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# Signal amplification by enzymatic tools for nucleic acids

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ABSTRACT

This review provides a comprehensive overview of current research involving the use of enzymatic tools for nucleic acids in the fabrication of signal amplification for biosensors. The enzymes include polymerases, nucleases, helicases, and ligases. They have the capability to mimic biological signal transduction or amplification processes, which can be applied in nucleic-acid assays by smart designs. Since most of the enzymatic tools have highly efficient catalytic effects, the sensitivity of the assays can be guaranteed. Moreover, DNA can be used as a promising material for nanobiotechnology, and the nanostructures of DNA can be engineered to recognize a variety of molecules. Thus, such enzymatic tools can be largely used for biosensing. Enzymatic tools for nucleic acids may have great practical utility in cutting-edge analytical methods and protocols.



**Contents**



*Abbreviations:* λ exo, λ exonuclease; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline)- 6-sulfonic acid; AP, Alkaline phosphatase; AuNP, Gold nanoparticle; BHQ, Black hole quencher; CL, Chemiluminescence; DNase, Deoxyribonuclease; dsDNA, Double-stranded DNA; DSN, Duplex-specific nuclease; ECL, Electrochemiluminescent; ExoIII, Exonuclease III; FRET, Fluorescence resonance-energy transfer; GCE, Glassy-carbon electrode; HDA, Helicase-dependent amplification; LAMP, Loop-mediated isothermal amplification; LSPR, Localized surface-plasmon resonance; MB, Molecular beacon; MMP, Magnetic microparticle; MWCNT, Multi-walled carbon nanotube; NEase, Nicking endonuclease; OTA, Ochratoxin A; PCR, Polymerase chain reaction; PDDA, Poly(diallyldimethyl ammonium chloride); PNK, Polynucleotide kinase; QD, Quantum dot; qRT-PCR, Quantitative real-time PCR; RCA, Rolling-circle amplification; $[Ru(NH_3)_6]^{3+}$, Hexaammineruthenium (III) chloride; RNase, Ribonuclease; SA-HRP, Streptavidin-horseradish peroxidase; SDA, Strand-displacement amplification; SELEX, Systematic evolution of ligands by exponential enrichment; SERS, Surface-enhanced Raman spectroscopy; ssDNA, Single-stranded DNA; $T_m$, Melting temperature.

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## 1. Why enzymatic tools for signal amplification of biosensors?

Biosensors are analytical devices to convert the information of analytes (e.g., identity, concentration, and reactivity) to measurable signals (e.g., optical, fluorimetric, electrical, electrochemical, and mechanical), involving the use of biological materials, biological derived materials or biomimics. Currently, the investigation of biomolecular functions and biological pathways largely depends on the sensitivity and the selectivity of biosensors. Common target analytes of biosensors include small molecules (e.g., ions and ATP) and biomacromolecules (e.g., nucleic acids, polysaccharide, and proteins) [1–3]. They may carry out a vast array of biological functions and some can be used as drug candidates, vectors and biomarkers for diseases [4,5].

In most cases, two critical components are required in a biosensor. One is a recognition element and the other is a signal reporter. To date, a large number of molecular pairs have been discovered, such as antibody and antigen [6], affibody and target [7], enzyme and substrate [8], aptamer and target [9], nucleic acids and complementary strands [10], and streptavidin and biotin [11]. However, different signal reporters have also been developed with modern techniques {e.g., colorimetric [12], electrochemical [13], fluorescent [14], and surface-enhanced Raman spectroscopy (SERS) [15]}.

The sensitivity of a biosensor usually relies on the relationship established between the concentration of the target analyte and the signal. However, certain analytes to be detected are in very low abundance, such as circulating cell-free DNA and other tumor biomarkers. They can hardly be distinguished by traditional sensing, which generates insufficient signals. To solve the problem, much effort has been devoted to enhancing the strength of the signals obtained, e.g.:

- the extinction coefficient is increased in colorimetric measurements; and,
- the quantum yield is raised in fluorimetric assays.

However, such improvements are not always significant and have their limitations. Powerful signal-amplification strategies may become more promising solutions [14,16]. So far, lots of signal-amplification systems have been exploited for biosensors, including strategies based on enzymatic tools for nucleic acids [17,18], DNA-templated reactions [19], DNA-supersandwich structures [20], macromolecules [21] and nanomaterials [22]. Among them, enzymatic tools for nucleic acid-mediated signal amplification have been studied in detail and developed.

The mechanism of enzyme-mediated signal amplification was described previously [23,24]. Herein, we discuss general enzyme-catalyzed reaction processes and dynamics in detail (Fig. 1).

