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Polydopamine as a versatile optical indicator for colorimetric and fluorescence-based biosensing

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Beyond their well-established adhesive properties, polydopamine (pDA) and pDA-like materials are emerging as superior alternatives to conventional optical indicators in biosensing applications due to their exceptional biocompatibility, tunable optical properties, and high sensitivity, arising from their eumelanin-like physicochemical characteristics. These materials attract significant attention for their ability to function as optical probes and transducers, enabling precise and sensitive detection in complex biological environments. This review highlights recent advancements in developing pDA-based optical probes, emphasizing strategies for fine-tuning synthetic parameters to optimize material properties, clarifying the fundamental sensing mechanisms underlying pDA-based systems, and exploring their potential roles in addressing global healthcare challenges. By facilitating early disease detection, real-time monitoring, and targeted therapeutic intervention, pDA-based optical probes offer transformative solutions to pressing biomedical needs. Through a comprehensive examination of cutting-edge research, this review aims to illuminate how the unique attributes of pDA materials drive innovation in biosensing technologies and contribute to improved healthcare outcomes.

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1. Introduction

Biosensing techniques are crucial for identifying specific biomolecules from various specimens, serving a significant role in disease treatment, drug discovery, environmental monitoring, and other essential applications. In particular, *in vitro* diagnostics that detect biomarkers in biofluids, such as blood, saliva, sweat, and tears, have gained considerable attention in personalized healthcare. These techniques enable more targeted therapies and improve patient outcomes by facilitating early diagnosis, treatment monitoring, and prognosis.^{1,2}

Various detection principles have been developed to produce measurable signals upon biomarker recognition. In line with advancements in novel materials (such as MXene, graphene, and Borophene), which are known for their high surface reactivity as well as excellent thermal and electrical conductivity, bioassays based on electrochemical, magnetic, and mass-based methods have also seen significant progress.^{3,4} However, many of these detection techniques require expensive instruments and highly trained operators, limiting their accessibility for real-world applications. Optical sensing, in particular, has been extensively studied due to its convenience and rapid readout capabilities. Colorimetric

detection is widely utilized among optical methods, particularly in home-based commercial kits, as exemplified by lateral-flow immunoassays (LFIA). This approach allows results to be visually interpreted without requiring specialized equipment, making it highly accessible for self-testing.⁵ Similarly, fluorescence-based sensing is a preferred strategy due to its high sensitivity for detecting low-abundance molecules, utilizing both commercially available devices and compact, custom-built equipment.⁶

In both colorimetric and fluorescence-based techniques, developing novel colorants and fluorophores is essential for enhancing sensitivity and specificity, which are critical to improving the overall accuracy of these assays. Small molecular chromophores have been extensively explored for biosensing due to their well-defined optical properties and the capacity to fine-tune spectral ranges through precise structure–property modifications.^{7,8} Despite their potential, challenges such as photobleaching and low aqueous solubility limit their use in complex biological and environmental contexts.^{9,10} Nanomaterials have emerged as a robust alternative, providing distinct advantages, including tunable spectral properties, enhanced optical stability, and improved robustness under diverse conditions.^{2,11,12} Examples include quantum dots, gold nanoparticles, and upconversion nanoparticles, each suited to biosensing applications in various physiological and environmental settings. In addition, their high surface area facilitates functionalization with bioreceptors, enabling target-specific recognition and expanding their utility.¹³ However,

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challenges persist. Variability in nanomaterial synthesis often results in batch-to-batch inconsistencies, adversely affecting assay reproducibility and reliability.^{14,15} In addition, concerns about the cytotoxicity of specific nanomaterials limit their direct application in biological systems, necessitating the development of safer, biocompatible alternatives.^{14,16} Large-scale production poses significant challenges, as scalability often compromises material quality and performance.¹⁵ Overcoming these issues requires innovative strategies to engineer materials with enhanced stability, biocompatibility, and scalability, ensuring they meet the practical demands of biosensing applications while addressing current limitations.

