Electronic Supplementary Material (ESI) for Dalton Transactions.
This journal is © The Royal Society of Chemistry 2014

Supplemental Data

Bifunctional Zn(II) Complexes for Recognition of Non-Canonical Thymines in DNA Bulges and G-Quadruplexes

*Kevin E. Siters,† Stephanie A. Sander,† Jason Devlin and Janet R. Morrow**

Department of Chemistry, University at Buffalo, State University of New York, Buffalo, New York 14260, United States

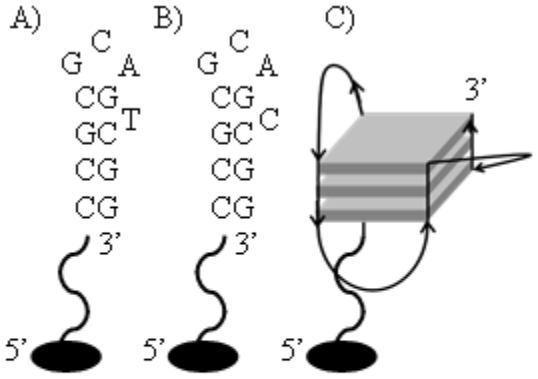
† contributed equally to manuscript

EXPERIMENTAL METHODS

The oligonucleotides that formed the duplex or hairpin structures were dissolved in 100 mM NaCl. H-Telo was dissolved in 0.10 M KCl. Solutions of DNA were annealed by heating solutions up to 70-75 °C for 5-10 minutes, followed by slow cooling (8-12 h) to room temperature. The oligonucleotide concentrations were determined by UV-Vis spectroscopy at 25 °C using a molar extinction coefficient provided for each oligonucleotide at 260 nm. Solutions of Zn(DSC) and Zn(MCC) were standardized by using their extinction coefficients of 5,558 L mol^{-1} cm^{-1} at 335 nm and 8,770 L mol^{-1} cm^{-1} at 314 nm with 17,260 L mol^{-1} cm^{-1} at 275 nm, respectively.

Surface Plasmon Resonance Measurements

The binding affinity of Zn(II) complexes to T-Bulge, C-Bulge and H-Telo G-quadruplex (Scheme 3) was determined. Streptavidin was immobilized via standard amine coupling of EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) and NHS (N-hydroxy succinimide) onto both channels of a Carboxymethyl Dextran sensor chip. The Biotinylated DNA samples were captured over the sample channel at a concentration of 200 μM in running buffer. The surface was then blocked over both channels by injecting biotin for 2 minutes. Sensorgrams were collected by injecting dilutions of Zn(II) complexes in 0.10 M NaCl (for H-Telo 0.10 M KCl), 0.020 M HEPES, pH 7.5 and 0.05% Tween. The samples were flowed over the sample and reference channels at 25 μl/minute. Data overlays were performed using Scrubber 2.0a (Biologic Software Pty, Australia). The data were double referenced using the reference channel along with buffer blank injections. Kinetic data were fit to a 1:1 binding model. Langmuir isotherms were also obtained and fit either to a 1:1 binding model or a 2-site model.



Scheme S1 5' Biontinlyated DNA used for SPR:
A) Thymine Bulge DNA (5'-Biotin-dCCGCGCAGTCGG 3')
B) Cytosine Bulge DNA (5'-Biotin-dCCGCGCAGCCGG 3')
C) Telomeric G-Quadruplex (5'-Biotin-$dA(G_3T_2A)_3G_3$)

Isothermal Calorimetry

10 - 20 μM oligonucleotide solutions were prepared in 0.020 M HEPES (pH 7.5) and 0.10 M NaCl (or 0.10 M KCl for G-quadruplexes). Solutions of Zn(DSC), Zn(MCC), Zn(QMC), Zn(CQC) and Zn(ATQ) were prepared in double distilled H_2O or 0.020 M HEPES (pH 7.5) and 0.10 M mM NaCl (0.10 M KCl for G-Quadruplex DNA), depending on the solubility of the complex. 37 to 44 injections were used for studies using G-quadruplex DNA and for hairpin DNA. Injections of 4 - 7 μL were made every 225 to 300 seconds at a rate of 2 seconds per μl to a solution of DNA, stirring at 116 rpm, at 25 °C. A blank run was also conducted under identical conditions to correct for heat signatures observed for the solvation of the complex into the buffer if needed. The resulting data was fit by using Origin software provided by MicroCal. The heat in μcal/second was integrated and subtracted against the blank run. The resulting data was fit by using a single type site binding equation (Eq. S17) or sequential binding equation (Eq. S18).

pH Potentiometric Titrations

5 μL or 10 μL of 0.1034 M NaOH was titrated into an aqueous solution of 1.00 mM solution of QMC or CQC macrocycle and 1.00 mM $ZnCl_2$ (I = 0.10 M NaCl, 25°C). Changes in pH were monitored from approximately pH 4 to pH 9. The 5 μL or 10 μL aliquots of base were added every 120 seconds. The program HYPERQUAD 2008 was used to determine the protonation states and formation constants of the complexes from the pH data. Speciation diagrams were obtained by using the program HYSS.

Optical Thermal Melting

Aqueous solutions of 2.2 μM of H-telo were prepared in 0.02 M HEPES (pH 7.5) and 0.10 M KCl, with 1 or 2 equivalents of Zn(II) complex, for optical thermal melting studies. Absorbance readings at 260 nm were taken every minute as the temperature was raised at a rate of 0.5 °C/min. Melting temperatures (T_m) were obtained by fitting the data to the Meltwin 3.5 program available at http://www.meltwin3.com.

