SUPPLEMENTARY INFORMATION

Improved DNA Equilibrium Binding Affinity Determinations of Platinum(II) Complexes using Synchrotron Radiation Circular Dichroism

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Input Data File CSV format

The *Mathematica* notebook accepts a standard CSV file; the path of which must be specified within the notebook. The format is as follows. Cell A1 contains the DNA concentration [M]. The first column contains the wavelengths. Subsequent columns contain the blank/buffer, DNA, followed by the DNA + MC data at the various concentrations.

An example is shown below.

[DNA conc]				
	Blank/buffer	DNA, no MC	DNA + MC conc 1	DNA + MC conc 2
Wavelength 1				
Wavelength 2				
Wavelength 3				
Wavelength 4				

0.000035				
	0	0	0.00000166	0.00000332
400	0.366	0.475	0.397	0.415
399	0.355	0.442	0.426	0.467
398	0.346	0.455	0.412	0.437
397	0.345	0.457	0.443	0.441
396	0.384	0.511	0.418	0.416

Equation Derivation

In what follows we use the following labels for description: L_F (free metal complex concentration), S_F (free binding site concentration), L_B (bound metal complex concentration), S_B (bound/occupied binding site concentration), L_T (total metal complex concentration) and S_T (total binding site concentration).

In the original development of the theory the binding equilibrium rate equation is given by,

$$L_F + S_F \underset{K}{\leftrightarrow} L_B$$

Giving generally,

$$K = \frac{L_B}{L_F \times S_F} \qquad (1)$$

The value of K is assumed to be a constant at a given wavelength and independent of L_B. Substituting for $L_F = L_T - L_B$ and $S_F = S_T - S_B$ into Eq. (1) and making a reasonable assumption that $S_B = L_B$, results in a quadratic equation for L_B which has a solution of

$$L_B = 0.5R\left(\frac{1}{K} + L_T + S_T - \sqrt{\left(\frac{1}{K} + L_T + S_T\right)^2 - 4S_T L_T}\right) \qquad (2)$$

where R is a scaling constant, and L_B is directly proportional to the measured normalized molar absorption coefficient, ε_M . The value of K can be found by fitting the titration data directly in Eq. (2), which is the preferred approach.[1] The number of DNA binding sites, n, is related to the total DNA concentration and calculated where n = [DNA]/S_T.

In the literature, however, Scatchard,[2] Schmechel and Crothers[3] and others[4-6] have attempted to linearize Eq. (1) by approximation. Substituting the above values in equation (1) gives

$$K = \frac{L_B}{(L_T - L_B)(S_T - L_B)} \qquad (3)$$

A rearrangement results in

$$\frac{1}{L_B} = \frac{1}{K(L_T - L_B)L_T} + \frac{1}{L_T} \qquad (4)$$

At this point a further assumption is made that at low total ligand concentration $L_B \rightarrow L_T$ on the RHS of Eq. (4). This implies that by this method K is an extrapolation as $L_B \rightarrow 0$; this is at best artificial because K is determined when there is effectively no binding. Such an assumption is not made by Eq. (2). In the literature

Eq. (4) leads to a more traditional form in order to determine *K* by using ε_M (measured absorbance), ε_B (bound absorbance), ε_F (free absorbance), and the identity

$$\frac{1}{L_B} = \frac{(\varepsilon_B - \varepsilon_F)}{(\varepsilon_M - \varepsilon_F)} \frac{1}{L_T}$$

To obtain

$$\frac{1}{\varepsilon_M - \varepsilon_F} = \frac{1}{K(\varepsilon_B - \varepsilon_F)} \times \frac{1}{(S_T - L_B)} + \frac{1}{(\varepsilon_B - \varepsilon_F)} \quad (5)$$

Plotting the experimentally derived $\varepsilon_M - \varepsilon_B$ versus $S_T - L_B$ yields a value of slope and intercept from which *K*, again extrapolated to $L_B \rightarrow 0$, is found. If the plot is not a straight line (as mathematically it is not), it is argued that a value of *K* can be found from the initial slope of the graph. Both approaches determine artificially the binding constant at very low bound ligand concentrations.

Binding Data obtained from SRCD experiments

Table S1 Complex **1**, $[Pt(phen)(en)]^{2+}$ binding data, experiment B.

