CAN YOU PREDICT THE THERMODYNAMIC STABILITY OF A DNA DUPLEX?

YES

For example, how do you design DNA oligonucleotides for PCR, site-directed mutagenesis, or sequencing?

There are tables of “Nearest Neighbor” energies that are used by software packages from IDT (for example).

How do they work?

Table 1: Nearest-Neighbor Thermodynamic Parameters for Watson–Crick Base Pair Formation in 1 M NaCl[a]

propagation sequence	ΔH° (kcal/mol)	ΔS° (eu)	ΔG°_{37} (kcal/mol)
AA/TT	-7.9 ± 0.2	-22.2 ± 0.8	-1.00 ± 0.01
AT/TA	-7.2 ± 0.7	-20.4 ± 2.4	-0.88 ± 0.04
TA/AT	-7.2 ± 0.9	-21.3 ± 2.4	-0.58 ± 0.06
CA/GT	-8.5 ± 0.6	-22.7 ± 2.0	-1.45 ± 0.06
GT/CA	-8.4 ± 0.5	-22.4 ± 2.0	-1.44 ± 0.04
CT/GA	-7.8 ± 0.6	-21.0 ± 2.0	-1.28 ± 0.03
GA/CT	-8.2 ± 0.6	-22.2 ± 1.7	-1.30 ± 0.03
CG/GC	-10.6 ± 0.6	-27.2 ± 2.6	-2.17 ± 0.05
GC/CG	-9.8 ± 0.4	-24.4 ± 2.0	-2.24 ± 0.03
GG/CC	-8.0 ± 0.9	-19.9 ± 1.8	-1.84 ± 0.04
init. w/term. G–C[b]	0.1 ± 1.1	-2.8 ± 0.2	0.98 ± 0.05
init. w/term. A–T[b]	2.3 ± 1.3	4.1 ± 0.2	1.03 ± 0.05
symmetry correction	0	-1.4	0.4

[a] Errors are resampling standard deviations (see text). [b] See text for how to apply the initiation parameters.

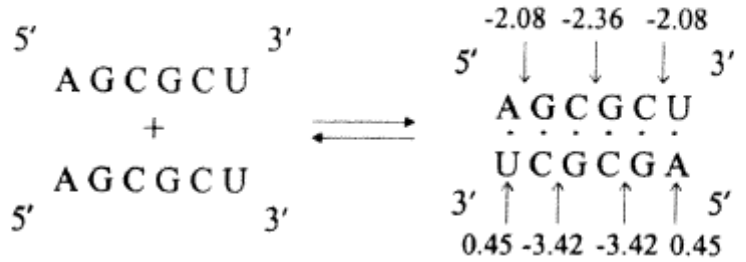
The experimental observation was that thermodynamic stability of a given base pair depends on its sequence **and** the flanking base pairs (hence the nearest neighbor).

So: Synthesize DNA and RNA oligos, and measure their thermodynamic stability. [How many oligos?]

Measure their enthalpy and calculate the entropy and free energy. Use 1 M NaCl. pH 7.0

AN RNA EXAMPLE

(a)



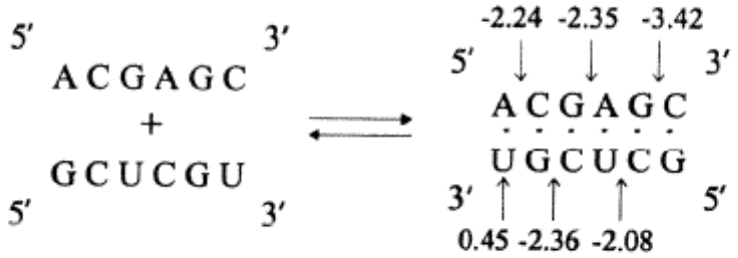
$$\Delta G^\circ_{TOT} = \Delta G^\circ_{INIT} + \Delta G^\circ_{SYM} + \sum \Delta G^\circ_{NN} + 2\Delta G^\circ_{TERM-AU}$$

$$= 4.09 + 0.43 + (-13.36) + 2 \times 0.45$$

$$= -7.94 \text{ kcal / mol}$$

Strands are self-complementary

(b)



