

Surface-Enhanced Raman Scattering Sensing of Transition Metal Ions in Waters

Luca Guerrini* and Ramon A. Alvarez-Puebla*



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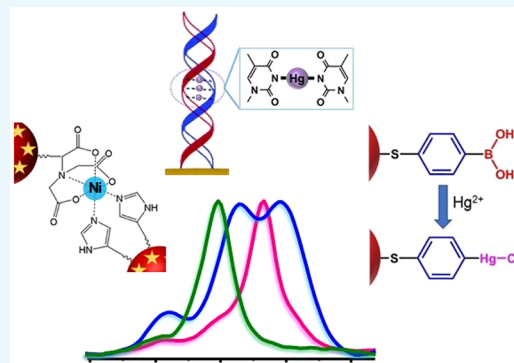
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ABSTRACT: In this mini-review, we provide a coherent discussion on the sensing schemes exploited in the surface-enhanced Raman scattering (SERS) analysis of transition metal ions in waters. A critical approach was used where illustrative examples are selected to discuss key drawbacks and challenges associated with various experimental configurations and the employed enhancing substrates.



INTRODUCTION

Recent years have witnessed growing ecological and public health concerns associated with transition metal contamination.¹ Although these metals are naturally occurring elements, anthropogenic activities have caused a dramatic increase in human exposure. Furthermore, even when transition metals are essential nutrients for multiple biochemical and physiological functions, acute and chronic exposures can cause major adverse health effects, including the development of neurodegenerative and cancer diseases.^{1,2}

The extent of their toxicity and carcinogenicity is strictly related to the type and speciation of the transition metal, as well as exposure time and dose.^{1,2} Accordingly, their maximum allowable concentrations in the environment significantly vary from element to element. However, guidelines for drinking water quality, recommended by the World Health Organization, the European Union, or the Environmental Protection Agency, typically set maximum contaminant limits between the pm and ppb regimes.²

A great effort has been devoted to the exploitation of the unique properties of nanoscale materials for the design of advanced analytical tools fulfilling sensitivity, selectivity, and reproducibility requirements while simultaneously enabling their implementation into cost-effective miniaturized devices for real-time monitoring of these pollutants.^{3,4} A paradigmatic example of a nanoscale analytical technique is surface-enhanced Raman scattering (SERS) spectroscopy. SERS exploits the strong electromagnetic fields generated at the surface of plasmonic nanostructures upon excitation with light (i.e., localized surface plasmon resonances, LSPRs) to magnify the

Raman scattering of molecules located in their close proximity by many orders of magnitude.⁵ This high sensitivity granted by the plasmonic-mediated intensification combines with the high specificity and experimental flexibility of Raman spectroscopy making SERS an ultrasensitive technique. At the same time, the possibility of engineering integrated portable SERS-based sensors paved the way for the implementation of SERS as a potential tool to perform rapid on-field quantification of analytes in real-life environmental and biological media.^{6,7}

SERS detection of transition metals in waters is almost a 15 years old field of research which has been exponentially expanding in recent years.⁸ This growth is built upon the advances in nanofabrication techniques as well as the design of molecular ligands and receptors which are progressively facilitating the transition from basic proof-of-concept studies on simple artificial matrices to on-field quantification of transition metals via portable, multifunctional, optical devices.^{9,10} This minireview is mainly devoted to a critical overview and coherent discussion of the different sensing schemes devised to enable the SERS-based quantification of transition metals in aqueous media. In this context, the general features and ideal requirements of the employed plasmonic substrates are

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discussed. Also, representative examples are selected to illustrate key concepts and applications.

■ PLASMONIC PLATFORMS

Colloidal spherical particles synthesized via wet-chemistry methods are typically low-cost substrates that can be easily produced in large batches. However, isolated round nanoparticles do not provide sufficient enhancements for ultra-sensitive detections. Thus, nanofabrication methods have been devised to tackle this issue mostly by engineering: (i) anisotropic individual colloidal nanoparticles;¹¹ (ii) ordered assemblies of nanoparticles stable in solution;¹² (iii) assemblies of nanoparticles on solid inert surfaces;¹³ and (iv) nanostructured 2D and 3D surfaces via either colloidal assembly¹⁴ or nanolithography.¹⁵

The strong dependence of the LSPR on the nanoparticle composition, size, and shape facilitates the tunability of its plasmonic response. Single-particle morphologies such as nanorods, nanocubes, or nanostars localize large electromagnetic fields at the corners and tips, making them far more efficient enhancers than their spherical counterparts.¹¹ Even more outstanding improvements of the enhancing performances can be achieved by promoting the assembling of nanoparticles into superstructures with closely spaced configurations.^{12,13} In this scenario, giant electromagnetic (EM) fields are concentrated at the narrow interparticle gaps (hot spots) as a result of the particle plasmon coupling, yielding enhancements of the Raman signal up to 10^{10} – 10^{11} for those molecules positioned at these interstitial volumes. It is also worth noting that further intensifications of the SERS signals can be determined by an increase of the Raman cross-section of the adsorbed molecule at the metal surface. This phenomenon is known as chemical enhancement and its contribution to the overall signal magnification is typically much smaller than the EM one (commonly within the 10^1 – 10^3 range).⁵

While sensing platforms relying on colloidal nanoparticles, including the anisotropic ones, profit from the relative simplicity of their fabrication, their direct applicability as on-site ultrasensitive and quantitative analytical tools can be challenging. In fact, nanocolloidal stability in complex media with high ionic strength and rich in organic matter is a cause of concern that can severely impact the signal reproducibility. This limitation can be circumvented by organizing the nanostructures into fixed configurations onto solid supports. Such plasmonic films can be generated both from colloidal suspensions and by physical methods. In both cases, the production of aggregated films by casting, or silver islands by physical vapor deposition, is simple and inexpensive. However, these techniques commonly face major difficulties at precisely controlling the nanoparticle organization which, in turn, limits the degree of reproducibility. On the other hand, refined methods such as the controlled fabrication of colloidal supercrystals¹⁴ or the physical evaporation on patterned substrates¹⁵ make possible the production of uniform and highly reproducible plasmonic substrates with well-defined nanostructured features but commonly to the increment of production costs.

