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An Ionic Liquid Form of DNA: Redox-Active Molten Salts of Nucleic Acids

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Abstract: Ionic liquids are described that contain duplex DNA as the anion and polyether-decorated transition metal complexes based on $M(MePEG\text{-}bpy)_3^{2+}$ as the cation (M = Fe, Co; MePEG-bpy = 4,4′-$(CH_3(OCH_2CH_2)_7OCO)_2$-2,2′-bipyridine). When the undiluted liquid DNA—or molten salt—is interrogated electrochemically by a microelectrode, the molten salts exhibit cyclic voltammograms due to the physical diffusion (D_{PHYS}) of the polyether−transition metal complex. When M = Co(II), the cyclic voltammogram of the melt shows an oxidative wave due to the Co(III/II) couple at $E_{1/2} = 0.40$ V (versus Ag/AgCl) and a D_{PHYS} of 6×10^{-12} cm^2/s, which is significantly lower than that for $Co(MePEG\text{-}bpy)_3(ClO_4)_2$ ($D_{PHYS} = 2.6 \times 10^{-10}$ cm^2/s) due to greater viscosity provoked by the DNA polymer. When a 1:1 mixture is made of the $Co(MePEG\text{-}bpy)_3\cdot$DNA and $Fe(MePEG\text{-}bpy)_3(ClO_4)_2$ melts, two redox waves are observed. The first is due to the Co(III/II) couple, and the second is a catalytic wave due to oxidation of guanine in DNA by electrogenerated Fe(III) in the undiluted melt. Independent experiments show that the Fe(III) form of the complex selectively oxidizes guanine in duplex DNA. These DNA molten salts constitute a new class of materials whose properties can be controlled by nucleic acid sequence and that can be interrogated in undiluted form on microelectrode arrays.

The ability of nucleic acids to store and transfer information through Watson−Crick base pairing has intriguing parallels with microelectronic circuitry.[1−3] Binding of complementary DNA sequences has been suggested as a method for forming connections between circuit elements,[3−5] directing assembly of nanoscale structures,[6,7] and computing.[1,8] In addition to providing a molecular self-assembly platform, DNA can also provide specific electronic signals through electron-transfer reactions of the guanine nucleobase.[9−11] Herein, we report on novel ionic liquid materials based on DNA salts of polyether-decorated transition metal complexes (Figure 1) that, in undiluted form, undergo electrochemical reactions and act as catalytic electron relays between a microelectrode and DNA. The DNA remains double-stranded in the ionic liquids, and the electrochemistry is performed under vacuum. The semisolid-state nature of the new DNA materials—which can be regarded as molten salts—may be useful in microelectronic circuits that utilize DNA for *both* self-assembly and electronic connections. Further, the ability to control DNA sequence and secondary structure will allow creation of new classes of molten materials that undergo well-defined structural changes programmed by sequence and monitored by electrochemical signals.

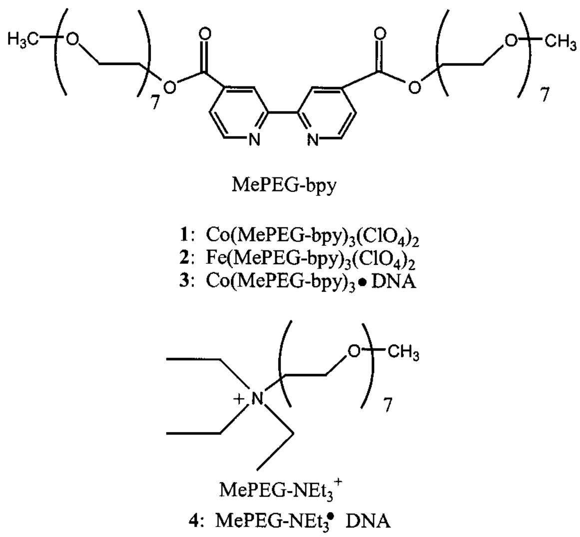

Figure 1. Structures of the hybrid redox polyether melts. In the melt formed between $Co(MePEG\text{-}bpy)_3^{2+}$ and DNA (**3**), there is one Co complex for each base pair of the DNA. In the melt formed with the $MePEG\text{-}NEt_3^+$ cation (**4**), there are two ammonium cations per base pair. Elemental analysis of the DNA melts shows that the composition is >90% of the indicated cation−DNA pair with <10% of the perchlorate or chloride salt of the polyether cation. Very little sodium ion was detected in either **3** or **4**, showing that the polyether cation replaced all of the DNA cations during the dialysis.

