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Strategies to Bring Conjugated Polymers into Aqueous Media

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1.1 Introduction

Conjugated polymers (CPs) are organic macromolecules with extended π-conjugation along the molecular backbone [1, 2]. Their unique optoelectronic properties that result from the highly delocalized π-electrons can be easily manipulated through modification of the conjugated backbones. As a result, CPs have been widely used in various research fields related to organic optoelectronic devices [3–5], chem/biosensors [6, 7], and medical diagnosis and therapy [7–9]. For biology-related applications, the main obstacle is to render CPs water soluble or water dispersible. So far, mainly three strategies have been used to bring CPs into aqueous media, which include the design and synthesis of conjugated polyelectrolytes (CPEs) and neutral water-soluble conjugated polymers (WSCPs), as well as the fabrication of water-dispersible conjugated polymer nanoparticles (CPNPs) (Scheme 1.1).

CPEs are a kind of macromolecules characterized by π-conjugated backbones and ionic side chains [10]. Their solubility in aqueous media can be fine-tuned by modification of the ionic side chains. Although neutral WSCPs do not possess any charge, they have amphiphilic segments, for example, oligo(ethylene glycol) [11], that compensate for the hydrophobic nature of the conjugated backbones. These two strategies require the chemical modification of each polymer to bring them into water. A more general and straightforward method is to prepare for the CPNPs, which can in principle bring any organic soluble polymers into aqueous media [12]. To simplify our discussion, in this chapter, we only discuss CPNPs that are prepared from neutral CPs. The water solubility of CPNPs is largely determined by the polymer matrix used and the nanoparticle size, while their optical properties are associated with the neutral CP.

This chapter aims to provide readers with an overview of the strategies that can be used to bring CPs into aqueous media for potential biological applications. In this chapter, we will discuss the synthetic approaches for CPEs first, which is followed by the neutral WSCPs and CPNPs. The section on CPEs is organized
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According to the charge sign. For each type of charge, we mainly select three types of CPEs (polythiophenes, poly(phenylene)s, and polyfluorenes) as examples to describe how the conjugated backbones can be synthesized and how the charged chains can be incorporated. Similar strategies will be discussed on neutral WSCPs. For the section on CPNPs, we will mainly introduce three strategies for CPNP preparation (e.g., reprecipitation, miniemulsion, and nanoprecipitation) with specific examples. Owing to the limited space, we apologize that we cannot cover every example, and only the most representative ones are selected for discussion.

1.2 Synthesis of CPEs

In the past decades, a large number of CPEs have been successfully developed. According to their chemical structures, the synthesis of CPEs involves two aspects: construction of the conjugated backbones and incorporation of charged side chains. Many well-established polymerization methods have been employed to build the conjugated backbones (Scheme 1.2), which were typically catalyzed with organometallic complexes or bases, including Suzuki, Yamamoto, Stille coupling reaction and FeCl$_3$-catalyzed oxidative reaction for single bond formation; Heck, Witting, Knoevenagel, and Gilch coupling reactions for double bond formation; and Sonogashira coupling reaction for triple bond formation. In addition, CpCo(CO)$_2$-catalyzed homopolycycloctrimethylenization has also been used to synthesize hyperbranched CPEs [13]. Rational design of the conjugated backbones allows facile manipulation of their optical properties, such as absorption, emission, and quantum yield. The charges can be incorporated via direct polymerization of charged monomers or postfunctionalization of neutral CPs into CPEs (Scheme 1.3). According to the charge sign of the ionic side chains, CPEs can be categorized into three groups: anionic CPEs, cationic CPEs, and zwitterionic CPEs. The anionic groups generally include sulfonate [14], carboxylate [15], and phosphonate [16], while the cationic groups include quaternary ammonium [17], pyridinium [18], and phosphonium [19]. In the following section, we will use specific examples to show the synthetic approaches of CPEs with different charges. We start each section with polythiophenes, as they are commonly synthesized via electropolymerization and FeCl$_3$-catalyzed oxidative polymerization methods. The other CPEs are generally introduced following the sequence of single, double, and triple-bonded CPEs.
1.2 Synthesis of CPEs

Scheme 1.2 Polymerization methods most widely employed to construct conjugated backbones. \( \text{Ar}_1 \) and \( \text{Ar}_2 \) represent aromatic units.

Scheme 1.3 Representative strategies for synthesis of CPEs through incorporation of charges via direct polymerization (a) and postpolymerization (b) method.
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1.2.1 Anionic CPEs

1.2.1.1 Sulfonated CPEs

Sulfonated polythiophenes are generally synthesized through electropolymerization or FeCl₃-catalyzed oxidative polymerization methods. As shown in Scheme 1.4a, the first sulfonated polythiophene P₂ was reported by Wudl’s group in 1987 [14]. 2-(Thiophen-3-yl)ethanol 1 reacting with methanesulfonyl chloride yielded methyl 2-(thiophene-3-yl)ethanesulfonate 2. Electropolymerization of 2 led to a neutral polythiophene P₁, which was subsequently treated with NaI in acetone to give sulfonated P₂. Using the same electropolymerization method, another sulfonated polythiophene P₃ was developed by Zotti’s group (Scheme 1.4b) [20]. The key sulfonated monomer 4 was synthesized by alkylation of 4H-cyclopenta[2,1-b:3,4-b‘]dithiophene 3 in the presence of 1,4-butanesultone and n-BuLi. The direct electropolymerization of 4 afforded P₃. Unlike P₂ prepared via postpolymerization strategy, the sulfonated groups of P₃ are inherited from the key monomer 4. In addition, Leclerc’s group reported a sulfonated polythiophene P₄ by oxidative polymerization method (Scheme 1.4c) [21]. Briefly, the alkoxylation of 3-bromo-4-methylthiophene 5 was performed in N-methyl-2-pyrrolidone in the presence of sodium methoxide and copper bromide, leading to 3-methoxy-4-methylthiophene 6, which reacted with 2-bromoethanol and sodium sulfite in toluene to yield 3-(2-bromoethoxy)-4-methylthiophene 7. Subsequent treatment of 7 with sodium hydroxide gave sulfonated P₄.

Scheme 1.4 Synthesis of sulfonated polythiophenes P₂–P₄.
1.2 Synthesis of CPEs

Sulfite in a mixture of water/acetone led to sodium 2-(4-methyl-3-thiophenyl-1-oxy) ethanesulfonate 8, which underwent FeCl₃-catalyzed oxidative polymerization to afford P₄.

