Convergence?

Stability of RNA hairpins

Joanna Sarzynska &Tadeusz Kulinski, Poznan, Poland

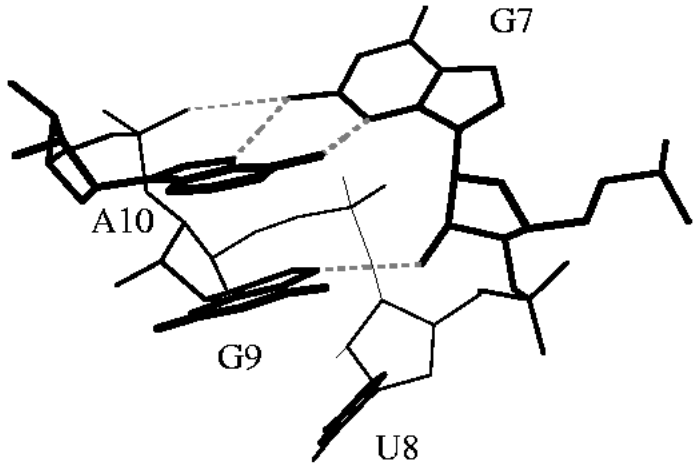
Lennart Nilsson, Karolinska Institutet

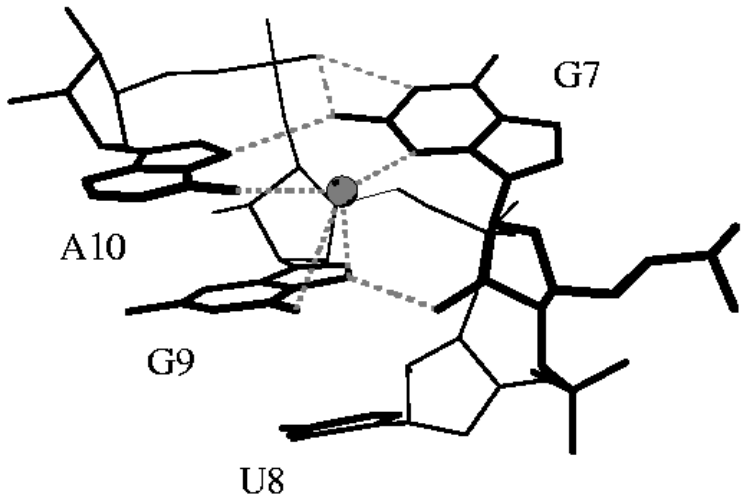
RNA Tetraloops – common motifs involved in tertiary contacts, protein binding, folding initiation

Of 256 possible tetraloops, 12 dominate in nature.

