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Synthetic Approaches to Flexible Fluorescent Conjugated Polymers

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

SYNTHETIC APPROACHES TO FLEXIBLE FLUORESCENT
CONJUGATED POLYMERS

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Tereza Vokatá

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Tereza Vokatá, and entitled Synthetic Approaches to Flexible Fluorescent Conjugated Polymers, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Anthony McGoron

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Date of Defense: March 19, 2015

The dissertation of Tereza Vokatá is approved.

Dean Michael R. Heithaus
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Florida International University, 2015

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DEDICATION

To all the bonds formed, may they never be broken.

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ABSTRACT OF THE DISSERTATION

SYNTHETIC APPROACHES TO FLEXIBLE FLUORESCENT CONJUGATED POLYMERS

by

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Florida International University, 2015

Miami, Florida

Professor Joong Ho Moon, Major Professor

Conjugated polymers (CPs) are intrinsically fluorescent materials that have been used for various biological applications including imaging, sensing, and delivery of biologically active substances. The synthetic control over flexibility and biodegradability of these materials aids the understanding of the structure-function relationships among the photophysical properties, the self-assembly behaviors of the corresponding conjugated polymer nanoparticles (CPNs), and the cellular behaviors of CPNs, such as toxicity, cellular uptake mechanisms, and sub-cellular localization patterns.

Synthetic approaches towards two classes of flexible CPs with well-preserved fluorescent properties are described. The synthesis of flexible poly(*p*-phenylenebutadiynylene)s (PPBs) uses competing Sonogashira and Glaser coupling reactions and the differences in monomer reactivity to incorporate a small amount (~10%) of flexible, non-conjugated linkers into the backbone. The reaction conditions provide limited control over the proportion of flexible monomer incorporation. Improved synthetic control was achieved in a series of flexible poly(*p*-phenyleneethynylene)s (PPEs) using modified Sonogashira conditions. In addition to controlling the degree of flexibility, the linker provides disruption of backbone conjugation that offers control of the length of conjugated segments within the polymer chain. Therefore, such control also results in the modulation of the photophysical properties of the materials.

CPNs fabricated from flexible PPBs are non-toxic to cells, and exhibit subcellular localization patterns clearly different from those observed with non-flexible PPE CPNs. The subcellular localization patterns of the flexible PPEs have not yet been determined, due to the toxicity of the materials, most likely related to the side-chain structure used in this series.

The study of the effect of CP flexibility on self-assembly reorganization upon polyanion complexation is presented. Owing to its high rigidity and hydrophobicity, the PPB backbone undergoes reorganization more readily than PPE. The effects are enhanced in the presence of the flexible linker, which enables more efficient π - π stacking of the aromatic backbone segments. Flexibility has minimal effects on the self-assembly of PPEs. Understanding the role of flexibility on the biophysical behaviors of CPNs is key to the successful development of novel efficient fluorescent therapeutic delivery vehicles.

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ABBREVIATIONS AND ACRONYMS

AA	acetic acid
Boc	<i>t</i> -butyl carbamate
CP	conjugated polymer
CPE	conjugated polymer electrolyte
CPN	conjugated polymer nanoparticle
DBU	1,8-diazabicycloundec-7-ene
DCM	dichloromethane
DIPA	<i>N,N</i> -diisopropylamine
dppf	1,1'-bis(diphenylphosphino)ferrocene
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EtOH	ethanol
FRET	fluorescence resonance energy transfer
FT-IR	Fourier transform infrared spectroscopy
GPC	gel permeation chromatography
GSH	glutathione
HA	hyaluronic acid
HOMO	highest occupied molecular orbital
HR-MS	high-resolution mass spectrometry
ⁱ PrOH	isopropyl alcohol
LUMO	lowest unoccupied molecular orbital
MeCN	acetonitrile
MeOH	methanol

M_n	number average molecular weight
M_w	weight average molecular weight
m/z	mass-to-charge ratio
NMR	nuclear magnetic resonance
NTA	nanoparticle tracking analysis
OD	optical density
PA	poly(acetylene)
PDI	polydispersity index
$\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$	bis(triphenylphosphine)palladium(II) dichloride
$\text{Pd}(\text{OAc})_2$	palladium(II) acetate
$\text{Pd}[(\text{PPh}_3)_4]$	tetrakis(triphenylphosphine)palladium(0)
PPB	poly(<i>p</i> -phenylenebutadiynylene)
PPE	poly(<i>p</i> -phenyleneethynylene)
PPP	poly(<i>p</i> -phenylene)
PT	poly(thiophene)
PTFE	polytetrafluoroethylene
PPV	poly(<i>p</i> -phenylenevinylene)
QY	quantum yield
R	substituent
rt	room temperature
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
UV-Vis	ultraviolet-visible

CHAPTER I. Introduction to Conjugated Polymers

1.1 General Introduction to Conjugated Polymers

Conjugated polymers (CPs) have gained much interest in the scientific community since the discovery of the unique semiconducting properties of doped polyacetylene by Shirakawa et al. in 1977.¹⁻³ Conjugated polymers possess the electronic properties of inorganic materials, such as metals and semiconductors, while also exhibiting the mechanical and processing properties of organic materials. According to a SciFinder search conducted at the end of 2014, the number of peer-reviewed articles published on the topic of “Conjugated polymers” exceeded 12,000 (Figure 1.1), and is expected to rise further as new applications for this unique class of macromolecular materials are discovered.

Figure 1.1. Number of publications featuring the term “conjugated polymers” according to a SciFinder search conducted in December of 2014.

Conjugated polymers have received the most attention as semiconducting materials for electronic applications, for example in liquid crystal displays (LCDs)⁴⁻⁶ and organic photovoltaic (OPV) devices⁷⁻⁹, and as fluorescent turn-on and turn-off sensors for the detection of various biological¹⁰⁻¹⁶ and chemical¹⁷⁻²² analytes. The main research focus of the Moon group is the use of CPs for biological applications including cellular imaging²³ and *in vitro* delivery of biologically active substances.²⁴⁻²⁶ The biophysical behaviors and cellular uptake pathways of CPs depend on their

chemical structure, the degree of aggregation, and the properties of the resulting conjugated polymer nanoparticles (CPNs). This dissertation will emphasize the synthetic aspects of developing new flexible, biodegradable CPs. These materials will aid the understanding of the effects of polymer chemical structure and flexibility on the aggregation and biophysical properties of CPNs.

1.2 Structure of Conjugated Polymers

Conjugated polymers are polyunsaturated macromolecular materials consisting of alternating single and double or triple bonds. The π -conjugation is a result of the overlap of unhybridized orbitals of the sp and sp^2 hybridized backbone carbons. The delocalized π -electrons extend along the polymer backbone, giving rise to a semiconducting band, which is responsible for the inherent fluorescence of these materials.²⁷⁻²⁸ The valence band of the semiconductor is formed from the interaction and mixing of the highest occupied molecular orbitals (HOMO) of the polymer. The empty conduction band is formed from the mixing of the lowest unoccupied molecular orbitals (LUMO). The magnitude of the HOMO-LUMO gap drives the conductive and photophysical properties of CPs.

Examples of several classes of CPs are depicted in Figure 1.2. Polythiophenes (PTs) and poly(*p*-phenylenevinylene)s (PPVs) have been used extensively for photonic applications, while poly(*p*-phenyleneethynylene)s (PPEs) emerged as materials suitable for sensing applications.²⁹ As a consequence of their low solubility and processability, poly(*p*-phenylenebutadiynylene)s (PPBs) have remained largely unexplored.

Figure 1.2. Common examples of conjugated polymers.

1.3 Photophysical Properties of Conjugated Polymers

The property that sets CPs apart from other macromolecular materials is their intrinsic fluorescence. When irradiated, CPs absorb photons of energy corresponding to the magnitude of the HOMO-LUMO band gap. Photon absorption leads to the excitation of an electron from the valence band to the conduction band, forming an exciton, i.e., an electrostatically bound electron-hole pair.³⁰ The migration of this exciton species along the conjugated backbone forms the basis of the use of CPs in photovoltaics and as fluorescence sensors. Exciton deactivation through radiative processes and non-radiative relaxation then returns the electron back to the ground state with or without the emission of a photon.

Figure 1.3. Jablonski diagram illustrating the electronic transition processes leading to fluorescence and phosphorescence.

The absorption and emission process can be illustrated using a Jablonski diagram (Figure 1.3). Upon absorption of a sufficiently energetic photon, the electron in the ground state S_0 is promoted to any of the vibrational levels of the excited state S_1 . Upon relaxation of the excited electron to the lowest vibrational level of the excited state by non-radiative decay processes, the electron can either return to the ground state via non-radiative processes with no observable light emission, or it can relax to the ground state with the emission of a photon, giving rise to luminescence. The main luminescence pathway in CPs is the direct radiative relaxation of the excited electron from S_1 to S_0 , leading to fluorescence. Alternatively, phosphorescence, which involves electron intersystem crossing and spin-forbidden electronic transitions, is a much slower process and is not considered to be a significant contributor to the photophysical properties of CPs.

The fluorescence brightness, or emission efficiency, is quantified using quantum yield (QY). Quantum yield (Φ) is defined as the ratio of the number of emitted photons relative to the photons absorbed (Eq. 1.1).

$$\Phi = \frac{\text{photons emitted}}{\text{photons absorbed}} \quad (\text{Eq. 1.1})$$

The value of experimental QY is always less than 1 (or 100%) because of the loss of some photons through non-radiative processes. For new materials, QY is typically determined relative to a highly bright standard with a known QY value.

As a result of the loss of energy through vibrational relaxation of the excited electron into the lowest level of the S_1 excited state, the emitted photon is of lower energy than the photon absorbed. This process results in the fluorescence spectrum having longer wavelengths than the corresponding absorption spectrum of the material. Further shifts towards longer wavelengths can occur through polymer aggregation, where the excited state can be delocalized over more than one polymer chain.³¹ The effect of aggregation is discussed in more detail in Section 1.5. Because of the inherent polydispersity of the polymer material, localized aggregation, and different backbone conformations, the absorption and emission spectra of CPs are generally broader than the spectra of small fluorescent molecules. This phenomenon is especially true for PPVs whose backbone allows for much more conformational flexibility compared to PPEs. The spectral features of the more rigid PPEs and PPBs are, therefore, comparatively much more defined than those of PPVs.

The absorption and emission wavelength maxima exhibit a bathochromic shift with an increasing length of the polymer chain. The increased number of π -orbitals in longer conjugated systems increases the energy of the resulting HOMO, while the energy of the LUMO decreases. The HOMO-LUMO convergence results in a decreasing energy gap with increasing conjugation length, and consequently in red-shifted emission. Because of distortions in the polymer backbone

structure, the π -electrons do not delocalize completely over the entire conjugated π -system. The HOMO-LUMO gap can, therefore, never become zero, and eventually reaches a point of saturation. The number of conjugated aromatic units (n) that are required to reach the saturation point, beyond which no further shifts of absorption maxima are observed, defines the effective conjugation length.³² The effective conjugation length varies, among other factors, with the structure of the backbone. The length has been determined to be approximately $n = 10$ for PPBs,³³ $n = 11$ for PPVs,³⁴ and $n = 12$ for PPEs.³⁵ The importance of conjugation length is investigated in detail in Chapter 4.

The polymer conjugation length is, however, not the only variable that affects the size of the HOMO-LUMO band gap. The electronic structure of the polymer backbone, its substitution, and polymer aggregation are also key contributors to the CP photophysical properties, as discussed in the following sections.

1.4 Conjugated Polymer Structural Modifications

Tailoring CPs for specific applications requires structural modifications of the conjugated backbone and the pendant side-chains in order to modulate their physical, biological, and optical properties. Among CPs, PPE and PPB conjugated backbones are the most rigid, symmetrical materials. They have relatively low conformational flexibility, sharp spectral features, and highly bright fluorescence emission properties. The work presented herein focuses on modulating the photophysical and biophysical properties of these two backbone types.

Between PPE and PPB materials, PPE is comparatively more studied. The unsubstituted PPE backbone is very hydrophobic, and therefore, exhibits poor solubility in most common solvents. Side-chain modifications provide a virtually unlimited way to improve solubility, and at the same time, introduce specificity of the material for the desired application. For biological applications, water solubility of PPEs is typically enhanced by the introduction of ionic side-chains. For

example, positive charge can be provided by amines, and negative charge is achieved through the use of carboxylates, phosphonates, or sulfonates. Water-soluble CPs bearing multiple ionic functional groups are described as conjugated polymer electrolytes (CPEs). Functional, application-specific side-chain modifications include folate-substituted PPEs for the detection of cancer cells,³⁶ or sugar-substituted PPEs for the sensing of metals, toxins, and sugar-binding proteins.³⁷ A 2009 review by Bunz provides a more detailed overview of the use of side-chain modifications of PPEs for sensing applications.³⁸

An unsubstituted or an alkyl-substituted PPE backbone exhibits blue-green fluorescence with a maximum around 430 nm.³⁹ Many research efforts have focused on producing red-shifted CPs for both electronic and biological applications through side-chain substitution and mainly through structural modifications of the polymer backbone itself. The backbone aromatic rings or the CP backbone itself can be substituted with electron-donating and electron-withdrawing groups, which influence the magnitude of the HOMO-LUMO band gap, thus affecting the photophysical properties of the polymers.⁴⁰⁻⁴⁷

1.5 Polymer Aggregation

The photophysical properties of CPs, as outlined in Section 1.3, represent an ideal polymer sample, which is dissolved in good solvent and exhibits no other interactions with its surroundings. However, for biological applications, CPs are typically fabricated into conjugated polymer nanoparticles (CPNs) by precipitating a dilute polymer solution in organic solvent into water. The self-assembly of the CP into nanoparticles is governed by two main processes, both driven by the hydrophobic nature of the CP backbone: the inter-chain aggregation and the intra-chain collapse. Among other factors, the size of the nanoparticles is determined by the competition between these two processes. When using dilute stock solutions during particle formation, inter-chain aggregation is reduced and intra-chain collapse is favored, resulting in

smaller CPN size. The size and extent of CPN aggregation are also affected by the nature of the organic stock solvent and the identity of the ionic side-chain counter-ion. For example, treatment of amine-containing cationic PPEs with various organic acids, prior to CPN formation, results in particles of different sizes dependent on the nature of the acid.⁴⁸ Conjugated polymers with ionic side chains typically form nanoparticles with a hydrodynamic diameter of around 50-100 nm.⁴⁹ Aggregation events are typically associated with changes in the material's photophysical properties. Aggregates formed in CPs through π - π stacking are often likened to small-molecule dye aggregates of the J- and H-type. The π - π stacking interaction of the chromophores, which are regarded as point dipoles, results in the splitting of their excited state into two energy levels because of the interaction of their transition dipoles. Depending on the geometry of the π - π stacking and the resulting dipole interaction,⁵⁰ the transitions to each energy level are either allowed or forbidden (Figure 1.4).

Figure 1.4. A schematic diagram illustrating the spectral shift in H- and J-type aggregates based on chromophore arrangement.

The H-type aggregates are formed by parallel, plane-to-plane π - π stacking of the neighboring chromophores. Transitions to the upper level, higher energy excited state are allowed and result in a blue shift in absorbance. The J-type aggregates are formed through a head-to-tail, end-to-end

stacking. The allowed transition to the lower energy level gives rise to a red shift.⁵¹⁻⁵² Since the transitions to the lower level excited state are forbidden in H-aggregates, any fluorescence emission, following an electron relaxation event from the higher to the lower excited level, will be weak. The H-aggregates, therefore, lead to fluorescence quenching. On the other hand, lower excited level transitions are allowed in J-aggregates and fluorescence is, therefore, conserved.⁵³

In the context of CPs, inter-chain π - π stacking leads to the formation of H-aggregates. In contrast, intra-chain interactions favor J-aggregation behavior. As a consequence of the many polymer conformations and a range of effective conjugation lengths, the photophysical processes induced by aggregation are complex and result in broad spectral features. The effect of inter-chain interactions decreases with increasing conjugation length. Polymers with short conjugation length, which favors inter-chain π - π stacking, therefore, exhibit H-aggregation behavior. The matter is further complicated by changes in effective conjugation length. For example, the strong H-type π - π interactions between aromatic rings can lead to the planarization of the polymer backbone. The resulting increased effective conjugation length gives rise to a red shift in the absorbance spectrum, despite the blue-shifting nature of the H-aggregates.⁵⁴⁻⁵⁵ Typical experimental evidence of polymer aggregation is the red-shifting of the material's photophysical properties combined with a decrease in fluorescence quantum yield. The formation of large aggregates can be reduced by minimizing the inter-chain π - π interactions through the introduction of long side-chains,⁵⁶ bulky backbone substituents,¹⁷ or addition of surfactants.⁵⁷

The aggregation structure of cationic CPs can undergo rearrangement upon polyanion complexation. For example, the Schanze research group demonstrated large changes in spectral properties of oligo(*p*-phenyleneethynylene)s when complexed with carboxymethylcellulose and carboxymethylamylose.⁵⁸⁻⁵⁹ More recently, Twomey et al. fabricated core-shell nanoparticles by treating CPNs with hyaluronic acid, showing structural changes by atomic force microscopy.⁶⁰ The extent of changes in CPN aggregation upon polyanion complexation depends on the

hydrophobic inter- and intra-chain polymer interactions, as well as the strength of interactions of the cationic CPN side-chains with the anionic residues of the polyanion. The effect of backbone connectivity and polymer flexibility on aggregation upon treatment of CPNs with polyanions is investigated in Chapter 5.

1.6 Flexibility and Biodegradability

The systematic investigation of the effect of flexibility on the behavior of CPs is at the forefront of interest because of the potential to affect a number of CPN properties, including aggregation, size and shape, cellular uptake pathways, and subcellular localization. Flexibility has been introduced into other CP backbone types such as polythiophenes and PPVs in the form of a non-conjugated, flexible hydrocarbon spacer in an effort to achieve better film processability of materials in the OPV industry,⁶¹⁻⁶² and to gain higher QYs with an increasing length of the non-conjugated spacer.⁶³⁻⁶⁴ The synthesis of polymers with a flexible component typically involves multiple steps. First, the conjugated segment of desired length is prepared, containing reactive functional groups on both ends. These reactive sites are then coupled with the flexible aliphatic spacer in a polycondensation reaction. The advantage of this approach is the synthetic control over the length of the conjugated segments. The major disadvantage is their time-consuming, low-yielding, and often also expensive multi-step preparation. An alternative one-pot synthesis involving copolymerization of two or more monomers at variable ratios provides a rapid, less expensive alternative to the incorporation of flexible units into the CP backbone at the expense of the precise control of the length of the conjugated segments.⁶⁵⁻⁶⁸ The copolymerization strategy for the synthesis of flexible PPEs is discussed in Chapter 4.

With the emergence of CPs as attractive materials for biological applications, efforts have been made to mitigate the slow polymer excretion rate and related toxicity through the introduction of stimuli-responsive biodegradable functionality. The most popular moieties used in aliphatic

synthetic polymeric carriers for the delivery of therapeutic material are esters, such as poly(lactic-co-glycolic acid) (PLGA), which undergo slow, pH-dependent hydrolytic cleavage,⁶⁹⁻⁷² and disulfide bonds, which are stable in the extracellular region and during circulation, but respond relatively fast to the reducing intracellular environment provided by glutathione (GSH).⁷³⁻⁷⁴ The 10- to 1000-fold higher localization of GSH inside the cell compared to the extracellular environment,⁷⁵ combined with the fast kinetics of the GSH-mediated thiol-disulfide exchange, make the disulfide functionality an attractive option for controlled-release intracellular delivery of therapeutic materials in vivo.

The field of biodegradable CPs remains largely unexplored. The largest obstacle to introducing biodegradable linkages directly into the conjugated backbone is the partial or complete loss of fluorescence resulting from the break in the conjugation of the aromatic segments, as discussed in previous sections. However, a few notable exceptions describe the synthesis of polymers with ester connectivity in the backbone; the Schmidt research group reported the synthesis of biodegradable, electrically conducting oligo thiophenes⁷⁶⁻⁷⁷ and the Wang group demonstrated the synthesis of water-soluble biodegradable polyfluorenes.⁷⁸ The synthesis is typically accomplished in multiple steps: first by preparing the conjugated oligomer segments functionalized on both ends, and then linking these segments in a polycondensation reaction. The use of disulfide bonds in CP backbones has not been reported thus far.

It follows that both flexibility and biodegradability are desirable properties that can be introduced into existing polymer backbones. The two characteristics are not mutually exclusive – flexible spacers, which are exemplified by aliphatic hydrocarbons, can also contain a cleavable linkage. Since the ultimate application of the new flexible polymers presented in this dissertation is for the targeted delivery of substances of biological interest into cells, the incorporation of a biodegradable moiety within the flexible aliphatic “linker” offers itself to consideration. To

utilize the fast kinetics of disulfide bond cleavage and to preserve biocompatibility to the largest extent possible, a modified L-cystine building block was selected as the flexible linker.

Figure 1.5. Chemical structures of L-cysteine and L-cystine.

The biomolecule L-cystine originates from the dimerization of the naturally occurring amino acid L-cysteine (Figure 1.5). Disulfide bonds formed by cysteine dimers give rise to the tertiary structure of proteins.⁷⁹ The acid terminus of L-cystine can be used for the attachment of ester- or amide-linked reactive sites useful for subsequent polymerization chemistry. The use of the different analogs of this cystine-based linker will be discussed in more detail throughout the following chapters concerned with the synthesis of novel biodegradable PPEs and PPBs.

1.7 Synthetic Preparation of poly(*p*-phenyleneethynylene)s

The synthesis of PPEs is typically achieved via the palladium-catalyzed Sonogashira coupling reaction between aryl halides and terminal aryl alkynes in the presence of palladium(0), a copper salt, and a base. The polymerization under these conditions proceeds in a step-growth fashion, is tolerant of a wide variety of functional groups, and results in an alternating A-B-type polymer (Scheme 1.1) with a relatively large polydispersity index. Typical sources of Pd(0) include Pd[(PPh₃)₄], Pd[Cl₂(PPh₃)₂] and Pd(OAc)₂. Copper iodide is generally used as the copper co-catalyst. A wide range of bases has been successfully utilized, although a tertiary amine, such as trimethylamine, is typically employed.⁸⁰

Scheme 1.1. A general scheme for the formation of PPEs via Sonogashira coupling.

