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Review

Functionalization of Water-Soluble Conjugated Polymers for Bioapplications

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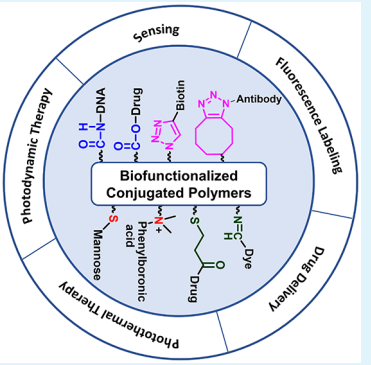
ABSTRACT: Water-soluble conjugated polymers (WS-CPs) have found widespread use in bioapplications ranging from *in vitro* optical sensing to *in vivo* phototherapy. Modification of WS-CPs with specific molecular functional units is necessary to enable them to interact with biological targets. These targets include proteins, nucleic acids, antibodies, cells, and intracellular components. WS-CPs have been modified with covalently linked sugars, peptides, nucleic acids, biotin, proteins, and other biorecognition elements. The objective of this article is to comprehensively review the various synthetic chemistries that have been used to covalently link biofunctional groups onto WS-CP platforms. These chemistries include amidation, nucleophilic substitution, Click reactions, and conjugate addition. Different types of WS-CP backbones have been used as platforms including poly(fluorene), poly(phenylene ethynylene), polythiophene, poly(phenylenevinylene), and others. Example applications of biofunctionalized WS-CPs are also reviewed. These include examples of protein sensing, flow cytometry labeling, and cancer therapy. The major challenges and future development of functionalized conjugated polymers are also discussed.



KEYWORDS: *conjugated polymer, biofunctionalization, biosensor, fluorescence, flow cytometry, cancer therapy*

1. INTRODUCTION

Conjugated polymers (CPs) make up a class of organic polymers with π-conjugated backbones and versatile side-chains. Building on advances in synthesis and understanding the properties of conjugated polymers that occurred in the 1970s,[1] the field of conjugated polymers has flourished over the past few decades.[2−10] Because of their unique electronic and optical properties, combined with their polymeric nature and facile preparation and processing, CPs display attractive advantages for application in optoelectronic devices including polymer light-emitting diodes (PLEDs),[11] polymer solar cells (PSCs),[12,13] polymer field-effect transistors (PFETs),[14] optical sensors (chemo- and biosensors),[15] and next-generation wearable electronic devices.[16]

With the introduction of water-soluble ionic groups onto the polymer side-chains, conjugated polyelectrolytes were developed, extending the application of conjugated polymers to biological systems.[17−19] Conjugated polyelectrolytes are conjugated polymers with water-soluble ionic side-chains, which not only inherit the advantages of CPs including high molar absorptivity, energy transfer efficiency, fluorescence quantum yield, and photostability but also provide ionic interactions with analytes in aqueous solution. In addition, oligo(ethylene glycol) groups were also introduced onto CPs, improving their aqueous solubility and reducing undesired nonspecific interactions.[20,21] Throughout this review, water-soluble conjugated polymers will be abbreviated as "WS-CPs"; this abbreviation refers to both conjugated polyelectrolytes and nonionic, water-soluble conjugated polymers substituted with oligo(ethylene glycol) groups. Over the past two decades, a variety of WS-CPs have been developed with tunable chemical structures, bandgaps, energy levels, and solubility tailored to attain desired properties.[22−24] Some of the most prominent examples of WS-CPs are poly(fluorene-*co*-phenylene) (PFP), poly(phenylenevinylene) (PPV), poly(phenylene ethynylene) (PPE), polythiophene (PT), and polydiacetylene (PDA). The chemical structures of these polymer backbones and commonly used water-soluble side-chains are summarized in Scheme 1. Cationic WS-CPs can interact with negatively charged phosphate groups through electrostatic interactions and have been used for nucleic acid and cell membrane binding.[25−27] Anionic WS-CPs have shown advantages on metal ion sensing by electrostatic and coordination interactions.[28,29]

As the fields of chemical biology, biochemistry, and biomedical engineering continue to develop, the demand for materials that can interact strongly and specifically with biomolecules, biomacromolecules, and biomaterials is growing.

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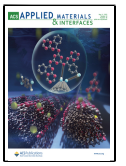
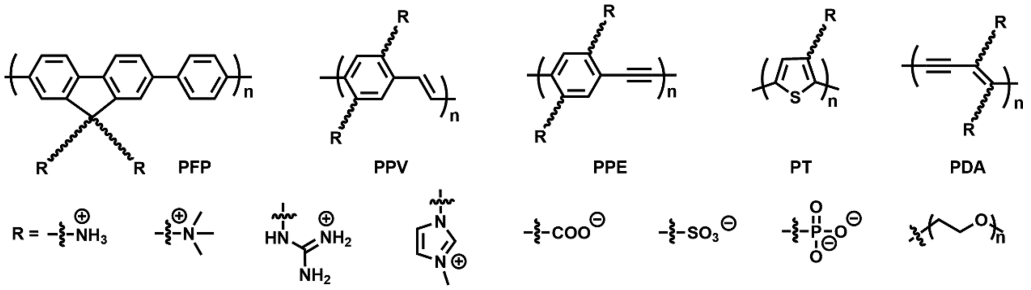


Scheme 1. Backbones and Water-Solubilizing Groups of Common Examples of WS-CPs



WS-CPs with intriguing properties and good biocompatibility are promising candidates for these purposes.[30] However, the traditional WS-CPs described above can only bind to biological targets through nonspecific electrostatic, van Der Walls, and hydrophobic interactions. Further functionalized WS-CPs are required to allow more specific interactions between the polymers and biological targets including proteins, nucleic acids, cells, and tissues. Encouragingly, functionalization of WS-CPs has been realized by rational design and fabrication strategies. Many different kinds of intelligent functionality have been linked to WS-CPs, ranging from small molecules such as biotin,[31,32] folic acid,[33] mannose,[34] lipids,[35] drugs,[36] dyes,[37,38] and ligands[39] to biomacromolecules including enzymes,[40] antibodies,[41] peptides,[42] and nucleic acids.[43] Functionalized WS-CPs further improve the high performance of CPs, opening up significant opportunities for customizing materials for various biomedical applications, particularly for sensing, imaging, drug delivery, and therapy.

Despite the fact that many papers have given detailed reviews on the design and applications of WS-CPs,[22,23,44−57] there are only a few reviews that specifically and systematically introduce the synthetic strategies and bioapplications for biofunctionalized WS-CPs.[30,58] This review aims to provide a broad overview on the development of biofunctionalized WS-CPs, with a specific focus on optimal synthetic strategies for incorporation of functional groups, followed by highlighting some prominent biomedical applications of biofunctionalized WS-CPs, specifically for sensing and therapy. The review concludes with a summary and discussion of the major challenges and future development of biofunctionalized WS-CPs.

