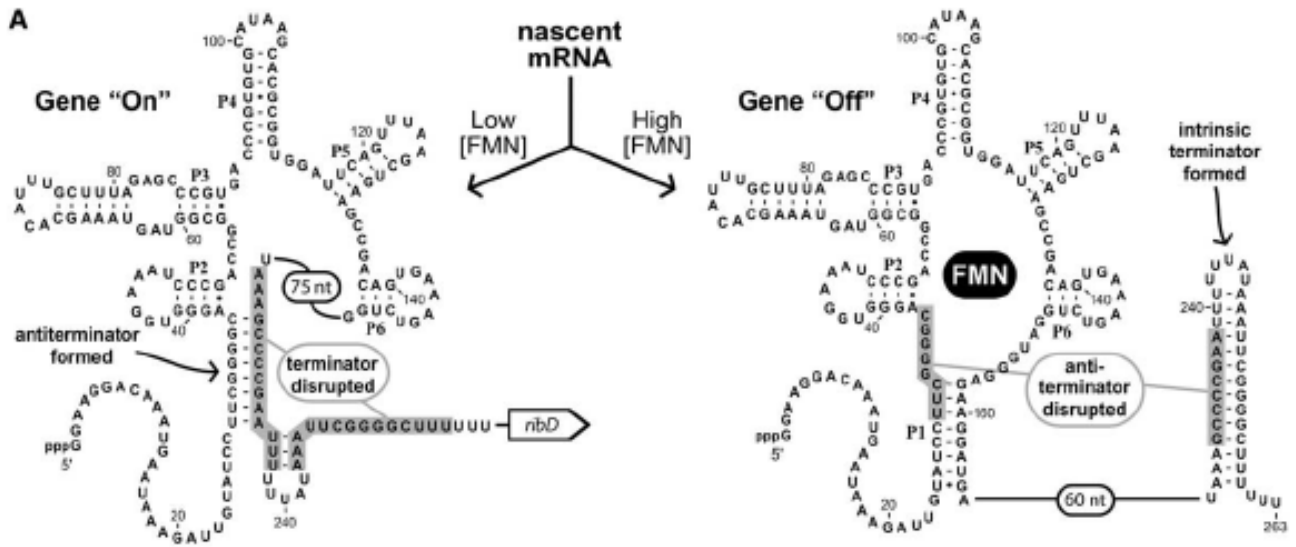
Lecture 4. 2023
Riboswitch decisions and RNA interactions

An example of aptamer binding a ligand

An mRNA structure that controls gene expression by binding FMN.

Winkler, Cohen-Chalamish, Breaker. 2002. PNAS 99:15908-15913



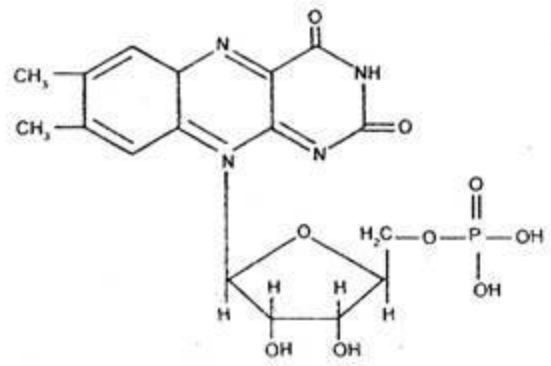
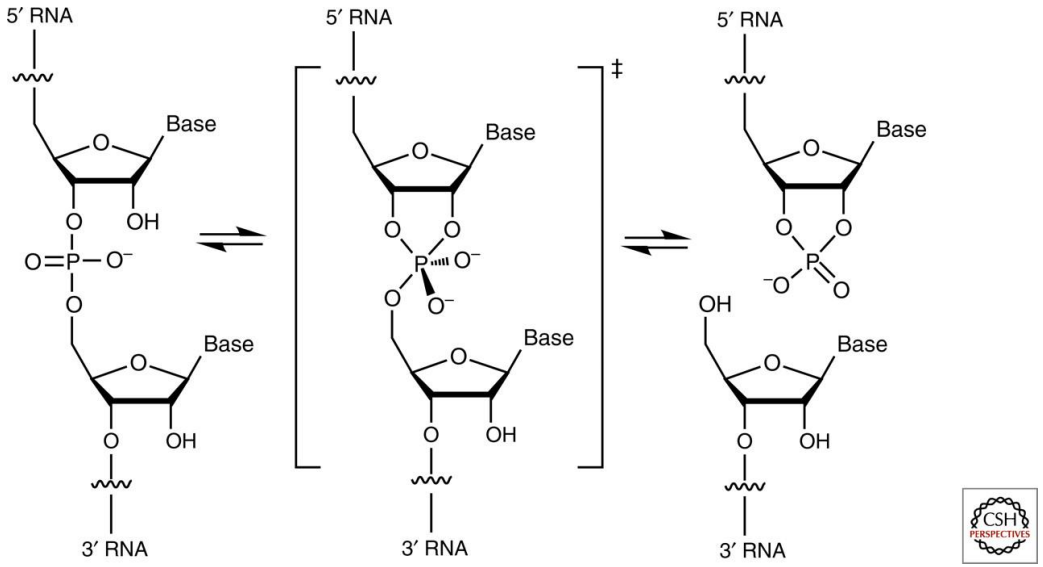


Fig. 1.37 Structure of Flavin mononucleotide (FMN).

How did the Breaker lab show that an RNA could specifically bind a ligand?

In-line probing

The principle: spontaneous cleavage of the phosphodiester backbone in the presence of Mg^{+2}.

