Electronic Supplementary Information

Washing-free *In-situ* Self-desalting and Enriched Peptide Analysis by Block Copolymer in MALDI-TOFMS

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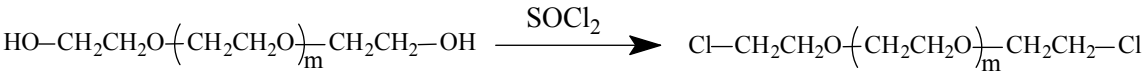
1. Synthesis and characterization validation of PSF-b-PEO

Materials

The 4,4'-Dichlorodiphenyl sulfone (DCPS) was purchased from Aldrich and purified by recrystallization from ethanol before use. Poly(ethylene oxide) (PEO) with an average molecular weight of 1500 was purchased from Acros. 4,4'-Dihydroxybiphenyl (DHBP) were purchased from TCI. PEO and DHBP were used without further purification. Other materials were purchased from Shanghai Chemicals Co. Thionyl chloride was purified by distillation prior to use. N,N-dimethylacetamide (DMAC) was distilled under reduced pressure and dried over a molecular sieve (type 4A) before use. Anhydrous potassium carbonate was further dried at 160 ºC for 10 h in a vacuum.

Synthesis of Chlorine-terminated PEO (Cl-PEO)

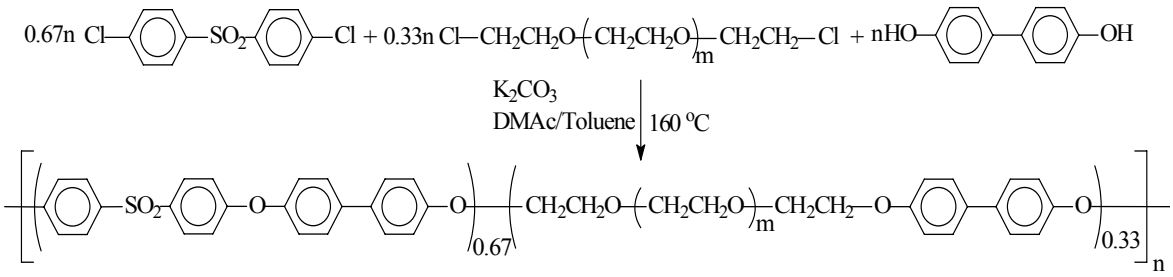
Scheme S1



To a dry 3-neck flask equipped with a condenser and a calcium chloride drying tube 15.0 g (10 mmol) of PEO was added, and the flask was heated till PEO melted. Then 3.0 mL of thionyl chloride was added to the flask and the mixture was stirred and heated at 80 ºC for 3 h and 120 ºC for 5 h. The flask was connected to a pump to remove the residual thionyl chloride. Pale-yellow product was obtained in a quantitative yield. The resulting product was characterized with fourier transform infrared (FT-IR) spectrum (KBr, cm-1): 2890, 1636, 1476, 1346, 1278, 1240, 1118, 962, 844, 668, 530. 1H NMR (in CDCl3), δ (ppm): 3.74 (4H), 3.69-3.60 (~120H). 13C NMR (in CDCl3), δ (ppm): 71.51, 70.81, 70.72, 42.89 (data not shown).

Polymerization

Scheme S2



PSF-*b*-PEO

To a dry 3-neck flask 0.2878 g (1.0 mmol) of DCPS, 0.75 g (0.5 mmol) of Cl-PEO,0.2793 g (1.5 mmol) of

DHBP, 0.25 g of K_2CO_3, 5 mL of DMAc and 4 mL of toluene were added under nitrogen flow. The reaction mixture was stirred and heated to reflux, and toluene and water were evaporated and collected in a Dean-Stark trap. Then the reaction temperature was raised to 160 ºC and the reaction was kept at this temperature for 20 h. After cooling to room temperature, the resulting highly viscous solution mixture was poured into water and the precipitate was collected by filtration, thoroughly washed with deionized water and dried in vacuum. The yield is quantitative. The resulting block copolymer was characterized with FT-IR and ^{1}H NMR spectrum (see supplementary Figure 1) and its molecular weight was also measured as shown in supplementary Table 1.

