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Abstract

Water pollution is a global concern for human and environmental health. As technology and industries have developed over the past decades, increasingly more complex and diverse pollutants are found even in treated waters. For better management of water resources, continuous and efficient monitoring is needed to detect the broad range of contaminants.

Biosensors have the potential to meet this challenge and to overcome the limitations of the conventional methods used for water analysis. They combine a biological recognition element to a transducer in a sensitive and robust device, capable of specific detection of molecules of interest. DNA-based sensing technologies meet this set of specifications and benefit from the progress made in nanoscience and nanotechnology. This mini-review proposes an overview of this upcoming new generation of DNA-based biosensors, focusing on promising innovations having for portable, stable, rapid and sensitive devices for water quality monitoring.

Keywords

Biosensor; Environmental contaminant; Nanotechnologies; Aptamer; DNAzyme; Single molecule detection

1. Introduction

The demand for novel monitoring methods for the control of water status was pointed out by the EU parliament and council in the founding Directive of 2013 regarding priority substances in the field of water policy (EUdirective 2013). This directive announced the establishment of the first watch list of substances and was followed by vast Union-wide monitoring programs. Among contaminants posing threat to aquatic environment and human health are i) the microorganisms (bacteria, viruses and parasites) and ii) micropollutants with negative effects at low concentrations, ranging from µg/L to ng/L. The latter include an immense variety of inorganic and organic chemicals: metal ions, in particular heavy metal ions, hydrocarbons, plasticizers, pesticides, pharmaceuticals, personal care products, detergents and sanitizer products...

Massive analytic, ecotoxicological and toxicological data are collected and reviewed on the basis of what risks are assessed for determining new priority substances and environmental quality standards (EQS). The discharge of micropollutants in surface waters comes for a large part from the wastewater treatment plants which are rarely designed to eliminate micropollutants. In addition, an important diffuse pollution exists also with contamination of ground and surface waters from rainfall runoff draining pollutants from agriculture and livestock. Pesticides, for example, are among the contaminants for which the EQS are the most frequently exceeded (Petit and Michon 2015). Climate change and, since micropollutants are mainly of anthropogenic origin, demographic growth will increase water stress, the ratio between water withdrawals and the available renewable water supplies. Preserving the quality of water has hence become a major worldwide concern and monitoring devices will help to set regulatory limits for micropollutants and enable their continuous control with the most extensive coverage.

The disclosure of micropollutants occurrence in surface water owes much to the progress of analytical methods, in particular liquid chromatography coupled to mass spectrometry (LC-MS) which has become a gold standard of water micropollutant analyses (EUdirective 2013). Thanks to the sensitivity of the technique and its capacity of identification of a large multiplicity of compounds in a complex matrix (Cotton, Leroux et al. 2016), massive campaigns of measurements revealed the extent of the contamination and also of the variety of the compounds (Petrie, Barden et al. 2015). While LC-MS is extremely useful to unveil this environmental concern, such a technique is not adapted for a regular and systematic control of catchment stations, urban and industrial WWTP effluents, … To meet the future challenges, in addition to high sensitivity, the pre-requisites for devices adapted to such a mass use are: portability, automation, rapidity, versatility, reduced cost, ease of use and, ideally, sustainability.

The use of antibodies has led to the development of a panel of fast and cost-effective alternatives to this analytic approach relies on the detection of analytes through their specific recognition. The well-known ELISA tests (for Enzyme Linked Immuno Sorbent Assay) allow to reach detection limits comparable to LC-MS/MS in some cases and sometimes even below the European Union limit for drinking water and. Antibody-based commercial kits are widely used in biology and biomedical laboratories, where conditions are met to perform those assays routinely but their format is not usually suitable for field applications.

Nanoscale or nanotechnology-enabled sensors offer the potential of integration in miniaturized and automated devices. Based on a nanomaterial, a recognition element and a mechanism for the transduction of the detection event efficient at the molecular level, they promise high performance with extremely low detection limit and rapid analysis (Vikesland 2018). Over the past decades, DNA, apart from its obvious interest for species identification, has emerged as a highly potent recognition element, with enormous advantages: versatility, robustness and cost-effective mass synthesis (Palchetti and Mascini 2008). DNA has also made a breakthrough in the domain of nanotechnology as a building block for the construction of refined nanostructures (Abu-Salah, Ansari et al. 2010). Devices embarking DNA-based nanobiosensors are most promising candidates.

This mini-review depicts the essential features of recent achievements in the field of DNA-based nanobiosensors, focusing on functional solutions for the monitoring of ions, pesticides and pathogens and discusses the future developments in progress.

2. Detection principles of DNA-based nanosensors

Biosensors can be classified according to the different components employed for their design: the recognition element used, the signal transduction method, or the analyte of interest (IUPAC definition) (Bhalla, Jolly et al. 2016). The selectivity of a sensor is provided by the recognition element chosen. While it is commonly thought that the biorecognition element is the most important part of a biosensor, we will also discuss how choosing the transducer affects the device's performance. Accordingly, biosensors can be designed as electrochemical, optical, or piezoelectric devices. Here we present another classification, showing how different assay configurations can be designed to exploit the polymorphism of DNA.

2.1 Conformational change

DNA molecules, contrary to protein recognition elements, present a potential for malleability often exploited in the conception of bioassays. Conformational change tests are based on the

measurement of a distance shift consecutive to the recognition event. To the polymorphism of DNA is added the versatility of surfaces used in sensors: modified electrodes, noble metal nanoparticles, quantum dots, carbon nanotubes, graphene sheets,… offering a wide range of experimental designs (Dolatabadi, Mashinchian et al. 2011).