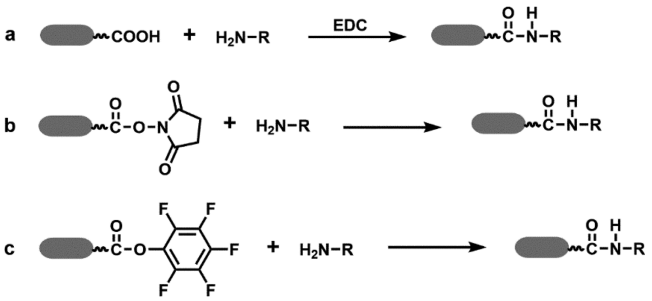
2. SYNTHETIC STRATEGIES FOR BIOFUNCTIONALIZATION OF WS-CPS

The development of bioconjugation reactions and bioorthogonal reactions provides a variety of strategies for the modification of conjugated polymers. In this section, we summarize these synthetic strategies. Most strategies that have been previously used can be divided into four general types: (1) amidation or esterification reactions between carboxylic acid groups and amine or hydroxyl groups; (2) 1,3-dipolar cycloaddition reactions between azides and alkynes; (3) nucleophilic substitution reactions, specifically referring to the reactions of amino, hydroxyl, or mercapto groups with alkyl halides; (4) nucleophilic addition reactions including the reactions of amino and mercapto groups with α,β-unsaturated carbonyl compounds, and Schiff base formation reactions.

2.1. Amidation Reactions. Amine, hydroxyl, and carboxylic acid groups are widely present in bioactive substances such as amino acids, proteins, nucleic acids, carbohydrates, drugs, and ligands. It is also convenient to introduce these groups onto polymers or functional compounds by straightforward synthetic protocols. Amides are formed from the condensation reaction of carboxylic acids and amines. However, the reaction of these two functional groups cannot take place spontaneously at room temperature. To overcome this problem, carboxylic acids are usually activated by coupling reagents that transform them into reactive electrophiles, which are readily attacked by the nucleophilic amine groups at room temperature.[59]

Carbodiimides are often used coupling reagents for amidation reactions in the synthesis of functionalized WS-CPs. N-Substituted carbodiimides react with carboxylic acids to form *o*-acylisourea derivatives that can further react with amines to form amide linkages. *N,N′*-Dicyclohexyl carbodiimide (DCC) is generally used for coupling in organic media, and the byproduct dicyclohexylurea is insoluble and is removed by filtration. 1-Ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDC) is a water-soluble carbodiimide, and the byproduct of the reaction, isourea, is also water-soluble, which facilitates purification of the target. Thus, EDC has been used extensively as the coupling reagent of choice for amidation reactions in aqueous solution (Scheme 2a).

Scheme 2. Amidation Reactions for Functionalization of WS-CPs[1]



[1](a) Amide bond formation by the reaction of carboxylic acid and amine with EDC as the coupling reagent. (b) Amide bond formation by the reaction of *N*-succinimidyl activated ester and amine. (c) Amide bond formation by the reaction of pentafluorophenyl activated ester and amine.

Active ester-amine chemistry is another approach to activate formation of amide bonds (Scheme 2b,c). Indeed, when EDC is used as the coupling reagent, *N*-hydroxysuccinimide (NHS) or sulfo-NHS (Scheme 3) is often used as an additive to improve amidation efficiency or produce dry-stable NHS activated ester derivatized WS-CPs. NHS can rapidly convert unstable *o*-acylisourea intermediates to more stable NHS esters while promoting amide bond formation under mild conditions. By using this method, Swager et al.[60] covalently attached a

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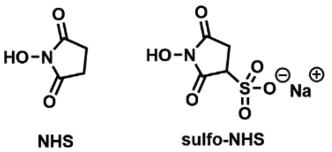
Scheme 3. Chemical Structures of NHS and Sulfo-NHS



fluorescence-quenching peptide and a dinitrophenyl chromophore onto a PPE-type polymer (**1a** and **1b**, Scheme 4). Kim et al.[61] further introduced oligonucleotides and antibodies on PPE type CPEs (**2** and **3**, Scheme 4) by using EDC and sulfo-NHS as coupling reagents. By controlling the stoichiometric amount of PPE during the bioconjugation reaction, PPE-antibody conjugates were prepared with different PPE/antibody ratios.[41]

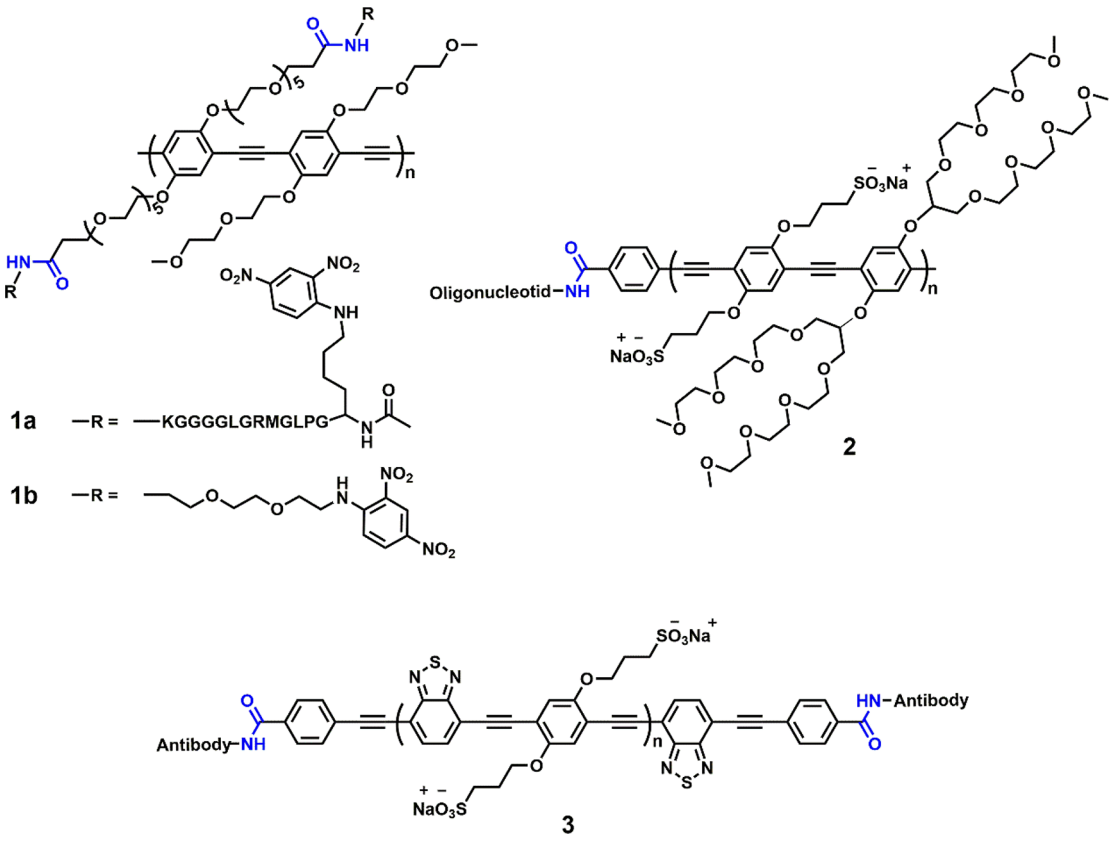
Sun and co-workers optimized the efficiency of amide coupling reactions between a water-soluble PPE and biotin-ethylenediamine by systematically exploring a variety of coupling reagents and reaction conditions (Scheme 5).[62] It was found that a two-step reaction in a solution with sequentially adjusted pH gave rise to the highest biotin loading onto the PPE polymer. In step 1, PPE reacted with EDC and NHS in 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer (pH = 5) for 1 h for the formation of active NHS ester. In step 2, after biotin-ethylenediamine was added, the pH was increased to 8, which benefits the nucleophilic addition of biotin-ethylenediamine to the NHS ester. NMR analysis of the product polymer revealed that coupling yields were ∼50% based on the stoichiometry of the carboxylate groups in the PPE. On the basis of this optimized method, a family of functionalized PPEs was prepared that contained biotin (**4a**), rhodamine (**4b**), cholesterol (**4c**), mannose (**4d**), and folate (**4e**) moieties in the polymer side-chains (Scheme 5).

