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TRENDS

ICP-MS for multiplex absolute determinations of proteins

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Abstract In the last few years MS-based proteomics has been turning quantitative because only the quantity of existing proteins or changes of their abundance in a studied sample reflect the actual status and the extent of possible changes in a given biological system. So far, however, only relative quantifications are common place. Recently, the ideal analytical features of ICP-MS that allow robust, accurate and precise absolute determinations of heteroelements (present in proteins and their peptides) have opened the door to its use, as a complementary ion source of MALDI- and/or ESI-(MS), in achieving the "absolute" quantification of a protein. Unfortunately, so far such "heteroatom-tagged proteomics" applications deal with only single-heteroatom measurements. Thus, the outstanding capability of ICP-MS for multi-element (-isotope) simultaneous determinations is somewhat wasted. On the other hand, multiplexed determinations of proteins (e.g. in common or new multiplexed formats) today constitute a pressing need in medical science (e.g. to determine accurately many biomarkers at a time). This is a clear trend in analytical science where ICP-MS could eventually play an important role. Therefore, reported approaches to multiplex protein determinations using ICP-MS, with liquid sample nebulisation and with laser direct sampling from a solid, are discussed here. Apart from such multiplex bioassays for absolute protein determinations, efforts to simultaneously quantitate enzyme activities are also discussed. It appears that the time is ripe to combine the multi-isotopic character of ICP-MS with well-known multi-analyte separation techniques (e.g. HPLC or multiplex immunoassays) to tackle the challenge of analysing abundances and activities of several proteins and enzymes, respectively, in a single assay. Many attractive opportunities for creative work and interdisciplinary developments for analytical atomic spectroscopists seem to lie ahead related to multiplexed quantitative targeted proteomics via ICP-MS.

Keywords Mass spectrometry/ICP-MS · Amino acids/peptides · Bioanalytical methods · Absolute determination · Multiplex protein analysis

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Introduction

Derivatization of analytes to render them detectable by a given analytical technique is common practice in analytical chemistry. For instance, reactions with coloured or fluorescent reagents have been widely employed in many fields and particularly in separation science [1]. Similarly, the use of "tracers", both using radioactive and enriched stable isotopes, has been widely exploited in environmental, biological and nutritional investigations [2].

In any case, the common ground of all labelling methods consists of adding an appropriate label (molecule, element or isotope) which binds to or associates selectively with the sought analyte allowing its "visualization" (detection) by the technique, in such a way that it can be distinguished from all the rest of the components and selectively detected in the sample (e.g. a biological system).

In the field of protein analysis, in particular, the development of new, simple and efficient methods for proteins detection and determination is urgently needed.

Common mass spectrometry (MS)-based methods in proteomics (e.g. MALDI-MS or ESI-MS) have been most successful in characterising proteins in complex mixtures, opening new biological and clinical avenues, but results so far have largely been qualitative and not so reliable [3]. The great difficulties encountered when aiming at "absolute"[1] and accurate determinations of the amount of protein(s) "buried" in a real-life biological mixture have been repeatedly pointed out [4]. To make matters worse, conventional MS-based techniques used in proteomics provide analytical signals whose intensities are strongly affected by the species considered (e.g. amino acid sequence), sample matrix or used solvents. Using MALDI or ESI "soft" ion sources, to preserve the molecular information needed, the price to pay is that a linear dependence between the sought protein (peptide) concentration in real sample analysis and the corresponding MS signal measured is not observed.

To get around the difficult problem of protein quantifications most quantitative proteomic research available in the literature uses "relative" approaches, comparing amounts of protein expression between two (or more) different biological states [5, 6]. The most versatile and powerful MS-based techniques for that purpose rely on the use of differential internal labelling of the desired proteins or its peptides, with an appropriate stable isotope. For instance, in isotope-coded affinity tags (ICAT) and similar techniques, a chemical labelling reagent is used for this purpose (see Fig. 1 which illustrates the concept).

