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Surface-immobilized DNAzyme-type biocatalysis

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The structure of the double helix of deoxyribonucleic acid (DNA, also called duplex-DNA) was elucidated sixty years ago by Watson, Crick, Wilkins and Franklin. Since then, DNA has continued to hold a fascination for researchers in diverse fields including medicine and nanobiotechnology. Nature has indeed excelled in diversifying the use of DNA: beyond its canonical role of repository of genetic information, DNA could also act as a nanofactory able to perform some complex catalytic tasks in an enzyme-mimicking manner. The catalytic capability of DNA was termed DNAzyme; in this context, a peculiar DNA structure, a quadruple helix also named quadruplex-DNA, has recently garnered considerable interest since its autonomous catalytic proficiency relies on its higher-order folding that makes it suitable to interact efficiently with hemin, a natural cofactor of many enzymes. Quadruplexes have thus been widely studied for their hemoprotein-like properties, chiefly peroxidase-like activity, *i.e.*, their ability to perform hemin-mediated catalytic oxidation reactions. Recent literature is replete with applications of quadruplex-based peroxidase-mimicking DNAzyme systems. Herein, we take a further leap along the road to biochemical applications, assessing the actual efficiency of catalytic quadruplexes for the detection of picomolar levels of surface-bound analytes in an enzyme-linked immunosorbent (ELISA)-type assay. To this end, we exploit an innovative strategy based on the functionalization of DNA by a multitasking platform named RAFT (for *regioselectivity addressable functionalized template*), whose versatility enables the grafting of DNA whatever its nature (duplex-DNA, quadruplex-DNA, *etc.*). We demonstrate that the resulting biotinylated RAFT/quadruplex systems indeed acquire catalytic properties that allow for efficient luminescent detection of picomoles of surface-bound streptavidin. We also highlight some of the pitfalls that have to be faced during optimization, notably demonstrating that highly optimized experimental conditions can make DNA pre-catalysts catalytically competent whatever their secondary structures.

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Introduction

The "RNA world" hypothesis, formally articulated in 1986 by W. Gilbert, posits that a common misconception was to consider that ribonucleic acid (RNA) had an accessory role only in the evolutionary process.[1] RNA is not merely the molecular link between DNA (the repository of the genetic information) and proteins (which catalyze complex tasks). Instead, RNA probably preceded both DNA and enzymes in the evolution, thriving on the challenge of exhibiting both informational and catalytic – so-called ribozyme – properties, the former to hold genetic information and ensure an exact mother-to-daughter transmission, the latter to ensure self-replication *via* enzyme-mimicking copying and self-reproducing processes. Thus, DNA probably appeared after RNA and was subsequently selected as the privileged recipient of the genetic information thanks to its error-correcting double-stranded nature and intrinsic stability. In the evolution timescale, DNA thus somewhat unfairly overshadowed RNA. The basis for this conclusion was uncompromisingly sound, but the question of the catalytic capabilities of DNA was a bit too quietly shelved since less than a decade after, Breaker & Joyce demonstrated that DNA is not solely holding the genetic information but could be enzymatically proficient as well.[2] The enzyme-like activity of DNA – termed deoxyribozyme or DNAzyme – was demonstrated through the study of the RNA-cleaving activity of a DNA strand included in a hybrid DNA/RNA duplex. Later on, the scope of the DNAzyme biocatalysis was further extended by Li & Sen, *via* the demonstration that a single-stranded DNA can acquire enzymatic activity when it serves as an aptamer for hemin,[3] a known cofactor of many hemoprotein enzymes (including peroxidases). The higher-order folding of this G-rich aptamer, established as a quadruplex structure,[4] was found to offer a privileged binding site to hemin (one of its external G-quartets); upon addition of a stoichiometric oxidant (H_2O_2), the hemin/quadruplex complex promotes peroxidase-like oxidation reactions, notably the easily

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monitored oxidation of chromogenic substrates. Dozens of new examples of quadruplex-based DNAzymes are now reported each year,[5] thereby giving credit to the possibility of a "DNA world" echoing Gilbert's "RNA world".

In the present article, we wish to fathom precisely the ins and outs of the quadruplex-based DNAzyme, trying to answer the yet simple but eminently important following questions: what is the actual efficiency of quadruplex-based DNAzyme? In terms of sensitivity? DNA detection threshold? DNA structure selectivity? Could they be efficient enough to detect picomoles of analytes (as usually required by routine biochemical assays)? Practically convenient enough for the detection of immobilized analytes?, *etc.* The conclusions drawn from the reported results unambiguously indicate that DNA-based pre-catalysts indeed yield to proficient biocatalytic systems (providing a reliable catalytic response in the picomolar range, in both solution and under surface-immobilized conditions), which might also be subjected to caution since the optimized employed experimental conditions make DNA pre-catalysts catalytically competent whatever their secondary structures. We also demonstrated the interest of a DNA functionalization strategy by a RAFT (for *regioselectivity addressable functionalized template*) molecular platform, which enables the implementation of catalytic DNA experiments whatever the nature of the oligonucleotide (duplex-DNA, intra- and inter-molecular quadruplexes, *etc.*), in a fully comparable manner. Finally, we demonstrate the real potential of RAFT-based biotinylated quadruplex/hemin catalytic complexes that allow for efficient and reliable luminescent detection of picomoles of surface-bound streptavidin, according to a convenient, short and easy-to-use ELISA-inspired catalytic protocol.