Wavelength nm	Binding Constant $K \times 10^5$	Estimated Binding sites per complex, n
297	1.1 ± 0.54	2.4 ± 0.1
320	5.8 ± 0.40	4.3 ± 0.2

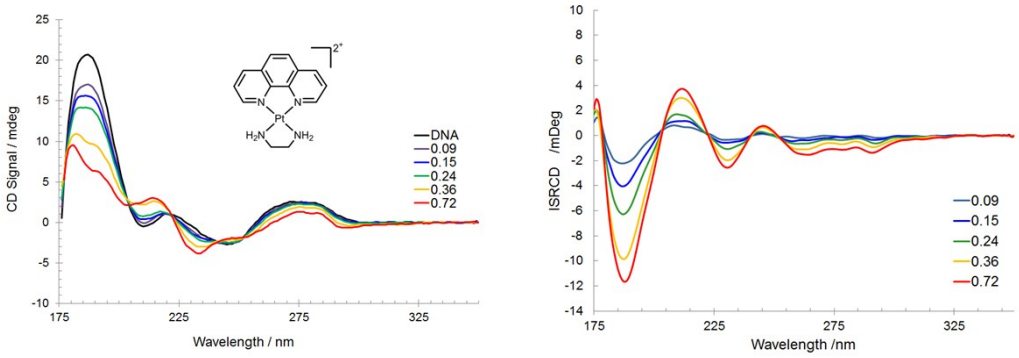

Figure S1 **Expt B –** SRCD and ISRCD spectra at different concentrations of metal complex **1**, into calf thymus DNA in PS buffer.

Table S2 Complex **2**, $[Pt(4\text{-Mephen})(en)]^{2+}$ binding data, experiment A.

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
181	2.14 ± 0.84	2.6 ± 0.1
185	0.76 ± 0.09	3.6 ± 0.1
186	1.08 ± 0.15	3.3 ± 0.1
190	1.75 ± 0.25	2.8 ± 0.1
192	1.79 ± 0.14	2.4 ± 0.1
194	3.37 ± 0.95	2.1 ± 0.1
195	1.46 ± 0.21	2.1 ± 0.5

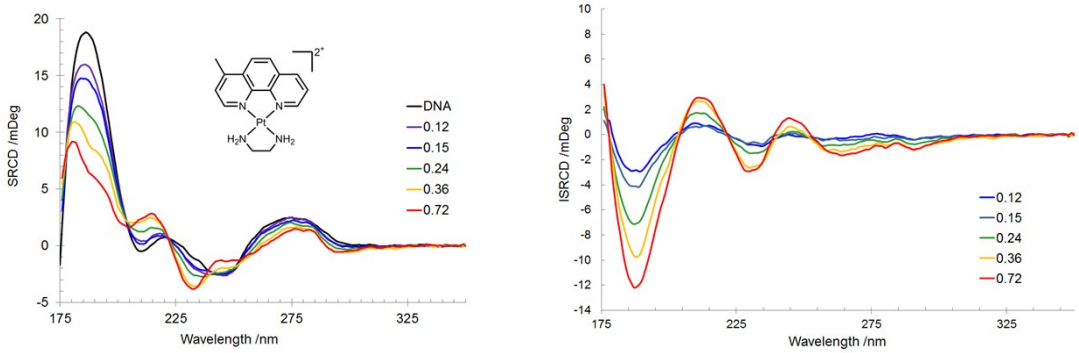

Figure S2 Expt A – SRCD and ISRCD spectra at different concentrations of metal complex **2**, into ct-DNA in PS buffer.

Table S3 Complex **2**, $[Pt(4\text{-Mephen})(en)]^{2+}$ binding data, experiment B.

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
177	11.6 ± 3.0	2.4 ± 0.2
184	0.95 ± 0.52	1.6 ± 0.0
186	1.8 ± 0.74	1.7 ± 0.1
192	3.4 ± 0.52	1.6 ± 0.0
194	6.3 ± 3.5	1.5 ± 0.0