Of course, a chemical reaction is today the most used labelling approach, but enriched stable-isotope tags can be introduced in the analyte biomolecule by enzymatic or metabolic reactions as well, as widely documented in the excellent review on this topic by Bantscheff et al. [5]. ICAT, and many ICAT modified versions reported in the last decade, still have serious limitations [6]. In fact, such elemental and molecular MS methods based on internal labelling present inherent severe disadvantages for absolute quantifications:

(a) There is a need for quantitative labelling reactions (the reaction should be stoichiometric, fast and reproducible) with known stoichiometries.
(b) The labelling should be selective enough (ideally specific).
(c) The excess of reagents should be readily separated from the bioconjugated compound.

Another limitation to consider in ICAT relative measurements is that peptide isoforms obtained with heavy and light ICAT reagent labelling did not exactly coelute in reversed-phase chromatography and their isotope ratio varied during elution.

Although reports and efforts to achieve absolute quantifications are available in the literature, most of the papers published on applications relate to "relative" protein content measurements, comparing two states of a cell or tissue (e.g. differential expression between cancer and a normal cell, which are compared to identify possible cancer biomarkers) as noted before.

The ICP-MS promise

A possible alternative to such quantification problems, such as those encountered using soft ion sources (MALDI or ESI), is to resort to a hard ion source such as ICP-MS (see Table 1 detailing the specific unique features of ICP-MS detection for proteomics).

Admittedly, ICP-MS-guided proteomics today relies on the presence of a detectable heteroatom (i.e. an element different from C, H, O and N) in the sought protein(s) and so a previous knowledge of the biocompound to be determined is needed (targeted proteomics). However, the analytical potential of ICP-MS for element/isotopes detection (Table 1) for quantitative "targeted" proteomics is today undeniable and this fact seems to be ignored by common molecular MS users [5].

In fact, the great analytical potential of ICP-MS (in combination with appropriate separation techniques) to determine with extreme sensitivity and selectivity non-metals (e.g. P, S, I, Br), semimetals (Se, As) and metals (Fe, Cu, Zn, Cd, etc.) associated with important biomolecules (and eventually the whole metal–biomolecule) has been already widely demonstrated [7].

Of course, the straightforward field of applications of ICP-MS-guided "heteroatom-tagged proteomics" involves the detection and quantification of proteins naturally having such ICP-MS-detectable heteroatoms (e.g. metals in metalloproteins, Se in selenoproteins, P in phosphoproteins, etc.).

Today, however, a few authors are trying to extend the exceptional features of ICP as ion source for MS to almost every protein determination by using "labelling" principles (similar to those discussed earlier for molecular MS). If an appropriate labelling with an element or isotope is accomplished, ICP-MS detection advantages could be obtained, opening the door to a great number of new ICP-

[1] Note: The term "absolute" here refers to its usual meaning in the proteomics world (i.e. absolute amount of the protein determined without the need for a relative comparison between two different biological states). Of course, as an internal standard is still needed here for quantification, this term is not equivalent to the common meaning in analytical chemistry where "absolute" determinations refer to quantifications without the need for chemical standards.

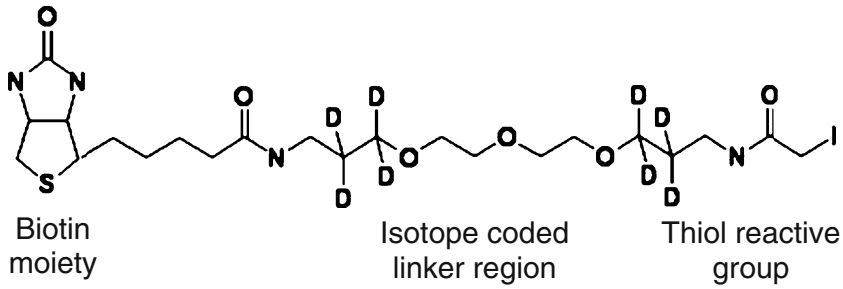

Fig. 1 Isotope-coded affinity tag (ICAT) original reagent, showing the three basic parts: biotin moiety for purification on avidin columns, deuterium isotope-coded linker region and reactive group for thiols of proteins (hydrogen atoms are omitted for clarity)

MS applications related to absolute quantification of peptides and eventually of targeted proteins [7].