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Experimental Section

Materials and Measurements. Compounds **1** and **2** were prepared according to a published procedure[12] and characterized by NMR and electrochemistry. The MePEG-NEt$_3$Cl salt was prepared according to a published procedure.[13] Electrochemical measurements were conducted under vacuum at 67 °C on 3-electrode arrays with a 3.9 μm radius Pt working electrode as previously described.[12] CD spectra were acquired on an Aviv Model 62DS circular dichroism spectrometer with a 0.1 cm cell. Values of D_{PHYS} were determined from chronoamperometry data plotted as current versus $t^{-1/2}$; and linear fits were used to determine D_{PHYS} as described previously.[12] The bulk concentration of the Co complex was 0.4 M in **3** and 0.2 M in the 1:1 **3**/**2** melt.

Preparation of DNA Ionic Liquids. Herring testes DNA was obtained from Sigma and sheared to a size range of 50−100 bp as determined by agarose gel electrophoresis. To form compound **3**, 41.0 mL of 49 mM sheared HT DNA (2.0 mmol) was added to 1.0 mmol of **1**. The solution was diluted to 300 mL and added to Millipore brand 500 MWCO dialysis tubing that had been soaked twice for 30 min in Nanopure water. Dialysis was executed for 1 week during which the 8-L reservoir was replaced every 12 h. Following dialysis, the solution was removed from the dialysis tubing, and the water was removed by rotary evaporation at ambient temperature. The resulting melt was rinsed repeatedly with Nanopure water. Removal of water produces a viscous, transparent material that was further dried under vacuum. Elemental analysis gave P = 1.90%, Na = 0.04%, Cl = 0.77%, and Co = 1.90%. Complete conversion to Co(MePEG-bpy)$_3$·DNA would give P = 1.75%, Na = 0%, Cl = 0%, and Co = 1.66%. Compound **4** was prepared by the same method except that 2 mmol of MePEG-NEt$_3$Cl was used as was 100 MWCO dialysis tubing. Elemental analysis of **4** gave P = 4.63%, Na = 0.20%, and Cl = 0.87%. Complete conversion to MePEG-NEt$_3$·DNA would give P = 3.90%, Na = 0%, and Cl = 0%.

Gel Electrophoresis. Compound **2** was oxidized to the Fe(III) form by reaction with 1 equiv of Ce(ClO$_4$)$_4$ + 2HClO$_4$ in an acetonitrile/perchloric acid solution (GFS Chemicals, Powell, OH). Complete oxidation to the Fe(III) form was confirmed by absorbance spectroscopy. In the cleavage reactions, a 10-μL solution of 5′-^{32}P labeled oligonucleotide (5′-AAAAATATAGTATAAAAAA-3′) and 1 equiv of calf thymus DNA was mixed with 10 μL of either a 1 mM solution of oxidized **2**, **2** that had not been oxidized, or Ce(IV) alone. The reaction was allowed to proceed until the Fe(III) was converted to Fe(II) (the color of the solution changed from green to violet). The samples were ethanol precipitated, piperidine treated, and electrophoresed on a denaturing polyacrylamide gel according to a published procedure.[14] A single cleavage site was detected on the gel at the guanine nucleotide upon reaction with oxidized **2** (the site of reaction was determined by comparison with a Maxam−Gilbert G reaction). No cleavage was observed upon reaction with **2** that had not been oxidized or with Ce(IV) in the absence of **2**.

Results and Discussion

Room temperature melts are reliably formed when one partner in a cation/anion pair is decorated with poly(ethylene glycol) tails of appropriate length (Figure 1)[12,13] and are redox-active molten salts when one of the partners is also capable of electron transfer.[12,13] The polyether tail can be attached to either the redox-active partner or the redox-inert counterion.[13] For example, the perchlorate salt Co(MePEG-bpy)$_3$(ClO$_4$)$_2$ (**1**), containing a redox-active metal complex with polyether tails, is a molten material.[12] When this highly viscous, amorphous compound is placed on a three-electrode array containing a 3.9 μm radius Pt microelectrode, an electrochemical signal due to the Co(III/II) oxidation reaction (0.18 V, all potentials in the text are versus Ag/AgCl) is observed in the neat liquid (Figure 2A).

