Self-Assembly Hot Paper

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Reversible Supramolecular Assembly at Specific DNA Sites: Nickel-Promoted Bivalent DNA Binding with Designed Peptide and Bipyridyl–Bis(benzamidine) Components**

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Dedicated to Professor Javier de Mendoza on the occasion of his 70th birthday

Abstract: *At specific DNA sites, nickel(II) salts promote the assembly of designed components, namely a bis(histidine)-modified peptide that is derived from a bZIP transcription factor and a bis(benzamidine) unit that is equipped with a bipyridine. This programmed supramolecular system with emergent properties reproduces some key characteristics of naturally occurring DNA-binding proteins, such as bivalence, selectivity, responsiveness to external agents, and reversibility.*

Transcription factors (TFs) are specialized proteins that regulate gene expression by binding to specific DNA regulatory sequences.[1] TFs are grouped into families according to the structure of their DNA binding domains, and in many cases, their interaction with the DNA occurs as part of multimeric complexes. The DNA recognition process by many TFs is coupled to the folding of their DNA binding domains into well-defined secondary structures, typically α-helices, which create a complementary contacting surface with the DNA major groove.[2] In some cases, such as in the zinc-finger family, the α-helical folding is mediated by coordination of a metal ion (Zn^{2+}) to amino acid side chains, typically Cys and His residues.[3]

As a result of their central biological role, there has been a great interest in the development of miniaturized models of TFs that are capable of reproducing the DNA-binding properties of the natural proteins.[4,5] The more successful approaches have relied either on engineering the DNA-binding regions into pre-folded secondary structures[6] or on the conjugation of these peptidic modules to other DNA-binding units, such as minor-groove binders.[7] Also, in the context of mimicking nature, several groups have developed synthetic constructs whose DNA recognition properties can be modulated by application of external stimuli, such as light or metal ions.[8]

Herein, we present a new approach for the specific recognition of DNA sequences, which combines many of the attributes of these previous designs in a single system: bivalence, conditional folding, responsiveness to external stimuli (metal ions), and even reversibility. The strategy, which relies on a planned, dual role for nickel(II) species as both a dimerizing agent and a folding promoter, involves a programmed self-assembly of dissimilar components.

As a starting point for our design, we chose the yeast transcription factor GCN4, an archetypical bZIP TF that specifically binds to ATF/CREB (5′-ATGA(c/g)TCAT-3′) or AP1 (5′-ATGA(c)TCAT-3′) sites as a leucine zipper-mediated dimer of uninterrupted α-helices.[9] The DNA interaction occurs through the N-terminal basic regions, which undergo a transition from a disordered structure to an α-helix upon DNA binding.[10] Whereas an isolated basic region peptide is unable to interact with its DNA consensus site, we have shown that appropriate conjugation to other DNA binders, such as distamycin analogues or bis(benzamidines), results in hybrid systems that can recognize composite target sites.[7]

Relying on these precedents, we considered an alternative method for connecting the basic region peptide and the minor-groove binding unit based on their coordination to a transition metal and formation of a heterodimeric complex. Moreover, we envisioned that an appropriate modification of the basic region might allow the use of the metal not only as a tethering unit but also as a promoter of the required α-helical folding.[11] Therefore, we decided to make a GCN4 basic region derivative in which two amino acids that are located in consecutive helical turns are mutated to histidines (His). In particular, we synthesized the peptide brHis_2, featuring the amino acids Asp226–Arg249 of the GCN4 DNA binding domain, but with the residues Leu230 and Arg234 replaced by His. The addition of Ni^{II} or related metal ions to this peptide might induce the formation of an α-helix by nucleating the N-terminal turns, while simultaneously providing a coordination site for bis(benzamidine) derivatives equipped with a bipyridine ligand.[12] Given that the bis(benzamidine) binds to the minor groove of adenine/thymine-rich (A/T-rich) DNA sites,[13] the bipyridine unit might be able to

