THE DESIGN, SYNTHESIS AND REDOX PROPERTIES OF METALLATED ARTIFICIAL OLIGOPEPTIDES

A Thesis in
Chemistry
by
Kristi Ohr

© 2007 Kristi Ohr

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2007
The thesis of Kristi Ohr was reviewed and approved* by the following:

Mary Elizabeth Williams  
Assistant Professor of Chemistry  
Thesis Advisor  
Chair of Committee

Thomas E. Mallouk  
DuPont Professor of Materials Chemistry and Physics

Mark Maroncelli  
Professor of Chemistry

Theresa S. Mayer  
Professor of Electrical Engineering

Ayusman Sen  
Professor of Chemistry  
Head of the Department of Chemistry

*Signatures are on file in the Graduate School
ABSTRACT

This work details investigations of ligand-substituted artificial oligopeptides that form well-ordered supramolecular structures upon metal complexation. The structures are tailorable based on choice of ligand, metal and sequence, and the arrangement and spacing between the metal centers can be controlled. These unique materials provide a means of controlling and directing charge transport and are important candidates for the assembly of molecular circuits.

Chapter two presents the synthesis and characterization of pyridine-substituted artificial oligopeptides of varying length. UV-Visible absorption spectrophotometric titrations of the oligopeptides with Cu(II) and Pt(II) complexes of 2,2’:6’,2”-terpyridine and pyridine 2,6-dicarboxylic acid show stoichiometric binding based on the number of pyridines per peptide strand. Cyclic voltammetry of [Pt(terpyridine)]^{2+} containing oligopeptides reveal two sequential one-electron reductions at formal potentials independent of oligopeptide length. The diffusion coefficients decrease linearly with increasing oligopeptide length.

Chapter three presents the synthesis and characterization of metallated oligopeptide duplex assemblies composed of phenyl-terpyridine ligands cross-linked by Co(II) and Fe(II) ions. Cyclic voltammograms are consistent with one-electron oxidative reactions without strong coupling between the metal complexes. The diffusion coefficients decrease linearly with increasing oligopeptide length, suggesting the primary products are metal-linked oligopeptide duplex assemblies. Larger metallated
oligopeptides yield irreversibly adsorbed electroactive films during cyclic voltammetry which are chemically reversible but kinetically quasi-reversible.

Chapter four presents the synthesis of metal-crosslinked oligopeptide duplexes containing Co(II) and Fe(II), functionalized with cysteine and adsorbed to Au electrode surfaces. Cyclic voltammetry of mixed monolayers containing metallated oligopeptide and propanethiol reveals facile electron transport in Fe(III/II) and Co(II/I), but a decrease in conductivity of the Co(III/II) reaction, as the length of the oligopeptide increases, suggesting that these structures behave as molecular wires.

Chapter five presents electrochemical investigations of the rates of electron transfer in the SAMs described in chapter four. Rates are measured by Laviron analysis of cyclic voltammograms and AC impedance spectroscopy and in all cases are larger than $10^4 \text{ s}^{-1}$. Values for the transfer coefficients of the films show a dependence on oligopeptide length for the Co (III/II) couple, but not for the Co(II/I) or Fe(III/II).
# TABLE OF CONTENTS

LIST OF FIGURES......................................................................................................................vi

LIST OF TABLES ........................................................................................................................xi

LIST OF ABBREVIATIONS ......................................................................................................xii

Chapter 1  Introduction ............................................................................................................. 1

  1.1 Introduction......................................................................................................................... 1
  1.2 DNA ................................................................................................................................... 4
  1.3 Modified DNA ..................................................................................................................... 5
  1.4 Modified PNA ...................................................................................................................... 7
  1.5 iDNA .................................................................................................................................. 8
      1.5.1 Electron Transfer Properties of iDNA ........................................................................ 11
      1.5.2 Measuring Charge Transfer ...................................................................................... 15
      1.5.3 Summary of the Current Work ................................................................................... 19
  1.6 References.......................................................................................................................... 22

Chapter 2  Pyridine-Substituted Oligopeptides as Scaffolds for the Assembly of
Multimetallic Complexes: Variation of Chain Length ............................................................... 26

  2.1 Introduction........................................................................................................................... 26
  2.2 Experimental ...................................................................................................................... 28
      2.2.1 Chemicals .................................................................................................................. 28
      2.2.2 Instrumentation and Analysis .................................................................................... 28
      2.2.3 Synthesis .................................................................................................................. 29
      2.2.4 pH Titrations ............................................................................................................. 33
      2.2.5 Addition of Metal Complexes ................................................................................... 35
  2.3 Results and Discussion ....................................................................................................... 37
      2.3.1 Oligomer Characterization ....................................................................................... 37
      2.3.2 Reaction with Cu and Pt Complexes ........................................................................ 40
      2.3.3 Metal Coordination to Acetyl Tetrapeptides ............................................................. 45
      2.3.4 Molecular Modeling ................................................................................................. 47
      2.3.5 Electrochemistry ....................................................................................................... 49
  2.4 Conclusion ........................................................................................................................... 56
  2.5 References.......................................................................................................................... 57

Chapter 3  Redox Behavior of Phenyl-Terpyridine Substituted Artificial
Oligopeptides Cross-Linked by Co and Fe ............................................................................. 59

  3.1 Introduction........................................................................................................................... 59
  3.2 Experimental ...................................................................................................................... 62
      3.2.1 Chemicals .................................................................................................................. 62
LIST OF FIGURES

Figure 1-1: Cartoon depicting the assembly of a molecular circuit from component units (spheres) which are chemically functionalized (remaining shapes) to specifically bind to other components. ................................................3

Figure 1-2: Structure of the metal complex formed between 2,6-pyridyldicarboxylic acid and pyridine tethered to the phosphoester backbone of DNA on coordination to Cu (II). .................................................................6

Figure 1-3: (A) Possible binding motifs of ligand containing artificial oligopeptides where the number indicates the denticity of the ligand and M is the metal used. (B) Structures of the artificial oligopeptides and the ligands which have been used. ......................................................................................10

Figure 1-4: Cartoons depicting the behavior of (A) a molecular wire and (B) a molecular diode. The green semi-circle represents bipyridine, and the red represents terpyridine ................................................................................................12

Figure 1-5: Theoretical cyclic voltammograms ($\nu = 500$ mV/s) calculated with CHI660A digital simulation software for surface bound oligopeptide assemblies ($\Gamma = 2 \times 10^{-10}$ mol/cm$^2$) containing (A) (―) one Fe bis-terpyridine complex and (--) two Fe bis-terpyridine complexes ($k^0 = 1 \times 10^5$ cm$^{-1}$s$^{-1}$, $k_{EX} = 1 \times 10^6$ M$^{-1}$s$^{-1}$) and (B) a Fe bis-terpyridine complex sandwiched between the electrode and a Cu bis-bipyridine complex with (―) a fast rate ($k_H = 1 \times 10^{11}$ M$^{-1}$s$^{-1}$) of electron transfer from the Cu (I) to the Fe (III), (--) a slower rate ($k_H = 1 \times 10^8$ M$^{-1}$s$^{-1}$) and (—) with competing super-exchange ($k^0_{Cu} = 100$ cm$^{-1}$s$^{-1}$, $k_H = 1 \times 10^8$ M$^{-1}$s$^{-1}$) for the Cu center. ............................................................................................................16

Figure 2-1: A) Monomer syntheses; i) 1. CH$_2$Cl$_2$, EDC, HOBt, DIPEA, 4-pyridylacetic acid hydrochloride; 2. 3 M HCl; ii) 1. CH$_2$Cl$_2$, EDC, HOBt, DIPEA, glacial acetic acid; 2. TFA/CH$_2$Cl$_2$, B) Structures of the pyridyl and acetyl oligomers. .................................................................................................................................34

Figure 2-2: (A) Titration curve of the change in pH as a function of the amount of added 99.4 $\pm$ 0.1 mM NaOH to 12.0 $\mu$M oligomer 6, plotted in terms of the relative molar quantities of base and oligomer 6 (■); (B) Plot of the equivalence point versus the number of pyridyl groups on each oligopeptide, with linear regression. ..............................................................................39

Figure 2-3: Pyridyl Oligopeptide Coordination to M = Cu (II) or Pt (II) Complexes ..............................................................................................................................................................................41

Figure 2-4: UV-Visible absorption spectra acquired during the reaction of pyridine tetramer (4) with metal complexes: A) 3.875mM peptide (4) with
3.25 mM [Cu(pda)] in MeOH; B) 2.09 mM peptide (4) with 29.9 µM [Cu(tpy)]^{2+} in 2.5% H₂O in MeOH; and C) 0.124 mM peptide (4) with 99.9 µM [Pt(tpy)]^{2+} in 25% H₂O in MeOH. The insets of each plot show the increases in absorbance at 662, 630, and 336 nm for A, B, and C, respectively. ..........................................................................................................43

**Figure 2-5:** Change in UV-visible absorption spectra during the titration of acetyl tetramer, (4Ac, —) and py tetramer (4, - - -) with metal complexes: (A) 4.0 mM peptide 4Ac with 3.25 mM [Cu(pda)] in MeOH; (B) 2.1 mM peptide 4Ac and 29.9 µM [Cu(tpy)]^{2+} in 2.5% H₂O in MeOH; and (C) 0.13 mM peptide 4Ac with 99.9 µM [Pt(tpy)]^{2+} in 25% H₂O in MeOH. The insets compare the change in absorbance for 4Ac (●) and 4 (○) at the indicated wavelengths...........................................................................................................46

**Figure 2-6:** Energy minimized structures of [Pt(tpy)]^{2+}-coordinated series of pyridine oligopeptides, with the H atoms omitted for clarity. Scale bar is 2 nm..........................................................................................................................48

**Figure 2-7:** Cyclic voltammograms of (A) 1.35 mM [Pt(tpy)(1)]^{2+} and (B) 0.258 mM [(Pt(tpy))₅(5)]^{10+} solutions containing 0.2 M TBAP in DMF, using a glassy carbon working electrode and potential scan rate of 50 mV/s. Insets contain reductive scan differential pulse voltammograms....................................50

**Figure 2-8:** Cyclic voltammograms of the metallated oligopeptides [(Pt(tpy))₄(4)]^{8+} (---); [(Pt(tpy))₆(6)]^{12+} (- - -) and [(Pt(tpy))₁₀(10)]^{20+} (—), normalized for the concentration of [Pt(tpy)]^{2+}, in solutions containing 0.2 M TBAP in DMF. Potential scan rates were 50 mV/s. Inset is plots of the peak cathodic currents versus the square root of the scan rate for 4 (○), 6 (▼), and 10 (●) with bound [Pt(tpy)]^{2+} ..............................................................................53

**Figure 2-9:** Linearized current-time transients resulting from an applied potential step of -1.0V for the metallated oligopeptides [Pt(tpy)(1)]^{2+} (●), [(Pt(tpy))₅(5)]^{10+} (○), and [(Pt(tpy))₁₀(10)]^{20+} (□), with linear regressions. Inset: Plot of the measured diffusion coefficient vs the number of pyridine ligands per oligopeptide, with a line to guide the eye..................................................................................55

**Figure 3-1:** Representation of metal-chelation based molecular recognition of artificial oligopeptide strands: (A) tridentate-monodentate and (B) bidentate-bidentate ligand pairs are selectively linked by tetracoordinate metals. (C) The tridentate-tridentate ligand pair is crosslinked by hexacoordinate metals.....61

**Figure 3-2:** Monomer Synthesis i) NBS, AIBN, Benzene; ii) 1. NaCN, DMSO, 2. conc. HCl; iii) 1. Fmoc-aeg-otBu, EDC, HOBt, DIPEA, CH₂Cl₂, 2. 2.5% triisopropylsilane in TFA......................................................................................63

**Figure 3-3:** Structures of Oligopeptides 1 - 4..................................................................................................................72
Figure 3-4: Temperature dependent $^1$H NMR (400 MHz) spectra of 1 in $d_6$-DMSO .............................................................73

Figure 3-5: Structures of Metallated Oligopeptides 1 - 4 ........................................75

Figure 3-6: Titration curves for the change in absorbance upon addition of oligopeptide to (A) Co (II) at 514 nm and (B) Fe (II) at 567 nm for oligopeptides (■) 1, (●) 2, (♦) 3, (▼) 4 in methanolic solutions. Insets show representative absorbance difference spectra acquired during titration with 3......76

Figure 3-7: Cyclic voltammograms obtained for solutions containing (A) Co and (B) Fe complexes of the oligopeptides 1 (····), 2 (· –), 3 (– – –), and 4 (——) in 80:20 ACN:H$_2$O with 0.15 M TBAP supporting electrolyte, acquired at a potential scan rate of 50 mV/s. Currents are normalized for the metal complex concentrations determined separately from the solution absorbances. ........................................81

Figure 3-8: Linearized charge transients of the (A) Co and (B) Fe complexes of 2 in 80:20 ACN:H$_2$O with 0.15 M TBAP. Insets show the calculated $nD^{1/2}$ of the metallated complexes of oligopeptides I - 4 vs. the number of ligands on each strand, with linear regression .................................................................83

Figure 3-9: (A) Sequential cyclic voltammograms of [Fe$_4$(4)$_2$]$_{8+}$ using a Pt electrode and potential scan rate of 50 mV/s. (B) Cyclic voltammogram of the same film in 0.2 M TBAP in ACN using a scan rate of 50 mV/s. (C) Peak current vs. scan rate for films deposited on a Pt electrode at 50 mV/s in 0.2 M TBAP ACN solutions following (■) 5, (▲) 20, (▼) 40, and (●) 60 deposition cycles. (D) Surface coverage of metal complexes for [Fe$_4$(4)$_2$]$_{8+}$ on the Pt electrode as a function of cycle number .................................................................84

Figure 3-10: (A) Absorbance at 567 nm for a film of [Fe$_3$(3)$_2$]$_{6+}$ on ITO coated glass in 0.2 M TBAP in ACN as the potential is swept from 0.35 to 135 V at 50 mV/s. (B) Absorption of the film at 567 nm as a function of applied potential for 1 cycle .................................................................89

Figure 4-1: Structures of phenyl-terpyridine substituted oligopeptides 1-3 ..........96

Figure 4-2: Representation of the structure of [M$_3$(3)]$_{6+}$, where M is Co or Fe, calculated using molecular modeling .................................................................97

Figure 4-3: Cartoon depiction of the two methods employed to deposit the monolayers of the metallated oligopeptides onto Au electrode surfaces. (A) in situ metal coordination sequentially adsorbs 1 onto the electrode and adds metal ion. (B) ex situ coordination reacts oligopeptides 1-3 with metals, and then adsorbs the product onto the surface .................................................................99
Figure 4-4: Cyclic voltammograms obtained during in situ metal coordination according to Scheme 2A for (A) the second cycle of Fe addition to monopeptide 1 and (B) the fourth cycle of Co addition to monopeptide 1. Potential scan rate is 500 mV/s.

Figure 4-5: Series of cyclic voltammograms obtained during in situ metal coordination of 1 (Scheme 2A) by the metal ions (A) Fe and (B) Co. Potential scan rates are 500 mV/s in 1M TBAP solutions of ACN. Insets contain plots of the integrated peak current vs. the number of cycles of deposition.

Figure 4-6: Cyclic voltammograms following adsorption of the Fe complex of 1 (ex situ formation, Scheme 2B) at the indicated times of exposure to the 0.22 mM Fe complex solution. Voltammograms were obtained in solutions containing only 1M TBAP in ACN at potential scan rates of 500 mV/s. Inset contains a plot of the integrated peak current as a function of adsorption time.

Figure 4-7: Plots of the fraction of initial surface coverage (from integrated peak currents in the CVs) as a function of the exposure time to 1-propanethiol. (A) Surface coverage of Fe complexes 1 – 3 exposed to 1 mM propanethiol in 20:80 DMF:ACN. (B) Surface coverage of Fe complex of 1 following exposure to 1 mM propanethiol solutions in ACN (●) and 20:80 DMF:ACN (♦).

Figure 4-8: Cyclic voltammograms for Au electrodes modified with mixed monolayers containing Co complexes of 1 (-), 2 (--), or 3 (...) together with 1-propanethiol diluent ($\Gamma_1 = 7.0 \times 10^{-11}$; $\Gamma_2 = 7 \times 10^{-11}$; $\Gamma_3 = 6 \times 10^{-11}$ mol/cm$^2$). CVs are normalized to the Co(II/I) cathodic peak current ($i \propto$ metal complex surface concentration). Potential scan rates are 500 mV/s in 1 M TBAP in ACN solutions. Inset: Plot of the relative peak currents of the Co(III/II) oxidation to Co(II/I) reduction as a function of the number of metals per peptide duplex.

Figure 4-9: Depiction of electron transfers in adsorbed multi-redox oligopeptides. Heterogeneous electron transfer ($k^\text{h}$) between the electrode and closest metal complex creates a mixed valent layer that can further transfer electrons by self-exchange ($k_{\text{EX}}$).

Figure 5-1: Cartoons depicting the different schemes of electron transfer for dipeptide assemblies containing Co (II) bis phenyl-terpyridine centers. (A) A monometallic system for the Co (III/II) couple. (B) A dimetallic system for the Co (III/II) couple, which cannot self-exchange. (C) A dimetallic system for the Co (II/I) couple, which can self-exchange.

Figure 5-2: Cyclic Voltammograms of the Co (II) complex of 2 at the indicated scan rates in 1 M TBAP ACN.
Figure 5-3: Representative Plots to Determine the Transfer Coefficient for Metal Complexes of 2 for: ● Fe (III/II), ■ Co (II/I), ▲ Co (III/II). The surface coverage for each in terms of metal concentration is $1 \times 10^{-11}$ mol/cm$^2$. ...............127

Figure 5-4: Cyclic Voltammograms at 500 mV/s of Fe (─) and Co (--) complexes of 3 on a 1.37 cm$^2$ Au electrode in 1 M TBAP in acetonitrile. Surface coverages are approximately $1 \times 10^{-11}$ mol/cm$^2$ with respect to the metal centers for each. .............................................................................................................132

Figure 5-5: Laviron plots for complexes of 3 for the redox couple: (A) Fe (III/II), (B) Co (II/I) and (C) Co (III/II). Surface coverages are approximately $1 \times 10^{-11}$ mol/cm$^2$ in terms of redox center concentration. .................................................................134

Figure 5-6: (A) Nyquist and (B) cot ($\phi$) vs. $\omega^{1/2}$ plots for Co (III/II) couple of ~1mM Co 1 in 80:20 acetonitrile:water with 0.2 M TBAP. Applied DC potential is 0.265 V (vs. AgQRE) and frequencies are 1 Hz to 0.1 MHz. ...............137

Figure 5-7: Nyquist plot for surface bound complex of Co 3 for the Co (III/II) couple. Surface coverage is approximately $1 \times 10^{-11}$ mol/cm$^2$ in terms of redox center concentration. Applied DC potential is 0.374 V and frequencies are 1Hz to 0.1 MHz. .............................................................................................................139
## LIST OF TABLES

**Table 2-1:** Characterization data for the pyridyl oligopeptides 1, 4, 5, 6, and 10........32

**Table 2-2:** Stoichiometry of Metal Complexation with the Oligopeptides.................44

**Table 2-3:** Electrochemical Data for Pyridine Oligopeptides with Bound
\[
[\text{Pt(tpy)}]^2^+\\
\]
........................................................................................................51

**Table 3-1:** Phenyl Terpyridine Substituted Oligopeptide Characterization Data......68

**Table 3-2:** Solution Phase Electrochemical Data for Metallated Oligopeptides.........78

**Table 3-3:** Electrochemical Data for Deposited Films on Pt.................................87

**Table 5-1:** Values for the Transfer Coefficient for Each Oligopeptide and Redox
Couple..................................................................................................................128

**Table 5-2:** Apparent Rate Constants determined by Laviron Analysis.................135
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>hydrodynamic radius</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>transfer coefficient</td>
</tr>
<tr>
<td>$A$</td>
<td>electrode area</td>
</tr>
<tr>
<td>Ac</td>
<td>acetate</td>
</tr>
<tr>
<td>AC</td>
<td>alternating current</td>
</tr>
<tr>
<td>ACI</td>
<td>alternating current impedance</td>
</tr>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>aeg</td>
<td>N-(2-aminoethylglycine)</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2’-azobisobutyronitrile</td>
</tr>
<tr>
<td>anhyd</td>
<td>anhydrous</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>$C$</td>
<td>concentration</td>
</tr>
<tr>
<td>conc</td>
<td>concentrated</td>
</tr>
<tr>
<td>COSY</td>
<td>two-dimensional NMR correlation spectroscopy</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>$D$</td>
<td>diffusion coefficient</td>
</tr>
<tr>
<td>DC</td>
<td>direct current</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DIPCDI</td>
<td>N,N’-diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ds</td>
<td>double-stranded</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>molar absorbtivity</td>
</tr>
<tr>
<td>$E^0$</td>
<td>formal potential</td>
</tr>
<tr>
<td>$E_{pa}$</td>
<td>anodic peak potential</td>
</tr>
<tr>
<td>$E_{pc}$</td>
<td>cathodic peak potential</td>
</tr>
<tr>
<td>EDC</td>
<td>1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride</td>
</tr>
<tr>
<td>$E_{HW}$</td>
<td>peak width at half the maximum value</td>
</tr>
<tr>
<td>Eq.</td>
<td>equation</td>
</tr>
<tr>
<td>ESI+</td>
<td>positive ion electrospray ionization mass spectrometry</td>
</tr>
<tr>
<td>Et$_2$O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>$\Delta E_p$</td>
<td>peak splitting</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant, 96485 C</td>
</tr>
<tr>
<td>Fc</td>
<td>ferrocene</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenylethoxycarbonyl</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>$\Gamma'$</td>
<td>electrode surface coverage</td>
</tr>
<tr>
<td>$\eta$</td>
<td>viscosity</td>
</tr>
<tr>
<td>HBTU</td>
<td>2-((1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
</tr>
</tbody>
</table>
HMOC  heteronuclear correlation through multiple quantum coherence
HOBT  1-hydroxybenzotriazole
HPLC  high performance liquid chromatography
i  current
iDNA  inorganic DNA
ILIT  indirect laser induced temperature jump
i_p  peak current
ITO  indium tin oxide
k^0  standard heterogeneous rate constant
k_B  Boltzmann constant, 1.38065×10^{-23} J/K
k_{EX}  homogeneous, or self-exchange, rate constant
KHP  potassium hydrogen phthalate
k_s  apparent rate constant for surface species
MALDI-TOF  mass assisted laser desorption ionization-time of flight mass spectrometry
M-DNA  DNA coordinated to metal cations through natural base pairs
Me  methyl
MeOH  methanol
min  minutes
MLCT  metal-to-ligand charge transfer
MM+  molecular mechanics
MS  mass spectrometry
m/z  mass to charge ratio

n  number of electrons transferred per reaction

v  potential scan rate

NBS  N-bromosuccinimide

NIR  near infrared

NMR  nuclear magnetic resonance

OrBu  tert-butyloxy

PAL  polyamide linker

pda  pyridine-2,6-dicarboxylate

PEG  polyethylene glycol

ph or Φ  phenyl

PNA  peptide nucleic acid

PS  polystyrene

py  pyridine

Q  charge

Q_{dl}  double layer charge

Q_{ads}  charge of adsorbed species

QRE  quasi-reference electrode

R  gas constant, 8.314 J/(mol) K^{-1}

RC  time constant, product of resistance and capacitance

RNA  ribonucleic acid

SAMs  self-assembled monolayers

SCE  saturated calomel electrode
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPPS</td>
<td>solid phase peptide synthesis</td>
</tr>
<tr>
<td>ss</td>
<td>single-stranded</td>
</tr>
<tr>
<td>$t$</td>
<td>time</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature</td>
</tr>
<tr>
<td>TBAH</td>
<td>$n$-tetrabutylammonium hexafluorophosphate</td>
</tr>
<tr>
<td>TBAP</td>
<td>$n$-tetrabutylammonium perchlorate</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TIS</td>
<td>triisopropylsilane</td>
</tr>
<tr>
<td>tpy</td>
<td>2,2’:6’,2”-terpyridine</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>phase angle</td>
</tr>
<tr>
<td>Vis</td>
<td>visible</td>
</tr>
<tr>
<td>$\omega$</td>
<td>frequency</td>
</tr>
<tr>
<td>$Z'$</td>
<td>real component of impedance</td>
</tr>
<tr>
<td>$Z''$</td>
<td>imaginary component of impedance</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1 Introduction

The field of molecular electronics has exploded in the last 30 years after it was postulated by Aviram and Ratner in 1974\textsuperscript{1} as a means of overcoming the limitations inherent in silicon chip technology. Single molecules capable of functioning as wires,\textsuperscript{2} switches,\textsuperscript{3} transistors\textsuperscript{4} and other circuit components have all been examined. Indeed a plethora of materials capable of functioning as components of a molecular scale circuit are known.

