

Ribozyme evolution in an RNA-mineral world

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Minerals and RNA in early life

Minerals

Bind, concentrate, and
protect/react with organics

Structural similarities to
many inorganic cofactors

RNA

Highly conserved RNA
structures support central
biological functions

Monomers present in many
coenzymes

Minerals + RNA

Bind RNA

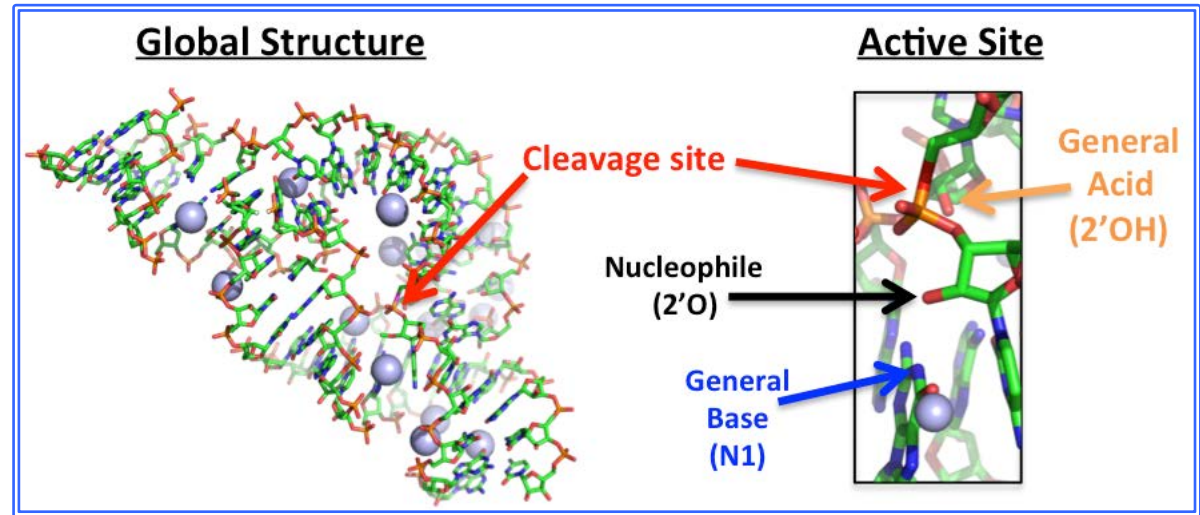
Protect RNA from damage and degradation

Polymerize activated RNA monomers

Biopolymer folding determines function

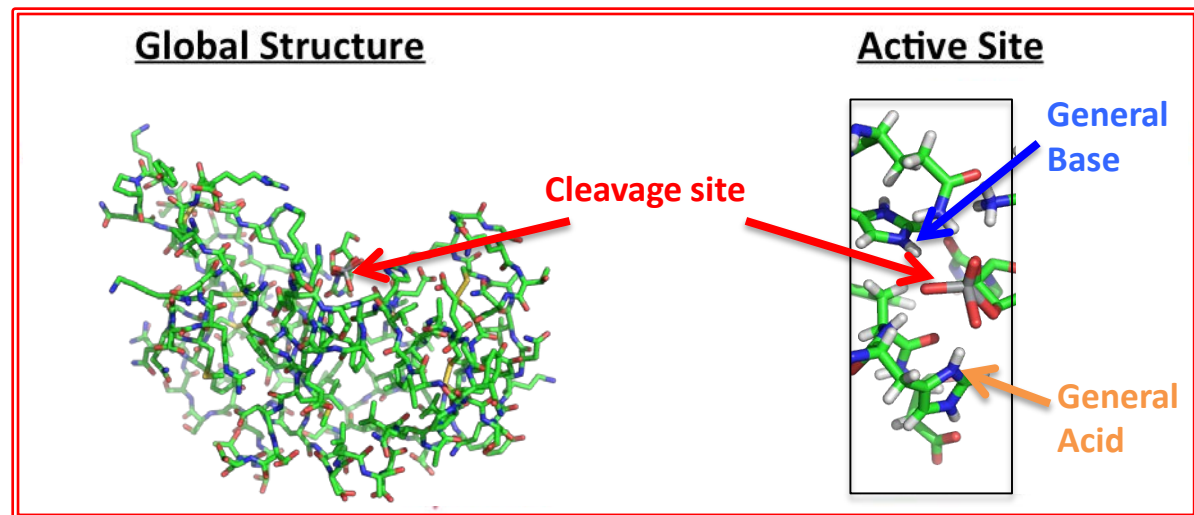
RNAs fold into complex structures to function

