Responsive Supramolecular Assemblies Based on Amphiphilic Polymers and Hybrid Materials

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RESPONSIVE SUPRAMOLECULAR ASSEMBLIES BASED ON AMPHIPHILIC POLYMERS AND HYBRID MATERIALS

A Dissertation Presented

by

LONGYU LI

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To my wife Zhu, my parents, and my elder sister
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ABSTRACT
RESPONSIVE SUPRAMOLECULAR ASSEMBLIES BASED ON AMPHIPHILIC POLYMERS AND HYBRID MATERIALS
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The design and synthesis of responsive supramolecular assemblies are of great interest due to their applications in a variety of areas such as drug delivery and sensing. We have developed a facile method to prepare self-crosslinking disulfide-based nanogels derived from an amphiphilic random copolymer containing a hydrophilic oligo-(ethylene glycol)-based side-chain functionality and a hydrophobic pyridyl disulfide functional group. This thesis first provides a concept of studying the influence of Hofmeister ions on the size and guest encapsulation stability of a polymeric nanogel. The size and core density of nanogel can be fine-tuned through the addition of both chaotropes and kosmotropes during nanogel formation. We demonstrate that the change in core density can affect the guest encapsulation stability and stimuli-responsive character of the nanogel. Fluorescence resonance energy transfer (FRET) has been used as the tool to interrogate the guest-exchange process among varieties of host-guest assemblies, which has proved to be quite a robust method to gain insights regarding the guest encapsulation stability in these host assemblies. We studied the effect of host and guest environment upon the guest-exchange dynamics. By systematically comparing the behavior of pH-sensitive and pH-insensitive nanogels, we show that size, concentration, and hydrophobicity can all play a critical role in guest-exchange dynamics. More importantly, these studies reveal that the dominant mechanism of guest exchange can intimately depend on environmental factors. Nanocarriers that can be effectively transported across cellular membranes have potential in a variety of biomedical applications. We report a facile route to prepare nanogels, which generate surface charge with pH
as stimulus due to the slightly acidic conditions observed in the extracellular environment of solid tumor. We show that the pH at which the charge is generated, *i.e.* the isoelectric point (pI) of the nanogel, can be easily adjusted. Intracellular delivery of these nanogels was greatly enhanced in an acidic pH environment due to the surface charge generation. This study demonstrates the versatile nature of the nanogels to introduce specific functionalities with relative ease to achieve desired functional behavior. Further, we have taken advantage of photo-induced heterodisulfide metathesis to develop a reagent-free synthetic method to generate self-crosslinking disulfide-based nanogels crosslinked polymer nanoparticles.

In addition, we report on a simple method to prepare monodisperse polymeric nanoparticles through sequential boronate esterification of boronic acids and bifunctional catechols under ambient conditions. Our results suggest that the initial polymer formation, serving as the nucleus for monodisperse nanoparticle assembly, involves a cooperative polymerization, wherein the dative bond between the nitrogen in the imine building blocks and the boron in the boronate ester plays a critical role. The dynamic nature of the dative interaction in this equilibrium self-assembly has been shown to endow these nanoparticles with thermal responsive characteristics. Further, hollow metal-organic nanoparticles (MOPs) were synthesized from these polymeric nanoparticles using a simple metal-comonomer exchange process in a single step. The Kirkendall effect has been identified as the underlying mechanism for the formation of these hollow MOPs, which also allows a unique opportunity to tune the shell thickness of the MOPs. The generality of the methodology is evident from that it is applied for a variety of metal ions with different coordination geometries.
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CHAPTER 1
INTRODUCTION


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1.1 Introduction

Self-assembly of amphiphilic copolymers are of great interest for many decades because these materials are able to offer a rich variety of morphologies and transitions as well as their potential applications in many fields, such as biomedical, micro-electronic, photoelectric and optical materials.¹⁻⁵ Significant progress has been made in the design and synthesis of a variety of amphiphilic copolymers owing to advances in controlled polymerization techniques, such as nitroxide mediated radical polymerization (NMP), atom transfer radical polymerization (ATRP), reversible addition–fragmentation chain transfer polymerization (RAFT) and ring-opening mediated radical polymerization (ROMP).⁶⁻¹¹ Copolymers can be broadly classified into two categories: block copolymers and random copolymers. In the case of block copolymers, the monomers are arranged systematically in the form of blocks where each block is a repetition of a certain monomer species, whereas in the case of random copolymers different monomeric components of the polymer are randomly arranged where the probability of finding a given monomeric unit at any given location on the polymer is independent of the nature of the adjacent units. Until recently, much focus has been directed towards understanding the self-assembly of block copolymers, due to their unique and excellent assembly behaviours.¹²⁻¹⁷ However, their synthetic methods can be tedious and time-consuming, as it involves sequential controlled polymerization or post-polymerization treatments such as grafting, substitution, hydrolysis and “click” chemistries.¹⁸⁻²⁰ Compared to block copolymers, preparation of random copolymers is
relatively easy, as they are typically achieved in a one step co-polymerization of two (or more) different monomers. Therefore, it is intriguing to highlight the supramolecular capabilities of amphiphilic random copolymers in self-assembly.

1.2 Simple nanogels based on amphiphilic random copolymers

Our group has had a longstanding interest in using random copolymers for self-assembly. Amphiphilic random copolymers, containing triethylene glycol as the hydrophilic part and an alkyl chain connected by the disulfide bond as the hydrophobic part, were prepared by free radical polymerization. These polymers were able to form micelle-like nanoassemblies in water and encapsulate hydrophobic guests inside their core (Figure 1.1). These nanoassemblies disintegrate in the presence of a reducing environment, leading to the release of encapsulated guest molecules. This stimuli responsive behavior is due to the cleavage of the disulfide bond that connects the hydrophobic moiety to the polymer backbone. Thus, under the reducing conditions, the self-assembling amphiphilic polymer is converted to a hydrophilic polymer that no longer has the ability to self-assemble, leading to the release of guest molecules.

![Figure 1.1](image_url)

**Figure 1.1** Schematic representation of drug release via disassembly of amphiphilic polymer micelle triggered by GSH (reproduced with permission from reference 21).

Considering that micelle-type assemblies can be destabilized upon dilution, strategies to crosslink the core of these nanoassemblies have been developed. Apart from retaining their structural integrity upon dilution, these nanogels can also stably encapsulate guest molecules and release them only in response to a redox trigger, such as glutathione (GSH). The nanogel is
based on a random copolymer that contains hydrophilic oligoethylene glycol (OEG) and hydrophobic pyridyl disulfide (PDS) units as side chain functionalities. This random copolymer forms nanoaggregates in water; addition of a deficient amount of dithiothreitol (DTT) to this solution leads to an intra/inter polymer chain disulfide exchange reaction to afford the core crosslinked polymeric assembly (Figure 1.2). Hydrophobic guest molecules were stably encapsulated inside, while they were released in a reducing environment due to the redox-sensitive disulfide bonds. The size of these polymeric aggregates could be tuned by varying the properties of the polymer, such as molecular weight of the polymer and the relative percentages of OEG units and PDS units incorporated into the polymer. Because the procedure for the nanogel with high encapsulation stability is quite simple and release of guest molecules is tunable, this polymer nanogel scaffold holds great potential for drug delivery, especially for chemotherapeutics.

Figure 1.2 (a) Structure of the polymer precursors and nanogels: (i) cleavage of specific amount of PDS group by DTT, and (ii) nanogel formation by inter/intrachain cross-linking. (b) Schematic representation of the preparation of the biodegradable nanogels (reproduced with permission from reference 22).
To monitor the guest exchange dynamics, which can provide insight into the encapsulation stability of these nanogels, a fluorescence resonance energy transfer (FRET) based method was developed. A lipophilic FRET pair, 3,3’-dioctadecyloxacarbocyanine perchlorate (DiO, donor) and 1,1’-dioctadecyl-3,3’,3’,3’-tetramethylindocarbocyanine perchlorate (DiI, acceptor) were independently encapsulated inside nanogels. As shown Figure 1.3a, if the encapsulation stability of dye molecules is pretty high, the two dye molecules will continue to be in two separate nanogels when the nanogels containing the dye molecules were mixed in solution. No FRET would be observed because the distance between the two dye molecules is much higher than their Förster radius. However, it is likely that the dye molecules will equilibrate between the two containers, if the dye molecules are not stably encapsulated, causing DiI and DiO molecules to occupy the same container, resulting in the increased FRET (Figure 1.3b). The FRET ratio $I_a/(I_d + I_a)$, where $I_a$ and $I_d$ are the fluorescence intensities of the acceptor (DiI) and the donor (DiO).
respectively, was plotted against time. The slope of the linear fit is defined as the leakage coefficient ($\Lambda$), which shows the dynamics of the guest exchange (Figure 1.3c). It showed that the encapsulation stability of nanogels depends on the degree of cross-linking.

The unique advantage of these assemblies made from random copolymers is that the surface of our nanogel can be conveniently functionalized by free thiol containing molecules via thiol–disulfide exchange. Unlike the block copolymer assemblies where the hydrophobic units are completely buried in the interior, random copolymer aggregates have a certain portion of surface exposed functional hydrophobic PDS moieties, which have been exploited for nanogel decoration with cysteine-modified ligands including folic acid, cyclic arginine-glycine-aspartic acid (cRGD) peptide, and cell-penetrating peptide (Figure 1.4). The results showed that these functionalized nanogels caused rapid uptake by the cells, whereas the unfunctionalized nanogels are taken up very poorly by various cells. RGD-modified nanogels were selective uptake by cells over-expressing integrin and folic-acid decorated nanogels were selective uptake by cells over-expressing FRs. Nanogels with cell-penetrating peptides caused rapid nonspecific uptake by the cells, independent of the receptor.

Figure 1.4 (a) Schematic Representation of the One-Pot Synthesis of T-NGs, (b) Chemical Structure of Polymer and T-NGs, and (c) Cysteine-Containing Targeting Ligands (reproduced with permission from reference 25).
Supramolecular nanoassemblies, which are capable of binding and delivering either lipophilic small molecules or hydrophilic molecules, are of great interest. These polymeric nanogels indeed could not only be used to deliver hydrophobic drugs, but also be used to bind proteins on their surface through electrostatic interactions, resulting in the concurrent delivery of proteins and hydrophobic small molecules (Figure 1.5). Tri-arginine was chosen as the functional group due to two reasons: (i) providing the positively charged surface to bind to the negatively charged surface of protein; (ii) exhibiting cell penetrating peptide characteristics for efficient uptake by cells. It showed that complexation of the protein with the nanogel does not alter the activity of the protein. The complex exhibits efficient uptake by cells, where both the lipophilic small molecule and the protein are concurrently taken up by the cells. The conjugation of protein at the nanogel exterior by covalent attachment was also determined. After modification with a thiol linker, the protein was readily conjugated to the nanogels via disulfide

Figure 1.5 Structures of the nanogel’s polymer precursor and tri-Arginine peptide as well as nanogel–protein complexation by complementary electrostatic interactions (reproduced with permission from reference 26).
exchange reactions with pyridyl disulfide moieties at the nanogel exterior (Figure 1.6). The results further suggest that this nanogel system is a simple and effective platform for development of sophisticated drug delivery systems. From the application standpoint, the results indicate the possibility to combine therapeutic proteins and antibodies to the nanogels, which are important for selective targeting of the nanogels.

Amphiphilic random copolymers with polyethylene glycol methacrylate (PEGMA) as the hydrophilic unit and the pentafluorophenyl acrylate (PFPA) as the lipophilic unit were synthesized. The addition of diamine into the polymer solution could cause inter- and intrachain cross-linking amidation reactions to afford the nanogel, wherein the lipophilic guest molecules were encapsulated within the cross-linked interiors. Both THF and water can be used as the solvent for the nanogel formation. The size of the nanogel can be tuned via the change in polymer concentration.

**Figure 1.6** (a) BSA modification with SATP linker, (b) NG-Dil–BSA conjugate formation. (BSA structure PDB: 3V03) (reproduced with permission from reference 29).

Besides disulfide cross-linked systems, a simple reaction between the lipophilic activated PFP ester and diamines was also used to achieve polymeric nanogels (Figure 1.7). Amphiphilic random copolymers with polyethylene glycol methacrylate (PEGMA) as the hydrophilic unit and the pentafluorophenyl acrylate (PFPA) as the lipophilic unit were synthesized. The addition of diamine into the polymer solution could cause inter- and intrachain cross-linking amidation reactions to afford the nanogel, wherein the lipophilic guest molecules were encapsulated within the cross-linked interiors. Both THF and water can be used as the solvent for the nanogel formation. The size of the nanogel can be tuned via the change in polymer concentration.
Polymer nanoparticles were also prepared using amphiphilic random copolymers containing 2-aminooethyl methacrylamide and 3-(9-methylcoumarinoxo)propyl methacrylamide (Figure 1.8). These copolymers self-assembled in to a micelle aggregate in water, where the hydrophilic amino functional groups are exposed on the surface, while the coumarin moieties are buried in the hydrophobic interior. The coumarins groups can undergo photochemically driven [2 + 2] cycloaddition reaction, resulting in the inter- and intrachain cross-linking reaction to achieve the polymer nanoparticles. Hydrophobic guest molecules can be noncovalently encapsulated, while the amine functionality can be used as a handle for surface functionalization of the nanoparticles. Since the pH of the solution changes the hydrophilic–lipophilic balance of the polymer, leading to the formation of polymer aggregates with different sizes, polymer nanoparticles with different sizes can be achieved by simply photochemically locking these aggregates.
1.3 Complex polymeric aggregates based on amphiphilic random copolymers

In order to prepare smart materials that have additional features such as stealth characteristics and stable encapsulation of guest molecules in addition to stimuli responsive behavior towards multiple environmental triggers, multiple polymers or polymer assemblies have been integrated to form self-assembled structures.

To mask the surface properties viz., surface charge on the nanoparticle surface and increase the guest encapsulation stability, polymer coating on nanogels were achieved through electrostatic complementarity (Figure 1.9). First a polycationic nanogel was achieved using intra-aggregate self-crosslinking strategy with PDS functionalities using amphiphilic random copolymer containing pyridyldisulfide units, quaternary ammonium moieties and N-isopropyl acrylamide units. These disulfide crosslinked nanoassemblies were coated with a pH-sensitive...
anionic polymer through the electrostatic interactions. This resulted in masking of the positive surface charge of the nanogel and also led to an increase in the encapsulation stability of the non-covalently sequestered guest molecules inside the nanogels. The pH-sensitivity of the coating polymer is such that there is a charge conversion in the polymer in response to lower pH. The pH-induced charge conversion causes electrostatic repulsion between the coating polymer and the polymer nanogel to reveal the positive charge on the nanogel surface. Moreover, the encapsulation stability of the nanogel is also weakened due to the pH-induced decoating, which is further accentuated using a redox stimulus.

Figure 1.9 Schematic representation of the polymer structure, the coating and decoating processes (reproduced with permission from reference 32).

Nanocomposites can also be formed by integration of more than one pre-formed nanostructures. For example, the combination of two independent supramolecular assemblies resulted in a novel, dynamic composite nanostructure (Figure 1.10). These composite structures are developed by using polymeric micelles from a block copolymer (poly(2-(diisopropylamino)ethylmethacrylate-b-2-aminoethyl methacrylate hydrochloride)) and nanogels formed from a random copolymer poly(oligoethyleneglycol monomethylether methacrylate-co-
glycidylmethacrylate-co-pyridyldisulfide ethyl methacrylate). These two supramolecular assemblies were integrated together by utilizing the covalent reaction between primary amines on the surface of the micellar assemblies and epoxide functionalities present on the nanogel surface to produce a composite nanostructure between the two nanoassemblies. The diisopropylamine (DIPA) block, which constitutes the hydrophobic core of the polymer micelle, endows the composite assembly with pH sensitivity. Under physiologically neutral pH (around pH 7.4 or higher) the DIPA block would be mostly unprotonated and therefore would be hydrophobic. However, when the pH is lowered below 6.5, these functional groups are protonated, which convert the hydrophobic core to a hydrophilic one, causing a disassembly of micellar aggregates. The nanogels on the other hand are redox responsive. In the composite assembly, each of these assemblies retains their individual stimulus-responsive characteristics. But, there exists a synergy. Since the composite nanostructure is formed based on a reactive self-assembly event, when the micelle at the core of this composite assembly breaks, the disassembled polymer chain becomes covalently attached to the nanogel. This feature endows the nanogel with a positively charged
surface that was previously unavailable on the nanogel. The pH-induced charge generation that leads to rapid cellular uptake and the possibility of encapsulating and releasing two different molecules at two different times and locations potentially lend themselves for applications in cancer therapy.

Considering the significant role that the size of drug delivery vehicles plays in enhanced permeability and retention (EPR) effect based tumour targeting,\textsuperscript{34,35} it is interesting to be able to design composite nanostructures that change size in response to microenvironments that are unique to cancer tissues. For example, a larger nanoparticle size is desired for tumour homing, while much smaller nanoparticle sizes are desired for tissue penetration. This combined with the fact that the tumour pH is lower; it is interesting to design a system that exhibits one size at neutral pH and reduces in size when subjected to lower pH conditions. Accordingly, a system of complex aggregates was designed to exhibit variations in size and charge in response to slight changes in pH.\textsuperscript{36}

This design involves preparation of stimuli responsive nanoclusters by crosslinking multiple nanoparticles, utilizing the pH sensitive dynamic covalent imine bond between them (Figure 1.11). This is achieved by reversibly crosslinking the nanoassemblies using a small molecule crosslinker. These nanogels are prepared using self-crosslinked nanogel system with a slight modification by using a random copolymer poly(oligoethyleneglycol monomethylether methacrylate-co-aminoethyl methacrylate hydrochloride-co-pyridyldisulfide ethyl methacrylate). The interparticle crosslinking between these nanogels was achieved by reacting the nanogels with a calculated amount of hexaethyleneglycol dibenzaldehyde crosslinker at pH 7.4. This results in the formation of nanoclusters due to the imine formation. These nanoclusters break down into smaller nanogel particles at slightly acidic pH 6.5, presumably due to hydrolysis of imine bonds. This also leads to a simultaneous exposure of free primary amines to present positive charges on the nanogel surface that accelerate cellular uptake.
1.4 Thesis overview

This thesis work focuses on responsive supramolecular assemblies based on amphiphilic random copolymers. In Chapter 2, influence of Hofmeister ions has been investigated on the size and guest encapsulation stability of a polymeric nanogel. While variations in macroscopic phase transitions have been observed in response to the presence of salts, changes in the size and host–guest behavior of polymeric aggregates in the presence of salts have not been explored in any detail. The size and core density of nanogel can be fine-tuned through the addition of both chaotropes and kosmotropes during nanogel formation. The change in core density affects the guest encapsulation stability and stimuli-responsive character of the nanogel. These studies not only have practical applications in areas such as drug delivery and sensing but also have

Figure 1.11 Schematic representation of nanocluster formation at physiological pH and reversal at lower pH (reproduced with permission from reference 36).
fundamental implications in the future design and syntheses of polymer nanoassemblies and nanoparticles.

The guest-exchange mechanism in supramolecular hosts was studied to probe the possible pathways for guest exchange in these polymeric nanogels in Chapter 3. Dynamic exchange of guest molecules, encapsulated in host assemblies, is a phenomenon in supramolecular chemistry that has important implications in several applications. While the mechanism of exchange in micellar assemblies has been previously investigated, the effect of host and guest environment upon the guest-exchange dynamics has received little attention, if any. By systematically comparing the behavior of pH-sensitive nanogels along with pH-insensitive nanogels as a control, size, concentration, and hydrophobicity can all play a critical role in guest-exchange dynamics. More importantly, these studies reveal that the dominant mechanism of guest exchange can intimately depend on environmental factors.

Nanocarriers that can be effectively transported across cellular membranes have potential in a variety of biomedical applications. Among these, materials that are capable of changing their surface properties and thus gain entry into a cell, in response to a specific tissue environment, are of particular interest. In Chapter 4, a facile route was designed to prepare nanogels, which generate surface charge with pH as stimulus. This is achieved by designing a polymeric nanogel containing 2-diisopropylamino (DPA) moieties. The pH at which the charge is generated, i.e. the isoelectric point (pI) of the nanogel, can be adjusted by varying the percentage of DPA units in the nanogel, its preparation process and crosslinking density. Intracellular delivery of these nanogels was greatly enhanced in an acidic pH environment due to the surface charge generation. This study demonstrates the versatile nature of the nanogels to introduce specific functionalities with relative ease to achieve desired functional behavior.

Photo-crosslinking chemistry has significant importance owing to its advantages, such as no crosslinking agents or reaction catalysts are needed and the photo-crosslinking procedure can be
easily tuned by turning on or off the light. However, the problem is that the de-crosslinking for
the current methods always involves the use of high-energy ultra-violet (UV) or visible light,
which limits the practical application of these nanocarriers. In Chapter 5, photoinduced
heterodisulfide metathesis of pyridyl disulfide (PDS) functionalities is an irreversible reaction.
The micelle aggregates based on random copolymer containing PDS can be successfully
crosslinked to form polymer nanoparticles based on redox-responsive disulfide bonds. Since the
crosslinks generated here are biologically relevant, this method could also have implications in
generating new materials of interest in biology and medicine.

This thesis also describes a novel and facile approach to generate well-defined, hollow
metal-organic nanoparticles (MOPs) from polymeric organic nanoparticles. In Chapter 6,
polymeric organic nanoparticles were prepared through sequential boronate esterification of
boronic acids and bifunctional catechols under ambient conditions. The nucleation step involves
the cooperative polymerization, wherein the covalent dative interaction between nitrogen in imine
and boron center from boronate ester was used as the secondary. In Chapter 7, these polymeric
organic nanoparticles based on boronate ester were extended to prepare the hollow MOPs using a
simple metal-comonomer exchange process in a single step. The Kirkendall effect has been
identified as the underlying mechanism for the formation of these hollow MOPs. This non-
reciprocal diffusion process based effect has not been utilized for hollow metal-organic
composites, which are of great interest in a variety of applications because of their low density
and high surface area.

Finally, in Chapter 8 general conclusions and several future research directions are provided.
We hope that our work will have some impact on the design of responsive supramolecular
assemblies based on amphiphilic polymers. Our ability to reliably generate organic-inorganic
hybrid nanoparticles using polymeric nanostructure templates will open up a fount of
opportunities for hybrid materials nanotechnology.
1.5 References


CHAPTER 2

EFFECT OF HOFMEISTER IONS ON THE SIZE AND ENCAPSULATION STABILITY OF POLYMER NANOGELS


2.1 Introduction

Inorganic salts have the ability to enhance solubilization or precipitate proteins in an aqueous solution, based on a phenomenon observed by Hofmeister in the late 19th century.\(^1\)\(^-\)\(^3\) While biological applications of these findings are well-established,\(^4\)\(^-\)\(^6\) its implications in chemical applications are relatively underexplored.\(^7\) For example, it has been shown that addition of salts can significantly alter the lower critical solution temperature (LCST) of water-soluble polymers and that the effect is dependent on the nature of the salt; that is, they follow the Hofmeister series.\(^8\)\(^-\)\(^1\)\(^1\)\(^1\) The LCST behavior, or the macroscopic phase separation of the polymer from the aqueous phase, is attributed to the reduced hydrogen bonding driven interaction between water and the polymer at elevated temperatures. While it is well-studied in macroscopic hydrogels and surfaces,\(^1\)\(^2\)\(^,\)\(^1\)\(^3\) we do not know of any studies that take advantage of the subtle changes that are likely to occur due to altered hydration or dehydration of functional groups in the presence of Hofmeister ions in nanosized gels. We have been interested in probing this phenomenon, with fine-tuning the size and guest encapsulation stability in oligo(ethylene glycol)-functionalized polymer nanogels as the context.

We hypothesized that although LCST transitions in polymers are often sharp, it is likely that there are also subtle changes in the aggregation states of these polymers at temperatures well below their LCST. Considering that Hofmeister ions could influence LCST, it is also likely that these ions would influence these aggregation states and thus the nanogel itself. We then
envisaged the possibility of tuning the size and the host–guest properties of polymer nanogels by simply varying the nature and concentration of the salt ions in the aqueous solution in which they are prepared (illustrated in Figure 2.1).

Figure 2.1 Schematic illustration of the possibility of varying size and encapsulation stability in polymeric nanogels using Hofmeister ions.

In this paper, we use a recently reported set of polymer nanogels derived from an amphiphilic random copolymer.\textsuperscript{14,15} This polymer precursor was based on a hydrophilic oligo(ethylene glycol)-functionalized comonomer and a hydrophobic pyridyl disulfide-functionalized comonomer. The key feature here was that the size of the amphiphilic aggregate is fully retained in the chemically crosslinked nanogel, suggesting that there was minimal or no inter-aggregate crosslinking and that the intra-aggregate crosslinking dominated the nanogel formation. This feature made this an ideal system to test our hypothesis for ion-based control over size and guest encapsulation stability in polymer nanogels.

