Electronic Supplementary Material (ESI) for Dalton Transactions

SUPPORTING MATERIAL

DNA Nuclease Activity of Rev-Coupled Transition Metal Chelates

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Complex	With Attached Rev		Without Attached Rev	
	k_{nick} for DNA nicking (min^{-1})	k_{lin} for DNA linearization (min^{-1})	k_{nick} for DNA nicking (min^{-1})	k_{lin} for DNA linearization (min^{-1})
Fe-DOTA	0.06 ± 0.01	0.0008 ± 0.0002	––[a]	0.0008 ± 0.0001
Co-DOTA	0.05 ± 0.01	0.0012 ± 0.0002	––[a]	0.0005 ± 0.0001
Ni-DOTA	0.016 ± 0.004	0.0008 ± 0.0006	0.030 ± 0.007	0.0029 ± 0.0008
Cu-DOTA	0.043 ± 0.007	0.0025 ± 0.0008	––[a]	0.0013 ± 0.0001
Fe-DTPA	0.025 ± 0.005	0.0011 ± 0.0002	––[a]	0.0012 ± 0.0001
Co-DTPA	0.015 ± 0.001	0.0009 ± 0.0002	––[a]	––[a]
Ni-DTPA	0.10 ± 0.01	0.0021 ± 0.0007	––[a]	––[a]
Cu-DTPA	0.11 ± 0.01	0.0083 ± 0.0008	––[a]	––[a]
Fe-EDTA	0.07 ± 0.02	0.0021 ± 0.0007	0.044 ± 0.009	0.0005 ± 0.0001
Co-EDTA	0.0177 ± 0.0008	0.005 ± 0.003	0.08 ± 0.02	––[a]
Ni-EDTA	0.041 ± 0.007	0.0011 ± 0.0004	0.037 ± 0.002	0.00019 ± 0.00002
Cu-EDTA	0.076 ± 0.005	0.0032 ± 0.0007	0.032 ± 0.003	0.00030 ± 0.00003
Co-GGH	0.037 ± 0.005	0.0021 ± 0.0002	––[a]	< 0.0002
Ni-GGH	0.067 ± 0.008	0.0015 ± 0.0007	––[a]	––[a]
Cu-GGH	0.069 ± 0.006	0.0013 ± 0.0001	0.06 ± 0.01	0.0012 ± 0.0002
Co-KGHK	0.03 ± 0.01	0.0013 ± 0.0005	0.014 ± 0.002	0.0042 ± 0.0008
Ni-KGHK	0.024 ± 0.007	0.0016 ± 0.0004	––[a]	0.0003 ± 0.0001
Cu-KGHK	0.17 ± 0.04	0.0062 ± 0.0006	0.055 ± 0.008	0.00054 ± 0.00004
Fe-NTA	0.04 ± 0.02	0.002 ± 0.001	0.05 ± 0.01	0.0008 ± 0.0002
Co-NTA	0.04 ± 0.04	0.0010 ± 0.0007	0.1 ± 0.1	0.005 ± 0.003
Ni-NTA	0.08 ± 0.01	0.0016 ± 0.0004	––[a]	< 0.009
Cu-NTA	0.60 ± 0.06	0.012 ± 0.001	0.148 ± 0.007	0.0032 ± 0.0004
Background	0.006 ± 0.001	0.00024 ± 0.00003	0.006 ± 0.001	0.00024 ± 0.00003

Table SM1. Summary of observed first-order rate constants (k_{obs}) for consecutive DNA nicking (k_{nick}) and subsequent linearization (k_{lin}) by each M-chelate-Rev complex (this work) and by each M-chelate lacking Rev (Joyner et al., 2011),[1] arranged by chelating species (arranged by metal in the main manuscript). [a] Below detection limit (0.0097 min^{-1} for nicking; 0.00033 min^{-1} for linearization).

