Biomolecule-Functionalized Smart Polydiacetylene for Biomedical and Environmental Sensing

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Abstract: Polydiacetylene (PDA) has attracted interest for use as a sensing platform in biomedical, environmental, and chemical engineering applications owing to its capacity for colorimetric and fluorescent transition in response to external stimuli. Many researchers have attempted to develop a tailor-made PDA sensor via conjugation of chemical or biological substances to PDA. Here, we review smart bio-conjugates of PDA with various biomolecules such as carbohydrates, lipids, nucleic acids, and proteins. In addition, materialization and signal amplification strategies to improve handling and sensitivity are described.

Keywords: biomolecules; polydiacetylene; conjugation; sensor

1. Introduction

Polydiacetylene (PDA), first prepared by Wegner in 1969, can be easily synthesized by polymerization of diacetylenic monomers under photo-irradiation, free radical induction, or plasma treatment [1–3]. In general, when adjacent diacetylenes are stacked at a distance of about 5 Å and the angle of monomer with stacking axis is about 45°, 1,4-addition-polymerization is possible by UV light (254 nm) at room temperature without the need for a catalyst or initiator, and thus, no by-products are produced (Figure 1) [4]. The resultant PDA has an ene-yne alternating backbone structure and a deep blue color (absorption $\lambda_{\text{max}} = \sim 640$ nm), and the extended $\pi$-electron delocalization gives it excellent electronic and optical properties. When the blue-phase PDA encounters heat, organic solvent, mechanical stress, or molecular recognition, a blue-to-red color shift (the wavelength shift of maximum absorption from ca. 640 to ca. 540 nm) occurs and fluorescence switches on [5]. The switch is induced by a decrease in the effective conjugation length of the PDA backbone under lipid chain distortion and disorder. Based on these core characteristics, many scientists have attempted to apply PDA assembly to various biomedical, environmental, and nanotechnological applications [6].

![Figure 1](image-url)  
**Figure 1.** Polydiacetylene (PDA) synthesis scheme via self-assembly and polymerization of diacetylene monomers.
For structural variation, the length of the acyl chain or the diacetylic position can be changed. Although 10,12-pentacosadiynoic acid (PCDA), 10,12-tricosadiynoic acid (TRCDA), 5,7-docosadiynoic acid (DCDA), and 5,7-tetracosenoic acid (TCDA) are all utilized as monomeric units [1], PCDA is the most widely used monomer. Given this basic unit, head group modification has been used to endow the polymer backbone with specific functions. In this respect, intelligent design using the PDA platform is achievable and valuable for researchers in many disciplines. Although various groups and methods for tethering to the diacetylene lipid have been investigated, this review focuses on the combination of biomolecules and PDA. These hybrid materials possess bio-induced specificity combined with the robustness/stimuli-responsiveness of a synthetic polymer.

Biomolecules include carbohydrates, lipids, proteins, nucleic acids, and small molecules such as vitamins, hormones, and metabolites. They are associated with various molecular interactions under physiological conditions, and their interactions macroscopically impact our body. For example, hemagglutinin anchored in the influenza viral envelope specifically binds the sialic acid terminating glycan on the surface of cellular membranes in a human host, permitting viruses to replicate inside the body, causing infection [7]. Similarly, bacterial toxins interact with a ganglioside moiety on host cells, eventually giving rise to inflammation and other related diseases via invasion [8,9]. In addition, biomembranes, which are comprised of lipids, carbohydrates, and proteins, have a dynamic structure, participating in numerous interfacial and cellular processes [10]. Focused on these biological issues, PDA has been modified and applied in various sensing fields (Figure 2). Biomolecule-functionalized PDA shows promise for many biomedical and environmental applications. Herein, this review deals mainly with the PDA bio-conjugates producing sensing signal, while the physicochemical aspects of PDA materials are not critically covered in this review.

