Epitope imprinted polyethersulfone beads by self-assembly for target protein capture from a plasma proteome

Supporting Information

1. Chemicals

Polyethersulfone (PES, Ultrason E 6020P, CAS No.:25608-63-3) was purchased from BASF chemical company (Ludwigshafen, Rhineland-Palatinate, Germany) and was used to prepare the porous particles. The transferrin (TRF, MW 77 kDa, pI 5.5), ribonuclease B (RNB, MW 11.7 kDa, pI 8.8), cytochrome C (CYC, MW 12.3 kDa, pI 10.6) and β-lactoglobulin (β-LG, MW 17.5 kDa, pI 5.2) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The transferrin epitope, a peptide with the sequence MRLAVGALL, was obtained from GL Biochem (Shanghai, China) and was used as the template. The ammonia solution, sodium hydroxide, N,N-dimethylacetamide (DMAc) and ferric chloride hexahydrate were obtained from Tianjing Kermel Chemical Reagents Development Centre (Tianjin, China). Iron (II) sulfate heptahydrate was purchased from Shenyang Chemical Reagent Co., Ltd. (China). The HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany), and 1-(3dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), trifluoroacetic acid (TFA), dithiothreitol (DTT) and iodoacetamide (IAA) were obtained from Acros Organics (Geel, Belgium) and applied to the shotgun proteomic analysis and quantification. All reagents were of analytical reagent grade, unless otherwise specified. The water was purified using a Milli-Q system (Millipore, Milford, MA).

2. Preparation of the Fe₃O₄ magnetic nanoparticles

The Fe₃O₄ magnetic nanoparticles were prepared by the co-precipitation method.^[1] A 25-mL mixture containing 5.406 g FeCl₃· $6H_2O$, 2.780 g FeSO₄· $7H_2O$ and 3 vol% concentrated hydrochloric acid was prepared in water (Solution A). A 250-mL

ammonia solution (40 vol%), Solution B, was prepared in a 500-mL three-necked bottle and purged with nitrogen for 15 min. Solution A was then added dropwise to Solution B under a nitrogen atmosphere. Upon addition, the solution turned black and was stirred mechanically at 1000 rpm for 2 h. The Fe_3O_4 magnetic nanoparticles were washed three times with water prior to their suspension in DMAc.

3. Preparation of the epitope-imprinted polyethersulfone magnetic beads via polymer self-assembly

The PES-imprinted particles were prepared using the self-assembly method based on a PES phase inversion in its non-solvent. A 200-mg aliquot of PES was dissolved in 800 mg DMAc at room temperature. Then, 30 mg transferrin epitope, MRLAVGALL, employed as the model template, was dispersed in the aforementioned PES solution. Next, a 100-mL magnetic Fe_3O_4 nanoparticle solution containing 23 mg Fe_3O_4 magnetic nanoparticles was dispersed in the mixture. The resultant polymer solution was added dropwise to the distilled water using a 0.6-mm diameter needle at room temperature to fabricate the epitope-imprinted particles via phase inversion. Then, these particles were incubated in water over 24 h to remove the DMAc from the microspheres. The particles were prepared after the exchange between the solvent and the non-solvent was complete. The template molecules were then extracted from the solidified polymers by washing the polymers with methanol and a 1 M sodium hydroxide solution for several days at 40°C until the epitope peak disappeared from the HPLC chromatogram.

4. Morphology

The beads were dried at room temperature prior to SEM observation. The beads were then quenched with liquid nitrogen, cut with a single-edged razor blade, attached to the sample supports and coated with a gold layer. A JSM-6360 LV (JEOL, Tokyo, Japan) was used for the morphological analysis. SEM photographs of the cross-sections of NIPs are shown in Fig. S1.

The transmission electron microscopy (TEM) was performed for the Fe_3O_4 magnetic nanoparticles on a JEM-2000 EX (JEOL, Tokyo, Japan). The nanoparticles are shown in Fig. S2.



Figure S1. SEM photographs of the cross-sections of NIP: the entire particle (a), the skin layer (b) and the porous internal structure (c). Voltage: 20 kV



Figure S2. TEM image of the Fe₃O₄ magnetic nanoparticles.

5. Magnetism of the imprinted particles

The control particles were prepared using the same method without the addition of the magnetic Fe_3O_4 nanoparticles. As shown in Fig. S3, the control particles and magnetic imprinted particles were combined in one tube. Magnetic iron was applied onto one side of the tube. Then, the imprinted particles were aggregated together under a magnetic field.



Magnetic iron

Figure S3. Magnetism of the imprinted particles: Control, pure PES particles (white); magnetic imprinted particles (brown).