The detailed mechanistic cycle of the Sonogashira reaction is depicted in Figure 1.6. It is believed that the Sonogashira coupling involves two cycles – the palladium-mediated coupling and the copper-catalyzed formation of the copper acetylide. In the palladium cycle, the active Pd(0) species is formed in situ from Pd(II) sources through reduction with phosphines and/or amines used as ligands, solvents, and bases in the reaction. The oxidative addition step of the aryl halide to the palladium complex is relatively fast, and is heavily influenced by the nature of the aryl halide bond. Aryl iodides and aryl triflates perform much faster than aryl bromides, and the rate can be significantly enhanced in the presence of electron-withdrawing substituents on the aromatic ring, which reduce electron density in the carbon-halide bond. The transmetallation step is believed to be the rate-determining step; it involves the transfer of the acetylide species from the copper metal to the palladium complex. Electron-withdrawing substituents on the aryl alkyne have been shown to slow down the rate of transmetallation, although the mechanism of this step is not yet fully understood.⁸¹ The acidity of the terminal alkyne proton is also thought to play an important role in the transmetallation of the resulting copper acetylide to palladium. The final step in the palladium cycle, the reductive elimination of the Sonogashira product, leads to the regeneration of the Pd(0) species, and the cycle is repeated.

Figure 1.6. Sonogashira cycle mechanism.

Since the Sonogashira reaction requires a catalytic amount of copper salt as a co-catalyst, the generation of copper acetylides can lead to the formation of acetylene dimers. These homocoupling defects in the polymer backbone are especially prominent at elevated temperatures and high copper content. Because any step-growth polymerization requires a high degree of stoichiometric balance to generate a polymer with large molecular weight, the formation of homocoupling defects, which consume only the aryl alkyne monomers, is detrimental to the synthesis of high-molecular weight polymers. Additionally, if a three-monomer system is employed for the synthesis of structurally diverse polymer materials, the differences in reactivity between structurally different aryl halide monomers lead to preferential incorporation of one monomer over the other into the polymer chain, therefore, disrupting any efforts for statistical control of monomer incorporation into the polymer.

Achievement of precise control over the conjugation length in PPEs is challenging due to the step-growth nature of the polymerization reaction. Unlike in chain-growth polymerization, where

the polymer chain length grows linearly with the addition of a monomer to the end of the polymer chain, in step-growth polymerization the reaction between the reacting species is random. A monomer can react with another monomer, or with an already formed dimer. Dimers, trimers and longer oligomers can subsequently react among themselves to produce oligomers and polymers with a broad distribution of molecular weights. Eventually, longer and longer fragments combine to form high-molecular weight polymers. Since the molecular weight growth is exponential and the incorporation of different monomers is random, the precise control of molecular weight and, therefore, conjugation length cannot be achieved. However, recently, Kang et al. demonstrated the use of controlled catalyst transfer polycondensation method for the synthesis of PPEs with controlled degree of polymerization under Sonogashira-type conditions in a chain-growth fashion.⁸² While this technique is still in its infancy, it opens up new avenues in the synthesis of flexible PPEs with precisely controlled conjugated block length through careful design of monomers and reaction conditions.

The majority of the work presented in the following chapters takes advantage of the competing reactions within the Sonogashira system. The competing rates of Sonogashira and Glaser coupling give rise to the research efforts presented in Chapter 2 for the synthesis of functional PPBs, whereas Chapter 4 takes advantage of the relative rates of the oxidative addition and transmetallation steps to produce novel PPE materials with a statistically controlled length of conjugated segments.

1.8 Synthetic Preparation of poly(*p*-phenylenebutadiynylene)s

Poly(*p*-phenylenebutadiynylene)s are typically prepared by the homocoupling reaction of terminal diacetylene monomers. The copper-catalyzed homocoupling between two terminal acetylenes is the oldest reported metal-catalyzed reaction, published by Carl Glaser in 1869.⁸³ The

reaction involves the formation of a copper acetylide species, which then dimerizes in the presence of an oxidant to produce a butadiyne product (Scheme 1.2).⁸⁴

Scheme 1.2. Original Glaser oxidative coupling reaction between two phenylacetylenes.

Since the time of the first publication, many alternative reaction conditions have been reported to improve the rate and the yield of the reaction, most notably by Hay et al., who used TMEDA as a ligand to solubilize the copper species.⁸⁵ The mechanism for this reaction was proposed by Bohlmann et al. in 1964,⁸⁶ and is depicted in Scheme 1.3.

Scheme 1.3. Mechanism of the Glaser-Hay coupling proposed by Bohlmann et al.⁸⁶

Kijima et al. reported the first use of oxidative homocoupling of acetylenes for the synthesis of CPs.⁸⁷ While the Hay coupling conditions (CuCl/TMEDA) yielded low-MW substituted poly(*p*-phenylenebutadiynylene) (PPB) polymers, modified conditions using Pd(PPh₃)₄ and CuI in the

presence of I₂ oxidant provided slightly better yields and a higher degree of polymerization.⁸⁸ Williams and Swager improved upon the palladium-catalyzed reaction conditions by using hydroquinone as a more effective oxidant, yielding PPBs with even higher molecular weight.⁸⁹ Despite the introduction of solubilizing substituents on the aromatic ring and improvement in reaction conditions, the inherent issue with the synthesis of PPBs is their limited solubility at higher molecular weights because of the rigid and hydrophobic nature of the backbone. Chapter 2 addresses this problem by introducing a small amount of flexible units along the PPB backbone, which improves solubility and leads to polymers with high molecular weight and photophysical properties similar to the fully-conjugated PPB backbone.

The downside of PPB synthesis via the Glaser-Hay coupling is the lengthy synthesis of the aryl alkyne monomers from their aryl halide precursors. The two-step conversion leads to unnecessary experimental losses associated with monomer purification. Chapter 3 explores an alternative method towards the synthesis of PPBs via the direct decarboxylative coupling of aryl halides with propiolic acid, thus eliminating the need for the conversion of the aryl halides into aryl alkynes prior to polymerization.

1.9 Polymer Characterization Techniques

Several common laboratory techniques routinely used for the characterization of polymers and nanoparticles are discussed below.

Polymer molecular weight is typically measured by *gel permeation chromatography* (GPC), which is a form of physical chromatographic separation of a mixture of components based on size. The sample is injected into a continuous stream of inert solvent and passed through a column filled with a porous resin. Sample components are eluted at different times depending on their interaction with the column – larger molecules pass through the column more quickly, while smaller molecules are retained in the pores of the column and elute more slowly. The polymer

molecular weight is determined from a calibration curve prepared from a set of standard samples, typically polystyrene. The calibration curve is a linear plot of the logarithm of molecular weight of the standard sample against the corresponding retention time, as depicted in Figure 1.7.

Figure 1.7. GPC calibration curve using a set of polystyrene standards (Mw 1,200 – 275,000 Da).

The distribution of molecular weights in a sample is characterized by the statistical treatment of the relative amount of each species (N_i) and the corresponding molecular weight (M_i). The two most common parameters used to describe the molecular weight distribution of polymers are the weight average molecular weight (M_w) (Eq. 1.2) and the number average molecular weight (M_n) (Eq. 1.3). The M_w/M_n ratio determines the polydispersity index (PDI) of the sample.

$$M_w = \frac{\sum M_i^2 N_i}{\sum M_i N_i} \quad (\text{Eq. 1.2})$$

$$M_n = \frac{\sum M_i N_i}{\sum N_i} \quad (\text{Eq. 1.3})$$

The photophysical properties of CPs are measured using *absorption* and *fluorescence spectroscopy*. The principles of fluorescence spectroscopy are described in Section 1.3. UV-

Visible absorption spectroscopy measures the absorption of radiation by a sample in the approximate range of 200-800 nm. A UV-Vis spectrum is acquired by sequentially irradiating the sample in a cuvette with light of a range of wavelengths and measuring the amount of light that passes through the sample. The absorbance value is defined as the logarithm of the ratio of the incident power of radiation (P_0) to the radiant power transmitted by the sample (P) (Figure 1.8).

Figure 1.8. A schematic representation of the measurement of UV-Vis absorbance.

According to the Beer-Lambert law (Eq. 1.4), the sample absorbance is directly proportional to the path length (l , i.e., length of the cuvette), sample concentration (c), and molar absorptivity (ϵ , i.e., intrinsic ability of sample to absorb light). As a consequence of this direct relationship, the concentration of an unknown sample can be determined from a calibration curve relating the absorbance to the concentration of standard samples.

$$A = \epsilon lc \quad (\text{Eq. 1.4})$$

The average chemical structure of a polymer sample can be ascertained by *nuclear magnetic resonance* (NMR) spectroscopy. The technique is based on the changes of magnetic moment of magnetically active nuclei, such as ^1H , ^{13}C or ^{19}F , upon exposure of the sample to a short, broad pulse of radio waves. The frequency of the energy emitted upon relaxation of the excited nuclei is translated into a spectrum of signals, which are dependent on the applied magnetic field and the

magnetic effect of nearby electrons and nuclei. The area under each spectral peak is proportional to the number of nuclei giving rise to that signal. The splitting pattern (i.e., peak shape) is indicative of the presence and nature of the neighboring nuclei. Therefore, the analysis of polymer NMR spectra can give quantitative information about the relative proportion of each monomer in the polymer chain. However, the technique only provides information about the average sample, and thus cannot provide accurate information about specific sections of a polymer chain.

The presence of numerous functional groups in a polymer sample can be detected using *Fourier transform infrared spectroscopy* (FTIR). The detection principle behind this technique is quite similar to UV absorption spectroscopy in that when the sample is irradiated, it absorbs some of the incoming energy, and the remaining transmitted radiation is recorded by a detector. The intensity of the radiation is low, with wavelengths in the infra-red region (typically 2,500 – 20,000 nm). The supplied energy is sufficient to cause vibrational excitation of covalent bonds present in most organic molecules. The absorption region of certain bonds, for example C=O, C=C, O-H, N-H etc., is characteristic of the given functional group, and can, therefore, be used as a confirmatory tool for the presence of specific monomers in a polymer sample, albeit typically not in a quantitative fashion.

Dynamic light scattering (DLS) is used for the characterization of particle size. When a particle in the sample is irradiated with a laser beam, it scatters light in all directions. The scattering is randomized as a result of the Brownian motion of the particle. The scattered light undergoes either constructive or destructive interference with light scattered from neighboring particles. The fluctuation of the scattered light is monitored over time and correlated to the diffusion coefficient of the particles in the solvent. As large particles move slowly, the light fluctuation pattern is also changing slowly. Smaller particles move more quickly, resulting in faster changes in the light pattern. The speed of the Brownian motion of the particles can be described by the diffusion

coefficient, which, in turn, is related to the particle's hydrodynamic radius through the Stokes-Einstein equation (Eq. 1.5),

$$r_h = \frac{kT}{6\pi\eta D} \quad (\text{Eq. 1.5})$$

where r_h is the hydrodynamic radius of the particle, k is the Boltzmann constant, T is temperature in Kelvin, η is the solvent viscosity, and D is the diffusion constant. The downside of using DLS for the determination of the distribution of particle sizes in a polydisperse polymer sample is that the intensity of the scattered light is not the same for all particles. Since larger particles have a larger cross-section, they allow more light to be scattered. The intensity is proportional to the sixth power of the particle diameter, which leads to the overestimation of the contribution of larger particles in a polydispersed sample, thereby skewing the overall sample distribution.⁹⁰

Single particle light scattering analysis, also known as *nanoparticle tracking analysis* (NTA), is an alternative way to measure particle size. In NTA the sample is irradiated with a laser and the scattered light is detected with a CCD camera mounted on a microscope. The scattering from the sample is recorded over time. The software then identifies each individual particle and tracks its motion throughout the duration of the recorded video. The measured particle displacement is a function of Brownian motion, which is related to the particle size through the Stokes-Einstein equation as shown previously (Eq. 1.2). Compared to DLS, NTA gives a more accurate representation of the particle size distribution, because due to the direct detection of each nanoparticle, the calculation which involves the Rayleigh scattering factor favoring larger particles is eliminated.

1.10 Biological Applications of Conjugated Polymers

PPE-based CPNs undergo cellular uptake *in vitro*,²³ exhibit high photostability with little or no toxicity, and can be used as delivery vehicles for targeted gene knockdown using small

interfering RNA.²⁶ The side-chain and backbone chemical structure of the polymers affect their cellular uptake mechanism and sub-cellular localization,⁹¹⁻⁹² and more comprehensive studies to determine the structure-activity relationships are under way.

The ultimate goal of the use of CPs in the Moon research group is the targeted delivery of compounds of biological importance into cancer cells. A successful drug and/or gene delivery agent needs to exhibit, among other characteristics, excellent water solubility, high cellular uptake, a rapid response to local environments to ensure intracellular release of therapeutic material upon cellular entry, and rapid metabolic clearance.⁹³ The successful use of CPNs in *in vivo* systems starts with the design of the chemical structure of the polymer. This dissertation does not attempt to describe or investigate the biological behaviors of CPs, but rather it is concerned with the development of new synthetic approaches towards flexible CPs. With those requirements in mind, the synthetic methodology employs monomers with carefully thought out structural design, allowing for the investigation of the CP biological function in the future.

1.11 Summary

The work presented herein is concerned with the structural modifications of PPEs and PPBs for biological applications. Chapter 2 reports the synthetic methodology towards flexible, soluble PPB-type polymers with unique aggregation behaviors upon polyanion complexation, and cellular uptake pathways clearly distinct from the uptake mechanism of conventional PPEs. Chapter 3 complements Chapter 2 by describing a shorter one-pot synthesis of PPB-type polymers from simpler monomer precursors. Chapter 4 describes a synthetic methodology towards improved control of the amount of flexibility along the CP backbone, and offers a systematic investigation of the effect of flexibility on the biophysical properties of PPEs. Finally, Chapter 5 uses the new PPE and PPB polymer materials, both with and without the flexible

component, to demonstrate the effect of backbone nature and flexibility on aggregation properties of CPNs.

1.12 References

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CHAPTER II

Synthesis of Phenyleneethynylene-doped Poly(p-phenylenebutadiynylene)s for Live Cell Imaging

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2.1 Abstract

A new synthetic approach to high molecular weight poly(*p*-phenylenebutadiynylene)s (PPBs) was developed by increasing backbone flexibility. The introduction of a small amount of flexible units along the backbone improved both the physical and photophysical properties of the polymers. These materials were successfully fabricated into conjugated polymer nanoparticles (CPNs) and used for fluorescent live cell imaging for the first time.

2.2 Introduction

Conjugated polymers (CPs) are intrinsically fluorescent materials exhibiting the excellent photophysical properties (i.e., high brightness and photostability) necessary for various biological imaging,¹⁻³ sensing,⁴⁻¹¹ and delivery¹² applications. The high extinction coefficient, fluorescent quantum yields (QYs), and facile synthetic versatility of CPs make them promising materials for various biological applications. Recently, CPs with water-soluble side chains¹³⁻¹⁴ and hydrophobic CPs blended with amphiphilic polymers have been used as immunofluorescence labels for live-cell imaging.¹⁵⁻¹⁶ Protein detection has also been demonstrated with hybrid gold nanoparticle-CP conjugates.¹⁷ Using weakly positively charged CPs, small interfering RNA (siRNA) delivery and target gene knockdown was also demonstrated.^{12, 18}

Among CPs, poly(*p*-phenylenebutadiynylene)s (PPBs) are relatively less studied and used for biological applications, even though their synthesis is generally less sensitive to reaction environments. The resulting PPBs exhibit similar or better photophysical properties to their counterpart poly(*p*-phenyleneethynylenes) (PPEs).¹⁹⁻²⁰ PPB synthesis is commonly accomplished via the homocoupling reaction between two terminal alkynes under Glaser or Hay coupling conditions,²¹ as depicted in Scheme 2.1. Because the polymerization only involves alkyne monomers, there is no need for a precise stoichiometric balance to yield a high molecular weight polymer, which is a critical requirement for many step-polymerizations including the synthesis of

PPEs and poly(*p*-phenylenevinylenes) (PPVs). Because of the similarity in the electronic properties of conjugated backbones, the absorption and emission profiles of both PPEs and PPBs are similar. However, PPBs are known to be less susceptible to oxidation due to the high oxidation potentials,²²⁻²³ which contribute to their increased photostability under microscopic imaging conditions.

Scheme 2.1. Formation of PPB under (a) Glaser and (b) Hay coupling conditions.

Despite the synthetic and photophysical advantages of PPBs, a limited number of PPB syntheses have been reported. Kijima et al. synthesized a series of PPBs and examined their semiconducting, fluorescence, and thermotropic liquid crystalline properties.²⁴⁻²⁵ However, the PPBs exhibit low molecular weights (~5,000-9,000 g/mol) and poor solubility. Baier et al. prepared PPB nanoparticles in water using the miniemulsion polymerization technique.²⁶ While the authors reported that the molecular weight of the particles was ~20,000-40,000, such measurements may be overestimated due to chain aggregation within the nanoparticles. A common approach to increasing the solubility of CPs, as demonstrated in PPEs or PPVs, is the introduction of long and flexible side chains on the constituent monomers. However, the side chain modification is not enough to improve the solubility of PPBs, as the butadiene units along the backbone provide an elongated structure prone to interpolymer interactions (i.e., interlocking). Williams et al. demonstrated various PPB syntheses with high molecular weights by the oxidative Sonogashira reaction of two alkynes with different side chains. In addition to the efficient oxidation of palladium by benzoquinone, the PPBs' randomness obtained from two

alkynes is believed to improve the solubility and molecular weight of the block copolymers.²⁷ The same group also demonstrated that high molecular weight PPBs (up to ~124,000 g/mol) were synthesized by using bulky iptycene monomers, minimizing interchain stacking.²⁸ The rigid iptycene scaffolds are also known to minimize π - π stacking in solid films, preserving the photophysical properties suitable for ultrasensitive detection of chemicals.²⁹

This chapter reports a new synthetic approach to achieving high molecular weight PPBs by increasing backbone flexibility. A series of deactivated aryl halides containing a flexible linker was synthesized and polymerized with an alkyne under the palladium/copper-mediated coupling conditions. High molecular weight PPBs (~38,000 g/mol) were successfully synthesized when the deactivated aryl bromide linker was reacted with an alkyne monomer. The incorporation of a small amount (4-6%) of Sonogashira products [i.e., phenyleneethynylenes (PEs)] into the PPB backbone (i.e., PE-doped PPB, PE-d-PPB) is responsible for the improved physical and photophysical properties of PPBs. Finally, CPNs were fabricated by treating the PPB with organic acids followed by dialysis and used for fluorescent microscopic imaging of live cells.

2.3 Results and Discussion

In order to improve the PPB backbone flexibility, the structure of the aryl halide linker necessitated the introduction of a flexible, non-conjugated moiety. Therefore, to synthesize the PE-d-PPB without compromising the characteristic photophysical properties of the fully conjugated PPB, only a minimal amount of the non-conjugated linker was intended to be incorporated in the conjugated backbones. To accomplish this, it was hypothesized that if the aryl halides are relatively inactive (i.e., electronically deactivated aryl bromides) under modified Sonogashira reaction conditions with high copper content, the alkyne homocoupling will be predominant with a minor incorporation of Sonogashira reaction product.³⁰⁻³¹ This concept is the

opposite of PPE synthesis, in which the cross coupling between alkynes and aryl halides is predominant, with minor incorporation of homocoupling products.

Scheme 2.2. Synthesis of aryl halide monomers **M2** and **M3**.

Monomer Synthesis. To test the hypothesis, two aryl halides (**M2** and **M3**) were synthesized and reacted with an alkyne (**M1**) under conditions allowing both competing coupling reactions to occur. Both aryl halides were synthesized using a commercially available N-Boc-protected L-cysteine, which was reacted with bromoaniline and iodoaniline to afford monomers **M2** and **M3**, respectively (Scheme 2.2). As the electron donating amide groups deactivate the C_{sp^2} -halide bonds of the aryl halides, the reactivity of the aryl bromide (**M2**) is expected to decrease significantly compared to that of the corresponding aryl iodide (**M3**). Under the high copper content in a typical Sonogashira reaction, the relatively inactive aryl bromides will not participate in the Sonogashira coupling cycles, allowing for the homo-coupling of alkynes to occur preferentially with a limited amount of Sonogashira coupling.

Polymer synthesis. A series of polymers was prepared under the palladium/copper catalytic conditions outlined in Scheme 2.3. To create a competing environment between the cross (i.e., Sonogashira) and homo (i.e., Glaser) coupling of the monomers, relatively high amounts of palladium [0.2 molar equivalent (eq)] and copper (0.9 eq) catalysts were used. The reaction was carried out at 70°C overnight in a nitrogen environment.

Scheme 2.3. Synthesis of PPB₁, PE-d-PPB, and PPE.

Table 2.1. Comparison of the average physical and photophysical properties of PPE, PPB, and PE-d-PPBs.

Polymer	Type	M1:M2 ratio	M _w (g/mol) ^a	M _n (g/mol) ^a	PDI ^b	λ _{max, abs} (nm) ^c	λ _{max, em} (nm) ^{c,d}	QY (%) ^e	M2 monomer incorporation (%) ^f
PPB ₁	PPB	1 : 0	37,400	16,600	2.3	447	479	17	-
PPB _{2a}	PE-d-PPB	1 : 0.2	23,400	11,200	2.1	434	477	9	n.d.
PPB _{2b}	PE-d-PPB	1 : 1	97,400	38,400	2.5	453	478	32	6
PPB _{2c}	PE-d-PPB	1 : 2	97,900	40,100	2.4	453	478	34	4
PPB ₃	PE-d-PPB	1 : 1 ^h	71,100	32,400	2.2	455	478	31	-
PPE	PPE	1 : 1 ⁱ	14,100	8,100	1.7	376	408/445	16	-

^a Determined by gel permeation chromatography in THF. ^b PDI (polydispersity index) = M_w/M_n.

^c Measured in DMF. ^d PPB₁ and PPB₂ excitation wavelength 430 nm, PPE excitation wavelength 370 nm in DMF. ^e Quantum yield in DMF measured relative to diphenylanthracene standard. ^f Determined by ¹H NMR peak integration. ^g Not determined due to low signal to noise ratio of the ¹H NMR signals. ^h M4:M2 ratio 1:1. ⁱ M1:M3 ratio 1:1.

Physical and photophysical properties of the polymers were averaged using several independent batches of polymers and are summarized in Table 2.1. **PPB₁** synthesized by the homocoupling of the monomer **M1** (in the absence of aryl halide) exhibit poor solubility in both DMF and DMSO and moderate molecular weights (number averaged molecular weight, M_n ~16 kg/mol) that can be achieved by other catalytic systems. When the aryl halides were reacted with **M1** under the

same polymerization conditions, two different polymers (**PPE** and **PPB₂**) were obtained. While a PPB was obtained when the less reactive aryl bromide was used, polymerization with the aryl iodide led to the formation of a PPE. The chemical structure of the resulting polymers was assessed by ¹H NMR spectroscopy. An example of the quantitative assessment of the presence of linker is illustrated on the structure of **PPB₂** (Figure 2.1). **PPB₂** clearly contains the Boc group from linker **M2** at 1.37 ppm, and the integration ratio between the aromatic (from the PPB backbone) and Boc protons indicates that the incorporation of the flexible non-conjugating units was approximately 4-6%.