UNCG, GNRA, CUYG

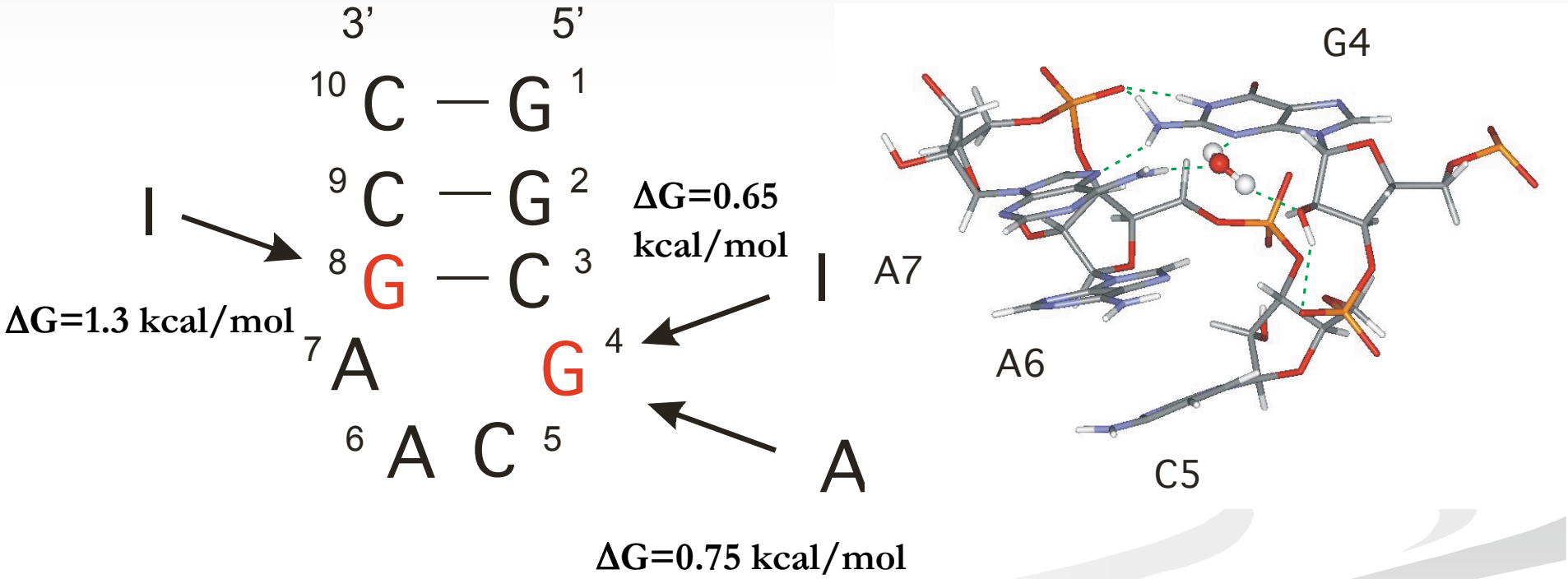
~50% of rRNA tetraloops are GNRA loops:





Stability of a GNRA tetraloop

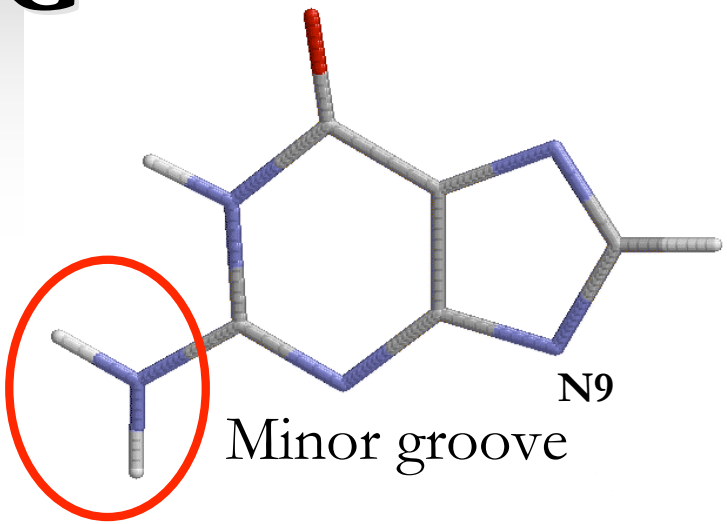
experimental destabilizing effects of point mutations



Themodynamics: SantaLucia Jr., J., Kierzek, R. & Turner, D.H. (1992) Science **256**, 217-219.

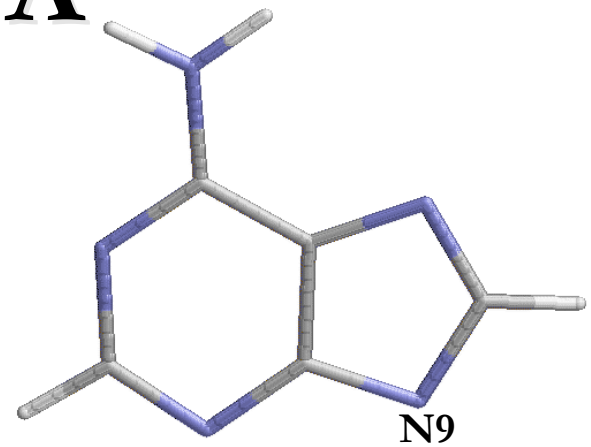
NMR (wt 1zih): Jucker FM, Heus HA, Yip PF, Moors EHM &Pardi A. (1996) J. Mol. Biol. **264**, 968-980.