Pentafluorophenol (PFP) active esters (Scheme 2c), which feature higher reactivity and improved hydrolytic stability compared to NHS esters,[63−65] have also been used for functionalization of WS-CPs. Wang et al.[66] prepared an oligo(*p*-phenylenevinylene) derivative (**5** in Scheme 6) functionalized with pentafluorophenol active esters via an esterification reaction of the carboxylic acid with PFP by using DCC and 4-dimethylaminopyridine (DMAP) as the coupling reagents. Because of the perfluoro effect and the π-electron structure of pentafluorophenol moiety, compound **5** displayed spectral changes with the departure of the pentafluorophol active ester groups during reactions with amines. Taking advantage of the mild reaction conditions and high reactivity of the active ester-amine chemistry, coupled with the fluorescence spectral changes of **5** during the amidation reactions, amine reactivity profiling in living cells was realized by monitoring the fluorescence signal of **5**.

Although active ester-amine chemistry is a powerful tool for the modification of WS-CPs, the hydrolytic stability of active esters can limit their use in specific reaction media and also limits their long-term storage. For example, the half-life of alkyl NHS esters is typically 4−5 h at 0 °C at pH 7.0.[67] However, at pH 8.0 and 25 °C, the half-life is only 1 h.[68] To minimize the effects of hydrolysis and maximize the modification, a high concentration of the amine nucleophile is necessary in the reaction medium.

2.2. Huisgen 1,3-Dipolar Cycloaddition (Click) Reactions. *2.2.1. Cu(I)-Catalyzed 1,3-Dipolar Cycloaddition Reactions.* Click chemistry has been widely used in many areas including drug discovery and materials chemistry since it

Scheme 4. Chemical Structures of 1−3



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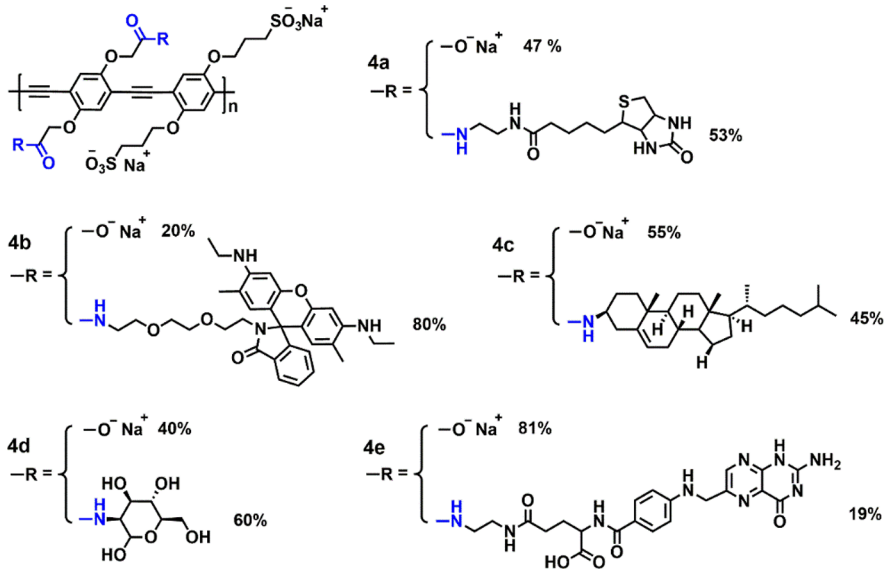
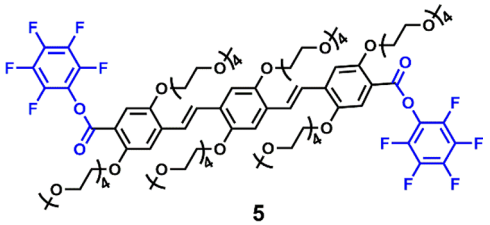
Scheme 5. Chemical Structures of 4a–4e



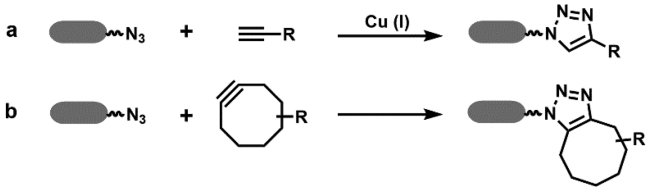
Scheme 6. Chemical Structure of 5



5

was introduced by Sharpless in 2001.[69,70] Click reactions need to meet the following criteria: (1) modularity; (2) compatibility with a broad range of functional groups and conditions; (3) high yield reaction, producing benign byproducts that can be easily separated without chromatography; (4) stereospecificity; (5) easy to perform. The Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction of azides with alkynes is the most widely used Click reaction (Scheme 7). In addition to the merits

Scheme 7. (a) Cu(I)-Catalyzed Huisgen 1,3-Dipolar Cycloaddition Reaction between Azides and Alkynes for Functionalization of WS-CPs; (b) Strain-Promoted [3 + 2] Cycloaddition of Azides and Cyclooctynes



mentioned above, the functional groups needed for this reaction are easy to introduce, and the reaction can be carried out in organic or aqueous media.[71,72] The azide/alkyne Click reaction is highly specific with 1,4-disubstituted-1,2,3-triazoles as the only product in the reaction. Several methods for introducing Cu(I) species *in situ* to the reaction system have been studied.[73,74] Direct addition of a Cu(I) salt, for example, CuI or CuBr, requires anaerobic conditions and is usually performed in organic solvents. An effective way to overcome the influence of O_2 uses Cu(II)SO_4 in combination with sodium ascorbate or ascorbic acid. The ascorbate reduces Cu(II) to Cu(I) making it available to catalyze the Click reaction. This type of reaction can be performed under aerobic conditions in aqueous solution. In addition, bacterial redox systems have also been shown to reduce Cu (II) to Cu (I) to activate the Click reaction.[75,76]

Bunz and co-workers synthesized a series of functionalized PPEs by using the Huisgen 1,3-dipolar cycloaddition reaction.[77−79] The sequence was realized by first preparing a PPE with pendent triisopropylsilyl-protected ethynyl groups. Subsequently, the triisopropylsilyl groups were removed by tetrabutylammonium fluoride *in situ*, unmasking the terminal alkynes to react with functional azides in the presence of Cu(II)SO_4 and sodium ascorbate. The Click reaction was carried out for 48 h at 60 °C. Five functionalized PPEs (**6a–6e**, Scheme 8) were obtained in high yield via this postpolymerization modification strategy. On the basis of this approach, they further synthesized a series of water-soluble PPEs functionalized with mannose units and studied their applications for biosensing and bacterial detection.[78,79] In more recent work, additional WS-CPs and oligomers functionalized with saccharides were prepared by using the 1,3-dipolar cycloaddition reaction with sodium ascorbate and Cu(II)SO_4 at room temperature.[34,80,81] Instead of attaching functional moieties on the side-chains of WS-CPs, Bazan and co-workers introduced biotin onto the end of poly(fluorene) chains.[82] This was accomplished by first preparing a silylacetylene-terminated water-soluble poly(fluorene); following desilylation, the terminal alkyne was reacted with azide functionalized biotin to afford the triazole-linked biotin end groups. Because of the high yield and mild condition of this reaction, the same approach could also be used for introduction of a variety other functional groups onto WS-CPs including dihydropyridine, folic acid, oligo(ethylene glycol), and drugs.[83−86]

