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Recent progress and advancement in detecting methylmercury using a battery of biosensors and biomolecular-based techniques: An updated overview

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ABSTRACT

Methylmercury (MeHg) represents the most toxic form of mercury, owing to its ability to permeate both the blood-brain and placental barriers, leading to bioaccumulation in organisms. In the marine food web, MeHg concentrations can reach levels millions of times higher than those found in the surrounding environment, posing significant ecological and human health risks. This review provides a comprehensive overviews and critical evaluation of the available biosensor detection platforms for the detection of MeHg, with a focus on their performance based on key parameters such as (i) sensitivity, (ii) selectivity, (iii) response time, and (iv) adaptability to diverse environmental matrices. We examine recent advancements in MeHg biosensing technologies, emphasizing innovative approaches that surpass current methodologies regarding detection limits, reversibility, response time, and operational stability. Furthermore, we present an in-depth discussion on future directions for the development of *in situ* MeHg detection platforms, with potential applications in both biomedical and environmental monitoring. The review concludes by outlining the challenges and opportunities for advancing MeHg sensing technologies to enhance real-time detection in aqueous environments.

1. Introduction

Mercury exists in various chemical forms including Elemental Mercury (Hg^0), Mercuric Ion (Hg^{2+}), Methylmercury (MeHg), Dimethylmercury (CH_3HgCH_3), Mercury Sulfide (HgS) and Ethylmercury ($C_2H_5Hg^+$). Among these, MeHg is the most toxic, posing a significant risk to both the environment and human health, even at concentrations as low as nanomolar (nM) levels. This concentration threshold is remarkably minuscule, diverging by two orders of magnitude from any other mercury species. Despite its minute presence, MeHg has the tendency for biomagnification within the food chain, thereby accumulating in organisms and resulting in neurological disorders, exemplified by Minamata disease [1]. Wild piscivorous fish, mammals, and birds, animals at the highest trophic level, are at risk for elevated dietary methylmercury intake and toxicity due to biomagnification through the food chain. Its neurotoxic effects are especially harmful to developing fetuses and young children, making precise quantification in food samples critical for public health. Monitoring MeHg levels in fish is essential for assessing exposure risks and ensuring food safety standards. Regulatory agencies rely on accurate MeHg detection to guide consumption advisories and protect vulnerable populations. Consequently, the demand for meticulous, sensitive methodologies to detect MeHg with precision has surged, especially in the context of evaluating water pollution dynamics. Biosensors for detecting methylmercury (MeHg) in food samples offer several advantages, including rapid detection and high sensitivity, making them effective at trace-level detection. These devices enable real-time monitoring, providing immediate feedback on MeHg levels, essential for ensuring food safety in vulnerable populations like pregnant women and fetuses. Their cost-effectiveness and potential for on-site application, especially in industries such as aquaculture and agriculture, make them suitable for widespread use. Additionally, integration with smartphone technology allows for automation and ease of

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use, improving overall food safety protocols [2–4].

Current conventional methods for MeHg detection employ techniques such as atomic fluorescence spectrometry (AFS) with gold traps and automated systems based on cold vapour atomic fluorescence spectrometry (CVAFS). The CVAFS mechanism involves the conversion of MeHg into volatile ethylmercury through the use of sodium tetraethyl borate. After this conversion, alkyl forms of mercury are purged using nitrogen gas and captured on Tenax columns. After desorption at an elevated temperature, gas chromatography is employed to identify alkyl mercury compounds post-pyrolysis [5–7]. This method currently stands as the most sensitive for MeHg, with an impressively low limit of detection (LOD) at 0.004 ng/L. However, it necessitates the laborious task of sample collection from sites and transport to laboratories, which can be time-consuming and resource-intensive. In addition to sample collection challenges, laboratory-based MeHg measurement entails the extraction of MeHg from complex matrices, including organisms and cells. Specific sample preparation steps are crucial, and tailored to the physicochemical properties of each matrix. Even seemingly simple samples like water require meticulous processing, involving pH adjustment to 4.5 before the addition of sodium tetraethyl borate and overnight derivatization into volatile ethylmercury.

Although the necessary steps to obtain MeHg from biota or naturally occurring complexes cannot be omitted, the specific preparation of samples in laboratories might be simplified, resulting in rapid usage, fewer experts and decreased costs, if sensors can be used instead of using expensive laboratory equipment. Therefore, sensors that can enable measurement of MeHg in the environment are of high priority to increase the amount of data that will precisely map the routes of MeHg for thwarting toxicity reaching the global nutrient cycle and to enable us to determine contaminated food. This will yield biologically relevant information about the biomagnification and bioaccumulation of MeHg under real environmental conditions. Since the processes of methylation and demethylation are continuous, the onsite measurement will give us dense time points, resulting in time and spatially dependant distribution when followed by the precise sampling locations, since laboratory measurements can give results only for a limited number of samples.

Among the several developed platforms, biosensors for detecting methylmercury (MeHg) in food samples offer several advantages, including rapid detection and high sensitivity, making them effective at trace-level detection [8]. These devices enable real-time monitoring, providing immediate feedback on MeHg levels, essential for ensuring food safety in vulnerable populations like pregnant women and fetuses. Their cost-effectiveness and potential for on-site application, especially in industries such as aquaculture and agriculture, make them suitable for widespread use. Additionally, integration with smartphone technology allows for automation and ease of use, improving overall food safety protocols. Microfluidic devices offer high sensitivity and accuracy but may require technical expertise. Lateral flow assays and paper-based assays provide moderate to high sensitivity with ease of use but less accuracy in quantification. Smartphone-based sensors, electrochemical sensors, and optical biosensors achieve high sensitivity and accuracy, with the latter two being highly precise and user-friendly for real-time monitoring.

Although sensors for measuring inorganic mercury (Hg^{2+}) or Total Mercury (THg) have been developed, most MeHg sensors remain at the proof-of-concept stage [1]. To advance the development of MeHg sensors, this review focuses on available detection methods in the literature for MeHg detection, categorising them into six major types: Whole-cell biosensors (WCB), Immunostrip sensors, Small molecule probe-based (SMP) sensors, Metal-Organic Framework (MOF) sensors, Nanoparticle (NPs)-based sensors, and Nanoarchitectonics sensors. This comprehensive overview provides insights and strategies for advancing MeHg sensors by integrating biological, chemical, and nanotechnological approaches. A critical bottleneck in the development of MeHg sensors is the need for a well-integrated system that includes a receptor with high specificity for MeHg, efficient signal transduction, low noise amplifier, and reporters capable of showing realtime high quality data. Addressing these challenges is essential for creating effective and reliable MeHg sensing solutions.

The current state of sensor development underscores the critical role of receptor, transducer, and reporter components in designing highly effective MeHg detection systems. Receptors, which includes proteins, aptamers, and engineered molecules, serve as the primary recognition elements due to their high affinity and selectivity for MeHg binding. Beyond traditional fluorescence, reporters can leverage various detection principles, enhancing versatility across different sensing platforms. The selection of an appropriate transducer is vital, with factors such as sensitivity, matrix compatibility, and detection limits shaping the choice of the sensor architecture.

Electrochemical transducers, for example, enable precise quantification of MeHg concentrations across diverse environmental matrices [9]. In enzymatic sensors, electrochemical transduction is commonly employed, capitalizing on the enzymatic conversion of MeHg into electroactive species [10]. Immunosensors, on the other hand, utilize antibodies or aptamers as receptors and often integrate surface plasmon resonance (SPR) transducers to enable real-time detection [11]. The choice of transducer is pivotal, as it directly impacts the sensor's sensitivity, specificity, and adaptability to different environmental and biological matrices.

This review provides a comprehensive overview of detection platforms for MeHg, with a particular focus on recently developed sensors capable of trace-level detection. The advancements in sensing technologies are highlighted, emphasizing innovative approaches that surpass currently available analytical methods in terms of sensitivity and specificity. Moreover, the review outlines future perspectives to guide further research and development in this field, offering insights into potential improvements and innovations for MeHg detection. A brief conclusion is provided to summarize key findings and suggest pathways for advancing sensor technologies in this direction [9].

Fig. 1 illustrates a range of detection techniques employed in MeHg sensors, including small molecule probes, whole-cell biosensors (WCB), immunostrip assays, metal-organic frameworks (MOFs), and nanoarchitectonics-based sensors. The central diagram highlights the core components of currently known MeHg sensors, emphasizing the interaction between the analyte and the recognition element, followed by signal transduction. These diverse approaches showcase the integration of biological components with advanced materials, significantly enhancing the performance of MeHg detection technologies.

2. Whole cell biosensor platforms

Whole-cell biosensors (WCB) use microbial cell components as the receptors and transducers integrated inside the microbial cell to respond to the presence of chemicals (analyte of interest) and produce detectable output signals. The analytical signals are generally either fluorescence or luminescence. Fluorescence involves the emission of light following excitation by an external light source, commonly utilizing fluorescent proteins or dyes to detect target analytes. Luminescence, on the other hand, is based on light emission resulting from biochemical reactions, such as those catalyzed by luciferase enzymes, which occurs without external excitation. The cells are genetically engineered in a way that genes that are producing sensing components, usually proteins that specifically bind the analyte, are introduced either in the chromosome or kept on plasmids. In many cases, genes encoding reporter proteins or those producing reporter molecules are added to the same operon along with the sensing component, allowing the reporters to indicate the activity of the operon. This combination of sets of genes allows for the detection, recording, and quantification of the target analyte. The advancements in genetic engineering and synthetic biology have substantially enhanced the robustness of WCBs through introduction of genetic constructs into different microbial species that are more relevant for environmental conditions or targeting specific biogeochemical

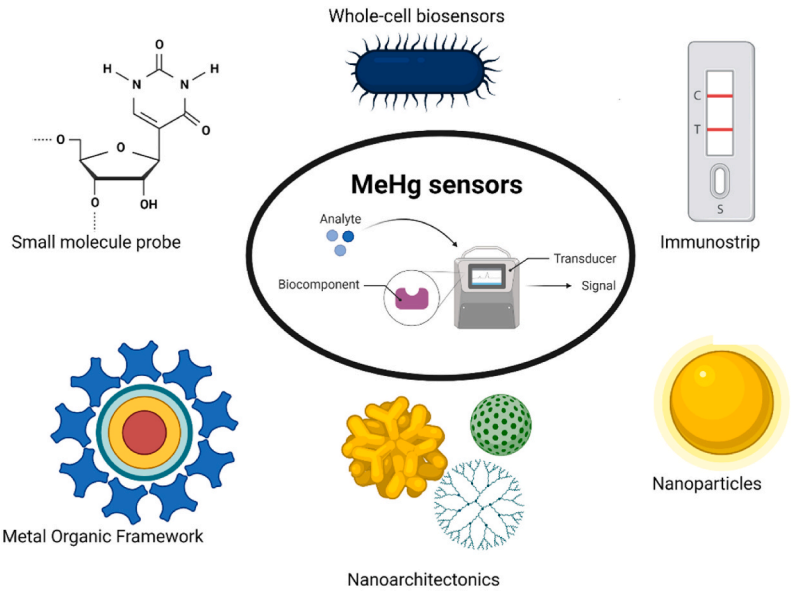

Fig. 1. The schematic illustration presents the various methods for MeHg detection reported in the literature, which include: (1) whole-cell biosensors (WCB), (2) immunostrip sensors, (3) small molecule probe-based (SMP) sensors, (4) metal-organic framework (MOF) sensors, (5) nanoparticle (NP)-based sensors, and (6) nanoarchitectonics sensors. Created with BioRender.com.

processess. Genetically engineering bacterial sensing modules that are introduced into surrogate hosts offers a versatile platform for the development of synthetic receptors with diverse applications (Fig. 2a) [12]. Based on these new approaches it resulted in expanded applicability of WCBs, enabling their use not only for analyzing laboratory-prepared artificial samples but also for measuring analytes directly in real environmental samples such as directly in the river water [11] or in collected contaminated water samples [13]. Based on our comparisons of data obtained from literature the sensitivity of WBC is at least one order of magnitude lower in both the environmental samples as well as in spiked media. However, this lower sensitivity might be due to the lower bioavilability of Hg species since they can get complexed with organic material [14] and as a result it is measured a fraction of relevant information that is representing bioavailable Hg concentrations [14]. Estimates of "bioavailable" mercury, the fraction that can be taken up by microorganisms in the environment, is especially important in oceanic environment since this fraction can play a pivotal role as it represents the bioavailable mercury at the particular niche in the ecosystem and is important for evaluation of potential further biomagnification processes.

Unlike laboratory procedures that often require additional sample preparation, biosensor platforms can achieve maximum sensitivity within 30 min of incubation in most cases. However, for certain samples, such as human urine, increased incubation times of several hours have been reported to enhance sensitivity [15]. Despite these advantages, maintaining stationary cultures of sensing cells for extended periods remains challenging due to factors such as unstable genetic elements, contamination risks, and the high energy demands of reactors. Nonetheless, successful attempts have been made to develop detection devices incorporating whole-cell biosensors (WCBs) in various forms, including fully integrated or non-integrated designs, utilizing microreactors or immobilized cells. These devices are miniaturized, field-deployable, and capable of multiplexed detection [16].

To the best of our knowledge, most whole-cell biosensors (WCBs) for the detection of MeHg exploit genetic constituents of the Hg-resistance operon, which enables microbes to detoxify Hg^{2+} and MeHg. In nature, within bacterial cells, the MeHg detoxification system is comprised of merB gene that is always present together with the merA gene, the gene for Hg^{2+} reductase transforming Hg^{2+} to Hg0, and a gene for the merR sensor protein. Therefore merR-based sensors employing merB genes are detecting Hg^{2+} after MerB protein demethylates MeHg and releases Hg^{2+} of MMHg. In such systems the specificity toward MeHg is problematic and both Hg^{2+} and MMHg are detected. Researchers solved this problem by combining two principles, demethylation with Hg^{2+} sensing, where merB, merR-containing cells and cells having only merR genes were exposed to the same samples separately. The levels of responses on Hg^{2+} are then deducted from signals obtained in cells containing merB together with merR. This was further enhanced by omitting Hg^{2+} transporters or mutating naturally occurring exporting mechanisms that increase specificity and sensitivity to MeHg. Since the genetic sensing elements are responding relatively fast the WCB can be also used in determining dynamics of methylation and demethylation in the culture of Hg methylating strains and conditions enabling their growth giving insights in conditions and ecological interactions that are stimulating or inhibiting MeHg production. Accordingly, Colin et al., 2018 have reported the approach to determine the Hg methylation potential of sulfate-reducing bacteria by using a luminescent biosensor for screening methylmercury production by co-culturing the reporter strain to either methylating or non-methylating *Desulfovibrio* strains [17].