G

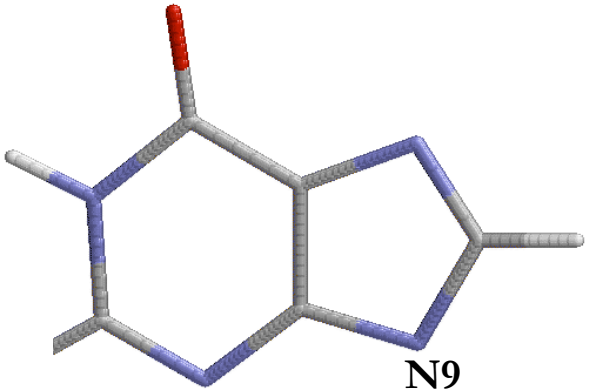


Minor groove

A



Minor groove



Minor groove

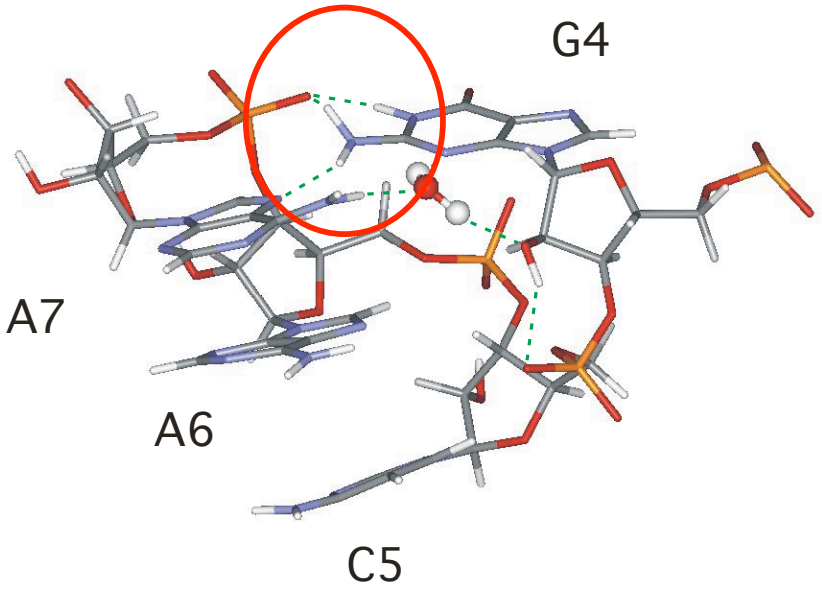
I

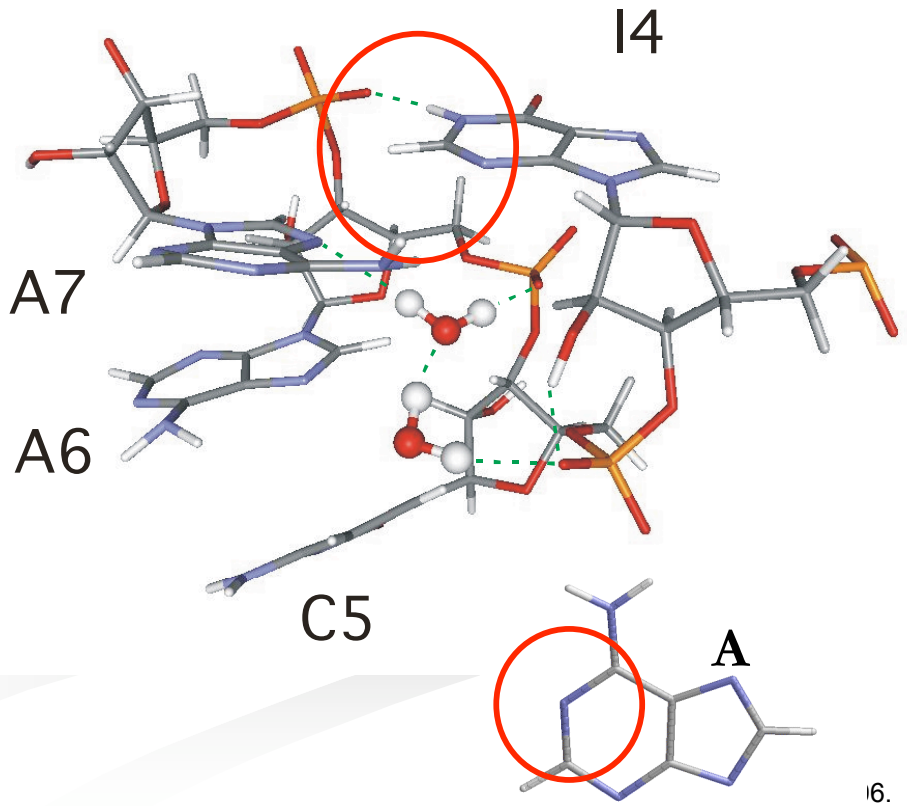
What is happening?