Experimental section

UV-Vis experiments were carried out in a 96-well plate with a ThermoScientific Multiskan GO microplate spectrophotometer. RAFT-free oligonucleotides were purchased from Eurogentec (Belgium) in OligoGold® purity grade at ~200 nmol scale (purified by RP-HPLC). RAFT-oligonucleotides were synthesized according to previously reported procedures (see ref. 6). Hemin, TMB (3,3′,5,5′-tetramethylbenzidine) and SigmaScreen™ Streptavidin High Capacity coated 96-well Plate were purchased from Sigma-Aldrich; all the chemicals were used without further purification.

Sequences were as follows (in the 5′-to-3′ direction): (a) duplex-DNA: *hp*-duplex: $CGCGCGCGT_4CGCGCGCG$; ds12: $CGCGA_2T_2CGCG$ (self-complementary); ds17: C_2AGT_2CGTAG-TA_2C_3/$G_3T_2ACTACGA_2CTG_2$; ds26: $CA_2TCG_2ATCGA_2T_2C$-$GATC_2GAT_2G$ (self-complementary); (b) quadruplex-DNA (or G-quadruplex, GQ): *intra*-GQ: $G_3T_2AG_3T_2AG_3T_2AG_3T_2$; *inter*-GQ: $(T_2AG_3T)_4$; (c) RAFT-oligonucleotides: RAFT-*intra*-GQ: $G_3T_2AG_3$-$T_2AG_3T_2AG_3T_2$-RAFT; RAFT-*inter*-GQ: $(T_2AG_3T)_4$-RAFT′; RAFT-*hp*-duplex: $CGCGCGCGT_4CGCGCGCG$-RAFT, with RAFT: Biotine-c [Lys-Ala-Pro-Gly-Ala-Ala-Lys*-Pro-Gly-Ala] and RAFT′: Biotine-c [Lys-Lys*-Pro-Gly-Lys*-Ala-Lys*-Pro-Gly-Lys*] (* indicates the amino acids on which the oligonucleotides are anchored *via* oxime bond formation; see ref. 6). All DNA structures were prepared in a Caco.K buffer, comprised of 10 mM lithium cacodylate buffer (pH 7.2) plus 10 mM KCl/90 mM LiCl. The final concentrations were theoretically 250, 125 and 83.3 μM (expressed in motif concentration) for mono-, bi- and tetramolecular DNA structures, respectively. The actual concentration of each DNA was determined according to the nearest-neighbor model, *via* dilution to 1 μM theoretical concentration (expressed in motif concentration) for monomolecular and bimolecular structures, and to 0.2 μM for tetramolecular structure, through UV spectral analysis at 260 nm (after 5 min at 90 °C) with the molar extinction coefficients provided by the manufacturer. High-order DNA structures (with or without RAFT) were folded according to two procedures: (a) for the monomolecular architectures, solutions were heated (90 °C, 5 min), cooled on ice (7 h) and then stored at least overnight (4 °C); (b) for the folding of all other structures, the solutions were heated (90 °C, 5 min), gradually cooled (over 6 hours) to 25 °C and then stored at least overnight (4 °C).

All the experiments were carried out at 25 °C with Caco.KTD buffer, comprised of 10 mM lithium cacodylate buffer (pH 4.4 for most of the investigations, *vide infra*) plus 10 mM KCl/90 mM LiCl, 0.05% Triton X-100 and 0.1% dimethylsulfoxide (DMSO). Stock solutions varied as a function of the experiments, between 2 and 100 mM (in DMSO) of TMB and 0.2 to 600 mM (in H_2O) of H_2O_2 for optimizing TMB and H_2O_2 concentrations, respectively. Stock solution of hemin was 100 μM in DMSO and of RAFT-*intra*-GQ (used for optimization) was 20 nM in Caco.KTD (pH 4.4).

Experimental conditions for: (a) pH optimization: RAFT-*intra*-GQ (10 nM), TMB (0.25 mM), hemin (1 μM) and H_2O_2 (6 mM) in Caco.KTD buffer at pH 3.48, 3.99, 4.41, 5.03, 5.57, 6.01, 6.49, 7.07, 8.00 and 10.01; (b) for TMB optimization: RAFT-*intra*-GQ (10 nM), TMB (0.02, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50 and 0.60 mM), hemin (1 μM) and H_2O_2 (6 mM) in Caco.KTD buffer at pH 4.4; (c) H_2O_2 optimization: RAFT-*intra*-GQ (10 nM), TMB (0.4 mM), hemin (1 μM) and H_2O_2 (0.02, 0.06, 0.20; 0.60, 2, 6, 20 and 60 mM) in Caco.KTD buffer at pH 4.4; (d) DNA detection threshold: RAFT-*intra*-GQ (0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 2, 5, 7 and 10 nM), TMB (0.4 mM), hemin (1 μM) and H_2O_2 (2 mM) in Caco.KTD buffer at pH 4.4; (e) the evaluation of the RAFT influence: oligonucleotides (RAFT-*intra*-GQ, *intra*-GQ-*biot* and *intra*-GQ, 10 nM), TMB (0.4 mM), hemin (1 μM) and H_2O_2 (2 mM) in Caco.KTD buffer at pH 4.4; (f) of the DNA nature: oligonucleotides (RAFT-*intra*-GQ, *intra*-GQ, RAFT-*inter*-GQ, *inter*-GQ, RAFT-*hp*-duplex and *hp*-duplex 10 nM), TMB (0.4 mM), hemin (1 μM) and H_2O_2 (2 mM) in Caco.KTD buffer at pH 4.4; (g) of the duplex-DNA catalytic capability: oligonucleotides (*hp*-duplex, ds12, ds17 and ds26, 10 nM), TMB (0.4 mM), hemin (1 μM) and H_2O_2 (2 mM) in Caco.KTD buffer at pH 4.4; (h) the immobilized DNAzyme: oligonucleotides (RAFT-*intra*-GQ and *intra*-GQ, 10 nM), TMB (0.4 mM), hemin (1 μM) and H_2O_2 (2 mM) in Caco.KTD buffer (pH 4.4).

