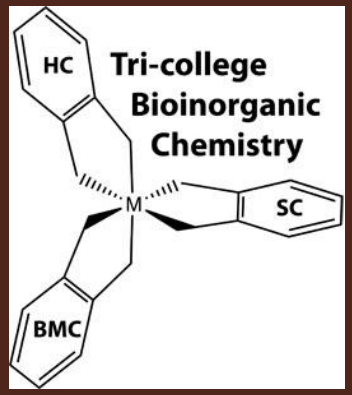
Investigating the Interactions between a Platinum Metalloporphyrin and Quadruplex DNA

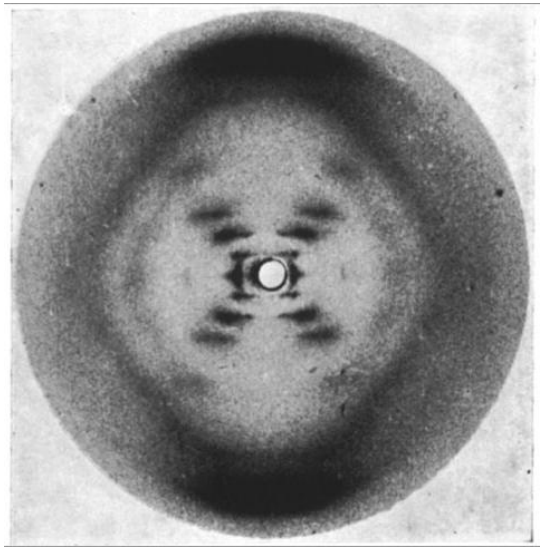


TBIC Meeting - April 3, 2012

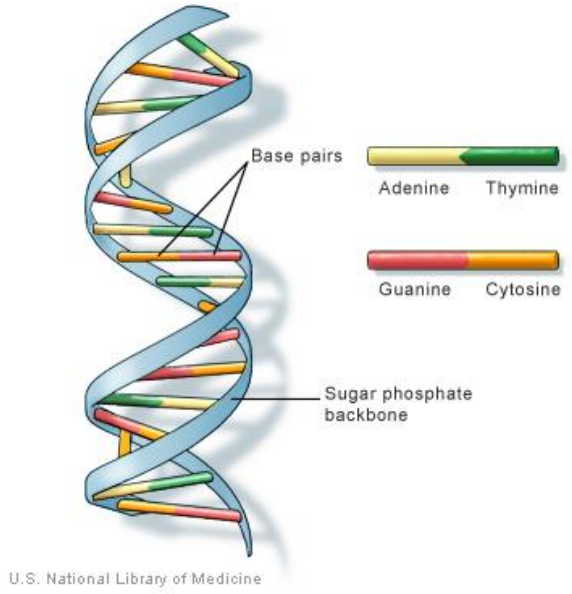
By Navin Sabharwal, Yatsunyk Laboratory
Swarthmore College

But first, some background…

"This structure has novel features which are of considerable biological interest" -Watson and Crick

