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A Robust Two-Dimensional Separation of Intact Proteins for Bottom-Up Tandem Mass Spectrometry of the Human CSF Proteome

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Abstract

The cerebrospinal fluid (CSF) is produced in the brain by cells in the choroid plexus at a rate of 500mL/day. It is the only body fluid in direct contact with the brain. Thus, any changes in the CSF composition will reflect pathological processes and make CSF a potential source of biomarkers for different disease states. Proteomics offers a comprehensive view of the proteins found in CSF. In this study, we use a recently developed non-gel based method of sample preparation of CSF followed by liquid chromatography high accuracy mass spectrometry (LC-MS) for MS and MS/MS analyses, allowing unambiguous identification of peptides/proteins. Gel-eluted liquid fraction entrapment electrophoresis (Gelfree) is used to separate a CSF complex protein mixture in 12 user-selectable liquid-phase molecular weight fractions. Using this high throughput workflow we have been able to separate CSF intact proteins over a broad mass range 3.5 kDa-100 kDa with high resolution between 15 kDa and 100 kDa in 2 hours and 40 min. We have completely eliminated albumin and were able to interrogate the low abundance CSF proteins in a highly reproducible manner from different CSF samples in the same time. Using LC-MS as a downstream analysis, we identified 368 proteins using MidiTrap G-10 desalting columns and 166 proteins (including 57 unique proteins) using Zeba spin columns with 5% false discovery rate (FDR). Prostaglandin D2 synthase, Chromogranin A, Apolipoprotein E, Chromogranin B, Secretogranin III, Cystatin C, VGF nerve growth factor, Cadherin 2 are a few of the proteins that were characterized. The Gelfree-LC-MS is a robust method for the analysis of the human proteome that we will use to develop biomarkers for several neurodegenerative diseases and to quantitate these markers using multiple reaction monitoring.

Introduction

Cerebrospinal fluid (CSF) is produced mainly by the cells found in the choroid plexus at the rate of 500 ml/day and contains approximately 0.3% plasma proteins, mainly albumin and immunoglobulins.[1–15]. CSF functions are mainly for neuroprotection, buoyancy and chemical stability. Found in direct contact with the brain, CSF is a potential indicator of abnormal central nervous system states such as inflammation, infection, neurodegeneration and tumor growth. There is a huge advantage in knowing which proteins are present, absent or change concentrations in the CSF. These proteins not only guide diagnosis or disease prognosis but also give valuable insights regarding the pathophysiology of these diseases.

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Proteomics studies have shown that we can identify not just one but a large number of proteins in a single experiment. In our laboratory there is a specific interest in utilizing the CSF proteome for a number of neurology-based diseases or conditions, including multiple sclerosis, Parkinson's disease and HIV-associated dementia. However, there are several challenges that must be solved in proteome analysis such as: the vast number of proteins, the dynamic range, the variation in protein levels depending on gene expression, the wide variety of post-translational modifications, the relative abundance of protein components, the intrinsic protein properties such as hydrophobicity, size, charge, chemical moiety and additionally the low abundance of signaling proteins. Therefore, it is of a great importance to develop methods that overcome all these limitations and allow an accurate qualitative and quantitative analysis.

