

# Understanding the Folding of a Small RNA Hairpin

## A Replica Exchange Molecular Study

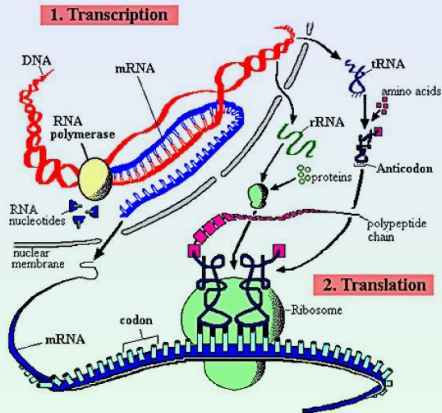
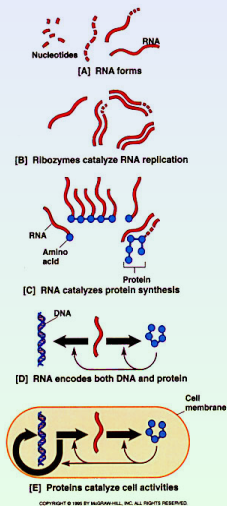
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April 28, 2009

# RNA world: Before the Central Dogma

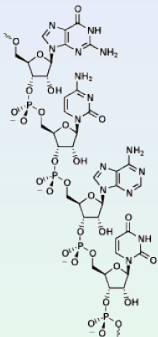
## 154 Proposed RNA world origins



Crick and Watson, Nobel Prize in Medicine  
 Figures Obtained from Internet

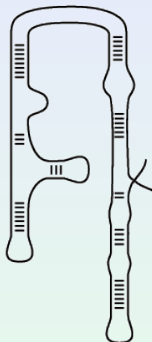
# Hierarchy of RNA Folding

1° structure



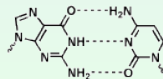
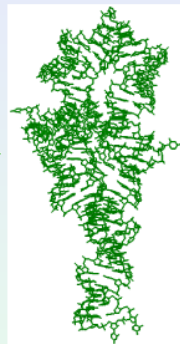
→  
form 2°  
structure

2° structure

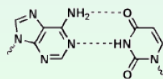


→  
Mg<sup>2+</sup>  
form 3°  
structure

3° structure

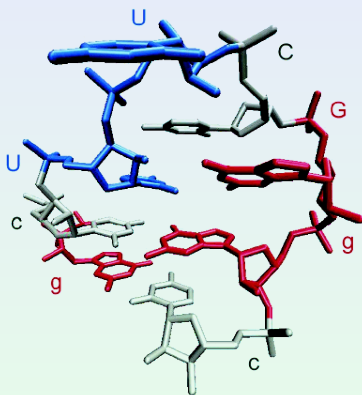


G-C base pair



A-U base pair

# The RNA Hairpin

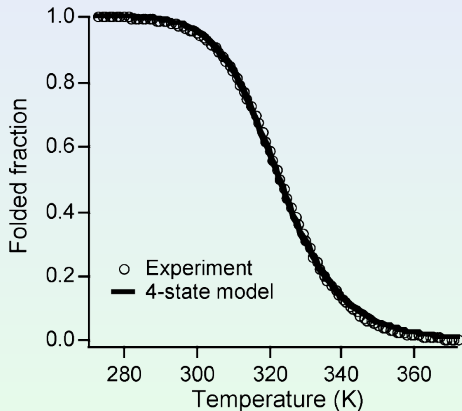


## Property

- ▶ The smallest RNA hairpin (8nt, 5'-gcUUCGgc).
- ▶ Stable and well-formed hairpin, a tetraloop with a two base pair stem (stack).
- ▶ Folding in a rugged free energy landscape, have multiphase kinetics.

