Evolution of Nanomaterial Electrochemiluminescence Luminophores Towards Biocompatible Materials

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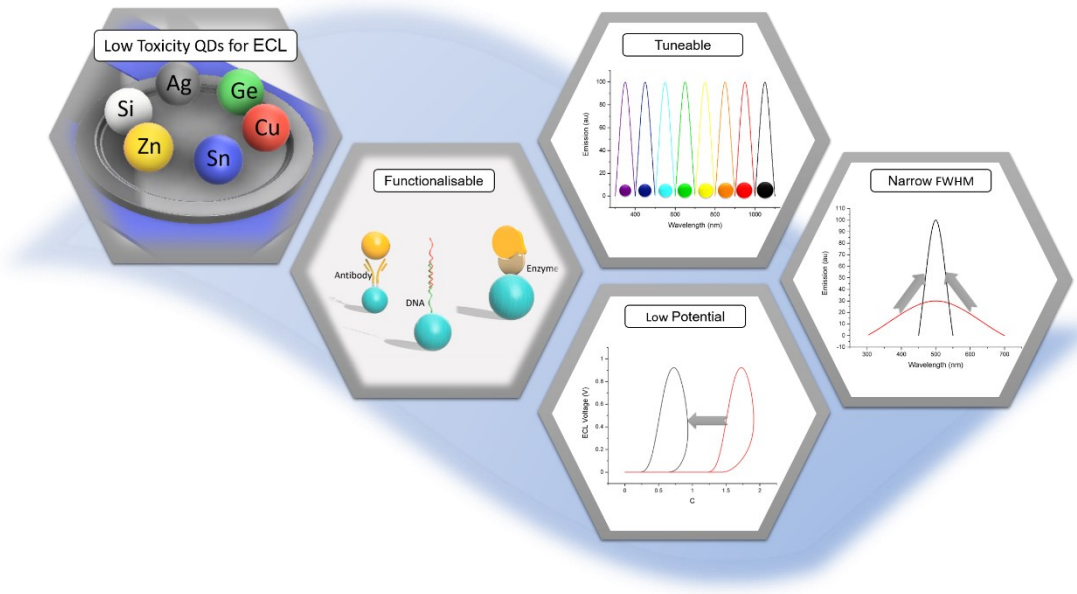
Abstract



Electrochemiluminescence (ECL) is a powerful electrochemical technique for the detection and quantification of molecules both synthetic and biological in origin. Traditional ECL luminophores are based on organic or organometallic compounds, however nanoparticle-based materials offer the benefits of tuneable wavelengths and narrow emission profiles. Materials based on cadmium have been the most extensively studied ECL nanoparticle to date. Cadmium based nanoparticles exhibit high levels of toxicity thereby impacting their suitability for mass produced sensing

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applications. As such alternative materials with reduced toxicities are required. This review focuses on the innovations and applications of low toxicity semi-conductor quantum dots (SCQDs) utilised as ECL luminophores within biosensors. These materials include silver, copper, zinc, tin, silicon and germanium. This contribution presents an evaluative overview of these materials for use as ECL luminophores in terms of toxicity, tunability of emission, potential for amplification, and water dispersibility. Capacity for functionalisation and multiplexing potential is also explored.

1 Introduction

Electrochemiluminescence or electrogenerated chemiluminescence (ECL) occurs when light is emitted in the course of an electrochemical reaction.[1] In recent times, ECL has gained increased popularity as an analytical tool due to the unique combination of electroanalytical and spectroscopic data it provides, and the respective advantages that each of these can offer. Electroanalytical techniques are cost-efficient and adaptable with wide linear ranges.[2] ECL offers the sensitivity of photonic detection without requiring an excitation light source,[3] thereby providing high sensitivity alongside temporal and spatial control, a significant advantage for analytical applications.[4] In addition ECL based platforms have been commercialised by Roche Diagnostics[5] and MesoScale.[6]

The main component of any ECL sensor is the ECL luminophore which emits light upon interaction with a target analyte, typically via the co-reactant pathway. The vast majority of luminophores are based on ruthenium complexes, specifically tris(bipyridine)ruthenium(II) chloride ($[Ru(bpy)_3]^{2+}$).[7, 8, 9] Extensive work has been conducted on ruthenium based ECL biosensing and similar complexes, including aptasensing and immunosensing.[10, 11] In more recent times, cadmium based nanoparticles (NPs) have begun to rival ruthenium due to their narrow emission bandwidths and tuneable emission profiles.[4, 12, 13, 14] However, both materials exhibit high toxicity which has raised concerns about their application to mass production of point of care biosensing in the longer term. Therefore, researchers have begun to look at lower toxicity NPs equally capable of acting as ECL luminophores. Lower toxicity NPs are also advantageous from an environmental perspective, particularly given the move towards greener and recyclable materials in all industries. The expected increase in use of diagnostic kits will have significant environmental impacts if this issue is not addressed. This can be keenly felt with the regular use

of disposable antigen tests used during the Covid-19 pandemic. While materials such as metal nanoclusters[15, 16] and carbon nanomaterials[17, 18] have previously been investigated as ECL luminophores, semi-conductor quantum dots (SC QDs) have not received the same attention. QDs are NPs with all dimensions on the nanoscale and their diameter less than twice their Bohr exciton radius, imbuing them with quantum confinement effects.[19] Quantum confinement effects are discrete and discontinuous properties that occur due to delocalised electrons. This results in discrete energy spectra and emission, thus allowing colour tuning NPs by changing their size. Adjusting the bandgap of NPs by altering their size, shape or composition changes the energy and frequency of light emitted, resulting in different colours.[20]

In many industries, there has been a conscious move away from toxic cadmium-based NPs to less toxic heavy-metal free NPs with similar optical and electrical properties. This review describes the emergence of less toxic, biocompatible SC QDs that are suitable as ECL luminophores in biosensing.

2 Electrochemiluminescence (ECL) for Biosensing

Figure 1A outlines the fundamental methods of ECL based biosensors while Figure 1B highlights the most common ECL pathways namely the annihilation pathway and the co-reactant pathway. The annihilation pathway involves oxidation or reduction of a luminophore at the working electrode. The co-reactant pathway involves oxidation or reduction of a luminophore which undergoes electron transfer reactions with a co-reactant. Both of these processes emit light after the excited luminophore falls to the ground state.[1, 21]

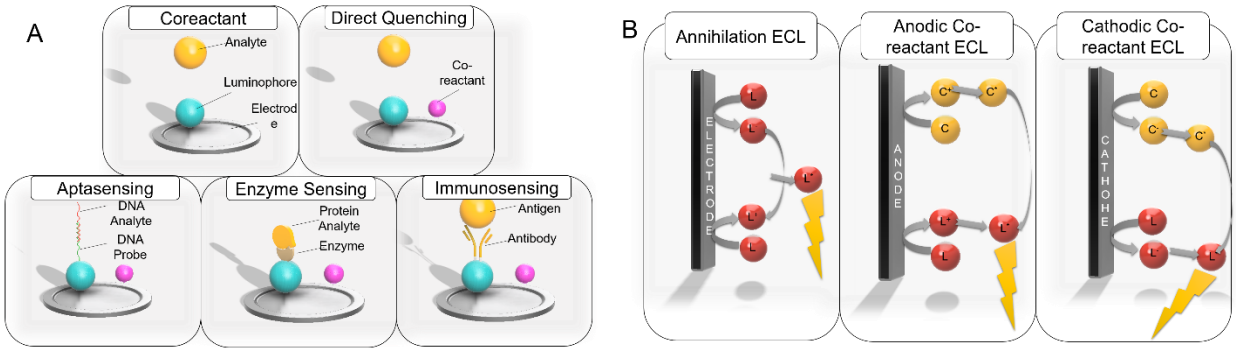

Figure 1: A. Fundamental methods of ECL biosensors and B. Succinct schematic of the annihilation, anode co-reactant and cathode co-reactant ECL where L represents the luminophore and C represents the co-reactant

The diagnostic approach taken is usually dependant on the nature of the target analyte, and can include bio-recognition agents such as DNA,[22] enzymes,[14] antibodies and / or aptamers.[23] For the vast majority of these systems, the co-reactant pathway is employed, wherein the energetics of the interaction between the target analyte and the ECL luminophore is utilised for quantitative analysis. Competitive processes can also be exploited when using the co-reactant pathway, resulting in a quenched response which is proportional to analyte concentration. This has been shown to be effective for small molecules such as dopamine,[24] amino acids[25] and metal ions.[26] Table 1 highlight the different ECL parameters as well as a brief description of each mechanism.

Table 1: Summary of different mechanisms for ECL biosensing

Mechanism	**Bio-recognition Agent**	**Target**	**Description**
Co-reactant Mechanism	None	Small molecule	The analyte acts as the co-reactant, thus the luminescence will be directly proportional to the concentration of the analyte.
Direct Quenching	None	Small molecule	The analyte interferes with a co-reactant-luminophore reaction and quenches luminescence. Thus, the luminescence is inversely proportional to the luminescence.
Aptasensing	DNA	DNA	Single strands of DNA are attached to the surface of the luminophore which are used to selectively interact with the target DNA.[27]
Enzyme Sensing	Enzyme	Protein	Enzymes are used to selectively detect proteins. Oxidases such as glucose oxidase and cholesterol oxidase can be used to produce H_2O_2 which acts as a co-reactant in many ECL systems.[28]
Immunosensing	Antibody	Antigen	The antibody selectively interacts with the antigen. The luminophore can be labelled

			with the antibody or competitive (non-labelled) immunoassay can occur.[29]

2.1 Nanoparticle Luminophores

More recently, to expand the potential applications of ECL based biosensors and facilitate potential multiplexing which has proven challenging for organometallic complexes, research into nanoparticles (NPs) as alternative ECL luminophores has gained traction. This is primarily due to the unique properties that NPs such as QDs possess. By controlling the particle size and composition, the wavelength of ECL emission can be altered, through quantum confinement effects.[4, 13] Furthermore, QDs have properties such as good quantum yields (QYs), a resistance to photobleaching,[3] ability to functionalise with bio-recognition agents and low production cost.[13]

When considering the properties of NPs for biosensing applications, it is important to differentiate between the properties exhibited by an isolated single NP and the properties of multiple NPs used together. In biosensing, the latter is typically used for analytical applications. Single particle NP ECL has been explored and offers the potential to retain the inherent benefits of NPs. Single-molecule spectroelectrochemistry (SMS-EC) has been reported for NPs including Au NPs, Pt NPs and $Ru(bpy)_3^{2+}$-SiO_2 NPs. From a biosensing perspective, single molecule events are desirable due to their high sensitivity but more complex to achieve.[30, 31] This is an emerging field and there is much work to be done to exploit the potential of single particle ECL. Challenges facing this technique include the synthesis and characterisation of single particles due to their nano-size as well as difficulties associated with measuring tiny currents and charges.[32] Therefore, the rest of this review will discuss multiple NPs used together in biosensors.

For an NP employed as a transduction material in an ECL biosensor, a typical approach is to immobilise the NPs on or close to the electrode and functionalise with a biorecognition agent. This is specifically relevant to quenching based ECL. Alternatively, in a sandwich immunoassay, the luminophore is tethered close to the electrode through interaction with selective biomolecules and the target analyte. The functionalisation of a NP with a biorecognition agent can have subsequent effects on the luminescence as these large molecules often have low conductivity and large steric hindrance.[23] The biorecognition agent could also increase the distance between the NP and the electrode or the NP and the co-reactant which can affect the emission. Functionalisation of the NP

with biomolecules mostly uses the physical adsorption method but can also be achieved through encapsulation, cross-linking and covalent bonding.[16, 33, 34] These methods, which chemically alter the surface of the NP are likely to affect the emission as ECL is a surface state phenomenon.

Immobilisation involves tethering the NP to the working electrode (WE) through ligands, conductive polymers, microbead loading and nanocomposites as outlined in Figure 2.[13] Immobilisation of the NP at the WE ensures that a high concentration of NP is confined within the diffusion layer of the electrode resulting in enhanced electron transfer through the conductive immobilisation compound. This causes increased luminescence due to the proximity of the NP to the electrode. This is a concern for NPs that exhibit a highly water-soluble nature which tend to migrate from the surface of the WE and into the solution, often diminishing the ECL produced.

