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Control of biological processes using metallopeptides

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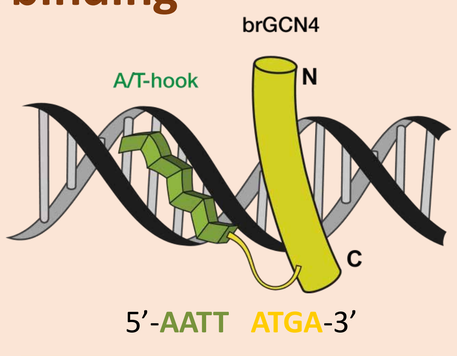
SUMMARY We have relied on transition metal chemistry to develop new tools for the controlled, specific recognition of DNA. We describe the potential of using nickel complexes as tethering coordination systems to promote DNA recognition and the use of a Pd-mediated cleavage to modulate the DNA-binding of a dimeric peptide.

Nickel-promoted DNA binding

1. Previous work

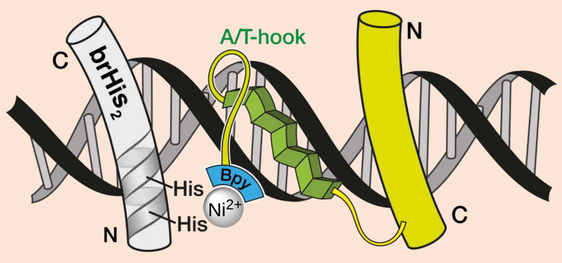
GCN4 TF binds to its consensus DNA as a homodimer of uninterrupted α-helices. Whereas an isolated basic region peptide fails to interact with its consensus DNA, we have shown that conjugation to an AT-hook anchor, allows the specific recognition of composite DNA sites featuring the binding sequences of the GCN4br and the AT-hook in tandem.



Chem. Sci., **2015**, 6, 4767.

2. Design of the metal-chelating peptide

We envisioned that incorporating a metal-chelating bipyridine moiety at the N-terminus of the fully peptidic GCN4br/AT-hook conjugate might allow, in the presence of an appropriate metal, the trapped of a designed GCN4br mutant featuring two coordinating histidines (**brHis$_2$**) and bind a cognate target DNA site with an extended palindromic TCAT-A$_2$T$_2$-ATGA sequence.



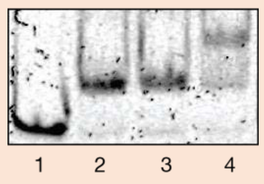
	226	230	234			245	249
GCN4br:	DPAAL		KRARN		TEAARRSRA		RKLQR
brHis$_2$:	DPAA**H**		KRA**H**		NTEAARRSRA		RKLQR
brK:	DPAAL		KRARN		TEAARRSRA	**K**	KLQR
AT-hook:	KPRGRPKK						

3. DNA binding properties

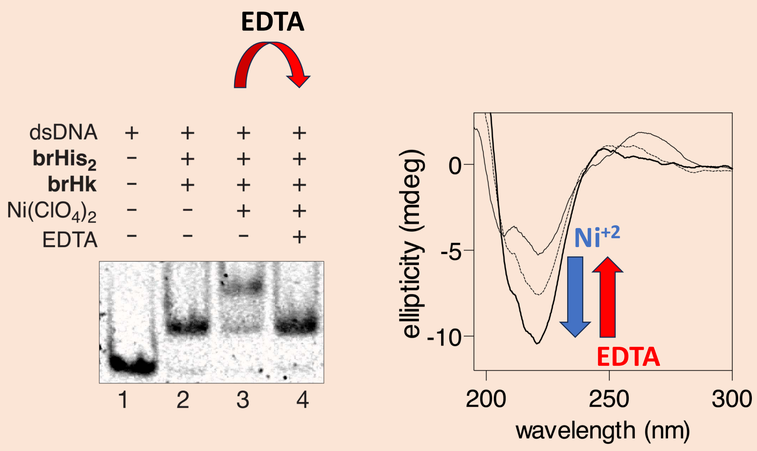
Incubation of **brk** with its consensus DNA, induces the formation of a retarded band corresponding to the expected DNA-peptide complex. Addition of Ni(ClO$_4$)$_2$ to this mixture does not generate new retarded bands, but subsequent addition of the metal-chelating **brHis$_2$** gives rise to a new, more retarded band that must correspond to the desired ternary complex.

