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Supporting Information

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Nickel-promoted Recognition of long DNA sites by designed Peptide Derivatives

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Abbreviations

DEDTC: sodium diethyldithiocarbamate

HATU: 2-(1H-7-aza- benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HBTU: 2-[(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

TMR: tetramethylrhodamine dye

TFA: trifluoroacetic acid

TIS: triisopropylsilane

General

The bipyridine ligand was synthetized according to previously described methods.[1]

General peptide synthesis procedures

All peptide synthesis reagents and amino acid derivatives were purchased from GL Biochem (Shanghai) and Novabiochem; amino acids were purchased as protected Fmoc amino acids with the standard side chain protecting scheme: Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Glu(O*t*-Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH, Fmoc-Thr(*t*-Bu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH and Fmoc-Asp(O*t*-Bu)-OH except for the orthogonally protected Fmoc-Lys(Alloc)-OH, which was purchased from *Bachem*. All other chemicals were purchased from *Aldrich* or *Fluka*. All solvents were dry and synthesis grade, unless specifically noted.

Peptides were synthesized using an automatic peptide synthesizer from *Protein Tecnologies PS3 PeptideSynthesizer*. Peptide synthesis was performed using standard Fmoc solid-phase method on a PAL–PEG–PS resin (0.19 mmol/g) using HBTU/HOBt (4 equiv) as coupling agent, DIEA as base (6 equiv) and DMF as solvent. The deprotection of the temporal Fmoc protecting group was performed by treating the resin with 20% piperidine in DMF solution for 20 min. For **brHk** and **brHis$_2$**, after the N-terminal aspartic acid, we coupled p-ABA chromophore.

Side chain deprotection: The resin containing the alloc peptide (75 mg, aprox. 0.015 mmol) was suspended in DMF and shaken for 1 h to ensure a good swelling. Then $Pd(OAc)_2$ (1 mg, 0.3 equiv), 4-methylmorpholine (16 µL, 10 equiv), PPh_3 (6 mg, 1.5 equiv), $PhSiH_3$ (18 µL, 10 equiv) and 1 mL CH_2Cl_2 were added, and the mixture shaken overnight. The resin was then filtered and washed with DMF, DEDTC and DMF again.

Coupling of bipyridine acid derivative: The resin containing the peptide conjugate with the N-terminal AT-hook (50 mg, aprox. 0.01 mmol) was suspended in DMF and shaken for 1 h to ensure a good swelling. A solution of the bipyridine carboxylic acid derivative (7 mg, 0.02 mmol, 2 equiv), HATU (9 mg, 0.02 mmol, 2 equiv) and DIEA (250 µL, 0.195 M in DMF, 0.04 mmol, 4 equiv) was added. The reaction mixture was shaken for 1 h. The resin was washed with DMF (3 ×, 5 min) and DCM (2 ×, 5 min).

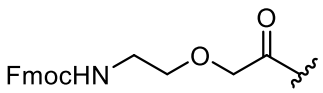
The cleavage/deprotection step was performed by treatment of the resin-bound peptide for 1.5–2h with the following cleavage cocktail: 900 µL TFA, 50 µL CH_2Cl_2, 25 µL H_2O and 25 µL TIS (1 mL of cocktail / 40 mg resin).

The crude products were purified by RP−HPLC, 4 mL/min, gradient 10 to 50% B over 40 min. (A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA) and identified as the desired peptides.

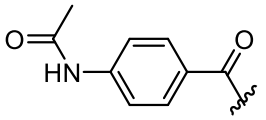
High-Performance Liquid Chromatography (HPLC) was performed using an *Agilent 1100* series Liquid Chromatograph Mass Spectrometer system. Analytical HPLC was carried out using a *Eclipse XDB-C18 analytical column (4.6 x 150 mm, 5 µm)*, 1 mL/min, gradient 5 to 75% B over 30 min. Purification of the peptides was performed on a semipreparative *Phenomenex Luna–C18* (250 × 10 mm) reverse-phase column.

Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an *Agilent 1100* Series LC/MSD VL G1956A model in positive scan mode.

Fmoc–O1Pen–OH =



p-ABA =



TMR =

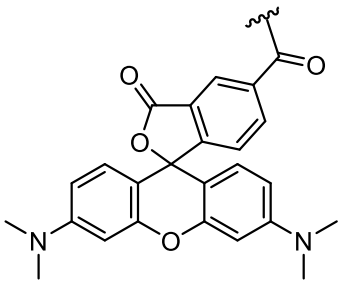


Fig. S1 Structures of Fmoc-O1Pen-OH, p-ABA and TMR.

brHk: was isolated with an approx. yield of 22%.