![Biomolecule-functionalized smart polydiacetylene](image1)

**Figure 2.** Biomolecule-functionalized polydiacetylene (PDA) based biomedical and environmental sensing.

2. Carbohydrate-Functionalized Polydiacetylene (PDA)

The first PDA sensing system using the viral lectin and hemagglutinin-sialic acid interaction was developed by Charych et al. [11]. Sialic acid, a neuraminic acid derivative with a nine-carbon backbone, is ubiquitous in animal cells. This sensor provides a direct colorimetric method for detection of influenza using a 2–5% sialoside PDA monolayer coated on an octadecylsilane layer. The sialoside group is attached to a diacetylene monomer with a triethylene glycol linker, and the sialoyl lipid is utilized for the liposome and Langmuir–Blodgett (LB) film–type PDA influenza virus sensor [11–13]. Sensing specificity has been demonstrated using a competitive inhibition assay with lactose-attached lipids and bovine serum albumin [11]. The mechanism of the thermochromic blue-to-red shift of the modified PDA thin film was also investigated at the molecular level [14].

Mannose-derivatized PCDA has been utilized to create a PDA LB film that can recognize *Escherichia coli* [15]. Even in the incorporation of mannoside lipid (MC16) into PDA, the color change...
was triggered by the specific bio-interaction of mannose and a toxin secreted by *E. coli* [16]. Interestingly, the color response could be controlled and monitored in-situ by a third factor (TiO$_2$ sterilization colloid). While the mannose- and lactose-tethered diacetylenic monomers, through click reaction, were synthesized with different arms, only the mannose-linked PDA displayed a color transition in the presence of concavalin A. Furthermore, the longer spacer was more effective for the colorimetric response, permitting the optimal disposition of mannose for interaction with the lectin, and a longer arm acting as a lever causes more stress on the PDA backbone [17].

Recently, oligosaccharides have been incorporated into PDA assembly and applied to the detection of small molecules [18–20]. In 2015, succinoglycan monomer 1, isolated from *Sinorhizobium meliloti*, was directly derivatized to PCDA. The resulting modified PDA liposome exhibits color change and fluorescence within 1 min in the presence of some highly hydrophobic flavonoids [18]. In this case, the succinoglycan octasaccharide functions as a flexible molecular agent for capture of alpha- and beta-naphthoflavones. Subsequently, succinoglycan octasaccharide-functionalized PDA-doped alginate beads were developed for sensing ions of the toxic heavy metal, barium [19]. Since the succinoglycan octasaccharide is a pyruvyl and succinyl substituted linear glycan, the carboxyl and hydroxyl groups contribute to multilateral interactions of succinoglycan octasaccharide with Ba$^{2+}$, producing a color signal via the triggering of the PDA array. For the cyclic oligosaccharide, a $\beta$-cyclodextrin-functionalized PDA vesicle was prepared, allowing selective visualization of cationic amino acids (arginine and lysine) among twenty amino acids [20]. Although the detection limit is not low (millimolar concentration), the cavity type $\beta$-cyclodextrin working as a membrane channel for target molecules is noted.

Some researchers have attempted to improve the time-consuming synthesis of carbohydrate-attached diacetylene lipids. If the interacting glycolipid itself is incorporated into the diacetylenyl assembly, interacting analytes can be detected. For example, G$_{M1}$ and G$_{T1b}$ gangliosides, which are present on the surface of intestinal cells and at neuromuscular junctions, were utilized for color-based detection of cholera toxin and botulinum neurotoxin [21,22]. For this application, 5% ganglioside lipid was used, since a higher concentration inhibits polymerization of the diacetylene assembly. This modified PDA system mimics cell membranes, and could be used to detect toxin translocation. Next, the inexpensive diocetyl glycerylether-$\beta$-glucoside was co-assembled into PDA, and *E. coli* detection was achieved [23]. *E. coli* was also recognized with 70–90 colorimetic response (CR%) within 20 s, using a PDA platform consisting of 2,4-heneicosadiynoic acid (HCDA) and a cholesterol-linked glucoside [24]. This report also notes the influence of spacer length, in which a longer spacer is more sensitive in detecting *E. coli*. In another study, the size effect was investigated in concanavalin A detection using $N$-acetamide-$\beta$-glucoside; smaller PDA vesicles showed stronger detection ability [25]. This effect is explained by steric hindrance and a higher rate of membrane rupture derived from the greater curvature of small PDA vesicles. The *E. coli* and glycolipid binding event was also successfully transmuted into color change using a combined liposome comprised of phospholipids:PCDA:glycolipid (1,2-dihexadecanoyl-3-O-$\beta$-maltotriosyl-glycerol) 5:5:0.2 [26]. In another example, multi-head glycolipids analogous to the native cell surface were embedded in the PDA array, where a complex PDA system of 5% G$_{M1}$/5% sialic acid derivatized PCDA/90% PCDA was reported to target choleratoxin [27]. In a similar way, this combination surface system gave rise to good selectivity, with a 10 ng/mL detection limit for avian influenza, owing to the synergetic effect of the active sialic acid-$\beta$-glucoside and the inactive lactose-$\beta$-glucoside glycolipids [28]. In a PDA system, size and charge of head-groups can affect the color of the polymer due to the differential packing properties, planarity, and conformation of PDA [1,29,30]. Considering the sensor application, blue-phase PDA system is desirable for the starting material [31]. Thus, it is also important to use the appropriate portion of biomolecules for the construction of PDA bio-conjugate as a sensing platform. The carbohydrate-functionalized PDAs are listed in Table 1.
Table 1. Components for carbohydrate functionalized PDA system.

