Development of copper nanoclusters for *in vitro* and *in vivo* theranostic applications

Wing-Fu Lai, Wing-Tak Wong, and Andrey L. Rogach*

Dr. W. F. Lai
School of Life and Health Sciences,
The Chinese University of Hong Kong, Shenzhen, P. R. China
E-mail: rori0610@graduate.hku.hk

Dr. W. F. Lai, Prof. W. T. Wong
Department of Applied Biology and Chemical Technology,
Hong Kong Polytechnic University, Hong Kong SAR

Prof. A. L. Rogach
Department of Materials Science and Engineering, and Centre for Functional Photonics (CFP),
City University of Hong Kong, Hong Kong SAR; Shenzhen Research Institute, City University of Hong Kong, Shenzhen, P. R. China

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Abstract

Theranostics refers to the incorporation of the therapeutic and diagnostic capacity into one material system. An important class of nanomaterials exploited for theranostics nowadays is metal nanoclusters (NCs). Contrary to gold and silver NCs, copper is an essential trace element for humans, which can be more easily removed from a body. Along with the low cost of copper that offers potential for large-scale nanotechnology applications, copper NCs have attracted great interest in recent years. This article reviews the latest advances in the design, synthesis, surface engineering, and applications of copper NCs in disease diagnosis, monitoring and treatment. Strategies to control and enhance the emission of copper NCs are considered. With the synopsis of the up-to-date development of copper NCs as theranostic agents, it is hoped that insights and directions for translating current advances from the laboratory to the clinic can be further advanced and accelerated.

1. Introduction

The term "*theranostics*" combines therapeutics and diagnostics, and largely represents a transition from conventional medicine to personalized medicine. Since the turn of the last century, the development of theranostic agents has been facilitated by advances in research on nanomaterials and nano-formulations, ranging from semiconductor quantum dots and carbon nanotubes to metal and upconversion nanoparticles.[1] For instance, by combining Gd^{3+}-doped upconversion nanoparticles with poly(ethylene glycol) (PEG) and poly(ethylenimine) (PEI),[2] a luminescent system for multimodal imaging and serum-enhanced gene delivery is developed. Quantum dots coated with mesoporous silica followed by diverse surface modifications (including amine functionalization of the silica surface, PEGylation, and ligand conjugation) display dual emissive properties and enhance magnetic resonance imaging (MRI) contrast,[3] with the silica pores enabling the loading of doxorubicin for chemotherapy.[4] More recently, by incorporating Gd_2O_3 nanocrystals and gold nanoclusters (NCs) together into a protein-stabilized hybrid system, drug delivery and imaging-guided cancer therapy are made possible.[5] These selected examples demonstrate the use of nanomaterials in theranostics. Among different classes of nanomaterials, light-emitting metal NCs show favorable properties partly because of their lack of heavy metals (and hence lower toxicity) as compared to semiconductor quantum dots in many of which heavy metals are used, and of their excellent photostability and low

toxicity as compared to organic dyes. In addition, due to their comparatively simple chemical composition, NCs are easier to be engineered for optimization than those of multi-component systems (e.g., upconversion nanoparticles, and liposomes). As far as metal NCs are concerned, gold and silver NCs have been widely studied and used.[6] In recent years, however, copper (Cu) NCs begin to attract increasing interest, not only because of their high yield in mild synthetic conditions, but also due to the abundance and low cost of Cu, which offers potential for large-scale nanotechnology applications (**Figure 1**).[7] Moreover, contrary to gold, silver and platinum, Cu is an essential trace element in a human body. The excess of Cu can be effectively removed. This gives Cu NCs an additional advantage when being applied to theranostics.

The practical potential of Cu NCs has attracted extensive research attention in the past few decades.[8] Despite this, efforts devoted to comprehensively exploiting and reviewing the prospects of Cu NCs in theranostics are scant till now. Filling this gap is in need to properly evaluate the latest progress in the development of theranostic agents based on Cu NCs, and to identify future directions for translation of current research into practicable interventions. This article starts with a synopsis of current methods of synthesizing Cu NCs for theranostic purposes. Based on the latest understanding of the physicochemical and optical properties of Cu NCs, the design and working principles, as well as specific examples on theranostic applications, of Cu NCs are discussed. It is hoped that this article cannot only provide a synopsis of the up-to-date development of Cu NCs for theranostic applications in the current scientific literature, but also offers important insights for further research pursuance to translate research on Cu NCs from the laboratory to the clinic.

2. Synthesis of Cu NCs as theranostic agents

Methods of synthesizing and engineering Cu NCs as theranostic agents are often designed based on the mechanisms governing the thermodynamics of NCs. Nucleation is one of the important steps to be taken into account when NC synthesis is considered. At the nanoscale, biological properties (including the cellular internalization efficiency and blood circulation time) of metal NCs are easily affected by the size and shape of the clusters. One of the goals of research, therefore, is to generate clusters with a narrower size distribution profile. A fundamental understanding of NC nucleation and growth enables more precise control over the size and shape of the clusters. The cluster free energy is an important parameter governing the nucleation process. It can be defined in multiple ways, in terms of either the cluster radius[9] or the number of atoms.[10] Regardless of the way the cluster free energy is expressed, it possesses two competing terms. One is the negative term that links to the bond formation process. The other one is the positive term that depicts the surface energy. Because of the competition between the two terms, the free energy has a maximum at a specific radius known as the critical radius.

In general, if a cluster has a radius smaller than the critical radius, growth will be unfavorable and the dissolution of the cluster will occur. On the contrary, if a cluster has a radius greater than the critical radius, cluster growth will be facilitated. Measures to properly control and manipulate the experimental conditions are, therefore, essential to the synthesis of Cu NCs with optimal physical properties for subsequent theranostic use. This is, however, easier said than done. Compared to the bulk metal whose thermodynamics is determined predominately by atoms of the volume, effects of the fluctuations and contributions from the surface on the overall properties of the system become significant in clusters. Alternations by only one atom in a NC can remarkably change the physical, optical, and electronic properties of the system.[11] Furthermore, in a bulk metal, different possible symmetries (e.g., the body centered cubic structure, the face centered cubic structure, and the hexagonal close packed structure) are separated by a very large energy barrier in the state space.[11] This barrier can be overcome only at the time of melting. Different symmetries inherent to NCs, however, are often separated from

each other by an energy barrier that is lower than the melting energy.[11] Solid-to-solid transitions can, therefore, occur even before melting. This is one of the challenges to be tackled when NCs are generated for theranostics, which is stringent on the uniformity and properties of the clusters to be adopted. Apart from the thermodynamic factors, compared to clusters of noble metals, Cu has high oxidation tendency because of its lower reduction potential. Strategies for the synthesis of Cu NCs have to take all these factors into account in order to generate stable products. In this section, our discussions will focus only on the strategies that have been reported to synthesize NCs as agents for treatment and diagnosis.

2.1 "Bottom-up" synthetic strategies

"Bottom-up" strategies for the synthesis of Cu NCs can be roughly divided into two categories. The first one is the electrochemical approach. In this approach, a sacrificial anode, which serves as a source of metal ions by undergoing anodic dissolution, is involved. Metal ions formed are reduced at the cathode to generate metal NCs. This method has been adopted in a previous study,[12] in which Cu NCs were generated in a thermostatted three-electrode electrochemical cell, with an aqueous solution of tetrabutylammonium nitrate serving as the electrolyte. The anode, the cathode, and the reference electrode were a Cu sheet, a platinum sheet, and Ag/AgCl, respectively.[12] Due to the large band gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), the product is resistant to oxidation, and shows the same emission spectra after storage at ambient conditions for more than one year.[12, 13] It has an average size of around 0.6 nm, with the size easily controlled by manipulating the current density, purification methods, and heat treatment conditions.[12, 13] Despite the possibility of manipulating the NC size, less flexibility is available to the chemical properties of the NCs when the electrochemical method is used. Reports on the use of this strategy in theranostics have been scant till now.

The other category of "bottom-up" strategies is wet chemical reduction, which offers much higher flexibility to the tuning of the chemical and physical properties of Cu NCs. During synthesis, proper selection of a reducing agent is important. Some commonly used reducing agents include sodium borohydride,[14] hydrazine hydrate,[15] tetrakis(hydroxymethyl)phosphonium chloride,[16] and ascorbic acid.[17] In general, a suitable reducing agent is the one with strong reducing capacity. This explains the extensive use of sodium borohydride in the synthesis of metallic clusters. Mild reducing agents, however, are more preferable when a strong stabilizer is adopted. This facilitates sequestering by the stabilizer for the generation of luminescent clusters.[18] While the reductant and the stabilizer in a synthetic protocol can be of two different entities, sometimes one agent is used to serve both purposes. One good example is folic acid (FA), which can serve as a reducing agent, a stabilizer, and also a targeting ligand for receptor-mediated endocytosis.

FA is known to have negligible toxicity, low immunogenicity, and high affinity to the folate receptors (FRs). Currently, there are three isoforms known in the family of FRs: FRα, FRβ, and FRγ.[19] As FRα is often overexpressed in cancer tissues (including malignant nasopharyngeal, breast, renal, colon, testicular, and ovarian carcinomas) but not in normal tissues,[20] it gains the most attention among different isoforms. Because of the overexpression of FRs, FA conjugation is a well-documented approach for directing nanoconstructs to FRs for cellular internalization via a mechanism similar to that followed by free folate.[21] FA-stabilized Cu NCs can be prepared by mixing an aqueous solution of $CuCl_2$ with an alkaline solution of FA, followed by incubation at 50°C for NC formation.[22] Due to the presence of amine groups in the pteridine ring, FA can bind to Cu via the N-Cu bond.[22] On the other hand, the carboxyl groups of FA give the NCs a negative surface charge.[22] The generated NCs have an average diameter of around 0.9 nm, and show negligible cytotoxicity in HeLa cells and A549 cells.[22] Together with

their capacity of emitting blue light upon UV irradiation and their target specificity to FR-overexpressed cancer cells,[22] these Cu NCs have good potential for use in cancer cell detection and targeted cancer therapy.

