

①

## RNA Function & Structure:

- RNA is a chain like polymer built from 4 possible monomers: G, C, A, U
- An RNA molecule is specified by its sequence  
e.g. 5'-AAGCGUAGUU-3'

## Functions in cell:

- The number of functions that RNA molecules carry out inside the cell is more than being just a messenger molecule.

① mRNA  $\equiv$  messenger RNA

- RNA's main function in the cell is to act as the intermediate chemical message between DNA and the making of protein

DNA (gene)  $\xrightarrow{\text{transcription}}$  mRNA  $\xrightarrow{\text{translation}}$  protein

② tRNA  $\equiv$  transfer RNA

- transfer RNAs are used in translation
- there is one tRNA for each of the 64 codons

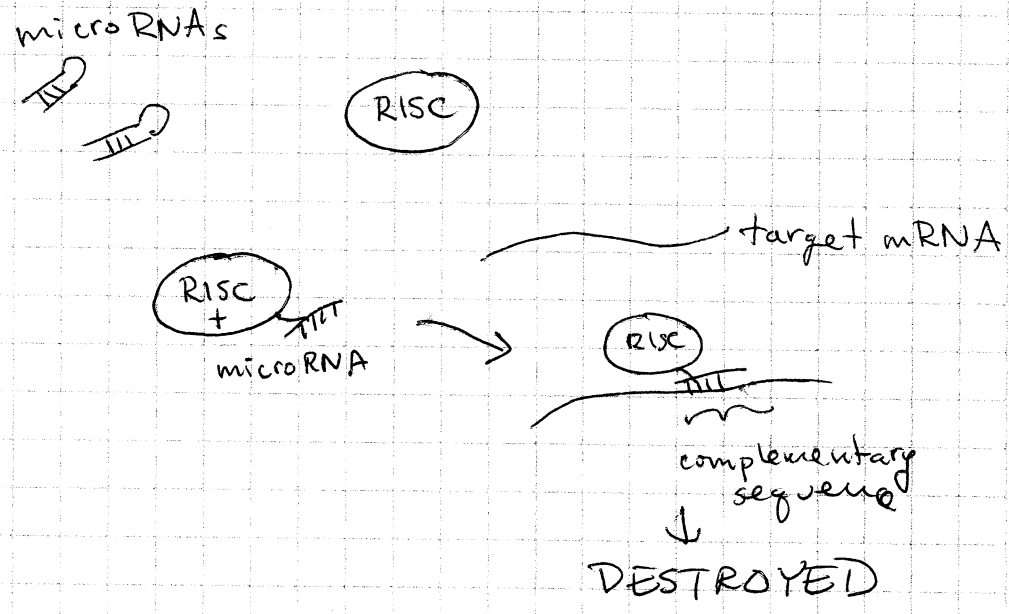
② rRNA — ribosomal RNA

— RNA molecules that are part of the giant molecular machine known as the ribosome

Functions discovered in the last 10 years

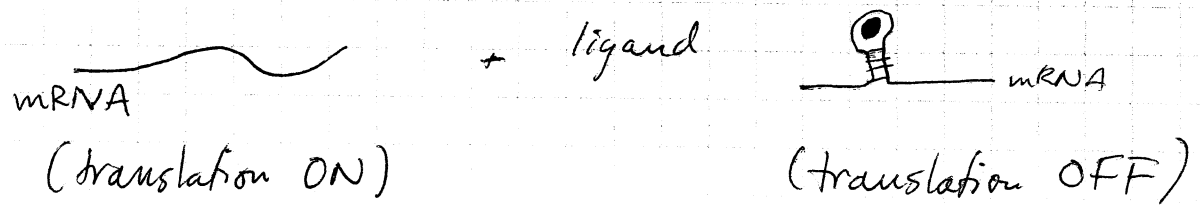
① microRNAs — small 21-23 ~~nt~~ long RNAs that are used by the RNA interference pathway to regulate gene expression

RNA Interference (Nobel Prize)



