

## Proteomics With Two-Dimensional Gel Electrophoresis and Mass Spectrometry Analysis in Cardiovascular Research

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### Summary

Proteomics is a large-scale, comprehensive study of the proteins of a cell or organism. It is a unique means of characterizing proteins that are expressed in a cell or tissue at any given time-point and of identifying any modifications that they may undergo. Thus, it is a powerful technology that can detect and identify the changes of the structure and function of proteins in response to intra- and extracellular environmental signals or disease states. As proteomics can establish a link for genes and proteins with a disease, it will play an important role in defining the molecular determinants of a disease and in identifying targets for drug discoveries and diagnostics. We have carried out the first proteomics study for coronary artery disease (CAD) and found that the expression of the ferritin light chain was significantly increased in CAD tissues. In this chapter, we use the CAD study as an example to demonstrate the procedures involved in proteomics analysis. The proteome is visualized by two-dimensional gel electrophoresis, a powerful and widely used method for proteomics, and the proteins of interest are then identified by mass spectrometry. This technique should be useful in characterizing cardiovascular diseases and in defining signaling pathways for cardiovascular development and physiology.

**Key Words:** Proteomics; two-dimensional (2D) gel electrophoresis; proteome; cardiovascular disease; signaling; mass spectrometry; protein structure and function.

### 1. Introduction

Proteomics is the large-scale analysis of the structure and function of proteins expressed in cells, tissues, and fluids (*1*). The proteome, the total protein output encoded by a genome, is far more complex than the genome because there are more proteins than genes as a result of the alternative splicing of genes and posttranslational modification of proteins (~22,000 genes vs

~400,000 proteins). This discrepancy makes proteomics a necessary tool to characterize the complex network of proteins involved in cellular regulation and signaling.

Proteomics permits the detection of proteins that are associated with specific cellular functions by means of their altered levels of expression. It allows a comparison of two or more different states of a cell or an organism (e.g., diseased vs nondiseased tissues) in order to identify specific qualitative and quantitative protein changes (2). In addition, proteomics can be applied in basic research, for example, the profiling of drug effects, molecular diagnostics, and various other therapeutic areas.

The experimental strategy most often employed in proteomics is to separate the proteins expressed in comparable systems (e.g., diseased vs nondiseased tissues) using two-dimensional (2D) gel electrophoresis and quantify the amounts of each protein in each cell system by the density of staining of each respective protein band (**Fig. 1**). The 2D gel electrophoresis possesses a sufficient resolving power for proteome analysis (3,4). This technique separates proteins in two steps: the first-dimension and the second-dimension gel electrophoresis. In the first dimension, proteins are separated by their isoelectric point (pI), the pH at which a protein carries no net charge and will not migrate in an electrical field. The technique is also called isoelectric focusing (IEF) electrophoresis (5). A sample preparation is the key to successful IEF. In the second dimension, proteins after IEF are further resolved by their molecular weight (MW) using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The resulting gel is then stained with Coomassie Blue or silver to visualize the protein spots.

Protein patterns on 2D gels are analyzed using software programs to statistically and scientifically determine meaningful spots. Proteins of interest can then be excised from the gel for further identification and full characterization using mass spectrometry (MS).

## 2. Materials

### 2.1. Sample Preparation (see Note 1)

1. Lysis buffer: 8 M urea, 1% Triton X-100, 0.1 M dithiothreitol (DTT), 0.1 M NaCl, 0.045 M Tris-HCl, pH 7.4, 4% “complete” protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN). Dissolve with shaking, but do not use any heat (see Note 2).
2. Tris stock buffer: 0.5 M Tris-HCl, pH 8.0, 50 mM MgCl<sub>2</sub>.
3. DNase stock: 10 mg/mL bovine pancreatic DNase (Sigma, St. Louis, MO) in Tris stock (see Note 3).
4. RNase stock: 10 mg/mL bovine pancreatic RNase (Sigma) in Tris stock.
5. Nuclease reagent: 1 mg/mL DNase, 1 mg/mL RNase in Tris stock.
6. Tri-*n*-butylphosphate:acetone:methanol (1:12:1).