Polydopamine (pDA) and pDA-like materials have gained recognition as versatile materials for biosensing applications due to their unique physicochemical properties and biomimetic characteristics. A key feature of pDA in biosensing is its effectiveness as an interfacial layer for immobilizing bioreceptors onto transducers.^{17,18} The adhesive characteristics of pDA facilitate the robust attachment of proteins, nucleic acids, or small molecules, improving the stability and reproducibility of biosensor performance. Beyond its role as an adhesive layer, pDA's broadband light absorption, spanning the ultraviolet to near-infrared (NIR) regions and resembling natural eumelanin biopigments, unlocks novel optical signal transduction possibilities.^{19–21} For instance, its visible light absorption supports colorimetric sensing, enabling visually detectable biomarker quantification. Simultaneously, pDA's NIR absorption facilitates advanced techniques such as photoacoustic signal generation and photothermal therapies. In addition, its broadband absorption allows pDA to function as a universal fluorescent quencher, enhancing its utility in fluorescence-based biosensor platforms. Traditionally, pDA NPs have been considered non-fluorescent. However, recent advancements in synthetic methods have resulted in fluorescent pDA analogues, significantly expanding their application potential. These fluorescent derivatives have introduced new opportunities in biosensing, bioimaging, and *in vitro* diagnostics. These developments highlight the multifaceted nature of pDA and its derivatives, bridging material science and biomedical applications to address diverse biosensing requirements.

This review investigates advanced strategies utilizing pDA and pDA-like organic materials as optical probes in biosensing. The focus is on optimizing synthetic conditions to enhance material properties, clarifying the operational principles of pDA-based optical sensing, and examining their potential applications in healthcare. The review is structured into three main sections to provide a comprehensive analysis:

- (1) **pDA-based colorimetric sensing:** Highlighting applications that use pDA's visible light absorption for simple and direct biomarker detection.
- (2) **Biosensing utilizing pDA as fluorescent quenchers:** Exploring the utility of pDA in fluorescence-based platforms through its quenching capabilities.
- (3) **Fluorescent pDA analogues in biosensing:** Discussing the emerging applications of fluorescent pDA derivatives in bioimaging and diagnostics.

The aim is to provide insights into how nature-inspired pDA materials can address critical challenges in biosensing by exploring these advancements. These innovations are poised to enable more accurate, cost-effective, and accessible health-care solutions, with significant implications for the future of personalized medicine and diagnostics.

2. pDA as visible indicators

2.1. Fundamentals in the absorption spectrum of pDA and its connection to eumelanin

Eumelanin, a class of organic biopigments responsible for brown-to-black coloration in nature, exhibits broad light absorption across UV-Vis-NIR wavelengths, which monotonically increases toward high-energy regions. This unique property, rarely observed in natural organic chromophores, is often regarded as semiconductor-like behavior.²² Such behavior is attributed to eumelanin's highly complex and disordered chemical structure and hierarchical nanoscale assembly. In nature, eumelanin synthesis involves the enzymatic oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), followed by the formation of intermediates such as DOPochrome, DHI, and DHICA (Fig. 1A). These intermediates grow into heterogeneous oligomeric chromophores, commonly called protomolecules, which define the primary absorption characteristics across various wavelengths. However, this alone does not fully explain the monotonic increase in absorption across the wide spectral range extending from UV to NIR. Several studies indicate that excitonic and/or electronic coupling between protomolecules, facilitated by π -stacking and higher-order aggregation, broadens the absorption spectrum, introducing new transition energies distinct from those of individual chromophores (Fig. 1B).^{22–24} Partial proton transfer from catechol hydroxy groups to the solvent has also been proposed as a contributing factor.²⁴ These molecular interactions enable eumelanin to dissipate absorbed energy as heat through rapid non-radiative relaxation processes, resulting in an extremely low fluorescence quantum yield (<0.05%).^{25,26} This mechanism is critical to the photoprotective function of eumelanin in nature.²⁴

pDA is widely recognized as the most acceptable functional mimic of eumelanin due to its remarkable similarities in synthetic pathways and resulting material properties.^{19–21} The synthesis of pDA closely mirrors that of eumelanin, beginning with the oxidation of catechol-containing precursors, followed by the formation of protomolecules that further assemble into nanoscale structures. As a result, pDA forms brown-black, water-insoluble aggregates with broadband absorption spanning the UV to NIR regions, similar to eumelanin.²⁸ Although the DA precursor used in pDA synthesis lacks the carboxyl group necessary for generating DHICA-based protomolecules, the inclusion of 5,6-dihydroxyindole (DHI), a major intermediate extensively discussed in prior eumelanin studies, strongly supports the designation of pDA as a functional mimic of eumelanin (Fig. 1A). These similarities highlight the potential of pDA to replicate the biological roles of eumelanin, such as