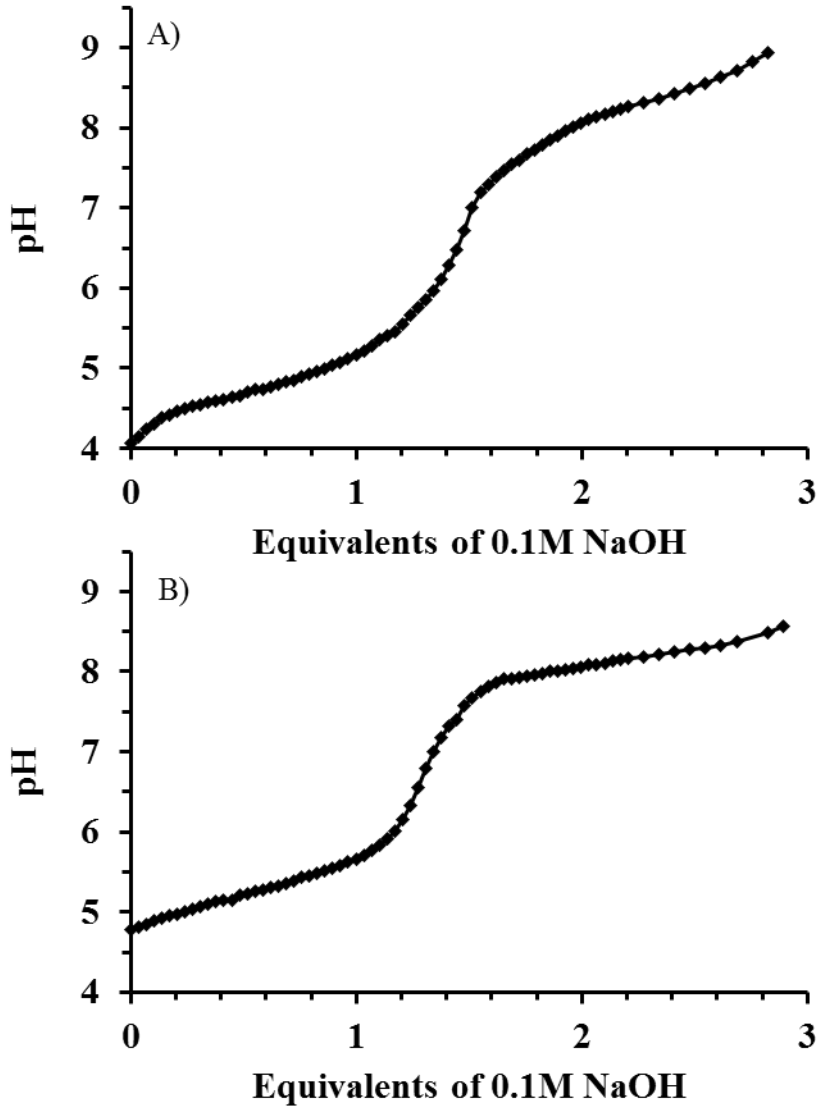


Figure S1a. pH-potentiometric titration of equimolar concentrations of a solution of 1.0 mM A) QMC and B) CQC complexes with Zn(II) at 25°C in 0.10 M NaCl.

