Thiol-Ene Click Chemistry for Solid State Triplet-Triplet Annihilation Upconversion and Parts-Per-Billion Pyrophosphate Sensing in Artificial Seawater

Abagail K. Williams

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THIOL-ENE CLICK CHEMISTRY FOR SOLID STATE TRIPLET-TRIPLET ANNIHILATION UPCONVERSION AND PARTS-PER-BILLION PYROPHOSPHATE SENSING IN ARTIFICIAL SEAWATER

by

Abagail Katharine Williams

A Thesis
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Polymer Science and Engineering
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

Approved by:

Dr. Jason Azoulay, Committee Chair
Dr. Derek Patton
Dr. Yoan Simon

Dr. Jason Azoulay
Committee Chair
Dr. Derek Patton
Director of School
Dr. Karen S. Coats
Dean of the Graduate School
ABSTRACT

Thiol-ene click chemistry is a robust approach to molecularly engineering polymers for many applications. Within this work, thiol-ene click chemistry is used to fabricate thiol-ene networks for TTA-UC and to synthesize a conjugated polyelectrolyte (CPE) used as a pyrophosphate (PPi) sensor in complex aqueous media. Chapter I focuses on the synthesis and upconversion performance of rubbery networks fabricated using thiol-ene click photopolymerization. The advancement of triplet-triplet annihilation based upconversion (TTA-UC) in emerging technologies necessitates the development of solid-state systems that are readily accessible and broadly applicable. We demonstrate that thiol-ene click chemistry can be used as a facile cure-on-demand synthetic route to access elastomeric films capable of TTA-UC. Photopolymerization of multifunctional thiols in the presence of a thiol-functionalized 9,10-diphenylanthracene (DPA) emitter results in covalent DPA integration and homogenous crosslinked polymer networks. The palladium(II) octaethylporphyrin (PdOEP) sensitizer is subsequently introduced into the films through solution immersion. Upon excitation at 544 nm, green-to-blue upconversion is observed with compositional tuning resulting in an optimal upconverted emission intensity at 1.0 wt% DPA and 0.02 wt% PdOEP. The effectiveness of thiol-ene networks to function as robust host materials for solid-state TTA-UC is further demonstrated by improved photostability in air. In Chapter II, a thiol-ene click post polymerization modification is used to construct a conjugated polyelectrolyte capable of sensing pyrophosphate in a complex aqueous environment. The evolution of CPEs that transduce analyte-receptor interactions into detectable fluorescent responses in complex aqueous environments is predicated on advancements in molecular design and improved
synthetic accessibility. Here, we demonstrate a simple post-polymerization modification protocol based on thiol-ene click chemistry that results in the rapid installation of sodium sulfate terminated side chains to a poly(fluorene-co-ethynyl) scaffold. The fluorescence of the resulting water-soluble CPE is quenched by Fe$^{3+}$, dequenched selectively by pyrophosphate (PPI), and accurately quantifies PPI within ±6 nM in artificial seawater. The broad utility of thiol-ene click chemistry should offer the straightforward integration of diverse sensing elements.
I would like to thank Dr. Jason Azoulay for assuming the role as chair of my graduate committee following the leave of Dr. Joseph Lott, and for his constant motivation and support. I would like to thank my committee – Drs. Jason Azoulay, Matthew Sfeir, Robson Storey, Derek Patton, and Yoan Simon. I especially would like to thank Matthew Sfeir assuming the role of my scientific advisor and offering his guidance, technical training, and moral support while I was conducting research at Brookhaven National Laboratory (BNL).

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I thank the members of the Azoulay Research Group, especially Naresh, Alex, and Lifeng for supplying me with polymers to characterize, and offering supporting analysis while I was at BNL. I would also like to thank my undergraduate student Erin Crater for her help performing experiments for a number of projects we worked on together.
DEDICATION

I would like to dedicate this thesis to the ones that were there for me through the hardest part of my life. When my reality was twisted, and I had no more energy to give. You were the ones that helped carry me to the finish line. My parents, Doug and Lisa Gourley, thank you for becoming frequent fliers and lifting me up at my lows. To the best Sister-in-law a sister could dream of, Amanda Williams. Thank you for being the one I can talk to about anything and everything. To my Brother, the smartest person I’ve ever met, Chistopher Williams. I miss you like crazy. To my best New York friends, Dee and Kate. Thank you for some of the best memories I made in grad school. I will always love you guys. Lastly, to Patrick Metz, for showing me what love really is and being the one by my side walking across the finish line.
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<th>Description</th>
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<tbody>
<tr>
<td>$^0S$</td>
<td>Ground state</td>
</tr>
<tr>
<td>$^1S^*$</td>
<td>First singlet excited state</td>
</tr>
<tr>
<td>$^3S^*$</td>
<td>Triplet state</td>
</tr>
<tr>
<td>$^{(AA)}^*$</td>
<td>Encounter complex</td>
</tr>
<tr>
<td>$\lambda_{em}$</td>
<td>Emission wavelength</td>
</tr>
<tr>
<td>$\lambda_{ex}$</td>
<td>Excitation wavelength</td>
</tr>
<tr>
<td>$\lambda_{max}$</td>
<td>Absorption maximum</td>
</tr>
<tr>
<td>$\phi_F$</td>
<td>Fluorescence yield</td>
</tr>
<tr>
<td>$\phi_{ISC}$</td>
<td>Intersystem crossing yield</td>
</tr>
<tr>
<td>$\phi_{TTA}$</td>
<td>Triplet-triplet annihilation yield</td>
</tr>
<tr>
<td>$\phi_{TTET}$</td>
<td>Triplet-triplet energy transfer yield</td>
</tr>
<tr>
<td>$\phi_{UC}$</td>
<td>Upconversion yield</td>
</tr>
<tr>
<td>ATR-FTIR</td>
<td>Attenuated total reflectance Fourier transform infrared</td>
</tr>
<tr>
<td>CPE</td>
<td>Conjugated polyelectrolyte</td>
</tr>
<tr>
<td>CP</td>
<td>Conjugated polymer</td>
</tr>
<tr>
<td>$D$</td>
<td>Polydispersity</td>
</tr>
<tr>
<td>DPA</td>
<td>9,10-Diphenylanthracene</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>ISC</td>
<td>Intersystem crossing</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>Term</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>MLCT</td>
<td>Metal-to-ligand charge transfer</td>
</tr>
<tr>
<td>$M_n$</td>
<td>Average molecular weight</td>
</tr>
<tr>
<td>NLO</td>
<td>Nonlinear optical</td>
</tr>
<tr>
<td>PdOEP</td>
<td>Palladium (II) octaethylporphine</td>
</tr>
<tr>
<td>PPi</td>
<td>Pyrophosphate</td>
</tr>
<tr>
<td>SHG</td>
<td>Second harmonic generation</td>
</tr>
<tr>
<td>Ksv</td>
<td>Stern-Volmer constant</td>
</tr>
<tr>
<td>SEC</td>
<td>Size exclusion chromatography</td>
</tr>
<tr>
<td>TCSPC</td>
<td>Time-correlated single-photon counting</td>
</tr>
<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>TPA</td>
<td>Two-photon absorption</td>
</tr>
<tr>
<td>TTA</td>
<td>Triplet-triplet annihilation</td>
</tr>
<tr>
<td>TTET</td>
<td>Triplet-triplet energy transfer</td>
</tr>
<tr>
<td>UC</td>
<td>Upconversion</td>
</tr>
</tbody>
</table>

