Tunable Energy Transfer Rates via Control of Primary, Secondary, and Tertiary Structure of a Coiled Coil Peptide Scaffold

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Supporting Information

ABSTRACT: Herein we report energy transfer studies in a series of Ru(II) and Os(II) linked coiled-coil peptides in which the supramolecular scaffold controls the functional properties of the assembly. A general and convergent method for the site-specific incorporation of bipyridyl Ru(II) and Os(II) complexes using solid-phase peptide synthesis and the copper-catalyzed azide–alkyne cycloaddition is reported. Supramolecular assembly positions the chromophores for energy transfer. Using time-resolved emission spectroscopy we measured position-dependent energy transfer that can be varied through changes in the sequence of the peptide scaffold. High level molecular dynamics simulations were used in conjunction with the spectroscopic techniques to gain molecular-level insight into the observed trends in energy transfer. The most efficient pair of Ru(II) and Os(II) linked peptides as predicted by molecular modeling also exhibited the fastest rate of energy transfer (with $k_{\text{TR}} = 2.3 \times 10^7$ s$^{-1}$ (42 ns)). Additionally, the emission quenching for the Ru(II) and Os(II) peptides can be fit to binding models that agree with the dissociation constants determined for the peptides via chemical denaturation.

I. INTRODUCTION

The design of molecular materials capable of performing complex functions is pivotal to “bottom-up” approaches in molecular electronics,† sensing,1a,2 and solar energy conversion.3,4 The most common strategy for building artificial assemblies uses covalent bond formation to connect molecular components with rigid linkers that dictate both distance and orientation. While this approach provides exquisite control over spatial parameters,5 the optimization of functional performance often requires the development of new synthetic routes making the implementation very difficult, especially as the number of molecular components increases. Alternatively, chromophores have been placed on easily synthesized scaffolds such as polymers,6 dendrimers,1a,6,7 and organogels.8 However this approach can yield assemblies that are not monodisperse in molecular weight or chemical composition and incorporate many different morphological constituents. While large systems are readily made and some control over the primary structure is possible, the flexible scaffolds result in solution structures that vary from one assembly to the next.

Another approach to achieve functional architectures draws inspiration from natural systems, which combine simple molecular building-blocks to form highly complex functional systems. Nature exploits relatively weak noncovalent interactions to achieve functional architectures with a hierarchical control, in which sequence defines structure and self-assembly, which defines function. Proteins, lipids, and oligonucleotides form the structural framework that organize functional elements in spatial proximity and with well-defined orienta-

tions.9 To this end, functionalized biological molecules such as oligonucleotides,10 amyloid-like peptide fibrils,11 and even derivatized virus coated proteins have been designed and investigated as functional materials.12 These types of designed systems13−15 that mimic the organizational strategies of biomolecules provide a number of advantages, including design flexibility, ease of synthesis, and spatial control of functionality through supramolecular architectures that allow for fine-tuning of materials properties.

Herein we report the design of an artificial polypeptide system based on a heterodimeric coiled-coil architecture in which the primary sequence defines both the secondary and the tertiary structure, resulting in self-assembly, which provides fine control of the positioning of octahedral tris(bipyridyl) transition metal complexes [M$^\text{II}$(bpy)$_3$]$^{2+}$ (M = Ru or Os, bpy = 2,2’-bipyridyl). Coiled-coils are a common protein motif and provide structural architecture for many important protein scaffolds including α-keratin16 and tropomyosin.17 Moreover, the sequence-structure rules are well-defined, allowing for the design of highly tunable supramolecular architectures by control of the primary sequence.18 In this study we demonstrate the ability of the peptide secondary structure to control self-assembly and hence relative positioning of the octahedral complexes, resulting in systematic tuning of the energy transfer properties of the system. The Ru(II) and Os(II) metal complexes are positioned near the midpoints of two...
complementary peptide chains, each consisting of 28 residues (Figure 1). The primary sequence of each chain is chosen such that they adopt a dimeric supramolecular structure consisting of two α-helical coils, in which the hydrophobic residues are shielded from the aqueous environment and the hydrogen bonding and ionic interactions are maximized. Photoexcitation of the Ru(II) complex in the folded assembly results in energy transfer to the lower energy Os(II) acceptor on the opposing chain. Since the metal complexes are placed on different peptide chains, energy transfer is only possible if the two chains associate in solution, making this system particularly sensitive to the secondary and tertiary structure of the peptide scaffold.

The peptide structures studied here take advantage of two flexible synthetic methodologies: solid-phase peptide synthesis (SPPS) and the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC or "click reaction"). SPPS is advantageous as it allows for exact positioning of the chromophores in the primary sequence. The use of click chemistry as an orthogonal linkage strategy between the chromophores and the peptides has several advantages. First, it avoids issues with formation of statistical mixtures of species, as was obtained in the electron transfer coiled-coil systems developed by Ogawa, in which the chromophore linkage was accomplished via nonspecific coordination chemistry. Second, click chemistry provides advantages over the direct amide linkage used in the electron-transfer oligoproline systems reported by Meyer, in which orthogonal protecting group strategies had to be employed.

We have examined a series of peptide assemblies that systematically vary the placement of the complexes along the peptide backbone, results in predictable changes in the energy transfer rate, which are measured using time-resolved emission methods. Variation in the rate by almost an order of magnitude across the series, as well as denaturation studies, confirm that energy transfer is the direct result of folding into a well-defined tertiary structure. All-atom molecular dynamics simulations provide insight into the microscopic environment, revealing an assembly with a dynamic, yet robust, tertiary structure that effectively controls the relative positioning of the two complexes.

II. RESULTS AND DISCUSSION

System Design. Since energy transfer between Ru(II) and Os(II) polypyridine complexes has not been previously studied using this type of chemical scaffold, a number of design elements were considered crucial for the proper development of such a system, as discussed below: (1) the design of the coiled-coil peptide scaffold, (2) the attachment chemistry including its utility, versatility, and compatibility with standard conditions for automated peptide synthesis, (3) the nature of the metal complexes including their chemical stability and photophysical properties, (4) the nature of the linking group and how it may affect coiled-coil secondary and quaternary structure, and (5) the positions for Ru(II) and Os(II) modification within the coiled-coil peptides.

Coiled-Coil Peptide Scaffold Design. The coiled-coil peptide scaffold was adopted from the heterodimeric self-assembling fiber (SAF) system originally reported by Woolfson and co-workers (Figure 2). Both 28-residue peptides contain the canonical repeating heptad sequence (designated abcddefg) that most coiled-coil peptides display. The a and d sites are reserved for hydrophobic residues which provide the primary driving force for dimerization. Isoleucine and leucine residues were specifically chosen for the a and d sites, respectively, because they have been shown superior promoters for the desired parallel dimeric structure. The e and g positions contain oppositely charged residues such as lysine and glutamate that provide complementary interactions only when the heterodimeric and parallel partnering is considered. An asparagine residue is included at a single a position within each sequence. Polar residues included at these positions must satisfy their hydrogen bonding potential, and impart folding specificity.
through mutual alignment to give a parallel coiled-coil exclusively.\textsuperscript{19,20b,21} Although the incorporation of two asparagines residues within the hydrophobic core of the parallel dimer interface is slightly destabilizing compared to more hydrophobic residues at these positions, their incorporation is more destabilizing within undesired structures including trimers, tetraters, antiparallel dimers, and misaligned dimers.\textsuperscript{19,20b,21} Thus, this sequence design provides parallel heterodimeric coiled-coils to the exclusion of other supramolecular architectures. Of significance for this study is the fact that the monomeric peptides with this type of sequence design typically do not fold into helical structures to any great extent. This is attributed to the fact that such a monomeric helix would display a large hydrophobic patch on one face of the helix, which is unfavorable in aqueous solution.