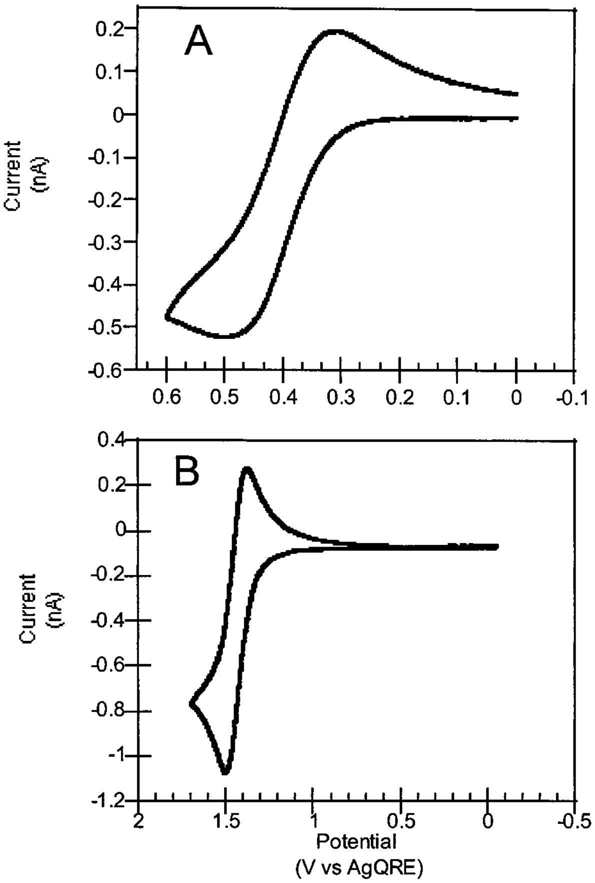

Figure 2. Cyclic voltammograms for (A) **1** and (B) **2** at 25 mV/s on the 3.9 μm Pt microelectrode with a silver quasireference and a Pt auxiliary electrode. Measurements were performed at 67 °C under vacuum using a home-built potentiostat capable of detecting 0.1 pA of current. The potentials in the figure are as-collected versus the silver quasireference, but those quoted in the text are versus aqueous Ag/AgCl after conversion with a ferrocene standard.

Diffusion-controlled currents in polyether melts reflect the summed rates of physical diffusion (D_{PHYS}) of the metal complex to the microelectrode and of electron hopping (self-exchange) between oxidized and reduced forms of the complex in the melt,[12] as embodied in the Dahms−Ruff equation:[15]

$$D_{\mathrm{APP}} = D_{\mathrm{PHYS}} + \frac{k_{\mathrm{EX}}\delta^2 C}{6} \tag{1}$$

where D_{APP} is the overall (apparent) diffusion coefficient, k_{EX} is the rate constant for self-exchange, and C and δ are the concentration and average center-to-center distances between the complexes in the melts, respectively. For the Co(III/II) wave observed in **1**, electron hopping is slow, and the observed current is solely a function of D_{PHYS}. The value of D_{PHYS} for **1** is 2.6 × 10^{-10} cm^2/s; such slow diffusion is typical of the polyether melts.[12] When Co is replaced with Fe to form Fe(MePEG-bpy)$_3$(ClO$_4$)$_2$ (**2**), the Fe(III/II) oxidation wave appears at 1.04 V and electron hopping is much faster (while D_{PHYS} remains constant), giving higher overall currents (Figure 2B). The larger k_{EX} for Fe compared to Co is a well-understood chemical difference.[12,16] We will show below analogous electrochemical behavior of

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metal complex molten salts containing DNA and exhibiting evidence of its electron-transfer chemistry.

One-electron oxidation of the guanine base has been extensively scrutinized as a partial origin of oxidative DNA damage[17,18] and as a probe of long-range electron transfer along the DNA helix.[9,11,19−22] Transient formation of oxidized guanine bases appears to play a critical role in the ability of DNA to mediate remote electron transfer between two attached circuit elements where those circuit elements can either be molecular donors and acceptors or electrodes.[4,11,22−24] While direct transfer of electrons from guanine to solid electrodes in fluid, homogeneous solutions is slow, guanine oxidation can be studied indirectly by electrochemically generating small molecule redox catalysts.[10,25] Transition metal complexes with potentials ≥1.0 V, such as $\mathrm{Ru(bpy)_3^{2+}}$ (bpy = 2,2′-bipyridine), mediate guanine electron transfer from intact DNA molecules according to the reaction scheme:[26−28]

