

Engineering the Bio–Nano Interface Using a Multifunctional Coordinating Polymer Coating

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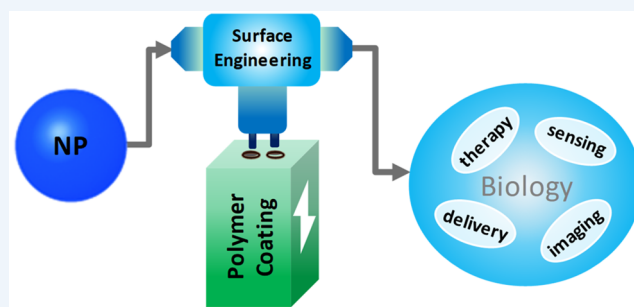
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Supporting Information

CONSPECTUS: In the past three decades, interest in using nanoparticles as diagnostic tools to interrogate various biosystems has witnessed remarkable growth. For instance, it has been shown that nanoparticle probes enable the study of cellular processes at the single molecule level. These advances provide new opportunities for understanding fundamental problems in biology, innovation in medicine, and the treatment of diseases. A multitude of nanoparticles have been designed to facilitate *in vitro* or *in vivo* sensing, imaging, and diagnostics. Some of those nanoparticle platforms are currently in clinical trials or have been approved by the U.S. Food and Drug Administration. Nonetheless, using nanoparticles in biology is still facing several obstacles, such as poor colloidal stability under physiological conditions, nonspecific interactions with serum proteins, and low targeting efficiency in biological fluids, in addition to issues of uncontrolled biodistribution and cytotoxicity. All these problems are primarily controlled by the surface stabilizing coating used.

In this Account, we summarize recent progress made in our laboratory focused on the development of multifunctional polymers as coordinating ligands, to tailor the surface properties of nanoparticles and facilitate their application in biology. We first detail the advantageous features of the coating strategy, followed by a discussion of the key parameters in the ligand design. We then describe the synthesis and use of a series of multicoordinating polymers as ligands optimized for coating quantum dots (QDs), gold nanoparticles (AuNPs), and magnetic nanoparticles (MNPs), with a focus on (i) how to improve the colloidal stability and antifouling performance of materials in biological conditions; (ii) how to design highly compact coating, without compromising colloidal stability; and (iii) how to tailor the surface functionalities to achieve conjugation to target biomolecules. We also highlight the ability of a phase transfer strategy, mediated by UV irradiation, to promote rapid ligand exchange while preserving the integrity of key functional groups. We then summarize the bioconjugation approaches applied to polymer-coated nanoparticles, with emphasis on the ability of metal–histidine self-assembly and click chemistry, to control the final nanoparticle bioconjugates. Finally, we demonstrate the use of polymer-coated nanoparticles for sensor design based on redox-active interactions and peptide-mediated intracellular delivery. We anticipate that the coating design presented in this Account would advance the integration of nanoparticles into biology and medicine.



1. INTRODUCTION

There has been an unprecedented growth in nanoparticle research over the past few decades.^{1–7} The unique physical and chemical properties exhibited by these materials have made them attractive for use as signal transducers and diagnostic tools to interrogate various biological processes.^{8–10} Nanoparticles can be engineered with diverse surface functionalities that allow one to integrate them into arrays of biological areas including the detection of biomolecules, tracking of cellular events, identifying enzyme functions, probing protein–receptor interactions, imaging specific biomarkers, and as drug delivery vehicles.^{3–5,7,10} Designing optimized nanoparticle bioconjugates can allow both a better understanding of fundamental problems and treatment of diseases.

Integration of these materials into biology requires precision engineering of the nanoparticle surfaces, in order to control their colloidal stability, surface functionality, and interactions with the

complex nature of biosystems. Designing a carefully optimized surface coating can provide compact nanoparticles that exhibit antifouling properties, robust colloidal stability, extended *in vivo* circulation, and controlled biodistribution.^{3,11–13} Ligand exchange is a commonly employed strategy for the functionalization of nanoparticles. It relies on the competitive substitution of the native cap with hydrophilic metal-coordinating ligands. Over the past two decades, a variety of coordinating ligands have been designed to achieve the desired nanoparticle properties,

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which include small molecules, dendrimers, oligomers, polymers, and biomolecules.^{11,14} Among these, the use of judiciously designed multifunctional coordinating polymers can allow fine control over the nanoparticle surface properties and their interactions with biological systems, which is paramount for their successful use in biomedicine. In this Account, we will summarize research strategies developed mainly by our group for controlling the nanoparticle interface, via ligand exchange strategy, with a particular focus on the use of multifunctional coordinating polymers.

2. MERITS OF POLYMERS AS COATING LIGANDS

Surface functionalization of nanoparticles using coordinating polymers provides several key advantages. First, it is possible to install multiple metal anchoring groups in a single chain, which promotes ligand binding via multisite coordination, yielding enhanced affinity to the nanoparticle surfaces (as depicted in Figure 1A). This shifts the coordination equilibrium to lower concentrations, greatly reducing the rate of ligand desorption from the nanoparticle surface, and thereby substantially improving the dispersion colloidal stability. For instance, gold nanoparticles (AuNPs) capped with multithiol polymers exhibit stronger resistance against dithiothreitol (DTT) competition and chemical digestion by cyanide ions, compared to molecular dithiol ligands (Figure 1).¹⁵ Second, the wealth of available polymerization reactions can yield coating materials with controlled complex architectures, where distinct but complementary functionalities can be combined within the same chain. This “all in one” design offers better control over the structure and stoichiometry of the coating on a nanoparticle surface. Third, small ligands presenting weakly binding anchors tend to yield modest to mediocre colloidal stability for the nanocrystals. However, when introduced in a polymer ligand, the multi-dentate interactions can still impart much enhanced long-term steric stability for these materials. This certainly broadens the choices of anchoring groups to explore when designing a coating ligand.

3. CONSIDERATIONS FOR LIGAND DESIGN

Two properties inherent to the ligand structure ultimately determine the colloidal stability of hydrophilic nanoparticles in biological environments: (1) strength of the coordination interaction of the anchoring group onto the nanoparticle surfaces and (2) affinity of the hydrophilic moieties to the surrounding aqueous environment.

3.1. The Metal-Chelating Groups

Selecting the best anchoring group to graft along the polymer chain requires a clear understanding of the metal-coordination chemistry involved for a particular nanoparticle system; a common guide relies on considerations predicted by the hard–soft acid–base (HSAB) theory.¹⁶ According to this theory, high binding affinity occurs between hard acid and hard base or between soft acid and soft base. A representative list of acid and base groups with coordination affinity toward nanoparticle surfaces discussed in this Account is summarized in Table 1. Based on HSAB, certain specific anchors can be identified, if the nature of the atoms/ions on the nanocrystal surfaces is classified as Lewis acid or Lewis base.

Figure 2A shows a list of anchoring groups most frequently incorporated in ligand designs for coordination onto three representative nanoparticle cores. Several of them are shared by these nanoparticles. For example, carboxylate- and amine-

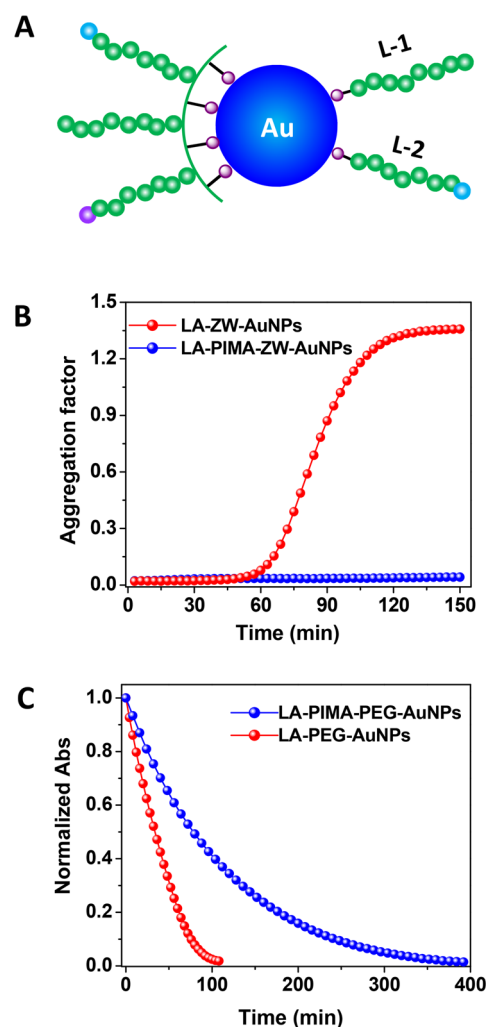


Figure 1. (A) Schematic representation showing side-by-side either a polymer ligand or a mixture of two molecular scale ligands coordinated on the surface of a AuNP. The terminal blue and purple dots in the ligands designate reactive groups. (B) Comparison of the colloidal stability data acquired from dispersions of AuNPs coated with lipoic acid- and zwitterion-modified polymer (LA-PIMA-ZW) or zwitterion-terminated lipoic acid (LA-ZW) in the presence of 1 M DTT. (C) Results of a sodium cyanide digestion test applied side-by-side to dispersions of AuNPs stabilized with LA-PIMA-PEG polymer or its monomer counterpart LA-PEG. Reproduced with permission from ref 15. Copyright 2017 American Chemical Society.

