Supporting Information

Remote control of charge transport and chiral induction along a DNA-metallohelicate

Mohammad S. Askari, Christopher Lachance-Brais, Felix J. Rizzuto, Violeta Toader, and Hanadi Sleiman*

S1. General Information

40% bis:acrylamide solution (19:1), H_3BO_3, PBS buffer (phosphate buffered saline, pH = 7.4) and tris(hydroxymethyl)-aminomethane (Tris) were purchased from BioShop Canada, Inc. and used as received. Acetic acid, ammonium hydroxide, ammonium persulfate (APS), tetramethylethylenediamine(TEMED), tetrakis(acetonitrile)copper(I) hexafluorophosphate ($[(CH_3CN)_4Cu]PF_6$) and urea, were purchased from Sigma Aldrich. Gel Red was purchased from VWR. Anhydrous acetonitrile, 1000Å LCAA CPG pre-packed, universal columns, activator (0.25M ETT in acetonitrile), oxidizer (0.02 *M* iodine in tetrahydrofuran (THF)/Pyridine/H_2O), Cap A mix (THF/Lutidine/ acetic anhydride), Cap B mix (16% 1-methylimidazole in THF), and Deblock (3% dichloroacteic acid in dichloromethane) solutions were all used as received from Glen Research. 2-cyanoethyldiisopropylchloro phosphoramidite and derivatized nucleosides (dA, dC, dG and dT) were purchased from Glen Research. The latter were dissolved in 25 mL anhydrous acetonitrile prior to use. Sephadex G-25 (superfine, DNA grade) used for DNA desalting was purchased from Glen Research. Diphenylphenanthroline phosphoramidite used in this study was synthesized according to previously published procedure.[1] Unless otherwise noted all experiments were done in 1xPBS buffer. For Tm measurements 100 μL of 3 μM DNA solutions were used. For CD titration and kinetic studies 120 μL of 10 μM DNA solutions were used. Amount of DNA was quantified by absorption measurement at 260 nm and correcting for dpp absorption (section S3).

S2. Instrumentation

Standard automated oligonucleotide solid-phase synthesis was performed on a MerMade 6 Column DNA / RNA Automated Synthesizer from Bioautomation. Gel electrophoresis experiments were carried out on a 20 x 20 cm vertical Hoefer 600 electrophoresis unit at 25 °C. Strand annealing was performed in an Eppendorf Vapo Protect PCR machine. UV measurements were carried out using a Varian Cary Bio300 dual beam UV/Vis spectrophotometer equipped with a Peltier temperature controller. 150 μL quartz sample cells with 1 cm path length were used. Circular dichroism (CD) spectra were recorded on a JASCO J-800 spectropolarimeter in quartz cells (0.1 cm) at constant temperatures using a Peltier temperature controller and a circulating water bath. Reverse phase high-performance liquid chromatography (RP-HPLC) were done using a Hamilton PRP-1 5 μm 100 Å pore 2.1 x 150 mm column and a mixture of acetonitrile and Triethylammonium acetate (TEAA) buffer (pH 7.8) as the mobile phase; elution gradient: 3% to 50% acetonitrile in TEAA gradient over 30 minutes. Elution of the modified oligonucleotides was monitored at 260 nm (absorption of DNA). For each separation approximately 0.8 OD_{260} of crude DNA was injected as a 100 μL solution in 18.2 MΩ MilliQ water).

S3. DNA sequence and Synthesis of the Oligonucleotides

X = diphenylphenanthroline ligand.

Table S 1: Oligonucleotide Sequences 5'-3'

Name	Sequence	Extinction Coefficient $L \cdot mol^{-1} \cdot cm^{-1}$	Exact Mass $g \cdot mol^{-1}$ Calculated [Found]
S	ACGACATAGGACATCAGTCCTT GATATCTC	293900	9225.53
S'	GATGTCCTATGTCGT	141400	4651.75
P1	**X**ACGACATAGGACATCAGTCCT TGATATCTC	293900	9752 [9752.1]
P1'	GATGTCCTATGTCGT**X**	141400	5176 [5175.9]
P2	**XX**ACGACATAGGACATCAGTCC TTGATATCTC	293900	10354 [10354.2]
P2'	GATGTCCTATGTCGT**XX**	141400	5778 [5778.1]