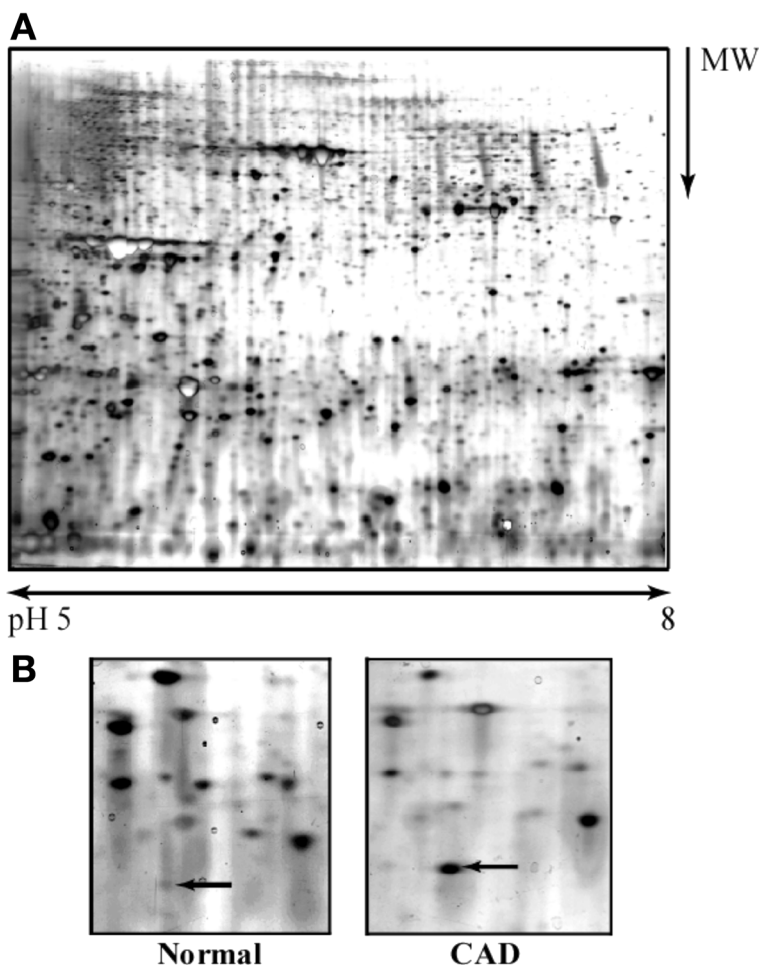


Fig. 1. Two-dimensional (2D) map of human coronary artery proteins. **(A)** Protein extract was prepared from a normal human coronary artery using the delipidation method. Reproducible 2D patterns were obtained from protein samples from other coronary arteries. The resolution of protein spots was optimized using pH 5.0–8.0 immobilized pH gradient strips. **(B)** Enlarged areas from 2D gel images of a normal individual and a coronary artery disease (CAD) patient. The protein spot indicated by the arrow shows a higher level of expression in CAD than in their normal counterparts. This result is reproducible from many other samples. The protein spot has an isoelectric point of 5.5 and molecular mass of about 20 kDa, and has been identified as the ferritin light chain by mass spectrometry.

7. Tri-*n*-butylphosphate (Sigma).
8. Methanol: high-performance liquid chromatography (HPLC)-grade.
9. Acetone: HPLC-grade.

10. Boiling buffer: 0.325 *M* DTT, 4% CHAPS, 0.045 *M* Tris-HCl, pH 7.4 (*see Note 4*).
11. Dilution buffer: 8 *M* urea, 4% CHAPS, 0.1 *M* DTT, 0.045 *M* Tris-HCl, pH 7.4.
12. Bio-Rad protein assay kit I: contains 450 mL of dye reagent concentrate and a bovine  $\gamma$ -globulin standard (Bio-Rad, Hercules, CA) (*see Note 5*).
13. Whatman no. 1 filter paper or equivalent.
14. Concentrated HCl to make a 0.12 *N* stock.

