Summer 2020

Unpacking Pi Stacking: How electrostatic interactions and aromaticity can be utilized to aid triplex formation in double helical RNA

John M. Talbott
Elizabethtown College, talbottj@etown.edu

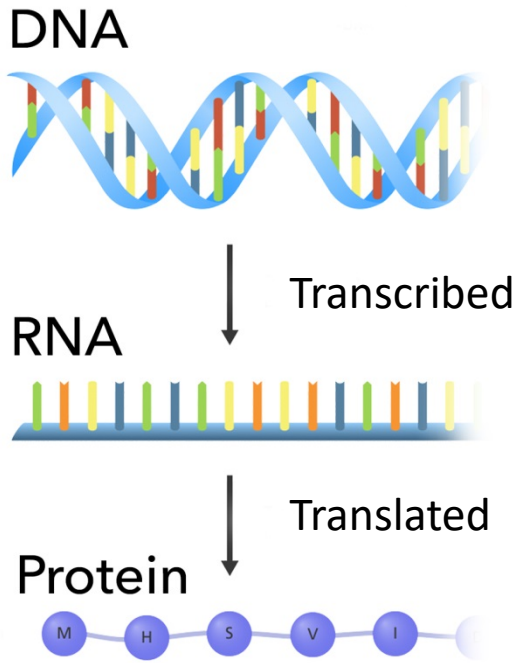
Unpacking Pi Stacking: How electrostatic interactions and aromaticity can be utilized to aid triplex formation in double helical RNA

Presented by: John Talbott

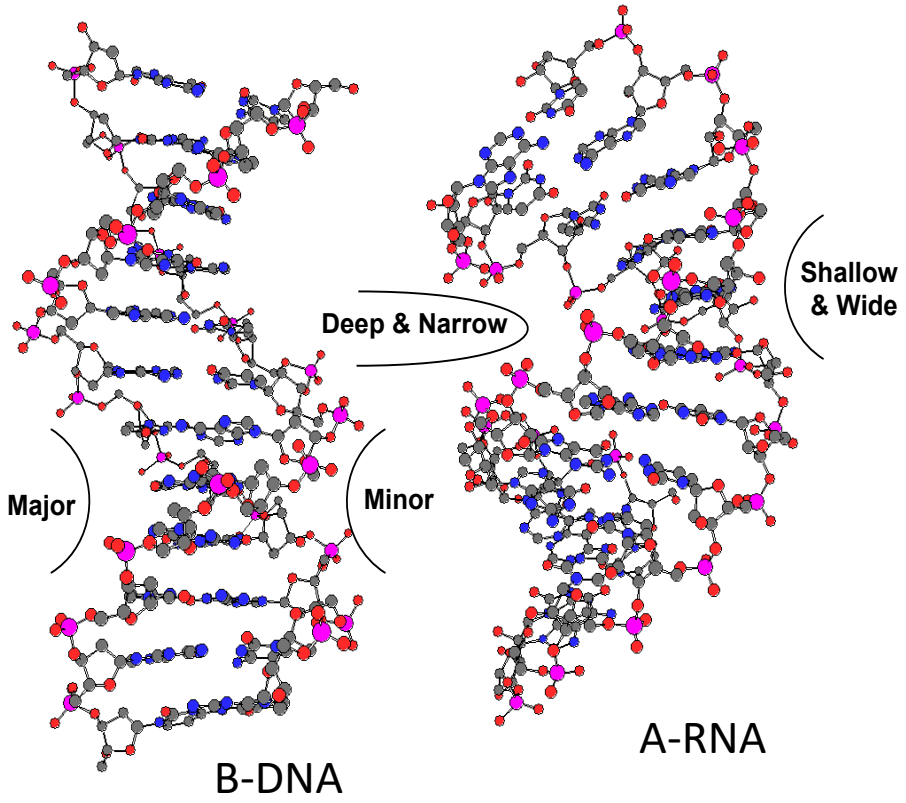
Mentor: Dr. James MacKay

SCARP 2020

Central Dogma

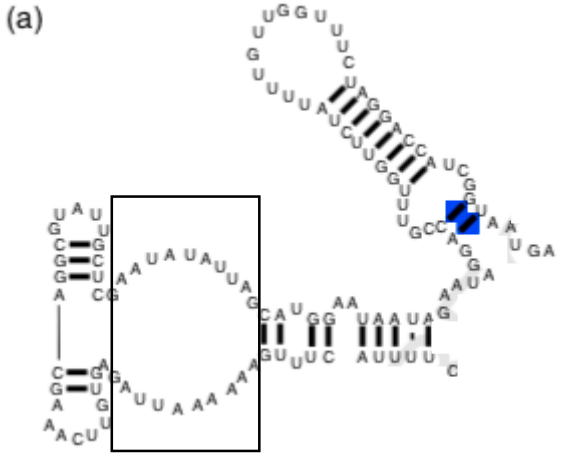


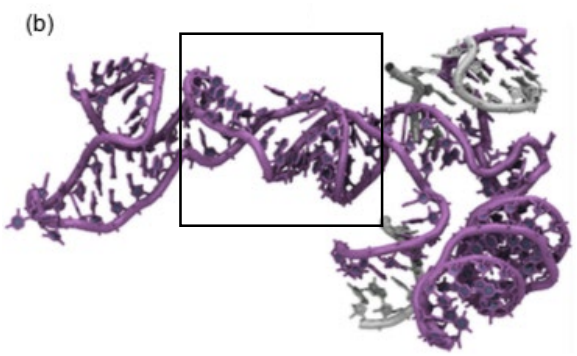
- About 80% of DNA in humans is transcribed into RNA
- However, only ~1.5% of DNA transcribes coding RNA
- RNA that does not translate into proteins is considered ‘non-coding’ RNA



