

**SYNTHESIS AND CHARACTERIZATION OF CROSS-LINKED WATER-
DISPERSIBLE CONJUGATED POLYMER NANOPARTICLES**

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MASTER OF SCIENCE

By

ŞEYMA EKİZ

APRIL 2012

I certify that I have read this thesis and in my opinion it is fully adequate, in scope and in quality, as a thesis of the degree of Master of Science.

Assoc. Prof. Dr. Dönüş TUNCEL

I certify that I have read this thesis and in my opinion it is fully adequate, in scope and in quality, as a thesis of the degree of Master of Science.

Prof. Dr. Engin Umut AKKAYA

I certify that I have read this thesis and in my opinion it is fully adequate, in scope and in quality, as a thesis of the degree of Master of Science.

Assist. Prof. Dr. Salih ÖZÇUBUKÇU

Approved for the Graduate School of Engineering and Science

Prof. Dr. Levent ONURAL

Director of Graduate School of Engineering and Science

ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF CROSS-LINKED WATER- DISPERSIBLE CONJUGATED POLYMER NANOPARTICLES

ŞEYMA EKİZ

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Supervisor: Assoc. Prof. Dr. Dönüş TUNCEL

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In this study, a novel synthetic method was demonstrated for the water-dispersible crosslinked light-emitting conjugated polymer nanoparticles with enhanced stability.

In order to synthesize the novel conjugated polymer nanoparticles, thiophene-based monomers were synthesized with different functional groups such as bromine, hydroxyl and azide groups. These monomers were characterized by ^1H -NMR spectroscopy.

After the synthesis of the monomers, various polymers were synthesized via Suzuki coupling and oxidative polymerization. Their structural and optical properties were fully characterized by spectroscopic techniques such as ^1H -NMR spectroscopy, FT-IR spectroscopy and Gel Permeation Chromatography (GPC).

Finally, crosslinked conjugated polymer nanoparticles were synthesized by a diaminoalkyne crosslinker and various useful functional groups were introduced to the nanoparticles such as triazoles and amine groups. Incorporation of the hydrophilic functional groups to the conjugated polymer nanoparticles resulted with patchy, janus-like nanoparticles. CB6 was used as a catalyst for the first time in nanoparticle synthesis for 1,3-azide alkyne Huisgen cycloaddition which formed a conjugated polymer-based nanosized rotaxanes. Crosslinking

of the conjugated polymer nanoparticles was also achieved by the irradiation of the nanoparticles under UV light in order to get shape-persistent nanoparticles.

Various functional groups of the conjugated polymer nanoparticles make them highly versatile for biological studies such as cell imaging and drug delivery in biological systems. Synthesized nanoparticles were fully characterized by dynamic light scattering (DLS) measurement, transmission electron microscopy (TEM), FT-IR spectroscopy and UV-Vis spectroscopy.

Keywords: Conjugated polymers, water dispersible nanoparticles, crosslinking, click chemistry.

ÖZET

SUDA DAĞILABİLİR ÇAPRAZ BAĞLI IŞIYAN POLİMER NANOPARÇACIKLARIN SENTEZİ VE KARAKTERİZASYONU

ŞEYMA EKİZ

Kimya Bölümü Yüksek Lisans Tezi

Tez Yöneticisi: Doç. Dr. Dönüş TUNCEL

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Bu çalışmada, suda dağılabilen çapraz bağlı ışık saçan konjüge polimerlerin kararlılıkları geliştirilerek orijinal sentez metodu gösterilmiştir.

Işıyan konjüge polimerleri sentezlemek için bromin, hidroksil ve azit gibi farklı fonksiyonel gruplarla türevlendirilmiş monomerler sentezlenmiştir. Sentezlenen monomerler ¹H-NMR spektroskopisi ile karakterize edilmiştir.

Monomer sentezinden sonar, Suzuki kenetlenme reaksiyonu ve oksidatif polimerizasyon ile çeşitli polimerler sentezlenmiştir. Sentezlenen polimerlerin yapısal ve optik özellikleri ¹H-NMR spektroskopisi, FT-IR spektroskopisi ve jel geçirgenlik kromatografisi ile karakterize edilmiştir.

Son olarak, çapraz bağlı, ışık saçan konjüge polimer nanoparçacıklar diaminoalkin çapraz bağlayıcı kullanılarak sentezlenmiş ve triazol, amin grupları gibi fonksiyonel gruplar nanoparçacıklara katılmıştır. Hidrofilik fonksiyonel grupların sentezlenen konjüge polimer nanoparçacıklara katılması, yamalı, iki yüzölçümlü nanoparçacık oluşumuyla sonuçlanmıştır. Bu çalışmada, CB6 ilk defa nanoparçacık sentezinde 1,3-azitalkin Huisgen siklo katılma reaksiyonlarında katalizör olarak kullanılmış ve konjüge polimer-bazlı nano boyutta rotaksanlar elde edilmiştir. Şekilleri kalıcı konjüge polimer nanoparçacıkların çapraz bağlanması aynı zamanda UV ışığı altında da gerçekleştirilmiştir.

Işık saçan konjüge polimer nanoparçacıklardaki çeşitli fonksiyonel gruplar, nanoparçacıkları hücre görüntüleme ve biyolojik sistemlere ilaç taşıma gibi biyolojik çalışmalarda oldukça kullanışlı yapmıştır. Sentezlenen nanoparçacıklar, dinamik ışık saçılımı (DLS) ölçümleri, aktarmalı elektron mikroskobu (TEM), FT-IR spektroskopi ve UV-Vis spektroskopi ile karakterize edilmiştir.

Anahtar Kelimeler: Konjüge polimerler, suda dağılılabılır nanoparçacıklar, çapraz bağlanma, klik kimyası.

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I am dedicating my thesis to myself who will do the best for humanity.

ABBREVIATIONS

$^1\text{H-NMR}$	Proton-Nuclear Magnetic Resonance
FT-IR	Fourier Transform-Infrared
GPC	Gel Permeation Chromatography
UV-Vis	Ultraviolet-visible
PL	Photoluminescent
DLS	Dynamic Light Scattering
TEM	Transmission Electron Microscopy
CDCl_3	Deuterated chloroform
D_2O	Deuterim oxide
TBAB	Tetra-n-butylammonuim bromide
CB6	Cucurbit[6]uril
CPN	Conjugated polymer nanoparticle

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CHAPTER 1

INTRODUCTION

1.1 Conjugated Polymers

For many years, it has been well-known that conjugated polymers have various important properties such as optical, magnetic and electrical. So the scientists widely work on the development of conjugated polymers.

Conjugated polymers can be conductive, or semiconductive depending on their structure. They are formed from the covalently bonded methyne ($=CH-$) groups which form a linear carbon chain. The important point to be a conductive polymer is to have a conjugated system on the backbone of the polymer (Figure 1.1). In this system, every bond has a localised sigma (σ) bond which provides a strong chemical bond. Also each double bond has a less strongly localized pi (π) bond that provides a less strong chemical bond compared with the sigma bond.¹

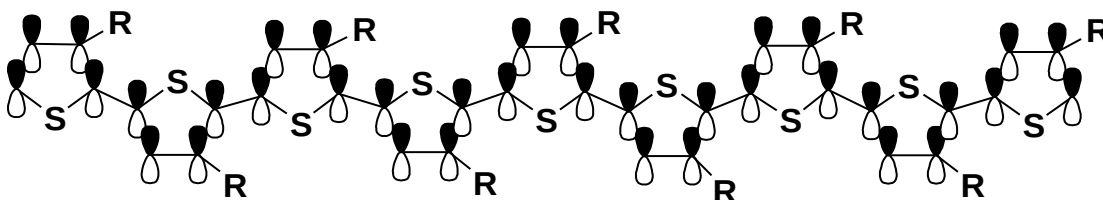


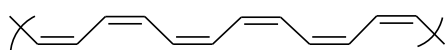
Figure 1.1: Conjugated π System of Polythiophene.²

In order to be conductive, conjugation is not enough for a polymer. In addition to the conjugation, extra electrons or extra holes should be introduced into the polymer. This is the work of a dopant. If there is a missing electron in the polymer system, the position of the missing electron is called as the hole. These holes are filled by electrons that are migrating in the pi system of the polymer which creates a new hole. By the help of this electron migrating, the charge can easily travel a long conjugated system.³

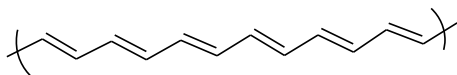
Today, many chemists are working on the conjugated polymers in order to further develop them. They are widely used in transistors, light-emitting diodes, television screens and solar cells.

Polyacetylene is the oldest impressive conjugated polymer. It was first discovered by Natta and coworkers in 1958.⁴ Natta and co-workers synthesized polyacetylene by the polymerization of acetylene in hexane. They used $\text{Et}_3\text{Al}/\text{Ti}(\text{OPr})_4$ (Et= ethyl, Pr=propyl) as a catalyst. The resulting material was strongly crystalline with a regular structure. It was a black, air-sensitive, infusible and insoluble powder. Ziegler-Natta polymerization was discovered for the alkene polymerization like ethylene. In this polymerization, an unsaturated molecule is inserted into the carbon-titanium bond of the growing macromolecule.

Polyacetylene had a long conjugated backbone but it didn't attract much interest in the chemistry world. The important point was the discovery of polyacetylene films. They were prepared by Shirakawa and co-workers from acetylene in 1974.⁵ They used Ziegler-natta catalyst for the synthesis of polyacetylene. However, the resulting polyacetylene wasn't a conductor. Figure 1.2 illustrates the all-cis and all-trans polyacetylene synthesized by Shirakawa and co-workers.



all-*cis*-polyacetylene
(copper colored)



all-*trans*-polyacetylene
(silver colored)

Figure 1.2: All-cis and all-trans Polyacetylene.⁵

The conductive polyacetylene films were discovered by Shirakawa, MacDiarmid and Heeger in 1977. They showed that oxidation of the polyacetylene films by chlorine, bromine or iodine vapor increases the conductivity of the polyacetylene films by 10^9 times. This treatment of the conjugated polymers via halogens was called as 'doping'. The state of the art of the conductivity of polymers was doping the polyacetylene which provides the highest conductivity as 10^5 siemens/meter.³ Figure 1.3 illustrates the conductivity of conductive polymers compared to other materials.

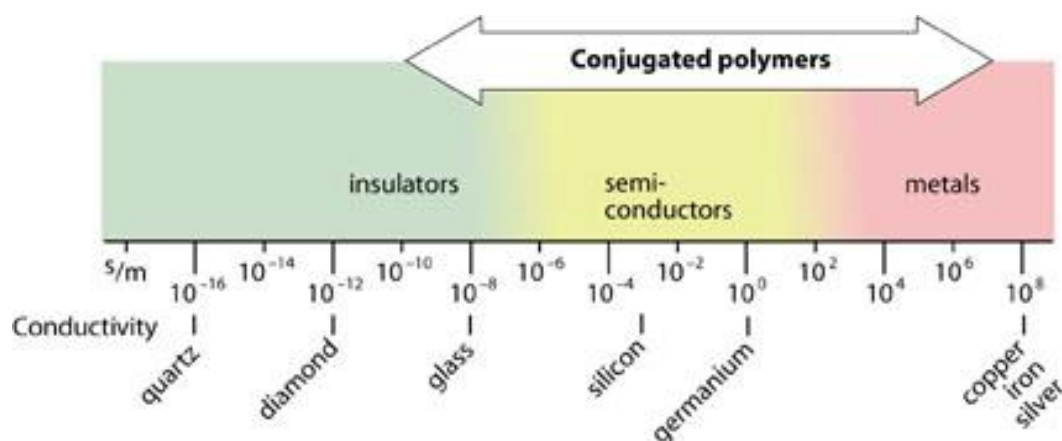


Figure 1.3: Conductivity of Conductive Polymers Compared to Other Materials.³

After the discovery of highly conductive polyacetylene films, scientists started to design several conjugated polymers which have conductive properties from the beginning of 1980s such as polypyrrole, polythiophene, polyphenylenevinylene and polyaniline. Then, the second generation electroluminescent semiconductive polymers were synthesized. They were useful in field-effect transistor (FET), photodiodes and light-emitting diodes (LED).³

Today, polymer LEDs show impressive characteristics. Efficient light from LEDs is a well-known example of this. Conjugated polymers are widely studied because of their rapid, low-cost processing by using polymer solutions that form films.

After the discovery of the conductivity of conjugated polymers, they became a hot topic in chemistry world. Their properties are coming from their conduction molecular orbitals and valence molecular orbitals, i.e. bonding π orbitals and antibonding π^* orbitals (Figure 1.4). Molecular orbital diagrams indicate the energy levels of the orbitals in a molecule. Energy difference between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) gives the energy gap of the molecule. This energy gap determines the conductivity of a material. Molecules with large energy gaps are insulators. When the energy gap decreases, conductivity of the material increases.⁶

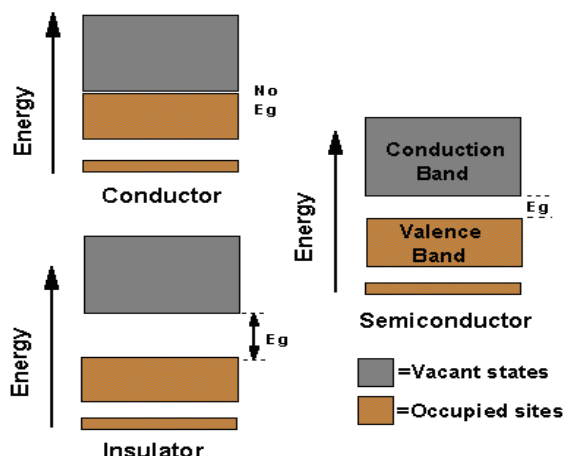


Figure 1.4: Idealized representation of energy bands and gaps. ⁷

Conjugated polymers have electroluminescent properties. Electroluminescence is an optical and an electrical term which means the emission of light with the treatment of electric current. The first report about the electroluminescent properties of the conjugated polymers was published in 1990 by J.H.Burroughes and co-workers. ⁸ They used poly(p-phenylene vinylene) PPV as a semiconducting polymer. Electroluminescent polymers are widely used in light-emitting diodes. These diodes' first layer contains a semiconductor polymer. One side of the polymer is covered by a hole injection electrode, ITO (indium tin oxide), and the other side is covered by an electron-injecting metal contact (i.e. aluminium). By the application of a proper bias, electrons and holes are injected into the system. Subsequently they meet at the polymer bulk film. After the radiative charge carrier recombination in the semiconductive polymer film, emission of light is observed. PPV has a 2.5eV energy band gap between its HOMO and LUMO level and produces a yellow-green luminescence. Depending on the structure of the polymer, the energy band gap varies and different emission color can be obtained. Figure 1.5 shows a general example of the cross section of a polymer light-emitting diode.

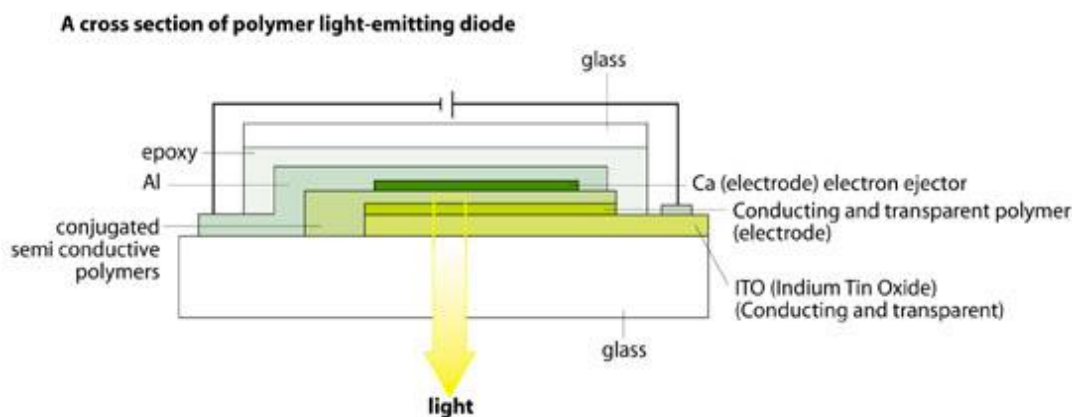


Figure 1.5: A Cross Section of Polymer Light-emitting Diode.³

1.1.1 Synthesis of Conjugated Polymers

After the discovery of the conductive polyacetylene, chemists started to develop various organic semiconductive polymers which have a conjugated backbone. Many synthetic procedures have been developed in this area. There are two main synthetic approaches for conjugated polymers which are electrochemical synthesis and synthesis in solution.

1.1.1.1 Electrochemical Synthesis

Most of the conjugated polymers are synthesized via electrochemical polymerization. Especially pyrrole and thiophene are synthesized by this method. In this method, the monomer and the electrolyte are put in a suitable solvent such as acetonitrile and oxidized at a mild potential such as 0.5. The oxidation (p-doping) is the halogen doping which converts the polymer to a good conductor. At the anode, a polymer film starts to grow. This reaction occurs in a heterogeneous environment which is the electrode surface. The polymerization is occurred by the coupling of radical cations.⁹

The prepared polymer film at the anode is doped by the excess charge which is passed in the polymerization reaction and contains a counterion from the electrolyte solution (Figure 1.6). This film can be undoped by passing current from the reverse direction.⁹

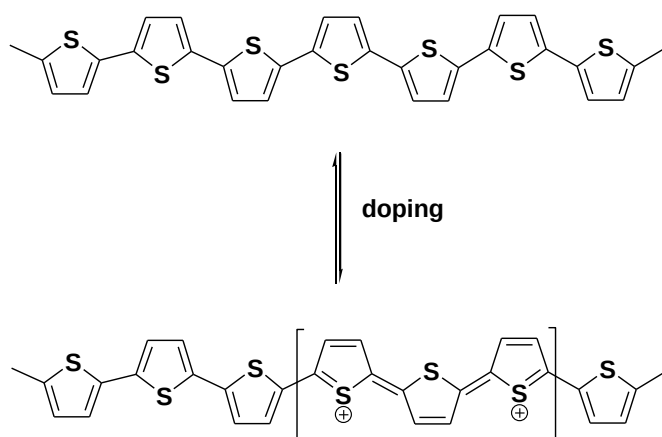


Figure 1.6: Polythiophene Doping.²

After this method, many other conjugated polymers were synthesized. Polyparaphenylene, polyaniline and polyfluorene derivatives are the examples of the synthesized polymers via electrochemical synthesis.

1.1.1.2 Synthesis in Solution

The formation of a carbon-carbon bond is very important in organic chemistry. Highly important molecules can be synthesized by the assembly of the carbon atoms into chains. Up to now, many carbon-carbon bond syntheses were awarded by the Nobel Prize such as the Grignard reaction (1912), the Diels-Alder reaction (1950), the Wittig reaction (1979), and olefin metathesis to Y. Chauvin, R. H. Grubbs, and R. R. Schrock (2005).

Transition metals play an important role in carbon-carbon bond formation syntheses because they easily activate the various organic compounds and catalyze the new bond formation. After the discovery of the oxidation of ethylene to acetaldehyde by air in a palladium-catalyzed reaction, palladium metal became popular in organic chemistry. After the detailed research on palladium-catalyzed carbonylation, new synthetic approaches were found for the carbon-carbon bond formation. In 2010, formation of carbon-carbon single bonds through palladium-catalyzed cross-coupling reactions was awarded by the Nobel Prize.¹⁰

Palladium-catalyzed cross couplings are based on the assembly of the two molecules on the metal by the metal-carbon bond formation. Carbon atoms which are bound to palladium come closer to each other by this way. After that they couple to each other and new carbon-carbon bond formation occurs.¹⁰

Step-growth polymerization method is one of the most common method to synthesize conjugated polymers. In this polymerization, bi-functional or multifunctional monomers first react to form dimers, then trimers and at the end of the reaction long-chained polymers. The most significant examples of the polymers synthesized via this way are polyaniline, poly(phenylene sulfide), polythiophene and its derivatives.⁹

Polythiophenes are widely synthesized by organometallic step-growth polymerization. This synthesis is generally performed by using nickel-catalyzed coupling of the di-Grignard of thiophene, Friedel-Crafts alkylation, coupling of the di-halide using a Ni(0) catalyst, and oxidation with FeCl₃ (Figure 1.7).⁹

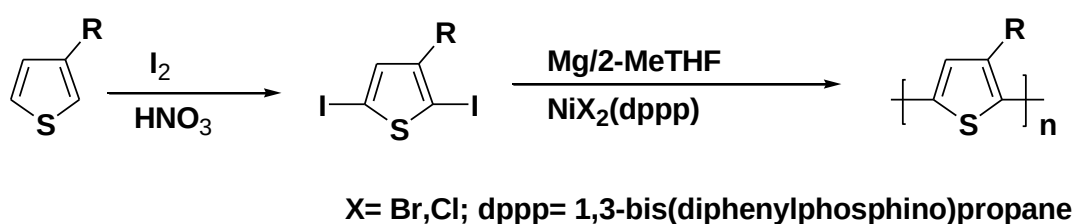


Figure 1.7: Nickel-catalyzed Polymerization of Thiophene. ⁹

The most common polymerization method in this group is the Suzuki-Miyaura coupling polymerization. This polymerization method contains an arylpalladium(II) halide complex catalyst as an initiator. The initiator unit of the polymer is aryl group of the arylpalladium(II) halide complex. The polymerization of the fluorene was achieved in the presence of ^tBu₃PPd(Ph)Br catalyst (Figure 1.8). By this method, polymers with a narrow polydispersity can be obtained. It is determined that the molecular weight of the synthesized polymer increased proportionally to the conversion of the monomer to polymer indicating that the polymerization method is the example of the chain-growth polymerization. ¹¹

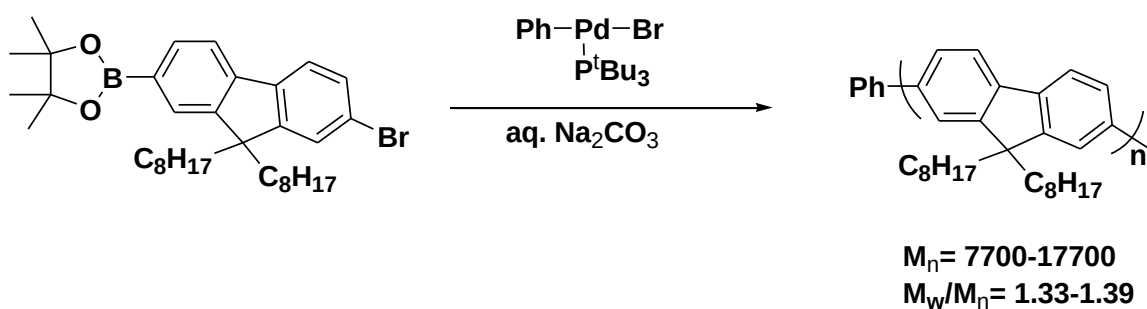


Figure 1.8: Polymerization of Fluorene from Suzuki-Miyaura. ¹¹

Reynolds and coworkers demonstrated the one-pot Suzuki-Miyaura coupling polymerization starting from 2,7-dibromo-9,9-dioctylfluorene and bis(pinacolato)diboron (Figure 1.9).