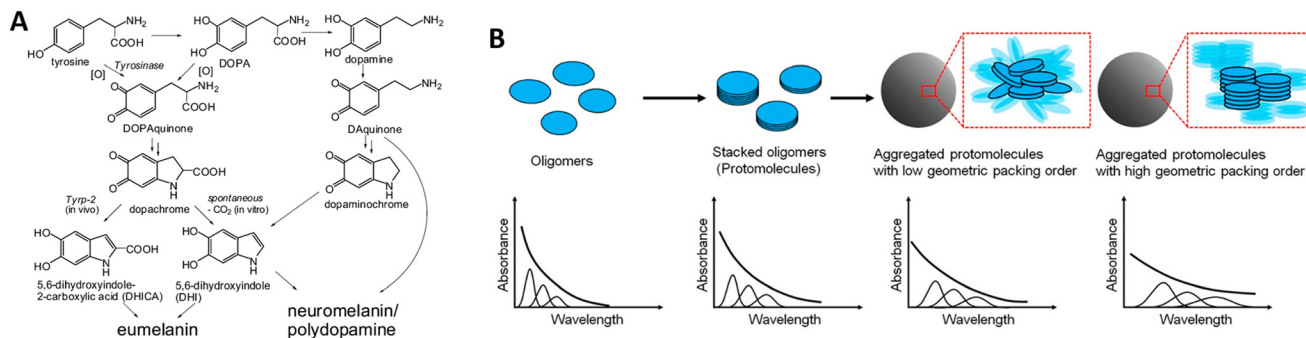


Fig. 1 Fundamentals in eumelanin synthesis. (A) The initial stages of eumelanin synthesis begin with the oxidation of tyrosine to DOPA, which is further converted into the key intermediates DHICA and DHI. The DHI-involved pathways in eumelanin synthesis overlap with those of pDA synthesis. Adapted from ref. 27, Copyright 2014, ACS. (B) A proposed structure–property relationship of eumelanin, highlighting spectral broadening toward low energies observed during hierarchical assembly. Adapted from ref. 22, Copyright 2018, ACS.

photoprotection and antioxidation, while serving as an innovative material for optical and biomedical applications. In biosensing, the absorption properties of pDA in the visible range are particularly advantageous, facilitating the development of colorimetric and fluorescence-based readouts for point-of-care testing (POCT).

2.2. pDA NP-based colorimetric assays

Colorimetric bioassays represent one of the most accessible detection methods, allowing results to be directly observed with the naked eye without requiring specialized analytical equipment. This accessibility renders colorimetric assays particularly suitable for point-of-care (POC) applications, facilitating their use in real-world settings outside conventional laboratories.

pDA nanoparticles (NPs) have been successfully integrated into LFIA for POC diagnostics. For instance, Tong *et al.* developed a colorimetric LFIA for COVID-19 detection, utilizing pDA NPs as visible indicators.²⁹ Their study demonstrated that pDA NPs exhibit superior absorption in the visible range compared to AuNPs at the same mass fraction (4.5%), resulting in greater sensitivity for the pDA-based LFIA kits than commercial Au-based alternatives. Similarly, Zhang *et al.* synthesized pDA-coated metal–organic frameworks (MOFs) as visible probes in LFIA. Their system achieved a detection limit of 0.045 ng mL⁻¹ for enrofloxacin, which improved upon the 0.095 ng mL⁻¹ limit observed in conventional AuNP-incorporated systems.³⁰ Xu *et al.* also reported that pDA coatings on AuNPs enhance color brightness, increasing the molar extinction coefficient by 1.68-fold and grayscale intensity by 1.87-fold.³¹ This enhancement improved the sensitivity of LFIA while providing high dispersibility across a wide pH range (6–14) and in NaCl-rich environments, broadening its compatibility with diverse bioassay conditions.

The versatility of pDA as a colorant extends beyond LFIA applications. For example, its application in enzyme-linked immunosorbent assays (ELISA) has shown considerable potential. Horseradish peroxidase (HRP)-accelerated pDA deposition significantly enhanced sensitivity, as demonstrated by Xu

et al., who observed a 100-fold improvement in sensitivity compared to conventional enhanced chemiluminescence (ECL) kits.³²

2.3. Spatial marking of biomarkers

Two primary strategies are employed to generate optical signals in bioassays. One involves using pre-prepared colorants, such as quantum dots and AuNPs, which bind specifically to target molecules (*via* antibody–antigen interactions), enabling visualization of biomarker distribution. However, this approach often suffers from limited signal amplification because the optical density of these materials is fixed during preparation. Alternatively, enzyme-incorporated strategies amplify signals by converting numerous substrate molecules into detectable indicators. Although this method provides substantial amplification, it is typically restricted to solution-based assays, as the generated colorants tend to diffuse in aqueous environments, making precise spatial localization challenging.