2.2 Results and Discussion

2.2.1 Effect of Hofmeister ions on the LCST of polymers

To investigate the possibilities of size control and encapsulation stability variations by use of Hofmeister ions, it is essential that these polymers do exhibit an LCST behavior. Oligo(ethylene glycol)-based polymers and assemblies have been shown to exhibit LCST, because these
functionalities can dehydrate at elevated temperatures.\textsuperscript{16-19} To probe our polymer nanogel precursor, we first screened the cloud point of a precursor polymer, containing 30\% OEG methacrylate and 70\% PDS derived methacrylate (Scheme 2.1) in the presence of several salts. We used dynamic light scattering (DLS) at different temperatures to assess the onset of cloud point in the aqueous solution. While variation of cations had no effect on the cloud point (Figure 2.2b), Figure 2.2a shows that the LCST decreases with the addition of anion and LCST trend follows the Hofmeister series SCN\textsuperscript{−} > Cl\textsuperscript{−} > SO\textsubscript{4}\textsuperscript{2−}. Note that one would expect the cloud point to increase in NaSCN, since this is a salting-in chaotropic anion. However, at low SCN\textsuperscript{−} concentrations, the cloud point onset decreased, relative to water (Figure 2.2a). Nonetheless, there are several indications that this behavior is indeed consistent with the Hofmeister effect on these amphiphilic polymer aggregates: (i) This anomalous behavior has been previously observed with chaotropic anions at low salt concentrations due to the pyridine groups in the polymer. The pyridine will be partially quaternized due to the weak acid-base interaction. Thus the ion-pairing between pyridinium cations and anions could screen the electrostatic repulsion inside and among the polymers, thereby promoting the salting-out behavior.\textsuperscript{19} To test if our results are consistent with those observations, we tested the cloud point at a much higher salt concentration of 1 M NaSCN (Figure 2.2d). The polymer did exhibit a much higher cloud point temperature, consistent with the salting-in capability of this anion. (ii) Increase in concentration of the salt ions affords higher cloud points with chaotropic (salting-in) anions and lower cloud point transitions in
kosmotropic (salting-out) anions (Figure 2.2c, d). (iii) Contrary to the effect of high concentrations of SCN$^-$, polymers precipitate out at ambient temperatures in the presence of a high concentration of salting-out anions (SO$_4^{2-}$).

**Figure 2.2** (a) LCST trend of polymer in salts with different anions solution, the salt concentration is 30 mM/mL (b) LCST trend of polymer in salts with different cations solution, the salt concentration is 30 mM/mL (c) LCST trend of polymer in Na$_2$SO$_4$ solution with different concentrations (d) LCST trend of polymer in NaSCN solution with different concentrations. All the concentration of polymer solution is 10 mg/mL.

### 2.2.2 Effect of Hofmeister ions on the size of nanogels

Encouraged by these observations, we investigated the possibility of varying the size of the nanogels based on salt concentrations. We have previously observed that the size of the self-crosslinking nanogels, formed from the DTT-induced disulfide exchange, is determined by the size of the amphiphilic aggregate formed in the aqueous phase.\textsuperscript{15} Therefore, we hypothesized that the sizes of nanogels made in Na$_2$SO$_4$ solution would be larger even below the LCST, since SO$_4^{2-}$ shows the salting-out effect. Indeed, as shown in Figure 2.3 and Table 2.1, the nanogel sizes can be varied systematically from 8 to 24 nm by simply varying the Na$_2$SO$_4$ concentration. Also, note that there is a systematic change in the size of the amphiphilic aggregate, which translates to size tunability in the nanogel. At concentrations above 100 mM Na$_2$SO$_4$, the polymer precipitated out
of solution. On the other hand, while the size of the aggregate itself did not change, size of the final nanogel increased with NaSCN concentration. We attribute this to the possibility that SCN$^{-}$ renders the polymer more soluble, which results in less stable aggregates in solution. This loose aggregation could induce interaggregate crosslinking, resulting in larger size of the nanogel. For example, the nanogel size jumped to about 97 nm, while the polymer aggregate was still only about 11 nm.

**Figure 2.3** Size of polymer aggregates (a) in Na$_2$SO$_4$ solution (b) in NaSCN solution; Size of nanogels after dialysis (c) made in Na$_2$SO$_4$ solution (d) made in NaSCN solution.

**Table 2.1** Sizes of the Polymer Aggregates and Nanogels

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration</th>
<th>Size of polymer aggregates /nm</th>
<th>Size of nanogel /nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 mM</td>
<td>7±0.4</td>
<td>8±0.2</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>2 mM</td>
<td>9±0.4</td>
<td>10±0.3</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>13±0.3</td>
<td>14±0.4</td>
</tr>
<tr>
<td></td>
<td>50 mM</td>
<td>22±0.3</td>
<td>22±0.4</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>22±0.2</td>
<td>24±0.3</td>
</tr>
<tr>
<td>NaSCN</td>
<td>2 mM</td>
<td>11±0.4</td>
<td>10±0.1</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>11±0.3</td>
<td>13±0.3</td>
</tr>
<tr>
<td></td>
<td>50 mM</td>
<td>11±0.4</td>
<td>19±0.5</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>10±0.6</td>
<td>21±0.4</td>
</tr>
<tr>
<td></td>
<td>1M</td>
<td>11±0.4</td>
<td>97±1.0</td>
</tr>
</tbody>
</table>
2.2.3 Effect of Hofmeister ions on the encapsulation stability of nanogels

If the hypotheses suggested above were correct and nanogel sizes increased with salt concentration in both cases, we conceived that it should follow that the guest encapsulation stability of nanogels formed in the presence of kosmotropic anions (Na$_2$SO$_4$) should be considerably different from those formed in the presence of chaotropic anions (NaSCN) at similar size and crosslink density. Note that kosmotropic anions would dehydrate the OEG units, making some of these units behave as if they are hydrophobic. These OEG units would be tucked within the hydrophobic interiors of the nanogels during their synthesis. When these nanogels are redistributed in saltless water solution, these OEG units are likely to be rehydrated and present themselves on the surface. This structural reorganization is likely to make the nanogel interior more porous, thus making the nanogels leaky. With NaSCN nanogels, on the contrary, the guest encapsulation stability should increase with salt concentration used during the nanogel preparation.

Figure 2.4 Fluorescence emission spectra of mixed NGs encapsulated DiI/DiO. (a) NG made in 100 mM Na$_2$SO$_4$(aq), (b) NG made in 100 mM NaSCN(aq). (c) Comparing the dynamics of leakage/interchange of NGs made in different solution.
We used a recently reported fluorescence resonance energy transfer (FRET) based method to measure the leakage coefficient (Λ) and gain insights regarding the guest encapsulation stability in these nanogels.\textsuperscript{21} For this purpose, we prepared nanogels at identical crosslink densities (6% crosslinked) containing 1 wt % FRET donor dye (DiO) or FRET acceptor dye (DiI) as the guest molecule at different salt concentrations. Figure 2.4a shows that there is a rapid evolution of FRET with time in the nanogels prepared with Na\textsubscript{2}SO\textsubscript{4}, compared to those prepared without salt. On the other hand, the nanogels prepared with NaSCN exhibited little change in FRET ratio with time and thus very high encapsulation stability (Figure 2.4b). The extent of encapsulation stability can be systematically tuned. For example, the nanogels made from 2 mM and 100 mM Na\textsubscript{2}SO\textsubscript{4} solution exhibited Λ values of 0.0092 and 0.0393 min\textsuperscript{-1}, respectively, while those from NaSCN solutions exhibited Λ values of 0.0034-0.0011 min\textsuperscript{-1} (Figure 2.4c and Table 2.2).

<table>
<thead>
<tr>
<th>Table 2.2 Leakage coefficient of nanogels made in different conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Pure water</td>
</tr>
<tr>
<td>0 mM</td>
</tr>
<tr>
<td>2 mM</td>
</tr>
<tr>
<td>10 mM</td>
</tr>
<tr>
<td>50 mM</td>
</tr>
<tr>
<td>100 mM</td>
</tr>
<tr>
<td>Na\textsubscript{2}SO\textsubscript{4}</td>
</tr>
<tr>
<td>2 mM</td>
</tr>
<tr>
<td>10 mM</td>
</tr>
<tr>
<td>50 mM</td>
</tr>
<tr>
<td>100 mM</td>
</tr>
<tr>
<td>NaSCN</td>
</tr>
<tr>
<td>2 mM</td>
</tr>
<tr>
<td>10 mM</td>
</tr>
<tr>
<td>50 mM</td>
</tr>
<tr>
<td>100 mM</td>
</tr>
</tbody>
</table>

To further test this, we first prepared the nanogels with three different crosslink densities (6%, 13%, and 25%) in salt-free water. We then tested their encapsulation stability directly by monitoring the absorption spectrum of the guest molecule, DiI. Here we measure the leakage and precipitation of the dye molecule, instead of the dynamic exchange of the dye molecule. Since the nanogel is already prepared in water, the solution that contains Na\textsubscript{2}SO\textsubscript{4} should dehydrate the
exposed OEG groups, thus rendering the nanogel less leaky. On the contrary, NaSCN should render the nanogels more leaky, because more OEG units will be hydrated and become more exposed to the solvent, thereby leaving the core of the nanogel less dense. Indeed, Figure 2.5 shows that this is indeed the case. In all three nanogels, there is no leakage of the dye molecules in the presence of Na₂SO₄. However, the 6% and 13% crosslinked nanogels were leaky in the presence of NaSCN, while the 25% crosslinked one encapsulates the guest molecule sufficiently tightly that it was unperturbed by the presence of the SCN⁻ anions. A similar trend was observed for the nanogels with DiO as the guest molecule (Figure 2.5).

We rationalize the above results on the basis of salt dependent dehydration or rehydration of the OEG units: NaSCN would rehydrate the OEG units and thus render any buried OEG units to be presented at the surface. This rehydration should leave behind a relatively hollow interior. To further test this possibility, we investigated the nanogels by atomic force microscopy (AFM). If Na₂SO₄ and NaSCN dehydrate and rehydrate the nanogels, respectively, then the density of the nanogel from the former salt should be denser. If this were the case, it is possible that the

Figure 2.5 Absorbance of the nanogel loaded with DiI after the addition of Na₂SO₄ or NaSCN. Nanogel was made in salt free water, and concentration of both the measured nanogel solutions is 0.2 mg/mL. (a-b) Nanogels with 6% crosslinking degree, (c-d) Nanogels with 13% crosslinking degree, (e-f) Nanogels with 25% crosslinking degree.
nanogels are more rigid in the presence of Na$_2$SO$_4$ and therefore would not collapse on a solid surface. On the other hand, NaSCN should cause these nanogels to collapse on the surface. We examined this possible difference via AFM height images. Figure 2.6b shows the height profile obtained from these nanogels in the presence of Na$_2$SO$_4$. Although they are obtained from the same sample, the height of the nanogels is greater in the presence of Na$_2$SO$_4$ (Figure 2.6b) and lesser in the presence of NaSCN (Figure 2.6c), compared to the salt-free nanogels (Figure 2.6a). In addition to the height images in Figure 2.6, more quantitative analyses of the heights of the nanogels in these images are shown in the Figure 2.7. These results support our hypotheses that Na$_2$SO$_4$ rigidifies the nanogels and NaSCN loosens the nanogels, due to the differential hydration of the OEG units.

**Figure 2.6** Change in morphology of the nanogels due to the presence of salts (a) nanogel without salts (b) nanogel after adding Na$_2$SO$_4$ (c) nanogel after adding NaSCN from AFM(height mode).
Figure 2.7 Analysis of the nanogel AFM height images of the nanogels (a) in the absence of salt (b) in the presence of Na$_2$SO$_4$ (c) in the presence of NaSCN.
2.2.4 Effect of Hofmeister ions on the release behavior of nanogels

Finally, we tested the relative release rate of the guest molecules from nanogels prepared under different salt conditions in the presence of a stimulus. Note that the disulfide bonds, which form crosslinked network inside nanogels, make them susceptible to an uncrosslinking reaction in the presence of glutathione (GSH). This led us to test whether the nanogels prepared under various salt conditions would exhibit differential guest release rates in the presence of GSH. For this, we prepared a series of nanogels in the presence of 30 mM Na$_2$SO$_4$, NaOAc, NaCl, or NaSCN. All nanogels were prepared at 25% crosslink density to ensure significant guest encapsulation stability in the absence of GSH, and the loading of DiI was 1 wt %. When these nanogels were subjected to 10 mM GSH concentration, the release rates observed were indeed consistent with the Hofmeister series, which followed the order Na$_2$SO$_4$ > NaOAc > NaCl > no salt > NaSCN, as shown in Figure 2.8.

![Figure 2.8](image)

Figure 2.8 GSH-induced (10mM) DiI release rate from the nanogels made in different salt solutions.

2.3 Summary

In summary, we have demonstrated the following: (i) There are subtle differences in the aggregation states of OEG-functionalized amphiphilic polymers in aqueous phase, endowed by
the presence of various salts. (ii) While anions provide variations as predicted by the Hofmeister effect, variation in cations does not affect the aggregation of the polymer. (iii) In the case of kosmotropic $\text{SO}_4^{2-}$, the size of the nanogel can be systematically varied with concentration, since the size is determined by the aggregate size. (iv) In the case of chaotropic anion $\text{SCN}^-$, interaggregate crosslinking occurs due to the rather loose nature of the aggregates. (v) Encapsulation stability of guest molecules is dependent on the nature of the salt used for preparation of the nanogels; chaotropic anions afford nanogels with greater guest encapsulation stability. (vi) Salt-dependent hydration of the OEG units in the nanogel causes variations in density, which then determine the leaky character of the nanogel. From a practical perspective, utilizing Hofmeister ions provides unprecedented control in fine-tuning the size of the polymer nanogel. In addition to size, we have concurrent control over altering the guest encapsulation stabilities. These studies not only have practical applications in areas such as drug delivery and sensing but also have fundamental implications in the future design and syntheses of polymer nanoassemblies and nanoparticles.

2.4 Experimental

2.4.1 General

2,2'-Dithiodipyridine, 2-mercaptopropanol, poly(ethylene glycol) monomethyl ether methacrylate (MW 450), D,L-dithiothreitol (DTT), 1,1’-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid, and other conventional reagents were obtained from commercial sources and were used as received, unless otherwise mentioned. Pyridyl disulfide ethyl methacrylate (PDSEMA) was prepared by a previously reported procedure. $^1$H NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer with the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). Molecular weights of the polymers were estimated by gel-permeation
chromatography (GPC) with a refractive index detector, with poly(methyl methacrylate) (PMMA) as a standard. Dynamic light scattering (DLS) measurements were performed on a Malvern Nanozetasizer. UV–visible absorption spectra were recorded on a Varian (model EL 01125047) spectrophotometer. Fluorescence spectra were obtained from a Jasco FP-6500 spectrofluorometer. Atomic force microscopy (AFM) images were collected on a Digital Instruments Nanoscope III in tapping mode under ambient conditions by use of silicon cantilevers (spring constant 0.58 N/m).

2.4.2 Synthesis of Random Copolymer.

Polymers were prepared by reversible addition–fragmentation chain transfer (RAFT) polymerization. A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (3.0 mg, 0.02 mmol), PDSEMA (1.25 g, 4.9 mmol), poly(ethylene glycol) monomethyl ether methacrylate (1.0 g, 2.1 mmol), and 2,2′-azobis(isobutyronitrile) (AIBN; 2.5 mg, 0.014 mmol) was dissolved in tetrahydrofuran (THF) (5 mL) and degassed by performing three freeze–pump–thaw cycles. The reaction mixture was sealed and then transferred into a preheated oil bath at 65 °C for 10 h. To remove unreactive monomers, the resultant mixture was precipitated in cold ethyl ether (20 mL) to yield the random copolymer as a waxy liquid. GPC (THF) $M_n$, 13 kDa; polydispersity, 1.3. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.46, 7.68, 7.11, 4.35-4.09, 3.94-3.37, 3.03, 2.04-1.64, 1.43-0.87. The molar ratio between two blocks was determined by integrating the methoxy proton in the poly-(ethylene glycol) unit and the aromatic proton in the pyridine and was found to be 3:7 (PEO:PDSEMA).

2.4.3 Synthesis of Nanogels Containing DiI/DiO.

As shown in Scheme 1, the polymer (10 mg) was dissolved in 1 mL of water or salt solution. A 0.02 mL DiI stock solution (5 mg/mL) or 0.04 mL DiO stock solution (2.5 mg/mL) in acetone was added into the polymer solution. The mixture was stirred overnight at room temperature, open to the atmosphere to allow the organic solvent to evaporate. Then, to control the
crosslinking density of nanogel (6%, 13%, and 25% by molar equivalents), a measured amount of DTT (0.4, 0.8, and 2.0 μmol for 10, 20, and 50 mol % against PDS groups, respectively) was added. After 4 h of stirring, any insoluble DiI/DiO residue was removed by filtration and pyridothione was removed from the nanogel solution by extensive dialysis through a membrane with a molecular weight cutoff of 7000 g/mol.

2.4.4 Dynamic Light Scattering Measurement.

Dynamic light scattering experiments were performed by using a Malvern Nanozetasizer. The scattered light is detected at an angle of 173°. For cloud point measurement, the polymer (10 mg/mL) in water or salt solution was used. The temperature was increased from 10 to 60 °C in 2 °C increments. Equilibration time at each temperature was 10 min. Above the LCST, the polymer chains collapse into a globule and produce a more compressed particle with a higher refractive index, so scattering intensity was monitored as a function of temperature to determine the LCST behavior of polymer solution. For size distribution measurement, we used the polymer (10 mg/mL) or nanogel (1 mg/mL) solution in water or each salt solution, which contains a specific salt concentration. The measurement was kept constant at 25 °C throughout the experiment. Dust was eliminated by filtering the solution through 0.45 μm membrane filter.

Nanogel Encapsulated Dye Mixing. A solution of nanogel containing DiI (100 μL) was mixed with a solution of nanogel containing DiO (100 μL) in a cuvette, and then Milli-Q water (800 μL) was added to adjust the volume. The fluorescence intensity was recorded (λ_{exc} = 450 nm).

2.5 References


CHAPTER 3
ENVIRONMENT-DEPENDENT GUEST EXCHANGE IN SUPRAMOLECULAR HOSTS


3.1 Introduction

Nanocontainers that can bind and hold hydrophobic molecules have attracted significant interest due to implications in areas such as self-healing materials and drug delivery.1−3 Among the factors that are taken into account while arriving at a molecular design for these containers, encapsulation stability is often considered a critical one.4,5 Encapsulation itself is often defined by the loading capacity of the host, i.e., the amount of guest molecule that a host assembly can hold. This capacity is dictated by the thermodynamic distribution coefficient of the guest molecule between the host and the solvent.6−8 A feature that has not received much attention involves the guest exchange dynamics. This is important, as this is a direct and arguably the most rigorous indicator of encapsulation stability of a host assembly.9 Considering the diversity of the environments that a supramolecular assembly encounters in a typical biological system, it is important that we understand the influence of these external environmental factors upon encapsulation stability. In this paper, we show that the mechanism of guest-exchange dynamics and thus the factors that affect encapsulation stability can greatly vary with environmental changes.

Three limiting scenarios exist for the mechanism of guest exchange: (i) collision−exchange−separation mechanism, (ii) exit−re-entry mechanism, and (iii) fission−recombination mechanism.10−16 The first mechanism is collision-based, where the guest exchange occurs only because of a collision between two host assemblies. The rate of this process mostly depends on the effective collision frequency. The second pathway is diffusion-based,
where the guest-exchange rate depends on the ability of guest to exit and enter from the host assemblies. The third mechanism involves fragmentation of the host assembly into two smaller entities as the first step, followed by a recombination to regain the original host assembly. To simplify the possibilities, we chose to use host assemblies that are based on cross-linked polymer nanogels, which do not lend themselves to the third possibility. The cross-linking feature of nanogels obviates the fission possibility, and thus, the third mechanism can be ruled out in this case. The two viable pathways for the host assembly are illustrated in Figure 1. Between these pathways, although the kinetic orders of these mechanisms are different, it is difficult to quantify the order of a molecular exchange process, as this is not a chemical reaction. Here, we design a series of experiments to probe the possible pathways for guest exchange in these polymeric nanogels.

We use the recently reported fluorescence resonance energy transfer (FRET) as the tool to interrogate the guest-exchange process,\textsuperscript{17} which has proved to be quite a robust method for a variety of host-guest assemblies.\textsuperscript{18-25} Briefly, a hydrophobic FRET pair, 3,3′-dioctadecyloxycarbocyanine perchlorate (donor) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (acceptor), were independently encapsulated in otherwise similar nanogel hosts. When these solutions were mixed, the emission spectrum of the...
mixed solution in response to donor excitation at 450 nm was monitored over time. No FRET would be observed if the two dye molecules are stably encapsulated and continue to be in their separate nanocontainers, since the distance between the two dye molecules is much higher than their Förster radii. On the other hand, if the guest exchange between the host and its surroundings was significant, the extent of FRET would evolve, as the guest composition in the nanogel changes over time.

3.2 Results and Discussion

3.2.1 Design and synthesis of nanogels

Scheme 3.1 Structure of polymers P1 and P2; nanogels NG-C and NG; and the dye molecules DiO and Dil.
Two cross-linked polymeric nanogels were used in this study. The first nanogel (NG-C) is based on a random copolymer (P1), formed from a hydrophilic oligoethylene glycol (OEG) functionalized methacrylate monomer and a hydrophobic pyridyl disulfide (PDS) functionalized methacrylate monomer (Scheme 3.1). The second nanogel (NG) and its precursor (P2) contain a diisopropylamine (DPA) functionalized methacrylate as a comonomer in addition to the OEG- and the PDS-functionalized monomers. The size of these two types of nanogels at neutral pH was found to be very similar (Figure 3.2).

![Size distribution of nanogel at different pH values via DLS measurements. a) NG with 30% crosslinking density, b) NG-C with 20% crosslinking density.](image)

**Figure 3.2** Size distribution of nanogel at different pH values via DLS measurements. a) NG with 30% crosslinking density, b) NG-C with 20% crosslinking density.

### 3.2.2 Collision-based mechanism for control nanogels NG-C

First, we were interested in understanding the mechanism of guest exchange in the control nanogel NG-C, which is not sensitive to the external environment, as this provides the baseline for our study. Among the collision- and diffusion-based pathways, the former pathway should clearly depend on the concentration of the nanogel. One would expect strong and positive correlations between the collision frequency and the concentration of nanogels in solution. We used the FRET based method, which provided a measure of the dye exchange rate in the form of the leakage coefficient (Λ), which can be obtained as the initial slope of the linear fit (earlier four points in the linear regime) by plotting the FRET ratio $I_a/(I_a + I_d)$ against time, where $I_a$ and $I_d$ are the fluorescence intensities of the acceptor (DiI) and the donor (DiO) respectively. The
concentrations of the nanogels were varied as 0.05, 0.10, and 0.15 mg/mL for both NG-C containing DiI and NG-C containing DiO at pH 7.1. Figure 3.3c shows that there was indeed a faster evolution of FRET with time in the nanogels with a concentration of 0.15 mg/mL, compared to those with a concentration of 0.05 mg/mL (Figure 3.3a) and 0.10 mg/mL (Figure 3.3b). A values of 0.0192, 0.0284, and 0.0315 min$^{-1}$ were observed for the concentrations of 0.05, 0.1, and 0.15 mg/mL, respectively, indicating that the guest exchange between nanogels indeed increased with increasing concentration (Figure 3.3d). These data provide the initial evidence that the guest exchange in these control nanogels happens via the collision-based mechanism as the dominant pathway.

**Figure 3.3** Fluorescence evolution when NG-C containing DiI and DiO are mixed in solutions at pH 7.1. a) 0.05 mg/mL, b) 0.10 mg/mL, c) 0.15 mg/mL. d) Comparison of the dynamics of guest-exchange between NG-C with different concentration at pH 7.1.

**3.2.3 pH-dependent guest-exchange mechanism**

Additionally, the size of the nanogel would also impact guest exchange, if the collision-based mechanism was the dominant mechanism for guest exchange, since the collision frequency
would be affected by the size of nanogels. The pH-sensitive nature of the DPA moieties endows NG with pH-dependent size variations in this nanogel. The size of NG increases with decreasing pH, while the size of NG-C does not change with pH (Figure 3.2). Figure 3.2a shows that the sizes of NG increased from 10 to 18 nm when the pH changed from 8.0 to 5.1. The observed size increase in NG is attributed to the protonation of the DPA units, which presumably results in the swelling of the nanogels due to electrostatic repulsion between these positively charged DPA groups inside. As the size of NG varies with pH, we expected that the dye-exchange rate would also change with the pH.

![Graphs and diagrams](image)

**Figure 3.4** Fluorescence evolution when NG containing DiI and DiO are mixed in solutions at different pH values. a) pH 8.0, b) pH 6.4, c) pH 5.1. d) Guest-exchange rates between NG at different pH values. The concentration of each nanogel was 0.10 mg/mL.

**Table 3.1** Leakage coefficient (min⁻¹) of 0.10 mg/mL NG of at different pH values.

<table>
<thead>
<tr>
<th>pH</th>
<th>Leakage Coefficient</th>
<th>pH</th>
<th>Leakage Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>0.0833</td>
<td>6.8</td>
<td>0.0023</td>
</tr>
<tr>
<td>5.6</td>
<td>0.0495</td>
<td>7.1</td>
<td>0.0154</td>
</tr>
<tr>
<td>6.1</td>
<td>0.0131</td>
<td>7.5</td>
<td>0.0485</td>
</tr>
<tr>
<td>6.4</td>
<td>0.0037</td>
<td>8.0</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Figure 3.4a shows that there is a rapid evolution of FRET with time for NG at pH 8.0. On the other hand, NG at pH 6.4 exhibits little change in FRET ratio with time and thus shows very slow dye exchange (Figure 3.4b). Thus, the exchange rate indeed decreased from a Λ value of 0.080 min$^{-1}$ at pH 8.0 to 0.004 min$^{-1}$ at pH 6.4 (Figure 3.4d and table 3.1). These results are consistent with the collision-based mechanism, as the size of NG indeed affected the rate of guest exchange, providing further evidence that the dominant guest exchange in nanogels might happen via the collision-based mechanism. It should be noted, however, that while the collision frequency is expected to decrease with increase in size, because of the slower diffusion (via the Stokes–Einstein equation), it is also known that the collision frequency can increase with an increase in size.$^{27}$ One possible explanation is that the number of effective collisions, *i.e.*, collisions that result in guest exchange, decrease with an increase in size. This is reasonable, as the effect of the reduced diffusion could potentially reduce the effective collisions. This explanation is consistent with the results but remains provisional, as there are no established quantitative relationships to this effect at this point.

