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Rapid enzymatic assays for fecal contamination in aquatic environment: challenges, advances and prospects

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Abstract

Routine monitoring of sanitation and hygiene to identify fecal contamination in aquatic environments is an effective means to prevent threatening disease transmission. Compared to immunological or genetic methods performed in a central lab, enzymatic assays are considered simple, quick, cost-effective and thus promising for in-field (near) real-time detection. However, the long detection time for mildly polluted samples is a major obstacle to its deployment as an early warning system. Here, the challenges faced by the assays in real environmental sample measurements are summarized, followed by the current status of their field applications. Furthermore, the likelihood and ways are discussed for significant assay improvements using state-of-the-art synthetic biology technologies. Rapid advances in synthetic biology such as various new enabling tools for precise biomolecular manipulation and cell-free expression systems have great potential to address the present bottlenecks of the enzymatic assays, paving the way for better early warning strategies and performance.

Keywords: Rapid enzymatic assays, fecal contamination, *E. coli*, total coliform, synthetic biology, field application, aquatic environment, β-D-galactosidase, β-D-glucuronidase

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Introduction

Frequent outbreaks of waterborne bacterial gastrointestinal diseases such as cholera and typhoid stem from feces-contaminated water. WHO reported that 26% of people globally accessed contaminated drinking water sources in 2020, causing an estimated 829,000 deaths from diarrhoeal disease each year [1]. Severe water shortages and the increasing worldwide use of reclaimed water for irrigation means that, in addition to improving public sanitation facilities, routine monitoring of water quality hygiene for early warning purpose has become more crucial than ever. Since direct detection of trace amounts of various pathogenic microorganisms using current technology is quite expensive, technically demanding, and time-consuming [2, 3], Gram-negative (G-) total coliform and *Escherichia coli* (*E. coli*) are widely used fecal indicator bacteria (FIB) to assess the potential presence of enteric-pathogens. Monitoring both total coliform and *E. coli* together is beneficial for the ease and accuracy of testing (though *E. coli* is considered the better proxy [4, 5]), and is the 'gold' standard in national and international guidelines for the microbiological quality assessment of water resources [6].

Traditional tests are growth-based methods, tedious, costly, and time-consuming, taking at least 48-72 hours before final results are obtained [7]. Significant interference from non-target organisms is the main concern when trying to avoid high false-positive results. To this end, enzymatic assays were developed that combined detection of specific enzymes that are produced by total coliform and *E. coli* with a selective medium to inhibit the growth of non-target microorganisms [8, 9]. Lebaron et al. tested *E. coli* contamination in seawater in this way [10], resulting in a total detection time of less than 6 h, and good correlation was obtained between enzyme activity and culture methods ($r^2 = 0.81$, in agreement with other studies [11]). Encouraged by these, at least ten commercial enzymatic assay kits have been approved for drinking water analysis by USEPA. However, their performance with real samples depends on both the microorganisms present and the sample matrix.

In early warning practice, since samples contain a tiny amount of FIB, enzyme assays may need a long pre-culture time to boost enzyme expression for significant enzymatic hydrolysis, e.g. the detection of 32 and 1 *E. coli* spiked in 100 ml type water took 17 and 24 hours, respectively [12]. In these cases, the assays could become extremely delayed or even useless in tracking contamination

sources. Detection speed is also critical especially in emergencies that require immediate response [11, 13]. Continuous and near 'real-time' monitoring of fecal pollution within 4 h, which has been defined as "rapid" [14], are therefore preferred to allow same-day decision-making.

To this end, many efforts have been based on the molecular detection methods associated with immunology, genetics, or enzyme functions. Among them, immunological methods have great advantages when used with pre-enrichment, purification, target detection, and so forth. For example, target bacteria are purified and enriched using immunomagnetic beads and then identified with second antibody modified with strong fluorescent quantum dots (QDs, Fig. 1a) or multiple fluorophore (Fig. 1b), which significantly improved the sensitivity of bacterial detection by enhancing the signal strength. Wu's group built an similar ELISA-like sandwich structure to test *E. coli* from 8 water samples in 10 mins, which showed a linear relationship with counting results, leading to the limit of detection (LOD) of 57 CFU/mL [15]. One major obstacle of the method when applied to FIB is the lack of sufficient specificity because polyvalent antibodies that cover the global *E. coli* population do not yet exist. As a genetic method, qPCR is extremely beneficial in source tracking when monitoring for event-based contamination (Fig. 1c), especially when combined with immune pre-enrichment to further reduce the LOD (Fig. 1d), though several concerns merit careful attention: 1) PCR primers lack enough specificity; 2) Inhibitors to polymerase exist in many environmental samples [16]; 3) Extracellular or dead cell DNA lead to false positive estimations [6]; 4) Starved or stressed bacteria contain low ribosome content [17].

In comparison, enzymatic assays enable the determination of viable but non-culturable (VBNC) bacteria [7, 18-20] and are therefore a better-adapted detection method for FIB, especially in mildly contaminated water that nevertheless contains significant numbers of VBNC bacteria e.g. seawater [10, 11, 21]. Usefully, results from enzyme assays can often be easily distinguished visually and quantified using affordable digital imaging and image processing techniques [22]. Considering that a field test requires a simple, low-cost, field-deployable instrument and the rapid screening technique may be performable by an untrained individual, cheap and portable enzymatic assays can be more amenable to resource-limited settings [11]. Therefore, in this review paper, we will pay close attention to the enzymatic assays, focusing mainly on the challenges faced by the assays in practical aspect for early warning purpose and the recent progress made in field applications for a variety of aquatic

environments. Following this, we further boldly predict the future prospects for fecal contamination assays in aquatic environments from a synthetic biology perspective, aiming to provide several new clues and directions to address the present bottleneck issues of the assays.

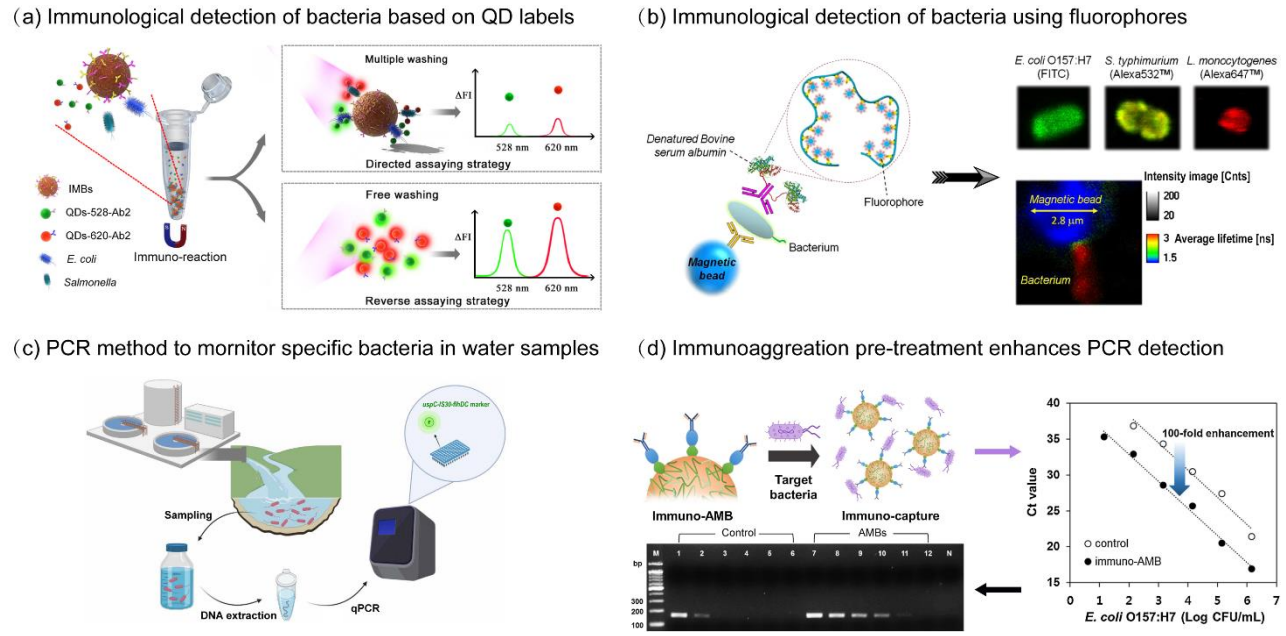

Figure 1. Examples of immunofluorescence (a, b) and genetic methods (c, d) for bacterial detection. Adapted with permission from Refs. [23-25].

2. Enzymes and related substrates for enzymatic assays

β-D-glucuronidase (β-GLUase) and β-D-galactosidase (β-GALase) are recognized as unique marker enzymes with the both being produced by *E. coli*, but only the latter by total coliform [14, 26-28]. As a member of total coliform, *E. coli* could be also identified by β-GALase [29, 30] , but β-GLUase shows superior specificity [18, 19]. As listed in Table 1, even though the majority of *E. coli* excrete β-GLUase this does not confer absolute specificity for FIB because the enzyme may also originate from other G- bacteria (e.g. *Shigella*, *Salmonella*, a minority of *Yersinia*), some Gram-positive (G+) species, etc. Such nontarget interference always exists when considering the large number of micro-organisms in aquatic environments and assays tend to correlate with the presence of both targets and nontargets in a sample, though in heavily polluted water the latter is negligible [31].

Table 1 Examples of well-known organisms often existing in natural or artificial aquatic environment that produce the marker enzymes. The numbers in round brackets indicate the proportion of the species being reported to produce the enzymes.