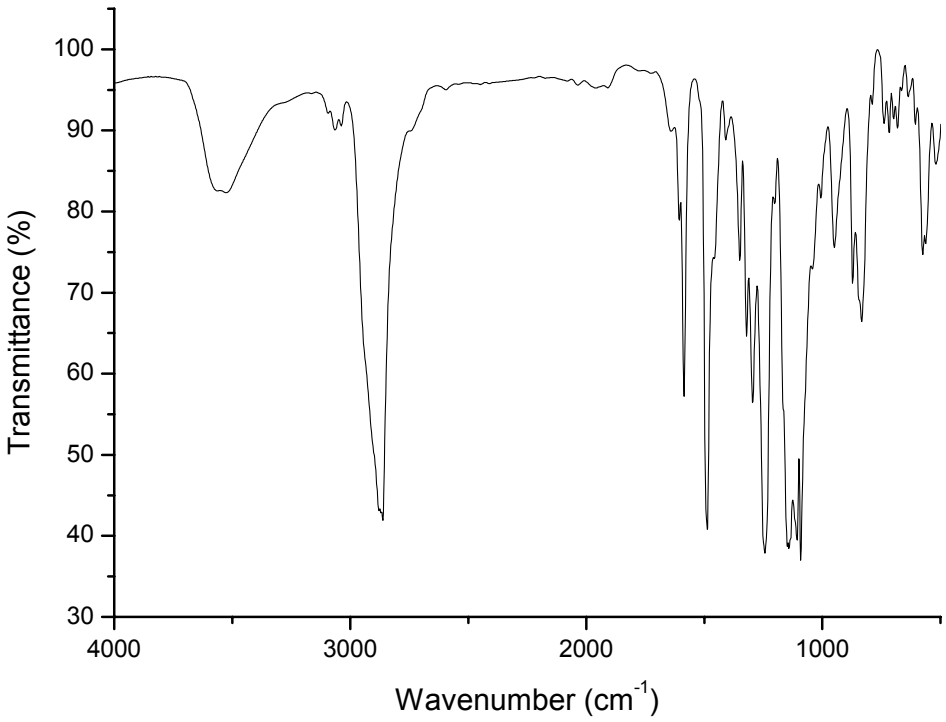
Table S1: Molecular weight and polydispersity of PSF-b-PEO[a]