## 2.2. First-Dimension IEF

1. Rehydration buffer: 7 *M* urea, 2 *M* thiourea, 1% DTT, 1% CHAPS, 1% ampholytes, 1% Triton X-100. Dissolve with shaking, but do not use any heat (*see Note 6*).
2. 1% bromophenol blue (BPB): 1% BPB in water.
3. Immobilized pH gradient (IPG) gel strips (Bio-Rad) (*see Note 7*).
4. Mineral oil.
5. Wick (Bio-Rad) (*see Note 8*).
6. PROTEAN<sup>®</sup> IEF cell (Bio-Rad): a first-dimension instrument.

## 2.3. Second-Dimension Gel Electrophoresis

1. Equilibration buffer: 5.4 g urea, 0.3 g SDS, 3.8 mL 1.5 *M* Tris-HCl, pH 8.8, 3 mL glycerol in a 50-mL centrifuge tube. Adjust the total volume to 15 mL with water. Dissolve with shaking, but do not use any heat (*see Note 9*).
2. Reducing reagent: 120 mg DTT in 7.5 mL equilibration buffer.
3. Alkylation reagent: 150 mg iodoacetamide in 7.5 mL equilibration buffer, 100  $\mu$ L 1% BPB.
4. Agarose sealing solution: 0.5 g agarose, 10 mL of 10X Tris-glycine running buffer (Bio-Rad), 30 mL glycerol. Adjust the total volume to 100 mL with water. Add 1 mL of 1% BPB. This reagent can be stored at room temperature and used repeatedly over several months.
5. Running buffer (1X): 100 mL of 10X Tris-glycine buffer, 900 mL water. Cool on ice before use.
6. Criterion gel (Bio-Rad): the gels are stored at 4°C (*see Note 10*).
7. Mini-PROTEAN 3 cell (Bio-Rad): a second-dimension instrument.

## 2.4. Silver Staining (*see Note 11*)

1. Fixing solution: 50% methanol, 5% acetic acid in water (v/v).
2. Washing solution: 50% methanol in water (v/v).
3. Sensitizing solution: 0.02% sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) in water (make fresh).
4. 0.1% silver solution: 0.1% silver nitrate in water. It takes time to completely dissolve silver nitrate in water.
5. Developing solution: 0.04% formalin, 2% sodium carbonate in water (make fresh).
6. Terminating solution: 5% acetic acid in water (v/v).
7. Storing solution: 1% acetic acid in water (v/v).

### 2.5. Protein Sequencing and Identification

1. Gel washing solution: 50% acetonitrile, 50 mM ammonium bicarbonate.
2. 50 mM ammonium bicarbonate: 3.96 mg/mL.
3. Protease digestion solution: resuspend lyophilized trypsin (20 µg/vial, Promega, Madison, WI) in 20 µL of the 50 mM acetic acid solution provided with trypsin, yielding a 1 µg/µL stock solution. Dilute that stock to 1 µg/50 µL with 50 mM ammonium bicarbonate (50-fold dilution, 20 ng/µL). Aliquot and store at -70°C. Do not repeat freeze-thaw of trypsin stock solutions more than once.
4. Reducing reagent: 10 mM DTT in 0.1 M ammonium bicarbonate.
5. Alkylation reagent: 50 mM iodoacetamide in 0.1 M ammonium bicarbonate.
6. Extraction solution: 50% acetonitrile, 5% formic acid.

## 3. Methods

Sample preparation is the key to successful 2D gel electrophoresis (6–8). Sample preparation and solubilization of any protein mixture for subsequent 2D separation is of major importance, as it will affect the overall performance of the technique. It should follow three important rules. First, as many proteins as possible, including hydrophobic proteins, must be solubilized. Second, protein aggregates must be solubilized. Third, sample preparation must be reproducible in order to reduce misleading results.