While improvements in mass spectrometric instrumentation and on-line HPLC separation methods are enabling high-throughput proteomics experiments, there continues to be a need for a more effective protein separation method for reducing the high-complexity of the initial protein samples. Currently there are several orthogonal, multidimensional separation strategies that are used for proteome fractionation. Charge-based protein separation is usually combined with initial separations based on protein size, hydrophobicity, or affinity. Cation-exchange chromatography followed by reverse phase liquid chromatography (RPLC) is a two dimensional LC system that separates based on charge and hydrophobicity, and used to characterize mixtures of proteins and peptides. [6, 7] An integrated protein separation system has been used based on isoelectric focusing (IEF) and CE (capillary electrophoresis).[8] A solution isoelectric focusing (sIEF) based separation platform was recently tested to separate intact proteins.[9] Other research groups have used IEF followed by RPLC to resolve a complex sample of cellular proteins. [10] Ion exchange chromatography (IEC) has been an effective separation approach for peptide mixtures. In the case of proteins, IEC presents some limitations in pH range. [11] Size-based proteome separation complements existing chromatographic platforms that are generally used on-line with the mass spectrometer. Frequently used in combination with RPLC, size-exclusion chromatography (SEC) separates proteins in solution based on their size but not their mass. [10, 12] A two-dimensional separation system has been developed to study proteins consisting of SEC and capillary zone electrophoresis (CZE). [13] SEC was used successfully to separate brain proteins using a filter-assisted sample preparation method. [14] SEC is especially useful as an effective step to separate the impurities or protein aggregates from the components of interest. However, it has a low resolution compared with other chromatographic methods. [15, 16] An important intrinsic protein property is molecular weight. The best known and most used molecular weight-based separation is two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), which separates proteins by isoelectric point (IP) and molecular weight (MW). [17–19] This method is used in multiple studies directly followed by MS or combined with RPLC. [19] Despite the high degree of resolution, the 2 D-PAGE has limitations regarding low-abundance proteins, proteins with extreme pI values and size and in addition, it has a low protein recovery. [20, 21] As an alternative, an in-solution size separation platform, gel-eluted liquid fractionation entrapment electrophoresis (Gelfree) has been developed to study complex biological samples.[15, 22] This is a high-throughput technique that separates intact proteins over a broad mass range ensuring fast separation, sample enrichment, reproducibility, and unbiased high protein recovery. In this study, we analyze human CSF using the Gelfree system followed by LC- MS on a high performance LTQ/Orbitrap mass spectrometer. Using this workflow we have been able to separate CSF intact proteins from different samples in the 3.5kDa–100kDa mass range with high resolution between 15kDa and 100kDa in 2 hour and 40 min. We have completely eliminated albumin and we were able to interrogate the low abundance CSF proteins in a highly reproducible manner. Using LC-MS as a downstream analysis we identified with high confidence 112 proteins from PD-10 desalting columns and

spin columns. Prostaglandin D2 synthase, Chromogranin A, Apolipoprotein E, Chromogranin B, Secretogranin III, Cystatin C, VGF nerve growth factor, Cadherin 2 were a few of the proteins that were characterized with above 50% coverage.

Material and Methods

Solutions, Reagents, and Protein Standards

All proteins standards including trypsin as well as formic acid, ammonium bicarbonate, dithiothreitol (DTT), iodoacetamide were purchased from Sigma (St. Louis, MO). Milli-Q water grade was purified to 18 MΩ cm^{-1}.

Cerebrospinal Fluid

The CSF specimens were collected by spinal tap for diagnostic purpose and cell-free CSF (cells were removed by centrifugation at 3000 rpm for 10 min) was aliquoted and stored immediately at −80 C. The patients presented with mild neuroinflammatory diseases. In this study, we used 1 mL of clear unpooled CSF per experiment. All samples underwent a single freeze thaw cycle. The samples were provided by the CSF bank from the Neurology Department at the Johns Hopkins Medical Institutions.

CSF sample preparation

The CSF samples were desalted and cleaned up using PD MidiTrap G-10 with 700 Da cutoffs (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) or Zeba desalting columns with 7 kDa molecular weight cutoffs (MWCO). All samples were prepared according to the protocol provided by the desalting columns. 50 mM ammonium bicarbonate was used as buffer exchange. The desalted proteins were then separated by molecular weight using the Gelfree 8100 system (Expedon/Protein Discovery, San Diego CA). 100–150 μg of proteins were measured and loaded into the Gelfree chamber for separation using the 10% Tris-Acetate cartridge (Protein Discovery, Knoxville, TN) with a mass range 3.5 – 100 kDa, and resolution between 15 kDa and 100 kDa. The system can separate up to 8 different biological samples in the same time. The samples are loaded in different loading chambers and run in different channels so that there is no carryover or cross contamination. Samples were collected in 12 in-solution fractions over 2.4–3 hours, each fraction containing a volume of 150 μL. In order to visualize the separation we used 4–20% Tris-Glycine Invitrogen (Carlsbad, CA) Silver Staining for Mass Spectrometry. Further details on operation of the Gelfree 8100 system and sample preparation can be obtained from Witkowski and Harkins, 2009.[23] After separation, we removed the 0.1% SDS using the detergent removal spin column (Pierce, Rockford, IL). 50 mM Ammonium bicarbonate was used as buffer exchange. After SDS removal, samples were concentrated until dry and reconstituted with 100 μL 50 mM ammonium bicarbonate. Proteins were reduced with 0.5 μL of 1 M DTT (60°C for 30 min), then alkylated with 1.5 μL of 1 M iodoacetamide (room temperature in dark for 30 min) and digested overnight with trypsin in a ratio of 1:20 protein: trypsin in each vial.

