# Thermodynamic and Structural Studies of the Interactions between c-MYC G4 DNA and Minor Groove Binders as an Anticancer Approach

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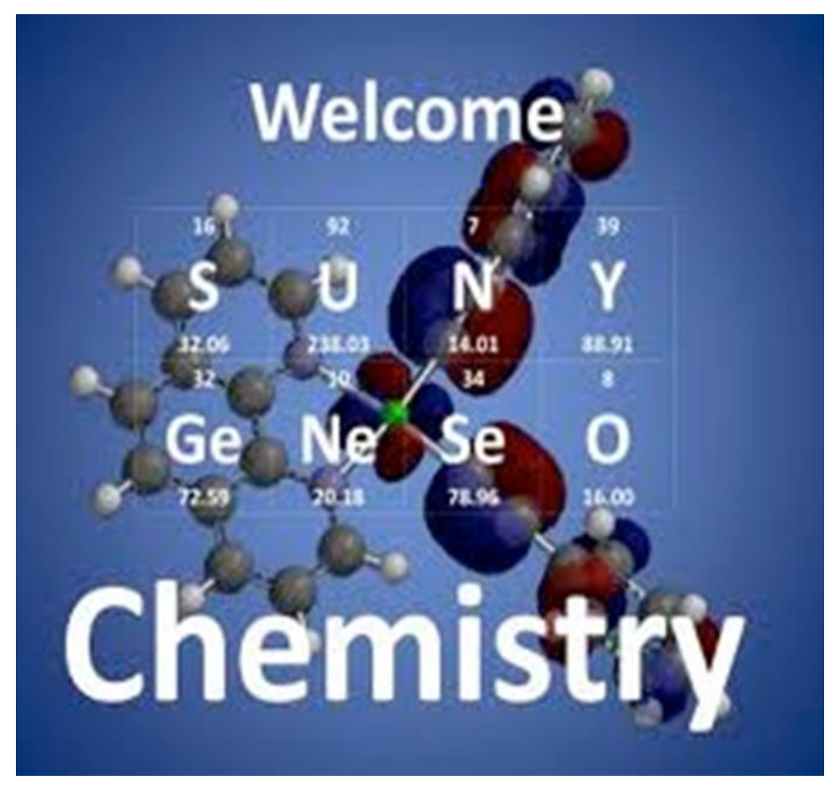

## Introduction

G-quadruplex (G4) DNA are non-canonical higher order structures formed from guanine rich sequences, consisting of stacked G-tetrads stabilized by non-Watson-Crick (Hoogsteen) base pairing. Recently, G4 has been shown to be overrepresented in the promoter regions of oncogenes (e.g., *c-myc and ras*). As a result, G4 represents a viable target for possible anti-cancer therapeutic agents to treat previously “undruggable” targets such as the *c-myc* and *ras* oncogenes[1]. Most traditional approaches to targeting G4 have involved using compounds with planar frameworks that are expected to either stack on top, and/or intercalate between the G4 tetrads. However, there have been reports that groove binding compounds may also target G4[2]. These compounds would be expected to lie within the grooves typically present in the G4 structure. In this work, G4 structures formed by *c-myc* were investigated by targeting it using four known duplex DNA minor groove binders (Distamycin[3], DAPI, Berenil, Hoechst 33258[4,5]).