G → I: removal of NH_2 in minor groove

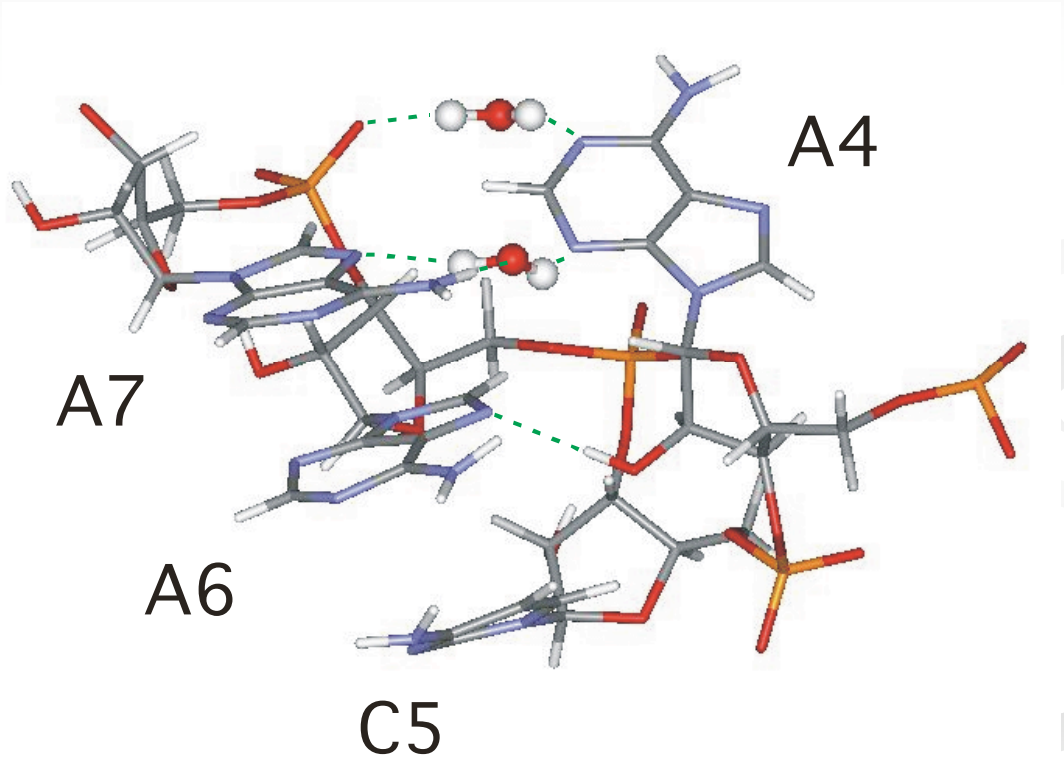
G →A: also loss of N-H at position 1

- G8 → I8 in the stem, loss of one H-bond (1.3 kcal/mol)
- G4 → I4 in the loop, loss of two H-bonds (0.65 kcal/mol)
- G4 → A4 in the loop, loss of three H-bonds (0.75 kcal/mol)

