



Advanced early diagnostics for infectious diseases through electrochemiluminescence

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ABSTRACT

Rapid, sensitive and accessible diagnosis is central for improving the management of infectious diseases, particularly in vulnerable populations and settings where access to centralized testing is limited. Hence, this review examines recent advances in electrochemiluminescence (ECL) biosensing for early pathogen detection, with a focus on the coordination chemistry of typical luminophores, metal-based nanostructures and hybrid materials that underpin assay performance. Particular attention is devoted to transition-metal complexes, coordination polymers, supramolecular and porous hosts, and metal-organic and inorganic nanoplateforms capable of acting as ECL emitters, as well as on coreactant carriers and recognition scaffolds in immunoassays, aptamer- and oligonucleotide-based sensing, on imaging strategies and portable formats such as paper-based and microfluidic devices. Across representative examples targeting whole viruses and bacteria, pathogenic nucleic acids or clinically relevant biomarkers, these architectures have achieved high analytical sensitivity and, in several cases, are compatible with point-of-care implementation. Overall, the work critically discusses structure–property relationships governing ECL efficiency, signal amplification and assay robustness, and outlines design principles for next-generation ECL platforms aimed at bridging the gap between analytical performance and practical clinical implementation in both high- and low-resource healthcare contexts, supporting earlier diagnosis, improved treatment decisions, and broader infection surveillance.

1. Introduction

The evolutionary interplay between humans and pathogens has long posed a serious threat to mankind health and survival. Over the past 150 years, high-income countries (HICs) have mitigated this threat through consistent improvements in hygiene conditions and nutrition, introducing in addition the administration of antimicrobial drugs and vaccines, as well as the development and continuous improvement of microbiological diagnostic tools. These interventions resulted to be extremely effective in the reduction of infant mortality and in the increase of life expectancy. In contrast, low- and middle-income countries (LMICs) continue to experience a thought burden of infectious diseases, due to limited access to healthcare resources, poor hygienic practice, malnutrition, and insufficient epidemiological surveillance. [1] In 2019, infectious diseases caused an estimated 13.7 million deaths worldwide,

with bacterial infections accounting for 7.7 million deaths (13.6% of all global deaths), particularly in sub-Saharan Africa. [2] Moreover, the resurgence of diseases such as tuberculosis (TB) demonstrates how infectious threats remain globally interconnected. In 2023, indeed, global TB incidence reached 134 cases per 100,000 population, with rates of 24 and 33 per 100,000 in Europe and the Americas, respectively. The reported data thus indicate a growing burden even in HICs, likely influenced by migration and antimicrobial resistance, [3] showing a worldwide interdependence between countries requiring coordinated Global Health strategies, as emphasised by COVID-19 pandemic.

In particular, a major consequence of globalization has been the emergence and spread of zoonotic and transboundary animal diseases. [4] As a matter of fact, there is a continue interexchange of microbial populations between humans, animals, and the environment they share, [5] as conceptualised in the One Health perspective. [6–8]

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Consequently, accurate and rapid diagnostics are crucial for guiding clinical decisions, requiring coordinated Global Health actions. [9] This is especially true, as previously mentioned, in the case of respiratory viruses, which increasingly impact public health and economies. [10,11] More in detail, the initial phase of the last pandemic outbreak revealed the main limitations still present in our diagnostic capacity. Such limitations were already highlighted in 1974 by Dr. Raymond C. Bartlett, who observed that “our technical capabilities are exceeding our ability to apply them [the diagnostic tools] effectively and economically to human problems”, [12] a reflection that remains pertinent. Thus, bridging the gap between technological innovation and clinical implementation is essential to improve preparedness against future outbreaks. At the same time, antimicrobial resistance (AMR) is becoming increasingly central to global health, making early and accurate pathogen detection crucial for timely clinical decision-making and outbreak control.

1.1. Global and growing threats to human health

Nowadays, AMR is rapidly escalating and has been identified by the World Health Organization as one of the ten leading global public health threats. [13] Driven primarily by genetic adaptations and the excessive use of antibiotics, AMR has promoted the emergence of multidrug-resistant pathogens, including the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*), associated with high mortality and healthcare costs, [14] a situation further exacerbated by the recent COVID-19 pandemic. [15] Nowadays, it is estimated that AMR is responsible for approximately 136 million hospital-associated drug-resistant infections (HARIs) worldwide annually, highlighting the need for early diagnosis, rational antibiotic use, and effective surveillance systems. [16] One of the most severe consequences is sepsis, a life-threatening organ dysfunction caused by infection, which was responsible for 11 million deaths in 2017. [17] This figure may seem disproportionately high compared to the 13.7 million infectious disease deaths in 2019, but it also includes cases where sepsis arose as a complication in patients initially hospitalised for non-infectious causes, such as immunocompromised individuals, highlighting the broad and complex impact of infections. It is noteworthy that the reported numbers are underestimated owing to limited diagnostic capacities, especially in LMICs. Even though not all the reported cases resulted to be linked to resistant microorganisms, the presence of multidrug-resistant organisms (MDROs) has been shown to significantly complicate clinical outcomes. Indeed, AMR in sepsis is associated with higher mortality and longer intensive care unit stays, primarily due to delays in administering effective empirical therapies, since there are no effective diagnostic tools to quickly screen the correct drug-pathogen (including possible resistance) combination. [18,19] Accordingly, it has been demonstrated that each hour of delay in initiating appropriate antimicrobial therapy increases the risk of death by approximately 10%. [20] Taken together, these observations emphasize the importance of rapid diagnostics and targeted therapy, especially in resource-limited settings, where sepsis-related mortality remains alarmingly high. [21,22]

1.1.1. Advanced early diagnostics for improved infection management

Rapid qualitative and quantitative pathogen identification is essential for effective infection management, especially in vulnerable populations such as elderly individuals, cancer patients, transplant recipients, and immunocompromised patients. More in detail, cancer patients face a tenfold higher risk of infection than the general population, with a sepsis prevalence of 16 cases per 1000 and a hospital mortality rate of 37.8%. [23] Resistant pathogens, including *Pseudomonas aeruginosa*, frequently contribute to these infections. [24] Furthermore, over 50% of transplant patients are known to develop infections within six months post-surgery – 80% bacterial and 18% viral – due to immunosuppressive therapies. [25,26]

Nosocomial infections remain a growing concern worldwide. The World Health Organization (WHO) and the Organization for Economic Co-operation and Development (OECD) estimates suggest that, without effective preventive measures, annual mortality in HICs alone could reach 3.5 million deaths. [13] Challenges are even greater in LMICs, where inadequate infrastructure, limited training, and poor hygiene facilitate pathogen transmission. Consequently, accessible, portable, and user-friendly diagnostic tools would be of great help in tackling this challenge.

Nowadays, *gold standard* molecular technologies, such as Polymerase Chain Reaction (PCR) and metagenomic Next-Generation Sequencing (mNGS), enable rapid and precise pathogen identification, facilitating optimized antimicrobial therapy. [27] However, their widespread implementation remains limited by their high costs and insufficient standardization, particularly in LMICs. Improving accessibility, standardization, and integration with conventional diagnostic methods is therefore essential to maximize their clinical impact. Emerging ultrasensitive diagnostic systems with high signal-to-noise ratios may further improve diagnostic performance and help bridge the gap between high- and low-resource settings.

1.1.2. Clinical impact of pathogens detectable via new ultrasensitive systems

Advanced pathogen detection is particularly relevant for chronic infections, many of which contribute to cancer development and adversely affect immunocompromised individuals. Since the discovery of major oncogenic viruses, such as HBV and Epstein–Barr virus (EBV) in the 1960s, the role of infectious agents in carcinogenesis has become increasingly evident. [28] Infection-attributable cancers show substantial geographical variability, with the highest incidence rates observed in eastern Asia and sub-Saharan Africa. [29] To be highlighted that, according to a global analysis by the International Agency for Research on Cancer, in 2018 an estimated 2.2 million new cancer cases (*i.e.*, 13% of the global cancer burden) were attributable to infections. [29] The major contributors were *Helicobacter pylori*, Human Papillomavirus (HPV), HBV and HCV. Notably, HBV and HCV are responsible for approximately 60–70% of hepatocellular carcinoma (HCC) cases worldwide through mechanisms involving chronic inflammation, fibrosis, and genomic instability. [30] HPV, particularly genotypes 16 and 18, is strongly associated with cervical, anal, and oropharyngeal cancers through the action of viral oncoproteins E6 and E7. [31] Vaccines against HPV provided a great effect in containing HPV prevalence and, consequently, the associated cancers. However, HPV infection should be continuously monitored, as viral strains not covered by vaccines may spread by exploiting the available ecological niche.

Beyond carcinogenesis, infectious diseases significantly affect outcomes in immunocompromised patients. Several bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, represent important targets for the development of ultrasensitive diagnostic platforms. In this context, methicillin-resistant *Staphylococcus aureus* (MRSA) is particularly relevant in oncology settings: 76% of *S. aureus* isolated from patients with solid or haematological malignancies were found to be methicillin-resistant and frequently multidrug-resistant. [32] Mortality associated with MRSA bloodstream infections reached a median of 36.1% and was consistently higher than that observed for susceptible strains. [33] Furthermore, transplant recipients are also highly vulnerable to infections due to immunosuppressive treatments. Cytomegalovirus (CMV) remains one of the most clinically significant pathogens, since it can directly damage the transplanted organs, promote graft rejection, and predispose patients to secondary infections. [34] More in detail, CMV reactivation is a critical risk in the early post-transplant period and is associated with increased hospitalization and mortality rates. In addition, emerging pathogens such as *Francisella tularensis* may be difficult to diagnose because of non-specific clinical manifestations. A documented case of suspected donor-derived transmission resulted in severe complications and death in one

recipient, highlighting the limitations of conventional diagnostics and the importance of rapid nucleic acid-based testing. [35]

Collectively, the increasing burden of infectious diseases, AMR, and infection-related complications in vulnerable populations underscores the urgent need for innovative, rapid, and highly sensitive diagnostic systems capable of improving pathogen detection and clinical decision-making worldwide.

1.2. Electrogenenerated chemiluminescence (ECL): A useful tool in biomedical diagnostics

For all the reasons presented above, in the context of improving the sensibility and accuracy of easy-to-handle diagnostic systems, PCR-free approaches represent an appealing resource, since they do not require any analyte-amplification reaction, allowing to significantly simplify the instrumentation needed and thus lowering the associated costs, including those related to the need for trained personnel. [36] Between all the PCR-free detection systems that have been currently developed, biosensors have particularly found enormous applications in pathogens detection, providing more sensitive, specific, fast, and reproducible results than conventional techniques. [37] More in detail, as defined by Gronow in 1982, [38] biosensors are analytical tools or systems consisting of an immobilized biological material in contact with a suitable transducer device, converting the biochemical signal into a quantifiable electrical signal. The term “biosensor” was first coined by Cammann, and its definition then accepted by IUPAC, [39] while the concept of a biosensor itself has been pioneered in the 1960s by Clark and Lyons. [40] At present, we can identify biosensors as highly selective and accurate measurement analytical devices to determine ultralow analyte concentrations in biological samples, consisting of a selective biological recognition element and a physicochemical signal transducer for the readout of the corresponding signal, [41] which must be highly specific and independent of other chemical and physical parameters, such as pH and temperature. [39] In addition, they must present significant advantages over other common analytical devices, as fast response,

simplicity, stability, portability, high sensibility, low cost and not operable even by not highly specialised personnel. [42] In these bio-affinity sensors, to produce a measurable signal even at low concentrations, there is the need to use biomolecules (such as antibodies, aptamers or oligonucleotides, membrane receptors, and so on) endowed with high affinity and selectivity, coupled with the target analyte. [43]

Regarding the output signal obtained, the transducing system in biosensing devices can involve various signal transduction mechanisms, e.g., piezoelectric, optical, electrochemical and so on. [41] Among all the different techniques used for biosensor transduction, electrogenerated chemiluminescence or electrochemiluminescence (usually abbreviated as ECL) results to be very attractive. This technique consists of a redox-induced light emission, in which species generated at the surface of an electrode and chemical species in solution undergo high-energy electron-transfer (eT) reactions to form the excited state of one of them, the so-called luminophore, that subsequently deactivates through light emission. [44] Being ECL based on a luminescent phenomenon induced by an electrochemical stimulus, it represents an ideal marriage between electrochemical and spectroscopic methods, allowing to overcome photoluminescence (PL) – due to the ECL reduced background noise, providing high sensitivity (fM range) and broad dynamic range [36] – and chemiluminescence (CL) – allowing the time and position of the light-emitting reactions to be accurately controlled, which provides excellent reproducibility and sensitivity, as well as superior temporal and spatial control on light emission. A systematic comparison in terms of cost, throughput, scalability and portability of the abovementioned techniques has been reported and summarised in Table 1.

Therefore, ECL-based biosensors can offer a good alternative to classic methodologies for pathogens detection, presenting outstanding advantages over other common analytical techniques, [50,51] and allowing their application to trace target detection of biomolecules, clinical diagnostics, environmental and food monitoring, [43] resulting in commercial ECL immunoassays and DNA probe assays able to provide sensitive, selective and rapid response (as schematically reported in the comparative Table 2 with respect to other *gold standard* detection

Table 1

Comparison of ECL with different transduction methodologies applied to detection strategies (PL, colorimetry, CL, electrochemical methods, SPR, SERS), reporting the related detection ranges, cost- and time-effectiveness, scalability and/or portability of the instrumentation (comprising further application for point-of-care, PoC, diagnostics), and technique overall reliability. [45–49].

Detection method	Detection range	Cost/time	Scalability/portability	Reliability
ECL	nM – fM	< 20€ per sample, few mins per sample	Large scale analysis, compact and suitable for PoC diagnostics	High signal-to-noise ratio, increasing preciseness in quantitative analysis
Photoluminescence (usually, fluorescence)	nM – pM	< 20€ per sample, few mins per sample	Large scale analysis, costly and bulky instrumentations	High background, unselective photoexcitation
Colorimetry	μM – pM	< 2€ per sample, < 1 h per sample	Large scale analysis, adaptable to PoC diagnostics	Often suffers from false-positive or false-negative results
Chemiluminescence	pM – aM	< 50€ per sample, < 1 h per sample	Requires controlled laboratory conditions, challenging for applications in large scale analysis and PoC	Reliable quantitative analysis
Electrochemistry	nM – pM	< 10€ per sample, < 1 h per sample	Large scale analysis, compact and adaptable to PoC diagnostics	Reliable quantitative analysis, but depends on methods or electrodes functionalization
Surface plasmon resonance (SPR)	nM – pM	< 300€ per sample, ~15 mins per sample	Requires precise surface functionalization, costly and bulky instrumentations	Highly reliability for real-time and quantitative analysis
Surface-enhanced Raman spectroscopy (SERS)	nM – zM	< 200€ per sample, ~30 mins per sample	Possible for large scale analysis, costly and bulky instrumentations	Poor signal reproducibility at lower concentrations

Table 2

Representative values from recent literature comparing newly developed ECL-based biosensors with more “classical” approaches, *i.e.*, PCR, mNGS, CRISPR-based assays, enzyme-linked immunosorbent assays (ELISA) and lateral flow immunoassays (LFI). Methods are typically compared within the same target class (*e.g.*, nucleic acids vs proteins or whole pathogens). The table outlines key strengths and weaknesses, including typical detection ranges, cost- and time-effectiveness, portability and laboratory requirements, and barriers to commercial translation. Cost estimates are indicative and can vary markedly between high-income and low-/middle-income settings. [45–48].

Biosensing strategy	Sensitivity	Detection target	Cost/time	Commercial translation barriers	Test complexity	Laboratory requirements
ECL-based biosensor	High to ultra-high	Nucleic acid, antigen, antibody, whole pathogen	~20€ per sample, few mins per sample (bench prototypes)	Large scale analysis, commercial ECL immunoassay platforms already available, while other next-generation ECL biosensors under development	Most are complex, a few are easy to use, generally dedicated to trained personnel	Average to high (from benchtop instruments to potential PoC devices)
PCR assay	Very high	Nucleic acid	~20–100€ per sample, several hours per run	Challenging for large scale analysis (requires laboratory setting and well-trained personnel), <i>gold standard</i> commercial devices available but costly	Complex, dedicated to trained personnel	High
mNGS assay	Very high	Nucleic acid	Up to 2000€ per sample, 1–2 days per sample	Challenging for large scale analysis (requires laboratory setting and well-trained personnel), <i>gold standard</i> commercial devices available but costly	Complex, dedicated to trained personnel	High
CRISPR-based assay	High (potentially very high with optimal design)	Nucleic acid	< 50€ per sample, < 1 h per sample	Challenging for large scale analysis (requires CAS enzyme and crRNA design)	Simple to moderate, requires CAS enzyme and crRNA design, usually dedicated to trained personnel	Average to high (molecular laboratories)
ELISA	Moderate	Antigen, antibody, toxin	< 50€ per sample, few hours per sample (assay-dependent)	Challenging for large scale analysis (requires laboratory setting and well-trained personnel), various commercial devices already available	Simple, easy to use, generally dedicated to trained personnel	Average to low
LFI	Moderate	Antigen, antibody, toxin	< 5€ per sample, ~30 mins per sample	Possible application to large scale analysis, various commercial devices already available	Simple, easy to use	Low

methodologies commonly employed in the healthcare sector). [52–54] The principle of ECL biosensing is based on detecting interactions between the biological recognition element and the corresponding target *via* changes in a diagnostic ECL signal intensity – not easily affected by other interfering factors, such as the environment and the substrate [55] – enabling the quantitative detection of the analyte. [56] Additionally, from the practical consideration, they can perform rapid measurement (*i.e.*, few seconds) with low volumes (≤ 1 mL), being ECL typically confined at the electrode surface, and are characterized by simplified optical setup compared with PL. [54,57–59] Commercial ECL systems have been developed to detect several clinically important analytes, *e.g.*, alpha fetoprotein (AFP), carcino-embryonic antigen (CEA), calcitonin, ferritin, and so on, [60–62] attracting much attention due to their great potential for point-of-care (PoC) diagnosis.

Nonetheless, it is noteworthy that, despite their portability and potential suitability for point-of-care applications, ECL-based diagnostic devices may still face barriers to implementation in LMICs. These include the cost of the device and consumables, challenges associated with supply-chain sustainability, and the limited capacity of healthcare systems to fully exploit diagnostic information for patient management. Furthermore, in resource-constrained settings, immediate access to treatment often remains a higher priority than pathogen-specific diagnosis. Nevertheless, the deployment of sensitive portable diagnostic technologies in LMICs could provide substantial benefits for global infectious disease surveillance, particularly for emerging pathogens and antimicrobial resistance monitoring. In this context, international investments in diagnostic capacity may generate public health benefits extending far beyond the local setting.

1.2.1. Fundamentals of electrochemiluminescence

The first known record of ECL dates back to 1929, when it was first

documented by N. Harvey. [63] Successively, important works reporting the real beginning of this electrochemical technique, as it is known today (mainly by Hercules, [64] Chandross, [65] and Bard [66]), were traced back to 1960s. Here, it is worth mentioning that original research on ECL entirely begun as an academic curiosity, [57,67] even though, nowadays, it has important industrial applications mainly focused on medical diagnostics. [68–72] At present, ECL applied to immunoassays represents a leading transduction technique for ultrasensitive bioanalysis, [53] with the most representative commercial example constituted by a ruthenium complex-based immunoassay (Elecys®) developed by Roche Diagnostics and Hitachi High Technologies. [73] Other commercialized ECL systems currently exist, developed by *e.g.* Xi'an Remax Electronic Co.Ltd. [74] and MesoScale Diagnostic. [75,76]

1.2.2. ECL generation: Mechanisms

Since the 1970s, ECL was observed by reacting electrogenerated tris-2,2'-bipyridylruthenium(II) ($[\text{Ru}(\text{bpy})_3]^{2+}$, where bpy refers to 2,2'-bipyridine) with tri-*n*-propylamine (TPRA) to create an electronically excited state that emits at approximately 620 nm, allowing the use of water as solvent. [77] In most of the systems, in order to generate ECL, two species, namely D, an electron donor, and A, an electron acceptor (both produced through redox reactions at the electrode surface) undergo an electron-transfer process in the diffusion layer, in order to obtain the excited state of one of them (the so-called ECL luminophore), which emits light (as from Fig. 1). [54]

Looking more in detail at the mechanism characteristic of the presented technique, there are two dominant pathways through which ECL can be produced, namely the “annihilation” and “coreactant” pathway. [68]

Particularly, ion annihilation (or radical ion annihilation) ECL involves the formation of excited state as a result of exergonic eT (in the

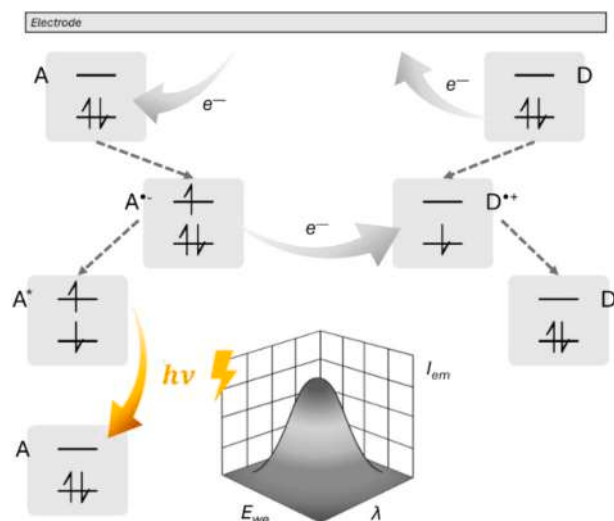


Fig. 1. Schematic representation of the generation of electrochemiluminescence, starting from an electron donor species (D) and an electron acceptor species (A). To be noted that A and D could also be the same species.

diffusion layer) between electrochemically generated species (typically, radical ions derived from subsequent oxidation and reduction of the same single species) at the surface of electrodes during potential pulsing or cycling. [78] In general, the generation of the radical ions – highly reactive or unstable – and subsequent annihilation reaction must be carried out in pure and aprotic solvents with large potential windows. [79,80]

On the other hand, exploiting the coreactant pathway (introduced by Bard and co-workers in 1977, paving the way toward the wide ECL exploitation in clinical analysis), [81] ECL emission can take place upon application of a single direction potential sweep: here, both ECL luminophore and coreactant can be oxidised (“oxidative-reduction”) or reduced (“reductive-oxidation”) at the electrode to form radical ions and intermediate species, followed by decomposition and formation of excited states that emit light. [78] Compared with the annihilation process, the use of coreactant ECL presents unique advantages in all bioanalytical applications, such as (i) facilitated ECL generation in aqueous solution (*i.e.*, where hydrogen evolution and oxygen reduction may strongly compete with the generation of the reduced luminophore), [68] (ii) higher number of possible ECL reporters, since high stability of both the oxidised and reduced forms of the luminophore is no longer required, [57] (iii) the possibility to operate only in the region of positive potentials (“oxidative-reduction” mechanism), avoiding any possible interference of oxygen (*i.e.*, a reducible species) in the generation of ECL partners, thus permitting to perform analysis in air-equilibrated solutions. [82]

1.2.3. ECL luminophores and coreactants

It is well known that the most used system for coreactant ECL mechanism is represented by the combination in aqueous media of TPrA as coreactant and $[\text{Ru}(\text{bpy})_3]^{2+}$ as emitter. Even if the second one still constitutes one of the benchmarks in ECL, there is extensive research activity in developing new inorganic complexes, organometallic species or organic molecules able to give rise to ECL, either freely diffusing in solution or embedded within nanosystems. [82–85] In this context, nanomaterials play a crucial role that ranges from the investigation of the complex mechanisms for ECL generation to the study of the electrochemical behaviour of new highly efficient dyes. In this text, we will avoid performing a general and extensive description of the most commonly employed ECL luminophores and coreactants during time, as further detailed discussions on this topic have already been excellently reviewed elsewhere. [54,69,82,86,87] Instead, as a difference with recently

reported reviews, [88] we mainly focus on the most recently exploited coordination complexes as well as – in some cases – organic luminophores as ECL luminophore/coreactant couples, applied to the quantification of several pathogens either in a direct or indirect way, through the building of immunoassays and/or the application of magnetic beads. As the reader can derive from Table 3 and Table 4 reported in the next section, attention is still currently focused on the employment of Ru(II) complexes as luminophores, *e.g.*, $[\text{Ru}(\text{bpy})_3]^{2+}$ (tris(2,2'-bipyridyl)ruthenium(II)), $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (bis(1,10-phenanthroline)dipyrido[3,2-a,2',3'-c]phenazine-ruthenium(II)), $[\text{Ru}(\text{dcbpy})_3]^{2+}$ ([tris(4,4'-dicarboxylic acid-2,2'-bipyridyl)ruthenium(II)]. However, some common drawbacks for these compounds are usually constituted mainly by their limited water solubility, [89,90] their absence of stability during the electrochemical switching, and also by the challenge of introducing binding sites without affecting the previously existing photophysical/electrochemical properties. [91] Therefore, in recent years, cyclometallated Ir(III) complexes (as from Fig. 2) have risen as promising ECL luminophores alternatives due to their higher quantum yields and water solubility, [91–93] and their ability to exhibit excellent colour tunability across the visible spectrum (which can be readily achieved by ligands modification), [94] thus some of them have been applied to the detection of pathogens, *e.g.*, $[\text{Ir}(\text{ppy})_3]$ (tris(2-phenylpyridine)iridium(III)) and $[\text{Ir}(\text{dmpq})_2](\text{acac})$ (bis(2-(3,5-dimethylphenyl)quinoline-C,N)(acetylacetonato)iridium(III)), as well as other systems (always depicted in Fig. 2) such as quantum dots (QDs; *e.g.*, carbon dots, CdSe, CdS, CdTe@CdS, MXene QDs), luminol, tetraphenylethylene, EuS nanoparticles (NPs), polydopamine NPs, and some cyanoarene derivatives manifesting Thermally Activated Delayed Fluorescence (TADF). [95]

To be mentioned that cyclometallated iridium complexes (water-soluble and freely diffusing in solution) has been further exploited also as novel methods to amplify electrochemiluminescence, first enabling – operating as redox-mediators – the amplification of the homogeneous ECL emission, [96] and later to enhance the heterogeneous coreactant ECL signal generation in microbeads-based assays. [97] Recently, a systematic investigation has been performed by Fracassa et al. [98] to elucidate the influence of three different Ir complexes, *i.e.*, $[\text{Ir}(\text{sppy})_3]^{3-}$ (*fac*-tris(2-phenylpyridinato)iridium(III)), $[\text{Ir}(\text{dfppy})_2(\text{pt-TEG})]^+$ (bis(2-(2,4-difluorophenyl)pyridine)-1-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-1-(4-pyridyl)-1,2,3-triazole)iridium(III)), and $[\text{Ir}(\text{bt})_2(\text{pt-TEG})]^+$ (bis(1,3-benzothiazole)(1-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-1-(4-pyridyl)-1,2,3-triazole)iridium(III)). They demonstrated that this so-called “redox-mediated” mechanism was able to finely tune the heterogeneous ECL, enhancing the signal up to 107%.