Category	Organisms	β-GALase	β-GLUase	Reference
FIB	*Escherichia coli*	+	(94-97%)	[9, 26, 30]
	Total coliform	+	-	
Non-coliform (G-)	*Shigella*	+	(44-58%)	[9, 32, 33]
	Salmonella	+	(20-29%)	
	Yersinia	+	+	
	Flavobacteria		+	
Non-target bacteria (G+)	*Staphylococcus*	+	+	
	Streptococcus spp.	+	+	
	Clostridia		+	
Others	Algae and plants	+	+	

Table 2 Widely used substrates for enzymatic assays and their common features

Type	Substrates		Advantages	Disadvantages
	β-GALase	β-GLUase		
Chromogens	*o*-Nitrophenyl-β-D-galactopyranoside (ONPG) 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) Chlorophenol red β-galactopyranoside (CPRG) 6-Chloro-3-indoxyl-β-D-galactopyranoside (Red-Gal)	*p*-Nitrophenyl-β-D-glucuronide (PNPG) 5-Bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Glu or X-Gluc or BCIG)	· Low-cost; · Rapid naked-eye detection; · Easy operation; · Field applicable;	· Insensitive; · Hard for quantitative measurement;
Fluorogens	4-Methylumbelliferone-β-D-galactoside (MUGA or MUGal)	4-Methylumbelliferyl-β-D-glucuronide (4-MUG)	· Sensitive; · Rapid detection; · Suitable or both quantitative and qualitative analysis;	· Costly; · Need special instruments;

A wide range of synthesized compounds release chromogenic or fluorogenic molecules when cleaved by the enzymes as shown in Fig. 2, then these signaling molecules are either enclosed in bacteria to label them (Fig. 3b) or changed the whole medium (Fig. 2) to report the result. Table 2 summarizes normally used such substrates with their common pros and cons. Among them, chromogenic substrates are in general much cheaper than fluorogenic ones, but rarely as sensitive as the latter [19].

However, colorful signals facilitate high throughput screening, for example, multiplexing chromogenic substrates has been used to identify multiple pathogenic strains simultaneously, which makes the assay especially beneficial in food tests [34, 35]. In comparation to X-Glu or X-Gal producing an insoluble blue indigo dye inside cells with no impact on their viability [8], and OPNG or PNPG, which produces yellow-colored nitrophenol suffering extensive extracellular diffusion [9], CPRG is a better substrate with regards to the cost, sensitivity, and kinetic characteristics, with a significant color change from yellow to dark red occurring in 10 min (Figs. 2a, 3a) [36], such rapid, simple, naked-eye detection is more preferred for field applications. Other synthetic substrates have also been reported for low cost and/or sensitivity [37].

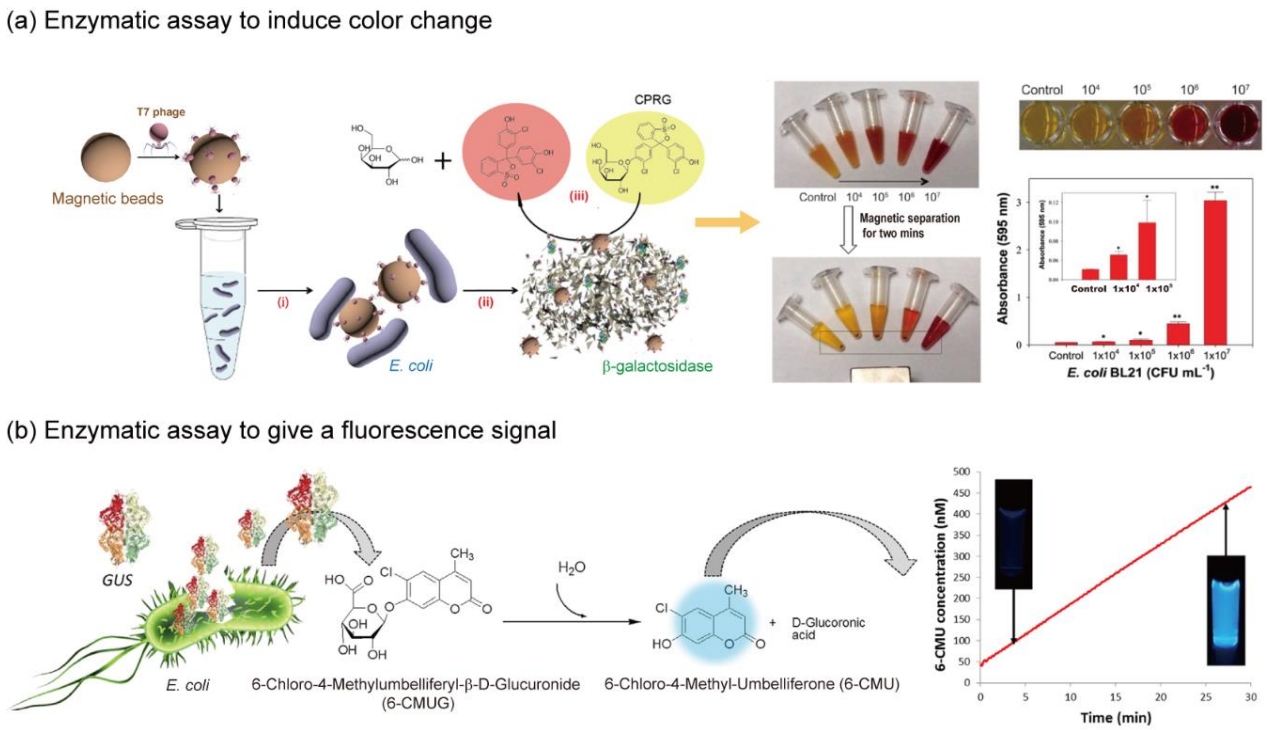

Figure 2. Specific enzyme substrates with its enzymatic reaction, product, and signal detection mode. (a) CPRG gives different color signals dependent on the abundance of *E. coli*, facilitating a quick qualitative test: (i) bacteria purification, (ii) cell lysis, (iii) enzyme reaction; (b) 6-CMUG gives a fluorescence signal and could be used to detect the reaction kinetics. Adapted with permission from Refs. [19, 22].

Aqueous insoluble coumarin derivatives MUGA and 4-MUG release highly fluorescent product of 4-MU [7, 29, 38], which tends to autohydrolyze quickly and then causes a high background readings in low FIB samples, that would otherwise correspond to 100 to 1000 CFU/100 ml [39] or 60-100 CFU/100 ml [42] for 15 min assay at 41.5°C. Several other features of 4-MU also detract from its

performance: 1) Optimal pH (> pH10) for a maximum fluorescence intensity is far from that of most culture conditions [11, 26]; 2) Inhibits bacterial growth; 3) Diffuses easily into surrounding medium resulting in lower and delayed signals [8, 29]. Recently, two more substrates containing umbelliferone variants (6-CMUG and 3-CUG) have become commercially available. These both produce fluorophores with a lower p*K*a value [21, 38] and 6-CMUG can produce a fluorescent signal 6 times higher than that of 4-MUG (Fig. 2b), making the method almost 10 times more sensitive and more reproducible (relative standard deviation (RSD) = 4.88%) [21]. (As a side note, in contrast to fluorogenic substrates, chemiluminescent ones are less suited for real sample detection due to the conflict between sensitivity and specificity [40])

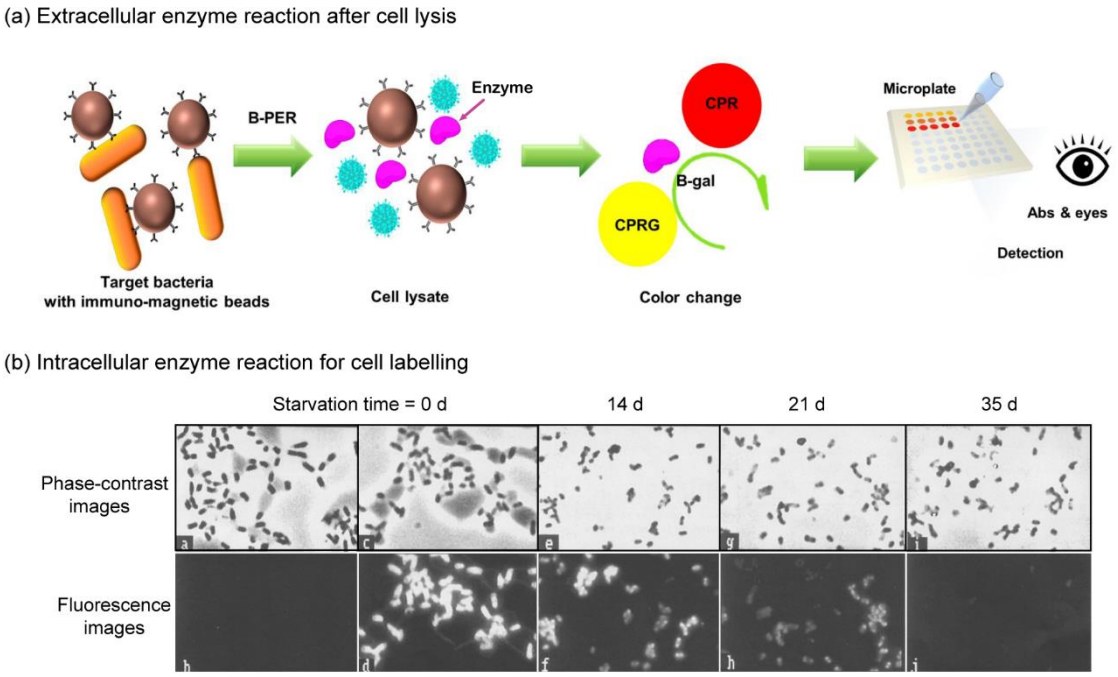

Figure 3. Two ways to perform the enzymatic assays: (a) through an extracellular enzyme reaction after cell lysis to release the enzymes, and (b) through an intracellular enzyme reaction after IPTG induction to produce more marker enzyme and then substrate diffusion into the cells. Dissimilar to the others in (b), sample a was un-induced by IPTG before the enzyme reaction; Samples e, g, and i are those starved for 14, 21, and 35 days, respectively, separately containing only nearly 1%, 0.3%, and 0.04% of culturable bacteria. Adapted with permission from Refs. [41, 42].

3. Factors affecting the performance of rapid enzymatic assays

Enzymatic assays have many limitations in monitoring real samples. For example, with marine water a Colilert-18 kit produced false-positive results that were 1 to 3 orders of magnitude higher than

estimates from membrane filtration counts [43], and sewage normally contains many non-target substances that hydrolyze enzyme substrates [6, 27]. These issues are further complicated by the extent and kind of interference encountered being dependent on sample origins or environmental localities [7]. The high probability of autochthonous enzyme-positive non-coliform species being present in field samples is another big challenge faced. This, and other factors meriting special attention in the context of field testing, are discussed below.