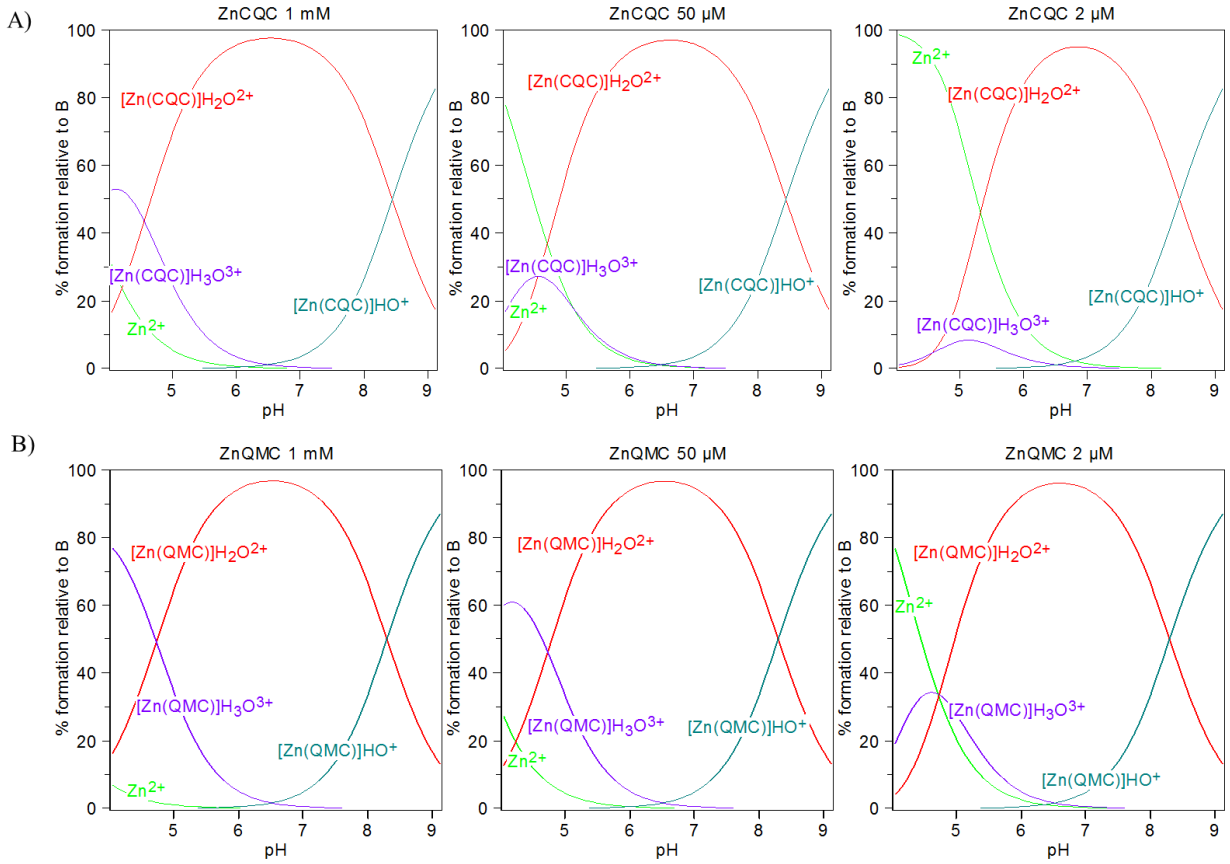


Figure S1b. Speciation diagram for solutions containing equimolar amounts of A) CQC B) QMC and $ZnCl_2$ in 0.10 M NaCl, 25 °C at 1.00 mM, 50 µM and 2 µM metal ion and ligand.

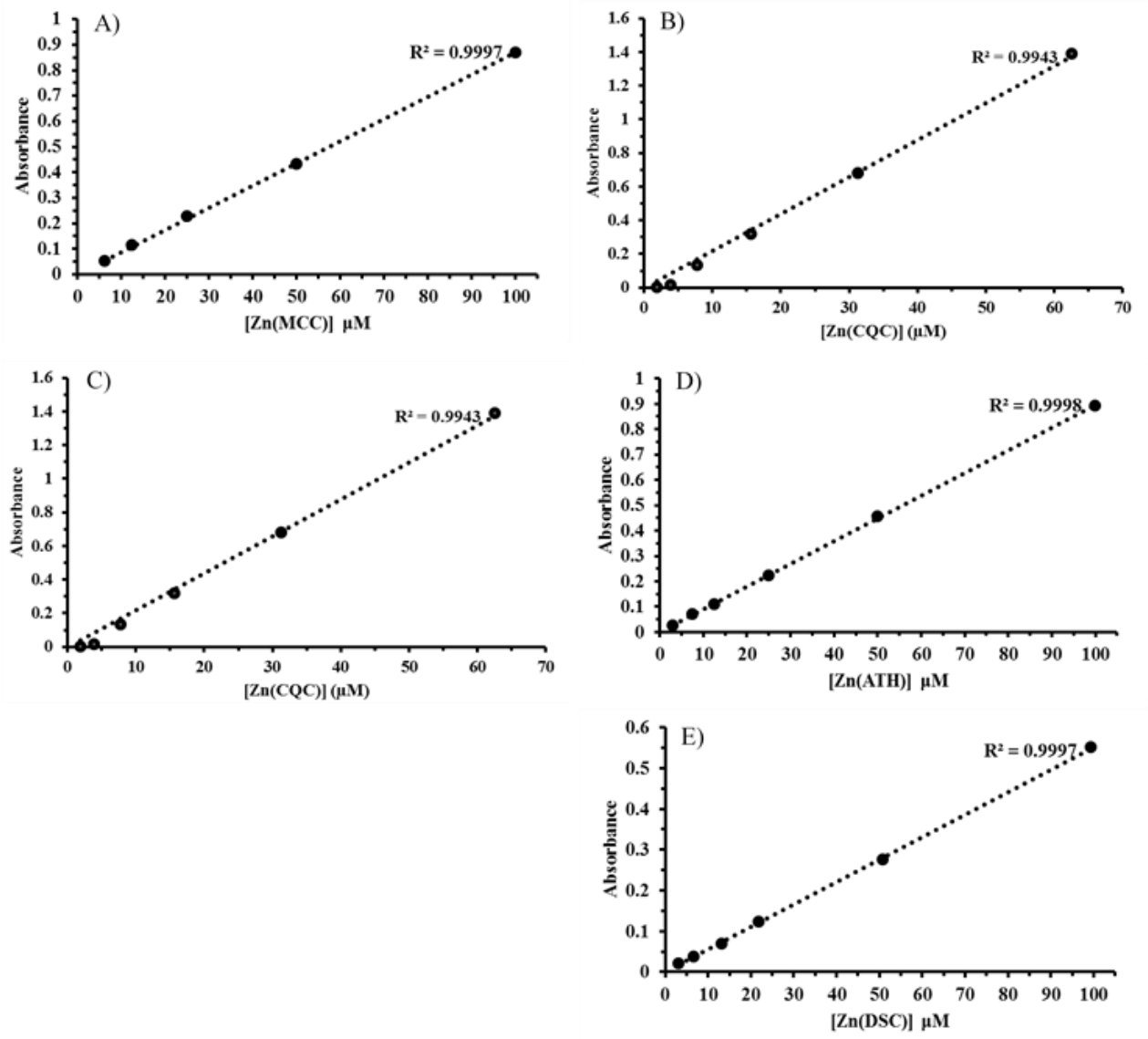


Figure S2. Plots of concentration vs. absorbance for solutions of A) Zn(MCC), B) Zn(QMC), C) Zn(CQC), D) Zn(ATQ) and Zn(DSC), in 0.10 M NaCl and 0.020 M HEPES (pH 7.5).