Apart from FA, small-molecule compounds that can be adopted as stabilizers for the synthesis of Cu NCs include cysteine,[23] glutathione,[24] proline,[25] cysteamine,[26] adenosine,[27] tannic acid,[28] phosphinothiolates,[29] and trypsin.[30] The high versatility of small-molecule compounds renders the chemical properties of NCs tunable and flexible; however, due to their small molecular mass, sometimes these compounds fail to form a perfect protective layer on the surface of Cu cores, causing the generated NCs prone to oxidation. To address this issue, polymeric materials (e.g., dendrimers, and polyelectrolytes) are adopted to replace the small-molecule stabilizers. The polymers here serve as a template within which Cu NCs are formed. By manipulating the cavity dimensions of the template, the core size, as well as the size distribution profile of the clusters, can be controlled. NCs generated using this method can be exemplified by the case of oligonucleotide-hosted Cu NCs, which can be prepared from $Cu(NO_3)_2$ by using a DNA duplex as a template and ascorbic acid as a reducing agent.[31] During synthesis, interactions between Cu^{2+} ions and the DNA duplex vary with the metal ion concentration. At a low metal ion concentration, the ions tend to interact with the phosphate backbone through nonspecific electrostatic interactions. In this case, no NC can be formed. When the concentration increases, Cu^{2+} ions effectively bind to DNA bases, within which they are further reduced by ascorbic acid to form luminescent NCs. The diameter of the NCs generated are less than 2 nm.[31] Lately, by using the artificial peptide CLEDNN as a template, Cu NCs with excitation- and temperature-dependent fluorescence are also prepared.[32] After incubation of the NCs with HeLa cells for 24 hours, fluorescence in the plasma membrane and cytosol was observed. The success in cellular uptake, along with the emissive properties of those peptide-templated NCs, enable the use of the clusters in drug delivery and imaging-based diagnosis.

In drug delivery research, transferrin is an extensively used ligand for cancer targeting.[33, 34, 35] It has been widely exploited for the generation of Cu NCs as theranostic agents, too. Transferrin-templated Cu NCs have been generated from Cu(II) sulfate, which, when mixed with transferrin in an aqueous medium, undergoes a reduction reaction mediated by hydrazine hydrate under alkaline conditions. During cluster fabrication, not only can transferrin serve as a targeting ligand, but it can also function as a stabilizer via strong metal-ligand interactions between Cu^{2+} ions and some residues (particularly cysteine, and tyrosine) of transferrin.[15] In addition, under alkaline conditions, tyrosine residues can provide a reducing environment for cluster formation.[15] The transferrin-templated Cu NCs have an average size of around 2 nm, and emit blue light at 460 nm (λ_{ex} = 375 nm).[15] By using quinine sulfate as a standard, the photoluminescence quantum yield (PLQY) of the NCs is estimated to be around 7.5%.[15] As revealed by the matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) based mass spectrometric measurements and X-ray photoelectron spectroscopic analysis, each of the NCs consist of 3, 5, or 7 Cu atoms, and comprise only Cu(0) and Cu(I) species.[15] Although a slight reduction in the availability of the α-helix conformation (from 28.6 to 26.2%) and the β-sheet content (from 21.3 to 20.3%) occurs during NC formation,[15] the targeting capacity of transferrin is retained. To tune the emission profile of transferrin-templated Cu NCs for imaging, the reducing agent can be changed from hydrazine hydrate to ascorbic acid, which not only enables the synthetic process to proceed at ambient conditions at a higher rate[36] but also leads to the generation of Cu NCs that give red fluorescence with an emission peak at 670 nm.[36] The PLQY of these NCs has been estimated to be around 6%, which is larger than that of the previously reported bovine serum albumin (BSA)-templated red fluorescent Cu NCs (4.1%)[37] and near-infrared thiolate-protected Cu NCs (0.6%).[38] Upon incubation with HeLa cells (in

which the transferrin receptor is overexpressed) for only 6 h, which is much shorter than the incubation time previously adopted when BSA-templated Cu NCs were used as imaging probes in HeLa cells,[37] intense red fluorescence is detected. The enhancement of the cellular internalization efficiency is attributed to the occurrence of receptor-mediated endocytosis.

In addition to biomacromolecules, synthetic polymers can be used as templates. This can be exemplified by the case of poly(vinylpyrrolidone) (PVP)-templated Cu NCs.[39] During synthesis, PVP is dissolved in a saturated sodium chloride solution, followed by the addition of Cu(II) chloride. The excessive amount of Cl^- ions facilitates the formation of Cu(I) chloride, which is subsequently reduced to Cu(0) in the presence of ascorbic acid. This solution is then mixed with the dihydrolipoic acid (DHLA) solution, which is generated by reducing α-lipoic acid to DHLA using sodium borohydride, to generate Cu NCs that are stabilized by PVP via weak coordination bonds.[39, 40] The NCs, however, are stable under aqueous conditions for only around two days. If the clusters are to be applied to biomedical use, their stability may need to be enhanced for long-term storage in practice. Another polymer that shows enormous potential for the generation of Cu NCs as theranostic agents is PEI, which is an aziridine polymer that exists as a polycation with a high proton buffering capacity over a broad range of pH.[41] Over the years, PEI and its derivatives have been widely studied for delivering nucleic acid materials (ranging from plasmids[42] and oligonucleotides[43] to ribozymes[44]) *in vivo*. It has also been adopted to generate metal nanoconstructs as theranostic carriers for both drug delivery and imaging,[1, 45] and in particular for the preparation of Cu NCs.[46] PEI-templated Cu NCs can be generated simply by dissolving Cu(II) sulfate in an aqueous solution of PEI, followed by the addition of hydrazine hydrate and incubation at 95 °C for 12 h. The generated NCs has a diameter of around 1.8 nm, and can emit blue luminescence.[46] Although at the moment studies on the performance of PEI-templated Cu NCs in theranostics are lacking, taking the track record of the applications of PEI in drug delivery research into account,[35, 47] these Cu NCs have a high chance to grow in importance in theranostic research in the near future.

2.2 "Top-down" synthetic strategies

Besides constructing clusters from ions, Cu NCs can be generated in a "top-down" manner by using chemical etching that converts nanoparticles into NCs. Compared to the "bottom-up" approach in which the biological and physicochemical properties of the NCs can be easily tuned by changing the stabilizer adopted, the process of chemical etching exhibits far less versatility. However, because of the growing sophistication of techniques for the preparation of Cu nanoparticles, the use of chemical etching in NC generation can take full advantage of those well-documented advances. One commonly used etchant is ammonia.[48] It reacts with Cu^{2+} and Cu^+ to produce $Cu(NH_3)_4^{2+}$ and $Cu(NH_3)_2^+$ complexes, respectively. $Cu(NH_3)_4^{2+}$ complexes, which can also be generated from the oxidation of $Cu(NH_3)_2^+$, can at the end oxidize metallic Cu to etch Cu nanoparticles. By using this method, non-luminescent Cu nanoparticles with an average diameter of 3.7 ± 0.5 nm can be reduced to luminescent NCs with a mean diameter of 1.2 ± 0.3 nm.[48] Another widely used etchant is glutathione. During the etching process, the presence of excess glutathione can lead to the removal of Cu atoms, in the form of Cu(I)-glutathione complexes, from the surface of the Cu nanocrystal. The complexes generated can subsequently form Cu NCs via $Cu^+\cdots Cu^+$ cuprophilic interactions.[49] By using glutathione as an etchant, the mean diameter of the Cu nanocrystal has been successfully reduced from 4.2 ± 1.1 nm to 1.3 ± 0.4 nm.[49]

More recently, by using the interfacial etching approach, Cu NCs capped with hyperbranched PEI have also been prepared.[50] Synthesis of the clusters starts with the generation of oleyl amine (OA)-stabilized Cu nanoparticles, which are obtained by adding $Cu(NO_3)_2{\cdot}6H_2O$ into OA and 1-octadecene (ODE), followed by heating at 240 °C (**Figure 2**).[50] OA functions as both a reducing agent and a stabilizer, and enables Cu nanoparticles to be readily dispersed in

chloroform and toluene.[51] After mixing the chloroform solution of the nanoparticles with an aqueous solution containing PEI and formaldehyde, the etching reaction is initiated at 50 °C.[50] The generated Cu NCs show good aqueous dispersivity, pH-sensitive fluorescence, good biocompatibility, and low cytotoxicity.[50] During cell imaging, the fluorescent signals of the NCs are distributed not only in the cytoplasm but also in the nucleus.[50] Owing to their positive surface charge, the NCs were shown to condense nucleic acids during the gel retardation assay.[50] The capacity of condensing nucleic acids, however, is not sufficient for gene delivery, which involves other processes such as cellular internalization and endo-lysosomal escape.[52] Detailed evaluation of the *in vitro* and *in vivo* transfection efficiency of the NCs is required before the application potential of the NCs for gene therapy can be more conclusively determined.