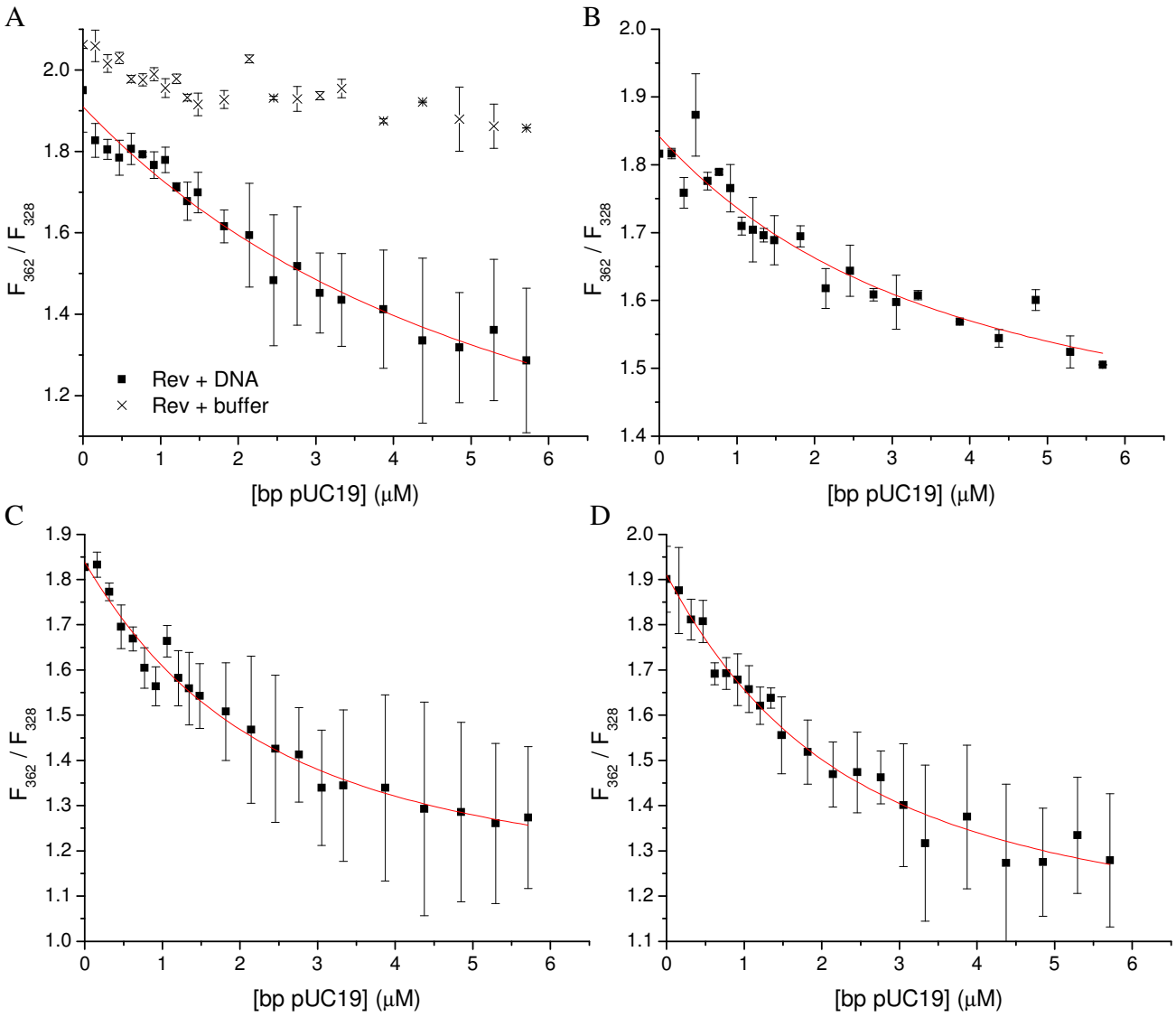

Figure SM1. Titration response curves for (A) titration of 500 μL 1 μM Rev peptide with either a solution containing 20 μM base pairs supercoiled pUC19 plasmid DNA or with a control solution containing only buffer, (B) titration of 500 μL 1 μM Ni-DOTA-Rev with a solution containing 20 μM base pairs supercoiled pUC19 plasmid DNA, (C) titration of 500 μL 1 μM Ni-DTPA-Rev with a solution containing 20 μM base pairs supercoiled pUC19 plasmid DNA, and (D) titration of 500 μL 1 μM Ni-EDTA-Rev with a solution containing 20 μM base pairs supercoiled pUC19 plasmid DNA. All titrations were performed in a binding buffer containing 20 mM HEPES, 100 mM NaCl, pH 7.4 at 37 °C.