Table 1. Classification of Representative Hard and Soft Acids and Bases

category	examples
hard acids	Fe ³⁺ , Cr ³⁺ , Mg ²⁺ , Ga ³⁺ , Co ³⁺ , In ³⁺
borderline acids	Fe ²⁺ , Ni ²⁺ , Co ²⁺ , Pb ²⁺ , Cu ²⁺ , Zn ²⁺
soft acids	Cu ⁺ , Ag ⁺ , Au ⁺ , Hg ²⁺ , Cd ²⁺ , M ⁰
hard bases	R-CO ₂ ⁻ , R-PO(OH)O ⁻ , R-NH ₂ , R-catechol
borderline bases	R-pyridine, R-imidazole
soft bases	R-S ⁻ , R ₃ -P, R ₃ -PO, (RO) ₃ -P

terminated ligands have been used to surface-passivate quantum dots (QDs), AuNPs, and magnetic nanoparticles (MNPs) alike; however, being strong bases, their coordination onto these materials is relatively weak since the NPs present rather soft or borderline acid surfaces (see Table 1). Nonetheless, these ligands are still adopted as hydrophobic capping molecules

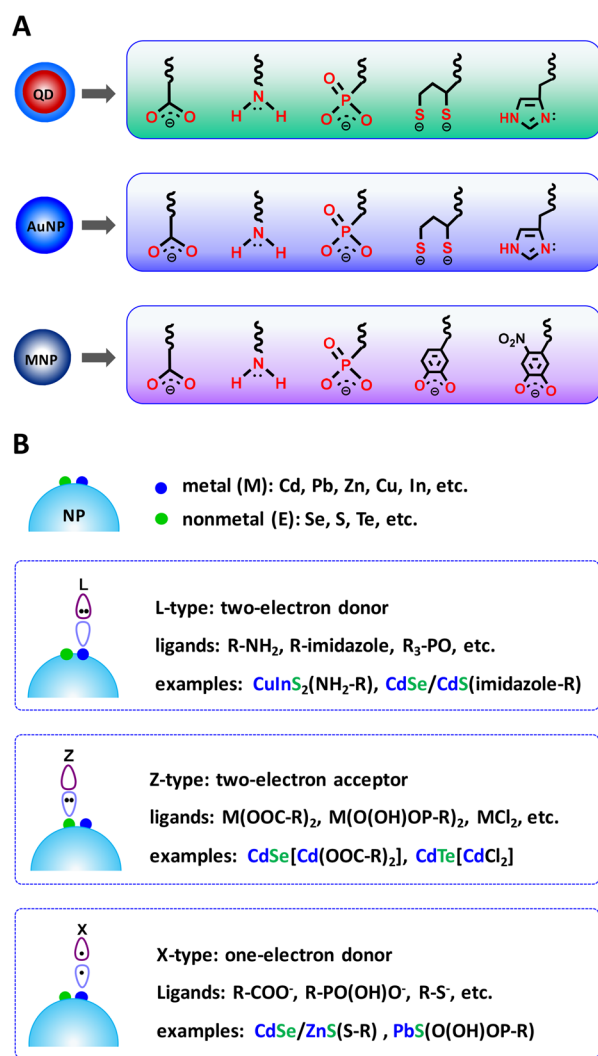


Figure 2. (A) Summary of the various metal-anchoring groups frequently employed in ligand design for three representative nanoparticles. (B) Nanoparticle–ligand binding motifs according to the CBC method and associated examples.

during the controlled growth of nanoparticles. Conversely, phosphonate ligands, being hard bases, can coordinate onto a wide range of nanoparticles, though they have been more widely applied for coating MNPs made of Fe₂O₃ and Fe₃O₄, where their affinity is comparable to that of catechols.¹⁷ Imidazole has been shown to coordinate onto QDs and AuNPs with high effectiveness if used in a multicoordinating polymer.^{18,19} In contrast, thiolates as soft bases show exceptionally high affinity to metals (e.g., Au and Ag) and semiconductor surfaces (e.g., Cd- and Zn-rich), which are considered as soft or borderline acids. This has motivated many research groups to develop thiol-based ligands for functionalizing metallic nanoparticles and semiconductor QDs.^{8,11,14} In general, stronger coordination tends to reduce the rate of ligand dissociation, providing better colloidal stability.

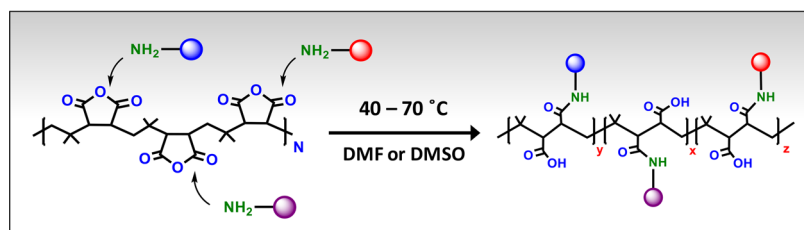
The key for understanding how ligands coordinate onto the nanoparticle surface is to elucidate the type of interaction between anchoring groups and surface sites. For this, covalent bond classification (CBC) has been used to organize the NP–ligand binding motifs.^{20–23} The CBC considers that every bond can be presented in terms of two-center–two-electron interaction. As such, three types of coordinating ligands have

been identified following the classification proposed by Green and Parkin,²⁴ namely, L-, Z-, and X-type, depending on the number of electrons contributed by a ligand to form the NP–ligand bond.^{20,21} Figure 2B shows three types of NP–ligand binding motifs and representative ligands, using semiconductor QDs as a model NP system. (1) L-type ligands donate two electrons to electrophilic sites on the NP. They typically coordinate onto surface metal atoms (e.g., Cd, Zn, Pb, etc.) through a NP(L) binding motif. Examples of L-type ligands include phosphine oxides (R₃-PO), primary amines (R-NH₂), imidazole derivatives (R-imidazole), and pyridine derivatives (R-pyridine). (2) Z-type ligands accept two electrons from nucleophilic sites on the NP surface; they typically coordinate onto nonmetal atoms (e.g., Se, S, Te, etc.) in a NP(Z) binding motif. Metal carboxylates, metal phosphonates, and metal chlorides are the examples of Z-type ligands. (3) X-type ligands interact with either metal or nonmetal atoms by sharing one valence-shell electron with a surface atom. These X-type ligands can be neutral radicals binding neutral surface sites or monovalent ions binding oppositely charged sites on the surface via a NP(MX₂) binding motif. Examples include carboxylates (R-COO⁻), thiolates (R-S⁻), and phosphonates (R-PO(OH)O⁻). Understanding these NP–ligand binding motifs allows one to rationalize the ligand selection, predict ligand exchange reaction, and anticipate its ability to passivate surface trap sites, which in turn provide a better control over the colloidal stability and physical/chemical properties of the materials.

3.2. The Hydrophilic Motifs

The hydrophilic motif plays a key role for using nanoparticles in biology, since it defines the colloidal characteristics and controls interactions with biological systems. Poly(ethylene glycol) (PEG) has been a popular hydrophilic motif applied for a variety of colloids.²⁵ Indeed, PEGylation provides effective shielding of the cores while promoting affinity to water, yielding materials with great colloidal stability and antifouling surfaces. These properties combined have made PEG a ubiquitous hydrophilic motif used by various groups. Nonetheless, high molecular weight PEG blocks are often required, which tends to affect affinity to water and increase the hydrodynamic size of the nanoparticles. Recently, using zwitterion (ZW) groups as hydrophilic motifs has generated much attention.²⁶ Zwitterion groups present an anion and a cation in the same structure, making the entire group charge-balanced. A coating based on zwitterions creates a thick hydration layer around the nanoparticles, which drastically reduces nonspecific protein absorption. Additionally, given their molecular scale, zwitterion-presenting ligands generate compact nanoparticles, a feature that is difficult to realize with PEGylated coatings. However, most zwitterion groups cannot be readily activated for further conjugation (e.g., sulfobetaine and phosphocholine), and a secondary ligand is often employed to address this limitation. Carboxybetaine in comparison allows further reaction via the carboxyl group. It loses its zwitterionic nature, nonetheless.²⁷ From the synthetic point of view, it is more challenging to prepare zwitterion-modified ligands, due to the limited solubility of zwitterion groups in common organic solvents. Overall, a rational selection of the hydrophilic motif for inclusion in the ligand design should ultimately be motivated by the desired application.

A. General Synthetic Scheme



B. Functionalities

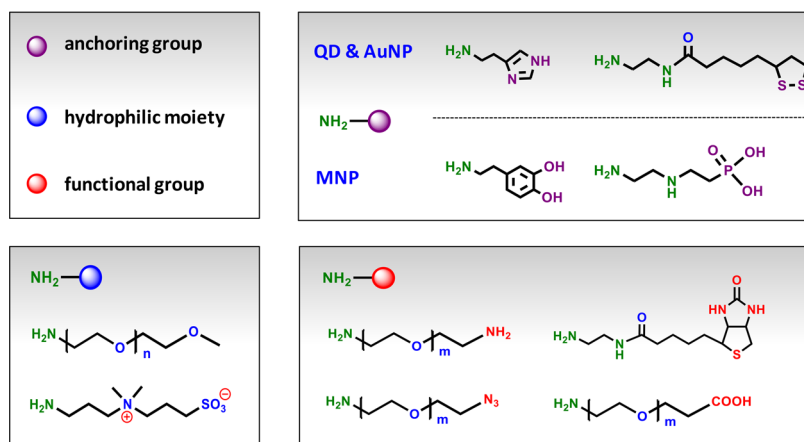


Figure 3. (A) General synthetic scheme of the nucleophilic addition reaction starting with PIMA precursor, used to prepare various multifunctional polymer ligands. (B) Structures of the building blocks used in the synthesis of those ligands: anchoring groups, hydrophilic moieties, and reactive functionalities.