We distinguish two categories of sensors: one using (de)hybridization, and the other nucleic acid probes (aptamers and DNAzymes). In the first case, the capacity of nucleic acids chains to shift from single-stranded molecules to double-stranded helices in the presence of a complementary strand is exploited. Alternatively, a single-stranded oligonucleotide can be functionalized with a fluorophore and its complementary sequence carries the quencher or vice versa.

Another, more often used type of DNA probe, is the aptamer, first introduced in 1990 by the laboratories of Szostak and Gold (Ellington and Szostak 1990; Tuerk and Gold 1990). Aptamers are single-stranded oligonucleotides ranging from 20 to 100 nucleotides in length, which can bind specifically to the target of interest (Figure 1). They are obtained by a process called Systematic Evolution of Ligands by EXponential enrichment (SELEX), developed by the two aforementioned teams. A randomized DNA (or RNA) library of around 10^{14} sequences, flanked by specific primers is incubated with the immobilized target to allow binding. The unbound sequenced are washed, the rest is eluted and amplified by Polymerase Chain Reaction (PCR), dehybridized before going through another round of incubation with the target. The cycle is repeated between 8 to 30 times with increasing stringency, and the enrichment of bound sequences is followed by sequencing after each round. Some of the aptamers are consequently tested to single out the best ones. Since the 90s, several researchers have been trying to improve SELEX and adapt it to a wide variety of applications (Sharma, Bruno et al. 2017; Wang, Chen et al. 2019). Indeed, aptamers can recognize targets such as ions, small molecules, proteins, pathogens and eukaryotic cells. Aptamers are therefore considered as the nucleic acid analogs of antibodies, and their affinity and selectivity can sometime rival those of the more commonly known recognition molecules. Aptasensors can be found in as many different assay configurations as immonusensors based on antibodies, and benefit from the advantages inherent to the molecular nature of aptamers, including versatility in target recognition (even non-immunogenic molecules), cost-effective development, long shelf-life and possible chemical modifications. The latter allows to graft aptamers on various nanomaterials (Nguyen, Kwon et al. 2017; Liu, Khan et al. 2019).Aptamers adopt a three-dimensional structure when they recognize their target, a conformational change that is taken advantage of for sensing methods. It is interesting to note that, while many RNA aptamers have been generated by SELEX, very few can be found in the water quality field as they are more fragile than DNA, despite the various chemical modifications made to improve their stability (Meek, Rangel et al. 2016). .

Alongside the development of aptamers, it is also possible to carry out the *in vitro* selection of oligonucleotides called DNAzymes , which possess catalytic properties that only work in the presence of metal ions as co-factors for which they have high specificity (McGhee, Loh et al. 2017) (Figure 1). When functionalized with a fluorophore-quencher pair on each end of the DNA strand, these DNAzymes allow on-site and real-time detection of picomolar concentrations of metal ions including $UO2^{2+}$, Pb^{2+}, Hg^{2+}, Cu^{2+}, Ag^{+}, Cr^{3+}, and Ce^{3+}, Ni^{2+}, Zn^{2+} in different aqueous media.

This list of the different types of nucleic acid-based sensors is far from being exhaustive. Starting from the same type of biomolecule, a wide variety of assays can be designed. Reminding us of building blocks, researchers can combine nucleic acids with different nanomaterials and different signal transducers. This is how, and why, hundreds of different biosensors have been developed; some more fit than others for the specific detection of a target of interest. The complexity of this field is then easily understood when we realize that it comes from its wealth in sensors architecture.

When we take an interest into what makes a biosensor fit for commercialization and used by the public, we find that optical based devices are the most successful. Based on plasmonic resonance, colorimetry or fluorescence (Damborský, Švitel et al. 2016), their coupling to nanomaterials such as nanoparticles strongly enhances signal transduction (Table 1). Among noble metals, gold nanoparticles (AuNPs) are by far the most popular (Aldewachi, Chalati et al. 2018). Smaller than 100 nm, they offer an ease of preparation and biocompatibility, making them perfect candidates for bioassay designs, all the more so since their unique optical, chemical, and electric properties can be explored for signal amplification. Their most common utilization is as platforms for colorimetric sensors: a color shift is observed according to their shape, size and aggregation state (Jain, Lee et al. 2006). The amino function groups found in the bases of nucleic acids non-covalently bind to the negative charges of AuNPS, stabilizing the particles' suspension. When a molecule of interest is added to the suspension, the DNA will preferentially bind to its target, leaving the AuNPS bare and susceptible to aggregation due to the presence of high salt concentration. The extent of the resulting absorbance shift being target concentration dependent (Li and Rothberg 2004). While simple visual observation can be used to detect this phenomenon, the resonance behavior of AuNPs provides an alternative transduction method. As AuNPs present unique Localized Surface Plasmon Resonance (LSPR) properties, sensitive to the mean interparticle distance, transduction is often coupled to Surface Enhanced Raman Spectroscopy (SERS). SERS enhances Raman scattering by a 10^{10} factor when molecules are adsorbed on metal surfaces, allowing us to reach single molecule sensitivity, a perfect example of how nanotechnology can be used to improve detection limits (Blackie, Le Ru et al. 2009; Wei, Hossein Abtahi et al. 2015).

Despite their numerous advantages, sensors based on particle aggregation are yet to be used for *in situ* studies as further development is still needed. Since their properties are shape and size-dependent, a better control of these parameters is required during the particles' preparation. On the other hand, progresses made for point-of-care (POC) devices using such colorimetric assays could be transposed to environmental applications.