Table S1. Ligand protonation constants (Log K) and Zn(II) binding constants (log K_{Zn}) at 25°C in 1.00 mM NaCl. See Eqs. S1-S16 for reference.

Equilibria	QMC	CQC
Log K_1	6.77	5.56
Log K_2	9.57	10.81
Log K_3	4.31	3.97
Log K_4	2.35	2.11
Log K_{ZnL}	12.03	10.59
Log K_{ZnLH}	4.75	4.56
pK_a (ZnL-OH_2)	8.3	8.44

Table S2. T-Bulge rate constants from SPR

Complex	k_a	k_d	K_d (μM)
Zn(CQC)	3170 ± 354	0.0.0961 ± 0.02	30.4 ± 1.8
Zn(QMC)	5595 ± 672	0.18 ± 0.03	38.6 ± 1.2
Zn(MCC)	3449 ± 793	0.14 ± 0.02	31.5 ± 1.0
Zn(CYC)	1000	.096	NB[a]
Zn(DSC)	649 ± 627	0.49 ± 0.25	1100 ± 400

[a]Binding was too weak to accurately determine K_d

Table S3. H-Telo rate constants from SPR

Complex	k_a	k_d	K_d (μM)
Zn(CQC)	3695 ± 525	0.0232 ± 0.0034	6.5 ± 1.9
Zn(QMC)	3120 ± 260	0.0844 ± 0.0018	27.2 ± 1.7
Zn(MCC)	4855 ± 245	0.0564 ± 0.0184	11.4 ± 3.1
Zn(CYC)	286	0.7273	2.54 mM
Zn(DSC)	1180 ± 110	0.1019 ± 0.0105	86.0 ± 1.0

