2D Gel Electrophoresis Training

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Proteomics and Mass Spectrometry Facility

DONALD DANFORTH PLANT SCIENCE CENTER

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2D gel sample preparation

- Protein degradation, add protease inhibitors
- Contaminations: skin, hair etc., use gloves, clean glassware
- Sample components: lipid, salt etc.
- Whole tissue, organelle purification and protein fractionation
- Sample solubilization: particulates cause smearing and block gel pores

2 D gel sample solubilization:

Many ways: sonication, a drop of 0.1 M NaOH, ultracentrifugation

2 D sample buffer:

8 M urea, 2% CHAPS, 50 mM DTT, 0.2% ampholytes and Bromophenol

- **Chaotrophe**: urea and thiourea to disrupt hydrogen and hydrophobic bonds
  * urea forms an equilibrium with CN which causes carbamylation. Do not heat over 30 ^{o}C

- **Detergents** (nonionic or zwitterionic): solubilise lipids, disrupt membranes and hydropobic interactions

- **Reductants**: DTT to reduce disulphide bridges

- **Ampholytes**: help solubilization, scavenging CN and inhibiting interactions between sample proteins and immobilines of IPG strips.

Protein loading into IPG strip :

- **Passive rehydration**: strips are placed in contact with the protein sample diluted in sample buffer and covered with mineral and left overnight.

- **Active rehydration:** facilitate the entry of large proteins into the gel, is carried out in a similar fashion except 50 V is applied throughout the rehydration period.

- **Cup-loading**: beneficial when using basic IPG strips, when the samples are contaminated with nucleic acids or other large non-proteinaceous molecules, or when running serum that has not been depleted of albumin.

Isoelectric focusing (IEF):

- **Temperature:** 20 ^{0}C
- **Current limit:** 50 mA
- **Program:** first at low voltage, then ramp up to higher voltages after the current has settled down. Here is an example.

Step	Voltage	Time	Volt-Hours	Ramp
1	250	20 min		linear
2	8000	2.5 h		linear
3	8000		20,000	Rapid

IPG strip equilibration:

- transitional step from IEF to SDS-PAGE

- **Equilibration buffer:**
  6M urea, **2% SDS**, 0.375 M Tris-Cl pH 8.8, 20% glycerol

- **Equilibration buffer I:** 10 min
  2% DTT to ensure any reformed disulphide bonds are reduced

- **Equilibration buffer II:** 10 min
  2.5% iodoacetamide to alkylate proteins and react with any unreduced DTT

Reduction/alkylation facilitate downstream protein in-gel digestion

SDS-PAGE and gel staining:

- precast gels to increase reproducibility

- Gel staining:

1. **Colloidal Coomassie Blue (Bio-Safe Coomassie):**
   broadest spectum of proteins, 2 orders of magnitude, sensitivity to 10 ng.

2. **SYPRO Ruby fluorescent staining:** (Ext. 280 nm and 450 nm, Em: 610 nm)
   little background staining and very sensitive (1 ng), linear over 3 orders of magnitude, detection of glycoproteins, lipoproteins and metalloproteins.

3. **Silver Staining:**
   very sensitive (1 ng), but the linearity is low (1 order magnitude).

Protocol for 2D Gel Electrophoresis:

Day One:

- Sample Preparation:

  185 ul (~ 200 ug) E. coli crude protein extract in sample buffer.

- Sample Application and Rehydration:

  1. Remove a dry strip from -20°C and allow to equilibrate at RT for 10 – 15 min.

  2. Load the sample into the rehydration tray, leave about 1 cm at each end.

  3. Note the basic end of the strip and position it at the left side of the tray. Place IPG strip gel-side down on the top of the sample, no bubbles.

  4. Overlay with 1.6 ml minearl oil. Cover the tray with lid, RT O/N.

Protocol for 2D Gel Electrophoresis:

Day Two, early morning:

- Isoelectric Focusing:

1. Place a paper wick at both ends of the focusing try channel, wet each one with 8 ul water.
2. Take out IPG strip and hold vertically for about 10 seconds the drain the oil.
3. Place the IPG strip gel side down and with basic end to the left in the tray
4. Cover each strip with 2 ml minear oil.
5. Place the tray into IEF cell and run the following program.

Step	Voltage	Time	Volt-Hours	Ramp
1	250	20 min		linear
2	8000	2.5 h		linear
3	8000		20,000	Rapid

Protocol for 2D Gel Electrophoresis:

Day Two, after lunch:

- Buffer and gel preparation:

1. **1x SDS-PAGE gel running buffer:** dilute 10x with MilliQ water.
2. **Equilibration buffer I:** add 13.35 ml 30% glycerol, stir and mix until all the solids have dissolved. Do not heat above 30 ^{o}C.
3. **Equilibration buffer II:** add 13.35 ml 30% glycerol, stir and dissolve as above. Note 0.5 g iodoacetamide is already added.
4. **Gel well rinsing:** rinse the gel well with nanopure water.

