

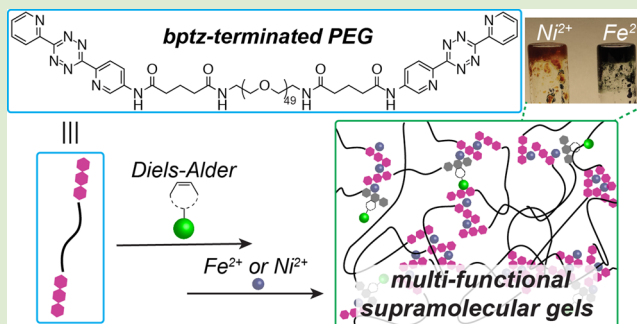
Dual Role for 1,2,4,5-Tetrazines in Polymer Networks: Combining Diels–Alder Reactions and Metal Coordination To Generate Functional Supramolecular Gels

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S Supporting Information

ABSTRACT: The inverse-electron demand Diels–Alder cycloaddition of tetrazines and olefins has emerged as a powerful coupling reaction for the formation of polymer gels with diverse applications. Tetrazines are also excellent ligands for metal atoms. For example, 3,6-bis(2-pyridyl)-1,2,4,5-tetrazines (bptz) have been used to generate discrete supramolecular M_xbptz_y , metal clusters and extended 2D grid structures. We reasoned that both the Diels–Alder and the metal-coordination modes of reactivity of bptz derivatives could be leveraged in the context of hydrogel design to yield novel hybrid materials. Here we report on the formation of supramolecular hydrogels via substoichiometric Diels–Alder functionalization of bptz ligands bound to the ends of poly(ethylene glycol) (PEG) chains followed by metal-coordination-induced gelation in the presence of Ni^{2+} and Fe^{2+} salts. Our results show that simple bptz-based polymers are versatile precursors to a diverse range of novel functional materials.



Interest in tetrazine reactivity has surged in recent years due to its application in bioconjugation,¹ coordination,² and soft materials chemistry.³ Thanks to its “click”-like efficiency,^{3d,4} the inverse electron-demand Diels–Alder reaction of tetrazines with strained olefins⁵ (Figure 1A, *i*) has proven particularly

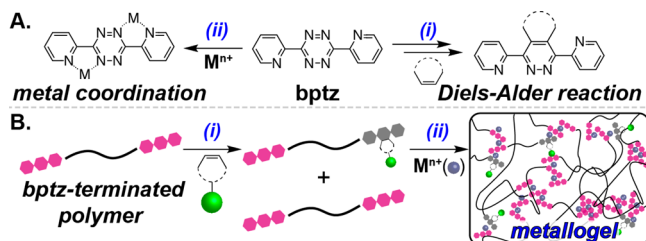


Figure 1. (A) Diels–Alder reaction (*i*) and metal coordination (*ii*) of bptz. (B) Schematic of our approach, which combines these two modes of reactivity for the synthesis of functional supramolecular gels. Green sphere = functional species of interest.

useful for the synthesis of covalent polymer gels. For example, in 2012 we reported on the synthesis of poly(ethylene glycol) (PEG)-based gels via end-linking of a norbornene-terminated PEG macromer with a tris-tetrazine monomer.^{3c} Since our initial report, we⁶ and others⁷ have constructed a variety of diversely functionalized covalent gels using this chemistry.

Tetrazines are also well-known for their ability to coordinate with metal ions (Figure 1A, *ii*).^{2,8} In particular, 3,6-bis(2-

pyridyl)-1,2,4,5-tetrazine (bptz) and related derivatives have been widely applied as ligands for the formation of discrete metallosupramolecular assemblies.⁹ For example, Dunbar and co-workers have reported on the anion-templated synthesis of molecular triangles, squares, and pentagons through the use of bptz and various metal ions including Fe^{2+} , Ni^{2+} , and Ag^+ , respectively.^{2c} These fascinating complexes are composed of multiple metals and bptz ligands arranged in a counteranion-dependent “ M_xbptz_y ” stoichiometry.