3. Surface engineering of Cu NCs for theranostics

Surface engineering is a generic term depicting various methods of manipulating the surface properties of NCs. Manipulation of the NC surface may help increase the cluster stability, enhance the target specificity of the cluster, improve the *in vitro* and *in vivo* drug delivery performance, and boost the fluorescence intensity. Discussions in this section will focus only on the first three aspects, while advances in the use of surface engineering in luminescence enhancement will be covered in the later part of this article.

3.1 Enhancement of the cluster stability

One of the parameters determining the usability of the NCs in theranostic applications is the cluster stability, especially in an environment full of chemically and biochemically active species. This is particularly true when Cu NCs are concerned, owing to the susceptibility of Cu(0) to oxidation (E_0 = 0.34 V) in comparison with Au (E_0 = 1.50 V) and Ag (E_0 = 0.80 V).[39] To address the instability issue, one strategy is to modify the surface of an NC with long-chain alkanethiols [e.g., 1-octanethiol, 1-decanethiol, and 1-dodecanethiol (DT)]. Monolayer-protected Cu NCs can be easily formed by first mixing the respective alkanethiol with Cu(II) nitrate, followed by the addition of sodium borohydride.[53] During synthesis, the alkanethiol can chemisorb the NC surface via the −SH group to form a monolayer, with the packing density increasing with the alkyl chain length.[53] This monolayer serves as a barrier layer to protect the Cu core from oxidation. Density functional theory (DFT) calculations estimate that the length of the Cu-S bond is around 2 Å. Through interdigitation or intercalation of the alkanethiol chains chemisorbed on Cu NCs, superlattice structures can be formed.[53] A similar observation has been made previously on monolayer-protected Au NCs.[54] Apart from long-chain alkanethiols,[53] penicillamine[55] and 2-mercapto-5-*n*-propylpyrimidine[56] can function as protecting ligands for surface engineering of Cu NCs, and are worth further exploitation for theranostic applications.

NCs generated from Cu-containing compounds can benefit from the stabilizing effects offered by surface engineering as well. This is shown in the case of $[DBFDP]_2Cu_4I_4$ (**Figure 3**), whose chelate phosphine ligand 2,9-di(diphenylphosphine)-dibenzofuran (DBFDP) enhances the solution processability of the NC.[57] The ground state (S_0), as well as the first singlet (S_1) and triplet (T_1) excited states, of the NC have been studied using DFT and time-domain DFT (TDDFT) simulations. The ground and excited state configurations of the NC were found to be almost identical, indicating the high structural rigidity of the bis-phosphine chelated cluster.[57] Although the performance of $[DBFDP]_2Cu_4I_4$ in theranostics has not yet been evaluated, the evidence supporting the role of the ligand in NC stabilization is extendable to the development of Cu NCs as theranostic agents in future research.

3.2 Incorporation of the target specificity

Another important function of surface engineering is to enhance the target specificity of the NC. One commonly used reagent for surface modification is lipoamido-dPEG$_{12}$-2,3,5,6-tetrafluorophenyl (TFP) ester. It has been adopted to modify the surface of Cu NCs with a clinically used CXCR4-specific binding peptide FC131.[58] During synthesis, the peptide is mixed with lipoamido-dPEG$_{12}$-TFP ester in *N*,*N*-dimethylformamide, followed by the addition of *N*,*N*-diisopropylethylamine. The TFP ester group can react with the amine group of FC131; whereas the lipoamide group can form stable dative bonds with Cu during subsequent NC fabrication. Upon the addition of this product, as well as m-dPEG$_{12}$-lipoamide, to an aqueous solution of $CuCl_2$ and $^{64}CuCl_2$, NCs consisting of around 960 Cu atoms and 65 FC131 peptides can be formed under the action of sodium borohydride.[58] The intracellular fate of the FC131-incorporated NCs was studied upon conjugation with Texas Red.[58] Compared to the non-targeted counterparts, targeted NCs were found to be internalized into murine mammary carcinoma 4T1 cells more effectively, and to largely colonialize with the lysosome marker, lysosomal-associated membrane protein 1 (LAMP-1) (**Figure 4**A-D).[58] This confirms the occurrence of CXCR4-mediated internalization of Cu NCs through specific binding of FC131.

In a mouse model of triple negative breast cancer (TNBC), where CXCR4 upregulation occurs in the tumor tissue (Figure 4E-F), the level of tumor accumulation of the FC131-incorporated NCs was shown to be much higher than that of the unmodified ones (Figure 4G-H), with around 60% of the tumor uptake of the FC131-incorporated NCs attributed to CXCR4 binding.[58] The target specificity of the NCs was further corroborated upon competitive receptor blocking, in which an excess amount of non-radioactive targeted NCs was co-injected with the radiolabeled ones. The tumor uptake of the radiolabeled targeted NCs was found to be significantly blocked after competitive receptor blocking (Figure 4I).[58] Apart from using the cell line-derived xenograft model, a patient derived xenograft (PDX) model, in which human TNBC cells had been implanted into immunodeficient mice, was adopted to examine the imaging efficiency of the NCs. Human malignant tissues with high and low CXCR4 levels, as characterized by global gene expression array analysis, were used for model establishment. Non-targeted NCs led to a low level of tumor accumulation in PDX models, regardless of the CXCR4 level of the tumor tissue (Figure 4J).[58] In contrary, targeted NCs experience significant tumor uptake in a model that has a high CXCR4 level but not in the one with low CXCR4 expression (Figure 4K-L).[58] This further corroborates the target specificity of the NCs after surface modification with the targeting ligand.

3.3 Enhancement of the molecular function

Apart from enhancing the cluster stability and target specificity, surface modification can improve the molecular function, particularly the catalytic activity, of the NCs. This is shown by an earlier study, which used a cyclodextrin (CD) derivative, mono-6-thio-β-CD, as a template to synthesize Cu NCs and as a modulator to increase the catalytic activity of the clusters.[59] During synthesis, sodium hydroxide is first added to an aqueous solution containing mono-6-thio-β-CD and Cu(II) nitrate, followed by the addition of hydrazine hydride to generate β-CD protected NCs, whose maximum fluorescence excitation and emission wavelengths are 360 nm and 450 nm, respectively.[59] The size of the NCs is estimated to be around 2 nm.[59] Due to the presence of Cu(0) and Cu(I) in the NCs,[59] the O-O bond of hydrogen peroxide (which adsorbs on the NC surface) can be broken up into OH• radicals. β-CD protected NCs can thus catalyze the hydrogen peroxide-mediated oxidation of tetramethylbenzidine. The apparent Michaelis–Menten constant (K_m) of β-CD protected NCs with hydrogen peroxide is around 33 mM,[59] which is much higher than that of horseradish peroxidase (HRP, 3.70 mM). This suggests that those Cu NCs have higher affinity for hydrogen peroxide as compared to HRP.

Intriguingly, compared to BSA-templated Cu NCs, β-CD protected NCs display higher catalytic activity.[59] This is probably because substrate molecules have to get through the layer of stabilizer molecules before they can reach the cluster core, causing an impediment to the start of the catalytic reaction in BSA-templated NCs. In β-CD protected NCs, however, the less polar upper rim (primary side) of the CD molecule can bind to the apolar surface of the NC, with the more polar lower rim (secondary side) of the CD molecule exposed outwards.[59] The CD molecules can complex with substrate molecules. This brings the substrate molecules closer to the NC core, thereby increasing the probability of the occurrence of catalytic oxidation. With the combined use of glucose oxidase (GOx), which is an oxido-reductase that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone, β-CD protected NCs show the potential to detect glucose, with the detection limit being 0. 4 μM under the signal-to-noise ratio of 3.[59] If functions in treatment can be further incorporated into the NCs, applications are envisaged in theranostics.

4. Working principles of Cu NCs as theranostic agents

Owing to their molecule-like features (i.e., the availability of discrete electronic states, and size-dependent fluorescence) and other favorable properties (e.g., small size, and reasonable photostability), Cu NCs become an emerging class of fluorophores for theranostic use. Although reports on Cu NCs that possess both the therapeutic and diagnostic capacity concomitantly are limited, the practical potential of Cu NCs for disease diagnosis and treatment is evidenced in the literature.

4.1 Disease diagnosis

With the advent of technologies for NC synthesis and optimization, Cu NCs have been exploited in diagnosis and drug monitoring for different diseases, ranging from cancer to epilepsy (**Table 1**).[60-69] Diagnosis using Cu NCs is performed largely based on the detection of abnormal molecular events in the pathological site. In cancer cells, abnormal molecular events are usually manifested in terms of alternations in protein expression,[52] changes in the level of reactive oxygen/nitrogen species,[70] and alternations in intracellular pH.[1, 71] Cu NCs can possibly be developed as probes for these molecular events because some of them exhibit an aggregation-induced emission (AIE) effect in liquid media.[55] This enables intracellular complex interactions to be detected. Unfortunately, at the moment the AIE of Cu NCs is largely induced by solvent-induced aggregation[72] or cation-induced aggregation;[73] neither of these mechanisms can be translated into applications in a biological system. This results in a lack of AIE-based probes based on Cu NCs for bioimaging in the literature. In recent years, the possibility of rendering Cu NCs pH-responsive has been demonstrated by the NCs generated in the presence of DHLA and PVP.[39] Those NCs display pH-tunable optical behavior, which is attributed to changes in the dispersivity of the clusters at different pH values. When the pH is 4.5, the carboxyl group and one of the thiol groups of DHLA undergo protonation, leading to the precipitation of the NCs from the dispersion. When the pH changes from 4.5 to 11.0, deprotonation of the carboxyl and thiol groups occur, leading to an increase in the luminescence intensity. Furthermore, owing to the charge transfer from the ligand to the metal core through Cu-S bonds, the color of the dispersion changes from red to orange, with a shift in the emission maximum observed. All these demonstrate the pH responsiveness of the Cu NCs. With the pH-responsive behavior, induction of the AIE of Cu NCs by intracellular pH is now possible. This has been shown by cysteine/chitosan-co-stabilized Cu NCs,[61] which give the orange-red emission at pH 4.5 and undergo aggregation at pH 7.4 to provide the cyan-green emission. This pH responsive behavior is partly attributed to the presence of chitosan (pKa = 6.5), which loses its aqueous solubility (as well as reactive amine groups) when the pH of the NC dispersion is above 7.[61] *In vitro* studies showed that the rate constant for first-order aggregation of the NCs in MCF-7 cells is higher than that in HEK-293 cells,[61] possibly due to the higher level of

protein expression in cancer cells.[61] This reveals the potential for using the NCs to differentiate cell types based on the cell content.