3.1 Impact of environmental factors on enzyme activity

Marker enzymes in their purified forms are often used when developing a test and as internal references. However comparison between commercial β-GALase (purified form) and that excreted by *E. coli* in a river (environmental form) found that optimal enzymatic conditions occurred at dissimilar pH's and temperatures, with the different ions and compounds that commonly exist in aquatic environments also contributing to variations in enzyme activity [28]. An example of such interference is illustrated by the altered response of β-GALase (from pure *E. coli*) when metallic ions (cadmium and lead), nontarget organisms, and certain sodium salts are present [29]. This highlights the necessity to establish the range of variation in enzyme activity caused by the local environment in a real sample and identify differences in the kinetics of the environmental and commercial forms of the enzyme. Both of these complications detract from the simplicity of using enzymatic signals in a 'rapid' assay.

Aside from the above considerations, another strong influencing factor when monitoring bacteria loads in drinking and waste water systems is the presence of chlorine, used as a routine disinfectant. Here, it has been found that the marker enzymes or their encoding genes could be damaged during the process, leading to the assay detection rate of *E. coli* dropping by half [44].

3.2 Enzyme induction to improve detection limit and accelerate enzyme reactions

Inducing enzyme activity is a crucial advantage of enzymatic assays. This can effectively promote intracellular enzyme expression without cell proliferation or enzyme consumption (Fig. 3b). For example, β-GALase is commonly induced by isopropyl-β-D-thiogalactoside (IPTG) [7, 45], a molecular mimic of allolactose, which triggers the expression of *lac* operon to promote the

transcription of *lacZ* gene encoding β-GALase [46]. Similarly, methyl-β-D-glucuronide (Met-Glu) targets β-GLUase expression[47]. Importantly, nontarget bacteria were found to be less inducible, showing several orders of magnitude lower β-GALase activity [31]; this variation in inducibility can also facilitate discrimination between viable and non-viable cells of the same species.

Such induction processes improves both sensitivity and specificity of the assays, e.g. the β-GALase activity of *E. coli* increases ~100-fold after IPTG induction for 30 min [48]. (Note, while lactose is a cheaper inducer than IPTG [49], it is unsuitable for enzyme accumulation on account of the competition with other substrates through lactose fermentation.) However, it should be noted that a real sample which species originating from sewage or the human gut, may already contain molecules that have induced FIB enzyme expression, and in such cases, further induction treatments have no effect [49].

3.3 Permeability of bacterial envelope facilitates intracellular uptake of substrates

When the test is performed on whole bacteria, substrates must transfer across the bacterial envelope to access intracellular enzymes with the signal-reporting product remaining either inside (and so enhancing the bacteria's "visibility") or released outside (Fig. 3). The cell wall permeability is a decisive parameter both in a test's performance and in selecting the measurement instrumentation to be used. For example, the addition of chloroform mixed with 1% sodium dodecyl sulfate (SDS) permeabilizes *E. coli* effectively, significantly enhancing the electrochemical signal generated by an intracellular enzyme reaction with an otherwise impermeable substrate [48]. Many reagents (toluene, 6-thiatetracycline, colistin, lysozyme, polymyxin B, etc.) and their combinations with different permeabilization mechanisms have been adopted for this purpose. Yang et al. [50] compared the permeability of *E. coli* after being treated with lysozyme-EDTA mix, chloroform-SDS mix, and osmotic shock (using 1 mM disodium EDTA), respectively. The first two treatments exposed cells directly to the environment whereas osmotic shock did not damage the cell wall sufficiently or for long enough for substrates to enter the cell. Optimal permeabilizers should balance the permeabilization effect with the efflux of enzymes from the cells before interaction with the substrate has occurred.

3.4 Enzyme extraction via cell lysis

To eliminate the above-mentioned envelope barrier, cell lysis has been adopted to extract enzymes directly for the reaction; this shortens reaction times remarkably. The commercial B-PER reagent allows enzyme extraction from *E. coli* within seconds and as a result *E. coli* can be qualitatively identified within a few minutes (4×10^6 to 4×10^5 CFU/mL) [29]. Such enzyme-extraction however warrants extra attention considering enzyme denaturation [30]. Compared to other optical, electrical, or mechanical methods, simple and cost-efficient chemical lysis is device-independent and preferable for enzymatic assays. Briciu-Burghina et al. [38] have evaluated relevant parameters in detail, such as lysing agents, lysing time and volume, temperature, etc. The strong ionic detergent SDS or the milder non-ionic detergent of Triton X-100 are more beneficial to enzyme recovery and/or maintaining its activity compared to other frequently used chemicals (Triton X-114, Tween 20, Tween 80, NDSB-21, Nonidet P-40, etc.); In addition, Triton X-100 shows less tendency to denature proteins along with acceptable lysis time (e.g. 30 s for 0.1% Triton X-100) [51]. Prominent candidates also include B-PER and 1mg/mL lysozyme in PELB™ buffer [38] (Fig. 3a).

3.5 Media for enzymatic assays

Despite components of assay mediums being complex and diverse, all of them in principle perform two functions: supply substrates and suppress the interference from autochthonal bacteria or other disrupting substances, such as cell-free components that have been released from damaged cells or cell-free enzymes that induce similar substrate reactions. Keeping the 'background' signal to the lowest level is critical for both sensitivity and specificity, especially given the impossibility of eliminating all substrate cleavage from autochthonal and other sources without employing time-consuming pretreatments. To this end, the addition of inhibitory compounds like bile salts and sodium heptadecylsulfate (a powerful and mild growth inhibitor of Gram-positive organisms, respectively) has been found to be a simple way to afford the media superior selectivity. Nevertheless, no medium is omnipotent when used for samples coming from any type of source, and even preferred additives may cause uncertainty in testing, e.g. SDS suppresses *E. coli* growth at a higher concentration but reduces medium selectivity at a lower concentration enabling autochthonal bacteria to thrive. Hence, promising media for rapid enzymatic assays should be niche-targeted, with consideration being given

to the optimal concentrations of substrate, growth media, enzyme inducer, etc., especially when used together in a 'one-pot' process.

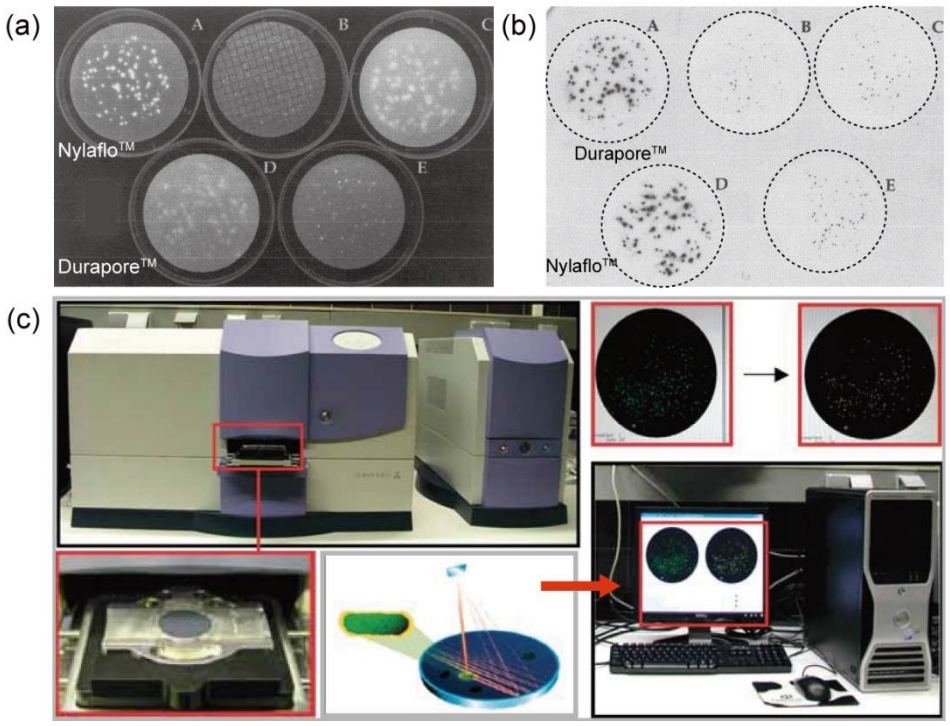

Figure 4. Visually and instrumentally detecting FIB on filter membranes. Detection of fluorescent (a) and chemiluminescent (b) *E. coli* on different types of membrane filter after 5 h preincubation, then labeled based on the β-GLUase reactions. (a) A: Nylaflo™, B: nitrocellulose, C: Supor™, D: Durapore™, E: Vericel™; (b) A: Durapore™, B: nitrocellulose, C: Supor™, D: Nylaflo™, E: Vericel™. Adapted with permission from Refs. [52]. (c) Illustration of an SPC instrument that rapidly scans bacteria on a filter membrane. Adapted with permission from Refs. [53].

3.6 Detection procedure

There are two ways to perform enzymatic assays: a one-step process and a two-stage approach. In the former, the whole procedure is carried out in the same 'pot', under the same conditions [7, 10]. This may not be well suited for each aspect of the assay, or even can be deleterious. For instance, permeabilizers generally must be absent in growth media due to their bactericidal effect [54]. Thus, a two-stage assays have been proposed in which, enzyme induction is separated from subsequent enzymatic signaling. For example, allowing FIB to grow selectively during an enzyme induction step through a relatively long cultivation process can be used to enhance test sensitivity, though at the cost of end-end assay time. This approach minimizes possible competition between enzyme inducer and substrates [54], such as that between lactose and MUG [55]. Performing the two-stage assay on a

filter membrane has also been found to be more advantageous than in liquid media since it can prevent the growth of nontarget microcolonies on the membrane from confusing colony counting [54]. In this case, fast growth of FIB to produce microcolonies on the membrane and a low background signal of the membrane to enhance the contrast from colonies, similar as that of the Nylaflo™ and Durapore™ membranes shown in Figs. 4a and 4b, are preferred for rapid colony counting by naked eyes.