In this context, pDA has emerged as an innovative adhesive colorant capable of addressing these limitations. Its local generation near biomarkers ensures precise spatial marking while enabling signal amplification. For example, Li *et al.* developed a pDA-based immunoassay named EASE, involving the *in situ* generation of pDA near biomarkers pre-labeled with HRP.³³ This approach produced a detectable brownish color under bright-field imaging. In addition, the secondary immobilization of quantum dots (QDs) or HRP on the pDA layer further enhanced sensitivity (Fig. 2A). Using this method, an ELISA demonstrated remarkable sensitivity, detecting HIV antigens in blood samples at concentrations below 3 fg mL⁻¹. Kim *et al.* employed *in situ* deposition of pyrocatechol-based adhesive colorants, showing the localized formation of pigments near HRP, in contrast to the diffusion of TMB in solution (Fig. 2B).³⁴ Both studies were validated in cellular systems (Fig. 2C and D). In addition, Li *et al.* exploited the spectral shift in localized surface plasmon resonance (LSPR) of AuNPs induced by pDA coating as a visible marker (Fig. 2E). This method effectively detected food contaminants such as bio-



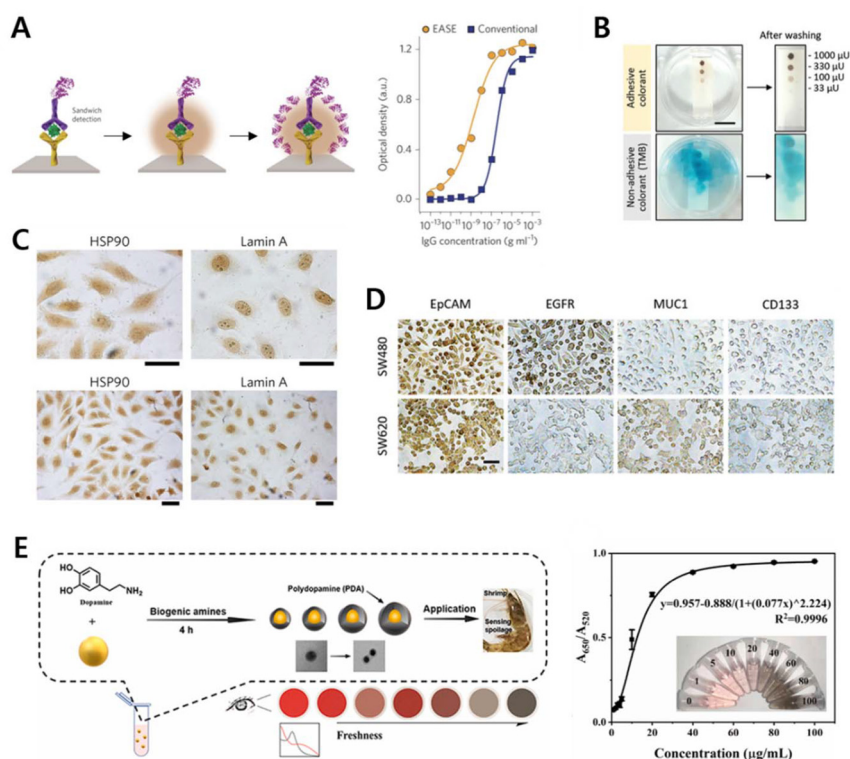


Fig. 2 Colorimetric bioassays enabled by localized *in situ* generation of pDA and pDA-like materials. (A) A pDA-based immunoassay with enhanced sensitivity was achieved by secondary incorporating HRP (purple) onto pDA generated around biomarker-associated sites (brown). Adapted from ref. 33, Copyright 2017, Springer Nature. (B) A comparison of color localization between pDA-like adhesive colorants and conventional TMB activated by HRP. Adapted from ref. 34, Copyright 2020, Wiley. (C and D) Spatial marking of biomarkers in a cellular system. (C) was adapted from ref. 33, Copyright 2017, Springer Nature, and (D) was adapted from ref. 34, Copyright 2020, Wiley. (E) *In situ* pDA coating in the presence of food contaminant biogenic amines, tuning the LSPR of AuNPs as a visible indicator. Adapted from ref. 35, Copyright 2021, Elsevier.

genic amines, with the pDA coating occurring significantly faster in the presence of organic amines.³⁵ These advancements highlight the unique advantages of pDA and pDA-like materials in spatially marking biomarkers, providing precise localization and robust signal amplification for various biosensing applications.

