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DNA Recognition

Metal-Ion-Regulated Miniature DNA-Binding Proteins Based on GCN4 and Non-native Regulation Sites

Emmanuel Oheix and Anna F. A. Peacock*[a]

Abstract: The design of artificial peptide dimers containing polypyridine switching domains, for which metal-ion coordination is shown to regulate DNA binding, is reported. Short peptides, based on the basic domain of the GCN4 transcription factor (**GCN4bd**), dimerised with either 2,2′-bipyridine (**bipy**(**GCN4bd**)$_2$) or 2,2′:6′,2″-terpyridine (**terpy**(**GCN4bd**)$_2$) linker units, undergo a conformational rearrangement on Cu^{II} and Zn^{II} coordination. Depending on the linker substitution pattern, this is proposed to alter the relative alignment of the two peptide moieties, and in turn regulate DNA binding. Circular dichroism and UV–visible spectroscopy reveal that Cu^{II} and Zn^{II} coordination promotes binding to DNA containing the CRE target site, but to a differing and opposite degree for the two linkers, and that the metal-ion affinity for **terpy**(**GCN4bd**)$_2$ is enhanced in the presence of CRE DNA. Binding to DNA containing the shorter AP1 target site, which lacks a single nucleobase pair compared to CRE, as well as half-CRE, which contains only half of the CRE target site, was also investigated. Cu^{II} and Zn^{II} coordination to **terpy**(**GCN4bd**)$_2$ promotes binding to AP1 DNA, and to a lesser extent half-CRE DNA. Whereas, **bipy**(**GCN4bd**)$_2$, for which interpeptide distances are largely independent of metal-ion coordination and less suitable for binding to these shorter sites, displays allosteric ineffective behaviour in these cases. These findings for the first time demonstrate that biomolecular recognition, and specifically sequence-selective DNA binding, can be controlled by metal-ion coordination to designed switching units, non-native regulation sites, in artificial biomolecules. We believe that in the future these could find a wide range of applications in biotechnology.

Introduction

In nature, allosteric regulation involves the binding of an effector molecule to the regulation site in a protein or enzyme, which is distinct from the active site. This binding event can trigger a conformational change, which modifies the activity of the global system. The ability to identify the regulation mechanism provides an opportunity to control protein or enzyme activity. Alternatively, a non-native regulation site can be synthetically incorporated into proteins, in an effort to artificially control their activation, and represents an interesting challenge for chemists and biochemists.[1]

Transcription factors are activated and regulated through various allosteric mechanisms.[2] Notable examples which are relevant to this work are metal-sensing transcriptional regulators which trigger expression of the machinery required for heavy metal detoxification or regulation, in response to accumulation of specific metal ions. For example, SmtB and ArsR are bound to gene promoter sites in the absence of Zn^{II} and As^{III}, respectively, but a conformational change is triggered on metal-ion coordination resulting in dissociation from DNA and expression of metallothioneins.[3] In contrast, MerR is bound to DNA in both the absence and presence of Hg^{II}, but Hg^{II} binding induces a conformation change which triggers DNA unwinding and subsequent transcription of the *mer* operon, responsible for Hg^{II} detoxification.[4]

GCN4 is a yeast transcription factor involved in amino acid regulation, stimulating the transcription of more than 30 amino acid biosynthetic genes. Miniature artificial proteins consisting of the basic leucine zipper (b-ZIP) domain (ca. 60 out of the 281 native residues) of GCN4 have been reported to retain similar DNA affinity and selectivity to the native protein.[5] The basic (DNA binding) domain makes specific contact with the target sequence, through electrostatic and hydrogen bonding interactions to the phosphate backbone and exposed nucleobases in the major groove. Notably this domain is only folded into an α-helix when bound to the target site.[6] The leucine zipper domain is responsible for dimerisation of the peptide, which is important for strong DNA binding.[7]

One strategy adopted by chemists to control GCN4 DNA binding, is to perturb folding of the leucine zipper domain by incorporation of non-natural residues bearing photoswitchable[8] or metal-chelating ligands[9] as side-chains. Some of these stimuli-responsive leucine zipper mimics have subsequently been shown to regulate AP1 transcription activity in vivo, attributed to an inhibition of b-ZIP peptide dimerisation.[10] Alter-

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natively Kim and co-workers prepared a synthetic peptide dimer, in which the entire leucine zipper domain was replaced by a disulfide bond between two cysteine (Cys) residues. This first artificially dimerised miniature GCN4 basic domain peptide was reported to retain similar DNA binding ability to the native protein.[11] A number of different linkers have subsequently been investigated.[12,13] Of particular relevance to this work are the reports by Schepartz and co-workers on a bis(terpyridyl)iron(II) complex as the dimerisation domain, where the two terpyridyl ligands are substituted on the central pyridine ring with a basic domain peptide, and a non-metallated bipyridyl peptide dimer.[14] Importantly they were able to establish a relationship between the distance separating the two peptide basic domains and target DNA affinity.[14] This was further exploited by Mascareñas and co-workers, who developed the only system with a switchable linker, a photoswitchable azobenzene group.[15] The *trans* azobenzene linker could be converted to the *cisoid* form upon light irradiation at 365 nm, thus shortening the distance between the two basic domains and enhancing DNA binding. However, the switch is not fully reversible as the azobenzene *cisoid*-to-*transoid* reconversion over time is prevented when bound to target DNA.

Though the conformational rearrangement of chelating ligands on metal-ion coordination to promote DNA binding of small synthetic molecule ligands has been reported,[16,17] to the best of our knowledge no reports exist of their introduction into larger biomolecules for controlling DNA binding. Here we investigate the potential of utilising non-native polypyridine linkers as an allosteric regulation site, by exploiting their *transoid*-to-*cisoid* conformational rearrangement upon metal-ion complexation. Quantum mechanical calculations in the gas-phase estimate the enthalpy of the *cisoid*-conformation of 2,2′-bipyridine (**bipy**) and 2,2′:6′,2″-terpyridine (**terpy**) to be 26.5 and 52.5 kJ mol^{-1} higher than their respective *transoid* conformations, principally due to steric repulsion between two central hydrogen atoms.[18,19] However, the *cisoid* conformation is favoured upon metal-ion complexation, due to the formation of highly stable 5-membered chelate rings. Rebek and co-workers first took advantage of the reorientation of 2,2′-bipyridine substituents on metal-ion complexation, to introduce an allosteric regulation site into a crown ether, thus regulating its alkali-metal-ion binding capacity.[20] The metal switching behaviour of substituted bipyridine and terpyridine has subsequently found many applications, primarily in aprotic solvents,[20–23] and has been introduced into a range of macromolecular synthetic (e.g., polymers)[24] and biological structures (e.g., DNA).[25] Despite the latter there are no reports of their introduction into peptides and their subsequent switching behaviour, beyond the work by Kelly and co-workers who introduced a bipyridine unit into a peptide backbone and reported that Cu^{II} coordination triggers a *transoid*-to-*cisoid* conformational change, but only under basic conditions (pH 9.5).[26] We recently reported on the development of model switching units consisting of either a disubstituted bipyridine or terpyridine unit as the dimerisation domain for peptides through the thiol side-chain of Cys, and investigated their Cu^{II} and Zn^{II} coordination chemistry.[27] The bipyridine linker was designed such that the resulting *transoid*-to-*cisoid* conformational transition on metal-ion coordination would not alter the distances between the peptide units, and was proposed to be allosteric ineffective. In contrast the terpyridine linker was designed so that the distance between the two peptides would be highly sensitive to metal-ion coordination.

Here we extend this work to larger protein fragments capable of biomolecular recognition. We propose to test the applicability of our metal-ion regulation sites as artificial dimerisation units for GCN4, which represents a well-studied system with a plethora of mutagenesis and (minimal) sequence studies identifying features important for selective DNA binding.[28–32] This report therefore describes the synthesis and characterisation of miniature DNA binding proteins, based on the basic domain of GCN4, dimerised by metal chelating bipyridine and terpyridine units. The Cu^{II} and Zn^{II} binding properties are evaluated and how pyridine ring rotation and subsequent reorientation of the peptide substituents, regulates DNA binding is reported. Though this work focuses on GCN4, we propose that this strategy, of dimerisation sites capable of regulating peptide alignment, could be applied to a large number of biomolecular recognition processes.