Nevertheless, environmental FIB is always severely stressed or injured and hard to form visually detectable microcolonies in a short period. Therefore, a technique that could capture both microcolonies and single cells at the same time would help enable testing in less than 4 h. Such an instrument is a solid laser cytometer (SPC) (Fig. 4c). It takes merely 3 min to scan a 25 mm diameter membrane filter with 1 to 1000 viable fluorescing cells resulting in a total assay time of around 3.5-5 h and a theoretical LOD of one cell per liter [53]. However, the cost and the need of an epifluorescence microscope for result validation make SPC testing more useful in 'emergency' situations, when timely information about drinking water safety is critical. The terms "rapidity" and "sensitivity" when applied to enzymatic assays are often incompatible or contradictory attributes, especially in the context of lightly polluted environmental samples.

4. Recent progress in on-site applications

No matter whether in developing or developed countries, early warning to prevent outbreaks of infectious disease has been the focus for proactive and sustainable water management over the last two decades. To achieve this, either simple field deployable tests or automated field systems for (near)real-time detection are in urgent demand for regular water-quality monitoring. Simple and rapid field tests are particularly desired in resource-poor countries and regions.

4.1 Potential portable sensors for on-site applications

Up to now, no satisfactory field-deployable enzyme assay-based sensors have emerged, and neither have many potential candidates. It reflects the enormous challenges faced in filed applications as described in *Section 3*. Mitra's group developed a Mobile Water Kit (Fig. 5iv) for qualitative monitoring of drinking water in Canada and India [56]. It dramatically improved response time (e.g. 50 min detection for 3-5 CFU per 100 ml of well water) by collecting bacteria for enzyme extraction

on a syringe filter, showing visible signals after 1 h incubation at 37°C. They further constructed an easy-to-operate plunger-tube assembly based on the same concept (Fig. 5i) [29]. Quantitative analysis of river water and seawater quality was performed similarly by measuring fluorescence signals on a fluorimeter, resulting in the total detection time of less than 75 min and a low LOD's of 26 MPN and 110 MPN per 100 ml, respectively (Fig. 5iii) [38]. Therefore, rugged miniaturized signal detection components could play a key role in developing fast and sensitive on-site tests. For example, Heery et al. [19] determined *E. coli* in the Tolka and Liffey Rivers (Dublin) at a concentration lower than 250 MPN per 100 ml within 75 min with a portable fluorimeter (Fig. 5ii); Wildeboer et al. [26] obtained a low LOD of 7 CFU/ml of *E. coli*. in the River Thames using a hand-held confocal fluorimeter. Despite these promising results with portable optical systems, image analysis tools that enable in-field quantitative analysis or even remote signal transmission could be a more preferred route for simplifying on-site applications [30, 57, 58].

4.2 Near real-time automated online monitoring systems

An automated online system typically consists of a sample intake, reagent stocks, a temperature-controlled reaction chamber, a UV emitter, an optical sensor, as well as a control unit and a user interface (Fig. 5v). Major obstacles for such systems are sufficient sensitivity and robustness in view of the fluctuation in field conditions (e.g. temperature variations and event-caused disturbances). Ryzinska-Paier et al. [59] have studied the β-GLUase activity in groundwater in Austria for two years with monthly services to change filters and refill reagents. The results suggested a lower β-GLUase activity than either reported literature values or growth-based *E. coli* measurements (correlation coefficient (r) = 0.53). This discrepancy, caused probably by discharge and surface runoff conditions, highlights a strong need of further detailed investigations when using *E. coli* as a proxy for FIB estimations in complex catchments.

Koschelnik et al. [60] built an outdoor measurement station named ColiMinder (Fig. 5vii) to monitor the β-GLUase activity in sediment-laden creek water in Petzenkirchen (Austria) for two months in 2014. Based on over 2100 automated measurements, the activity is compatible with the level of *E. coli* except for the observation of daily fluctuations and event-caused extreme increases. Another station (BACTcontrol) built at the same location found that the β-GLUase activity linked to runoff

events and particle transport to some extent, but became more consistent over a long period of time, which suggests a high potential for the assays to be implemented in early warning systems[61]. Another online study combined with isotope hydrology found that the resuspension of streambed sediments was the main source of the enzyme activity [62]. A mobile version of the ColiMinder (Fig. 5vi) was also developed to investigate daily fluctuations in the β-GLUase activity at a remote karst spring in Northern Vietnam for ten days [20]. Besides the *E. coli* level, the results correlated well with 2-3 μm and 5-10 μm particles, implying the attachment of bacteria to such particles.

Lately, Baptiste Burnet et al. for the first time directly compared the performance of automated systems to established culture and molecular-based methods [14]. They adapted the autonomous ColiMinder® technology to test the raw water from drinking water treatment plant intake in Quebec (Canada) and found significant ($p < 0.05$) correlations between β-GLUase activity and the results of other assays, including the defined substrate technology ($r = 0.77$), membrane filtration ($r = 0.73$), qPCR ($r = 0.55$), and automated microbial detection system ($r = 0.50$). However, further research is still required to correlate the β-GLUase activity to the presence of *viable E. coli*. Online monitoring systems are currently not suited to replace proven surveillance practices but rather complement existing microbiological standards.

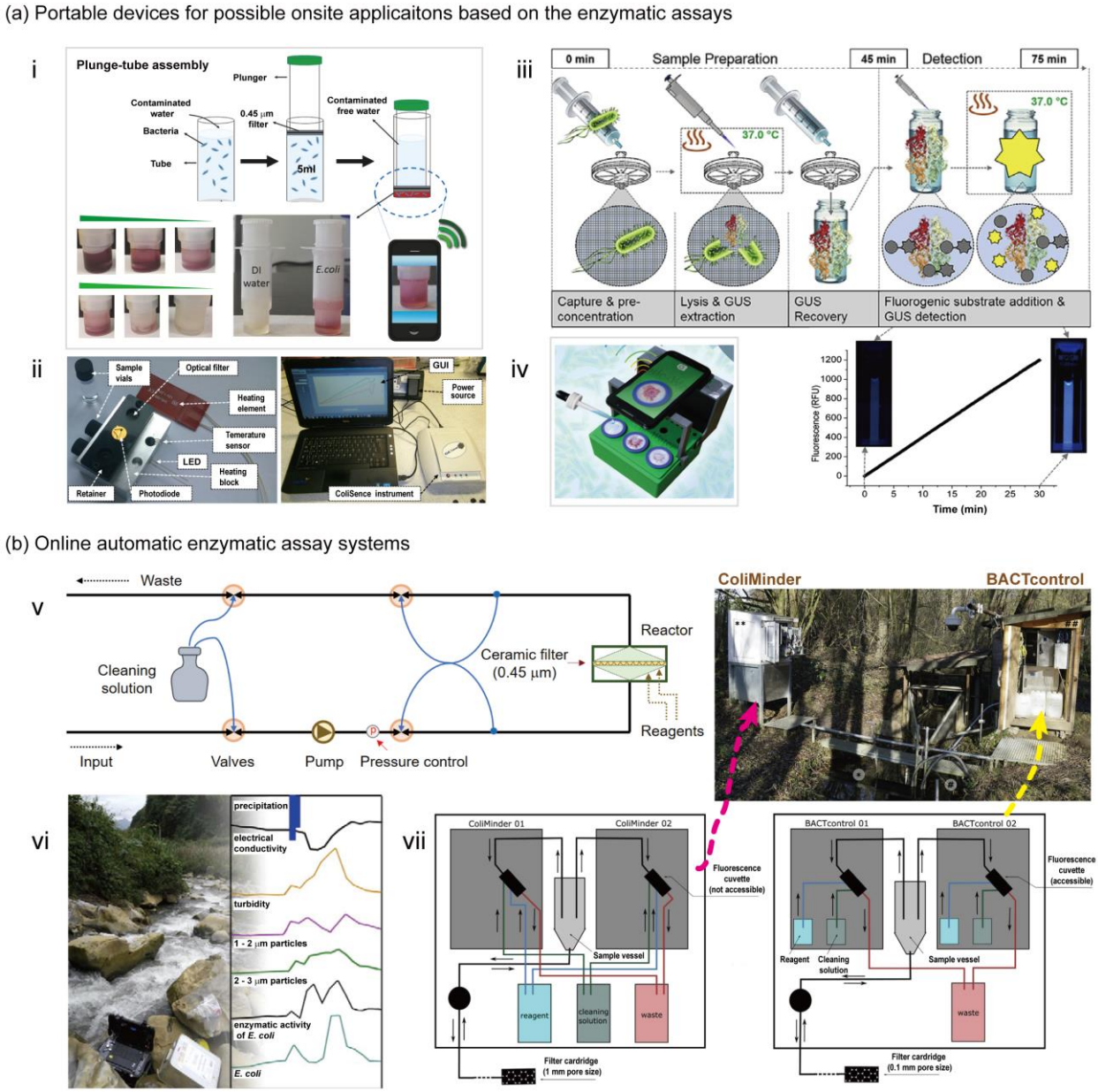

Figure 5. Examples of onsite applications of the enzymatic assays. (i, iii, iv) Mobile water kits developed for rapid detection of FIB in real samples; (ii) Miniaturized field signal detecting device. Adapted with permission from Refs. [19, 29, 38, 56]. (v) Common design of an automated online system for rapid enzymatic assays; (vi) Field-portable miniaturized automated ColiMinder system. Adapted with permission from Refs. [20]; (vii) Photography of two measurement stations, ColiMinder (marked by asterisks) and BACTcontrol (marked by hashtags), that are located on either side of a stream for a long period of water quality monitoring, along with the corresponding schematics of the basic construction. Adapted with permission from Refs. [59, 61].

5. Future prospects of rapid enzymatic assays

The insufficient sensitivity of FIB detection accounts for a delay in the detection speed of field-deployable enzymatic assays, in particular for mildly contaminated samples such as drinking water.

To address the issue and achieve (near) real-time monitoring of *E. coli*, new sensing strategies utilizing more sensitive electrochemical signals have emerged in recent years [30]. Innovative substrates that produce electroactive products via the enzyme reaction have been developed for simple and rapid electrochemical analysis [45, 48]. Rochelet et al. first took the idea to detect *E. coli* in wastewater on a disposable screen-printed carbon electrode in a 3 h test [63], certifying the applicability in turbid media. Despite this improvement in detection time, however, it did not solve the essential problem: Insufficient marker enzymes in mildly polluted samples decisively hamper the application of the assays, far from fulfilling the purpose of early warning. There is an urgent need for new technologies or new ideas to break through this limitation.

The enzymatic assays report results by sensing colored, fluorescent, or electrical products, therefore any factor that disturbs the enzyme reaction (see *Section 3)* will impact the test greatly and reduce the robustness in field applications. Given that the marker enzymes indicate FIB by themselves, directly sensing the molecules by employing sensitive probes or performing signal amplification could be a promising solution. For example, aptamers with a high affinity for the outer-membrane proteins or lipopolysaccharides [64] have been developed to recognize *E. coli* [65, 66]. Although no aptamer has been used to probe the marker enzymes for FIB detection yet, the aptasensors monitoring thrombin (a serine protease) have been successfully developed since a long time before [67, 68]. The unique characteristics (inexpensiveness, stability, chemical synthesis in high purity, and relative ease to modify) and flexible targets (including proteins, small molecules, cells, antibiotics, and viruses) [69-71] make aptamers a preferred structure-sensitive probe for the marker enzymes released from FIB.

Encoding genes of *uidA* for β-GLUase and of *lacZ* for β-GALase have already been used for *E. coli* detection [6, 7], which are intriguing alternatives of the marker enzymes deserving more attention. With the rapid development of synthetic biology tools (e.g. CRISPR-Cas systems and split-inteins [72]), a completely new concept for building biosensors have emerged [73]. That is utilizing synthetic biology techniques, such as various gene expression tuning strategies [74-76] and signal amplification methods [75, 77], to construct engineered sensing-processing-signaling genetic circuits for probing various targets within or outside a cell [78], which could significantly enhance the performance and accuracy of a whole cell-based biosensor [79, 80] (Fig. 6a). Multi-input genetic logic gate circuit are

alternative efficacious solutions to overcome the problem of sensor selectivity [80, 81]. Moreover, in contrast to the detection of small molecules and ions that are normally controlled by allosteric transcription regulators, specific RNA molecules could be directly targeted by a 'toehold switch' circuit (Fig. 6b) through breaking its hairpin barrier structure upstream a reporter gene [82]. As a result, more flexible, simpler, less burdensome, and fast responsive RNA-based biosensors have been created [83].

The emergence of cell-free expression systems is a landmark achievement of synthetic biology. Since free from a cell-chassis, such systems profoundly alleviate the biosafety concerns associated with whole-cell sensors [84, 85]. It can be lyophilized for a prolonged shelf-life and rehydrated to initiate immediate use (Fig. 6d). Moreover, it is an "open" system to accept direct addition of any necessary pre-mixed ingredients for biosensing, the circuits thus become simpler with more options, e.g. aptamers can be involved in the circuits as recognition elements to identify protein molecules (Fig. 6h). Meanwhile no fluorescent protein reporter is in absolute demand any more (Fig. 6g), e.g. Lucks' group developed a biosensor relying on RNA-level outputs for quick detection of multiple contaminants in municipal and lake water for a 4 h-reaction [86]. More critically, the cell-free expression systems have been proven as a potential platform for developing field-deployable biosensors by producing easy-to-interpret outputs either directly by naked eye or by smartphone [87-89].

The CRISPR-Cas immune systems of prokaryotes, which contain either DNA-recognizing Cas9, Cas12, and Cas14 or RNA-recognizing Cas13 effectors, have paved the way to new sensing strategies due to their strong modular nature, especially in a cell-free expression system [90]. In principle, the Cas effectors recognize nucleic acid targets efficiently via a cognate guide RNA's (gRNA's). Modifying the sequence of gRNA's such as the re-engineered gRNA pairings crRNA-tracrRNA (Fig. 6c) would therefore lead to specific target identifications [91]. In addition, the Cas effectors are excited when combined with a genetic target while promiscuously shearing surrounding RNA or DNA molecules. This collateral cleavage phenomenon has been used smartly to produce a mass of fluorescence signals (Fig. 6e) [92-94], highly conducive to a sensitive detection. Affordable gene preamplification before such activation of Cas effectors can further reduce the LOD, due to increased

RNA amount by a million-fold in less than 2 h at 41°C [88] and greatly mitigated false positive outputs if combined with a toehold switch as a "filter" to select a proper target for signaling (Fig. 6f) [95]. Combining gene amplification treatments (normally less than 2 h) with multiple synthetic biology enabling techniques has become a common strategy to develop new ultrasensitive, ease-to-use, and field-deployable biosensors.

In summary, the widely used enzymatic assays experience great challenges in early warning applications, especially in mildly feces-contaminated aqueous environment, which highlights an urgent requirement for new sensing techniques and strategies. The emergence of diverse synthetic biology enabling tools has shown great prospects in field applications [96], for instance, it may detect the occurrence of genetically modified *E. coli* (developed in clinical or environmental settings) effortlessly via screening various resistance genes or others inserted. Combined with widely introduced emerging interdisciplinary techniques such as microfluidics [97, 98] and machine learning [99, 100], rapid and continuous technological advances in synthetic biology make us believe that, the marker enzymes itself is a potent alternative analysis target and construction of engineered genetic circuits for sensing it will probably enhance the sensitivity and robustness of FIB measurements that meet the practical requirement for early warning in diverse field applications, thus bringing new vitality to the enzymatic assays.

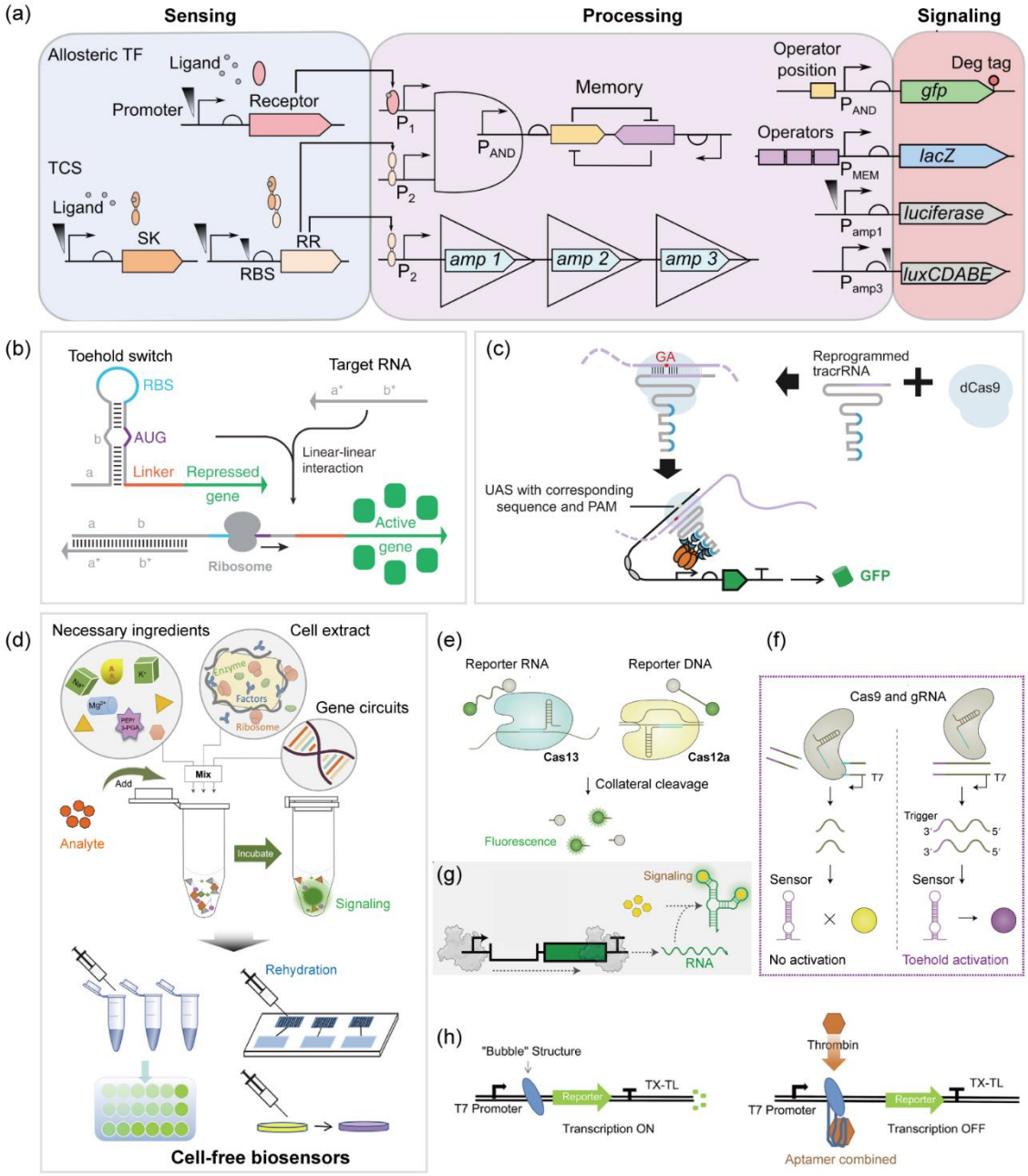

Figure 6. Representative synthetic biology enabling tools for achieving field-deployable biosensors for various contaminants. (a) Design of synthetic cell-based biosensors programmed with engineered signal processing genetic circuits for customized input-output functions. Adapted with permission from Ref. [79]. (b) Working principle of a toehold switch using RNA as a target. Adapted with permission from Ref. [82]. (c) Reprogrammed tracrRNA for dCas9 could directly hijack various RNAs as crRNA to trigger output reporting genetic circuits. Adapted with permission from Ref. [91]. (d) The workflow of cell-free biosensors. Adapted with permission from Ref. [84]. Signal generation is based on the collateral cleavage effect of Cas effectors after being activated (e) or relies on the Cas effector that is coupled with a toehold switch for target selection (f). Adapted with permission from Ref. [94]. (g) RNA is designed as an output reporter of genetic circuits, which undergoes conformational change to produce a fluorescent signal quickly. Adapted with permission from Ref. [86] (h) Thrombin-bound aptamer (ssDNA) combines with the genetic circuit through the "bubble" structure to block transcription of T7 RNA polymerase. Adapted with permission from Ref. [84].

