

Tetraplatinated Artificial Oligopeptides Afford High Affinity Intercalation into dsDNA

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Experimental

Chemicals and Materials.

All materials were purchased from Acros and were used as received unless otherwise noted. Water was obtained from a nanopure system (Barnstead, 18.2 M Ω). Single strand DNA oligomers (sequences 5'CAG CTG GTC ACG3' and 5'CGT GAC CAG CTG3') were purchased from Integrated DNA Technologies Inc with standard desalting. The pyridine substituted aeg monomer (Fmoc-aeg(py)-OH·HCl) was synthesized as reported previously.^{S1} The synthesis of (2,2':6',2''-terpyridine-N, N', N'')platinum (II) dperchlorate (i.e., [Pt(tpy)]²⁺) was adapted from a literature procedure using AgClO₄ instead of AgBF₄.^{S2} The [Pt(tpy)(pic)](BF₄)₂ was prepared according to the literature.^{S3}

Sample Preparation

A stock solution of phosphate buffer (pH = 6.95) was prepared using 1 mM sodium monobasic and 1 mM potassium dibasic solutions and 50 mM sodium acetate. A stock solution of 30 mM HEPES (N-[2-Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]) buffer (pH = 6.95) was prepared containing 100 mM sodium acetate.

Separate stock solutions of DNA (250 μ M) in HEPES and phosphate buffers were prepared by combining 250 nmoles of each of the ssDNA oligos in 500 μ L of buffer. Single strand solutions (500 μ M) were combined, heated to 90°C for 2 min, and allowed to cool to room temperature to ensure complete hybridization. Following standard procedures, the HEPES buffer solutions were used for the ITC experiments, and phosphate buffer solutions for the CD and melt experiments.

Spectrophotometric titration of Pyridine Tetrapeptide with $[\text{Pt}(\text{tpy})]^{2+}$: The formation of the metallated $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ tetrapeptide was monitored as previously reported^{S1} by UV-Visible absorption spectroscopy using a Cary 500 UV-Vis NIR spectrophotometer. Briefly, a solution containing 500 μM aqueous solution of the tetrapyridine oligopeptide was titrated with a stock solution of 100 μM $[\text{Pt}(\text{tpy})(\text{CH}_3\text{CN})](\text{ClO}_4)_2$. Figure S1 shows the change in absorption during sequential additions: the inset shows that the absorption at 265 nm increases and levels off at a molar ratio of 4 Pt^{2+} ions per tetrapeptide. The solution was then back-titrated to the stoichiometric equivalence point with a solution of tetrapyridine oligopeptide in water. The product was dried by lyophilization for a minimum of 48 hours prior to use; purity was confirmed as before using ^1H NMR spectroscopy.^{S1}

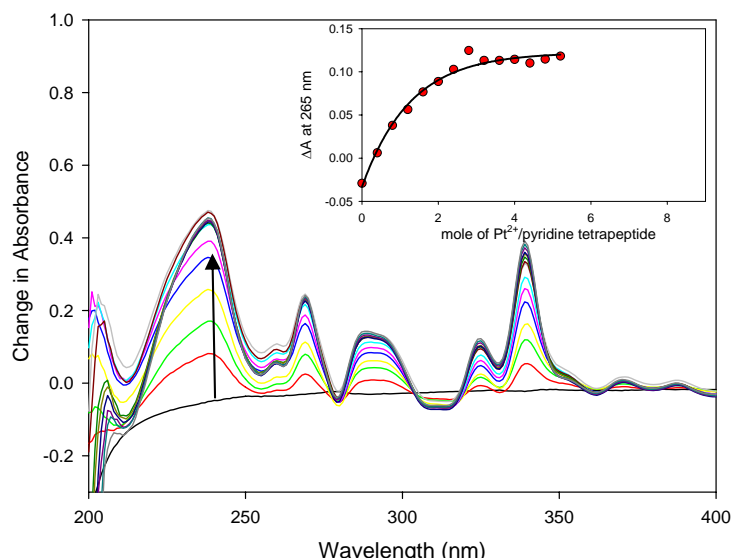


Figure S1. Difference spectra acquired during the titration of $[\text{Pt}(\text{tpy})]^{2+}$ with pyridine tetrapeptide. Inset: plot of the change in absorption at 265 nm.