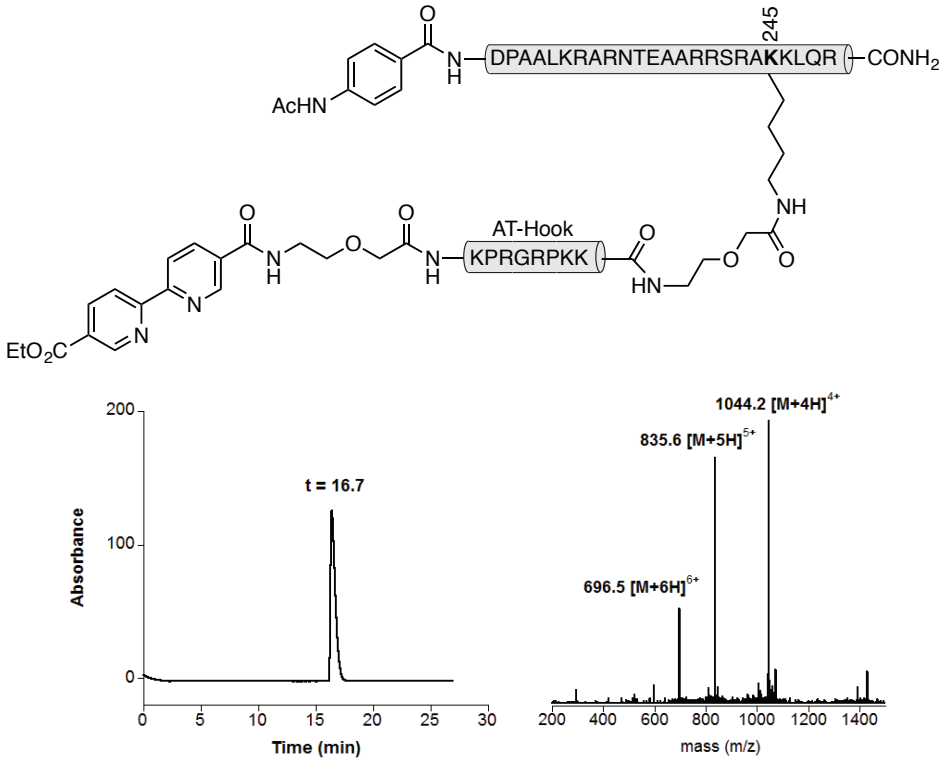


Fig. S2 Left) HPLC chromatogram of the purified peptide. Gradient 5 to 75% B over 30 min. Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. for $C_{181}H_{303}N_{65}O_{49}$: 4171.32. Found: 1044.2 $[M+4H]^{4+}$; 835.6 $[M+5H]^{5+}$; 696.5 $[M+6H]^{6+}$.

brHis$_2$ was isolated with an approx. yield of 17%.

AbaHN—DPAAHKRAHNTEAARRSRAKKLQR—$CONH_2$

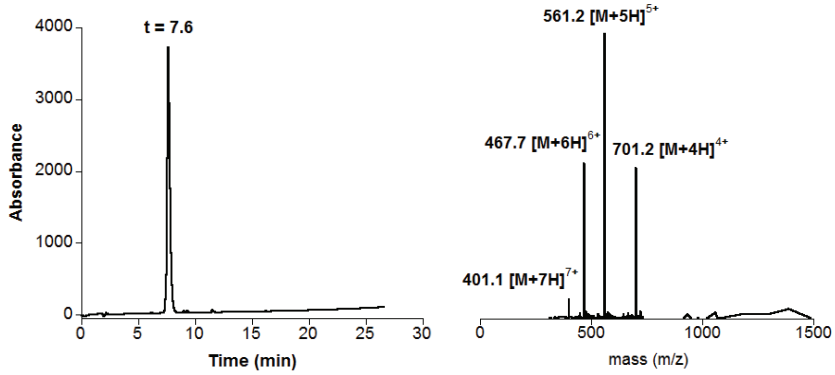


Fig. S3 Left) HPLC chromatogram of purified peptide. Gradient 5 to 95% B over 30 min. Right) Right) Mass spectrum of the purified peptide.

EM–ESI$^+$ (m/z): Calcd. for $C_{117}H_{193}N_{47}O_{34}$: 2800.5. Found: 701.2 $[M+4H]^{4+}$; 561.2 $[M+5H]^{5+}$; 467.7 $[M+6H]^{6+}$; 401.1 $[M+7H]^{7+}$.