Inspired by these studies, we wondered if it would be possible to form gels not through Diels–Alder reactions of tetrazine-functionalized polymers, but instead via metal-tetrazine coordination. In general, gels formed via coordination of polymeric ligands with metal atoms, metallogels, display unique dynamic mechanical properties.¹⁰ We suspected that bptz-based metallogels might possess similar properties with the distinct advantage that the bptz moiety could also operate as a reactive handle for covalent gel modification via Diels–Alder reactions. Herein, we demonstrate that indeed bptz can facilitate both supramolecular gelation and covalent functionalization to provide a diverse range of functional metallogels with potential controlled release applications (Figure 1B).

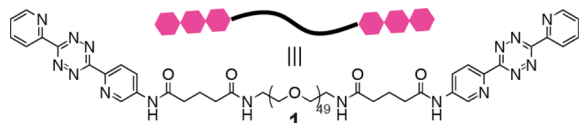
Our studies commenced with the synthesis of macro-bptz ligand **1**, which was prepared via carbodiimide-mediated

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Chart 1. Bis-bptz PEG Macromer 1



coupling of a previously reported bptz-COOH derivative^{3c} onto the ends of 2 kDa diamino-PEG (see Supporting Information, SI, for synthetic details). Dunbar and co-workers have shown that bptz forms discrete M_4L_4 square complexes with Ni^{2+} and Fe^{2+} templated by perchlorate.^{9e,f} We reasoned that analogous complexation between **1** and Ni^{2+} or Fe^{2+} could give rise to metallogels. Indeed, exposure of **1** (100 mg/mL) to $Ni(ClO_4)_2 \cdot 6H_2O$ or $Fe(ClO_4)_2 \cdot xH_2O$ in a 1:1 metal to ligand ratio in MeCN or water resulted in qualitatively fast gelation with an accompanying color change (Figure 2). These gels dissolved

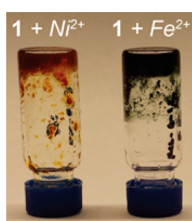


Figure 2. Images of gels formed upon mixing **1** (1.0 equiv, 100 mg/mL) with either $Ni(ClO_4)_2 \cdot 6H_2O$ (left) or $Fe(ClO_4)_2 \cdot xH_2O$ (right) in MeCN, 150 μ L.

immediately upon addition of a strong metal chelator (25 μ L of 0.05 M aqueous K_4EDTA). Furthermore, solutions of **1** do not gel in the absence of metal, and solutions of PEG without bptz ligands do not gel in the presence of metal. These observations lead us to conclude that metal-bptz coordination is the key interaction responsible for gelation and that the bptz end-groups and metal ions form M_xbptz_y assemblies with x and y values greater than 1; such assembly is necessary to achieve network branching, which is a prerequisite for gelation.¹¹

Based on model studies, we do not believe that the junctions of these gels are composed of precise M_4L_4 Dunbar-type complexes. We prepared an unsymmetrically substituted monobptz derivative (TEGbptz, see SI) via coupling 2-(2-(2-methoxyethoxy)ethoxy)ethanamine to bptz-COOH. This ligand did not form well-defined assemblies in the presence of Ni^{2+} and Fe^{2+} (Figure S1). Thus, though we are confident that metal complexation between **1** and Ni^{2+} or Fe^{2+} is responsible for gelation, the exact M_xbptz_y stoichiometry of the junctions is currently not known.

We characterized the mechanical properties of these metallogels by oscillatory rheology. Frequency sweeps for Ni^{2+} and Fe^{2+} -based materials (Figure 3A) revealed linear viscoelastic storage moduli of approximately 22 kPa over the frequency range examined. These data suggest that these two metallogels have similar degrees of network branching. While the linear viscoelastic storage moduli of both gels were similar, strain sweeps (Figure 3B) indicate that their nonlinear properties are different. Ni^{2+} -based gels maintained their elastic nature at larger strains than Fe^{2+} -based gels, as measured by their linear viscoelastic range limits ($\sim 10\%$ for Fe^{2+} and $\sim 20\%$ for Ni^{2+}) and by their fluidization strains (18 and 42%, respectively).