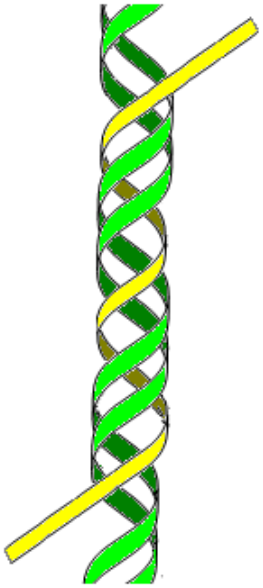


Rosalind Franklin's Photo 51

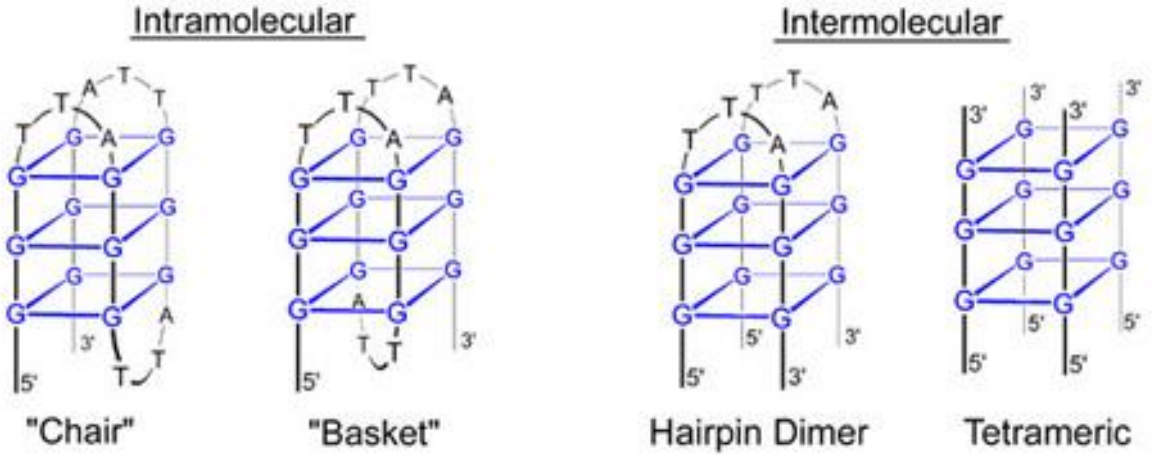


Canonical Watson-Crick Duplex DNA

Novel DNA Structures

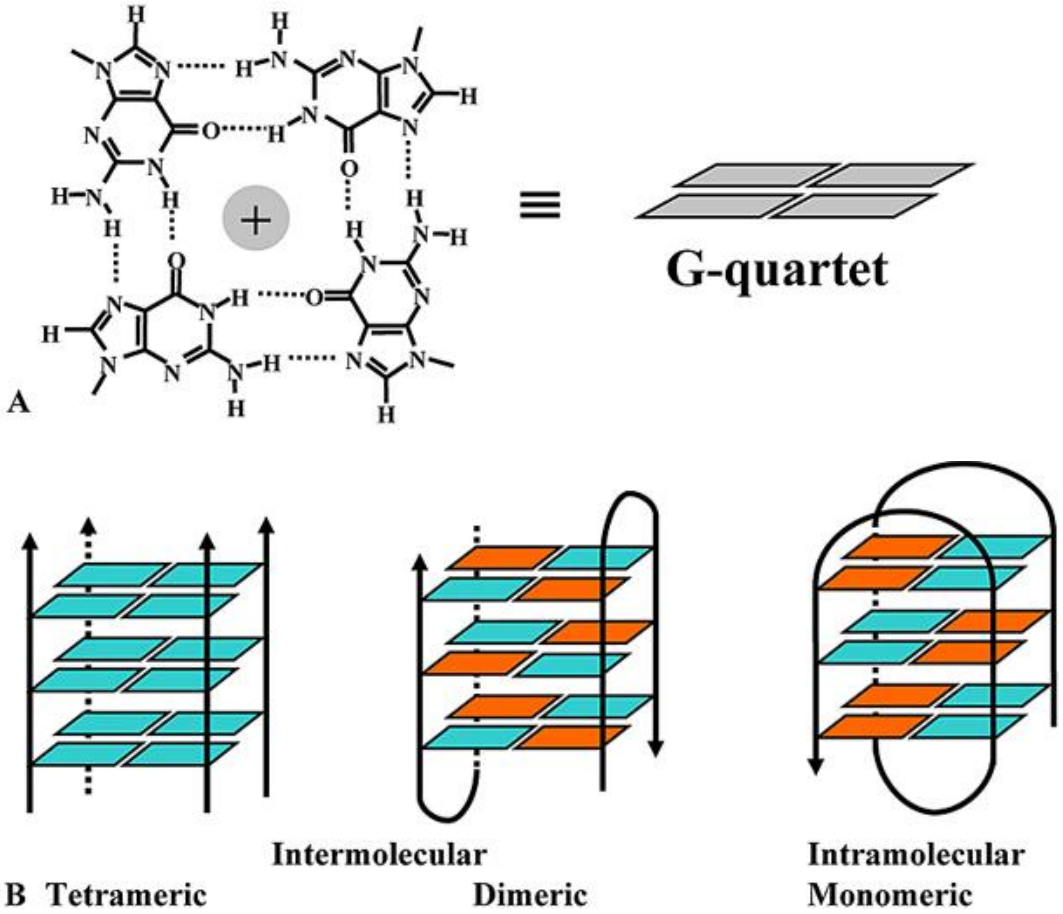


Triplex DNA

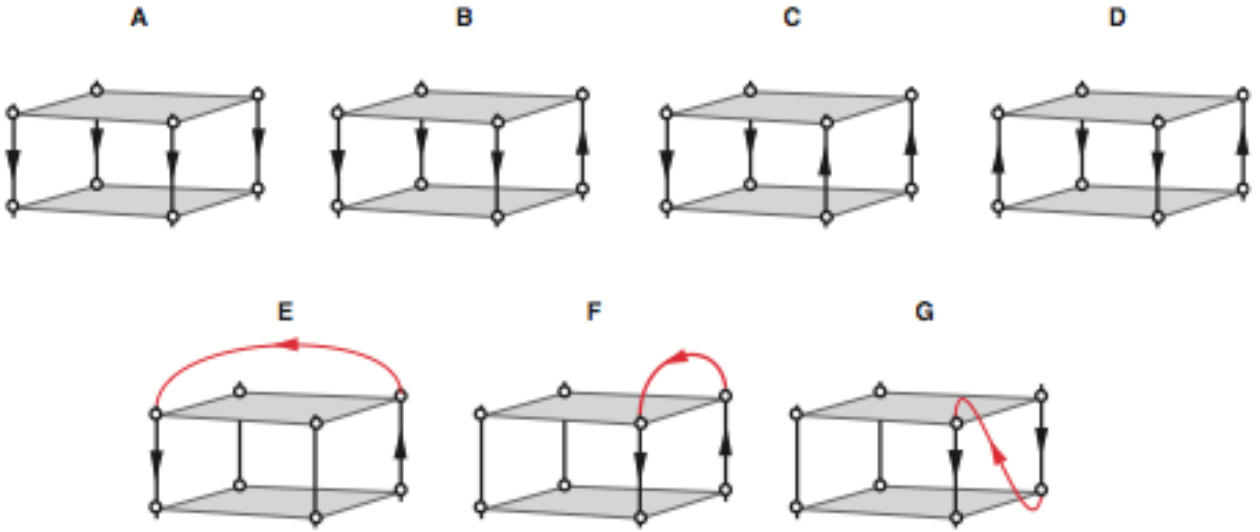


Quadruplex DNA

G-Quadruplex (GQ) DNA