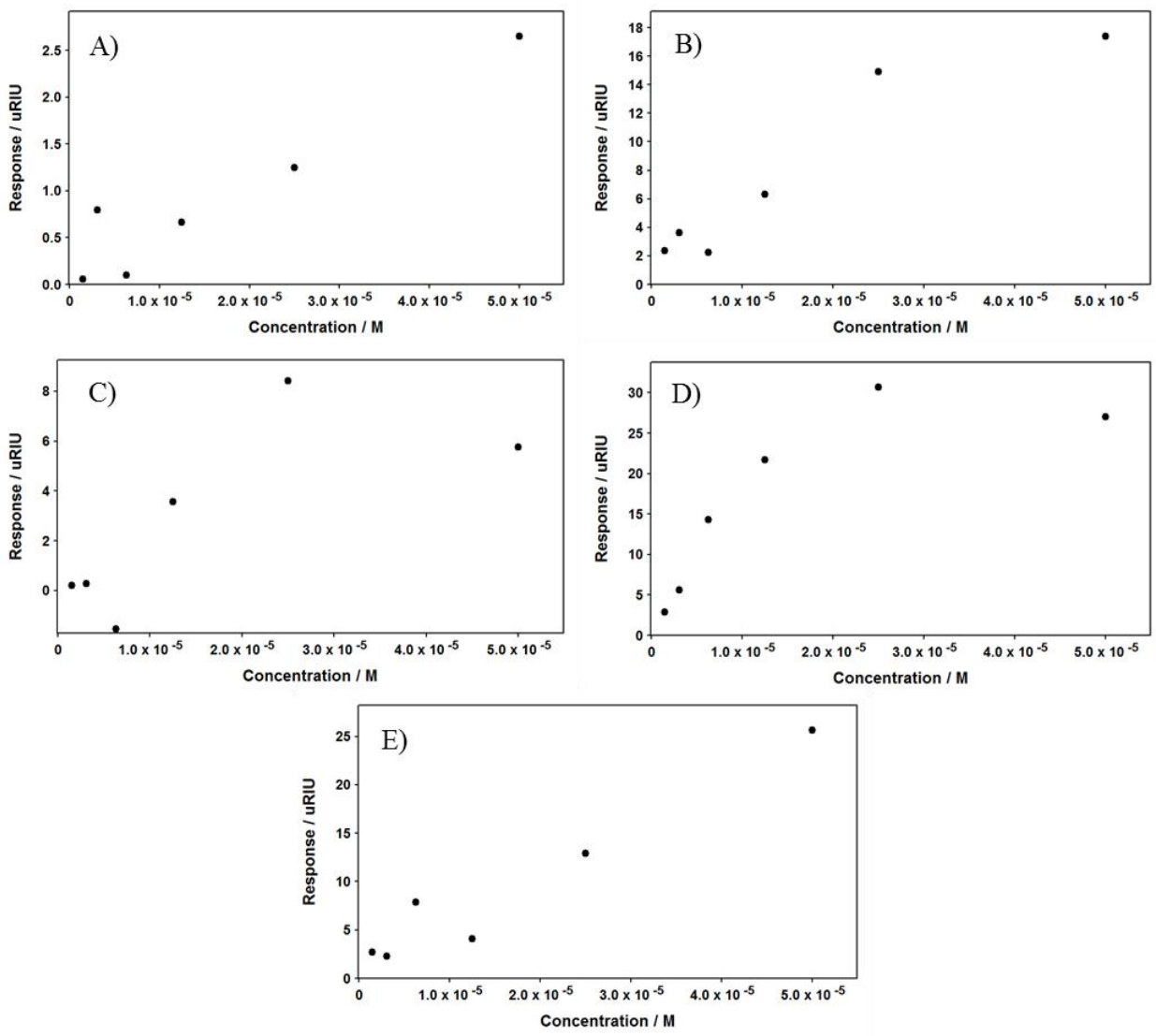


Figure S3. Response of Zn(II) complexes to immobilized C-bulge A) Zn(CYC), B) Zn(MCC), C) Zn(MQC), D) Zn(CQC) and E) Zn(DSC). All samples were prepared with 0.10 M NaCl, 0.020 M HEPES, pH 7.5, 0.05 % Tween 20.

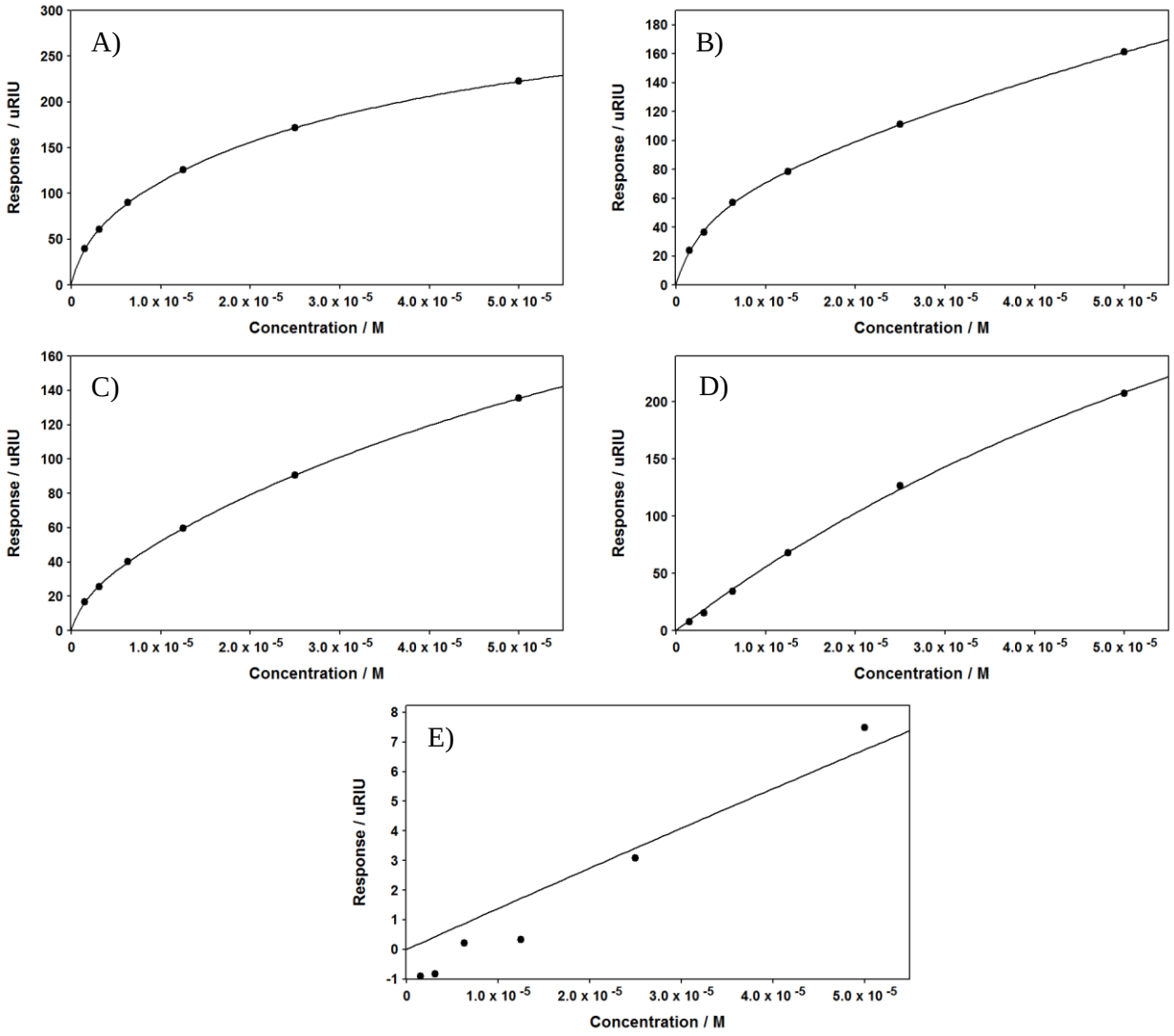


Figure S4. H-Telo Binding curves from SPR studies A) Zn(CQC) B) Zn(MQC) C) Zn(MCC) D) Zn(DSC) E) Zn(CYC). Concentration of Zn(II) complex ranged from 1.5 µM – 50 µM. All samples were prepared in 0.10 M KCl, 0.020 M HEPES (pH 7.5) and 0.05% Tween 20.