The other frequently developed type of sensor is based on electrochemistry. Ease-of-use and facile miniaturization are the main advantages of this sensing method (Willner and Vikesland 2018). The interaction of an analyte of interest with an electrode is measured, using one of four main modes: amperometric (change in current), potentiometric (change in potential), conductometry or impedance, and finally *field-effect* methods (Thévenot, Toth et al. 2001) (Table 1). The last one is based on transistor technology and measures current as a result of a potentiometric effect at a gate electrode. The surface architecture of the electrodes has benefited from the development of nanotechnology, improving detection limits and lowering signal-to-noise ratios (Hammond, Formisano et al. 2016). Overall, electrochemical sensing presents the most advantageous detection limits, and the possibility of reuse as regeneration of the surfaces is possible. Portability in most cases is also feasible making them interesting candidates for their use in environmental monitoring. However, they do require instrumentation and complex, expensive fabrication steps when nanotechnology is involved.

Other transduction methods are not discussed in this review: piezoelectric effect, sensitive to mass change, and thermal-based sensors that rely on the energy released by biochemical reactions. Since both are too sensitive to environmental conditions and not adapted for onsite use, we will not discuss them further here.

2.2 Nucleic acid amplification

Stemming from a growing need in the biomedical field, gene amplification techniques have been developed alongside biosensing strategies. The most popular, Polymerase Chain Reaction (PCR), was created by K. Mullis in 1983 (Mullis, Faloona et al. 1986): a specific region of a DNA strand is amplified by a polymerase enzyme after repeated and cyclic steps of denaturation, specific primers hybridization to the target DNA strand (annealing) and elongation (Figure 1). PCR-based conventional methods rely on nucleic acid amplification of a specific target sequence, and are used for detection of pathogens at low concentration (Ahmed, Rushworth et al. 2014). A limit of this strategy is the potential amplification of a contaminant sequence due to non-specific or mismatched hybridization of PCR primers. Therefore, a precise knowledge of the genetic information of interest is vital. Other

downsides to its use on the field are the length and complexity of the sample preparation steps (Lazcka, Campo et al. 2007) and the potential amplification of genetic material from non-viable cells (Velusamy, Arshak et al. 2010). The limitations of conventional PCR should be overcome by lab-on-chip platforms integrating solid-phase PCR and recently proven to work for the detection of pathogens (Hung, Chin et al. 2017).

Isothermal Amplification of nucleic acids have been developed as a simpler alternative to the former technique (Zhao, Chen et al. 2015), allowing the detection of a wide spectrum of targets with higher sensitivities. They are commonly divided into three categories: linear amplification, cascade amplification and exponential amplification. Hybridization Chain Reaction (HCR), is an example of enzyme-free amplification process where an initiator sequence triggers a cascade of hybridization between two populations of DNA hairpins. The latter can be directly functionalized with labels or signaling molecules or incorporated to nanostructured platforms such as NPs for the detection of a broad range of targets (Bi, Yue et al. 2017). Another method, called Exponential Amplification Reaction (EXPAR) has been thoroughly explored using a simple template, a polymerase and a nicking endonuclease achieving amplification up to a 10^8 fold in less than 30 min (Reid, Le et al. 2018). Although the detection of nucleic acids (miRNA, genomic DNA, ...) is the most straightforward application of isothermal amplification techniques, research has been led to incorporate them in assays for a broader range of targets by linking the oligos with recognition molecules and DNA probes, increasing the sensitivity of the detection of molecules that cannot be chemically amplified such as proteins or metal ions. To this versatility is the added bonus of a wide choice in the mode of detection of the amplification products, from simple colorimetry (AuNPs), to fluorescence and electrochemical transducers, displaying the important potential in developing amplification techniques to carry out on-site tests.

3. Proven sensing solutions

Among the abundant literature reporting a multiplicity of detection sensing solutions, it is worth emphasizing the following few examples of advanced or very successful developments commercially available (see also Table 2).

3.1 Metal ions

The real-time and on-site detection of metal ions is of primary importance as they have strong negative impact on human health. Lead, mercury, cadmium, chromium, copper, zinc, arsenic… produced by industrial activity or released by various equipment can compromise the quality of water resources. These elements are easily and efficiently identified by standard laboratory

techniques, but those are not adapted to field measurements. Although aptamers have been successfully selected against a variety of metal ions (Abu-Ali, Nabok et al. 2019), DNAzymes sensors indisputably took the lead in the field of metal ion sensing due to their extremely high selectivity. Several methods with limits of detection (LOD) in the pM range have been developed using the ease of DNA functionalization, to label it with fluorophores, electrochemical agents, or attach it to nanomaterials followed by various detection modes: fluorescence, SERS, colorimetry, electrochemistry or electroluminescence.

DNAzymes are synthetic single-stranded DNA sequences consisting of a substrate strand, with a ribonucleoside adenosine at the cleavage site for the RNA-cleaving DNAzymes, and an enzyme strand with catalytic activity in the presence of the analyte (Figure 1). The group of Yi Lu (Li and Lu 2000) was the first to report the utilization of DNAzymes as metal ion biosensors, starting with Pb^{2+}, which could be detected down to 10 nM with a high selectivity over other metal ions. After this breakthrough obtained using a fluorophore-quencher pair as a signaling method, alternative methods for the detection were investigated, aiming at reducing the required instrumentation and cost and/or enhancing the sensitivity, such as colorimetry with gold nanomaterials (Liu and Lu 2004) and SERS spectroscopy with Ag nanoparticles-on-a-film (Fu, Xu et al. 2014). Nowadays fluorescent DNAzyme-based sensors have reached LOD in the pM range. This is well below the guideline value in drinking water of 50 nM for lead for example which is one of the more stringent prescription with arsenic and selenium (WHO 2017). Commercially available in a portable format (ANDalyze) they are successfully applied to an extended list of metal ions in a large range of water types.

