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Fast human serum profiling through chemical depletion coupled to gold-nanoparticle-assisted protein separation

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ABSTRACT

The use of chemical protein depletion in conjunction with gold-based nanoparticles for fast matrix assisted laser desoption ionization time of flight mass spectrometry-based human serum profiling was assessed. The following variables influencing the process were optimized: (i) amount of nanoparticles, (ii) sample pH, (iii) amount of protein and (iv) incubation time. pH was found the most important factor to be controlled, with an optimum range comprised between 5.8 and 6.4. The minimum incubation time to obtain an adequate profiling was 30 min. Using this approach, serum from five patients with lymphoma, five patients with myeloma and from two healthy volunteers were correctly classified using Principal component analysis.

1. Introduction

Matrix Assisted Laser Desorption/Ionization, MALDI, mass spectrometry, MS-based profiling of serum is a fast and easy way to find altered abundances of human serum components. Such alterations can be linked to human diseases, particularly cancer. Profiling of human serum with MALDI demands a sample pretreatment to avoid the serum's high abundance of proteins, as they create a large amount of signals that mask the ones corresponding to other less abundant proteins, which are yet more important in terms of information [1,2]. Depletion and equalization are the strategies currently in use to avoid major serum components [3].

Magnetic beads and other type of nanoparticles (NPs) have been pointed out in medicine, since the mid-1970s, as powerful tools to obtain a reduction in the complexity of human samples [4,5]. NPs have been used to separate targeted proteins [6–9] as well as for protein pre-concentration in human serum and plasma [10–13].

This work shows the potential of gold NPs, GNPs, to be utilized as fast tools to obtain human serum profiling in combination with depletion with ACN in an easy and straightforward way. This is done by first using a cheap and fast acetonitrile-based procedure to deplete the serum's high abundance proteins. Then, a rapid gold NPs-based protocol is applied to separate the remaining protein content into two sub-samples: the GNPs-based pellet and the supernatant. If the major proteins were not depleted, the gold nanoparticles would remove them also. This means that the fingerprinting would be a spectrum with the dominant peaks belonging to such major proteins. As for the fingerprinting, it was anticipated that the sample fingerprinting-based classification would become compromised. By separating the major proteins before the utilization of the gold nanoparticles it was expected a better classification of the samples. With this approach, one fingerprinting is obtained from each sub-sample, the GNPs-based

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pellet and the supernatant. Finally, data sets are statistically treated for classification purposes. This protocol was applied to comparative peptide profiling of patients with lymphoma, patients with myeloma and healthy individuals.

2. Materials and methods

2.1. Chemicals and reagents

All reagents used were HPLC-grade or higher. Sodium citrate tribasic, trypsin, trifluoroacetic acid, bovine serum albumin, carbonic anhydrase DL-Dithiothreitol (DTT), Iodoacetamide (IAA), acrylamide/bis-acrylamide 30% solution (37.5:1), Glycerol 86–88%, Tris-base, Coomassie Brilliant Blue R250 (CBB), sodium carbonate, sodium thiosulfate, Na_2-EDTA, silver nitrate solution and the SigmaMarker wide range 6.5–200 KDa were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) and formaldehyde were purchased from Panreac (Barcelona, Spain). β-mercaptoethanol was purchased from Merck (Hohenbrunn, Germany) and bromophenol-blue was purchased from Riedel-de Haen (Seelze, Germany). Hydrogen tetrachloroaurate (III) hydrate (99.9%-Au) (49% Au) at 10%w/v was purchased from Strem Chemicals (Newburyport, MA, USA).

α-Cyano-4-hydroxycinnamic acid, ammonium bicarbonate (AMBIC) and formic acid were purchased from Fluka (Steinheim, Germany). ZipTip® was purchased from Millipore.

2.2. Human serum samples

Human serum samples were purchased from Patricell Ltd (BioCity Nottingham, UK). In this study, serum samples from five patients diagnosed with lymphoma and from five patients diagnosed with multiple myeloma were used. For control purposes, a pool of two individuals (mixed gender) was also employed (Table 1).