### 3.1. Protein Delipidation and Extraction From Tissues

1. 0.1 g of tissue is homogenized in 1 mL of lysis buffer.
2. Incubate for 15 min at 34°C and cool on ice for 10 min.
3. Add nuclease reagent, mix well, and incubate on ice for 10 min.
4. Centrifuge the homogenate at 4°C for 15 min at 10,000g.
5. Collect the aqueous phase between the upper lipid phase and lower cellular debris phase.
6. Mix the collected aqueous phase with 14 mL of ice-cold tri-*n*-butylphosphate: acetone:methanol (1:12:1) and incubate at 4°C for 90 min.
7. Centrifuge at 2800g for 15 min and remove supernatant.
8. Wash the pellet sequentially with 1 mL of tri-*n*-butylphosphate, acetone, and methanol, and then air-dry.
9. Boil the precipitate in 0.1 mL of boiling buffer and cool to room temperature.
10. Dilute the cooled sample in 1.5 mL of dilution buffer and incubate at 34°C for 15 min.
11. To quantify total proteins, prepare the bovine  $\gamma$ -globulin standard at 14 mg/mL by reconstituting the lyophilized protein in 1 mL of water. This is 10X of the concentration that is recommended in the kit instructions.
12. Prepare a 1:4 dilution of the dye reagent concentrate by mixing one part dye with three parts water, and filter the dye through Whatman no. 1 filter paper. Each assay point requires 3.5 mL of diluted dye reagent.
13. Prepare 0.12 N HCl (nominal) by diluting concentrated HCl.

14. Prepare standards covering the range of 0.1–14  $\mu\text{g}$  protein/ $\mu\text{L}$  by diluting the 14 mg/mL standard in the same buffer as the sample.
15. Mix 20  $\mu\text{L}$  of each standard or sample with 80  $\mu\text{L}$  of 0.12 *N* HCl in separate assay tubes. It is a good idea to make duplicates for each sample or standard.
16. Add 3.5 mL of diluted dye reagent to each tube. Vortex gently.
17. After 5 min, measure the absorbance of each sample or standard at 595 nm.
18. Plot the absorbance values vs the amount of protein (in micrograms) to generate the standard curve. Expect a nonlinear relationship.
19. Compare the absorbance reading for each sample with the standard curve to determine the concentration of the sample.

### 3.2. First-Dimension IEF

1. Prepare the samples in 1.5-mL microcentrifuge tubes. For silver-stained gels, add 30  $\mu\text{L}$  of sample (5 mg/mL) to 220  $\mu\text{L}$  of rehydration buffer and 10  $\mu\text{L}$  of 1% BPB (see **Note 12**).
2. Transfer the samples to a well in the IEF tray. Place all of samples at one end of the well and coat the entire well by tipping the tray and slowly allow the sample to move to the other end of the tray. Repeat to go back and forth several times. Pop any bubbles with a Kimwipe.
3. Place the IPG gel strip side down in the channel of a focusing tray that contains the sample. Gently move back and forth a bit to ensure good wetting of the gel surface. Force out any bubbles under the gel with a pair of forceps.
4. Cover the strip with oil to prevent evaporation.
5. Rehydrate the strip overnight at room temperature, applying 50 V (program the PROTEAN IEF cell for active rehydration). Typical rehydration time is 12–16 h.
6. Stop the rehydration. Take the tray out of the IEF cell.
7. Take the IPG strips out of the channel of a focusing tray and put them on wet Kimwipe (put the gel side up) (see **Note 13**).
8. Wet the electrode wicks with water. Put the wicks on both positive and negative ends of the well.
9. Put the IPG strips back in the wells (face down).
10. Cover the strips and adjacent wells with mineral oil.
11. Set voltage. IEF is conducted at 10°C at 300 V for 3 h, followed by 1500 V for 3 h, and finally 3000 V for 18 h (see **Note 14**).

### 3.3. Second-Dimension Gel Electrophoresis

1. After the first dimension gel electrophoresis, the IPG strip is equilibrated with equilibration buffer (see **Note 15**). Place the strip face up in the equilibration tray.
2. Cover each strip with approx 3.5 mL of the reducing reagent.
3. Incubate the strip at room temperature with shaking for 15 min (time is important).
4. Remove the reducing agent.
5. Cover the strip with approx 3.5 mL of the alkylation reagent.
6. Incubate the strip at room temperature with shaking for 15 min (time is important).
7. Proceed directly to the gel assembly. While the strips are equilibrating, prepare the running buffer and cool it on ice.