Nuclease ribozyme



Proteins fold into complex structures to function

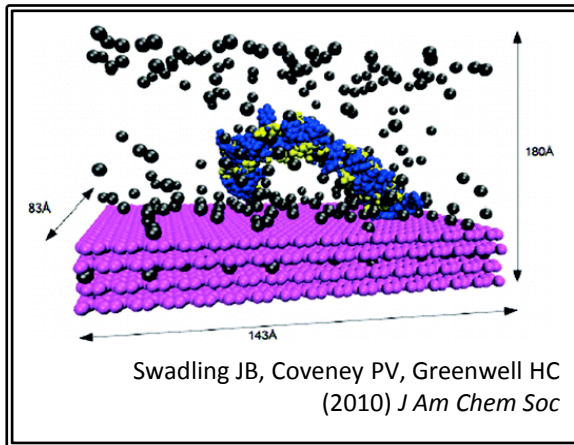
Nuclease enzyme



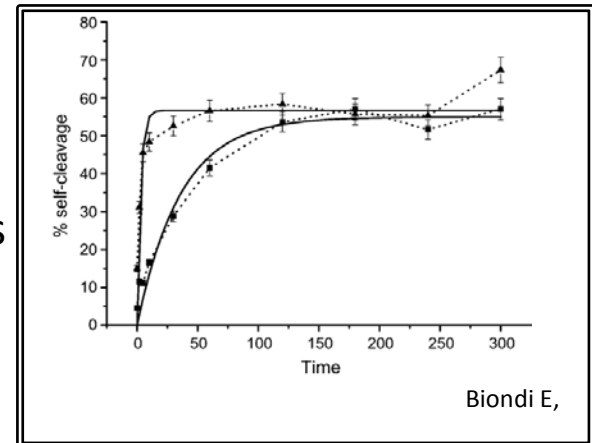
Minerals can impact RNA folding

Directly through contact between minerals and RNA

Altered folding in Molecular Dynamics simulations



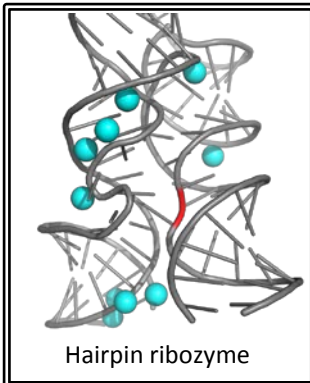
Altered catalytic activity of ribozymes



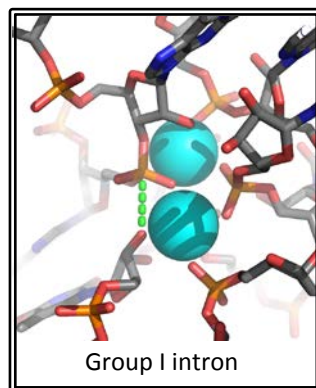
Indirectly through impact on solution conditions

Metal ions

Structural role

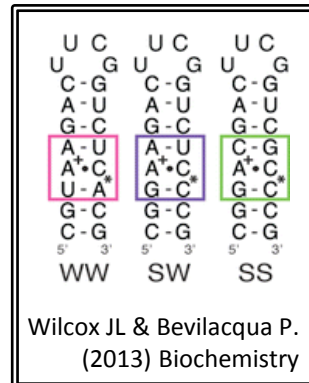


Catalytic role

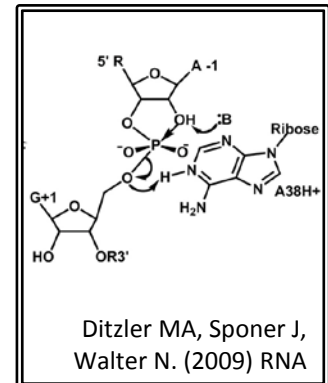


pH

Structural role



Catalytic role



In vitro evolution of RNA

In vitro evolution can be used to ask...