Table S4. Apparent Dissociation constants ($K_{d\ (app)}$ X 10^{-6} M) of Zn(II) complexes to H-Telo using multisite binding equations.

Complex	$K_{d\ (app)}$ by SPR[1]	$K_{d\ (app)}$ by ITC[2]
Zn(CQC)	2.2 ± 0.3; 55.1 ± 17.1	9.7 ± 4.3; 1.3 ± 0.1
Zn(QMC)	2.8 ± 0.6; 23 ± 3.0	24.7 ± 6.0; 3.9 ± 1.4
Zn(MCC)	2.4 ± 0.5; 118.5 ± 21.5	32.9 ± 16.3; 2.3 ± 1.4
Zn(DSC)	Weak	6.3 ± 5.6; 13.6 ± 4.6[3]
Zn(ATQ)	nd[4]	29.0 ± 13.6; 160.3 ± 207; 7.4 ± 9.4

[1] Apparent dissociation constants of Zn(II) complexes binding to immobilized H-Telo in 100 mM KCl, 20 mM HEPES (pH 7.5) and 0.05% Tween 20 at 25 °C. [2] Apparent dissociation constants of Zn^{2+}complexes binding to a solution of H-Telo in 100 mM KCl and 20 mM HEPES (pH 7.5) at 25 °C. [3] From reported work (Ref 1). [4] Not determined using SPR.

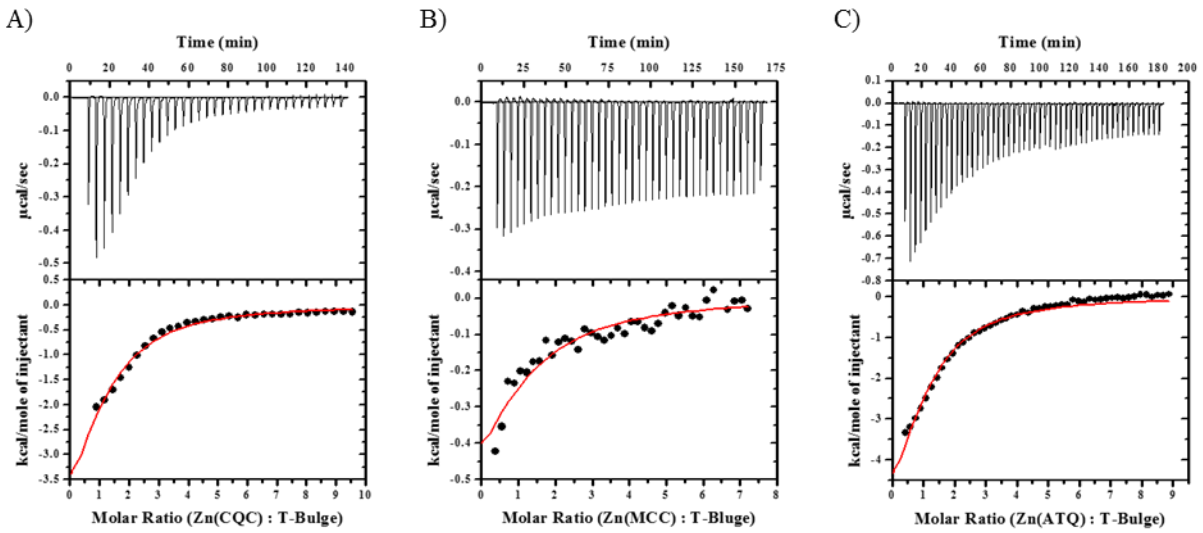


Figure S5. (Top) Isothermal Calorimetric Plots for titration of: A) Zn(CQC), B) Zn(MCC) and C) Zn(ATQ) into 20 µM T-Bulge, in 100 mM NaCl and 20 mM HEPES (pH: 7.5). (Bottom) Plots of heat evolved (kcal/mol) versus molar ratio (Complex: T-Bulge) fit to a single-site binding mode.

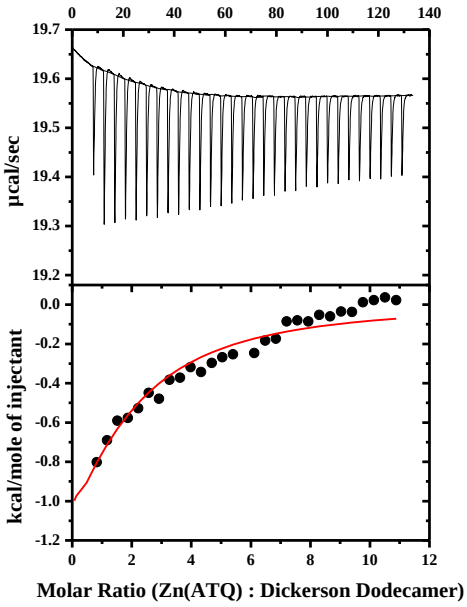


Figure S6. (Top) Isothermal Calorimetric Plots for titration of Zn(ATQ) into 20 µM Dickerson Dodecamer, in 100 mM NaCl and 20 mM HEPES (pH: 7.5). (Bottom) Plots of heat evolved (kcal/mol) versus molar ratio (Complex: Dickerson Dodacemer) fit to an equivalent site binding equation (S17).