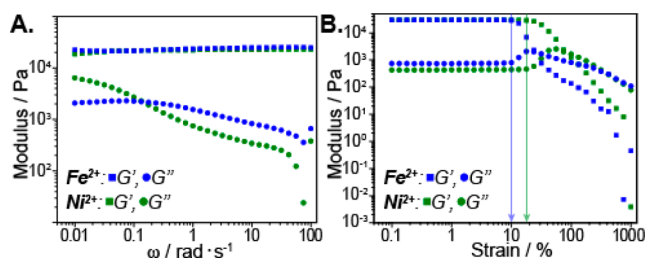


Figure 3. Frequency (A) and strain ($\omega = 10$ rad/s) (B) sweeps for metallogels derived from **1** and Ni^{2+} (green) or Fe^{2+} (blue). Strain amplitude (γ_0) and frequency (ω) values of 1% and 10 $rad \cdot s^{-1}$ were used for (A) and (B), respectively. Arrows in (B) correspond to the observed cutoff of linear response.

We also studied the effect of solvent on gelation of the Ni^{2+} -based metallogels (Figure 4). As discussed above, a 1:1 ratio of

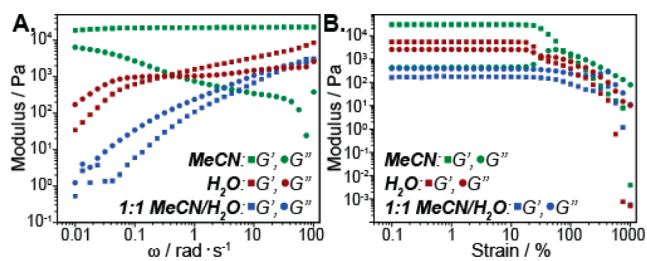


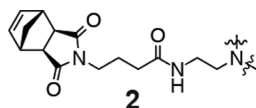
Figure 4. Frequency (A) and strain (B) sweeps for metallogels derived from **1** and Ni^{2+} in different solvents. Strain amplitude (γ_0) and frequency (ω) values of 1% and 10 $rad \cdot s^{-1}$ were used for (A) and (B), respectively.

Ni^{2+} and **1** (100 mg/mL) in acetonitrile resulted in an elastic gel at all measured frequencies; the G'/G'' crossover point lies beyond the leftmost point on the frequency axis. In contrast, gelation in water provided dynamic materials that behave as viscous fluids at low frequencies and elastic solids at high frequencies. This behavior is very similar to related “bioinspired” metallogels based on Fe^{3+} -catechol interactions.^{10f} Interestingly, 1:1 acetonitrile/water provided elastic materials only at the highest frequencies tested. This unexpected result suggests that the rates of metal–ligand association/dissociation are perturbed in a nonlinear fashion in different solvents, which could indicate the formation of different M_xbptz_y assemblies. We seek to investigate the origin of this behavior in future studies.

We next sought to combine Diels–Alder reactions with bptz-metal coordination to generate hybrid metallogels. We chose to use *exo*-norbornene-imide dienophiles as they are easy to synthesize, and they react with tetrazines at suitable rates for gelation.^{3c} Model studies revealed that Fe- and Ni-bptz complexes do not readily react with *exo*-norbornene-imides. Thus, we pursued a stepwise Diels–Alder followed by metal addition strategy. Macro-ligand **1** was combined with tris-norbornene cross-linker **2** in MeCN to generate hyperbranched polymers (HBPs) with 25% and 50% of their bptz groups reacted based on stoichiometry. Without purification, these HBPs (100 mg/mL) were mixed with $Ni(ClO_4)_2$ in MeCN to provide a 1:1 bptz/ Ni ratio. Immediate gelation was observed.