Interestingly, however, an unexpected change in the FRET evolution occurs below pH 6.4, even though the size-increase is observed through the entire pH range from 8.0 to 5.1. From pH 6.4 to 5.1, the FRET evolution was found to increase (Figure 3.4c). Figure 3.4d shows that the exchange rate systematically increases with decreasing pH from 6.4 to 5.1. The Λ value of 0.004 min$^{-1}$ at pH 6.4 increased back to 0.083 min$^{-1}$ at pH 5.1 (table 3.1). Since the size of the nanogel increases with decreasing pH, including at this pH range, these results are not consistent with the collision-based mechanism. Therefore, it is possible that the diffusion-based pathway is the dominant operating mechanism in this scenario, providing the first indication that there might be an environment-dependent change in mechanism. However, a few alternating possibilities need to be considered prior to reaching this conclusion.

First, it is possible that the dye molecules themselves have a certain pH-dependent solubility
and thus is affecting the guest molecule exchange in the collision state. To test this possibility, we investigated the pH-dependence of guest-exchange dynamics in NG-C, where the size of the nanogel does not change with pH. Figure 3.5a,b shows that there was indeed similar evolution of FRET with time in the nanogels at pH 8.0 and 7.1, while a slightly faster FRET evolution was observed at pH 6.1 (Figure 3.5). We attribute the small pH-dependence to the possibility that the solubility of DiI and DiO molecules may vary at different pH values. The solubility may be improved a bit at low pH due to the presence of tertiary amine (Scheme 3.1), leading to easier dye exchange during the collision. Note that we do not observe any significant dye loss in the nanogel during our FRET experiments, which suggests that the repulsion between these positively charged dyes and protonated NG at lower pH might be very small. Two features are noteworthy in these results: first, the magnitude of difference in exchange dynamics is too small compared to those observed with NG and, therefore, does not account for the results observed with NG. Second, the guest-exchange kinetics continues to increase with pH and there is no change in the trend after pH 6.4. These results suggest that the results observed in NG are not due to the inherent difference in dye molecule behavior at different pH values.

![Figure 3.5](image_url)

**Figure 3.5** Comparison of the dynamics of guest-exchange between NG-C with 0.10 mg/mL at different pH values.
We hypothesized next that the observed change in pH dependence trend at pH 6.4 could perhaps be explained by a change in the hydrophobicity of the nanogel interior, i.e., the host properties. It is reasonable to anticipate that the protonation of DPA groups would decrease the hydrophobicity of the nanogel. Pyrene’s fluorescence properties are greatly dependent on its microenvironment. Specifically, the ratio of the intensities between the first and the third peaks ($I_1/I_3$) in the pyrene emission spectrum can be used to determine the polarity of the dye’s microenvironment. The value of this ratio can range from 1.9 in polar solvents to 0.6 in certain hydrocarbon solvents. We utilized this dye as the guest molecule to probe the hydrophobicity of the microenvironment within the nanogel interiors. Aqueous buffer solutions of NG containing 2 wt% pyrene were prepared for this purpose at different pH values. A sample fluorescence spectrum of nanogel is shown in Figure 3.6a. The hydrophobicity of nanogel at different pH values was studied by calculating the intensity ratio of the first and third emission peaks of pyrene, commonly referred to as the $I_1/I_3$ ratio. Figure 3.6b shows the variations in $I_1/I_3$ of pyrene encapsulated in NG at different pH values. When the pH was reduced from 8.0 to about 6.4, the $I_1/I_3$ remained constant around 1.25. This value suggests that the nanogel interior is quite hydrophobic. However, when the pH was further decreased, a steady increase in the $I_1/I_3$ value was observed, suggesting that the microenvironment of the dye is becoming increasingly polar. The $I_1/I_3$ ratio at pH 5.1 was found to be about 1.37. On the contrary, pyrene in the control

![Figure 3.6](image-url)  
Effect of pH on the hydrophobicity. a) Fluorescence emission spectrum measured for nanogel NG-C loading 2 wt% pyrene and b) calculated $I_1/I_3$ ratios for nanogels at different pH values, (black, NG; red, NG-C).
nanogel NG-C (that lacks the DPA units) did not exhibit any discernible change in the $I_1/I_3$ ratio over the same pH range of 5.1-8.0, suggesting that the hydrophobicity of these nanogels was not pH-dependent.

While the environment inside NG was quite hydrophobic between pH 8.1 and 6.4, it became less hydrophobic when the pH value decreased from 6.4 to 5.1, which indicates that the ability of NG at these pH values to encapsulate the DiI or DiO molecules could be greatly depleted. Our studies with the control nanogel NG-C also suggest that the solubility of these dye molecules can be slightly improved at lower pH. Therefore, the dye might be able to exit one nanogel and diffuse through the solvent to re-enter another; i.e., the diffusion-based mechanism is possible in this case. Thus, these results suggest that the guest exchange is primarily based on the collision based mechanism from pH 8.0 to 6.4, but changes to a diffusion-based mechanism below pH 6.4.

**Figure 3.7** Comparison of the dynamics of guest-exchange between NG of different concentrations (0.05 mg/mL, 0.10 mg/mL and 0.15 mg/mL) at a) pH 8.0, b) pH 7.1, c) pH 6.1 and d) pH 5.1
To further test this possibility, we investigated the effect of nanogel concentration on the guest-exchange rate at different pH values in NG (Table 3.2). We varied the concentrations of NG samples (0.05, 0.1, and 0.15 mg/mL) and studied their FRET evolution at pH 5.1, 6.1, 7.1, and 8.0. Figure 3.7a, b shows that the exchange rates increase with nanogel concentration at both pH 7.1 and 8.0. This suggests that the guest exchange at these pH values indeed occurred mostly via the collision-based mechanism. However, while there is no clear trend at pH 6.1 (Figure 3.7c), the guest-exchange rate became slower with increasing concentration at pH 5.1, as Λ values of 0.098, 0.083, and 0.046 min⁻¹ were observed for nanogel concentrations of 0.05, 0.1, and 0.15 mg/mL, respectively (Figure 3.7d). Note that in a diffusion-based mechanism, the diffusion rate of dye out from the nanogel should depend on the concentration of nanogels in solution. At higher concentrations, the concentration of the host nanogel is higher while the concentration of the dye is very low at all these concentrations. In this case, the driving force for the guest molecules to diffuse into the solvent from the host nanogel is lower at higher host concentrations. Therefore, the observed decrease in guest-exchange rate with the increasing nanogel concentration supports the assertion that a diffusion-based mechanism is dominant at pH 5.1.

**Table 3.2** Leakage coefficient of NG of different concentrations (0.05 mg/mL, 0.10 mg/mL and 0.15 mg/mL) at different pH values (8.0, 7.1, 6.1 and 5.1).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>pH 8.0</th>
<th>pH 7.1</th>
<th>pH 6.1</th>
<th>pH 5.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 mg/mL</td>
<td>0.0442</td>
<td>0.0101</td>
<td>0.0119</td>
<td>0.0981</td>
</tr>
<tr>
<td>0.10 mg/mL</td>
<td>0.08</td>
<td>0.0154</td>
<td>0.0131</td>
<td>0.0833</td>
</tr>
<tr>
<td>0.15 mg/mL</td>
<td>0.0918</td>
<td>0.0408</td>
<td>0.0126</td>
<td>0.0464</td>
</tr>
</tbody>
</table>

If our hypotheses were correct, it should also follow that there should not be any change in the mechanism of guest exchange in NG-C, as the hydrophobicity of its interior does not change with pH. To perform this control experiment, we investigated the pH-dependent trends at different concentrations of NG-C. The concentration dependence of guest exchange was similar
for this nanogel at all pH values. Indeed, the FRET evolution increased with concentration at all four pH values (Figure 3.8 and table 3.3), indicating that the guest-exchange rate indeed increased with the increasing concentration. This suggests that the operating mechanism is collision-based and is not dependent on pH in the pH-insensitive nanogel NG-C. The results from the control nanogel are consistent with our conclusions for the pH-sensitive nanogels NG.

Figure 3.8 Comparison of the dynamics of dye exchange between NG-C of different concentrations (0.05 mg/mL, 0.10 mg/mL and 0.15 mg/mL) at a) pH 8.0, b) pH 7.1, c) pH 6.1 and d) pH 5.1.

Table 3.3 Leakage coefficient of NG-C of different concentrations (0.05 mg/mL, 0.10 mg/mL and 0.15 mg/mL) at different pH values (8.0, 7.1, 6.1 and 5.1).

<table>
<thead>
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<th>Concentration</th>
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<th>pH 7.1</th>
<th>pH 6.1</th>
<th>pH 5.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 mg/mL</td>
<td>0.0195</td>
<td>0.0192</td>
<td>0.0259</td>
<td>0.0558</td>
</tr>
<tr>
<td>0.10 mg/mL</td>
<td>0.0262</td>
<td>0.0284</td>
<td>0.0363</td>
<td>0.0614</td>
</tr>
<tr>
<td>0.15 mg/mL</td>
<td>0.0302</td>
<td>0.0315</td>
<td>0.0407</td>
<td>0.0719</td>
</tr>
</tbody>
</table>
3.3 Summary

In summary, we have designed a series of experiments to study the guest-exchange mechanism in pH-sensitive nanogels along with pH-insensitive nanogels as a control. We have shown that the dominant mechanism for guest exchange in these pH insensitive nanogel hosts is collision-based. Perhaps, the most important take-home message of this work is that the mechanism of guest exchange in the pH-sensitive host-guest assemblies can change on the basis of the microenvironment of the host. When the nanogel interior is hydrophobic, the collision-based mechanism is the dominant pathway. However, when the interior is sufficiently hydrophilic, the dominant mechanism changes to a diffusion-based one. From an even broader perspective, it is important to recognize that both the intrinsic factors, such as size and interior environment of the host assemblies, and the extrinsic factors, such as pH and concentration, can have significant impact on encapsulation stability. From the application standpoint, these findings could have implications in many areas. For example, in drug delivery applications, the drug-loaded nanocarrier experiences both concentration and environmental changes upon biodistribution to diseased tissues. The mechanistic variations in two different environments provide insights into molecular designs that can afford stable encapsulation in one environment and release of the molecules in another.\textsuperscript{31,32} Similarly, the results from this study may also provide new opportunities for designing nanoreactors\textsuperscript{33,34} in which catalysts encapsulated in a host can be used to reversibly turn a chemical reaction on or off, because of the environment-dependent diffusion of reactant molecules.

3.4 Experimental

3.4.1 General Methods

2,2'-Dithiodipyridine, 2-mercaptoethanol, poly(ethylene glycol) monomethyl ether methacrylate (PEGMA, Mw 450 g/mol), 2-(diisopropylamino)ethyl methacrylate (DPA), D,L-dithiothreitol (DTT), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI),
3,3′-dioctadecyloxacarbocyanine perchlorate (DiO), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid, and other conventional reagents were obtained from commercial sources and were used as received without further purification. 2,2′-Azobis(isobutyronitrile) (AIBN) was purified by recrystallization from ethanol. Pyridyl disulfide ethyl methacrylate (PDSEMA) was prepared using a previously reported procedure. Phosphate buffer solutions were prepared by using monosodium phosphate and disodium phosphate. The buffer strength was constant at 10 mM. The pH value was determined via the Accumet AB 15/15+ benchtop pH meter. 1H NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). Molecular weights of the polymers were estimated by gel permeation chromatography (GPC) using PMMA standard with a refractive index detector. Dynamic light scattering (DLS) measurements were performed using a Malvern Nanozetasizer. UV–visible absorption spectra were recorded on a Varian (model EL 01125047) spectrophotometer. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter. Transmission electron microscopy (TEM) images were taken using a JEOL 2000FX at 200 kV. All experiments were carried out at ambient temperature, unless otherwise mentioned.

3.4.2 Synthesis of Random Copolymer
Random copolymer P2, containing DPA groups, was synthesized according to a previously reported procedure. A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (12.0 mg, 0.043 mmol), PDSMA (150.0 mg, 0.588 mmol), PEGMA (215.0 mg, 0.453 mmol), DPAMA (130.0 mg, 0.609 mmol), and AIBN (1.5 mg, 0.010 mmol) was dissolved in THF (1.0 mL) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then transferred into a preheated oil bath at 65 °C and stirred for 10 h. To remove unreacted monomers, the resultant mixture was precipitated in cold diethyl ether (20 mL) and redispersed in
THF three times to yield the random copolymer P2 as a waxy solid. GPC (THF) Mn: 10.6 kDa. D: 1.44. ¹H NMR (400 MHz, CDCl₃) δ: 8.46, 7.67, 7.10, 4.35-4.09, 3.94-3.37, 3.02, 2.62, 2.04-1.64, 1.43-0.87. The molar ratio (PDS/PEG/DPA) was determined to be 34%/28%/38% by integrating the methoxy protons in the polyethylene glycol unit (δHₓ = 3.37 ppm), the aromatic protons in the pyridine (δHₓ = 8.46, δHᵧ = 7.67, δHᵨ = 7.10), and the methylene protons next to the nitrogen in DPA units (δHᵣ = 2.62, δHᵩ = 3.02).

3.4.3 Preparation of Nanogels Containing DiI/DiO

Polymer (10 mg) was dissolved in 1 mL of water, and the pH was adjusted to around 10 through the addition of NaOH aqueous solution (1 M). Then 0.04 mL of DiI in acetone stock solution (5 mg/mL) or 0.08 mL of DiO in acetone stock solution (2.5 mg/mL) was added into the polymer solution (0.2 mg of each dye for 10 mg of polymer in 1 mL of water). The final dye concentration was about 0.2 mmol/L. The mixed solution was stirred overnight at room temperature, open to the atmosphere to allow the organic solvent to evaporate. Then a measured amount of DTT (0.2 µmol for 15 mol % against PDS groups) was added. After stirring for 4 h, insoluble DiI/DiO was removed by filtration and the pyridothione byproduct was removed from the nanogel solution by extensive dialysis using a membrane with a molecular weight cutoff of 7000 g/mol. Finally, nanogel stock solutions with a concentration of 1 mg/mL were prepared for further studies.

3.4.4 Mixing of Nanogel-Encapsulated Dyes

Nanogel containing DiI and nanogel containing DiO were mixed in phosphate buffer solution at ambient temperature and at a certain pH value such that the total volume was 1 mL. The pH did not change after mixing. The fluorescence spectra were recorded using a 450 nm excitation wavelength. At the same time, absorption spectra were also measured for all samples to ensure that there is no loss of dye molecules due to precipitation. The FRET ratio Iₛ/(Iₛ + Iᵰ), where Iₛ and Iᵰ are the fluorescence intensities of the acceptor (DiI) and the donor (DiO),
respectively, was plotted against time to show the dynamics of the dye exchange in nanogel solution. The leakage coefficients ($\Lambda$) were calculated on the basis of the slopes from four earlier points in the linear regime. In the case of very fast guest exchange, the time frame for obtaining these four data points is shorter.

3.5 References


4.1 Introduction

Nanocarriers with cationic surface charge are readily internalized by the cells because of their higher affinity for the negatively charged phospholipid bilayer of cell membranes.\textsuperscript{1,2} Since cellular internalization can often be a stumbling block in therapeutic approaches, positively charged nanoparticles are often used to enhance cellular uptake.\textsuperscript{3} However, the positively charged nature of carriers may also induce non-specific interactions in serum, leading to their severe aggregation and rapid clearance from the circulation by the reticuloendothelial system following systemic administration.\textsuperscript{4} To address this problem, recent efforts have been dedicated to the development of stimuli-responsive charge conversional nanoparticles.\textsuperscript{5-7} Typically, it is desirable that the surface charge of nanoparticles be neutral or even negatively charged under normal physiological conditions. Then, under the aberrant conditions that simulate the disease site, the surface charge of these environment responsive nanoparticles become positive. The hypothesis is that such a change could result in rapid uptake of the nanocarriers by the cells.

Environmental stimuli that were exploited in the development of stimuli-responsive drug delivery systems include pH,\textsuperscript{8-10} temperature,\textsuperscript{11-13} enzymatic expression,\textsuperscript{14-16} redox reaction,\textsuperscript{17-19} and light.\textsuperscript{20-22} Many of these designs were motivated by the environment around solid tumors and thus have been targeted to ultimately impact cancer therapy. Among these, pH responsiveness is most frequently utilized, because the extracellular pH (pH\textsubscript{e}) of tumor tissue (~6.5) is slightly
lower than that of normal tissue and blood (~7.4), which is due to the hypoxia-induced production of excess lactic acid in the tumor microenvironments.  

For example, nanocarriers that are capable of reversing the surface charge from negative to positive at pH_e have been designed, where the pH-induced cleavage of the amide bond between an amino and 2,3-dimethylmaleic anhydride causes the charge conversion. Similarly, nanoparticles containing a pH-sensitive outer stealth layer have been designed using layer-by-layer techniques. In this case, the nanoparticle sheds a layer in response to acidity and reveals a charged nanoparticle surface that can be readily internalized by tumor cells.

In this manuscript, we report on a system that contains a few important and new complementary features. In our design, we stipulated that: (i) the system should be a charge generation process (neutral to positive), instead of a charge conversion process (negative to positive). This design has the potential to further reduce non-specific binding, prior to the charge-generation step; (ii) the charge generation should be rapid; (iii) the pH-induced charge-generation step is reversible, i.e. the surface charge is environment-dependent, but not permanent; (iv) the scaffold that exhibits these features should be nanoscale and be able to non-covalently bind hydrophobic guest molecules; and (v) the charge-generation step should not produce any small molecule by-products. Polymer nanogels have the potential to satisfy these requirements and thus afford pH-activated cellular uptake.

4.2 Results and Discussion

4.2.1 Design and synthesis of pH-sensitive nanogels

To afford pH-activated cellular uptake and satisfy the requirements above, we utilize our recently reported self-crosslinking disulfide-based nanogels as the scaffold. Disulfide based nanogels systems have gained significant interest because of their triggered encapsulated guest release capabilities in the cytosol. Our polymeric disulfide nanogels can be achieved by taking advantage of amphiphilic random copolymers that form nanoscale aggregates and by trapping
them as nanogels through a crosslinking reaction in the hydrophobic component of the amphiphilic assembly. In our current work, we have developed a series of random copolymers by copolymerizing 2-(diisopropylamino) ethyl methacrylate (DPA), polyethyleneglycol methacrylate (PEGMA) and 2-(pyridyldisulfide) ethyl methacrylate (PDSMA) monomers. Nanogels were generated by crosslinking the pyridyl disulfide (PDS) moieties within the hydrophobic interior of the amphiphilic aggregates.

![Scheme 4.1](image)

**Scheme 4.1** Schematic illustration and chemical structure of polymeric nanogels for pH-induced surface charge generation and activated cellular uptake.

Our design hypothesis is that the DPA moieties are hydrophobic and are buried within the interior of the nanogels at neutral pH, but are protonated at pH$_{c}$ to generate a positively charged surface (Scheme 4.1), thus providing an opportunity for activated cellular uptake. The main reason for choosing the diisopropylamino moiety, as the pH-sensitive moiety, is its pK$_a$. The pK$_a$ of the protonated form of the amine is around 6.2, which suggests that at neutral pH this amine will be mostly unprotonated and thus will be hydrophobic. However, at a pH of 6.2, about 50% of the functional groups would be protonated. We anticipate this protonation event to be rapid and reversible; the transformation also should not provide any byproducts. Moreover, we expected that the protonation event to render the DPA functional group to be sufficiently hydrophilic to be
displayed on the surface of the nanogel. In fact, such a pH-dependent change in hydrophobicity has been utilized to disassemble amphiphilic block copolymer micelles, where the DPA units are incorporated within the hydrophobic component of the assembly.\(^{35,36}\) In our case, we were interested in retaining the nanoscale structure, but only change the surface charge of the assembly. We hypothesized that incorporation of the DPA within the crosslinked polymeric nanostructure should make this a possibility, as shown in Scheme 1. Note also that the nanogel surface would be decorated with PEG moieties, which is likely to be charge neutral. Therefore, the pH-induced presentation of the protonated DPA moieties on the surface should convert the assembly from a charge neutral nanogel to a positively charged one. Finally, since these polymeric nanostructures are assembled from an amphiphilic aggregate, executing the crosslinking reaction in the presence of hydrophobic guest molecules provides the opportunity to incorporate them within the nanogel interior. Thus, the targeted polymer nanogel has the potential to satisfy all the design criteria mentioned above.

The PEGMA-co-DPAMA-co-PDSMA copolymers were synthesized by a reversible addition-fragmentation chain transfer (RAFT) polymerization reaction (Figure 4.1). Four different copolymers with varying comonomer compositions were targeted. Copolymers P1-P4 were synthesized by simply varying the feed ratio of the monomers during the polymerization. Copolymer P4 differs from the polymers P1-P3, as P4 is synthesized by using a PEGMA with shorter length polyethylene glycol side chain (~350 g/mol). After polymerization, the polymers were purified by precipitation in hexane, filtered, and washed to remove unreacted monomers. The polymers were characterized by NMR and GPC. We used the characteristic resonances of PDS (\(\delta H_a = 8.46, \delta H_b = 7.67, \delta H_c = 7.10\)), PEGMA (\(\delta H_d = 3.37\) ppm) and DPA (\(\delta H_e = 2.62, \delta H_f = 3.02\) ppm) moieties to calculate the relative ratios of the monomer incorporated in the polymer (Figure 4.1). The number-average molecular weights (\(M_n\)) and dispersity (\(D = M_w/M_n\)) of the copolymers were evaluated by GPC. The results are summarized in Table 4.1.
Next, these polymers were converted into the corresponding nanogels, NG1-NG4, by first dispersing these polymers in aqueous phase. The nanoscale aggregates are locked-in as nanogels by generating intra-aggregate disulfide crosslinks. The crosslinking reaction is executed by adding a calculated amount of dithiothreitol (DTT), which cleaves the disulfide bond of corresponding amount of PDS moieties. The free thiols generated in this process then react with remaining PDS functionalities in the polymer chain (both intra- and interchain) to provide the

**Table 4.1** Characteristics of PEGMA$_x$-co-DPA$_y$-co-PDS$_z$ copolymers (P1–P4).

<table>
<thead>
<tr>
<th>Sample code</th>
<th>$x/y/z$</th>
<th>$M_n$ (kDa)$^b$</th>
<th>$D^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0.28/0.19/0.53</td>
<td>8.26</td>
<td>1.29</td>
</tr>
<tr>
<td>P2</td>
<td>0.28/0.36/0.36</td>
<td>10.2</td>
<td>1.34</td>
</tr>
<tr>
<td>P3</td>
<td>0.26/0.49/0.25</td>
<td>8.36</td>
<td>1.23</td>
</tr>
<tr>
<td>P4$^c$</td>
<td>0.26/0.32/0.42</td>
<td>12.3</td>
<td>1.51</td>
</tr>
</tbody>
</table>

$^a$ Calculated weight ratio by $^1$H NMR  
$^b$ Determined by GPC in THF using PMMA as standards  
$^c$ The molecular weight of PEGMA is 350 g/mol
crosslinked polymer nanogel. The intra-aggregate nature of the crosslinking reaction insures that the nanogel size is tightly controlled by the size of the nanoscale aggregate. In all our nanogels, we used about 50% DTT (relative to the PDS units) to cause the PDS units to be exhausted in the crosslinking reaction; the extent of the PDS cleavage is conveniently monitored by the absorption peak that corresponds to the 2-thiopyridone by-product.

Next, we investigated the pH-dependent charge-generation features in these nanogels through zeta potential measurements. The zeta potential of the PEG-decorated nanogel at neutral pH is negative. Although this seems surprising, there have been several reports that suggest that the surface charge of PEG-decorated nanostructures are indeed negative.\textsuperscript{37,38} The origins of this charge are not clear. Upon decreasing the pH of the solution, the surface charge of the nanogel changes to positive with the transition from negative to positive charge at a pH \(\sim 6.2\). Considering that there is no formal charge in the PEG moieties, we hesitate to ascertain that the generation of the pH induced positive charge generation as charge-conversional. Nonetheless, the apparent negative surface charge of the PEG moieties on the surface of the nanogel served as a useful starting point for us to conveniently ascertain the pH at which the surface charge of the nanogel becomes positive.