In addition to detecting cancer cells, Cu NCs can be used to diagnose diseases at the molecular level. This has been demonstrated by the case of oligonucleotide-templated clusters,[31] which have succeeded in detecting G-to-A mutations in DNA. This type of mutation occurs in the genome of patients suffering from hereditary tyrosinemia type I. Upon the interaction with mismatched DNA, the NCs show enhanced luminescence, whose relative intensity is three-fold higher than that caused by the interaction with matched DNA.[31] This phenomenon is proposed to be partially governed by the electron donation capability of the ligand base pairs located in the major grooves of the DNA duplex used for NC formation.[31] As deoxyribonucleotide units of DNA display different charge-donating capability (dG > dA > dT > dC),[31] their quenching efficiency towards the fluorescence of the Cu NCs varies. This allows for the detection of the mismatch-mediated fluorescence enhancement.

Finally, Cu NCs have potential to be used for different imaging modalities *in vivo* upon appropriate modification. For instance, by using a one-pot sonochemical route in which glutathione is used as both a reducing agent and a stabilizer, luminescent and magnetic Cu NCs can be generated for bimodal MRI/fluorescence imaging of cancer cells.[74] Recently, ultrasmall chelator-free radioactive ^{64}Cu-incorporated Cu NCs can also be produced by using BSA, in the form of a conjugate with luteinizing hormone releasing hormone (LHRH), as a scaffold.[75] Upon intravenous administration to mice bearing orthotopic A549 lung tumors, the generated NCs were found to display high radiolabeling stability and to largely deposit in the left lung inoculated orthotopic A549 tumors and kidney (**Figure 5**).[75] The tumors were shown to be clearly delineated with a very little local background in whole-body positron emission tomography (PET) imaging.[75] Compared to near-infrared fluorescence imaging, the use of the Cu NCs as PET imaging agents enables more accurate, sensitive, and deep-penetration imaging of orthotopic lung cancer *in vivo*.[75] Because PET imaging has the merits of high temporal resolution, high sensitivity, and unlimited tissue penetration, upon further incorporation of the Cu NCs with various therapeutic agents (e.g., chemotherapeutic drugs and antisense oligonucleotides), it is expected that the NCs may offer potential for theranostic use in the treatment of lung cancer.

4.2 Disease treatment

Taking into account the attractive emissive properties of Cu NCs, by incorporating the NCs into existing drug carriers, a multifunctional carrier enabling both drug delivery and imaging can be developed. This has been demonstrated by an earlier study, which used Cu NCs to dope hydroxyapatite nanoparticles to generate a system that cannot only be loaded with kanamycin but can also serve as an imaging probe for bacterial cells.[76] Apart from simply incorporating Cu NCs into other carriers, there are two more ways of using Cu NCs in disease treatment. One is to use the NC directly as a carrier to deliver therapeutic agents into pathological sites. The other one is to make use of the intrinsic properties of Cu NCs to elicit therapeutic effects. Both methods can benefit from the emissive properties of Cu NCs, and can enable the combination of treatment and diagnosis in one system.

4.2.1 Functioning as drug carriers

To function as a carrier, a Cu NC-based theranostic agent should be able to be loaded with drug molecules. Drug loading can be performed by using different strategies, including physical entrapment,[77] surface adsorption,[78] and chemical conjugation.[78] By the therapeutic effects of the loaded drug and the emissive properties of NCs, a foundation is constituted for the establishment of a theranostic treatment. The possible use of Cu NCs in this manner has been

demonstrated by an earlier study, which incorporated DHLA-decorated clusters into a PVP/poly(vinyl alcohol) (PVA) hydrogel to form composite particles, with the hydrodynamic diameter being around 280 nm (**Figure 6**A).[39] These particles were further loaded with cisplatin,[39] with the size of the particles changed to be 350 nm upon drug loading. Although these nanocomposites were found to be successfully internalized into HeLa cells for living cell imaging (Figure 6B),[39] and to enable the sustained release of cisplatin to induce cancer cell death,[39] deposition of the NCs in tumor tissues relied on the enhanced permeation and retention (EPR) effect. Incorporation of targeting ligands may help enhance the target specificity of the nanocomposites in clinical use.

The potential of Cu NCs as drug carriers has been studied not only *in vitro* but also *in vivo*. The latter is partly revealed by the case of ^{64}Cu-incorporated Cu NCs, whose surface is functionalized with temozolomide (TMZ) and the FC131 peptide for the treatment of glioblastoma multiforme.[79] The integrity of TMZ molecules conjugated to ^{64}Cu-labelled Cu NCs is confirmed by high performance liquid chromatography (HPLC) analysis, with around 17 FC131 peptides and 28 TMZ molecules found on the surface of each NC.[79] Apart from TMZ and the FC131 peptide, a similar surface functionalization approach has been adopted to functionalize ^{64}Cu-incorporated Cu NCs with PEF monomethyl ether. *In vivo* pharmacokinetic analysis showed that ^{64}Cu-Cu NCs surface-modified with PEF monomethyl ether exhibit effective liver uptake and blood retention at 1 h post-injection, followed by rapid clearance at 24 h post-injection.[79] Taking their biocompatibility and multiple functionalities into consideration, these NCs have potential to be further developed for both glioblastoma multiforme imaging and therapy.

The emissive properties of Cu NCs can be exploited not only to image target cells but also to track and monitor the fate of delivered pharmaceuticals in real time. This can be achieved by making use of the interactions between specifically designed NCs and drug molecules (or biomacromolecules), as those interactions may lead to changes in the emissive properties of NCs via various optical mechanisms [including photoinduced electron transfer, and Förster resonance energy transfer (FRET)]. This has been illustrated by a recent study,[15] which complexed doxorubicin with transferrin-templated Cu NCs (**Figure 7**A). The complexation process is proposed to be mediated by two types of interactions. One is the hydrogen bonding interactions and π–π stacking interactions between drug molecules and transferrin. The other one is the electrostatic interactions between the negatively charged transferrin-templated Cu NCs and the positively charged drug molecules. After complexation with doxorubicin (λ_{ex} = 480 nm and λ_{em} = 590 nm) and upon UV irradiation, red luminescence can be obtained from the originally blue-emitting transferrin-templated NCs, owing to the occurrence of FRET from the NC as a donor to doxorubicin as an acceptor.[15] At the same time, gradual activation of the blue emission of the transferrin-templated NCs happens in the cytoplasm of the HeLa cell in which the transferrin receptor is overexpressed (Figure 7B).[15] This enables real-time monitoring of the gradual release of doxorubicin from the NCs upon cellular uptake. Moreover, compared to transferrin-templated NCs and doxorubicin alone, the NC/doxorubicin complexes induce a higher level of oxidative stress in the treated cancer cells.[15] In Swiss albino mice bearing Dalton's lymphoma ascites (DLA) tumorigenesis, the complexes were found to be more effective than doxorubicin in reducing the tumor volume.[15] This high efficiency is attributed to the higher tumor targeting capacity of the complexes, as well as the longer blood circulation time which enables a larger amount of the complexes to be deposited in tumor cells via the EPR effect.

4.2.2 Functioning as therapeutic agents per se

While existing efforts to adopt Cu NCs in treatment are largely confined to the use of NCs either as a modifier of a drug carrier or as a carrier *per se*, Cu NCs may actually elicit physiological, and potentially therapeutic, effects due to their intrinsic redox properties. This has been shown by the use of BSA-templated Cu NCs in catalyzing the hydrogen peroxide-mediated oxidation of 3,3',5,5'-tetramethylbenzidine.[80] This catalytic process is mediated first by the adsorption of hydrogen peroxide on the NC surface. After that, hydrogen peroxide is converted to OH• radicals under the action of Cu(0) and Cu(I).[80] Cu NCs are by themselves therapeutic in the sense that they can induce the formation of reactive oxygen species (ROS),[39] partly due to the redox activity of Cu and also to the presence of pro-oxidant functional groups on the NC surface. In C2C12 cells, Cu NCs lead to a reduction in the mitochondrial membrane potential, an increase in the Bax/Bcl-2 ratio, and an increase in the caspase-3/9 activity.[81] Because mitochondria are the primary site of ROS production, it is therefore possible that Cu NCs can induce oxidative stress in cells not only via its chemical properties *per se*, but also via disruption of the mitochondrial function. Taken this into consideration, Cu NCs may possibly display synergistic effects with chemotherapeutic drugs to enhance the level of cancer cell death.[39] This reveals the future prospects of applying Cu NCs to cancer therapy.

