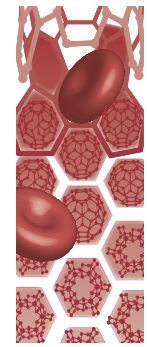
Editorial

Nanomedicine



Nature-inspired DNA switches: applications in medicine

"With their ability to bind and respond to a variety of specific molecular markers combined to their capacity to be adapted in a nanoswitch format that reports an easily measurable fluorescent signal, DNA switches can be transformed in efficient biosensors with numerous applications in fundamental research."

First draft submitted: 3 October 2016; Accepted for publication: 17 October 2016; Published online: 12 January 2017

Keywords: biosensors • DNA switches • drug delivery • fluorescent-guided surgery • point-of-care molecular diagnosis

Arnaud Desrosiers
Laboratory of Biosensors & Nanomachines, Département de Chimie, Université de Montréal, Québec, Canada
and
Département de Biochimie et Médecine Moléculaire, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec H3C 3J7, Canada

Alexis Vallée-Bélisle
Author for correspondence:
Laboratory of Biosensors & Nanomachines, Département de Chimie, Université de Montréal, Québec, Canada
and
Département de Biochimie et Médecine Moléculaire, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec H3C 3J7, Canada
a.vallee-belisle@umontreal.ca

Life is built on biomolecular switches and most, if not all, diseases arise from dysfunctions of these switches. Biomolecular switches typically change their structure or conformation, and thus their activity, in response to various stimuli, including temperature, light, pH changes, small molecules or proteins, and translate these signals into sophisticated and specific biological outputs. A better understanding of these switches is crucial in the field of medicine. For example, studies on G protein–coupled receptors switches, a membrane protein family containing >800 identified members [1], has led to key advances in medicine with >50% of all modern drugs targeting G protein–coupled receptors [2]. In addition to studying natural molecular switches, dozens of research groups have also started to engineer artificial molecular switches with applications ranging from biosensing to guided surgery and drug delivery. In this perspective, we present a rapid overview of recently developed artificial molecular switches and focus our attention on the specific case of DNA switches for applications in medicine.

DNA switches

Biomolecular switches are molecules that change between two (or more) conformations (typically 'OFF' and 'ON') in response to specific molecular inputs (Figure 1, center). In living organisms, for example, most proteins contain a switching functionality that controls their activity in response to environmental or molecular changes. Artificial molecular switches, on the other hand, are typically programmed either to generate an easily measurable signal (e.g., fluorescent and electrochemical) or to trigger a precise activity including drug release in response to a specific molecular target.

Despite the fact that most natural biomolecular switches are made of proteins (and sometimes RNA), most recent advances in the field of artificial switches have been realized using DNA chemistry [3]. Traditionally, DNA has been mainly studied for its role as a genetic carrier, but since the early 1990, however, many research groups have started to explore the incredible programmability of DNA to build specific 3D structures [4,5] or to create molecules with novel binding [6] and catalytic activities [7]. There are mainly four reasons that render DNA an ideal material to build switches: first, their simple A–T and G–C base paring code allows us to rationally create a variety of nanostructures [8,9] and structure-switching mechanisms [10,11] with predictable thermodynamics [12]. Sec-

