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Isothermally-Responsive Polymers Triggered by Selective Binding of Fe^{3+} to Siderophoric Catechol End-Groups

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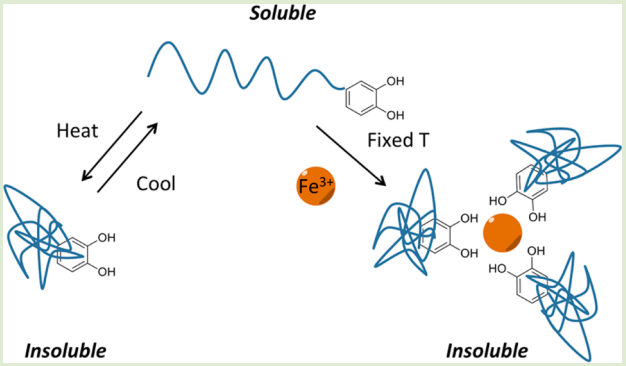
Supporting Information

ABSTRACT: Thermoresponsive polymers have attracted huge interest as a way of developing smart/adaptable materials for biomedicine, particularly due to changes in their solubility above the LCST. However, temperature is not always an appropriate or desirable stimulus given the variety of other cellular microenvironments that exist, including pH, redox potentials, ionic strength, and metal ion concentration. Here, we achieve a highly specific, isothermal solubility switch for poly(*N*-isopropylacrylamide) by application of ferric iron (Fe^{3+}), a species implicated in a range of neurodegenerative conditions. This is achieved by the site-specific incorporation of (Fe^{3+}-binding) catechol units onto the polymer chain-end, inspired by the mechanism by which bacterial siderophores sequester iron from mammalian hosts. The ability to manipulate the hydrophilicity of responsive systems without the need for a temperature gradient offers an exciting approach toward preparing increasingly selective, targeted polymeric materials.



The design of systems which respond to small changes in local environment has been mastered by Nature. Within the human body, for instance, the pancreas reacts to modulate our blood sugar levels; we intrinsically respond to repair wounds and muscular reflex reactions help us to recognize pain. Inspired by this, synthetic stimuli-responsive or "smart" materials have increasingly found themselves at the forefront of scientific and technological innovation.[1] Such systems can undergo significant macroscopic changes in response to a wide range of stimuli, such as redox potentials, pH, and temperature, where aqueous polymer solutions can reversibly precipitate, often upon heating through a Lower Critical Solution Temperature (LCST).[2] This property has been applied to a number of areas such as for catalysis,[3] purification,[4] and to control cell culture and adhesion.[5] Moreover, the biological potential of these systems either for controlled release[6] or to modulate interactions with biological membranes[7,8] is obvious given the wide range of microenvironments found in the body. These environments can also be combined to produce increasingly complex materials eliciting multiple responses in a parallel or sequential manner.[9] To extend the utility of these systems, thermoresponsive polymers can also be manipulated without the need for a temperature change (i.e., isothermally) by using secondary triggers to alter the hydrophilic−hydrophobic balance. This has been achieved using stimuli such as redox potentials,[10,11] light,[12] pH,[13,14] and ionic strength.[15]

A lesser studied stimulus is that of host−guest complexation based on metal ion binding. Metal ions are critical in a range of biological functions and have also been implicated as biomarkers in certain disease states, providing a new target for responsive systems.[16] Chiper et al. have prepared terpyridine-terminated p(*N*-isopropylacylamide), pNIPAM, samples where the LCST is dependent on the addition of Zn^{2+} and Fe^{2+} to trigger metallo-supramolecular dimer formation.[17] Sharma and Srivatstava have used metal ions to cross-link thermosensitive polyaspartamides containing propylimidazole pendants, lowering the LCST through stabilization of the globular form.[18] Crown ethers have been used to modulate the LCST depending on the binding affinity of the metal ion employed,[19,20] and thermoresponsive ratiometric fluorescent indicators have been prepared for the detection of Cu^{2+}, Zn^{2+}, Pb^{2+}, and K^{+}.[21−23]

Another metal of biological interest is iron given its role in functions such as oxygen transport, metabolism, and conditions such as anemia, Alzheimer's, and Parkinson's.[24,25] Ferric iron (Fe^{3+}) predominates in aerobic conditions but, given its propensity to form poorly aqueous soluble compounds in these conditions ($\sim 10^{-18}$ M), requires proteins like transferrin or heme for efficient transportation.[26] Bacteria are also dependent on Fe^{3+} to survive where it is obtained through competitive abstraction from mammalian hosts using siderophores.[27] These low molecular weight compounds contain functional groups such as α-hydroxycarboxylates, hydroxamates, and catecholates capable of binding Fe^{3+} with association constants in excess of 10^{50}.[28,29] Enterobactin remains one of the best-understood siderophores, where three catecholate functionalities, linked to a triserine macrocycle, are used to