Eventually, more unconventional ECL-active luminophore/coreactant combinations have also been proposed for advanced imaging, such as a mixture of rubrene (confined to organic phase) and oxalate (in aqueous phase) forming a triphasic ECL system that emits only at the liquid|liquid|electrode interfaces and enables direct visualisation of microdroplet dynamics and interfacial morphology, allowing to interrogate complex, multiphase systems in real time through ECL microscopy. [99]

1.2.4. Bead-based assays for ECL biosensing

Herein, it is important to further note that the overall ECL efficiency is principally controlled by the diffusion of the reactive species in solution, particularly by the coreactant and intermediates diffusion within a few micrometres from the electrode surface. Hence, ECL intrinsically constitutes a surface-confined process, incorporating concomitant steps for the generation of the analytical signal. [100] For this reason, relatively large structures such as nanoparticles (NPs) are currently extensively employed in ECL, owing (also) to their diffusion coefficients, which are orders of magnitudes lower than those typical of molecular species. [82]

Furthermore, magnetic microbeads (MMBs)-based ECL has been widely used in clinical diagnostics [101] for obtaining both a rapid and efficient separation tool and a high-throughput detection platform.

Table 3

Representative summary of the most recently developed applications of ECL-based biosensing platforms for bacteria detection, which have been here classified according to the approach the biosensor is based on, and the ECL luminophore/coreactant couple used. These examples have been reported with the outcoming Limit of Detection (LoD).

Detected pathogen	Detection approach	Luminophore/ coreactant	Luminophore modification	LoD, detection range	Ref.
<i>Escherichia coli</i> (<i>E. coli</i>)	Whole bacteria as target. Electropolymerization of dopamine in the presence of bacteria, then removed to generate polydopamine-based <i>E. coli</i> imprint on electrode surface followed by specific binding of <i>E. coli</i> , then labelling with antibody-conjugated luminophore	Nitrogen-doped graphene quantum dots (N-GQDs)/K ₂ S ₂ O ₈	Antibodies labelled with the chosen luminophore	8 CFU/mL, 10–10 ⁷ CFU/mL	[106]
	Whole bacteria as target. Aptamer modification on the electrode surface for capturing <i>E. coli</i> , then labelling with TPE-based cationic luminophore nanoaggregates	Tetraphenyl-ethene/ K ₂ S ₂ O ₈	Luminophore nanoaggregates enhance the ECL signal by 17-times	1.99 CFU/mL, 10–10 ⁷ CFU/mL	[107]
	Whole bacteria as target. Aptamer modification on the electrode surface for capturing <i>E. coli</i> , then labelling with ECL-active tetraphenylethene-covalent organic polymer-based NPs	Tetraphenyl-ethene/ K ₂ S ₂ O ₈	TPE-COP NPs (8-fold higher ECL than [Ru(bpy) ₃] ²⁺)	0.2 CFU/mL, 10 ² –5·10 ⁶ CFU/mL	[108]
<i>Francisella tularensis</i> (<i>F. tularensis</i>)	Bacterial lipopolysaccharide and whole inactivated live vaccine strain as targets. Antibodies modification on the electrode surface for the capturing, then labelling with detection antibody-conjugated luminophore-silica NP	[Ru(bpy) ₃] ²⁺ /TPPrA	Encapsulated in silica NPs and conjugated with the antibody	0.4 ng/mL (LPS), 70 bacteria/mL (LVS + Ab), 40 bacteria/mL (LVS + F(ab)), –	[109]
<i>Lactobacillus salivarius</i> (<i>L. salivarius</i>)	Whole bacteria as target. Electropolymerization of dopamine in the presence of bacteria, then removed to generate polydopamine-based bacterial imprint on electrode surface and specific bacteria binding	Polydopamine/H ₂ O ₂	Polydopamine acts as ECL luminophore	10 CFU/mL, 10–10 ⁷ CFU/mL	[110]
<i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>)	Bacteria nucleic acid as target. Electrode modification by enzyme-cleavable oligonucleotide, then labelling with DNA functionalized magnetic-luminescent NPs	[Ir(dmpq) ₂](acac)/TPPrA, ECL/PL dual-mode probe	Luminophore-doped magnetic-silica NPs conjugated with oligonucleotide	73 fM, 100 fM–10 nM	[111]
<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	Whole bacteria as target. Cellulose paper modified with aptamer and DNA-glucose oxidase, then incubated with bacteria for quantitative release of DNA-glucose oxidase, followed by ECL on an electrode modified with luminophore-incorporated porous MOF	[Ru(bpy) ₃] ²⁺ /TPPrA	Incorporated into metal-organic framework NPs	1 CFU/mL, 5–10 ⁸ CFU/mL	[112]
	Whole bacteria as target. Bacterium recognition via Fe ₃ O ₄ -aptamer and aptamer amplification through PCR. Electrode modification with the resulting 3D DNA networks	[Ru(bpy) ₃] ²⁺ /TPPrA	–	0.78 CFU/mL, 10–10 ⁸ CFU/mL	[113]
	Bacteria nucleic acid as target. Electrode modification with branched DNA for enzyme-free signal amplification, streptavidin-coated MMBs for capturing target DNA and labelling with luminophore	[Ru(bpy) ₃] ²⁺ /TPPrA	Conjugated with oligonucleotide	2 pM, 20 pM–100 nM	[114]
	Whole bacteria as target. Aptamer-modified Fe ₃ O ₄ NPs for bacteria capturing and extraction followed by DNA amplification, their capturing at electrode, labelling with luminophore and Au:Ag NPs-based ECL signal quenching	Luminol/H ₂ O ₂	–	17 CFU/mL, 10 ² –10 ⁸ CFU/mL	[115]
	Whole bacteria as target. Binding of aptamer with bacteria releases a primer chain that initiates hybridization chain reaction amplification. Electrode modified with MoS ₂ NF@Au NPs for signal amplification and functionalized with DNA for selectivity	[Ru(bpy) ₃] ²⁺ /TPPrA	–	1 CFU/mL, 1–10 ⁷ CFU/mL	[116]
	Whole bacteria as target. Electrode modification by polydopamine bacterial surface-imprinted polymer. Capturing of bacteria followed by ferrocene-aptamer labelling to modulate ECL response	[Ru(bpy) ₃] ²⁺ /TPPrA	–	10 CFU/mL, 10–10 ⁶ CFU/mL	[117]
<i>Vibrio parahaemolyticus</i> (<i>V. parahaemolyticus</i>)	Whole bacteria as target. Electrode modification with AuNPs@PEI/MWCNTs, then binding with bacterium and labelling with luminophore-loaded ordered mesoporous carbon-DNA2	[Ru(bpy) ₃] ²⁺ /TPPrA	Incorporated into ordered mesoporous carbon and conjugated with the probe DNA2	2.11 CFU/mL, 7.04–1·10 ⁷ CFU/mL	[118]
Procalcitonin (biomarker)	Procalcitonin as target for bacterial infection and sepsis marker. Electrode modification with ECL coordination polymer, then with antibody-1 for capturing, then binding with multiwalled carbon nanotubes-Au-antibody-2 to quench the signal	[Ru(dcbpy) ₃] ²⁺ / ethylenediamine	Dual-ligand self-enhanced ECL coordination polymer with Zn(II) nodes	0.47 fg/mL, 10 ^{–6} ng/mL–10 ng/mL	[119]
Bacteria imaging	Whole bacteria as target. Bacteria deposited at the electrode, then exposed to luminophore-coreactant prior to ECL imaging of bacteria along with their microstructure analysis	[Ru(bpy) ₃] ²⁺ /2-(dibutylamino)ethanol	–	Single-bacterium imaging, –	[120]

Table 4

Representative summary of the most recently developed applications of ECL-based biosensing platforms for viruses detection, which have been here classified according to the approach the biosensor is based on, and the ECL luminophore/coreactant couple used. These examples have been reported with the outcoming Limit of Detection (LoD).

Detected pathogen	Detection approach	Luminophore/ coreactant	Luminophore modification	LoD, detection range	Ref.
Hepatitis B virus (HBV)	Viral nucleic acid as target. Disposable paper-based BPE array modified with hairpin DNA, followed by recognition of target DNA and binding with Pt NPs-oligonucleotide	[Ru(bpy) ₃] ²⁺ /TPrA	–	0.2 fM, 0.2–10 ² fM	[121]
	Viral HBsAg gene as target. Target recognition through a polymer-amplified ECL assay via construction of linear [Ru(bpy) ₃] ²⁺ -polymer, then capture on the electrode surface with MMB-oligonucleotide	[Ru(bpy) ₃] ²⁺ /TPrA	Luminophore- and DNA probes-conjugated polymer	100 aM, –	[122]
	Viral surface antigen as target. Luminophore-conjugated antibody-1 for viral antigen recognition, then capture through antibody-2-conjugated MMBs, using low-cost paper-based electrode	[Ru(bpy) ₃] ²⁺ /N-butyl-diethanolamine	Luminophore-conjugated antibody	34.2 pg/mL, 34.2 pg/ mL–34.2 ng/ mL	[123]
	Viral surface antigen as target. PAMAM dendrimer with many amine functional groups used to immobilize QDs and antibody-2 (conjugation), then viral antigen recognition and capture with magnetic NPs-antibody-1	CdTe@CdS QDs/TPrA	Luminophore-conjugated antibody	0.8 fg/mL, 3 fg/mL–0.3 ng/mL	[124]
	Viral nucleic acid as target. Electrode modification with capture oligonucleotide, Pt nanoflowers and ECL quencher, then exonuclease III-induced target DNA amplification	Boron and nitrogen co-doped carbon dots/K ₂ S ₂ O ₈	–	18 aM, 10 ² aM–1 nM	[125]
	Viral nucleic acid as target. Electrode modification with two oligonucleotides to capture viral NA, then luminophore intercalation into the formed NA double strand	[Ru(phen) ₂ dppz] ²⁺ /K ₂ S ₂ O ₈	–	0.05 cps/μL, 5–10 ⁴ cps/ μL	[36]
	Viral nucleic acid as target. Dye-doped SiNPs conjugated with oligonucleotides for capturing the target NA and associated hybridization chain reaction-based amplification	[Ru(bpy) ₃] ²⁺ /TPrA	Dye-doped silica NPs, conjugated with the NA	7.41 fM, 10 fM–10 nM	[126]
Hepatitis C virus (HCV)	Viral protein E2 as target. Electrode is modified with peptide to capture viral protein, then labelled with antibody-conjugated composite of Pluronic block copolymer-Nb ₂ C nanosheet prior to ECL measurement	F-127@Ir(ppy) ₃ /g-C ₃ N ₄	Ir(ppy) ₃ introduced into Pluronic F-127 and used for antibody conjugation	0.84 pg/mL, 1 pg/ mL–10 ² ng/ mL	[127]
	Viral nucleic acid as target. Electrode modified with luminophore-silica NPs-graphene oxide and conjugated with molecular beacon, then binding with target DNA for enhanced ECL response	[Ru(bpy) ₃] ²⁺ /TPrA	Doped in silica-graphene and conjugated with molecular beacon	14 aM, 0.1 fM–0.1 nM	[128]
	Virus envelop E2 protein as target. Peptide-modified electrode for capturing target DNA, then labelling with luminophore-antibody.	MXene QDs/–	Coated with polydopamine and conjugated with antibody	33 fg/mL, 0.1 pg/ mL–10 ng/ mL	[129]
Hepatitis A/C virus (HAV/HCV)	Viral nucleic acid as target. Electrode modification with MOF and oligonucleotide-1 for capturing target DNA, followed by binding with luminophore-oligonucleotide-2	D-MOF/–, CdSe QDs/K ₂ S ₂ O ₈	Conjugated with oligonucleotide	0.05 pM (HAV), 0.2 pM (HCV), 0.1 pM–10 ² nM	[130]
Human immunodeficiency virus (HIV)	Viral nucleic acid as target. Electrode modification with luminophore, Ag NPs and oligonucleotide for capturing target DNA, followed by exonuclease III-assisted multiple recycling amplification	Fluorine-nitrogen co-doped carbon dots/S ₂ O ₈ ^{2–}	Fluorine and nitrogen doping of CDs to enhance their the ECL signal	6.34 aM, 10 aM–10 pM	[131]
	Viral nucleic acid as target. Electrode modification with ECL-active nanoaggregates and oligonucleotide for capturing target DNA, followed by DNA nanomachine-mediated multi-output amplification	Ultrasmall SiO ₂ NPs/S ₂ O ₈ ^{2–}	Zn ²⁺ /SiO ₂ NPs aggregates used for ECL signal generation in the presence of oxygen vacancies	50.48 aM, 10 ² aM–0.1 nM	[132]
Human herpesvirus type 6 (HHV-6 A)	Whole virus as target. Electrode modification with MXene@AuNPs and antibody for capturing virus, then labelling with luminophore-antibody.	Bistridentate Ir(III) complex comprising the N-heterocyclic carbene ligand /TPrA	Encapsulated in silica NPs and conjugated with antibody	42 cps/μL, 10 ² –10 ⁷ cps/ μL	[133]
Monkeypox virus (MPXV)	Viral nucleic acid as target. Electrode modification with microfluidic compartment and oligonucleotide for capturing target DNA, then rapid recombinase polymerase amplification of DNA and luminophore intercalation in double strand RPA products prior to ECL analysis	[Ru(phen) ₂ dppz] ²⁺ /TPrA	–	10 copies/ μL, –	[134]
	Viral nucleic acid as target. Oligonucleotide-1 conjugated MMBs used for target DNA binding, then labelling with double-layered nanotag	Double-layered nanotag ([Ru(bpy) ₃] ²⁺ , [Ru(dcbpy) ₃]Cl ₂)/TPrA, both ECL and colourimetry probe	Doped in metal organic framework-based NPs, conjugated with Pt NPs and oligonucleotide	10 aM, 0.1 fM–10 ⁴ pM	[135]

(continued on next page)

Table 4 (continued)

Detected pathogen	Detection approach	Luminophore/ coreactant	Luminophore modification	LoD, detection range	Ref.
Rabies virus (RABV)	luminophore–oligonucleotide-2 and magnetic capture on electrode Viral glycoprotein (surface) as target. Electrode modification with luminophore@SiNPs–antibody-1 for capturing, then labelling with MnO _x NPs–antibody-2	[Ru(bpy) ₃] ²⁺ /H ₂ O ₂	Loaded in dendritic mesoporous SiNPs and conjugated with antibody	88 fg/mL, 0.1 pg/ mL–10 ng/ mL	[136]
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Viral Spike S1 protein as target. Electrode modification with carbon dots (CNDs) and antibody, then labelling with target protein and luminophore	[Ru(bpy) ₃] ²⁺ /Ab- CNDs (ECL enhancer and coreactant)	–	1.2 pg/mL, –	[137]
	Viral nucleocapsid protein as target. Antibody-1–conjugated Au@Pt/Au NPs used as ECL acceptor. Antibody-2–modified MMBs used for capturing	ECL-RET [Ru(bpy) ₃] ²⁺ –Au@Pt/Au NPs/TPrA	–	1.27 pg/mL, –	[138]
	Viral nucleocapsid protein as target. Electrode modification with antibody-1–conjugated luminophore nanostructures, then binding with target protein and Au-ZnO NPs–antibody-2	Tetraphenyl-ethylene/K ₂ S ₂ O ₈	Doped in zeolite imidazole backbone to induce AIECL	0.52 fg/mL, 10 ng/ mL–10 ² ng/ mL	[139]
	Viral spike protein as target. Electrode modification with DNA–fluorophore for capturing target protein, then DNAzyme–based signal amplification	[Ru(bpy) ₃] ²⁺ /TPrA, ECL and PL dual-mode probe	Incorporated inside MOF and conjugated with DNAzyme	0.58 fg/mL, –	[140]
	SARS-CoV-2 pseudovirus as target. Electrode modification with bipolar silica nanochannel arrays loaded with luminophore, Au NPs and functionalized antibody for target binding	[Ru(bpy) ₃] ²⁺ /TPrA	Incorporated into nanoporous arrays and modified with antibody	31 TU/mL, 50–50 ³ TU/ mL	[141]
	Viral nucleocapsid protein as target. Electrode modification with antibody-1 for capturing target protein, then binding with luminophore–antibody-2 and detection with lateral flow immunoassay platform	[Ru(dcbpy) ₃] ²⁺ /2,2-bis(hydroxymethyl)-2,2',2''-nitrilotriethanol (BIS–TRIS)	Incorporated into dendritic mesoporous silica nanospheres and conjugated with antibody	0.52 ng/mL, 1–10 ⁴ ng/ mL	[142]
Tobacco mosaic virus (TMV)	Viral nucleic acid as target. Electrode modification with hairpin capture DNA, then target binding followed by polymerization at the electrode surface to enhance ECL response	Luminol/H ₂ O ₂	–	2.61 fM, 0.1 pM–10 nM	[143]
Zika virus (ZIKV)	Viral antigen as target. Silica microparticles conjugated with antibody-1 used for virus capture and deposition at the electrode, followed by labelling with antibody-2–conjugated QD	CdS QDs/S ₂ O ₈ ²⁻	3-mercaptopropionic acid capped CdS QDs conjugated with Zika virus antibody	0.3 fg/mL, 1.0 fg/ mL–1.0 ng/ mL	[144]

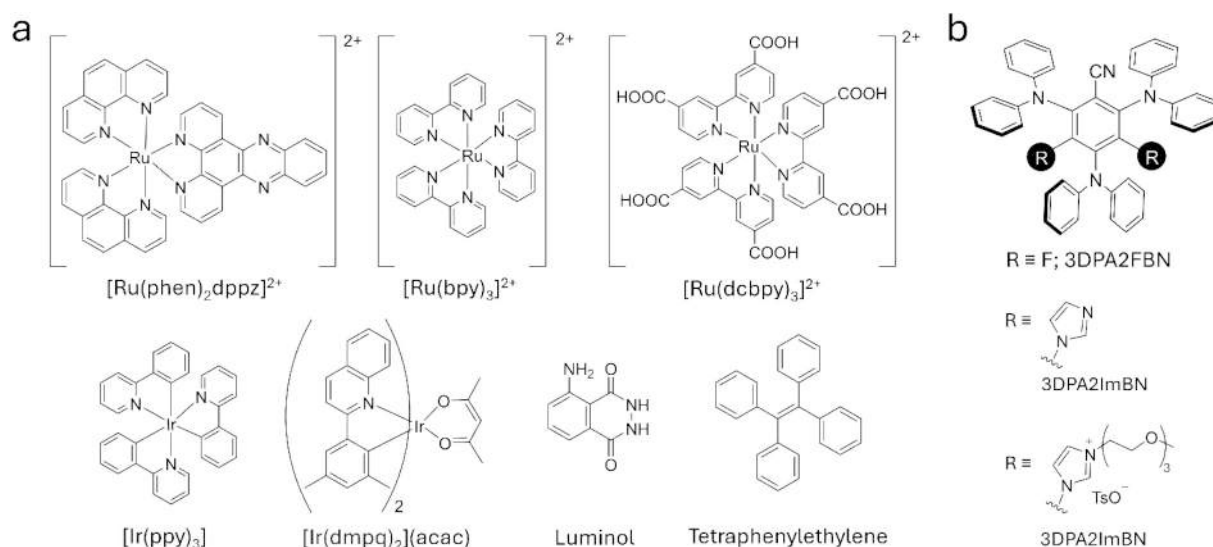


Fig. 2. a) Summary scheme of the most recently exploited ECL luminophores for the detection of pathogens, mainly involving coordination complexes of either Ru (II) or Ir (III). b) Example of the molecular structures of a TADF scaffold, namely 3DPA2FBN, which underwent further modification by derivatizing the cyanoarene accepting core with fluorine atoms (i.e., 3DPA2FBN), imidazole heterocycles (i.e., 3DPA2ImBN), or imidazole units bearing a triethylene glycol chain to provide water solubility (i.e., [3DPA2(ImTEG)BN](OTs)₂).

[102] More in detail, most of the commercialized ECL biosensors exploit antibodies–based sandwich assays, which can be directly built with the MMBs technology and adapted to a wide range of immunoassays,

[69,75,87,103] as well as directly imaged through ECL microscopy [104,105] after target immobilization, resulting in a non-homogeneous ECL emission, as from the scheme reported in Fig. 3. This approach

constitutes the basis of the Elecsys® technology, commercialized by Roche Diagnostics, which counts more than 2 billion tests performed each year. [73] In 2020, Zanut et al. [100] further demonstrated the possibility to obtain an increase of 128% of the ECL signal (compared to the standard TPra) upon using Ru-functionalized MMBs (in TPra 180 mM solution), with 30 mM of *N*-dipropyl isobutyl amine (DPIBA) as additive. This result confirmed the enhancement of reactivity at small distances, due to the involvement of MMBs in the system.