The true need for investigation does not therefore rely in necessarily developing new materials as components of molecular circuits, but rather, how to arrange these into circuits, the so-called “bottom-up” approach.\textsuperscript{5} A variety of techniques have been employed, including conventional photo- and electron beam lithography.\textsuperscript{5,6} However, these methods are rather complex, expensive, limited in resolution and frequently the components of the circuit are damaged during these processes. Softer lithographies, such as nanoimprinting, have also been employed with some success. Nanoimprinting\textsuperscript{7} creates nanoscale features by mechanically deforming polymer materials, so the resolution is not limited to the wavelength of light or electrons like conventional lithographies. While this technique is very promising, it still has some important drawbacks, like difficulty with creating complex patterns and varied feature density.\textsuperscript{7} Several novel non-lithographic
techniques have also been utilized. Lieber used a flow-through channel technique to
deposit criss-cross patterns of 10 nm long (and longer, up to microns) semiconducting
nanowires. But this technique is only applicable to these large circuit components; it
could never be used to arrange molecular scale components. Ideally, we need a simple,
generally applicable method for assembling pre-arranged architectures of specific, chosen
electron transfer properties.

Chemical functionalization of subunits which can be arranged into larger
architectures in a specific scheme based upon the functional groups employed is an
attractive option. Such a scheme would not only provide a means of creating complex
arrangements of molecules with well defined spatial arrangement and molecular scale
resolution, but also allow for modular construction of these assemblies. This scheme is
depicted by the cartoon in Figure 1-1. The various components of the circuits (i.e. wires,
switches, etc.) can be multifunctionalized with various chemical groups such that under
appropriate conditions, a covalent, coordinative or intermolecular bond is selectively
made between two of the groups, thus effectively connecting the components of the
circuit. The ability to construct more complex architectures from a “toolbox” of
functional subunits means that a large variety of structures could be created from a small
number of these precursory pieces.
Figure 1-1: Cartoon depicting the assembly of a molecular circuit from component units (spheres) which are chemically functionalized (remaining shapes) to specifically bind to other components.
1.2 DNA

Materials capable of selective recognition and self-assembly are obviously attractive candidates for such endeavors. Indeed, many have investigated DNA for its well known ability to self-assemble and be functionalized for such roles. Single-stranded DNA (ssDNA) can be easily chemically functionalized on the termini of the phosphoester backbone, so that molecules of interest can be covalently bound to these termini. The inherent self-recognition and assembly of complementary strands of DNA, based on hydrogen bonding between base pair complements to form double-stranded DNA (dsDNA) may then be employed to spatially arrange these molecules. Many groups have employed the self recognition and assembly properties of DNA in this way, to align nanoparticles, quantum dots, redox moieties, carbon nanotubes and fluorescent and/or quencher species (i.e. commercially available molecular beacons).

But there are several drawbacks to using DNA in molecular electronic devices. The very same molecular force that provides the basis for self-assembly in DNA also makes the double-stranded structure inherently unstable. The complementary hydrogen bonding between base pairs is a rather weak intermolecular force. As such, it is sensitive to solvent, pH and temperature conditions, and is only stable in a fairly narrow range of these. Furthermore, the ability of DNA to carry a charge is a hotly debated topic, with claims ranging from DNA behaves as an insulator, to others calling it a superconductor. Theoretical treatments have shown that these properties are largely sequence dependent, with the number of and distance between guanine units as the primary factor governing charge mobility and mechanism. While this dependence on
guanine units provides a means for tuning the charge transfer properties of DNA, the onset potential of this behavior cannot be varied because it is an inherent property of the guanine oxidation. Furthermore, dsDNA is well known to decompose upon oxidation. In practicality, this inability to tune the potential range over which a charge is carried and instability under a variety of conditions makes DNA a poor candidate for real world applications of molecular electronics.

1.3 Modified DNA

While some groups have modified dsDNA after duplex assembly to enhance stability and conduction, many groups have synthesized structures of DNA which contain some alternate, stronger binding motifs and better defined charge transfer properties. An example is M-DNA, DNA to which metal ions, such as Zn (II), Co (II) and Ni (II), have been added. The metal cations are found to form coordination complexes with some of the base pairs in DNA, which function as ligands. These materials are found in some cases to possess enhanced conduction properties compared to natural dsDNA. Other groups have substituted some of the naturally occurring base pairs in DNA for chelating ligands, like pyridine and 2,6-pyridyldicarboxylic acid, among others, which can specifically coordinate metal cations based on the denticities of the ligands used and the coordination number of the specific metal cations. The structure of the pyridine (a monodentate ligand) and 2,6-pyridyldicarboxylic acid (a tridentate ligand) metal complex that links the sugar-phosphoester backbone of dsDNA is shown in Figure 1-2. Coordination to a tetracoordinate metal (like Cu (II)) satisfies the
**Figure 1-2:** Structure of the metal complex formed between 2,6-pyridyldicarboxylic acid and pyridine tethered to the phosphoester backbone of DNA on coordination to Cu (II).\textsuperscript{22a}
coordination number of the metal and the denticities of the ligands employed. Thus, the metal-ligand coordination chemistry provides a means of complementary recognition and self-assembly analogous to that afforded by the hydrogen bonding motif in naturally occurring base pairs. The resulting overall stability of these structures is generally greater than natural dsDNA when multiple metal-ligand units are incorporated into the structures.

While these systems present the potential to broadly tune the electron transfer properties by choice of metal and ligand, and to also improve the duplex stability, the systems are not ideal for molecular electronic applications. It is well known that addition of metal cations to DNA causes non-specific coordination via electrostatic interactions with the phosphoester backbone of the DNA. Furthermore, the nucleoside bases function as ligands for the metals as well, and these assemblies have shown coordination complexes between both the bases and the ligands. Therefore the structures and properties of these systems cannot be precisely controlled, so they are not good candidates for aligning components of circuits.

### 1.4 Modified PNA

To alleviate the issue of non-specific interactions with the charged backbone, some have focused on the use of a charge-neutral backbone, a peptide, to which nucleic acid bases are tethered, called peptide nucleic acid (PNA). The peptide backbone (an amino-ethyl glycine repeating unit, aeg) is designed such that the spacing between the base pairs exactly matches that in naturally occurring DNA. PNA is known to form more
stable duplexes than DNA due to the lack of electrostatic repulsion in the neighboring backbones. Additionally, some groups have been able to substitute some of the base pairs for ligands, like hydroxyquinoline and bipyridine. UV-Visible spectrophotometric titrations of these ligand-substituted PNAs show that they complex metal cations, like Ni (II) and Cu (II), though the stoichiometry demonstrates that the base pairs participate in this coordination as well. UV melting experiments show that the assemblies have higher stability than strictly natural base pair containing PNA duplexes. However, these materials typically contain no more than three ligands per strand of PNA, which are usually at least ten units long. Thus, the primary means for assembly and duplex stability in these materials is still hydrogen bonding, and they are not stable enough to be used in real world applications of molecular circuitry. Additionally, because of the presence of natural bases, the electron transfer properties of these materials likely resembles that of natural dsDNA, and is thus limited to the redox properties of guanine, instead of the rich and diverse properties offered by use of only transition metal complexes.

1.5 iDNA

This apparent need for employing hydrogen bonding of natural base pairs to retain the complementary and self-assembly properties of these materials into controlled structures is logistically a false assumption. Metal-ligand coordination chemistry alone can also provide a means of recognition and assembly into regular architectures. By appropriate choice of ligands and metals, the denticity of the ligands and coordination
numbers of the metals provides and inherent scheme for selective binding. **Figure 1-3A** illustrates some of the possible binding motifs. For example, juxtaposed bidentate ligands are complementary in the presence of a tetracoordinate metal cation (a 2+2 binding motif). Similarly, a tridentate ligand is complementary to a monodentate ligand in the presence of a tetracoordinate metal cation (3+1). Two tridentate ligands are complementary in the presence of an octahedral metal (3+3). Thus, in addition to providing a more stable structure based upon stronger bonds, metal-ligand coordination chemistry also provides a basis for recognition and self-assembly.

Use of only transition metal complexes in these assemblies also provides access to unique charge transfer capabilities that are not possible with systems which contain only or mostly natural base pairs. The potential range over which charge transfer can occur and the direction of transfer can be easily controlled by choice of ligands, metals and sequence. Therefore, assemblies containing only transition metal complexes also present the possibility of tuning the coupling properties between elements of the molecular circuit, and also present the potential of acting as more than simple wires within the circuit. With careful choice of ligands, metals and sequence, these assemblies could function as more integral parts of the circuits (*i.e.* diodes, switches, etc.).
Figure 1-3: (A) Possible binding motifs of ligand containing artificial oligopeptides where the number indicates the denticity of the ligand and M is the metal used. (B) Structures of the artificial oligopeptides and the ligands which have been used.
To these ends, we have synthesized a series of ligand-containing peptides, using the aeg backbone, and the respective metal complexes\textsuperscript{27} depicted in Figure 1-3B which employ only metal-ligand coordination chemistry to self-assemble into larger architectures. These can be chemically functionalized on the termini to provide future points of attachment in electronic circuits. We refer to this class of structures as inorganic DNA, or iDNA. The ability of these materials to form higher order, regular structures based on the formation of metal coordination compounds, and the well defined arrangement and spacing of these metal complexes with respect to one another within these materials, makes them ideal candidates for self-assembled components of molecular circuits.

1.5.1 Electron Transfer Properties of iDNA

The charge transfer properties of these materials are dictated by choice of metals, ligands and sequence. The specific identity and arrangement of the metal complexes constituting the core of the assemblies will govern the direction and mechanism, and therefore rate, of charge transfer. For example, a simple 1D wire could be formed by a columnar arrangement of metal complexes in close proximity, each of which could transfer electrons to its nearest neighbor. This is depicted in Figure 1-4A, where an oligopeptide assembly possessing a core of identical metal complexes which can self-exchange ($k_{EX}$) is tethered on one end to an electrode surface. From our toolbox of ligands and metals shown in Figure 1-3B, one appropriate choice for the metal complexes composing the core in these systems would be bis-terpyridine complexes of
Figure 1-4: Cartoons depicting the behavior of (A) a molecular wire and (B) a molecular diode. The green semi-circle represents bipyridine, and the red represents terpyridine.
Fe (II), because the Fe (III/II) couple is well known to transfer electrons by self-exchange. Thus, if a sufficiently large positive potential is applied to the electrode to cause oxidation of the first metal complex (i.e. heterogenous electron transfer, with rate constant $k^0$) a mixed valent system will result with an Fe (III) neighboring an Fe (II) complex. A self-exchange transfer (with rate constant $k_{EX}$) could then occur where an electron from the Fe (II) complex is transferred to the Fe (III) complex. For longer assemblies than that depicted, this process would repeat until all of the metal centers were oxidized. Thus electrons would be shuttled down the core of the assembly, by hopping from adjacent metal centers, constituting a simple wire. The direction of electron transfer in these cases (i.e. up or down the column) is governed by the position of the potential source. Thus attaching the electrode to the other end of the assembly would cause the electron flow to occur in the opposite direction. Alternatively, once all of the metal centers are oxidized, reversing the polarity of the applied potential would cause the process to occur in reverse, with sequential reductions occurring.

This wire-like behavior would be observed any time homometallic assemblies capable of self-exchange are utilized to form the core of the assembly. Use of heterometallic systems, where each metal complex would have different formal potentials for oxidation/reduction would give rise to assemblies with diode-like behavior, in which charge transfer occurs unidirectionally, if the sequence of the metal complexes was such that the charge transfer events occurred in a sequential thermodynamically downhill fashion. This is depicted in Figure 1-4B. Appropriate choices for the metal complexes in this case could be a bis-terpyridine complex of Fe (II) and a bis-bipyridine complex of Cu (I). The oxidation of the Fe (II) species occurs at a much more positive
(approximately 1 V) potential than that of the Cu (I) complex.\textsuperscript{27a,f} Thus, an oligopeptide assembly such as that depicted in Figure 1-4B, where the Fe complex is placed proximal to the electrode surface, between the Cu complex and the electrode, will only allow current to flow in one direction upon application of a sufficiently positive potential to cause oxidation of the Fe species. Once the Fe species is oxidized to the (III) state, the Cu center can transfer an electron to the Fe (III) center (with rate constant $k_{\text{II}}$), because this is an energetically favorable event. If, however, the assembly were aligned in the opposite direction, (i.e. the Cu complex proximal to the electrode and between the electrode and Fe complex) or if the polarity of the applied potential were reversed following oxidation, the Fe (II) would not transfer an electron to the oxidized Cu (II), because this is an energetically uphill event. Thus, this system functions as a simple diode, allowing charge transfer in only one direction.

Other sorts of arrangements, and therefore electron transfer functionalities, are possible for these assemblies and derivatives of these structures. For example, functionalized dendritic structures could be used as switches. Because these materials can be functionalized on the oligopeptide termini, they can be used to provide means of attachment to potential sources (as discussed in the examples above) or other circuit components. Thus, they are able to function as components of the circuit, and to align other pre-existing constituent circuit elements as well.
1.5.2 Measuring Charge Transfer

The specific charge transfer properties of these systems can be tailored based on what is known of the electron transfer properties of the constituent metal complexes and their arrangements with respect to one another, as discussed in the examples above. These properties can be probed electrochemically or spectroscopically. Because the oligopeptides can be chemically functionalized on the termini, the metallated assemblies can be tethered to electrode surfaces to investigate the directionality, mechanism and rate of charge transfer. For example, in the case of the wire-type assemblies in Figure 1-4A, cyclic voltammetry would show more current for the wave corresponding to the Fe (III/II) couple for longer assemblies vs. shorter assemblies (if the surface coverages were the same) because there are more redox centers in the longer assemblies, all of which are electrochemically accessible because of the inherent fast self-exchange capability of the constituent metal complexes. This is shown in the calculated voltammogram in Figure 1-5A where the magnitude of the current observed for an oligopeptide assembly containing two Fe bis-terpyridine centers is twice that for an assembly containing only one at the same surface coverage. Moreover, the magnitude of the currents observed for the anodic and cathodic peaks should be identical, because of the lack of directional dependence for self-exchange in these materials. If these systems did not self-exchange, there would be no difference in the magnitude of current observed for the same surface coverages of metallated oligopeptide assemblies of varying lengths, because only the redox centers immediately next to the electrode surface would be accessible.
Figure 1-5: Theoretical cyclic voltammograms ($v = 500$ mV/s) calculated with CHI660A digital simulation software for surface bound oligopeptide assemblies ($\Gamma = 2 \times 10^{-10}$ mol/cm$^2$) containing (A) (—) one Fe $\text{bis}$-terpyridine complex and (--) two Fe $\text{bis}$-terpyridine complexes ($k_0^B = 1 \times 10^5$ cm$^{-1}$ s$^{-1}$, $k_{EX} = 1 \times 10^6$ M$^{-1}$ s$^{-1}$) and (B) a Fe $\text{bis}$-terpyridine complex sandwiched between the electrode and a Cu $\text{bis}$-bipyridine complex with (—) a fast rate ($k_H = 1 \times 10^{11}$ M$^{-1}$ s$^{-1}$) of electron transfer from the Cu (I) to the Fe (III), (--) a slower rate ($k_H = 1 \times 10^8$ M$^{-1}$ s$^{-1}$) and (—) with competing super-exchange ($k_0^{Cu} = 100$ cm$^{-1}$ s$^{-1}$, $k_H = 1 \times 10^8$ M$^{-1}$ s$^{-1}$) for the Cu center.
In contrast, the calculated voltammetric behavior of a heterometallic assembly, such as that depicted in Figure 1-4B would be quite different. If the rate of electron transfer from the Cu (I) to the Fe (III) were fast, the current for the anodic peak corresponding to the Fe (III/II) wave would be twice the size of the corresponding cathodic peak, because it would correspond to two electrons, one from the Fe (II) and one from the Cu (I), and there would be no wave associated with the Cu (II/I) couple. This is shown in Figure 1-5B. If the rate of electron transfer from the Cu (I) to the Fe (III) were very slow, we might expect to see an anodic post-wave appear. The post-wave would correspond to the reoxidation of the Fe (II) complex following the slow electron transfer from the Cu (I) complex, and would likely be embedded in the normal anodic peak for the Fe (III/II) couple. In Figure 1-5B, this post-wave appears as such, giving rise to a tailing structure on the anodic peak. Additionally, if the Cu (II/I) couple were electrochemically accessible through a super-exchange mechanism then we would expect waves for both the Fe (III/II) and Cu (II/I) couples, in which case it would not be a diode. This is also shown in Figure 1-5B, where waves for both couples are shown, and the magnitude of the Cu (II/I) wave is slightly less than that for the Fe (III/II) wave and its peak splitting is larger because of its slower heterogenous kinetics due to its distance from the electrode surface. Thus, simple electrochemical experiments can be used to differentiate between mechanisms of electron transfer in these molecules.

We can also use electrochemical investigations to more quantitatively probe the rates of charge transfer in these systems. Nominally fast rate constants (~ 10⁴ s⁻¹) for electron transfer processes of surface confined species can be measured through a number of electrochemical techniques including cyclic voltammetry, AC impedance.
spectroscopy\textsuperscript{30} and chronoamperometry.\textsuperscript{31,30g} Faster rate constants require more specialized techniques, such as the laser-induced temperature jump method (ILIT) pioneered by Chidsey,\textsuperscript{32} but can be measured.

Spectroscopic techniques can also be employed to elucidate rates and mechanisms of charge transfer. The advantage of spectroscopic techniques over electrochemical ones is that the materials need not be confined to a surface to probe the directionality or mechanism of charge transfer, and faster rates of charge transfer can be easily measured. For mixed-valent systems which transfer electrons by a self-exchange mechanism, the presence of an absorbance band in the NIR region can be used to extract values for the self-exchange rate constant ($k_{EX}$).\textsuperscript{33} Time-resolved absorption spectroscopy can also be used to monitor the timescale of charge transfer events by monitoring the changes in intensities of metal-to-ligand (MLCT) or ligand-to-metal (LMCT) charge transfer bands as a function of time, since these bands are characteristic of the metal complexes and their specific oxidation states.\textsuperscript{34} This technique also provides access to extraordinarily fast rate constants, depending on the specific components of the apparatus. Rate constants on the order of $10^{12}$ s\textsuperscript{-1} are routinely measured.

Thus, by use of electrochemical and spectroscopic techniques we can probe the directionality, mechanism and rate of charge transfer in the metallated oligopeptide systems. We can then make comparisons with what we expect for the behavior of these systems based upon the charge transfer properties of the constituent small molecule metal complexes, and test the efficacy of these materials to function as components of molecular circuitry.
1.5.3 Summary of the Current Work

This work presents most of our initial efforts in this area, demonstrating modular construction of the ligand containing peptides, specific self-assembly of the peptides into higher order structures based only on metal complexation and the examination of their electrochemical and charge transfer properties. The synthetic control over sequence and therefore, arrangement of donor-acceptor complexes makes these materials truly unique.

Chapter 2 presents the synthesis and characterization of varying length oligopeptides containing a monodentate ligand, pyridine, and the metal complexes of these species with tetracoordinate metals to which a tridentate ligand is already bound. The formation of these single strand oligopeptide metal complexes demonstrates: (1) the oligopeptides stoichiometrically complex metals based upon the number of ligands per strand, even when this leads to the formation of highly charged species; (2) the peptide backbone does not interact with the added metal complexes; (3) the metallated oligopeptides have electrochemical properties similar to those of the corresponding small molecule metal complexes; and (4) electrochemical techniques can be used to compared the mass transport properties of metallated oligopeptides as a function of length, and therefore can be used to distinguish between the sizes of these molecules.

Chapter 3 presents the synthesis and characterization of varying length oligopeptides containing tridentate ligands, phenyl-terpyridine. Addition of Fe and Co metal cations to these peptides gave stoichiometric complexation. Measurement of the mass transport properties of these molecules showed that they are consistent with duplex, and not polymeric structures. Electrochemical characterization of these complexes
revealed that they form electrodeposited films, and analysis of these films showed them to be chemically and electrochemically stable. Measurement of the mass transport properties of these molecules showed that they are consistent with duplex, and not polymeric structures.

