Supporting Information

Brain extracellular fluid protein changes in acute stroke patients

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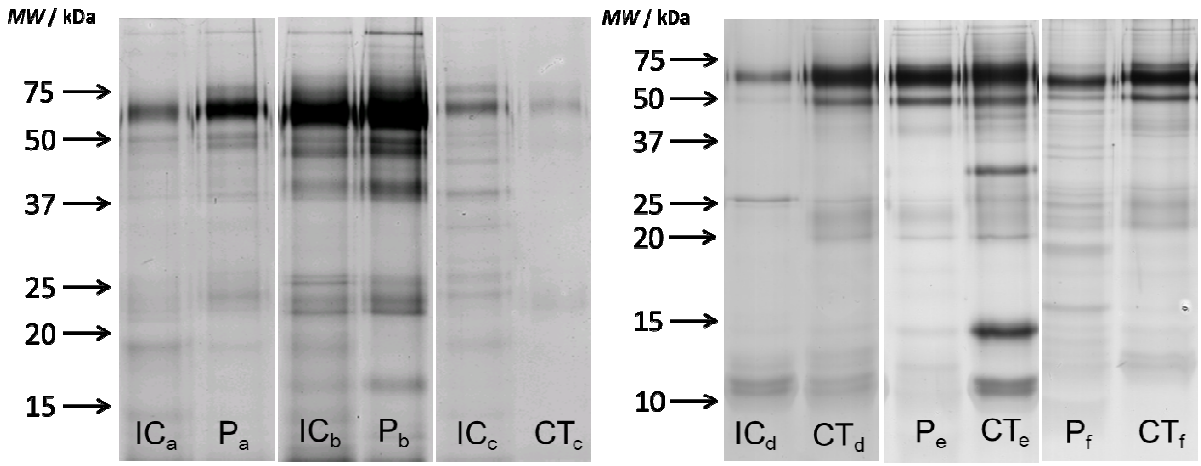
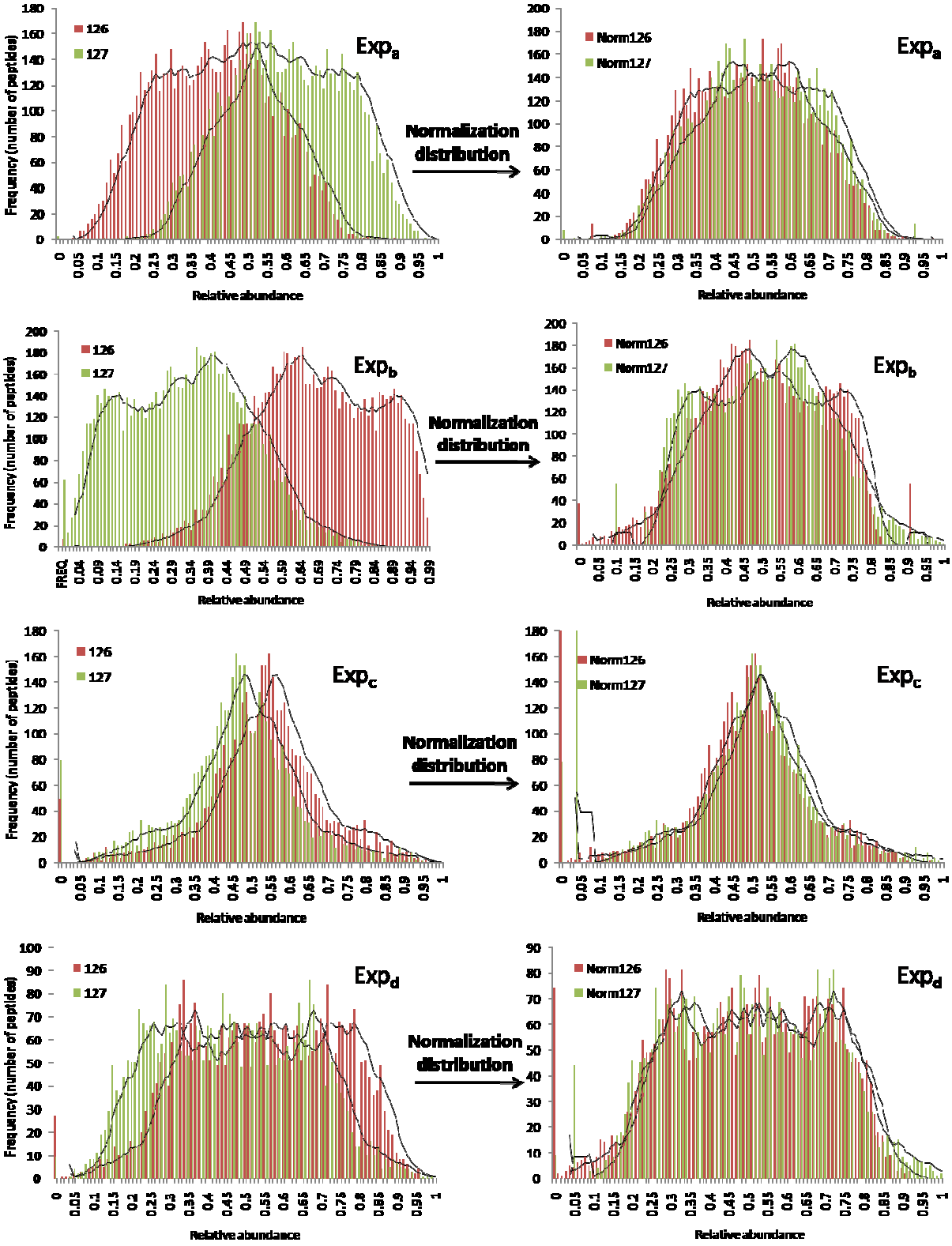
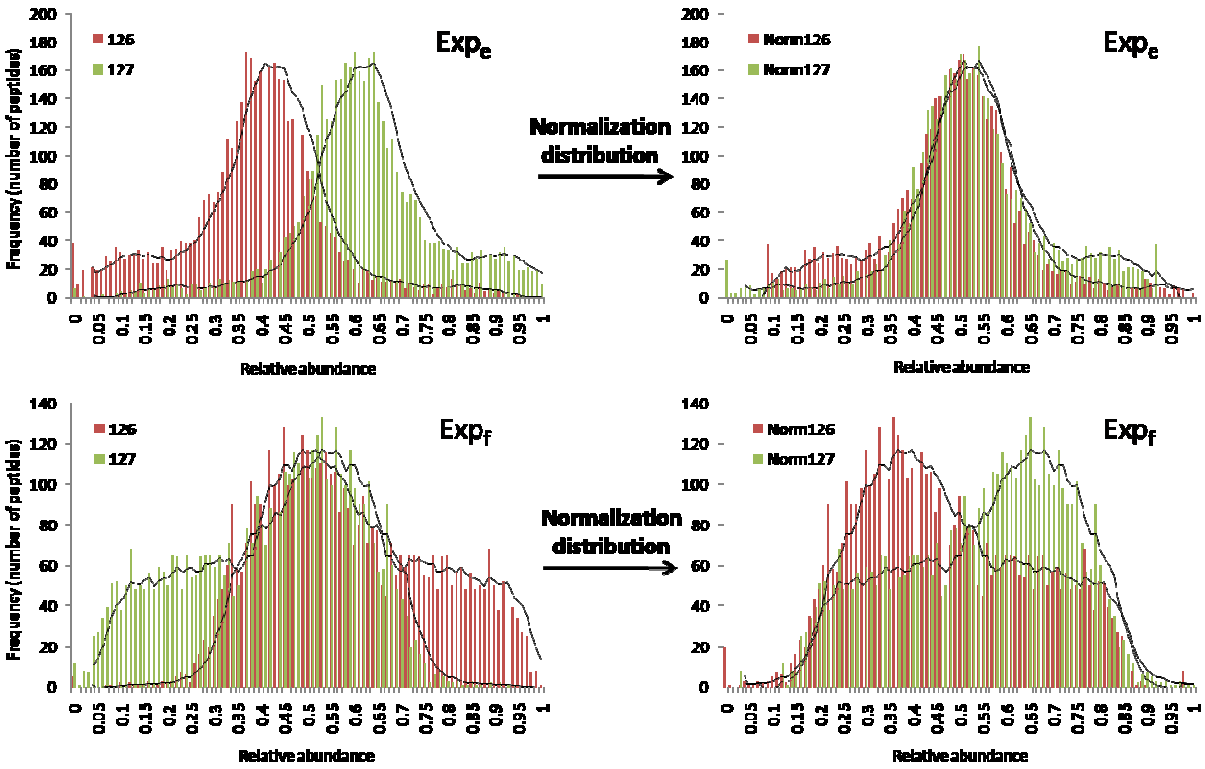
SI1.