4.2.2 Effect of monomer ratio on pI

We first hypothesized that the pH at which the surface charge is generated in the nanogel can be conveniently tuned by simply altering the ratio of DPA unit in the copolymer. To test this possibility, we used the nanogels NG1-NG3 and investigated their isoelectric point (pI). Due to the apparent negative charge on the PEG surface, we named the pH at which the surface charge of the nanogel becomes neutral as the nanogel's pI. We found that the pI indeed increased with the ratio of DPA in the copolymer, as shown in Figure 4.2. The pI of the NG1 was found to be 6.2, while those of the NG2 and NG3 were found to be 6.7 and 6.8 respectively. These results are taken to suggest that the surface charge generation is driven by the protonation induced charge
build-up within the interior of the nanogel. After a certain charge build-up, the electrostatic repulsion is likely relieved by presenting the protonated DPA units on the surface of the nanogel. This change is achieved at higher pH in the case of larger number of DPA units, because the critical charge built-up can be achieved within the nanogel interior relatively quickly.

Next, we were interested in investigating the effect of length of OEG units on the charge conversion. It is reasonable that OEG moieties can be used to shield the DPA units. If this were the case, then the length of the OEG functionalities should affect the pI of the nanogel. To test this possibility, we used the nanogel NG4, which differs from other nanogels only in the length of OEG units; these are shorter. We found that the pI of the nanogel from NG4 was indeed slightly higher than those of NG2 and NG3, even though the relative ratio of DPA units was smaller in the case of NG4. Note however that in the case of NG4, there is also another factor that is concurrently changed, viz. crosslink density due to the percentage of the PDS units in the polymer P4.

**Figure 4.2** Change in the zeta potential of nanogels as a function of pH. NG1-NG4 were made by using P1-P4 at pH 10. All nanogel crosslinking densities were 100%, achieved by adding 50 mol% DTT with respect to PDS groups.
4.2.3 Effect of crosslink density on pI

Note that the crosslink density of NG4 is higher than that of NG2 and NG3 in the experiment above. The pI of NG4 is higher than those of NG2 and NG3. However, if crosslink density were to have an effect on the pI, we anticipated that the nanogels with lower crosslinking density to exhibit higher pI. This is because the DPA groups that are buried inside the core can be more easily presented on the nanogel surface upon protonation, when the crosslink density is lower, due to the greater flexibility of the polymer chains. To test this further, nanogels of three different crosslink densities were prepared using the polymer P1 by adding different amounts of dithiothreitol (DTT) (30, 40 and 50 mol% against the precursor PDS groups) during the crosslinking reaction. These nanogels were labeled as NG1-60x, NG1-80x, and NG1-100x respectively, indicating the percent crosslink densities in each of the nanogels. When examining the change in surface charge with pH (Figure 4.3), the pI for the NG1-60x is much higher than that of NG1-80x, which was higher than that of NG1-100x. These results suggest that: (i) the pH-induced surface presentation of the DPA units, buried in the core of the nanogel when hydrophobic and unprotonated, is influenced by the flexibility of the polymer chains; and (ii) the

Figure 4.3 Change in the zeta potential of nanogels with different crosslinking density as a function of pH. All three nanogels were made by using P1 at pH 10, and the crosslinking density was varied by using appropriate amount of DTT.
higher pI of NG4, compared to NG2 and NG3, is not due to the crosslink density variation and is indeed due to the shorter OEG units on the surface of NG4.

Considering our observations above, we were next interested in investigating the effect of nanogel preparation conditions upon its pI; more specifically the pH of the solution at which the crosslinking reaction was carried out. If the chain flexibility is indeed the reason for the observed variation in pI, then it should follow that at same crosslink densities, the percentage of the hydrophobic and unprotonated DPA units presented on the surface vs. the core of the nanogel should influence the nanogel's pI. This presentation can be tuned because: (i) if the pH were lower during the crosslinking step of the nanogel preparation, there will be a greater percentage of DPA units that are protonated and therefore hydrophilic to be presented on the surface (the pKa of the protonated DPA tertiary amine moiety is ~ 6.2); (ii) although at neutral pH, the originally protonated DPA units would be unprotonated and hydrophobic, it is unlikely that these units can be tucked inside the core of the nanogel after crosslinking; and (iii) the longer OEG units, compared to the DPA moieties, can allow for the unprotonated and hydrophobic DPA units to be presented outside the nanogel and yet not exhibit significant hydrophobicity induced aggregation.

4.2.4 Effect of preparation condition on pI

To test this possibility, we synthesized nanogels at pH 5, 6, 8, and 10 using P1 and 50% DTT to obtain NG1-5, NG1-6, NG1-8, and NG1-10 respectively. After syntheses, all these nanogels were brought to identical solution conditions through extensive dialysis. Then, we investigated the charge-generation possibility of the nanogel by measuring the zeta potential at different pHs (Figure 4.4). Indeed, we found the pI of the nanogel to be dependent on the preparative conditions. For example, the pI for NG1-8 was found to be ~ 6.4, while the pI of the NG1-5 and NG1-10 were found to be 6.5 and 6.2 respectively. We were also interested in ascertaining that the observed differences are not due to any adventitious effects and are indeed due to the DPA units. For this purpose, we also prepared a structurally similar nanogel NG5, but
without the DPA units. The zeta potential of the nanogel without DPA units remained at ~10 mV at whole pH range. This clearly suggests that the observed pH-dependence is indeed due to the DPA units in the nanogel.

4.2.5 pH-dependent cellular uptake

One of the key motivations in altering surface charges in response to pH is the ability to demonstrate pH-dependent cellular uptake. Since the change in surface charge is close to the slightly acidic tumor extracellular environment (the so-called pH_e), exploration of this possibility is relevant. For this approach to be useful in applications such as drug delivery, it is also essential that these nanogels are able to carry a hydrophobic cargo. A key advantage of the self-crosslinking nanogels is that these scaffolds can non-covalently and stably encapsulate hydrophobic guest molecules. We used 1,10-dioctadecyl-3,3,30,30-tetramethylindocarbocyanine perchlorate (DiI) as the guest molecule due to its hydrophobicity and fluorescence emission wavelength that is suitable for studying the cellular uptake using confocal laser scanning microscopy (CLSM). We investigated the internalization behavior of DiI-encapsulated NG2 at

Figure 4.4 Change in the zeta potential of nanogels as a function of pH. NG1-x nanogels were made by using P1 at different pHs, represented as NG1-x where x stands for the pH at which the nanogel was prepared. Crosslinking density in all nanogels was fixed at 100%. For comparison, nanogel without DPA units (NG5) was prepared; structure of NG5 is also shown.
the normal extracellular pH of 7.4 and pHe of 6.5 with the HeLa cell line. Significantly different cellular uptake was observed for the DPA nanogels under these conditions. As shown in Figure 4.5, the red fluorescence is significantly higher at pH 6.5, compared to pH 7.4. We attributed this difference to the possibility that the DPA nanogel would become positively charged upon the protonation of DPA groups at lower pH and thus enhance the cellular internalization. First, we

Figure 4.5 (a) and (b) are confocal images of NG2, containing DiI at pH 7.4 and 6.5 respectively, after incubation with HeLa cells for 2 hours. The nanogels used were 100% crosslinked. Cells were imaged using a 63 × oil-immersion objective. Within each image set, left panel is the red channel that shows DiI emission; middle panel is the DIC image, and right panel is an overlap of both.

Figure 4.6 (a) and (b) are confocal images of the control nanogel NG5 (without DPA units) loaded with DiI at pH 7.4 and 6.5 respectively after incubation with HeLa cells for 2 hours. The nanogels used were made from polymer without DPA units. Cells were imaged using a 63 × oil-immersion objective. Within each image set, left panel is the red channel that shows DiI emission; middle panel is the DIC image, and right panel is an overlap of both images.
wished to confirm that this is indeed due to the DPA moieties. Therefore, we carried out cellular internalization experiments with NG5 at both pHs for the same amount of time (2 hours). At both pH 7.4 and 6.5, very little internalization was observed (Figure 4.6). These results support our assertion that the pH-induced surface charge change can affect cellular uptake due to the DPA units.

The results above do not necessarily show that the difference in fluorescence is due to the cellular uptake differences, because it is possible that the DiI leaks out at lower pH (in response to DPA protonation). This small molecule can then be rapidly taken up by the cells, as DiI is hydrophobic. In this case, difference in cellular uptake is not necessary to explain the difference in fluorescence observed at different pH. We carried out two different sets of experiments to gain further insight: (i) we tested the stability of encapsulation at different pH; and (ii) we covalently attached a fluorophore to the nanogel, where the leakage of the fluorophore possibility is circumvented.

4.2.6 Encapsulation capability and redox-responsive behavior

To test encapsulation stability at different pH, we prepared DiI-incorporated nanogel solutions and monitored their fluorescence over time at pH 7.4 and 6.0. We found the fluorescence to be unchanged even over a 24 hours time period (Figure 4.7a and b). This suggests that although the surface charge of the nanogels change with pH, their host–guest capabilities do not change with pH. Note that this assay is based on the expectation that any leaked dye from the nanogel will precipitate out of solution. However, since these nanogels also have certain amphiphilic character, it is also possible that there is a driving force to keep the dye molecules within its interiors at both pHs. Therefore, to more rigorously test whether there is a stable encapsulation of the guest molecules within the nanogel, we utilized a recently developed fluorescence resonance energy transfer (FRET) experiment. Here, we prepare two different solutions one with a FRET donor and another with a FRET acceptor. When these solutions are
mixed, if the extent of FRET increases with time, then the nanogel is leaky. If there is no change in FRET, then the encapsulation is stable. In this experiment, the measure of encapsulation stability is independent of the ability of the nanogel to solubilize the dye molecules through amphiphilic driving forces. 3, 3’-Dioctadecyloxacarbocyanine perchlorate (DiO) is a well-known higher energy FRET donor partner for DiI. The FRET evolution was investigated at both pH 7.4...
and 6.0, where we found that the extent of FRET did not change over a 24 hours time period (Figure 4.7c and d). This confirms that the guest molecules are indeed stably encapsulated, independent of whether the DPA units are protonated or not.

While stable encapsulation is desirable, it is also important that these guest molecules be released in response to a specific stimulus. Since these are disulfide-based crosslinks, the nanogels are sensitive to reactive thiols such as DTT and glutathione. In the presence of 5 mM DTT, the nanogels were found to be quite leaky, with a large extent of the guest molecules released within 2 hours (Figure 4.7e and f). Again, this feature was found to be independent of the pH. To insure that the observed change is not an adventitious pH response to fluorescence, we also monitored the absorbance of the solution under these conditions. Similar results were observed for the DiI release, via absorption spectra (see Figure 4.8).

![Figure 4.8 Absorption spectra for DiI release experiment at a) pH 6.0 with 5 mM DTT and b) pH 7.4 with 5 mM DTT.](image)

Finally, to further confirm that the pH-dependent internalization is indeed due to change in surface charge, nanogels with covalently attached fluorescein were prepared and incubated with the HeLa cells for 2 hours, at both pH 6.5 and pH 7.4. From the optical microscopy images, it is clear that the cells treated with nanogels at pH 6.5 exhibit much higher cellular uptake, compared to the same nanogels at pH 7.4 (Figure 4.9). These results confirm that the difference in fluorescence images at two different pHs, with the DiI-encapsulated nanogels, is not due to the
non-covalently encapsulated guest leakage. These results also support our assertion that the difference in cellular uptake is indeed due to the greater nanogel uptake at pH 6.5, most likely due to the difference in surface charge. These results also suggest that the current nanogel system can be used in drug delivery via both physical encapsulation and chemical conjugation.

4.3 Summary

In summary, we report on a nanogel that is capable of transforming into a positively charged nanogel at a pH that is relevant to the slightly acidic tumor extracellular environment. We have shown that: (i) the co-incorporation of DPA units to PEG based nanogels can lead to effective pH-dependent charge generation; (ii) the pI of the DPA nanogel can be systematically tuned by varying the (a) percentage DPA units present in the nanogel, (b) percentage of DPA units that are initially exposed on the nanogel surface, and (c) crosslink density; (iii) the charge generation process can be utilized to enhance cellular uptake at pHe, compared to the normal extracellular pH. It is remarkable that this charge generation event results in enhanced cellular uptake, even though the length of the OEG units are much higher than the side chains that contain the DPA moieties; and (iv) non-covalently encapsulated guest molecules are stably encapsulated at both pH and are thus effectively taken up by the cells at lower pH without any leakage.

**Figure 4.9** (a) and (b) are confocal images of fluorescein-labeled DPA nanogel at pH 7.4 and 6.5 after incubation with HeLa cells for 2 hours. The concentration of the nanogels used was 1mg/mL. Cells were imaged using a 63 × oil-immersion objective. Within each image set, left panel is the green channel that shows FITC emission; middle panel is the DIC image, and right panel is an overlap of both.
that there exist a number of ternary amines with very different pKₐ from 5 to 7, it is also possible to prepare nanogels with diverse pI. Thus, the surface charge can be selectively modulated not only for cellular uptake, but also for different endocytic compartments such as early endosomes (pH 5.9-6.2) and lysosomes (5.0-5.5). Overall, the reported facile synthetic method for preparing the pHₑ-responsive charge generation nanogels, combined with stable encapsulation and tunable pI, should open up new avenues for the design of the next generation nanogels.

4.4 Experimental

4.4.1 General

2,2’-dithiodipyridine, 2-mercaptoethanol, polyethylene glycol monomethylether methacrylate (MW 450 g/mol and 350 g/mol) (OEG), 2-(diisopropylamino) ethyl methacrylate (DPA), D,L-dithiothreitol (DTT), 1,1’-dioctadecyl-3,3,3’,3’-tetramethyl indocarbocyanine perchlorate (DiI) and 3,3’-dioctadecyloxacarbocyanine perchlorate (DiO), 4-cyano-4-(phenylcarbonothioylothio)pentanoic acid, fluorescein isothiocyanate (FITC) and other conventional reagents were obtained from commercial sources and were used as received, unless otherwise mentioned. Polymers were synthesized using RAFT polymerization and then purified by precipitation. Pyridyl disulfide ethyl methacrylate (PDS) was prepared using a previously reported procedure.¹² ¹H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). Molecular weights of the polymers were estimated by gel permeation chromatography (GPC) using PMMA standard with a refractive index detector and THF as the elution. Dynamic light scattering (DLS) measurements and zeta potential measurements were performed using a Malvern Nanozetasizer. UV-visible absorption spectra were recorded on a Varian (model EL 01125047) spectrophotometer. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter.
4.4.2 Synthesis of random copolymer P1-P4

A mixture of 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (5.9 mg, 0.021 mmol), PDS (107.4 mg, 0.421 mmol), polyethylene glycol monomethyl ether methacrylate (100 mg, 0.21 mmol), 2-(diisopropylamino) ethyl methacrylate (27mg, 0.126 mmol) and AIBN (0.7 mg, 0.00421 mmol) was dissolved in THF (0.5 mL) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then transferred into a pre-heated oil bath at 65 °C and stirred for 10 h. To remove unreactive monomers, the resultant mixture was precipitated in cold ethyl ether (20 mL) to yield the random copolymer P1 as a waxy solid. GPC (THF) $M_n$: 8.3 KDa. D: 1.3. $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.46, 7.67, 7.10, 4.35-4.09, 3.94-3.37, 3.02, 2.62, 2.04-1.64, 1.43-0.87. Other polymers P2-P4 were prepared by a similar method using the appropriate molar ratio of the co-monomers. The molar ratio between three monomer units was determined by integrating the methoxy protons in the polyethylene glycol unit, the aromatic protons in the pyridine and methylene protons contacted to amino groups.

4.4.3 Synthesis of random copolymer P5 without DPA units

A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (3.0 mg, 0.02 mmol), PDS (1.25 g, 4.9 mmol), polyethylene glycol monomethyl ether methacrylate (1.0 g, 2.1 mmol) and AIBN (2.5 mg, 0.014 mmol) was dissolved in THF (5 mL) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then transferred into a pre-heated oil bath at 65 °C and stirred for 10 h. To remove unreactive monomers, the resultant mixture was precipitated in cold ethyl ether (20 mL) to yield the random copolymer as a waxy liquid. GPC (THF) $M_n$: 13 kDa; PD: 1.3. $^1$H NMR (400 MHz, CDCl$_3$) δ: 8.46, 7.68, 7.11, 4.35-4.09, 3.94-3.37, 3.03, 2.04-1.64, 1.43-0.87. The molar ratio between two monomer units was determined by integrating the methoxy protons in the polyethylene glycol unit and the aromatic protons in the pyridine and found to be 3:7 (PEG:PDS).
4.4.4 Synthesis of random copolymer with primary amine groups

A mixture of 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (5.5 mg), PDS (75 mg), polyethylene glycol monomethyl ether methacrylate (107 mg), 2-(diisopropylamino) ethyl methacrylate (65 mg), 2-Aminoethyl methacrylate hydrochloride (4 mg) and AIBN (0.6 mg) was dissolved in DMF (0.5 mL) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then transferred into a pre-heated oil bath at 65 °C and stirred for 10 h. To remove unreactive monomers, the resultant mixture was precipitated in cold ethyl ether (20 mL) to yield the random copolymer as a waxy solid.

4.4.5 Encapsulation of DiI/DiO in nanogels

The polymer (10 mg) was dissolved in 1 mL water. To this solution, either a 0.02 mL of DiI stock solution (5 mg/mL in acetone) or 0.04 mL DiO stock solution (2.5 mg/mL in acetone) was added into the polymer solution. The pH was adjusted by adding drops of HCl or NaOH solution (1M). The mixed solution was stirred overnight at room temperature, open to the atmosphere allowing the organic solvent to evaporate. Then a measured amount of DTT was added. After stirring for 4 hours, insoluble DiI/DiO was removed by filtration and pyridinethione was removed from the nanogel solution by extensive dialysis using a membrane with a molecular weight cutoff of 7,000 g/mol.

4.4.6 Synthesis of fluorescein-labeled DPA nanogels

The blank DPA nanogel was made by using amine group containing polymer (10 mg/mL) using the procedures outlined above. Then excess FITC was added into the nanogel solution. After stirring for 24 hours, excess FITC was removed from the nanogel solution by extensive dialysis using a membrane with a molecular weight cutoff of 7,000 g/mol.

4.4.7 DLS measurement

Dynamic light scattering experiments were performed by using a digital correlator and goniometer. The light source was solid-stat laser system, operating at 514 nm. For size
measurements, the nanogels in deionized water (1 mg/mL) was kept constant at 25 °C throughout the experiment. Dust was eliminated by filtering the solution through 0.45 μm polycarbonate filter. All measurements were done at a correlation time of 30 seconds. For zeta potential measurements, the pH was adjusted by adding drops of HCl or NaOH solution (1M). The nanogels solution was also kept constant at 25 °C throughout the experiment.

4.4.8 Dye exchange experiment

A solution of nanogel containing DiI (100 μL) was mixed with a solution of nanogel containing DiO (100 μL) in a cuvette, and then milliQ water (800 μL) was added to adjust the volume. The fluorescence spectra were recorded using the excitation wavelength of 450 nm.

4.4.9 DiI release experiment

A solution of nanogel containing DiI (100 μL) was mixed with a buffer solution with pH 6 or 7.4 (800 μL) in a cuvette, and then DTT stock solution (100 μL) was added. The fluorescence spectra were recorded using the excitation wavelength of 530 nm.

4.4.10 Confocal experiments

Confocal experiments were done using Zeiss510 META confocal laser scanning microscope. 100k HeLa cells were incubated in glass bottomed dishes, purchased from Mat tek corp, at 37 °C overnight in 5% CO₂ incubator allowing the cells stick to the bottom of dish. Then, the nutrient medium was taken out and cells were washed with 5 mL of pH 7.4 PBS buffer. In one of the confocal dish, the pH of 1 mL of nutrient media was adjusted to 6.5. Then, 100uL of 1mg/mL solution of pH sensitive nanogels were added to each of the confocal dishes maintained at pH 6.5 and at pH 7.4. The cells were incubated for 2 hrs at 37 °C and the florescence was observed at 560 nm by laser excitation at 543 nm for the DiI encapsulated nanogels and florescence was observed at 501nm by exciting at 480nm for fluorescein-attached nanogels. All images were taken using 63x oil immersion objective.
4.5 References


CHAPTER 5
PHOTOINDUCED HETERODISULFIDE METATHESIS FOR REAGENT-FREE SYNTHESIS OF POLYMER NANOPARTICLES


http://pubs.rsc.org/en/content/articlehtml/2015/cc/c4cc08000a

5.1 Introduction

Metathesis reactions have found utility in many synthetic and supramolecular chemistry strategies. For example, diene metathesis has found use in the syntheses of polymers and small molecules,\textsuperscript{1-7} while strategies such as imine\textsuperscript{8-13} and hydrazone\textsuperscript{14-18} metathesis reactions have found use in supramolecular chemistry and dynamic combinatorial chemistry, among others. All these reactions are reversible under the reaction conditions and this provides the opportunity to obtain the thermodynamically favorable structures, under a given set of conditions. For example, the host identification process for a guest molecule in a dynamic combinatorial chemistry reaction is driven by the templation by the guest molecule to select for a specific host structure among the myriad possibilities. Disulfide metathesis reactions have also been used in this context both by Mother Nature for stabilizing the desired protein secondary and tertiary structures\textsuperscript{19-21} and for identifying optimal host geometries in supramolecular chemistry.\textsuperscript{22-25} More recently, the photolabile nature of disulfides has been exploited to produce bulk hydrogels from oligomeric disulfidemolecules and polymeric disulfides.\textsuperscript{24} Although this has provided very interesting materials in their own respect, this observation also indicates a rather uncontrolled nature of photoinduced disulfide metathesis reactions. We sought to develop a photoinduced disulfide metathesis reaction that can provide greater control such that we can achieve well defined, crosslinked polymeric nanostructures and have outlined our findings in this chapter.
Photochemically driven reactions are interesting, because these are reagentless reactions. Photoreactions, based on the dimerization of thymine\textsuperscript{26} and coumarin\textsuperscript{27-29} or benzophenone moieties,\textsuperscript{30} have been used quite extensively. The dimerization reactions are reverted by a higher energy photochemical irradiation, while the reactive radicals generated from benzophenone often provide irreversible products. The photoinduced disulfide formation reaction has the potential of being initiated by a photochemical reaction, but being reverted by a biologically relevant stimulus, such as the redox potential of the intracellular environment.\textsuperscript{31-35}

Photochemical reactivity of disulfides has been known for several decades.\textsuperscript{36-42} Disulfides are thought to undergo homolytic cleavage, giving thio radicals, which can attack nearby disulfide bonds, resulting in disulfide exchange under photoirradiation.\textsuperscript{24, 37, 40} We were interested in exploring this photoinduced metathesis of disulfide molecules containing pyridyl disulfide (PDS) units. PDS units are well known for their unreactive byproduct, pyridothione, formed during disulfide exchange reactions, which provides the opportunity for reliably generating unsymmetrical disulfides.\textsuperscript{43, 44} However, it is not clear whether such a possibility exists in a photochemical disulfide metathesis reaction. In fact, ultraviolet (uv) irradiation of 2,2'-dipyridyldisulfide (DPDS) results in the formation of pyridine-2- sulfonic acid, presumably due to oxidation.\textsuperscript{45, 46} This suggests that hydrogen abstraction by the thio radical is much slower than the oxidation reaction. Considering that pyridyl groups are relatively electron poor compared to the alkyl groups, we were also concerned that the homolytic cleavage of the disulfide bond between an alkyl group and a pyridyl group will result in the formation of two sulfonic acids.

5.2 Results and Discussion

5.2.1 Photo-induced disulfide metathesis of 2-hydroxyethyl-o-pyridyl disulfide

To investigate the possibility, we first used the photochemical reaction of 2-hydroxyethyl-o-pyridyl disulfide (PDS-OH, 1) as a model system. A solution of 2.5 mg/mL of 1 in CD\textsubscript{3}OD was irradiated using a UV lamp (15W) equipped with a 350 nm light source. If this reaction were a
purely disulfide exchange reaction due to thio radical formation and then recombination, molecule 1 would have been in equilibrium with bis(2-hydroxyethyl) disulfide (2) and DPDS (3). Ideally, a statistical ratio of 2 : 1 : 1 of products 1-3 would be obtained (Scheme 5.1). However, the starting material 1 was being continuously consumed with a concurrent increase in the concentration of 2. As shown in the $^1$H NMR spectra over the irradiation time (Figure 5.1), the integrated signal intensity of the triplet at 2.94 ppm, arising from the CH$_2$ protons attached to sulfur in 1, decreased with the corresponding increase in the intensity of the triplet at 2.83 ppm (the same CH$_2$ protons in 2). Finally, the disappearance of the peak at 2.94 ppm showed that no starting material (1) remained in the reaction mixture. Also, there is no evidence of the formation
of DPDS (3) in the reaction mixture. However, we did find that the formation of 2 was accompanied by the formation of pyridine-2-sulfonic acid (4) (Scheme 5.2). Finally, we found that the percent conversion in this reaction was lower at higher concentrations (Figure 5.2). This is typical of photochemical reactions as the fluence of light was kept the same in all these reactions. Two features are readily discerned from these observations: (i) no equilibrium is observed in this photoinduced disulfide metathesis and (ii) while the pyridylthio radical is readily oxidized, the alkylthio radical undergoes a radical recombination reaction to produce 2. These initial observations are encouraging for the use of heterodisulfides containing PDS units for controlled polymer nanoparticle synthesis.