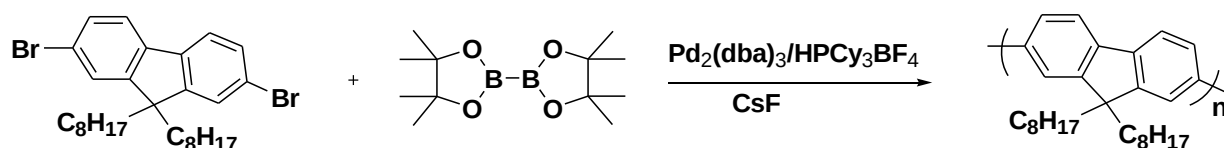


Figure 1.9: Polymerization of Fluorene from Reynolds and Co-workers.¹¹

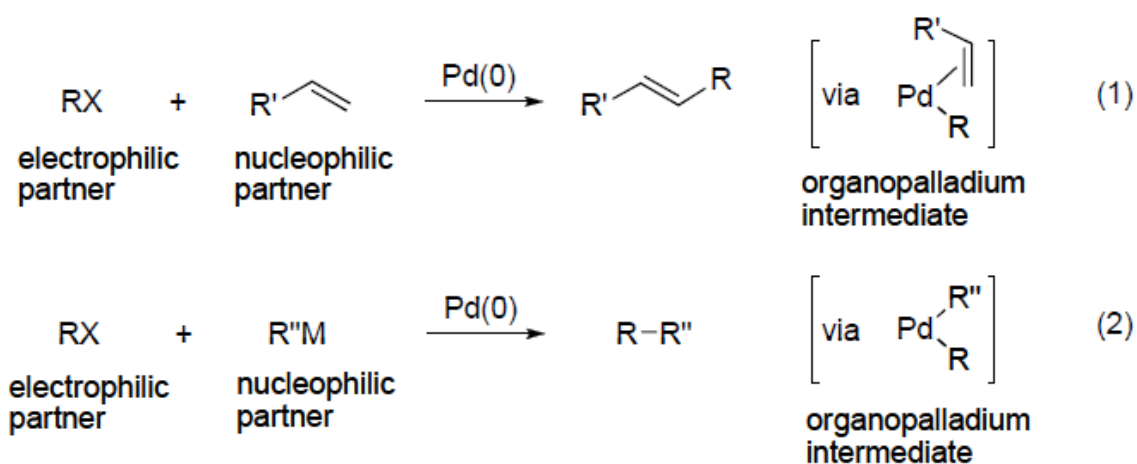


Figure 1.10: Two Types of Palladium-Catalyzed Carbon-Carbon Bond Formation.¹⁰

Heck, Negishi and Suzuki were awarded by the Nobel Prize in 2010 by their palladium catalyzed C-C coupling reactions which opened a new door for many organic synthesis.

In 1972, Heck developed cross coupling reactions involving olefins (Figure 1.11).¹² He generated an organopalladium complex from an organohalide in an oxidative addition.

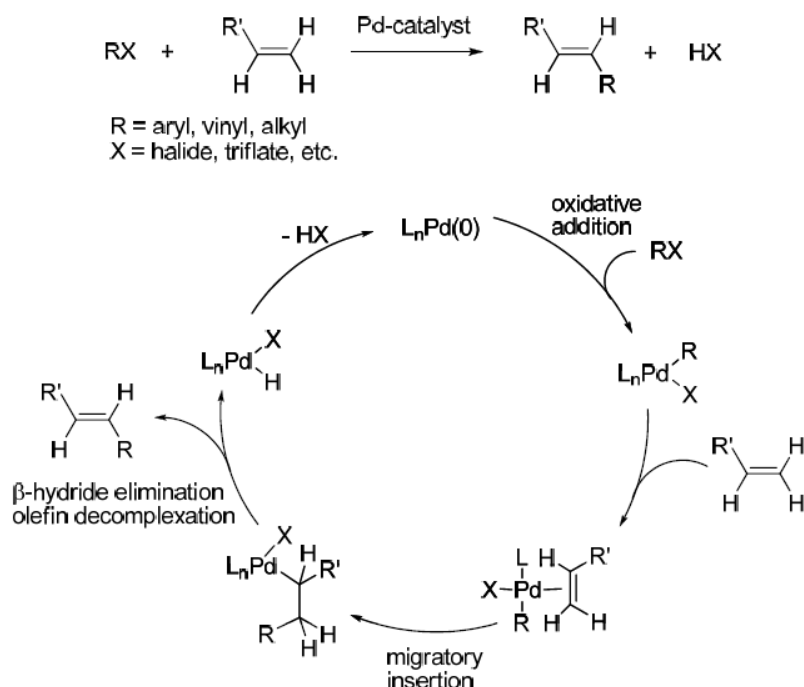


Figure 1.11: Mechanism of the Heck Reaction. ¹² Reprinted with permission from ref. 12 (Copyright 2012 American Chemical Society)

In 1977, Negishi developed the organozinc compounds as the nucleophilic coupling reagents in palladium-catalyzed cross coupling reactions (Figure 1.12). ¹³ Organozinc compounds give very high yields when they are compared to the different organometallic compounds. They are also highly mild and selective compounds. Organozinc compounds allow for the presence of various functional groups in the palladium-catalyzed cross-coupling reaction.

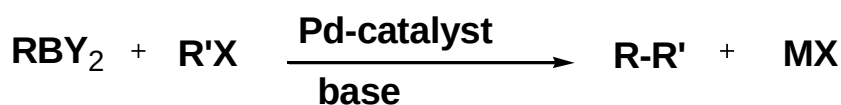


R, R' = aryl, vinyl, alkyl
X = halide, triflate, etc.

Figure 1.12: Negishi Reaction Scheme. ¹³ Reprinted with permission from ref. 13 (Copyright 2012 American Chemical Society)

In 1979, Suzuki and co-workers reported that organoboron compounds can easily be used as coupling compounds if there is a base in the reaction medium in palladium-catalyzed cross coupling reactions with vinyl and aryl halides (Figure 1.13). ¹⁴ Organoboron reagents are activated by the base in the reaction medium that is resulted with a boronate

intermediates which provides the organic group transfer from boron to palladium. This process is called as transmetallation. After the development of this reaction, coupling with also alkyl groups were demonstrated.



R, R' = aryl, vinyl, alkyl
X = halide, triflate, etc.

Figure 1.13: Palladium-catalyzed Cross Coupling with Vinyl and Aryl Halide.¹⁴

In Suzuki cross-coupling reactions, an organoboron compound reacts with an organohalide and forms a couple. Palladium(0) catalyzes these reactions. The reaction is resulted with a new carbon-carbon bond formation (Figure 1.14).

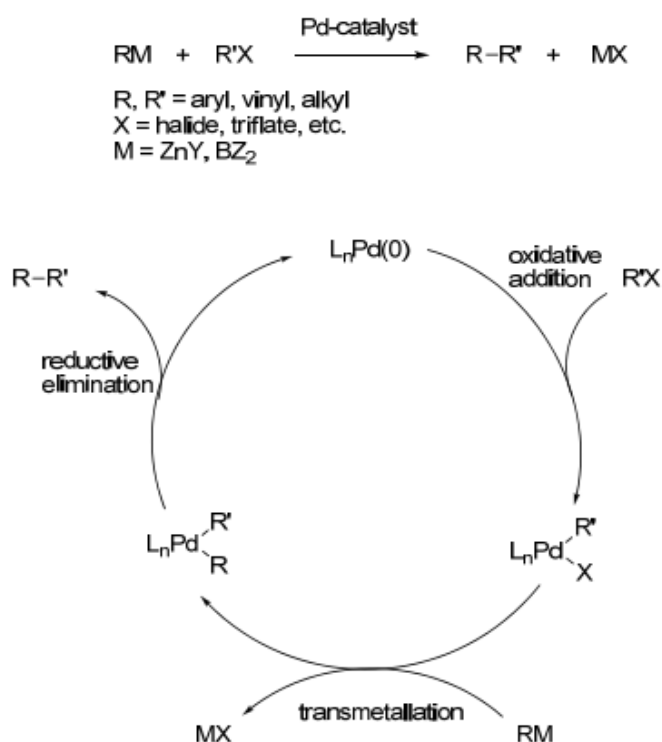


Figure 1.14: Mechanism of the Suzuki Cross-coupling Reaction. ¹⁵ Reprinted with permission from ref. 15 (Copyright 2012, Elsevier)

1.1.2 Properties and Applications of the Conjugated Polymers

Conjugated polymers have a wide range of applications. They are used in polymer-light emitting devices, electroluminescent displays, field-effect transistors (FET), and in

various sensing devices.⁸ They are also widely used in supercapacitors. A high number of conjugated polymers have electrochromic properties. These kind of polymers such as polyaniline are used in smart windows which absorb sunlight.

1.1.3 Water soluble Conjugated Polymers

Water soluble conjugated polymers have a wide range of use in biological applications. They are generally prepared by the incorporation of the water-soluble moieties. Water soluble conjugated polymers are formed from the two parts. One part contains the conjugated backbone which provides the good optical properties. Other part contains the hydrophilic or charged functional groups such as ammonium, sulfonate which provides polar groups for the solubility of the polymer in water (Figure 1.15).¹⁶

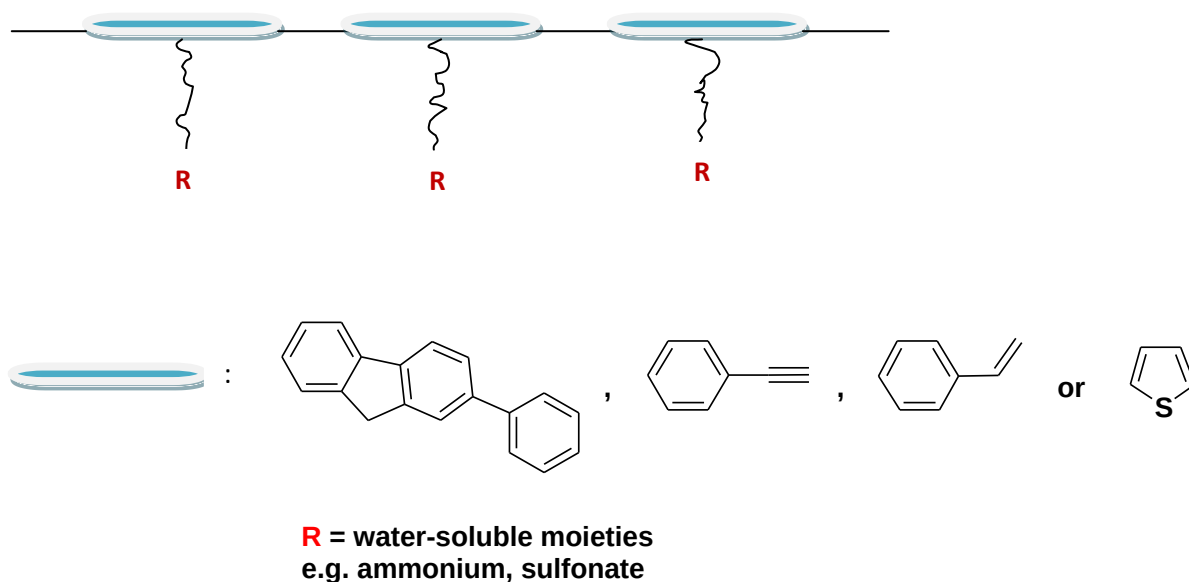


Figure 1.15: Typical Structures of Water-soluble Conjugated Polymers.¹⁶

Water soluble functional groups are generally charged. They provide the tunable interactions between the conjugated polymers and the biomacromolecules. As a result of the electrostatic interactions which provides the target recognition, scientists achieved a lot of specific biological detections. Water soluble conjugated polymers have also wide range of use in fluorescence imaging in vivo and in cell level.¹⁶

Poly(thiophene) (PT), poly(p-phenylenevinylene) (PPV), poly(p-phenyleneethynylene) (PPE) and poly(fluorene) (PF) are widely used in fluorescent biosensing applications. Poly(thiophene) is one of the oldest synthesized water-soluble polymer by Wudl and

coworkers. The methyl sulfonate monomers were polymerized by electropolymerization and then converted into the sodium salts. Leclerc and Inganas groups have also demonstrated synthesis of new water soluble poly(thiophene) derivatives. They were synthesized by oxidation polymerization of monomers in chloroform in which FeCl_3 was used as the oxidizing agent (Figure 1.16).¹⁶

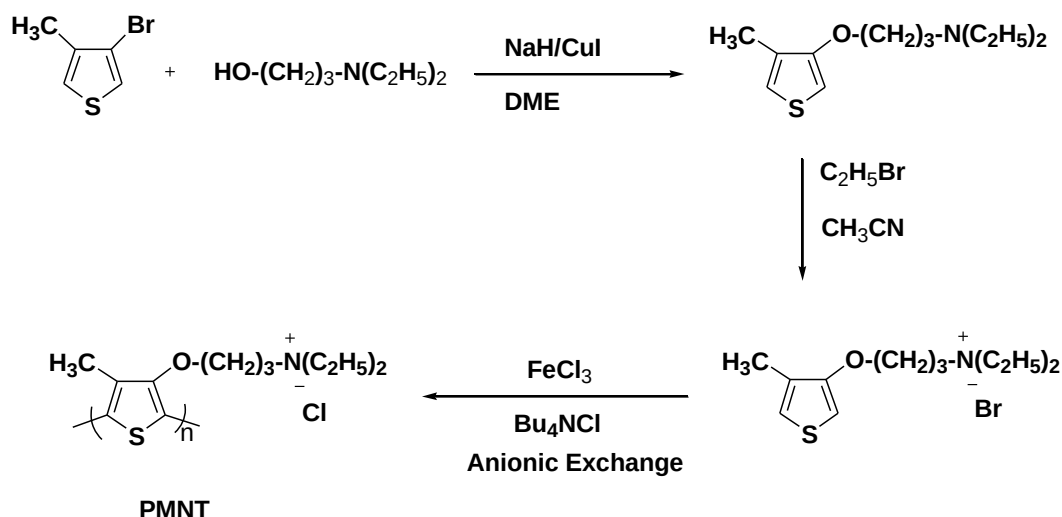


Figure 1.16: Synthetic Route of Cationic Poly(thiophene) by Oxidation Polymerization Reaction.¹⁶

Polyfluorenes also attracted the interest of many scientists because of their various applications in biological and chemical sensing areas. They provide an easy substitution at the C9 position fluorene, they have high chemical and thermal stability. They also have high fluorescence quantum yields in water.

For the water soluble polyfluorene synthesis, the palladium-catalyzed Suzuki coupling reaction is widely used. Bazan and co-workers have reported a cationic polyfluorene that was synthesized by quaternization with methyl iodide of pendant $-\text{NMe}_2$ groups on a neutral precursor polymer in THF–DMF–water (Figure 1.17).¹⁷

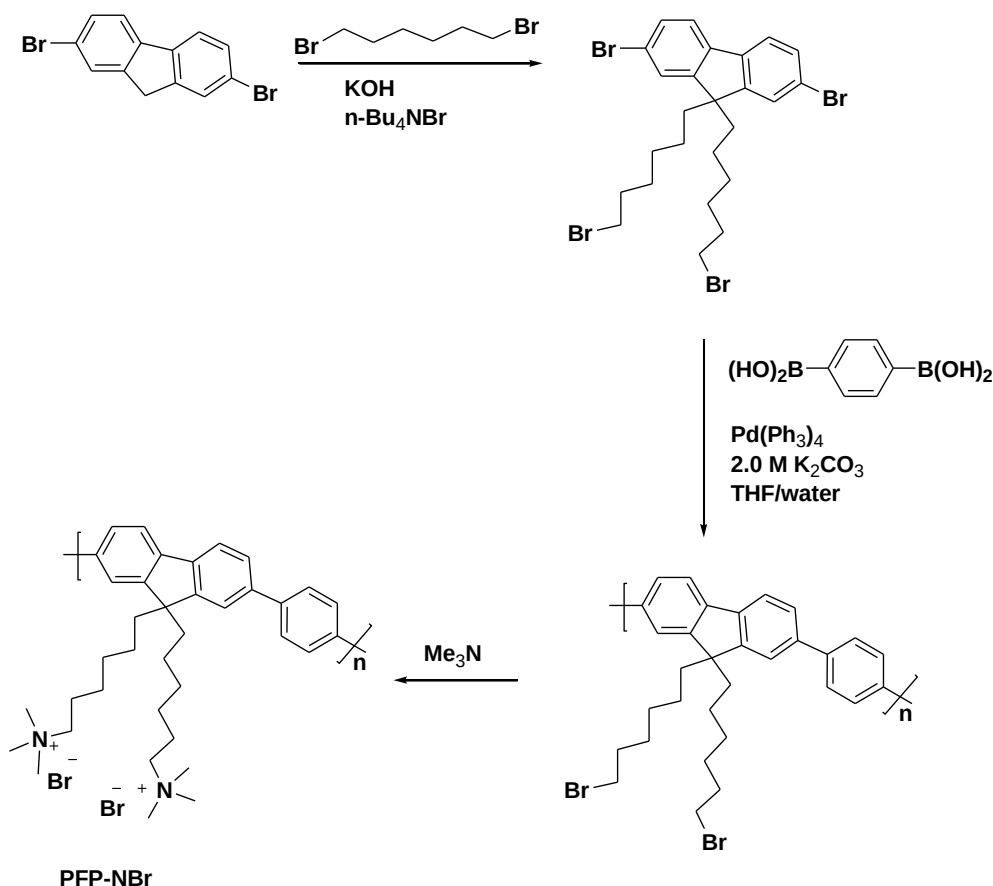


Figure 1.17: Synthesis of Cationic Polyfluorene.¹⁷

1.2 Conjugated Polymer Nanoparticles

Conjugated polymer nanoparticles are highly versatile nanoparticles with their high number of applications in various branches of chemistry. They provide important opportunities in optoelectronics, photonics, bioimaging, biosensing and nanomedicine. These nanoparticles have attracted the interest of the scientists for several reasons. First of all, they have a straightforward synthetic route. Their properties can be easily tuned by changing their structures which makes them easily modified for different applications. They are also very advantageous in biological applications because of their low toxicity and biocompatibility compared to the inorganic nanoparticles.¹⁸

1.2.1 Preparation of the Conjugated Polymer Nanoparticles

Conjugated polymer nanoparticles are commonly synthesized via postpolymerization dispersion technique. In this technique, polymer solutions dissolved in an organic solvent is the beginning part. Nanoparticles are generally formed by the solvent removal from the

mini-emulsion or by the addition of a polymer solution to an excess amount of continuous phase which is resulted as a precipitation.¹⁹

1.2.1.1 Miniemulsion Technique

This method is widely used in conjugated polymer nanoparticle synthesis. Here, the polymer is dissolved in organic solvent which is not soluble in water. Then, the resulting organic solution is injected into the water which contains an a suitable surfactant. The mixture is stirred rapidly with the ultrasonication in order to form stable miniemulsions which contains small polymer solution drops. Then, the organic solvent is evaporated and resulting solution contains well-dispersed conjugated polymer nanoparticles (Figure 1.18).

It is easy to change the size of the nanoparticles by changing the concentration of the nanoparticles. Their sizes vary from 30nm to 500nm¹⁸.

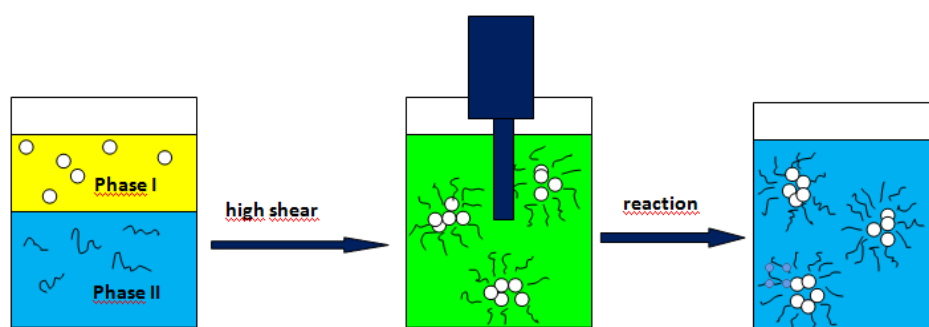


Figure 1.18: Preparation of Nanoparticles by Miniemulsion Technique.

Ostwald ripening may cause the destabilization of the droplets by solvent diffusion through the aqueous phase. In order to prevent this, hydrophobes are used. The hydrophobe's job is to promote the osmotic pressure formation inside the droplets. This osmotic pressure counteracts the Laplace pressure which prevents the diffusion from one of the polymer droplet to the aqueous medium around the droplets¹⁸.

Most of the examples of the conjugated polymer nanoparticle synthesis via miniemulsion technique involve the synthesis of nanoparticles by using the polymers with an oil-in-water system. In contrast to this system, Muller et al. developed the synthesis of nanoparticles which started from the monomers in oil-in-oil emulsions.²⁰ They used cyclohexane as a continuous phase and acetonitrile as a dispersed phase. Polyisoprene-block-poly(methyl methacrylate) (PI-b-PMMA) was used as an emulsifying agent.

Poly(3,4-ethylenedioxythiophene) (PEDOT), polyacetylene and poly(thiophene-3-yl-acetic acid) were synthesized as polymeric nanoparticles by oxidative and catalytic polymerization using this method. PI-b-PMMA emulsifying agent was sent away by washing with THF after the formation of nanoparticles. DLS results showed that the number-average diameter of the particles is 43 nm (± 10 nm).

Another synthetic approach for the nanoparticle synthesis was reported by Mecking and co-workers in 2006.²¹ They synthesized polyacetylene nanoparticles by the acetylene polymerization in aqueous miniemulsion technique. First of all, they dissolved a Pd catalyst in a minimum amount of hexane-ethanol mixture. Then they added this mixture into a surfactant-organic acid solution which contains sodium dodecylsulfate and methane sulfonic acid. After the sonication, a miniemulsion was formed and by stirring the miniemulsion under an acetylene atmosphere, they obtained a black dispersion of polyacetylene nanoparticles. The size of the nanoparticles were determined by TEM technique which was recorded as approximately 20 nm.