2. Application of ECL to the early diagnosis of infectious diseases

2.1. ECL-based biosensing platforms: Recent developments

Being the increasing spreading of pathogens a serial threat to human well-being, as previously highlighted, in this text we aim to review and outline the most recent and valuable applications of ECL-based biosensing systems to this field, in the context of improving the sensibility and accuracy of easy-to-handle diagnostic technologies, significantly simplifying the instrumentation needed and thus lowering the related costs, and allowing their use also to not specialised personnel. On this topic, the presented manuscript will mainly focus on describing the progresses performed in the development of biosensors' recognition elements (including antibodies, peptides, oligonucleotides, aptamers, and molecular beacons), the advances of the nucleic acid-based pathogen detection methods, as well as the ECL luminophores modifications performed to enhance the detection sensitivity/selectivity. In the presented content, we will also discuss the main detection mechanism strategies employed, as well as the problems and challenges faced by ECL detection methods. Two representative summaries of the most relevant biosensing platforms built for the quantification of bacteria and viruses (either in their entire form or through the detection of e.g. their nucleic acid or a specific antigen/antibody) have been listed below in [Table 3](#) and [Table 4](#), respectively.

As the reader can derive, a wide variety of viruses and bacteria have been recently detected and quantified thanks to the application of ECL, including threatening viruses such as HIV, HAV, HBV, HCV, ZIKV, TMV, coronaviruses (e.g., SARS-CoV-2), RABV, HHV and MPXV, as well as several bacteria, e.g., *Francisella tularensis*, *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus salivarius*, and *Vibrio parahaemolyticus*. The reported detection limits were usually very low, most commonly ranging

from pg/mL to fg/mL. In operational terms, the ECL platforms collected in [Table 3](#) are particularly suited to applications ranging from food and water safety monitoring to the surveillance of clinically relevant bacteria in high-risk patients, where robustness in complex matrices is critical. By contrast, many of the virus-oriented strategies collected in [Table 4](#) favour extremely low LoDs and sophisticated amplification schemes (*vide infra*), making them especially competitive for early infectious diseases detection and highly sensitive screening in clinical or reference laboratories, with selected formats already approaching true point-of-care implementation.

Furthermore, the presence in [Table 3](#) and [Table 4](#) of both widespread and emerging pathogens highlights the versatility of ECL-based biosensors in covering a wide range of infectious threats relevant for oncology, transplant medicine, and global public health. This evidence, combined with the global disparities in diagnostic accessibility between HICs and LMICs, as already discussed in the introduction, further supports the urgent need for rapid, sensitive, and cost-effective diagnostic platforms based on ECL.

2.2. ECL-based immunoassays and whole virus detection

Generally, it is known that the direct detection of a whole virus could avoid some of the pretreatment processes usually needed for the infected samples, such as complicated nucleic acid isolation/denaturation and associated steps. [145] However, this approach requires the addition into the system of high-affinity molecules able to selectively interact with the virus, e.g. antibodies, as well as their appropriate handling and management. Therefore, considering these issues, different ECL approaches have been applied for the detection of either the whole virus or a specific surface antigen (as listed in Table 4 and described in detail in the following sections). In most of the cases, sandwich immunoassays have been applied, designing different types of antibodies-conjugated ECL luminophores, including semiconductor NPs and molecular ECL luminophore-based NPs. Moreover, amplification of the ECL signal has been achieved in several ways, e.g., upon using MXene or metal NPs as electron-transfer agents, allowing the encapsulation of a large number of luminophores inside the nanoparticle, and magnetic/centrifuge-based enrichment of virus.

2.2.1. Micro- And nanoparticles-based ECL immunosensors

Even if $[\text{Ru}(\text{bpy})_3]^{2+}$ still constitutes one of the benchmarks in ECL, we have already introduced the current need for extensive research in developing new inorganic complexes, organometallic species or organic molecules able to yield high ECL intensities, especially embedded within nanosystems, [82–85] *e.g.*, in silica nanoparticles, [92] cucurbit[*n*]urils, [93,146] or cyclodextrins. [94] These systems are endowed with multifunctional features, such as specific molecular recognition capabilities, tuneable photophysical responses to external stimuli, [94] and higher quantum yields. [91–93] In this context, nanomaterials play a crucial role that ranges from the investigation of the complex mechanisms for ECL generation to the study of the electrochemical behaviour of new highly efficient dyes. In particular, the ECL properties of QDs and porous nanomaterials (mainly based on silicon and silica) [133,144,147,148] has attracted widespread attention since the ECL of silicon QDs was reported by Bard and coworkers in 2002. [149]

For example, QDs-generated ECL is nowadays widely applied in several fields, ranging from drug detection and clinical diagnosis to environmental analysis. [150–154] One recent work reported by Zhang et al. [144] has thus exploited the ECL signal enhancement provided by CdS QDs capped with 3-mercaptopropionic acid (MPA@CdS QDs) and derivatised with suitable antibodies to construct a sandwich immunosensor for Zika virus (ZIKV) detection, a mosquito-borne flavivirus which has become a major global health problem since 2017. [155] In this approach, silica structures (here, microspheres) were used as platform and conjugated with antibody-1 (Ab₁) for capturing ZIKV antigen, followed by a centrifuge-based extraction for their isolation. Eventually,

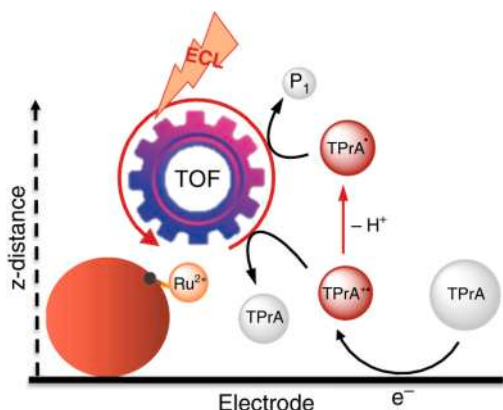


Fig. 3. Scheme of the remote ECL mechanism exploiting beads-based assay. In this case, TPrA is oxidised to the radical cation (TPrA^{*+}), which has a half-life of ~ 200 μs , and deprotonates to the radical form. TPrA^{*+} and $\text{TPrA}^{\bullet}$ react then with the $[\text{Ru}(\text{bpy})_3]^{2+}$ (Ru^{2+}) on the magnetic bead (red sphere), giving rise to surface generation–bead emission. The turnover frequency (TOF) is the number of photons emitted in 1 s by a single luminophore. Reproduced with permission from ref. [100] Copyright 2020, SpringerNature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

they were deposited on the electrode surface, and the antibody-2 conjugated QDs (QDs-Ab₂) were bound to the antigen, followed by ECL detection in the presence of persulfate as the coreactant (as from Fig. 4a). A linear response was observed from 1.0 fg/mL to 1.0 ng/mL, and the obtained LoD was as low as 0.3 fg/mL.

Switching from the use of silica microspheres to silica nanoparticles (SiNPs), instead, Dai et al. [133] has recently detected the human herpesvirus type 6 A (HHV-type 6 A). Since HHV-6 A may activate in case of impaired immunity (especially in young children) [156,157] and cause serious health hazards, e.g., fever and encephalitis, [158,159] there is a urgent need for a unique, sensitive, and convenient detection method different from the widespread used RT-PCR, as plenty highlighted in the course of the introduction. In this work, authors investigated the potential application of efficient ECL detection to this aim, exploiting novel BisL_T-Ir-NHC, where the tridentate Ir ligand possesses a distribution of electronic states more favourable for electron-transfer processes, providing higher stability and rigidity, hence reducing non-radiative deactivation pathways and enhancing the quantum efficiency and brightness of metal complexes. [160] Therefore, as evident in Fig. 4b, a classical sandwich immunoassay technique has been applied to the ECL-based detection of HHV-6 A, with BisL_T-Ir-NHC as highly efficient luminophore embedded into SiNPs functionalised with antibody-2 (BisL_T-Ir-NHC@SiO₂-Ab₂). In this case, the HHV-6 A antigen has been

detected upon modification of the ECL sensing interface by combining chitosan (CS) and MXene@Au through surface self-assembly, and then labelling the surface with HHV-6 A antibody-1 (Ab₁). BisL_T-Ir-NHC@SiO₂-Ab₂ was added to record ECL response in the presence of TPrA, observing a wide linear range of 10² to 10⁷ cps/μL and a LoD about 41.93 cps/μL. Hence, these results demonstrated the significant potential of the bis-tridentate iridium(III) complex containing a NHC ligand in developing ECL-based sensitive analytical methods for virus detection and exploring novel kinds of efficient iridium-based ECL luminophores in the future.

Furthermore, from a practical perspective, it is evident how the presence of antibodies in both configurations shown in Fig. 4 is used to ensure target selectivity, while coordination-based luminophores are embedded in micro/nanostructured supports in order to amplify the resulting ECL signal. However, in the ZIKV system, even though the use of silica microspheres combined with CdS QDs enables extremely low LoDs in serum-like matrices, the entire detection workflow requires multiple conjugation and centrifugation steps that are more compatible with centralized laboratories than with fully decentralised PoC testing. Similarly, the BisL_T-Ir-NHC complex confined in SiNPs for HHV-6 A detection provides high ECL efficiency and stability, yet at the price of a more demanding synthetic effort and stricter control over NPs functionalization. Taken together, these workflows highlight how micro/

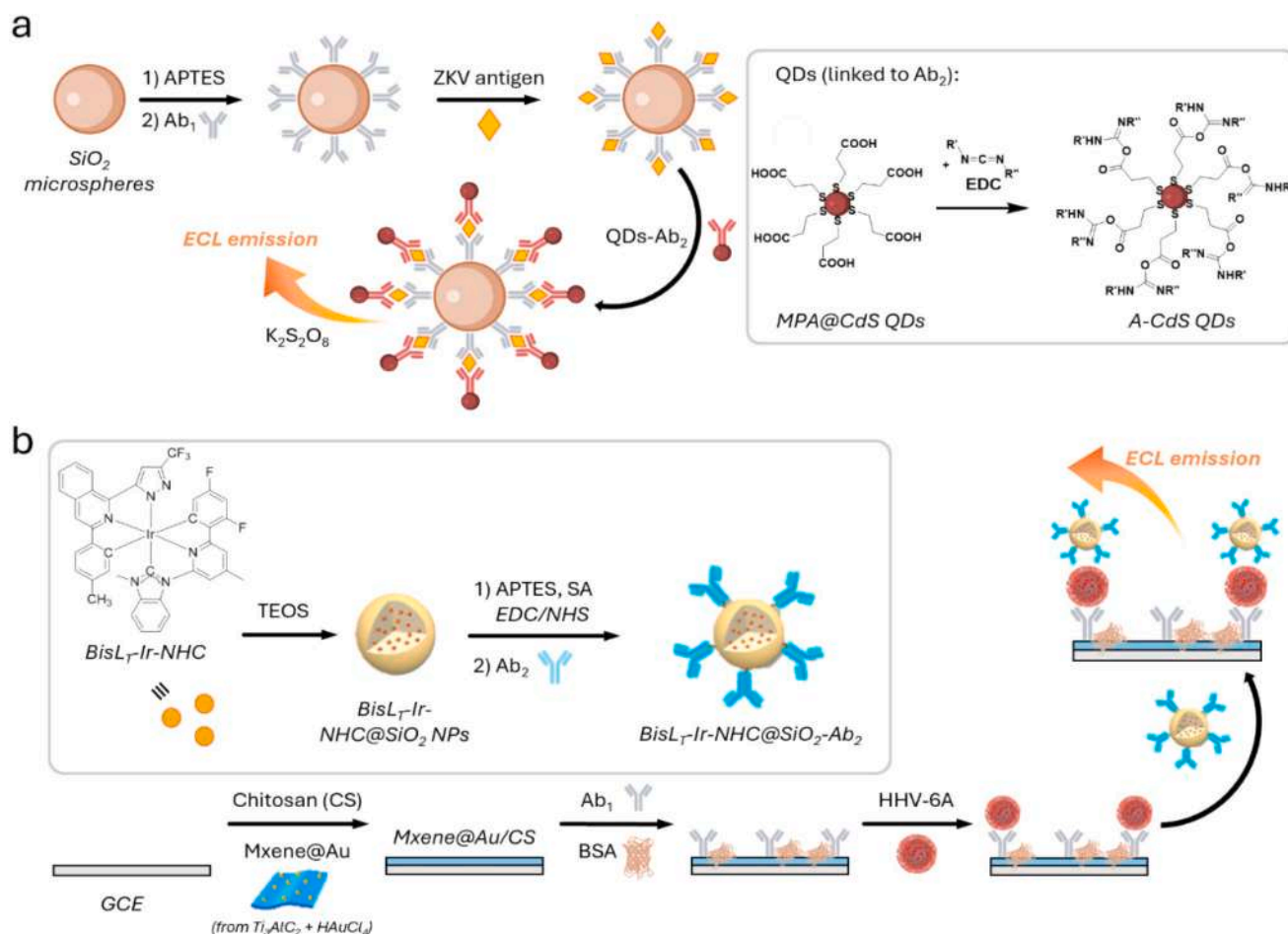


Fig. 4. a) Schematic of the fabrication process of the proposed ECL immunosensor, with preparation of the activation of MPA@CdS QDs reported in the grey square (based on ref. [144]). b) ECL-based detection and quantification of whole human herpesvirus. From left, schematic representation of the ECL-based quantification of human herpesvirus that involved CS-based electrode modification with MXene@Au (prepared via HF etching of Ti₂AlC₂ and reaction with H₂AlCl₄), antibody (Ab₁) conjugation, bovine serum albumin (BSA) blocking, virus recognition, luminophore NPs labelling and ECL detection. In the grey square, preparation of Ir(III) complex-based NPs via tetraethylorthosilicate (TEOS) hydrolysis in the presence of Ir(III) complex is reported, followed by reaction with 3-aminopropyltrimethoxysilane (APTES), succinic hydride (SA), and antibody (Ab₂) conjugation via EDC coupling. Adapted with permission from ref. [133] Copyright 2024, American Chemical Society.

nanostructured coordination systems can be integrated into classically established immunoassay formats to push sensitivity, while current implementations remain best suited to well-equipped analytical settings.

Moreover, mesoporous nanostructures – in particular, vertically-ordered mesoporous silica films (VMSF) [161,162] – have been employed as well for the development of wireless and real-time monitoring electrochemical (EC) and ECL sensors. [163,164] As a recent example, in 2024 Zhang et al. [141] designed a cost- and time-effective ECL sensor for the quantitative detection of the worldwide known respiratory virus SARS-CoV-2 in saliva and pond water samples, exploiting bipolar silica nanochannel array (bp-SNA). As highlighted in Fig. 5, bp-SNA consists of homogeneous two-layer mesoporous silica films bearing inner silanol groups and outer amino groups, generating a solid “electrostatic nanocage” for stable confinement of the ECL luminophore (i.e., $[\text{Ru}(\text{bpy})_3]^{2+}$) and Au NPs, and further providing functional sites for covalent modification of SARS-CoV-2 IgG antibody, here used as the biorecognition element ($\text{Ab}/\text{Ru}@\text{AuNPs}/\text{bp-SNA}/\text{ITO}$). In this case, the confinement of Au NPs has been used for providing 24% ECL intensity enhancement with respect to the only $[\text{Ru}(\text{bpy})_3]^{2+}/\text{TPrA}$ couple, due to the catalytic oxidation effect of Au NPs toward TPrA. [165,166] Hence, owing to the preconcentration capacity of bp-SNA and amplified effect of Au NPs, EC and ECL signals of $[\text{Ru}(\text{bpy})_3]^{2+}$ can be remarkably promoted and thereby increase the analytical performance, which can be then diminished by immunorecognition of target SARS-CoV-2 pseudoviruses on the sensing interface. Thus, the developed integrated ECL/EC sensor based on $\text{Ru}@\text{AuNPs}/\text{bp-SNA}$ modified solid indium tin oxide (ITO) electrode enabled the sensitive analysis of SARS-CoV-2 pseudoviruses with a linear range of 50 TU/mL–5000 TU/mL and a LoD of 31 TU/mL. Moreover, the designed sensor showed satisfactory results in the analyses of saliva and pond water samples, and, when a flexible electrode substrate such as polyethylene terephthalate was employed, $\text{Ru}@\text{AuNPs}/\text{bp-SNA}$ showed great potential to be integrated with KN95 face masks for direct detection of SARS-CoV-2 pseudoviruses produced from breathing, talking and coughing processes, which could in principle provide an efficient platform for point of care diagnosis. The architecture and sensing workflow reported in Fig. 5 hence simplifies the assay operation to a sequence of electrode modification, antibody immobilization, virus binding, and ECL readout, and takes advantage of the preconcentration effect and catalytic activity of Au NPs to enhance the resulting analytical signal. At the same time, the fabrication of well-defined bp-SNA films and their integration on flexible substrates still

require controlled deposition procedures and instrumentation that are more typical of research or specialised laboratories. As such, this strategy exemplifies how advanced mesoporous coordination-related architectures can bridge high performance and increasing portability, while further simplification will be needed before widespread PoC deployment.

Overall, from the analysis of the examples collected in this section, ECL-based immunoassays for whole virus detection result to be still mainly suited to centralized laboratories and high-throughput clinical testing, where robust antibody panels and stringent sample handling can be guaranteed. In these formats, sandwich architectures and micro/nanoparticles-based amplification reliably push detection limits into the low pg/mL or even fg/mL range, but they remain constrained by antibody availability, cross-reactivity, and the need for cold-chain logistics. Therefore, whole virus ECL immunoassays are ideal for confirmatory diagnostics and quantitative monitoring in well-equipped settings, whereas their use at the point-of-care level or in low-resources environments is still limited by cost, instrumentation, and assay complexity. Particularly, in the broader context of infectious disease diagnostics, these technologies complement, rather than replace, simpler serological tests and nucleic acid-based assays.

2.3. ECL detection of whole bacteria

Concerning instead the direct detection of whole bacteria, this approach possesses the advantage of avoiding any additional step in the biosensing workflow, mainly related to the isolation of nucleic acid or proteins, coupled with a minimal use of costly chemicals/biochemicals, which renders it suitable for rapid identification. [167] Moreover, antibodies or other high-affinity molecules, whose reliability is commonly verified by several studies, are usually already available for the selective detection of many of the most threatening bacteria. [168] Therefore, taken all together, ECL-based biosensors exploiting such approach have been developed for the detection of many different bacteria (as from Table 3), including *S. aureus*, *F. tularensis*, *E. coli* and *V. parahaemolyticus*. Various ECL-active probes have been used in these methodologies, including conventional $[\text{Ru}(\text{bpy})_3]^{2+}/\text{TPrA}$ or luminol/ H_2O_2 couples, as well as newly designed ECL luminophores, such as graphene QDs or aggregated tetraphenylethylene derivatives. All of them can be either directly used as such or incorporated into silica NPs, metal-organic frameworks (MOFs), or porous carbon NPs. In addition, ECL

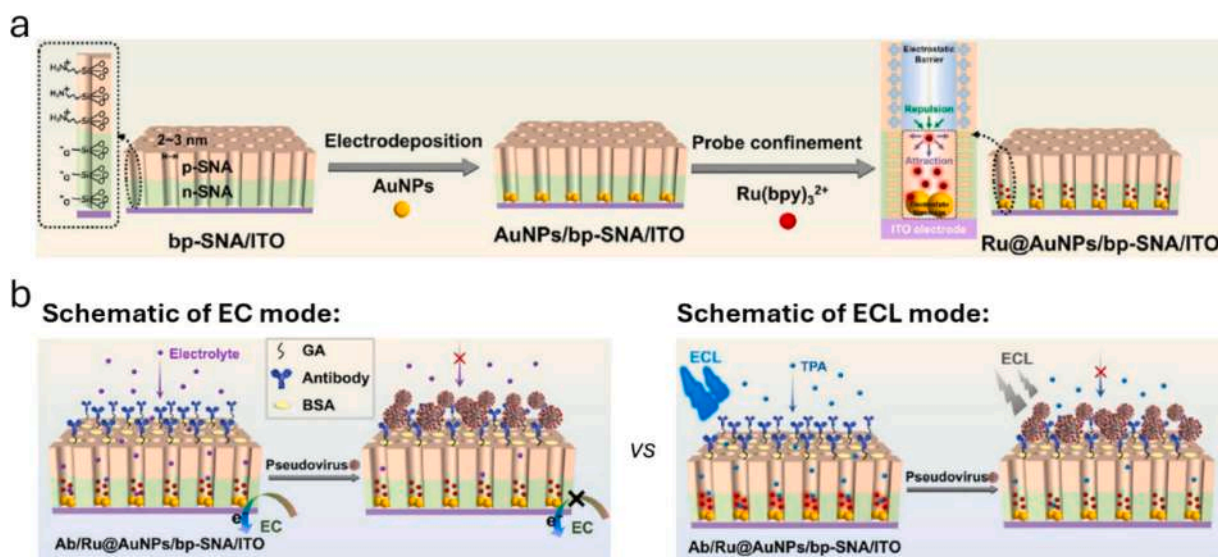


Fig. 5. Schematic illustration of a) preparation of $[\text{Ru}(\text{bpy})_3]^{2+}$ and Au NPs incorporated bipolar silica nanochannel array (bp-SNA) as electrode material, and b) analytical mechanism of ECL sensors for SARS-CoV-2 determination that involved antibody functionalization of electrode using glutaraldehyde (GA), BSA blocking, virus recognition and ECL detection. Adapted with permission from ref. [141] Copyright 2024, Elsevier B.V.

imaging-based approaches have been also explored as an advanced detection modality. [167] To be mentioned that, in many of the reported cases, the developed biosensors have been further tested for the real-time monitoring of selected pathogens directly present in contaminated food/water/juice/milk, and extended toward point-of-care applications. [113]

2.3.1. Oligonucleotide-based approach

In recent years, the detection of several pathogens has been effectively advanced by the efficient identification of various bacteria-specific aptamers, where an aptamer is identified as a short single-stranded DNA/RNA sequence obtained through the systematic evolution of ligands by exponential enrichment (SELEX) technique, which enables it to recognise and bind a target pathogenic receptor with high specificity and affinity. [169,170] Aptamers-based recognition of bacteria thus holds the ability to significantly enhance the detection selectivity and sensitivity, similarly to what is performed with antibodies, holding the possibility to be further coupled with conventional PCR-based signal amplification approaches.

For example, Feng et al. [112] detected *S. aureus* (a methicillin-resistant pathogen causing a wide range of infections, additionally releasing enterotoxins that induce food poisoning) by employing an *S. aureus* specific aptamer, $[\text{Ru}(\text{bpy})_3]^{2+}$ as the ECL-active luminophore and TPrA as the coreactant (as reported in Fig. 6). Particularly, *S. aureus*-responsive cellulose paper was prepared by employing the aptamer as the recognition element and a DNA probe tagged with glucose oxidase (probe DNA-GOD) as the signal amplification unit. The recognition of *S. aureus* mediated then the quantitative release of the DNA probe, used for ECL-based quantification of the target pathogen. Furthermore, in order to improve ECL performance, porous zinc-based MOFs were synthesized by mixing zinc salt and terephthalic acid,

following which a large number of $[\text{Ru}(\text{bpy})_3]^{2+}$ molecules were embedded inside the pores via heating for several hours (Ru-MOF-5 NFs). Next, the electrode was modified with this material via electrostatic interaction with poly(diallyldimethylammonium chloride) (PDDA). The developed approach shows a linear relationship of the ECL signal with *S. aureus* concentrations ranging from 5 CFU/mL to 10^8 CFU/mL, with a LoD of 1 CFU/mL, and its detection abilities have been demonstrated in real-time monitoring of *S. aureus*-contaminated food samples. Moreover, excellent flexibility and skin adherence of the sensor enabled evaluation of *S. aureus* contamination on rough sample surfaces and can be extended for PoC testing of foodborne pathogenic bacteria.

