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Engineered nanoparticle surfaces for improved mass spectrometric analyses

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Engineering of nanoparticle surface functionality provides controlled interactions with biomolecules such as cell membrane lipids, proteins and nucleic acids. Concurrently, this surface chemistry control also opens up new avenues for improving mass spectral analyses. In this Minireview, we highlight some of the emerging work that integrates surface-engineered nanoparticles with mass spectrometry to improve the analysis of a wide variety of chemical and biological systems.

Introduction

The unique properties of nanoparticles (NPs) are being exploited in a wide range of areas such as imaging,¹ protein recognition,² drug/gene delivery,^{3–5} biosensing,⁶ and clinical diagnostics.^{7,8} Nanoparticles have also been used for many years to facilitate analyses by mass spectrometry (MS). Early work by Tanaka *et al.*⁹ showed that cobalt particles (~30 nm) suspended in glycerol facilitated the ionization of biomacromolecules such as proteins. Subsequently, C,¹⁰ Ag,^{11,12} Au,^{13–17} and Si¹⁸ micron- or nano-sized particles have been demonstrated as desorption/ionization matrices with different degrees of success. The unique properties of NPs have also caused some researchers to implant these NPs into tissues, such as brain^{19,20} and plant leaf tissues,¹² to facilitate the mass spectral imaging of biomolecules of interest.

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In general, when NPs are used as matrices, they provide little interference in the low mass-to-charge ratio (m/z) range, facilitating the analysis of small molecules.

With advances in synthetic methods has come the ability to tailor the size and surface chemistry of NPs, thereby providing greater control over the physical and chemical properties of the particle surface.^{8,21} For example, organic ligands can be readily introduced onto the surfaces of a wide range of AuNPs to stabilize them against aggregation and endow the particles with unique surface properties.²¹ Being able to engineer NP surfaces provides better tunability of the interactions of particles with biomolecules leading to new applications in biosensing, drug delivery and clinical diagnostics.^{1,7,8} Control over NP surface chemistry also opens up new avenues for mass spectral analyses. In this Minireview, we highlight some of the emerging approaches that use NP surface chemistry to improve MS-based analyses.

Mass 'barcodes' on NP surfaces

Mass spectrometry is an important technique for characterizing the structures of two-dimensional (2D) surfaces. Self-assembled

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monolayers (SAMs) of alkanethiols chemisorbed onto 2D Au surfaces can be cleaved and ionized directly, in many cases, upon laser irradiation.^{22,23} Mrksich and Su^{24–26} have taken this idea further by combining SAMs with matrix-assisted laser desorption/ionization (MALDI)-MS to directly monitor chemical and biological reactions on 2D SAMs. SAMs were engineered with functional groups that reacted with specific molecules of interest, and the resulting reactions were directly monitored by MS. For example, in a SAM-based enzyme activity assay, an enzyme substrate is grafted onto the alkanethiol monolayer and then treated with the enzyme to give the corresponding product. This product has a different m/z ratio than the substrate, essentially allowing the reaction product to be directly ‘read out’ by MALDI-MS.²⁴ This surface readout method enables applications to a broad range of problems in chemistry and biology, including enzyme assays, protein–protein interactions, and chemical screening.²⁶

Similarly, introducing monolayers onto the surfaces of NPs enables a similar ‘read out’ to be performed by MS. The core materials of most metal NPs readily absorb at the common laser wavelengths (e.g. 337 nm from a N₂ laser or 355 nm from a Nd:YAG laser) used in commercial mass spectrometers, eliminating the need to add an organic matrix. The laser energy absorbed by the core material can cleave the metal core–ligand bond(s) and ionize the liberated surface ligands. In effect, these surface ligands can then act as mass ‘barcodes’ for the NP. This idea has been used in several recent biological studies. Nagahori and Nishimura²⁷ tagged AuNP with carbohydrate-functionalized alkanethiol monolayers (e.g. *N*-acetylglucosamine (GlcNAc)), which acted as substrates for glycosyltransferases. Once treated with the proper glycosyltransferase enzyme (e.g. β -1,4-galactosyltransferase), the mass change of alkanethiol ligands was monitored by LDI-MS (Fig. 1), providing a very rapid and direct detection of the enzymatic reaction. Also examined with this approach were stepwise sugar-elongation reactions by sequential enzymatic glycosylation reactions and an inhibition assay using uridine 5'-diphosphate. Because the Au core acts to ionize the surface ligands directly and no additional

organic matrix is used, ionization and spectral interference from other molecules such as salts or proteins is very limited. This lack of interference allowed the carbohydrate-functionalized AuNP assay to be used to directly detect the glycosyltransferase activity of crude cell extracts from *Escherichia coli*. In comparison to the 2D SAMs assays described above, the AuNPs offer an additional advantage. Because the AuNPs are dispersed in solution, adsorbed analytes are efficiently and readily transferred between instruments and can be easily manipulated in biological assays with minimal sample loss. One potential drawback, though, is the limited ability of LDI-MS to ionize high molecular weight ligands in comparison to MALDI-MS.

