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REVIEW

# Oligonucleotide–Polymer Conjugates: From Molecular Basics to Practical Application

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## Abstract

DNA exhibits many attractive properties, such as programmability, precise self-assembly, sequence-coded biomedical functions, and good biocompatibility; therefore, DNA has been used extensively as a building block to construct novel nanomaterials. Recently, studies on oligonucleotide–polymer conjugates (OPCs) have attracted increasing attention. As hybrid molecules, OPCs exhibit novel properties, e.g., sophisticated self-assembly behaviors, which are distinct from the simple combination of the functions of DNA and polymer, making OPCs interesting and useful. The synthesis and applications of OPCs are highly dependent on the choice of the polymer block, but a systematic summary of OPCs based on their molecular structures is still lacking. In order to design OPCs for further applications, it is necessary to thoroughly understand the structure–function relationship of OPCs. In this review, we carefully categorize recently developed OPCs by the structures of the polymer blocks, and discuss the synthesis, purification, and applications for each category. Finally, we will comment on future prospects for OPCs.

**Keywords** Oligonucleotide–polymer conjugates · DNA block copolymers · Functional nucleic acid · Self-assembly · Drug delivery

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**Abbreviations**

| | |
|---|---|
| DNA | Deoxyribonucleic acid |
| ODN | Oligonucleotide |
| OPC | Oligonucleotide–polymer conjugate |
| DX | Double-crossover |
| siRNA | Small interference RNA |
| RISC | RNA-induced silencing complex |
| PLA | Polylactic acid |
| PGA | Polyglycolic acid |
| PLGA | Poly (lactic-co-glycolic acid) |
| PCL | Polycaprolactone |
| PASP | Polyaspartic acid |
| PNIPAM | Poly[*N*-isopropylacrylamide] |
| PEG | Polyethylene glycol |
| DMSO | Dimethyl sulfoxide |
| DMF | Dimethylformamide |
| PS | Polystyrene |
| CPG | Controlled pore glass |
| PPE | Poly-(phenylene–ethynylene) |
| HE | Dodecanediol phosphoramidite |
| ATRP | Atom transfer radical polymerization |
| RAFT | Reversible addition-fragmentation chain transfer polymerization |
| CPADB | 4-Cyano-4-(phenylcarbonothioylthio) pentanoic acid |
| BTPA | 2-(Butylthiocarbonothioyl) propionic acid |
| EY | Eosin Y |
| AscA | Ascorbic acid |
| APS | Ammonium persulfate |
| TEMED | Tetramethylethylenediamine |
| PAGE | Polyacrylamide gel electrophoresis |
| FDA | Food and Drug Administration |
| PEI | Polyethyleneimine |
| SNA | Spherical nucleic acid |
| shRNA | Short hairpin RNAs |
| RCT | Rolling circle transcription |
| MDR1 | Multidrug resistance protein 1 |
| DOX | Doxorubicin |
| PPT-g-PEG | Peptide-grafted poly (ethylene glycol) |
| Fc | Ferrocene |
| MNP | Magnetic nanoparticle |
| PNB | Polynorbornene |
| PPO | Polypropylene oxide |
| LCST | Low critical solution temperature |
| VPTT | Volume phase transition temperature |
| DMT | Dimethoxytrityl |
| HPLC | High performance liquid chromatography |
| PPE | Poly (phenyleneethynylene) |

| | |
|---|---|
| HEX | Hexachlorofluorescein |
| FRET | Fluorescence Resonance Energy Transfer |
| SWNT | Single-walled carbon nanotube |
| PFO | Polyfluorene |
| PT | Polythiophene |
| PFP | Poly [fluorine-co-phenylene fluorene] |
| ACQ | Aggregation caused quenching |
| APPV | (2,5-Dialkoxy) paraphenylene vinylene |
| PAM | Polyacrylamide |
| ROMP | Ring-opening metathesis polymerization |
| pRNA | Passenger-stranded RNA |

## 1 Introduction

Nucleic acids are bio-macromolecules that encode genetic information for living organisms. As solid-phase nucleic acid synthesis techniques are now well developed, their unique molecular structure and conformation make nucleic acids interesting molecules in the field of materials science. Due to specific Watson–Crick base pair interactions, nucleic acids not only specifically recognize complementary sequences, but can also conduct precise self-assembly and organize other molecules or nanomaterials in a well-defined manner at the nanoscale [1–4]. Therefore, nucleic acid-based nanotechnology has attracted significant attention in recent years (Fig. 1) [5, 6]. For instance, as a kind of DNA self-support nanostructure, DNA origami shows unparalleled advantages in precise controlling size and position at the nanoscale. However, DNA origami depends on a complicated and costly fabrication technique, which also exhibits susceptible stability (most structures require an environment with a high concentration of $Mg^{2+}$). Moreover, a large number of drugs and contrast agents with low-solubility in water need to be incorporated into nanostructures with hydrophobic domains—a feature lacking in DNA origami. Therefore, the application of DNA origami in certain directions will be limited.

Oligodeoxynucletide (ODN) is a general term for a class of short-chain nucleic acids. Plenty of functional ODNs have been screened and well investigated, and show sequence-dependent functions, such as CpG islands (DNA sequences containing a large amount of "-C-phosphate-G-") for immune activation [7], aptamers for targeted recognition [8], DNAzymes for catalysis [9], antisense ODNs and small interference RNAs (siRNA) for gene silencing (Fig. 2a, b) [10, 11]. Additionally, certain sequences can undergo conformational changes in reaction to external stimuli, including the quadruplex folding of cytosine-rich sequences at low pH [12] and G-quadruplex formation in the presence of monocations (Fig. 2c) [13]. More importantly, ODNs can be modified easily by well-developed chemical methods to increase their functionality and intelligence. These excellent properties of ODNs make them attractive in the fields of controllable self-assembly, diagnostics, and therapeutics. However, as for free functional nucleic acids, they display some inherent disadvantages for biomedical applications, including poor biological stability, non-specific immunogenicity, and unsatisfactory cellular uptake.

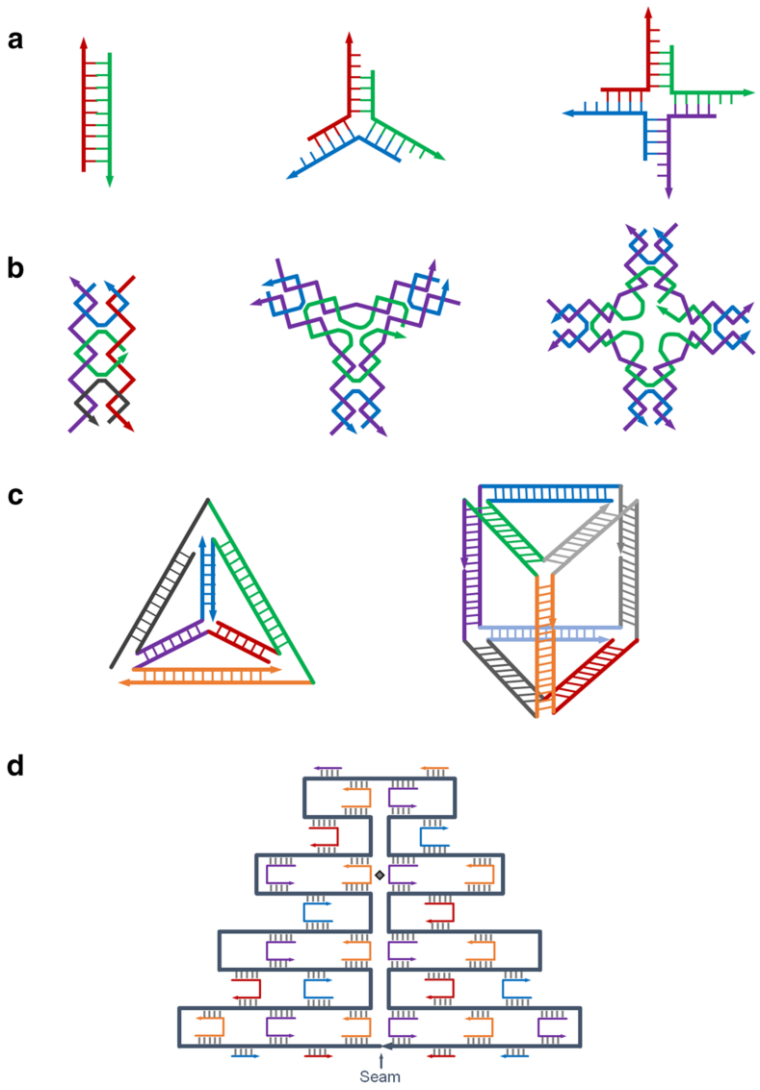


**Fig. 1** Nucleic acid-based nanotechnology [5]. **a** The schematic diagram from left to right represents: linear, Y-shaped, X-shaped nucleic acid tiles. Nucleic acid tiles with single-stranded ends ("sticky ends") can self-assemble into complex structures. **b** The schematic diagram from left to right represents rectangular double-crossover (DX) tiles, a Y-shaped DX tile, and an X-shaped DX tile. Unlike the structures in **a**, these double-crossover nucleic acid tiles have enhanced rigidity and highly planar structures that can assemble into a higher-order structure. **c** Three-dimensional nucleic acid structures. Left A nucleic acid tetrahedron constructed from four single strands. Right Nucleic acid cube constructed from five single stands. **d** Schematic representation of DNA origami. Long genomic DNA is folded with the help of small staple strands to give the desired, computationally designed, structure

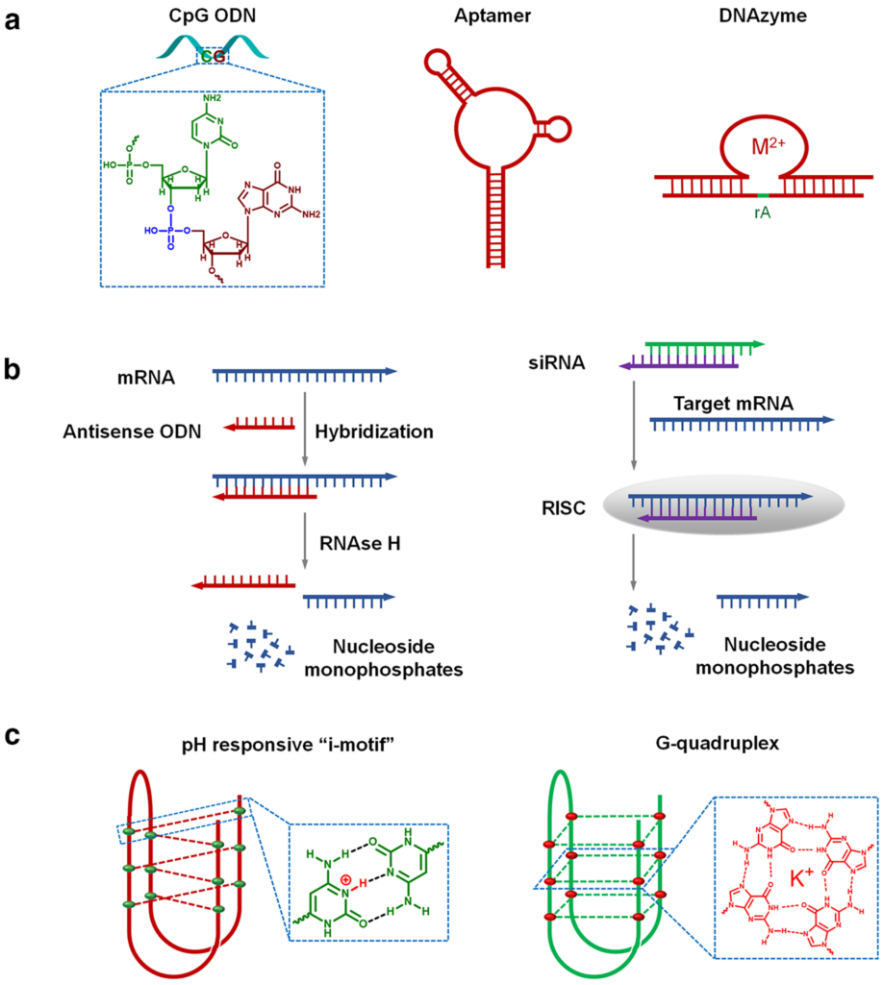


**Fig. 2** Functional nucleic acids. **a** A CpG oligodeoxynucletide (ODN) containing a "C–G" sequence can elicit an immune response in a mammal through the TLR9 signaling pathway, thereby enhancing immunotherapy as an immunological adjuvant [7]. The nucleic acid aptamer is referred to as a "chemical antibody" that can specifically bind to a target substance for targeted delivery and therapeutic purposes [8]. With the aid of metal ions, specific nucleic acid sequences have a catalytic effect [9]. **b** Both the antisense ODN and the small interfering RNA (siRNA) can regulate gene expression in a target cell [10, 11]. The most common mechanism of action is that RNase H or RISC (RNA-induced silencing complex) complexes inhibit expression of target genes. **c** Some specific sequences can form stable quadruplex structures under acidic conditions or under metal ion-mediated conditions [12, 13]

Synthetic polymers are attractive to researchers because of their enriched chemical structure and tunable properties. To avoid confusion, in this review, "polymers" are defined as chemically synthesized macromolecules, while "DNA" is a naturally occurring biomacromolecule; although DNA can be chemically synthesized nowadays, it originally exists in nature, whereas synthetic "polymers" do not. Polymers,

including biodegradable polymers, amphiphilic block copolymers, graft copolymers, and π-conjugated conductive polymers, have been well developed in recent years. Research into these polymers is changing rapidly in the fields of macromolecular self-assembly [14], drug delivery [15], biosensing [16], bioimaging [17], phototherapy [18], and polymer solar cells (Fig. 3) [19]. However, conventional synthetic polymers are used mainly as passive matrix materials to deliver hydrophobic drugs and contrast agents, which generally lack biological functions, such as targeting and stimuli-responsiveness to biomolecules.