SI1. Gel images of the MDs (n = 12) under study after separation with 1-D PAGE and silver staining. Ten µL of each MD was loaded on the gels.

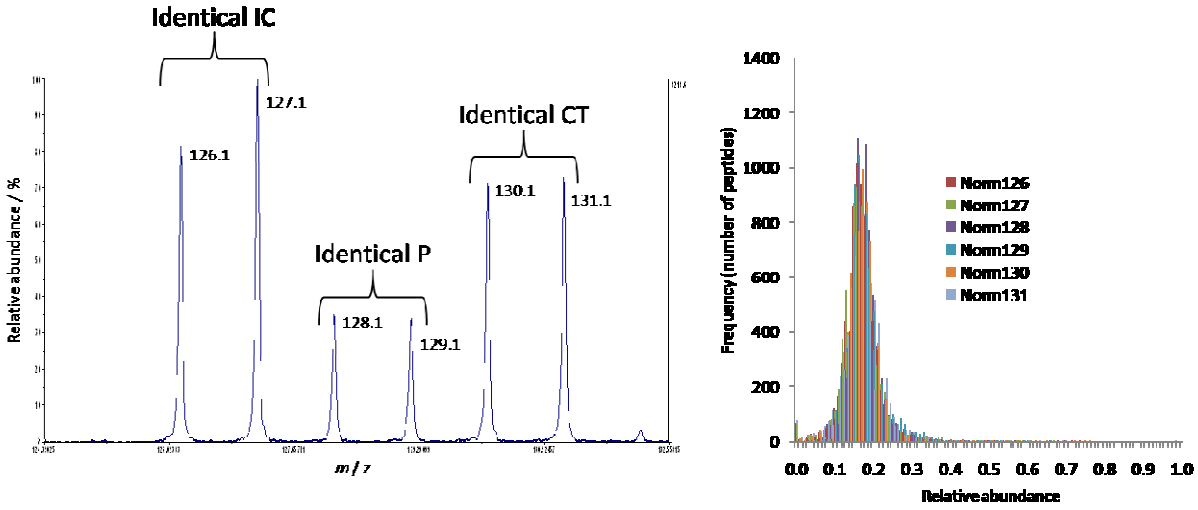
SI2.


126
127
Exp$_a$
Frequency (number of peptides)
Relative abundance
Normalization distribution
Norm126
Norm127
Exp$_a$
Relative abundance
126
127
Exp$_b$
Frequency (number of peptides)
FREQ
Relative abundance
Normalization distribution
Norm126
Norm127
Exp$_b$
Relative abundance
126
127
Exp$_c$
Frequency (number of peptides)
Relative abundance
Normalization distribution
Norm126
Norm127
Exp$_c$
Relative abundance
126
127
Exp$_d$
Frequency (number of peptides)
Relative abundance
Normalization distribution
Norm126
Norm127
Exp$_d$
Relative abundance



SI2. Distribution of relative abundance of TMT^2 reporter-ions for Exp$_{a\text{-}f}$ before and after final normalization steps. Basically, a translation was operated on the relative abundances for both data for reporter-ions at m/z = 126.1 (distribution in red) and 127.1 (distribution in green) in order that the common area between both distribution was maximal (*i.e.*, to minimize the quantitative differences between both populations). The normalization coefficients were obtained on the entire reporter-ion dataset (*i.e.*, even when peptides were not matched to any sequences).