Secondary ion mass spectrometry (SIMS) can also be used to monitor surface ligands of NPs. Kim *et al.*²⁸ demonstrated that peptides adsorbed to AuNPs are more efficiently ionized in SIMS than peptides adsorbed to 2D gold surfaces with SAMs. This enhanced ionization effect enabled the sensitive detection of peptide modifications caused by enzymatic reactions (e.g. phosphorylation) of peptide substrates. This same approach was used later by Kim *et al.*²⁹ to design a label-free protein kinase assay based on peptide-conjugated AuNPs and SIMS. The AuNPs acted as both signal enhancers and target concentrators. The kinase reaction resulted in phosphorylation of the peptides bound to the AuNPs, and the extent of phosphorylation was readily and sensitively probed by monitoring the resulting 80 Da mass increase of the adsorbed peptide substrates. Phosphorylation efficiency was determined in a single spectrum by taking the ratio of the phosphorylated and unphosphorylated peptide ion signals. This label-free kinase assay was further used to quantitatively screen kinase inhibitors. When compared to conventional fluorescence assays of kinase activity, the approach based on AuNPs and SIMS is a higher throughput method because the mass spectral readout easily allows multiple substrates to be simultaneously monitored. A similar approach was also used to construct a label-free activity assay for the detection of matrix metalloproteinase (MMP) in human serum. The peptide cleaved from the AuNP surface by MMP-7 was characterized by SIMS. This method showed a detection limit for MMP-7 as low as 20 ng mL⁻¹ (1 pmol mL⁻¹) in human serum.³⁰ Currently, however, the SIMS approach is limited to analytes that have molecular weights less than 1300 Da.²⁹

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Fig. 1 Schematic illustrations of carbohydrate-conjugated AuNP for the glycosyltransferase activity assay (a) and readout by LDI-MS (b).

The ease with which NP surface ligands can be desorbed and ionized enabled our group to recently develop a new technique for simultaneously identifying and quantifying the cellular uptake of multiple AuNPs using LDI-MS (Fig. 2).³¹ The tunable surface functionality of NPs provides design flexibility to facilitate specific targeting, efficient cellular uptake and controllable release of NPs.^{1,3,4} Techniques such as TEM and fluorescence spectroscopy are commonly used to follow cellular uptake of NPs, but these techniques are not readily able to track multiple NP types simultaneously. In our work,³¹ AuNPs were tagged with readily ionizable cationic or neutral surface functionalities that acted as mass barcodes for the specific AuNPs (Fig. 2c). Upon laser irradiation of these AuNPs, the surface functionalities were simply read out by LDI-MS, providing characteristic peaks for identifying the AuNPs (Fig. 2b). A key advantage of this approach is that it allows a side-by-side comparison of the influence of NP surface chemistry on NP cellular uptake efficiency. In one set of experiments, the uptake of four different AuNPs by mammalian kidney cells (COS-1) was quantified. These experiments indicated that NPs with positively-charged surfaces were more readily taken up than neutral NPs. In addition, subtle changes to the surface hydrophobicity of the positively-charged NPs lead to significant changes in cellular uptake propensities, with moderately hydrophobic NPs being taken up more efficiently than very hydrophobic NPs.³¹ Such multiplexed screening of AuNPs should be valuable for rapidly assessing the chemical and physical parameters that influence NP uptake by cells. Another important aspect of this approach was its sensitivity. We found that different NPs could be simultaneously identified and quantified at levels as low as 30 pmol.

