Dedicated to our dear friend Dr. Joseph Wang for his 70th birthday, a scientist unrepeatable and still better person

Janus particles for (bio)sensing

P. Yánez-Sedeño[a], S. Campuzano[a,*], J.M. Pingarrón[a,b,*]

[a] Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid. Avda. Complutense s/n, E-28040 Madrid, Spain. Fax:

[b] IMDEA Nanoscience, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain

E-mails: yseo@quim.ucm.es; susanacr@quim.ucm.es; pingarro@quim.ucm.es.

Tel. 34913944315, fax 34 913944329.

* to whom correspondence should be addressed

Abstract

This review article sheds useful insight in the use of Janus nanoparticles for (bio)sensing in connection with optical and electrochemical transduction. After a brief introduction of the main properties, types and fabrication strategies of Janus nanoparticles, selected applications for their use in electrochemical and optical biosensing are critically discussed. Highlighted examples illustrate the great versatility and interesting possibilities offered by these smart multifunctional nanoparticles for (bio)sensing of relevant analytes operating both in static and dynamic modes. Progress made so far demonstrate their suitability for designing single- or multiplexed (bio)sensing strategies for target analytes of different nature (organic and inorganic compounds, proteins, cells and oligomers) with relevance in clinical (H_2O_2, glucose, cholesterol, CEA, human IgG, propranolol, bacterial and tumor cells) and environmental (lead and organophosphorous nerve agents) fields. Key future challenges and envisioned opportunities of the use of Janus nanoparticles in the (bio)sensing field are also discussed.

Keywords: Janus particles; Janus micromotors; biosensing; optical; electrochemical.

1. Introduction

Janus particles are the simplest case of patchy or non-centrosymmetric particles [1]. These particles, whose name derives from the two-faced Roman God Janus, possess two or more spatial regions that have different surface makeups, structures or material properties [2]. The concept of Janus particles was first raised by P. G. de Gennes in his Nobel Prize address in 1991 [3]. Unlike conventional particles used in material and analytical sciences, two-faced Janus particles provide asymmetry and directionality, and can combine different or even incompatible properties within a single particle, which makes them a unique category of materials in contrast to other particles [4]. The surface anisotropy of these particles spatially decouples analytical functions (e.g. targeting and sensing) that would otherwise be difficult to combine within single particles, giving them interesting properties and functions not possible for particles of uniform composition and opening up opportunities for novel multimodal analytical methods. Specifically, the ease of functional modification on the distinct surface, which is the key step for the utilization of nanosystems in the field of (bio)sensing and other bionanotechnology, renders Janus nanoparticles truly multifunctional entities [5].

The asymmetry of the particle surface, known as the Janus balance (quantified as the ratio of surface area devoted to different surface types on the two sides of the particle), can vary from interspersed small patches to half-and-half hemispheres. The structural asymmetry of Janus particles that contain compartments with different chemical, optical, electrical, or magnetic properties enables to perform many functions such as dual-targeting, molecular sensing, and *in vivo* imaging, which are incompatible when they are combined on the same surface, in a single structural unit [5,6]. Although individual components coexist together in Janus particles, however the intrinsic optical, magnetic, and electronic properties of each component are not often altered, interfered with, or lost [7] thereby exhibiting improved physical/chemical properties [8] and great potential in numerous applications [9]. This

exceptionality makes Janus particles a unique category of materials in contrast to other particles. In addition to combining imaging and targeting functions, these asymmetric particles have also been employed in (bio)sensing and, to date, various types of Janus nanoparticles have been reported as synthesized for such applications.

As a result of the simultaneous presence of two different regions, which can be designed to have different hydrophobicity and thereby mimic the behavior of surfactants, they can form stable clusters with defined size, and substantially reduce the interfacial tension between two different phases. Additionally, these particles can bring together different materials in a segregated manner at the nanoscale, thus combining widely different properties in a single entity, as in the case of heterodimers [10-15]. Furthermore, Janus nanocomposites with spatially separated functionalities, uniform size, tunable composition, and efficient stimuli-responsive features have been synthesized whose properties usually rely on the composition, size and shape of the particles, as well as their appropriate surface modification. Such asymmetric architectures are composed of two incompatible hemispheres with different chemistry, polarity, or other physicochemical properties on opposite sides [11,16]. Due to these intrinsic characteristics, Janus particles have been found to exhibit diverse potential applications in materials science, biomedicine and in the field of highly specific biosensors [15,17].

The properties and applications of Janus particles have been reviewed in various articles [1,6,9,15,17,18]. However, the use of these particles in the (bio)sensing field has been little emphasized. Although the number of examples is still relatively scarce, this review article attempts to draw attention to the wide possibilities offered by ~~of~~ Janus nano- and microparticles for the sensing of species of interest using different types of strategies and modes of detection ranging from electrochemistry to optical techniques such as fluorimetry or Raman spectroscopy.

2. Types of Janus nanoparticles and fabrication strategies

Janus nanoparticles can be prepared with different morphologies and shapes (Fig. 1A) and can be classified mainly according to the three different preparation strategies: self-assembly, masking and phase separation (Fig. 1B) [6,15].

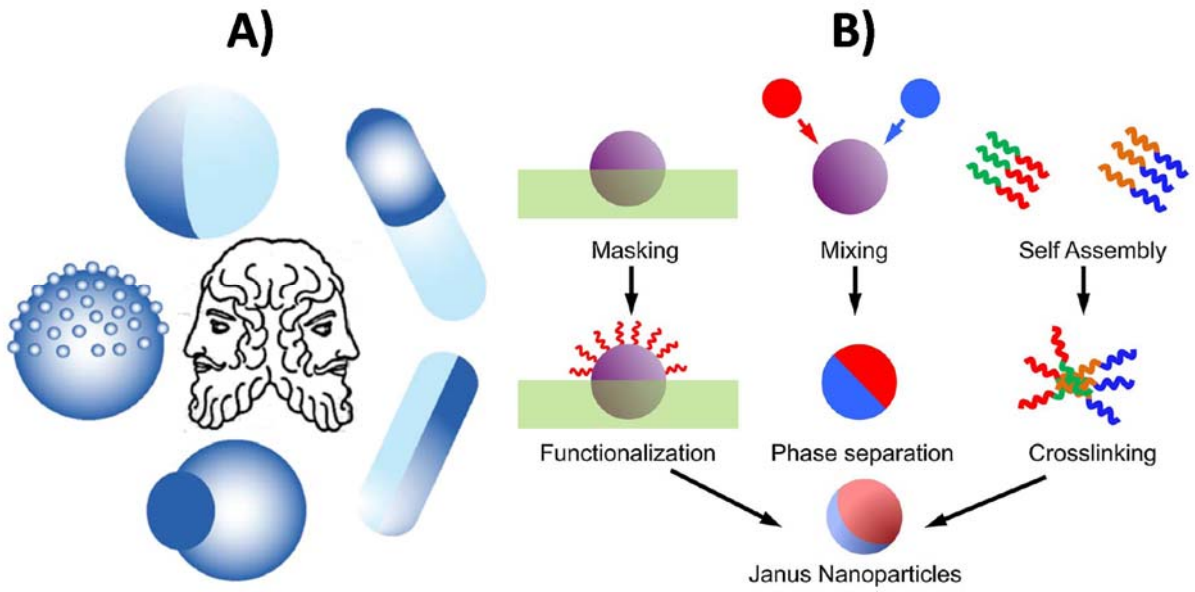

Fig. 1. Schematic diagrams illustrating Janus particles of different morphologies and shapes (A) and the three main strategies used for the preparation of Janus nanoparticles: masking, phase separation and self-assembly (B). Reprinted from [6] (A) and [15] (B) with permission.

Janus nanoparticles can be prepared through self-assembly of their components, typically block copolymers and mixtures of ligands [15]. The block copolymers self-assembly is a flexible strategy applied to many different types of polymers and allows the preparation of block copolymers with well-defined architecture, composition and narrow molecular weight distribution. However, the preparation of Janus nanoparticles using this approach requires a good knowledge of the polymers mixture thermodynamics as well as the effect of all parameters affecting the self-assembly process (temperature, and pH and ionic strength in the

case of polyelectrolytes). Moreover, this approach has a limited scalability since assembly of block copolymers at high concentrations is not always a well-controlled process.