Different conductive polymers such as nafion[35] and chitosan[36] can be used to immobilise the ECL NPs to the electrode surface. The micro-structured environment in which NPs are encapsulated can result in an enhancement or inhibition of desirable NP properties. The quantity of NPs can also have an effect as high concentrations of NPs, followed by subsequent agglomeration, can result in self-quenching for many systems. Single NPs have a higher quantum yield (QY) than an agglomeration of NPs due to reduced surface area and the size distribution of the NPs can also result in broadened emission, with different sized NPs emitting at different wavelengths. A single NP will have very sharp, pronounced emission but with a high concentration of NPs, the wider the size distribution, the wider the emission profile observed.[37]

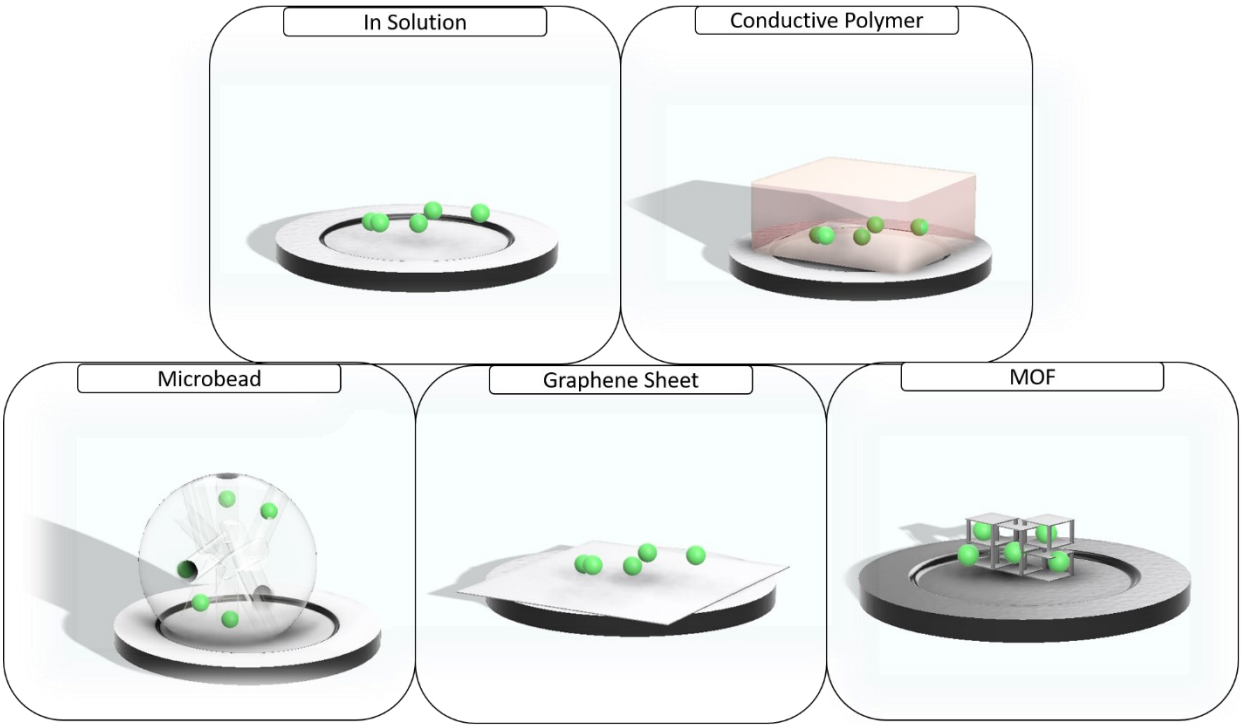

Figure 2: Luminophores in different microstructured environments

Numerous studies have examined the impact of surface confinement on the bulk compound, $Ru(bpy)_3^{2+}$.[38, 39] These studies indicate that careful consideration of a suitable polymer, luminophore distribution and optimum charge transfer rates are required for optimum ECL efficiency. These studies should be replicated for NPs to investigate the effect of the surface confinement.

2.2 Cadmium NPs as Luminophores

Since Bard's pioneering work on CdSe QDs for ECL,[12] there has been an exponential increase in cadmium containing materials reported for ECL applications. Cadmium based NPs, however, are highly toxic, a significant drawback that limits their application in mass produced biosensors. CdX QDs, where X = S, Se or Te, have been extensively investigated for ECL applications.[40, 41, 42, 43] There have been several comprehensive reports on Cd based nanomaterials for ECL, highlighting the advantages of these materials, including high QYs, narrow and tuneable emission and low oxidation potential.[3, 4, 13, 30, 44] A key advantage of using Cd QDs for ECL is their high PLQYs (photoluminescence quantum yields). PL and ECL are closely related and, as there is no analogous

quantitative measurement for ECL, PLQY can be used to give an indication of the ECL emission.[45] Another advantage of Cd QDs is the wide range over which their ECL emission has been tuned. As shown in Figure 3, Cd QDs give strong, ECL emission over the visible[46] and near infrared (NIR) regions, from 650 to 900 nm.[47]

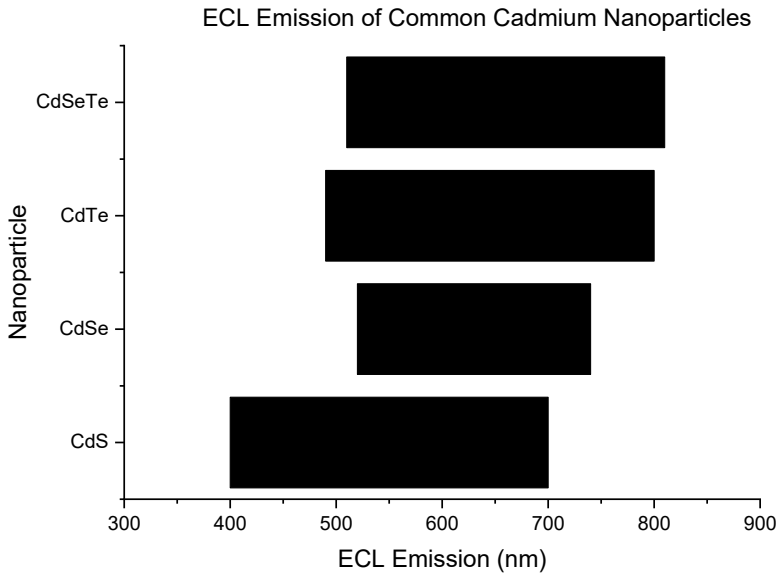

Figure 3: ECL emission wavelengths of common cadmium nanoparticles including CdS,[33, 48] CdSe,[12, 46] CdTe[49, 50] and CdSeTe[21,31]

Tuneable ECL is achieved in QDs due to quantum confinement effects which are discrete and discontinuous properties in QDs that occur due to delocalised electrons. This results in discrete energy spectra and emission that can be tuned to different wavelengths through tuning the composition, size and shape of the QDs.[51] Cd QDs can be thus tuned to emit in the NIR window facilitating analysis of whole blood samples,[24] thereby offering significant advantages for Point of Care (POC) diagnostics.[52] The ability to tune ECL emission wavelengths also paves the way for spectral multiplexing.

The reported PL and ECL emissions wavelengths of various QDs are shown in Figure 4 where Cd QDs have shown ECL signals over the broadest range. Other QDs have been tuned to emit PL emission over wide ranges but the same studies have not been carried out for ECL. The PL of samples generally tends to be narrower and red-shifted when compared to the ECL. This because PL predominantly originates from band-gap transitions while ECL occurs due to both band-gap

transitions and surface state phenomena. As these QDs, especially Ag, Cu, and Si, have shown PL signals over wide ranges, their ECL should be investigated over the same ranges.

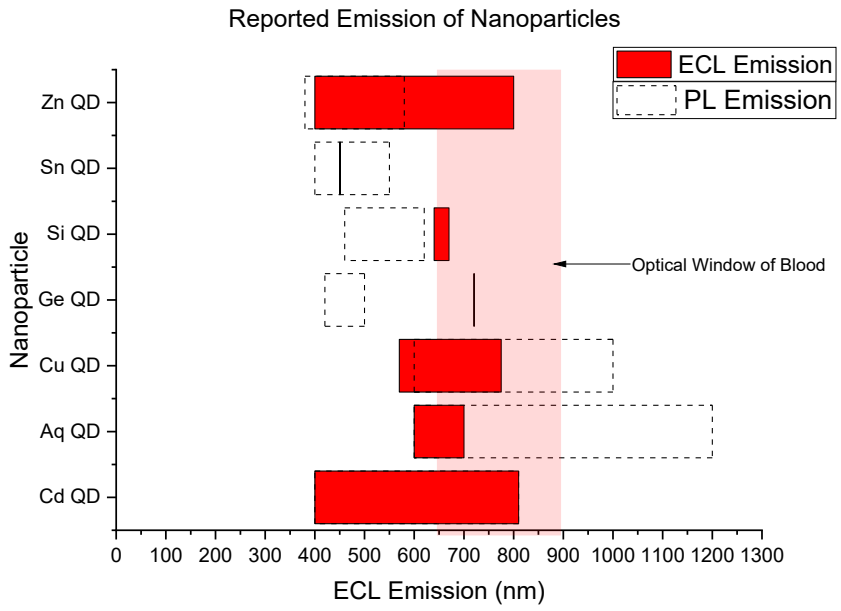

Figure 4: Reported electrochemiluminescence and photoluminescence of various nanoparticles compared to cadmium QD ECL compiled from Table 2 and Figure 3

In addition, the lower oxidation potential of Cd QDs is another favourable characteristic for ECL applications. The ECL oxidation or reduction potential is the potential at which the luminophore forms a free radical and is determined by the core NP, the surrounding ligands and the choice of co-reactant. Unexpected secondary reactions can occur at higher potentials which interfere with the selectivity of a sensor and biomolecules are susceptible to adverse conditions including high potentials. Proteins and enzymes have been shown to denature at 1.2 V *vs Ag/AgCl* in water[53] and DNA undergoes oxidative stress at similar potentials.[54] TGA-CdSe QDs have exhibited low oxidation potential at ≈ 0.9 V *vs Ag/AgCl* with TPrA[55] and sulphite.[56] Therefore, luminophores that luminesce at relatively low potentials are highly desirable for ECL biosensor applications.

The most significant limitation of Cd QDs as ECL luminophores is their toxicity. Cadmium has been shown to leach as Cd^{2+} from the nanomaterial and have cytotoxic effects on biological material. In addition, the toxicity effect is compounded by the materials size. The small size of

these materials permits them to penetrate cell walls and tissues, a phenomenon that is not observed for the bulk material.[57] Both core and core-shell QDs have been found to be cytotoxic, however factors such as dose and exposure also contribute significantly.[58] If widespread use of POC sensing is to be realised, there will be an increase in the number of sensors produced. If all of these were based on cadmium NPs, there would be serious environmental and health implications. To increase the use and research into POC sensors, low toxicity alternatives to cadmium NPs need to be investigated.

3 Existing Low Toxicity Nano-Luminophores

Some groups of low toxicity NPs have already been investigated for ECL biosensing, which are shown in Figure 5 and discussed below.

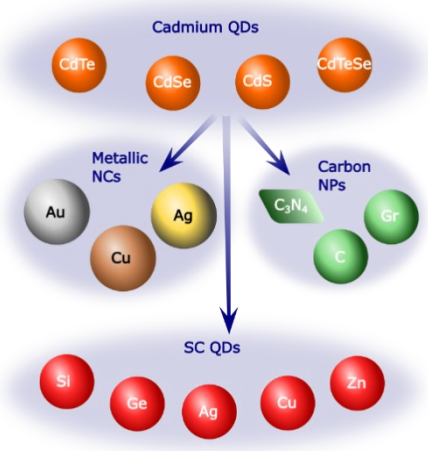

Figure 5: Evolution of nano-luminophores towards lower toxicity materials

3.1 Metal Nanoclusters

Nanoclusters (NCs), clusters of up to a hundred atoms whose size is comparable to the metal's Fermi wavelength, have previously been used for ECL applications.[15, 16, 59, 60] The most common NCs for ECL are made of Au or Ag but Cu, Pt and Pd have also been explored.[16] Water soluble NCs can be synthesised in a variety of ways including chemical/photochemical-reduction, etching or template assisted synthesis,[60-62] and have been used to detect and quantify a number of analytes such as DNA,[22] enzymes,[63] and dopamine[64]. However, the range of analytes detectable is less than Cd based ECL platforms.

The most ubiquitous example, Au_{25} (25 atoms of gold) is often used for ECL due to its quantum confinement, two-photon absorption and high quantum yield.[63] The Au NC is capped with ligands, such as glutathione[65] or N-acetyl-l-cysteine,[66] which can be bonded to DNA strands, enzymes or other labels. Au NCs have been shown to have tuneable emission wavelengths from approximately 650 nm[65] to 900 nm[66] which is highly dependent on the surface ligand. Large, well-defined organic ligands are required for NIR ECL however due to the complexity of the role of the ligand in ECL, there have been few reports on NIR ECL with Au NCs.[67]

Other NCs that have been investigated for ECL applications include Ag NCs.[68] Ag NCs, however, require high cathodic potentials which can often have unpredictable side reactions and result in over-oxidation of analytes. Ag NCs are also less stable than Au.[15] Cu NCs have only been shown to emit from 300 nm[69] to 500 nm[64] which limits their potential for ECL biosensing. Therefore, other morphologies of low toxicity NPs aside from nanoclusters such as quantum dots, nanorods, nanoparticles, have been explored for ECL applications.

3.2 Carbon Nanomaterials

Carbon nanomaterials are another NP luminophore with lower toxicities relative to Cd QDs. Carbon and graphene QDs,[13, 59, 60, 70, 71] carbon nanotubes (CNTs),[16, 30, 60, 71, 72] graphene oxide, [16, 70] carbon nitride[59, 60, 71] and fullerene[16] NPs have been used extensively for ECL applications. Carbon and graphene QDs, and in some cases carbon nitride sheets, act as luminophores themselves whereas other aforementioned carbon materials have been shown to act as ECL catalysts, employing a separate luminophore.

Carbon and graphene QDs are attractive materials for sensing applications as they are water-soluble, biocompatible and relatively easy to functionalize[72] and can be exploited for both anodic and cathodic ECL.[59] In addition, due to the well-defined variety of synthetic routes for functionalisation,[73] they have been successfully employed to quantify small molecules through direct ECL enhancement,[74] ECL quenching,[75] immunoassays[76] and aptamers.[77] Despite these advances, carbon NPs for ECL applications exhibit a limited emission range, with the minimum recorded at 300 nm[78] and the maximum at 600 nm.[73] This inhibits the application of carbon QDs

to whole blood samples as the optical window of blood is between 650 – 1000 nm as shown in Figure 6. Near infrared (NIR) QDs have shown promising results with respect to addressing this issue.[79] However, this work is in its infancy compared to other facets of ECL research.