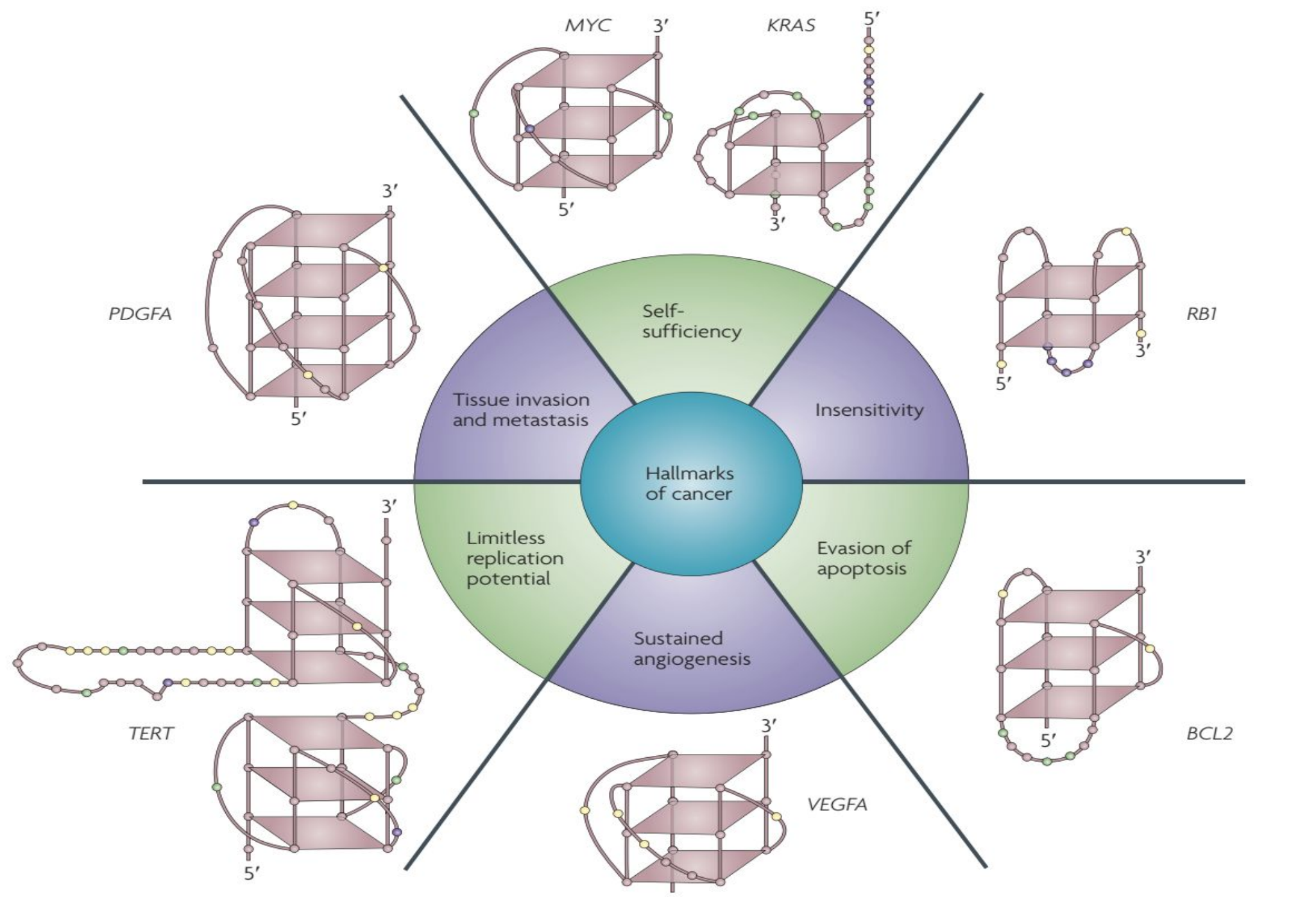

**Figure 1**. G4 exhibit diversity in their folding patterns and loop lengths. The six hallmarks of cancer are shown with the corresponding G4[6].

## Methodology

PTI Quantamaster™ 40 Spectrofluorometer[7]

**Buffer**

All trials were prepared with a 10mM phosphate buffer with 0.3mM EDTA and added $K^+$ concentrations over a range of 5mM to 25mM.

**Thioflavin-T (ThT) Fluorescence Displacement**

Studies were performed from 400-600 nm. Thioflavin-T (ThT) was used as a fluorescent indicator for the quadruplexes. The solution was excited at 425 nm and fluorescence intensity was measured.

Microcal VP ITC[8]

**Circular Dichroism (CD)**

Conducted over the range of 200-400 nm, using a nitrogen purge at around 20°C.