To synthesize CPEs with single bonded backbones, Suzuki polymerization was often used due to its high reaction yield and good selectivity toward various functional groups [22]. As shown in Scheme 1.5a, the first sulfonated poly(p-phenylene) P₆ was synthesized by Wegner’s group via the Suzuki polymerization method [23]. The key monomer 10 was prepared via the chlorosulfonation of 1,4-dibromobenzene 9 with chlorosulfonic acid in dichloromethane, followed by treatment with p-cresol in the presence of pyridine. Then, Pd-catalyzed Suzuki polymerization between 10 and 2,2′-(2-dodecyl-5-methyl-1,4-phenylene)bis(1,3,2-dioxaborinane) 11 in the presence of sodium carbonate yielded a neutral poly(p-phenylene) P₅. Subsequent solvolysis of P₅ in a mixture of sodium butanolate/1-butanol followed by the addition of water, gave the sulfonated poly(p-phenylene) P₆ in quantitative yield. Only one of the two possible positional isomeric structures of the repeated unit is shown in P₅ and P₆ for the sake of simplicity in illustration.

**Scheme 1.5** Synthesis of sulfonated CPEs P₆ and P₇ with single-bonded backbones through Suzuki polymerization method.
A direct polymerization method for synthesizing sulfonated poly(p-phenylene) P7 through Suzuki polymerization was reported by Reynolds's group via three steps (Scheme 1.5b) [24]. 2,5-Dibromohydroquinone 13 was synthesized via bromination of 1,4-dimethoxybenzene 12 using bromine in tetrachloromethane, followed by the treatment with boron tribromide in anhydrous dichloromethane. Subsequent sulfonation of 13 with 1,3-propanesultone and sodium hydroxide in absolute ethanol led to the key sulfonated monomer 14, which directly reacted with 1,4-phenylenediboronic acid 15 through Suzuki polymerization to yield P7. Unlike P5 and P6, there is no isomeric structure for P7 due to its symmetric chemical structure.

To synthesize sulfonated CPEs with double-bonded backbones, various polymerization methods have been employed. Herein, we choose the widely studied poly(p-phenylenevinylene)s as the examples. The first sulfonated poly(p-phenylenevinylene) P10 was synthesized by Wudl's group in 1990 [25], starting from the key monomer 16 (Scheme 1.6a). 16 was self-polymerized either in methanol or in water with the help of sodium methoxide or sodium hydroxide, respectively, yielding a reactive p-xylylene intermediate P8. Further hydrolysis followed by base-assisted elimination led to the sulfonated poly(phenylene vinylene) P10. The purity of 16 is very crucial to ensure that P10 can be obtained with narrow polydispersity and high molecular weight.

Another approach to synthesize P10 was reported by Gu et al. [26]. As shown in Scheme 1.6b, potassium 3-(4-methoxyphenoxy)propanesulfonate 18 was prepared through the esterification of 4-methoxyphenol 17 with 1,3-propanesultone in anhydrous ethanol under basic conditions. Further chloromethylation of 18 with paraformaldehyde in acidic aqueous at 40 °C led to the key monomer 19. Gilch dehydrohalogenation polymerization of 19 was performed using t-BuOK as catalyst to give P10.

The same group also employed Witting polymerization to synthesize poly(p-phenylenevinylene) derivatives (Scheme 1.6c) [27]. A mixture of 19 and triphenylphosphine in anhydrous toluene was kept at reflux conditions leading to the monomer 20, which was copolymerized with terephthalaldehyde in tert-butyl alcohol through Witting condensation with potassium tert-butoxide as catalyst to yield alternating sulfonated poly(p-phenylenevinylene) P11.

Heck polymerization is also an important approach to synthesize sulfonated poly(p-phenylenevinylene) derivatives. As shown in Scheme 1.6d, the sulfonated diiodo-substituted monomer 22, which was synthesized with a similar approach as for 18, copolymerized with 1,4-dimethoxy-2,5-divinylbenzene 21 using palladium acetate and tri(o-tolyl)phosphine as cocatalysts in dimethyl sulfoxide under basic conditions affording the alternating sulfonated poly(p-phenylenevinylene) P12 [28].

In addition to the abovementioned approaches for synthesizing poly(p-phenylenevinylene)s, Knoevenagel polymerization has also been used to prepare cyano-substituted poly(p-phenylenevinylene) derivatives [29]. As shown in Scheme 1.6e, 4-methoxyphenol 17 subsequently underwent alkylation, chloromethylation, and cyanide exchange reactions to yield 23. Subsequently, the hydroxyl groups of 23 underwent sequential methanesulfonylation, iodination, and sulfonation, affording the key monomer 24. Knoevenagel condensation reaction between 24 and the neutral dialdehyde monomer 25 using t-BuONa as catalyst in a mixture of t-BuOH/DMF led to the sulfonated P13.
To synthesize CPEs with triple-bonded backbones, Sonogashira coupling reaction is commonly employed. This reaction involves a palladium-catalyzed sp²–sp coupling reaction between aryl or alkenyl halides or triflates and terminal alkynes, with or without a copper(I) cocatalyst [30]. It can be performed under mild conditions, such as room temperature, in aqueous media. Several
monomers could also be synthesized with sulfonate functional groups. A direct polymerization approach to synthesize sulfonated polyfluorene P14 was reported by Liu’s group (Scheme 1.7a). It only requires one step to synthesize the key monomer 2,7-dibromo-9,9-bis(4-sulfonatobutyl)fluorene 27 via direct alkylation of 2,7-dibromofluorene 26 with 1,4-butane sultone and NaOH in DMSO [31]. The copolymerization of 27 and 4,7-diethynyl-2,1,3-benzothiadiazole 28 under Sonogashira coupling reaction conditions led to the sulfonated poly(fluorene vinylene) P14 [32]. In addition, as shown in Scheme 1.7b, the key sulfonated monomer 30 can be prepared using procedures similar to 18 and 22. Sonogashira reaction between 1,4-diethynylbenzene 29 and 30 in basic aqueous/DMF solution using tetrakis(triphenylphosphine)palladium and copper iodide as co-catalysts led to P15. In 2006, by utilizing the above mentioned strategy, Schanze’s group developed a series of sulfonated poly(phenylene ethynylene)s with variable band gaps based on the monomer 30 [33].

Scheme 1.7 Synthesis of sulfonated CPEs P14 and P15 with triple-bonded backbones through Sonogashira reaction.