Liquid Chromatography/Mass Spectrometry analysis of Gelfree fractions

Peptide fractions were separated by an online Eksigent Technologies (Dublin, CA) NanoLC-2D reverse-phase liquid chromatograph (LC) interfaced to an electrospray tandem mass spectrometer. The samples were injected onto 10 cm fused silica capillary column emitter (assembled in-house) with an inner diameter of 75 μm, and packed with Pur C_{18} material (YMC Co, Ltd. Kyoto, Japan). 10 μL sample volume at a flow rate of 5 μL/min was injected on a 40μL sample loop over a period of 70 min retention time. The gradient from 0.1% formic acid in water (solvent A) to acetonitrile (ACN) with 0.1% formic acid (solvent

B) was as follows: 10 min flush column with 5% solvent B, increase to 40 % solvent B over 30 min time period, increase to 95% solvent B over 10 min time period, hold at 95% solvent B for 10 min, decrease solvent B to 5% over 10 min, hold at 5% solvent B for 10 min. A 60 min run of solvent A was used to flush the LC-MS system between each fraction to avoid any carryover from the previous sample. The electrospray ionization mass spectrometry (ESI-MS) experiments were carried using a LTQ-Orbitrap from Thermo Scientific (Thermo Scientific, San Jose, CA,) and operated in a positive mode with a spray voltage of +2.1 kV. The instrument was calibrated using caffeine (m/z 195), MRFA (m/z 524) and ultramark 1621 according to LTQ Orbitrap calibration protocol. For all studies the ion transfer tube was held at 200 °C. Resolution was set at 60,000 and the m/z range was set 200–4000. The spectra were analyzed using the acquisition software XCalibur 2.0.7 (Thermo Scientific, San Jose, CA). The MS/MS fragmentation was run in a data dependent mode selecting the 5 most abundant molecular ion masses in each chromatographic window) using collision induced dissociation (CID) with energy of 35 % in the ion trap analyzer (LTQ) and detection in the Orbitrap in centroid mode.

Data analysis

Raw files were uploaded into Proteome Discoverer (PD) 1.2 Software (Thermo Scientific, San Jose, CA, USA). The files were searched individually for each fraction and merged combining all fractions using multidimensional protein identification technology (MudPIT) scoring. The search parameters for dynamic post-translational modifications (PTMs) included oxidation of methionine and static PTMs included carbamidomethylation of cysteine. We used RefSeq_40_Complete database with the decoy option and one missed cleavage. Precursor mass tolerance was 15 ppm, and fragment mass tolerance was 0.8 Da. The peptides were filtered based on a cut-off score of 10 with a strict false discovery (FDR) of 1% and a relaxed FDR of 5%. After PD analysis the files were exported as proXml and uploaded into Protein Center for further statistical analysis.

Results and Discussion

In this study, we used the Gelfree system for proteome fractionation by molecular weight of CSF proteins prior to MS analysis in a workflow shown in Figure 1. Prior to using the separation system the samples were desalted using gravity flow columns or spin desalting columns to avoid the interference of salts with the electrophoretic current. Gravity flow columns removed salts, lipids, and products with a molecular weight lower than 700 Da. Figure 2 shows that there is no sample loss in the flow through that represents the washing solution. At this stage we can also observe an increase in protein concentration in the desalted sample lane, with albumin detected as the most prominent band. Further separation enabled us to completely eliminate albumin from our sample analysis and allowed detection of lower abundance proteins. After desalting the Gelfree system was used for intact protein molecular weight fractionation, isolation and purification allowing liquid-phase recovery without band or spot cutting. The system incorporates a 10% Tris-Acetate cartridge kit designed for separation in the mass range 3.5 – 100 kDa, with resolution between 15 kDa and 100 kDa. 100–150 μg of protein were loaded in the loading chamber after mixing with the sample buffer provided by the kit. When the electric current is applied, charged molecules migrate into the stacking gel and then separate according to their electrophoretic mobility. Figure 3 shows an aliquot of each of the combined or single Gelfree fractions from two CSF samples partitioned using the 10% cartridge kit and run out on a 1D gel for visualization. The two samples are separated and collected in the same time. An important advantage of this system is sample reproducibility. Fig. 3 shows the reproducibility of this technique. The fractions of CSF with a low concentration of proteins were combined for a better visualization on the 1D gel. Another advantage of this technique is that it is very robust, allowing the separation of 8 samples in parallel and each lane is collected in 12 in-