4. SYNTHESIS OF MULTIFUNCTIONAL POLYMER LIGANDS

Synthesis of multicoordinating polymer ligands can be roughly classified into three routes: carbodiimide-mediated condensation, radical polymerization, and nucleophilic addition reaction with poly(maleic anhydride) copolymers (as starting precursors). We briefly describe those methods, with a focus on the nucleophilic addition reaction mainly developed by our group.

4.1. Carbodiimide-Mediated Condensation

The polymer ligands can be prepared via condensation reaction between amine-presenting functionalities and carboxyl-rich polymer, or vice versa. This method is widely employed because of the ubiquitous nature of these two functional groups in chemistry. Efficient condensation reaction of amines and carboxylic acids generally requires the use of additional reagents (e.g., 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide, EDC; 1,1'-carbonyldiimidazole, CDI; *N,N*-dicylohexylcarbodiimide, DCC) to increase the yield and reduce side reactions. Several commercially available polymers, such as poly(acrylic acid) and polyethylenimine, can be utilized as starting precursors. Using this route, a variety of ligands appended with the desired anchoring groups and hydrophilic moieties have been developed for coating different nanoparticles. A few representative works are listed in Table S1.

4.2. Radical Polymerization

Radical polymerization, such as reversible addition–fragmentation chain-transfer polymerization (RAFT) and atom transfer radical polymerization (ATRP), provides a powerful tool for the synthesis of complex polymer structures. A key feature of this technique is its ability to polymerize an extensive range of

functional monomers. The majority of RAFT- and ATRP-mediated polymer ligands are synthesized from controlled polymerization of (meth)acrylate- or (meth)acrylamide-derivatives using RAFT chain transfer agents (e.g., thiocarbonylthio compounds) and ATRP catalysts (e.g., transition metal complexes), respectively. The distribution of the functional groups within the polymer chain can be either random or adopt a block architecture, depending on the synthetic strategy used. Representative ligands prepared using radical polymerization are listed in Table S2.

4.3. Nucleophilic Addition Reaction Starting with Poly(maleic anhydride) Copolymers

Exploiting the effectiveness of the nucleophilic addition reaction with copolymers presenting anhydride rings has provided chemists with a great platform to prepare an array of multifunctional materials. Reaction with molecules presenting a primary amine allows one to introduce a variety of lateral functionalities along the polymer backbone. This chemical route has been utilized by several groups to prepare amphiphilic polymers used for encapsulation of nanoparticles (see Table S3). Our group has developed a set of new multicoordinating polymers starting with poly(isobutylene-*alt*-maleic anhydride) (PIMA, MW ~ 6000 Da), and applied them for the functionalization of several sets of nanoparticles. This platform offers a few unique advantages: (1) The nucleophilic addition reaction between anhydride rings and primary amines is highly efficient (with near 100% yield), without requiring any coupling reagents. (2) The presence of multiple anhydride rings (~39) along the polymer backbone allows one to install large but controllable numbers of anchoring groups, hydrophilic moieties, and specific reactive groups within the same polymer chain. (3)

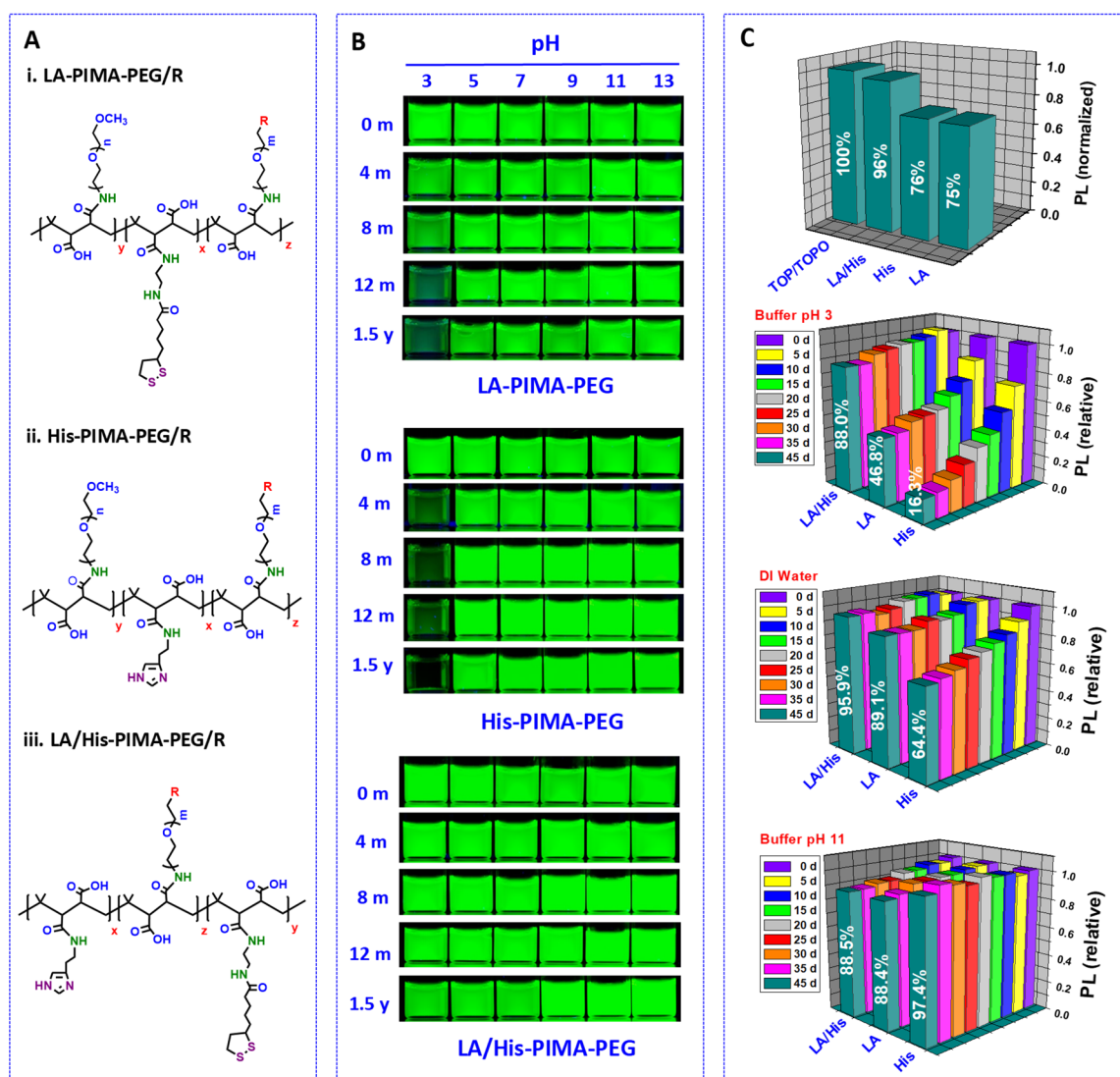


Figure 4. (A) Structures of a few representative PEGylated ligands used for QD functionalization: LA-PIMA-PEG/R, His-PIMA-PEG/R, and LA/His-PIMA-PEG/R. (B) Colloidal stability tests applied to QDs ligated with the above polymer ligands dispersed in buffer at different pHs. (C) Effects of varying the polymer ligand coordination on the QD stability and fluorescence properties were tested in comparison with hydrophobic QDs (as a reference). Also shown is the time progression of the PL measured from polymer-coated QDs dispersed in different pH buffers. The intensity is normalized with respect to the value measured for freshly prepared samples. Reproduced with permission from ref 28. Copyright 2015 American Chemical Society.

Varying the nature of the anchoring groups, one can design ligands optimized for coating nanoparticles with distinct core compositions, including semiconductor, metallic, and magnetic nanomaterials. Figure 3 schematically summarizes the general synthetic scheme employed by our group.