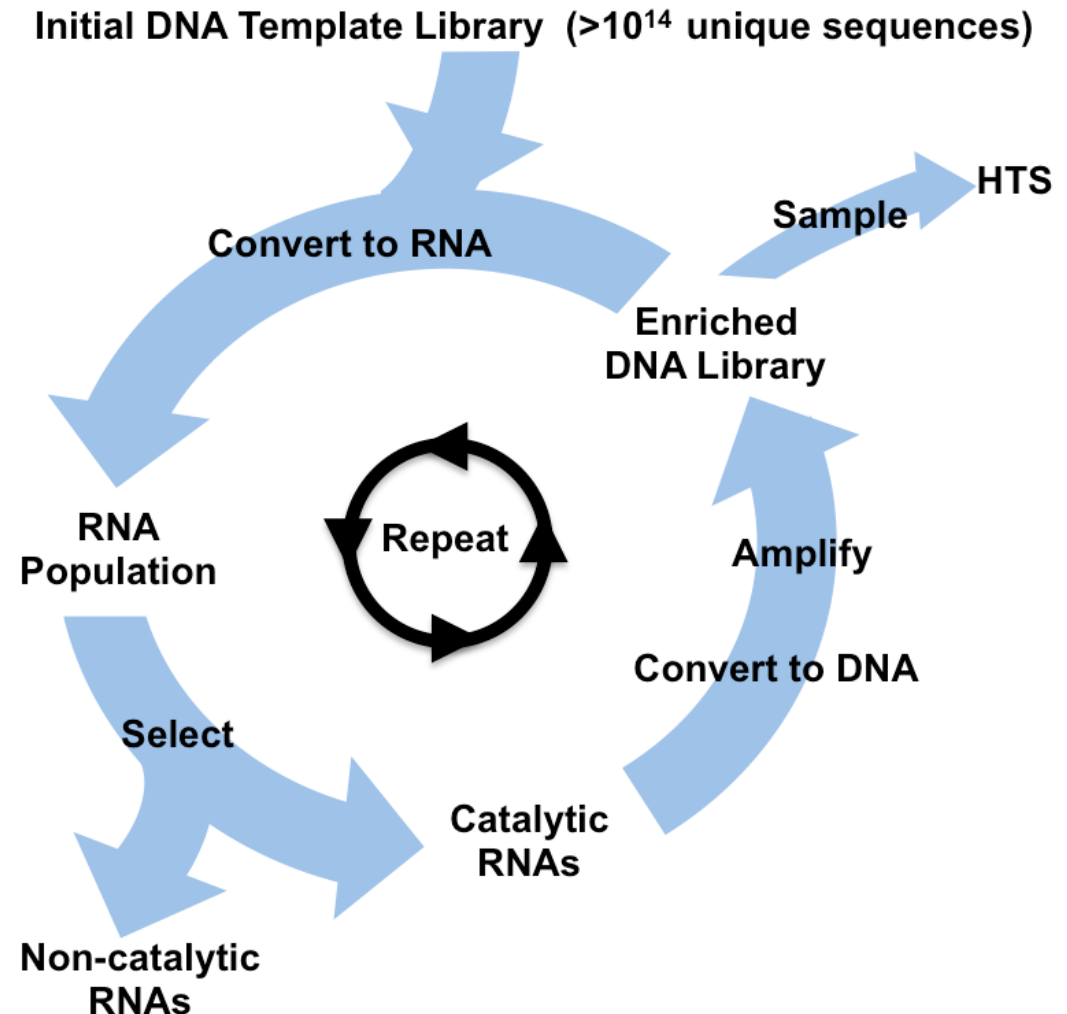
What RNA can do?

How common are functional RNAs in sequence space?

How complex do functional RNAs need to be?

How RNA can evolve new functions?

How does the environment impact the evolution of functional RNAs?



T7 promoter **5' fixed** Variable sequence (90bp) **3' fixed** **Cleavage** **3' fixed**
sequence
 6x10¹⁴ DNA templates

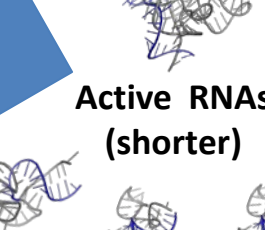


In vitro
transcription



Size selection (PAGE)