The detection of methylmercury with whole-cell biosensors is influenced by factors such as cell characteristics and their viability including cell type and any additional genetic or phenotypic modifications or characteristics; environmental and experimental conditions like temperature, pH, nutrient availability, and sample matrix; and detection and calibration factors, which encompass MeHg concentration, presence of interferences, and the necessity for proper calibration for each cell type or even batch of culture used in the sensing [18].

The mercury methylation rates generally depend on the activity of the anaerobic Hg methylating microorganisms and the bioavailability of inorganic Hg(II) that can be converted by these bacteria [19]. The uptake of Hg(II) may involve specific Hg(II) complexes or forms of mercury that can bind to a nonspecific transmembrane transport system [19]. We should take into account that dissolved organic matter (DOM) affects mercury methylation due to its influence on bacterial cell physiology (as a potential nutrient) and its influence on Hg(II) speciation in the environment (as a complexing agent), therefore controlling Hg bioavailability [20]. As shown in Table 1, the WCB has proven to be sensitive with a detection limit of 2.5 nM. Further, the effects of chlorides, humic

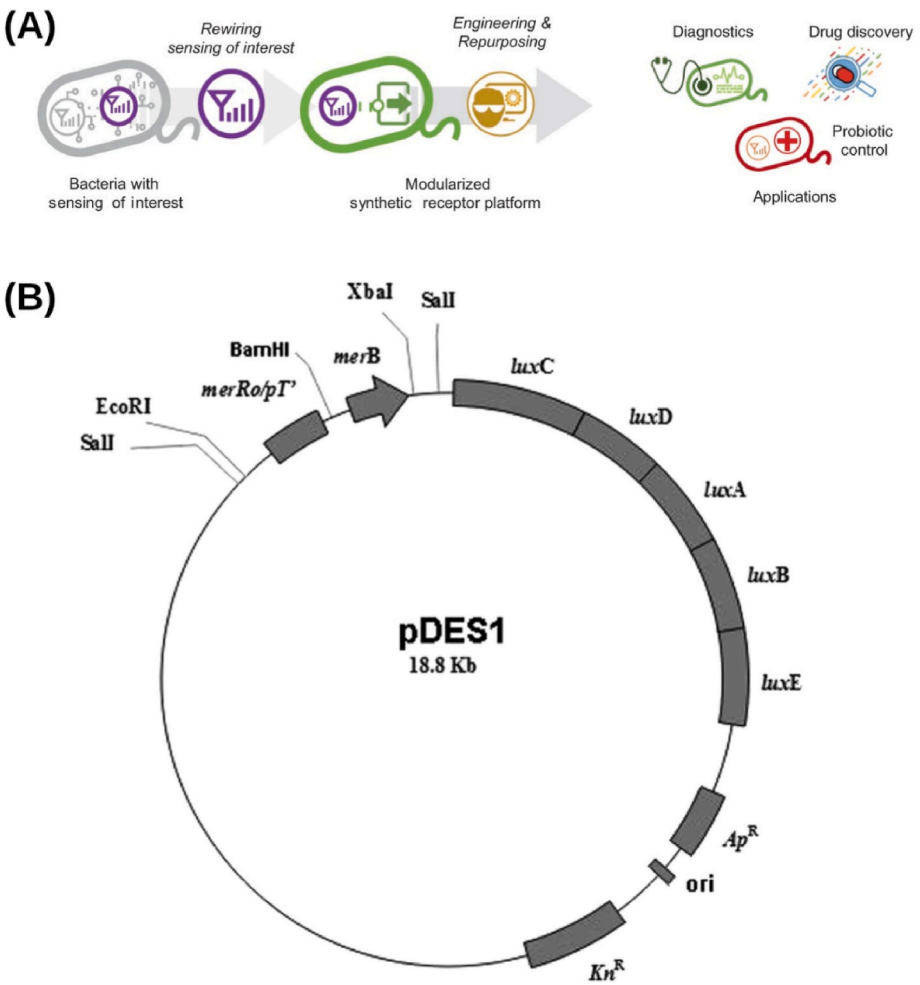

Fig. 2. a) Illustration for genetically engineering bacterial sensing modules into surrogate hosts for the development of a synthetic receptor platform that can be used for multiple application. Reproduced with permission from Ref. [12], Copyright (2021) Springer Nature. b) Diagram of the modification of Prb28 by inserting the merB gene into the *Bam*HI-*Xbal* region under the control of the mer promotor Reproduced with permission from Ref. [21], Copyright (2012) American Society for Microbiology.

acids, and thiols on the bioavailability of MeHg in *E. coli* were also investigated. Here, the concentration of chlorides increased MeHg detection, suggesting that there was passive diffusion of the neutral complex ($MeHgCl_2$) through the cell wall and membrane. In contrast, humic acids reduce the bioavailability of MeHg. Complexation with cysteine resulted in increased bioavailability of MeHg. The bioavailability of MeHg decreased with increasing glutathione concentrations [21]. In this regards, due to the interferences of cellular structures, which are varying among different strains, with the MeHg sensing, researchers should use cell types that are environmentally relevant to ensure accurate and applicable results as it was reported by Rijavec et al. [16]. The performance of different microbial host cells varies based on environmental conditions such as pH, temperature, the analyte of interest, and the specific gene chosen for the operon system [22–24]. The host strain selected must be able to function in different conditions of the regulatory genes [25]. Here, the optimization of the regulatory gene and RBS (Ribosome Binding Site) sequences should be considered. Since in most cases the plasmids are used for DNA cloning, genome editing, recombinant gene expression, and construction of genetic circuits all applicable in sensor construction, therefore, the plasmid copy number (PCN) is the primary consideration for quantitative metabolic flux enhancement as well as quantitative gene expression. The blossoming of synthetic biology not only needs plasmids with fixed PCN to maintain stable and permanent gene expression but also longs for plasmids with dynamically adjusted PCN to cope with changes in the life cycle and cellular metabolism [26]. Dynamic control of PCN will break the limitation of canonical plasmids with fixed PCN. So far, dynamic PCN control has only been achieved for pSC101 and ColE1 plasmids, which are both designed for *Escherichia coli*. We can envision that dynamic PCN control can facilitate the dynamic spatial organization of metabolism by adjusting the number of scaffolds for enzyme binding. With these updates, dynamic PCN control may produce a profound effect on calibration of the WCB since the activity of sensing genetic construct would be less impacted by the number of genetic elements.

Moreover, genetic systems exhibit population based response variety, a consequence of both stochastic processes and the mutability of underlying genetic programs. Improvement in the ability to monitor the behaviour of single cells has provided insight into how stochastic processes generate noises in isogenic population [27]. Fluctuations in the availability of shared resources of the host, such as polymerase and ribosomes, have influence on expression levels. In contrast, the biochemical processes responsible for the expression of individual genes cause further variation. In prokaryotes, the balance between transcription and translation strength strongly influences expression noise, with low transcription and high translation, which generates the most variability [28].

In addition, besides the internal cellular or population effects on the WCB the environmental factors are also making the biased responses. As an example, the WCB that can have growth optima in mild conditions cannot be used in extreme environments. However, if we prepare the

Table 1
Biological-based detection platform for MeHg.

No.	Hg species	(LOD)	Sample matrix	Sensor components			Sensing mechanism	References
				Bioreceptor	Transducer	Reporter		
1.	MeHg	10 pM with 1 ml sample	Contaminated environmental water samples	*MerB* gene	*MerR* of the mer-operon from Pseudomonas K-62 plasmid pMR26, *Vibrio harveyi*	Lux-AB	Luminescence based biosensor	[13]
2.	MeHg	2 nM	Model waters	pmerR	*Desulfovibrio* strains	$pmerR_{BS}$BPmerlux	Bacterial luminescent biosensor	[17]
3.	MeHg	2.5 nM	Model waters	mer-lux operon	*E. coli*	mer-lux WCB	Bacterial bioreceptor	[21]
4.	MeHg	0.238 nM	Natural water samples	pmerR	*E. coli* MC1061	lux	Luminescent bacterial based sensor	[11]
5.	MeHg	0.11 pM	Model waters	$pmerR_{BS}$BPmerlux	19sp (luxCDABE)	lux	Recombinant whole-cell luminescent bacterial sensors	[34]
6.	Hg^{2+} and MeHg	1.6×10^{-7} M and 5.0 $\times 10^{-7}$ M for Hg^{2+} 8.0×10^{-7} M and 1.0 $\times$ 10−6 M for MeHg	Model water	Anti-MeHg monoclonal antibody (mAb)	Immunostrip	immunochromatographic reporter	Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) and an immunochromatographic reporter	[35]
7.	MeHg	5.0 nM	Environmental samples	Aptamer (H_{T7})	AuNPs	Colourimetric detection	Chromogenic reaction of 3,3,5,5-tetramethylbenzidine (TMB)-H_2O_2	[36]
8.	MeHg and ethyl-mercury (C_2H_5Hg)	5000 nM, 600 nM	Model water and Fish sample	T-rich aptamers (HT_5 and H_{T7})	DNA-templated alloy Ag–Au nanoparticles	Color change in the solution	The aptamers specifically bind to MeHg and C_2H_5Hg, thus facilitating the formation of alloy Ag–Au NPs, which display a color change in the solution	[37]
9.	Hg^{2+} and MeHg	1000 nM	Environmental samples	Thymine moiety	AuNP	Naked-eye detection	Functionalized AuNPs with thymine moiety that sandwiches single mercuric species between two thymine molecules on the surface of the NPs	[38]
10.	MeHg	0.4 nM	Model water and Fish Samples	aptamer (H_{T7})	DNA functionalized AgNPs	Fluorescence	Metal amalgamation between mercury atoms and certain metal atoms with MeHg-specific scaffolds. They have also utilized the potential of aptamers (H_{T7}) that have a specific affinity towards MeHg than Hg^{2+}	[39]
11.	Hg^{2+} and MeHg	Hg^{2+} and MeHg were 3 pM and 4 nM, respectively	Seawater	Lysozyme type VI	lysozyme type VI-stabilized gold nanoclusters (Lys VI-AuNCs)	Fluorescence quenching mechanism	High affinity between Hg^{2+} and Au^{+} on the Au surface	[40]
12.	Hg^{2+} and MeHg	≥0.1 nm	Model waters	Adenine (A) conjugated small organic semiconductor (BNA) and deoxyribooligothymidine (dT_n)	Thin films containing bioreceptor	Conductivity measurement	Mutually templated co-assembly (BNA_ndT_n) of an organic semiconductor (BNA) and deoxyribo-oligothymidine (dT_n)	[41]

recombinant host adapted to the toxic environment it can be then used in sensing pollutant in extreme conditions. For example, *Acidothiobacillus ferrooxidans* BY-3 was used for the bioleaching of As(III) in extremely acidic conditions (pH < 1.8) [29,30]. It is also important to consider interfering pollutants when applying whole-cell biosensors (WCBs) because such pollutants can disrupt cell-cell interactions, substrate exchange, and signal transduction. However, we should not exclude possible horizontal gene transfer between cells that can in turn increase their tolerance to these interfering pollutants especially if used in longer sensing experiments [20].

During cell-cell interaction of the host in the environment and *in vitro* applications when cells are used in the biosensing application, secreted diffusible siderophores and other shared resources leading to cooperative, exploitative and competitive interactions between individuals that can affect sensor performance. These social interactions, in turn, can spur co-evolutionary arms races between strains and other species in the environmental matrices for WCBs, leading to ecological dependencies between them. The negative ecological interaction such as bacterial predation can decrease the number of WCS and give false low concentrations especially during the long incubation times during the induction. All the above phenomena should be taken into account for further WCB for MeHg detection [31].

Not only sensing components but also the reporting ones of the WCB are important to get proper intensity of the signal. Reporter genes can be

engineered into chromosomal DNA, inserting in the chromosome and disrupting specific gene. It can increase the sensitivity of biosensors but makes cells more vulnerable if the insertion of the sensing genes is in the region that have important cell function. Sometimes, reporter genes under the control of the corresponding promoters are introduced into the chromosome using mobile genetic elements or transposons. On other hand, a transcriptional fusion of reporter genes with an inducible promoter cloned in a plasmid is more frequently used in WCB platforms. In this case the construct is introduced into the appropriate plasmid compatible cells.

According to the type of the produced reporter proteins it is also expected to obtain different noise to signal ratios, sensitivity for physical conditions and time for obtaining appropriate signal. Several reporter genes are used in biosensing applications [16], including β-Galactosidase (lacZ) [32], luciferase (Luc), and various fluorescent proteins such as Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), and Yellow Fluorescent Protein (YFP). Each reporter has specific advantages and disadvantages: lacZ offers sensitivity dependent on the substrate and requires no ATP but has low permeability and can be used if cells are lysed. Luciferase is highly sensitive and provides rapid responses but requires substantial oxygen and ATP; in bioreactors, real-time detection can be hindered by simultaneous stirring that is giving additional noise when measured using PMT or photodiode. GFP is stable, moderately sensitive, and substrate-independent with no ATP requirement but can exhibit fluorescence after cell death, the protein needs approximately a few hours to form appropriate secondary structure, and in some cells the autofluorescence background might increase noise. Additional drawback represent also that the induction of recombinant protein expression typically occurs when cells reach a specific optical density, which is dependent on their generation time. Selecting an appropriate host for quicker protein expression can significantly reduce production costs.

Additionally, for monitoring environment and further enhancement either sensing proteins or reporters the directed evolution is a valuable method for biosensor development, involving the creation of a diverse gene library through targeted or random mutagenesis, followed by screening to identify variants with enhanced properties. This approach can lead to the development of robust enzymes for environmental sensing applications. Other strategies include engineering thermostable proteins through ancestral sequence reconstruction or incorporating thermostable protein motifs into other functional genes [33]. These approaches can be used especially in developing WCB for extreme environments such as environments with higher or extremely low temperatures, strict anaerobic conditions, salt water, etc.

