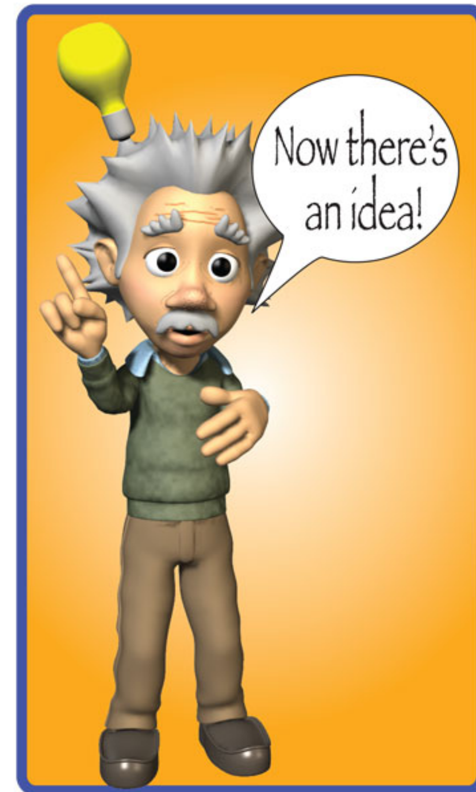
Desalting/Clean-up



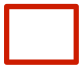
	Cell/Tissue: Solubilize	Enrichment (protein)	Digestion	Fractionation (peptide)
Global Proteome	Detergents. Chaotropes. Physical disruption. ***May include subcellular fractionation.***	Not common.	In solution.	Most common. Depth of coverage is determined by how much fractionation.
Global PTM	Detergents. Chaotropes. Physical disruption ***Important to inhibit enzymes that affect PTM.***	Not common ***Sometimes used to enrich for PTM-modified proteins.***	In solution.	Most common. ***Coupled to enrichment*** of peptides with the desired PTM by anti-PTM antibodies.
Affinity Enrichment	Mild solubilization. ***Need to maintain interactions and complexes***! ***May include subcellular fractionation.***	Common	In gel.	Depends on the complexity of the sample ***after affinity enrichment*** with the bait.

What is (y)our goal?





A catalog of proteins that may (or not) make sense?



A prioritized list of candidates to generate (or answer) hypotheses?

Quantitative Proteomics