Isothermal Titration Microcalorimetry (ITC): A Microcal VP-ITC isothermal titration calorimeter operating at 30°C, a reference power of 20 $\mu\text{cal}/\text{sec}$, and a stirring speed of 310 rpm was used for all experiments. All solutions were prepared in 30 mM HEPES containing 100 mM sodium acetate. Injections of 400 μM $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ in HEPES buffer were titrated in 5 μL aliquots in 10 second injection times into a 10 μM DNA solution. A 200 sec interval between injections was used to ensure a complete return to baseline after each injection. Data were corrected for background signal by performing similar titrations with buffer solutions, and fit to a single-site binding model using Origin 7 SR4, and are shown in Figure S2. Binding constants are converted to M^{-1} by taking the square root of the trimolecular K_B (in M^{-2}). A minimum of three titrations were performed for each sample, and the fit parameters

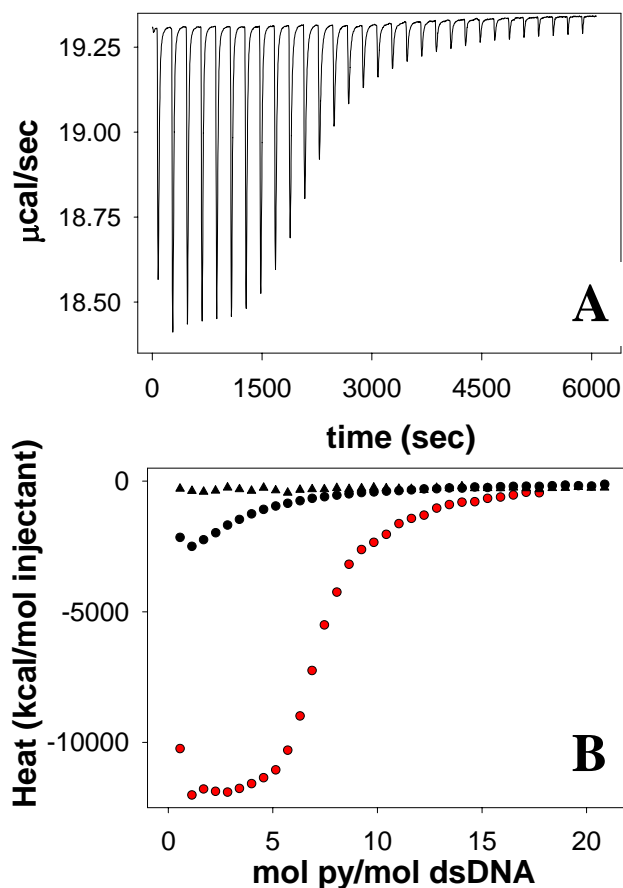


Figure S2. (A) Raw ITC data during the titration of 12bp dsDNA with $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ tetrapeptide. (B) ITC curves for the titration of 12bp dsDNA with $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ tetrapeptide (●); $[\text{Pt}(\text{tpy})(\text{pic})]^{2+}$ (●); and pyridine tetrapeptide (▲), versus the ratio of mol of pyridyl ligand/mol ds DNA.

reported as the mean value \pm the standard deviation. Also shown in Figure S2 are the ITC curves for titrations performed with 400 μM unmetallated pyridine tetrapeptide (▲) solution or with a 1.6 mM $[\text{Pt}(\text{tpy})(\text{pic})]^{2+}$ (●) solution.

Circular Dichroism (CD): All spectra were obtained using a Jasco J-810 circular dichroism spectrophotometer, a 2 mm cuvette, a step time of 4 sec, and a wavelength range of 400 to 205 nm. All solutions were prepared from the phosphate buffer stock solution. Separate samples were prepared, each with 40 μM dsDNA, containing molar ratios of the metallated oligopeptide to dsDNA of 0:1, 1:0, 1:1, 2:1, and 3:1.