Data treatment: the characteristic UV-Vis signal of oxidized TMB (at 370 nm) was recorded every 2 min over 120 min, with 20s shaking sequence between each point, and was plotted as a function of time with OriginPro® 8 software (OriginLab Corp. Northampton, MA, USA). Raw data were first subtracted of the

control experiment (carried out under the same conditions but without pre-catalyst) and subsequently zeroed at their very first point for the sake of comparison.

Results and discussion

To gain insight into the actual applicability of quadruplex-based DNAzyme biocatalysis, an array of representative DNAs were selected as pre-catalysts (Fig. 1A, complete sequences are given in the Experimental section): two quadruplexes of different nature (*i.e.*, a tetramolecular and a monomolecular quadruplex-DNA, termed *inter*-GQ and *intra*-GQ, respectively) as well as their immobilisable – biotinylated – counterparts (named RAFT-*inter*-GQ and RAFT-*intra*-GQ, respectively), along with several DNA controls, including a RAFT-free immobilisable monomolecular quadruplex (*intra*-GQ-*biot*), a hairpin duplex-DNA (*hp*-duplex) and its biotinylated analogue (RAFT-*hp*-duplex). The immobilization step being envisioned to be based on the classical high-affinity biotin/streptavidin association (Fig. 1), the macrocyclopeptidic RAFT was selected since this template uniquely offers the possibility of assembling DNA whatever their strandness (mono-molecular duplex-DNA and both mono- and tetra-molecular quadruplex-DNA) while exhibiting a biotin anchorage free of steric constraint on its other face.[6] This template ensures an identical DNA coating, in a totally DNA-structure independent manner, thus enabling a reliable comparison of the resulting DNAzyme-type biocatalyses, and affords also a higher stability to the templated quadruplex architectures.

The principle of the catalytic reaction studied herein as a quadruplex-based surface-bound DNAzyme model is schematically represented in Fig. 1B:[7] briefly, hemin (an iron(III)-porphyrin chloride) interacts with DNA through end-stacking interactions with the accessible G-quartet of the quadruplex architecture; once in its hydrophobic binding site, hemin reacts with hydrogen peroxide (H_2O_2) to provide an oxidized hemin (iron(IV)-oxo-porphyrin), which interacts with the substrate, herein 3,3′,5,5′-tetramethylbenzidine (TMB), concomitantly oxidizing it and returning to its iron(III) resting state (a complete description of the detailed DNAzyme catalytic cycle has been thoroughly discussed elsewhere).[8]