Results and Discussion

Design of a peptide allosteric regulation site

Our goal was to prepare a miniature DNA binding protein which responds to the presence of metal ions (Figure 1). The leucine zipper dimerisation domain of GCN4 was therefore replaced with a polypyridine linker (bipyridine or terpyridine) capable of binding to metal ions, such as Cu^{II} and Zn^{II}. Our approach was to take advantage of the reactivity of the cysteine (Cys) thiol side-chain to couple di(bromomethyl) derivatives of bipyridine and terpyridine, through a nucleophilic substitution reaction. The two metal chelating linker units selected, 5,5′-dibromomethyl-2,2′-bipyridine and 6,6″-dibromomethyl-2,2′:6′,2″-terpyridine, were synthesised as reported previously,[14c,27] and subsequently reacted with the single Cys amino acid introduced for this purpose towards the C terminus of peptide sequences based on GCN4.

The substitution of bipyridine along the axis of the pyridine–pyridine bond (5,5′-position) should place the peptide substituents about 10 Å apart, independent of whether the bipyridine adopts the *cisoid*[33] or *transoid* conformation.[34] Though the bipyridine *cisoid*-to-*transoid* conformational transition would result in reorientation of substituents, the free rotation of the thioether motif should be able to compensate for this. In contrast, placement of substituents at the 6,6″-position of terpyridine would result in substituents located either about 5 (*cisoid*)[22] or 12 Å (*transoid*)[35] apart (Figure 2). These linkers therefore represent the two extremes, where intersubstituent distance is independent (allosteric ineffective) or highly dependent (allosteric effective) on the polypyridine linker conformation. Such comparison should allow for important relationships between intersubstituent distance and the regulation process, to be established.

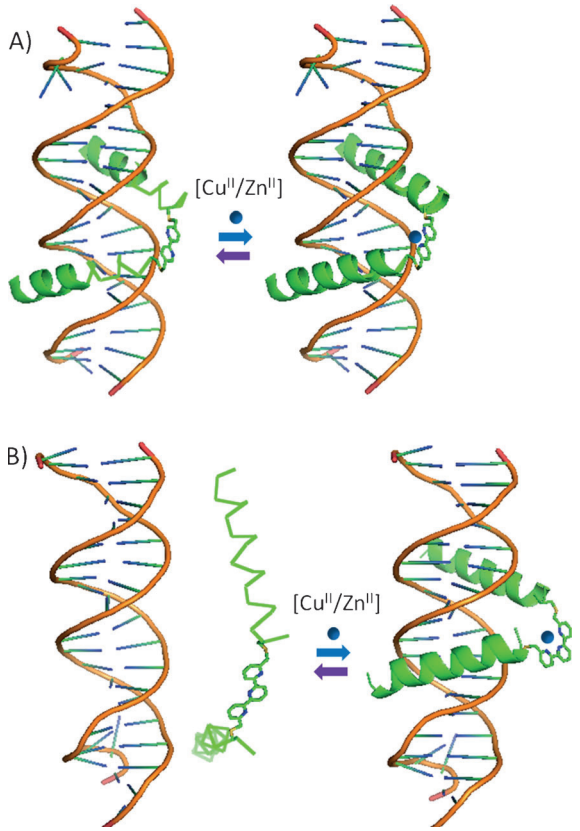

Figure 1. Cartoon illustration of the designed metal-ion-dependent DNA binding of: A) the bipyridine, and B) terpyridine dimerised GCN4 DNA binding domains. A conformational change, achieved on metal-ion coordination, is most important for terpyridine binding to DNA.

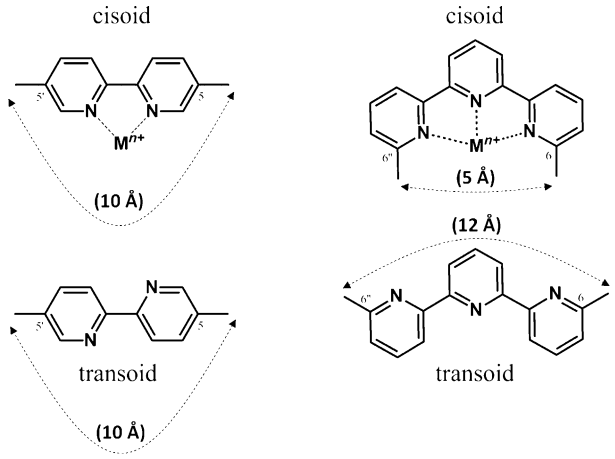

Figure 2. Scheme illustrating the impact of the *cisoid–transoid* conformational transition on the intersubstituent distance for the 5,5′-disubstituted 2,2′-bipyridine, and 6,6′′-disubstituted 2,2′;6′,2″-terpyridine linker.

DNA binding peptide sequence design

It has previously been demonstrated that the leucine zipper domain of GCN4 can be replaced by a chemical linker and still retain similar DNA binding.[11–15] For example, a study by Kim and co-workers, compared a library of peptide dimers (of different lengths) derived from the basic domain of the GCN4 transcription factor. These peptides all bore a GGC motif toward the C terminus to allow dimerisation through thiol oxidation. Both DNase I footprinting and circular dichroism (CD) melting experiments revealed that the 15 amino acid sequence KRARNTEAARRSRAR contains the necessary conserved residues required for the formation of specific contacts with the DNA target site CRE, whereas binding to AP1 required additional conserved residues.[13] The linker region LQRMKQL, separating the basic and leucine zipper domains and which allows for the formation of an uninterrupted α-helix,[36] has been reported to be important for thermal stability on binding to both CRE and AP1 DNA.[11,13]

Based on this literature our initial design, Ac-ALKRARNTEAARRSRARKLQRMKQLGG**C**G-NH_2, included both the basic domain and the flexible linker region so as to achieve strong and selective DNA binding. However, DNA binding was found to be sufficiently strong so that both the bipyridine and terpyridine peptide dimers bound to DNA in the absence of any metal ions (confirmed by gel electrophoresis), and the addition of Cu^{II} or Zn^{II} did not alter DNA binding.[37] This lack of DNA binding regulation in response to the presence of Cu^{II} and Zn^{II} ions is most likely due to the peptide sequence adopted, which contains both a flexible linker domain and Gly residues which precede the Cys. This flexible section may be able to buffer conformational changes arising from the allosteric site, thus reducing its ability to influence DNA binding. Therefore, the peptide sequence adopted for this work, **GCN4bd** Ac-ALKRARNTEAARRSRAR**C**G-NH_2, contains 17 conserved GCN4 residues (corresponding to the domain located between residue 229 and 245 of GCN4) which is predominantly the basic domain and which lacks the flexible linker domain (thought to be important in the stability of the DNA complex). The location of Cys (equivalent to Lys246 of native GCN4) is such that the two thiols from the side-chains should be positioned about 7.1 Å apart in the complex formed between GCN4 and DNA containing the CRE target site, most suitable for either a 5,5′-dimethyl-2,2′-bipyridine (*cisoid* and *transoid*) or for the *cisoid* form of 6,6′′-dimethyl-2,2′:6′,2′′-terpyridine (measurements based on crystal structure PDB code 2DGC; Figure 3).[38] In the similar complex formed between GCN4 and DNA containing the related AP1 target site, which lacks one nucleobase pair compared to CRE, these are positioned closer together at about 5.7 Å apart (Figure 3), a more suitable distance for only the *cisoid* form of the 6,6′′-dimethyl-2,2′:6′,2′′-terpyridine linker unit (measurements based on crystal structure PDB code 1YSA).[39] This minimal peptide sequence, **GCN4bd**, has been designed such that the alignment of folded α-helices, the DNA binding domain, will be highly dependent on the conformation of the linker unit. This peptide was synthesised, purified and characterised by routine methods.[40]

Preparation of GCN4 peptide dimers containing allosteric regulation sites

The linkers, 5,5′-dibromomethyl-2,2′-bipyridine and 6,6′′-dibromomethyl-2,2′:6′,2′′-terpyridine, were successfully coupled to

