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Expanding the scope of chemiluminescence in bioanalysis with functional nanomaterials

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Abstract

Nanomaterial-enabled chemiluminescence (CL) detection has become a growing area of interest in recent years. We review the development of nanomaterial-based CL detection strategies and their applications in bioanalysis. Much progress has been achieved in the past decade, but most attempts still remain in the proof-of-concept stage. This review highlights recent advances in nanomaterials in CL detection and organizes them into three groups based on their role in detection: as sensing platform, as signal probe, and applications in homogeneous systems. Furthermore, we have discussed the critical challenges we are facing and future prospects of this field.

Introduction.

The broad category of nanomaterials refers to materials that have at least one dimension in a three-dimensional space at the nanometer level or as a basic unit (e.g. nanowires, nanofilms). Nanotechnology has been recognized as one of the most promising technological developments of the century and has been widely used in the further research and development of nanomaterials. In order to combine signal amplification technology with the bioanalysis of nanomaterials, various nanomaterials have been applied to the fixation of immunological reagents at a sensor interface. In recent years, signal marking and amplification techniques for nanomaterials have also made breakthroughs, resulting in the use of nanomaterials for sensors becoming a research hotspot.

Chemiluminescence (CL) is an advantageous detection tool because of the high signal-to-noise ratio of its optical-signal readout, which does not require an external excitation source. CL bioanalysis has gained increasing attention in different fields due to its positive characteristics of high sensitivity, wide linear range, good selectivity, fast analysis speed and low instrument price. For a typical CL events, the release of energy during a chemical reaction (redox reaction in particular) excites the luminescent materials to radiate visible light. The most popular CL substrates are luminol, lucigenin, 4-methoxy-4-(3-

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Conflicts of interest

There are no conflicts to declare.

phosphatephenyl)-spiro-(1,2-dioxetane-3,2-adamantane) (AMPPD), and their derivatives. CL systems have been used in conjunction with other technologies such as immunoassay (IA), flow injection (FIA), and high performance liquid chromatography (HPLC), which are used in a wide variety of applications such as genetic, pharmaceutical, clinical, food testing and analysis. Various nanomaterials that catalyze CL systems have been widely explored and used to detect different biological substances, such as hydrogen peroxide,[1] amino acids,[2] thrombin,[3] C-reactive protein,[4] IgG[5] and isoniazid[6].

Efficient immobilization of biomolecules and development of new sensitive CL bioanalysis methods are important steps for advancing sensing platform technology. The physical and chemical properties of the biosensing interface play a crucial role in obtaining excellent assay performance,[7] which can be enhanced by nanomaterials. Conventionally, micro-scaled materials such as microbeads and membranes are widely used in CL bioanalysis for the immobilization of proteins.[8] In recent years, the modification of biomolecules on the nanostructured sensing surface has opened up new potential avenues in the fabrication of CL biosensors.[9] numerous types of nanomaterials, such as metal nanoparticles, magnetic nanomaterials, carbon nanomaterials and semiconductor nanomaterials, have been exploited as solid support for proteins immobilization to develop CL biosensing systems, to take advantage their unique physical and chemical properties. The resulting analytical capacities of CL bioassays have, as a result, been greatly improved.

The sensitivity of the signal probe is another key factor to develop sensitive CL bioanalysis methods. For traditional CL analysis, naturally occurring enzyme molecules such as horseradish peroxidase (HRP) have been widely used to catalyze CL substrates for high sensitivity CL detection.[10] Nanomaterials have been used to enrich both the signals themselves and high levels of catalyst for signal measurement and tracking. As an enzyme platform or catalyst, nanomaterials greatly increase the number of tags associated with a single biometric event, thereby greatly increasing sensitivity and reducing detection limits. This opens the door for many high-sensitivity analytical diagnostic studies using these new solid-phase or homogeneous nanomaterial sensing platforms, taking advantage of the various characteristics of the nanomaterials, and the development of labelling and related technologies. As the original CL system has become more refined and new CL systems and mechanisms have steadily matured, CL detection has gradually become a more routine analysis method. For the purposes of immunosensing, there is also the potential for substitution of conventional enzyme-linked immunoassays. Since the concept of nanozymes was introduced by Pasquato, Scrimin and co-workers,[11] its definition has been extended to encompass artificial enzymes based on nanomaterials: in short, nanomaterials with enzyme-like activity. Compared to natural enzymes, the properties of nanozymes are generally more stable, easy to obtain, low cost and controllable. Nanozymes have thus gradually become another area of increased interest and have developed into new research fields. Yan and co-workers'[12] seminal work on the topic reported that Fe_3O_4 magnetic nanomaterials exhibit intrinsic peroxidase-like activity. As of now, a range nanomaterials have been shown to have intrinsic peroxidase-like activities, such as metal oxides, metal hydroxides, metal sulphides, metal-organic framework materials (MOFs), carbon-based nanomaterials and their complexes, all of which have important positions and applications in CL biosensing.[13]

To the best of our knowledge, few publications summarize recent advances in nanomaterial-based sensitive CL detection and their analytical applications. Nanomaterial-based CL detection has developed into a variety of new strategies in which nanomaterials play different roles. In this review, we classify the role of nanomaterials as signal probe, as sensing platform and in applications in homogeneous CL detection. Herein, recent advances in nanomaterial applications for CL analysis in a subset of systems were summarized (Fig. 1). In addition, some typical examples of nanomaterial-based CL detection and their applications were listed (Table 1).

Nanomaterial-based CL sensing platform

Magnetic nanomaterials

Magnetic nanomaterials have been widely used in many fields. Magnetic nanoparticles (MNPs) and their composites, with their controllable morphology, uniform particle size and excellent performance, have gradually become a central avenue of research in magnetic nanomaterials. Due to their nanoscale-order sizes, magnetic nanomaterials have macroscopic quantum tunnelling and small size effects, and thus exhibit different properties from conventional magnetic materials, and have unique magnetic properties and better biological compatibility. In a typical separation process, biological entities are immobilized on superparamagnetic nanomaterials and then separated by an external magnetic field.

Agarose gels, nitrocellulose membranes, and adsorptive microplates are commonly used as carriers to immobilize targets in SELEX (systematic evolution of ligands by exponential enrichment) processes, but subsequent separation steps are cumbersome and time consuming. Xi et al.[14] instead used MNPs as a carrier to immobilize the target, which facilitates rapid magnetic separation (Fig. 2). First, DNA aptamers against hepatitis B surface antigen (HBsAg) were selected by immobilizing HBsAg on the surface of carboxylated MNPs. The ssDNA library for each selection round was prepared by asymmetric PCR amplification for the next round of selection. Selected aptamers were used to construct CL aptamer sensors based on magnetic separation and immunoassays to detect HBsAg from pure protein or actual serum samples. Bi et al.[15] used G-quadruplex subunit sequence and the fluorophore fluorescein imine (PAM) are tightly encoded in DNA-4WJ, stimulating a CRET process in the presence of hemin/K+ to form horseradish peroxidase (HRP)-mimicking DNAzyme that catalyzes the generation of luminol/H_2O_2 CL, which further transfers to FAM. Background interference and extraneous information could be easily removed via magnetic separation using magnetic graphene oxide (MGO), which helps to improve detection sensitivity.