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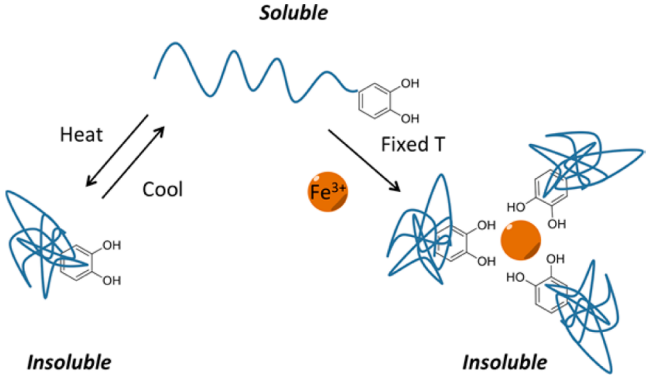
coordinate iron.[30] One of the few examples of polymers exhibiting a response to Fe^{3+} has been demonstrated by Yin and co-workers who prepared an isothermal, thermochromic sensor based on a copolymer containing porphyrin groups in the side-chain. This system exhibited thermochromic characteristics in the presence of the ion,[31] however, multiple side-chain interactions were required to achieve the observed response. Alternatively, polymer function can be elegantly manipulated using simple alterations in polymer end-group functionality. In this fashion, absolute control over a polymer property, such as solubility, can be maintained using only one or two functionalization events, rather than the multiple reactions required on a polymer side chain. This concept is also easily accessible given the development of controlled radical processes such as Reversible Addition−Fragmentation Chain Transfer (RAFT) polymerization, which afford high levels of control over polymer structure.[32] End-groups can therefore be installed with high degrees of fidelity and can be easily accessed based on the chain transfer agent used or through post-polymerization modification techniques.[33]

Despite this, reports pursuing this approach remain few and have focused on light,[34] bacterial binding,[35,36] pH[37] and redox environments as triggers.[38,39] Herein, we seek to exploit isothermal polymer technologies in combination with the biologically relevant catechol binding motif to prepare systems whereby the solubility can be controlled in response to the presence of Fe^{3+} as an alternative to temperature changes (Scheme 1).

Scheme 1. Isothermal Concept: An LCST and Polymer Conformational Change Can Be Induced through Catechol-Fe^{3+} Binding, Rather Than by a Temperature Change



Catechol-functional polymers were prepared using a chain-transfer agent containing a catechol unit as the R group. This was prepared following a simple two-step procedure from a carboxylic acid-terminated, trithiocarbonate starting material (Supporting Information).[40] *N*-Isopropylacrylamide (NIPAM) was then polymerized to give polymers of two different molecular weights, **pNIPAM-1** (M_n (SEC) = 14000 g·mol^{-1}, M_w/M_n = 1.12) and **pNIPAM-2** (M_n (SEC) = 5100 g·mol^{-1}, M_w/M_n = 1.09) (Supporting Information). The polymerization kinetics for **pNIPAM-1** are shown in Figure 1, reaching high conversion quickly (>70% after 1 h) and following first order kinetics. Good agreement between the theoretical and SEC molecular weights was observed.

The MALDI-ToF spectrum for **pNIPAM-2** (Figure 2) confirmed the successful installation of the desired polymer end-groups as sodium adducts, and was corroborated by ^{1}H