solution fractions. After collection of each fraction from the collection chamber, the samples contain a 0.1 % SDS. Because SDS suppresses ion signal in the mass spectrometer our workflow then includes SDS removal. In this study, we used resin containing columns to remove any detergent in our samples. As an alternative method for SDS removal other studies have successfully used chloroform/methanol/water precipitation protocol. [24]

Table 1 contains the number of proteins detected in each fraction. The number of proteins in each fraction of the CSF sample desalted using gravity flow columns is higher than the number of proteins detected in each fraction of the CSF sample desalted with spin column except fraction 12 which contains the same number of proteins. This can be due to the sample loss during the washing procedure that requires extra steps when spin desalting columns are used or the sample analytes remain bounded to the resin column. The complete listings of the proteins identified in each fraction, including the Mascot score, sequence coverage and peptide count, are presented in the two supplemental tables. As noticed in Figure 3, fractions 1–8 are completely albumin free while fractions 9–12 contain albumin in addition to other proteins. For a complete elimination of the albumin in these fractions further steps can be adopted in the protocol. However, the albumin content in these fractions did not impact the detection of other proteins (Table 1 and supplemental information). In Figure 3 the concentration of proteins visualized in lane 1 and 2 are at or below the limit of detection of the silver staining method (for silver staining the limit of detection is 1 ng proteins). Nevertheless, in Table 1 the number of proteins detected in fraction 1 and 2 is 40, respectively 39 for the first sample and 34, respectively 33 for the second sample. Hence, there is a clear advantage in combining this separation technique with mass spectrometry.

This is the first study that shows a multiplex molecular weight-based protein purification of CSF with liquid phase recovery combined with a bottom up MS analysis. A total of 425 proteins with 5% FDR and a p-value ≤ 0.06 were detected with MS using the Gelfree system. Our sample preparation includes different desalting procedures of CSF followed by intact protein separation. Using gravity flow columns we characterized 368 proteins (Figure 4). As an alternative method we also used spin desalting columns and we detected 166 proteins, including 57 not observed with the gravity flow columns. Both samples were prepared and analyzed in the same time. Proteins prepared the gravity flow columns outnumber the proteins desalted with spin desalting columns (Figure 4). The two procedures have different MWCO, 700 Da for gravity flow columns and 7 kDa for spin desalting columns, which explains the difference in the number of the detected proteins. There is an interesting correlation in the majority of proteins detected by both methods: the majority of them are cytoplasmic components (Figure 5a) and most of them have binding functions (Figure 5b). Apolipoprotein E, chromogranins, osteopontins, secretogranins, VGF nerve growth factor, prostaglandins, cystatin C, calmodulin are several families of proteins detected in both samples.

Several groups have adopted the MudPIT approach to study CSF. Wetterhall et al. studied CSF using shotgun proteomics, in combination with isoelectric focusing (IEF) and nano-LC/MS-MS identified 339 proteins ($p \leq 0.05$) in two different ventricular CSF samples from patients with traumatic brain injuries.[25] The samples were analyzed with a hybrid triple quadrupole/ion trap. The number of proteins detected in the samples varied as 265 in the first sample and 205 in the second sample. 130 proteins overlapped between samples. [25] A different research group examined three different affinity and immunoaffinity subtraction spin columns: Montage spin column, ProteomeLab column, and ProteoPrep column. [26]This method was investigated for the removal of the most abundant proteins in CSF. The samples were subjected to 1D gel electrophoresis and nanoLC-MALDI-TOF/TOF-MS. Overall, 173 proteins were identified on a 95% MudPIT confidence scoring level. For the intact CSF, 91 unique proteins were identified, 128 proteins for the Montage spin column,

