MATERIALS AND METHODS

The c-MYC-24mer WT, 1-2-1, and 1-6-1 sequences were obtained from Oligos Etc (Wilsonville, OR). The primary sequence synthesized was the native 24mer of the polypurine sequence found in the c-MYC P1 promoter. The construct sequence of the WT was (5'-TGGGGAGGGTGGGGAGGGTGGGGA-3'). The sequences of the two mutated constructs, 1:2:1 and 1:6:1, are shown in Scheme 1. The 24mer stock solutions were prepared by dissolution of weighed amounts of lyophilized oligonucleotide into [K^+] BPES buffer at two different salt concentrations, 30 mM [K^+] and 130 mM [K^+] (1). Approximately 1 mL of the oligonucleotide was exhaustively dialyzed (1000 molecular-weight cutoff membrane) with two changes of buffer solution (1 L, 24h each) at 4°C. The concentrations of all DNA solutions were verified using ultraviolet-visible spectrophotometry (UV-Vis) as well as verified for quadruplex structure using circular dichroism. All molar extinction coefficients used to determine concentration were determined using a nearest-neighbor method for single stranded DNA (1). The extinction coefficients calculated for the three sequences at 260 nm were 248200, 241000, and 24100 cm-1 M-1 for the WT, 1:2:1, and 1:6:1, respectively.

TMPyP4 was obtained from Frontier Scientific (Logan, UT). All TMPyP4 solutions were prepared by dissolution of a known amount of TMPyP4 into a measured volume of the dialysate buffer solution from the corresponding oligonucleotide. All TMPyP4 concentrations were verified using UV-Vis with molar extinction coefficient of ε_{424}=2.26 X 10^5 $M^{-1}cm^{-1}$ found in the literature (26).

Differential Scanning Calorimetry (DSC) experiments were performed using a Microcal VP-DSC instrument (Microcal, Northampton, MA). The experimental scan rate was set at 90°C/h with a temperature range from 10° to 110° C. The thermograms for each of the oligonucleotides exhibited one or more independent "two-state" melting transitions. The thermograms were deconvoluted using Origin 7.1 software into the minimum number of "two-state" transitions needed to fit the thermogram within expected experimental error. DSC data was analyzed based on a model assuming that each "two-state" transition observed in each thermogram represents a unique structure or folded conformation. Through Origin analysis, values for the melting temperature, *T*m, and the calorimetric ΔH_{cal}, and Van't Hoff enthalpies ΔH_{VH}, for each transition observed was reported (1).

Isothermal Titration Calorimetry (ITC) experiments were performed using a Microcal VP-ITC (Microcal, Northampton, MA). All titrations were performed by filling the ITC cell with ~1.5 mL of oligonucleotide solution concentration ranging from ~25-50μM. The cell was overfilled by adding ~57-5μL injections of TMPyP4 ranging from ~1.2 X 10^{-3} to 1.6 X 10^{-3} M to obtain a ligand concentration approximately twenty times greater than oligonucleotide. All titrations were run at 25°C and at the two supporting electrolyte concentration of 30 mM [K^+] and 130 mM [K^+]. Three replicate titration experiments were typically performed. The ITC data were fit using an independent sites model with either two or three sites required to fit the data within experimental error. The nonlinear regression fitting was done using Mathematica 5.0 and algorithms developed in our lab. The ITC fitting procedures used here have been described previously (1, 27-30).

UV-VIS spectroscopy titrations were performed using an Agilent 8453 diode array (Agilent, Santa Clara, CA). DNA and ligand solutions for the UV-Vis titrations were prepared at relatively equal concentration of 10μM using BPES buffer to dilute to desired concentration. Each titration experiment was performed in two parts, each with a separate procedure. In the

first half of the experiment, 2mL of the dialysate buffer solution was placed in a standard quartz cuvette to be used as the blank, and 2mL dilute c-MYC 24mer was placed in another standard quartz cuvette. 100µL of buffer was removed manually from the first cuvette and 100µL of the DNA solution was removed manually from the second cuvette. 100µL of the dilute TMPYP4 solution was subsequently added to both cuvettes, keeping the cuvette volume constant after each injection. The buffer cuvette was run as the blank and the cuvette containing the DNA run as the sample each time. This procedure was repeated for a total of 15 injections. In the second half of the titration, 2mL of the dilute TMPYP4 solution was placed into two separate standard quartz cuvettes. 100µL of the TMPYP4 solution was removed manually from both cuvettes and 100µL of buffer was added to the first cuvette and 100µL of the dilute c-MYC 24mer solution was subsequently added to the second cuvette, keeping the cuvette volume constant through each injection. This procedure was repeated for a total of 15 injections. Absorbance spectra were collected from 200 to 600 nm and recorded after each injection in both portions of the titration experiment. Using this procedure the entire mole fraction range of TMPYP4 to DNA of 0-1 was collected with two overlapping points at the end of each "half-titration." The job plot was analyzed to determine the mole fractions corresponding to the formation of the TMPyP4/DNA complex.

CD analysis was performed using either a JASCO 810 CD Spectrometer (Jasco, Tokyo, Japan) or an Olis DSM 20 CD Spectrophotometer (Olis, Bogart, GA). Spectra were collected over a wavelength range of 200-500 nm. The samples were prepared at a concentration that produced an absorbance of 1.0 for titration experiments. Concentrations of oligonucleotide and TMPyP4 were verified using UV-Vis absorbance measurements. Titrations were all performed at 25°C. For the titration, 2mL of dilute DNA was placed into a standard quartz cuvette and TMPyP4 was manually injected to saturation. CD spectra were collected at 1 mole ratios of 2:1, 1:0, 0:1, 1:1, 2:1, 3:1, 4:1, 5:1 and 6:1 for TMPyP4 and each 24mer oligonucleotide respectively