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CHAPTER I – ROBUST TIPLET-TRIPLET ANNILHILATION UPCONVERSION USING THIOL-ENE NETWORKS

1.1 General Background and Motivation

Upconversion (UC) describes the transformation of low energy photons into higher energy photons. This anti-Stokes shift in wavelength can occur by many methods including second harmonic generation (SHG), two-photon absorption (TPA) and triplet-triplet annihilation (TTA). TPA and SHG are nonlinear optical (NLO) processes (scale with the square of light intensity ($I^2$)) that are in competition with linear absorption processes (linearly proportional to $I$). For NLO mechanisms at low power excitations, the linear processes dominate the optical response and upconversion is not achieved. In order to observe the NLO-based behavior, a high intensity, coherent light source is required, limiting the applicability of the techniques. Conversely, upconversion through TTA employs a cascade of linear photophysical processes that can be activated using non-coherent low power energy sources (<0.1 W/cm²) such as sunlight. As such, TTA-UC has uses in applications ranging from photocatalytic water splitting,¹ solar cells,²–⁴ photo-stimuli responsive systems,⁵ and bioimaging.⁶,⁷

A schematic of the TTA process is illustrated in Figure 1.1 and mathematically depicted in Equations 1.1 – 1.7. TTA-UC uses a paired chromophore system in which a sensitizer (also known as a donor) absorbs an incident photon and an emitter (also called the acceptor or annihilator) ultimately releases a photon of higher energy than the incident source. To begin the UC process, a sensitizer in its ground state ($^0S$) absorbs a photon and undergoes excitation to the first singlet excited state ($^1S^*$) (Equation 1.1). Following photoexcitation, $^1S^*$ can fluoresce back down to the ground state (Equation
1.2) or undergo intersystem crossing (ISC) to the triplet state ($^3S^*$) (Equation 1.3). From the triplet state, the donor transfers its triplet energy to the acceptor though a triplet-triplet energy transfer (TTET) (Equation 1.4). If TTET does not occur then $^3S^*$ will phosphoresce back down to the ground state (Equation 1.7). As a result, the magnitude of the sensitizer’s phosphorescence intensity provides information on the TTET efficiency. Following TTET, TTA can occur when two emitters in their triplet state ($^3A^*$) encounter one another. For each (AA)$^*$ encounter complex one of the emitters will return to its ground state and the other emitter can form an excited quintet, triplet or singlet as noted in Equation 1.5a-c, respectively. Only when the singlet excited state is formed will the emitter undergo fluorescence decay and emit a photon of higher energy than the incident light source (Equation 1.6).

![Energy level diagram of the triplet-triplet annihilation upconversion process.](image)

Figure 1.1 Energy level diagram of the triplet-triplet annihilation upconversion process.

Colored solid lines represent radiative processes, whereas the dashed lines represent non-radiative processes.
Absorption: $0S + h\nu_1 \overset{k_A}{\rightarrow} 1S^*$  

Equation 1.1

Fluorescence: $1S^* \overset{k_{Fl}}{\rightarrow} 0S + h\nu_2$  

Equation 1.2

ISC: $1S^* \overset{k_{ISC}}{\rightarrow} 3S^*$  

Equation 1.3

TTET: $3S^* + 0A \overset{k_{TTET}}{\rightarrow} 0S + 3A^*$  

Equation 1.4

TTA: $3A^* + 3A^*$  

Equation 1.5a

Upconverted Fluorescence: $1A^* \overset{k_F}{\rightarrow} 0S + h\nu_2$  

Equation 1.6

Phosphorescence: $3S^* \overset{k_{ph}}{\rightarrow} 0S$  

Equation 1.7

Upconversion efficiency, $\phi_{UC}$, is described by Equation 1.8, where $\phi_{ISC}$, $\phi_{TTET}$, $\phi_{TTA}$, and $\phi_F$ represent the yields of the ISC, TTET, TTA, and upconverted fluorescence processes, respectively. The bimolecular nature of the TTA process is accounted for in the $\phi_{TTA}$.  

$\phi_{UC} = \phi_{ISC} \phi_{TTET} \phi_{TTA} \phi_F$  

Equation 1.8

These photo physical processes can be split into two categories: intramolecular and bimolecular. The intramolecular processes, $\phi_{ISC}$ and $\phi_F$, are independent of concentration and largely unaffected by molecular mobility. Specifically, $\phi_{ISC}$ is an intrinsic property of the chosen sensitizer. Typically sensitizers that contain heavy atoms are chosen because they have strong spin-orbit coupling interactions which promote ISC. For example, when using porphyrin sensitizers (such as palladium (II) octaethylporphine (PdOEP)), $\phi_{ISC}$ is assumed to be unity and does not limit $\phi_{UC}$ in solution or solid systems.  

Interestingly, solid materials can promote ISC because reduced molecular mobility limits
vibrational quenching and therefore lengthens triplet lifetimes in solid materials. The fluorescence quantum yield is primarily dictated by the chosen emitter. As such, compounds that naturally have high fluorescent yields, such as polycyclic aromatic compounds, are often employed. Even with a good emitter choice, singlet transfer back to the sensitizer (also known as back transfer) (Equation 1.9) can be problematic and decrease the $\phi_F$, particularly at high sensitizer concentrations and in single molecule TTA systems.$^{10-12}$

**Singlet Back Transfer:** $^{1}A^* + ^0S \xrightarrow{k_{TTE}} ^0A + ^1S^*$  
Equation 1.9

The $\phi_{TTA}$ is a result of the interplay between fundamental mechanistic aspects and environmental factors. From one view, $\phi_{TTA}$ has an inherent limitation based on spin statistics.$^{8,13}$ When two triplet acceptors undergo TTA, three different encounter complexes can form: quintet, triplet, or singlet (Equations 1.5a-c). According to spin statistics, only one out of nine encounter complexes will be of singlet multiplicity (the only state capable of radiative decay) resulting in a maximum $\phi_{TTA}$ of only 11.1%. However, forward dissociation of the quintet encounter species cannot occur due to high energy barriers, and the complex will instead dissociate back into the original two triplet states. In addition, when a triplet encounter complex dissociates forward, $^3A^*$ and $^0A$ are formed, and the resulting triplet can then subsequently be recycled and undergo TTA with another triplet. Taking these parameters into account, the generally accepted $\phi_{TTA}$ maximum is 40%.$^{8,13}$ This efficiency limit is supported by the fact that several groups have reported TTA efficiencies in excess of 11.1%.$^{8,14}$ From a practical perspective, TTA yield can also be affected by external factors such as triplet quenching processes. Emitter
triplets are primarily quenched by other emitters, sensitizers and oxygen. The former results in no net change in the total $^3A^*$ concentration, and in fact, triplet migration along a polymer backbone can ultimately be beneficial to increasing TTA-UC efficiency in solid-state systems.\textsuperscript{15} On the other hand, quenching by oxygen effectively reduces the triplet populations that drive the TTA process thereby reducing efficiency.

The main challenge to overcome in solid-state systems is to improve the bimolecular photophysical processes, $\phi_{\text{TTET}}$ and $\phi_{\text{TTA}}$. These bimolecular photophysical processes occur through a Dexter-type energy transfer, which involves an exchange of electrons between molecules and can be described by the Perrin approximation.\textsuperscript{16} For this interchange to transpire, the molecular orbitals of the molecules involved must overlap (be within 10 Å).\textsuperscript{16} In solution, molecules can easily diffuse and collide with one another, leading to efficient TTET and TTA yields. However, this efficiency greatly decreases when transitioning to solid-state systems because during the sensitizer and emitter triplet lifetimes the molecules’ translational motions are reduced in elastomers and essentially fixed in glassy polymers. At low chromophore concentrations the energy transfer processes are highly dependent upon molecular mobility and are drastically hindered in solid materials. However, at higher concentrations (i.e. the distance between chromophores is within the Perrin limit) the molecular mobility becomes inconsequential.