Apart from tackling cancer, Cu NCs display antibacterial effects. This has been illustrated by the case of tannic acid-capped Cu NCs (**Figure 8**).[28] After incubation with these NCs for 10 min, the growth of *Staphylococcus aureus* and *Bacillus subtilis* was found to be completely inhibited.[28] The cellular integrity of those treated gram-positive bacteria was drastically lost, leading to cell rupture and a substantial change in the morphology of the bacterial cells.[28] The same cell treatment, however, showed little effect on the growth of gram-negative bacteria (including *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa*).[28] This selective bactericidal activity is partially attributed to the comparatively positive surface charge, which enables more effective electrostatic interactions with the negatively charged NCs, of gram-positive bacteria. Regarding the fact that the NCs show high biocompatibility and strong excitation-dependent fluorescence, they may possibly be further developed into a selective antibacterial drug with a side-function in bioimaging.

Here it is worth noting that when the intrinsic redox properties of Cu NCs are employed to elicit the therapeutic effect, the high accessibility of the metal core is required. With this in mind, "stabilizer-free" Cu NCs, in which the contact between exogenous agents and the metal core is shortened due to the absence of bulky surface ligands, may be good candidates for future use in theranostics. Generation of such NCs has been reported by an earlier study,[82] in which the NCs were synthesized by the addition of an aqueous solution of Cu(II) nitrate trihydrate to *N*,*N*-dimethylformamide at 140 °C under vigorous stirring, followed by reflux for 9 h. NCs formed exhibit intense blue fluorescence with an emission maximum at 430 nm upon excitation at 350 nm.[82] In addition, the emission intensity of the NCs was shown to be quenched only by Fe^{3+} (**Figure 9**), but not by other single (Li^+, Na^+, K^+), double (Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}), and triple (Cr^{3+}, Al^{3+}) cations or anions (F^-, Cl^-, Br^-, I^-, NO_3^{2-}, OAc^-, SO_4^{2-}, $P_4O_7^{2-}$).[82] This allows the NCs to be used not only to elicit therapeutic effects by themselves, but also to function as a probe to detect alternations in the concentrations of hemin, cytochrome C, and ferritin, with the limit of detection being around 68 nM, 0.8 μM, and 17 nM, respectively.[82] Despite this, compared to conventional Cu NCs that contain stabilizers, "stabilizer-free" Cu NCs generally show lower stability. This is one of the challenges to be overcome before the clinical use of "stabilizer-free" Cu NCs in theranostics can be made possible.

5. Design of Cu NCs for theranostic use

The biological body is a complex system, in which multiple factors (e.g., blood retention, and tissue distribution) significantly affect the efficiency of a therapeutic agent. With this in mind, when Cu NCs are designed as theranostic agents, the synthetic method and the chemical composition of the NCs are two major areas to be considered because they directly influence the performance of the clusters generated. The former has been discussed in earlier parts of this article; whereas the latter can be exemplified by the case of Zn^{2+}-incorporated Cu NCs,[83] which can be produced by first mixing zinc sulfate with a solution containing egg white, followed by the addition of copper(II) sulfate, sodium hydroxide, and hydrazine. The presence of Zn^{2+} in egg white-protected Cu NCs was found to increase the fluorescence intensity of the NCs by 3.5-fold,[83] and to extend the average luminescence lifetime from 3.6 μs to almost 4 μs.[83] This enhancing effect is led by the binding of the Zn^{2+} ions with the hydroxyl groups on the NC surface, leading to the occurrence of the surface confinement effect and the spatial localization of excitons.[83] This strategy, although having been studied only to enhance the emission intensity of egg white-protected Cu NCs, may potentially be applicable to other types of NCs consisting of Cu.

Despite the possibility of improving the performance of NCs by designing the chemical composition, further optimization is required. Taking the above case as an example, if the amount of Zn^{2+} ions added is suboptimal, hydrated oxide precipitates will be formed during NC synthesis,[83] leading to interference with fluorescence due to the higher turbidity of the NC solution and hence causing an impediment to subsequent theranostic applications. While the chemical properties of Cu NCs matter during the design process, the physical properties of NCs should not be overlooked, too. In general, design of physical properties is manifested in either the size or the shape of the NC, as will be considered in the following discussions.

5.1 Size effects

Size is one of the most important parameters to be considered when Cu NCs are designed because it influences the surface-to-volume ratio, which at the end affects not only the drug loading efficiency and drug release sustainability but also the chemical activity of the clusters.[78, 84] The latter has been evidenced in a previous study,[84] which employed DFT to study the size evolution of the chemical activity of Cu NCs. The chemical hardness of the NC was found to be inversely related to the size.[84] This is caused by a reduction in the number of valence levels that are close in energy (and hence an increase in the relaxation energy and a decrease in polarizability) when the size of the NC decreases.[84] Apart from chemical hardness, the effect of size on the catalytic activity of Cu NCs has been shown by a recent study,[13] which adopted the reduction of methylene blue by hydrazine to leucomethylene blue as a model. Because of its reversible color change, this model enables the study of the recycling of nanocatalysts. Cu NCs of three different sizes (0.42 ± 0.21 nm, 0.60 ± 0.27 nm, and 0.98 ± 0.41 nm) were used. The smallest one displayed the highest efficiency as a catalyst, whereas the largest one showed the least catalytic activity.[13] This is probably because cluster-mediated electron transfer from the donor (hydrazine) to the acceptor (methylene blue) has to proceed through the conduction band of the NCs, in which the position of the Fermi level affects the position of the HOMO/LUMO orbitals.[13] In medium- and small-sized NCs, the LUMO is located above the redox potential of methylene blue and below that of hydrazine.[13] This allows the NCs to serve as a catalyst. In large-sized NCs, the LUMO, however, is located below the redox potential of methylene blue, rendering the clusters catalytically inactive.[13] Taking this into account, when the intrinsic redox properties of Cu NCs are applied to elicit therapeutic effects, size is a factor that has to be carefully considered.

Apart from the chemical activity, the size of NCs influences the pharmacokinetic profile when Cu NCs are used as drug carriers. Nanoconstructs having a size of around 5 nm or less, in

general, can be easily eliminated from the body by renal excretion.[85] Those having a larger size may get accumulated in the bone marrow,[86] heart,[87] stomach,[88] and kidney;[89] whereas nanoconstructs having a size of 150-300 nm are often largely present in the spleen[90] and liver.[91] As Cu NCs usually have a size of only few nanometers or less, they are susceptible to renal excretion.[58] This is supported by the case of Cu NCs with PEG-containing ligands.[58] Although PEG is known to be able to extend the blood circulation time of various systems (including polymeric nanoparticles,[35, 71, 78] and viral vectors[92]) by rendering them less prone to opsonization, the NCs were found to have a half-life of less than 2 h in blood circulation, partly due to their small size and hence high susceptibility to renal clearance.[58] At the cellular level, the efficiency of cellular uptake of the NCs is linked with the cluster size, too. This has been suggested by an earlier study,[93] which observed no cytotoxicity in A549 cells after 24-h exposure of the cells to 30-nm-diameter CuS clusters at a concentration of 800 μM. Upon an increase in the size of the clusters to around 5 nm, exposure of the cells to a concentration of 50 μM was found to cause the cell viability to drop to 15%.[93] This suggests that a reduction in size may cause an increase in the efficiency of cellular internalization of the NCs,[52] thereby increasing the cellular content of the clusters and hence the effect of the clusters on the physiology of the cells. Despite the close relationship between the NC size and the physiological activity, ultra-small size is an intrinsic characteristic of Cu NCs. Changing it to manipulate the pharmacokinetic profile may not be practical sometimes. In this case, adoption of alternative methods (e.g., surface engineering) to improve the performance of NCs may be more feasible in reality.

Finally, the absorption and fluorescence properties of Cu NCs are closely linked with the cluster size. Due to the molecular-like characteristics of Cu NCs, which have the size comparable to the Fermi wavelength of electrons,[94] electronic transitions between the HOMO and the LUMO are possible.[95] This renders Cu NCs luminescent. Based on the simple spherical Jellium model, the relationship between the cluster size and the emission energy can be expressed as Eq. 1:

$$E_g = \frac{E_{fermi}}{N^{-1/3}} \quad (1)$$

where E_g is the emission energy, E_{fermi} is the Fermi energy of the bulk metal, and N is the number of atoms in a cluster. This equation has successfully fitted the experimental observations made in some series of Cu NCs,[12, 13, 96] but has deviated substantially from the size measurements done by mass spectrometry for Cu NCs stabilized by mercaptobenzoic acids,[97] poly(methacrylic acid) (PMAA),[98] PVP/DHLA,[39] and penicillamine.[55] It is possible that the Jellium model is more accurate only when non-protected NCs or those protected by weakly bound ligands are concerned.[49] To effectively control and enhance the emissive properties of Cu NCs by size manipulation, more extensive studies devoted to the establishment of comprehensive models linking the cluster size with the emission wavelength seem to be a pre-requisite.