With ICP-MS the real efficiency of present methods used worldwide for sample preparations, preconcentrations, separations, etc. in proteomics analysis could be assessed [8] due to the "absolute" character of such determinations. Such measurements could be of enormous value in high-throughput proteomics, where such reliable quantification approaches are urgently and widely demanded [9, 10].

In spite of the bright future of ICP-MS detection in the field, design and evaluation of labelling reagents to extend elemental (isotopic) MS analysis of proteins are scarce and rather preliminary. Only a few reports have been published on the intentional introduction of a heteroatom into a specific amino acid, or other active group, to render the protein detectable by ICP-MS [10].

Basic approaches reported

Several strategies have been reported so far, the first being the use of a bifunctional reagent with a selective antibody at one end, for biochemical recognition of the biocompound, and an europium chelate at the other for ICP-MS detection [11]. This concept was later extended to using bifunctional reagents in which the recognition end was another chemical group able to react selectively with the biomolecule as in the so-called metal-coded affinity tags (MeCAT) [12] (see Fig. 2 for the used bifunctional chemical reagent). The second, operationally much simpler approach, uses a low mass chemical reagent, which is known to provide a straightforward reaction with a particular group of the protein or peptide (e.g. –SH or phenol groups) and contains the desired heteroatom in its structure (e.g. Hg [13] or I [14]).

A third approach, demonstrated by Zhang et al. [15] in 2002, consists of using antibodies for recognition but, in this case, bioconjugated with colloidal gold nanoparticles to allow detection by ICP-MS.

It is worth noting, however, that most previously cited labelling applications are intended to introduce one heteroatom in the desired biomolecule (as usually occurs for natural tags). Thus, the exceptional capability of ICP-MS for multi-element (multi-isotope) simultaneous determinations is lost. In other words, such uni-elemental uses of ICP-MS are a long way from the common high-throughput approaches of today's proteomics or even from targeted proteomics where simultaneous determinations of several biologically relevant biomolecules (e.g. biomarkers) are sought for. However, as ICP-MS provides elemental (isotopic) detection with extreme selectivity and sensitivity for around 80 stable elemental isotopes, up to nine orders of

Table 1 Unique features of ICP-MS detection for proteomics

1. Specificity to the heteroatom (metals, semimetals and non-metals except C, H, O and N)
2. Compound and matrix-independent detection
3. High elemental sensitivity
4. Sample preparation and purity requirements comparably low (robustness)
5. Direct isotopic information (heteroatoms with multiple isotopes) allowing one to carry out very accurate and precise quantitative isotope dilution analysis (IDA)
6. Versatility to be coupled to HPLC, CE and GC (e.g. for elemental speciation)
7. Great potential for species (molecules) quantitative metabolism and nutritional studies using enriched isotopes and HPLC separations
8. Multi-element (-isotope) capabilities

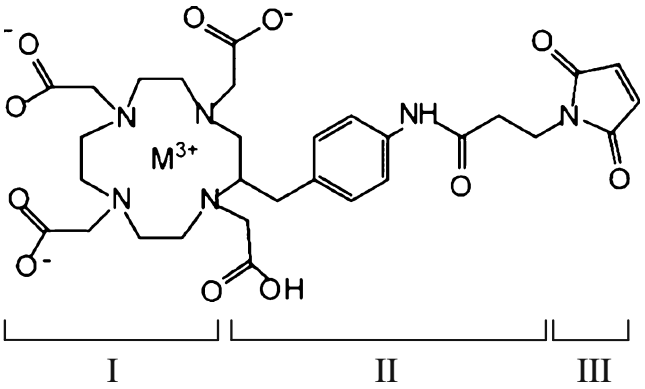

Fig. 2 Metal-coded affinity tag (MeCAT) label, showing the metal chelate complex (*I*) based on DOTA (1,4,7,10-tetraazacyclododecane *N*,*N*′,*N*″,*N*‴-tetraacetic acid) reagent, a spacer (*II*) which connects the macrocycle and, finally, the maleimide reactive group (*III*) for thiol-specific labelling [12]

magnitude dynamic range, and with low biological analysis background, ICP-MS-based detection may be ideal for multiplex bioassays [16] provided that adequate labelling strategies are available.