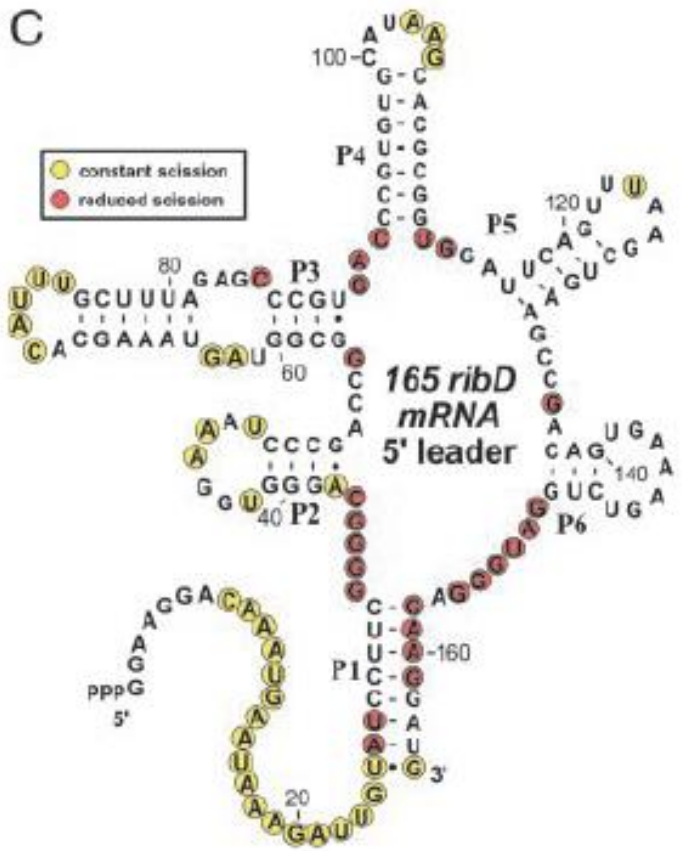


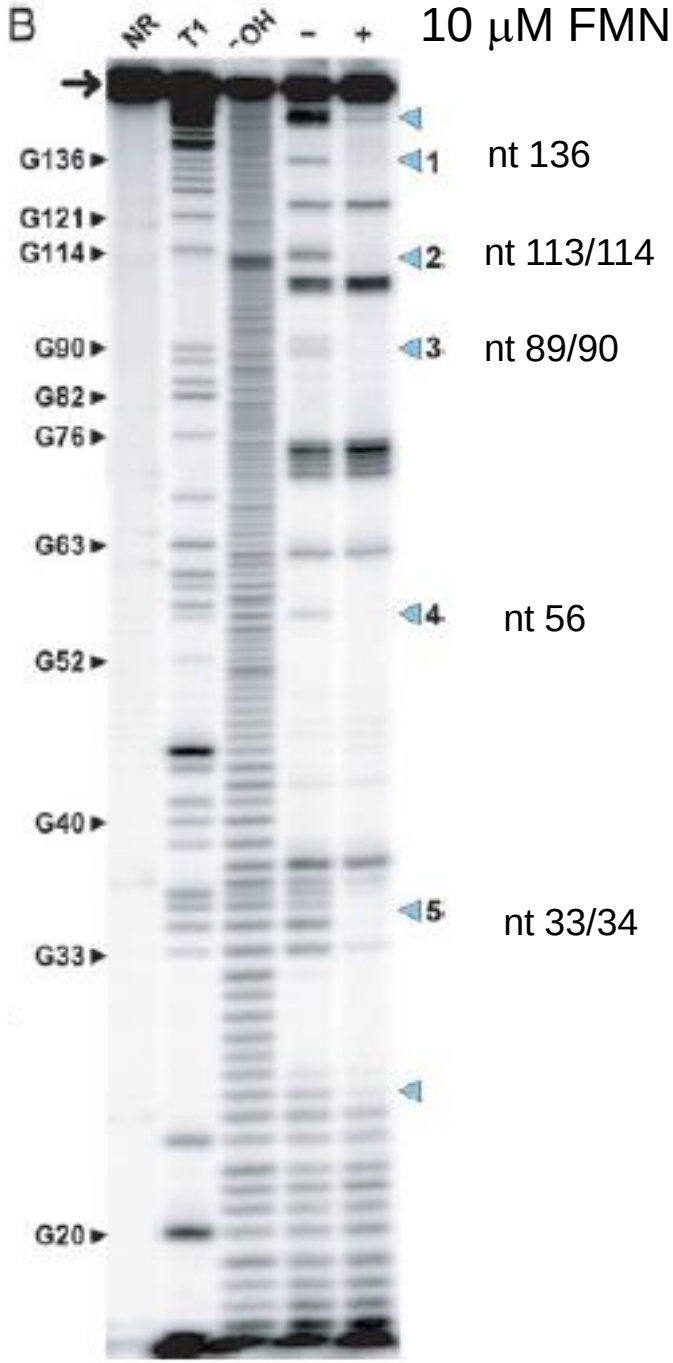
These are internal transesterification reactions that involve the 2’ OH and Mg^{2+}.

This reaction requires a specific geometry (SN_2-inline displacement reaction) that does not correspond to typical conformations in structured regions of an RNA (especially duplex regions).
However, flexible regions of the RNA can access this geometry, at least transiently with some probability.
To enhance the efficiency of the transesterification reaction and thus backbone cleavage, the reactions contain 20 mM Mg^{2+} and are run at room temperature for 40 hours.

Why room temperature for 40 hours?

≈1 nM 5′ ^{32}P-labeled RNA was incubated for ≈40 h at 25°C in 20 mM $MgCl_2$/50 mM Tris·HCl (pH 8.3 at 25°C)/100 mM KCl in the presence or absence of added ligand (FMN, FAD, or riboflavin) at concentrations that are indicated for each experiment.





10 μM FMN

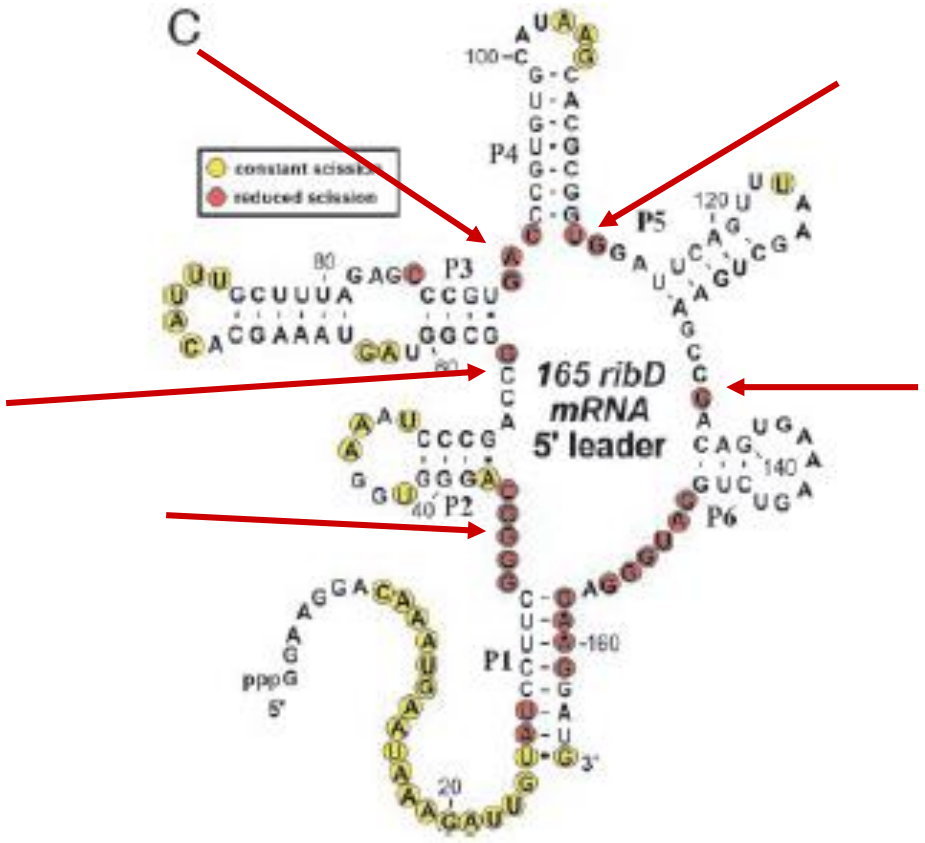
nt 136

nt 113/114

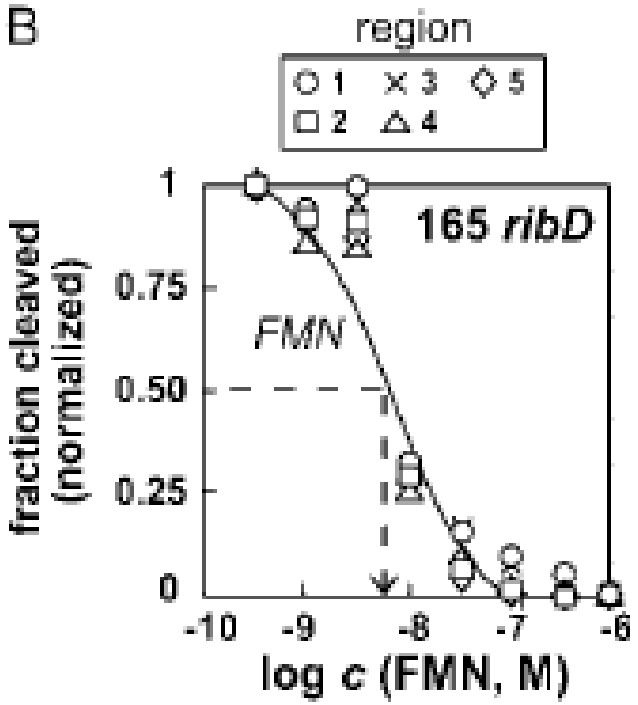
nt 89/90

nt 56

nt 33/34

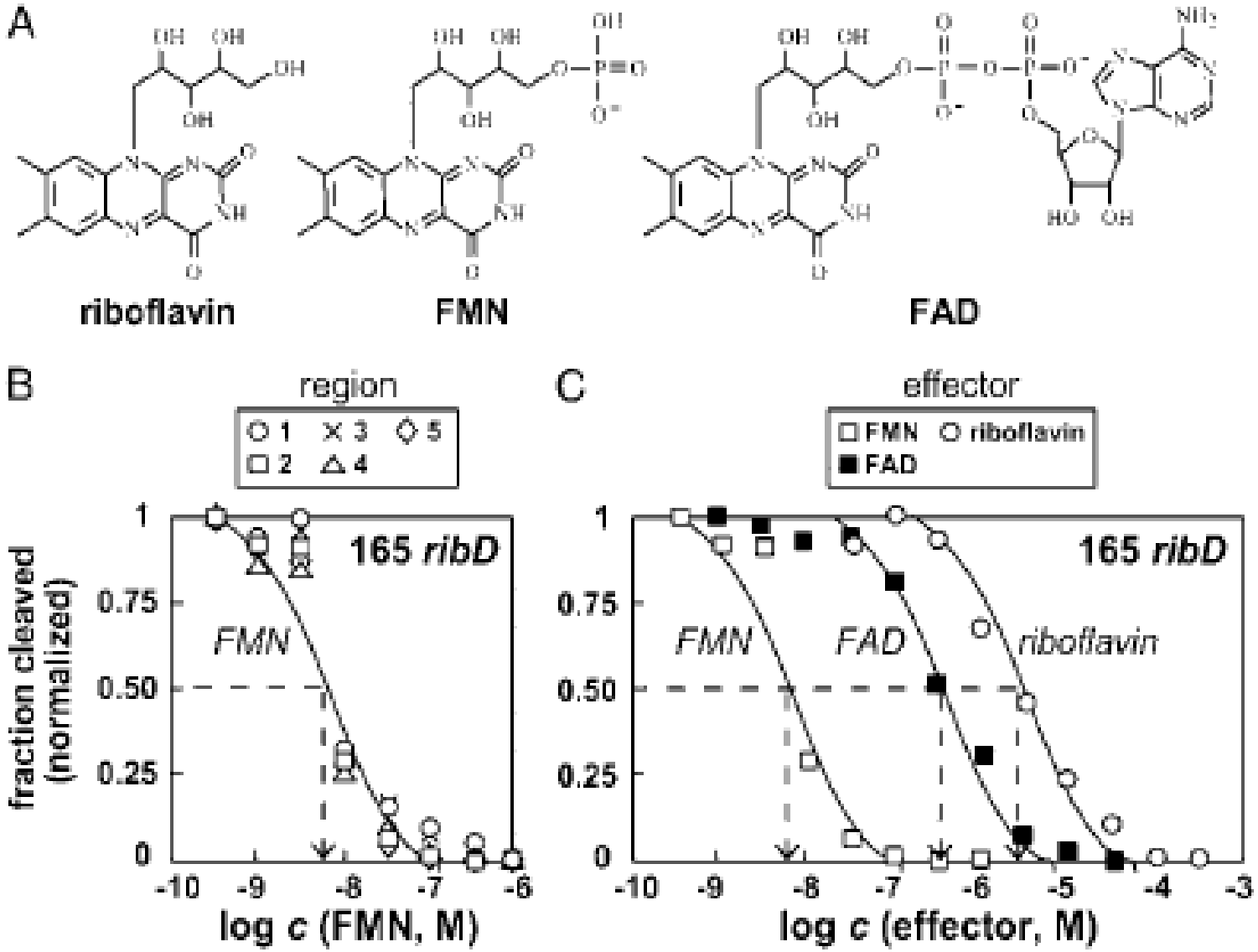


Nucleotides that become less reactive when FMN is bound.



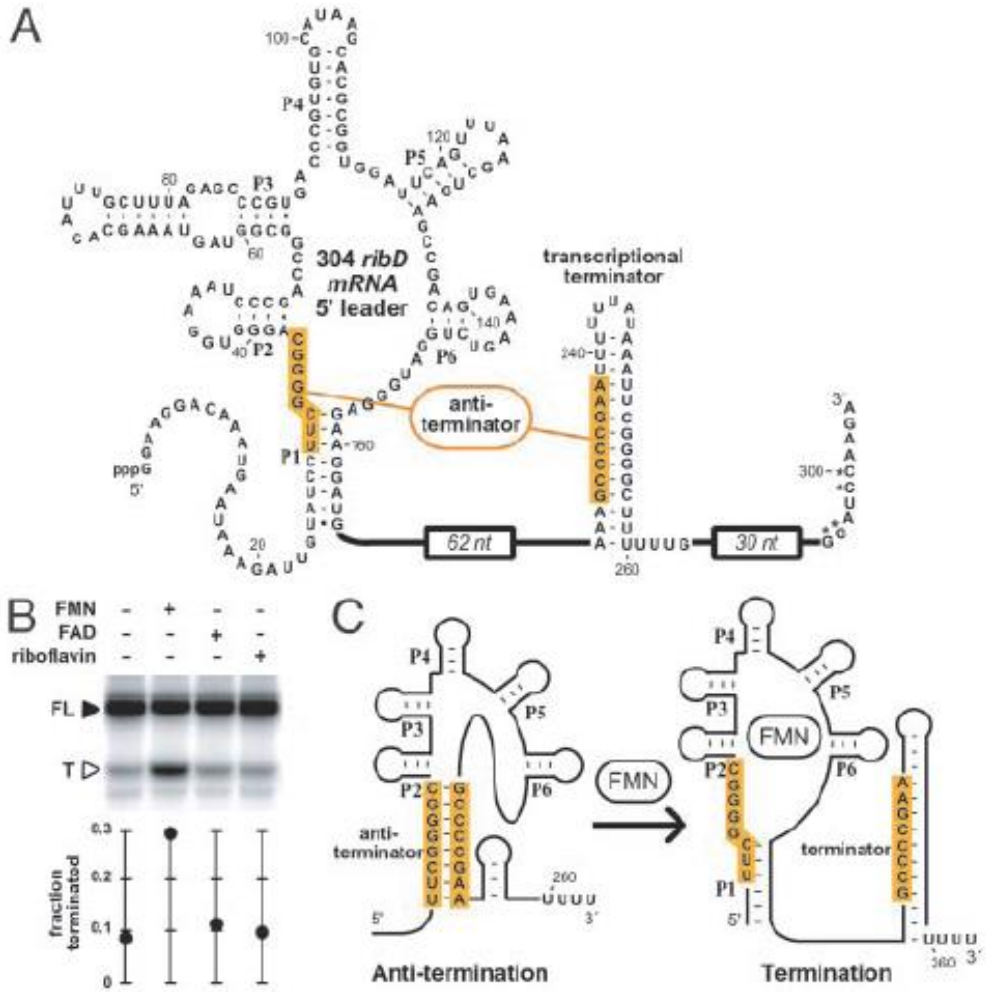
All regions show the same FMN concentration-dependence.
Therefore, the conformational change is global and cooperative.

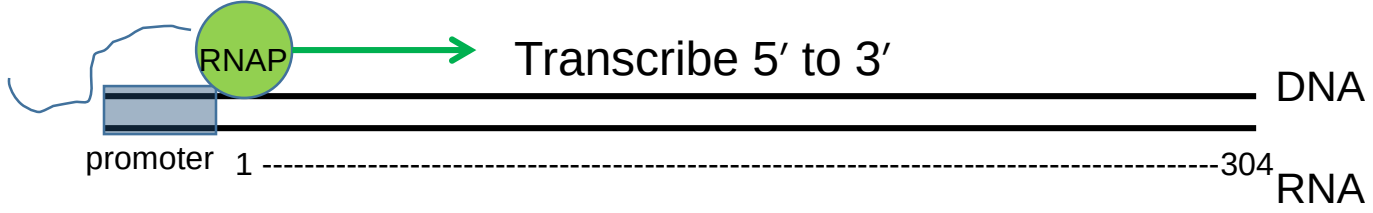
Binding is specific to FMN.

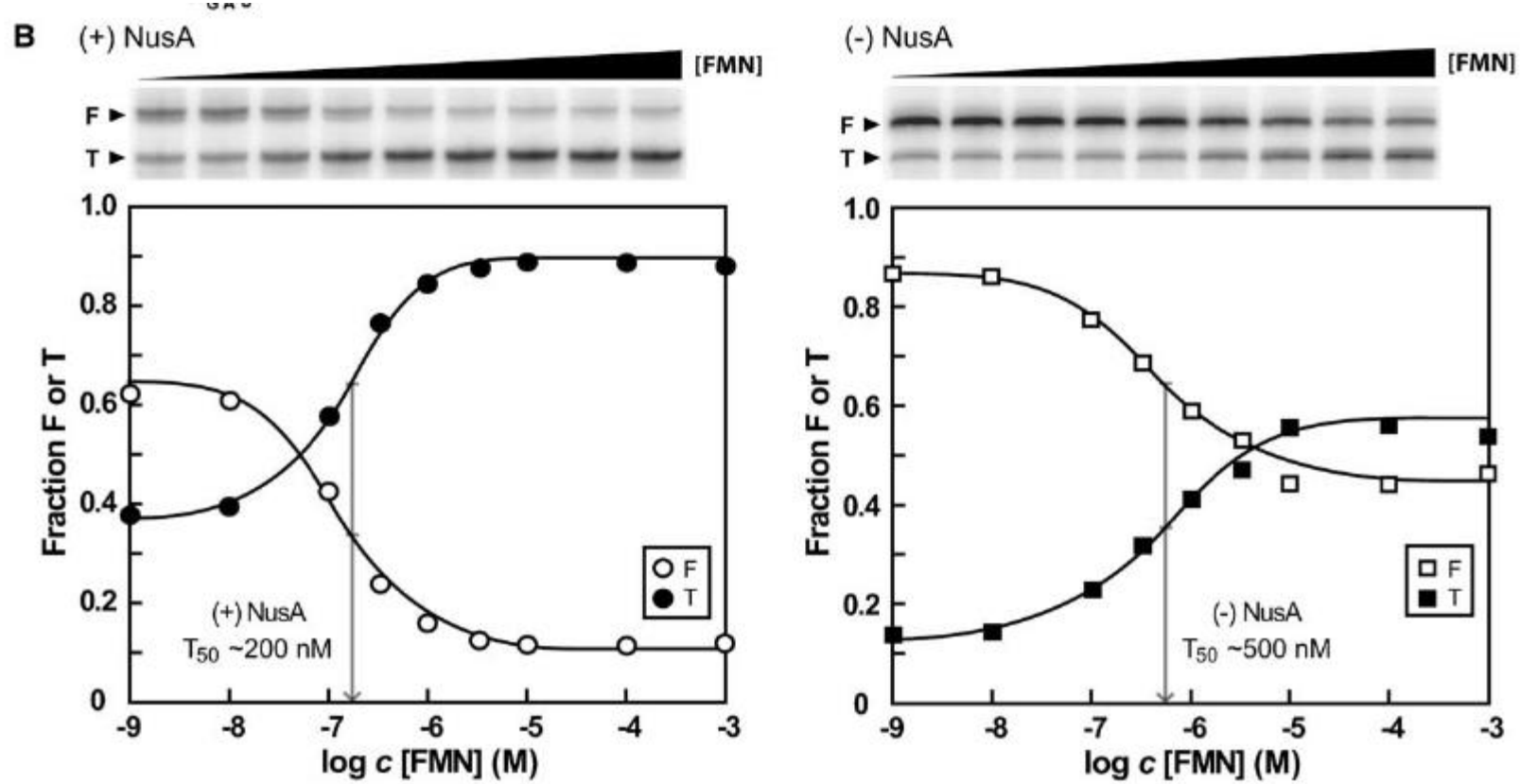


The Speed of RNA Transcription and Metabolite Binding Kinetics Operate an FMN Riboswitch

Wickiser, Winkler, Breaker, Crothers. 2005. Mol Cell. 18:490-60.