Another group of Janus nanoparticles is that prepared using a masking step in which one portion of a nanoparticle surface is temporarily made inaccessible to some reagent usually by contact with another surface, so that a chemical reaction, a polymerization or a functionalization step, can be carried out exclusively on the other portion of the nanoparticle surface. Although this is the most straightforward method for fabricating Janus particles several requirements must be fulfilled to be adequately performed. The solid support used, preferably with a high surface area to maximize the number of NPs that can be supported, must hold the nanoparticles throughout the process, be inert to the chemicals that react with nanoparticles and be either removed by filtration, disaggregated then washed off, or preferably recycled for further reuse [19]. This method typically involves three to four steps as follows: (a) the particles are immobilized at an interface (spherical or planar) via reversible physical or chemical means; (b) metal vapor is then applied directionally and sequentially to the solvent-exposed surface to create Janus particles with thin metal caps (from a few to hundreds of nanometers of thickness) controlled by the deposition time; (c) the modified JPs are removed from the surface and separated; and (d) an optional modification of the previously masked site can be performed [6,9]. Using this protocol, Janus nanoparticles are fabricated commonly by catalytic metallic (e.g., Ir, Au, Pt or Ti) thin films deposition (by electron-beam evaporation, atomic layer deposition or sputtering processes) or polymeric coating onto the surface of nano- or micro-beads of different materials (e.g., SiO_2, polystyrene, Mg) uniformly distributed on glass slides or silicon wafers. The thickness of the metal cap is controlled by the deposition time and can range from a few to hundreds of nanometers. This fabrication method is excellent for the control of the amount of area functionalized and particle-to-particle reproducibility, and it is applicable for a variety of

particle materials, such as silica and polymer, as long as the particles can form a monolayer on a surface. The main limitation of this approach is the generally low yield due to the limits of surface area, especially for NPs smaller than a few hundred of nanometers, which tend to be "bridged" by the metal coating [1].

The third group comprises Janus nanoparticles formed by a phase separation of two different polymers, a phase segregation of an inorganic material inside or outside a polymer matrix, or the phase separation of two inorganic components, such as to form nanocrystalline heterodimers [15].

In summary Janus particles are typically synthesized using either surface functionalization of homogeneous particles or via phase separation methods. However, surface functionalization techniques produce limited quantities of Janus particles because they are required to be stabilized in monolayers to perform spatially selective chemical or physical modification. Phase separation mechanisms require unique synthesis conditions since the final particle composition and morphology is highly dependent on the interaction energies between precursors [20]. In order to overcome the limitations of these conventional methods, click chemistry has been recently used also for the fabrication of Janus particles with diverse chemical compositions. [5,20,21]. Click chemistry allows both tailoring the surface chemistry and surface charge of Janus particles, and controlling the extent of modification [20].

3. Janus particles for electrochemical (bio)sensing

The design and fabrication of electrochemical biosensors with improved analytical performance by exploring new concepts and sensor platforms where the interactions between biomaterials and nanoscale structures play key roles is a burgeoning area of research [22]. In this context, Biji et al. [22] reported the use of Janus monolayer gold nanoclusters-modified with tetraoctylammonium ion (TOA^+) and tryptophan onto glassy carbon electrodes (GCE) to

develop effective electrochemical sensors for dopamine (DA). A remarkable enhancement in the differential pulse voltammetric peak current value, a noticeable lowering in the LOD value (0.5 vs 6,400 nM), and better antifouling was found working with the Janus nanoparticle-modified electrode compared to the bare GCE. The improved analytical performance was attributed to the electro-oxidation of DA by the Janus gold nanocluster favored by the H-bonding interaction between L-tryptophan and DA, which made the tryptophan at the peripheral Janus cluster hemisphere acted as a DA pre-concentrating layer (Fig. 2).

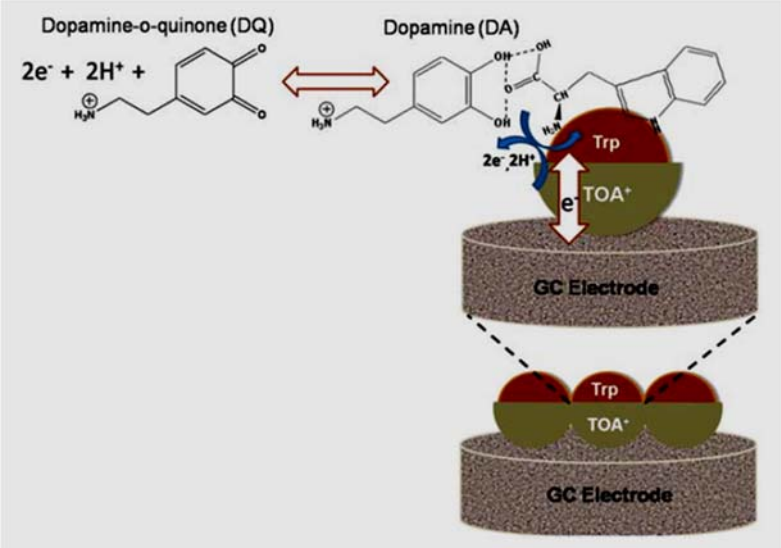

Fig. 2. Schematic illustration of the Janus gold nanocluster modified GCE for the electrocatalytic oxidation of DA. Reprinted from [22] with permission.

Although Janus nanoparticles offer the advantage for biosensing of two different faces with different chemical compositions allowing toposelective immobilization of complementary bioreceptors on these faces by using different specific ligands and different linking strategies [23], Pingarrón´s group was the first to explore the use of these nanoparticles for the development of electrochemical biosensors.

Sánchez et al. [24] used Janus Au-mesoporous silica nanoparticles (Au-MS JNP) as scaffolds to design an integrated electrochemical biorecognition-signaling system. They demonstrated the concept by covalent immobilization of HRP on the mesoporous silica face as enzymatic signaling element, and modification of the Au face with streptavidin (Stv) and polyethylenglycol (PEG) chains as biorecognition and solubilizing agents, respectively (Au-MS JNPHRP-Stv-PEG). To achieve these goals, the mesoporous silica face of Au-MS JNP was first enriched with primary amino groups by treatment with (3-aminopropyl)triethoxysilane (APTES). After activation of the amino groups with glutaraldehyde, the covalent immobilization of HRP on the mesoporous silica face was accomplished. On the other hand, streptavidin was reacted with 3,3′-dithiobis (sulfosuccinimidylpropionate) (DTSSP) and further treated with $NaBH_4$ to provide the protein surface with reactive thiol groups, allowing the protein immobilization on the gold face of Au-MS JNP through a chemisorption process. Results presented demonstrated that Au-MS JNPHRP-Stv-PEG particles have the ability to both specifically biorecognize biotin residues on an Au surface and to produce an enzyme-mediated electroanalytical signal by cyclic voltammetry upon addition of H_2O_2.

Same group proposed the deposition of Au–mesoporous silica Janus nanoparticles on GCEs coated with single-walled carbon nanotubes for the preparation of a bienzyme amperometric biosensor for D-glucose through toposelective immobilization of glucose oxidase (GOx) and horseradish peroxidase (HRP) on the Au and mesoporous silica faces, respectively (Fig. 3) [23]. The developed biosensor (GOx–HRP–JNP/CNT/GCE) demonstrated an excellent electroanalytical performance with a wide linear range of 490 nM–600 μM, a high sensitivity of 4.3 mA M^{-1}, and a low LOD (360 nM) for glucose, and successful applicability to the determination of D-glucose in commercial soft drink samples.