Polymer	Mn	Mw	Polydispersity
PSF-*b*-PEO	56000	67000	1.19

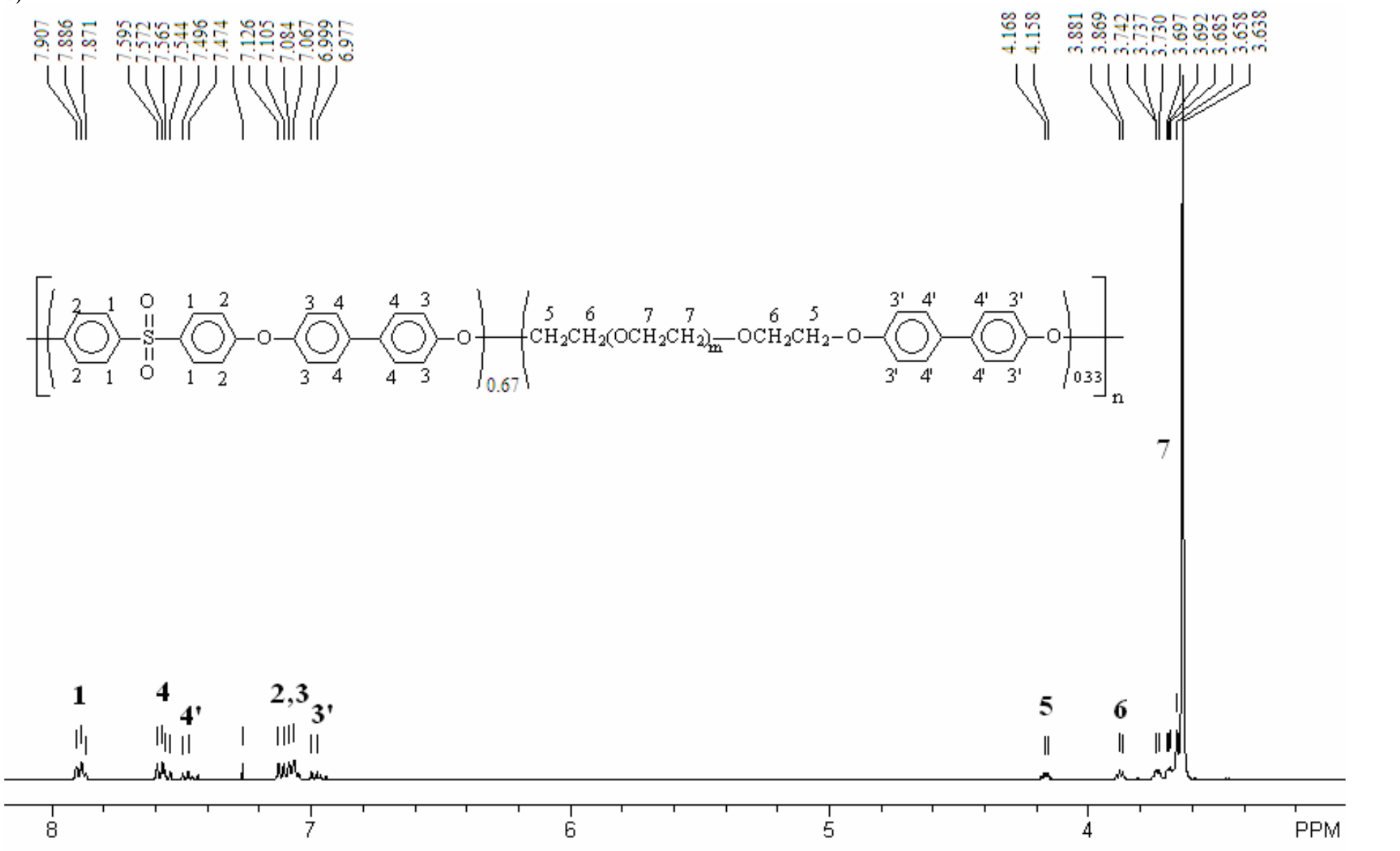
[a]Obtained by Gel permeation chromatography (GPC) combined with light scattering which was performed with a Waters 515HPLC (column: Waters Styragel® HR4) and a DAWN EOS light scattering apparatus. Tetrahydrofuran (THF) was used as the eluant at a flow rate of 1.0 mL/min. Polymer solutions were filtered through a 0.45 μm PTFE filter prior to injecting into the column.

Figure S1. (a) Fourier transform infrared (FT-IR) spectrum and (b) ^{1}H NMR spectrum of PSF-*b*-PEO.

a)



b)


7.907
7.886
7.871
7.595
7.572
7.565
7.544
7.496
7.474
7.126
7.105
7.084
7.067
6.999
6.977
4.168
4.158
3.881
3.869
3.742
3.737
3.730
3.697
3.692
3.685
3.658
3.638
0.67
033
n
7
1
4
4'
2,3
3'
5
6
8
7
6
5
4
PPM

2. Validation experiments of the WISE method

Table S2: The enrichment effect of PSF-b-PEO and spectrum-to-spectrum reproducibility of MYO digestion[a].