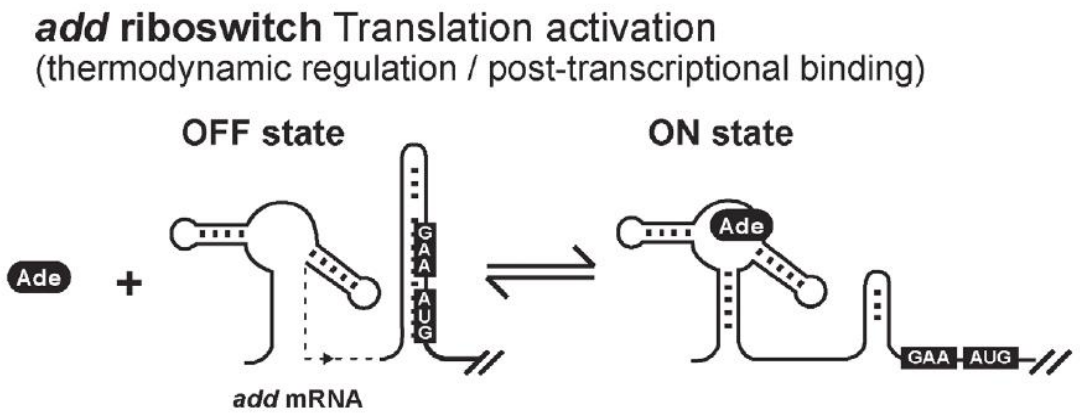




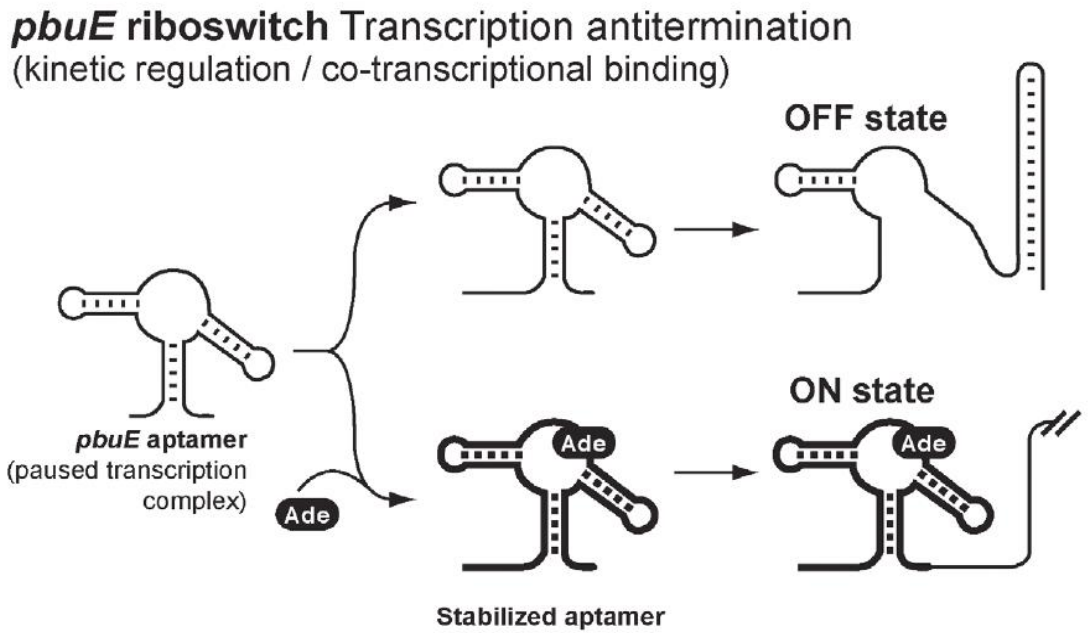
NusA is a protein that binds to bacterial RNAP and slows transcription.

This riboswitch is not at thermodynamic equilibrium at the time the choice is made to transcribe or terminate. Therefore, this decision is kinetically driven.

The **Purine riboswitch** operates in two modes.

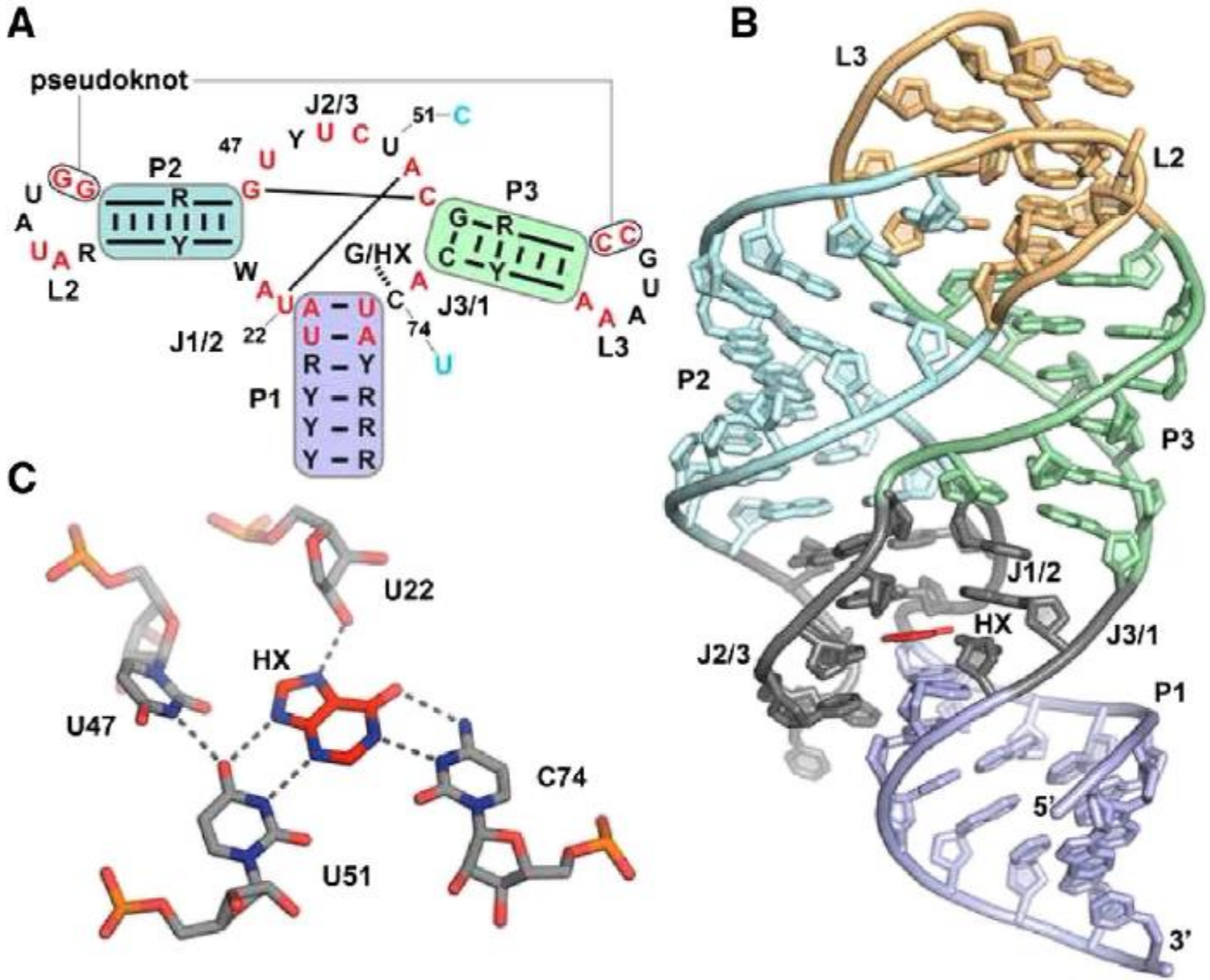


Shine-Delgarno (GAA) and AUG start are paired in a stem. Adenine binding shifts the equilibrium.