![Scheme 5.2 Scheme of photo-induced chemistry reaction of PDS-OH.](image)

**Scheme 5.2** Scheme of photo-induced chemistry reaction of PDS-OH.

![Figure 5.2 Concentration dependent of reaction rate of PDS under photoirradiation.](image)

**Figure 5.2** Concentration dependent of reaction rate of PDS under photoirradiation.

Prior to utilizing this in nanoparticle synthesis however, we were interested in investigating the reasons for the observed oxidation of the pyridylthio radical. Presumably, this oxidation is
caused by the oxygen in the reaction mixture. To check this hypothesis, we excluded oxygen from the reaction by performing three freeze-pump-thaw cycles. Analysis of the PDS-OH (1) photochemical reaction under these conditions shows that the product generation has become seemingly slower than that in the reaction where oxygen was not rigorously removed (Figure 5.3).

Even after 4 hours of irradiation, there was still 50% of starting material 1 left in the reaction, consistent with a reaction in equilibrium. Note that the starting material 1 was completely consumed in about 2 hours without oxygen exclusion. In addition to the expected DPDS (3) product, there were additional peaks in the aromatic region for this reaction mixture, as shown in Figure 5.3. NMR analysis of this reaction mixture indicates that the formation of the byproduct in this reaction is indeed due to hydrogen abstraction by the pyridylthio radical to generate pyridothione (Scheme 5.3). In fact, we have shown that direct irradiation of DPDS (3), after the freeze–pump–thaw cycles, cleanly produces pyridothione rather than 4 (Figure 5.4). Several observations are noteworthy here: (i) the thio radical from pyridine undergoes oxidation to form pyridine-2-sulfonic acid (4) under ambient conditions; (ii) this reaction can be mitigated by
rigorously excluding oxygen from the reaction mixture; and (iii) in the absence of oxygen, the pyridylthio radical undergoes both radical recombination (evidenced by the formation of DPDS from 1) and hydrogen abstraction (evidenced by the formation of pyridothione from 1 and from DPDS irradiation).

Scheme 5.3 Scheme of photo-induced chemistry reaction of 2-pyridyl disulfide (PDS-OH) under anaerobic condition.

Figure 5.4 Time-dependent $^1$H NMR spectra of 2, 2'-dipyridyl disulfide (DPDS) under the photoirradiation without O$_2$.

Overall, photochemical irradiation of PDS-OH (1) results in the homolytic cleavage of the disulfide bond to generate a pyridyl and an aliphatic thio radical. The aliphatic radical undergoes rapid recombination to generate the corresponding disulfide. However, the stability provided by resonance in the thiopyridyl radical seems to be sufficient to slow down radical recombination or hydrogen abstraction, but is not sufficient to inhibit oxidation to the corresponding sulfonic acid 4. In the absence of the oxidative pathway, this radical ultimately undergoes radical recombination
and hydrogen abstraction. These latter processes also provide the opportunity for the photochemical reaction of 1 to be in equilibrium and thus slow down the conversion of the starting material to the product. In other words, the oxidized byproduct serves to drive the equilibrium towards the right in the reaction of 1. Therefore, in reactions where we need the thiopyridyl byproduct to be continuously removed, the reaction should be carried out without excluding oxygen (vide infra).

5.2.2 Preparation of nanogels via photo-induced crosslinking reaction

Encouraged by these observations, we then envisaged the possibility of utilizing this methodology for preparing crosslinked polymer nanoparticles. We wanted to test this for a well-characterized polymer and polymer nanoparticles so that the validation of this methodology is robust. A 3 : 7 random copolymer of the oligoethylene glycol methacrylate monomer (5) and the PDS-ethylmethacrylatemonomer (6), shown as structure 7 in Scheme 5.4, has been shown to form micelle-like assemblies in water. These assemblies have been previously shown to be also crosslinked to form polymer nanogels, triggered by the thiol exchange reaction with dithiothreitol (DTT).

![Scheme 5.4 Representation of photo-induced crosslinking reaction of random copolymers containing PDS groups.](image)

A solution of polymer 7 (1 mg/mL) in water was found to aggregate to form assemblies with a hydrodynamic diameter of 9 nm, as discerned by dynamic light scattering (Figure 5.5). This
solution was subjected to irradiation in a photochemical reaction chamber at 350 nm for 2 hours. At this time, the size of the assemblies was assessed by DLS again and was found to be 13 nm, which is close to the size of the aggregates prior to crosslinking (Figure 5.5b). This observation suggests that the disulfide metathesis occurs sufficiently fast such that the crosslinking reaction is mostly intra-aggregate. The slight increase in the size could be the result of a small amount of inter-aggregate crosslinking or the swelling that is likely to occur in crosslinked nanoparticles, when the hydrophobic components are reduced. Alternatively, this increase in the size could also be attributed to the adventitious heating that occurs during photochemical irradiation of the sample. This latter possibility is consistent with the increase in the size of PEG-based assemblies at higher temperatures.\textsuperscript{44,47}

If it is indeed due to temperature variations and not due to the inter-aggregate reaction, we should be able to systematically tune the size of the nanoparticles by varying the concentration of the polymer. Indeed, when we varied the sample concentrations from 1 mg/mL to 10 mg/mL, the size of the micelle aggregates increased from 9 nm to 20 nm with increasing concentrations (Figure 5.5a). Upon irradiation, the size of the nanogels was only slightly increased from 20 nm to 38 nm even at a concentration of 10 mg/mL (Figure 5.5b). If the observed increase in the size at 1 mg/mL were due to inter aggregate crosslinking, then with an increase in the concentration

\textbf{Figure 5.5} DLS sizes in hydrodynamic diameter, a) micelle aggregates prepared with polymer solutions with different concentrations, b) nanogels prepared with polymer solutions with different concentrations, the photoirradiation time was 2 h for all samples.

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by an order of magnitude, the size of the nanoparticles should have been substantially increased or even formed bulk gels. Instead, there was only a slight increase in size. This suggests that the observed increase in the size upon crosslinking is most likely due to the increase in solution temperature, and the photoinduced crosslinking is predominantly intra-aggregate.

Next, we were interested in estimating the crosslink density in the nanoparticles. We utilized the residual PDS units present in the polymer nanoparticles to estimate this. According to our analysis, upon using the small molecule model system mentioned above, the pyridylthio radical gets converted to pyridine-2-sulfonic acid 4. The unreacted PDS in the polymer can be treated

![Figure 5.6](image)

**Figure 5.6** a) Absorption spectra of pyridothione in UV-vis. Pyridothione, which is a byproduct by the reaction between PDS with DTT and shows characteristic absorption at 343 nm wavelength, is monitored in each nanogel (2 mg/mL) prepared. b) Time dependent crosslinking density of the nanogels with different concentrations of polymer solution, from 1 mg/mL to 10 mg/mL.

**Table 5.1** Calculated crosslinking densities for nanogels.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Crosslinking density/%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>1 mg/mL</td>
<td>16</td>
</tr>
<tr>
<td>2 mg/mL</td>
<td>13</td>
</tr>
<tr>
<td>5 mg/mL</td>
<td>10</td>
</tr>
<tr>
<td>10 mg/mL</td>
<td>7</td>
</tr>
</tbody>
</table>

Next, we were interested in estimating the crosslink density in the nanoparticles. We utilized the residual PDS units present in the polymer nanoparticles to estimate this. According to our analysis, upon using the small molecule model system mentioned above, the pyridylthio radical gets converted to pyridine-2-sulfonic acid 4. The unreacted PDS in the polymer can be treated
with a thiol to reliably generate pyridothione, which can be readily monitored spectroscopically
due to its distinct absorption peak at 343 nm. The concentration of pyridothione generated then
gives us the amount of unreacted PDS in the mixture. As shown in Figure 5.6a, there was very
little difference between the absorption spectra before and after photoirradiation. However, the
addition of excess DTT into both samples resulted in a significant difference between the
intensities of the absorption peak at 343 nm due to the difference in the extent of pyridothione
generated. The crosslinking density was then calculated using the ratio of these intensities. If \( I_N \) is
the increased intensity at 343 nm of the irradiated sample obtained by adding excess DTT, while
\( I_M \) is that of the unirradiated sample, then the crosslinking density was calculated from \( I_N/I_M \).
Thus, the crosslinking density of the nanoparticles was found to be 33\% after photoirradiation for
2 hours, when the concentration of the polymer was 2 mg/mL. The crosslinking density could be
systematically varied using different irradiation times, as shown in Figure 5.6b and table 5.1.
Interestingly, the crosslink density scales much slower with irradiation time at higher
concentrations (Figure 5.6b). This might seem to be counterintuitive at first, because reactions
seem to be slower at higher concentrations. This is indeed consistent with photochemical
reactions between small molecules (Figure 5.2), because we are measuring the percent conversion
of the starting material and the reagent here is light, which is constant at all different
concentrations.

### 5.2.3 Encapsulation capability and redox-responsive behavior

To test if the difference in the extent of PDS generation is indeed due to crosslinking, we
incorporated hydrophobic guest molecules inside these nanogels prior to the photoirradiation.
With the dye molecules, DiI, incorporated into the nanoparticles, the guest molecules were found
to be stably encapsulated in the nanoparticles over a time period of 24 hours, even after a 10-fold
dilution (Figure 5.7a). However, when glutathione (GSH) was added to this solution at 10 mM
concentration, the guest molecules seem to be releasing over time (Figure 5.7b).
To further confirm that the encapsulation stability could be easily tuned by simply varying the irradiation time, a fluorescence resonance energy transfer (FRET) based encapsulation stability assay was carried out. In this experiment, a faster FRET evolution suggests leaky nanoparticles, while an unchanged FRET over time suggests stably encapsulated guests inside the nanoparticles. Indeed, we observed that there was a rapid evolution of FRET with time in the micelle aggregates without any photoirradiation (Figure 5.7c), compared to that in the nanoparticles prepared after 1 hour of irradiation (Figure 5.7d). The evolution of FRET was even slower after 2 hours of irradiation (Figure 5.7e), suggesting that the encapsulation stability can indeed be tuned by varying the irradiation time.

5.3 Summary

In summary, we have demonstrated that: (i) PDS-containing asymmetrically substituted disulfides can be homolytically cleaved by photochemical irradiation; (ii) although the alkyl substituted thio radical consistently undergoes radical recombination, the reactivity of the 

Figure 5.7 Dye release from the nanogels prepared via photoirradiation for 2 h in response to varied GSH concentrations, a) 0 mM; b) 10 mM. Sample concentration was 0.1 mg/mL. FRET experiment based DiI/DiO exchange, c) micelle aggregates; d) nanogels prepared via photoirradiation for 1 h; e) nanogels prepared via photoirradiation for 2 h. Sample concentration was 0.1 mg/mL.
pyridylthio radical is dependent on the presence of oxygen; (iii) in the presence of oxygen, the pyridylthio radical gets converted to pyridine-2-sulfonic acid, while it undergoes hydrogen abstraction or radical recombination in deoxygenated solutions; (iv) the oxidation reaction also provides a pathway for driving the equilibrium towards the desired product; (v) this feature was used for the reagentless synthesis of crosslinked polymer nanoparticles; (vi) the size of the nanoparticles can be conveniently varied by tuning the size of the nanoaggregates, because the photo-induced crosslinking was predominantly intra-aggregate; and (vii) the crosslink density and encapsulation stability of these nanoparticles can be simply varied by altering the irradiation time. In addition to the typical advantages of a reagentless reaction, the fact that this reaction is carried out in water and that the byproduct of this reaction is a highly water-soluble sulfonic acid that can be easily removed by dialysis suggests that this methodology offers a promising new approach for nanoparticle synthesis. Since the crosslinks generated here are biologically relevant, this method could also have implications in generating new materials of interest in biology and medicine.

5.4 Experimental

5.4.1 General Methods

2,2'-Dithiodipyridine, 2-mercaptoethanol, polyethyleneglycol monomethylether methacrylate (PEGMA, $M_w$ 450 g/mol), D,L-dithiothreitol (DTT), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO), 4-Cyano-4-(phenylcarbonothioylthio)-pentanoic acid and other conventional reagents were obtained from commercial sources and were used as received without further purification. The 2,2’-azobisisobutyronitrile (AIBN) was purified by recrystallization from ethanol. $^1$H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). Molecular weights of the polymers were estimated by gel permeation chromatography (GPC) using PMMA standard with a refractive index detector. Dynamic light scattering (DLS)
measurements were performed using a Malvern Nanosetasizer. UV-visible absorption spectra were recorded on a Varian (model EL 01125047) spectrophotometer. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter.

5.4.2 Synthesis of 2-hydroxyethyl 2-pyridyl disulfide (PDS-OH) and 2-thiopyridone

PDS-OH was synthesized according to the previous report. 49 2, 2'-dipyridyl disulfide (DPDS) (10.3 g, 0.047 mol) was dissolved in 100 mL of methanol. Then 1.2 mL of glacial acetic acid, used as catalyst, was added. To this mixture, a solution of mercaptoethanol (4.4 g, 0.056 mol, 4 mL) in 100 mL methanol was added drop-wise at room temperature with continuous stirring. Once the addition was over, the reaction mixture was stirred at room temperature overnight. The stirring was stopped; solvent was evaporated to get the crude product as yellow oil which was purified by flash column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. When the polarity of the eluent was increased to 40% ethyl acetate/hexane, the desired product came out as colorless oil. Yield: 80 %. 1H NMR: (CD3OD, 400 MHz), δ (ppm): 8.40 (m, 1H, aromatic proton ortho-N), 7.84 (m, 1H, aromatic proton meta-N), 7.80 (m, 1H, aromatic proton para-N), 7.22 (m, 1H, aromatic proton, ortho-disulfide linkage), 3.77(t, 2H, -CH2OH), 2.94 (t, 2H, -SS-CH2-).

2-thiopyridone can also be prepared at the same time by continually increasing the polarity of the eluent up to 55% ethyl acetate/hexane to get the desired product. After concentrating the solution, light yellow Needle-shaped crystals were got. 1H NMR: (CD3OD, 400 MHz), δ (ppm): 7.67 (m, 1H), 7.47 (m, 2H), 6.85 (m, 1H).

5.4.3 Synthesis of PDS monomer

PDS monomer was also synthesized according to the previous report. 49 To a solution of PDS-OH (12 g, 64.1 mmol) in 50 mL of dry dichloromethane was added 7.8 g (76.9 mmol) of triethylamine. The mixture was firstly cooled in an ice-bath. To this cold mixture, a solution of methacryloyl chloride (6.7 g, 64.1 mmol) in 25 mL dichloromethane was then added drop-wise
with continuous stirring. After the addition was over the reaction mixture was stirred at room temperature overnight. The stirring was stopped and the reaction mixture was washed with 3x100 mL distilled water and then with 100 mL of brine. The organic layer was collected, dried over anhydrous Na₂SO₄ and concentrated to get the crude product as yellow oil. It was purified by column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. The pure product was collected at 30% ethylacetate/hexane. Yield: 80% ¹H NMR: (CDCl₃, 400 MHz), δ (ppm): 8.52 (m, 1H, aromatic proton ortho-N), 7.62-7.83 (m, 2H, aromatic proton meta-N and para-N), 7.15 (m, 1H, aromatic proton, orthodisulfide linkage), 6.23 (d, 1H, vinylic proton, cis-ester), 5.65(d, 1H, vinylic proton, trans-ester) 4.41 (t, 2H, -S-S-CH₂CH₂O-), 3.13 (t, 2H, -S-S-CH₂CH₂O-), 1.91(s, 3H, methyl proton of the methacryloyl group).

5.4.4 Synthesis of random copolymer containing PDS groups

Random copolymers were prepared by reversible addition–fragmentation chain transfer (RAFT) polymerization.⁴⁷ A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (24 mg, 0.086 mmol), poly(ethylene glycol) monomethyl ether methacrylate (0.4 g, 0.84 mmol), PDS (0.537 g, 2.1 mmol), and 2,2′-azobis(isobutyronitrile) (AIBN; 2.8 mg, 0.017 mmol) was dissolved in tetrahydrofuran (THF) (2 mL) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then transferred into a preheated oil bath at 65 °C for 10 h. To remove unreactive monomers, the resultant mixture was precipitated in cold ethyl ether (20 mL) to yield the random copolymer as a waxy liquid. GPC (THF) Mₘ, 12 kDa; polydispersity, 1.2. ¹H NMR (400 MHz, CDCl₃) δ 8.46, 7.68, 7.11, 4.35-4.09, 3.94-3.37, 3.03, 2.04-1.64, 1.43-0.87. The molar ratio between two blocks was determined by integrating the methoxy proton in the poly-(ethylene glycol) unit and the aromatic proton in the pyridine and was found to be 1:2.70 (PEO:PDS).
5.4.5 Time-dependent $^1$H NMR measurement

In 5mm NMR tubes, 1 mL of samples with different concentrations was added. $^1$H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. These tubes were put inside UV chamber (SYLVANIA F15T8/350/BL, 15w). After each 1 hour’s photoirradiation, another NMR spectrum for each sample was taken with the same parameters. The integrations of peak at 2.94 ppm and peak at 2.83 ppm were used to determine the percent of remaining PDS-OH.

5.4.6 Nanoparticles preparation

1 mL of polymer solution of different concentrations was added to a 7 mL vial. These vials were then put inside an UV chamber (SYLVANIA F15T8/350/BL, 15w). After photoirradiation for certain times, nanoparticles with different sizes were formed in solutions. The crosslinking density could be determined by taking some volume of polymer solution from the above solutions into cuvettes for UV-vis measurement. The concentration of these samples was all 0.1 mg/mL. Absorption spectra were taken first. Then, excess DTT were added into these samples and then the solution was observed using absorption spectroscopy again after 3 hours, during which all PDS functionalities could be changed to 2-thiopyridine by DTT.

5.4.7 Determination of crosslinking density

In 7mL small glass vial, 1 mL of polymer solution with different concentrations was added. From them, certain volume of polymer solution was taken and then added into cuvettes for UV-vis measurement. The volume was made to 1 mL by adding water. The concentration of these samples was all 0.1 mg/mL. Absorption spectra were taken. Excess DTT were added into these samples, one more absorption spectra were taken after 3 hours, during which all PDS functionalities could be changed to 2-thiopyridine by DTT.

Then these vials were put inside UV chamber (SYLVANIA F15T8/350/BL, 15w). After each 1 hour’s photoirradiation, certain volume of polymer solution was taken from the above
solutions and then added into cuvettes for UV-vis measurement. The volume was made to 1 mL by adding water. The concentration of these samples was all 0.1 mg/mL. Absorption spectra were taken. Excess DTT were added into these samples, one more absorption spectra were taken after 3 hours, during which all PDS functionalities could be changed to 2-thiopyridine by DTT.

5.4.8 Redox-responsive guest release experiment

A solution of nanogel containing DiI (100 µL) was mixed with water (800 µL) in a cuvette, and then GSH stock solution (100 µL) was added. The fluorescence spectra were recorded using the excitation wavelength of 530 nm.

For control experiment, A solution of nanogel containing DiI (100 µL) was mixed with water (900 µL) in a cuvette. The fluorescence spectra were recorded using the excitation wavelength of 530 nm.

5.4.9 FRET experiment

A solution of nanogel containing DiI (100 µL) was mixed with a solution of nanogel containing DiO (100 µL) in a cuvette, and then milliQ water (800 µL) was added to adjust the volume. The fluorescence spectra were recorded using the excitation wavelength of 450 nm.

5.5 References


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CHAPTER 6

THERMORESPONSIVE POLYMERIC NANOPARTICLES: NUCLEATION FROM
COOPERATIVE POLYMERIZATION DRIVEN BY DATIVE BONDS


6.1 Introduction

Cooperative polymerization, also called nucleation-elongation (N-E) polymerization, has become a new and interesting paradigm in the field of supramolecular materials.1-3 Although it has been observed and relatively well studied in biological systems (e.g. actin, tubulin, bacterial flagellin, and tobacco mosaic virus),4,5 these are not common in artificial material assemblies. It is believed that systems governed by the N-E mechanism can provide access to materials with novel properties, which are otherwise not available with classical synthetic polymerization techniques.6-8 For example, although a polymer is achieved through a condensation-type reaction, the N-E system could produce polymers with substantially high molecular weights even when the stoichiometry of the monomers is imbalanced.9 The molecular origin of cooperative growth of supramolecular polymers is regarded as the result of secondary interaction between units, which usually involves a non-covalent interaction arising due to electronic (both short-range polarization and longer-range electrostatic effects), structural (e.g. helix formation and allosteric conformational changes), or hydrophobic effects.1 However, there are no reports on utilizing dative bonds, a covalent interaction, as the driving force for the cooperative polymerization. Demonstration of such a possibility will greatly expand the repertoire of nucleation-elongation polymerization in materials science, as exemplified in this report.
Boronate ester formation is the reaction between boronic acids and diols to form boronate esters, which is a covalent, yet reversible, bonding interaction. Recently, considerable attention has been devoted to the use of this dynamic ester formation as a popular strategy to build boron-containing multicomponent systems, including self-assembling macrocycles, capsules, covalent organic frameworks, reversible linear polymers and particles. On the other hand, it is well known that boronate esters can act as Lewis acids, which enable them to form donor–acceptor complexes with N-donor ligands through a so-called coordinative or dative N→B bond and have been exploited to achieve several self-assembled systems.

In this paper, we report on a simple method to prepare monodisperse polymeric nanoparticles through sequential boronate esterification of boronic acids and bifunctional catechols under ambient conditions. We find that the nucleation step for the formation of the particle involves a N-E polymerization step, wherein the dative bond between the nitrogen in the imine building blocks and the boron in the boronate ester plays a critical role. The controlled polymerization induced nucleation of the particle formation also allows a unique opportunity for significant control over the size of the self-assembled nanoparticles. Since the dative covalent B-N bond is relatively weak, these boronate nanoparticles exhibit a unique reversible, thermal responsive behavior. We expect that this finding will have a considerable impact on the development of dynamic supramolecular materials.

6.2 Results and Discussion

6.2.1 Design and preparation of nanoparticles

To introduce the imine building blocks, we designed two monomers based on boronic acid and on catechol, which were synthesized through a simple condensation reaction between a p-phenylenediamine and an aryl carboxaldehyde (Figure 6.1a). The latter molecule was based on 4-formyl-phenylboronic acid or 3, 4-dihydroxybenzaldehyde to obtain the boronic acid monomer Im-BA or the catechol monomer Im-Ca respectively. When a methanol solution of Im-BA was
added dropwise to a methanol solution of \textbf{Im-Ca}, the resultant mixture became deeper in color compared to the monomer solutions and a turbid suspension. Analyses of these mixtures using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Figure 6.1b and 6.1c) revealed formation of spherical and monodisperse nanoparticles with a smooth surface. In the case of the nanoparticles shown in Figure 6.1, the concentrations of the \textbf{Im-BA} and \textbf{Im-Ca} were 13.4 mM (5.0 mg/mL). We also noticed that the size and dispersity of the particles is independent of the order of addition of the two solutions.

![Figure 6.1](image)

\textbf{Figure 6.1} Synthesis and characterization of boronate nanoparticle (BNP). a) Scheme presents the syntheses of \textbf{Im-Ca} and \textbf{Im-BA} monomers, as well as polymer P1 via the polyboronate esterification between \textbf{Im-Ca} and \textbf{Im-BA}. b) TEM image of the BNP, the scale bar is 500 nm. c) SEM image of \textbf{Im-Ca} and \textbf{Im-BA}. d) TEM and SEM samples were taken immediately after the addition. d) FTIR spectra of molecule \textbf{Im-BA}, \textbf{Im-Ca} and solid BNP, and e) Zoomed-in FTIR spectra from (d).

Considering the chemical structure of both starting molecules, we hypothesized that the observed nanoparticles are composed of boronate ester polymer P1, which are formed through
sequential boronate esterification. To test this, we first investigated the particles using infrared absorption spectroscopy (Figure 6.1d-6.1e). Contrary to the starting Im-BA and Im-Ca monomers, the product nanoparticles did not exhibit a distinct peak for the hydroxyl stretch frequencies at 3470 and 3217 cm\(^{-1}\), suggesting the formation of the dehydrated boronate ester products. Moreover, the peak corresponding to B-O stretch in boronate ester was also observed at 1350 cm\(^{-1}\).\(^{29,30}\) Similarly, the new peak centered at 1658 cm\(^{-1}\) is regarded as an IR marker for the formation of phenylboronate ester.\(^{31}\)

![Image]

**Figure 6.2** Particle size and absorption characteristics variation. a) DLS of the BNP prepared with different monomer concentration in methanol. TEM images of the BNP, prepared with different monomer concentration in methanol, b) 1.5 mg/mL, c) 3.0 mg/mL, d) 5 mg/mL. The scale bar is 500 nm. e) Normalized absorption spectra for Im-BA, Im-Ca and BNP prepared with different monomer concentration in methanol. Temporal evolution of absorption spectra for BNP prepared with f) 3 mg/mL in methanol, g) 5 mg/mL in methanol. All samples were prepared by adding 0.01 mL reaction mixture into 1 mL methanol.

Remarkably, we found that the size of these monodisperse particles can be tuned by simply altering the concentrations of the monomers used for the polymer formation. The spherical nanoparticles were about 150 nm in size, when the concentrations of the monomers were about 4.0 mM (1.5 mg/mL). This size could be increased to 300 nm and 500 nm, while maintaining the monodispersity of the nanoparticles, when the concentrations were increased to 8.0 mM (3.0
mg/mL) and 13.4 mM (5.0 mg/mL) respectively. Thus, we found that the sizes of the polymer nanoparticles systematically increase with concentration. The rather uniform dispersity of the particles is evident from the TEM images shown in Figure 6.2. These results were also consistent with the size measurements in solution using dynamic light scattering (DLS) (Figure 6.2a). Lack of diffraction peaks, except for an ill-defined broad peak at about 2θ=20, in powder X-ray diffraction (PXRD) (Figure 6.3) suggests that these particles were mostly amorphous. Interestingly, there is a need for a critical concentration, below which there was no discernible nanoparticle formation. We noticed that particles could not be formed when the initial concentration of both monomers (1:1 ratio) was lower than 1.0 mg/mL, as discerned from TEM. The poor correlation function, observed in DLS, further confirmed this assertion.