Declaration of competing interests

The authors declare no competing financial interests.

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References

[1] World Health Organization, Drinking-water, https://www.who.int/news-room/fact-sheets/detail/drinking-water, 2023 (accessed 27 August 2023).

[2] World Health Organization, Protecting surface water for health. Identifying, assessing and managing drinking-water quality risks in surface-water catchments, http://www.who.int/iris/handle/10665/246196, 2016 (accessed 07 December 2023).

[3] U.E.P. Agnecy *Recreational water quality criteria*; USEPA Office of Water, Washington DC.: 2012.

[4] S. Edberg, E. Rice, R.J. Karlin, M. Allen, *Escherichia Coli*: the best biological drinking water indicator for public health protection, Symposium series (Society for Applied Microbiology) 88 (2000) 106S-116S. https://doi.org/10.1111/j.1365-2672.2000.tb05338.x

[5] A. Havelaar, U.J. Blumenthal, M. Strauss, D. Kay, J. Bartram, Guidelines: the current position in: L. Fewtrell and J. Bartram (Eds.), World Health Organization Water Series. IWA Publishing. London, UK., 2001, pp. 17-42.

[6] N. Wery, C. Lhoutellier, F. Ducray, J.P. Delgenes, J.J. Godon, Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR, Water Research 42 (2008) 53-62. https://doi.org/10.1016/j.watres.2007.06.048

[7] H. Satoh, K. Kikuchi, Y. Katayose, S. Tsuda, R. Hirano, Y. Hirakata, M. Kitajima, S. Ishii, M. Oshiki, M. Hatamoto, M. Takahashi, S. Okabe, Simple and reliable enumeration of *Escherichia coli* concentrations in wastewater samples by measuring β-D-glucuronidase (GUS) activities via a microplate reader, Science of the Total Environment 715 (2020) 136928. https://doi.org/10.1016/j.scitotenv.2020.136928

[8] M. Manafi, New developments in chromogenic and fluorogenic culture media, International Journal of Food Microbiology 60 (2000) 205-218. https://doi.org/10.1016/s0168-1605(00)00312-3

[9] M. Manafi, Fluorogenic and chromogenic enzyme substrates in culture media and identification tests, International Journal of Food Microbiology 31 (1996) 45-58. https://doi.org/10.1016/0168-1605(96)00963-4

[10] A.H. Farnleitner, L. Hocke, C. Beiwl, G.G. Kavka, R.L. Mach, Hydrolysis of 4-methylumbelliferyl-β-D-glucuronide in differing sample fractions of river waters and its implication for the detection of fecal pollution, Water Research 36 (2002) 975-981. https://doi.org/10.1016/S0043-1354(01)00288-3

[11] P. Lebaron, A. Henry, A.S. Lepeuple, G. Pena, P. Servais, An operational method for the real-time monitoring of *E. coli* numbers in bathing waters, Marine Pollution Bulletin 50 (2005) 652-659. https://doi.org/10.1016/j.marpolbul.2005.01.016

[12] S. Edberg, M. Edberg, A defined substrate technology for the enumeration of microbial indicators of environmental pollution, The Yale Journal of Biology and Medicine 61 (1988) 389-399.

[13] A.S. Madoux-Humery, S. Dorner, S. Sauve, K. Aboulfadl, M. Galarneau, P. Servais, M. Prevost, Temporal variability of combined sewer overflow contaminants: evaluation of wastewater micropollutants as tracers of fecal contamination, Water Research 47 (2013) 4370-4382. https://doi.org/10.1016/j.watres.2013.04.030

[14] J.B. Burnet, Q.T. Dinh, S. Imbeault, P. Servais, S. Dorner, M. Prevost, Autonomous online measurement of β-D-glucuronidase activity in surface water: is it suitable for rapid *E. coli* monitoring?, Water Research 152 (2019) 241-250. https://doi.org/10.1016/j.watres.2018.12.060

[15] S. Ma, Y. Tang, J. Liu, J. Wu, Visible paper chip immunoassay for rapid determination of bacteria in water distribution system, Talanta 120 (2014) 135-140. https://doi.org/10.1016/j.talanta.2013.12.007

[16] K.S. Reynolds, C.P. Gerba, I.L. Pepper, Rapid PCR-based monitoring of infectious enteroviruses in drinking water, Water Science and Technology 35 (1997) 423-427. https://doi.org/10.1016/S0273-1223(97)00297-7

[17] R.I. Amann, W. Ludwig, K.H. Schleifer, Phylogenetic identification and in situ detection of individual microbial cells without cultivation, Microbiological reviews 59 (1995) 143-169. https://doi.org/10.1128/mr.59.1.143-169.1995

[18] K. Demeter, J.-B. Burnet, P. Stadler, A. Kirschner, M. Zessner, A.H. Farnleitner, Automated online monitoring of fecal pollution in water by enzymatic methods, Current Opinion in Environmental Science & Health 16 (2020) 82-91. https://doi.org/10.1016/j.coesh.2020.03.002

[19] B. Heery, C. Briciu-Burghina, D. Zhang, G. Duffy, D. Brabazon, N. O'Connor, F. Regan, ColiSense, today's sample today: A rapid on-site detection of β-D-Glucuronidase activity in surface water as a surrogate for *E. coli*, Talanta 148 (2016) 75-83. https://doi.org/10.1016/j.talanta.2015.10.035

[20] A. Ender, N. Goeppert, F. Grimmeisen, N. Goldscheider, Evaluation of β-D-glucuronidase and particle-size distribution for microbiological water qualitymonitoring in Northern Vietnam, Science of the Total Environment 580 (2017) 996-1006. https://doi.org/10.1016/j.scitotenv.2016.12.054

[21] C. Briciu-Burghina, B. Heery, F. Regan, Continuous fluorometric method for measuring β-

glucuronidase activity: comparative analysis of three fluorogenic substrates, Analyst 140 (2015) 5953-5964. https://doi.org/10.1039/c5an01021g

[22] J. Chen, S.D. Alcaine, Z. Jiang, V.M. Rotello, S.R. Nugen, Detection of *Escherichia coli* in drinking water using T7 bacteriophage-conjugated magnetic probe, Analytical Chemistry 87 (2015) 8977-8984. https://doi.org/10.1021/acs.analchem.5b02175

[23] I.H. Cho, L. Mauer, J. Irudayaraj, In-situ fluorescent immunomagnetic multiplex detection of foodborne pathogens in very low numbers, Biosensors and Bioelectronics 57 (2014) 143-148. https://doi.org/10.1016/j.bios.2014.02.012

[24] B. Yin, Y. Wang, M. Dong, J. Wu, B. Ran, M. Xie, S.W. Joo, Y. Chen, One-step multiplexed detection of foodborne pathogens: Combining a quantum dot-mediated reverse assaying strategy and magnetic separation, Biosensors and Bioelectronics 86 (2016) 996-1002. https://doi.org/10.1016/j.bios.2016.07.106

[25] M.C. Lim, G.H. Lee, D.T.N. Huynh, C.E. Hong, S.Y. Park, J.Y. Jung, C.S. Park, S. Ko, Y.R. Kim, Biological preparation of highly effective immunomagnetic beads for the separation, concentration, and detection of pathogenic bacteria in milk, Colloids and Surfaces B: Biointerfaces 145 (2016) 854-861. https://doi.org/10.1016/j.colsurfb.2016.05.077

[26] D. Wildeboer, L. Amirat, R.G. Price, R.A. Abuknesha, Rapid detection of *Escherichia coli* in water using a hand-held fluorescence detector, Water Research 44 (2010) 2621-2628. https://doi.org/10.1016/j.watres.2010.01.020

[27] M.S. Little, S.J. Pellock, W.G. Walton, A. Tripathy, M.R. Redinbo, Structural basis for the regulation of β-glucuronidase expression by human gut Enterobacteriaceae, Proceedings of the National Academy of Sciences 115 (2018) E152-E161. https://doi.org/10.1073/pnas.1716241115

[28] V.C. Wutor, C.A. Togo, B.I. Pletschke, The effect of physico-chemical parameters and chemical compounds on the activity of β-D-galactosidase (B-GAL), a marker enzyme for indicator microorganisms in water, Chemosphere 68 (2007) 622-627. https://doi.org/10.1016/j.chemosphere.2007.02.050

[29] N.S.K. Gunda, R. Chavali, S.K. Mitra, A hydrogel based rapid test method for detection of *Escherichia coli* (*E. coli*) in contaminated water samples, Analyst 141 (2016) 2920-2929. https://doi.org/10.1039/C6AN00400H

[30] J.A. Adkins, K. Boehle, C. Friend, B. Chamberlain, B. Bisha, C.S. Henry, Colorimetric and electrochemical bacteria detection using printed paper- and transparency-based analytic devices, Analytical Chemistry 89 (2017) 3613-3621. https://doi.org/10.1021/acs.analchem.6b05009

[31] I. Tryland, L. Fiksdal, Enzyme characteristics of β-D-galactosidase and β-D-glucuronidase-

positive bacteria and their interference in rapid methods for detection of waterborne coliforms and *Escherichia coli*, Applied and Environmental Microbiology 64 (1998) 1018-1023. https://doi.org/10.1128/AEM.64.3.1018-1023.1998

[32] L. Fiksdall, I. Tryland, Application of rapid enzyme assay techniques for monitoring of microbial water quality, Current Opinion in Biotechnology 19 (2008) 289-294. https://doi.org/10.1016/j.copbio.2008.03.004

[33] M. Manafi, W. Kneifel, S. Bascomb, Fluorogenic and chromogenic substrates used in bacterial diagnostics, Microbiological Reviews 55 (1991) 335-348. https://doi.org/10.1128/mr.55.3.335-348.1991