In particular, the ability of the reported configuration to separate recognition and signal-generation steps is extremely attractive for on-site or irregular surfaces detection, where the mechanical adaptability of paper improves sampling, while the use of MOFs ensures high local luminophore loading and signal amplification. Nevertheless, preparation of the Ru-MOF-5 and the need for glucose oxidase stability and careful calibration still argue for use in semi-centralized laboratories or in well-controlled PoC devices rather than fully disposable, ultra-low-cost strips.

In another work, *S. aureus* has been detected by Wu et al. [115] using luminol/ H_2O_2 as ECL couple, and an *S. aureus*-specific aptamer (T strand) was conjugated with Fe_3O_4 NPs and used for target hybridization and extraction. Similarly, Sun et al. [116] exploited an *S. aureus*-specific aptamer and $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPrA as ECL couple. In these works, various amplification strategies were employed for sensitive ECL detection, including strand-displacement reaction, hybridization chain reaction (HCR) amplification, DNA walker approach, and Au:Ag NPs or MoS_2 nanoflowers (NFs)-based enhancement. These approaches can detect *S. aureus* in a range of 1 CFU/mL– 10^7 CFU/mL and have been applied to the detection of *S. aureus* in contaminated real samples, such as water/

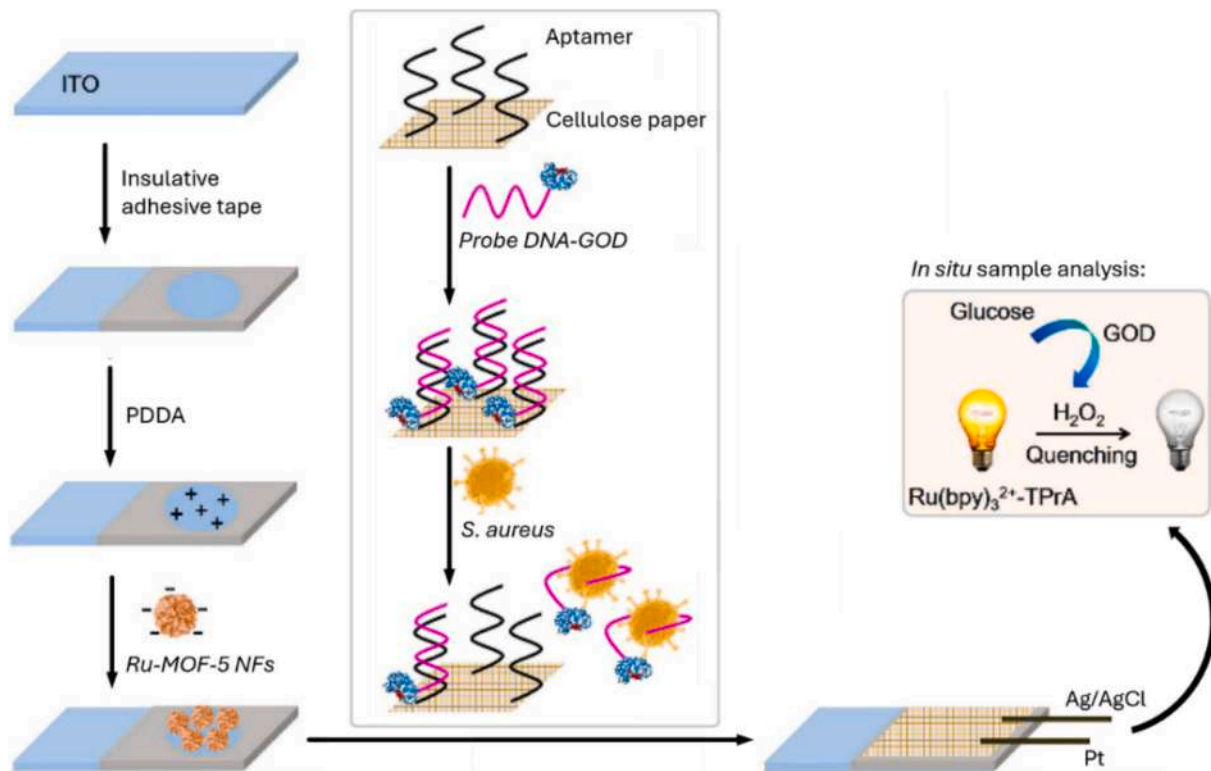


Fig. 6. Schematic illustration of a paper-based ECL biosensor for *S. aureus* in real samples. $[\text{Ru}(\text{bpy})_3]^{2+}$ molecules were embedded into porous zinc-based metal-organic frameworks (Ru-MOF-5 NF) and then ITO electrode was modified with this material via electrostatic interaction with poly(diallyldimethylammonium chloride) (PDPA). Cellulose paper was modified with aptamer and glucose oxidase (GOD) tagged probe DNA (probe DNA-GOD) via chitosan coating. The formation of aptamer-*S. aureus* complex mediated the quantitative release of probe DNA-GOD. The remaining probe DNA-GOD on the paper was then activated by glucose which resulted in a decrease in ECL signal. Adapted with permission from ref. [112] Copyright 2024, Elsevier B.V.

juice/milk.

In a similar approach, in 2024 the bacterium *E. coli* has been detected through the use of an *E. coli*-specific aptamer coupled with tetraphenylethene (TPE)-based nanostructures as ECL-active luminophores by He et al. [107] and Lian et al. [108]. More specifically, the two methodologies commonly relied on the modification of a gold electrode surface with a suitable aptamer for *E. coli* capturing, followed by labelling with TPE-based nanostructures. These two examples further showed that tetraphenylethene derivatives-based ECL can represent a powerful alternative to conventional ruthenium/iridium complexes-based emitters. Being TPE derivatives well known aggregation-induced emitters, they have also recently been reported with aggregation-induced ECL (AIECL) property. However, controlling the aggregation states of tetraphenylethene derivatives in order to obtain uniform aggregates with intense ECL emission is challenging. Therefore, the reported examples (*vide infra*) illustrate the development of significantly novel strategies, where aggregation states of TPE derivatives have been controlled either by exploiting their molecular amphiphilicity – inducing the aggregation between molecules or leading to assembly formation – or *via* covalent organic polymerization, *i.e.*, the process of linking organic monomers *via* covalent bonds that generate porous solid

polymers. Particularly, Lian et al. [108] used tetraphenylethene-based covalent organic polymer (TPE-COP) nanoparticles as ECL luminophores, resulting in an 8-fold higher ECL signal with respect to the one of typical tris(bipyridine)ruthenium(II) emitters (see Fig. 7a). Here, gold electrodes were modified with an *E. coli* aptamer for target capturing, followed by passivation and ECL luminophore adsorption. The synthesized TPE-COP NPs were positively charged, hence enabling their high affinity toward negatively charged bacterial surfaces *via* electrostatic attraction. This resulted in strong ECL signals that were closely associated with the *E. coli* concentration, detected in a linear range of 10^2 – $5 \cdot 10^6$ CFU/mL, with a LoD of 0.2 CFU/mL. In contrast, He et al. [107] synthesized three different tetraphenylethene derivatives carrying a pyridinium group, an alkyl chain, and a quaternary ammonium group, exhibiting a significantly red-shifted luminescence and enhanced ECL emission due to the tuneable reduction of the HOMO–LUMO energy gap (the best one being the C₃ nanoaggregate in Fig. 7b). Here, the amphiphilicity of the as-developed molecules enabled their spontaneous self-assembly into well-controlled spherical nanoaggregates, reporting an ECL intensity 17-fold enhanced compared to that of the original 4-(4-(1,2,2-triphenylvinyl)phenyl)pyridine molecule. [107] These cationic nanoaggregates demonstrated high affinity toward the target bacteria as

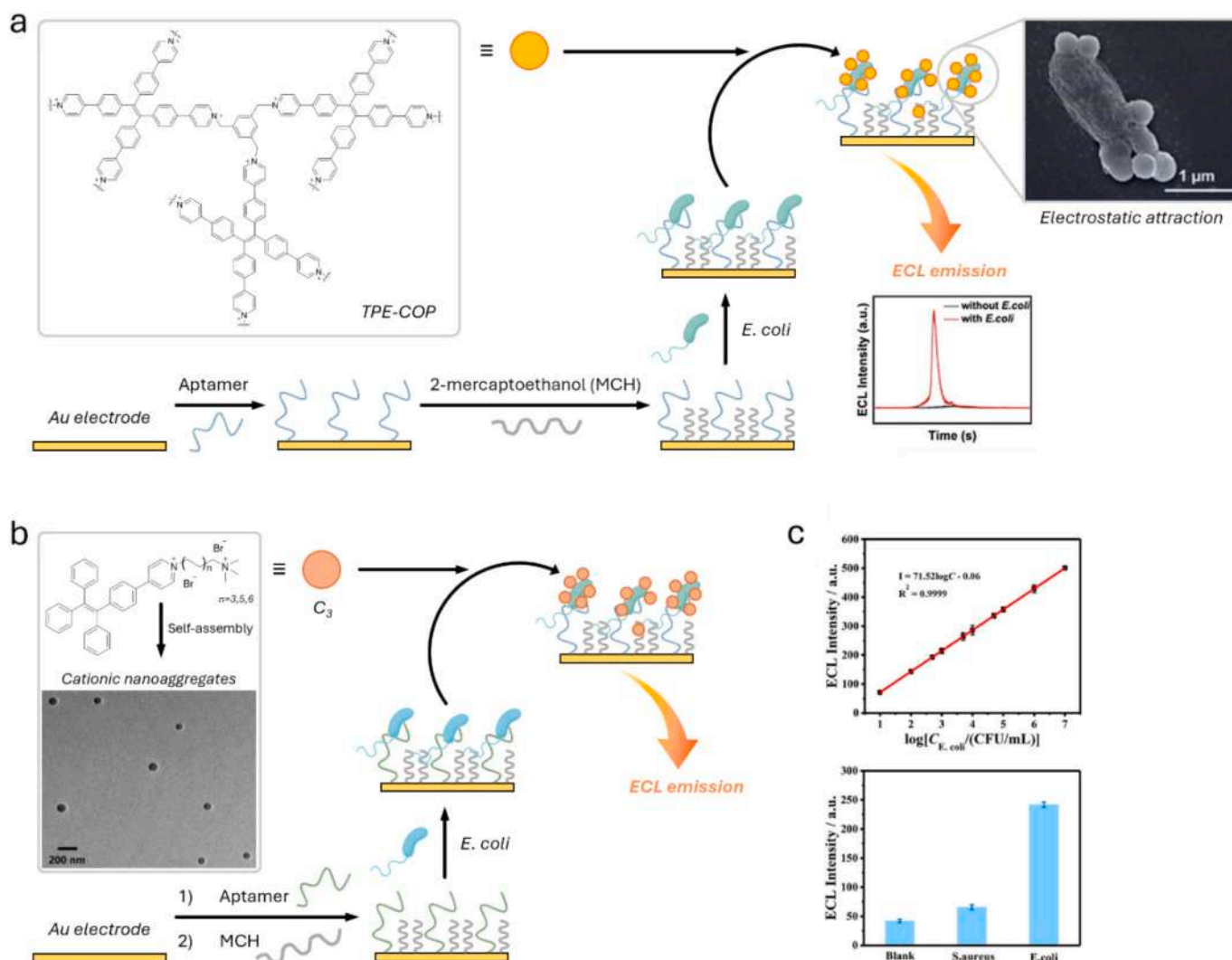


Fig. 7. a) Chemical structure of TPE-COP (grey square) and schematic mechanistic illustration of the construction and detection of the TPE-COP-based *E. coli* biosensor. Adapted with permission from ref. [108] Copyright 2024, American Chemical Society. b) Chemical structure of C₃ (and related cationic nanoaggregates, TEM image prepared in mixed water/THF solvents, $v/v = 90\%$, in grey square), with corresponding ECL-based detection mechanism of *E. coli*. c) On the top, related calibration curve between the ECL intensity and the logarithm of *E. coli* concentrations ($n = 3$). On the bottom, compared ECL responses of 10^3 CFU/mL *E. coli* and *S. aureus*, respectively. Adapted with permission from ref. [107] Copyright 2024, American Chemical Society.

well, thus an ECL sensor was developed with a broad linear range ($10\text{--}10^7$ CFU/mL) and good selectivity in the presence of an *E. coli*-specific aptamer (LoD = 1.99 CFU/mL).

Therefore, from the comparison of the two workflows reported in Fig. 7a and b, it is evident that both platforms efficiently demonstrated how AIECL luminophores can effectively substitute classical e.g. Ru(II)-based ECL-active complexes, offering higher brightness and tuneability, even though their performance remains tightly linked to controlling aggregate size, surface coverage, and the chemical stability of the resulting organic framework. Consequently, they appear particularly well suited for high-sensitivity detection in research and reference laboratories, and may serve as a basis for future PoC devices once synthetic and surface-engineering steps will have been further streamlined.

In another example, Li et al. [117] engineered a bipolar-electrode (BPE) ECL platform for the detection of *Salmonella typhimurium* (a Gram-negative foodborne pathogen able to infect a broad range of hosts, constituting the leading cause of food poisoning worldwide) [171,172] by combining the aptamer-based selective detection with a molecular imprinting approach. More in detail, polymerization of dopamine (DA) was performed in the presence of the target bacterium as “template”; after template removal, a cavity working as “negative image” of the target was left on the resulting polydopamine (PDA) bacterial surface-imprinted polymer (SIP) on the cathode. This system was combined with a ferrocene (Fc)-labelled aptamer as the recognition/signal unit: the Fc-aptamer complex was thus bound to the captured cells on the modified cathode, modulating the reaction occurring at the spatially separated anode upon addition of the $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPrA ECL-active couple. It should be mentioned here that this device operates by applying a high driving bias (5.2 V) across the closed BPE, first enabling Fc oxidation at the cathode and subsequently driving $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPrA oxidation and light emission at the anode within the same configuration. Under optimized conditions, a logarithmic linear range from 10 CFU/mL to 10^6 CFU/mL, with a LoD of 10 CFU/mL were reported, although the relatively elevated operating voltage may limit compatibility with more delicate electrode materials or low-power setups.

Furthermore, other two works built ECL biosensors for the detection of *Vibrio parahaemolyticus* and *Staphylococcus aureus* through the use of functionalized DNA as luminescent probes. In particular, Zhang et al. [118] detected *V. parahaemolyticus* (a common pathogenic bacterium in aquatic products) using Au NPs decorated with polyethyleneimine/multi-walled carbon nanotubes (AuNPs@PEI/MWCNTs), which improved both the ECL signal and the conductivity of the biosensor, providing a larger surface area to load more luminescent groups. Moreover, ordered mesoporous carbon (OMC) was used to increase the loading/incorporation of $[\text{Ru}(\text{bpy})_3]^{2+}$, absorbing it and creating OMC@[$\text{Ru}(\text{bpy})_3]^{2+}$ -DNA2 systems used as luminescent and capture probes for specific recognition of *V. parahaemolyticus* and for generating enhanced ECL signals. This method displayed detection sensitivity in the range $7.04\text{--}1\cdot 10^7$ CFU/mL, a LoD of 2.11 CFU/mL, and it has been successfully applied to the detection of *V. parahaemolyticus* in scallops, suggesting the practical utility of the developed method. Concerning instead *S. aureus* detection, Liu et al. [113] exploited always the $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPrA ECL-active couple, first building a BPE modified with capture probes able to bind to the three-dimensional structured DNA network resulting from the bacterium recognition. Indeed, target addition stimulated the release of T strand from the double-stranded DNA on Fe_3O_4 NPs, which initiated a PCR amplification resulting in the DNA network creation upon Au NPs-labelled probes addition and subsequent polymethylene blue NPs (PMB NPs) loading, leading to the enhancement of ECL intensity. This approach displayed a *S. aureus* detection limit of 0.78 CFU/mL and concentration-dependent linearity in the range $10\text{--}10^8$ CFU/mL. This work hence showed how coupling of ECL with PCR can be adapted for sensitive and accurate detection of pathogenic bacteria.

2.3.2. Antibody-based approach

As from the general spread of immunoassays applied to the field of medical diagnostics, it is commonly known that antibodies can be generally applied to various transduction methodologies to enhance the procedure's detection selectivity. Thus, antibody-based approaches have been commonly adapted for the detection of various bacteria using different ECL-active systems, especially the well-known $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPrA couple [109] or nitrogen-doped graphene quantum dots (N-GQDs) with $\text{K}_2\text{S}_2\text{O}_8$ as coreactant. [106] In particular, *F. tularensis* was detected in 2016 by Spehar-Délèze et al. [109] using $[\text{Ru}(\text{bpy})_3]^{2+}$ -encapsulated silica NPs linked to a secondary antibody (for the signal transduction). The screen-printed gold electrode arrays were instead modified with the related capture antibody. Thus, after bacterium addition and $[\text{Ru}(\text{bpy})_3]^{2+}$ -labelled reporter antibody introduction, the system was washed and the ECL signal recorded upon TPrA addition. The assay was performed in a fluidic chip reporting a LoD of 0.4 ng/mL for the detection of lipopolysaccharide (LPS) from *F. tularensis* and of 70/45 bacteria/mL for the whole inactivated *F. tularensis* live vaccine strain (LVS) bacteria (depending on whether the capturing molecule was the whole Ab or the F(ab) fragment). This is one of the earlier reports showing how ECL biosensing can overcome the sensitivity typical of conventional enzyme-based immunosorbent assays (ELISA) or even of electrochemical assays.

Similarly, *E. coli* O157:H7 was detected by Chen et al. [106] using an antibody-based detection approach where molecular imprinting was combined with N-GQDs as efficient ECL-active probes (reported in Fig. 8). In particular, the electrode was modified with PDA via electropolymerization of dopamine in the presence of bacteria. Next, the bacteria were removed to generate PDA-based *E. coli* imprint on the electrode surface and for specific binding of *E. coli*. Separately, N-GQDs were synthesized and conjugated to the reporter polyclonal antibody (pAb). For ECL measurement, then, *E. coli*-imprinted electrode was treated with *E. coli*, followed by labelling with pAb-N-GQDs. This approach showed a linear relationship between ECL intensity and *E. coli* concentration in the range $10\text{--}10^7$ CFU/mL, with a limit of detection of 8 CFU/mL, demonstrating that graphene QDs can effectively serve as novel, sensitive and selective ECL probes. Furthermore, it has demonstrated the applicability of imprinting approach for selective *E. coli* detection in the presence of other bacteria.

2.3.3. ECL imaging approach

In recent years, ECL imaging was identified as a possible alternative approach to detect whole bacteria with high sensitivity. Indeed, ECL has lately gained significant attention in the microscopy field due to its ability to provide useful faradaic and optical information (current and light emission, respectively) about the investigated system, [173,174] operating without external light sources. [175] Moreover, since the emitted light can be spatially resolved, ECL combined with optical microscopy (ECLM) constitutes a powerful imaging method, capable of visualizing objects that participate in or alter the ECL reactions near the electrode surface. [176,177] For these reasons, ECLM has been implemented to resolve single (biological) objects, and, in specific cases, even single molecules. [71,178–182] Thus, imaging-based approaches represent one of the most advanced detection approaches, which can be further coupled with other conventional detection methodologies, obtaining sensitivity down to the single bacterium.

This particular imaging technique has been already presented in 2017 [176] for visualizing and mapping cell membranes; successively, in 2023, it was adapted by Zhou et al. [120] for the detection of whole bacteria with a sensitivity down to the single bacterium (as reported in Fig. 9). In this work, the ECL luminophore $[\text{Ru}(\text{bpy})_3]^{2+}$ was coupled with 2-(dibutylamino)ethanol (DBAE) as coreactant, adding the resulting solution to the target bacteria deposited on the electrode surface, followed by ECL imaging. Since the deposition of bacteria weakens the electron-transfer probability between the ECL system and the electrode, ECL imaging down to the single bacterium can be already achieved with

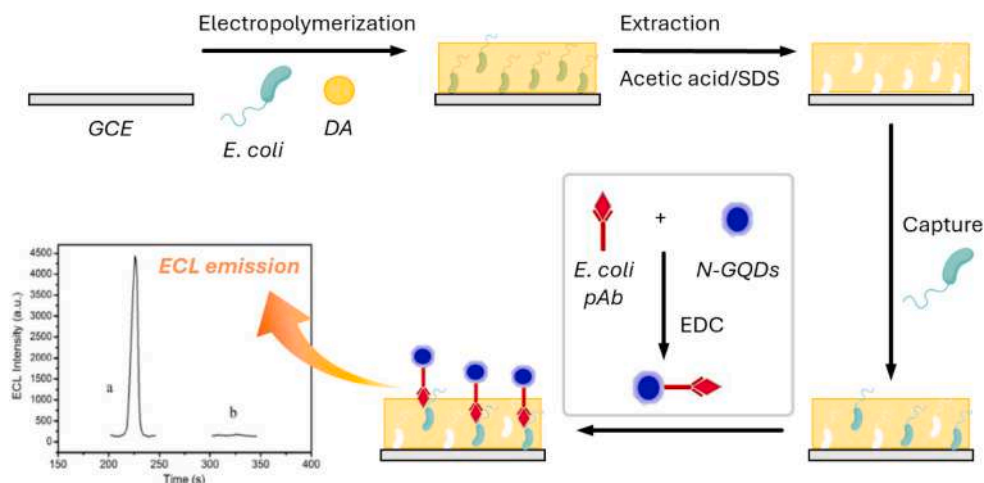


Fig. 8. Schematic representation of the fabrication procedure of the ECL biosensor for *E. coli*. Dopamine was electropolymerized directly on the electrode surface in presence of *E. coli* and then *E. coli* was removed in the presence of acetic acid and a surfactant (sodium dodecyl sulfate, SDS), generating the *E. coli* imprinted polydopamine at the electrode surface. Next, *E. coli* was captured at the electrode via specific recognition with the *E. coli* imprint. N-GQDs were conjugated with polyclonal antibody (*E. coli* pAb) via EDC-coupling and used to label *E. coli* at the electrode via interaction with pAb. Finally, ECL was measured in the presence of $K_2S_2O_8$ as coreactant, obtaining a good ECL intensity response (curve a, *E. coli* O157:H7 10^4 CFU/mL). On the other hand, upon addition of a non-specific target, such as *Salmonella* (curve b, 10^4 CFU/mL), no ECL increase was observable. Adapted with permission from ref. [106] Copyright 2017, American Chemical Society.