![Figure 6.3 PXRD pattern of the BNP. A broad bump at about 2θ=20 was resulted from crystalline parts of polymers.](image)

The absorbance spectra for these particles in methanol are also shown in the Figure 6.2e. Compared to the starting Im-BA and Im-Ca, the nanoparticle solutions exhibit new peaks at longer wavelengths. This is taken to be an indicator for the formation of boronate ester between Im-BA and Im-Ca, which could lead to a longer π-conjugated polymer. Conjugated systems extending through the boronate ester is reasonable. For example, poly (dioxaborole)s, in which
the empty p-orbitals on the boron atoms and the lone pairs of electrons on the oxygen atoms are in conjugation with the phenyl rings \(^{32-36}\), are considered to provide an opportunity for extending \(\pi\)-conjugation and thus have attracted attention as a novel class of conjugated polymers. The red shift in the new peak, resulting from the polymerization reaction, increased with concentration. In a condensation polymerization, it is reasonable to assume that the polymer length increases with increasing concentration and therefore it is reasonable that the increase in red shift is taken to be an indicator of the resultant increase in \(\pi\)-conjugation. Unfortunately, we could not measure the molecular weight of the polymer from the resultant nanoparticles by size-exclusion chromatography because of the disassembly in response to dilution.

![Absorption spectra for Im-BA and Im-Ca as the time.](image)

**Figure 6.4** Absorption spectra for **Im-BA** and **Im-Ca** as the time. The concentration for both solutions is 0.02 mg/mL.

As shown in Figure 6.2f and 6.2g, a time-dependent blue shift in the low energy peaks was also observed upon large dilution (about 100 times), along with the expected commensurate decrease in the absorption intensity. We attributed this to the dynamic nature of the boronate ester bonds and the dilution-induced depolymerization. To insure that imines are not unstable under these conditions, the same time-dependent absorption measurements were carried out for both molecules **Im-BA** and **Im-Ca** by themselves at an even higher dilution and no change in the absorption spectra was observed (Figure 6.4). This suggests that the observed change in absorption spectra was indeed due to the reversibility of the boronate ester. It should be noted
however that the hydrolytic stability of the imine bonds did compromise the nanoparticle morphology, when these were left in solution over a long time (over three days). Nonetheless, these nanoparticles were found to be stable in both morphology and size over several months in the solid state (see Experimental section).

6.2.2 Mechanism study of the particle formation

It has been reported that micron-sized particles can be prepared by adding 1.0 equiv pyridine into a 1:1 mixture of benzene-1,4-diboronic acid and 1,2,4,5-tetrahydroxybezene in THF. The authors proposed that the condensation polymerization between these two molecules could be promoted under these conditions, leading to nucleation in the early stages for the particle formation. In their experiments, the ratio of boron molecule to catechol molecule was set as 1:1, resulting in the formation of high $M_w$ polymers via condensation polymerization for the nucleation. In our case however, we hypothesized that the polymerization based on boronate esterification in our particle formation may not be a simple condensation polymerization, but a so-called nucleation-elongation polymerization which greatly enhances the materials tunability.

To understand the mechanism of the formation of the nanoparticles, we added different volumes (0.1, 0.3, 0.5, 0.65, 0.8, 1.0 mL) of a solution of Im-Ca in methanol (5 mg/mL) dropwise into a 1 mL of Im-BA (5 mg/mL in methanol). These solutions could be assumed as intermediate stages of the fast particles formation. As shown in Figure 6.5, DLS measurements and TEM suggest that the nanoparticle size systematically increased with increase in the amount of Im-Ca in the mixture. Since the molecular weights of the two monomers are similar, the relative stoichiometry of the first five solutions is below 1:1. It is interesting to note that small nanoparticles were formed even at a 1:10 ratio and that their sizes from all these mixtures were quite uniform. These observations provided the first indication that polymers are likely formed through the nucleation-elongation polymerization, rather than the classical step growth mechanism. At the beginning of the dropwise addition, the polymers formed via this specific polymerization could form the
nucleus that serve as the template for further nanoparticle growth with increasing amounts of Im-Ca. To make sure that short oligomers could not lead to the formation of particles, we synthesized molecule mono-Im-Ca containing only one catechol groups via reaction between aniline and 3, 4-dihydroxybenzaldehyde. The addition of this molecule should result in shorter oligomers in solution. No particles could be observed, suggesting that short oligomers indeed could not lead to the formation of particles. Note that Im-BA and Im-Ca in beginning of the Im-Ca addition were under sub-stoichiometric conditions. If the mechanism involves a simple step growth formation

Figure 6.5 Mechanism study of the particle formation. a) Photographs of the BNP solution with different ratios of Im-Ca into Im-BA in methanol. b) DLS of the BNP prepared with different addition volumes of Im-Ca into 1 mL of Im-BA solution. TEM images of the corresponding solutions, c) 0.10 mL Im-Ca + 1.0 mL Im-BA, d) 0.30 mL Im-Ca + 1.0 mL Im-BA, e) 0.65 mL Im-Ca + 1.0 mL Im-BA, f) 1.0 mL Im-Ca + 1.0 mL Im-BA. The scale bar is 500 nm. Absorption spectra of the reaction mixture with different initial concentrations during the dropwise addition, g) 1 mg/mL, h) 2 mg/mL, i) 3 mg/mL. Note that there were flat lines between 500 and 525 nm, because samples had an absorbance of more than dynamic range of instrument.
of the boronate ester, then only oligomers would form in solution and there should not be significant nanoparticle formation at imbalanced ratios of the starting monomers.

To further determine that it is the polymer formed but not oligomer formation, leading to the nucleation step, we investigated the absorption spectra of these reaction mixtures after small additions of \textbf{Im-Ca} into \textbf{Im-BA}, since the red shift in absorption likely indicates increase in conjugation, \textit{i.e.} polymer chain length. First, a solution of \textbf{Im-Ca} (1 mg/mL) was added in 0.1 mL increments to a 5 mL solution of \textbf{Im-BA} (1 mg/mL) and the evolution of the absorption spectrum of the resultant solution was monitored after each addition. As shown in Figure 6.5g, \textbf{Im-BA} solution with 1 mg/mL did not have any absorption while \textbf{Im-Ca} solution with 1 mg/mL had a small absorption from 500 nm to 550 nm. After 0.2 mL \textbf{Im-Ca} was added into 5 mL \textbf{Im-BA}, a significant absorption was noticed even though the concentration of \textbf{Im-Ca} in this mixture was only around 0.04 mg/mL, suggesting the formation of oligomers, which have a much stronger absorption from 500 nm to 550 nm. As more \textbf{Im-Ca} was added, the absorption intensity of the low energy peak between 500 nm and 550 nm keeps slowly increasing and red-shifting throughout the addition process. Interestingly however, no particle formation was observed via both DLS and TEM at this monomer concentration. The solution was also clear, although the color became deep orange. This is taken to suggest that the 1 mg/mL concentrations of the monomers are lower than the critical concentration for the nucleation step in the nucleation-elongation polymerization. On the contrary, when the concentrations of the monomers are 2 mg/mL, there was a sharp increase in absorption after 550 nm at a sub-stoichiometric ratio of monomers (2 mL of \textbf{Im-Ca} into 5 mL of \textbf{Im-BA}, the ratio of \textbf{Im-Ca} to \textbf{Im-BA} is 0.4/1), as shown in Figure 6.5h, suggesting the formation of polymers with larger conjugation. At this ratio, the solution became a turbid suspension and small spherical particles were also observed by TEM. Similar results were found at an even lower stoichiometry of \textbf{Im-Ca} (only 0.9 mL of \textbf{Im-Ca} into 5 mL of \textbf{Im-BA}), when the initial concentration was 3 mg/mL (Figure 6.5i).
Several observations are noteworthy in this reaction: 

(i) there is a critical concentration above which nucleation for nanoparticle formation seems to occur (>1 mg/mL in this case). 

(ii) Conjugated polymers with strong absorption above 550 nm are achieved even when there is a significant imbalance in the stoichiometry of the constituent monomers. 

(iii) Uniformly dispersed nanoparticles are formed at these imbalanced stoichiometry conditions, and size of particles increased with the continuing addition of monomer. 

(iv) The sharp increase in low energy absorption at a certain ratio is indicative of the possibility that the reaction rate is much faster after a slower nucleation process. All these observations are consistent with the nucleation-elongation (N-E) polymerization pathway.

The cooperative growth of supramolecular polymers, seen in such a N-E pathway, often has a secondary interaction as its molecular origin. We hypothesized that the possible dative N→B bond from the nitrogen of the imine and the boron from the boronate ester could be the reason for chain fold and collapse, which has been often proposed to drive the high-polymer formation in the reversible polymerization.\(^{37,38}\) Similarly, the La Mer model suggests that the common route to achieve monodisperse NPs in the solution phase is to separate nucleation and growth steps during the synthesis.\(^{39}\) Here, we hypothesize that the N-E polymerization first leads to polymers, inducing particle nucleation in the early stage. Further particle growth continues using the initially formed aggregates as the template. Finally, the resulting particles have to be spherical owing to the requirement for minimization of the interfacial free energy between the particles and solvent used.\(^{40,41}\)

To first test whether there is a dative interaction between the imine nitrogen and boronate ester boron atoms, we focused on the distinct structural change from trigonal planar to tetrahedral geometry at the boron atom associated with such an interaction.\(^{21}\) Indeed, a sharp peak was observed at 21 ppm in solid state \(^{11}\)B NMR for the particles, indicative of the tetrahedral geometry. This peak is distinct from the typical signal at ~30 ppm, observed for the trigonal planar boronate esters in the solid state (Figure 6.6).\(^{42}\) In fact, in the case of the boronate
nanoparticles, a small percentage of the trigonal boronate ester was seen in the NMR, most likely arising from the surface boronate moieties that are not datively satisfied. In addition, we also note that Im-BA by itself exhibits both trigonal-planar and tetrahedral geometries at the boron atom. This is attributed to the fact that boronic acids could also form dative N-B bonds with the imine groups.

To investigate further whether the imine bonds really play an important role in the formation of the nanoparticles, we synthesized two structurally similar stilbene-based monomers St-BA and St-Ca, wherein the imine bond was replaced with C=C double bond (Scheme 6.1). Since the solubility of St-BA in methanol is low, we used ethanol as the solvent in these experiments. No discernible nanoparticles were found, when the experiments were carried out with St-BA and St-Ca. However, the corresponding imine-based monomers did indeed afford nanoparticles in ethanol, supporting our assertion that the presence of imine is important. Interestingly, mixing the stilbene-based boronic acid monomer with the imine-based catechol monomer or vice versa did not result in any nanoparticle formation (Figure 6.7). These indicate that there seems to be a critical density of the imines that is essential for the observed nanoparticle formation.

Figure 6.6 Solid $^{11}$B-NMR spectra of Im-BA, boronate ester from 4-formylphenylboronic acid and 3,4-dihydroxybenzaldehyde, and solid BNP.

To investigate further whether the imine bonds really play an important role in the formation of the nanoparticles, we synthesized two structurally similar stilbene-based monomers St-BA and St-Ca, wherein the imine bond was replaced with C=C double bond (Scheme 6.1). Since the solubility of St-BA in methanol is low, we used ethanol as the solvent in these experiments. No discernible nanoparticles were found, when the experiments were carried out with St-BA and St-Ca. However, the corresponding imine-based monomers did indeed afford nanoparticles in ethanol, supporting our assertion that the presence of imine is important. Interestingly, mixing the stilbene-based boronic acid monomer with the imine-based catechol monomer or vice versa did not result in any nanoparticle formation (Figure 6.7). These indicate that there seems to be a critical density of the imines that is essential for the observed nanoparticle formation.
Figure 6.8 a) Typical photos of the mixtures using Im-BA react with Im-Ca2 and Im-Ca6 in methanol, and their TEM pictures of BNP using b) Im-Ca2, c) Im-Ca6. The scale bar is 500 nm.
To further test this, we introduced spacers between the catechol moieties by reacting ethylenediamine and hexamethylene diamine to react with 3,4-dihydroxybenzaldehyde to obtain Im-Ca2 and Im-Ca6 respectively (Scheme 6.1). In these cases, we dilute the nitrogen-boron contacts. Indeed, mixing these molecules with Im-BA did afford nanoparticles. However, these nanoparticles were not as well-defined and were less monodisperse compared to those obtained with Im-BA and Im-Ca (Figure 6.8), supporting our assertion that a critical density of imines might indeed be needed.

![Image](image.png)

**Figure 6.9** Solvent effects on the particle formation. TEM images of the BNP prepared with methanol (M) and DMSO (D) mixture solution, a) M/D=4/1, b) M/D=6/1, c) M/D=9/1, d) Pure M. The scale bar is 500 nm.

Next, we were interested in studying the effect of solvent on the formation of the particles to further investigate the effect of interrupting secondary interactions upon the formation of the nanoparticles. Since solvent could significantly influence the rather weak secondary interactions, this could play a critical role on the stability and dynamics of a supramolecular polymer.43, 44
While the alcoholic solvents such as methanol, ethanol and isopropanol afforded the nanoparticles, no nanoparticle formation was observed with DMSO as the solvent. It is likely that DMSO interrupts the dative interaction between the imine nitrogen and boron. To empirically test the effect of DMSO, we tested the formation of the boronate ester particles in a mixture of methanol and DMSO. Indeed, particle with around 30 nm diameter formed in the mixture when the ratio of methanol to DMSO is 4/1, while the same mixture afforded a particle size of 450 nm in pure methanol. There were no discernible particle formation, when the presence of DMSO was much higher. In fact, the size of the nanoparticles systematically decreased from pure methanol to mixture solvent with different percentages of DMSO, and the size also decreased with the increasing presence of DMSO (Figure 6.9a-6.9d).

6.2.3 Responsive behavior of BNP based on dative N→B bond

![Image](bee02.png)

Figure 6.10 Responsive behavior of BNP based on dative N→B bond. a) Photographs of the BNP with the addition of pyridine, imidazole, 4-dimethylaminopyridine, b) Photographs of the BNP with changing temperature and their TEM images of the BNP, c) before heating, d) after first heat-cool cycle, e) after second heat-cool cycle, f) after third heat-cool cycle. The scale bars are 500 nm.

To further test whether competing binders for boron could interrupt the dative N→B interaction and thus interrupt the observed cooperative supramolecular assembly, we chose to
study the effect of amine-based Lewis bases, such as pyridine, imidazole, 4-dimethylaminopyridine (DMAP) (Figure 6.10a). We found that the addition of a strong base such as imidazole and DMAP causes the turbid nanoparticle solution to become clear. This is attributed to the possible breakage of the dative N→B interaction by these Lewis bases. Without the secondary interaction, the formed nanoparticles could disassemble. Indeed no particles were observed in these solutions after the addition of these Lewis bases. On the other hand, addition of pyridine did not cause any change to the nanoparticles, most likely because the N→B interactions were unaffected by the rather weak Lewis base.

Finally, we were interested in testing the effect of temperature upon the nanoparticles for two reasons: first, it is possible that the relatively weak N→B dative bond could be broken at higher temperatures\(^{26}\), which provides an opportunity to test the possibility of thermal reversibility in these nanoparticles. Second, if we were able to disassemble the particles at higher temperature in solution, investigation of the morphology and size of the particles re-formed allows us to test whether the initially formed nanoparticle is the result of equilibrium self-assembly. As shown in Figure 6.10b, the methanolic solution of nanoparticles became clearer upon heating it to 60 °C and the solution turned turbid again when cooled down to ambient temperature. This indicates that the nanoparticles could be losing its nanoassembly at higher temperature, but is re-formed upon cooling. To test whether the morphology and size of the nanoparticles were similar before and after the heat-cool cycle, the samples were investigated using TEM. Indeed, both these features remain unchanged even after three heat-cool cycles. We also further tracked the reformation of these nanoparticles by looking at the samples for TEM at different time points during the cooling step (Figure 6.11). It is clear that the nanoparticles are small initially, finally reaching the particle size similar to that observed before heat-cool cycle. These results show that these nanoparticles are thermally reversible and that the formation of the nanoparticles is likely due to equilibrium self-assembly.
In summary, we report on a supramolecular nanoparticle that is autonomously self-assembled by conjugated polymers based on boronate ester. We have shown that: (i) the polymers inducing nucleation for the formation of the particle involves a N-E polymerization step; (ii) dative bond between the nitrogen in the imine building blocks and the boron in the boronate ester is regarded as the necessary secondary interaction for the specific reaction; (iii) their particle size could be easily adjusted by controlling the mixing concentration; (iv) imine density and solvent can affect the particle formation; and (v) these boronate nanoparticles exhibit thermally responsive disassembly behavior due to the relatively weak dative covalent N→B bond. To our knowledge, this is the first time for a cooperative polymerization to use covalent dative bond as

**Figure 6.11** TEM images of the BNP, a) before the heating and cooling at room temperature for b) 1 min, c) 5 mins, d) 10 mins.
the driving force. We believe that this will open new doors for autonomous self-assembly strategies to achieve not only monodisperse, but also functionally diverse nanoassemblies.

6.4 Experimental

6.4.1 General Procedures

$p$-Phenylenediamine, ethylenediamine, hexamethylene diamine, 3,4-dihydroxybenzaldehyde, 4-formylphenylboronic acid, tert-butyldimethylsilyl chloride, imidazole, 4-Dimethylaminopyridine, pinacol, 1,4-bis (bromomethyl) benzene, triethylphosphite and other conventional reagents were obtained from commercial sources and were used as received unless otherwise mentioned. $^1$H-NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using the residual proton resonance of the solvent as the internal standard. $^{13}$C-NMR spectra were recorded on a Bruker 100 MHz NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. Solid state $^{11}$B-NMR spectra were recorded at 13 kHz on a Bruker DSX300 spectrometer with 4 mm rotor. Mass spectra were taken on Bruker MicrOTOF-II. Dynamic light scattering (DLS) measurements were performed by using a Malvern Nanozetasizer. UV/visible absorption spectra of the samples in solutions were measured on a Unico UV/Vis 2802PCS instrument. Transmission electron microscopy (TEM) images were taken from JEOL 2000FX at 200 KV. FT-IR spectra were acquired in a Bruker Alpha-P Diamond ATR-FTIR. Scanning electron microscopy (SEM) images were taken from FESEM Magellan 400. Thermogravimetric analysis (TGA) was performed in a nitrogen atmosphere on a DuPont TGA 2950 at a heating rate of 10 °C/min.

6.4.2 Synthesis of Im-Ca

Scheme 6.2 Synthesis of Im-Ca.
The preparation of **Im-Ca** was achieved following a literature procedure with a minor modification. The scheme is shown above. To a solution of p-phenylenediamine (0.15 g, 1.39 mmol) in anhydrous ethanol (10 mL) was added 3,4-dihydroxybenzaldehyde (0.40 g, 2.90 mmol). The clear brown mixture was stirred at room temperature under an argon atmosphere and protected from light. The product precipitated out of the reaction mixture. The brown precipitate was filtered and washed with cold ethanol three times. Final product was vacuum dried to obtain a brown solid (yield: 70%) $^1$H-NMR (400MHz, DMSO-6D) δ(ppm): 9.57 (s, 2H), 9.29 (s, 2H), 8.41 (s, 2H), 7.38 (d, 2H), 7.23 (s, 4H), 7.17 (q, 2H), 6.82 (d, 2H). $^1$H-NMR (400MHz, CD$_3$OD and DMSO-6D) δ(ppm): 8.39 (s, 2H), 7.39 (d, 2H), 7.22 (s, 4H), 7.17 (q, 2H), 6.82 (d, 2H). $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 159.89, 149.57, 146.09, 128.56, 122.91, 122.30, 115.83, 114.51. ESI/MS m/z 349.11 [M+H]$^+$ (expenct m/z=349.35).

### 6.4.3 Synthesis of **Im-BA**

**Im-BA** was synthesized from condensation reaction between p-phenylenediamine and 4-formylphenylboronic acid according to the synthesis of **Im-Ca**. The scheme is shown above. To a solution of p-phenylenediamine (0.05 g, 0.46 mmol) in 20 mL MeOH was added 4-formylphenylboronic acid (0.15 g, 1.00 mmol). The clear yellow mixture became cloudy after stirring at room temperature overnight. The yellow precipitate was filtered and washed with cool methanol three times. Final product was vacuum dried to obtain a yellowish solid (yield: 80%) $^1$H-NMR (400MHz, DMSO-6D) δ(ppm): 8.67 (s, 2H), 8.22 (s, 4H), 7.89 (m, 8H), 7.36 (s, 4H). $^1$H-NMR (400MHz, CD$_3$OD and DMSO-6D) δ(ppm): 8.65 (s, 2H), 7.88 (m, 8H), 7.34 (s, 4H). $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 160.67, 149.83, 137.76, 134.90, 128.06, 122.54.
ESI/MS m/z 431.16 [M+59] (expect m/z=431.19), m/z 445.20 [M+73] (expect m/z=445.21), m/z 459.22 [M+87] (expect m/z=459.23).

6.4.4 Synthesis of mono-Im-Ca

Scheme 6.4 Synthesis of mono-Im-Ca.

Mono-Im-Ca was synthesized according to a literature procedure. In brief, aniline (0.466 g, 5 mmol) and 3,4-dihydroxybenzaldehyde (0.691 g, 5 mmol) were mixed in water (10 mL) and stirred at room temperature for 1 hour. Then the solid insoluble precipitate was filtered and washed with water for three times. The crude product was recrystallized from toluene to give a pale yellow solid (yield: 60%). 1H-NMR (400MHz, DMSO-6D) δ(ppm): 9.40 (s, 2H), 8.35 (s, 1H), 7.38 (m, 3H), 7.15 (m, 4H), 6.83 (d, 1H). 13C-NMR (100MHz, DMSO-6D) δ(ppm): 160.67, 152.44, 149.69, 146.10, 129.58, 128.45, 125.72, 123.00, 121.33, 115.82, 114.65.

6.4.5 Synthesis of Im-Ca2

Scheme 6.5 Synthesis of Im-Ca2.

The preparation of Im-Ca2 is similar to that of Im-Ca by using ethylenediamine instead. The scheme is shown above. To a solution of 3,4-dihydroxybenzaldehyde (0.2 g, 1.45 mmol) in methanol (5 mL) was added ethylenediamine (0.046 mL, 0.041 g, 0.68 mmol). The mixture was stirred at room temperature under an argon atmosphere and protected from light. The product precipitated out of the reaction mixture. The precipitate was filtered and washed with cool methanol three times. Final product was vacuum dried to obtain a pale yellow solid (yield: 80%). 1H-NMR (400MHz, CD3OD) δ(ppm): 8.03 (s, 2H), 7.17 (d, 2H), 7.02 (q, 2H), 6.71 (d, 2H), 3.82
(s, 4H). $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 161.75, 148.72, 145.79, 128.55, 121.67, 115.63, 114.07, 61.36, 48.98. ESI/MS m/z 301.11 [M+H]$^+$ (expect m/z=301.31).

6.4.6 Synthesis of Im-Ca6

![Scheme 6.6 Synthesis of Im-Ca6.](image)

The preparation of Im-Ca6 is also similar to that of Im-Ca by using hexamethylene diamine instead. The scheme is shown above. To a solution of 3, 4-dihydroxybenzaldehyde (0.2 g, 1.45 mmol) in methanol (5 mL) was added hexamethylene diamine (0.08 g, 0.69 mmol). The mixture was stirred at room temperature under an argon atmosphere and protected from light. The product precipitated out of the reaction mixture. The precipitate was filtered and washed with cool methanol three times. Final product was vacuum dried to obtain a pale yellow solid (yield: 85%).

$^1$H-NMR (400MHz, CD$_3$OD) δ(ppm): 8.02 (s, 2H), 7.17 (s, 2H), 7.05 (q, 2H), 6.71 (d, 2H), 3.52 (m, 4H), 1.70 (m, 4H), 1.40 (m, 4H). $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 160.31, 148.73, 146.06, 128.43, 121.69, 115.79, 113.87, 60.55, 31.12, 27.06. ESI/MS m/z 357.17 [M+H]$^+$ (expect m/z=357.42)

6.4.7 Synthesis of St-BA and St-Ca

Both molecules were prepared through Horner-Wadsworth-Emmons reaction between tetraethyl (1,4-phenylenebis(methylene))bis(phosphonate) and benzaldehyde molecules. Pinacol protected 4-formylphenylboronic acid and TBS protected 3, 4-dihydroxybenzaldehyde were used as the aldehydes in the syntheses of St-BA and St-Ca respectively.
6.4.8 Synthesis of tetraethyl (1,4-phenylenebis(methylene))bis(phosphonate)

Scheme 6.7 Synthesis of tetraethyl (1,4-phenylenebis(methylene))bis(phosphonate).