| Active Component      | Analytes                  | Chemical Structures | Ref  |
|-----------------------|---------------------------|---------------------|------|
| Sialic acid           | virus                     | ![Chemical Structure](image1) | [11–14,21,27] |
| Mannose               | E. coli                   | ![Chemical Structure](image2) | [15]  |
|                       | E. coli                   | ![Chemical Structure](image3) | [16]  |
|                       | lectin                    | ![Chemical Structure](image4) | [17]  |
| Succinoglycan octasaccharide | flavonoids               | ![Chemical Structure](image5) | [18]  |
|                       | Ba²⁺                      | ![Chemical Structure](image6) | [19]  |
| β-cyclodextrin        | arginine, lysine          | ![Chemical Structure](image7) | [20]  |
| GM1 glycoside         | cholera toxin             | ![Chemical Structure](image8) | [21,22,27] |
| GTb1 glycoside        | botulinum neurotoxin      | ![Chemical Structure](image9) | [21]  |
| β-glucoside           | E. coli                   | ![Chemical Structure](image10) | [23]  |
|                       | E. coli                   | ![Chemical Structure](image11) | [24]  |
| N-acetamide-β-glucoside | lectin                   | ![Chemical Structure](image12) | [25]  |
| β-maltotriose         | E. coli                   | ![Chemical Structure](image13) | [26]  |
| Sialic acid-β-glucoside | hemagglutinin (HA1)      | ![Chemical Structure](image14) | [28]  |

3. Lipid-Functionalized PDA

Other lipid moieties can be incorporated into PDA assembly, and lipid decoration is generally performed without covalent attachment (Table 2). Overall, the lipid portion inserted in the PDA system is larger than the carbohydrate portion, since the lipids interact well with diacetylene lipids. Interestingly, 40% dimyristoyl phosphatidylcholine (DMPC)/PDA vesicles display a color response in the presence of phospholipases [32,33]. The insertion of sphingomyelin and cerebroside into PDA also permits detection of sphingomyelinase and galactosidase [34]. These interfacial catalysts are ubiquitous in biomembranes, and the prepared systems can be used to detect the lipolytic process with the naked eye. The phospholipase action was detected on the silica microbead without nonspecific color change using PDA/DMPC and CaCl₂ [35]. Enzyme inhibitor assay using this system also provides potential for novel therapeutic insight.

Furthermore, various combinations of phospholipids (DMPC, dimyristoyl phosphoethanolamine (DMPE), dimyristoyl phosphatidylglycerol (DMPG), and cardiolipin) and PDA have been utilized for the detection of antimicrobial peptides (mellitin, magainin, and alamethicin) [36,37]. The peptide-membrane association has a direct relationship with the blue-to-red transition. In phosphatidylinositol-4,5-phospholipids (PIP₂)/PDA systems, aminoglycosidic antibiotics have been
visually and fluorescently expressed with a detection limit of 61 ppb for neomycin [38]. The mechanism follows the inhibition of PIP$_2$-phospholipase C signaling pathway by PIP$_2$ binding of neomycin. This biomimetic membrane system was also used to study penetration enhancers, membrane binding of casein oligomers, α-lactoalbumin, and β-lactoglobulin [39–41]. The effect of the physiological lipid molecule’s cholesterol and cardiolipin on membrane fluidity and thermal stability has been investigated using this biomimetic lipid/PDA system [42]. Using cholesterol-incorporated PDA, the bacterial pore-forming toxin streptolysin O was colorimetrically detected [43]. Since Streptolysin O (SLO) creates pores in biomembranes in a cholesterol-dependent manner, PDA surface disruption can be visually detected in a cholesterol-containing biomimetic PDA composition. Jelinek et al. have also evaluated the extent of membrane interactions of lipoproteins using a lipid/PDA platform [44]. In cases of disease related to lipid oxidation, reduced membrane–lipoprotein interactions may be a novel marker for oxidative stress-related diseases or provide a therapeutic methodology.