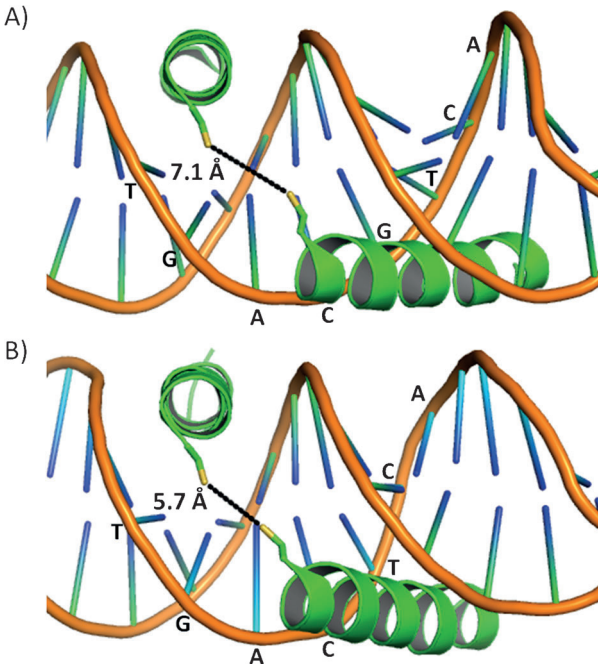

Figure 3. Cartoon illustrating the distances between sulfur atoms from a theoretical Cys, introduced as the best aligned rotamer, in place of residue Lys246 of the GCN4 homodimer, when complexed with duplex DNA containing the: A) CRE (based on PDB ID 2DGC[38]), or B) AP1 (based on PDB ID 1YSA[39]) target site, which differ in a single nucleobase pair. The DNA bases are indicated with single letters.

the Cys side-chain to generate GCN4 peptide dimers (**bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$) which contain a metal-ion chelation site in place of the leucine zipper dimerisation domain. Though 5,5′-disubstituted-2,2′-bipyridine compounds have previously been introduced into peptides,[14c,26,41,42] this and our recently reported model compounds with glutathione[27] constitute, to the best of our knowledge, the first describing the insertion of a disubstituted terpyridine unit as a linker between two peptide strands.

A control peptide dimer, which lacks a metal chelating unit (bipyridine or terpyridine), was prepared by dimerising two equivalents of **GCN4bd** by formation of a disulfide bond to generate (**GCN4bd**)$_2$.

All peptide dimers were purified by reversed phase C18-HPLC and characterised by ESI-MS (Figures S1–S6 in the Supporting Information).

Circular dichroism: folding of GCN4 peptide dimers in the presence of DNA

Peptides containing basic amino acids can non-specifically bind in the presence of the negatively charged DNA phosphate backbone.[43] However, peptides derived from the basic domain of GCN4 have been shown to display sequence-specific folding into α-helices in the presence of DNA containing a GCN4 target site (such as CRE).[13] Therefore, CD, and specifically the ellipticity at 208 and 222 nm, can be used to infer DNA binding. This is particularly important as we were unable to obtain band retardation for any of the **GCN4bd** peptide dimer DNA complexes in gel electrophoresis band-shift assays in either the absence or presence of metal ions, even at CD relevant concentrations. Whereas bipyridine dimers of the longer peptides, vide supra, did display band retardation when bound with target CRE DNA.[37] This is consistent with a shorter peptide design and previous reports indicating that truncation of the hinge area of GCN4, results in a decrease in the stability of the peptide–DNA complex, preventing successful separation under electrophoresis conditions.[11,12a,13]

The CD spectrum of a 5 μM solution of (**GCN4bd**)$_2$, lacking the chelating polypyridyl dimerisation unit, in 10 mM phosphate buffer pH 7.4 is consistent with a random coil structure. On addition of 1 equivalent of non-specific (NS) DNA, there was a small increase in the negative signal (Θ_{222} $-3.4\times10^3\rightarrow -5.6\times10^3$ deg dmol^{-1} cm^2 per residue). In contrast the addition of 1 equivalent of target CRE DNA, resulted in a spectrum with intense minima at 208 and 222 nm (Θ_{222} -14.6×10^3 deg dmol^{-1} cm^2 per residue), consistent with the formation of an α-helix and binding to the DNA (Figure 4A).

Similar results were obtained for the analogous experiments performed with the dimers containing the polypyridyl linker domains, **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$. The CD spectra of 5 μM solutions of either **bipy**(**GCN4bd**)$_2$ or **terpy**(**GCN4bd**)$_2$ in 10 mM phosphate buffer pH 7.4 are also consistent with random coil structures, which are largely unchanged in the presence of 1 equivalent of NS DNA (Figure 4B and C). Addition of 1 equivalent of target CRE DNA to a 5 μM solution of **bipy**(**GCN4bd**)$_2$ yields a CD spectrum with the characteristic α-helix minima at 208 and 222 nm (Θ_{222} -13.1×10^3 deg dmol^{-1} cm^2 per residue; Figure 4B). This is comparable to that obtained for (**GCN4bd**)$_2$ confirming that DNA binding can be retained on replacing a disulfide bond with a bipyridine unit.[14c] In contrast, the addition of 1 equivalent of target CRE DNA to a 5 μM solution of **terpy**(**GCN4bd**)$_2$ yields a CD spectrum with minima at 204 and 222 nm (Θ_{222} -9.9×10^3 deg dmol^{-1} cm^2 per residue; Figure 4C). Though this is consistent with a more folded peptide domain, this is notably less α-helical than either the disulfide or bipyridine dimerised peptide analogues, which suggests reduced binding to CRE DNA.

The **bipy**(**GCN4bd**)$_2$ peptide domains fold in the presence of CRE DNA, consistent with our design that both the *cisoid* and *transoid* bipyridine conformation would be capable of binding to CRE DNA. However, the **terpy**(**GCN4bd**)$_2$ peptide dimer is less well folded in the presence of CRE DNA, suggesting that the distance between the peptides is less appropriate for the peptide alignment required for binding to the CRE target site. This is most likely due to the terpyridine dimerisation unit not fully adopting a conformation which is optimal for binding to CRE DNA, which could be consistent with either the *transoid* (Figure 2) or a mixed *cisoid–transoid* conformation. These results were obtained in the absence of metal ions, and so it therefore remained for us to evaluate if this folding is sensitive to the presence of metal ions, such as Cu^{II} and Zn^{II}, and specifically whether these can be exploited to regulate DNA binding.

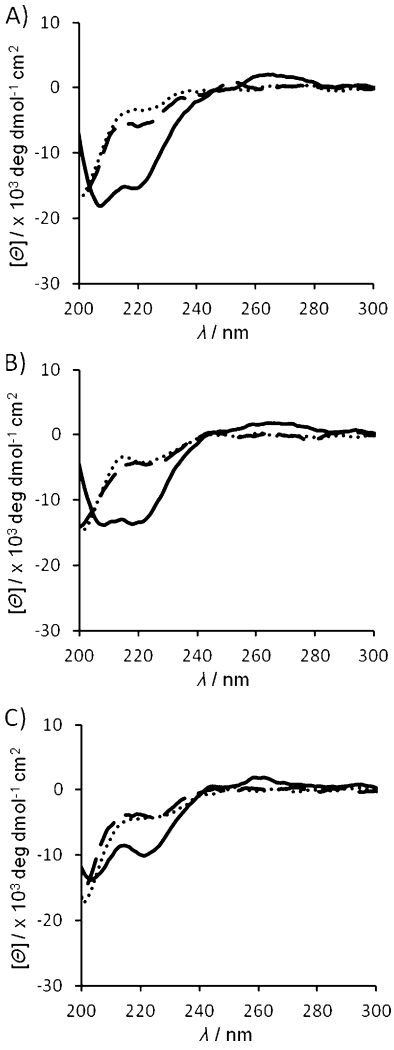

Figure 4. CD spectra of 5 μM solutions of: A) (**GCN4bd**)$_2$ oxidised dimer, B) **bipy**(**GCN4bd**)$_2$, and C) **terpy**(**GCN4bd**)$_2$ in 10 mM phosphate buffer pH 7.4, in the absence (•••••) of duplex DNA, and in the presence of 5 μM NS (-----) or CRE DNA (——).