Among reporter genes the most suitable represent luminiscence based reporting mechanisms, which might be either indigenous or needed to be added as a luciferin component externally. As reported by Rantala et al. (2011), the luciferin-luciferase system (lux operon) was utilized as a reporter for MeHg detection in their study. This system requires no substrate additions, enabling homogeneous, real-time monitoring of reporter gene expression. They developed a WCB strain, *Escherichia coli* MC1061, containing the recombinant plasmid (pmerRBlux), which responds to total mercury content in samples. The MerR operon, which regulates genes involved in bacterial mercury resistance, controls this system. In the absence of Hg^{2+}, MerR binds to DNA and represses transcription [11]. Upon Hg^{2+} binding, the DNA bound to MerR undergoes untwisting and unbending which facilitates open complex formation. Since the environmental matrix might be toxic due to other substances or inappropriate physiological conditions, researchers were normalizing sensor readings using the non-inducible, constitutively expressing lucifein/luciferase system in a toxicity control setup. Here the *Escherichia coli* MC1061 with the recombinant construct (pmerRBlux), which offers resistance toward MeHg (Fig. 2b) was used as a WCB. The merB mediates the cleavage of the carbon–mercury bond of MeHg to yield Hg^{2+}, which binds to MerR sensing component, induces the reporter genes and produces a self-luminescent organism due to the expression of lux enes. To decrease interferences of available Hg^{2+} present in the environment the ethylenediaminetetraacetic acid (EDTA) has been employed as a chelating agent to bind inorganic mercury (Hg^{2+}) from the sample, using an optimized concentration. *E. coli* MC1061 (pmerRBlux) can detect methylmercury (MeHg) at subnanomolar levels with high sensitivity under optimized assay conditions. The limit of detection (LOD) of 0.238 nM enables the measurement of MeHg even in complex real samples (see Table 1) [11].

In another study, Ivask et al. (2009) developed "lights-on" sensors, which are typically recombinant microbial cells containing a metal-response unit fused to a promoterless reporter gene that encodes a reporting signal with addition of external luciferin substrate. These bioluminescent strains detect metals, with expression levels indicating sub-toxic metal concentrations, allowing for the quantitative determination of bioavailable metals. Among these sensors, one was specifically designed for the detection of methylmercury (MeHg) in aqueous samples, achieving a detection limit of 0.11 pM [34]. Similar approach as reported by Ivask et al. (2009), Ndu et al. (2012) have developed a mer-lux WCB to determine the bioavailability of MeHg in *E. coli*.

There is another report in which Nagata et al. (2010) developed a WCB that carries a luciferin-luciferase gene constructs, luxAB, from *Vibrio harveyi* as a reporter under the control of the mercury-inducible MerR of the mer-operon from Pseudomonas K-62 plasmid pMR26 using similar principles as described above where merB gene that encodes organomercurial lyase cleaves the C–Hg bond of MeHg to give Hg^{2+} and is co-expressed with MerR in the sensor. The mer-promoter is activated when Hg^{2+} released from MeHg binds to the regular MerR protein. Light is emitted when mercurials in this specific form are present in the cytoplasm of the sensor bacteria, which merR did not recognize CH_3Hg^+ as an inducer. The concentration of CH_3Hg^+ can be determined by measuring the intensity of luxAB-specified luminescence. The resulting bacterial sensor responded specifically to MeHg, and the lowest detectable concentration of MeHg was 10 pM (Table 1) with a 1 ml sample in the optimized assay conditions. This detection limit is enough to detect many contaminated and pristine environmental samples [13]. Additionally, Colin et al. (2018) developed a luminescent biosensor for screening MeHg production and validated it by exposing the reporter strain to both methylating and non-methylating *Desulfovibrio* strains. Methylation activity, often associated with sulfate-reducing bacteria under anoxic conditions, was assessed using this biosensor. The sensitivity of the biosensor to MeHg was highly dependent on the growth conditions of sulfate-reducing bacteria. Sulfide concentrations ranging from 1 to 10 mM resulted in a 40–70 % decrease in luminescence as sulfide levels increased. Among inorganic ligands, sulfides are considered the most effective under anoxic conditions, as they significantly influence mercury speciation in ecosystems [17].

Besides WCB, there are reported also other attempts using bio-based approaches to detect MeHg. The use of monoclonal antibodies against conjugated MeHg with immunogenic molecules was reported. Indirect enzyme-linked immunosorbent assay (ic-ELISA) as the potential immobilization platform for biomolecules was developed for the detection of MeHg. As compared to other analytical methods, the further developed ic-ELISA in a immunostrip-based detection of methylmercury (MeHg) showed low sensitivity, with a limit of detection (LOD) of 1.6×10^{-7} M for Hg^{2+} and 8.0×10^{-7} M for MeHg, as shown in Table 1 [35].

Towards the development of rapid visual color change platforms, Chen et al. (2018) reported the use of T-rich aptamers (H_{T5} and H_{T7}) for the detection of MeHg, achieving a LOD 5000 nM. In their approach, DNA-templated alloy Ag–Au nanoparticles functionalized as transducers with color changes in the solution serving as the reporting mechanism.

Employed a nanoarchitectonics-based approach using adenine-rich sequences instead of aptamers, significantly improving sensitivity by more than four orders of magnitude under controlled laboratory conditions. However, this method failed to demonstrate efficacy in environmental samples [37,41].

Additionally, Lin and Tseng (2010) demonstrated an indirect detection mechanism for MeHg in the nanomolar range using lysozyme

type VI (Lys VI) in combination with the formation of gold nanoclusters (Lys VI-AuNCs). This method utilized fluorescence quenching as the transduction and reporting mechanism [40].

Further details on these platforms, including their working principles and detection capabilities, are presented in Chapter 3 (immunostrips) and Chapter 6 (aptamers), with specific examples provided for the measurement of MeHg.

3. Immunostrip based MeHg detection

Immunostrips assays are widely used for their simplicity, affordability, and portability, enabling rapid, on-site detection with high specificity and sensitivity. They consist of a sample pad, conjugate pad (with labeled antibodies), nitrocellulose membrane (test and control lines), and absorbent pad to ensure efficient operation (Fig. 3). These features make them ideal for environmental monitoring, food safety, and diagnostics. Immunostrip assays are based on the reaction between immobilized antibodies or conjugate on the membrane and the binding of the analyte of interest [42]. Advancements in immunostrips have been used in combination with novel nanomaterials, such as gold nanoparticles (GNPs), quantum dots, and upconversion materials. Immunostrips are widely employed in medical diagnostics due to their rapid, simple, and cost-effective nature.

Lateral flow assays (LFIA) offers several advantages, including rapid results, ease to use, and the ability to function in resource poor settings without extensive laboratory infrastructures [43]. LFIA provide quantitative and semiquantitative testing applications such as detection and determination of nucleic acids [44], proteins, whole-cell bacteria [45, 46] and also in rapid biosensing technology in point-of-care testing (POCT). Gold nanoparticles as reporters are often used in most of the assays and in recent years much attention has been paid to upconversion nanoparticles (UCNPs), for instance, lanthanide-doped nanocrystals as fluorescence signal nanomaterials [47–49]. This is mainly because of their high anti-Stokes shifts, optical stability, multiple emission bands and long lifetime. However, imunostrips are an ideal tool for the visual and fast estimation of analyte in “yes” and “No” forms, there are numerous challenges that need to be addressed for the future development of Immunostrips, such as selectivity, sensitivity, and response time.

In comparison to other MeHg sensing approaches, only one report has been published in this direction. Zou et al. (2017) described an immunostrip approach for the detection of MeHg utilizing MeHg-specific antibodies [35]. The study developed an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) and an immunochromatographic strip assay for detecting methylmercury (MeHg) in tap water. The authors have reported high specificity with an IC50 of 16.64 ng/mL and a limit of detection (LOD) of 2.03 ng/mL for ic-ELISA. The strip assay had a cut-off value of 500 ng/mL and a LOD of 11.3 ng/mL, with recovery rates of 98.13 % and 107.87 %, respectively. The most important feature of this study was its high specificity for MeHg without cross-reactivity with other metal ions and the convenience of not requiring sample derivatization. However, drawbacks include the need for optimization of assay conditions and potential variability in monoclonal antibody performance, with the strip assay possibly lacking quantitative accuracy compared to ic-ELISA. The assay can be improved by enhancing sensitivity and specificity, exploring alternative antibody production methods (eg. egg yolk antibodies), and integrating and interfacing these strips with reflectometer with portable detection devices for on-site testing.

4. Small molecule probes (SMP) in MeHg detection

Small molecule probes (SMP) are low molecular weight chemical compounds designed to selectively detect specific biological, chemical, or environmental targets through measurable changes, such as fluorescence, luminescence, or color shifts [50–57]. These probes often consist of a recognition moiety that interacts with the target and a reporter unit that produces a detectable signal upon binding or reaction. SMPs are extensively being used in diverse fields such as biology, physiology, medicine, pharmacology, and environmental sciences for their high specificity, sensitivity and versatility in detecting small molecules, ions, or biomolecules in complex systems. These fluorescent probes exhibit changes in intensities/emission wavelengths through possible sensing mechanisms which include Förster resonance energy transfer (FRET), intramolecular charge transfer (ICT), photoinduced electron transfer (PeT), excited-state intramolecular proton transfer (ESIPT) [57], and aggregation-induced emission (AIE) (Fig. 4a) [58]. The development of SMP has been rapidly increasing due to advancements in its application, such as g-fluorescence imaging modalities.

An ideal system of SMP-based fluorescent sensing platforms would provide a reliable signal read-out response under analytical conditions, which in turn helps us to measure the concentration of the analyte. The SMP that forms a reversible interaction between the analyte and the probe is categorized as a chemosensor measuring the accurate concentration of the analyte over time. If the sensing machinery is irreversible when contacted with the analyte, the probe is categorized as a chemodosimeter, in which the response will indicate only the maximum concentration of the analyte present in a time unit. Taking account of the above properties of SMP various MeHg detection platforms have been reported in recent years.

A simple turn-on fluorescence probe in real samples was developed by Shu et al. (2015) which demonstrated the design and synthesis principle. The group has developed a 4-hydroxynaphthalimide-derived fluorescent probe (1) to detect both organic mercury and Hg^{2+} in the aqueous medium. The mechanism of action for the sensing is with the dimethyl-thio carbamide ester group acting as the recognition unit, and N-butyl-4-hydroxy-1,8-naphthalimide acting as the fluorophore (Fig. 4b). The developed platform can be used as a “naked-eye” indicator for mercury species with the help of hydrogen peroxide (H_2O_2), which acts as a fluorescent enhancement. The study demonstrates that dimethyl-thiocarbamic ester moiety is a new design strategy for the

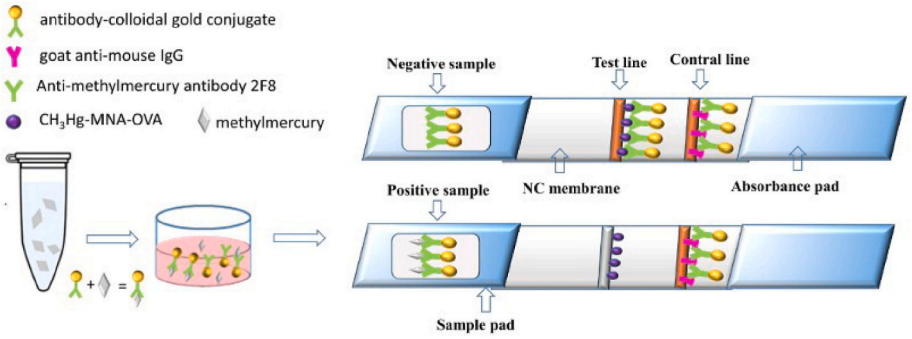

Fig. 3. Principle illustration of the immunochromatographic strip assay for MeHg detection. Reproduced with permission from Ref. [35], Copyright (2017) Taylor & Francis.

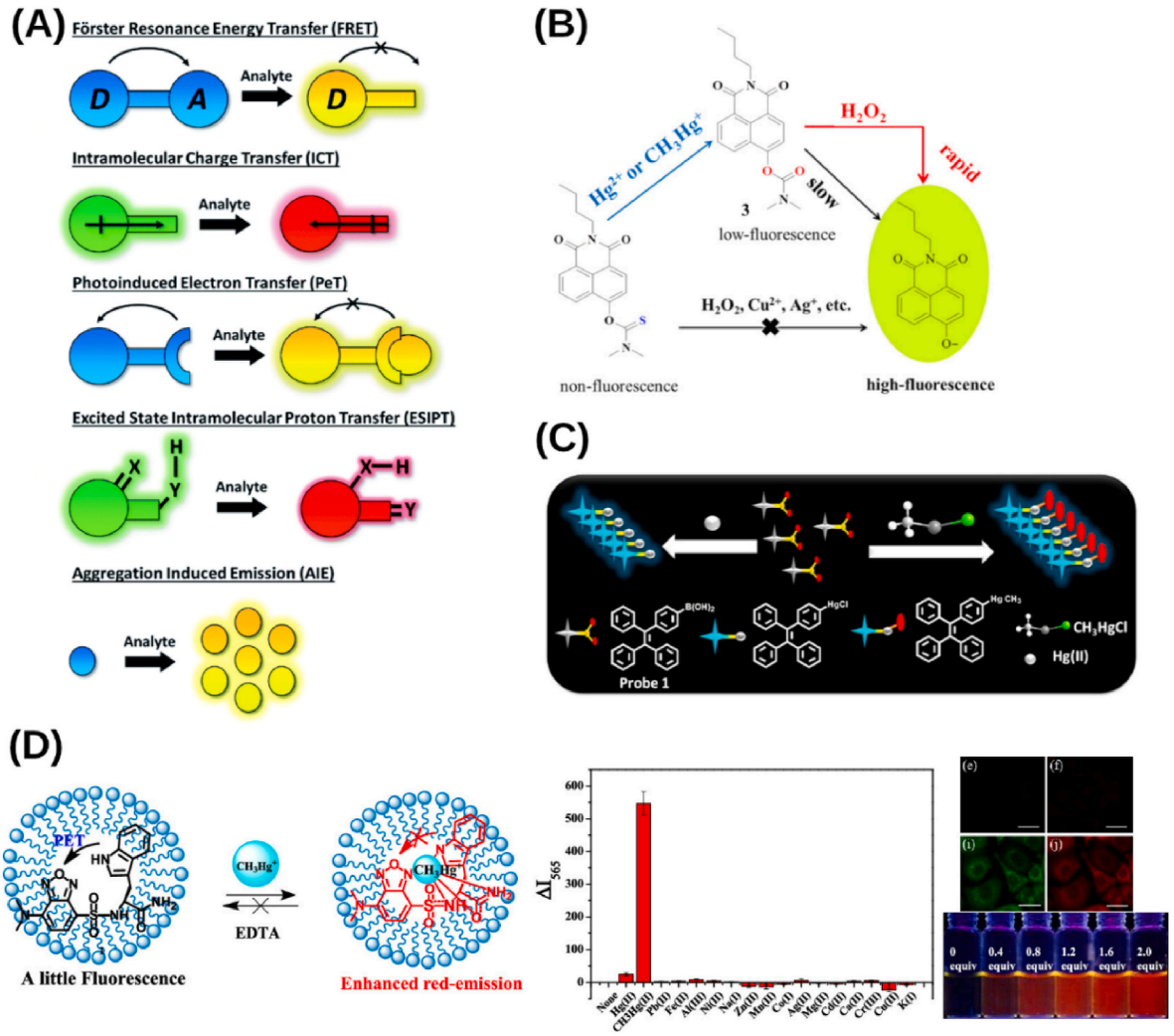

Fig. 4. a) General scheme for recent advances in SMP. Reproduced with permission from Ref. [58]. Copyright (2021) Royal Society of Chemistry. b) Reaction mechanism of probe 1 for mercury species with the help of H_2O_2. Reproduced with permission from Ref. [59]. Copyright (2015) American Chemical Society. c) A schematic representation of the sensing process of probe 1 towards Hg^{2+} and CH_3Hg^+ by turn-on type AIE. Reproduced with permission from Ref. [60]. Copyright (2017) American Chemical Society. d) Fast and sensitive fluorescent detection method of CH_3Hg^+ and Hg^{2+} using a reversible fluorescent molecular probe and SDS micelles. Reproduced with permission from Ref. [62] Copyright (2020) American Chemical Society.

construction of highly selective and sensitive probes for the simultaneous detection of inorganic and organic mercury species with the amplification reagent of H_2O_2. The sensitivity of the developed method was attained with the LOD of 2.4 nM for Hg^{2+} and 5.8 nM for MeHg in an aqueous solution [59].