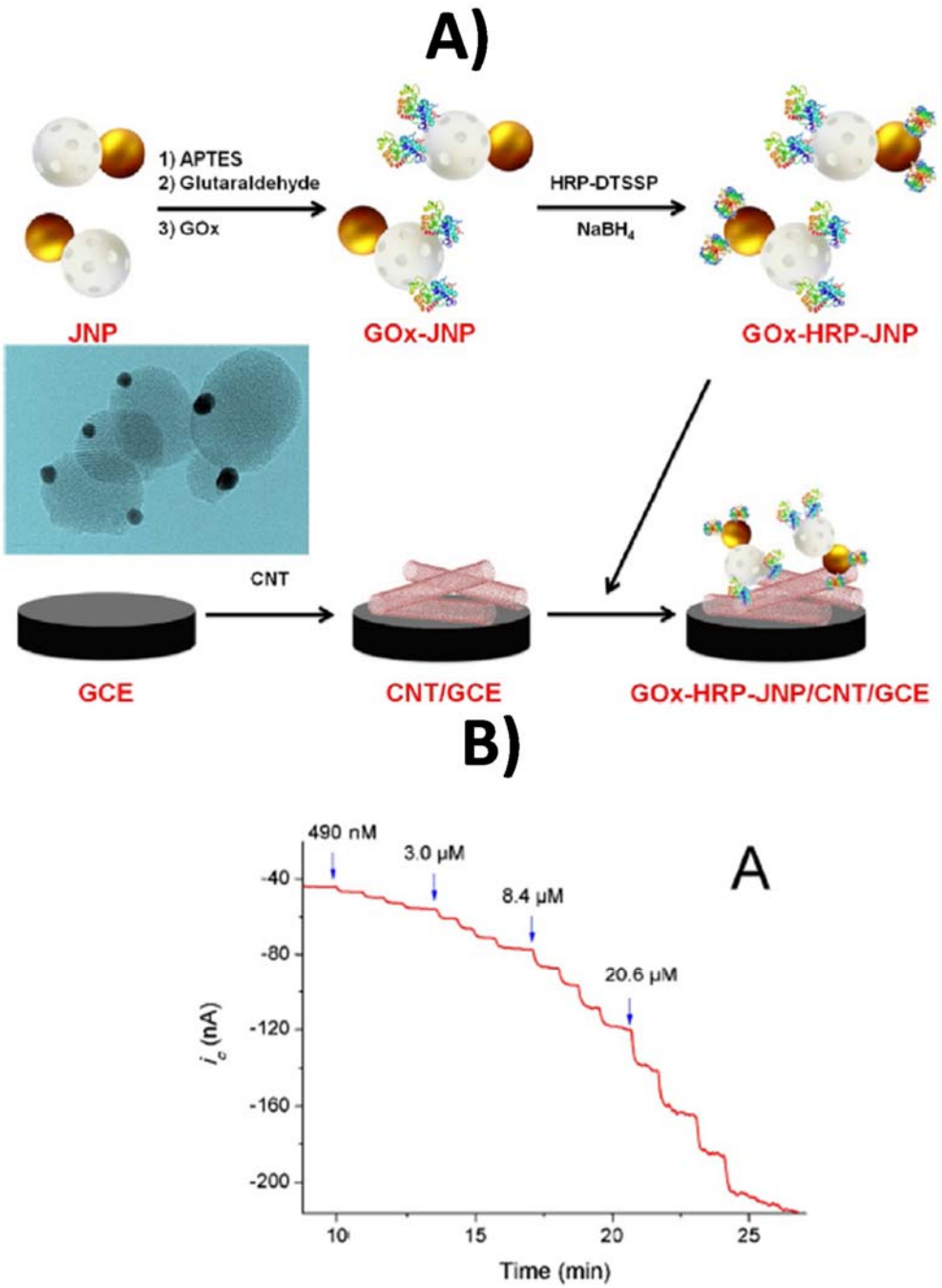

Fig. 3. Schematic display of the steps involved in the assembly of the GOx–HRP–JNP/CNT/GCE bienzyme biosensor (A) and amperometric response obtained for glucose (B). Reprinted and adapted from [23] with permission.

Various metals catalyze electrochemical reactions that generate gases, such as the decomposition of H_2O_2 catalyzed by platinum. When such metals are incorporated into one or

more building blocks of a Janus particle, the particle becomes a chemically powered motor that exhibits autonomous bubble propulsion enabled by the particle surface asymmetry [25] in the presence of the appropriate chemical fuel and even in microfluidic channels [26]. A couple of electrochemical biosensing strategies have been reported based on such self-propelled Janus motors. For example, Mg microspheres efficiently propel in the presence of water and chloride because the Mg surface is readily oxidized, by combination of galvanic and pitting corrosion processes, which results in the generation of hydrogen microbubbles and hydroxyl ions [27]. These self-propelled Janus particles, also known as Janus microengines, have been exploited also as "autonomous stirrers" which is especially relevant in connection to the use of screen-printed electrodes limited to quiescent solutions and hence solely to diffusion transport for electrochemical sensing [28]. In comparison with other types of motors, Janus particles offer interesting advantages for biosensing applications such as the distinct chemical or physical properties of their surface which allow their easy functional modification [28,29] and their large surface to capture/adsorb more (bio)chemicals [30]. Moreover, the use of non-catalytic Janus micromotors driven by light [31,32] or biocompatible fluids such acid [33,34] or water [35,36] avoids the interference of H_2O_2 in some (bio)sensing approaches and greatly expand the scope of practical and biomedical applications impeded by the requirement of H_2O_2 [29,37]. The composition of the motor and the fuel makes Janus micromotors highly biocompatible and environmentally friendly compared to others. Indeed they can be easily made of transient self-destroyed materials and they can perform biosensing while autonomously disappeared in the media leaving non-toxic residues [38].

The first example of the remarkable capabilities offered by Janus micromotors in electrochemical sensing was reported by Wang´s group [39] by using water driven magnetic Au-Ni-Mg Janus micromotors, confined onto the surface of printable sensor strips. Janus Mg-

microengines served as artificial enzymes toward the alkaline and selective hydrolysis of non-detectable pesticide paraoxon into the electroactive and non-hazardous p-nitrophenol (see mechanism and reactions involved in Fig. 4A). Results demonstrated the improved amperometric detection accomplished by fixing the micromotors onto the strip surface through an intermediate Ni layer which prevented their movement to the electrode area avoiding interference during the electrochemical measurements. Moreover, the presence of bubble-generating magnesium Janus microengines led to a 15-fold increase in the sensitivity towards paraoxon detection compared to a bare screen-printed electrode, results attributed to the enhanced mixing induced by the motion of self-propelled Janus particles [40].

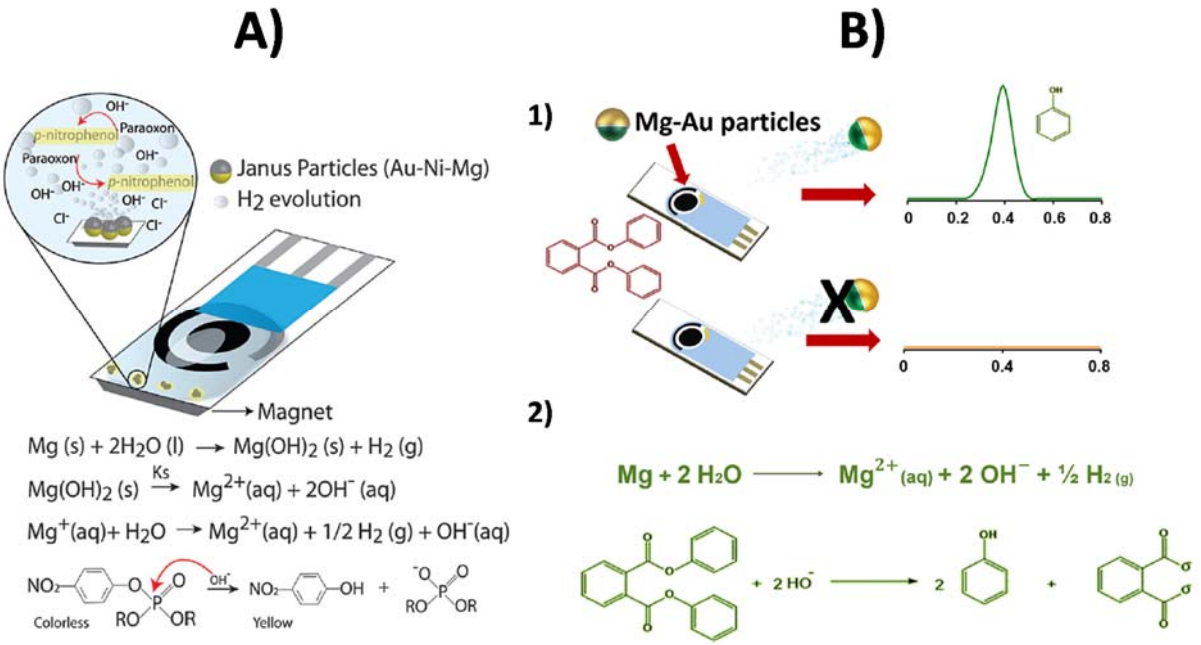

Fig. 4. Mechanism and reactions involved in the paraoxon OP nerve agents degradation to p-nitrophenol (A) and diphenyl phthalate (DPP) to phenol at Janus nanoparticles-modified sensor trips (B). Reprinted from [39] (A) and [27] (B).