The majority of Woolfson’s SAF peptides contain complementary interactions that promote a staggered heterodimer structure referred to as the “sticky-ends” design.\textsuperscript{18b} The P1 peptide sequence is a permutation which has been rearranged to discourage longitudinal association, and therefore fiber formation.\textsuperscript{18b} Natural coiled-coil peptides all share this “blunt” end assembly.\textsuperscript{22} The P2 apopeptide sequence remains unchanged from several of Woolfson’s reports.\textsuperscript{18}

**Synthesis of Metallopeptides Using CuAAC.** A number of redox-active amino acids containing Ru(II) and Os(II) bipyridyl complexes have previously been reported.\textsuperscript{14,23} These amino acid derivatives are convenient because they can be incorporated into metallopeptides during traditional linear solid-phase peptide synthesis (SPPS), although they often include longer coupling times, more exotic coupling reagents, and decreased yields. We chose a convergent synthetic strategy because it would allow for the peptide, linker, and bipyridyl complexes to be easily varied for later optimization and investigation. The Cu(I)-catalyzed azide–alkyne 1,3-dipolar cycloaddition provides general and robust conditions for the conjugation of biomolecules.\textsuperscript{24} Several alkynyl-functionalized Ru(II) and Os(II) complexes have been reported,\textsuperscript{10a–c,25} having previously been used for oligonucleotide modification with Sonogashira Pd(0) cross-coupling chemistry.\textsuperscript{26} The Ru(II) and Os(II) bipyridyl complexes 1a and 1b (Figure 3) were selected,\textsuperscript{25} as opposed to previously described phenanthroline complexes,\textsuperscript{10a–c} because the sp$^3$-hybridized carbon within the propargyl amide group increases flexibility and electronically decouples the metallobipyridine from the triazole ring formed during the CuAAC reaction.

Lysine ε-azide was selected to provide the tether for 1a and 1b since it contains four aliphatic methylene units, and was anticipated to be less destabilizing toward the coiled-coil peptide structure compared to shorter tether lengths (Figure 3).\textsuperscript{18,27} The linker was also selected to be sufficiently flexible so as to allow rotational freedom for the metal complexes since orientation and angular relationships between donor and acceptor modules have a strong influence on energy transfer efficiencies.\textsuperscript{10c,d,13a}

Azidopeptides were synthesized by incorporating Fmoc-lysine ε-azide (2) at the desired point of (donor/acceptor) attachment during SPPS (Scheme 1). After the azidopeptides were cleaved from the solid-phase support and purified using RP-HPLC, they were conjugated to 1a or 1b using the CuAAC reaction (Scheme 1). Reactions containing a tris-(triazolylmethyl)amine ligand (3) demonstrated shorter reaction times and increased yields compared to reactions lacking the ligand.\textsuperscript{28} A more basic reaction medium (pH 8.5) provided shorter reaction times compared to lower pH media.\textsuperscript{24} After conjugation was complete, the metallopeptides were purified using a combination of size-exclusion chromatography and RP-HPLC as described below.

**Donor/Acceptor Placement in the Coiled-Coil.** The coiled-coil peptide scaffold contains a multitude of possible positions for the attachment of polypyridyl donor/acceptor complexes. The positions selected for modification were chosen to provide a range of well-defined donor/acceptor distances in the self-assembled coiled-coil for measuring excited-state energy transfer. Substitutions on the first and last heptad repeat units of the peptides could potentially result in complications from end fraying near the termini, and were therefore avoided. Conjugation of 1b (Os(II)) at the 2f, 2c, or 2g position within the P1 peptide sequence gave the excited state energy acceptor metallopeptides 2f-Os-P1, 2c-Os-P1, and 2g-Os-P1, respectively.

**Figure 3.** (a) Alkynyl-functionalized Ru(II) and Os(II) containing complexes 1a and 1b, respectively. (b) Fmoc-lysine ε-azide 2 used in the synthesis of azidopeptides. (c) tris-(triazolylmethyl)amine ligand 3 used to accelerate the CuAAC conjugation reaction.
Similarly, conjugation of 1a (Ru(II)) at the 2f, 2b, or 2e position within the P2 peptide sequence provided the donor metallopeptides 2f-Ru-P2, 2b-Ru-P2, and 2e-Ru-P2, respectively (Table 1). Three specific pairs of metallopeptides were selected for structural analysis, photophysical characterization, and all-atom molecular dynamics simulation. The 2f-Os/2f-Ru metallopeptide pair refers to the heterodimer formed by the 2f-Os-P1 and 2f-Ru-P2 metallopeptides and was expected to provide the largest donor/acceptor separation distance, based on the analysis of parallel dimeric coiled-coils with crystal structures reported in the protein database (PDB code: 3NM6, 1UIX, and 3M9B). The distance between the α-carbons of aligned f positions to 14.9 Å in these crystal structures. Similar analysis indicated that b-c separations were 12.7−13.5 Å, and e-g separations were 9.6−10.3 Å. Although these measurements do not account for the length or flexibility of the azidolysine linker, they qualitatively represent the general trends that may be expected for the rates of energy transfer in the 2f-Os/2f-Ru, 2b-Os/2b-Ru, and 2e-Os/2e-Ru metallopeptide pairs.

**Table 1. Ru(II) and Os(II) Metallopeptide Sequences**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>EIDALEY-ENDALEQ-KIAALKQ-KIASLKQ</td>
</tr>
<tr>
<td>2f-Os-P1</td>
<td>EIDALEY-ENDALEQ-KIAALKQ-KIASLKQ</td>
</tr>
<tr>
<td>2c-Os-P1</td>
<td>EIDALEY-ENDALEQ-KIAALKQ-KIASLKQ</td>
</tr>
<tr>
<td>2g-Os-P1</td>
<td>EIDALEY-ENDALEQ-KIAALKQ-KIASLKQ</td>
</tr>
<tr>
<td>H2f-Ru-P2</td>
<td>KIRRKLQ-KNARLQ-EIALEY-EIAALEQ</td>
</tr>
<tr>
<td>2b-Ru-P2</td>
<td>KIRRKLQ-KNARLQ-EIALEY-EIAALEQ</td>
</tr>
<tr>
<td>2e-Ru-P2</td>
<td>KIRRKLQ-KNARLQ-EIALEY-EIAALEQ</td>
</tr>
</tbody>
</table>

*The primary sequences for the peptides synthesized and characterized in this study are given using the single letter amino acid code (in capitals). X represent the artificial amino acid lysine ε-azide used for the attachment of Os(II) (green) or Ru(II) (red) complexes.