Figure 1 illustrates representative examples of different classes of plasmonic substrates employed in the fabrication of SERS sensors for transition metal ions detection. Round colloidal gold nanoparticles of ~ 50 nm diameter (Figure 1A) display a characteristic LSPR centered around 540 nm (Figure 1C, green curve),¹⁶ while gold nanostars of ~ 80 nm diameter (Figure 1B) support a more intense plasmon resonance centered

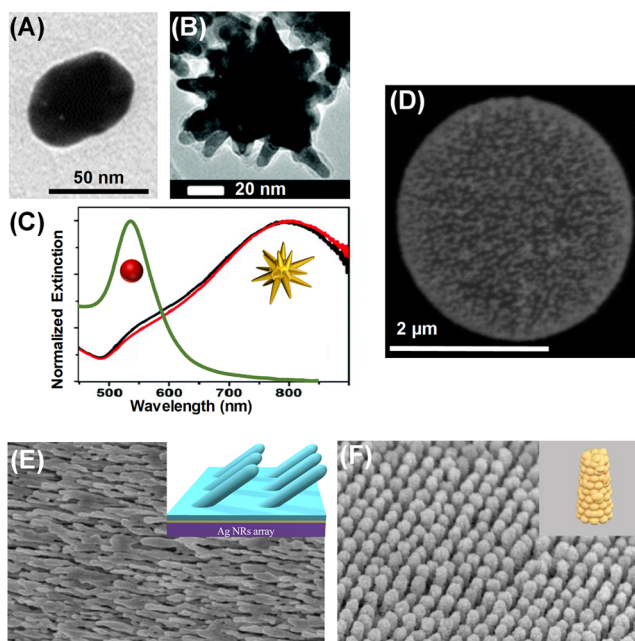


Figure 1. Representative examples of plasmonic substrates employed in the SERS detection of transition metal ions in waters. (A, B) Representative TEM images of a round gold nanoparticle and a gold nanostar, respectively. (C) Normalized extinction spectra of the corresponding colloidal dispersion (black curve, gold nanostars as synthesized; red curve, gold nanostars labeled with malachite green isothiocyanate and coated with a ~ 3 nm silica shell. Adapted with permission from ref 16. Copyright 2014 Royal Society of Chemistry. Adapted with permission from ref 17. Copyright 2015, Royal Society of Chemistry). (D) Hybrid plasmonic particles comprising a non-plasmonic microbead and a dense collection of gold nanoparticle satellites: representative SEM image of a single hybrid microparticle (Adapted with permission from ref 16. Copyright 2014 Royal Society of Chemistry). (E) Silver nanorod (Ag NRs) array prepared by the oblique angle deposition: a representative SEM image (Adapted with permission from ref 19. Copyright 2017 Elsevier). (F) Gold nanorod array prepared by template-assisted reactive ion etching and subsequent sputtering: a representative SEM image (Adapted with permission from ref 20. Copyright 2020 Elsevier).

at 785 nm which is further slightly red-shifted upon coating with a 3 nm silica layer as a result of the change in refractive index of surrounding medium (Figure 1C, black and red curves, respectively).¹⁷ Spherical nanoparticles ($\sim 50\text{ nm}$ diameter, gold) have been also used upon assembly onto polystyrene microbeads ($3\text{ }\mu\text{m}$) via a layer-by-layer protocol (Figure 1D).¹⁶ The resulting hybrid plasmonic microstructures are colloidally stable microcarriers of highly localized 3D collections of closely spaced nanoparticles, thereby yielding intense and averaged SERS signals at the single-particle level. When the polymeric core is replaced with silica-coated Fe_3O_4 microspheres, the hybrid construct is further equipped with magnetic properties that enable the straightforward separation of the SERS platform from the media by simply applying a magnet.¹⁸ Finally, plasmonic ordered arrays have also been exploited as SERS substrates for their outstanding signal reproducibility, structural uniformity, and excellent SERS enhancing properties.^{19,20} For instance, Song et al. fabricated silver nanorods array prepared by oblique angle deposition technique (Figure 1E).¹⁹ With this approach, the authors, rather than organizing colloidal particles onto a solid surface, physically evaporated and deposited silver on top of a rotating surface (a 200 nm thick, smooth Ag film)

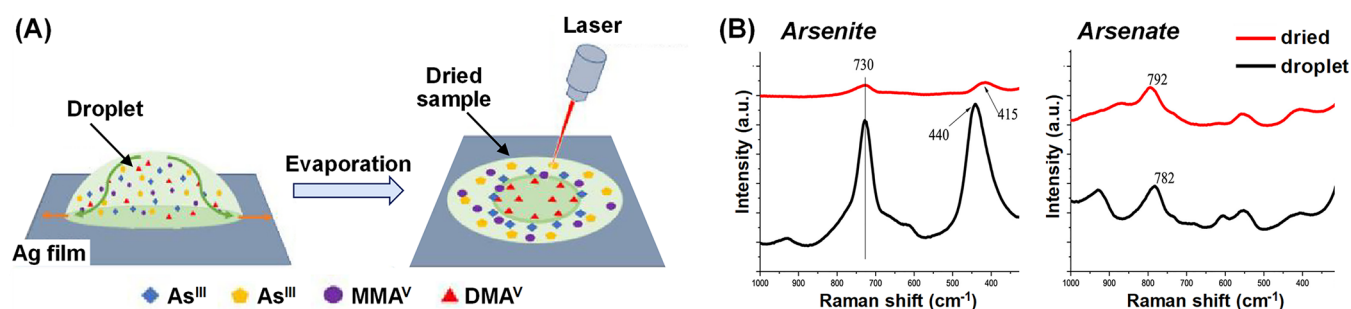


Figure 2. Direct SERS speciation of arsenic forms. (A) Outline of the sensing scheme for physical separation and SERS speciation of four arsenic forms: arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA(V)), and dimethylarsinic acid (DMA(V)). (B) SERS spectra of separate solutions of arsenite and arsenate on Ag film (black curves, signal obtained at the center of the droplet; red curves, signal obtained at the edge of the dried sample). The spectra were acquired by focusing a 785 nm laser with a $\times 20$ optical lens ($100\ \mu\text{m}$ spot size) (Adapted with permission from ref 26. Copyright 2019 American Chemical Society).

until a 2000 nm layer of obliquely aligned Ag nanorods was generated. On the other hand, gold nanorod arrays were prepared by Zhao et al. via sputter deposition of gold onto a sacrificial template consisting of an ion-etched 2D colloidal crystal of hexagonally close-packed polystyrene particles (120 nm diameter).²⁰ The resultant nanostructured features at each nanorod surface provide a large number of highly SERS active sites (Figure 1F).