3.2 Chemicals and drugs

Synthetic chemicals are introduced in the water cycle by industrial and urban WWTP effluents and agriculture. While advanced processes can be used to improve the rate of chemicals' abatement at a WWTP output, and thus reduce the contamination of surface waters by chemicals of domestic and industrial origin, the application of crop protection products and fertilizers in agriculture is followed by their penetration in groundwater and their runoff in rivers in case of heavy rains. Pesticide contamination is one of the major problem of public health worldwide and will remain so for a long time. Indeed, pesticides can have long break down times and products banned more than ten years ago are still found in rivers and groundwater (Cotton, Leroux et al. 2016). Excessive concentrations of pesticides and fertilizers is the major cause of abandonment of drinking water catchments. The demand for frequent and rapid control of pesticide content is strong and devices to meet this great challenge are eagerly expected.

This context has motivated massive research efforts for the development of aptasensors. The first and essential step in this direction is the selection of fully potent aptamers. A dozen of aptamers are presently available for a variety of pesticides with good (Kd around 1 µM) to very good affinities (Kd between 10 to 100 nM), the best candidate being the aptamer against atrazine with a Kd of 0.6 nM (Liu, Khan et al. 2019).

Colorimetric platforms using aptamers and gold nanoparticles allowing simple visual detection (see section 2) proved to be functional. In one version of these assays, DNA aptamers are used as stabilizers of a gold nanoparticle suspension and desorb from the particles surface in the presence of the analyte. Such assays are susceptible to be biased by the ion composition of matrices.

Here again sensors based on fluorescence detection have been designed following various strategies. Typically, a fluorophore is attached at one end of the aptamer and the other end is bound to a quencher, or to a surface acting as a quencher, leading to a loss of fluorescence as the analyte binds to the aptamer and changes its conformation. More complex designs, involving a complementary DNA sequence functionalized with the quencher that is released upon analyte binding, result in an increase of fluorescence signal upon aptamer conformational change. Validated in a large variety of aqueous matrices, the main limiting step of such aptasensors is the optimization of the design which becomes all the more difficult the longer the aptamer is.

Finally, an original impedimetric biosensor was proposed that is highly selective and has the lowest LOD of electrochemical sensors for atrazine equal to 10 pM (Madianos, Tsekenis et al. 2018). Conventional Ti interdigitated electrodes are bridged by Pt nanoparticles microwires deposited along patterns designed by electron beam lithography. The sensor surface is then silanized to immobilize the thiol modified aptamers that hinder the charge transfer between electrodes upon binding of the analyte. This method which has been tested with two pesticides in tap and bottle mineral waters can be easily integrated in portable devices and could be exploited for the control of drinking water.

3.3 Pathogens

According to the World Health Organization, a plethora of bacteria, viruses, protozoa and parasites are recognized as harmful and are classified by health significance in their water quality guidelines. Conventional methodologies such as colony counting do not allow the continuous, real-time monitoring of the presence of pathogens in the environment, and while their use is still widespread researchers all over the world work to develop portable, rapid and sensitive biosensing platforms to overcome their limitations (Sutarlie, Ow et al. 2017; Rajapaksha, Elbourne et al. 2019). Waterborne pathogen sensing relies on either the detection of whole cells, a representative epitope (a membrane protein for example), genetic material or pathogen product such as toxins.

In terms of specificity, detecting the genetic material of a microorganism is by far the most secure way to determine its presence in a sample. As was discussed above, nucleic acid amplification techniques, particularly isothermal and enzyme-free methods, have taken over this particular field. Paradoxically, however, most of the research published using these techniques is for the detection of cancer biomarkers, tumor cells, and pathogens for diagnostics and point-of-care purposes (Mayboroda, Katakis et al. 2018). The problem with genetic material, however, is that it does not provide information on the viability of a pathogen detected. Combining this type of assay with the detection of toxins released or RNA expression would offer better and more informative alternatives for water quality analysis. Here we present a few examples.

Several aptasensors have been developed for the detection of whole bacteria, viruses or spores (Hong and Sooter 2015; Davydova, Vorobjeva et al. 2016). Based on aptamers hybridized with complementary DNA, grafted on AuNPs and Upconversion Nano Particles respectively, an aptasensor reached a detection limit of 3 cfu/mL for the ATCC 8739 strain of *E. coli* in tap and pond water (Jin, Wang et al. 2017). This sensitivity does not meet the requirements for drinking water (WHO 2017) but is suitable to evaluate the quality of recreational waters (EUdirective 2006; Khang, Kim et al. 2016).

Detection of toxins produced by pathogens is also useful. Of special concern are the toxins released by cyanobacteria because of their potential lethal effect. One of them, the microcystin-LR is hepatotoxic and can be detected using an aptamer developed against the toxin in 2012 (Ng, Chinnappan et al. 2012) leading to many different nanomaterial-based aptasensors (Bostan, Taghdisi et al. 2018) reaching detection limits in the ng/L range, well below the guideline value set by WHO (WHO 2017) for drinking water.

Ozkan-Ariksoyal et al designed an electrochemical sensor targeting the conserved 16s-rDNA region of E. *coli* using DNA wrapped multi-walled carbon nanotubes from PCR amplified real samples, in less than 20 minutes and with a detection limit as low as 0.5 pmol (Ozkan-Ariksoysal, Kayran et al. 2017). Melaine et al successfully and simultaneously detected the RNA of *Pseudomonas aeruginosa*, *Legionella pneumophila* and *Salmonella typhimurium* in a multiplex SPR imaging platform based on DNA probes coupled to AuNPS, applicable to a wider range of waterborne bacteria, with a LOD of 10 $pg.mL^{-1}$ (Melaine, Saad et al. 2017). These methods require additional preparation steps, for which many integrative sensors are being tested.

Several methodologies were developed for diagnostics, assessment of water and food contaminations, using paper, microfluidics or lateral flow (Kumar, Nehra et al. 2019), showing promising future breakthroughs for rapid *in situ* microorganisms detection. For example, Feng Xu's

lab created a paper-based device combining all the steps necessary for genetic material detection and analysis, including extraction and amplification (Tang, Yang et al. 2017).