Using ICP-MS allows one to overcome some of the serious limitations of currently used fluorescent tags [6, 17] particularly those derived from the spectral overlap of the different dyes used for multiplexing and the difficulty in simultaneously measuring analytes (targets) differing by one or more orders of magnitude in their actual concentrations in the sample solution.

In the last 3 years several reports have demonstrated successful multiplexed bioassays with ICP-MS detection. One of the most promising examples is the recent use of the pioneering labelling concept [11] but improved by using a water-soluble polymer bearing multiple metal-chelating ligands, forming high affinity complexes with lanthanide ions [17], to increase the sensitivity. Monoclonal antibodies to five leukaemia cell surface markers were labelled with five different lanthanide elements (Pr, Tb, Ho, Eu and Tm), illustrated in Fig. 3 for one antibody and one metal. By ICP-MS final detection of the corresponding metals the possibility of multiplexed quantitative analysis of the five sought proteins in cells was demonstrated. Another recent example of such successful use of the elemental detection for multiplex bioassay includes the detection of glycoproteins [18]. Here lectins, instead of antibodies, are bioconjugated to the metal-chelating polymer tag. Such element-labelled lectins are able to distinguish between glycoproteins (although lectins are less specific than antibodies) and when multiple different lectins are employed with different sugar specificities (in a multiplexed assay with ICP-MS elemental detection) a fingerprint of lectin activities can be used to identify

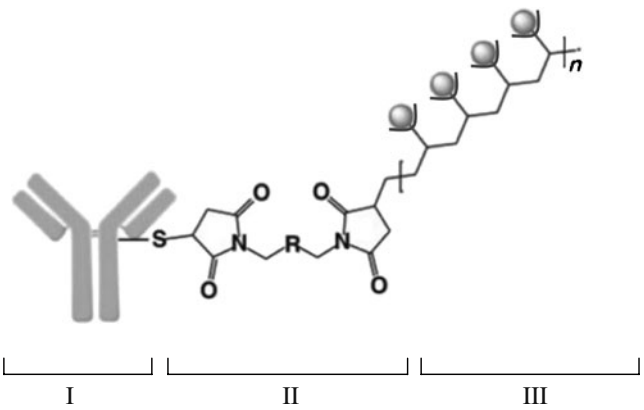

Fig. 3 Polymer-based elemental tag for antibody bioconjugation: the reactive –SH groups of the selected antibody (*I*) react with the terminal maleimide groups (*II*) of a polymer bearing many metal-chelating ligands along the polymer backbone (*III*). Each type of antibody desired is labelled with a different element [17]

