Recognition and Encapsulation of an Electroactive Guest within a Dynamically Folded Polymer

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Abstract: Diaminotriazine-functionalized polymer **1** folds in nonpolar media into unimolecular "micelles", in which the diaminotriazine units are inwardly directed to form a polar pocket analogous to the active sites found in proteins. To explore the possibility of site isolation within this pocket we synthesized the redox active guest 6-ferrocenyluracil **2**. Encapsulation of guest **2** by polymer **1** was established by NMR studies; functional site isolation was demonstrated through cyclic voltammetry studies.

Site isolation of redox centers is a functional requirement for metalloenzyme activity.[1] In biomolecular systems, the dynamically assembled polypeptide scaffolding isolates electroactive centers from unwanted interactions and actively controls redox processes. Site isolation is also a powerful tool in materials chemistry, where encapsulation of redox-active centers has been used to create data storage units,[2] sensors,[3] catalysts,[4] and magnets.[5]

In recent synthetic systems,[6] rigid dendrimer cages[7] have been used to sequester electroactive species. These complex polymeric systems mimic the site isolation associated with metalloenzymes through covalent assembly of three-dimensional macromolecular structure around redox-active cores.[8,9] This mode of assembly, however, is fundamentally different from that of biological systems where noncovalent assembly is used to control encapsulation and micelles.[10] The dynamic attributes inherent in biomolecular systems make this mode of encapsulation an attractive proposition for the creation of sensors, catalysts, and other devices by noncovalent assembly.

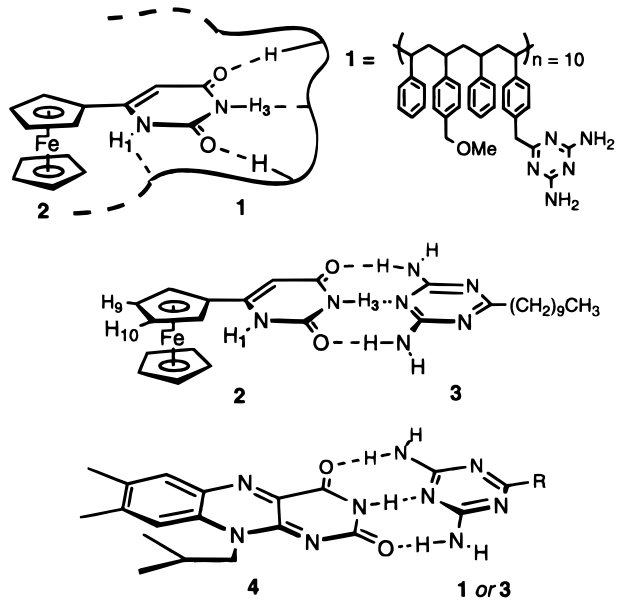

Figure 1. Schematic showing the binding processes for **2·1** and **4·1** polymer systems and **2·3** and **4·3** monomer systems. Polymer **1** is a random atactic copolymer, with M_w of 6000 g mol^{-1} containing the functionality and stoichiometry shown.

Recently, we have created biologically inspired polymer **1** (Figure 1), where three-dimensional structure is provided by hydrogen bond-mediated folding.[11] Using gel permeation chromatography and IR spectroscopy, we established that polymer **1** folds into a compact "inverse micellar"[12] structure, with the triazine moieties converging to form a polar core. This

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engineered arrangement of converging functionality provides a potential means for encapsulation of guests through specific noncovalent interactions. We report here the application of this strategy to the structural and functional site isolation of electroactive guest **2** within the core of globular polymer **1**.

Experimental Section

Materials. Ferrocene, ethyl malonyl chloride, aluminum chloride, thiourea, and chloroacetic acid were purchased from Aldrich and used as received. Ethyl 3-ferrocenyl-3-oxopropionate (**5**) was prepared according to the method of Rinehart et al.[13] All solvents were obtained from VWR, and were Reagent grade. Dichloromethane was dried over CaH_2 prior to use. Thin-layer chromatography (TLC) and column chromatography were carried out on glass precoated TLC plates with silica gel 60 and silica gel 60 (230−400 mesh), respectively. All ^{1}H NMR spectra (200 MHz) were recorded using $CDCl_3$ and d_6-DMSO as solvent. IR spectra were recorded on KBr pellets. The melting points presented are uncorrected.