Because of the attractive properties of both nucleic acids and polymers, the idea of integrating the two naturally came into being. Compared with pure DNA nanostructures or polymer self-assemblies, oligonucleotide–polymer conjugates (OPCs) can combine the advantages of both and result in more capable nanomaterials due to the synergetic effects: (1) in addition to providing programmability, the DNA block

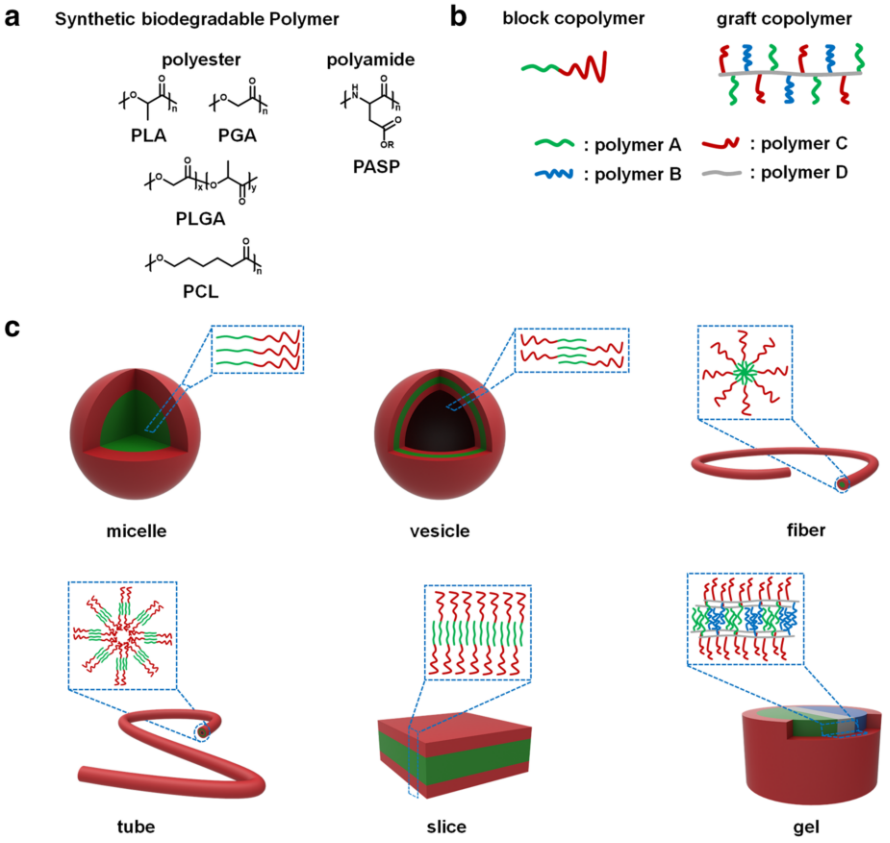


**Fig. 3** Synthetic polymers and their self-assembly. **a** Chemical structure of biodegradable polymers. There are two main types: polyester and polyamine. *PLA* Polylactic acid, *PGA* polyglycolic acid, *PLGA* poly (lactic-co-glycolic acid), *PCL* polycaprolactone, *PASP* polyaspartic acid. **b** Structure of two common copolymers: block copolymers and graft copolymers. **c** Some interesting macromolecular self-assemblies

also renders many distinctive biological functionalities to OPCs, such as gene regulation, targeting, and stimuli-responsiveness. (2) The polymer block provides diverse interactions, including, but not limited to, hydrophobic interactions, π–π stacking, and host–guest interactions, which will bring many other organic functional materials into OPCs. (3) In particular, amphiphilic OPCs will self-assemble into stable nanostructures through hierarchical supramolecular interactions. On the one hand, the self-assembly structure will drive DNA blocks to be closely packed together, which will make DNA more resistant to nuclease degradation and enhance the cellular penetration capability. On the other hand, the self-assembly structures may show more intelligent stimuli-responsive properties; for instance, both the conformational changes of DNA blocks (e.g., pH-sensitive DNA i-motifs) and phase-transition of the synthetic polymer blocks (e.g., temperature-sensitive polyNIPAM) will change the amphiphilicity of OPCs, and result in morphological or functional variations. Therefore, OPCs will surely set off a revolutionary wave in the fields of supramolecular chemistry and biomedicine. In recent years, plenty of excellent related work has been reported. Although several reviews have reported on OPC research [20–26], this comprehensive review will first classify OPCs through the coupled polymer block, and then systematically summarize the synthesis and purification methods of various OPCs. A perspective for the future development of OPCs is also provided.

## 2 Synthesis of OPCs

### 2.1 Direct Coupling of Polymer and ODN

Obviously, the most straightforward method to synthesize OPCs is through direct coupling reactions between functionalized ODNs and polymers. Various ODNs with reactive functional groups (“X” in Fig. 4) can be synthesized on a DNA synthesizer via phosphoramidite chemistry; polymers with reactive functional groups (“Y” in Fig. 4) can be synthesized by certain chemical methods. The two kinds of functional groups (X and Y) can react with each other to form the desired OPCs (“X–Y” in Fig. 4).

To date, a number of coupling reactions have been applied to the synthesis of OPCs (Fig. 4). Each coupling reaction has its own characteristics: (1) for the amidation reaction between the amine group and the carboxyl group, the two functional groups are easy to introduce to ODN and the polymer, and the coupling condition is mild [27, 28]; however, the resultant amide bond is unstable and easily hydrolyzed. (2) The OPCs prepared by disulfide bonds are responsive to the reducing environment, and this characteristic can be used to realize stimuli-responsiveness in tumor environments [29, 30]. (3) The chemical bonds formed by other reactions, including Michael addition [31, 32] and copper-catalyzed [33] (or copper-free [34, 35]) cycloaddition reactions, are relatively stable. Overall, a proper coupling reaction can be selected according to different research priorities and purposes.

The coupling reaction in Fig. 4 can be broadly classified into two types: (1) reactions in solution; (2) reactions on solid supports.

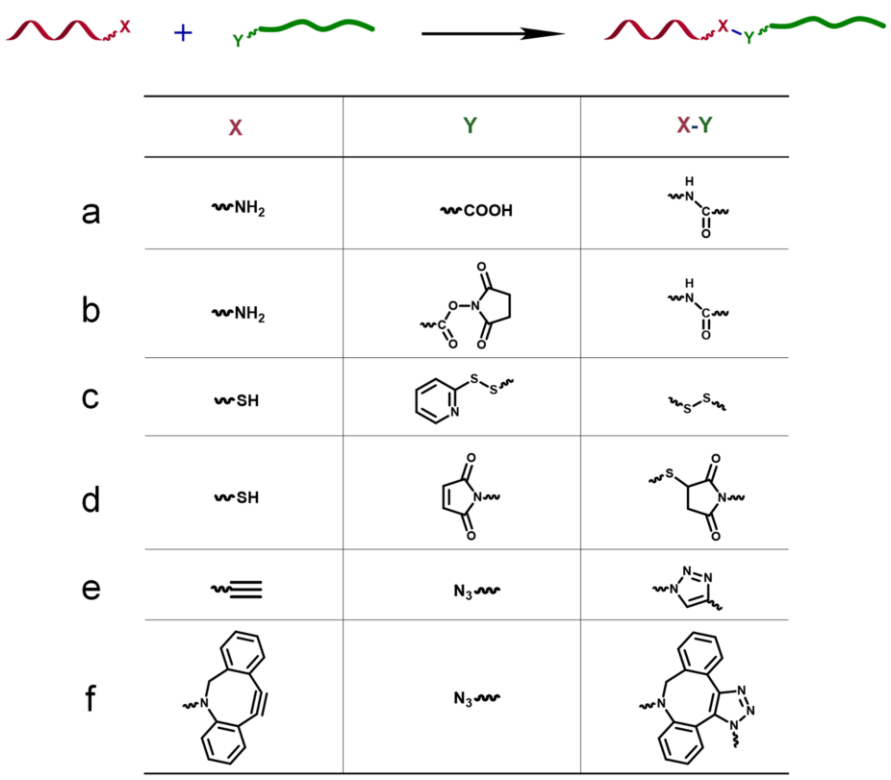


**Fig. 4** Various synthetic methods for direct coupling of ODNs and polymers. **a** Amidation of amine and carboxyl groups [27, 28]. **b** Direct coupling of amine and NHS (*N*-hydroxysuccinimide) ester-activated polymers [67]. **c** 2-Pyridyldithiol and sulfhydryl groups can form cleavable disulfide bonds [29, 30]. **d** Michael addition reaction of mercapto and maleimide [31, 32, 125]. **e** Copper-catalyzed cycloaddition reaction of alkynyl and azide [33]. **f** Copper-free "click" reaction of cyclooctyne and azide [34]

### 2.1.1 Reactions in Solution

A high coupling efficiency generally results from the reaction in a homogeneous system. DNA is a fully hydrophilic molecule, which readily couples with hydrophilic polymers, such as polypeptide chains [36, 37], polyethylene glycol (PEG) [38], and polyacrylamide [39], resulting in fully hydrophilic OPCs.

Due to the heterogeneity of the reaction, coupling hydrophobic polymer to hydrophilic ODN becomes more difficult. Herrmann's group has attempted to introduce surfactants to transfer ODN into the organic phase to promote the coupling reactions with hydrophobic polymers [40]; however, additional steps are needed to remove the organic surfactant, which may induce biosafety risks. Another attempt was to synthesize OPCs in a mixed solvent that can dissolve both ODNs and organic polymers. Generally, ODN has the highest solubility in water, high solubility in DMSO (dimethyl sulfoxide), and moderate solubility in DMF (dimethylformamide). Some hydrophobic polymers, such as PCL, PLA, or PS (polystyrene), have good solubility

in DMSO or DMF. Therefore, mixed solvents, such as DMSO/water [34], DMSO/DMF mixed solvents [41], and even pure DMF solvents [33], were used in the synthesis of amphiphilic OPCs with satisfactory yields.

#### 2.1.2 Reactions on Solid Support

The solid phase support, controlled pore glass (CPG), is a carrier for synthesizing ODN on a DNA synthesizer. After synthesizing the ODN with a reactive functional group, the polymer to be coupled can be conjugated to the ODN on the CPG. Compared with the solution-phase reaction, the solid-phase reaction shows advantages in wider solvent choice and more convenience in the subsequent purification.

The most classic solid-phase reaction is based on phosphoramidite chemistry [42–44], as shown in Fig. 5a, b, which is highly efficient with no need for any catalysts. However, such reactions are sensitive to water; the incorporation of a small amount of water will greatly reduce the efficiency of the reaction. Therefore, phosphoramidite synthesis is generally performed "on-line" on a DNA synthesizer that can provide a highly anhydrous environment [45, 46]. "Click" (Fig. 5c) [47, 48] and amidation reactions (Fig. 5d) [49, 50] are also widely used solid-phase synthesis methods. However, as solid-phase synthesis involves an aminolysis step to cut ODNs from CPG supports, reaction products with chemical bonds that are sensitive to the alkaline conditions, are not suitable for solid-phase methods.

### 2.2 In Situ Polymerization from the End of ODN

According to the collision theory of reaction kinetics, conjugation reactions from the single end of a polymer will be less efficient. In order to improve conjugation yields, new polymerization strategies have been developed to prepare OPCs, one of which is to perform polymerization in situ from the end of the ODN/polymer with a functionalized group.

Yang et al. [51] carried out the direct polymerization of poly-(phenylene–ethynylene) (PPE) derivatives from the end of the ODN (Fig. 6a), which was capped with a 5I-dU functional group to initiate PPE polymerization and finally obtain ODN–PPE conjugates. It is worth mentioning that the reaction was carried out on a CPG solid support, which has convenient advantages in product synthesis and purification.

Also to take advantage of the solid-phase phosphoramidite chemistry, Sleiman's group developed a method for the synthesis of sequence-defined polymers appended to ODNs (Fig. 6b) [52, 53]. They used a commercially available DMT-protected dodecanediol phosphoramidite (HE), which is sequentially coupled to the 5′ terminus of an ODN strand. The resulting OPCs consisted of ODN portions conjugated with 1–12 HE units punctuated by phosphate moieties, showing distinct amphiphilic properties.

Matyjaszewski's group [54–56] utilized atom transfer radical polymerization (ATRP) reactions to polymerize polymer from the end of an ODN. In their method, an ATRP initiator was bonded to an ODN during solid-phase synthesis, from

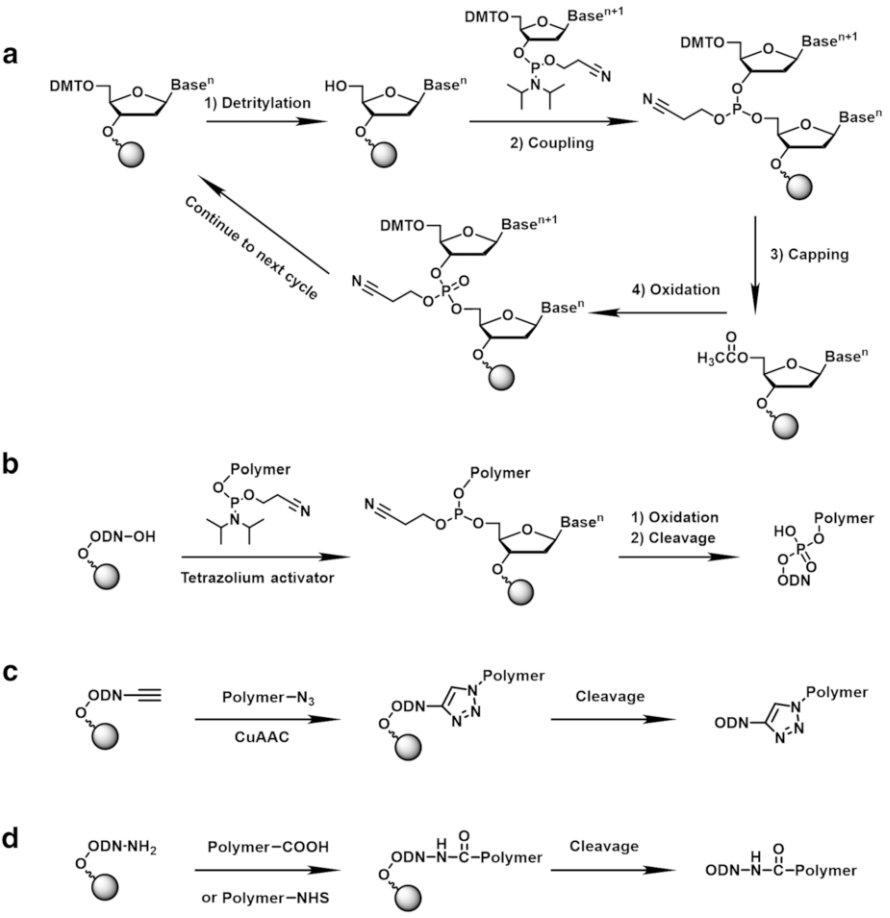


**Fig. 5** **a** Schematic diagram of the synthesis of ODN by a DNA synthesizer. **b** The polymer with a hydroxyl group may be modified with a phosphoramidite; further, OPCs are chemically synthesized by phosphoramidite chemistry. **c** A copper-catalyzed cycloaddition reaction carried out on solid support [47, 48]. **d** Amidation reaction carried out on solid support [49, 50]

which a polymer chain was grown to produce OPCs (Fig. 6c). The authors claimed that ATRP polymerization can be carried out in solution after the ODN-initiator sequence is cut from the solid support, or directly on the solid support. Therefore, it is a versatile and high-yield method of preparing OPCs, wherein both fully hydrophilic polymers and amphiphilic polymers can be applied.