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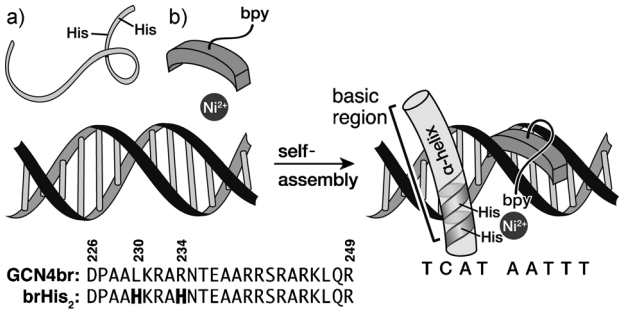
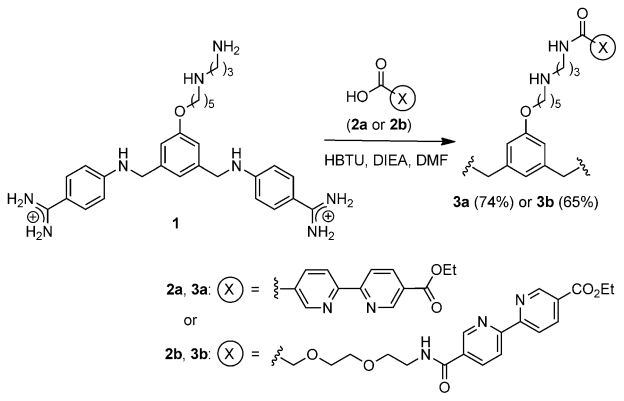


Figure 1. Outline of the cooperative assembly strategy. a) Unfolded $brHis_2$, which will fold into an α-helix in the complex (right). b) Bis(benzamidine) minor-groove binder. $brHis_2$: Aba-DPAA**H**KR A**H**NTEAARRSRARKLQR-NH_2, where Aba = 4-acetamidobenzoic acid; bpy: bipyridine chelator tethered to the bis(benzamidine).

recruit the modified GCN4 basic region ($brHis_2$) into the adjacent major groove that features a consensus DNA binding sequence (Figure 1).[14]

The GCN4-based peptide $brHis_2$ was synthesized following standard solid-phase peptide synthesis (SPPS) procedures. On the other hand, we synthesized two bis(benzamidine)–bipyridine derivatives featuring linkers of different lengths between the DNA-binding bis(benzamidine) and the bipyridine ligand (**3a** and **3b**); both constructs were assembled from aminobis(benzamidine) **1**, which was synthesized in three steps from commercially available starting materials (Scheme 1; see also the Supporting Information).



Scheme 1. Synthesis of bipyridine–bis(benzamidine) derivatives for coordination with the GCN4-based peptide. DIEA = diisopropylethylamine, DMF = *N*,*N*-dimethylformamide, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

The DNA binding properties of the synthetic constructs were studied by electrophoretic mobility assays (EMSA) in polyacrylamide gel under non-denaturing conditions and using SybrGold as the DNA stain. As expected, incubation of the peptide $brHis_2$ in the presence of bis(benzamidines) **3a** or **3b** with the double-stranded (ds) oligonucleotide $AP1^{hs}$·A/T, which contains the required AP1 half-site ($AP1^{hs}$) next to an A/T-rich sequence, does not induce the formation of retarded bands in the gel (Figure 2a, lane 2).