Similarly, a highly sensitive and selective aggregation-induced emission (AIE)-based turn-on probe for both inorganic mercury ions and organicmercury species was reported by Chatterjee et al. The probe's mechanism is based on the mercury ion-promoted transmetallation reaction of aryl boronic acid. The probe, a tetraphenylethylene (TPE)-monoboronic acid (1), was used for the detection of mercury species (Fig. 4c). Both Hg^{2+} and MeHg ensued a fast transmetallation of TPE-boronic acid, causing a drastic reduction in the solubility of the resulting product (TPE-HgCl/TPE-HgMe) in the working solvent system. At the dispersed phase, the aggregated form of TPE-mercury ions recovers planarity as a result of restricted rotational freedom, promoting aggregation-induced emission [60]. This was the first report on the AIE based fluorescence imaging study on MeHg contaminated live cells and zebrafish. However, based on our knowledge and literature information the TPE stability with prolonged time may be compromised in extreme environmental conditions and cannot be appropriately used in various environments.

As previously seen in the work of Chatterjee et al. using the AIE turn-on probe strategy for dual detection of mercury species, Neupanea et al. (2020) developed a novel fluorescent probe for the detection of Hg^{2+} and MeHg in aqueous solutions using the displacement reaction of aryl boronic acid with mercury species. This probe demonstrated effective sensing properties for both Hg^{2+} and MeHg by forming a covalent adduct. The aryl boronic acid derivatives used in the displacement reaction enabled the development of a sensor with limits of detection (LOD) of 4.02 nM in distilled water, 1.82 nM in aqueous buffered solutions, and 10 nM in drinking water (Table 2). The major advantage of this platform is the detection time of less than 2 min for Hg^{2+} and less than 5 min for MeHg, and runs effectively in aqueous matrices [61].

Most of the SMPs are based on the fluorescence probe and researchers are trying to use different synthetic molecules to enhance the detection capabilities in coming years. In yet another report, Oh et al. (2020) developed a fluorescent detection method for Hg^{2+} ions and MeHg using an amino-acid-based fluorescent probe in combination with Sodium Dodecyl Sulfate (SDS) micelles (Fig. 4d). The fluorescent probe in SDS micelles sensitively and selectively detected Hg^{2+} ions and MeHg by the enhancement of the red emission at 575 nm in a purely aqueous solution, and the detection of MeHg was completed within 1 min which showed comparatively fast responses to the reported results of

Table 2
Chemical-based detection techniques for methylmercury.

No.	Hg species	(LOD)	Sample matrix	Sensor components			Sensing mechanism	References
				Bioreceptor	Transducer	Reporter		
1.	Hg^{2+} and MeHg	2.4 nM - Hg^{2+} 5.8 nM - MeHg	River water samples	Dimethyl-thioc varbamide ester group	4-hydroxynaphthalimide	Fluorescence	Dimethyl-thiocarbamide ester group acts as the recognition unit, and N-butyl-4-hydroxy-1, 8-naphthalimide acts as the fluorophore	[59]
2.	Hg^{2+} and MeHg	598.2 nM	Fish samples	TPE-monoboronic acid	Aggregate induced emission (AIE)	Fluorescence	Dimethyl-thiocarbamide ester group acts as the recognition unit, and N-butyl-4-hydroxy-1, 8-naphthalimide acts as the fluorophore	[60]
3.	Hg^{2+} and MeHg	4.02 nM 1.82 nM 10 nM	Environment samples	Displacement reaction of aryl boronic acid	Fluorescent probe based on displacement reaction of arylboronic acid	Fluorescence	Displacement reaction of aryl boronic acid with mercury species	[61]
4.	Hg^{2+} and MeHg	9.1 nM for Hg^{2+} and 206 nM for MeHg	Model water	Aamino-acid-based (indole, sulfonamide, and amide groups) and Sodium Dodecyl Sulfate (SDS) micelles.	Amino acid – based fluorescent probe (1) and SDS micelles	Fluorescence	Mercury ion-promoted transmetallation reaction of aryl boronic acid	[62]
5.	Hg^{2+} and MeHg	Hg^{2+} is 20 nM MeHg is 50 nM	Cells and living systems	pyridyl group	Coordination-induced emission	Fluorescence	The rotation of 1,8-naphthalimide moieties would be restricted due to the chelation between 1,8-naphthalimide and MeHg, which results in enhanced fluorescent emission	[63]
6.	Hg^{2+} and MeHg	94 nM	Model water	1,1,2,2-tetrakis[4-(3-methyl-1H-benzimidazol-1-yl) phenyl ethylene tetraiodide (Tmbipe)	1,1,2,2-tetrakis[4-(3-methyl-1H-benzimidazol-1-yl) phenyl ethylene tetraiodide (Tmbipe)	Fluorescence	Displacement reaction of aryl boronic acid with mercury species	[64]
7.	Hg^{2+} and MeHg	54 nM 19.08 nM	Model water	zeolitic imidazolate framework-7 (ZIF-7) and zeolitic imidazolate framework-60 (ZIF-60)	Coordinated induced emission	Fluorescence	Strict cavity confinement towards Hg^{2+} and MeHg	[65]
8.	Hg^{2+} and MeHg	1.79 nM	Model water	MIL-53(Fe) with CCl_4	MIL-53(Fe)	Fluorescence	Amino-acid-based fluorescent probe and Sodium Dodecyl Sulfate (SDS) micelles with enhancement of the red emission at 575 nm	[66]
9.	Hg^{2+}, Hg_2^{2+} and MeHg	5.0 pM	Environmental water	Piperazine derivative, HEPPSO, i.e., N-(2-Hydroxyethyl) piperazine-N′-(2-hydroxypro-panesulfonic acid)	catalysis-reduction in an aqueous solution through the cooperative effect of AuNP-catalyzed properties and the formation of gold amalgam	Absorption using UV-Spectrophotometer	Organic reaction catalyzed by AuNP and measured the change in the catalytic product induced by the deposition of Hg atoms on the surface of AuNPs	[67]
10.	Hg^{2+} and MeHg	10 nM for Hg^{2+} and 15 nM for MeHg	Model waters	$CuDDTC_2$ Complex; diethyldithiocarbamate (DDTC)	Au-NPs; AIE	Naked-eye detection	The rotation of 1,8-naphthalimide moieties would be restricted due to the chelation between 1,8-naphthalimide and MeHg, which results in enhanced fluorescent emission	[1]
11.	MeHg	5.9 nM	Environmental samples	PEG	Carbon dots	Fluorescence	Recognition event of the MeHg is dependent on its hydrophobicity and its ultrasound-assisted permeation via PEG	[68]

(*continued on next page*)

Table 2 (*continued*)

No.	Hg species	(LOD)	Sample matrix	Sensor components			Sensing mechanism	References
				Bioreceptor	Transducer	Reporter		
12.	MeHg and Hg^{2+}	1.7 nM for DAOC-AgNPs and 7 nM for RhB@DAOC-AgNPs	Contaminated water	amide-modified oxacalix [4] arene derivative viz. Di-acetamido-oxacalix [4] arene (DAOC) (a heteracalixarene host)	Silver nanoparticles (Oxacalix [4]arene templated) - (DAOC-AgNPs)	Fluorescence	The fluorescent dye could rapidly coordinate with Hg2+ species to form a dinuclear Hg^{2+} tetracarbene complex which can then self-aggregate to turn on AIE fluorescence	[69]
13.	MeHg and other toxic cations	~1 aM (atto molar)	Environmental matrices (Lake water)	Stripped layers of Hexathiol and PEG	Au gold film sputter coated on a glass slide	Conductivity measurement	Changes in the tunneling current across films of NPs protected with striped monolayers of organic ligands	[70]

Chatterjee and Neupanea group previously. The interference studies of the probe were selectively tested with 16 other metal ions and found that the probe in SDS micelles with EDTA exhibits highly sensitive and selective turn-on detection for MeHg over Hg^{2+} [62].

The chemodosimeter, a newly developed method in the area of SMPs, paved the way for the detection of MeHg and Hg^{2+} reported by Zou and Tian (2010). The work includes the synthesis of two novel fluorescent chemodosimeters that displayed selectivity involving receptor and sensing mechanisms consisting of 2,1,3-benzothiadiazole and thiourea signalling and recognition moieties, respectively. The chemodosimeter's spectral and optical properties exclusively distinguish between Hg^{2+} and MeHg and open new opportunities for monitoring. The developed method was able to detect Hg^{2+} with LODs (1.6×10^{-7} M and 5.0×10^{-7} M) and MeHg (8.0×10^{-7} M and 1.0×10^{-6} M) as reflected in Table 2 [71]. However, based on the understanding of the sensing reaction based on the above mentioned constituents the detection of mercury species can be only single use type due to their irreversible reaction. This is also the main reason why this approach can be used in dosimeters and are not suitable for real-time monitoring.

More recently, Yuan et al. (2019) developed a new fluorescent dye for the selective detection of Hg^{2+} ions and organic mercury including MeHg and phenylmercury ($PhHg^{+}$). The sensing mechanism involves the rapid coordination of dye with Hg^{2+} species to form a dinuclear Hg^{2+} tetracarbene complex, which self-aggregate to activate turn on AIE fluorescence. The dye 1,1,2,2-tetrakis[4-(3-methyl-1H-benzimidazol-1-yl) phenyl ethylene tetraiodide (Tmbipe) featured four positively charged methylated benzimidazole groups, conferring excellent water solubility. The fluorescence activation occurs within 3 min driven by synergistic rigidification of the tetraphenylethylene-bridged Tmbipe core through chelation ring formation with mercury, further enhanced by probe aggregation. This system achieves an LOD of 94 nM, as summarized in Table 2 [64]. As in the case of chemodosimeter studies, this work restricts the multiple analysis and may face interference in complex matrices which is not sufficient for ultratrace mercury detection in a strict regulatory context.

With AIE based principle and turn-on fluorescence probe as seen from the above studies for the detection of dual mercury species, Yang et al. (2009) developed a dual fluorescent platform which utilizes intramolecular rotational restriction to designed a molecular probe incorporating a pyridyl group as the chelating unit and 1,8-naphthalimide as the fluorescent unit. This probe produces a very weak fluorescence in the absence of an analyte of interest since the free intramolecular rotations of the 1,8-naphthalimide moieties non-radiatively annihilate its excited state. However, in the presence of analytes (Hg^{2+} or MeHg), the rotation of 1,8-naphthalimide moieties would be restricted due to the chelation between 1,8-naphthalimide and MeHg, which results in enhanced fluorescent emission. The limitation of the sensor is actually the response induced by Hg^{2+} since it is much stronger than the response caused by the MeHg. However, in this type of sensor the problem was tackled by using T-rich DNA that masks Hg^{2+} in solution and therefore enable MeHg detection. With this method, the sensitivity in detection of two mercury species was reached with the LODs of 50 nM (MeHg) and 20 nM (Hg^{2+}). The method has an advantage in terms of selectivity and has shown less or no interference with other metal ions [72].

5. Metal-organic framework (MOF) for MeHg detection

Metal-organic frameworks (MOFs) are crystalline porous materials composed of metal ions or clusters coordinated to organic linkers. According to the International Union of Pure and Applied Chemistry (IUPAC) definition, these are like porous coordination polymers showing crystalline structures and potential voids [73]. MOFs are most compatible with organic and aqueous media with additional advantages such as defined porosity due to the larger spaces such as 544 $m^2\ g^{-1}$ and cost-effectiveness. Nitrogen adsorption porosimetry, electronic, optic and atomic force microscopies, powder X-ray diffraction, solid-state NMR, UV–vis and IR spectroscopies are some of the defined techniques that can be used for the characterization of the MOFs [74,75]. Development of MOFs is achieved using autoclaves and fabrication using microwave-assisted techniques to accelerate the kinetics of the coordination or mixing of precursors [76]. In recent years, various new polymerization techniques have been used for fabrication to fine-tune the size and morphology based on the electrosynthesis of the conducting material [77]. For preparation of a MOF different metal ions or clusters are mixed with organic linkers using a convenient solvent. Coordination polymerization takes place between the precursors, resulting in a cross-linked network showing potential voids (Fig. 5) [78].