**Isothermal Titration Calorimetry (ITC)**

Experiments were conducted at 30°C and results were analyzed using Origin 7.0 software. Injections of drug into DNA occurred in 7 to 10 μL increments.

Jasco-815 Spectropolarimeter[9]

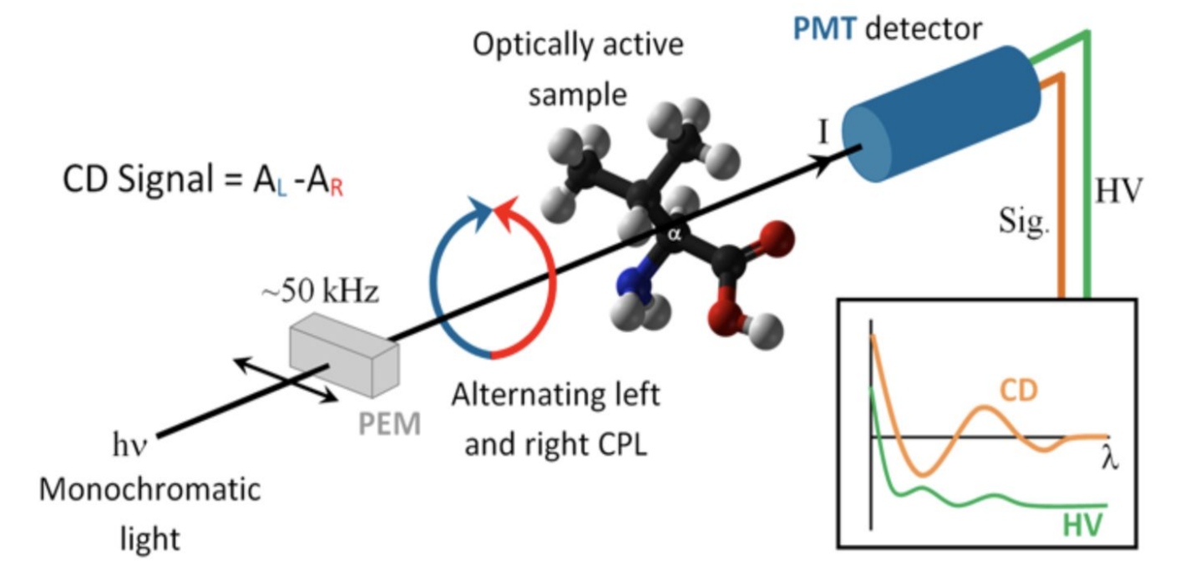

**Figure 2**. A schematic of the principles behind circular dichroism spectroscopy[10].

## Results

### Circular Dichroism (CD)

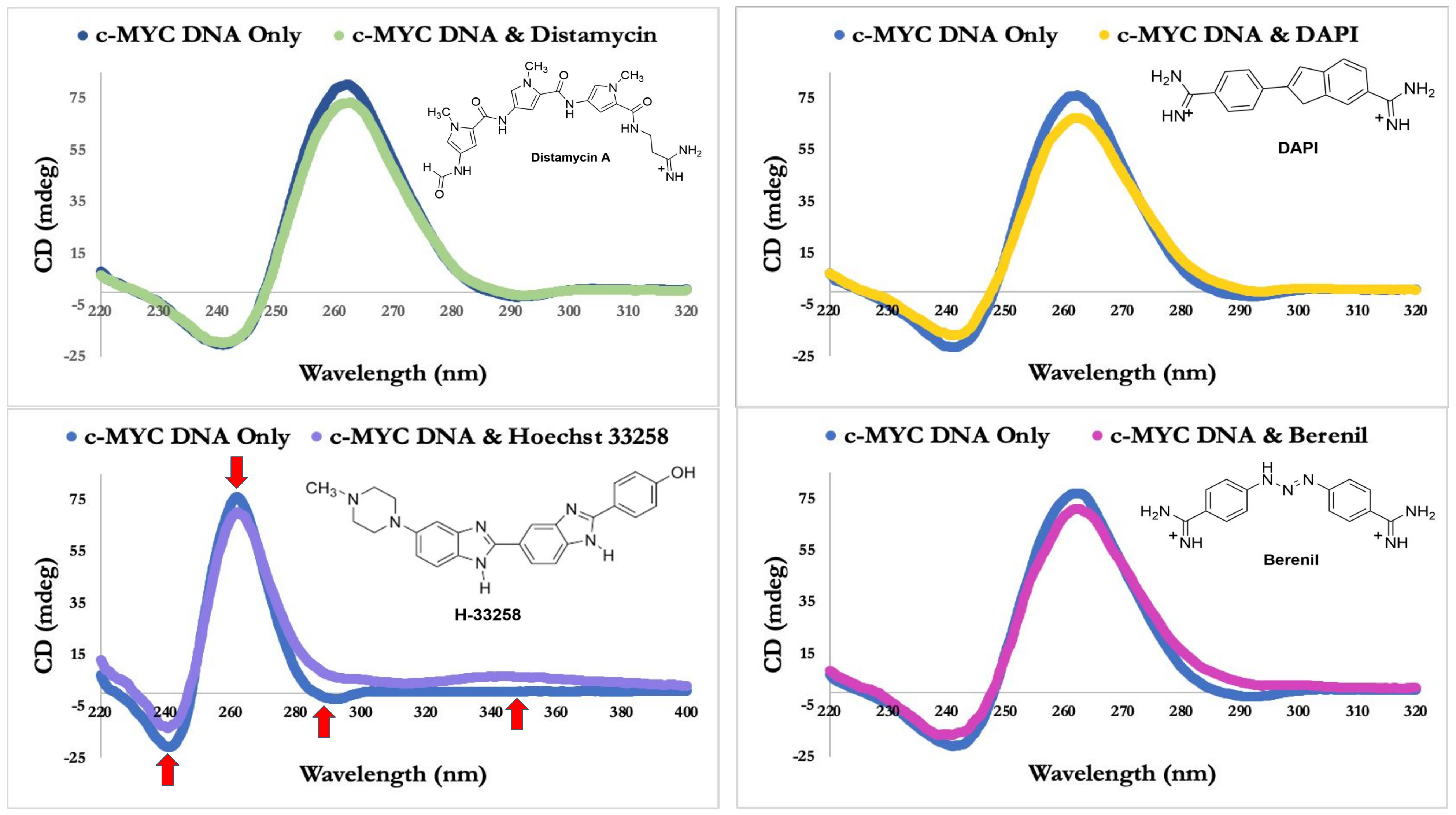

**Figure 3.** Changes in G4 structure at ~240, 262, 292, & 344 nm. Compounds injected in 10 μL increments; shown is G4 structural changes after 20 injections (total 200 μL of drug). Performed with 10 μM c-MYC and drugs ranging from 60 -300 μM.

### ThT Fluorescence Displacement

| Compound | Δ Fluorescence |
|---|---|
| Distamycin | 9.57 x $10^4$ |
| DAPI | 1.44 x $10^5$ |
| Hoechst 33258 | 1.59 x $10^5$ |
| Berenil | 2.95 x $10^5$ |