2.3. Apparatus

A PowerPac Basic power supply from Bio-Rad (CA, USA) was used for SDS-PAGE protein separation. Protein quantification was accomplished by measuring the absorbance at 280 nm with the use of a NanoDrop 1000 Spectrophotometer from Thermo Scientific. Gold nanoparticles were characterized by UV spectroscopy using a spectrophotometer from JASCO V-650 UV–vis (Easton, MD, UK) and Transmission Electron Microscopy, Philips CM20. MS profiles were acquired using an Ultraflex II matrix-assisted laser desorption/ionization tandem time-of-flight (MALDI-TOF-TOF) instrument (Bruker-Daltonics, Bremen, Germany) equipped with a LIFT cell and 50 Hz nitrogen laser. A peptide calibration standard II from Bruker was used for the calibration of the mass spectrometer. Gel image acquisition was carried out with a ProPicII-robot (Digilab-Genomic Solutions, USA).

2.4. Synthesis of citrate-capped gold nanoparticles

Gold nanoparticles, GNPs, were synthesized by the citrate reduction method of chloroauric acid ($HAuCl_4$) in aqueous solution [14,15]. 60 ml of sodium citrate tribasic solution (0.075% w/v) was heated briefly to 100 °C, and then 54 ml of 10% w/v of hydrogen tetrachloroaurate (III) hydrate solution was added. The reaction mixture was kept under refluxing for 15 min, until a deep red color was detected. Citrate-capped GNPs solution was stored at 4 °C. GNPs were characterized by UV spectroscopy and transmission electron microscopy (Fig. 1 of Supplementary material, Fig. 1SM-A and B).

2.5. BSA/CA reduction and alkylation

Two model proteins, bovine serum albumin, BSA, and carbonic anhydrase, CA, were selected for optimization of GNPs-protein separation. A stock solution containing 2.5 mg of BSA and 2.5 mg of CA type 2 from bovine erythrocytes was prepared in 1 mL of ammonium bicarbonate, AMBIC, 25 mM (pH 8.5). Aliquots of 20 µl of the protein stock solution were reduced for 1 h at 37 °C with 2 µl of DTT 110 mM, prepared in AMBIC 25 mM. Then proteins were alkylated for 45 min at room temperature in the darkness with 2 µl of IAA 600 mM prepared in AMBIC 25 mM.

2.6. BSA/CA separation using GNPs

After protein reduction and alkylation, 100 µl of GNPs were added to the sample, followed by 40 µl of citrate/citric acid buffer to a final pH of 5.8. The optical density (OD) of the GNPs solution used was 96.36% (Fig. 2SM). Then, the GNPs-protein solution was incubated at 37 °C in a thermostatic bath until a pink suspension was noted by naked eye. The pellet was then harvested by centrifugation at 14000 rpm during 30 min in a CM · 50M centrifuge from ELMI (Riga, Latvia). Afterwards, the pellet was washed with a citrate/citric acid buffer and harvested again by centrifugation at 14,000 rpm during 30 min. Supernatant was vacuum-dried in a centrifuge UNIVAPO 150 ECH from UniEquip (Martinsried, Germany) coupled with a refrigerated aspirator vacuum pump, model Unijet II (UniEquip). Both were then reconstituted in 10 µl of citrate/citric acid buffer for analysis by gel electrophoresis or in 10 µl of AMBIC 25 mM for protein digestion and MALDI-MS analysis. Prior to analysis, 0.5 µL of sample was hand-spotted onto a ground steel MALDI target (MTP 348) and subsequently, 1 µL of a 5 mg/mL solution of α-cyano-4-hydroxycinnamic acid matrix in 0.1% v/v TFA and 50%v/v ACN was added to the peptide digest spots and allowed to air dry.

Table 1
Classification of the patients used in the study.

Patient	Diagnosis	Age	Gender	Medication	Stage/other comments
I	Lymphoma	90	Female	–	Pretreatment
II	Lymphoma	77	Female	–	Pretreatment
III	Lymphoma	69	Male	–	Pretreatment
IV	Lymphoma	53	Female	–	Pretreatment
V	Lymphoma	83	Female	–	Pretreatment
VI	M. Myeloma	57	Male	Revlimid, velcade	–
VII	M. Myeloma	57	Male	Revlimid, velcade	–
VIII	M. Myeloma	57	Male	Revlimid, velcade	Stable
IV	M. Myeloma	57	Male	Revlimid, velcade	Stable
V	M. Myeloma/High Cholesterol	57	Male	Aredia, revlimid	Stable