6-Ferrocenyl-2-thiocarbonyluracil (6). A 50 mL two-necked RB flask, fitted with a reflux condenser, was purged with Ar. Na metal (0.077 g, 3.3 mmol) was added to the flask and subsequently dissolved by adding 6 mL of absolute ethanol. This was followed by addition of ferrocene ketoester **5** (0.45 g, 1.5 mmol) and thiourea (0.190 g, 2.5 mmol), and the mixture was heated to reflux for 12 h. After the mixture was cooled to room temperature, dilute HCl was added until the solution was slightly acidic (pH paper), and the ethanol was removed in vacuo. The reaction mixture was then filtered, washed with distilled H_2O, and dried to provide **6** as an orange crystalline solid in 89% yield (0.415 g). The product was used without further purification. Mp = 220 °C dec. ^{1}H NMR (200 MHz, d_6-DMSO): δ 4.21 (s, 5H, Cp_1), 4.54 (s, 2H, Cp_2), 5.20 (s, 2H, Cp_2), 6.10 (s, 1H, CH), 11.86 (s, 1H, N_3−H), 12.32 (s, 1H, N_1−H). IR (KBR): ν (cm^{-1}) 3097, 3005, 2925, 2855, 1647, 1608, 1539, 1157.

6-Ferrocenyluracil (2). Into a 50 mL RB flask was placed thiouracil derivative **5** (0.40 g, 1.3 mmol), chloroacetic acid (0.242 g, 2.5 mmol), and 6 mL of H_2O. A condenser was added and the system was refluxed for 6 h. HCl (4 mL, 2 M) was then added, and the solution was refluxed overnight. After the mixture was cooled, the solid was filtered, washed with H_2O, and dried to afford **2** as an orange solid in 98% yield (0.38 g), which could be further purified by recrystallization from MeOH/EtOH. Mp = 247 °C dec. ^{1}H NMR (200 MHz, d_6-DMSO): δ 4.20 (s, 5H, Cp_1), 4.51 (t, J = 1.7 Hz, 2H, Cp_2), 5.07 (t, J = 1.4 Hz, 2H, Cp_2), 5.75 (s, 1H, CH), 10.66 (s, 1H, N_1−H), 10.94 (s, 1H, N_3−H). IR (KBR): ν (cm^{-1}) 3163, 3100, 3007, 2922, 1700, 1654, 1430. HRMS (FAB^+) *m/e* calcd for $C_{14}H_{12}N_2O_2Fe$ (M^+) 296.1024, found 296.0242. Anal. Calcd for $C_{14}H_{12}FeN_2O_2$: C, 56.79; H, 4.08; N, 9.46. Found: C, 56.48; H, 4.19; N, 9.25.

Electrochemical Studies. Investigations were performed on a Cypress Systems Potentiostat. CH_2Cl_2 was dried over CaH_2 prior to use. Tetrabutylammonium perchlorate (TBAP), obtained from Acros chemicals, was used as electrolyte. The electrochemical measurements were performed under an Ar atmosphere.

Results and Discussion

To provide a redox-active guest specifically engineered for encapsulation, we synthesized 6-ferrocenyluracil **2** (Scheme 1).[14] Guest **2** possesses diverging donor−acceptor−donor−acceptor (DADA) functionality complementary to the converging triazines[15] in the core of polymer **1**. Target **2** was synthesized starting with the Friedel−Crafts acylation of ferrocene with ethyl malonyl chloride to provide the ketoester **5**. Cyclization with thiourea under basic conditions provided the thiouracil **6**, which was hydrolyzed under acidic conditions to provide ferrocenyluracil **2** (41% yield from ferrocene).[16]