Figure 2.1. Illustration of the ¹H NMR determination of the percent incorporation of monomer **M2** into the PPB polymer backbone.

Full NMR spectra for polymers **PPB₁**, **PPB_{1b}**, **PPB₂** and **PPB₃** are included in the Experimental Section. The integration ratio also supported the formation of PPE when the **M1** monomer reacted with **M3**. The integration ratio between the aryl and Boc protons corresponds to the formation of 1:1 coupling between the two monomers (i.e., trimeric aryl conjugated units). When the more reactive aryl iodide (**M3**) was employed under the Sonogashira conditions, the oxidative addition of aryl iodide to Pd(0) was favorable (i.e., the reaction followed Sonogashira coupling cycles despite high copper concentration). Since the efficiency of the oxidative addition of aryl bromide to Pd(0) is relatively poor, Glaser coupling among copper coordinated-alkynes was observed when the deactivated aryl bromide (**M2**) was used. If the polymerization reaction contained an oxidant, the homocoupling of two alkynes from Pd(II) could be possible, since the Pd(0) catalyst generated after the reductive elimination of butadiynes can be oxidized to Pd(II). The polymerization results indicate that Pd(0) was the active catalytic center for the cross coupling reaction (i.e., PPE) and the homocoupling reaction (i.e., PPB) occurred by the copper-mediated coupling reaction.

Figure 2.2. Normalized absorption (A) and emission (B) spectra of polymers **PPB₁**, **PPB_{2b}**, and **PPE** in DMF.

The absorption and emission spectra of PPE and PPBs were also representative (Fig. 2.2). Since the **PPE** mainly contains trimeric conjugation units (confirmed by NMR analysis) due to the non-conjugating aryl iodides incorporated stoichiometrically along the chain, characteristic blue absorption from the trimers was observed. The presence of the shoulder at around ~425 nm indicates that the **PPE** may contain a small portion of butadiynyls. Because the stoichiometric balance between **M1** and **M3** was broken by the homocoupling of **M1**, the resulting **PPE** exhibits low molecular weight ($M_n \sim 14,000$ g/mol). However, when the less reactive aryl bromide **M2** was reacted with **M1** under the same conditions, completely different results were obtained. Even though an equivalent amount of the non-conjugating aryl bromide was used, the resulting polymer exhibits a red-shifted absorption (~450 nm) and emission (~478 nm), implying that the main backbones of the polymer are phenylenebutadiynyls, and not phenyleneethynyls.

The PPBs synthesized by the homo-coupling of an alkyne in the absence (i.e., **PPB₁**) or presence (**PPB₂**) of aryl bromides exhibit different physical and photophysical properties: **PPB₂** has higher molecular weight (38,000 g/mol), solubility in organic solvents, and QY than those of **PPB₁**.

FT-IR spectroscopy also provides evidence that **PPB₂** and **PPE** contain the carbonyl functional group found in the linker **M2**. The carbamate C=O stretch band, typically observed in the 1690-1630 cm^{-1} region, was clearly observed at 1689 cm^{-1} in the PPE FT-IR spectrum (Figure 2.3). This band was absent in the case of the homo-polymer **PPB₁**. The FT-IR spectrum of **PPB₂** exhibited a shoulder at ~1692 cm^{-1} consistent with a low degree of **M2** linker incorporation.

Figure 2.3. FT-IR spectra for polymers **PPE** and **PPB₂**.

High molecular weights, solubility, and QYs were consistently obtained only when the aryl bromide was used for the polymerization reaction. To confirm the flexibility effects on the physical properties, we co-polymerized monomer **M1** with 1,4-diethynylbenzene, which lacks the flexible moiety of the linker **M2** but mimics its aromatic group (Scheme 2.4). Similar to the conventional PPB (i.e., **PPB₁** that was synthesized in the absence of the linker), the reaction solution produced insoluble materials, and the resulting polymer exhibited poor solubility in DMF during the purification processes. The soluble fraction of the resulting polymer **PPB_{1b}** exhibited molecular weight similar to that of the homo-polymer **PPB₁**, and its ¹H NMR spectrum showed approximately 22% 1,4-diethynylbenzene incorporation (Figure 2.8). It can be, therefore, concluded that the incorporation of a small quantity of non-conjugating but flexible PE units in the polymer backbone (i.e., **M2** doping) increases the flexibility of the resulting PPBs and decreases interchain aggregations, resulting in high molecular weight PPBs with preserved photophysical properties.

Scheme 2.4. Synthesis of PPB co-polymer **PPB_{1b}**.

Decreasing the relative amount of monomer **M2** (0.2 eq) compared to **M1** in the polymerization produces PE-d-PPBs with a reduced PE incorporation (**PPB_{2a}**, percent incorporation could not be determined due to low signals), while an excess amount of **M2** (2.0 eq) did not proportionally increase the incorporation ratio in the PE-d-PPB (**PPB_{2c}**). Based on these observations, it can be concluded that in order to achieve the desired PE doping effect during polymerization, the deactivated aryl bromide flexible linker must have at least an equimolar concentration.

Cellular imaging. Since cellular membranes contain negatively charged proteoglycans and hydrophobic lipid bilayers, weakly positively charged hydrophobic CPNs exhibit high interaction with the membranes³² and subsequent cellular entries through various endocytosis pathways.³³ Unlike polymers containing quaternary ammonium salts, which cause cellular toxicity, primary amine-containing CPNs exhibit no cellular toxicity owing to the low charge-to-molecular weight ratio (i.e., 1/43 and 1/360 for polyethyleneimine and CPN, respectively).³⁴

To apply the PE-d-PPBs for live cell imaging, we polymerized a Boc-protected amine-containing alkyne **M4** in the presence of the aryl bromide **M2** (Scheme 2.2). The resulting amine-containing PE-d-PPB **PPB₃** exhibits similar physical and photophysical properties to those of PE-d-PPB **PPB₂** (Table 2.1). After deprotection of the Boc groups upon treatment with trifluoroacetic acid, CPNs were fabricated by dialysis.³⁵ The CPNs exhibited a hydrodynamic diameter of ~50 nm with a broad size distribution (Figure 2.4).

Figure 2.4. Dynamic light scattering (DLS) data – PPB₃ CPN. Z-Average = 52 nm (PDI = 0.38).

The metabolic activities of HeLa cells incubated with CPNs were monitored using WST-1 assay. Succinate-tetrazolium reductases in viable cells reduce tetrazolium salts to colored (540 nm) formazan, and the viability inhibition can be assessed by comparing formazan concentrations between control (no treatment) and sample (CPN treated) cells. As shown in Figure 2.5, formazan production from the cells treated with CPNs was similar to the control cells, indicating that CPNs exhibit no viability inhibition under the treatment conditions.

Figure 2.5. Cell viability evaluation by WST-1 assay (n = 3). CPNs cause no viability inhibition.

CPNs were accumulated in cytosolic compartments shown as green dots [Figure 2.6 (b)], not in the nucleus. To identify the subcellular localization of CPNs, the CPN-containing HeLa cells were co-stained with a golgi-specific fluorescent probe [red, BODIPY-TR C5-ceramide-bovine serum albumin (BSA) complex]. As shown in Fig. 2.6 (d), high overlaps (yellow or orange dots) between CPNs and BODIPY-TR C5-ceramide-BSA complex were observed, implying that CPNs were trafficked by an endocytosis mechanism (i.e., caveolae-mediated endocytosis³⁶) to the golgi apparatus.

Figure 2.6. Microscopic images of HeLa cells incubated with **PPB₃** CPNs. Green fluorescent CPNs (b) were accumulated in the golgi apparatus (c), confirmed by overlapped signals (yellow) in the composed image (d). A contrast image is shown in (a).

2.4 Conclusion

In conclusion, a new synthetic method was developed to improve the physical and photophysical properties of PPBs by increasing the flexibility of the polymer backbone. The incorporation of a small quantity of non-conjugating, flexible units in the PPB backbone leads to the successful synthesis of high molecular weight PE-d-PPBs (~38,000 g/mol). It was also demonstrated that PE-d-PPBs are useful materials for fluorescent live cell imaging. The newly developed doping technique is well suited for the synthesis of polar side chain-containing PPBs, showing promise for future use in biological sensing and labeling.

2.5 Outlook

The synthetic methodology described in this chapter makes use of the different reactivity of the monomers under a combination of Sonogashira and Glaser coupling conditions. Unlike in the PPE series presented in Chapter 4, variation of monomer ratios and reaction conditions does not provide a means for controlling the amount of incorporation of the flexible linker into the polymer backbone. If adapted to other side-chain and flexible linker structures, the methodology might yield copolymers with very different properties to those described here, depending on the relative monomer reactivity.

Since the publication of this work in *Macromolecules*, a more systematic sub-cellular localization study confirmed that compared to regular amine-containing PPE materials, the semi-flexible backbone structure of polymer **PPB₃** contributes towards increased Golgi uptake of the CPNs.³⁷ Further studies on polymer **PPB₃** have revealed the unique ability of the material to undergo self-assembly reorganization into core-shell nanoparticles upon polyanion complexation. The resulting polyanion/CPN complex showed high cellular uptake specificity towards cancerous cell lines such as HeLa and Panc-1 cells over regular HEK cells, providing a possible avenue towards target-specific cancer cell labeling.³⁸

2.6 Experimental Section

2.6.1 General. Chemicals, including solvents, were purchased from Fisher Scientific and used as received. *N*-*tert*-butoxycarbonyl (Boc)-protected L-cystine was purchased from Aldrich. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Cambridge, MA). The average molecular weight (M_n) and polydispersity index ($PDI = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC) against polystyrene standards using a Shimadzu high performance liquid chromatography (HPLC) system fitted with PLgel 5 μ m MIXED-D columns and SPD-20A ultraviolet-visible (UV-vis) detector. UV-vis spectra were recorded using a Varian Cary 50 Bio spectrophotometer. Fluorescence spectra were obtained using a FluoroLog-3 Spectrofluorometer (Jobin Yvon/Horiba). 9,10-diphenylanthracene (QY = 1.0) in cyclohexane was used as a fluorescence standard for QY determination. Fourier transform infrared (FT-IR) spectra were recorded on a PerkinElmer Spectrum 100 FT-IR Spectrometer. Fine polymer powders were directly mounted on an attenuated total reflection (ATR) cell of the spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz Avance Bruker NMR spectrometer. Chemical shifts were reported in parts per million (ppm) for ^1H NMR on the δ scale based on the middle peak ($\delta = 2.50$ ppm) of the dimethylsulfoxide (DMSO)- d_6 solvent as an internal standard. Dialysis and solvent exchange of CPNs were conducted using an Ultrafiltration Stirred Cell (Millipore) with membrane filters [Ultracel Ultrafiltration Disc, molecular weight cut-off (MWCO): 30 kDa]. Hydrodynamic radii were determined by the dynamic light scattering technique using Zetasizer nano-ZS (Zen 3600, Malvern Instruments Ltd).

2.6.2 Synthesis of aryl halide linkers **M2** and **M3**.

Aryl bromide linker M2: Boc-protected L-cystine **1** (1.00 g, 2.27 mmol) was dissolved in anhydrous tetrahydrofuran (THF) (25 mL) in a round bottom flask, which was evacuated and filled with nitrogen. *N*-methylmorpholine (0.52 mL, 4.77 mmol) was added, and the suspension was allowed to stir until all the solid was dissolved. Isobutyl chloroformate (0.60 mL, 4.60 mmol) was added, and the mixture was allowed to stir at room temperature for 15 min. A solution of 4-bromoaniline (0.82 g, 4.77 mmol) in anhydrous THF (10 mL) was prepared under a N₂ atmosphere and transferred into the reaction flask using a cannula. The reaction was allowed to proceed for 3 h, after which the reaction mixture was filtered. The filtrate was concentrated *in vacuo*, and the resulting solid was washed with dichloromethane and precipitated overnight from the THF/ dichloromethane (DCM) solvent system. Yield: 0.50 g (29%). High-resolution mass spectrometry (MS) (ESI⁺): theoretical 771.0316 m/z; experimental 771.0322 m/z [M+Na⁺]. ¹H NMR (400 MHz, DMSO-d₆): δ 10.22 (s, 1H), 7.60 – 7.52 (m, 2H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 7.9 Hz, 1H), 4.39 – 4.28 (m, 1H), 3.16 (dd, *J* = 13.3, 4.8 Hz, 1H), 3.00 – 2.89 (m, 1H), 1.37 (s, 9H). ¹³C NMR (150 MHz): δ 169.3, 155.2, 138.1, 131.4, 121.4, 115.1, 78.4, 54.5, 28.1.

Aryl iodide linker M3: Following the procedure for the preparation of **M2**, the reaction between Boc-protected L-cystine **1** (0.50 g, 1.13 mmol) and 4-iodoaniline (0.52 g, 2.38 mmol) afforded the aryl iodide linker **M3**. Yield: 0.28 g (29%). High-resolution MS (ESI⁺): theoretical 865.0058 m/z; experimental 865.0084 m/z [M+Na⁺]. ¹H NMR (400 MHz, DMSO-d₆): δ 10.19 (s, 1H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 1H), 4.33 (q, *J* = 8.0 Hz, 1H), 3.15 (dd, *J* = 13.3, 4.8 Hz, 1H), 3.01 – 2.89 (m, 1H), 1.37 (s, 9H). ¹³C NMR (150 MHz): δ 169.3, 155.2, 138.5, 137.3, 121.7, 87.0, 78.4, 54.5, 28.1.

2.6.3 Synthesis of aromatic monomers M1 and M4.

Scheme 2.5. Synthetic route towards precursor compound **3**.

Compound 3 (Scheme 2.5). Compound **3** was synthesized according to literature procedures.³⁹ More specifically, compound **2** (33.1 g, 79.8 mmol) was dissolved in DCM (300 mL), degassed with a stream of N_2 for 15 mins and the mixture was placed in an ice/water bath. BBr_3 (100 g, 399 mmol) was added and the reaction mixture was allowed to stir at room temperature overnight. The reaction was quenched with DCM/methanol mixture and concentrated *in vacuo*. The resulting solid was washed with DCM on Buchner funnel. Compound **3** was obtained as a light beige solid (26.7 g, 87 %). ^1H NMR (400 MHz, DMSO-d_6): δ 9.80 (s, 2H), 7.14 (s, 2H).

Scheme 2.6. Synthetic route towards precursor compound **5**.

Compound 5 (Scheme 2.6). Compound **5** was synthesized according to literature procedures.⁴⁰ More specifically, compound **4** (20.0 g, 122 mmol) was dissolved in dichloromethane (DCM) (100 mL) and triethylamine (25.5 mL, 183 mmol) in a round-bottom flask and the flask was placed in an ice/water bath. Tosyl chloride (22.1 g, 116 mmol) was dissolved in DCM (100 mL) and placed in an addition funnel. The solution was added dropwise while stirring, and the reaction was allowed to stir at room temperature overnight. The reaction

was quenched with H₂O, the two layers were separated and the organic layer was washed with H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo* to yield compound **5** as a yellow oil (30.9 g, 79 %). ¹H NMR (400 MHz, CDCl₃): δ 7.79 (d, 2H, *J* = 8.34 Hz), 7.33 (d, 2H, *J* = 8.08 Hz), 4.16-4.14 (m, 2H), 3.69-3.66 (m, 2H), 3.61-3.58 (m, 6H), 3.53-3.51 (m, 2H), 3.36 (s, 3H), 2.44 (s, 3H).

Scheme 2.7. Synthetic route towards monomer **M1**.

Compound 6 (Scheme 2.7). Compound **3** (5.0 g, 13.8 mmol), compound **5** (9.2 g, 29.0 mmol), and potassium carbonate (7.6 g, 55.3 mmol) were dissolved in acetonitrile (100 mL) in a round bottom flask equipped with a stir bar and a condenser. The mixture was degassed with N₂ before refluxing overnight. The reaction mixture was then taken up in ethyl acetate, washed with H₂O (2x) and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The resulting solid was purified by column chromatography under 0-40 % ethyl acetate in hexane to yield compound **6** as a beige solid (4.23 g, 47 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.38 (s, 1H), 4.10 (t, 2H, *J* = 4.58 Hz), 3.74 (t, 2H, *J* = 4.56 Hz), 3.64-3.62 (m, 2H), 3.59-3.52 (m, 4H), 3.48-3.41 (m, 2H), 3.24 (s, 3H).

Compound 7 (Scheme 2.7). A Schlenk flask was charged with compound **6** (2.0 g, 3.06 mmol), Pd[Cl₂(PPh₃)₂] (215 mg, 0.306 mmol) and CuI (29.1 g, 0.153 mmol). The Schlenk flask was evacuated and filled with N₂ (3x). A solution of THF (8 mL) and triethylamine (2 mL) was degassed, and the mixed solution was transferred to the Schlenk flask via a cannula. Trimethylsilylacetylene (TMSA) (2.2 mL, 15.3 mmol) was degassed in a separate vial and transferred into the Schlenk flask using a syringe. The reaction was stirred at room temperature overnight. The solvent was then removed *in vacuo*, the solid was redissolved in ethyl acetate, washed with H₂O (2x) and brine, dried over Na₂SO₄ and the solvent was removed *in vacuo*. The resulting solid was purified by column chromatography under 20-40 % ethyl acetate in hexane to yield compound **7** as a beige solid (780 mg, 43 %).

Monomer M1 (Scheme 2.7). Compound **7** (780 mg, 1.31 mmol) and K₂CO₃ (453 g, 3.28 mmol) were dissolved in MeOH and allowed to stir at room temperature. The reaction was closely monitored by TLC for the formation of fluorescent impurities. Upon reaction completion, the reaction mixture was filtered at once and concentrated in vacuo. Column chromatography under 50-100 % of ethyl acetate in hexane yielded monomer **M1** as a brown solid (335mg, 57 %). ¹H NMR (400 MHz, CDCl₃): δ 6.99 (s, 1H), 4.99 (br s, 1H), 4.14 (t, 2H, *J* = 4.94 Hz), 3.86 (t, 2H, *J* = 4.94 Hz), 3.77 (m, 2H), 3.68-3.64 (m, 4H), 3.56-3.53 (m, 2H), 3.37 (s, 3H), 3.33 (s 1H).

Scheme 2.8. Synthetic route towards precursor compound **10**.

Compound 9 (Scheme 2.8). Compound **9** was synthesized according to literature procedures.⁴¹ More specifically, compound **8** (20 g, 95.1 mmol) was dissolved in DCM (300 mL) in a round-bottom flask. Di-*tert*-butyl dicarbonate (Boc₂O) (39.4 g, 90.4 mmol) was dissolved in DCM (200 mL) and placed in an addition funnel. The solution was added dropwise while stirring, and the reaction was allowed to stir at room temperature overnight. The reaction was quenched with H₂O, the two layers were separated and the organic layer was washed with H₂O and brine, dried over Na₂SO₄ and concentrated *in vacuo* to yield compound **9** as a colorless oil (34.6 g, 93 %). ¹H NMR (400 MHz, CDCl₃): δ 5.11 (br s, 1H), 3.72 (m, 2H), 3.57-3.53 (m, 4H), 3.34-3.30 (m, 4H), 2.68 (br s, 1H), 1.43 (s, 9H).

Compound 10 (Scheme 2.8). Compound **10** was synthesized according to literature procedures.³⁷ More specifically, compound **9** (33.9 g, 165 mmol), tosyl chloride (31.5 g, 165 mmol), triethylamine (46.1 mL, 330 mmol) and DCM (500 mL) were reacted according to the procedure for the preparation of compound **5** above. Following extraction, the crude mixture was recrystallized from the tetrahydrofuran (THF)/ether solvent system to yield compound **10** as a white solid (41.7 g, 70 %). ¹H NMR (400 MHz, CDCl₃): δ 7.82 (d, 2H, *J* = 8.34 Hz), 7.36 (d, 2H, *J* = 8.08 Hz), 4.81 (br s, 1H), 4.18-4.16 (m, 2H), 3.65-3.63 (m, 2H), 3.46 (t, 2H, *J* = 5.31 Hz), 3.25 (q, 2H, *J* = 4.97 Hz), 2.46 (s, 3H), 1.46 (s, 9H).

Scheme 2.9. Synthetic route towards monomer **M4**.

Compound 11 (Scheme 2.9). Following the synthetic procedure for compound **6**, the reaction of compound **3** (8.0 g, 22.1 mmol), compound **10** (17.5 g, 48.6 mmol), and potassium carbonate (12.2 g, 88.4 mmol) gave a crude product, which was purified by recrystallization from ethyl acetate to yield compound **11** as a beige crystalline solid (9.4 g, 58 %). ^1H NMR (400 MHz, CDCl_3): δ 7.30 (s, 1H), 5.06 (br s, 1H), 4.13 (t, 2H, $J = 4.55$ Hz), 3.87 (t, 2H, $J = 4.80$ Hz), 3.69 (t, 2H, $J = 5.05$ Hz), 3.40-3.39 (m, 2H), 1.48 (s, 9H).

Compound 12 (Scheme 2.9). Following the synthetic procedure for compound **7**, the reaction of compound **11** (2.0 g, 2.72 mmol), $\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$ (191 mg, 0.272 mmol), CuI (26.0 mg, 0.136 mmol), and TMSA (1.93 mL, 13.6 mmol) yielded compound **12** as a beige crystalline solid (1.39 g, 75 %). ^1H NMR (400 MHz, CDCl_3): δ 6.92 (s, 1H), 4.95 (br s, 1H), 4.11 (t, 2H, $J = 4.74$ Hz), 3.82 (t, 2H, $J = 4.74$ Hz), 3.65 (t, 2H, $J = 5.14$ Hz), 3.34 (m, 2H), 1.57 (s, 9H), 0.25 (s, 9H).

Monomer M4 (Scheme 2.9). Following the synthetic procedure for monomer **M1**, the reaction of compound **12** (1.36 g, 2.00 mmol) and K_2CO_3 (552 mg, 4.00 mmol) in MeOH yielded monomer

M4 as a beige crystalline solid (679 mg, 64 %). ^1H NMR (400 MHz, CDCl_3): δ 6.99 (s, 1H), 4.99 (br s, 1H), 4.13 (t, 2H, $J = 4.70$ Hz), 3.82 (t, 2H, $J = 4.68$ Hz), 3.63 (t, 2H, $J = 5.06$ Hz), 3.39 (s, 1H), 3.36-3.32 (m, 2H), 1.43 (s, 9H).