123 proteins for the ProteomeLab IgY-12 spin column, and 104 proteins for the ProteoPrep 20 spin column. Capillary electrophoresis (CE), interfaced off-line to MALDI-TOF/TOF MS, was the first time used to monitor protein content in CSF with iTRAQTM labeling in patients with traumatic brain injury. [27] The analysis of ventricular CSF yielded to 43 significantly ($p \leq 0.05$ MudPIT scoring). Previously CSF has been characterized using a liquid phase isoelectric focusing in the Rotofor cell in combination with electroelution in the Mini whole cell gel eluter. [28] Using matrix-assisted laser desoption/ionization mass spectrometry (MALDI-TOF MS), 6 CSF proteins were characterized: transfferin, α1-acid-glycoprotein, Zn-α2-glycoprotein, apolipoprotein A1, apolipoprotein E and β-trace. Although this technique used a similar strategy of electroelution across the entire gel and fractionated CSF over a broad size-based separation, it has a low number of collected fractions that limits the flexibility to optimize resolution. In addition the system is not performing in a high-throughput format. In comparison, our study represents a robust proteomics approach of CSF analysis detecting hundreds of proteins.

Conclusion

In this study, our strategy of CSF analysis enables and introduces a robust and fast characterization of any complex protein human body fluid carrying intact endogenous proteins. The liquid phase is an asset that allows this method to be easily combined with affinity based selection for targeted proteomics approaches. At the same time, it offers MS flexibility that allows top down and bottom up analysis. In combination with non-label quantitation and bioinformatics workflows, the technique can be used as validation tool for disease specific biomarkers. Applying this technique will provide comprehensive and efficient solutions to characterize brain-specific proteins in CSF that will help us to investigate pathophysiology of different brain disorders. While the Gelfree system in this case has been applied to the analysis of proteins in cerebral spinal fluid, it can be expected to be applicable as well to more complex body fluids such as serum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Merril CR, et al. Ultrasensitive Stain for Proteins in Polyacrylamide Gels Shows Regional Variation in Cerebrospinal-Fluid Proteins. Science. 1981; 211(4489):1437–1438. [PubMed: 6162199]
2. Maurer MH, et al. The proteome of human brain microdialysate. Proteome science. 2003; 1(1):7. [PubMed: 14675487]
3. Ames A 3rd, Sakanoue M, Endo S. Na, K, Ca, Mg, and C1 Concentrations in Choroid Plexus Fluid and Cisternal Fluid Compared with Plasma Ultrafiltrate. Journal of neurophysiology. 1964; 27:672–81. [PubMed: 14194965]
4. Maurer MH. Proteomics of brain extracellular fluid (ECF) and cerebrospinal fluid (CSF). Mass spectrometry reviews. 2010; 29(1):17–28. [PubMed: 19116946]
5. Felgenhauer K. Protein size and cerebrospinal fluid composition. Klinische Wochenschrift. 1974; 52(24):1158–64. [PubMed: 4456012]
6. Opiteck GJ, et al. Comprehensive on-line LC/LC/MS of proteins. Analytical chemistry. 1997; 69(8): 1518–1524. [PubMed: 9109352]