[34] H.J. Kim, C. Kwon, H. Noh, Paper-based diagnostic system facilitating *Escherichia coli* assessments by duplex coloration, Acs Sensors 4 (2019) 2435-2441. https://doi.org/10.1021/acssensors.9b01034

[35] H.J. Kim, C. Kwon, B.S. Lee, H. Noh, One-step sensing of foodborne pathogenic bacteria using a 3D paper-based device, Analyst 144 (2019) 2248-2255. https://doi.org/10.1039/c8an02151a

[36] O.R. Miranda, X. Li, L. Garcia-Gonzalez, Z.J. Zhu, B. Yan, U.H. Bunz, V.M. Rotello, Colorimetric bacteria sensing using a supramolecular enzyme-nanoparticle biosensor, Journal of the American Chemical Society 133 (2011) 9650-9653. https://doi.org/10.1021/ja2021729

[37] G. Magro, R.E.S. Bain, C.A. Woodall, R.L. Matthews, S.W. Gundry, A.P. Davis, Synthesis and application of resorufin β-D-glucuronide, a low-cost chromogenic substrate for detecting *Escherichia coli* in drinking water, Environmental Science & Technology 48 (2014) 9624-9631. https://doi.org/10.1021/es502319n

[38] C. Briciu-Burghina, B. Heery, F. Regan, Protocol for the recovery and detection of *Escherichia coli* in environmental water samples, Analytica Chimica Acta 964 (2017) 178-186. https://doi.org/10.1016/j.aca.2017.02.035

[39] L. Fiksdal, M. Pommepuy, M.P. Caprais, I. Midttun, Monitoring of fecal pollution in coastal waters by use of rapid enzymatic techniques, Applied and Environmental Microbiology 60 (1994) 1581-1584. https://doi.org/10.1128/AEM.60.5.1581-1584.1994

[40] S.O. Van Poucke, H.J. Nelis, Limitations of highly sensitive enzymatic presence-absence tests for detection of waterborne coliforms and *Escherichia coli*, Applied and Environmental Microbiology 63 (1997) 771-774. https://doi.org/10.1128/aem.63.2.771-774.1997

[41] J.Y. Park, K. Park, G. Ok, H.J. Chang, T.J. Park, S.W. Choi, M.C. Lim, Detection of *Escherichia coli* O157:H7 using automated immunomagnetic separation and enzyme-based colorimetric assay, Sensors 20 (2020) 1395. https://doi.org/10.3390/s20051395

[42] C.E. Nwoguh, C.R. Harwood, M.R. Barer, Detection of induced β-galactosidase activity in individual non-culturable cells of pathogenic bacteria by quantitative cytological assay, Molecular Microbiology 17 (1995) 545-554. https://doi.org/10.1111/j.1365-2958.1995.mmi_17030545.x

[43] J.M. Pisciotta, D.F. Rath, P.A. Stanek, D.M. Flanery, V.J. Harwood, Marine bacteria cause false-positive results in the Colilert-18 rapid identification test for *Escherichia coli* in Florida waters, Applied and Environmental Microbiology 68 (2002) 539-544. https://doi.org/10.1128/aem.68.2.539-544.2002

[44] J. Li, L. Liu, D. Yang, W.L. Liu, Z.Q. Shen, H.M. Qu, Z.G. Qiu, A.M. Hou, D.N. Wang, C.S. Ding, J.W. Li, J.H. Guo, M. Jin, Culture-dependent enumeration methods failed to simultaneously detect disinfectant-injured and genetically modified *Escherichia coli* in drinking water, Environmental Science-Processes & Impacts 19 (2017) 720-726. https://doi.org/10.1039/c6em00625f

[45] J. Kwon, H.Y. Kang, H. Yang, Permeabilization-free β-galactosidase-induction-based electrochemical detection of *Escherichia coli*, Sensors and Actuators B-Chemical 337 (2021) https://doi.org/10.1016/j.snb.2021.129768

[46] T. Bigham, J.S.G. Dooley, N.G. Ternan, W.J. Snelling, M.C. Héctor Castelán, J. Davis, Assessing microbial water quality: Electroanalytical approaches to the detection of coliforms, Trends in Analytical Chemistry 121 (2019) 115670. https://doi.org/10.1016/j.trac.2019.115670

[47] K. Zuser, J. Ettenauer, K. Kellner, T. Posnicek, G. Mazza, M. Brandl, A sensitive voltammetric biosensor for *Escherichia coli* detection using an electroactive substrate for β-D-glucuronidase, Ieee Sensors Journal 19 (2019) 7789-7802. https://doi.org/10.1109/jsen.2019.2917883

[48] S. Noh, Y. Choe, V. Tamilavan, M.H. Hyun, H.Y. Kang, H. Yang, Facile electrochemical detection of *Escherichia coli* using redox cycling of the product generated by the intracellular β-D-galactosidase, Sensors and Actuators B: Chemical 209 (2015) 951-956. https://doi.org/10.1016/j.snb.2014.12.073

[49] S.C. Apte, C.M. Davies, S.M. Peterson, Rapid detecion of fecal-coliforms in sewage using a colorimeteric assay of β-D-galactosidase assays, Water Research 29 (1995) 1803-1806. https://doi.org/10.1016/0043-1354(95)00010-i

[50] C. Yang, H. Xie, Y. Li, J.K. Zhang, B.L. Su, Direct and rapid quantum dots labelling of *Escherichia coli* cells, Journal of Colloid and Interface Science 393 (2013) 438-444. https://doi.org/10.1016/j.jcis.2012.10.036

[51] G. Ocvirk, H. Salimi-Moosavi, R.J. Szarka, E.A. Arriaga, P.E. Andersson, R. Smith, N.J. Dovichi, D.J. Harrison, β-Galactosidase assays of single-cell lysates on a microchip: a complementary

method for enzymatic analysis of single cells, Proceedings of the IEEE 92 (2004) 115-125. https://doi.org/10.1109/JPROC.2003.820551

[52] S.O. Van Poucke, H.J. Nelis, Rapid detection of fluorescent and chemiluminescent total coliforms and *Escherichia coli* on membrane filters, Journal of Microbiological Methods 42 (2000) 233-244. https://doi.org/10.1016/s0167-7012(00)00193-7

[53] L.M. Vanhee, H.J. Nelis, T. Coenye, Detection and quantification of viable airborne bacteria and fungi using solid-phase cytometry, Nature Protocol 4 (2009) 224-231. https://doi.org/10.1038/nprot.2008.228

[54] N.J.C.F. Hans Enzymatic method for detecting coliform bacteria or *E. coli* 5861270, 01,19, 1999.

[55] K. Venkateswaran, A. Murakoshi, M. Satake, Comparison of commercially available kits with standard methods for the detection of coliforms and *Escherichia coli* in foods, Applied and Environmental Microbiology 62 (1996) 2236-2243. https://doi.org/10.1128/aem.62.7.2236-2243.1996

[56] N.S. Kumar Gunda, S. Naicker, S. Shinde, S. Kimbahune, S. Shrivastava, S. Mitra, Mobile Water Kit (MWK): a smartphone compatible low-cost water monitoring system for rapid detection of total coliform and *E. coli*, Analytical Methods 6 (2014) 6236-6246. https://doi.org/10.1039/C4AY01245C

[57] Q. Lin, Z. Yu, L. Lu, X. Huang, Q. Wei, D. Tang, Smartphone-based photoelectrochemical immunoassay of prostate-specific antigen based on Co-doped Bi_2O_2S nanosheets, Biosensors and Bioelectronics 230 (2023) 115260. https://doi.org/10.1016/j.bios.2023.115260

[58] R. Ren, G. Cai, Z. Yu, D. Tang, Glucose-loaded liposomes for amplified colorimetric immunoassay of streptomycin based on enzyme-induced iron(II) chelation reaction with phenanthroline, Sensors and Actuators B: Chemical 265 (2018) 174-181. https://doi.org/10.1016/j.snb.2018.03.049

[59] G. Ryzinska-Paier, T. Lendenfeld, K. Correa, P. Stadler, A.P. Blaschke, R.L. Mach, H. Stadler, A.K.T. Kirschner, A.H. Farnleitner, A sensitive and robust method for automated on-line monitoring of enzymatic activities in water and water resources, Water Science and Technology 69 (2014) 1349-1358. https://doi.org/10.2166/wst.2014.032

[60] J. Koschelnik, W. Vogl, M. Epp, M. Lackner, Rapid analysis of β-D-glucuronidase activity in water using fully automated technology, Water Resources Management VIII 1 (2015) 471-481. https://doi.org/10.2495/wrm150401

[61] P. Stadler, G. Bloschl, W. Vogl, J. Koschelnik, M. Epp, M. Lackner, M. Oismuller, M. Kumpan, L. Nemeth, P. Strauss, R. Sommer, G. Ryzinska-Paier, A.H. Farnleitner, M. Zessner, Real-time

monitoring of β-D-glucuronidase activity in sediment laden streams: A comparison of prototypes, Water Research 101 (2016) 252-261. https://doi.org/10.1016/j.watres.2016.05.072

[62] P. Stadler, G. Bloschl, L. Nemeth, M. Oismuller, M. Kumpan, J. Krampe, A.H. Farnleitner, M. Zessner, Event-transport of β-D-glucuronidase in an agricultural headwater stream: Assessment of seasonal patterns by on-line enzymatic activity measurements and environmental isotopes, Science of the Total Environment 662 (2019) 236-245. https://doi.org/10.1016/j.scitotenv.2019.01.143

[63] M. Rochelet, S. Solanas, L. Betelli, B. Chantemesse, F. Vienney, A. Hartmann, Rapid amperometric detection of *Escherichia coli* in wastewater by measuring β-D-glucuronidase activity with disposable carbon sensors, Analytica Chimica Acta 892 (2015) 160-166. https://doi.org/10.1016/j.aca.2015.08.023

[64] Y.W. Zhao, H.X. Wang, G.C. Jia, Z. Li, Application of aptamer-based biosensor for rapid detection of pathogenic *Escherichia coli*, Sensors (Basel) 18 (2018) 2518. https://doi.org/10.3390/s18082518