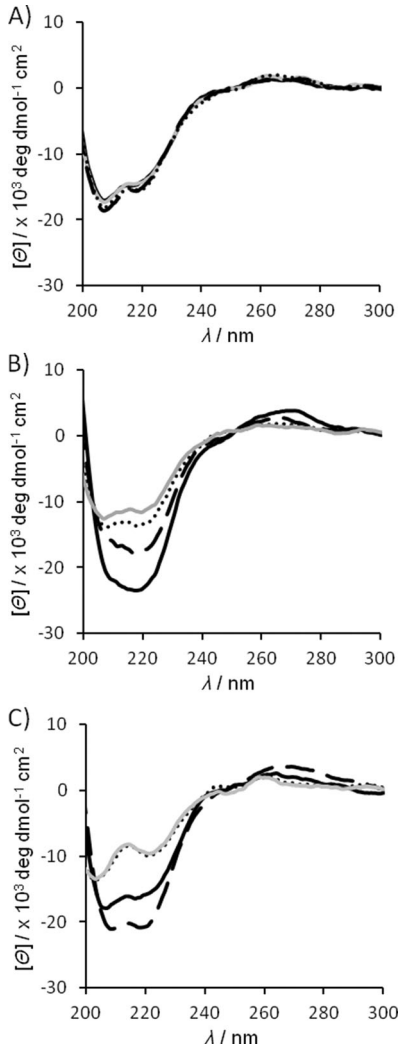

Figure 5. CD spectra of 5 μM solutions of CRE DNA and: A) (**GCN4bd**)$_2$ oxidised dimer, B) **bipy**(**GCN4bd**)$_2$ and C) **terpy**(**GCN4bd**)$_2$ in 10 mM phosphate buffer pH 7.4, in the absence (•••••) and presence of 2 equivalents of either $CuCl_2$ (-----) or $ZnCl_2$ (——). Reversibility was monitored by addition of 10 equivalents of EDTA (——).

Circular dichroism: influence of metal ions on DNA binding

CD spectra recorded of all three peptide dimers, (**GCN4bd**)$_2$, **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$, display poor α-helicity in the absence of DNA or in the presence of NS DNA. The addition of two equivalents of either $CuCl_2$ or $ZnCl_2$ resulted in only minor changes (Figure S7 in the Supporting Information). However, in the presence of DNA containing the CRE target site, the three peptides display a notable increase of the negative ellipticity at 222 nm, suggesting partial or full folding into α-helices. The addition of two equivalents of either $CuCl_2$ or $ZnCl_2$ to a 5 μM solution of (**GCN4bd**)$_2$ in the presence of CRE DNA, does not result in any further change in the CD spectrum, consistent with a design which lacks a metal-ion-chelating polypyridine linker unit (Figure 5A). However, the helicity of **bipy**(**GCN4bd**)$_2$ in the presence of CRE DNA, increases by 29% upon addition of two equivalents of $CuCl_2$ (Θ_{222} $-13.1\times10^3\rightarrow-17.0\times10^3$ deg dmol^{-1} cm^2 per residue) and 70% upon addition of two equivalents of $ZnCl_2$ (Θ_{222} $-13.1\times10^3\rightarrow-22.3\times10^3$ deg dmol^{-1} cm^2 per residue; Figure 5B). In contrast, the peptide helicity of **terpy**(**GCN4bd**)$_2$ in the presence of CRE DNA doubles upon addition of two equivalents of $CuCl_2$ (Θ_{222} $-9.9\times10^3\rightarrow-20.0\times10^3$ deg dmol^{-1} cm^2 per residue), but only increases by a more modest 55% upon addition of two equivalents of $ZnCl_2$ (Θ_{222} $-9.9\times10^3\rightarrow-15.4\times10^3$ deg dmol^{-1} cm^2 per residue; Figure 5C).

CD spectra indicate that both **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$ experience an increase in the negative ellipticity at 222 nm upon metal-ion addition, which in turn is highly dependent on the nature of the metal ion, that is, Cu^{II} versus Zn^{II}. The percentage change in molar ellipticity at 222 nm suggests a higher affinity for DNA containing the CRE target site for Cu–**terpy**(**GCN4bd**)$_2$ and Zn–**bipy**(**GCN4bd**)$_2$ complexes, and a lower affinity for the Zn–**terpy**(**GCN4bd**)$_2$ and Cu–**bipy**(**GCN4bd**)$_2$ complexes (Figure 5). These changes are accompanied by the appearance of a positive band at 268 nm in the difference CD spectra, appearing upon addition of peptide

conjugates to CRE DNA and growing in intensity upon metal-ion addition. This can be assigned to the bending of the DNA which has previously been reported to occur upon binding of basic zipper peptides.[6,44]

These experiments indicate that binding to DNA containing the CRE target site is sensitive to the presence of metal ions, when the linker domain contains a chelating ligand. The enhancement in DNA binding for **terpy**(**GCN4bd**)$_2$ on coordination of metal ions is consistent with the terpyridine adopting the *cisoid* conformation, which we propose is more appropriate for DNA binding. However, we also observe an enhancement, though to a lesser extent, for the bipyridine linker and therefore not only the *transoid–cisoid* conformational change, but other factors, such as enhanced electrostatic attraction with the DNA, may also contribute.

Circular dichroism: reversible DNA binding

In order to assess reversibility, EDTA was added to solutions of the metal–peptide conjugates in the absence and presence of DNA. Addition of excess EDTA (10 equivalents per metal ion) resulted in no significant change to the CD spectra recorded in either the absence or presence of NS DNA (Figure S7 in the Supporting Information). However, a decrease in the negative ellipticity at 222 nm was observed for both **bipy**(**GCN4bd**)$_2$ (Cu Θ_{222} $-17.0\times10^3\rightarrow-11.0\times10^3$; Zn Θ_{222} $-22.3\times10^3\rightarrow-11.0\times10^3$ deg dmol^{-1} cm^2 per residue) and **terpy**(**GCN4bd**)$_2$ (Cu Θ_{222} $-20.0\times10^3\rightarrow-9.5\times10^3$; Zn Θ_{222} $-15.4\times10^3\rightarrow-8.0\times10^3$ deg dmol^{-1} cm^2 per residue) in the presence of CRE DNA (Figure 5 B and C). The resulting spectra are similar to those recorded prior to metal-ion complexation, which is importantly consistent with a fully reversible process. In contrast, no change was observed in the spectrum of (**GCN4bd**)$_2$ after addition of EDTA, consistent with the oxidised dimer not coordinating a metal ion in the linker domain.

UV–visible Cu^{II} and Zn^{II} titrations

It remained necessary to evaluate the Cu^{II} and Zn^{II} coordination chemistry of the two new peptide dimers, **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$. The UV–visible spectrum of a solution of **bipy**(**GCN4bd**)$_2$ in 20 mM phosphate buffer pH 7.4 displays two transitions with λ_{max} at 299 and 246 nm, assigned as $\pi\rightarrow\pi^*_1$ and $\pi\rightarrow\pi^*_2$ transitions, respectively (Figure 6).[45] The analogous spectrum of **terpy**(**GCN4bd**)$_2$ shows a peak with λ_{max} 298 nm attributed to $\pi\rightarrow\pi^*_1$, however, the $\pi\rightarrow\pi^*_2$ transition overlaps with that for the peptide bond (Figure 6). These spectra are consistent with a predominantly *transoid* conformation for both the bipyridine and terpyridine linker unit.[27,46,47]

The bands shift to lower energy upon Zn^{II} and Cu^{II} complexation, allowing estimation of an apparent binding constant.[41] Aliquots of a stock solution of $ZnCl_2$ were titrated into a 5 μM solution of **bipy**(**GCN4bd**)$_2$ or **terpy**(**GCN4bd**)$_2$ in 20 mM phosphate buffer pH 7.4, up to 3 equivalent of $ZnCl_2$. This resulted in the steady decrease in the absorbance at 299 (**bipy**(**GCN4bd**)$_2$) and 298 nm (**terpy**(**GCN4bd**)$_2$), and an increase in the absorbance at 313 and 320 nm (**bipy**(**GCN4bd**)$_2$), and 328 and 341 nm (**terpy**(**GCN4bd**)$_2$; Figure 6). Continuous-variation plots are consistent with predominant formation of a 1:1 complex between Zn^{II} and either **bipy**(**GCN4bd**)$_2$ or **terpy**(**GCN4bd**)$_2$ under these conditions (Figure S8 in the Supporting Information). The isosbestic points at 308 (**bipy**(**GCN4bd**)$_2$) and 318 nm (**terpy**(**GCN4bd**)$_2$) are consistent with the clean conversion to a metal bound complex. A plot of the absorbance of the new transitions versus Zn^{II} concentration was fitted to a 1:1 binding equation (Figure 6), and the extinction coeffi-