Mass barcodes on AuNPs have also been used to report and amplify the signal associated with DNA hybridization, allowing for ultrasensitive detection of DNA. Qiu *et al.*³² tagged AuNPs with an alkanethiol monolayer as well as probe DNA strands. A three-component DNA hybridization assay was then designed (Fig. 3). First, capture DNA strands were covalently immobilized on a glass or silicon wafer chip. Next, the modified chip was

hybridized with target DNA strands, followed by washing to remove free target DNA. The chip was then incubated with probe-strand-capped AuNPs that allowed the probe DNA to hybridize with the overhanging region of target DNA sequence. Hybridization caused the AuNPs to be captured and immobilized on the chip. The small alkanethiol monolayers, rather than DNA strands, on the AuNPs were then directly read out by LDI-MS, thereby reporting on the target DNA strands. The surrogate alkanethiol monolayer provides an amplified signal because the binding of a single AuNP with the complementary DNA target molecule leads to the release of multiple AuNP surface ligands upon laser irradiation. As a result, detection limits as low as 100 pM were achieved. Multiplexed analyses of several DNA strands with different sequences were also achieved by using distinct mass barcodes on each AuNP that carried a different probe DNA sequence. Micron-sized Au particles have also been used in a very similar manner by Lee *et al.*³³ to achieve attomolar level detection of antigen–antibody binding. Presumably, the larger particles with an increased number of reporter ligands provided an even more amplified signal.

Most of the studies to date that have used the mass barcode approach have relied on AuNPs because of the ease with which these NPs can be synthesized and functionalized and because of the biocompatibility of Au. Other functionalized NPs that readily absorb UV laser light, however, are also expected to be amenable to this mass barcode approach.

Functionalized NPs for selective extraction and concentration

MS is a versatile and effective method for analyzing a wide array of compounds. When samples are very complex, however, ion suppression can limit the overall utility of the technique. Consequently, on-line chromatographic techniques are often used to fractionate complex mixtures before mass spectral analysis. An alternate approach is to use sample cleanup methods that provide either selective extraction of the desired

Fig. 2 Multiplexed tracking of AuNP cellular uptake by LDI-MS. (a) Schematic illustration of the analysis of the AuNPs in cell lysates by LDI-MS. (b) Illustration of an LDI mass spectrum. (c) Structural representation of the AuNPs. The mass/charge ratios (m/z) attached to each AuNP act as mass barcodes for identification of the AuNPs.

Fig. 3 Mass barcoded AuNPs for a DNA hybridization assay with LDI-MS readout.

analytes or selective rejection of interfering species. NPs have many desirable attributes for the creation of selective extraction agents, and their small size makes them inherently effective as concentration agents. When compared to micron-sized particles used in most solid-phase extraction methods, NPs have much higher surface area-to-volume ratios. As particle radius decreases from 10 μm to 10 nm, for example, the surface area-to-volume ratio increases 1000-fold. This increase translates into much greater extraction capacity. If NPs can be dispersed into solution, then analytes can have ready access to NP binding sites in a way that is not possible with micron-sized particles that readily settle in solution. Finally, when compared to micron-sized particles, NPs should be able to more efficiently concentrate analytes because they can pack more closely together, thereby bringing their attached analytes into a much smaller volume (Fig. 4).

Because of their inherent advantages, numerous researchers have investigated NPs as extraction/concentration agents for coupling with MS. Some bare metal and metal oxide NPs have intrinsic affinities for certain functional groups, and so they can be used to extract and enrich analytes with these functional groups. Some examples include TiO_2 NPs that can be used for phosphopeptide enrichment³⁴ and AuNPs that have been used for selective enrichment of thiol compounds or cysteine-containing peptides.¹⁵ The tunable surface chemistry of NPs, however, increases the scope of analytes that can be targeted for extraction. Here, we focus on studies that describe the rational design of NP surfaces for selective extraction of specific analytes. NP surfaces have been designed for both non-covalent and covalent extractions (Fig. 4).

Non-covalent selective extractions have relied on either non-specific or bio-specific interactions between NPs and targeting analytes. Non-specific extractions are mainly based on electrostatic or hydrophobic interactions. For example, our group recently employed AuNPs with cationic or anionic ligands to extract peptides according to their pI values.³⁵ Cationic and anionic AuNPs selectively targeted negatively- or positively-charged peptides, respectively, with efficiencies that were found to be dependent upon peptide pI and solution pH. The extracted peptides were then directly analyzed by MALDI-MS after aggregating the solution-dispersed NPs, and detection limits as low as 500 pM from a volume of 250 μL were achieved. This electrostatic-based extraction strategy was also successfully used to extract peptides from a tryptic digest of myoglobin. Similarly,