The nucleophilic addition reaction with PIMA yields lateral functionalities attached to the polymer chain via amide bonds. The composition of a polymer ligand can be tuned by simply controlling the molar fraction of each functionality with respect to the succinic anhydride groups in the PIMA chain. Typically, in order to provide strong ligand coordination, good water dispersibility while guaranteeing reactivity, a stoichiometric mixture of anchoring groups (~50%), hydrophilic motifs (~40%) and reactive groups (~10%) is reacted with PIMA, which can introduce ~20 anchoring groups, ~16 hydrophilic motifs, and ~4 reactive groups per ligand.²⁸ Employing this platform, we have designed a series of ligands with variable architectures and coordinating anchors that were optimally

adapted for different nanoparticle surfaces, with the aim of providing nanomaterials that are compact and exhibit excellent colloidal stability and tunable surface reactivity.^{15,19,28–33}

5. POLYMER COATING OF NANOCRYSTALS

5.1. Coating of Luminescent QDs

Hydrophilic ligands presenting thiol anchors have been widely used to functionalize semiconductor QDs. However, such ligands tend to negatively affect the QD fluorescence by reducing the radiative rate of exciton recombination.³⁴ In addition, most thiolate ligands tend to suffer from oxidation, which can induce ligand desorption from the QD surfaces.³⁵ To address some of these limitations, ligands presenting imidazole groups have been developed;¹⁸ using imidazole coordination has also been found to enhance QD emission.³⁶ Nonetheless, the coordination affinity of imidazole-modified ligands to QD surface is relatively weak, resulting in hydrophilic nanoparticles that are only colloidal stable at pH > 5. Building on those

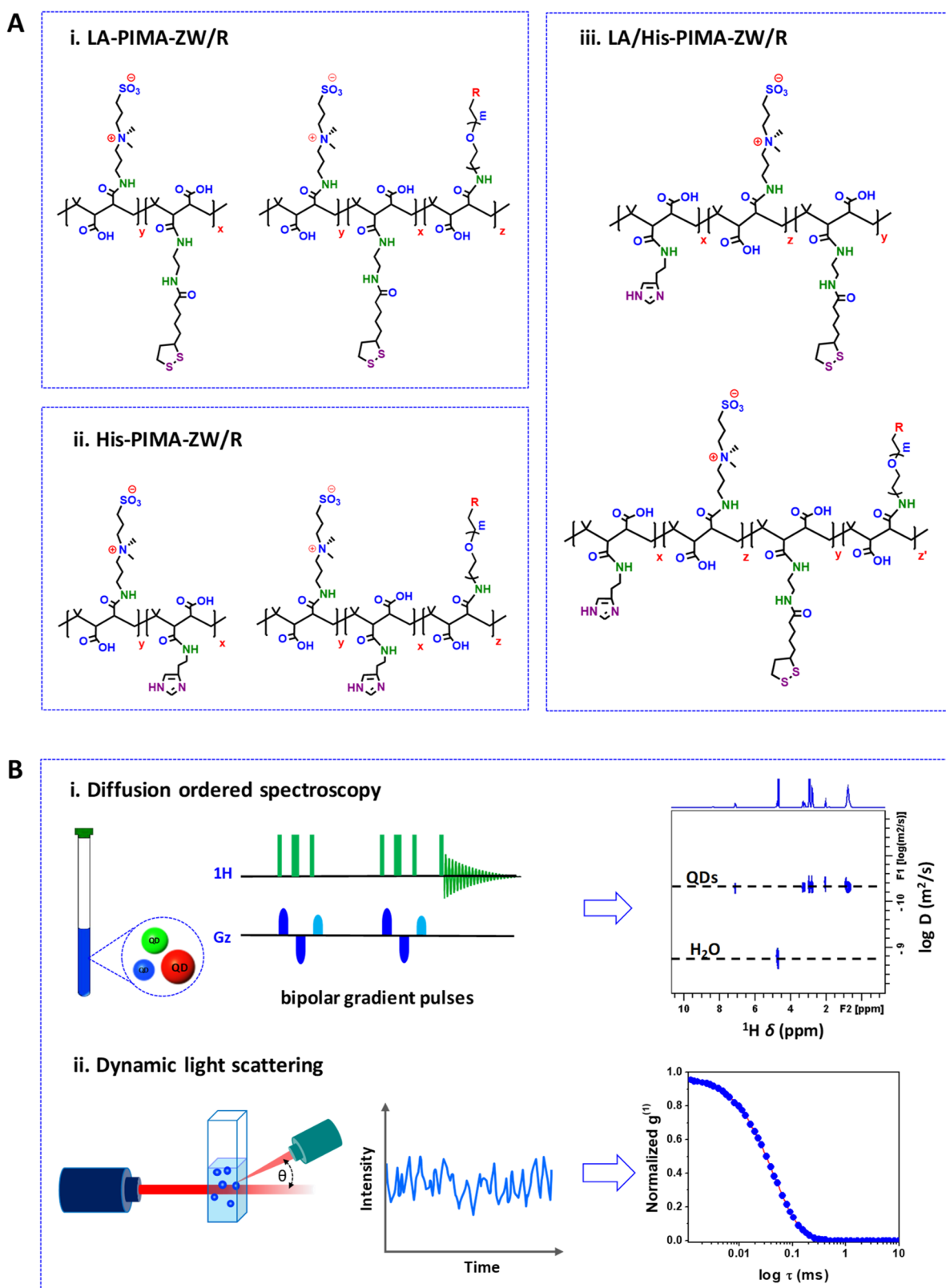


Figure 5. (A) Structures of few representative ligands based on the zwitterion motif, used for QD functionalization: LA-PIMA-ZW/R, His-PIMA-ZW/R, and LA/His-PIMA-ZW/R. (B) Schematics of the DOSY-NMR and DLS experiments applied to measure the hydrodynamic size of polymer-capped QDs. Reproduced with permission from refs 28–30 and 38. Copyright 2015, 2016, 2015, and 2020, respectively, American Chemical Society.

findings, we proposed that combining thiol and imidazole groups within the same ligand structure would enhance the overall ligand-to-QD affinity, reducing the issues of thiol oxidation and weak coordination affinity of imidazole. To

prove this hypothesis, we designed three sets of PEGylated PIMA ligands that present lipoic acid (LA), histamine (His), or a mixture of both (Figure 4).²⁸ The colloidal stability of QDs coated with these polymers was compared side-by-side over a

broad range of conditions and for extended periods of time. The data shown in Figure 4B and C confirm that indeed LA/His-PIMA-PEG provides QDs with higher fluorescence and better long-term colloidal stability across the tested conditions, compared to those coated with ligands appended with thiol or imidazole only anchors. Overall, mixed coordination polymers exploit the benefits of each group, which synergistically address the limitations encountered by ligands presenting only one type of anchors.

Developing nanoprobe with compact size is highly desired, since large hydrodynamic sizes limit their efficiency in sensor design and negatively affect the nanoparticle's *in vivo* behavior.^{3,4,13} Additionally, compact QDs permit probing of biological processes in rather tight spaces, such as the synaptic regions in neuron cells.³⁷ To achieve this goal, we extended the above synthetic rationales to prepare a set of compact polymer ligands based on the zwitterion motif (Figure 5A).^{29,30,38} The one-step reaction of PIMA with a stoichiometric mixture of amine-modified nucleophiles can be conducted without requiring coupling reagents, which makes it highly suitable for preparing zwitterion polymers given the stringent solubility of these charged groups. It also streamlines the purification steps of the final compounds. We found that substituting PEG blocks with zwitterion groups did not compromise the optical properties and colloidal stability of QDs but greatly reduced their dimensions. The hydrodynamic radius extracted from diffusion ordered NMR spectroscopy (DOSY-NMR) and dynamic light scattering (DLS) measurements confirmed that QDs coated with zwitterionic polymers are markedly smaller than their PEGylated counterparts, i.e., 5–6 nm vs 10–11 nm (Figure 5B and Table 2).^{28–30,38,39} Such compact coating has allowed metal–histidine assembly of polyhistidine-tagged proteins directly on the QDs (see below).

Table 2. Diffusion Coefficients and Hydrodynamic Radii of Nanoparticles Coated with PEGylated or Zwitterion-Modified Polymers, Extracted from the DLS and DOSY-NMR Analyses^a

QD	capping ligands	diffusion coefficient (<i>D</i>) [m ² s ⁻¹]	hydrodynamic radius (<i>R_H</i>) [nm]
QD537	LA-PIMA-PEG	1.89 × 10 ⁻¹¹	11.3
QD537	His-PIMA-PEG	2.08 × 10 ⁻¹¹	10.3
QD537	LA/His-PIMA-PEG	1.96 × 10 ⁻¹¹	10.9
QD537	LA-PIMA-ZW	3.22 × 10 ⁻¹¹	6.6
QD537	His-PIMA-ZW	4.16 × 10 ⁻¹¹	5.2
QD537	LA/His-PIMA-ZW	3.36 × 10 ⁻¹¹	6.4

^aReproduced with permission from refs 28–30 and 38. Copyright 2015, 2016, 2015, and 2020, respectively, American Chemical Society.

To modulate interactions with cellular systems, engineering nanoparticle surfaces that allow conjugation with biomolecules is critically important. Designing nanoparticles presenting controllable numbers of functional groups can be easily achieved relying on this synthetic route. Replacing a fraction of inert hydrophilic moieties (i.e., PEG-methoxy or zwitterion) with NH₂-PEG-R (e.g., R = carboxy, amine, azide, or biotin) during the addition reaction step, we have synthesized a set of ligands with distinct but controllable functionalities (Figures 4A and 5A).^{28–30,38} Such coatings can generate hydrophilic nanoparticles that display distinct reactive groups, making them ideally adapted for implementing bioorthogonal conjugation strategies.

5.2. Coating of Gold Nanocrystals

As thiolates and thiyl radicals show strong affinity to gold surfaces, we applied lipoic-acid-modified polymers, LA-PIMA-PEG and LA-PIMA-ZW, to an array of gold nanostructures, including nanoparticles, nanorods (AuNRs) and nanoshells (Figure 6).^{15,32} We found that both PEGylated and zwitterion coatings promote excellent long-term colloidal stability in various conditions, including a broad pH range (Figure 6C). Furthermore, these polymer coatings are highly effective in preventing corona formation on the nanoparticle surfaces even at high protein concentration (~50 mg/mL), as confirmed by gel electrophoresis and DLS measurements.^{15,32} However, we found that the ability of PEGylated and zwitterion coatings to “protect” the metallic cores against digestion by cyanide ions is very different. Our experiments showed that PEGylated ligands provide substantially better protection of nanoparticles against cyanide etching compared to those stabilized with a zwitterion coating.¹⁵ The etching decay constant extracted for LA-PIMA-PEG-capped AuNPs was ~5 times longer than that measured for their LA-PIMA-ZW-capped counterparts (Figure 6D). This result can be attributed to the more densely packed PEG shell around the nanoparticle surfaces, which shields the cores from ions in the surrounding solution. In contrast, the small and highly hydrated zwitterion groups are less effective in preventing access of the cyanide ions to the gold surfaces, resulting in faster digestion kinetics.