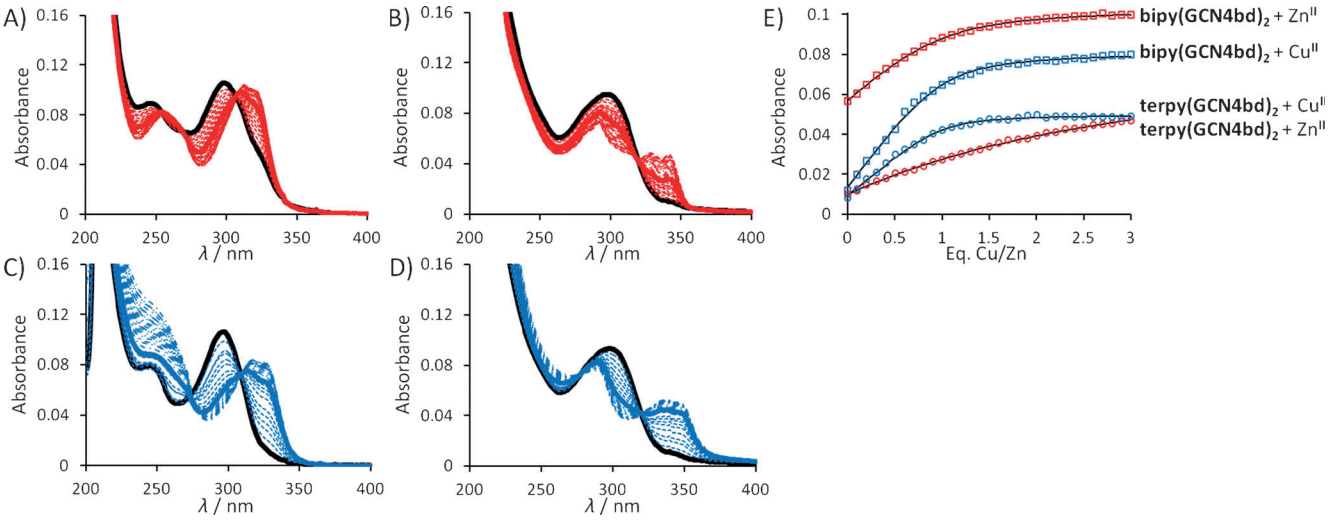

Figure 6. UV–visible spectra for the metal titration of peptide conjugates. $ZnCl_2$ (A and B) or $CuCl_2$ (C and D) were added to solutions containing 5(±0.6) μM of either **bipy**(**GCN4bd**)$_2$ (A and C) or **terpy**(**GCN4bd**)$_2$ (B and D) in 20 mM phosphate buffer pH 7.4. ——: 0 equivalent metal added; ——: 1 equivalent $CuCl_2$ added; ——: 1 equivalent $ZnCl_2$ added; ----- or -----: between 0 and 1 equivalent of $CuCl_2$ or $ZnCl_2$ added; –•–•– or –••–••–: more than 1 equivalent added. C) Buffer concentration was 100 mM phosphate and 20 mM glycine was added as competitor. E) Plot of absorbance (monitored at 320 (□), 328 nm (□) for **bipy**(**GCN4bd**)$_2$ and 341 (○), 348 nm (○) for **terpy**(**GCN4bd**)$_2$) versus the equivalence of metal ion (with $ZnCl_2$ (——) and $CuCl_2$ (——)). Line represents best fit for a 1:1 metal/peptide dimer conjugate binding ratio.

Table 1. Summary of the UV–visible data obtained for Cu^{II} and Zn^{II} coordination to polypyridyl peptide conjugates, **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$, in the absence and presence of DNA (NS and CRE).

		Peptide conjugate	λ [nm]	ε_{ML} [$M^{-1}\,cm^{-1}$]	K_{app} [M^{-1}]	K_M [M^{-1}]	R^2
$ZnCl_2$		**bipy**(**GCN4bd**)$_2$	320	1.95 ± 0.01 E + 04	1.38 ± 0.10 E + 06	8.29 ± 0.60 E + 06	0.9986
		terpy(**GCN4bd**)$_2$	341	1.89 ± 0.06 E + 04	7.74 ± 0.53 E + 04	4.66 ± 0.32 E + 05	0.9989
$ZnCl_2$	NS	**bipy**(**GCN4bd**)$_2$	320[a]	1.74 ± 0.08 E + 04	7.20 ± 1.27 E + 04	4.34 ± 0.77 E + 05	0.9939
	DNA	**terpy**(**GCN4bd**)$_2$	341	1.80 ± 0.03 E + 04	2.43 ± 0.15 E + 05	1.46 ± 0.09 E + 06	0.9987
$ZnCl_2$	CRE	**bipy**(**GCN4bd**)$_2$	320	1.90 ± 0.02 E + 04	1.29 ± 0.32 E + 06	7.77 ± 1.95 E + 06	0.9904
	DNA	**terpy**(**GCN4bd**)$_2$	341	1.62 ± 0.02 E + 04	6.94 ± 0.57 E + 05	4.18 ± 0.34 E + 06	0.9976
$CuCl_2$		**bipy**(**GCN4bd**)$_2$	328	1.55 ± 0.01 E + 04	2.94 ± 0.39 E + 06	1.48 ± 0.20 E + 13[b]	0.9966
		terpy(**GCN4bd**)$_2$	348	1.14 ± 0.01 E + 04	2.67 ± 0.27 E + 06	8.72 ± 0.89 E + 07	0.9972
$CuCl_2$	NS	**bipy**(**GCN4bd**)$_2$	328	1.54 ± 0.01 E + 04	7.34 ± 0.57 E + 05	9.25 ± 0.72 E + 11[c]	0.9979
	DNA	**terpy**(**GCN4bd**)$_2$	348	1.32 ± 0.01 E + 04	3.40 ± 0.60 E + 06	1.11 ± 0.20 E + 08	0.9941
$CuCl_2$	CRE	**bipy**(**GCN4bd**)$_2$	328	1.47 ± 0.01 E + 04	1.45 ± 0.20 E + 06	1.82 ± 0.25 E + 12[c]	0.9945
	DNA	**terpy**(**GCN4bd**)$_2$	348	1.35 ± 0.07 E + 04	4.80 ± 0.88 E + 06	2.57 ± 0.47 E + 09[d]	0.9950

[a] Absorbance at 320 nm corrected by subtracting the absorbance at 400 nm. Selected titrations performed in the presence of: [b] 20, [c] 10, or [d] 0.2 mM glycine as competitor.

cients of the peptide dimers and the resulting Zn^{II} complexes determined and reported in Table 1. Taking into account the competitive metal-ion binding of the phosphate buffer employed in these experiments,[48] binding constants, $\log K_M$, were calculated to be 6.92 ± 0.03 for formation of the Zn–**bipy**(**GCN4bd**)$_2$ complex and 5.67 ± 0.03 for the analogous Zn–**terpy**(**GCN4bd**)$_2$ complex.

The addition of increasing aliquots of $CuCl_2$ into a 5 μM solution of **bipy**(**GCN4bd**)$_2$ or **terpy**(**GCN4bd**)$_2$ in 20 mM phosphate buffer pH 7.4, resulted in a similar decrease in the absorbance at 299 nm (**bipy**(**GCN4bd**)$_2$) and 298 nm (**terpy**(**GCN4bd**)$_2$), and an increase in the absorbance at 317 and 328 nm (**bipy**(**GCN4bd**)$_2$) and at 335 and 348 nm (**terpy**(**GCN4bd**)$_2$). Again the isosbestic points at 309 (**bipy**(**GCN4bd**)$_2$) and 320 nm (**terpy**(**GCN4bd**)$_2$) are consistent with clean formation of the complex, which has been identified as a 1:1 complex by a continuous-variation plot (Figure S8 in the Supporting Information). The plot of absorbance as a function of increasing Cu^{II} concentration was fitted to a 1:1 binding equation, from which extinction coefficients were determined (Table 1). Titration of **bipy**(**GCN4bd**)$_2$ with Cu^{II} had to be performed in the presence of a competitor (20 mM glycine), in order to accurately estimate the Cu^{II} affinity (Table 1). Formation constants, $\log K_M$, were determined to be 13.17 ± 0.06 for the Cu–**bipy**(**GCN4bd**)$_2$ complex and 7.94 ± 0.04 for the Cu–**terpy**(**GCN4bd**)$_2$ complex.