4. Ongoing developments

4.1 DNA origami

As was stated previously, the polymorphism of DNA allows us to use it not only as a recognition element (probes) or a signal transducer (amplification), but also as the building block of a sensor architecture. In this section, we present DNA as a nanoscale platform upon which it is possible to graft recognition elements. The interactions between the recognition element and the target can be observed directly on the nanostructure, or indirectly by the modification of the shape of the platform. This last concept counts as an emerging tool for the development of bioassays and biosensors.

The *in vitro* constructs of DNA nanostructures have evolved during the last forty years (Seeman 1982; Seeman 2003; Goodman, Schaap et al. 2005) to be used as sophisticated large scaffolds called origami with broad potentials. Using bioinformatics and the large single stranded DNA of the M13 phage, combined to short ssDNA called “staples”, it is now possible to create a diverse catalog of DNA structures (Rothemund 2006). Many studies demonstrated the potential use of origami for target drug delivery and diagnostics (for a complete review concerning recent progress in origami studies see Ijäs (Ijäs, Nummelin et al. 2018) and Sakai (Sakai, Islam et al. 2018). We focus here on the evolution of DNA origami for biosensing applications.

Ke and al. designed tiles as arrays (built like DNA chips) with a 2D origami on which DNA probes were grafted to detect (RNA) target in solution and observed by Atomic Force Microscopy (AFM) to quantify hybridization events (Ke, Lindsay et al. 2008). This paved the way to use origami in sensing devices, demonstrated in 2016 by grafting the aptamer targeting the malaria biomarker PfLDH on origami tiles (Godonoga, Lin et al. 2016). Beyond the simple use of origami as a support for recognition molecules, Andersen showed that it was possible to have a conformational transition of a 3D origami (a box) in response to stimuli to detect DNA (or RNA) in solution, observable by AFM, TEM or FRET (Andersen, Dong et al. 2009). Recently the same origami box was designed to detect plasmodium using the malaria biomarker aptamer (Tang, Shiu et al. 2018).

Other designs of DNA origami that fold differently in response to various stimuli were published by Kuzuya (Kuzuya, Sakai et al. 2011). In this article authors constructed origami pliers that could detect metal ions by zipping, small molecules (miRNA and ATP) by unzipping and proteins by pinching

mechanisms, at the single molecule resolution. In a similar manner, Ke and al. (Ke, Meyer et al. 2016) designed an elegant tunable DNA origami device named “nanoactutor” responding to different stimuli ranging from small ions, to large molecules like miRNA and proteins.

These studies demonstrated the potential of origami as a signal transducer. However reaching single molecule detection still has to be improved, and the most important problem is of course the signal readout, as AFM is not transposable to carry out *in situ* studies and demands expertise. Another limitation is the growing cost of some of these complex structures compared to the more basic sensors discussed in this review. It is too early to have an idea about the reproducibility and the selectivity of biosensors based on origami. In terms of sensitivity that could be reached, Selnihhin et al. (Selnihhin, Sparvath et al. 2018) recently developed a high-throughput, optical DNA origami nanobiosensor based on strand displacement by the target DNA sequence at concentrations down to 100 pM, with future multiplexing development (Figure 2). We believe DNA origami is still in the early stages of its development for application purposes, but presents an important potential that still needs to be explored.

4.2 Single DNA sensing

In contrast to the complex DNA origami structures, single molecule approaches emerge that propose simple designs of detection assays. Single molecule sensing is an exciting and extremely active field bearing the promise of very high sensitivity. After a few decades of instrumental developments in optics, scanning probe microscopy and nanofabrication, measurements at the single molecule level are now part of the scientist’s toolbox. DNA has been one of the favorite object of interest for the pioneers of single molecule methods. With a view to elucidate biological behaviors by unmasking the effects of averaging inherent to conventional methods, a panel of techniques have been developed to visualize and manipulate DNA leading to important contributions in the detailed understanding of the cellular processes involving DNA (Kapanidis and Strick 2009). Remarkable technological achievements are the third generation sequencing methods, single molecule long-read methods, which took only about 10 years to go successfully from the bench to the market (van Dijk, Jaszczyszyn et al. 2018).

One family of these methods where the motion of a particle linked via a DNA tether to a surface is observed by video-microscopy can be diverted for a use as a biosensor for the analysis of water pollutants. Indeed, the efforts devoted to the development of these new tools have led to progress in nanoscale engineering leading to reliable, accurate and robust techniques. Pulling on the particle, while measuring the extension of the DNA molecule determined by tracking the particle’s position in

real time, informs about DNA conformation and elasticity. It is referred to as single molecule force spectroscopy measurements (Bustamante, Bryant et al. 2003).

Halvorsen et al designed a clever approach using DNA origami to construct a DNA tether with two distant functional sites (Halvorsen, Schaak et al. 2011) and optical tweezers to exert a force on the DNA tether. This simple method gives in principle access to measurements of force-dependent bond rupture and bond formation of pairs of interacting molecules which can be performed repeatedly for the analysis of molecular interactions (Figure 3 A). A simpler design of DNA tethered particle pulling assay is proposed by Silver and Neuman (Silver, Li et al. 2015) as sandwich immunoassay. The sensor uses DNA tethers attached by one end to a flow cell surface, particles bind the other end of the DNAs via an immune sandwich formed in case of capture of the analyte (Figure 3 B). The number of tethered particles determined by the particles displacement upon application of a flow allows to quantify the concentration of analyte with a detection limit down to pM, at the prototype stage.

Even more simple but effective is the so-called particle mobility sensing by Visser et al (Visser, Yan et al. 2018) wherein no force is applied on the DNA tethered particle. In this sandwich assay, the particle and the surface are functionalized with affinity molecules (Figure 3 C). The particle experiences successive reversible binding events, leading to immobilization periods upon recognition of the analyte, the duration of which is related to the analyte concentration. Demonstrated on model biomarkers, single stranded DNA and a protein in complex media, the technology has a picomolar range sensitivity and the potential for a broad range of applications.