Chapter 4 presents the formation of metallated oligopeptide self-assembled monolayers (SAMs) by modification of the assemblies described in chapter 3 with a cysteine terminus. The thiol terminated peptides and metal complexes of these can be assembled on Au surfaces. Oligopeptide monolayers form the metal complexes on surfaces after exposure to octahedral metal cations (Fe (II) and Co (II)) and a potential sweep through the formal potential. The surface coverages of the metallated assemblies can be controlled by desorbing some of the monolayers from the surface, and backfilling with a diluent alkane thiol. Electrochemical investigations suggest that these materials can behave as 1D molecular wires.

Chapter 5 presents initial electrochemical investigations of the rates of electron transfer in the metallated oligopeptide SAMs described in Chapter 4. We employed Laviron analysis of cyclic voltammograms and AC impedance spectroscopy to probe the rates. From analysis of the observed peak splittings in cyclic voltammetry, we were able to extract values for the transfer coefficients of the metallated oligopeptide films. The measured values for the transfer coefficients do not show a dependence on oligopeptide length for the oligopeptide assemblies containing redox couples which can self-exchange. However, films of these assemblies containing redox couples which do not self-exchange yield transfer coefficients which demonstrate a dependence on the length of the oligopeptides. We find that the rates of electron transfer in all cases are in excess of $10^4$ s$^{-1}$.
1, which are too large to be measured more accurately with our electrochemical system. These studies together form the basis for our current understanding of how to make, assemble and study iDNA structures. Possible future directions are described in the conclusion of chapter 5.
1.6 References


Chapter 2

Pyridine-Substituted Oligopeptides as Scaffolds for the Assembly of Multimetallic Complexes: Variation of Chain Length

2.1 Introduction

The molecular recognition by complementary sequences of DNA provides a unique and challenging benchmark, in terms of both binding specificity and affinity, for the creation of analogous supramolecular inorganic materials. In the initial steps toward the creation of synthetic analogs, the synthesis and stabilities of peptide nucleic acids (PNAs) in which one nucleic acid is replaced with a ligand capable of metal complexation,\(^1\) similar to studies done with DNA,\(^2\) have recently been reported. However, these studies primarily rely on hydrogen bonding to achieve molecular recognition, and focus on the enhanced stabilities of duplexes that result from the metal complexation of the single incorporated ligand-for-nucleotide substitution.

Alternatively, if duplex (and higher order) formation is based solely on metal-ligand complexation, this would provide a more robust linkage than hydrogen bonding. This strategy has been used to mimic the structure of DNA (and RNA) by incorporation of the ligands onto the anionic sugar phosphoester backbone.\(^3\) While PNA is a structural analog of DNA,\(^4\) the amide linkages are charge neutral so that there are no interstrand electrostatic repulsive forces. Complementary PNA-DNA duplexes in fact have greater binding affinity than DNA-DNA duplexes.\(^{4a,5}\) Adaptation of the metal-coordination strategy to the aminoethylglycine (aeg) backbone should therefore provide an even more tightly bound duplex structure. Despite the much greater stability that would be imparted
by such a binding motif, there have been no examples in the literature that utilize only metal complexation as the basis for molecular recognition in oligopeptides.

We recently reported the synthesis of pyridine-substituted hexapeptides and bipyridine-substituted tripeptides, and demonstrated that these molecules can be used to bind stoichiometric amounts of metal ions. In the case of the bipyridine tripeptide, complexation of Cu (II) and Fe (II) created interstrand crosslinks that led to the formation of duplex structures based solely on metal coordination. This chapter builds on that initial work by examining the structural and functional properties of the single-strand pyridine oligopeptides as a function of the number of attached pyridyl ligands on a series of peptide lengths. Spectrophotometric titrations using Pt (II) and Cu (II) complexes of two tridentate ligands, 2,2’:6’,2”-terpyridine (tpy) and pyridine 2,6-dicarboxylic acid (pda), are employed to quantitatively determine the reactivity of this family of pyridine-substituted oligopeptides toward metal complexation. These experiments demonstrate synthetic control over a wide range of multimetallic structures. We investigate the degree of interaction of the metal ions with the peptide backbone in analogous titrations with an acetyl substituted derivative of the oligopeptide. In the case of the [Pt(tpy)]^{2+} oligopeptide complexes, we present electrochemical characterization of their solution phase transport and electron transfer properties to further understand their solution structures and show their unique properties.
2.2 Experimental

2.2.1 Chemicals

All materials were purchased from Acros and were used as received unless otherwise noted. Water was obtained from a nanopure water system (Barnstead, 18.2 MΩ). N,N-dimethylformamide (DMF) and diisopropylethylamine (DIPEA) were purified by distillation under N₂ from CaH₂. Tetrabutylammonium hexafluorophosphate (TBAH) was recrystallized from ethyl acetate (EtOAc) three times and dried under vacuum prior to use. Ferrocene (Fc) was purified by sublimation.

2.2.2 Instrumentation and Analysis

Automated peptide synthesis was performed on a flow-through synthesizer (Applied Biosystems Pioneer). Preparatory and analytical scale reverse-phase high performance liquid chromatographies (HPLC) were performed with a Varian system equipped with two quaternary pumps (Model 210), an autosampler (Model 410), UV-Vis detector (Model 320), fraction collector (Model 701) and C-18 columns. Oligomer elution was monitored at the pyridine absorbance at 254 nm.

Molecular structures were calculated using Hyperchem 6.0 using molecular mechanics (MM+) with atomic charge based electrostatic repulsions, and a Polack-Ribiere conjugate gradient to a minimum energy gradient of 0.01 kcal/mol. Solvent molecules and anions were not considered in the calculation.
The UV-Visible absorption spectra were obtained with a double beam spectrophotometer (Varian, Cary 500). Positive ion electrospray mass spectrometry (ESI+) was performed at the Penn State Mass Spectrometry Facility using a Mariner mass spectrometer (Perseptive Biosystems.) $^1$H-NMR spectra were collected on a 300 MHz spectrophotometer (Bruker).

All electrochemical measurements were obtained using a CH Instruments potentiostat (Model 660) with a 0.31 cm diameter glassy carbon working and Pt wire counter electrodes with a Ag/Ag$^+$ reference electrode. Solutions were prepared from distilled DMF containing 0.2 M TBAH supporting electrolyte. The solutions were prepared, stored and analyzed in an N$_2$ saturated environment. After obtaining voltammograms, Fc was added as an internal potential reference to convert to the SCE scale.$^7$ Chronoamperometric measurements were corrected for background currents by subtraction of the current transients obtained from potential steps of equal magnitude applied in solutions containing solely supporting electrolyte.

### 2.2.3 Synthesis

The synthesis of aqua(pyridine 2,6-dicarboxylato)copper (II)$^8$ (i.e., [Cu(pda)]), aqua(2,2′:6′,2″-terpyridine)copper (II) diperchlorate$^9$ (i.e., [Cu(tpy)]$^{2+}$), aqua(pyridine 2,6-dicarboxylato)platinum (II)$^{10}$ (i.e., [Pt(pda)]), and N-[2-(Fmoc aminoethyl)glycinate tert-butylester hydrochloride$^{11}$ (i.e. Fmoc-aeg-OtBu·HCl) have been reported elsewhere. The synthesis of aqua(2,2′:6′,2″-terpyridine-N, N’, N″)platinum (II) diperchlorate (i.e., [Pt(tpy)]$^{2+}$) was adapted from a literature procedure using AgClO$_4$ instead of AgBF$_4$.$^{12}$
The pyridine substituted aeg monomer (Fmoc-aeg(py)-OH·HCl) was synthesized as reported previously.\(^6\)

*Fmoc-aeg(Ac)-OH (Acetyl monomer):* Glacial acetic acid (1.00 mL, 0.0174 mol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 3.34 g, 0.0174 mol), 1-hydroxybenzotriazole hydrate (HOBt, 2.35 g, 0.0174 mol), Fmoc-aeg-OtBu·HCl (6.03 g, 0.0139 mol) and DIPEA (5.46 mL, 0.0313 mol) were combined in 200 mL of CH\(_2\)Cl\(_2\) and stirred for 24 h under N\(_2\). The solution was washed with H\(_2\)O (5 ×100 mL) and evaporated to dryness. The resulting oil was chromatographed on silica gel with 25 % acetone/75 % CH\(_2\)Cl\(_2\) and the t-Bu protected product was isolated as the major band. The t-Bu group was cleaved by stirring in 100 mL of 25% trifluoroacetic acid (TFA) in CH\(_2\)Cl\(_2\) for 30 min. and evaporating to dryness. The pure product was precipitated as a white solid from cold CH\(_2\)Cl\(_2\) by addition of Et\(_2\)O, and collected by vacuum filtration. Yield: 1.09 g (21 %) (\(^1\)H NMR, 300 MHz, CD\(_3\)Cl): 2.05 (d, 3H); 3.25 (m, 2H); 3.40 (m, 2H); 3.88 (d, 2H); 4.11 (m, 1H); 4.31 (m, 2H); 5.51,5.88 (m, 1H); 7.25 (t, 2H); 7.35 (t, 2H); 7.55 (d, 2H), 7.69 (d, 2H)

*Pyridine Oligomers (1, 4, 5, 6, 10):* The oligomers were prepared from the pyridine monomer (Fmoc-aeg(py)-OH·HCl) as described previously.\(^6\) Briefly, the oligopeptides were prepared via automated solid-phase peptide synthesis in DMF using an Fmoc protection scheme at a 0.1 mmol scale using Fmoc-PAL-PEG-PS resin (Applied Biosystems). Deprotection was performed for 5 min with 20% piperidine in DMF. Couplings were carried out using a four-fold molar excess of oligomers for 30 min with separate solutions of 0.5 M diisopropylcarbodiimide (DIPCID) and 0.5 M DIPEA.
Capping steps were performed after each coupling using 0.5 M benzoic anhydride and 0.5 M DIPEA in DMF for 5 min.

Oligopeptides were cleaved from the resin using 2.5% triisopropylsilane and 2.5% water in TFA. Upon filtration into cold ether, the peptides and deletions were obtained as white to off-white precipitates. These were collected by centrifugation and washed with ether (3 x 10 mL). The pure oligopeptides were obtained by preparatory scale reverse-phase HPLC by elution with a solvent gradient ramp of 5%:95% to 15%:85% (v/v of 0.1% TFA in acetonitrile/0.1% aqueous TFA) for 10 min with a total flow rate of 20 mL/min. In each case, the oligopeptide was found to be the major peak. The oligopeptides were isolated as white solids following flash evaporation and lyophilization. Yields are given in Table 2-1. Purity was assessed with analytical scale HPLC by elution with a solvent gradient from 0.1% aqueous TFA to 0.1% TFA in acetonitrile (ramped over 100 min) and a flow rate of 1 mL/min. Only one peak was observed in the chromatogram in each case, and the percentage purity from these is given in Table 2-1. Identity of the oligopeptides was confirmed with ESI+ mass spectrometry, and the calculated and observed m/z are given in Table 2-1. Purity, identity, and dryness (following extensive lyophilization) were confirmed with $^1$H NMR. ($^1$H NMR, 300 MHz, d$_6$-DMSO): Compound (1): 3.1-3.65 (m, 6H), 3.85 (d, 2H), 3.98 (s, 1H), 4.05 (s, 1H), 6.75-7.10 (s-s, 1H), 7.22 (m, 4H), 7.54 (m, 4H), 7.75-8.41 (m-m, 1H), 8.60 (t, 2H); Compound (4): 3.10-3.79 (m, 19H), 3.80-4.30 (m, 15H), 7.11 (s, 1H), 7.25 (s, 1H), 7.45 (m, 5H), 7.77 (m, 9H), 8.03 (m, 1H), 8.19-8.59 (m-m, 3H), 8.80 (m, 8H); Compound (5): 3.02-3.73 (m, 23H), 3.51-4.31 (m, 19H), 7.11 (s, 1H), 7.21 (s, 1H), 7.48 (m, 6H), 7.82 (m, 11H), 8.05 (m, 1H), 8.33 (m, 1H), 8.50 (m, 1H), 8.72 (m, 11H); Compound (6): 3.15-
**Table 2-1:** Characterization data for the pyridyl oligopeptides 1, 4, 5, 6, and 10

<table>
<thead>
<tr>
<th>Oligopeptide</th>
<th>1</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>41 mg</td>
<td>45 mg</td>
<td>69 mg</td>
<td>92 mg</td>
<td>192 mg</td>
</tr>
<tr>
<td>Purity (%)</td>
<td>99.1 %</td>
<td>99.0 %</td>
<td>99.8 %</td>
<td>98.0 %</td>
<td>99.3 %</td>
</tr>
<tr>
<td>(M+H)⁺ Found (Calculated)</td>
<td>398.3</td>
<td>1056.6</td>
<td>1274.5</td>
<td>1493.8</td>
<td>1186.7⁵</td>
</tr>
<tr>
<td>Titration Equivalence Point</td>
<td>1.27</td>
<td>4.06</td>
<td>4.88</td>
<td>6.17</td>
<td>10.1</td>
</tr>
<tr>
<td>pKₐ ⁶</td>
<td>5.04</td>
<td>4.45</td>
<td>3.53</td>
<td>3.38</td>
<td>3.8</td>
</tr>
</tbody>
</table>

**Notes:**

a. Percent yield of the final, purified product based on the loading level of the resin.

b. Percentage purity of the product following separation by preparatory scale HPLC, based on the area of the integrated peak on a subsequent analytical scale HPLC injection.

c. Observed m/z peak in the positive ion electrospray ionization mass spectrum versus the predicted value.

d. (M+2H)²⁺ peak.

e. Molar equivalence point (mol OH⁻/mol oligopeptide) determined from the pH titration curve

f. The acid dissociation constant, determined from the half equivalence point of the pH titration curve.
3.75 (m, 26H), 3.75-4.30 (m, 24H), 7.08 (s, 1H), 7.21 (s, 1H), 7.50 (m, 4H), 7.71 (m, 14H), 8.01 (m, 1H), 8.30 (m, 3H), 8.50 (m, 2H), 8.70 (m, 12H); Compound (10): 3.05-3.78 (m, 45H), 4.78-4.30 (m, 37H), 7.10 (s, 2H), 7.25 (s, 2H), 7.45 (m, 11H), 7.78 (m, 19H), 8.00 (m, 1H), 8.28 (m, 4H), 8.47 (m, 3H), 8.70 (m, 16H).

*Acetyl Tetrimer Synthesis (4Ac)*: The acetyl tetramer was prepared using analogous coupling steps by hand with the acetyl monomer (Fmoc-aeg(Ac)-OH) in Figure 2-1. The tetramer was purified by preparatory scale HPLC with a gradient elution of 20%:80% to 50%:50% (v/v 0.1 % TFA in CH$_3$CN/0.1 % TFA in H$_2$O) ramped over 13 min with a total flow of 20 mL/min. The major peak was isolated and dried as above and determined to be the desired tetramer product using mass spectrometry and NMR. ESI$^+$ mass spectrometry: Calculated, (M+H)$^+$ = 865.42; Found (M+H)$^+$ = 865.5. $^1$H NMR, 300 MHz, d$_6$-DMSO: 1.32 (m, 9H), 1.99 (m, 4H), 2.68 (m, 2H), 3.40 (m, 12H), 3.92 (m, 5H), 4.28 (m, 6H), 7.13 (m, 1H), 8.39 (m, 3H), 8.64 (m, 3H), 7.93 (m, 4H), 8.23 (m-m, 1H), 8.85 (m, 3H)

### 2.2.4 pH Titrations

Following lyophilization for up to 72 hours, the dryness of the oligomers 1, 4, 5, 6, and 10 was confirmed by $^1$H NMR; these were then weighed and dissolved in water to a total volume of 5.00 mL. The solutions were then titrated with 0.0994 ± 0.0001 M NaOH (standardized against potassium hydrogen phthalate, KHP) and the pH monitored with an electrode (Accumet, Fisher Scientific) calibrated versus standard buffers until the pH reached ~ 11 - 12. The equivalence point was determined from the x intercept of the
**Figure 2-1:** A) Monomer syntheses; i) 1. CH$_2$Cl$_2$, EDC, HOBT, DIPEA, 4-pyridylacetic acid hydrochloride; 2. 3 M HCl; ii) 1. CH$_2$Cl$_2$, EDC, HOBT, DIPEA, glacial acetic acid; 2. TFA/CH$_2$Cl$_2$, B) Structures of the pyridyl and acetyl oligomers.
second derivative of the titration curve, and the $pK_A$ as the pH value at half-equivalence; these are given in Table 2-1.

2.2.5 Addition of Metal Complexes

Following lyophilization, solutions of 1, 4, 5, 6, and 10 were prepared in spectroscopic grade MeOH and the pyridine concentrations were determined from a calibration curve generated for the pyridine monomer as described previously, using $\varepsilon = 5310 \, \text{M}^{-1}\text{cm}^{-1}$ at 255 nm. The complexation of $[\text{Pt(tpy)}]^{2+}$, $[\text{Pt(pda)}]$, $[\text{Cu(pda)}]$, $[\text{Cu(tpy)}]^{2+}$ with each of the oligomers was monitored by UV-Visible absorption spectroscopy. Solutions of known concentration of $[\text{Cu(pda)}]$ in MeOH; $[\text{Cu(tpy)}]^{2+}$ in 2.5% H$_2$O in MeOH; $[\text{Pt(tpy)}]^{2+}$ in 25% H$_2$O in MeOH; and $[\text{Pt(pda)}]$ in H$_2$O were prepared. Typical concentrations for metals were $\sim 10$ mM for the Cu compounds and 0.1 mM for the Pt species. For each experiment, the same metal complex solution was placed in the sample and reference cuvettes of a double beam spectrophotometer. The oligomer solution was then added in 25 µL increments to the sample beam cuvette; an equivalent volume of solvent was added to the reference cell. In the case of the Cu complexes, solutions were stirred for a minimum of 5 min at room temperature for each iterative addition; the Pt solutions were stirred for 5 min at 50ºC, and then 5 min at room temperature. The UV-Visible absorbance difference spectrum was measured after each addition. Because the oligomer absorbance is similar in wavelength to the Pt complex, the difference spectra were manually corrected for this additional background absorbance.
The Cu-complexed oligopeptides were isolated as powders by precipitation upon addition of ether and dried for a minimum of 2 days under vacuum prior to structural analysis. [Pt(tpy)]^{2+} containing oligomers were precipitated by the addition of saturated aqueous ammonium hexafluorophosphate (NH_4PF_6), washed with water, isopropanol, and ether, and dried in vacuum at 70ºC for 3 days prior to structural analysis and electrochemical characterization. (^1H NMR, 300 MHz, d_6-DMSO):

**[Cu(pda)] containing oligomers:** (1): 3.18-4.30 (m-m, 10H), 6.85-8.79 (m-m, 9H); (4): 2.78-4.49 (m-m, 34H), 6.59-8.35 (m-m, 12H); (5): 2.82-4.40 (m-m, 42H), 6.80-8.95 (m-m, 13H); (6): 2.55-4.19 (m-m, 50H), 6.68-8.73 (m-m, 14H); (10): 2.62-4.30 (m-m, 82H), 6.81-8.78 (m-m, 18H).

**[Cu(tpy)]^{2+} containing oligomers:** (1): 3.02-4.45 (m-m, 10H), 6.49-8.20 (m-m, 9H); (4): 2.90-4.38 (m-m, 34H), 6.76-8.79 (m-m, 12H); (5): 3.05-4.40 (m-m, 42H), 6.91-8.90 (m-m, 13H); (6): 2.90-4.35 (m-m, 50H), 6.88-8.79 (m-m, 14H); (10): 2.78-4.20 (m-m, 82H), 6.87-8.79 (m-m, 18H).

**[Pt(tpy)]^{2+} containing oligomers:** (1): 3.40-3.89 (m, 6H), 4.05 (s, 1H), 4.15 (s, 1H), 4.20 (s, 1H), 4.35 (s, 1H), 7.20 (s-s, 1H), 8.46 (m, 3H), 8.88 (m, 5H), 8.97 (m, 2H), 8.29 (m, 1H), 8.65 (m-m, 10H), 8.95 (m, 1H), 9.05 (m, 2H); (4): 2.85-3.50 (m, 18H), 3.50-4.20 (m, 16H), 6.61-7.82 (m-m, 36H), 7.82-8.92 (m-m, 36H); (5): 2.45-4.15 (m-m, 42H), 7.15-7.92 (m-m, 18H), 8.08-8.91 (m-m, 60H); (6): 3.02-4.42 (m-m, 50H), 7.11-8.10 (m-m, 32H), 8.22-9.15 (m-m, 72H); (10): 2.90-4.25 (m-m, 82H), 6.93-7.89 (m-m, 70H), 7.99-9.00 (m-m, 98H).
2.3 Results and Discussion

2.3.1 Oligomer Characterization

The pyridine substituted artificial oligopeptides were prepared via standard automated solid-phase peptide synthesis (SPPS) using an Fmoc protection scheme. The oligomer yields, based on the loading of the solid phase support, range from 29 – 52%, and are equal to or better than those for natural oligopeptides synthesized via SPPS. The oligomer identities were verified with electrospray ionization mass spectrometry, and the purities were assessed by slow gradient ramping analytical scale HPLC after bulk purification (Table 2-1). Lyophilization of the peptides was continued until there was no evidence for the presence of solvent in the $^1$H NMR spectrum. $^1$H NMR data are qualitatively complicated due to the presence of rotamers, however based on the peak integrations these confirm the identity and purity of the desired oligopeptide sequence.\textsuperscript{6b}

The oligopeptides are isolated and purified in the presence of excess amounts of trifluoroacetic acid, so that the pyridyl units are fully protonated as their TFA salts. Because the oligomers are isolated as dry solids following lyophilization, their masses may be measured with reasonable accuracy (and used together with their molecular weights as TFA salts) to prepare solutions of known concentration. Protonated pyridines are weak acids that may be deprotonated by equivalent addition of a strong base, so that neutralization provides a means to accurately determine the amount acidic pyridyl protons per oligomer. Thus titrations are a tool for verification of the identity and relative purity of the oligomer, since significant amounts of impurities or an incorrect oligopeptide molecular weight \textit{(i.e. its identity)} would cause a deviation in the predicted
amount of base required. We therefore utilize pH titrations with NaOH to determine the identity and purity of the oligomers; a representative titration curve is shown in Figure 2A for the pyridyl hexapeptide 6. For oligomers 1, 4, 5, 6, and 10, the titration curves have a single inflection point that indicates the pH at which the concentrations of base and pyridyl acid are the same (i.e. the titration equivalence point). The existence of only one equivalence point for each oligomer indicates that all of the pyridines on the oligomer are simultaneously neutralized, and therefore act independently. The equivalence points for oligomers 1, 4, 5, 6, and 10 are determined from the second derivative of their titration curves, and are given in Table 2-1. These values agree well with those predicted based on the length of the oligopeptides; the small errors in these values most likely arise from uncertainties associated with the oligopeptide concentration due to small amounts of water or salt that were not detectable by NMR. We find that there is a linear relationship between the equivalence point and the number of pyridyl ligands on each oligomer with a slope of 0.99 (Figure 2-2B). We therefore are able to utilize straightforward titrations to quickly assess oligopeptide synthesis products.

