Application of the ProteomeLab™ PF2D protein fractionation system in proteomic analysis for human genetic diseases

Li Wang¹,², Nanbert Zhong¹,²,³,*

¹Peking University Center of Medical Genetics, Peking University, Health Science Center, Beijing, China
²Department of Medical Genetics, Peking University, Health Science Center, Beijing, China
³Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA

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Abstract: Proteomic analysis has been widely used in elucidating the mechanism of diseases. As a classical proteomic approach, two-dimensional gel electrophoresis (2DGE) has been commonly applied in finding differentially expressed proteins through a first dimension of separation by the isoelectric point (pI) of proteins and a second dimension of separation according to the molecular weight (MW) of proteins. Compared to 2DGE, a recently developed commercial system from Beckman Coulter, the two-dimensional protein fractionation (PF2D), separates proteins according to the pI of proteins in the first dimension followed by a second dimension of separation according to the degree of protein hydrophobicity. As a liquid-based fractionation system, PF2D could facilitate the extraction and separation of broader protein categories and improve reproducibility and quantification as well as be less labor-intensive, which are usually identified as limitations of a gel-based 2DGE platform. This review evaluates the applications of the PF2D system and discusses the perspectives and advantages of PF2D in the investigation of cancer and genetic disorders and in protein mapping in human biological fluids and cell cultures.

Keywords: ProteomeLab™ PF2D Protein Fractionation System, • Cancer research • Pediatric neurodegenerative disorder • Premature aging disease

1. Introduction

As the basis of modern two-dimensional (2D) electrophoresis, 2D polyacrylamide gel electrophoresis (2D-PAGE), first introduced by O’Farrell in 1975 for separating cellular proteins under denaturing conditions, enabled the resolution of hundreds of proteins [1]. Since then, 2D gel electrophoresis (2DGE) has been widely accepted and greatly improved [2-6]. Generally, this technique separates proteins into two dimensions, according to two independent properties of proteins: the first dimension is isoelectric focusing (IEF), which separates proteins according to their isoelectric points (pI); the second dimension is a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which separates proteins according to their molecular weights (MWs). In this way, complex mixtures consisting of thousands of different proteins can be resolved into discrete spots, and the relative amount of each protein can be determined. Combined with mass spectrometry (MS) technology [7], resolved proteins could be further identified and characterized with an accurate MW.

2DGE is a well-established technique and is considered one of the most powerful methods for conducting proteomic studies, i.e., the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples such as sera, plasma, amniotic fluid, ascites, saliva, urine, etc., to look for novel
biomarkers and investigate disease pathogenesis [8-13]. However, severe limitations have greatly restricted the application of 2DGE, such as lack of reproducibility, difficulty in automating, poor resolution for proteins of extreme hydrophobicity, and limited pI ranges [14,15]. In addition, proteins are embedded in the gel, requiring labor-intensive procedures to excise the spots for further analysis by mass spectrometry.

Multidimensional liquid chromatography (MDLC) coupled with MS has subsequently emerged as the technique of choice for large-scale protein studies due to its superior throughput and sensitivity [16]. Generally, MDLC proteomic methods have focused on separating, by high pressure liquid chromatography (HPLC), complex mixtures of peptides obtained following digestion of the proteome, which are then fragmented and sequenced through MS to identify the parent proteins. This peptide-based MDLC analysis, also called bottom-up proteomics, shows inherent selectivity and sensitivity, and is widely used in most laboratories [16].

Peptide-based MDLC is also restricted by a few major drawbacks [17]. This proteomic analysis is performed at the peptide level and requires sample proteolysis with enzymes, which is time-consuming. Moreover, huge amounts of peptides generated from protein complexes make it difficult for the current separation methods to resolve so many components in a single analytical dimension prior to the MS analysis, which subsequently results in the reduced efficiency and accuracy of MS analysis. Shared peptides interference problem is another challenge in bottom-up proteomics and can lead to ambiguities in determining the identities of proteins in the sample [18]. The major fundamental drawback is that peptide-base MDLC cannot address the post-translational modifications (PTMs) of proteins, as protein information such as identification and quantification is all deduced from the detected peptides. PTMs of proteins such as glycosylation or phosphorylation may lead to functional modification. Therefore, fractionating complex protein mixtures while maintaining intact proteins in the liquid phase is a most desirable feature for use in further analyses. For these reasons, an alternative approach, top-down proteomics, has attracted attention in the last few years [19-22]. Top-down proteomics allows intact protein molecular ions to be separated directly by liquid chromatography (LC) and then analyzed by the mass spectrometer. The top-down strategy has the potential to identify a larger fraction of protein sequences and the ability to locate and characterize PTMs.

Recently, a newcomer in top-down proteomics platforms and also two-dimensional LC (2D-LC) from Beckman Coulter, the Proteome-Lab™ PF2D platform (PF2D), has shown advantages in circumventing some limitations of fractionation using 2DGE [23,24] and MDLC.

As shown in Fig. 1, PF2D is an automated fractionation platform that separates intact proteins in the first dimension according to their pI using chromatofocusing based on charge, followed by fractionation according to hydrophobicity, using nonporous reversed-phase chromatography in the second dimension. In combination with ProteoVue and DeltaVue software, a highly detailed pI versus hydrophobicity protein expression map can be established in a 2-D “lane and band” format. Both dimensions of analysis use standard HPLC equipment designed to reproducibly handle large numbers of samples in the liquid phase. This equipment also uses UV absorption for detection so that quantitative comparisons of protein expression can be performed between samples. The signal of the first dimension was recorded at 280 nm, and the signal of the second dimension was recorded at 214 nm. The pH gradient was generated using start buffer (pH 8.5) and elution buffer (pH 4), which meant that the general pH range of PF2D is from 4 to 8.5 [23]. Lee et al. established an efficient PF2D system for analysis and rapid semi-quantification of membrane proteins present in a wide pH range (4.0-10.5) by adjusting the pH of start buffer to 10.5 and adjusting the elution buffer to pH 7.0 [25]. In addition, this system also allows the separation of particular proteins with 9-40 kDa MW [26].

PF2D facilitates the extraction and separation of membrane-associated proteins, allows broader pI (4.0-10.5) and MW ranges, and improves run-run reproducibility. PF2D also provides a precise quantification of protein and is highly automated and seamless in association with mass spectrometry (MS) for further identification. Thus, PF2D enhances the precise detection of isofoms or PTMs that can alter the pI or hydrophobicity of a protein. Also, by resolving protein complexes on the basis of their intact characteristics prior to mass spectrometry analysis, PF2D allows a high degree of sequence coverage for more accurate identification in the case of PTMs. This is in contrast to peptide-based MDLC, in which protein identifications can be made on a single observed peptide with no information about the nature of the parent protein. Several recent proteomic studies have shown that a 2D-LC platform allows more efficient identification of proteins than 2D gels and may be more applicable for novel protein discovery [27,28]. Coupled with direct online MS analysis, PF2D facilitates the effective implementation of a 2D-LC system and has been used increasingly in proteomic studies, including human diseases, especially in research on cancer and genetic disorders. This review consists of three sections that