Future Medicine part of fsg

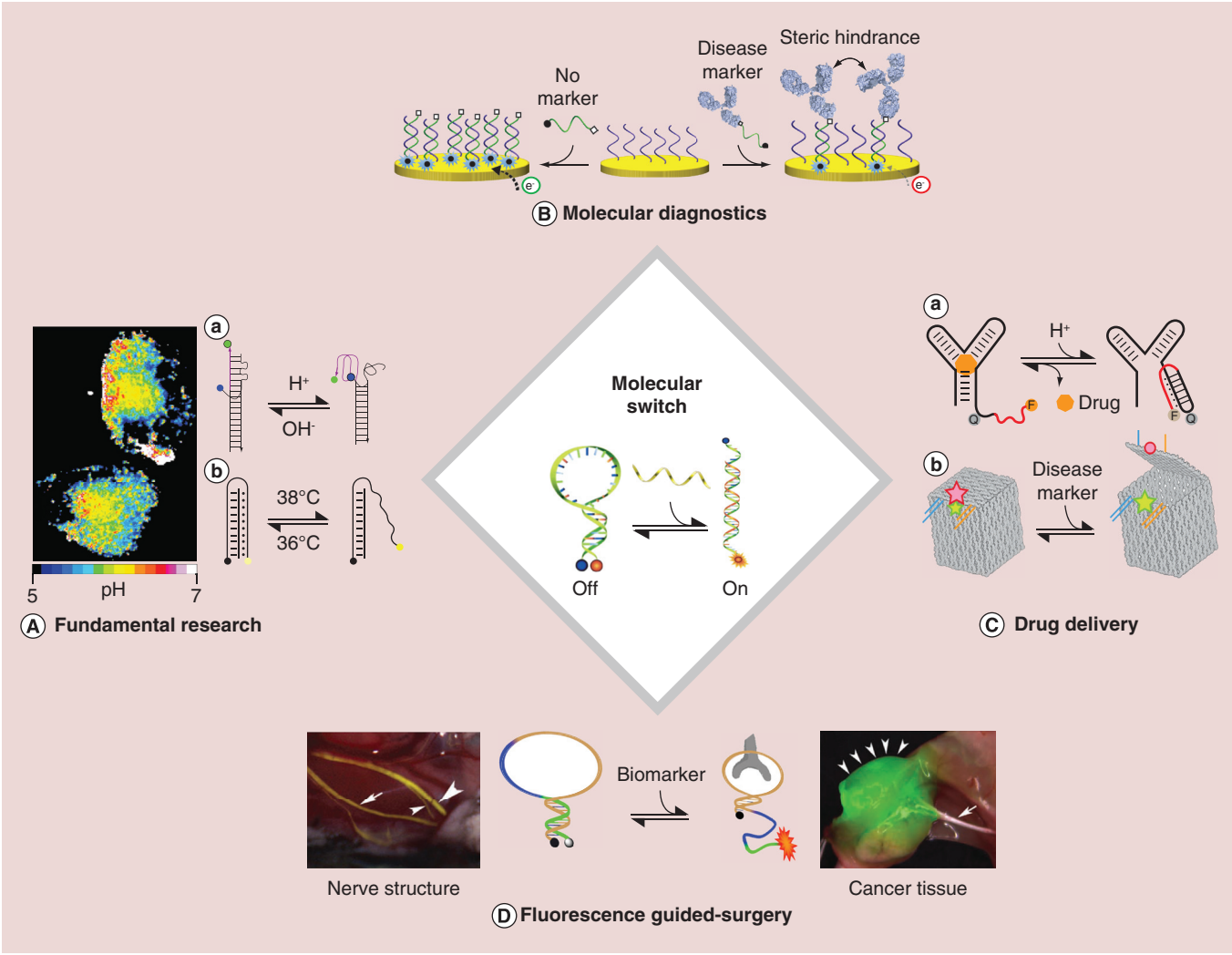


Figure 1. Various applications of DNA switches in medicine. **(Center)** A DNA switch (molecular beacon) that opens up and fluoresces in presence of a specific DNA sequence [26]. **(Aa)** DNA i-motif adapted into a fluorescent pH-meter allows us to map pH inside living cells (adapted with permission from [22] © MacMillan Publishers Ltd. *[Nat. Nanotechnol.]* [2013]). **(Ab)** DNA triplex nanothermometers can monitor temperature variations down to 0.05°C [13]. **(B)** DNA-based diagnostic devices that use steric hindrance to detect large protein biomarkers [33]. **(Ca)** pH-triggered, aptamer-based drug releasing nanomachine (adapted with permission from [35] © 2015 ACS); **(Cb)** DNA boxes can be triggered to release drugs in response to specific and localized molecular signals (adapted with permission from [36] © MacMillan Publishers Ltd. [*Nature*] [2009]). **(D)** Activatable fluorescent DNA switches [44] could be used to highlight anatomical structures, such as nerves and cancer tissue (reprinted with permission from [39] © Macmillan Publishers Ltd. [*Nat. Biotechnol.*] [2012]).