A similar approach was developed by Rojas et al. [27] for the degradation of the non-electroactive diphenyl phthalate (DPP) into phenol directly measured by difference pulse

voltammetry at Mg/Au Janus micromotors-modified disposable screen-printed electrodes (Fig. 4B). In this case, the use of propelled Mg/Au micromotors provided a ~20 times improvement in sensitivity compared with that achieved under static conditions. This Janus micromotors-based electrochemical sensor demonstrated applicability for the determination of DPP in spiked water, milk, whiskey, and raw human serum samples without sample pretreatment and in just 5 min.

4. Janus particles for optical (bio)sensing

Wang´s group was pioneer in using non-spherical Janus particles for optical (bio)sensing. In particular, they took advantage on selective acceleration of chemically-powered Pt–Au bi-segmented nanowires (Fig. 5A) in the presence of chemical stimuli for developing new motion-based sensing [41] and biosensing [42] strategies which relied on the use of an optical microscope for tracking changes in the speed/distance of nanowire motors in the presence of the target analytes. Directed magnetic alignment accomplished by incorporating a ferromagnetic nickel segment into the nanowire motors provided a convenient and attractive method to quantify target analytes based on straight-line distance signals of 'racing nanomotors'.

The idea of motion as a new transduction principle was introduced in connection with the detection of silver ions which led to a specific and dramatic acceleration of catalytic Pt-Au nanowires, attributed to the increase in the electrocatalytic activity of the Pt segment for H_2O_2 oxidation after underpotential deposition of silver (Fig. 5B) [41]. Such nanomotor-based silver sensing allowed the construction of a calibration graph by plotting the speed vs. silver ion concentration over the 10^{-9} to 10^{-4} M range. This motion-driven analytical readout was utilized also for the detection of nucleic acids and bacteria (*Escherichia coli, E. coli*) in a typical sandwich hybridization assay involving the incubation of the target nucleic acid

(synthetic DNA and *E. coli* 16S rRNA) with a surface-bound oligonucleotide probe, followed by the capture of a silver nanoparticle-tagged detector probe (Fig. 5C) [42]. The silver nanoparticles were then rapidly dissolved by adding the hydrogen peroxide fuel and the entire solution was added to the nanomotors. Silver-induced changes in the nanomotor speed led to a well-defined dependence of the speed signal on the concentration of the nucleic-acid target allowing LODs of 40 amol (synthetic DNA) and 2,000 colony forming units (cfus) μL^{-1} *E. coli*. These motion-driven (bio)sensing approaches represented a paradigmatic shift in bioanalysis, proposing for the first time speed and distance as analytical readouts. These concepts opened a new avenue for the detection of a broad range of target biomolecules in connection with other biomolecular interactions and motion transduction principles.

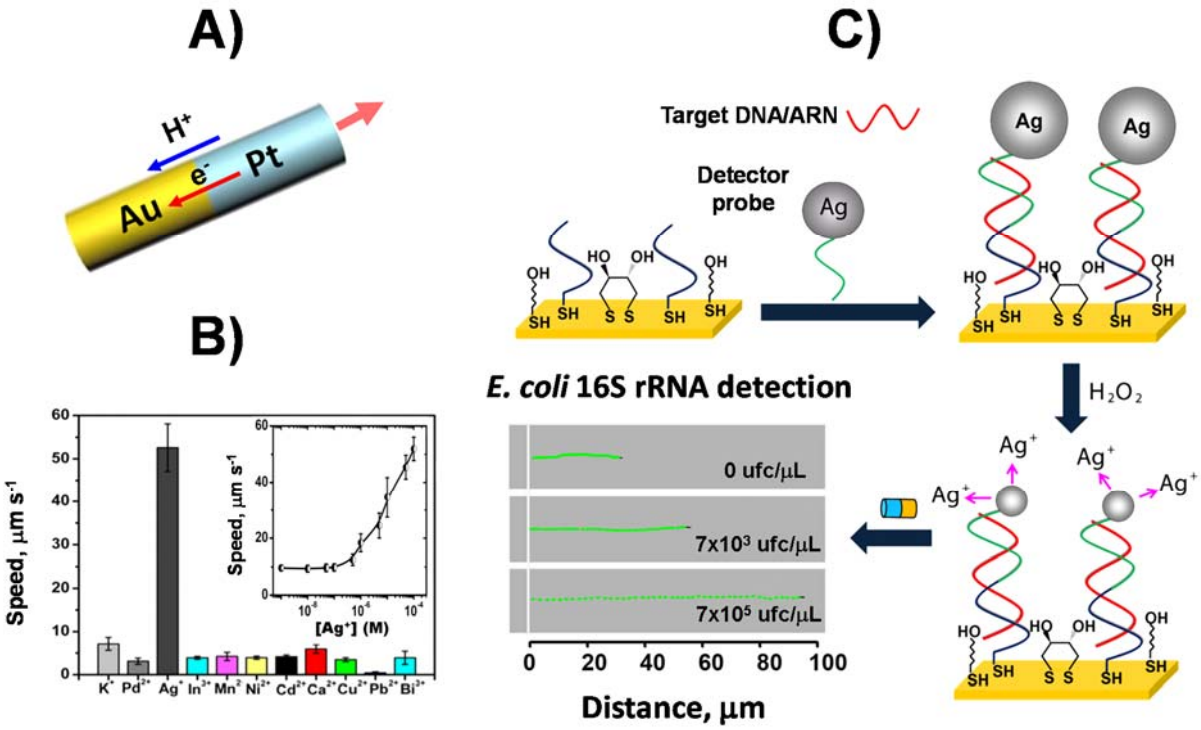

Fig. 5. Pt–Au bisegment nanowires (A). Bar chart comparing the average speed of 20 nanomotors in 100 µM solutions of each of the specified ions and the calibration curve obtained for Ag^+ determination (insert) (B). Basis of the methodology developed for nucleic acid determination and visual detection of the movement of catalytic nanomotors in the

resulting Ag^{+} enriched H_2O_2 solutions after hybridization with the complementary nucleic acid (C). Reprinted and adapted from [41] (a, b) and [42] (c) with permission.

More recently, the excellent ability of Janus particles to reinforce the optical properties of different materials, as well as the possibility of designing multitarget detection schemes has aroused the interest of using these particles for the optical sensing of diverse species. In fact, Janus particles can allow an independent biochemical conjugation with the possibility of optical detection based on dyes or contrast agents located within the two sides in absence of interferences [43]. Techniques such as fluorescence, SERS, or visible spectrophotometry have been used so far. However, despite the many potential advantages envisaged, the number of sensors and biosensors developed until now is still very low, and so, many more are likely to be developed in the coming years.

Polydiacetylenes (PDA) have been utilized in numerous colorimetric sensing systems due to their convenient spontaneous color change and fluorescent emission development under various external stimuli [44]. PDA-based sensors are commonly prepared by self-assembly of rationally designed PDA monomers into liposomes. Immobilization of PDA liposomes on a solid substrate or in microparticles and fibers have some important advantages related to stability and sensitivity. Lee and Kim [45] prepared multiphasic sensing alginate-based Janus particles with PDA liposomes (Fig. 6). Alginate, an anionic polysaccharide constituent of the algae cell membrane characterized by its biocompatibility, porosity, and gelation property with multivalent cations, such as Ca^{2+} ions, has been widely used for the encapsulation of various chemicals and drugs. This approach took advantage of the gelation and porous nature of the cross-linked alginate microparticles for the encapsulation of PDA liposomes able to produce a sensing signal as result of freely access of analytes to them. Biphasic Janus particles containing two different PDA liposomes, biotin- 10,12-pentacosadiynoic acid

(PCDA) and 2-(2-(2-bromoethoxy)ethoxy)-ethyl pentacosa-10,12-diynoate (PCDA-EG-CA)/PCDA (9/1), selectively interacted with avidin-fluorescein isothiocyanate (FITC) and melamine, respectively, the latter through cyanuric acid (CA). Hemispherical green/red fluorescence originated for each interaction observed by fluorescence microscopy confirmed that each liposome component of the biphasic alginate detected selectively the corresponding target without interference from the other. The developed system was found to be selective and sensitive for the detection of melamine down to 50 ppb level in aqueous solution.