8. Melt the agarose sealing solution in a microwave oven. Heat to  $>65^{\circ}\text{C}$ , but do not boil.
9. This reagent must be used at approx  $45^{\circ}\text{C}$ , but it will begin to solidify at approx  $40^{\circ}\text{C}$ . Therefore, some timing is needed. It is easier to cool the reagent just before use than to heat it just before use.
10. Prepare the gel. Remove the Criterion gel from its package and wash briefly with water. Place the gel in a stand. Remove the green comb and rinse the well (five times) with running buffer. Leave the well covered with running buffer.
11. At the end of equilibration, check the temperature of molten agarose. Carefully cool the agarose sealing solution on ice as needed, until the temperature reaches approx  $45^{\circ}\text{C}$ .
12. Pour buffer out of the gel. Remove the strip from the alkylaton reagent. Blot away any excess reagent and place the strip in the well. Note the orientation (+) end of the strip.
13. Cap the well with agarose. Using a glass pipet, force the agarose over the strip, filling the well. A vigorous action will help minimize the number of bubbles that are trapped in the agarose. These bubbles may disturb protein migration and must be removed. Cool the agarose briefly with ice (*see Note 16*).
14. Add approx 5 mL of cold running buffer to the top chamber.
15. To assemble the gel system, pour the buffer out of the top chamber of the gel.
16. Remove the tape covering the bottom of the gel.
17. Place the gel in the criterion cell, and fill each bottom chamber with cold running buffer to the fill lines.
18. Fill each top chamber with cold running buffer to the top of the chambers.
19. Place the ready precast gels in the Mini-PROTEAN 3 cell, taking care to properly orient the electrodes.
20. Following Bio-Rad's instructions, run the gel at 200 V constant voltage. The total running time will be approx 70 min. Stop the run when the tracking dye just leaves the bottom of the gel. If two gels are being run at the same time, it is possible to pause the run, remove one gel, and restart the run if one gel gets ahead of the other.

### 3.4. Silver Staining

1. Place 300 mL of the fixing solution in the clean Pyrex dish.
2. When the SDS-PAGE run is complete, remove the gel from the cassette.
3. Break the Criterion gel cassette using the green plastic comb taken out of the IPG well.
4. Immerse the broken cassette in the fixing solution and begin removing one plate of the cassette. Being very careful so as not to tear the gel, remove one plate from the cassette.
5. Cut the gel in the top corner at the acidic (+) end of the strip.
6. Carefully cut the gel along the top to remove the IPG strip. Discard the strip.
7. Immerse the second plate, with the gel on it, in the fixing solution. Being very careful so as not to tear the gel, remove the gel from the second plate.
8. Fix the gel for 20 min at room temperature with gentle shaking.
9. Aspirate the fixing solution off of the gel. Be careful so as not to tear the gel.
10. Add 200 mL of washing solution to the gel and wash for 10 min.

11. Aspirate the washing solution off the gel. Be careful so as not to tear the gel.
12. Add 500 mL of water for 2 h. Additional washing overnight will reduce background staining.
13. Aspirate the water off of the gel. Be careful so as not to tear the gel.
14. Add 200 mL of sensitizing solution to the gel and incubate for 1 min.
15. Aspirate the sensitizing solution and wash the gel in 500 mL of water for 1 min.
16. Aspirate the water and wash the gel in 500 mL of water for 1 min.
17. Aspirate the water, add 200 mL of silver solution to the gel, and incubate the gel for 20 min.
18. Aspirate the staining solution and wash the gel with 500 mL of water for 1 min.
19. Transfer the gel to new glass chamber, and wash the gel with 500 mL of water for 1 min.
20. Aspirate the water and add 200 mL of developing solution to the gel. Observe the color and change the solution when the developer turns yellow. Terminate when the staining is sufficient.
21. Aspirate the developing solution and add 200 mL of terminating solution to the gel. Change the solution a couple of times.
22. Leave the gel at 4°C in storing solution.