ond, DNA sequences may specifically bind or respond to a whole variety of molecular inputs, including temperature [13], pH [14], ions [15], small molecules [16] and proteins [17]. In addition to many DNA structures that have evolved naturally to bind specific molecular targets (e.g., transcription factors), thousands of artificially evolved and selected DNA sequences have been identified by systematic evolution of ligands by exponential enrichment methods to bind almost any molecular target [18]. Third, artificial synthesis of DNA is simple and inexpensive (US$0.05–0.15 per nucleotide) and can support a myriad of modified nucleotides and functional moieties [19]. Finally, DNA switches only measure a few nanometers, are readily life compatible and require very little modifications to escape the immunological response [20].

Applications in fundamental research

With their ability to bind and respond to a variety of specific molecular markers combined to their capacity to be adapted in a nanoswitch format that reports an easily measurable fluorescent signal, DNA switches can be transformed in efficient biosensors with numerous applications in fundamental research. For instance, DNA switches based on i-motif [21] or triplex DNA structures [14] have been adapted into nanoscale pH-fluorescent sensors and used to monitor pH variations inside living cells (Figure 1A, panel a) [22]. Using such

pH sensors, for example, Krishnan and colleagues have provided in real time new molecular insights on the mechanism of Brefeldin A, an antibiotic known to cause extensive tubulation of the *trans* Golgi network [22]. Programmable DNA switches based on stem-loops [13,23] and DNA triplex structures [13] have also been recently utilized to monitor temperature variations in real time at the nanoscale (Figure 1A, panel b). These fluorescent nanothermometers hold much promises for temperature sensing inside or outside living cells, which could improve our knowledge on various pathologies involving metabolism deregulations, such as cancer [24]. Other fluorescent DNA switches targeting mRNA [25], transcription factors [17] or any other molecules (through the use of aptamer-based switches) are likely to provide new molecular insights into the real-time functions of healthy and deregulated cells.

Molecular diagnostics

DNA switches also represent a material of choice to build medical diagnostic devices. One of the earliest examples of diagnostic technology based on DNA switches came from Tyagi and Kramer who developed a fluorescent stem-loop DNA that opens up and fluoresces in presence of a specific DNA sequence (Figure 1, center) [26]. This DNA sensor is routinely employed in many diagnostic facilities for the rapid identification of single-nucleotide polymorphism and the diagnostic of various diseases including viral and bacterial infections using real-time PCR techniques [27]. Fluorescence output, however, is not ideally suited for marker detection in complex biological matrix, such as whole blood (which usually absorbs or diffracts light) and typically remains relatively expensive for adaptation in cheap point-of-care formats. To overcome these limitations, DNA switches can also be adapted into an electrochemical format which translates DNA conformational changes into electrochemical outputs by varying the distance between a redox element and a conducting surface, such as gold electrodes [28–30]. Using this approach, Soh, Plaxco and colleagues have recently developed a potentially universal platform named MEDIC that uses electrochemically adapted aptamers for real time drug monitoring applications [31]. This instrument could, for example, drastically improve the treatment of various conditions by enabling real-time dosage of toxic drugs to their optimal therapeutic concentrations [31]. Similar electrochemical antibody-activated DNA switches have also been developed for the rapid detection of antibodies directly in whole blood in <10 min [32]. More recently, DNA-based sensors that exploit steric hindrance generated by the binding of large protein markers to small peptide epitopes attached on redox-active DNA strands are also being developed for the rapid detection of protein markers directly in whole blood (Figure 1B) [33].