5.2 Shape effects

Apart from size, shape is an important factor determining the drug release sustainability because of its impact on the kinetics of diffusional mass transport, which is a predominate mechanism controlling the release of drug molecules out of a dosage form.[99] In addition, drug carriers with an optimal shape display higher cellular uptake efficiency and a longer circulation half-life.[100, 101] This has been hinted at by the case of hydrogen-bonded tannic acid/PVP hemispherical capsules, which were found to be internalized by macrophages two times more effectively as compared to the spherical counterparts.[101] The impact of the shape of a carrier on its biological performance has recently been verified by the case of red blood cell-mimicking multilayer

hydrogel discoidal capsules of PMAA/PVP, which exhibited 60% lower efficiency in cellular internalization as compared to the spherical ones.[102] All these observations suggest that the shape of a carrier plays an important role in determining the performance in drug delivery. At the moment, reports on the manipulation of the shape of Cu NCs as theranostic agents are scant, but such a possibility has been illuminated by a study which successfully converted polyhydrido Cu NCs into rhombus-shaped Cu nanoparticles by making use of hydride addition to manipulate the growth of Cu NCs.[103] This demonstrates the technical feasibility of designing and changing the shape of Cu NCs to meet practical needs.

As the structural stability of Cu NCs is inversely related to the surface energy, shape manipulation is a method of improving the NC stability, too. This has been reported by Yang and coworkers,[104] who induced the formation of free-standing ribbons from $Cu_{12}DT_8Ac_4$ NCs via self-assembly for stability enhancement (**Figure 10**). The NCs were generated by reducing Cu(II) acetylacetonate ($CuAc_2$) in a solvent mixture of dibenzyl ether (BE) and liquid paraffin (LP), with DT adopted as both a reducing agent and a stabilizer.[104] Self-assembly of the generated NCs was initiated by dipolar attraction, and was reinforced by van der Waals interactions between DT. Nanowires, namely Wire-26, with an average diameter of 26 nm, were formed after the self-assembly process.[104] Upon further annealing treatment that converted $Cu_{12}DT_8Ac_4$ into Cu_8DT_8, free-standing ribbons, namely Ribbon-1.3, were produced.[104] Although this method has not yet been applied to manipulate the shape of Cu NCs for theranostic use, it may emerge as an approach alternative to conventional ligand-based NC stabilization for the development of "surfactant-free" Cu NCs as theranostic agents.

6. Strategies for luminescence control and enhancement

Regarding the important roles played by the emission of Cu NCs in disease diagnosis and treatment as presented in the preceding sections, the practicality of the NCs in theranostics is significantly determined by the luminescence efficiency. Over the years, different techniques have been adopted to manipulate the optical properties of Cu NCs (**Table 2**).[36, 39, 61, 62, 105-110] Besides manipulating the chemical composition,[83] the emission of Cu NCs can be controlled and enhanced by changing the cluster size, the dispersion medium, and the surface ligands. Because the size effect has already been discussed, here we will focus only on the latter two factors.

6.1 Change of the dispersion medium

The emissive properties of Cu NCs can be modulated by manipulating the dispersion medium before administration of the NCs *in vitro* or *in vivo*. For example, changing the concentration of tetrahydrofuran (THF) in a water-THF mixture from 10% (v/v) to 60% (v/v) has been reported to cause an enhancement in the luminescence intensity of PEI-templated Cu NCs.[46] This is attributed to the AIE effect induced by the low solubility of PEI in THF. Because of the restrictions on molecular movements, the probability of NCs to undergo nonradiative decay is reduced and the occurrence of radiative decay is stimulated. Apart from the intensity of the emission, the position of an emission peak can be modulated by changing the dispersion medium. This is supported by the observation that dispersions of PEI-templated Cu NCs in THF and 1,4-dioxane display a blue shift of the emission peak relative to the counterparts in an aqueous solution.[46] PEI is rich in amine groups, and can function as an electron donor when the Cu core serves as an electron acceptor. This enables the establishment of an internal charge transfer state. In a solution, the general solvent effect is largely governed by the orientation polarizability (Δf),[111] which is derived from the dielectric constant (ε) and the refractive index (n) of the medium (Eq. 2):

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \quad (2)$$

This orientation polarizability, along with the excited-state (μ*) and ground-state (μ) dipole moments of NCs, determines the Stokes shift, which is defined as a difference between the frequency of the absorption maximum (v_a) and that of the emission maximum (v_f). The relationship between these parameters is depicted in Eq. 3, where *h* is Planck's constant, *c* is the speed of light, and *a* is the solute cavity radius.

$$v_a - v_f = \Delta f \cdot \frac{2(\mu^* - \mu)^2}{hca^3} \quad (3)$$

In general, a decrease in *n* or an increase in ε results in a larger Stokes shift. This leads to a shift in the emission peak when NCs are dispersed in different media. In addition, the availability of hydrogen bonding interactions in a dispersion medium may change the energy gap between the singlet excited state and the ground state of Cu NCs, resulting in a change in the emission wavelength.[46] Despite the technical plausibility of manipulating the emissive properties of Cu NCs by designing the dispersion medium, sometimes this approach is not favorable. This is because water is the medium predominately used for drug administration in preclinical and clinical settings. Addition of any organic solvents may cause acute or chronic toxicity *in vivo*, not to mention its influence on the pharmacokinetic profile of the delivered agent. Furthermore, upon administration of an NC dispersion into a body, the contact of the dispersion with extracellular and body fluids, and hence a change in the composition of the dispersion medium, can hardly be avoided. More investigations on the impact of those fluids on the emissive properties of Cu NCs are, therefore, vital to the translation of Cu NC-based theranostic agents from the laboratory to the clinic in the future.

6.2 Surface modification

Apart from changing the dispersion medium, the emission of Cu NCs can be manipulated by surface modification, which, as presented in the preceding sections, can also enhance the cluster stability,[53, 55-57] improve the target specificity,[58] and enhance the redox activity.[59] Luminescence control and enhancement in Cu NCs can be achieved by using metal-organic frameworks (MOFs), which are porous materials generated from metal ions and organic linkers. *In situ* encapsulation, in which a nanomaterial and an MOF are generated simultaneously, is a typical method of loading the framework with the nanomaterial.[112] This method has been adopted to encapsulate glutathione-protected Cu NCs in an MOF.[24] Compared to bare Cu NCs, the fluorescence intensity of those inside the MOF was found to display a 35-fold increase, with the stability prolonged from days to months.[24] This is attributed to the restriction of the molecular movements of the protecting groups on the NC surface upon the encapsulation process,[24] resulting in a reduction in non-radiative transitions. Apart from *in situ* encapsulation, the ship-in-bottle strategy (where a nanomaterial is assembled inside an MOF), the bottle-around-ship strategy (in which an MOF is assembled around a nanomaterial), and the sandwich assembly strategy (in which a nanomaterial is embedded between layers of the MOF) have been adopted to encapsulate Cu NCs.[24] The luminescence intensity of the products prepared by these strategies, however, is far from satisfactory.[24] Further studies are required to advance the technical basis for confining Cu NCs into such frameworks.

Besides MOF encapsulation, changing the isomerism of the ligand on the NC surface may help modulate the emissive properties of the clusters. This has been shown by an earlier study, in which Cu NCs were generated by using different stabilizers [including 3-mercaptobenzoic acid (3-MBA), 4-MBA, and thiosalicylic acid (TA)].[97] Owing to π–π stacking of the surface ligands, TA-stabilized NCs form aggregates with a size of around 3.7 ± 0.5 nm.[97] A similar

phenomenon also occurs in 3-MBA-stabilized and 4-MBA-stabilized NCs, which form net-like aggregates and slat-like aggregates, respectively.[97] Aggregates of NCs stabilized by TA and 4-MBA emit blue and red light respectively upon UV irradiation, whereas aggregates of 3-MBA-stabilized NCs are weakly fluorescent.[97] When the pH of the NC solution is changed from 3.0 to 7.0, the luminescence intensity of the aggregates formed by 4-MBA-stabilized NCs decreases, while that of the aggregates formed by TA-stabilized NCs increases.[97] This demonstrates that by manipulating the isomerism of the surface ligand, significant changes can be achieved in the emissive properties of the NCs. Besides ligands with different isomeric structures, electron-rich ligands can be used to modify the surface of the Cu NC to enhance the luminescence intensity.[113] The success of this method, however, relies on proper optimization of the amount of ligands present on the NC surface, because an excess amount of ligands may hamper the cluster stability and the cellular internalization efficiency.[1, 78] Despite this, regarding the sophistication of techniques for structural design and ligand modification, along with the track record of applications of surface engineering in the development of drug carriers,[1, 34, 52, 114] manipulation of the structure of the surface ligand is expected to be an important method of adding versatility to Cu NCs and controlling luminescence in future theranostic research.

7. Conclusions and outlook

Based on the evidence presented so far, it is not difficult to see how the advances in the chemistry and engineering of Cu NCs have facilitated the biomedical use of Cu NCs. Despite this, right now Cu NCs that can enter clinical trials are absent. This is partly because of the still unsophisticated techniques for combining diagnostic and therapeutic functions. The lack of a comprehensive understanding of the physiological fate of Cu NCs, and hence the technical difficulty in precisely controlling the performance of the clusters as both a diagnostic probe and a therapeutic agent, also plays a role. In fact, at the moment our knowledge of the biological effects of Cu NCs is fragmentary and sometimes even controversial. For example, Cu is thought to be more easily removed from the human body than noble metals (e.g., gold and silver) and hence should be biocompatible, but some studies call this understanding into question. One of these studies has been performed in human lung epithelial cells, in which Cu-zinc alloy nanoparticles have been found to induce not only chromosomal damage but also damage to single- and double-stranded DNA.[115] Cu(I) oxide nanoparticles have also been reported to display systemic toxicity, partly due to their ability to target mitochondria to activate the activity of caspase-3/9 in treated cells.[116] Similar contradictory observations on the biological safety of Cu exist in Cu NCs, too. While those NCs have been widely reported in the literature as non-toxic,[117] they have also been found to induce atrophy in C2C12 myotubes, causing impairment in Akt phosphorylation and an increase in the expression of autophagy-related genes (including *beclin-1*, and *bnip-3*).[81] These findings have undermined the validity of conventional beliefs about the high safety profile of Cu NCs in theranostics, and are calling for future research for calcification.