- IPG Equilibration:

1. Take out IPG strip and drain the oil for 5 second and put them gel side up in a clean tray.
2. Add 3.8 ml Equilibration buffer I and shake for 10 min.
3. Discard buffer I, add 3.8 ml buffer II and shake for 10 min.
4. During this incubation, melt overlay agrose for about 30 second.
5. Discard equilibration buffer II.

Protocol for 2D Gel Electrophoresis:

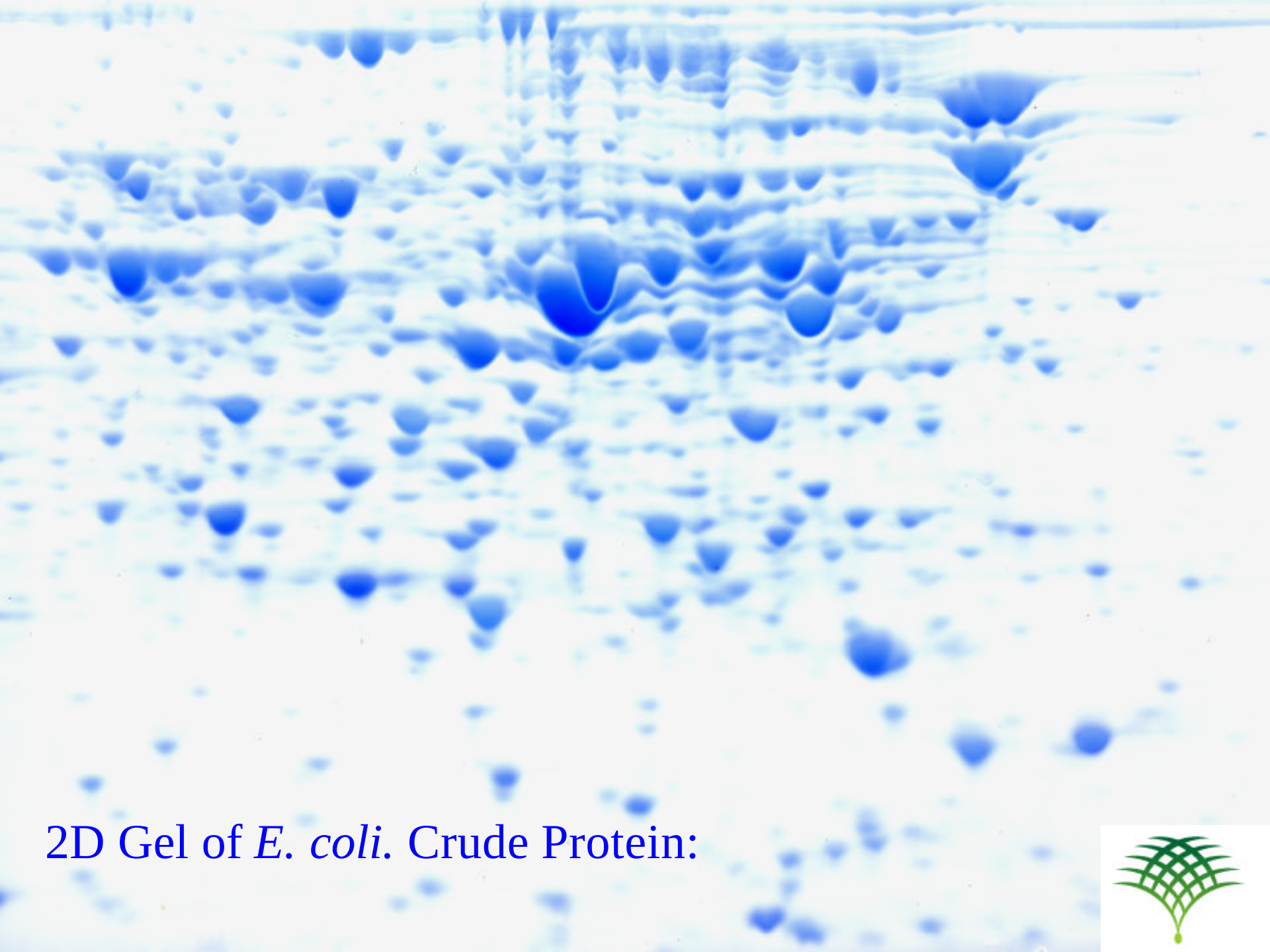
Day Two, after lunch:

- SDS-PAGE:

1. Blotting away water from the IPG well of SDS-PAGE gel.
2. Dip the IPG strip briefly in 1x SDS-PAGE gel running buffer, and lay the strip gel side up onto the back plate of the SDS gel lying on the bench.
3. Stand the gel plate vertically, fill the well first with agarose. Add marker, and then push the IPG strip into the well carefully. Be sure that the forcep is pushing on the plastic backing to the strip, not the gel matrix.
4. Place the gel in the gel box and let agarose to solidify for 5 min.
5. Fill the gel box with running buffer and run the gel at 60 volt for 15 min, then at 198 volt for about 1 h.

- BioSafe Coomassie Staining:

1. Take out the gel and wash 3 times with water for 5 min each wash.
2. Add 50 ml stain, and shake 1 h to O/N.
3. Discard the stain, wash gel several times with water. The gels can be stroed in water for several days.



2D Gel of *E. coli.* Crude Protein: