

# A Novel Molecular and Cellular Study on Curcumin

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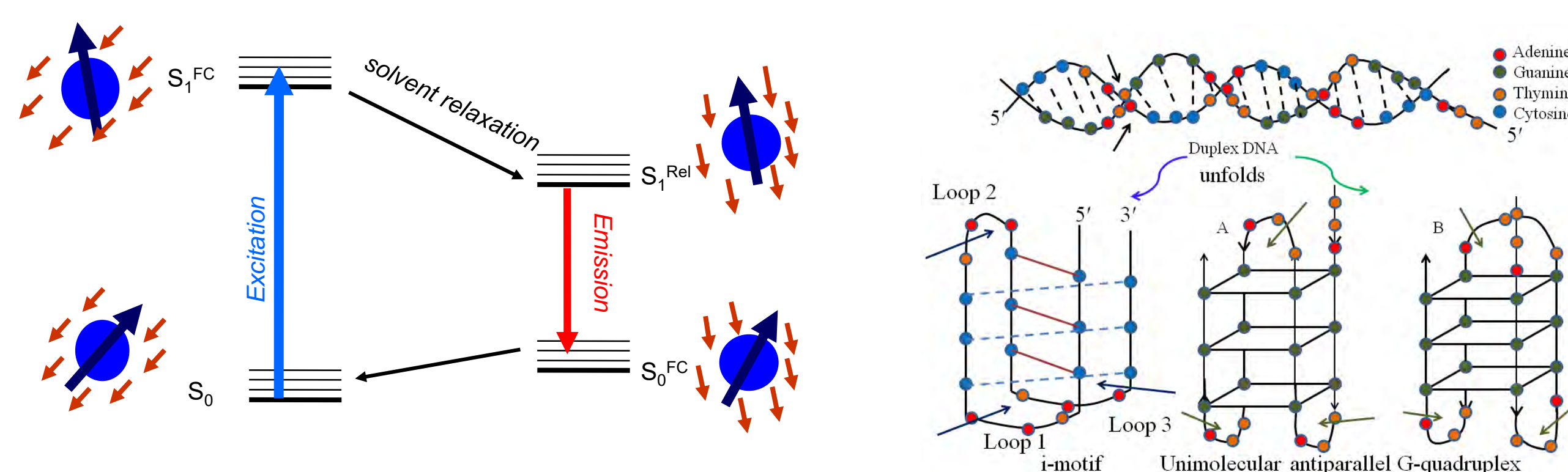
## Background

Since the discovery of G-quartet (G<sub>4</sub>) by M. Gellert in 1962, much attention has been given on G<sub>4</sub> and C<sub>4</sub> (also called i-motif) as important drug design targets for the treatment of various human disorders. G<sub>4</sub> forming sequences are prevalent in human genome, which includes many important regions of the eukaryotic genome, such as telomere ends, regulatory regions of many oncogenes c-kit, proto-oncogene c-myc, Kirsten rat sarcoma viral oncogene homolog (KRas). Curcumin (diferuloylmethane), an antiinflammatory and antioxidant compound, is found in the rhizomes of the plant *Curcuma longa*. The phyto polyphenolic chemical curcumin has been in the prominence due to its diverse pharmacological activities. Here, we studied the binding of curcumin with G-quartet and duplex DNA as well as protein bound DNA. Curcumin showed inclination toward binding with G<sub>4</sub> than C<sub>4</sub> and duplex DNA. Furthermore, cellular studies have been initiated on HeyA8 ovarian cancer cells. Curcumin treatment inhibited the proliferation of HeyA8 cells in a dose responsive manner demonstrated by MTT assay. studies on the binding of curcumin with HeyA8 DNA are underway.

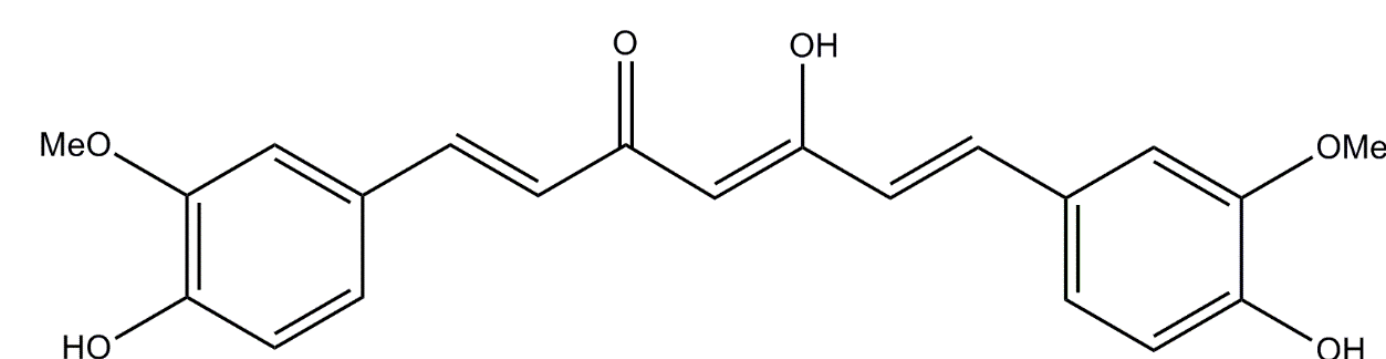
## Hypothesis and Objective

1. Curcumin is an excellent fluorophore.
2. Curcumin stabilizes the unusual DNA structures.
3. Curcumin shows antioxidative properties.

## Experimental Setup



The red shift is larger: the more polar the solvent is, the bigger the dipole moment of the fluorophore is and the bigger its change upon excitation is.



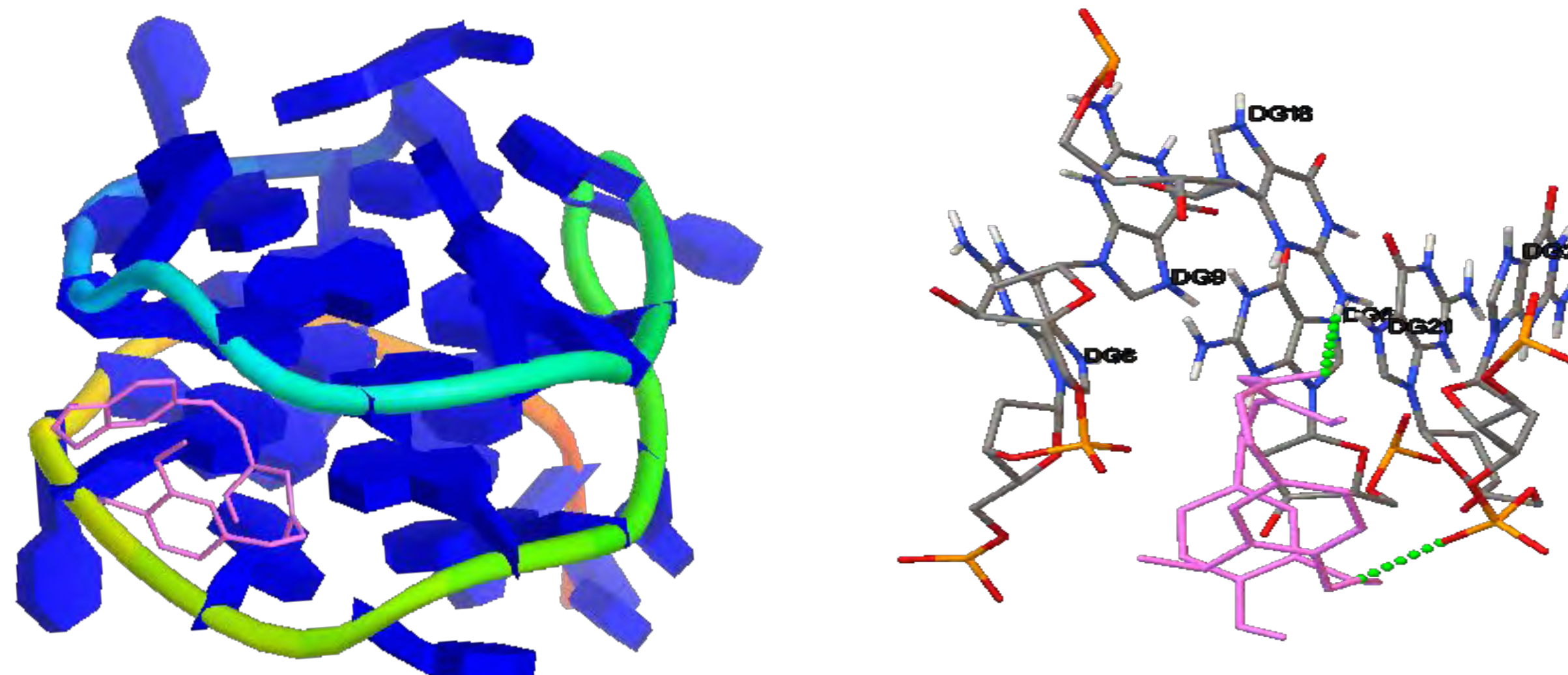
Chemical Structure of Polyhydroxy phenolic Curcumin

Curcumin is a naturally occurring polyphenol found in the rhizome of turmeric (*Curcuma Longa*).

## Acknowledgement:

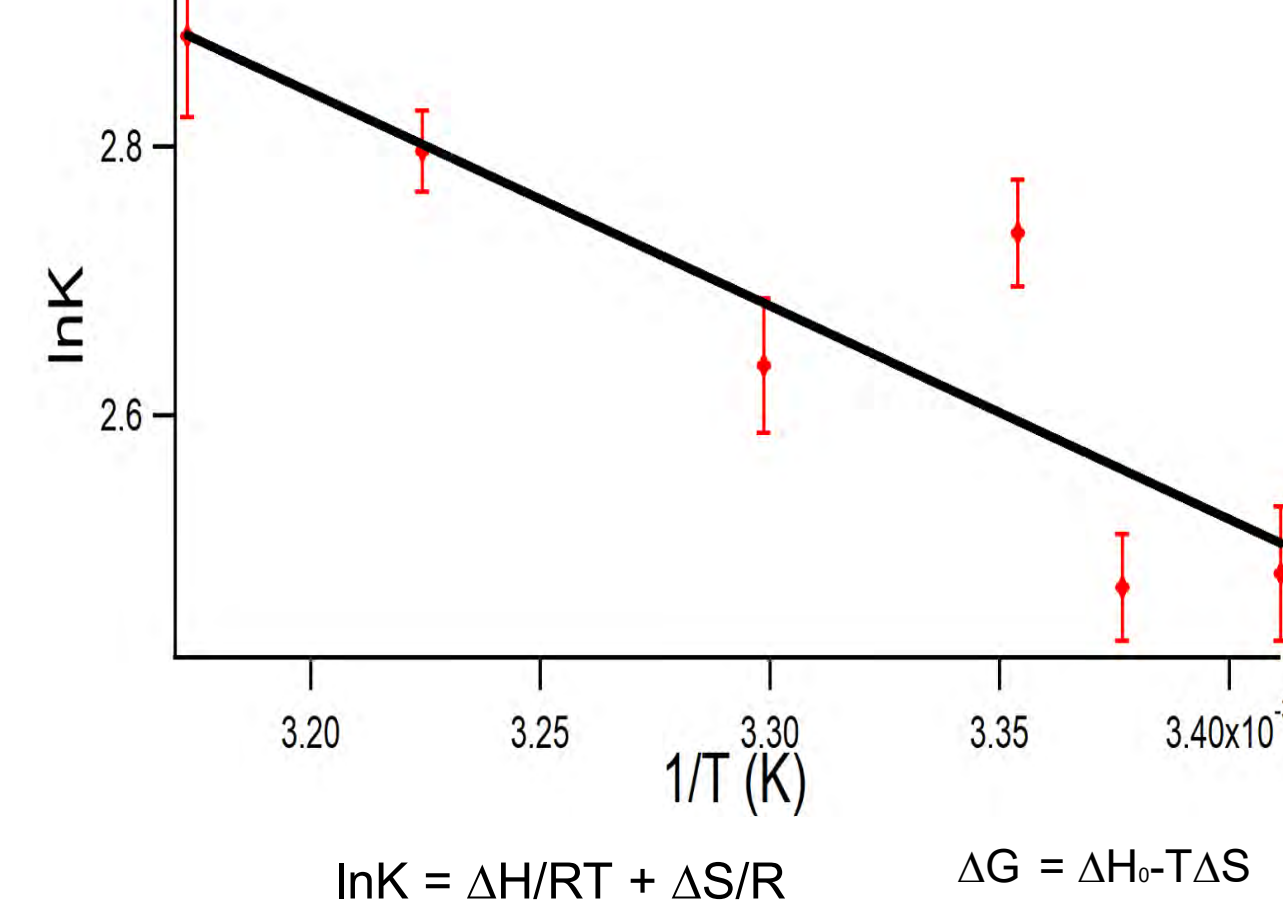
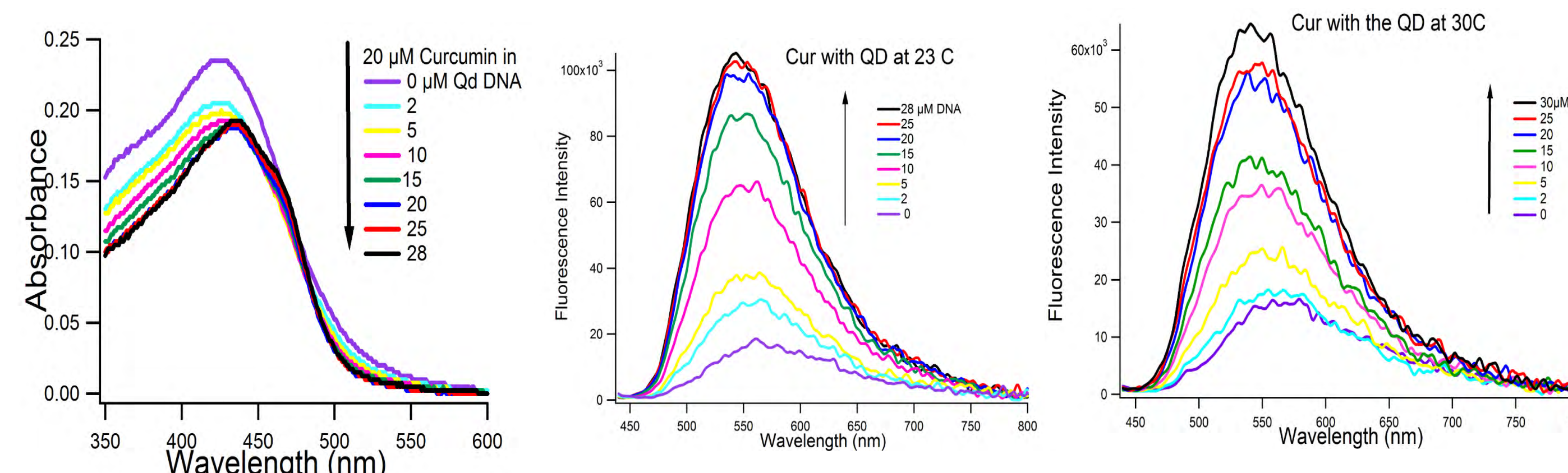
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## Docking Studies



Audocking studies of curcumin in G4-QD-DNA shows it binds in the loop region and makes 2-Hydrogen bonds

## Fluorescence Emission Spectra of Curcumin in DNA



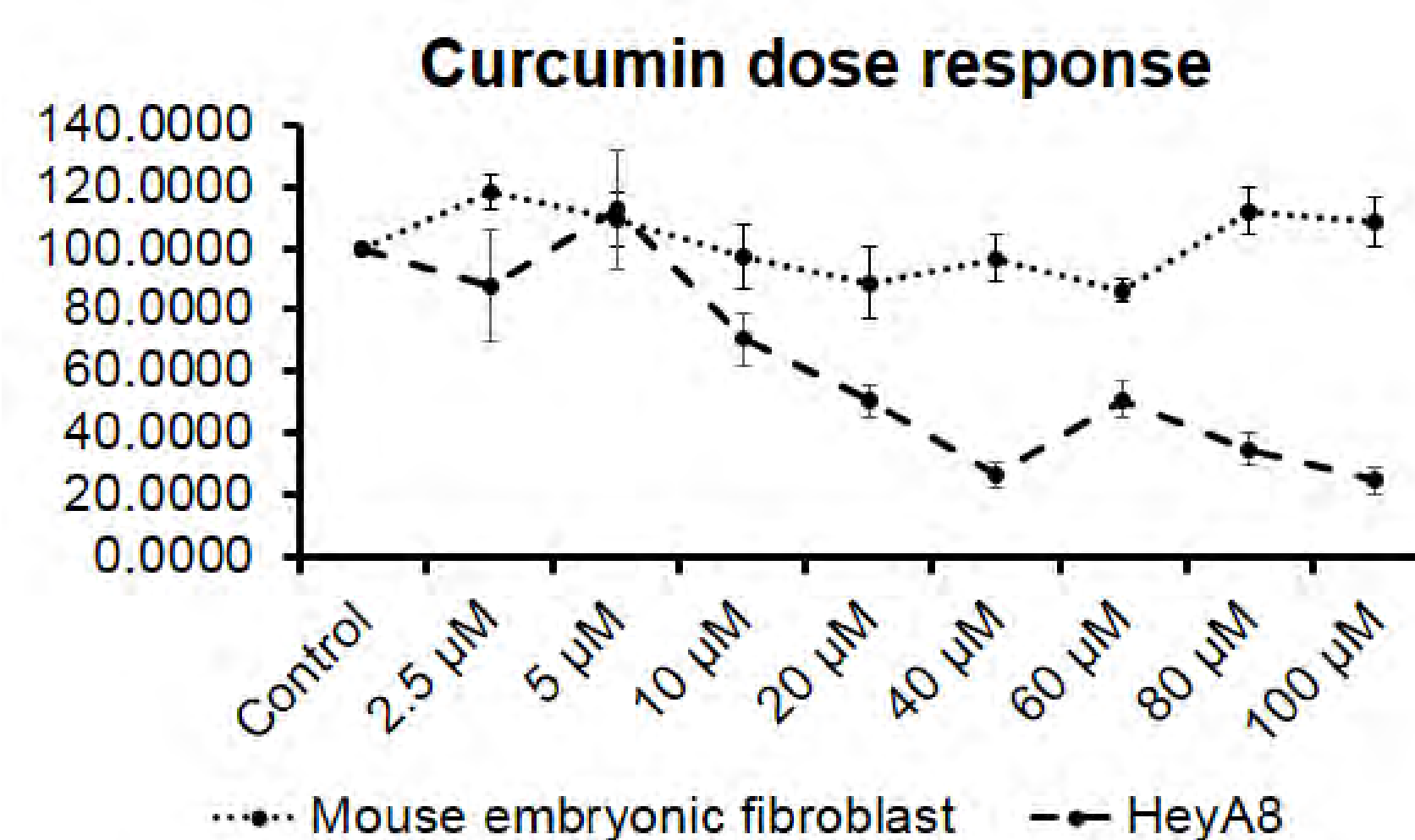
Absorption and Fluorescence emission studies of Curcumin (20 μM) in increasing concentration of G4 DNA (sequence GGGTTAGGGTTAGGGTTAGGGTTA) at temperatures 20, 23, 25, 30, 37 and 42C. Decrease in absorbance and increase in fluorescence of curcumin clearly suggest that curcumin is binding with the DNA. The binding constant K is calculated using the formula [6] at various temperature.

$$\ln K = \Delta H/RT + \Delta S/R$$

$$\Delta G = \Delta H - T\Delta S$$

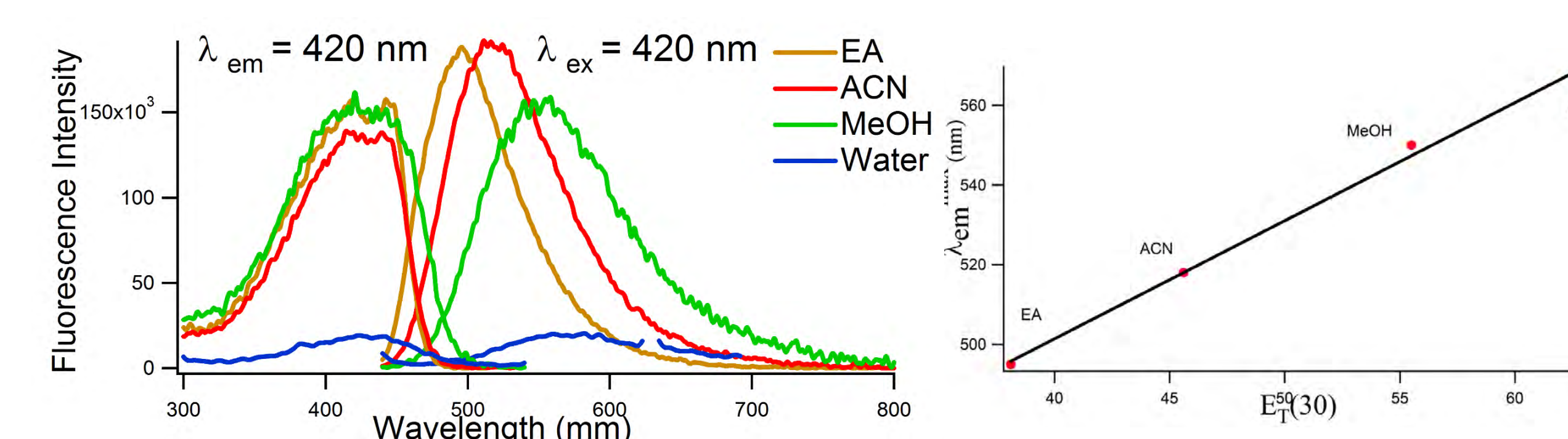
$$\log (F-F_0)/F = n \log K - n \log (1/([D] - (F-F_0) \cdot Pt / F))$$

## Curcumin in Ovarian Cancer Cells (IC50 study)



MTT assay was performed on ovarian cancer cells HeyA8 and normal fibroblast cells. Curcumin kills the cancer cells in a dose dependent manner where at 20 μM concentration, half of the cells were dead. Curcumin did not influence the normal cells.

## Solvent Dipolar Relaxation Studies



- We have used the empirical polarity index ET(30) (developed by Dimroth and Reichardt [5], which is based on the transition energy for the solvatochromic intramolecular charge transfer absorption of the betain dye 2, 6-diphenyl-4-(2, 4, 6-triphenyl-1-pyridino) phenolate) in order to obtain quantitative measures of the polarity of the local environments of curcumin in DNA.
- Fluorescence emission and excitation scans were collected on Curcumin in homogeneous solvents to understand the effect of polarity on Cur. The emission and excitation wavelengths are significantly different in solvents of varied polarity, an important characteristic of a fluorescence sensor.

Environment	Em Peak (nm)	ΔG (kcal/mol)	ΔH (kcal/mol)
Water	568		
G <sub>4</sub> Tetraplex DNA	550	-8.03	-3.15
Duplex DNA	466		
MeOH	549		
EA	495		

## Summary and Conclusions

1. Excited state is stabilized by solvent interactions in polar solvents, which is absent in less polar solvent like EA. Curcumin displays excellent solvent dipolar relaxation mechanism highlighting it as an excellent fluorescence sensor.
2. Curcumin binds with G4-DNA spontaneously as is evident from ΔG of -8.03 kcal/mol in the loop region and makes 2H-bonds.
3. The IC50 of curcumin is found to be 20 μM in human ovarian cancer cells.

## Future Studies

- We plan to perform studies in duplex DNA and proteins..
- We will study the secondary structures of the macromolecules using CD spectroscopy.
- Computational simulations (docking and molecular dynamics) are underway.

## References

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