One of the reasons explaining the discrepancies regarding the biological safety is the differences in experimental conditions adopted by different studies. In fact, while Cu is essential for all organisms, excess Cu has been shown to be toxic due to its ability to promote free radical formation.[118] The toxicity of Cu varies with the dose, the method of administration, the treatment time, the synthetic approach, the chemical composition, and the surface properties of the adopted nanoconstruct. This is demonstrated by the fact that the median lethal dose (LD_{50}) of Cu^{2+} ions for mice is 110 mg kg^{-1} upon oral administration; whereas that of Cu nanoparticles is 413 mg kg^{-1}.[119] This may partially explain the variation in the reported toxicity of Cu nanoconstructs among studies. In addition, the low toxicity seen with Cu NCs in *in vitro* and *in vivo* applications may be because the concentration of NCs used for those applications is usually

much lower than that for toxicological research. Variations in the stability of Cu NCs prepared by different methods may also account for the variation in toxicity because the toxicity of Cu NCs is partly caused by the presence of Cu^{2+} ions, which can bind to cellular proteins (e.g., Ca-ATPases), to interfere with intracellular signaling, and to impair the cytoskeletal organization.[120] The toxicity of Cu NCs, however, cannot be totally explained by the activity of Cu^{2+} ions because the biological properties of these two species are different. This is suggested by the observation, while ROS production is reduced in haemocytes treated with Cu(II) chloride dihydrate at a concentration of 4 μg mL^{-1},[120] exposure of C2C12 cells to Cu NCs leads to the induction of ROS formation and the reduction in the activities of catalase and glutathione.[81] Further studies are, therefore, required before the actual mechanisms governing the variation in the biological activities of different Cu NCs can be elucidated. This understanding is also vital to the design of Cu NCs as theranostic agents in the future.

As a matter of fact, although different methods are available for the synthesis of Cu NCs, currently the clusters that can be obtained often show a large size distribution profile and excitation-dependent photoluminescence behavior. This limits the transition of the clusters into further *in vivo* theranostic applications. Search for methods of large-scale production of Cu NCs with low polydispersity will, therefore, continue to be in focus. Another direction that is worth exploring in future research is the development of "smart" clusters that can be "turned on" upon arrival at target sites. The viability of this research area has already been shown by Josephson and coworkers,[121] who have successfully generated superparamagnetic iron oxide nanoparticles with enzyme-activated near-infrared fluorescence. Although reports on "smart" Cu NCs are lacking at the moment, it is anticipated that research efforts in this direction can enhance the target specificity of NC-based theranostics in the future. In addition, right now imaging of a diseased region relies largely on the detection of the subtle difference between the abnormal tissue and the subset of surrounding cells. To enhance the diagnostic accuracy and specificity, one common strategy is to combine moieties for multiple imaging modalities within a single NC. The success of this strategy has been partly demonstrated by the combination of MRI with optical imaging, in which the anatomical resolution of the former and the sensitivity of the latter work together to enable the imaging of tumors and the monitoring of atherosclerosis.[122] It is envisaged that multimodal will become one of the most prominent areas on which future research on Cu NC-based theranostics will focus. When such NCs are designed, proper control of the ratio of different imaging moieties incorporated into the NC is vital. For instance, as far as combined MRI/fluorescence imaging is concerned, the concentration of the MRI moiety should generally be higher than that of the moiety for fluorescence imaging, owing to the difference in the sensitivity of the two imaging techniques. In addition, interference between the moieties for different imaging modalities has to be avoided, otherwise the efficiency of the NC in theranostics will be reduced. Finally, although most of the studies reported in the literature involve the use of Cu NCs alone, the possibility of integrating multiple fluorophores together should not be ruled out. Such a possibility has been illuminated by an earlier study, which combined silver NCs and Cu NCs for the detection of biomacromolecules with different charges.[123] More recently, 3-aminophenylboronic acid (APBA)-modified carbon dots have also been covalently linked to BSA-stabilized red-emitting Cu NCs via carbodiimide-activated coupling to generate blue-emitting nanohybrids for dopamine detection.[124] Regarding the wide diversity of types of fluorophores available in the literature, the combined use of them and Cu NCs may provide a route to enhance the versatility and functionality of Cu NC-based theranostic systems in the future.

In summary, Cu NCs are an emerging class of fluorophores whose biomedical applications have attracted extensive research interest since the turn of the last century. This is manifested by the large number of reports in the literature on Cu NCs with different properties and structures. To

promote the clinical translation of Cu NCs, techniques for synthesizing and engineering Cu NCs shall be further advanced so that the chemical, physical, and biological properties of the clusters can be more effectively controlled. Our understanding of the mechanisms governing the physiological fate of Cu NCs shall be enhanced as well. Last but not least, theranostics represents a transition from conventional medicine to personalized medicine. It exploits an integration of multiple imaging and treatment technologies into one single system, and also aims at personalizing drug regimens to meet patient needs. To achieve this goal, future efforts should be directed to deciphering the impact of environmental factors and epigenetic factors on the pharmacokinetic profile of NCs administered to a biological body. It is true that a long way is ahead before the use of Cu NCs can be integrated into the routine clinical practice; however, the rapid progress in the chemistry and engineering of Cu NCs over the years has been shortening the distance between research and practice. Along with the similarities of concepts adopted in the theranostic use of metal NCs and the foundation already established by studies on other metal NCs (e.g., gold NCs, and silver NCs) in disease diagnosis and treatment, it is envisaged that insights provided in the literature on other NCs will continue to fuel the rapid development of Cu NCs as theranostic agents in the forthcoming decades.

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Conflict of Interest
The authors declare no conflict of interest.

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Figure Legends

Figure 1 Advantages and limitations of major types of metal NCs

Figure 2 (A) A schematic diagram depicting the process of interfacial etching of Cu nanoparticles and the formation of water-soluble Cu NCs. (B) Photographs showing the color change of the NCs in buffers at different pH values under (i) visible light and (ii) UV irradiation. (C) (i) Bright field, (ii) confocal fluorescence, and (iii) overlay images of 293T cells incubating with Cu NCs for 12 h. Scale bar = 20 μm. Abbreviation: NPs: nanoparticles; OA: oleyl amine; ODE: 1-octadecene; PEI: poly(ethylenimine). Reproduced with permission.[50] 2016, Elsevier

Figure 3 (A) The synthetic procedure, and (B) the single-crystal structure and packing diagram of $[DBFDP]_2Cu_4I_4$. Reproduced with permission.[57] 2017, American Chemical Society.

Figure 4 (A, B) Cellular internalization of (A) non-targeted and (B) targeted Cu NCs in 4T1 cells. (C, D) The co-localization of the (C) non-targeted and (D) targeted Cu NCs with LAMP-1 in 4T1 cells. (E) Hematoxylin-eosin and (F) CXCR4 immunohistochemical staining of the 4T1 tumor (original magnification: ×20). (G-I) PET images of (G) non-targeted and (H, I) targeted Cu NCs, in the (H) absence and (I) presence of non-radioactive targeted NCs, in the tumor tissue of the 4T1 mouse TNBC model. (J-L) PET images of (J) non-targeted and (K, L) targeted Cu NCs in the PDX model, where the CXCR4 level of the tumor tissue is either (J, L) high or (K) low. Reproduced with permission.[58] 2019, American Chemical Society.

Figure 5 (A) Fluorescence microscopy images of tissue sections taken at 4 h after intravenous administration of the NCs to orthotopic A549 lung tumor bearing mice. In order to enable intracellular localization, the NCs are conjugated with the near-infrared fluorescent dye CF680R before use. Blue fluorescence shows nuclei stained with DAPI. Red fluorescence shows the location of the NCs. (B) Representative PET images of coronal single slices on orthotopic A549 lung tumor bearing mice after intravenous administration of 6.7 MBq of the NCs. The location of the tumor is indicated by white arrows. Reproduced with permission.[75] 2015, American Chemical Society.

Figure 6 (A) A schematic diagram showing the generation of cluster-incorporated nanocomposites and the loading of cisplatin into the composites. (B) (i) Bright field images, (ii) fluorescence images under the excitation filter of green (540/25 nm), (iii) fluorescence images under the excitation filter of blue (465-495 nm), and (iv) merged images of HeLa cells after 2-hr incubation with the nanocomposites. Scale bar is 100 μm. Abbreviation: PVP: poly(vinylpyrrolidone); PVA: poly(vinyl alcohol); AA: ascorbic acid; RT: room temperature. Reproduced with permission.[39] 2015, American Chemical Society.

Figure 7 (A) A schematic diagram showing the generation and use of Cu NC/doxorubicin complexes in theranostics. Abbreviations: DLA: Daltons lymphoma ascites; Dox: doxorubicin; FRET: Förster resonance energy transfer; PL: photoluminescence; Tf: transferrin; Tf-Cu NCs: transferrin-templated Cu NCs; Dox-Tf-Cu NCs: complexes formed between Dox and Tf-Cu NCs. (B) Confocal fluorescence images of HeLa cells at different time intervals (0 h, 2 h, 4 h, and 6 h) after administration of the Cu NC/doxorubicin complex. First column shows the bright-field image, and the second and third columns show the fluorescence images collected in the range of 580–700 and 380–490 nm, respectively. The fourth column shows the merged image. The scale bar is 10 μm. Reproduced with permission.[15] 2018, American Chemical Society.