An improvement of these single molecule sensing methods is their controlled parallelization by self-assembly of the DNA tethered particles on regular nanoarrays. Using a standard microcontact printing technique, Plénat, Tardin et al (Plénat, Tardin et al. 2012) successfully developed a single DNA biochip for the simultaneous acquisition of data from more than 500 DNA molecules, strongly increasing the yield and quality of the output (Figure 3 D). Together with the possible miniaturization of the readout system using mobile phone-based wide field fluorescence or dark-field imaging (McLeod, Wei et al. 2015), these capabilities make single DNA tethered particle methods promising platforms for the development of systems for water monitoring in either laboratory or field conditions.

5. Concluding remarks

Biosensor development for environmental contamination is growing rapidly, benefiting from the advances made in new nanotechnologies and single molecule assays. As demonstrated by a first

successful industrialization based on DNAzymes, DNA nanobiosensors are excellent candidates for the implementation of highly sensitive, specific and rapid methods into portable and cost-effective devices. We soon expect to see more to arrive on the market, combining DNA with nanomaterials in creative ways, continuously enriching the field.

The ideal assay would be a fully integrated device, something already conceptualized with lab-on-chip and paper-based sensors, overcoming the tedious preparation steps necessary in conventional methods (Mahadeva, Walus et al. 2015). Another benefit would be the accessibility of these cheaper, one-shot devices for low income countries.

We foresee that new solutions, transposable to environmental monitoring, inspired from biomedical devices will emerge in the future. Indeed, sensors developed for point-of-care diagnostics and personalized medicine share similar technical specifications with water quality monitoring systems.

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Table 1: Overview of nanomaterial-based biosensors transduction methods. For further information, see (Sutarlie, Ow et al. 2017).

Transduction	Methods	Nanomaterials associated
Optical	Colorimetry	
	Raman spectroscopy (SERS) and infrared spectroscopy (FTIR)	Noble metal nanoparticles
	Surface Plasmon resonnace (SPR)	
	Fluorescence Chemiluminescence	Quantum dots, carbon nanotubes, graphene
	Single-molecule microscopy	Polystyrene or magnetic nanoparticles
Electrochemical	Amperometry	
	Conductimetry	
	Impedance spectroscopy	Modification of the electrode itself (gold, silver…) Carbon nanotubes, graphene…
	Potentiometry	
	Field-effect transistors	

Table 2: Performances of DNA-based nanobiosensors for the detection of micropollutants and microorganisms in water, with regard to EU and WHO guideline values for drinking water and commercial ELISA tests. Examples of targets were selected to represent the following types of contaminants: metal ions, chemicals, microorganisms. ([a]) Abcam, ([b]) Creative Diagnostics, ([c,d]) MyBioSource. N.A. not available.

Micropollutant/ microorganism	WHO Guideline values (WHO 2017)	EU quality standards (EUdirective 2015)	DNA-based nanobiosensors tested in natural water			ELISA commercial test
	µg/L (in drinking water)		LOD (range) µg/L	Method	Reference	LOD (range) µg/L
Lead	10	10	0,002 (0,01-20)	Chronocoulometric DNAzyme sensor using AuNPs.	(Zhang, Lai et al. 2016)	N.A.
			0,1 (0,5-20)	Portable DNAzyme sensor with magnetic beads and Platinum NPs.	(Huang, Lin et al. 2019)	
Mercury	6	1	0,3 (0,5-20)	Colorimetric aptasensor using Rolling Cycle Amplification	(Wu, Yu et al. 2020)	$1,6 \times 10^3$ ($1,6 \times 10^3$-100×10^3) [a]
			2 (2-50)	Fluorimetric DNAzyme sensor	ANDalyze.com	
Atrazine	100	0,1	$2,5 \times 10^{-6}$ (10^{-3}-0,064)	Aptamer grafted on graphene in a photoelectrochemical sensor.	(Sun, Liu et al. 2019)	0-10 [b]
			0,002 (0,02-200)	Aptamer and platinum NPs grafted on microwires for impedance spectroscopy.	(Madianos, Tsekenis et al. 2018)	
Microcystin-LR	1	1	0,03	Steric hindrance initiated signal amplification effect	(Du, Jiang et al. 2016)	0,005 (0,03-1) [c]
			0,002 (0,004-0,512)	Interaction with DNA-modified electrodes in impedimetric sensor	(Zhang, Ma et al. 2018)	
E. coli	0/100mL	0/100mL	3 cfu/mL (5–10^6 cfu/mL)	AuNPs and Upconverstion particles FRET aptasensor	(Jin, Wang et al. 2017)	$<10^3$ cfu/mL (6×10^3 - 4×10^5 cfu/ml) [d]
			10 cfu/mL (10-10^6 cfu/mL)	Graphene functionalized impedimetric aptasensor	(Kaur, Shorie et al. 2017)	

Figure captions

Figure 1: Main features of DNA nanobiosensors for water monitoring. The various water contaminants (metal ions M^{x+}, chemicals and pathogens) can be detected through different processes via DNA-based recognition elements: target induced conformational changes for DNAzymes and aptamers and, nucleic acid amplification for oligos (primers).

Figure 2: DNA origami sensing platform. Two arrays made of DNA origami harbor multiple donor and acceptor fluorophore pairs for enhanced FRET signal. The device immobilized on a glass slide switches from closed to opened state upon binding of the target to the recognition elements (sensor modules) on the free edges of the arrays. Reproduced and adapted from Selnihhin et al ACS Nano 2018 (Selnihhin, Sparvath et al. 2018) with permission of the American Chemical Society and from Xavier and Chadrasekaran Nanotechnology 2018 (Xavier and Chandrasekaran 2018) with permission of IOP Publishing Ltd.