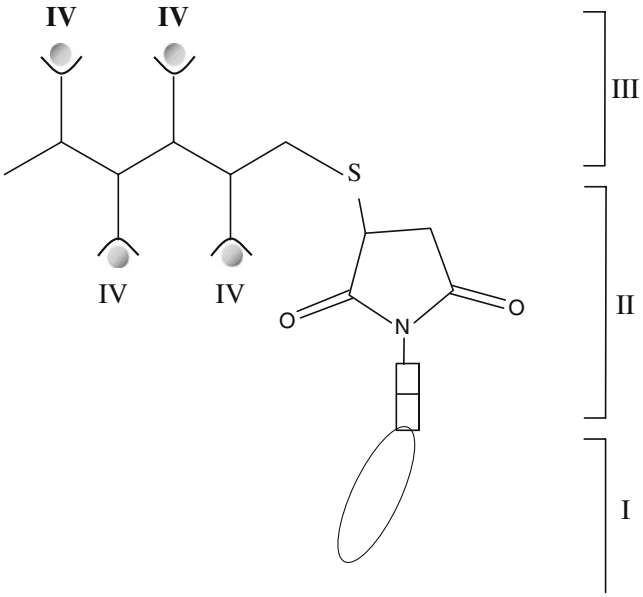

Fig. 4 Polymer-based elemental tag for lectin (shown as an *ellipse* for clarity) bioconjugation. In this case, the lectin (*I*), with appropriately modified surface lysine residues to allow maleimide activation (*II*), is bioconjugated to a thiol-containing polymer (*III*), previously loaded with the appropriate lanthanide ion (*IV*) [18]

glycosylation patterns in proteins (see Fig. 4 illustrating this clever bifunctional reagent for glycoproteins).

Most of the above described multiplex methods are based on a typical heterogeneous reaction (e.g. a microtiter plate assay [18]) requiring extensive and error-prone procedures of sample preparation and washings to separate the excess labelling reagent. A recent multiplex determination of cancer protein biomarkers, however, describes bioassays carried out in solution. Again, soluble metal-tagged antibodies in a bifunctional reagent labelled with different lanthanides complexed by DOTA [19]are used, but the critical immunocomplex and excess unbound antibody separation is accomplished by conventional size exclusion chromatography (SEC) coupled on-line to the ICP-MS. Such a typical "trace element speciation" approach has proved most useful for multiplex bioassay purposes. Its analytical potential and advantages have been discussed and its first application to discriminate between ovary and uterus tumour tissue samples from those of healthy subjects is described in ref. [19].

As ICP-MS is typically a liquid solution detector, most applications dealing with the determination of proteins by elemental labelling have been carried out with final ICP-MS measurements of a liquid solution (e.g. after appropriate separation, and dissolution if needed, of the labelled complex). However, there are important bioassays using classical immunological reac-

tions (mainly for protein identification but also for relative expression assessment) where the proteins are appropriately separated in a solid phase (e.g. 2D gel electrophoresis spots and immunomicroarrays).

The Western blot or immuno-blot procedure is usually applied after general 1D or 2D gel electrophoresis separation of the proteins of the sample. The proteins in the separated "spots" of the gel are typically transferred to a nitrocellulose or poly(vinylidene difluoride (PVDF) polymer membrane and, once there, they are detected by using specific antibodies which bind only to the sought protein (the antigen). The exceptional separative capabilities of 2D gel electrophoresis and so the practical applications of this method in immunology, biochemistry, biology and clinical chemistry are today commonplace for proteomic researchers. Thus, it would be desirable to have a solid sample introduction device able to transport directly the separated protein(s) from the spots to the ICP-MS for detection.

The application of laser ablation (LA) to such purposes (LA-ICP-MS) for the analysis of 1D sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)-separated proteins, after the blotting process and subsequent immunolabelling with nanogold-labelled antibodies, was reported 5 years ago [20]. Jakubowski et al. [21], in their third paper reporting the use of such a technique for detection of proteins after SDS-PAGE separation, compared two strategies for elemental labelling of antibodies, in combination with Western blot experiments. They reported a three-plex assay based on simultaneous LA-ICP-MS detection of three DOTA-complexed lanthanide elements (Ho, Tb and Tm). Results observed by LA-ICP-MS of these three elements after SDS-PAGE separation of three model proteins (lysozyme, bovine serum albumin and casein) agreed well with results obtained by conventional Coomassie stained bands technique [21].

Microarray-based multiplexed detection with ICP-MS

The high-throughput demands of present proteomics studies have driven developments in the fabrication and detection modes of microarrays for proteins. Labelled probe methods for detection of many captured target proteins (evolved from classic clinical immunoassay techniques and concepts) in an array is usually carried out by fluorescence using a charge coupled device (CCD) camera. Of course, there is room for technical improvement and for alternative detection modes, e.g. ICP-MS. The advantages of conventional ICP-MS for immunoassays with an element-tagged antigen, or antibody, have already been detailed and assessed [16] but are hampered if the formed antigen–antibody complex has to be broken by acids for dissolution, before final ICP-MS measurement in the microplate wells [22].