2.2.2. Strain-Promoted [3 + 2] Azide–Alkyne Cycloaddition Reactions. To alleviate the need to use Cu(I) to activate the alkyne, the release of ring-strain in cyclooctyne can be used to provide the driving force for the [3 + 2] azide–alkyne cycloaddition (Scheme 7b).[87] Although this Click reaction can be carried out in the absence of Cu(I) catalyst, the strain-promoted [3 + 2] azide–alkyne cycloaddition is slower than the

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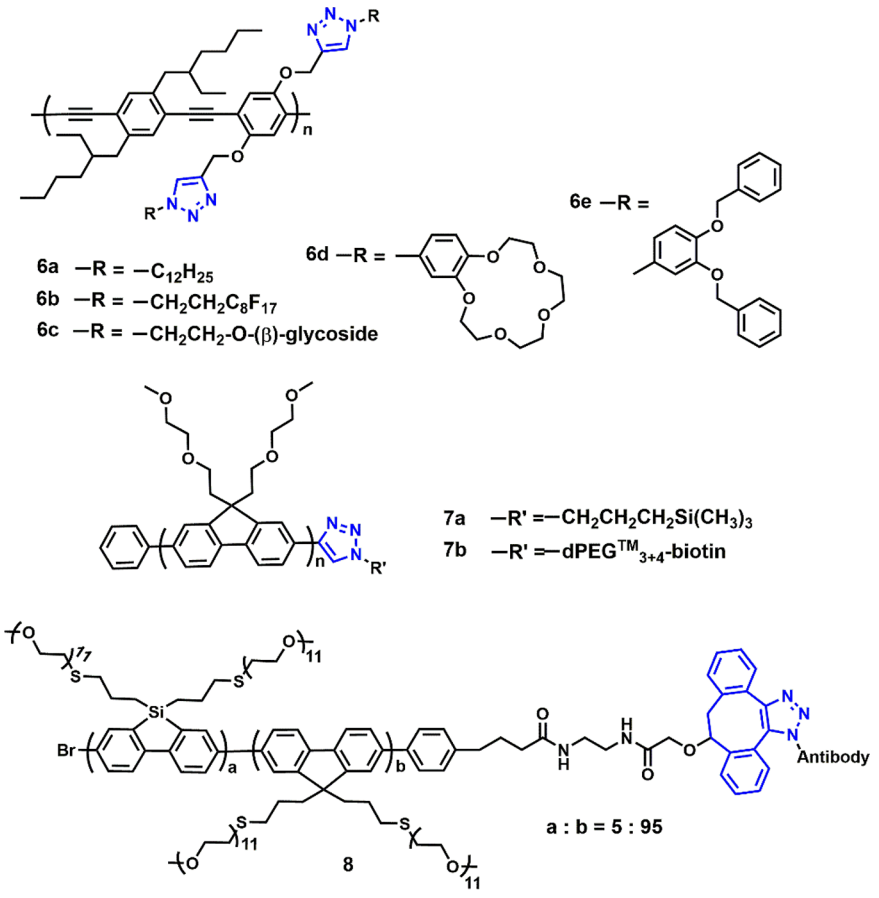
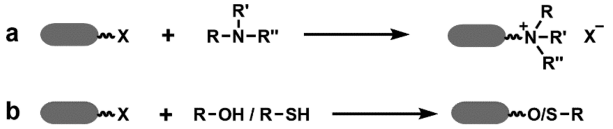
Scheme 8. Chemical Structures of 6−8



Cu(I)-catalyzed 1,3-dipolar cycloaddition reactions with terminal alkynes.[87] The convenience of strain-promoted [3 + 2] azide−alkyne cycloaddition reactions is that the reactions can be carried out in living systems *in situ* without Cu(I) so as to decrease cytotoxicity. Moreover, with the development of cyclooctyne derivatives, the reaction can be accelerated.[88−90] Gee and co-workers prepared a water-soluble poly(2,7-dibenzosilole) that is capped on one end with a dibenzocyclooctyne (DIBO) functional group.[91] The subsequent bioconjugation with an azido-modified antibody was carried out via the strain-promoted [3 + 2] azide−alkyne cycloaddition to afford WS-CP (**8**, Scheme 8), which was further used as an ultrabright fluorescent label for antibody-based flow cytometry.

2.3. Nucleophilic Substitution Reactions. Nucleophilic substitution reactions have been applied extensively for the modification of conjugated polymers (Scheme 9). Taking advantage of the nucleophilic nature of nitrogen, oxygen, and sulfur atoms, functional units can be efficiently attached to the polymers via nucleophilic substitution reactions. The halides or sulfonate esters are most often employed as the leaving groups.

Scheme 9. Nucleophilic Substitution Reactions for Functionalization of WS-CPs



Wang and co-workers introduced a phenylboronic acid moiety onto a polyfluorene backbone WS-CP (**9**, Scheme 10) via a nucleophilic substitution reaction between tertiary amino groups on the polymer side-chains with 2-[4-(bromomethyl)-phenyl]-1,3,2-dioxaborinane.[92] The quaternary ammonium salt that is formed by the substitution reaction not only provides the linkage to the functionalized groups but also improves the polymer's solubility in polar solvent. This is an example in which tertiary amino groups on the polymer side-chains react with alkyl bromide functionalized moieties. It is also feasible to use alkyl bromide functionalized polymers to introduce nitrogen containing functional groups via nucleophilic substitution. Thus, in a separate study, Wang and coauthors attached lapatinib, an anticancer drug, to a polythiophene backbone through reaction between a phenolic hydroxyl in the lapatinib derivative and iodoethyl groups in the polymer side chains (**10**, Scheme 10).[93] Liu and co-workers prepared poly(fluorene-*co*-phenylene) polymers featuring glucose functional groups through a postpolymerization reaction of the primary alkyl bromide side chains with thio-glucose tetraacetate (**11**, Scheme 10).[94] Formation of the thioether linkage was demonstrated by nuclear magnetic resonance spectroscopy (NMR) with a substitution ratio of 98%. On the basis of this method, they further prepared a series of functionalized WS-CPs pendent with glucose, mannose, and cyclic RGD peptide residues on the side-chains.[42,95]

2.4. Nucleophilic Addition Reactions. *2.4.1. Michael-type Addition Reactions.* The Michael addition involves the nucleophilic attack of soft nucleophiles such as thiols, amines, and carbanions on unsaturated electrophiles featuring electron