Nanocomposites are emerging complex nanoscale materials that are composed of different phases and are widely recognized for their widespread impact on many fields.[16] Xing et al.[16 (a)] designed a PAA-coated Au/Fe_3O_4 composite magnetic nanoparticle (NP) with monodispersity and high biostability for the development of a highly sensitive C-reactive protein (CRP) CLIA. An improved and highly sensitive CLIA biosensing system was demonstrated by using monodisperse PAA coated Au/Fe_3O_4NP (PAA-Au/Fe_3O_4NPs) as a magnetic carrier for detecting CRP in clinical serum. Fan and co-workers[17] reported that mesoporous material-loaded $CoFe_2O_4$ magnetic nanoparticles have unique

peroxidase-like activity and react with luminol to generate new CL without H_2O_2. Strong electrostatic interaction between the positively charged mesoporous material carrier $CoFe_2O_4$ nanoparticles and the negatively charged luminol anion then generates more active free radicals, which results in the surface of the $CoFe_2O_4$ NPs producing more $^1O_2$ with a strong CL emission.

Gold nanomaterials

Gold nanomaterials have attracted considerable interest due to their own unique physical properties, excellent stability, good biocompatibility and ease of functionalization.[18] Novel functional gold nanomaterials with special properties can be synthesized, thanks to the rational design of the surface chemistry of gold nanoparticles (AuNPs) with various functional molecules. Being composed of a largely inert metal, AuNPs can effectively overcome the common problem of lack of chemical stability of nanoparticles and thus play an important role in nanoscience and nanotechnology. Compared with other common metal nanomaterials, gold nanomaterials tend to have better stability on the nanometer scale, so researchers give priority to precious metal gold in the choice of designing nanomaterial sensing platforms. AuNPs can provide unique and controllable surface chemistry via both the strong interactions in gold-sulfur bonds and natural self-assembly to couple the desired functionalized polymers or groups on the surface of gold to construct the desired nanodevices, enabling detection, analysis, imaging, etc. AuNPs can effectively enhance the Rayleigh scattering and Raman scattering signals, and obtain information on common optical signals that are difficult to obtain using standard biological materials. AuNPs have a high specific surface area to provide a binding site for the desired ligand, providing a wide range of bioprobe elements for the specific detection of small biomolecules and tumor markers using CL.

Huang and co-works[19] reported a general assembly strategy for multifunctional gold nanoparticles (MF-GNP) with CL, catalytic and immunological activity, which can be directly used in CL immunoassays (Fig. 3). N-aminobutyl-N-ethylisoluminol functionalized gold nanoparticles (ABEI-GNPs) were used as a platform for detection by serial assembly of antibodies, bovine serum albumin (BSA) and Co^{2+}. It has been shown that the specific binding of human IgG (hIgG) and the corresponding anti-hIgG causes a decrease in the CL signal of MF-GNP, since this immune reaction leads to the polymerization of GNP. Based on the reduction of CL intensity, the label-free CL immunosensor based on this MF-GNP platform with high sensitivity and selectivity was established for antigen detection.

Qin et al.[20] developed an effective AuNPs sensing platform based on CRET for the detection of biomolecules. The aptamer was covalently labelled with the CL reagent N-(4-aminobutyl)-N-ethylisoluminol (ABEI). The ABEI-labelled aptamer was then hybridized with AuNPs-functionalized ssDNA, which was complementary to the aptamer, to obtain an aptamer sensor. CRET between ABEI and AuNPs in aptamer sensors results in CL quenching of ABEI. In the presence of the target analyte, it forms a complex with the aptamer and releases the ABEI-aptamer from the surface of the AuNPs, resulting in CL recovery of ABEI.

Carbon nanomaterials

Carbon nanomaterials have attracted considerable attention in the manufacture of biosensors due to their extraordinary physical and electronic properties. Carbon nanotubes are a one-dimensional quantum material with a special structure: the radial size is on the order of nanometers, and the axial dimension is on the order of micrometers. It can be subdivided into single-walled carbon nanotubes and multi-walled carbon nanotubes (MWCNT). Carbon nanotubes (CNTs), one of the most interesting carbon nanostructures, have unique physical and properties such as extremely high surface/volume ratio and unique electronic properties that promote electron transfer.[21] As shown in Fig. 4, the ultrasensitive CLIA system for detecting tumor markers was developed.[22] For the first time, a MWCNT platform based on streptavidin functionalization was designed. The biofunctionalized MWCNTs platform exhibited a large reaction surface area and excellent biocompatibility. Based on the highly selective recognition of biotinylated antibodies by streptavidin, capture antibodies can be efficiently immobilized on the surface of the biosensing platform. Compared to immunosensors that do not use MWCNTs, the CLIA system showed a 7.9-fold increase in detection sensitivity.

Graphene oxide (GO), an atomically thin sheet of graphite, is increasingly attracting chemists for its own unique characteristics.[23] The composites exhibit improved electrical conductivity, mechanical strength, or enhanced functionalities such as loading capacity and catalytic activity. To date, several multifunctional graphene-based nanomaterials with CL activity have been explored. Cui and co-works[24] report the first graphene-based composite material noncovalently functionalized with ABEI (ABEI-CCG). They have demonstrated a facile approach to prepare CL functionalized graphene composites via the reduction of GO by CL reagents with aromatic rings such as ABEI, luminol and isoluminol. Furthermore, based on the novel CL property of ABEI-CCG, they have developed a CL sensor for the detection of H_2O_2 to demonstrate its direct usage. Yang et al.[25] developed a new CLIA method coupled with flow-through system for quantitative determination of ChIFN-γ. The biocompatible graphene oxide nanosheet as a sensing platform was introduced into CL immunoassay for highly efficient immobilization of capture antibody. The GO-chitosan film provided a very large surface area for high-capacity loading of proteins and displayed a biocompatible microenvironment for long activity retention of the biomolecules.

Nanocomposites with CL activity are ideal candidates for nanoscale assembly platforms due to their atomic-level flat surface, CL properties and excellent catalytic performance. He et al.[26] proposed in situ preparation of graphene oxide/Ag nanoparticles (GO-AgNPs) nanocomposites by reducing $AgNO_3$ with CL reagent luminol in the presence of GO (Fig. 5). The nanocomposite exhibits excellent CL activity when reacted with H_2O_2. It was found that glutathione can enhance the CL intensity between the GO-AgNPs nanocomposite and H_2O_2. On this basis, a sensitive selective glutathione detection method was developed. In the work of our research group,[27] a novel streptavidin functionalized GO/AuNPs composite is prepared and for the first time used to construct sensitive CL immunosensor for the detection of a tumor marker. The GO/AuNPs biofunctionalized composite platform has a large reactive surface area and excellent biocompatibility, thus the capture antibody

can be efficiently immobilized on its surface based on the highly selective recognition of streptavidin to biotinylated antibody.

Other nanomaterials

In addition to magnetic nanomaterials and gold nanomaterials, various other nanomaterials or nanocomposites have been used as sensing platforms for CL-based detection. Chen et al.[28] synthesized Zn/Cu bimetallic nanoclusters (NCs) using bovine serum albumin (BSA) by a simple chemical reduction method (Fig. 6). Zn/Cu@BSA NCs significantly enhanced the ultra-weak CL produced by the decomposition of HCO_4^- in hydrogen peroxide and sodium bicarbonate systems, and studied the mechanism of CL. The amplified CL was induced by the catalysis of Zn/Cu@BSA NCs and the metal surface plasmon coupling emission of $(CO_2)_2$*. This Zn/Cu@BSA NCs-amplified CL system was successfully used for the selective detection of hydrogen peroxide in environmental samples. Our group[29] constructed an innovative label-free CL immunosensing method using biofunctional CuS nanoparticles (CuSNPs), which were used here as peroxide-mimicking enzymes and immobilization support materials. CuSNPs with high catalytic activity and stability were synthesized by simple co-precipitation method. Compared to label-based CL immunoassays, the proposed label-free assay mode is simpler, cheaper and faster. In addition, the label-free CL immunoassay system based on the CuS nanozyme sensing platform showed good specificity, acceptable repeatability and good accuracy.