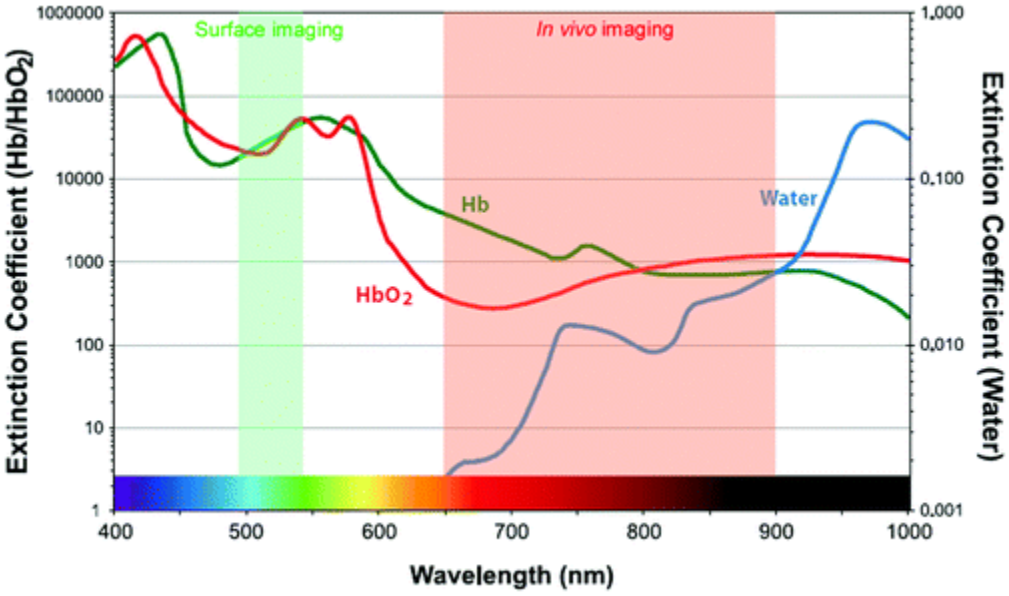

Figure 6: Extinction coefficients of water oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hb) showing window where optical interference from blood is reduced (reprinted with permission from [80]. Copyright 2010 American Chemical Society)

Carbon nanotubes (CNTs) are primarily used as an amplifier or immobilization agent in ECL applications. The relative ease with which they can be labelled with biomolecules has the potential to incur great specificity.[70] When coupled with luminophores, such as ($[Ru(bpy)_3]^{2+}$)[30] or CdSe,[72] immobilized to the CNT surface, ECL enhancement is observed. Amplification is achieved by the high electrical conductivity of the CNTs which acts to increase rates of the electron transfer.[49] Similarly, graphene oxide can be used to increase sensor performance[70] and has advantages such as high tensile strength, high electrical conductivity and low toxicity.[16]

Carbon nitride (C_3N_4) are 2D sheets similar to graphene but doped with nitrogen. They are relatively low cost, easy to make and display good electrochemical properties.[81] They are chemically and thermally stable.[71] These are also primarily utilised to enhance ECL[82] or immobilise biomolecules.[83] They have been used to detect epinephrine[84] and copper ions[81] through direct quenching as well as other analytical applications.[17, 18]

4 Emerging Low Toxicity Semi-Conductor Nanomaterials for Electrochemiluminescence

While low toxicity semi-conductor QDs have been mentioned in some review articles in recent years, they have not been reviewed exclusively and deserve more attention as an emerging type of luminophore given their potential benefits relative to other systems. Semi-conductor QD materials have successively been employed in ECL sensing applications. Table 2 collectively summaries the application of these materials in such applications.

4.1 Non Cd based Luminophores

Below is a list of non-Cd based semi-conductor nanoparticles which are less toxic that have been used as luminophores.

Table 2: List of semi-conductor nanomaterials for ECL

Luminophore	Type	Ligand	Synthesis	Amplification	ECL	ECL, FWHM (λ)	Analyte	Linear Range	Limit of Detection	Mechanism	Ref
Ag_2S-Mn	D QD	MPA	Aq	No	Cathodic	610, 105	Laminin	10^{-8} - 10^{-4} kg m^{-3}	3 x10^{-9} kg m^{-3}	Im	[85]
Ag_2S@ CdSe	NN	MAA	Aq	PPy-NH_2GO	Cathodic	NG	CA72-4	10^{-3} - 20 U/mL	2 x10^{-5} U/mL	Im	[86]
Ag_2Se	QD	L-ala	Aq	MWCNT	Cathodic	695, 75	Dopamine	NG	1 x10^{-7} M	DQ	[87]
AgInZnS/ ZnS	NP	L-cyst	Aq	No	Anodic	605, 150	microRNA141	10^{-16} - 2 X10^{-11} M	5 x10^{-17} M	Ap	[88]
AgInS, AgGaInS	NP	GSH	Aq	NA	Anodic	744, 250	PSA	5x10^{-14} – 1x10^{-9} g/mL	1x10^{-14} g/mL	Im	[89]
Zn-$AgIn_5S_8$	QD	L-cyst	Aq	NA	Anodic	NG	NA	NA	NA	C-L	[90]
$AgInS_2$/ZnS	NP				Anodic	695	CA125	5x10^{-6} – 5x10^{-3} U/mL	1x10^{-6} U/mL	Im	[91]
$CuInS_2$	NP	DDT	HU	No	Anodic	775, 150	No	NG	NG	C-L	[92]
$CuInS_2$/ ZnS	NP	GSH	Aq	No	Anodic	775, 130	VEGF gene	10^{-13} - 10^{-9} M	5 x10^{-14} M	Ap	[93]

$CuInS_2$/ ZnS	NP	GSH/ citrate	Aq	No	Anodic	731, 140	Cu(I)/Cu(II)	10^{-9} – 2 x10^{-6} M	5 x10^{-9} M	DQ	[94]
$CuInS_2$/ ZnS	NP	MBA, citrate	Aq	No	Anodic	730, 144	No	NA	NA	C-L	[95]
CuInZnS	QD	MPA, CA, GSH	Aq	Au NPs	Anodic	568, 602, 595, 150	EGFR gene	5 x10^{-11} - 10^{-9} M	4 x10^{-12} M	Ap SEECL	[96]
CuInZnS	QD	MPA	Aq	Au NPs	Cathodic	560 (PL), 70 (PL)	P53 gene	10^{-10} - 2 x10^{-8} M	3 x10^{-11} M	Ap SEECL	[97]
CuZnInS	QD	AGM	Aq	Gr	Anodic	575, 220 (RLS)	LPA	2 x10^{-6} – 8 x10^{-5} M	7 x10^{-7} M	DE	[98]
CuInZnS	QD	DDT	HI	No	Cathodic	650, 110	No	NG	NG	C-L	[99]
CuInZnS	NP	MPA	HU-LE	Au NPs + semi carbazide	Cathodic	690, 200 (PL)	FLT3 gene	10^{-18} - 10^{-12} M	10^{-18} M	Ap	[100]
Ge	NP	OC	SS	No	Annih.	720, 140	No	NG	NG	C-L	[101]
Si	NP	ODE	LP	No	Anodic, Cathodic	670, 160	No	NG	NG	C-L	[102]
Si	NP	MPO	SS	No	Cathodic	640, 180	No	NG	NG	C-L	[103]
Si	QD	MAA	MW	Au NPs	Cathodic RET	475 (PL), 75 (PL)	Target DNA	10^{-16} – 10^{-12} M	2 x10^{-17} M	Ap RET	[104]
SnS_2	QD	L-cyst	SA	Ag NF	Cathodic	450, 210	antiCMV pp65	10^{-15} – 10^{-7} M	3 x10^{-16} M	Ap	[105]
SnO_2	QD	NG	SA	MnO_2 NF, Ag NPs	Cathodic	650, 200	microRNA	1x10^{-17} – 1x10^{-10} M	3x10^{-18} M	Ap	[106]
ZnO	NP	NG	TD	MWCNT	Cathodic	600, 225	No	5 x10^{-7} – 3 x10^{-4} M	NG	C-L	[107]

ZnO	NP	NG	Aq	NA	Cathodic	NG	epinephrine	8 x10^{-10} – 2 x10^{-7} M	10^{-11} M	DQ	[108]
ZnO	NR	NG	Aq	NA	Cathodic	440 – 660	Cytochrome C	10^{-12} – 5 x10^{-9} M	5 x10^{-12} M	DQ	[109]
ZnO	D-NR	NG	Aq	BPQD	Cathodic	450 - 600	Cytochrome C	10^{-10} - 5 x10^{-7} M	10^{-10} M	DQ	[110]
ZnO	NR	NG	Aq	MoS_2 sheets	Cathodic	350 – 550 (PL)	cysteine	10^{-9} - 10^{-7} M	7^{-9} M	DQ	[111]
ZnS	NP	NG	Aq	No	Annih., Cathodic	460, 90	No	NG	NG	C-L	[112]
ZnS	NP	NG	ED	Go	Cathodic	NG	Cu^{2+}	1x10^{-10} – 1x10^{-7} M	2x10^{-11} M	QD	[113]
ZnS/Mn^{2+}	D QD	NG	Aq	No	Cathodic	570 (PL), 50 (PL)	No	NG	NG	C-L	[114]
ZnSe	QD	GSH	Aq	No	Cathodic	380 (PL), 25 (PL)	Co-reactant	6 X10^{-7} - 3 X10^{-4} M	2 x10^{-7} M	C-L	[115]
ZnSe	wz NC	OLA	HU	No	Cathodic	800, 160	No	NG	NG	C-L	[116]
$ZnIn_2S_4$	NS	L-cyst	Aq	RGO	Cathodic	551, 200	Insulin	1x10^{-13} – 8x10^{-8} g/mL	3E-14 g/mL	Im	[117]
Zn_2SnO_4	NR	APTES	Aq	Au NPs	Cathodic	555, 150	AFP	1x10^{-12} – 5x10^{-8} g/mL	3x10^{-12} g/mL	Im	[118]
Type		D QD: doped quantum dot, NC: nanocube NN: nanoneedle, NP: nanoparticle, NR: nanorod, NS: nanosheet NW: nanowire, QD: quantum dot									

Ligands		AGM: agmatine, CA: cysteamine, DDT: 1-dodecanethiol, GSH: glutathione, L-ala: L-alanine, L-cyst: L-cysteine, MAA: mercaptoacetic acid, MBA: 4-mercaptobenzoic acid, MPA: 3-mercaptopropionic acid, MPO: 1-mercoptooctane, OC: octanol, ODE: octadecyl, OLA: oleylamine, Qu: quinolinolate
Synthesis		Aq: hydrothermal, ED: electrodeposition HI: hot injection, HU: heat up, LE: ligand exchange, LP: laser pyrolysis, MW: microwave SA: self assembly, SC: sonochemical, SS: supercritical solvent, TD: thermal decomposition
Amplification		GO: graphene oxide, NF: nanoflower, MWCNT: multiwalled carbon nanotube, RGO: reduced graphene oxide
ECL		Annih.: annihilation
Analyte		LPA: lysophosphatidic acid
Mechanism		Ap: aptamer, C-L: co-reactant-luminophore interaction, DE: direct enhancement, DQ: direct quenching, Im: immunosensing

As illustrated in Table 2, there are several new materials emerging to address the issue of toxicity in ECL luminophores. The main QDs being examined are those based on Ag, Cu, Si, Ge, Sn and Zn. These exhibit essential parameters including low toxicity, narrow tuneable emission, low ECL potential, water dispersibility and potential for multiplexing.

4.2 Toxicity

As the demand for POC biosensing increases, the toxicity of biosensing materials must be considered. Low toxicity materials are important in the manufacture, use and disposability of biosensors. The toxicity of QD materials is dependent on elemental composition, particle size, shell, ligand and concentration. Elements that have higher toxicity associated with them include heavy metals such Cd and Pb. Hence, Ag, Cu, Zn, Cd and Sn QDs have received attention as ECL luminophores for biosensing due to their low toxicity as well as being environmentally benign.[98, 105, 119, 120] These low-toxicty NPs explored for ECL are given in Table 2. Non-metallic compounds have also been explored such as carbon, silicon and biopolymer nanoparticles.[121]

A strategy to reduce the toxicity of NP materials is to modify the NP with a less toxic outer shell. This can be achieved by growing the shell of another semiconductor material around the core NP. This shell may consist of a material with a wider bandgap resulting in enhanced luminescence. This strategy has also been shown to increase the stability and make the QD less prone to oxidation and photobleaching. This in turn prevents degradation and leaching of the core, reducing overall toxicity.[58]

Another approach to reduce the overall NP toxicity is the use of ligands to stabilise the QD. Specific functional groups can be selected for subsequent coupling of biomarkers or probes. Non-toxic biomolecules can also be employed as stabilising ligands.[121] The effect of ligands on cytotoxicity was seen for Si and Ge NPs, where careful choice of carboxylic acid, dextran or PEG based ligands exhibited little or no cytotoxicity validating this approach as a promising technique for low toxicity biosensing.[122] The incorporation of a stabilising ligand offers additional benefits with respect to aqueous solubility as outlined in Figure 7. As biomolecules are predominantly aqueous based, it is preferred that the luminophores that interact with them can be dispersed in

water. NPs can be rendered water dispersible with water soluble ligands as can be seen in Figure 7. This can be achieved through either a direct aqueous synthesis method or by ligand exchange/ encapsulation following an organic synthesis method. Water dispersibility also facilitates the use of NPs in *in vivo* biosensing.[123] The stabilising ligands are largely independent of the core material and common ligands exist that can be used on all SC QDs.