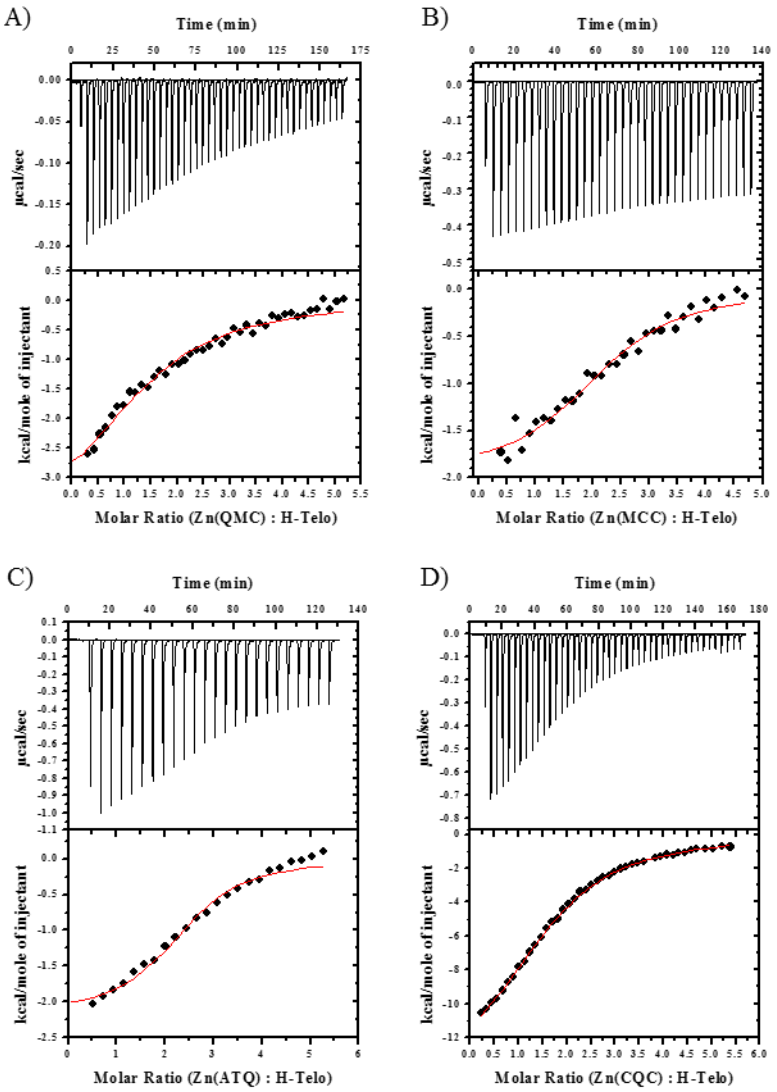


Figure S7. (Top) Isothermal Calorimetric Plots for titration of: A) Zn(QMC), B) Zn(MCC), C) Zn(ATQ), D) Zn(CQC) into 20 μM H-Telo, in 100 mM KCl and 20 mM HEPES (pH: 7.5). (Bottom) Plots of heat evolved (kcal/mol) versus molar ratio (Complex: H-Telo) fit to an equivalent site binding model (Eq. S17).

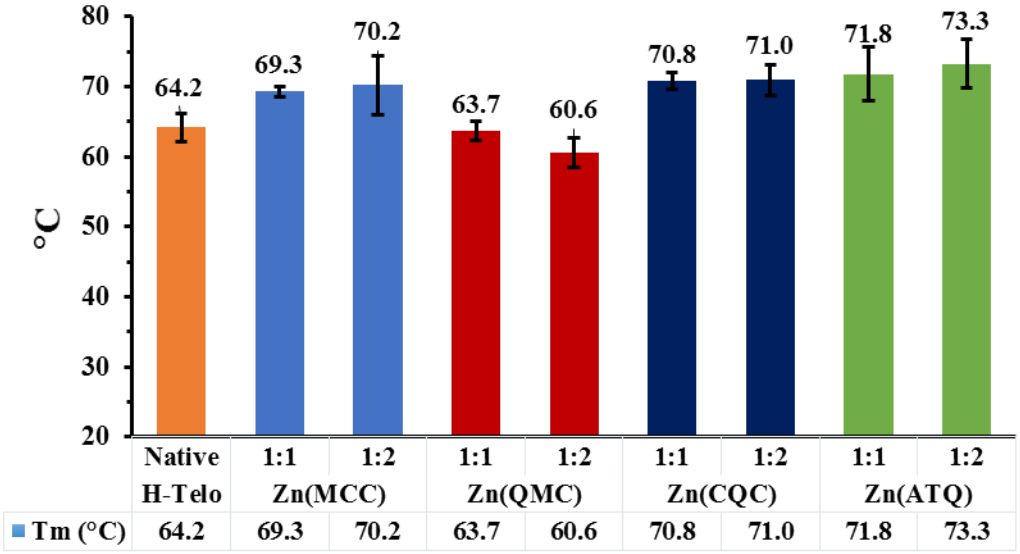


Figure S8. Thermal melting temperatures of 2.2 µM H-Telo solutions, in 0.10 M KCl and 0.20 M HEPES (pH 7.5), containing 1 and 2 equivalents of Zn(II) complex.