Several hypotheses are advanced:

- the reaction rate is nearly negligible with an inactivated enzyme or in the absence of the analyte;
- the concentration of the enzyme is saturable for the interaction with the analyte without any inhibition;
- the amount of substrate is also excessive; and,
- the equilibrium of the above interaction is established quickly enough ($k_1 >> k_2$).

After competitive equilibrium recognition between analyte and inactivated enzyme ($E_I$), enzyme features, such as structural or electrical characteristics, alter. The enzyme is then activated ($E_A$) and catalyzes the substrate into the product. According to Equations (1) and (2) in Fig. 1A, the $E_A$ generated has a concentration very close to the initial analyte ($[A]_0$). The final concentration of the product can then be calculated [Equations (3) and (4)], and has linear relationship with $[A]_0$ or the reaction duration.

Fig. 1B shows a self-amplification mechanism, in which product P can also activate the enzyme in a similar way to (or the same way as) the analyte. From Equations (5) and (6), the calculated concentration of P after the reaction has an exponential relationship with reaction duration. Since the output signals of biosensors usually

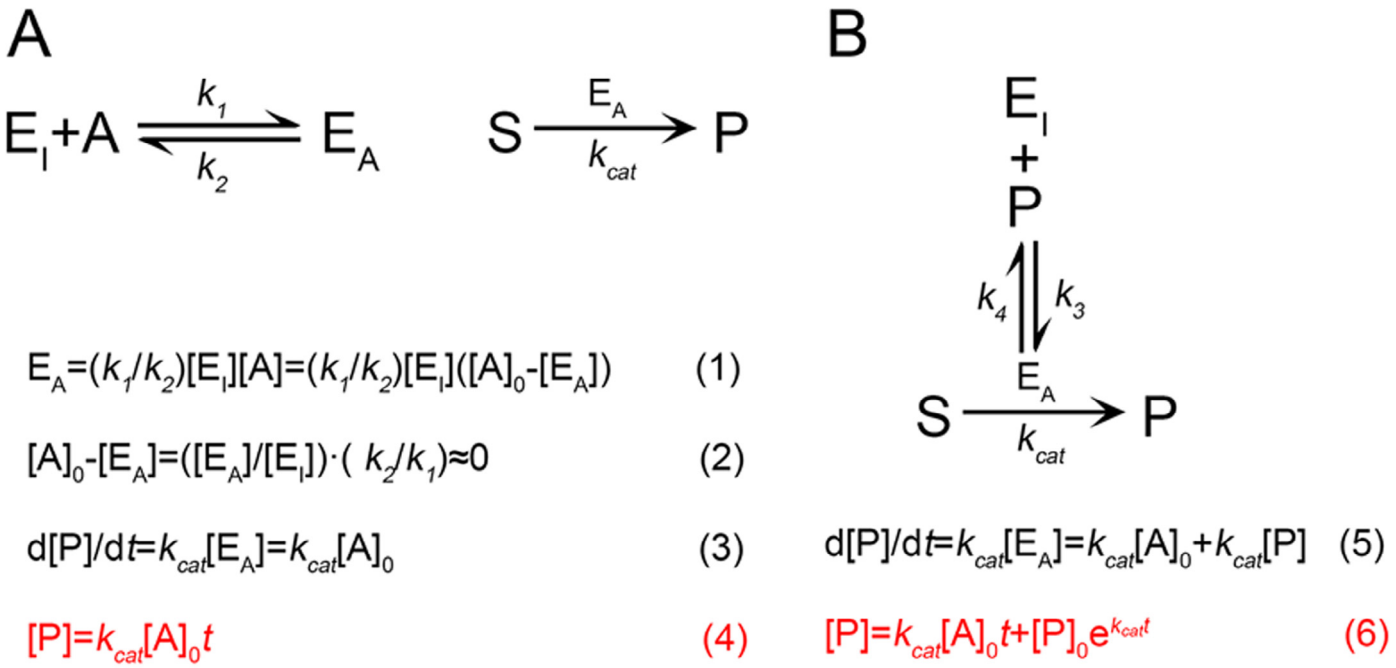


**Fig. 1.** (A) Two steps of enzyme catalytic reactions. (B) Product P has the ability to activate the enzyme reactions. A, Analyte; $[A]_0$, Initial analyte concentration; $E_A$, Activated enzyme; $E_I$, Inactivated enzyme; $k_{cat}$, Catalytic turnover; P, Product that can be directly read out; S, Substrate; t, Reaction duration.