Reversible addition-fragmentation chain transfer polymerization (RAFT) is another kind of controlled radical polymerization method, which provides similar flexibility in monomer scope and end-group functionalization. Moreover, unlike ATRP methods that rely on toxic transition metal catalysts, the RAFT approach typically does not require metal catalysts and can be photo-initiated, which can offer a biocompatible polymerization platform for the synthesis of OPCs. Weil and

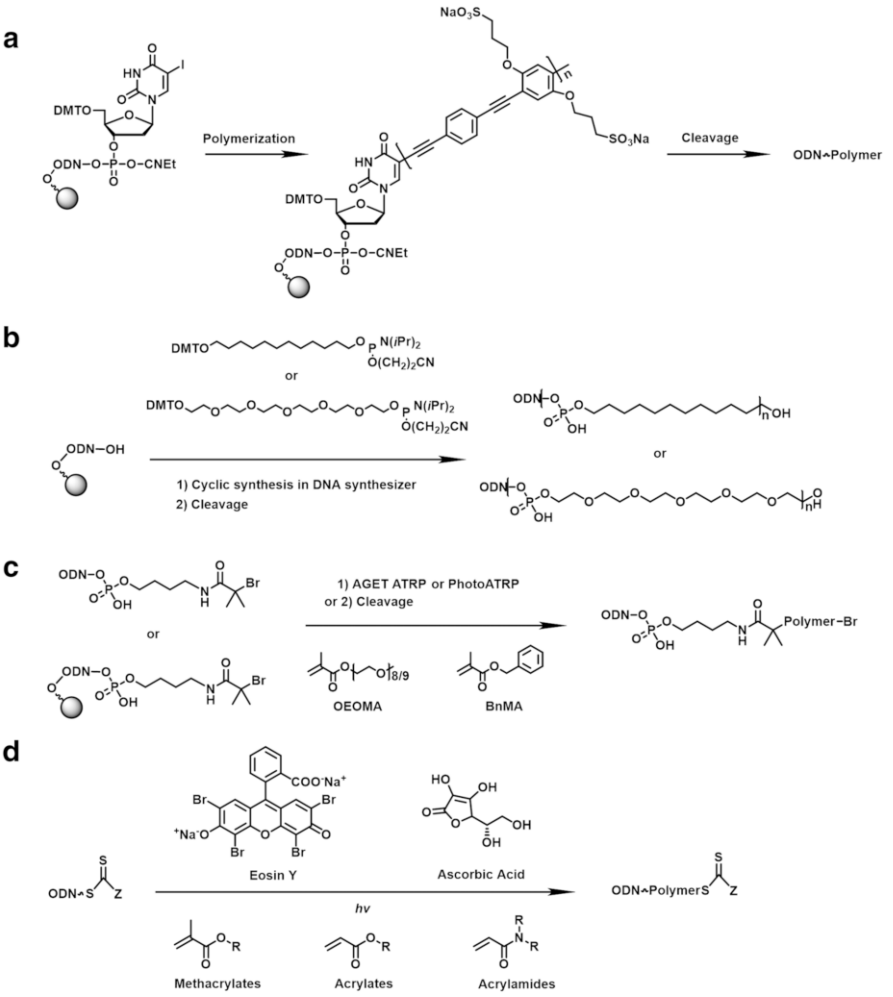


**Fig. 6** Polymers were polymerized directly at the end of the ODN to prepare OPCs. **a** Polymerization of water-soluble PPE on controlled pore glass (CPG) solid support [51]. **b** Sequence synthesis of well-defined OPCs on a DNA synthesizer [52, 53]. **c** The atom transfer radical polymerization (ATRP) reaction was directly carried out at the end of the ODN to synthesize OPCs [54–56]. **d** In the solution, the ODN containing the reversible addition-fragmentation chain transfer polymerization (RAFT) initiator was subjected to RAFT polymerization to prepare OPCs [57]

colleagues [57] developed a new "graft-from" approach based on photo-induced RAFT polymerization, by which OPCs can be prepared in solution (Fig. 6d). First, ODNs were functionalized by the established RAFT agents 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPADB) and 2-(butylthiocarbonothioyl) propionic acid (BTPA), and then subjected to light-induced RAFT polymerization. It should be noted that: (1) RAFT reagents are unstable in alkaline media and cannot be synthesized directly on CPG solid support. (2) The deoxygenation of this

ultra-small-volume reaction system is a challenge. The use of Eosin Y (EY) and ascorbic acid (AscA) as reducing agents can replace the deoxygenation step in the polymerization process [57].

### 2.3 Acrydite-Functionalized ODN for Free-Radical Polymerization

In order to efficiently obtain ODN-graft polymer conjugates, one approach is to prepare polymerizable macromonomers containing ODNs and then copolymerize the macromonomers with other functional monomers (Fig. 7). Acrydite-functionalized ODN is the macromonomer used most widely to prepare OPCs such as ODN-g-PNIPAM (poly[*N*-isopropylacrylamide]) [58], and ODN-g-polyacrylamide [39, 59, 60]. These OPCs are used widely for preparing stimuli-responsive DNA hydrogels.

## 3 Separation and Purification of OPCs

Whether used in therapeutic, diagnostic, or self-assembly research, the purity of OPCs is critical. The unreacted hydrophobic polymer in the crude product can be removed by centrifugation due to its low solubility in aqueous solution. Another impurity is the unreacted ODNs. Briefly, there are several methods that can be used to remove free ODNs (Fig. 8): (1) dialysis [41, 61]; (2) ultrafiltration [27, 62]; (3) electrophoresis-cutting [48]; (4) reversed-phase column chromatography [34, 53]; (5) size exclusion chromatography [63–66]; and (6) anion exchange chromatography [46].

Most of the free ODN can be removed by selecting a dialysis bag or ultrafiltration tube with a suitable molecular weight cutoff. However, inevitably, there

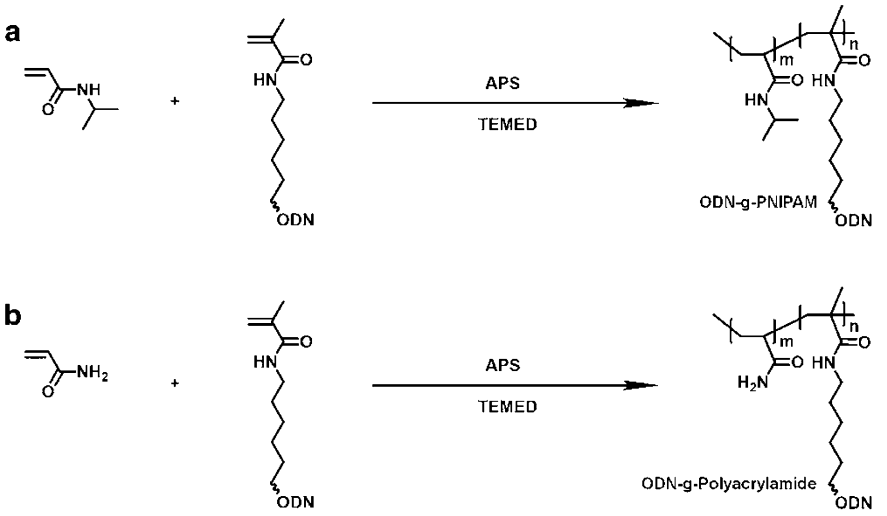


**Fig. 7** Synthetic route of two representative ODN-graft polymer conjugates. **a** ODN-g-PNIPAM [58]. **b** ODN-g-Polyacrylamide [39, 59, 60]. *APS* Ammonium persulfate, *TEMED* tetramethylethylenediamine

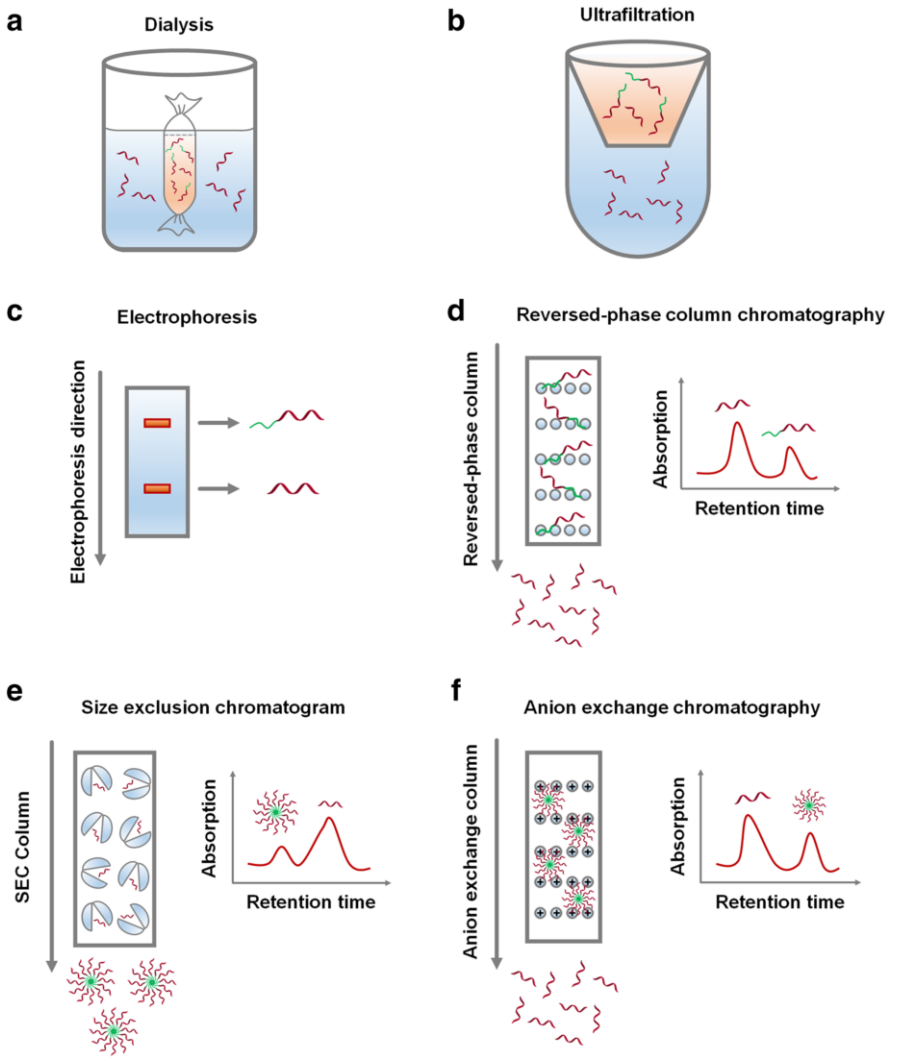


**Fig. 8** Separation and purification of OPCs. **a** Dialysis. ODNs with small molecular weights will pass through the dialysis bag, while OPCs with large molecular weights will be trapped in the dialysis bag [41, 61]. **b** Ultrafiltration. Its separation principle is similar to dialysis, but the processing time is shorter compared with dialysis [27, 62]. **c** Electrophoresis-cutting purification. Separation by polyacrylamide gel electrophoresis (PAGE) or agarose gel electrophoresis, then cutting the target bands to obtain the purified OPCs [48]. **d** Purification by reversed-phase column chromatography. Separation and purification via differences in hydrophilicity [34, 53]. **e** Size exclusion chromatography purification: separation and purification by the difference in size [63–66]. **f** Anion exchange chromatography purification: separation and purification by the difference in charge density [46]

will still be a portion of the residual ODN in the sample of OPCs. These residual ODNs can potentially affect the properties of OPCs in subsequent studies.

Gel electrophoresis is the most common method for nucleic acid purification, and can separate products with very small differences in molecular weight. However, it requires the use of nucleic acid dyes to trace nucleic acids, which introduces new impurities to the OPCs.

Purification of OPCs by column chromatography is the most ideal separation method. For reversed-phase column chromatography, OPCs consisting of a hydrophobic-polymer-block will show longer retention time compared with the hydrophilic ODNs. Using this principle, we can get relatively pure OPCs. Notably, when separating OPCs using such methods, the hydrophobicity of the coupled polymer should not be too strong, otherwise the OPCs will not be eluted in the reversed-phase chromatographic column.

Another type of column chromatography that can be used for OPC purification is size exclusion chromatography. The principle of this method is that the substance with a larger size is eluted first, and the small-size one is eluted later. For highly hydrophobic polymers, the corresponding OPCs are likely to form large assembled structures due to the hydrophobic interactions, which will have larger sizes than the free ODNs. Using this principle, OPCs can be purified thoroughly.

The principle of anion exchange chromatography is to use the differences in charge densities of the substances to be separated. Amphiphilic OPCs containing hydrophobic moieties will form aggregates in aqueous solution. These aggregates have higher charge densities than free ODNs. Thus, free ODNs show shorter retention time in the anion exchange column compared to OPCs.

## 4 Classification of OPCs

Many types of OPCs have been reported. In the past, most review articles classify OPCs by their application directions. Here, we summarized various OPCs according to their coupled polymers. So far, the coupled polymers can be divided roughly into the following categories: biodegradable polymers, strong hydrophobic polymers, polymers with weak hydrophobicity, π-conjugated polymers, and other polymers. These polymers with different structures show very different properties; therefore, the research priorities and applications of the corresponding OPCs will be very different.

### 4.1 Biodegradable Polymer-Based OPCs

When applying OPCs to therapeutics, there is no doubt that biodegradable polymers are preferred. Currently, biodegradable polymers coupled with ODNs include mainly PLGA, PCL, and PLA.

### 4.1.1 ODN–PLGA Conjugates

PLGA is approved for clinical use by the Food and Drug Administration (FDA) of many countries due to its good biocompatibility and tunable degradation rate. PLGA contains two repeating units: lactic acid and glycolic acid. The degradation rate of PLGA can be regulated by adjusting the molecular weight of PLGA or the ratio between the lactic acid and glycolic acid units. Compared to PLA and PCL, PLGA has a faster degradation rate due to its good hydrophilicity. These excellent properties make PLGA a promising material in the biomedical field.