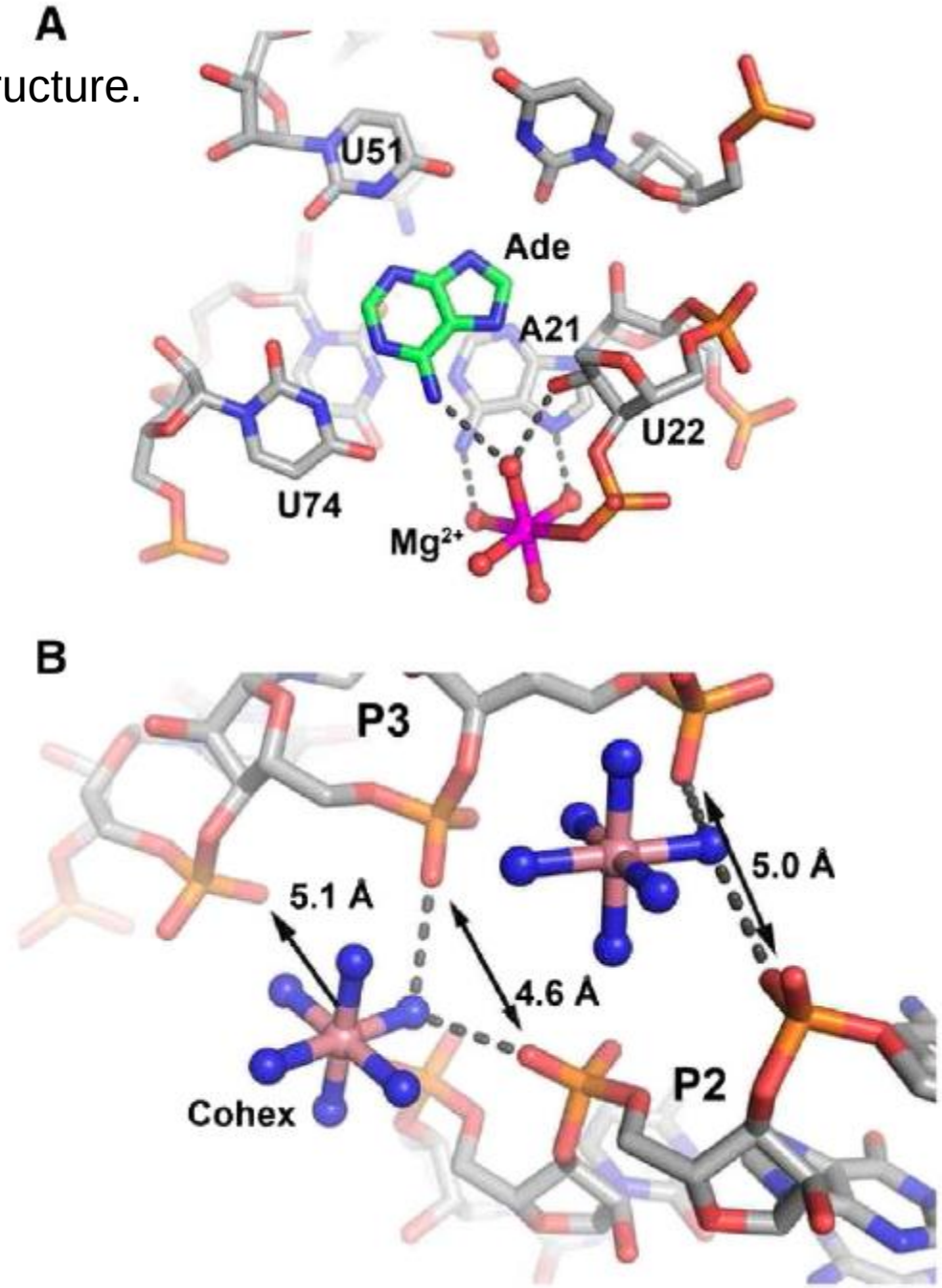
Low Adenine concentration leads to OFF state. High concentration can co-transcriptionally bind leading to the ON state.

The purine riboswitch



Porter et al., 2014. Biochem Biophys Acta 1839:919-930.

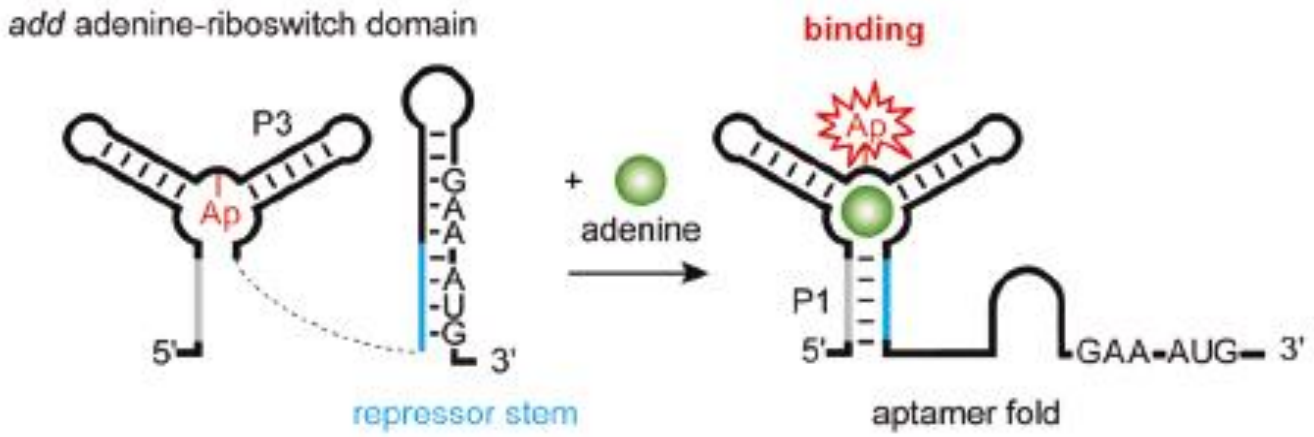
Ions in the crystal structure.
What do they do?



A spectroscopic method to study the ligand binding process

Examine adenine binding by the purine riboswitch. Replace a single nucleotide with the fluorescent nucleotide 2-aminopurine (Ap).

When the riboswitch undergoes a conformational change, Ap fluorescence could increase or decrease (or not change), reporting on the timescale of the binding and also the folding pathway.



Haller, A, Souliere MF, Micura R (2011) The Dynamic Nature of RNA as Key to understanding riboswitch mechanisms. Accts Chem Res. 44:1339-1348.

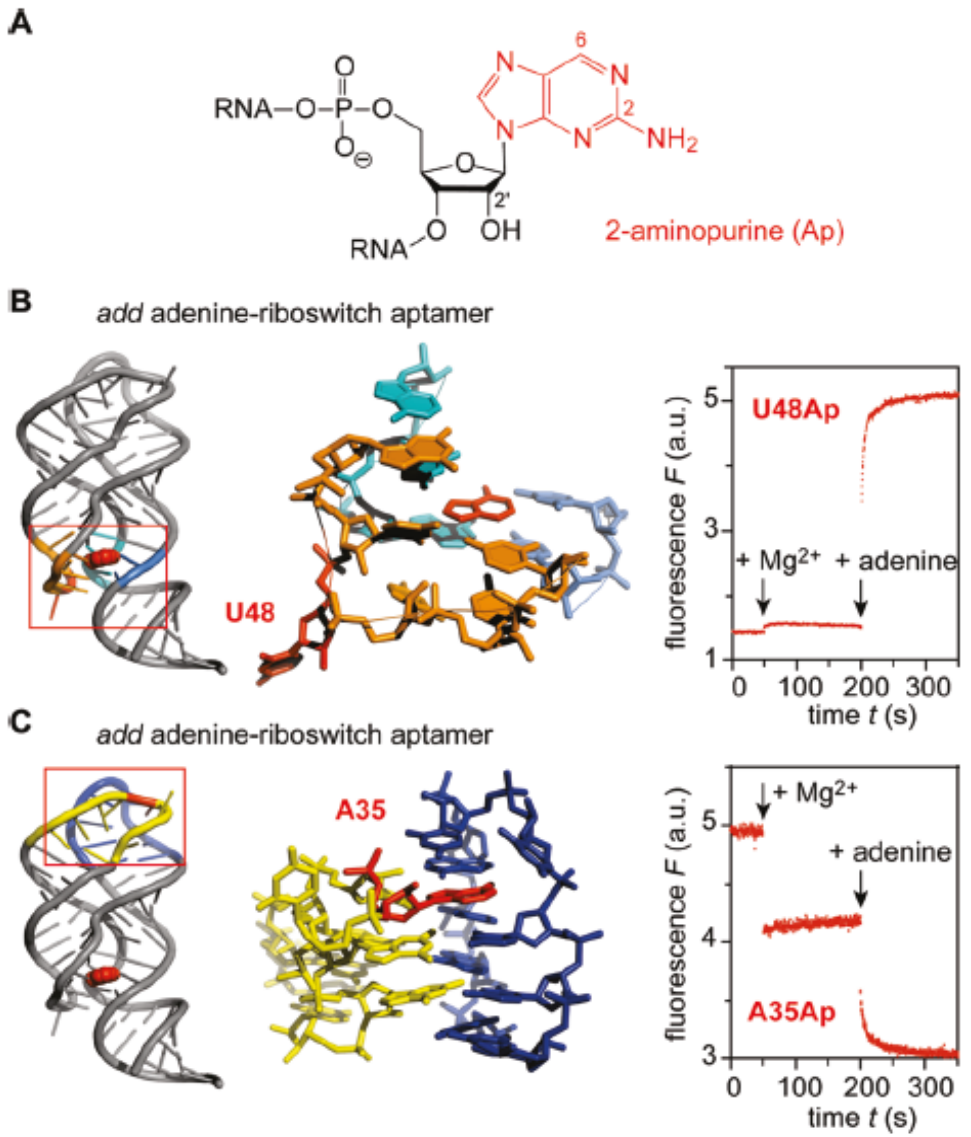


FIGURE 1. 2-Aminopurine (2Ap) labeling to study Mg^{2+}- and ligand-induced RNA folding (2ApFold approach). (A) Chemical structure of 2Ap. (B,C) Structure-based selection of 2Ap nucleoside replacement exemplified for U48 (B) and A35 (C) of the adenine riboswitch, and corresponding fluorescence response.