**Structural Characterization of the Metallopeptide Heterodimers by Circular Dichroism.** Circular dichroism (CD) is a common technique for the characterization of peptide secondary structure.29 A well-formed α-helix exhibits a maximum in the CD spectrum below 200 nm and minima at 208 and 222 nm. The CD spectra of the monomeric peptides each exhibit relatively weak minima at 208 and 222 nm, characteristic of a dynamic partially folded helical structure, as expected (Figure 4). Because peptide CD spectra are typically plotted with concentration-independent mean residue ellipticity on the y-axis, a 1:1 mixture of complementary peptides is expected to exhibit deeper minima at 208 and 222 nm if an α-helical coiled-coil structure is formed, whereas if dimerization does not occur, the spectrum of the mixture will be identical to the average of the spectra for the two individual peptides. The CD spectra for all three heterodimeric metallopeptide pairs indicate that they form coiled-coil structures when mixed in a 1:1 ratio in aqueous buffer at μM concentrations (Figure 4).29 The ellipticity at 222 nm is a quantitative measure of α-helicity and can be used to monitor coiled-coil dimerization. All three pairs display maximum α-helicity when equimolar mixtures are measured, indicating that heterodimeric coiled-coil structures are being formed (see Supporting Information). The 2f-Os/2f-Ru peptide pair exhibits the largest negative signal at 222 nm, with an ellipticity ratio for the 2f-Os/2f-Ru pair at 208 and 222 nm, θ222/θ208, equal to 1.00. Similarly, the 2c-Os/2b-Ru and 2g-Os/2e-Ru peptide pairs both have θ222/θ208 values equal to 1.03. This ratio is characteristic of dimeric coiled-coil peptides, as single-stranded α-helices have values closer to 0.85.30

**Thermodynamic Stability Determined Using Guanidinium Denaturation.** Chemical denaturation using guanidinium
Guanidinium chloride (GdnHCl) provides a reliable tool for measuring coiled-coil dissociation constants.\textsuperscript{20c,31} The method has been used to compare small structural variations within families of similarly designed coiled-coils.\textsuperscript{20c} Gibbs free energy change of unfolding ($\Delta G_{\text{unf}}$) was measured for each of the metallopeptide dimers using the method of linear extrapolation (Figure 5).\textsuperscript{31} The different donor/acceptor modification points for the metallopeptide pairs resulted in only subtle differences in structural stability. As may be expected, the 2f-Os/2f-Ru pair formed the most thermodynamically favorable heterodimer (Table 2). The value of $\Delta G_{\text{unf}}$ measured for the 2f-Os/2f-Ru pair, 8.6 ± 0.10 kcal/mol, corresponds to a dissociation constant ($K_d$) equal to 0.49 ± 0.09 $\mu$M. The $\alpha$-helicity of the fully folded 2f-Os/2f-Ru dimer was calculated to be 80%, based on molar ellipticity measurements at 222 nm (−28,600 ± 800 deg·cm$^2$·dmol$^{-1}$). The $\alpha$-helical content determined for the 2f-Os/2f-Ru dimer falls within the range reported for similar sequences since values from 69 to 96% are common for three to five heptad-repeat coiled-coils.\textsuperscript{8,52} The fact that it is less than 100% helical likely arises from end-fraying.

The 2c-Os/2b-Ru peptide pair showed a minor destabilization when compared to the 2f-Os/2f-Ru pair. The difference in $\Delta G_{\text{unf}}$ (8.2 ± 0.10 kcal/mol) for the 2c-Os/2b-Ru peptide pair corresponds to an increase in the extrapolated $K_d$ (0.97 ± 0.20 $\mu$M). The maximum ellipticity for the 2c-Os/2b-Ru pair (−26,400 ± 700 deg·cm$^2$·dmol$^{-1}$) corresponds to 74% $\alpha$-helicity, indicating slightly less $\alpha$-helical character when compared to the 2f-Os/2f-Ru dimer. Not unexpectedly, the 2g-Os/2e-Ru peptide pair was found to be the least stable heterodimer, as this pair places the Ru(II) and Os(II) closest to the dimerization interface, although the magnitude of the destabilization was again quite minimal. The extrapolated $\Delta G_{\text{unf}}$ (8.0 ± 0.10 kcal/mol) value for the 2g-Os/2e-Ru pair is within error of that reported for the 2c-Os/2b-Ru pair. The calculated $K_d$ (1.4 ± 0.3 $\mu$M) value for 2g-Os/2e-Ru pair is therefore also within error. The maximum ellipticity (−25,900 ± 300 deg·cm$^2$·dmol$^{-1}$) for the 2g-Os/2e-Ru dimer corresponds to 72% $\alpha$-helicity.

Although the thermodynamic parameters ($\Delta G_{\text{unf}}$, $K_d$, and $\% \alpha$-helicity) for the 2b-Ru/2c-Os and 2c-Ru/2g-Os metallopeptide pairs are within error, the GdnHCl denaturation midpoints ($[\text{GdnHCl}]_1/2$) = 2.0 ± 0.05 M for the 2b-Ru/2c-Os and 1.8 ± 0.05 M for the 2c-Ru/2g-Os) for the two pairs analyzed at the same total peptide concentrations are well outside of error. Chemical denaturation midpoints are often used to compare structurally similar peptide sequences since $\Delta G_{\text{unf}}$ values are extrapolated and inherently prone to error.\textsuperscript{31,33} The difference in free energy of unfolding between the 2b-Ru/2c-Os and 2c-Ru/2g-Os metallopeptide pairs ($\Delta \Delta G_0$) was calculated to be 0.3 kcal·mol$^{-1}$ based on the difference between the $[\text{GdnHCl}]_1/2$ values (see Experimental Section).\textsuperscript{31,33}

Solubility issues prevented full analysis of the parent sequences lacking Ru(II) or Os(II) complexes by GdnHCl denaturation. On the basis of the relatively small degree of destabilization within the different metallopeptide pairs, it is expected that the 2f-Os/2f-Ru pair and the parent sequences would not be measurably different. Qualitative examination of CD spectra for the parent sequences also indicated a similar folding behavior (see Supporting Information). This conclusion

![Figure 5](https://dx.doi.org/10.1021/ic300669t.Inorg.Chem.2012,51,11324-11338)

**Figure 5.** (a) Guanidinium chloride denaturation curves at 25 °C in 20 mM sodium phosphate buffer, 100 mM NaCl, pH 7.4. Data plotted for the 2f-Os/2f-Ru pair (black circles, 300 $\mu$M), the 2c-Os/2b-Ru pair (red squares, 200 $\mu$M), and the 2g-Os/2e-Ru pair (blue diamonds, 200 $\mu$M). The calculation of fraction folded is described within the experimental procedures section. (b) Gibbs free energy of unfolding is plotted as a function of guanidinium chloride concentration ($\Delta G = m[\text{GdnHCl}] + \Delta G_{\text{unf}}$). Linear extrapolation (Experimental Section) gives the value of $\Delta G_{\text{unf}}$ at zero denaturant concentration. Data shown for the 2f-Os/2f-Ru pair (black circles, $m = -1.7$ kcal·mol$^{-1}$·M$^{-1}$, $R^2 = 0.997$), the 2c-Os/2b-Ru pair (red squares, $m = -1.5$ kcal·mol$^{-1}$·M$^{-1}$, $R^2 = 0.999$), and the 2g-Os/2e-Ru pair (blue diamonds, $m = -1.7$ kcal·mol$^{-1}$·M$^{-1}$, $R^2 = 0.997$).

| Table 2. Thermodynamic Stability of the Metallopeptide Heterodimers |
|-----------------|-----------------|-------------|------------|
| peptide pair | $\Delta G_{\text{unf}}$ (kcal/mol)$^a$ | $K_d$ [\muM]$^b$ | $\alpha$-helicity$^c$ |
| 2f-Os/2f-Ru | 8.6 | 0.49 ± 0.09 | 80% |
| 2c-Os/2b-Ru | 8.2 | 0.97 ± 0.2 | 74% |
| 2g-Os/2e-Ru | 8.0 | 1.4 ± 0.3 | 72% |

$^a$Gibbs free energy of unfolding ($\Delta G_{\text{unf}}$) was measured for each peptide pair using the linear extrapolation method (Experimental Section). Error estimated to be ±0.10 kcal/mol based on error due to linear extrapolation. $^b$Dissociation constants ($K_d$) were calculated using the equation $\Delta G_{\text{unf}} = -RT \ln K_d$, using $\Delta G_{\text{unf}}$ values obtained by the linear extrapolation method. $^c$Maximum ellipticity values for each pair were measured using the CD signal at 222 nm for multiple trials. The predicted maximum molar ellipticity for a 28 residue helix ($X_{\alpha}$) is calculated to be $-35,900$ deg·cm$^2$·dmol$^{-1}$ using the equation: $X_{\alpha} = X_{\alpha}^m(1 - k/n)$, where $X_{\alpha}^m$ is the molar ellipticity for a helix of infinite length and is equal to $-39,500$ deg·cm$^2$·dmol$^{-1}$, $n$ is equal to 28 residues, and $k$ is wavelength-dependent constant equal 2.57 at 222 nm.\textsuperscript{33}
agrees with Ogawa’s studies of de novo designed electron transfer metallopeptides, where very little destabilization was measured when large transition metal complexes were attached at f positions within the sequence. The \( \Delta G_{\text{fold}} \) and \( K_d \) values reported for the three metallopeptide diners were similar to those reported by Ogawa, and other previously reported values corresponding to four-heptad coiled-coils, taking into account that the two asparagine substitutions are expected to be slightly destabilizing.