Double Helix Structure of DNA and RNA

Double Helical RNA





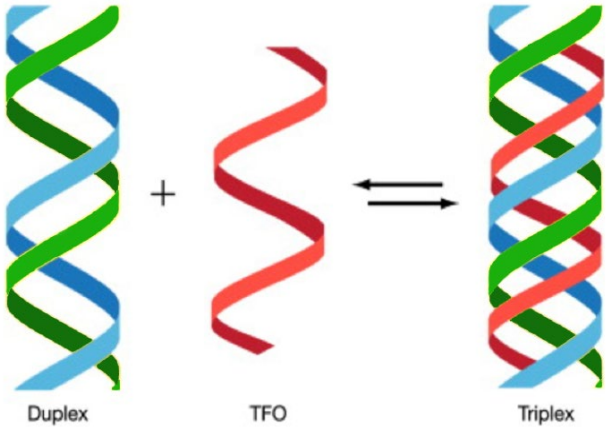
(a) Secondary structure of segment ES6 of the yeast ribosomal subunit
(b) X-ray crystallography of the tertiary structure of the E6S yeast unit

*the boxed areas represent the same sections

Non-Coding RNA has multitude of functions including:

- Catalyzing chemical reactions
- Regulating gene expression
- Post-transcription modification
- Mutations could be linked to diseases

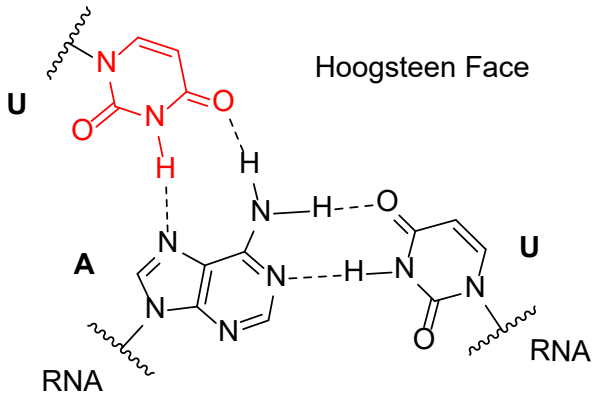
Triplex Formation



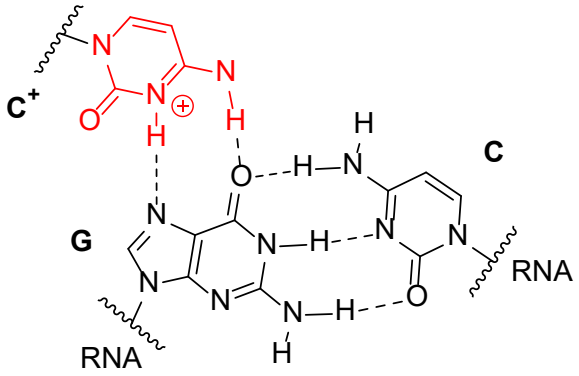
A triplex forming oligonucleotide (TFO) can be used to sequence selectively bind to double helical RNA.

TFOS bind through Hoogsteen hydrogen bonds

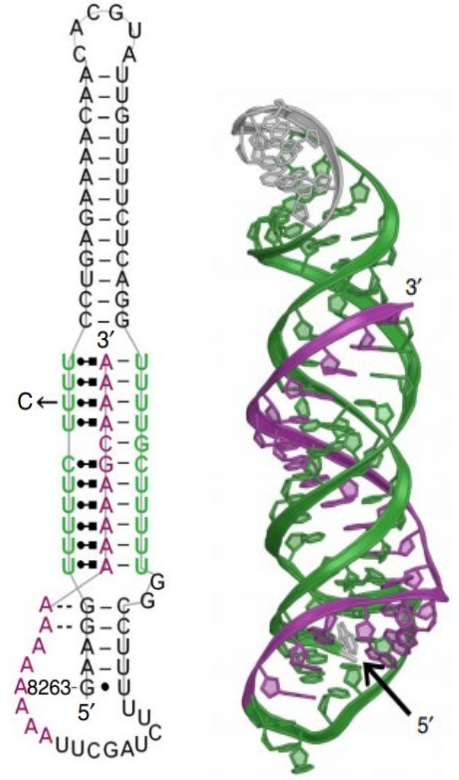
Natural precedents exist with the U*AU and C^+*GC triplets