7. MacNair JE, et al. Rapid separation and characterization of protein and peptide mixtures using 1.5 mu m diameter non-porous silica in packed capillary liquid chromatography mass spectrometry. Rapid Communications in Mass Spectrometry. 1997; 11(12):1279–1285. [PubMed: 9276975]
8. Wang YC, Choi MN, Han JY. Two-dimensional protein separation with advanced sample and buffer isolation using microfluidic valves. Analytical chemistry. 2004; 76(15):4426–4431. [PubMed: 15283583]
9. Doucette AA, Tran JC, Wall MJ. Evaluation of a solution isoelectric focusing protocol as an alternative to ion exchange chromatography for charge-based proteome prefractionation. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences. 2009; 877(8–9): 807–813.
10. Hanash S, Wang H. Multi-dimensional liquid phase based separations in proteomics. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences. 2003; 787(1): 11–18.
11. Ottens AK, et al. A multidimensional differential proteomic platform using dual-phase ion-exchange chromatography-polyacrylamide gel electrophoresis/reversed-phase liquid chromatography tandem mass spectrometry. Analytical chemistry. 2005; 77(15):4836–4845. [PubMed: 16053296]
12. Pasa-Tolic L, et al. Using size exclusion chromatography-RPLC and RPLC-CIEF as two-dimensional separation strategies for protein profiling. Electrophoresis. 2006; 27(13):2722–2733. [PubMed: 16732621]
13. Lemmo AV, Jorgenson JW. 2-Dimensional Protein Separation by Microcolumn Size-Exclusion Chromatography Capillary Zone Electrophoresis. Journal of Chromatography. 1993; 633(1–2): 213–220.
14. Wisniewski JR, et al. Brain Phosphoproteome Obtained by a FASP-Based Method Reveals Plasma Membrane Protein Topology. Journal of Proteome Research. 2010; 9(6):3280–3289. [PubMed: 20415495]
15. Doucette AA, Tran JC. Multiplexed Size Separation of Intact Proteins in Solution Phase for Mass Spectrometry. Analytical chemistry. 2009; 81(15):6201–6209. [PubMed: 19572727]
16. Buchacher A, et al. Improved performance of protein separation by continuous annular chromatography in the size-exclusion mode. Journal of Chromatography A. 2001; 921(1):15–24. [PubMed: 11461008]
17. Beeley JA, et al. Sodium Dodecyl Sulfate-Polyacrylamide Gel-Electrophoresis of Human Parotid Salivary Proteins. Electrophoresis. 1991; 12(12):1032–1041. [PubMed: 1815955]
18. Beeley JA, Khoo KS, Lamey PJ. 2-Dimensional Electrophoresis of Human Parotid Salivary Proteins from Normal and Connective-Tissue Disorder Subjects Using Immobilized Ph Gradients. Electrophoresis. 1991; 12(7–8):493–499. [PubMed: 1717250]
19. Fisher SJ, et al. Toward defining the human parotid gland salivary proteome and peptidome: Identification and characterization using 2D SDS-PAGE, ultrafiltration, HPLC, and mass spectrometry. Biochemistry. 2005; 44(8):2885–2899. [PubMed: 15723531]
20. Nagele E, et al. 2D-LC/MS techniques for the identification of proteins in highly complex mixtures. Expert Review of Proteomics. 2004; 1(1):37–46. [PubMed: 15966797]
21. Aebersold R, et al. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(17):9390–9395. [PubMed: 10920198]
22. Doucette, J.C.T.a.A.A. Gel-Eluted Liquid Fraction Entrapment Electrophoresis: An Electrophoretic Method for Broad Molecular Weight Range Proteome Separation. Analytical chemistry. 2008; (80):1568–1573. [PubMed: 18229945]
23. Witkowski C, Harkins J. Using the Gelfree 8100 Fractionation System for molecular weight-based fractionation with liquid phase recovery. Journal of visualized experiments : JoVE. 2009; (34)
24. Botelho D, et al. Top-down and bottom-up proteomics of SDS-containing solutions following mass-based separation. Journal of Proteome Research. 2010; 9(6):2863–70. [PubMed: 20377267]
25. Sjodin MO, Bergquist J, Wetterhall M. Mining ventricular cerebrospinal fluid from patients with traumatic brain injury using hexapeptide ligand libraries to search for trauma biomarkers. Journal

of chromatography. B, Analytical technologies in the biomedical and life sciences. 2010; 878(22): 2003–12.

26. Wetterhall M, et al. Assessment of the partitioning capacity of high abundant proteins in human cerebrospinal fluid using affinity and immunoaffinity subtraction spin columns. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 2010; 878(19): 1519–30.
27. Zuberovic A, et al. CE MALDI-TOF/TOF MS for multiplexed quantification of proteins in human ventricular cerebrospinal fluid. Electrophoresis. 2009; 30(10):1836–43. [PubMed: 19441030]
28. Davidsson P, Nilsson CL. Peptide mapping of proteins in cerebrospinal fluid utilizing a rapid preparative two-dimensional electrophoretic procedure and matrix-assisted laser desorption/ ionization mass spectrometry. Biochimica et biophysica acta. 1999; 1473(2–3):391–399. [PubMed: 10594376]

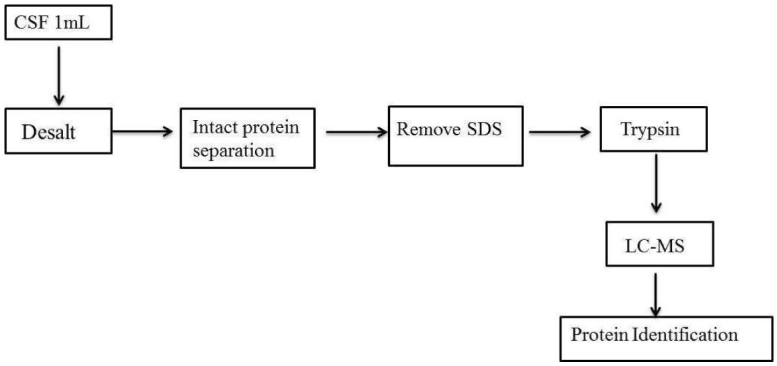

Figure 1.
Schematic representation of CSF sample preparation including the Gelfree protein separation system.