Nanomaterial as signal probe in CL detection

Nanomaterials such as gold nanoparticles, magnetic nanoparticles, CuO nanoparticles, and some composite nanostructures have been used to enrich signals and high-content catalysts, thereby enabling signal measurement and tracing of CL detection. This greatly increases the number of detectable markers associated with a single biometric event, further increasing the sensitivity and reducing the detection limit, thus opening the door to many highly sensitive analytical diagnostic studies.

The good biocompatibility of these nanomaterials allows labelling probes that use them the advantage of usability in organic environments. Information about cellular and single molecule events that drive many biological functions can be easily measured in actual living systems when the signal probe is both biocompatible and on the nanometer scale. Some nanomaterials can be used to load a large number of biological signal markers such as enzymes for enzyme-catalyzed CL reactions for signal amplification. A gold nanoparticle-based bioconjugate of HRP having a high molar ratio was used to detect antibodies for signal amplification. Under a sandwich immunoassay, HRP-triggered CL signals captured on each sensory cell are collected by a charge coupled device for simultaneous measurement of the combined diagnosis of biomarkers and certain tumor markers. As a proof of concept, immunosensor arrays were used to detect AFP, CA 125, CA 153 and CEA. This method, reported by Zong et al.,[30] showed a broad linear range of more than 5 orders of magnitude and a lower detection limit compared to previously report multiplex immunoassays.

Nanoparticles (NPs) can serve as innovative and powerful new tags whose properties can be controlled and modulated in a predictable manner to meet the requirements of

specific applications. Pal and Bhand[31] demonstrated an ultrasensitive hybrid sandwich immunoassay for the determination of CEA in human serum based on functionalized zinc oxide nanoparticles (ZnONPs) probes. Sun and co-works[32] have developed a new SiC nanoparticle (SiC NPs) - based CL probe for the selective and sensitive detection of ·OH in PM2.5. The mechanism for sensing ·OH was based on radiative recombination of $\cdot OH^-$injected holes and electrons in SiC NPs to produce excited SiC NPs that emitted photons to produce a strong CL phenomenon.

As previously mentioned, nanomaterials can not only act as enzyme carriers, but also act as mimic enzymes to catalyze CL reactions. This property can also contribute directly to high-sensitivity CL detection systems and provide an opportunity for their development. Our research group[33] reported a novel concept in chemiluminescence imaging nanozymes immunoassay (CINIA) in which nanozymes are used as catalytic labels (Fig. 7). In this work, CuSNPs were first proposed as mimetic catalytic tags in the CINIA method to achieve multiplex detection using a combination of CL array sensors and cooled low-light CCDs. Two nanozyme probes were prepared by direct conjugation of CuSNPs to specific secondary antibodies for sensitive detection of two chicken cytokines. CINIA provides a new universal nanozymes-labelled multiplex immunoassay strategy for high-throughput detection of relevant biomarkers and further disease diagnosis.

MNPs have low catalytic activity, which limits their use in CLIA. The work of Yang et al.[34] potentially widens the applicability of MNPs as a CLIA label. This work found that $K_4Fe(CN)_6$ can improve the catalytic activity of Fe_3O_4 MNP in a luminol CL reaction (Fig. 8). It is interesting to note from this study that potassium ferrocyanide reacts with MNP, resulting in the *in situ* formation of Prussian blue. The resulting Prussian blue showed a high catalytic activity for the luminol CL reaction.

CRET takes advantage of the energy transfer process of the non-radiative dipole-dipole generated by the CL material participating in the redox reaction as an energy donor when it is very close to the energy acceptor. Compared with fluorescence resonance energy transfer, CRET does not require excitation of the light source, which reduces the interference of the background light. Liu et al[35] proposed a novel CRET-based nanoprobe for in vivo imaging of drug-induced alkaline phosphatase (ALP). As shown in Fig. 9, a nanoprobe called MSN @RhB @ b-CD @ AMPPD contains three segments. AMPPD (specific CL substrate for ALP) was selected as an energy donor and the energy receptor rhodamine B (RhB) was loaded into mesoporous silica nanoparticles (MSN). The probe has excellent sensitivity and specificity, is able to monitor ALP levels in real time, and can subsequently directly evaluate drug-induced liver damage.

In the context of CL analytical strategies, nanomaterials can play a variety of roles, acting as carriers to load large quantities of matter and also as reporter labels. Bi and co-workers[36] reported a synergistic enhanced chemiluminescence (SECL) strategy (Fig. 10). Magnetic nanoparticle (MNP)-based multiple nanoprobes (CuS / DNA / Au / DNA / MNP) were used both as DNA molecular carriers and reporter markers. A luminol-H_2O_2-Cu^{2+}-Fe^{3+}-SECL system was implemented, which was achieved by these multi-component nanoparticle

probes. The combination of the SECL method and MNP amplification enables detection of specific DNA sequences and Ramos cells.

Nanomaterial-based in homogeneous CL detection

Homogeneous CL detection takes the luminescent signal of the bulk solution and so forgoes the separation step seen in the previous applications. If the apparent properties of some nanomaterials can be greatly enhanced or significantly reduced after combination regardless of the surrounding medium, it is only then necessary to determine the degree of increase or decrease in the apparent performance of the entire solution after the reaction, reflecting the amount of the bound analyte. Homogeneous CL is therefore relatively simple operationally but is susceptible to interference from complex samples.

Precious metal nanoparticles, especially gold nanoparticles, have high electron density, display dielectric and catalytic properties, and can bind to a variety of biomacromolecules without affecting their biological activities. Because their preparation methods are typically simple and rapid, with uniform and controllable particle size along with high stability and dispersity, it is easy to obtain a stable nanocomposite by functionalization of a macromolecule containing a -SH functional group reagent and a protein. Therefore, gold nanoparticles have become the most commonly used nanomaterials in homogeneous CL research.

Qi et al.[37] designed a gold-nanoparticle-based CL system for label-free and homogeneous DNA hybridization detection that took advantage of changing catalytic properties of different forms of a nanomaterial. They found that aggregated gold nanoparticles have much higher catalytic activity towards the luminol-H_2O_2 CL system than individual nanoparticles. Single stranded oligonucleotides prevent AuNPs aggregation in 0.5 M NaCl while double-stranded oligonucleotides do not, so hybridization that occurs between a probe DNA and target DNA strand can result in aggregation AuNPs, creating a strong CL emission. The assay used here is notable for not requiring any covalent functionalization or immobilization, making the analysis very straightforward.

Zhou and co-workers[38] developed a CL assay based on AuNPs for DNAzyme with low background noise in homogeneous solutions and without the need for separation (Fig. 11). In the analysis, the target molecule was recognized by the ligated DNA. This recognition disrupted the hybridization and released the DNAzyme moiety from the surface of the nanoparticle into the solution. The DNAzyme half bound to the cofactor hemin and was converted to a catalyzed hemin/G quadruplex structure that uses luminol oxidation as a CL signalling. Qi et al.[39] reported a CL method based on label-free AuNPs for propranolol determination. The interaction of AuNPs with propranolol changed the surface charge properties of AuNPs and induced the aggregation of AuNPs, and the AuNP pair then produced strong CL signals after aggregation. Covalent functionalization of AuNPs was also not required during this assay. This assay was also a homogeneous system that avoided the standard separation and washing steps. This CL method based on AuNPs has the advantages of being simple, inexpensive, fast and sensitive.