Further analysis of the titration curves allows comparison of the acid dissociation constants (pKₐ, the pHs at their half equivalence points) of the pyridine oligopeptides. The pKₐ of the “unimer”, 1, which contains only a single pyridine ligand, is 5.04 and agrees with the known pKₐ for pyridine, 5.23.¹³ Table 2-1 indicates that the pKₐs of the oligopeptides generally decrease as the number of pyridyl monomers increase. This is likely a result of electrostatic repulsions between positively charged, protonated pyridines; as the chain lengthens the larger number of cations increases the electrostatic
**Figure 2-2:** (A) Titration curve of the change in pH as a function of the amount of added 99.4 ± 0.1 mM NaOH to 12.0 µM oligomer 6, plotted in terms of the relative molar quantities of base and oligomer 6; (B) Plot of the equivalence point versus the number of pyridyl groups on each oligopeptide, with linear regression.
force that pushes the pyridines farther apart. Similar observations have been reported for pyridine containing polymers.\textsuperscript{14} The lower pK\textsubscript{A}s of longer oligopeptides suggest an increase in deprotonation efficiency or lower stability of the protonated oligopeptide. This is consistent with the observed changes in the equivalence points, where the pyridyl groups must be far enough apart to deprotonate independently. In the case of 10, which deviates from this trend, the peptide may be long enough so that it can twist to decrease these repulsive forces, and therefore the pK\textsubscript{A} is slightly larger.

2.3.2 Reaction with Cu and Pt Complexes

The oligopeptides 1, 4, 5, 6, and 10 contain pyridyl groups that can also function as ligands in metal complexes. To examine the ability of the pyridine-containing oligopeptides to bind metals, we selected four metal complexes containing either Pt (II) or Cu (II) with one tridentate ligand, [Cu(tpy)]\textsuperscript{2+}, [Pt(tpy)]\textsuperscript{2+}, [Pt(pda)] and [Cu(pda)]. The Pt (II) and Cu (II) in each of these are known to prefer tetracoordinate geometries, so that in the presence of a monodentate pyridine ligand, each should bind in one-to-one fashion. This strategy allows us to confirm that the pyridyl ligands act independently during metal coordination, analogous to the neutralization titrations above, as shown in Figure 2-3.

Formation of a metal-pyridine bond typically leads to the formation of an absorbance peak in the visible region of the spectrum, and we therefore monitored metal complexation using UV-Visible absorption spectroscopy as a function of the relative molar ratios of oligopeptide and metal complex. Representative examples of the change
Figure 2-3: Pyridyl Oligopeptide Coordination to M = Cu (II) or Pt (II) Complexes
in the UV-Visible absorbance during these titrations are shown in Figure 2-4 for each of the metal complexes with pyridine tetramer, 4. In Figure 2-4A, the broad peak at 662 nm (associated with a Cu d-d transition) increases in intensity and levels at a constant absorbance value. Analogous spectra are shown for the [Cu(tpy)]^{2+} (Figure 2-4B) and [Pt(tpy)]^{2+} (Figure 2-4C) complexes. In each case, the Figure 2-4 insets show that the absorbance intensities increase and reach a limiting value at a molar ratio of 4 metal complexes:1 oligopeptide. These data indicate that each of the four pyridine ligands on the oligopeptide are capable of binding one metal complex in a stoichiometric fashion.

We have performed similar titrations using each of the oligopeptides in the series 1, 4, 5, 6, and 10 using the metal complexes [Cu(tpy)]^{2+}, [Pt(tpy)]^{2+}, and [Cu(pda)]. Titrations of oligopeptides 4, 5, 6, and 10 with [Pt(pda)] resulted in precipitation from solution, even with solvents such as DMF and dimethylsulfoxide (which readily dissolve the other metallated oligopeptides), precluding collection of the UV-Visible titration data. The equivalence points for the reaction of each of the oligopeptides and metal salts were determined from the titration curves by least squares fitting of the data and are listed in Table 2-2.

The empirical equivalence points agree well with the predicted stoichiometries for the metal complex ligation of the pyridine substituents. Even the longest oligopeptides are able to bind stoichiometric quantities of metals to create large, multinuclear complexes. Importantly, these titration data show that complexation is independent of metal complex charge, since the [Pt(tpy)]^{2+} and [Cu(tpy)]^{2+} di-cation complexes bind equivalently to the charge-neutral [Cu(pda)] complexes. For the longest oligopeptide, 10, the total charge for the Pt (II) and Cu (II) tpy complexes is +20.
**Figure 2-4:** UV-Visible absorption spectra acquired during the reaction of pyridine tetramer (4) with metal complexes: A) 3.875 mM peptide (4) with 3.25mM [Cu(pda)] in MeOH; B) 2.09 mM peptide (4) with 29.9 µM [Cu(tpy)]²⁺ in 2.5% H₂O in MeOH; and C) 0.124 mM peptide (4) with 99.9 µM [Pt(tpy)]²⁺ in 25% H₂ in MeOH. The insets of each plot show the increases in absorbance at 662, 630, and 336 nm for A, B, and C, respectively.
**Table 2-2:** Stoichiometry of Metal Complexation with the Oligopeptides

**Stoichiometric Equivalent of Metal Complex**

(mol complex/mol oligopeptide)

<table>
<thead>
<tr>
<th>Oligopeptide</th>
<th>[Cu(pda)]</th>
<th>[Cu(tpy)]&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>[Pt(tpy)]&lt;sup&gt;2+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>4.2 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>5.3 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>5.9 ± 0.1</td>
<td>6.0 ± 0.1</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>9.8 ± 0.2</td>
<td>10.3 ± 0.2</td>
<td>9.9 ± 0.2</td>
</tr>
</tbody>
</table>
Purity of the titration products was confirmed using $^1$H NMR, and while these spectra are broadened (compared to the unmetallated oligomers) by the presence of metals, the spectra of all metal-oligopeptide complexes give the expected integrations for the pure, stoichiometrically complexed oligopeptides. In the case of the Cu (II) containing molecules, the aromatic pyridyl protons disappear from the low field region of the spectrum due to the paramagnetic nature of the bound Cu (II) metal center. This result also suggests that attachment of the metals occurs at the desired ligand rather than to the peptide backbone.

2.3.3 Metal Coordination to Acetyl Tetrapeptides

Metal ions are known to bind weakly to secondary amine and amide groups and carbonyl oxygens,\(^{15}\) all of which are plentiful on the oligopeptide backbone. To test the specificity of metal complex coordination with the pyridine ligand sites rather than to these groups on the peptide, we prepared an acetyl-functionalized tetrapeptide, 4Ac (Figure 2-1). The identity and purity of the acetyl tetramer were confirmed with mass spectrometry and $^1$H NMR spectroscopy. This tetramer is equivalent in length to oligopeptide 4 (Figure 2-1) but contains methyl substituents in place of the pyridine ligands, and should not contain any moieties with particularly high affinity for transition metals. To test this, titrations with each of the metal complexes were performed analogously to the experiments with the pyridine oligomers. Figure 2-5 compares titrations of the acetyl and pyridine tetrapeptides. Minor increases in the UV-Visible
Figure 2-5: Change in UV-visible absorption spectra during the titration of acetyl tetramer, (4Ac, —) and py tetramer (4, - - -) with metal complexes: (A) 4.0 mM peptide 4Ac with 3.25 mM [Cu(pda)]in MeOH; (B) 2.1 mM peptide 4Ac and 29.9 µM [Cu(tpy)]$^{2+}$ in 2.5% H$_2$O in MeOH; and (C) 0.13 mM peptide 4Ac with 99.9 µM [Pt(tpy)]$^{2+}$ in 25% H$_2$O in MeOH. The insets compare the change in absorbance for 4Ac (●) and 4 (〇) at the indicated wavelengths.
absorption spectra were observed upon reaction with [Cu(tpy)]^{2+}, but no observable change was seen for the [Pt(tpy)]^{2+} or [Cu(pda)].

The specific nature of the interaction of [Cu(tpy)]^{2+} with the oligopeptide backbone is not known, however, as the inset of Figure 2-5B shows, the absorbance change for titration with 4Ac is far less than that observed for the titration with the pyridine oligomer. The absorbance change associated with backbone interaction is sufficiently minor that it does not affect the stoichiometry of complexation with the pyridine oligomers, which clearly shows a 1:1 ratio for pyridine:[Cu(tpy)]^{2+} bond formation for each length of oligopeptide (Table 2-2). This is most likely due to a competitive equilibrium between binding the backbone and pyridine ligand (in the pyridine oligopeptides), where bond formation with the pyridine ligand would be thermodynamically favored.

### 2.3.4 Molecular Modeling

The titration products are isolated as fine powders, and while their purity is confirmed by NMR spectroscopy, we have been unable to grow crystallographic quality crystals to date. To understand the structures of the oligopeptides, we have turned to molecular modeling. Simple molecular mechanics calculations were performed to obtain the energy minimized structures of both the protonated pyridine oligopeptides and [Pt(tpy)]^{2+}-coordinated structures; the latter of these are shown in Figure 2-6 for the series 1, 4, 5, 6, and 10. Note that charge compensating counteranions are not shown in
Figure 2-6: Energy minimized structures of \([\text{Pt(tpy)}]^2\)\(^{2+}\)-coordinated series of pyridine oligopeptides, with the H atoms omitted for clarity. Scale bar is 2 nm. See section 2.2.2 for parameters.
these structures, but would contribute to their overall hydrodynamic size, and enable folding.

The calculated structures reveal that despite favorable $\pi-\pi$ interactions of the aromatic ligands, electrostatic repulsions between the $[\text{Pt(tpy)}]^2^{\text{+}}$ complexes push these apart to distances of $\sim 1.5 \text{ nm}$. While the models have linearly extended structures of increasing length as the number of pyridyl units increase, the peptide backbone is expected to be highly flexible in solution, allowing for conformationally dynamic structures. The models in Figure 2-6 indicate that the long axis of the multimetallic peptides increases by a factor of $\sim 7$ from compound 1 to 10 in the series.

2.3.5 Electrochemistry

While the electrochemical characterization of the Cu (II) complexed oligopeptides was precluded by apparent adsorption and electrochemical irreversibility, the cyclic voltammetry of each of the $[\text{Pt(tpy)}]^2^{\text{+}}$ decorated oligopeptides 1, 4, 5, 6, and 10 contains two sequential reduction waves at formal potentials of $-0.6$ and $-1.1 \text{ V vs. SCE}$. Representative cyclic and differential pulse voltammograms are shown in Figure 2-7 for the complexes $[(\text{Pt(tpy)})(1)]^{2^{\text{+}}}$ and $[(\text{Pt(tpy)})(5)]^{10^{\text{+}}}$. Based on reported voltammetry for the small molecule analog $[\text{Pt(tpy)(py)}](\text{PF}_6)_2$, these reactions are tpy-centered reductions (with some possible mixing of Pt character);$^{16,17}$ the formal potentials are given in Table 2-3 for each of the complexes. For the series of metallated oligopeptides, the peak currents for the cathodic and anodic reactions are equivalent, indicating that the two reactions are chemically reversible. It is noteworthy that there is no evidence for
Figure 2-7: Cyclic voltammograms of (A) 1.35 mM [Pt(tpy)(1)]²⁺ and (B) 0.258 mM [(Pt(tpy))₅(5)]¹⁰⁺ solutions containing 0.2 M TBAP in DMF, using a glassy carbon working electrode and potential scan rate of 50 mV/s. Insets contain reductive scan differential pulse voltammograms.
Formal potential measured as the average peak potential for the reaction in V vs SCE, measured using a potential scan rate of 50 mV/s.

Difference in the cathodic and anodic peak potentials in the cyclic voltammogram, measured at a potential scan rate of 50 mV/sec.

Separation between the first and second reduction reactions, measured as the difference in formal potential.

Calculated from the slopes of the Cottrell plots from chronoamperometry (Figure 2-8) and analyzed using Eq. (2.2).

Calculated from the diffusion coefficient and viscosity of DMF (0.794 mPa·s)\(^{19}\) using Eq. (2.3).

### Table 2-3: Electrochemical Data for Pyridine Oligopeptides with Bound [Pt(tpy)\(^{2+}\)]

| Oligomer | [Pt(tpy)]\(^{2+/1+}\) | [Pt(tpy)]\(^{1+/0}\) & \(\Delta E^o\) (V) & \(\Delta E_p\) (mV) & \(D \times 10^6\) (cm\(^2\)/s) & \(a\) (Å) |
|----------|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1        | 1                         | -0.59           | 74              | -1.14           | 74              | 0.55            | 11 ± 1          | 2.5 ± 0.3       |
| 4        | 4                         | -0.58           | 85              | -1.13           | 74              | 0.55            | 8 ± 1           | 3.4 ± 0.6       |
| 5        | 5                         | -0.60           | 80              | -1.14           | 72              | 0.54            | 9 ± 1           | 3.1 ± 0.5       |
| 6        | 6                         | -0.60           | 83              | -1.14           | 68              | 0.55            | 6.2 ± 0.8       | 4.4 ± 0.7       |
| 10       | 10                        | -0.57           | 67              | -1.13           | 84              | 0.56            | 1.2 ± 0.4       | 22 ± 8          |

\(^a\) Formal potential measured as the average peak potential for the reaction in V vs SCE, measured using a potential scan rate of 50 mV/s.

\(^b\) Difference in the cathodic and anodic peak potentials in the cyclic voltammogram, measured at a potential scan rate of 50 mV/sec.

\(^c\) Separation between the first and second reduction reactions, measured as the difference in formal potential.

\(^d\) Calculated from the slopes of the Cottrell plots from chronoamperometry (Figure 2-8) and analyzed using Eq. (2.2).

\(^e\) Calculated from the diffusion coefficient and viscosity of DMF (0.794 mPa·s)\(^{19}\) using Eq. (2.3).
adsorption to the electrode surface for even the largest oligopeptide, which undergoes a change in charge from +20 to neutral upon reaction with 20 electrons. In all cases, the average difference between peak potentials ($\Delta E_p$) is $\sim 75$ mV, and neither $E^0$ nor $\Delta E_p$ were found to be dependent on oligopeptide length (Table 2-3). Together, these data lead to the conclusion that the attached $[\text{Pt(tpy)}]^2^{+}$ redox centers are electronically isolated and behave independently; that is, each of the two reactions are electrochemically quasi-reversible, one-electron reductions. These data imply that the greater charge of the Pt complexes creates electrostatic repulsions that push the dicationic redox centers to distances that would prevent electronic communication, consistent with the calculated molecular models in Figure 2-6.

Since the redox centers are apparently reacting with the electrode surface independently (i.e., $n = 1$ reactions), it is expected that the amount of observed current should increase as the peptide chain length and attached $[\text{Pt(tpy)}]^2^{+}$ increases. In Figure 2-8, the cyclic voltammograms of the metallated complexes of oligopeptides 4, 6 and 10 obtained at the same potential scan rate (50 mV/s) are normalized for the concentration of $[\text{Pt(tpy)}]^2^{+}$ and overlaid. The relative amount of current observed during the voltammetry decreases with the size of the multimetallic oligopeptides. To understand this, plots such as that shown in the Figure 2-8 inset examine the relationship between cathodic peak current ($i_p$) of the first reduction reaction as a function of scan rate. For an electrochemically reversible reaction, these are related by the equation:

$$i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C V^{1/2}$$

Eq. (2.1)
Figure 2-8: Cyclic voltammograms of the metallated oligopeptides \([(\text{Pt(tpy)})_4(4)]^{8+} (- - -); [(\text{Pt(tpy)})_6(6)]^{12+} (- - -)\) and \([(\text{Pt(tpy)})_{10}(10)]^{20+} (---)\), normalized for the concentration of \([\text{Pt(tpy)}]^{2+}\), in solutions containing 0.2 M TBAP in DMF. Potential scan rates were 50 mV/s. Inset is plots of the peak cathodic currents versus the square root of the scan rate for 4 (○), 6 (▼), and 10 (●) with bound \([\text{Pt(tpy)}]^{2+}\).
where \( n \) is one electron; \( A \) is the surface area of the working electrode (cm\(^2\)); \( C \) is the concentration of the reactant (mol/cm\(^3\)); \( D \) is the diffusion coefficient (cm\(^2\)/s); and \( \nu \) is the potential scan rate (V/s). Based on the linearity of the plots in the Figure 2-8 inset, the reduction reactions are diffusion controlled.

However, because the reactions are quasi-reversible, the use of Eq. (2.1) to evaluate the slopes in Figure 2-8 ultimately underestimates \( D \), and we instead use potential step chronoamperometry to analyze the mass transport rate of each of the [Pt(tpy)]\(^{2+}\) metallated oligomers. Figure 2-9 shows the linearized current-time transients that are the result of application of a large overpotential to the mass transport limited region of the first reduction wave (-1.0V) for the [Pt(tpy)]\(^{2+}\)-complexed 1, 5 and 10 oligopeptides. The linearity of these plots is again indicative of diffusion-controlled reactions. These data were used to calculate the diffusion coefficients of each molecule using the Cottrell equation:

\[
i = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}}
\]

using the known concentration of [Pt(tpy)]\(^{2+}\) in each oligopeptide sample, and are listed in Table 2-3. As shown in the Figure 2-9 inset, the measured diffusion coefficients decrease as the number of pyridine ligands (i.e. length) on the oligomer increases. This relationship is expected, since the mass transport of molecules is inversely proportional to their size and is described by the Stokes-Einstein equation:

\[
D = \frac{k_BT}{6\pi\eta a}
\]
Figure 2-9: Linearized current-time transients resulting from an applied potential step of -1.0V for the metallated oligopeptides [Pt(tpy)(1)]^{2+} (●), [(Pt(tpy))_{5}]^{10+} (○), and [(Pt(tpy))_{10}(10)]^{20+} (□), with linear regressions. Inset: Plot of the measured diffusion coefficient vs the number of pyridine ligands per oligopeptide, with a line to guide the eye.
where $k_b$ is Boltzman’s constant; $T$ is the absolute temperature; $\eta$ is the solution viscosity; and $a$ is the hydrodynamic radius of the molecule. It is evident from the molecular modeling that the length of the molecule increases by $\sim 7$ fold; the electrochemical data show that over the same series of molecules, the calculated $D$ decreases by an order of magnitude, so that there is a slight deviation from the relationship described by Eq. (2.3). This could arise from the large number of counteranions and solvent molecules that diffuse with the metallated oligopeptides and contribute to their overall hydrodynamic radii, but is most likely a result of the roughly cylindrical molecular shape of these multimetallic structures. Moreover, the calculated hydrodynamic radii in Table 2-3 are physically reasonable and likely reflect this convolution of the different axes lengths.

2.4 Conclusion

We have presented the facile construction of a series of supramolecular structures containing multiple and variable metal complexes tethered to pyridine-substituted oligopeptide scaffolds. These self-assembled structures may provide new strategies for chelation-based molecular recognition, and their unique optical and electronic properties could find utility in biomolecular recognition.
2.5 References


18. Concentration of [Pt(tpy)]^{2+} is taken as the known concentration of oligopeptide multiplied by the number of attached Pt complexes.

3.1 Introduction

Over the last 30 years\(^1\) the burgeoning field of molecular electronics has encompassed materials ranging from conducting polymers\(^2\) to porphyrin-based multichromophore assemblies\(^3\) to self-assembled monolayers\(^4\) to be utilized in the construction of wires, switches, and gates. Double-stranded DNA (dsDNA) is a particularly attractive candidate for molecular wires because its self-recognition is ideally suited for bottom-up construction of potentially long, linear structures.\(^5\) The incorporation of metal atoms into the dsDNA duplex\(^6\) (or even the peptide nucleic acid, PNA, analogs)\(^7\) might be expected to increase the number of addressable redox couples, improve electron transport kinetics, and improve the conductivity of dsDNA by insertion of redox sites. However, metal ion chelation with nucleic acids and the phosphoester backbone is known to occur, which would create defect sites along the desired molecular wire. In addition, the redox chemistry of nucleic acids limits the accessible voltage range that can be applied since irreversible oxidative cleavage occurs at anodic potentials.\(^8\) We have therefore sought to create analogous supramolecular structures that self-assemble solely upon metal chelation, creating more robust bonds and less dynamic structures than DNA and PNA, while simultaneously providing accessible and reversible electron transfer pathways.
In our inorganic self-assembly motif, molecular recognition between artificial oligopeptides is achieved when the denticity of the ligands provide coordinative saturation for a metal ion. For example, tetracoordinate metals would be expected to form interstrand links between oligopeptides containing bidentate ligands (i.e. $2 + 2$) or a monodentate and a tridentate ligand (i.e. $1 + 3$) (Figure 3-1A and B). Six-coordinate metals would instead crosslink oligopeptides containing tridentate ligands (i.e. $3 + 3$) as in Figure 3-1C. Selective crosslinking of two strands in this manner is analogous to base pairing in DNA: by tethering “complementary” ligands to oligopeptides, metal chelation forms double-stranded structures. The artificial oligopeptides are scaffolds that hold these complexes in a geometry that should enable electron transfers between proximal metals; the metal-linked oligopeptide duplexes can thus serve as model molecular wires.