Drug delivery

With their ability to specifically bind to a variety of therapeutic drugs, DNA molecules have been increasingly used as drug transporters that may, in some cases, specifically unload their cargo at desired tissue locations in response to specific disease markers. Tan and colleagues, for example, have designed DNA 'nanotrains' for the targeted transporting of doxorubicin to neoplastic tissue using tissue-specific DNA aptamers [34]. Although this mechanism enables enrichment of the drug in the vicinity of a specific tissue, it does not, however, facilitate drug release nor does it preclude drug release in other unspecific tissue locations. To overcome this, Ricci and colleagues have recently developed binding-induced aptamer-based drug delivery nanomachines that unload their payload in response to more acidic pH, a common biomarker of tumor environment (Figure 1C, panel a) [35]. Other research groups have also built 3D DNA structures (DNA origami [4,5]) to sequester drugs in DNA boxes [36] or DNA cubes [37]. These drug-filled DNA containers can then be opened via specific DNA switches that respond to specific disease markers. Using this approach, Church and colleagues, for example, have created DNA-based drug containers that open up only in the presence of two specific biomarkers (Figure 1C, panel b) [38].

> "Biomolecular switches are molecules that change between two (or more) conformations (typically 'OFF' and 'ON') in response to specific molecular inputs."

Fluorescence-guided surgery

Surgery, one of the most ancient medical specialities, represents a promising field of application for DNA switches. More specifically, fluorescence-guided surgery, in which fluorescent probes are employed to highlight specific anatomical structures (Figure 1D) [39,40], has recently emerged as a powerful approach to reduce cancer recurrence in mouse [41] and was tested with freshly removed human bladder cancer [42]. Current fluorescent probes, however, such as fluorescent-labeled tissue-binding peptides developed by Tsien and colleagues, only display contrast enhancements *in vivo* of typically threefold due to the high background of these 'always on' probes [43]. DNA switches, on the other hand, can be engineered to adopt a fluorescent 'OFF' state, which is reversed to a fluorescent 'ON' state upon binding to specific tissue markers (Figure 1D). Tan and colleagues, for example, have developed an aptamer-based fluorescent switch that, upon binding to protein tyrosine kinase-7, a well-characterized cancer marker,

10.2217/nnm-2016-0349

changes its structure and separate a fluorophore–quencher pair leading to dramatic contrast enhancement [44]. Similar fluorescent DNA switches could, in principle, be adapted for the detection of virtually any molecular marker for tissue imaging.

Future perspective

DNA switches are expected to have a great impact on the field of medicine. On one hand, DNA switches will provide physicians with a deeper understanding of molecular mechanisms underlying a variety of diseases. DNA-based inexpensive, point-of-care, diagnostic tests may also become routinely accessible for patients in physician's offices or even at home, thus increasing testing frequency and also providing new accessible diagnostic possibilities in developing countries. DNA switches may also contribute to optimize the efficiency of many drugs through novel drug delivery systems, as well as enabling high-resolution fluorescent-guided surgery, which could drastically reduce cancer recurrence. While new mechanisms and applications of DNA switches for medicine are growing at an increasing rate, more efforts, however, will be needed to translate these novel promising technologies into useful tools for practicing physicians.

Acknowledgements

The authors would like to thank L Pedro for useful comments on this manuscript.

Financial & competing interests disclosure

This work was supported by the National Sciences and Engineering Research Council of Canada through grant no. 2014-06403 (NSERC; to A Vallée-Bélisle). A Vallée-Bélisle holds the Canada Research Chair in Bioengineering & Bio-nanotechnology Tier II, A Desrosiers is a Fonds Nature et Technologie fellow. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Lagerström MC, Schiöth HB. Structural diversity of G protein coupled receptors and significance for drug discovery. *Nat. Rev. Drug. Discov.* 7(4), 339–357 (2008).