Individually, imidazole groups tend to exhibit weaker affinity to gold surfaces than thiolates. However, we have demonstrated that when multiple imidazole anchors are introduced in a polymer, the resulting ligand can easily ligate onto oleylamine- or citrate-stabilized nanospheres as well as cetyltrimethylammonium bromide (CTAB) stabilized nanorods, promoting colloidal stability in buffers at pH ≥ 5 and in the presence of high concentration of electrolytes (Figure 7A).¹⁹ These findings provide an equivocal proof that metal–polyhistidine conjugation directly on gold nanoparticles can be effectively implemented.^{40,41}

5.3. Coating of Other Core Materials

The present ligand design can be readily expanded to other types of inorganic cores by simply substituting the anchoring groups. As such, polymers modified with dopamine anchors have been prepared and applied for coating iron oxide nanoparticles with high efficiency (Figure 7B).^{33,42} The strong affinity of catechol to iron-rich surfaces is attributed to the improved orbital overlap of the five-membered enediol-iron coordination.⁴³ Introducing specific reactive groups into the polymer structure allows conjugation of the nanoparticles with biomolecules, yielding magnetic probes suitable for sensing and imaging applications.^{33,44} These ligands have also been adopted by other groups to functionalize upconverting nanoparticles (UCNPs) and metal–organic frameworks (MOFs).^{44,45} More recently, we prepared a family of PIMA polymer ligands containing phosphonate anchoring groups to promote the phase transfer of several hydrophobic nanoparticles to water, including QDs, AuNPs, and MNPs (Figure 7B).³¹ We found that the colloidal stability of MNPs functionalized with these ligands is comparable to that offered by dopamine-modified polymers. Nonetheless, our data showed that phosphonate coordination affinity to QD and AuNP surfaces is weaker than that of ligands presenting lipoic acid anchors, for example.³¹

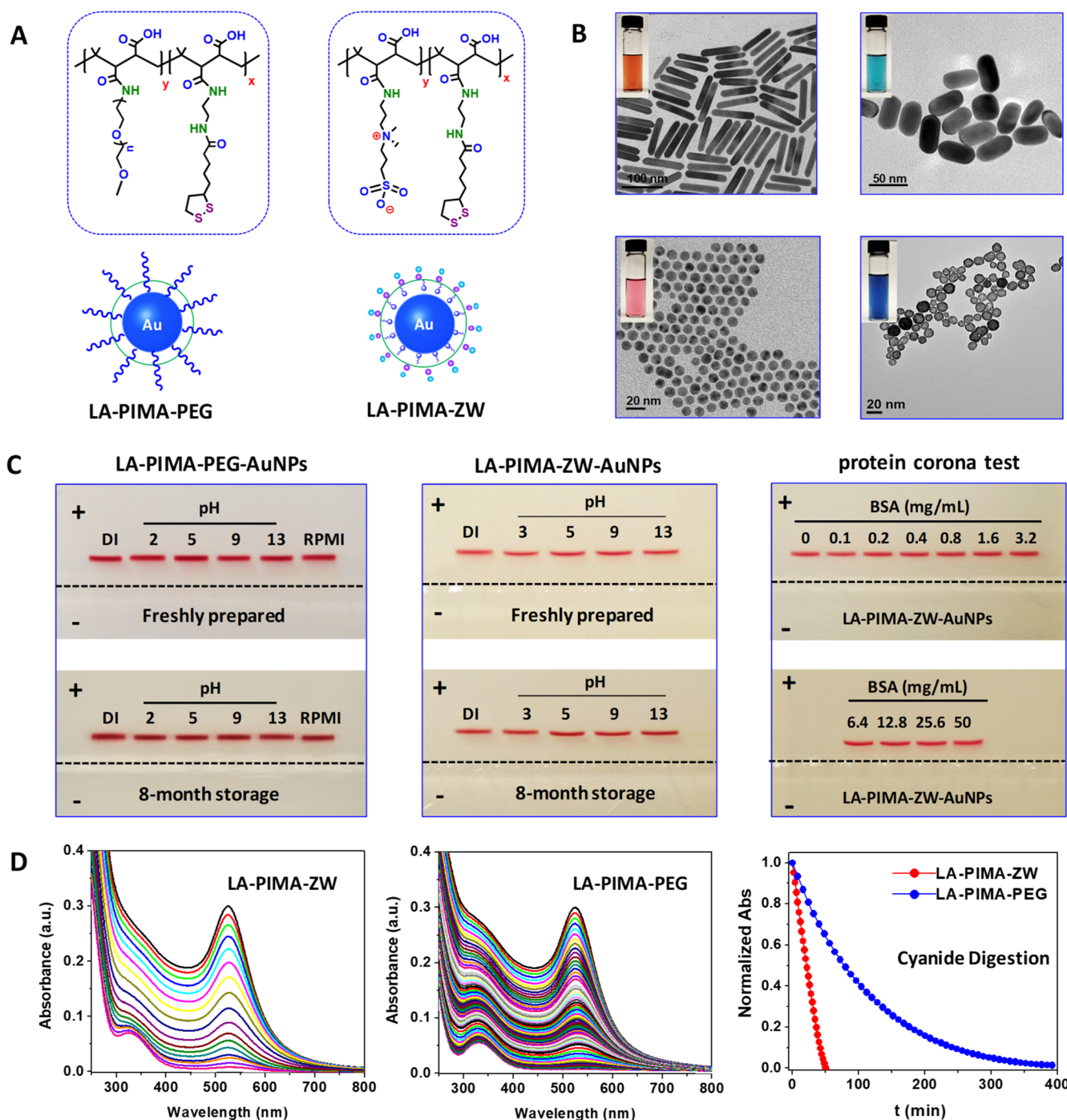


Figure 6. (A) Surface functionalization of AuNPs with LA-PIMA-PEG and LA-PIMA-ZW. (B) Transmission electron microscopy images of representative gold nanostructures, along with white light images of aqueous dispersion of these nanocolloids. (C) Representative agarose gel electrophoresis images of AuNPs ligated with LA-PIMA-PEG and LA-PIMA-ZW dispersed in buffers at different pHs, along with protein corona test for LA-PIMA-ZW-coated AuNPs after incubation with different concentrations of BSA (0–50 mg/mL) for 1 h. (D) Side-by-side comparison of the sodium cyanide digestion tests applied to PEGylated and zwitterionic polymer-coated AuNPs; the blue profile in the digestion plot was also shown in Figure 1C. Reproduced with permission from refs 15 and 32. Copyright 2017 American Chemical Society and Copyright 2016 The Royal Society of Chemistry, respectively.

5.4. Photoligation of QDs and Au Nanocrystals with LA-Based Polymers

Ligation of luminescent QDs with LA-based ligands generally requires chemical reduction of 1,2-dithiolane to dihydroliipoic acid, as the ligand coordination is driven by availability of thiolate groups. However, chemical reduction of LA to dihydroliipoic acid often carried under harsh conditions tends

to alter the integrity of certain functional groups that are highly desired in biological applications (e.g., azide and aldehyde). To circumvent this problem, we developed a phase transfer strategy starting with LA-based ligands (no chemical reduction conditions), where ligand exchange is promoted by UV irradiation (Figure 8).⁴⁶ The effectiveness of this route stems from the photochemical sensitivity of the strained dithiolane

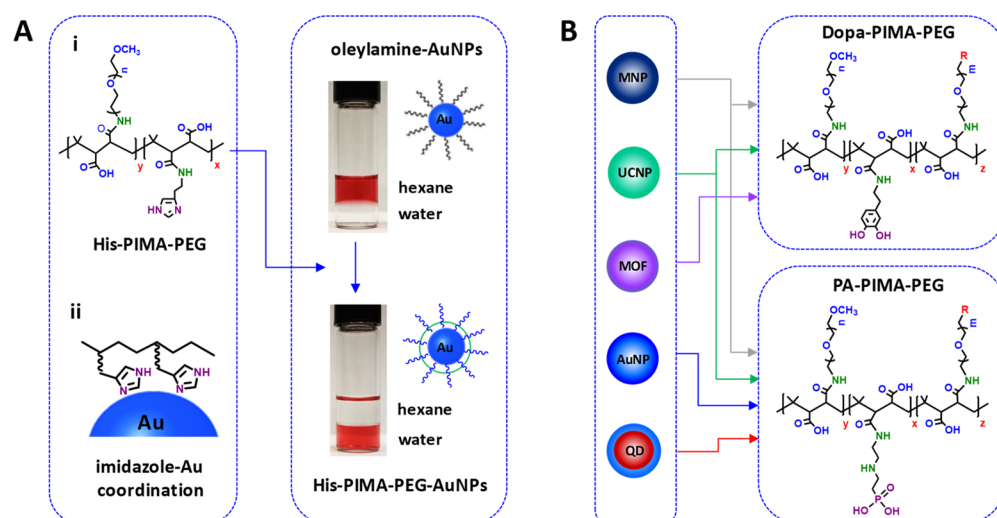


Figure 7. (A) Ligand exchange and phase transfer of oleylamine-coated AuNPs with His-PIMA-PEG promoted by imidazole–Au coordination. (B) Versatility of dopamine and phosphonate coordination onto various core materials. Dopa-PIMA-PEG promotes functionalization of MNPs, UCNPs, and MOFs, while and PA-PIMA-PEG can be applied for the coating of MNPs, UCNPs, AuNPs, and QDs. Adapted with permission from refs 19, 31, and 33. Copyright 2014 Royal Society of Chemistry and Copyright 2018 and 2014, respectively, American Chemical Society.