We have recently reported the synthesis of a series of ligand-substituted oligopeptides based on an aminoethylglycine (aeg) backbone, which is superior to the phosphoester framework of DNA in terms of enhanced stability and solubility in a variety of environments and lower affinity with metal species. These artificial oligopeptides were used to stoichiometrically complex metals and form supramolecular structures; double-stranded duplexes formed when bipyridine-containing artificial tripeptides were crosslinked by Cu(II) ions. While these metal-oligopeptide assemblies possess unique spectroscopic and electrochemical properties that are dictated by the identities of the metal and ligand and the oligopeptide length, the slow electron transfer kinetics of the Cu(II/I) reaction limits the use of that peptide duplex. To study the dynamics of transport in these one-dimensional molecular wires, different redox centers with a range of electron transfer rates and formal potentials are necessary.
Figure 3-1: Representation of metal-chelation based molecular recognition of artificial oligopeptide strands: (A) tridentate-monodentate and (B) bidentate-bidentate ligand pairs are selectively linked by tetracoordinate metals. (C) The tridentate-tridentate ligand pair is crosslinked by hexacoordinate metals.
We have therefore begun to incorporate six-coordinate metals, with their well-known rich and widely varying redox chemistry, into the double-stranded oligopeptide scaffolds. To accomplish this, new artificial oligopeptides containing the tridentate ligand phenyl terpyridine (Figure 3-2) have been prepared. These are reacted with either Co(II) or Fe(II) to create metal bis(phenyl terpyridine) complexes that cross-link the peptide strands. This work presents the synthesis and characterization of the oligopeptides and their Fe and Co complexes, and investigates their solution and film electrochemical and spectroelectrochemical behavior as a function of the length (i.e. number of metals) of the oligopeptides. These experiments represent the first demonstration of these inorganic DNA mimics as electrochemically and chemically reversible structures and pave the way to their use as molecular wires.

3.2 Experimental

3.2.1 Chemicals

All materials were reagent grade, purchased from Acros and used as received unless otherwise noted. Water was obtained from a nanopure water system (Barnstead, 18.2 MΩ). N’,N-diisopropylethylamine (DIPEA), acetonitrile (ACN) was distilled over CaH₂ under N₂. Tetrabutylammonium perchlorate (TBAP) was recrystallized 3x from ethyl acetate and dried in vacuo. Ferrocene (Fc) was purified by sublimation.
**Figure 3-2:** Monomer Synthesis i) NBS, AIBN, Benzene; ii) 1. NaCN, DMSO, 2. conc. HCl; iii) 1. Fmoc-aeg-otBu, EDC, HOBt, DIPEA, CH₂Cl₂, 2. 2.5% triisopropylsilane in TFA.
3.2.2 Instrumentation and Analysis

Preparatory and analytical scale reverse-phase high performance liquid chromatographies (HPLC) were performed with a Varian system equipped with two quaternary pumps (Model 210), an autosampler (Model 410), UV-Vis detector (Model 320), fraction collector (Model 701) and C-18 columns. Oligomer elution was monitored at 254 nm.

The UV-Visible absorption spectra were obtained with a double beam spectrophotometer (Varian, Cary 500). Positive ion electrospray mass spectrometry (ESI+) was performed at the Penn State Mass Spectrometry Facility using a Mariner mass spectrometer (Perseptive Biosystems.) All NMR spectra were collected on either 300 or 400 MHz spectrophotometers (Bruker). Diffuse reflectance infrared spectra were collected on a Varian FTS 7000 spectrophotometer using KBr.

All solution phase electrochemical measurements were obtained with a CH Instruments potentiostat (Model 660) with a 0.22 cm diameter Pt working and Pt wire counter electrodes with a Ag/Ag+ reference electrode. Solutions were prepared from distilled ACN containing 0.15 M TBAP supporting electrolyte and 20% H\textsubscript{2}O. After obtaining voltammograms, Fc was added as an internal potential reference to convert to the SCE scale.\textsuperscript{11}

Electrochemical analysis of films was conducted on either 0.22 cm diameter Pt or indium-tin oxide (ITO) coated glass working electrodes with Pt wire counter and Ag/Ag+ reference electrode. Following film deposition, the coated electrodes were washed
copiously with ACN, and immersed in solutions containing only supporting electrolyte (0.2 M TBAP in ACN) for electrochemical and spectroelectrochemical measurements.

3.2.3 Synthesis

The synthesis of N-[2-(Fmoc aminoethyl]glycinate tert-butylester hydrochloride (Fmoc-aeg-OtBu·HCl)\textsuperscript{12} and 4’-(4-bromomethyl-phenyl)-[2,2’;6’2’’]terpyridine (tpy-ph-CH\textsubscript{2}Br)\textsuperscript{13} have been previously reported.

4’-p-tolyl-[2,2’;6’2’’]terpyridine (tpy-ph-CH\textsubscript{3}). The synthesis of tpy-ph-CH\textsubscript{3} follows a modified literature preparation.\textsuperscript{14} p-Tolualdehyde (14 mL, 0.12 mol, Alfa Aesar), 2-acetylpyridine (29 mL, 0.26 mol), 200 mL of methanol, 200 mL of conc ammonium hydroxide and 20 mL of 15% KOH were refluxed for 2 days. The mixture was cooled to room temperature, diluted with water (300 mL), and extracted with ether (6 x 200 mL.) The combined ether layers were extracted with water (2 x 100 mL) and evaporated to yield a brown sludge. The product was isolated as white crystals by recrystallization from 95:5 ethanol:water. Yield = 22.2 g (58.1\%) \textsuperscript{1}H NMR (300 MHz, d\textsubscript{6}-DMSO): 2.31 (s, 3H), 7.30 (d, 2H, J = 6 Hz), 7.42 (t, 2H, J = 6 Hz), 7.75 (d, 2H, J = 12 Hz), 7.95 (t, 2H, J = 15 Hz), 8.51 (d, 2H), 8.56 (s, 2H), 8.67 (d, 2H).

(4-[2,2’;6’2’’]terpyridin-4’-yl-phenyl)-acetic acid (tpy-ph-CH\textsubscript{2}COOH). A 7.42 g amount of tpy-ph-CH\textsubscript{2}Br (0.0185 mol) and 5.00 g NaCN (0.102 mol) in 125 mL DMSO were stirred at 70 °C for 18 hr. The mixture was cooled to room temperature and unreacted
NaCN was decomposed by addition of conc HCl (ca. 10 mL). The mixture was brought to a pH = 7 with 6 M NaOH and poured over 600 g ice. The resulting yellow precipitate was collected by filtration and washed with water. The solid was suspended in 50 mL conc. HCl and refluxed for 4 h. The mixture was poured over ice water (500 mL) and brought to a pH = 5 with 6 M NaOH. The tan solid was collected, rinsed with water, isopropanol, and ether, and then dried at 100 °C in vacuo for 24 h. Yield = 5.14 g (75.9 %) 

\(^1\)H NMR (300 MHz, d\textsubscript{6}-DMSO): 3.59 (s, 2H), 7.41 (d, 2H), 7.50 (t, 2H), 7.87 (d, 2H, J = 15 Hz), 8.02 (t, 2H, J = 9 Hz), 8.61 (d, 2H), 8.67 (s, 2H), 8.72 (d, 2H), 12.39 (s, 1H); FTIR (KBr, cm\textsuperscript{-1}): ν 2401-3552 (COOH), 2360 (COO), 2339 (COO), 1716 (C=O)

\{2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-[2-(4-[2,2';6',2"]-terpyridin-4'-yl-phenyl)-acetyl]-amino\}-acetic acid, trifluoroacetic acid salt (Fmoc-aeg(ph-tpy)-OH·TFA). A 5.14 g quantity of the tpy-ph-CH\textsubscript{2}COOH (0.0140 mol), 2.75 g EDC (0.0144 mol), and 2.01 g HOBt (0.0149 mol) were suspended in 300 mL CH\textsubscript{2}Cl\textsubscript{2} and the mixture stirred for 15 min. Fmoc-aeg-OtBu·HCl (4.85 g, 0.0112 mol) was then added, followed by 8.0 mL DIPEA (0.046 mol.) The mixture was allowed to stir for 24 h and then extracted with H\textsubscript{2}O (3x100 mL). The organic layer was dried over anhyd Na\textsubscript{2}SO\textsubscript{4}, separated from the drying agent, and flash evaporated to yield a brown oil. The oil was chromatographed on a silica column eluting with a gradient (CH\textsubscript{2}Cl\textsubscript{2} to 5% methanol in CH\textsubscript{2}Cl\textsubscript{2}). The tBu protected product was isolated as the major band containing tpy. Following flash evaporation and drying in vacuo, the foam product was stirred in 2.5% triisopropylsilane in 20 mL TFA for 3 h. The carboxylic acid product was obtained as a yellow powder by precipitation from 300 mL of sonicating ether, washed with ether and
dried in vacuo. Yield = 6.69 g (86.5 %) ESI+: found (cald.) 690.27 (690.27) 

$^1$H NMR (300 MHz, d$_6$-DMSO): 3.23 (bm, 2H), 3.51 (m, 2H), 3.79 (s, 1H), 3.91 (s, 1H), 4.05 (s, 1H), 4.32 (m, 2H), 4.41 (d, 1H), 7.32-7.60 (m-m, 7H), 7.75 (m, 5H), 7.92 (d, 4H), 8.28 (t, 2H), 8.88 (m, 6H): FTIR (KBr, cm$^{-1}$): $\nu$ 3333 (OH), 2389-3477 (COOH), 2359 (COO), 2333 (COO), 1651 (C=O)

**Oligomer Synthesis (1-4):** The oligomers 1 - 4 were prepared by hand via solid-phase peptide synthesis. The oligopeptides were prepared in DMF at a 0.1 mmol scale using Fmoc-PAL-PEG-PS resin (Applied Biosystems). Fmoc deprotection was performed for 15 min with 20% piperidine in DMF. Couplings were carried out using a four-fold molar excess of monomers for 18 h with solutions of 0.5 M HBTU and 1 M DIPEA. Capping steps were performed after each coupling step using 0.5 M benzoic anhydride and 0.5 M DIPEA in DMF for 15 min.

Oligopeptides were cleaved from the resin using 2.5% triisopropylsilane and 2.5% water in TFA by stirring the resin for 3 h in this solution. Upon filtration into 160 mL cold ether, the crude products were obtained as off-white precipitates. These were collected by centrifugation and washed with ether (3 x 40 mL). The oligopeptides were purified by preparatory scale reverse-phase HPLC by elution with a solvent gradient ramp of 15:85 to 37:63 (v/v of 0.1% TFA in ACN/0.1% aqueous TFA) for 25 min with a total flow rate of 5 mL/min. In each case, the oligopeptide was found to be the major peak. The oligopeptides were isolated as films following flash evaporation and were further dried by lyophilization (72 h) to yield off-white fluffy solids. Yields are provided in **Table 3-1.** Identity of the purified oligopeptides was confirmed with ESI+, and the
<table>
<thead>
<tr>
<th>Length</th>
<th>Yield (mg)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30.7 (41.4 %)</td>
<td>29.6 (22.7 %)</td>
<td>41.0 (21.9 %)</td>
<td>54.5 (22.4 %)</td>
<td></td>
</tr>
<tr>
<td>ESI+</td>
<td></td>
<td>628.2</td>
<td>1077.5</td>
<td>1526.6</td>
<td>1974.8</td>
</tr>
<tr>
<td>Found</td>
<td>(Calculated)</td>
<td>(628.26)</td>
<td>(1077.44)</td>
<td>(1526.63)</td>
<td>(1974.81)</td>
</tr>
</tbody>
</table>
calculated and observed m/z are also given in Table 3-1. Purity, identity and dryness (following extensive lyophilization) were confirmed with $^1$H NMR. Additional characterization was performed by HMQC NMR and variable temperature $^1$H NMR for I.$^{15}$ $^1$H NMR (400 MHz, d$_6$-DMSO): (I): 3.44 (m, 1H), 3.54 (m, 2H), 3.64 (m, 2.5H), 3.72 (m, 1.5H), 3.87 (s, 1H), 4.03 (s, 1H), 4.21 (s, 1H), 7.10 (m, 1H), 7.21 (bs, 0.5H), 7.39 (m, 2.5H), 7.50 (m, 3H), 7.62 (t, 2H), 7.83 (m, 4H), 8.14 (t, 2H), 8.28 (t, 0.5H), 8.42 (t, 0.5H), 8.48 (t, 0.5H), 8.75 (m, 4.5H), 8.82 (m, 2H); (2): 3.35-3.77 (m, 10H), 3.82-3.93 (m, 3H), 3.94-4.08 (m, 4H), 4.20 (m, 1H), 7.11 (m, 1H), 7.22 (bs, 0.5H), 7.26-7.60 (m, 12H), 7.61-7.90 (m, 6H), 7.93-8.13 (m, 4H), 8.23-8.37 (mm, 1.5H), 8.42-8.85 (m, 13H); (3): 3.21-3.71 (m, 16H), 3.75 (m, 2H), 3.85 (bs, 1H), 3.91 (bs, 1H), 4.04 (m, 4H), 4.20 (m, 2H), 7.12 (bs, 1H), 7.19-7.68 (m, 19H), 7.68-7.96 (m, 6.5H), 7.96-8.22 (m, 6.5H), 8.22-8.92 (m, 20H); (4): 3.21-3.77 (m, 19H), 3.80-4.25 (m, 15H), 7.12 (bs, 1H), 7.18-8.07 (m, 37H), 8.10-8.85 (m, 29H)

3.2.4 Metal Complexation

Following lyophilization, solutions of I - 4 were prepared in spectroscopic grade methanol (MeOH) and the phenyl terpyridine concentrations were determined from a calibration curve generated for the phenyl terpyridine monomer (Fmoc-aeg(ph-tpy)OH·TFA) as described previously,$^{9a}$ using its measured molar extinction value of 39540 M$^{-1}$cm$^{-1}$ at 279 nm. The complexation of cobalt (II) perchlorate hexahydrate (Co(ClO$_4$)$_2$·6H$_2$O, Aldrich) and iron (II) perchlorate hexahydrate (Fe(ClO$_4$)$_2$·6H$_2$O, Aldrich) with each of the oligomers was monitored by UV-Visible absorption
spectroscopy at their respective MLCT bands, 514 and 567 nm. Solutions of metal complex in MeOH were prepared and the metal complex concentrations measured with their extinction coefficients (2.74 x 10^4 and 2.45 x 10^3 M^-1 cm^-1 for the Fe and Co species, respectively). For each experiment, the same metal solution was placed in the sample and reference cuvettes of a double beam spectrophotometer. The oligomer solution was then added in 25-50 µL increments to the sample beam cuvette; an equivalent volume of solvent was added to the reference cell. For each iterative addition the solutions were stirred for a minimum of 5 min at room temperature, after which the UV-Visible absorbance spectra were measured. The MeOH solutions of the metal-oligomer complexes were evaporated to dryness and lyophilized for a minimum of 72 h prior to electrochemical analysis and structural characterization. ESI+ found (cald.):

[Co(I)_2](ClO_4)_2: M^{2+} = 656.7 (656.7); [Co_2(2)_2](ClO_4)_4: M^{4+} = 567.99 (567.69);

[Fe(I)_2](ClO_4)_2: M^{2+} = 655.3 (655.2); [Fe_2(2)_2](ClO_4)_2: M^{4+} = 566.49 (566.19); ^1H NMR (400 MHz, d_6-DMSO): [Fe(I)_2](ClO_4)_2: 3.56-3.63 (m, 1.5H), 3.66-3.74 (m, 4H), 3.77-3.93 (m, 8H), 3.96 (s, 2.5H), 4.08 (s, 2.5H), 4.32 (s, 1.5H), 6.40 (bs, 1.5H), 6.68 (bs, 2H), 7.10-7.35 (m, 11H), 7.45-7.73 (m, 10.5H), 7.82 (d, 1.5H), 7.93 (m, 6H), 8.15 (d, 2H), 8.23 (m, 3.5H), 8.61 (m, 4H), 9.12 (s, 2H), 9.18 (s, 2H); Co(I)_2(ClO_4)_2: 3.52-4.15 (m 6H), 4.25 (m, 6.5H), 4.37 (m 3H), 4.55 (t, 1.5H), 4.65 (s, 1.5H), 4.72 (s, 0.5H), 5.02 (s, 1H), 6.35 (m, 1.5H), 6.60 (s, 0.5H), 6.80 (s, 0.5H), 7.15 (m, 1.5H), 7.30 (m, 0.5H), 7.35-7.68 (m 2.5H), 7.75-8.09 (m, 7H), 8.13-8.39 (m, 2.5H), 8.55 (d, 2.5H), 8.68 (bs, 1H), 8.80-9.05 (m, 5H), 9.20 (s, 0.25H), 9.25 (s, 0.25H), 9.38 (bs, 2.5H), 13.63 (m, 3H).
3.3 Results and Discussion

3.3.1 Oligopeptide Synthesis and Characterization

To use octahedral metals to form crosslinks between artificial oligopeptide strands, the tridentate phenyl terpyridine ligands were incorporated onto the aminoethylglycine scaffold. Following synthesis of the acetic acid phenyl terpyridine ligand (tpy-ph-CH$_2$COOH) by acid-catalyzed hydrolysis of the nitrile derivative, this was coupled to the secondary amine of Fmoc-aeg-OtBu·HCl using EDC/HOBt, and the tBu removed by acid hydrolysis to give the new tridentate monomer Fmoc-aeg-(ph-tpy)OH·TFA (Figure 3-2). The artificial oligopeptides 1 - 4 shown in Figure 3-3 were then synthesized using solid phase peptide synthesis and obtained in yields of 20 - 40 % (Table 3-1), only slightly lower than those observed for peptide coupling of pyridine-substituted aeg monomers.$^9b$ Following purification of oligopeptides 1 - 4 by preparatory scale HPLC, the purity and identity of the products was confirmed by NMR and positive ion electrospray mass spectrometry (Table 3-1).

The $^1$H NMR spectra yielded the expected relative $^1$H integrations for each species. However, similar to our pyridine-substituted analogs$^{15}$ and PNA$^{16}$ structures, the NMR spectra are complicated by the presence of rotamer species. Figure 3-4 contains a series of $^1$H NMR spectra obtained at variable temperatures$^{15}$ to demonstrate the effect of rotamers on the spectra of I. As the temperature is increased, coalescence of the peaks to the number that is expected (i.e., in the absence of rotamers) is indicative of rotamer structures at low temperature that give rise to chemically non-equivalent $^1$H with two distinct signals. For example, this is clearly shown between 3.4 - 4.2 ppm, where a
Figure 3-3: Structures of Oligopeptides 1 - 4
Figure 3-4: Temperature dependent $^1$H NMR (400 MHz) spectra of $I$ in d$_6$-DMSO.$^{15}$
series of 8 peaks observed at room temperature collapse at high temperature to the 5 sharp peaks that are expected in this region. The complexity of the room temperature spectra of the oligopeptides is exacerbated as the oligopeptide length, and corresponding number of chemically non-equivalent protons, increases. $^1$H NMR spectra of 2 - 4 therefore consist of broad multiplets that are analyzed by comparison of the $^1$H integrations: in all cases, the spectra for 1 - 4 yield the expected relative (i.e. aromatic and amide vs. aliphatic) number of protons. To conclusively confirm their identity and purity, complete assignment of the peaks observed in HMQC spectra (and COSY in the case of 1) obtained for 1 - 4 was performed.\textsuperscript{15}

3.3.2 Metal Complexes

Terpyridine ligands are known to chelate Co(II) and Fe(II) to form [M(tpy)$_2$]$^{2+}$ complexes;\textsuperscript{17} addition of Co(II) and Fe(II) to the phenyl terpyridine substituted oligopeptides was expected to similarly cause formation of six-coordinate metal complexes and form multimetallic supramolecular structures. Therefore, each of the artificial oligopeptides 1 - 4 was reacted with Co(ClO$_4$)$_2$ and Fe(ClO$_4$)$_2$ in methanolic solutions with the aim of producing the metal-linked peptide duplexes depicted in Figure 3-5. Metal complexation was followed by monitoring the change in the absorbance of the solution at wavelengths associated with the metal-to-ligand charge transfer (MLCT) bands for [Co(ph-tpy)$_2$]$^{2+}$ (514 nm) and [Fe(ph-tpy)$_2$]$^{2+}$ (567 nm),\textsuperscript{18} as shown in Figure 3-6. In each case, the MLCT absorbance increased and leveled at values that are
Figure 3-5: Structures of Metallated Oligopeptides 1 - 4

M = Co, Fe
Figure 3-6: Titration curves for the change in absorbance upon addition of oligopeptide to (A) Co (II) at 514 nm and (B) Fe(II) at 567 nm for oligopeptides (■) 1, (●) 2, (♦) 3, (▼) 4 in methanolic solutions. Insets show representative absorbance difference spectra acquired during titration with 3.
consistent with the stoichiometric reaction to make \([\text{M(ph-tpy)}_2]^{2+}\) complexes. The equivalence points were determined from the plots in Figure 3-6 as described previously.\textsuperscript{9b} For example, in the reaction of Co(II) with 3, the equivalence point is reached when the molar ratio is 1.5 Co: 1 oligopeptide, or 3 Co: 2 oligopeptides. The calculated equivalence points for each of the oligopeptides upon reaction with Fe and Co are listed in Table 3-2. These data do not distinguish between the single-stranded structures, crosslinked peptide duplexes, or polymers that are possible for 2 – 4. To analyze the products of these reactions, the titration products were isolated and dried before characterization by spectroscopic and electrochemical methods.

In the case of the Fe and Co complexes with 1, in which polymer and intrastrand bond formation is not possible, ESI\textsuperscript{+} and \textsuperscript{1}H NMR data were obtained.\textsuperscript{15} Similar to the oligopeptide \textsuperscript{1}H NMR, the metallated species spectra are complicated by the presence of rotamers and further broadened and split by metal complexation and the likely through-space oligopeptide interstrand proton interactions. In the case of the paramagnetic Co(II) complex, the aromatic ligand protons are shifted downfield. Nonetheless, the relative integrations of the \textsuperscript{1}H NMR spectra of the Co and Fe complexes of 1 are consistent with the pure \([\text{M(1)}_2]^{2+}\) species; observation of the molecular ion peak in the ESI+ mass spectra additionally identify these products. ESI\textsuperscript{+} mass spectra were also obtained for the products of the reaction of Fe(II) and Co(II) with oligopeptide 2. In each of these cases, the molecular ion peaks (the \textsuperscript{M}^{4+} ion) conclusively identified the product as two cross-linked oligopeptides linked by two metal ions (\textit{i.e.} \textsuperscript{M}_2(2)_2^{4+}) to form the dimetallic peptide duplexes.
From UV-Vis titration curves at 514 nm and 567 nm for the Co and Fe complexes, respectively.

Formal potential of the oxidation reaction, using the average of the oxidative and reductive peak potentials, obtained from cyclic voltammograms acquired at a potential scan rate of 50 mV/sec in 0.15 M TBAP in ACN.

Difference in oxidative and reductive peak potentials during cyclic voltammetry as in b.

Diffusion coefficients obtained from chronocouletry of the charge transients using Eq. (3.2).

Calculated from the diffusion coefficient and viscosity for acetonitrile (0.369 mPa·s) using Eq. (3.1).