P3	**XXX**ACGACATAGGACATCAGTC CTTGATATCTC	293900	10956.5 [10957.1]
P3’	GATGTCCTATGTCGT**XXX**	141400	6380.5 [6379.9]
P1(GC)1	**X**GCGACATAGGACATCAGTCCT TGATATCTC	290300	9768.18 [9767.8]
P1(GC)1’	GATGTCCTATGTCGC**X**	139000	5161.18 [5160.9]
P1(GC)4	**X**AAAGAAAAAAAAAAA	242700	5254.38 [5254.03]
P1(GC)4’	TTTTTTTTTTTTTTTCTTT**X**	121200	6609.08 [6609.06]
P1(GC)8	**X**AAAAAAAGAAAAAAA	242700	5254.38 [5254.03]
P1(GC)8’	TTTTTTTTTTTCTTTTTTT**X**	121200	6609.08 [6609.06]
P1(AT)	**X**AAAAAAAAAAAAAA	162600	5238.38 [5238.0]
P1(AT)’	TTTTTTTTTTTTTTTTTTT**X**	183400	6624.18 [6624.06]
P1(GCGC)2	**X**AGGAAATTTAATTATAGTCC	208200	6738.26 [6738.20]
P1(GCGC)2’	ATAATTAAATTTCCT**X**	149800	5124.99 [5125.06]
P1(GCGC)3	**X**AAGGAATTTAATTATAGTCC	208200	6738.26 [6738.16]
P1(GCGC)3’	ATAATTAAATTCCTT**X**	149800	5124.99 [5125.18]

DNA solutions were quantified based on their OD at 260 nm and their extinction coefficients. For strands containing diphenylphenanthroline (dpp) ligand the following correction was used to account for dpp absorption at 260nm:

$$OD_{corr}^{260} = \left(\frac{\varepsilon_{DNA}^{260}}{\varepsilon_{DNA}^{260} + n \cdot \varepsilon_{dpp}^{260}} \right) \cdot OD_{DNA}^{260}$$

Where n is the number of dpp ligands, ε_{DNA}^{260} is DNA absorption coefficient from table above, OD_{DNA}^{260} is the measured optical density of DNA solution, and $\varepsilon_{dpp}^{260} = 18790\ L \cdot mol^{-1} \cdot cm^{-1}$ is the absorption coefficient of dpp at 260 nm.

S4. Preparation of duplexes

ssDNA were quantified by measuring the optical density (OD) at 260nm. Equimolar ratios of complementary single–stranded DNA were combined in PBS buffer with final duplex concentration of 3 μM (Tm experiments) or 10 μM (CD experiments). The strands were annealed at 95 °C for 5min followed by heating gradient from 80 °C to 4 °C with one degree decrement per 5 min.

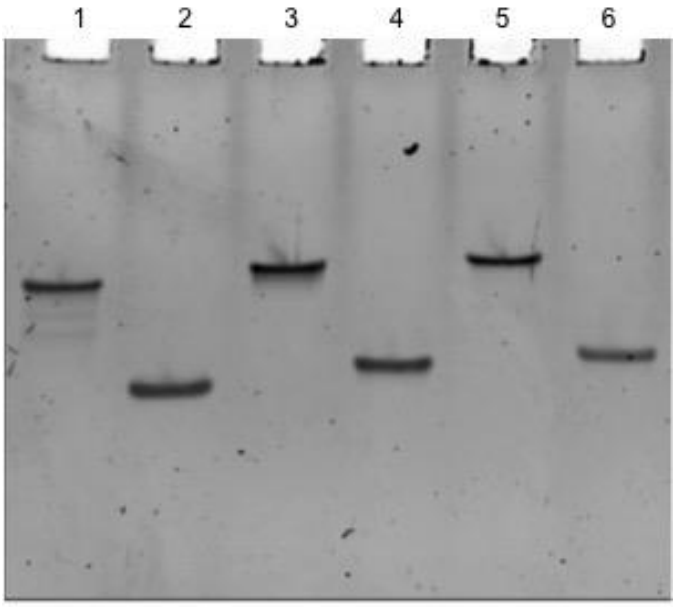

Figure S 1: Denaturing PAGE (20% acrylamide, 1xTBE, 8*M* urea) of (1) P1, (2) P1', (3) P2, (4) P2', (5) P3, (6) P3'.