Chen and Chen³⁶ modified magnetic iron oxide NPs with negatively-charged functionality to selectively trap positively-charged insulin (pI 5.3) by adjusting the solution pH to 5. The group found that higher solution pH values prevented the NPs from extracting insulin because the NPs and protein were both negatively charged at pH values >8 . In another example of the use of hydrophobicity to target analytes, AgNPs tagged with dodecanethiol and octadecanethiol were used to extract and pre-concentrate peptides and proteins prior to atmospheric pressure MALDI analysis.³⁷ The value of this approach was demonstrated by extracting gramicidin from urine and plasma samples. Extraction time, pH, ionic strength, temperature and alkyl chain length were found to be the governing factors in the extraction. The optimum extraction efficiency of gramicidin was observed at pH 7.0 for 1.5 h of extraction time and the addition of 7% NaCl. The AgNPs modified with octadecanethiol showed better performance than those AgNPs modified with dodecanethiol. The limits of detection for gramicidin using these AgNPs were 0.13 and 0.16 μM in urine and plasma, respectively.

Non-covalent extractions using bio-specific interactions, such as antibody–antigen, ligand–protein, and protein–protein interactions have also been achieved by introducing one of the binding partners onto the NP surfaces. For example, magnetic NPs conjugated with antibodies have been used as affinity probes with mass spectrometric analysis to profile biomarker proteins in human plasma.³⁸ A multiplexed immunoassay was constructed using various antibody-conjugated NPs. Serum amyloid P (SAP), C-reactive protein (CRP) and serum amyloid A (SAA) in 1 μL of human plasma were selectively and simultaneously captured, separated and analyzed by MALDI-MS. Methoxy-ethyl-terminated ethylene glycol (MEG) ligands grafted on the NP surfaces together with the antibodies significantly suppressed non-specific binding during the separation of the protein biomarkers. Lin *et al.*³⁹ have conjugated concanavalin A on magnetic NPs to specifically extract mannose from human plasma for detection by MALDI-MS. Similarly, NPs immobilized with the disaccharide moiety $\text{Gal}(\alpha 1-4)\text{Gal}\beta$ were used for affinity capture of uropathogenic P fimbriated *E. coli* through

Fig. 4 Schematic illustrations of selective extraction and concentration with functionalized NPs.

disaccharide–protein interactions (e.g. Gal(α 1–4)Gal β –P fimbriae interaction).⁴⁰ The specificity of *E. coli* capture was confirmed by measuring *E. coli*-specific proteins by MALDI-MS. Recently, Li *et al.*⁴¹ tagged magnetic NPs with a nitrilotriacetic acid derivative (NTA) to facilitate selective MALDI-MS analysis. Ni(II) was immobilized on the surfaces of the NTA-magnetic NPs, and these particles were shown to be very effective at selectively trapping His-tagged peptides and proteins from cell lysates. When either Zr(IV) or Ga(III) ions were immobilized on the surfaces of the NTA-magnetic NPs, the NPs were able to selectively enrich phosphorylated peptides from tryptic digests.

Covalent extractions are achieved by forming covalent bonds between functional groups on the NP surface and the targeted analytes. The relative irreversibility of such interactions allows non-specifically bound analytes to be more effectively removed *via* more rigorous washing procedures. As an example, Palani *et al.*⁴² functionalized disulfanylpuridine, a thiol-specific and reactive functional group, onto the surface of superparamagnetic Fe₃O₄@SiO₂ NPs. These NPs were used to selectively capture cysteine-containing peptides *via* the formation of a disulfide bond with almost no contamination from non-specifically interacting peptides. The captured cysteine-containing peptides were released from the NPs by reduction of the disulfide bond by tris(2-carboxyethyl)phosphine (TCEP), followed by LC/MS/MS analysis. The covalent extraction strategy was also successfully used to extract cysteine-containing peptides from tryptic digests of enolase and depleted human serum. Another covalent capture example involved the use of aminoxy-functionalized AuNPs to efficiently capture glycosphingolipids.⁴³ Glycosphingolipids extracted from cells or mouse brains were converted to aldehydes by selective ozonolysis of carbon–carbon double bonds in glycosphingolipids. The generated glycosphingolipid aldehydes were enriched by forming an oxime bond upon exposure to the aminoxy-functionalized AuNPs. The captured glycosphingolipids were then directly analyzed by MALDI-MS through the cleavage of oxime bonds under laser irradiation. This AuNP capture approach appears to have widespread utility for identifying and characterizing whole glycosphingolipids present in living cell membranes.