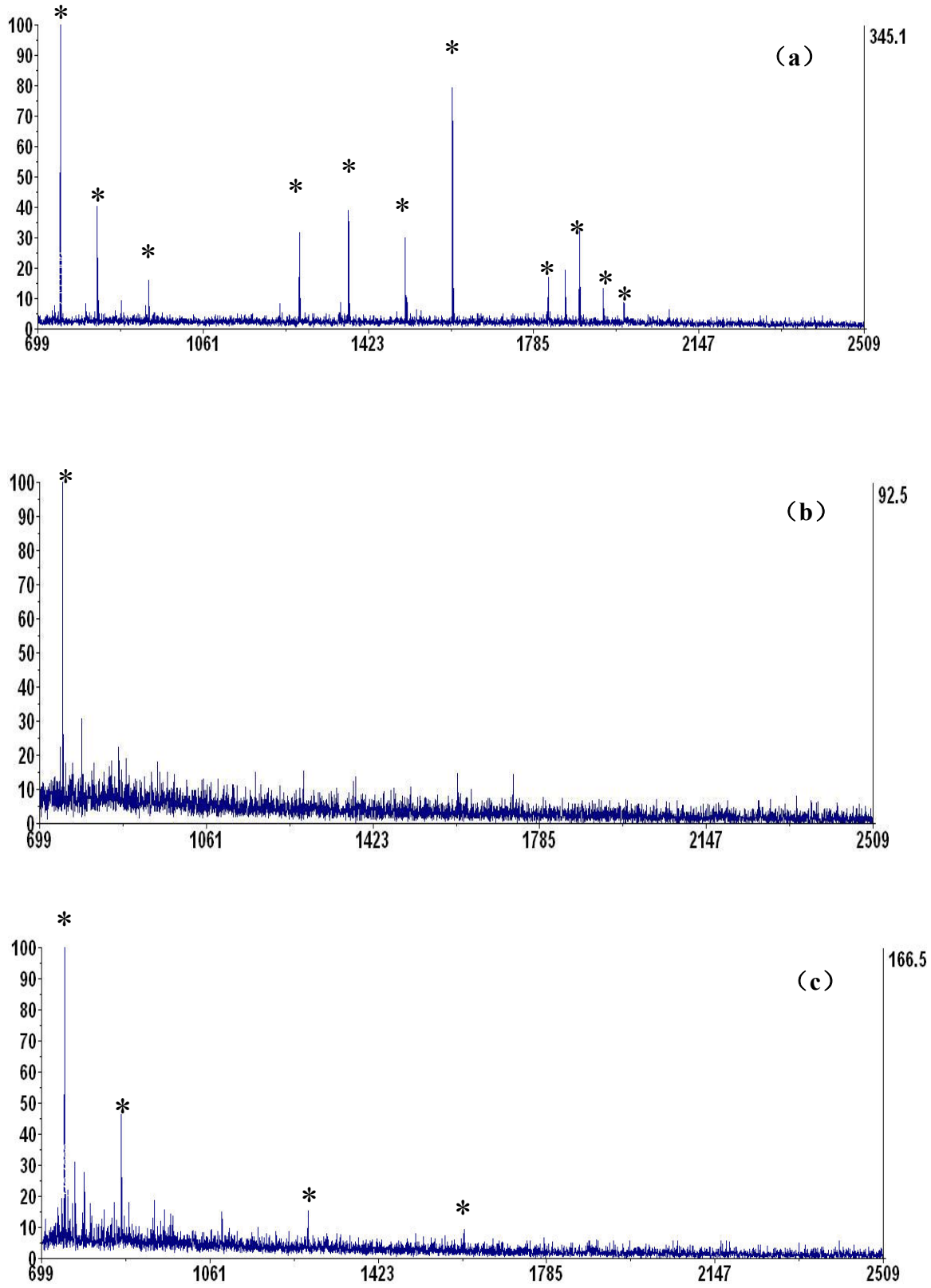
Number of mass spectra	Intensity (m/z 1606.85)[b]	Intensity (m/z 1606.85)[c]
1	294	1053
2	566	987
3	712	960
4	365	1215
5	427	1107
6	240	911
7	423	1091
8	289	1118
9	528	1079
10	1024	1224
11	319	944
12	328	1168
13	489	1265
14	612	1190
15	285	992
16	577	1196
17	971	1092
18	472	1085
19	398	1210
20	627	1086