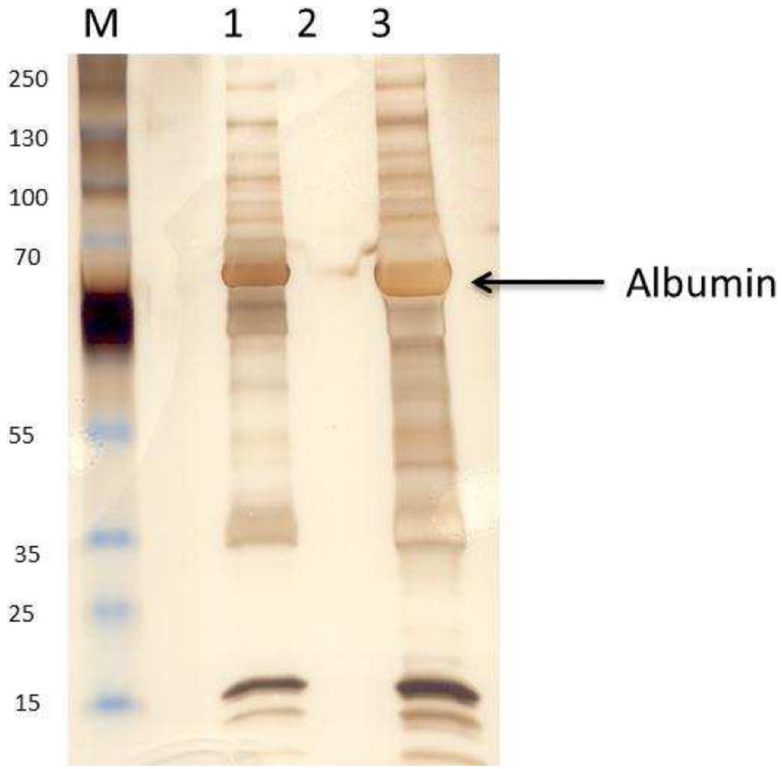

Figure 2.
Silver staining gel of: (M) protein marker, (1) undesalted CSf, (2) flow-through and (3) desalted CSF.

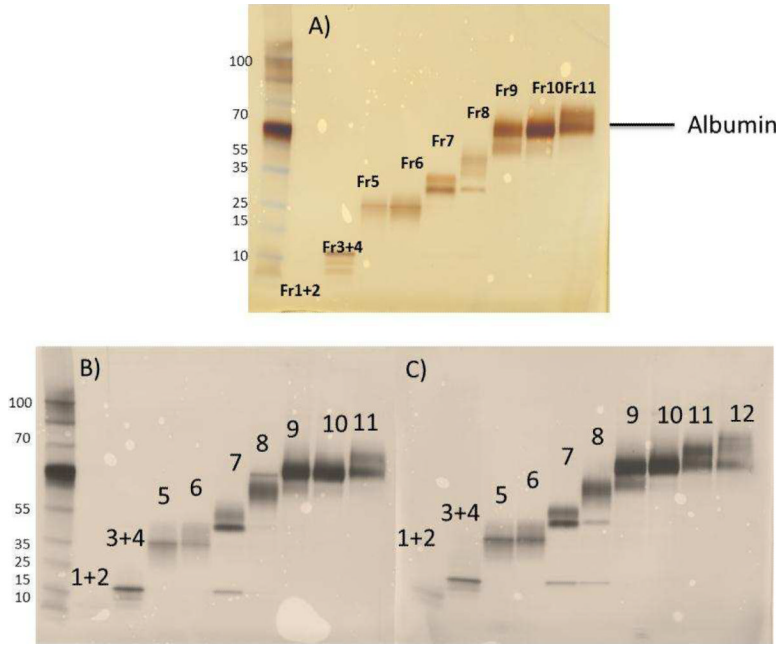

Figure 3.