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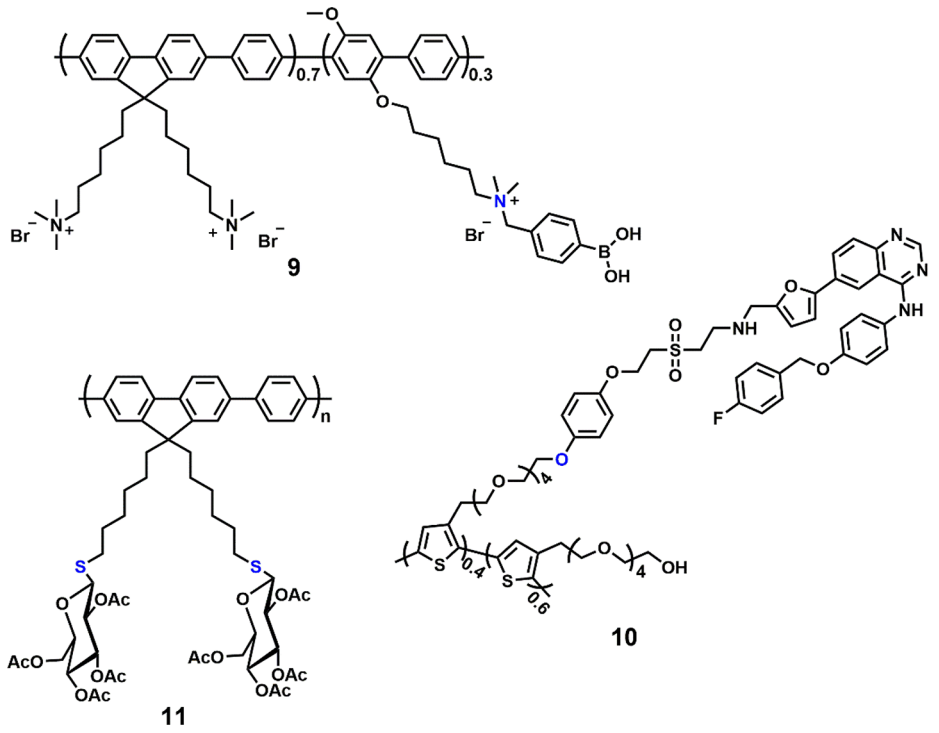
Scheme 10. Chemical Structures of 9−11

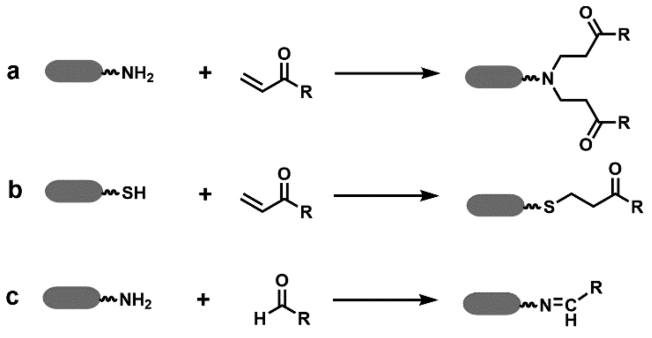


withdrawing substituents (Scheme 11a,b). It is a versatile approach for the mild preparation of numerous complex molecules in a simple, convenient manner with a quantitative conversion yield.

Wang et al.[96] introduced four carboxylate groups in each repeat unit of a polyfluorene backbone by the Michael addition of primary amino groups to methyl acrylate. The subsequent hydrolysis of the ester units afforded the final water-soluble carboxylic acid substituted polyfluorene (**12**, Scheme 12). By employing the same method, they also prepared a polythiophene with multiple carboxyl groups on the side-chains for chelation with Gd^{3+} ions.[43] A multiple carboxylate functionalized poly(phenylene ethynylene) was also synthesized by this reaction.[97]

The Michael addition between thiols and activated alkenes is also an attractive method for functionalization of WS-CPs. The reaction is one order of magnitude faster than that of amines at neutral pH values.[98] Zeng and co-workers incorporated a thiol-modified mannose onto polythiophene units featuring quinones through the 1,4-reductive Michael addition (**13**, Scheme 12).[99] Using a similar method, Wang and co-workers synthesized paclitaxel-oligo(*p*-phenylene vinylene) conjugates in a controlled manner such that an equivalent amount of acrylyl-functionalized paclitaxel was bound to one thiol terminal of an oligo(*p*-phenylene vinylene) over a total of four −SH in the side-chains (**14**, Scheme 12).[100] Swager et al. designed a protection and deprotection strategy to modify PPE type WS-CPs by the addition of thiol to maleimide groups.[101] They first masked the maleimide groups with furan using Diels−Alder chemistry. Thus, the maleimide was protected from participating in the polymerization process. Then furan can be removed in thermal conditions via a cycloreversion reaction, exposing maleimide to react with thiol containing moieties.

Scheme 11. Nucleophilic Addition Reactions for Functionalization of WS-CPs[1]



[1](a, b) Reaction of amino and mercapto group with α,β-unsaturated carbonyl compound. (c) Schiff base reaction.

2.4.2. Schiff Base Reactions. The Schiff base reaction was discovered by Hugo Schiff in 1864, and it usually refers to the condensation of amine bases (primary amines, hydroxylamines, hydrazines) and active carbonyls (aldehydes and some ketones) by nucleophilic addition (Scheme 11c).[102,103] Aromatic carbonyl compounds are more active than aliphatic carbonyls, and the aldehydes are more reactive than ketones.[104] The target products can be easily acquired in a high yield by simply mixing and stirring the primary amines with aromatic aldehydes. Tang et al.[105] attached a chemiluminescent group onto WS-CP side-chains through the Schiff base reaction (**15**, Scheme 13). The reaction was performed in ethanol, and formation of the imine C═N bond was confirmed by Fourier-transform infrared spectroscopy (FT-IR) signal at 1723 cm^{-1}.

It should be noted that the stability of the formed Schiff base imine is affected significantly by the pH in aqueous solution. The imine is stable in basic solution; however, it undergoes hydrolysis at low pH.[106,107] This property provides opportunities for the development of stimuli-responsive conjugated polymers based on Schiff base linkers.[108]

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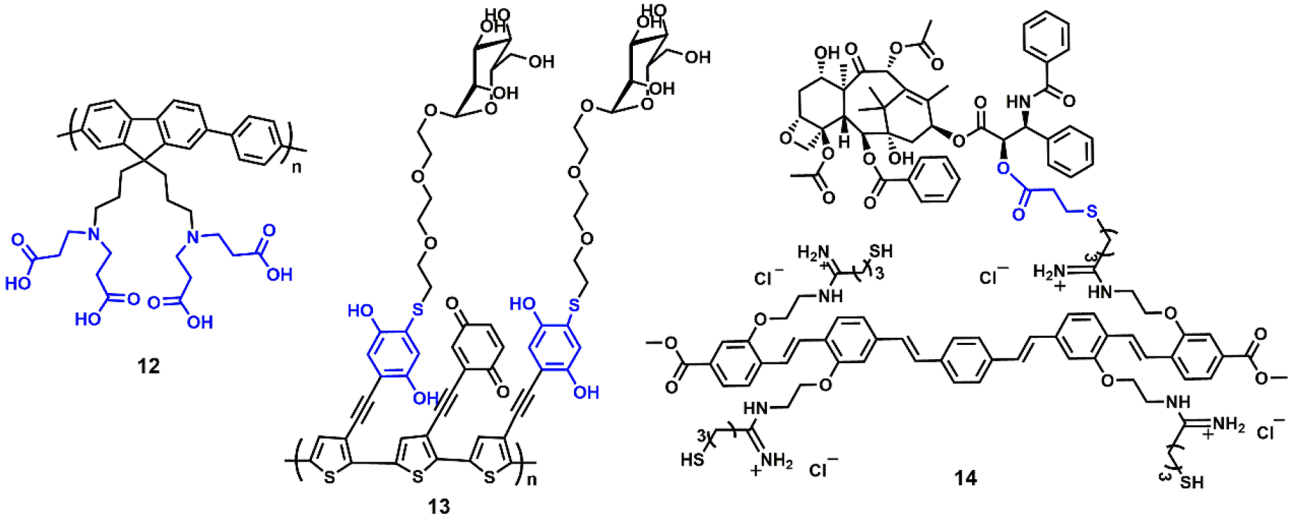
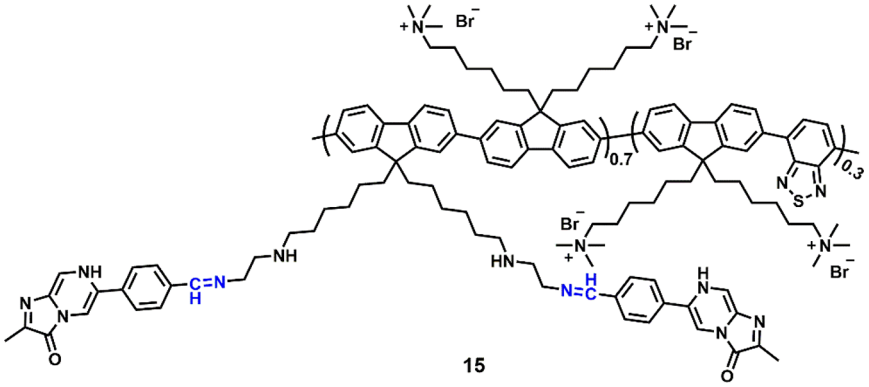
Scheme 12. Chemical Structures of 12−14