[a] Each spectrum was taken from signal averaging of 1000 laser shots.
[b] The signal intensity of peak at m/z 1606.85 Da acquired from 1.0μL 50 fmol μL^{-1} MYO digestion spotted on stainless steel target.
[c] The signal intensity of peak at m/z 1606.85 Da acquired from 1.0μL 50 fmol μL^{-1} MYO digestion spotted on PSF-b-PEO film.

3. Sample preparation of ZipTipC_{18}:

The sample preparation of peptides prior to MALDI-TOF MS using ZipTipC_{18} pipette tips was according to the standard procedure provided by the technical note from the Millipore Corporation (http://www.millipore.com/publications.nsf/docs/tn224). Briefly: 1. The tip was prewet by depressing the pipetter plunger to a dead stop using the maximum volume setting of 10 µL. The wetting solution (50% ACN in TFA in Milli-Q water) was aspirated into the tip and dispenced to waste. This process was repeated. 2. The tip was equilibrated for binding by washing it twice with the equilibration solution (0.1% TFA in Milli-Q water). 3. Peptides were bound to the ZipTip by fully depressing the pipette plunger to a dead stop. Aspiration and dispensing of 5 µL sample solution was carried out for 8 cycles. 4. the tip was washed and the washing solution discarded using at least 2 cycles of wash solution (0.1% TFA in Milli-Q water). 5. Elution was carried out using 5 µL of elution solution (50% ACN/0.1% TFA in Milli-Q water) using a standard pipette tip. The eluant was carefully aspirated and dispensed through the ZipTip at least 3 times without introducing air. 6. Then 0.5 µL of the eluant containing the purified sample was mixed with 0.5 µL of α-CHCA saturated solution (50% ACN/0.1% TFA in Milli-Q water) on the MALDI plate and applied for MALDI-TOF-MS analysis.

Figure S2. MALDI-TOF mass spectra of (a) 10 fmol μL^{-1} MYO digests with 50 mM ABC spotted on PSF-*b*-PEO film, (b) normal 10 fmol μL^{-1} MYO digests spotted on stainless steel target, (c) 10 fmol μL^{-1} MYO digests with 50mM ABC desalted by ZipTipC18 pipette tip. Asterisks (*) represent for peaks assigned to MYO digests.



4. Real sample analysis

Sample preparation and two-dimensional electrophoresis (2DE):