Oligonucleotide sequence

Double stranded (only one strand is shown) oligonucleotides used for EMSA experiments were supplied by *Thermo Fischer* and their sequences were:

TCAT-A_2T_2-ATGA	5´- GCGAGTCAT*AATT*ATGAAGGCG-3´
ctctc-A_2T_2-gagag	5´- CGCGCTCTC*AATT*GAGAGCTC-3´
TCAT-A_2T_2-gaga	5´- CGCG TCAT*AATT*GAGAGCGC -3´
TCAT-cagg-ATGA	5´-GCGAGTCAT*CAGG*ATGAAGGCG -3´
TCAT-TA_2T_2-ATGA	5´- GCGAGTCAT*TAATT*ATGAAGGCG-3´

EMSA experiments

EMSAs were performed with a BioRad Mini Protean gel system, powered by an electrophoresis power supplies PowerPac Basic model, maximum power 150 V, frequency 50-60 Hz at 140 V (constant V). Binding reactions were performed over 30 min in 18 mM Tris-HCl (pH 7.5), 90 mM KCl, 1.8 mM $MgCl_2$, 0.2 mM TCEP, 9% glycerol, 0.11 mg/mL BSA, 2.2% NP-40 and 0.01 mM of $Ni(ClO_4)_2$. In the experiments we used 75 nM of the ds−DNAs and a total incubation volume of 20 µL. After incubation for 40 min products were resolved by PAGE using a 10% non-denaturing polyacrylamide gel and 0.5x TBE buffer for 50 min at 4 ºC, and analyzed by staining with SyBrGold (Molecular Probes: 5 µL in 50 mL of 1x TBE) for 10 min. and visualized by fluorescence.

5x TBE buffer (0.445M Tris, 0.445 M Boric acid)

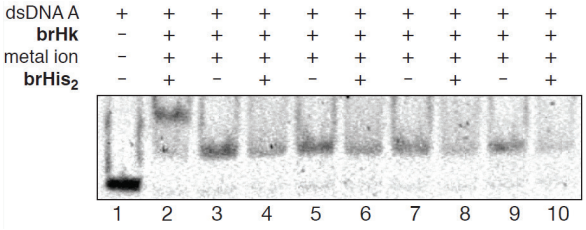


Fig. S4 EMSA analysis of the DNA binding recognition properties using different metals. Lanes 1-10: TCAT-A_2T_2-ATGA (75 nM). Lane 2: $Ni(ClO_4)_2$; lanes 3-4: $ZnSO_4$; lanes 5-6: $CuSO_4$; lanes 7-8: $Co(ClO_4)_2$, lane 9-10: $Fe(NH_4)_2(SO_4)_2$.

Fluorescence Anisotropy

Measurements were made with a Jobin-Yvon Fluoromax-3, (DataMax 2.20) coupled to a Wavelength Electronics LFI−3751 temperature controller, using the following settings: integration time: 2.0 s; excitation slit width: 5.0 nm; emission slit width: 20.0 nm; excitation wavelength 559 nm; emission wavelength 585 nm.

TMR-5'–GCGAGTCAT*TAATT*ATGAAGGCG–3' (one strand shown) (5 µL, 5 µM) was added to 995 µL of Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, 300 nM of **brHk** and 300 nM **br(His)$_2$** and the anisotropy was measured. Aliquots of a stock solution in water of the compound $Ni(ClO_4)_2$ (25 µM) were successively added to this solution, and the anisotropic value was recorded after each addition.

CD measurements

Circular Dichroism experiments were made with a *Jasco-715* coupled with a thermostat *Nestlab* RTE-111. The settings used were: Acquisition range: 300-195nm; band width: 2.0 nm; resolution: 0.2 nm; accumulation: 5 scans; sensitivity 10 mdeg; response time: 0.25 s, speed: 100 nm/min. Measurements were made in a 2 mm cell at 20 ºC. Samples contained 10 mM phosphate buffer pH 7.5 and 100 mM of NaCl, 5 µM peptide and 5 µM of corresponding dsDNA (when present).

The mixtures were incubated for 5 min before registering. The CD spectra of the peptides (when measured in the presence of DNA) were calculated as the difference between the spectrum of the peptide/DNA mixture and the measured spectrum of a sample of the DNA oligonucleotide. The spectra are the average of 5 scans and were processed using the "smooth" macro implemented in the program *Kaleidagraph* (v 3.5 by Synergy Software).

[1] H. Ishida, Y. Maruyama, M. Kyakuno, Y. Kodera, T. Maeda, S. Oishi, *ChemBioChem* **2006**, *7*, 1567-1570.