Although TTA-UC was established in the 1960’s,\textsuperscript{17, 18} it has only been in the past decade that solid-state systems have been realized,\textsuperscript{19, 20} enabling practical application toward technologies such as photovoltaics,\textsuperscript{2-4, 21} optical data storage,\textsuperscript{22} and organic light-emitting diodes.\textsuperscript{23, 24} Polymeric host materials have been at the forefront of these advances owing to their wide range of chemical compositions, tunable mechanical
Examples of TTA-UC in diffusion restricted polymer matrices demonstrate that higher molecular mobility more effectively facilitates intermolecular energy transfers (TTET and TTA) and leads to greater upconversion efficiencies.\textsuperscript{9, 30-33} Excess mobility, however, results in dye leaching and phase separation resulting in nonradiative decay.\textsuperscript{16} In glassy polymeric hosts, where the diffusion of dye molecules is restricted, very high emitter concentrations are required to facilitate TTET and TTA and result in aggregation and component separation.\textsuperscript{34, 35}

The design of solid-state systems is further complicated by the sensitivity of excited triplet states to the presence of very small amounts of molecular oxygen.\textsuperscript{36} Only a few strategies have been demonstrated to address this challenge in the solid-state such as employing specific matrices to reduce the oxygen concentration,\textsuperscript{37} or by incorporating oxygen scavengers.\textsuperscript{38} Recently, robust TTA-UC was demonstrated in air-saturated solvent mixtures containing thioether functionalities which exhibit efficient oxygen scavenging.\textsuperscript{39}

The dependence of TTA-UC on the chromophore concentration, their relative proximity, and air instability continues to hamper the realization of practically applicable solid-state systems. In this work, we demonstrate that thiol-ene click chemistry can be used as a facile cure-on-demand synthetic route to fabricate homogenous, optically transparent networks with covalently attached emitters. The sensitizer is subsequently introduced into the network via a simple solution immersion process resulting in films capable of TTA-UC. This strategy synergistically combines the favorable molecular diffusion within rubbery polymers,\textsuperscript{9, 40-43} hinders performance limiting dye aggregation,\textsuperscript{44-46} imparts facile control over emitter and sensitizer concentrations, and affords fine-
tuning of crosslink density (mechanical properties) and chemical functionality. Lastly, a high-density of thioether linkages results in improved photostability in air enabling broad applicability.

1.2 Synthesis and Characterization of monomers and Thiol-ene Networks

In this work, solid-state TTA-UC was achieved by covalently incorporating a 9,10-diphenylanthracene (DPA) derivative (2), along with unbound PdOEP, into elastomeric networks prepared using thiol-ene click chemistry. To prevent aggregation of the DPA units, emitters bearing two reactive thiols were synthesized allowing covalent integration within the polymer network upon curing (Figure 1.2). Our synthetic approach begins with the functionalization of 1 to promote solubility in the thiol-ene monomer formulation. This was achieved through the reaction of 1 and 3,6-dioxo-1,8-octanediithiol (50 equiv.) in the presence of AIBN at 60 °C in CHCl₃ to give 2 in 78% yield. The absorption and emission spectra of PdOEP and 2 are shown in Figure 1.3. PdOEP has two regions of absorption, a high-energy Soret band centered at 393 nm and lower-energy metal-to-ligand charge transfer (MLCT) bands at 512 and 546 nm. Upconversion occurs when the MLCT band of PdOEP is excited and blue emission from the DPA is observed. The characteristic absorption fingerprint and strong blue emission of 2 are similar to unmodified DPA, indicating that the solubilizing groups do not interfere with the optical properties of the DPA core and 2 can act as an efficient emitter when paired with PdOEP.

The method by which the thiol-ene films were prepared is shown in Figure 1.2. The thiol-ene monomer mixture containing 2 was cured via photopolymerization under ambient conditions using a Cure-Tek UVA-400 lamp. The thiol-ene resin and solid networks were analyzed using attenuated total reflectance (ATR) Fourier transform
infrared (FTIR) spectroscopy to determine the degree of conversion. Prior to irradiation, the =C-H and –S-H stretches were detected between 3130 - 3100 cm\(^{-1}\) and 2675 - 2500 cm\(^{-1}\), respectively as shown in Figure 1.4a. After curing, the peaks disappear indicating the thiol-ene reaction achieved full conversion. The resulting films were optically clear, flexible, and exhibited glass transition temperature (\(T_g\)) values at -53 ± 1 °C as measured by differential scanning calorimetry (DSC) (Figure 1.4).

**Figure 1.2 Synthetic Scheme for upconverting thiol-ene Networks.**

Synthesis of 2 and schematic representation of the process employed to produce thiol-ene networks. Thiol-ene monomers, photoinitiator and 2 were mixed, and cured under a Cure-Tek UVA-400 lamp for 8 min. The crosslinked networks were subsequently submerged in PdOEP CHCl\(_3\) solutions to incorporate the sensitizer.
Figure 1.3 Absorption and Emission of PdOEP and DPA.

Normalized absorption (solid lines) and photoluminescence spectra (dashed lines) of PdOEP and 2 in CHCl₃ solutions. The photoluminescence from PdOEP and 2 were collected using excitation wavelengths of 393 and 375 nm, respectively.

Figure 1.4 FTIR spectra, photograph, and DSC thermograph of DPA containing thiol-ene networks.

a) ATR-FTIR spectra of thiol-ene monomer mixture before (solid lines) and after (dotted lines) UV-curing. The areas around the –C=H (blue) and –S-H (orange) stretches are highlighted. b) Picture of a cured thiol-ene film to show the film flexibility and optical clarity. c) DSC thermograph of thiol-ene films using a 10 °C min⁻¹ heating rate.
UV-vis spectroscopy was used to quantitatively determine the DPA concentrations in the thiol-ene networks. For samples with greater than 0.5 wt% DPA, the film thickness in combination with the large extinction coefficient of DPA resulted in absorbances that were too high for quantitative measurement. Analysis of the 0.25 wt% DPA film was in excellent agreement with theoretical values (Figure 1.5 and Figure 1.6).

To confirm covalent attachment of 2 into the polymer network, dye leaching experiments were performed. The DPA containing thiol-ene films were submerged in CHCl₃ for 24 h and Beer-Lambert law was used to calculated the % DPA leached into solution via UV-vis analysis of the supernatant. It was found that there was >95% covalent incorporation of the thiol functionalized DPA for all samples (Table 1.1). The results from both the leaching experiments and UV-vis indicate that concentration values used in the synthetic preparation accurately reflect the emitter concentrations in the films.

Figure 1.5 Absorption spectra of thiol-ene film with 0.25 wt% DPA.

The absorbance at 376 nm is used to calculate the DPA concentration.
Once the DPA-bearing films were prepared and characterized, the sensitizer was incorporated into the thiol-ene films by overnight immersion into CHCl$_3$ solutions of PdOEP. The films were subsequently dried under vacuum for 24 h to remove any residual solvent and analyzed by UV-vis to determine quantitative PdOEP concentrations (Figure 1.5). Soaking the films in solutions with PdOEP concentrations of 0.038, 0.075 and 0.15 mg mL$^{-1}$ repeatedly resulted in 0.005, 0.01 and 0.02 wt% PdOEP in the polymer network, respectively (Table 1.2). A linear trend between the solution concentration and
wt% PdOEP in the thiol-ene films demonstrates that sensitizer concentration can be controlled (Figure 1.7).