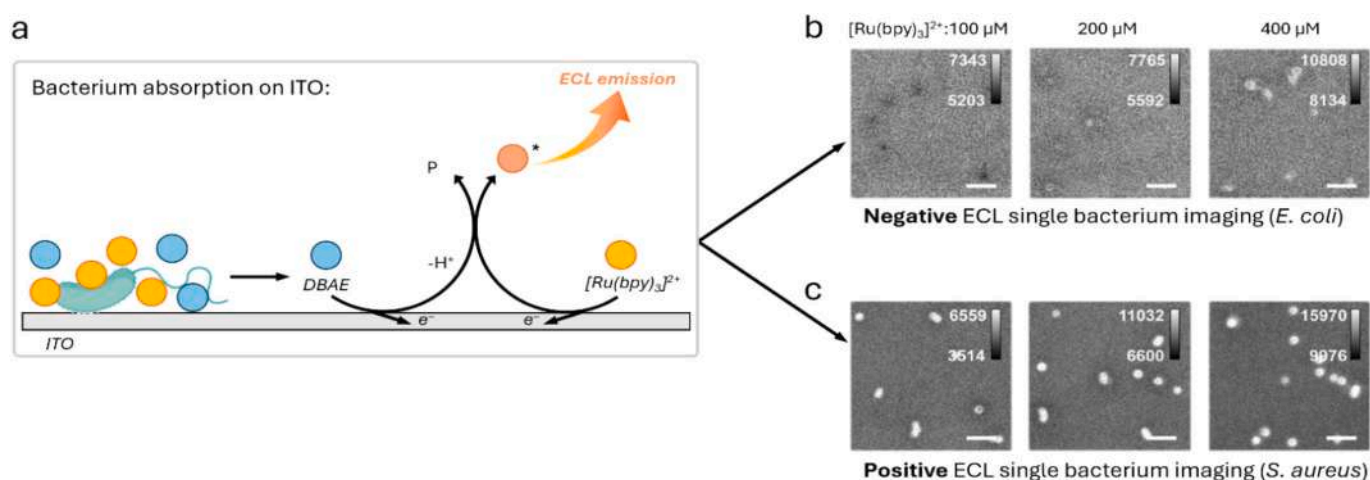


Fig. 9. a) Schematic illustration of tuneable single-bacterium imaging using ECL microscopy. Bacteria are deposited on an ITO electrode used as working electrode, with Ag/AgCl as reference electrode (RE), and Pt as counter electrode (CE), and ECL imaging is performed in the presence of 100 mM 2-(dibutylamino)ethanol (DBAE) as coreactant and $400 \mu\text{M}$ $[\text{Ru}(\text{bpy})_3]^{2+}$ in PB 0.2 M, by applying a voltage of 1.4 V. Reported are, from left to right, ECL images of b) *E. coli* and c) *S. aureus*, upon increasing $[\text{Ru}(\text{bpy})_3]^{2+}$ concentration (100, 200, $400 \mu\text{M}$) in PB 0.02 M, with an EM gain 50 and an exposure time of 10 s. Scale bar: $5 \mu\text{m}$. In b), ECL imaging with negative contrast has been performed (without luminophore, deposition of bacteria weakens the electron-transfer probability between the ECL system and the electrode compared with the exposed ITO surface), while ECL imaging with positive contrast has been reported in c) images (absorption of positively charged luminescent molecules on the bacterial cell wall, mostly negatively charged). Adapted with permission from ref. [120] Copyright 2023, American Chemical Society.

a negative contrast. In addition, positive imaging contrast of bacteria was effectively performed by decreasing the concentration of the phosphate buffer and increasing the concentration of the luminophore, as this condition favours the adsorption of $[\text{Ru}(\text{bpy})_3]^{2+}$ on the bacterial surface. The presented ECL microscopy thus further offers high spatio-temporal resolution and rapid bacterial counting and classification. However, selection of the appropriate ECL luminophore, optimization of labelling and imaging protocol, and the development of imaging set up are usually critical. This work therefore demonstrates the application of ECL for structure-specific imaging of microorganisms along with the quantitative information of chemicals/biochemicals. However, a major limitation of this approach over conventional fluorescence imaging is represented by the coupling of the electrochemical setup with the biological sample, which is practically difficult in most cases. Nevertheless, high sensitivity with nearly zero background noise from the biological

sample offers a great advantage for ECL imaging and thus indigenous approaches would be developed in near future to overcome the existing limitations. Furthermore, elements for increasing the selectivity (e.g., the use of antibodies) are still needed.

A recent work by Wang et al. [183] has further shown that, beyond classical Ru(II) complexes, polymer dots can be also engineered to deliver exceptionally high ECL efficiencies for cellular imaging. Particularly, authors introduced an aggregation-induced resonance energy transfer (AET) mechanism by using AIE-active 6,6'-(9H-fluorene-9,9-diyl)bis(*N,N*-diethylhexan-1-amine) as energy donor and a typical TADF molecule, DMAC-TRZ, as energy acceptor to simultaneously enhance exciton utilisation and photoluminescence quantum yield of ECL emitters. The resulting self-enhanced systems achieved an ECL efficiency of 92.6% (at about +0.96 V), leading to hydrazide-functionalised ECL probes to image cell-surface glycans with improved signal-to-noise

ratios compared to matched fluorescence probes, highlighting how advanced emitter design can expand the scope of ECL bioimaging.

Hence, taken together, all the reported ECL-based detection strategies generally targeting whole bacteria span from highly specific oligonucleotide- and antibody-based recognition to imaging approaches able to map spatial distributions at the single-molecule or single-cell level. Overall, oligonucleotide probes and aptamers offer tuneable specificity and are well suited to multiplexing, but they often require careful optimisation of hybridisation conditions and may be sensitive to matrix effects in complex clinical samples. Antibody-based strategies, in turn, provide straightforward assay formats and strong affinity, yet they share the same limitations as the previously reported viral immunoassays in terms of reagents' cost and stability. On the other hand, ECL imaging approaches add valuable mechanistic and spatial information to the target detection itself – including multiplexing – and, fuelled by recent advances in highly efficient nanoemitters such as ARET-enabled polymer dots, are increasingly capable of delivering high-contrast visualisation of bacterial surfaces and cell-associated structures. At present, however, they still demand sophisticated optical-electrochemical instrumentation and remain best positioned in research or reference laboratories rather than routine (and possibly PoC) diagnostics.

2.4. Pathogenic nucleic acid as target for ECL-based detection

In the last years, many viruses and bacteria detection strategies have been reported using the respective pathogenic nucleic acid as target, some of the most recent examples collected in Table 3 and Table 4. Especially, a key advantage of directly detecting the pathogenic genome is that each virus/bacterium possess a specific nucleic acid sequence that offers extremely good detection selectivity. In some cases, this approach has been further coupled with conventional PCR-based amplification to increase even more the sensitivity of the methodology, but at the expense of the entire detection workflow simplicity, typical of point-of-care systems. In most of these approaches, conventional $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPRA has been used as ECL-active couple, even though various new generations of ECL luminophores, such as heteroatom doped carbon dots, Ir-complexes, CdSe QDs, MXene QDs, EuS NPs, and ultrasmall SiO_2 NPs aggregates, have also been designed (see Table 4). The main focus was to achieve an efficient ECL signal enhancement by incorporating multiple ECL-active molecular probes inside a single porous nano-architecture, coupled with MMB-based extraction, or metal NPs/graphene/MXene-based signal enhancement. In some cases, the developed methodologies have also been tested on real clinical samples and for the analysis of serum samples of hospital patients. Moreover, disposable electrode-based detection and point-of-care testing of pathogenic nucleic acids have been proposed without requirements for trained personnel or specialised equipment.

2.4.1. Viral nucleic acid detection

An extremely popular approach, especially for the diagnosis of infectious diseases, relies on the “indirect” detection of a virus using its (extracted) nucleic acid as diagnostic target. This particular approach joins the advantages of extremely selective detection, as each virus has obviously a specific nucleic acid sequence, and of offering a generally higher sensitivity compared to the use of the whole pathogen or of some other biomarkers as targets. However, additional steps are necessary in the building of the biosensing system for the isolation/manipulation of the nucleic acid, which are generally time-consuming, costly, and could require highly-trained personnel. [184] As we have previously summarised in Table 4, this approach has been commonly applied especially to the quantification of threatening viruses like HIV, HCV, and TMV. Consequently, a significant number of works have been reported in literature for the ECL-based detection of these viral nucleic acids (NAs), mainly relying on the use of Ru-complexes as ECL luminophores, commonly enclosed in NPs to enhance the detection sensitivity. In these

systems, two different oligonucleotides strands are commonly employed, one for anchoring the selected analyte to a surface (including the one of MMBs), and the other one to perform the detection, carrying the ECL-active probe.

One representative example, reported in 2017 by Liao et al. [122] (and depicted in Fig. 10), focused on the quantification of the hepatitis B virus surface antigen (HBsAg) gene through a linear polymer (synthesized by using poly-L-lysine as binding skeleton) conjugated with multiple units of $[\text{Ru}(\text{bpy})_3]^{2+}$, further linked to a complementary DNA probe for viral NA recognition (signal probe). After target binding to the signal probe, a suitable biotin-labelled capture probe was further added, providing the capturing sites for streptavidin-functionalised magnetic beads, which were then magnetically attracted to the electrode for the generation of the ECL signal. The $[\text{Ru}(\text{bpy})_3]^{2+}$ -polymer has been also used, by the same group, to derivatize an antibody for carcinoembryonic antigen to demonstrate the validity of this amplification strategy in immunoassays. This work thus demonstrated the unique advantage of poly-L-lysine as compared to luminophore-conjugated/doped NPs. The repetitive amino groups of each L-lysine monomer in the polymer were used as binding sites for $[\text{Ru}(\text{bpy})_3]^{2+}$, while the single carboxyl group served as the site for the conjugation of oligonucleotides/antibodies. The resulting probe displayed higher water solubility due to the high solubility of the parent polymer. In contrast, the poor stabilities of the colloidal dispersions of luminophore-conjugated/doped NPs greatly limited the labelling processes. Hence, this new polymer-amplified ECL assay potentially represents a new paradigm for amplified ECL assays that may represent an additional tool in the field of ECL technologies.

In a similar and more recent approach, oligonucleotide-based molecular beacons were used for the detection of HBV/HCV DNA by Luo et al. [126] and Ding et al., [128] relying on the simultaneous conjugation with a fluorophore on one side and a fluorescence quencher on the other side of the probe, far away from each other. The hairpin-shaped structure of the beacon, predominant in the absence of the analyte, led the fluorophore in close proximity to the quencher, causing a strong quenching of its fluorescence. Emission intensity can be then restored when the molecular beacon binds to the target NA sequence, leading, upon recognition, to its elongation and breaking of the hairpin structure. Particularly, in the first reported example, [126] CRISPR/Cas12a was combined with hybridization chain reaction (HCR) amplification, using $[\text{Ru}(\text{bpy})_3]^{2+}$ -doped silica NPs conjugated with a “trigger” DNA, resulting in charge-controlled luminescent body ($\text{Ru}@\text{SiO}_2$). Upon target recognition, the trans-cleavage activity of Cas12a was triggered, inhibiting HCR amplification and consequently resulting in less electrostatic repulsion with the negatively charged electrode, coupled with removed hindering of electron and coreactant transport, and allowing a strong ECL signal generation. The background signal of this experiment was low, and the sensitivity was further improved (in the range of fM to nM), with detection limits down to 7.41 fM. Furthermore, in another work reported by Ding et al., [128] a DNA sequence from HCV was detected in the range of $1.0 \cdot 10^{-16} \sim 1.0 \cdot 10^{-10}$ M, with LoD of $1.4 \cdot 10^{-17}$ M, based on the efficient quenching effect of ferrocene cluster-functionalized gold NPs ($\text{Fc}@\text{AuNPs}$) on the ECL of electrodeposited silica@ $[\text{Ru}(\text{bpy})_3]^{2+}$ -chitosan-graphene oxide nanocomposite ($\text{SiO}_2@\text{Ru} - \text{CS} - \text{GO}$). These sensors have shown high accuracy in detecting clinical samples, holding significant potential for practical applications.

In another interesting approach, two capture oligonucleotides were used for enhanced NA binding and interaction of the ECL luminophore with the viral double strand DNA (dsDNA). In particular, the use of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ as ECL signal generation probe has been reported by Nikolaou et al. [36] for HBV genome detection (as from the scheme in Fig. 11). Here, gold electrodes were modified with two thiolated oligonucleotide probes, independently complementary to specific and different sequences of the viral genome, to capture the viral dsDNA. This step was then followed by $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ intercalation into probes-dsDNA, prior to ECL measurement. It is important to remind here

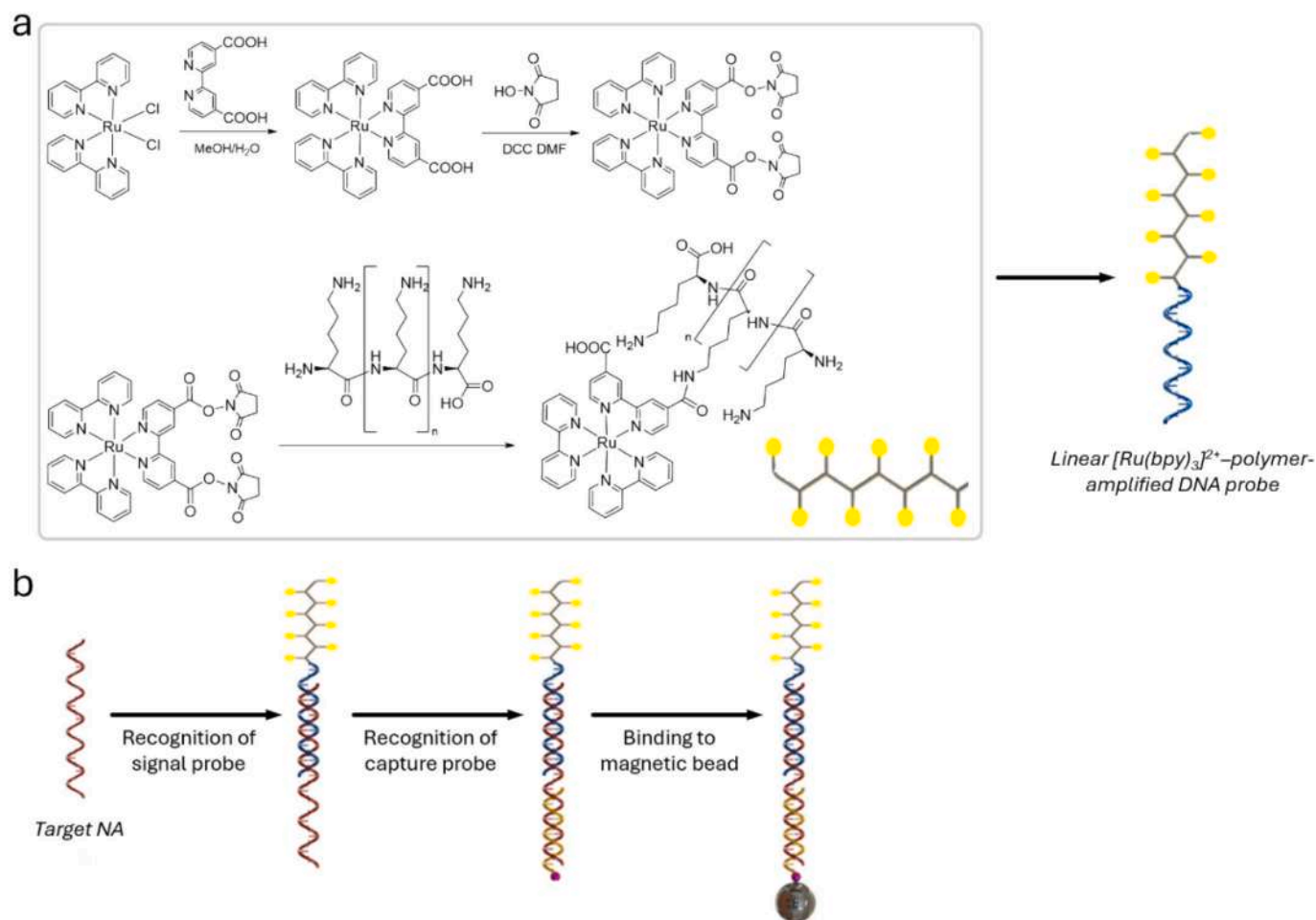


Fig. 10. Representative scheme of the polymer-amplified ECL assay applied to the detection of the sequence from HBsAg gene of the Hepatitis B virus (HBV). a) Reported are the synthetic routes to [Ru(bpy)₃]²⁺-NHS ester, and the linear [Ru(bpy)₃]²⁺-polymer upon poly-L-lysine (PLL) addition (PLL:[Ru(bpy)₃]²⁺ 1:10 M ratio). b) Formation of the polymer-amplified ECL assay for analysis of nucleic acids: formation of the polymer-amplified probe composed of the linear [Ru(bpy)₃]²⁺-polymer with some specific recognition domains; binding to the selected target NA sequence; addition of magnetic beads for efficient capturing at the electrode surface for ECL signal-production. Adapted with permission from ref. [122] Copyright 2017, American Chemical Society.

that [Ru(phen)₂dppz]²⁺ (as well as the analogous [Ru(bpy)₂dppz]²⁺, whose luminescent intensity is heavily quenched in water) interact with dsDNA or hybrid DNA–RNA with high affinities, with a concomitant large increase in emission intensity, thus allowing a high signal-to-noise ratio.

In a similar approach, HBV DNA was detected by Feng et al. [121] in combination with the syphilis (*Treponema pallidum*) gene and the immunodeficiency virus gene (HIV) using DNA hairpin via paper-based BPE array and [Ru(phen)₂dppz]²⁺ as ECL luminophore. In this case, a chitosan solution was cast onto the cathodic working zones of a carbon ink-printed paper-based electrode and dried at room temperature. Next, capture hairpin DNA was immobilized via the use of glutaraldehyde, and the prepared paper-based cathode was then exposed to the corresponding target NA, followed by incubation with the luminophore and TPrA coreactant, prior to ECL measurement. Here, Pt NPs were used to enhance the ECL signal via catalysed reduction of dissolved oxygen at the cathode, and detection limits down to 0.2 fM have been achieved for HBV.

In some other cases, nucleic acid detection has been coupled with the design of novel ECL probes, with the aim of achieving highly sensitive simultaneous detection of dual target. For example, in order to enhance the sensitivity of the transduction system, oligonucleotide-conjugated QDs have been exploited as ECL luminophores. In one example, the simultaneous HAV/HCV genomes detection have been reported by Wu et al. [130] in 2025 using self-luminescent Zn(II) dual-ligand metal

organic framework (D-MOF), cerium(III)-based MOF (Ce-MOF), and CdSe QDs. In this case, the electrode was modified with MOF and oligonucleotide-1 for the binding with target NA to occur, followed by interaction with oligonucleotide-2-conjugated QDs prior to the ECL measurement. More in detail, in one channel, one ligand of D-MOF acted as the luminophore and the other as the coreactant, so D-MOF could emit without requiring external coreactants, and the ECL intensity was decreased only upon addition of Ag NPs quenching probes through hybridization of HAV DNA, based on resonance energy transfer (RET). In the other channel, the CdSe QDs were introduced to Ce-MOF through hybridization of HCV DNA, increasing the negative potential signal upon target detection, due to the excellent ECL performance of CdSe QDs. The designed dual-channel sensor offered simultaneous detection of HAV and HCV genomes in concentrations ranging from pM to nM, and the feasibility of this method has been demonstrated in serum sample analysis of hospital patients.

Similarly, Guo et al. [125] made use of boron and nitrogen-co-doped carbon dots (BN-CDs) as highly efficient ECL emitters to detect HBV genome, involving the electrode modification with a capture oligonucleotide, Pt nanoflowers (Pt NFs), and the BN-CDs, subsequently followed by ECL signal generation using potassium persulfate as coreactant. Here, both Pt NFs (prepared by adding solution of H₂PtCl₆ into an aqueous mixture of glucose and ascorbic acid, followed by heating at 80 °C in a water bath) and boron radicals in the carbon dots were used to accelerate the reduction of persulfate coreactant to abundant SO₄^{•−},

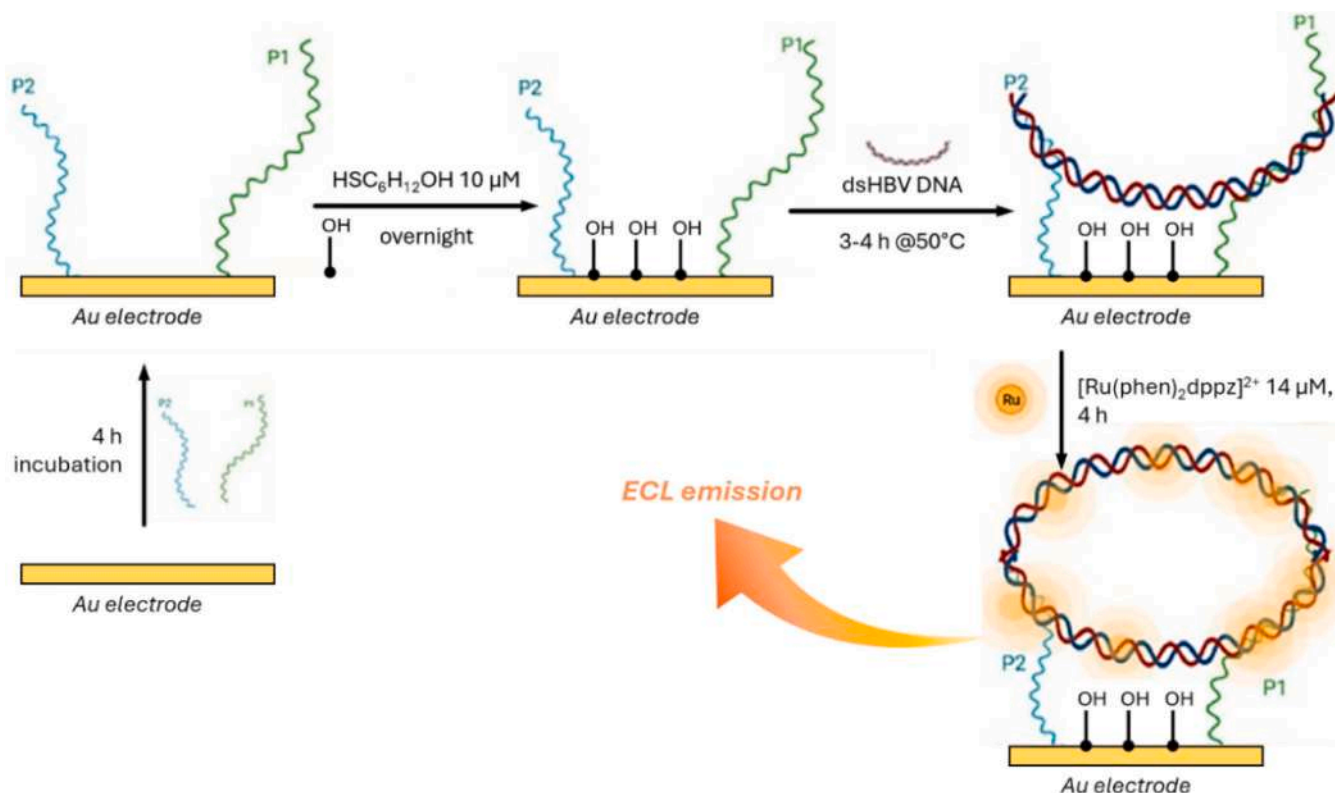


Fig. 11. Schematic representation of the ECL-based PCR-free recognition strategy: immobilization of specific probes P1 and P2, and of 6-mercapto-1-hexanol, on the surface of a previously polished gold electrode; recognition of double strand (ds) DNA by surface cooperative hybridization, and intercalation of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$, followed by viral whole genome ECL detection. Adapted with permission from ref. [36] Copyright 2022, Elsevier B.V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly enhancing the ECL performance. In practice, the authors have adopted a protocol starting with electrode modification with platinum nanoflowers and followed by the addition of the capture oligonucleotide and the ECL quencher, and finally by the exonuclease III-induced target DNA amplification. This method offered detection of HBV genome in the concentration range 10^2 aM–1 nM, with a LoD of 18.08 aM, relevant for clinical disease analysis.

Later, Yang et al. [135] reported the detection of Monkeypox virus NA using detection oligonucleotide-conjugated luminophore NPs and capture oligonucleotide-conjugated MMBs. Here, a dual-signal probe was constructed by integrating double-layered ECL luminophores and peroxidase-like nanozyme Pt NPs, using Zr-MOF ($\text{UiO}-66-\text{NH}_2$) as the carrier. In particular, $[\text{Ru}(\text{bpy})_3]^{2+}$ (U6–Ru) and $[\text{Ru}(\text{dcbpy})_3]\text{Cl}_2$ (Ru@U6-Ru) were used to prepare dye-doped multifunctional nanoprobes, coupled with the use of magnetic beads for the target DNA extraction (as from Fig. 12). Indeed, it has been shown that the two layers of $[\text{Ru}(\text{bpy})_3]^{2+}$ and $[\text{Ru}(\text{dcbpy})_3]\text{Cl}_2$ inside the NPs enhanced the ECL signal by 10 times. The Ru@U6-Ru/Pt NPs were conjugated with a detection oligonucleotide and mixed with capture oligonucleotide-conjugated magnetic beads. Magnetic separation was used for collection and deposition onto the electrode for ECL measurement. This approach offered both colorimetric and ECL-based readout platforms, where the colorimetric mode allowed virus screening with a visual LoD of 0.1 pM, while the ECL mode supported virus quantitative detection with a LoD as low as 10 aM. This approach has been tested in a variety of clinical samples, showing great promise for rapid, sensitive, and accurate diagnosis of Monkeypox viral NA, and may be adapted to other infectious pathogens.