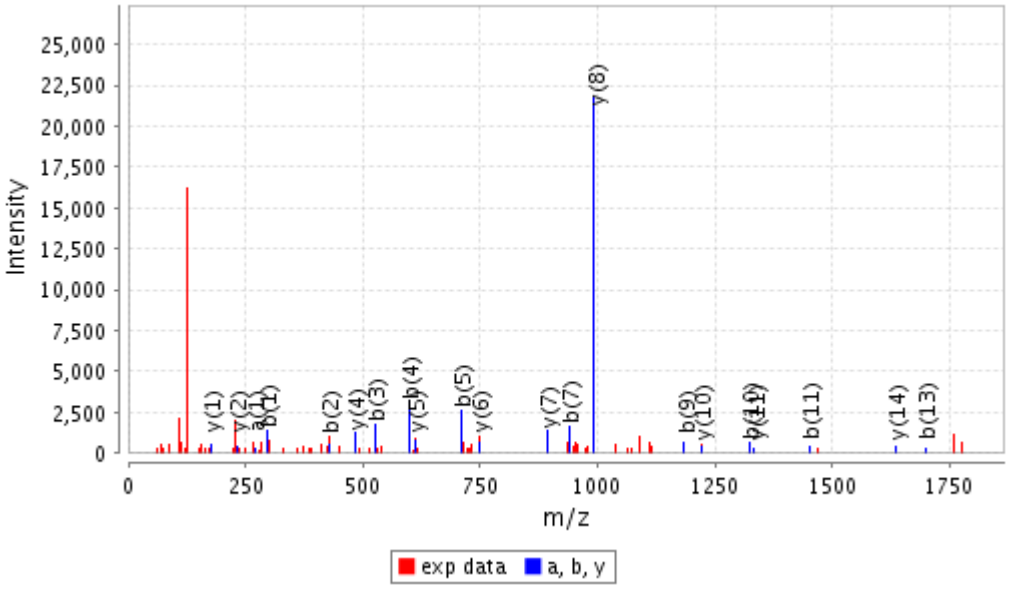
SI3.



	Average on 10 peptides	
False discovery rate	1%	5%
Lower cut-off	0.61	0.74
Higher cut-off	1.68	1.48

SI3. Experimental evaluation of the cut-off values to set for the TMT^2-based quantitative experiments of the human brain microdialysates. Following the experimental procedure detailed in the article (*i.e.*, tryptic digestion, isobaric tagging, IEF fractionation, and RP-LC MS/MS), TMT^6 were used to tag identical samples of IC, P, and CT microdialysates. Because no difference was expected between identical samples (*e.g.*, the two IC samples), deviations from 1:1 ratio were evaluated in term of false positive. The relative abundances provided in each TMT channel were mixed randomly. Ratios were then calculated between identical samples, and geometrical means were obtained from clusters of 10 ratio data points. These mean ratios were then used to evaluate the cut-off values at a given false positive rate. The final cut-off values were averaged from the IC, P, and CT results. To have symmetrical cut-off values at 1% FDR, 1.68 and 0.59 (instead of 0.61) cut-off ratios had to be chosen.

SI11.



SI11. Tandem mass spectrum matching AMVALIDVFHQYSGR peptide of protein S100-B.

SI12a. Decreased ratios IC/P in microdialysis samples.