S5. Cu(I) binding and characterization

To a 3 μ*M* solution of duplex **P1**, **P2**, and **P3**, a solution of $[(MeCN)_4Cu]PF_6$ in acetonitrile was added in 2, 4, and 6 equivalents, respectively. For **P2**, and **P3**, the strands were annealed after addition of Cu(I) by three cycles of heating to 95 °C for 10 minutes followed by rapid cooling (within 1 min) to 10 °C. The metallated duplexes **P2** and **P3** were purified by native PAGE electrophoresis followed by band excision and extraction into 1xPBS buffer.

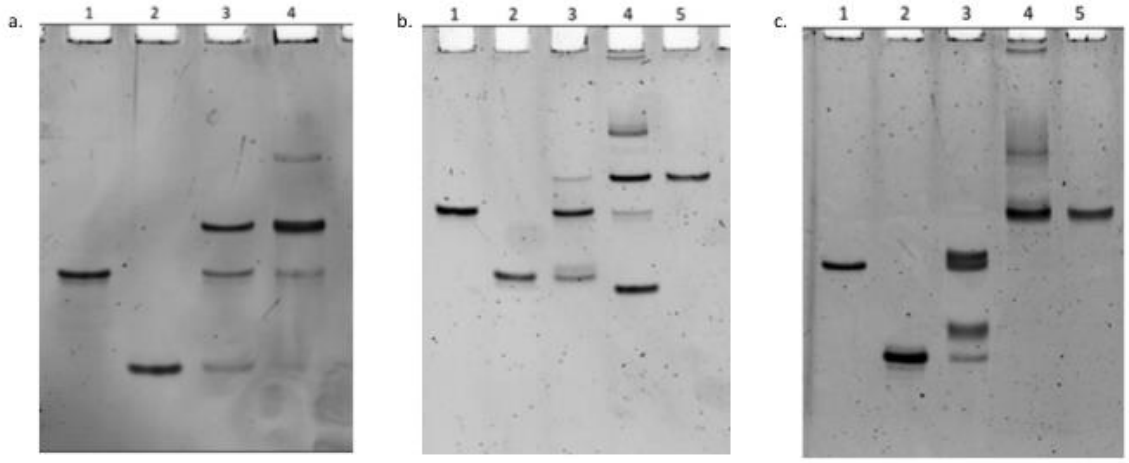

Figure S 2: Denaturing gel electrophoresis (15%); a. P1, P1' (lane 1, 2); dsP1 (lane 3); P1 duplex plus 2 equivalents of Cu(I). b. P2, P2' (lane 1, 2); dsP2 (lane 3); dsP2 plus 4 equivalents of Cu(I) before purification (lane 4) and after purification (lane 5). c. P3, P3' (lane 1, 2); dsP3 (lane 3); dsP3 plus 6 equivalents of Cu(I) before purification (lane 4) and after purification (lane 5).

S6. Melting Temperature measurements

3μ*M* solution of the duplexes was metallated through addition of 50 equivalents of $CuBr_2$ and heating the mixture at 40 °C for 24h. The complete formation of the Cu(I)-metallated duplexes was verified by CD measurement prior to melting experiments.