These results are consistent with the coordination of Cu^{II} and Zn^{II} to the polypyridyl chelating linker unit, which is associated with a *transoid*-to-*cisoid* conformational rearrangement. Changes in the ligand $\pi\rightarrow\pi^*$ bands is consistent with formation of a 1:1 complex and these data were fitted and binding constants were extracted. However, due to the lower extinction coefficient for the metal-to-ligand charge transfer (MLCT) and d–d transitions, we are unable to rule out the possibility that formation of a 1:1 complex is followed by the formation of a 1.5:1 or 2:1 complex as observed previously for related small model compounds with glutathione (GS) in place of **GCN4bd** (**bipyGS$_2$** and **terpyGS$_2$**).[27]

The results summarised in Table 1 are in good agreement with previous reports for related polypyridyl Cu/Zn coordination,[41,42] displaying a higher affinity for Cu^{II} than for Zn^{II}. The binding constants reported for the Zn–**bipy**(**GCN4bd**)$_2$ and Cu–**terpy**(**GCN4bd**)$_2$ complexes, are similar to those we previously reported for the analogous glutathione dimers, **bipyGS$_2$** and **terpyGS$_2$**.[27] However, the Cu^{II} affinity of **bipy**(**GCN4bd**)$_2$ is 10-times greater than that for the analogous **bipyGS$_2$**, whereas the Zn^{II} affinity of **terpy**(**GCN4bd**)$_2$ is three-times lower than that of **terpyGS$_2$**. These differences highlight the non-innocent role of the peptide substituents, where amino acid side-chains may be able to contribute to and/or compete for metal-ion coordination. Intriguingly binding constants are largely unchanged for Zn–**bipy**(**GCN4bd**)$_2$ and Cu–**terpy**(**GCN4bd**)$_2$ which are the same species that display the best binding to CRE DNA, as indicated by CD. These results could indicate that **GCN4bd** contributes to, or competes for metal-ion coordination in Cu–**bipy**(**GCN4bd**)$_2$ and Zn–**terpy**(**GCN4bd**)$_2$, making important amino acid side-chains unavailable for, and thereby disrupting, DNA binding.

Cu^{II} and Zn^{II} titrations in the presence of DNA

Similar $CuCl_2$ and $ZnCl_2$ titrations of **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$ monitored by UV–visible and CD spectroscopy were carried out in the presence of one equivalent of duplex DNA (5 μM) containing either the CRE or NS site, so as to investigate metal binding to the polypyridine linkers under these conditions, and to evaluate if metal binding to these linkers and DNA binding of the peptide substituents is cooperative. The UV–visible titrations all display a shift of the $\pi\rightarrow\pi^*$ transition towards lower energies, consistent with Cu^{II} and Zn^{II} binding to the polypyridyl linkers (Figures S9 (CRE DNA) and S10 (NS DNA) in the Supporting Information). Changes in absorbance were plotted as a function of metal-ion concentration (Figures S9E (CRE DNA) and S10E (NS DNA) in the Supporting Information), the data were fitted to a 1:1 model and binding constants were calculated, the results of which are reported in Table 1. Considering a thermodynamic cycle linking the four main species (peptide, peptide/metal, peptide/DNA and peptide/metal/DNA) in equilibrium, any change in the metal-binding affinity of the peptide dimer conjugates is due to the DNA, as DNA binding could alter the polypyridyl linkers conformation.[49,50] The affinity of **terpy**(**GCN4bd**)$_2$ in the presence of NS DNA is slightly greater for Zn^{II} ($\log K_M$ 6.16 ± 0.03) and unchanged for Cu^{II} ($\log K_M$ 8.04 ± 0.08) compared to the affinity determined in the absence of DNA (Zn^{II}, $\log K_M$ 5.67 ± 0.03; Cu^{II}, $\log K_M$ 7.94 ± 0.04). However, the affinity for both Cu^{II} and Zn^{II} is

considerably enhanced in the presence of DNA containing the CRE target site (Zn^{II}, $\log K_M$ 6.62 ± 0.04; Cu^{II}, $\log K_M$ 9.41 ± 0.07). Therefore, the presence of the target DNA site increases the metal-ion affinity of the terpyridine linker in a cooperative fashion (Figure S11 in the Supporting Information). We propose that **terpy**(**GCN4bd**)$_2$ binds to DNA through the *cisoid* conformation (Figure 1B), which is more preorganised for chelation, resulting in enhanced metal-ion affinity. To the best of our knowledge, this is the first example in which the presence of a target DNA site promotes recruitment by an artificial switching device of its effector molecule.

In contrast, the Zn^{II} affinity of **bipy**(**GCN4b**)$_2$ decreases very slightly in the presence of NS DNA ($\log K_M$ 5.63 ± 0.08), and remains largely unchanged in the presence of DNA containing the CRE target site ($\log K_M$ 6.89 ± 0.10). The Cu^{II} affinity of **bipy**(**GCN4bd**)$_2$ decreases in the presence of DNA, regardless of the presence of the target site (NS, $\log K_M$ 11.97 ± 0.03; CRE, $\log K_M$ 12.26 ± 0.06). Despite **bipy**(**GCN4bd**)$_2$ binding to CRE DNA being enhanced in the presence of metal ions (presumably due to a combination of electrostatics and conformational changes), binding to CRE DNA decreases the metal-ion affinity. These results suggest that DNA binding does not necessarily promote the more favourable *cisoid* conformation for metal-ion chelation, consistent with a model in which both the *cisoid* and *transoid* conformation are appropriate for DNA binding (Figure 1A). Instead the DNA behaves like a competitive ligand for the metal ions, either directly (metal binding to DNA), or indirectly (peptide donor atoms which contribute to the metal-ion coordination sphere are no longer available when bound to DNA).

A Cu^{II} and Zn^{II} metal-ion titration of **bipy**(**GCN4bd**)$_2$/**terpy**(**GCN4bd**)$_2$ in the presence of CRE DNA was also monitored by CD spectroscopy (Figure S12 in the Supporting Information). A plot of molar ellipticity at 222 nm, a measure of the α-helical content and thereby DNA binding, as a function of metal-ion equivalence is consistent with the formation of 1:1 complexes. However, an exception could be the Cu–**bipy**(**GCN4bd**)$_2$–CRE system in which the smaller signal change is less conclusive (Figure S12E in the Supporting Information).

Both peptide dimers display a higher affinity for Cu^{II}, and peptide folding in the presence of CRE DNA, an indication of DNA binding, is greatest for **terpy**(**GCN4bd**)$_2$ when bound to Cu^{II}. However, for **bipy**(**GCN4bd**)$_2$ this is the case when bound to Zn^{II}, for which it has a lower affinity both in the absence and presence of the CRE DNA target site. These differences are likely due to coordination geometry preferences, where Zn^{II} is known to favour a tetrahedral[51,52] coordination geometry whereas Cu^{II} tends to adopt a square planar[53] coordination geometry, when bound to bipyridine. Although the terpyridine *transoid*-to-*cisoid* conformation is required for binding to DNA, and this is dependent on the affinity for the metal ion, which is greatest for Cu^{II}, the bipyridine unit does not require this conformational change. Rather greater DNA binding is likely to be due to enhanced electrostatics on coordinating a metal ion. Though the affinity of the bipyridine is greatest for Cu^{II}, the forked alignment of GCN4 basic domains bound to DNA (Figure 1) appears more suitable for a metal ion which adopts a tetrahedral coordination geometry when bound to the bipyridine dimerization domain.