U*A-U Hoogsteen Triplet

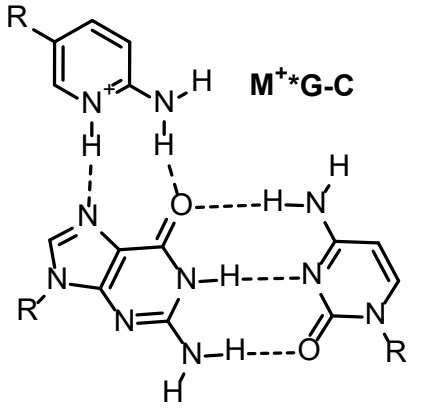


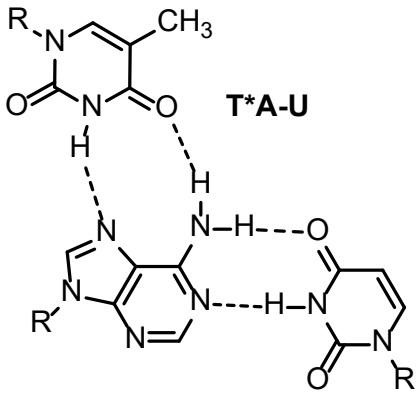
C^+*G-C Hoogsteen Triplet

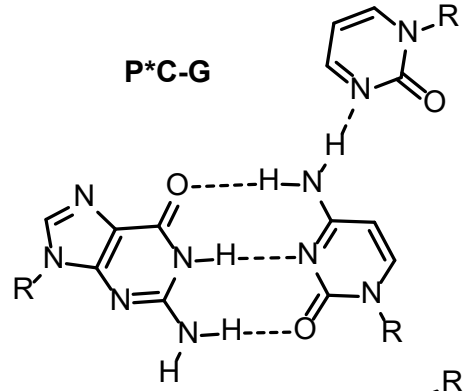


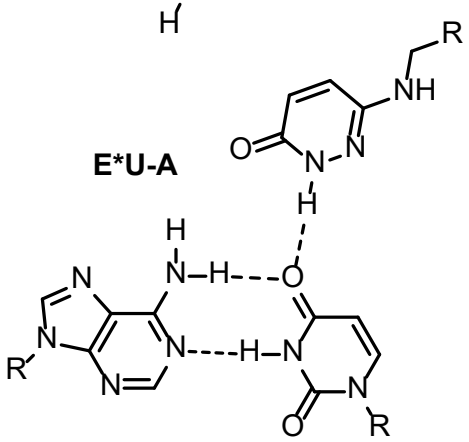
Secondary and Tertiary Structure of MALAT1

Pyrimidine Predicament



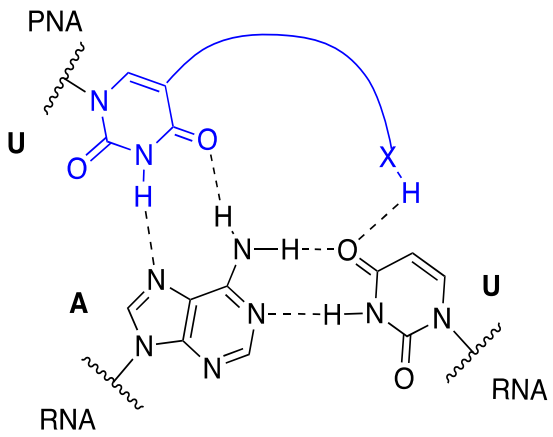




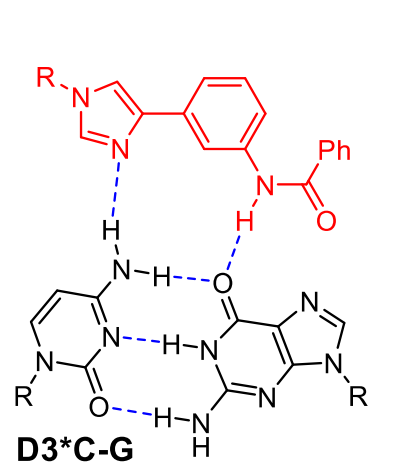


- Purines (A&G) offer 2 sites for hydrogen bonding while pyrimidines (U&C) only offer one
- This leads to difficulty selectively binding to pyrimidines

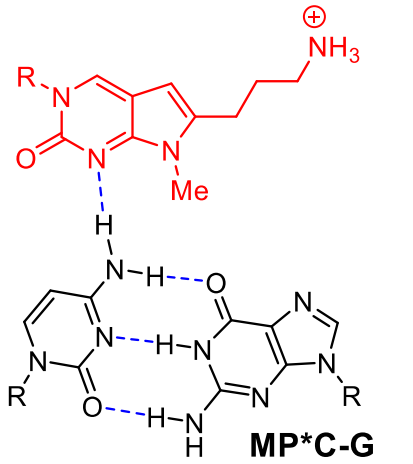
Extended Nucleobases



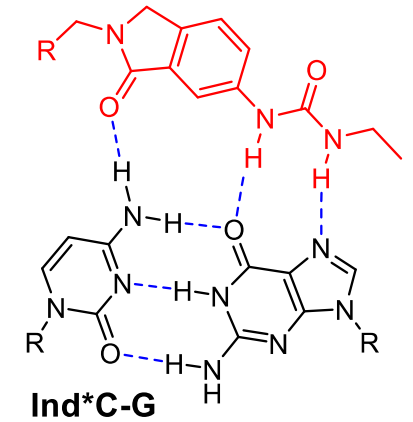
- Bind across the Watson-Crick base pair to afford 3 hydrogen bonds
- This has been previously attempted by multiple groups with mixed results