Antisense ODN can block the expression of specific proteins to treat a particular disease, but its most serious problem is its low cell permeability. In 2001, Park's group reported the coupling of antisense ODN and PLGA (Fig. 9a) [67]. The amphiphilic ODN-b-PLGA block copolymers could self-assemble into stable micelles in aqueous solution. More importantly, these ODN-b-PLGA micelles can enter cells via endocytosis without the need for a cationic transfection agent. In addition, the controlled degradation of PLGA allows the sustainable release of antisense DNA to efficiently inhibit the expression of certain genes [67].

siRNA can target mRNA and mediate the degradation of the target mRNA to realize gene silencing. Likewise, intracellular delivery of siRNA also requires the utilization of toxic cationic polymeric carriers. In order to realize self-delivery, siRNA was chemically conjugated to PLGA via a cleavable disulfide bond [29, 30] to form siRNA–PLGA conjugates, which would subsequently self-assemble into micelles in aqueous solution with a high density of siRNAs at the surface (Fig. 9a,

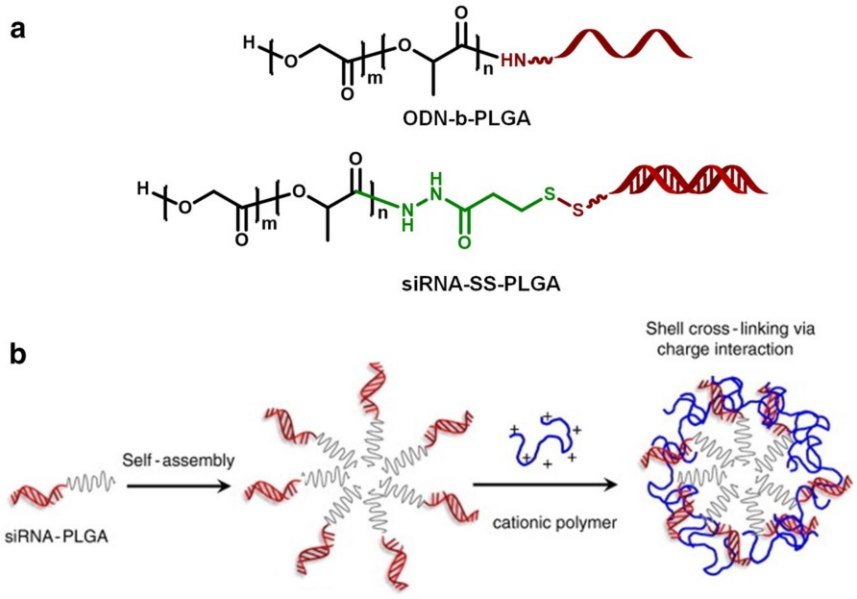


**Fig. 9** **a** Structural formula of two common ODN–PLGA conjugates. **b** Self-assembled siRNA–PLGA conjugates for intracellular delivery of siRNA (adapted with permission from [30])

b). Compared to the free siRNA, siRNA-b-PLGA was more readily complexed by linear-low-molecular-weight PEI (polyethyleneimine), which shows comparatively lower cytotoxicity. In addition, the siRNA-b-PLGA/PEI complex exhibited improved cell permeability and gene silencing efficiency than the siRNA/PEI complex [30].

#### 4.1.2 ODN–PCL Conjugates

PCL is also a biodegradable polymer that is approved by the FDA for biomedical applications. PCL can be prepared by the ring-opening polymerization of ε-caprolactone monomers catalyzed by a metal anion complex. By optimizing the polymerization conditions, PCL of different molecular weights can be synthesized. Compared to PLGA and PLA, modifications of PCL are more readily performed.

According to the theory of spherical nucleic acids (SNAs) [68, 69], densely packed and highly oriented nucleic acids in a spherical geometry show properties that differ markedly from their linear cousins. SNAs can enter cells more efficiently to induce gene regulation or detect biological targets in live cells. Generally, SNAs are constructed on spherical nanoparticle cores, to the surfaces of which ODNs were covalently attached; in addition to this, the self-assembled OPCs can also build SNA structures. In 2015, Zhang et al. [61] reported two ODN–PCL conjugates: linear ODN-b-PCL and ODN-g-PCL conjugates (Fig. 10a, b). They believe that the nucleic acid micelles formed by ODN-g-PCL conjugates have a higher surface density of ODNs than the micelles formed by linear ODN-b-PCL. Compared with ODN-b-PCL, ODN-g-PCL showed more negative surface charge, higher melting temperatures, higher cell uptake efficiency, and more efficient gene suppression efficiency [61].

In a follow-up work, Zhang's group developed a method of using siRNA as a cross-linker to mediate further self-assembly of the ODN-g-PCL conjugates, forming spherical and nanosized hydrogels. The siRNAs were fully embedded in the nanogel, which showed good bio-stability during the systemic delivery. This size-adjustable cross-linked siRNA@DNA-g-PCL nanogel could deliver siRNAs efficiently to different cells without the need for any transfection agents, and achieved efficient gene silencing both in vitro and in vivo. Through this, significant inhibition of tumor growth was realized in the anticancer treatment (Fig. 10a, c) [41].

#### 4.1.3 ODN–PLA Conjugates

PLA is also an FDA-approved biodegradable material that degrades at a slower rate than PLGA. PLA can be synthesized by the controlled ring-opening polymerization of lactide. To synthesize ODN–PLA conjugates, a "Click" reaction can be performed using azide-terminated PLA and alkynyl-terminated ODN [33]. Similarly, ODN–PLA conjugates could self-assemble into spherical micelles in aqueous solution. Chen's group used in situ rolling circle transcription (RCT) to prepare short hairpin RNAs (shRNA) from the amphiphilic ODN–PLA micelles, using the peripheral ODNs as primers. The shRNAs were applied to inhibit the expression of multidrug resistance protein 1 (MDR1) in breast cancer. In addition, the hydrophobic

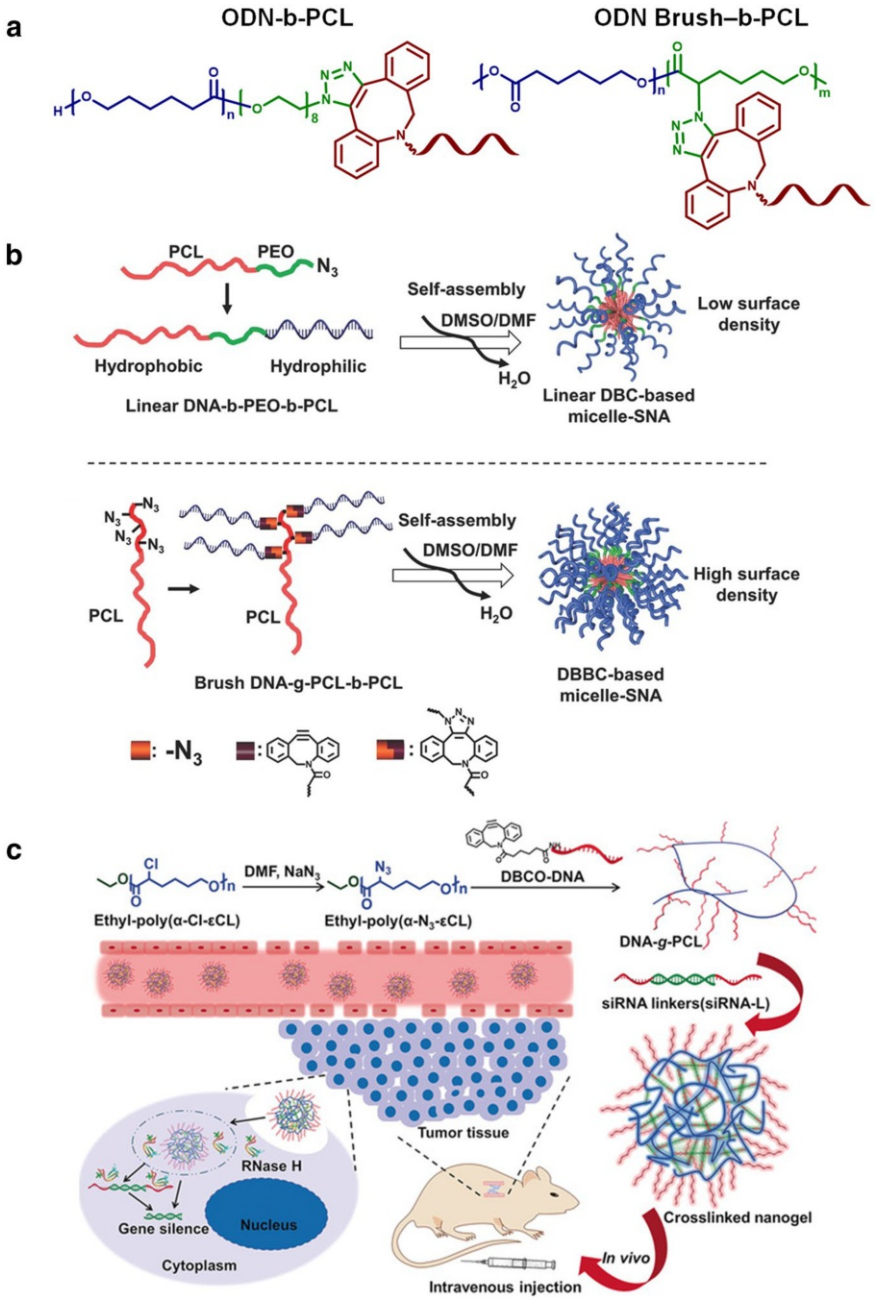


**Fig. 10** Assembly and application of ODN–PCL conjugates. **a** The chemical structures of two typical ODN–PCL conjugates [61]. **b** Two ODN–PCL micelles with different surface nucleic acid densities are used for intracellular gene silencing (reproduced with permission from [61]). **c** siRNA@ODN-g-PCL nanogel prepared using siRNA as a crosslinker for siRNA delivery and anti-tumor therapy (reproduced with permission from [41])

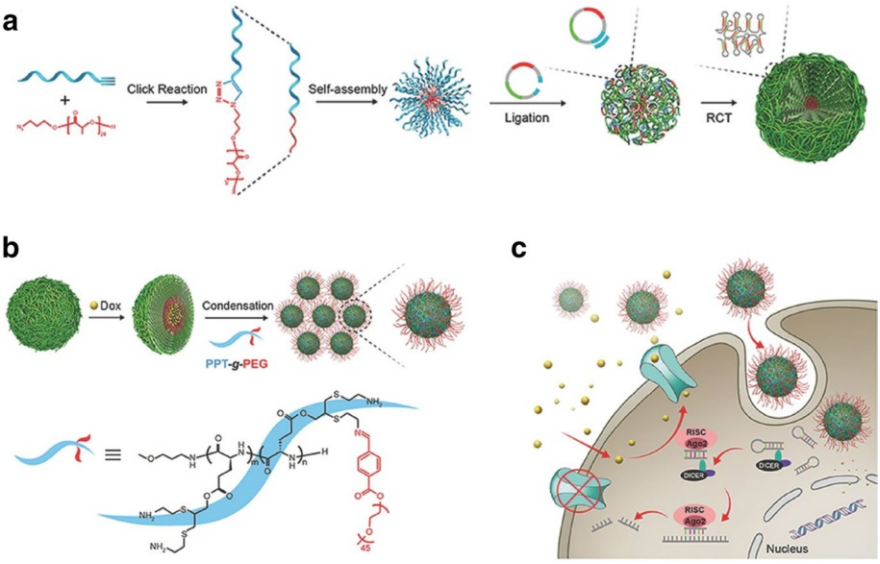


**Fig. 11** Assembly and application of ODN–PLA conjugates (reproduced with permission from [33]). **a** Self-assembly of ODN-b-PLA synthesized by a copper-catalyzed "click" reaction to form spherical micelles. Rolling circle transcription (RCT) was then performed in situ on the micelles to prepare a large amount of shRNA. **b** shRNA@ODN–PLA encapsulates hydrophobic doxorubicin (DOX) and is further condensed by PTT-g-PEG to obtain nanoparticles for the synergistic treatment of multidrug-resistant breast cancer (**c**)

PLA core can carry hydrophobic drugs, such as doxorubicin (DOX). The product of shRNA@ODN–PLA was then condensed by positively charged peptide-grafted poly (ethylene glycol) (PPT-g-PEG) into nanoparticles. This multifunctional composite nanoparticle could be efficiently delivered to cancer cells and accumulate in xenograft tumors, enabling multi-modal therapy for MDR breast cancer (Fig. 11) [33].

### 4.2 DNA–Strong Hydrophobic Polymer Conjugates

Generally, polymers bearing hydrocarbon chains ($–CH_2$) and aromatic units (benzene, etc.) show strong hydrophobic properties. Examples of this include polystyrene and polynorbornene. When these highly hydrophobic polymers are coupled to hydrophilic ODNs, the resulting amphiphilic OPCs typically form compact and small micelles with "solid" cores. This type of micelle is more suitable for encapsulating substances that we do not want to leak from the micelles, such as toxic contrast agents and organic dyes.

#### 4.2.1 ODN–Polystyrene Conjugates

The strong hydrophobicity of polystyrene (PS) makes the synthesis of ODN–PS conjugates difficult. Several approaches that have been applied to the synthesis of

ODN–PS conjugates include phosphoramidite chemistry [70, 71], amidation reactions [72, 73], Michael addition reactions [32], and copper-catalyzed Click reactions [48].

Due to the strong hydrophobicity of PS, ODN–PS conjugates are able to self-assemble into very stable micelles, which can be used to encapsulate hydrophobic dyes and drugs with high efficiency and stability. Park's group co-assembled magnetic nanoparticles (MNP) and ODN–PS conjugates into hybrid nanostructures, which show potential applications in magnetic separation and handling of DNA molecules, magnetic resonance imaging, local drug delivery, and treatment of diseases by magnetic hyperthermia therapy. The surfaces of such nanostructure consist of high-density ODN chains, resulting in SNAs-like properties. Therefore, these hybrid nanostructures display excellent DNA hybridization properties including a

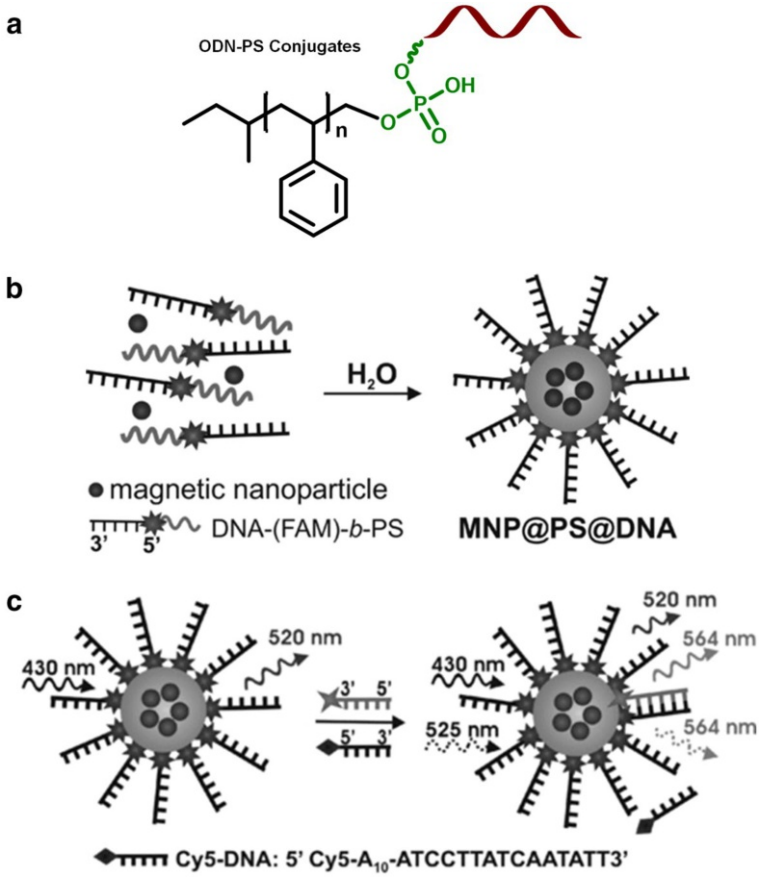


**Fig. 12** Assembly and application study of ODN–PS conjugates (reproduced with permission from [71]). **a** Chemical structure of ODN–PS conjugates prepared by phosphoramidite chemistry. **b** Preparation of ODN–PS assembly with magnetic nanoparticles. **c** Cy3-labeled target DNA preferentially binds to MNP@ODN–PS in the presence of Cy5-labeled competing ODN (i.e., the free ODN having the same sequence as that on MNP@PS–DNA)

high DNA binding constant for DNA detection and delivery applications (Fig. 12) [71].