Glass-supported lipid (DMPC, sphingomyelin, cholesterol)/PDA films have been employed for visual detection of membrane active molecules such as polymixin B and for bacterial fingerprinting [45,46]. Further, to diagnose human diseases, an array-based lipid/PDA gel was developed, and the blue-to-red transition was induced by plasma molecular components [47]. An omics-based approach can clearly distinguish disease states using a prepared robust and stable sensing gel matrix. For materialized PDA systems, the color change can be determined using a digital color analysis (DCA) algorithm instead of CR%.

Conventional PDA solutions employ nano-sized vesicles, but micrometer-sized giant phospholipid/PDA vesicles have also been utilized to detect and analyze membrane processes [48]. This system does not require sonication of the lipid suspension in water and provides easy analysis using optical and microscopic methods. The time course of vaccinia virus internalization was traced using fluorescence microscopy imaging. Local interactions of vesicles with polymyxin B or uniform interactions of the membrane with lipophilic pharmaceuticals could also be observed. Used not only for a specific purpose, membrane lipid components are also generally co-incorporated into various PDA platforms to mimic biomembrane condition or modulate the PDA assembly.

### Table 2. Biomimetic Lipids-PDA systems for detection.

| Biomimetic PDA                  | Detection Fields                                      | Ref       |
|--------------------------------|-------------------------------------------------------|-----------|
| DMPC $^1$/PDA                  | Phospholipase action                                   | [32–35]   |
|                                | Penetration enhancers (azone, oleic acid etc.)         | [39]      |
|                                | Casein oligomers                                       | [40]      |
|                                | α-Lactoalbumin, β-lactoglobulin                        | [41]      |
| DMPE $^2$, DMPC/PDA            | Antimicrobial peptides (mellitin, maganin, alamethicin, polymixin B) | [36,37,45] |
| DMPC,sphingomyelin,cholesterol/PDA | Polymixin B                                            | [45]      |
|                                | Bacterial fingerprinting                               | [46]      |
| DMPC,DMPE,DGS $^3$,DMPG $^4$/PDA | Lipoproteins                                           | [44]      |
|                                | Plasma molecular components                            | [47]      |
| DMPC, DMPE, DMPG/PDA giant vesicles | Vaccina virus, polymixin B, lipophilic pharmaceutical (nortriptyline) | [48]      |
| PIP$_2$ $^5$/PDA               | Aminoglycoside antibiotics (neomycin)                  | [38]      |
| Sphingomyelin/PDA              | Sphingomyelins action                                  | [34]      |
| Cerebroside/PDA                | Galactosidase action                                   | [34]      |
| Cholesterol/PDA                | SLO $^6$                                               | [43]      |

$^1$ dimyristoyl phosphatidylcholine; $^2$ dimyristoyl phosphoethanolamine; $^3$ 1,2-dipalmitoyl-sn-glycero-3-succinate; $^4$ 1,2-dimyristoyl-sn-glycero-3-phosphorylglycerol; $^5$ phosphatidylinositol-4,5-phospholipids; $^6$ streptolysine O.

### 4. Protein (Antibody) Functionalized PDA

Antibodies protect cells against foreign antigen by recognizing antigens with high affinity and selectivity [49]. Specific molecular recognition is achieved by noncovalent interactions,
including hydrophobic interactions, Van der Waals forces, electrostatic interactions, and hydrogen bonds. The interaction can be visualized by using hybridization of antibodies with a PDA domain. Jiang et al. reported a chromatic immunoassay based on antibody-functionalized PDA [50]. Vesicles of DMPC/TRCDA were linked with goat anti-h-IgG via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling, bovine serum albumin (BSA) blocking was used to block non-specific absorption, and then polymerization was performed. The resulting PDA produces a discernible color change in the presence of 1 ng/mL antigen. Recently, influenza detection was conducted using antibody-modified PDA [51,52]. The antibody for the H5 influenza virus was modified using EDC/NHS coupling after polymerization of diacetylene vesicles [51]. In another case, the antibody was conjugated with NHS-functionalized PCDA [52]. The modified vesicle and polydiacetylene difluoride (PVDF) supported one were polymerized. Although antibody conjugation methods differ slightly by case, DMPC was incorporated for enhanced sensitivity.