The manufacturing cost, while very marginally considered at the academic level, represents a major obstacle in the implementation of SERS into routine real-life applications.²¹ While steady advances in nanofabrication techniques are continuously improving the rational development of affordable plasmonic nanomaterials with the desired performances, current limitations still impose a trade-off between the ideal requirements (e.g., high sensitivity and reproducibility, robustness, cost, easy operation, and convenient modification with molecular elements to impart the necessary chemical functions). It is also important to highlight that the analytical performances of the substrate are also dependent on the measuring setup. For instance, acquiring SERS spectra via simultaneous interrogation of large portions of substrates by using, for instance, macrolenses will provide highly averaged signals with improved reproducibility but decreased sensitivity as compared to experimental set ups that favor the illumination of fewer molecules concentrated at smaller and highly electromagnetically active locations of the plasmonic platform. Nonetheless, it is worth stressing that SERS mapping of large areas with high spectral resolution can be a time-consuming process unless using state-of-the-art techniques such as SERS holography.²²

As a matter of fact, the application of SERS to *in situ* monitoring of transition metals demands the implementation of the plasmonic substrates into all-in-one portable devices. In addition to a compact Raman spectrometer for optical detection, these systems must integrate additional functionalities that would enable, besides optical detection, sample processing such as filtration, preconcentration, and separation when the complexity of the sample matrix would require it (e.g., wastewaters, freshwaters). In fact, while the intrinsic sensitivity and selectivity of a SERS method are primarily defined by the enhancing ability of the plasmonic substrate and the molecular properties of the surface receptors, respectively, competitive adsorptions of matrix interferences may significantly deteriorate the sensing performances. This issue is particularly relevant when the target analyte is present at very low concentrations. To this end, recent years have witnessed multidisciplinary

approaches to integrate SERS sensing with microfluidics, paper-based substrates, magnetic functionalities, and capillary-based chips to facilitate automated sample handling and processing.²³ For instance, magnetic-based extraction and preconcentration of the target analyte from complex media enable high throughput sample processing with the additional improvement of the limit of detection (LOD) by several orders of magnitude.²⁴ On the other hand, separation tools such as capillary techniques are beneficial for the selective quantification of multiple target analytes.²⁵ However, it has to be stressed that excessively extensive and laborious sample processing makes far less attractive the use of nanotechnology in decentralized analysis.

■ DIRECT SENSING

The simplest approach to discriminate and quantify the presence of a target species in a mixture by SERS is via the acquisition of the intrinsic SERS signal of the analyte and measurement of its intensity (direct SERS sensing). Evidently, this approach is not suitable for vibrationless species such as atomic ions and, thus, is limited to the detection of transition metals involved in inorganic, metalorganic, or organometallic compounds. For instance, arsenic in waters commonly exists as a combination of relatively harmless organoarsenic compounds and the toxic inorganic species such as arsenite (AsO_2^{2-}) and arsenate (AsO_4^{3-}).²⁶ The relative toxicity of the arsenic species is tightly related to the oxidation state of the metal cation (where As(III) is much more toxic than As(V)). Thus, chemical speciation of the different forms is key for environmental analysis. However, the high chemical reactivity of As(III) poses important challenges for laboratory methods to perform a reliable speciation analysis due to the favorable oxidation of arsenite species during the steps of sample collection, storage, and transportation. For instance, Yang et al.²⁶ described a simple qualitative approach for the direct SERS speciation of arsenic compounds based on the physical separation achieved by exploiting the coffee ring effect. A $2\ \mu\text{L}$ portion of an arsenic solution in phosphate buffer was placed on a silver film prepared by electrostatic deposition of $\sim 45\ \text{nm}$ silver nanoparticles onto an amino-modified glass slide (Figure 2A). As evaporation occurs, arsenic species migrate to different extents toward the outer edge promoting, therefore, their partial separation and preconcentration in preferential area of the Ag film (a surfactant, SDS, was also added to the sample to extend the movement of the solvent onto the film). SERS spectra collected at a different radii from the center of the drop reveal the characteristic

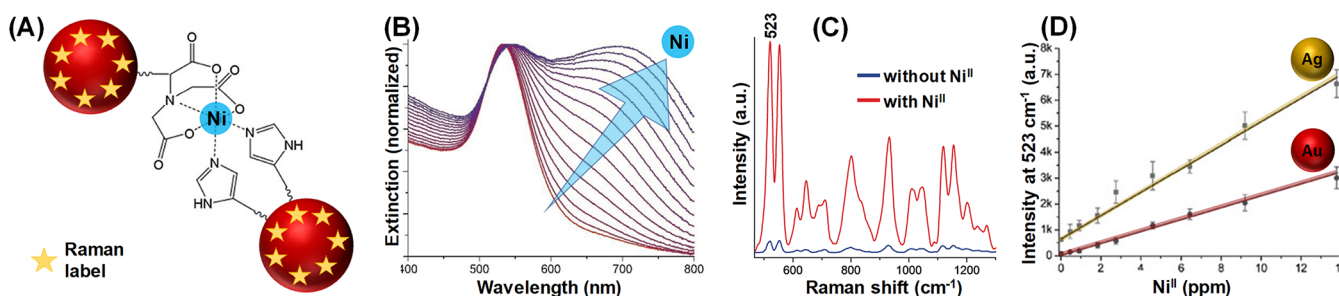


Figure 3. Indirect SERS sensing via transition metal ion mediated assembly of colloidal nanoparticles. Dual colorimetric-SERS sensing of Ni(II): (A) Outline of the sensing scheme. (B) Normalized extinction spectra of mixtures of NTA- and His-gold nanoparticles at increasing Ni(II) concentration (from 0 to 70 ppm). (C) SERS spectra of the NIR-797 Raman label before (blue curve) and after (red curve) the addition of Ni(II). (D) Intensity of the 523 cm^{-1} band obtained in the 0–14 ppm of Ni(II) concentration range using ca. 45 nm diameter gold or silver nanoparticles. The SERS spectra were acquired by focusing a 785 nm laser with a $\times 20$ long-working distance objective (Adapted with permission from ref 27. Copyright 2012 Wiley-VCH).

vibrational fingerprints of each molecular species. Figure 2B shows the characteristic SERS fingerprints of As(III) and As(V) species in the 350–900 cm^{-1} spectral range of both the droplet and the dried sample (black and red curves, respectively). Besides the speciation of the arsenite and arsenate forms, this approach also allowed the discrimination of the structurally analogous organoarsenic species monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}).