In addition to precious metals, other materials have also been explored for their special properties for homogeneous CL detection. For example, Lin et al.[40] demonstrated for the first time that g-C_3N_4 nanosheet (NS) can directly and efficiently quench the CL emission of the luminol-H_2O_2 system by photoinduced electron transfer from the excited state luminol to g-C_3N_4 NS (Fig. 12). Based on this new discovery, a label-free and homogeneous CL aptasensor platform was designed for sensitive detection of detect CEA and ATP. The process was in a homogeneous liquid phase without any labelling and modification, which was easy, cost effective and avoided cumbersome separation procedures. Chen et al.[41] developed a homogeneous CL sensing system based on graphene oxide, using the ultra-high CRET quenching efficiency of graphene oxide and the amplification strategy that utilized exonuclease III-assisted target recycling. This assay was a homogeneous CL system and occurred in the liquid phase, which made it easy to automate through standard robotic manipulation of the microplate. This assay also avoided any separation and washing steps.

Conclusions and perspectives

In this review, recent developments in nanomaterial-based CL detection and its applications have been comprehensively summarized. As can be seen from the above discussion and our literature survey, nanomaterials play a diverse and important role in CL detection and are widely used in various fields by immunological or hybridization assays. We attempted to classify and discuss nanomaterial-based on CL analysis, and expect that readers can get meaningful information more clearly based on classification. In this connection, we have discussed the existing problems and prospects.

- A variety of nanomaterials with peroxidase-like activity have been explored but so far have been limited to implementation in colorimetric assays. Nanozymes have the potential to replace natural enzymes in CL systems due to their excellent catalytic activity, low cost, high stability, and easy regulation. A variety of nanomaterial-related properties could serve to make CL detection with these nanozymes more widely attractive: 1) a CL system can be catalyzed by the peroxidase activity of nanomaterials; 2) rapid separation by the magnetic properties of some nanomaterials can reduce the analytical burden; 3) modification of the nanomaterial by functional groups or polymers can provide strong ability to capture the target and increase its specificity for the analyte.
- The detection and application of CL in in vivo optical imaging has up to now been severely hindered, and the development of CL imaging in situ has been slow, largely due to its short wavelength and low CL intensity. However, unlike in situ fluorescence imaging, CL does not involve additional light sources and does not cause photo damage to tissues. Fluorescence also generally has the problem of strong background fluorescent signal interference, while CL assays can detect with high signal-to-noise ratio and are not affected by the autofluorescence of the enzyme or tissue background. Therefore, there is high potential upside in developing CL imaging detection strategies for in situ applications. CRET can be efficiently transferred to matched materials to produce longer wavelengths and higher CL intensity. However, it is necessary

to carefully control the stability of nanomaterials to harsh conditions such as extreme pH, temperature, and salt, as well as limit biological toxicity and aggregation. It is expected that CL, when combined with biocompatible and signal-amplifying nanomaterials, can achieve sensitive, accurate and highly specific in-situ CL imaging methods.

- Integrated nanomaterial-based CL detection could be a primary strategy for multi-analyte determination. Due to the rapid development of microfabrication technology, nanomaterials could be assembled into microchips or microarrays, and then measured by CL imaging to develop miniaturization, integration, integration and automated analysis.

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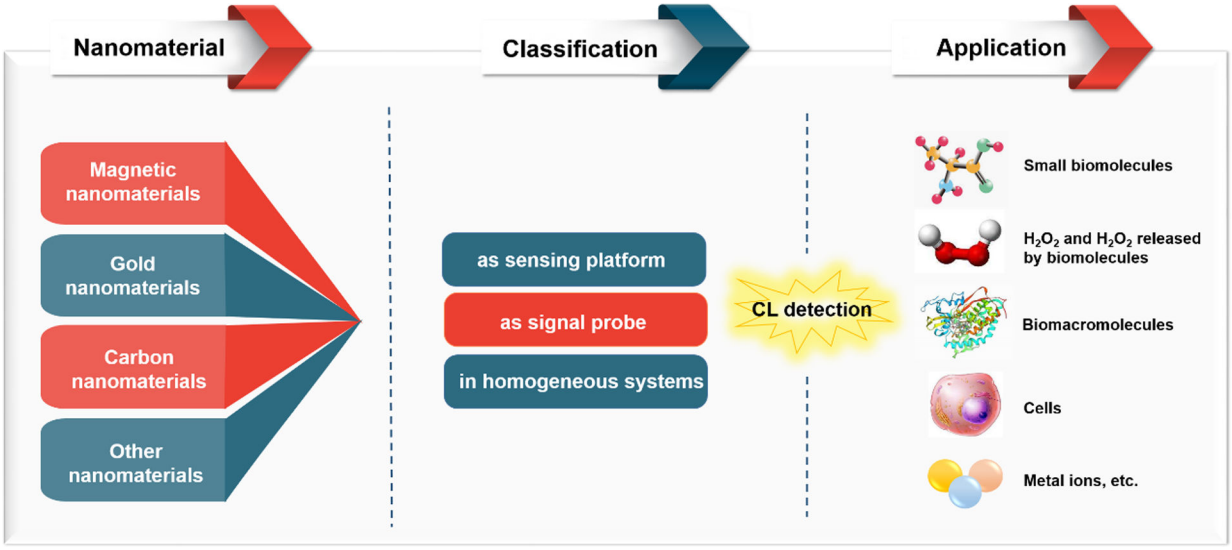

Fig. 1.
Scheme showing classification and application of the nanomaterial-based CL detection.