In addition, Herrmann's group [32] incorporated ferrocene (Fc) into micelles formed by ODN–PS conjugates. They found that the introduction of Fc molecules into the hydrophobic core would not affect the micellar morphology. Moreover, Fc encapsulation significantly changes the electrical properties of the micelles, which are expected to be applied to nanoelectronics or biosensing [32].

### 4.2.2 ODN–Hydrophobic PNB Conjugates

In order to build a polymer micellar SNA to improve the biostability of ODN for intracellular and in vivo applications, Gianneschi's group conjugated a carboxylic

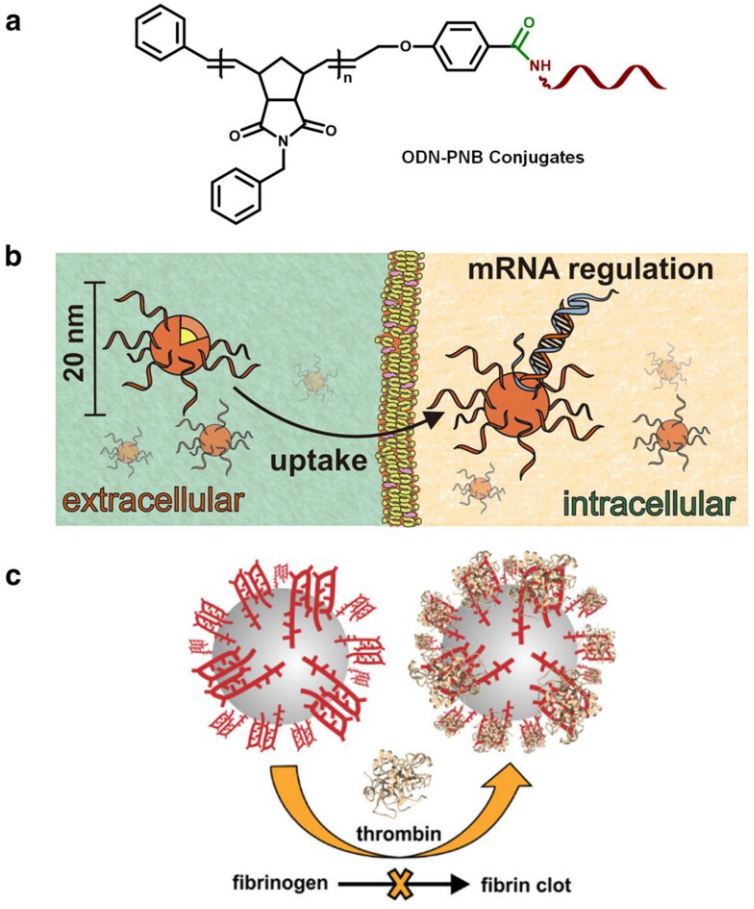


**Fig. 13** **a** Chemical structure of ODN–PNB conjugates. **b** Amphiphilic ODN–PNB conjugates self-assemble into micellar nanoparticles for intracellular gene regulation (reproduced with permission from [65]). **c** Micellar thrombin-binding aptamers for anticoagulation (reproduced with permission from [64])

acid terminated polynorbornene (PNB) with an amine-modified ODN through solid-phase coupling reaction (Fig. 13a). Due to the strong hydrophobicity of PNB, ODN–PNB conjugates self-assembled into solid micelles [63–66]. Initially, they grafted long ODNs to the side chain of PNB to form an amphiphilic brush copolymer. When the long ODN strand is cleaved by the DNAzyme, the assembly of the amphiphilic copolymer is converts from a spherical to a cylindrical structure. This cylindrical structure could reconvert into a spherical structure by adding a long complementary strand, which was a reversible process by strand competition [49]. The same group also explored a variety of applications for these kinds of ODN–PNB based SNAs including gene regulation (Fig. 13a, b) [63, 65] and micellar thrombin-binding aptamers (Fig. 13c) [64].

### 4.3 ODN–Dynamic Polymer Conjugates

Polymers show tunable hydrophobicity upon response to environmental changes, and are thus defined as "dynamic polymers". These polymers generally have low glass transition temperatures; therefore, the corresponding OPCs could form micelles showing properties different from "solid" micelles, which could go through dynamic changes under different conditions. Therefore, smart micelles fabricated from this kind of OPCsshow interesting application potentials in the biomedical field, and are able to respond to pH, enzymes, and temperature.

#### 4.3.1 ODN–PPO Conjugates

Polypropylene oxide (PPO) shows a glass transition temperature ($T_g = -$ 70 °C) much lower than that of PLGA and PS. PPO is a polymer that shows temperature-dependent properties: it is hydrophilic at low temperatures (below 20 °C) and changes to hydrophobic at room temperature. Therefore, OPC micelles with PPO as the hydrophobic core show "dynamic" characteristics. Moreover, the synthesis of ODN–PPO conjugates is very accessible, as PPO polymers with hydroxyl end groups can be readily coupled with ODN through the phosphoramidite chemistry on a DNA synthesizer (Fig. 14a).

The amphiphilic block copolymer ODN-b-PPO can self-assemble in aqueous solution to form dynamic spherical micelles. Thus, these micelles, which display a recognition function at the ODN shell and an encapsulation function at the PPO core, were applied to DNA-templated organic synthesis [45], targeted drug delivery (Fig. 14b) [74, 75], and virus loading [76]. In addition, ODN-b-PPO could be inserted into the lipid vesicle layer [77], or co-assembled with other amphiphilic micelles [78] to prepare hybrid materials. Making use of the hydrophilicity of PPO at low temperatures, ODN-b-PPO was inserted into the network of a DNA hydrogel to study the process of molecular self-collapse (due to the phase transition of PPO) [79]. In another work, the authors claimed that the dynamic micelles of ODN-b-PPO could switch from spherical to rod-shaped structures via hybridization with cDNA (Fig. 14c) [80]. In addition, the use of a stimuli-responsive ODN sequence could also induce the dynamic change of ODN-b-PPO micelles. For example, the

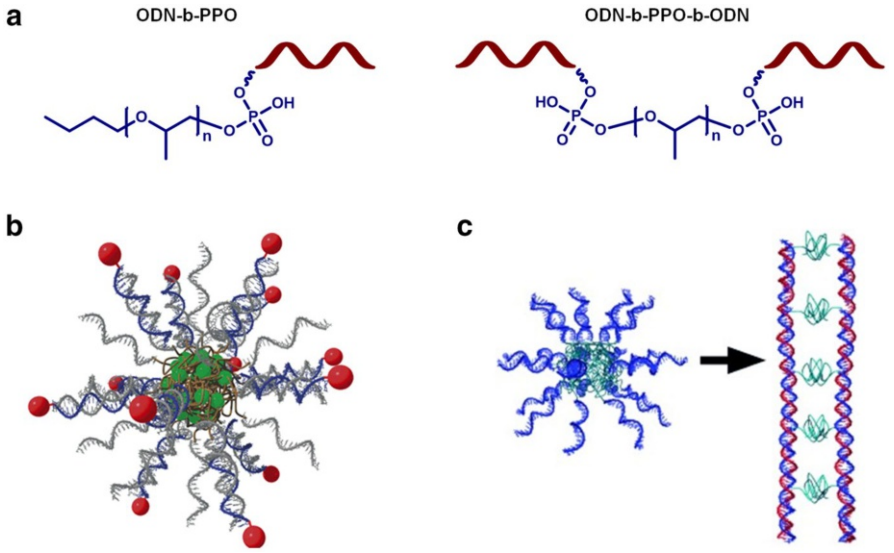


**Fig. 14** **a** Structures of ODN-b-PPO [74] and ODN-b-PPO–ODN [79]. **b** Micelles formed by the self-assembly of ODN-b-PPO amphiphilic block copolymer are used for the treatment of cancer (reproduced with permission from [74]). (Green ball hydrophobic drug, red ball targeting group.) **c** Morphological changes of ODN-b-PPO with thermodynamic equilibrium induced by long-chain DNA hybridization (reproduced with permission from [80])

pH-responsive "i-motif" sequence will fold at acidic pH conditions, which would drive the morphology change of the self-assembly from spherical micelles to long fibers [81]. The biological methods, such as enzymatic polymerization or ligation [82–84], can also be used to change the properties of ODNs and subsequenty control the morphology changes of the OPC self-assemblies.

### 4.3.2 ODN–PNIPAM Conjugates

Poly(*N*-isopropylacrylamide) (PNIPAM) is a widely studied temperature-sensitive polymer. At room temperature, linear PNIPAM dissolves well in aqueous solution, but when the temperature reaches a certain temperature, PNIPAM will undergo a transition from hydrophilic to hydrophobic in nature. The critical temperature for hydrophilic–hydrophobic transition is called low critical solution temperature (LCST). Accordingly, a PNIPAM-based microgel exhibits a high degree of swelling (hydration state) at room temperature, and, when the temperature is raised to about 32 °C, the PNIPAM microgel will convert into a dehydrated state, resulting in significant shrinkage. The transition temperature of PNIPAM microgel is defined by the volume phase transition temperature (VPTT). The fast-response and temperature-sensitive properties of PNIPAM make it suitable for biomedical applications such as controlled drug delivery and biosensing.

Based on the reversible phase-transition properties of PNIPAM, ODN–PNIPAM conjugates are expected to show new and interesting properties (Fig. 15a).

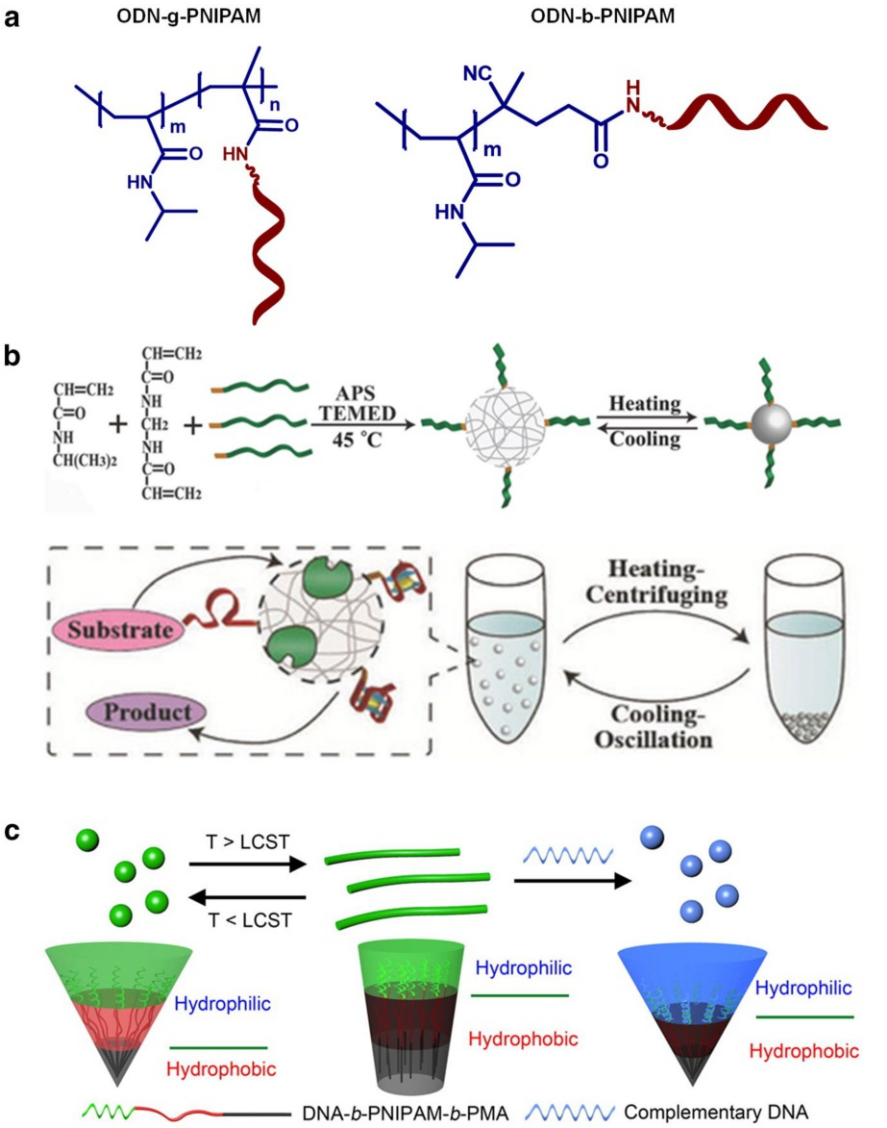


**Fig. 15** Application and assembly behavior of ODN–PNIPAM conjugates. **a** Structures of ODN-g-PNIPAM [86] and ODN-b-PNIPAM [91]. **b** A multifunctional poly-*N*-isopropylacrylamide/DNAzyme microgel as a highly efficient and recyclable catalyst for biosensing (reproduced with permission from [58]). **c** Multimodal shape transformation of a dual responsive ODN block copolymer (reproduced with permission from [91])

For example, the charge of ODN would affect the phase transition behavior of ODN–PNIPAM conjugates [85], and the molecular recognition capability, stimuli-responsiveness, and therapeutic functions of ODNs would greatly broaden the properties of PNIPAM. Various materials (such as hydrogels [86]) have been developed

based on ODN–PNIPAM conjugates, which have been applied in the separation of nucleic acids [87, 88] and proteins [89], as well as recyclable biocatalysts [58] (Fig. 15b). ODN–PNIPAM conjugates also showed more versatile self-assembly properties. For example, the molecular hybridization capability of ODN allows PNIPAM to form layer-by-layer assemblies [90] or aggregate by non-covalent crosslinking [31]; Some switchable self-assemblies can also be prepared by using the phase-transition properties of PNIPAM (Fig. 15c) [91]. In addition, PNIPAM can be combined with classical DNA nanostructures (such as DNA tetrahedral [92]) to produce smart nano-materials.