■ INDIRECT SENSING VIA SELECTIVE NANOPARTICLE ASSEMBLING

Designing efficient direct sensing strategies for analytes in complex media presents major problems that are often impossible to overcome. First of all, even if the target displays a good affinity for the plasmonic substrate, the presence of additional compounds in the sample matrix that compete with the analyte for surface adsorption can drastically reduce both the sensitivity and selectivity of the method. Thus, prepurification steps are typically required to isolate the target analyte from complex environments. However, this prerequisite essentially precludes the viability of SERS as a rapid technique for remote sensing. Furthermore, as previously mentioned, direct SERS has the intrinsic inability of detecting “silent” species such as free metal cations.

Far more suited for an environmental sensing scenario are indirect SERS approaches relying on the use of molecular receptors bound to the plasmonic substrate that are capable of selectively interact with high affinity with the target transition metal via covalent and/or noncovalent specific interactions or even selectively undergo metal ion catalytic reactions. In indirect methods, the measurable readout signal is provided by an external molecular source that yields an intense SERS spectrum whose spectral variations can be quantitatively correlated with the amount of the target contaminant in the media. Broadly speaking, such spectral variations can be classified into two distinct groups: changes of the overall SERS intensity (the vibrational pattern remains unperturbed) and alterations of the spectral profile (e.g., band shifts, changes in relative band intensities, the emergence of new spectral features, etc.). In the first case, molecular receptors typically act as “SERS silent” ligands for transition metal ion binding while a secondary molecular source (Raman label) is exploited to generate the SERS output signal. Raman labels are selected among the pool of molecules that, in addition to being firmly bound to the metallic surface, display high Raman cross-section and well-defined vibrational profile. This is intended to further improve the

sensitivity and reliability of the SERS response. In the second case (i.e., alterations of the spectral profile), it is required that both roles of transition metal receptor and Raman label are executed by the same molecular entity, which we refer to it as the “chemoreceptor”.

The most straightforward use of indirect SERS approaches builds on an extension of colorimetric methods that measure color changes, including by naked eye, resulting from the analyte-dependent modulation of the plasmonic coupling between colloidal Au or Ag nanoparticles.^{27–30} In the example illustrated in Figure 3, two batches of ca. 45 nm gold nanoparticles were derivatized with either nitrilotriacetic acid (NTA) or histidine (His) functionalities via EDC/Sulfo-NHS coupling reaction, to provide anchoring sites for nickel ions binding (l-carnosine was used as a source of histidine residues). Subsequently, the infusion of a near-infrared dye (NIR-797 isothiocyanate) onto the metallic surface of the NTA-gold nanoparticles equipped the system with a Raman label that yields resonant Raman scattering when irradiated with the 785 nm laser (i.e., resonant Raman label).²⁷ This phenomenon entails an additional enhancement to the SERS signal that enables the acquisition of intense spectra even for very low NIR-797 surface coverages, thereby minimizing the risk of colloidal destabilization of the individual particles. The two sets of NTA- and His-gold nanoparticles were combined, and upon addition of Ni(II) solutions in deionized water, rapid aggregation takes place (less than 3 min). This results from the interparticle linking due to the formation of an octahedral metal complex coordinating the NTA moiety and the closely packed histidines present on the nanoparticles (Figure 3A). Interparticle plasmon coupling resulting from Ni(II) binding is both highlighted by a change of the extinction profile (Figure 3B) and an increase of the SERS intensity, resulting from the concentration of high EM fields at the interparticle junctions (Figure 3C), which can be quantitatively correlated with the metal ion content. Specifically, colorimetric sensing provided a wider linear response for the Ni(II) concentration (ca. 20–100 ppm, approximately equals to 0.2–2.0 μM) while the SERS read-out displays a limit of detection approximately 1 order of magnitude lower ($\text{LOD} = 8.5 \text{ nM}$). Commonly, gold nanoparticles are preferred in colorimetric assays over their silver counterparts because of the higher extinction coefficients and distance-dependent optical properties which enhance the overall sensitivity (in addition to their higher chemical stability and ease of functionalization). On the other hand, silver nanoparticles are typically better SERS enhancers over a wider spectral range of excitation as compared

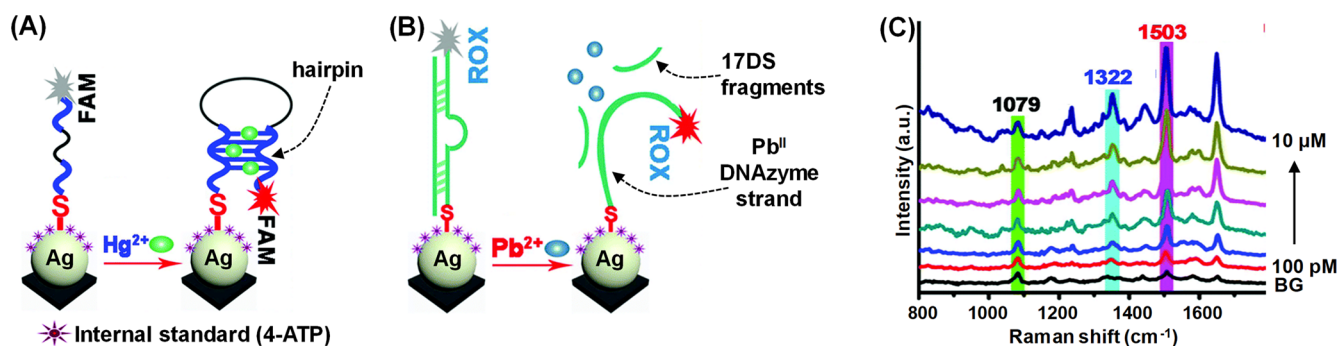


Figure 4. Indirect SERS sensing based on the conformational reorganization of Raman labeled DNA aptamers onto solid plasmonic supports. (A–C) Simultaneous detection of $\text{Hg}(\text{II})$ and $\text{Pb}(\text{II})$ on Ag nanoparticle-decorated silicon wafer: (A, B) schematic outline of the aptamer based Hg^{II} and Pb^{II} detection, respectively; (C) SERS spectra of the sensing platform in the presence of equimolar $\text{Pb}(\text{II})$ and $\text{Hg}(\text{II})$ buffer solutions at increasing concentrations (from 100 pM to 10 μM). Background (BG) stands for distilled water. SERS spectra were collected using a 633 nm laser (Adapted with permission from ref 31. Copyright 2018 Royal Society of Chemistry).