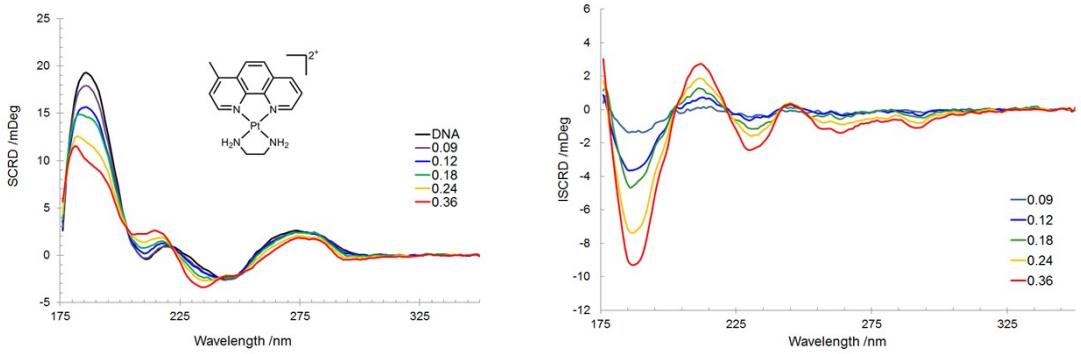

Figure S3 Expt B - SRCD and ISRCD spectra at different concentrations of metal complex **2**, into ct-DNA in PS buffer.

Table S4 Complex **3**, $[Pt(5\text{-Mephen})(en)]^{2+}$ binding data, experiment A.

Wavelength nm	Binding Constant $K \times 10^5$	Estimated Binding sites per complex, n
192	2.33 ± 0.18	2.2 ± 0.0
193	3.34 ± 0.22	2.2 ± 0.0
221	1.32 ± 0.78	6.5 ± 1.0

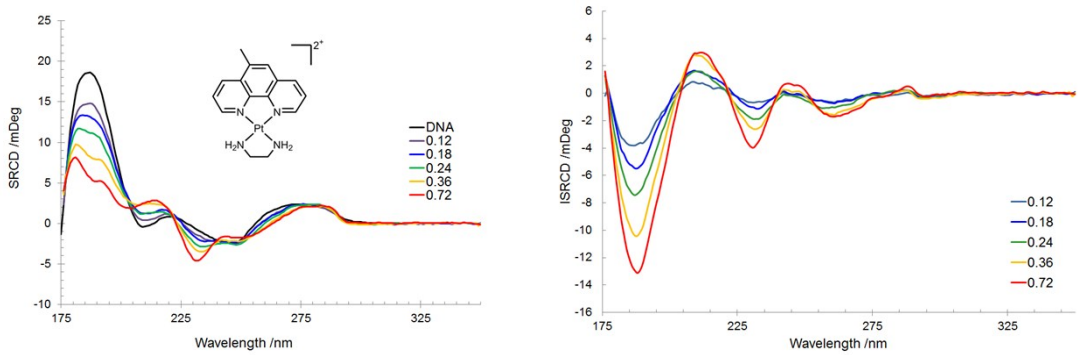

Figure S4 Expt A - SRCD and ISRCD spectra at different concentrations of metal complex **3**, into ct-DNA in PS buffer.

Table S5 Complex **3**, $[Pt(5\text{-Mephen})(en)]^{2+}$ binding data, experiment B.

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
181	4.5 ± 1.9	2.5 ± 0.1
185	5.8 ± 1.2	2.5 ± 0.1
191	8.5 ± 0.73	2.4 ± 0.1
208	5.2 ± 1.2	4.0 ± 0.2
210	5.6 ± 0.87	3.6 ± 0.2
213	2.7 ± 0.86	3.9 ± 0.1
214	2.2 ± 0.42	4.0 ± 0.2
215	2.4 ± 0.75	3.8 ± 0.1
218	1.2 ± 0.47	6.0 ± 0.2
279	0.46 ± 0.10	1.9 ± 0.0
330	7.0 ± 0.78	3.9 ± 0.3

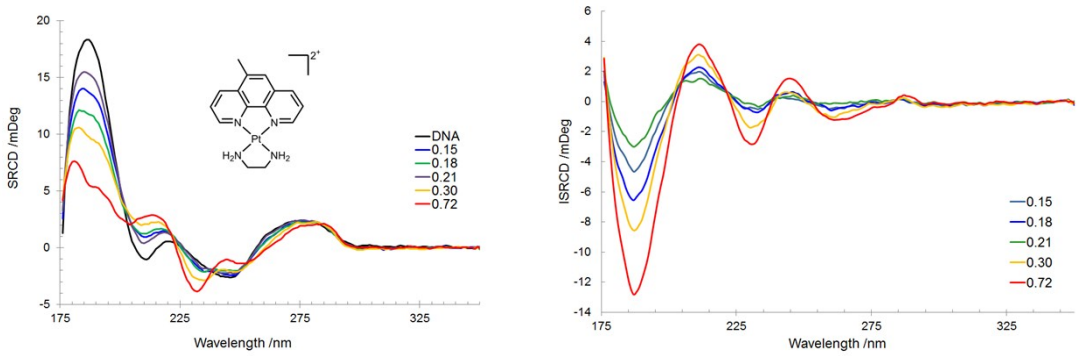

Figure S5 Expt B - SRCD and ISRCD spectra at different concentrations of metal complex **3**, into ct-DNA in PS buffer.