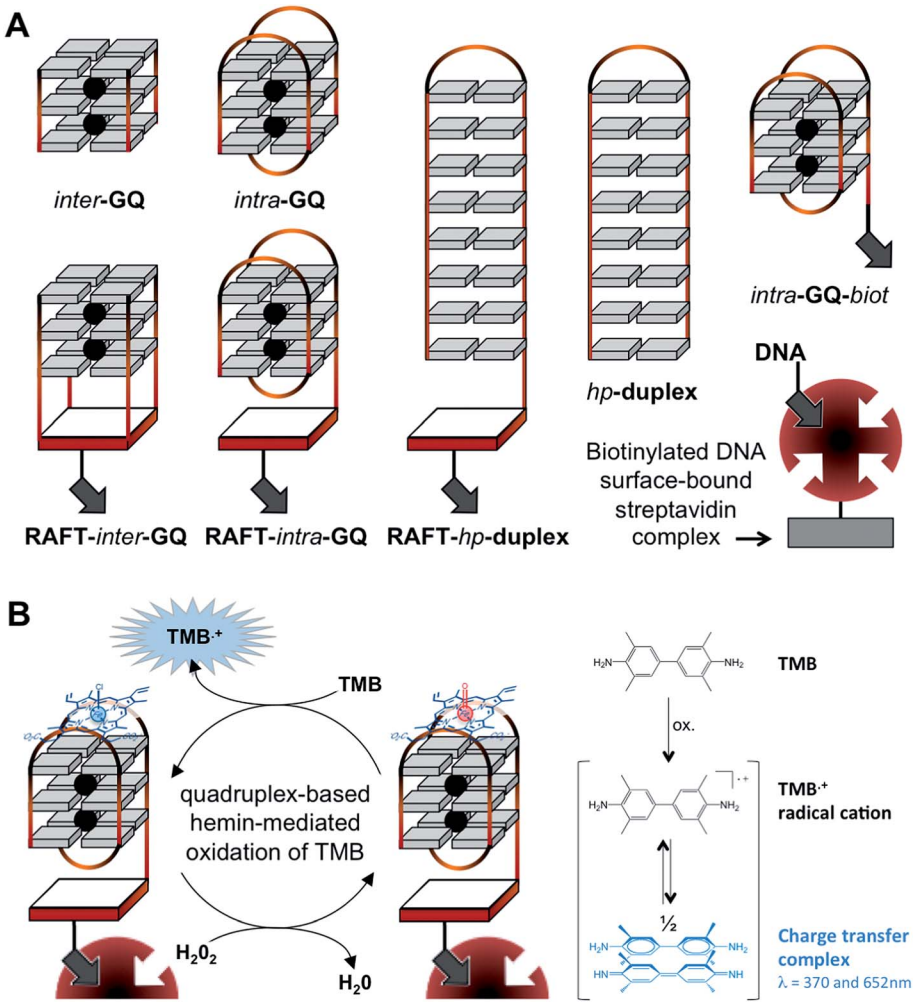

Fig. 1 (A) Schematic representation of DNA pre-catalysts used in the present study: DNA bases and RAFT template are shown as grey and red rectangular prisms respectively, DNA backbone as black-to-yellow lines, biotin as dark grey arrows and streptavidin as a red sphere. (B) Schematic representation of the quadruplex-based hemin-mediated DNAzyme catalysis and chemical structure of TMB.

Numerous experimental variables influence the catalysis efficiency,[9] including pH, DNA-precatalyst and H_2O_2 concentrations. Each of these parameters was first individually fine-tuned to determine the best possible experimental conditions to be implemented to assess the DNA detection threshold of the present assay. As seen in Fig. 2, the buffer's pH, the concentrations of TMB and H_2O_2 were sequentially adjusted on the basis of a previously reported quadruplex-based hemin-mediated catalytic protocol,[10] *i.e.*, 10 nM DNA (herein, RAFT-*intra*-GQ), 0.25 mM TMB, 6 mM H_2O_2, 1 μM hemin in 10 mM lithium cacodylate buffer pH 7.2 plus 90 mM KCl, 0.05% triton-X100 and 0.1% DMSO (known as Caco.KTD buffer). The catalytic capability of the resulting system was monitored through the appearance of a UV-Vis signal at 370 nm, characteristic of the charge transfer complex that results from the first TMB oxidation step (Fig. 1B).[11] Of note, this intermediate was selected since it provides a more sensitive response than both the final TMB oxidation product and the other classically implemented chromogenic probe 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS)), for short reaction times. Firstly, the impact of buffer's pH was evaluated, in the course of 2 hour experiments at 25 °C, from buffer pH 3 to 10: as seen in Fig. 2A, better catalytic performances were obtained for buffer pH below 5; the best signal-to-noise ratio (compared to experiments carried out without DNA pre-catalyst) being obtained for pH 4.4, this value was selected for subsequent experiments. Secondly, the influence of the concentration of TMB was investigated, from 0.02 to 0.6 mM, in the course of 2 hour experiments at 25 °C at pH 4.4: as seen in Fig. 2B, the relationship between catalytic performances and TMB concentration was straightforward (*i.e.*, the more, the better); however, precipitation occurred in experimental mixtures with TMB concentration higher than 0.6 mM, consequently 0.4 mM TMB was selected for subsequent experiments. Finally, the impact of the H_2O_2 concentration was studied, from 0.02 to 60 mM, in the course of 2 hour experiments at 25 °C at pH 4.4 with 0.4 mM TMB: as seen in Fig. 2C, the relationship between catalytic performances and H_2O_2 concentration was again straightforward (*i.e.*, the more, the better); however, the better signal-to-noise ratio (highlighted by the difference of performances between DNA-catalyzed and uncatalyzed reactions, orange and red bars, respectively) is obtained for concentrations below 6 mM, and the best compromise was found for 2 mM H_2O_2.

Altogether, these investigations enabled us to define the optimized catalytic conditions, comprised of 0.4 mM TMB and 2 mM H_2O_2 in Caco.KTD pH 4.4, for 2 hour experiments at 25 °C. It was thus of interest to assess the actual efficiency of this protocol, lowering the DNA pre-catalyst RAFT-*intra*-GQ concentration (from 10 nM to 20 pM), in order to delineate the lower limit of DNA detection of the assay. As seen in Fig. 2D, only catalytic mixtures with DNA concentrations higher than 0.2 nM display an efficiency whose error bars did not collide with that of the control experiments (carried out without DNA pre-catalyst, symbolized by a blue dotted line), thereby implying

