Protein Extraction

Medicago truncatula

Shinorhizobium medicae root nodules

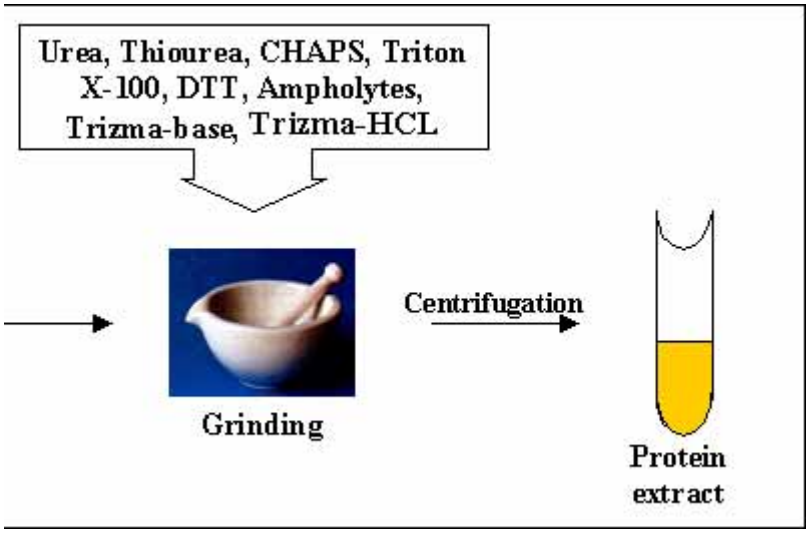
Plant Harvest:

drought stress

Control

Liquid-N2!

Grinding and solubilizing:



variouse extraction buffers and detergents exists!

Protein Extraction

Medicago truncatula

Sinorhizobium medicae root nodules

drought stress

Control

Plant Harvest:

Medicago truncatula (Rhizobium inoculated) : **leaves**

Group 1) wt

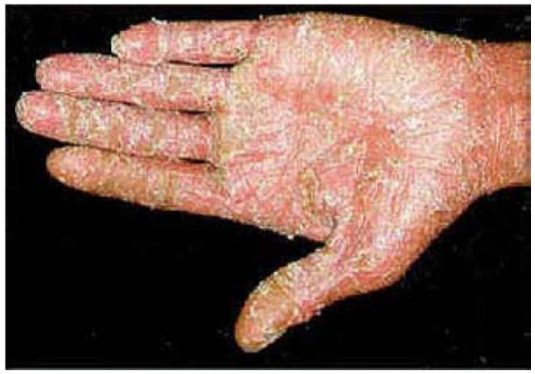
Group 2) dnf5-2 (N-fixation deficient)

Group 3) NF11301 (ferritin F3)

Group 4) NF9644 (ferritin F2)

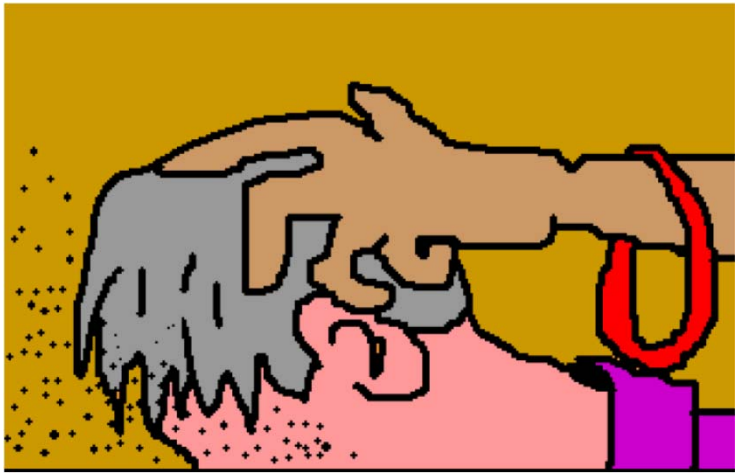
each: control and drought stress (7 days)