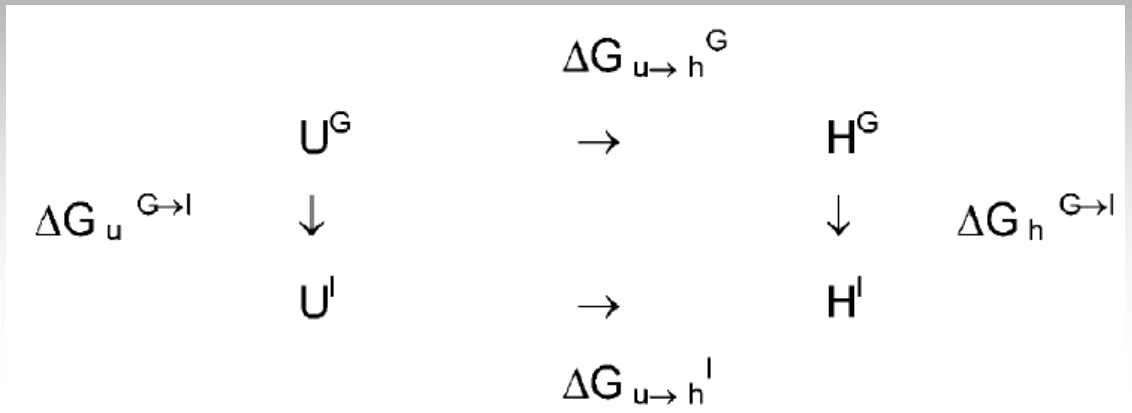




ACAA loop
no specific A4-A7 interactions



FEP simulations



Unfolded *vs*

Hairpin

Models of unfolded state	**Average ΔG_u [kcal/mol]**	
	G→I	G→A
Single Guanosine	73.6±0.1	74.5 ±0.1
CGC triplet in A-RNA conformation	73.9±0.2	74.3±0.4
CGC triplet from the loop	74.4±0.2	75.3±0.1
GCGCA in A-RNA conformation	73.6±0.1	

PERT, PBC w/ or w/o PME, spherical geometry, 101 windows, 0.4 – 0.8 ns total time

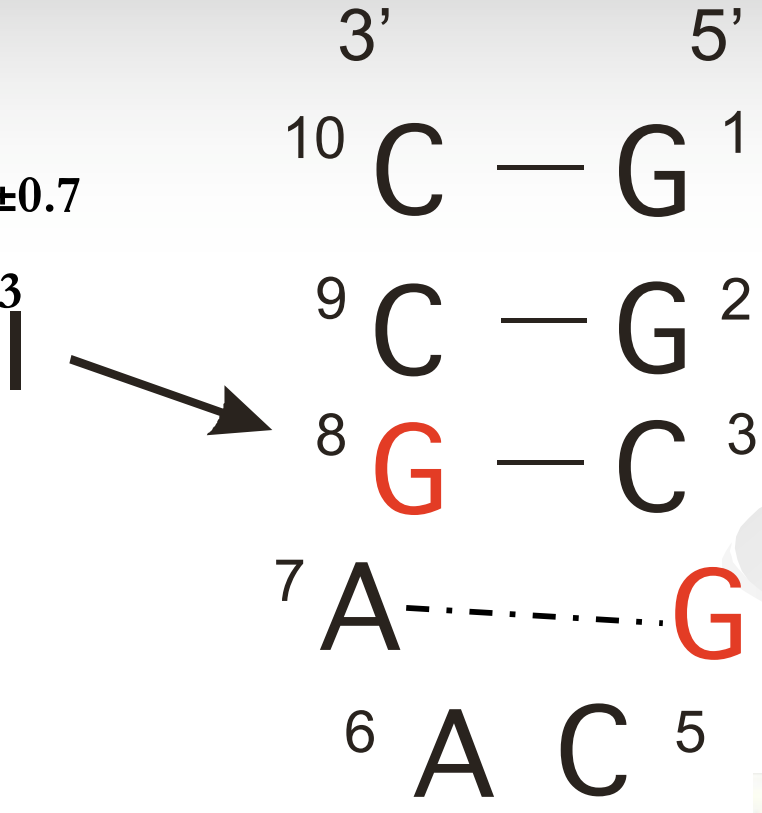
Sarzynska J, Nilsson L, Kulinski T. (2003). Effects of Base Substitutions in an RNA Hairpin from Molecular Dynamics and Free Energy Simulations. Biophys. J. **85**(6):3445-3459.

Calculated (PERT) hairpin stabilities

Sensational!

$\Delta\Delta G_{calc}$=1.7±0.7

$\Delta\Delta G_{exp}$= 1.3



$\Delta\Delta G_{calc}$=3.3±0.7

$\Delta\Delta G_{exp}$= 0.65

OK!