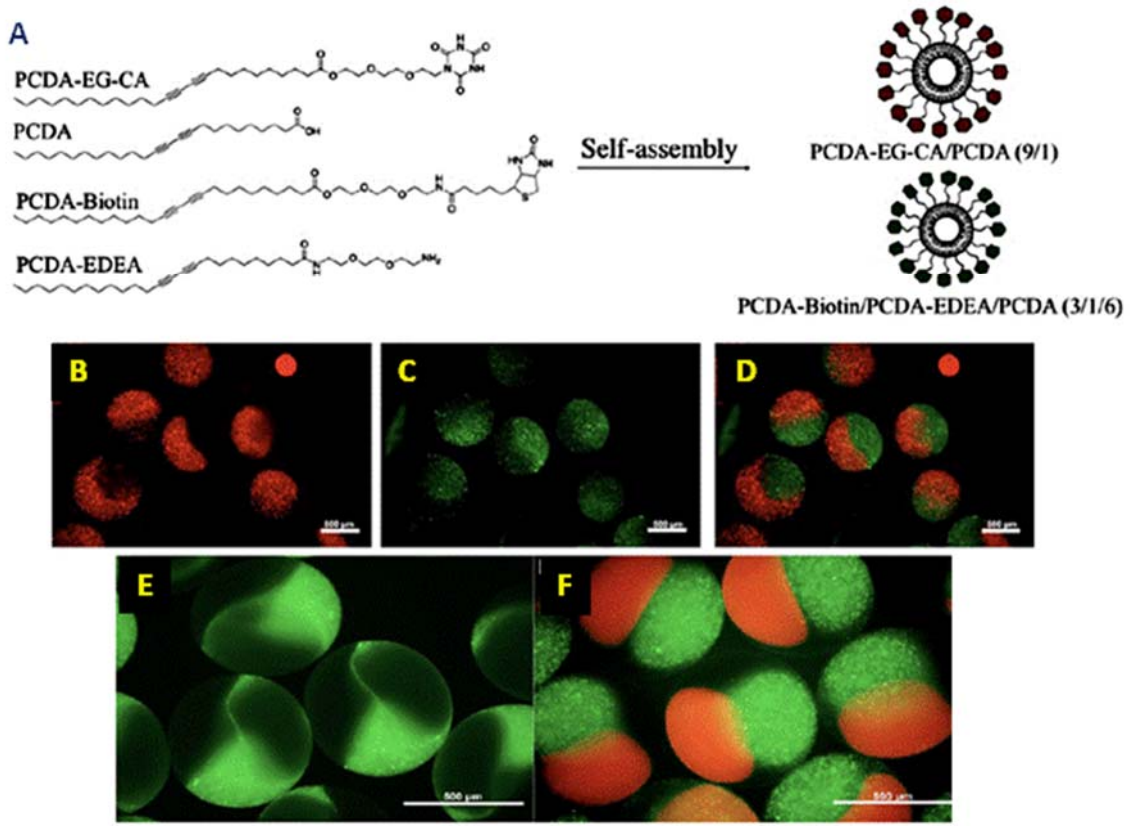

Fig. 6. Chemical structures of the investigated PDA molecules to form self-assembled PDA liposomes (A); fluorescence microscope image of biphasic Janus alginate microparticles with two different PDA liposomes (PCDAEG-CA/PCDA (9/1) and PCDA/PCDA-EDEA/PCDA-biotin (6/1/3)) after incubation in melamine solution (B); green fluorescence emission after incubation in an avidin-FITC solution (C); overlay of the two fluorescence images representing the biphasic property of the Janus alginate particles (D); triphasic image of PDA

liposomes loaded with alginate particles after incubation with avidin-FITC (E); subsequently with melamine solution (F). Reprinted and adapted from [45] with permission.

Janus-compartmental microbeads composed of two divided phases with sensing PDA liposomes and magnetic nanoparticles were prepared for the selective detection of lead. The Janus microbeads were fabricated by cross-linking alginate solutions containing sensing PDA liposomes and magnetic nanoparticles, respectively, with calcium ions as shown in Fig. 7. PDA liposomes were prepared with the lipid 10,12-pentacosadiynoic acid and 1,2-dipalmitoyl-sn-glycero-3-galloyl (DPGG) which is known to form phenolic metal complexes with lead (II). Integration of sensing PDA and efficient alginate absorption into the magnetic Janus microbeads permitted the sensitive detection of the metal ion. In this way, PDA-DPGG liposome in the Janus microbeads showed sharp color change and red fluorescence upon exposure to lead(II) ions (Fig. 7D). A good correlation between the fluorescence signal intensity and the analyte concentration was found between 0.1 and 1.0 mM, with a LOD of 0.1 mM Pb(II) [44].

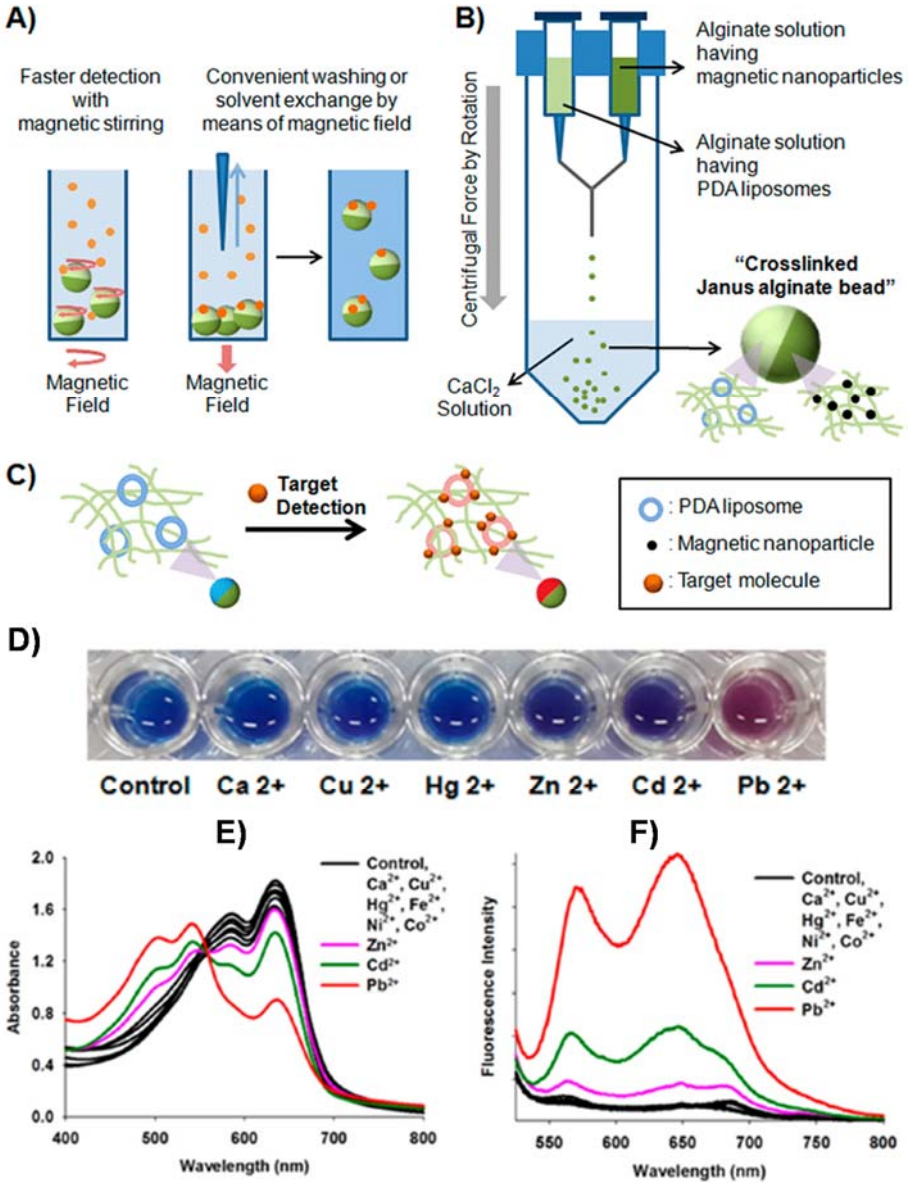

Fig. 7. Schematic illustration of Janus magnetic microbeads in solutions (A); scheme of the fabrication procedure of the Janus-compartmental microbeads with sensing PDA liposomes and magnetic nanoparticles (B); colorimetric detection of target lead(II) ions (C); optical images of PDA-DPGG liposomes (0.66 mM) after 1 h incubation with different metal ions (0.26 mM) (D), and corresponding UV−vis (E) and photoluminescence (F) spectra. Reprinted and adapted from [44] with permission.