Given that these gels are comprised of HBPs connected via coordination bonds, there is not an extended covalent network, we hypothesized that they would display mechanical properties similar to the unmodified networks described above. Indeed,

Chart 2. Tris-norbornene Crosslinker 2



oscillatory rheology (Figure 5A,B) showed small (less than 2-fold) differences between 0, 25, and 50% modified materials.

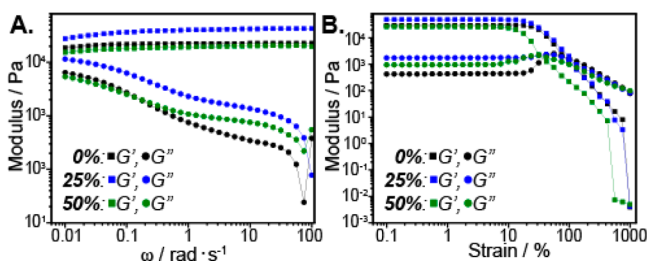


Figure 5. Frequency (A) and strain (B) sweeps for metalogels prepared from Ni^{2+} and HBPs. Samples are referred to by their % of consumed bptz groups. Strain amplitude (γ_0) and frequency (ω) values of 1% and 10 $\text{rad}\cdot\text{s}^{-1}$ were used for (A) and (B), respectively.

The fluidization strains (Figure 5B) for the 0 and 25% modified gels were roughly the same; the 50% modified gel showed a ~ 2 -fold lower value. These results suggest that consumption of a significant fraction of bptz groups via Diels–Alder reaction with **2** is not deleterious to the mechanical properties of these gels.

PEG-based materials and Fe^{2+} salts are typically nontoxic. Indeed, macromer **1** displayed no in vitro HeLa cell cytotoxicity at relevant concentrations (Figure S2). Thus, we sought to exploit the versatility of this system to create a Fe-based metallo-hydrogel that could serve as a scaffold for drug release. We employed *exo*-norbornene-imide doxorubicin (DOX) prodrug **3** (Figure 6A) that we used previously¹² for the UV-triggered release of DOX. Exposure of **1** (1.0 equiv) to **3** (0.1 equiv) for 24 h at room temperature in MeCN provided a statistical mixture of bifunctionalized (1%), monofunctionalized (18%), and unfunctionalized (81%) **1**. The completion of this reaction was verified by ^1H NMR (Figure S3). This mixture was dried under vacuum, dissolved in water, and then combined with $\text{Fe}(\text{ClO}_4)_2$ such that the ratio of metal to bptz was 1:1. Qualitative gelation, as determined by vial inversion, occurred within seconds (Figure 6A, inset image).