#### 4.3.3 ODN–$HE_n$ Conjugates

The structure and properties of DNA can be well-defined by its sequence. Inspired by this, synthesized organic molecules can be coupled sequentially to the ODN strand on the solid support using a DNA synthesizer. Sleiman's group [53] used commercially available dimethoxytrityl (DMT)-protected dodecanediol phosphoramidite, which corresponds to the hexamer portion of polyethylene (HE) for the synthesis of sequence-controlled OPCs. Due to highly efficient phosphoramidite chemistry reactions, the degree of polymerization can be fully controlled (up to 72 units). Upon purification by reverse-phase high-performance liquid chromatography (HPLC), monodispersed ODN–$HE_n$ conjugates can be obtained (Fig. 16a) [53]. The presence of the phosphate moiety in the polymer backbone does not affect the hydrophobic nature of the HE moiety. Moreover, the hydrophobicity of the OPCs increased as the degree of polymerization increased. When the number of HE units reached six, ODN–$HE_6$ conjugates formed spherical micelles in the presence of magnesium ions ($Mg^{2+}$). The more HE units incorporated, the more stable the resultant micelles became, with a higher loading capability for hydrophobic guest molecules. It should be noted that all ODN–$HE_n$ conjugates retained hybridization capability to cDNAs. Taking advantage of the controlled self-assembled properties of ODN–$HE_n$ conjugates, as well as the precise hybridization of ODNs, a large number of newly assembled structures or materials were prepared [93]. Examples include the superstructure formed by DNA nanocages (Fig. 16b) [94] and DNA cage-based ring structures formed through hydrophobic interactions (Fig. 16c) [95]. With regard to the application, the micelles of ODN–$HE_n$ conjugates were used as nano-reactors to improve the efficiency of the coupling reaction between hydrophobic molecules and ODNs [96]. Similar to other OPCs, ODN–$HE_n$ conjugates were also applied to drug delivery [97, 98] and gene regulation [99, 100].

### 4.4 ODN–Conjugated Polymer Conjugates

The π-conjugated polymer is one of the most important organic functional materials, which shows extended π-conjugation along the molecular backbone with delocalized π-electrons. Due to their excellent light-harvesting and light-amplifying properties, π-conjugated polymers have been used widely in the biomedical and biosensing fields [101]. In addition, π-conjugated polymer-based OPCs show strong π–π and

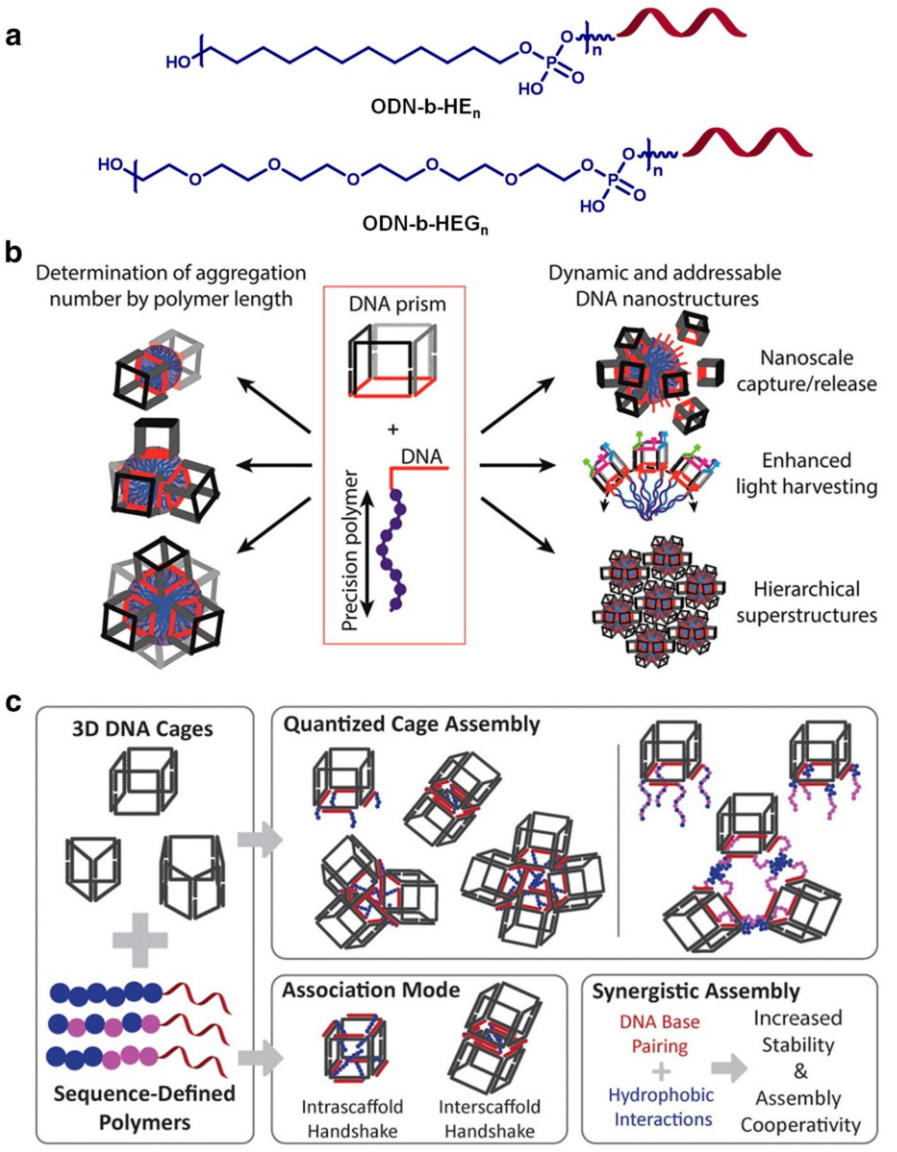


**Fig. 16** Self-assembly of sequence-defined ODN–HE$_n$ conjugates. **a** Chemical structure of ODN–HE$_n$ and ODN–HEG$_n$ conjugates [53]. **b** Precision assembly of ODN–HE$_n$ conjugates with 3D DNA cages into DNA cage-micelles or hierarchical superstructures (reproduced with permission from [94]), as well as the ring structure of DNA cages (**c**) (reproduced with permission from [95])

hydrophobic interactions, which would result in unique self-assembly behaviors and interesting optical and electronic properties [102].

#### 4.4.1 ODN–PPE Conjugates

Water-soluble poly(phenyleneethynylene) (PPE) is easy to synthesize and has a high fluorescence quantum yield in aqueous solution, so it has been applied widely to fluorescence biosensing. Yang et al. [51] developed a molecular beacon system using PPE polymer as the fluorescence reporter. Due to the super-quenching property of their π-conjugated polymer, the fluorescence of PPE was completely quenched when the molecular beacon was folded to a hairpin structure. When hybridized with the target DNA, the hairpin structure was opened to generate fluorescence signal. This molecular beacon based on a π-conjugated polymer shows a high sensitivity for nucleic acid detection [51], due to the good light-harvesting capability and efficient energy transfer along the molecular backbone of PPE. A DNA molecular beacon modified with PPE as the energy donor and the HEX (hexachlorofluorescein) dye as the energy acceptor has also been developed, which would detect nucleic acids through FRET (fluorescence resonance energy transfer) signals, resulting in a more specific and quantitative detection capability (Fig. 17) [27].

#### 4.4.2 ODN–PFO Conjugates

The single-walled carbon nanotube (SWNT) is one of the most important carbon nanomaterials, exhibiting excellent mechanical, electrical, thermal and optical properties. SWNTs intrinsically tend to bundle together due to van der Waals interactions. Surface functionalization with amphiphilic dispersant provides an efficient method to disperse and purify SWNTs with minimal introduction of defects. It is interesting to note that polyfluorene (PFO) can selectively dissolve semiconducting SWNTs rather than the conductive counterpart with a narrow size distribution.

Herrmann's group [46] coupled PFO and ODN to synthesize ODN–PFO conjugates (Fig. 18), which could effectively and selectively disperse SWNTs as well as provide a platform for precise operation. Using the ODN–PFO conjugates, electronic devices can be fabricated by the bottom-up method on an extended surface, resulting in high yields. Electrostatic repulsion between ODNs and the strong interaction between PFO and the sidewalls of the SWNTs make ODN–PFO a good dispersion agent for SWNTs. In addition, ODN-modified gold nanoparticles could be readily functionalized to the surface of the nanodevice through hybridization with ODN–PFO dispersed SWNTs [46].

#### 4.4.3 ODN–PT Conjugates

Due to the liquid crystal properties of rigid–rod type polymers, rod–coil type block copolymers show higher structural diversity than conventional coil–coil type polymers. Conjugates of ODN and rigid–rod polymers were thus predicted to have unusual assembly behaviors; they could be either rod–coil type block copolymers (single-stranded ODN with a flexible structure) or rod–rod type

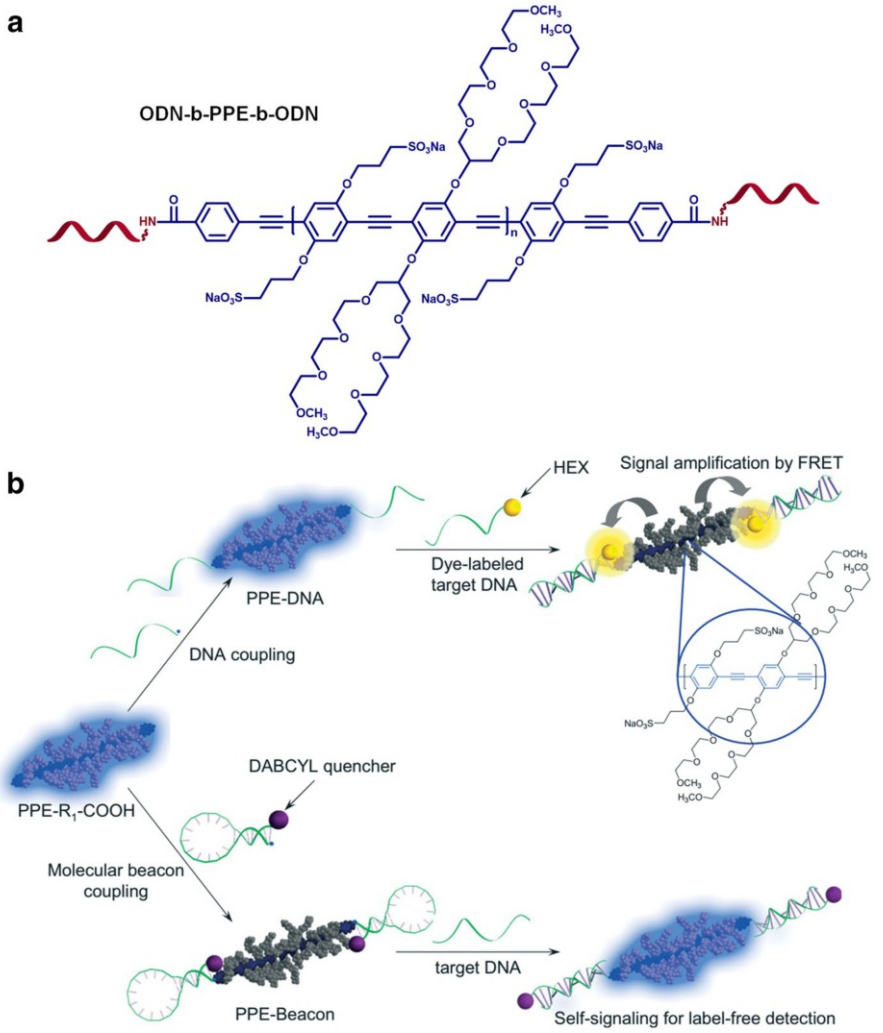


**Fig. 17** **a** Chemical structure of water-soluble poly(phenyleneethynylene) (PPE)–ODN conjugates. **b** Molecular beacons and signal amplification systems based on conjugated polymers (reproduced with permission from [27])

block copolymers (duplex nucleic acid with a relatively rigid structure). Park's group coupled the rigid–rod polymer PT (polythiophene) with ODN to synthesize ODN-b-PT conjugates (Fig. 19). In general, ODN amphiphilic block copolymers tend to form simple spherical micelles. However, due to the rigid structure of PT and its strong π–π interaction, ODN-b-PT conjugates formed a hollow vesicle structure. It should be noted that, due to the dense stacking of PTs, the vesicles formed by ODN-b-PT show very weak fluorescent signals in water. The size of the vesicles formed by self-assembly is regulated by adjusting the concentration

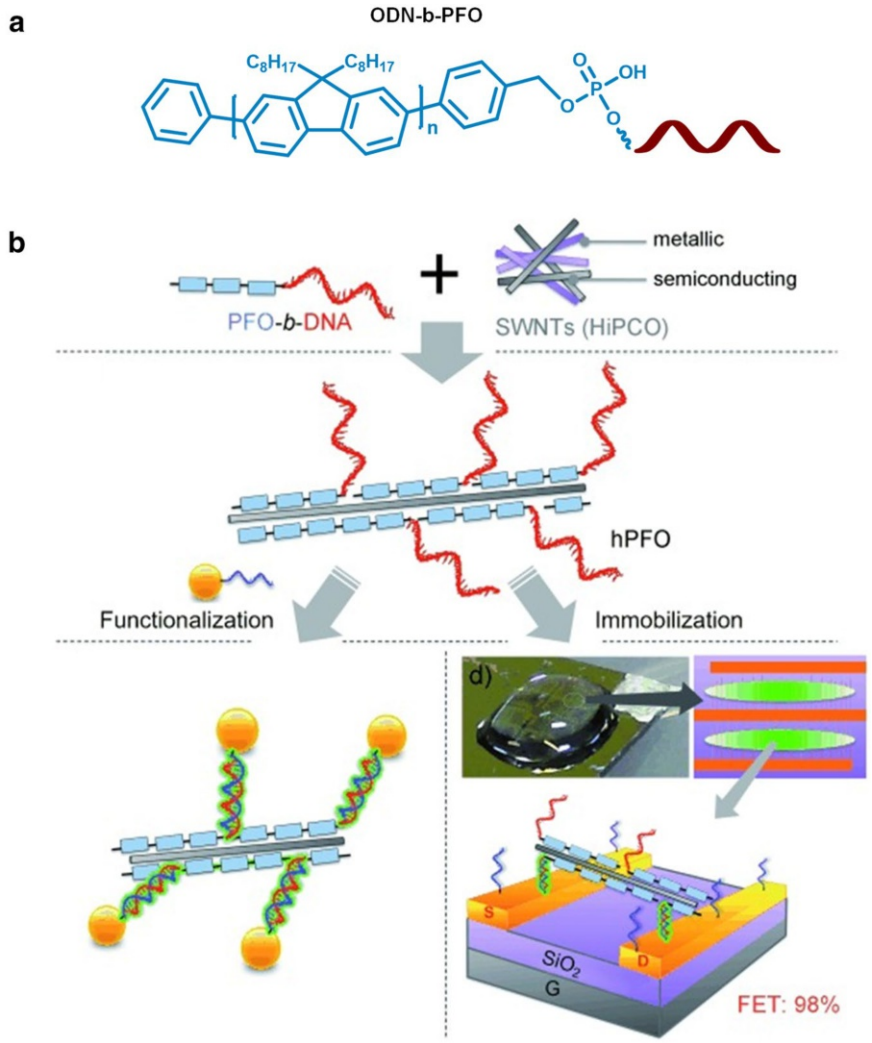


**Fig. 18** **a** Chemical structure of ODN–PFO conjugates. **b** Co-assembly of ODN–PFO conjugates and single-walled carbon nanotubes (SWNTs) and their application in nanoelectronics (reproduced with permission from [46])

of ODN-b-PT in aqueous solution. In addition, increasing the salt concentration in solution converted ODN-b-PT from a vesicular morphology to a sheet structure; this change was reversible. Interestingly, the researchers incorporated ODN-b-PT into a one-dimensional nanoribbon assembled from PEG-b-PT to functionalize the nanoribbon with AuNPs. The ODN-conjugated polymer prepared by coupling ODN with a rigid conjugated polymer show interesting morphologies due to the strong π–π interactions, and new properties due to the optoelectronic properties of conjugated polymers [103].