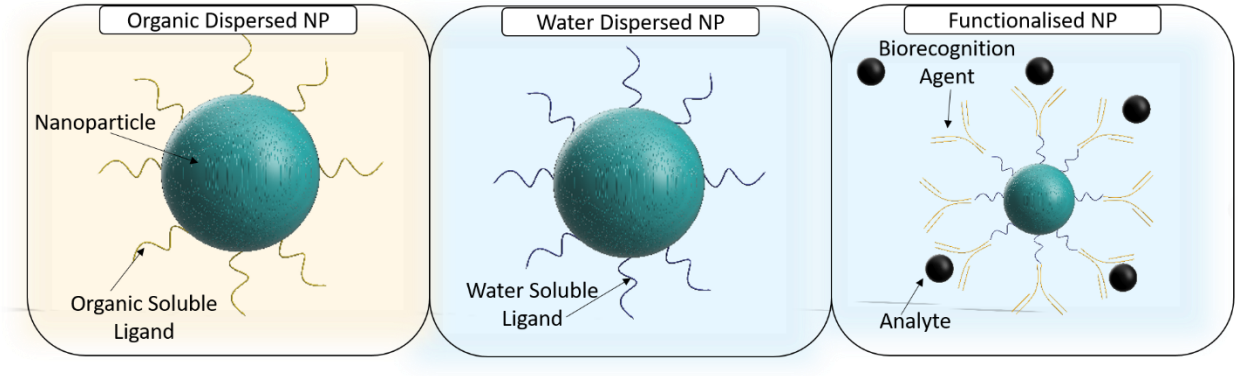

Figure 7: Nanoparticles with different ligands imbuing them with different properties, from left to right: organic ligands on NPs in organic media, aqueous ligands on NPs in aqueous media and aqueous ligands on NPs attached to biorecognition agents selectively binding to analytes in aqueous media

In addition to lowering toxicity of the NP, ligand exchange permits NPs to be functionalized with antibodies, aptamers, DNA, RNA and enzymes. This paves the way for immunosensing, aptasensing and enzyme sensing as described in Table 1. Functionalisation has been achieved in many types of luminophores including ruthenium complexes as well as NPs.[10] The stabilising ligands often facilitate surface modification to allow attachment of the recognition biomolecules as shown in Figure 8.

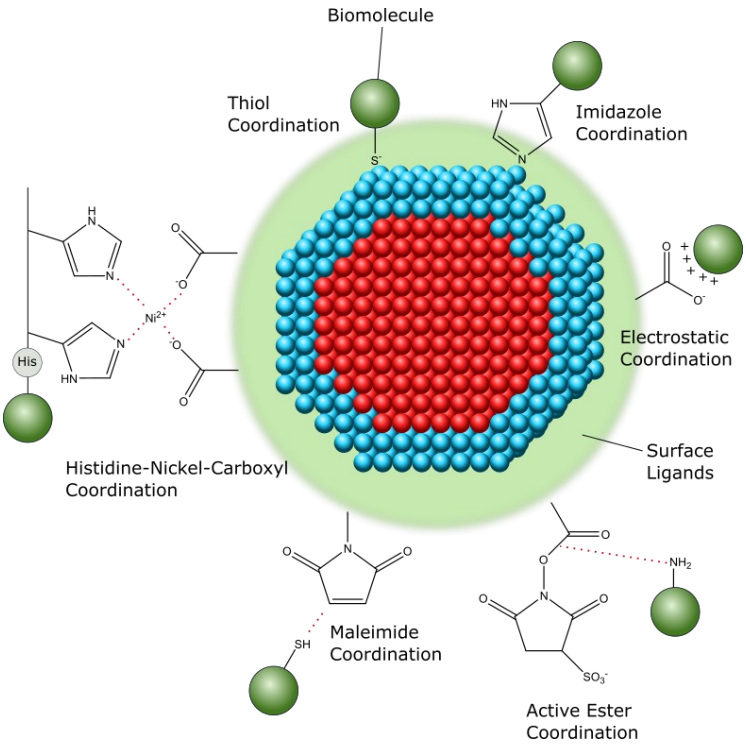

Figure 8:An overview of ligand facilitated NP functionalisation where biomolecules are bound through different chemistry

As outline in Table 2, there are numerous methods in which conjugation or surface modification can take place, however, the most common is EDC/NHS involving active ester coordination.[89]

The concentration of the NP material contributes significantly to overall toxicity. As would be expected, the higher the concentration of NPs, the higher the collective toxicity.[121] Therefore, biosensors should endeavour to use low concentrations of luminophores. Low concentrations of NP in ECL application is possible, provided strongly emitting luminophores are employed. In the case of weakly emitting materials, amplification strategies can be used as well to improve the ECL efficiency, which are discussed in Section 4.4.

As a final note, irrespective of the material used, there are still concerns surrounding the inherent toxicity of NPs due to their size.[120] The nanoscale size allows them to penetrate cell walls and tissues unlike bulk materials.[57] The smaller the NP, the more cytotoxic they can be. It has been shown that smaller, 2.3 nm, CdTe QDs were more toxic than larger, 5.7 nm, QDs to PC12 and N9 cells.[121] The toxicity of any material used for biosensing applications should be fully investigated. In some cases, the presence of low toxicity elements in the QDs may not be sufficient and the

inclusion of a shell is important. Alarmingly, recent reports have found core only $CuInS_2$ QDs have high toxicity, while core shell CIS/ZnS QDs and quaternary CIZS QDs have much lower toxicity and higher biocompatibility.[121] Therefore, thorough investigation of the toxicity of the QD should be carried out, before application in biosensing systems.

4.3 Strong, Narrow ECL Emission

High ECL efficiency and narrow defined emission profiles (identified by low full width half maxima (FWHM))) are desirable for ECL luminophores for improved selectivity and performance. Additionally, distinct, narrow ECL signals facilitate spectrally resolved multiplexing by ensuring different spectral emissions from different analytes do not overlap or obscure each other, thereby enhancing accuracy. The FWHM of QD ECL peaks is typically ≈ 100 nm and can be narrowed through surface passivation and higher monodispersity of the NP. Completely passivated surfaces will have identical ECL and PL spectra. Utilising two stabilising ligands on the surface of the QD can improve surface passivation as Liu *et al* showed with both HMP and MPA ligands on CdSe QDs.[34] Monodispersity can be achieved through careful synthesis, cleaning and separation procedures. Highly monodisperse and passivated Cd QDs can have a FWHM ECL as low as 45 nm[124] and 28 nm[34] respectively.

Ag QDs have similar FWHMs as cadmium QDs, however, FWHMs as low as 75 nm have been reported.[87] Unfortunately, they exhibit weak luminescence alone but this has been improved by doping with Mn,[85] paired with Cd QDs[86] or amplified as described in Section 4.4. Surrounding the inner core of a ZnAgInS nanocrystal with a ZnS shell is another way improve luminescence and is a common method employed in nanocrystal synthesis to reduce surface defects.[88] Therefore, to achieve the narrow emission profiles preferred for ECL biosensing, additional synthesis steps and materials may be required.

The ECL FWHM of low-toxicity Cu QDs is about 150 nm in most cases, which is wider than that of high-toxicity Cd systems at 100 nm.[92 - 99] The wide FWHM is a limiting factor in the use of Cu QDs as ECL luminophores and originates from the Cu-state involved carrier recombination. This recombination effect results in “red shifting” of the spectrum as Cu concentrations increase.[125]

Similar to Ag QDs, the incorporation of Zn into the ternary CIS nanocrystal can increase the luminescence, facilitate ligand exchange and allow greater control over optical properties.[126] ZnS shells are also used to eliminate surface traps which improves quantum yields and photostability.[127] Interestingly, mercaptopropionic acid capped QDs exhibited better ECL than glutathione or cysteamine capped materials.[96] There have been very few studies relating the capping ligand to ECL and this route could prove a valuable method to increase the ECL of poorly emitting NPs. Incorporation of additional amplification components to improve the ECL emission can broaden the ECL FWHM.[100]

The reported FWHMs of the Si and Ge QD emission are between 140 – 180 nm[101 - 104] and the Sn QDs are quite broad at 210 nm.[105] However, there have been limited reports on the ECL emission profiles of these materials to date.

Zinc ZnO NPs have shown catalytic activity on the ECL of other luminophores such as luminol as well as exhibiting their own ECL activity.[109] ZnS and ZnSe NPs have displayed narrow ECL similar to that of cadmium NPs, ranging from 90 nm – 160 nm.[112, 116] Where the ECL FWHM is not mentioned, the PL is quite narrow from 25 nm – 50 nm.[114, 115] There is a tendency for the ECL emission of all NPs to become red-shifted and broader with respect to the PL emission as outlined for the ZnSe NPs in Figure 9. This is due to ECL being produced as a surface state emission in addition to band-gap transitions.[45]

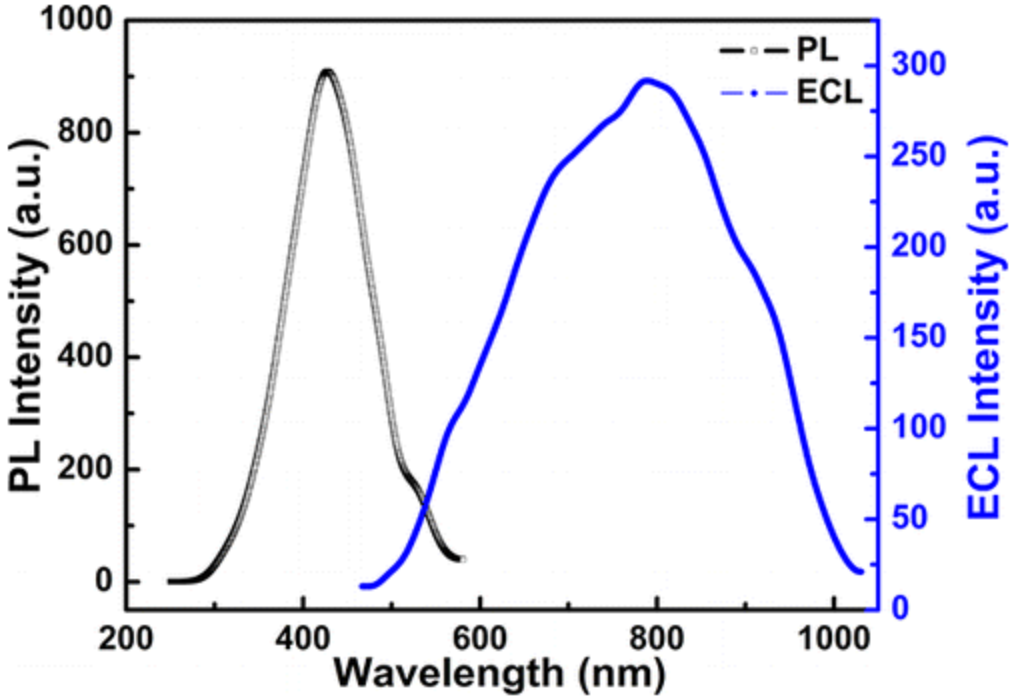

Figure 9: PL and ECL spectra of ZnSe NPs in 0.1 M KOH with 0.1 M $K_2S_2O_8$ (reprinted with permission from [116] Copyright 2016 American Chemical Society)

ZnX NPs have less intense luminescence than cadmium NPs. During the course of an ECL reaction they can also form unstable electrochemical products.[107]

Interestingly, ZnO nanorods have also been reported to give ECL emission.[109] - [111] This morphology has not been explored for other materials. ZnO is a promising ECL luminophore due to its wide band-gap, high exciton binding energy and facile synthesis. The rods exhibit a broad emission profile with several localised peaks while QDs of the same material emit over a narrower wavelength. This is due to native and intrinsic defects as well as zinc and oxygen vacancies.[128] While there could be potential to engineer rods with different functional groups or biorecognition agents on different surfaces of the rods, nanorods might not be suitable for spectrally resolved multiplexing due to the wide, convoluted spectra.

4.4 Amplification

Often, there is a trade-off between low-toxicity and strong, narrow ECL emission. Many emerging low-toxicity luminophores suffer from low ECL emission profiles. Therefore, signal amplification may be required to achieve biosensors capable of measuring analytes with low detection limits and

within clinically significant ranges. This is especially relevant for biosensors that detect analytes via an ECL quenching mechanisms as the output signal is even further reduced. The materials used in amplification strategies should exhibit low-toxicity. Common methods of amplification include immobilising the luminophore with a conductive polymer, employing a nanosheet such as graphene and using Au NPs to achieve surface enhanced ECL (SEECL) or resonance transfer ECL (ECL-RET). A mixture of these can be used to optimise the luminescence. Amplification techniques such as these are carried out for bulk luminophores such as luminol and $Ru(bpy)_3^{2+}$ as well.[10] These techniques are further discussed below including their respective toxicity.