Figure 8 (A) Photographs of different bacterial colonies grown on the agar plates containing tannic acid-stabilized Cu NCs at a concentration of 30 μg mL^{-1}. (B) Atomic force microscopic images of (i) *S. aureus*, (ii) *B. subtilis*, (iii) *E. coli*, and (iv) *P. aeruginosa*, after treatment with phosphate buffer or after 10-min incubation with tannic acid-stabilized Cu NCs. Reproduced with permission.[28] 2019, Elsevier.

Figure 9 (A) Photographs of the aqueous solutions of Cu NCs under UV light (top) and normal light (bottom) in the presence of different ions: (a) Cr^{3+}, (b) Mn^{2+}, (c) Fe^{2+}, (d) Fe^{3+}, (e) Co^{2+}, (f) Ni^{2+}, (g) Cu^{2+}, (h) Zn^{2+}, (i) Hg^{2+}, (j) Pb^{2+}, (k) Cd^{2+}, (l) Al^{3+}, (m) Li^{+}, (n) Na^{+}, and (o) K^{+}. (B) TEM images of (a) the aqueous solution of Cu NCs alone, (b) the aqueous solution of Cu NCs in the presence of 0.5 mM Fe^{3+}, (c) the aqueous solution of Cu NCs in the presence of 1 mM Fe^{3+}, and (d) the aqueous solution of Cu NCs in the presence of 1 mM Fe^{3+} and 1.2 mM ascorbic acid. (C) The proposed mechanism of Fe^{3+}-induced quenching and the reversal of the quenching process by the use of ascorbic acid. Reproduced with permission.[82] 2019, Elsevier.

Figure 10 (A) Evolution of the self-assembled structure of the DT-stabilized Cu NCs from Wire-26 to Ribbon-1.3. (B) (a-c, e-g) TEM and (d, h) tapping-mode atomic force microscopic images of (a-d) Wire-26 and (e-h) Ribbon-1.3. Insets in (a) and (e) show the viscous gel generated upon the assembly of $Cu_{12}DT_8Ac_4$ NCs into Wire-26 and the colloidal solution containing Ribbon-1.3, respectively. Reproduced with permission.[104] 2014, John Wiley & Sons, Inc.

Table 1 Examples of diseases studied for the diagnostic use of Cu NCs

Disease	Type of application	Example of use	Working principle	Ref.
Cancer	Diagnosis	The process of the *in situ* generation of Cu NCs has been exploited for the development of a label-free method of detecting the level of miRNA-21 in cancer cells	The presence of miRNA-21 leads to NC formation. The concentration of miRNA-21 in cells can then be quantified based on the fluorescence change of the NCs.	60
		The intracellular aggregation-induced emission (AIE) kinetic rate of Cu NCs has been exploited to probe cancer cells.	Upon internalization into living cells, the NCs display a fluorescence change, which is largely affected by the pH of the cellular environment. Different cell lines can then be differentiated based on the differences in the intracellular AIE kinetic rate.	61
		The process of the AIE of Cu NCs has been exploited for the detection of VEGF165.	The presence of VEGF165 in serum can affect the nucleation and the fluorescence intensity of Cu NCs, thereby enabling the concentration of VEGF165 to be monitored.	62
Diabetes	Diagnosis	Cu NCs have been incorporated into the development of glucose sensors to increase the sensor performance by increasing the electrocatalytic active area and promoting electron transfer for the reaction of glucose oxidation	Cu NCs are electrochemically deposited on an electrode modified with Nafion-solubilized multiwall carbon nanotube (CNTs) for the development of a sensor for the detection of glucose in blood serum samples.	63
Duchenne muscular dystrophy (DMD)	Diagnosis	The process of the *in situ* generation of Cu NCs has been exploited for the development of a method of detecting the deletion or duplication genotypes of DMD	Double-strand DNA is used as a template for the formation of Cu NCs, whose variations in the luminescence intensity enables different genotypes of DMD to be distinguished.	64
Hepatitis	Diagnosis	Cu NCs have been used to develop a colorimetric method of detecting the DNA of hepatitis B virus	In the presence of the target DNA, Cu NCs can be formed. They can then be oxidized to Cu^{2+}, which later forms a complex with creatine and leads to the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)	65
Infection	Diagnosis	The process of the *in situ* generation of Cu NCs has been exploited for the determination of micrococcal nuclease (MNase) and hence the pathogenicity of *Staphylococcus aureus*	In the absence of MNase, double-stranded DNA with AT-rich regions and protruding 3'-termini can function as a template for the generation of Cu NCs, giving bright yellow fluorescence. In the presence of MNase, the template is digested and no NC can be formed.	66
Rheumatoid arthritis	Drug monitoring	Cu/molybdenum bimetallic NCs have been generated using cysteine as both a capping agent and a reducing agent, and have been adopted to determine the concentration of methotrexate (MTX)	The fluorescence of the NCs can be affected by the presence of MTX, which exerts a strong inner filter effect on the emission of the NCs, in spiked urine of patients.	67
Disorders of the uterus	Drug monitoring	Cu NCs have been used as a probe for the detection of gossypol	The fluorescence quenching mechanism induced by interactions between BSA and gossypol is adopted to determine the concentration of gossypol in clinical samples	68
Epilepsy	Drug monitoring	Cu NCs coated with cetyl trimethylammonium bromide have been adopted to determine the amount of carbamazepine (CBZ) in the exhaled breath condensate of patients receiving the drug	Interactions of the NCs with CBZ lead to the blocking of non-radiative e^-/h^+ recombination defect sites on the NC surface, resulting in the enhancement of the blue-green fluorescence of the NCs	69

Table 2 Major techniques for engineering the optical properties of Cu NCs

Property	Mechanism	Possible technique	Example	Implications for theranostics	Ref.
Absorption	The continuous density of states in NCs breaks up into discrete energy levels, causing a loss of plasmonic properties. NCs can interact with light through electronic transitions among energy levels, leading to sharp absorption.	• Manipulate the NC size • Engineer the ligand on the NC surface • Manipulate the properties of the surrounding medium	By changing the concentration of $VEGF_{165}$ in the surrounding medium, the absorption wavelength of the aptamer-templated Cu NCs has been found to display a shift by 400 nm.	The sharp absorption band enables more effective control over the spectral sensitivity of the theranostic system during imaging.	62
Fluorescence emission	When the size of a metal nanoparticle approaches the Fermi wavelength of electrons, electronic transitions among occupied d bands and states above the Fermi level are possible. In addition, electronic transitions between the HOMO and the LUMO can occur. All these account for the fluorescence emission of NCs.	• Manipulate the NC size • Engineer the ligand on the NC surface • Manipulate the properties of the surrounding medium	The emission of transferrin-templated Cu NCs has been changed from blue to red after the reducing agent has been changed from hydrazine hydrate to ascorbic acid.	The fluorescence emission of NCs enables applications in molecular imaging, image-guided tumor resection, and the detection of biomarkers.	36
			The emission of Cu NCs generated in the presence of DHLA and PVP has been changed from red to orange, with a shift in the emission maximum observed, when the pH of the surrounding medium has been changed from 4.5 to 11.0.		39
			By changing the pH of the surrounding medium from 4.5 to 7.4, the emission of the cysteine/chitosan-co-stabilized Cu NCs has been changed from orange-red to cyan-green.		61
			The emission of lysosome-templated Cu NCs has been shifted from 410 to 575 nm when the excitation wavelength has been changed from 325 to 525 nm.		105
Two-photon absorption	Two photons of identical or different frequencies are simultaneously absorbed, leading to the excitation of an NC from the lower energy electronic state to the higher energy one.	• Manipulate the NC size • Design the composition of the NC to enable two-photon absorption (e.g., incorporation of lanthanides)	Cu NCs have been fabricated upon implantation of Cu ions into fused silica. The two-photon absorption coefficients of the NCs have been shown to be size-dependent.	In comparison with one-photo excitation, the possibility of exciting a molecule using two photons in the near-infrared region is beneficial to *in vivo* imaging and photodynamic therapy, due to the increase in the penetration depth, the decrease in scattering (and the resulting increase in spatial resolution), and the reduction in auto-fluorescence.	106
			Crystals containing Cu and lanthanide elements (e.g., Yb, and Er) have been generated using a solvothermal method. They have been found to display the upconversion emission.		107
Electrochemi-luminescence	A co-reactant is used to generate a reactive intermediate, which can react with the NCs generated at electrodes, causing them to commence an excitation pathway or to be in the excited state that emits light.	• Design the structure of the co-reactant	By using Cu NCs, which have been generated *in situ* electrochemically, as luminophores and TiO_2 as a co-reaction accelerator, electrochemiluminescence has been made possible for the development of a biosensor for microRNA detection.	The application potential of this property in *in vivo* imaging is limited; however, it might be used to develop devices to detect biomarkers.	108
Solvatochromism	Changes in the solvent properties lead to the induction of electronic energy splitting and electron redistribution on the NC surface.	• Engineer the ligand on the NC surface	Owing to the solute-solvent interaction between the chelate cation and the solvent molecules, Cu(II) complexes of 3-acetylcoumarine and dinitrogen bases have been reported to be solvatochromic, displaying different colors in different organic solvents.	These properties enable NCs to detect changes in the surrounding medium (e.g., cell milieu), and to be exploited for the detection of biomarkers and molecular imaging.	109
			Dinuclear oxalato-bridged Cu(II) complexes have been found to be solvatochromic, with the change in the absorption maximum determined by solvent parameters.		110