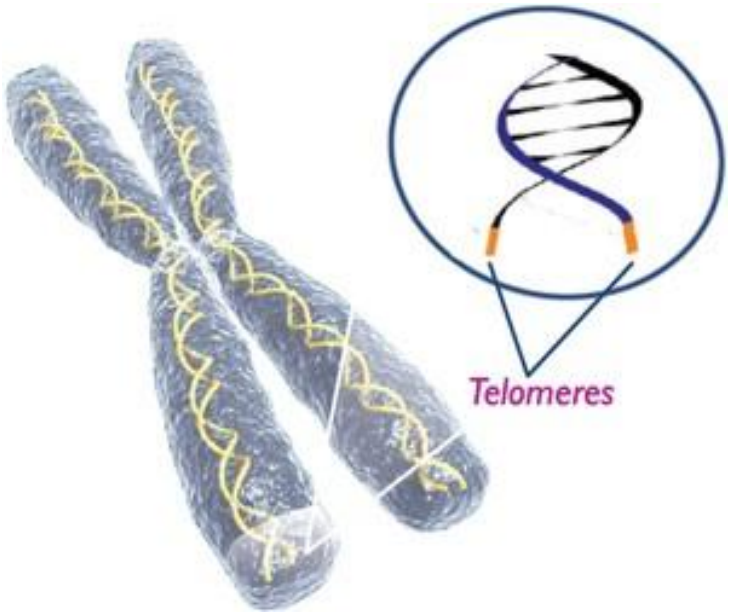


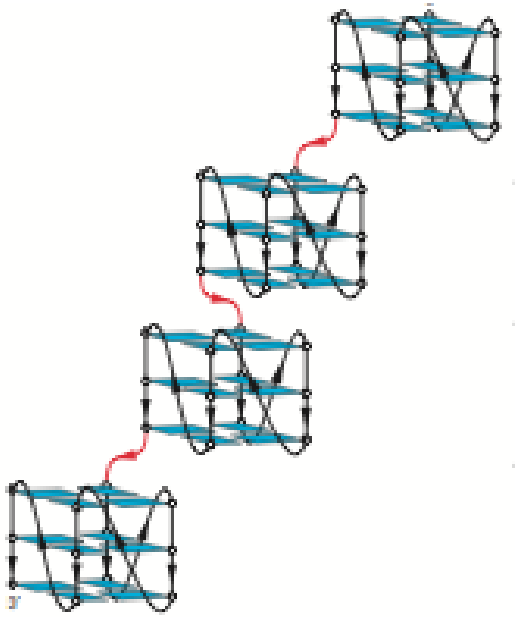
Diversity of GQ DNA



(A) Parallel G-Tetrad Core (B) (3+1) G-Tetrad Core (C) Antiparallel G-Tetrad Core (up-up-down-down) (D) Antiparallel G-Tetrad Core (up-down-up-down) (E) Diagonal Loop (F) Edgewise Loop (G) Double-chain Reversal Loop

Why study GQ DNA?

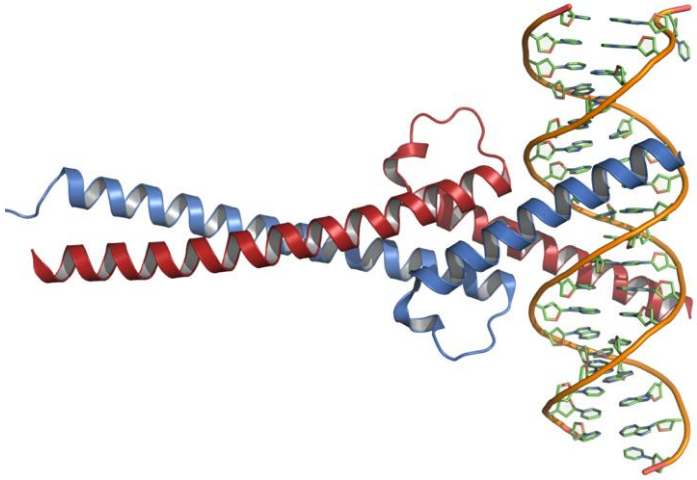




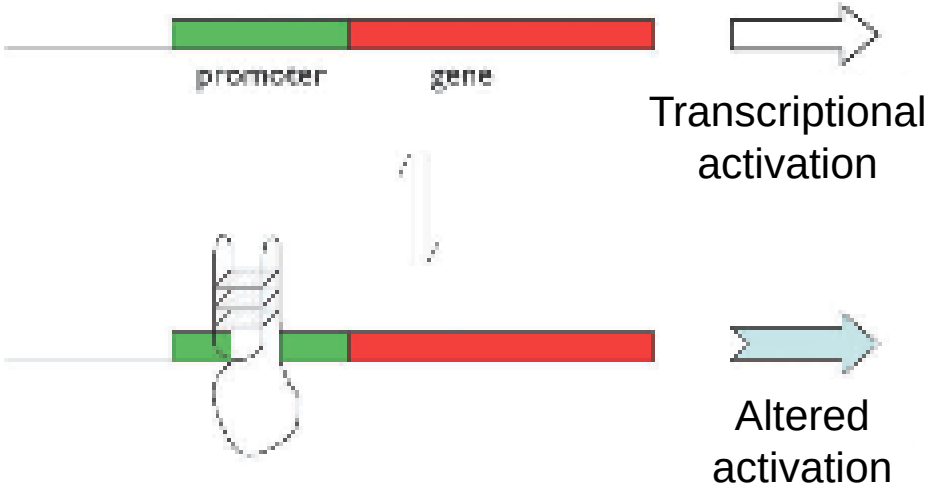
•Telomeres are found at the end of chromosomes.

•Beads-on-a-string model for GQ presence in telomeric DNA sequences.

Regulation of oncogene transcription



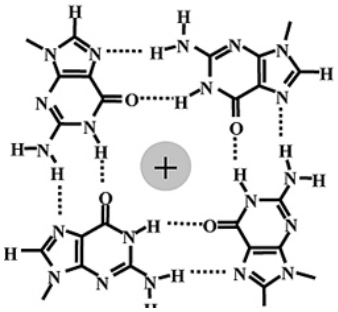
C-myc, an oncogene which regulates expression in 15% of genes. This oncogene is guanine rich and can consequently form GQ DNA

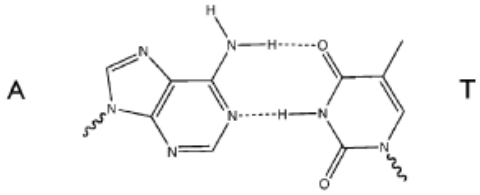


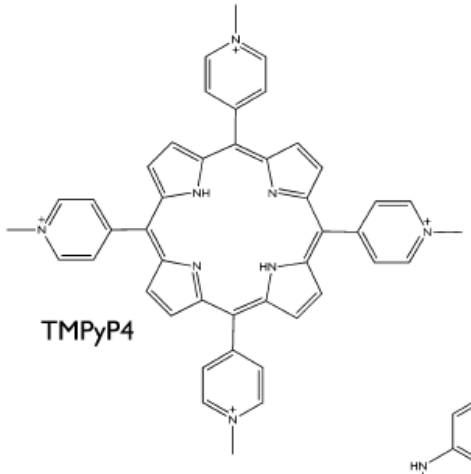
Model for hindrance of oncogene transcription through GQ formation.

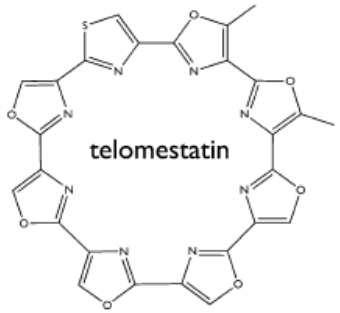
Ligand binding to GQ DNA

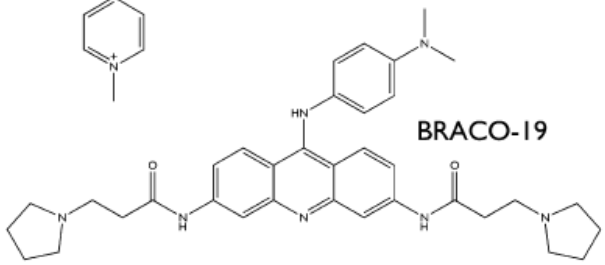
- GQ structures have a large π surface
  - Twice that of duplex DNA
  - Four co-planar bases instead of 2
- Small molecules tend to have large π surfaces
  - Maximize π- π interactions
  - Porphyrins
- GQ DNA has a strong negative charge
  - Cationic ligands will bind tightly
  - Non-specific



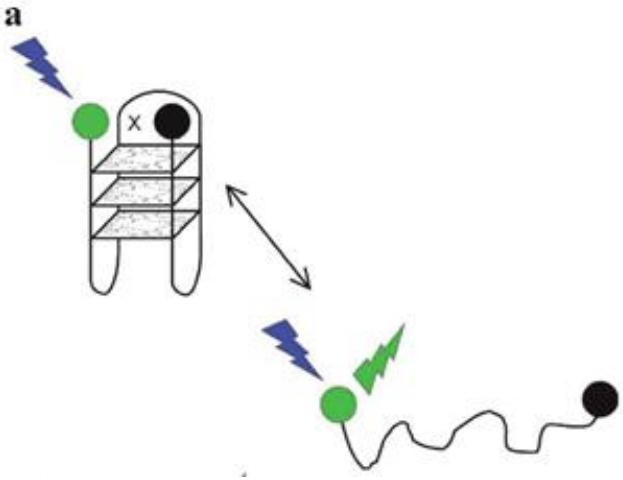


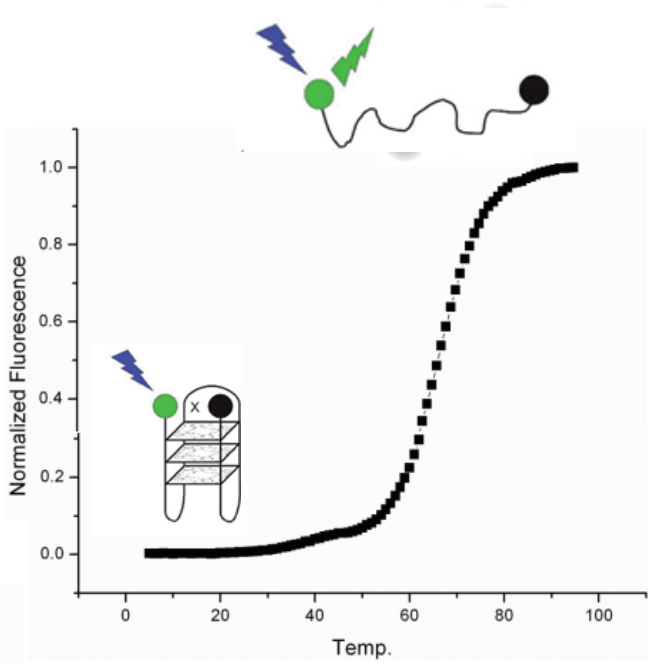


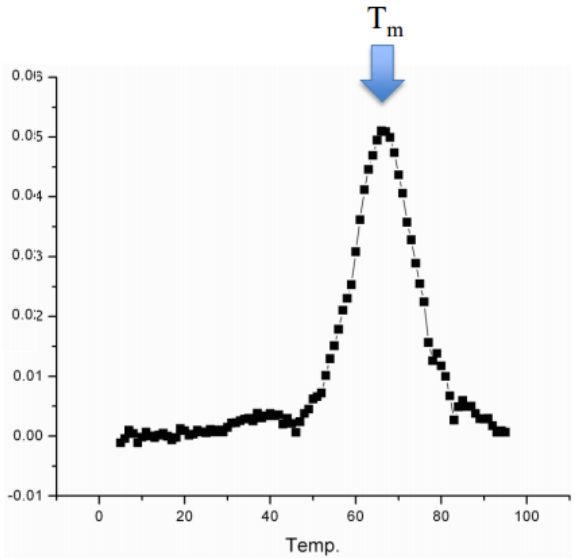




Fluorescent Resonance Energy Transfer (FRET)

