ProteomeLab PF 2D
Frequently Asked Questions (FAQs)

Automation

Q: How long does an automated run take?
A: With collection at 0.3 pH intervals and running all fractions it could take 36 hours for a complete profile. However, not all pI fractions collected contain protein and running only the protein fractions will take 19-20 hours. The ProteomeLab PF 2D software provides a means to run only the fractions of interest.

Q: What tubes are used for collection and injection?
A: 96 deep well plates.

Q: Does the technology lend itself to automation and integration into existing proteomics platforms? How about the link with MS technologies? Structural studies (x-ray, NMR, etc.)?
A: The ProteomeLab PF 2D system is an all-liquid phase technique for mapping and fractionation of proteins resulting in liquid fractions that can be analyzed with standard techniques. Fractions can be collected in appropriate formats, such as microwell plates, to facilitate automation. Direct interface of the liquid fractions for MS analysis, as well as spotting plates for MALDI, and in addition to whatever other characterization techniques you might want to perform, are possible. The same holds true for structural studies. Although on line MS is possible, off line transfer to MALDI-MS or ES-MS/MS appears to have advantages with regards to sample conservation.

Capacity

Q: What is the minimum quantity of sample required?
A: There really is no minimum quantity other than to ensure that there is enough material to be detected. This will be determined by the limit of detection (LOD) of a given analyte and the complexity of the sample. Also it will be dependent on the method of detection. In the second dimension with detection at 214nm, the average LOD for proteins is ~500 pg. Considering the complexity of most samples , it is probably necessary to inject at least 100 µg total protein. However, the recommended range of 1-5 mg of protein will maximize detection of low abundance proteins.

Q: What is the maximum quantity of sample that can be loaded?
A: Up to 5 mg of total protein is recommended. Injection of >5 mg protein may result in loss of resolution and/or clogging of the first dimension chromatofocusing column.

Q: What are the limits of the types of proteins, i.e., basic, membrane bound, small and low abundance, that can be fractionated?

A: The ProteomeLab PF 2D system works ideally with proteins <150 kD. It has particularly high resolution (as compared to 2D gels) for proteins <80kD. Because of the nature of membrane proteins, there is a possibility that some membrane proteins will not be soluble in the Start Buffer for the chromatofocusing dimension.

Chemistry

Q: If pH is overshot with iminodiacetic acid for the Start or Eluent Buffer, what can be used to adjust back?
A: Use 1M NH_4OH.

Q: What is the composition of the chromatofocusing buffers?
A: The composition of the buffers is proprietary information. The buffers contain little salt. They were designed to have high buffering capacity but low ionic strength. This is best for use with the HPCF column.

Q: What is the chemistry used in the HPCF column?
A: That is proprietary information.

Q: Are there any corrosive reagents in the HPCF buffers?
A: No. However, if the HPCF buffers come in contact with eyes or skin, wash with water for 15 minutes and seek medical attention if pain or irritation occur. First aid measures and toxicology information can be found in the MSDS for each buffer.

Column filters

Q: Is it necessary to change the filters on the columns?
A: Not recommended.

Q: Are they available separately?
A: No

Q: How do you change them?
A: See above.

Column lifetime - HPCF

Q: What is the lifetime of the HPCF column?
A: It is guaranteed for 5 profiles.

Q: What is the lifetime of the HPRP column?
A: It is guaranteed for 5 profiles.

Q: How many experiments are possible with each kit?

A: It is guaranteed for 5 profiles.

Q: How many experiments are guaranteed?
A: See above.

Column storage

Q: How should HPCF column be stored?
A: The HPCF column can be stored for <2days in 100% water (with a low flow rate of 0.1 mL/min) and for >2 days in 100% isopropanol. The HPCF column should be stored at room temperature.

Q: How should HPRP column be stored?
A: The HPRP column can be stored for <2days in 100% of 0.08% TFA in acetonitrile and for >2 days in 100% acetonitrile. The HPRP column should be stored at room temperature.

Computer software

Q: Is there deconvolution software to resolve peaks in the second dimension?
A: No.

Q: If both UV and MS detection are used, can ProteoVue handle that?
A: Not at the present time. Other than a total ion chromatogram, which can be treated as any other ASCII chromatographic file.