What do you think you would observe if adenine were added first, then Mg^{2+}?

The lock and key paradigm of substrate binding clearly fails for these riboswitches.
Instead, there is a coupled binding/folding that needs a new formalism to describe it. There are two popular models that you'll find in the literature. Induced Fit, and Conformational Selection. Are they mutually exclusive mechanisms, or are they the same mechanism with different names, or can both be present in the same system?

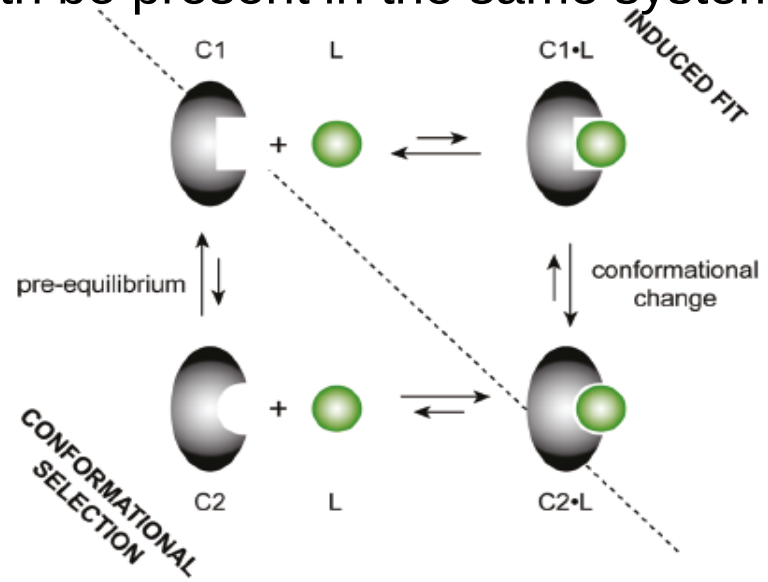
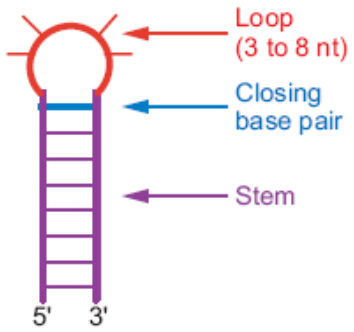
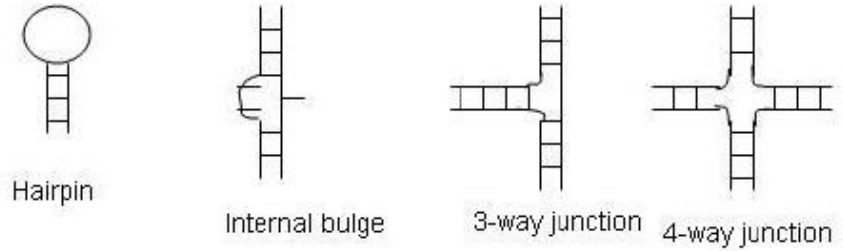


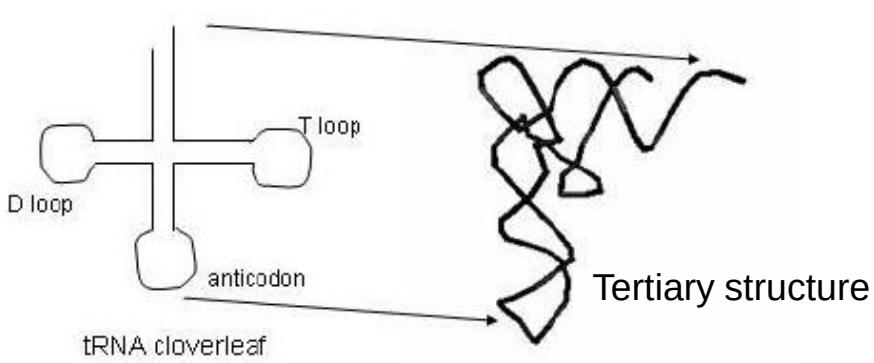
FIGURE 5. Two models for molecular recognition: induced fit and conformational selection.[63] In conformational selection, the binding-competent conformation (C2) is pre-existing in solution before the addition of ligand (L). In induced fit, initial binding contacts (C1•L) between ligand and receptor induce conformational rearrangements to achieve the conformation C2•L of the complex.

Vogt & Di Cera . Biochemistry. 2012 51(30):5894-902.

An RNA molecule is more than a duplex.







Secondary structure

Tertiary structure

Experiment: Not all loops are the same.

RNA GGAX(NNNN)X'UCC Hairpin Parameters

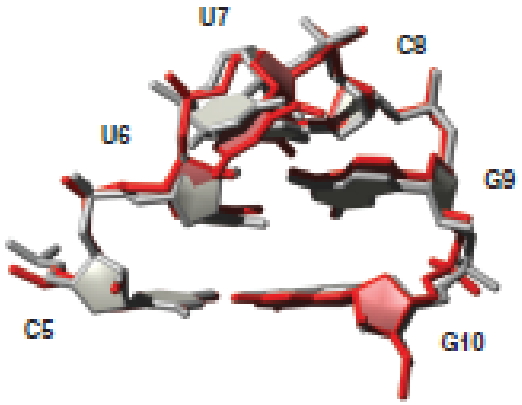
RNA Loop Sequence	Tm (°C)	ΔH° (kcal/mol)	ΔS° (e.u.)	ΔG°(37) (kcal/mol)
C(UUCG)G	76.2	−55.9	−159.9	−6.3
C(UUUG)G	70.3	−44.0	−128 0	−4.2
C(UUUU)G	69.6	−44.3	−129.3	−4.2
G(UUCG)C	67.7	−44.8	−131.4	−4.0
G(UUUG)C	*	*	*	*
G(UUUU)C	*	*	*	*
G(CUUG)C	*	*	*	*
C(GCUU)G	70.9	−45.0	−130.8	−4.4
C(UACG)G	73.8	−53.6	−154.5	−5.7

*Evidence for duplex formation was seen in the melting curves

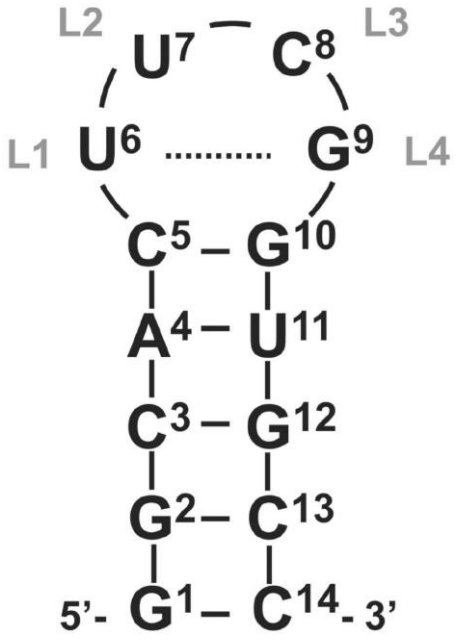
WHY?