Figure 3: Single DNA tethered particle mobility sensing. The displacement of the particle under an applied force using either magnetic or optical tweezers (A) or flow (B) allows, with *ad hoc* functionalizations of the DNA tether, to analyze interactions between receptor and ligand grafted to distant locations on the DNA (A, adapted from Halvorsen et al Nanotechnology 2011 (Halvorsen, Schaak et al. 2011) with permission of IOP Publishing Ltd or to identify the presence of an analyte (B, adapted from Silver et al Biosensors and Bioelectronics 2015 (Silver, Li et al. 2015) with permission of Elsevier). In the absence of force, target binding events between the recognition elements on the particle and the substrate are detected through immobilization periods of the particle (C, reproduced from Visser et al Nature Communications 2018 (Visser, Yan et al. 2018)). The parallelization of the Tethered particle Motion technique by the controlled positioning of the tethered particles on regular nanoarrays drastically improves the throughput capacity (D, reproduced from Plénat, Tardin et al Nucleic Acids Research 2012 (Plénat, Tardin et al. 2012) with permission of Oxford University Press). Dark field microscopy images of single DNA tethered particles scale bars equal to 50 µm and 20 µm for upper and lower frames, respectively.

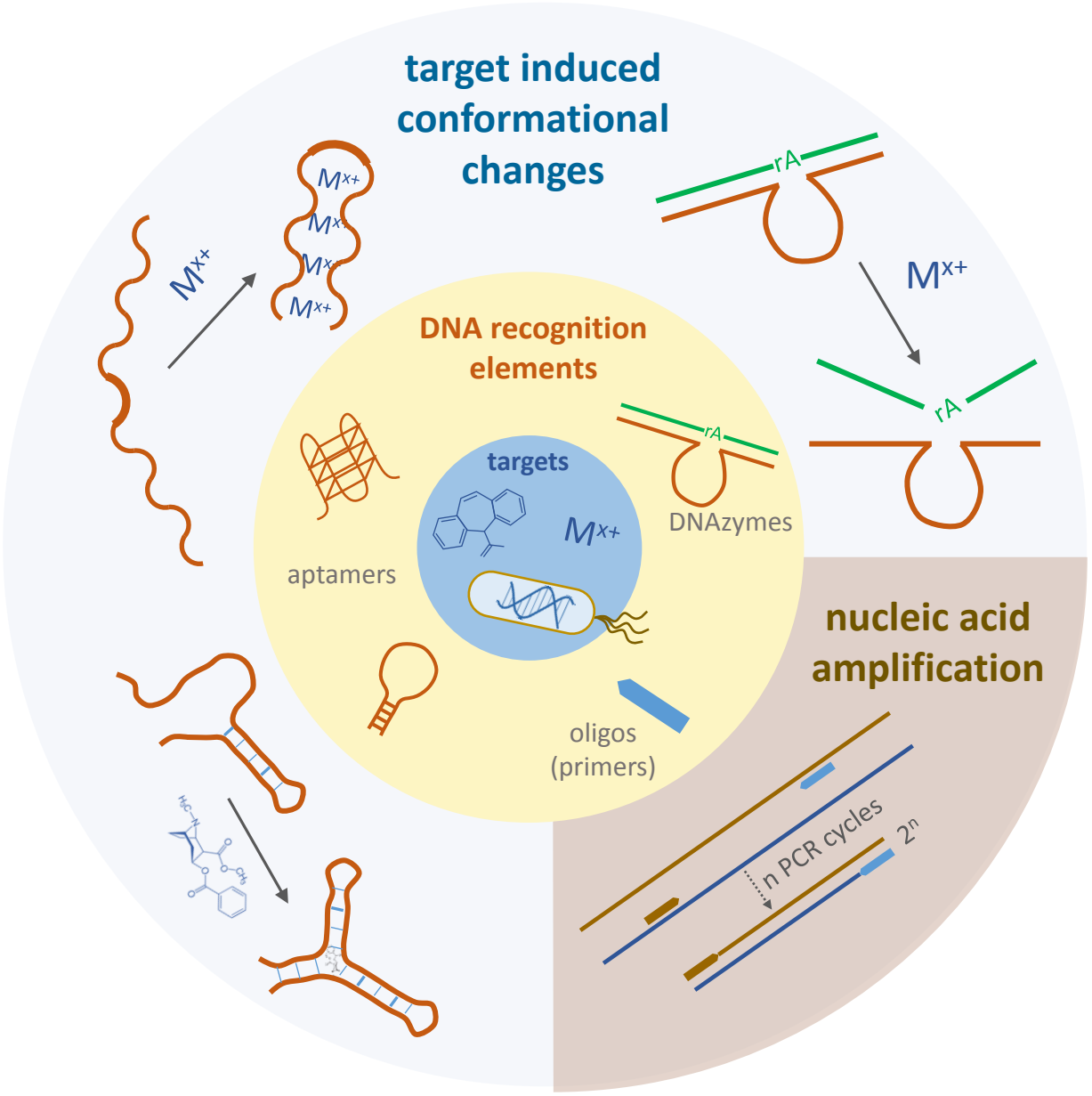
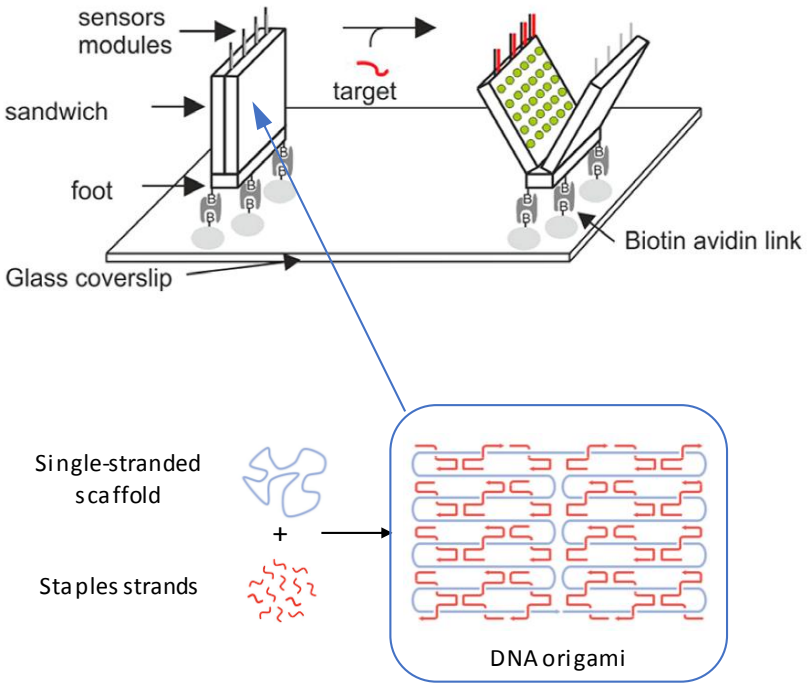