Mecking et al. also developed the nanoparticle synthesis of poly(arylene diethynylene) derivatives by the miniemulsion technique.²² GPC recorded the molecular weights of the nanoparticles between M_n 10^4 to 10^5 g/mol. TEM analysis showed their sizes around 30 nm.

1.2.1.2 Reprecipitation Technique

This nanoparticle synthesis technique is also widely used. In this technique, very dilute (e.g., several tens of parts per million) polymer solution is added to an excess volume of nonsolvent such as water. Mixing of the solvent with the nonsolvent causes a decrease of solvent quality. This situation is resulted with a precipitation of the polymer and polymer nanoparticles are formed (Figure 1.19). In order to be sure the nanoparticle formation, rapid mixing is done by ultrasonication. Poly(arylenevinylene) and polyfluorene nanoparticles are widely synthesized by this method. After the rapid stirring of their solutions at elevated temperatures, their nanoparticles are formed in water. When THF solvent is evaporated under reduced pressure, conjugated polymer nanoparticles in water are obtained without any surfactant. Their average sizes vary from 5 to 50nm.²³

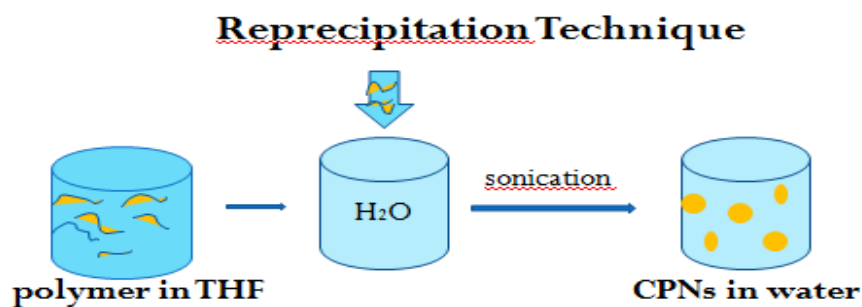
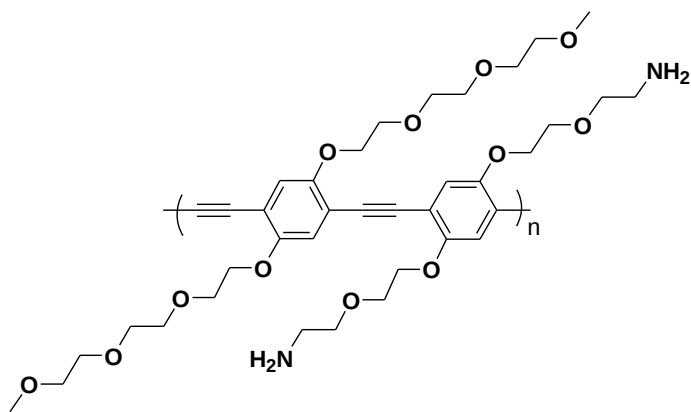


Figure 1.19: Preparation of the Nanoparticles by Reprecipitation Technique.

The nanoparticle size can be tuned easily by changing the concentration of the organic polymer solution. In reprecipitation technique, colloidal stabilization of the nanoparticles are not fully understood compared to the miniemulsion technique. In reprecipitation technique, no surfactant is used and the polymer does not contain a hydrophilic moiety. Charge accumulation at the particle-dispersing medium interface may be a one reason for the stabilization toward particle aggregation and coalescence. Another reason for the steric and electrostatic stabilization could be the impurities at low levels can adsorb to their surface.¹⁹

The nanoparticles which are prepared by the reprecipitation technique are formed by the overlap of the polymer chains which is resulted with a spherical-shaped nanoparticles. They are thermodynamically favorable.

In the reprecipitation technique case, Moon et al. reported the synthesis of nanoparticles from poly-(phenylene ethynylene) (PPE) derivatives by phase inversion precipitation (Figure 1.20).²⁴ According to the report, polymer is dissolved in DMSO and then added into the aqueous SSPE buffer (saline, sodium phosphate, EDTA). According to the DLS measurements, a mean particle size is 400–500 nm.



PPE-2

Figure 1.20: Structures of Poly(phenylene ethynylene) (PPE) Derivative Used in Nanoparticle Synthesis.²⁴

Nanoparticles are mainly formed by the hydrophobic effect. When the organic polymer solution is poured into water, polymer chains do not want to contact with water. As a result, they gain spherical shapes in order to decrease the interaction with water. So, the amount of the hydrophilic side groups significantly affects the nanoparticle formation. If the side chain contains the protonated amine group and the short chain contains the nonionic diethylene oxide group, aggregation and precipitation is prevented by the stabilization of the nanoparticle surface.¹⁸

1.2.2 Properties and Applications of the Conjugated Polymer Nanoparticles

Photophysical properties of nanoparticles make them very desirable materials for many applications. One of the most important applications of these nanoparticles is in optoelectronics.

Before using the CPNs in the fabrication of optoelectronics, it should be known if the photophysical and optical properties of the polymers affect the nanoparticle formation. In addition, in the miniemulsion technique of the nanoparticles synthesis, some surfactants and hydrophobes are used. It is very important to determine the influence of these surfactants and hydrophobes on the photophysical properties of the nanoparticles. CPNs.

In order to demonstrate the photophysical properties of CPNs which are synthesized via the miniemulsion technique, Piok et al. studied on the methyl-substituted ladder-type poly(para-phenylene) (m-LPPP).²⁵ They worked on the nonequilibrium photoexcitation dynamics of CPNs and then compared them with the same polymer's film. According to results, synthesis of nanoparticles from m-LPPP isn't resulted with a significant change in the optical properties of the polymer. Absorption and emission spectra are not change again with the change of size.

Photophysical properties of nanoparticles synthesized by the reprecipitation method was also studied. Masuhara and co-workers synthesized nanoparticles from poly(3-[2-(N-dodecylcarbamoyloxy)ethyl]thiophene-2,5-diyl) (P3DDUT) with different sizes changing from 40 nm to 400 nm.²⁶ They controlled the spectroscopic and thermochromic behavior of the nanoparticles in water. They also made films from their water dispersions. In the absorption and emission spectra, a blue shift was observed.

In the applications related to the energy transfers, conjugated polymer nanoparticles are highly used again. Fluorescence energy transfer has many applications in molecular biosensors and optoelectronic devices. In order to observe a FRET, excitation energy of the fluorophore which acts as a donor should be transferred to the fluorophore that acts as an acceptor which follows a nonradiative pathway. Energy transfer is widely used in conjugated polymers in order to increase the quantum efficiency and change the emission color of LEDs.²⁷ DNA and protein sensing are also well-known examples of FRET applications.

Grigalevicius and co-workers synthesized the first dye-doped conjugated polymer nanoparticles in 2006.²⁸ Energy transfer from the excited nanoparticle chromophore to the fluorescent dye was shown. They have synthesized nanoparticles with negative charges from blue-emitting polyfluorene derivatives. They used the anionic surfactant sodium dodecyl sulfate. They incorporated Rhodamine 6G and tetramethylrhodamine ethyl ester perchlorate (Rhodamine TM) as cationic fluorescent dyes. Results showed that intensity of the blue fluorescence of the dye-coated polyfluorene nanoparticles decreased after they were doped with the dyes. After the energy transfer, emission band of the dye (Rhodamine 6G or Rhodamine TM) which is bound to surface is observed between the 530 nm and 600 nm. This results indicates that excitation energy from PF chromophores was transferred successfully to the fluorescent dye.

Conjugated polymer nanoparticles are also widely used in the biological sensor applications. In these systems, water soluble materials are required. For this aim, Moon and co-workers synthesized CPNs from PPE that contains amine groups via ultrafiltration.²⁹ They showed the usage of CPNs for 2P imaging of endothelial cells in a tissue model. 2P-action cross section of CPNs were measured between 1000-11000 GM. The maximum value was about 730 nm. This value is significantly higher than the recent organic fluorophores and very close to the quantum dots (QDs).

For the purpose of livecell imaging, amine containing poly(p-phenylene ethynylene) (PPE) water-soluble nanoparticles were synthesized. In order to determine the cell permeability, cellular toxicity and photostability of CPNs, baby-hamster kidney (BHK) and BALB/C 3T3 (mouse embryonic fibroblast) were used. They were incubated with CPNs in culture media with different time periods. Results showed that CPNs were cell permeable. Also they accumulate exclusively in the cytosol without inhibiting the cell viability (Figure 1.21).

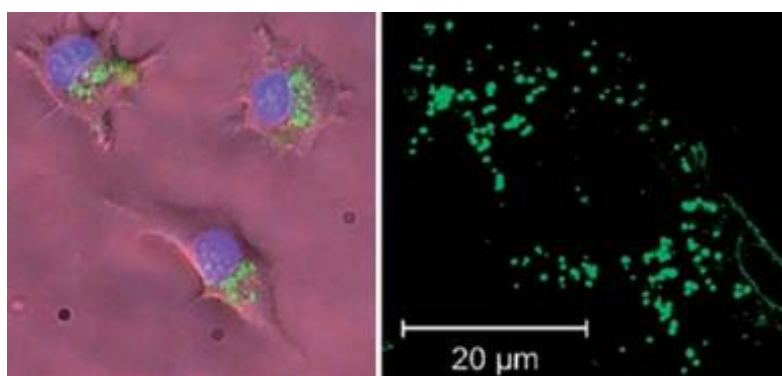


Figure 1.21: Fluorescence Images of Live and Fixed Cells.³⁰ Reprinted with permission from ref. 30 Copyright 2012 John Wiley and Sons

1.3 Click Chemistry in Polymers

Click chemistry is a chemical concept which was introduced by K. Barry Sharpless in 2001. It is based on the generation of the chemical substances in a quick and reliable way by joining small units together. Sharpless was inspired by the nature. Nature creates substances by combining small molecules together. The huge natural molecules are usually constructed from a small set of building blocks using a few types of reactions for connecting them together.³¹

One of the most important part of the click chemistry is the cycloaddition reactions which involve the heteroatoms. Hetero-Diels-Alder reaction is an example of this cycloaddition reaction. The most significant reaction is 1,3-dipolar cycloadditions (Figure 1.22). These cycloaddition reactions need two unsaturated reactants and they provide various five- and six-membered heterocycles. In these click reactions, Huisgen dipolar cycloaddition of azides and alkynes play an important role. This reaction occurs between C–C triple, C–N triple bonds and alkyl-/aryl-/sulfonyl azides. The products are tetrazoles, 1,2,3-triazoles and 1,2-oxazoles, respectively.³²

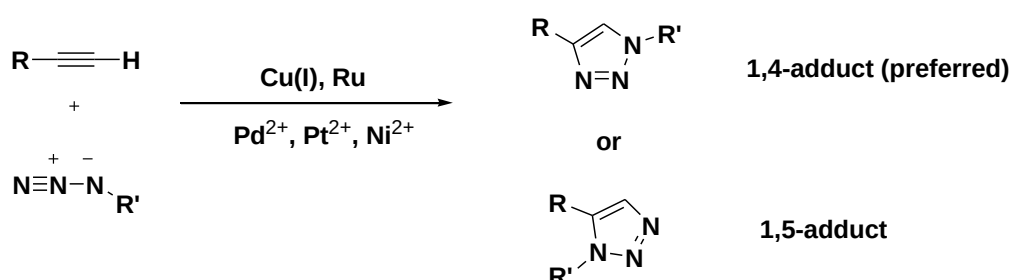


Figure 1.22: Azide-alkyne Click Reaction.³²

The reaction mechanism was determined by computational methods and demonstrated by Bock et al (Figure 1.23).

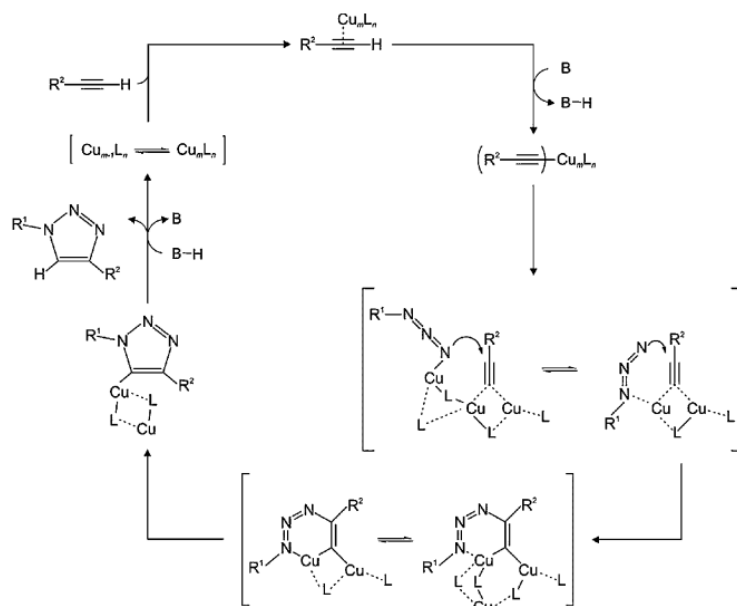


Figure 1.23: Azide-alkyne Click Reaction Mechanism.³² Reprinted with permission from ref. 32 Copyright 2012, John Wiley and Sons

Click Chemistry is also widely used in polymer reactions and polymeric applications. Its reaction efficiency is quite high and has a high functional group tolerance. This reaction is very active in water which makes it an important reaction in biological studies. Click chemistry concept demonstrates a solution to a lot of problems related to the polymer science. First of them is the poor degree of functionalization with many well-known techniques. If the polymer involves multiple functional groups (i.e.: at graft-, star-, and block copolymers, dendrimers, as well as on densely packed surfaces and interfaces), this becomes a much more serious problem. Second problem is the purification problems related with the often emerging partially functionalized mixtures. Harsh reaction conditions of conventional methods are also a big problem that can be overcome by click chemistry.

The click chemistry opens a new door to a various researches related to the new functionalized polymeric architectures, hitherto unreachable by the polymerization methods themselves. Cu-catalyzed azide-alkyne coupling reaction provides a small-molecule organic chemistry in polymer science. It brings the functional broadness, molecular addressability, and structural integrity.³³

1.4 Crosslinked Polymer Nanoparticles via Click Chemistry

Cu(I)-catalyzed azide-alkyne coupling (CuAAC) is very popular in nanochemistry. It has various applications in nanoscale studies. A lot of research groups have utilized CuAAC for the biological, polymeric, inorganic and carbon nanoscale objects. Wooley and coworkers have studied on the synthesis of shell and core crosslinked polymeric nanoparticles (Figure 1.24).^{34, 35} They were synthesized from amphiphilic PS-PAA diblock copolymers. Mentioned copolymers contain alkyne groups on the hydrophilic PAA block for shell crosslinked nanoparticles or the hydrophobic PS block for core crosslinked nanoparticles. They had a micelle-like shapes in water and were crosslinked by CuAAC by reacting with bifunctional azide groups and multifunctional dendritic azides. The reaction was resulted with the formation of the polymeric nanoparticles. After the formation of the nanoparticles, they were further functionalized with fluorescent groups.

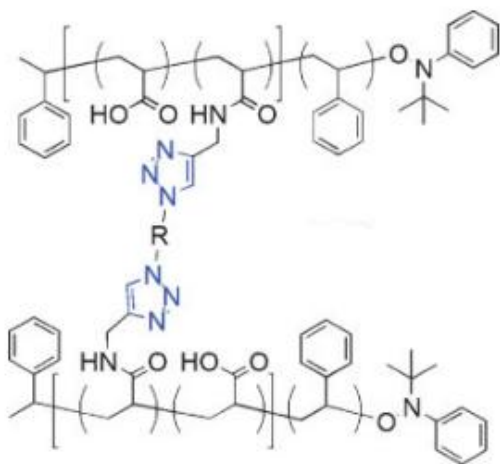


Figure 1.24: Molecular Structure of Shell-CuAAC-crosslinked Polymeric Nanoparticles Prepared by Wooley and Co-workers.³⁴ Reprinted with permission from ref. 34 Copyright 2012 American Chemical Society

Finn and coworkers have demonstrated the synthesis of a highly crosslinked polymer by using tripropargyl amine and 1,6-diazidohexane in a solution phase CuAAC reaction (Figure 1.25).³⁶

Polymer synthesized by Finn and coworkers was swelling and deswelling reversibly when it was mixed with trifluoroacetic acid (Figure 1.25). This observation was the result of the amine functional groups in the polymer.

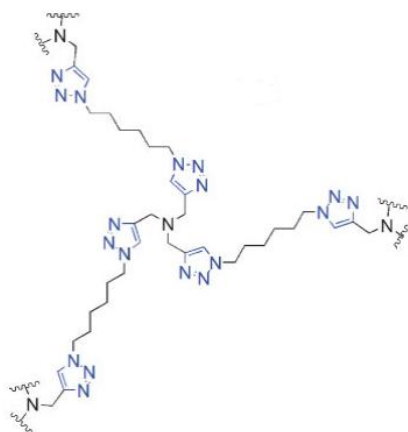


Figure 1.25: Acid-swelling CuAAC-crosslinked Network.³⁶ Reprinted with permission from ref. 36 Copyright 2012, John Wiley and Sons

Liu and coworkers have followed a similar approach with the synthesis of core crosslinked nanoparticles comprised of poly(N,N-dimethylacrylamide) (PDMA) and

poly(*N*-isopropylacrylamide-co-azidopropylacrylamide).³⁷ Caruso and coworkers demonstrated an attractive synthesis of highly thin microcapsules via facile layer-by-layer (LBL)/CuAAC approach.³⁸ First of all, the authors used the electrostatic and hydrogen bonding interactions between PAA and silica to deposit a thin layer of PAA. Some of its carboxylic acid units were converted to azides (PAA-N₃), onto the silica particle. After that, PAA layers containing alkynes (PAA-alkyne) were coated to the previous layer via CuAAC. Mentioned process was repeated several times in order to make the yield desirable.

Click chemistry is also widely used for biodegradable polymer nanoparticles. As it is all known that the triazole group formed by azide-alkyne cycloaddition reaction has significant chemical stability. However, biodegradable NPs can be obtained when biodegradable polymers are used for crosslinking. As demonstrated by Harth and coworkers, using biodegradable polyesters as precursors, discrete functionalized NPs have been obtained via a controlled intermolecular click crosslinking process³⁹

1.5 Aim of the Thesis

In this study, a novel method was demonstrated for the synthesis of water-dispersible conjugated polymer nanoparticles with enhanced stability. Fluorene and thiophene-based conjugated polymer nanoparticles were synthesized that can be used in biological applications such as cell imaging and drug delivery.

For cell imaging and drug delivery, conjugated polymer nanoparticles attracted much interest in chemistry, biology and medicine world. In this study, the aim is to synthesize water-dispersible, multifunctional and stable conjugated polymer nanoparticles and contribute to the biological studies with a new aspect.

Crosslinked conjugated polymer nanoparticles were synthesized by 1,3-dipolar cycloaddition reaction between an azide group and a diaminodialkyne crosslinker. By this crosslinking, various useful functional groups were introduced to the nanoparticles such as triazole and amine groups. This provides the formation of water-dispersible nanoparticles which is desired for cell imaging and biomolecule delivery studies.

In 1,3-dipolar cycloaddition reaction, Cu(I) and cucurbituril (CB6) were used as a catalyst. The idea of using CB6 as a catalyst was to get more biocompatible nanoparticles for biological applications. It also provided rotaxane structures in the nanoparticles.

Crosslinking of the conjugated polymer nanoparticles was also achieved by the irradiation of the nanoparticles under UV light. All CPNs synthesized with different crosslinking techniques were designed as novel candidates of biomolecule delivery agents and cell imaging.

CHAPTER 2

RESULTS AND DISCUSSION

This chapter is divided into three main parts. In the first part, synthesis and characterization of the monomers as a precursor of the polymers are discussed. In the second part, synthesis and various characterization techniques of the facile polymers are demonstrated. In the final part, preparation and fully characterization of the water-dispersible crosslinked polymer nanoparticles are explained in detail.

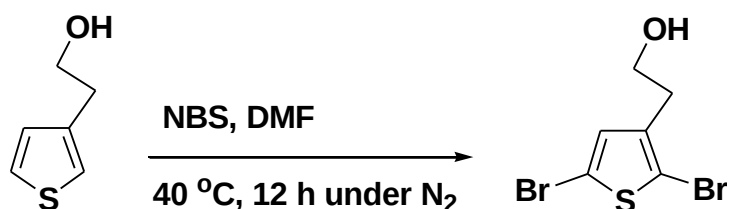
This project demonstrates a novel synthetic method for the synthesis of water-dispersible conjugated polymer nanoparticles with enhanced chemical and mechanical stability. Synthesis of the polymers and their nanoparticle preparation via reprecipitation technique were studied in detail. The synthesized nanoparticles in this project can be utilized for biological studies such as cell imaging, drug delivery in biological system and theranostic nanomedicine. This project opens an important door for the conjugated polymer nanoparticle synthesis and applications.

2.1 Synthesis and Characterization of Monomers

2.1.1 Synthesis and Characterization of 2-(2,5-dibromothiophen-3-yl)ethanol (M1)

2-(2,5-dibromothiophen-3-yl)ethanol (**M1**) was synthesized by the bromination of alpha positions of thiophene with N-bromosuccinimide (NBS). After the reaction at 40 °C under N₂, product was extracted with diethylether and purified by column chromatography by using hexane with the yield of 64%.

The synthesis of the monomer M1 was illustrated in Scheme 2.1.



Scheme 2.1: Synthesis of the monomer 2-(2,5-dibromothiophen-3-yl)ethanol (**M1**) demonstrates the bromination with NBS at 40 °C for 12 h under N₂.

Monomer M1 was characterized by ¹H-NMR spectroscopy. Figure 2.1 shows the ¹H-NMR spectrum of monomer M1.

According to the ¹H-NMR spectrum, triplets at 4.19 ppm and 2.84 ppm labeled as (a) and (b) belong to the -CH₂ protons nearer to the hydroxyl group and thiophene, respectively. -CH₂ protons nearer to the hydroxyl group were deshielded because of the electronegative hydroxyl group. A sharp singlet at 2.04 ppm is coming from the proton of hydroxyl group. The proton at the beta position of thiophene was detected as a singlet at 6.81 ppm.