GLP



Skin flakes

Dandruff



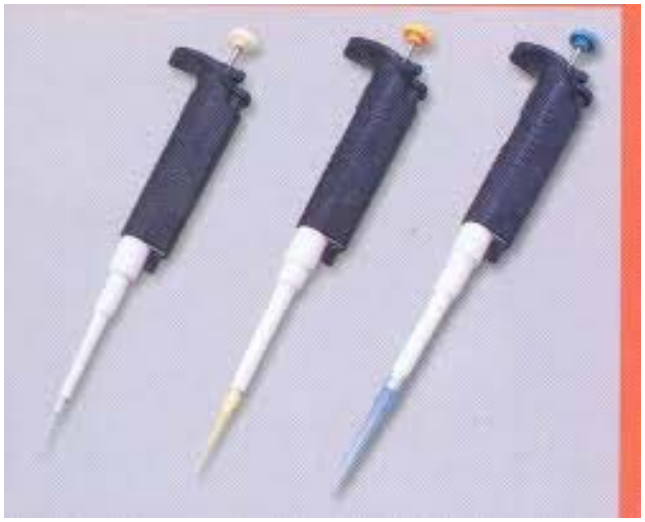
GLP

clean your bench!

use the gloves!

GLP

Handle with care!!!



NEVER !!!

Safety Instructions!!!

Lena Fragner

Different Extraction strategies

- **Volatile buffers preferable (e.g. ammonium bicarbonate)**
  - can be removed by vacuum centrifugation or desalting
  - reconstituted in aqueous organic solvent + acid
- **Soluble samples**
  - 50 % aqueous MeOH or CH_3CN + 1-5 % formic/acetic acid
- **Insoluble samples (membrane proteins)**
  - chloroform, higher percentage of acid

There is no„one and only“ extraction!

Protein Extraction

- Urea for protein solubilization; does not bind to proteins – can cause modifiction at temperatures above 37°C!
- PMSF (**Phenylmethylsulfonylfluorid**) = Protease inhibitor
- Acetone Precipitation for desalting and removal of hydrophobic and other MS-unsuitable buffer components
- ß-Mercaptoethanol like DTT for irreversible disulfide reduction

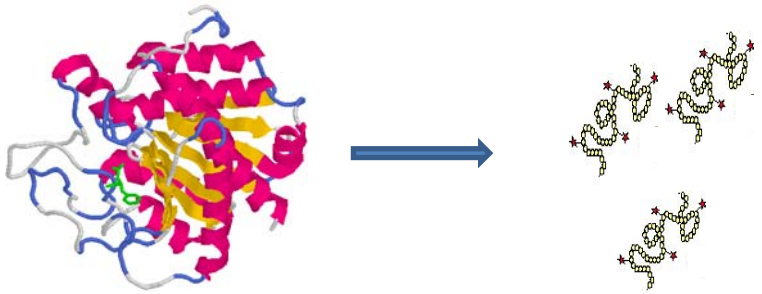
Gel-free
vs.
Gel-based

Shotgun-MS Analysis

Protein Digestion

For gel-free Shotgun-MS Analysis

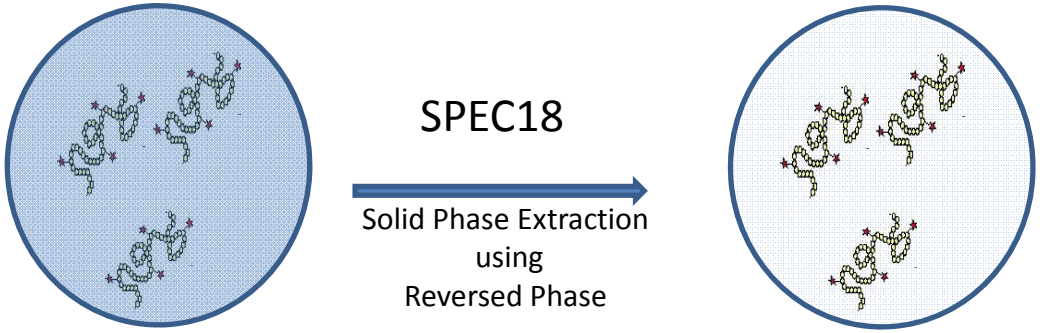
In Solution
Protein digestion:



Protein

Peptides

Peptide desalting:



Peptides in Urea
(Salt) Buffer

Protein Extraction

- **Removal of non-volatile buffers i.e. NaCl, phosphate, etc**
  - reverse phase HPLC (off-line)
  - desalting cartridges
  - centrifugal filtration
  - passive dialysis
  - interface chromatographic or desalting cartridges with ESI source (LC-MS)

- **Removal of detergents or surfactant**
  - they quench and obscure ESI response (e.g. SDS from gel)
  - difficult to remove from protein samples, SCX
  - best to avoid if sample is for ESI analysis

Peptide Desalting

Sample purification

a) activate – MeOH

b) equilibrate – solution A

c) load you sample

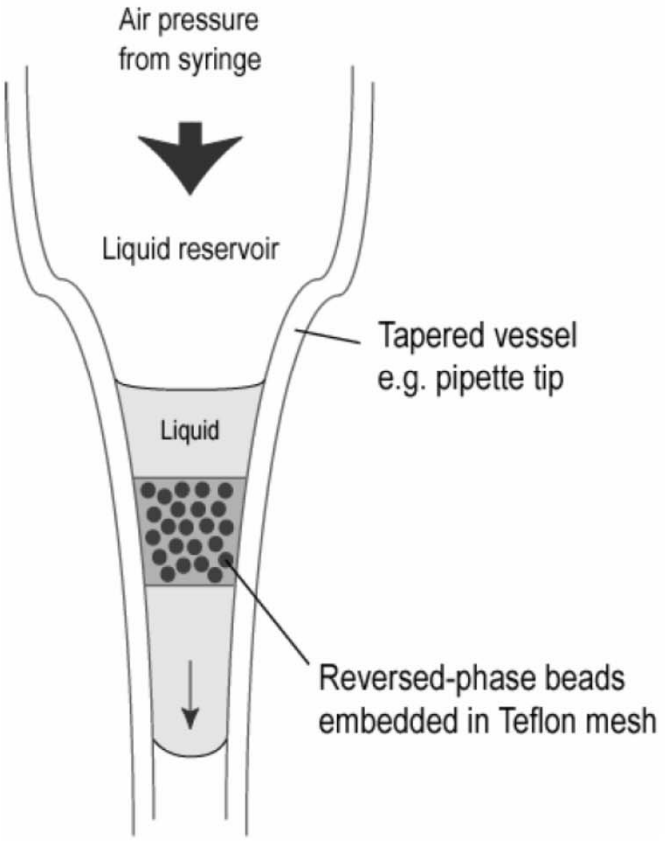
d) wash – solution A

b) elute – solution B

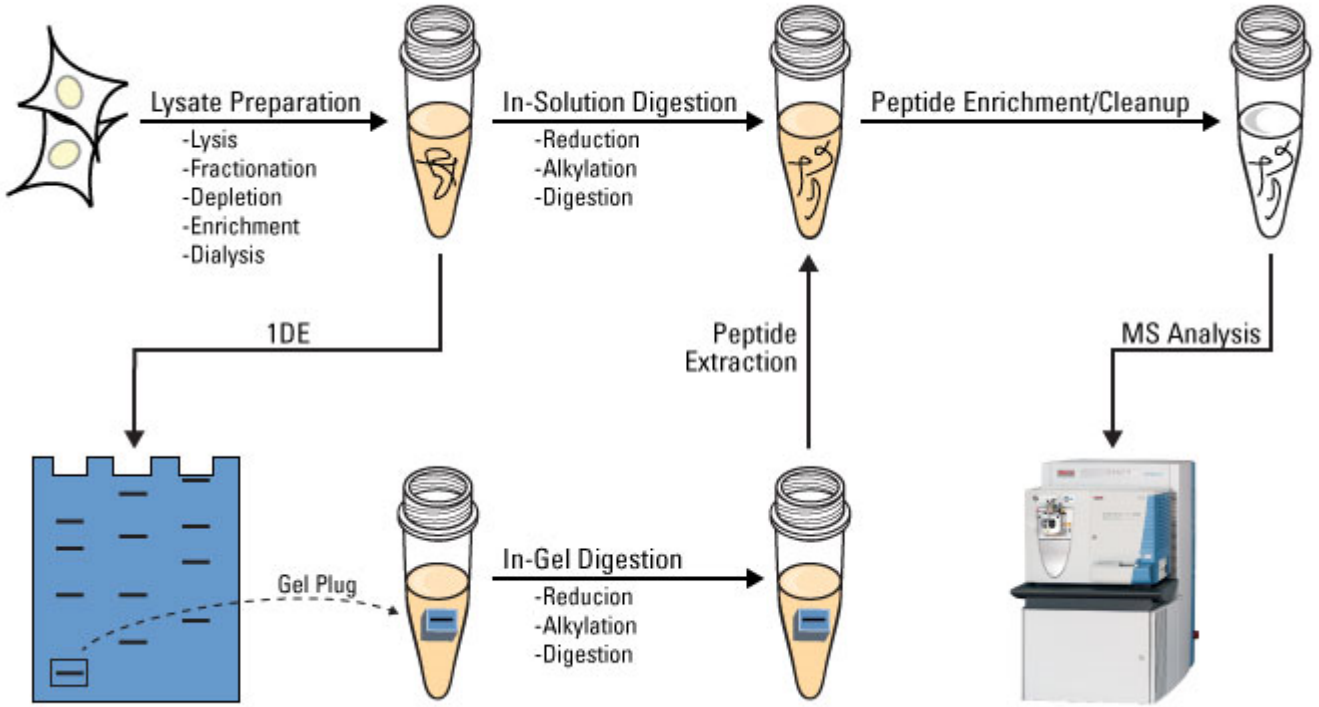
solution A:
0.5% acetic acid

solution B:
0.5% acetic acid
80% acetonitrile

How it works ?



Sample preparation workflow



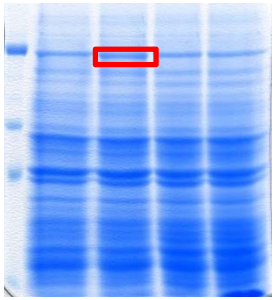
Lysate samples are prepared from biological specimens or cultured cells by a customized protocol that may include cell lysis, subcellular fractionation, depletion of high abundant proteins, enrichment of target