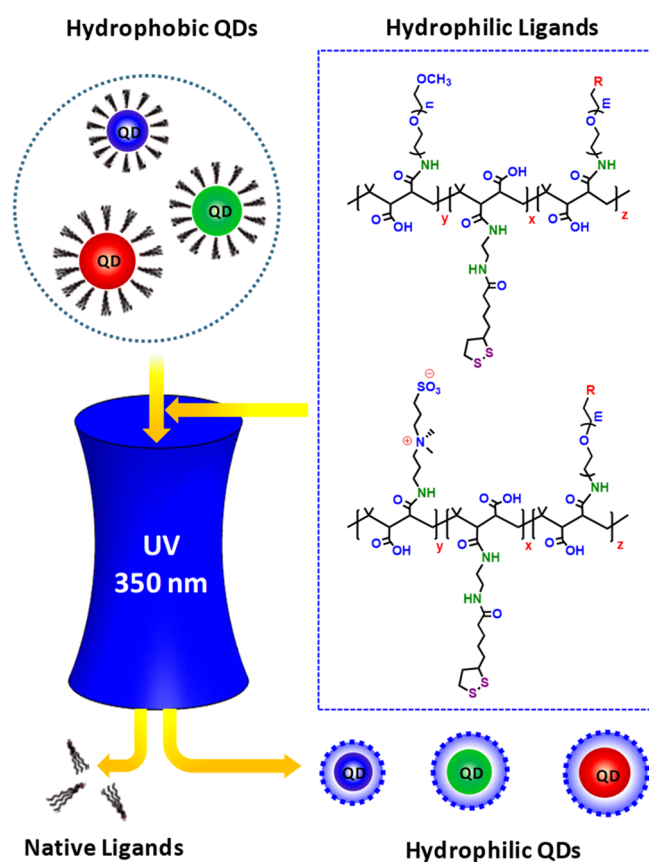


Figure 8. *In situ* ligand exchange and phase transfer of hydrophobic QDs with LA-based ligands promoted by UV irradiation at 350 nm.

hydrophobic QDs, which can be converted to thiol-containing species under continuous UV irradiation, promoting spontaneous coordination onto the QD surfaces.⁴⁷

Though our efforts developing this method have initially focused on molecular-scale ligands, such as LA-PEG and LA-zwitterion,⁴⁶ we found that this approach is equally applicable to LA-based polymer ligands, regardless of the ligand stoichiometry

and hydrophilic motifs used.^{28,29} Photoligation combined with polymer ligands provides hydrophilic QDs with excellent optical properties and great colloidal stability. More importantly, we found that the integrity of sensitive functional groups is fully preserved.^{29,38} This strategy has also been extended to other nanoparticles, such as AuNPs.⁴⁸ The photoligation strategy undoubtedly offers a range of exciting possibilities for nanoparticle manipulation, which will advance their integration within biology.

6. NANOPARTICLE BIOCONJUGATION

In addition to the importance of achieving colloidal stability, equally important is one's ability to conjugate the nanoparticles to biomolecules of interest, in order to facilitate their use in biology. The polymer-based coating approach developed by our group and others offers great benefits and a high degree of flexibility, yielding hydrophilic nanoparticles with surface reactive functionalities that are compatible with a broad range of conjugation techniques, such as carbodiimide reaction, thiol-maleimide coupling, hydrazone ligation, and biotin–streptavidin interaction. We particularly highlight two conjugation approaches: metal–polyhistidine coordination and click chemistry, which can provide a unique control over the assembly of nanoparticle–biomolecule systems.

6.1. Metal–Polyhistidine Self-Assembly

After the early demonstration by our group that C- or N-terminal polyhistidine sequences on proteins can promote direct protein binding onto Zn-rich QDs, this conjugation approach has been expanded by different groups to an array of biomolecules.^{36,49} Polyhistidine-mediated conjugation of nanomaterials is highly desirable due to a few key factors: (1) functional simplicity and high binding affinity ($K_d \sim 10^{-9}$ M);⁵⁰ (2) the ubiquitous nature of polyhistidine-tagged biomolecules in biology; (3) control over the conjugate valence and biomolecule orientation on the nanoparticles.³⁶

While this conjugation route is easy to implement, one key requirement must be satisfied: rather compact coatings are needed in order to allow the polyhistidine direct access to the nanoparticle surfaces. Such coatings have been often realized

using molecular ligands (Figure 9A), but the colloidal stability of the nanoparticles is often limited. Recently, we found that hydrophilic polymer coating based on the zwitterion motif is compatible with this conjugation route. In particular, we

demonstrated that QDs coated with His-PIMA-ZW exhibited excellent colloidal stability while allowing easy conjugation with polyhistidine-tagged full-size proteins, such as maltose-binding proteins and fluorescent mCherry proteins (Figure 9B and C).³⁰ Nonetheless, such conjugation involves ligand rearrangement since polyhistidine competes for accessible sites to bind to the QD surfaces. Requirement for ligand coordinating strength must be also considered.⁴⁰ Our study revealed that polyhistidine-tagged protein can self-assemble onto QDs capped with His-PIMA-ZW but conjugation with LA-PIMA-ZW-capped QDs was inefficient.²⁹ This is due to the fact that thiolate coordination onto the QD surfaces is stronger than imidazole, which prevents the polymer rearrangement induced by polyhistidine tag competition. These results provide insight into the interplay between several parameters controlling this conjugation method.

6.2. Click Chemistry

Click reaction between two abiotic groups with exclusive mutual reactivity has made great impact in the chemistry and biology fields. The advantages offered by this bioorthogonal conjugation include rapid reaction rate, high efficiency, and unprecedented selectivity even within complex conditions.⁵¹ Prototypical examples are represented by copper-catalyzed azide–alkyne cycloaddition (CuAAC), strain-promoted azide–alkyne cycloaddition (SPAAC), and *trans*-cyclooctene–tetrazine inverse-electron-demand Diels–Alder (IEDDA) reaction (see Figure 10A).

Implementing click chemistry on nanoparticle surfaces potentially provides researchers with an excellent tool to investigate various biological processes. It promises to combine the nanoparticle unique photophysical properties with selective conjugation in complex conditions. Exploiting the multifunctional nature of polymer-based ligands, clickable groups can be introduced into the ligand structure through precise synthesis and brought onto the nanoparticle surface in a controlled manner. We and others have designed a series of polymer ligands incorporating azide, dibenzocyclooctyne, or norbornene groups, which were utilized for nanoparticle bioconjugation, allowing for *in situ* labeling or *in vitro* sensing and imaging.^{29,44,52} For example, by introducing azide functionality into the PIMA ligand, we demonstrated that QDs can be conjugated to cyclooctyne-labeled anti-tropomyosin receptor kinase B antibody (α -TrkB) via copper-free click reaction, providing a fluorescence probe to visualize the distribution of TrkB in pyramidal neurons within cortical tissue (Figure 10B).³⁸ Optimizing these conjugation techniques can offer a better means for controlled interfacing of nanoparticle with bio-systems, in general.

7. BIOLOGICAL APPLICATIONS

Engineering nanoparticles as diagnostic and therapeutic tools has the potential to revolutionize current medicine and offers many exciting possibilities, where significant advances could be made in molecular imaging, sensing, diagnostics, and the treatment of diseases. Here, we summarize our recent works using polymer-coated nanoparticles for sensing and intracellular delivery.