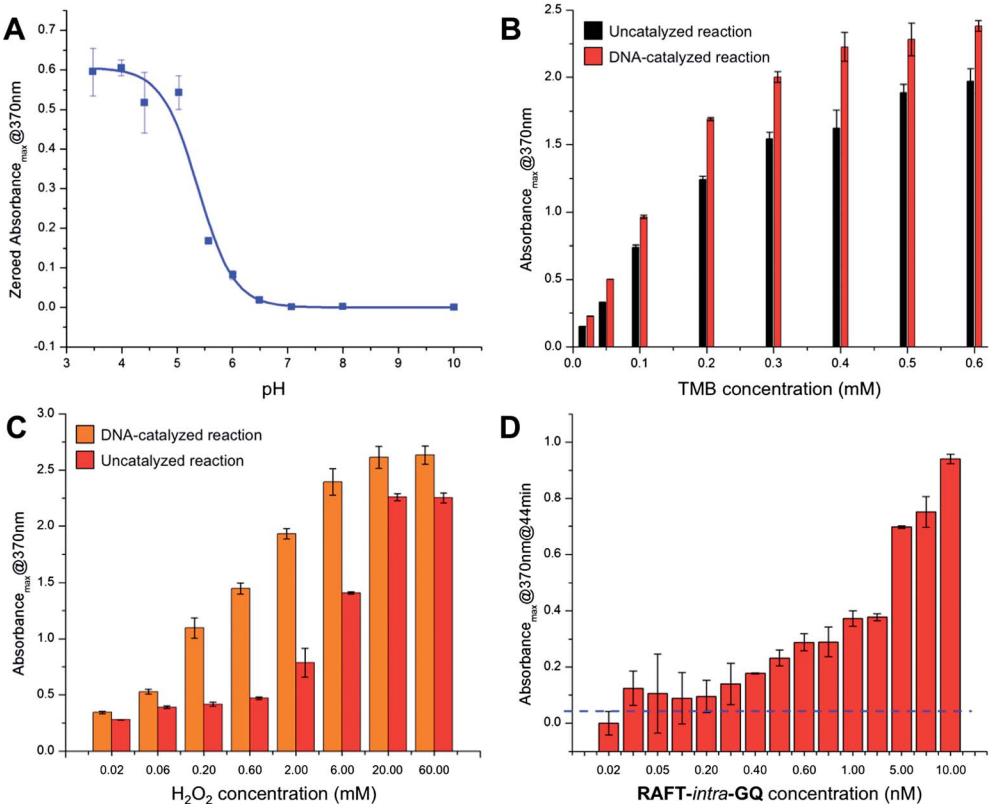

Fig. 2 TMB oxidation results for experiments carried out with RAFT-*intra*-GQ pre-catalyst as a function of the buffer's pH (A), then TMB (B) and H_2O_2 concentration (C). (D) Determination of the DNA detection limits of the present catalytic assay.

that the lower limit of the present catalytic system reliably lied in the picomolar range (*i.e.*, 200 pM DNA).

Before investigating the actual proficiency of these optimized catalytic conditions in surface-bound systems, the influence of the RAFT template was also precisely assessed. To this end, the catalytic capabilities of RAFT-*intra*-GQ and *intra*-GQ were compared, for experiments carried out with 10 nM DNA pre-catalyst, 0.4 mM TMB, 2 mM H_2O_2, 1 μM hemin in Caco.KTD buffer (pH 4.4). As seen in Fig. 3A, no noticeable differences were found between the enzyme-like capabilities of systems with DNA pre-catalysts with (brown bar) or without (orange bar) RAFT appendage. Biotin itself was not influential as well, as underlined by the slight difference between the catalytic properties of *intra*-GQ and *intra*-GQ-*biot* systems (orange and cherry bars, respectively). To further demonstrate this, three series of experiments were performed to compare the catalytic capabilities of systems with RAFT-based *versus* RAFT-free pre-catalysts, within each DNA series, *i.e.*, RAFT-*intra*-GQ *vs.* *intra*-GQ, RAFT-*inter*-GQ *vs.* *inter*-GQ and RAFT-*hp*-duplex *vs.* *hp*-duplex. Results shown in Fig. 3B clearly enlighten that in each instance the RAFT template did not affect the catalytic properties of the DNA. Interestingly, the catalytic efficiencies of RAFT-*intra*-GQ and RAFT-*inter*-GQ were not significantly different: the former was herein selected for subsequent experiments in light of its overall better performance but these results clearly indicate that the latter can also be considered as an interesting candidate for further studies since its preparation and folding is less sensitive to ionic (and temperature) conditions, making it a more robust catalytic system.[6] More surprising however was the observation that, under these conditions, quadruplex-based systems (*intra*-GQ and *inter*-GQ, with and without RAFT) were not markedly superior to the duplex-based system (*hp*-duplex, with and without RAFT). This was somewhat unexpected, but in agreement with a recent report from J. Wu *et al.* in which it was demonstrated than a manganese porphyrin/duplex-DNA complex could be indeed catalytically competent.[12] To gain further insight into this observation, we decided to investigate the catalytic performances of 4 different hemin/duplex complexes, with hemin interacting with either *hp*-duplex or three other random duplex-forming sequences ds12, ds17 and ds26, respectively comprised of 12, 17 and 26 base pairs. As seen in Fig. 3C, the four hemin/duplex-DNA systems were found to be catalytically efficient under these conditions: these results clearly emphasize that a great caution must be exercised in carrying out DNAzyme-type