2.7. Gel electrophoresis

Volumes of 5 μl of (i) protein marker SigmaMarker wide range 6.5–200 KDa, (ii) BSA-CA mixture, (iii) supernatant and (iv) pellet were separately mixed with 5 μl of sample loading buffer (5 ml of Tris-base 0.5 M, 8 ml of SDS at 10% w/v, 1 ml of β-mercaptoethanol, 2 ml of glycerol, and 4 mg of bromophenol-blue, all diluted into 20 ml of water). Then, they were loaded into a 5% acrylamide/bis-acrylamide, stacking gel/12.5% acrylamide/bis-acrylamide running gel, of 1 mm thickness, and separated at 200 V for 50 min. After gel electrophoresis, gels were rinsed with Milli-Q water and then incubated for 2 h in CBB staining solution at room temperature (previously prepared dissolving 1 g of CBB in 200 ml of a mixture of 45% v/v methanol and 7.5% v/v glacial acetic acid). After incubation, the staining solution was removed: the gel was rinsed with Milli-Q water and then incubated for 3 h at room temperature in distaining solution (45% v/v methanol, 7.5% v/v glacial acetic acid). For the case of the clinical samples, MS compatible silver staining was used. Brieffly gels were incubated for 30 min in a fixing solution containing 40% v/v ethanol and 5% v/v acetic acid and then rinsed 2×10 min with wash solution containing 20% v/v ethanol. Gel sensitizing solution (0.02% w/v sodium thiosulfate, $Na_2S_2O_3$) was added to the gels and left to incubate for 1 min, following by 2×1 minute wasing with water. Then gels were incubated with 0.1% w/v silver nitrate for 30min. After this step gels were rinsed once in water for 30 s and once with 2.5% w/v sodium carbonate for 1 min. The stain was developed in 2.5% w/v sodium carbonate, 0.01% v/v formaldehyde solution for 10 min. To stop the staining development, gels were incubated in an aqueous solution containing 1.5% w/v Na_2EDTA for 20 min. Gel image acquisition was carried out with a ProPicII-robot.

2.8. Sample treatment for serum samples

20 μl of serum was diluted to 60 μl with Milli-Q water. Then, acetonitrile, which was cooled to −20 °C before use, was added drop by drop to a final concentration of 60% (v/v) acetonitrile/water. Then, samples were vortexed and sonicated in an ultrasonic bath for 10 min. Supernatant was collected after centrifugation at 10,000 rpm for 20 min, and then evaporated until dry, as described by Fernández et al. [3]. Pellet was then resuspended in 20 μl of AMBIC 25 mM and protein concentration was calculated using absorbance at 280 nm with UV-Nanodrop. Finally, the sample was reduced and alkylated as described in 2.5, and then GNPs were added as explained in 2.6. Then, the solution was incubated at 37 °C in a thermostatic bath until a pink suspension was noted by naked eye. Supernatant and pellet were separated by centrifugation at 14000 rpm during 30 min and they were treated as follows. The pellet containg the GNPs was washed with a citrate/citric acid buffer, and then the GNPs were harvested again by centrification at 14,000 rpm during 30 min. The GNPs were then resuspendend in 10 μl of AMBIC 25 mM. Then 10 μl of trypsin 1:20 were added to digest the sample. Digestion was performed using ultrasonic energy (ultrasonic cup horn) for 5 min at 50% ultrasonic amplitude [16]. The supernatant (83 μl) was treated with 2 μl 30% v/v ammonia and then with 10 μl of trypsin 1:20 [17]. Digestion was performed using ultrasonic energy as described above. Digestion was stopped by the addition of 1 μl of concentrated formic acid. Samples were desalted with ziptips and peptides were eluted with 76 μl of 90% v/v ACN/water [18] . Prior to analysis, 0.5 μL of sample was hand-spotted onto a ground steel MALDI target (MTP 348) and subsequently, 1 μL of a 5 mg/mL solution of a-cyano-4-hydroxycinnamic acid matrix in 0.1% v/v TFA and 50%v/v ACN was added to the peptide digest spots and allowed to air dry.

2.9. MALDI-MS analysis

The mass spectrometer was operated in positive ion mode using a reflectron, and thus, spectra were acquired in the *m/z* range of 600–3500. A total of 500 spectra were acquired for each sample at a laser frequency of 50 Hz. External calibration was preformed with the [M+H]+ monoisotopic peaks of bradykinin 1–7 (*m/z* 757.3992), angiotensin II (*m/z* 1046.5418), angiotensin I (*m/z* 1296.6848) substance P (*m/z* 1758.9326), ACTH clip 1–17 (*m/z* 2093.0862), ACTH 18–39 (*m/z* 2465.1983) and somatostatin 28 (*m/z* 3147.4710). Peptide mass fingerprints were searched with the MASCOT search engines with the following parameters: (i) SwissProt Database 2012_04 (535698 sequences; 190107059 residues); (ii) molecular weight of protein: all; (iii) one missed cleavage; (iv) fixed modifications: carbamidomethylation (C); (v) variable modifications: oxidation of methionine and (vi) peptide tolerance up to 50 ppm after close-external calibration. A match was considered successful when protein identification score is located out of the random region and the protein analyzed scores first.