CD Melt Spectra: Scans were obtained using a solution containing 80 μM $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$

tetrapeptide and 40 μM 12mer DNA in phosphate buffer every two degrees heating from 25°C to 95°C and cooling back to 5°C on a JASCO J810 spectrophotometer. Scans were taken using a one minute holding time with a two minute equilibrium time, scanned from 370 to 210 nm in 4 sec integration times. Background scans using pure buffer solutions were acquired in the same cuvette, and subtracted from the initial change in ellipticity.

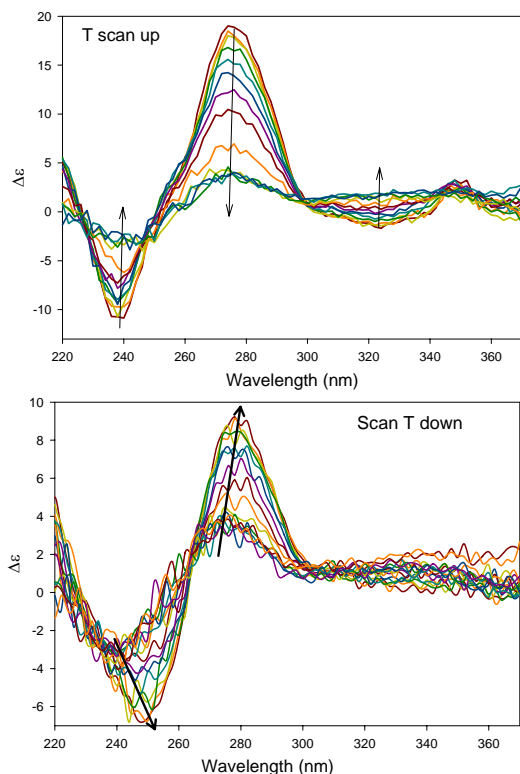


Figure S3. Temperature dependent CD spectra for a solution containing 40 μM dsDNA and 80 μM $[\text{Pt}(\text{tpy})(\text{py})_4]^{8+}$ tetrapeptide in phosphate buffer. Temperature was initially increased from 25 to 95°C (top) and then decreased from 95 to 5°C in 2°C increments.

previous literature reports; upon cooling, T_m decreases to 58°C, the same as for a solution containing only DNA.

For a sample containing 2:1 Pt tetramer:dsDNA, a second set of experiments were performed (using fresh solutions) in which the sample was heated from 30 to 95°C, cooled to 5°C, warmed to 30°C and then allowed to sit for 1 – 24 hours at this temperature. Following this rest period, the absorbance data was again

UV Melts: The UV absorption at 260 nm was monitored as a function of temperature for each of the samples from the CD spectroscopy experiments using a Gilford Response II UV-Vis Spectrophotometer and a 5 mm cuvette. Data were acquired in three sequential scans: 30 to 95°C, 95 to 5°C, and 5 to 95°C. Analogous experiments were performed using a 1 mm cuvette with the small molecule $[\text{Pt}(\text{tpy})(\text{pic})]^{2+}$:12mer dsDNA ratios of 1:1, 2:1 and 3:1. Melting temperatures were determined from the peaks of the derivative of the melting curves. Figure S4 shows the melting curves for the solution containing 2:1 $[\text{Pt}(\text{tpy})(\text{pic})]^{2+}$:dsDNA during sequential heating, cooling, and reheating cycles. Two inflections are observed in the initial heating cycle (at 65 and 79°C), consistent with