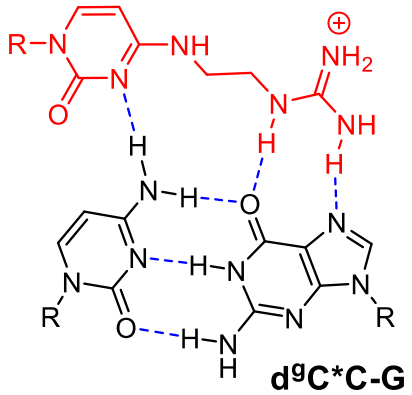
Dervan and co-workers, 1992



Fox, Brown and co-workers, 2005

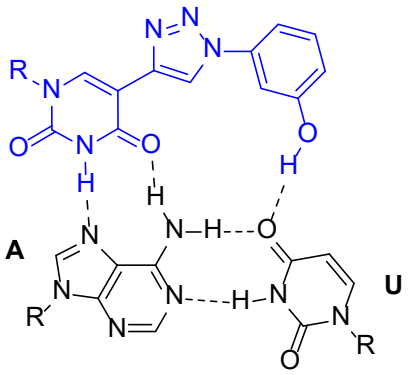


Zimmerman and co-workers, 2004

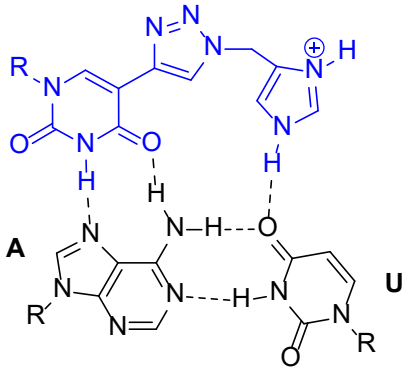


Sekine and co-workers, 2015

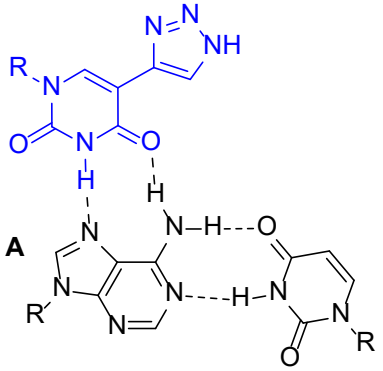
First Generation: T-series



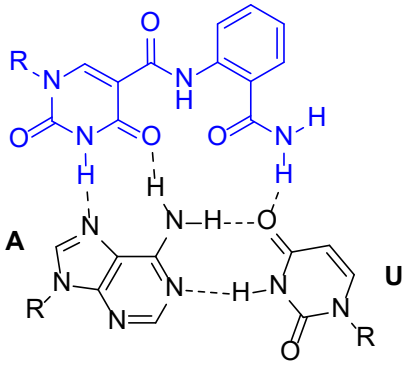
T1*A-U



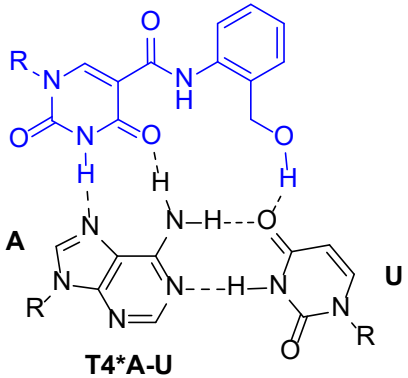
T2*A-U



Tr*A-U



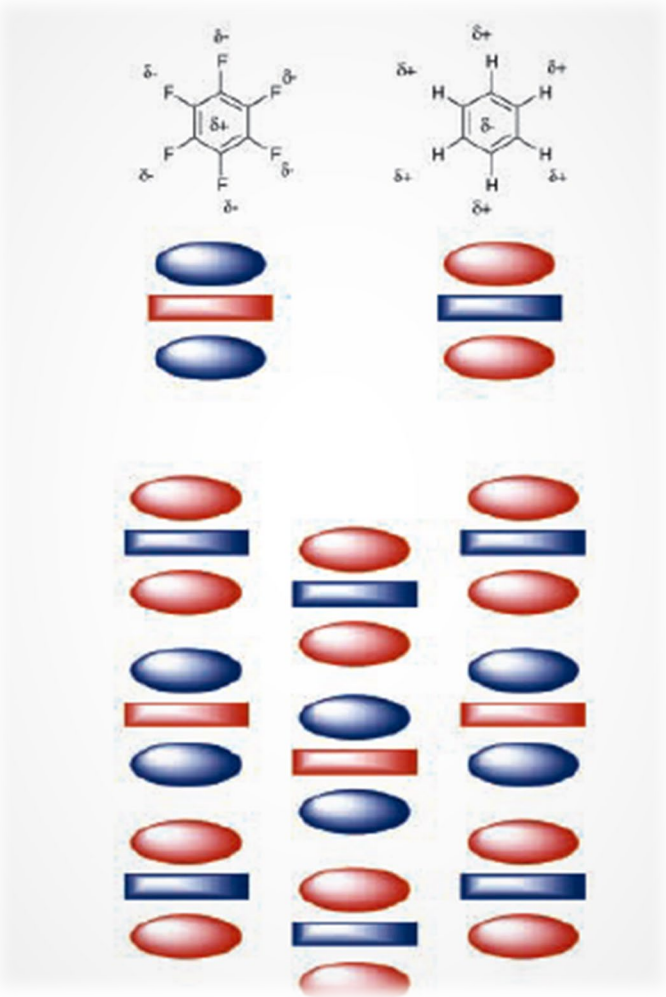
T3*A-U



T4*A-U

- All bases had mediocre binding affinity compared to the control (T)
- Interestingly, Tr without the 3rd hydrogen bond had similar binding affinity to T1-T4

Benzene and hexafluorobenzene



Purple= slightly negative electrostatic area
Red = slightly positive electrostatic area

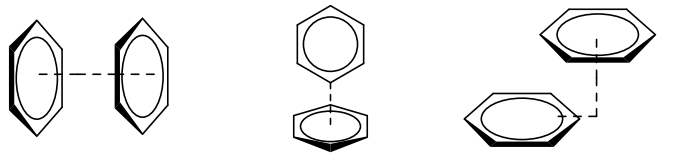
Hexafluorobenzene melting point = 5.2 °C