4.4.1 Nanosheets – Carbon Materials

Carbon nanomaterials are low-toxicity materials, the robustness of which have been proven in electrochemical biosensors.[72] Graphene oxide (GO) and reduced graphene oxide (rGO) have been used as a bridging material to allow better flow of electrons between luminophore and electrode therby overcoming the reduced ECL observed with less toxic materials.[129] GO and rGO contain more defects than pristine graphene and exhibit reduced mechanical properties including tensile strength. However, they are easier to synthesise, scale-up and work with due to these defects. rGO is preferred over GO due to larger surface areas, better conductivity and electrochemical properties.[130] Reduced graphene nanosheets have been used to attract analytes such as LPA by hydrophobic interaction.[98] Other nanosheets including Mo_2S and C_3N_4 have also been investigated. The ECL of silver Ag_2S QDs was amplified with a conductive polymer/graphene oxide complex. Aminated GO (NH_2-GO) facilitated better conductivity and hydrophilicity. The conductive polymer (polypyrrole) was incorporated as the substrate to attach the luminophores due to its low cost and high conductivity.[86] Similarly, the ECL signal of Ag_2Se QDs has been amplified with MWCNT and polyethleneimine (PEI). MWCNTs facilitated fast electron transfer and low background signal while PEI immobilised the anion QDs in a film.[87]

Nanosheets such as MWCNT,[107] graphite[108] or MoS_2[111] or more recently, black phosphorus,[110] stabilise the ZnO NRs and amplify the ECL. MoS_2 nanosheets can be decorated with ECL luminophores due to the presence of active sites and high electrical conductivity. The MoS_2 was decorated with ZnO NRs which restricted their shape to cubes and enhanced their ECL. It has been proposed that the enhanced ECL could be due to the suppression of electron-hole pair recombination by the MoS_2.[111] ZnO NPs were doped with black phosphorus QDs, which are black

phosphorus nanosheets, to form ZnO cubic morphologies with BPQDs doped into the crystal lattice. Strong ECL emission was observed from these materials due to enhanced P doping.[110] Another approach involved using a CuZnInS QD/$K_2S_2O_8$ system, whereby ECL was enhanced using MoS_2/GO/o-MWNTs nanohybrids which were shown to immobilise the QDs and facilitate enhanced electron transfer in the reaction. Coupling carbon nanomaterials with high surface area MoS_2 sheets helps to increase its conductivity.[100]

An added advantage of using graphene was demonstrated in an interesting case with CIZS QDs, where they were used to selectively capture the analyte. The hydrophobic analyte was strongly adsorbed on the nanosheets, which also increased the electrochemical activity of the biosensor.[98]

4.4.2 Surface Enhanced Electrochemiluminescence – Au NPs

Another way to enhance ECL in lower toxicity materials is through surface enhanced electrochemiluminescence (SEECL). SEECL occurs when there is an energy transfer between surface plasmons and excitons which can increase PL and ECL. Au NPs enhance ECL due to Surface Plasmon Response (SPR) with a controlled distance between QD and Au NP.[97] Surface plasmons (oscillating electrons) of Au NPs interact with excitons from QDs to amplify luminescence. Separation distance, spectral overlap and magnetic field are all relevant parameters to consider.[131]

Gold NPs have been used in a variety of electrochemical biosensors and bioassays due to their biocompatibility, excellent electrochemical properties, high extinction coefficient and broad absorption spectrum. They have been employed alone in biosensors, but more commonly feature as catalytic agents in ECL sensors due to their high surface to volume ratio and availability of active sites. The electroanalytical limit of detection can thus be reduced improving sensitivity.[132]

Excitons from QDs and surface plasmons from Au NPs can be separated by DNA strands, PDA polymers[97] or silica encapsulation.[133] The ECL of the QDs triggers the SPR of the Au NPs thereby enhancing the ECL. The distance between the QD and the Au NPs is of utmost importance. If they are too close, electrochemiluminescence Resonance Energy Transfer (ECL-RET) will occur resulting in quenching. ECL-RET occurs when energy transfers from an excited donor to a ground state acceptor through long-range dipole-dipole interactions, similar to Förster Resonance Energy Transfer (FRET). ECL-RET can be used as a label free method for detection of biomolecules,

where the analyte quenches the ECL. QDs can act in pairs with Au NPs or other QDs.[104] To achieve SEECL preferentially and avoid ECL-RET quenching, longer distances of approximately 12 nm should be used to separate Au NPs and QDs which can be achieved with DNA strands.[13]

The SEECL amplification strategy has been used for CIZS QDs. The Au NPs and CIZS were separated through poly(dopamine)[97] and DNA,[96] observing an increase in ECL in both cases. Au NPs are relatively simple to synthesise via the citrate reduction method. They can then be used as a powerful amplifier of ECL.

4.4.3 Co-reactant Accelerators

Co-reactant accelerators can be used in the case of $S_2O_8^{2-}$ and peroxide co-reactant systems. The $S_2O_8^2$ ion is reduced to form a free radical which can inject holes into negatively charged QDs and lead to enhanced ECL. However, high concentrations of the ions are difficult to achieve due to their poor solubility and high oxidizing power which can destroy biosensors. Therefore, co-reactant accelerators are employed to accelerate the reduction of the co-reactant, increasing the number of QD excited states and thus enhancing ECL.[134] They are used to catalyse the decomposition of the co-reactant to the active species. This speeds up the ECL reaction and prevents highly reactive species from destroying the biosensor.

Some of the low-toxicity systems in Table 2 include low-toxicity co-reactant accelerators. Tin SnS_2 QDs employed silver nanoflowers as co-reactant accelerators to enhance the ECL. They efficiently boosted the ECL between the QDs and the $S_2O_8^{2-}$ as $S_2O_8^{2-}$ was converted into $SO_4^{\bullet-}$ faster.[105] Silver NPs exhibit low reactivity and have antibacterial properties which make them suitable for biosensing applications. However, high concentrations could be cytotoxic, so care should be taken when employing these materials.[135]

Similarly, a semicarbazide was used as a co-reactant accelerator for a CZIS/$K_2S_2O_8$ system. Semicarbazide is associated with seizures and hypotension.[100] Therefore, other co-reactant accelerators would be more suitable.

Some novel co-reactant accelerators include metal organic frameworks (MOFs) which could further enhance the ECL of SC QDs.[136] As MOFs are neither intrinsically safe or toxic, investigation should be done on a case-by-case basis, taking size, shape and surface

functionalisation into account. There is not much information available on the toxicity of MOFs, so this should be considered before incorporating into a biosensor.[137]

4.5 Multiplexing Potential

For biomedical diagnostics, the identification of several key biomarkers is usually required for robust, sensitive and specific diagnostics to be made. This is often due to the fact that many biomarkers are indicators of multiple disease states and many diseases have several characteristic biomarkers. Advantages of multiplexed biosensors include increased accuracy, reduced false positives, sample volume and reagent use and increases confidence in the resulting diagnosis.[149] To date, multiplexing in ECL has been difficult to achieve but many strategies have been explored. These include spectral, potential and spatial resolved multiplexing. Successful multiplexing methodologies employing low toxicity luminophores would maximise the potential of biosensors. In spatial multiplexing, different WEs are modified with different luminophores to detect different analytes. Spatial multiplexing can be complex due to multiple preparation steps but shared REs and CEs can be used to simplify the set-up. Arrays of nanoelectrodes can be used to reduce the working area. Spatial multiplexing can be expensive and impracticable.[138]

In spectral multiplexing, luminophores with different emission wavelengths are employed to detect different analytes. For spectral multiplexing, luminophores with different wavelengths are required. $Ru(bpy)_3^{2+}$ only emits at 620 nm but this can be altered via the use of selective ligands.[139] Similarly, other organic and organometallic materials can be tuned via chemical modifications. In some cases ruthenium and iridium complexes have been used simultaneously for multiplex analysis as their spectra are well resolved.[138] However, bulk luminophores can only target a limited amount of available wavelengths. Due to NPs size properties, they can be easily tuned across visible, NIR and IR wavelengths. Simply adjusting their size by reaction time can result in different emission wavelengths. Therefore, NP luminophores have an advantage over bulk luminophores.[138] Low toxicity SC QDs have not yet been used for spectral multiplexing but have potential as, in Table 2, ECL emission from 450 nm to 800 nm is recorded which spans almost the entire visible spectrum as well as some of the NIR spectrum. As well as this, some of these materials show narrow FWHMs especially silver and zinc which was discussed in Section 4.3.

In potential multiplexing, luminophores with different ECL voltages are used to detect different analytes. This avoids the need for a wavelength detector such as a CCD or filter. Luminophores should have different ECL voltages and ideally work with the same co-reactant to simplify the system. While the ECL potential for bulk luminophores can be adjusted, it involves convoluted chemical manipulations. NP ECL potentials can be more facilely altered through the core NP, the ligands and the co-reactant. NP ligands can be changed during or post synthesis and open an avenue for variable ECL potentials.[138] Although this has not be done yet for any low toxicity SC QDs, there is great potential to carry this out as the materials in Table 2 have shown a wide range of voltages from -2.3 V – +2.7 V, with both cathodic and anodic luminescence. Low potentials are useful for potential multiplexed ECL as they have high selectivity and result in low interfering reactions. These could include thiol capped Cu QDs with hydrazine as the co-reactant which had anodic ECL at 0.45 V.[95]

While there are no examples to date of low toxicity SC QDs used for multiplexing, several Cd QDs at different wavelengths have been used for spectral and potential multiplexing.[138] Other techniques such as logic gates, dual gears[138] and resolution by employing different pHs[140] have also been explored. However, there still exists many stumbling blocks for efficient, well-defined multiplexed ECL especially for low emitting low toxicity NPs.

4.6 Trends in Other Applications

To aid the exploration of novel SC QD luminophores for ECL beyond what is discussed in this review, trends in other applications should be considered. Especially interesting are applications that require emissive QDs with good electrochemical, biocompatible and safe properties. These include photovoltaic and bioimaging applications. Materials that are mass produced for other applications could also lead to development of ECL materials. These include some of the following mentioned materials.

Silver colloidal NPs are low toxicity NPs used in photocatalysis, bioimaging, solar cells and antimicrobial agents. Binary Ag_2S, Ag_2Se and Ag_2Te and ternary $AgInSe_2$ and $AgInS_2$ QDs have been synthesised via organic, aqueous and cation exchange methods with tuneable PL and relatively narrow FWHMs. These systems display PL in the NIR region making them suitable for biosensing. Their application in bioimaging also points to them as suitable materials for *in vivo* biosensing.[119]

A variety of other copper based colloidal NPs are being synthesised and utilised for applications including photovoltaic, LED, catalytic and energy storage applications. Incorporating elements such as bismuth, tin, gallium and silicon can yield materials with desirable properties. These materials are also being used due to their lower toxicity, reduced environmental impact and lower cost. The incorporation of gallium can result in green emitting materials and allows for extra tunability of wavelength. Other morphologies could also prove interesting for ECL. Nanorods, nanobullets and tetrapods of copper NPs have been synthesised. However, NPs with elongated morphologies tend to have broad emission spectra which would not be ideally suited to monochromatic biosensing.[141]

Germanium NPs have good electrical, optical properties and exhibit low toxicity however they have not been widely applied in biosensing. This could be due to the limited emission range or the high cost of material.[142]

Colloidal Si QDs have been used in other bio-applications, such as fluorescent imaging, through various methods to form Si QDs with various surface ligands. Common synthesis methods include supercritical fluid synthesis, layer pyrolysis and plasma synthesis. Moieties such as hydrides, halogens, alkanes, alkenes and carboxylic acids have been used as the surface ligands. Choice in surface ligands is crucial for functionalisation, water dispersibility and control over ECL potential and emission.[143]

Tin nanomaterials, especially tin oxide NPs, have found applications in gas sensors and photodetectors.[105] SnO_2 NPs have a wide band gap of 3.62 eV and can be synthesised through chemical vapour deposition or thermal evaporation. They tend to have low PLQYs. To overcome this, they can be doped with various materials such as caesium or manganese.[144] Alternatively, ternary NPs including gallium can facilitate tuning the emission wavelengths and increasing PLQYs. Emission can be tuned in the visible region from 390 – 550 nm.[145]

5 Conclusions and Future Perspectives

In the past few years, various semi-conductor nanoparticles have emerged in response to demand for materials with improved biocompatibility and reduced toxicity. Materials based on copper, silver, zinc and silicon have shown promising results to date in ECL biosensing. If such materials

are to rival the excellent luminophore performance of cadmium, further targeted research should be performed, in the areas highlighted below.

(1) **NP composition, shape and size:** Additional efforts to control NP parameters such as core element, size, shell, and concentration should be explored to reduce the toxicity of NPs for ECL applications. Narrow, strong emission is required for clear, distinct peaks and for spectral multiplexing. This has been achieved through incorporation of doping materials,[85] including a shell on the core QD,[127] selective choice of capping agent[96] and by ensuring a narrow monodispersity. QDs such as silver and zinc exhibit extremely narrow FWHMs and should be investigated further.

(2) **Signal Amplification:** ECL can also be increased through amplification. Amplification methods have been explored for various luminophores such as ruthenium complexes, cadmium NPs and low toxicity SC QDs. There are several well understood and proven methods including the incorporation of nanosheets, Au NPs and co-reactant accelerators. The area of co-reactant accelerators is especially interesting with novel materials such as MOFs. MOFs can be used to house SC QDs to further amplify their emission.