MOFs offer high surface area, tunable porosity, and chemical versatility, enabling applications in gas storage, catalysis, sensing, and drug delivery [52,54,79,80]. MOFs emerged in the 1990s, with pioneering examples including MOF-5, HKUST-1, MIL-101, and ZIF-8., The developed 1D, 2D, and 3D MOFs have paved the way for the advanced MOF-based sensor systems. Although the integration of MOFs into electronics is still in its infancy, promising applications have been demonstrated in devices such as field-effect transistors (FETs) and resistive random-access memories (RRAM) [9]. In MOF-based electronic sensors, sensitivity is defined as the smallest fluctuation in an external stimulus that induces a measurable signal variation.

In sensors, MOFs function as a receptor or recognition element, enabling the design of MOFs with superior selectivity. MOFs can detect analytes, including MeHg, through mechanisms such as size exclusion (based on porosity) and interactions engineered via specific linkers or precursors. Selectivity is achieved by tailoring the MOFs interaction with the target analyte, leveraging forces such as hydrogen bonding, π-π interactions, and electron donor-acceptor coordination [81,82].

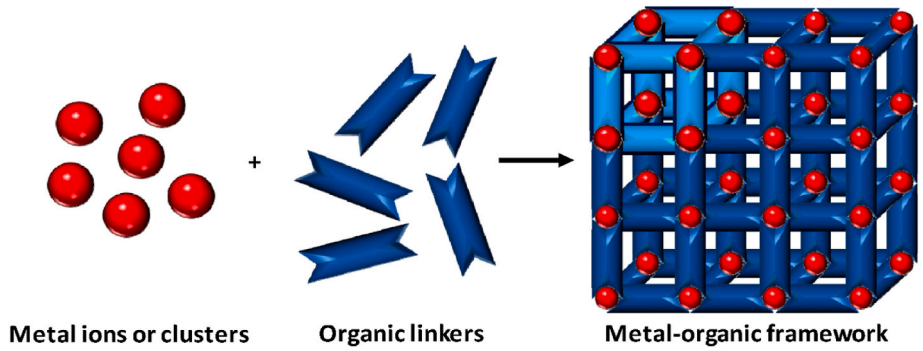

Fig. 5. Scheme for the preparation of a MOF. Different metal ions or clusters are mixed together with organic linkers using a convenient solvent. Over the past few decades, over 100,000 structures have been reported in the ''MOF subset'' of the Cambridge Structural Database (CSD) Reproduced with permission from Ref. [78] Copyright (2018) MDPI.

Compared to biomolecules MOFs offer advantages as recognition materials, including customizability, cost-effectiveness, high stability, and compatibility with harsh conditions like extreme temperatures and pH levels.

Based on insights we gained through our thorough literature review, metal-organic frameworks (MOFs) can be broadly categorized into four major types: 1) mass-loaded, 2) filtration, 3) electronic function, and 4) optical sensitivity.

Mass-loaded: The porous nature of MOFs can adsorb and preconcentrate the analytes in complex sample matrices, increasing the possibility of host-guest interactions and further improving the sensitivity of the sensors. The selective nature of this MOF type is mainly based on its unique and specific host–guest interaction which is also beside specificity also improves the sensitivity of the sensor. However, the introduction of MOFs into electronic sensors as mass-loaded layer that are sensitive to mass change seems to be more feasible. Integration of MOFs with Quartz crystal microbalance (QCM), surface acoustic wave sensors (SAWS) and microcantilevers have been explored in recent years [9]. MOFs based electronic sensors can only serves as the adsorption layer for different molecules such as gases (CO_2, CO, CH_4) and volatile organic chemicals (VOC) [9].

Filtration: The MOFs filtration properties captures a targeted analyte and serve as the filtration layer to shield other interferences. With this property of MOFs, different platforms were developed to screen, filter and purify mixtures including gases, pollutants and organics [9]. The best example in this direction is ZIF-8 which has high hydrogen permeability due to its pore size (0.34 nm), allowing selective penetration of H_2 molecules (0.289 nm) and excluding their larger gas molecules (diameter >0.34 nm) [9]. In recent years, Koo et al. and Zhou et al. fabricated MOFs designed for H_2 and CH_4 detection by functionalized ZIF-8 as a filtering layer assembled on Pd NWs and ZnO nanorods [83]. The major drawback of the system is that the detection range for analytes is still limited because of mechanisms of filtration adopted for selectivity.

Electronic Function: The long-range order in MOF crystal structures leads to charge transport possibly through the metal ions and the organic linkers. However, MOFs are confronted with the opposite condition where most MOFs suffer from a low degree overlap between the p orbitals in the linker groups and the metal d orbitals since the oxygen atoms of carboxylate ligands coordinate to the metal ions through σ bonds. Therefore, conventional MOFs with low atomic density plus strong electron charge transfer are considered as insulators (conductivity $<10^{-10}$ S $cm^{-}1$) [84]. Accordingly, most MOFs in the electronic sensors are designed on the on-chip thin film grown directly on electrodes, as a paste or freestanding pellets, and used for sensing gases and other volatiles such as alcohols.

Optical Sensitive: MOFs exhibit superior optical properties deriving from its d–d transition in metal ions and electron transfer in the ligand or metal-to-ligand/ligand-to-metal electron transfer under external stimuli, including light, temperature and pressure. The optical sensing performance of MOF-based sensors stems from the host-guest interaction which converts the recognition interaction into an electronic signal or optical signal [9]. The receptors in MOFs are clustered molecules, such as organic linkers or metal ions, that, in response to analytes like MeHg, induce changes in the structural framework of the MOF [73,85–87]. The reporting system for the MOFs is usually based on the difference in the fluorescence signal detected before the analyte interacts with MOFs and after when the MOF coordination changes. Most of the MOFs reported optical sensors are based on the utilization of monoloyer nanofilms and oleylamine (OLA) which provide the water stability of the MOF layer.

To the best of our knowledge, based on a review of the literature, only a few published reports are available on the specific detection of MeHg using MOFs at sensitive and trace levels. Utilizing dual zeolitic imidazolate frameworks (ZIF-7) and (ZIF-60), Xu et al. (2013) have developed a fast, sensitive and facile sensing method on the basis of the fluorescence based mechanism and speciation analysis of inorganic Hg^{2+} and MeHg. The synthesis protocol was simple and based on a microwave-ultrasound-assisted approach. The sensing mechanism is based on the strict cavity confinement of ZIF-7 and ZIF-60 structures, which exhibited excellent selectivity for Hg^{2+} and MeHg even in the presence of various cations or anions. The developed method showed LODs of 54 nM and 19.08 nM for Hg^{2+} and MeHg, respectively (Table 2). The platform was also tested with real samples and the best performance of the sensor was reported at pH 7, with a recovery of 100 % and 96.2 % for Hg^{2+} and MeHg, respectively. respectively [65]. Another pioneering work by Jia et al. (2013) developed a rapid and facile microwave ultrasound-assisted synthesis method for the preparation of MIL-53(Fe) MOF for the direct, rapid, highly selective and ultrasensitive sensing of MeHg. The sensor achieved the detection limit of 1.79 nM, where MIL-53(Fe) suspension, with CCl_4 as the dispersant, served as a fluorescence sensing platform, demonstrating a significant increase in fluorescence intensity upon exposure to MeHg, with minimal interference from other tested Hg species [66].

The complexity of sensor fabrication, which may hinder scalability and reproducibility potentially affects applicability in diverse environments.

6. Nanoparticle (NP) based biosensor

Nanoparticle (NP) based biosensors leverage the high surface area, tunable chemistry, and sensitivity of NPs for precise analyte detection in fields like diagnostics, environmental monitoring, and food safety. Nanomaterials are different in surface effects compared to bulk materials, due to three reasons; (a) large surface area and high particle number per mass unit, (b) the fraction of atoms at the surface is increased, and (c) the atoms situated at the surface have fewer direct

neighbours (Fig. 6a). Among various nanoparticles, gold nanoparticles (AuNPs) and Silver (Ag) are widely used for the detection of methylmercury (MeHg) and mercury (Hg). This is mainly because they are often functionalized with spacers and functional groups to enable selective and specific binding. Their localized surface plasmon resonance (LSPR) enables optical detection with high sensitivity, while their conductivity supports electronic sensing applications. A few examples of functionalized nanoparticles are outlined below.

(i) **AuNPs surface functionalized with diethyldithiocarbamate (DDTC):** AuNPs functionalized with DDTC are highly effective for binding Hg due to the strong affinity of sulfur in DDTC for mercury ions. This binding relies on the soft-soft interaction principle of Hard and Soft Acids and Bases (HSAB) theory. Functionalization enhances the selectivity and sensitivity of AuNPs for Hg detection, with binding causing measurable changes in optical or electronic properties. Based on this principle, Chen et al. (2024) developed a colorimetric nanosensor strategy for various species of Hg in the aqueous phase (Fig. 6b). The proposed method involves $CuDDTC_2$ complex assisted AuNP-Based nanosensors that act as specific ligands to recognize the Hg species. Since Hg has a high affinity for soft donors such as sulfur, in the presence of Hg species, they can immediately displace the Cu^{2+} in the CuDDTC2 complex by promptly forming Hg−DDTC complexes containing two residual thiol groups for Hg^{2+} and one for MeHg. The citrate ions, which keep AuNPs from aggregation on the surfaces, can be easily displaced by thiol groups. Thus, the Hg−DDTC complex can be attached to the surface of AuNPs through Au–S linkages. This interaction destabilizes the system, causing the aggregation of AuNPs and resulting in a colour change in the solution. EDTA was used as a masking agent in the nanosensing mechanism to selectively mask Hg^{2+} from other mercury species. With this method, the dual mercury species were simultaneously determined with the LODs of 10 nM for Hg^{2+} and 15 nM for MeHg. They also tested the selectivity of the sensing mechanisms and found out that the mechanism is very stable toward mercury species against other metal ions [1].

(ii) **Lysozyme type VI-stabilized gold nanoclusters:** This type of functionalization renders the effective binding of Hg via thiol, amine and carboxyl functional groups, which form stable complexes with Hg ions. The use of lysozyme type VI-stabilized gold nanoclusters (Lys VI-AuNCs) provides significant advantages in maintaining fluorescence stability, enhancing detection capabilities, and ensuring the reliability of the sensing method. A dual-mode detection platform was designed based on the above nanocluster phenomena developed by Lin and Tseng (2010). The method utilizes a one-step approach to prepare Lys VI-AuNCs for the ultrasensitive detection of Hg^{2+} and MeHg based on a fluorescence quenching mechanism. The study reported the use of 25 mg/mL Lys VI as a reducing agent, which formed Lys VI-AuNCs (denoted as Au-631). Au-631 was highly stable in a high concentration of glutathione or high salinity solution such as NaCl. Au-631 was capable of sensing Hg^{2+} and MeHg through the interaction between Hg^{2+}/MeHg and Au^{+} on the Au surface. When EDTA was introduced which acts as the Hg^{2+} masking agent, Au-631 was able to discriminate between Hg^{2+} and MeHg. The LODs obtained in this study were 3 pM and 4 nM for Hg^{2+} and MeHg, respectively. The selectivity of this probe is highly efficient and, in fact, more than 500-fold for Hg^{2+} over any metal ions. The authors have also used bovine serum albumin-stabilized AuNCs, with Au-631, in which Au-631 provided an approximately 330-fold improvement in the detection of Hg^{2+}. Importantly, they have successfully applied this probe for the determination of Hg^{2+} and MeHg in the seawater matrix [40]. In another study, Xie et al. (2018) have developed a simple, rapid and cost-effective colourimetric method for selective and sensitive detection of MeHg. The sensing mechanism is based on the increasing surface deposition of Hg, enhancing the catalytic effect

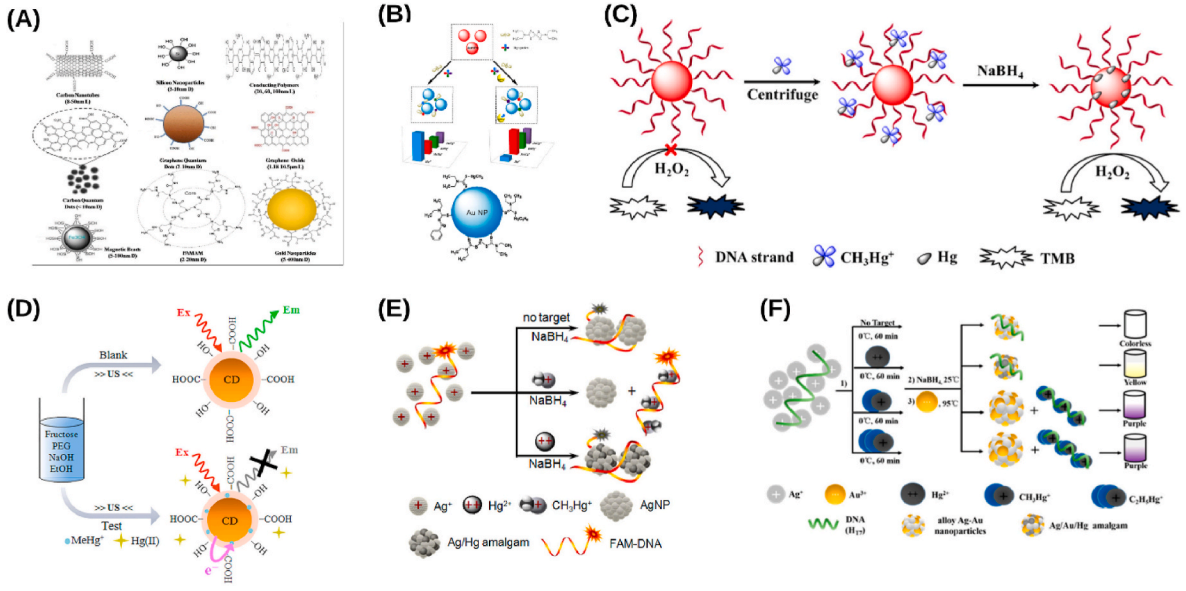

Fig. 6. a) Commonly used nanomaterials in various kinds of sensors fabrication with their sizes. L: length; D: Diameter. Reproduced with permission from Ref. [91] Copyright (2020) MDPI. b) Illustrations of AuNP-Based colorimetric sensing strategy for Hg Species and Hg− DDTC complex attached to the surface of AuNPsc) CH_3Hg^{+} sensing mechanism. Reproduced with permission from Ref. [1] Copyright (2014) American Chemical Society. c) CH_3Hg^{+} sensing mechanisim. Reproduced with permission from Ref. [89] Copyright (2018) MDPI. d) Schematic representation of the mechanism involved in the fluorescence quenching caused by the presence of MeHg. Reproduced with permission from Ref. [68] Copyright (2014) American Chemical Society. e) Schematic illustration for fluorescent detection of MeHg based upon a dye-labeled T-rich DNA (FAM-DNA). Formation of AgNPs or Ag/Hg amalgams on FAM-DNA template quenches the FAM fluorescence emission; the presence of MeHg suppresses formation of the metal nanostructures, revealing fluorescence enhancement. Reproduced with permission from Ref. [39] Copyright (2015) The Royal Society of Chemistry. f) Schematic illustration of the experimental principle for the colorimetric detection of MeHg and C_2H_5Hg. Reproduced with permission from Ref. [37] Copyright (2018) American Chemical Society.

of AuNPs. The aptamer (H_{T7}) was used as a bioreceptor toward MeHg (Fig. 6c) which was used to capture and separate MeHg by using centrifugation. This capturing of MeHg using aptamers functionalized in the AuNPs increased the Hg deposition on Hg onto the surface of AuNPs. This enabled and enhanced the catalytic activity of the AuNPs toward the chromogenic reaction of 3, 3,5,5-tetramethylbenzidine (TMB)-H_2O_2. The LOD of the nanobiosensor was 5.0 nM with a linear range of 10–200 nM [89].