1.2.1.2 Carboxylated CPEs
Carboxylated polythiophenes have been prepared through Yamamoto coupling polymerization, Stille coupling polymerization, and FeCl₃-catalyzed oxidative polymerization. As shown in Scheme 1.8a, the neutral poly(methyl thiophene-3-carboxylate) P16 was synthesized via the Yamamoto polymerization of methyl 2-(2,5-dichlorothiophen-3-yl)acetate 31 [15]. Subsequently, the hydrolysis of P16 in 2.0 M NaOH aqueous led to poly(sodium thiophene-3-carboxylate) P17. During the purification process, filtration was needed to remove the insoluble fraction. In addition, a CuO-mediated Stille coupling polymerization of 32 was carried out to give poly(4,5-dihydro-4,4-dimethyl-2-(2-(thiophen-3-yl)ethyl)
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oxazole) \( \text{P18} \) (Scheme 1.8b) [34], which was converted to \( \text{P19} \) after acid-assisted hydrolysis and base treatment. Compared to \( \text{P17} \) and \( \text{P19} \), which only have one carboxylate group on each repeat unit, \( \text{P20} \) reported by Wang’s group possesses two carboxylate groups on each repeat unit (Scheme 1.8c) [35]. The monomer 34 was synthesized by reacting 2-(3-thienyl)ethylamine hydrochloride 33 with methyl acrylate under basic conditions in the presence of boric acid. Oxidative polymerization of 34 in chloroform using \( \text{FeCl}_3 \) as oxidizing agent followed by hydrolysis in \( \text{NaOH} \) aqueous solution yielded \( \text{P20} \).

Suzuki polymerization is generally used to synthesize carboxylated CPEs with single-bonded backbones. The first carboxylated poly(\( p \)-phenylene) \( \text{P21} \) was reported by Wallow and Novak in 1991 [36]. As shown in Scheme 1.9a, \( \text{Pd}(0) \)-catalyzed Suzuki coupling reaction between 4,4′-dibromo-[1,1′-biphenyl]-2,2′-dicarboxylic acid 35 and 4,4′-di(1,3,2-dioxaborolan-2-yl)-1,1′-biphenyl followed by treatment with dilute hydrochloric acid yield \( \text{P21} \) with free acid. \( \text{P21} \) was completely insoluble in all common organic solvents, but was soluble in dilute aqueous base as its sodium, potassium, or triethylammonium salt.

A postfunctionalization approach to synthesize carboxylated poly(\( p \)-phenylene) was later reported by Rehahn’s group starting from the precursor polymer \( \text{P22} \) (Scheme 1.9b) [37]. Owing to the high reactivity of bromide methylene group on \( \text{P22} \), etherification of \( \text{P22} \) with ethyl \( p \)-hydroxyl benzoate in a mixture of toluene/DMF in the presence of \( t \)-BuONa produced \( \text{P23} \) in nearly quantitative yield. During the reaction process, the ester groups were kept intact. Almost all the ester groups of \( \text{P23} \) was cleaved in a homogeneous solution of toluene in the presence of 10 equiv. of \( t \)-BuOK and only 2 equiv. of water, followed

\[ \text{Scheme 1.8 Synthesis of carboxylated polythiophenes P17, P19, and P20.} \]
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By acidification with hydrochloric acid to yield $P_{24}$. However, unexpectedly, no hydrolysis of $P_{23}$ was observed in a two-phase system of water/toluene in the presence of NaOH.

Another postpolymerization approach to synthesize carboxylated polyfluorene was reported by Liu’s group (Scheme 1.9c) [38]. Direct alkylation of $26$ via Michael addition of the 9-position carbon with tert-butylacrylate afforded fluorene ester $36$, which was converted to the corresponding diboronate ester $37$ under Suzuki–Miyaura reaction conditions in the presence of bis(pinacolato)diborane, Pd(dppf)$_2$Cl$_2$, and KOAc with anhydrous DMF as the solvent. In the next step, Suzuki polymerization between $36$ and $37$ led to the neutral precursor polymer $P_{25}$. Contrary to the preparation of $P_{24}$ where the hydrolysis was performed under basic condition, $P_{26}$ was obtained by the hydrolysis of $P_{25}$ in acid condition followed by the neutralization with aqueous Na$_2$CO$_3$.

Carboxylated CPEs with double-bonded backbones have been synthesized through Heck and Gilch polymerization methods. As shown in Scheme 1.10a, Heck coupling polymerization between $38$ and the diiodo-substituted monomer $39$ in DMF using Pd(OAc)$_2$ and P(o-Tolyl)$_3$ as cocatalysts yielded the precursor polymer $P_{27}$, which was hydrolyzed into carboxylated $P_{28}$ in THF with $t$-BuOK.
as the base [39]. P28 is only soluble in DMSO and dilute aqueous bases such as NaOH and NH4OH but is insoluble in CHCl3. Similarly, Gilch dehydrochlorination polymerization was also used to synthesize carboxylated poly(phenylene vinylene) P29 (Scheme 1.10b) [40]. The key monomer 40 was prepared by etherification of 4-methoxyphenol 17 with 1-bromohexanoic acid ethyl ester and MeONa, followed by chloromethylation with formaldehyde and hydrochloric acid. In the next step, Gilch dehydrochlorination polymerization of 40 in the presence of t-BuOK yielded the carboxylated P29.

Sonogashira coupling reaction is the most commonly used method to synthesize carboxylated CPEs with triple-bonded backbones. As shown in Scheme 1.11a, P30 was directly obtained by polymerization of 3,5-diiodo benzoic acid 41 with acetylene gas in water in the presence of a water-soluble Pd(0) catalyst, CuI co-catalyst, 1 equiv. of NaOH and 3 equiv. of Et3N [41].

Bunz’s group synthesized P32 by a postpolymerization method (Scheme 1.11b) [42]. Starting from 2,5-diiodo-hydroquinone 42, reaction with 2-bromoethyl acetate in butane in the presence of K3CO3 yielded the ester-protected diiodo-monomer 43. Subsequent alkylation utilizing trimethylsilylacetylene and the catalysts of Pd(PPh3)2Cl2/CuI with trimethylamine as the solvent
furnished 44 after desilylation with tetrabutylammonium fluoride in THF. It is important to note that the ester groups were not cleaved under such basic conditions. Copolymerization between 43 and 44 under Sonogashira reaction conditions yielded the neutral P31, which was converted to P32 through hydrolysis with NaOH in methanol [42].

To increase the number of carboxylate groups on each repeat unit, Wang’s group reported P33 from the key monomer 46 (Scheme 1.11c). Starting from the alkylation of 2,7-dibromofluorene 26 with ethyl bromoacetate under basic conditions followed by treatment with HCl solution, the obtained intermediate further reacted with L-aspartic acid dimethyl ester hydrochloride via N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (EDC) hydrochloride-catalyzed coupling reaction at room temperature to yield 45 [43]. The key monomer 46 can be easily obtained by reaction between 45 and trimethylsilylacetylene using Pd(PPh₃)₂Cl₂/CuI as catalyst
followed by desilylation with tetrabutylammonium fluoride in THF. Subsequently, Sonogashira coupling reaction between 46 and Pt(PMe_3)_2Cl_2 followed by hydrolysis of the ester groups using 2M KOH solution as the base yielded the desired carboxylated P33 with four carboxylate groups on each repeat unit [44].