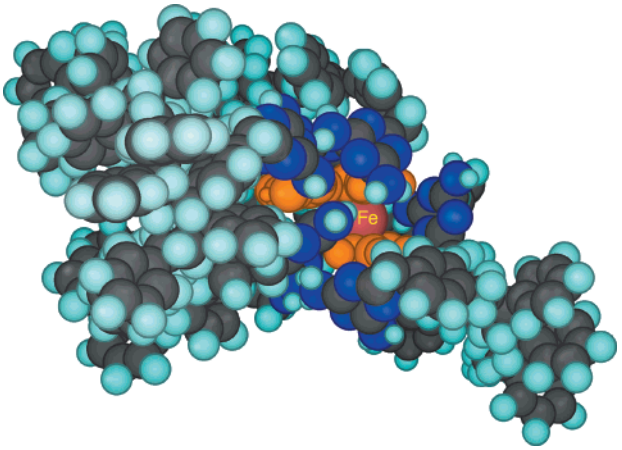
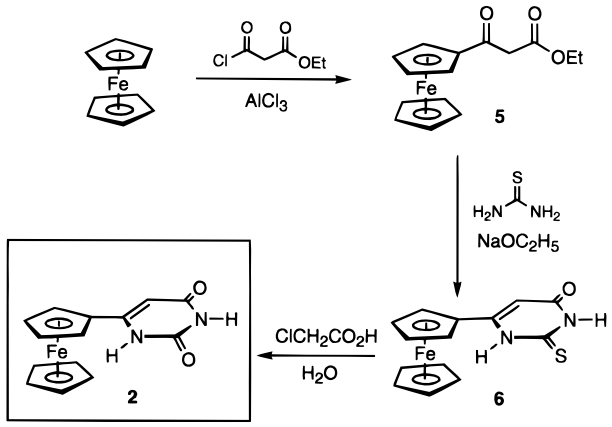


Figure 2. Sampled structure from a final 20 ps dynamics simulation (300 K, Amber force field, $CHCl_3$) predicting encapsulation of guest **2** (in orange) by atactic polystyrene (40 monomer units total) with methylene−diaminotriazine substitution at every fourth carbon.

Scheme 1. Synthesis of 6-Ferrocenyluracil **2**



Extensive molecular dynamics simulations of guest **2** with a model triazine-functionalized polymer system (Figure 2)[17] predict internalization of the ferrocene moiety into the polar polymer core (Figure 1). From the predicted structure, it is apparent that the internalization is driven by both hydrogen bonding and π-stacking interactions between polymer **1** and guest **2**.

Proton NMR investigations were performed to demonstrate the encapsulation of guest **2** within the polar core of polymer **1**. These studies compared the interactions of **2** with polymer **1** and the monomeric control receptor **3**, which is incapable of encapsulation. During these experiments, distinct differences

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(17) Calculations were performed in vacuo using the Amber* force field, as implemented by Macromodel. Macromodel 4.0: Mohamadi, F.; Richards N.; Guida, W.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. *J. Comput. Chem.* **1990**, *11*, 440−448. A rigid structure was used for ferrocenyluracil **2**, and molecular motion of the polymer was limited to dihedral angle rotations. Dynamics simulations were run in vacuo at 700, 500, and 300 K for 20 ps at each temperature. These runs were followed by continuum solvent runs ($CHCl_3$) of 10 ps (300 K) followed by the final 20 ps (300 K) simulation shown in Figure 1. For a description of the solvation method used, see: Still, W.; Tempczyk, A.; Hawley, R.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.