Subsequent photophysical measurements, including the appropriate control experiments, were conducted at concentrations well above the measured \( K_d \) values.

**Photophysics. Steady-State Spectroscopy.** The ground state absorption spectrum for the 2g-Os/2e-Ru metallopeptide pair exhibits a peak at 450 nm and a lower energy band that is centered at 650 nm (Figure 6). The peak centered at 450 nm is the singlet metal-to-ligand charge transfer (1MLCT) for both the Ru(II) and Os(II) bipyridyl complexes, while the lower energy band corresponds to direct excitation of the 1MLCT in the Os(II) complex which is optically accessible because of large spin–orbit coupling. When excited at 450 nm, the steady state emission spectrum for the 2g-Os/2e-Ru peptide exhibits a higher energy band (\( \lambda_{\text{max}} \) at 660 nm) that corresponds to Ru(II) 1MLCT emission after fast intersystem crossing from the 1MLCT, while the peak centered at 800 nm is Os(II) 1MLCT emission (Figure 6). The steady-state emission spectra show an increase in the Os(II) emission as the chromophores are moved closer to each other, providing direct evidence for Ru(II) to Os(II) energy transfer (see Supporting Information, Figure S3).

**Time-Resolved Spectroscopy.** Photoexcitation of the Ru(II) center at 450 nm is followed by rapid relaxation into the 3MLCT band, and potentially triplet–triplet energy transfer to the lower energy Os(II) site. In principle, energy transfer can be observed either through the quenching of Ru(II) emission at 660 nm or the appearance of the Os(II) emission at 800 nm. In practice, however, the Os(II) emission due to energy transfer is obscured by Ru(II) emission in the low energy tail and phosphorescence arising from the direct excitation of Os(II) at 450 nm. Both these contributions make a quantitative analysis of the Os(II) emission difficult. While Ru(II) emission contaminates the emission of Os(II) at 800 nm, the converse is not true, and both the time-resolved and steady-state emission measurements show no detectable Os(II) emission at 660 nm. Thus, Ru(II) emission provides the clearest window through which to view Ru-to-Os energy transfer.

The P1/2e-Ru coiled-coil was used as a control to measure the Ru lifetime in the absence of energy transfer (Figure 7a).

The decay is single exponential with a lifetime around 450 ns of the Os(II) emission. While Ru(II) emission contaminates the emission of Os(II) at 800 nm, the converse is not true, and both the time-resolved and steady-state emission measurements show no detectable Os(II) emission at 660 nm. Thus, Ru(II) emission provides the clearest window through which to view Ru-to-Os energy transfer. The P1/2e-Ru coiled-coil was used as a control to measure the Ru lifetime in the absence of energy transfer (Figure 7a).

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chemical additives. Temperature induced denaturation is more problematic because of the high thermal stability of the coiled-coil peptides. The analysis would be further complicated by the fact that the Ru(II) 3MLCT excited state lifetime is diminished with increasing temperature because of the thermally accessible metal-centered (3d^d) excited states that undergo rapid nonradiative decay. Chemical denaturation with GndHCl provides a viable alternative method for studying the denatured state at the same concentration used for time-resolved experiments.

The 660 nm decays for the P1/2-Ru and 2g-Os/2c-Ru systems in the presence of guanidine denaturant were measured and compared to the nondenatured complex (Figure 7b). The excited state lifetime of the 2c-Ru peptide is slightly longer in the highly polar 5 M GndHCl denaturation medium (τ = 520 ns compared to 450 ns in buffer), but is unaffected by the presence of up to 2 equiv (50 mM) of the 2g-Os peptide.

The ability of the chemical denaturant to turn off energy transfer confirms that the Ru(II) emission quenching arises from the folded coiled-coil peptide structure and not from nonspecific interactions between the peptide chains.

In addition, an Os(II) “control complex” (4, Figure 8) was designed to mimic the acceptor module, while lacking any molecular recognition elements that would allow for association with the partner peptide. When the control complex (4) was mixed with the 2b-Ru peptide, no change in the excited-state lifetime was observed with up to 2 equiv present. This differs from similar studies of Ru(II) and Os(II) modified oligonucleotides, which show that the addition of a noncovalently attached Os(II) complex ([Os(bpy)_2(phen)]^2+) to a Ru(II)-containing oligonucleotide solution results in some quenching (~8%) of the Ru(II) based phosphorescence, even at much lower Os(II) concentrations. This observation could be a consequence of the difference in the net charge on the two scaffolds or intercalation. The oligonucleotides used in that work are polyanionic molecules, and may have attractive charge–charge interactions with bipyridyl complexes that result in aggregation in solution. The 2b-Ru peptide on the other hand contains a net positive (+5) charge at pH 7, resulting in a repulsive charge–charge interactions with the control complex. In addition, weak intercalation of the bpy ligands into the DNA duplex may be responsible for the observed quenching in the DNA system, which is not possible in this peptide scaffold.

The results of the two control experiments clearly demonstrate that energy transfer within the peptide system occurs between coiled-coil dimer partners, and requires the folded self-assembled peptide scaffold for structural organization.

**Positional Variation and Energy Transfer.** We compared the Ru(II) emission quenching for the three coiled-coil metallopeptide pairs: 2f-Os/2f-Ru, 2c-Os/2b-Ru, and 2g-Os/2c-Ru (Figure 9), along with the transients from three coiled-coils containing the Ru(II) metallopeptides paired to the P1 apopeptide. All three of the P1/Ru(II) systems exhibit qualitatively similar monoeponential decay kinetics (Table 3). Each transient in the mixed Ru(II)/Os(II) systems is biexponential, where the slow component arises from the free Ru chains in solution and the fast component reflects Ru quenching due to energy transfer. The peptide pair that places the complexes the farthest apart, 2f-Os/2f-Ru (Figure 9 blue line), has the largest distance between alpha carbons (14.0 Å), and results in the slowest energy transfer. The 2g-Os/2c-Ru peptide pair (Figure 9, green line) places the complexes closest to each other with an α-carbon distance of 9.6–10.3 Å, and has the fastest energy transfer. Lastly, the 2c-Os/2b-Ru peptide pair has an intermediate spacing (12.7–13.5 Å) and its energy transfer rate falls in the middle (Figure 9, red line). The trend of the quenching rates indicates that the peptide assembly influences the relative positions of the Ru(II) and Os(II) complexes, and thus their ability to undergo energy transfer, in a predictable manner.