Hematite−silica hybrid (γ-Fe_2O_3/SiO_2) Janus nanoparticles were prepared to construct a multifunctional biosensing platform for sensitive colorimetric detection of H_2O_2 and glucose.

The prepared nanoparticles exhibited an intrinsic peroxidase-like catalytic activity, could be used over a wider range of pH and temperatures and were more stable over time than the natural enzyme. Importantly, the asymmetric properties of the Janus particles enabled them for multiple functional utilities and various biosensing applications, including the ease of surface modification without deactivation of catalytic activity and reusability by magnetic separation [46]. Glucose oxidase was conjugated onto the silica side of nanoparticles for the glucose conversion whereas the Fe_2O_3 side catalyzed the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 into a blue product. In addition, the magnetic property of this hemisphere enabled easy separation and recycling of the nanoparticles. A linear range between 1 and 100 μM and a LOD value of 10.6 nM were achieved for the determination of H_2O_2, while a linear response between 0 and 20 mM, with a LOD of 3.2 mM was found for glucose.

Recently, a method for the determination of glucose and cholesterol was developed based on the distinct hemispheres of Janus particles. Single-phase and Janus hydrogel microparticles were fabricated using a centrifugal microfluidic chip. Concanavalin A and FITC-labeled dextran were used for competitive binding assay and were encapsulated in alginate particles, whose fluorescence was positively correlated with glucose concentration (Fig. 8). Regarding cholesterol detection, microparticles were embedded with γ-Fe_2O_3 NPs used as catalyst for the oxidation of TMB by the H_2O_2 generated by enzymatic hydrolysis of cholesterol. Advantageously, the color transition was demonstrated to be more sensitive in the microparticles than in solution. Apart from the possibility of manipulation by an external magnetic field, another advantage was that multitarget sensing of glucose and cholesterol could be performed in the two hemispheres without obvious interference. Application to the analysis in human serum confirmed the potential utility of these microparticles in real sample detection [47].

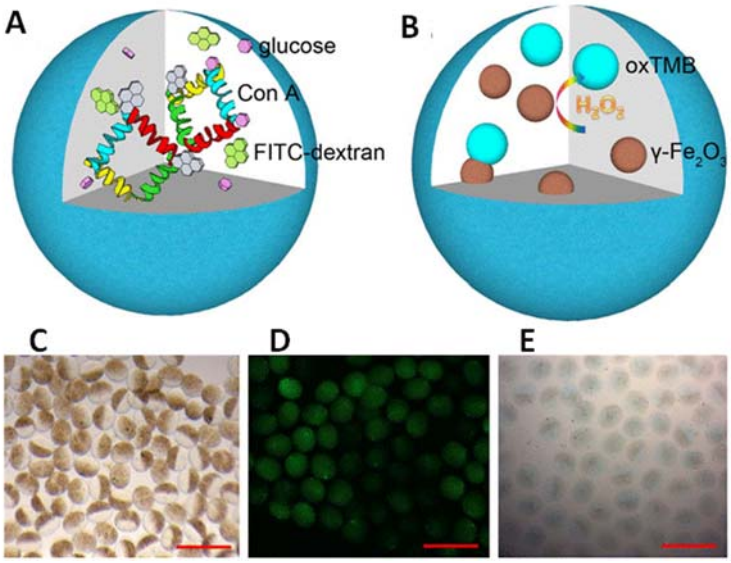

Fig. 8. Upper: Schematic diagrams of sensing mechanisms for glucose (A) and cholesterol (B) within the Janus microparticles (JMPs). Down: bright field image of JMPs (C), fluorescence image of JMPs after addition of 400 mg dL^{-1} glucose sample (B) and bright field image of JMPs after subsequent addition of 0.3 mM cholesterol sample (C). Scale bars represent 500 μm. Reprinted and adapted from [47] with permission.

Metal nanoparticles that improve molecular spectral information with techniques such as surface-enhanced Raman spectroscopy (SERS) are promising since the read-out signal is intrinsic to molecules in proximity to the nanoprobe surface. This molecular fingerprint then offers the potential for providing label-free multichannel information of local biochemical composition. Wu et al. reported the first study that simultaneously employed Janus particles for targeting and SERS sensing of tumor cells [48]. A stand-alone cellular probe called nanocoral, which combined cellular specific targeting with biomolecular sensing, was developed. This consisted on Janus particles (100–800 nm in diameter) with a roughened gold coating on one side and polystyrene on the other. After roughening the polystyrene particles using oxygen plasma etching, a thin layer of gold was coated on one hemisphere. By selectively functionalizing the exposed polystyrene surface with anti-HER-2 antibodies, the

Janus particles were shown to be selectively attached to the breast cancer cells. The combination of cell-targeting ligands on the polystyrene hemisphere and high SERS signal from roughened gold surface could potentially be used to map the distribution of biomolecules at the cell surface.

Using an equivalent strategy, Hsieh et al. prepared dual-faced and tri-functional nanoparticles for sequentially recognizing tumor cells, drug delivery and real-time monitoring of biological responses. Similar to the previous work, the Janus particles consisted on fluorescent polystyrene beads tailored by oxygen plasma process into a corrugated upper hemisphere. Simultaneously, the entire surface was modified with carboxylic groups, and gold was further deposited onto the corrugated surface for SERS. Nanoparticles were functionalized with sulfo-NHS-SS-biotin disulfide linkers and anti-CD44 antibodies used for the detection of glycoproteins CD44 on the surface of cancer cells and for the release of their loads (strep-QDs, Qdot® 585 streptavidin, were used as model) in the cytoplasm via the cleavage of disulfide bonds [49].

Anisotropic polymeric microparticles modified with maleimide or acetylene were developed to spatio-selectively conjugate two different antibodies into each compartment for fluorescence multiplexed biosensing of carcinoembryonic antigen (CEA) and human IgG using two different fluorescent dyes. The antibodies were immobilized through separate maleimide-thiol coupling reaction and Huisgen 1,3-dipolar cycloaddition ("click" chemistry) resulting in antibodies ordered orientation due to the spatio-specific molecular interaction. As Fig. 9 shows, sandwich-type immunoassays were performed by biofunctionalization of specific antibodies onto poly(dimethylsiloxane) (PDMS) glass substrates after immunocomplexation with the respective antigen [50].

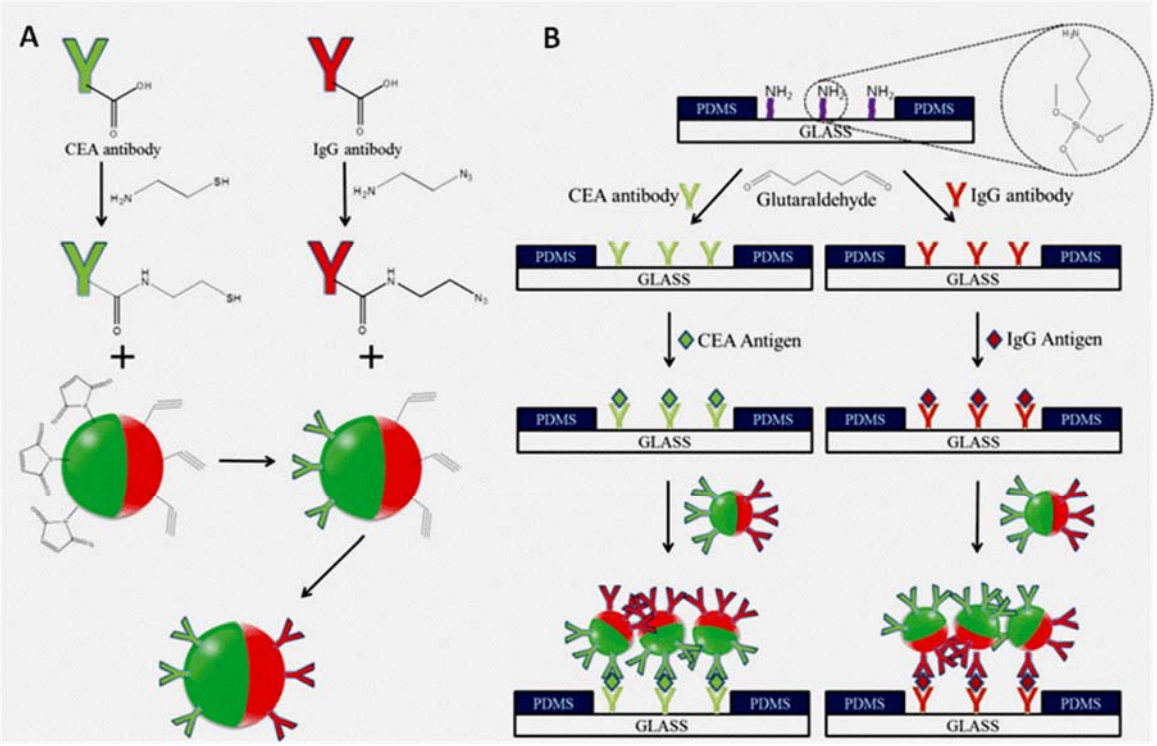

Fig. 9. Schematic illustration of spatio-selective bioconjugation on each compartment of anisotropic polymer microparticles with anti-CEA via maleimide-thiol coupling, and anti-IgG via copper (I)-catalyzed Huisgen 1,3-dipolar cycloaddition (A); sandwich-type immunocomplex formation for fluorescence-based, multiplexing detection of CEA and IgG antigens (B). Reprinted from [50] with permission.