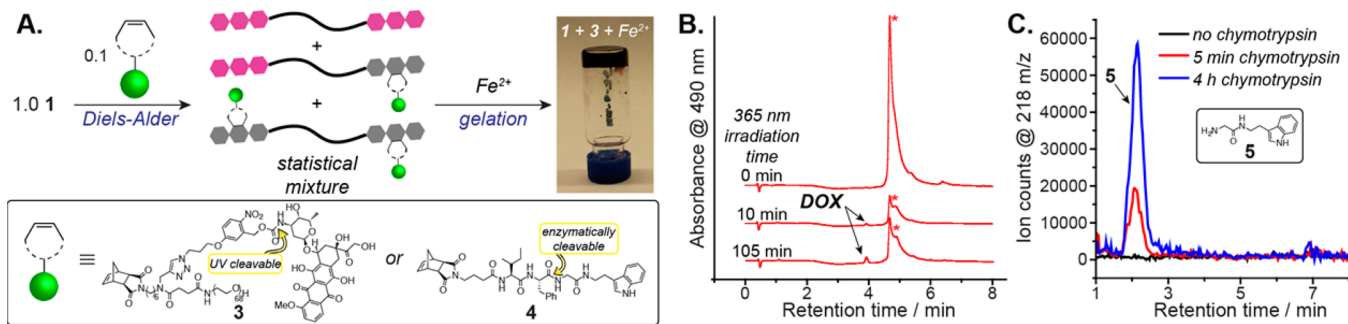


Figure 6. (A) Substoichiometric Diels–Alder functionalization of **1** with DOX- and peptide-norbornene conjugates **3** and **4**, respectively, provides statistical mixtures of covalently coupled products. Exposure of these mixtures to Fe^{2+} in water provides metallo-hydrogels (image of **3**-functionalized gel shown). (B) LC/MS traces demonstrating phototriggered release of free DOX from **1** + **3** + Fe^{2+} gel. Peak labeled “*” corresponds to the statistical mixture of **1** and **1** + **3** coupled products, which are observed due to gel erosion. (C) LC/MS traces demonstrating chymotrypsin-triggered release of **5**.

To test the release of DOX, this **1** + **3** + Fe^{2+} metallo-hydrogel was covered with 100 μL of water and irradiated with a 365 nm UV lamp. The water layer was removed and replaced with fresh water at various time intervals. DOX release was monitored by liquid chromatography–mass spectrometry (LC/MS, Figure 6B). At 0 min, no free DOX was observed; a peak at ~ 5 min (labeled “*”) is observed that corresponds to a mixture of **1** and **1** + **3** coupled products. After irradiation for 10 min, a peak for free DOX appears at a retention time of ~ 4 min. Irradiation for 105 min led to increased DOX release. The photorelease of DOX from these materials was slow, which is likely due to competitive light absorption by the strongly colored gel. This result suggests the intriguing possibility of tuning rates of photorelease through the use of alternative metals or ligands with different optical properties.

Notably, though the metallo-hydrogel used for DOX release remained intact throughout the course of the photolysis experiments, **1** and **1** + **3** products were continuously extracted, which is indicative of gel erosion. When these materials were submerged in water for an extended time, complete dissolution was observed after ~ 1 week. This result suggests that these materials could be used for applications that require mechanical integrity over several days, yet eventual degradation into nontoxic PEG and iron species.

To further demonstrate the versatility of this system, **1** was coupled to chymotrypsin-cleavable norbornene-Ile-Phe-Gly peptide **4** (Figure 6A) to provide enzymatically responsive metallo-hydrogels. Compound **4** was coupled to **1** in the same fashion as described for DOX-conjugate **3** (Figure S4). The statistical mixture of **1** and **1** + **4** was combined in a 1:1 metal–ligand ratio with $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ to provide gels. We found that iron sulfate formed gels in water with a similar storage modulus as that of $\text{Fe}(\text{ClO}_4)_2\cdot x\text{H}_2\text{O}$ in MeCN (Figures S5–S7). This gel was placed in 100 μL of a buffered solution (100 mM Tris, 10 mM CaCl_2 , pH 7.8) and treated with chymotrypsin at a concentration of 1.9 μM . After 45 min, the release of tryptamine glycinamide (**5**) was observed by LC/MS (Figure 6C, observed $[\text{M} + \text{H}]^+$, 218.0; expected $[\text{M} + \text{H}]^+$, 218.1), which confirmed enzymatic cleavage between the Phe and Gly residues. Though this model cleavage product (**5**) has no known biological function, the retention of enzymatic activity in these gels suggests that the same material platform could be used to display an array of biologically active species.

To summarize, we have described the use of bptz ligands for the synthesis of supramolecular gels based on metal–ligand

coordination. These materials display the dynamic mechanical properties of metallogels; they offer the additional advantage that they can be readily functionalized via efficient inverse electron-demand Diels–Alder reactions. We make use of this dual reactivity to prepare metallo-hydrogel scaffolds for photo- and enzymatically triggered small molecule release. The nontoxic nature of the components and the ease of functionalization make these bptz-based PEG gels a promising platform for materials design with potential applications in controlled-release, self-healing, sensing, and heterogeneous catalysis.

■ ASSOCIATED CONTENT

● Supporting Information

Synthetic procedures, methods, instrumentation, and characterization details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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