### 1.2.1.3 Phosphonated CPEs

As compared to sulfonated and carboxylated CPEs, the studies concerning phosphonated CPEs are less reported [16, 45, 46]. As shown in Scheme 1.12a, 3-(3-bromopropoxy)thiophene 49 was synthesized from 3-methoxythiophen 48.

![Scheme 1.12](image_url)

**Scheme 1.12** Synthesis of phosphonated CPEs P35, P36, and P38.
and 3-bromopropanol in toluene in the presence of NaHSO$_4$. Treatment of 49 with triethyl phosphite yielded the phosphonic acid diethyl ester 50. Direct electropolymerization of 50 led to the precursor polymer P34, which was hydrolyzed into phosphonated P35 using bromotrimethylsilane [16].

A phosphonated polyfluorene P36 was also synthesized by Wang’s group via direct Suzuki polymerization method (Scheme 1.12b) [46]. Starting from 2,7-dibromoacetone 26, reaction with 1,3-dibromopropane in aqueous NaOH yielded 2,7-dibromo-9,9-bis(3-bromopropyl)fluorene 51, which reacted with triethylphosphite to afford 52 in a quantitative yield. Treatment of 52 with trimethylsilyl bromide and subsequently water yielded 2,7-dibromo-9,9-bis(3-diethoxylphosphorylpropyl)fluorene 53. Direct Suzuki polymerization between 53 and 1,4-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene using Pd(dppf)Cl$_2$ as catalyst in DMF and Na$_2$CO$_3$ aqueous solution gave P36 with molecular weight higher than 15 kDa.

Phosphonated CPE P38 with triple-bonded backbones was also synthesized. As shown in Scheme 1.12c, bromination of 54 in acetonitrile using carbon tetrabromide and triphenylphosphine yielded 55, which underwent iodination to give the compound 56. The key monomer 57 was synthesized from 56 using a similar procedure to 50 and 52. P38 was then prepared via Sonogashira reaction between 57 and 1,4-diethynylbenzene to yield P37, which was followed by trimethylsilyl bromide treatment to promote the hydrolysis of the butylphosphonate ester groups (Scheme 1.12c) [45]. However, Sonogashira reaction between the hydrolysis product of 57 and 1,4-diethynylbenzene could not afford high molecular weight for P38, presumably because the ionic phosphonate groups can coordinate with and deactivate the catalysts during the polymerization.

### 1.2.2 Cationic CPEs

#### 1.2.2.1 Ammonium CPEs

Ammonium polythiophene P39 was reported by Leclerc’s group through direct oxidation of cationic thiophene monomer 59 (Scheme 1.13a) [47]. The key monomer 59 was prepared from the Williamson reaction between 3-bromo-4-methylthiophene 5 and 3-(diethylamino)propanol to yield 58, which was followed by quaternization with bromoethane. Oxidative polymerization of 59 in chloroform using FeCl$_3$ as the oxidizing agent yielded P39. Alternatively, a postpolymerization method to synthesize ammonium polythiophene was also reported by McCullough’s group [48]. As shown in Scheme 1.13b, a neutral polythiophene P40 was synthesized from 2-bromo-3-hexylbromothiophene 60 via Ni(dppp)Cl$_2$ catalyzed coupling reaction. Quaternization of P40 with methylamine in a mixture of THF/methanol yielded the cationic P41. It should be pointed out that the chemical structure of P40 should be written as P42 with a bromide on the conjugated backbone terminal, according to the reaction. Using P42 as a macromonomer and 2-bromo-(9,9-dioctylthiophene)-7-pinacolato boronate 61 as AB-type monomer under Suzuki cross-coupling conditions, Scherf’s group has successfully synthesized all-conjugated cationic diblock copolythiophenes P44 through a “grafting from” approach (Scheme 1.13c) [49].
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To synthesize single-bonded ammonium CPEs, a direct polymerization strategy was used to synthesize P45 using Suzuki polymerization (Scheme 1.14a) [50]. Compound 62 was synthesized through Williamson etherification of 13 by refluxing in acetone with 2-chloroethyl diethylamine and K₂CO₃. The key monomer 63 was obtained by quaternization of 62 with bromoethane. Subsequent Suzuki polymerization between 63 and 1,4-bis(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzene 64 afforded the ammonium P45.

The same group also employed Stille coupling reaction to synthesize thiophene-containing poly(p-phenylene) P47 [51]. As shown in Scheme 1.14b, the key monomer 65 was synthesized using a similar procedure to 62. It is noted that the diiodo-substituted monomer was chosen over a dibromo-substituted monomer due to its higher reactivity in Pd-catalyzed coupling reaction. Stille coupling reaction between 65 and 2,5-bis(trimethylstannyl)thiophene 66 led to the neutral polymer P46, which was treated with bromoethane to afford ammonium poly(p-phenylene) P47.

In 2002, Bazan's group reported an ammonium polyfluorene via postpolymerization method. P49 was obtained by the sequence of reactions shown in Scheme 1.14c [52]. Deprotonation of 2,7-dibromofluorene 26 with NaOH in a
Scheme 1.14 Synthesis of ammonium CPEs P45, P47, P49, P51, P52, and P56 with single-bonded backbones.
two-phase mixture of water and 1,6-dibromohexane provided 2,7-dibromo-9, 9-bis(6′-bromohexyl)fluorene 67, which was treated with dimethylamine to give 2,7-dibromo-9,9-bis(6′-(N,N-dimethylamino)hexyl)fluorene 68. Direct Suzuki polymerization between 68 and 1,4-phenyldiboronic acid 15 at a stoichiometric ratio yielded the neutral precursor polymer P48. Quaternization of P48 with iodomethane in a mixture of THF/DMF/water led to the alternating ammonium P49 in a moderate yield.

The same group also used another postpolymerization method to synthesize homopolyfluorene with different counterions [53]. As shown in Scheme 1.14d, Suzuki–Miyaura reaction between 67 and bis(pinacolato)diboron in the presence of AcOK and Pd(dpff)2Cl2 in anhydrous dioxane afforded the fluorene boronate ester 69. The Suzuki polymerization between 67 and 69 using Pd(PPh3)4 as catalyst and Na2CO3 as base in a two-phase mixture of toluene/water yielded the neutral precursor polymer P50. Owing to the highly efficient reaction between alkyl bromide and the trimethylamine, subsequent reaction between P50 and trimethylamine resulted in P51 with more than 95% degree of quaternization. Upon treatment of P51 with an excess of different salts, P52 was obtained with different counter ions.