target induced conformational changes
Mx+
rA
Mx+
rA
DNA recognition elements
targets
Mx+
aptamers
rA
DNAzymes
oligos (primers)
nucleic acid amplification
n PCR cycles
2n

Figure 1


sensors
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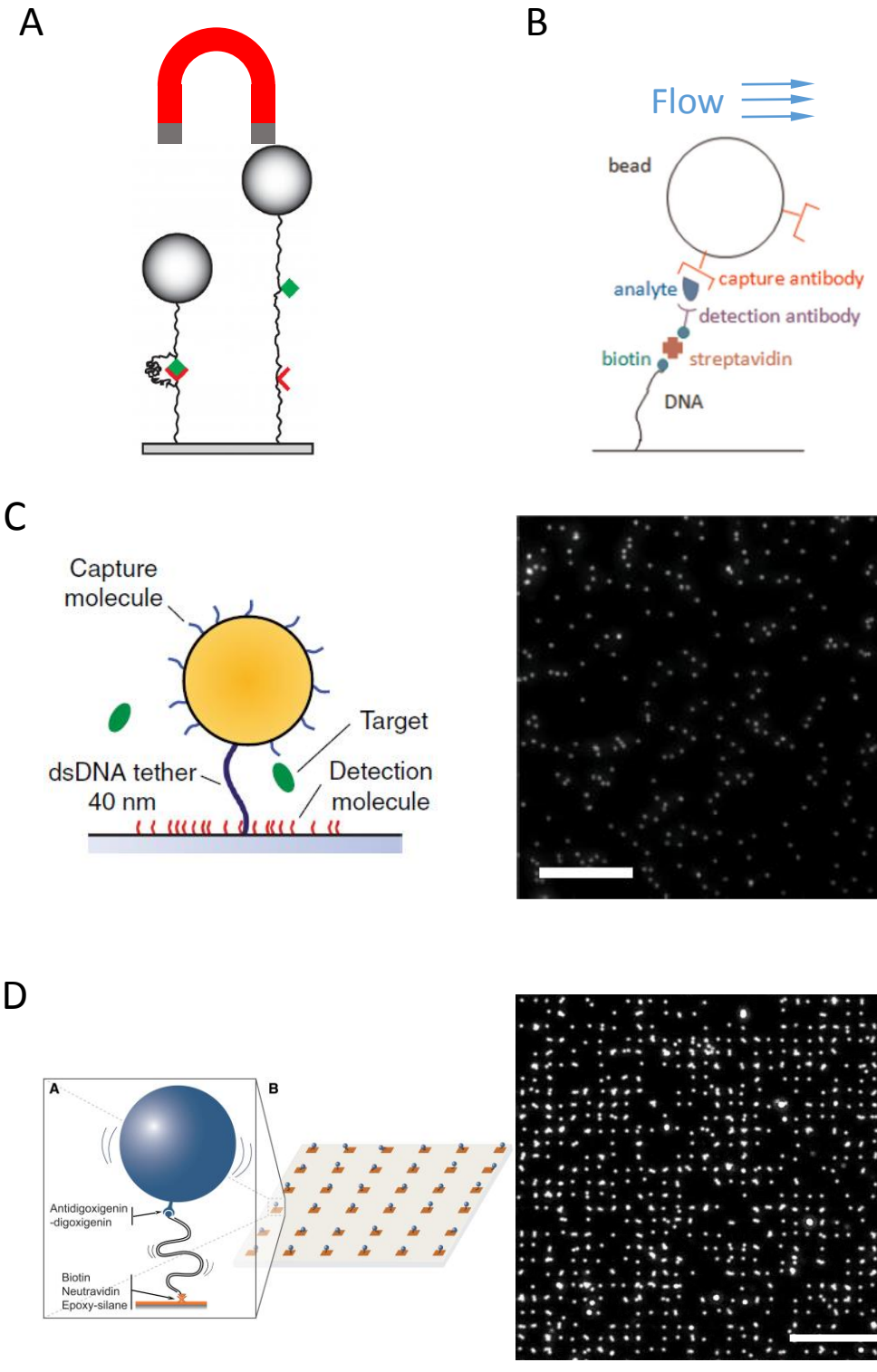
Glass coverslip
Single-stranded
scaffold
+
Staples strands
DNA origami

Figure 2


A
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Flow
bead
analyte
capture antibody
detection antibody
biotin
streptavidin
DNA
C
Capture molecule
Target
dsDNA tether 40 nm
Detection molecule
D
A
B
Antidigoxigenin -digoxigenin
Biotin
Neutravidin
Epoxy-silane

Figure 3