Scheme 13. Chemical Structure of 15



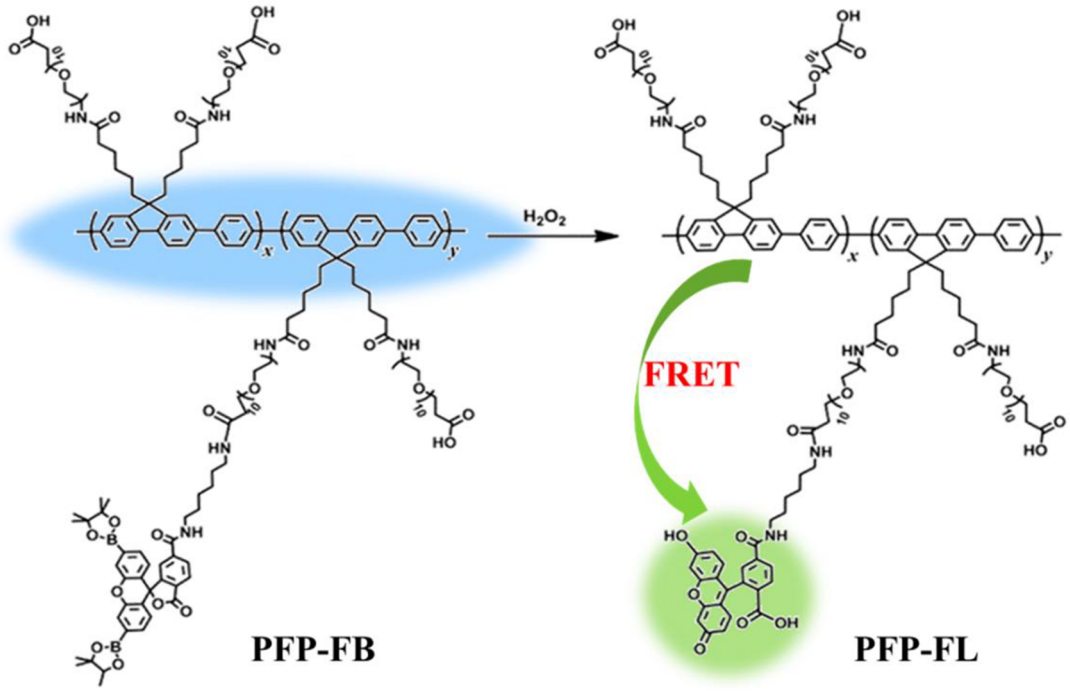


Figure 1. Reaction of PFP-FB with H_2O_2. Reproduced with permission from ref 109. Copyright 2015 American Chemical Society.

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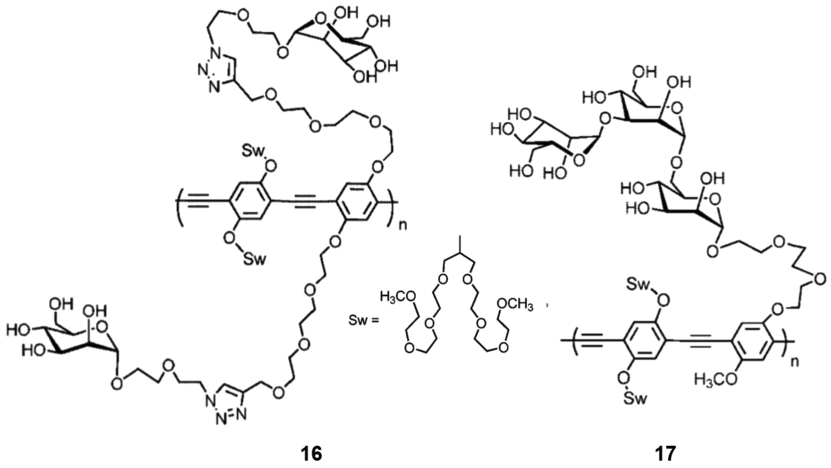
Scheme 14. Chemical Structures of 16 and 17[1]



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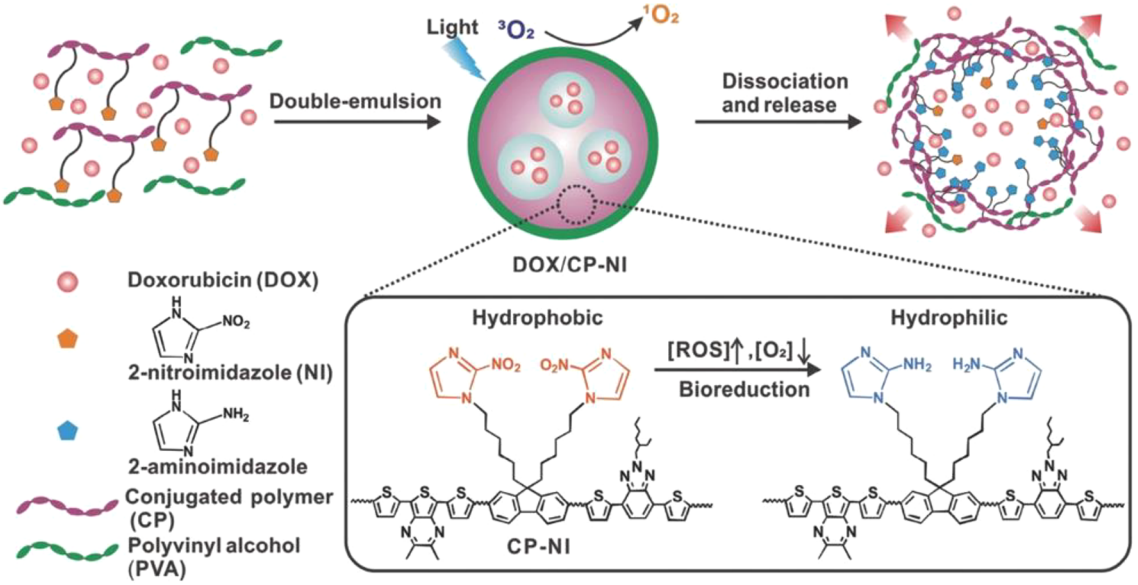

Figure 2. Preparation of DOX/**CP-NI** nanoparticles and the mechanism of the light-activated hypoxia drug-delivery system. (Reproduced with permission from ref 113. Copyright 2016 John Wiley and Sons.