Ma, et al., JACS 2006

# Folding Transition



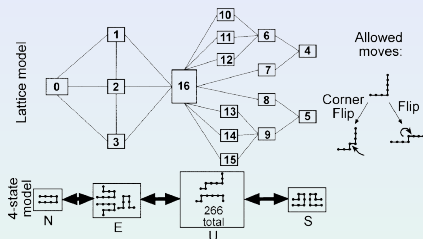
## Methods

- ▶ Heat denature
- ▶ UV absorption
- ▶ 2D Lattice Model

## Results

- ▶  $T_m = 323K$

# Four States



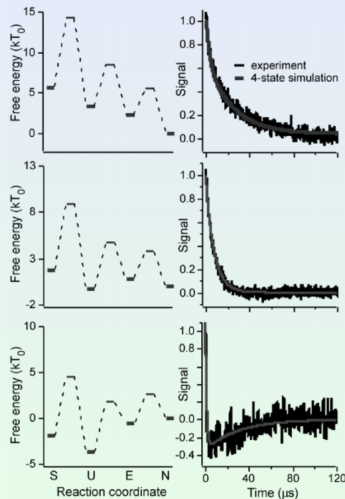
## Four States

**N:** Native State

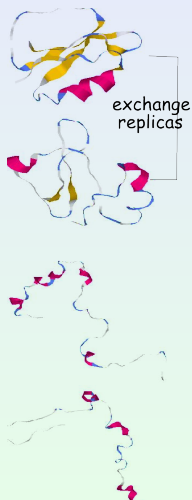
**I:** On-pathway Intermediate

**U:** Unfolded States

**S:** Off-pathway Intermediate



# Replica-Exchange Molecular Dynamics (REMD)



## Motive

Efficiently sample the conformation space.

## Method

- 1 M noninteracting trajectories with  $T_m$ .
- 2  $\tau$  independent MD simulation.
- 3 exchange neighbor replicas with

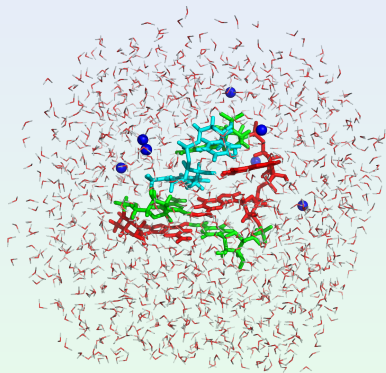
$$P(i \leftrightarrow j) = \min\{1, \exp[(\beta_j - \beta_i)(U_j - U_i)]\}$$

- 4 Repeat steps 2 and 3.

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Sugita & Okamoto, Chem. Phys. Lett. 1999

# MD simulation

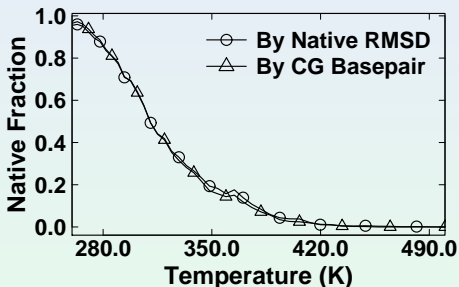


## MD parameters

- ▶ Amber 8, Parm99.RNA, Periodic Boundary.
- ▶ PME, SHACK,  $d_{cutoff} = 12.0$
- ▶ Octahedral box,  $7Na^+$ , 1347 Water(TIP3P).
- ▶ 8000 steps minimization and 100 ps constant pressure before.
- ▶  $M = 44$ ,  $\tau = 2ps$

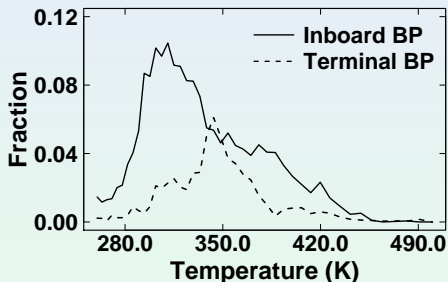


# Native State and Basepair



- ▶ Histogram sensitive to temperature.
- ▶ Folded transition midpoint  $T_m = 310\text{K}$ .
- ▶ 2 basepairs formed, RNA hairpin folded.

# Intermediate: Two Folding Pathways



- ▶ 2 basepairs, 2 substates, 2 pathways.
- ▶ Inboard basepair stabler, forming inboard basepair first is primary.