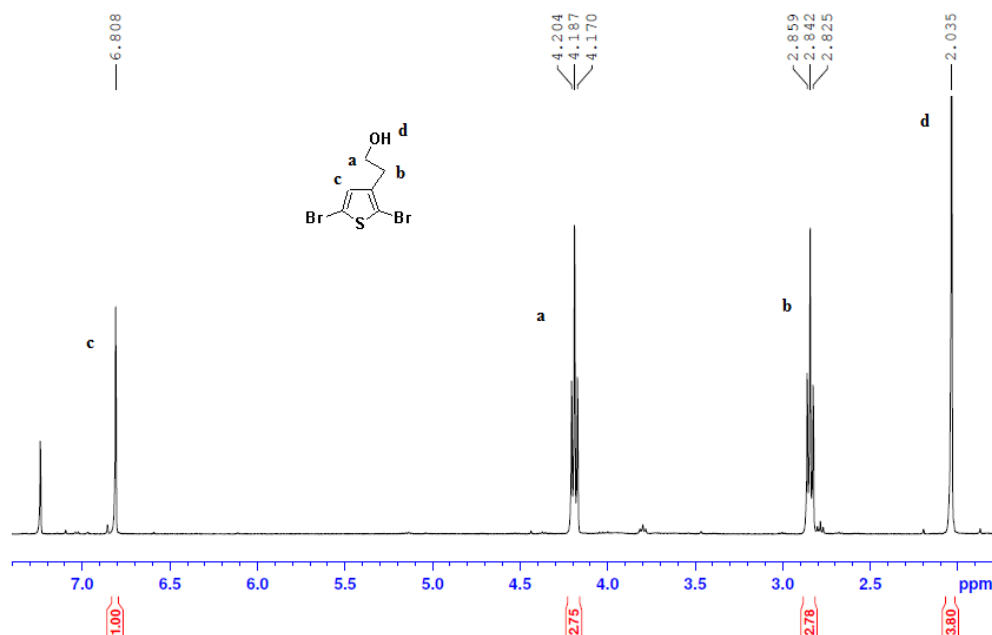
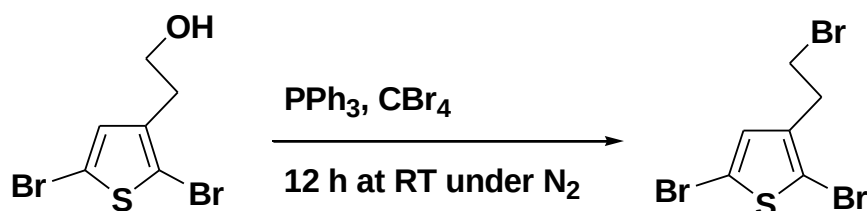


Figure 2.1: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of 2-(2,5-dibromothiophen-3-yl)ethanol (**M1**).

2.1.2 Synthesis and Characterization of 2,5-dibromo-3-(2bromoethyl)thiophene (**M2**)

2,5-dibromo-3-(2-bromoethyl)thiophene (**M2**) was synthesized by the Appel Reaction.⁴⁰ Hydroxyl group of **M1** was converted into bromide by using triphenylphosphine and carbontetrabromide. Carbontetrabromide was used as the bromide source and added to the cooled **M1** and triphenylphosphine solution in THF under N_2 . After the reaction was over, the product was isolated by extraction with diethylether and brine solution. Purification was done by column chromatography with hexane as an eluent and product yield was calculated as 34%.

Synthesis of the monomer **M2** was illustrated in Scheme 2.2.



Scheme 2.2: Synthesis of the monomer 2,5-dibromo-3-(2-bromoethyl)thiophene (**M2**) demonstrates the bromination of the hydroxyl group with CBr_4 for 12 h at RT under N_2 .

Monomer M2 was characterized by ^1H -NMR spectroscopy. Figure 2.2 shows the ^1H -NMR spectrum of monomer M2.

According to the ^1H -NMR spectrum, triplets at 3.47 ppm and 3.08 ppm labeled as (a) and (b) belong to the $-\text{CH}_2$ groups nearer to the bromine and the thiophene, respectively. $-\text{CH}_2$ protons nearer to the bromine group were deshielded because of the electronegative bromine group. A sharp singlet coming from the proton of alcohol group disappeared in the spectrum which indicates the successful bromination of the hydroxyl group. The proton of the thiophene was detected as a singlet at 6.84 ppm.

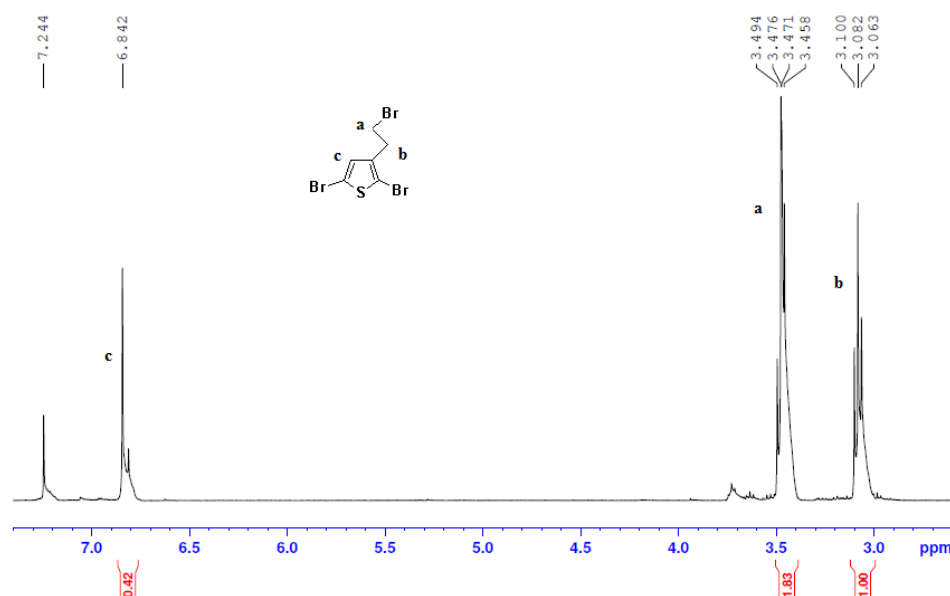


Figure 2.2: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of 2,5-dibromo-3-(2-bromoethyl)thiophene (M2).

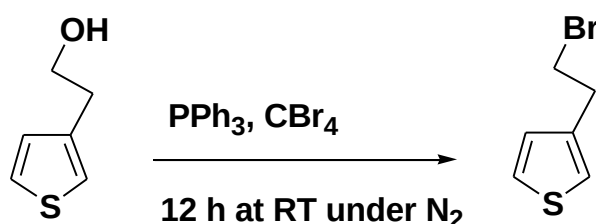
2.1.3 Synthesis and Characterization of 3-(2-bromoethyl)thiophene (M3)

3-(2-bromoethyl)thiophene (M3) was synthesized by the Appel Reaction in order to convert the hydroxyl group of 2-(3-thienyl)ethanol into bromide by using triphenylphosphine and carbontetrabromide.⁴⁰ Carbontetrabromide was used as the bromide source and added to the cooled 2-(3-thienyl)ethanol and triphenylphosphine solution in THF under N_2 . After the reaction was over, the product was isolated by

extraction with diethylether and brine solution. Purification was done by column chromatography with hexane as an eluent and the product yield was calculated as 79%.

This monomer was synthesized as a precursor of the trimethyl amine functionalized polymer P4 which was synthesized via oxidative polymerization. For this reason, the hydroxyl group of the 2-(3-thienyl)ethanol was brominated by the Appel Reaction without bromination of the alpha positions of thiophene.

Synthesis of the monomer M3 was illustrated in Scheme 2.3.



Scheme 2.3: Synthesis of the monomer 3-(2-bromoethyl)thiophene (**M3**) demonstrates the bromination of the hydroxyl group with CBr₄ for 12 h at RT under N₂.

Monomer M3 was characterized by ¹H-NMR spectroscopy. Figure 2.3 shows the ¹H-NMR spectrum of monomer M3.

According to the ¹H-NMR spectrum, triplets at 3.55 ppm and 3.19 ppm labeled as (a) and (b) belong to the -CH₂ groups nearer to the bromine group and the thiophene, respectively. A sharp singlet coming from the proton of hydroxyl group disappeared in the spectrum which indicates the successful bromination of the alcohol. Aromatic protons of thiophene labeled as (c), (d) and (e) were coming at 7.27 ppm, 7.05 ppm and 6.96 ppm as multiplets and doublet of doublets, respectively.

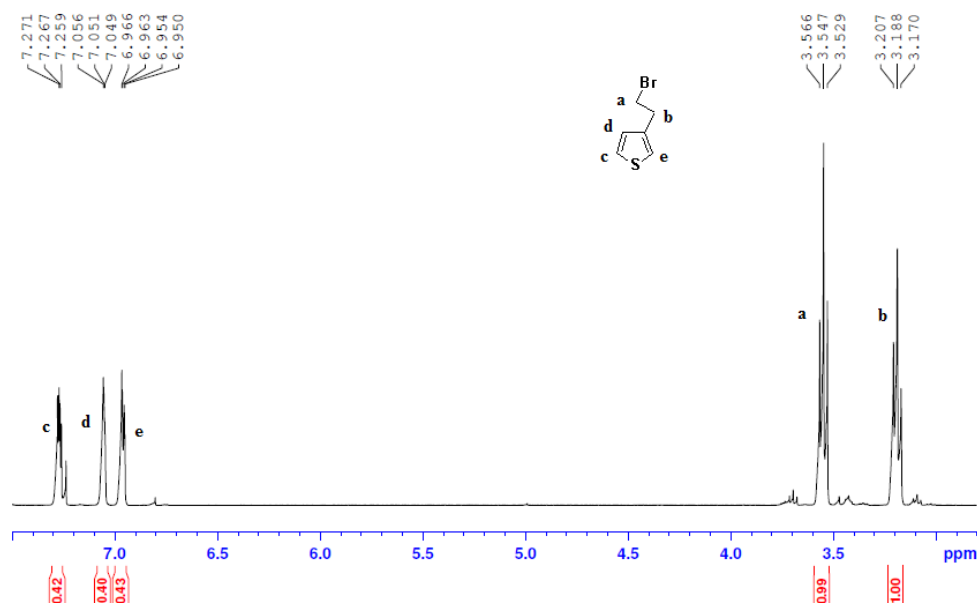
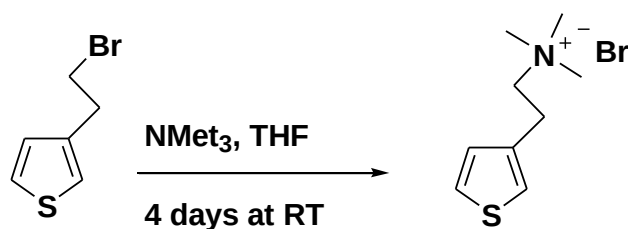


Figure 2.3: ¹H-NMR (400 MHz, CDCl₃, 25 °C) spectrum of 3-(2-bromoethyl)thiophene (M3).

2.1.4 Synthesis and Characterization of 2-(thiophen-3-yl)-N,N,N trimethylethanammoniumbromide (M4)

2-(thiophen-3-yl)-N,N,N trimethylethanammoniumbromide (**M4**) was synthesized by the nucleophilic substitution reaction between the trimethylamine and the bromine group of 3-(2-bromoethyl)thiophene.⁴⁰ 3-(2-bromoethyl)thiophene (M3) was dissolved in a minimum amount of THF and added to 25% trimethylamine solution. After 4 days stirring, the excess trimethylamine solution was evaporated and the product was purified by hexane:acetone washing with the yield of 59%.

Synthesis of the monomer M4 was illustrated in Scheme 2.4.



Scheme 2.4: Synthesis of the monomer 2-(thiophen-3-yl)-N,N,N trimethylethanammoniumbromide (**M4**) demonstrates the amination of the bromine group with NMe₃ at RT.

Monomer M4 was characterized by ^1H -NMR spectroscopy. Figure 2.4 shows the ^1H -NMR spectrum of monomer M4.

According to the ^1H -NMR spectrum, multiplets at 3.60 ppm and 3.21 ppm labeled as (a) and (b) belong to the $-\text{CH}_2$ groups nearer to trimethylamine group and the thiophene, respectively. A sharp singlet coming at 3.17 ppm belong to the methyl protons of the trimethylamine group. Aromatic protons of thiophene labeled as (c), (d) and (e) were coming at 7.47 ppm, 7.28 ppm and 7.10 ppm as multiplets and doublet of doublets, respectively.

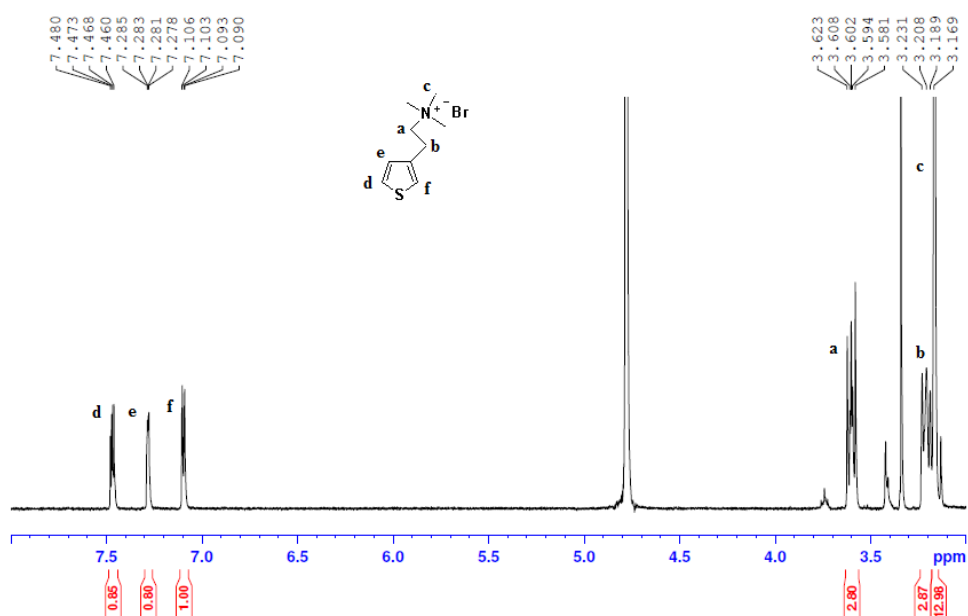


Figure 2.4: ^1H -NMR(400 MHz, D_2O , 25 $^\circ\text{C}$) spectrum of monomer 2-(thiophen-3-yl)-*N,N,N*-trimethylethanammoniumbromide (**M4**).

Chemical structure and mass to charge ratio of the monomer M4 was elucidated by electrospray ionization mass spectrometry (ESI-MS). Figure 2.5 illustrates the mass spectrum of the monomer M4. According to the spectrum, molecule with the molecular weight of 170.1 g was detected significantly. This molecular weight equals to the molecular weight of the monomer M4 which confirmed the successful amination of monomer M3.

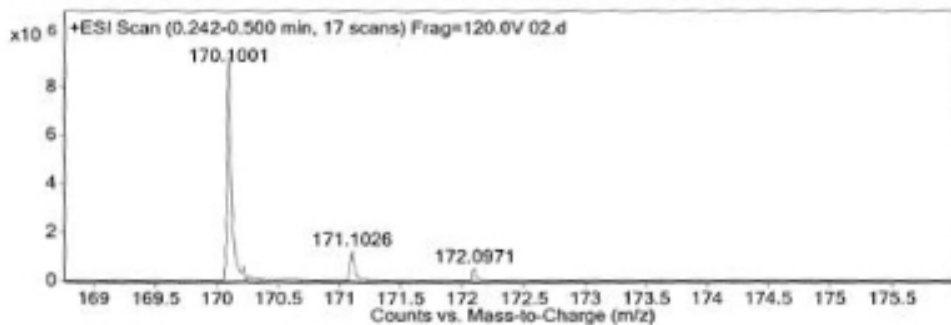
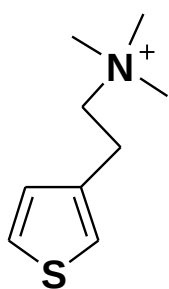


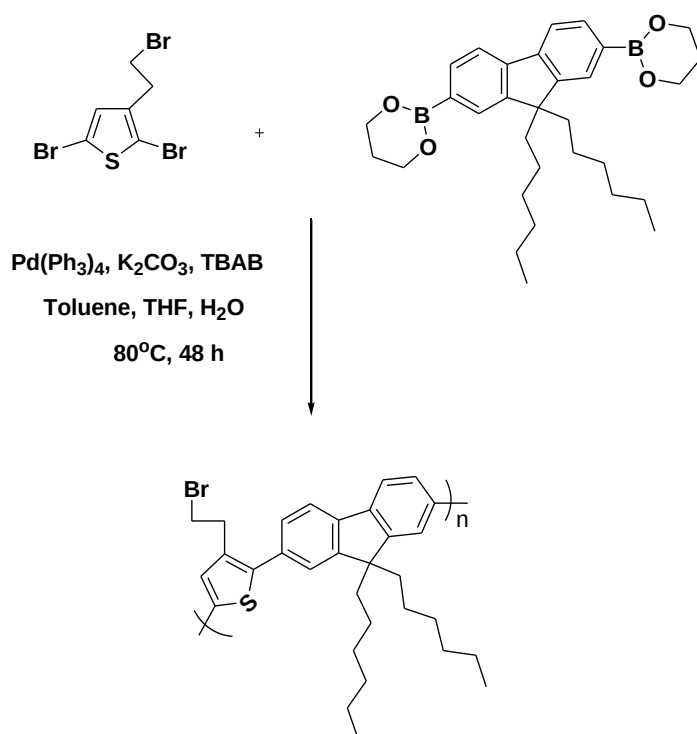
Figure 2.5: Mass spectrum of the monomer **M4**.

2.2 Synthesis and Characterization of Polymers

2.2.1 Synthesis and Characterization of poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**)

Poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**) was synthesized via Suzuki Coupling of monomer M2 with purchased boronic ester of fluorene by using $\text{Pd}(\text{Ph}_3)_4$ as a catalyst. Starting materials were dissolved in degassed toluene, water and THF. After adding TBAB, K_2CO_3 and palladium catalyst, reaction was stirred for 48 h. After the reaction was over, the mixture was extracted with CHCl_3 and the polymer solution was precipitated into excess MeOH. The product was obtained as yellow powder in 59% yield.

Synthesis of the polymer P1 was illustrated in Scheme 2.5.



Scheme 2.5: Synthesis of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**) via Suzuki coupling with $\text{Pd}(\text{Ph}_3)_4$ catalyst.

P1 was characterized by various spectroscopic techniques which are ^1H -NMR, FT-IR, UV-VIS Spectroscopy and Fluorescence Spectroscopy. Molecular weight of P1 was determined by GPC analysis.

Structure of the polymer P1 was characterized by ^1H -NMR spectroscopy. Figure 2.6 shows the ^1H -NMR spectrum of polymer P1.

According to the ^1H -NMR spectrum, methylene groups nearer to the bromine group and the thiophene were splitted into doublet at 3.53 ppm and 3.25 ppm, respectively different than the monomer M2 NMR spectrum. This could be the result of the electronic interaction of bromine and the unsaturated thiophene ring which provide a different electronic environment for the protons of methylene groups between them and resulted as a further splitting.

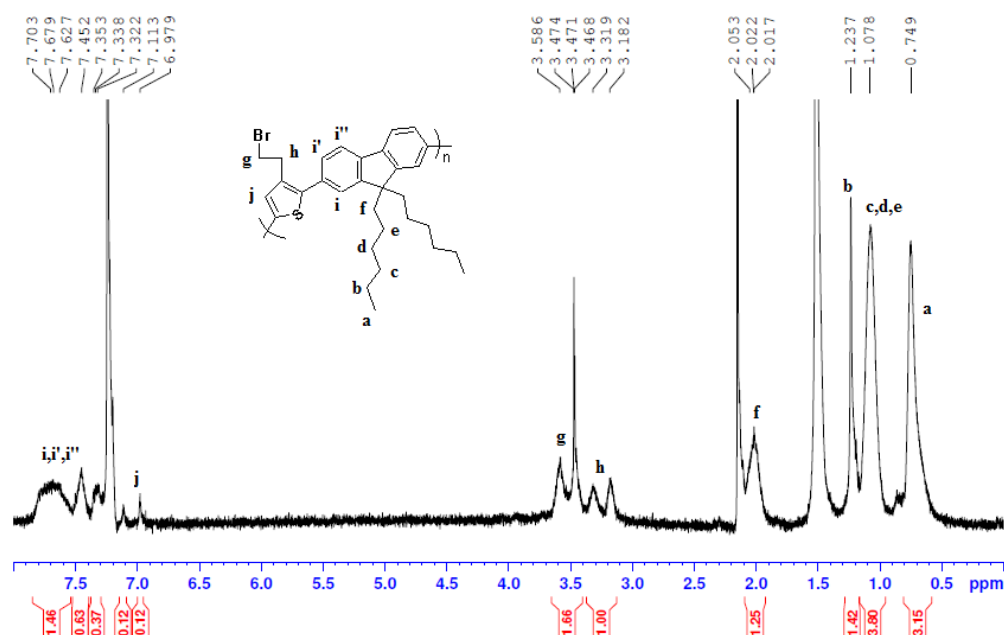


Figure 2.6: ^1H -NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$) spectrum of poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**).

Structure of P1 was also characterized by FT-IR spectroscopy. Figure 2.7 shows the FT-IR spectrum of polymer P1.

According to the FT-IR spectrum, alkyl chain stretchings of 9,9-dihexylfluorene were observed at around 2928 cm^{-1} . A strong peak at 1464 cm^{-1} was seen because of the C-C stretching of the aromatic fluorene backbone of the polymer. C-Br stretching was observed at 818 cm^{-1} .

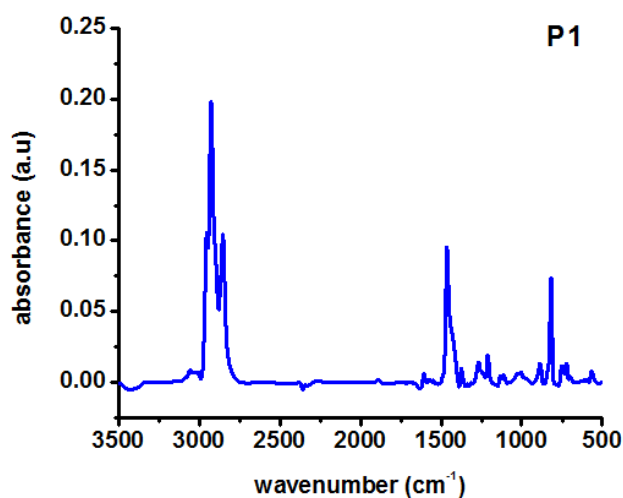


Figure 2.7: FT-IR (KBr pellet, cm^{-1}) spectrum of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**).

The optical properties of P1 were determined by UV-Vis and fluorescence spectroscopy (Figure 2.8). Absorption spectrum of P1 was obtained by dissolving P1 in THF. Absorption maxima was observed at 383 nm. Emission intensity of P1 was determined by fluorescence spectroscopy. P1 was dissolved in THF and emission spectrum was obtained. The emission maxima of P1 was observed at 471 nm.