Table S6 Complex **4**, $[Pt(4,7\text{-}Me_2phen)(en)]^{2+}$ binding data, experiment A.

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
194	4.7 ± 0.61	2.6 ± 0.2
195	3.4 ± 0.70	2.4 ± 0.1
199	0.35 ± 0.09	1.9 ± 0.0
307	33 ± 1.5	2.4 ± 0.1
312	24 ± 1.4	2.2 ± 0.1
336	1.4 ± 0.2	2.1 ± 0.1

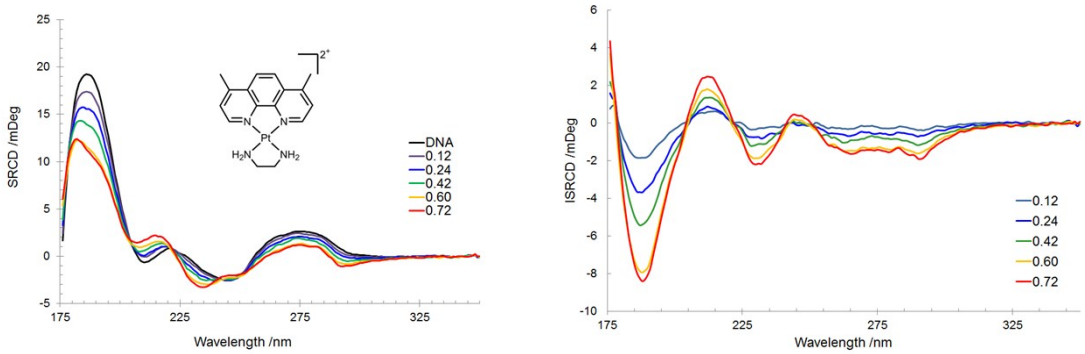

Figure S6 Expt A - SRCD and ISRCD spectra at different concentrations of metal complex 4, into ct-DNA in PS buffer.

Table S7 Complex **4**, $[Pt(4,7\text{-}Me_2phen)(en)]^{2+}$ binding data, experiment B.

Wavelength nm	Binding Constant $K \times 10^5$	Estimated Binding sites per complex, n
184	3.5 ± 0.28	2.0 ± 0.1
209	1.9 ± 0.12	3.2 ± 0.1
311	8.6 ± 0.76	3.6 ± 0.2

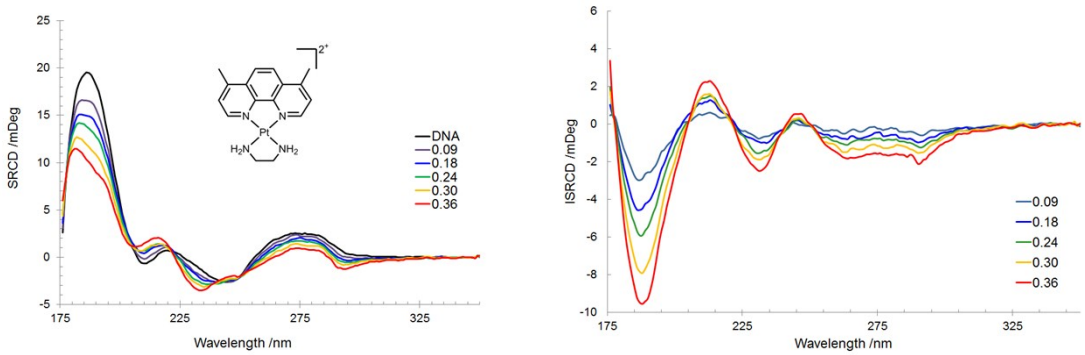

Figure S7 Expt B - SRCD and ISRCD spectra at different concentrations of metal complex 4, into calf thymus DNA in PS buffer.

Table S8 Complex **5**, $[Pt(5,6\text{-}Me_2phen)(en)]^{2+}$ binding data, experiment A.