**Data Analysis.** The emission decay was measured in a series of 2g-Os/2c-Ru samples in which the 2c-Ru peptide
concentration is kept constant at 25 μM and the 2g-Os peptide concentration is incrementally increased from 0 μM to 50 μM (Figure 10). As the 2g-Os peptide concentration is increased the slow component decreases in amplitude, consistent with a diminishing amount of free Ru(II). The relative amplitudes of the two kinetic components reflect the fractions of Ru(II)/Os(II) dimer (fast) and free Ru(II) peptide (slow), resulting in an intensity decay that can be expressed as

\[
I(t) = \left( \frac{[RuOs]}{[Ru]_0} \right) e^{-(k_{Ru}+k_{enT})t} + \left( \frac{[Ru]_0 - [RuOs]}{[Ru]_0} \right) e^{-k_{enT}t}
\]

(1)

where \( k_{enT} \) is the energy transfer rate and \( k_{Ru} \) is the rate of Ru(II) excited state decay. The relative amplitudes of the two components are dependent on the concentration of associated heterodimers, [RuOs], which is determined by the ground state equilibrium:

\[
Ru + Os \rightleftharpoons RuOs
\]

(2)

\[
K_g = \frac{[RuOs]}{[Ru][Os]}/\frac{[Ru]_0 - [RuOs]}{(Os)_0 - [RuOs]}
\]

(3)

where \([Ru]_0\) and \([Os]_0\) are the total concentrations of the two peptides and \(K_g\) is the equilibrium constant.

The solid lines in Figure 10 are the result of a global nonlinear least-squares fit of the series of decays obtained at different Os(II) concentrations to eqs 1–3 with \( k_{enT}, k_{Ru}, \) and \( K_g \) being adjustable parameters. Table 3 summarizes the fitting results for the three peptide pairs. The 2g-Os/2c-Ru peptide pair exhibits the fastest energy transfer with \( k_{enT} = 2.3 \times 10^7 \) s\(^{-1}\) (42 ns) and the 2f-Os/2f-Ru peptide pair has the slowest with \( k_{enT} = 1.0 \times 10^6 \) s\(^{-1}\) (816 ns), and the 2c-Os/2b-Ru pair fell in the middle at \( 3.0 \times 10^6 \) s\(^{-1}\) (304 ns). The relatively slow energy transfer times in comparison with the lifetime of the Os(II) excited state (16 ns, \( 6.21 \times 10^7 \) s\(^{-1}\)) prevents a build-up of Os(II) excited state population. Hence a delayed rise in the Os emission, which has been observed in other systems, is not observed here. The long linker between the chromophore and the peptide scaffold will cause the attached chromophores to experience a variety of conformations that interconvert on the time scale of the excited state lifetime. Because energy transfer will be more favorable when the two chromophores are in close proximity, the observed rate will likely also reflect the time scale for structural fluctuations.

All-atom molecular dynamics simulations (discussed below) depict the bipyridyl complexes in the 2g-Os/2c-Ru and 2c-Os/2b-Ru systems in direct contact, and therefore the energy transfer occurs through Dexter energy transfer mechanism.\(^{35}\) On the other hand the simulations show the complexes in the 2f-Os/2f-Ru system remaining at a significant metal–metal separation (3 nm). Therefore the energy transfer mechanism for the 2f-Os/2f-Ru system is unlikely to be through space Dexter energy transfer. Förster, energy transfer, which occurs through a dipole–dipole mechanism, can occur over larger donor–acceptor separations.\(^{35}\) Although Förster energy transfer is formally forbidden for triplet–triplet energy transfer, the large spin–orbit coupling in these late transition metal complexes, particularly the Os(II), may provide it with some allowed character.\(^{35}\) The presence of Förster energy transfer at longer separations is consistent with observations made in other Ru(II)/Os(II) energy transfer systems.\(^{6c,10a–c}\) Regardless of the mechanism, the coiled-coil peptide system is an effective scaffold for controlling the donor/acceptor placement and hence energy transfer rate in molecular assemblies.

All-Atom Molecular Dynamics Simulations. All atom molecular dynamics simulations were performed on each of the three coiled-coils to gain insight into the molecular level interactions in these systems. Since Ru(II) and Os(II), when enveloped by the bipyridyl ligands, are practically identical from a molecular dynamics perspective, Ru(II) was used as the central atom for simulation in both bipyridyl complexes. For the sake of concise comparisons with experiments, we retain the same nomenclature for the metallopeptide pairs. It is important to mention that since a single metal–metal distance can map into a variety of different conformational arrangements between two metal complexes, we present below detailed trajectory analysis, with supplementary movies providing additional structural information (see Supporting Information).

2f-Os/2f-Ru Heterodimer. Both bipyridyl complexes in the 2f-Os/2f-Ru system start from a distal position relative to the peptide backbone. During the equilibration phase the complexes were frequently found in the proximity of the coiled-coil peptide backbone, likely driven by favorable hydrophobic interactions. To overcome the bias of the deliberately chosen initial conditions, the first 250 ns of the simulation were not included when calculating the distance distributions. The subsequent data collection phase was run for approximately 500 ns. The dynamics of the bipyridyl complexes and their unnatural side chains can be described by two regimes. First, there are large-scale conformational rearrangements during which the tethered complexes escape from their states bound to the coiled-coil and are free to explore the phase space to find new collapsed conformations. Second, there are small-scale oscillations within these conformations, which occur on a much faster time scale, but do not result in significant displacement of the metal complexes.

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Figure 10. Time-resolved emission for 1/12Ru-2c peptide (black: 25 μM 2c-Ru and 50 μM 1) and 2g-Os/2e-Ru peptide pair containing various amounts of the 2g-Os peptide (blue: 6.25 μM, red: 12.5 μM, green: 18.75 μM, gray: 25 μM, and purple: 50 μM with all containing 25 μM 2c-Ru) in a 10 mM phosphate buffer solution at pH 7 and 25 °C. The increase in 2g-Os peptide concentration drives the equilibrium to heterodimer formation and thus greater Ru(II) emission quenching by energy transfer. The time-resolved emission data were collected at 660 nm with an excitation wavelength of 444 nm.

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broad (over a 3 nm range) and non-Gaussian. Throughout the course of the simulation, the metal-center displacements observed for the 2f-Os/2f-Ru system are relatively large and would be expected to limit the efficiency of energy transfer. As discussed above, these simulations indicate that the Förster energy transfer mechanism may play an important role for this complex.

Interestingly, the bipyridyl complexes appear to influence the stability of the 2f-Os/2f-Ru coiled-coil structure as illustrated in the Supporting Information, Movie 6. α-Helical peptides have an increased propensity toward fraying near the termini, and when the metal complex and the 2f-Os/2f-Ru peptide terminus approach each other, the complex can entrain the ends of the peptides by providing competing hydrophobic interactions which result in further fraying. This partial folding and unfolding can be observed in the timeline plot of the helicity index α which is equal to the ratio of the number of residues in the α-helical conformation compared to the total number of residues in a sequence (see Figure 12 and Table 4). The implications of this observation are elaborated below.

2c-Os/2b-Ru Heterodimer. After extensive equilibration for approximately 300 ns, the initial conformation for the data collection corresponded to spatially separated bipyridyl complexes with relaxed linkers and a slightly perturbed structure for the coiled-coil scaffold. During the first 20 ns of the production run, the bipyridyl complexes do not form any stable close contact, with one complex actively exploring the surface of the peptide scaffold. At ~25 ns the two complexes contact each other and form a loose association, in which a single bipyridyl ring aligns with the triazole ring of the complementary peptide linker (see Figure 13c). This was followed by close packing of the metal complexes, and resulted in π−π stacking of bipyridine rings (see Figure 13b). The metal complexes showed relatively fast (~0.2 ns) conformational rearrangement of the bipyridine rings between them, often including a non-parallel, ~45° metastable π−π stacking (see Supporting Information, Movies 1 and 2).