Benzene melting point = 5.5 °C

1:1 mixture melting point = 23.7 °C

Hunter, C. A.; Lawson, K. R.; Perkins, J.; Urch, C. J. *J. Chem. Soc., Perkin Trans. 2*, **2001**, 651–669.

Pi Stacking

Pi Stacking: electrostatic interaction between the pi systems of two molecules



Parallel-displaced is prevalent in helices due to aromatic nature of nucleic acids

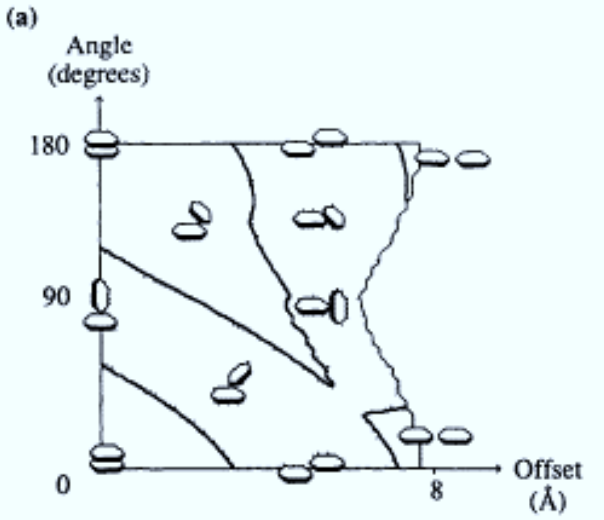
To maximize pi stacking:

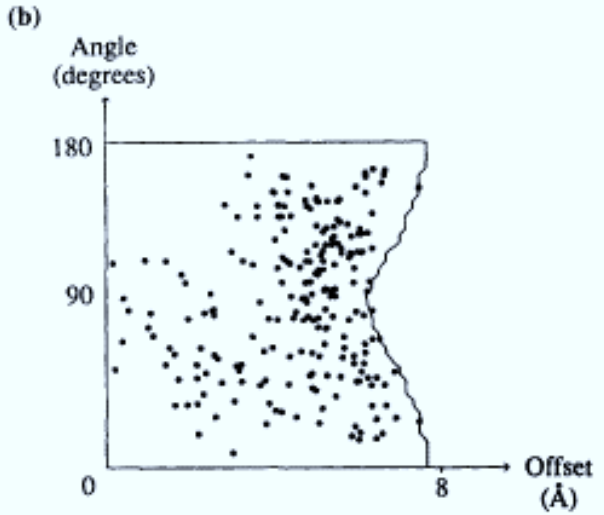
- Planarity
- Extended aromatic systems

Unclear factors:

- Electron withdrawing vs Electron donating groups
- Polarity
- Offset distance

Pi Stacking- Not so simple



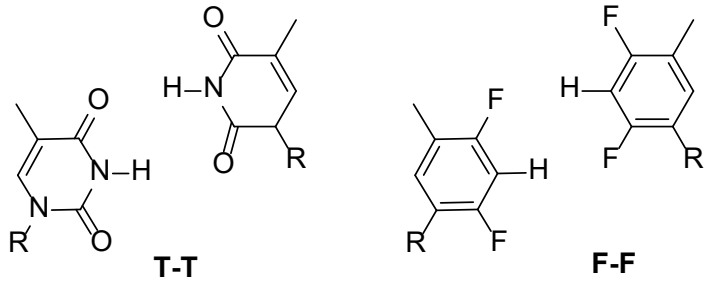


No clear relationship between offset distance (Å) and offset angle for two benzene molecules

Hunter, C. A. *Chem. Soc. Review* **1994**, 101-109.