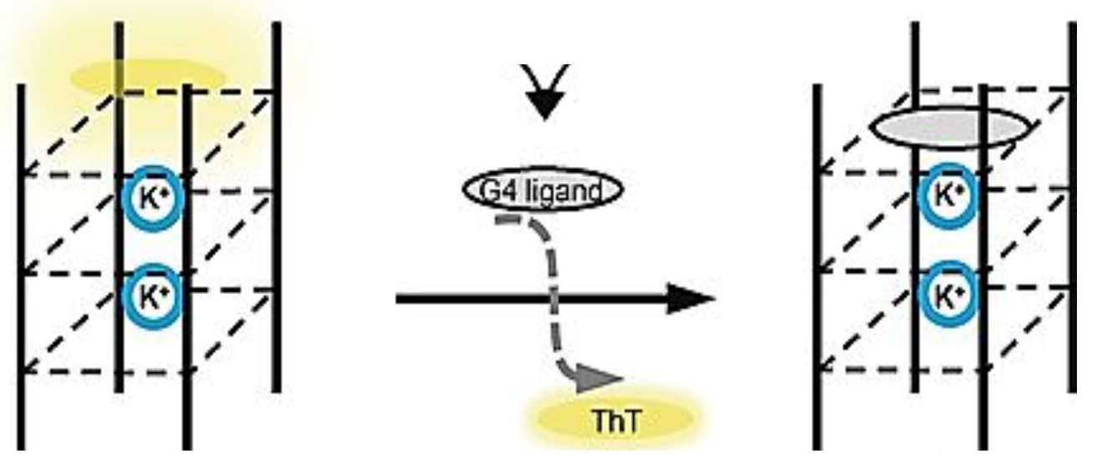

**Figure 4.** A diagram demonstrating the mechanism of the Thioflavin-T displacement assay[11].

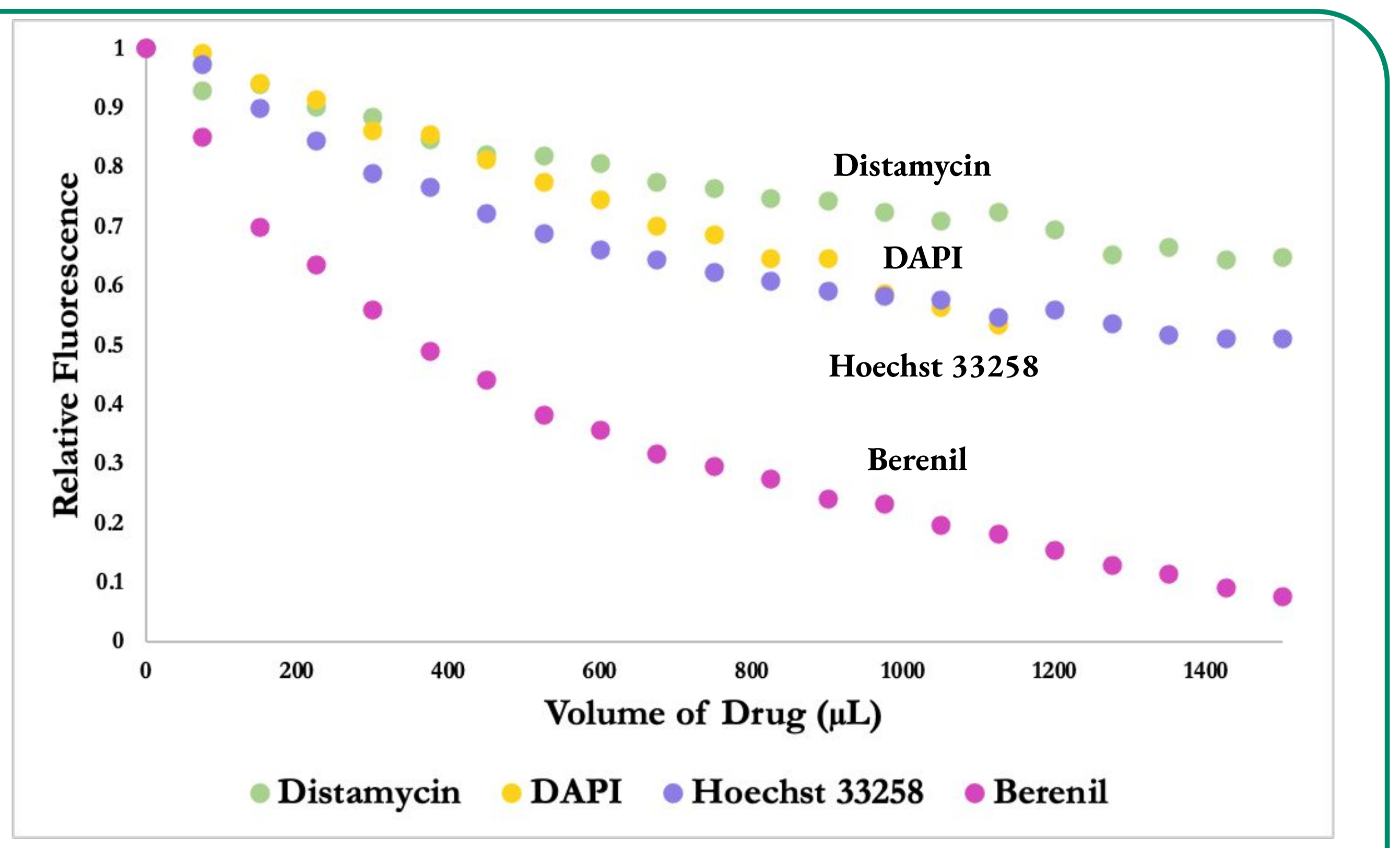

**Figure 5.** ThT displacement comparing various compounds. Drug was added in 75 μL increments. Fluorescence was measured at the maximum wavelength from 200-400 nm. Performed with 20 μM c-MYC DNA.

### Isothermal Titration Calorimetry

| | Distamycin | DAPI |
|---|---|---|
| **n** | 0.772 | 0.598 |
| **K ($M^{-1}$)** | 1.15 x $10^5$ | 5.08 x $10^5$ |
| **ΔH (cal/mol)** | -1.024 x $10^4$ | -2.154 x $10^4$ |
| **ΔS (cal/mol K)** | -10.6 | -44.9 |