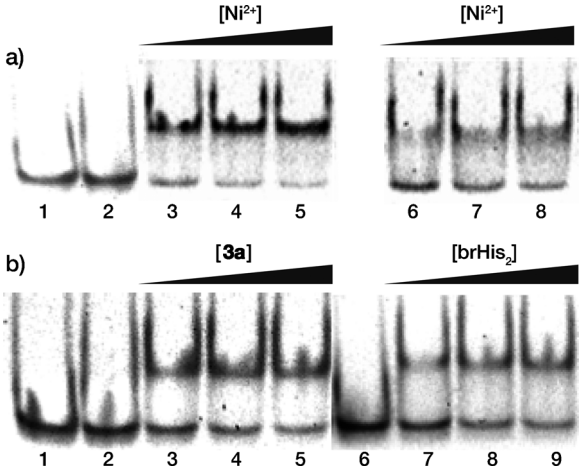


Figure 2. Comparative EMSA analysis of the DNA-binding processes. a) Lanes 1–8: $AP1^{hs}$·A/T (0.1 μM); lane 2–5: $brHis_2$ (1 μM) and **3a** (1 μM); lanes 3–5: 0.6, 1, and 1.6 μM of $Ni(ClO_4)_2$; lanes 6–8: $brHis_2$ (1 μM), **3b** (1 μM), and 0.6, 1, and 1.6 μM of $Ni(ClO_4)_2$, respectively. b) Lanes 1–9: $AP1^{hs}$·A/T (0.1 μM); lane 2–5: $brHis_2$ (0.8 μM) and $Ni(ClO_4)_2$ (1.6 μM); lanes 3–5: 0.8, 1, and 1.6 μM of **3a**; lanes 6-9: **3a** (0.8 μM) and $Ni(ClO_4)_2$ (1.6 μM); lanes 7–9: 0.8, 1, and 1.6 μM of $brHis_2$. The procedure for fluorescence EMSA experiments involved the addition of peptide solutions in Tris-HCl (20 mM, pH 7.5) to a solution of Tris-HCl (18 mM, pH 7.5) containing KCl (90 mM), $MgCl_2$ (1.8 mM), glycerol (9%), BSA (0.11 mg·mL^{-1}), NP-40 (2.25%), and DNA (100 nM; 4 °C, 30 min) and loading into the gel. Products were resolved by PAGE analysis on a 10% non-denaturing polyacrylamide gel and 0.5X tris/borate/EDTA (TBE) buffer over 40 minutes at 4 °C and by staining with SybrGold (5 μL in 50 mL of TBE) for 10 minutes, followed by fluorescence visualization. Note that we needed to run the EMSA experiments in the absence of EDTA to avoid sequestration of the nickel cation; this is the reason for the smearing of the bands. Sequence of $AP1^{hs}$·A/T: 5′-ACGAACG *TCAT*·AATTT CCTC-3′ (peptide binding site in italics and minor-groove binding site underlined, only one strand shown).

However, the addition of increasing concentrations of $Ni(ClO_4)_2$ to the previous mixtures generates clear retarded bands (Figure 2a, lanes 3–5). In the case of **3b**, these bands are fainter and show some smearing (Figure 2a, lanes 6–8), and therefore, bis(benzamidine) **3a** was chosen for further characterization. In agreement with the proposed interaction model, no new bands that migrate more slowly were observed when the mixtures do not include the minor-groove binding component **3a** (Figure 2b, lane 2), but the formation of the retarded band was again restored when **3a** was added to the mixture of the other components (Figure 2b, lanes 3–5). This result confirms the requirement of the bipyridine-equipped minor-groove anchor for specific DNA binding of the peptide moiety. It was also interesting to observe that the complex can also be assembled upon addition of increasing amounts of $brHis_2$ to a mixture of $AP1^{hs}$·A/T, the Ni^{II} salt, and **3b** (Figure 2, lanes 7–9).

Taken together, these results indicate that the system dynamically assembles independently of the order in which the components are mixed, and any potential competitive homodimeric $[(brHis_2)_2Ni]^{2+}$ or $[(\mathbf{3a})_2Ni]^{2+}$ species are kinetically labile and evolve towards the desired $[(brHis_2)(\mathbf{3a})Ni]^{2+}$

complex in the presence of the target DNA oligonucleotide AP1^{hs}·A/T.

We next performed a forward titration experiment by adding premixed combinations of equimolecular amounts of brHis$_2$, **3a**, and Ni(ClO$_4$)$_2$ (5 equiv) to a ^{32}P-labeled AP1^{hs}·A/T dsDNA oligonucleotide. Considering the [(brHis$_2$)(**3a**)Ni]$^{2+}$ complex as a single species binding to the DNA, we could calculate an approximate dissociation constant of 0.45 μM at 4 °C (Figure 3a), even though we acknowledge that this is an oversimplification of a much more complex equilibrium.[7e]