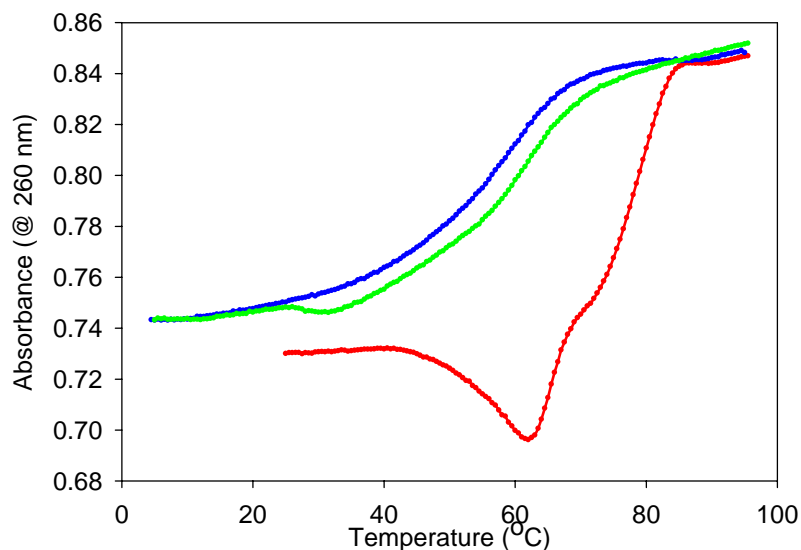


Figure S4. Temperature dependent absorbance at 260 nm for a phosphate buffer solution containing 40 μM dsDNA and 80 μM $[\text{Pt}(\text{tpy})(\text{pic})]^{2+}$ while heating (red), and subsequently cooling (blue line) and reheating the solution (green).

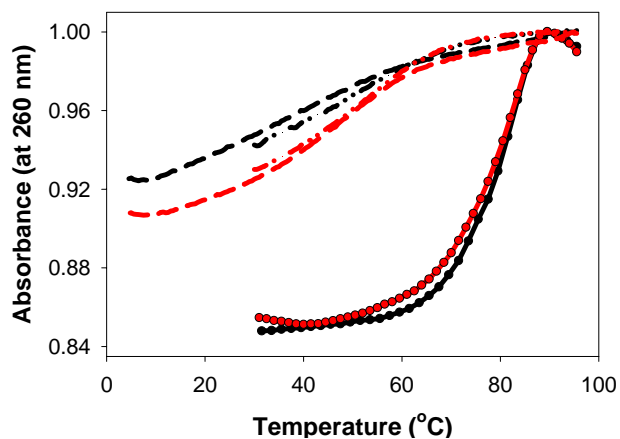


Figure S5. Temperature dependent absorption at 260 nm for a solution containing 40 μM dsDNA and 80 μM $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ (red lines) and a solution containing 40 μM of each of the ssDNA oligos and 80 μM $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ (black lines). Initial heating step (—), initial cooling cycle (---), and subsequent re-heating cycle (- · -).

The temperature-dependent absorption was monitored as before, and the melt data is also shown in Figure S5 (black lines).

Temperature Dependent Absorbance Spectra: 3D Melts.

All spectra were obtained using a Beckman-Coulter DU 650 over a temperature range from 25 $^{\circ}\text{C}$ to 95 $^{\circ}\text{C}$ and back to 25 $^{\circ}\text{C}$, measured every 5 $^{\circ}\text{C}$ from 370 to 220 nm with 0.5 cm cuvettes. After the cell reached the desired temperature, it was allowed to equilibrate for a minimum of 2 min. The concentration in each of the solutions of platinum terpyridine tetrapeptide was 40 μM . For solutions containing DNA the 12mer concentration was 20 μM .

collected over the temperature range 30 – 95 $^{\circ}\text{C}$: these data are shown in Figure S5 (red lines).

A separate experiment was performed by preparing a solution containing 80 μM of the ssDNA (5'CGT GAC CAG CTG 3') and 160 μM of the metallated tetrapeptide in phosphate buffer, which was allowed to sit for one hour. To this was added an equal volume of an 80 μM solution of the ssDNA complement (5'GCG CTG GTC ACG3') so that the final concentration of each ssDNA oligo was 40 μM and the $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ was 80 μM (a 2-fold excess).

