Sample Preparation

IDeA National Resource for Proteomics

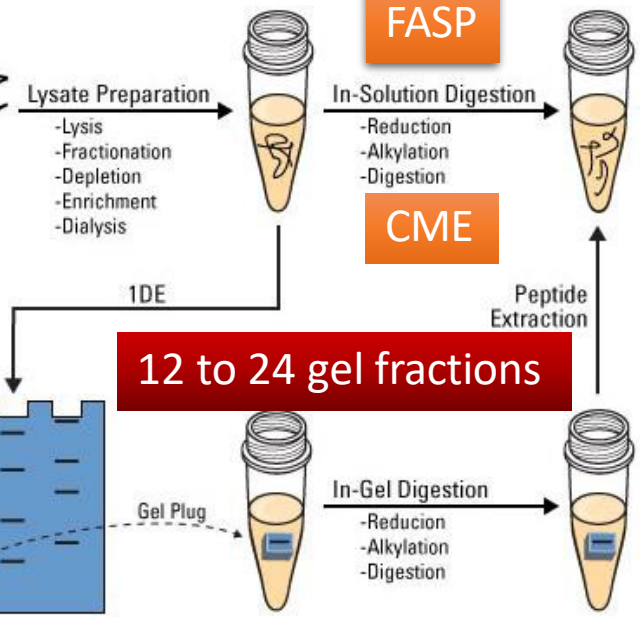
Workshop for Students and Faculty
February 2020

Proteomics Workflow: UAMS Proteomics Core

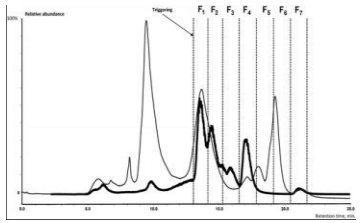
Sample preparation → Enrichment → UHPLC-MS/MS → Data analysis

Serum depletion

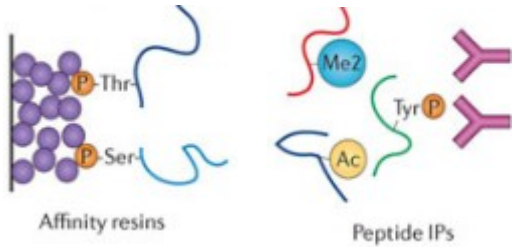
SILAC

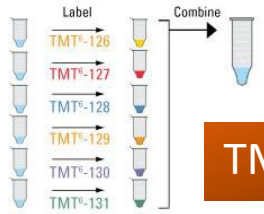


Off-line high pH Fractionation (13)



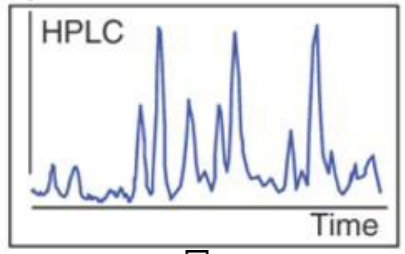
Peptide enrichment



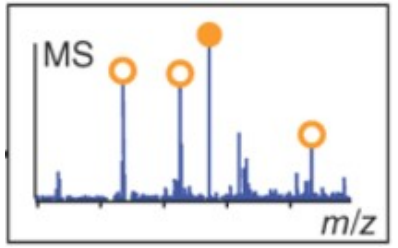


TMT labeling

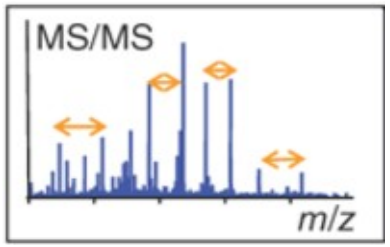
UHPLC separation



MS1: full spectra



MS/MS: fragments



Comparative proteomics

Scaffold Persus R....