A

$\Delta\Delta G_{calc}$= 2.9 – 6.2

$\Delta\Delta G_{exp}$= 0.75

Auguste Compte, Philosophie Positive (1830):

"Every attempt to employ mathematical methods in the study of chemical questions must be considered profoundly irrational and contrary to the spirit of chemistry. If mathematical analysis should ever hold a prominent place in chemistry - an aberration which is happily almost impossible - it would occasion a rapid and widespread degeneration of that science."

(mis?)calculated hairpin stabilities

TABLE 8 Free energy perturbation results from simulations in water sphere [kcal/mol]

Mutation	ΔG^* (forward/backward)	ΔG_{AVG}	$\Delta\Delta G_{CALC}$
Unfolded[†] G4→A4[‡]	74.4/−74.2	74.3	
Hairpin G4→A4[§]	79.6/−74.7	77.2	2.9
Hairpin G4→I4	77.0/−78.0	77.5	
Hairpin I4→A4	4.2/−1.8	3.0	
Hairpin G4→A4 via I4	81.2/−79.8	80.5	6.2
Hairpin I4→G4[¶]	−74.0		
Hairpin A4→G4 via I4[¶]	−75.8		4.2

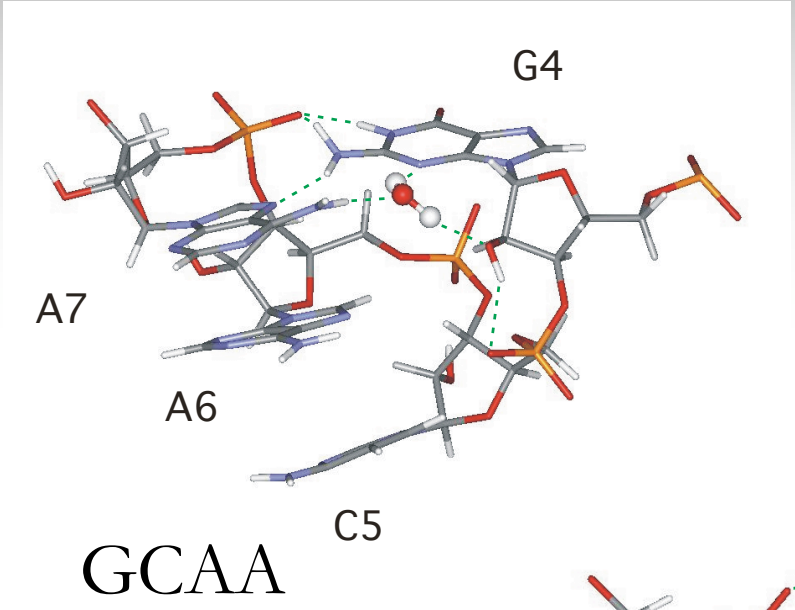
Conformational heterogeneity of the **A**CAA loop (when imposed on a GCAA structure)

Conclusion

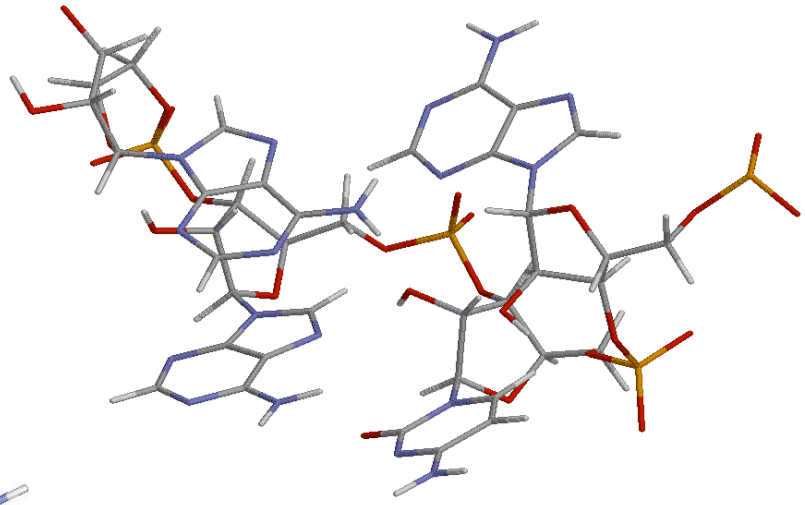
crucial for the rational design of RNA-derived molecules of therapeutic application. Our study suggests that GNRA tetraloops where G is replaced by I or A may have other low-energy conformations distinct from the GNRA fold, which were not reached during FEP simulations. This observation is consistent with the idea that the phylogenetic preference