(3) **Ligand Modification and selective co-reactant choice:** Some QDs have achieved very low ECL potentials using ligands and selective co-reactants. Thiol capped copper QDs with hydrazine show exceptionally low ECL at 0.45 V.[95] Further investigation should be performed with a view to reducing the ECL potential through using different reducing co-reactants with low oxidation potentials, or ligands that facilitate charge transfer.

(4) **Trends in other Applications:** Research in Photovoltaic and bio-imaging applications share many materials with ECL biosensing applications and aim to reduce material toxicity. Innovations in these fields may offer solutions for improved ECL NP biocompatibility.

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7 References

1. Zoski, C. G., *Handbook of Electrochemistry*. Elsevier: 2007; p 892.
2. Wang, J., *Analytical Electrochemistry*. Wiley-VCH: 2006; p 250.
3. Wang, J.; Han, H., Near-infrared electrogenerated chemiluminescence from quantum dots. *Reviews in Analytical Chemistry* **2013,** *32*, 91-101.
4. Bertoncello, P.; Ugo, P., Recent Advances in Electrochemiluminescence with Quantum Dots and Arrays of Nanoelectrodes. *ChemElectroChem* **2017,** *4*, 1663-1676.
5. Roche ElectroChemiLuminescence (ECL).
6. Mesoscale Why Electrochemiluminescence? https://www.mesoscale.com/en/technical_resources/our_technology/ecl/.
7. McCord, P.; Bard, A. J., Electrogenerated chemiluminescence: Part 54. Electrogenerated chemiluminescence of ruthenium(II) 4,4'-diphenyl-2,2'-bipyridine and ruthenium(II) 4,7-diphenyl-1,10-phenanthroline systems in aqueous and acetonitrile solutions. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1991,** *318*, 91-99.
8. Yuk, J. S.; O'Reilly, E.; Forster, R. J.; MacCraith, B. D.; McDonagh, C., Demonstration of surface plasmon-coupled emission using solid-state electrochemiluminescence. *Chemical Physics Letters* **2011,** *513*, 112-117.
9. O'Reilly, E. J.; Keyes, T. E.; Forster, R. J.; Dennany, L., Deactivation of the ruthenium excited state by enhanced homogeneous charge transport: Implications for electrochemiluminescent thin film sensors. *Electrochemistry Communications* **2018,** *86*, 90-93.
10. Li, L.; Chen, Y.; Zhu, J.-J., Recent Advances in Electrochemiluminescence Analysis. *Analytical Chemistry* **2017,** *89*, 358-371.
11. O'Reilly, E. J.; Conroy, P. J.; Hearty, S.; Keyes, T. E.; O'Kennedy, R.; Forster, R. J.; Dennany, L., Electrochemiluminescence platform for the detection of C-reactive proteins: application of recombinant antibody technology to cardiac biomarker detection. *RSC Advances* **2015,** *5*, 67874-67877.
12. Myung, N.; Zhifeng, D.; Bard, A. J., Electrogenerated Chemiluminescence of CdSe Nanocrystals. *Nano Letters* **2002,** *2*, 1315-1319.
13. Chen, X.; Liu, Y.; Ma, Q., Recent Advances in Quantum Dot-Based Electrochemiluminescence Sensors. *Journal of Materials Chemistry C* **2018,** *6*, 942-959.
14. Stewart, A. J.; O'Reilly, E. J.; Moriarty, R. D.; Bertoncello, P.; Keyes, T. E.; Forster, R. J.; Dennany, L., A Cholesterol Biosensor Based on the NIR Electrogenerated-Chemiluminescence (ECL) of Water-Soluble CdSeTe/ZnS Quantum Dots. *Electrochimica Acta* **2015,** *157*, 8-14.
15. Li, J.; Guo, S.; Wang, E., Recent advances in new luminescent nanomaterials for electrochemiluminescence sensors. *RSC Advances* **2012,** *2*, 3579.
16. Rizwan, M.; Mohd-Naim, N. F.; Ahmed, M. U., Trends and Advances in Electrochemiluminescence Nanobiosensors. *Sensors (Basel, Switzerland)* **2018,** *18*.
17. Chen, Y.; Cao, Y.; Ma, C.; Zhu, J. J., Carbon-based dots for electrochemiluminescence sensing. *Materials Chemistry Frontiers* **2020,** *4*, 369-385.
18. Adhikari, J.; Rizwan, M.; Keasberry, N. A.; Ahmed, M. U., Current progresses and trends in carbon nanomaterials-based electrochemical and electrochemiluminescence biosensors. *Journal of the Chinese Chemical Society* **2020,** *67* (6), 937-960.
19. Cassette, E.; Helle, M.; Bezdetnaya, L.; Marchal, F.; Dubertret, B.; Pons, T., Design of new quantum dot materials for deep tissue infrared imaging. In *Advanced Drug Delivery Reviews*, Elsevier: 2013; Vol. 65, pp 719-731.

20. Mei, S.; Zhu, J.; Yang, W.; Wei, X.; Zhang, W.; Chen, Q.; He, L.; Jiang, Y.; Guo, R., Tunable emission and morphology control of the Cu-In-S/ZnS quantum dots with dual stabilizer via microwave-assisted aqueous synthesis. *Journal of Alloys and Compounds* **2017,** *729*, 1-8.
21. Dennany, L., Electrochemiluminescence fundamentals and analytical applications. *SPR Electrochemistry* **2019,** *15*, 96-146.
22. Zhou, Y.; Wang, H.; Zhang, H.; Chai, Y.; Yuan, R., Programmable Modulation of Copper Nanoclusters Electrochemiluminescence via DNA Nanocranes for Ultrasensitive Detection of microRNA. *Analytical Chemistry* **2018,** *90*, 3543-3549.
23. Yan, P.; Zhang, J.; Tang, Q.; Deng, A.; Li, J., A quantum dot based electrochemiluminescent immunosensor for the detection of pg level phenylethanolamine A using gold nanoparticles as substrates and electron transfer accelerators. *The Analyst* **2014,** *139*, 4365-4372.
24. Stewart, A. J.; Hendry, J.; Dennany, L., Whole Blood Electrochemiluminescent Detection of Dopamine. *Analytical Chemistry* **2015,** *87*, 11847-11853.
25. Dennany, L.; O'Reilly, E. J.; Keyes, T. E.; Forster, R. J., Electrochemiluminescent monolayers on metal oxide electrodes: Detection of amino acids. *Electrochemistry Communications* **2006,** *8*, 1588-1594.
26. Zhao, G.; Li, X.; Zhao, Y.; Li, Y.; Cao, W.; Wei, Q., Electrochemiluminescence assay of Cu2+ by using one-step electrodeposition synthesized CdS/ZnS quantum dots. *The Analyst* **2017,** *142*, 3272-3277.
27. Shi, Z.; Li, G.; Hu, Y., Progress on the application of electrochemiluminescence biosensor based on nanomaterials. *Chinese Chemical Letters* **2019,** *30* (9), 1600-1606.
28. Zhang, Y.; Zhang, R.; Yang, X.; Qi, H.; Zhang, C., Recent advances in electrogenerated chemiluminescence biosensing methods for pharmaceuticals. *Journal of Pharmaceutical Analysis* **2019,** *9*, 9-19.
29. Benoit, L.; Choi, J. P., Electrogenerated Chemiluminescence of Semiconductor Nanoparticles and Their Applications in Biosensors. *ChemElectroChem* **2017,** *4*, 1573-1586.
30. Bertoncello, P.; Forster, R. J., Nanostructured materials for electrochemiluminescence (ECL)-based detection methods: Recent advances and future perspectives. *Biosensors and Bioelectronics* **2009,** *24*, 3191-3200.
31. Wang, Y.; Cao, Z.; Yang, Q.; Guo, W.; Su, B., Optical methods for studying local electrochemical reactions with spatial resolution: A critical review. *Analytica Chimica Acta* **2019,** *1074*, 1-15.
32. Fan, F. R. F.; Bard, A. J., Observing single nanoparticle collisions by electrogenerated chemiluminescence amplification. *Nano Letters* **2008,** *8*, 1746-1749.
33. Zhang, Y.; Deng, S.; Lei, J.; Xu, Q.; Ju, H., Carbon nanospheres enhanced electrochemiluminescence of CdS quantum dots for biosensing of hypoxanthine. *Talanta* **2011,** *85*, 2154-2158.
34. Liu, S.; Zhang, X.; Yu, Y.; Zou, G., A Monochromatic Electrochemiluminescence Sensing Strategy for Dopamine with Dual-Stabilizers-Capped CdSe Quantum Dots as Emitters. *Analytical Chemistry* **2014,** *86*, 2784-2788.
35. Dennany, L.; Gerlach, M.; O'Carroll, S.; Keyes, T. E.; Forster, R. J.; Bertoncello, P., Electrochemiluminescence (ECL) sensing properties of water soluble core-shell CdSe/ZnS quantum dots/Nafion composite films. *Journal of Materials Chemistry* **2011,** *21* (36), 13984.
36. Stewart, A. J.; Brown, K.; Dennany, L., Cathodic Quantum Dot Facilitated Electrochemiluminescent Detection in Blood. *Analytical Chemistry* **2018,** *90*, 12944-12950.

37. Yeo, J. C.; Kenry; Zhao, Z.; Zhang, P.; Wang, Z.; Lim, C. T., Label-free extraction of extracellular vesicles using centrifugal microfluidics. *Biomicrofluidics* **2018,** *12*, 024103.
38. O'Reilly, E. J.; Keyes, T. E.; Forster, R. J.; Dennany, L., Insights into electrochemiluminescent enhancement through electrode surface modification. *The Analyst* **2013,** *138*, 677-682.
39. O'Reilly, E. Conjugated metallopolymer systems for electrochemiluminescence enhancement: new approaches and materials. Dublin, 2008.
40. Huang, H.; Li, J.; Zhu, J. J., Electrochemiluminescence based on quantum dots and their analytical application. *Analytical Methods* **2011,** *3*, 33-42.
41. Wang, Z.; Qian, Y.; Wei, X.; Zhang, Y.; Wu, G.; Lu, X., An "on-off" Electrochemiluminescence Biosensor Based on Molecularly Imprinted Polymer and Recycling Amplifications for Determination of Dopamine. *Electrochimica Acta* **2017,** *250*, 309-319.
42. Jie, G.; Huang, H.; Sun, X.; Zhu, J.-J., Electrochemiluminescence of CdSe quantum dots for immunosensing of human prealbumin. *Biosensors and Bioelectronics* **2008,** *23*, 1896-1899.
43. Liu, L.; Ma, Q.; Li, Y.; Liu, Z.; Su, X., A novel signal-off electrochemiluminescence biosensor for the determination of glucose based on double nanoparticles. *Biosensors and Bioelectronics* **2015,** *63*, 519-524.
44. Valenti, G.; Rampazzo, E.; Kesarkar, S.; Genovese, D.; Fiorani, A.; Zanut, A.; Palomba, F.; Marcaccio, M.; Paolucci, F.; Prodi, L., Electrogenerated chemiluminescence from metal complexes-based nanoparticles for highly sensitive sensors applications. *Coordination Chemistry Reviews* **2018,** *367*, 65-81.
45. Wu, P.; Hou, X.; Xu, J.-J.; Chen, H.-Y., Electrochemically Generated versus Photoexcited Luminescence from Semiconductor Nanomaterials: Bridging the Valley between Two Worlds. *Chemical Reviews* **2014,** *114*, 11027-11059.
46. Wang, T.; Zhang, S.; Mao, C.; Song, J.; Niu, H.; Jin, B.; Tian, Y., Enhanced electrochemiluminescence of CdSe quantum dots composited with graphene oxide and chitosan for sensitive sensor. *Biosensors and Bioelectronics* **2012,** *31*, 369-375.
47. Russell, R.; Stewart, A. J.; Dennany, L., Optimising electrogenerated chemiluminescence of quantum dots via co-reactant selection. *Analytical and Bioanalytical Chemistry* **2016,** *408*, 7129-7136.
48. Wang, X.; Zhou, J.; Yun, W.; Xiao, S.; Chang, Z.; He, P.; Fang, Y., Detection of thrombin using electrogenerated chemiluminescence based on Ru(bpy)32+-doped silica nanoparticle aptasensor via target protein-induced strand displacement. *Analytica Chimica Acta* **2007,** *598*, 242-248.
49. Zhang, L.; Cheng, Y.; Lei, J.; Liu, Y.; Hao, Q.; Ju, H., Stepwise Chemical Reaction Strategy for Highly Sensitive Electrochemiluminescent Detection of Dopamine. *Analytical Chemistry* **2013,** *85*, 8001-8007.
50. Liang, G.; Liu, S.; Zou, G.; Zhang, X., Ultrasensitive Immunoassay Based on Anodic Near-Infrared Electrochemiluminescence from Dual-Stabilizer-Capped CdTe Nanocrystals. *Analytical Chemistry* **2012,** *84*, 10645-10649.
51. Buzea, C.; Pacheco, I. I.; Robbie, K., Nanomaterials and Nanoparticles: Sources and Toxicity. *Biointerphases* **2007,** *2*, MR17-MR71.
52. Zou, G.; Tan, X.; Long, X.; He, Y.; Miao, W., Spectrum-Resolved Dual-Color Electrochemiluminescence Immunoassay for Simultaneous Detection of Two Targets with Nanocrystals as Tags. *Analytical Chemistry* **2017,** *89*, 13024-13029.