The mixture of 1,4-bis (bromomethyl) benzene (1.3 g, 4.93 mmol) and triethylphosphite (20 mL) was heated to reflux for 5 hours. Afterwards, excess triethylphosphite was removed under the reduced pressure. The remaining slurry was poured into a large amount of hexane to extract residual remains of triethylphosphite. The precipitate was filtered and washed with hexane three times. Final product was vacuum dried to obtain a white solid (yield: 90%). $^1$H-NMR (400 MHz, CDCl$_3$) δ(ppm): 7.24 (s, 4H), 4.00 (m, 8H), 3.12 (d, 4H), 1.23 (t, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ(ppm): 130.25, 129.96, 62.24, 34.13, 32.51, 16.40.

6.4.9 Protection of 4-formylphenylboronic acid

Scheme 6.8 Protection of 4-formylphenylboronic acid.

The boronic acid group was protected using pinacol. At room temperature, 10.0 mmol boronic acid and anhydrous pinacol (11.0 mmol) were mixed in anhydrous diethyl ether (10 mL) and the reaction mixture was stirred for 24 hours. After washing with water, the ether phase was dried with MgSO$_4$ and concentrated to dryness in vacuo to give the final product (yield: 95%). $^1$H-NMR (400MHz, CDCl$_3$) δ(ppm): 10.05 (s, 1H), 7.95 (d, 2H), 7.87 (d, 2H), 1.36 (t, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ(ppm): 192.83, 138.56, 135.30, 128.86, 84.26, 25.34.
6.4.10 Protection of 3, 4-dihydroxybenzaldehyde

The hydroxyl groups were protected using tert-butyldimethylsilyl chloride. To a solution of 3, 4-dihydroxybenzaldehyde (2.05 g, 15 mmol) in 25 mL DMF was added imidazole (3.05 g, 45 mmol), DMAP (183 mg, 1.5 mmol) and tert-butyldimethylsilyl chloride (6.80 g, 45 mmol). The mixture was kept stirring at room temperature for 1 hour. Then the solution was extracted with 25 mL diethyl ether for three times. The combined organic solution was washed with brine, and then dried over anhydrous MgSO$_4$. The remaining solvent was removed under reduced pressure. Final product was vacuum dried to obtain a white solid (yield: 98%). $^1$H-NMR (400MHz, CDCl$_3$) δ(ppm): 9.80 (s, 1H), 7.35 (m, 2H), 6.93 (d, 1H), 0.99 (s, 18H), 0.23 (s, 12H). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ(ppm): 190.99, 153.41, 147.77, 130.72, 125.30, 120.43, 25.59, 18.61.

6.4.11 Synthesis of St-BA

First, 1.72 mmol aldehyde was added to a solution of the tetraethyl (1,4-phenylenebis(methylene))bis(phosphonate) in anhydrous DMF (5 mL) under argon atmosphere. 

Scheme 6.10 Synthesis of St-BA.
Potassium tert-butanolate (5.17 mmol) was added to the clear solution at 0 °C. The mixture was stirred for 24 hours and allowed to warm up to room temperature. The reaction mixture was poured into water (20 mL) and neutralized with 1M HCl. The white precipitate formed was collected by filtration, washed thoroughly with water, and dried under vacuum. The residue was recrystallized from petroleum ether to give the pre-product as colorless needles (yield: 90%).

The crude pre-product was dissolved in THF (11mL) and some sodium periodate (2.22 g) and water (2.80 mL) was added. After stirring for 30 mins, 1M HCl (1.1 mL) was added. After 2 more hours, additional sodium periodate (1.17 g) was added and the solution was stirred for another 2 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water. The organic phase was dried over anhydrous MgSO₄. The remaining solvent was removed under reduced pressure. Final product was vacuum dried to obtain a light yellow solid (yield: 50%). ¹H-NMR (400MHz, DMSO-6D) δ(ppm): 8.03 (s, 4H), 7.78 (d, 4H), 7.62 (s, 4H), 7.56 (d, 4H), 7.28 (q, 4H). ¹³C-NMR (100MHz, DMSO-6D) δ(ppm): 139.04, 136.93, 135.03, 129.01, 128.91, 127.41, 125.97. ESI/MS m/z 429.18 [M+59]⁻ (expect m/z=429.20), m/z 443.21 [M+73]⁻ (expect m/z=443.22), m/z 457.22 [M+87]⁻ (expect m/z=457.24).

6.4.12 Synthesis of St-Ca

Scheme 6.11 Synthesis of St-Ca.
The preparation of **St-Ca** is similar to that of **St-BA** by using protected 3, 4-dihydroxybenzaldehyde instead. 1.06 mmol aldehyde was added to a solution of tetraethyl (1,4-phenylenebis(methylene))bis(phosphonate) in anhydrous DMF (5 mL) under argon atmosphere. Potassium tert-butoxide (3.17 mmol) was added to the clear solution at 0 °C. The mixture was stirred for 24 hours and allowed to warm up to room temperature. The reaction mixture was poured into water (20 mL) and neutralized with 1M HCl. The mixture was kept stirring overnight. The white precipitate formed was collected by filtration, washed thoroughly with water, and dried under vacuum. The residue was recrystallized from petrolether to give the pre-product as colorless needles (yield: 60%). $^1$H-NMR (400MHz, DMSO-6D) δ(ppm): 9.10 (s, 2H), 8.94 (s, 2H), 7.49 (s, 4H), 6.90 (m, 8H), 6.72 (d, 2H), $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 146.11, 145.84, 136.60, 129.15, 128.85, 126.80, 125.13, 119.10, 116.13, 113.68. ESI/MS m/z 369.10 [M+Na]$^+$ (expect m/z=369.38).

**6.4.13 Preparation of Nanoparticles via dropwise addition**

First, **Im-Ca** and **Im-BA** were dissolved in methanol with the help of heating. After cooling down by setting at room temperature, one solution was added into the solution drop by drop, the mixture solution because deep orange and suspension was finally got. The resultant particles were purified by repeating centrifugation, decantation and resuspension. The composite particles were then dried in a vacuum oven at 50 °C overnight.
6.4.14 Stability of nanoparticles in solution and in solid state.

![Image of TEM images showing nanoparticles](image)

**Figure 6.12** Aging of the BNP in methanol. TEM images of the samples taken after a) 5 mins after the mixing, size is 330 nm, b) 3 days after the mixing, size is 320 nm. The scale bar is 500 nm.

As noted in the manuscript, the nanoparticles disassemble at large dilutions in solution. Here we compare the stability in solution and dry solid state over a prolonged period. In solution, the particles seem to turn porous after 3 days (Figure 6.12). One possible explanation is that imine bond might hydrolyze, resulting in the disassembly of the particles. In fact, we observed that the imine bond in the **Im-BA** could hydrolyze back to the amine and aldehyde molecules slowly. In the $^1$H NMR spectra over time, the intensity of the singlet at ~10 ppm, arising from the aldehyde CH proton, increased with a concurrent decrease in the intensity of the singlet at 8.6 ppm from the imine CH proton (Figure 6.13). By contrast, the dried particles retained its spherical morphology and size, when stored under dry conditions and redispersed in methanol for analysis.
Additionally, we characterized the thermal stability of the particles by TGA (Figure 6.14).

The nanoparticles were found to be quite stable thermally with >70% of the residue remaining even at the high temperature of 600 °C. There was about 10% weight decrease at ~150 °C indicating the possibility that there were some solvents trapped in these nanoparticles.

**Figure 6.13** NMR spectra showing stability of the imine bond in **Im-BA** and **Im-Ca** as time.
6.5 References


Figure 6.14 TGA curves of Im-BA, Im-Ca and solid BNP.


CHAPTER 7
HOLLOW METAL ORGANIC NANOPARTICLES FROM POLYMERIC
NANOPARTICLES USING KIRKENDALL EFFECT

7.1 Introduction

The rapidly developing field of nanotechnology depends on versatile strategies that provide access to the next generation of nanomaterials\(^1\).\(^3\). Majority of current nanomaterials are either purely inorganic\(^4\),\(^5\), such as silica nanoparticles, metal and metal oxide nanocrystals, or purely organic\(^6\),\(^7\), such as polymer nanoparticles and supramolecular assemblies. There is a recent surge in methods for generating hybrid metal-organic coordination materials, constructed from metal ions and polyfunctional organic ligands\(^8\)-\(^11\). One of the important determinants of their versatility emanates from the opportunity for diversity in these materials through functional group variations of the inorganic or organic building blocks. This chemical design feature has opened up the opportunities for these materials in a number of diverse applications such as catalysis, gas storage, biosensing, and biomedicine\(^12\)-\(^14\).

In all these applications, hollow nanoparticles have increasingly attracted special interest, because of their low density, high surface area, material economy, and lower cost compared to their solid counterparts. Several assembly techniques, including liquid-liquid interfacial growth\(^15\), spay-drying\(^16\), solvothermal approach\(^17\),\(^18\) Ostwald ripening,\(^19\) and template synthesis\(^20\)-\(^22\), have been used to prepare hollow metal-organic nanoparticles (MOPs). Similarly, hollow inorganic nanoparticles have been accessed using a range of related techniques\(^23\)-\(^26\). Among the methods used for hollow inorganic nanoparticles, Kirkendall effect is particularly interesting, as this provides a simple and robust access to pristine and hybrid hollow inorganic materials\(^24\). Despite the advantages, this non-reciprocal diffusion process has not been used for achieving hollow MOPs.
In this manuscript, we report a simple, rapid, and robust strategy for achieving hollow MOPs from high-quality organic polymer nanoparticles using the Kirkendall effect. The well-defined solid nature of the starting organic polymer nanoparticles translates to well-defined hollow MOPs after the addition of metal ions. We show that (i) the underlying mechanism for the formation of the hollow MOP is the Kirkendall effect, which is the first time to be applied for metal-organic composites, rather than for metallic nanocrystals; (ii) a variety of metal ions with different coordination geometries can result in the formation of the hollow MOPs, indicating the generality of the methodology; (iii) this Kirkendall effect is then conveniently exploited to demonstrate control over the thickness of the nanoparticle walls; and (iv) the size of the hollow MOPs can be easily tuned by using the precursor organic polymer nanoparticles of different sizes.

7.2 Results and Discussion

7.2.1 Preparation and characterization of hollow metal organic nanoparticles (MOPs)

Scheme 7.1 Scheme presents the syntheses of BNP via Im-Ca and Im-BA, and the preparation of hollow MOP by adding metal chloride salts to the BNP solution.

In a typical synthesis, a calculated amount of FeCl₃ is simply added to a methanolic suspension of the pre-made organic boronate nanoparticle (BNP). After stirring the solution for about 12 hours, a solid product was collected by centrifugation and washed with methanol. The BNP itself was prepared using a simple condensation-driven cooperative polymerization of a bis-imido boronic acid (Im-BA) molecule and a bis-imido catechol (Im-Ca) molecule in methanol. The reaction scheme and the MOP formation are illustrated in scheme 7.1.
Figure 7.1a shows transmission electron microscopy (TEM) images of BNP with diameter of \(~300\) nm. Upon addition of FeCl$_3$, the color of the solution immediately changed and the formation of nanoparticles with distinct contrast between the center and corona were evident from TEM (Figure 7.1b). This provided the first indication of the hollow nature of the resultant nanoparticles. The hollow morphology was further investigated using field emission scanning electron microscopy (SEM, Figure 7.1c). Broken nanoparticles indeed showed empty inner spaces (inset of Figure 7.1c), providing direct evidence for the formation of hollow nanoparticles.

**Figure 7.1** a) TEM image of the BNP; b) TEM image of the hollow Fe$^{3+}$-MOP; c) SEM image of the hollow Fe$^{3+}$-MOP; Inset is a SEM image of one broken hollow Fe$^{3+}$-MOP. All the scale bar is 500 nm.

**Figure 7.2** a) EELS mapped images of BNP; b) EELS mapped images of hollow Fe$^{3+}$-MOP. All the scale bar is 500 nm.
The presumption in this experiment is to test the effect of the strong coordination interaction between catechol moieties and Fe$^{3+}$ upon the polymer nanoparticle. This coordination interaction presumably replaces the boronic acid monomer in the polymer to result in a crosslinked nanoparticle. Note that Fe$^{3+}$ complexes are octahedral and therefore require three catechol moieties for binding, which should result in a crosslinked nanoparticle. This possibility was investigated using Energy Filtered Transmission Electron Microscopy (EFTEM). The EFTEM maps show clearly that boron, carbon, nitrogen and oxygen are all homogenously distributed throughout the entire BNP (Figure 7.2a). In contrast however, iron was found along with carbon, nitrogen and oxygen in the hollow nanoparticles (Figure 7.2b). There was no discernible presence of boron in these particles, implying that the hollow nanoparticles might indeed be due to the iron-catechol coordination and thus the observed nanostructures could be hollow MOPs. To further investigate the hollow nature of these MOPs, EFTEM cross-sectional profiles were

Figure 7.3 Fe, C, N and O line-scanned EELS profiles of two nanoparticles in Figure 1f. EELS images and line-scan profiles confirm the hollow structure of hollow Fe$^{3+}$-MOP.
analyzed across two individual particles (Figure 7.3). Elemental mapping by energy dispersive X-ray absorption spectroscopy (EDS) further confirmed similar distribution of iron and carbon throughout the entire hollow nanoparticles (Figure 7.4). The intensity of the mapped images shows the characteristic properties of a hollow structure with contrast between the core and shell of the nanoparticles.

![Figure 7.4](image)

**Figure 7.4** Bright-field STEM images and EDS maps of hollow Fe$^{3+}$-MOP, element C and Fe included.

### 7.2.2 General method for hollow metal organic nanoparticles (MOP)

The versatility of this method of generating hollow MOPs was investigated using other metal ions, Cu$^{2+}$, Sn$^{4+}$, and Al$^{3+}$. These metal ions were chosen, because they provide not only variations in their preferred coordination geometry (4- or 6-coordinate), but also variations in their ability to crosslink. Since only two catechol units are needed for the 4-coordinate distorted tetrahedral geometry, it is not necessary that the resultant metal organic material is crosslinked. We investigated whether similar hollow MOP formation could be observed for these other metal ions as well. As shown in Figures 7.5a-7.5c, hollow MOPs are also obtained for all of these ions. EDS analysis of the Sn-based MOP also showed the presence of both tin and carbon in the hollow nanoparticles (Figure 7.6a). In addition, the possibility of preparing hollow dual-metal MOPs was
Figure 7.5 a) TEM image of the hollow Cu$^{2+}$-MOP by adding Cu$^{2+}$; b) TEM image of the hollow Sn$^{4+}$-MOP adding Sn$^{4+}$; c) TEM image of the hollow Al$^{3+}$-MOP adding Al$^{3+}$; All the scale bar is 500 nm.

Figure 7.6 a) Bright-field STEM images and EDS maps of hollow Sn$^{4+}$-MOP, element C and Sn included; b) HAADF STEM images and EDS maps of dual Fe$^{3+}$/Sn$^{4+}$-hollow MOP, element C, Fe and Sn included.
also tested by adding a mixture of FeCl₃ and SnCl₄ in a 1:1 ratio to the BNP solution. EDS of samples indeed showed a homogenous distribution of element Fe and Sn in the hollow nanoparticles (Figures 7.6b).

7.2.3 Electron paramagnetic resonance spectroscopy characterization

The hypothesis for describing the formation mechanism of the hollow MOPs is that the metal ions replace the boronate functionalities to form a stable metal-catecholate complex. Electron paramagnetic resonance (EPR) spectroscopy was used to investigate this ligand field environment around the high-spin (hs, d5) Fe³⁺. EPR spectroscopy is a sensitive probe of the number of catecholate ligands coordinated to the Fe³⁺ center²⁸. Figure 3 shows the EPR spectra of the metal-free BNP and three metal-containing (Fe³⁺, Al³⁺, and dual Fe³⁺ - Al³⁺) hollow MOPs. The spectrum of as-prepared BNP shows a single sharp feature at $g = 2.00$ that is readily assigned to an organic radical species. The spectrum of the Al³⁺ hollow MOP displays the same $g = 2.00$ resonance. However, the spectrum of the Fe³⁺ hollow MOP displays two broad features centered at $g' = 4.25$ and ~ 9.6 in addition to the significantly broadened $g = 2.00$ resonance. These peaks in the spectrum of pure Fe³⁺-MOPs are rather broad and indistinguishable from the ferromagnetic resonances that are typical for Fe₂O₃ nanoparticles²⁹ or mixed-valent iron centers³⁰. To conclusively rule out the formation of these undesired phases, we prepared hollow MOPs using only 5 mol % Fe³⁺ diluted in Al³⁺. The features in the EPR spectrum of these hollow MOPs, containing diluted Fe³⁺ centers, are much narrower which is consistent with the absence of condensed Fe-O phases. Interestingly, an additional weak resonance at $g' ~ 9.6$ is resolved in the spectrum of the Fe³⁺-doped Al³⁺ hollow MOPs that is occluded by the broad $g' = 4.25$ resonance in the pure Fe³⁺ hollow MOPs. The EPR spectrum of the mixed metal hollow MOPs displays the same features of Fe³⁺ with tris(catecholate) coordination²⁸. The pseudo-octahedral geometry of [Fe(catecholate)₃]³⁺ produces a rhombic EPR spectrum with resonances at $g' = 4.22$ and a weak resonance at $g' ~ 9.6$.²⁹ In addition to the rhombic Fe³⁺ features at $g$-values > 3, a rather intense
radical signal \((g = 2.00)\) can also be observed. A similar radical signal was also reported in a paramagnetic \(\text{Fe}^{3+}\) complex with three substituted catecholate ligand, DOPA (3,4-dihydroxyphenylalanine)\(^{31,32}\).

**Figure 7.7** EPR spectra of BNP and hollow MOP. 295 K, X-band (9.8 GHz) EPR spectrum measured at room temperature for BNP (black), hollow \(\text{Al}^{3+}\)-MOP (green), hollow \(\text{Fe}^{3+}\)-MOP (blue), dual hollow \(\text{Fe}^{3+}/\text{Al}^{3+}\)-MOP (red) (starting composition was 0.05 mol fraction of FeCl\(_3/\text{AlCl}_3\)). Inset: \(g' \approx 9.6\) transition of the dual hollow \(\text{Fe}^{3+}/\text{Al}^{3+}\)-MOPs on an expanded scale.

### 7.2.4 Mechanism study of the hollow MOP formation

The mechanism of the hollow MOP formation was probed. A reasonable hypothesis is that the formation of hollow MOPs is due to Kirkendall effect, which is a phenomenon that was originally observed in metallurgy\(^{33}\). Here, the nonreciprocal mutual diffusion process through an interface of two metals results in the creation of vacancies to compensate from the inequality of the material flow. This feature has been utilized to generate hollow \(\text{Co}_3\text{S}_4\) and \(\text{CoO}\) nanocrystals\(^{24}\) and later to obtain hollow nanoparticles of oxides and chalcogenides\(^{34}\). Thus, this effect has previously been limited to the chemical transformation of metallic nanocrystals. To investigate if the Kirkendall effect is responsible for the observed formation organic-inorganic composite nanostructures, we monitored the temporal evolution of the conversion of the BNPs to hollow MOPs after addition of FeCl\(_3\). Aliquots were taken from the reaction mixture at various times and
monitored by TEM. A few small cavities in interior of the solid nanoparticles (Figure 7.8b) are clearly observed within 20 minutes. After 1 hour, the particle cores became patchy and the surface of the nanoparticles became concurrently more prominent (Figure 7.8c). Fully hollow nanoparticles were formed after 6 hours (Figure 7.8d) and no further evolution of morphology was observed, suggesting that the formation of hollow nanoparticles before 6 hours. A similar trend in the temporal evolution was also observed for the Sn^{4+}-based hollow MOPs (Figure 7.9).

**Figure 7.8** TEM images taken at a) 0 min, b) 20 min, c) 1 h and d) 6 h after the addition of FeCl₃ into BNP; the scale bar is 500 nm. Conditions: 0.1 mL FeCl₃ methanol solution was added into 1 mL BNP methanol solution prepared using 3 mg/mL Im-BA and Im-Ca, the ratio of Fe^{3+} to Im-Ca was 1/2. The TEM samples were taken by drop-casting. The scale bar is 500 nm.

An important implication of understanding the operating mechanism is that it offers a pathway for additional control elements. A rendition of the Kirkendall effect to explain the formation of hollow MOPs would suggest that the diffusion of the boronate ester polymer to the surface of the nanoparticle, where the reaction between Fe^{3+} and the boronate ester takes place, is faster than the diffusion of Fe^{3+} from the solution to the interior of the particle (Scheme 7.2). If
the opposite were true (diffusion of Fe\(^{3+}\) into the interior of the BNP is faster than boronate ester polymer out-diffusion), then the resultant product will be a solid nanoparticle.

**Scheme 7.2** Schematic illustration showing the transformation of solid polymer nanoparticles to hollow or solid metal organic particles, depending on the relative diffusivities.

![Scheme 7.2 Schematic illustration showing the transformation of solid polymer nanoparticles to hollow or solid metal organic particles, depending on the relative diffusivities.](image)

**Figure 7.10** The thickness of shell could be tuned by changing the ratio of Fe\(^{3+}\) to Im-Ca, a) 1/8, b) 1/2, c) 3/4. However, when the ratio increased up to 1/1, only solid MOPs were observed as shown TEM in figure d). EDS maps of solid Fe\(^{3+}\)-MOP, element C (e) and Fe (f) included. , all scale bar is 500 nm except e) and f) with 250 nm scale bar.

We hypothesized then that one can gain control over the thickness of the shell in the hollow MOPs by simply tuning the concentration of Fe\(^{3+}\) in solution, as these variations will generate an intermediate scenario between the solid and the hollow nanoparticles. Accordingly, the ratio of
metal ions to the polymer repeat units was varied. Indeed, this variation was found to affect not only the thickness of the shells, but understandably also the rate of the formation of the hollow MOPs. For example, when the concentration of FeCl$_3$ is 1/8th of that of the Im-Ca repeat unit in the polymer, it took more than 12 hours to complete the formation of hollow MOP. However, this process was completed within 2 hours, when this ratio was changed to 1:2. As anticipated, the thickness of the shell was also observed to increase consistently with the increasing ratio of FeCl$_3$/Im-Ca, from about 15 nm to 90 nm when the ratio increased from 1/8 to 3/4 (Figure 7.10a – 7.10c). Similar to Fe$^{3+}$, both the rate of formation and the thickness of the shell increase with the increasing amount of Sn$^{4+}$ ions. The formation of solid MOPs in the presence of an even higher concentration of Fe$^{3+}$ ions (ratio of FeCl$_3$/Im-Ca is 1:1) further supports that the operating mechanism as one based on Kirkendall effect. EDS analysis of the samples showed a homogenous distribution of Fe in the respective solid nanoparticles (Figure 7.10d – 7.10f).

**Figure 7.11** The size of hollow MOP could be varied by tuning the size of BNP used, a, d) 1.5 mg/mL monomers, b, e) 3.0 mg/mL monomers, c, f) 5.0 mg/mL monomers. The ratio of Fe$^{3+}$ to Im-Ca was 1/2; all scale bar is 500 nm.

Finally, it is also important to identify whether the starting size of the boronate nanoparticle will be preserved during the transformation to hollow MOPs. The BNPs size can be tuned by
simply varying the concentrations of the monomers. Three BNP s with diameters of 210, 410, and 550 nm were used for the formation of hollow MOPs (Figure 7.11a – 7.11c). Our results indeed show that the resulting size of the hollow MOPs depends on the size of the primary BNP (see Figure 7.11d – 7.11f). Overall, hollow MOPs can be obtained with an excellent control over size, kinetics of formation and shell thickness.

![Image](image1.png)

**Figure 7.12** Preparation and application of hollow Fe@C particles. TEM measurement of a) hollow Fe@C particles after the temperature thermal treatment of MOPs, b) hollow carbon particles after the acid treatment of Fe@C; c) Optical photograph of the MB solution without (left) and with (right) the addition of Fe@C applying an external magnet; d) UV-vis absorption measurement before and after the magnetic separation.

To demonstrate a potential application of these hollow MOPs, hollow carbon particles loaded with metal nanoparticles (M@C) were prepared by direct pyrolysis. For example, the high temperature thermal treatment of hollow Fe$^{3+}$-MOPs at 800 °C under a N$_2$ atmosphere for 1 hour resulted in the formation of light color hollow carbon spheres loaded with small dark
nanoparticles as shown in TEM of Figure 7.12a. The iron content of the small nanoparticles was confirmed from the EDX line scanning data (Figure 7.13). These small nanoparticles can be removed by the addition of hydrogen chloride with the formation of small bubbles in the solution (Figure 7.12b), further indicating that these small nanoparticles are likely to be pure Fe nanoparticles. These magnetic-functionalized carbon particles with encapsulated Fe nanoparticles have been successfully used as a magnetically separable adsorbent for removing organic waste from aqueous solution using an organic dye (methylene blue, MB). The photograph in Figure 7.12c and the absorption spectra in Figure 6d demonstrate the separation of MB using these hollow Fe@C. Absorption spectra, measured before and after mixing aqueous solutions of MB with Fe@C nanoparticles, indicate that MB has been almost quantitatively removed from the solution (Figure 7.12d). The magnetic property of the Fe@C particles is then utilized to remove them from the aqueous solution. Redistribution of these particles in methanol releases the MB into methanol, which allows for the particles to be reused. We show that MB can be quantitatively removed from the aqueous solution and then released into methanol.