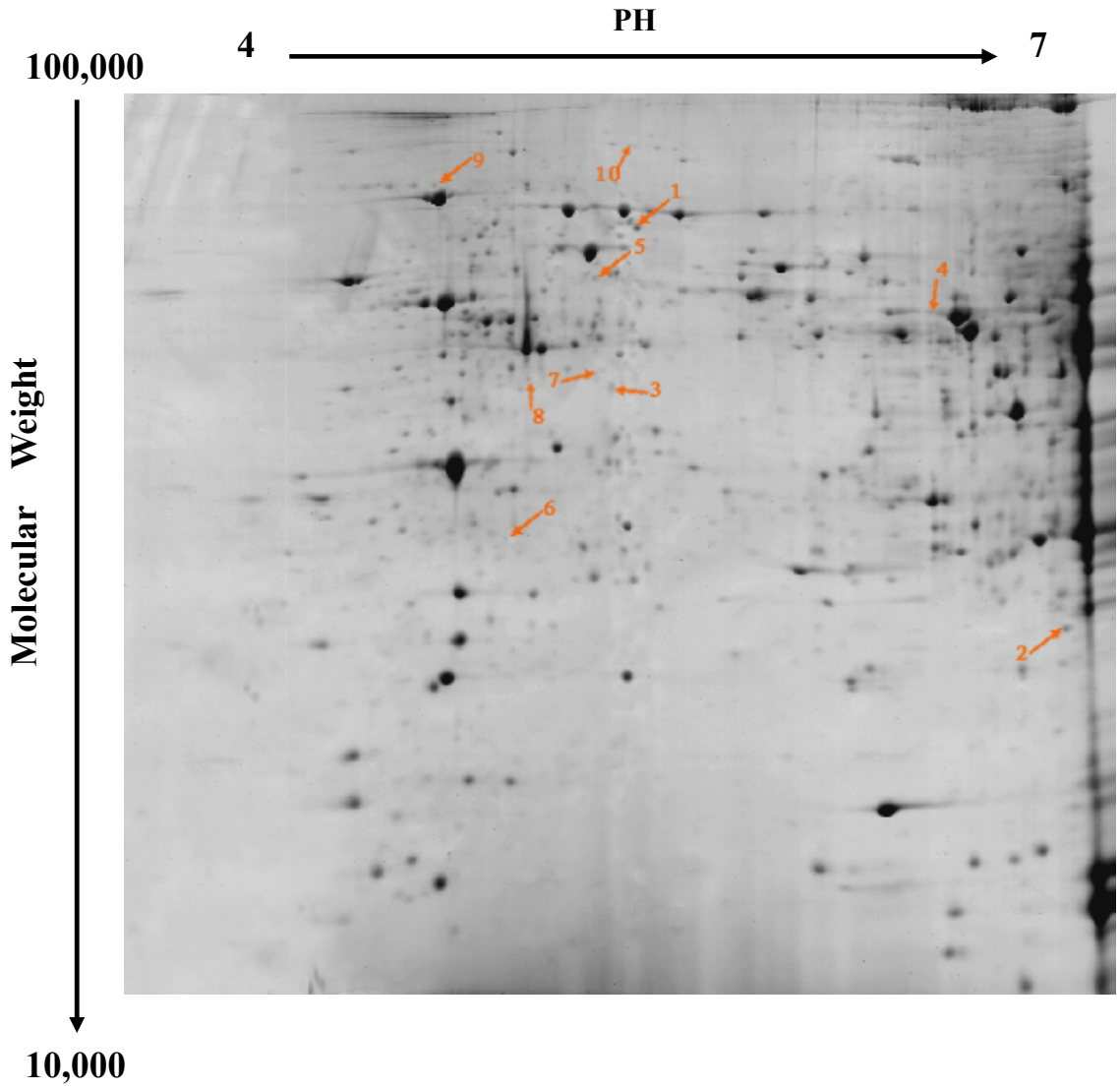
Fresh house mouse liver was cut into small pieces and washed several times with 0.9% NaCl to remove blood. After that, the tissues were dried by pledget and weighed, then homogenized at 0 ℃°.A lysis buffer of 7 M urea, 2 M thiourea, 4%CHAPS, 1% protein inhibitor was added to extract the total proteins with a sample/buffer ratio of 1/8 (w/v). Extraction was aided by vortex of 30 min. Samples were centrifuged at 20 000 g at 4 ℃ for 1h, and the supernatant was kept at -80 ℃ until use. 2-DE was performed using nonlinear pH 3–10 IPG strip. 700 μg proteins were applied to 18 cm strip. IEF was performed using an IPGphor apparatus (Amersham Pharmacia). Second dimensional electrophoresis was performed by using a Protean IIxi system (Bio-Rad). Gel was Coomassie blue stained. The stained gels were scanned using an ImageScanner (Amersham Pharmacia) and analyzed with ImageMaster software (Amersham Pharmacia).