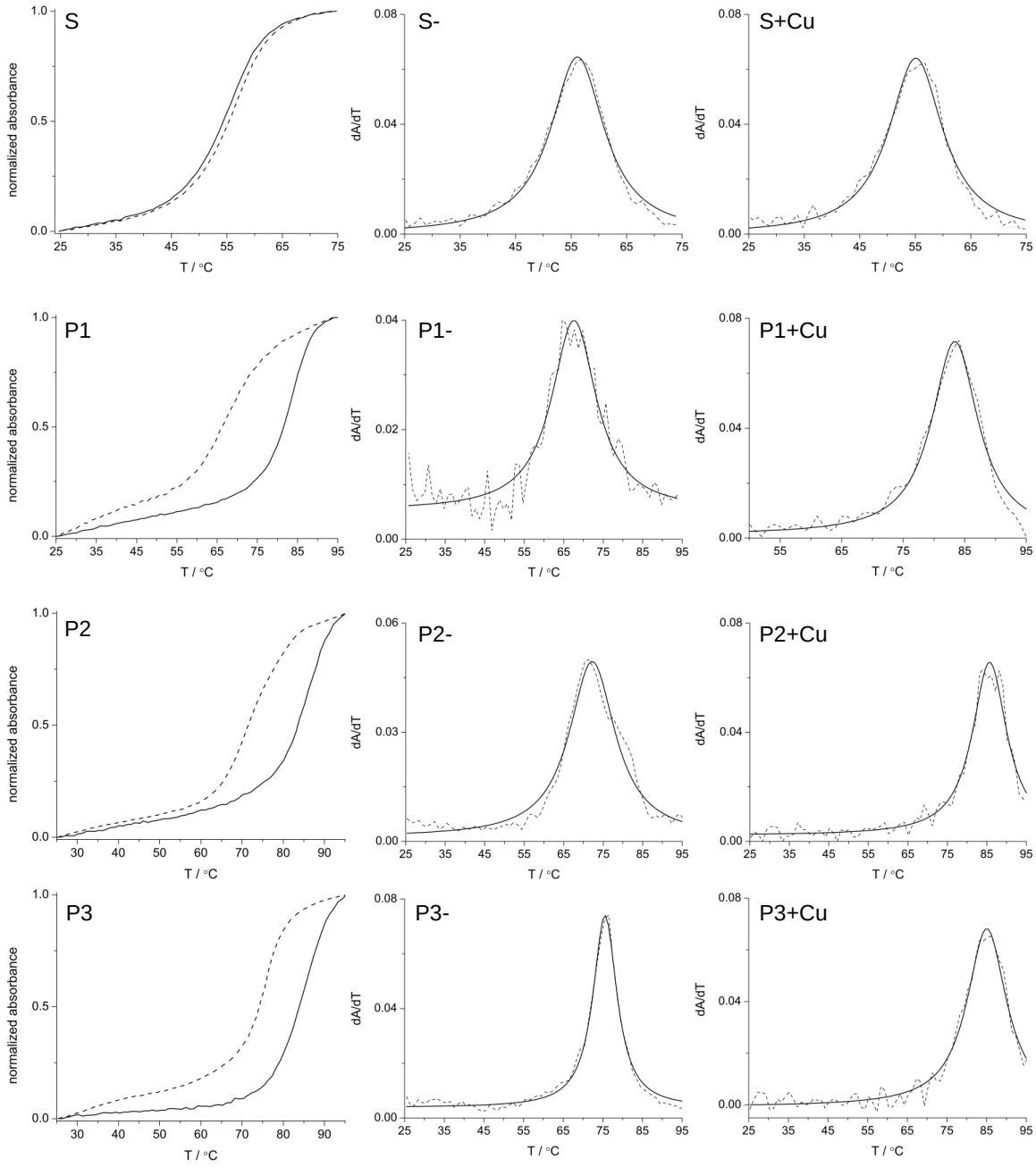

Figure S 3: Melting curves, (––) Metal free duplexes, (—) After metallation and first derivative analyses by fitting to Lorentzian function.