~ 8×10^{16} RNAs



**Active RNAs
(shorter)**

Reverse transcription
PCR

CAT ACG
ATC CTGC
ATA TGC

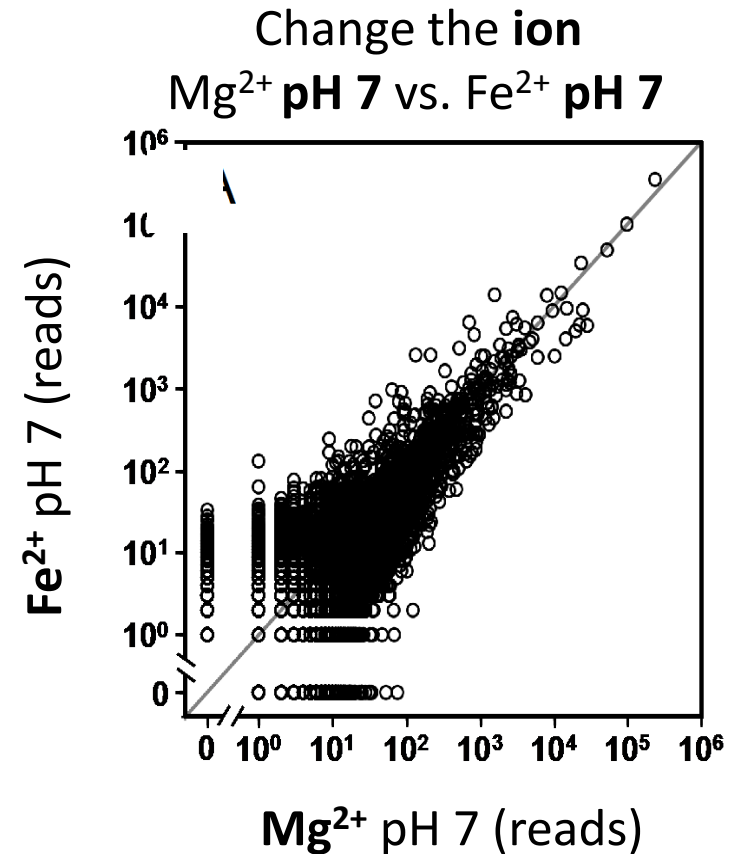
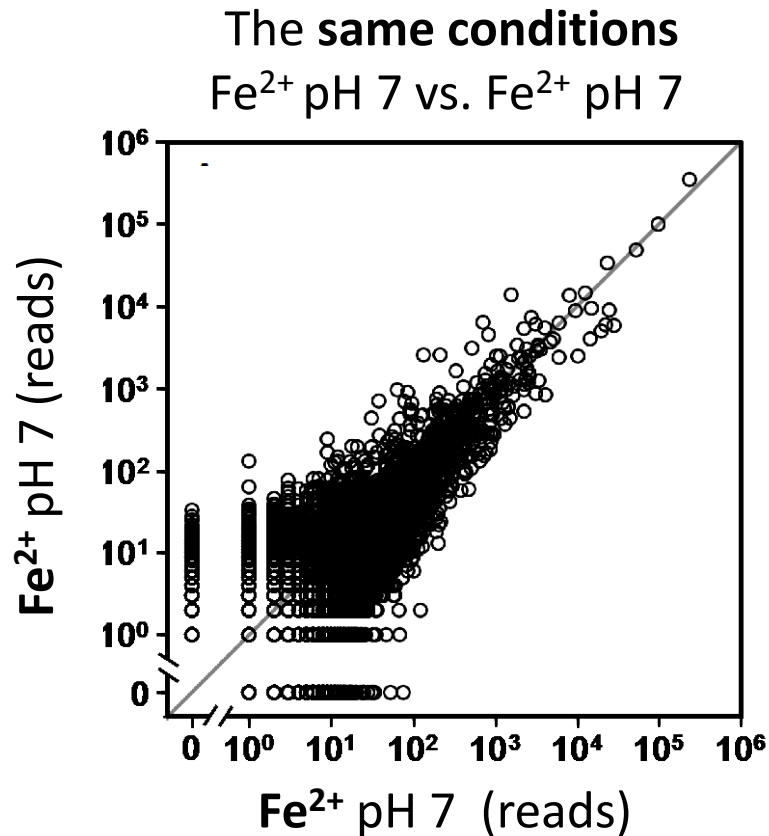
▶ ATGCTAGCTAGCTAGCTAGCTACGTCGT
ACCTGTACGTACGCTGCATCGTCGT
AGTACTGCTGTGACGTAGCAAGCG
TATGATATGATGATGATCCCTGG
ACTG CGA
TCGA CTA