2.6.4 Polymer Synthesis. *General procedure:* A Schlenk flask was charged with monomer **M1** (or **M4**), monomer **M2** (or **M3**), $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (0.2 eq.) and CuI (0.95 eq). The Schlenk flask was evacuated and filled with N_2 . A solution of anhydrous dimethylformamide (DMF) (4 mL) and freshly distilled triethylamine (1 mL) was degassed, and 1 mL of the mixed solution was transferred to the Schlenk flask via a cannula. The reaction was heated at 70°C for 14 h. The solution was then cooled to room temperature and transferred dropwise to cold ether, resulting in precipitation. After centrifugation (2 min, 4,000 rpm), the supernatant was decanted, and the precipitate was re-dissolved in DMF (1 mL) for further purification.

PPB₁: Using the general procedure described above, the polymerization of monomer **M1** (5.0 mg, 0.0111 mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (1.6 mg, 0.00222 mmol) and CuI (2.0 mg, 0.0105 mmol) yielded PPB polymer **PPB₁** (3.2 mg, 64 %). ^1H NMR (400 MHz, DMSO-d_6): δ 7.31 (s, 1H), 4.20 (br m, 2H), 3.76 (br m, 2H), 3.65-3.62 (m, 2H), 3.56-3.50 (m, 4H), 3.43-3.39 (m, 2H), 3.20 (s, 3H). FT-IR (neat): 3476, 2872, 2200, 1243, 1600, 1495, 1453, 1402, 1351, 1274, 1216, 1096, 1053, 944, 848, 722 cm^{-1} . GPC: $M_w = 43,700\text{ g/mol}$, $M_n = 18,100\text{ g/mol}$, $\text{PDI} = 2.4$. UV $\lambda_{\text{max}} = 447\text{ nm}$, Fluo $\lambda_{\text{max}} = 479\text{ nm}$, QY = 22%.

Figure 2.7. ^1H NMR (400 MHz, DMSO-d_6) spectrum of PPB polymer **PPB₁**.

PPB_{1b}: Using the general procedure described above, the co-polymerization of monomer **M1** (5.0 mg, 0.0111 mmol) and 1,4-diethynylbenzene (1.4 mg, 0.0111 mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (1.6 mg, 0.00222 mmol) and CuI (2.0 mg, 0.0105 mmol) yielded PPB co-polymer **PPB_{1b}** (1.5 mg, 23 %). Precipitation in the polymerization solution and poor solubility in DMF during the purification steps were responsible for the relatively poor yield. ^1H NMR (400 MHz, DMSO-d_6): δ 7.33 (s, 0.34H), 7.31 (s, 0.77H), 4.19 (br s, 2H), 3.77 (br s, 2H), 3.64-3.62 (m, 2H), 3.55-3.50 (m, 4H), 3.39 (m, 2H), 3.20 (s, 3H). FT-IR (neat): 2871, 2201, 1597, 1493, 1452, 1408, 1350, 1273, 1216, 1098, 1053, 940, 837, 720 cm^{-1} . GPC: $M_w = 36,400$ g/mol, $M_n = 15,700$ g/mol, PDI = 2.3. UV $\lambda_{\text{max}} = 435$ nm, Fluo $\lambda_{\text{max}} = 474$ nm, QY = 31%.

Figure 2.8. ^1H NMR (400 MHz, DMSO-d_6) spectrum of PPB co-polymer **PPB_{1b}**.

PPB₂: Using the general procedure described above, the polymerization of monomer **M1** (10.0 mg, 0.0222 mmol) and monomer **M2** (16.6 mg, 0.0222 mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (3.1 mg, 0.00444 mmol) and CuI (4.0 mg, 0.0211 mmol) yielded the PE-d-PPB polymer **PPB₂** (8.9 mg, 78 %). ^1H NMR (400 MHz, DMSO-d_6): δ 7.58 (d, 0.11H), 7.47 (d, 0.12H), 7.30 (s, 1H), 4.19 (br s, 2H), 3.76 (m, 2H), 3.63 (m, 2H), 3.54–3.51 (m, 4H), 3.40 (m, 2H), 3.20 (s, 3H), 1.37 (s, 0.57H). FT-IR (neat): 2872, 2203, 2142, 1693 cm^{-1} (carbamate C=O), 1592, 1495, 1455, 1397, 1351, 1275, 1219, 1200, 1097, 1053, 946, 848, 633 cm^{-1} . GPC: M_w = 69,000 g/mol, M_n = 30,300 g/mol, PDI = 2.3. UV λ_{max} = 453 nm, Fluo λ_{max} = 478 nm, QY = 35%.

Figure 2.9. ^1H NMR (400 MHz, DMSO-d_6) spectrum of PE-d-PPB polymer **PPB₂**.

PPB₃: Using the general procedure described above, the polymerization of monomer **M4** (10.0 mg, 0.0188 mmol) and monomer **M3** (14.1 mg, 0.0188 mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (2.6 mg, 0.00375 mmol) and CuI (3.4 mg, 0.0178 mmol) yielded Boc-protected amine PE-d-PPB polymer **PPB₃** (10.5 mg, 95 %). ^1H NMR (400 MHz, DMSO-d_6): δ 7.57 (br m, 0.05H), 7.48 (br m, 0.07H), 7.30 (s, 1H), 6.73 (t, 1H), 4.18 (br s, 2H), 3.74 (br s, 2H), 3.49 (m, 2H), 3.11 (m, 2H), 1.35 (s, 9H). FT-IR (neat): 3343 (amide N-H), 2976, 2932, 2877, 2203, 1696 (carbamate C=O), 1494, 1455, 1391, 1364, 1272, 1248, 1219, 1165, 1123, 1054, 941, 779, 759 cm^{-1} . GPC: M_w = 75,900 g/mol, M_n = 34,000 g/mol, PDI = 2.2. UV λ_{max} = 456 nm, Fluo λ_{max} = 478 nm, QY = 25%.

Figure 2.10. ^1H NMR (400 MHz, DMSO-d_6) spectrum of PE-d-PPB amine polymer **PPB₃**.

PPE: Using the general procedure described above, the polymerization of monomer **M1** (5.0 mg, 0.0111 mmol) and monomer **M3** (9.4 mg, 0.0111 mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (1.6 mg, 0.00222 mmol) and CuI (2.0 mg, 0.0105 mmol) yielded PPE polymer (8.8 mg, 75%). ^1H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H, NH), 7.68 (d, 2H), 7.47 (d, 2H), 7.27-7.22 (m, 1H), 7.16 (s, 1H), 4.37 (br s, 1H), 4.16 (br s, 2H), 3.78 (br s, 2H), 3.66 (m, 2H), 3.51-3.46 (m, 4H), 3.38-3.28 (m, 2H), 3.18 (s, 3H), 3.10-2.92 (br m, 2H), 1.38 (s, 9H). FT-IR (neat): 3287 (amide N-H), 2984, 2939, 2874, 2208, 1689 cm^{-1} (carbamate C=O), 1591, 1516, 1493, 1406, 1365, 1311, 1278, 1245, 1216, 1199, 1160, 1097, 1050, 1021, 944, 837, 775 cm^{-1} . GPC: $M_w = 14,100$ g/mol, $M_n = 8,100$ g/mol, PDI = 1.7. UV $\lambda_{\text{max}} = 376$ nm, Fluo $\lambda_{\text{max}} = 408$ nm and 445 nm, QY = 16%.

Figure 2.11. ^1H NMR (400 MHz, DMSO-d_6) spectrum of **PPE** polymer.

2.6.5 CPN formation. A solution of polymer **PPB₃** in DMSO-d_6 was mixed with acetic acid (2 mL) and trifluoroacetic acid (1 mL) and allowed to stir at room temperature for 14 days. The mixture was then added to acetic acid (20 mL), allowed to stir overnight, and centrifuged, and supernatant was added dropwise (2 drops/s) to 500 mL water (18 Ω) while stirring. Using a solvent-resistant stir cell fitted with a 30 kDa-MWCO membrane, the solution was concentrated to approximately 10 mL, and dialyzed against 1 L of water. The resulting solution was further dialyzed in a 10 KDa membrane for three days. The solution was subsequently filtered through a Teflon (PTFE) syringe filter (0.45 μm) and stored for future use. UV $\lambda_{\text{max}} = 448 \text{ nm}$ and 482 nm, Fluo $\lambda_{\text{max}} = 532 \text{ nm}$ (broad), QY = 0.5%.

2.6.6 Cell culture, microscopic imaging, and toxicity assay. ~10,000 HeLa cells (human cervical cancer, purchased from ATCC) were seeded into a glass-bottomed eight-well chamber slide (Lab-Tek, Thermo Scientific) and cultured in a minimum essential medium (MEM) / Earle's balanced salt solution (EBSS) (400 μL , HyClone, SH30024) medium containing 10% fetal bovine serum (FBS) and 100 U/m penicillin for 24 h under 5% CO_2 at 37°C. 80 μL of 20 μM CPNs in

water was added to the culture medium directly, and the cells were further cultured for 24 h (final CPN concentration: 4 μ M). For the golgi apparatus staining, BODIPY-TR C₅-ceramide-BSA complex (final 10 μ M, Molecular probes, USA) was incubated for 30 min at 4°C. After washing with fresh medium, the cells were further incubated for 30 min at 37°C. 1 μ L of Hoechst (5 μ g/ml) was added to the culture medium and incubated with the cells for 10 min at 37°C, and washed two times with phosphate buffered saline (PBS). The cells were fixed with 4% paraformaldehyde for 10 min. Fluorescent images of the cells were obtained using a DeltaVision Elite Microscope System (Applied Precision, Issaquah, Washington) equipped with bandpass filters such as blue (410-460 nm, Hoechst) and green (500-550 nm, CPNs).

HeLa cells (~10,000 cells/well) in 200 μ L of complete medium were seeded into a 96-well plate and cultured for one day in a 5 % CO₂ incubator at 37 °C. CPNs with various concentrations (5 to 40 μ M) were added and incubated for 24 h. To measure toxicity, 10 μ L of WST-1 [2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] (CytoScan) solution was added into the each well, and the plate was further incubated for 4 h at 37 °C. Cell viability was compared by measuring absorbance values at 540 nm using a microplate well reader (Synergy 2, BioTek, USA). Relative cell viability as a function of CPN concentration was obtained by subtracting absorbance values of each sample well with control CPN absorbance at 540 nm.

2.7 References

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CHAPTER III

Synthesis of Poly(phenylenebutadiynylenes) Using the Decarboxylative Coupling of Propiolic Acids to Aryl Halides

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3.1 Abstract

Conjugated random copolymers containing phenylenebutadiynylenes and phenyleneethynylenes were synthesized from the palladium- and copper-catalyzed decarboxylative coupling reaction of propiolic acid with aryl iodides. This one-step synthetic approach provides a facile route to conjugated polymers whose synthesis typically requires a multistep conversion of the starting diiodoarenes into diacetylenes prior to polymerization.

3.2 Introduction

Palladium-catalyzed carbon-carbon bond formations have been widely used in the synthesis of natural products, bioactive molecules, and conjugated polymers (CPs).¹ A variety of named coupling reactions have been developed, such as Kumada, Negishi, Stille, Suzuki, Hiyama and Sonogashira.² Although these reactions are applicable to a broad scope of substrates with good reactivity and tolerance of functional groups, their drawback is the production of organometallic waste in the coupling reaction. To overcome this fundamental problem, the direct C-H activation³ and decarboxylative couplings⁴ have been developed. The synthetic chemistry field has paid more attention to the latter due to the greater ease of controlling the reaction site, and the release of the environmentally benign carbon dioxide by-product. The decarboxylative coupling reactions of aryl carboxylic acids have been intensively studied by Goossen and other groups.⁴ Recently, palladium-catalyzed decarboxylative coupling reaction was developed between propiolic acid and aryl halides, which provided an efficient tool in the synthesis of aryl alkynes.⁵⁻¹⁵ Since the first report, a variety of coupling partners such as aryl boronic acids, amines, phosphines and C-H activated arenes have been employed in the decarboxylative coupling of alkynyl carboxylic acid.¹⁶⁻¹⁹

Propiolic acid is a useful alkyne source because of its easy storage and handling. It has been employed in the synthesis of diaryl alkynes, aryl alkynes, aryl carboxylic acids and diaryl diynes.

However, it has never been used in the synthesis of CPs despite aryl alkyne being an important building block in the synthesis of poly(*p*-phenyleneethynylene)s (PPEs) and poly(*p*-phenylenebutadiynylene)s (PPBs) whose structure is depicted in Figure 3.1. The synthesis of PPEs relies on the reaction between an aryl halide and an aryl alkyne under Sonogashira coupling conditions, which require precise stoichiometric balance of monomers and inevitably lead to the incorporation of minor homocoupling (i.e. butadiynylene) defects,²⁰ giving mostly a PPE product with minor PPB content. The synthetic route to PPBs involves the coupling of two terminal alkynes typically accomplished under Hay or Glaser coupling conditions.²¹ PPBs exhibit similar photophysical properties to PPEs and the coupling reactions are less sensitive to the reaction environment.²²

Figure 3.1. Chemical structure of poly(*p*-phenyleneethynylene) (PPE) and poly(*p*-phenylenebutadiynylene) (PPB).

However, the synthesis of dialkyne monomers, which is central to both types of reactions, is time-consuming and low yielding as it generally involves the coupling of an aryl halide with TMS-acetylene and subsequent TMS deprotection. The isolation of dialkyne monomers from these reactions is often complicated by the presence of polar side chain substituents. As a necessary feature of the monomers, polar side-chains increase aqueous solubility of the final polymer, making the polymer suitable for biological applications. A one-pot reaction facilitating the direct conversion of aryl halides into a polymer containing the alkyne moiety is therefore desirable.

A previous study on small molecules reported the one-pot synthesis of diaryl diynes from the coupling reaction of propiolic acid and aryl iodides.²³ The reaction consists of two steps. The first step is the formation of aryl alkynyl carboxylic acid and the second step is the decarboxylative

homocoupling of the newly formed aryl alkynyl carboxylic acid. This successful one-pot approach yields itself to application in the field of polymer synthesis. This chapter describes the one-pot synthesis of conjugated co-polymers containing both aryl ethynylene and butadiynylene units under optimized coupling reaction conditions.

3.3 Results and Discussion

In order to find the optimized conditions for the synthesis of the conjugated polymer, the diiodoarene **1** was chosen as a monomer for the coupling reaction with propiolic acid, because the monomer exhibits good solubility in DMSO owing to its long ethylene oxide side chains. The reaction conditions are depicted in Scheme 3.1, and the corresponding results are summarized in Table 3.1. Successful polymer synthesis was indicated by high molecular weight (MW), represented by the value of M_n , and the material's absorption/emission profile characterized by the UV ($\lambda_{\text{max, abs}}$) and fluorescence ($\lambda_{\text{max, em}}$) maxima. High MW polymers exhibit red-shifted absorption/emission, which reaches a saturation value when the conjugation length is around 7-10 units.²⁴⁻²⁵ The polymerization yield is not indicative of the efficiency of a single coupling reaction event; rather it represents the amount of high-MW polymer material isolated after the removal of unreacted monomers and low-MW oligomers. The reported percent yield represents the polymer yield per butadiynylene-containing repeating unit.

To investigate the optimum reaction conditions, the previously reported conditions for the synthesis of diyne from aryl iodide and propiolic acid were first employed. The result under $\text{Pd(PPh}_3)_2\text{Cl}_2$, dppb, DBU, CuI, K_2CO_3 and 2.4 equiv. of propiolic acid in DMSO is displayed as entry 1 in Table 1. The resulting homopolymer **3** exhibited low MW (M_n 5,600 g/mol). As expected, in the absence of CuI only low-MW material (M_n 2,500 g/mol) was obtained (entry 3). Most of the polymerizations that followed, including decreasing the amount of propiolic acid to 2.2 equiv. (entry 2), substituting the reaction solvent for DMF (entry 4), the use of organic bases

instead of K_2CO_3 (entry 6 and 7), increasing the amount of solvent (entry 8), or decreasing the reaction temperature (entry 9), were somewhat successful, yielding materials with M_n between 5,500 and 8,600 g/mol. While this is indicative of the decarboxylative coupling reaction taking place, the MW of the materials does not indicate significant degree of polymerization desirable for a conjugated polymer. A notable exception was the use of Et_3N as a base leading to the formation of a gel in the reaction mixture (entry 5). Gelation is a characteristic property of high-MW CPs. Indeed, the soluble fraction of the polymer exhibited a markedly improved M_n of 31,900 g/mol. The absorption maximum at 442 nm is also red-shifted compared to the low MW materials. The polymerization yield of this reaction was low at 34% since it only accounts for the material obtained after purification of the soluble fraction. The optimum reaction conditions of entry 5 were used for the copolymerization experiment. The addition of comonomer **2** to the reaction under these conditions, however, afforded copolymer **4** with M_n of only 8,600 g/mol (entry 10). An improvement was observed when a 4/1 ratio of solvent to base was used, exhibiting gelation in the reaction mixture and yielding the copolymer **4** with M_n of the soluble fraction of 16,500 g/mol (entry 11).

Scheme 3.1. The synthesis of polymers **3** and **4**.

Table 3.1. The synthesis of polymers **3** and **4**.^a

Entry	Solvent	Base	monomer	M _n (g/mol) ^b	λ _{max, abs} (nm) ^c	λ _{max, em} (nm) ^{c,d}	Degree of polymerization	Polymerization yield (%) ^e
1	DMSO	K ₂ CO ₃	1	5600	428	479	21	57
2 ^f	DMSO	K ₂ CO ₃	1	6200	430	479	23	64
3 ^g	DMSO	K ₂ CO ₃	1	2500	410	475	9	90
4	DMF	K ₂ CO ₃	1	5500	432	477	20	59
5	DMF	Et ₃ N	1	31900 ^h	442	477	117	34 ^h
6	DMF	Morpholine	1	7400	431	478	27	83
7	DMF	DIPA	1	5200	422	477 ⁱ	19	48
8	DMF	Et ₃ N ^j	1	7700	437	477	28	50
9 ^k	DMF	Et ₃ N	1	6300	434	477	23	56
10	DMF	Et ₃ N	1 + 2	8600	435	477	11	71 (1.3:2) ^l
11	DMF	Et ₃ N ^j	1 + 2	16500 ^h	433	477	21	78 ^h (1:2) ^l

^aReaction conditions : monomer **1** (x mmol), propiolic acid (2.4 equiv.), Pd[Cl₂(PPh₃)₂] (20 mol%), dppb (40 mol%), CuI (20 mol%), DBU (9.6 equiv.) were reacted in solvent at 80 °C. ^bDetermined by gel permeation chromatography in THF. ^cMeasured in reaction solvent. ^dExcitation wavelength 430 nm. ^eYield determined based on the corresponding PPB repeating unit. ^f2.2 eq of propiolic acid. ^gNo CuI added to reaction. ^hData for soluble fraction of the resulting gel. ⁱExcitation wavelength 420 nm. ^j4:1 solvent:base ratio. ^kReaction at 30 °C and then 80 °C. ^lRelative **1**:**2** monomer incorporation determined by ¹H NMR.

Scheme 3.2. Possible pathways of the decarboxylative coupling reaction leading to two different products: ethynylene (pathway a), or butadiynylene (pathway b).

The resulting polymers **3** and **4** exhibited properties similar to conjugated PPE and PPB polymers synthesized using traditional approaches. According to an earlier report, the coupling reaction proceeds in two steps. First, the Sonogashira coupling between propiolic acid and aryl iodide yields the intermediate aryl alkynyl carboxylic acid. The decarboxylative coupling of this intermediate can undergo two reaction pathways (Scheme 3.2). Sonogashira-like coupling with another aryl halide will give rise to an ethynylene link between the two aryl groups (i.e., Scheme 3.2, pathway a), while the decarboxylative coupling between two aryl alkynyl carboxylic acids gives rise to a 1,3-diyne (butadiynylene) linkage (i.e., Scheme 3.2, pathway b). The rates of these two respective reactions will depend on the nature of the aryl iodide substitution. It has been shown previously on model small-molecule systems that the reaction under optimized conditions of *ortho* alkoxy-substituted iodobenzene is not very selective towards the butadiynylene linkage (4:3 butadiynylene:ethynylene product), while the alkoxy- substituent in the *meta*-position gives almost exclusively the butadiynylene product (13:1). Monomers **1** and **2** contain two alkoxy chains each, substituted in both the *ortho*- and the *meta*-position. Based on our previous observations and the monomer ring substitution pattern, it is assumed that the decarboxylative coupling exhibits some selectivity towards the homocoupling product giving rise to the formation of butadiynylene linkages. The resulting polymers **3** and **4** hence contain predominantly butadiynylene units with a smaller proportion of ethynylene links.

Figure 3.2. Normalized absorption (solid line) and emission (dotted line) profile of copolymer **4** in DMF.

The absorption and emission spectrum of copolymer **4** (Table 1, entry 11) is shown in Figure 3.2. The absorption and emission maxima at 433 nm and 477 nm, respectively, are consistent with earlier PPE and PPB investigations but not indicative of the relative proportion of each moiety within the polymer. This is because there is no significant difference in the absorption/emission profile of PPEs and PPBs as concluded by Williams et al.²⁶ The results are also consistent with the observation that higher molecular weight polymers exhibit a red shift in the absorption maximum, as evidenced by the high-MW polymer **3** (entry 5).

Polymers **3** and **4** were characterized by ¹H NMR spectroscopy. The spectrum of homopolymer **3** (Figure 3.3) exhibits the broadening of aliphatic chain proton signals b and c (4.21 and 3.81 ppm respectively) characteristic of a polymeric structure.

Figure 3.3. ^1H NMR (400 MHz, DMSO-d_6) spectrum of homopolymer **3**.

Figure 3.4. ^1H NMR (400 MHz, DMSO-d_6) spectrum of random copolymer **4**.

Figure 3.4 shows the spectrum of copolymer **4** (entry 11) which is indicative of the presence of both monomers (**1** and **2**) within the polymer chain. Both monomers contribute to the signals at 7.16 ppm, 4.21 ppm, 3.81 ppm (peaks a, b, and c). The peak at 3.21 ppm (d) is characteristic of the tetraethyleneglycol monomethyl ether-containing monomer **1**, while the *t*-butylcarbamate

group of monomer **2** gives rise to an amide proton signal (e) at 6.71 ppm and the Boc-group protons (f) at 1.35 ppm.