Table 1.2 Data used to calculate the concentration of PdOEP infused into DPA containing thiol-ene films.

<table>
<thead>
<tr>
<th>PdOEP Solution Concentration (mg mL(^{-1}))</th>
<th>DPA Concentration (wt%)</th>
<th>Absorbance at 546 nm</th>
<th>PdOEP Concentration in Film (M)</th>
<th>PdOEP Concentration in Film (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
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Figure 1.7 Linear trend between PdOEP concentration of CHCl\(_3\) solutions and doped films.

Calculated wt% PdOEP infused into thiol-ene films as a function of the PdOEP concentration of the CHCl\(_3\) solution the films were submerged in.
1.3 Optical Characterization of Films

Normalized direct fluorescence spectra of thiol-ene films prior to PdOEP incorporation are shown in Figure 1.8. As the DPA concentration is increased, the maximum fluorescence wavelength shifts from 439 to 448 nm. The decrease in emission at the blue edge can be attributed to reabsorption,\(^\text{48}\) whereas, the increase in lower energy emission, most pronounced in the 5.0 wt% DPA sample, is commonly attributed to the formation of excimer states.\(^\text{49, 50}\) Direct fluorescence measurements of PdOEP-doped films were also conducted (Figure 1.9), and negative peaks centered around 515 and 546 nm appear, consistent with reabsorption from PdOEP.

![Normalized direct fluorescence spectra of DPA containing thiol-ene films.](image)

Figure 1.8 Normalized direct fluorescence spectra of DPA containing thiol-ene films.

Emission measurements were taken prior to doping with PdOEP (\(\lambda_{\text{ex}} = 375\) nm).
Figure 1.9 Direct fluorescence spectra of thiol-ene networks doped with PdOEP.

a) 0.005, b) 0.01, c) 0.02 wt% PdOEP ($\lambda_{ex} = 375$ nm).
As shown in Figure 1.10b, upon irradiating the PdOEP-doped thiol-ene films with green light (532 nm), blue emission (440 nm) can clearly be observed without the use of a 500 nm short-pass filter. Upconversion performance was analyzed via steady-state photoluminescence spectroscopy using a 544 nm excitation wavelength to selectively excite the MLCT band of PdOEP. As an example, the photoluminescence spectra from the films comprised of 1.0 wt% DPA are shown in Figure 1.10.a. Areas under the upconverted emission from DPA (400 – 520 nm) and phosphorescence from PdOEP (630 – 800 nm) are plotted in Figure 1.10c and Figure 1.10d for all samples, respectively.

Figure 1.10 Photoluminescence data from PdOEP doped DPA containing thiol-ene films. 

a) Photoluminescence emission from PdOEP doped thiol-ene networks comprised of 1.0 wt% DPA showing the upconverted emission (400 – 520 nm) from DPA and phosphorescence from PdOEP (630 – 800 nm) ($\lambda_{ex} = 544$ nm). A break from 520 to 630 nm is present to exclude the excitation peak for clarity. b) Picture of upconverting thiol-ene film upon irradiation with 532 nm laser in natural room light without aid from a bandpass filter. Integrated upconverted emission (c) and phosphorescence (d) intensities from thiol-ene films with various PdOEP concentrations as a function of DPA concentration.
The overall upconversion quantum efficiency is determined by the efficiencies of ISC of the photosensitizer (which can be assumed to equal 1), \(^9\) TTET from the sensitizer to emitter, TTA between two emitters, and fluorescence quantum yield (\(\Phi_{Fl}\)) of the emitter.\(^{51, 52}\) As DPA concentration is increased the phosphorescence diminishes indicating the TTET from PdOEP to DPA becomes more efficient with maximum upconverted emission intensity observed at 1.0 wt% DPA (Figure 1.10.c). To rationalize the decrease in emission intensity above this point, the \(\Phi_{Fl}\) of the thiol-ene films without sensitizer were measured using an integrating sphere. When progressing from 0.25 to 5.0 wt% DPA the \(\Phi_{Fl}\) decreases from 68 to 56% (Figure 1.11), which falls within the same reported range for similar systems and can be attributed to self-quenching effects.\(^{53}\)

![Graph](image)

**Figure 1.11** Fluorescent quantum yields and lifetimes for DPA containing thiol-ene films.  

a) Direct fluorescence quantum yield of thiol-ene films prior to PdOEP addition as a function of DPA concentration (\(\lambda_{ex} = 375\) nm). b) Direct fluorescent lifetimes of thiol-ene films with various PdOEP concentrations as a function of DPA concentration (\(\lambda_{ex} = 400\) nm, \(\lambda_{em} = 425\) nm).

To further investigate these results and the effect of PdOEP, the direct fluorescence decay behavior of DPA was examined using time-correlated single-photon counting (TCSPC). Upon excitation at 400 nm, the time resolved emission at 425 nm was
recorded. As shown in Figure 1.11.b, films without PdOEP exhibit a linear decrease in fluorescence lifetime which further supports the self-quenching effects observed in the \( \Phi_{\text{Fl}} \) data. When PdOEP is present, fluorescence lifetimes shorten, signifying back energy transfer from DPA to PdOEP.\(^{54}\) Interestingly, the fluorescence decay lengthens from 0.25 to 1.0 wt% DPA. This result may be due to the overlap area, between the PdOEP Soret band and DPA emission, decreasing as the emission red shifts upon increasing emitter concentration. This result demonstrates that spectral overlap between sensitizer absorption and emitter fluorescence is an important factor in governing the performance of TTA-UC systems.\(^{55,56}\)

1.4 Oxygen Scavenging Behavior

Fabricating upconverting materials via thiol-ene click chemistry is advantageous because the thioether linkages formed upon curing can act as oxygen scavengers to improve photostability. To investigate this, the upconversion photostability of thiol-ene networks with thioether linkages was compared to thiol-ene films where the thioether groups were oxidized to sulfone groups. For these samples, thiol-ene films (without covalently tethered DPA) were synthesized and the DPA and PdOEP were infused into the network through solution immersion (1.0 wt% DPA and 0.01 wt% PdOEP). Thioether groups were oxidized prior to doping with PdOEP and DPA using a hydrogen peroxide solution according to previously reported procedures.\(^{57,58}\) As a secondary comparison, DPA and PdOEP doped Tecoflex films, a well-studied aliphatic polyether-based thermoplastic polyurethane host material for TTA-UC, were also prepared according to previously reported methods.\(^{25}\) Figure 1.12 shows the upconverted emission intensity at 440 nm measured under continuous irradiation at 532 nm in ambient conditions. The
thiol-ene films with thioether functionality exhibit considerably better photostability compared to the thiol-ene networks with sulfone functionality and Tecoflex films, potentially indicating that the thioether linkages scavenge molecular oxygen and can aid in long term upconversion performance.

Figure 1.12 Photostability of upconverting thiol-ene films.

Normalized upconverted emission intensity from samples comprised of 1.0 wt% DPA and 0.01 wt% PdOEP under constant irradiation with a 10 mW 532 nm laser. Each plot corresponds to a different host material: thiol-ene network with thioether functionality (blue), thiol-ene network and sulfone groups (black), and Tecoflex (red).