(iii) **AuNPs with thymine moiety:** AuNPs functionalized with thymine are effective for Hg binding due to the high affinity of thymine to Hg ions. Thymine forms stable T-Hg^{2+}-T complexes, where Hg^{2+} bridges two thymine molecules, leading to selective recognition. Based on this, Aulsebrook et al. (2018) developed a tandem colourimetric, temperature-dependent naked eye sensing machine for Hg^{2+} and MeHg. In this study, the authors have functionalized AuNPs with a thymine moiety that sandwiches a single mercuric species between two thymine molecules on the surface of the NPs, and this enables the aggregation of NPs when it is introduced to Hg^{2+} and MeHg species. The interaction/reaction of Hg^{2+} and MeHg caused a significant change in the visible absorbance spectrum, which resulted in a naked eye distinguished colour change from red to blue. Though they were successful in employing the basic chemistry for mercury-induced aggregation in the functionalized AuNPs, the LOD of this approach is much higher than the permissible level of mercury in the environment (1000 nM), which makes it unusable in environmental matrices [38]. The platform was rapid, sensitive and simple it often suffers potential interference with limited detection range due to the stability and complex functionalization process.

(iv) **HEPPSO, N-(2- Hydroxyethyl) piperazine-N′-(2-hydroxypropanesulfonic acid) functionalized AuNPs:** Functionalization Au surface with HEPPSO showed higher affinity towards Hg ions due to strong chelating properties of the sulfonic acid group (-SO_3H) and –OH present in HEPPSO. These functional groups interact specifically with Hg, forming stable complexes. The functionalization enhances the selectivity and sensitivity of AuNPs for Hg detection. Recently, Li et al. (2017) have developed a dual detection platform for sensing Hg^{2+}, and MeHg using catalysis-reduction in an aqueous solution through the cooperative effect of AuNP-catalyzed properties and the formation of gold amalgam. The method utilized an organic reaction catalyzed by AuNP and measured the change in the catalytic product induced by the deposition of atoms of various Hg species on the surface of AuNPs. Piperazine derivative, HEPPSO has been used to functionalize AuNPs to construct a catalysis-reduction assay. HEPPSO specifically reduces Hg species (such as Hg^{2+}, Hg^{2+} and MeHg) into Hg^0. The signal reporter for detecting Hg with an absorption peak at about 340 nm is based on the product of AuNPs that can catalyze HEPPSO. The LOD of the developed sensor was 5.0 pM which was 3 orders of magnitude lower than the U.S. Environmental Protection Agency (EPA) limit value of Hg for drinking water [67]. The method showed promising results with a new functionalization method with broad applicability, often lacks large scale synthesis and possibly faces practical challenges compared with WCB and MOFs based detection platforms.

(v) **Silver NP functionalized with amide-modified oxacalix** [4] **arene derivative viz. Di-acetamido-oxacalix** [4] **arene (DAOC) (a heteracalixarene host)**: This type of surface functionalization are highly effective for Hg detection due to host-guest interaction between Hg ions and the unique structure of DAOC. The amide group in DAOC provide selective binding sites, allowing for the effective complexation with Hg ions. Similarly, Dey et al. (2020) have developed a sensor methodology using amide-modified oxacalix [4] arene derivative viz. Di-acetamido-oxacalix [4] arene. DAOC (a heteracalixarene host) was synthesized for the stabilization of silver nanoparticles (DAOC-AgNPs). The surface of DAOC-AgNPs was functionalized with Rhodamine B (RhB), yielding RhB@DAOC-AgNPs to induce fluorescence activity. DAOC-AgNPs and RhB@DAOC-AgNPs were found to be selective and sensitive sensors with chromogenic mechanisms for the detection of MeHg with an LOD of 1.7 nM and 7 nM respectively. The basic underlying mechanism is postulated as mercury-induced oxidation of DAOC-AgNPs leading to the formation of Ag–Hg nanoalloy, which is the reason for the disappearance of the SPR (Surface plasmon resonance) band. PXRD, SEM, TEM and EDX have been used to characterize the sensors and their sensing mechanism. Cysteine, the amino acid, was also selectively detected with the aid of DAOC-AgNPs and MeHg based on the soft-soft interaction between the thiol group of cysteine and MeHg ions. Utilizing the masking nature of EDTA towards Hg^{2+}, a multilevel INHIBIT-OR logic gate assembly was designed to discriminate MeHg and Hg^{2+} ions. The sensing machinery was employed for onsite detection of MeHg using three different kinds of portable sensors (cellulose fibre strip, hybrid alginate bead and ZIF-8 based nanocomposite); this was accomplished by immobilization of DAOC-AgNPs into cellulose fibre, sodium alginate beads and zeolite imidazole framework (ZIF-8). RhB@DAOC-AgNPs were also additionally employed for *invivo* MeHg detection in brine shrimp (Living system-aquatic crustacean *Artemia salina*) [69]. The developed method has limitations in large scale applications and requires complex functionalization process.

6.1. Carbon dots

Carbon dots (CD) are gaining popularity in biosensing due to their excellent biocompatibility, optical properties, and ease of functionalization. CDs exhibit strong fluorescence, photostability, and minimal cytotoxicity, making them ideal for real-time detection in environmental applications [90]. CDs possess abundant functional groups such as –OH, –COOH, and –NH_2, which enable efficient interactions with Hg^{2+} ions through coordination or electrostatic interactions. These features makes CD an ideal candidate to develop sensor platforms. An in situ fluorescent assay for MeHg detection using CDs as optical nanoprobes for the detection of MeHg was developed by Costas-Mora et al. (2014). The recognition event is based on the hydrophobicity of MeHg and its ultrasound-assisted permeation through the passivation coating made of polyethelyne glycol (PEG). The fluorescent quenching of MeHg is measured using Microvolume fluorospectrometry. The assay uses low amounts of organic precursors (fructose, PEG, and ethanol), and is completed within less than 1 min. The mechanism of fluorescence quenching caused by the presence of MeHg interacted with CD are presented in Fig. 6d. This green synthesis of CDs can substitute various tedious sample preparation methods and the involvement of various analytical instruments. The CDs synthesized in this study has an average size of 2.5 nm used as optical nanoprobes for sensing MeHg with a LOD of 5.9 nM (Table 2). The optical nanoprobe demonstrates high selectivity with detection completed within 1 min with excitation at 470 nm and emission at 517 nm. Fluorescence, AAS, and Fourier transform infrared spectrometry (FTIR) were applied for the determination of sensor machinery and are suggested as the basis of the recognition event. With the help of a portable fluorospectrometer, these nanoprobes can have extended applications in various environmental matrices such as tap water, river water and sea-water [68]. To the best of our knowledge, CDs have demonstrated high sensitivity, eco-friendliness, and cost-effective preparation methods with great versatility. However, despite these advantages, CDs are prone to stability issues in long-term studies.

6.2. Aptamer based detection

Aptamers are short, single stranded DNA or RNA sequences that bind selectively with Hg ions through electrostatic interactions, hydrogen bonding and conformational changes. Upon binding Hg, aptamers undergo structural changes, which can be detected using various analytical methods. This method for detecting Hg is highly effective due to high affinity and specificity of aptamers towards Hg. In another detailed report, Deng et al. (2015) have developed a sensor for MeHg detection and easy discrimination of MeHg from other Hg species based on the interaction of DNA aptamer with Ag ions that can direct formation of three types of nanostructures either (i) Ag-DNA nanoparticles (AgNP) in the absence of MeHg, (ii) Amalgamed AgHg-DNA nanoparticles in the presence of Hg^{2+} ions and just Ag nanoparticles and separated MeHg-DNA complexes. In all of the DNA-NP adducts the fluorescence signal from DNA aptamer is quenched leading to the strong fluorescent signal when MeHg is present in the solution (see Fig. 6e). They have utilized high specificity potential of aptamers (H_{T7}) that have much stronger affinity towards MeHg than Hg^{2+}. With a fluorophore-labeled DNA aptamer, the sensing machinery can detect MeHg down to the picomolar level, which is > 125 times more sensitive than Hg^{2+}. As shown in Table 1, the LODs for the sensing mechanisms are 0.4 nM. The sensor had over 50 times superior selectivity towards MeHg than Hg^{2+} and 106 times than other metal ions. Interestingly they have employed this sensor for testing its capability in monitoring MeHg accumulation in tissues and organs from three kinds of daily consumed fishes; the results show the accumulated order of snakehead > bighead carp > grass carp through the food chain [39].

Recently, Chen et al. (2018) developed a sensor that visually detects MeHg and C_2H_5Hg using DNA-templated alloy Ag–Au NPs. They employed two T-rich aptamers (H_{T5} and H_{T7}) to specifically recognize MeHg, and C_2H_5Hg. When Au^{3+}, Ag^{+} and T-rich aptamer, MeHg and C_2H_5Hg all are present in a solution, the aptamers specifically bind to MeHg and C_2H_5Hg, thus facilitating the formation of alloy Ag–Au NPs, which display a color change in the solution (Fig. 6f). The developed platform has a visuable detection range of 5000 nM, and when using UV–visible spectrometry, the LOD can reach as low as 600 nM of MeHg. The developed sensor system was tested with fish muscles showing a recovery rate of 101–109 % [37]. Few examples of recently developed aptamers sequences for MeHg are listed in Table 3.

Table 3
List of reported aptamers specific to MeHg.

Type	DNA Seuences	References
H_{T5}	5′-CTTTGTTAAAAATTCTTTG-3′	[37]
H_{T7}	5′-GTTCTTTGTTAAAAATTTGTTC-3′	
H_{T9}	5′-TTGTTCTTTGTTAAAAATTCTTTGTTCTT-3′	
H_{T10}	5′-TTTGTTCTTTGTTAAAAATTCTTTGTTCTTT-3′	
H_{T12}	5′-TTCTTTGTTCTTTGTTAAAAATTCTTTGTTCTTTGTT-3′	
H_R	5′-CTGCTGCTGCAAAAAGCAGCAGCAG-3′	[39]
H_{T7}	5′-GTTCTTTGTTAAAAATTCTTTGTTC-3′	
FAM-H_R	5′-FAM-CTGCTGCTGCAAAAAGCAGCAGCAG-3′	
FAM-H_{T5}	5′-FAM-CTTTGTTAAAAATTCTTTG-3′	
FAM-H_{T7}	5′-FAM-GTTCTTTGTTAAAAATTCTTTGTTC-3′	
FAM-H_{T9}	5′-FAM-TTGTTCTTTGTTAAAAATTCTTTGTTCTT-3′	
dT_6	5′-TTTTTT-3′	[41]
dA_6	5′AAAAAA-3′	
dA_{10}	5′-AAAAAAAAAA-3′	
dT_{10}	5′-TTTTTTTTTT-3’a	
dG_{10}	5′-GGGGGGGGGG-3′	
dC_{10}	5′-CCCCCCCCCC-3′	
dT_{20}	5′-TTTTTTTTTTTTTTTTTT-3′	
dA_{20}	5′-AAAAAAAAAAAAAAAAAAAA-3′	
Apt1	5′CTTTGTTAAAAATTCTTTG-3′	[92]
Apt-com	5′GAAACAATTTTTAAGAAAC-3′	
Apt2	5′CTATGTTAAAAATTCTTTG-3′	
Apt3	5′CTTAGTTAAAAATTCTTTG-3′	
Apt4	5′CTTTCTTAAAAATTCTTTG-3′	
Hp-Fc	5′-SH-GGCGATGTTTTTTCGCC-Fc-3	[93]
S1	5′-CGGATTTTTCTTGC-3′	
S2	5′-SH-GTTCTTTGTTAAAAATTCTTTGTTC-3′	
SA−MB	5′-GAACAAAGAAAAAAAAACAAAGAAC-MB-3′	
ST	5′-GAACAAAGAATTTTTAACAAAGAAC-3′	

AH_{T5}, H_{T7}, and H_{T9} represent CH_3Hg^+ - Specific DNA with different T bases, H_g represents random DNA, FAM - 6-carboxyfluorescein.

7. Nanoarchitectonics based detection platforms

Nanoarchitectonics represents an advanced conceptual paradigm for designing and synthesising of functional materials with nano-sized structural features (Fig. 7a). The term, nanoarchitectonics, was first coined by Masakazu Aono, who proposed this concept in the conference title, 1st International Symposium on Nanoarchitectonics Using Supra-interactions in 2000 [94,95]. The terms "nanoarchitectonics" and "nano + architectonics" have appeared frequently in scientific literature, as evidenced by the publication title by Hecht (2003) [96], and in the names of research centres in Tsukuba (2001) [97] and at the University of California, Los Angeles (2003).

Nanoarchitectonics is a confluence of advanced nanotechnology with scientific disciplines, including supramolecular chemistry and materials processing [98,99]. In the nanoscale regime, accommodating the uncertainties and unexpected disturbances through statistic distributions and thermal fluctuations of functionalized materials and structures that are architecturally designed with atomic/molecular/nanoscopic elements through various processes, including atom/molecular-level manipulations, chemical modification and self-assembly/self-organization, defines the nanoarchitectonics foundations [100,101]. The molecular-level regulations in biology and biomedical fields are still different from the atom/molecular-level controls in nanotechnology, although they both interact on the same scale. The nanoarchitectonics approach seeks to realize precise control over atoms and molecules in manipulations and reactions.