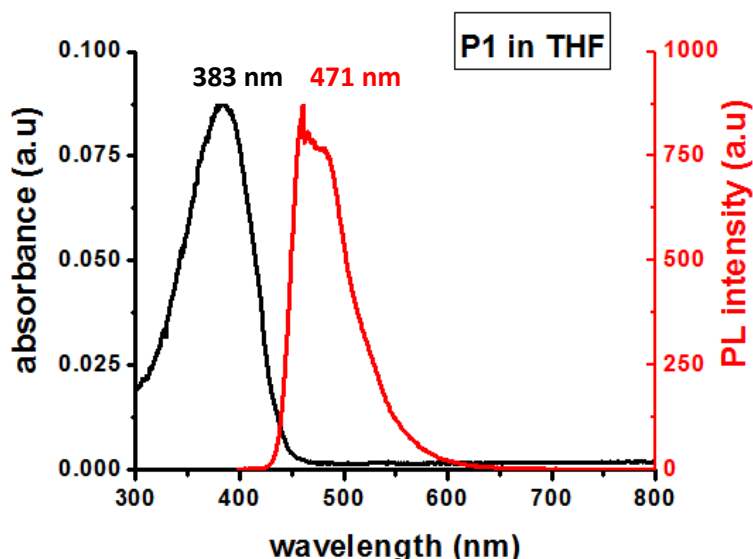
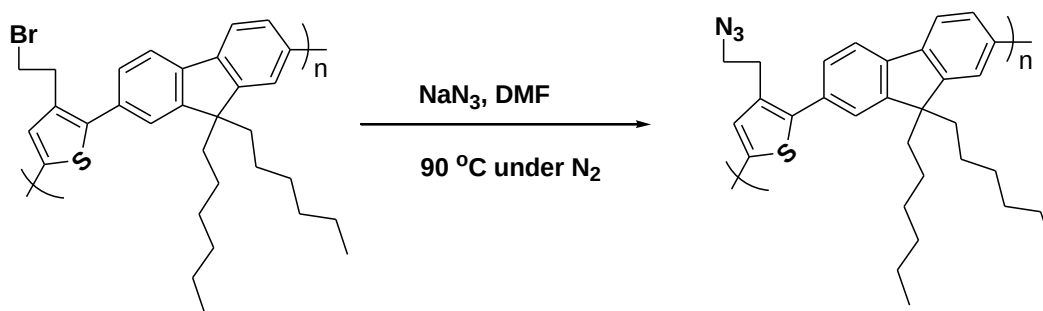


Figure 2.8: Absorption and emission spectra of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**) in THF ($\lambda_{\text{abs}} = 383 \text{ nm}$, $\lambda_{\text{exc}} = 383 \text{ nm}$, $\lambda_{\text{em}} = 471 \text{ nm}$).

2.2.2 Synthesis and Characterization of poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**)

Poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) was synthesized via conversion of $-\text{Br}$ groups of **P1** into azide groups by nucleophilic substitution reaction with sodium azide in DMF. After the reaction at 90°C , solvent was evaporated and the desired product was dissolved in THF and precipitated into water; yellow powders were obtained. The yield of the reaction was 87%.

The reaction scheme 2.6 illustrates the synthesis of polymer **P2**.



Scheme 2.6: Synthesis of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) by the functionalization of the polymer P1 with NaN_3 .

P2 was characterized by various spectroscopic techniques which are ^1H -NMR, FT-IR, UV-Vis and fluorescence spectroscopy. Molecular weight of P2 was determined by GPC analysis.

Structure of the polymer P2 was determined by ^1H -NMR spectroscopy. Figure 2.9 shows the ^1H -NMR spectrum of polymer P2.

According to the ^1H -NMR spectrum, methylene peaks at the secondary position of the azide group were shifted 0.2 ppm to upfield. This could be due to the location of the methylene protons nearer to the thiophene which is in the anisotropic cone of the azide group. These methylenic protons are located in the cone-shaped shielding zone of the azide group and shifted to the upfield.

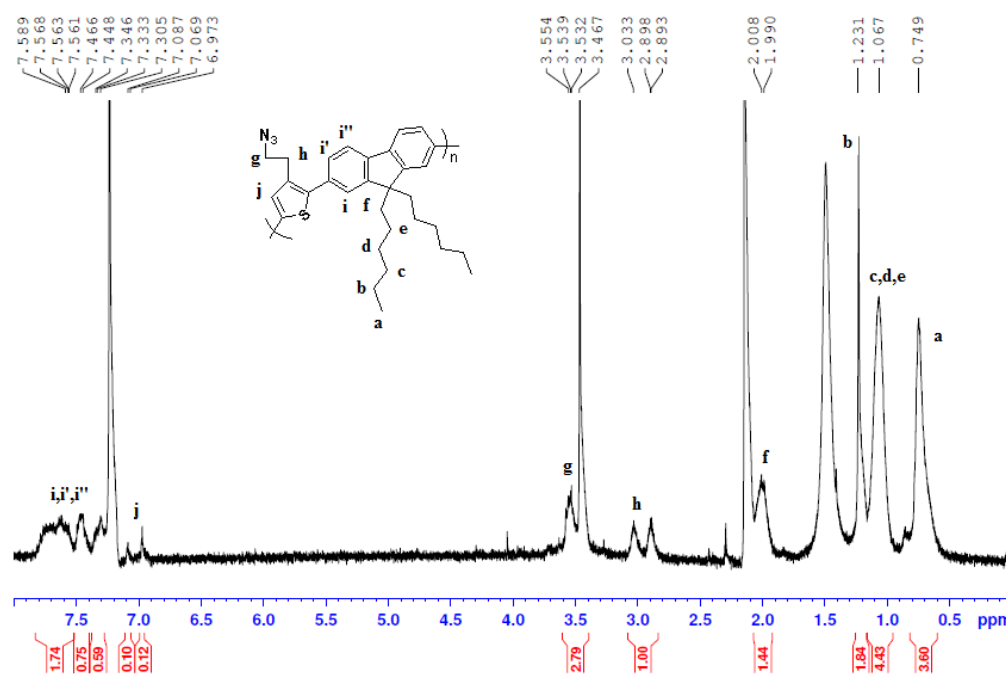


Figure 2.9: ¹H-NMR (400 MHz, CDCl₃, 25 °C) spectrum of poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**).

Figure 2.10 shows the superposed NMR spectra of the polymer P1 and P2.

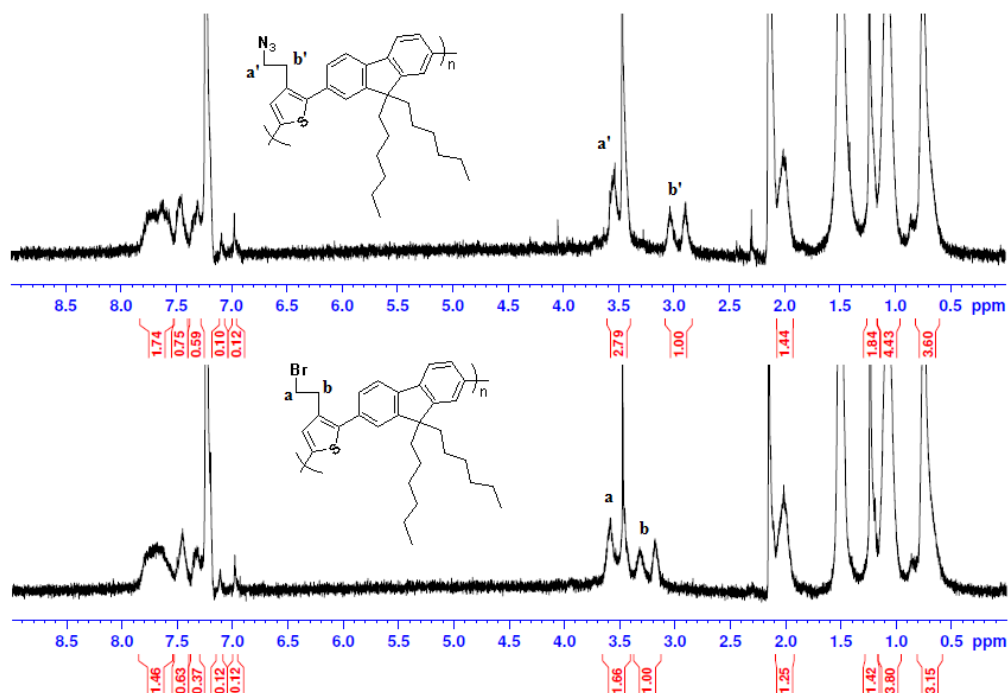


Figure 2.10: ¹H-NMR spectra of the polymer P1 and P2.

Structure of P2 was also determined by FT-IR spectroscopy. Figure 2.11 shows the FT-IR spectrum of polymer P2.

According to the FT-IR spectrum, alkyl chain stretchings of 9,9-dihexylfluorene were observed at around 2924 cm^{-1} . A strong peak at 2094 cm^{-1} indicates the characteristic azide stretching peak. The weaker peak seen at 1252 cm^{-1} is the C-H wagging of $-\text{CH}_2\text{Br}$. At 1462 cm^{-1} , C-C stretching of the aromatic fluorene was observed. C-Br stretching was observed at 818 cm^{-1} .

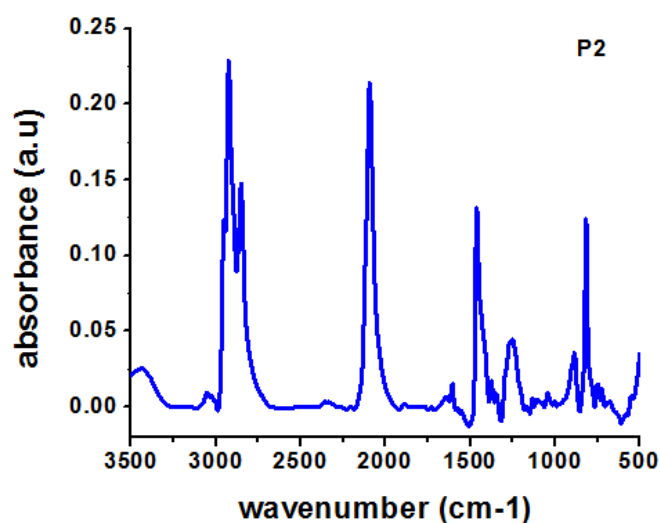


Figure 2.11: FT-IR (KBr pellet, cm^{-1}) spectrum of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] (**P2**).

Conversion of the bromine group of polymer P1 to azide group in polymer P2 was confirmed easily when the two FT-IR spectra of P1 and P2 were imbricated. A sharp azide peak of polymer P2 at 2094 cm^{-1} confirm the successful conversion of bromide to azide (Figure 2.12).

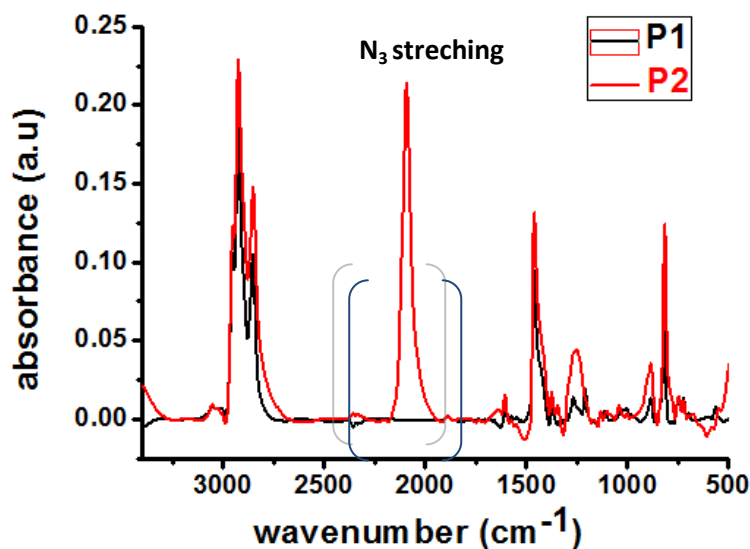


Figure 2.12: Confirmation of the successful functionalization of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene)] (**P1**) to polymer polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) by the FT-IR (KBr pellet, cm^{-1}) spectra of the polymers.

Optical properties of P2 were determined by UV-Vis and fluorescence spectroscopy. Absorption maxima of P2 was observed at 383 nm. Emission intensity of P2 was determined by fluorescence spectroscopy and emission maxima was observed at 457 nm (Figure 2.13).

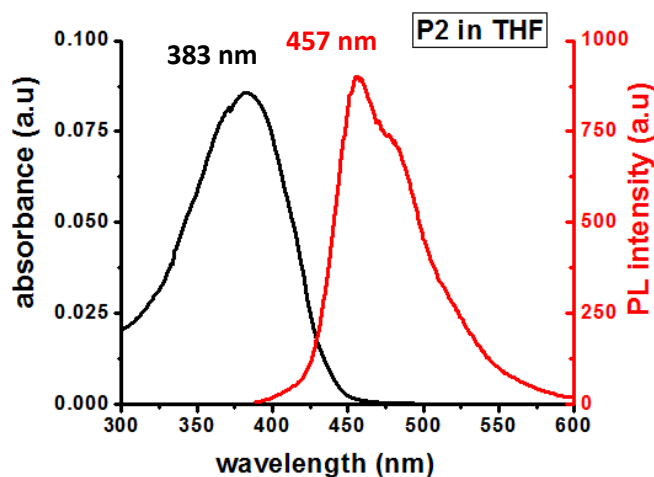


Figure 2.13: Absorption and emission spectra of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) in THF ($\lambda_{\text{abs}} = 383 \text{ nm}$, $\lambda_{\text{exc}} = 383 \text{ nm}$, $\lambda_{\text{em}} = 457 \text{ nm}$).

The fluorescence quantum yield of polymer P2 was calculated in order to determine the emission efficiency of the conjugated polymer. All measurements were done in THF. P2 is a green fluorescent polymer and fluorescein in ethanol ($\lambda_{\text{abs}} = 484 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$, $Q = 97\%$)

was used as a standard for the quantum yield calculation of P2. 83% quantum yield was calculated according to the PL intensity vs absorbance graph of polymer P2 (Figure 2.14).

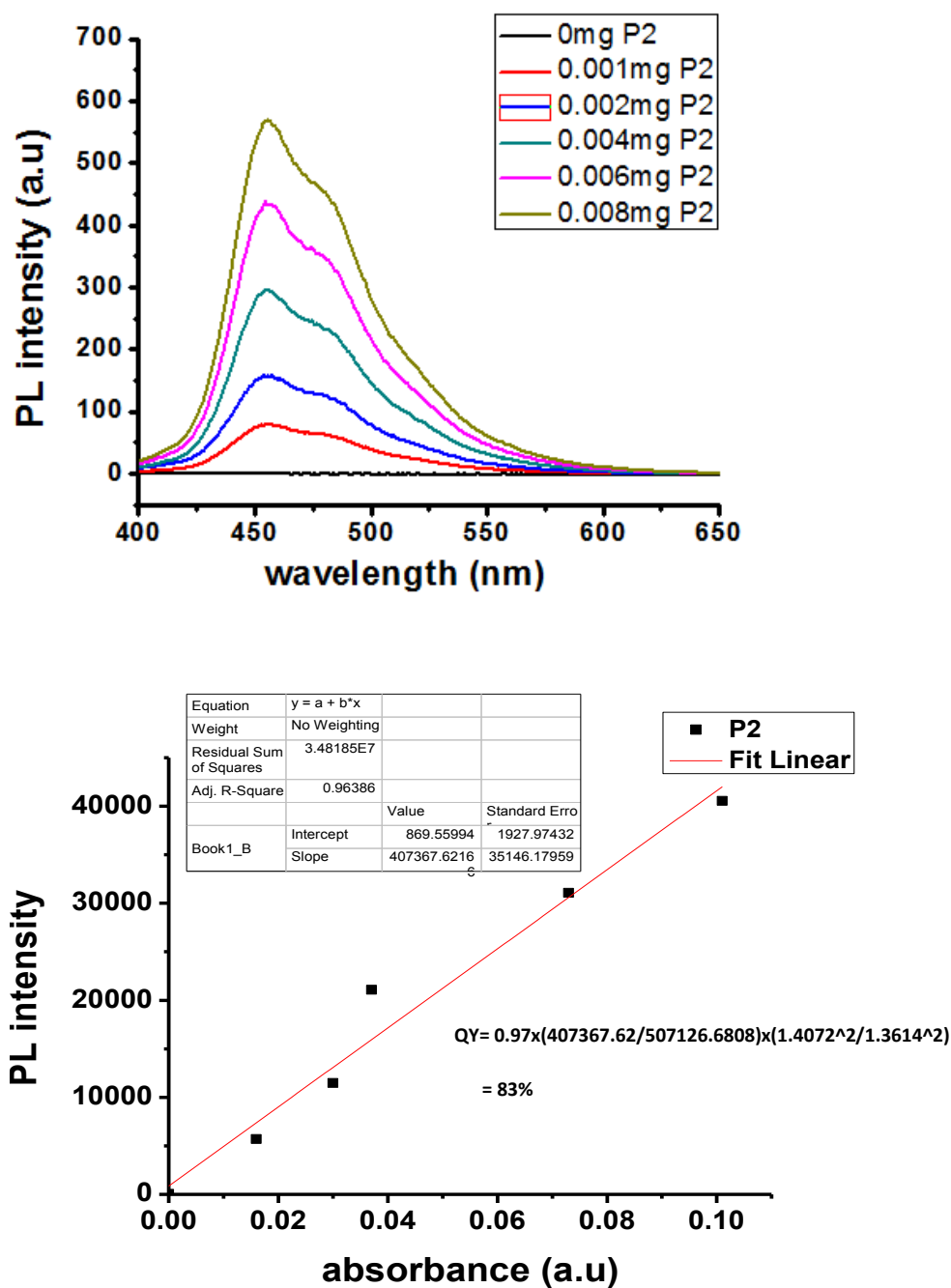


Figure 2.14: Fluorescence quantum yield of poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (P2).

UV-Vis absorption spectrum of the solvent background was recorded first which was THF. Then absorption spectra of the sample were determined at different concentrations and absorption maxima of the polymer P2 was determined. By using the absorption maxima for excitation, fluorescence spectra of the sample were recorded at different concentrations. With increasing the concentration of the sample, absorption and fluorescence spectra of polymer P2 were recorded. Finally, integrated fluorescence intensity vs absorbance graph was plotted.

Quantum yield of P2 polymer was calculated by the Equation 1.

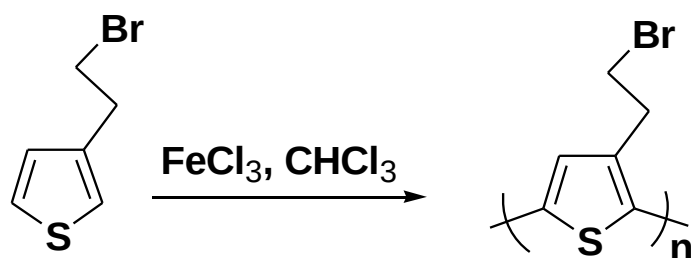
$$\Phi_X = \Phi_{ST} \left(\frac{\text{Grad}_X}{\text{Grad}_{ST}} \right) \left(\frac{\eta_X^2}{\eta_{ST}^2} \right)$$

Equation 1: In this equation, ϕ_X and ϕ_{ST} indicate the fluorescence quantum yield of the standard and test, respectively. Grad is the gradient from the plot of integrated fluorescence intensity vs absorbance. Finally, η indicates the refractive index of the solvent.

2.2.3 Synthesis of poly[3-(2-bromoethyl)thiophene] (P3)

Poly[3-(2-bromoethyl)thiophene] (**P3**) was synthesized via oxidative polymerization with FeCl_3 .⁴¹ Monomer M3 and FeCl_3 were reacted in dry CHCl_3 . After 24 h stirring under nitrogen, CHCl_3 was evaporated under reduced pressure. The product was dissolved in MeOH and the polymer solution was precipitated into MeOH by adding hydrazine. The polymer was collected as brown powder in 7% yield.

Synthesis of the polymer P3 was illustrated in **Scheme 2.7**



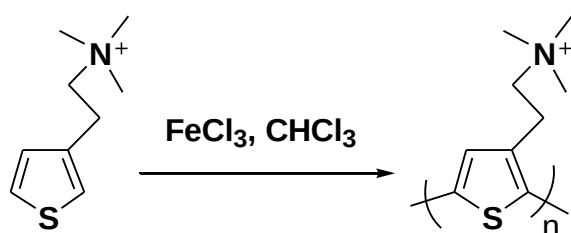
Scheme 2.7: Synthesis of the polymer poly[3-(2-bromoethyl)thiophene] (**P3**)

This polymer could not be characterized by ^1H -NMR spectroscopy due to the solubility problems. So, it could not be used for further studies.

2.2.4 Synthesis of poly[2-(thiophen-3-yl)-*N,N,N* trimethylethanammonium] (P4)

Poly[2-(thiophen-3-yl)-*N,N,N* trimethylethanamine] (**P4**) was synthesized via oxidative polymerization with FeCl_3 .⁴¹ Monomer M4 and FeCl_3 were reacted in dry CHCl_3 . After 24 h stirring under nitrogen, CHCl_3 was evaporated under reduced pressure. The product was dissolved in MeOH and the polymer solution was precipitated into MeOH by adding hydrazine. The polymer was collected as brown powder in 27% yield.

Synthesis of the polymer P4 was illustrated in Scheme 2.8.



Scheme 2.8: Synthesis of the polymer poly[2-(thiophen-3-yl)-*N,N,N* trimethylethanammonium] (**P4**).

Chemical structure and mass to charge ratio of the polymer P4 was elucidated by electrospray ionization mass spectrometry (ESI-MS). Figure 2.15 illustrates the mass spectrum of the polymer P4. According to the spectrum, molecule with the molecular weight of 421.1 g was detected significantly. This molecular weight equals to the molecular weight of the dimer form of the monomer M4. At the end of the oxidative polymerization of M4 with FeCl_3 , a dimer form of the monomer was obtained not the high molecular weight polymer.