Of course, a solid sampling introduction system such as LA, able to volatilise analytes in a spot of a few microns on the solid microarray, could get around that limitation.

Zhang's group, in a further development of their pioneering work [15] on antibody elemental labelling for ICP-MS, more recently reported the detection of multiple proteins on one spot by LA-ICP-MS to demonstrate the viability of ICP-MS in carrying out multiplexed protein analysis in immunomicroarrays [23]. It was possible to detect three model proteins: α-fetoprotein IgG (AFP), carcinoembryonic antigen (CEA) and human IgG on the basis of sandwich-type immunoreactions on a microarray with appropriate Sm^{3+}, Eu^{3+} and Au^{0} labelled antibodies and final LA-ICP-MS specific detection (Fig. 5). The ICP-MS detection of multiple analytes in each spot of the microarray with micron spatial resolution was thereby demonstrated. Thus, the present technical needs to fabricate high density arrays for multiplex assays can be mitigated

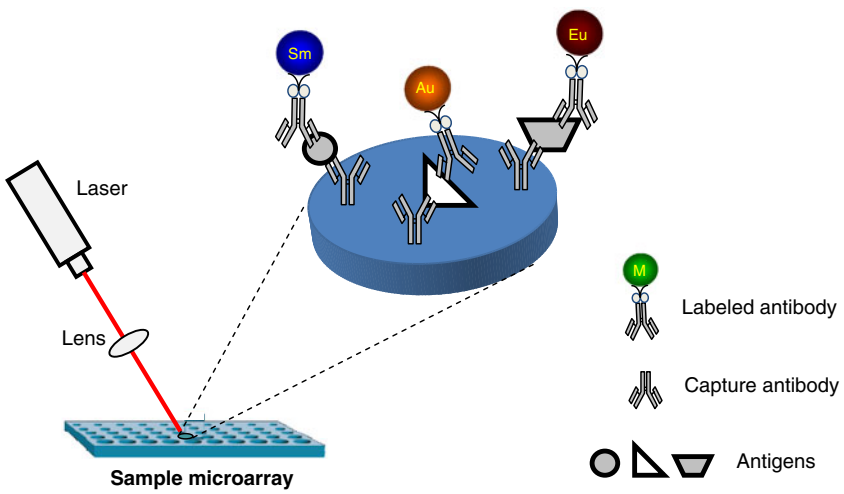

Fig. 5 Laser ablation scheme for multiplexed direct sampling analysis from a microarray. Detection of three different analytes (antigens) on each spot of the microarray is schematically depicted [23]

when LA-based ICP-MS strategies are used for protein microarray readout [23].

ICP-MS-based protein activity measurements

Most recently, Lathia et al. [24] described a novel ICP-MS-based bioassay to measure the activity of proteases. The developed methodology is based again on the use of a lanthanide-tagged protease substrate as labelling reagent containing a biotin label at the C- terminus and the lanthanide chelator at the other end (N-terminus). After incubation with the protease (analyte) the cleaved products were separated from the uncleaved peptide substrate via the biotinylated end reacting with streptavidin agarose beads and separation by centrifugation. Lanthanide determinations of the soluble cleaved products by ICP-MS provided an analytical signal proportional to chymotrypsin enzyme activity. The detection limit achieved in this way was better than that of a similar fluorometric assay and the successful use of that ICP-MS-based chymotrypsin activity assay was demonstrated in HeLa cell lysates [24].