In 2010, Huang’s group successfully synthesized a water-soluble grafted CPE P56 via atom transfer radical polymerization (ATRP) for the first time (Scheme 1.14e) [54]. Starting from P53, which was synthesized by Suzuki polymerization, reaction with 2-bromoisobutyryl bromide afforded the macroinitiator P54, which was used to prepare P55 via the ATRP approach. Subsequent reaction with methyl iodide gave P56. The brush-like side-chain architecture of polyfluorenes P56 endows it with an extremely high ammonium ion density, consequently giving rise to high water solubility (28 mg ml⁻¹) and high quantum efficiency (52%).

To synthesize ammonium CPEs with double-bonded backbones, Heck coupling reaction was used. As shown in Scheme 1.15a, 9,9-bis(6′-bromohexyl)-2,7-divinylfluorene 70 was obtained by heating the mixture of 67 and tributylvinyltin in toluene using Pd(PPh3)2Cl2 as catalyst and 2,6-di-tert-butylphenol as the inhibitor. Subsequent quaternization of 70 with trimethylamine in a mixture of THF/water afforded the monomer 9,9-bis(6′-(N,N,N-trimethylammonium)hexyl)-2,7-divinylfluorene dibromide 71. Finally, the ammonium P57 was obtained via Pd(OAc)2/P(o-tolyl)3-catalyzed direct Heck coupling polymerization of 71 and 4,7-dibromo-2,1,3-benzothiazole 72 in a solvent mixture containing DMF/H2O/TEA (v/v/v = 2/1/2) [55].

A postpolymerization method was also reported by Wang’s group using Heck coupling reaction. As shown in Scheme 1.15b, alkylation of 42 with 1,6-dibromohexane and K2CO3 in acetone afforded 73. Heck reaction between 73 and the divinyl monomer 74 led to the neutral polymer P58. The presence of the active bromide atom of P58 allows the facile synthesis of P59 through quaternization with trimethylamine [56].

Another approach to synthesize poly(fluorene vinylene)s is through the Witting polymerization method [57]. As shown in Scheme 1.15c, the key monomer 2,7-dicarbaldehyde-9,9-bis(6′-bromohexyl)fluorene 75, was obtained by treatment of 67 with BuLi followed by the addition of anhydrous DMF. The Witting–Horner condensation reaction between 75 and 1,4-bis(diethylphosphinatymethyl)phenylene
Scheme 1.15 Synthesis of ammonium CPEs P57, P59, P61, and P63 with double-bonded backbones.
76 in dry THF in the presence of t-BuOK led to the precursor P60, which was converted to ammonium P61 via quaternization with trimethylamine in chloroform/methanol.

Gilch coupling reaction was also employed by Shen’s group to synthesize ammonium poly(p-phenylene vinylene)s [58]. As shown in Scheme 1.15d, 3-(4-methoxyphenoxy)-N,N-dimethylpropan-1-amine 77 was synthesized through Williamson etherification of 17 by refluxing in acetone with 3-chloropropylidemethylamine and K₂CO₃. Chlorination of 77 with a 37% formalin solution in concentrated hydrochloric acid resulted in the key monomer 78. Gilch reaction of 78 was performed in THF in the presence of t-BuOK to yield the precursor polymer P62, which was treated with bromoethane in a mixture of THF/DMSO to give the ammonium poly(p-phenylene vinylene) P63.

Sonogashira reaction was often used to prepare ammonium CPEs with triple-bonded backbones. As shown in Scheme 1.16a, quaternization of 67 with trimethylamine in THF/water yielded the ammonium monomer 79, which was directly copolymerized with 4,7-diethynyl-2,1,3-benzothiadiazole 28 to yield P64 via Sonogashira reaction using Pd(PPh₃)₄/CuI as catalysts in a mixed solution of DMF/H₂O/diisopropylamine (v/v/v = 2/1/1.5) [32].

Another approach to synthesize poly(fluorene ethynylene)s is via postpolymerization. As shown in Scheme 1.16b, the reaction between 67 and trimethylsilyl acetylene using Pd(PPh₃)₃Cl₂/CuI as catalysts followed by trimethylsilyl deprotection in a basic solution led to 9,9-bis(6-bromohexyl)-2,7-diethynylfluorene 80. The ethynyl and bromide groups were kept intact during the reactions. 80 was copolymerized with 1,4-diiodobenzene 81...
through Pd(PPh₃)₄/CuI-catalyzed Sonogashira reaction in a mixture of toluene/diisopropylamine (v/v = 2/1) to yield P₆₅. Quaternization of P₆₅ with trimethylamine afforded the ammonium P₆₆ [59].

Apart from the above-discussed linear cationic CPEs, Liu’s group also reported hyperbranched ammonium polyfluorenes. For example, P₆₇ was synthesized through 2 steps from 80. As shown in Scheme 1.17, CpCo(CO)₂- catalyzed homopolycyclotrimerization of 80 with UV irradiation led to hyperbranched neutral P₆₇, which was converted to P₆₈ after treatment with trimethylamine [60].

1.2.2.2 Pyridinium CPEs

Pyridinium CPEs are generally obtained through quaternization between pyridine and active halogen atoms (e.g., Br). As shown in Scheme 1.18a, Fukuhara and Inoue reported pyridinium polythiophene P₆₉ through treatment of P₄₀

![Scheme 1.17 Synthesis of hyperbranched CPE P₆₈.](image)

![Scheme 1.18 Synthesis of pyridinium CPEs P₆₉, P₇₁, and P₇₂.](image)
1.2 Synthesis of CPEs

with pyridine in $N,N$-dimethylacetamide (DMA) [61]. Bazan’s group also reported pyridinium CPEs $P71$ and $P72$ with narrow band gap (Scheme 1.18b) [62]. Starting from 3, alkylation with 1,6-dibromohexane and KOH followed by bromination of the intermediate using $N$-bromosuccinimide yielded the dibromide-substituted cyclopentadithiophene 82. Suzuki polymerization between 82 and the bis-boronate ester of benzothiadiazole generated the neutral precursor polymer $P70$. Quaternization of $P70$ with pyridine led to the pyridinium $P71$. In addition, bromide counter ion in $P71$ has also been further exchanged with the large tetrakis(1-imidazolyl)borate (BIm$_4^-$) to yield $P72$.