proteins, dialysis and desalting. In-solution digestion entails irreversibly breaking disulfide bonds via reduction and alkylation followed by protein digestion into peptide fragments. An alternative approach is to first resolve proteins by 1D or 2D electrophoresis (1DE or 2DE, respectively) and then collect gel slices that contain the desired band(s). The proteins in these gel plugs are then reduced, alkylated and digested in situ. After peptides are extracted from the gel matrix, the peptides are enriched and salts and detergents removed and prepared for MS analysis. While this diagram encompasses the most common steps of sample preparation, the protocol must be tailored to specific samples and MS techniques for optimal results.

Protein Extraction

For gel-based Shotgun-MS Analysis

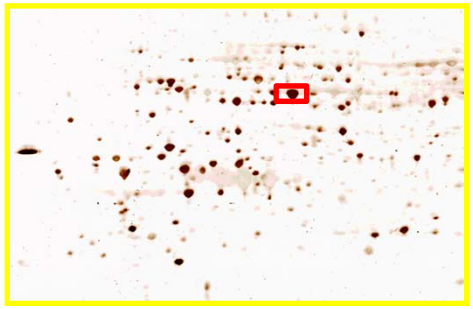
In Gel
Protein digestion:

SDS- PAGE



or

2D-(IF-SDS) GelElectrophoresis



1) Cutting gel band/spot
2) Wash gel pieces
3) In-Gel Tryptic Digestion
4) Peptide extraction from gel
5) Desalting with STAGE Tips
6) MS-Analysis

Sample preparation workflow

digest

Cut the band out

↓

Wash — Volatile buffers - ammonium bicarbonate!