Metal-ion affinity and the ability to regulate DNA binding are therefore not mutually inclusive. A parallel can be drawn with two natural transcription factors involved in metal-ion regulation, Nmtr and SmtB, which belong to the same family, but are capable of sensing different metal ions. This is achieved due to the metal-ion coordination geometry offered by the allosteric site, and not the metal-ion affinity. Robinson and co-workers report that, within a cyanobacterial cytosol, the regulatory metalloprotein *Synechococcus* PCC7942 SmtB functions in response to Zn^{II} coordination but not Co^{II}, even though in vitro studies have shown that Zn^{II} and Co^{II} both bind to the same tetrahedral allosteric site.[54] In contrast, the Ni^{II} and Co^{II} responsive metalloprotein NmtR from *Mycobacterium tuberculosis* regulating the nmt operator, contains an allosteric metal-binding site on helix α5 of the winged helix–turn–helix motif, similarly to SmtB, but which offers two additional ligands for potential metal-ion coordination in an octahedral fashion. Coordination of these two additional ligands would require reorganisation, leading to a structural transition which in turn inhibits DNA binding. Zn^{II} binds to NmtR with a greater affinity than Co^{II}, but in a tetradentate rather that an hexadentate fashion, and is therefore unable to regulate DNA binding.[3,55] These examples illustrate that metal-ion allosteric regulation can be highly dependent on preferred metal-ion coordination geometry and how that might alter protein structure, rather than metal-ion affinity. Therefore, our examples above need to be considered not in terms of enhanced metal-ion affinity and resulting DNA affinity, but rather in combination with preferred coordination geometry and resulting DNA affinity.

DNA sequence selectivity

To gain further insight into the sequence-selective DNA binding of our three artificial peptide dimers, (**GCN4bd**)$_2$, **bipy**(**GCN4bd**)$_2$, and **terpy**(**GCN4bd**)$_2$, similar CD experiments were carried out with DNA containing either the AP1 or half-CRE site. AP1 lacks a central G–C nucleobase pair, in contrast to the palindromic CRE site, and is in fact the natural binding site of GCN4.[56,57] However, much of the artificially dimerised GCN4 literature has focused on CRE DNA,[12a,15,58] which has been shown to better accommodate the bulk resulting from artificial dimerisation linkers.[14c] In contrast, half-CRE contains half of the palindromic repeat of CRE, and therefore represents the docking domain for only one of the two peptide α-helices of GCN4. Previous studies have demonstrated that homodimeric peptides based on GCN4 which contain both the conserved basic and zipper domains, display similar binding affinities for CRE, AP1 and half-CRE DNA.[59]

A slight increase in the helicity of (**GCN4bd**)$_2$ and **bipy**(**GCN4bd**)$_2$ is observed in their CD spectra recorded in the presence of either AP1 or half-CRE DNA, similar to that in the presence of CRE DNA (but in the absence of metal ions), and to previous reports (Figure 7A and B).[14c] However, almost no change is observed for **terpy**(**GCN4bd**)$_2$ (Figure 7C). As expect-

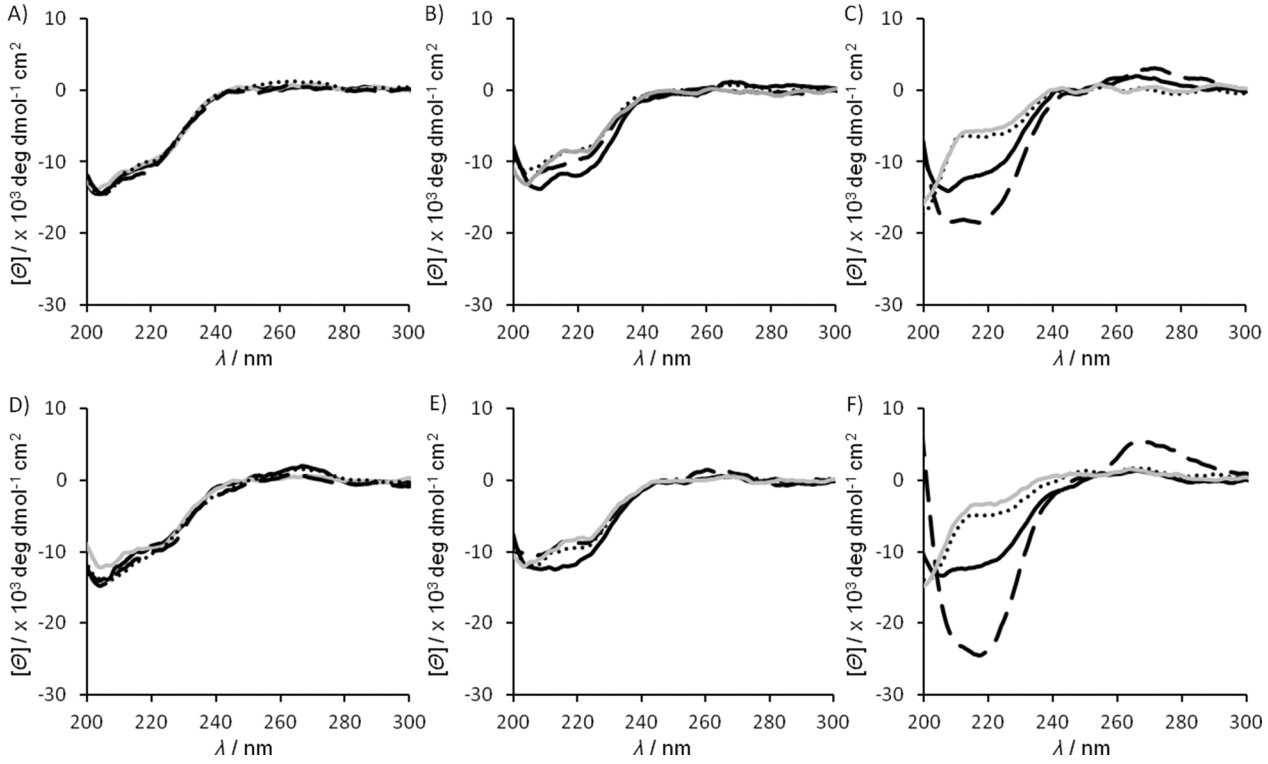

Figure 7. CD spectra of 5 μM solutions of half-CRE (A, B and C) and AP1 (D, E and F) DNA and (**GCN4bd**)$_2$ oxidised dimer (A and D), **bipy**(**GCN4bd**)$_2$ (B and E) and **terpy**(**GCN4bd**)$_2$ (C and F) in 10 mM phosphate buffer pH 7.4, in the absence (•••••) and presence of 2 equivalents of either $CuCl_2$ (-----) or $ZnCl_2$ (——). Reversibility was monitored by addition of 10 equivalents of EDTA (——).

ed the addition of either $CuCl_2$ or $ZnCl_2$ does not alter the CD spectrum of (**GCN4bd**)$_2$ which lacks a metal chelating linker unit. More intriguingly, however, is that the addition of metal ions does not significantly alter the CD spectra of **bipy**-(**GCN4bd**)$_2$ in the presence of either AP1 or half-CRE DNA, despite being sensitive to the presence of these metal ions in the presence of CRE DNA. In contrast, the helicity of **terpy**-(**GCN4bd**)$_2$ in the presence of half-CRE and AP1, is very sensitive to the presence of metal ions. For both half-CRE and AP1 DNA the addition of Zn^{II} is consistent with a more α-helical peptide (half-CRE Θ_{222} $-6.6\times10^3\rightarrow-11.0\times10^3$; AP1 Θ_{222} $-6.3\times10^3\rightarrow-11.3\times10^3$ deg dmol^{-1} cm^2 per residue), of similar α-helicity as both (**GCN4bd**)$_2$ and the **bipy**(**GCN4bd**)$_2$ analogue. However, the addition of Cu^{II} is accompanied by a very significant increase in the α-helical signal at 222 nm (half-CRE Θ_{222} $-6.1\times10^3\rightarrow-16.9\times10^3$; AP1 Θ_{222} $-4.9\times10^3\rightarrow-22.6\times10^3$ deg dmol^{-1} cm^2 per residue), in much the same fashion as was previously observed in the presence of CRE DNA (Figures 5 and 7), but is greater in the presence of DNA containing the AP1 target site.