2.10. Statistical analysis

2.10.1. Jaccard analysis

In order to evaluate the similarity of the spectra, the Jaccard Index (JI) [19] was employed. This statistic is commonly used as a similarity coefficient to compare the likeness and diversity of two sample sets. As formula 1 demonstrates, it is defined as the ratio between the size of the intersection and the size of the union of the sets. JI takes values between 1, for identical sets, and 0, for sets without common elements.

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \quad (1)$$

When calculating the similarity of the two spectra, each spectrum is treated as a set of m/z, ignoring their peak intensities. A previous peak alignment is needed in order to determine the peak correspondence among different spectrums. The alignment between spectra was done adhering to the subsequent criteria: (i) *m/z* error allowed: 250 ppm; (ii) minimum peak intensity: 1.75; (iv) minimum signal to noise ratio: 9 and (v) for an alignment to be considered a *m/z* value, it must be present in at least 80% of the spectra.

2.10.2. Principal component analysis

The Principal Component Analysis (PCA) [20] was applied so as to reduce the data dimensionality, allowing a visual analysis of the sample separation in a three dimensional space. PCA was configured to reduce the variables (*m/z* values) to three principal components [21]. PCA was done taking as variables the *m/z* peaks as well as their respective intensities. A previous peak alignment is needed in order to determine the peak correspondence among different spectrums. The alignment between spectra was done adhering to the subsequent criteria: (i) *m/z* error allowed: 250 ppm; (ii) minimum peak intensity: 1.75; (iv) minimum signal to noise ratio: 9 and (v) for an alignment to be considered a *m/z* value, it must be present in at least 80% of the spectra.

3. Results and discussion

3.1. Optimization of the separation procedure

GNPs can separate proteins containing cysteine amino acids from other proteins. The sulfur contained in the cysteine binds to the gold of the NPs. A number of variables influence the protein capture efficiency, namely (i) the GNPs/protein ratio, (ii) the pH at

which the reaction takes place and (iii) the GNPs-sample contact time [14–22]. These parameters were optimized with a simple mixture of proteins (bovine serum albumin, BSA, and carbonic anhydrase, CA) before applying the procedure to human serum samples.

3.1.1. Influence of the GNPs/protein ratio

To investigate the influence of the amount of GNPs during the separation process, a series of solutions containing the same amount of reduced and alkylated BSA and CA (100 μg total protein) was prepared. These two model proteins were chosen because BSA has a total of 33 cysteine residues, whilst CA does not have cysteine residues. Volumes of GNPs ranging from 50 μL to 200 μL, as shown in Fig. 3SMA, were added to the BSA/CA solutions in order to get the following protein/GNPs ratios: 10, 5, 3.5, and 2.5. After 30 min of incubation, the supernatant and the pellets were separated via centrifugation and the respective protein content was assessed through the use of 1D gel electrophoresis. Fig. 3SM-A shows the result of this set of experiments. The first line (marked as Mix) corresponds to the mixture of BSA/CA. In this line, both proteins are visible. The lines marked as S correspond to the supernatants. Each of these lines shows the band corresponding to CA, thus demonstrating that this protein was not bound to the GNPs. P-named lines correspond to the pellets. In these lines the band corresponding to BSA is noted, indicating that BSA was removed from the protein mixture by the GNPs. On the Overall, the results shown in Fig. 3SM-A suggest that BSA and CA are effectively separated, even for the lowest amounts of GNPs tested (50 μL). Therefore, for further experiments it was decided to use a GNPs/protein ratio of 5.

The next step was to test the loading capacity of the GNPs. To this end a series of BSA/ CA solutions with total protein content ranging from 20 μg to 500 μg were prepared. Figs. 3SM-B and C present the results of these series, which suggest good efficiency in the separation of BSA through the use of GNPs, even at the highest assessed protein amount, 500 μg. However, in order to get bands with a reasonable density (not overloaded), 100 μg was the amount of total protein chosen to work with.