The comparative graph of distance distributions in Figure 11 shows that 2c-Os/2f-Ru system has three main peaks dominated by the close-packed geometry at less than 1.5 nm. The timeline of Ru–Os distance has a feature of switching from single bipyridyl ring aligns with the triazole ring of the complementary peptide linker (see Figure 13c).

Figure 11. (a) Comparative plot of Ru–Os distance histograms (normalized) for different chromophore placements, including 2f-Os/2f-Ru, 2c-Os/2b-Ru, and 2g-Os/2e-Ru. (b) Ru–Os distance evolution in time for the 2f-Os/2f-Ru, 2c-Os/2b-Ru, and 2g-Os/2e-Ru metallopeptide pairs.

Figure 12. Fraction of α-helical content in each peptide as a function of time shown for the 2f-Os/2f-Ru (top), 2c-Os/2b-Ru (middle), and 2g-Os/2e-Ru (bottom) metallopeptide pairs. Each peptide actively explores its conformational space, while predominantly staying in an α-helical state. The exception is 2g-Os/2e-Ru system (see bottom graph), where an unfolding event is caught during MD. Visualization of the trajectory showed that peptide termini started interacting with the bipyridyl complexes and significantly disrupted the overall coiled-coil ternary and secondary structure.

Table 4. Dependence of the Coiled-Coil Secondary Structure and Metal-Center Displacement on Substitution Position

<table>
<thead>
<tr>
<th>X-Y</th>
<th>helicity index α, Ru–Os average distance</th>
<th>helicity index α, X-Os–P1</th>
<th>helicity index α, Y-Ru–P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2f</td>
<td>0.458 ± 0.097</td>
<td>0.559 ± 0.079</td>
<td>3.100 ± 0.336</td>
</tr>
<tr>
<td>2c</td>
<td>0.631 ± 0.139</td>
<td>0.593 ± 0.124</td>
<td>1.444 ± 0.319</td>
</tr>
<tr>
<td>2e</td>
<td>0.249 ± 0.097</td>
<td>0.070 ± 0.116</td>
<td>1.195 ± 0.048</td>
</tr>
</tbody>
</table>
another, yet not in direct contact. These conformations correspond to the 2c-Os bipyridine ring π−π stacking with the triazole ring of 2b-Ru linker, as shown in Figure 13c. However the contribution from loose packing conformations is statistically larger, as can be illustrated by comparing the heights of the first peak at < 1 nm (π−π stacking, see Figure 13b) and the second peak at approximately 1.2 nm (loose packing, see Figure 13c) in the Figure 11a.

In this system, we did not observe a similar perturbation of the peptide scaffold by the bipyridyl complexes as was seen in the 2f-Os/2f-Ru system. The peptide termini dynamically unfold and refold during the course of simulations (see Supporting Information, Movies 2 and 3), but not because of association with the bipyridyl complexes in this case. The helicity index of the 2c-Os-P1 and 2b-Ru-P2 peptides is shown in Figure 12. Interestingly, the standard deviation of the helicity index is approximately 2-fold higher for the 2c-Os/2b-Ru system, indicating that peptides are more dynamic and actively explore their phase space (see Table 4).

2g-Os/2e-Ru Heterodimer. In a manner very similar to the two previous systems, the bipyridyl complexes in the 2g-Os/2e-Ru system quickly collapsed onto the coiled-coil peptide scaffold during the equilibration phase ~250 ns. The two metal complexes came into van der Waals contact with each other in a conformation very similar to the intermediate chromophore packing arrangement that was observed in the 2c-Os/2b-Ru system (Figure 13c). For 30 ns the bipyridyl complexes fluctuate between the π−π stacking (see Figure 13b) and looser ligand arrangements, as shown in Figure 11b. Finally, we observe one more major conformational rearrangement where a tight “neck” stacking is achieved, which is characterized by the bipyridyl ring and a part of the connected linker of one complex aligning with the corresponding structural parts of the other. As a result, the complexes are facing away from each other (see Figure 13a and Supporting Information, Movie 4). This conformation, which was not observed in the 2c-Os/2b-Ru system, persists until the end of the simulation. As shown in Figure 13a and Supporting Information, Movie 5, the bipyridyl complexes in the 2g-Os/2e-Ru system also maintain a close proximity with the bipyridyl ligands spending most of their time in van der Waals contact.

This “neck” stacking creates a significant amount of strain for the peptide scaffold, as well as an additional hydrophobic surface consisting of the linker side chains pulled together. We show in Figure 13a and Supporting Information, Movie 5 that the peptide termini closest to the bipyridyl complex detach from the coiled-coil interface and bind to the groove between linkers or wrap around the bipyridyl complexes themselves, leading to more disruption of the coiled-coil structure, compared with the other two systems (see Figure 13). The analyses of the simulation data for all three systems suggest that the various placements of the complexes results in dissimilar complex-peptide interactions, where the latter influence the corresponding coiled-coil stabilities (see Table 4). Furthermore, although the trends predicted from the MD simulations are qualitatively consistent with the corresponding thermodynamic measurements, the quantitative extents of the coiled-coil disruption seen in simulations are likely overestimated. The following possibilities could have contributed to producing extra fraying in our MD simulations: (1) overestimating the metal-complex-peptide interactions, (2) underestimating the strength of the interpeptide bonding, or (3) preparing the initial coiled-coil conformations in an imperfect way, which did not allow tight enough packing of side-chains or accurate enough alignment of hydrophobic interactions (see the Experimental Section).

In summary, comparison of the dynamics for all three metallopeptide systems shows that the 2g-Os/2e-Ru pair has the narrowest Ru(II) to Os(II) distance distribution with the smallest probable separation (~1 nm), and would therefore be the best promoter for energy transfer. A metal–metal distance of 1 nm suggests that the complexes are in close contact with each other and would imply efficient energy transfer, regardless of which mechanism is considered. This is in agreement with the photophysical measurements made on the metallopeptide systems, which also indicated the 2g-Os/2e-Ru pair to be the most efficient energy transfer promoter, as discussed above. Importantly, we have not observed unfolding of the coiled-coil near the linker attachment points for any of the three systems. This suggests that the dynamics of the bipyridyl complexes themselves do not impose a significant stress along the peptide backbone in our scaffold design. To gain deeper insights into
the mechanisms of energy transfer in these systems, subsequent quantum mechanical calculations can be carried out on selected structural snapshots of stable stacking conformations observed in the MD simulations. This, in turn, may help to improve the design of peptide scaffolds, allowing for more precise control over molecular components.

III. CONCLUSIONS
The ability to control the positioning and organization of molecular components is central to the design of functional molecular-based materials. However, architectures that rely solely on covalent bonding for structure (e.g., polymers, dendrimers) have limited control over the assembly geometry and higher-order spatial control. Using peptides as scaffolds, we have designed an artificial self-assembling system that utilizes weak forces to control the relative placement of Ru(II) and Os(II) complexes. The assemblies incorporate an α-helical coiled-coil peptide scaffold consisting of α-helical heterodimers in which each coil is functionalized with either a Ru(II)- or Os(II)-containing energy donor or with an Os(II)-containing energy acceptor. This architecture differs from many other types of molecular assemblies in that it uses both intra- and intermolecular noncovalent interactions to adopt well-defined secondary and tertiary structures that control the placement of the energy transfer complexes. Moreover, the use of “click” chemistry allows for straightforward and well-defined control of the placement of the covalently linked chromophores.