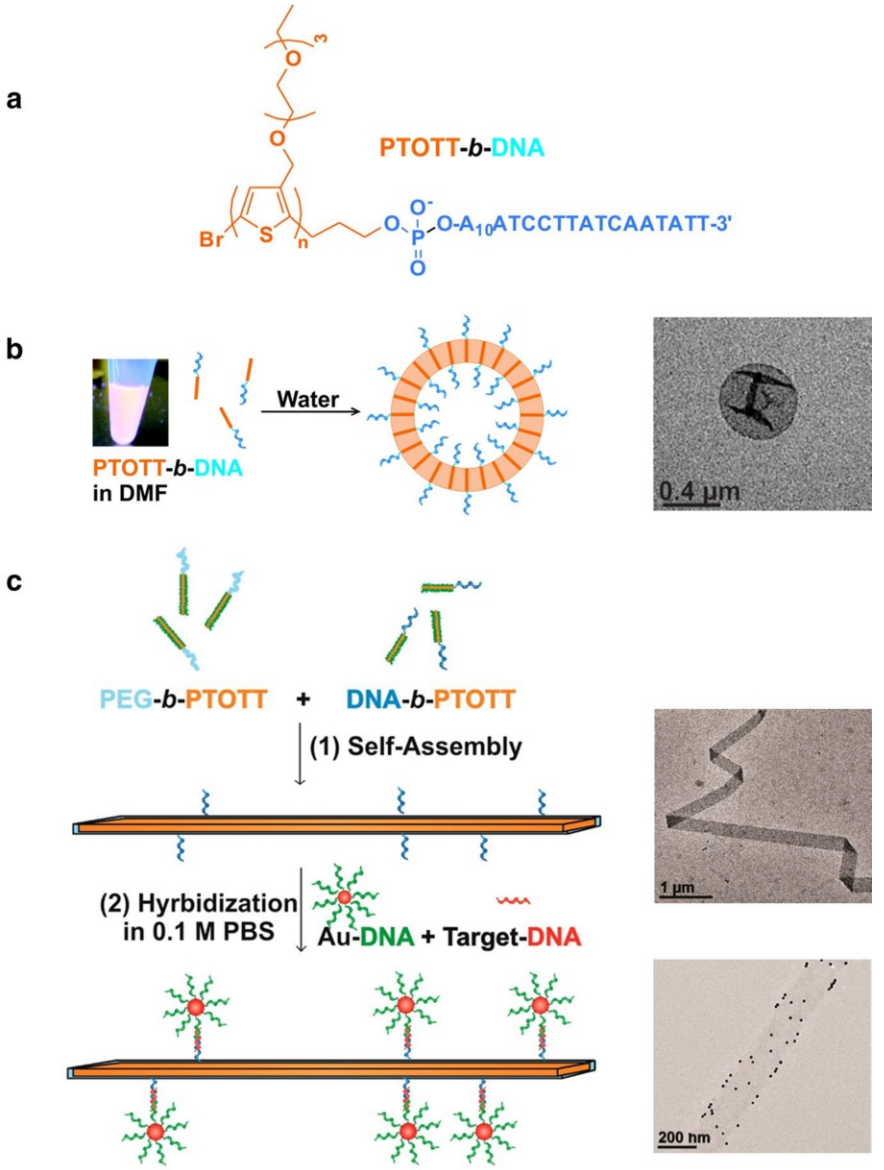


**Fig. 19** **a** Chemical structure of ODN–PT conjugates. **b** ODN–PT conjugates self-assemble into vesicles in water; transmission electron microscopy (TEM) images of vesicles at the right. **c** ODN-b-PT conjugates can be co-assembled with PEG-b-PT to achieve the functionalization of the nanoribbons. TEM images at the right. Insets **b**, **c** are adapted with permission from [103]

#### 4.4.4 ODN–PFP Conjugates

PFP (poly[fluorine-co-phenylene fluorene]) is a typical fluorescent polymer that displays an effect called "aggregation caused quenching" (ACQ). Xia's group [28] prepared ODN-b-PFP conjugates by coupling ODN and PFP via amidation reactions. Due to the hydrophobicity of the benzene ring on the PFP backbone and the hydrophilicity of the ODN phosphate backbone, ODN-b-PFO formed a micellar structure in aqueous solution. In the presence of $Hg^{2+}$ ions, the formation of T-$Hg^{2+}$-T complex leads to the further aggregation of the micelles, which would quench the fluorescence of the PFP due to the ACQ effect. After addition of the chloride anion, the formation of $HgCl_2$ reduced the concentration of $Hg^{2+}$ in solution, which could efficiently recover the ODN-b-PFP conjugates. In another study, a strand of ODN that could be recognized by telomerase was used, and the resultant ODN-b-PFP tightly self-assembled into micelles showing quenched fluorescence. In the presence of telomerase, the length of ODN increased via the addition of GGGTTA repeats, which increased the hydrophilicity of the ODN-b-PFP conjugates. Due to this, the aggregation state of the micelles became unstable and loose, and the PFP fluorescence was increased. These interesting ODN–PFP conjugates can be applied in bioassays and environmental analysis (Fig. 20) [28].

#### 4.4.5 ODN–APPV Conjugates

Molecular conformation is crucial to the performance of nanodevices based on single polymers; however, this needs precise control at the molecular scale and is still very challenging for recent techniques. Gothelf et al. [104] prepared hydroxyl-group-modified (2,5-dialkoxy) paraphenylene vinylene (APPV) conjugated polymers. ODNs were directly synthesized on the side chains of APPV by automated solid-phase synthesis to obtain ODN-grafted APPV (ODN-g-APPV; Fig. 21a). DNA origami technology can produce unique two- or three-dimensional nanostructures with very precise addressable capability. Anchor DNA chains with a specific shape were predesigned on the DNA origami template, and then the conformation of the individual APPV was controlled at the nanoscale by DNA hybridization manipulation. By virtue of this method, an individual APPV can be assembled into arbitrary shapes in 2D or 3D geometry (Fig. 21b). Therefore, this method was the first to use a DNA origami technique to control the geometry of a π-conjugated polymer at the molecular scale, which may generate interesting electric or optical properties.

### 4.5 ODN–Other Polymer Conjugates

#### 4.5.1 ODN–PAM Conjugates

Polyacrylamide (PAM) was initially used in electrophoresis. More recently, due to its low cost and availability, PAM has been usedwidely in enzyme immobilization, drug delivery, and other biomedical applications. Acrylamide can

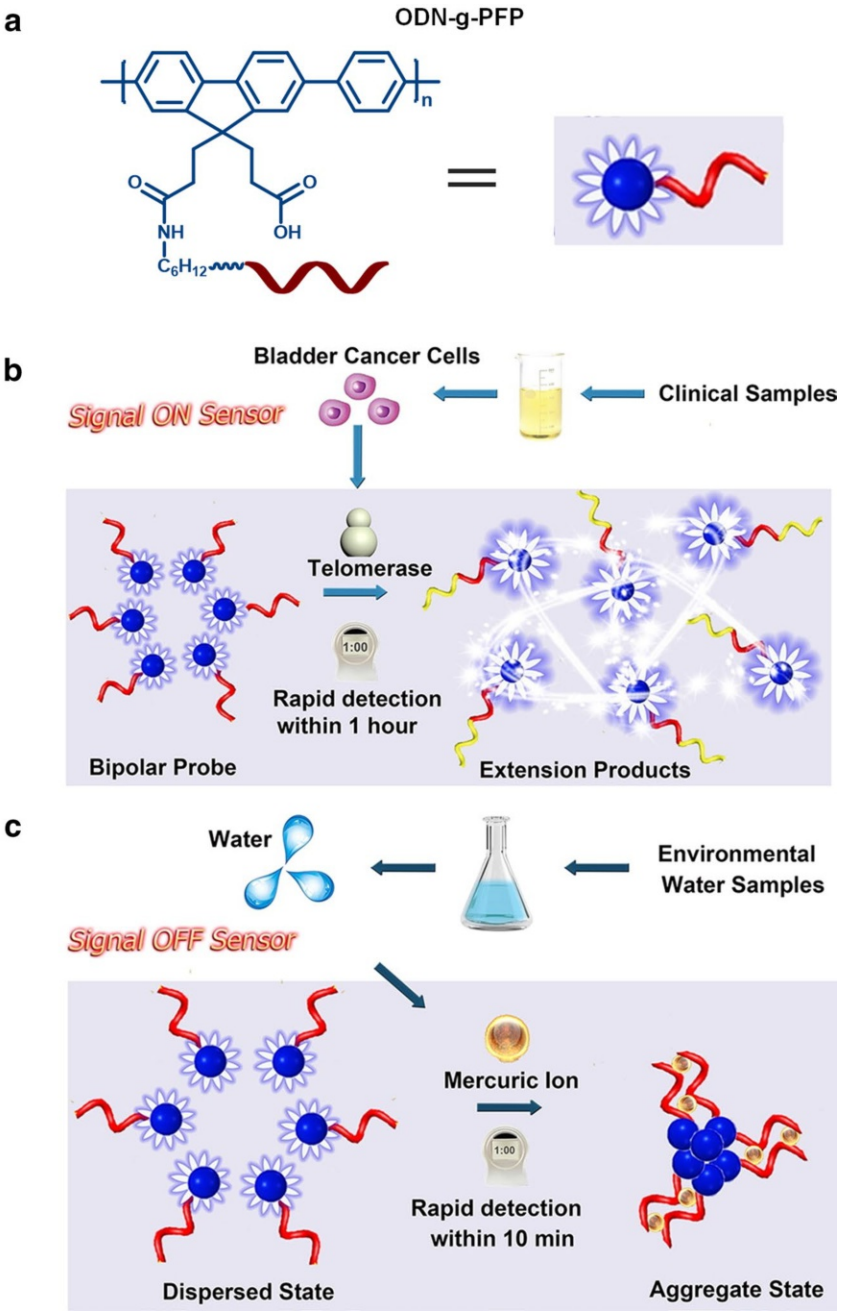


**Fig. 20** **a** Chemical structure of ODN–PFP conjugates. **b** Detection of telomerase by prolonging the ODN chain to release the fluorescence of PFP. **c** Decreased fluorescence due to increased aggregation by the addition of $Hg^{2+}$ (adapted with permission from [28])

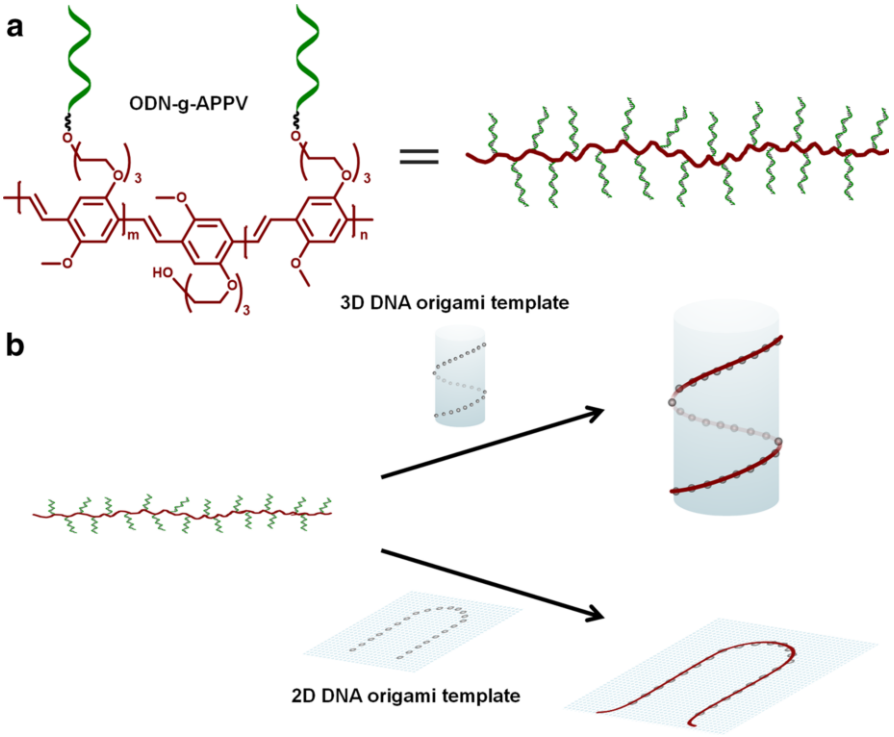


**Fig. 21** **a** Chemical structure of ODN-g-(2,5-dialkoxy) paraphenylene vinylene (APPV). **b** Illustration of the organization of an individual ODN-g-APPV by DNA origami template

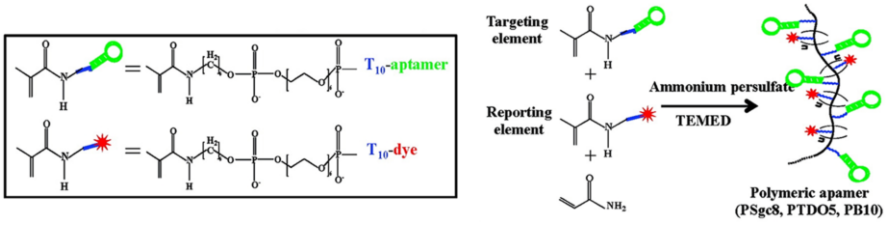


**Fig. 22** Synthesis of polymeric aptamers via ODN–PAM conjugates (reproduced with permission from [39])

polymerize into linear polyacrylamide, and can also copolymerize with many bifunctional crosslinking agents (the most commonly used is diacrylamide) to give gelation. Gel polymerization is usually initiated by ammonium persulfate (APS), and the reaction rate can be accelerated by adding catalysts such as *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) [105]. Since PAM can be prepared easily, among many OPCs, ODN–PAM conjugates were readily synthesized and processed.