1.5 Conclusions

In summary, thiol-ene click chemistry was successfully used to create robust materials for solid-state TTA-UC. Functionalized DPA chromophores with pendant thiols allowed for near quantitative covalent incorporation into homogeneous, elastomeric polymer networks. This synthetic strategy resulted in optically transparent films using a quick solventless photopolymerization method. Upon addition of a PdOEP sensitizer,
green-to-blue upconversion was observed with optimal upconverted emission intensity detected at 1.0 wt% DPA and 0.02 wt% PdOEP. Lastly, by using thiol-ene click chemistry, a high concentration of thioether linkages were present in the material resulting in increased photostability. These results provide a clear demonstration of the versatility of the thiol-ene approach toward creating upconverting polymers.

1.6 General Remarks

All solvents and reagents were purchased from commercial sources and used as received. 9,10-Dibromoanthracene and 4-vinylbenzeneboronic acid were purchased from Alfa Aesar. Palladium(II) octaethylporphyrin and poly(ethylene glycol)divinyl ether were obtained from Sigma-Aldrich. Pentaerythritol tetrakis(3-mercaptopropionate) and 3,6-dioxa-1,8-octanedithiol were purchased from TCI, and 2,2-dimethoxy-2-phenylacetophenone was purchased from Acros. 9,10-Bis(4-vinylphenyl)anthracene (1) was prepared according to literature procedures.59

1H and 13C NMR spectra were recorded on a Bruker Ascend 600 MHz spectrometer and chemical shifts were referenced to the residual solvent peak. Fourier transform infrared measurements were obtained using a Nicolet 6700 spectrometer equipped with an ATR accessory. The Tgs were measured by DSC using a TA Instruments Q200 with heating and cooling rates of 10 °C min⁻¹. Tg was defined as the maximum of the first derivative plot of the first heating cycle from -80 °C. UV-vis absorption spectra were recorded using an Agilent Technologies Cary 5000 UV-vis-NIR spectrophotometer. Steady-state photoluminescence measurements were recorded using a PTI-Horiba QuantaMaster 400 spectrofluorimeter equipped with a 75 W Xe arc lamp. Quantum yields were obtained using a PTI K-Sphere “Petite” integrating sphere.
Fluorescence decay profiles were recorded using a Horiba PPD-850 TCSPC detector using a Fianium WhiteLase SC-400 laser at a repetition rate of 2 MHz.

1.6.1 Synthesis of 2.

9,10-Bis(4-vinylphenyl)anthracene (0.500 g, 1.31 mmol), 3,6-dioxa-1,8-octanedithiol (12 mL, 65 mmol) and 2,2'-azobis(2-methylpropionitrile) (6.4 mg, 0.039 mmol) were added to a Schlenk tube, dissolved in CHCl₃ (20 mL), and stirred for 1 h at 60 °C. The solution was cooled to room temperature and poured into methanol (100 mL). Upon cooling to 0 °C light yellow crystals formed, which were filtered, washed with excess methanol, and dried under vacuum to afford 2 (0.55 g, 78%). ¹H NMR (600 MHz, CDCl₃): δ (ppm) 7.73 (4H, dd, J = 6.8, 3.3 Hz), 7.47 – 7.45 (8H, m), 7.36 (4H, dd, J = 6.9, 3.2 Hz), 3.78 (4H, t, J = 6.8 Hz), 3.70 (8H, m), 3.67 (4H, t, J = 6.4 Hz), 3.12 (4H, m), 3.03 (4H, m), 2.87 (4H, t, J = 6.9 Hz), 2.73 (4H, dt, J = 8.2, 6.4 Hz), 1.62 (2H, t, J = 8.2 Hz). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) 139.72, 137.05, 136.90, 131.43, 129.96, 128.54, 126.99, 124.96, 72.98, 71.23, 70.38, 70.31, 36.44, 34.17, 31.83, 24.32. EA Found: C, 67.35; H, 6.72. Calc. for C₄₂H₅₀O₄S₄: C, 67.50; H, 6.75%.
Figure 1.13 $^1$H NMR spectra of 2.

Figure 1.14 $^{13}$C NMR spectra of 2.
1.6.2 Synthesis of Thiol-ene Polymer Networks with DPA

When calculating the DPA concentration for the various networks only the DPA core was considered and the amounts of 2 were chosen such that the resulting films contained 0, 0.25, 0.5, 1.0, 1.8, 2.5 and 5.0 wt% DPA. The following is a typical experimental procedure performed for the network containing 1.0 wt% of DPA. Dithiol 2 (39 mg, 0.052 mmol), 3,6-dioxa-1,8-octanedithiol (0.495 g, 2.72 mmol), poly(ethylene glycol) divinyl ether (0.940 g, 4.60 mmol), and pentaerythritol tetrakis(3-mercaptopropionate) (0.459 g, 0.1 mmol) (to achieve a 3:1 dithiol to tetrathiol molar ratio) were combined in a 1:1 thiol to alkene molar ratio in the presence of 1 wt% photoinitiator (2,2-dimethoxy-2-phenylacetophenone). The monomer solution was pipetted between glass slides separated by a 0.265 mm Teflon spacer and cured 14 inches away from a Cure-Tek UVA-400 lamp for 8 min. The films were subsequently soaked overnight in CHCl₃ solutions containing 0, 0.038, 0.075, and 0.15 mg mL⁻¹ PdOEP and dried under vacuum for 24 h prior to optical characterization.

1.6.3 Synthesis of Neat Thiol-ene Polymer Networks

3,6-Dioxa-1,8-octanedithiol (0.516 g, 2.83 mmol), poly(ethylene glycol) divinyl ether (0.961 g, 4.75 mmol), and pentaerythritol tetrakis(3-mercaptopropionate) (0.461 g, 0.943 mmol) (to achieve a 3:1 dithiol to tetrathiol molar ratio) were combined in a 1:1 thiol to alkene molar ratio in the presence of 1 wt% photoinitiator (2,2-dimethoxy-2-phenylacetophenone). The monomer solution was pipetted between glass slides separated by 0.265 mm Teflon spacers and cured 14 inches away from a Cure-Tek UVA-400 lamp for 8 min.
1.6.4 Oxidation of Neat Thiol-ene Polymer Networks and Chromophore Doping

Thiol-ene networks were oxidized following a previously reported procedure. Thiol-ene films were submerged for 24 h in 30% H₂O₂ in water. The samples were immersed in neat DI water 3 times for 1 h to remove any H₂O₂. The films were dried on a hotplate at 150 °C until no mass loss was observed. The neat and oxidized thiol-ene networks were submerged in CHCl₃ solutions containing 1.33 mg mL⁻¹ DPA and 0.075 mg mL⁻¹ PdOEP for 24 h. The films were dried under vacuum for 24 h to remove any residual solvent prior to optical characterization.

1.6.5 Preparation of Tecoflex Films

DPA and PdOEP containing Tecoflex films were prepared following previously reported methods.²⁵,⁴⁰ To a Teflon beaker was added 2.0 g of a Tecoflex EG-80A solution (5 wt% in DMF), 2 mL DPA solution (0.5 mg mL⁻¹ in DMF), and 1 mL PdOEP solution (0.01 mg mL⁻¹ in DMF). The beaker was placed on a hotplate at 90 °C for 2 h and further dried at 90 °C under vacuum overnight.
CHAPTER II – THIOL-ENE CLICK POST-POLYMERIZATION MODIFICATION OF A FLUORESCENT CONJUGATED POLYMER FOR PARTS-PER-BILLION PYROPHOSPHATE DETECTION IN SEAWATER

2.1 General Background and Motivation

Pyrophosphate (PPI; P$_2$O$_7^{4-}$) has been found to be an essential anion with roles in energy transduction, biomacromolecule synthesis, extracellular signal mediation, etc. While microbes in coastal wetlands readily use PPI for energy, recent industrial activities have led to excessive concentrations of PPI in agriculture run-off and eutrophication of these aquatic ecosystems, thereby negatively impacting human health and the economy. There remains a critical need for simple and robust phosphate sensors that reduce the high-cost and complexity of collecting data and better capture intricacies associated with how phosphates exist in biological processes and within ecosystems. While several chemosensors have been developed, selective and sensitive anion recognition in water has been a challenge due to competitive solvation, and the required sensitivity to probe biologically relevant concentrations.