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
207	13.4 ± 0.96	3.3 ± 0.1
210	6.9 ± 0.41	3.2 ± 0.0
212	5.6 ± 0.33	3.1 ± 0.0
214	7.3 ± 0.27	3.3 ± 0.0
215	9.1 ± 0.52	3.3 ± 0.0
216	9.8 ± 0.34	3.2 ± 0.0
220	3.3 ± 0.21	4.8 ± 0.1
241	0.53 ± 0.10	1.6 ± 0.0
242	2.0 ± 0.35	2.0 ± 0.1
298	2.2 ± 0.22	2.5 ± 0.1
300	0.81 ± 0.20	2.8 ± 0.1

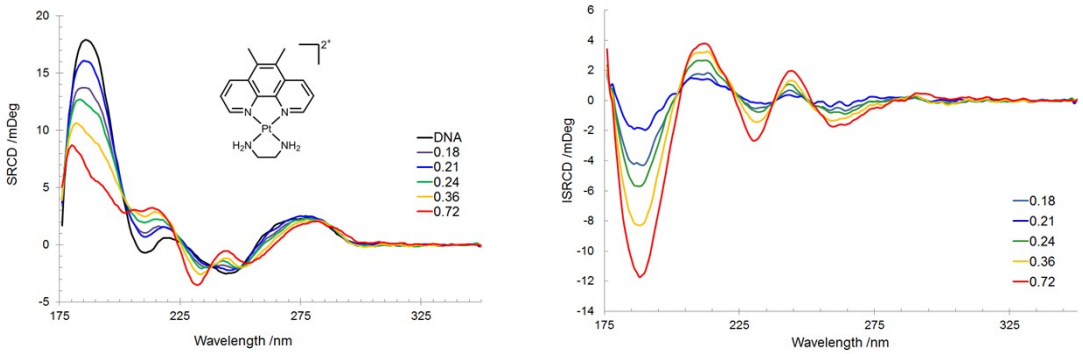

Figure S8 Expt A - SRCD and ISRCD spectra at different concentrations of metal complex 5, into ct-DNA in PS buffer.

Table S9 Complex **5**, $[Pt(5,6\text{-}Me_2phen)(en)]^{2+}$ binding data, experiment B.

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
176	6.2 ± 0.98	1.4 ± 0.1
181	7.4 ± 1.5	2.3 ± 0.1
183	2.9 ± 0.37	2.4 ± 0.1
185	2.5 ± 0.13	2.4 ± 0.1
186	3.5 ± 0.35	2.3 ± 0.1
191	4.0 ± 1.4	2.2 ± 0.1
193	5.1 ± 0.39	2.0 ± 0.1
235	10 ± 0.73	2.7 ± 0.1
259	5.3 ± 0.54	2.8 ± 0.1
260	1.7 ± 0.20	3.2 ± 0.1
261	1.8 ± 0.16	3.0 ± 0.1
262	3.8 ± 0.73	2.7 ± 0.1
269	8.5 ± 0.43	2.6 ± 0.0
304	2.4 ± 0.23	2.1 ± 0.0
329	3.6 ± 1.9	3.0 ± 0.2

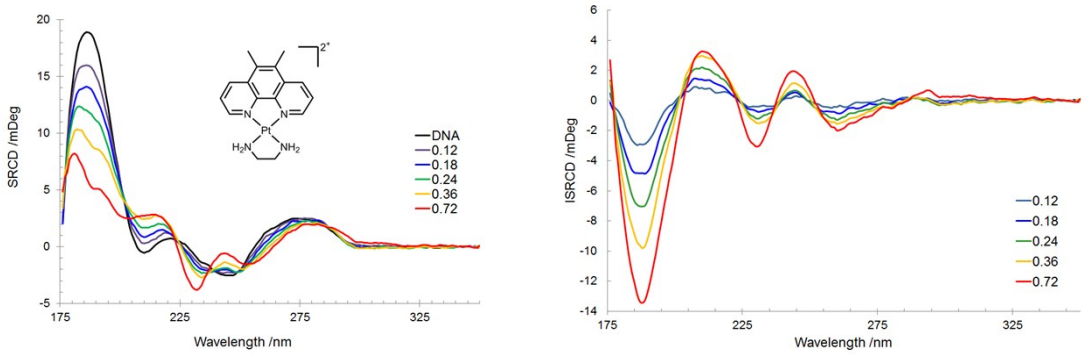

Figure S9 Expt B - SRCD and ISRCD spectra at different concentrations of metal complex 5, into ct-DNA in PS buffer.