5'- TCAT *AATT* ATGA -3'

dsDNA	+	+	+	+
brHk	–	+	+	+
Ni(ClO$_4$)$_2$	–	–	+	+
brHis$_2$	–	–	–	+

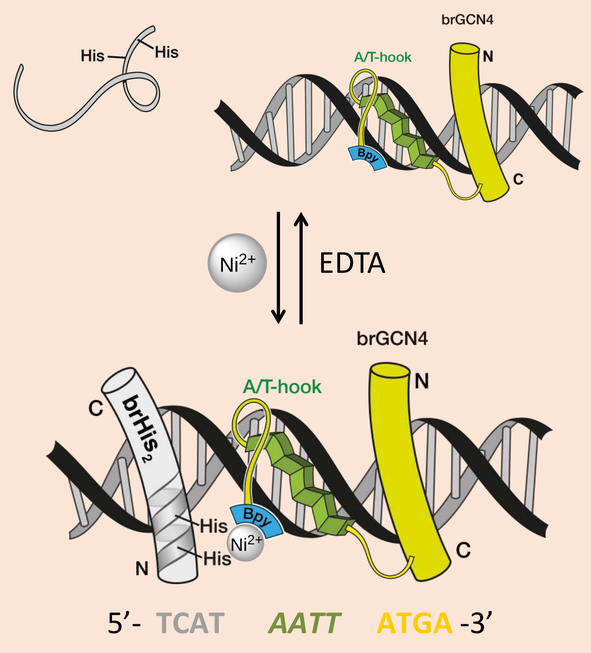


The key role of the Ni(II) ion prompted us to examine the possibility of disassembling the [(**brHis$_2$**)(**brK**)Ni]$^{2+}$/DNA complex by using an external chelator: addition of EDTA to the preformed metallosupramolecular complex with the consensus DNA promoted the disassembly of the complex. CD also showed a decrease in the degree of helicity.



4. Conclusions

We have introduced a new approach for achieving a conditional and selective trivalent recognition of a long 12 base-pair DNA site. The strategy involves the nickel-promoted assembly of a supramolecular DNA binder



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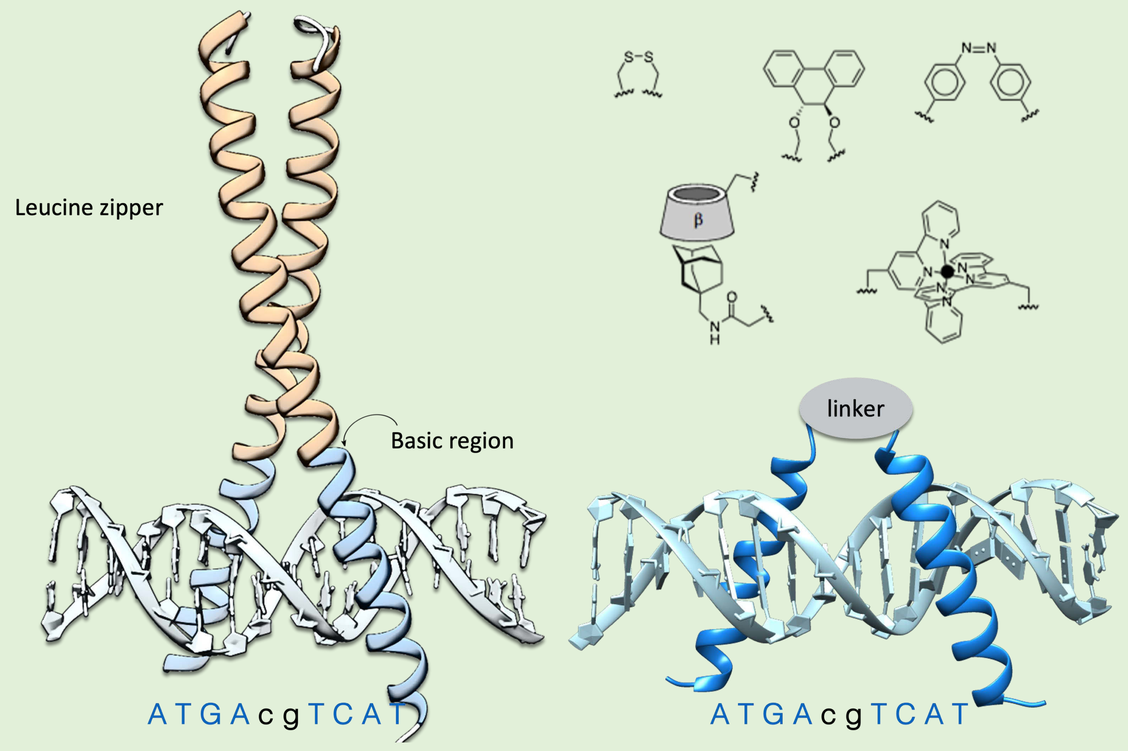
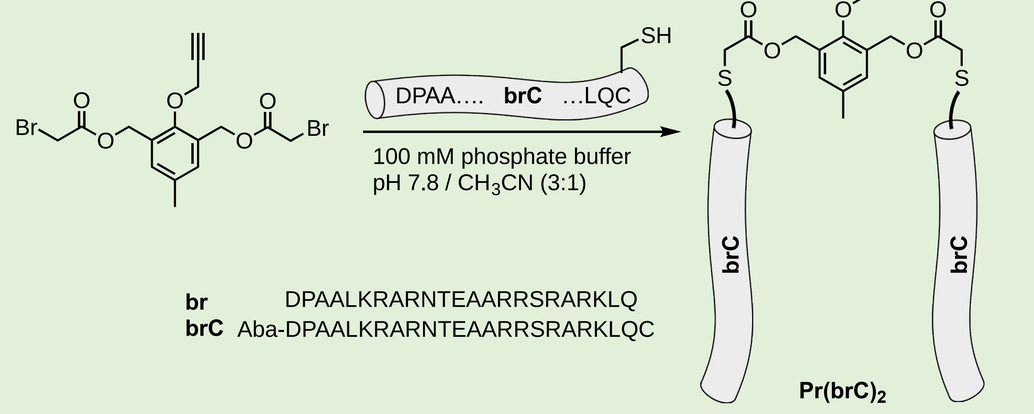
Pd-mediated deactivation of DNA binding