7.1. Nanoparticle-Based Sensing

The intense photophysical responses to external stimuli (e.g., light excitation or application of a magnetic field) and the ability to modulate such responses in the presence of target analytes make nanoparticles excellent signal transducers. Among these,

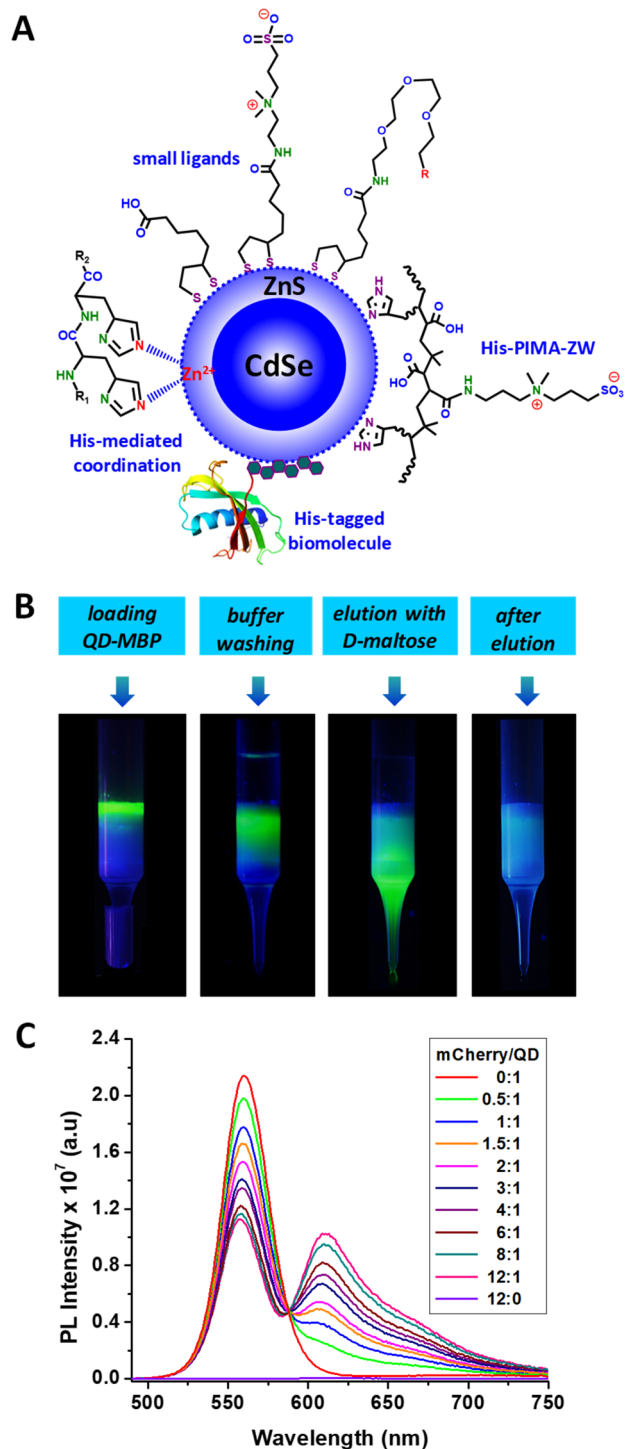


Figure 9. (A) Schematic of the metal-polyhistidine conjugation: small ligand vs polymer ligand. (B) Amylose affinity chromatography assay directed conjugation of His-PIMA-ZW-QDs with MBP-His₇. (C) Fluorescent resonance energy transfer (FRET) measurement: evolution of the PL spectra of mCherry-His₆-QD conjugates as a function of the protein-to-QD ratio, varied from 0:1 to 12:1. Reproduced with permission from ref 30. Copyright 2015 American Chemical Society.

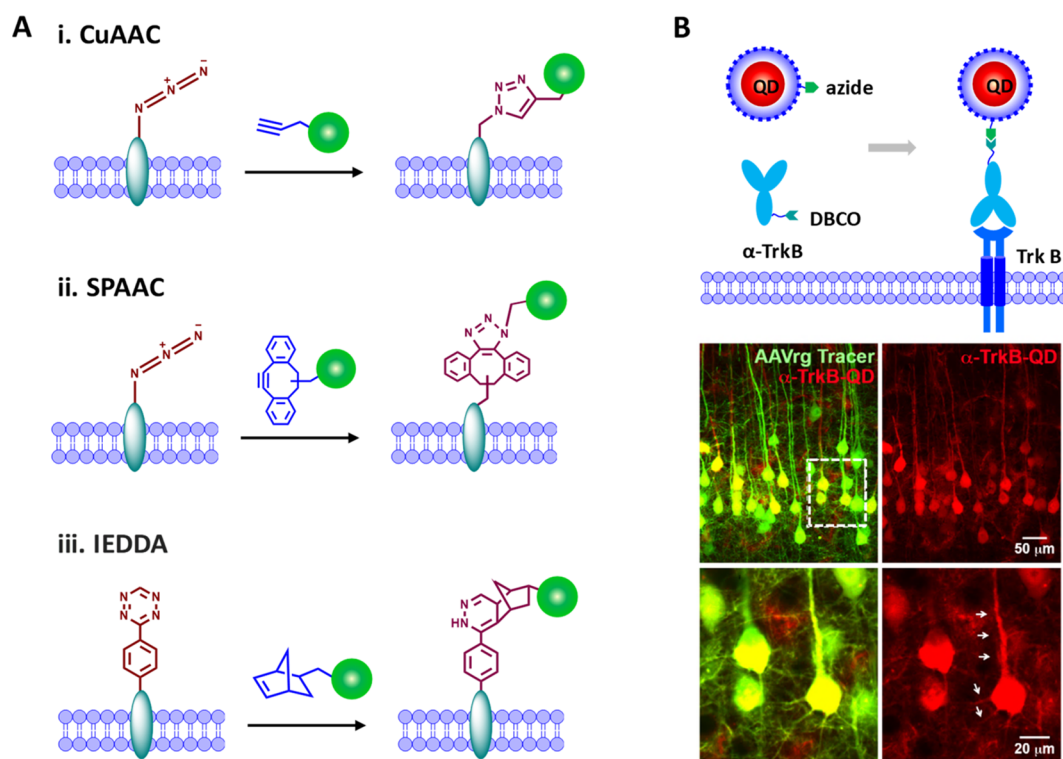


Figure 10. (A) Three representative click chemistries applied to nanoparticle bioconjugation: (i) copper-catalyzed azide–alkyne cycloaddition, (ii) strain-promoted azide–alkyne cycloaddition, and (iii) inverse-electron-demand Diels–Alder. (B) Conjugation of QDs with anti-TrkB antibody via click reaction (top), along with the immunofluorescence staining of the pyramidal neurons within cortical tissue to visualize the distribution of the TrkB through the sample. Reproduced with permission from ref 38. Copyright 2020 American Chemical Society.

QDs, AuNPs, and MNPs are most commonly used in nanoparticle biosensor designs. Signal transductions include (i) QD-based resonance energy or charge transfer interactions;⁴ (ii) AuNP-based localized surface plasmon resonance and surface-enhanced Raman scattering;⁵ and (iii) MNP-based magnetic resonance.⁶ Quantitative sensing relying on these formats has been used to probe various *in vitro* and *in vivo* biological processes, including pH and ion concentration changes, enzymatic activity, oligonucleotide hybridization, and ligand–receptor interactions.

Our group has designed an array of fluorescence-based QD sensors to detect enzyme activity, pH changes, and ligand–receptor interactions.⁴ Recently, we explored the capacity of a redox-active platform constructed by covalently attaching several dopamines to the polymer coated QDs, where the PL could be modulated by charge transfer interactions between the central QD and proximal dopamines.^{29,30} We showed that this conjugate can be used to sense pH changes, Fe ion concentration, and the presence of thiolate molecules. Figure 11 shows a schematic representation of the platform along with modulation of the QD PL realized using any of the four pathways: (1) pH-induced PL changes, attributed to a shift in the chemical equilibrium between reduced catechol (electron donor) and oxidized quinone (electron acceptor) combined with a change in the oxidation potential of catechol;⁵³ (2) cysteine-induced PL recovery promoted by formation of 5-S-cysteinyl-dopamine, leading to a reduction in the rate of charge transfer as the quinone concentration is decreased; (3) Fe ion-induced PL loss, promoted by Fe-catalyzed oxidation of catechol to quinone, which enhances the electron transfer rates; and (4) PL recovery caused by competitive interactions between cysteine and Fe-catalyzed quinone, promoting an irreversible

transformation of 5-S-cysteinyl-dopamine. These sensing concepts may provide possibilities to study the oxidative metabolism of dopamine.

7.2. Intracellular Delivery

Delivering nanoparticles to intracellular regions of interests is an active research area yet with great challenges, since nanoparticles are commonly uptaken by endocytosis, where they end up enveloped within endosomal compartments.⁵⁴ To reach the cytosol, nanoparticles must escape those endosomes. Displaying cell-penetrating peptides on the nanoparticle surfaces is an attractive strategy that can promote nanoparticle transport across the cell membrane without disrupting the lipid bilayer.⁵⁵

We recently reported on the ability of a synthetic peptide, SVS-1, to facilitate the rapid delivery of nanoparticles into live cells (Figure 12).^{56,57} SVS-1 is a lysine-rich peptide that can interact with the negatively charged surfaces of cancer cells, inducing their lytic destruction.⁵⁸ It has been shown that, at concentrations below its IC_{50} , this peptide does not affect cell viability but promotes entry into cytoplasm via membrane translocation.⁵⁸ Motivated by these findings, we explored the potential of SVS-1 to facilitate the intracellular delivery of nanoparticles. We found that SVS-1 mediates a rapid and pronounced uptake of nanoparticles into live cells, regardless of nanoparticle size, composition, shape and surface coating.^{56,57} Epifluorescence and confocal scanning microscopy revealed that the internalized nanoparticles are distributed across the cells and do not colocalize with endosome markers (Figure 12B). Additional inhibition assays strongly suggested that the cell entry mechanism is not attributed to common endocytic pathways (Figure 12C). These findings indicate that SVS-1