2 Zhang R, Xie X. Tools for GPCR drug discovery. *Acta Pharmacol. Sin.* 33(3), 372–384 (2012).

3 Seeman NC. DNA in a material world. *Nature* 421(6921), 427–431 (2003).

4 Rothemund PW. Folding DNA to create nanoscale shapes and patterns. *Nature* 440(7082), 297–302 (2006).

5 Chen JH, Seeman NC. Synthesis from DNA of a molecule with the connectivity of a cube. *Nature* 350(6319), 631–633 (1991).

6 Elbaz J, Lioubashevski O, Wang F, Remacle F, Levine RD, Willner I. DNA computing circuits using libraries of DNAzyme subunits. *Nat. Nanotechnol.* 5(6), 417–422 (2010).

7 Ellington AD, Szostak JW. Selection *in vitro* of single-stranded DNA molecules that fold into specific ligand-binding structures. *Nature* 355(6363), 850–852 (1992).

8 Veneziano R, Ratanalert S, Zhang K *et al.* Designer nanoscale DNA assemblies programmed from the top down. *Science* 352(6293), 1534 (2016).

9 Jones MR, Seeman NC, Mirkin CA. Programmable materials and the nature of the DNA bond. *Science* 347(6224), 1260901 (2015).

10 Nutiu R, Li Y. Structure-switching signaling aptamers. *J. Am. Chem. Soc.* 125(16), 4771–4778 (2003).

11 Vallée-Bélisle A, Plaxco KW. Structure-switching biosensors: inspired by Nature. *Curr. Opin. Struct. Biol.* 20(4), 518–526 (2010).

12 Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31(13), 3406–3415 (2003).

13 Gareau D, Desrosiers A, Vallée-Bélisle A. Programmable quantitative DNA nanothermometers. *Nano Lett.* 16(7), 3976–3981 (2016).

14 Idili A, Vallée-Bélisle A, Ricci F. Programmable pH-triggered DNA nanoswitches. *J. Am. Chem. Soc.* 136(16), 5836–5839 (2014).

15 Li L, Li B, Qi Y, Jin Y. Label-free aptamer-based colorimetric detection of mercury ions in aqueous media using unmodified gold nanoparticles as colorimetric probe. *Anal. Bioanal. Chem.* 393(8), 2051–2057 (2009).

16 Famulok M. Oligonucleotide aptamers that recognize small molecules. *Curr. Opin, Struct. Biol.* 9(3), 324–329 (1999).

17 Vallée-Bélisle A, Bonham AJ, Reich NO, Ricci F, Plaxco KW. Transcription factor beacons for the quantitative detection of DNA binding activity. *J. Am. Chem. Soc.* 133(35), 13836–13839 (2011).

18 Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249(4968), 505–510 (1990).

19 Kosuri S, Church GM. Large-scale de novo DNA synthesis: technologies and applications. *Nat. Methods.* 11(5), 499–507 (2014).

20 Surana S, Shenoy AR, Krishnan Y. Designing DNA nanodevices for compatibility with the immune system of higher organisms. *Nat. Nanotechnol.* 10(9), 741–747 (2015).

21 Lannes L, Halder S, Krishnan Y. Tuning the pH response of i-motif DNA oligonucleotides. *Chembiochem* 16(11), 1647–1656 (2015).

22 Modi S, Nizak C, Surana S, Halder S, Krishnan Y. Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell. *Nat. Nanotechnol.* 8(6), 459–467 (2013).

23 Ke G, Wang C, Ge Y, Zheng N, Zhu Z, Yang CJ. L-DNA molecular beacon: a safe, stable, and accurate intracellular nano-thermometer for temperature sensing in living cells. *J. Am. Chem. Soc.* 134(46), 18908–18911 (2012).

24 Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. *Nat. Rev. Drug Discov.* 10(9), 671–684 (2011).

25 Bratu DP, Cha BJ, Mhlanga MM. Visualizing the distribution and transport of mRNAs in living cells. *Proc. Natl Acad. Sci. USA* 100(23), 13308–13313 (2003).