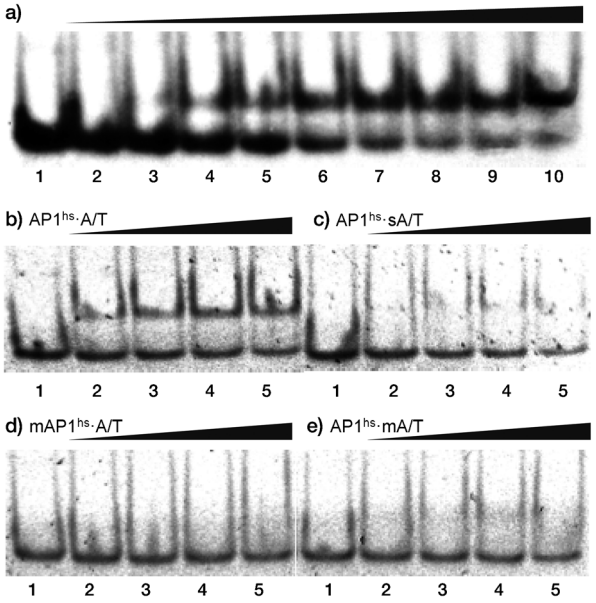


Figure 3. a) EMSA titration of the mixture of the target dsDNA with a mixture of brHis$_2$, **3a**, and Ni(ClO$_4$)$_2$ with radioactively labeled DNA: Lanes 1–10: AP1^{hs}·A/T (50 nM, ca. 100 pM P^{32}-labeled); Lanes 2–10: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 1 μM of an equimolecular mixture of brHis$_2$ and **3a**, in the presence of Ni(ClO$_4$)$_2$ (5 equiv). b–e) EMSA analysis (SybrGold staining) of the DNA selectivity. Lanes 1–5 contain the indicated ds-oligonucleotide (0.1 μM) and 0, 0.2, 0.4, 0.6, and 0.8 μM of an equimolecular mixture of brHis$_2$ and **3a** with Ni(ClO$_4$)$_2$ (5 equiv). b) AP1^{hs}·A/T. c) AP1^{hs}·sA/T. d) mAP1^{hs}·A/T. e) AP1^{hs}·mA/T. Oligonucleotide sequences (only one strand is shown): AP1^{hs}·sA/T: 5′-ACGAACG *TCAT*·AATT CCTC-3′, mAP1^{hs}·A/T: 5′-ACGAACG *TCGT*·AATTT CCTC-3′, AP1^{hs}·mA/T: 5′-ACGAACG *TCAT*·AGTTT CCTC-3′.

We next studied the sequence selectivity of the system by using other dsDNAs that contain specific mutations (Figure 3d and 3e). Not surprisingly, with DNAs such as AP1^{hs}·mA/T (*TCAT*·A**G**TTT), which lack the A/T-rich tract required for binding of the minor-groove agent, we did not observe the formation of retarded bands. Moreover, we also did not observe stable DNA complexes with oligonucleotides mutated in the recognition site of the peptide (mAP1^{hs}·A/T, *TC**G**T*·AATTT). This result contrasts with previously studied covalent derivatives that do bind to these DNAs with substantial affinity.[7c,d]

The system is even capable of discerning sequences that feature shorter A/T-rich binding regions, such as in AP1^{hs}·sA/T (*TCAT*·AATT; Figure 3c). This selectivity could be ascribed to the lower affinity of **3a** for AATT versus AATTT, as revealed by fluorescence titration experiments ($K_D \approx 0.17$ μM for AP1^{hs}·sA/T vs. $K_D \approx 0.035$ μM for AP1^{hs}·A/T; see the Supporting Information).[15] The above observations highlight the higher specificity that is attainable through cooperative recognition in noncovalent multicomponent systems.

Not surprisingly, a truncated derivative of the basic region lacking the N-terminal capping motif (Asp-Pro-Ala)[16] failed to promote the formation of retarded bands in EMSA experiments (see the Supporting Information). Likewise, a related GCN4 basic region peptide that featured a cysteine residue instead of the histidine at position 230 also failed to yield the desired DNA-binding assembly in the presence of both nickel or other metal ions (see the Supporting Information).

We also studied the selectivity of the system with regard to the metal ion (Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, and Fe^{2+} salts). Using comparable conditions, we only observed the formation of more slowly migrating bands in the presence of Ni^{2+} cations (see the Supporting Information). Therefore, it seems that nickel has the right coordination properties to promote an effective heterodimeric assembly in the presence of the cognate DNA.[17]

As expected for a poorly structured peptide, the circular dichroism spectrum of brHis$_2$ presents a relatively weak negative signal at 222 nm, even in the presence of **3a** and AP1^{hs}·A/T (Figure 4 left, curve b); the addition of Ni(ClO$_4$)$_2$ to the mixture promoted a considerable increase in the negative ellipticity intensity at 222 nm, which is consistent