Assessing the importance of Pi Stacking

- Removing hydrogen bonding capabilities allows for observation of pi stacking interactions



- Kool's lab synthesized nucleobase **F**, an isostere of **T**
- They have nearly identical electrostatics

Schweitzer, B. A. & Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*(7), 1863-1872.

duplex	T_m (°C) [a]	$-\Delta G°_{25}$ (kcal)
5'-C T T T T C T T T C T T 3'-G A A A A G A A A G A A	39.4	12.3
5'-C T T T T C T T T C T T 3'-G A A A A G C A A G A A	26.4	8.7
5'-C T T T T C T T T C T T 3'-G A A A A G G A A G A A	30.7	9.3
5'-C T T T T C T T T C T T 3'-G A A A A G T A A G A A	27.1	8.9
5'-C T T T T C F T T C T T 3'-G A A A A G A A A G A A	21.4	7.4
5'-C T T T T C F T T C T T 3'-G A A A A G C A A G A A	25.0	8.2
5'-C T T T T C F T T C T T 3'-G A A A A G G A A G A A	23.0	8.0
5'-C T T T T C F T T C T T 3'-G A A A A G T A A G A A	20.2	7.3
5'-C T T T T C T T T C T T 3'-G A A A A G A A A G A A	39.4	12.3
5'-F F C T T T T C T T T C T T F F 3'-F F G A A A A G A A A G A A F F	50.2	14.9
5'-C T T T T C F F F F T T T C T T 3'-G A A A A G F F F F A A A G A A	37.7	11.5

- Internal **F** was very destabilizing, but external **F** was stabilizing
- Interestingly, 1 internal **F** was extremely destabilizing, but 4 internal **F** was only minimally destabilizing

Takeaway

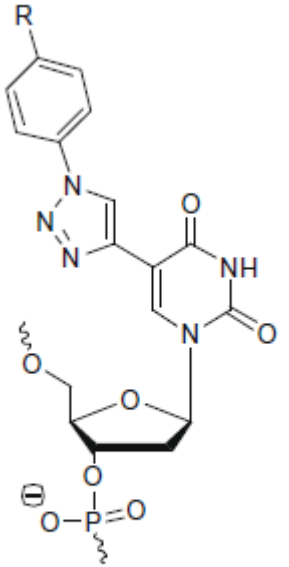
- Nonpolar bases require too much energy
- Need to utilize a more polar base to avoid spending energy expelling H_2O molecules

Schweitzer, B. A. & Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*(7), 1863-1872.

Impact of EWG vs EDG

		T_m (ΔT_m/mod.)/°C[b]		
		B = X	Y	Z
ON1	5′-dGTG TBT TGC	29.0[c] (−2.0)	30.5 (−0.5)	30.0 (−1.0)
ON2	5′-dGTG BTT TGC	30.0 (−1.0)	29.0 (−2.0)	29.0 (−2.0)
ON3	5′-dGTG BBT TGC	35.0 (+2.0)	37.0 (+3.0)	35.5 (+2.3)
ON4	5′-dGTG TBB TGC	37.5 (+3.3)	39.0 (+4.0)	39.0 (+4.0)
ON5	5′-dGTG BBB TGC	43.0 (+4.0)	45.0 (+4.7)	46.0 (+5.0)
ON6	5′-dGTG BBB BGC	51.5[c] (+5.1)	51.0 (+5.0)	55.5 (+6.1)

EWG (Z) had better binding compared to neutral (Y) and EDG (X)



X R = H
Y R = OH
Z R = SO_2NH_2

Takeaway

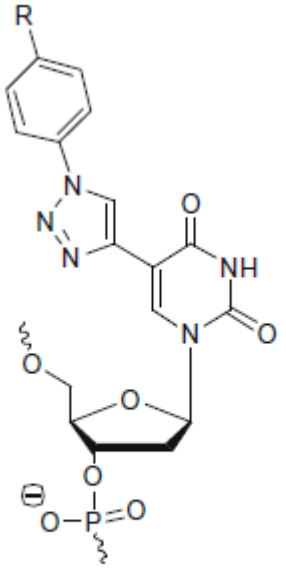
- EWG potentially better but only minimal difference

Anderson, N. K.; Chanduk, N.; Bruilikova, L.; Kumar, P.; Jensen, M. D.; Jensen, F.; Sharma, P. K.; Nielsen, P. *BioOrg. Med. Chem.* **2010**, *18*, 4702-4710.