Protein description	*Ratio IC/P (Exp_a)*	*Ratio IC/P (Exp_b)*
Alpha-1-acid glycoprotein 1 precursor	**0.48**	**0.47**
Alpha-1-antitrypsin precursor	0.61	0.40
Alpha-1B-glycoprotein precursor		0.46
Carbonic anhydrase 1	0.45	1.60
Ceruloplasmin precursor		0.44
Fibrinogen beta chain precursor		0.46
Fibrinogen gamma chain precursor		0.41
Haptoglobin precursor	**0.46**	**0.36**
Hemoglobin subunit alpha	0.34	0.70
Hemoglobin subunit beta	0.37	0.80
Ig kappa chain C region	0.51	0.28
Keratin, type I cytoskeletal 10	0.72	0.39
Serotransferrin precursor	**0.42**	**0.50**
Serum albumin precursor	**0.40**	**0.33**

Empty cases derive from the lack of the protein identification/quantification in the studied sample. The values reported in bold indicate significantly decreased ratios (*i.e.*, inferior to 0.5) for both patients (*i.e.*, patients a and b).

SI12b. Decreased ratios IC/CT in microdialysis samples.

Protein description	*Ratio IC/CT (Exp_c)*	*Ratio IC/CT (Exp_d)*
Alpha-1-antitrypsin precursor		0.49
Alpha-2-macroglobulin precursor		0.36
Beta-2-microglobulin precursor	0.80	0.44
Carbonic anhydrase 1	0.29	4.68
Chromogranin-A precursor	0.29	
Complement C3 precursor		0.43
Cystatin-C precursor	0.62	0.03
Dermcidin precursor	0.85	0.35
Fibrinogen alpha chain precursor	0.46	2.33
Fibrinogen beta chain precursor		0.38
Glial fibrillary acidic protein	4.13	0.37
Haptoglobin precursor	0.83	0.14
Ig gamma-2 chain C region		0.18
Keratin, type I cytoskeletal 10	0.99	0.48
Keratin, type II cytoskeletal 2 epidermal	0.97	0.43
Lambda-chain precursor		0.21
Myelin basic protein		0.30
Prostaglandin-H2 D-isomerase precursor	**0.27**	**0.46**
Putative uncharacterized protein		0.15
Serotransferrin precursor	1.47	0.42
Serum albumin precursor	1.17	0.38
SNC73 protein		0.30

Empty cases derive from the lack of the protein identification/quantification in the studied sample. The values reported in bold indicate significantly decreased ratios (*i.e.*, inferior to 0.5) for both patients (*i.e.*, patients c and d).

SI12c. Decreased ratios P/CT in microdialysis samples.

Protein description	*Ratio P/CT (Exp_e)*	*Ratio P/CT (Exp_f)*
Alpha-1-antitrypsin	1.05	0.35
Alpha-1-acid glycoprotein 1 precursor	1.38	0.38
Alpha-2-macroglobulin precursor	0.68	0.36
Alpha-enolase	0.45	
Apolipoprotein E precursor	0.30	
Beta-2-microglobulin	0.92	0.25
Beta-Ala-His dipeptidase precursor	0.32	
Ceruloplasmin precursor		0.45
Clusterin	0.49	
Complement component 3	0.72	0.48
Complement factor B precursor	1.15	0.36
Cystatin-C precursor	**0.17**	**0.38**
Dermcidin precursor		0.34
Ectonucleotide pyrophosphatase/phosphodiesterase family member 2 precursor	0.46	
Glial fibrillary acidic protein	0.31	2.33
Haptoglobin precursor		0.45
Hemoglobin subunit alpha	0.11	0.55
Hemoglobin subunit beta	0.11	0.59
Ig gamma-2 chain C region	0.87	0.22
Insulin-like growth factor-binding protein 7 precursor	0.02	
Kallikrein-6 precursor		0.41
Keratin, type II cytoskeletal 2 epidermal	1.08	0.33
Lambda-chain precursor	0.89	0.35
Neutrophil defensin 1 precursor	0.17	
Pigment epithelium-derived factor precursor	0.15	
Prostaglandin-H2 D-isomerase precursor	0.20	0.64
Putative uncharacterized protein DKFZp686I15212		0.37
Serotransferrin precursor	0.91	0.36

Empty cases derive from the lack of the protein identification/quantification in the studied sample. The values reported in bold indicate significantly decreased ratios (*i.e.*, inferior to 0.5) for both patients (*i.e.*, patients e and f).