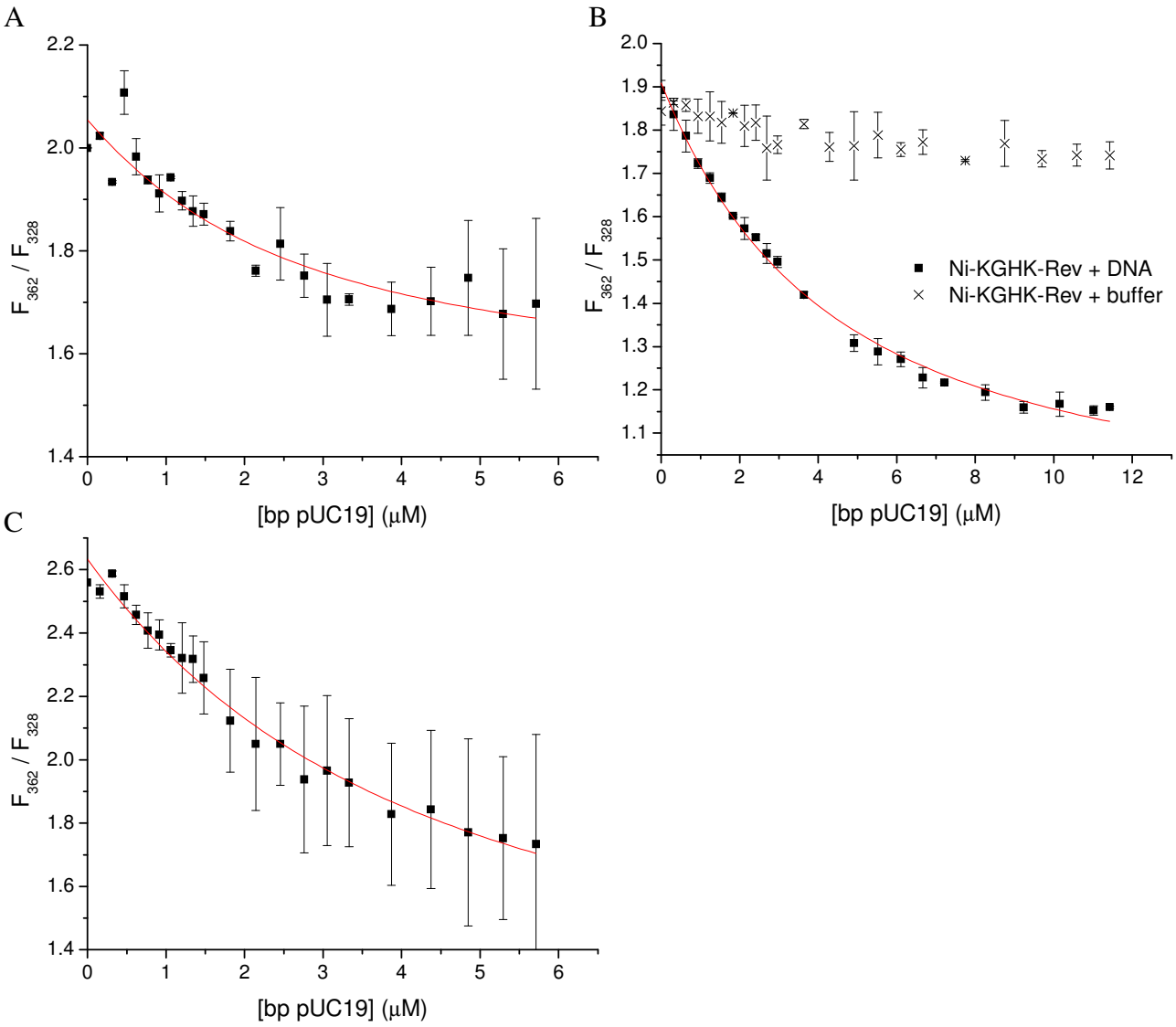

Figure SM2. Titration response curves for (A) titration of 500 μL 1 μM Ni-GGH-Rev with a solution containing 20 μM base pairs supercoiled pUC19 plasmid DNA, (B) titration of 500 μL 1 μM Ni-KGHK-Rev with either a solution containing 40 μM base pairs supercoiled pUC19 plasmid DNA or a control solution containing only buffer, and (C) titration of 500 μL 1 μM Ni-NTA-Rev with a solution containing 20 μM base pairs supercoiled pUC19 plasmid DNA. All titrations were performed in a binding buffer containing 20 mM HEPES, 100 mM NaCl, pH 7.4 at 37 °C.