### Table 3-2: Solution Phase Electrochemical Data for Metallated Oligopeptides

<table>
<thead>
<tr>
<th>Equivalence Point&lt;sup&gt;a&lt;/sup&gt; (mol metal/ mol oligomer)</th>
<th>Co</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E&lt;sup&gt;0&lt;/sup&gt; (III/II)&lt;sup&gt;b&lt;/sup&gt; (V vs. SCE)</td>
<td>ΔE&lt;sub&gt;p&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (mV)</td>
</tr>
<tr>
<td>1 0.50 ± 0.01</td>
<td>0.257</td>
<td>74</td>
</tr>
<tr>
<td>2 0.95 ± 0.01</td>
<td>0.266</td>
<td>90</td>
</tr>
<tr>
<td>3 1.44 ± 0.01</td>
<td>0.268</td>
<td>99</td>
</tr>
<tr>
<td>4 2.0 ± 0.1</td>
<td>0.277</td>
<td>83</td>
</tr>
<tr>
<td>1 0.497 ± 0.001</td>
<td>0.979</td>
<td>79</td>
</tr>
<tr>
<td>2 1.019 ± 0.004</td>
<td>0.991</td>
<td>66</td>
</tr>
<tr>
<td>3 1.53 ± 0.02</td>
<td>1.01</td>
<td>59</td>
</tr>
<tr>
<td>4 2.04 ± 0.09</td>
<td>0.987</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined from UV-Vis titration curves at 514 nm and 567 nm for the Co and Fe complexes, respectively.

<sup>b</sup> Formal potential of the oxidation reaction, using the average of the oxidative and reductive peak potentials, obtained from cyclic voltammograms acquired at a potential scan rate of 50 mV/sec in 0.15 M TBAP in ACN.

<sup>c</sup> Difference in oxidative and reductive peak potentials during cyclic voltammetry as in b.

<sup>d</sup> Diffusion coefficients obtained from chronocouletry of the charge transients using Eq. (3.2).

<sup>e</sup> Calculated from the diffusion coefficient and viscosity for acetonitrile (0.369 mPa·s) using Eq. (3.1).
The $^1$H NMR spectra\textsuperscript{15} for the metal titration products of 2 - 4 was overwhelmed by the presence of water even after extended periods of lyophilization (2 weeks). Moreover, we were unable to observe molecular ion peaks in the ESI+ spectra of 3 and 4. There are two possible explanations for the absence of the molecular ions: (i) the resulting multimetallic duplexes are too unstable in ESI+ conditions to be observed, or (ii) the products are polymeric species which are inefficiently ionized (no higher molecular weight ions were observed by either ESI+ or MALDI-TOF mass spectrometry). The former of these is an ongoing challenge in the characterization of inorganic supramolecular compounds\textsuperscript{19} and is reasonable to expect for the highly charged, larger tri- and tetrametallic structures.

3.3.3 Solution Electrochemistry

To therefore distinguish between duplexes and polymers, we turned to quantitative analysis of their mass transport rates by electrochemical methods. Since metal-linked peptide duplexes would have substantially smaller dimensions than polymeric species, this would lead to their faster mass transport (\textit{i.e.} diffusion) rates according to the Stokes-Einstein equation:\textsuperscript{20}

$$D = \frac{k_B T}{6\pi \eta a} \quad \text{Eq. (3.1)}$$

in which the diffusion coefficient ($D$) is inversely related to the hydrodynamic radius ($a$) of the molecule and the viscosity of the medium ($\eta$), and where $k_B$ is Boltzman’s constant.
and \( T \) is the temperature. Thus, the measured \( D \) values in the same solvent are expected to be directly related to changes in the molecular radii.

In unstirred electrochemical solutions, the anodic and cathodic currents can be quantitatively related to \( D \) so that this analysis may be made. Figure 3-7 compares the oxidative cyclic voltammograms obtained for each of the Co(II) and Fe(II) complexes of oligopeptides 1–4,\(^{21}\) which have been normalized for the concentration of metal complex. In each case, only a single oxidative wave, which is attributed to either the Co (III/II) at \( \sim 0.26 \) V or the Fe(III/II) at \( \sim 0.99 \) V reactions, is observed. The formal potentials (\( E^o \)) of these reactions are listed in Table 3-2, and appear to slightly increase with increasing oligopeptide length, which could reflect differences in local environment of the metal complexes for the larger structures. However, we simultaneously observe that the longer oligopeptides have sharper wave shapes and the difference between the anodic and cathodic peaks (\( \Delta E_p \)) decreases, each of which are indicative of adsorption to the electrode surface and could account for the shift in \( E^o \). Each of the Co species has a larger \( \Delta E_p \) than their isostructural Fe analogs, which is due to the known slower heterogeneous kinetics for Co(II) oxidations.\(^{22}\) In all cases, only one oxidative peak is apparent indicating that there is a lack of electronic coupling between redox centers, which is expected for metal complexes that are not bridged by conjugated ligands.

The amount of current observed in the cyclic voltammograms in Figure 3-7 is inversely related to the length of the oligopeptide. Since these data are normalized to the metal complex concentration and these are one-electron oxidations (\( n = 1 \)), the decreased anodic current is a result of slower mass transport to the electrode surface \( (i.e. \ i \propto D^{1/2}) \).
Figure 3-7: Cyclic voltammograms obtained for solutions containing (A) Co and (B) Fe complexes of the oligopeptides 1 (・・・), 2 (・・ --), 3 (－－), and 4 (——) in 80:20 ACN:H₂O with 0.15 M TBAP supporting electrolyte, acquired at a potential scan rate of 50 mV/s. Currents are normalized for the metal complex concentrations determined separately from the solution absorbances.
Analysis of the peak currents ($i_p$) as a function of the potential scan rate ($\nu$) are consistent with mixed diffusion and electrode adsorption in these experiments,\textsuperscript{11} so that this data cannot properly be used to determine $D$. That is, plots of $i_p$ vs. $\nu$ and $\nu^{1/2}$ are both linear. However, to accurately measure $D$ of species that tend to adsorb to electrode surfaces, chronocoulometry was used. Application of anodic potential steps to the diffusion limited portion of the oxidative wave evolves charge ($Q$) over this time ($t$) according to:\textsuperscript{11}

$$Q = (2nFAD^{1/2}C/\pi^{1/2})t^{1/2} + Q_{dl} + Q_{ads}$$  \hspace{1cm} \text{Eq. (3.2)}

where $Q_{dl}$ is the double layer charge and $Q_{ads}$ is the charge contribution from the adsorbed species. The linearized charge transients for the Fe and Co complexes of 2 are shown in Figure 3-8; the slopes of these lines are used together with the known concentrations of metal complexes to calculate the term $nD^{1/2}$. Since in these cases $n = 1$, the diffusion coefficient of each species is determined and given in Table 3-2; the measured values of $D$ are within an order of magnitude of mass transport rates of monometallic, small molecule inorganic complexes.\textsuperscript{11} In Table 3-2, the measured $D$ for the Co-containing species are smaller than those measured for the Fe species, which is likely a convolution due to oxidation of the peptide overlapping the Fe (III/II). The mass transport rates decrease with increasing oligopeptide length (Figure 3-8 insets) as predicted by Eq. (3.1), and demonstrating that the primary species in solution are metallated oligopeptides are \textit{not} polymeric structures. Using Eq. (3.1) and the calculated diffusion coefficients, the calculated hydrodynamic radii support the formation of dipeptide assemblies and not polymeric materials. Using the mass spectra and
Figure 3-8: Linearized charge transients of the (A) Co and (B) Fe complexes of 2 in 80:20 ACN:H₂O with 0.15 M TBAP. Insets show the calculated $nD^{1/2}$ of the metallated complexes of oligopeptides 1 - 4 vs. the number of ligands on each strand, with linear regression.
electrochemical data, a reasonable conclusion is that the majority of the soluble metallated oligopeptides are double-stranded duplex structures, and that if any polymeric species form during metal complexation, their relative solution concentrations are not appreciable.

3.3.4 Film Electrochemistry and Spectroelectrochemistry

Since the metallated oligopeptides were found to adsorb to the surface of the working electrode, the electrochemical and spectroelectrochemical properties of these films in the absence of solution phase species were also investigated. To deposit the films, sequential cyclic voltammograms were performed. The typical response during 20 sequential cycles is shown in Figure 3-9A for the Fe complex of 4 using a Pt working electrode, in which the peak current increases incrementally and $\Delta E_p$ decreases as material is deposited onto the electrode. The electrode was then removed from the metallated oligopeptide solution, rinsed thoroughly with ACN, and placed in a solution of ACN containing only 0.2 M TBAP electrolyte. Figure 3-9B contains the cyclic voltammograms obtained with this electrode: a chemically reversible oxidative wave that is attributed to the Fe(III/II) couple indicates that the $[\text{Fe}_2(4)_2]^{2+}$ complex is adsorbed and remains electrochemically accessible. Over the course of the repetitive cycles, the magnitude of the current does not decrease, evidence that the adsorbed layer does not desorb from the electrode. In contrast to the voltammograms obtained of the solution phase species in Figure 3-7, there is no shift in the formal potential in Figure 3-9B and the difference in peak potentials is 44 mV. During potential cycling of similar films of
Figure 3-9: (A) Sequential cyclic voltammograms of $[\text{Fe}_4(\mathcal{L})_2]^{8+}$ using a Pt electrode and potential scan rate of 50 mV/s. (B) Cyclic voltammogram of the same film in 0.2 M TBAP in ACN using a scan rate of 50 mV/s. (C) Peak current vs. scan rate for films deposited on a Pt electrode at 50 mV/s in 0.2 M TBAP ACN solutions following (■) 5, (▲) 20, (▼) 40, and (●) 60 deposition cycles. (D) Surface coverage of metal complexes for $[\text{Fe}_4(\mathcal{L})_2]^{8+}$ on the Pt electrode as a function of cycle number.
the Co and Fe complexes of 3 and 4, desorption from the electrode was not observed. In contrast, the cyclic voltammetric currents of films of the metal complexes of 1 and 2 slowly decrease while sequentially cycling the potential as these molecules desorb and diffuse away from the surface. Adsorption is thus a function of the oligopeptide length, and it is likely a result of the decreased solubility in pure ACN for the larger and more highly charged species.

The peak currents continue to increase during sequential cycling in Figure 3-9A as a result of continued deposition of larger quantities of material onto the electrode surface. We therefore used this approach to deposit a series of films of varying thickness of the [Fe₄(4)₂]⁸⁺ complex on the Pt electrode by controlling the number of potential cycles. In each case, the resulting voltammograms of the modified electrodes revealed adsorbed films that remained adhered to the electrode surface. Figure 3-9C contains plots of the peak current as a function of the potential scan rate for a series of films of [Fe₄(4)₂]⁸⁺ deposited for increasing numbers of deposition cycles. In each case, and for all the Co and Fe complexes of 3 and 4, the plots are linear. The slopes of the lines are used to calculate the surface coverages (Γ) of adsorbed metal complexes (i.e. n = 1), which are listed in Table 3-3 for various cycle numbers for the Co and Fe complexes of 3 and 4.¹¹ Figure 3-9D shows that the surface coverage exponentially increases and levels off (after ~60 cycles). Leveling off may result from decreased conductivity as the film thickness increases by rate-limiting ion transport through the film, and is typical for charged inorganic redox oligomers and polymers.²³

The ΔEₚ values were determined for each of the four film types as a function of cycle number (i.e. surface coverage, Table 3-3): the values of these are consistent with
Table 3-3: Electrochemical Data for Deposited Films on Pt

<table>
<thead>
<tr>
<th>Number of Cycles</th>
<th>$\Gamma \times 10^{11}$</th>
<th>$\Delta E_p$</th>
<th>$\Gamma \times 10^{11}$</th>
<th>$\Delta E_p$</th>
<th>$\Gamma \times 10^{11}$</th>
<th>$\Delta E_p$</th>
<th>$\Gamma \times 10^{11}$</th>
<th>$\Delta E_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mol/cm$^2$)</td>
<td>(mV)</td>
<td>(mol/cm$^2$)</td>
<td>(mV)</td>
<td>(mol/cm$^2$)</td>
<td>(mV)</td>
<td>(mol/cm$^2$)</td>
<td>(mV)</td>
</tr>
<tr>
<td>5</td>
<td>2.25 ± 0.06</td>
<td>49</td>
<td>2.8 ± 0.1</td>
<td>43</td>
<td>8.4 ± 0.3</td>
<td>46</td>
<td>3.2 ± 0.1</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>2.88 ± 0.09</td>
<td>55</td>
<td>3.0 ± 0.1</td>
<td>47</td>
<td>93 ± 0.3</td>
<td>33</td>
<td>4.8 ± 0.4</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>3.6 ± 0.3</td>
<td>69</td>
<td>3.9 ± 0.1</td>
<td>52</td>
<td>12.0 ± 0.3</td>
<td>33</td>
<td>5.2 ± 0.4</td>
<td>21</td>
</tr>
<tr>
<td>40</td>
<td>6.6 ± 0.3</td>
<td>62</td>
<td>4.4 ± 0.4</td>
<td>53</td>
<td>20.4 ± 0.6</td>
<td>36</td>
<td>8.8 ± 0.4</td>
<td>27</td>
</tr>
<tr>
<td>60</td>
<td>7.8 ± 0.6</td>
<td>65</td>
<td>4.4 ± 0.4</td>
<td>50</td>
<td>22. ± 0.6</td>
<td>34</td>
<td>10.2 ± 0.4</td>
<td>34</td>
</tr>
</tbody>
</table>

a. Electrode potential was swept between 0.7 and 1.2 V (Fe complexes) or 0 and 0.5 V (for Co complexes), at a rate of 50 mV/sec using a Pt working electrode.

b. Surface coverage of metal complex, assuming $n = 1$ per complex, determined from the plot of $i_p$ vs scan rate for films deposited on Pt electrodes and immersed in 0.2 M TBAP in ACN solutions.

c. Difference between the anodic and cathodic peak potentials at a scan rate of 200 mV/sec for films on Pt electrodes immersed in 0.2 M TBAP in ACN.
electrochemically quasi-reversible reactions. The Co species again have larger $\Delta E_p$ values than those for the Fe complexes because of the slower heterogeneous kinetics for the Co oxidation. There is no clear trend in $\Delta E_p$ as a function of surface coverage for the Fe complexes, although their Co analogs appear to show a slight increase in $\Delta E_p$ for thicker films. While the Fe oxidation kinetics are expected to be electrochemically reversible, rates are also related to counterion permeation and diffusion within these films, resulting in the observed quasi-reversible peak splittings.

Films of the metallated complexes of 3 and 4 could also be deposited irreversibly on optically transparent ITO coated glass using the same method. The coated slides were then transferred to a cuvette containing 0.2 M TBAP in ACN and counter and reference electrodes, and used as the working electrode in UV-Visible spectroelectrochemical experiments. The absorbances of the films were measured at the MLCT wavelengths of the Fe complexes as a function of time while the potential was anodically cycled. Representative data obtained from these experiments is shown in Figure 3-10 for $[\text{Fe}_3(3)_2]^{6+}$: as the potential of the electrode becomes more positive, a decrease in the absorbance at the MLCT is observed when the overpotential is sufficiently large to generate Fe(III). Upon reversal of the potential, the MLCT absorbance returns to its initial value, a process that is reproducible for many cycles. Thus, electrochemical cycling of the adsorbed films as in Figure 3-10 does not cause either desorption or chemical reactions that result in observable changes in the electronic spectra. By using the scan rate for the potential sweep to convert from time to potential, absorbance vs. potential plots, such as those in Figure 3-10B, were generated for the species. The
Figure 3-10: (A) Absorbance at 567 nm for a film of [Fe$_3$(J)$_2$]$_6^{6+}$ on ITO coated glass in 0.2 M TBAP in ACN as the potential is swept from 0.35 to 135 V at 50 mV/s. (B) Absorption of the film at 567 nm as a function of applied potential for 1 cycle.
hysteresis in the plots again reflects the quasi-reversible kinetics of the oxidation that likely represents both electron and ion diffusion within the films.

3.4 Conclusions

We have created a new class of artificial oligopeptides containing pendant tridentate ligands which are crosslinked upon chelation of Co (II) and Fe (II). UV-Vis spectrophotometric titrations confirm stoichiometric addition of the metals; the electrochemical data quantitatively demonstrate via measurement of the diffusion coefficients that these are primarily oligopeptide duplex structures. These remain chemically stable, electroactive species that have quasi-reversible to reversible electron transfer kinetics. The larger metallated oligopeptides form electrodeposited films of controllable surface coverage that enable interrogation by spectroelectrochemical methods. While the kinetics of the Co(II) oxidations appear to be slower than the Fe(II) species, analysis of the electron transfer kinetics, including assessment of transfers between metal complexes in the same duplex or between duplexes, is precluded because of the interplay of adsorption and counterion transport. This structural motif provides the basis for a new type of multimetallic structure in which one-dimensional electron transport can be controlled and studied. Our ongoing investigations seek to further modify the conditions to better control the structure on the electrode surface and quantitatively measure the operative transport mechanisms.
3.5 References


21. The need to use water to solubilize the materials precludes observation of the Co(II/I) reduction.


24. The lower molar absorptivity of the Co complexes of 3 and 4 precluded spectroelectrochemical investigation of these films.


Chapter 4

Molecular Wire Behavior in Monolayers of Metal-Linked Artificial Oligopeptides

4.1 Introduction

Redox active materials are important for a range of potential applications, including molecular electronic components such as wires, transistors, and switches. The complexity of these functions will rely on intricate molecular structure, and elegant examples of linked multi-redox structures have begun to address electron transport and charge separation in complex assemblies. Unlike synthetically daunting, covalently-linked donor-acceptor arrays, the design and implementation of molecules that self-assemble into larger, functional architectures is an appealing approach. Bottom-up construction of well-defined nanoscale structures containing multiple redox centers provides an advantage that may ultimately allow application to real world devices.

Our approach has been to use metal coordination to self-assemble ligand-containing artificial peptides into multimetallic architectures. We recently reported the synthesis and properties of a series of artificial oligopeptides containing pyridine, bipyridine, terpyridine, and phenyl terpyridine ligands. These chelate metal ions and in some cases form peptide duplexes in which multiple metal complexes are linked by two oligopeptide strands. The resulting structures are unique because the lack of bridging ligands between redox sites means that multi-electron transfers between weakly coupled but adjacent metal complexes can occur by electron hopping. All of our investigations to date have examined the electrochemical behavior of solution phase species or electrodeposited films. However, these important initial architectures and experiments
have not allowed us to probe electron transfers down the length of the multimetallic structure and their use as molecular wires.

We have therefore modified the oligopeptide duplexes to enable their attachment to electrode surfaces and subsequently investigate directional electron transport. Figure 4-1 shows the structure of phenyl terpyridine (Φ-tpy) substituted oligopeptides containing cysteine termini (I – 3); the presence of ligands and thiols on the chain provides a means of orthogonal self-assembly. We have previously shown that artificial peptides containing these tridentate Φ-tpy ligands form duplexes upon coordination of Fe (II) and Co (II). The oligopeptides serve as scaffolds to tether together the metal complexes, as shown in the model of a trimetallic Φ-tpy peptide duplex molecule in Figure 4-2. In this paper, the thiol termini are employed to attach these architectures to surfaces using Au-S bonds. Attachment of these complexes to Au electrode surfaces provides a means for controlling the order in which the metal complexes are oxidized or reduced. Few examples of self-assembled monolayers containing multiple redox centers have been reported. In our architectures, such as shown in Figure 4-2, the redox centers are separated by ~9 Å and are linked by two peptide strands. Using the known differences in electron transfer kinetics of the Fe(III/II), Co(II/I) and Co(III/II) couples, this work demonstrates incoherent electron hopping is the dominant mechanism for transport in the molecules in only the former two reaction couples, the first evidence that these structures act as molecular wires.
Figure 4-1: Structures of phenyl-terpyridine substituted oligopeptides 1 - 3.
Figure 4-2: Representation of the structure of $[\text{M}_3(3)]^{6+}$, where M is Co or Fe, calculated using Hyperchem 6.0 with MM+ with previously reported parameters.
4.2 Results and Discussion

The cysteine termini of the phenyl-terpyridine substituted oligopeptides allow these to be deposited as molecular monolayers onto Au electrode surfaces by formation of Au-S bonds. Figure 4-3 depicts the two approaches that can be employed to form self-assembled monolayers (SAMs) of oligopeptide-metal complexes on Au. We first investigated deposited films using the first of these methods (Figure 4-3A), in which unmetallated Φ-tpy monopeptide (I) is exposed to the Au surface to form a SAM. This modified electrode is then treated with a solution containing either Co (II) or Fe (II) cations to form the metal complexes \textit{in situ} on the Au surface. After rinsing, the electrode is placed in a solution containing only supporting electrolyte (\textit{i.e.} without added Φ-tpy peptide or metal); the electrochemical response of the film is measured before the deposition process is repeated as shown in Figure 4-3A.

4.2.1 \textit{In Situ} Metal Complexation and Deposition

Sequential cyclic voltammograms that were obtained following film deposition are shown in Figure 4-4 for reaction of I with the Fe or Co. In Figure 4-4A these voltammograms were obtained after two deposition cycles of I and Fe (II), while Figure 4-4B is the response after four deposition cycles of I and Co (II). The currents in the voltammograms change and shift (indicated by the arrows) during potential cycling until they reach a steady and reproducible signal. In both cases, the stable cyclic voltammograms contain oxidative peaks at positive potentials due to the metal-centered one electron reactions of the Fe(III/II) and Co(III/II) couples; a cathodic peak in
Figure 4-3: Cartoon depiction of the two methods employed to deposit the monolayers of the metallated oligopeptides onto Au electrode surfaces. (A) *in situ* metal coordination sequentially adsorbs 1 onto the electrode and adds metal ion. (B) *ex situ* coordination reacts oligopeptides 1 – 3 with metals, and then adsorbs the product onto the surface.
Figure 4-4: Cyclic voltammograms obtained during *in situ* metal coordination according to Scheme 2A for (A) the second cycle of Fe addition to monopeptide 1 and (B) the fourth cycle of Co addition to monopeptide 1. Potential scan rate is 500 mV/s.
**Figure 4-4B** is the one electron Co(II/I) reduction couple. For the Co complex, the Co(II/I) wave is always larger in magnitude than the Co(III/II) wave, which is expected based on the known difference in the heterogeneous electron transfer rate constants for these couples. Changes in the current and shifting of peaks has also been observed with analogous thiolated terpyridine SAMS, and was attributed to initial formation of [Co(tpy)]^{2+} complexes followed by rearrangements that led to the [Co(tpy)_2]^{2+} complexes upon potential cycling. Given the mobility of thiols on Au surfaces, this is a reasonable expectation for our complexes and the behavior in **Figure 4-4** implies that analogous processes take place.