The presence of monomer **2** within copolymer **4** is also evident from the FTIR spectra of the two polymers (Figure 3.5). The C=O peak at 1702 cm^{-1} is clearly indicative of the presence of *t*-butyl carbamate protecting groups originating from the side-chains of monomer **2**. This peak is absent in the FTIR spectrum of homopolymer **3**, which only contains tetraethylene glycol side chains.

Figure 3.5. FTIR Spectrum overlay of polymers **3** and **4**.

The integration ratios of peaks in the ^1H NMR spectrum in Figure 3.4 can provide an estimate of the relative proportion of the two monomers within the copolymer chain. While the sole contributor to the Boc-peak at 1.35 ppm (f) is monomer **2** (9H), the signal for peak b at 4.21 ppm is a combination of protons from both monomers (contributing two protons each). Given that the integral of the Boc-proton peak f in Figure 3.4 has an intensity of 9.00, monomer **2** must contribute to the integration of proton b (overall intensity 3.02) with a magnitude of 2.00. The remaining contribution in peak b (i.e. 1.02) is due to the two aliphatic protons in monomer **1**, and consequently it can be concluded that the ratio of monomers **1** and **2** is 1 : 2. The proportion

calculation can be generalized using the following formulas: $x = b/2 - f/9$, and $y = f/9$, where x is the proportion of monomer **1**, y is the proportion of monomer **2**, and b and f are the integration intensities of the corresponding spectral peaks. When this formula is applied to the spectrum of the low-MW copolymer **4** (entry 10 in Table 1), the monomer ratio is calculated to be 1.3:2, and can therefore be considered relatively independent of the molecular weight of the polymer. The higher rate of incorporation of the Boc-protected amine-containing monomer **2** suggests its higher reactivity under the experimental conditions compared to the tetraethyleneglycol monomethyl ether-substituted monomer **1**.

In an attempt to provide a quantitative assessment of the relative proportion of the ethynylene vs the butadiynylene linkages within the polymers, other characterization techniques were employed, since neither ^1H NMR spectra nor the photophysical profiles can provide that information. To qualitatively observe the presence of both ethynylenes and butadiynylenes in the polymers, ^{13}C NMR spectrum of polymer **4** (Table 3.1 entry 5) was acquired. Despite a large number of scans (40,000), the sp -carbon signals from the ethynylene and butadiynylene linkages could not be observed. It is likely that in addition to the low concentration of the NMR sample due to limited solubility of the PPB polymers, the low signals were a result of the quaternary nature of the carbons, which do not generally produce strong signals in the ^{13}C spectrum.

Table 3.2. Elemental analysis results for polymer **4**.

	Polymer 4 (experimental)	PPE (predicted)	PPB (predicted)
C (%)	58.78	60.68	62.44
H (%)	7.15	8.23	7.86
C/H ratio	8.22	7.37	7.94

The quantification of the relative proportion of the ethynylene and butadiynylene linkages was also attempted based on the elemental analysis of the polymer samples. The only difference between the PPE and PPB polymer is the additional two carbon atoms from the butadiynylene linkage compared to the ethynylene. Therefore, theoretically the elemental C/H ratio of polymer **4** should fall within the range between the theoretical C/H ratio of PPE and that of PPB. Table 3.2 summarizes the results of the elemental analysis on polymer **4** carried out by Galbraith Laboratories. It also lists the predicted C and H content for pure PPE and pure PPB polymers based on the structure of their repeating unit. Unfortunately, the experimental C/H ratio result did not correspond to the range of expected values. The inconsistency could be a result of the presence of traces of unidentified impurities within the polymer that are difficult to remove during purification due to low polymer solubility. Furthermore, the experimental error associated with the elemental analysis measurement itself may not be able to distinguish the very small relative differences between the carbon content in PPEs and PPBs with sufficient accuracy.

3.4 Conclusion

In summary, a one-pot synthesis of conjugated homo- and copolymers containing phenyleneethynylenes and phenylenebutadiynylenes was developed using the palladium catalyzed decarboxylative coupling reaction of propiolic acid and iodoarenes. It was found that the choice of organic base such as Et₃N was important in the polymerization to obtain polymers with increased molecular weight. Random copolymerization of monomers with different side chains resulted in a polymer containing a reproducible ratio of the two monomers indicative of their reactivity under the experimental conditions. While the relative proportion of the ethynylene and butadiynylene units remains unknown, this decarboxylative coupling polymerization reaction provides a facile synthetic route to phenylene-containing conjugated polymers without the need to control the stoichiometric balance.

3.5 Outlook

The synthetic methodology outlined in this chapter can be adapted to yield mixed PPE/PPB polymers for a range of applications depending on the side-chain. In order to fully characterize the nature of the polymer backbone, characterization techniques other than the conventional organic chemistry tools outlined above will need to be employed. The quantitation of the butadiynylene component may be possible through chemical modifications of the backbone, taking advantage of the selective reactivity of the butadiynylene bridge towards cycloadditions. For example, it has been demonstrated that small-molecule 1,3-diynes undergo cycloadditions with amines or water to yield substituted pyrroles or furans, respectively.²⁷⁻²⁹ A successful quantitative conversion of butadiynes in the polymer chain into heterocycles will introduce heteroatoms into the polymer backbone, thus red-shifting the polymer's photophysical properties. Additionally, the proposed modification will introduce alkene protons into the backbone, which are detectable by ¹H NMR spectroscopy. A proposed example is illustrated in Figure 3.6.

Figure 3.6. Proposed approach towards the selective modification of the PPE/PPB polymer backbone for the quantification of the relative amount of ethynylene and butadiynylene units.

3.6 Experimental Section

3.6.1 General. Chemicals, including solvents, were purchased from Fisher Scientific and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Cambridge, MA). UV-vis spectra were obtained using a Varian Cary 50 Bio spectrophotometer. Fluorescence spectra were recorded using a FluoroLog-3 Spectrofluorometer (Jobin Yvon/Horiba). 9,10-

diphenylanthracene (QY = 1.0) in cyclohexane was used as a fluorescence standard for QY determination. The average molecular weight (M_n) and polydispersity index ($PDI = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC) against polystyrene standards using a Shimadzu high performance liquid chromatography (HPLC) system fitted with PLgel 5 μ m MIXED-D columns and SPD-20A ultraviolet-visible (UV-vis) detector. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz Avance Bruker NMR spectrometer. Chemical shifts were reported in parts per million (ppm) for ^1H NMR on the δ scale based on the middle peak ($\delta = 2.50$ ppm) of the dimethyl sulfoxide (DMSO)- d_6 solvent as an internal standard. Fourier transform infrared (FT-IR) spectra were recorded on a PerkinElmer Spectrum 100 FT-IR Spectrometer. Fine polymer powders were directly mounted on an attenuated total reflection (ATR) cell of the spectrometer.

3.6.2 Polymer Synthesis. *General procedure:* A Schlenk flask was charged with solids – monomer **1**, monomer **2** (if applicable), CuI, $\text{PdCl}_2(\text{PPh}_3)_2$, 1,4-bis(diphenylphosphino)butane (dppb) and base (in reactions utilizing K_2CO_3). The Schlenk flask was evacuated and filled with N_2 . A solution of the appropriate solvent, base (in all reactions other than those using K_2CO_3), 1,8-diazabicycloundec-7-ene (DBU) and propiolic acid was degassed for 15 minutes and then transferred to the Schlenk flask via a cannula. The vial was rinsed with copious amounts of degassed solvent which was then transferred into the Schlenk flask. The reaction was heated at 80°C for 14 h. The solution was then cooled to room temperature and transferred dropwise to cold ether/methanol mixture (9:1), resulting in precipitation. After centrifugation (2 min, 4000 rpm), the supernatant was decanted, and the precipitate was redissolved in DMF (1 mL) for further purification. Yield measurement was based on the amount of dried precipitate following re-precipitation of the DMF solution in cold ether. The reported percent yield represents the

polymer yield per butadiynylene-containing repeating unit with molecular weight of 272 (entries 1-9) and 803 (entries 10-11).

Synthesis of homopolymer 3. Using the general procedure described above, the polymerization of monomer **1** (10.0 mg, 0.0135 mmol) in the presence of CuI (0.5 mg, 0.00269 mmol), dppb (1.1 mg, 0.00269 mmol), Pd[(PPh₃)₂Cl₂] (0.9 mg, 0.00135 mmol), DMF (0.5 mL), Et₃N (4.5 μ L, 0.0323 mmol), DBU (9.7 μ L, 0.0647 mmol) and propiolic acid (2.0 μ L, 0.0323 mmol), yielded 2.5 mg of dried solid material (34%). ¹H NMR (400 MHz, DMSO-d₆): δ 7.16 (s, 1H), 4.21 (s, 2H), 3.81 (s, 2H), 3.52-3.30 (br m, 4H), 3.21 (s, 3H). FT-IR (solid): 2866, 2164, 1606, 1494, 1455, 1419, 1350, 1206, 1091, 1032, 935, 845, 701 cm⁻¹. GPC: M_w = 45 100 g/mol; M_n = 31 900 g/mol; PDI = 1.4. UV λ_{max} = 442 nm; fluo λ_{max} = 477 nm; QY = 12%.

Synthesis of copolymer 4. Using the general procedure described above, the polymerization of monomer **1** (10.0 mg, 0.0135 mmol), monomer **2** (9.9 mg, 0.0135 mmol), CuI (1.0 mg, 0.00539 mmol), dppb (2.3 mg, 0.00539 mmol), Pd[(PPh₃)₂Cl₂] (1.9 mg, 0.00269 mmol), propiolic acid (4.0 μ L, 0.0647 mmol), DBU (19.3 μ L, 0.129 mmol) and Et₃N (0.2 mL) yielded copolymer **4** (11.3 mg, 78%). ¹H NMR (400 MHz, DMSO-d₆): δ 7.16 (s, 1.5H), 6.70 (br s, 1H, Boc-NH), 4.21 (s, 3H), 3.81 (s, 3H), 3.50-3.30 (br m, 6H), 3.21 (s, 1.5H), 1.35 (s, 9H). FT-IR (solid): 3414, 2920, 2872, 2251, 1702 (carbamide C=O), 1602, 1493, 1452, 1365, 1273, 1250, 1215, 1104, 1050, 1024, 1004, 821, 757, 696 cm⁻¹. GPC: M_w = 28 300 g/mol; M_n = 16 500 g/mol; PDI = 1.7. UV λ_{max} = 433 nm; fluo λ_{max} = 477 nm; QY = 6%.

3.7 References

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CHAPTER IV

Synthesis of Biodegradable Conjugated Polymers with Controlled Backbone Flexibility

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4.1 Abstract

A series of flexible, highly bright fluorescent poly(*p*-phenyleneethynylene)s (PPEs) was prepared by employing a disulfide-containing non-conjugated monomer at various ratios under Sonogashira reaction conditions. PPEs with flexible linkers exhibited fluorescence properties comparable to those of a fully conjugated PPE when less than 50% of flexible monomers were incorporated into the backbone. To evaluate the self-assembly properties of PPEs, a series of conjugated polymer nanoparticles (CPNs) was fabricated by treating PPEs with organic acids followed by dialysis. CPNs containing linkers exhibited different complexation behavior with polysaccharides, warranting further investigation into how flexibility and biodegradability of CPNs influence their cellular interaction and entry.

4.2 Introduction

Aromatic π -electron conjugated polymers (CPs) are synthetic organic materials exhibiting intrinsic fluorescence and unique structural properties that have been used for various biological applications including sensing,¹⁻³ imaging,⁴⁻⁷ and delivery⁸⁻¹⁰ of biologically active substances. CPs contain two unique components: the backbone and the side chain. The backbone is responsible for the intrinsic fluorescence, although the hydrophobicity and stiffness present a challenge for some applications in aqueous environments. Nonspecific and uncontrolled backbone aggregation in an aqueous medium will diminish both physical and photophysical merits dramatically. To increase the aqueous compatibility, the hydrophobic backbones are often modified with hydrophilic side chains containing charges, which reduce interpolymer aggregation.¹¹ Depending on the nature and functionality of the charged side chains and the nature of the backbones (i.e., flexibility), the photophysical and physical properties of CPs are closely related to the side chain interaction with the stimuli, resulting in fluorescence changes through backbone aggregation (i.e., sensor application¹²) or modulated cellular behaviors through

the balanced hydrophobicity and hydrophilicity (i.e., imaging and delivery application¹³). Using relatively flexible polythiophene (PT)-based CP, several researchers have used the concept of backbone reorganization for biosensing, in which the elongation and planarization of the PT backbones are directly related to the interaction of the side chains with biomolecules.¹⁴ For cellular applications of CPs, the Moon research group has demonstrated that conjugated polymer nanoparticles (CPNs) fabricated by controlled aggregation of non-aqueous soluble, amine-containing poly(p-phenyleneethynylene)s (PPEs) in an aqueous solution exhibit efficient uptake and delivery of small interfering RNA to live cells.⁸ Furthermore, CPNs fabricated from a poly(p-phenylenebutadiynylene) (PPB) containing a small amount (~10%) of flexible, non-conjugated units (described in detail in Chapter 2)¹⁵ exhibit unique self-assembly behaviors with polyanions¹⁶ and modulated subcellular localization patterns.¹³

These observations prompt a systematic investigation of the relationship between the conjugated backbone structure and the photophysical and biophysical properties, the understanding of which will have a high impact on the design of CPs for improved cellular labeling, sensing, and delivery. While side-chain modifications have been extensively investigated and successfully employed for increasing the solubility and introducing functional moieties for sensing and labeling of chemical and biological substances,¹¹ backbone modulations have been mainly limited to the tuning of photophysical properties (i.e., color tuning) by incorporating conjugated aromatic π -electron donors or acceptors.¹⁷ Although the backbone modifications with biodegradable, non-conjugating, flexible units are perceived as an attractive approach for fine-tuning of the physical (i.e., aggregation control) and biophysical (i.e., biodegradability and cellular behavior) properties of CPEs, the backbone modulations of CPs have not been widely explored because the incorporation of non-conjugation units interrupts the π -electron delocalization through the backbone, resulting in a complete loss or dramatic reduction of the excellent photophysical properties of the materials. Therefore, there is an urgent need for developing synthetic methods

towards CPs with flexibility and biodegradability while preserving their photophysical excellence.

This chapter reports a novel synthetic technique to control flexibility in the backbone of PPEs by careful design of monomers and polymerization conditions without sacrificing their photophysical properties. The PPE was chosen as a model backbone to explore the flexibility structure-function relationships because the cross-coupling chemistry offers good experimental control of the resulting polymer structures. By co-polymerizing a flexible, biodegradable monomer with fully aromatic co-monomers under optimized Sonogashira cross-coupling reaction conditions, a series of PPEs containing flexible linkers along the backbone was successfully synthesized. For cellular applications, CPNs were fabricated by treating the polymers with organic acids followed by dialysis. Upon complexation of the corresponding nanoparticles with biologically relevant polyanion derivatives such as carboxymethyl cellulose (CMC), a clear difference in nanoparticle self-assembly behavior was observed between the rigid and flexible polymers.

4.3 Results and Discussion

Design concepts. The strategy towards improved experimental control over the length of the conjugated segments within the polymer chain employs the use of the flexible diacetylene monomer **B'** in a ternary monomeric system (Scheme 4.1). The copolymerization of the conjugated aromatic diacetylene **B** and the flexible diacetylene **B'** with the aryl halide monomer **A** will lead to the formation of PPEs containing conjugated blocks connected via the flexible functionality of **B'**. Under the assumption of equal reactivity of the two diacetylene monomers, the length of the conjugated blocks will be a function of the B/B' monomer feed ratio. For example, if the molar ratio of B/B' is 1 (a 50:50 mixture), statistically the resulting polymer should contain all pentameric conjugated blocks along the backbone (Figure 4.1, **P-50**, where the

number 50 in the polymer nomenclature denotes an average of 50% content of linker monomer **B'** as a proportion of the total amount of diacetylene monomers used). Due to the non-living character of the catalytic system and a subtle reactivity difference between the monomers, the formation of a small amount of shorter (i.e., trimer) and longer (i.e., heptamer) segments is also expected in addition to the majority of pentamers, giving rise to a statistical distribution of lengths. By controlling the B/B' monomer feed ratio it is still, however, possible to achieve a relative control over the average conjugation length due to the random statistical incorporation of the non-conjugated linker **B'**. The predicted average lengths of the conjugated segments as a function of the monomer ratios are summarized in Table 4.1.

Scheme 4.1. Synthesis of polymers **P-0** to **P-100**.

P-0 ($y = 0$) - fully conjugated PPE, **P-10** ($y = 0.10$), **P-25** ($y = 0.25$), **P-33** ($y = 0.33$), **P-50** ($y = 0.50$), **P-75** ($y = 0.75$), **P-100** ($y = 1$)

Figure 4.1. An illustration of conjugation length variation and relationship with UV absorbance maxima for **P-0**, **P-50** and **P-100**.

Table 4.1. Prediction of conjugation length (n) as a function of variable monomer ratios.

A (eq)	B (eq)	B' (eq)	n	y	Polymer
1	0	1	3	1	P-100
2	1	1	5	0.5	P-50
3	2	1	7	0.33	P-33
4	3	1	9	0.25	P-25
5	4	1	11	0.2	
6	5	1	13		
7	6	1	15		
8	7	1	17		
9	8	1	19		
10	9	1	21	0.1	P-10

Monomer **A** was decorated with ethylene oxide side-chains containing a pendant guanidinium group to enhance biocompatibility. This positively charged amine-rich functionality consisting of two delocalized primary amines and one secondary amine with a high pKa of around 13 has been employed in a wide variety of synthetic materials to improve solubility and enhance cellular uptake.¹⁸⁻²⁰ An aromatic diacetylene monomer **B** has been decorated with long ethylene oxide chains to preserve polymer solubility. The structure of the flexible, non-conjugated linker **B'** is derived from the naturally occurring biomolecule L-cystine featuring a cleavable disulfide bond, which provides the biodegradable feature of monomer **B'** in addition to offering flexibility in the backbone.²¹

Since the electronic and steric effects of aryl halide substituents govern the monomer reactivity under the Sonogashira reaction, the stoichiometry of the aryl iodide monomer **A** was kept constant throughout the polymer series. The reactivity of the acetylene-containing monomers **B** and **B'** is largely dependent on the acidity of the acetylene protons, which is similar for both diacetylene monomers. The Glaser homocoupling side-reaction among acetylene-containing

monomers **B** and **B'** was minimized by using a catalytic amount of copper (5%) and reaction temperatures not exceeding 50°C.

Polymerization. A series of polymers with a nearly controlled flexible linker content was prepared by the coupling of aryl iodide **A**, aryl alkyne **B** and flexible alkyne linker **B'** in variable proportions according to Scheme 4.1. Initial attempts to synthesize a polymer with the highest possible flexible content (i.e. **P-100**) using Sonogashira conditions typically employed for the formation of PPE polymer **P-0** ($\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$, CuI, morpholine, DMF) did not yield a polymer with considerable molecular weight, an observation contrary to polymerizations containing a structurally similar flexible monomer lacking the disulfide bond. Due to the lack of examples using disulfide-containing reactants in Sonogashira coupling reactions in the literature, together with reported complexation of thiols and disulfides with the palladium metal,²²⁻²³ we hypothesized that the presence of monomer **B'** in the Sonogashira coupling cycle inhibits the reaction through possible coordination of the palladium species, where the monomer acts as a bidentate ligand coordinating through the sulfur and nitrogen atoms. Sonogashira reactions often use ligands to accompany the palladium source for improved catalytic cycle efficiency.²⁴ Therefore the introduction of a ligand with stronger affinity than **B'** towards palladium should lead to efficient polymerization in the reaction system despite the presence of the disulfide-containing monomer.

A screening of reaction conditions using monomers **A** and **B'** under systematically varied combinations of $\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$, $\text{Pd}[(\text{PPh}_3)_4]$ and $\text{Pd}(\text{OAc})_2$ with bidentate ligands 1,4-bis(diphenylphosphino)-butane (dppb), 1,1'-bis(diphenylphosphino) ferrocene (dppf), and 2,2'-bipyridyl (bpy) showed that the presence of dppf in the coupling reaction greatly improves the resulting polymer molecular weights. The optimized reaction conditions (i.e., $\text{Pd}(\text{OAc})_2$, dppf, CuI, DMF/THF/DIPA) were, therefore, used to prepare the series of polymers from **P-0** to **P-100** (Scheme 4.1).

Polymer Characterization. All polymers with less than 50% of flexible content (i.e., **P-0** to **P-50**) are readily soluble in polar organic solvents such as DMF and DMSO, and exhibit high molecular weights and acceptable experimental yields as listed in Table 4.2. With higher flexible **B'** content, however, the polymer solubility is reduced, resulting in polymers **P-75** and **P-100** having significantly lower molecular weights. The experimental yields of **P-75** and **P-100** are correspondingly lower due to the removal of low-MW fragments during the polymer purification process by precipitation.

Table 4.2. Comparison of the average physical and photophysical properties of polymers **P-0** to **P-100**.

Polymer	A equiv.	B equiv.	B' (= y)	n ^a	M _n (g/mol) ^b	PDI ^c	Yield (%) ^d	λ _{max, abs} (nm) ^e	λ _{max, em} (nm) ^{e,f}	QY (%) ^g
P-0	1	1	0	PPE	46,700	1.79	54	447	476	21
P-10	1	0.90	0.10	21	41,800	1.85	61	434	475	20
P-25	1	0.75	0.25	9	34,200	1.69	66	422	474	15
P-33	1	0.67	0.33	7	22,200	2.44	61	416	473	20
P-50	1	0.50	0.50	5	21,400	1.69	48	363, 397	467	15
P-75	1	0.75	0.25	3 + 5	6,400	1.57	27	361	453	5
P-100	1	0	1	3	5,700	1.69	15	361	408	3

^a Statistically predominant number of phenylene rings within a conjugated segment. ^b Determined by gel permeation chromatography in THF. ^c PDI (polydispersity index) = Mw/Mn. ^d Percent yield per repeating unit after repeated purification by precipitation. ^e Measured in DMF. ^f Excitation wavelength 430, 430, 420, 415, 400, 360 and 360 nm in DMF. ^g Quantum yield in DMF measured relative to diphenylanthracene standard.