(A) Silver stained images showing GelfrEE fractions for visualization of protein separation found in each fraction. (B) and (C) show the reproducibility of the separation technique for two additional aliquots separated on parallel channels.

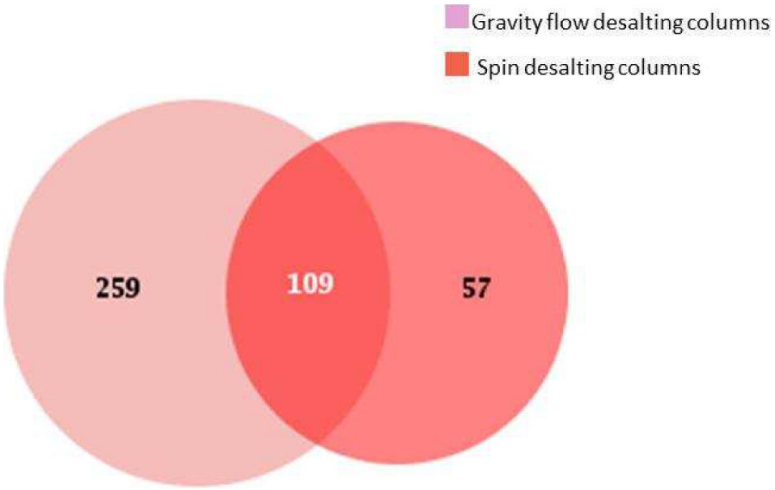

Figure 4.
Number of unique proteins characterized with GelFrEE system using two different desalting methods: gravity flow desalting columns and spin desalting columns.

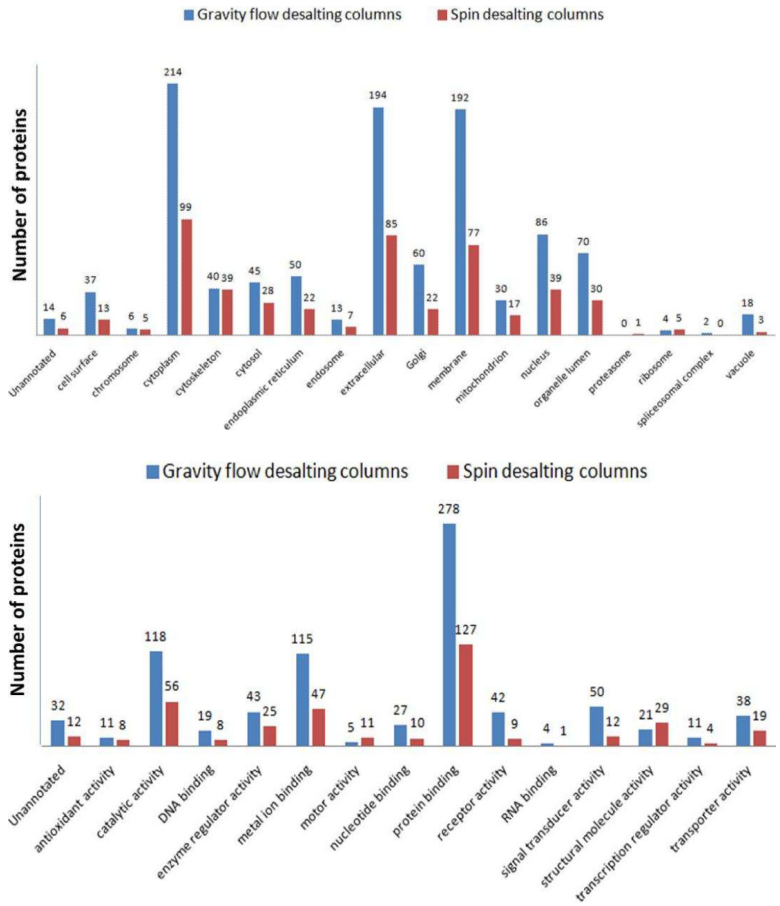

Figure 5.

(a) Cellular components of CSF proteins characterized with GelFrEE system, (b)Molecular functions of CSF proteins characterized with GelFrEE system.

Table 1

The number of proteins detected in each fraction using gravity flow desalting and spin desalting columns

Fraction No.	Gravity flow desalting columns	Spin desalting columns
1	39	34
2	37	33
3	84	53
4	135	67
5	160	99
6	108	37
7	90	28
8	87	29
9	66	23
10	103	22
11	124	93
12	28	12