Engineered NP surfaces for improved ionization

Early work by Tanaka *et al.* demonstrated the ability of NP dispersions in glycerol to ionize large molecules.⁹ More recently, NPs and nano-structured surfaces^{44–47} have been used to analyze small or large molecules directly. The key advantage of such approaches for producing analyte ions is the reduced interference in the low *m/z* region of the mass spectrum, facilitating small molecule analysis and somewhat simplifying sample preparation. Initial work by Russell and co-workers illustrated that low concentrations of AuNPs could be used to directly ionize peptides.¹⁴ However, the use of AuNPs as the matrix results in more analyte fragmentation and abundant alkali-adducted analyte ions (e.g. [M + Na]⁺, [M + K]⁺). Furthermore, the sensitivity of this approach is much lower than conventional MALDI with organic matrices. To be more effectively used as matrices for MALDI, NPs need to meet the three criteria that conventional organic matrices fulfill: (i) absorb the laser energy; (ii) prevent analyte aggregation; and (iii) provide a source of charging (e.g. H⁺). AuNPs automatically fulfill the first two roles, but in an

attempt to meet the third criterion, 4-aminothiophenol (4-ATP)- and 4-mercaptobenzoic acid (4-MBA)-functionalized AuNPs were recently investigated as matrices (Fig. 5).^{48,49} The premise was that the organic monolayer would act as a proton source to promote efficient production of protonated analytes ([M + H]⁺). This approach was successful as, for example, the [M + H]⁺ ion signal of the peptide ACTH(18–39) was found to be 3 orders of magnitude greater when using the 4-ATP-capped AuNPs than when using citrate-capped AuNPs. In addition, the mass spectra with both new matrices were found to be less complicated due to the drastic reduction of alkali-adducted analyte ions and fewer fragment ions. In a related approach, Lin *et al.*³⁹ functionalized magnetic NPs with 2,5-dihydroxybenzoic acid (DHB), which is a typical stand-alone organic matrix used in the analysis of peptides and proteins. It was demonstrated that the DHB-functionalized NPs allowed small molecules to be readily ionized with minimal interferences from DHB molecules in the low *m/z* region, which is in marked contrast to analyses done in the presence of DHB alone. An additional benefit of the DHB-functionalized NPs was the reliable quantitation of small molecules without resorting to internal standards. NPs with silicon cores have also been used to enhance ionization. Wysocki and co-workers¹⁸ derivatized silicon NPs with several functional groups to deactivate and stabilize the NP surfaces, and found that a fluorinated compound, [(pentafluorophenyl)propyl]dimethylchlorosilane, enhanced ionization efficiency and reduced the background signal in the low mass range when compared to underivatized silicon NPs.

Summary and outlook

Engineering NP surfaces allows us to exploit the unique chemical and physical properties of NPs. In this review, we have highlighted three ways in which designed NP surfaces can be utilized to enhance biochemical detection when coupled with MS. The strong UV absorption cross-section of metal NPs and the ease with which this energy can be transferred to desorb and ionize surface ligands provide an opportunity to use the surface ligands as mass barcodes for detecting and monitoring NPs and the chemistry they undergo. There are numerous potential ways to exploit such mass barcodes, but we predict that this approach will prove very useful for tracking and perhaps imaging NPs in complex biological samples such as tissues.

Engineering NP surface chemistry also enables the development of selective and highly efficient extraction agents. Because

Fig. 5 Schematic representation of ionization-enhancing 4-aminothiophenol (4-ATP)-capped AuNP and AuNP-based LDI-MS analysis of peptides.

of their small size and ability to pack very closely together, the inherent extraction capabilities of NPs are accompanied by excellent concentration factors. A potentially exciting application of such surface-engineered NPs is the extraction/detection of compounds in live cells. NPs of the appropriate size and surface chemistry can spontaneously cross cell membranes.^{1,4,31} If these NPs are functionalized with appropriate surface groups, they could be used to monitor the level of given intracellular compounds under different cell states.

NP surface chemistry has recently been used to improve the MALDI ionization efficiency of peptides in the absence of traditional organic matrices, thus enabling low interference detection of such compounds. An intriguing extension of such an approach would be the functionalization of NPs with surface groups that can both extract compounds of interest and allow for direct, and perhaps selective, ionization of the captured compounds with minimal sample workup. Taken together, the versatility of NP chemistry coupled with the inherent sensitivity and high specificity of MS will enable many other creative approaches to be developed for the analysis of *in vivo* and *in vitro* systems.

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