S7. Metal titration of P1, P2, and P3

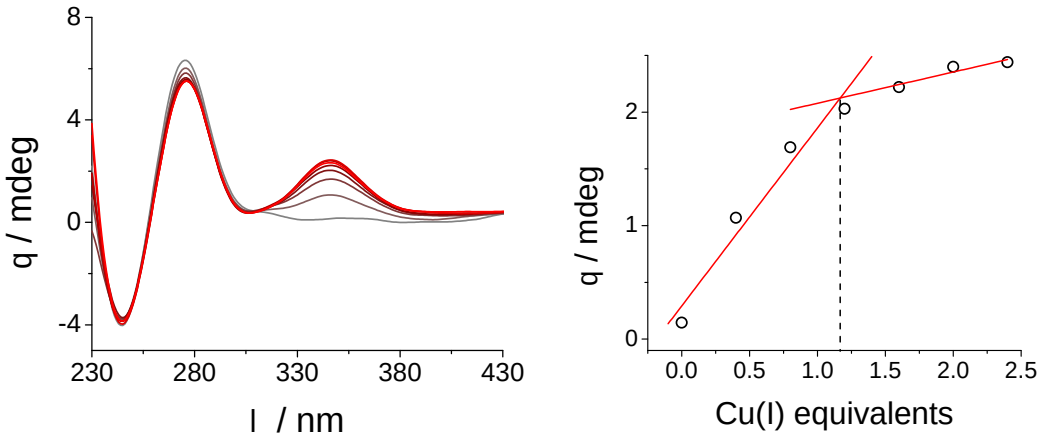

Figure S 4: Titration of a 10µM solution of **P1** in 1xPBS with a solution of $[(MeCN)_4Cu]PF_6$ in acetonitrile at 25 °C. Left, CD spectra after addition of Cu(I) aliquot, right, profile at 345nm.

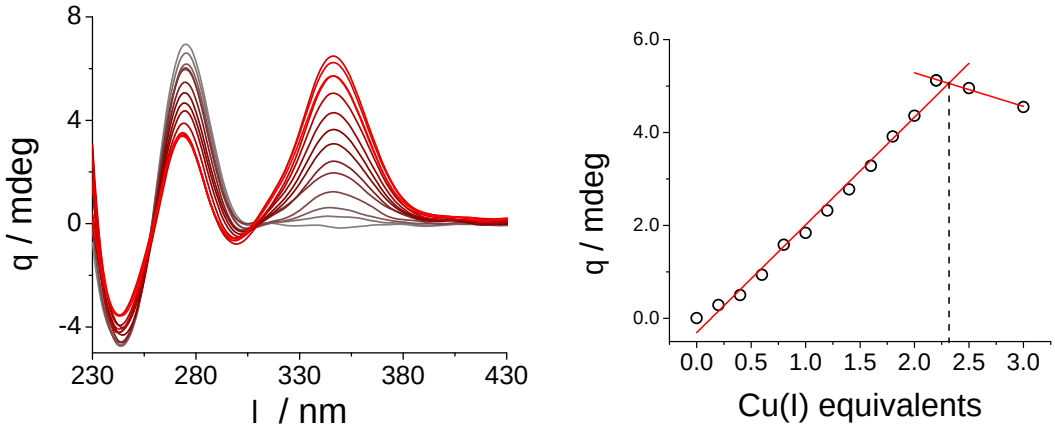

Figure S 5: Titration of a 10µM solution of P2 in 1xPBS with a solution of $[(MeCN)_4Cu]PF_6$ in acetonitrile at 25 °C. Left, CD spectra after addition of Cu(I) aliquot, right, profile at 345nm