Signal improvement could also be obtained by using an ethylenediamine interlinker between individual antibody-PDA liposomes for target pathogens [53]. Subsequently, a hybrid stimulus strategy was introduced by the same group (Sim et al.), in which the primary response by a monoclonal antibody-conjugated PDA chip was amplified by the force generated by polyclonal antibody-conjugated magnetic beads, for prostate cancer detection [54]. The resulting sensitivity was as low as 0.1 ng/mL of the target complex. Employing a similar principal, polyclonal antibody–conjugated gold nanoparticles were utilized for signal amplification in micro-arrayed PDA sensors for human immunoglobulin E (hIgE) detection, amplifying the signal up to 100-fold over the primary response [55]. The mechanical stress was provided by the magnetic force.

To detect phosphinothricin acetyltransferase (PAT) in genetically modified crops, a microbead-assisted PDA sensor tethering anti-PAT was developed [56]. DMPC/TRCDA vesicles were immobilized on amine-coated silica beads, coated with anti-PAT using EDC coupling, and protected using ethanolamine, followed by polymerization. With respect to the same antibody, a recent study suggested another immunohydrogel bead–type PDA sensor using a polyethylene glycol diacrylate hydrogel matrix [57]. Anti-hepatotoxin microcystin-leucine-arginine (MC-LR) was also conjugated to PDA using EDC/NHS, and the PDA immunosensor showed a detection limit of 1 ng/mL, which is the maximum content recommended by the World Health Organization (WHO) for human drinking water [58]. Salmonella detection was performed using EDC crosslinked antibody-PDA vesicles [59]. Several kinds of immunoglobulin-PDA-phospholipids, synthesized using another cross linker sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), were encapsulated in hydrophilic siloxane sol-gels for use as a solid-state colorimetric biosensor [60].

Although conjugation generally employs the amine group of the antibody protein, a few reports have described methods using the sulfhydryl group of the antibody and PCDA-maleimide (Figure 3) [61,62]. The prepared PDA vesicle was coated on nanoporous membranes such as PVDF, mixed cellulose esters (MCE), cellulose nitrate (CN), nylon, and polycarbonate (PC) [62]. Coating was conducted using a syringe and positive pressure with possible pretreatment of polylysine. Furthermore, a noncovalently linked antibody-PDA array system was developed using the avidin-biotin interaction (Figure 3) [63].
were added to PDA composed of DMPC:TRCDA 4:6, permitting detection of the physiologically
was 2
and polymerized for detection of glucose. For ionophore-modified PDA, valinomycin and monesin
conformational change for a solid-type PDA sensor was published in 1997 [69]. Hexokinase
to detect glucose and specific cations, respectively [69,70]. The first example to utilize protein
L7A7K4G) was synthesized, with the epitope displayed at the
−2 Hemagglutinating Units (HAU) based on red fluorescence emission. They suggested that the
sterol repulsion between probe and target is more important for PDA sensing signal amplification
Another antibody (100 μg/mL) to BVDV IgG antibody (~150 kDa), and selectivity was
that generates a greater stress on the PDA backbone. There is still room for
improvement in terms of direct detection, colorimetric response (CR%), and the detection time before it
is ready for application in a real sensing format. Kim et al. expanded the idea in their next report using an
influenza A virus M1 peptide tethered PDA liposome microarray [66]. The resulting detection limit
was 2
−
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steric repulsion between probe and target is more important for PDA sensing signal amplification
than binding strength. This viewpoint is similar to the sialic acid functionalized PDA system
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detected in the range of millimolar concentration, and the high concentration range is regarded to be due to the target and probe size effects mentioned above. Alternatively, it is thought the conformational change before and after target binding might not be enough to perturb the PDA backbone. Unstructured proteins undergoing induced conformational change on binding can be effective for coupling with the signaling domain, PDA.