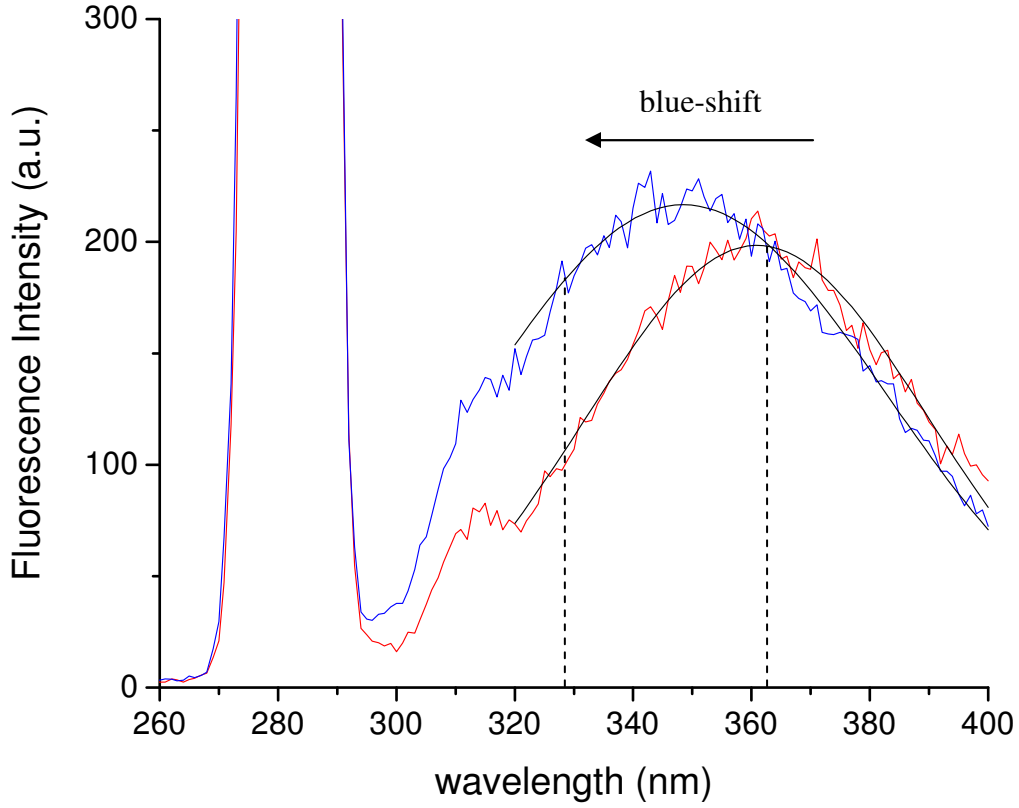

Figure SM3. Fluorescence emission spectra for the beginning (red) and end (blue) of a titration of 1 μM Rev peptide with supercoiled pUC19 plasmid DNA. A blue-shift in tryptophan fluorescence, with a shift in λ_{max} from ~ 361 nm to ~ 349 nm (peaks fit to Gaussian equations), was observed upon binding of DNA. The fluorescence intensities at 362 nm and 328 nm (marked by dashed lines above) were recorded at each point during each titration of Rev and M-chelate-Rev complexes with supercoiled pUC19 plasmid DNA. The ratio of these intensities (F_{362}/F_{328}), which decreased upon binding of DNA, provided a measure of the extent of binding. The observed blue-shift in tryptophan fluorescence occurred most likely as a result of decreased solvent exposure of the tryptophan upon Rev/DNA complex formation, consistent with previous reports of blue-shifts in tryptophan fluorescence following reduction of the dielectric constant (decreased solvent exposure) within the surrounding microenvironment.[2-4]

Complex	n_2/n_1 with attached Rev	n_2/n_1 without attached Rev [1]	k_{lin}/k_{nick} with attached Rev	k_{lin}/k_{nick} without attached Rev [1]	IC_{50} (mM DMSO) with attached Rev
Fe-DOTA	0.018 ± 0.007	~ 0	0.014 ± 0.004	~ 0	30 ± 50
Co-DOTA	0.021 ± 0.003	~ 0	0.023 ± 0.006	0.13 ± 0.02	400 ± 700
Ni-DOTA	0.021 ± 0.006	0.05 ± 0.01	0.05 ± 0.04	0.10 ± 0.05	800 ± 600
Cu-DOTA	0.015 ± 0.005	~ 0	0.06 ± 0.02	~ 0	200 ± 700
Fe-DTPA	0.037 ± 0.003	~ 0	0.05 ± 0.01	~ 0	<5000
Co-DTPA	~0.04	~ 0	0.06 ± 0.01	~ 0	1100 ± 700
Ni-DTPA	~0	~ 0	0.020 ± 0.007	~ 0	7 ± 7
Cu-DTPA	0.103 ± 0.005	~ 0	0.073 ± 0.009	~ 0	2000 ± 3000
Fe-EDTA	0.045 ± 0.005	0.03 ± 0.02	0.03 ± 0.01	0.01 ± 0.05	1400 ± 500
Co-EDTA	0.10 ± 0.02	~ 0	0.3 ± 0.2	~ 0	3000 ± 9000
Ni-EDTA	0.034 ± 0.005	0.0073 ± 0.0001	0.03 ± 0.01	0.01 ± 0.02	10 ± 10
Cu-EDTA	~0.06	0.01 ± 0.01	0.043 ± 0.009	0.01 ± 0.02	500 ± 100
Co-GGH	0.060 ± 0.006	~ 0	0.057 ± 0.008	~ 0	1400 ± 900
Ni-GGH	0.028 ± 0.009	~ 0	0.02 ± 0.01	~ 0	500 ± 300
Cu-GGH	0.0150 ± 0.0001	0.01 ± 0.01	0.019 ± 0.002	0.02 ± 0.03	540 ± 90
Co-KGHK	0.039 ± 0.008	0.11 ± .03	0.04 ± 0.02	0.30 ± 0.03	900 ± 400
Ni-KGHK	0.042 ± 0.008	~ 0	0.07 ± 0.02	0.03 ± 0.05	310 ± 50
Cu-KGHK	~0	0.017 ± .005	0.036 ± 0.009	0.01 ± 0.02	2000 ± 4000
Fe-NTA	0.01 ± 0.01	0.03 ± 0.01	0.04 ± 0.03	0.02 ± 0.05	7000 ± 1000
Co-NTA	0.11 ± 0.03	~ 0	0.02 ± 0.03	< 0.2	70 ± 60
Ni-NTA	0.07 ± 0.02	~ 0	0.021 ± 0.006	~ 0	1000 ± 200
Cu-NTA	0.010 ± 0.005	0.016 ± .003	0.022 ± 0.002	0.02 ± 0.02	>>1000
Background	~0	~ 0	0.03 ± 0.02	0.03 ± 0.02	180 ± 50

Table SM2. Summary of n_2/n_1 and k_{lin}/k_{nick} ratios for M-chelate-Rev complexes (this work) and M-chelates lacking Rev (Joyner et al., 2011),[1] which provides a relative measure of the concertedness of nicking and linearization for each reaction. IC_{50} values for DMSO inhibition of DNA cleavage by M-chelate-Rev complexes and coreactants are also shown. Attachment to Rev generally provided higher n_2/n_1 and/or k_{lin}/k_{nick} ratios, which were attributed to tighter DNA binding, although in a limited number of cases, attachment to Rev actually decreased these ratios; this decrease was attributed to Rev-induced geometric/steric constraint of the metal center, relative to the DNA backbone.