Authors also investigated the application of a second approach, [134] where $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}/\text{TPrA}$ was used as the ECL-active couple together with rapid recombinase polymerase amplification (RPA). In this case, the electrode was modified with microfluidic

compartments conjugated with the oligonucleotide for rapid capturing of the target DNA, followed by luminophore intercalation into the double-stranded RPA products. Here, microfluidic compartments worked as tiny, enclosed spaces for precise control over the microenvironment, enabling high-throughput analysis. The developed sensing platform effectively detected Monkeypox virus with detection limits as low as 10 cps/ μL , and the detection sensitivity and specificity have been further validated using clinical samples from pustules, throat, urine, blood, saliva, and anus of Mpox-positive patients. Moreover, the developed hand-held detection device and platform can facilitate the point-of-care testing of Monkeypox virus without trained personnel and specialised equipment.

In another interesting approach, tobacco mosaic virus (TMV) RNA was detected by Guo et al. [143] via electrode modification with a hairpin DNA capture oligonucleotide (hDNA) followed by target RNA (tRNA) binding, forming linear tRNA/hDNA hybrids able to initiate a click chemistry-based polymerization reaction, i.e., activators regenerated by electron-transfer-atom-transfer radical polymerization (ARGET-ATRP). Here, luminol/hydrogen peroxide was used as the ECL-active couple, and the polymerization led to increased luminol binding, further enhancing the ECL signal. This method displayed a good linear relationship between ECL intensity and the logarithm of the target NA concentration in the range of 0.1 pM–10 nM, with a LoD of 2.61 fM, suggesting possible practical applications.

Eventually, Liu et al. [131] applied fluorine-nitrogen co-doped carbon dots (FNCDs, with improved ECL intensity due to the doping of fluorine and nitrogen in the carbon dots – CDs – which both hinder the free rotation of CDs and lead to a narrower energy gap) in combination with peroxide as alternative ECL-active couple for the detection of HIV DNA fragments. Here, the electrode was modified with FNCDs, Ag NPs and the capture oligonucleotides, followed then by exonuclease III (Exo III)-assisted multiple recycling amplification in the presence of the target

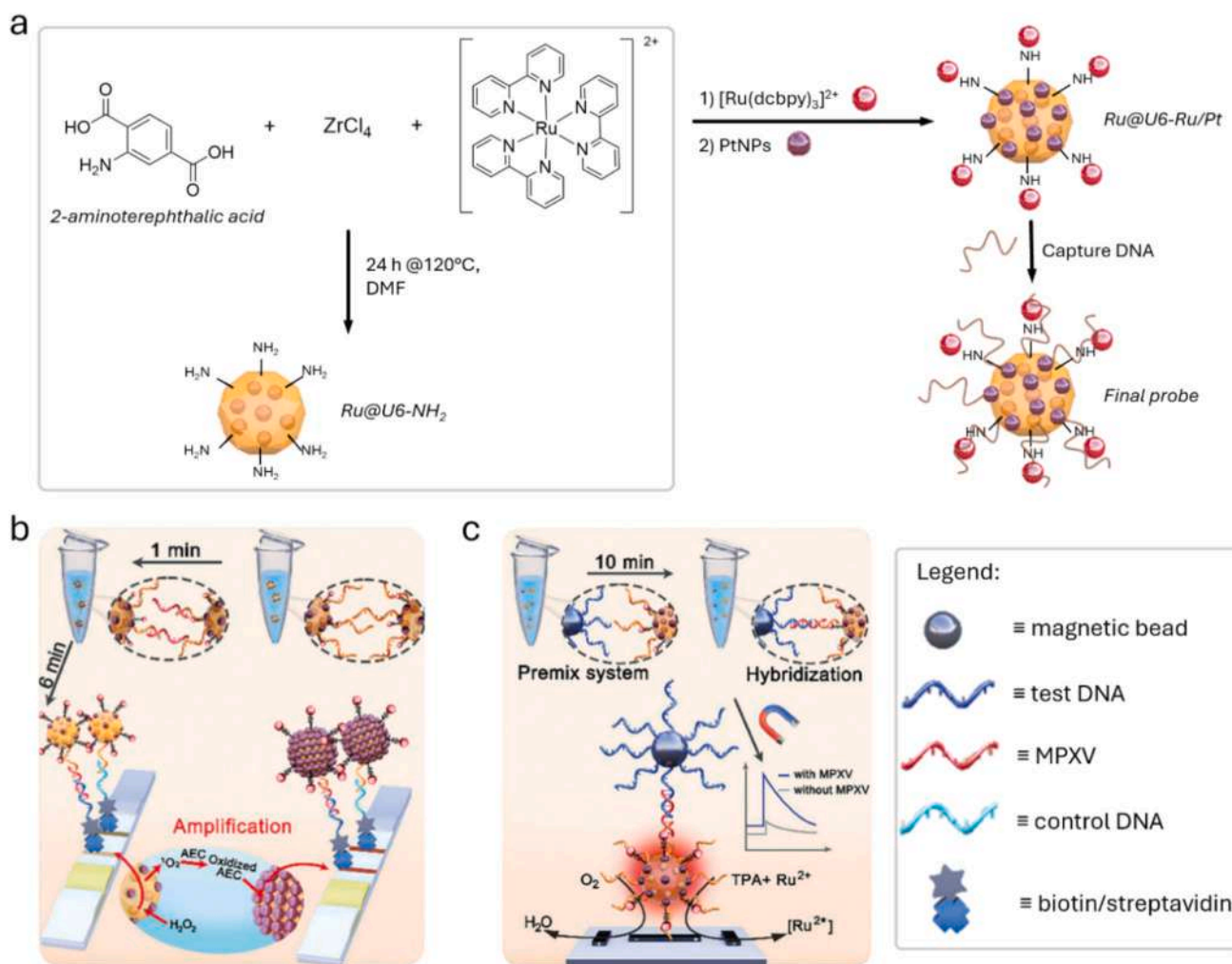


Fig. 12. a) Schematic illustration of the synthesis of Ru@U6-Ru/PtNPs. First, the Zr-based MOF Ru@UiO-66-NH₂ (Ru@U6-NH₂) was prepared via a facile solvothermal method using 2-aminoterephthalic acid (BDC-NH₂) as the ligand, and ZrCl₄ as the metal centre. The ECL luminophore [Ru(bpy)₃]Cl₂ was then encapsulated to generate Ru@U6-NH₂ nanostructures. Next, [Ru(dcbpy)₃]Cl₂ was further conjugated on the outer surface via EDC coupling, generating Ru@U6-Ru. Next, PtNPs were loaded via sonication, producing Ru@U6-Ru/PtNPs. Finally, the obtained system was incubated with thiolated capture oligonucleotide to prepare Ru@U6-Ru/Pt NPs-DNA nanoprobe. b) Schematic illustration of colorimetric lateral flow assay. Test strip is functionalized with control DNA/test DNA via biotin-streptavidin interaction approach, incubated with designed nanoprobe in the presence of Monkeypox viral NA (MPXV), followed by colour development with 3-amino-9-ethylcarbazole (ACE)-H₂O₂. c) Schematic illustration of ECL sensing platform. Nanoprobe was mixed with MPXV and DNA-conjugated magnetic beads for hybridization followed by magnetic separation and deposition onto the electrode for ECL measurement. Adapted with permission from ref. [135] Copyright 2024, Wiley-VCH GmbH.

DNA, further leading to a three-dimensional network DNA nanostructure in which doxorubicin-ferrocene carboxylic acid can be inserted to quench the ECL signal. This approach offered heteroatom-doped CDs as promising ECL nanoprobes, displaying ultrasensitive detection of HIV genome in the concentration range 10 aM–10 pM, with a LoD of 6.34 aM and potential for early clinical disease diagnosis.

In another work, oligonucleotide-conjugated ultrasized SiO₂ NPs (u-SiO₂ NPs, < 5 nm) have been used by Duan et al. [132] as novel ECL emitters for HIV genome detection. In particular, efficient ECL generation was achieved from u-SiO₂ NPs – as opposed to conventional SiO₂ NPs – due to the autocatalytic activity and electron-hole confinement in ultrasized volumes, and further ameliorated through the use of Zn²⁺-induced u-SiO₂ nanoaggregates with abundant oxygen vacancy (Zn/u-SiO₂-Ov nAGG), where the chelation of Zn²⁺ with (3-aminopropyl)trimethoxysilane (APTMS) triggered the self-growth of u-SiO₂ NPs and their aggregation, resulting in an efficient AIECL through the hindering of molecular motion, suppressing non-radiative deactivation. This approach offered SiO₂ NPs as novel ECL nanoprobes, and HIV detection was achieved in the concentration range 10² aM–0.1 nM, with a LoD of

50.48 aM.

2.4.2. Bacterial nucleic acid detection

Performing a comparison with the previously reported systems, we can assess that bacterial nucleic acid detection via ECL results to be relatively less popular, mainly due to the fact that the antibodies (or other affinity molecules or alternative markers) primarily used for whole bacteria quantification through ECL are generally easily available in most of the cases, [185] thus avoiding the requirement of additional steps for nucleic acid isolation. However, as a first recent example, *P. aeruginosa* NA detection was operated by Xu et al. [111] (as reported in Fig. 13), coupling a dual-mode ECL/PL transduction with a CRISPR/Cas12a system. More in detail, in the reported work, Ir(dmpq)₂(acac) and Fe₃O₄ NPs were encapsulated into silica spheres to build the ECL luminophore Fe₃O₄@SiO₂@Ir/SiO₂. These NPs were then linked to the detection oligonucleotide conjugated with ferrocene units, providing quenching of the luminescent signal. Incubation with the target DNA then led to specific recognition by CRISPR/Cas12a units, thus activating the cleavage function (DNA-Fc) and leading to the recovery of

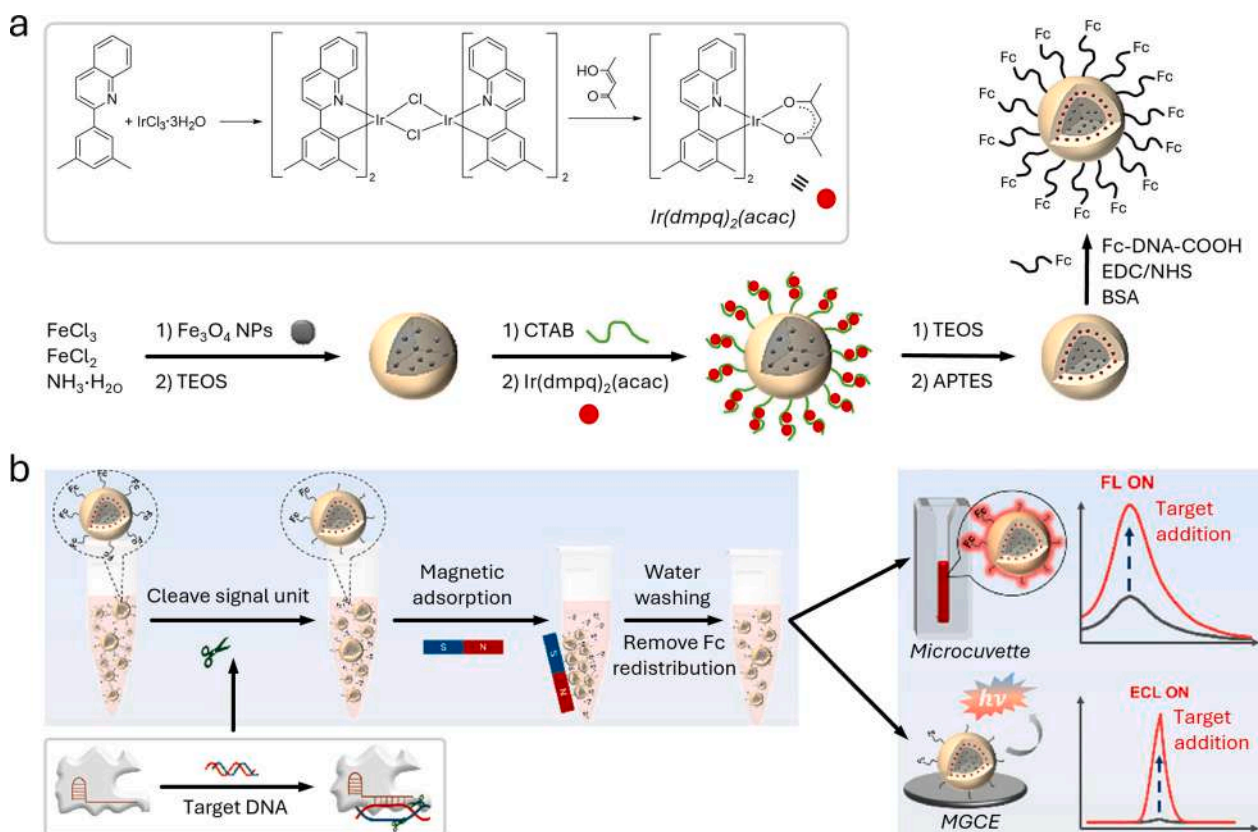


Fig. 13. a) Preparation of signal units involving encapsulation of $\text{Ir}(\text{dmpq})_2(\text{acac})$ and Fe_3O_4 NPs onto silica spheres prepared by hydrolysis of silica precursors (TEOS , APTES) in presence of cetyltrimethylammonium bromide (CTAB) as surfactant, followed by conjugation with oligonucleotide-ferrocene (Fc-DNA-COOH) via EDC coupling. b) Schematic diagram of the dual-mode biosensor for detecting *P. aeruginosa*, involving incubation with the target DNA followed by specific recognition with CRISPR/Cas12a units (Cas12a-crRNA, for activating the cleavage of DNA-Fc) and magnetic deposition of nanoprobe at magnetic glassy carbon electrode (MGCE). Adapted with permission from ref. [111] Copyright 2024, Elsevier B.V.

luminescence. The designed Ir-complex exhibited both ECL and PL properties, thus offering a dual-mode detection option. In particular, ECL detection worked in the range 10^2 fM–10 nM, with LoD of 73 fM, while photoluminescence mode detection displayed a linear response in the range 1 pM to 10 nM, with a LoD of 0.126 pM. This proposed dual-mode biosensor has been used for the detection of *P. aeruginosa* in real samples, underscoring its practical application potential.

Hence, this strategy elegantly couples the sequence-specificity of CRISPR/Cas systems with the brightness of an Ir(III) coordination complex. Nonetheless, it remains more compatible with well-equipped molecular laboratories rather than on-site or low-resource settings, due to the introduction of multiple biochemical steps and the requirement for precise control of nuclease activity. Thus, it currently represents a powerful tool for confirmatory testing and method development rather than a straightforward screening assay for decentralised contexts.

In another work, Liu et al. [114] detected *S. aureus* NA exploiting $[\text{Ru}(\text{bpy})_3]^{2+}/\text{TPrA}$ as the ECL-active couple, demonstrating how enzyme-free branched DNA-based ECL signal amplification can be an effective alternative to enzyme-based signal amplification. Here, the electrode was modified with branched DNA, and streptavidin-coated magnetic beads were used to bind with biotin-modified target DNA and magnetically extract it prior to ECL measurement. This method can be used for the detection of *S. aureus* in the range 20 pM– 10^2 nM, with LoD of 2 pM, showing that branched DNA can effectively be employed for enzyme-free signal enhancement with high sensitivity.

Overall, in comparison to whole pathogen assays, ECL-based platforms targeting pathogenic NAs are thus particularly attractive when very low copy numbers must be quantified or when clinically relevant genotype information is necessary (especially when dealing with

bacterial NA detection). By combining capture probes, amplification schemes, and coordination-based molecular luminophores or nanomaterials, these systems approach or even surpass the analytical sensitivity of conventional PCR assays, while enabling more compact and/or integrated device designs. However, many of these strategies still rely on prior NA extraction, precise temperature control, and complex multi-step protocols that can limit true point-of-care applications, especially in LMICs. These approaches are therefore most competitive when integrated into simplified workflows or when their inherent sequence selectivity provides clear advantages over immunoassays or biomarker-based formats.

2.5. Pathogens identification through biomarkers' detection

The efficient detection of threatening pathogens, such as viruses and bacteria, using specific diagnostic markers (or biomarkers) is a popular approach in the healthcare sector for early diagnosis and prevention. As introduced in previous sections, biomarkers are molecules able to accurately predict relevant clinical outcomes or disease presence. [186] Thus, considering that the early diagnosis of pathogenic infections is a critical aspect for healthcare, considerable efforts have been made in finding biomarkers able to accurately predict their outcome. Common examples of such diagnostic markers include inflammatory responses proteins (or cytokines), cell surface marker proteins, metabolic activity marker proteins, virus component proteins and metabolites. Identifying such biomarkers and enhancing their reliability via differential diagnosis of pathogenic infections, as well as their comparison with multiple biomarkers and with alternative approaches, are important. [45] In addition, their extension to point-of-care platforms could be promising

in solving some of the major problems concerning early detection, time effectiveness, sensitivity and cost of measurements. [187] In the majority of these approaches, conventional $[\text{Ru}(\text{bpy})_3]^{2+}$ has been used as the most effective ECL luminophore, even though other ECL-active systems have also been explored, such as Ir-complexes, CdTe-CdS QDs, and tetraphenylethene aggregates (as reported in Table 3). In many cases, a high number of luminophores is incorporated inside suitable porous nanostructures, in order to enhance the resulting ECL signal intensity, and they are often conjugated with an antibody to achieve higher detection selectivity. Moreover, magnetic beads-based extraction or enzyme-based signal enhancement is usually coupled with ECL detection. Especially, various approaches have been tested for SARS-CoV-2 detection through this methodology in human saliva samples/river water/urban wastewater, and for rabies virus detection in brain tissue. Furthermore, some approaches have been further transformed into cost-effective, rapid and point-of-care detection methodologies for e.g. SARS-CoV-2. [142]

2.5.1. Viral biomarkers detection

There are many cases in which viral antigens have been used as efficient targets for infectious diseases diagnostics, as it is the case with e.g. HBV surface antigen, HCV E2 protein, RABV glycoprotein, SARS-CoV-2 nucleocapsid (N-) protein, and SARS-CoV-2 spike (S-) protein (as previously reported in Table 4). Such biomarker-based detection has indeed the advantage of avoiding additional treatments of the sample (e.g., isolation of the nucleic acid), while specific biomarkers are readily available for most viruses. [45] Moreover, this approach can be adapted by scientists of allied disciplines and be extended for dealing with a wide variety of disease conditions that avoid direct handling of the virus itself. [188]

A clear example in this context has been reported by Li et al. [136] (and summarised in Fig. 14), where ECL-based quantification using $[\text{Ru}(\text{bpy})_3]^{2+}$ as ECL luminophore has been applied to RABV glycoprotein

quantification. Herein, a ECL resonance energy transfer (ECL-RET) and electrochemical (EC) dual-mode immunosensor was developed using dendritic mesoporous SiNPs (DMSNs) loaded with $[\text{Ru}(\text{bpy})_3]^{2+}$ (Ru@DMSNs) to enhance the ECL intensity, in combination with manganese oxide NPs (MnO_x) to quench the ECL signal via RET upon target recognition and building of the sandwich assay. With this method, RABV glycoprotein can be detected from 0.10 pg/mL to 10 ng/mL, with a LoD of 88 fg/mL (in ECL). Further catalytic properties of MnO_x NPs were then used for electrochemical mode of detection via catalytic oxidation of *o*-phenylenediamine, reporting a detection limit of 0.10 pg/mL (in EC). Moreover, to further extend the applicability of this approach, RABV has been directly analysed in brain tissue.

In addition, immunosensors based on ECL-RET have also been extensively applied to the detection of SARS-CoV-2. Indeed, after the pandemic outbreak of 2020, SARS-CoV-2 has been commonly detected by a variety of different approaches, focusing in recent years on the targeting of the SARS-CoV-2 N-protein, as reported e.g. by Villa-Manso et al. [138] In this work, $[\text{Ru}(\text{bpy})_3]^{2+}$ acted as energy donor and Au inlays/Pt-coated/Au core NPs (Au@Pt/AuNPs , synthesized as from previously optimized protocols applied to other Au NPs) [189] acted as acceptors, while MMBs have been used for magnetic extraction-based signal enhancement. The immunoreaction with the antigen was carried out by resuspending the antibody-conjugated MMBs in a mixture solution containing SARS-CoV-2 N-protein and the antibody-conjugated Au@Pt/AuNPs . After washing, the MMBs modified with the sandwich immunocomplex were used for ECL measurements in the presence of $[\text{Ru}(\text{bpy})_3]^{2+}$ as energy donor. The proposed approach offered a LoD of 1.27 pg/mL and has been used for the quantification of SARS-CoV-2 N-protein in human saliva samples.

In another approach, SARS-CoV-2 spike S1 protein was detected by Guerrero-Esteban et al. [137] in 2022, using N-rich carbon nanodots (CNDs)-amplified ECL in the presence of $[\text{Ru}(\text{bpy})_3]^{2+}$ as luminophore. Here, carbon nanodots displayed a double function, i.e., (i) amplification

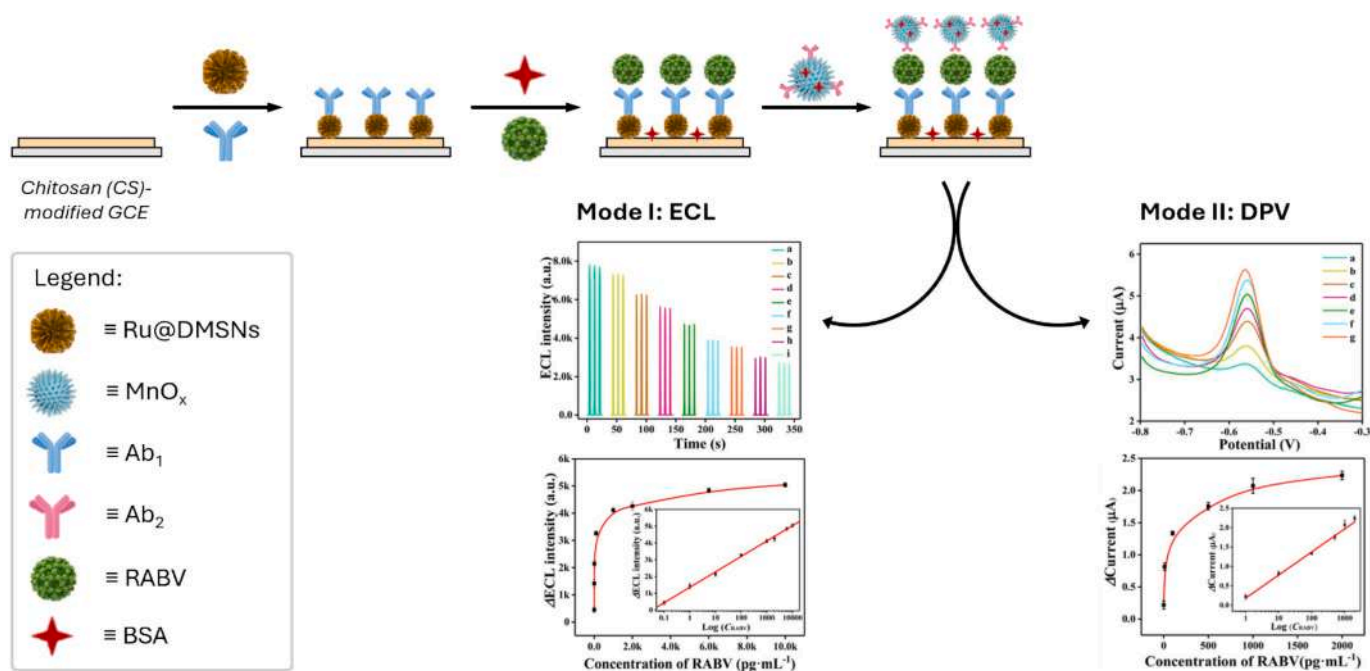


Fig. 14. Schematics of ECL quantification of rabies virus (RABV) glycoprotein using $[\text{Ru}(\text{bpy})_3]^{2+}$ -based NPs conjugated with antibody-1 (Ab_1). In particular, DMSNs were synthesized and reacted with APTES and succinic anhydride, followed by loading with $[\text{Ru}(\text{bpy})_3]^{2+}$ to generate the starting ECL nanoprobe (Ru@DMSNs). Ru@DMSNs were used to decorate the glassy carbon electrode (GCE) surface using chitosan (CS), followed by modification with Ab_1 . RABV was specifically recognized and captured by Ab_1 , causing the decline of the ECL signal due to the obstruction of electron-transfer. Successively, manganese oxide nanoparticles (MnO_x) were prepared and modified with antibody (Ab_2) and added to the system, since they can further quench the ECL signal of Ru@DMSNs via the resonance energy transfer between Ru@DMSNs and MnO_x . The resulting two different detection strategies are shown, where mode II used MnO_x -based catalytic oxidation of *o*-phenylenediamine (*o*-PD). Adapted with permission from ref. [136]. Copyright 2022, American Chemical Society.

of the ECL signal of $[\text{Ru}(\text{bpy})_3]^{2+}$ by providing nanostructured electrodes (CNDs/SPCE) more conductive than the bare corresponding electrode, and (ii) antibody supports by providing functional groups capable of covalent conjugation (carbon nanodots-modified electrodes were functionalized with a suitable antibody *via* EDC coupling chemistry). Furthermore, authors reported that functional groups, such as -OH and amino groups carried by CNDs, acted as coreactants in addition to the benefits of nanostructuring the electrode surface. The ECL response was collected in the presence of $[\text{Ru}(\text{bpy})_3]^{2+}$, displaying a LoD of 1.2 pg/mL, and this methodology has been successfully used for the detection of SARS-CoV-2 S1 protein in river and urban wastewater, further suggesting its reliability for early virus detection.