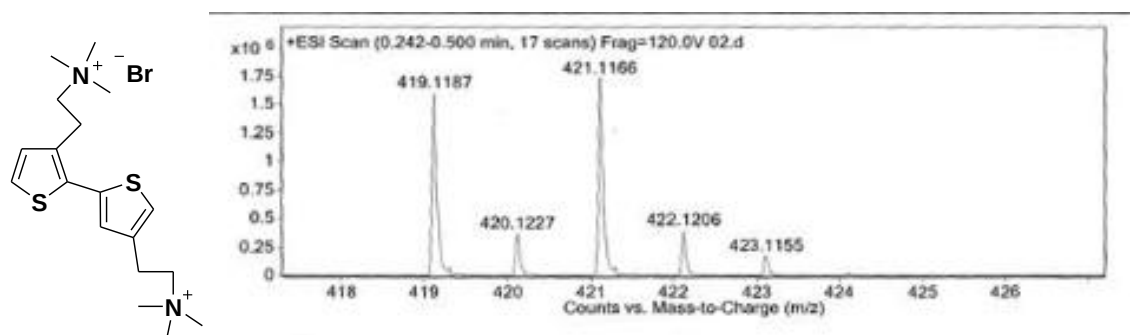


Figure 2.15: Mass spectrum of the monomer 2-(thiophen-3-yl)-*N,N,N* trimethylethanammoniumbromide (**M4**) and its dimer form.

2.3 Synthesis and Characterization of Water Dispersible Conjugated Polymer Nanoparticles.

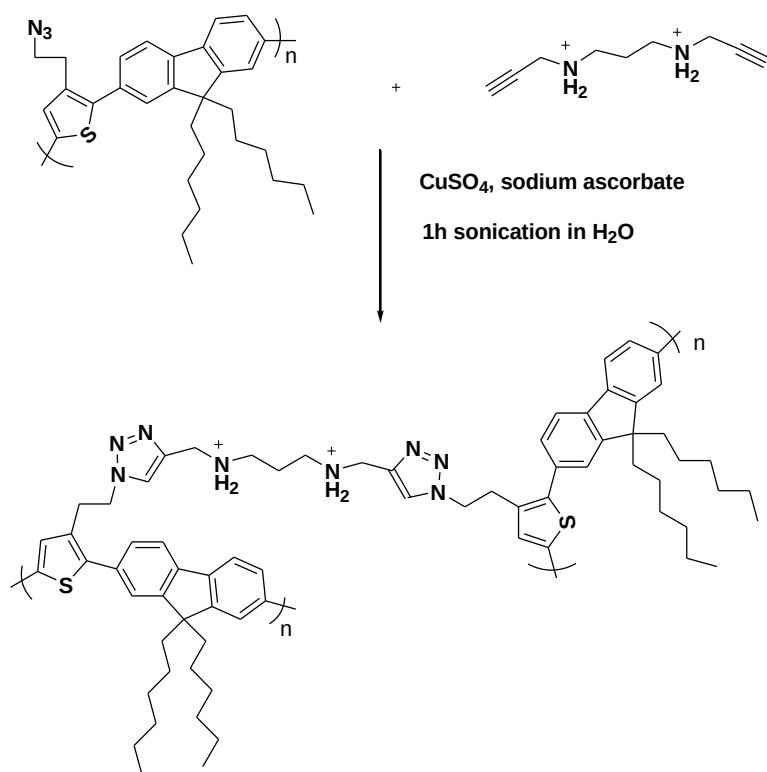
Polymer P2 based conjugated polymer nanoparticles (**CPNs**) were synthesized by the reprecipitation technique. In this technique, polymer dissolved in a good solvent which is THF is added to an excess volume of a poor solvent such as water. Mixing of the solvent with the nonsolvent causes a decrease of solvent quality. This situation is resulted with a folding of the polymer chains and formation of polymer nanoparticles. In order to be sure the nanoparticle formation, rapid mixing is done by ultrasonication. After the evaporation of THF, water-dispersible CPNs are obtained.

Stability of the CPNs were provided by the crosslinking of the azide groups of polymer P2 with diaminoalkyne crosslinker. Azide groups were also crosslinked under UV-light through the decomposition of the azide groups into nitrene and then reaction with the alkyl chains of the polymer P2.

2.3.1 Synthesis and Characterization of CPNs via Cu(I) -catalyzed Click Reaction

Water dispersible conjugated polymer nanoparticles synthesized via Cu(I)-catalyzed click reaction were synthesized by using P2 polymer and Cu(I) (CuSO_4 and sodium ascorbate) as a catalyst for click reaction. P2 was dissolved in THF which is miscible in water and injected into the Cu(I) and diaminoalkyne solution in water. After the sonication for 1h, CPNs in water synthesized via Cu(I)-catalyzed click reaction were left overnight. Then THF was evaporated under the reduced pressure and the remaining nanoparticles in water were purified via dialysis for 1 day in a cellulose membrane in order to remove unreacted diaminoalkyne and excess Cu(I).

Diaminoalkyne was used as a crosslinker and by the formation of a triazole with the alkyne group of diaminoalkyne and azide functional group of P2 polymer, crosslinked, shape-persistent conjugated polymer nanoparticles were obtained. (Scheme 2.9)



Scheme 2.9: Crosslinking of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) nanoparticles via Cu(I)-catalyzed click reaction.

The optical properties of CPNs synthesized via Cu(I)-catalyzed click reaction were determined by UV-Vis spectroscopy and fluorescence spectroscopy. Absorption maxima of synthesized nanoparticles was recorded as 391 nm and emission maxima was recorded as 511 nm (Figure 2.16).

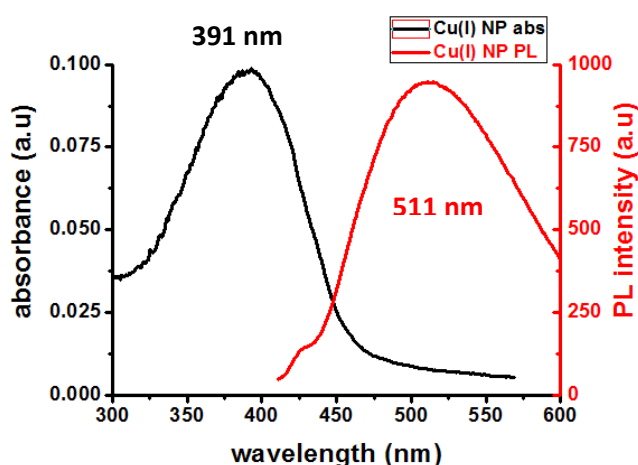


Figure 2.16: Absorption and emission spectra of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) nanoparticles in water synthesized via Cu(I)-catalyzed click reaction ($\lambda_{\text{abs}} = 391 \text{ nm}$, $\lambda_{\text{exc}} = 391 \text{ nm}$, $\lambda_{\text{em}} = 511 \text{ nm}$).

Synthesis of nanoparticles was resulted with a significant change in the emission spectrum when it was compared with the emission spectrum of P2 polymer. A distinct red-shift in the emission spectrum was observed. This red-shift is due to the chain-chain interactions of the polymer converted to nanoparticles which is the result of pi-pi stacking between the aromatic groups of the polymer in the backbone (Figure 2.17).

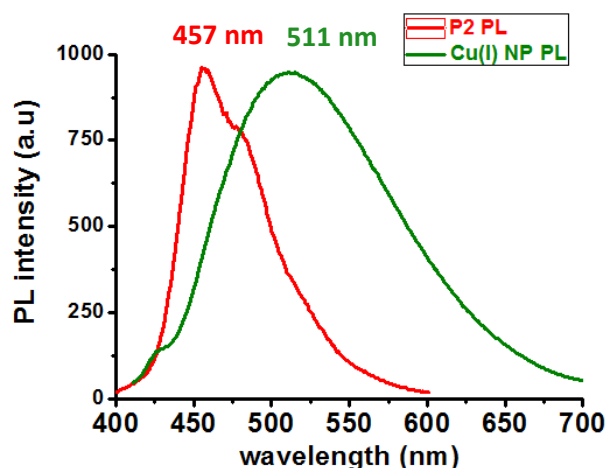


Figure 2.17: Emission spectra of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (**P2**) in THF and P2 nanoparticles in water synthesized via Cu(I)-catalyzed click reaction.

FT-IR spectroscopy was used to characterize the CPNs synthesized via Cu(I)-catalyzed click reaction in order to determine if the triazole formation was successful or not. Figure 2.18 illustrates the FT-IR spectrum of the CPNs synthesized via Cu(I)-catalyzed click reaction. The samples were prepared by dropping the nanoparticle solution in water on the silicon wafer. After drying of silicon wafers, FT-IR spectrum was recorded. In the FT-IR spectrum, the characteristic azide peak at 2094cm^{-1} should have decreased as a result of the triazole formation. At 1628 cm^{-1} C=C stretching of the triazole was observed. According to spectrum, click reaction was successful and desired triazole was formed because the azide peak of P2 polymer was disappeared in the FT-IR spectrum (Figure 2.18).

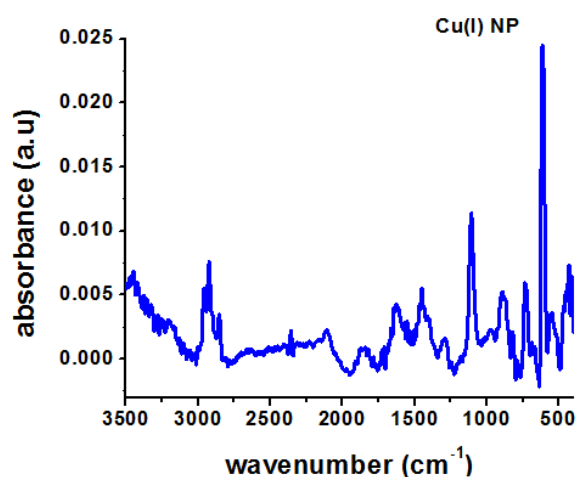


Figure 2.18: FT-IR (silicon wafer, cm^{-1}) spectrum of the CPNs synthesized via Cu(I)-catalyzed click reaction.

Figure 2.19 illustrated the superposed FT-IR spectra of the polymer P2 and the CPNs synthesized via Cu(I)-catalyzed click reaction. A sharp decrease at 2094 cm^{-1} belong to the azide peak of the polymer P2 indicated the successful triazole formation

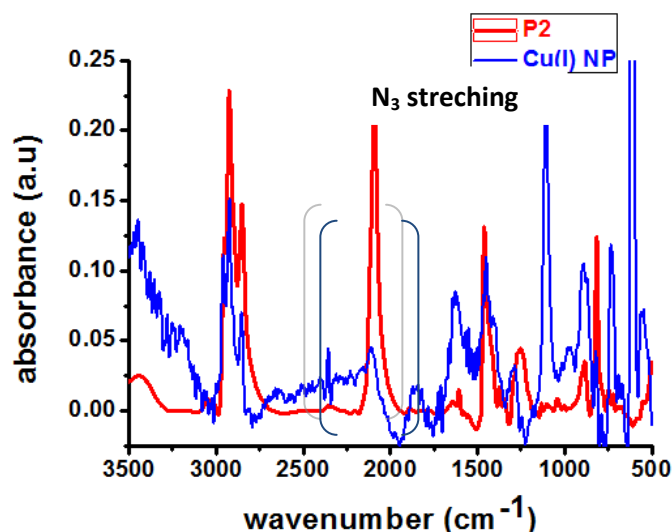


Figure 2.19: FT-IR (silicon wafer, cm^{-1}) spectrum of the CPNs synthesized via Cu(I)-catalyzed click reaction.

The hydrodynamic size of the CPNs synthesized via Cu(I)-catalyzed click reaction was determined by using dynamic light scattering (DLS). Diameter of the NPs was recorded as 72 nm (Figure 2.20).

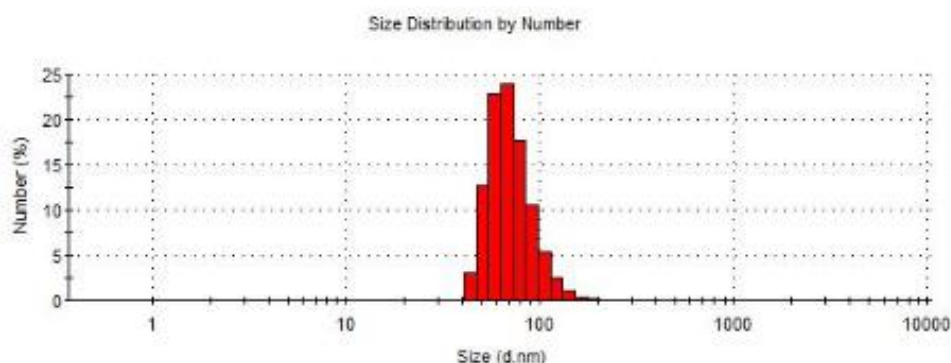


Figure 2.20: DLS data of the CPNs synthesized via Cu(I)-catalyzed click reaction.

Potential difference between the water and the stationary layer of fluid which was attached to the nanoparticle surface was determined by zeta potential. Zeta potential shows the degree of repulsion between the similarly charged particles in a dispersion. A high zeta potential indicates the stability of the nanoparticles. If the zeta potential is low, attraction exceeds the repulsion between the nanoparticles and the dispersion breaks. In this study, zeta potential was measured in order to observe the magnitude of the electrical charge at the double layer and determine the stability of the nanoparticles in water. Zeta potential of the CPNs was measured as -4.86 mV (Figure 2.21).

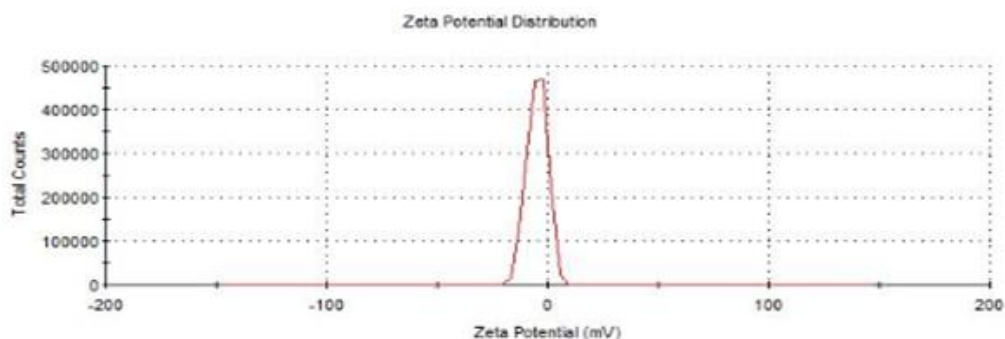


Figure 2.21: Zeta-potential of the CPNs synthesized via Cu(I)-catalyzed click reaction.

CPNs were also characterized by TEM. CPNs synthesized via Cu(I)-catalyzed click reaction were synthesized by using hydrophilic diaminoalkyne as a crosslinker and a hydrophobic polymer P2. TEM images demonstrated the centrosymmetric nanoparticle formation . (Figure 2.22).

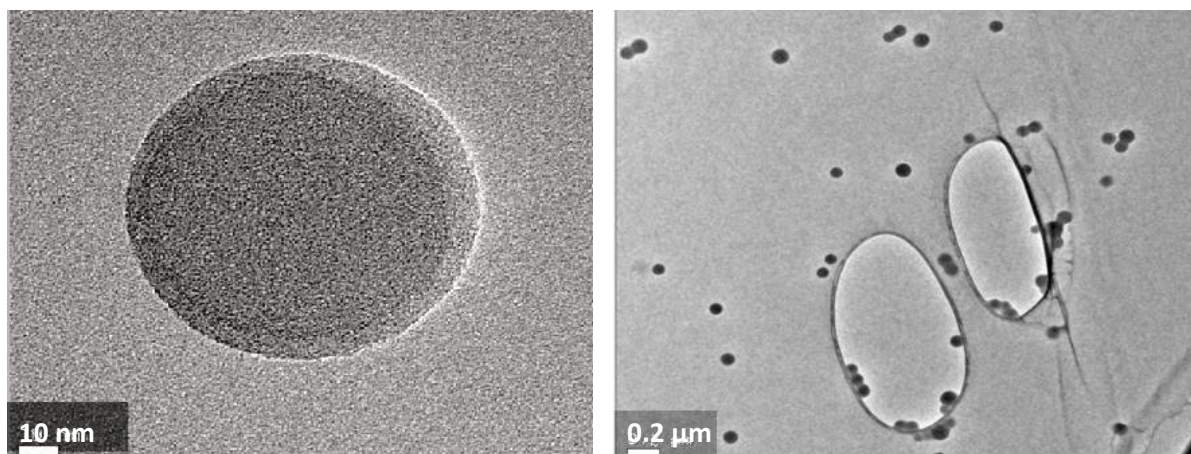


Figure 2.22: TEM images of the CPNs synthesized via Cu(I)-catalyzed click reaction.

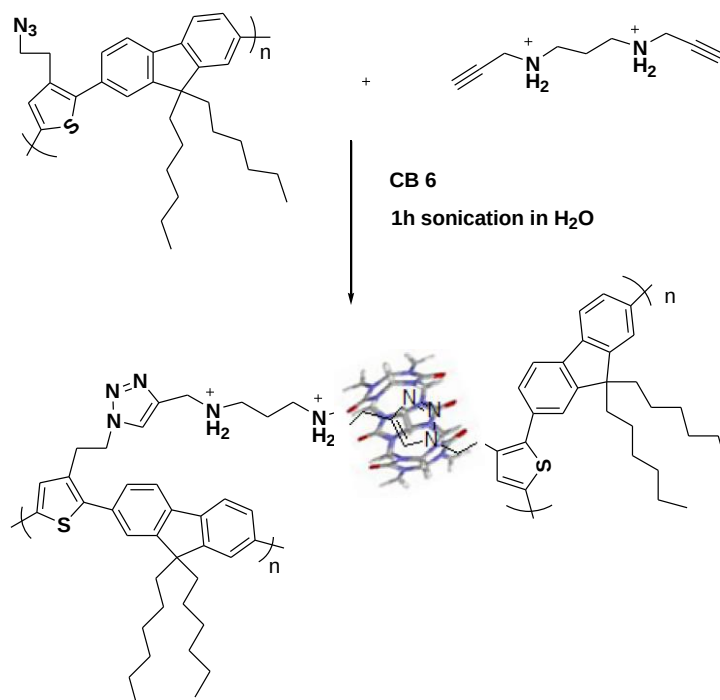
2.3.2 Synthesis and Characterization of CPNs via CB6-catalyzed Click Reaction

Water dispersible conjugated polymer nanoparticles were synthesized via CB6-catalyzed click reaction using P2 polymer. P2 was dissolved in THF which is miscible in water and the solution was injected into the CB6 and diaminoalkyne containing water. After the sonication for 1h, CPNs in water synthesized via CB6-catalyzed click reaction were left overnight. Then THF was evaporated under the reduced pressure and the remaining nanoparticles in water were purified via dialysis for 1 day in a cellulose membrane in order to remove unreacted diaminoalkyne and excess CB6.

Diaminoalkyne was used as a crosslinker for the crosslinked CPNs synthesis. When CB6 is dissolved in diaminoalkyne containing water, it encapsulates the alkyne group of diaminoalkyne and provides a hydrophobic environment for triazole formation via click reaction. CB6 has a hydrophobic cavity and provides a convenient environment for alkyne group. When the dissolved azide functionalized P2 polymer in THF is injected into the CB6 and diaminoalkyne containing water, azide groups are reacted with alkyne groups in the hydrophobic cavity of CB6. This cavity is a good guest for triazole (Scheme 2.10).

At low pH, CB6 encapsulates triazoles as a result of the ionic interaction between the carbonyl portals of CB6 and amine groups of the diaminoalkyne. By changing the pH, it is possible to change the location of CB6 because of the difference in the ionic properties of the system. When the pH increases, lower ion-dipole interaction is observed between the carbonyl

group of CB6 and amine groups of the diaminoalkyne. As a result, CB6 weakly encapsulates the triazole ring or do not encapsulates and changes the location on the crosslinker.



Scheme 2.10: Crosslinking of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] (**P2**) nanoparticles via CB6-catalyzed click reaction.

CB6-catalyzed nanoparticles were characterized by several techniques such as UV-VIS absorption spectroscopy, fluorescence spectroscopy, FT-IR, DLS, Zeta sizer and TEM.

Optical properties of CB6-catalyzed NPs were determined by UV-Vis absorption spectroscopy and fluorescence spectroscopy. Absorption maxima of synthesized nanoparticles was recorded as 392 nm and emission maxima was recorded as 494 nm (Figure 2.23).

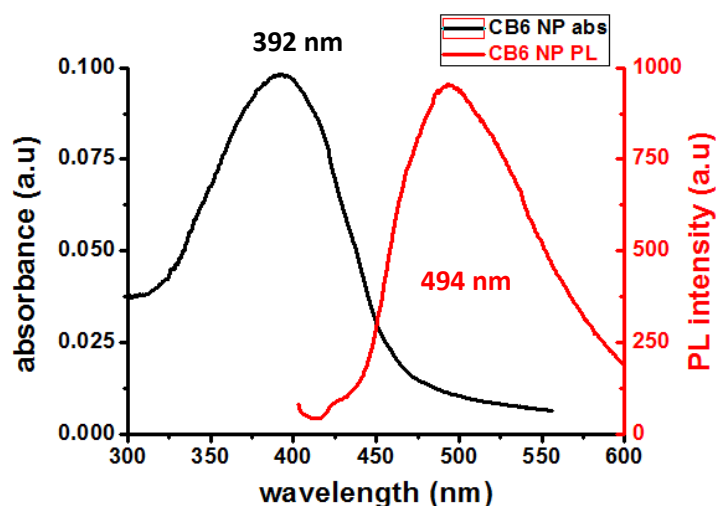


Figure 2.23: Absorption and emission spectra of polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] (**P2**) nanoparticles in water synthesized via CB6-catalyzed click reaction ($\lambda_{\text{abs}} = 392 \text{ nm}$, $\lambda_{\text{exc}} = 392 \text{ nm}$, $\lambda_{\text{em}} = 494 \text{ nm}$).

Converting P2 polymer into nanoparticles resulted in a significant change in the emission spectrum when it was compared with the emission spectrum of P2 polymer. A distinct red-shift in the emission spectrum was observed as in the emission spectrum of the CPNs synthesized via Cu(I)-catalyzed click reaction. This red-shift is due to the chain-chain interactions of the polymer converted to nanoparticles which is the result of pi-pi stacking between the aromatic groups of the polymer in the backbone. However red-shift in the emission spectrum of CPNs synthesized via Cu(I)-catalyzed click reaction is much more significant when it is compared with the emission spectrum of CPNs synthesized via CB6-catalyzed click reaction. CB6 units which act as a guest for the formed triazole after the click reaction decrease the chain-chain interactions of the aromatic groups in the polymer and cause a less significant red-shift in the emission spectrum of the CPNs (Figure 2.24).