53. Ramanaviciene, A.; Kausaite-Minkstimiene, A.; Oztekin, Y.; Carac, G.; Voronovic, J.; German, N.; Ramanavicius, A., Visualization of red-ox proteins on the gold surface using enzymatic polypyrrole formation. *Microchimica Acta* **2011,** *175*, 79-86.
54. Kudo, K. i.; Ito, H.; Ihara, S.; Terato, H., Quantitative analysis of oxidative DNA damage induced by high-voltage pulsed discharge with cavitation. *Journal of Electrostatics* **2015,** *73*, 131-139.
55. Liu, X.; Guo, L.; Cheng, L.; Ju, H., Determination of nitrite based on its quenching effect on anodic electrochemiluminescence of CdSe quantum dots. *Talanta* **2009,** *78*, 691-694.
56. Liu, X.; Cheng, L.; Lei, J.; Ju, H., Dopamine detection based on its quenching effect on the anodic electrochemiluminescence of CdSe quantum dots. *The Analyst* **2008,** *133*, 1161.
57. Su, Y.; Hu, M.; Fan, C.; He, Y.; Li, Q.; Li, W.; Wang, L.-h.; Shen, P.; Huang, Q., The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties. *Biomaterials* **2010,** *31*, 4829-4834.
58. Rzigalinski, B. A.; Strobl, J. S., Cadmium-containing nanoparticles: Perspectives on pharmacology and toxicology of quantum dots. *Toxicology and Applied Pharmacology* **2009,** *238*, 280-288.
59. Zhai, Q.; Li, J.; Wang, E., Recent Advances Based on Nanomaterials as Electrochemiluminescence Probes for the Fabrication of Sensors. *ChemElectroChem* **2017,** *4*, 1639-1650.
60. Li, S.; Liu, Y.; Ma, Q., Nanoparticle-based electrochemiluminescence cytosensors for single cell level detection. *TrAC Trends in Analytical Chemistry* **2019,** *110*, 277-292.
61. Wang, T.; Wang, D.; Padelford, J. W.; Jiang, J.; Wang, G., Near-Infrared Electrogenerated Chemiluminescence from Aqueous Soluble Lipoic Acid Au Nanoclusters. *Journal of the American Chemical Society* **2016,** *138*, 6380-6383.
62. Chen, A.; Ma, S.; Zhuo, Y.; Chai, Y.; Yuan, R., In Situ Electrochemical Generation of Electrochemiluminescent Silver Naonoclusters on Target-Cycling Synchronized Rolling Circle Amplification Platform for MicroRNA Detection. *Analytical Chemistry* **2016,** *88*, 3203-3210.
63. Chen, S.; Fan, Y.; Zhang, C.; He, Y.; Wei, S., Quenched solid-state electrochemiluminescence of gold nanoclusters and the application in the ultrasensitive detection of concanavalin A. *Electrochimica Acta* **2017,** *228*, 195-202.
64. Zhao, M.; Chen, A.-Y.; Huang, D.; Zhuo, Y.; Chai, Y.-Q.; Yuan, R., Cu Nanoclusters: Novel Electrochemiluminescence Emitters for Bioanalysis. *Analytical Chemistry* **2016,** *88*, 11527-11532.
65. Kim, J. M.; Jeong, S.; Song, J. K.; Kim, J., Near-infrared electrochemiluminescence from orange fluorescent Au nanoclusters in water. *Chemical Communications* **2018,** *54*, 2838-2841.
66. Peng, H.; Jian, M.; Deng, H.; Wang, W.; Huang, Z.; Huang, K.; Liu, A.; Chen, W., Valence States Effect on Electrogenerated Chemiluminescence of Gold Nanocluster. *ACS Applied Materials & Interfaces* **2017,** *9*, 14929-14934.
67. Swanick, K. N.; Hesari, M.; Workentin, M. S.; Ding, Z., Interrogating Near-Infrared Electrogenerated Chemiluminescence of Au 25 (SC 2 H 4 Ph) 18 + Clusters. *Journal of the American Chemical Society* **2012,** *134*, 15205-15208.
68. Díez, I.; Pusa, M.; Kulmala, S.; Jiang, H.; Walther, A.; Goldmann, A. S.; Müller, A. H. E.; Ikkala, O.; Ras, R. H. A., Color Tunability and Electrochemiluminescence of Silver Nanoclusters. *Angewandte Chemie International Edition* **2009,** *48*, 2122-2125.

69. Liao, H.; Zhou, Y.; Chai, Y.; Yuan, R., An ultrasensitive electrochemiluminescence biosensor for detection of MicroRNA by in-situ electrochemically generated copper nanoclusters as luminophore and TiO2 as coreaction accelerator. *Biosensors and Bioelectronics* **2018,** *114*, 10-14.
70. Bertoncello, P.; Stewart, A. J.; Dennany, L., Analytical applications of nanomaterials in electrogenerated chemiluminescence. *Analytical and Bioanalytical Chemistry* **2014,** *406*, 5573-5587.
71. Chen, Y.; Zhou, S.; Li, L.; Zhu, J.-j., Nanomaterials-based sensitive electrochemiluminescence biosensing. *Nano Today* **2017,** *12*, 98-115.
72. Deng, S.; Ju, H., Electrogenerated chemiluminescence of nanomaterials for bioanalysis. *Analyst* **2003,** *138*, 43-61.
73. Dong, Y.; Chen, C.; Lin, J.; Zhou, N.; Chi, Y.; Chen, G., Electrochemiluminescence emission from carbon quantum dot-sulfite coreactant system. *Carbon* **2013,** *56*, 12-17.
74. Chen, S.; Chen, X.; Xia, T.; Ma, Q., A novel electrochemiluminescence sensor for the detection of nitroaniline based on the nitrogen-doped graphene quantum dots. *Biosensors and Bioelectronics* **2016,** *85*, 903-908.
75. Li, J.; Wang, N.; Tran.T, T.; Huang, C. a.; Chen, L.; Yuan, L.; Zhou, L.; Shen, R.; Cai, Q., Electrogenerated chemiluminescence detection of trace level pentachlorophenol using carbon quantum dots. *The Analyst* **2013,** *138*, 2038.
76. Lu, J.; Yan, M.; Ge, L.; Ge, S.; Wang, S.; Yan, J.; Yu, J., Electrochemiluminescence of blue-luminescent graphene quantum dots and its application in ultrasensitive aptasensor for adenosine triphosphate detection. *Biosensors and Bioelectronics* **2013,** *47*, 271-277.
77. Li, L.-L.; Liu, K.-P.; Yang, G.-H.; Wang, C.-M.; Zhang, J.-R.; Zhu, J.-J., Fabrication of Graphene-Quantum Dots Composites for Sensitive Electrogenerated Chemiluminescence Immunosensing. *Advanced Functional Materials* **2011,** *21*, 869-878.
78. Wang, X.; Zhang, M.; Huo, X.; Zhao, W.; Kang, B.; Xu, J.-J.; Chen, H., Modulating the electronic structure of a semiconductor to optimize its electrochemiluminescence performance. *Nanoscale Advances* **2019,** *1*, 1965-1969.
79. Jiang, K.; Feng, X.; Gao, X.; Wang, Y.; Cai, C.; Li, Z.; Lin, H., Preparation of multicolor photoluminescent carbon dots by tuning surface states. *Nanomaterials* **2019,** *9*.
80. Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y., New strategies for fluorescent probe design in medical diagnostic imaging. *Chemical Reviews* **2010,** *110*, 2620-2640.
81. Cheng, C.; Huang, Y.; Tian, X.; Zheng, B.; Li, Y.; Yuan, H.; Xiao, D.; Xie, S.; Choi, M. M. F., Electrogenerated Chemiluminescence Behavior of Graphite-like Carbon Nitride and Its Application in Selective Sensing Cu2+. *Analytical Chemistry* **2012,** *84*, 4754-4759.
82. Jiang, L.; Mo, G.; Yu, C.; Ya, D.; He, X.; Mo, W.; Deng, B., Based on reduced graphene oxide-copper sulfide-carbon nitride nanosheets composite electrochemiluminescence sensor for determination of gatifloxacin in mouse plasma. *Colloids and Surfaces B: Biointerfaces* **2019,** *173*, 378-385.
83. Guo, Z.; Wu, L.; Hu, Y.; Wang, S.; Li, X., Potential-resolved "in-electrode" type electrochemiluminescence immunoassay based on functionalized g-C3N4 nanosheet and Ru-NH2 for simultaneous determination of dual targets. *Biosensors and Bioelectronics* **2017,** *95*, 27-33.
84. Sun, Y.; Wang, Y.; Yang, Y.; Yang, M., An electrochemiluminescent sensor for epinephrine detection based on graphitic carbon nitride nanosheet/multi-walled carbon nanotubes nanohybrids. *Chemistry Letters* **2019,** *48*, 215-218.

85. Wu, F.-F.; Zhou, Y.; Wang, J.-X.; Zhuo, Y.; Yuan, R.; Chai, Y.-Q., A novel electrochemiluminescence immunosensor based on Mn doped Ag2S quantum dots probe for laminin detection. *Sensors and Actuators B: Chemical* **2017,** *243*, 1067-1074.
86. Lv, X.; Pang, X.; Li, Y.; Yan, T.; Cao, W.; Du, B.; Wei, Q., Electrochemiluminescent Immune-Modified Electrodes Based on Ag ₂ Se@CdSe Nanoneedles Loaded with Polypyrrole Intercalated Graphene for Detection of CA72-4. *ACS Applied Materials & Interfaces* **2015,** *7*, 867-872.
87. Cui, R.; Gu, Y.-P.; Bao, L.; Zhao, J.-Y.; Qi, B.-P.; Zhang, Z.-L.; Xie, Z.-X.; Pang, D.-W., Near-Infrared Electrogenerated Chemiluminescence of Ultrasmall Ag ₂ Se Quantum Dots for the Detection of Dopamine. *Analytical Chemistry* **2012,** *84*, 8932-8935.
88. Zhang, B.; Zhang, F.; Zhang, P.; Shen, D.; Gao, X.; Zou, G., Ultrasensitive Electrochemiluminescent Sensor for MicroRNA with Multinary Zn–Ag–In–S/ZnS Nanocrystals as Tags. *Analytical Chemistry* **2019,** *91*, 3754-3758.
89. Fu, L.; Fu, K.; Gao, X.; Dong, S.; Zhang, B.; Fu, S.; Hsu, H. Y.; Zou, G., Enhanced Near-Infrared Electrochemiluminescence from Trinary Ag-In-S to Multinary Ag-Ga-In-S Nanocrystals via Doping-in-Growth and Its Immunosensing Applications. *Analytical Chemistry* **2021,** *93*, 2160-2165.
90. Zhang, D.; Mao, B.; Li, D.; Liu, Y.; Li, F.; Dong, W.; Jiang, T.; Shi, W., 0D/2D Z-scheme heterojunctions of Zn-AgIn5S8 QDs/α-Fe2O3 nanosheets for efficient visible-light-driven hydrogen production. *Chemical Engineering Journal* **2021,** *417*.
91. Yin, M.; Wang, Y.; Gao, X.; Du, S.; Cheng, Y.; Yu, S.; Zou, G.; Xue, F., Electrochemiluminescence ultrasensitive immunoassay for carbohydrate antigen 125 based on AgInS2/ZnS nanocrystals. *Analytical and Bioanalytical Chemistry* **2021,** *413*, 2207-2215.
92. Long, X.; Tan, X.; He, Y.; Zou, G., Near-infrared electrochemiluminescence from non-toxic CuInS 2 nanocrystals. *Journal of Materials Chemistry C* **2017,** *5*, 12393-12399.
93. Long, X.; Zhang, F.; He, Y.; Hou, S.; Zhang, B.; Zou, G., Promising Anodic Electrochemiluminescence of Nontoxic Core/Shell CuInS 2 /ZnS Nanocrystals in Aqueous Medium and Its Biosensing Potential. *Analytical Chemistry* **2018,** *90*, 3563-3569.
94. Fu, L.; Zhang, B.; Long, X.; Fu, K.; Gao, X.; Zou, G., Promising Electrochemiluminescence from CuInS 2 /ZnS Nanocrystals/Hydrazine via Internal Cu(I)/Cu(II) Couple Cycling *Analytical Chemistry* **2019,** *91*, 10221-10226.
95. Li, Z.; Wu, S.; Zhang, B.; Fu, L.; Zou, G., Promising Mercaptobenzoic Acid-Bridged Charge Transfer for Electrochemiluminescence from CuInS2@ZnS Nanocrystals via Internal Cu+/Cu2+ Couple Cycling. *Journal of Physical Chemistry Letters* **2019,** *10*, 5408-5413.
96. Chen, X.; Gui, W.; Ma, Q., Ultrasensitive Detection of EGFR Gene Based on Surface Plasmon Resonance Enhanced Electrochemiluminescence of CuZnInS Quantum Dots. *Analytica Chimica Acta* **2018,** *1009*, 73-80.
97. Liu, Y.; Chen, X.; Ma, Q., A novel amplified electrochemiluminescence biosensor based on Au NPs@PDA@CuInZnS QDs nanocomposites for ultrasensitive detection of p53 gene. *Biosensors and Bioelectronics* **2018,** *117*, 240-245.
98. Chen, X.; Gui, W.; Liu, H.; Ma, Q., A novel CuZnInS quantum dot-based ECL sensing system for lysophosphatidic acid detection. *The Analyst* **2017,** *142*, 4142-4149.
99. Feng, J.; Xu, H., ECL emission of non-stoichiometric quaternary Zn–Cu–In–S QDs. *Micro & Nano Letters* **2018,** *13*, 347-350.