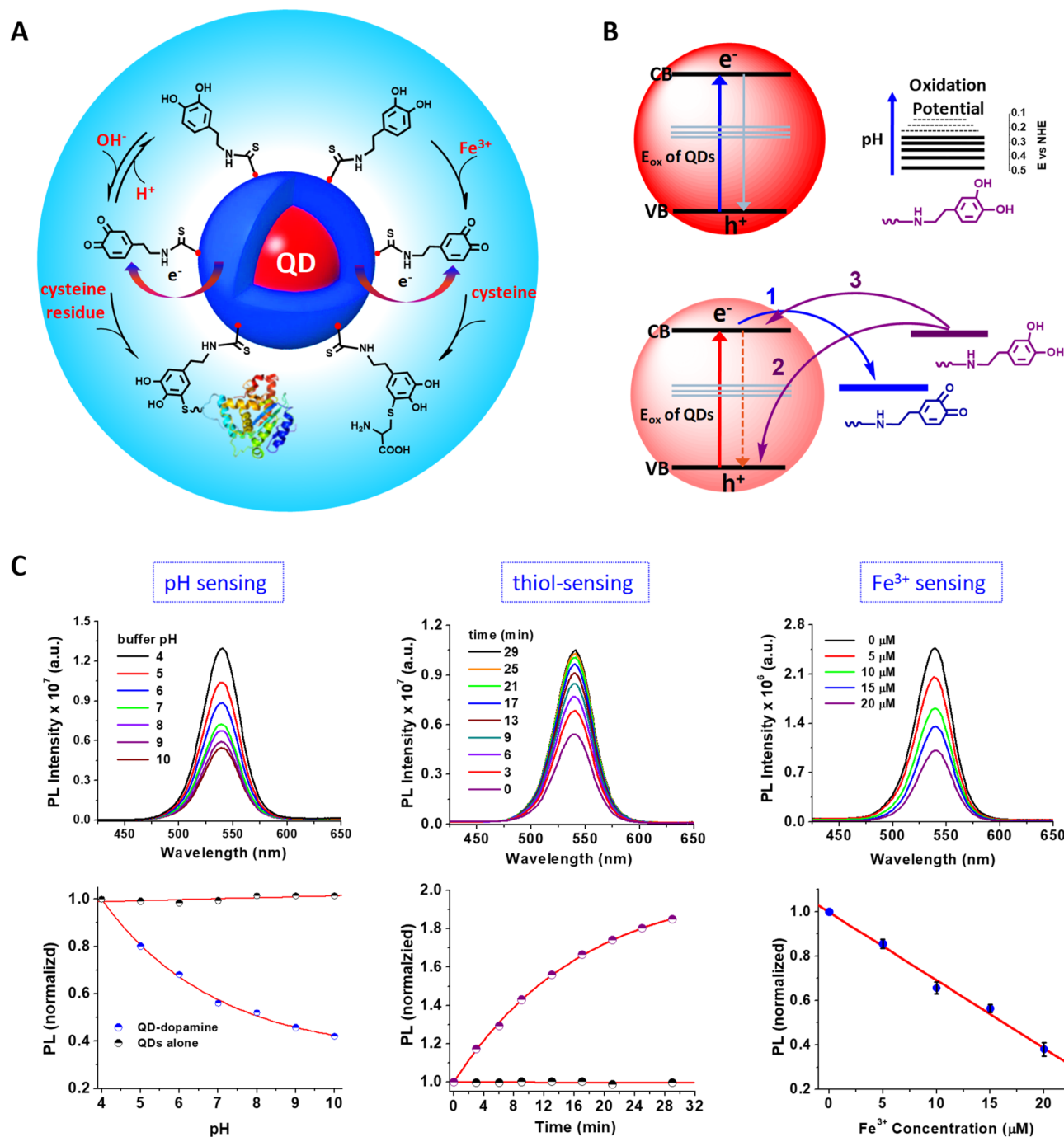


Figure 11. (A) Schematic of the QD–dopamine sensing platform, relying on charge transfer interactions between the fluorescent QD and redox-active dopamine. (B) Proposed mechanisms for the electron and hole transfer in a QD–dopamine conjugate system: (1) electron transfer from a photoexcited QD to quinone; (2) electron transfer from dopamine to the valence band of a photoexcited QD; (3) electron transfer from dopamine to the LUMO of nonexcited QD. (C) QD–dopamine conjugates employed for sensing pH changes, and the presence of cysteine molecules and iron ions. Reproduced with permission from refs 29, 30, and 53. Copyright 2016, 2015, and 2012, respectively, American Chemical Society.

offers a promising strategy for the intracellular delivery of nanoparticles.

Though the toxicity of nanomaterials surface-stabilized with our multicoordinating polymers has not been discussed in this report, *in vitro* cell viability data applied to several types of cells along with *in vivo* imaging of *Drosophila melanogaster* embryogenesis, acquired by our group using luminescent QDs, MNPs, or AuNPs functionalized with this family of polymer ligands, have shown no measurable toxicity.^{33,38,56,57}

8. OUTLOOK

In this Account, we presented a set of versatile high affinity ligands as a platform for tailoring the bio–nano interface, facilitating nanoparticle bioconjugation and their application in biology. This platform offers synthetic advantages, and it can be applied to various nanoparticles, endowing them with compact size, antifouling surfaces, excellent colloidal stability, and tunable conjugation. We envisage that further optimization of these coating platforms will broaden their scope and eventually make

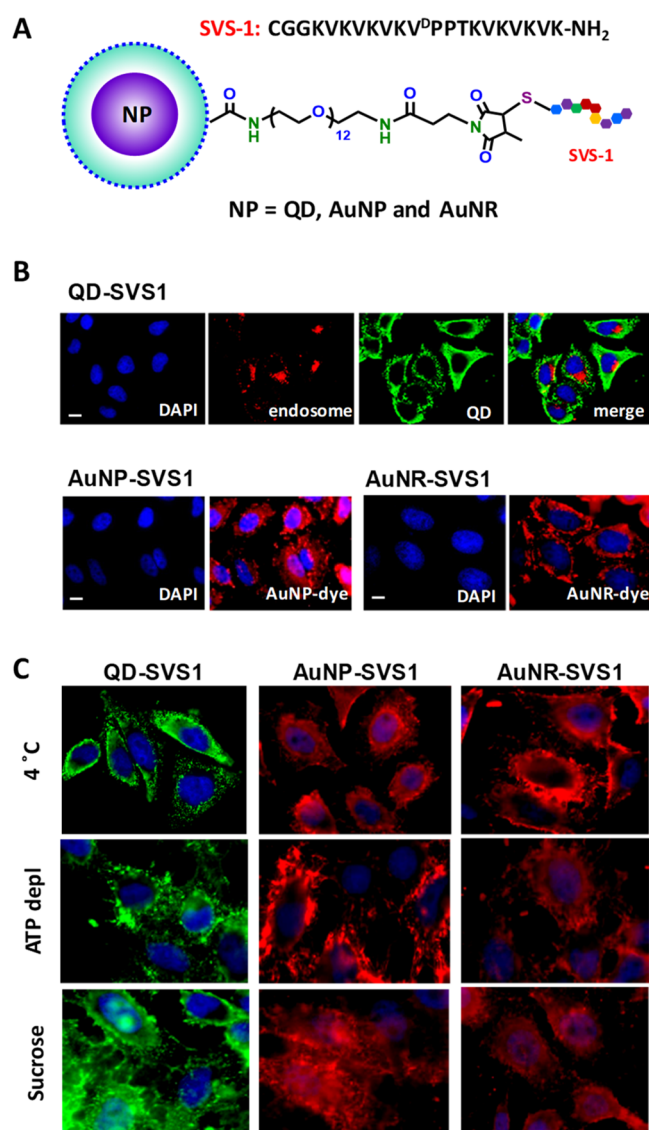


Figure 12. (A) Schematic of a nanoparticle–SVS-1 conjugate along with the peptide sequence. (B) Representative fluorescence images showing conjugate internalization by HeLa cells. Cells were incubated with 50 nM QD–SVS-1, 2 nM AuNP–SVS-1/dye, and 0.2 nM AuNR–SVS-1/dye, respectively, for 1 h at 37 °C. (C) Endocytosis inhibition experiments: HeLa cells incubated with nanoparticle–SVS-1 conjugates at 4 °C and following pretreatment with either NaN_3 or a hypertonic sucrose solution. Reproduced with permission from refs 56 and 57. Copyright 2018 American Chemical Society.

them reliable tools for facilitating the use of nanoparticles in biomedicine.

In vitro diagnostics (IVD) is likely to be one of the first areas where use of multifunctional nanoparticles could have a substantial impact. Nanoparticle-based point-of-care diagnostics, such as glucose monitoring, HIV detection, and pregnancy testing, have already been commercialized. Nonetheless, there are still challenges for a broader use of nanoparticles in applications involving human subjects. First, the stability and safety of nanoparticles are still important concerns. New candidates with safer core materials and reliable coating strategies will continue to evolve. Second, the community is still seeking a thorough understanding of the “structure–function” relationship governing use of nanoparticles in biology. The complexities of the material characteristics are constantly

varying. Thus, developing standards for reproducible synthesis of high-quality nanoparticles combined with reliable surface functionalization strategies and nanomaterial characterizations are needed. Third, efficiency of nanoparticle delivery for cancer-targeting applications is still poor.⁵⁹ The clear path for improving the efficiency relies on active targeting, which still requires optimized surface engineering methods and conjugation approaches, in order to maximize the nanoparticle performance, bioactivity, and targeting *in vivo*. Overcoming these hurdles demands careful consideration of nanoparticle cores, surfaces, and interactions with various biosystems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.accounts.9b00641>.

Representative polymer ligands prepared via carbodiimide-mediated condensation, representative polymer ligands synthesized by radical polymerization, and representative amphiphilic polymer ligands for the encapsulation of nanoparticles (PDF)

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Notes

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