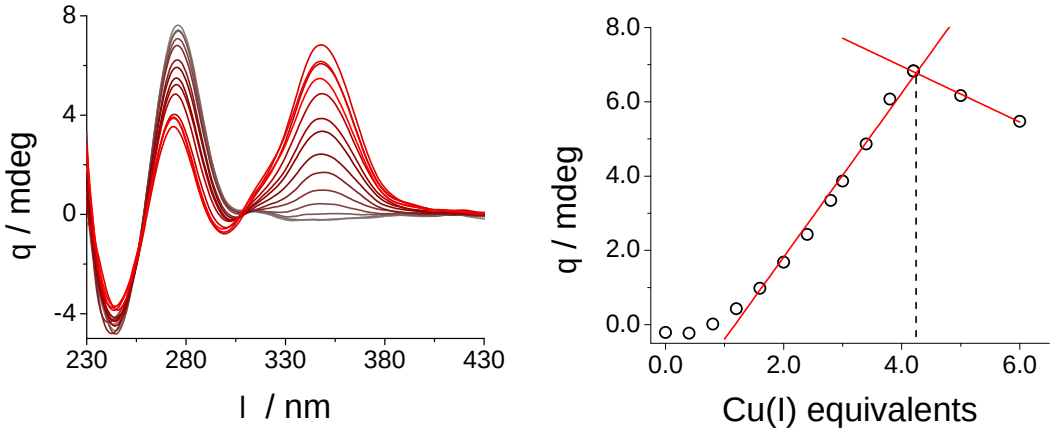

Figure S 6: Titration of a 10µM solution of P3 in 1xPBS with a solution of $[(MeCN)_4Cu]PF_6$ in acetonitrile at 25 °C. Left, CD spectra after addition of Cu(I) aliquot, right, profile at 348nm.

S8. Kinetic Analyses and Eyring plots

Experimental procedure: In a typical experiment 120 μL of 10 μM solution **P1**, **P2**, or **P3** in 1xPBS (or in 1x Sorenson) was placed in a quartz cuvette having 1mm path length. To this solution 50 equivalent of $CuBr_2$ (or $CuSO_4$ in Sorenson) was added and CD spectra were recorded. Time evolution of the spectra at the maximum was then fit into pseudo-first order kinetics and rate constants were extracted.

Table S 2: Measured rate constants for P1, P2, and P3 used for the Eyring analysis.

T °C	k / 10^{-4} (s^{-1})		
	P1	**P2**	**P3**
20	0.62	1.26	-
25	1.54	3.18	-
30	1.86	4.32	-
35	2.92	6.76	0.68
40	5.55	12.7	0.77
45	14.2	26.5	1.33
50	16.0	28.9	1.47
55	-	-	3.58

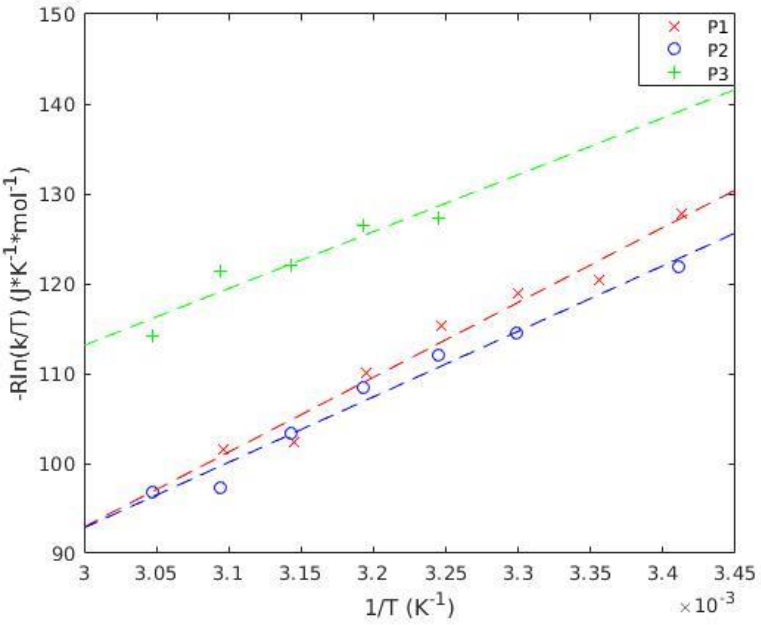

Figure S 7: Eyring Plots for **P1**, **P2**, and **P3** with the linear regression fitting.

Table S 3: Measured rate constants for P1 in PBS or in Sorenson buffer used for the Eyring analysis.

T °C	k / 10^{-4} (s^{-1})	
	PBS	**Sorenson**
10	-	0.60
15	-	2.06
20	0.62	3.77
25	1.54	4.01
30	1.86	8.43
35	2.92	-
40	5.55	18.91
45	14.2	-
50	16.0	-