Small-molecule fluorophores have been used for PPI detection through indicator displacement, photoinduced electron transfer, and monomer-excimer formation assays. However, these approaches have been limited by ratiometric quenching, failure to discriminate between various oxyanions, multi-step synthetic approaches, and limited sensitivity. Several supramolecular structures have been developed for phosphorus oxyanion detection, but selective binding in water continuously remains a challenge due to competitive solvation. These failings have made the transduction of
small-molecule binding events into signals with the required sensitivity for biological, physical, and environmental applications a challenge.

Intrinsically fluorescent conjugated polymers (CPs) and conjugated polyelectrolytes (CPEs) can simultaneously perform molecular recognition and signal transduction processes for the detection of analytes. These systems take advantage of unique signal amplification that, when compared to small molecules, gives way to orders of magnitude improvements in sensitivity. In addition, CPs and CPEs transduce electronic signal through exciton migration, which is relatively immune to electrostatic and dielectric variations, allowing their successful implementation for sensing applications within ion rich aqueous environments. The exciton diffusion process and correlated optical response are closely related to the electronic and structural conformation of the polymer backbone, which can be synthetically tuned to incorporate molecular design features that enhance intra- and intermolecular exciton delocalization leading to stronger amplified signals and detectivity at nanomolar concentrations. The modular nature of CPs allows fine-tuning of backbone planarity, solubility, functionality, quantum yield, etc. to optimize sensor performance.

The hallmark signal amplification, coupled with the ability to tailor the optical response to various analytes with a diverse set of compatible receptor chemistries, has enabled the development of selective and sensitive CP-based sensors. CPE-based chemosensors have been reported for the selective detection of PPI in aqueous media, such as an on-off-on fluorescent assay based on a sulfonate functionalized poly(fluorene-co-phenylene) copolymer. This assay incorporates the strong selectivity of molecular recognition elements, high sensitivity from CP signal amplification, and low signal-to-
noise ratio characteristic of a “turn-on” output process. Many applications, however, require additional orders of magnitude improvements in sensitivity, more elaborate control over chemical structure, and the eventual integration of more complex recognition elements.

Arduous multi-step synthetic procedures, scalability, and a restricted understanding of structure-function-property relationships remain a limiting factor in the development of CPE-based materials and sensors.\textsuperscript{73, 82} This motivated our investigation of synthetic strategies that afford facile and rapid access to diverse CPEs applied in the context of current sensing platforms.\textsuperscript{83-85} Thiol-ene click reactions are simple, tolerant of most functional groups, and ene- and thiol- functionalized monomers are inexpensive and readily available.\textsuperscript{86, 87} Integration of the ethynyl structural units into P2 was hypothesized to improve assay sensitivity through enhanced backbone rigidity and planarity, which leads to extension of the exciton diffusion length and increases in the intrinsic tendency of the CPE to aggregate in water.\textsuperscript{88}

\subsection*{2.2 Synthesis and Characterization of P2}

The sulfonate functionalized polyfluorene-co-ethynyl CP (P2) was rapidly synthesized using sequential microwave-mediated polymerization and post-polymerization modification reactions. This bottom-up post polymerization modification approach afforded a conjugated polyelectrolyte (CPE) without harsh reagents such as sulfuric acid, and circumvented common arduous techniques such as dialysis and lyophilization (Figure 2.1). The conjugated polymer backbone scaffold (P1) is synthesized using a microwave-mediated Stille coupling polymerization between 2,7-dibromofluorene and bis(trimethylstanny)acetylene. The fluorene bridgehead is
difunctionalized with pentene groups to facilitate a post polymerization modification thiol-ene “click” reaction with sodium 3-mercapto-1-propanesulfonate to afford the sulfonate functionalized polyfluorene-co-ethynyl CP (P2) in 92% yield. The Fourier-transform infrared (FTIR) spectrum of P1 and P2 are shown in Figure 2.1. P1 has peaks at 909, 993, and 1471 cm\(^{-1}\) corresponding to out-of-plane bending and in-plane scissoring modes of the terminal \(=\text{C-H}_2\) groups. Following the thiol-ene click post-polymerization modification these peaks disappear and new peaks at arise 1173 and 1046 cm\(^{-1}\), characteristic of the S=O stretching vibrations of sulfonate salts. While alkyne stretching modes typically appear between 2,100 – 2,200 cm\(^{-1}\), there are no peaks present within this region due to the weak vibrational dipole moment of internal alkynes. The complete conversion of terminal olefins by thiol-ene chemistry was also observed via NMR spectroscopy through the disappearance of peaks between 4.8 – 5.8 ppm (Figure 2.2 and Figure 2.3).

![Synthetic scheme and FTIR for P2](image)

Figure 2.1 Synthetic scheme and FTIR for P2.

(Top) Synthesis of P2: (i) Pd(PPh\(_3\))\(_4\) (5 mol %), xylene/DMF, 200 mW, 125 °C, 10 min; (ii) Irgacure 819 (2 wt%), tetrahydrofuran/methanol/water, UV, 2 h. (Bottom) FTIR spectra of P1 and P2 that highlights the disappearance of alkene groups and appearance of sulfonate groups following the thiol-ene click post-polymerization modification reaction.
Figure 2.2 $^1$H NMR (600 MHz, CDCl$_3$) of P1 at 328 K.

Figure 2.3 $^1$H NMR (600 MHz, D$_2$O) of P2 at 353 K.
2.3 Optical Sensing Characterization

The fluorescence quenching and dequenching behavior of P2 in aqueous solutions was evaluated through a series of titrations using Fe$^{3+}$ and PPi, respectively (Figure 2.4). For all optical characterization and assay experiments the concentration of P2 is based on the molarity of polymer repeat units where the polymer repeat unit extinction coefficient was measured to be 9,200 L mol$^{-1}$ cm$^{-1}$. Upon excitation at 380 nm, P2 exhibits a strong emission between 400 and 700 nm, with maximum intensity centered at 463 nm. Upon titration with Fe$^{3+}$, the fluorescence of P2 was strongly quenched with maximum quenching, defined as the point at which the fluorescence intensity does not decrease upon further addition of Fe$^{3+}$ to the P2 solution, observed at 1.3×10$^{-5}$ M Fe$^{3+}$ (Figure 2.4b). A Stern-Volmer plot was obtained by plotting $I_o/I$ against the Fe$^{3+}$ concentration, where $I_o$ and I are the fluorescence intensities of P2 in the absence and presence of Fe$^{3+}$, respectively, and the slope is the Stern-Volmer constant ($K_{SV}$) (Figure 2.4c). The plot has a linear range between 0 and 7.3 × 10$^{-6}$ M Fe$^{3+}$ with a $K_{SV}$ of 3.4 × 10$^5$ M. The strong linearity and high $K_{SV}$ indicate a static and amplified quenching mechanism, and there is no observable variation in the fluorescence lifetimes of P2 and P2-Fe$^{3+}$, which further indicates a static quenching mechanism (Figure 2.5). The evolution in the absorbance of P2 upon Fe$^{3+}$ addition is shown in Figure 2.4f. The absorption maximum ($\lambda_{max}$) only slightly blue shifts 5 nm from 378 to 383 nm, indicating that binding of Fe$^{3+}$ has little effect on the electronic structure of P2. Upon addition of PPi (up to 5.3 × 10$^{-6}$ M) to P2-Fe$^{3+}$ the quenched fluorescence is almost fully recovered (Figure 2.4e). We hypothesized that the incorporation of the ethynyl linker in the polymer backbone would promote planarity and extend the conjugation length to improve assay sensitivity. Indeed, the
absorption and emission maxima of P2 are significantly redshifted compared to those reported for a CPE with a fluorene-alt-phenylene backbone (334 and 411 nm, respectively), and a two orders of magnitude enhancement in the sensitivity is observed.81, 89