to their analogous gold counterparts, thus improving the overall sensitivity of the SERS-based method. This is exemplified in Figure 3D where the SERS intensity of the NIR-797 band at 523 cm^{-1} , for two colloidal systems comprising spherical gold or silver nanoparticles of identical size, is plotted against the $\text{Ni}(\text{II})$ concentration. However, the sensing system displayed a similar affinity for $\text{Cu}(\text{II})$ ions, which prevents its applicability as $\text{Ni}(\text{II})$ SERS sensors in natural waters where the copper content is typically much higher than the nickel. On the other hand, selectivity can be primarily improved by designing receptors with higher specificity for the target analyte. Further improvements can be also achieved by adjusting the sample conditions (e.g., pH) as well as upon addition of water-soluble masking agents that can selectively sequester the interfering ion from the medium (e.g., EDTA).

■ INDIRECT SENSING VIA CONFORMATIONAL CHANGES OF THE MOLECULAR RECEPTOR

The use of DNA aptamers for the SERS quantification of Hg^{II} has been extensively reported in the literature,^{5,6,31,32} as it combines the detection of an analyte of high interest with the availability of extremely selective surface receptors (i.e., thymine-rich aptamers) which are readily amenable to chemical synthesis and *a la carte* modifications. Most notably, the implementation of DNA aptamers onto solid substrates for the quantification of $\text{Hg}(\text{II})$ via a “turn-on/turn-off” response undoubtedly represents the most widespread application of SERS-based sensing of transition metals in real water systems.³¹ In this approach, one extremity of the aptamer is typically conjugated with a resonant dye which relative proximity to the plasmonic surface is determined by the $\text{Hg}(\text{II})$ -mediated conformational change of the DNA chain. Therefore, DNA structural reorganization to different forms (e.g., single strand, duplex, hairpin) exposes the Raman label to higher electromagnetic fields (turn-on SERS), when the label is closer to the metal surface, or to lower enhancements (turn-off SERS), when the relative distance between the two components is increased. The work by Shi et al.³¹ affords an illustrative example of this approach. A thymine rich single-stranded DNA labeled with carboxyfluorescein (FAM) is covalently attached to a silver nanoparticle film on a silicon wafer via sulfur bond (Figure 4A). For sufficiently high oligonucleotide surface coverages, the single strands preferably adopt a tilted geometry with the Raman label spaced away from the metal surface (i.e., weak SERS background signal). Upon $\text{Hg}(\text{II})$ addition, the formation of a

hairpin structure forces the close approximation of the label to the substrate, which is revealed by an increase of the SERS intensity. A second oligo receptor was also introduced onto the Ag film, consisting of a DNA duplex obtained by hybridization of the surface-bound $\text{Pb}(\text{II})$ -specific DNAzyme strand labeled with 6-carboxy-X-rhodamine (ROX) with the corresponding substrate strand (17DS). The rigid duplex structure, that places the ROX label far from the silver film, is disrupted in the presence of lead ions via cleavage of the substrate strand 17DS by the $\text{Pb}(\text{II})$ activated DNAzyme strand (Figure 4B). The acquired flexibility of the DNAzyme strand allows the ROX moiety to approach the plasmonic film. The catalytic reaction was completed after ~ 1 h, which was set as the experimental incubation period for metal ion quantification. Notably, the Ag film was also decorated with 4-aminothiophenol (4-ATP) molecules, which provides an easily distinguishable SERS signal with an intensity comparable to those of ROX and FAM labels. Figure 3C shows the SERS spectra of obtained in the presence of equimolar buffer solution of $\text{Hg}(\text{II})$ and $\text{Pb}(\text{II})$ at increasing concentration, where three bands at 1079 (4-ATP), 1322 (FAM), and 1503 cm^{-1} (ROX) can be discerned. For quantification purposes, rather than plotting the absolute intensities of the FAM and ROX marker bands (I_{1322} and I_{1503}) vs the metal ion concentration, ratiometric values were calculated using the 4-ATP marker band as the internal standard (I_{1322}/I_{1079} and I_{1503}/I_{1079}). Such an approach has been shown to significantly reduce the signal fluctuation associated with interferences in the local environment as well as instrumental and procedural errors.²¹ Ratiometric analysis allows a broader linear dynamic range (from 1 nM to 10 μM for $\text{Hg}(\text{II})$ and from 100 pM to 10 μM for $\text{Pb}(\text{II})$) and lowers the limits of detection (LODs) (~ 1 nM for $\text{Hg}(\text{II})$ and 0.1 nM for $\text{Pb}(\text{II})$) as compared to noncalibrated SERS data (in buffer solutions).³¹ Finally, the authors demonstrated the viability of the sensing tool to real-life applications by detecting $\text{Pb}(\text{II})$ and $\text{Hg}(\text{II})$ spiked into industrial wastewater, previously filtered to remove large-sized particles, using a portable hand-held Raman instrument. Unfortunately, the design of specific oligonucleotide sequences for selective capturing of transition metal ions is currently restrained to very few species, which significantly limits their broader use in environmental sensing.