1.2.2.3 Phosphonium CPEs

In addition to the ammonium and pyridinium salt, cationic CPEs with other types of charges (e.g., phosphonium) have also been reported. As shown in Scheme 1.19a, quaternization of the neutral polymer $P40$ with 1-methyl-imidazole or trimethylphosphine led to $P73$ or $P74$, respectively [63]. The obtained CPEs are insoluble in common organic solvents (e.g., THF, chloroform, dichloromethane, and toluene) but readily soluble in water. Using a similar strategy, $P75$ was treated with PPh$_3$ to yield the corresponding phosphonium $P76$ (Scheme 1.19b) [64].

![Scheme 1.19 Synthesis of cationic CPEs $P73$, $P74$, and $P76$.](image)

1.2.3 Zwitterionic CPEs

Different from anionic and cationic CPEs, zwitterionic CPEs contain side groups with anionic and cationic functionalities that are covalently linked with each other. Zwitterionic polythiophenes have been synthesized by Konradsson’s
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As shown in Scheme 1.20a, 1 was tosylated with \( p \)-TsCl in the presence of pyridine to give a thiophene derivative 83. Alkylation of 83 with a Boc-protected amino acid, \( N-t \)-Boc-Thr, yielded the key monomer 84. After Boc-deprotection with trifluoroacetic acid, FeCl₃-catalyzed oxidative polymerization of the product gave the zwitterionic polythiophene P77 [65].

Another type of zwitterionic polythiophene P79 was also synthesized. As shown in Scheme 1.20b, the precursor polymer P40 first reacted with diethylamine in a mixture of THF/DMF to give P78, which was converted to P79 using 1,4-butane sultone as the quaternization agent and solvent [66].

In 2011, Huck’s group reported a zwitterionic polyfluorene as the charge injection layer for high-performance polymer light-emitting diodes [67]. As shown in Scheme 1.20c, the Pd-mediated Suzuki polymerization between 85 and di-boronate ester fluorene yielded a neutral polymer P80. After quaternization of P80 using 1,4-butane sultone in a THF/methanol solvent mixture, P81 was obtained with near 100% conversion of the tertiary amines into sulfobetaine zwitterionic groups [67].
1.3 Neutral WSCPs

In the previous section, we have described the various approaches to synthesize CPEs with different charge signs. In this section, we will briefly discuss the synthesis of neutral WSCPs. Neutral WSCPs contain neutral polar functionalities, for example, oligo(ethylene glycol) segments, that compensate for the hydrophobic nature of the conjugated backbones. Neutral WSCPs often exhibit high fluorescence quantum yield, good water solubility, and strong resistance to nonspecific interactions. As shown in Scheme 1.21a, Bazan’s group reported the synthesis of a neutral WSCP \( P_{82} \) using the complex cis-(bromo)(phenyl) [1,2-bis(diphenylphosphino)ethane]nickel as the initiator and 86 as the monomer in the presence of isopropylmagnesium chloride–lithium chloride, followed by quenching the polymerization with [2-(trimethylsilyl)ethyl]magnesium bromide [11]. Owing to the incorporation of octakis(ethylene glycol) side chains, \( P_{82} \) exhibited good solubility in water and high-fluorescence quantum yield (80%). In addition, each \( P_{82} \) chain possesses one terminal silylacetylene group, which allowed further conjugation with functional components via click reaction. This provides a versatile strategy to fabricate ideal fluorescent probes for biosensing applications.

![Scheme 1.21 Synthesis of neutral WSCPs \( P_{82} \) and \( P_{85} \).](image_url)

Meanwhile, Huang’s group reported a postpolymerization method to synthesize WSCP \( P_{85} \) with oligo(ethylene glycol) side chains [68]. As shown in Scheme 1.21b, Suzuki polymerization of monomers 36, 37, and 88 followed by deprotection of ester groups with trifluoroacetic acid yielded \( P_{83} \). Subsequently, \( P_{83} \) was modified with 2-(pyridyldithio)ethylamine through amidation to yield \( P_{84} \), which was converted to \( P_{85} \) through the replacement of pyridine units with PEG-SH chains.
Bunz’s group also used Sonogashira reaction to prepare neutral water soluble poly(\(p\)-phenylene ethynylene) \(\text{P87}[69]\). As shown in Scheme 1.22, Pd-catalyzed Sonogashira coupling between 89 and 90 furnished the neutral polymer \(\text{P86}\), which was deprotected \textit{in situ} and subjected to a cupper catalyzed 1,3-dipolar cycloaddition with azide-functionalized sugar resulting in \(\text{P87}\). Because of the presence of polar side chains (sugar and oligo(ethylene glycol)), \(\text{P87}\) can be freely dissolved in water.

\begin{align*}
\begin{array}{c}
\text{OR}_1 \quad \text{I} \quad \text{OR}_2 \quad \text{I} \quad \text{Pd(PPh}_3)_2\text{Cl}_2, \text{CuI, THF, piperidine} \\
\text{Pd(PPh}_3)_2\text{Cl}_2, \text{CuI, THF, piperidine} \\
\text{OR}_1 \quad \text{I} \quad \text{OR}_2 \quad \text{I} \quad \text{Pd(PPh}_3)_2\text{Cl}_2, \text{CuI, THF, piperidine} \\
\text{OR}_1 \quad \text{I} \quad \text{OR}_2 \quad \text{I} \quad \text{Pd(PPh}_3)_2\text{Cl}_2, \text{CuI, THF, piperidine} \\
\end{array}
\end{align*}

\text{P87}

\text{Scheme 1.22 Synthesis of neutral WSCP P87.}

Liu’s group developed another strategy to prepare WSCPs using hyperbranched polyglycerol as brush due to its good biocompatibility \[70\]. This method involves the combination of “grafting from” strategy and living ring-opening polymerization technique. As shown in Scheme 1.23, Starting from \(\text{P88}\), reaction with \(\text{NaN}_3\) in a mixture of THF/DMF afforded \(\text{P89}\). Subsequent click reaction with propargyl alcohol led to the macronitiator \(\text{P90}\), which was used to incorporate hyperbranched polyglycerol as brush via ring-opening polymerization technique, yielding \(\text{P91}\) with good water

\begin{align*}
\begin{array}{c}
\text{P88} \quad \text{NaN}_3 \quad \text{THF, DMF} \\
\text{P88} \quad \text{NaN}_3 \quad \text{THF, DMF} \\
\text{P88} \quad \text{NaN}_3 \quad \text{THF, DMF} \\
\text{P88} \quad \text{NaN}_3 \quad \text{THF, DMF} \\
\end{array}
\end{align*}

\text{P91}

\text{Scheme 1.23 Synthesis of neutral WSCP P91 with HPG brush.}
solubility. In addition, the same group also reported many CPs with poly(ethylene glycol) brush through click reaction between poly(ethylene glycol) and neutral precursor CP [71–73].