Figure 2.4 Scheme, photograph, and optical data depicting the fluorescence quenching and dequenching behavior of P2 upon addition of Fe3+, and PPi, respectively.

(a) Scheme depicting the fluorescence quenching and dequenching behavior of P2 upon the addition of Fe3+ and PPi, respectively. (b) Fluorescence quenching behavior of an aqueous solution of P2 (2 × 10⁻⁵ M) upon addition of Fe³⁺ (up to 1.3 × 10⁻⁵ M) (λ_ex = 380 nm). (c) Stern-Volmer plot showing the quenching behavior of P2 as a function of Fe³⁺ concentration (λ_ex = 380 nm, λ_em = 463 nm). The Ksv value is determined from the slope of the line of best fit. (d) Picture of P2 solutions in DI water showing the on-off-on fluorescence behavior upon subsequent additions of Fe³⁺ and PPi. (e) Fluorescence dequenching of P2-Fe³⁺ upon addition of PPi (up to 5.3 × 10⁻⁶ M) (λ_ex = 380 nm). (f) The evolution in the absorbance of P2 (2 × 10⁻⁵ M) upon addition of Fe³⁺ in water.
Figure 2.5 Normalized fluorescence decay profiles of P2 and P2-Fe$^{3+}$ ($\lambda_{ex} = 400$ nm, $\lambda_{em} = 475$ nm).

The instrument response function (IRF) is also plotted for reference.

2.4 Anion and Cation Selectivity Experiments

To accurately determine PPi concentration in complex aqueous environments it is important that the on-off-on fluorescent behavior of P2 is not strongly influenced by cations and anions other than Fe$^{3+}$ and PPi. Therefore, a series of selectivity tests were performed to individually assess the effect of various cations and anions on the fluorescent properties of P2 and P2-Fe$^{3+}$, respectively. With the intent of utilizing P2 as a PPi sensor in marine environments, we investigated the effects of Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, and Sr$^{2+}$, as these are the most prevalent cations in seawater (>1 ppm), as well as a variety of minor cations found in seawater including: Co$^{2+}$, Fe$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$. Figure 2.6a plots $I_q/I_{oq}$ where $I_{oq}$ is the fluorescence intensity of neat P2 in DI water, and $I_q$ is the intensity of P2 in the presence of the various cations combined in a 1:100 molar ratio P2 to metal cations. Negative values correspond to fluorescence quenching,
whereas positive values represent fluorescence enhancement. The presence of Fe$^{3+}$ and Co$^{2+}$ induced the greatest reduction of the emission intensity at 80% and 40%, respectively. Some cations, such as Fe$^{2+}$ and Mn$^{2+}$, slightly enhanced the fluorescence. In comparison, Fe$^{3+}$ most significantly quenched the fluorescence of P2, demonstrating that the fluorescence quenching of P2 is selective toward Fe$^{3+}$ and that cations present in marine and brackish environments would have minimal effect of the fluorescent signal of P2.

**Figure 2.6** Bar graphs summarizing the effect of cations and anions on the fluorescence intensity of P2 and P2-Fe$^{3+}$, respectively.

The initial fluorescence intensities, $I_{oq}$ and $I_{od}$, are defined as the emission intensity of neat P2 and P2 following the addition of Fe$^{3+}$ to quench the fluorescence by 85%, respectively, and $I_{q}$ and $I_{d}$ are the fluorescent intensities of P2 and P2-Fe$^{3+}$ in the presence of the various cations and anions, respectively. Negative values correspond to fluorescence quenching, whereas positive values indicate fluorescence dequenching. Pictures showing the fluorescent responses of P2 and P2-Fe$^{3+}$ solutions, under UV irradiation ($\lambda_{ex} = 365$ nm), in the presence of cations and anions, respectively, are also included. ($\lambda_{em} = 380$ nm, $\lambda_{em} = 463$ nm)

Using a similar analysis, the fluorescence response of P2-Fe$^{3+}$ to a variety of anions was also evaluated. Anions ranged from the major anionic constituents in seawater; $\text{F}^-$, $\text{Br}^-$, $\text{Cl}^-$, $\text{H}_2\text{BO}_3^-$, $\text{HCO}_3^-$, and $\text{SO}_4^{2-}$, trace and minor anionic components in
seawater; I, C₂H₃O₂⁻, CO₃²⁻, and NO₃⁻, and other phosphates; H₂PO₄⁻ and PO₄³⁻. In these experiments the fluorescence of P₂ was quenched by 85% with Fe³⁺ and the molar ratio P₂ to anions was adjusted to 1:100. In Figure 2.6b, I_d/I_od-1 is plotted, where I_od is the fluorescence intensity of P₂-Fe³⁺ (PPi fluorescence quenched to 85% with Fe³⁺) and I_d is the fluorescence intensity following the addition of the various anions. In the presence of PPi the fluorescence of P₂-Fe³⁺ rapidly increases by 4-fold, whereas, the addition of other phosphate anions, H₂PO₄⁻ and PO₄³⁻, only slightly dequenched the fluorescence. The other anions have minimal effect on the emission intensity of P₂-Fe³⁺ indicating the dequenching behavior of P₂-Fe³⁺ is highly selective toward PPi and could potentially function as a PPi sensor in more complex systems.

2.5 PPi Sensing in Artificial Seawater

To demonstrate the potential of P₂ to serve as a PPi sensor in complex aquatic environments, a fluorescence assay was designed to measure PPi concentrations in artificial seawater. A calibration curve of the fluorescence intensity of P₂-Fe³⁺ with respect to PPi concentration is shown in Figure 2.7a, with a linear response observed from 0 to 40 nM PPi. The limit of detection (LOD), defined as the lowest concentration of analyte that can be detected, was calculated to be 4 nM using the equation LOD = 3 δ/m, where δ and m are the standard deviation and slope of the calibration line, respectively. Artificial seawater solutions with known amounts of PPi (6, 15, and 30 nM) were prepared with Instant Ocean Sea Salt. In a typical assay, 3 µL P₂-Fe³⁺ (0.2 mM P₂, 0.1 mM Fe³⁺) was added to a 3 mL seawater sample and the fluorescence intensity at 463 nm was measured (λ_ex = 380 nm). The averaged results from three trials are plotted in
Figure 2.7a, which demonstrates that the experimental data are in good agreement with
the theoretical values. The limit of quantification (LOQ), defined as the lowest
concentration of analyte that can be quantified, was determined using a precision profile
approach to account for any potential variability in the fluorescence measurements due to
the presence of cations and anions in the artificial seawater. The LOQ was found to be 6
nM within a 10% relative standard deviation, demonstrating the ability of P2 to
accurately measure PPI in a complex environment at parts-per-billion concentrations.