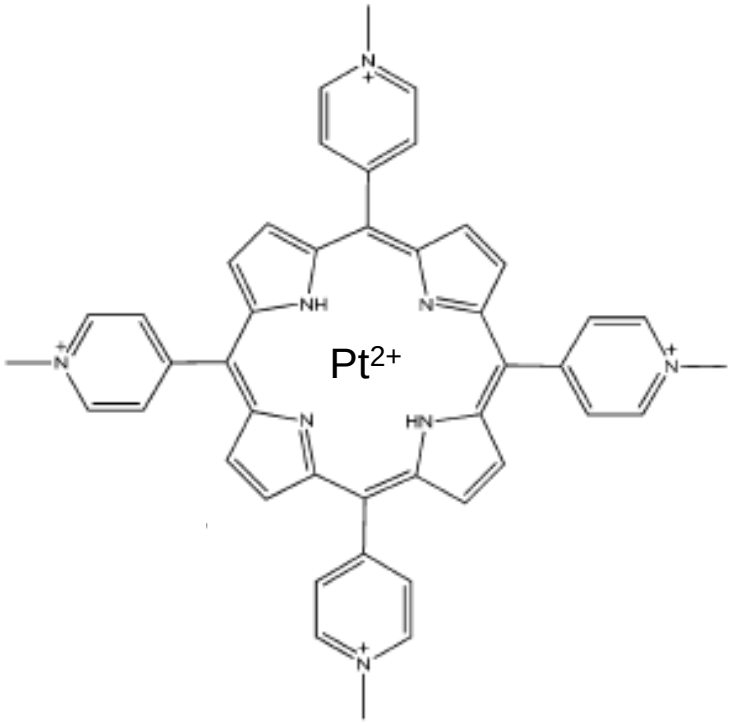






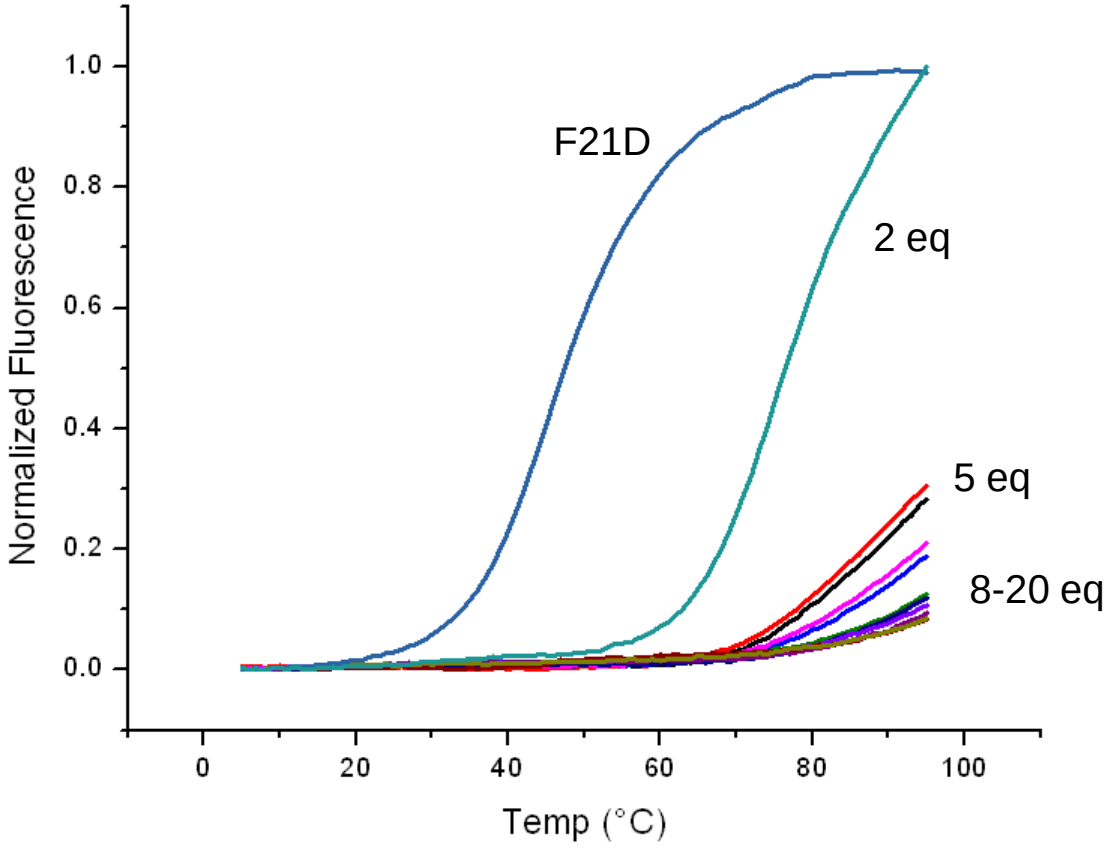
First derivative curve

My ligand – PtT4



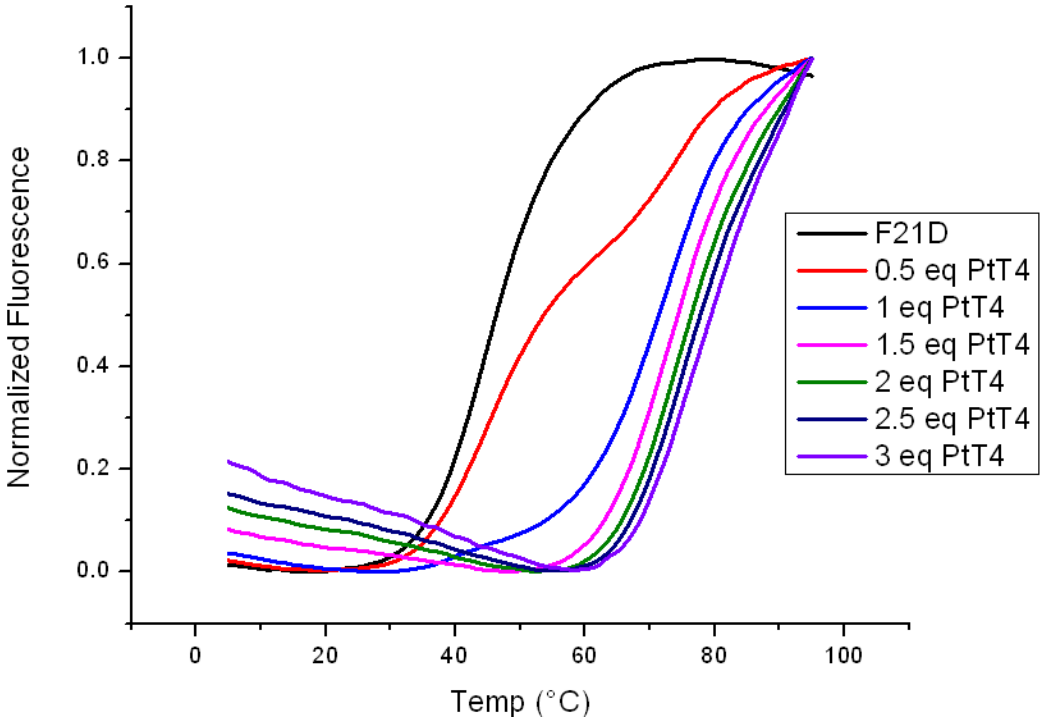
Pt(II)5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21H23H-porphine (PtT4)