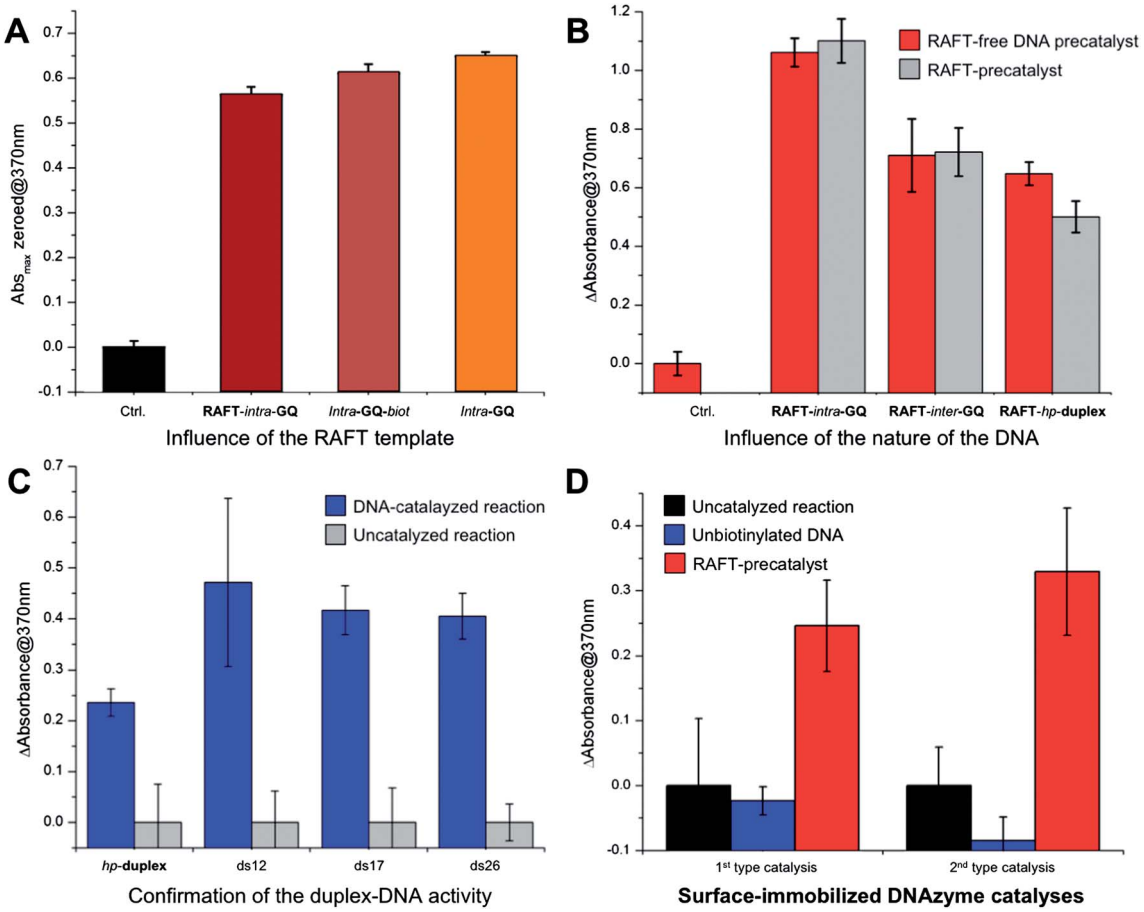

Fig. 3 TMB oxidation results for experiments carried out under various conditions to assess the influence of the presence of the RAFT template (A), of the nature of the DNA pre-catalyst (B) and the duplex-DNA catalytic efficiency (C). (D) Results of TMB oxidation for experiments carried out with surface-bound pre-catalysts.

biocatalyses since the quest of the best experimental conditions possible can be achieved at the expense of a firm DNA structure discrimination.

Finally, the applicability of the present DNAzyme systems under biochemically relevant conditions was assessed. The parent – and inspiring – catalytic system, horseradish peroxidase, was thoroughly used in biochemical applications, notably ELISA-type assays, owing to its ability to reliably detect very weak protein concentrations *via* strong amplification of the resulting luminescent signal. Routinely used ELISA kits enable a reliable detection of analytes (chiefly antibodies) in a typical $\mu g\ mL^{-1}$ to $ng\ mL^{-1}$, that is, nM to pM concentration range. We thus decided to use the RAFT-based catalytic systems described herein to detect streptavidin that is surface bound in a 96-well microplate. The biotin binding capacity of the commercially available used microplate being 300 pmol per well, we decided to use a default of RAFT-based pre-catalysts to avoid unspecific associations, lowering the DNA loading to 2 pmol per well. Given that the final volume of the experiments was 200 μL, this implied a 10 nM DNA concentration, fully in line with the aforementioned catalytic proficiency of the RAFT-*intra*-GQ system (Fig. 2D).