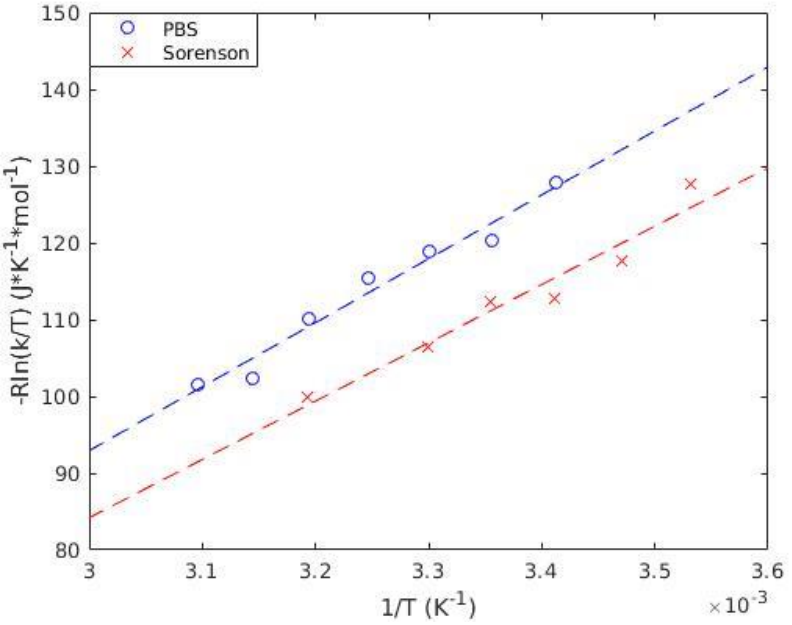

Figure S 8: Eyring Plots for **P1** in PBS or in Sorenson with the linear regression fitting.

S9. Piperidine Digestion experiments

General procedure: To a 10 μM solution of **dsDNA** in 1xPBS with and without metal ion piperidine was added such that the final concentration of piperidine was 3M. The mixture

was heated at 90 °C for 30 minutes. 5 μL aliquot of the solution was diluted in 10 μL of 8 M aqueous urea solution and loaded into a denaturing gel (TBE buffer).

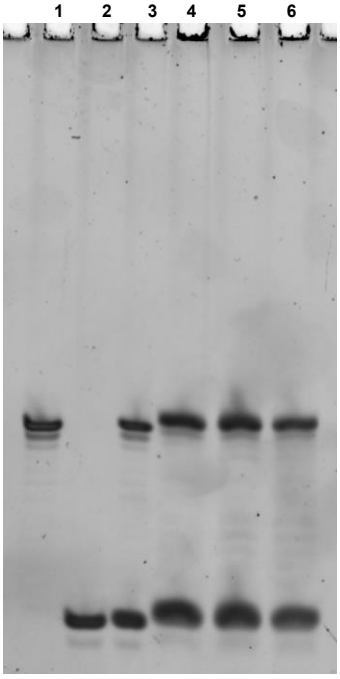

Figure S 9: Denaturing PAGE for the analysis of piperidine-induced DNA cleavage after oxidation with Cu(II). Digestion of **S**; Lane 1: S, 2: S', 3: **S**, 4: **S**+piperidine, 5: **S**+Cu(I)+piperidine, 6: **S**+Cu(II) +piperidine.

S10. Synthesis of metallated strands using Cu(II) followed by reduction

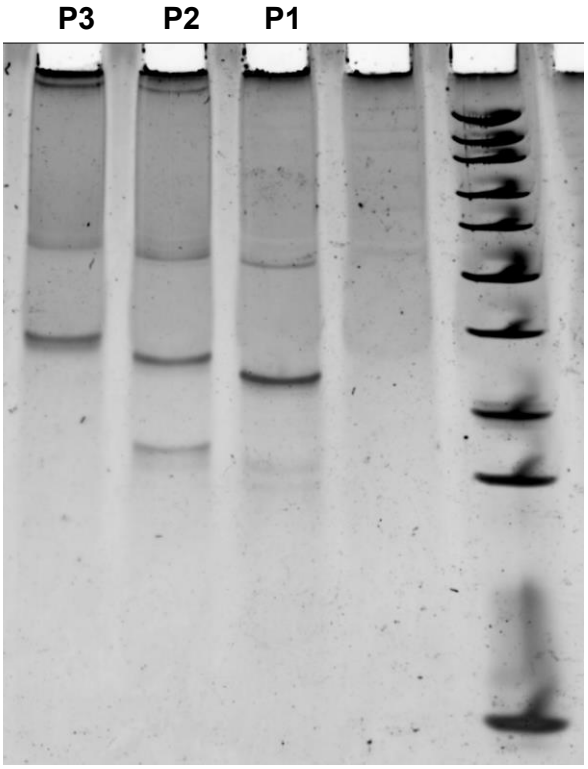

Figure S 10: Denaturing PAGE of **P1**, **P2**, and **P3** after incubation with Cu(II) and reduction.