The nanoarchitectonics concepts have myriad applications in various research fields, such as fabrication of functional materials [102–105], energy and environmental science [106,107], and biological and biomedical applications [103,108,109]. Among these fields, sensing applications represents one of the most crucial areas that can benefit from the nanoarchitectonics approach [94,110]. By tailoring the structure, composition, and surface properties of nanomaterials, such as nanoparticles, nanoclusters, or nanocomposites, researchers can develop highly efficient sensors for Hg detection. Nanoarchitectonics enables the controlled assembly of functional components, such as receptors, ligands, or functional groups, that selectively bind to Hg ions, resulting in measurable changes in optical, electrical, or electrochemical signals.

Since interesting approaches can be built for sensor development using a nanoarchitectonics approach, such as the one that Pandeeswar et al. (2016) have developed, where instead of conventional T and A nucleotides of the DNA are used as a recognition element, they have, using self-assembly, synthesized adenine (A) conjugated small organic semiconductor (BNA) and deoxyribooligothymidine (dT_n) as a recognition element for MeHg detection. Nanoarchitectonics also offers possibilities to develop self-assembly based solid-state sensors developed by Cho et al. (2012), which was the only approach that was able to detect MeHg in the real concentration from various environmental matrices such as fish samples and lake water samples [41,70]. Pandeeswar et al. (2016) group have developed a nanoarchitechtonics based ultra-sensitive detection of Hg^{2+} and organometallic mercury, driven by a novel chemical design principle that allows strong mercury thymine interaction. The novel optoelectronic approach based is on nanoarchitectonics of a small molecule templated DNA system that consists of an adenine (A) conjugated small organic semiconductor (BNA) and deoxyribooligothymidine (dT_n) (Fig. 7b). The mutually templated co-assembly (BNA_ndT_n) of an organic semiconductor (BNA) and deoxyribo-oligothymidine (dT_n) has tunable chiroptical, morphological and electrical properties tapped in to enable ultrasensitive and selective

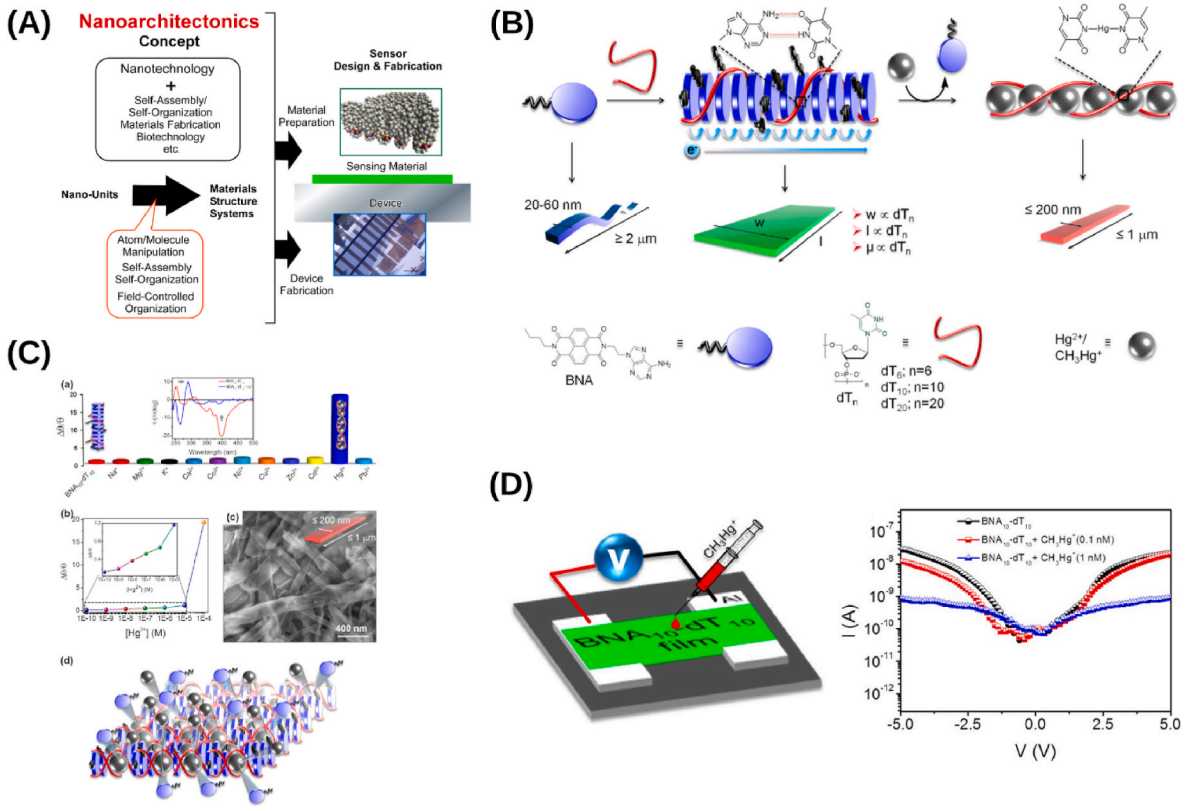

Fig. 7. a) Basic concept of nanoarchitectonics. Reproduced with permission from Ref. [111] Copyright (2019) Beilstein Journal of Nanotechnology. b) Schematic illustration of the nanoarchitectonics of BNA and dT_n coassembly as well as the corresponding molecular structures. Reproduced with permission from Ref. [41] Copyright (2017) American Chemical Society. c) Chiroptical sensing of Hg^{2+}. Schematic representation of Hg^{2+} induced displacement of BNA from the BNA_n-dT_n 2D assembly via formation of a metallo-DNA duplex $[dT\text{-}Hg\text{-}dT]_n$ and self-assembled BNA 1D tapes. Reproduced with permission from Ref. [41] Copyright (2017) American Chemical Society. d) Conductometric sensing of CH_3Hg^+. Schematic representation of CH_3Hg^+ detection device structure. BNA_{10}-dT_{10} device response before and after the addition of different CH_3Hg^+ concentrations (0.1 nM and 0.5 mM). Reproduced with permission from Ref. [41] Copyright (2017) American Chemical Society.

detection of Hg^{2+} and organometallic mercury in water. The rapid conformational change of a BNA_n-dT_n co-assembly into a metallo-DNA duplex [dT-Hg-dT] n in the presence of Hg^{2+} and organometallic mercury is utilized for a chiro-optical and conductivity (Fig. 7c and d) based rapid and sub-nanomolar sensitivity (≥0.1 nM) to mercury ions in water, as noted in Table 1. The correlation in terms of the dTn sequence length and the stability, integrity and functional properties of the BNAn-dTn co-assembly was obtained from a range of microscopic and spectroscopic measurements. The strategy that they used to advance the nano-approach is redefining the simple interaction of mercury with thymine bases using altered bases i.e. adenine (A) conjugated small organic semiconductor (BNA) and deoxyribooligothymidine (dT_n). Moreover, they have used aptamers which can clearly differentiate MeHg and Hg^{2+}. Rather than using the interaction of the recognition molecule and mercury species in an aqueous phase, they had the novel approach of developing a film that acts as a transducer which selectively hops BNA in the presence of MeHg to influence the conductivity measurement. This design strategy is anticipated to advance the development of novel templated DNA nanoarchitectonics approaches, enabling the creation of bio-optoelectronic devices and sensors for detecting toxic cations such as MeHg [41]. As from our knowledge, and detailed in the publication, this method does not distinguish between inorganic and organic mercury sources, limiting its applicability. Furthermore, the results may be influenced by external factors like pH and ionic strength, affecting reliability and also validation in real-world scenarios is necessary to confirm the robustness of the detection system.

With all the above-based detection platforms, the majority of the platforms are not reproducible, and encounter interference issues. This may be because of the selection of films and lack of integration with appropriate sensing components. Cho et al. (2012) have developed a simple solid-state sensor for the ultrasensitive detection of MeHg in model solutions and environmental matrices. In this study, AuNPs coated with binary mixtures of n-hexanethiol (HT) and glycol (EG) that generates supramolecular structure (HT/EG3) in a solid-state sensor of unmatched sensitivity towards MeHg with a detection limit of ~1 aM [88]. This HT/EG3 NPs structure sense CH3Hg + selectively and with a detection limit of ~1 aM. The complexity of sensor fabrication, which may hinder scalability and reproducibility potentially affecting applicability in diverse environments.

The mechanism of the sensor is based on the changes in the tunneling current across films of NPs protected with organic ligands-striped monolayers. The sensitivity advancement is enabled by the ligand shell organization of the NPs. When MeHg binds with the metal cation, the electronic structure of the molecular bridges between proximal NPs changes. This will lead to a tunnelling current increase resulting in highly conductive paths, thus resulting in the percolation of the entire film. The nanoscale heterogeneity of the film structure increases the possibilities of the cation-binding constants, which leads to a wide range of sensitivity (remarkably, over 18 orders of magnitude in MeHg concentration) [70]. This study introduced reduced selectivity of the sensors when exposed to mixtures of cations with similar binding characteristics, which can complicate detection in real-world samples. While the sensors demonstrate high sensitivity, their performance may vary based on the specific ligand-shell organization of the nanoparticles, potentially limiting their effectiveness for certain cations. The reliance on specific conditions, which is hard to achieve in environmental samples, may

hinder practical applications in diverse settings.

8. Future perspectives, challenges and conclusions

8.1. Whole-cell biosensors (WCB)

This type of sensor is especially important in determining the ecologically relevant bioavailable fraction of mercury. However, the bioavailable fraction is dependent on the type of the cell and environmental conditions and therefore it is hard to clearly differentiate real bioavailable fraction from the low sensitivity. Based on the current synthetic biology tools the repertoire of the hosts for introducing genetically engineered genes is expanded and should be the concept of bioavailability tackled by combining and comparing (i) chemical laboratory based tools with (ii) the outcome in the environment, e.g. bioaccumulation and (iii) results of using a battery of different WCB environmentally relevant hosts, similar as it was shown by using native marine and freshwater WCB hosts [16], but it should be utilized many different hosts relevant for particular either geochemical cycle or foodchain transformations.

Moreover, the WCB can determine the level of MeHg in the environment in contrast to the standardized approaches therefore minimal disturbance of the environment can be much easier achieved, but still in most cases the samples are tested in laboratory conditions since there are not many attempts to use WCB directly in the field. Therefore, it should be further developed as self sustainable WCB platform bearing alive cells and should be biologically safe not deploying genetically modified strains in the environment. The miniaturisation and portable platforms are set to make on-site analysis more desirable but this is facing many challenges such as preserving low fluxes of nutrients and samples such as in microreactors since small designs are prone to clogging, preserving suspended or immobilized cells for monthly operations as well as appropriate calibration. Perhaps advances in cell encapsulation, microfluidics and multi-cell systems are expected to enhance, on one hand the stability and reproducibility and on the other hand the extension of the environmentally relevant information. In this regards the key organisms in the environment must be first identified using state-of-the-art metagenomic approaches, then utilizing advanced culturomics to be able to obtain strains that can be then at the end available for introducing genetic components by using genetic engineering tools and transforming them into WCB.

As described in Chapter 2, the WCB often might have lower genetic stability which hampers the performance over time and calls for either approach to replenish the cells using lyophilized cultures or monitoring sensitivities by appropriate on site calibration.

Although various factors such as complexation with cysteine would increase the uptake of MeHg and the addition of humic acid can reduces the MeHg uptake within the cells, we do not know the precise mechanisms that are aiding in MeHg uptake and WCB can serve us not only as a sensor platform but also to determine key environmentally relevant aspects of MeHg bioavailability. Therefore, further advances need to be carried out in research that clarifies the meaning and applicability of the use of WCB in ecological and human health risk assessment. WCB are best in conditions similar to their natural environment [112] and the temperature, incubation time, medium type, pH, and reagents can all have effects on the performance of the biosensors that can only be understood with further research and testing. Employing advanced cell encapsulation techniques to enhance stability, and optimizing growth conditions to ensure cell functionality will prolong the WCB performance.

Since WCB are also prone to the high background noise that is affecting LOD it would be very important to incorporate the currently developed advanced AI tools or autocorrelative mathematical models to better distinguish signal from the cellular, genetic or environmentally introduced noises resulting in updated sensing platform. By addressing these factors, WCBs can become more reliable, sensitive, and effective for accurate MeHg detection, standing out as the only type of sensor capable of providing environmentally relevant data.

8.2. Immunostrip based MeHg detection

Immunostrips colourimetric detection platforms for MeHg often suffer sensitive limitations, especially for trace levels. They also encounter non-specific bindings or cross-reactivity with other metal ions that can affect the accuracy. The strips can degrade over time, affecting the stability of immobilized biomolecules (antibodies, aptamers, and enzymes). Though the platforms bring advantages such as simplicity, speed and cost-effectiveness, making them suitable for on-field detection platforms, they often encounter lower sensitivity compared with other methods are susceptible to matrix interference and limited ability to distinguish from other mercury species.

Employing MeHg-specific antibodies in conjunction with an improved reporter system could provide enhanced solutions for future MeHg sensors. Integrating antibodies against MeHg with mesoporous materials or AuNPs could significantly improve sensitivity. Additionally, leveraging advanced techniques such as Surface Plasmon Resonance (SPR) or Surface-Enhanced Raman Spectroscopy (SERS) could enable sensors with detection limits several orders of magnitude lowe [35].

As detailed in Chapter 3, the sensitivity of immunostrips can be enhanced by incorporating nanomaterials into the conjugate pads and selecting biomolecules with high affinity for MeHg. A further approach to improve platform sensitivity involves integrating a reflectometer to enhance pixel detection of gold nanoparticle test lines, facilitating more precise intra-sample comparison. Since this platform is useful in semi-quantitative analysis and can be used for rapid onsite “yes” and “No” type detection of MeHg.