6. Loss on drying (LOD) measurements

The diameter and the porosity of the particles were calculated from the density of the polymer and the change in the sample weight with drying, or the drying loss measurements, using the following equations:^[2]

Diameter =
$$\begin{bmatrix} \frac{6[W_A/\rho_P + (W_B - W_A)/\rho_W]}{\pi} \end{bmatrix}^{1/3}$$
(1)
Porosity =
$$\frac{(W_R - W_A)/\rho_W}{W_A/\rho_P + (W_B - W_A)/\rho_W}$$
(2)

where W_B is the weight of the sample before drying in g; W_A is the weight of the sample in g after drying; ρ_W is the density of water equal to 1.0 g/cm³; and ρ_P is the density of the polyethersulfone, which is equal to 1.43 g/cm³. To calculate the diameter and the porosity, the experiments were conducted in triplicate with 10 stochastic particles included in each experiment.

7. Binding capacity, recognition coefficient and quantities bound to the recognition sites

The template quantities bound to the imprinted and non-imprinted particles,

[S]_(imprinted) and [S]_(non-imprinted) (µmol/g), were calculated using the following equation:

$$[S]_{(imprinted) or (non-imprinted)} = 1000(C_0 - C_t)V/WM$$
(3)

where C_0 and C_t are the template concentrations (mg/L) in the solutions measured initially and after an interval, time *t*, respectively. The volume of the incubation

solution is V(L), and W(g) is the weight of the dry PES particles. M(g/mol) is the molecule weight of the target molecular.

The recognition coefficient (α) was used to evaluate the recognition ability, which was defined as follows:

$$\alpha = [S]_{(imprinted)} / [S]_{(non-imprinted)}$$
(4)

The quantity of the template bound to the recognition sites $([S]_{(sites)})$ on the imprinted microspheres was calculated according to the following equation:

$$[S]_{(sites)} = [S]_{(imprinted)} - [S]_{(non-imprinted)}$$
(5)



8. Efficiency of the epitope imprinted sites for protein recognition

Scheme S1. Calculation of the efficiency of the epitope imprinted sites for protein recognition.

9. Proteomic analysis and quantification

9.1 Sample treatment

The MIPs and NIPs were incubated separately with 3 mL human plasma (diluted 50fold) for 72 h. Then, 1 mL supernatant was collected from each sample and heated in a 90°C water bath for 30 min. An 8- μ L aliquot of 100 mM DTT was added to 1 mL of the diluted serum. The protein solution was incubated at 56°C for 2 h. Then, 20 μ L of 1.0 M IAA was added, and the solution was shaken and kept in darkness for 30 min. A 32- μ L aliquot of a 1 mg/mL trypsin solution was subsequently added to the mixture, which was incubated at 37 °C overnight. Prior to analysis, the tryptic digests were desalted with a C18 solid-phase cartridge.

9.2 RPLC-ESI-MS/MS analysis

The peptide analysis was performed using RPLC-ESI-MS/MS on an LTQ-Orbitrap. A 15-cm-long capillary (75 µm i.d.) with a pulled spray tip was packed with C18 particles (5 μm, 300 Å, XBP) at 5–6 MPa by a gas pressure pump overnight. Meanwhile, a 2cm-long capillary (150 µm i.d.) packed with the same particles was prepared as the precolumn. The ESI voltage was set at 2.2 kV for the LTQ-Orbitrap, and the spray capillary was heated to 250°C. The total ion current chromatograms and mass spectra ranging from m/z 400 to 2000 were recorded using the Xcalibur software (v 2.1). The MS acquisition cycle was set as one full MS scan followed by twenty MS/MS scans. The dynamic exclusion function was set as follows: repeat count, 1; repeat duration, 30 s; and exclusion duration, 60 s. All ions with a charge state of +1 were excluded from the collision-induced dissociation (CID) fragmentation in the linear ion trap (LTQ). The MS/MS collision energy was fixed at 35%, and the fragment ions were detected in the Orbitrap analyser at a resolution of 60,000. The two mobile phase solutions were (A) H_2O with 2% (v/v) ACN and 0.1% (v/v) formic acid and (B) ACN with 2% (v/v) H_2O and 0.1% (v/v) formic acid. The gradient was set as follows: 0-15 min, 0% B (v/v); 15-20 min, 0-5% B (v/v); 20-115 min, 5-35% B (v/v); 115-125 min, 35-80% B (v/v); 125–135 min, 80% B (v/v); and 135–152 min, 0% B (v/v). The flow rate from 0–15 min was 8 μ L/min; 150 μ L/min from 15–150 min; and 8 μ L/min from 150–152 min.