3. pDA-based fluorescent quenchers in bioassays

3.1. Fundamentals of pDA-mediated fluorescence quenching

pDA has been proposed as a multi-wavelength quencher for various fluorophores; however, its fluorescence quenching mechanisms remain not fully understood and appear to vary depending on the type of fluorophores involved and their proximity to pDA. Qiang *et al.* suggested that the quenching of small molecular organic dyes, such as Cy5, results from a combination of dynamic and static mechanisms, primarily involving fluorescence resonance energy transfer (FRET). This conclusion was supported by a decrease in fluorescence decay time and the observation of a nonlinear Stern–Volmer plot with an upward curvature (concave toward the *y*-axis).³⁶ In addition, they noted that an electron transfer mechanism

cannot be excluded, as monomeric dopamine (DA), which lacks spectral overlap with the fluorophore, also induced some fluorescence quenching. Medintz *et al.* previously confirmed the redox coupling between quantum dots and the quinone state of DA, demonstrating its applicability in pH sensing.³⁷ Similarly, Wang *et al.* investigated the quenching mechanism of upconversion fluorescent nanoparticles (UCNPs) by pDA, identifying photoinduced electron transfer (PET) as the primary mechanism.³⁸

Chen *et al.* proposed that the primary quenching mechanism of carbon dots by pDA is the inner filter effect (IFE), based on their calculation of the contribution of IFE to quenching efficiency.³⁹ They also suggested that neither dynamic nor static quenching mechanisms contributed to the quenching of carbon dots, as the fluorescence lifetime of carbon dots remained unchanged in the presence of pDA, and no evidence was found for forming non-fluorescent complexes between pDA and carbon dots. Similarly, Wang *et al.* argued that IFE, rather than FRET, is the more plausible quenching mechanism for pDA with three tested fluorescent particulate donors, aggregation-induced emission fluorescent microspheres, fluorescent microspheres, and quantum dot beads, based on the observation that the fluorescence lifetime decay showed no significant changes after mixing with pDA.⁴⁰



**Table 1** Summary of bioassays utilizing pDA-based fluorescent quenchers

Nucleic acid probe	Year	Mechanism	Quencher	Fluorescent dye (used Ex/Em)	Target biomarker	Sensitivity	Ref.
	2014	Turn-on	Spherical pDA NPs (diameter: 336 nm)	AMCA (335/448 nm), FAM (470/518 nm), TAMRA (535/575 nm), Cy5 (625/660 nm)	Model DNA Thrombin	Linear range: 0.78–25 nM, LOD: 0.1 nM Linear range: 1.56–37.5 nM, LOD: 0.5 nM	36
	2015	Turn-on (Exo III-assisted amplification)	Spherical pDA NPs with a diameter of 297.3 ± 20.4 nm	FAM (470/518 nm)	Model DNA ATP	Linear range: 0.078–5 nM, LOD: 5 pM Linear range: logarithm of 0.39–1600 μM, LOD: 180 nM	42
	2016	Turn-on (Exo III-assisted amplification)	pDA nanotubes	FAM	HIV DNA ATP	Linear range: 10–200 pM, LOD: 3.5 pM Linear range: logarithm of 0.35–800 μM, LOD: 150 nM	43
	2017	Turn-on (DNase I-assisted amplification)	Mesoporous pDA NPs (diameter: 70 nm)	FAM (480/525 nm)	Let-7a miRNA-21	LOD: 40 pM LOD: 32 pM	44
	2022	Turn-on (DNase I-assisted amplification)	DA-PEI co-polymerized nanodots (diameter: 6.7 nm)	AMCA (365/452 nm)	miRNA-21	Linear range: 0.8–50 nM, LOD: 0.52 nM	45
<i>In situ</i> pDA generation	2023	Turn-off	<i>In situ</i> pDA coating	Fluorescent carbon dots (370/500 nm)	DA	Linear range: 0.1–15 μM, LOD: 37 nM	41
Immunoassays	2015	Turn-on	<i>In situ</i> pDA coating	Upconversion fluorescent NPs (980/540 nm)	Total antioxidant capacity	Recoveries: 94.1%–100.1%	38
	2019	Turn-off	<i>In situ</i> pDA coating	Fluorescent carbon dots (350/452 nm)	Acid phosphatase	Linear range: 1–60 U L ⁻¹ , LOD: 0.45 U L ⁻¹	39
	2022	Turn-on (LFIA)	Spherical pDA NPs (diameter: 205 nm)	AIEFM (320/505 nm), FM (495/530 nm), QB (354/615 nm)	Sulfamethazine	Linear range: 0.05–10 ng mL ⁻¹ , LOD: 0.043 ng mL ⁻¹	40
	2018	Turn-off (sandwich)	pDA coated mesoporous SiO ₂	ECL (luminol-O ₂ system)	Insulin	Linear range: 0.0001–50 ng mL ⁻¹ , LOD: 26 fg mL ⁻¹	46
	2024	Turn-on (competitive)	Hybrid pDA@ZIFs NPs	ECL (g-C ₃ N ₄ /K ₂ S ₂ O ₈ system)	Ochratoxin A	Linear range: 10.0 fg mL ⁻¹ –1.0 ng mL ⁻¹ , LOD: 4.8 fg mL ⁻¹	47
	2023	Turn-on (LFIA)	Fe(m)-complexed pDA NPs	Fluorescent quantum dots (365/625 nm)	Enrofloxacin	LOD: 0.016 ng mL ⁻¹	48