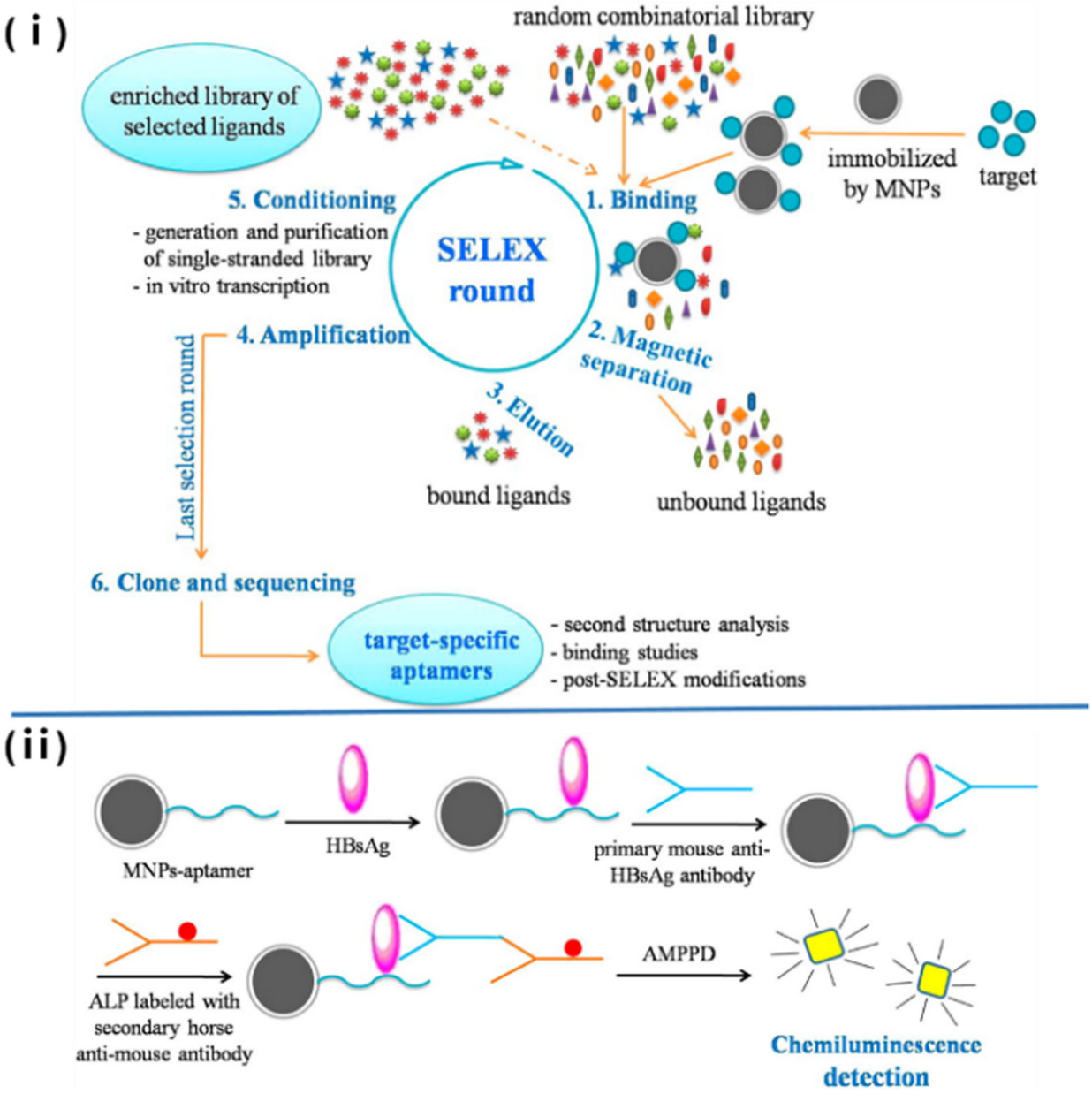

Fig. 2.

In Vitro Selection of target-specific aptamers based on magnetic separation by SELEX (i); schematic representation of the construction of a CL aptasensor based on magnetic separation and immunoassay (ii).[14]

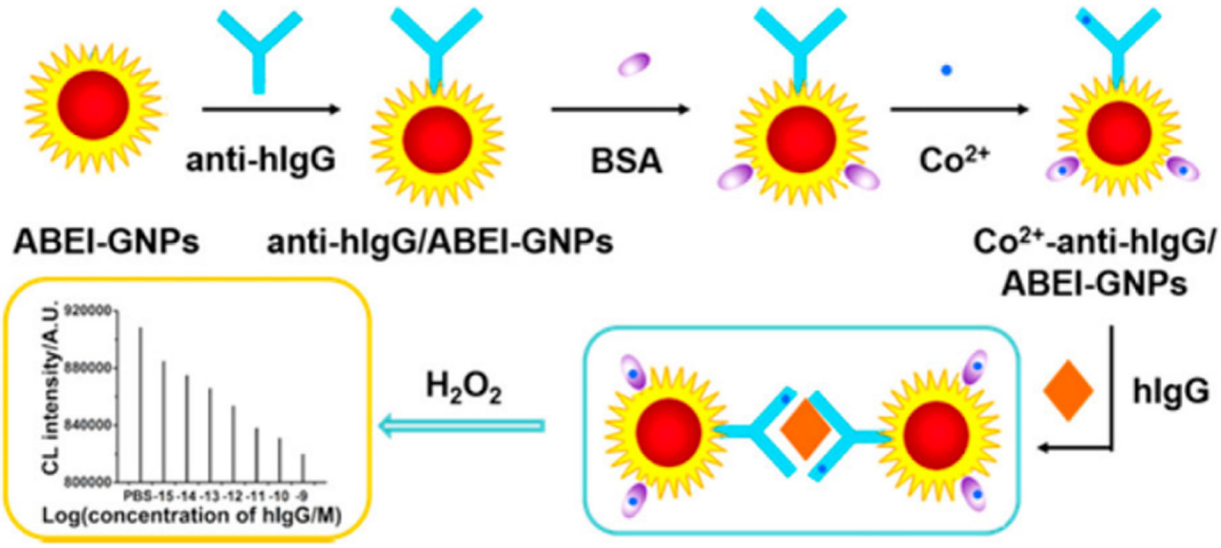

Fig. 3.

Schematic for fabrication of Co^{2+}-BSA / anti-hIgG / ABEI-GNPs nanocomposites.[19]

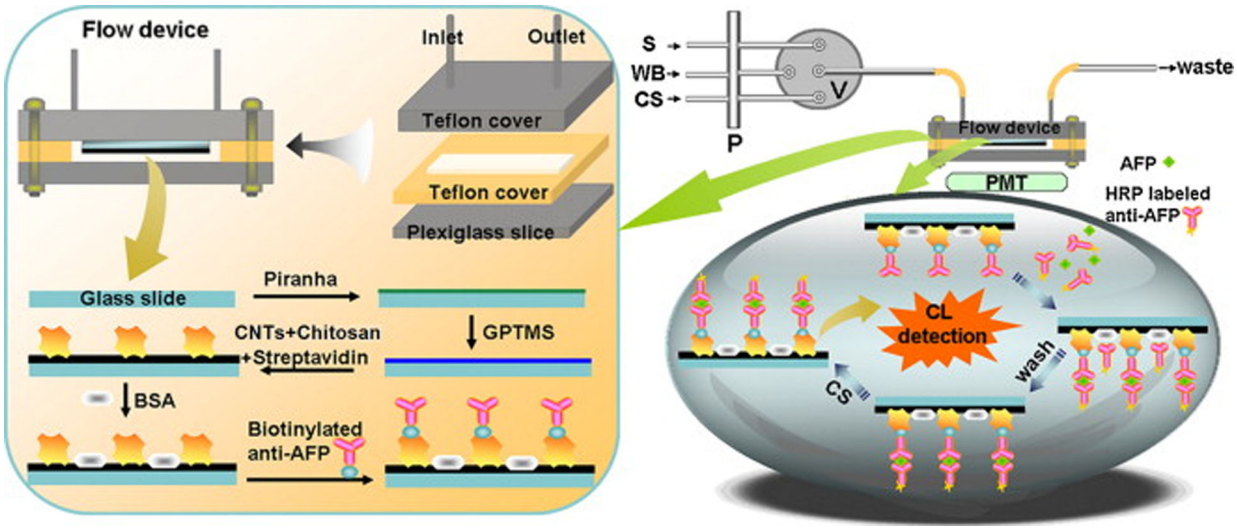

Fig. 4.