1.4 Fabrication of CPNPs

Compared to the chemical synthesis of CPs, preparation of CPNPs directly from organic solvent-soluble CPs represents another strategy to bring CPs into aqueous media. To simplify the discussion, all the NPs prepared from organic soluble CPs are termed as CPNPs in this chapter. The most widely used approaches for CPNPs synthesis include reprecipitation, miniemulsion, and nanoprecipitation [9, 12, 74]. In the following section, we choose some specific examples to illustrate each method by discussing their advantages and disadvantages. Scheme 1.24 shows the chemical structures of representative CPs and amphiphilic materials used for CPNP preparation.

Scheme 1.24 Representative chemical structures of CPs and amphiphilic materials used for CPNP preparation.
1.4.1 Reprecipitation

Reprecipitation method was first applied to CPs by Masuhara’s group [75]. In a typical reprecipitation method schematically shown in Scheme 1.25, a small amount of hydrophobic CP is dissolved in a water-miscible solvent (e.g., THF). Next, the organic solution is rapidly injected into excess water under vigorous stirring or sonication. The large solubility discrepancy of the CP in two solvents as well as the hydrophobic interactions between polymer chains induces the nanoparticle formation during the organic solvent evaporation. This method does not involve the use of any additives such as surfactants. Using this method, Masuhara’s group prepared P92 NPs with sizes ranging from 40 to 420 nm by injecting a solution of P92 in THF into vigorously stirred distilled water [75]. The size can be tuned by changing the concentration of P92 in THF or the temperatures of water (e.g., from 20 to 80 °C). The lower concentration provided the smaller size. The obtained CPNPs exhibited size-dependent spectroscopic properties.

Subsequently, McNeill’s group reported a modified procedure to prepare CPNPs with the sizes ranging from 5 to 30 nm [76]. In this specific case, 2 ml of P93 in THF with a very low concentration (0.005 wt%) was added quickly to 8 ml of deionized water under sonication conditions. After evaporation of the THF under vacuum, filtration with a 0.2 µm membrane filter was performed to afford P93 NPs with major sizes between 5 and 10 nm. P92 and P93 NPs exhibit spherical shape. This method is applicable to a wide range of CPs that are soluble in water-miscible organic solvents. In addition, it is possible to adjust the particle size via tuning the CP concentration or selecting CPs with appropriate molecular weight. However, due to the inherent hydrophobic nature of CP, the obtained CPNPs tend to aggregate into large sized particles, thus precipitating from water. Therefore, these CPNPs were only obtained with a very low concentration (e.g., 0.005%), and could not be stored for a long period.

1.4.2 Miniemulsion

Miniemulsions are specially formulated heterophase systems consisting of stable nanodroplets in a continuous phase (e.g., water). In a typical miniemulsion procedure illustrated in Scheme 1.26, the CP dissolved in a water-immiscible organic...
1.4 Fabrication of CPNPs

Scheme 1.26 Schematic illustration of the preparation of CPNPs using miniemulsion method.

solvent (e.g., dichloromethane, chloroform) is added to an aqueous solution containing an appropriate surfactant. The mixture is treated with ultrasonication to form stable miniemulsions containing small organic droplets of the CP solution. After solvent evaporation, the droplets collapse to form stable water-dispersible CPNPs. Compared to the reprecipitation method, the miniemulsion method employs surfactant to form and stabilize droplets. The sizes of formed CPNPs via this method vary from 2 to 500 nm, depending on the nature and concentration of the polymer and surfactant used.

Using the miniemulsion method, Liu’s group reported a generic strategy to prepare a series of multicolor CPNPs with emission spanning from 400 to 700 nm, using Food and Drug Administration (FDA)-approved poly(DL-lactide-co-glycolide) (PLGA) as the encapsulation matrix, and poly(vinyl alcohol) (PVA) as the emulsifier [77]. Herein, we still choose P93 as an example. In this case, a dichloromethane solution containing P93 and PLGA was poured into an aqueous solution containing PVA. After sonicating the mixture, the resulting emulsion was stirred at room temperature to remove the solvent. Then, P93 NPs were obtained after careful washing and centrifugation to remove the emulsifier and free P93 molecules. Although the resultant CPNPs show good colloidal stability in water, their sizes are in the range of 240–270 nm and the encapsulation efficiencies are relatively low (~45%).

Meanwhile, Green’s group reported a simple strategy to synthesize a series of PEG encapsulated CPNPs with emission spanning from blue to green, yellow, and red [78]. Using P94 as an example, the dilute P94 solution in dichloromethane (40 ppm by weight) was added dropwise to an aqueous PEG solution while stirring vigorously with ultrasonication. After sequential evaporation of the remaining dichloromethane, filtration with filter paper, and centrifugation, the P94 NPs were obtained with mean diameters ranging from 2 to 3 nm and a quantum yield of ~12%. The presence of PEG is essential to stabilize the formed NPs, as the P94 NPs prepared without PEG are not stable. However, a considerable amount of fluorescence quenching was observed in the small CPNPs.

Compared to the straightforward strategy to synthesize CPNPs from preprepared CPs, another strategy to synthesize CPNPs is miniemulsion polymerization. This method generally employs water-tolerance reactions, such as Sonogashira, Suzuki, and FeCl₃-catalyzed oxidative polymerization reactions.
Generally, the appropriate monomers are dispersed in surfactant-containing aqueous solution under vigorous stirring to form stable nanodroplets. During this process, the hydrophobic monomers are embedded in the interior. After addition of appropriate catalyst, polymerization and nanoparticle formation are achieved concurrently. This strategy will be discussed in detail in the next chapter.

1.4.3 Nanoprecipitation

Nanoprecipitation is a modification of the reprecipitation method, and has become the most commonly used approach for the preparation of CPNPs. In a typical procedure as shown in Scheme 1.27, CP and amphiphilic encapsulation matrix are first dissolved well in a water-miscible organic solvent (e.g., THF). The resulting solution is then quickly added into a water phase under sonication to form a stable nanoparticle dispersion. After organic solvent removal, the matrix encapsulated CPNPs were obtained. During nanoparticle formation, the hydrophobic components of the matrix and CPs are likely embedded into the NP core while the hydrophilic segments of the matrix are oriented to the aqueous environment. The NPs prepared by this method show better colloidal stability than those obtained from reprecipitation. The particle size and optical and surface chemical properties of the CP NPs can be adjusted by changing the CPs, amphiphilic matrix, and other related parameters.