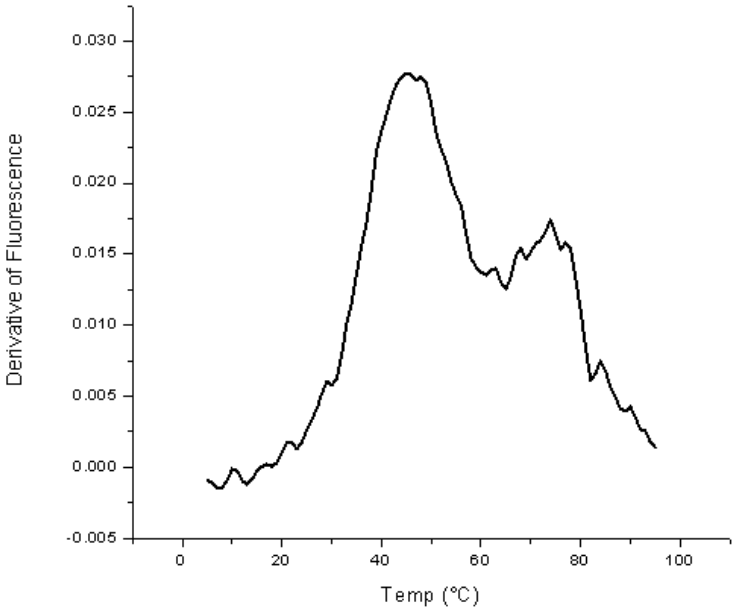
Preliminary FRET Results



Melting curves for 2 to 20 eq PtT4 added are indicative of exceptional GQ stabilizing

Reduced Concentration FRET Results

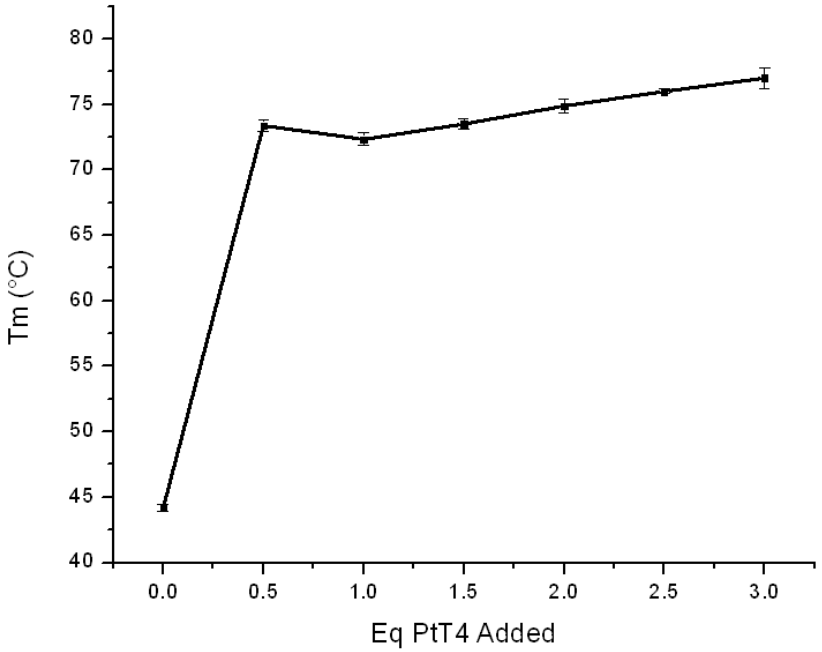




Melting curves for 0 to 3 eq PtT4 added suggest excellent GQ stabilization even at low concentrations.

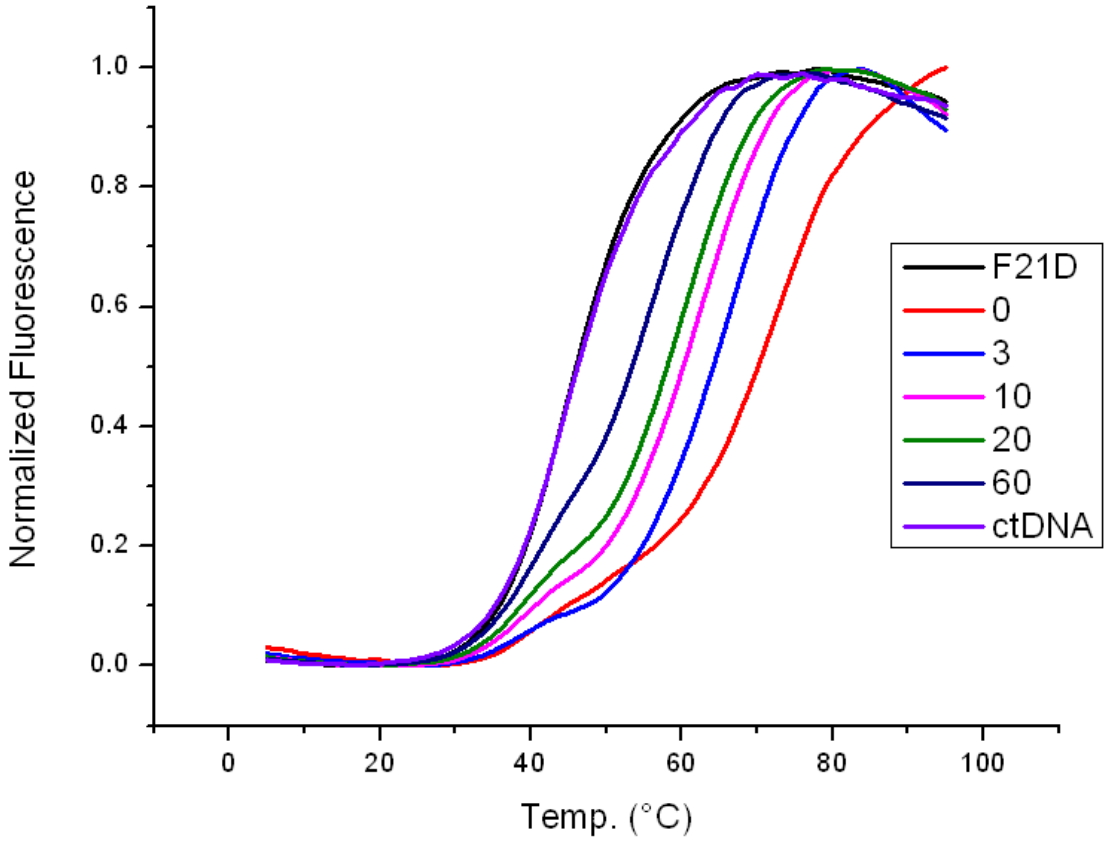
Reduced Concentration FRET results (cont.)