1. Previous work

The leucine zipper in the GCN4 TF can be replaced by other dimerizing agents without significantly altering the DNA binding properties.



2. Synthesis of the dimer Pr(brC)$_2$

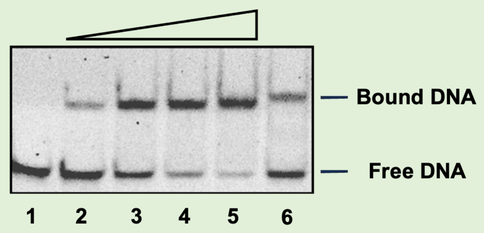


br DPAALKRARNTEAARRSRARKLQ
brC Aba-DPAALKRARNTEAARRSRARKLQC

3. DNA binding properties

When a ds-oligonucleotide containing the target ATF/CREB site was incubated with increasing concentrations of **Pr(brC)$_2$**, we observed the appearance of a new retarded band consistent with the formation of a specific DNA/**Pr(brC)$_2$** complex.

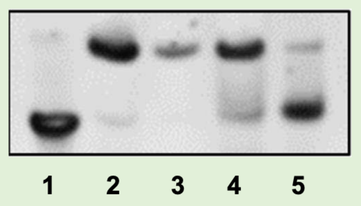
5´- ATGA CG TCAT -3'



4. Palladium-promoted cleavage

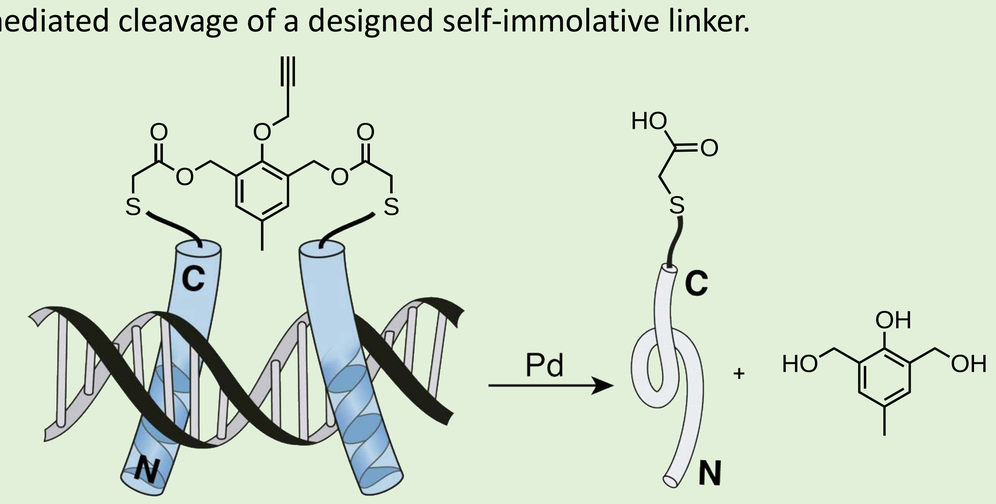
When the **Pr(brC)$_2$**-DNA complex was treated with [Pd(allyl)Cl]$_2$ (**Pd1**) for 3 h, we observed the disappearance of most of the retarded band corresponding to the **Pr(brC)$_2$**-DNA complex (lanes 4-5). A control experiment with the disulfide dimer **(brGCC)$_2$** revealed that the DNA binding is retained after incubation with **Pd1** (lanes 2-3), which confirms that the cleavage/disassembly sequence only occurs with our responsive peptide dimer.

(brGCC)$_2$	-	+	+	-	-
Pr(brC)$_2$	-	-	-	+	+
Pd1	-	-	+	-	+



5. Conclusions

We describe the on-demand disassembly of dimeric peptides using a Pd-mediated cleavage of a designed self-immolative linker.



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