An alternative approach for such activity determinations, based on ICP-MS measurements, takes advantage of enzymes in which the heteroatom is eventually responsible for the protein activity. Using this concept of heteroatom-tagged proteomics [7], we have most recently published [25] the use of HPLC-ICP-MS hybrid techniques to simultaneously obtain the Cu, Zn-superoxide dismutase (SOD) absolute concentration and its enzymatic activity. HPLC-ICP-MS with post-column IDA of Cu has been applied for quantification, after appropriate evaluation of the protein sample preparation and column recoveries. A good linear correlation was observed between such Cu-SOD determination (concentration of the protein) and alternative activity results using the spectrophotometric pyrogallol autoxidation method. Interpolation in such previously obtained curves allowed us, from only IDA-ICP-MS measurements, to obtain, in addition to the absolute total concentration of Cu, Zn-SOD, its enzymatic activity in red blood cells of a control individual and patients with metallic total hip arthroplasty [25].

Outlook

Protein activity assays could be easily extended to multiplex protease determinations in a single ICP-MS assay if enough knowledge of the desired proteases, to be simultaneously determined, is available (in order to select or synthesize appropriate substrates for each analyte/protein).

In fact, multiplex capability for protease analysis is highly demanded today as proteases rarely act alone. Defining a key network of proteases and quantifying them in a single assay seems a much more reliable biomarker of disease (e.g. of a given type of cancer) than just single protease activity information.

The work by Lathia et al. [24] and our group's recent paper on Cu, Zn-SOD [25] show a route to relatively easy extensions to multiplexed detection of proteases and their absolute determinations. Of course, the individual enzymes to be followed simultaneously or the target protease substrates must be known and should contain the active heteroatom (an isotope easily and accurately followed by ICP-MS) in their structures.

Let me conclude by saying that this emerging application of ICP-MS for heteroatom (isotope) targeted proteomics [19, 26], in multiplexed formats to quantitate concentrations and activities of proteins, taking advantage of the multi-elemental (-isotopic) character of ICP-MS, can bring about new opportunities for creative work and interdisciplinary developments for analytical atomic spectroscopists, particularly those interested in expanding trace element speciation strategies to solve important bioscience challenges (e.g. new insights into largely uncharacterised metalloproteomes [27]).

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References

1. Rosenfeld JM (1999) Solid-phase analytical derivatization: enhancement of sensitivity and selectivity of analysis. J Chromatogr A 843:19–27
2. Lowe N, Jackson M (eds) (2001) Advances in isotope methods for the analysis of trace elements in man. CRC, Boca Raton, pp 1–39
3. Aebersold R (2009) A stress test for MS-based proteomics. Nat Methods 6(6):411–412
4. Bëtmer J, Montes Bayón M, Encinar JR, Fernández ML, Fernández de la Campa MR, Sanz-Medel A (2009) The emerging role of ICP-MS in proteómic analysis. J Proteomics 72:989–1005
5. Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B (2007) Quantitative MS in proteomics: a critical review. Anal Bioanal Chem 389:1017–1031
6. Rivera- Monroy Z, Bonn GK, Guttman A (2009) Fluorescent isotope-coded affinity tag 2: peptide labelling and affinity capture. Electrophoresis 30:1111–1118
7. Sanz-Medel A, Montes Bayón M, Fernández dela Campa MR, Encinar JR, Bëtmer J (2008) Elemental MS for quantitative proteomics. Anal Bioanal Chem 390:3–16
8. Pereira Navaza A, Ruiz Encinar J, Carrascal M, Abian J, Sanz-Medel A (2008) Absolute and site-specific quantification of protein phosphorylation using integrated elemental and molecular mass spectrometry: its potential to assess phosphopeptide enrichment procedures. Anal Chem 80:1777–1787
9. Ong SE, Mann M (2005) MS based proteomics turns quantitative. Nat Chem Biol 1:252–262