These results are in extremely good agreement with our original design. We proposed that 5,5′-disubstituted bipyridine, in both the *cisoid* and *transoid* conformation, and the *cisoid* conformation of 6,6′-disubstituted terpyridine, would separate peptide substituents by an appropriate distance for binding to the CRE DNA target site. The distance between peptides on binding to AP1 is shorter than when bound to CRE (Figure 3). Therefore, we proposed that the analogous binding to AP1 DNA would only be well satisfied by the *cisoid* conformation of the 6,6′-disubstituted terpyridine peptide dimer, **terpy**-(**GCN4bd**)$_2$, and that this conformation would be promoted on metal-ion coordination. Consistent with data for binding to CRE, binding to AP1 DNA is significantly enhanced on coordinating Cu^{II}, for which the terpyridine linker displays a higher affinity (compared to Zn^{II}). We have, therefore, managed to alter the metal-ion induced sequence selectivity, by changing the nature of a non-native regulation site, as opposed to the DNA binding peptide sequence.

Conclusions

Herein we report for the first time the design of artificial DNA binding peptide dimers which contain a non-native metal dependent switching domain as the linker unit. The polypyridine linker units have been selected such that metal-ion coordination will promote the *cisoid* conformation, which could trigger the alignment of peptide moieties capable of sequence-selective binding to DNA. Three peptide dimers; **bipy**(**GCN4bd**)$_2$, which contains an allosteric ineffective 2,2′-bipyridine unit, **terpy**(**GCN4bd**)$_2$, which contains the allosteric effective 2,2′:6′,2″-terpyridine unit, and (**GCN4bd**)$_2$, which contains a disulfide bond, which is insensitive to the presence of metal ions, are reported. CD studies are consistent with all three conjugates folding into α-helices in the presence of DNA containing the CRE target site, and that this is greatly enhanced on coordinating either Cu^{II} or Zn^{II} for those dimers containing

polypyridine linker units, **bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$. Metal-ion coordination and peptide realignment to promote DNA binding is fully reversible upon addition of excess EDTA. Notably a higher metal-ion affinity was not found to correlate with enhanced binding to CRE DNA, due to apparent coordination geometry requirements for allostery. Complementary studies performed in the presence of AP1 DNA, which differs from CRE in a single nucleobase pair, displays the most significant CD change, for metallated **terpy**(**GCN4bd**)$_2$ (greatest for Cu^{II}), which is consistent with a *cisoid*-terpyridine conformation which positions the two peptide moieties at an optimal distance for binding to the smaller AP1 DNA target site. In contrast, similar metal addition did not result in significant changes in the CD profile of **bipy**(**GCN4bd**)$_2$ recorded in the presence of AP1, where the interpeptide distance is too large for optimal binding to AP1 DNA.

Here we illustrate that switching units can be introduced into artificial DNA binding proteins, that can be regulated by coordinating metal ions, triggering a conformational change promoting sequence-selective DNA binding. Importantly the linker unit can be carefully designed so as to achieve discrimination between two very similar DNA target sites, which differ only in a single nucleobase pair. We propose that these metal dependent switches could be engineered into various designer proteins to control biomolecular recognition events which rely on a high level of specificity. Ultimately we envision that biomolecules containing these switches could find applications in the future as tools in biotechnology. Moreover, polypyridine ligands are routinely used as part of the inorganic chemist's toolbox, and can be found in a large number of complexes developed for biological applications, including luminescent probes, anticancer agents, and as catalysts. Therefore, the ligands reported here could offer opportunities to couple the attractive properties of these complexes with a high degree of control over biomolecular recognition.

Experimental Section

Materials, methods, and synthetic procedures are described in the Supporting Information.

UV–visible spectroscopy

UV–visible spectra were recorded in a 1 cm pathlength quartz cuvette at 298 K on a Shimadzu 1800 spectrometer. For a metal-ion titration of the peptide conjugates, aliquots of aqueous 0.3 mM stock solutions of $CuCl_2$ or $ZnCl_2$, were titrated into 600 μL of a 5(± 0.6) μM solution of polypyridyl–peptide dimer conjugate (5.28 ($bipy_{Zn}$), 5.24 ($bipy_{Cu}$), 4.51 ($terpy_{Zn}$) and 4.44 μM ($terpy_{Cu}$)) in 20 mM potassium phosphate buffer pH 7.4, containing 5 μM of double stranded oligonucleotide (either CRE or NS), if present. Spectra were recorded after 3 min equilibration. When the apparent Cu^{II} binding constant was too high to be accurately estimated, glycine was added as a competitor ligand; 20 mM glycine and 100 mM phosphate buffer were present for the titration of **bipy**(**GCN4bd**)$_2$ in the absence of DNA, 10 mM glycine and 50 mM phosphate buffer in the presence of both CRE and NS DNA, and 0.2 mM glycine for the titration of **terpy**(**GCN4bd**)$_2$ in the presence of CRE DNA. Nonlinear fitting was performed with Kaleidagraph software version 4.0 and K_{app} values were calculated as reported previously.[27]

For the continuous variation method experiment, solutions of varying ratio of **bipy**(**GCN4bd**)$_2$/**terpy**(**GCN4bd**)$_2$ and $CuCl_2$/$ZnCl_2$ were prepared from 120 μM stock solutions, so that the total concentration of $C_p + C_M$ was 10 μM (where C_p and C_M, correspond to the concentration of the peptide dimer conjugate and metal ion, respectively). UV–visible spectra were recorded and the absorbance at λ_{max}, for the metal complexes $\pi \rightarrow \pi^*$ transition, was plotted versus the fraction of ligand. Due to a contribution at this wavelength from the free ligand (**bipy**(**GCN4bd**)$_2$ and **terpy**(**GCN4bd**)$_2$), the absorbance values were corrected to account for this.[60] The fraction of peptide conjugates, χ **bipy**(**GCN4bd**)$_2$ and χ **terpy**(**GCN4bd**)$_2$, are defined as the ratio $C_p/(C_p+C_M)$.

Circular dichroism spectroscopy

CD spectra were recorded in a 1 mm pathlength quartz cuvette at 298 K on a Jasco J-715 spectropolarimeter. The observed ellipticities in millidegrees were converted into residual molar ellipticity, Φ_{RME}, reported in units of deg dmol^{-1} cm^2 per residue. For CD spectra of peptide conjugates in the presence and absence of DNA, a 300 μL blank solution containing 10 mM phosphate buffer pH 7.4 and 5 μM duplex DNA (if present) was recorded. Peptide dimer or peptide dimer conjugate solution (7.5 μL of a 200 μM solution) was added, and the data were collected after 15 min equilibration. Two equivalents of $CuCl_2$ or $ZnCl_2$ (2 μL, 1.5 mM) was added and the solution was allowed to equilibrate for 15 min prior to recording the spectra. Ten equivalents of EDTA per metal ion (3 μL, 10 mM) were added and the CD spectra were recorded, in order to investigate reversibility. Spectra are an average of 20 scans recorded between 185 and 400 nm, at 500 nm min^{-1} (0.5 nm pitch). Difference spectra were obtained by subtracting the CD spectrum of DNA alone (blank), from that of a mixture containing both peptide and DNA, in order to observe the CD contribution from the peptide component only.

Metal titrations of peptide/CRE DNA complexes were performed at a higher concentration (10 μM **bipy**(**GCN4bd**)$_2$/**terpy**(**GCN4bd**)$_2$, 10 μM DNA). To a 1 mm pathlength cuvette, 60 μL of a 50 μM solution of CRE DNA, 30 μL of 100 mM phosphate buffer pH 7.4 and 195 μL deionised water, were successively added. A blank spectrum was recorded, and 15 μL of **bipy**(**GCN4bd**)$_2$ or **terpy**(**GCN4bd**)$_2$ solution (200 μM) was added. Samples were allowed 15 min equilibration prior to recording the spectra. Aliquots of aqueous stock solutions of $CuCl_2$ or $ZnCl_2$ (0.3 mM) were then titrated into the solution containing 10 μM of the peptide–CRE DNA complex, and the CD were recorded after 7 min equilibration. Spectra are an average of eight scans recorded between 190 and 300 nm at 200 nm min^{-1} (0.5 nm pitch). The DNA blank was subtracted as previously described, and the molar ellipticity values were corrected to account for dilution.

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