3.1.2. Influence of pH in the efficiency of the separation process

pH is a variable that influences protein binding to the nanoparticles [23]. To assess the influence of pH in the separation process, a series of solutions containing GNPs (100 μL) and the mixture BSA/ CA (100 μg total protein) were prepared at different pHs comprised between 4.95 and 7.92. The results of these experiments are shown in Figs. 4SM-A–E. As may be seen, in the pH range of 5.85–6.33 the separation is achieved correctly, as the supernatant lane (S) has the band corresponding to CA whilst the pellet line (P) has the band corresponding to BSA. Those findings are in agreement with data reported previously in literature [23–25].

3.1.3. Influence of incubation time

Figs. 4SM-A–E show that incubation time is variable that does not influence the separation process. A time of 15 minutes is enough to guarantee an adequate BSA/CA separation. Another interesting finding was that separation was achieved even when the formation of a precipitate was not observed. It is usually common practice to end incubation should the formation of a precipitate be perceived [26,27]. In our conditions, however, formation of a precipitate was randomly obtained. 30 min was selected as the optimum incubation time.

3.2. Profiling of BSA bound to GNPs

Several experiments were devised in order to break the bound cysteine-GNPs to separate the BSA from the GNPs. To this end, the use of DTT, ultrasonication with probe or both at the same time was asessed. Fig. 4SM-F shows that BSA was not separated from the GNPs, as the BSA band still is observed in the pellets. However, this was not an inconvenience towards the purposes of this work, as we were interested in the peptide profile of the proteins separated by the GNPs. This profile could potentially be obtained by enzymatic digestion of the proteins while still bounded to the nanoparticles. Therefore, a tryptic digestion of 10 μl of BSA ($n=4$) bound to GNPs (ration enzyme/GNPs 1:20) was performed. To accelerate the process, the BSA cleavage was done using an ultrasonic cup-horn for 2.5+2.5 min (50% amplitude) [28]. Fig. 5SM shows a MALDI–MS profile corresponding to the aforementioned experiment. This result shows that proteins bound to GNPs can be digested without requiring their previous separation.

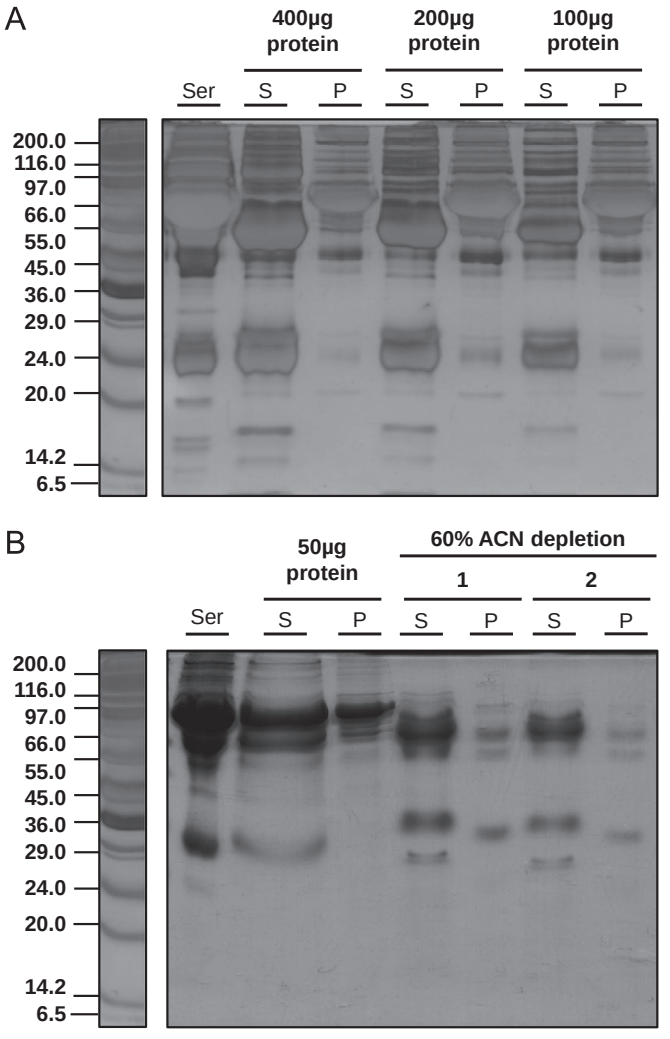

Fig. 1. (A) Influence of the amount of protein in the separation efficiency for non-depleted serum. 20 μl of sample containing amounts of protein ranging from 400 to 50 μg and a fixed volume of 100 μl of GNPs were used. (B) GNP-based separation of proteins for serum depleted with ACN. Total protein content before depletion was 880 ± 16 μg (20 μl) and the total protein content after depletion was 74 ± 11 μg (20 μl). Ser: crude serum. S1 serum after depletion with ACN. S2, serum first depleted with ACN and then treated with GNPs and treated. P pellet of GNPs.