Based on the stability and biocompatibility of PAM, Tan's group [39] incorporated acrydite-modified ODN into the PAM backbone by copolymerization (Fig. 22). The three components are copolymerized into ODN–PAM conjugates by a one-step process: 5′-acrydite-$T_{10}$-dye was used for tracking cell internalization; multiple targeting aptamers on the polymer chain can promote intracellular delivery through multivalent effects [39].

ODN–PAM conjugates also show unparalleled potential for the preparation of smart hydrogels. ODN–PAM hydrogels are more economical and adaptable than expensive full-DNA hydrogels [106]. In addition, by regulating the diversity of ODN, pH-responsive [107, 108], specific DNA sequence-responsive [109], or shape-memory hydrogels [59, 110, 111] were prepared. As for hydrogel swelling, the conformational change of the DNA chain can cause ODN–PAM hydrogels to swell by 10–15% of their original size, which is usually insufficient to alter the shape of the macroscopic gel system; thus Schulman's group prepared a DNA-triggered, deformable hydrogel with a significant degree of swelling, in which multiple DNA strands were inserted into the duplex. Moreover, a "terminator hairpin" can be created by modifying the sequence of the polymerizing hairpins. By adjusting the relative concentrations of polymerizing hairpins and terminator hairpins, the hydrogel swell could be well controlled to a certain degree [60].

#### 4.5.2 Brush PNB-Based OPCs

Ring-opening metathesis polymerization (ROMP) is a living polymerization reaction, in which a carbon–carbon double bond in a cyclic olefin is broken to form a new chemical bond in the presence of a metal catalyst. Norbornene and its derivatives are the monomers most studied and widely used for ROMP because of their high reactivity, abundant sources, and low price [112]. Moreover, many functional molecules can be coupled easily to the norbornene monomer, such as hydrophilic PEG [113], hydrophobic drugs [114], or charged ferrocene [115, 116], etc., to explore different possibilities for research.

Zhang's group designed and synthesized a new brush polymer/ODN conjugate [117–120]. The dense PEG side chain protects the ODN from protein degradation but allows accessibility of DNA hybridization. This ODN conjugate, protected by the dense PEG side chain, exhibits better biopharmaceutical properties compared to naked ODN, including better gene silencing efficiency and better physiological stability (Fig. 23a) [117].

More stable and controllable drug delivery effect can be obtained by chemically conjugating hydrophobic drug molecules to the micelles instead of physically encapsulation. Therefore, Zhang's group chemically conjugated paclitaxel to polynorbornene to form amphiphiles with hydrophilic ODN, which formed stable micelle nanoparticles enabling simultaneous intracellular delivery of drug molecules and therapeutic ODNs (Fig. 23b) [114].

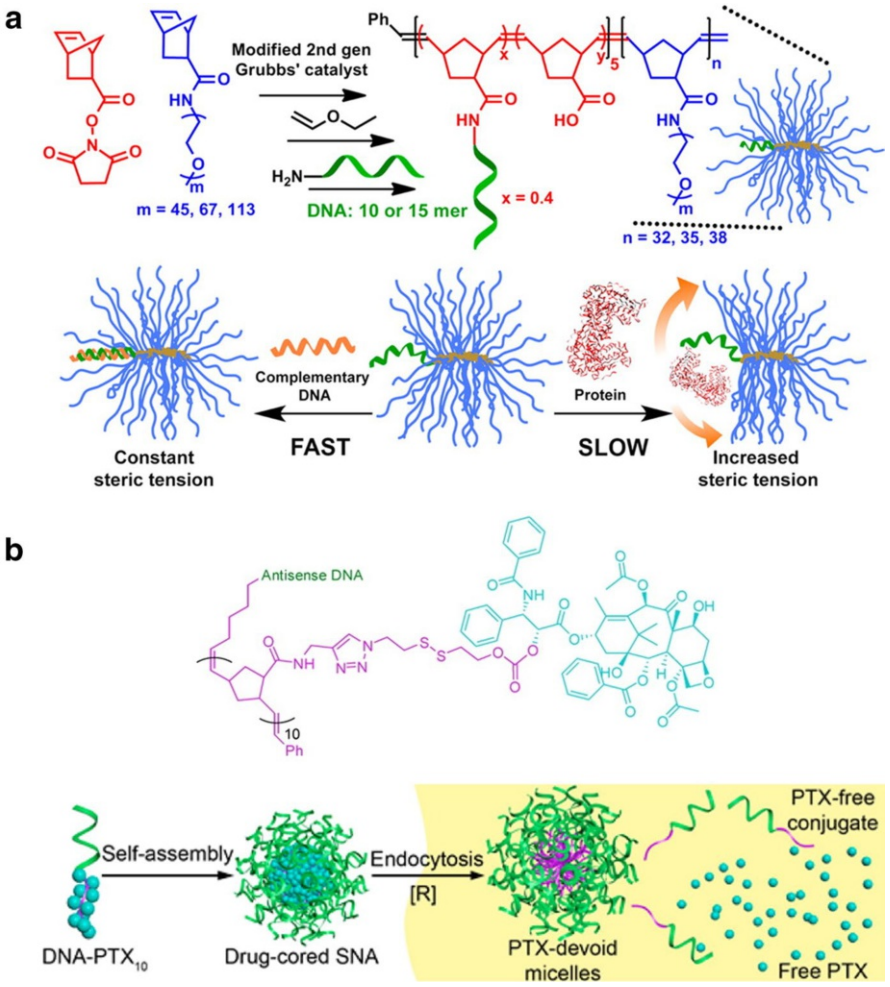


**Fig. 23** **a** Compact brush-polymers provide steric selectivity to the ODN (reproduced with permission from [117]). **b** Chemical conjugation of paclitaxel to the polynorbornene side chain for stable and controlled intracellular delivery (adapted with permission from [114])

### 4.5.3 ODN–ATRPolymer Conjugates

ATRP has been applied successfully to the preparation of various materials with controlled structures [121]. Using ATRP, it is possible to synthesize a variety of well-defined, multi-component polymers, such as block copolymers, graft copolymers, and hyper-branched polymers. In addition, the polymers synthesized by ATRP can be further functionalized by three synthetic strategies: (1) using functional ATRP initiators; (2) direct polymerization of functional monomers; (3) coupling chemistry utilizing end groups. Due to the attractive

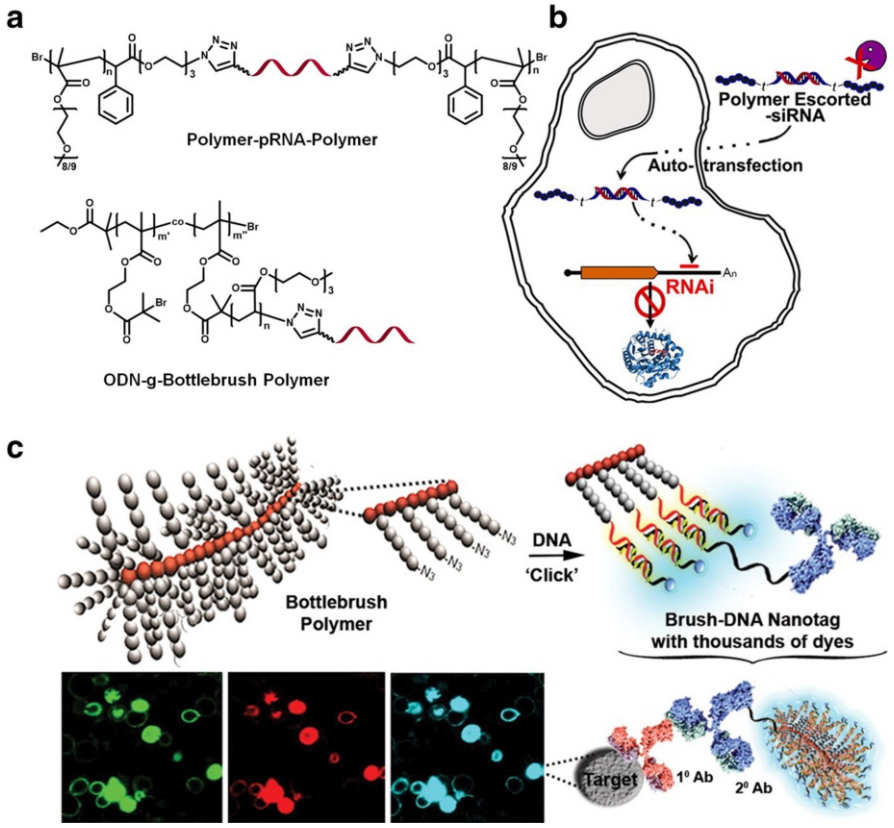


**Fig. 24** **a** The chemical structure of ODN brush/bottlebrush polymer conjugates. **b** Self-transfection of siRNA escorted by polymers (reproduced with permission from [122]). **c** ODN brush/bottlebrush polymer conjugates as a scaffold for functionality organization [123]

nature of ATRPolymers, the combination of ATRPolymers with ODN makes it easy to prepare special polymers with interesting structures or for some unusual applications.

In 2013, Das's group [122] used activators generated by electron ATRP to prepare a series of well-defined azide-terminated polymers for chemical conjugation with di-alkyne-functionalized passenger-stranded RNA (pRNA). This polymer-pRNA-polymer can escort the sense strand into the cell to realize RNA interference, and the polymer-pRNA-polymer structure could efficiently resist nuclease degradation (Fig. 24a, b) [122]. In 2015, the same group synthesized a bottlebrush polymer using ATRP, and then chemically conjugated the ODN to the bottlebrush polymer. These ODN-bottlebrush polymer conjugates can hold thousands of dye molecules, and, thanks to the array of ODN brushes, dye self-quenching could be efficiently avoided, resulting in a bright fluorescent signal (Fig. 24c) [123]. In addition, the ODN on this bottlebrush polymer, with a high local concentration, still retained its accessibility to endonucleases and kept chain displacement capability [124].

**Table 1** Various oligonucleotide–polymer conjugates (OPCs) and their applications in different research fields

| OPCs | | Research field |
|---|---|---|
| ODN–biodegradable polymer | ODN–PLGA | Micelles for ODN delivery [67], siRNA delivery [29, 30] |
| | ODN–PCL | ODN delivery [34, 61], Nanogel for siRNA delivery [41] |
| | ODN–PLA | Gene therapy and drug delivery [33] |
| ODN–highly hydrophobic polymer | ODN–PS | Loading inorganic nanoparticles [71] or organic molecules [32] |
| | ODN–PNB | Gene regulation [63, 65], micellar aptamers [64] |
| ODN–dynamic polymer | ODN–PPO | Template-based synthesis [45], drug delivery [74, 75], virus loading [76], self-assembly [77, 78, 80, 81], hydrogels [79] |
| | ODN–PNIPAM | Separation of nucleic acid [87, 88] and protein [89], self-assembly [31, 90–92], hydrogel [86], biocatalytic carrier [58] |
| | ODN–$HE_n$ | Self-assembly [93–95], drug delivery [97, 98], gene regulation [99, 100], nanoreactors [96] |
| ODN–conjugated polymer | ODN–PPE | Self-assembly [47], nucleic acid detection [27, 51] |
| | ODN–PFO | Nanoelectronics [46] |
| | ODN–PT | Self-assembly [103] |
| | ODN–PFP | Detection of ion and enzyme [28] |
| | ODN–APPV | Routing of individual polymers [104] |
| ODN–other polymer | ODN–PAM | Drug delivery [39], hydrogel [59, 110, 111] |
| | Brush PNB-based OPCs | Drug delivery [114], self-assembly [113], ODN delivery [117–120], electrochemistry [115, 116] |
| | ODN–ATRPolymer | Self-assembly [126], siRNA delivery [122], fluorescence labeling [123] |

## 5 Conclusion and Outlook

After nearly a century of development, a wide range of synthetic polymers with tunable physical, chemical, and biological properties are available for various applications. Since research on nucleic acids has extended from the biological applications to the material fields since 1980s, a wide variety of OPCs have been synthesized and used in supramolecular self-assembly, drug delivery, and biosensing. Table 1 summarizes the application of different types of OPCs in different fields.

Although many synthetic methods to produce OPCs have been developed, compared with conventional polymers, the synthesis of OPCs still needs special conditions or machines (solid-phase DNA synthesizer) and generally exhibits low efficiency due to the high heterogeneity between DNA and synthetic polymers. Thus, simple and efficient synthesis strategies that can be commonly applied to all kinds of OPCs are still urgently required. In particular, as for amphiphilic OPCs block copolymer, how to control and tune their self-assembly behavior by adjusting the ratio between hydrophobic and hydrophilic blocks has not been thoroughly investigated. In addition, compared with other blocks that are used to construct amphiphilic polymers, DNA shows many unique properties, including well-defined molecular weights and conformations, molecular recognition properties, stimuli-responsiveness, and therapeutic capability. All of these interesting properties make OPCs a promising branch of amphiphilic polymers. However, the application scope of OPCs is still relatively narrow, and it is necessary to continually explore more OPCs for new application purposes. As for in vivo application, many critical properties of OPCs, such as bio-stability, biocompatibility, immunogenicity, and intracellular penetration capability, need more in-depth characterization and further improvement. Last, but most importantly, the integration of DNA functions and polymer characteristics will be the future development direction of OPCs. For example, the programmability and molecular recognition of DNA can be used to regulate the aggregation state of polymers at the nanoscale; hydrophobic polymers can contain some functional molecules and their properties can be tuned through DNA hybridization; π-conjugated polymers with strong light-harvesting and energy transfer capabilities can enrich the application of DNA-based biomaterials in the field of optoelectronics. Therefore, we believe that there are still many opportunities for investigation of OPCs in future research, and that we require further efforts in this direction.

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### Compliance with Ethical Standasrd

**Conflict of interest** On behalf of all authors, Ying Bao and Leilei Tian state that there is no conflict of interest.

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