Q: Why do basic fractions have high lane numbers in ProteoVue when they are collected first?
A: To compare with conventional slab gel format.

Q: What is the accuracy of DeltaVue for determination of a difference by subtraction for a peak between two samples?
A: There needs to be at least a ~ 20 % difference between a peak from the two samples to see a difference with DeltaVue.

Detection

Q: What is the minimum detectable amount of protein by UV in the second dimension?
A: The limit of detection at 214 nm, which is three times signal to noise based on peak height, is ~500 pg.

Q: What is the minimum detectable amount of protein by MS?

A: According to manufacturers of MS instrumentation, ~500 fmole. We have not yet confirmed this.

Q: What is the minimum detectable concentration?
A: This is not a meaningful question as chromatofocusing is a concentrating technique. 3.5 ml is the maximum volume for the buffer exchange so as long as you have 500 pg in the solution you should see it.

Q: What is the maximum detectable amount without signal distortion?
A: In the second dimension, between 25-50 μg per protein.

Denaturation

Q: Does this process denature the proteins?
A: Yes.

Q: Does chromatofocusing chromatography denature the proteins?
A: Yes.

Q: Does reversed phase chromatography denature the proteins?
A: Yes.

Q: Is it difficult to keep proteins in their native state?
A: The proteins are denatured but not digested. There is no indication about the ability to refold and become active after the second dimension.

Dynamic range

Q: What is the dynamic range of detection by UV and MS?
A: Dynamic range of detection is ~ 10^4 for both UV and MS.

Flow rate

Q: What is the optimal flow rate for the first dimension?
A: 0.2 ml/min

Q: What is the maximum flow rate for the first dimension?
A: 0.2 ml/min

Q: What is the effect of working at more than optimal flow?
A: Loss of resolution and premature column degradation

What is the optimal flow rate for the second dimension?

A: 0.75 ml/min

Q: What is the maximum flow rate for the second dimension?
A: 0.75 ml/min

Q: Is it possible to sacrifice resolution for speed in the first dimension and recover it in the second dimension by reducing the flow rate or flattening the gradient?
A: Not recommended.

Fraction collection

Q: What is the volume of the fractions from the first dimension?
A: During timed collection, 1.0 mL and during pH collection between 0.45 mL and 0.99 mL.

Q: What is the volume of the fractions from the second dimension?
A: If collected by peak threshold, the range of fraction volumes is between 0.1 and 0.6 mL.

Q: What type of fraction collector should be used for the second dimension?
A: Whatever is appropriate, which depends on what is planned for the fractions. We recommend either the SC100 for limited collection or the Gilson FC204 for multi-plate collection.

Fraction storage

Q: How do you store fractions?
A: Freeze and store at -80 °C. If this is not available, -20 °C an be used with a non-frost free freezer.

Injection

Q: What is the recommended injection volume for the first dimension?
A: Depends on sample concentration but will typically be between 0.5 and 2 mL.

Q: What is the recommended injection volume for the second dimension?
A: Typically 0.2 mL injections are used for the second dimension.

Other

Q: Can you use a total ion chromatogram in place of UV for mapping?
A: Yes if it used to create a 32 K chromatogram file

Q: Can we use Fingertight fittings and PEEK tubing?
A: Fingertight fittings are used where appropriate. However, because of the possible back pressures on the system, stainless steel tubing is used. Do not use PEEK tubing.

Q: I am used to separating proteins based on molecular weight. Will I get this information with the ProteomeLab PF 2D system?
A: Hydrophobicity, which is the basis of separation in the second dimension, is a function of molecular weight but a direct comparison cannot be made. Once the protein is separated on the ProteomeLab PF 2D system, mass information can be obtained with MS analysis or SDS MW analysis with the ProteomeLab PA 800 can provide this. Moreover, the differential protein expression has much higher resolution with the reversed-phase chromatography technology of the ProteomeLab PF 2D system as compared to molecular weight separation in gels.

Q: What is the amount of labor time required to produce a two dimensional, pI/UV, map with the ProteomeLab PF 2D system?
A: Approximately three to four hours of "hands-on" time is required of a researcher to produce a two dimensional map.