iBAQ

SILAC TMT

Label Free

Quantification

Protein quant

Raw data

MaxQuant

Identification

Mascot

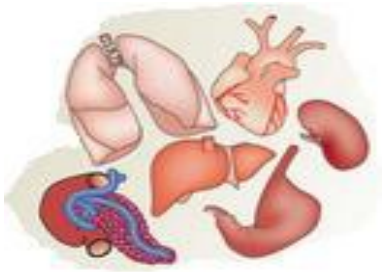
DB

Protein IDs

Cultured Cells

- cell pellet: 1-5 x 10^6 cells

Fresh-Frozen Tissue Samples



- 10-100 mg

FFPE Tissue Samples

- laser-capture microdissection

Serum/Plasma Samples

- 10-25 ul

Decision Tree of Sample Preparation in UAMS Proteomic Core

Serum depletion

SDS lysis

Serum Proteome

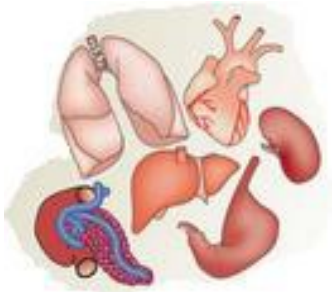
Frozen Cell Pellets

SDS lysis

Whole cell Proteome

RIPA lysis

Membrane Proteome



RIPA lysis

Cryo Bead Homogenizer

Tissue Proteome

8M urea

Tissue PTM

SDS lysis

Paraffin removal

FFPE tissue Proteome

CME

FASP

SILAC

TMT

Label Free

Phospho-Enrichment

Gel-Based Analysis

In-gel and in-solution digestion

PROTOCOL

In-gel digestion for mass spectrometric characterization of proteins and proteomes

Andrej Shevchenko[1,3], Henrik Tomas[1], Jan Havliš[1], Jesper V Olsen[2] & Matthias Mann[2,3]

In-gel digestion

- Robustness against impurities
- Fractionate proteins
- Lower peptide recovery
- Lower sequence coverage
- Total solubilization
- stained proteins and assess purity

Universal sample preparation method for proteome analysis

NATURE METHODS | VOL.6 NO.5 | MAY 2009 | 359

Jacek R Wiśniewski, Alexandre Zougman, Nagarjuna Nagaraj & Matthias Mann

In-solution digestion (FASP)

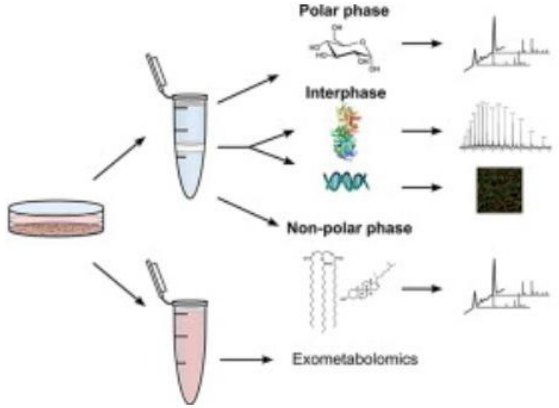
- Can’t handle detergents
- Fractionate peptides (high pH UHPLC)
- Higher peptide recovery
- Higher sequence coverage
- Incompletely solubilized

In-solution digestion (chloroform/methanol extraction)

Physiol Genomics. 2007 June 19; 30(1): 89.

Improved method for the analysis of membrane proteins by mass spectrometry

Shama P. Mirza, **Brian D. Halligan**, **Andrew S. Greene**, and **Michael Olivier**



MethodsX 1 (2014) 74–80

Simultaneous extraction of proteins and metabolites from cells in culture

Sean C. Sapcariu [a,c,*], Tamara Kanashova [b,c], Daniel Weindl [a], Jenny Ghelfi [a], Gunnar Dittmar [b,c,**], Karsten Hiller [a,c]

J Proteome Res. 2018 June 01; 17(6): 2226–2236.