Circular dichroism spectroscopy in conjunction with time-resolved emission spectroscopy confirms the importance of the heterodimeric α-helical coiled-coil structure for modulating energy transfer. Ru(II) to Os(II) energy transfer is only observed in the folded structures, and energy transfer rates measured across a series of supramolecular structures are consistent with a systematic variation of the metal complex molecular components is central to the design of functional materials that couples straightforward synthesis with function.

IV. EXPERIMENTAL SECTION
General Methods. Solvents were purchased from Fisher Scientific and were used as received unless noted otherwise. cis-Dichlorobis(2,2′-bipyridine) ruthenium(II) dihydrate was purchased from Strem Chemicals. All α-N-Fmoc-amino acids, including α-N-Fmoc-lysine, were purchased from Novabiochem. Tetrais(acetonitrile)copper(I) hexafluorophosphate, 2,2′-bipyridine, 4,4′-dimethyl-2,2′-bipyridine, and tripropargylamine were purchased from Aldrich.

Commercially available amino acids were coupled using double coupling cycles of 30–60 min each. The N-terminus of each peptide was acetylated using 5% acetic anhydride and 6% lutidine in DMF for 30 min. Cleavage of the peptides from the resin was performed in 95.0% TFA, 2.5% water, and 2.5% TIPS. TFA was evaporated with a stream of nitrogen and diethyl ether was added to precipitate the cleavage products. The peptides were extracted with water or collected as solids by centrifugation and lyophilized to dryness. Peptides were purified by RP-HPLC using an Atlantis Prep OBD dC18 semi-preparative column, with a gradient of 0–100% solvent B over 40 min, where solvent A was 95.5% water/ACN, 0.1% TFA, and solvent B was 95.5% ACN/water, 0.1% TFA. Purified samples were lyophilized and the peptide sequence was confirmed by ESI-MS. M was calculated as 3212.73 (exact) for the P1 parent sequence (C142H237N37O47). MS m/z observed: 1608.4 ([M + 2H]+), 1072.6 ([M + 3H]+), 804.7 ([M + 4H]+); M was calculated as 3363.95 (exact) for the P2 parent sequence (C143H240N40O45). MS m/z observed: 1123.0 ([M + 3H]+), 842.5 ([M + 4H]+), 674.0 ([M + 5H]+).

Azidepeptide Synthesis. Peptides containing azidolysine residues at desired positions were synthesized using an automated synthesizer as described above. α-Fmoc-c-azidolysine 2 was synthesized by the reported procedure. 16 α-Fmoc-c-azidolysine 2 was injected manually, and a single 3 h coupling reaction was performed. The azidepeptides were cleaved from the resin, and purified in a fashion identical to the parent sequences. Azidepeptide identities were confirmed by ESI-MS. M was calculated as 3238.76 (exact) for 2f-N1-P1 (C146H242N41O48). MS m/z observed: 16203.0 ([M + 2H]+), 10810.0 ([M + 3H]+), 8110.0 ([M + 4H]+); M was calculated as 3258.75 (exact) for 2e-N1-P1 (C147H242N41O48). MS m/z observed: 16493.0 ([M + 2H]+), 10980.0 ([M + 3H]+), 8252.0 ([M + 4H]+); M was calculated as 3237.77 (exact) for 2e-N1-P1 (C146H239N41O46). MS m/z observed: 16203.0 ([M + 2H]+), 10810.0 ([M + 3H]+), 8107.0 ([M + 4H]+); M was calculated as 3389.97 (exact) for 2f-N1-N2-P2 (C150H262N47O49). MS m/z observed: 11310.0 ([M + 3H]+), 8485.0 ([M + 4H]+), 6791.0 ([M + 5H]+); M was calculated as 3447.00 (exact) for 2f-N1-P1 (C146H242N41O48). MS m/z observed: 11503.0 ([M + 3H]+), 8630.0 ([M + 4H]+), 6906.0 ([M + 5H]+); M was calculated as 3389.94 (exact) for 2f-N1-P2 (C148H256N49O49). MS m/z observed: 11503.0 ([M + 3H]+), 8485.0 ([M + 4H]+), 6790.0 ([M + 5H]+).

Metallopeptide Synthesis. In a typical procedure 8–15 mg (∼2–4 μmol) of azidepeptide would be partially dissolved in 1–3 mL of 50% (v/v) DMF in 10 mM sodium phosphate buffer, pH 8.5, that was deoxygenated with N2 for 30 min. The metallopeptide was reacted with (2 equiv) of either the Ru(II) complex 1a or the Os(II) complex 1b in the presence of tetrakis(acetonitrile)copper(I) hexafluorophosphate (2 equiv) and tris(triazolylmethyl)amine ligand (3, 1 equiv). The mixtures were allowed to stir overnight for 12–48 h, and then diluted with water (10 mL). The aqueous solutions were frozen and lyophilized. The fully lyophilized residue was dissolved in 0.1 M aqueous TFA and passed through a Pierce polyacrylamide desalting column. The conjugates traveled through the size-exclusion media more quickly than 1a or 1b, and provided the final metallopeptides after purification by reversed-phase HPLC using the conditions listed.
above. Metallopeptide identities were confirmed by ESI-MS. M$^+$ was calculated as 3993.96 (exact) for 2f-OsP1 (C$_{23}$H$_{363}$N$_{62}$O$_{25}$). MS m/z observed: 1997.0 ([M$^+$/2$^+$/4$^+$], 1331.8 [M$^+$/2$^+$], 999.0 [M$^+$/2$^+$]), 799.4 [M$^+$/2$^+$/4$^+$], 666.4 [M$^+$/2$^+$/4$^+$/6$^+$]); M$^+$ was calculated as 4050.98 (exact) for 2c-OsP1 (C$_{23}$H$_{362}$N$_{62}$O$_{25}$). MS m/z observed: 2025.8 ([M$^+$/2$^+$]), 1350.7 ([M$^+$/2$^+$]), 1013.2 ([M$^+$/2$^+$/4$^+$]), 810.8 ([M$^+$/2$^+$/4$^+$/6$^+$]), 679.5 ([M$^+$/2$^+$/4$^+$/6$^+$]), M$^+$ was calculated as 3992.98 (exact) for 2g-OsP1 (C$_{23}$H$_{362}$N$_{62}$O$_{25}$). MS m/z observed: 1996.5 ([M$^+$/2$^+$]), 1131.3 ([M$^+$/2$^+$]), 998.7 ([M$^+$/2$^+$/4$^+$]), 799.2 ([M$^+$/2$^+$/4$^+$/6$^+$]), 666.2 ([M$^+$/2$^+$/4$^+$/6$^+$/8$^+$]); M$^+$ was calculated as 4055.12 (exact) for 2h-OsP1 (C$_{23}$H$_{362}$N$_{62}$O$_{25}$). MS m/z observed: 1352.0 ([M$^+$/2$^+$/4$^+$]), 1014.1 ([M$^+$/2$^+$/4$^+$/6$^+$]), 811.6 ([M$^+$/2$^+$/4$^+$/6$^+$/8$^+$]), 676.5 ([M$^+$/2$^+$/4$^+$/6$^+$/8$^+$]), 580.0 ([M$^+$/2$^+$/4$^+$/6$^+$/8$^+$]; M$^+$ was calculated as 4112.14 (exact) for 2h-OsP1 (C$_{23}$H$_{362}$N$_{62}$O$_{25}$). MS m/z observed: 1371.7 ([M$^+$/2$^+$/4$^+$]), 1029.0 ([M$^+$/2$^+$/4$^+$/6$^+$]), 823.6 ([M$^+$/2$^+$/4$^+$/6$^+$/8$^+$]); M$^+$ was calculated as 4055.09 (exact) for 2h-OsP1 (C$_{23}$H$_{362}$N$_{62}$O$_{25}$). MS m/z observed: 1352.6 ([M$^+$/2$^+$/4$^+$]), 1014.5 ([M$^+$/2$^+$/4$^+$/6$^+$]).