### **3.5. Protein Sequencing and Identification (see Note 17)**

1. Analyze the 2D gels with an image analysis software. Excise the protein spot of interest from the gel as closely as possible and transfer it into a clean 1.5-mL microcentrifuge tube.
2. Add 500  $\mu$ L of wash solution and incubate at room temperature for 15 min with gentle agitation. Remove the solution with pipet tips. Repeat this washing step up to three times or until the Coomassie dye has been completely removed.
3. Remove the solution and dehydrate gels in acetonitrile. At this point, the gel pieces should shrink and become an opaque-white color. If they do not, remove the acetonitrile and replace with fresh acetonitrile.
4. Discard the acetonitrile and vacuum centrifuge for 3–5 min (*see Note 18*).
5. Add 50  $\mu$ L of reducing solution at room temperature for 30 min.
6. Remove the reducing solution and add 50  $\mu$ L of alkylation solution at room temperature for 30 min.
7. Remove the alkylation reagent and dehydrate the gel pieces in 200  $\mu$ L of acetonitrile.
8. Remove the acetonitrile. The gel pieces should shrink and become an opaque-white color. If they do not, remove the acetonitrile and repeat the washing–dehydration cycle until they do.
9. Remove the acetonitrile and wash the gel pieces by rehydration in 200  $\mu$ L of 0.1 M ammonium bicarbonate.
10. Dehydrate the pieces in 200  $\mu$ L of acetonitrile and remove the acetonitrile.
11. Dry the gel pieces in a vacuum centrifuge for 3–5 min.
12. Rehydrate the gel pieces in 20  $\mu$ L of trypsin solution and place them on ice for 10 min.
13. Remove excess trypsin solution and add 5  $\mu$ L of 50 mM ammonium bicarbonate.



14. Incubate overnight at 37°C.
15. Extract the peptides from the gel in 60  $\mu$ L of 50 mM ammonium bicarbonate.
16. Spin down samples with brief centrifugation at 12,000g for 30 s and transfer supernatant to a clean microcentrifuge tube.
17. Extract the peptides with additional 60  $\mu$ L of extraction solution.
18. Spin down samples by brief centrifugation at 12,000g for 30 s and transfer the supernatant containing additional tryptic peptides to tube from **step 16**.
19. Repeat **steps 17 and 18**.
20. Combine the extracts and evaporate to <20  $\mu$ L for liquid chromatography (LC)-MS analysis. The LC-MS analysis is performed by a core facility with necessary expertise.
21. Analyze the data by searching the National Center for Biotechnology Information (NCBI)'s GenPept database using the computer program SEQUEST (see **Note 19**).

#### 4. Notes

1. This protocol is used to remove any interfering lipids in coronary artery tissues. Lipids can bind to proteins and increase artifactual migrations and streaking. This problem can be alleviated by a mixture of organic solvents (1 vol of Tri-*n*-butylphosphate:12 vol of acetone:1 vol of methanol) (**9**). Sample preparation methods may vary from sample to sample, but generally include reducing agents, chaotropes, and detergents.
2. Urea is the most commonly used chaotropic agent in sample preparation for 2D PAGE. It lowers the cohesion of water, making hydrophobic regions of proteins more soluble in aqueous solution (**10**). Urea should not be heated because carbamylation of the sample, which is a spontaneous nonenzymatic modification of proteins and amino acids by urea-derived isocyanate, may occur. DTT reduces cystine disulfide bonds within or between proteins (**11**). DTT is oxidized relatively quickly in aqueous solution. For best results in terms of the reproducibility of 2D-PAGE gels, DTT solutions should be prepared fresh.
3. The presence of nucleic acids, especially DNA, interferes with separation of proteins by IEF. DNA binds to proteins in the sample and causes artifactual migration and streaking.
4. CHAPS is a zwitterionic detergent based on a cholesterol ring. It assists urea in the solubilization of hydrophobic proteins (**10**).
5. Protein assays are generally sensitive to detergent or reducing agents used at the concentrations found in typical sample solutions. Modified Bio-Rad protein assay is used to determine the protein content in typical sample solutions used to load IPG strips.
6. Ampholytes are added to all IPG rehydration buffers and sample solubilization solutions to help keep proteins soluble. An ampholyte is a molecule possessing both positive and negative charge, or that can function as both an acid and a base. A large number of carrier ampholyte mixtures are available with different pH gradients. The choice of ampholytes is dependent on the pH range of the IPG strip.