SL-TMT: A Streamlined Protocol for Quantitative (Phospho)proteome Profiling using TMT-SPS-MS3

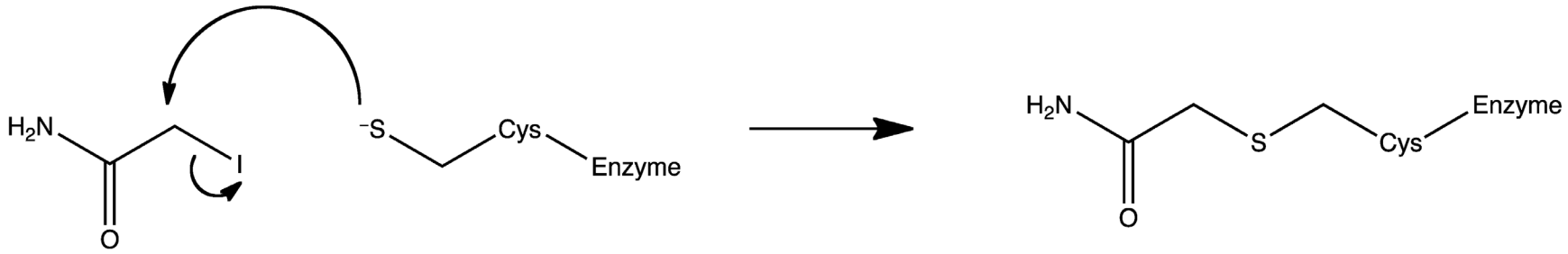
José Navarrete-Perea[†,#], **Qing Yu**[†,#], **Steven P. Gygi**[†,*], and **Joao A. Paulo**[†,*]

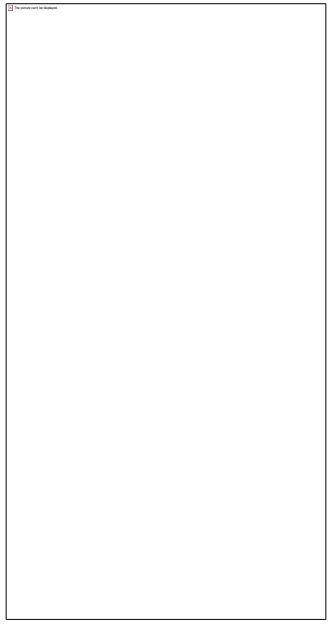
In-solution digestion (CME)

- Dilipidate proteins
- More detergent tolerance
- Favor membrane proteins
- Simultaneous extract lipids and metabolits

Reduction and alkylation of disulfide bonds

- Reducing agent: DTT or TCEP
- Alkylating reagent: iodoacetamide





Protease digestion of proteins for MS analysis

Trypsin

- most commonly used protease
- cleaves on C-terminal side of basic residues
  Arg and Lys unless next residue is Pro
- active near neutral pH
- inactive at low pH
- sequencing grade trypsin is methylated to
  protect against self-digestion