Dynamic restructuring during *in situ* metal complex formation and potential cycling is further supported by the observation that repeated monopeptide and metal deposition cycles result in a net increase in the amount of electroactive material on the electrode surface. **Figure 4-5** contains the cyclic voltammograms (after potential cycling to reach the constant response) for monolayers of 1 containing Fe or Co, in which the peak currents increase with the number of deposition cycles until they level off. Since the peak current is proportional to the surface coverage of redox-active metal complexes, these data imply that the number of metal bis(Φ-tpy) complexes increase and reach a maximum limit. The surface coverage (Γ) of metal complexes is quantitatively determined from the integrated area under the anodic peak (Q) by:

\[ Q = nFAG \]  
*Eq. (4.1)*
Figure 4-5: Series of cyclic voltammograms obtained during \textit{in situ} metal coordination of $I$ (Scheme 2A) by the metal ions (A) Fe and (B) Co. Potential scan rates are 500 mV/s in 1M TBAP solutions of ACN. Insets contain plots of the integrated peak current vs. the number of cycles of deposition.
where $A$ is the area of the electrode (cm$^2$), $F$ is Faraday’s constant (96485 C/mol), and $n$ is the number of electrons per reaction ($n = 1$ for these metal complexes). To compare different monolayers, the potential scan rate was held constant at 500 mV/s.$^{12}$ Plots of the peak charge for the Fe(III/II) and Co(II/I) waves (Figure 4-5 insets) show that these level off after $\sim 6$ deposition cycles.$^{13}$ Analysis using Eq. (4.1) shows that the maximum surface coverage is $\sim 3 \times 10^{-10}$ mol/cm$^2$ for monolayers of both [Fe(I)$_2$]$^{2+}$ and [Co(I)$_2$]$^{2+}$. This value is approximately twice the expected maximum surface coverage based on the footprint of the molecule (1.33x10$^{-14}$ cm$^2$/molecule, assuming an elliptical cross-section of 20 Å by 8 Å.) If, however, the maximum peak area of the Co (III/II) wave is considered, the corresponding surface coverage is 1.3x10$^{-10}$ mol/cm$^2$, which is in excellent agreement with the calculated molecular footprint and previous electrochemical estimations of surface coverage for similar molecules using the area of the Co (III/II) wave.$^{8i,10}$ The reason for the inflated peak areas of the Co (II/I) and Fe (III/II) couples versus that of Co (III/II) is not clear, however, we take this quantity calculated from the Co (III/II) wave as the amount of material necessary to form a complete monolayer on the Au electrode.

4.2.2 Ex situ Metal Complexation and Deposition

We utilized the method in Figure 4-3A to form layers of only metal complexes of 1 because the polyvalency of 2 and 3 would likely result in the formation of metal coordination networks on the electrode surface. The method in Figure 4-3B was therefore used to examine and compare the properties of monolayers of metal complexes
of 1 - 3. This approach involves the *ex situ* synthesis of the metallated oligopeptide duplexes prior to their deposition on the electrode surface. We have already demonstrated that metal coordination by Φ-tpy oligopeptides containing one to four ligands results in duplex formation, making the scheme in Figure 4-3B feasible in these experiments. While the scheme in Figure 4-3A has the advantage of forming a Au-S bond from both strands, *ex situ* synthesis of the complexes likely results in both parallel and antiparallel alignment of the oligopeptides. That is, the resulting structures have two thiol termini that are either on the same or opposite sides of the duplex; the stability of the monolayer and the mobility on the surface is expected to differ for these two isomers based on having one or two Au-S bonds.

We began by examining the voltammetric behavior of metal complexes of 1 that were prepared *ex situ* to compare to metal complexes formed *in situ* by sequential deposition. The amount of material that binds to the electrode is dependent on the length of time that these are allowed to adsorb. Figure 4-6 contains cyclic voltammograms that were obtained following adsorption of [Fe(I)$_2$]$^{2+}$ for varying times. A peak associated with the Fe(III/II) couple is again observed at ~ 1.0 V, and increases in magnitude with deposition time. The surface coverage of metal complexes was quantitatively determined from the integrated anodic current and using Eq. (4.1); the plot in the Figure 4-6 inset shows that the surface coverage increases with deposition time and again reaches a maximum. Analogous results were obtained for the deposition of all complexes. The timescale over which surface saturation occurs is highly dependent on the solution concentration of the metal complex. For both Fe and Co complexes of 1, the maximum
Figure 4-6: Cyclic voltammograms following adsorption of the Fe complex of 1 (ex situ formation, Scheme 2B) at the indicated times of exposure to the 0.22 mM Fe complex solution. Voltammograms were obtained in solutions containing only 1M TBAP in ACN at potential scan rates of 500 mV/s. Inset contains a plot of the integrated peak current as a function of adsorption time.
surface coverages of metal complexes formed \textit{ex situ} is $1.3 \times 10^{-10}$ mol/cm$^2$. In comparison with the maximum amount of metal complexes of 1 formed \textit{(in situ)} on the electrode surface ($1.3 \times 10^{-10}$ mol/cm$^2$), the maximum surface coverages obtained over long deposition times by the scheme in Figure 4-3 are approximately full monolayers.

Monolayers of $[M_2(2)_2]^{2+}$ and $[M_3(3)_2]^{6+}$ complexes were also adsorbed to the electrode using the approach in Figure 4-3B. In all cases, redox waves associated with Fe(III/II) or Co(II/I) were used to quantitatively determine the surface coverage as a function of time. Metallated complexes of 2 and 3 deposited in this fashion appear to result in full monolayers with slightly lower metal complex surface coverages ($\Gamma = 9 \times 10^{-11}$ mol/cm$^2$), although higher solution concentrations \textit{(i.e.} 1 mM metal complex) and deposition times as long as 10 hours were necessary to saturate the Au surface. Under identical conditions in which the concentrations of the Fe complexes of 1 - 3 were the same (0.22 mM complex) and deposition occurred for the same length of time, the maximum surface coverage decreases as a function of length. These differences are attributed to the increasing size and ionic charge for the metal complexes of the longer oligopeptides.

\subsection*{4.2.3 Mixed Monolayers}

Electron transfers in densely packed monolayers of redox active species can occur between metal complexes within the same molecule or between metal complexes of neighboring molecules. However, because of steric freedom and thiol mobility, using submonolayer coverages of these molecules can result in a variety of conformations on
the electrode surface. We therefore sought to dilute the surface with a redox inactive alkanethiol to reduce crosstalk between metal complexes and to prevent them from lying flat on the surface. This approach has been used in previous studies of redox active thiolated molecules.14

To accomplish this, metallated complexes are deposited on the electrode surface and the electrode exposed to a solution containing propanethiol, which displaces some of the metallated oligopeptide species. Cyclic voltammetry was used to quantify the amount of metal complex adsorbed to the electrode following exposure to propanethiol solutions. **Figure 4-7A** displays the surface coverages of (initially full) monolayers of the Fe complexes of 1 - 3 after soaking the films in 1 mM solutions of 1-propanethiol in 20:80 N,N'-dimethylformamide (DMF):ACN. These data show that the rate of desorption in the presence of a competing adsorbate is dependent on the length of the oligopeptides, where the larger multimetallic complexes desorb more slowly. The curves in **Figure 4-7A** appear to have multi-exponential decays, implying that several factors (such as solubility, number of Au-S bonds, steric) contribute to the overall rate. When the desorption solution was changed from 20:80 DMF:ACN to pure ACN, in which [Fe(I)₂]²⁺ is less soluble, desorption slowed considerably; **Figure 4-7B** compares the desorption rate of [Fe(I)₂]²⁺ in these two solvent systems. Monolayers of [Fe₂(2)₂]⁴⁺ and [Fe₃(3)₂]⁶⁺, neither of which is soluble in pure ACN, do not appreciably desorb under identical conditions. We conclude from these observations that desorption is primarily governed by the solubility of the metallated oligopeptides in the solvent containing the alkanethiol, rather than intrinsic differences in the nature of the Au-S attachments in each of the species.
Figure 4-7: Plots of the fraction of initial surface coverage (from integrated peak currents in the CVs) as a function of the exposure time to 1-propanethiol. (A) Surface coverage of Fe complexes 1 – 3 exposed to 1 mM propanethiol in 20:80 DMF:ACN. (B) Surface coverage of Fe complex of 1 following exposure to 1 mM propanethiol solutions in ACN (●) and 20:80 DMF:ACN (♦).
To therefore prepare mixed monolayers of the metal complexes of 1, 2, and 3, these were formed \textit{ex situ} and codeposited with propanethiol diluents from solution mixtures. Using the integrated current from the Fe(III/II) or Co(II/I) peaks, the resulting surface coverage of the metal complexes is always observed to be less than a full monolayer (\(< 9 \times 10^{10} \text{ mol/cm}^2\)) for complexes of 1. These surface coverages are variable based on the relative concentrations of propanethiol and metal complex, allowing us to control the molecular density on the electrode surface.

\textbf{4.2.4 Molecular Wire Behavior}

To minimize cross-talk between molecules, we compare the voltammetry of mixed monolayers of the metal complexes. While the cyclic voltammetry of all of the Fe complexes are essentially the same, dramatic differences in the voltammetric signatures of the Co complexes of 1 - 3 are apparent, as shown in Figure 4-8. These voltammograms are normalized to the cathodic peak current of the Co(II/I) wave to account for differences in both molecular surface coverage and the number of metal complexes per oligopeptide duplex. The cyclic voltammograms show a decrease in the relative size of the Co(III/II) wave with increasing oligopeptide length: if only one Co complex is oxidized in each duplex (because of a lack of self-exchange in this couple), an inverse first order decrease in the current relative to the Co(II/I) would be expected. However, the Figure 4-8 inset shows that the ratio of oxidation to reduction peak currents decreases \textit{exponentially} with the number of metals per molecule in the Co complexes of 1, 2, and 3.
Figure 4-8: Cyclic voltammograms for Au electrodes modified with mixed monolayers containing Co complexes of 1 (-), 2 (--), or 3 (..) together with 1-propanethiol diluent (Γ₁ = 7.0 x 10⁻¹¹; Γ₂ = 7 x 10⁻¹¹; Γ₃ = 6 x 10⁻¹¹ mol/cm²). CVs are normalized to the Co(II/I) cathodic peak current (i ∝ metal complex surface concentration). Potential scan rates are 500 mV/s in 1 M TBAP in ACN solutions. Inset: Plot of the relative peak currents of the Co(III/II) oxidation to Co(II/I) reduction as a function of the number of metals per peptide duplex.
To understand this change in the relative peak currents, the electron transport mechanisms for each of the reactions and transport within the metal-linked oligopeptide duplexes are considered. First, oxidation or reduction of the Co complexes requires a heterogeneous electron transfer, which will preferentially occur between the metal bis(Φ-tpy) complex closest to the surface and the electrode, with rate constant $k^\circ$ (Figure 4-9). For the multimetallic duplexes, reduction of the nearest Co results in the formation of a mixed valent adsorbed species containing adjacent Co (I) and Co (II) centers. Electron transfer between these two according to the reaction:

$$\text{Co (I)} + \text{Co (II)} \xrightarrow{k_{EX}} \text{Co (II)} + \text{Co (I)}$$

is known as electron self-exchange. Continued application of a reductive potential removes an addition electron from this species. In Figure 4-9, the fast self-exchange rate constant ($k_{EX}$) for Co(II/I) permits multiple electrons to be transferred for a total of one electron per metal complex. However for the converse Co(III/II) couple oxidation reaction, it is known that $k_{EX} \approx 0$ because it is spin-forbidden. Sequential electron hopping between adjacent complexes is therefore not possible for the Co oxidation reaction.

Competing transport mechanisms of electron self-exchange and tunneling are possible within the multimetallic complexes. Whereas tunneling is known to exponentially decrease with distance, electron transport by hopping between adjacent sites should be only weakly distant dependent. Heterogeneous kinetics and electron
Figure 4-9: Depiction of electron transfers in adsorbed multi-redox oligopeptides. Heterogeneous electron transfer ($k^o$) between the electrode and closest metal complex creates a mixed valent layer that can further transfer electrons by self-exchange ($k_{EX}$).
hopping in the Co(II/I) and Fe(III/II) couples are likely much faster than tunneling, enabling rapid and distant-independent charge transport when using these two redox couples. Alternatively, the relative exponential decrease in the current in the Co(III/II) reaction as a function of an increasing number of metals per duplex (i.e. distance) suggests that tunneling may play a key role in charge transport for this redox couple. For Co(II/I) and Fe(III/II), incoherent electron hopping between metal centers within the metal-linked oligopeptide duplexes allows these to behave as one-dimensional, multi-redox site molecular wires. Chapter 5 examines the details of the kinetics of these reactions to further study the relative roles of tunneling and hopping in charge transport.

4.3 Experimental

All materials were purchased from Acros and used as received unless otherwise noted. Water was obtained from a nanopure system (Barnstead, 18.2 MΩ.) Acetonitrile (ACN) was distilled over CaH₂ under N₂. N-tetrabutylammonium perchlorate (TBAP) was recrystallized from ethyl acetate.

4.3.1 Electrochemistry

All electrochemical data were acquired using a CH Instruments model 660B potentiostat, using a 0.104 cm² Au working, Pt wire counter, and Ag wire quasi-reference electrodes. The active working area of the electrode was determined from the chemisorption of iodine. Solutions were degassed with solvent-saturated nitrogen. The
Au electrodes were lightly polished with 0.05 μm alumina grit (Buehler) and washed with water. The electrodes were then dipped in 10:40:50 HCl:HNO₃:water for 30 s and washed copiously with water. The potential of the electrodes was then cycled from -0.3 to 1 V (vs. AgQRE) at 500 mV/s in 1 M H₂SO₄, after which they were rinsed with water, ACN, and dried under a stream of air.

4.3.2 Synthesis

Oligopeptides 1 - 3 were synthesized by solid-phase peptide synthesis using Fmoc protection as described previously, except cysteine was used as the first residue instead of glycine.⁷a Yields: 1: 23.5 mg (33%), 2: 24.7 mg (18%), 3: 44.4 mg (20%). Purity was assessed by analytical scale HPLC, in which only one peak is observed at the same retention time as in our earlier report.⁷a ESI+: (M+H)+ Found (Calc.) 1: 674.2 (674.2), 2: 1123.4 (1123.4), 3: 1572.6 (1572.6).

4.3.3 In situ Metal Coordination

Monopeptide 1 was attached to the electrode surface and then reacted with metal ions using the following protocol (shown schematically in Figure 4-3A). First, the electrode was soaked in a solution of 1 (0.8 mM in 4:1 ACN:water) for 15 min. The electrode was then removed from the solution, washed with ACN, and soaked in 100 mM Fe(ClO₄)₂ or Co(ClO₄)₂ ACN solution for 15 min. The electrodes were then washed thoroughly with ACN and placed in fresh supporting electrolyte (1 M TBAP in ACN)
and the potential swept for as many as 50 cycles. This process was sequentially repeated, beginning with soaking in the solution of 1.

**4.3.4 Ex Situ Metal coordination**

The oligopeptides were reacted with metal ions to pre-form the metallated duplex structures prior to placing them on electrode surfaces. This was accomplished as before\textsuperscript{7a} by spectrophotometric titration of the oligopeptide solutions with solutions of Co(ClO$_4$)$_2$ or Fe(ClO$_4$)$_2$ and monitoring duplex formation with visible absorbance at the MLCT bands (514 and 567 nm, respectively). Spectrophotometric titration curves were identical to those obtained in our prior report.\textsuperscript{7a}

Metallated oligopeptide duplexes were deposited by soaking the Au electrodes in 4:1 ACN:H$_2$O solutions containing (typically) 0.1 - 1 mM metal complexes for periods of approximately 1 - 10 h (**Figure 4-3B**). For co-deposition studies, 1 mM 1-propanethiol was also present in the ACN solutions.

**4.3.5 Metal-Oligopeptide Complex Desorption with Propanethiol**

Electrodes containing complete monolayers of the metal complexes were soaked in solutions of 1-propanethiol (1 mM in 20:80 DMF:ACN or ACN) for up to 8 h. The electrodes were then removed from these solutions, washed with ACN, and the potential cycled in a fresh solution of 1 M TBAP in ACN. This process was repeated until the redox peaks were no longer discernable in the voltammograms.
4.4 References


13. The number of deposition cycles required to saturate the surface depends on the solution concentrations of the monopeptide and metal ion, as well as the length of time that the electrode is exposed to these solutions. See experimental section for details.


15. While the voltammetry of the isostructural Fe complexes of I – 3 appears to be well-behaved and $k_{ex}$ of Fe(III/II) is approximately the same as Co(II/I), analogous comparison of redox response relative to a Co(III/II) internal standard is not possible.


Chapter 5

Electrochemical Investigations of the Nature and Rate of Electron Transfer in Co and Fe Linked Artificial Oligopeptides

5.1 Introduction

Redox active self-assembled monolayers (SAMs) comprise a class of materials which have a tremendous potential for application in molecular electronics. The ability of these systems to easily form well-ordered surface structures enables their application in creating architectures with defined spatial arrangements of electron donors and acceptors, and also provides a means of connection to an external power source. Despite this promise, most of the electroactive SAMs investigated have consisted of simple structures, with a single redox active moiety tethered by way of a functionalized organic spacer to an electrode surface, although some more complex systems have recently appeared. Electrochemical studies of the rates of electron transfer in these materials have yielded insight into the operative mechanisms of charge transfer in the organic linker species. Highly conjugated materials behave as molecular wires with fast rates (~$10^4$-$10^7$ s$^{-1}$) of electron transfer having little to no distance dependence on the length of the linker. Materials which are not highly conjugated have slower rates (typically less than $10^4$ s$^{-1}$, depending on the linker length) of electron transfer and exhibit an exponential distance dependence characteristic of a super-exchange, or tunneling, mechanism:

$$k^0 = A \exp(-\beta r) \quad \text{Eq. (5.1)}$$
where \( k^0 \) is the standard heterogenous rate constant at the formal potential \( (E^0) \) and some distance \( r \) from the electrode surface. \( A \) represents \( k^0 \) in the case where \( r = 0 \). \( \beta \) is a fall-off parameter that describes the distance dependence of \( k^0 \), and reveals information about the pathway through which the electron transfer occurs. For example, for electron tunneling through purely aliphatic bridges, \( \beta \) was measured to be about \( 1 \text{ Å}^{-1} \), whereas \( \beta \) through oligophenyleneethylene (OPE) bridges was between 0.3-0.8 \( \text{Å}^{-1} \), depending on the exact orientation of the OPE chain. Thus, conjugated materials typically give small \( \beta \) values, while saturated species give \( \beta \) values close to \( 1 \text{ Å}^{-1} \).

In our previous work, we introduced the synthesis, surface deposition and initial characterization of the thiol terminated, phthpy substituted peptides (Figure 4-1), as double stranded peptide assemblies linked with Co and Fe containing up to 3 addressable redox sites. In the case of the Co linked dipeptides, we showed that these complexes behave as molecular wires for the Co (II/I) couple, which can transfer electrons between Co centers by self-exchange. The Co (III/II) couple, however, self-exchanges slowly, and electrons are only transferred through the heterogeneous process. For dipeptide assemblies containing multiple Co centers, we showed that the peak currents for the (II/I) couple were larger than those for the (III/II) couple.

To understand this difference in current, we consider the potential electron transfer mechanisms of the dipeptide assemblies of Co (II) bis-phenyl terpyridine depicted in Figure 5-1. We will call the measured rate constants for these assemblies \( k_s \), the apparent rate constant. For a dipeptide assembly containing only one redox center, \( k_s = k^0 \). This is shown in Figure 5-1A. In the case of the dipeptide assemblies containing multiple redox centers, each with a \( k^0 \), and perhaps a self-exchange rate constant, \( k_{EX} \)
(depending on the specific identity of the couples), $k_s$ reflects the combined rates for all of those processes. **Figure 5-1B** depicts the oxidation of a dipeptide assembly containing two Co (II) $\text{bis}$-phenyl terpyridine centers. The self-exchange rate in the Co (III/II) couple is small, approximately 9 orders of magnitude slower than that for the Co (II/I) couple. Therefore, the only oxidation reaction which the center farthest from the electrode surface can undergo would proceed by a superexchange mechanism. Since the rate constant for this sort of a process has an exponential dependence described by Eq. (5.1) on the distance between the donor (the Co (II)) and the acceptor (the electrode), we expect that the rate constant for the Co center closest to the electrode should be larger than that for the center which is farther away. If the rates of these two superexchange processes are similar, the apparent rate will reflect each of these. The rates would be similar in the case where the $\beta$ value is small. For tunneling through peptides, the measured $\beta$ value is about 0.66 Å$^{-1}$. If we assume that tunneling in the present dipeptide assemblies occurs through the peptide backbone and that it has the same $\beta$ value, and a distance of about 9 Å between the redox centers,$^5$ we predict a decrease in rate of about 380 s$^{-1}$ in the standard rate constant for each metal center using Eq. (5.1). That is, for the assembly in **Figure 5-1B**, we expect $k_2^\beta = k_\beta/380$. The $\beta$ value for tunneling through space would be much larger, and thus, the differences between the heterogeneous rate constants for each metal center would be much larger. Given this estimation, we would expect that the heterogeneous rate constants for the individual metal centers as a function of the distance from the electrode (i.e. peptide length) would be significantly different. Thus, for very short time scale experiments, where what
**Figure 5-1:** Cartoons depicting the different schemes of electron transfer for dipeptide assemblies containing Co (II) \textit{bis}-phenyl terpyridine centers. (A) A monometallic system for the Co (III/II) couple. (B) A dimetallic system for the Co (III/II) couple, which self-exchanges slowly. (C) A dimetallic system for the Co (II/I) couple, which can self-exchange.
constituents “short” would be dependent on the magnitude of \( k^0 \), we would expect to see an apparent rate constant with magnitude equal to \( k^0 \). Thus, the assembly would kinetically behave like that in Figure 5-1A. However, at longer time scales, the \( k_s \) should show characteristics of both \( k^0 \) and \( k_{2}^{0} \).