![Scheme 1.27 Schematic illustration of the preparation of CPNPs using nanoprecipitation method.](image)

In 2010, Chiu's group synthesized CPNPs based on **P94** via the nanoprecipitation method using a functional, amphiphilic, comb-like poly(styrene-g-ethylene oxide) (PS–PEG-COOH) as an encapsulation matrix [79]. PS–PEG-COOH consists of a hydrophobic polystyrene backbone and several hydrophilic PEG side chains terminated with carboxylic acid. Briefly, a precursor THF solution with a constant **P94** concentration and PS–PEG-COOH/**P94** fractions ranging from 0 to 20wt% was quickly added to water in a bath sonicator to afford carboxylic-functionalized CPNPs of **P94**. During NP formation, the hydrophobic polystyrene segment and **P94** are most likely embedded inside the NPs, while the hydrophilic PEG chains and carboxylic acid groups extend outside into the aqueous environment. Moreover, the PEG chains not only function as a biocompatible layer to minimize nonspecific absorption, but also provide a steric barrier against nanoparticle aggregation and
carboxyl groups for further conjugation. The obtained CPNPs were determined by dynamic light scattering (DLS) to have an average diameter of 15 nm and a quantum yield of 30% in water. Replacement of PS–PEG-COOH with unfunctionalized PS–PEG did not cause any noticeable effects on particle size and morphology. In addition, increase of the concentration of P94 in THF solution produced larger sized NPs. The absorption and emission of NPs based on P94 show size-independent features.

Later on, the same group reported another strategy to prepare P94 NPs using poly(styrene-co-maleic anhydride) (PSMA) as an encapsulation matrix [80]. The preparation procedure is similar to that of PS–PEG-COOH encapsulated P94 NPs. During NP formation, the hydrophobic polystyrene units of PSMA and P94 were most likely localized on the NP surface and hydrolyzed in the aqueous environment to produce carboxylate groups on the NP surface for further conjugations. The obtained P94 NPs show comparable size and fluorescence quantum yield to PS–PEG-COOH encapsulated P94 NPs.

The nanoprecipitation method discussed above has two drawbacks: (i) The matrices are likely to dissociate from the formed NPs and (ii) it is difficult to achieve CPNPs with sizes smaller than 10 nm. To solve these problems, Chiu’s group further developed a cross-linking strategy to synthesize stable CPNPs based on P95. In this case, poly(isobutylene-alt-maleic anhydride) (PIMA) was chosen as matrix and cross-linker [81]. PIMA, which has multiple reactive units, first reacts with the amine-functionalized P95 to form covalent cross-links. Then, the resultant product dissolved in THF was injected quickly into Milli-Q water under sonication to afford P95 NPs with carboxylic groups on the particle surface. As compared to the P94 NPs, P95 NPs showed lower fluorescence quantum yield (21% vs 30%) but better colloidal stability.

PEG-functionalized lipid is another matrix commonly used for CP encapsulation. The first lipid-PEG encapsulated P94 NPs were reported by Christensen’s group in 2011 [82]. They synthesized a series of lipid-PEG (PEG \( M_r = 2000, 1000, 500 \)) with carboxy, biotin, or methoxy end groups. To synthesize P94 NPs, a dilute solution of P94 in THF was rapidly added to a ninefold volume of water containing lipid-PEG under continuous mild sonication. During this process, the aliphatic side chains on the P94 interact with the hydrophobic lipid-PEG tail and are inserted into the NP core, while the hydrophilic PEG groups protrude out into the aqueous media. The DLS diameters of the formed NPs are 20–30 nm, and are insensitive to the variation in the PEG length and end groups. However, the obtained P94 NPs have low concentration and moderate fluorescence quantum yield (~18%). Subsequently, Liu’s group modified this method to synthesize CPNPs based on a wide variety of preprepared CPs for bioapplications using the matrix of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE)-PEG\(_{2000}\) with different terminal functional groups, such as carboxylic acid, amine, azide, and maleimide [83].

Apart from PS- and lipid-derivatives, some proteins or peptides were also used as matrix to synthesize CPNPs via nanoprecipitation [84, 85]. For example, Liu’s group used bovine serum albumin (BSA) as a polymer matrix to prepare P96 NPs due to its good biocompatibility and nontoxicity [84]. The NP synthesis
started with the addition of a THF solution containing \textbf{P96} into a BSA aqueous solution under sonication, which was followed by cross-linking with glutaraldehyde and THF evaporation. The formed \textbf{P96} NPs exhibit DLS in the size of ~160 nm and carboxylic groups on the surface.

Encapsulation of CPs with silica shell is also an effective approach to synthesize CPNPs. Liu’s group first reported on the synthesis of SiO$_2$@CP@SiO$_2$ NPs at room temperature without specific CP design or silane modification [86]. Using \textbf{P94} as an example, the THF solution of \textbf{P94} was first poured into a mixture of ethanol/water (v/v = 9/1) under sonication. As THF evaporates, \textbf{P94} formed small dots. Subsequently, a silica precursor, tetraethyl orthosilicate (TEOS) as well as ammonia was injected into the resulting mixture with vigorous stirring. After 12 h, an amine-functionalized silica precursor, 3-aminopropyl triethoxysilane (APTES), was added to modify the obtained SiO$_2$@\textbf{P94}@SiO$_2$ NPs with amine groups on surface. It was noted that the resulting NPs were ~70 nm in diameter with a quantum yield of 27% in water. The authors used four different CPs to prepare silica encapsulated NPs in their study, demonstrating that this method can be applied to any CPs that are soluble in organic solvents.

1.5 Conclusion

This chapter describes the commonly used strategies to bring CPs into aqueous media for biological applications. The water-soluble or water-dispersible CPs can be realized via either chemical modification of the conjugated backbones and side chains for CPEs and neutral WSCPs or by preparation of CP-based CPNPs. According to their chemical structures, the synthesis of CPEs and neutral WSCPs involves two aspects: construction of conjugated backbones and incorporation of charged or polar side chains. The conjugated backbones have been built through well-established polymerization methods, which are typically catalyzed with organometallic complexes or bases, while the functional side chains can be obtained through either direct polymerization or postpolymerization methods. For the preparation of CPNPs, three strategies are generally employed to encapsulate organic-soluble CPs and bring them in aqueous media, namely, reprecipitation, miniemulsion, and nanoprecipitation. Despite the great success in bringing organic-soluble CPs into water in the past decades, there is much to be done to understand how molecular packing and polymer aggregation affect their optical properties and how CP structures and performance can be optimized in order to meet the requirements for existing as well as new applications.

References