Small peptides have also been used for the construction of PDA-based sensor systems. A specific tripeptide tryptophan-histidine-tryptophan (WHW) was functionalized to PDA, permitting detection of trinitrotoluene (TNT) explosives via multivalent binding mode [71]. Although the specific sequence for the TNT recognition motif was known to be WHWQRPLMPVSI based on phage display [72], only the truncated tripeptide PDA showed a colorimetric response. When using the full sequence, the large distance between the recognition site and the PDA backbone might pose an obstacle for electronic band change in the PDA backbone. The major driving force was considered to be pi-pi interactions between tryptophan and TNT’s aromatic ring. For sensitive and selective on-time monitoring, the system was further fused with single-walled carbon nanotube field-effect transistors (SWNT-FET) [73]. The combinational platform reaches 1 fM sensitivity. In addition, a mixed PDA assembly conjugated with fluorescent pentalysein and histidine was designed to mimic antibacterial (polymixin B)-lipopolysaccharide (LPS) interactions [74]. This system was utilized as a turn-on fluorescent sensor to detect LPS, a unique glycolipid produced by gram-negative bacteria, at low micromolar concentration. In a very recent study, a lysine- and arginine-rich pentapeptide, arginine-lysine-alanine-arginine-lysine (RKARK) was conjugated to TRCDA, producing two hydrophobic diacetylene chains at the C-terminal glutamic acids [75]. Based on its cell-penetrating properties, the cationic peptide-diacetylene permitted cell imaging by the polymerization that occurred during incubation with HeLa cells. In particular, endosomal uptake or membrane imaging could be controlled by different morphologies formed depending on the concentration; the assembly shows good biocompatibility.

Specific amino acid–derivatized PDA function in identification of LPS produced by different bacteria [76]. The amino acids tryptophan and tyrosine, which are essential residues for carbohydrate-binding proteins, were selected [77]. Glycine-linked PDA also exhibits a colorimetric response to Pb^{2+}, and when embedded in a polycrylonitrilne nanofibrous membrane, it can be applied as a colorimetric strip [78]. Recently, histidine-functionalized PDA was reported, optimized for pH-responsive small interfering RNA (siRNA) delivery [79]. When conjugated to PDA with a trioxatriedecane linker, the designed structure displayed a distinctive function compared with primary amine–linked PDA.

6. Nucleic Acid Functionalized PDA

Single-stranded deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) hybridize with complementary DNA or RNA. When a specific probe sequence is given (DMPC:TRCDA:probe

Figure 4. A schematic representation of antibody and antigen for conjugation with PDA.
sequence-cholesterol 29:70:1), a target sequence can be detected via hybridization [80]. Although the nucleic acid is not directly linked to TRCDA, 20 nM target DNA was detectable using the naked eye. As a practical application of specific DNA-RNA hybridization, a sequence-specific DNA probe was conjugated to the surface of a PDA vesicle on an α-cyclodextrin-coated chip [81]. After polymerization, bacterial cells with the target 16S ribosomal RNA (rRNA) were detected on this PDA biochip, which provides a reliable and label-free pathogen detection method.

Furthermore, given that a binding event can create a new structure, guanine-rich single-stranded DNA (ssDNA) is of interest because it forms a quadruplex upon binding with K⁺ [82]. Kim et al. have developed solution- and solid-type G-rich ssDNA-modified PDA via direct conjugation, which produces anionic repulsion at the PDA surface upon binding with K⁺ [83]. The platform exhibits selective potassium ion detection in the range of sub-millimolar concentrations without interfering with sodium ions (the physiological concentration of potassium ions is 3.5–5.3 mM). Following that, selective and sensitive Hg²⁺ detection was achieved using a PDA microarray conjugated with a thymine-rich ssDNA aptamer (5′-TTCTTTCTTCCCCCTTGGTTTTGTT-3′) [84]. The detection limit was 5 µM, which is much lower than the previous potassium detection range. An aptamer-based strategy was advanced using a bidentate aptamer-modified PDA for detection of thrombin protein [85]. The enhanced sensitivity and specificity was achieved by multisite binding and aggregation at a micromolar concentration. Another PDA-based aptasensor was designed targeting LPS of E. coli O157:H7 [86]. Using this aptamer-based colorimetric sensor, a range of 10⁴–10⁸ colony-forming units (CFU)/mL was specifically detected. Recently, Zn(II)-specific aptamers were also conjugated to PDA, to develop a PVDF strip aptasensor [87]. From the results, it is suggested that the increased aptamer length and unfolding of the hairpin aptamer cause greater steric repulsion to increase sensor sensitivity.