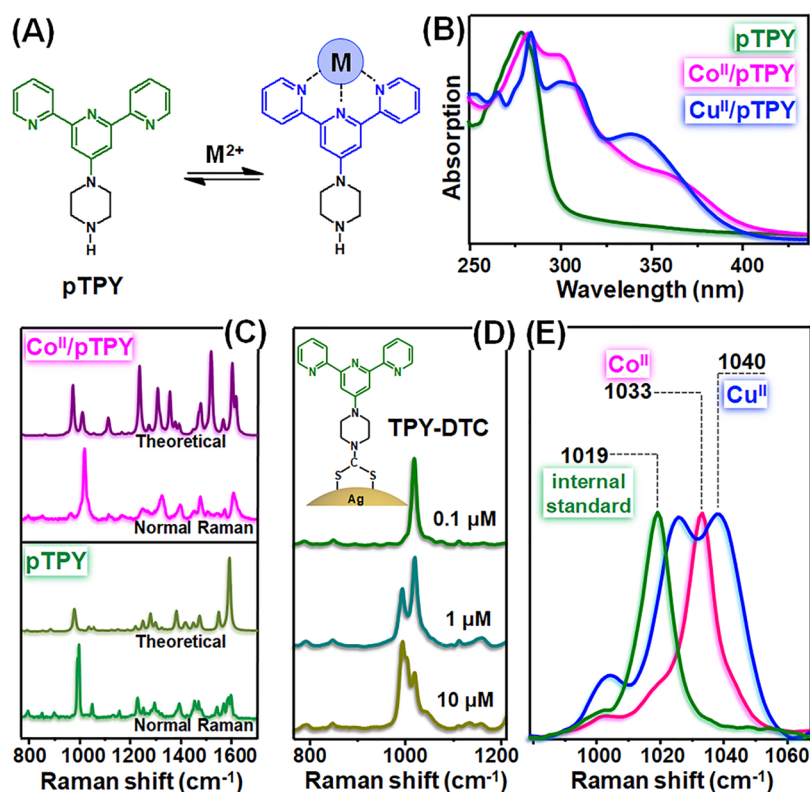


Figure 5. Indirect SERS chemosensing of Co(II) and Cu(II). (A) Molecular structures of 4-(N-piperazinyl)terpyridine (pTPY): free and coordinated with a metal ion $M(II)$. (B) Normalized electronic absorption spectra of pTPY and its equimolar complexes with Co(II) and Cu(II) in 50/50 CH_2Cl_2 /MeOH. (C) Theoretical and experimental Raman spectra of the solid samples. The spectra of Cu(II)/pTPY are not shown. (D) SERS spectra of TPY-DTC at different concentration on Ag colloids. (E) Normalized SERS spectra of TPY-DTC in the ring breathing region before (green line) and after incubation with Co(II) (30 ppb, pink line) and Cu(II) (6.4 ppm, blue line). SERS spectra were acquired on dried colloidal silver nanoparticles using a 785 nm laser with a 50 $\times$ objective (Adapted with permission from ref 34. Copyright 2013 Royal Society of Chemistry).

INDIRECT SENSING VIA ELECTRONIC CHANGES IN THE MOLECULAR RECEPTOR

The formation of T–Hg(II)–T base pairs not only induces structural reorganization and reorientation of oligonucleotides over the metallic surface but also can be detected by monitoring the spectral alterations of the intrinsic DNA vibrational profiles which, in turn, can be quantitatively correlated with the Hg(II) content.³³ Thus, DNA strands can act as chemoreceptors by simultaneously perform the role of selective molecular ligands and SERS transducers. Most notably, a key advantage of this approach is the use of the chemoreceptor itself as the internal reference, thereby enabling a straightforward implementation of ratiometric SERS sensing with improved sensitivity and reliability.

Nonetheless, to maximize the sensing performance, chemoreceptors are typically selected among small molecules: (i) with high Raman cross-section (i.e., molecules bearing one or more benzene rings with delocalized π -bonding in close contact to the plasmonic surface), yielding strong SERS signals with well-defined, narrow spectral features; (ii) that firmly bind the metallic surface (mostly via thiolated bonds); and (iii) undergo larger electronic and/or structural modifications upon binding of the target pollutant (which is reflected in larger alterations of the spectral profile).^{16,20,34,35} The methodological protocol for selecting and characterizing a chemoreceptor preferably involves several steps, which are exemplified by our work summarized in Figure 5A–D. In this study, we exploited a terpyridine (TPY-DTC) dithiocarbamate derivative as a chemoreceptor for the

simultaneous ultratrace SERS detection of Cu(II) and Co(II) in buffer solutions.³⁴ First, the electronic spectra of the piperazinyl TPY precursor (pTPY, Figure 5A) in solution were acquired via UV–vis spectroscopy prior and after the addition of transition metal ions. This affords a first indication of the selectivity of the chemoreceptor toward specific metals, which will be later assessed also by SERS. Moreover, it provides a clue of the extent of electronic perturbation that transition metal binding imposes (which broadly correlates with the alteration of Raman polarizability and, in turn, the degree of spectral alterations in SERS). Among different metal ions, Cu(II) and Co(II) have shown to produce major distinguishable alterations of the TPY absorption spectrum (Figure 5B). Second, density functional theory (DFT) calculations are conveniently performed to accurately identify the vibrational features of the chemoreceptor and determine those most sensitive to the metal ion coordination. Ideally, the experimental normal Raman spectra of the corresponding solids are obtained too for comparison (Figure 4C). Subsequently, the dithiocarbamate derivative (TPY-DTC) was obtained via a simple one-pot reaction with CS_2 equipping the chemoreceptor with a firm anchoring point onto the silver surface (Figure 5D). SERS spectra of the chemoreceptor on Ag colloids were then acquired at increasing surface coverages to optimize the sensing performance of the platform. SERS spectra of TPY-DTC at different concentrations display major changes, most notably in the ring breathing mode at 1019 cm^{-1} (Figure 5D). This has been associated with the conformational reshaping of the TPY unit due to lateral interactions, that negatively affects its metal ion coordination