Janus particles have also been designed to combine sensing and barcoding functions for multiplexed bioanalysis. For example, Janus disks composed of a fluorescent, graphically encoded region and a probe-loaded region were used to sensitively detect DNA oligomers [51]. Acrylate-modified oligonucleotide probes were designed to specifically detect DNA sequences. The Janus disks equipped with multiple probes were scanned in a flow through device, thus a high throughput analysis was achieved. Furthermore, DNA oligomers at 500 attomolar level could be detected without need for biotin-avidin-aided signal amplification.

This concept was also used in the capture and labelling of protein targets and the rapid microfluidic scanning of disk Janus particles for multiplexed detection [52]. Multifunctional

hydrogel particles made from poly(ethylene glycol) were prepared. These particles contained spatially segregated regions with a graphical barcode consisting of unpolymerized holes in the wafer structure of the gel particle, and one or more separate probe strips for multiplexed quantification of targets. The code served to identify the antibody probe covalently incorporated throughout a separate probe region of the particle. Hydrogel particles allowed the bulk immobilization of capture probes, thereby providing enhanced binding capacity over surface-functionalization techniques. By using this methodology, single- or multiple-probe particles can be reproducibly synthesized and used in customizable multiplexed panels to measure protein targets over a three-log range and at concentrations as low as 1 pg mL^{-1}.

Molecularly imprinted microcarriers based on Janus microparticles with specific molecular recognition ability, have been also developed. The resulting materials could be used not only as sensing systems but also as autonomous carriers for controlled drug delivery. Molecular imprinting is a well-known synthetic technology, which allows the preparation of homogeneous polymeric matrices with specific binding cavities. The main advantages of molecularly imprinted polymers (MIPs) are their high selectivity and affinity to the template molecules used in the imprinting procedure. MIP concept has found many practical applications including sensing and separation of diverse target analytes, offering great promise for biomedical and environmental applications [53]. In this context, Janus particles with specific molecular recognition ability towards propanolol were prepared. Monodisperse MIP particles containing amino groups were synthesized via a wax-water Pickering emulsion as it is illustrated in Fig. 10. Imprinted sites for the drug were introduced by crosslinking polymerization. Moreover, when the Janus MIP particles were exposed to the UV light in the presence of H_2O_2, an ion gradient was formed due to the production of Ag^+ and HOO^- ions in the solution, and the Janus MIP particles moved in the self-generated ion gradient along their axis with the Ag particle leading [54].

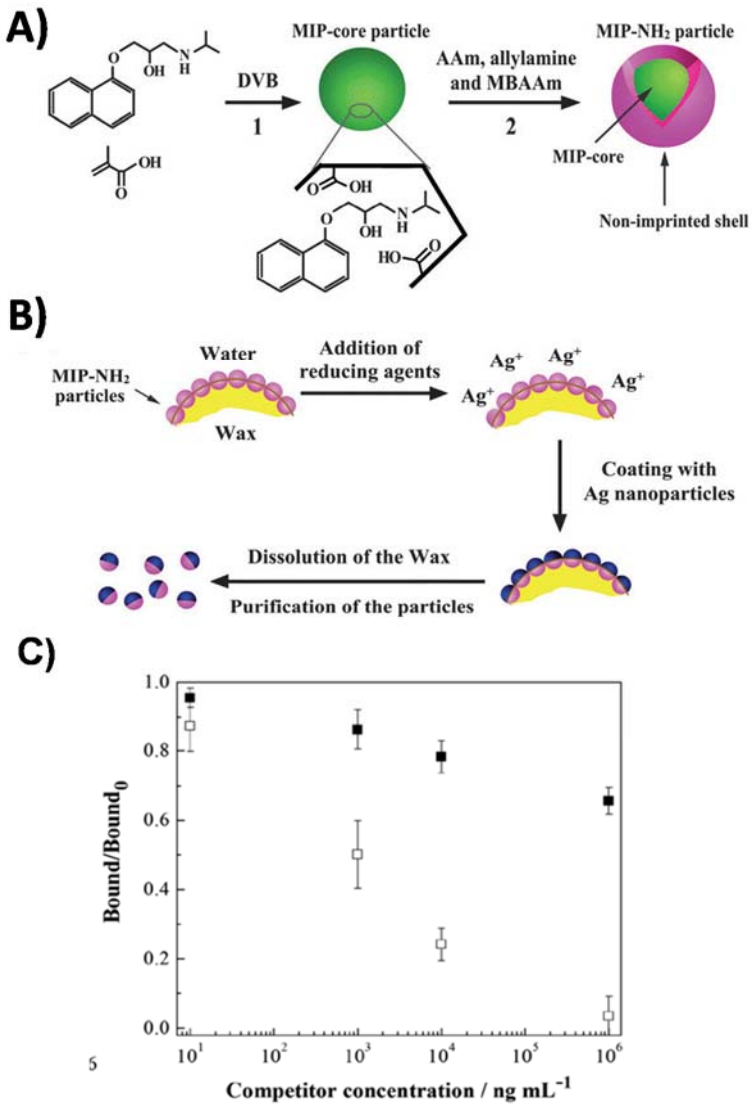

Fig. 10. Schematic representation of the synthesis of monodisperse MIP–NH_2 microspheres (A): 1) generation of propranolol-imprinted sites by cross-linking polymerization; 2) grafting amine groups by copolymerization. Fabrication of Janus colloidal particles (B). Displacement of radioligand binding to 0.5 mg of Janus MIP particles with increasing amount of atenolol (full squares) and propranolol (empty squares). $Bound_0$ and bound are the amount of [^{3}H]-(S)-propranolol bound by Janus MIP particles in the absence and presence of the competing compounds, respectively (C). Reprinted and adapted from [54] with permission.

As in the case of electrochemical detection, micro- and nanomotors based on Janus particles exhibiting useful optical properties have also been explored for monitoring target compounds and delivery applications. As an example, a highly efficient magnetocatalytic hybrid Janus micromotor was constructed by encapsulating graphene quantum dots (GQDs) modified with phenylboronic acid (PABA) for application as an ultrafast sensor for the detection of deadly bacteria endotoxins. An oil-in-water emulsion containing GQDs, PtNPs and Fe_3O_4 NPs was used to synthesize the asymmetric particles where PABA tags acted as highly specific recognition sites to detect endotoxin released from *E coli* bacteria (LPS0111:B4) showing fluorescence quenching upon the interaction of GQDs with the target lipopolysaccharides (LPS). Furthermore, in the presence of H_2O_2 or under a magnetic field, the two active differentiated regions in the particle enabled efficient propulsion. Importantly, continuous mixing induced by the motion of multiple micromotors across a contaminated sample resulted in greatly enhanced mass transport to increase the reaction rate between the LPS-contaminated solution and microparticles as compared to the use of static devices. Moreover, authors demonstrated the capabilities of GQDs-based micromachines for the screening of complex urine and human serum samples with high selectivity [55].