$$\mathrm{Ru(bpy)_3}^{2+} \rightarrow \mathrm{Ru(bpy)_3}^{3+} + \mathrm{e}^- \qquad (2)$$

$$\mathrm{Ru(bpy)_3}^{3+} + \mathrm{DNA} \rightarrow \mathrm{Ru(bpy)_3}^{2+} + \mathrm{DNA_{ox}} \qquad (3)$$

where $\mathrm{DNA_{ox}}$ is a DNA molecule with a guanine residue that is oxidized by one electron. Precise potentials are difficult to determine in the melts, so complex **2** was dissolved in ether and determined to have a potential of 1.04 V by use of an internal ferrocene standard. Thus, complex **2** should be capable of oxidizing guanine in DNA in reactions analogous to eqs 2 and 3.

A DNA melt was prepared by equilibrium dialysis of a solution of **1** and herring testes DNA at a stoichiometry of two nucleotides of DNA per one dicationic Co complex. After extensive dialysis to remove the perchlorate counterions from **1** and the sodium cations from DNA, the solution was placed on a rotary evaporator to yield a highly viscous melt, $\mathrm{Co(MePEG\text{-}bpy)_3 \cdot DNA}$ (**3**). Compound **3** was dried under vacuum, and elemental analysis showed a purity of ≥90% with virtually no detectable sodium ion present, indicating that all of the DNA counterions had been replaced by $\mathrm{Co(MePEG\text{-}bpy)_3^{2+}}$. The remainder of the composition of **3** is made up of some perchlorate anions in place of DNA.

The cyclic voltammogram of **3** showed a characteristic oxidation wave for the Co(III/II) couple at $E_{1/2} = 0.40$ V (Figure 3A) with currents considerably lower than those for the perchlorate melt **1**. Chronoamperometry experiments were performed, and the collected current was plotted as a function of $t^{-1/2}$. These plots were linear, and the slope was used along with the Cottrell equation as described previously[12] to give a D_{PHYS} for the complex in the DNA melt of 6×10^{-12} cm^2/s. The peak current in cyclic voltammograms was also a linear function of the square root of the scan rate and gave a D_{PHYS} of 5×10^{-12} cm^2/s. A smaller D_{PHYS} in the DNA melt (compared to the perchlorate melt) is not surprising, since the polymeric counterion provokes a qualitatively higher viscosity and the relatively rigid helices should impede transport of the metal complex to the electrode.

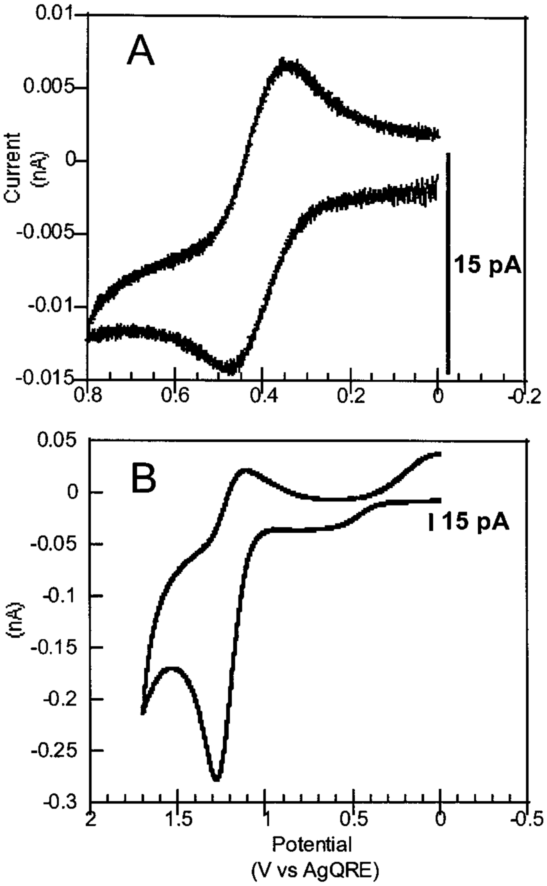

Figure 3. Cyclic voltammograms of (A) **3** and (B) a 1:1 mixture of **2** and **3**. Conditions were the same as in Figure 2.