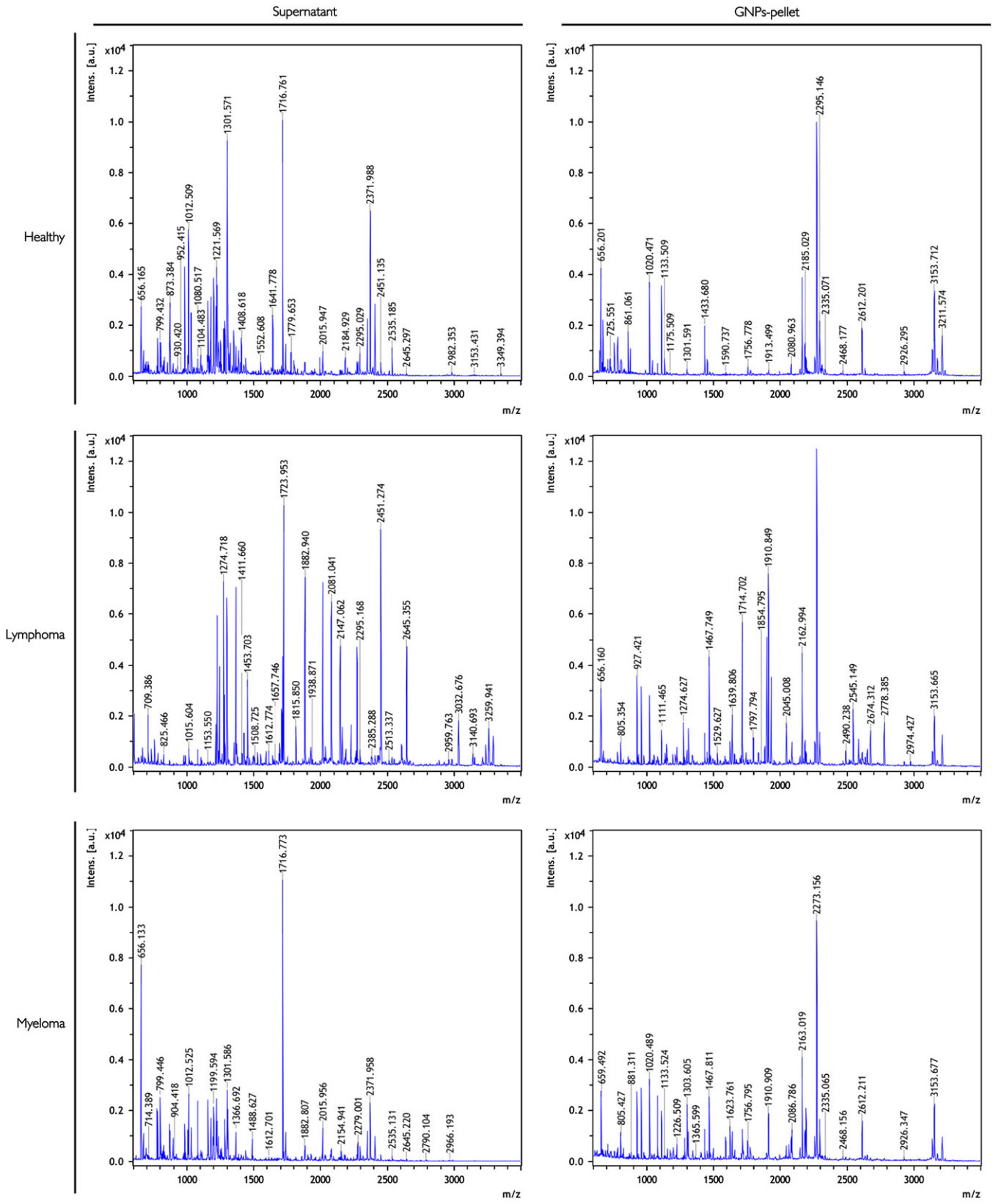

Fig. 2. MALDI–MS-based fingerprinting of serum from healthy individuals and patients with lymphoma and myeloma. For details about the sample treatment referes to the experimental section.