# Conformational Cluster

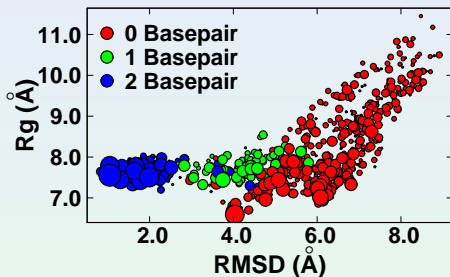


Figure: Clusters of  $T=310.6\text{K}$

by BasePairs &  $R_g$

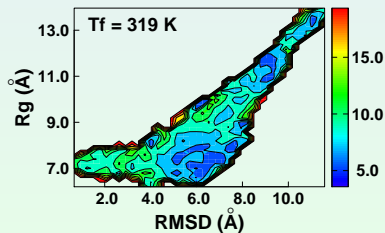
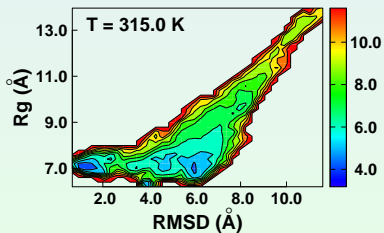
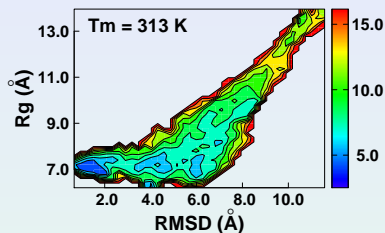
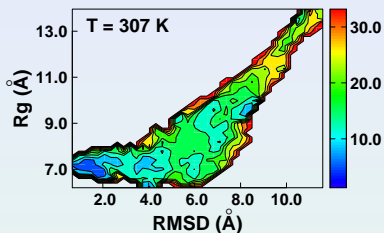
Native: 2 BP, Small  $R_g$

Intermediate: 1 BP, Small  $R_g$

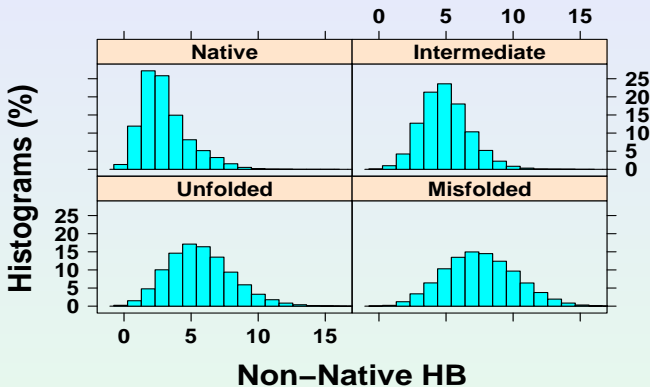
Unfolded: 0 BP, large  $R_g$

Misfolded: 0 BP, small  $R_g$

# Free Energy Landscape



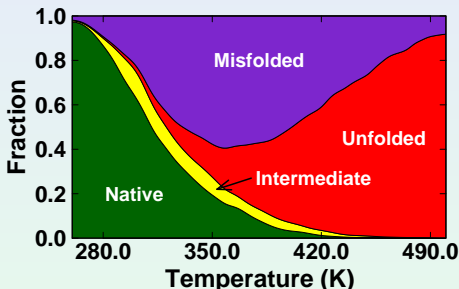
# Misfolded State



## Stable reason of misfolded state

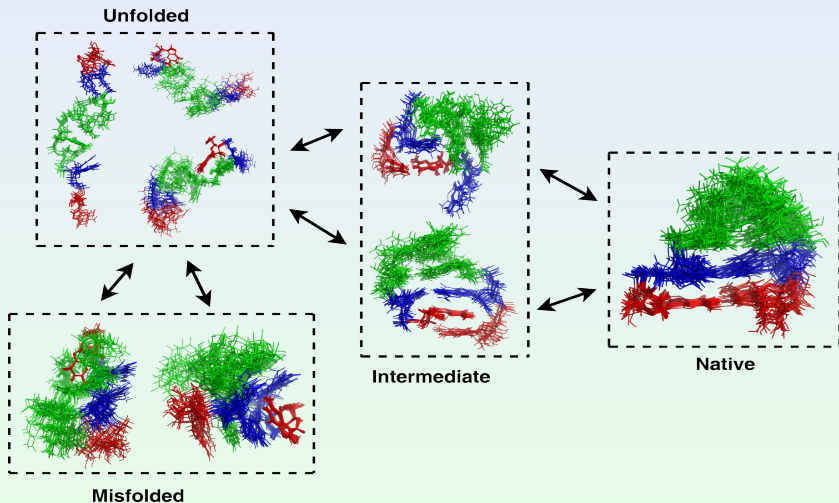
- ▶ Plenty of nan-native hydrogen bonds.
- ▶ Plenty of conformations.

# Four States



- ▶ Native state at low temperature.
- ▶ Intermediate is metastable.
- ▶ Misfolded state ( $345 < T < 400$  K).
- ▶ Unfolded state at high temperature.

# Folding Pathway Map



# Summary

- 1 Four states: native, intermediate, unfolded, and misfolded. Categorized by basepairs and  $R_g$ .
- 2 Folded transition midpoint  $T_m = 310$  K
- 3 Two folding pathways, two intermediate substates.
- 4 Inboard basepair formed first is primary.
- 5 Larger entropy and hydrogen bonds stable misfolded state.



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Prof. Wang Jin

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Thank you  
for your attention !