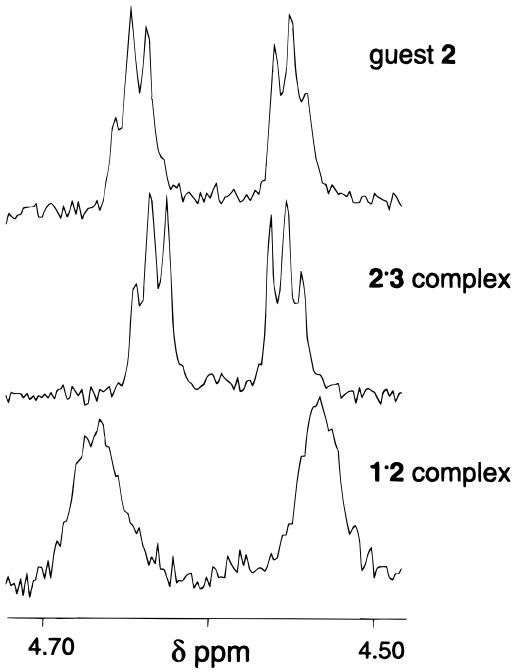

Figure 3. NMR spectra of ferrocene protons of the monosubstituted ring of **2** in $CDCl_3$ at 298 K: (a) guest **2** ([**2**] = 10^{-3} M); (b) **2**−monomer **3** complex ([**2**] = 8.7×10^{-4} M, [**3**] = 1.3×10^{-3} M); (c) **2**−polymer **1** complex ([**2**] = 1×10^{-3} M, [**1**] = 1×10^{-3} M, based on triazine equivalents).

between the two systems indicative of guest encapsulation in the **1·2** complex were observed: (1) All peaks arising from guest **2** were broadened in the presence of polymer **1** (Figure 3). This was not seen upon addition of monomer **3**, indicating that line broadening observed in the **1·2** complex arises from coupling of the guest motion with that of the polymer.[18] (2) In the presence of control **3**, H(3) of guest **2** moved downfield, while H(1) moved upfield, indicative of a lack of hydrogen bonding to H(1). In contrast, addition of polymer **1** resulted in strong downfield chemical shifts of *both* H(1)- and H(3)-peaks of **2**, confirming the presence hydrogen bonding at both of these positions. Given the divergent geometry of H(1) and H(3), convergence of the complementary triazines of polymer **1** is required for this effect. (3) No change was observed in the chemical shifts of the H(9)/H(10)-peaks of guest **2** upon addition of control receptor **3**. In contrast, addition of polymer **1** resulted in an upfield shift of these protons (Figure 3), diagnostic of edge-to-face/CH−π interaction of these protons with the phenyl rings of polystyrene-based polymer **1**.[19]

Perhaps the most revealing piece of data is afforded by the constant host titration for the guest **2**/triazine **1** system.[20] By monitoring the downfield shift of the H(9)/H(10)-protons of **2**, an association constant of 487 ± 52 M^{-1} was attained.[21] This figure corresponds to an over 13-fold enhancement in binding relative to that obtained for the flavin **4**/polymer **1** system recently studied (36 ± 3 M^{-1}).[11] Since the flavin **4** recognition surface features a 3-point DAD system (Figure 1), this observation indicates that the additional N(1)−H of **2** is intimately involved in H-bonding to the diaminotriazine moieties. For this

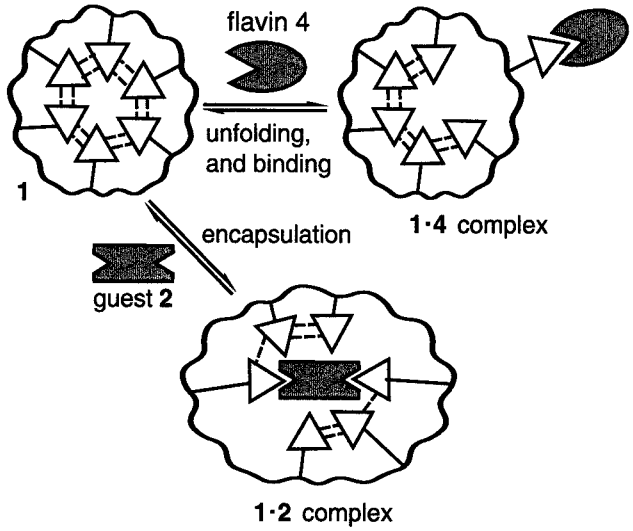

Figure 4. Illustration showing the difference in macroscopic conformational reorganization of polymer **1** during encapsulation and binding processes for the **2·1** and **4·1** systems, respectively. During encapsulation of guest **2**, *intra*molecular H-bonds of **1** are disrupted and are replaced by *inter*molecular H-bonds within the **2·1** complex.