In another work, Lu et al. [140] reported a dual-mode (ECL/PL) imaging platform for SARS-CoV-2 spike protein, based on the activation of a DNAzyme (single-stranded DNA catalyst) walker by the target SARS-CoV-2 spike protein, that provides the enhanced appearance of ECL spots generated by a $[\text{Ru}(\text{bpy})_3]^{2+}$ -doped MOF as nanoemitter (Ru@PCN-224, in combination with TPrA). Specifically, in the presence

of the target spike protein, the armDNA reported in Fig. 15 was free to perform a hybridization-cleavage-release cycle, resulting in the detachment of the carboxyfluoresceins (FAM), while the Ru@PCN-224 nanoemitters (functionalized with tDNAs) were specifically attached to the electrode surface due to the recognition with leftovers fragments of hairpin DNA (HDNA), thus detecting single ECL particle emission. More in detail, the MOF NPs were prepared by coordinating Zr(IV) with 5,10,15,20-tetra(4-carboxy) porphyrin as organic ligands. Next, they were mixed with an aqueous solution of $[\text{Ru}(\text{bpy})_3]^{2+}$ for their doping inside the pores of NPs. Next, functionalization with DNAs was achieved *via* chemical bonding between the vacant Zr^{4+} on the nanoparticle and the phosphate group at the 5' end of tDNAs. This method offered a LoD of 0.58 fg/mL (in ECL) and the dual-mode detection provided enhanced reliability on the virus detection.

In a recent work, simple, rapid, and low-cost lateral flow immunoassay has been adapted by Fu et al. [142] for the ECL-based detection of SARS-CoV-2 N-protein (as from Fig. 16). In this case, paper-like strip electrodes were fabricated on a nitrocellulose membrane, modified with

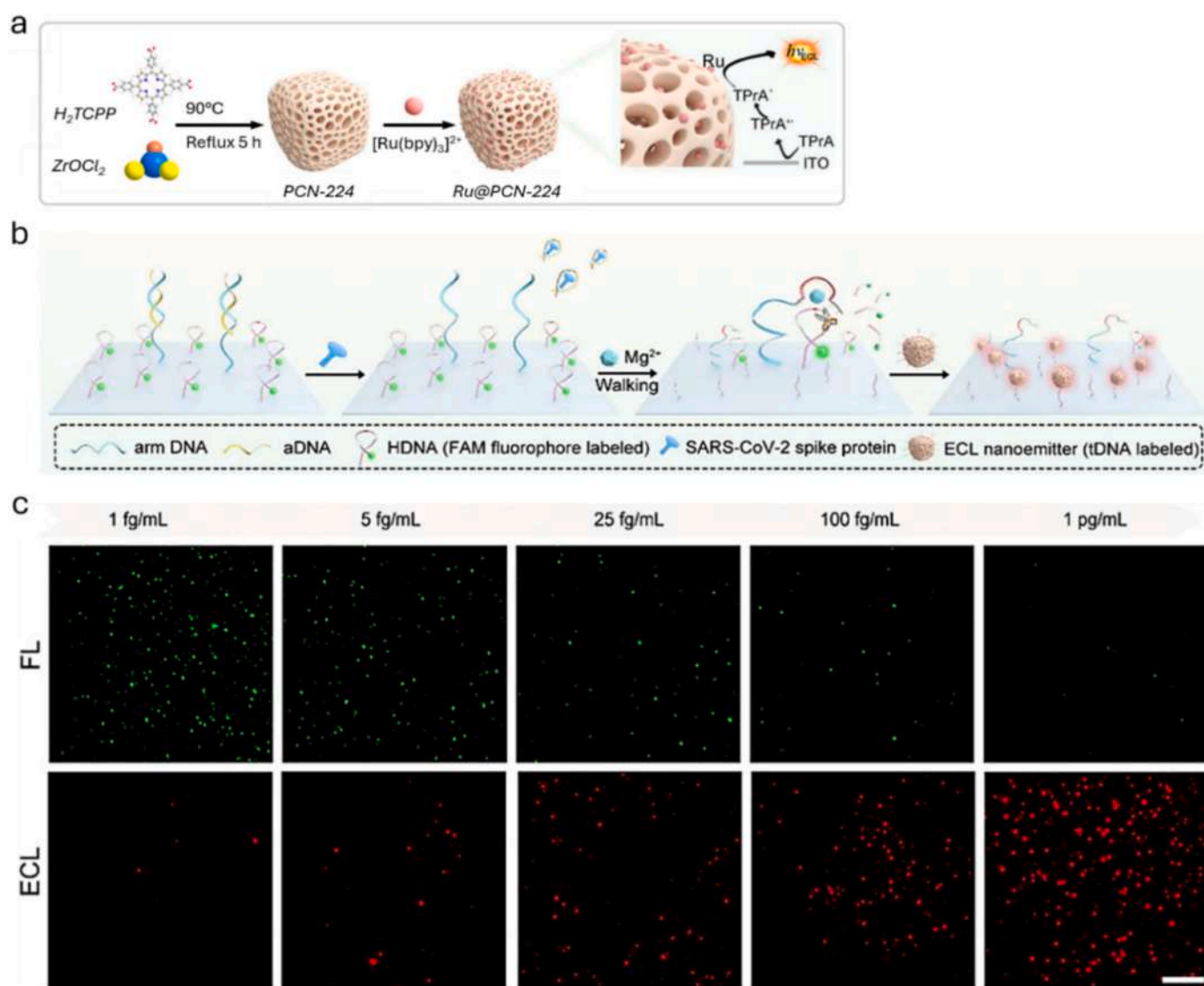


Fig. 15. a) Schematic diagram of the synthesis processes of the nanoemitter Ru@PCN-224 ($[\text{Ru}(\text{bpy})_3]^{2+}$ in a porous MOF material, designed by doping multiple numbers of $[\text{Ru}(\text{bpy})_3]^{2+}$ inside the metal organic framework, prepared *via* coordinating Zr(IV) with 5,10,15,20-tetra(4-carboxy) porphyrin as organic ligands, and its ECL imaging mechanism. b) Scheme of the dual-mode imaging detection platform based on self-powered DNAzyme walker. The electrode was modified with a hairpin DNA conjugated to a FAM fluorophore (FAM-HDNA). The specific activation by the target protein spike of the DNAzyme walker (armDNA, initially sequestered by the blocker aDNA) provides the power for the system's continuous running, enabling c) the simultaneous recording of the reduction in fluorescence spots and the appearance of ECL spots generated by the nanoemitter. Adapted with permission from ref. [140] Copyright 2024, American Chemical Society.

the capture antibody and used for ECL detection using 2,2-bis(hydroxymethyl)-2,2',2''-nitrotriethanol (BIS-TRIS) as coreactant. Separately, tris(4,4'-dicarboxylicacid-2,2'-bipyridyl)ruthenium(II)dichloride ($[\text{Ru}(\text{dcbpy})_3]^{2+}$) was loaded into dendritic mesoporous silica nanospheres (PEI-Ru/ dSiO_2) and then conjugated with the antibody, in order to be captured on the electrode surface *via* sandwich immunoreaction. More in detail, polyethyleneimine-conjugated $[\text{Ru}(\text{bpy})_3]^{2+}$ was prepared separately (*via* EDC coupling between the amine groups of the polymer and the carboxylic groups of $[\text{Ru}(\text{dcbpy})_3]^{2+}$) and mixed with water-dispersed NPs for $[\text{Ru}(\text{dcbpy})_3]^{2+}$ loading. The nanoparticles were again reacted with succinic anhydride for carboxylic acid termination, and then conjugated with antibody *via* EDC coupling chemistry. Using this method, SARS-CoV-2 N-protein was detected with a linear range of $1\text{--}10^4$ ng/mL and a LoD of 0.52 ng/mL. This approach hence merges the simplicity, rapidity, and low cost of paper-based lateral flow formats with the sensitivity and broad dynamic range of coordination complex-based ECL, making it particularly attractive for point-of-care testing. Its main limitations presently reside in the need for an external ECL reader and in the multi-step preparation of the PEI-Ru/ dSiO_2 , which may still restrict full decentralisation in resource-limited environments.

In another work, SARS-CoV-2 N-protein was detected by Wang et al.

[139] using the AIECL deriving from tetraphenylethylene doped in zeolite imidazole backbone-90 (TPE-ZIF-90), reported in Fig. 17. In this case, the electrode was modified with antibody-1-conjugated TPE-ZIF-90, and the ECL signal was recorded in the presence of the target protein and Au-ZnO nanoprobe functionalized with alkaline phosphatase and antibody-2 (ALP/Au-ZnO), with persulfate as coreactant, inducing the resonance energy transfer toward ALP/Au-ZnO composite and quenching the ECL signal. This system further catalysed the L-ascorbate-2-phosphate trisodium salt (AA2P) to produce ascorbic acid (AA) and PO_4^{3-} , which strongly coordinates to Zn^{2+} , leading to the disassembly of TPE-ZIF-90 and quenching even more the ECL signal. At the same time, the produced AA can reduce $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ to generate AuNPs, giving rise to a "triple quenching" of the ECL signal through RET within the shortening donor-acceptor distance. This method displayed a LoD of 0.52 fg/mL and has been applied for the detection of SARS-CoV-2 in real human samples. Moreover, the reported workflow synergically exploits the coordination chemistry of the ZIF-90 framework, the AIE properties of TPE derivatives, and the catalytic properties of metal oxide nanostructures, but requires careful optimisation of multiple reaction steps and material interfaces. Consequently, it currently appears most suitable for high-sensitivity assays in specialised laboratories, while it clearly showcases design principles that could inspire more streamlined

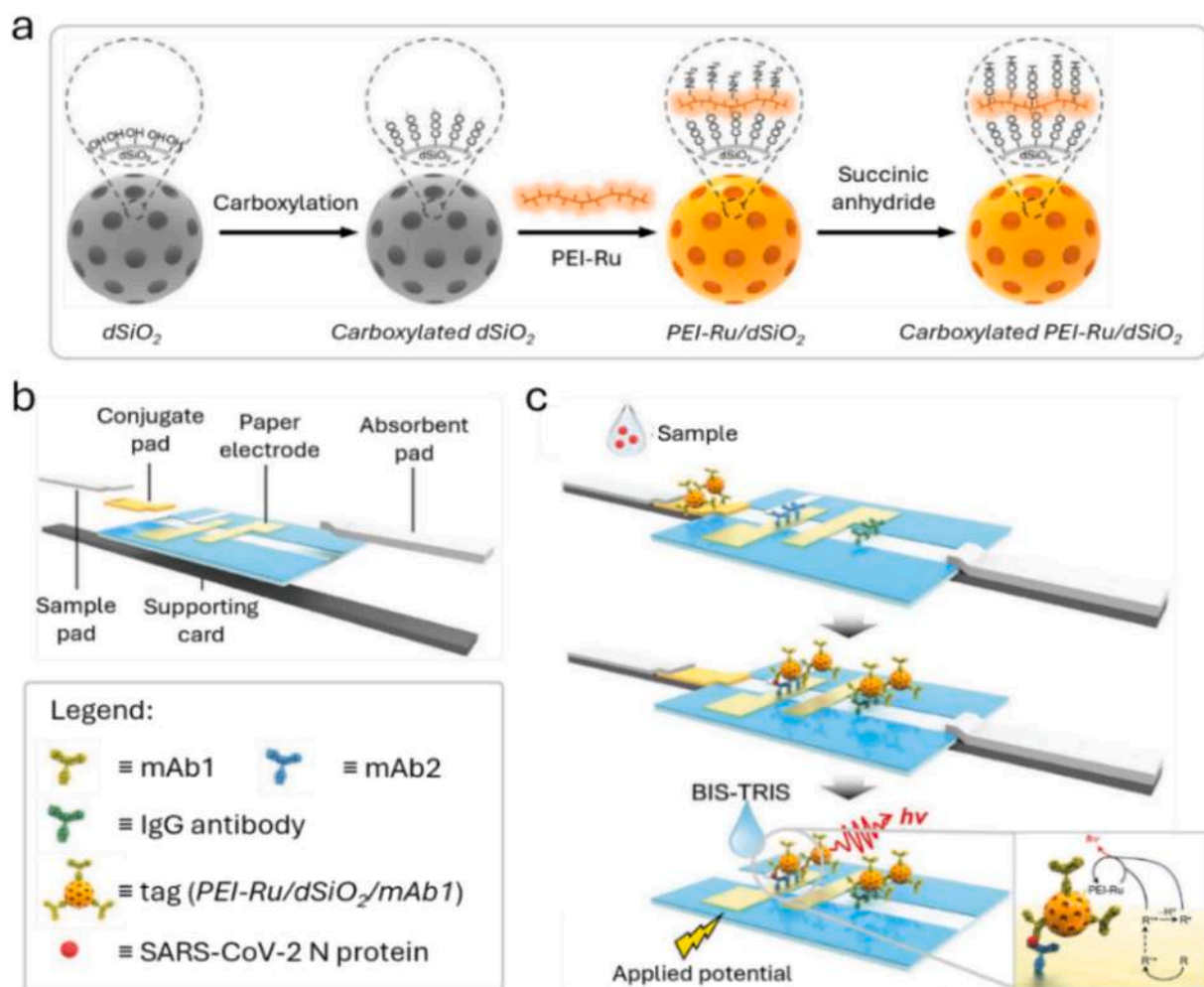


Fig. 16. a) Schematic illustration of the preparation of dendritic mesoporous silica spheres, embedding an ethyleneimine polymer (PEI)-tris(4,4'-dicarboxylicacid-2,2'-bipyridyl)ruthenium(II)dichloride ($[\text{Ru}(\text{dcbpy})_3]^{2+}$) system *via* electrostatic interaction (PEI-Ru/ dSiO_2). b) Illustration of ECL strip architecture for ECL-based detection. c) Operation process for the detection of SARS-CoV-2 N protein using ECL strips, with the related reaction mechanism for ECL generation in the grey square. Tags for ECL lateral flow immunoassays are prepared *via* loading $[\text{Ru}(\text{dcbpy})_3]^{2+}$ into dendritic mesoporous silica nanospheres and conjugating it with the chosen antibody (mAb1). The electrodes are fabricated using nitrocellulose membrane and functionalized with antibody (mAb2). Next, ECL tag is captured on the electrode surface in presence of SARS-CoV-2 N-protein *via* a sandwich immunoreaction, followed by ECL measurement in the presence of coreactant (BIS-TRIS). Adapted with permission from ref. [142]. Copyright 2024, Wiley-VCH GmbH.

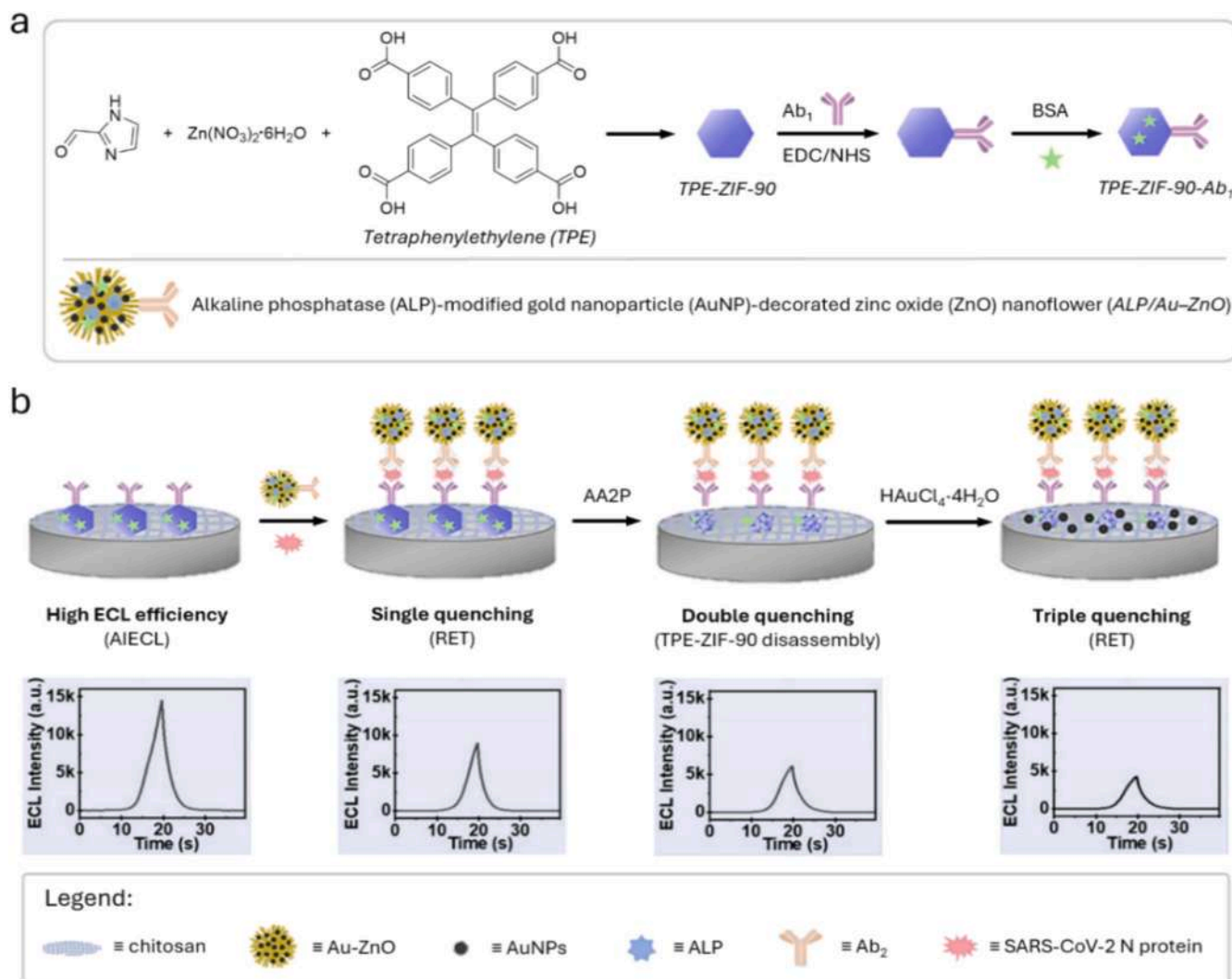


Fig. 17. a) Illustration of the preparation of ECL-active NPs that involve doping of tetraphenylethylene derivative inside zeolite imidazole backbone (TPE-ZIF-90), prepared using Zn(II) and imidazole derivative. Next, antibody (Ab_1) was conjugated via EDC coupling, followed by BSA blocking. The, Au-ZnO nanoprobe were prepared from ZnO nanoflower and functionalized with alkaline phosphatase (ALP) and antibody (Ab_2). b) Construction of the ECL immunosensor for SARS-CoV-2 N protein detection. The electrode is modified with ECL-active NPs using chitosan (CS), giving rise to aggregation-induced ECL (AIECL); then, upon the capture of the SARS-CoV-2 N protein by immune recognition, ALP/Au-ZnO composite was introduced, acting as RET receptor to quench the ECL signal and further catalysing the L-ascorbate-2-phosphate trisodium salt (AA2P) to produce PO_4^{3-} and ascorbic acid (AA). Adapted with permission from ref. [139]. Copyright 2024, American Chemical Society.

AIECL-based PoC platforms.

Moreover, HBV surface antigen (HBsAg) was detected using a paper-based ECL immunoassay, for example as reported by Chen et al., [123] exploiting an antibody-1-conjugated $[\text{Ru}(\text{bpy})_3]^{2+}$ as ECL luminophore to detect the viral antigen, and antibody-2-conjugated MMBs-based magnetic extraction for capturing and depositing on the electrode surface (with *N*-butyldiethanolamine as ECL coreactant). Detection was achieved in the range 34.2 pg/mL–34.2 ng/mL, reporting a LoD of 34.2 pg/mL. Here, paper-based ECL was used to join more rapid analysis with acceptable sensitivity, selectivity, and reproducibility, showing better performances than chemiluminescence-based immunoassays and ELISA. Similarly, HBsAg was also detected by Babamiri et al. [124] using signal amplification derived from dendrimeric-QDs structures (CdTe@CdS QDs), where the dendrimer was used to increase the loading of CdTe@CdS and worked as platform for the conjugation with antibody and immunoassay development. This sensor resulted in a LoD of 0.8 fg/mL with a linear detection range from 3 fg/mL to 0.3 ng/mL.

In a separate work, Huang et al. [127] achieved an efficient detection

of the HCV E2 protein using $\text{Ir}(\text{ppy})_3/\text{g-C}_3\text{N}_4$ as ECL-active couple. Herein, tris(2-phenylpyridinato)iridium ($\text{Ir}(\text{ppy})_3$) encapsulated into pluronic block copolymers ($\text{F-127@Ir}(\text{ppy})_3$) acted as the anode emitter (with enhanced ECL signal generation), while $\text{g-C}_3\text{N}_4$ as the cathode emitter. The resulting ratiometric ECL signal could be mediated by using multifunctional Nb_2C nanosheets (Nb_2C NSs) with excellent electrocatalytic activity and superoxide dismutase activity, so as to enhance the ECL intensity of anode and quench the response of cathode ECL, obtaining a specific immunorecognition process with a linear detection range spanning 1 pg/mL– 10^2 ng/mL, and a LoD of 0.84 pg/mL. Furthermore, this work demonstrated the application of water-soluble Ir (III) complex-derived NPs as potential ECL probes.

2.5.5. Bacterial biomarkers detection

In comparison with the previously reported section, nowadays it has been observed that biomarkers-based detection of bacteria is relatively less popular, especially through ECL-based methodologies. Nonetheless, a consistent number of reliable bacterial biomarkers is known and commonly used in other biosensing platforms, [45] hence we expect that

more and more biomarkers-based ECL strategies will be adapted to bacteria detection in the next future.