The first implemented protocol, schematically represented in Fig. 4, was comprised of 5 steps: (i) the DNA pre-catalyst (10 nM) was incubated in Caco.KTD buffer (pH 4.4) within coated wells, enabling the fixation of RAFT-based compounds only; (ii) washing steps (3 × 300 μL Caco.KTD, pH 7.2) were performed to remove all unbound material; (iii) hemin (1 μM) was thus incubated in Caco.KTD buffer (pH 4.4) and clung only to quadruplex-DNA (if present); (iv) washing steps (3 × 300 μL Caco.KTD, pH 7.2) were again performed to remove all unfixed hemin; (v) finally, the addition of both H_2O_2 (2 mM) and TMB (0.4 mM) in Caco.KTD buffer (pH 4.4) triggered the catalysis, *i.e.*, yielded to a luminescent signal. Two control experiments were also implemented, the first one without DNA pre-catalyst (upper line), the second one with a DNA pre-catalyst devoid of RAFT moiety (*i.e.*, *intra*-GQ, lower line). As seen in Fig. 3D (columns "1st type catalysis"), results obtained following this protocol were entirely satisfactory, since the luminescent signal detected in wells with a complete catalytic system (red bars) was far more intense than that of the control wells (black and blue bars).

These interesting results were further confirmed *via* an alternative, significantly shorter protocol (schematically represented in Fig. 5), since comprised of only 3 steps: (i) both the DNA pre-catalyst (10 nM) and hemin (1 μM) were incubated in Caco.KTD buffer (pH 4.4) within coated wells, thus enabling the fixation of RAFT-based DNA/hemin complexes only; (ii) washing steps (3 × 300 μL Caco.KTD, pH 7.2) were performed to remove all unbound material; (iii) finally, the addition of both H_2O_2 (2 mM) and TMB (0.4 mM) in Caco.KTD buffer (pH 4.4) triggered the catalysis. Results obtained according to this simplified protocol (Fig. 3D, columns "2nd type catalysis") were even more interesting than the previous ones, notably for what concerns the difference of performances between the RAFT-based and RAFT-free catalytic systems (RAFT-*intra*-GQ *vs.* *intra*-GQ, red and blue bars, respectively).

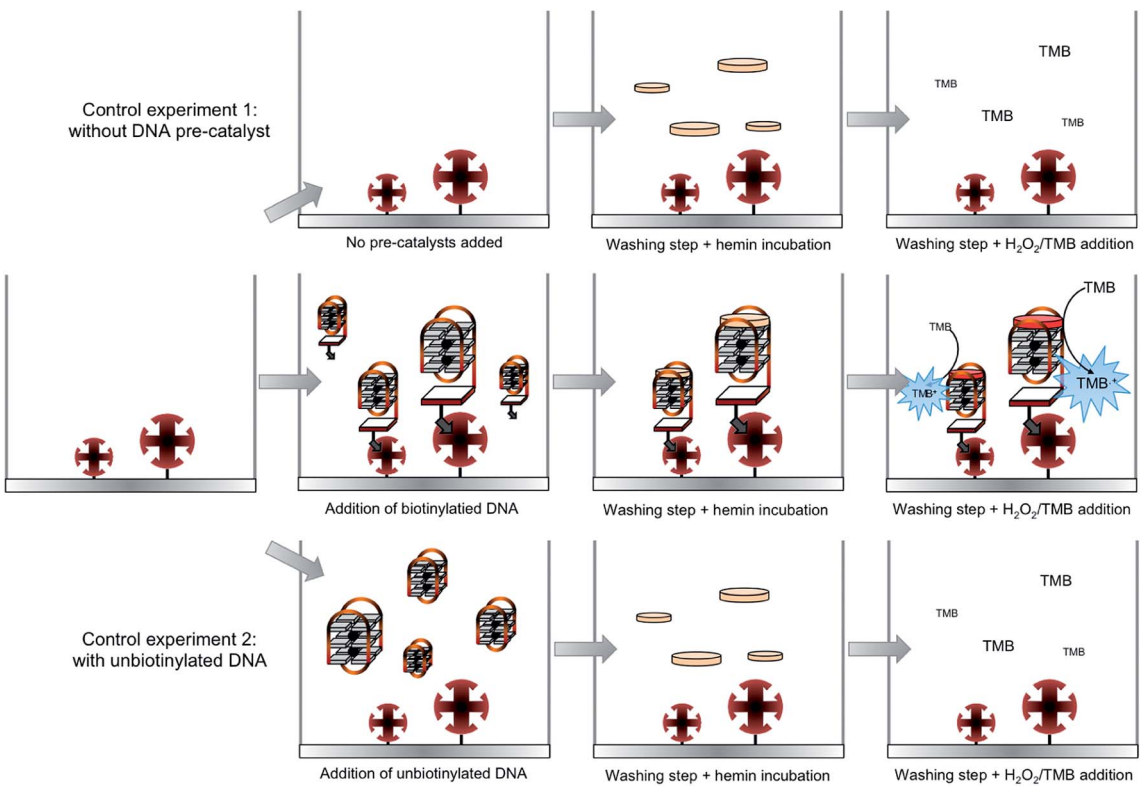

Fig. 4 Schematic representation of the 5-step protocol of surface-bound DNAzyme experiments.