7. IPG strips are commercially available and must be rehydrated with the appropriated additives prior to IEF because they are provided dry. The pH-gradient ranges of IPG strips vary; the use of broad-range strips (pH 3.0–10.0) allows the display of the most proteins in a single gel. With narrow-range strips (pH 3.0–6.0, pH 5.0–8.0, pH 7.0–10.0), resolution is increased by expanding a small pH range across the entire width of a gel.
8. Wicks collect salts and other contaminants in the sample. Without wicks, salts collect at the anode and cathode, producing high conductivity that can alter the gradient and cause discontinuities in the gel, “hot spots,” or burns.
9. Buffer must be freshly prepared.
10. Be aware that the gels have a limited shelf life. Note the expiration date.
11. To prevent carryover of contamination, staining of proteins must be performed with labware that has never been in contact with nonfat milk, BSA, or any other protein-blocking agent.
12. For Coomassie Blue-stained gels, use 150  $\mu\text{L}$  of sample (5 mg/mL) plus 100  $\mu\text{L}$  of rehydration buffer with 10  $\mu\text{L}$  of 1% BPB.
13. Wash the tray with soap and 95% ethanol. It is important to clean the focusing trays properly between runs. Channel-to-channel leakage is common when salts accumulate in the channels.
14. Focused IPG strips can be stored at  $-20^{\circ}\text{C}$  indefinitely without affecting the final 2D pattern.
15. Equilibration process reduces disulfide bonds and alkylates the resultant sulfhydryl groups of the cysteine residues. Concurrently, proteins are coated with SDS for separation on the basis of MW.
16. SDS-PAGE standards can be applied to gels that have no reference lane. Pipet 10  $\mu\text{L}$  of the SDS-PAGE standards onto the wick. Slip the wick into the slot in the gel sandwich next to the IPG strip.
17. Protein sequencing and identification is usually performed in an MS center. This protocol is a procedure for the preparation of Coomassie Blue-stained 2D gel spots. In general, the best results are obtained when using Coomassie Blue-stained 2D gels.
18. Care should be taken when handling the tube once the gel is dry, because the gel may “jump” out as a result of static electricity.
19. In the event that no matches in the databases are identified, the spectra are interpreted manually and further searching of the expressed sequence tag database is carried out using the FASTA program.
20. Always use nonlatex gloves when handling samples and gels; keratin and latex proteins are potential sources of contamination.
21. Never reuse any solutions; abundant proteins will partially leach out and contaminate subsequent samples.
22. The quantification of the amounts of each protein in a 2D gel by the density of staining of each respective protein band can be achieved using sophisticated image analysis systems. However, obvious protein bands that show differential differences between two systems (e.g., diseased vs nondiseased tissues) can be identified by direct visualization of the gels. Commercially available software packages can

be used to compare computer images of 2D gels to determine differential protein expression, and the user's guide should be followed from the specific manufacturer. For example, the Bio-Rad PDQuest image analysis software landmarks proteins for gel alignment and identifies subtle changes in the up- or downregulation of proteins based on the intensity of protein staining. Multiple gels can be compared using the image analysis systems when good reproducibility of the gels is maintained and when known landmark proteins can be employed to correct the positions of each protein band.

23. The MS experiments have the sensitivity to take any detectable protein band out of the gel, and determine the amino acid sequence of the peptides from the band. In general, identity of a protein band visible on a Coomassie Blue-stained gel, typically containing greater than 0.2 pmol of protein, can be defined easily and successfully by MS. Silver-stained bands, typically containing between 0.01 and 0.5 pmol of protein, can also be analyzed, but this is done less routinely.
24. The results from proteomics analysis should be validated using reverse-transcription PCR, Northern blot, or Western blot analyses (if antibodies are commercially available).
25. Proteomics holds great promise in cardiovascular research. It can be used to analyze differential protein expression in diseased hearts (e.g., atrial fibrillation, ischemic heart disease, and dilated cardiomyopathy) in comparison with normal hearts in animal models and humans. Furthermore, cells that are important for the pathogenesis of cardiovascular disease such as cardiac cells, endothelial cells, smooth muscle cells, and macrophages, can be challenged with various environmental agents (e.g., oxidized low-density lipoprotein and C-reactive protein, known risk predictors for atherosclerosis), and proteomics studies can be then used to identify proteins or signaling molecules involved in cell responses. These studies will be particularly helpful to better understand the relations between proteome changes and cardiovascular dysfunctions. Thus, proteomics allows us to examine global alterations in protein expression in the diseased hearts or vascular systems, and will provide new insights into the molecular mechanisms involved in cardiovascular dysfunction and disease.

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