The DNA in the melts is double-stranded. Compound **3** was dissolved in water, and the DNA fragments were separated on an agarose gel. The DNA fragments on the gel were 50−100 bp in length and stained well with ethidium bromide. Since heterogeneous polynucleotides such as herring testes DNA cannot reanneal, this experiment shows that the DNA in the melt was double-stranded. To confirm this point, another melt was prepared using a polyether-decorated ammonium cation, which formed a 1:1 melt with DNA nucleotides, $\mathrm{MePEG\text{-}NEt_3 \cdot DNA}$ (Figure 1, **4**). Compound **4** was characterized by elemental analysis and NMR and was found to give a CD spectrum in water that was identical with that for native herring testes DNA (Figure 4). Compound **3** could not be characterized by CD spectroscopy because the absorptions for the metal complex interfered with the DNA signals. This experiment further demonstrates that the DNA molecules in the melt remain double-stranded. Because **4** contains a countercation that does not exhibit optical absorption in the 250−300 nm range, the melt could be placed between glass plates and scanned in an optical spectrometer. The absorbance spectrum of the neat DNA melt is similar to that of DNA in solution. A pictorial representation

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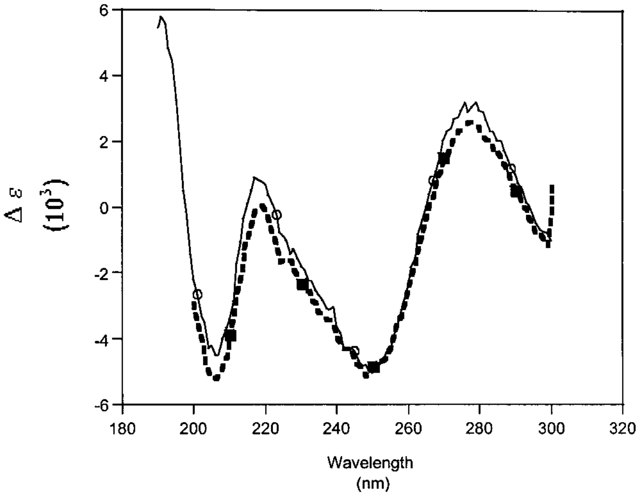

Figure 4. CD spectra of 500 μM herring testes DNA (dashed) and **4** (solid). Concentrations are in nucleotide phosphate.

of **3** showing the relative sizes of the polyether tails and DNA is shown in Figure 5.

The only electrochemical signal seen in melt **3** is that due to the Co(III/II) couple. Co(III) is not a sufficiently powerful oxidant to oxidize guanine. However, when a 1:1 mixture was made of **3** and the iron perchlorate melt **2**, two electrochemical signals were observed (Figure 3B). The first was due to the Co(III/II) wave, while the more positive wave for Fe(III/II) displayed an oxidation current much larger than the subsequent reductive current, which is characteristic of mediated electrocatalysis.[29] Faster potential scanning gave a more symmetrical Fe(III/II) wave, and repeated scanning led to smaller oxidation currents on successive scans; both of these features are indicative of electrocatalytic oxidation of guanine.[26,30,31] This behavior is ascribed to catalytic oxidation of guanine in DNA by the electrogenerated Fe(III) in the melt. The Co(III/II) couple was used in choronoamperometry experiments to determine a value of D_{PHYS} for the 1:1 **3**/**2** melt of 6×10^{-11} cm^2/s, which is higher than in compound **3** probably because of the higher ethylene oxide/DNA ratio.

To verify that guanines were the source of the electrons that gave the catalytic current in Figure 3B, we performed an experiment in which the tailed Fe(III) complex was reacted with a radiolabeled oligonucleotide containing a single guanine. This reaction was performed by oxidizing the Fe(II) complex with Ce^{4+} and mixing the resulting Fe(III) form with DNA in dilute aqueous solution. The DNA showed selective cleavage at the guanine residue following piperidine treatment and high-resolution gel electrophoresis. In addition, cyclic voltammetry of both **3** and **4** in the absence of Fe showed no faradaic current at potentials up to 1.5 V, showing that direct oxidation of guanine at the microelectrode does not occur at a detectable rate. We therefore ascribe the catalytic current observed in Figure 3B to oxidation of guanine by Fe(III) *in the neat DNA molten salt.*