Proton NMR spectra of all polymers were consistent with their predicted average structure. The analysis of predicted copolymer structures examined the alkoxy protons on the side-chain of monomers **A** and **B'** (H_{EO}, ~4.2 ppm), the guanidinium NH protons characteristic of monomer **A** (H_A, combination of ~11.4 and ~12.4 ppm), the methoxy protons characteristic of monomer **B**

(~3.2 ppm), and the aromatic protons ($H_{B'}$, ~7.45 and ~7.65 ppm) and amide NH ($H_{B''}$, ~10.4 ppm) characteristic of monomer **B'**. All proton peaks were integrated relative to the ethylene oxide proton peak H_{EO} , and were in good agreement with the integration values predicted by theoretical copolymer analysis. An example spectrum of polymer **P-10** is presented in Figure 4.2. The observed integration ratios for all polymers are reported in the Table 4.3 together with percent deviations from predicted values. While 1H NMR data point towards near stoichiometrically controlled **B'** incorporation, it is worth noting that the technique only provides information about the average conjugation length.

Figure 4.2. 1H NMR spectrum of polymer **P-10**. Based on the **B/B'** feeding ratio (0.9:0.1), the predicted polymer structure calls for H_{EO} proton integration of 3.8 ($0.9 \times 4H + 0.1 \times 2H$). The observed integrations of the remaining peaks correspond well to the predicted values: for H_A , predicted 1H ($0.9 \times 1H + 0.1 \times 1H$), observed 0.97 (0.23 + 0.74), for $H_{B'}$, predicted 0.2H ($0.1 \times 2H$), observed 0.32 (average of 0.23 and 0.41), and for $H_{B''}$, predicted 0.1, observed 0.1.

Table 4.3. ¹H NMR integration table of polymers **P-0** to **P-75**.

Polymer	x	Theoretical integration ^a				Observed integration ^b			
		H _{EO}	H _A	H _B	H _{B'}	H _{EO}	H _A (Δ ^c)	H _B (Δ ^c) ^d	H _{B'} (Δ ^c)
P-0	0	4.0	1	0	0	4.0	1.17 (+17%)	0	0
P-10	0.1	3.8	1	0.2	0.1	3.8	0.97 (-3%)	0.32 (+60%)	0.10 (0%)
P-25	0.25	3.5	1	0.5	0.25	3.5	0.99 (-1%)	0.43 (-14%)	0.18 (-28%)
P-33	0.33	3.34	1	0.67	0.33	3.34	1.03 (+3%)	0.67 (0%)	0.21 (-36%)
P-50	0.50	3.0	1	1	0.5	3.0	1.05 (+5%)	0.93 (-7%)	0.42 (-16%)
P-75	0.75	2.5	1	1.5	0.75	2.5	1.25 (+25%)	1.14 (-24%)	0.49 (-35%)

^a Based on B:B' feed ratio. ^b H_{EO} fixed at theoretical amount. H_A, H_B and H_{B'} are relative to H_{EO}. ^c Percent error relative to predicted values. ^d Value obtained by averaging the integration of peaks at ~7.45 and ~7.65 ppm.

Absorption Spectra. The increasing flexible linker content is apparent through a blue shift in the absorption maxima (Figure 4.3A) indicating the decrease in effective conjugation length of the phenylene ethylene segments (Figure 4.1). The fully conjugated PPE polymer (i.e., **P-0**) is composed of alternating A and B units and exhibits maximum absorbance at $\lambda_{\text{max}} = 447$ nm. On the other hand, polymer **P-100**, containing only alternating A and B' units which form electronically isolated species containing three phenylene rings conjugated through two ethylene bonds (i.e., trimers, $n = 3$) connected via non-conjugated cystine linkers, exhibits a characteristic blue-shifted maximum at 361 nm similar to conjugated compounds with short conjugation length. Polymer **P-50**, in which the two B-type acetylene monomers are in an equimolar ratio, is statistically expected to contain a majority of conjugated pentamers ($n = 5$), although segments of both shorter and longer conjugation length are predicted to be present due to the non-living characteristics of the polymerization and the different reactivity of the monomers.

Figure 4.3. Normalized absorption (A) and emission (B) spectra of polymers **P-0** to **P-100** in DMF.

As expected, **P-50** exhibits two distinct maxima, one comparable to **P-100** ($\lambda_{\text{max}} = 363$ nm, $n = 3$) and one red-shifted by 34 nm ($\lambda_{\text{max}} = 397$ nm) with a slight shoulder at even longer wavelengths, indicating the presence of conjugated segments with $n = 5$ and longer. Interestingly **P-75**, which is statistically expected to contain a mixture of $n = 3$ and $n = 5$ segments, exhibits two apparent shoulders at higher wavelengths comparable to the absorption maxima for **P-50** ($n =$

5) and **P-33** ($n = 7$), respectively. This observation indicates that segments of longer conjugation length are being formed in significant proportions despite statistical predictions, and suggests that the relative incorporation of **B** and **B'** may therefore not be fully random. However, the amount of these longer segments cannot be quantified from the UV spectra alone, as species with longer conjugation exhibit increasingly larger extinction coefficients. In polymers with less than 50% flexible linker content (such as **P-33**, $n = 7$) the contribution of the $n = 3$ absorption at ~ 361 nm is significantly reduced and the increased conjugation is indicated by a further gradual red-shift of the maximum absorbance from 416 nm (**P-33**), through 422 nm (**P-25**) and 434 nm (**P-10**) towards the fully-conjugated PPE **P-0** at 447 nm.

Fluorescence Spectra. Polymers with 50% or less linker **B'** content retain the emissive properties of a fully conjugated PPE backbone (Figure 4.3B). The quantum yields of polymers **P-50** to **P-10** fluctuate between 15 and 20% and are comparable to the quantum yield of PPE polymer **P-0** (21%). Similarly, there is only minimal red shift of 9 nm between the emission of **P-50** ($\lambda_{\text{max}} = 467$ nm, $n = 5$) and **P-0** ($\lambda_{\text{max}} = 476$ nm, PPE).

The trend in the emission profiles of polymers **P-100**, **P-75** and **P-50** is somewhat interesting. Polymer **P-100** exhibits blue fluorescence at 408 nm with a defined vibronic structure due to the fluorescence signal being emitted mainly from the shortest possible conjugated segments ($n = 3$). Polymer **P-50** is predicted to contain predominantly pentamer fragments ($n = 5$). Its emission maximum is significantly red-shifted at 467 nm (~ 60 nm Stokes shift) and virtually no emission is seen at the wavelength of the trimer species (408 nm) despite the UV profile of **P-50** indicating the presence of $n = 3$ segments. Polymer **P-75** ($n = 3$ and 5) shows two emission maxima, one at the wavelength of the trimer (408 nm) and one corresponding to pentamer with significantly higher intensity and appreciably red shifted (453 nm). In addition to possible different quantum yields between the $n = 3$ and $n = 5$ species, the discrepancy in the intensities of the two emission events in the spectrum of **P-75** can be explained by resonance energy transfer (RET), which

results from the overlap between the trimer emission and the pentamer absorption maxima (408 nm and 397 nm, respectively). The photoexcited states are transferred from the shorter, higher-energy trimer units to the longer, lower energy pentamers, resulting in the pentamer units being the major emitting species.²⁵ This explanation is consistent with the observed **P-75** emission spectrum, which features the predominant red-shifted emission at 453 nm. Previous studies of structurally related conjugated poly(*p*-phenylenevinylene)s (PPVs) observed a similar trend.²⁶ Furthermore, the emission peaks of the shorter polymers undergoing RET are very broad, while polymers with increased conjugation length and consequently decreased amount of RET (i.e., **P-0** and **P-10**) feature a clear vibrational structure indicating increased uniformity of the emissive species. No further significant red-shifting of emission maxima occurs with additional increase in conjugation length over pentamer (i.e., from **P-50** to **P-0**), suggesting that the effective conjugation length for this series of PPEs is reached with approximately 5 aromatic segments. Theoretical calculations on a series of oligo *p*-phenylene-ethynylenes agree that while in perfectly planar systems the effective conjugation length is saturated at around 10 aromatic units, this number decreases to around 5 when the planarity of the conjugated segment is disturbed.²⁷ Thus by keeping the amount of the flexible linker below 50% (i.e., **P-50** or longer), flexible polymers can be synthesized with good solubility and photophysical properties comparable to those of a fully-conjugated PPE polymer backbone, while providing additional biodegradability and controllable aggregation properties through the flexible linkers.

Mechanistic Considerations. Ideally, the length of the conjugated phenylene ethynylene fragments is determined by the **B/B'** feed ratio. However, due to the nature of the three-monomer system, the predicted fragment length will not form exclusively but instead there will be a distribution of lengths with an average determined by the feed ratio. The flexible monomer **B'** contains a biodegradable cystine moiety, and the degradation of its disulfide linkers in the

polymer chain under reducing conditions should therefore yield a mixture of thiol-capped conjugated fragments which can be separated, characterized and quantified to determine the exact composition of the copolymers.

Figure 4.4. Degradation experiment: Overnight incubation of a solution of **P-50** in THF with excess PBU_3 .

Contrary to expectations, the degradation experiments yielded a mixture of fragments, which were much larger than predicted by the monomer feed ratio (Figure 4.4), indicating that the incorporation of the linkers **B'** into the backbone did not proceed in a statistical, random fashion. Initially, this observation was ascribed to a possible difference in reactivity between the two acetylene monomers. The factors that determine their reactivity towards Sonogashira coupling are widely unexplored, but steric effects arising due to monomer size and flexible structure could slow down the rate of transmetallation of **B'** relative to **B**. Indeed, treating an equimolar mixture of **B** and **B'** with an excess of a model capping compound *p*-iodoanisole demonstrated that while all of **B** has undergone Sonogashira coupling, a significant amount of **B'** remained unreacted within the first hour of the time-course experiment.

Based on the reactivity difference, we hypothesized that in the polymerization reaction the fully conjugated monomer **B** undergoes preferential Sonogashira coupling with the halide monomer **A**, and a new A-B-A species will be formed before any **B'** monomer reacts to an appreciable extent. If allowed sufficient time, these A-B-A-type segments will subsequently be linked together into a

polymer chain through monomer **B'**. To test the hypothesis, the synthesis of polymer **P-25** was carried out with the sequential addition of monomers. **A** (1 eq) and **B** (0.75 eq) were allowed to react for 2 hours in the absence of linker **B'**. The resulting conjugated oligomers **P-25a** exhibited photophysical properties similar to the one-pot polymer **P-25**. When the remaining monomer **B'** (0.25 eq) was added and the polymerization was allowed to proceed overnight, polymer **P-25b** exhibited a significant increase in polymer molecular weight relative to oligomers **P-25a** but at the same time retained photophysical properties analogous to those of **P-25a** and the one-pot polymer **P-25** (Table 4.4). Additionally, the ^1H NMR spectrum of **P-25b** clearly demonstrates the incorporation of linker **B'** into the polymer backbone (Figure 4.5). All spectral evidence therefore supports our hypothesis that the A-B conjugated segments are formed first and then linked into a polymer through linkers **B'**.

Table 4.4. Comparative characteristics of sequential addition polymer **P-25b**.

Polymer	A (eq)	B (eq)	B' (eq)	M_n (g/mol)	PDI	$\lambda_{\text{max, abs}}$ (nm)	$\lambda_{\text{max, em}}$ (nm)	QY (%)
P-0	1	1	0	46,700	1.79	447	476	21
P-25	1	0.75	0.25	34,200	1.69	422	474	15
P-25a	1	0.75	-	5,000	1.46	429	474	15
P-25b	1	0.75	0.25	26,100	2.29	427	475	17

Figure 4.5. ^1H NMR spectrum of **P-25b** formed in the sequential addition experiment.

Figure 4.6. GPC trace illustrating conjugated oligomer distribution at an A:B monomer feed ratio of 4:1, statistically favoring the formation of trimers ($n = 3$).

The distribution of oligomers formed within the first two hours of the sequential addition experiment (**P-25b**) was heavily skewed towards larger molecular weights as observed by GPC, and prompted an investigation of this distribution as a function of the A/B monomer ratio. The ratio of acetylene monomer **B** relative to aryl iodide **A** was systematically decreased, and even at the A:B ratio of 4:1 (i.e., large excess of aryl iodides, which is conducive to the statistical

formation of conjugated trimers) the formation of several distinct larger molecular weight fragments in addition to trimers was observed by GPC (Figure 4.6).

In the polymer series reported in this chapter, the distribution of conjugated segment lengths is governed by the mechanism by which the conjugated A-B-A-type oligomers are formed before being linked into the polymer through **B'**. The formation of oligomers with MW distribution heavily skewed to large molecular weights therefore explains the larger-than-expected fragments observed in the degradation experiments. However, this phenomenon cannot be explained by statistical considerations involving the monomer feed ratios.

A possible explanation could be provided through end-group activation. According to recent reports,²⁸⁻²⁹ activation of the end groups of the new **A-B-A** species through extended conjugation could enhance the reactivity of the new species and propagate the growth of conjugated fragments in a chain-growth manner at the expense of the formation of lower molecular weight species. The propagation would occur until all available **B** monomers are depleted, at which time the larger, activated conjugated fragments will preferentially be linked together by reaction with the flexible linkers **B'**. Any low-MW fragments not incorporated into the polymer backbone will be removed during the purification procedure, resulting in polymers with longer-than-predicted conjugated fragments and lowered experimental yields as observed.

Cellular toxicity. The toxicity of the **P-0**, **P-25** and **P-50** CPNs was evaluated by MTT assay (Figure 4.7). All polymers exhibited cytotoxicity in HeLa cells at relatively low concentrations (>5 μ M). According to previous studies, regular rigid PPEs exhibit little or no cellular toxicity.¹³ In contrast, polymer **P-0**, which differs from these PPEs only by the structure of its side-chain, is toxic. It is, therefore, possible that the cellular toxicity of this series of polymers is caused by the presence of the guanidinium group and is not correlated to the linker content. There is no apparent correlation between the proportion of flexible linker and the measured degree of cell

viability. However, due to the relatively large standard deviations of the replicate measurements, no definitive conclusions can be made regarding the role of the linker in cellular toxicity without further investigation. The synthesis of a new series of polymers containing quarternary triethylamine salt in place of the guanidinium group is currently under way. The new series, which is predicted to have lower cellular toxicity, will allow for a more accurate investigation of the cellular behavior of the CPNs as a function of linker content.

Figure 4.7. Cell viability evaluation by MTT assay ($n = 3$). All polymers show toxicity at concentrations greater than 5 μM .

CPN/CMC Complex Formation. Naturally-derived, synthetically modified anionic polysaccharides such as carboxymethyl cellulose (CMC) are biocompatible materials with emerging use in the design of controlled-release drug delivery systems.³⁰ Cationic oligo *p*-phenyleneethynylenes (OPEs) with short conjugated segment lengths (up to $n = 7$) have been shown to exhibit unique self-assembly properties upon complexation with CMC. Changes in ionic and hydrophobic interactions of the OPEs upon CMC complexation contributed to dramatic shifts in absorbance and fluorescence maxima due to increased planarization of the

phenyleneethynylene backbone.³¹ Analogously, due to the polymer backbone flexibility and the controlled length of conjugated segments within our series of polymers, it could be expected that CPs with different amount of flexible linker will show different interaction with CMC.

Figure 4.8. Absorption, emission and hydrodynamic radius distribution spectra of **P-0** (top row), **P-25** (middle row) and **P-50** (bottom row) CPNs before and after complexation with 10 μM CMC.

Polymers **P-0**, **P-25** and **P-50** were fabricated into CPNs and complexed with 10 μM CMC for 10 min. Absorption and emission spectra and mean particle diameter were measured for each polymer before and after CMC complexation (Figure 4.8). The relatively broad emission spectra suggest the presence of different aggregation states, which makes unambiguous explanation of CPN-CMC interaction difficult without further studies using single-molecular spectroscopy.³² The Moon research group has shown previously that amine-containing PPEs form core-shell-like

structures with different levels of aggregation.³³ It is speculated that CMC partially intercalates between the CPN polymer chains in the shell, leading to better solvation, looser polymer aggregation in the shell, and consequently to an increased mean particle diameter. However, CMC complexation also leads to fluorescence quenching, presumably due to the increased hydrophobic interactions among PPEs within the CPN core caused by the addition of hydrophilic CMC into the shell. The backbone aggregation in the CPN core also gives rise to a slight red shoulder in the absorption spectrum of **P-0**.

Although the behavior of the three polymers towards CMC is somewhat similar, the complexation effects are most pronounced in **P-0**. **P-0** CPNs have a fully conjugated rigid backbone and exhibit high aggregation in aqueous environment, as evidenced by the strong excimer peak in the emission spectrum at around 500 nm (Figure 4). Upon CMC complexation, the excimer peak is significantly reduced, and the resulting spectrum resembles those of **P-25** and **P-50** CMC complexes. With the introduction of some flexibility into the CPN (i.e., **P-25**), the changes in aggregation upon CMC complexation are not as pronounced. This observation can be ascribed to the decreased hydrophobic interactions among flexible polymer chains. Previously, it was observed that in the case of flexible PE-d-PPBs, the presence of a small amount of linker functioned to increase interpolymer interactions upon complexation.¹⁶ In the case of PPBs, the hydrophobic interaction among CPs is relatively high owing to the intrinsic hydrophobicity of the backbone. In the PPE series presented in this work, the backbone is less hydrophobic and the effect of the flexible linker is therefore somewhat diminished. Consequently, CMC complexation of the most flexible polymer in the series, **P-50**, resulted in only minimal changes in photophysical and physical properties. It is speculated that the low impact of CMC complexation on the flexible CPN properties is likely due to the solvation effect of CMC and decreased CPN interpolymer hydrophobic interactions caused by the added flexibility. The CPNs of the flexible polymer **P-50** already exhibit a broad distribution of particle sizes, which is indicative of loose

aggregation. Upon complexation, CMC intercalation in between the loosely aggregated chains does not induce significant disaggregation and, therefore, results only in minimal changes in emission spectra and particle diameter distribution. Contrary to **P-0**, the UV spectra of **P-25** and **P-50** do not show a red-shifted shoulder. The flexible polymers contain relatively short conjugated segments, and any further aggregation effects on the core of CPNs resulting from CMC complexation are, therefore, diminished.

4.4 Conclusion

In conclusion, this chapter reports the synthesis of a series of PPE copolymers with nearly controlled amount of flexible component and preserved optical properties of a fully conjugated PPE backbone. Variable proportion of flexible linkers can be achieved by employing a ternary monomer coupling approach under Sonogashira reaction conditions. Increased amount of flexible linkers in the conjugated PPE backbone results in the formation of PE segments with shorter average conjugation length as evidenced by a clear hypsochromic trend in UV absorbance spectra. Materials with less than 50% of flexible linker exhibit fluorescence properties comparable to those of a fully conjugated PPE backbone. The self-assembly behavior of the corresponding nanoparticles is clearly different between the rigid and flexible polymers upon polyanion complexation. Since the degradation kinetics of these biodegradable materials is affected by their aggregation properties, the presented series of polymers lends itself to further investigation into the use of flexible PPEs in controlled-release cellular delivery of biologically active materials.

4.5 Outlook

Due to the cellular toxicity associated with the guanidinium side-chains, the potential of the reported series of polymers could not be fully realized. The synthetic methodology presented in

this chapter can be utilized to prepare a different series of polymers with variable linker content containing side-chains exhibiting lower toxicity, such as cleavable guanidinium-containing side-chains, regular primary amines, or perhaps other cationic moieties such as triphenylphosphines.

Another avenue, which can be pursued as a continuation of this project, is the investigation of the distribution of conjugated lengths within the polymer chain. The disulfide functionality can be cleaved under reducing conditions using tributyl phosphine,³²⁻³⁴ dithiotreitol (DTT),³⁵⁻³⁶ or glutathione (GSH).³⁷ Separation and isolation of the resulting thiol-capped conjugated fragments can provide a way to confirm their chemical structure and thus their conjugation length. The quantitation of the relative amount of each component can help advance the understanding of the mechanistic processes occurring during the polymerization reaction.

Better control over the conjugation length can be pursued through the combination of chain-growth approach and smart monomer design. A non-symmetrical, flexible non-conjugated linker can serve two purposes. Firstly, its highly reactive end will act as chain-growth initiator. Upon chain propagation, fragments of equal length will form via chain-growth of unsymmetrical conjugated monomer (Scheme 4.2, step 1). Secondly, the less reactive end of the linker will undergo Sonogashira coupling with the chain end, joining the chain-growth fragments together (Scheme 4.2, step 2). The resulting polymer will contain conjugated blocks of well-defined length formed through chain-growth polymerization, as well as the flexible, non-conjugated linker. The success of the proposed synthesis relies on the differences in reactivity among the monomers and the species formed in the reaction. Therefore, optimizing the monomer structures to satisfy all of these kinetic requirements would give rise to an entirely new research project.

Scheme 4.2. A suggested route to flexible polymers with controlled block conjugation length using chain-growth polymerization approach.

4.6 Experimental Section

4.6.1 General. Chemicals, including solvents, were purchased from Fisher Scientific and used as received. N,N' -Di-Boc-1H-pyrazole-1-carboxamidine was purchased from Ark Pharm, Inc. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Cambridge, MA). The average molecular weight (M_n) and polydispersity index ($\text{PDI} = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC) against polystyrene standards using a Shimadzu high performance liquid chromatography (HPLC) system fitted with PLgel $5\mu\text{m}$ MIXED-D columns and SPD-20A ultraviolet-visible (UV-vis) detector at a flow rate of 1.0 mL/min. UV-vis spectra were recorded using a Varian Cary 50 Bio spectrophotometer. Fluorescence spectra were obtained using a FluoroLog-3 Spectrofluorometer (Jobin Yvon/Horiba). 9,10-diphenylanthracene ($\text{QY} = 0.9$) in cyclohexane was used as a fluorescence standard for QY determination. Fourier transform infrared (FT-IR) spectra were recorded on a

PerkinElmer Spectrum 100 FT-IR Spectrometer. Fine polymer powders were directly mounted on an attenuated total reflection (ATR) cell of the spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz Avance Bruker NMR spectrometer. Chemical shifts were reported in parts per million (ppm) for ^1H NMR on the δ scale based on the middle peak ($\delta = 2.50$ ppm) of the dimethylsulfoxide (DMSO)- d_6 solvent as an internal standard. Nanoparticle hydrodynamic diameter was determined using single particle light scattering analysis. Measurements were performed with a LM10 HS (NanoSight, Amesbury, United Kingdom), equipped with a sCMOS camera, sample chamber with a 488 nm blue laser, and Viton fluoroelastomer o-ring. The samples were injected into the sample chamber with 1 mL sterile syringes (Restek Corporation, Pennsylvania, USA) until the liquid reached the tip of the nozzle. All measurements were performed in triplicate at 25°C using a LM14C temperature controller (NanoSight, Amesbury, United Kingdom).

4.6.2 Monomer Synthesis.

Scheme 4.3. Synthetic route towards monomer **A**.