8.3. Small molecule probe (SMP) detection platforms

SMP are extensively utilized in various sensing platforms due to their ability to selectively interact with the specific analyte through chemical and physical changes. Most of the SMP utilize fluorescence probes which exhibit lower detection limits compared to more sophisticated platforms like immunoassay or nanoparticle-based systems. The platforms also have limited stability with probes, potential interference from other Hg species and metal ions, and difficulty in achieving real-time detection in complex matrices. Though the SMP is designed for selective analysis, cross-reactivity with structurally similar molecules or ions can occur. The probes often degrade over time, particularly under harsh environmental conditions (eg. UV light, and temperature extremes). SMPs are usually tailored for single analyte detection, making them less suitable for multiplex detection of target analytes. SMPs often require precise environmental control or additional instrumentation for effective detection, such as fluorescence readers or spectrophotometers. Some SMPs may involve the use of organic solvents or chemicals that are less environmentally friendly. A chemodosimeter was developed and demonstrated potential for mercury detection; however, it is limited to single-use applications due to its reliance on an irreversible reaction mechanism.

Among the fluorescent SMP, near-infrared (NIR, 650–900 nm) probes have several advantages over other probes, such as reducing photo-bleaching due to the lower energy of excitation required [113]. As described by Denk et al., 1990 [114], the use of two-photon (TP) excitation has been predominantly used in NIR fluorescent probes [113–115]. Over the last few decades, there have been tremendous improvements in the SMP in the realm of biological sensing and bio-imaging. As described in Chapter 4 and Table 2, most of the SMPs utilize turn-on probe strategy for dual mode detection of mercury species and the major advantage was a shorter detection time (1–5 min), compared with available analytical methods. No SMP can measure MeHg in the seawater matrix and using FRET, ICT, PeT, ESIPT, AIE, and probes combining these modalities can be developed in the future for

MeHg onsite detection in seawater. Combinatorial fluorescence techniques have to be used to overcome the issue of interference and sensitivity for MeHg detection. Several boundaries have to be dismantled to push forward the development of enhanced SMP for MeHg detection in seawater or other environmental matrices beyond the current state-of-the-art.

Future direction in the line of SMPs functionalizing with nanomaterials and integrating into a hybrid platform combined with biological elements (eg aptamers and antibodies) would be an option to increase the sensitivity and stability. Development of SMPs with improved environmental stability and reduced cross-reactivity through advanced chemical synthesis techniques. Enhancing the chemical specificity of the probes and integrating advanced signal processing methods can also improve accuracy and reliability, making SMPs biosensors more effective for MeHg detection in diverse applications.

8.4. Metal organic framework (MOF)

MOFs can suffer from non-specific adsorption, leading to interference from other metal ions and compounds which restricts its limited selectivity. This is because of the porous structure which allows non-target analytes to occupy binding sites, reducing specificity for MeHg detection. Moreover, they encounter challenges including potential instability and degradation under varying environmental conditions, complex and costly synthesis and functionalization processes, and susceptibility to interference from complex sample matrices. The lack of sensitivity is a general barrier when using MOFs for the detection of MeHg as well as other analytes. MOFs often degrade under harsh conditions because of their metal-ligand bonds which are prone to hydrolysis or photodegradation. A complex and time-consuming synthesis process might hinder scalability and widespread use because controlled pore size, structure and functionality is required for selective MeHg sensing.

Most of the MOFs developed in the past are made for gas sensing applications because of their excellent adsorption capacities (chapter 5). Very limited structures of MOFs have been explored to date such as MOF-5,1 HKUST-1,2 MIL-101,3 and classical ZIF-8. As detailed in Chapted 5, MOFs have been used in different layers as selective applications. Although the MOFs offers sensitivity for the detection of MeHg (1 aM), then complicated and sophisticated nanoparticle thin films often limit its scalability and practicality for commercialization. The research should focus on developing more stable MOF materials with improved chemical and mechanical durability, refining synthesis and functionalization protocols to ensure reproducibility and efficiency, and integrating sample pre-treatment techniques to reduce matrix effects. MOFs can be engineered with specific ligands, functional groups, or structural modifications to enhance selectivity for MeHg. Combining MOFs with advanced nanomaterials such as graphene, fullerenes, carbon nitrides, and MXene along with signal amplification strategies, enables the use of different transducers and facilitates optical and electrochemical detection methods, thereby enhancing performance through improved sensitivity and selectivity. MOFs can be engineered with specific ligands, functional groups and should aim at creating portable, cost-effective sensors with improved stability and versatility for diverse environmental and industrial applications, thus expanding their practical use in precise MeHg detection [116–122]. [123].

Functionalized MOFs with specific sensing molecules might be the most suitable to target MeHg and would help reach lower sensitivity for MeHg detection. To the best of our knowledge there are no reports on using analyte-specific aptamers that can be used to link MOFs to build architectures that can specifically coordinate with MeHg. This approach and integration of MOF hybrid aptamer on microfluidic devices allow the high-performance biosensing and the controlled synthesis of MOFs. Develop robust MOFs using hydrophobic linkers, metal clusters, or post-synthetic modifications to withstand harsh environmental conditions. Integrate MOFs with nanomaterials (e.g., gold nanoparticles, carbon dots) or employ advanced detection techniques like SERS or SPR to amplify detection signals. Focus on cost-effective, scalable synthesis methods, such as microwave-assisted or mechanochemical approaches, to promote widespread use. Combine MOFs with other materials, such as polymers or biodegradable membranes, to create hybrid systems that improve selectivity, durability, and detection range in future MeHg based detection platforms.

8.5. Nanoparticles (NPs)

Nanoparticles such as AuNPs, carbon dots, silver nanoparticles, QDs, Magnetic Nanoparticles (MNPs), Carbon Nanotubes (CNTs), graphene, and nanofibres have been put forth for miniaturizing biosensing platforms to the nanoscale to improve the understanding of various properties like optical, electronic, and magnetic characteristics, which would pave the way for their use in bio-sensing systems [124–128]. However, some drawbacks such as cross-reactivity with non-targeted metal ions or compounds, reducing their specificity towards MeHg detection should be the main future focus since these are the main factors limiting their selectivity. These drawbacks are probably the result of unspecific binding to functional groups or surface ligands which interact with a range of analytes leading to false positive results. NPs are being used in various research fields and their potential release into the environment can pose ecological harm and health risks, especially metal-based ones, which are not biodegradable and can accumulate in organisms. NPs possess low stability and often tend to aggregate or degrade in complex matrices like biological fluids or wastewater affecting their performance. This is mainly because of changes in pH, ionic strength or the presence of organic matter. Cost and scalability are the biggest challenges for the synthesis and functionalization of NPs which can be expensive in maintaining the precise size, shape and surface chemistry for effective detection.

NPs integrated with fluorescence, colorimetry, SERS, SPR, and electrochemistry improve the sensitivity of MeHg detection. As discussed in Chapter 6, NPs (AuNPs and AgNP) functionalized with DDTC, Lysozyme, thymine, HEPPSO and DAOC display enhanced selectivity and sensitivity towards MeHg. This is mainly because of the selection of functional groups which interact with MeHg to form a special complex. However, though the methods are promising and can able to detect MeHg at a trace level, they often lack challenges such as large-scale synthesis, complex functionalization process and stability in relevant natural conditions. Attention has been made towards the development of aptamers-based conjugated NPs sensing platforms for the selective detection of MeHg (Section 6, Table 3). This type of approach is time-consuming and laborious in the production of aptamer specific towards MeHg.

To the best of our knowledge, bioconjugated NPs with highly specific biomolecules such as aptamers, antibodies or molecularly imprinted polymers (MIPs) very well minimise cross reactivity, but should be further evaluated in real samples or determining applicability. More research in the direction of the stability of NPs by modifying their surfaces with polymers such as PEG or hydrophobic coating to prevent aggregation and degradation in complex matric are required to be developed in future engineering approaches. Biodegradable NPs with less toxic formulations will mitigate the environmental and health risks. Research directions in the field of QDs [46,129,130], nanofibers [36,67, 131–134], MNPs [135,136][152], and CNTs [137] would remarkably increase the transduction sensitivity and detection capabilities. Cost-effective synthesis based on a greener approach (plant based or biomimetric routes) will reduce the production costs of the NPs for the detection of MeHg. Signal amplification might be better achieved by the integrating NPs with SERS platforms. Combining NPs with MOFs and 2D materials will enhance the performance of the sensors and add multi-functionality and specificity due to the filtration effects of MOFs or higher surface area for attaching sensing part that introduce more higher signal-to-noise ratio.

8.6. Nanoarchitectonics

Nanoarchitectonics involves designing, fabrication and assembling nanoscale structures in a controlled and purposeful manner to achieve special function and properties. Nanoarchitectonic structures enhance the sensitivity and selectivity of biosensors and chemical sensors. For MeHg detection, nanoarchitectonics helps create hybrid structures, such as combining metal nanoparticles with porous frameworks or functionalized surfaces. These tailored architectures enhance sensitivity, selectivity, and stability, providing effective platforms [70]. The complex and intricate design can be time-consuming and expensive, due to nanoscale interactions and self-assembly processes, requiring advanced techniques and resources. As discussed in Chapter 7, very few studies have been reported on the detection of mercury ions with high sensitivity using thymine interactions and ligands-striped monolayers. These sensors failed to distinguish between inorganic and organic mercury which limited its applicability and reduced selectivity of the sensors when exposed to mixtures of cations.

The large-scale production of nanoarchitectonic-based sensors with consistent quality remains challenging. This is mainly because small deviations in synthesis or assembly processes can lead to significant variations in performance. Simplified fabrication methods will scale up the production such as microwave assistant synthesis or templating to streamline the sensor production. One of the main disadvantages of nanoarchitectonics is the structural stability, which can enhanced by modifying using graphene derivatives, MOFs and polymer coating. Plasmonic signals of MOFs can be enhanced by integrating plasmonic nanostructures (e.g., gold or silver) to amplify localized electromagnetic fields, boosting optical detection sensitivity. Their porous structure supports high-density functionalization with selective agents like aptamers or thiols, minimizing cross-reactivity. MOF pre-concentration of analytes and reduced interference improve signal clarity, while their scaffolding enables multi-step signal amplification for superior detection performance.

9. Conclusion

For efficient MeHg sensors, prioritise the development of advanced materials and biorecognition elements with high specificity for MeHg, such as novel nanomaterials and selective aptamers/antibodies. Optimize signal processing using advanced technologies to improve accuracy and interpret complex data effectively. Implement real-time calibration and adaptive systems to maintain sensor performance under varying environmental conditions. Focus on increasing sensitivity to detect lower concentrations of MeHg and reducing response times for rapid, reliable measurements. Ensure the sensor's usability, cost-effectiveness, and scalability, and validate its performance through extensive field testing and adherence to regulatory standards.

Currently, methods such as Atomic Absorption Spectroscopy (AAS), Atomic Fluorescence Spectroscopy (AFS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and X-ray Fluorescence (XRF) Spectroscopy are widely used for mercury (Hg) detection across various matrices. However, these techniques often require extensive sample preparation, have lower sensitivity, perform poorly with liquid samples, are susceptible to matrix interferences, and are costly, requiring highly skilled operators. As a result, sensors for detecting MeHg represent a potential advancement in the field. Unfortunately, current detection methods often lack the necessary sensitivity and specificity, frequently failing to detect concentrations below environmentally relevant thresholds set by regulatory bodies (e.g., 0.1 ng/mL) or based on the effects of biomagnified MeHg, which necessitates lowering regulatory thresholds.

From a biological point of view, MeHg detection is particularly challenging: Even at extremely low concentrations, MeHg can cause biological amplification due to time-dependent or chronic exposure. This makes even current gold-standard laboratory methods not sensitive enough, as MeHg concentrations are often below the limit of detection (LOD). In contrast to the Hg^{2+} where biological responses tend to show concentration-dependent effects in living cells, the biological impact of MeHg is usually only evident in multicellular organisms at higher trophic levels, which is then separating the concentration levels in environmental matrices from the concentrations in organisms, for example ocean waters and fish, respectively, by more than five orders of magnitude.

Based on literature-gained knowledge and on our experiences, we have identified five key steps that should be specifically followed when developing an effective MeHg sensor.

1. Interdisciplinarity: Developing a sensor requires collaboration between analytical chemists, material scientists, physicists, and experts in biology (e.g., biochemists, molecular biologists). A bottom-up approach is needed, beginning with an understanding of interactions of the MeHg with the selected sensing component at the molecular level.
2. Matrix Consideration: The sensor development must begin by selecting the matrix (e.g. fish tissue, ocean water), as this determines the required sample pre-treatment, potential interferences (e.g. co-contaminants, extraction chemicals), expected concentration ranges, and physicochemical conditions such as ionic strength, pH, and temperature.
3. Relevance: Sensor relevance must be evaluated not just by concentration levels but also by the intended application –whether ecological (e.g. bioavailability), health-related, research-orientated, or commercial. The sensor's design should reflect its intended use.
4. Detection Modalities: The sensor should be able to measure concentration (quantitatively or semi-quantitatively), temporal changes, accumulation, or microspatial information (e.g. intracellular accumulation). The specific modality should align with the sensor's intended purpose.
5. Coupling Sensing Element with Transducer: The choice of transducer (electromagnetic, electrical, etc.) must be considered along with noise-to-signal ratio, signal range, and signal linearity. These factors impact signal amplification, filtration, correlation, and other signal-processing steps.

The recent surge in artificial intelligence (AI) has led to substantial advancement across various research domains. To date, most of the currently known MeHg sensing parts are designed based either on the known properties of selected sensing components or materials that preferentially interact with various Hg species, or by trial-and-error experiments. Currently, artificial intelligence tools offer new approaches in material design, discovery, and manufacturing, which can accelerate the development of sensors with improved sensitivity and especially increased selectivity towards MeHg [138]. Furthermore, based on our experiences working on WCBs and detecting very low levels of analytes [16], this process is often prone to a high noise-to-signal ratio. However, adopting machine learning approaches could help refine detection, possibly not for quantitative measurements, but more appropriately for qualitative information. This could be crucial for filtering out contaminated samples, ensuring reliable detection even in complex samples affected by matrix effects.

In conclusion, given the complexities discussed above, creating a universal sensor that functions across all matrices while providing relevant data would be extremely challenging. Therefore, work focused on matrix-specific sensors is necessary to achieve accurate and meaningful MeHg detection.

CRediT authorship contribution statement

A.F.P. Allwin Mabes Raj: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raghuraj Singh Chouhan:** Writing – review &

editing, Methodology, Formal analysis, Conceptualization. **Aljoša Košak:** Methodology, Formal analysis. **Milena Horvat:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition. **Aleksandra Lobnik:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition. **Tomaž Rijavec:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Data curation. **Aleš Lapanje:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

We declare No conflict of interest.

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Data availability

Data will be made available on request.

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