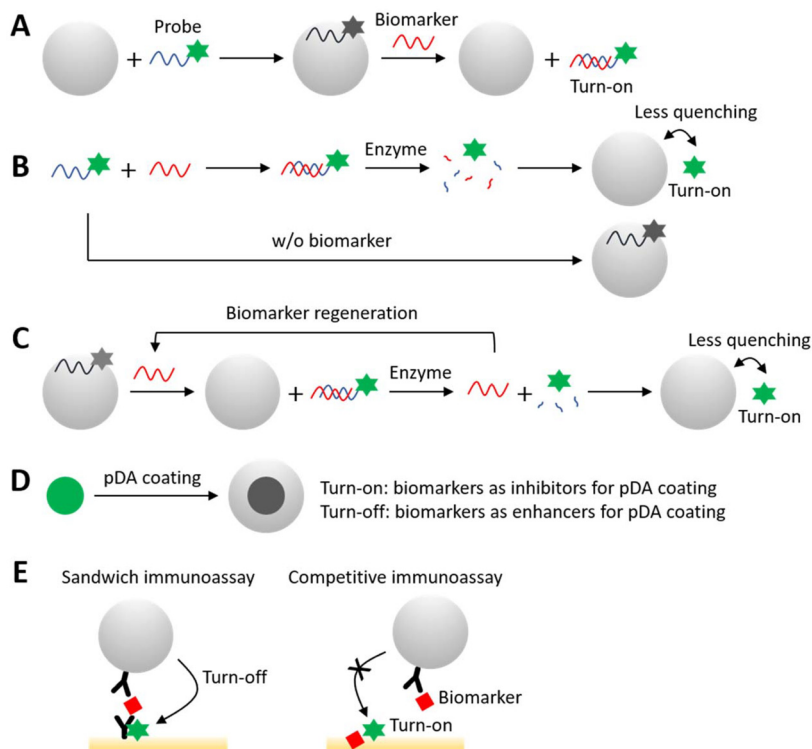


Fig. 3 Various types of fluorescence-based sensing schematics are mediated by pDA, utilizing “turn-on” and “turn-off” mechanisms.