26 Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* 14(3), 303–308 (1996).

27 Chakravorty S, Aladegbami B, Burday M. Rapid universal identification of bacterial pathogens from clinical cultures by using a novel sloppy molecular beacon melting temperature signature technique. *J. Clin. Microbiol.* 48(1), 258–267 (2010).

28 Fan C, Plaxco KW, Heeger AJ. Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc. Natl Acad. Sci. USA* 100(16), 9134–9137 (2003).

29 Lubin AA, Plaxco KW. Folding-based electrochemical biosensors: the case for responsive nucleic acid architectures. *Acc. Chem. Res.* 43(4), 496–505 (2010).

30 Labib M, Sargent EH, Kelley SO. Electrochemical methods for the analysis of clinically relevant biomolecules. *Chem. Rev.* 116(16), 9001–9090 (2016).

31 Ferguson BS, Hoggarth DA, Maliniak D *et al.* Real-time, aptamer-based tracking of circulating therapeutic agents in living animals. *Sci. Transl. Med.* 5(213), 213ra165 (2013).

32 Vallée-Bélisle A, Ricci F, Uzawa T, Xia F, Plaxco KW. Bioelectrochemical switches for the quantitative detection of antibodies directly in whole blood. *J. Am. Chem. Soc.* 134(137), 15197–15200 (2012).

33 Mahshid SS, Camiré S, Ricci F, Vallée-Bélisle A. A highly selective electrochemical DNA-based sensor that employs steric hindrance effects to detect proteins directly in whole blood. *J. Am. Chem. Soc.* 137(50), 15596–15599 (2015).

34 Zhu G, Zheng J, Song E *et al.* Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. *Proc. Natl Acad. Sci. USA* 110(20), 7998–8003 (2013).

35 Porchetta A, Idili A, Vallée-Bélisle A, Ricci F. General strategy to introduce pH-induced allostery in DNA-based receptors to achieve controlled release of ligands. *Nano Lett.* 15(7), 4467–4471 (2015).

36 Andersen ES, Dong M, Nielsen MM *et al.* Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* 459(7243), 73–76 (2009).

37 Edwardson TG, Carneiro KM, McLaughlin CK, Serpell CJ, Sleiman HF. Site-specific positioning of dendritic alkyl chains on DNA cages enables their geometry-dependent self-assembly. *Nat. Chem.* 5(10), 868–875 (2013).

38 Douglas SM, Bachelet I, Church GM. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* 335(6070), 831–834 (2012).

39 Whitney MA, Crisp JL, Nguyen LT *et al.* Fluorescent peptides highlight peripheral nerves during surgery in mice. *Nat. Biotechnol.* 29(4), 352–356 (2011).

40 Nguyen QT, Tsien RY. Fluorescence-guided surgery with live molecular navigation--a new cutting edge. *Nat. Rev. Cancer* 13(9), 653–662 (2013).

41 Nguyen QT, Olson ES, Aguilera TA *et al.* Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides decreases residual cancer and improves survival. *Proc. Natl Acad. Sci. USA* 107(9), 4317, 4322 (2010).

42 Pan Y, Volkmer JP, Mach KE *et al.* Endoscopic molecular imaging of human bladder cancer using a CD47 antibody. *Sci. Transl. Med.* 6(260), 260ra148 (2014).

43 Jiang T, Olson ES, Nguyen QT, Roy M, Jennings PA, Tsien RY. Tumor imaging by means of proteolytic activation of cell-penetrating peptides. *Proc. Natl Acad. Sci. USA* 101(51), 17867–17872 (2004).

44 Shi H, He X, Wang K *et al.* Activatable aptamer probe for contrast-enhanced *in vivo* cancer imaging based on cell membrane protein-triggered conformation alteration. *Proc. Natl Acad. Sci. USA* 108(10), 3900–3905 (2011).