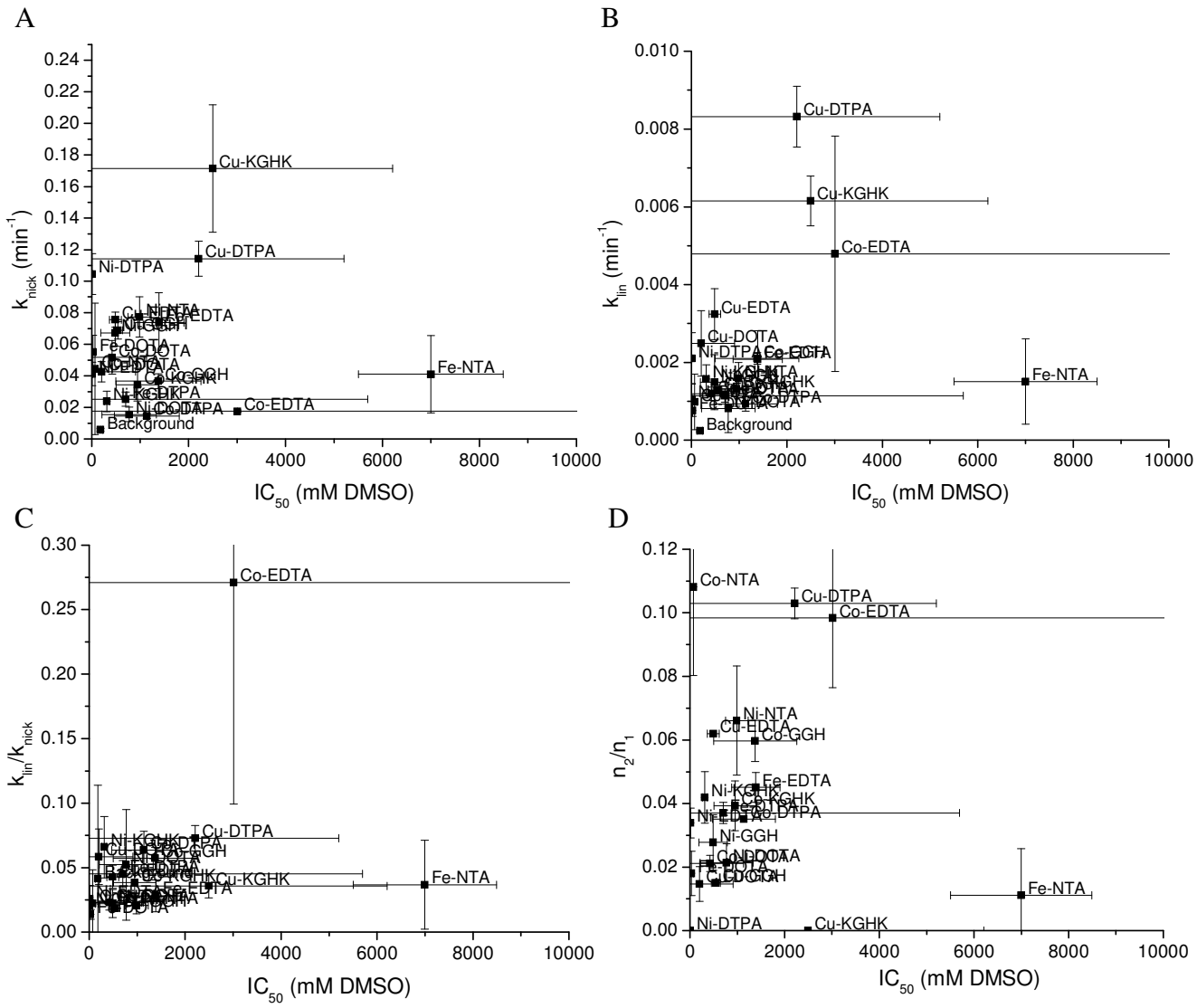

Figure SM4. There were no clear correlations between the IC_{50} of DMSO inhibition and (A) the rate constant for DNA nicking (k_{nick}), (B) the rate constant for DNA linearization (k_{lin}), (C) the k_{lin}/k_{nick} ratio, or (D) the n_2/n_1 ratio resulting from a Freifelder-Trumbo analysis for M-chelate-Rev complexes. The relatively high IC_{50} values (>100 mM DMSO), as well as the lack of any clear correlation are consistent with the primary involvement of metal-bound ROS, rather than diffusible radicals, in the mechanism of DNA cleavage, since only diffusible radicals are expected to be susceptible to DMSO inhibition.