10. Prange A, Pröfrock D (2008) Chemical label and natural element tags for the quantitative analysis of biomolecules. J Anal At Spectrom 23:432–459
11. Zhang C, Wu FB, Zhang YY, Wang X, Zhang XR (2001) A novel combination of inmunoreaction and ICP-MS for the determination of thyroid stimulating hormone (THS) in human serum. J Anal At Spectrom 16:1393–1396
12. Ahrends R, Pieper S, Kuhn A, Weisshoff H, Hamester M, Lindemann T, Scheler C, Lehmann K, Taubner K, Linscheid MW (2007) A metal-coded affinity tagged approach to quantitative proteomics. Mol Cell Proteomics 6:1907–1916
13. Guo Y, Xu M, Yang L, Wang Q (2009) Strategy for absolute quantification of proteins: CH_3Hg^+ labelling integrated molecular and elemental mass spectrometry. J Anal At Spectrom 24:1184–1187
14. Pereira Navaza A, Ruiz Encinar J, Ballesteros A, González JM, Sanz-Medel A (2009) Capillary HPLC-ICPMS and tyrosine iodination for the absolute quantification of peptides using generic standards. Anal Chem 81:5390–5399
15. Zhang C, Zhang ZY, Yu BB, Shi JJ, Zhang XR (2002) Application of the biological conjugated between antibody and colloid Au nanoparticles as analyte to ICP-MS. Anal Chem 74 (1):96–99
16. Ortnatsky OI, Kinach R, Bandura DR, Lou X, Tanner SD, Baranov VI, Nitz M, Winnik MA (2008) Development of analytical methods for multiplex bio-assay with inductively coupled plasma mass spectrometry. J Anal At Spectrom 23:463–469
17. Lou X, Zhang G, Herrera I, Kinach R, Ornatsky O, Baranov V, Nitz M, Winnik MA (2007) Polymer-based elemental tags for sensitive bioassays. Angew Chem Int Ed 46:6111–6114
18. Leipold MD, Herrera I, Ornatsky O, Baranov V, Nitz M (2009) ICP-MS-based multiplex profiling of glycoproteins using lectins conjugated to lanthanide-chelating polymers. J Proteome Res 8 (2):443–449
19. Terenghi M, Elviri L, Careri M, Mangia A, Lobinski R (2009) Multiplexed determination of protein biomarkers using metal-tagged antibodies and size exclusion chromatography−inductively coupled plasma mass spectrometry. Anal Chem 81(22):9440–9448
20. Müller D, Diaz-Bone RA, Felix J, Goedecke W (2005) Detection of specific proteins by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) using gold cluster labelled antibodies. J Anal At Spectrom 20(9):907–911
21. Waentig L, Roos PH, Jakubowski N (2009) Labelling of antibodies and detection by laser ablation inductively coupled plasma mass spectrometry PART III. Optimization of antibody labelling for application in a Western blot procedure. J Anal At Spectrom 24:924–933
22. Razumienko E, Ornatsky O, Kinach R, Milyavsky M, Lechman E, Baranov V, Winnik MA, Tanner SD (2008) Element-tagged immunoassay with ICP-MS detection: evaluation and comparison to conventional immunoassays. J Immunol Methods 336:56–63
23. Hu S, Zhang S, Hu Z, Xing Z, Zhang XR (2007) Detection of multiple proteins on one spot by laser ablation inductively coupled plasma mass spectrometry and application to immuno-microarray with element-tagged antibodies. Anal Chem 79(3):923–929
24. Lathia US, Ornatsky O, Baranov V, Nitz M (2010) Development of inductively coupled plasma-mass spectrometry-based protease assays. Anal Biochem 398(1):93–98
25. Nuevo Ordoñez Y, Montes-Bayón M, Blanco-González E, Sanz-Medel A (2010) Quantitative analysis and simultaneous activity measurements of Cu, Zn-superoxide dismutase in red blood cells by HPLC-ICPMS. Anal Chem 82:2387–2394
26. Sanz-Medel A (2005) From metalloproteomics to heteroatom-tagged proteomics. Anal Bioanal Chem 381:1–2
27. Cvetkovic A et al (2010) Microbial metalloproteomes are largely uncharacterized. Nature 466:779–782. doi:10.1038/nature09265