![Figure 7.13 HAADF STEM images and EDX line scanning data.](image-url)
7.3 Summary

In conclusion, a simple and versatile strategy for achieving hollow metal organic nanoparticles has been developed, where boronate ester based solid organic nanoparticles have been used as the precursor. The size of the hollow MOPs is dictated by the size of the precursor BNP, which itself exhibits excellent tunability. The formation of these hollow MOPs seem to be independent of the valence and the geometry of the metal ion, which also allows for the incorporation of more than one type of metal ion into the MOPs. Kirkendall effect has been shown to be the underlying mechanism for the formation of these hollow MOPs. For the first time, Kirkendall effect has been responsible for the formation of hollow metal organic nanoparticles from organic polymer nanoparticles. This understanding further lends itself to control in the shell thickness of the MOPs. Overall, the method reported here for achieving hollow MOPs with a high degree of control has the versatility to impact applications such as catalysis, sensing, storage, and biomedicine, as this approach should be extendable to other organic-inorganic molecular architectures. Moreover, the fields of polymer synthesis and self-assembly are orthogonally and rapidly evolving with its own versatility and control elements\(^{36}\). Our ability to reliably generate hollow organic-inorganic hybrid nanoparticles using polymeric nanostructure templates will open up a fount of opportunities for hybrid materials nanotechnology.

7.4 Experimental

7.4.1 General

\(p\)-Phenylenediamine, 3,4-dihydroxybenzaldehyde, 4-formylphenylboronic acid, Iron(III) chloride, Copper(II) chloride, Tin(IV) chloride pentahydrate, Aluminum chloride and other conventional reagents were obtained from commercial sources and used as received unless otherwise mentioned. \(^1\)H-NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using the residual proton resonance of the solvent as the internal standard. \(^13\)C-NMR spectra were recorded on a Bruker 100 MHz NMR spectrometer using the carbon signal of the deuterated
solvent as the internal standard. Mass spectra were taken on Bruker microTOF-II. Conventional Bright-Filed Transmission electron microscopy (TEM) images were taken using a JEOL JEM-2000FX operating at 200 KV. Scanning electron microscopy (SEM) images were taken using a FEI Magellan 400 FESEM in SE imaging mode. Energy Filtered Transmission Electron Microscopy was performed using a JEOL JEM-2200FS Energy Filtered Field Emission TEM operating at 200kV. Prior to generating the element distribution images (EFTEM maps) the presence of the respective elements was verified using Electron Energy Loss Spectroscopy (EELS) in the same instrument. Subsequently EFTEM maps were acquired using the three-window method in wide-field mode, which is based on the subtraction of two pre-edge images from one post-edge image for background subtraction\textsuperscript{1}. The following element edges where used: B\textsubscript{K}= 165eV, C\textsubscript{K}=284eV, N\textsubscript{K} = 401eV, O\textsubscript{K} = 532eV, Fe\textsubscript{L_{2,3}} = 708eV and an Energy Window width of E = 20eV was used for B and C while for N, O and Fe E was set to 30 eV. EDS mapping was performed in the same instrument in STEM mode utilizing an Oxford X-MAX 80mm\textsuperscript{2} detector and a probe size of 1.5nm. EDS maps were acquired until sufficient contrast was obtained (typically 15-30 min). Room temperature EPR spectra were collected on powdered samples using the TE102 mode of a X-band resonator (9.81 GHz, Bruker ELEXSYS-500).

7.4.2 Synthesis of Im-Ca

The preparation of Im-Ca was achieved following our previous report\textsuperscript{27}. To a solution of p-phenylenediamine (0.15 g, 1.39 mmol) in anhydrous ethanol (10 mL) was added 3,4-dihydroxybenzaldehyde (0.40 g, 2.90 mmol). The clear brown mixture was stirred at room temperature under an argon atmosphere and protected from light. The product precipitated out of the reaction mixture. The brown precipitate was filtered and washed with cold ethanol three times. Final product was vacuum dried to obtain a brown solid (yield: 70%) \textsuperscript{1}H-NMR (400 MHz, DMSO-6D) δ(ppm): 9.57 (s, 2H), 9.29 (s, 2H), 8.41 (s, 2H), 7.38 (d, 2H), 7.23 (s, 4H), 7.17 (q, 2H), 6.82 (d, 2H). \textsuperscript{1}H-NMR (400 MHz, CD\textsubscript{3}OD and DMSO-6D) δ(ppm): 8.39 (s, 2H), 7.39 (d,
2H), 7.22 (s, 4H), 7.17 (q, 2H), 6.82 (d, 2H). $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 159.89, 149.57, 146.09, 128.56, 122.91, 122.30, 115.83, 114.51. ESI/MS m/z 349.11 [M+H]+ (expect m/z 349.35).

7.4.3 Synthesis of Im-BA

Im-BA was synthesized from condensation reaction between p-phenylenediamine and 4-formylphenylboronic acid. To a solution of p-phenylenediamine (0.05 g, 0.46 mmol) in 20 mL MeOH was added 4-formylphenylboronic acid (0.15 g, 1.00 mmol). The clear yellow mixture became cloudy after stirring at room temperature overnight. The yellow precipitate was filtered and washed with cool methanol three times. Final product was vacuum dried to obtain a yellowish solid (yield: 80%) $^1$H-NMR (400MHz, DMSO-6D) δ(ppm): 8.67 (s, 2H), 8.22 (s, 4H), 7.89 (m, 8H), 7.36 (s, 4H). $^1$H-NMR (400MHz, CD$_3$OD and DMSO-6D) δ(ppm): 8.65 (s, 2H), 7.88 (m, 8H), 7.34 (s, 4H). $^{13}$C-NMR (100MHz, DMSO-6D) δ(ppm): 160.67, 149.83, 137.76, 134.90, 128.06, 122.54. ESI/MS m/z 431.16 [M+59]+ (expect m/z=431.19), m/z 445.20 [M+73]+ (expect m/z=445.21). m/z 459.22 [M+87]+ (expect m/z=459.23).

7.4.4 Preparation of Hollow Metal-Organic Particles

The preparation of hollow nanoparticles could be watched as the movie S1. First, Im-Ca and Im-BA were dissolved in methanol with the help of heating. After cooling down by setting at room temperature, one solution was added into the solution drop by drop, the mixture solution became deep orange and suspension was finally got. Then a methanolic solution of metal chloride salts (FeCl$_3$ in this example) was added to the suspension of BNPs once. After stirring for given time, the resultant hollow metal-organic particles were washed with methanol, collected by centrifugation, and dried in a vacuum oven at 50 °C overnight.

7.4.5 Preparation of Hollow Fe@C particles

Hollow Fe$^{3+}$-MOPs were placed in a tube furnace and let the nitrogen gas flow for 30 mins at room temperature. Then the furnace was calcined up to 800 °C quickly. After 1 hours, the
furnace was turned off and the resulting particles were cooled to room temperature with the nitrogen gas flow.

7.4.6 Adsorption of methylene blue (MB) from aqueous solution

Hollow Fe@C (2 mg) was added to 10 mL of MB aqueous solution (10 mg/L) and was sonicated to form the dispersed solution. After 5 mins, an external magnet was applied to removed the Fe@C particles from the aqueous solutions. UV-Vis spectra were measured before and after the separation.

7.4.7 Release of methylene blue (MB) from Fe@C particles

After collecting the Fe@C particles from the aqueous solution containing MB, methanol was used to wash the MB molecules out from these carbon based particles for four times. Each time, Fe@C particles were removed from the solution by applying external magnet, and methanol solution containing MB could be collected. The Fe@C particles can then be used for adsorption of MB again.

7.5 References


CHAPTER 8

SUMMARY AND FUTURE DIRECTIONS

8.1 Summary

To be an effective drug delivery system, the delivery vehicle should exhibit several key characteristics to achieve four functionalities (Figure 8.1): (i) to retain the carriers, which have encapsulated drug molecules, in the blood circulation after the injection, the delivery vehicle should have good drug loading capability, especially for hydrophobic drug, since most cancer drug are hydrophobic. The encapsulation stability should be good enough, so there is no release during the blood circulation; (ii) to evade the circulation system and accumulate in the tumor site, the particle size should be around 10-200 nm due to the requirement from the enhanced permeability and retention (EPR) effect; (iii) to be selectively taken up by the target cells, the surface should be easily modified with functional groups; (iv) finally, the drug should be able to be released upon the stimuli in the tumor cells, such as low pH and redox environment.

![Figure 8.1 Schematic representation of the functionalities for an effective drug delivery system after the injection.](image)

Our group has described the design and application of amphiphilic random copolymer to prepare self-crosslinked nanogels with single- or multi-stimuli responsive behaviors, providing non-covalent hydrophobic encapsulation capabilities with high encapsulation stability and
controlled release of the payload for drug delivery. In this thesis, we further fundamentally studied the structure-property correlations in self-crosslinked polymer nanogels and developed improved functionalities for drug delivery applications. In Chapter 2, we highlight unprecedented control in fine-tuning the sizes and encapsulation stability using Hofmeister ions. The size of the nanogel can be systematically varied with concentration of both kosmotropic and chaotropic anion. Encapsulation stability of guest molecules is also dependent on the nature of the salt used for preparation of the nanogels; chaotropic anions afford nanogels with greater guest encapsulation stability. The guest release kinetics of nanogels made with ions were also consistent with the Hofmeister series.

Fluorescence resonance energy transfer (FRET) based method has been applied to evaluate exchange dynamics of lipophilic guest molecules, providing important sights on the evaluation of the stability of these drug delivery vehicles. In Chapter 3, we have shown that the dominant mechanism for guest exchange in nanogel hosts is collision-based. In addition, the mechanism of guest exchange can change on the basis of the microenvironment of the host. When the nanogel interior is hydrophobic, the collision-based mechanism is the dominant pathway. However, when the interior is sufficiently hydrophilic, the dominant mechanism changes to a diffusion-based one. In drug delivery applications, the drug-loaded nanocarrier experiences both concentration and environmental changes upon bio-distribution to diseased tissues. The mechanistic variations based on environments provide insights into molecular designs that can afford stable encapsulation in one environment and release of the molecules in another.

It is important to have nanocarriers that can be effectively transported across cellular membranes for biomedical applications. In Chapter 4, we have developed a simple method to prepare a nanogel that is capable of transforming from a negatively charged form into a positively charged in the slightly acidic tumor extracellular environment. The reported facile synthetic method for preparing the pH-sensitive charge conversional nanogels, combined with stable
encapsulation and tunable pI, should open up new avenues in the design of advanced drug carriers for intelligent drug delivery. The study also highlights the opportunities to develop other functional nanogels using units with other functional group.

Reagent-free synthetic methods are needed for nanogel formation due to their simplicity and implications in green chemistry. In Chapter 5, we have demonstrated a new pathway for the preparation of disulfide based self-crosslinking nanogels using photoirradiation. The most important take-home message of this work is that pyridyl disulfide might be used as a photo-crosslinkable functional group, while this crosslinks would be redox responsive, which should play an important role in the design of drug delivery nanocarriers. From the application standpoint, changing the DTT-induced crosslinking method to photo-induced crosslinking method makes our nanogel preparation more cost-effective. The scale up of the process should become possible considering that the diffusion of crosslinking agents into the large volume of solution may result in heterogeneous crosslinking density.

Hybrid metal-organic coordination materials, constructed from metal ions and polyfunctional organic ligands, have received great attention due to their interesting applications. We have developed a simple, rapid, and robust method to produce hollow metal-organic particles with well controlled size and thickness from organic polymer particles. In Chapter 6, we prepared organic polymer particles through boronate esterification. Their size, absorption characteristic can be adjusted by controlling the mixing concentration. We pointed out that the nucleation step involves a nucleation elongation polymerization with N→B interaction as the driving force for the collapsing. In Chapter 7, we have demonstrated that hollow metal organic nanoparticles can be prepared by simply adding metal ions into purely organic nanoparticles. The Kirkendall effect is responsible for the formation of the hollow structure as shown by time dependent TEM measurements. The tunability of the original organic nanoparticles makes it easy to control the size of hollow nanoparticles, while the thickness can be tuned by simply altering the ratio of
metal ions to the catechol monomers. We further showed that various metal ions can be used to form hollow nanoparticles. We believe that this approach can be extended to the development of more diverse hollow metal organic nanoparticles.

8.2 Future directions

In this section future directions for two more projects are described.

8.2.1 Redox-activatable $^{19}$F-MRI nanoprobe

Magnetic resonance imaging (MRI) is of great interest in both basic biological research and clinical diagnosis of cancer and other diseases.$^{1-3}$ $^{19}$F MRI has received significant attention in MR imaging and spectroscopy studies because of two reasons: (i) $^{19}$F-MRI has little biological background, offering a means to detect diseases in deep tissue since there is low levels of endogenous fluorine in the body; (ii) $^{19}$F has 100% natural abundance and its gyromagnetic ratio (40.06 MHz/T) is second only to $^1$H, making it more sensitive for detection over other nuclei.$^4$

Here we are interested in redox-responsive $^{19}$F MRI nanoprobe, since it is important to monitor biological redox reactions.$^5-8$ Actually, biological redox reactions play important roles in many biological processes. Abnormal levels of redox reactions can result in various disease including liver damage, skin lesions, and slow growth.$^9-12$

A stimulus-responsive $^{19}$F-MRI nanoprobes have been usually prepared based on the strategy of using changes in spin-spin relaxations between the aggregation and free states to turn ON/OFF $^{19}$F signals in response to the pH values.$^{13-15}$ For example, pH-activatable $^{19}$F-MRI nanoprobes were prepared by introducing the fluoro groups into the ionizable block (PR) of amphiphilic copolymers containing a hydrophilic poly(ethylene oxide) (PEO) segment and tertiary amine/ammonium segment (Figure 8.2).$^{15}$ At pH > pK$_a$, the formation of micelle highly restricted chain motions, resulting in short spin–spin relaxation times (almost 0), which effectively broadened and eliminated the $^{19}$F signals. However, protonation of ammonium groups
at low pH dissembled the micelles into dissociated polymer chains with conformational flexibility, leading to reappearance of the previous $^{19}$F signal.

**Figure 8.2** a) pH-activatable ON/OFF $^{19}$F-MRI nanoprobes from ionizable diblock copolymers. At pH > pK$_a$, the hydrophobic segments selfassemble into a micelle core leading to $^{19}$F signal suppression as a result of restricted polymer chain motion. Upon pH activation (pH < pK$_a$), micelle disassembly leads to dissociated unimers and a strong $^{19}$F signal. b) Structural formula of three representative diblock copolymers containing different pH responsive segments and $^{19}$F reporter moieties, their pK$_a$ values and $^{19}$F chemical shifts (in ppm, relative to trifluoroacetic acid, or TFA), respectively, are shown in parenthesis (reproduced with permission from reference$^{15}$).

We have demonstrated the possibility to make nanogels desired functional behavior by introducing specific functionalities into the amphiphilic random copolymers. Herein, redox-activatable $^{19}$F-MRI nanoprobe is achieved by designing a polymeric nanogel containing trifluoroethyl groups. The PEGMA-co-TFEMA-co-PDSMA copolymers were synthesized by a reversible addition–fragmentation chain transfer (RAFT) polymerization reaction (Scheme 8.1). Three different copolymers with varying molecular weight were targeted. Copolymers P1–P3 were synthesized by simply varying the feed ratio of RAFT regent to the monomers during the
polymerization. After polymerization, the polymers were purified by precipitation in hexane, filtered, and washed to remove unreacted monomers. The polymers were characterized by NMR and GPC as shown in Table 1 and Figure 8.3. The relative ratios of the monomer incorporated in the polymer were determined by the characteristic resonances of PDS (δH_a = 8.46, δH_b = 7.67, δH_c = 7.10), PEGMA (δH_d = 3.37 ppm) and TFE (δH_e = 4.4 ppm) moieties to calculate the relative ratios of the monomer incorporated in the polymer (Figure 8.4).

Scheme 8.1 Synthetic scheme of PEGMA_x-co-PDSMA_y-co-TFEMA_z random copolymer.

Table 8.1 Characteristics of PEGMA_x-co-PDSMA_y-co-TFEMA_z copolymers (P1–P4)

<table>
<thead>
<tr>
<th>polymer</th>
<th>PEGMA</th>
<th>PDS</th>
<th>TFE</th>
<th>Mw</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>28%</td>
<td>56%</td>
<td>16%</td>
<td>7.6 kDa</td>
</tr>
<tr>
<td>P2</td>
<td>28%</td>
<td>56%</td>
<td>16%</td>
<td>13 kDa</td>
</tr>
<tr>
<td>P3</td>
<td>28%</td>
<td>56%</td>
<td>16%</td>
<td>28 kDa</td>
</tr>
</tbody>
</table>

Figure 8.3 GPC results of P1-P3.
To test the possibly of using these polymers for redox-activatable $^{19}$F MRI, $^{19}$F NMR spectra were taken for all three polymers before and after the addition of GSH in water containing 10% D$_2$O (Figure 8.5). There were almost no peaks observed due to the formation of micelle aggregates. However, we noticed that significant peaks appeared after adding GSH, since the disulfide bond can be broken, leading to the disassembly of micelle aggregates. The T2 was measured by employing the spin-echo method (Table 8.2). For control, the T2 was also measured for polymers in CDCl$_3$.

![Figure 8.4 Typical NMR spectrum of P2.](image)

$^{19}$F NMR spectrum of P2.

<table>
<thead>
<tr>
<th>polymer</th>
<th>T2 in CDCl$_3$</th>
<th>T2 in water with 10% D$_2$O</th>
<th>T2 in 10 mM GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>120 ms</td>
<td>~ 0</td>
<td>11 ms</td>
</tr>
<tr>
<td>P2</td>
<td>111 ms</td>
<td>~ 0</td>
<td>9 ms</td>
</tr>
<tr>
<td>P3</td>
<td>118 ms</td>
<td>~ 0</td>
<td>10 ms</td>
</tr>
</tbody>
</table>

Table 8.2 T2 measurement
The problem until now is that the T2 for the polymer solution with 10 mM GSH are too low to be useful for preclinical $^{19}$F imaging. Further step for this project is to increase the T2 number:

(i) try to decrease the amount of trifluoroethyl groups in the polymers. It is believed that the interaction between trifluoroethyl groups greatly decrease the motion of these groups, leading to low T2;

(ii) introduce another pH-responsive functional groups into the polymers. The protonation of these groups should be able to increase the motion of polymer chains after the disassembly. In addition, the cell-uptake will be also increased.

(iii) mix with degradable polymers, resulting in porous aggregation. It might decrease the density of trifluoroethyl groups among the particles, which should avoid the interaction between trifluoroethyl groups.

Figure 8.5 $^{19}$F NMR spectra were taken for all three polymers before and after the addition of GSH in water containing 10% D$_2$O.

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8.2.2 Hollow carbon particles loaded with metal nanoparticles

Nanoscale carbons is of great interest owing to their performance in various applications such as adsorption, supercapacitors, catalysis and biomedical applications.\textsuperscript{17-21} Many methods have been developed to this kind of materials, including carbide-derived carbon (CDC), template carbonization, and chemical or physical activation methods.\textsuperscript{22-25} Recently, porous coordination polymer (PCPs) or metal organic framework (MOFs), which are constructed from metal ions and polyfunctional organic ligands, have been demonstrated as novel templates to prepare microporous carbons by direct pyrolysis under an inert atmosphere.\textsuperscript{26-30}

Among the various carbon materials, hollow carbon particles have special interest, because of their low density, high surface area, materials economy, and lower cost compared to their solid counterparts. We hypothesize that hollow carbon particles can be easily prepared by direct pyrolysis of hollow metal organic particles (MOFs). Actually, in Chapter 7, hollow carbon particles loaded with iron nanoparticles (Fe@C) have been prepared by direct pyrolysis of hollow Fe$^{3+}$-MOPs at 800 °C under a N$_2$ atmosphere for 1 hour (Figure 8.6a). These magnetic-functionalized carbon particles with encapsulated Fe nanoparticles have been successfully used as a magnetically separable adsorbent for removing organic waste from aqueous solution using an organic dye (methylene blue, MB).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig86.jpg}
\caption{Hollow carbon particles loaded with metal nanoparticles (M@Fe), a) Fe@C, b) Cu@C, c) Sn@C.}
\end{figure}
As we have demonstrated that hollow Cu$^{2+}$-MOP and Sn$^{4+}$-MOP were also prepared using our Kirkendall effect based method, direct pyrolysis of these hollow particles were done at 800 °C under a N$_2$ atmosphere for 1 hour. TEM measurement was taken for both samples after the thermal treatment (Figure 8.6). Hollow structures were indeed observed. X-ray powder diffraction (XRD) data were collected to determine the state of metal. As shown in Figure 8.7, typical sharp peaks in all XRD patterns match well with the reported results, indicating the formation of pure Fe, Cu and Sn in these hollow carbon particles.

![XRD results of M@C. Typical peaks were marked.](image)

Figure 8.7 XRD results of M@C. Typical peaks were marked.

However, it is interesting to note that nanoscale structure was not observed for samples using only organic polymer particles. We first use thermogravimetric analysis (TGA) in N$_2$ to determine the thermal stability of hollow MOPs and organic polymer particles (BNP) (Figure 8.8a). There was a fast weight loss stage from 450 °C to 500 °C only detected for BNP, which was mainly caused by the decomposition of boronate ester, resulting in the crash of BNP. TGA in air for all M@C were also taken to measure the amount of metal in these carbon based particles.
(Figure 8.8b). All samples first have a mass increase from 250 °C to 300 °C, which was caused by the oxidizations. Then they stabilized at a temperature of 700 °C when 20-40 % by mass of the material remains, further showing the present of metal in all samples.

![Figure 8.8 TGA result, a) MOPs and BNP in N₂, b) M@C in air.](image)

Future directions for this project are to test the potential applications of these hollow carbon particles loaded with metal nanoparticles.

Cu nanoparticles have been used in various catalytic reactions such as the low-temperature oxidation of carbon monoxide, oxidation of alcohols, carbon–carbon bond formation reactions, carbon–heteroatom bond formation, various coupling reactions and so on.\(^{34-38}\) Here we studied the reduction of 4-nitrophenol (4-NP) into 4-aminophenol (4-AP) in the presence of NaBH\(_4\) using hollow Cu@C particles.\(^{39}\) In the typical reaction procedure, 1 mL solution of 4-NP (0.3 mg/mL) was added to a 10 mL freshly prepared solution of NaBH\(_4\) (1 mg/mL). To this mixture, 0.3 mg hollow Cu@C was added, the color of the solution faded as the reaction proceeded. UV-visible spectra were used to monitor the progress of the reaction. Every several minutes, 1 mL upper solution was transferred to a quartz cuvette for UV-visible spectra measurement. Once a spectrum was recorded, the solution was immediately transferred back to the previous vial and stirred for another few minutes for the sequential catalytic reaction. Blank experiments were also carried out to show that the reactions do not proceed without catalysts only in the presence of NaBH\(_4\). When
the reaction with Cu@C continues, the absorption peak at 400 nm from 4-NP gradually decreases in intensity while a small shoulder at 300 nm attributed to 4-AP gradually increases (Figure 8.9).

Sn nanoparticles have been widely studies as one of the promising anode materials for lithium batteries, since they have high theoretical specific capacity (994 994 mAh/g), which exhibit much higher capacity than the commercialized graphite anodes (~372 mAh/g). Unfortunately, the cycle life of Sn-based anode materials is commonly low, which mainly results from the pulverization of active materials caused by the huge volume change of Sn during lithium insertion/ extraction into/from Sn (about 300%). To potentially solve this issue, tin-encapsulated spherical hollow carbon has been designed and prepared, exhibiting higher capacity and better cycle performance than unencapsulated mixture materials. The reasons for that are: (i) the carbon shell has itself good electronic conductivity and prevents the aggregation of active Sn materials; (ii) the void space accommodates the strain of volume change during Li+ insertion/extraction. In addition, two more potential advantage using our Sn@C could be: (i) the

Figure 8.9 The time-dependent absorption spectra of the reaction solution in the presence of 0.3 mg of Cu@C.
carbon may be nitrogen-doped concerning the molecules used, which can improve the
electrochemical performance, exhibiting superhigh capacity and excellent rate capability\textsuperscript{44}; (ii)
Sn-based intermetallic compounds (MxSn; M = Cu, Fe, etc) and multiphase Sn–M alloys have
been explored to show better performance\textsuperscript{45}.

8.3 References

1. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, Randall B. Gadolinium (III) chelates as

2. Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller, R. N. Magnetic
iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations,

S.; Larson, S. M.; Grimm, J. Non-invasive mapping of deep-tissue lymph nodes in live animals

4. Ruiz-Cabello, J.; Barnett, B. P.; Bottomley, P. A.; Bulte, J. W. M. Fluorine (19F) MRS and
MRI in biomedicine. NMR Biomed. 2011, 24, 114-129

5. Hwang, C.; Sinskey, A. J.; Lodish, H. F. Oxidized redox state of glutathione in the

6. Chen, X.; Zhou, Y.; Peng, X.; Yoon, J.; Fluorescent and colorimetric probes for detection of

development of near-infrared fluorescent sensors for in vivo imaging. J. Am. Chem. Soc. 2012,
134, 13510-135123.

8. Jung, H. S.; Chen, X.; Kim, J. S.; Yoon, J. Recent progress in luminescent and colorimetric

Deresinski, S. C.; Herzenberg, L. A. Glutathione deficiency is associated with impaired survival

10. Shahrokhian, S. Lead phthalocyanine as a selective carrier for preparation of a cysteine-


2007, 39, 44-84.

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BIBLIOGRAPHY


He, J.; Tong, X.; Zhao, Y. Photoresponsive nanogels based on photocontrollable cross-links. Macromolecules 2009, 42, 4845-4852;