In-gel digestion and mass spectrometry analysis:

Gel was washed twice with water before spots were excised. Then, selected spots were excised from gels using Ettan Spot Picker. Excised spots were detained with a solution of 50 mM NH_4HCO_3 and ACN (1:1) for 20 min at room temperature. Then they were washed with Milli-Q water for 10min, shrunk by dehydration in 80 μL ACN twice. The 3 μL trypsin (sequencing level, 12.5 ng/μL, freshly diluted in 25 mM NH_4HCO_3; Roche, Switzerland) was added to the dried gel spots. The digestion was performed at 37°C overnight. 50 μL of 50% ACN/0.1% TFA was added to the gel and incubated for 20 min. The supernatant of extracted peptides was recovered and the operation was repeated once. All the steps of digestion were performed with the Ettan TA Digester (Amersham Sciences).

Every extraction solution of protein spots was dried with N_2 at 37°C. Then 1.5 μL solution of 50% ACN/0.1% TFA was added and purged several times to resolve the dried samples. For the conventional method, half the volume of the digested solution was spotted on the stainless steel target. After the sample was dried 0.5 μL of matrix solution (5 mg mL^{-1} CHCA in 50% ACN/0.1% TFA) was spotted. For the novel desalting-free method, half volume of the digests solution was spotted onto the film of PSF-b-PEO. After the sample was dried 0.5 μL of matrix solution (5 mg mL^{-1} CHCA in 50% ACN/0.1% TFA) was spotted. MS measurements were carried out on an ABI 4700 TOF-TOF MS. The mass instrument was calibrated using digested peptides of horse heart myoglobin in an internal mode, and the default calibration mode was applied for the sample peptides.

Figure S3. 2DE map of protein extracted from liver of healthy mouse. Electrophoresis conditions: IEF, pH 4-7, linear, 18 cm; 12% SDS-PAGE gel, stained with Coomassie blue.



Spot	Protein Name	Protein Score (A)[b]	Protein Score (B)	Sequence Coverage (A)[c]	Sequence Coverage (B)
1	unnamed protein product [Mus musculus] gi|74205924	274	277	31%	35%
2	Glutathione peroxidase 1 (GSHPx-1) (GPx-1) (Cellular glutathione peroxidase) gi|121666	259	283	39%	41%
3	vitamin D-binding protein [Mus musculus] gi|193446	141	176	28%	36%
4	PREDICTED: alpha-1-B glycoprotein [Mus musculus] gi|82957346	98	124	19%	17%

Table S3. The proteins identified from 10 protein spots from 2D-gel[a]

5	unnamed protein product [Mus musculus] gi|74143673	104	192	15%	18%
6	unnamed protein product [Mus musculus] gi|12844743	61	118	10%	16%
7	EndoA' cytokeratin (5' end put.); putative gi|309215	57	111	16%	25%
8	unnamed protein product [Mus musculus] gi|74212014	46	116	13%	19%
9	unnamed protein product [Mus musculus] gi|49728	28	104	16%	34%
10	unnamed protein product [Mus musculus] gi|74191884	66	177	11%	22%

Note: [a] Proteins were extracted from the liver of healthy house-mouse and separated by 2DE. Search parameters: database: NCBInr (Version: July 12 2005); digest used: Trypsin; maximum of missed cleavages: 1; peptide mass tolerance: 80 ppm; tandem mass tolerance: 0.5 Da. GPS Explorer software (Version 2.0) from Applied Biosystems with Mascot as a search engine was used to identify proteins. All proteins were identified using the peptide fingerprint mass spectra combined with tandem mass spectra. [b] Protein scores greater than 62 are significant ($p<0.05$). [c] Every extraction solution of protein spots was dried with N2 at 37°C. Then 1.5 μL solution of 50% ACN/0.1% TFA was added and purged several times to resolve the dried samples. The extraction solution was equally spotted on stainless steel target and on PSF-b-PEO film, respectively. The (A) represents for the results obtained on stainless target, and (B) for the results obtained on PSF-b-PEO coated target.