Synthesis of guanidinium-containing aryl halide monomer A (Scheme 4.3): Compound **1** (1.0g, 1.87 mmol, synthesized according to literature procedures³⁸) and N,N' -Di-Boc-1H-pyrazole-1-carboxamide (1.16g, 3.73 mmol) were placed under a nitrogen atmosphere in a two-neck round-

bottom flask fitted with a stir bar and a septum. A mixture of acetonitrile and dichloromethane (DCM) (1:2, 20 mL) was degassed with N₂ flow for 15 minutes and transferred into the reaction flask via cannula. The reaction mixture was allowed to stir at room temperature overnight, quenched with water and the two layers were separated. The aqueous layer was extracted with DCM and the combined organic layers were washed with brine, filtered and concentrated in vacuo. Column chromatography under 30% of ethyl acetate in hexane yielded monomer A as a white powder (597 mg, 31% yield). ¹H NMR (400 MHz, DMSO-d₆, δ): 11.46 (s, 1H), 8.42 (t, J = 5.3 Hz, 1H), 7.35 (s, 1H), 4.09 (t, J = 4.4 Hz, 2H), 3.77 (77, J = 4.3 Hz, 2H), 3.66 (t, J = 5.6 Hz, 2H), 3.49 (t, J = 5.2 Hz, 2H), 1.43 (s, 9H), 1.39 (s, 9H) (Figure 4.9). ¹³C NMR (150 MHz, δ): 163.0, 155.4, 152.5, 152.0, 122.7, 86.8, 82.9, 78.1, 69.7, 68.6, 68.5, 28.0, 27.6 (Figure 4.10) FT-IR (neat): 3329, 2978, 2929, 2851, 1719, 1665, 1639, 1614, 1573, 1516, 1482, 1466, 1406, 1394, 1326, 1276, 1242, 1155, 1125, 1048, 1024 cm⁻¹(Figure 4.11). HRMS (ESI⁺, m/z): [M+H⁺] calcd. for C₃₆H₅₈I₂N₆O₁₂, 1021.2275; found, 1021.2008.

Figure 4.9. ¹H NMR spectrum of monomer A.

Figure 4.10. ¹³C NMR spectrum of monomer A.

Figure 4.11. FT-IR spectrum of monomer A.

Synthesis of monomer B: Monomer **B** was synthesized according to literature procedures.¹³

Scheme 4.4. Synthetic route towards monomer **B'**.

*Synthesis of flexible linker **B'*** (Scheme 4.4): Boc-protected L-cystine **2** (800 mg, 1.82 mmol) was dissolved in anhydrous tetrahydrofuran (THF) (20 mL) in a round bottom flask, which was evacuated and filled with nitrogen. N-methylmorpholine (0.42 mL, 3.81 mmol) was added, and the suspension was allowed to stir until the entire solid was dissolved. Isobutyl chloroformate (0.48 mL, 3.67 mmol) was added, and the mixture was allowed to stir at room temperature for 15 min. A solution of 4-ethynylaniline (447 mg, 3.81 mmol) in anhydrous THF (10 mL) was prepared under a N₂ atmosphere and transferred into the reaction flask using a cannula. The reaction was allowed to proceed overnight, after which the reaction mixture was filtered. The filtrate was concentrated in vacuo, and the resulting solid was precipitated overnight from the DCM / diethyl ether solvent system. Yield: 419 mg (36%). ¹H NMR (400 MHz, DMSO-d₆, δ): 10.29 (s, 1H), 7.62 (d, J = 8.59 Hz, 2H), 7.41 (d, J = 8.59 Hz, 2H), 7.27 (d, J = 8.08 Hz, 1H), 4.35 (m, 1H), 3.17 (dd, J = 13.26, 4.93 Hz, 1H), 2.96 (dd, J = 12.88, 9.85 Hz, 1H), 1.37 (s, 9H) (Figure 4.12). ¹³C NMR (150 MHz, δ): 169.4, 155.3, 139.3, 132.3, 119.3, 116.4, 83.5, 80.0, 78.4, 54.5, 40.7, 28.1 (Figure 4.13). FT-IR (neat): 3323, 2982, 1699, 1675, 1665, 1608, 1586, 1516, 1407, 1393, 1369, 1307, 1272, 1250, 1161 cm⁻¹ (Figure 4.14). HRMS (ESI⁺, m/z): [M+Na⁺] calcd. for C₃₂H₃₈N₄O₆S₂, 661.2125; found 661.2085.

Figure 4.12. ¹H NMR spectrum of monomer B'.

Figure 4.13. ¹³C NMR spectrum of monomer B'.

Figure 4.14. FT-IR spectrum of monomer **B'**.

4.6.3 Polymer synthesis

General procedure: A Schlenk flask was charged with monomers **A**, **B** and **B'**, Pd(OAc)₂ (0.2 eq), 1,1'-bis(diphenylphosphino)ferrocene (dppf) (0.5 eq) and CuI (0.05 eq). The Schlenk flask was evacuated and filled with N₂. A solution of dimethylformamide (DMF) (3 mL), tetrahydrofuran (THF) (1 mL) and diisopropylamine (DIPA) (1 mL) was degassed with N₂, and 2 mL of the mixed solution was transferred to the Schlenk flask via a cannula. The reaction was heated at 50°C for 18 h. The solution was then cooled to room temperature and transferred dropwise to cold ether, resulting in precipitation. After centrifugation (5 min, 6,000 rpm), the supernatant was decanted, the precipitate was re-dissolved in DMF (1 mL) and the purification method was repeated using ether/methanol. The resulting polymer in DMF (1mL) was characterized by gel permeation chromatography (GPC) and its absorption/emission profile was measured. The material was then reprecipitated in pure ether, the supernatant was decanted and the precipitated polymer was allowed to dry under high vacuum for 4 hours prior to FTIR and ¹H NMR characterization.

P-0: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol) and **B** (13.2 mg, 0.0294 mmol) in the presence of Pd(OAc)₂ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PPE polymer **P-0** (19.4 mg, 54 %). ¹H NMR (400 MHz, DMSO-d₆): δ 12.71 (s, 0.32H), 11.45 (s, 0.85H), 8.43 (s, 0.84H), 8.10 (s, 0.32H), 7.13 (s, 2.16H), 4.20 (br m, 4.00H), 3.85-3.40 (br m, 18.04H), 3.19 (s, 4.31H), 1.41-1.36 (d, 19.67H) (Figure 4.15). FT-IR (neat): 3334, 2974, 2930, 2873, 1720, 1677, 1634, 1576, 1411, 1322, 1218, 1131, 1051, 1022 cm⁻¹. GPC: M_w = 83,800 g/mol, M_n = 46,700 g/mol, PDI = 1.79. UV-Vis (DMF) λ_{max} = 447 nm, Fluo λ_{max} = 476 nm, QY = 21%.

Figure 4.15. ¹H NMR spectrum of polymer **P-0**.

P-10: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol), **B** (11.9 mg, 0.0265 mmol) and **B'** (1.9 mg, 0.00294 mmol) in the presence of Pd(OAc)₂ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PE-d-PPE polymer **P-10** (22.0 mg, 61 %). ¹H NMR (400 MHz, DMSO-d₆): δ 12.71 (s, 0.23H), 11.44 (s, 0.74H), 10.37 (br s, 0.10H), 8.45 (s, 0.68H), 8.10 (s, 0.28H), 7.68 (s, 0.23H), 7.44 (s, 0.41H), 7.12 (s, 1.94H), 4.20 (br s, 3.81H), 3.85-3.47 (br m, 16.12H), 3.18 (s, 3.15H), 1.41-1.36 (s, 17.06H) (Figure 4.16). FT-IR (neat): 3336, 2975, 2930,

2872, 1718, 1634, 1583, 1411, 1322, 1218, 1131, 1049, 1022 cm^{-1} . GPC: $M_w = 77,400$ g/mol, $M_n = 41,800$ g/mol, PDI = 1.85. UV-Vis (DMF) $\lambda_{\text{max}} = 434$ nm, Fluo $\lambda_{\text{max}} = 475$ nm, QY = 20%.

Figure 4.16. ^1H NMR spectrum of polymer **P-10**.

P-25: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol), **B** (9.9 mg, 0.0220 mmol) and **B'** (4.7 mg, 0.00735 mmol) in the presence of $\text{Pd}(\text{OAc})_2$ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PE-d-PPE polymer **P-25** (24.5 mg, 66 %). ^1H NMR (400 MHz, DMSO-d_6): δ 12.71 (s, 0.26H), 11.45 (s, 0.73H), 10.37 (br s, 0.18H), 8.43 (s, 0.70H), 8.19 (s, 0.26H), 7.68 (s, 0.36H), 7.44 (s, 0.49H), 7.12 (s, 1.74H), 4.19 (br s, 3.49H), 3.84-3.46 (br m, 13.53H), 3.18 (s, 2.64H), 1.40-1.36 (d, 18.49H) (Figure 4.17). FT-IR (neat): 3332, 2975, 2930, 2873, 1718, 1634, 1583, 1410, 1220, 1131, 1050, 1022 cm^{-1} (Figure 4.18). GPC: $M_w = 57,600$ g/mol, $M_n = 34,200$ g/mol, PDI = 1.69. UV-Vis (DMF) $\lambda_{\text{max}} = 422$ nm, Fluo $\lambda_{\text{max}} = 474$ nm, QY = 15%.

Figure 4.17. ^1H NMR spectrum of polymer **P-25**.

Figure 4.18. FT-IR spectrum of polymer **P-25**. All other polymers gave similar IR spectra.

P-33: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol), **B** (8.9 mg, 0.0197 mmol) and **B'** (6.2 mg, 0.00970 mmol) in the presence of $\text{Pd}(\text{OAc})_2$ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PE-d-PPE polymer **P-33** (22.9 mg, 61 %). ^1H NMR (400 MHz, DMSO-d_6): δ 12.71 (s, 0.23H), 11.44 (s, 0.80H), 10.37 (br s, 0.21H), 8.42 (s, 0.79H), 8.08 (s, 0.25H), 7.67 (s, 0.55H), 7.44 (s, 0.79H), 7.12 (s, 1.64H), 4.19 (br s, 3.34H), 3.84-3.46 (br m,

12.66H), 3.18 (s, 2.18H), 1.40-1.35 (d, 19.51H) (Figure 4.19). FT-IR (neat): 3331, 2976, 2930, 2873, 1718, 1635, 1410, 1320, 1132, 1049, 1021 cm^{-1} . GPC: $M_w = 54,300$ g/mol, $M_n = 22,200$ g/mol, PDI = 2.44. UV-Vis (DMF) $\lambda_{\text{max}} = 416$ nm, Fluo $\lambda_{\text{max}} = 473$ nm, QY = 20%.

Figure 4.19. ^1H NMR spectrum of polymer **P-33**.

P-50: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol), **B** (6.6 mg, 0.0147 mmol) and **B'** (9.4 mg, 0.00147 mmol) in the presence of $\text{Pd}(\text{OAc})_2$ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PE-d-PPE polymer **P-50** (18.3 mg, 48 %). ^1H NMR (400 MHz, DMSO-d_6): δ 12.71 (m, 0.38H), 11.44 (d, 0.67H), 10.34 (br s, 0.42H), 8.39 (s, 0.66H), 8.07 (s, 0.41H), 7.67 (s, 0.88H), 7.42 (s, 0.97H), 7.12 (s, 1.46H), 4.18 (br s, 3.01H), 3.82-3.45 (br m, 11.49H), 3.18 (s, 2.10H), 1.39-1.35 (s, 21.49H) (Figure 4.20). FT-IR (neat): 3329, 2975, 2931, 2873, 1717, 1628, 1584, 1408, 1321, 1133, 1049, 1021 cm^{-1} . GPC: $M_w = 36,200$ g/mol, $M_n = 21,400$ g/mol, PDI = 1.69. UV-Vis (DMF) $\lambda_{\text{max}} = 398$ nm, Fluo $\lambda_{\text{max}} = 467$ nm, QY = 15%.

Figure 4.20. ^1H NMR spectrum of polymer **P-50**.

P-75: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol), **B** (3.3 mg, 0.00734 mmol) and **B'** (14.1 mg, 0.00220 mmol) in the presence of $\text{Pd}(\text{OAc})_2$ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PE-d-PPE polymer **P-75** (10.7 mg, 27 %). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 12.40 (m, 0.48H), 11.44 (d, 0.77H), 10.37 (br s, 0.49H), 8.40 (s, 0.60H), 8.17 (s, 0.35H), 7.66 (s, 1.07H), 7.42 (s, 1.21H), 7.05 (s, 0.99H), 4.17 (br s, 2.51H), 3.82-3.46 (br m, 12.21H), 3.18 (s, 1.31H), 1.39 (s, 24.19H) (Figure 4.21). FT-IR (neat): 3325, 2978, 2932, 2870, 1717, 1630, 1588, 1513, 1407, 1386, 1318, 1246, 1136, 1050, 1021 cm^{-1} . GPC: $M_w = 10,100$ g/mol, $M_n = 6,400$ g/mol, PDI = 1.57. UV-Vis (DMF) $\lambda_{\text{max}} = 361$ nm, Fluo $\lambda_{\text{max}} = 453$ nm, QY = 5%.

Figure 4.21. ^1H NMR spectrum of polymer **P-75**.

P-100: Using the general polymerization procedure described above, the polymerization of monomers **A** (30.0 mg, 0.0294 mmol) and **B'** (18.8 mg, 0.0294 mmol) in the presence of $\text{Pd}(\text{OAc})_2$ (1.3 mg, 0.00588 mmol), dppf (8.1 mg, 0.0147 mmol) and CuI (0.3 mg, 0.00147 mmol) yielded PE-d-PPE polymer **P-100** (6.2 mg, 15 %). ^1H NMR (400 MHz, CDCl_3): δ 12.27 (m, 0.28H), 11.43 (d, 0.76H), 8.67 (br s, 1.35H), 8.21 (s, 0.34H), 7.58 (s, 1.55H), 7.43 (s, 2.01H), 7.02-6.90 (m, 1.00H), 4.22-4.11 (m, 2.90H), 3.86-3.60 (m, 6.43H), 3.26-3.21 (m, 0.82H), 3.07 (m, 0.78H), 2.83 (m, 0.81H), 2.68 (m, 0.82H), 1.46-1.44 (m, 21.94H), 1.25 (m, 15.77H) (Figure 4.22). FT-IR (neat): 3331, 2977, 2932, 2873, 1718, 1623, 1592, 1514, 1408, 1367, 1323, 1248, 1159, 1140, 1050, 1023 cm^{-1} . GPC: $M_w = 9,600$ g/mol, $M_n = 5,700$ g/mol, PDI = 1.69. UV-Vis (DMF) $\lambda_{\text{max}} = 361$ nm, Fluo $\lambda_{\text{max}} = 408$ nm, QY = 3%.

Figure 4.22. ^1H NMR (CDCl_3) spectrum of polymer **P-100**.

4.6.4 CPN Formation.

General procedure: A polymer solution in DMSO-d₆ was added to a stirred mixture of trifluoroacetic acid (2 mL) and acetic acid (2 mL) and allowed to stir at room temperature for 2 days. The mixture was then diluted by addition of acetic acid (10 mL), and added dropwise (2 drops/s) to 500 mL water (18 Ω) while stirring. Using a solvent-resistant stir cell fitted with a 10 kDa-MWCO membrane, the solution was concentrated to approximately 10 mL, and dialyzed against 2 L of water. The solution was subsequently filtered through a cellulose syringe filter (0.45 μm) and stored for future use.

4.6.5 CPN/CMC complex formation.

Carboxymethyl cellulose (CMC, MW 90,000 g/mol, D.S. 0.70) was purchased from Aldrich Chemicals and used as received. A stock solution was prepared by dissolving 2.00 mg of CMC in 2.0 mL deionized water. The CPN/CMC complex was formed by mixing CPN (~0.1 optical density) with CMC (10 μ M) for 10 minutes.

P-0 CPN. UV-Vis (H₂O) $\lambda_{\text{max}} = 432$ nm, Fluo $\lambda_{\text{max}} = 495$ nm, QY = 0.6%. CPN hydrodynamic diameter $d = 107.9 \pm 4.2$ nm; CPN/CMC complex $d = 136.8 \pm 4.6$ nm.

P-25 CPN. UV-Vis (H₂O) $\lambda_{\text{max}} = 419$ nm, Fluo $\lambda_{\text{max}} = 487$ nm, QY = 1.3%. CPN hydrodynamic diameter $d = 95.3 \pm 4.4$ nm; CPN/CMC complex $d = 105.1 \pm 0.8$ nm.

P-50 CPN. UV-Vis (H₂O) $\lambda_{\text{max}} = 400$ nm, Fluo $\lambda_{\text{max}} = 488$ nm, QY = 1.1%. CPN hydrodynamic diameter $d = 141.6 \pm 2.2$ nm; CPN/CMC complex $d = 150.3 \pm 4.8$ nm.

Table 4.5. CPN characteristics.

Polymer	M_n (kDa)	$\lambda_{\text{max, abs}}$ (nm)	$\lambda_{\text{max, em}}$ (nm)	QY (%)	Hydrodynamic Diameter (nm)		% increase in diameter
					CPN only	CPN + 10 μ M CMC	
P-0	12.2	432	495	0.6	108 $\pm$ 4.2	137 $\pm$ 4.6	27
P-25	10.0	419	487	1.3	95 $\pm$ 4.4	105 $\pm$ 0.8	10
P-50	12.2	400	488	1.1	142 $\pm$ 2.2	150 $\pm$ 4.8	6

4.6.6 Cytotoxicity

HeLa cells (~10 000 cells per well) in 200 μ L of complete medium were seeded into a 96-well plate and cultured for one day in a 5% CO₂ incubator at 37 °C. CPNs with various concentrations (1 to 40 μ M) were added and incubated for 24 h. To measure toxicity, 10 μ L of MTT solution (5 mg mL⁻¹ in PBS) and 90 μ L of complete medium were then added into each well, and the plate was further incubated for 4 h at 37 °C. After the MTT conversion to insoluble formazan, the formazan crystals were dissolved in dimethyl sulfoxide (100 μ L), and the absorbance intensity was measured by a microplate well reader (infinite M1000 PRO, TECAN, Switzerland) at 540 nm. Relative cell viability (%) as a function of CPN concentration was expressed as a percentage relative to the untreated control cells. All measurements were carried out in triplicate and standard deviation was included in the error bar.

4.7 References

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CHAPTER V

Backbone-dependent Self-assembly Behavior of Phenylene-based Conjugated Polyelectrolytes

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5.1 Abstract

A set of four conjugated polyelectrolytes (CPEs) with both rigid and flexible poly(*p*-phenyleneethynylene) (PPE) and poly(*p*-phenylenebutadiynylene) (PPB) backbone connectivity is presented. Upon complexation with hyaluronic acid (HA), the PPBs undergo self-assembly reorganization which is clearly evident by a red-shift in their absorbance and emission maxima. The presence of the flexible linker in the PPB backbone further enhances this effect. Analogous PPE-type polymers do not show the same behavior. This unique property of the highly fluorescent PPB backbone warrants further investigation into the use of functionalized PPBs in biological applications.

5.2 Introduction

Aromatic conjugated polyelectrolytes (CPEs) are synthetic polymeric materials with excellent photophysical properties, intrinsic fluorescence, and structural versatility, which make these materials suitable for use in a variety of biological applications. Biological compatibility of CPEs is achieved through the introduction of positively (e.g., amine) or negatively (e.g., phosphate, carboxylate) charged side-chains.¹ Copolymerization of additional monomers with side-chains containing functional units allows for the tailoring of these materials to specific applications including cellular imaging,²⁻⁶ sensing,⁷⁻¹² and delivery¹³⁻¹⁶ of therapeutic substances. Among CPEs, poly(*p*-phenyleneethynylene)s (PPEs) and their derivatives have received much attention for biological applications due to their low toxicity and straightforward synthesis via palladium-mediated coupling chemistry. In contrast, reports of the use of poly(*p*-phenylenebutadiynylene)s (PPBs) for similar purposes are scarce,¹⁷⁻¹⁸ mostly due to solubility issues resulting from the presence of the rigid, hydrophobic butadiynylenes bridging the backbone phenylene units. Chapter 2 reported a synthetic approach towards soluble, semi-flexible high-MW PPBs,¹⁹ which were then demonstrated to undergo controlled self-assembly into core-shell nanoparticles upon

complexation with hyaluronic acid (HA),²⁰ a linear anionic polysaccharide with specific binding to cell surface receptors overexpressed in many types of cancer cells.²¹ The backbone of the semi-flexible PPB polymer differs from the widely studied PPE in two ways: firstly, through the substitution of the ethylene conjugated bridge with the longer, more rigid butadiynylene bridge, and secondly, by the presence of a flexible, non-conjugated linker at small proportions (~10%). Previous studies of a series of conjugated poly(*p*-phenylenevinylene)s (PPVs) with variable flexible linker proportion concluded that the added flexibility contributes to more ordered polymer conformations and thus a more efficient self-assembly of the conjugated segments. The effect is diminished with higher flexible content as the conjugated segments become shorter and their alignment becomes progressively less entropically favorable.²²⁻²³ Based on these observations, It was hypothesized that the CPE reorganization upon HA complexation was a result of the flexibility of the backbone stemming from the presence of the linker in small amounts. It was subsequently demonstrated that the presence of the linker also affects the subcellular localization patterns of the CPEs.²⁴ To investigate the effect of the linker on the biophysical behavior of CPEs, a series of semi-flexible linker-containing PPEs was synthesized in Chapter 4. However, the resulting polymers did not show the same dramatic self-assembly reorganization upon polyanion complexation that were expected from a CPE containing a semi-flexible backbone. The observations prompted a further investigation into the factors contributing to the self-assembly behavior of the cationic CPEs with polyanions,

This chapter reports the fabrication and self-assembly behavior of a set of four CPEs with PPE, flexible PPE, PPB, and flexible PPB structural features. The two PPB-type polymers undergo dramatic reorganization upon HA complexation, while the two PPE-type polymers exhibit only minimal aggregation changes. The complexation behavior of PPB with HA is further enhanced by the presence of the flexible linker, indicating that the self-assembly behavior of this class of CPEs can be controlled by regulating the proportion of the flexible linker in the polymer backbone.

5.3 Results and Discussion

Polymer Preparation. A set of four CPEs was synthesized, having PPE, flexible PPE, PPB, and flexible PPB backbone structural features, respectively. All four polymers contain aromatic phenylene units with pendant amine side-chains to provide aqueous solubility and positive charge. The PPE-type polymers were fabricated using the Sonogashira coupling synthetic methodology allowing for the control of the amount of linker incorporation through variable monomer feed ratios, as described in Chapter 4. The PPB-type polymers were synthesized by homo- or co-polymerization of acetylene monomers under palladium-assisted copper-catalyzed Glaser coupling conditions as outlined in Scheme 5.1. The structures of the CPEs after deprotection of the amine side-chains are depicted in Figure 5.1. CPEs bearing conventional backbone structure are labeled **PPE** and **PPB**, while **PPB-L** and **PPE-L** denote the presence of the flexible linker in the polymer.