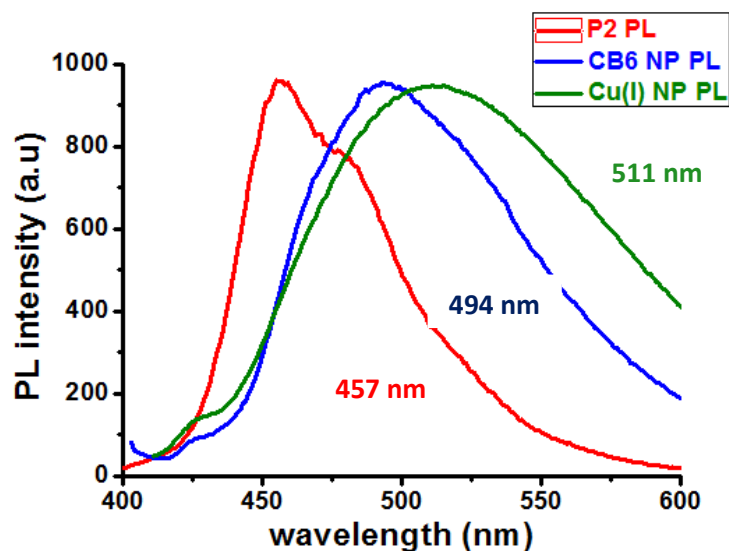


Figure 2.24: Emission spectra of the polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (P2) in THF, P2 nanoparticles in water synthesized via Cu(I)-catalyzed click reaction and P2 nanoparticles in water synthesized via CB6-catalyzed click reaction.

FT-IR spectroscopy was used to characterize the CPNs synthesized via CB6-catalyzed click reaction in order to determine if the triazole formation was successful or not. The samples were prepared by dropping the nanoparticle solution in water on the silicon wafer. After drying of silicon wafers, FT-IR spectrum was recorded. In the FT-IR spectrum, the characteristic azide peak at 2094cm^{-1} should have decreased and the characteristic carbonyl stretching band around 1730cm^{-1} should have detected because of the CB6 catalyst. According to spectrum, click reaction was successful and desired triazole was formed because the desired peaks were observed in the FT-IR spectrum. Azide peak significantly decreased and carbonyl stretching band detected in the spectrum. This FT-IR spectrum confirms the formation of a triazole and the presence of CB6 in the nanoparticle (Figure 2.25).

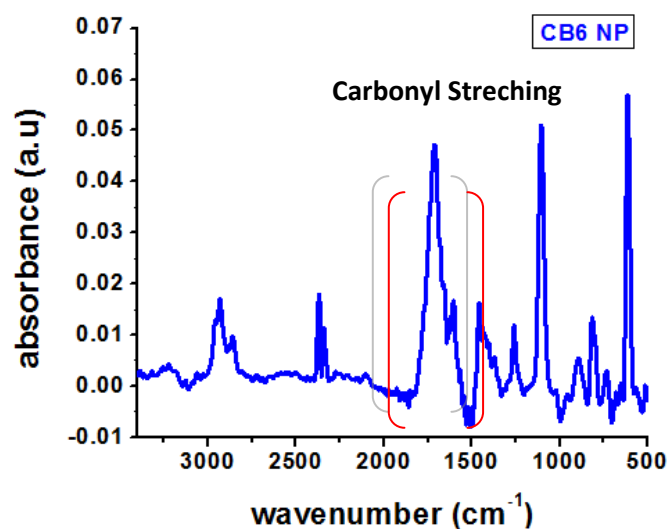


Figure 2.25: FT-IR (silicon wafer, cm⁻¹) spectrum of the polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (P2) nanoparticles synthesized via CB6-catalyzed click reaction.

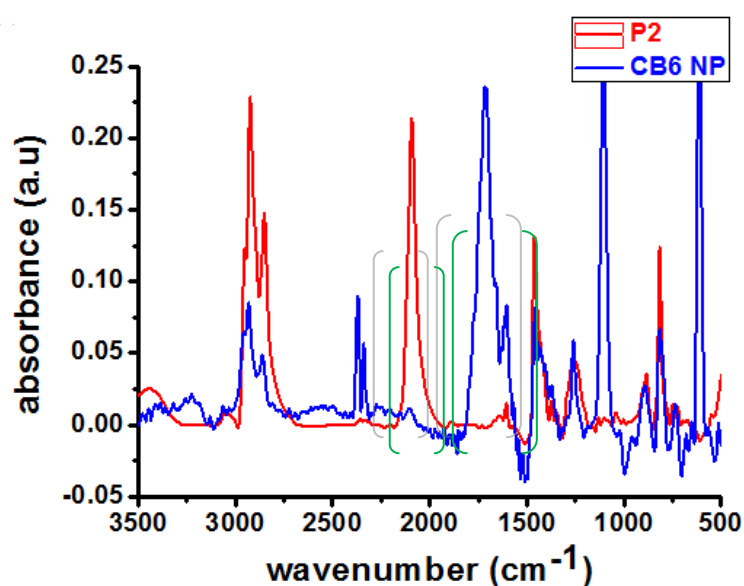


Figure 2.26: FT-IR (silicon wafer, cm⁻¹) spectra of the polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (P2) and P2 nanoparticles synthesized via CB6-catalyzed click reaction.

The hydrodynamic size of the CPNs synthesized via CB6-catalyzed click reaction was determined by using DLS. Sizes of the nanoparticles synthesized via Cu(I)-catalyzed click

reaction and CB6-catalyzed click reaction were almost the same according to DLS results. Diameter of the CB6-catalyzed nanoparticles was recorded as 71nm (Figure 2.27)

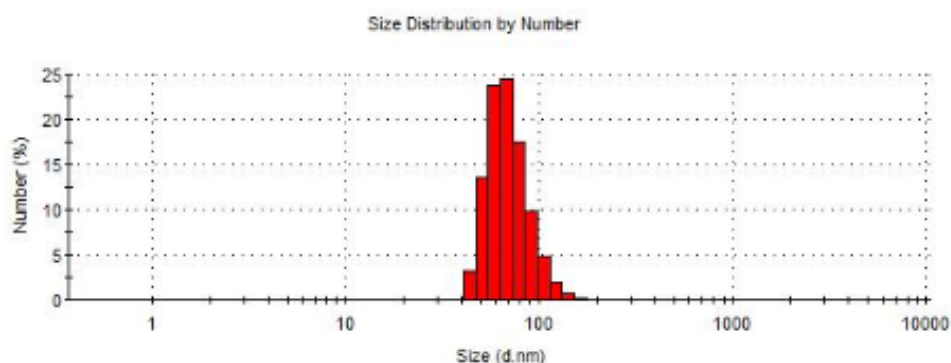


Figure 2.27: DLS data of of the polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] (**P2**) nanoparticles synthesized via CB6-catalyzed click reaction.

Potential difference between the water and the stationary layer of fluid which was attached to the nanoparticle surface was determined by zeta potential. Zeta potential of the CPNs was measured as -25.5 mV (Figure 2.28).

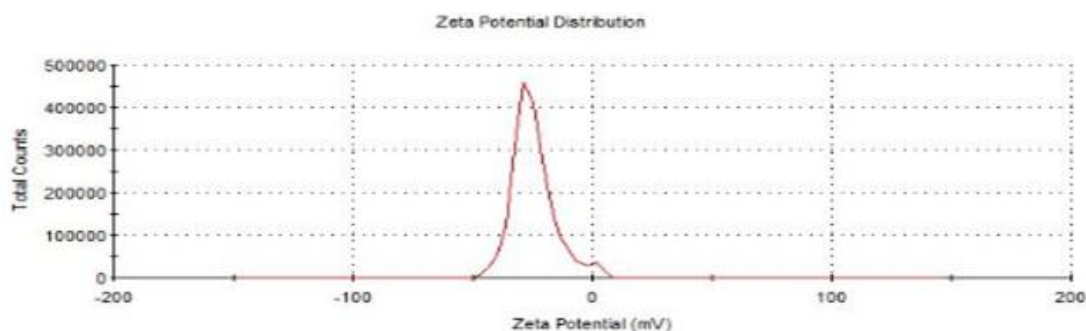


Figure 2.28: Zeta-potential of the polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] (**P2**) nanoparticles synthesized via CB6-catalyzed click reaction.

CPNs synthesized via CB6-catalyzed click reaction were also characterized by TEM. CPNs were synthesized by using hydrophilic diaminoalkyne. The click reaction between the hydropobic polymer P2 and hydrophilic crosslinker diaminoalkyne was resulted with a patchy, janus-like nanoparticles. Janus-like nanoparticles attract the variety of research areas because of their important role in theranostic nanomedicine (Figure 2.29).

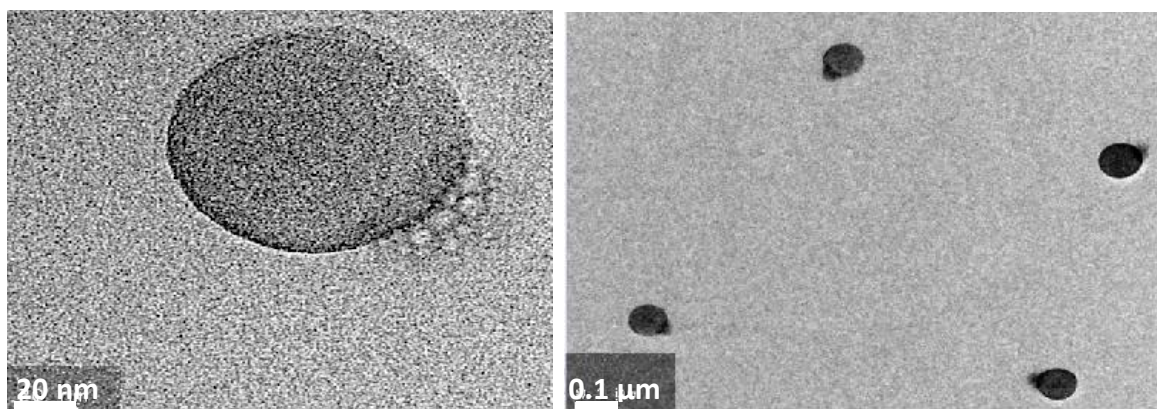
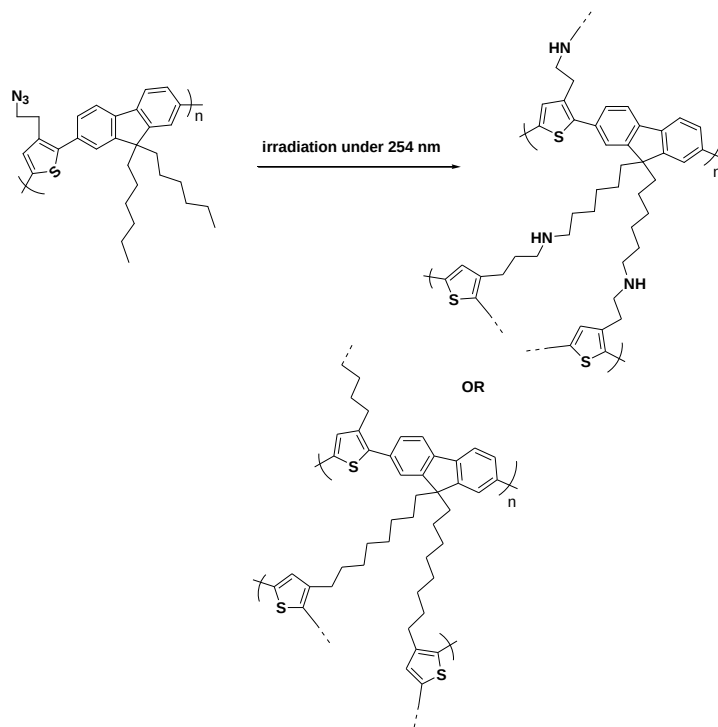


Figure 2.29: TEM images of the the polymer poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene)] (P2) nanoparticles synthesized via CB6-catalyzed click reaction.

2.3.3 Synthesis of Crosslinked P2 CPNs under UV-light

Shape-persistency and stability of the CPNs were provided by the crosslinking of the azide groups of polymer P2 with diaminoalkyne crosslinker via azide-alkyne Huisgen cycloaddition. Azide groups were also crosslinked under UV-light through the decomposition of the azide groups into nitrene species. Water dispersible polymer nanoparticles were synthesized by using P2 polymer. P2 polymer was dissolved in a good solvent which is THF and the resulting polymer solution was injected into a large excess of water. After 1h sonication, water dispersible P2 nanoparticles were obtained. The CPNs solution was left overnight and then, THF was evaporated under reduced pressure. Remaining water contained well dispersed CPNs. Then, this CPNs solution was irradiated by UV-light at 254 nm in order to achieve crosslinking. At 254 nm, azide groups of the P2 NPs were converted into nitrene groups and then these nitrene groups of the polymer were connected and crosslinked the polymer chains. After 4 h irradiation of the CPNs solution under UV-light, desired crosslinking was achieved.

Scheme 2.11 illustrates the synthesis of crosslinked P2 CPNs under UV-light.



Scheme 2.11: Synthesis of the crosslinked P2 CPNs under UV-light.

Optical properties of crosslinked P2 CPNs were determined by UV-Vis absorption spectroscopy and fluorescence spectroscopy. Absorption maxima of synthesized nanoparticles was recorded as 382 nm and emission maxima was recorded as 500 nm (Figure 2.30).

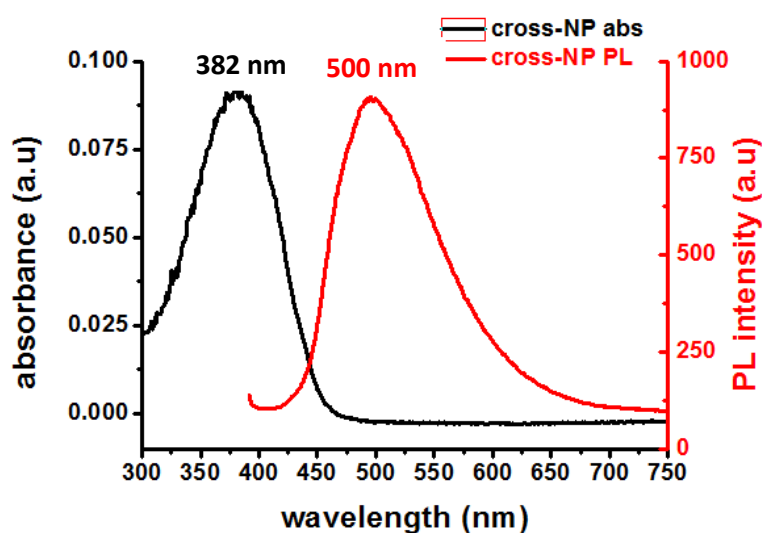


Figure 2.30: Absorption and emission spectrum of the crosslinked P2 CPNs in water under UV-light ($\lambda_{\text{abs}} = 382 \text{ nm}$, $\lambda_{\text{exc}} = 382 \text{ nm}$, $\lambda_{\text{em}} = 500 \text{ nm}$).

Crosslinking of the nanoparticles under UV-light was resulted with a red-shift in the emission spectrum because of more π - π stacking in the polymer backbone. Figure 2.31 illustrates the emission spectrum of crosslinked CPNs under UV-light.

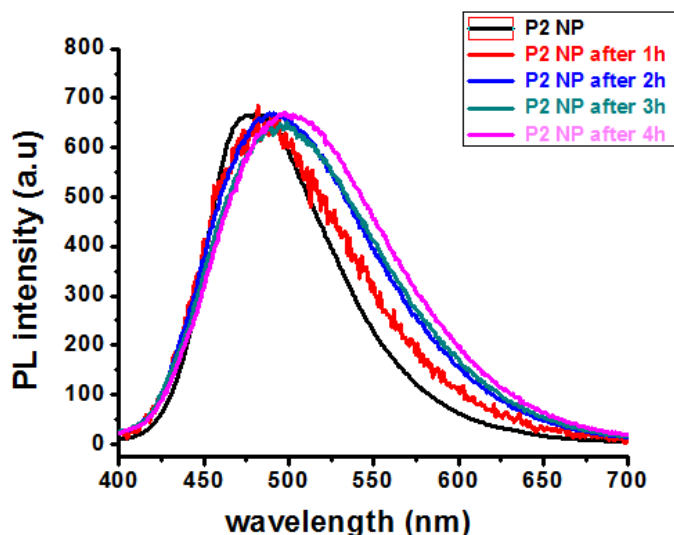


Figure 2.31: Emission spectra of the polymer P2 CPNs in water crosslinked under UV-light in 1 h, 2 h, 3 h, and 4 h, respectively.

FT-IR spectroscopy was used to characterize the crosslinked CPNs under UV-light in order to determine if the crosslinking was successful or not. The samples were prepared by dropping the nanoparticle solution in water on the silicon wafer. After drying of silicon wafers, FT-IR spectrum was recorded (Figure 2.32).

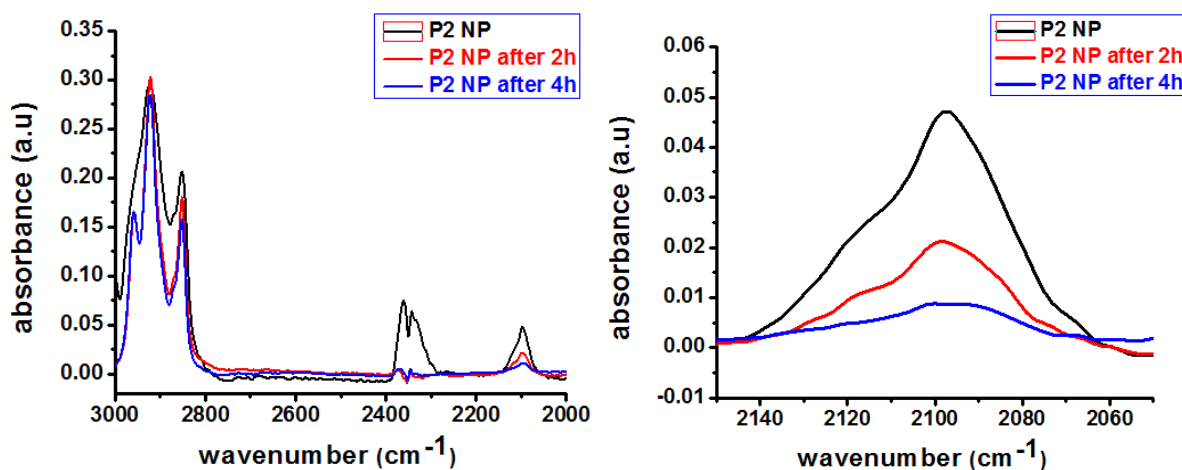


Figure 2.32: FT-IR (silicon wafer, cm^{-1}) spectrum of the polymer P2 and crosslinked P2 CPNs under UV-light.

In the FT-IR spectrum, the characteristic azide peak at 2094cm^{-1} should have decreased as a result of the crosslinking and nitrene formation. According to spectrum, crosslinking

was successful because the mentioned key peak was significantly decreased in the FT-IR spectrum. This confirms the successful crosslinking under UV-light.

The hydrodynamic size of the crosslinked CPNs under UV-light was determined by using DLS. Diameter of the nanoparticles were recorded as 34 nm (Figure 2.33).

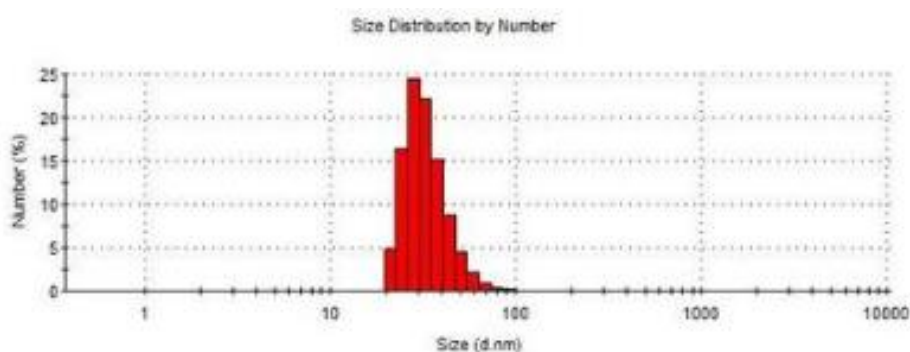


Figure 2.33: DLS data of the crosslinked CPNs under UV-light.

Potential difference between the water and the stationary layer of fluid which was attached to the nanoparticle surface was determined by zeta potential. Zeta potential of the crosslinked CPNs was measured as -33.3 mV (Figure 2.34).

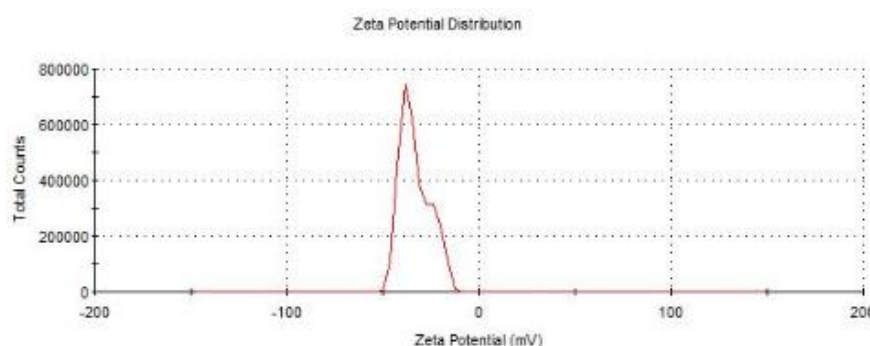


Figure 2.34: Zeta-potential of the crosslinked CPNs under UV-light.

The CPNs crosslinked under UV-light were also characterized by TEM. Spherical nanoparticles were obtained which agree with the DLS results (Figure 2.35).

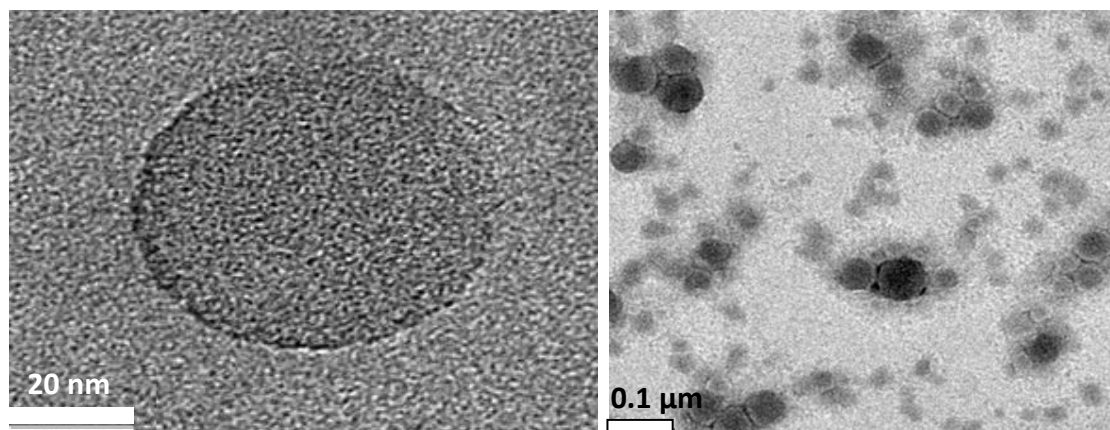


Figure 2.35: TEM images of the **crosslinked P2 CPNs under UV-light**.