Sequence

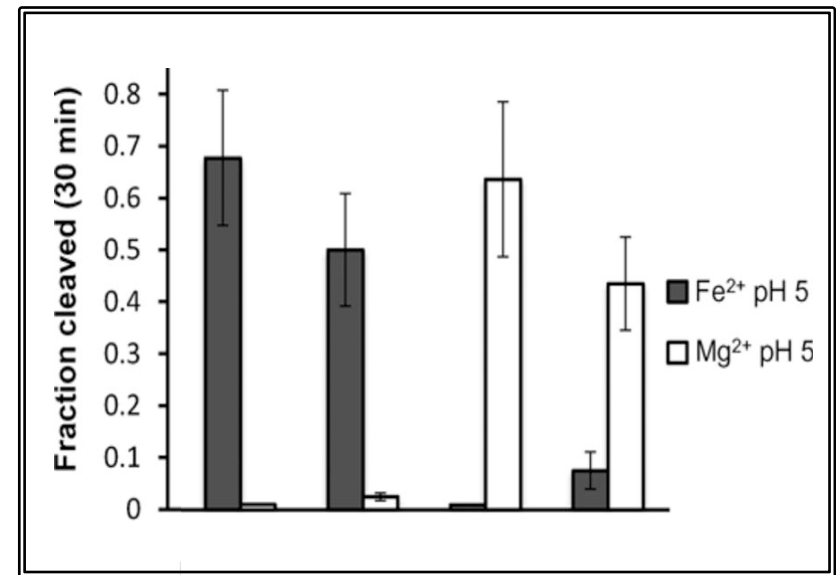
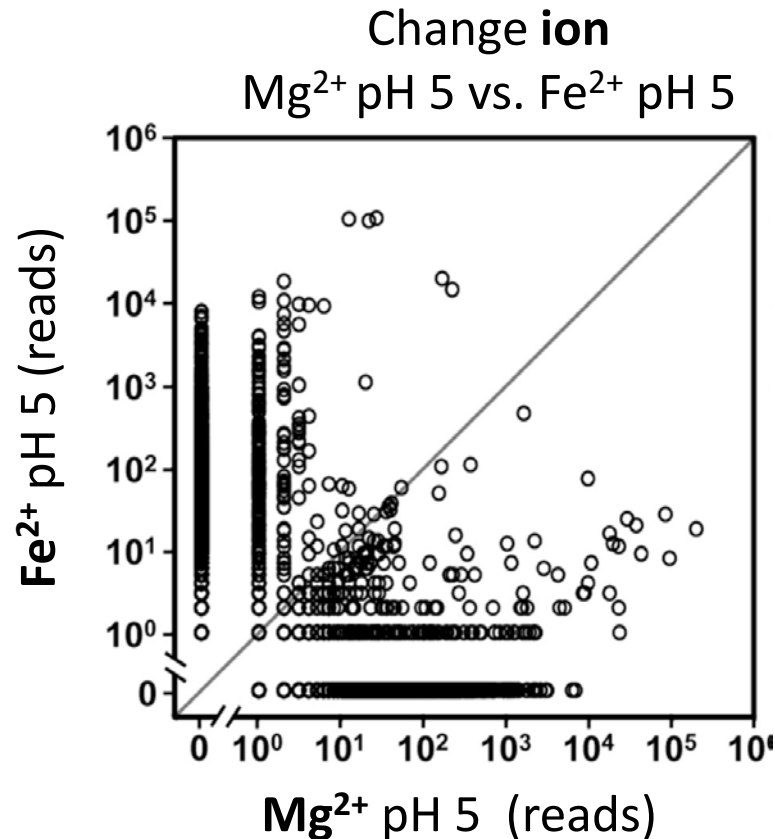
GCTAGCTAGCTAGCTAGCTAGTCA
GTCGATCGATCGATCGATCGTGCT
GTGACGTAGCTAGCTAGCTAGCTAGT
ATGATGATCGCTAGCTAGCTAGCTAG
GTCGAGATCGCTAGCTAGCTAGCTAG
CGATCGATCGCTAGCTAGCTAGCTAG
CTAGCTAGCTAGCTAGCTAGCTAGCT
TCGATCGATCGCTAGCTAGCTAGCTAG
GCTAGCTAGCTAGCTAGCTAGCTAGCT
TAAACCCAGTATGATCATCGA
TGCTACGTAGCTAGCTAGCTAGCTAG
GTACTAGCTAGCTAGCTAGCTAGCTAG
TCAGTACGTAGCTAGCTAGCTAGCTAG
AGCTAGCTAGCTAGCTAGCTAGCTAG
TACGTACGTAGCTAGCTAGCTAGCTAG
CTGCTGTGACCTAGCTAGCTAGCTAG
ATATGATGATCGCTAGCTAGCTAGCTAG