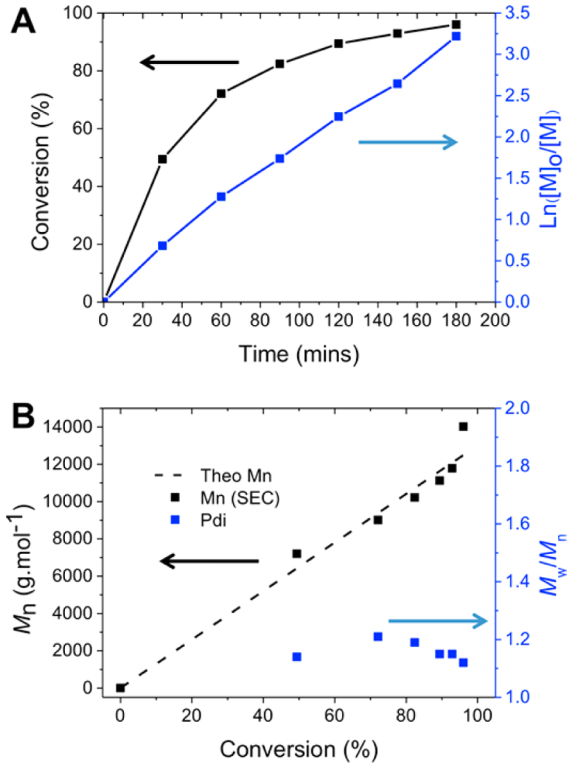


Figure 1. RAFT polymerization of **NIPAM-1**: (A)Time-dependent monomer conversion and pseudo first-order kinetics; (B) Evolution of M_n and M_w/M_n as a function of monomer conversion.

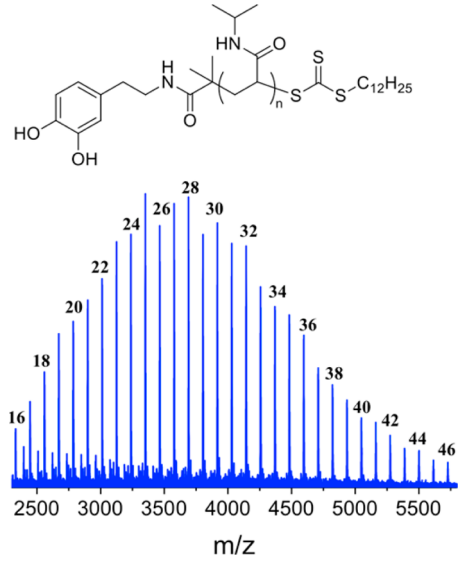


Figure 2. MALDI-ToF spectrum of **pNIPAM-2**. Each peak corresponds to a sodium adduct of *n*-repeat units (see Supporting Information for peak assignments).

NMR analysis (Supporting Information). A secondary distribution at low *m*/*z* related to sodium adducts of pNIPAM chains with no end-groups were presumably derived from the free radical initiation process.

PNIPAM-1 was dissolved at a concentration of 1 mg·mL^{-1} and the cloud point (the measurable property of the LCST) determined by turbidimetry to be 37.3 °C (Figure 3A). Next, the influence of Fe^{3+} on the polymer solution was investigated. The interaction between catechol and tribasic metal cations

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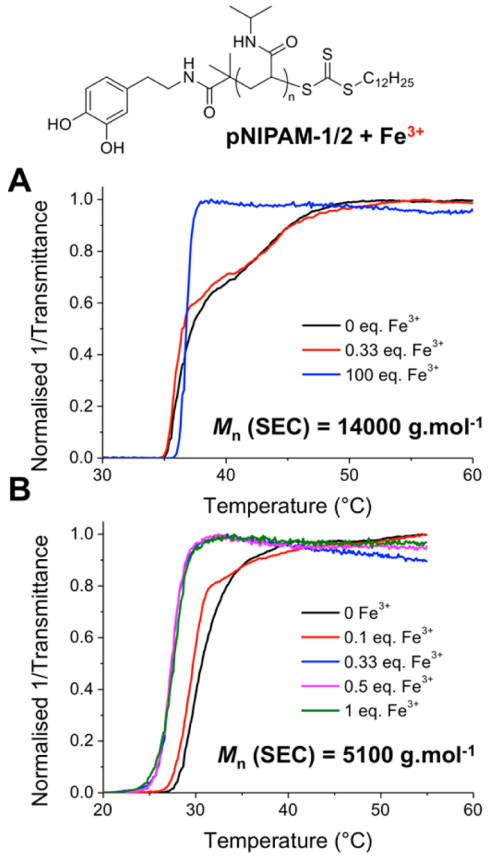

Figure 3. Cloud points and polymer structures of (A) **pNIPAM-1** and (B) **pNIPAM-2** with Fe^{3+}. Polymer concentration = 1 mg·mL^{-1}.