complex	ascorbate consumption rate with co-reactants (μM/min) [1]		TEMPO reaction rate with co-reactants (μM/min) [1]		reduction potential vs. NHE for M-chelate (mV) [5]
	reaction with H_2O_2	reaction with O_2	reaction with H_2O_2	reaction with O_2	
Fe-DOTA	–– [a]	–– [a]	0.0601 ± 0.0005	–– [a]	396
Fe-DTPA	–– [a]	–– [a]	0.0185 ± 0.0005	–– [a]	280
Fe-EDTA	80 ± 10	6.9 ± 0.2	0.078 ± 0.001	0.0222 ± 0.0006	391
Fe-NTA	130 ± 20	4.15 ± 0.04	0.145 ± 0.002	0.0449 ± 0.0005	464
Co-DOTA	–– [a]	–– [a]	–– [a]	–– [a]	142
Co-DTPA	–– [a]	–– [a]	–– [a]	–– [a]	1111
Co-EDTA	–– [a]	–– [a]	0.0185 ± 0.0003	–– [a]	146
Co-GGH	0.5 ± 0.2	4.9 ± 0.1	–– [a]	–– [a]	-119
Co-KGHK	0.5 ± 0.2	7.1 ± 0.2	–– [a]	–– [a]	-228
Co-NTA	–– [a]	–– [a]	0.075 ± 0.002	–– [a]	274
Ni-DOTA	–– [a]	–– [a]	–– [a]	–– [a]	-35
Ni-DTPA	–– [a]	–– [a]	–– [a]	–– [a]	-- [b]
Ni-EDTA	–– [a]	–– [a]	–– [a]	–– [a]	172
Ni-GGH	–– [a]	–– [a]	–– [a]	–– [a]	1000
Ni-KGHK	–– [a]	–– [a]	–– [a]	–– [a]	1055
Ni-NTA	–– [a]	–– [a]	–– [a]	–– [a]	176
Cu-DOTA	–– [a]	–– [a]	–– [a]	–– [a]	180
Cu-DTPA	–– [a]	–– [a]	–– [a]	–– [a]	148
Cu-EDTA	–– [a]	–– [a]	–– [a]	–– [a]	47
Cu-GGH	2.72 ± 0.08	–– [a]	–– [a]	–– [a]	1038
Cu-KGHK	–– [a]	–– [a]	–– [a]	–– [a]	1058
Cu-NTA	0.72 ± 0.05	3.46 ± 0.04	–– [a]	–– [a]	215
Background	0.6 ± 0.6	0.3 ± 0.1	0.007 ± 0.003	0.0039 ± 0.0007	

Table SM3. Summary of initial rates of ascorbate consumption, rates of radical generation (TEMPO-9-AC reaction), and reduction potentials for M-chelates.[1,5] Rates listed for reactions with H_2O_2 were the difference between rates for reactions with added H_2O_2 and reactions without added H_2O_2, while rates listed for reactions with O_2 were simply the rates observed without added H_2O_2. Redox couples are 3+/2+ for Fe, Co, Ni-ATCUN, and Cu-ATCUN complexes and 2+/1+ for all other Ni and Cu complexes. [a] Below detection limit. [b] Not determined. These data were used for the x-axis components of Figure SM5, Figure SM6, and Figure SM7 of the Supporting Information.

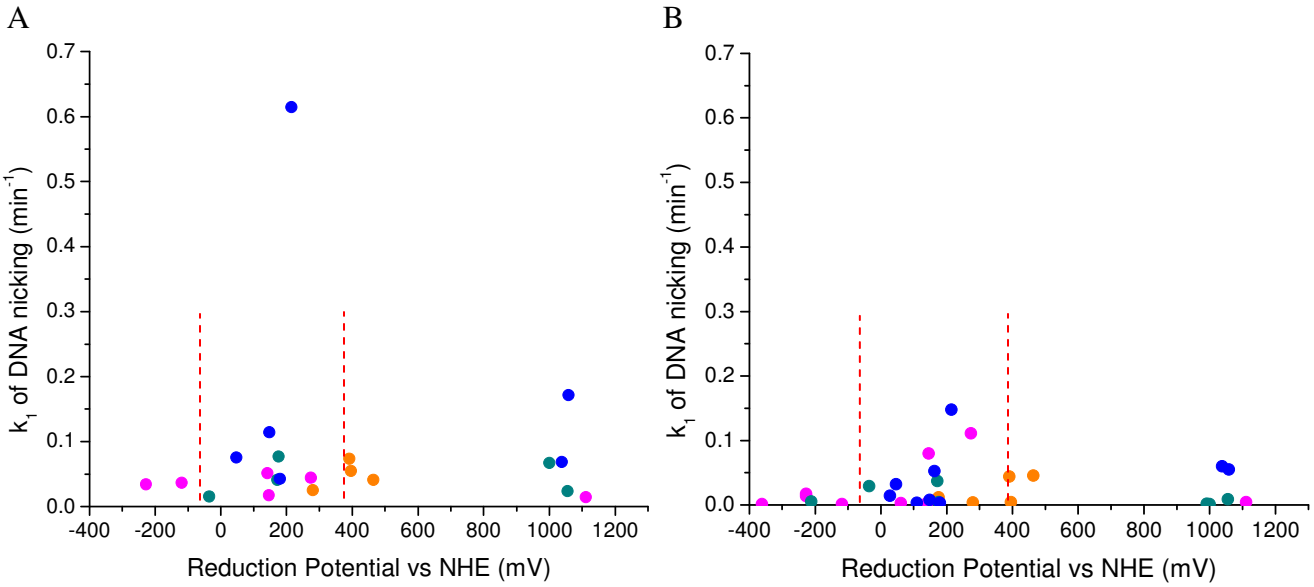

Figure SM5. Rate constants for DNA nicking by (A) M-chelate-Rev complexes (this work) and (B) the respective M-chelates lacking Rev (Joyner et al., 2011),[1] each with co-reactants H_2O_2/ascorbate, were highest for M-chelates with reduction potentials between (low potential region) those of ascorbyl radical/ascorbate (E^o = -66 mV) and H_2O_2/hydroxyl radical (E^o = +380 mV); modest rate constants were observed for reduction potentials near 1000 mV (high potential region). The reduction potentials for the ascorbyl radical/ascorbate (E^o = -66 mV) and H_2O_2/hydroxyl radical (E^o = +380 mV) half reactions are illustrated by the dashed lines. Orange = Fe; pink = Co; cyan/green = Ni; blue = Cu. M-chelate reduction potentials were determined previously.[5]