↓

Reduction — DTT

↓

Alkylation — Iodoacetamide

↓

Digestion — Trypsin or LysC

↓

Extraction — Acetonitrile

↓

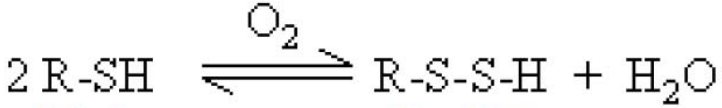
Purification — STAGE Tip

MS

Reduction and Alcylation

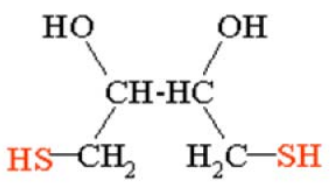
Digest

Reduction



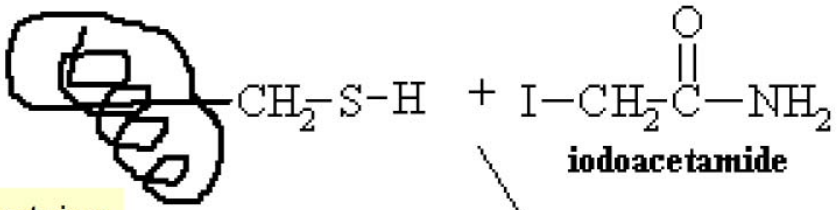
Cystein

DTT

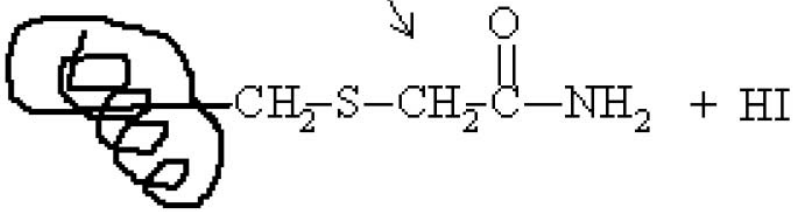


DTT to reduce SH-bridges

Alkylation



Carbamidomethylation of Cysteine



Peptide Desalting

SPE C18 (Solid Phase Extraction)

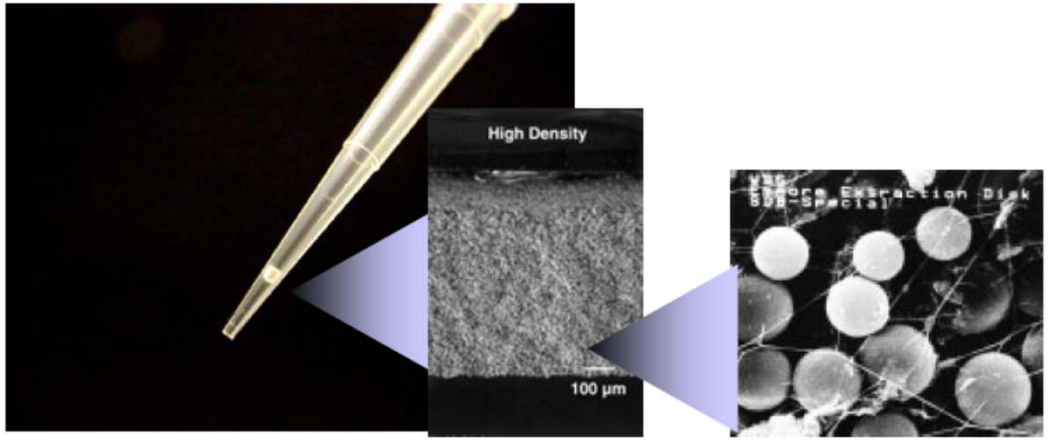
STop And Go Extraction Tip (StageTip) vs. 96-well-SPEC

STAGE tip is a reversed-phase column providing:

a) **Desalting**

b) **Filtration**

c) **Concentration**



From www.mmm.co.jp

Sample preparation workflow

LC/MS/MS Shotgun Analysis

homogenisation

Plant extraction

Protein Prefractionation

In-gel digestion

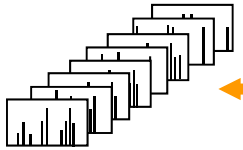
Liquid Chromatography

RP

ES I

MS/ MS

LTQ



MS/MS spectra

→ Screening for marker enzymes

Good Labratory Practice

BEFORE YOU START !!!

MAKE SURE !!!

THAT YOU AVOID !!!