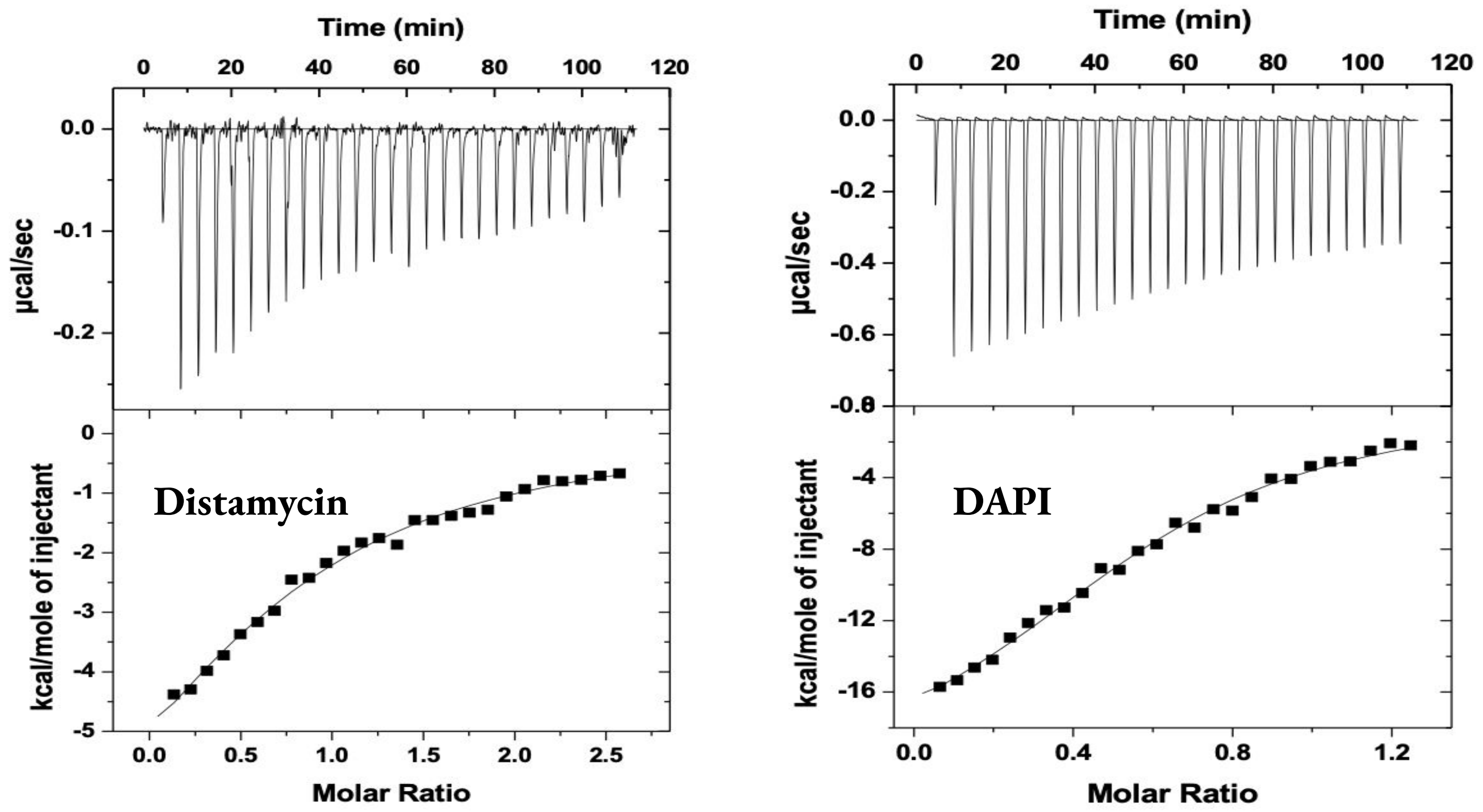

**Figure 6.** (Left) ITC calorimetric data of 150 μM distamycin and 10 μM c-MYC DNA. (Right) ITC calorimetric data of 64 μM DAPI and 10 μM c-MYC DNA.

## CD Results and G4 Validation

| Signature λ (nm) | Distamycin (Δ mdeg) | DAPI (Δ mdeg) | Hoechst 33258 (Δ mdeg) | Berenil (Δ mdeg) |
|---|---|---|---|---|
| ~ 240 | – | 4.61 | 7.47 | 4.35 |
| ~ 262 | 6.89 | 8.72 | 5.86 | 6.08 |
| ~ 292 | – | 3.51 | 8.44 | 4.36 |
| ~ 344 | – | – | 6.06 | – |