Analyze: Count, cluster, compare, predict structures

Different conditions favor the evolution of different sequences

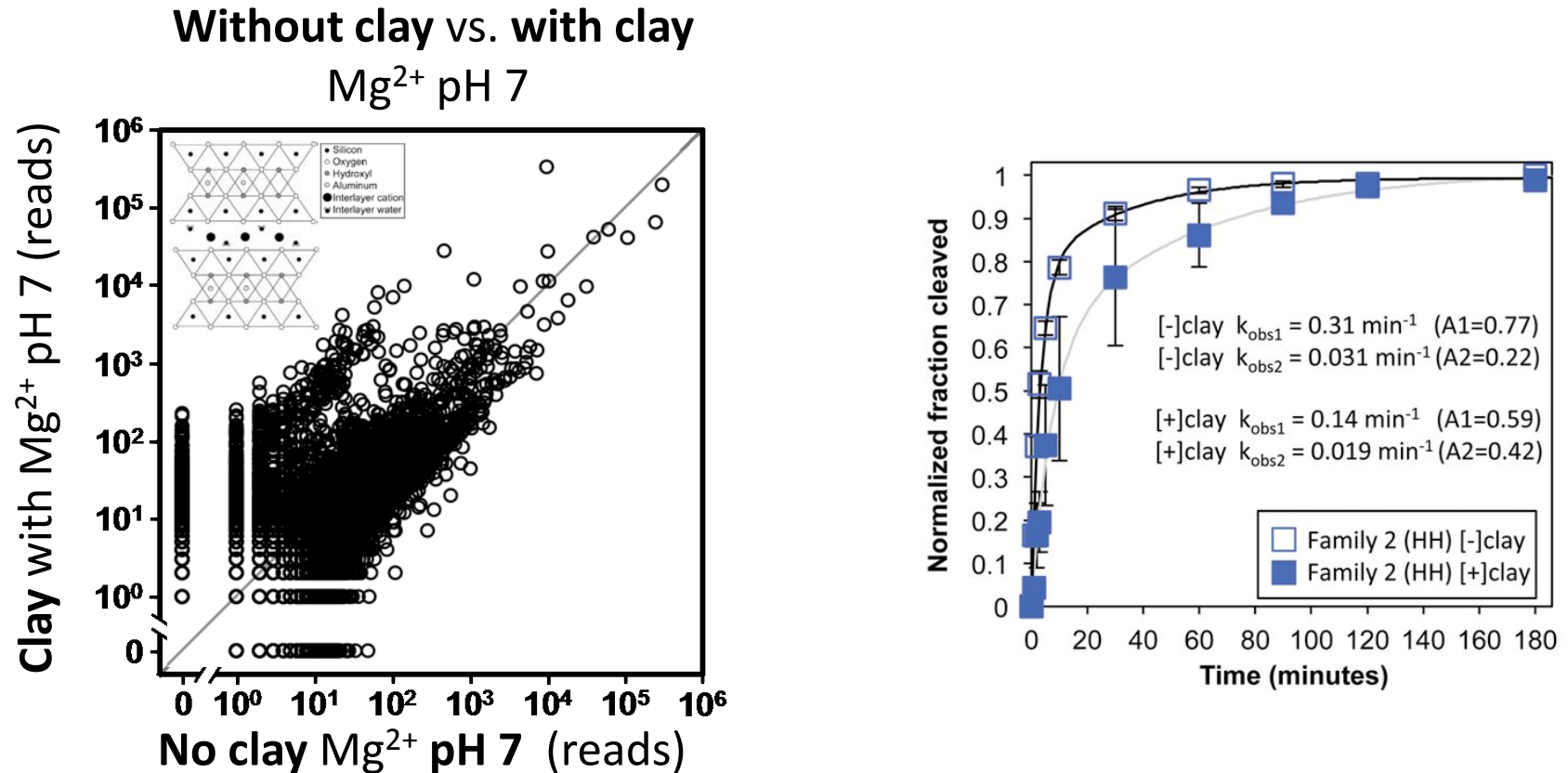


Different conditions favor the evolution of different sequences



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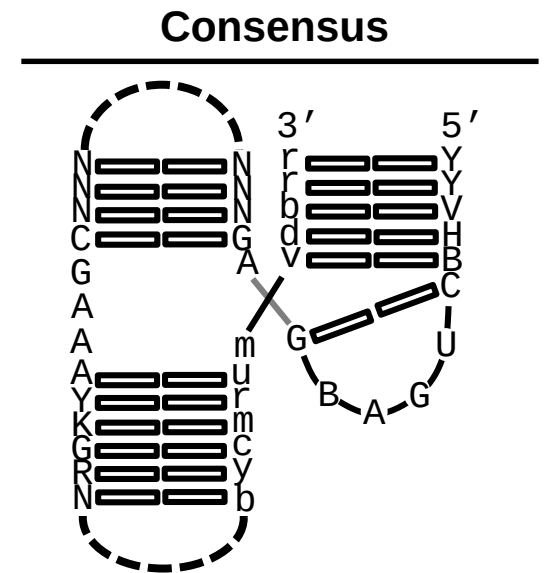
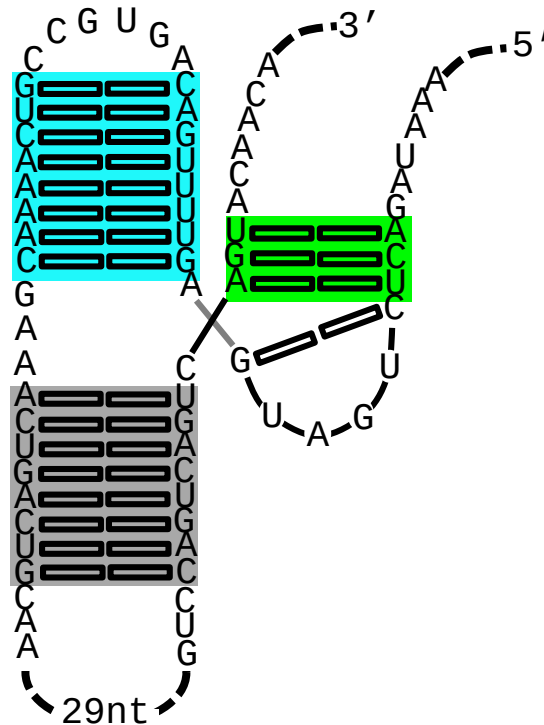
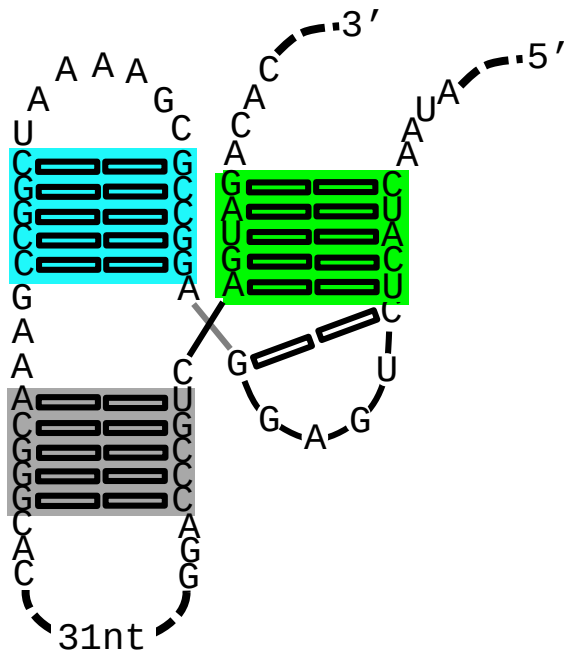
Different conditions favor the evolution of different sequences (sometimes)