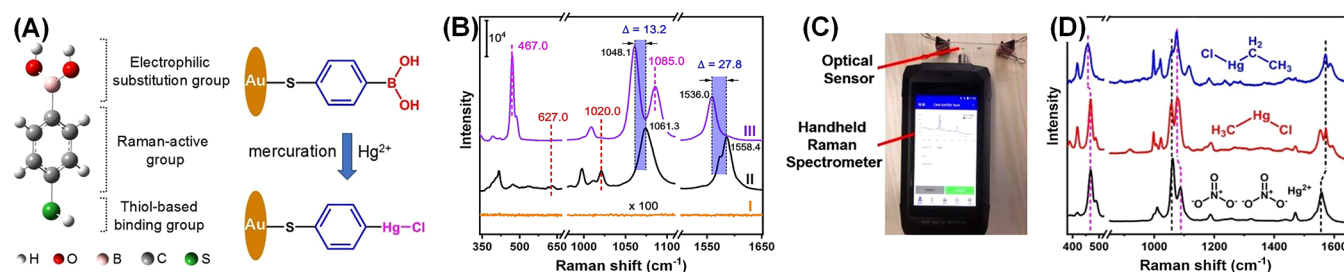


Figure 6. Indirect SERS chemosensing of Hg(II) via chemical reaction. (A) Molecular structure of 4-mercaptophenylboronic acid (4-MBA) and Hg(II) electrophilic substitution of the boronic group. (B) SERS spectra of (i) bare Au nanorod arrays after soaking in 1 mM ethanolic solution of HgCl₂, SERS spectra of 4-MBA on Au nanorod arrays (ii) before and (iii) after 3 h of immersion into 50 mL of 10 μ M ethanolic solution of HgCl₂. The substrate was rinsed with ethanol and water and completely dried with N₂ flow before measuring. (C) Experimental setup for simulated on-site SERS measurement using a hand-held Raman spectrometer (785 nm excitation). (D) SERS spectra of 4-MBA on Au nanorod arrays after immersion into 50 mL of 10 μ M ethanolic solutions of mercury nitrate, methylmercury chloride, and ethylmercury chloride. SERS spectra illustrated in this figure were acquired on dried Au nanorod arrays by illuminating a 1 μ m spot using a 785 nm laser (Adapted with permission from ref 20. Copyright 2020 Elsevier).

ability at higher surface coverages. Once identified the appropriate surface density of the chemoreceptor, SERS spectra are acquired at increasing concentration of metal ions in a buffer solution to experimentally identify the TPY vibrational features that undergo major alterations upon metal ion binding and, supported by the DFT calculations, identify the marker bands for ratiometric quantification of the target analytes (Figure 5E). Specifically, in this study, we used as spectral markers the ratios between the peak intensities at 1033 and 1040 cm⁻¹ (for Co(II) and Cu(II) detection, respectively), and the peak intensity at 1019 cm⁻¹ as the internal standard (IS), permitting a certain degree of multiplex capabilities for the simultaneous detection of the two transition metal cations. The SERS response of the chemoreceptor must be then tested in the presence of potential interfering analytes, such as transition metal ions dissolved at relatively high concentrations in buffer solutions, and, finally, in real sample matrices.

As for arsenic species, speciation of different Hg forms is essential to determine their bioavailability, mobility, and, thus, their environmental and toxic impact. Most notably, methylmercury MeHg(I) is the most potent mercuric toxin. As it is produced by aquatic microorganisms, MeHg(I) rapidly enters the aquatic food chain where it is subjected to biomagnification via progressive accumulation at the apex predators.^{16,36} The high volatility and tendency to undergo dynamic interconversion with other mercury species make the accurate quantification of MeHg(I) in aqueous environments a challenging task, in particular for off-site analysis that requires sample handling (i.e., collection, storage, and transport enhance the risk of methylmercury losses).³⁶ The speciation capability of SERS chemosensing for discriminating and quantifying Hg(II) and MeHg(I) in aqueous solution has been demonstrated using hybrid plasmonic microparticles modified with 4-mercaptopyridine (MPY) as the chemoreceptor (Figure 1D).¹⁶ Here, MPY forms a densely packed monolayer by binding the gold surface via a metal–sulfur bond while the pyridinic nitrogen sits further from the surface, therefore, remaining available for coordinating the two mercury species. SERS monitoring of the MPY-modified bead suspensions reveals major alterations upon exposure to the different mercury forms, which were tentatively ascribed to the formation of multidentate N-bonding of Hg(II), favored by the high MPY surface density, and unidentate coordination of MeHg(I). Ratiometric SERS analysis in deionized water reveals a linear correlation for an interval of $\sim$ 3 orders of magnitude for Hg(II), with a LOD of $\sim$ 0.25 nM. The relative position of the dynamic range of concentration can be easily controlled by

adjusting the concentration of the beads in the sample. On the other hand, the MeHg(I) quantification is restricted to a linear interval of from 0 to 70 nM, with a LOD of 7 nM. Interestingly, simultaneous quantification of the two species highlighted the dynamic interconversion between Hg(II) and MeHg(I) in water.

■ INDIRECT SENSING VIA CHEMICAL REACTIONS OF THE MOLECULAR RECEPTOR

In a very recent work, Zhao et al.²⁰ adopted 4-mercaptophenylboronic acid (4-MBA) as a SERS chemoreporter attached to ordered Au nanorod arrays (Figure 1F) as plasmonic substrates for ultratrace detection of Hg(II). In this case, an electrophilic substitution reaction takes place, in which the transition metal ion displaces the –B(OH)₂ functional group, leading to the chemical transformation to 4-mercaptophenyl mercury chloride (Figure 6A). Removal of the boronic moiety and the extensive perturbation of the polarizability of the phenyl ring determine a dramatic reshaping of the SERS spectra (Figure 6B), which includes the disappearance of the –B(OH)₂ modes at 627 and 1020 cm⁻¹, the striking blue-shifts of the 1061 cm⁻¹ ring breathing and 1558 cm⁻¹ ν (C=C) bands, and the appearance of two new intense features at 467 and 1085 cm⁻¹, ascribed to the stretching vibration of newly formed C–Hg bond and the ring deformation. This ensemble of dramatic spectral alterations equips the sensing platform with high discriminatory power, which is key to maximize accurate and quantitative SERS performances. Calibration curves showed a linear response over 5 orders of magnitude, with a LOD of 0.1 nM in deionized water, with insignificant interferences from other metal ions and pH conditions. Simulated on-site detection was carried out on the ground and lake waters spiked with Hg(II) (10 nM) and using a hand-held Raman spectrometer (Figure 6C), evidencing a consistent, repeatable response of the sensing platform in real-life aqueous media. Intriguingly, preliminary studies indicated the potential of 4-mercaptophenylboronic chemoreceptor for discriminating different mercury species (mercury nitrate, methylmercury chloride, and ethylmercury chloride; Figure 6D).

■ INDIRECT SENSING BY NANOPARTICLE BINDING OF NONAFFINITY METAL SELECTIVE DYES

An untapped wealth of potential SERS chemoreceptors can be found in classical qualitative analytical chemistry, which extensively describes multiple classes of organic reagents with

the ability to selectively discriminate, and even speciate, most metal ions. Nonetheless, their use in SERS has been significantly hampered by the inability of equipping the organic receptor with a mercapto group, as required for firmly anchoring the plasmonic surface while preserving its selectivity for the target transition metal. For instance, several anthraquinone and flavonoids derivatives have shown a diversity of excellent metal chelation capabilities with, often, emissive properties in the visible region. However, coordination to the target species mainly occurs through the same functional groups that enable their adsorption on silver and gold nanostructures, therefore commonly prevent their application as SERS chemoreceptors. To tackle this issue, we have recently illustrated that the structural and functional plasticity of DNA duplexes for noncovalently interacting with small aromatic molecules via intercalative binding can be exploited to retain the metal chelation capabilities of these classes of reagents onto the plasmonic surfaces.³⁷ Specifically, we demonstrated the validity of this approach using alizarin red S in combination with short DNA duplexes for the simultaneous Fe(III) and Al(III) quantification in spiked tap water.