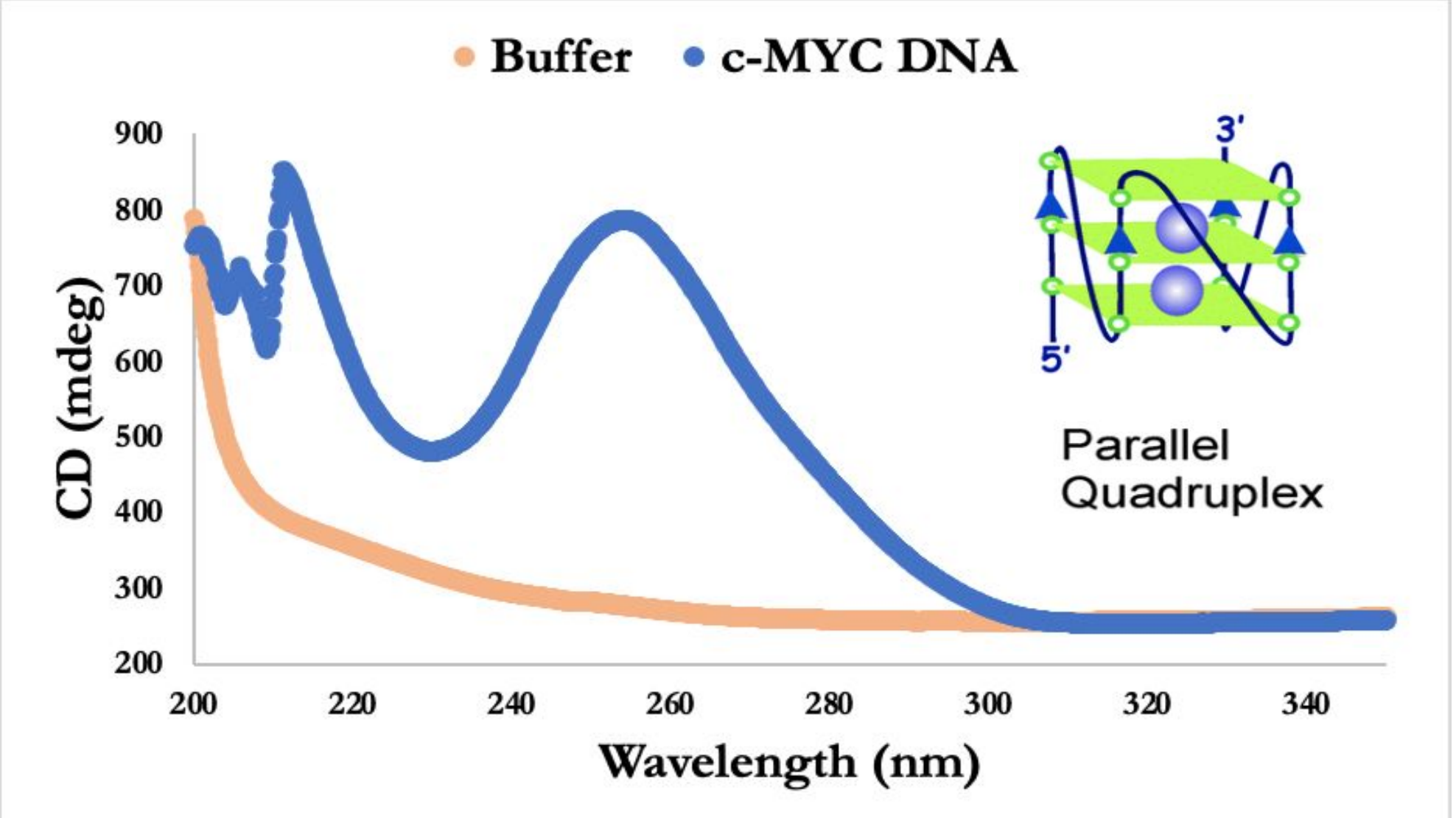

**Figure 7**. CD spectra comparing buffer only and c-MYC with an ion conc. of 10 mM. Results confirmed parallel confirmation of c-MYC G4.

## Conclusions

- All three approaches (ITC, CD, fluorescent displacement assays) show that the “minor groove” binding compounds used in this study (berenil, distamycin, DAPI, H33258) all bind to the c-MYC G4 DNA.
- A combination of the CD and ITC data suggests that both:
  (a) Multiple binding modes may be involved
  (b) The compounds do not exhibit the same binding modes
- CD studies shows a small ICD (induced CD signal) above 300 nm for H33258 suggesting it may adopt a groove binding mode. CD data also shows that distamycin perturbed only one of the signature bands for the G4 DNA, implying a single binding mode for distamycin. All other compounds elicited multiple band perturbations at G4 DNA signature bands: ~240, 262 and 292 nm.
- Fluorescent displacement assays revealed that berenil was able to displace ThT dye more readily than the other three compounds. This suggests that berenil may share the same binding sites as ThT, and/or berenil has the strongest affinity to the c-MYC G4 DNA.
- ITC data revealed binding affinities in the order of $10^5$ $M^{-1}$, with enthalpy changes being the major driving force. As is typical, entropy changes were smaller and unfavorable.

## What’s Next?

- Continue fine tuning fitting routines for the ITC data collected for berenil.
- Repeat ITC run for H33258 and extract the requisite thermodynamic data.
- Repeat DAPI ThT displacement assay at higher [DAPI].
- Explore other known DNA “minor groove” binders (e.g., netropsin) for comparison.

## References

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## Acknowledgements

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