100. Sun, Y.; Wu, X.; Zhang, K.; Ren, Q.; Xie, R., Electrochemiluminescent quaternary Cu-Zn-In-S nanocrystals as a sensing platform: Enzyme-free and sensitive detection of the FLT3 gene based on triple signal amplification. *Biosensors and Bioelectronics* **2018,** *100*, 445-452.
101. Myung, N.; Lu, X.; Johnston, K. P. B., Allen J., Electrogenerated Chemiluminescence of Ge Nanocrystals. *Nano Letters* **2003,** *4* (1), 183-185.
102. Bae, Y.; Lee, D. C.; Rhogojina, E. V.; Jurbergs, D. C.; Korgel, B. A.; Bard, A. J., Electrochemistry and electrogenerated chemiluminescence of films of silicon nanoparticles in aqueous solution. *Nanotechnology* **2006,** *17*, 3791-3797.
103. Ding, Z.; Quinn, B. M.; Haram, S. K.; Pell, L. E.; Korgel, B. A.; Bard, A. J., Electrochemistry and electrogenerated chemiluminescence from silicon nanocrystal quantum dots. *Science (New York, N.Y.)* **2002,** *296*, 1293-7.
104. Dong, Y.-P.; Wang, J.; Peng, Y.; Zhu, J.-J., Electrogenerated chemiluminescence of Si quantum dots in neutral aqueous solution and its biosensing application. *Biosensors and Bioelectronics* **2017,** *89*, 1053-1058.
105. Lei, Y.-M.; Zhou, J.; Chai, Y.-Q.; Zhuo, Y.; Yuan, R., SnS ₂ Quantum Dots as New Emitters with Strong Electrochemiluminescence for Ultrasensitive Antibody Detection. *Analytical Chemistry* **2018,** *90*, 12270-12277.
106. Yang, F.; Yang, F.; Tu, T. T.; Liao, N.; Chai, Y. Q.; Yuan, R.; Zhuo, Y., A synergistic promotion strategy remarkably accelerated electrochemiluminescence of SnO2 QDs for MicroRNA detection using 3D DNA walker amplification. *Biosensors and Bioelectronics* **2021,** *173*.
107. Bozorgzadeh, S.; Haghighi, B., Enhanced electrochemiluminescence of ZnO nanoparticles decorated on multiwalled carbon nanotubes in the presence of peroxydisulfate. *Microchimica Acta* **2016,** *183*, 1487-1492.
108. Zheng, R.; Zhao, C.; Zhong, J.; Qiu, Z.; Hu, Z., ELECTROCHEMICAL SCIENCE Determination of Epinephrine using a Novel Sensitive Electrochemiluminescence Sensor based on ZnO Nanoparticles Modified Pencil Graphite Electrode. *Int. J. Electrochem. Sci* **2019,** *14*, 9380-9390.
109. Zhang, X. L.; Tang, Z. R.; Dong, Y. P.; Wang, C. M., Electrogenerated chemiluminescence of ZnO nanorods and its sensitive detection of cytochrome C. *Talanta* **2018,** *179*, 139-144.
110. Ding, H. C.; Tang, Z. R.; Dong, Y. P., Synthesis of black phosphorus quantum dots doped ZnO nanoparticles and its electrogenerated chemiluminescent sensing application. *ECS Journal of Solid State Science and Technology* **2018,** *7*, R135-R141.
111. Tian, K. J.; Zhang, Y.; Zhang, S.; Dong, Y. P., Electrogenerated chemiluminescence of ZnO/MoS2 nanocomposite and its application for cysteine detection. *Journal of the Electrochemical Society* **2019,** *166*, H527-H533.
112. Shen, L.; Cui, X.; Honglan Qi, a.; Zhang, C., Electrogenerated Chemiluminescence of ZnS Nanoparticles in Alkaline Aqueous Solution. *The Journal of Physical Chemistry C* **2007,** *111* (23), 8172-8175.
113. Ye, Y.; Wang, L.; Liu, K.; Li, J., A label-free and sensitive electrochemiluminescence sensor based on a simple one-step electrodeposition of Go/ZnS modified electrode for trace copper ions detection. *Microchemical Journal* **2020,** *155*, 104749.
114. Wang, X.-F.; Xu, J.-J.; Chen, H.-Y., A New Electrochemiluminescence Emission of Mn2+ -Doped ZnS Nanocrystals in Aqueous Solution. *The Journal of Physical Chemistry C* **2008,** *112*, 17581-17585.

115. Hu, X.; Han, H.; Hua, L.; Sheng, Z., Electrogenerated chemiluminescence of blue emitting ZnSe quantum dots and its biosensing for hydrogen peroxide. *Biosensors and Bioelectronics* **2010,** *25*, 1843-1846.
116. Liu, S.; Zhang, Q.; Zhang, L.; Gu, L.; Zou, G.; Bao, J.; Dai, Z., Electrochemiluminescence Tuned by Electron–Hole Recombination from Symmetry-Breaking in Wurtzite ZnSe. *Journal of the American Chemical Society* **2016,** *138*, 1154-1157.
117. Khan, M. S.; Ameer, H.; Ali, A.; Li, Y.; Yang, L.; Ren, X.; Wei, Q., Electrochemiluminescence behaviour of silver/ZnIn2S4/reduced graphene oxide composites quenched by Au@SiO2 nanoparticles for ultrasensitive insulin detection. *Biosensors and Bioelectronics* **2020,** *162*.
118. Khan, M. S.; Ameer, H.; Chi, Y., Label-free and sensitive electrochemiluminescent immunosensor based on novel luminophores of Zn2SnO4 nanorods. *Sensors and Actuators, B: Chemical* **2021,** *337*, 129761.
119. Gui, R.; Jin, H.; Wang, Z.; Tan, L., Recent advances in synthetic methods and applications of colloidal silver chalcogenide quantum dots. *Coordination Chemistry Reviews* **2015,** *296*, 91-124.
120. Bahadar, H.; Maqbool, F.; Niaz, K.; Abdollahi, M., Toxicity of nanoparticles and an overview of current experimental models. *Iranian Biomedical Journal* **2016,** *20*, 1-11.
121. Zhu, C.; Chen, Z.; Gao, S.; Goh, B. L.; Samsudin, I. B.; Lwe, K. W.; Wu, Y.; Wu, C.; Su, X., Recent advances in non-toxic quantum dots and their biomedical applications. *Progress in Natural Science: Materials International* **2019,** *29*, 628-640.
122. Bhattacharjee, S.; Rietjens, I. M. C. M.; Singh, M. P.; Atkins, T. M.; Purkait, T. K.; Xu, Z.; Regli, S.; Shukaliak, A.; Clark, R. J.; Mitchell, B. S.; Alink, G. M.; Marcelis, A. T. M.; Fink, M. J.; Veinot, J. G. C.; Kauzlarich, S. M.; Zuilhof, H., Cytotoxicity of surface-functionalized silicon and germanium nanoparticles: The dominant role of surface charges. *Nanoscale* **2013,** *5*, 4870-4883.
123. Rong, G.; Corrie, S. R.; Clark, H. A., In Vivo Biosensing: Progress and Perspectives. *ACS Sensors* **2017,** *2*, 327-338.
124. Liu, S.; Zhang, X.; Yu, Y.; Zou, G., Bandgap engineered and high monochromatic electrochemiluminescence from dual-stabilizers-capped CdSe nanocrystals with practical application potential. *Biosensors and Bioelectronics* **2014,** *55*, 203-208.
125. Kim, H.-J.; Jo, J.-H.; Yoon, S.-Y.; Jo, D.-Y.; Kim, H.-S.; Park, B.; Yang, H., Emission Enhancement of Cu-Doped InP Quantum Dots through Double Shelling Scheme. *Materials* **2019,** *12*, 2267.
126. Yoon, H. C.; Oh, J. H.; Ko, M.; Yoo, H.; Do, Y. R., Synthesis and characterization of green Zn-Ag-In-S and red Zn-Cu-In-S quantum dots for ultrahigh color quality of down-converted white LEDs. *ACS Applied Materials and Interfaces* **2015,** *7*, 7342-7350.
127. Jiang, T.; Song, J.; Wang, H.; Ye, X.; Wang, H.; Zhang, W.; Yang, M.; Xia, R.; Zhu, L.; Xu, X., Aqueous synthesis of color tunable Cu doped Zn-In-S/ZnS nanoparticles in the whole visible region for cellular imaging. *Journal of Materials Chemistry B* **2015,** *3*, 2402-2410.
128. Janotti, A.; Van De Walle, C. G., Oxygen vacancies in ZnO. *Applied Physics Letters* **2005,** *87* (12), 122102.
129. Guo, Z.; Hao, T.; Du, S.; Chen, B.; Wang, Z.; Li, X.; Wang, S., Multiplex electrochemiluminescence immunoassay of two tumor markers using multicolor quantum dots as labels and graphene asconductingbridge. *Biosensors and Bioelectronics* **2013,** *44*, 101-107.

130. Compton, O. C.; Nguyen, S. T., Graphene Oxide, Highly Reduced Graphene Oxide, and Graphene: Versatile Building Blocks for Carbon-Based Materials. *Small* **2010,** *6*, 711-723.
131. Wang, J.; Shan, Y.; Zhao, W. W.; Xu, J. J.; Chen, H. Y., Gold nanoparticle enhanced electrochemiluminescence of CdS thin films for ultrasensitive thrombin detection. *Analytical Chemistry* **2011,** *83*, 4004-4011.
132. Guo, S.; Wang, E., Synthesis and electrochemical applications of gold nanoparticles. *Analytica Chimica Acta* **2007,** *598*, 181-192.
133. Jie, G. F.; Liu, P.; Zhang, S. S., Highly enhanced electrochemiluminescence of novel gold/silica/CdSe-CdS nanostructures for ultrasensitive immunoassay of protein tumor marker. *Chemical Communications* **2010,** *46*, 1323-1325.
134. Ma, M. N.; Zhuo, Y.; Yuan, R.; Chai, Y. Q., New Signal Amplification Strategy Using Semicarbazide as Co-reaction Accelerator for Highly Sensitive Electrochemiluminescent Aptasensor Construction. *Analytical Chemistry* **2015,** *87*, 11389-11397.
135. Pauksch, L.; Hartmann, S.; Rohnke, M.; Szalay, G.; Alt, V.; Schnettler, R.; Lips, K. S., Biocompatibility of silver nanoparticles and silver ions in primary human mesenchymal stem cells and osteoblasts. *Acta Biomaterialia* **2014,** *10*, 439-449.
136. Hu, X.; Zhang, H.; Chen, S.; Yuan, R.; You, J., A Signal-On Electrochemiluminescence Sensor For Clenbuterol Detection Based On Zinc-Based Metal-Organic Framework–Reduced Graphene Oxide–Cdte Quantum Dot Hybrids. *Analytical and Bioanalytical Chemistry* **2018,** *410*, 7881-7890.
137. Sajid, M., Toxicity of nanoscale metal organic frameworks: a perspective. *Environmental Science and Pollution Research* **2016,** *23*, 14805-14807.
138. Lv, W.; Ye, H.; Yuan, Z.; Liu, X.; Chen, X.; Yang, W., Recent advances in electrochemiluminescence-based simultaneous detection of multiple targets. *TrAC - Trends in Analytical Chemistry* **2020,** *123*, 115767.
139. Barbante, G. J.; Hogan, C. F.; Wilson, D. J. D.; Lewcenko, N. A.; Pfeffer, F. M.; Barnett, N. W.; Francis, P. S., Simultaneous control of spectroscopic and electrochemical properties in functionalised electrochemiluminescent tris(2,2′-bipyridine)ruthenium(II) complexes. *Analyst* **2011,** *136*, 1329-1338.
140. Brown, K.; Jacquet, C.; Biscay, J.; Allan, P.; Dennany, L., Tale of Two Alkaloids: PH-Controlled Electrochemiluminescence for Differentiation of Structurally Similar Compounds. *Analytical Chemistry* **2020,** *92*, 2216-2223.
141. Coughlan, C.; Ibáñez, M.; Dobrozhan, O.; Singh, A.; Cabot, A.; Ryan, K. M., Compound Copper Chalcogenide Nanocrystals. *Chemical Reviews* **2017,** *117*, 5865-6109.
142. Hu, J.; Lu, Q.; Wu, C.; Liu, M.; Li, H.; Zhang, Y.; Yao, S., Germanium nanoparticles: Intrinsic peroxidase-like catalytic activity and its biosensing application. *Talanta* **2019,** *195*, 407-413.
143. Cheng, X.; Lowe, S. B.; Reece, P. J.; Gooding, J. J., Colloidal silicon quantum dots: From preparation to the modification of self-assembled monolayers (SAMs) for bio-applications. *Chemical Society Reviews* **2014,** *43*, 2680-2700.
144. Gu, F.; Wang, S. F.; Lü, M. K.; Zhou, G. J.; Xu, D.; Yuan, D. R., Photoluminescence properties of SnO2 nanoparticles synthesized by sol-gel method. *Journal of Physical Chemistry B* **2004,** *108*, 8119-8123.
145. Ghodsi, V.; Radovanovic, P. V., Turning Weakly Luminescent SnO2 Nanocrystals into Tunable and Efficient Light Emitters by Aliovalent Alloying. *Chemistry of Materials* **2018,** *30*, 3578-3587.