Schematic illustration for fabrication process of the immunosensor and flow-through CL immunosensing system for AFP. (S) Sample, (WB) wash buffer, (CS) CL substrate for HRP, (P) peristaltic pump, (V) multiposition valve, (PMT) photomultiplier.[22]

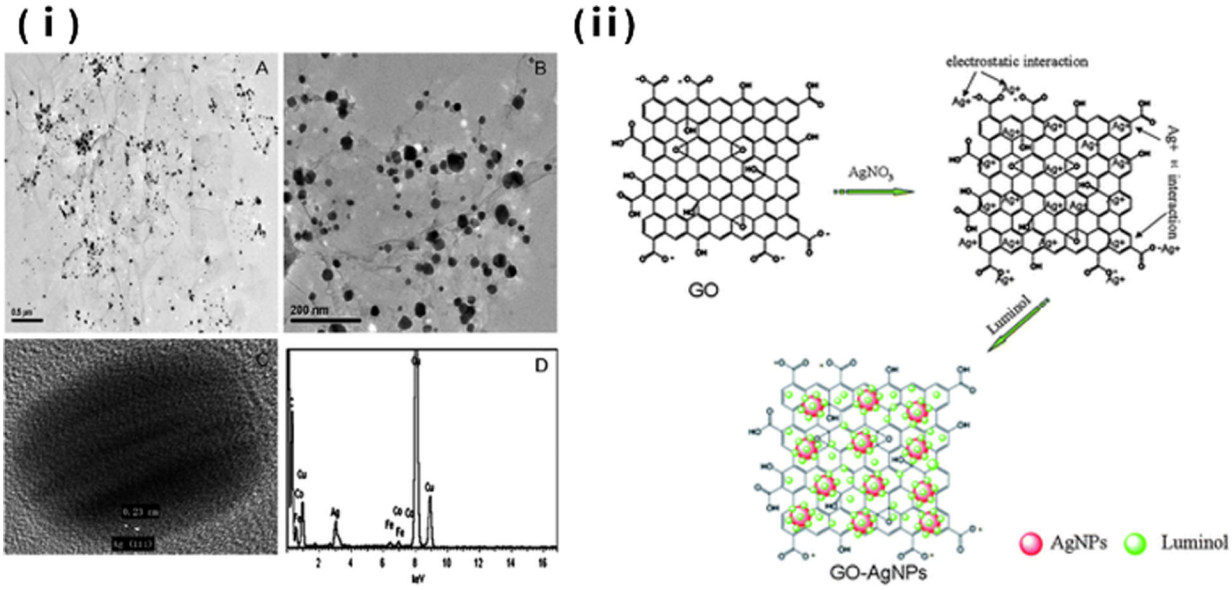

Fig. 5.

TEM images of GO-AgNPs nano-composites (A, B) at different magnifications. The HRTEM image of GO-AgNPs nano-composites (C). EDS pattern of GO-AgNPs nano-composites (D) (i); A possible formation mechanism for GO-AgNPs nano-composites (ii).[26]

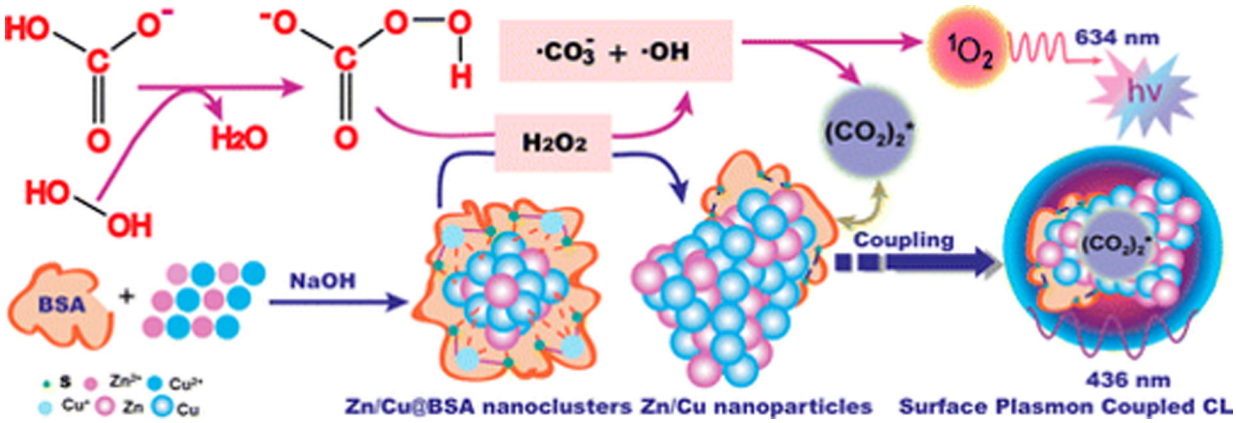

Fig. 6.

Schematic illustration of the CL mechanism of the $NaHCO_3$–Zn/Cu @ BSA NCs–H_2O_2 system.[28]

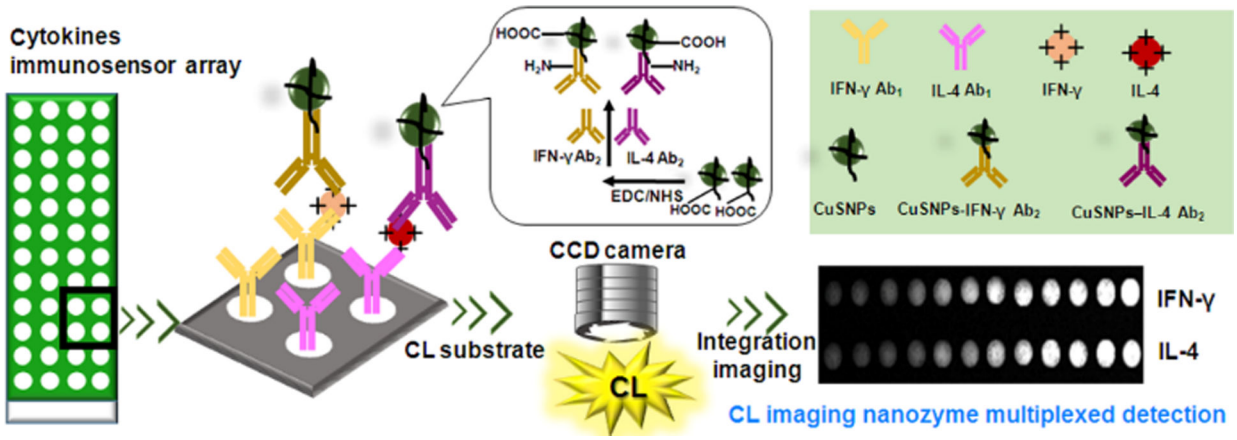

Fig. 7.

Schematic illustration of CL imaging nanozyme immunoassay of multiple chicken cytokines and immunosensor array with 4×12 sensing cells. Each column can be used for simultaneous detection of up to four targets in a single sample.[33]

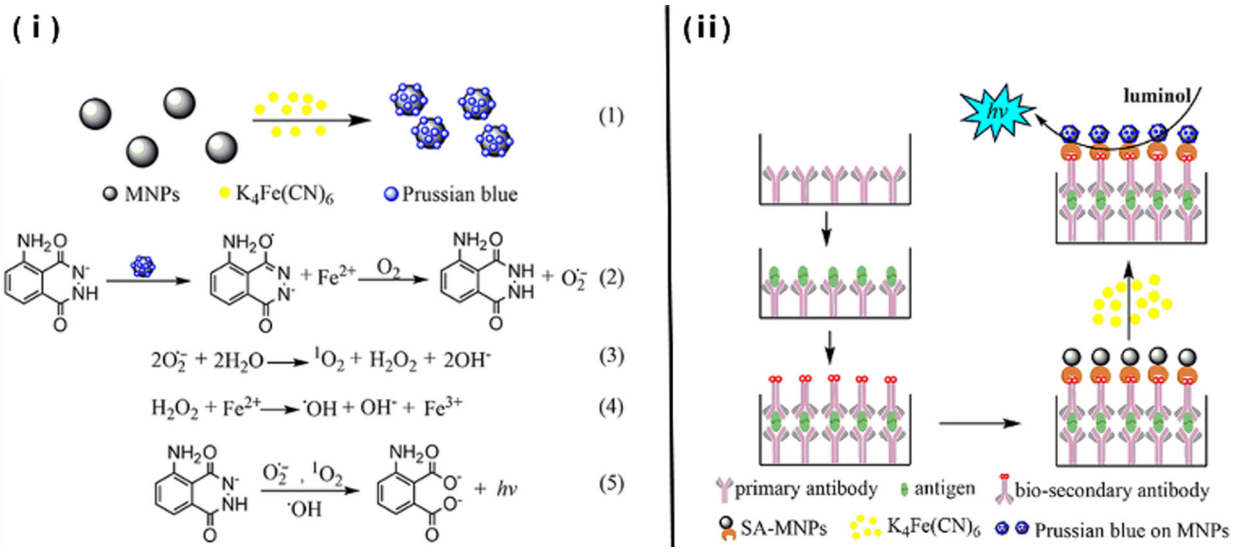

Fig. 8.