Sarin and soman are a particularly dangerous class of organophosphorous nerve agents. Janus microparticles were prepared for their detection by impregnation of fluoresceinamine (FLA) into silica microparticles followed by asymmetric deposition of Pt by sputtering. As illustrated in Fig. 11, the resulting FLA/silica-NH_2/Pt particles acted as a self-propelled micromotor-based fluorescent ‘‘On–Off’’ sensor of nerve agents. The approach, with inherent efficient solution mixing, resulted in the dramatic quenching of FLA fluorescence upon encountering nerve agents in solution [56]. The practical usefulness of the micromotor in environmental samples was evaluated in water without buffer using diethyl chlorophosphate (DCP) as a nerve agent simulant, as well as in sea, pool and lake waters. The specificity of the

developed micromotor was also confirmed by monitoring the fluorescence with non-reactive phosphonates, such as dimethyl methyl phosphonate (DMMP), which did not show fluorescence change at a 10^{-3} M concentration.

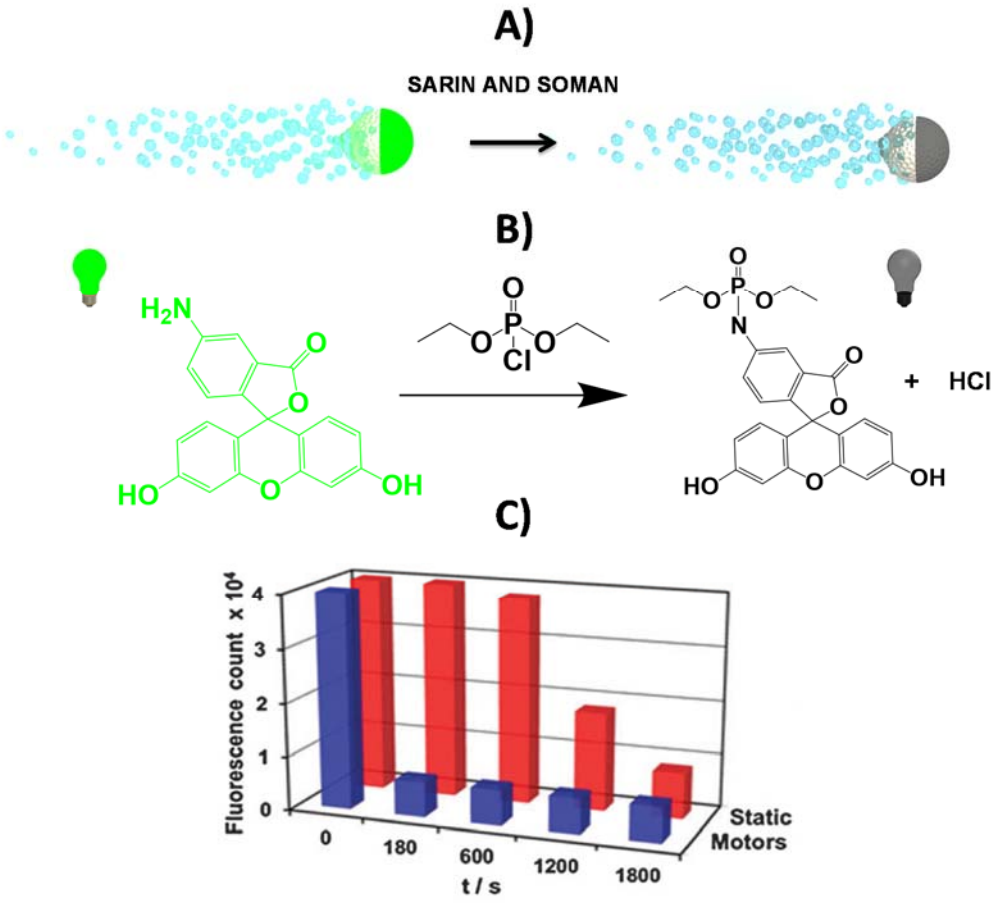

Fig. 11. Schematic representation of the micromotor based 'on-the-fly' fluorescent ''On–Off'' detection of nerve agent (A); reaction mechanism showing the reaction of FLA coated micromotor with DCP (B); comparison of fluorescent quenching with micromotor and static FLA-coated silica with 10^{-4} M DCP. Reaction conditions: H_2O^2 (2%), SDS (1%), λ_{ex} = 490 nm; λ_{em} = 510 nm (C). Reprinted and adapted from [56] with permission.

Very recently Zhang et al. [32] have highly efficient photocatalytic Au-WO_3@C Janus micromotors which display efficient propulsion in pure water. In the presence of UV light, Au-WO_3@C Janus micromotors move towards to Au coated side through a dominative

diffusiophoretic mechanism. The acceleration of the propulsion of the Janus micromotors due to enhanced diffusiophoretic effects by photocatalytic degradation of dyes such as sodium-2,6-dichloroindophenol (DCIP) and Rhodamine B (RhB) was exploited for monitoring of dyes rapid degradation in water. This dye-induced acceleration behavior offers the Janus micromotor a great potential for environmental applications such as photodegradation and detection at very low levels of dye pollutants (5×10^{-13} for RhB and 5×10^{-9} DCIP %) in water.

5. General conclusions, challenges and perspectives

The works highlighted in this article show as multi-faced, engineered Janus nanoparticles can be employed to open new possibilities for the design of advanced and tailor-made biofunctionalized nanomaterials for electrochemical and optical (bio)sensing purposes. Although scarcely used so far in electrochemical biosensing, Janus nanoparticles have been employed mainly as tailored supports for the co-immobilization of two complementary enzymes. Also, self-propelled particles have been integrated within electrochemical strip platforms for built-in mixing ultra-small samples while eliminating the need for additional stimuli, instrument or external stirrer. In this context, Janus micromotors have been mainly applied to the determination of non-electrochemically active hazardous compounds through their efficient degradation into electrochemically active non-hazardous substances. Nevertheless, Janus micromotors can be used in connection to thick-film and thin-film planar sensor strips for a wide variety of practical applications. Despite these attractive features, the relatively short lifetime of Janus nanomotors, which dissolves after 5 min movement in the sample, limits the possibility to increase further the sensitivity of pollutants determination through the generation of more readily detectable compound (i.e., higher degradation yield).

Regarding the applicability of Janus particles in optical (bio)sensing, they have demonstrated to offer very interesting alternative strategies for single or dual fluorescence

determination of relevant analytes such as melamine, lead, H_2O_2, glucose and cholesterol. Specific SERS biosensing of several antigens (CEA, human IgG) and tumor cells by targeting specific cell surface receptors such as HER-2 and CD44 upon modification of the Janus particles with specific antibodies represents interesting bioanalytical strategies. In addition, Janus particles have shown both sensing and barcoding functions to enable multiplexed determination of DNA oligomers and protein targets. The use of Janus micro- and nanomotors has been reported also in connection to optical transduction for sensing of LPS released by deadly bacteria, organophosphorous nerve agents, dyes and propanolol. It is worth to remark also the variability in the Janus particles preparation in the reported approaches. Janus particles containing PDA liposomes and MIP nanoparticles as sensing units and magnetic γ-Fe_2O_3 nanoparticles to provide them with magnetic properties have been described. Particularly attractive is the use of magnetic γ-Fe_2O_3 nanoparticles because, apart from enabling the easy separation and recycling of the Janus particles by an external magnetic field, they offer the possibility of exploiting the intrinsic peroxidase-like catalytic activity which is of great interest also in biosensing applications.

Moreover, although significant progress has been made in the preparation of well-defined Janus nanoparticles, such as heterodimers and polymeric Janus nanoparticles with only few steps, currently the combination of new materials, the creation of particles with well-defined compartments, each carrying its own functionalities, and the possibility to create objects with groups allowing for self-propulsion, is only achievable for large microparticles. Therefore, a major challenge ahead to take full advantage of their potential for biosensing, is to develop new methods able to produce high quantities of Janus nanoparticles in a precise and reproducible manner, and compatible with the diverse array of materials and functionalities that chemistry and material science offer. Indeed, it is envisaged that numerous applications of Janus nanoparticles will be increased further as the yield and control of the synthetic

methods is improves. Also, binary nanoparticles characterization must be improved for the unambiguous identification of Janus nanoparticles and to provide information about their polydispersity and quality. In this sense, it is worth to mention that the use of click chemistry to prepare Janus particles has been shown recently as a rapid and scalable method to design the chemistry, surface charge, morphology, and self-assembly of Janus particles.

In summary, given the large range of possibilities and interesting capabilities described so far and the main challenges to be facet yet, advances mainly in the preparation and modification of Janus particles with additional capabilities and in their practical applications in real-world systems are expected to drive researchers working in this exciting field.

Acknowledgements

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