Eq. PtT4 Added	T_m (°C)	**Δ** T_m (rel. to F21d) (°C)
0 (F21D)	44	0
0.5	72.9	28.9
1	72.9	28.9
1.5	73.4	29.4
2	74.5	30.5
2.5	75.9	31.9
3	76.3	32.3



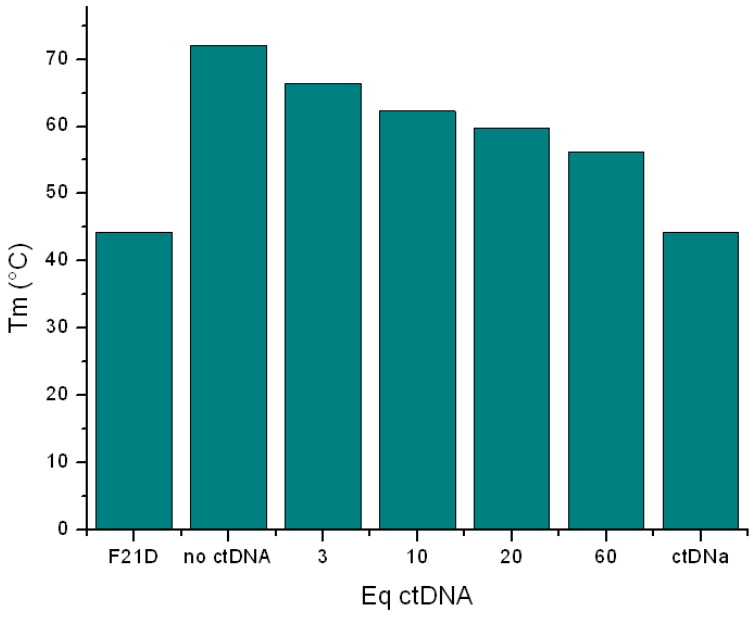
Results indicate excellent GQ stabilization at low PtT4 concentrations.

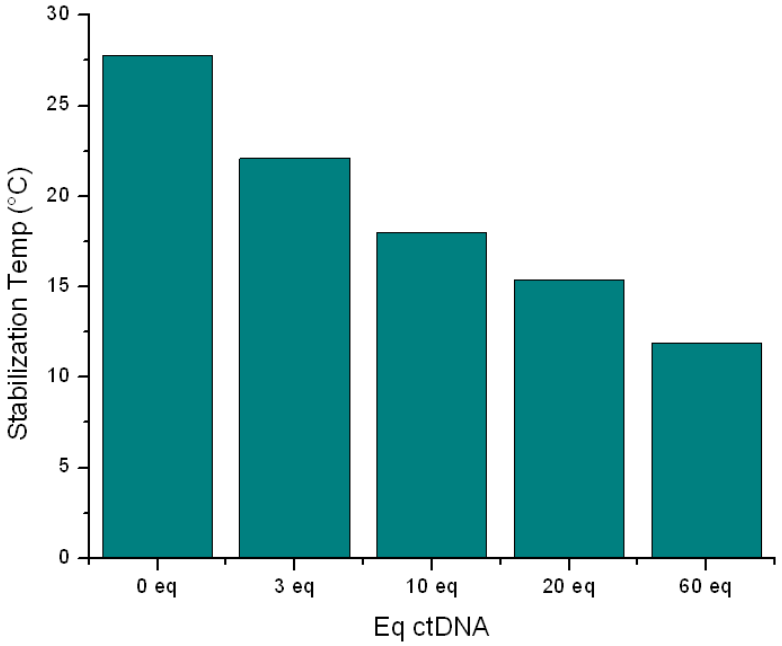
Competition Studies



Addition of duplex calf thymus (CT) DNA to F21D to evaluate PtT4's selectivity for GQ DNA

Competition Studies (continued)





PtT4 is mildly selective for GQ DNA

Acknowledgements

- All members of the Yatsunyk lab
  - Jack Nicoludis
  - Vienna Tran
  - Steven Barrett
  - Cole Harbeck
- Kaplinsky Lab
  - RT-PCR machine
  - Christina Rabeler
- Professor Yatsunyk