Possible mechanism of the $K_4Fe(CN)_6$-mediated luminol-MNPs CL reaction (i); Schematic Illustration of the establishment of CLIA (ii).[34]

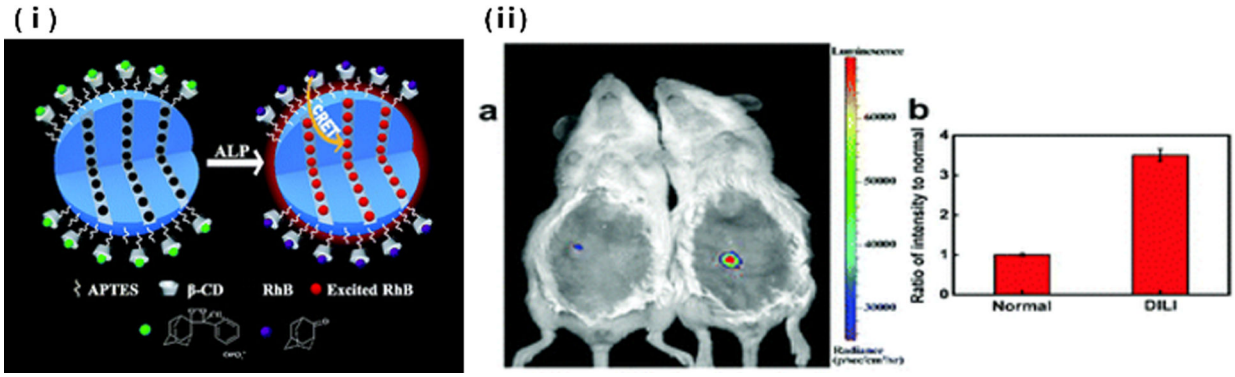

Fig. 9.

Schematic illustration of the nanoprobe MSN@RhB@β-CD@AMPPD for the recognition of ALP and the structure of AMPPD (i); (a) CL real-time imaging of ALP in normal (left) and DILI (right) mice after the nanoprobe administration using an IVIS Lumina II in vivo imaging system with an open filter. (b) Quantitative CL intensities of normal and DILI mice (ii).[35]

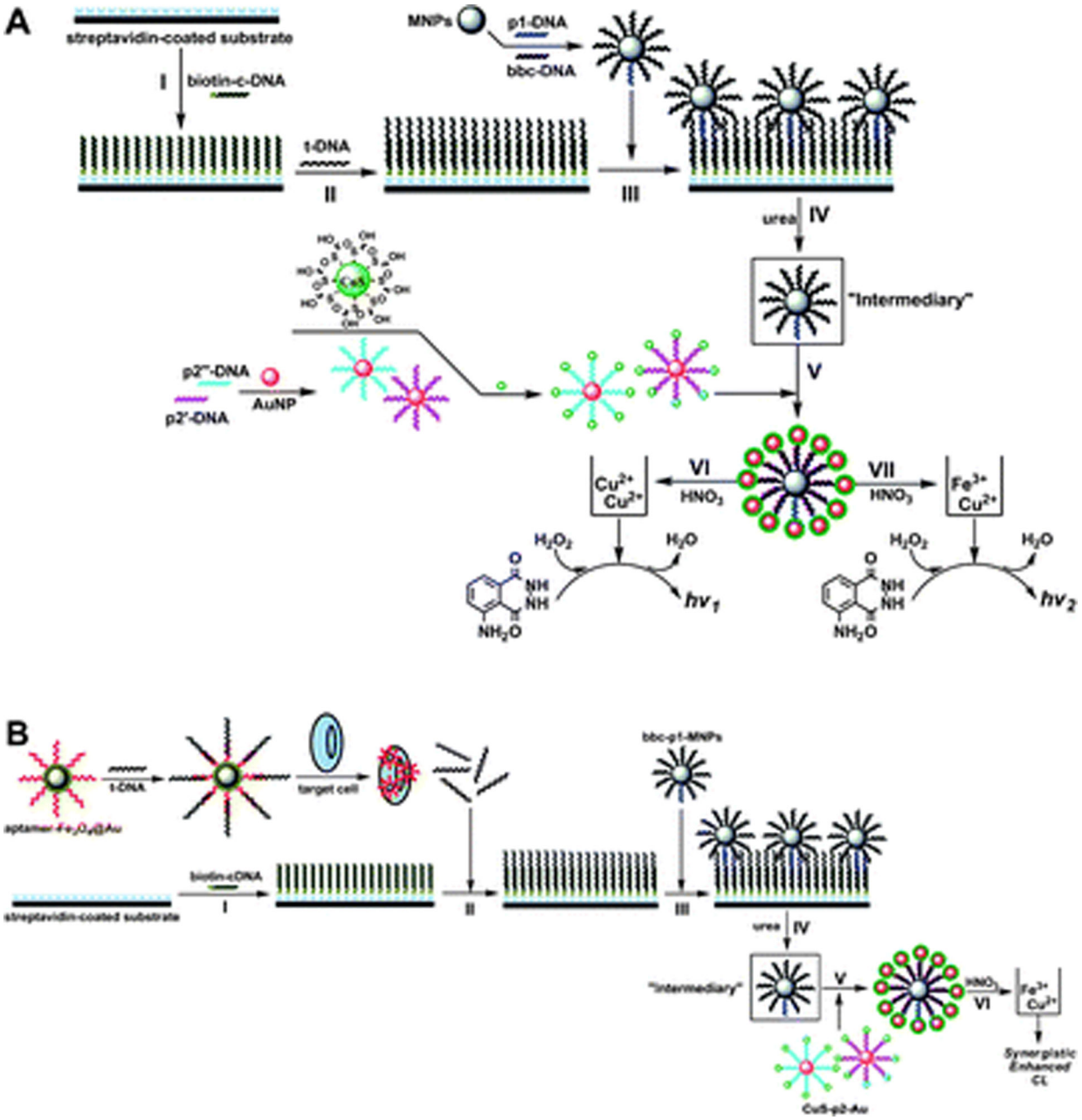

Fig. 10.