We can also consider more complex mechanisms. Figure 5-1C shows the reduction process for the same dipeptide assembly described above. The Co (II/I) couple in these systems can transfer electrons by self-exchange. The Co center closest to the electrode surface must undergo a heterogeneous exchange (\( k^0 \)). The next Co center, however, could transfer an electron through either a heterogeneous process (\( k_{2}^{0} \)), or a self-exchange reaction (\( k_{EX} \)) with the first Co center, following the reduction of this species. As above, \( k^0 > k_{2}^{0} \), but the relative magnitude of \( k_{EX} \) compared to \( k^0 \), and thus \( k_{2}^{0} \), is not known for this system for a surface confined process. If the sum of the rates for the \( k^0 \) and \( k_{EX} \) processes is larger than the \( k_{2}^{0} \) process, then self-exchange will be the predominant mechanism through which the second Co center reacts in a reduction reaction. If, however, the rate of the \( k_{2}^{0} \) process is faster than the sum of the \( k^0 \) and \( k_{EX} \) processes, then heterogeneous exchange will predominant. For the same reasons described above, the case of pure heterogeneous exchange would give \( k_s \) values that are dependent on the dipeptide assembly length. However, the self-exchange process should show no dependence on the dipeptide lengths, because all metal centers are separated by the same distance, regardless of assembly length. Thus, the self-exchange process would always proceed at the same rate, regardless of length.

We therefore sought to investigate the kinetics of electron transfer in these systems to determine the mechanism by which electron transfer proceeds, and the factors
which would alter those kinetics, such as surface coverage, type of diluent, identity and concentration of supporting electrolyte and solvent. To our knowledge, no such analogous investigations have been performed with SAMs containing multiple redox species. Two different electrochemical methods were employed: Laviron analysis of cyclic voltammograms (CV) and alternating current impedance spectroscopy (ACI) to probe the mechanisms of electron transfer in these systems. Values for the transfer coefficients ($\alpha$), which describe the symmetry for the barrier of the oxidation/reduction processes, of each complex and lower limits for the electron transfer rate constants were extracted.

5.2 Experimental

Synthesis of the metallated dipeptide assemblies and surface deposition has been described previously, except 1-octanethiol is used as the diluent. Electrochemical experiments and electrode preparation were also performed as described previously except that a 1.37 cm$^2$ Au electrode was used as the working electrode. Peak potentials from cyclic voltammetry were manually corrected for uncompensated resistance prior to Laviron analysis, using the measured solution resistance of approximately 10 $\Omega$ (measured by chronoamperometry for a potential step over a non-faradaic region, typically 0 to 0.1, or -0.1 V) and the scan rate. Rate constants reported for Laviron analysis are the averages of the rate constants obtained by analysis of the cathodic and anodic peaks for each wave, using Eqs (5.2) and (5.3) and the transfer coefficients in Table 5-1 and $n = 1$. RC time constants were measured by chronoamperometric
experiments used to measure the solution resistance. ACI measurements were performed by applying the DC formal potential (measured by CV) of the couple of interest and scanning the frequency range from 1 to $10^5$ Hz.

5.3 Results and Discussion

5.3.1 Laviron Analysis and Cyclic Voltammetry

The apparent rate constant for an electron transfer process of surface bound species can be estimated from the peak splittings of cyclic voltammograms at different scan rates according to the method developed by Laviron. Figure 5-2 shows representative cyclic voltammograms of the Co complex of 2 at varying potential scan rates ($\nu$), and demonstrates the effect of scan rate on peak splitting: as the scan rate is increased, the difference between the anodic and cathodic peak potentials increases (i.e. the system becomes more electrochemically irreversible relative to the experimental timescale). Laviron’s method exploits this dependence of peak splitting on scan rate to provide kinetic information about the surface bound system. The difference in the peak potentials (cathodic or anodic, $E_{pc}$ or $E_{pa}$, respectively) and formal potential ($E^0$) are plotted as a function of the natural logarithm of $\nu$ in the limit of electrochemical irreversibility (i.e. peak splittings ($\Delta E_p$) of 200 mV or more, for a one electron process). The relationship is expressed according to the following equations for the cathodic and anodic peaks:
Figure 5-2: Cyclic Voltammograms of the Co complex of 2 at the indicated scan rates in 1 M TBAP ACN.
\[
E_{pc} - E^0 = \frac{-RT}{anF} \ln \left( \frac{\alpha Fn \nu}{RTk_s} \right)
\]

Eq. (5.2)

\[
E_{pa} - E^0 = \frac{-RT}{(1 - \alpha)nF} \ln \left( \frac{(1 - \alpha) Fn \nu}{RTk_s} \right)
\]

Eq. (5.3)

where \( R \) is the gas constant, \( T \) is the temperature, \( n \) is the number of electrons transferred per redox center, \( F \) is Faraday’s constant and \( k_s \) is the apparent rate constant for the overall electron transfer process. The equations can be fit for \( n, \alpha \) and \( k_s \) simultaneously. However, a more direct method of obtaining \( k_s \) involves independently approximating \( n \) and \( \alpha \).

Laviron provides a means for estimating \( \alpha \) independent of \( k_s \) and \( n \). From Eqs (5.2) and (5.3), the term \(|E_{pc} - E^0|/|E_{pa} - E^0| \) approaches \((1-\alpha)/\alpha\) as the system becomes electrochemically irreversible (i.e. \( \nu \to \infty \), or \( \Delta E_p \geq 200 \text{ mV}/n \)). Thus, a plot of \(|E_{pc} - E^0|/|E_{pa} - E^0| \) vs. \( \Delta E_p \) should level at a value of \((1-\alpha)/\alpha\) as \( \Delta E_p \) becomes larger than 200 mV/n.\(^7\) Representative plots of this type are shown in Figure 5-3 for the three redox couples (Co (II/I), Co (III/II) and Fe (III/II)) of the metal complexes of 2, all at the same approximate surface coverage, which were measured from the area under the cyclic voltammetric peak at 500 mV/s as described previously.\(^5\) At fast potential scan rates, the data are approximately linear as predicted by Eqs (5.2) and (5.3). The linear regions of the plots are fit by a least squares method forcing the slope to 0, and the y intercept is taken as \((1-\alpha)/\alpha\). Table 5-1 gives \( \alpha \) values for all of the duplexes calculated in this fashion. The Fe (III/II) and Co (II/I) couples for all three peptides have \( \alpha \) values of...
Figure 5-3: Representative plots to determine the transfer coefficient for metal complexes of 2 for: • Fe (III/II), □ Co (II/I), ▲ Co (III/II). The surface coverage for each in terms of metal concentration is $1 \times 10^{-11}$ mol/cm$^2$. The diluent is 1-octanethiol.
Table 5-1: Values for the transfer coefficient for each oligopeptide and redox couple. The surface coverages are all approximately $1 \times 10^{11}$ mol/cm$^2$ in the metal center concentrations. 1-octanethiol is the diluent.

<table>
<thead>
<tr>
<th>Oligopeptide</th>
<th>Fe (II/III)</th>
<th>Co (II/I)</th>
<th>Co (II/III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.52±0.01</td>
<td>0.49±0.01</td>
<td>0.60±0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.50±0.01</td>
<td>0.48±0.01</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.50±0.01</td>
<td>0.50±0.02</td>
<td>0.70±0.03</td>
</tr>
</tbody>
</table>
approximately 0.5, and thus, the energy barrier for oxidation and reduction in these couples is nearly symmetric. The Co (III/II) couple, however, has $\alpha$ values of approximately 0.6 for the complexes of 1 and 2, and 0.7 for the complex of 3, which implies that these barriers are asymmetric. While the literature is somewhat sparse on the topic, the measured values for the transfer coefficients also agree well with values reported for similar small molecule species in solution.8

In all three dipeptide assemblies, we find that the Co (II/I) and Fe (III/II) couples show no variation in $\alpha$. The lack of distance dependence of the transfer coefficients for the Co (II/I) and Fe (III/II) couples suggests what the primary mechanism of electron transfer for these species might be. If the rate of self-exchange (on the order of $10^5$ s$^{-1}$) is faster than the heterogeneous exchange, we hypothesize that only the initial heterogeneous exchange is important since all subsequent electron transfer would occur by the faster self-exchange process. The redox centers are held at fixed distances with respect to one another by the peptide backbone, so that the degree of orbital overlap, and thus $\alpha$, between these should be the same. The initial heterogeneous exchange would also occur at the same distance for each double stranded assembly, since only the redox center proximal to the electrode surface should participate in this pathway. Thus, $\alpha$ should not exhibit a distance dependence for these couples as a function of length if self-exchange is the primary mechanism of electron transport.

Similarly, the distance dependence of the transfer coefficient for the Co (III/II) couple is likely a result of the mechanism of electron transfer for this species. Because the Co (III/II) couple self-exchanges very slowly, each individual redox center can only undergo an electron transfer by heterogeneous exchange with the electrode. Thus, as the
redox centers are farther removed from the electrode surface, it is likely that the overlap of the involved orbitals becomes worse, and thus the energy barrier is more asymmetric. Therefore, this observed asymmetry indicates that a super-exchange mechanism is likely important for these species, and that even the terminal Co (III/II) couple in the duplex of 3 is able to participate in this pathway. However, the exact pathway of super-exchange (i.e. through space, through the peptide backbone or some other means) is not clear from this analysis.

We also note that there does not appear to be a significant effect of surface coverage on the measured values for the transfer coefficient. Duplexes studied at a variety of surface coverages ranging from $5 \times 10^{-11}$ to $6 \times 10^{-10}$ mol/cm$^2$ (with respect to the metal centers) showed no statistically significant difference in the measured $\alpha$ values. If local effects, such as electrostatic repulsion, were responsible for the effects on $\alpha$, then we would expect to see a dependence on surface coverage.

Once $\alpha$ is known, $n$ can be approximated from the peak width at half the maximum value ($E_{HW}$). As a surface confined system diverges from complete electrochemical reversibility (i.e. tends towards $\Delta E_p \neq 0$), the $E_{HW}$ values show a dependence on both $\alpha$ and $n$. The width of the cathodic peak is:

$$E_{HW} = \frac{62.5}{n\alpha} \text{mV} \quad \text{Eq. (5.4)}$$

and that for the anodic peak is:

$$E_{HW} = \frac{62.5}{n(1 - \alpha)} \text{mV} \quad \text{Eq. (5.5)}$$
Figure 5-4 contains representative cyclic voltammograms of the Fe and Co complexes of 3 at 500 mV/s. The small peak around 0 V appears with potential cycling and is not present at fast scan rates. We hypothesize that this may be due to charge trapping, and we are currently investigating this phenomenon. The peak splittings are 45, 87 and 39 mV for the Co (II/I), Co (III/II) and Fe (III/II) couples, respectively, and are thus electrochemically quasireversible so Eqs (5.4) and (5.5) apply. The $E_{HW}$ values for the anodic and cathodic peaks for the Co (II/I) and Fe (III/II) couples are approximately 125 mV, which is the value expected for a one electron process with an $\alpha$ value of 0.5. For the Co (III/II) couple, the $E_{HW}$ for the anodic peak is 187 mV, close to the expected value of 209 mV, that corresponds to a one electron process and an $\alpha$ value of 0.67, which is within the error limits of the measured value (Table 5-1). The $E_{HW}$ value for the cathodic peak is 123 mV, larger than the predicted value of 89 mV. The discrepancy may be due to the small signal to noise ratio. However, the fact that $E_{HW}$ is larger than 89 mV most likely indicates that this is a one electron process. The voltammetry in Figure 5-4 of the Co and Fe complexes of 3 is characteristic of all the duplexes: regardless of length, it is found that $n = 1$. This is in agreement with the plots of Figure 5-3, where the leveling occurs at peak splittings of approximately 200 mV, or larger, exactly as predicted for a one electron process.
Figure 5-4: Cyclic Voltammograms at 500 mV/s of Fe (−) and Co (--) complexes of 3 on a 1.37 cm² Au electrode in 1 M TBAP in acetonitrile. Surface coverages are approximately 1×10¹¹ mol/cm² with respect to the metal centers for each.
The fact that \( n = 1 \) for all of these systems implies that each metal center behaves independently or is not strongly electronically coupled to its neighbor. This is consistent for proximal metal complexes which do not contain bridging ligands, and is in agreement with our electrochemical investigations of related solution phase species.\(^\text{10}\)

Using the measured \( \alpha \) and \( n \) values for each duplex, the \( k_s \) values can be obtained as described above from Eqs (5.2) and (5.3). Figure 5-5 shows representative Laviron plots for the complexes of 3. The linear regions (\( \Delta E_p \geq 200 \text{ mV} \)) are fit to Eqs (5.2) and (5.3), and the slopes and intercepts are used to calculate \( k_s \) (Table 5-2). We find that there is little difference in the apparent rate constants of all three redox couples, and the rate constants also appear to be independent of length (i.e. number of metals per duplex).

These results are surprising. Physically it is not possible for all three redox couples to have the same rate constant, because it is well known that the heterogeneous rate is much slower for the Co (III/II) couple than either the Fe (III/II) or the Co (II/I).\(^\text{11}\) Even for a surface confined system, in which all heterogeneous rates should increase, the Fe (III/II) and Co (II/I) should remain faster than the Co (III/II). Because the difference between these values is orders of magnitude, we expect to see a large difference in the measured rate constants of our analogous species. In light of these unexpected results, we chose to employ an alternative method of analyzing the rates.
Figure 5-5: Laviron plots for complexes of 3 for the redox couple: (A) Fe (III/II), (B) Co (II/I) and (C) Co (III/II). Surface coverages are approximately $1 \times 10^{-11}$ mol/cm$^2$ in terms of redox center concentration.
Errors are determined from the average (see section 5.2).

**Table 5-2:** Apparent Rate Constants determined by Laviron Analysis

<table>
<thead>
<tr>
<th>Oligopeptide</th>
<th>Fe (III/II)</th>
<th>Co (II/I)</th>
<th>Co (III/II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130 ± 10</td>
<td>150 ± 20</td>
<td>120 ± 10</td>
</tr>
<tr>
<td>2</td>
<td>135 ± 5</td>
<td>150 ± 20</td>
<td>120 ± 50</td>
</tr>
<tr>
<td>3</td>
<td>133 ± 7</td>
<td>140 ± 10</td>
<td>100 ± 20</td>
</tr>
</tbody>
</table>

\[ k_s (s^{-1})^a \]

\(^a\) Errors are determined from the average (see section 5.2).
AC Impedance Spectroscopy

AC impedance spectroscopy can also be used to measure rate constants for electron transfer processes, specifically in SAMs.\textsuperscript{12,13} In this method, the applied DC potential is held constant, generally at $E^0$ to simplify the properties of the system, and AC perturbations of different frequencies ($\omega$) are then applied. The measured impedance can then be examined as a function of the frequency of the applied perturbation. A Nyquist plot of such systems (the imaginary component of the impedance ($Z''$) vs. the real component of the impedance ($Z'$)) reveals kinetic information about the system. For diffusive systems, information about mass transport is also inherent to the data. A representative Nyquist plot for the solution phase complex of Co I for the Co (III/II) couple is shown in Figure 5-6A. At low frequencies (\textit{i.e.} long time scales) the plot is linear because the system is under the control of mass transport. At high frequencies (\textit{i.e.} short time scales) however, the plot is semi-circular, because the system is under kinetic control. For diffusive systems held at the DC formal potential over the high frequency range, the phase angle ($\phi$) between the AC current and voltage is related to the frequency by:\textsuperscript{6}

$$\cot(\phi) = -\left(1 + \frac{1}{k_s} \sqrt{\frac{D\omega}{2}}\right) \quad \text{Eq. (5.6)}$$
Figure 5-6: (A) Nyquist and (B) cot (φ) vs. ω^{1/2} plots for Co (II/III) couple of ~1mM Co 1 in 80:20 acetonitrile:water with 0.2 M TBAP. Applied DC potential is 0.265 V (vs. AgQRE) and frequencies are scanned from 1Hz to 0.1 MHz.
where $D$ is the diffusion coefficient given in Chapter 3, so that a plot of $\cot(\phi)$ vs. $\omega^{1/2}$ should be linear with a slope related to $k_s$. The plot in Figure 5-6B shows this plot for the solution phase complex of Co 1 for the Co (III/II) couple and yields a $k_s$ value of $3.8 \times 10^{-3}$ cm/s, which agrees with $k^0$ values reported for similar compounds.\textsuperscript{8b,c}

Similar analyses can be performed with molecules adsorbed to the electrode surface. For a surface confined system, in which there is no mass transport, the relationship between $\phi$ and $\omega$ is:\textsuperscript{13b}

$$\cot(\phi) = \frac{-\omega}{2k_s} \quad \text{Eq. (5.7)}$$

so that a plot of $\cot(\phi)$ vs. $\omega$ should be linear with a slope related to $k_s$.

Figure 5-7 shows a representative Nyquist plot for the complex of Co 3 for the Co (III/II) couple, which should have the slowest kinetics of all the complexes, since the electron transfer events would all proceed by a super-exchange mechanism, since self-exchange is very slow for this couple. The semi-circular region is absent from the plot, implying that the kinetics are too fast to be measured by this technique and with this experimental configuration (\textit{i.e.} electrochemical cell and potentiostat).
Figure 5-7: Nyquist plot for surface bound complex of Co 3 for the Co (III/II) couple. Surface coverage is approximately $1 \times 10^{-11}$ mol/cm$^2$ in terms of redox center concentration. Applied DC potential is 0.374 V and frequencies are swept from 1Hz to 0.1 MHz.
Based on the highest frequency ($1 \times 10^5$ Hz) we are able to use with our potentiostat, the upper limit for the measurable rate is less than $10^5$ s$^{-1}$. However, this upper limit is larger than the time constant of the cell, which is 40-90 µs for all of the experiments, and therefore corresponds to an absolute upper measurable rate constant of $\sim 10^4$ s$^{-1}$. Thus, we can safely say that the rate constants for these systems are larger than $10^4$ s$^{-1}$. It is likely that the lack of variation in the rate constants (Table 5-2) measured through Laviron analysis is a consequence of the fast rates and the limitations in the experimental configuration. The rate constants obtained through Laviron analysis clearly underestimate the actual rates when compared with the ACI analysis. We suspect that these slower rates reflect residual ion diffusion through the monolayer.

5.4 Conclusions

We have examined the rate constants of electron transfer and transfer coefficients for SAMs of metal complexes of Co and Fe of varying lengths of phenyl-terpyridine oligopeptides by electrochemical techniques. We find that the transfer coefficients are approximately the ideal symmetric values for the Fe (III/II) and Co (II/I) couples and are invariant with length. The transfer coefficients for the Co (III/II) couples, however, show a great deal of asymmetry and appear to have a length dependence. These results suggest that self-exchange may be the primary mechanism for electron transfer in the Co (II/I) and Fe (III/II) couples. Through ACI, we have shown that the rate constants for electron transfer are larger than $10^4$ s$^{-1}$. We cannot ascertain more quantitative values for the rate
constants because we are limited by the RC time constant of the electrochemical cell and potentiostat.

It is possible that by employing a carefully constructed cell and potentiostat with fast electronics so that the RC time constant were smaller, these measurements could be made by conventional techniques. Use of microelectrodes would decrease the double-layer capacitance, and therefore the RC time constant. However, the magnitude of the signal would decrease drastically, and may not be within the limits of detection of the experimental apparatus. Rate constants as fast as $10^7 \text{s}^{-1}$ have been measured electrochemically, through modified ACI and indirect laser induced temperature jump (ILIT)$^{1b,3b,4c-d}$ techniques. It may therefore be possible to measure the rate constants for these systems by employing similar types of set ups and experiments, so long as the rates are smaller than $10^7 \text{s}^{-1}$. Alternatively, conventional techniques could perhaps be used if the temperature of the systems were decreased during measurements to slow the rate.

Another option is to probe the rates spectroscopically, which would eliminate the timescale limitations of electrochemical analysis. Observation of an inter-valence charge transfer absorption band in the NIR region can be used to determine self-exchange rate constants.$^{15}$ NIR bands have been obtained for similar types of materials which contain mixed-valent Fe centers in close proximity.$^{16}$ Despite our efforts in these areas, we have not yet observed a band in the NIR region for any of these materials in spectroelectrochemical experiments. This could perhaps be due to the extraordinarily small extinction coefficients of these bands (typically $\varepsilon \sim 1-20$)$^{16}$ due to weak electronic coupling which makes their detection difficult even with sensitive spectrophotometers
and careful background subtraction. We are also limited by the solubility of these materials in NIR transparent solvents, but we continue our endeavors in this area.

5.5 Overall Conclusions and Future Directions

This work has demonstrated the facile synthesis of ligand containing artificial oligopeptides which can self-assemble into well defined architectures upon metal complexation. These supermolecular architectures have defined spacing and arrangement of metal centers, and the redox properties are dependent on metal-ligand identity and sequence. These materials can be used to direct and control charge transport.

We have demonstrated that homometallic oligopeptide assemblies containing metal complexes which behave as molecular wires, with facile electron transfer properties. We are currently collaborating with surface scientists to learn about the surface structures of these SAMs. Scanning tunneling microscopy (STM) investigations can be used to probe single molecules within the monolayer to examine their height and conductivity. STM can also be used to assess the mobility of the molecules in the monolayers. Infrared spectroscopy of the surfaces can also be used to determine the contact angle of the monolayers to the surface, which will tell us about the orientation of the monolayers on the surfaces.

The nature of the synthesis of these compounds also enables the placement of spacer groups between the ligand units on the oligopeptide strands. The addition of spacer units can be used to tune the rate and potentially mechanism of electron transfer between the metal centers. Additionally, by slowing these rates with spacer units,
modification of the oligopeptide strands in this way may enable measurements of the the rates, and therefore, important information about the nature of electron transfer mechanisms operative in these systems.

Heterometallic systems are currently being developed to create structures with more specialized charge transfer properties. Additionally, investigations have begun with attaching species to the termini of the oligopeptides and using the self-assembly properties to align these materials. These endeavors represent important steps towards the construction of functional molecular circuits.
5.6 References


5. Ohr, K.; Williams, M.E. 2007, Unpublished Results, Chapter Four of this work.


14. This is approximated from 1/(R*C), assuming that we could measure a rate constant that is 3 RC time constants slower than the RC time constant.

VITA

Kristi Ohr

Education

2001-2007 Ph.D., Chemistry
The Pennsylvania State University, University Park, PA
Thesis Title: “The Design, Synthesis and Redox Properties of Artificial Metallated Oligopeptides”
Graduate Advisor: Mary Elizabeth Williams

1996-2000 B.S., Chemistry (Minor in Mathematics)
Saint Vincent College, Latrobe, PA
Magna Cum Laude

Publications