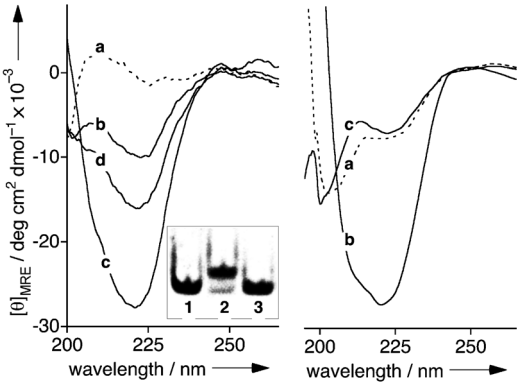


Figure 4. Left: Circular dichroism spectra (mean residue ellipticity, [θ]$_{MRE}$) of different components in presence of AP1^{hs}·A/T (5 μM) in Tris-HCl buffer (20 mM, pH 7.5) with NaCl (100 mM) at 20 °C. a) **3a** (5 μM). b) **3a** (5 μM) and brHis$_2$ (5 μM). c) **3a** (5 μM), brHis$_2$ (5 μM), and Ni(ClO$_4$)$_2$ (10 μM). d) Same conditions as in (c) after addition of 20 equivalents of EDTA; the addition of 30 equivalents of EDTA leads to full recovery of the initial helicity (data not shown). Inset: EMSA analysis (SybrGold staining). Lanes 1–3: AP1^{hs}·A/T (100 nM); lane 2: brHis$_2$ (1000 nM), **3a** (1000 nM), and Ni(ClO$_4$)$_2$ (5 equiv); lane 3: brHis$_2$ (1 μM), **3a** (1 μM), and Ni(ClO$_4$)$_2$ (5 equiv) after addition of EDTA (1.8 mM). Right: Circular dichroism spectra of **3a** (5 μM) and brHis$_2$ (5 μM) a) in the presence of Ni(ClO$_4$)$_2$ (2 equiv); b) in the presence of Ni(ClO$_4$)$_2$ (2 equiv) after addition of the consensus DNA AP1^{hs}·A/T; and c) in the presence of Ni(ClO$_4$)$_2$ (2 equiv) after addition of the mutated mAP1^{hs}·A/T oligonucleotide. The contribution of the AP1^{h}·A/T oligonucleotide has been subtracted from the spectra.

with the folding of the peptide chain into an α-helix (Figure 4 left, curve c).[18]

The key role of the nickel ion as an adaptor component that mediates the assembly of the system prompted us to examine the possibility of dismounting the DNA complex by using an external nickel chelator. Achieving stimuli-responsive reversibility in DNA binders is a challenge that cannot be easily accomplished.[8] Gratifyingly, the addition of EDTA to the supramolecular complex that resulted from mixing $brHis_2$, **3a**, the Ni^{2+} salt, and $AP1^{hs}$·A/T promoted a drastic decrease in the helicity of the peptide (Figure 4 left, curve d), which correlates with the disruption of the DNA complex, as demonstrated by EMSA experiments (inset). Therefore, whereas the presence of nickel is critical for the DNA interaction, the resulting multicomponent supramolecular complex can be dismounted by the addition of an external nickel chelator.

Importantly, control experiments showed that mixing $Ni(ClO_4)_2$ with the peptide $brHis_2$ does not promote an α-helical folding, even in the presence of the bipyridine partner **3a** (Figure 4 right, curve a). However, addition of the DNA $AP1^{hs}$·A/T to this mixture induces the formation of the α-helix (Figure 4 right, curve b). As expected, a mutated ds oligonucleotide ($mAP1^{hs}$·A/T) does not induce such folding (Figure 4 right, curve c). Therefore, the presence of Ni^{II} ions is not sufficient for inducing the folding transition of the peptide into an α-helix, which requires the template effect of the target DNA.

In summary, we have introduced a new approach for achieving a highly selective bivalent recognition of nine designed base-pair sequence DNAs. The strategy involves the nickel-promoted assembly of designed components consisting of a bis(histidine)-modified peptide derived from a bZIP transcription factor, and a bis(benzamidine) equipped with a bipyridine unit. Key for the success of the approach is the dual role of the metal as an α-helix-nucleating factor and as a heterodimerization staple. The multicomponent nature of the system and the kinetic lability of the metal coordination facilitate the disassembly of the supramolecular structure upon addition of external agents that sequester the nickel cation. Overall, we have devised a supramolecular system with emergent properties that reproduces some of the key characteristics of naturally occurring DNA-binding proteins, such as bivalence, selectivity, responsiveness to external agents, and reversibility. The system represents an infrequent case of self-assembly, as it involves four different components: a metal, a peptide, a small molecule, and a nucleic acid.

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[14] To design the synthetic targets we used the X-ray structural information of the DNA complexes of GCN4 (PDB: ID 1YSA) and pentamidine (PDB: ID 1D64) as a reference and information from previous work with other conjugates (Ref. [7]).

[15] These fluorescence experiments were carried out in aqueous buffers, under much less stringent conditions than those used in EMSA experiments.

[16] J. J. Hollenbeck, D. L. McClain, M. G. Oakley, *Protein Sci.* **2002**, *11*, 2740 – 2747.

[17] For examples of nickel(II)-promoted biomolecular folding or assembly processes, see: a) R. J. Radford, F. A. Tezcan, *J. Am. Chem. Soc.* **2009**, *131*, 9136 – 9137; b) M. Kalek, A. S. Madsen, J. Wengel, *J. Am. Chem. Soc.* **2007**, *129*, 9392 – 9400; c) M. R. Ghadiri, C. Choi, *J. Am. Chem. Soc.* **1990**, *112*, 1630 – 1632.

[18] J. T. Pelton, L. R. McLean, *Anal. Biochem.* **2000**, *277*, 167 – 176.