7. Other Biomolecule Functionalized PDAs

Other small molecules are useful to functionalize PDA for specific interactions and detection systems. Biotin, soluble vitamin B₇, binds to tetrameric avidin with high affinity (Kₐ = 10¹⁵ M⁻¹) [88]. A biotin-modified PCDA with an ethylene oxide spacer was designed, and streptoavidin interaction was colorimetrically detected with a large insoluble cluster by tetrameric association [89]. Moreover, this special interaction has been utilized for immobilization of PDA vesicles. For example, streptavidin-functionalized micropatterned PDA chips have been fabricated using biotinyl PCDA liposomes immobilized on a microchip [90]. Biotin-labeled target DNA transmitted the biotin-avidin interaction into fluorescence for pathogen detection. In this case, since a specific target sequence is not utilized, it can be universally applied to nucleic acid amplification–related detection systems in the fields of infectious diseases, forensic medicine, or genetically modified organisms. Another vitamin, B₉ or folate, was conjugated to PDA liposomes for tumor imaging and targeted drug delivery [91]. Since the surface of cancerous cells over-express folate receptors, folic acid is a good ligand for targeted drug delivery [92]. In this respect, folic acid was modified with dodecylamine, and it forms folate-PDA liposomes (egg phosphatidylcholine:PCDA:folate dodecyl amine 8:1:0.8). This novel system supports future theragnosis showing high docetaxel encapsulation as well as cancer cell-targeted fluorescence imaging. Biotinylated PDA might be an alternative to folated PDA, considering that biotin is a growth promotor.

The neurotransmitter dopamine has been used as a simple PDA sensor for lead (II) ions [93]. Based on the lead catecholate complex, co-assembly of dopamine-linked PCDA and PCDA permitted colorimetric and fluorogenic detection of Pb²⁺. This PDA liposome was further immobilized on alginate hydrogel microbeads. The hydrogel support added stability and sensitivity to the solution-based PDA platform, and the resulting detection limit was 200 ppb for lead ions. For detection of another toxic heavy metal, Hg²⁺, the nucleotide base thymine was incorporated into a PDA array [94]. T-Hg²⁺-T bonding creates liposomal aggregation on a 0.45-µm filter film, which permits naked-eye detection with a sensitivity of 0.1 µM. This system does not display any color change, but shows blue aggregated spot in the presence of 0–10 µM of Hg²⁺. Taken together, small targeting moieties such
as arginine-glycine-aspartic acid RGD, partial aptamers, and hormones have the potential for use in design of PDA-based sensors.

8. Conclusions and Outlook

This review summarized the progress in biomolecular functionalization of PDA. In terms of biomimetics, membrane interfacial events have been successfully realized by combining PDA with biomolecules. This improves our understanding of the organization of complex biological components and cellular processes. Above all, the shift from the invisible nano-world to the visible range remains attractive, and worthy of further exploration. This study guides us in developing new sensor platforms for biomedical and environmental applications. Since the stimuli-responsive color or fluorescence change property of PDA are successfully linked to bio-sourced macromolecules and small molecules, bioanalytical and biocompatible sensing tools can be fabricated. Identification of good combinations related to various cellular interactions and reactions will permit consistent development of novel sensor systems. In addition, the approach used in protein-PDA conjugates can be attempted in carbohydrate-PDA or lipid-PDA systems. To date, macromolecular proteins and nucleic acids have been utilized to modify PDA assemblies, due to their specific interactions with target molecules. Thus, the use of macromolecular carbohydrates such as polysaccharides may open a new area of PDA work. Although carbohydrates are crucially associated with various cellular interactions and recognition complexes, the potential, except for monosaccharides, has been overlooked. In addition, fusion with phage display and systematic evolution of ligands by exponential enrichment (SELEX) technology will permit fabrication of specific peptide and nucleic acid sequences for effective conjugation to PDA. Accordingly, technological support for carbohydrate sequence generation may make up for the gap with other biomolecule-conjugated polymers containing PDA. Another pioneering work of biomolecule-PDA conjugation is development as a theragnostic tool. PDA vesicles have an interior, which can encapsulate other drugs or active ingredients, as well as a fluorogenic exterior. To date, the functionality of the membrane itself has been the focus, and the inner portion shows promise for co-use for future work on PDA in theragnosis and monitoring. Without doubt, smart PDA materialization will also enrich studies on fused synthetic polymers-biopolymers.

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