9.3 Database search and post-processing of the search results

The proteins were identified by converting the raw data to mgf files using pXtract (version 1.0), a component of the pFind software package.^[3] The mgf files were used in the protein identification with a local installation of MASCOT (Matrix Science, version 2.3.2). The human database originated from the International Protein Index (IPI) (version 3.81, 92,111 sequences), and the reversed sequences were appended to the database to evaluate the false discovery rate (FDR). The cysteine residues were searched with the static modification of +57.0215 Da, and the methionine/asparagine residues were searched as variable modifications of +15.9949 Da. The precursor and fragment mass tolerances were 10 ppm and 1.0 Da, respectively. The acceptable charge states ranged from 2+ to 4+, while the number of missed cleavages allowed was set at 2.

The search results were filtered using pBuild, another component of the pFind software package,^[3] to maintain a peptide false discovery rate (FDR) of less than 1% and to group the identified proteins. The FDR was calculated using a reversed sequence strategy.^[4] When the same peptide(s) were assigned to multiple proteins, the multiple proteins were clustered into a "protein group". Furthermore, the proteins with the highest sequence coverage and a minimum of two distinct peptides in each "protein group" were extracted for proteomic quantification using the SI_N.

$9.4 \ SI_N$ calculation

For the SI_N calculations, the algorithm operated in a search engine-independent approach and ran in a manner developed in our previous study:^[5]

- Retrieve the MS/MS spectra and filter the fragment ions according to the S/N ratio threshold of 10.
- (2) Calculate the theoretical fragment ions:
- Calculate the theoretical b and y fragment ions and the MH precursor ions for the peptides at the charge states obtained from the search result.
- ✓ Calculate the mass losses of NH_3 and H_2O from the MH ion.
- \checkmark Calculate the NH₃ mass losses for the b and y fragment ions if they include R/K/N.
- ✓ Calculate H₂O mass losses for the b and y fragment ions if they include S/T/E/D.
- \checkmark For any of the aforementioned fragment ions, the highest charge state is set at 3+.

- ✓ Calculate the masses of the singly charged immonium ions (AA mass CO + proton)
- (3) Loop through the filtered fragment ions in the spectrum from most to least abundant. Check for a match to the theoretical ions within the specified mass tolerance.
- (4) Exclude the theoretical ion from further matches when a match occurs.
- 9.5 Quantitative changes in the top ten proteins in the human plasma

The SI_N was applied to quantify the human proteome. The quantitative changes in the top ten proteins were calculated and are listed in Table S1.

Table S1. Quantitative changes in the top ten proteins in the human plasma after treatment with the MIP and NIP particles.

| Protein Accession (Protein Name) | МІР | | NIP | | Original | ΔSI_{N} (in MIP)/ Δ |
|-------------------------------------|-----------------|------------------|-----------------|-----------------|-----------------|------------------------------------|
| | | | | | Solution | SI _N (in NIP) |
| | SI _N | ΔSI _N | SI _N | ΔSI_{N} | SI _N | |
| IPI00745872.2 | 0.7106 | -18.48% | 0.6356 | -5.97% | 0.5998 | 3.10 |
| (Serum albumin) | | | | | | |
| IPI00021841.1 | 0.0281 | 25.44% | 0.0329 | 12.63% | 0.0377 | 2.01 |
| (Apolipoprotein A-I) | | | | | | |
| IPI00022463.2 | 0.0271 | 25.97% | 0.0364 | 0.42% | 0.0366 | 61.57 |
| (Transferrin) | | | | | | |
| IPI00784985.1 | 0.0092 | 64.92% | 0.0071 | 73.07% | 0.0262 | 0.89 |
| (IGK@ protein) | | | | | | |
| IPI00550731.2 | | | | | | |
| (Putative uncharacterised | | 100% | 0.0070 | 73.17% | 0.0261 | 1.37 |
| protein) | | | | | | |
| IPI00448925.6 | | 100% | 0.0217 | 6.42% | 0.0233 | 15.57 |
| (IGHG1 44 kDa protein) | | | | | | |
| IPI00641737.1 | 0.0228 | -11.21% | 0.0302 | -46.85% | 0.0205 | 0.24 |
| (Haptoglobin) | | | | | | |

| IPI00022488.1 (Hemopexin) | 0.0132 | 23.04% | 0.0170 | 1.02% | 0.0172 | 22.56 |
|---|--------|---------|--------|---------|--------|-------|
| IPI00553177.1 (Alpha-1-antitrypsin) | 0.0284 | -84.77% | 0.0193 | -25.23% | 0.0154 | 3.36 |
| IPI00022429.3 (Alpha-1-acid glycoprotein 1) | 0.0117 | 18.44% | 0.0116 | 19.63% | 0.0144 | 0.94 |

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