Note added in proof: After this paper was accepted, the structure of an ACAA tetraloop was determined by NMR (Staple, D. W., S. E. Butcher. 2003. Solution structure of the HIV-1 frameshift inducing stem-loop RNA. *Nucl. Acids. Res.* 31:4326–4331) and found to be of the AGNN-tetraloop type, rather than of the GNRA-type. This confirms our suggestion that

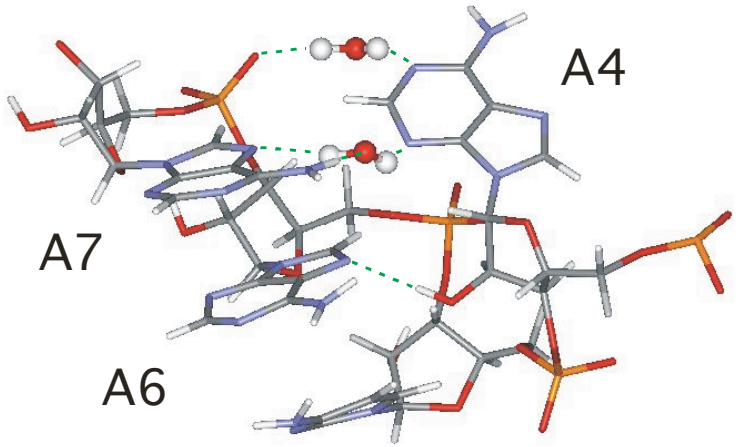
ACAA – sim vs real



GCAA



NMR ACAA

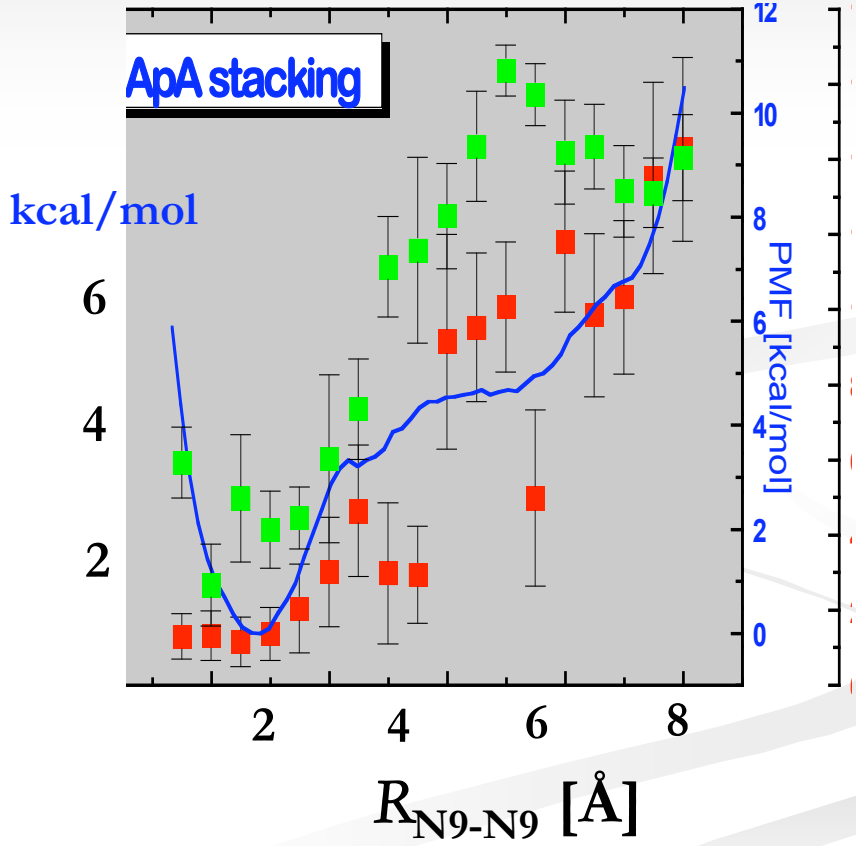


our ACAA

Conformational Equilibria

Stacking Free Energies from PMF Calculations on Dinucleotides

- Sequence
- Length
- Temperature
- DNA / RNA
- Solvent



Base-base angle