usually involves the displacement of six protons, hence, Fe^{3+} is widely accepted to bind in an octahedral geometry generated by three catechol functions.[27] The aqueous polymer solution was therefore doped with 0.33 equiv of $FeCl_3 \cdot 6H_2O$, giving a 3:1 ratio of catechol units/Fe^{3+} and the cloud point measured (Figure 3A). A slight decrease was observed (0.5 °C) that did not change further, even upon the introduction of a large amount (100 equiv) of the iron salt (Figure 3A). Interestingly, the shape of the turbidimetry curve in the latter case was noticeably sharper, potentially due to the extra water ordering afforded by the excess of salt in solution. To better assess whether the decrease described above was due to the desired binding motif and in a bid to promote a greater cloud point shift in the presence of iron, the shorter **pNIPAM-2** was next employed. This was selected given the effect of polymer end-group on the cloud point is generally observed to increase with decreasing molecular weight. This occurs as the end-groups contribute a higher percentage of the total structure and, hence, any effect is amplified.[41,42] The cloud point of this sample at 1 mg·mL^{-1} was determined to be 30.7 °C (Figure 3B), lower than that of **pNIPAM-1**, suggesting the large hydrophobic character of the catechol and dodecane end-groups outweighs the inversely proportional relationship previously reported between pNIPAM cloud point and molecular weight.[43,44] The addition of 0.1 equiv Fe^{3+} promoted a decrease in cloud point to 29.8 °C which was lowered further to 27.4 °C in the presence of 0.33 equiv Fe^{3+} (Figure 3B). This result provided strong evidence of catechol binding with the decreased cloud point potentially rationalized by either an increased ordering of the system afforded upon the formation of multimeric species bound to an Fe^{3+} core or due to an elevated local polymer concentration, as has been previously observed with branched polymers.[45] A final possibility is that if a supramolecular complex with three polymer chains is formed, then the effective molecular weight is increased, which should give a corresponding decrease in cloud point.[43] To directly probe the effect of the Fe^{3+}, ^{1}H diffusion-ordered NMR spectroscopy (DOSY) was used (Supporting Information). Upon addition of 0.33 equiv of Fe(III) there was a decrease in pNIPAM diffusion coefficient from 8.57 × 10^{-11} to 6.55 × 10^{-11} m^2·s^{-1}. The magnitude of this shift is consistent with previous reports for a 2−3-fold increase in molecular weight, suggesting that supramolecular association of multiple chains into larger branched architectures is occurring.[46] Interestingly, the addition of additional Fe^{3+} (up to 1 equiv) had no further effect on the cloud point. This suggests 0.33 equiv was sufficient to introduce maximum change and confirmed the decrease was not a simple "salting-out" effect.[47] Due to the acidic nature of the $FeCl_3$-containing solution, complete complexation to form a three-armed star-based structure is unlikely, which might imply a mixture of branched products are formed.[48] Nevertheless, binding was sufficient to promote the macroscopic changes discussed above.

To further probe the specificity of this response, **pNIPAM-2** was also investigated with $FeCl_2 \cdot 4H_2O$ (Figure 4A). The binding of catecholate-based siderophores to Fe^{2+} is signifi-

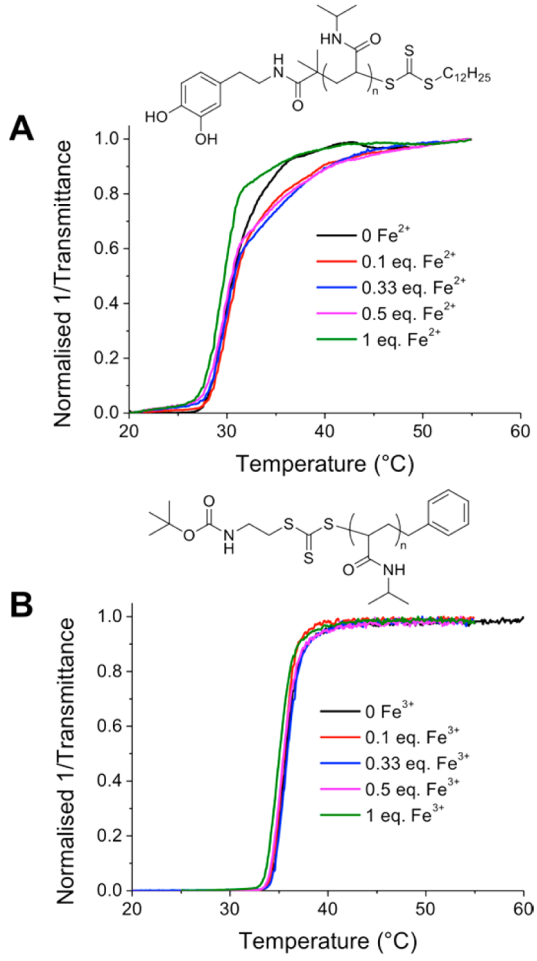

Figure 4. Cloud points and polymer structures of (A) **pNIPAM-2** with Fe^{2+} and (B) **pNIPAM-3** with Fe^{3+}. Polymer concentration = 1 mg·mL^{-1}.