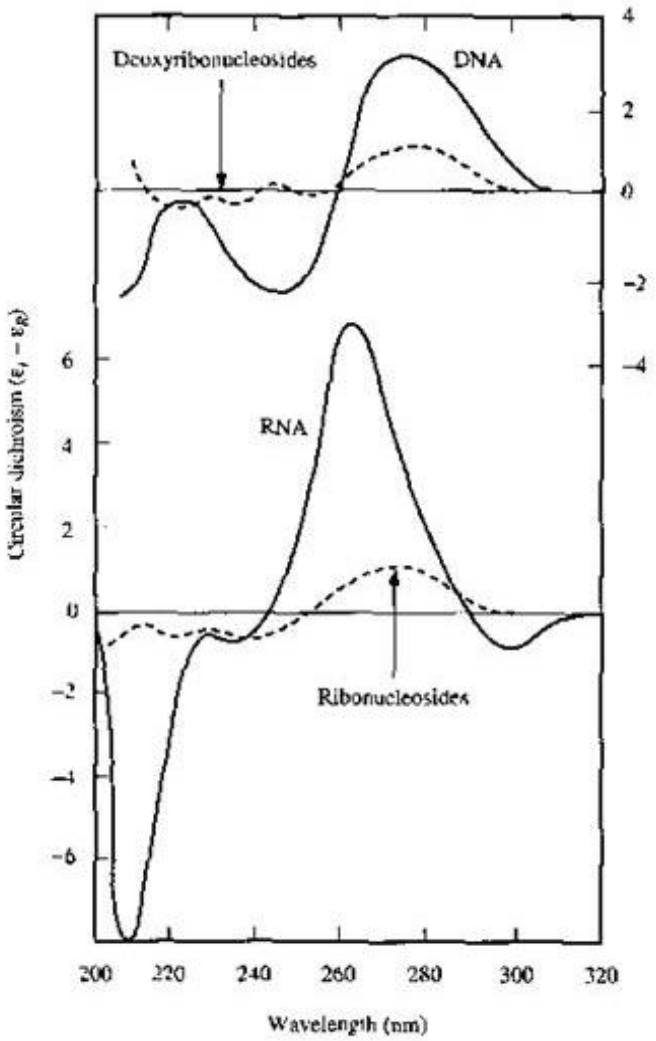
$$\Delta G^\circ_{TOT} = \Delta G^\circ_{INIT} + \Delta G^\circ_{SYM} + \sum \Delta G^\circ_{NN} + \Delta G^\circ_{TERM-AU}$$

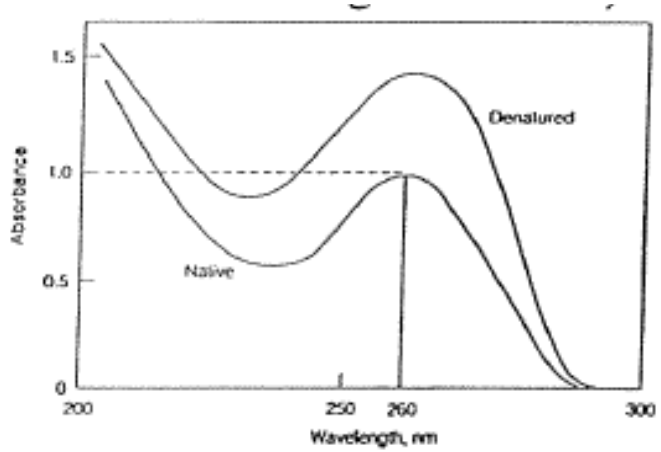
$$= 4.09 + 0 + (-12.45) + 0.45$$

$$= -7.91 \text{ kcal / mol}$$

Strands are non self-complementary

EXERCISE for Oct 20 Discussion Section
Replace U's with T's and do these calculations for DNA. Use the data from the table, and compare it with results from the IDT website. Be prepared to explain your results.





ASSIGNMENT For Discussion section Oct 20

- Could you use UV absorbance to distinguish between RNA and DNA in solution? Why or why not?
- Draw a CD spectrum of a single-stranded DNA.
- Draw a CD spectrum of a single-stranded RNA.
- How do you interpret the CD spectra of the ribo/deoxy nucleotides?
- Compare a typical absorbance spectrum of a purified protein (choose your favorite) with that of a dsDNA oligo from 350-200 nm. Assume 10 μM of each.
- Sketch the UV absorption spectra and the CD spectra of a native and denatured α/β sandwich protein (analogous to the DNA absorbance spectrum).
- Comment on your answers to explain your reasoning.