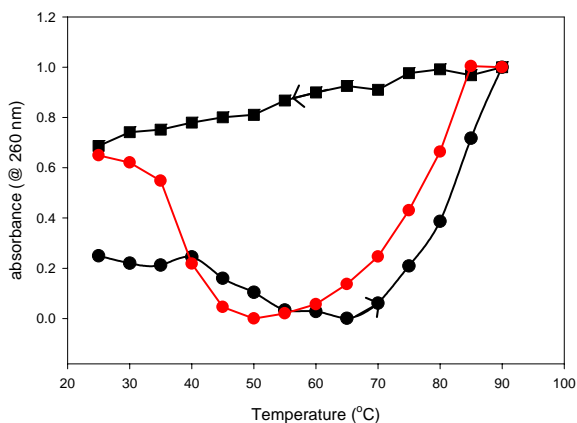


Figure S6. Temperature dependent absorbance at 260 nm for a solution containing 20 μM DNA and 40 μM $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ while heating from 25 to 95 $^{\circ}\text{C}$ (●) and subsequently cooling from 95 to 25 $^{\circ}\text{C}$ (■). An additional 6nm of $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ was added (40 μM) and the absorbance again monitored while heating this solution (●).

Temperature Dependent Spectra of $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ Peptide.

Temperature dependent absorption spectra of solutions containing $50 \mu\text{M}$ $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ oligopeptide in both pure water and phosphate buffer stock solution were obtained with a Gilford Response II UV-Vis spectrophotometer. Scans were acquired every 5°C over a range of $25 - 95^\circ\text{C}$, and are plotted as difference spectra (relative to the absorption at 25°C) in Figure S5. Similar changes in the electronic spectra as a function of increasing temperature are observed in both solutions, likely a result coordination of water ligands. However, in phosphate buffer solutions additional peaks at lower energy are observed that are associated with acetate anions.

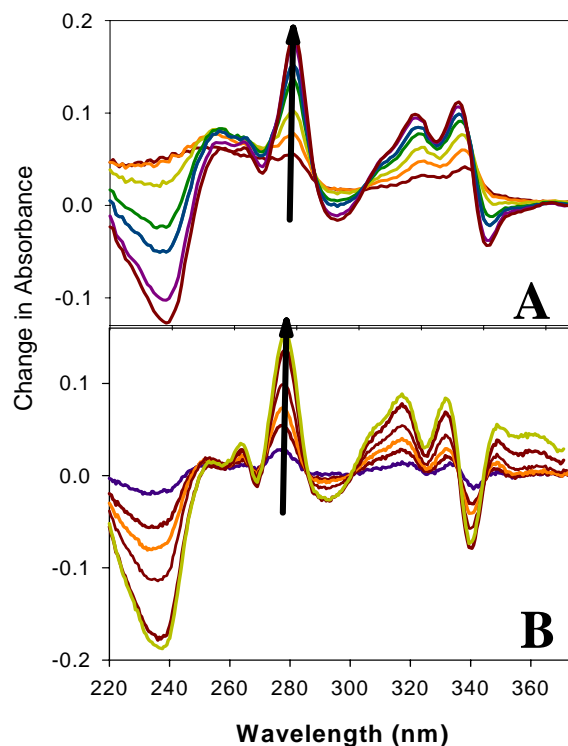


Figure S5. Change in UV absorption of $50 \mu\text{M}$ $[\text{Pt}(\text{tpy})(\text{py})]_4^{8+}$ oligopeptide in (A) water and (B) phosphate buffer solutions over the temperature range $25 - 95^\circ\text{C}$, normalized to the absorption at 25°C .

References.

S1 See Reference 6 in manuscript.

S2 McCoubrey, A.; Latham, H. C.; Cook, P. R.; Rodger, A.; Lowe, G. *FEBS Lett.* **1996**, *380*, 73.

S3 (a) Lowe, G.; Vilaivan, T. J. *Chem Research (S)* **1996**, 386. (b) Cusumano, M.; Di Pietro, M. L.; Giannetto, A. *Inorg. Chem.* **1999**, *38*, 1754.