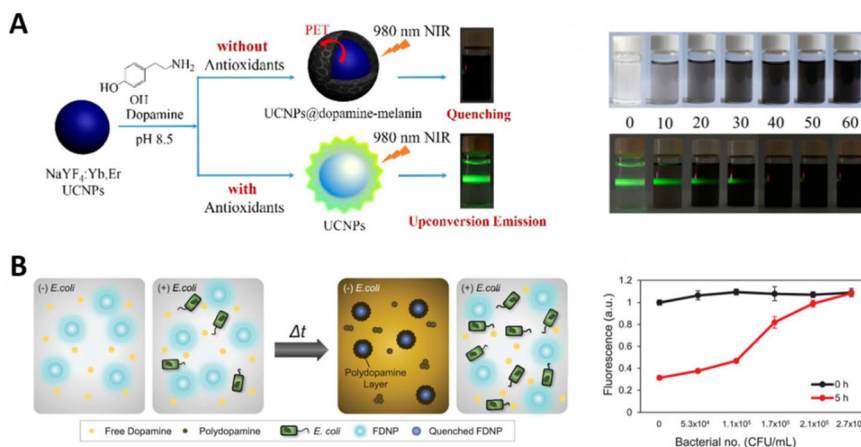


Fig. 4 *In situ* pDA generation for fluorescence quenching-based assays. (A) pDA quencher coupled to upconversion nanoparticles (UCNPs) for NIR-excited sensing of *in vitro* antioxidant capacity. Adapted from ref. 38, Copyright 2015, ACS. (B) pDA-based sensor platform for detecting bacterial hypoxia. Adapted from ref. 51, Copyright 2021, Wiley.

range of 0.0001–50 ng mL⁻¹ and a 26 fg mL⁻¹ detection limit. Similarly, Yang *et al.* employed pDA-incorporated ZIFs as detection probes for g-C₃N₄/K₂S₂O₈ ECL in a sandwich assay for mycotoxins, achieving a linear range of 10.0 fg mL⁻¹–1.0 ng mL⁻¹ and a detection limit of 4.8 fg mL⁻¹.⁴⁷ In addition, Lai *et al.* established an LFIA using iron-doped pDA (Fe-pDA) submicrobeads as quencher probes for pre-loaded quantum dots on the strip.⁴⁸ The incorporation of iron into pDA resulted

in a 14.9-fold higher fluorescence quenching constant than regular pDA due to the synergistic effects of FRET and the IFE. Based on this, a competitive assay was demonstrated in which antibody-decorated Fe-pDA beads were selectively bound to the pre-loaded target molecules near the quantum dots on the surface without the target in the medium, resulting in a negative signal. This approach achieved a detection limit of 0.016 ng mL⁻¹.



4. Fluorescent analogues of pDA

4.1. Synthesis of fluorescent pDA analogues

pDA NPs are typically synthesized as non-fluorescent spheres with sizes ranging from a few hundred nanometers. However, reports exist on synthesizing pDA analogues exhibiting fluorescence, making them valuable for biosensing and bioimaging applications. Understanding the mechanism behind this fluorescence emission remains challenging due to the complex chemical structure of pDA. Nevertheless, based on the non-radiative energy dissipation *via* electronic and/or excitonic coupling between stacked protomolecules, it is plausible that aggregation-caused quenching (ACQ) is linked to the growth of pDA NPs derived from intrinsically fluorescent protomolecules. Therefore, the synthesis of fluorescent pDA analogues (F-pDA) primarily focuses on reducing the degree of assembly and/or weakening the non-covalent interactions between protomolecules. This can be achieved using either a bottom-up approach, which involves enhanced oxidative conditions or the inclusion of additive molecules during synthesis, or a top-down approach, where pre-synthesized NPs are chemically degraded.

Non-fluorescent pDA is typically synthesized under mildly basic pH in aerobic aqueous conditions, where dissolved oxygen serves as the oxidant, eliminating the need for additional chemical oxidants. Enhanced oxidative conditions have been reported to facilitate the formation of F-pDA analogues. For example, Zhang *et al.* demonstrated that introducing H₂O₂ into a 15-minute pre-reacted pDA synthesis batch led to worm-like structured F-pDA analogues.⁵² This process required a relatively high concentration of H₂O₂, with 10 mL of concentrated H₂O₂ (30% w/w) added to 40 mL of the synthetic mixture. Then, Liu *et al.* utilized iron oxide NPs (Fe₃O₄) with peroxidase-like activity to significantly reduce the required H₂O₂ concentration for F-pDA synthesis to 5 mM (Fig. 5A),⁵³ while Li *et al.* employed the Fenton reaction, using Fe²⁺ and H₂O₂ to generate [•]OH, achieving a reduction in the required H₂O₂ concentration to as low as 2 mM.⁵⁴ Other inorganic oxidants, such as KMnO₄, MnO₂, and CoOOH, have also been employed to generate F-pDA, which can be further applied in various bioassays based on *in situ* F-pDA generation (section 4.3). Liu *et al.* reported a rapid and additive-free synthesis method using nonthermal air plasma treatment.⁵⁵ This