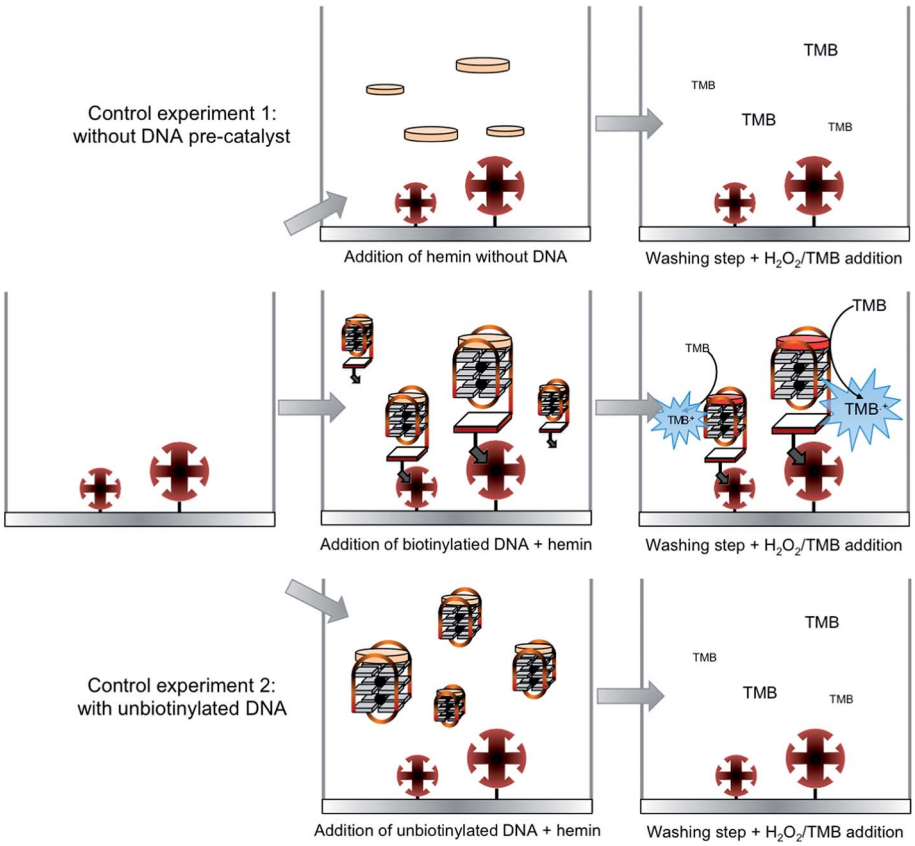

Fig. 5 Schematic representation of the 3-step protocol of surface-bound DNAzyme experiments.

Altogether, these results provide strong evidence that quadruplex-based DNAzyme-type biocatalysis can indeed be considered as a reliable component for the implementation of efficient biochemical assays. RAFT-based catalytic systems are readily immobilisable, without any loss of catalytic efficiency, making it one of the very first reported surface-bound quadruplex/hemin pre-catalytic systems.[13] The excellent signal-to-noise ratio reported herein for the luminescent detection of surface-bound streptavidin is also highly promising since it offers great scope of progress, notably in terms of detection limits.

Conclusions

Researchers evolving in the field of biocatalysis have become accustomed to the extremely rapid pace of progress in the development of DNA-based catalytic systems, and notably quadruplex-based DNAzymes. Dozens of new examples of quadruplex-mediated catalysis are now reported each year,[5] notably for applications as luminescent detection assays, including the detection of both monovalent (K^+,[14] or Ag^+)[15] or divalent cations (Cu^{2+},[16] Hg^{2+},[17] or Pb^{2+}),[18] proteins (nucleolin,[19] thrombin,[20] lysozyme,[21] vascular endothelial growth factor (VEGF)[22] and DNA binding proteins),[23] nucleic acid derivatives (DNA/RNA analytes,[24] single-stranded DNA,[25] miRNA,[26] adenosine triphosphate (ATP)[27] or cyclic diguanylate (c-di-GMP))[28] or nucleotide modifications (single nucleotide polymorphism[29] or single-base mutations).[30] Catalytic DNA has also been employed to implement more complex assays, including biochemical (to assess the telomerase,[31] methyltransferase,[32] glucose oxidase[33] or cholesterol oxidase[34] activity), biophysical (to identify G-quadruplex ligands),[35] histochemical[36] or immunological[37] assays. The only sour note of the field of catalytic quadruplexes is the lack of general, readily implemented strategy to make DNA pre-catalysts easily immobilisable. We address this issue herein, reporting on a strategy relying on a macrocyclopeptidic RAFT that can be concomitantly coated by DNA whatever its structure on one face and biotin free of steric hindrance on its other face. The generality of this approach has been firmly established not only in light of the broad diversity of studied RAFT-based DNA pre-catalysts, but also with respect to the luminescent assay described herein, which enables efficient detection of surface-bound streptavidin in the low nanomolar range according to a practically convenient, simple and rapid protocol. Of course, we will be remiss not to recognize that detecting immobilized streptavidin with biotinylated biolabels cannot be considered as a hard-won biophysical achievement; however, our results provide a solid framework to address the possibility of using quadruplex-based pre-catalysts as a valuable component for the development of biochemical assays. Now this trigger is removed, we must seize this opportunity to conceptualize and construct useful DNAzyme-based assays that will provide, hopefully, reliable answers to crucial health challenges.

Acknowledgements

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