ASA

Temperature dependenc of ApA stacking from PMF *calculations*

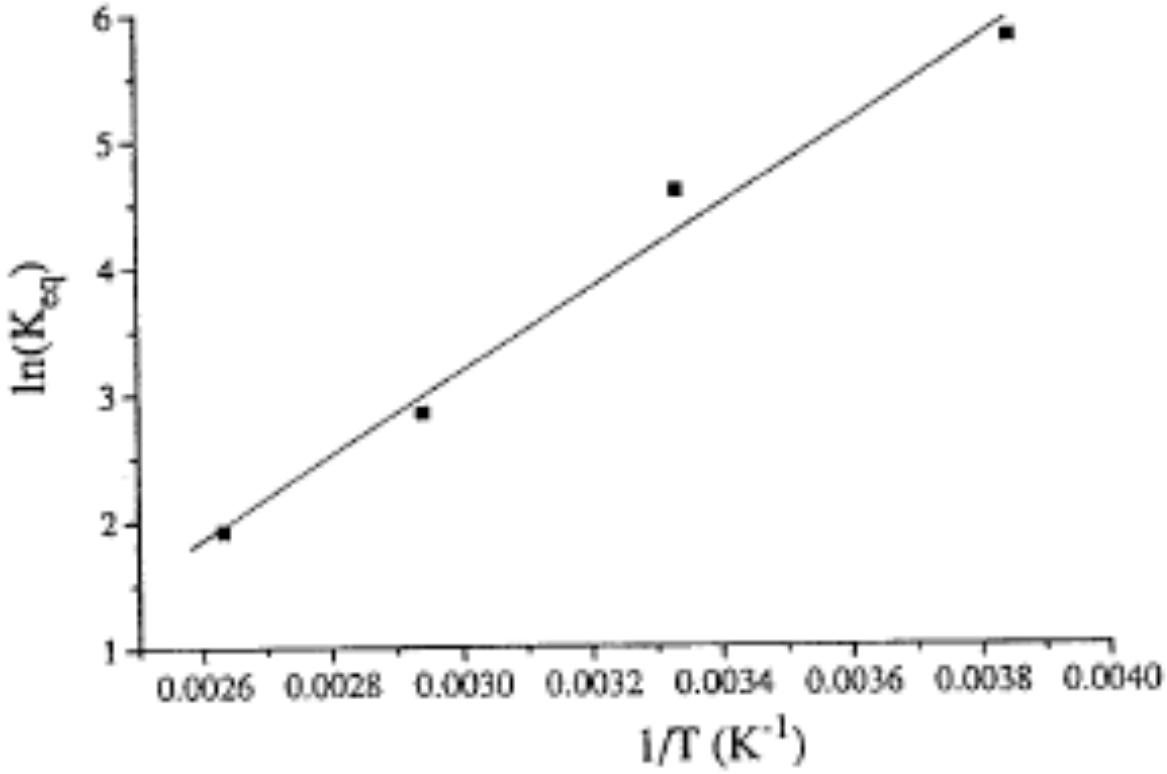


Figure 4. Van't Hoff plot for adenylyl-3′,5′-adenosine. The line is

Conformational Equilibria

- **Sequence**
  - **Pu-Pu > Pu/Py > Py-Py**
- **Temperature**
  - **Enthalpy driven**
  - **ΔH= -6kcal/mol**
  - **ΔS= -13 cal/mol/K**
- **DNA/RNA**
  - **T better than U**
  - **2'OH - slight favor of stack**

- Primarily nearest neighbor effect
- High dielectric solvents favor stacking
- Base mobility decreases stacking

Norberg&Nilsson, *Solvent Influence on Base Stacking.* Biophys J., 1998. **74**(1): 394-402 (and references therein)