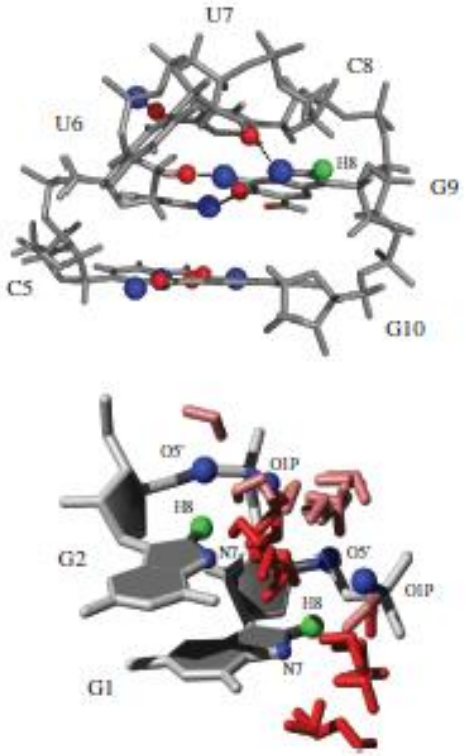
Experiments in 1 M NaCl, 0.01 mM EDTA, pH 7.0 to match conditions of thermodynamics parameters

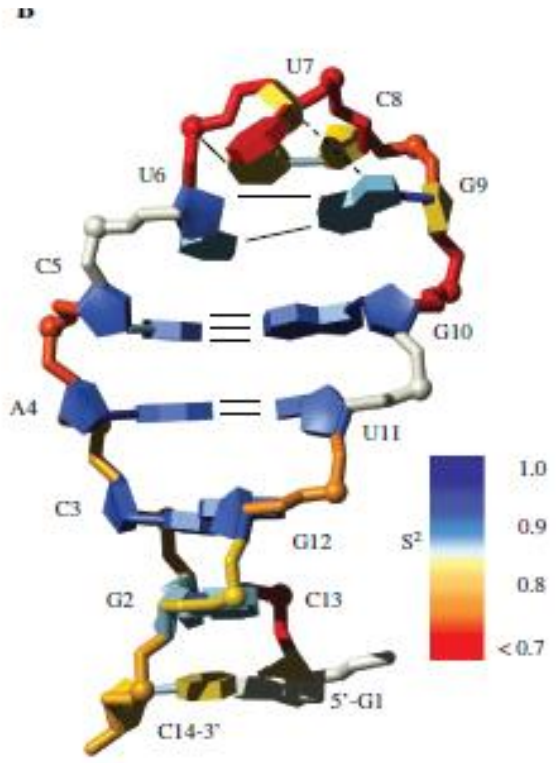
THE cUUCGg tetraloop is unique



New NMR solution structure (red)







Nozinovic S, Fürtig B, Jonker HR, Richter C, Schwalbe H. High-resolution NMR structure of an RNA model system: the 14-mer cUUCGg tetraloop hairpin RNA. Nucleic Acids Res. 2010;38(2):683-94.

Tertiary interactions fold RNA molecules.

COMMON THEMES

- ❑ Role of ions in structure and stability
- ❑ Structural dynamics, hydrogen bonding, and water
- ❑ Common motifs vs idiosyncratic interactions
- ❑ Predictions of tertiary structure

Kissing loops.

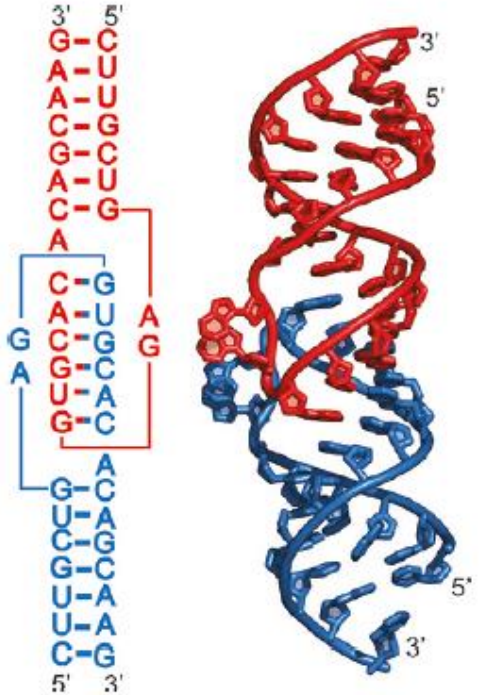
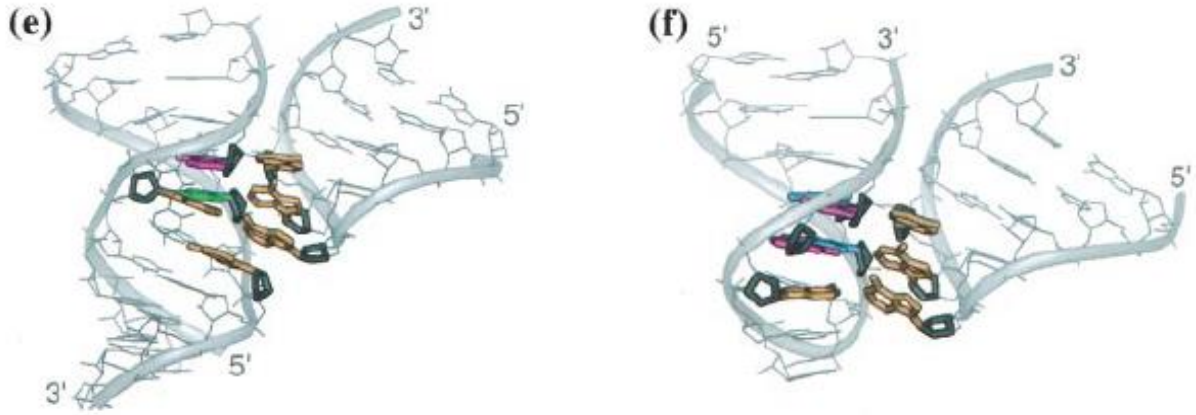


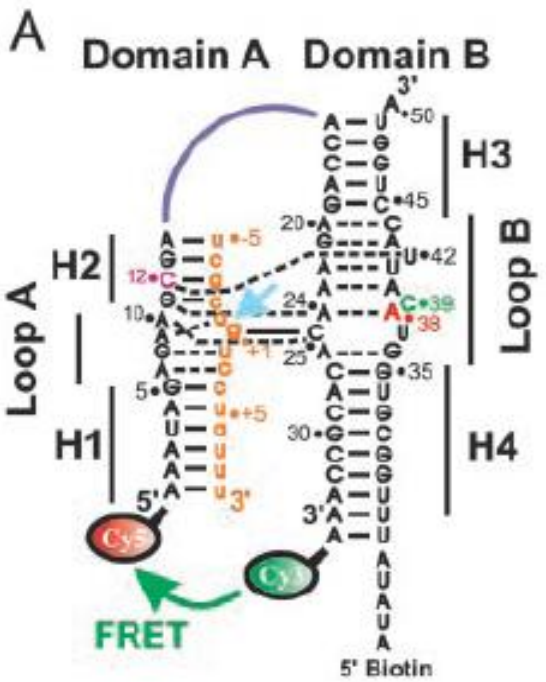
FIGURE 2. The HIV-1 dimerization initiation site kissing loop, PDB ID 1K9W. Hairpin loops are red and blue, respectively.

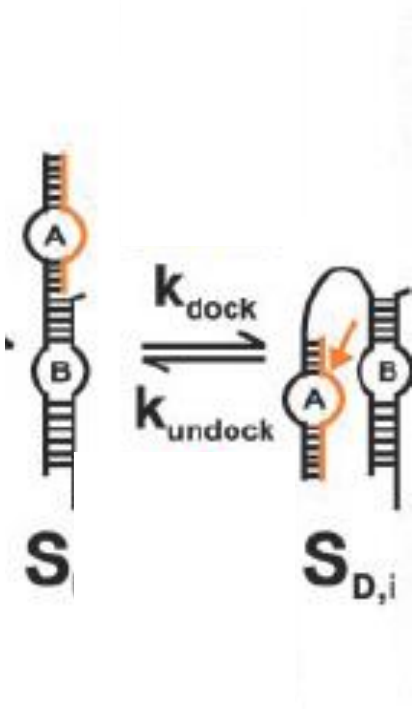
In more detail, you can see the bases juxtaposed to form hydrogen bonds.
Two base pairs are enough to make a stable kissing interaction.

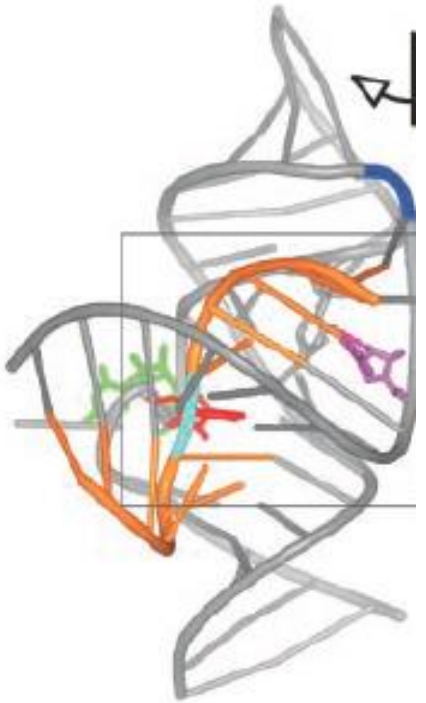


LOOP:LOOP INTERACTIONS ARE COMMON TERTIARY STRUCTURES

Interactions are exquisitely sensitive to hydrogen bonding







Rueda et al., 2004. PNAS 101:10066-10071

PSEUDOKNOTS

Typically do not require divalent cations to correctly fold.
Loop1 and Loop2 lengths are highly variable; as short as 1 nucleotide.