Figure 2.7 Optical data and photograph of pyrophosphate sensing in seawater.
(a) Peak fluorescence intensity of P2-Fe³⁺ with respect to PPI concentration (blue spheres), and intensities measured from seawater
samples with known amounts of PPI (red stars) (λ<sub>ex</sub> = 380 nm, λ<sub>em</sub> = 463 nm). Error bars correspond to standard errors measured
from 3 samples. (b) Picture of P2 solutions in artificial seawater showing the on-off-on fluorescence behavior upon subsequent
additions of Fe³⁺ and PPI.

In conclusion, we demonstrate a facile on-off-on fluorescent assay for PPI
detection in complex aqueous environments. A rigid water-soluble CPE with a
poly(fluorene-co-ethynyl) backbone and sulfate functionalized sidechains was rationally
designed to incorporate selective analyte-receptor interactions and promote enhanced
signal amplification. The target CPE was rapidly synthesized using a straightforward
approach employing a microwave-assisted Stille cross-coupling polymerization and thiol-ene click chemistry post-polymerization modification. This method enables facile access to the reported system P2 and other CPEs with an overall reduction in synthetic steps and purification procedures. It was shown that the polymer fluorescence is selectively quenched by Fe$^{3+}$ through an amplified and static quenching mechanism, and upon subsequent addition of PPi the fluorescence is almost completely restored at PPi concentrations as low as $5.3 \times 10^{-6}$ M. Accurate detection of PPi in complex aqueous environments was evaluated using artificial seawater samples with known amounts of PPi, and the LOD and LOQ were found to be 4 and 6 nM PPi, respectively. Results demonstrate that the designed assay could serve as a promising method for monitoring PPi in biological aquatic ecosystem, and the broad utility of thiol-ene click chemistry offers the straightforward integration of other diverse sensing elements.

2.6 General Remarks

2.6.1 Materials

Reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise specified. Deuterated solvents (CDCl$_3$ and D$_2$O) were purchased from Cambridge Isotope Laboratories and used as received. Tetrahydrofuran (THF) was degassed and dried over 4 Å molecular sieves. Monomer 1 was synthesized according to previously reported procedures.$^{91}$ Artificial seawater was prepared by dissolving Instant Ocean Sea Salt (15.5 mL) in DI water (500 mL). The solution was stirred for 24 h prior to spiking with PPi (6, 15, and 30 nM) and conducting assay experiments.
2.6.2 Instrumentation

$^1$H NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer and chemical shifts were referenced to the residual solvent peak. Microwave-assisted reactions were performed in a CEM Discover microwave reactor. The number average molecular weight ($M_n$) and polydispersity ($D$) were determined by gel permeation chromatography (GPC) relative to polystyrene standards at 160 °C in 1,2,4-trichlorobenzene (stabilized with 125 ppm of BHT) using an Agilent PL-GPC 220 High Temperature GPC/SEC system equipped with four PLgel 10 μm MIXED-B columns. Polymer samples were pre-dissolved (1.0 mg mL$^{-1}$) by stirring for 4 h at 150 °C. Fourier-transform infrared (FTIR) spectroscopy measurements were obtained using a Nicolet 6700 spectrometer equipped with an ATR accessory. UV-vis absorption spectra were recorded using an Agilent Technologies Cary 5000 UV-vis-NIR spectrophotometer. Steady-state photoluminescence measurements were recorded using a PTI-Horiba QuantaMaster 400 spectrofluorimeter equipped with a 75 W Xe arc lamp. Fluorescence decay profiles were recorded using a Horiba PPD850 time-correlated single-photon counting (TCSPC) detector with a Fianium WhiteLase SC-400 laser excitation source at a repetition rate of 2 MHz.

2.6.3 UV-vis and fluorescence measurements of P2 upon titration with Fe$^{3+}$.

In a cuvette, a 3.0 mL solution of P2 (2.0 × 10$^{-5}$ M in DI water (based on repeat unit)) was titrated with 10 μL aliquots of Fe$^{3+}$ (0.2 mM). After each addition, the solution was stirred until no further evolution is the spectra were observed ($\lambda_{ex} = 380$ nm).
2.6.4 Selectivity of fluorescence quenching of P2 with metal salts.

To obtain the initial fluorescence intensity, 10 µL of P2 (0.6 mM) was added to 3 mL of DI water and the peak fluorescence intensity was recorded. In a similar manner, the final fluorescence intensity was determined by adding an equivalent amount of the P2 to 3.0 mL of various metal salt solutions (0.2 mM) resulting in a 1:100 molar ratio of P2 to the cations. The solution was stirred for 2 min and fluorescence intensity measured (λ<sub>ex</sub> = 380 nm, λ<sub>em</sub> = 463 nm).

2.6.5 UV-vis and fluorescence measurements of P2-Fe<sup>3+</sup> upon titration with PPI.

An aqueous stock solution of P2-Fe<sup>3+</sup> was prepared by fully quenching the fluorescence of P2 (2.0 × 10<sup>−5</sup> M) to 85% with Fe<sup>3+</sup> (6.0 × 10<sup>−6</sup> M in final solution). A 3.0 mL solution of P2- Fe<sup>3+</sup> was titrated with 10 µL aliquots of an aqueous PPI stock solution (0.2 mM). After each addition, the solution was stirred until no further evolution is the spectra were observed (λ<sub>ex</sub> = 380 nm).

2.6.6 Selectivity of fluorescence dequenching of P2-Fe<sup>3+</sup> with anions.

The initial peak fluorescence intensity was defined as the emission of P2 (1.0 × 10<sup>−6</sup> M) following the addition of Fe<sup>3+</sup> to quench the fluorescence by 85%. The final fluorescence intensity was measured by adding an equivalent molar amount of P2 and Fe<sup>3+</sup> to 1.5 mL of DI water in a cuvette. Upon addition of 1.5 mL of an anion solution (0.2 mM) the repeat unit and anion concentrations were diluted to 1.0 × 10<sup>−6</sup> M and 0.1 mM, respectively, resulting in a 1:100 molar ratio of polymer repeat units to anions. The solution was stirred for 2 min and fluorescence intensity measured (λ<sub>ex</sub> = 380 nm, λ<sub>em</sub> = 463 nm).
2.6.7 Dequenching of P2-Fe\(^{3+}\) with PPI for calibration curve.

To 3 mL DI water was added 1 µL P2 solution (0.6 mM) and 2 µL Fe\(^{3+}\) solution (0.2 mM). The solution was stirred for 10 min or until no further decrease in emission was observed. The quenched solution was titrated with aliquots of PPI solutions (ranging from 2×10\(^{-8}\) to 2×10\(^{-4}\) M). In between each addition the solution was stirred until there was no further increase in emission (\(\lambda_{ex} = 380\) nm, \(\lambda_{em} = 463\) nm).

2.6.8 PPI sensing in artificial seawater.

Samples with known amounts of PPI were prepared by adding 3, 10, and 15 µL PPI solution (0.2 mM) to 100 mL artificial seawater resulting the PPI concentrations of 6.0 × 10\(^{-9}\), 2.0 × 10\(^{-8}\), and 3.0 × 10\(^{-8}\) M. In a typical assay, 3 µL P2-Fe\(^{3+}\) (0.2 mM P2, 0.1 mM Fe\(^{3+}\)) was added to a 3 mL seawater sample and the fluorescence intensity at 463 nm was measured.
REFERENCES