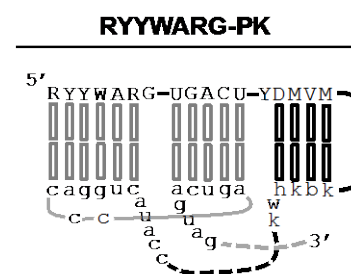
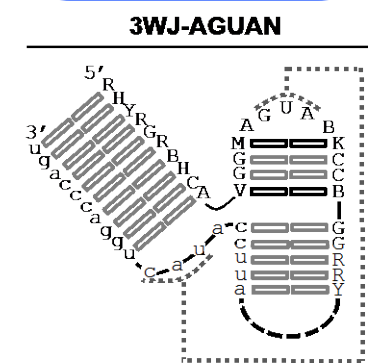
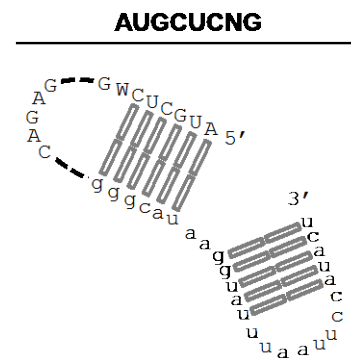
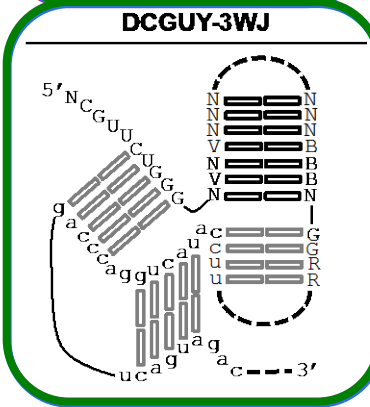
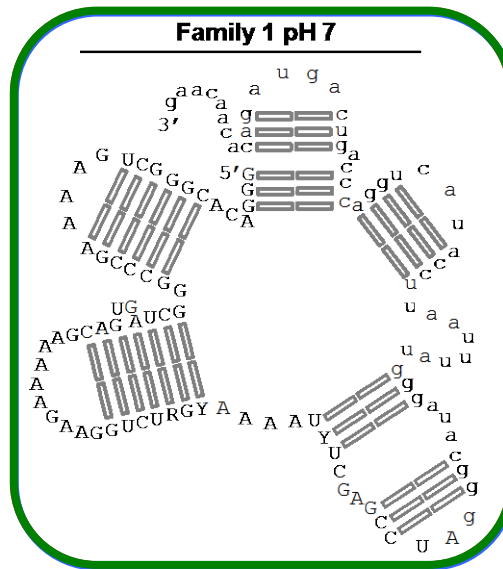
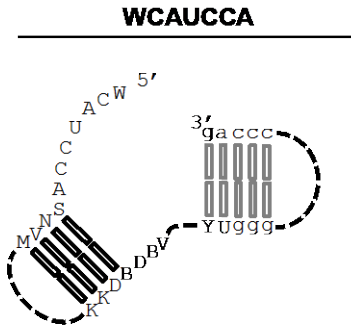
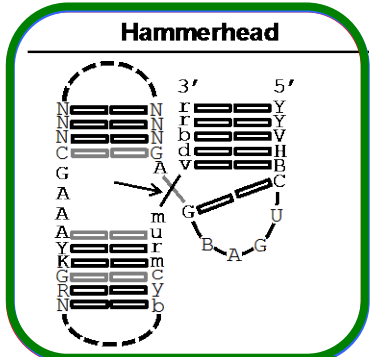
Identifying Structures

...AUAACUACUCUGAGGAGGCCCGA AAAU CGGCCGAAACGGGCAC[31nt]GACCCAGUCAGUAGACAC...

...AAUAGGUCCUGAUGAGUUUUGACAGUGCCGUCAAACGAAACUGACUGCAA[29nt]GUCCAGUCAGUAGACACAACA...