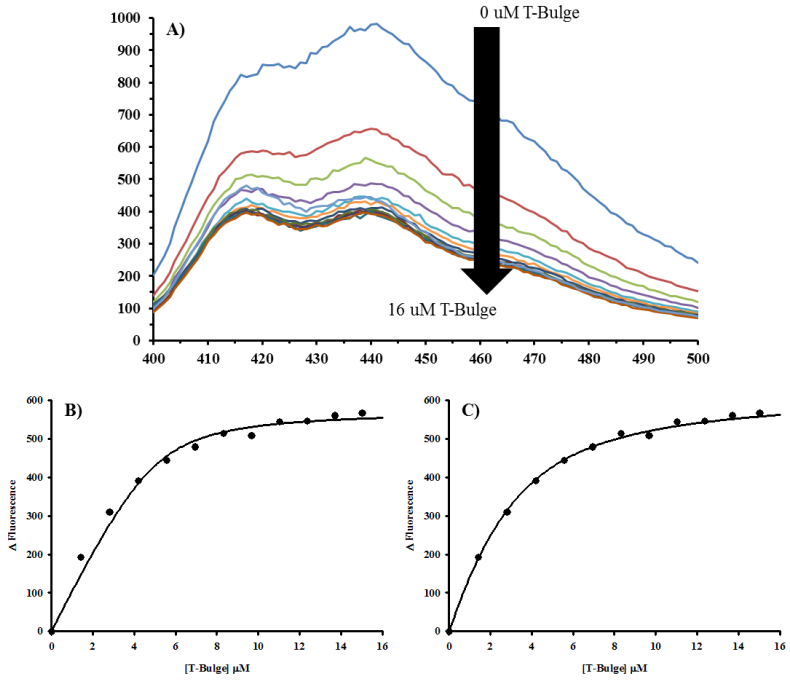


Figure S9: A) Representative fluorescence spectra of a solution containing 5 µM Zn(ACR) titrated with T-Bulge in 100 mM NaCl and 20 mM HEPES (pH 7.5) at 25 °C. B-C) Representative binding isotherms showing a plot of the change fluorescence intensity of a solution containing 5 µM Zn(ACR) at 435 nm using multiple values for stoichometry: n; B) n=1 ($K_{d(app)}$: <1 µM) and C) n=2 ($K_{d(app)}$: 2.2 µM)

The following equilibria are used in the fitting of pH potentiometric data. L = QMC or CQC

Eq.S1 $L + H^+ \leftrightarrows LH^+$

Eq. S2 $K_1 = [LH^+]/([L][H^+])$

Eq. S3 $LH^+ + H^+ \leftrightarrows LH_2^{2+}$

Eq. S4 $K_2 = [LH_2^{2+}]/([LH^+][H^+])$

Eq. S5 $LH_2^{2+} + H^+ \leftrightarrows LH_3^{3+}$

Eq. S6 $K_3 = [LH_3^{3+}]/([\ LH_2^{2+}][H^+])$

Eq. S7 $LH_3^{3+} + H^+ \leftrightarrows LH_4^{4+}$

Eq. S8 $K_4 = [LH_4^{4+}]/([\ LH_3^{3+}][H^+])$

Eq. S9 $LH_4^{4+} + H^+ \leftrightarrows LH_5^{5+}$

Eq. S10 $K_5 = [LH_5^{5+}]/([\ LH_4^{4+}][H^+])$

Eq. S11 $L + Zn^{2+} \leftrightarrows ZnL^{2+}$

Eq. S12 $K_{ZnL} = [ZnL^{2+}]/([\ Zn^{2+}][L])$

Eq. S13 $ZnL^{2+} + H^+ \leftrightarrows ZnLH^{3+}$

Eq. S14 $K_{ZnLH} = [ZnLH^{3+}]/([\ ZnL^{2+}][H^+])$

Eq. S15 $ZnL(H_2O)^{2+} + OH^- \leftrightarrows ZnLH(OH)^+ + H_2O$

Eq. S16 $K_{ZnLOH} = [ZnL(OH)^+]/([OH^-][\ ZnL(H_2O)^{2+}])$

The following equations were used to determine Zn(II) complex binding by ITC.

$$Q = n\Theta M_t \Delta H V_o$$

$$Q = \frac{nM_t\Delta HV_0}{2}\left[1 + \frac{X_t}{nM_t} + \frac{1}{nKM_t} - \sqrt{\left(1 + \frac{X_t}{nM_t} + \frac{1}{nKM_t}\right)^2 - \frac{4X_t}{nM_t}}\right]$$

Equation S17: Equivalent site binding equation used to determine Zn(II) complex binding with ITC.

$$F_n = \frac{K_1 K_2 \ldots K_n [X]^n}{P}$$

$$P = 1 + K_1[X] + K_1K_2[X]^2 + \ldots + K_1K_2 \ldots K_n[X]^n$$

$$F_1 = \frac{K_1[X]}{P}$$

$$P = 1 + K_1[X]$$

$$F_2 = \frac{K_1K_2[X]^2}{P}$$

$$P = 1 + K_1[X] + K_1K_2[X]^2$$

$$Q = M_t V_0 (F_1 \Delta H_1 + F_2[\Delta H_1 + \Delta H_2] + \cdots + F_n[\Delta H_1 + \Delta H_2 + \Delta H_3 + \cdots + \Delta H_n])$$

Equation S18: Sequential site binding mode equation used to determine Zn(II) complex binding with ITC.

References

1. K. E. Siters, M. A. Fountain and J. R. Morrow, *Inorg. Chem.*, 2014, **53**, 11540-11551.