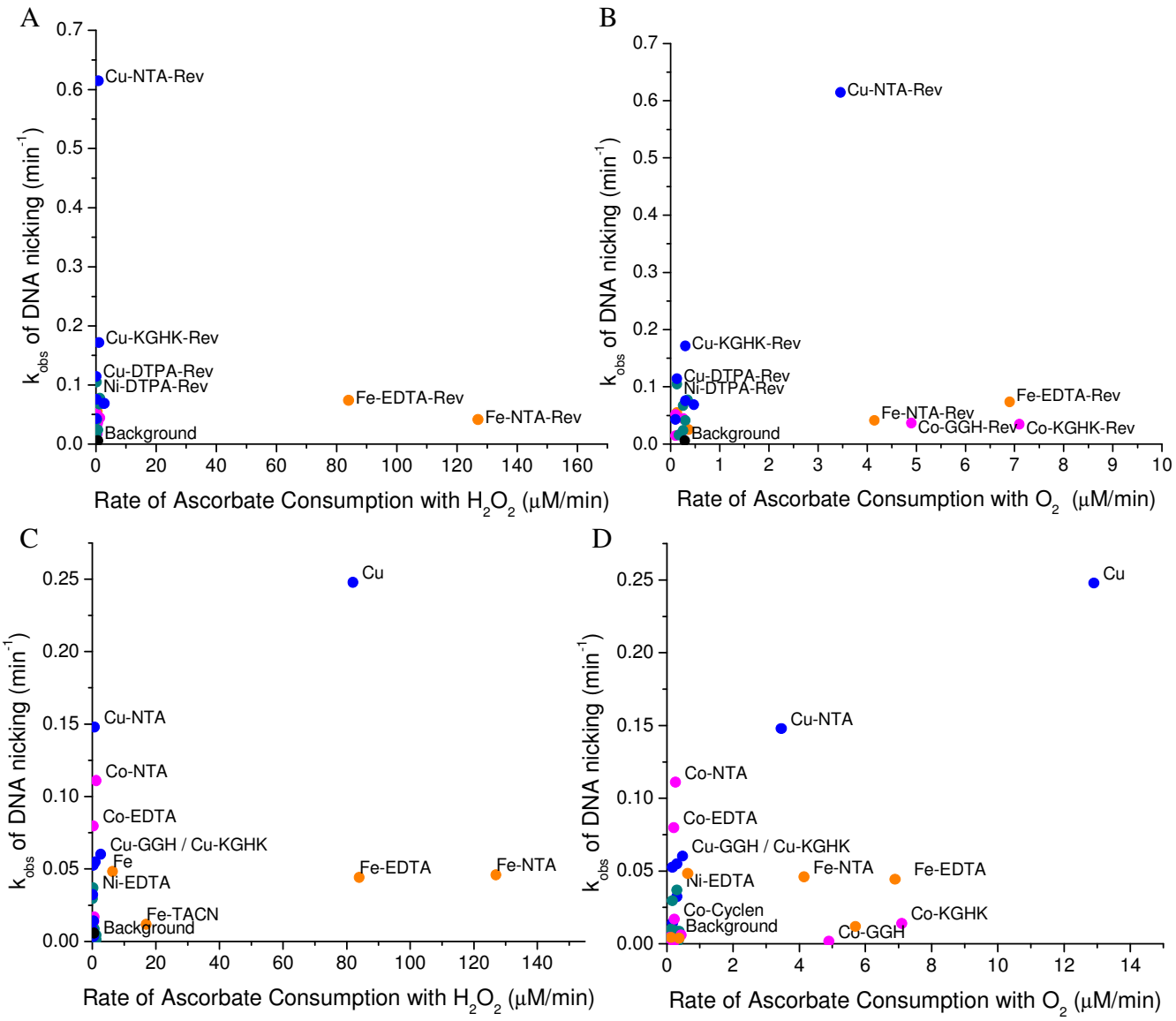

Figure SM6. Relationships between the rate constants for DNA nicking by M-chelate-Rev complexes and the rate for multiple turnover ascorbate consumption by respective M-chelates lacking Rev and with co-oxidants: (A) H_2O_2 and (B) O_2. Relationships between the rate constants for DNA nicking by metal complexes lacking Rev and the rate for multiple turnover ascorbate consumption by the same metal complexes and with co-oxidants: (C) H_2O_2 and (D) O_2. Orange = Fe; pink = Co; cyan/green = Ni; blue = Cu. Initial rates of ascorbate consumption (x-axes of graphs shown here) and rate constants for DNA nicking by M-chelates lacking Rev (y-axes of graphs in C and D) were determined previously.[1]