3.3. Application to human serum

The optimum conditions found in the preliminary experiments were used to reduce the complexity and large dynamic range of the human serum. A series of human serum samples were diluted to a protein content that was varied between 20 and 400 μg. The same volume of GNPs was added to all samples so as to produce the same final volume (100 μL of GNPs solution+20 μL

of protein solution+2 μL of DTT+2 μL IAA). After incubation (30 min), the GNP pellet was separated from the supernatant via centrifugation. Fig. 1A shows the results of those experiments. As the figure indicates, the lines corresponding to the pellet (named P) mainly show proteins with an aparent mass above 45 KDa. Most proteins possessing such mass range are included in the group of the most abundant proteins, such as albumin, serotransferrin, plasminogen, ceruloplasmin, Alpha-2-macroglobulin, complement C3, complement C4-A or apolipoprotein B-100 [3,28]. All those proteins are rich in cysteins, which explains the excellent separation achieved with the GNPs. Fig. 1 A also shows that the intensity of the bands decreases when the total amount of protein assessed is diminished. Once the success of the approach in terms of protein separation in crude serum was verified, the next step was to apply the GNPs fractionation to serum previously depleted of major proteins [3]. Recently, depletion with acetonitrile, ACN, has caught the attention of proteomic researchers, as it is fast, easy to handle and inexpensive [3,29]. Depletion of human serum with ACN renders a sample rich in apolipoproteins, promoting the precipitation of proteins with high molecular weight, over 75 KDa [30]. In Fig. 1B, the line named as "Ser" corresponds to crude serum, S1 corresponds to the depleted serum and S2 correspond to the supernatant of depleted serum after treatment with GNPs, Whilst line P corresponds to the pellet of GNPs. Bands present in P lines show that GNPs effectively extract proteins.

3.4. Fingerprinting of GNP-based treated serum

Once adequate conditions for the treatment of serum were found, the following step was to obtain the fingerprint of the pellet and the supernatant. The use of the GNPs to bind the proteins brings a number of benefits in terms of sample treatment. As GNP bound-to proteins are washed, salts and other contaminants are avoided. In addition, the use of GNPs provides simultaneous protein isolation and enrichment without additional steps. Furthermore, proteins bound to GNPs can be digested with trypsin. Then, peptides are easily separated from the GNPs through centrifugation. Finally, peptides are mixed with a matrix and spotted onto a MALDI plate. This procedure is fast, reproducible, amenable of automation, and renders an good fingerprinting as it is shown in Fig. 6SM-I.

Concerning the supernatant, as depicted in Fig. 6 SMA-H, after digestion, different treatments were done, focusing on making the sample treatment easy to handle in the shortest possible time (Figs. 6SMC and 6SMF) [18,31]. The sample treatment C in Fig. 6SM (with desalting) was selected as optimum for further studies as it renders a good matrix/sample crystallization, along with a spectra rich in signals.

3.5. Analytical figures of merit

The instrumental reproducibility, the within batch reproducibility and the between batch reproducibility was assessed using the same serum sample. The comparison was done using the Jaccard index, JI. This index is calculated as the ratio between common *m/z* values and the total *m/z* values obtained from different spectra. Ideally, this ratio should be 1, meaning all spectra are identical. For instance, a ratio of 0.5 means that 50% of the *m/z* values are present in different spectra. Fig. 7SM shows the results of this study. Concerning the supernatant, JI deviated between 55.2% and 73.4% for the instrumental reproducibility (dark-gray in Fig. 7SMA), which was calculated comparing the spectrum of the same sample spotted in five different spots. The JI for the within-batch reproducibility was calculated comparing the spectra of technical replicates done within the same day (5 technical replicates, each one spotted 5 times). JI was found to vary between the 44.6% and 62.6% in this case (medium gray in Fig. 7SMA). Finally, the JI for the between-batch reproducibility was calculated comparing the spectra of technical replicates done in different days (5 technical replicates, each one spotted 5 times). This JI was found to vary between 31.5% and 63.9% (clear gray in Fig. 7SMA). Similar JI values were found for the instrumental reproducibility, the within batch reproducibility and the between batch reproducibility for the pellet sample (Fig. 7SMB). Overall, the results obtained about reproducibility are in agreement with