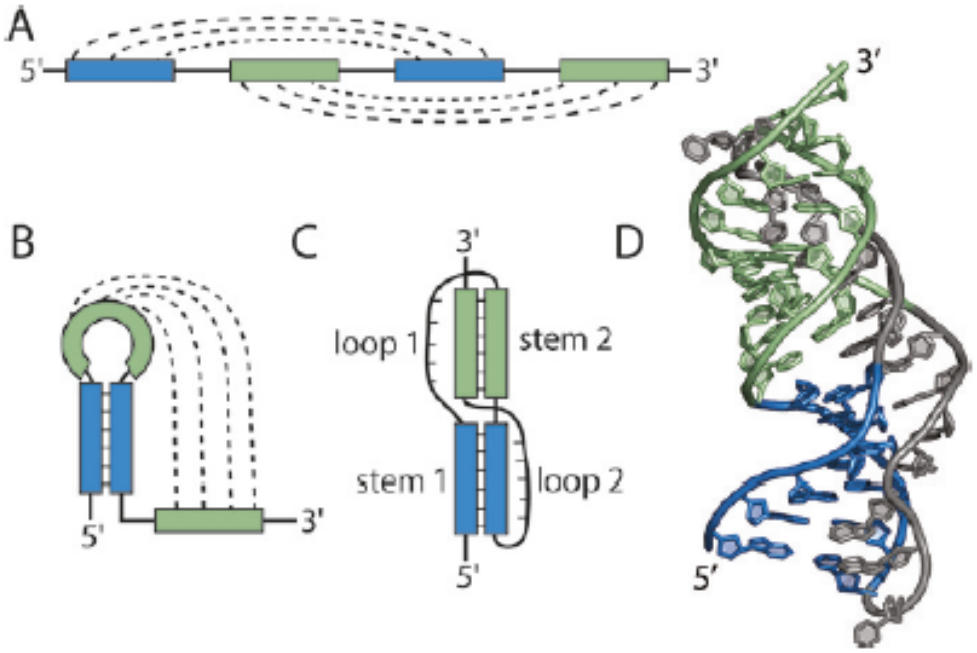


FIGURE 3. Pseudoknot topology and structure: (A) long-range base-pairing interactions; (B) hairpin secondary structure with long-range pseudoknot contacts; (C) coaxial stacking of pseudoknot helices; (D) the telomerase pseudoknot, PDB ID 2K96. Loops 1 and 2 (gray) form a series of base triples with stem 1 (blue) and stem 2 (green).

Triple strands

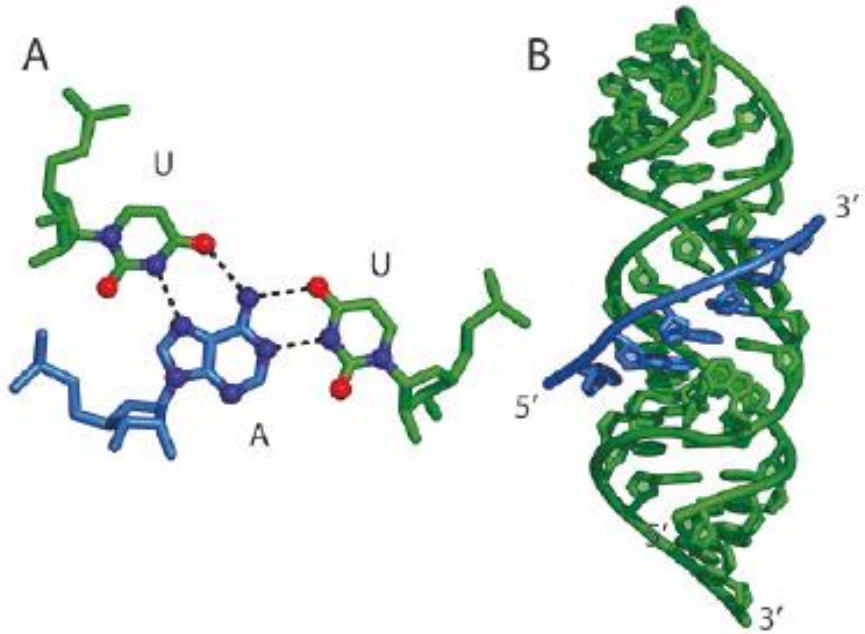


FIGURE 8. (A) Structure of an A–U·A triple base pair and (B) structure of the Kaposi's sarcoma-associated herpesvirus polyadenylated nuclear (PAN) RNA expression and nuclear retention element (ENE), showing a triple-stranded interaction between the ENE RNA (green) and poly-A (blue). From PDB ID 3P22.

Butcher & Pyle, 2011 Acc Chem Res. 44(12):1302-11.

G quartets

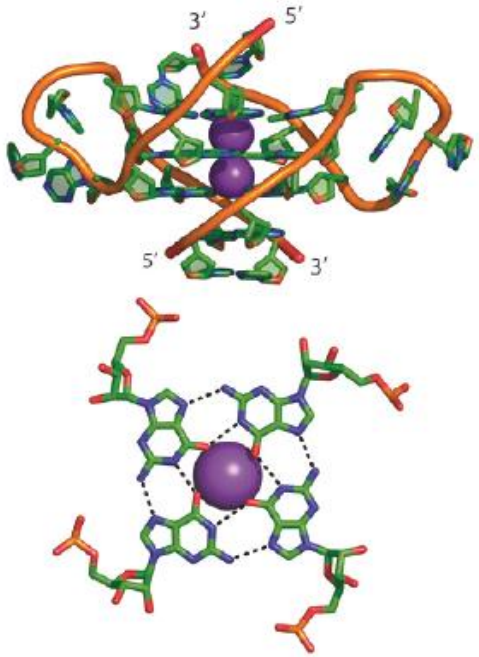
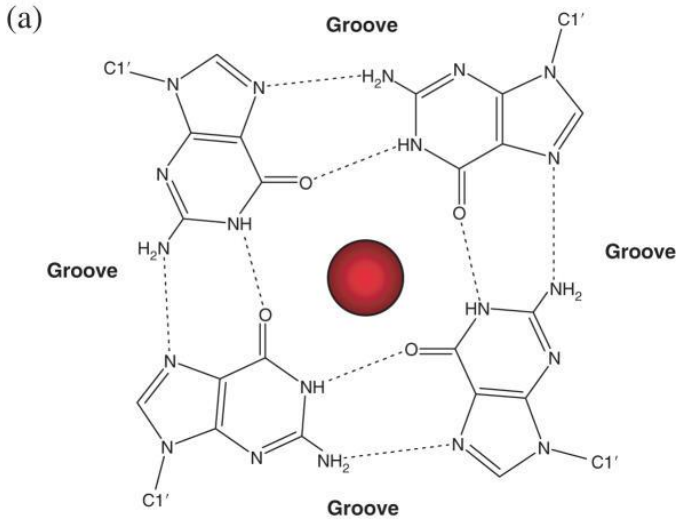
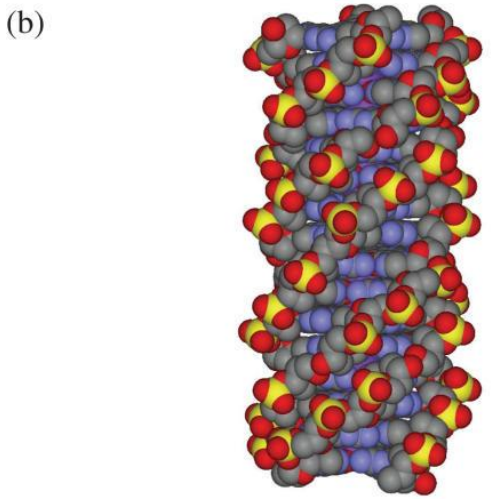
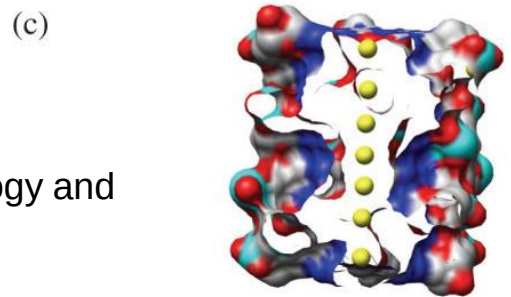


FIGURE 9. Structure of the human telomeric RNA (TERRA) quadruplex, PDB ID 3IBK: (top) side view of the TERRA quadruplex; (bottom) view of a central guanine quartet. Potassium ions are purple.

Butcher & Pyle, 2011 Acc Chem Res. 44(12):1302-11.







Burge et al., 2006. Quadruplex DNA: sequence, topology and structure.Nucleic Acids Res. 2006;34(19):5402-15.