Different conditions favor different structures
(except when they don't)



== Basepairs displaying covariation

Basepairs not displaying covariation

xx Constant sequence

XX Variable sequence

--- Single stranded region
of variable length

R = A or G B = C or G or U

Y = C or U D = A or G or U

W = A or U H = A or C or U

K = G or U V = A or C or G

M = A or C N = any base

- Fe^{2+} , pH 7

- Mg^{2+} , pH 7

- Mg^{2+} , pH 7 with montmorillonite clay

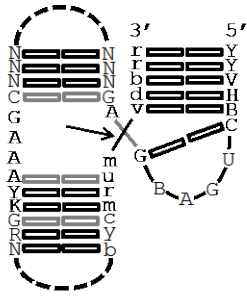
- Mg^{2+} , pH 5

- Fe^{2+} , pH 5

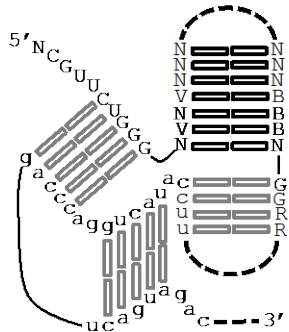
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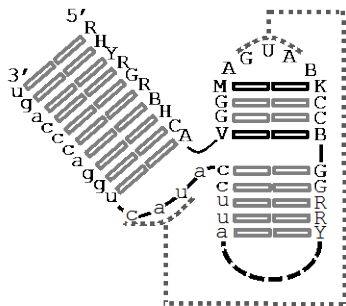
Hammerhead



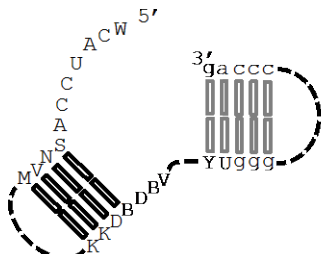
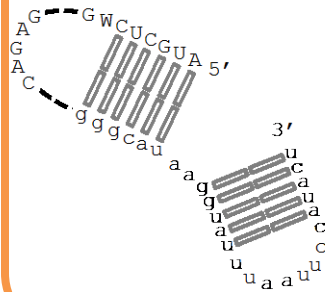
DCGUY-3WJ



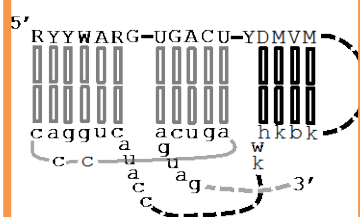
3WJ-AGUAN



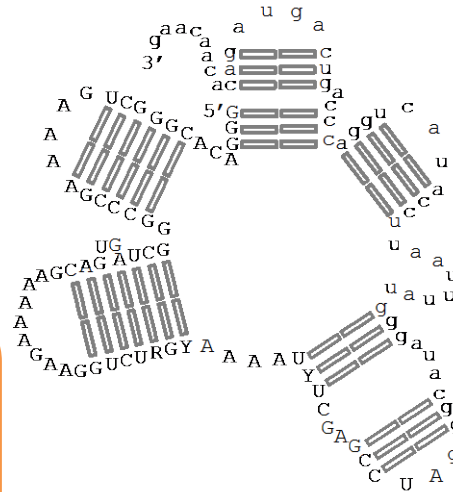
WCAUCCA

**AUGCUCNG**

RYYWARG-PK



Family 1 pH 7



- Fe^{2+} , pH 7

- Mg^{2+} , pH 7

- Mg^{2+} , pH 7 with montmorillonite clay

- Mg^{2+} , pH 5

- Fe^{2+} , pH 5

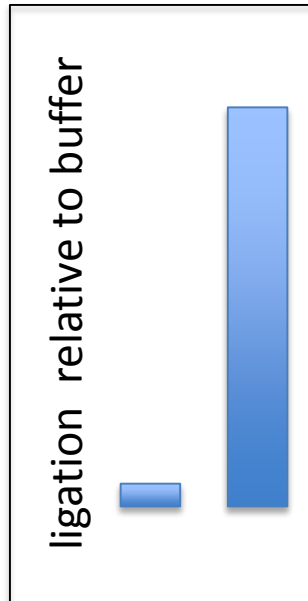
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Direct impact of minerals may be greater for inter-molecular functions

Preliminary L1 ligase ribozyme activity strongly inhibited by montmorillonite in trans but not in cis



Conclusions

Direct interactions with mineral surface have limited impact on RNA evolution (at least with respect to folding)

- Transition away from clay surface does **NOT** require evolution of new structures
- Conclusions based on prior in vitro evolution experiments **CAN** be applied to RNA on mineral surface

Mg²⁺ and Fe²⁺ support the evolution of similar structures at neutral pH

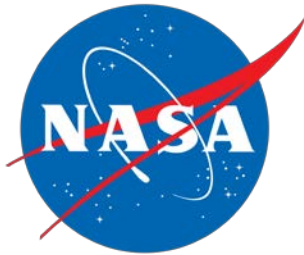
- Transition from Fe²⁺ to Mg²⁺ does **NOT** require evolution of new structures

pH has a large impact on RNA evolution and favors different structures

- Changes in pH would result in the evolution of **new structures**
- Many conclusions based on prior in vitro evolution experiments may **NOT** apply at low pH
- We need to pay close attention to how the mineral environment influences pH in an RNA world

Acknowledgments

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James Stephenson (clay)
Palmer Fliss (Fe and pH)
Thomas Bristow (clay)



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