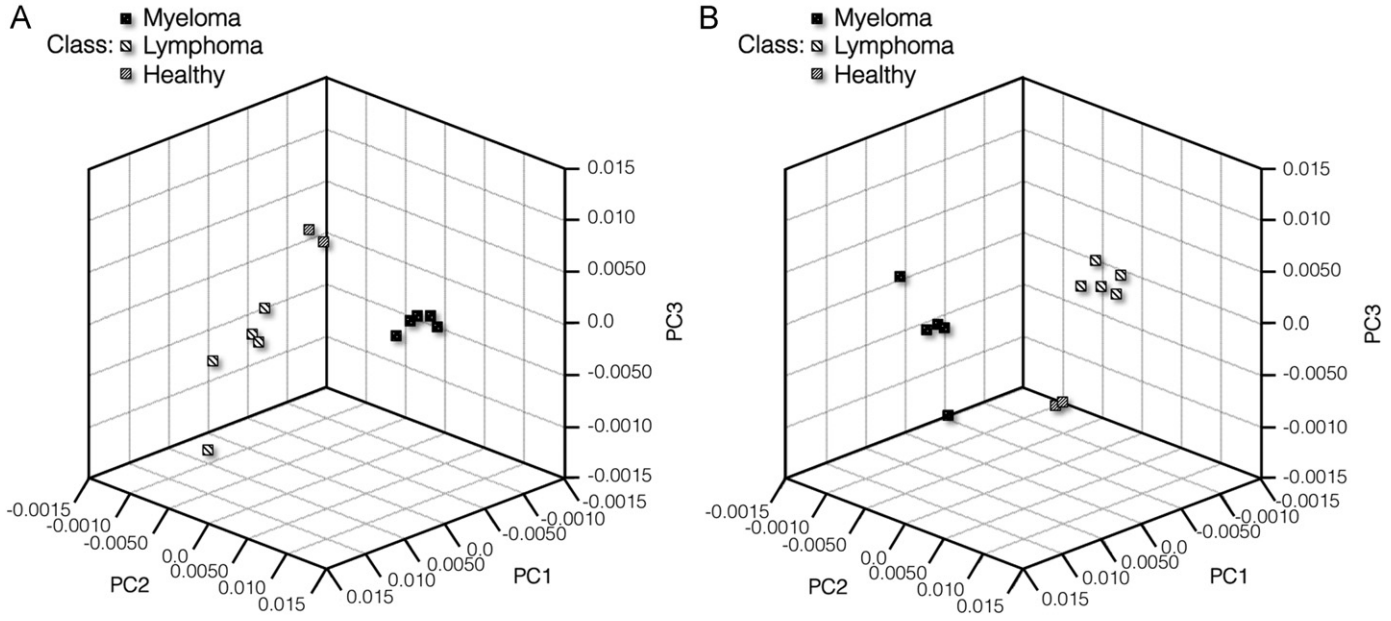

Fig. 3. Proof-of-concept: Principal Component Analysis (PCA) of the data obtained through the fingerprintings of sera samples from Myeloma, Lymphoma and Healthy donors. PCAs were done with data obtained from the analysis of supernatants (A) and pellets (B).

other similar outcomes reported, and validate the analytical performance of the proposed method [32,33].

3.6. Analysis of clinical samples

To assess the performance of the proposed method, we used serum from five patients with lymphoma, five patients with myeloma and two pools of healthy individuals. The typical MALDI spectra profiles of the supernatant and pellet are shown in Fig. 2. PCAs done with the data obtained from the analysis of the supernatants and pellets from the 12 individuals used in this proof-of-concept can be seen in Fig. 3A and B. In addition to these samples, the serum samples of the five individuals with lymphoma were pooled, as well as the five serum samples of the individuals with myeloma. Such pools we treated as extra samples. The PCAs done with the data corresponding to the pellet's and supernatants' fingerprinting show an excellent classification of the individuals in three classes: patients with lymphoma (including the pool), patients with myeloma (including the pool) and healthy individuals.

4. Conclusions

A method for fast human serum protein profiling that entails major protein depletion with acetonitrile and subsequent protein separation with gold nanoparticles has been developed. The new procedure is inexpensive, and the sample treatment can be completed in less than 15 min. Foremost, the method may be potentially automated, allowing high sample throughput. This method holds great assistive diagnosis potential over diseases, as the trials with the sera samples from the diverse patients with lymphoma and myeloma, and sera from two healthy individuals demonstrate. These mentioned serums have been correctly grouped using the described methodology in conjunction with mathematical statistics (Principal Component Analysis).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.08.020.

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