3. APPLICATIONS OF BIOFUNCTIONALIZED CONJUGATED POLYMERS

3.1. Sensing. The delocalized electronic structure of conjugated polymers endows them with the following unique properties. (1) Conjugated polymers have large light harvesting ability due to the large optical cross section provided by the extended electronic structure. (2) Excitons are transported along a conjugated polymer backbone and can be trapped by energy or electron acceptors. Thus, careful tailoring conjugated polymer backbones and modifying with suitable energy acceptors can facilitate efficient energy transfer between the two components, which offers a method for signal amplification.

Taking advantage of these properties, Wang and co-workers reported a water-soluble conjugated poly(fluorene-*co*-phenylene) derivative with boronate-protected fluorescein (**PFP-FB**, Figure 1). This polymer features a sensitive, ratiometric fluorescence response to H_2O_2.[109] As shown in Figure 1, fluorescein is generated by the reaction of H_2O_2 with the boronate-protecting groups. Then the generated fluorescein acted as the energy acceptor for intramolecular fluorescence resonance energy transfer (FRET), leading to a change of the blue to green fluorescence ratios. By using this method, choline and acetylcholine were detected with high sensitivity and specificity due to the production of H_2O_2 by the acetylcholine esterase/choline oxidase coupled reaction.

Bunz and co-workers synthesized two water-soluble, sugar-substituted poly(*p*-phenyleneethynylene)s (**16** and **17**, Scheme 14).[78] These polymers were prepared by Click reactions, and they exhibited strong interactions with concanavalin A (Con A) with Stern−Volmer quenching constant (K_{sv}) values exceeding 10^8 M^{-1}. They reported that either extending the linker length between the sugar functionality and conjugated backbone (**16**), or incorporating more complex sugars (trimannose functionality in **17**), increased sensitivity to sugar binding domains. Polymers **16** and **17** also strongly interact with mannose-binding *E. coli* and lead to their aggregation.

Chemiluminescence-based sensing has some advantages over traditional photoluminescence-based sensing such as the

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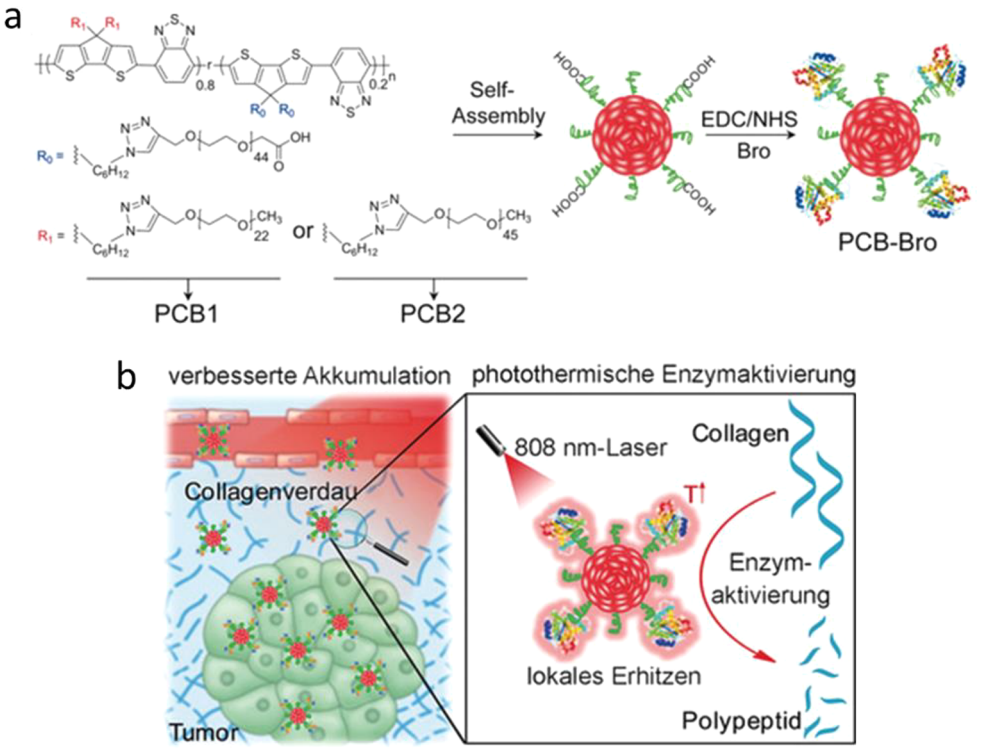

Figure 3. (a) Chemical structure of **PCB** and the preparation of **PCB-Bro**. (b) Illustration of the photothermic activation of **PCB-Bro** to degrade collagen I for enhanced accumulation of nanoparticles in tumors. Reproduced with permission from ref 40. Copyright 2018 John Wiley and Sons.

elimination of the need for an external light source, reduced background fluorescence, and reduced chance of phototoxicity. However, the low luminescence intensity and short luminescence time characteristic of chemiluminescence systems limit their broad applications. To overcome these limitations, Tang et al. constructed a chemiluminescence resonance energy transfer (CRET) system (**15**, Scheme 13) for detecting $O_2^{\bullet-}$ by covalent attachment of imidazopyrazinone groups on the polyfluorene side-chains.[70] Imidazopyrazinone is a well-established chemiluminescence sensor for $O_2^{\bullet-}$, which works as both $O_2^{\bullet-}$ recognition unit and the energy donor, while the WS-CP (**15**) acts as the energy acceptor and signal amplification matrix. This CRET system realized self-luminesce imaging for ultralow level $O_2^{\bullet-}$ *in vivo*.

3.2. Therapy. Photodynamic therapy (PDT), as a noninvasive and highly selective therapeutic method, is used in clinical therapy for cancer.[110] In the PDT process, a photosensitizer transfers excitation energy to surrounding O_2 or other substrates to generate reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) or free radicals, resulting in tumor cell death. Owing to their good light harvesting ability and efficient energy transport characteristics, conjugated polymers are promising photosensitizer agents, and they have attracted a great deal of attention.[111,112] Modifying conjugated polymers with functional groups or prodrugs enables the systems to achieve a synergistic therapeutic effect.[113]

During the PDT process in tumor tissue, the conversion of ground state oxygen ($^3O_2$) into singlet oxygen ($^1O_2$) and its subsequent reaction depletes the oxygen in the tissue and facilitates the generation of hypoxic condition in solid tumors.[114,115] Considering this, Gu and co-workers constructed a combination therapy system based on functionalized CPs, which integrates PDT with a hypoxia-responsive drug delivery strategy for enhanced anticancer therapy.[113] As shown in Figure 2, the backbone of the conjugated polymer (**CP-NI**) is composed of the dithiophene-thienopyrazine moiety alternating with dithiophene-benzotriazole moiety, which is employed for near-infrared (NIR) spectroscopy imaging and ROS generation. The polymer side-chains were modified with the hydrophobic group 2-nitroimidazole, which can be converted to hydrophilic 2-aminoimidazoles by a series of nitroreductases in a hypoxic environment. Doxorubicin hydrochloride (DOX), a chemotherapy drug, was encapsulated in the hydrophobic polymer and could be released when the hydrophobicity of CP was altered by the hypoxic environment. Such a light-activated hypoxia-responsive modality combines both PDT and chemotherapy, showing enhanced treatment efficiency *in vitro* and *in vivo*, providing a new strategy for the design of synergistic treatment.