Scheme 5.1. Synthesis of polymers **PPB**, **PPB-L**, **PPE** and **PPE-L**.

Figure 5.1. Chemical structures and graphical representations of CPEs **PPB**, **PPB-L**, **PPE** and **PPE-L**.

CPE Characterization. All four polymers exhibit a high degree of polymerization ($n > 20$) and photophysical properties consistent with previously published results. The physical and photophysical properties of the four CPEs are summarized in Table 5.1.

Table 5.1. Physical and photophysical properties of CPNs.

	% linker	M_n (g/mol) ^a	PDI ^b	$\lambda_{\text{max, abs}}$ (nm) ^c	$\lambda_{\text{max, em}}$ (nm) ^{c,d}	QY (%) ^e	CPE Diameter (nm) ^f	CPE/HA Diameter (nm) ^f
PPB	-	29,800	3.09	441	468	2	96.5 ± 0.6	96.4 ± 0.8
PPB-L	~4	16,900	2.28	445	469, 505	5	101.4 ± 1.7	103.9 ± 2.7
PPE	-	8,500	1.31	398	469	0.5	155.2 ± 14.3	104.2 ± 3.2
PPE-L	~16	11,000	1.79	435	502	0.3	140.0 ± 1.5	113.9 ± 1.5

^a Determined by gel permeation chromatography in THF. ^b PDI (polydispersity index) = M_w/M_n .

^c Measured in H₂O. ^d Excitation wavelength 400 nm in H₂O. ^e Quantum yield in DMF measured relative to diphenylanthracene standard. ^f Single particle tracking measurement at 25°C in water.

The relative linker content of **PPB-L** and **PPE-L** was determined by ¹H NMR spectroscopy through a simple deconvolutional analysis of the peak integration values between the aromatic protons originating from the fully conjugated phenylene units, and the protons coming from the flexible linker. **PPB-L** contains on average approximately 4% of linker (Figure 5.2), and the

PPE-L linker content is somewhat higher at 16% (Figure 5.3). The hydrodynamic diameters of the PPB-type CPEs are lower than those of the corresponding PPEs, indicating tighter polymer aggregation in PPBs. Since aggregation originating from face-to-face π - π stacking of aromatic segments typically leads to fluorescence quenching, the comparatively higher PPB quantum yields allude to possible different intra- and inter-chain organization to that of PPEs.

Figure 5.2. ¹H NMR spectrum of polymer **PPB-L**.

Figure 5.3. ^1H NMR spectrum of polymer **PPE-L**.

HA Complexation. Upon complexation of CPEs with hyaluronic acid (HA), distinct differences in photophysical properties and particle size were observed among the polymers. **PPB** and **PPB-L** exhibit a significant red-shift of their absorption maxima from 441 to 470 nm and from 445 to 473 nm, respectively (Figure 5.4). The newly formed red-shifted peak indicates a larger extent of interpolymer interactions brought about by chain reorganization resulting from the CPE complexation with the polyanion. This red-shifted aggregation shoulder is more pronounced in the semi-flexible **PPB-L**, which is in agreement with earlier studies reporting that the added flexibility in small proportions contributes to more efficient intra-polymer chain interaction resulting in more ordered structures.²²⁻²³ However, based on the results of **PPE** and **PPE-L** complexation with HA, in which only minimal change in absorption profile upon complexation was observed, it can be concluded that the main contributing factor towards the self-assembly of the CPEs appears to be the structure of the backbone itself. The longer, more hydrophobic

butadiynylene bridge in the PPB backbone gives rise to dramatic CPE reorganization upon polyanion complexation.

Figure 5.4. Absorption spectra of polymers **PPB**, **PPB-L**, **PPE** and **PPE-L** upon complexation with hyaluronic acid (HA).

Increased PPB aggregation upon HA complexation is further evidenced by the decreased emission intensity and red-shifted emission wavelengths of the PPB-based polymers, a phenomenon not observed in the PPE series (Figure 5.5). It is interesting to note that in both the PPE and the PPB polymers the decrease in emission intensity is much less pronounced in the semi-flexible CPEs containing the non-conjugated linker. Since conjugated polymers act as molecular wires, aggregation-induced quenching resulting from polyanion complexation is very effective in the fully conjugated **PPE** and **PPB** polymers. On the other hand, the **PPB-L** and **PPE-L** polymers, which contain isolated conjugated segments linked together through the

flexible functionality, exhibit a lower degree of quenching because a much higher concentration of quencher is required to elicit the same response.

Figure 5.5. Emission spectra of polymers **PPB**, **PPB-L**, **PPE** and **PPE-L** upon complexation with hyaluronic acid (HA).

The two backbone types also show marked differences in the hydrodynamic diameter of the CPEs (Figure 5.6). The PPB-type polymers form smaller particles than PPEs indicating tighter intra- and inter-chain interactions of the PPB backbone. Upon HA complexation, an interesting trend is observed. The mean hydrodynamic diameter of **PPB** and **PPB-L** remains virtually unchanged, although there is a slight shift in the size distribution towards larger particles. This observation can be ascribed to the formation of core-shell nanoparticles, which consist of the CPE core and an outer hydrophilic HA shell, as observed previously with an analogous flexible PPB-type polymer.²⁰ In contrast, there is a clear decrease in the mean hydrodynamic diameter of the PPE-

type CPEs upon HA complexation, accompanied by a more uniform particle size distribution shifted towards smaller sizes. The PPE-type CPEs, which are more loosely aggregated than the PPBs, can interact more efficiently with HA. Upon complexation, HA most likely intercalates in between the PPE chains. Further studies such as transmission electron microscopy imaging of the particles and their HA complexes will provide further explanation for the observed photophysical behavior and hydrodynamic diameter changes.

Figure 5.6. Particle hydrodynamic diameter of polymers **PPB**, **PPB-L**, **PPE** and **PPE-L** upon complexation with hyaluronic acid (HA).

Cellular Toxicity. The toxicity of **PPB**, **PPB-L**, **PPE**, and **PPE-L** CPNs was evaluated by MTT assay (Figure 5.7). All four polymers were shown to be non-toxic to cells at all concentrations tested (up to 30 μ M). These results are consistent with the cellular toxicity data previously reported by the Moon research group.^{19, 24} The observed biocompatibility opens up avenues

towards the systematic investigation of the effect of backbone structure and flexibility on sub-cellular localization patterns and delivery efficiency of biologically active substances into cells.

Figure 5.7. Cell viability evaluation of **PPB**, **PPB-L**, **PPE** and **PPE-L** CPNs by MTT assay. CPNs of all four polymers show no cell viability inhibition at concentrations up to 30 μM .

5.4 Conclusion

In summary, a set of conjugated polymer electrolytes with PPE and PPB-type connectivity was fabricated. The flexibility of the rigid backbone was modulated through the introduction of a flexible, non-conjugated linker at small proportions. The four resulting CPEs exhibit a distinctly different self-assembly behavior upon HA complexation. The PPB vs PPE connectivity is the main driving force for this behavior, with the presence of the non-conjugated linker further enhancing the effect in the PPB series because of the increased chain flexibility. Further investigations into the role of the amount of the flexible linker will reveal the enticing possibility to control the self-assembly of PPBs through linker content modulation. The results show that PPB CPEs are highly bright, biocompatible conjugated polymer materials with unique polyanion interaction capabilities and large potential for future use in biological applications.

5.5 Outlook

This chapter provides a systematic examination of the impact of backbone connectivity and flexibility on polymer aggregation properties. Due to their biocompatibility, the polymers prepared as part of this chapter can be used to investigate the backbone effects *in vitro*. Cellular uptake and sub-cellular localization studies can provide information about the localization patterns and cell uptake efficiency of the CPEs as a function of backbone structure.

Furthermore, the demonstrated differential interaction behavior with HA points towards possible differences in complexation efficiency of the CPEs with other polyanions such as small interfering RNA (siRNA) or plasmid DNA (pDNA). CPE/siRNA and CPE/pDNA complexes can be used to study complexation efficiency, cellular uptake, and gene knockdown efficiency as a function of the polymer backbone. The understanding of the role of backbone connectivity, flexibility and biodegradability in these cellular processes will aid the development of conjugated-polymer-based vehicles for highly efficient targeted delivery of biologically active substances to cells.

5.6 Experimental Section

5.6.1 General. Chemicals, including solvents, were purchased from Fisher Scientific and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Cambridge, MA). The average molecular weight (M_n) and polydispersity index ($PDI = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC) against polystyrene standards using a Shimadzu high performance liquid chromatography (HPLC) system fitted with PLgel 5 μ m MIXED-D columns and SPD-20A ultraviolet-visible (UV-vis) detector at a flow rate of 1.0 mL/min. UV-vis spectra were recorded using a Varian Cary 50 Bio spectrophotometer. Fluorescence spectra were obtained using a FluoroLog-3 Spectrofluorometer (Jobin Yvon/Horiba). 9,10-diphenylanthracene (QY = 0.9) in cyclohexane was used as a fluorescence

standard for QY determination. Fourier transform infrared (FT-IR) spectra were recorded on a PerkinElmer Spectrum 100 FT-IR Spectrometer. Fine polymer powders were directly mounted on an attenuated total reflection (ATR) cell of the spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz Avance Bruker NMR spectrometer. Chemical shifts were reported in parts per million (ppm) for ^1H NMR on the δ scale based on the middle peak ($\delta = 2.50$ ppm) of the dimethylsulfoxide (DMSO)- d_6 solvent as an internal standard. Nanoparticle hydrodynamic diameter was determined using single particle light scattering analysis. Measurements were performed with a LM10 HS (NanoSight, Amesbury, United Kingdom), equipped with a sCMOS camera, sample chamber with a 488 nm blue laser, and Viton fluoroelastomer o-ring. The samples were injected into the sample chamber with 1 mL sterile syringes (Restek Corporation, Pennsylvania, USA) until the liquid reached the tip of the nozzle. All measurements were performed in triplicate at 25°C using a LM14C temperature controller (NanoSight, Amesbury, United Kingdom).

5.6.2 Monomer Synthesis.

Monomer M1: The synthesis of monomer **M1** is described in Chapter 2 (referred to as compound **M4**).

Scheme 5.2. Synthetic route towards monomer **M2**.

Monomer M2: Dithiopropionic acid (1.0 g, 4.76 mmol) was dissolved in anhydrous tetrahydrofuran (THF) (20 mL) in a round bottom flask, which was evacuated and filled with nitrogen. *N*-methylmorpholine (1.10 mL, 9.99 mmol) was added, and the suspension was allowed

to stir until the entire solid was dissolved. Isobutyl chloroformate (1.25 mL, 9.61 mmol) was added, and the mixture was allowed to stir at room temperature for 15 min. A solution of 4-ethynylaniline (1.17 g, 9.99 mmol) in anhydrous THF (10 mL) was prepared under a N₂ atmosphere and transferred into the reaction flask using a cannula. The reaction was allowed to proceed overnight, after which the reaction mixture was hot-filtered. The filtrate was concentrated *in vacuo*, and the resulting solid was precipitated overnight from the DCM / THF solvent system. Yield: 775 mg (74%). High-resolution mass spectrometry (MS) (ESI⁺): theoretical 431.0858 m/z; experimental 431.0801 m/z [M+Na⁺]. ¹H NMR (400 MHz, DMSO-d₆): δ 2.75 (t, J=7.07 Hz, 2 H) 3.01 (t, J=7.07 Hz, 2 H) 4.08 (s, 1 H) 7.40 (d, J=8.60 Hz, 2 H) 7.60 (d, J=8.84 Hz, 2 H) 10.21 (s, 1 H). ¹³C NMR (150 MHz): δ ppm 169.5, 139.7, 132.4, 118.9, 116.1, 83.5, 79.7, 36.1, 33.4.

Monomer M3: The synthesis of monomer **M3** is described in Chapter 2 (referred to as compound **11**).

Monomer M4: The synthesis of monomer **M4** is described in Chapter 2 (referred to as aryl iodide linker **M3**).

5.6.3 Polymer synthesis. General procedure: A Schlenk flask was charged with appropriate monomers, Pd[Cl₂(PPh₃)₂] and CuI. The Schlenk flask was evacuated and filled with N₂. A solution of dimethylformamide (DMF) (4 mL) and triethylamine (TEA) (1 mL) was degassed with N₂, and 2 mL of the mixed solution was transferred to the Schlenk flask via a cannula. The reaction was heated at 70°C for 18 h. The solution was then cooled to room temperature and transferred dropwise to cold ether, resulting in precipitation. After centrifugation (2 min, 4,000 rpm), the supernatant was decanted, the precipitate was re-dissolved in DMF (1 mL) and the purification method was repeated. The resulting polymer in DMF (1mL) was characterized by gel permeation chromatography (GPC) and its absorption/emission profile was measured. The material was then reprecipitated in pure ether, the supernatant was decanted and the precipitated

polymer was allowed to dry under high vacuum for 4 hours prior to FTIR and ^1H NMR characterization.

PPB: Using the general polymerization procedure described above, the polymerization of monomer **M1** (10.0 mg, 0.0188 mmol) in the presence of $\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$ (2.6 mg, 0.00375 mmol) and CuI (3.4 mg, 0.0178 mmol) yielded polymer **PPB**. ^1H NMR (400 MHz, DMSO-d_6): δ 7.10 (s, 1H), 3.99 (br m, 4H), 3.55 (br m, 2H), 3.30 (br m, 2H), 2.91 (br m, 2H), 1.15 (s, 9H). GPC: M_w = 92,000 g/mol, M_n = 29,800 g/mol, PDI = 3.09. UV-Vis (DMF) λ_{max} = 452 nm, Fluo λ_{max} = 479 nm, QY = 21%.

PPB-L: Using the general polymerization procedure described above, the polymerization of monomers **M1** (10.0 mg, 0.0188 mmol) and **M2** (0.9 mg, 0.00209 mmol) in the presence of $\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$ (2.9 mg, 0.00417 mmol) and CuI (3.8 mg, 0.0198 mmol) yielded polymer **PPB-L**. ^1H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 0.02H), 7.73 (m, 0.04H), 7.56 (m, 0.09H), 7.29 (s, 1H), 6.71 (s, 1.03H), 4.18 (br m, 2.12H), 3.74 (br m, 2.19H), 3.49 (m, 3.02H), 3.11 (m, 2.28H), 1.34 (s, 10.68H). GPC: M_w = 38,600 g/mol, M_n = 16,900 g/mol, PDI = 2.28. UV-Vis (DMF) λ_{max} = 448 nm, Fluo λ_{max} = 478 nm, QY = 22%.

PPE: Detailed synthesis of polymer PPE is described elsewhere.²⁴

PPE-L: Using the general polymerization procedure described above, the polymerization of monomers **M1** (6.8 mg, 0.0127 mmol), **M3** (7.0 mg, 0.00951 mmol) and **M4** (2.7 mg, 0.00317 mmol) in the presence of $\text{Pd}[\text{Cl}_2(\text{PPh}_3)_2]$ (1.8 mg, 0.00254 mmol) and CuI (0.6 mg, 0.00301 mmol) yielded polymer **PPE-L**. ^1H NMR (400 MHz, DMSO-d_6): δ 10.281 (s, 0.07H), 7.77-7.70 (m, 0.24H), 7.48 (m, 0.24H), 7.16 (s, 1H), 6.70 (s, 1.25H), 4.21 (br m, 2.26H), 3.79 (br m, 2.36H), 3.52 (m, 2.59H), 3.09 (m, 2.90H), 1.41-1.35 (s, 14.40H). GPC: M_w = 28,700 g/mol, M_n = 11,000 g/mol, PDI = 1.79. UV-Vis (DMF) λ_{max} = 430 nm, Fluo λ_{max} = 475 nm, QY = 24%.

5.6.4 CPN Formation. *General procedure:* A polymer solution in DMSO-d₆ was added to a stirred mixture of trifluoroacetic acid (2 mL) and acetic acid (2 mL) and allowed to stir at room temperature for 2 days. The mixture was then diluted by addition of acetic acid (10 mL), and added dropwise (2 drops/s) to 500 mL water (18 Ω) while stirring. Using a solvent-resistant stir cell fitted with a 30 kDa-MWCO membrane, the solution was concentrated to approximately 10 mL, and dialyzed against 1.5 L of water. The solution was subsequently filtered through a cellulose syringe filter (0.45 μm) and stored for future use.

PPB CPN. UV-Vis (H₂O) λ_{max} = 441 nm, Fluo λ_{max} = 468 nm, QY = 2.0%.

PPB-L CPN. UV-Vis (H₂O) λ_{max} = 445 nm, Fluo λ_{max} = 469, 505 nm, QY = 5.1%.

PPE CPN. UV-Vis (H₂O) λ_{max} = 398 nm, Fluo λ_{max} = 469 nm, QY = 0.5%.

PPE-L CPN. UV-Vis (H₂O) λ_{max} = 435 nm, Fluo λ_{max} = 502 nm, QY = 0.3%.

5.6.5 Cytotoxicity. HeLa cells (~10 000 cells per well) in 200 μL of complete medium were seeded into a 96-well plate and cultured for one day in a 5% CO₂ incubator at 37 °C. CPNs with various concentrations (1 to 30 μM) were added and incubated for 24 h. To measure toxicity, 10 μL of MTT solution (5 mg mL⁻¹ in PBS) and 90 μL of complete medium were then added into each well, and the plate was further incubated for 4 h at 37 °C. After the MTT conversion to insoluble formazan, the formazan crystals were dissolved in dimethyl sulfoxide (100 μL), and the absorbance intensity was measured by a microplate well reader (infinite M1000 PRO, TECAN, Switzerland) at 540 nm. Relative cell viability (%) as a function of CPN concentration was expressed as a percentage relative to the untreated control cells. All measurements were carried out in triplicate and standard deviation was included in the error bar.

5.6.6 CPN/HA complex formation. Sodium hyaluronate (HA, MW 100,000 g/mol) was purchased from Lifecore and used as received. A stock solution was prepared by dissolving 2.00

mg of HA in 2.0 mL deionized water. The CPN/HA complex was formed by mixing CPN (~0.1 optical density) with HA (10 μ M) for 10 minutes.

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CHAPTER VI

General Conclusions

Synthesis of new functional conjugated polymers is of great interest for the development of CP-based delivery vehicles for use in disease therapy. The novel concept of the development of materials which exhibit flexibility, biodegradability, and fluorescence as a function of the polymer backbone is presented in this dissertation. The combined Sonogashira/Glaser coupling conditions provide a relatively straightforward synthetic pathway towards flexible PPBs with preserved fluorescent properties, even though the control over the amount of flexibility is limited due to the nature of the reactions involved. Modified Sonogashira coupling and smart monomer design allow for the statistical control over the flexibility in PPEs. Due to the non-conjugated nature of the flexible linker, the flexibility control also enables the modulation of the photophysical properties of the resulting materials.

Flexible PPBs were shown to be non-toxic to cells, and exhibited different cellular behavior than that which is typically observed in regular PPEs. Flexible PPBs were also subsequently demonstrated to undergo self-assembly changes upon complexation with polyanions as evidenced by photophysical spectral changes and particle size distribution measurements. Flexible PPEs did not show the same effect when complexed with polyanions. This observation prompted a systematic investigation into the effect of flexibility and backbone connectivity on polymer self-assembly behavior. The more rigid and hydrophobic PPB backbone was shown to be a key component necessary for polymer chain rearrangement upon complexation. The added flexibility enhanced the effect in the PPB series but only minimally influenced the PPE reorganization.

While basic understanding of synthetic control and the effect of backbone structural features on CPN behavior is the first step towards the development of efficient drug delivery vehicles, there

are many additional factors which affect the CPN behavior that have not been closely considered in this dissertation. One of the main factors is the chemical structure of the pendant ionic side-chains. The flexible PPBs presented in Chapter 2 were functionalized exclusively with side-chains containing primary amines. The PPE series presented in Chapter 4 was decorated with alternating non-polar (i.e., triethylene glycol) and ionic (i.e., guanidinium) side-chains. The hope for the guanidinium group was to introduce chemical functionality which would improve cellular uptake efficiency of the CPNs relative to the amine-only counterparts, and influence sub-cellular localization. Unfortunately, this series of polymers was toxic to cells even at very low concentrations, and a direct comparison of cellular behavior between the flexible PPB and PPE could, therefore, not be made at the time. For this reason, all polymers in Chapter 5 were designed to contain only primary amine side-chains, and are thus directly comparable. The future of this project lies in the investigation of the cellular uptake behavior, sub-cellular localization patterns, and delivery efficiency of CPNs prepared from the flexible and rigid PPEs and PPBs presented in Chapter 5.

The ability to control the length of the conjugated segments within a polymer chain in a one-pot fashion opens up interesting opportunities for further exploration of the modulation of the polymer photophysical properties. The current PPEs and PPBs have green emission which coincides with the autofluorescence of many biological substances. For biological applications, materials with red-shifted emission are, therefore, of great interest. Modification of linker design in the flexible synthetic methodology developed in Chapter 4 has the potential to yield such red-shifted materials. An asymmetrical, non-conjugated flexible linker containing an electron-donating aromatic moiety on one end and an electron-withdrawing one on the other will result in the formation of conjugated segments containing donor-acceptor pairs on the segment ends. The donor-acceptor interaction is known to decrease the magnitude of the HOMO-LUMO gap, pushing the emission towards the red.

Another avenue of future research, which has been alluded to on multiple occasions throughout this dissertation, is polymer biodegradability. The cystine-based linker was used to provide the non-conjugated building block, which introduces flexibility. At different proportions it also allows for the modulation of the length of the conjugated segments within the polymer. The degradation of polymer in organic solvent under disulfide-reducing experimental conditions should yield conjugated thiol-capped fragments of different lengths, which were formed during polymerization. Isolation of these fragments and quantitation of their length distribution will provide a better understanding of the mechanism of their formation. Since the cystine disulfide bond is known to be cleaved *in vivo* by intracellular glutathione, CPN degradation kinetics in aqueous environment can provide an estimate for the kinetics of drug release inside the cell. The effect of the biodegradable functionality on cellular toxicity and sub-cellular localization of polymers with and without the degradable linker can be assessed for better understanding of the structure-function relationship requirements for the successful development of novel fluorescent therapeutic delivery vehicles.

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PUBLICATIONS AND PRESENTATIONS

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