Schematic representation of SECL detection of DNA hybridization and Ramos target cells based on a dual-amplification strategy.[36]

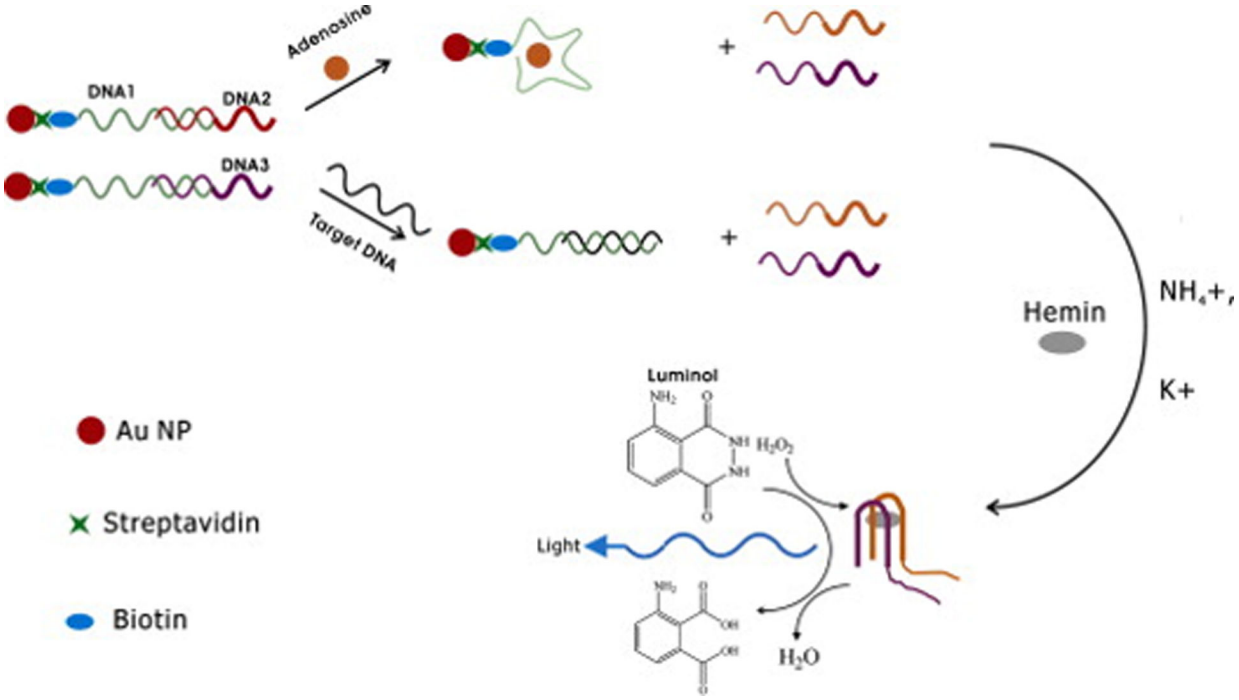

Fig. 11.
The design of DNAzyme-based aptasensor and DNA sensors.[38]

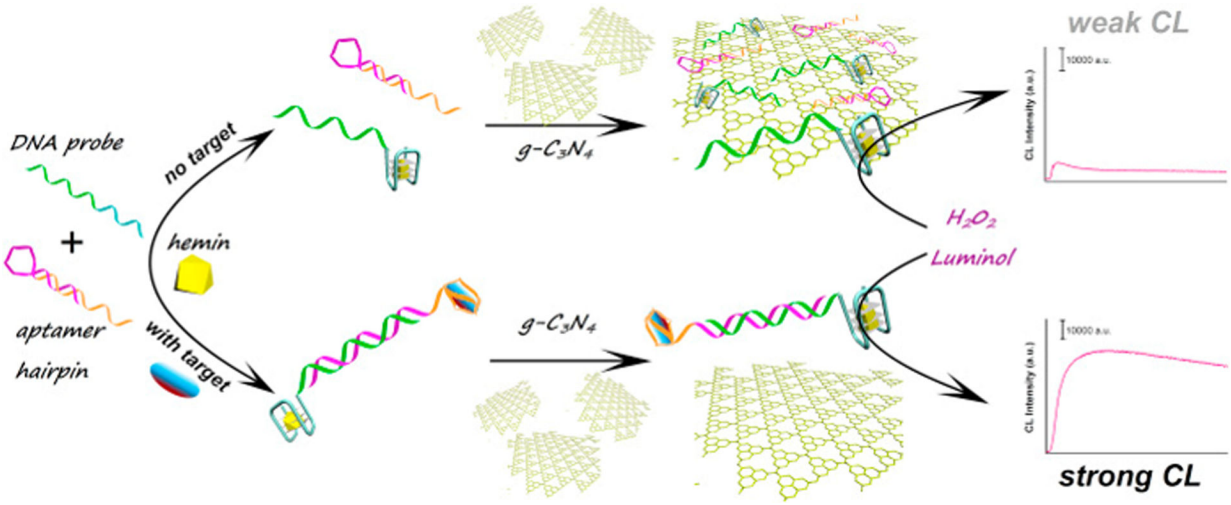

Fig. 12.

Schematic illustration of the g-C_3N_4 NS based luminol-hemin/G-quadruplex DNAzyme-H_2O_2 homogeneous CL aptasening platform for biomarkers detection.[40]

Table 1

Some typical examples of nanomaterial-based CL detection and their applications

Classification	Nanomaterials	Analytes	Applications	Ref.
as sensing platform	Magnetic nanoparticles (MNPs)	hepatitis B antigen	Serum samples	14
		Deoxynivalenol	Maize and wheat extract solution	42
	Graphene oxide (GO)	HIV sequence		43
		H_2O_2		24
		microRNA-let-7a		15
		ChIFN-γ	Chicken serum samples	25
	GO/Ag NPs	glutathione		26
	GO/Au NPs	AFP	Serum samples	27
	Carbon nanotubes	AFP	Serum samples	22
	Au NPs	human IgG	Serum samples	19
		human IgG	Serum samples	44
		AFP; folic acid	Serum samples; Milk Powder	10 (f)
		thrombin		20
		pyrophosphate ion	Plasma samples	45
	Au NPs/Metal−Organic Gels (MOGs)	Ethoprophos	Tap water and river water	46
	Au/Fe_3O_4 NPs	C-reactive protein	Serum samples	16 (a)
		fumonisin B_1	Corn and wheat samples	47
	CuO NPs	CEA	Serum samples	48
	CuS NPs	AFP	Serum samples	29
	Zn/Cu bimetallic nanoclusters	H_2O_2	Water samples	28
	Cu/Co bimetallic nanomaterials	CCRF-CEM cells	Serum samples	49
as signal probe	MNPs	Rabbit IgG	Rabbit serum samples	34
		Single-nucleotide polymorphisms and thrombin	Serum sample	50
		DNA sequences and Ramos cells	Blood samples	36
	Au NPs	AFP, CA 125, CA 153, CEA	Serum samples	30
		Cu(II) ions	Water and plasma samples	51
		ChIL-4, ChIFN-γ	Serum samples	52
	SiO_2 NPs	Staphylococcal and enterotoxin B	Milk, water and serum samples	53
		AFP	Serum samples	54
		Drug-induced ALP	Mice	35
		H_2O_2	RAW264.7 macrophages and mice	55
		cTnI, CK-MB, Myo	Serum samples	56
	TiO_2 NPs	Human IgG	Serum samples	57
	MnO_2 nanoflowers	Chlorpyrifos	Mock Samples	58

Classification	Nanomaterials	Analytes	Applications	Ref.
	Ag NPs	H1N1 influenza virus		59
	CuO NPs	HBs Ag	Serum samples	60
homogeneous system	Au NPs	Molecules adenosine and DNA		38
		histone	Serum sample	61
		IgG	Serum samples	62
		propranolol	Human urine samples	39
		DNA		37
		human IgG	Plasma samples	63
	GO	DNA		41
		CEA	Serum samples	64
	g-C_3N_4 nanosheet	CEA	Serum samples	40