Q: What skill level is required to run the ProteomeLab PF 2D system?
A: Virtually any scientist can run this system. Protocols have been provided that need to be followed step by step and the software of the system contains the method needed to run the hardware and obtain the data.

Q: What instruments are required to run my samples?
A: The ProteomeLab PF 2D has been designed as an integrated system of chemistry hardware and software that performs the technology to produce a two dimensional protein map.

pH range

Q: What pH range can be explored?
A: pH 8.5 to 4.0

Q: Could we go below pH 4?
A: Not at present.

Q: What is the upper pH limit of the HPCF column?
A: pH 8.5.

Pressure

Q: What is the normal pressure for a new HPCF column?

A: 0.5 to 1.2 kpsi.

Q: How does it change?
A: It will increase during use and should not exceed 2.0 kpsi

Q: What is the normal pressure for a new HPRP column?
A: 1.8-2.2 kpsi.

Q: How does it change?
A: Typically it may increase during use but unless performance deteriorates, there is no limit for the column.

Protein complexes

Q: Can complexes be fractionated by PF 2D?
A: Possibly, however the denaturing condition of the buffers and solvents currently used makes this unlikely.

Quantitation

Q: Could we quantify or semi quantify the proteins?
A: Yes.

Q: How?
A: Use integration features of 32 Karat software.

Q: Is an internal standard required?
A: No, instead one would normalize a peak's area to that of the total protein content.

Recovery

Q: What is the protein recovery from the first dimension?
A. > 95 %. (Lubman, et. al., Anal. Chem. **75**:2299-2308, 2003)

Q: Is there an exclusion effect in the first dimension?
A. Pore size is 300 angstroms so should be minimal.

Q: What is the protein recovery from the second dimension?
A. Between 60-90 %, which depends on the properties of the protein, e.g., hydrophilic proteins less than 40 kD have higher recoveries. (Wall, et. al., Anal. Chem. **71**:3894-3900, 1999)

Resolution

Q: What is the resolution of the chromatofocusing technique?
A: The resolution of the chromatofocusing column is within 0.2 pI units.

Reversed-phase modifiers

Q: Is trifluoroacetic acid a problem for MS?
A: Depends on concentration and mode of interface.

Q: If so, how can it be removed?
A: By evaporation.

Q: Can you use formic or acetic acid as modifiers in the second dimension?
A: Yes.

Q: How do they affect performance?
A: These could give inferior resolution.

Sample preparation

Q: How can we avoid losing proteins below 5kD on the PD 10 columns?
A: The PD cartridge can be obtained empty and packed with other grades of Sephadex that have a lower molecular weight cut-off (MWCO): G-15 with a 1.5 kD MWCO or G-10 with a 0.75 kD MWCO. It is essential to de-salt samples.

Q: Ampholytes aren't compatible with MS. When are they removed?
A: In the second dimension column.

Q: Can n-octylglucoside be avoided if there is no protein solubilization problem?
A: Yes.

Q: Is it necessary to filter my samples before running them? What about interfering salts and other components present in my samples?
A: A lysis protocol has been written, which addresses these challenges. A buffer exchange step is used for interfering components in the samples.

Temperature

Q: Is a cooled fraction collector required?
A: Yes, in order to minimize degradation of the proteins during fraction collection of the first dimension. This is standard with the system for the FC/I module.

Q: Is a cooled autosampler required?
A: Yes, because the injection into second dimension has a cycle time of 45 minutes between injections. Standard with the system for the FC/I module.

Q: What about operating temperature in the first dimension?
A: The first dimension can be run at ambient. It is not recommended to run the first dimension chromatofocusing in a chilled room as the buffers might precipitate. Also, the lysed sample is denatured with protease inhibitor, which prevents proteolysis, so there is little reason to run the first dimension chilled.

Q: What about operating temperature in the second dimension?
A: The second dimension is run at a column temperature of 50°C as provided with the system column heater.

Q: Should the column oven be turned off if there is no flow through the HPRP column?
A: Yes. It is not recommended to let the HPRP column stand at elevated temperatures without flow.