— many genes ~~also~~ ~~now~~ have their expression regulated by microRNAs

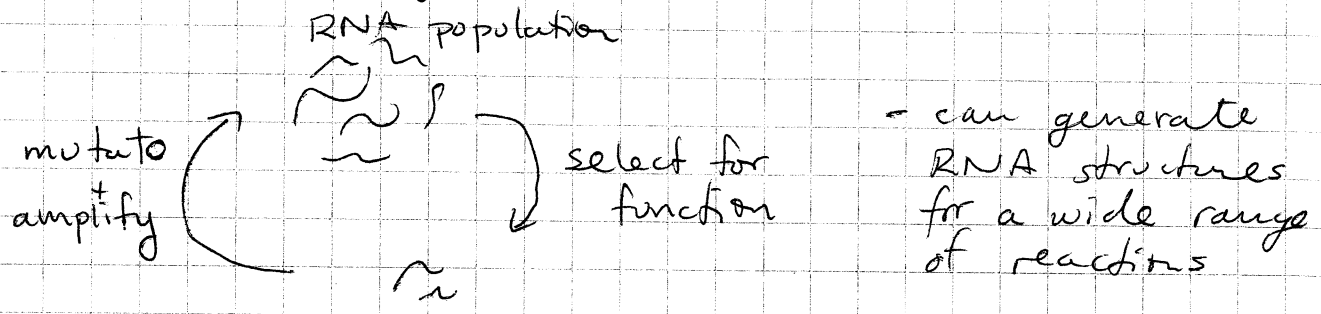
② Riboswitches — binding of small molecules (ligands) to RNA causes it to undergo a conformational change



### Other Functions (Synthetic):

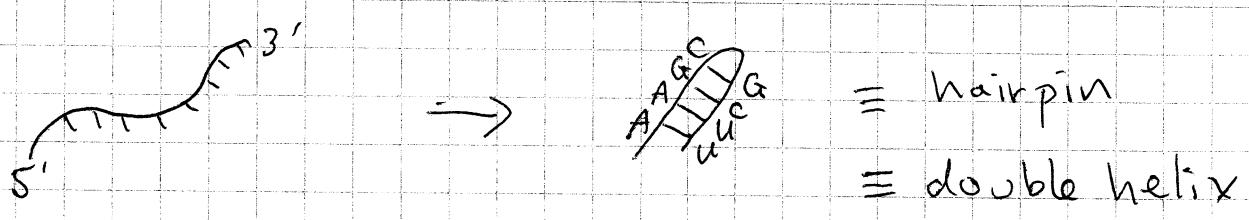
Ribozymes - RNAs that can catalyze chemical reactions

- in-vitro evolution experiments: evolve RNA molecules to carry out a selected chemical reaction

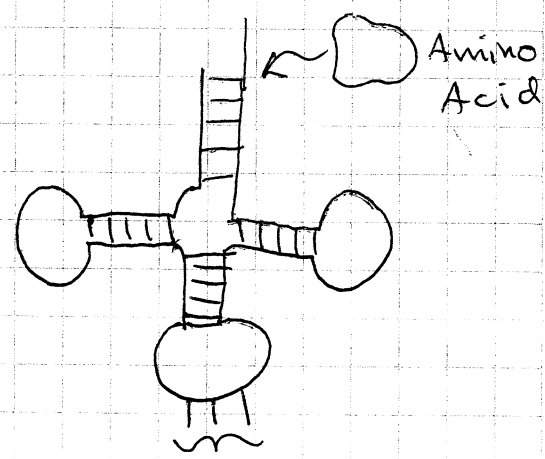


Q: can one select for a self-replicating RNA molecule?

### RNA Structure:



e.g. tRNA

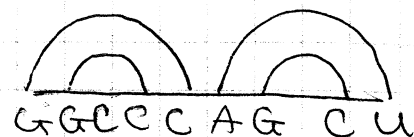
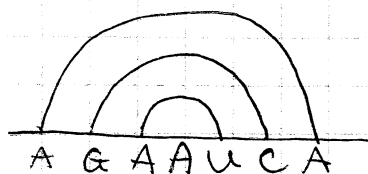


tRNAs ~ 76 - 100 nt long

anticodon - recognizes one of 64

# Structure Representation

Rainbow diagrams:



Paranthesical:

(( ( . ) ) )

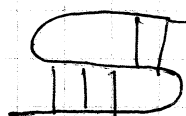
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String:

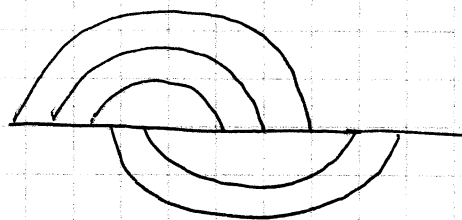
1110-1-1-1

110-1-111-1-1

Pseudo knots:



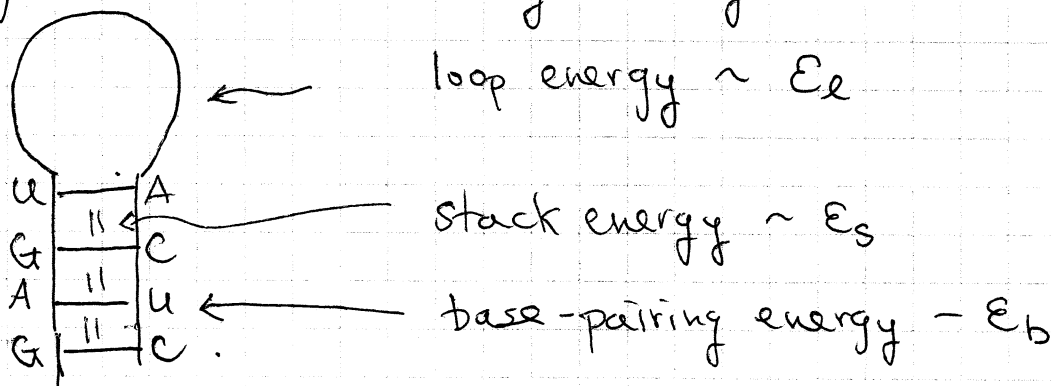
≡



- these occur in reality, but are computationally very hard to deal with.

## RNA Folding:

Energies involved in forming 2ndary structure



Turns out  $E_s \gg E_b$  - so stacking drives folding

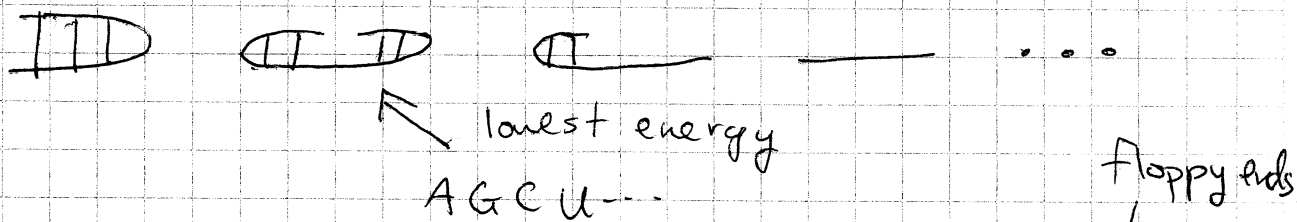
- Loop energy - due to floppy nature of loop  $\equiv$  entropy
  - each base in loop  $\approx \alpha$  conformations
  - for loop of length  $L \rightarrow$  # of conformations  $= \alpha^L$
  - So energy,  $E_L = k_B T \ln(\alpha^L) = L k_B T \ln \alpha$
- depends on temperature and length of loop.

Simple Model:

- just consider stacking energy,  $E_s > 0$

Problem: For a given RNA sequence, what is the ground state structure?

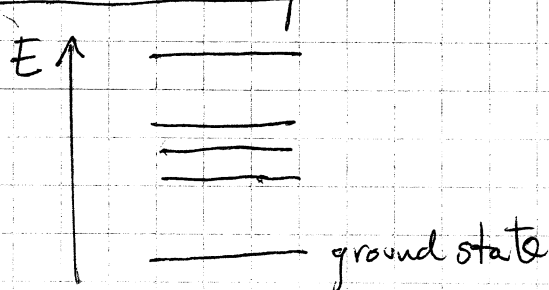
Brute force solution: enumerate all structures and see which has the lowest energy



Total energy:  $E = -n_s E_s$  [or  $F = E - TS$ ]