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cantly weaker than that of Fe^{3+} due to the reduced charge density on the coordinated cation.[27] This is important, as one of the main known mechanisms for the release of iron from siderophores comprises the reduction of siderophpore-bound Fe^{3+} to Fe^{2+} followed by spontaneous release or competitive sequestration of this reduced species.[49] As anticipated, minimal change in cloud point at Fe^{2+} concentrations up to 0.5 equiv was observed. A slight decrease of 0.7 °C occurred in the presence of 1 equiv Fe^{2+}, which, given these measurements, is performed in aerobic conditions, may be the result of partial oxidation to Fe^{3+}. As a final control to confirm the role of the catechol functionality, a benzyl-terminated pNIPAM of similar molecular weight to that of pNIPAM-2 (**pNIPAM-3**, M_n (SEC) = 5500 g·mol^{-1}, M_w/M_n = 1.08, Supporting Information) was prepared. The cloud point of this polymer was determined (Figure 4B) as 35.6 °C, with minimal change noted at any Fe^{3+} concentration. Taken together, these controls highlight that the change in cloud point observed in Figure 3B was due to selective catechol-Fe^{3+} binding rather than through nonspecific interactions.

The main aim of this study was to be able to trigger an "isothermal" shift upon specific binding of the polymers to Fe^{3+}. If **pNIPAM-2** is considered, addition of 0.33 equiv of Fe^{3+} shifted the cloud point by 3.3 °C, providing a window for an isothermal transition. To verify this, the following experiment was devised. A 1 mg·mL^{-1} polymer solution was transferred to two multiwell plates and equilibrated at 25 °C. This was selected to be below the cloud point of the polymer alone but above that observed in the presence of 0.33 equiv of Fe^{3+} (Figure 3B). The absorbance at 650 nm was then monitored for 25 min to confirm no spontaneous polymer precipitation, concurrently demonstrating good aqueous stability. After this time, the iron salt was added to one well leading to a rapid increase in absorbance, indicative of precipitation by a decrease in LCST to below the external temperature. By comparison, no change was observed in the absence of iron (Figure 5).

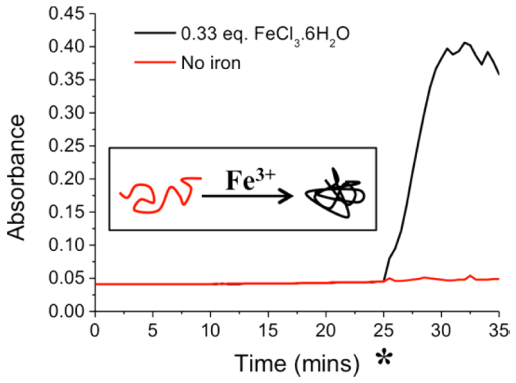

Figure 5. Isothermal turbidimetry data for **pNIPAM-2** (polymer concentration =1 mg·mL^{-1}). Temperature = 25 °C, $FeCl_3 \cdot 6H_2O$ added at the time indicated by an asterisk. Inset: Addition of Fe^{3+} triggers a coil-to-globule transition.

In conclusion, inspired by the action of siderophores, we have presented a method for controlling the cloud point of a polymeric system exploiting, for the first time, the powerful catechol-Fe^{3+} binding motif. This was achieved using an elegant, single chain-end binding event, with the effect amplified in lower molecular weight polymers. The addition of Fe^{3+} stimulated a decrease in transition temperature with maximum change observed with a catechol/Fe^{3+} ratio of 3:1. This phenomenon, not noted in the presence of Fe^{2+}, was subsequently exploited to promote isothermal polymer precipitation. Careful tuning of this temperature through judicial selection of polymer, molecular weight, and concentration is currently under way. A promising application of this system may lie in selective cell uptake given the pre-existing precedent for hydrophobic polymers to readily cross biological barriers.[7,8,50] Moreover, this trigger may facilitate the targeting of neurodegenerative disorders such as Alzheimer's and Parkinson's disease, where atypical iron concentrations are characteristic.[25]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional characterization and experimental 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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