[65] G. Wu, Z. Dai, X. Tang, Z. Lin, P.K. Lo, M. Meyyappan, K.W.C. Lai, Graphene field-effect transistors for the sensitive and selective detection of *Escherichia coli* using pyrene-tagged DNA aptamer, Advanced Healthcare Materials 6 (2017) 1700736. https://doi.org/10.1002/adhm.201700736

[66] R. Gupta, A. Kumar, S. Kumar, A.K. Pinnaka, N.K. Singhal, Naked eye colorimetric detection of *Escherichia coli* using aptamer conjugated graphene oxide enclosed Gold nanoparticles, Sensors and Actuators B: Chemical 329 (2021) 129100. https://doi.org/10.1016/j.snb.2020.129100

[67] C. Daniel, F. Mélaïne, Y. Roupioz, T. Livache, A. Buhot, Real time monitoring of thrombin interactions with its aptamers: Insights into the sandwich complex formation, Biosensors & Bioelectronics 40 (2013) 186-192. https://doi.org/10.1016/j.bios.2012.07.016

[68] K.Y. Fan, J.L. Zhu, X.W. Wu, X.H. Zhang, S.F. Wang, W. Wen, A flexible label-free electrochemical aptasensor based on target-induced conjunction of two split aptamers and enzyme amplification, Sensors and Actuators B-Chemical 363 (2022) 131766. https://doi.org/10.1016/j.snb.2022.131766

[69] R. Stoltenburg, C. Reinemann, B. Strehlitz, SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands, Biomolecular Engineering 24 (2007) 381-403. https://doi.org/10.1016/j.bioeng.2007.06.001

[70] R. Zeng, W. Wang, G. Cai, Z. Huang, J. Tao, D. Tang, C. Zhu, Single-atom platinum nanocatalyst-improved catalytic efficiency with enzyme-DNA supermolecular architectures, Nano Energy 74

(2020) 104931. https://doi.org/10.1016/j.nanoen.2020.104931

[71] Z. Qiu, J. Shu, D. Tang, Bioresponsive release system for visual fluorescence detection of carcinoembryonic antigen from mesoporous silica nanocontainers mediated optical color on quantum dot-enzyme-impregnated paper, Analytical Chemistry 89 (2017) 5152-5160. https://doi.org/10.1021/acs.analchem.7b00989

[72] T.Y.H. Ho, A. Shao, Z. Lu, H. Savilahti, F. Menolascina, L. Wang, N. Dalchau, B. Wang, A systematic approach to inserting split inteins for Boolean logic gate engineering and basal activity reduction, Nature Communications 12 (2021) 2200. https://doi.org/10.1038/s41467-021-22404-9

[73] X. Wan, B. Saltepe, L. Yu, B. Wang, Programming living sensors for environment, health and biomanufacturing, Microbial Biotechnology 14 (2021) 2334-2342. https://doi.org/10.1111/1751-7915.13820

[74] B. Wang, M. Barahona, M. Buck, Amplification of small molecule-inducible gene expression via tuning of intracellular receptor densities, Nucleic Acids Research 43 (2015) 1955-1964. https://doi.org/10.1093/nar/gku1388

[75] X. Wan, F. Volpetti, E. Petrova, C. French, S.J. Maerkl, B. Wang, Cascaded amplifying circuits enable ultrasensitive cellular sensors for toxic metals, Nature Chemical Biology 15 (2019) 540-548. https://doi.org/10.1038/s41589-019-0244-3

[76] X. Wan, F. Pinto, L. Yu, B. Wang, Synthetic protein-binding DNA sponge as a tool to tune gene expression and mitigate protein toxicity, Nature Communications 11 (2020) 5961. https://doi.org/10.1038/s41467-020-19552-9

[77] B. Wang, M. Barahona, M. Buck, Engineering modular and tunable genetic amplifiers for scaling transcriptional signals in cascaded gene networks, Nucleic Acids Research 42 (2014) 9484-9492. https://doi.org/10.1093/nar/gku593

[78] B. Wang, R.I. Kitney, N. Joly, M. Buck, Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology, Nature Communications 2 (2011) 508. https://doi.org/10.1038/ncomms1516

[79] M. Hicks, T.T. Bachmann, B. Wang, Synthetic biology enables programmable cell-based biosensors, ChemPhysChem 21 (2020) 132-144. https://doi.org/10.1002/cphc.201900739

[80] B. Wang, M. Barahona, M. Buck, A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals, Biosensors and Bioelectronics 40 (2013) 368-376. https://doi.org/10.1016/j.bios.2012.08.011

[81] B. Wang, M. Buck, Rapid engineering of versatile molecular logic gates using heterologous genetic transcriptional modules, Chemical Communications 50 (2014) 11642-11644. https://doi.org/10.1039/c4cc05264a

[82] A.A. Green, P.A. Silver, J.J. Collins, P. Yin, Toehold switches: de-novo-designed regulators of gene expression, Cell 159 (2014) 925-939. https://doi.org/10.1016/j.cell.2014.10.002

[83] A.J. Chiang, J. Hasty, Design of synthetic bacterial biosensors, Current Opinion In Microbiology 76 (2023) 102380. https://doi.org/10.1016/j.mib.2023.102380

[84] L. Zhang, W. Guo, Y. Lu, Advances in cell-free biosensors: principle, mechanism, and applications, Biotechnology Journal 15 (2020) e2000187. https://doi.org/10.1002/biot.202000187

[85] A. Tinafar, K. Jaenes, K. Pardee, Synthetic biology goes cell-free, BMC Biology 17 (2019) 64. https://doi.org/10.1186/s12915-019-0685-x

[86] J.K. Jung, K.K. Alam, M.S. Verosloff, D.A. Capdevila, M. Desmau, P.R. Clauer, J.W. Lee, P.Q. Nguyen, P.A. Pastén, S.J. Matiasek, J.-F. Gaillard, D.P. Giedroc, J.J. Collins, J.B. Lucks, Cell-free biosensors for rapid detection of water contaminants, Nature Biotechnology 38 (2020) 1451-1459. https://doi.org/10.1038/s41587-020-0571-7

[87] X. Lin, Y. Li, Z. Li, R. Hua, Y. Xing, Y. Lu, Portable environment-signal detection biosensors with cell-free synthetic biosystems, RSC Advances 10 (2020) 39261-39265. https://doi.org/10.1039/d0ra05293k

[88] K. Pardee, A.A. Green, M.K. Takahashi, D. Braff, G. Lambert, J.W. Lee, T. Ferrante, D. Ma, N. Donghia, M. Fan, N.M. Daringer, I. Bosch, D.M. Dudley, D.H. O'Connor, L. Gehrke, J.J. Collins, Rapid, low-cost detection of Zika virus using programmable biomolecular components, Cell 165 (2016) 1255-1266. https://doi.org/10.1016/j.cell.2016.04.059

[89] A. Lopreside, X. Wan, E. Michelini, A. Roda, B. Wang, Comprehensive profiling of diverse genetic reporters with application to whole-cell and cell-free biosensors, Analytical Chemistry 91 (2019) 15284-15292. https://doi.org/10.1021/acs.analchem.9b04444

[90] Z. Wang, W. Cui, CRISPR-Cas system for biomedical diagnostic platforms, View 1 (2020) 20200008. https://doi.org/10.1002/viw.20200008

[91] Y. Liu, F. Pinto, X. Wan, Z. Yang, S. Peng, M. Li, J.M. Cooper, Z. Xie, C.E. French, B. Wang, Reprogrammed tracrRNAs enable repurposing of RNAs as crRNAs and sequence-specific RNA biosensors, Nature Communications 13 (2022) 1937. https://doi.org/10.1038/s41467-022-29604-x

[92] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, V. Verdine, N. Donghia, N.M. Daringer, C.A. Freije, C. Myhrvold, R.P. Bhattacharyya, J. Livny, A. Regev, E.V. Koonin, D.T. Hung, P.C. Sabeti, J.J. Collins, F. Zhang, Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (2017) 438-442. https://doi.org/doi:10.1126/science.aam9321

[93] J.S. Gootenberg, O.O. Abudayyeh, M.J. Kellner, J. Joung, J.J. Collins, F. Zhang, Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6, Science 360 (2018) 439-444. https://doi.org/doi:10.1126/science.aaq0179

[94] M.M. Kaminski, O.O. Abudayyeh, J.S. Gootenberg, F. Zhang, J.J. Collins, CRISPR-based diagnostics, Nature Biomedical Engineering 5 (2021) 643-656. https://doi.org/10.1038/s41551-021-00760-7

[95] I.C. Koksaldi, S. Kose, R.E. Ahan, N. Haciosmanoglu, E. Sahin Kehribar, M.A. Gungen, A. Bastug, B. Dinc, H. Bodur, A. Ozkul, U.O.S. Seker, SARS-CoV-2 detection with de novo-designed synthetic riboregulators, Analytical Chemistry 93 (2021) 9719-9727. https://doi.org/10.1021/acs.analchem.1c00886

[96] B. Saltepe, L. Wang, B. Wang, Synthetic biology enables field-deployable biosensors for water contaminants, Trends in Analytical Chemistry 146 (2022) 116507. https://doi.org/10.1016/j.trac.2021.116507

[97] H. Huang, D. Densmore, Integration of microfluidics into the synthetic biology design flow, Lab on a Chip 14 (2014) 3459-3474. https://doi.org/10.1039/c4lc00509k

[98] Y. Yang, Y. Chen, H. Tang, N. Zong, X. Jiang, Microfluidics for biomedical analysis, Small Methods 4 (2020) 1900451. https://doi.org/10.1002/smtd.201900451

[99] B. Saltepe, E.U. Bozkurt, M.A. Gungen, A.E. Cicek, U.O.S. Seker, Genetic circuits combined with machine learning provides fast responding living sensors, Biosensors and Bioelectronics 178 (2021) 113028. https://doi.org/10.1016/j.bios.2021.113028

[100] H. Kim, W. Seong, E. Rha, H. Lee, S.K. Kim, K.K. Kwon, K.H. Park, D.H. Lee, S.G. Lee, Machine learning linked evolutionary biosensor array for highly sensitive and specific molecular identification, Biosensors and Bioelectronics 170 (2020) 112670. https://doi.org/10.1016/j.bios.2020.112670

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