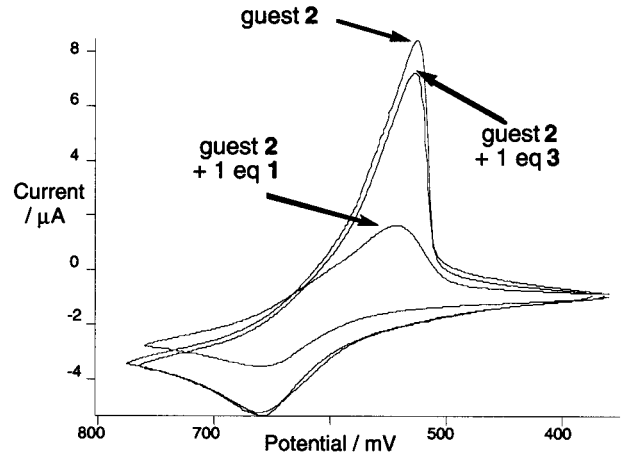

Figure 5. Voltammograms for **2**, the **2·1** polymer system, and the **2·3** monomer system. CH_2Cl_2 solvent, 0.1 M TBAP electrolyte, 296 K, 85 mV/s scan rate. [**2**] = [**3**] = 7.5×10^{-4} M. [**1**] = 7.5×10^{-4} M based on triazine equivalents.

to occur, receptor **2** must be encapsulated within a multi-point H-bonding environment. Consequently, a subtle but important feature manifests itself in the polymer **1**/receptor **2** system: the diverged N(1)−H proton of **2** induces a macroscopic conformational reorganization of polymer **1**. This "wrapping around" or encapsulation of receptor **2** by polymer **1** (Figure 4) differs from the interaction observed with the flavin **4**/polymer **1** system, in which it was established that the triazine-based polymer **1** unfolds a single recognition unit upon binding. The ensuing encapsulation of guest **2** by polymer **1** ensures maximized favorable contacts between host and guest, similar to the multidentate binding observed in biological systems.[22,23]

Functional demonstration of site isolation in the guest **2**−polymer **1** complex was obtained using cyclic voltammetry.

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(19) (a) Moriuchi, T.; Ikeda, I.; Hirao, T. *Organometallics* **1995**, *14*, 3578−3580. (b) Adams, H.; Carver, F. J.; Hunter, C. A.; Morales, J. C.; Seward, E. M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1542−1544.

(20) Equilibrium is rapidly established in the guest **2**/triazine **1** system; the on/off rate is faster than the NMR time scale.

(21) On the basis of triazine equivalents.

(22) For an excellent review on polyvalent interactions in biology, see: Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754−2794.

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The voltammogram of ferrocene derivative **2** (Figure 5) displays a sharp reduction peak arising from precipitation of the charged, oxidized ferrocenium species.[24] Addition of 1 equiv of monomer **3** to the solution of **2** had minimal affect on the electrochemical behavior, with only a slight decrease in the peak current of the reduction couple observed. In contrast, when an equimolar quantity of polymer **1** was added to a solution of **2**, the voltammogram of **2** became almost completely reversible. This demonstrates that encapsulation of guest **2** within polymer **1** effectively prevents aggregation of the oxidized species.[25,26]

In summary, we have demonstrated through NMR and electrochemical studies the site-isolation of electroactive target **2** using dynamically self-assembled polymeric receptor **1**. This provides a biomimetic approach to encapsulation, an approach we are currently applying to the development of biological models, drug delivery, and device-oriented systems.

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Supporting Information Available: ^{1}H NMR, IR, and mass spectra for **2**, ^{1}H NMR and IR spectra for **6**, and a NMR titration curve for the **1**/**2** system (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) This precipitation effect has been observed previously for a cobaltocene-based dendrimer/cyclodextrin system. González, B.; Casado, C. M.; Alonso, B.; Cuadrado, I.; Morán, M.; Wang, Y.; Kaifer, A. *Chem. Commun.* **1998**, 2569−2570.

(25) At the concentrations used for these electrochemical studies, approximately 25% of guest **2** is encapsulated, based on expected association constants. As shown by the electrochemical experiments, this 25% reduction in free guest **2** is sufficient to prevent aggregation and concomitant precipitation.

(26) In addition to preventing aggregation of oxidized target **2**, addition of polymer **1** caused a dramatic decrease in peak current, and an increase in peak-to-peak for guest **2**. This is a well-established behavioral pattern for encapsulated redox centers, resulting from inefficient electron transfer due to physical separation of the electroactive species from the electrode surface.