Impact of Consecutive Bases

		T_m (ΔT_m/mod.)/°C[b]		
		B = X	Y	Z
ON1	5′-dGTG TBT TGC	29.0[c] (−2.0)	30.5 (−0.5)	30.0 (−1.0)
ON2	5′-dGTG BTT TGC	30.0 (−1.0)	29.0 (−2.0)	29.0 (−2.0)
ON3	5′-dGTG BBT TGC	35.0 (+2.0)	37.0 (+3.0)	35.5 (+2.3)
ON4	5′-dGTG TBB TGC	37.5 (+3.3)	39.0 (+4.0)	39.0 (+4.0)
ON5	5′-dGTG BBB TGC	43.0 (+4.0)	45.0 (+4.7)	46.0 (+5.0)
ON6	5′-dGTG BBB BGC	51.5[c] (+5.1)	51.0 (+5.0)	55.5 (+6.1)

All 3 bases should increased binding with sequential bases



X R = H
Y R = OH
Z R = SO_2NH_2

Takeaway

- Need multiple extended bases in a row to afford good binding

Anderson, N. K.; Chanduk, N.; Bruilikova, L.; Kumar, P.; Jensen, M. D.; Jensen, F.; Sharma, P. K.; Nielsen, P. *BioOrg. Med. Chem.* **2010**, *18*, 4702-4710.

Impact of Consecutive Bases

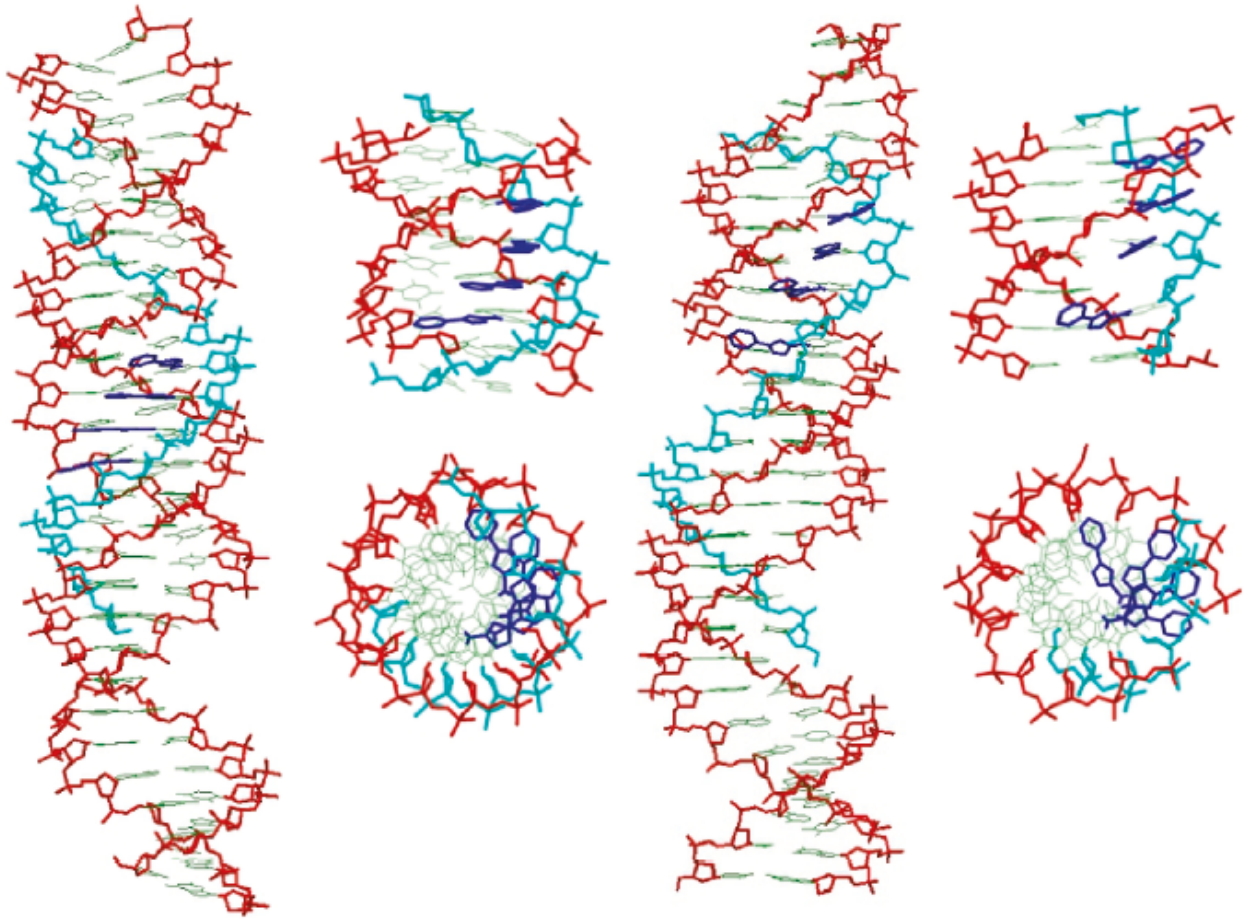


Figure 6. Modeling structures of modified triplexes. (a) ON7:dsDNA and (b) ON8:dsDNA; full structures, and modified sections in side view and top view. Cyan = backbone of the TFO; blue = 5-substituents of the TFO; red = backbone of the dsDNA; green = nucleobases.