Photothermal therapy (PTT) is emerging as another promising approach for treatment. Compared with PDT, which is limited by tumor hypoxia, the photothermal agents convert the energy from NIR light to heat via absorption followed by nonradiative decay. The resulting temperature increase in the cancer local environment causes irreversible cell damage. NIR absorbing WS-CPs with high photothermal conversion efficiency and good photostability have exhibited great advantages for PTT. Functionalized photothermal conjugated polymers modified with other therapeutic factors provide a strategy to achieve the improved tumoricidal efficacy. In this regard, Pu et al. attached a temperature sensitive collagen protease enzyme, bromelain (Bro), onto the conjugated polymer (**PCB**, Figure 3a) via an EDC/NHS activated amidation reaction.[40] At 808 nm irradiation, the prepared bioconjugated polymer (**PCB-Bro**) showed a good photothermal effect with a photothermal conversion efficiency and also good photothermal stability. The increased temperature caused by WS-CP increased the activity of Bro, promoting the collagen digestion in tumor extracellular-matrix, which facili-

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tated the accumulation of nanoparticles in tumor and better PTT effect (Figure 3b). Subsequently, they further built a hybrid semiconducting nanozyme composed of NIR conjugated polymer and chelated iron for photothermal ferrotherapy by coupling the photothermal property of WS-CP with the heat enhanced Fenton reaction.[116] In this system, the WS-CP showed nearly the highest photothermal conversion efficiency compared to all previously reported NIR-II photothermal agents. The increased temperature also amplified the Fenton reaction and obtained elevated •OH production. The seamless synergy of PTT and ferrotherapy has great potential for the complete tumor regression.

In addition to using PDT and PTT to produce reactive oxygen species and to create high-temperature environments to kill cancer cells directly, water-soluble conjugated polymers are also employed as optical platforms for phototriggered drug delivery. Most of the previous works are based on two strategies. One is to convert conjugated polymers from hydrophobic to hydrophilic by introducing photoresponse moieties on the polymer's side chains.[83,113] In this process, the hydrophobic drugs can be released from conjugated polymers/drug assemblies. The other strategy is to attach drugs to conjugated polymer backbones via ROS-cleavable groups.[117,118] Thus, the generated ROS upon the irradiation of conjugated polymers will trigger the drug release.

Recently, Sun and co-workers developed a novel approach for phototriggered drug delivery that operates by a different mechanism compared to the traditional strategies.[119] They designed and synthesized a water-soluble poly(phenylene ethynylene) that is functionalized with oxidized oxaliplatin Pt(IV) units (**PPE-Pt(IV)**, Figure 4a) attached via ester linkages. The ester linkages between the polymer side groups and the oxaliplatin precursor were produced by an EDC activated coupling reaction. The photoactivation is based on photoinduced electron transfer from the **PPE** backbone to oxaliplatin Pt(IV) as an electron acceptor; this process triggers the release of oxaliplatin Pt(II), which is a clinically used anticancer drug (Figure 4b). Cell studies showed that the cytotoxicity of **PPE-Pt(IV)** after irradiation (460 nm LED, 7.0 mW/cm^2, 20 min) is comparable to oxaliplatin alone as a control. More importantly, **PPE-Pt(IV)** can also be activated by 2-photon absorption at 725 nm with a moderate yield, suggesting potential *in vivo* applications of this approach.

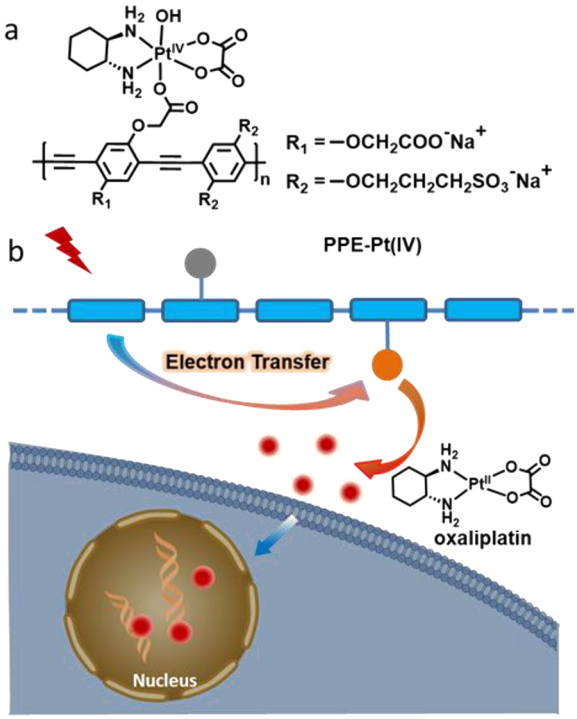

Figure 4. (a) Chemical structure of **PPE-Pt(IV)**. (b) Photoactivation of **PPE-Pt(IV)** for chemotherapy based on photoinduced electron transfer. Reprinted with permission from ref 119. Copyright 2022 American Chemical Society.

4. CONCLUSION AND PERSPECTIVE

Over the past several decades, research has demonstrated that WS-CPs are promising materials for a variety of biomedical applications because of their unique chemical and optoelectronic properties. Specific chemical functionalization methods such as amide bond formation, Click reaction, nucleophilic substitution, and addition reactions have been applied to integrate biofunctional molecules onto WS-CP platforms. This approach has led to significant advances in the application of WS-CPs for sensing and combination therapy.

This review provides a summary of recent advances with respect to biofunctionalized conjugated polymers. Critical synthesis strategies used for the functionalization have been systemically reviewed. While several approaches have been used for biofunctionalization of WS-CPs, more work is needed to optimize the conditions for the functionalization reactions and also to give rise to polymers that are fully characterized with respect to the degree of functionalization that is accomplished. A critical analysis of the work that has been done leads to the conclusion that amide coupling is probably the most versatile approach to functionalization of WS-CPs. This reaction can be carried out in organic and aqueous solutions and is tolerant of a variety of functional groups. Nevertheless, formation of active esters, which are important intermediates in amidation reactions, is sensitive to the reaction conditions, and consequently the yields of the functionalization reactions can vary widely.

Despite the progress that has been made, there are still limitations that impede the development and applications of biofunctionalized WS-CPs. Introduction of functional groups onto water-soluble conjugated polymers can cause decreased polymer solubility and give rise to altered, and in some cases, poor photophysical properties. Ideal chemistries for attaching biofunctional moieties onto conjugated polymers with all the desired properties (controlled loading, good solubility, unaffected fluorescence quantum yield, and unaltered biofunctions) will require further research and development. Addition of more than one type of functional molecule on a single conjugated polymer chain in a controlled manner remains a challenge. The development of functionalized WS-CPs with high molar absorptivity in the second NIR window is also needed for treating deep-tissue-buried diseases. Design of biodegradable WS-CPs is also needed considering longtime biosafety. Nevertheless, we are optimistic that the future of the field is bright and that the functionalized conjugated polymers with tailored material and biological properties will find broad uses for a variety of applications in research and clinical settings.

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Notes

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