Fig. 5 Fluorescent pDA analogues synthesized through various strategies. (A) Images of oxidized TMB (top), oxidized DA (middle), and fluorescence of oxidized DA under 470 nm excitation (bottom row) after reaction with each nanomaterial in the presence of H₂O₂. Adapted from ref. 53, Copyright 2016, RSC. (B) Sequence-specific enhancement of fluorescence in PEI-incorporated pDA dots. Adapted from ref. 56, Copyright 2022, ACS. (C) NPs emitting distinct fluorescence spectra ranging from 410 to 680 nm, synthesized by reacting DA and DA analogues as precursors with ethylenediamine (EDA). Reproduced under the terms of the CC-BY license,⁵⁷ Copyright 2024, The Authors, Published by Elsevier.



Table 2 Summary of fluorescent pDA analogues used in biosensing

Category	Year	F-pDA synthesis	Size of F-pDAs	Ex/Em for sensing	Sensing mechanism	Target biomarker	Sensitivity	Ref.
As-prepared F-pDA NPs	2016	Bottom-up (oxidant: iron oxide nanozyme/ H_2O_2)	A few to 15 nm	360/500 nm	Turn-on	Zn^{2+}	LOD: 60 nM (linear: up to 5 μM)	53
	2018	Top-down ($NaBH_4$ -induced reduction)	34.83 nm	335/423 nm	Turn-off	Fe^{3+}	LOD: 0.15 μM (linear: 0.5–20 μM)	58
	2022	Bottom-up (one-pot, polycytosine & PEI as additives)	2.5 ± 6 nm	400/550 nm	Turn-off	Cu^{2+}	LOD: 0.03 μM (linear: 0–10 μM)	56
	2023	Bottom-up (one-pot, folic acid as an additive)	1.9 ± 0.3 nm	360/around 440–450 nm (not specified)	Turn-off	Hg^{2+}	LOD: 0.18 μM (linear: 0–18 μM)	60
	2015	Top-down (degradation using hydroxyl radicals)		330/440 nm	Turn-off	Fe^{3+}	LOD: 0.3 μM (linear: 1–50 μM)	61
	2018	Bottom-up (GSH as an additive)	2.7–3.28 nm	360/450 nm	Turn-off	Fe^{3+}	LOD: 30 nM (linear: 0.1–3 μM)	62
Pre-quenched F-pDA NPs by protamine <i>In situ</i> F-pDA generation	2021	Top-down (degradation using hydroxyl radicals)	Diameter: 4.8 nm	340/435 nm	Turn-on (by removing Fe^{3+} from F-pDA)	Pyrophosphate	LOD: 0.6 μM (linear: 2–12 μM)	63
	2021	Bottom-up (Fe^{2+}/H_2O_2 as oxidants)	Monodispersed, about 8 nm	380/522 nm	Turn-on (by degrading protamine)	Trypsin	LOD: 6.7 ng mL^{-1} (linear: 0.01–0.1 $\mu g mL^{-1}$)	54
	2018	Bottom-up (Ficin/ H_2O_2 as oxidants)	Few tens to hundreds of nanometers	400/476 nm	Turn-on	DA	LOD: 0.09 μM (linear: 0.5–10 μM)	64
	2023	Bottom-up (MnO_2 as an oxidant)	Spherical, less than 50 nm	420/503 nm	Turn-on	H_2O_2	LOD: 0.07 μM (linear: 0–300 μM)	65
	2016	Bottom-up (MnO_2 as an oxidant)	Irregular, from several to tens of nanometers	400/485 nm	Turn-off (by consuming the oxidant)	Glucose (GOx-incorporated)	LOD: 0.49 μM (linear: 1 to 60 μM)	66
	2022	Bottom-up ($KMnO_4$ as an oxidant)	Spherical, 4 nm	400/480 nm	Turn-on	DA	LOD: 1.61 μM (linear: 0–100 μM)	67
	2017	Bottom-up (CoOOH as an oxidant)	Around 10–50 nm	400/495 nm	Turn-on	Quercetin	LOD: 5.5 nM (linear: 10 nM–5.0 μM)	68
	2021		About 5 nm with a wide distribution	400/490 nm		Alkaline phosphatase	LOD: 0.1 $U L^{-1}$ (linear: 0.5–300 $U L^{-1}$)	69
	2020		Irregular, about 10–50 nm	390/490 nm		Glutathione	LOD: 1.93 μM (linear: 10–100 μM)	70



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