Protease digestion of proteins for MS analysis

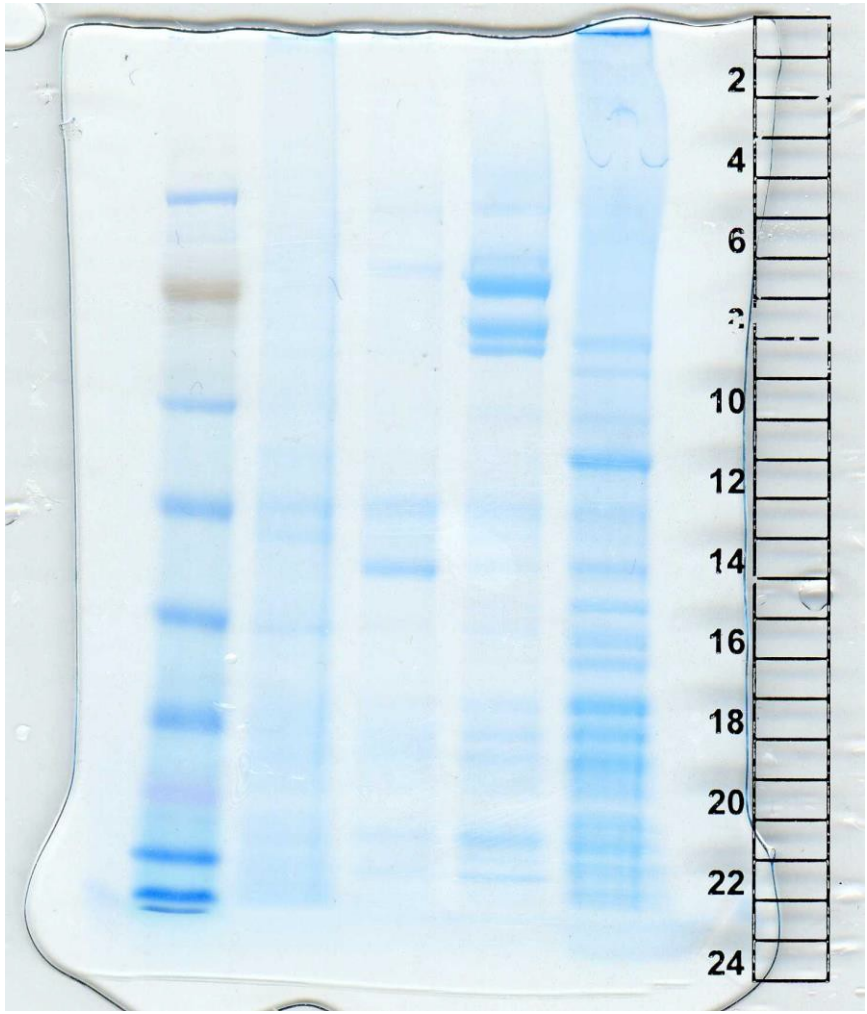
Commonly used alternate proteases

- AspN – cleaves on N-terminal side of Asp residues
- GluC – cleaves on C-terminal side of Glu residues
- LysC – cleaves on C-terminal side of Lys residues
- ArgC – cleaves on C-terminal side of Arg residues

- chymotrypsin – cleaves on C-terminal side of hydrophobic residues
- proteinase K – not sequence specific; used for limited digestion

Gel-Based Sample Preparation

- Excise gel bands
- Destain gel bands
- Reduction: TCEP
- Alkylation: Iodoacetamide
- Digestion: 100 ng of trypsin
- Quenching: 0.1% formic acid
- LC-MS/MS

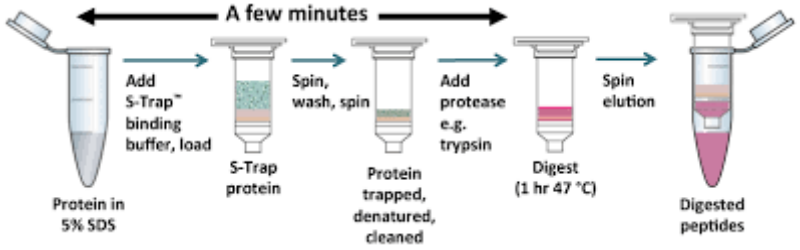


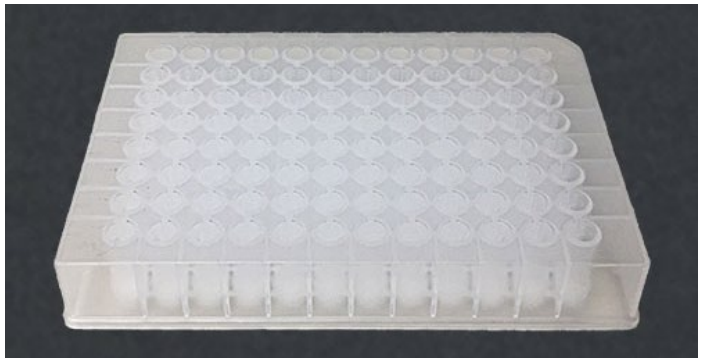
Gel-Free Sample Cleanup

- C18 reverse-phase resin
- Wash buffer: 0.5% ACN, 0.1% formic acid
- Elution buffer: 70% ACN, 0.1% formic acid

Higher-Throughput Sample Prep Options

Thermo EasyPep





Protifi S-Trap