where  $n_s = \#$  of stacks in structure

Probabilities of states

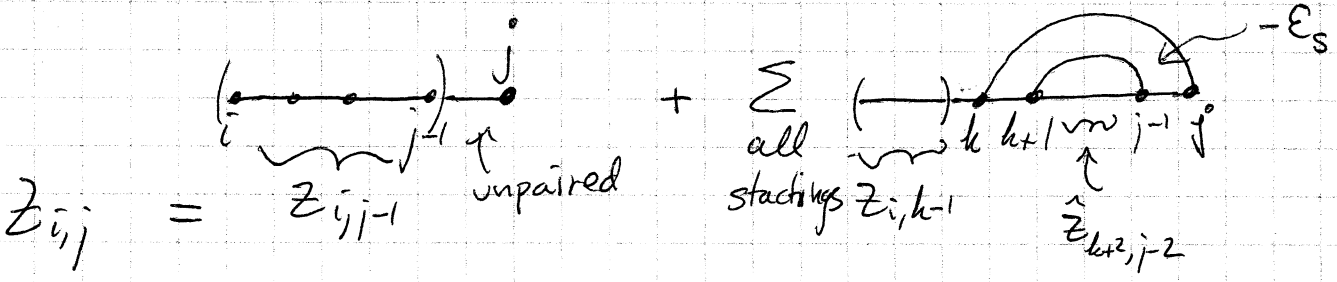


$$P(E_i) = \frac{e^{-\beta E_i}}{\sum_k e^{-\beta E_k}}$$

Partition function:  $Z = \sum_k g_k e^{-\beta E_k}$   
 degeneracy of state  $\equiv \#$  of conformations

Computing Z without Enumeration:

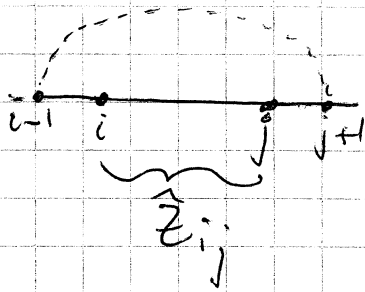
- enumerating structures becomes infeasible for structures longer than  $L > 50 \rightarrow$  too slow
- compute Z via transfer matrix / dynamic programming
- IDEA: ~~start with structure with~~ start with structure with  $i-1$  bases, and multiply by the number of conformations due to adding the next base,  $i$



- Algorithm:
- ① Start with  $Z_{\phi,\phi} = 1$
  - ② keep adding one at a time  $\rightarrow Z_{i,j}$
  - ③ stop when  $j = N$

$\hat{Z}_{i,j}$  is the same as  $Z_{i,j}$  except it has boundary condition that there is external base pairing.

z?



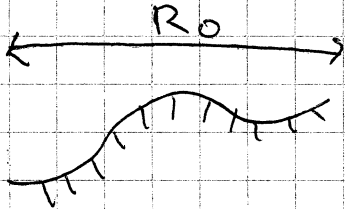
KEY

- if  $i$  can pair with  $j$  then another  $-E_s$  is given because of the pairing between  $i-1$  &  $j+1$

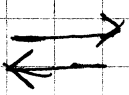
See RNA Primer for details

RNA Unfolding Experiments:

Let's consider the folding of a hairpin

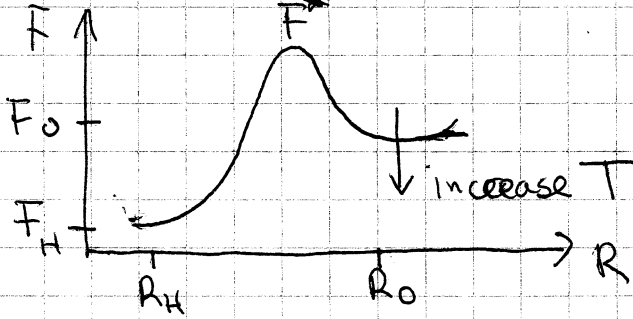


$F_0 = -TS$



$F_H = -n_s E_s$

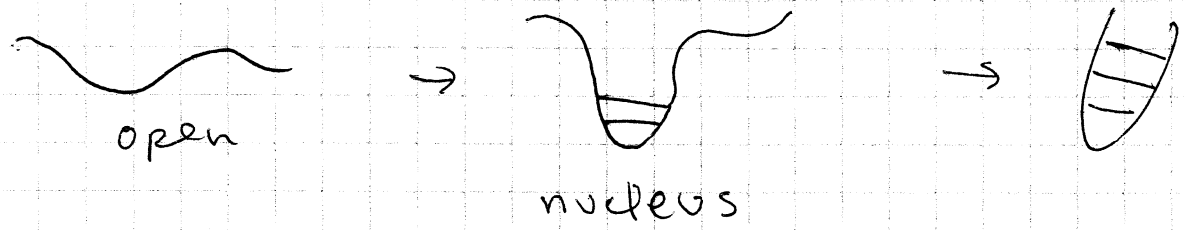
2 state system



$$P_H \approx \frac{e^{-F_H/kT}}{e^{-F_H/kT} + e^{-F_0/kT}}$$

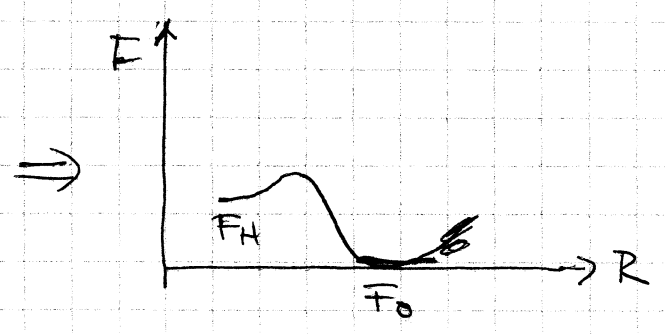
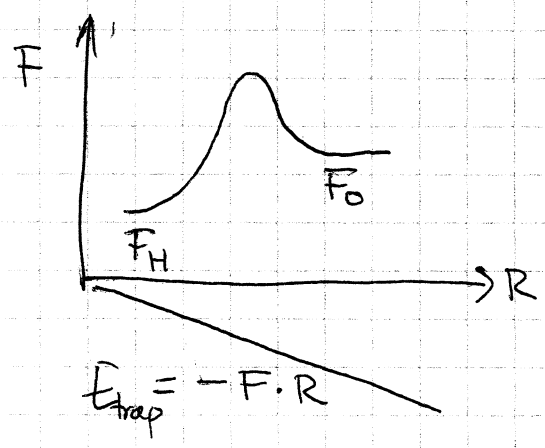
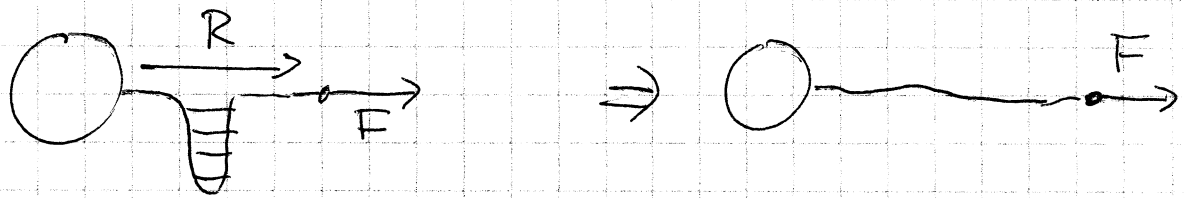
What is the barrier  $F^*$ ?

- energy cost to nucleate a stack



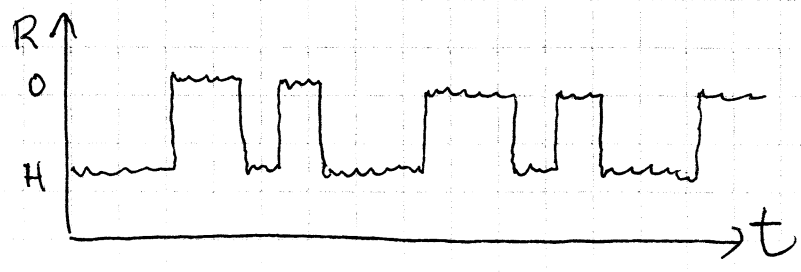
- Forming nucleus has an initial big entropy cost
- once nucleus forms, entropy loss is minimal compared to addition of more stacks.

RNA in Optical Trap



open state is now the ground state

Expt:

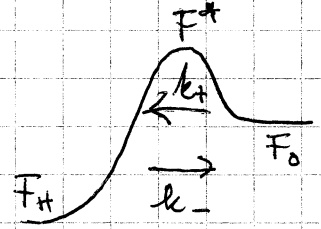


Constant Force

% total time in state  $\equiv P(E_0) \approx e^{-(F_H - F_0)/kT}$

What about duration time in states?

- dwell time depend on barrier  $F^*$



$$k_+ = C e^{-(F^* - F_0)/kT}$$

Dwell times are distributed exponentially

Why? Probability of no<sup>+</sup> hopping from 0  $\rightarrow$  H in time t is: didn't hop

solve  $P(t+dt) = (1 - k_+ dt) P(t)$   
 $P(t) = e^{-k_+ t}$

then hopping in next dt

$$\rightarrow P_{0 \rightarrow H}(t) dt = k_+ e^{-k_+ t} dt$$

so  $P_{0 \rightarrow H}(t) = k_+ e^{-k_+ t}$

Similarly  $P_{H \rightarrow 0}(t) = k_- e^{-k_- t}$