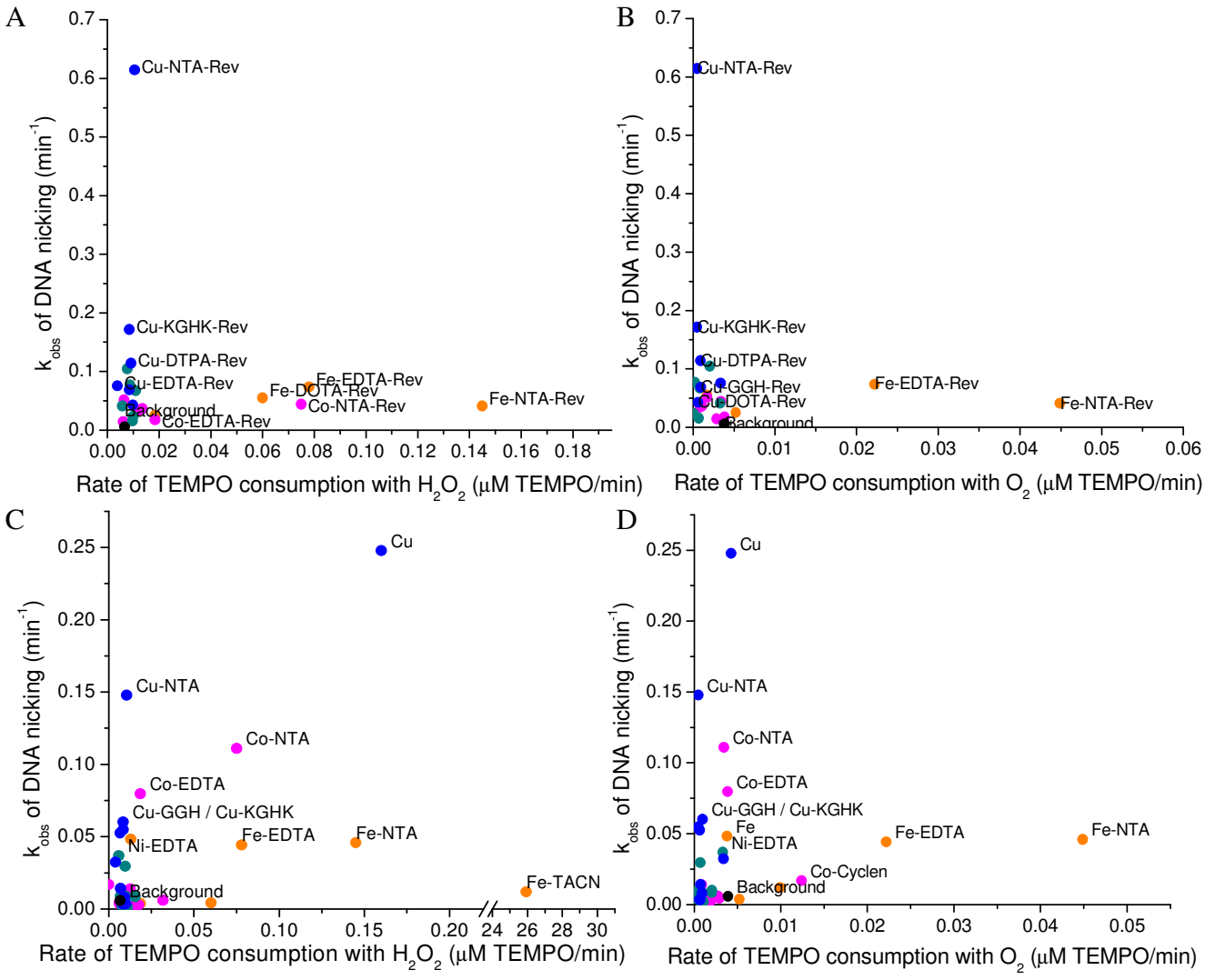

Figure SM7. Relationships between the rate constants for DNA nicking by M-chelate-Rev complexes and the rate of radical generation (TEMPO-9-AC reaction) by respective M-chelates lacking Rev and with co-oxidants: (A) H_2O_2 and (B) O_2. Relationships between the rate constants for DNA nicking by metal complexes lacking Rev and the rate of radical generation by the same metal complexes and with co-oxidants: (C) H_2O_2 and (D) O_2. Orange = Fe; pink = Co; cyan/green = Ni; blue = Cu. Rates of TEMPO-9-AC reaction (x-axes of graphs shown here) and rate constants for DNA nicking by M-chelates lacking Rev (y-axes of graphs in C and D) were determined previously.[1]

SM References

(1) Joyner, J. C.; Reichfield, J.; Cowan, J. A. *J. Am. Chem. Soc.* **2011**, *133*, 15613–15626.
(2) Vivian, J. T.; Callis, P. R. *Biophys. J.* **2001**, *80*, 2093–2109.
(3) Yuan, T.; Weljie, A. M.; Vogel, H. J. *Biochem.* **1998**, *37*, 3187-3195.
(4) Eftink, M. R.; Ghiron, C. A. *Biochem.* **1976**, *15*, 672-680.
(5) Joyner, J. C.; Cowan, J. A. *J. Am. Chem. Soc.* **2011**, *133*, 9912-9922.