■ CONCLUSIONS AND OUTLOOK

In summary, in this mini-review, we aimed at providing a coherent discussion on the sensing schemes exploited in the SERS analysis of transition metals in waters, using illustrative examples to describe the rationale of each approach, their most relevant aspects as well as the diverse classes of plasmonic nanomaterials utilized. Although the studies reported in the literature display a marked methodological diversity, we have broadly classified their sensing schemes into four main classes: (i) direct sensing, (ii) indirect sensing via nanoparticle assembling, (iii) indirect sensing via conformational changes of the molecular receptor in combination with Raman labels, and (iv) indirect chemosensing (either via coordination or chemical reaction of target ions with SERS active receptors). While the advantages and disadvantages of each approach are very case-specific, some general considerations can be drawn. First of all, direct sensing approaches commonly require, in addition to an intrinsic affinity of the target species for the metallic surface, the separation of the analyte from complex media. This largely prevents the applicability of such methods for remote SERS sensing of transition metal species in natural waters. To overcome this limitation, multiple indirect strategies have been developed to enable the selective capture of the target within its original matrix. The simplest indirect sensing design exploits the metal ion-induced assembly of Raman-labeled colloidal nanoparticles equipped with selective surface receptors for the target species. However, the complexity itself of natural matrices can put under significant stress the colloidal stability of the nanoparticles, thereby reducing the reliability of the sensor, especially at low target concentrations. This issue can be overcome by using nanostructured solid surfaces in combination with “SERS silent” molecular receptor such as DNA aptamers or “SERS active” chemosensors. Remarkably, the conformational reorganization of DNA aptamers upon binding of metal ions have been widely exploited in a variety of studies thanks to their high selectivity and reliable performance. Unfortunately, the current availability of aptamer receptors is limited to very few target metal ions (Hg(II), Pb(II), and Ag(I)). Alternatively, molecular receptors that act simultaneously as SERS transducers have recently gained a great deal of attention to increase the number of possible targets, while potentially enabling speciation

analysis. Furthermore, chemosensors intrinsically provide an internal standard for ratiometric SERS analysis.

Despite the extensive work carried out at the academic level, the successful design of SERS sensors for remote detection that combines ultrasensitivity and robust and reproducible response in complex and variable matrices with cost-competitiveness remains an unmet challenge. To address this issue, extensive efforts in applied research have been currently devoted to the development of low-cost, manageable, and portable Raman instruments for rapid data acquisition which can be easily integrated into multioperational platforms equipped with automated sample handling and processing functionalities. Concurrently, continuous advances are being pursued in the development of highly SERS efficient plasmonic materials with a reliable and quantitative response that can be fabricated at a large scale and low manufacturing cost using standardized procedures. On the other hand, expanding the range of surface elements, with a specific focus on the rational design of highly selective chemoreceptors, is key to improve the sensing performances in terms of selectivity, sensitivity, multiplexity and resilience against interference.

Finally, SERS technology appears specifically suited for the development of sensing devices for the remote and fast quantification of multiple contaminants with ultralow permissible limits that cannot be otherwise detected by more inexpensive methods. In fact, regardless of the potential improvements, we expect that SERS technique will not be able to compete on price with traditional colorimetric and fluorimetric methods for detection of transition metal ions when ultrasensitivity is not required.

■ AUTHOR INFORMATION

Corresponding Authors

Luca Guerrini – *Universitat Rovira i Virgili, Department of Physical and Inorganic Chemistry, 43007 Tarragona, Spain;*
● orcid.org/0000-0002-2925-1562; Email: luca.guerrini@urv.cat

Ramon A. Alvarez-Puebla – *Universitat Rovira i Virgili, Department of Physical and Inorganic Chemistry, 43007 Tarragona, Spain; ICREA, 08010 Barcelona, Spain;*
● orcid.org/0000-0003-4770-5756;
Email: ramon.alvarez@urv.cat

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acsomega.0c05261>

Notes

The authors declare no competing financial interest.

Biographies

Dr. Luca Guerrini received his Ph.D. degree from the Universidad Autonoma de Madrid (Spain, 2009). He later developed his postdoctoral activity first at the University of Strathclyde (Glasgow, UK, 2010–2012) and, then, at the Universidade de Vigo (Spain, 2012–2013). He was subsequently awarded a Marie Curie individual fellowship (Universitat Rovira I Virgili, Spain, 2013–2015). In 2015, he joined Medcom Advance SA, a spin-off company devoted to the development of biomedical devices based on nanotechnology, as a senior researcher. Since 2017, he has been a Ramon y Cajal researcher at the Universitat Rovira I Virgili (Tarragona, Spain). His scientific interests are mainly focused on the development of ultrasensitive nanophotonics-based devices for biomedical and environmental applications.

Prof. Ramon A. Alvarez-Puebla is an expert in surface science, nanoscience, and spectroscopy with emphasis on the manufacturing and characterization of plasmonic materials and their integration into advanced detection devices especially with applications in nanobiomedicine. He has a degree in chemistry (Universidad de Navarra, 2000) and a doctorate in surface science (Universidad Publica de Navarra, 2003, summa cum laude). He completed his postdoctorate at the University of Windsor (Windsor, ON, Canada) and General Motors Corporation (Warren, MI, USA) in nanofabrication and surface spectroscopy (SERS, SEFS, and SEIRA) with Prof. Ricardo Aroca (UWINDSOR) and Prof. Gholam Abbas Nazri (GMC). During 2006–2007, he worked as a Research Officer and Principal Investigator at the National Institute for Nanotechnology of the National Research Council of Canada (NINT-NRC, Edmonton, AB, Canada). In 2008 he returned to Spain as an associate Professor at the Universidade de Vigo. Since 2012, he has been the ICREA Professor at the Universitat Rovira i Virgili in Tarragona, where he leads the Plasmonics and Ultradetection Group (Zeptonic).

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