Melting value (T_m/mod)

Control: 28.0

ON7(left): 35.5 (+1.9)

ON8(right): No binding detected

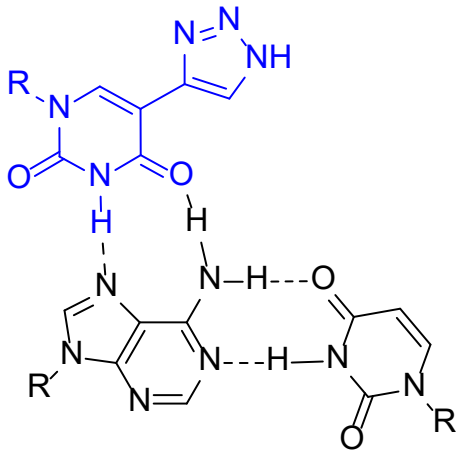
Takeaways

- Pi stacking requires the bases to overlap and thus we need multiple extended bases to overlap with each other
- Planarity is essential

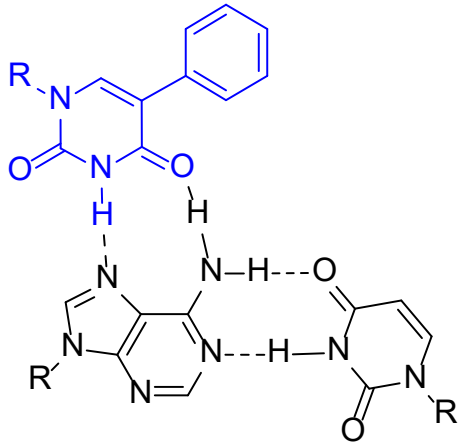
Anderson, N. K.; Dossing, H.; Jensen, F.; Vester, B.; Nielsen, P. *J. Org. Chem.* **2011**, *76*, 6177-6187.

Conclusions

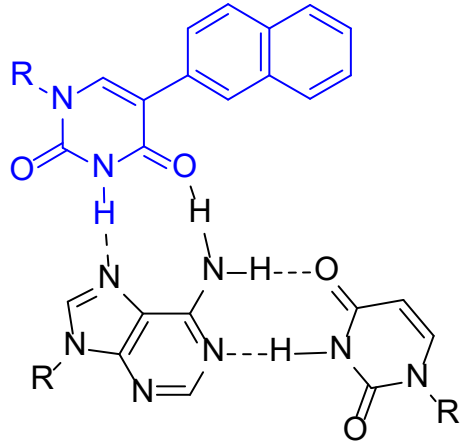
- Planarity is essential
- Multiple extended bases in a row is necessary to afford proper binding affinity
- Need bases with similar polarity



Tr*A-U



N-phenylisoorotamide*A-U

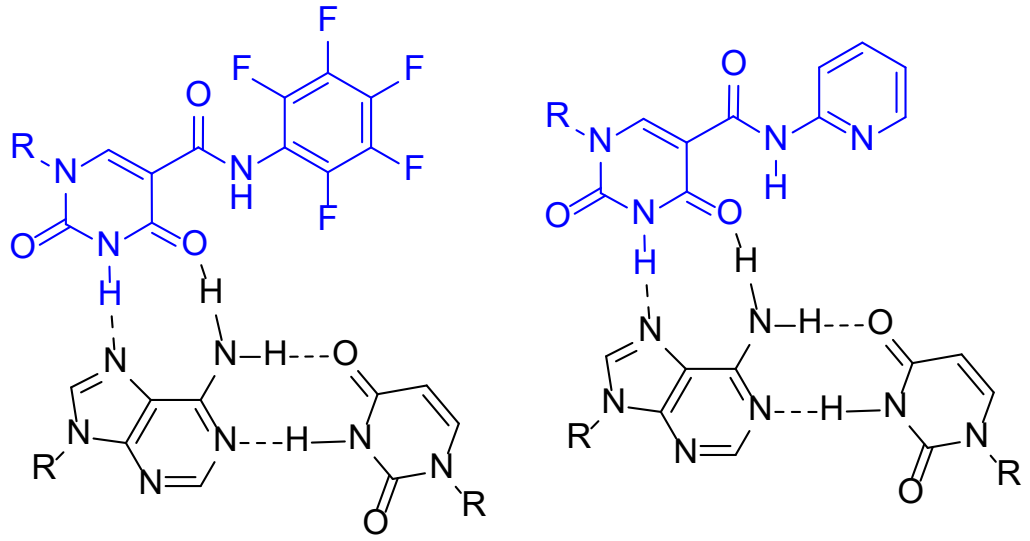


N-naphthaleneisoorotamide*A-U

- These bases have been or are currently being synthesized in Dr. MacKay's lab

Future Work

- Finish synthesis of N-phenylisoorotamide and N-naphthaleneisoortamide and complete binding studies
- Complete preliminary computational analysis on future bases below



Acknowledgements

- Elizabethtown College Department of Chemistry and Biochemistry
- MacKay Research Group
    - Emily Harding
    - Emily Kagarise
    - Aubrey Maryniak
    - Brandon Tessier
- Rozners Research Group, Binghamton University
- Summer Scholarship, Creative Arts and Research Program (SCARP)
- NSF CHE - 1708699