CHAPTER 3

CONCLUSION

In this study, a novel synthetic method was demonstrated for the water-soluble conjugated polymer nanoparticles with enhanced chemical and mechanical stability. Crosslinked conjugated polymer nanoparticles were synthesized by a diaminoalkyne crosslinker and various useful functional groups were introduced to the nanoparticles such as triazoles and amine groups which can be used in electrostatic drug-polymer binding. They make the synthesized nanoparticles highly versatile for biological studies such as cell imaging and drug delivery in biological systems.

Incorporation of the hydrophilic functional groups to the conjugated polymer nanoparticles resulted with patchy, janus-like nanoparticles because of the distinct phase separation. They can be utilized in theranostic nanomedicine studies. In this project, CB6 was used as a catalyst for the first time in nanoparticle synthesis for 1,3-azide alkyne Huisgen cycloaddition which formed a conjugated polymer-based nanosized rotaxanes.

Crosslinking of the conjugated polymer nanoparticles was also achieved by the irradiation of the nanoparticles under UV light. Synthesized nanoparticles can be used in cell imaging and theranostic nanomedicine.

CHAPTER 4

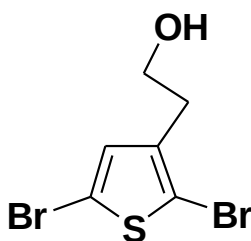
EXPERIMENTAL

Cucurbit[6]uril (CB6) and *N,N'*-di-prop-2-ynyl-propane-1,3-diamine hydrochloride were synthesized according to the literature.⁴² The reagents and solvents used in this project were purchased from Sigma-Aldrich Chemical Co. They were used as received without further purification. Purification of the monomers was established by the column chromatography with the silica gel (Kieselgel 60, 0.063-0.200 mm). Structural characterization of monomers and polymers was performed by nuclear magnetic resonance (NMR, Bruker Avance III 400 MHz spectrometer). CDCl₃ was used as a solvent in all NMR measurements. Structures of the polymers and nanoparticles were also verified by fourier transform infrared spectroscopy (FT-IR, Bruker TENSOR 27). Polymers dissolved in THF and nanoparticles in water were dropped onto the silicon wafers. The spectrum was recorded at 25 ± 1 °C in the mid-IR spectral range ($4000\text{ cm}^{-1} - 400\text{ cm}^{-1}$). 256 scans were accumulated for each sample with a resolution of 4 cm^{-1} . Molecular weights of the polymers were measured by gel permeation chromatography (GPC, Agilent 1200). THF was used as an eluent and polystyrene was used as a standard. Mass to charge ratio of the charged polymers were measured by electrospray ionization mass spectrometry (Agilent, 6224 TOF LC/MS). Optical properties of the polymers and nanoparticles were characterized by a UV-Vis spectrophotometer (Cary UV-Vis) and a fluorescence spectrophotometer (Cary Eclipse Fluorescent spectrophotometer). A xenon lamp was used as the excitation source. The sizes of the nanoparticles were determined by dynamic light scattering (DLS, Zetasizer Nano-ZS) measurements. All the measurements were carried out at 633 nm at room temperature. Laser was used as a light source during the measurements. Average particle diameters were calculated by the Marquardt method. All DLS measurements were repeated 3 times for each sample and the average values were reported. Zeta potentials of the nanoparticles were also measured with the same zetasizer (Zetasizer Nano-ZS). Morphological properties of the nanoparticles were characterized by transmission electron microscopy (TEM, FEI Tecnai G2 F30). The nanoparticle solution in water was purified via dialysis with a regenerated cellulose tubular membrane (Cellu.Sep T3, MWCO: 12 000–14 000, dry cylinder diameter: 21.0 mm, flat width: 33 mm, length: 30 m, vol cm⁻¹: 3.47 ml).

4.1 Synthesis of 2-(2,5-dibromothiophen-3-yl)ethanol (M1)

2-(3-thienyl)ethanol (2.5 g, 20 mmol) and *N*-bromosuccinimide (NBS) (7.1 g, 40 mmol) were dissolved in DMF (25 mL) as a mixture. Then it was stirred at 40 °C for 12 h under nitrogen. For the work-up, the mixture was cooled to room temperature. It was washed with diethyl ether and water. The organic layer was collected and dried over Na₂SO₄. Product was purified by silica packed column chromatography. Hexane was used as an eluent. Yield: 4.5 g, 64%

¹H-NMR (400 MHz, CDCl₃, δ): 6.81 (s, 1H), 4.19 (t, ³J = 6.8, 2H), 2.84 (t, ³J = 6.8, 2H), 2.04 (s, 1H).

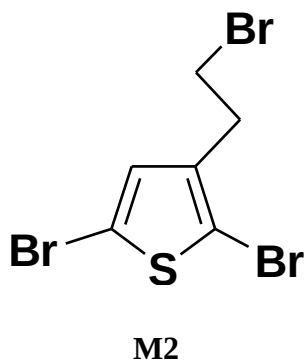


M1

4.2 Synthesis of 2,5-dibromo-3-(2-bromoethyl)thiophene (M2)

2-(2,5-dibromothiophen-3-yl)ethanol (M1) (4.5 g, 15.7 mmol) and PPh₃ (4.03 g, 15.40 mmol) were dissolved in 10mL THF as a mixture and cooled in the ice bath. To this mixture, CBr₄ (5.10 g, 15.40 mmol) in 5 mL THF was added dropwise and the reaction mixture was stirred for 12h under nitrogen. For the work-up, solvent was evaporated under reduced pressure and the remaining residue was washed with dichloromethane and water. Then organic layer was collected and washed with brine, dried over Na₂SO₄. Product was purified by silica packed column chromatography. Hexane was used as an eluent. Yield: 1.5 g, 27%

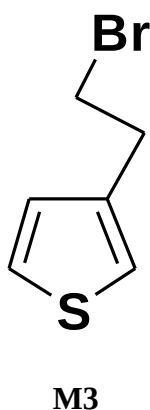
¹H-NMR (400 MHz, CDCl₃, δ): 6.84 (s, 1H), 3.47 (t, ³J = 7.2, 2H), 3.08 (t, ³J = 7.4, 2H).



4.3 Synthesis of 3-(2-bromoethyl)thiophene (M3)

2-(3-thienyl)ethanol (2.5 mL, 22.6 mmol) and PPh_3 (7.1g, 27.1 mmol) were dissolved in 10mL THF as a mixture and cooled in the ice bath. To this mixture, CBr_4 (9.0 g, 27.1 mmol) in 10 mL THF was added dropwise and the reaction mixture was stirred for 12 h under nitrogen. For the work-up, solvent was evaporated under reduced pressure and the remaining residue was washed with dichloromethane and water. Then organic layer was collected and washed with brine, dried over Na_2SO_4 . Product was purified by silica packed column chromatography. Hexane was used as an eluent. Yield: 2.3 g, 79%

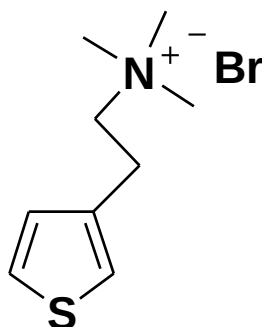
$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ): 7.27 (m, 1H), 7.05 (m, 1H), 6.96 (dd, 1H), 3.55 (t, $^3J = 7.6$, 2H), 3.19 (t, $^3J = 7.6$, 2H).



4.4 Synthesis of 2-(thiophen-3-yl)-N,N,N-trimethylethanammoniumbromide (M4)

3-(2-bromoethyl)thiophene (M3) (2.0 g, 0.01 mol) was dissolved in a minimum amount of THF (5 mL) and added to 25% trimethylamine solution in H₂O (100 mL). The resulting reaction mixture was stirred for 4 days at RT. For the work-up, the excess trimethylamine solution was evaporated under reduced pressure. Resulted precipitates were washed with hexane:acetone (1:1) mixture. Collected white precipitates were dried at 50 °C for 24h in vacuum. Yield: 1.0 g, 59%.

¹H-NMR (400 MHz, MeOD, δ): 7.47 (m, 1H), 7.28 (m, 1H), 7.10 (dd, 1H), 3.60 (m, 2H), 3.21 (m, 2H), 3.17 (s, 9H).



M4

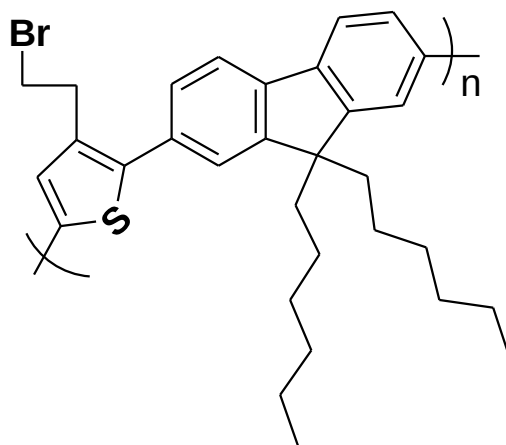
4.5 Synthesis of poly[(9,9-dihexylfluorene)-co-(2,5-(3-bromoethylthiophene) (P1)

2,5-Dibromo-3-(2-bromoethyl)thiophene M2 (500 mg, 1.43 mmol), the boronic ester of fluorene (720 mg, 1.43 mmol) and K₂CO₃ (1.88 g) were dried under vacuum for 30 min. To this mixture, degassed toluene (10 ml), water (10 ml) and THF (10 ml) were added. The reaction mixture was stirred for 10 min under nitrogen and Pd(Ph₃)₄ (17 mg, 0.0143 mmol) was added. Resulting mixture was heated to 80 °C for 3 h and then TBAB (46 mg) was added. The reaction mixture was stirred for 48 h at 80 °C under nitrogen. For the work-up, the reaction mixture was poured into a large amount of cold methanol. The precipitated product was collected by suction filtration and washed with water to remove K₂CO₃. The remaining water insoluble residues were dissolved in a minimum amount of THF and poured into cold methanol. The precipitates were collected and dried under vacuum for 6 h to yield a yellow powder. Yield: 715 mg, 59%

$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ): 7.79–7.32 (m, 6H), 6.98 (s, 1H), 3.53 (t, 2H), 3.25 (t, 2H), 2.02 (t, 4H), 1.24 (m, 8H), 1.08 (m, 8H), 0.75 (t, 6H).

GPC: $M_n = 0.25 \times 10^4 \text{ g mol}^{-1}$, $M_w = 3.8 \times 10^3 \text{ g mol}^{-1}$ (polystyrene was used as a standard).

FT-IR (KBr pellet, cm^{-1}): 2927.5 (CH_2 , s), 1463.8 (C-C, s), 817.7 (C-Br, s).



P1

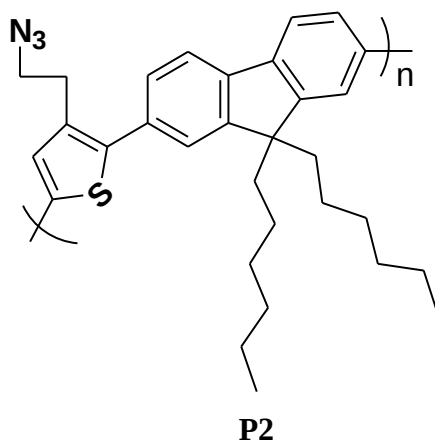
4.6 Synthesis of poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] (P2)

Poly[(9,9-dihexylfluorene)-co-(2,5-(3-azidoethylthiophene))] P1 (300 mg, 0.56 mmol) was dissolved in DMF (10 ml). Then NaN_3 (56 mg, 0.862 mmol) was added into this solution, and the mixture was stirred overnight at 90 °C. For the work-up, the solvent was removed under vacuum. The residue was dissolved in THF (10 ml). Then the solution was poured into cold water (150 mL) and precipitated. The precipitated product was collected with suction filtration and dried under vacuum to yield a yellow powder. Yield: 260 mg, 87%

$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ): 7.77–7.31 (m, 6H), 6.97 (s, 1H), 3.51 (t, 2H), 2.96 (t, 2H), 2.00 (t, 4H), 1.23 (m, 8H), 1.07 (m, 8H), 0.75 (t, 6H).

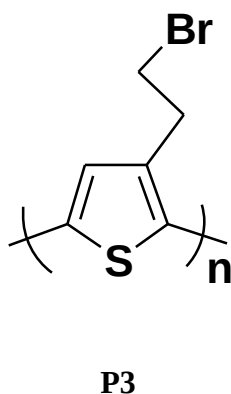
GPC: $M_n = 0.28 \times 10^4 \text{ g mol}^{-1}$, $M_w = 3.9 \times 10^3 \text{ g mol}^{-1}$ (polystyrene was used as a standard).

FT-IR (KBr pellet, cm^{-1}): 2923.7 (CH_2 , s), 2092.5 (N_3 , s), 1461.9 (C-C, s), 1251.6 (C-H, w), 815.8 (C-Br, s).



4.7 Synthesis of poly[3-(2-bromoethyl)thiophene] (P3)

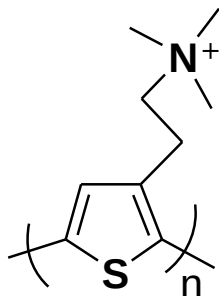
Monomer M3 (2 g, 0.01 mole) and anhydrous FeCl_3 (6.5 g, 0.04 mole) were dissolved in dry CHCl_3 (40 mL). The reaction mixture was stirred for 24 h under nitrogen at RT. For the work-up, CHCl_3 was evaporated under reduced pressure. To the crude product, MeOH was added and the product was dissolved. After the hydrazine addition to the dissolved polymer in MeOH, resulted polymer precipitates were collected. Yield: 135 mg, 7%.



4.8 Synthesis of poly[2-(thiophen-3-yl)-N,N,N trimethylethanammonium] (P4)

M4 (1.0 g, 5.9 mmol) and anhydrous FeCl_3 (3.7 g, 23 mmol) were dissolved in dry CHCl_3 (50 mL). The reaction mixture was stirred for 24 h under nitrogen at RT. For the work-up, CHCl_3 was evaporated under reduced pressure. To the crude product, MeOH was

added and the product was dissolved. After the hydrazine addition to the dissolved polymer in MeOH, resulted polymer precipitates were collected. Yield: 266 mg, 27%.

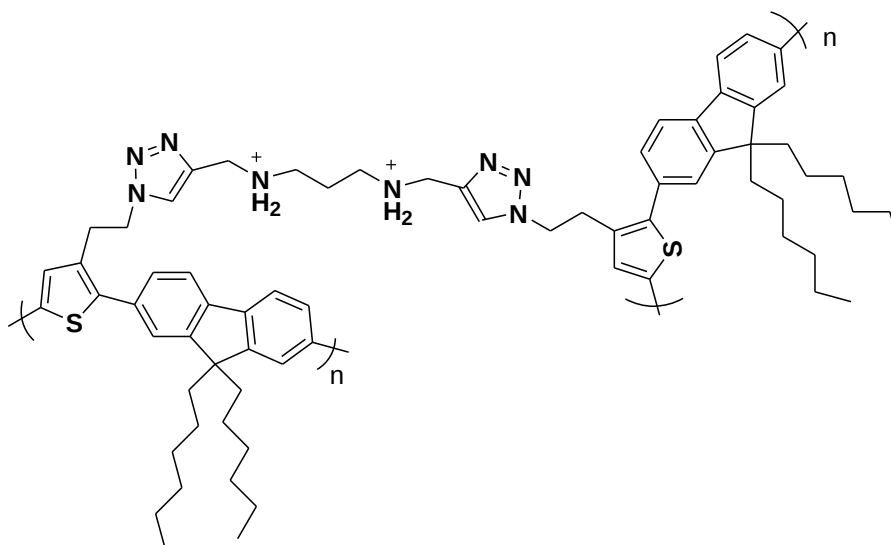


P4

4.9 Synthesis of CPNs via Cu(I)-catalyzed Click Reaction

P2 (0.01 mmol) was dissolved in THF (10 mL). Then the polymer solution was filtered through a 0.45 mm syringe filter. Crosslinker N,N'-di-prop-2-ynylpropane-1,3-diamine hydrochloride (0.005 mmol) was dissolved in water (500 ml). Then immediately CuSO₄ (5.0×10^{-5} mmol) and ascorbic acid (1.0×10^{-4} mmol) were added to this solution. The polymer solution in THF was injected rapidly into the CuSO₄ and ascorbic acid containing water solution under vigorous stirring. The resulting mixture was sonicated for 1h at room temperature. Synthesized nanoparticles were left overnight at room temperature and then THF was evaporated under reduced pressure. The remaining nanoparticle solution in water was concentrated to a total volume of 100 ml. Purification was done via dialysis for 24 h using a 14 kDa MWCO regenerated cellulose membrane to remove the catalyst and any unreacted species.

FT-IR (silicon wafer, cm⁻¹): 2925.6 (CH₂, s), 1859.1 (C=C, s), 1627.7 (C=C, s), 1450.3 (C-C, s), 1107.0 (C-N, s), 889.1 (N-H, w).

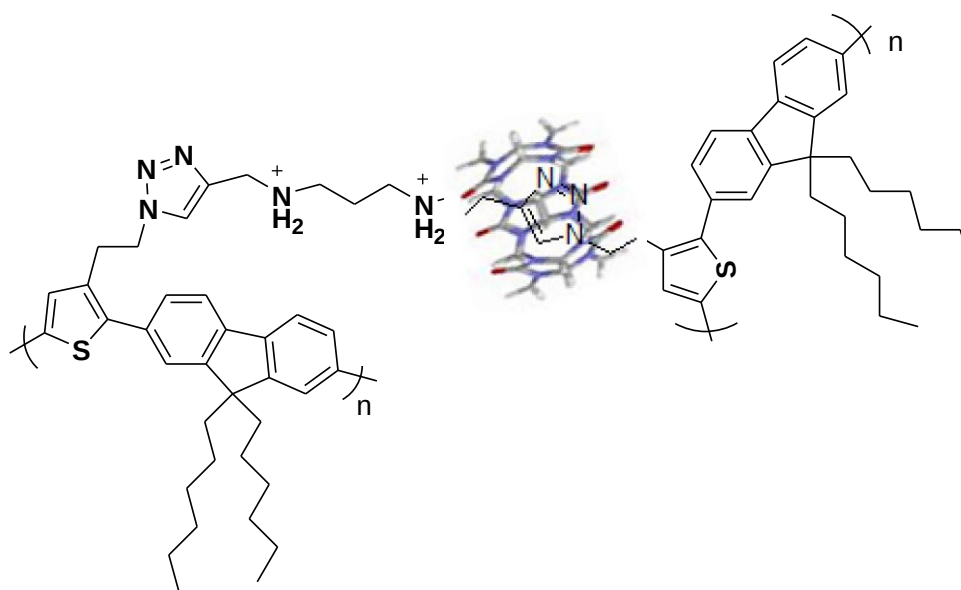


Cu(I) NP

4.10 Synthesis of CPNs via CB6-catalyzed Click Reaction

P2 (0.01 mmol) was dissolved in THF (10 ml). Then the polymer solution was filtered through a 0.45 mm syringe filter. Crosslinker N,N'-di-prop-2-ynylpropane-1,3-diamine hydrochloride (0.005 mmol) was dissolved in water (5 ml). Then immediately CB6 (0.005 mmol) was added to this solution. The mixture was stirred for 30 min to obtain a clear solution. The resulting solution was filtered through a 0.45 mm syringe filter and its volume was increased to 500 ml by adding deionized water. The polymer solution in THF was injected rapidly into the CB6 containing water solution under vigorous stirring. The resulting mixture was sonicated for 1 h at room temperature. Synthesized nanoparticles were left overnight at room temperature and then THF was removed under reduced pressure. The remaining nanoparticle solution in water was concentrated to a total volume of 100 ml. Purification was done via dialysis for 24 h using a 14 kDa MWCO regenerated cellulose membrane to remove the excess CB6 and any unreacted species.

FT-IR (silicon wafer, cm^{-1}): 2935.3 (CH_2 , s), 1716.4 (C=O , s), 1606.5 (C=C , s), 1458.0 (C-C , s), 1261.3 (C-H , w), 1107.0 (C-N , s), 815.8 (C-Br , s).

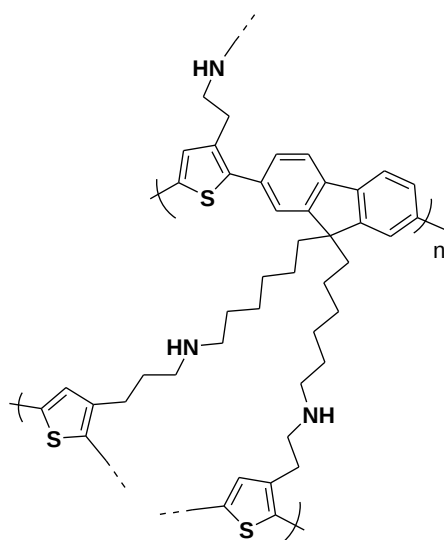


CB6 NP

4.11 Synthesis of Crosslinked P2 NPs under UV-light

P2 (0.01 mmol) was dissolved in THF (10mL). After 10 minutes sonication, P2 solution in THF was poured into the 500 mL water rapidly under vigorous stirring. The resulting mixture was sonicated 1h at room temperature. Synthesized nanoparticles were left overnight at room temperature and then THF was evaporated under reduced pressure. The remaining nanoparticle solution in water was concentrated to a total volume of 100 mL. Then nanoparticle solution was irradiated by UV-light at 256 nm for 4h. Resulting crosslinked nanoparticles were characterized by FT-IR spectrometer.

FT-IR (silicon wafer, cm^{-1}): 2921.8 (CH_2 , s), 2098.3 (N_3 , s).



Crosslinked NP

4.12 Calculation of Fluorescence Quantum Yield of P2 Polymer

Fluorescein in ethanol ($\lambda_{\text{abs}} = 484 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$, $Q = 97\%$) was used as a standard for the quantum yield calculation of P2. UV-Vis absorption spectrum of the solvent background was recorded first which was THF. Then absorption spectra of the sample were determined at different concentrations and absorption maxima of the polymer P2 was determined. By using the absorption maxima for excitation, fluorescence spectra of the sample were recorded at different concentrations. With increasing the concentration of the sample, absorption and fluorescence spectra of polymer P2 were recorded. Finally, integrated fluorescence intensity vs absorbance graph was plotted. 83% quantum yield was calculated according to the PL intensity vs absorbance graph of polymer P2.

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