**Compound 1a.** The propargyl amide derivatized Ru(II) complex was prepared according to the procedure reported by Khan and Grinstaff.25a

**Compound 1b.** The propargyl amide derivatized Os(II) complex was prepared according to the procedure reported by Khan and Grinstaff.25a A small round-bottom, equipped with a condenser, was charged with cis-dichlorobis(2,2'-bipyrinidino)osmium(II) (39.7 mg, 0.069 mmol), and 4-methyl-2,2'-bipyrinidino-4-propargylamide (19.1 mg, 0.076 mmol, 1.1 equiv). The reagents were heated at reflux in 50% aqueous EtOH for 24 h, and then concentrated under vacuum. The residue was suspended in H$_2$O (100 mL), and filtered to remove unreacted cis-dichlorobis(2,2'-bipyrinidino)osmium(II). The aqueous filtrate was washed with DCM (2 × 100 mL). A portion of saturated NH$_4$PF$_6$ was added to the aqueous solution and a green precipitate formed. The precipitate was extracted into EtOAc and then concentrated to a solid under vacuum. The solid was partially dissolved in DCM, filtered, and then concentrated to provide the product as a dark green solid (18.4 mg, 26%).$^1$H NMR (400 MHz, CD$_2$Cl$_2$): δ 8.80 (d, J = 1.2 Hz, 1H), δ 8.48 (s, 1H), δ 8.43–8.40 (m, 4H), δ 7.90–7.84 (m, 4H), δ 7.78 (t, J = 5.6 Hz, 1H), δ 7.70–7.65 (m, 4H), δ 7.65–7.59 (m, 4H), δ 7.41–7.34 (m, 4H), δ 7.44–7.41 (m, 4H), δ 7.21 (dd, J = 5.8, 1.0 Hz, 1H), δ 4.20 (dd, J = 5.6, 2.4 Hz, 2H), δ 2.69 (s, 3H), δ 2.28 (t, J = 2.4 Hz, 1H), UV–vis (CH$_3$CN) $\lambda_{max}$(e): 247 (44400), 291 (86000), 485 (100000), 593 (380000); High-resolution ESI-MS: m/z calculated for C$_{18}$H$_{24}$N$_{10}$O$_{6}$ (M$^+$), 337.6024; found 337.5952.

**Compound 3.** The tris-(triaziolyl)methane ligand was prepared according to the procedure reported by Chan and Fokin.25c A solution of tripropargylamine (233.0 mg, 1.777 mmol), methyl azidocateate (805.4 mg, 6.998 mmol, 3.94 equiv), and tetraakis(acetonitrile)copper(I) hexafluorophosphate (21.9 mg, 58.8 μmol) was stirred in a round-bottom and cooled in an ice bath before tetraakis(acetonitrile)copper(I) hexafluorophosphate (0.56 mg, 1.5 μmol, 20 mol %) was added. The solution was allowed to warm to room temperature slowly and stirred for 2 days under an inert atmosphere. The Os(II) complex was purified by RP-HPLC using methods identical to those described below for the metallopeptides.
pulsedwidth) operated at 200 kHz. A 495 nm long pass color filter was used for emission experiments.

The samples were placed in a 2.0 mm cuvette and placed at 45 degree angle from the incident laser beam. Samples were purged in Argon for >25 min just prior to emission experiments. All experiments were performed with Ab−atm < 0.2 OD. The solvent for each sample was 10 mM phosphate buffer at pH 7.

**Molecular Dynamic Modeling.** Since no crystal structure was available for the system, the initial structure was generated using PyMOL,38 which is a molecular visualization tool capable of constructing simple peptides. The P1 and P2 peptides were initially generated independently using PyMOL’s helical parameters and were then manually aligned with care taken to avoid steric clashes and satisfy the hydrophobic interface. The ε-triazolo-L-lysine linker segment was constructed using Gaussview, part of the Gaussian 03 suite.39 Ruthenium(II) and osmium(II) are very similar from an MD perspective. Since the primary focus of these simulations is peptide dynamics, ruthenium was used as the central atom in both complexes and osmium was not explicitly included. This is not expected to have any significant impact on the simulations, since the metal atoms are nearly completely shielded from the rest of the system by the bipyridyl ligands, and metal’s formal charge, which is the same for Ru(II) and Os(II), plays the most important role. To examine the effect of linker positioning on the complexes, the following three systems were created: System 1, which corresponds to the 2f-Os/2f-Ru metal-ligand pair, System 2 which corresponds to the 2c-Os/2h-Ru metal-ligand pair, and System 3 which corresponds to the 2g-Os/2c-Ru metal-ligand pair.

The simulations were prepared using the AMBER99SB-ff parameter set. Since the AMBER libraries do not possess parameters for the artificial amino acids used as tethers, or for the chromophores themselves, these values needed to be collected from literature or obtained through quantum calculations. Partial charges for the linker and chromophores were obtained from Gaussian calculations using restricted electrostatic potential (RESP) technique44 gave spurious results for ruthenium and the chelating nitrogen atoms in the bipyridyl ligands. RESP has difficulty predicting the correct charge for buried atoms since the charges are assigned in an effort to reproduce the external electrostatic potential.44 Because of this, Mulliken charges were used in lieu of RESP charges. In general, Mulliken charges tend to be slightly more exaggerated than RESP charges with an average difference in predicted charge of 0.1(±0.1)e for all atoms excluding the ruthenium and those atoms immediately surrounding it. There was insufficient memory to compute partial charges for the entire linker and complex. To deal with this issue, the partial charges were computed for the base of the peptide up to the γ-carbon of the side chain were extracted from the standard lysine amino acid residue. Force constant for Ru−N stretches, N−Ru−N (cis/trans) bends, C−C−N−Ru dihedrals, H−C−N−Ru dihedrals and van der Waals parameters were obtained from Brandt et al.39 Since AMBER does not explicitly support Octahedral geometry, chelating nitrogen atoms were divided into three distinctly named but chemically identical types to establish different bending force constants for cis and trans positions. Each of the three simulations were performed with ~13000 explicit TIP3P water molecules in a box with the dimensions ~75 × 75 × 75 Å under periodic boundary conditions. The charge of each system was neutralized by the addition of sodium counterions, followed by the subsequent introduction of an additional 10 mM NaCl. Each system was held at constant volume, and the peptides were frozen in place while the water and ions were minimized for 200,000 steps. Subsequently, all constraints were removed from the systems, and they were minimized for an additional 200,000 steps. The systems were gradually heated via Langevin temperature control to 300 K in incremental steps of 5 K every 50 ps. The production runs proceeded under the constant pressure, moderated by Langevin piston (set to 1 atm), with 2 fs time steps using the SHAKE algorithm and Ewald summation for long-range interactions. Short-range nonbonded interactions were calculated at each step, long-range interactions were only calculated on even steps, and the pair list was updated every 10 steps. System coordinates were saved every 2000 steps (4 ps) for analysis for a total simulation length of 300 ns for each system.

**ASSOCIATED CONTENT**

**Supporting Information**

Figures containing the continuous variation experiments, additional circular dichroism spectra, denaturation of parent sequence, additional time-resolved emission transients, NMR spectra, and the complete ref 39 and 40. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

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**REFERENCES**


(39) Frisch, M. J.; et al. Gaussian 03, Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.