Conclusions

The use of polyether-tailed cations to prepare ionic liquid forms of DNA raises many exciting possibilities. Tailed quaternary ammonium salts of DNA have been used to prepare aligned DNA films,[32] but these materials are not expected to possess favorable electrochemical characteristics because the tail contains only four polyether units with a C_{10} aliphatic unit on the end. The DNA materials **3** and **4** have long polyether tails terminated with only a methyl group, which facilitates ion transport and an ability to interrogate the neat liquid **3** electrochemically. Since the rate of electron hopping for Co(III/II) is slow, the currents in Figure 3 can be confidently assigned as arising from slow, physical diffusion of the Co complex in the melt. These experiments are carried out on dried materials under vacuum. The melts also show solubility in a wide range of fluid solvents, and both the dissolved forms and the neat melts are amenable to spectroscopic analysis. Recovery of the DNA from the melts and subsequent analysis shows that it is double-stranded. This new ionic phase of DNA is readily manipulated, and the cast films can be deposited onto microelectrode arrays, placed under vacuum, and still exhibit chemical reactions normally observed in dilute solution.

In these melts doped with the Fe complex, the polyether–metal complex serves a dual role: to provide a "solvent" in

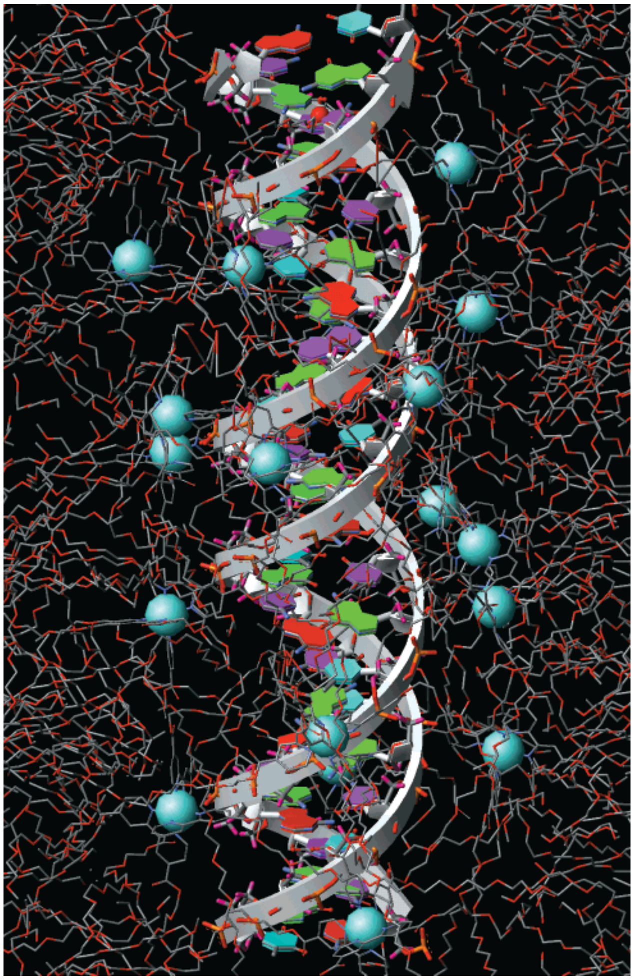

Figure 5. Model showing the structure of **3** with the tailed metal complexes and the DNA molecule rendered to scale. The DNA is shown in a cartoon form and the metal complexes are shown with the metal atoms as blue spheres and the MePEG ligands in CPK colors. The polyether chains provide considerable "solvent" for the DNA molecule and diffusion of the metal ions to the electrode. Note that, for clarity, the drawing shows fewer than the actual 1 metal ion per base pair present in the melt. The model was generated using the Cerius2 software package from MSI.

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which diffusive electron transfers can occur and to mediate electron transfer from guanine to the electrode. Numerous pathways for electron transfer exist in the melts, because electrons can transfer from the guanine to the metal, from guanine to an oxidized guanine, and from metal to metal. Guanine–guanine electron transfers may occur within single DNA molecules; however, because of the relatively high concentration of Fe in the melt and its ability to carry charge by electron hopping in the polyether medium, efficient electron transfer within DNA is probably not required. In the future, we believe that these processible ionic liquid materials will provide a simple basis for microstructures that utilize DNA as both a self-assembly partner and, when electron-transfer active polyethers such as **3** are used, specific electronic signals. In addition, sequence control of the DNA combined with the maintenance of the duplex structure in the melts provides pathways to new materials where the nucleic acid structure produces designed properties of the polymer melts. The electron-transfer reactivity provides a means to study these properties on small quantities of undiluted material.

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