Table S10 Complex **6**, $[Pt(3478\text{-}Me_4phen)(en)]^{2+}$ binding data, experiment A.

Wavelength nm	Binding Constant $K \times 10^5$	Estimated Binding sites per complex, n
208	0.77 ± 0.13	4.9 ± 0.4
254	0.71 ± 0.07	6.0 ± 0.4
261	0.10 ± 0.01	5.2 ± 0.1
323	2.5 ± 0.27	4.5 ± 0.2
330	1.1 ± 0.10	9.8 ± 0.5

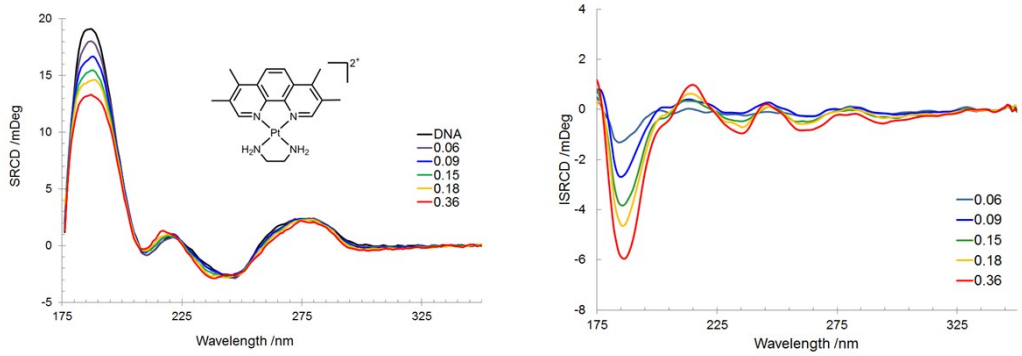

Figure S10 Expt A - SRCD and ISRCD spectra at different concentrations of metal complex 6, into ct-DNA in PS buffer.

Table S11 Complex **6**, $[Pt(3478\text{-}Mephen)(en)]^{2+}$ binding data, experiment B

Wavelength nm	Binding Constant $K \times 10^4$	Estimated Binding sites per complex, n
300	1.6 ± 0.15	3.5 ± 0.1
301	2.4 ± 0.20	4.7 ± 0.1
323	0.5 ± 0.19	5.4 ± 0.2

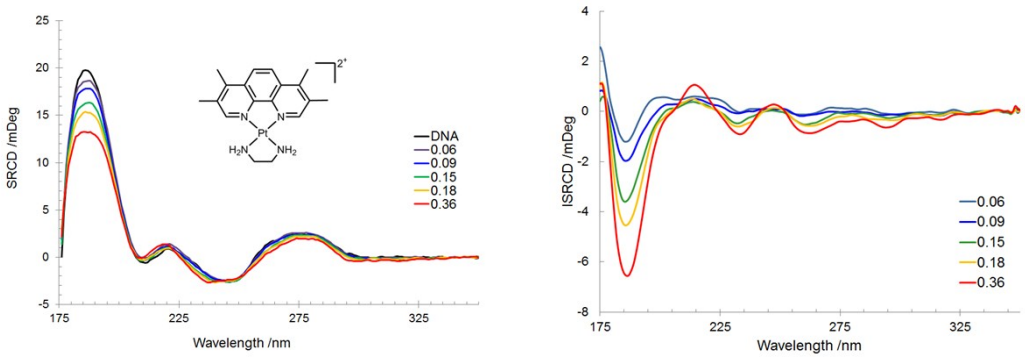

Figure S11 Expt B - SRCD and ISRCD spectra at different concentrations of metal complex 6, into calf thymus DNA in PS buffer.

References

1. F. H. Stootman, D. M. Fisher, A. Rodger and J. R. Aldrich-Wright, *The Analyst*, 2006, 131, 1145-1151.
2. G. Scatchard, *Ann. N. Y. Acad. Sci.*, 1949, 51, 660-672.
3. D. E. Schmechel and D. M. Crothers, *Biopolymers*, 1971, 10, 465-480.
4. S. Sarkar and B. Das, *Complex Metals*, 2014, DOI: 10.1080/2164232X.2014.887448, 80-87.
5. H. Wu, F. Kou, F. Jia, B. Liu, J. Yuan and Y. Bai, *Bioinorganic Chemistry and Applications*, 2011, 2011.
6. A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro and J. K. Barton, *Journal of the American Chemical Society*, 1989, 111, 3051-3058.