To double-stranded DNA in 1xPBS buffer a solution of $CuBr_2$ in water was added (>2 equivalents) and the strands were incubated at 40 °C for 24h. An aliquot of the solution were mixed with 8 *M* urea and the resulting solution was subjected to denaturing PAGE.

S11. Nuclease activity experiment

To ds GG2 strands is added either no copper, 1 equivalent of tetrakisacetonitrile Cu(I) bromide or 1 equivalent of Cu(II) sulfate in water. These strands are then diluted to 3 μM and mixed with either nothing, piperidine 1 *M* or a combination of 1 *mM* 3-mercaptopropionic acid, 3 *mM* H_2O_2 and 1 M piperidine according to table S3. MPA and H_2O_2 are added first to the strands and all strands are incubated at 40 °C for 18 hours. For the appropriate strands, piperidine is then added and heated at 90 °C for 45 minutes. From these solutions, 6 *pmol* of GG2 are imaged on a 20% polyacrylamide gel in TBE buffer 7 *M* urea with gel red. Cleavage is evident by a high mobility band forming. Cleavage is increased compared to the no copper control only when Cu(II) and piperidine are present, and MPA and H_2O_2 have no effect. Since Cu(I) doesn't cleave in presence of MPA and H_2O_2 , the system does not behave like previously reported nucleases. Lower mobility bands are formed probably because of associations between the hydrophobic dpp moieties.

Table S 3: Concentration of reactants in with either no copper, Cu(I) or Cu(II) and the strand GG2

Lane	***1***	***2***	***3***
[MPA] (*mM*)	0.0E+00	0.0E+00	1.0E+00
[H_2O_2] (*mM*)	0.0E+00	0.0E+00	3.0E+00
[Piperidine] (*mM*)	0.0E+00	1.0E+03	1.0E+03

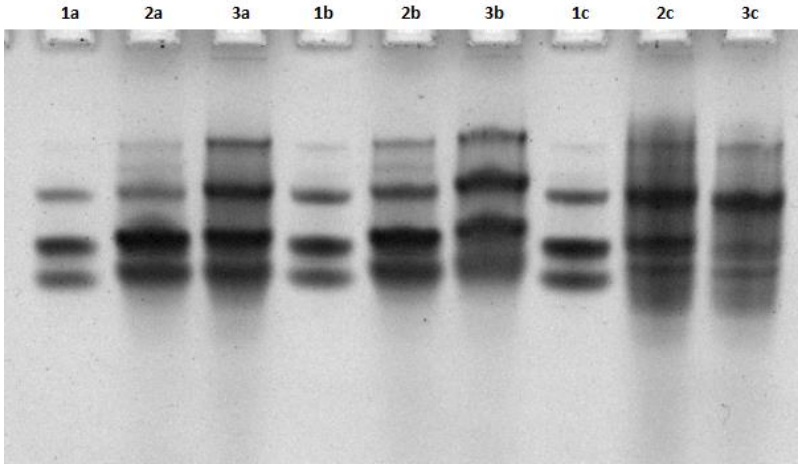

Figure S 11: Denaturing PAGE after **GG2** cleavage experiments with no copper (**a**) , Cu(I) (**b**) and Cu(II) (**c**). The last two bands are the 5' strand and the 3' strand of GG2 respectively. Higher mobility bands are due to cleavage. Lower mobility bands are assumed to be dimer, trimer and tetramer. Only Cu(II) bands with piperidine show cleavage.

References:

[1] H. Yang, H. F. Sleiman, *Angew. Chem., Int. Ed.* **2008**, *47*, 2443-2446.