In this section, we would like to mention a recent work in which an inflammatory indicator of bacterial infection and sepsis biomarker (*i.e.*, procalcitonin) has been detected by Bu et al. [119] using a dual-ligand self-enhanced ECL coordination polymer (Zn-Ru-EDA) composed of $[\text{Ru}(\text{dcbpy})_3]^{2+}$ as ECL luminophore and ethylenediamine (EDA) as corresponding coreactant (as schematised in Fig. 18). $[\text{Ru}(\text{dcbpy})_3]^{2+}$ and EDA were assembled in the presence of Zn^{2+} ions, then deposited on the electrode surface, which was then functionalized with the antibody to build a sandwich immunoassay for procalcitonin detection, using Au NPs and antibody-2-conjugated multiwalled carbon nanotubes (Au@MWCNTs-Ab_2) as ECL quencher. The sensor was designed by depositing polymeric luminophore NPs followed by deposition of Pt NPs and functionalization with antibody-1. This was then followed by recognition of procalcitonin and addition of Au@MWCNTs-Ab_2 prior to ECL measurement. This method offered sensitive detection in the range 10^{-6} ng/mL–10 ng/mL, with outstanding selectivity and a LoD of 0.47 fg/mL.

In conclusion, the overall detection of pathogen-associated biomarkers through ECL-based strategies occupies a “complementary niche” between whole pathogen and nucleic acid assays. On the one hand, biomarkers such as inflammatory cytokines or procalcitonin can

be accessed with relatively simple immunoassay formats, leveraging mature ECL-active luminophores and nanostructured supports to achieve clinically relevant detection limits. On the other hand, biomarkers levels are often influenced by other complementary factors, *e.g.*, disease stage and co-morbidities, which may blur the link between ECL signal intensity and pathogen load. As a result, these ECL platforms are particularly valuable for risk stratification, treatment monitoring and – in line with what highlighted in the course of the introduction – sepsis management, whereas definitive etiological diagnosis may require combination with direct pathogen or nucleic acid detection.

2.6. Emerging approaches for ECL-based detection of pathogens

It must be brought to the reader's attention that, nowadays, there are several arising approaches that deserve special attention in the field of ECL-based detection and quantification of various pathogens. Indeed, with the aim of increased sensitivity, ECL luminophores are commonly conjugated to polymers and/or incorporated into porous/mesoporous nanostructures, as well as used for doping various NPs. Particularly, in this last case, since multiple dye molecules can be grouped together in a single NP, the ECL detection sensitivity can be highly increased, a fact that has already been observed also when dealing with luminophores giving rise to aggregation-induced enhancement of the ECL signal.

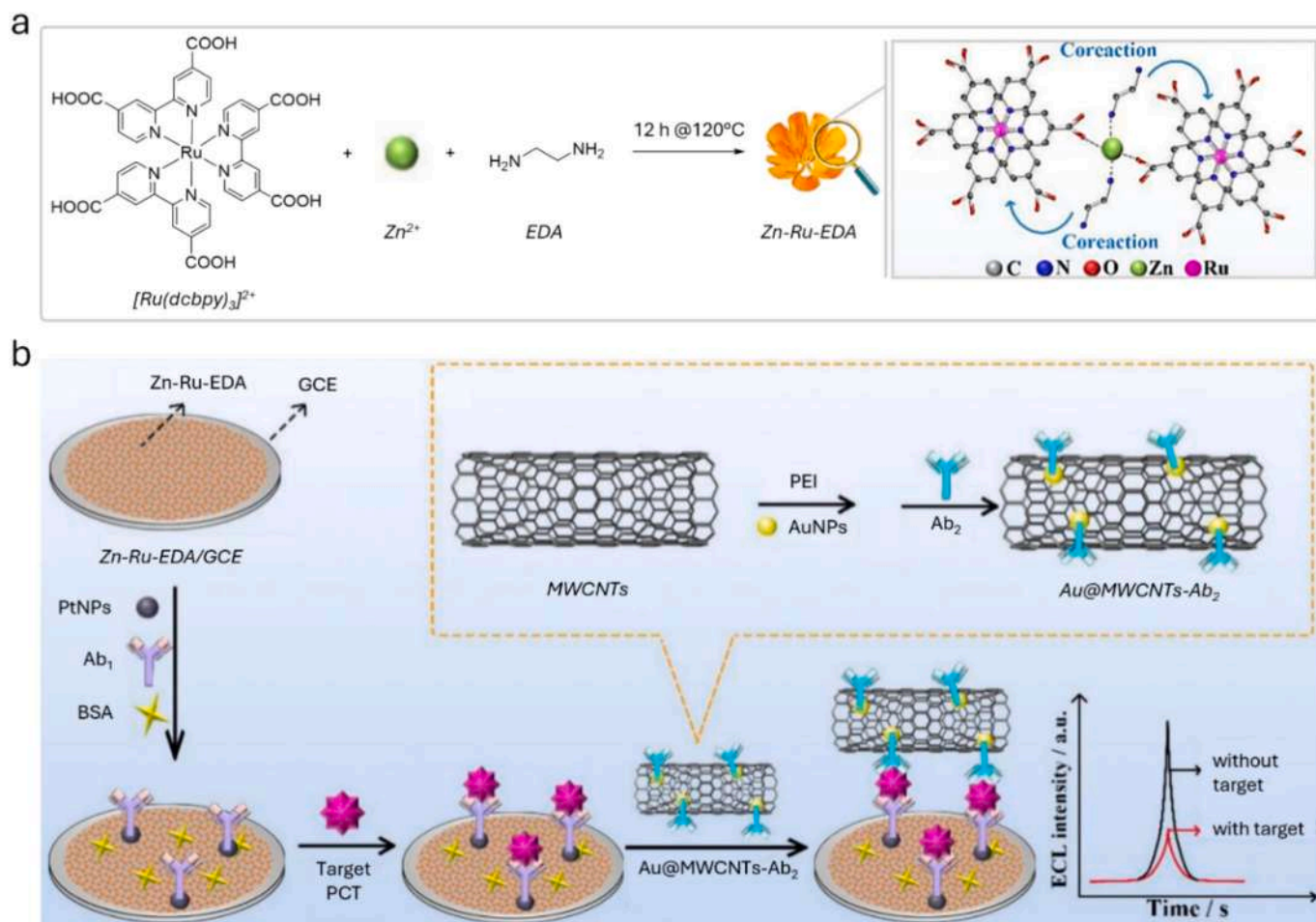


Fig. 18. Schematic fabrication of the ECL-based biosensor for procalcitonin (PCT). a) Synthesis of the dual-ligand coordination polymer Zn-Ru-EDA by using $[\text{Ru}(\text{dcbpy})_3]^{2+}$ as ECL reagent and EDA as coreactant via coordination with Zn^{2+} ions. b) Schematic illustration depicting the assembly of the sandwich ECL immunosensor for PCT detection. Zn-Ru-EDA is deposited on the electrode surface using Nafion polymer, then PtNPs were deposited to increase the conductivity. Next, electrode is functionalized with the antibody (Ab_1), followed by BSA blocking and finally sandwich immunoassay was used for procalcitonin detection. In the yellow square, preparation of the quenching element (Au@MWCNTs-Ab_2) is reported via deposition of AuNPs on multi-walled carbon nanotube (MWCNTs) and conjugation with Ab_2 using PEI. Adapted with permission from ref. [119]. Copyright 2024, American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[107,119] As previously emphasised, luminescent molecules usually employed to create ECL-active NPs are mainly based on Ru(II) and Ir(III) complexes, as well as TPE-based aggregation-induced emission luminophores, which has received increasing attention in the last years. The synthesized NPs are then conjugated to antibodies or oligonucleotides, to be used then for the quantitative detection of several pathogens.

Another interesting approach is represented by the assembly of ECL luminophore and coreactant into a single nanostructure to increase the ECL sensitivity upon limiting their diffusion. [119,130] This is particularly important as the ECL mechanism involves coreactant-generated short lived radical intermediates, and the close proximity of the luminophore and coreactant may favour the ECL mechanism. For example, $[\text{Ru}(\text{dcbpy})_3]^{2+}$ and the coreactant (ethylenediamine) were incorporated into polymeric forms able to increase the ECL sensitivity. [119,130] Furthermore, beyond classical Ru(II) complexes, it was recently demonstrated the possibility to build self-enhanced Au nano-clusters (protected by the ligand 6-aza-2-thiothymine) engineered through host-guest interactions with the coreactant *N,N*-dimethyl-1,3-propanediamine (DMPDA). This supramolecular architecture enabled large gains in ECL efficiency, allowing fg/mL-level detection of protein biomarkers when combined with dual-catalyst hairpin assembly and DNA network quenching strategies. [190] Alternatively, ECL luminophores that do not require a coreactant can be developed: in one work, self-luminescent dual-ligand MOF was prepared, where one ligand acted as the luminophore and other one as the coreactant, generating an ECL signal without requiring external coreactants. [119,130]

In addition, as already introduced in paragraph 1.2.4, commercial ECL-based immunoassays are currently exploiting the immobilization of the biomarker directly on the working electrode through the use of MMBs, which can then be collected toward the electrode itself through the application of a magnetic field. In this way, a magnetic separation-based ECL signal enhancement can be obtained in the considered system. As an example, Valenti and coworkers [103] built a simplified immunoassay model based on MMBs (schematization of the system reported in Fig. 19) to test the ECL performances of dye-doped silica NPs (DDSNPs), reporting a 150–400% ECL signal enhancement due to the dye distribution effect inside the NPs.

To be further mentioned that other important detection methodologies include design of novel ECL luminophores with enhanced performance, [133] amplification of ECL signal using MXene or metallic NPs as electron-transfer agents, [118,133] and coupling ECL with lateral flow immunoassay platform for point-of-care detection. [142] These approaches have been currently used for sensitive detection of HBV, [123] HCV, [128] Monkeypox virus, [135], *P. aeruginosa*, [111] and SARS-CoV-2. [138] In the context of portable and user-friendly devices,

recent efforts have also addressed both the optical readout and the sensing substrate. On the detection side, the research group of Francisco Javier del Campo [191] showed that carefully controlling smartphone image acquisition and processing can significantly improve the analytical figures of ECL imaging, resulting in an Android-based ECLiCare workflow able to restore proportionality between pixel intensity and emitted radiance, and to report limits of detection and quantification in the nM range. On the substrate side, instead, Polo, Cinti and coworkers [192] systematically compared filter and office paper as supports for screen-printed ECL electrodes, exploiting paper porosity to preload TPrA and create ready-to-use $[\text{Ru}(\text{bpy})_3]^{2+}$ /TPrA sensors that do not require manual coreactant addition. Their study demonstrated improved reproducibility, simplified workflows and at least 10 days of storage stability for TPrA-functionalised electrodes (on office paper) under ambient conditions. Together, these contributions illustrate how integrating optimized smartphone imaging with sustainable paper-based substrates can advance truly portable ECL platforms for PoC applications.

Therefore, joining the research on new ECL-active luminophores to the overcome of the currently existing limitations related to the use of antibody-based sandwich immunoassays (such as ELISA), the research group of Valenti and coworkers [193] investigated and developed an ultrasensitive platform allowing the detection of pathogenic microorganisms upon substituting target NA amplification with novel signal amplification strategies. Particularly, a novel stimuli-responsive ECL MMBs-based immunoassay has been designed (reported in Fig. 20), yielding an impressive signal enhancement. Here, rapid detection of SARS-CoV-2 Spike (S1) protein was achieved in whole virus samples from swab formulations, incorporating a cleavable Ru(II)-based luminophore for an effective ECL signal enhancement of up to 613%. The stimuli (i.e., cleavage) response originated from the derivation of MMBs with a luminophore able to exploit the more efficient homogeneous ECL mechanism for signal generation upon incorporation of a disulfide moiety on the lateral chain of an ancillary ligand. Hence, applying a sufficiently high anodic potential for oxidizing both TPrA and the luminophore, the disulfide bridge was cleaved by electrogenerated TPrA radicals, thereby releasing the Ru(II) complex into the solution and activating the homogeneous ECL mechanism.

Overall, it can be concluded that the emerging approaches discussed in this section extend the capabilities of ECL-based biosensing by encoding luminophores and coreactants into coordination polymers, metal-organic frameworks, and nanocomposites, or by coupling ECL readout to lateral-flow or MMBs-based formats. These architectures routinely deliver substantial gains in signal-to-noise ratio and detection limits, even though at the expense of more complex syntheses, stability

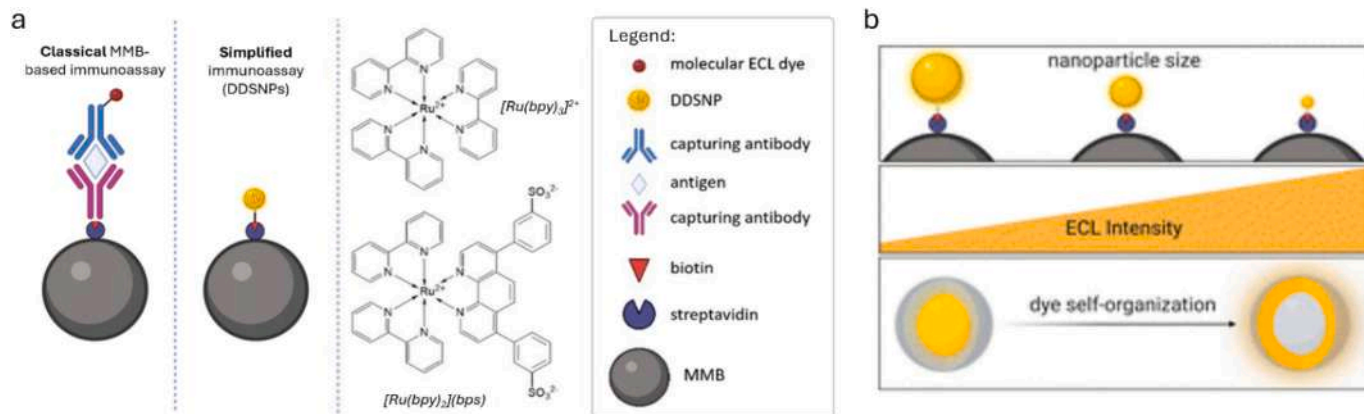


Fig. 19. a) Schematization of a classical magnetic microbead (MMB)-based immunoassay with an antibody labelled by molecular ECL emitter vs the simplified immunoassay model based on MMBs used in the work to test the ECL performances of DDSNPs, with the related molecular structures of the employed dyes, i.e., $[\text{Ru}(\text{bpy})_3]^{2+}$ and $[\text{Ru}(\text{bpy})_2]\text{bps}$. b) Dye distribution effect on ECL signal intensity, resulting in 150–400% signal enhancement for the NPs doped with the $[\text{Ru}(\text{bpy})_2]\text{bps}$ complex, compared to the $[\text{Ru}(\text{bpy})_3]^{2+}$ -doped ones. Adapted with permission from ref. [103]. Copyright 2024, Royal Society of Chemistry.

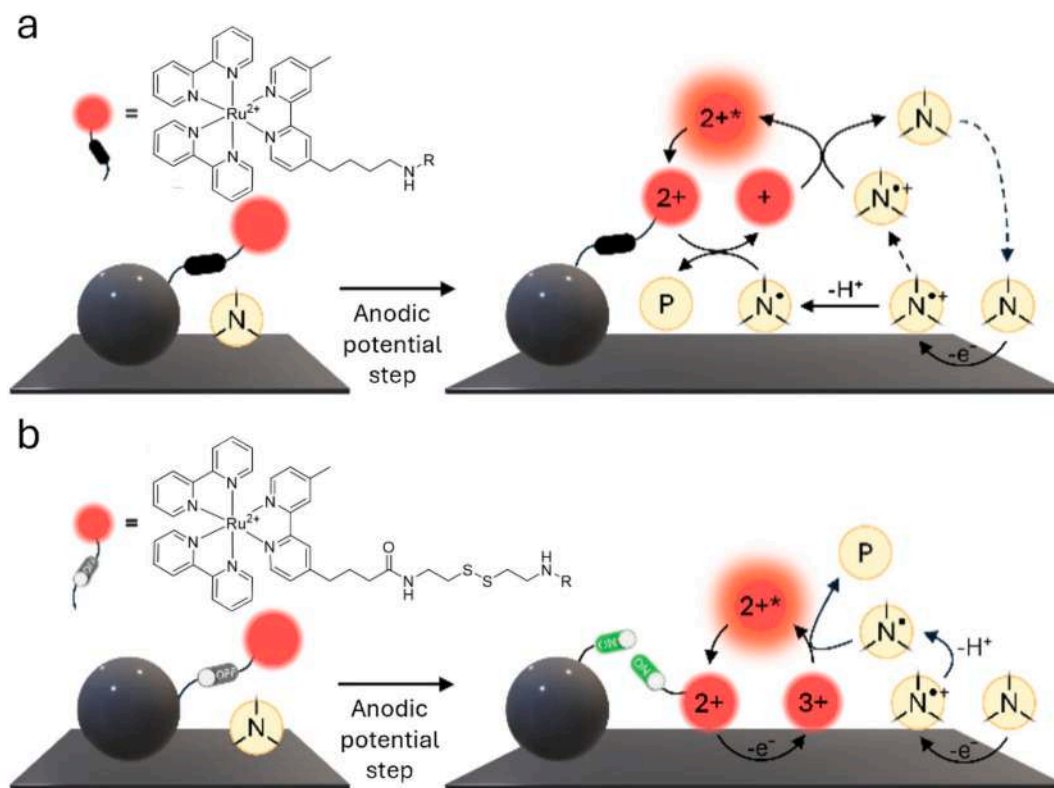


Fig. 20. Representation of a) a conventional ECL MMBs-based immunoassay system (exploiting the traditional heterogeneous ECL mechanism) in a TPrA solution; b) the cleavable MMBs-based immunoassay system in a TPrA solution, where a more effective homogeneous ECL mechanism takes place. Red spheres labelled $2+$, $3+$, and $+$ represent the different redox states of Ru(II) complex involved in the ECL process, while $2+^*$ denotes its emissive excited state. Yellow spheres labelled N, N^{*+} , and N^* correspond to TPrA, its radical cation, and its neutral radical, respectively. Adapted with permission from ref. [193] Copyright 2025, The Authors. Published by American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

issues, or stricter control over surface chemistry. At present, they are therefore best placed in advanced research and development pipelines, where their performance can be carefully benchmarked and simplified before real-world translation. Alongside these advancements, recent work on smartphone-based imaging workflows and ready-to-use paper-based electrodes shows that device engineering, substrate selection and image processing can be as critical as the underlying chemistry in determining analytical performance. Therefore, in the longer term, the design principles demonstrated herein are expected to feed into the next generation of clinically viable ECL devices, bridging the gap between laboratory prototypes and deployable diagnostics in both high- and low-resource settings.

3. Conclusions and perspectives

In conclusion, we have extensively emphasised how the application of ECL-based biosensing systems is nowadays essential to consistently improve the sensibility, accuracy, and cost-effectiveness of diagnostic systems, with the aim of obtaining easy-to-handle assays, significantly simplifying the instrumentation needed and making it suitable to be employed also in challenging contexts, as it is the case when dealing with infections diagnosis and treatment in LMICs. In this context, many different sensing approaches have been considered, ranging from the detection of the whole pathogen to the analysis of its nucleic acid or some specific biomarkers. In some cases, these systems could be already coupled with microfluidic devices, where low-cost electrodes and lateral flow immunoassays are currently used as well in point-of-care detection. [135] Particularly, disposable paper electrodes can also be exploited to make the method cost-effective. [121] Moreover, in order to enhance the detection selectivity, ECL-active dyes (either in their free form or included in NPs) can be conjugated to antibodies or oligo/

polynucleotides in order to enhance the resulting signal. In addition, in some recent works, imprinted polymers have further been used as an alternative to antibodies for achieving higher selectivity, such as polydopamine with an imprint of *L. salivarius* [110] and polymers with HIV imprint. [194]

Therefore, future developments in this field will likely focus on the advanced design of luminophores and hybrid architectures simultaneously combining high ECL efficiency with improved signal stability, water solubility, and straightforward bioconjugation. On this topic, rationally designed Ru(II) and Ir(III) complexes, MOFs, covalent organic polymers (COPs), and AIECL-active systems are particularly promising scaffolds to control local microenvironments, tune emission wavelengths, and enable multi-colour or multiplexed detection. Nonetheless, their synthetic routes and batch-to-batch reproducibility must be further simplified to avoid becoming a barrier to scale-up and regulatory approval. On the device level, then, integrating these coordination-based emitters and nanostructured supports into compact, user-friendly platforms – including microfluidic chips, paper-based devices, lateral flow formats, and portable potentiostats – will be crucial to fully exploit their potential in PoC and decentralised settings. In parallel, further work is still needed to couple ECL transduction with upstream sample preparation, eventual biochemical amplification, such as nucleic acid or CRISPR-based recognition in a seamless fashion, thereby minimising manual steps and limiting the risk of contamination or user-dependent variability.

In addition, we have clearly emphasised the importance of early and accurate detection of oncogenic and opportunistic pathogens in vulnerable and immunocompromised populations, where long latency periods, antimicrobial resistance, and complex clinical courses make timely diagnosis particularly challenging. As we have discussed, the development and implementation of rapid, sensitive, and minimally

invasive technologies – such as ECL-based biosensors – offer a promising avenue for enhancing pathogen detection, especially when dealing with vulnerable populations. The ability to rapidly identify viral or bacterial pathogens with high specificity and low sample requirements can directly influence clinical decisions, enabling timely treatment, reducing complications, and ultimately improving patient survival, addressing several unmet medical needs. Under these circumstances, there is still plenty of space for improvements in the application of ECL-based technologies to clinically demanding targets such as Epstein–Barr virus, *Clostridioides difficile*, and other pathogens that critically impact transplant recipients and cancer patients, for which current diagnostic workflows are often time-consuming, costly or poorly accessible. [195–197] These examples, together with the persistent disparities in access to molecular testing for HPV, HBV and HCV between high- and low-income countries, clearly highlight priority areas where robust, low-cost, and minimally instrumented ECL platforms could have a transformative role, especially when coupled with simplified sampling strategies, e.g., dried blood spots (DBS) sampling and rapid diagnostic tests (RDTs), that already show promising sensitivity and specificity in field studies, though broader validation and laboratory support are still needed. [198]

Overall, these global disparities and the clinical urgency of rapid diagnosis underscore the need for innovative detection platforms. At present, several efforts have been made to verify the reliability of ultralow detection limits of ECL-based systems (varying mainly in the range from pg/mL to fg/mL, as shown in Table 3 and Table 4) using dual-mode detection platforms, [135,140] allowing to consistently diminish the arising of false-positive or false-negative results and improving the detection accuracy, and further compared with other detection platforms. [199] At this point, it would be of major importance that some of these technologies could reach a level of accuracy, reproducibility, simplicity, and cost that would allow them to successfully enter into the market. In parallel, future studies should increasingly adopt more standardised evaluation frameworks, systematically reporting assay time, total cost per test, stability under storage, operating conditions, and performance in blinded or inter-laboratory trials, together with “classically” reported limits of detection and linear ranges, so as to clarify the specific niches where ECL biosensors can provide the greatest added value with respect to existing diagnostic technologies.

Authors contribution

All authors contributed to the writing, reviewing, and revising of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Luca Prodi reports a relationship with Bionys s.r.l. that includes: board membership and equity or stocks. Giovanni Valenti reports a relationship with Bionys s.r.l. that includes: equity or stocks. Paolo Garagnani reports a relationship with Bionys s.r.l. that includes: equity or stocks. Luca Prodi has patents #WO2023047306, #WO2024069527, #WO2023286095, #PCT/IB2023/053007 issued to University of Bologna. Giovanni Valenti has patents #WO2023286095 and #PCT/IB2023/053007 issued to University of Bologna. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Elements in the graphical abstract and in Figs. 1, 4, 7, 8, 9 were created with the help of BioRender (<https://BioRender.com>).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ccr.2026.218314>.

Data availability

All data supporting the findings in this review are available from the referenced sources listed in the manuscript.

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