Imaging RNA structures and folding intermediates using electron cryo-microscopy

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RNA can form irregular tertiary structures

Sarcin Ricin loop

P RNA subdomain

Ribosome

tRNA

Group I Intron

How do they obtain these structures?
Kinetics --- Thermodynamics --- Structure
Only the sequence is needed!

Primary Sequence (nucleotides, amino acids) → Unfolded → “Mother Folding” → RNA → Native
Metal requirement: **Tertiary RNAs** typically require divalent cations to fold.

Two classes of cation-RNA interactions
1. non-specific binding (counter-ion condensation, Me\(^+\) or Me\(^{2+}\) )
2. High occupancy metal sites are observed in crystal structures of tertiary RNAs (often Me\(^{2+}\)).

Non-specific binding occurs at low [Me], stabilizes secondary structures
Specific binding at higher [Me\(^{2+}\)] is involved in tertiary folding transitions.
Bacillus stearothermophilus Rnase P RNA

Specificity
S-domain
(~150 nt)

Catalytic
C-domain
(~255 nt)

Pace and coworkers, PNAS 2005
Mg$^{2+}$-induced equilibrium folding of C-domain of P RNA

At low [Me$^{2+}$], non-specific (and specific) binding,

At high [Me$^{2+}$], energetics of dominated by specific binding

Fang, Pan & Sosnick
Biochemistry, 1999
Summary of C-domain folding

\[ \text{U}_{\text{urea}} \]

Limited by Me\(^{2+}\) binding site formation: Defines tertiary structure

Fast, local conformational search

\[ R_g = 180 \text{ Å} \]

\[ R_g = 46 \text{ Å} \]

\[ R_g = 39 \text{ Å} \]

\[ R_g = 38 \text{ Å} \]

\[ I_{eq} \]

\[ I_{1k} \]

\[ I_{2k} \]

\[ \leq 1 \text{msec collapse, } 2^\circ + \text{some } 3^\circ, \]

non-specific and some specific Mg\(^{2+}\)

\[ \text{Limiting step} \]

Consolidation of specific metal binding site

Fang, Pan & Sosnick, PNAS, 2002
Structural characterization of intermediates

U \rightleftharpoons \text{Mg}^{2+} \rightleftharpoons I \rightleftharpoons \text{Mg}^{2+} \rightleftharpoons N
B. subtilis Specificity domain of RNase P RNA

Krasilnikov et al, Nature 2003
Site-resolved information from chemical and nuclease cleavage

**U to I transition**

- Four-way junction

**I to N transition**

- I<sub>eq</sub> contains J11/12 module & four-way junction

- TL-receptor

- Core

- P10

- P12

- P11

- P8

- P9

- P7

- J11/12

- J12/11

- G

- C

- A

- U

- •

- V1

- T1

- KE

- DMS

- DEPC
$P(r)$ – general shape, from small-angle X-ray scattering

$R_g$ – overall size

Experimental SAXS

Crystal Structure

$R_g \sim 30 \, \text{Å}$

APS, BioCat beamline
Start from the crystal structure…

Modeling the intermediate with experimental constraints
(SAXS, Nuclease and chemical mapping)

With Eric Westhof
Baird et al. JMB 2005

Disrupt TL-receptor interaction
\[ \Delta R_g \approx 2 \text{ Å} \]

Rotate P10.1 further
\[ \Delta R_g \approx 5 \text{ Å} \]

Rotate both P10.1
and P12 arm

\[ \Delta R_g \approx 8 \text{ Å} \]
Intermediate Structure

$I_{eq}$

$N$
Role of metal ions in folding cooperativity

Direct mediation of long-range contacts

Indirect mediation - metal binding coupled to large-scale conformational change

S-domain

U

I_{eq}

N
Intermediate Structure (model)
Large molecules light up on EM

But nothing beats a real picture…

• Ribosome – 3 MD

• Proteasome – 750 kD

• Generally accepted lower limit ~200 kD
Direct imaging of ‘small’ RNAs
W. Chiu & S. Ludtke, Baylor

Start with molecules with known crystal structures to assess feasibility

82 kD
Catalytic domain RNase P RNA

Side
Top
Front

(blind reconstructions)
When the intermediate is stably populated it can be directly imaged!

Native S-domain
$I_{\Delta 25}$ comparison with reconstruction
Model vrs SAXS & CryEM reconstructions

Model
CryoEM
SAXS
Add an extension to enhance image

Native

Folding behavior unchanged

I_{eq} \text{ with } P9_{ext}
$I_{eq}$ with $P9_{ext}$
I_{eq} dimensions: no salt dependence below 1 M NaCl

Why doesn’t flexibility blur the image?

Not just electrostatics holding I_{eq} in an extended state:
Defined thermodynamic well

Structure in the core?

All-atom simulations
RNA structure determination using CryoEM + modeling + all-atom simulations:

A rapid alternative to crystallography?
Thoughts:

Include “folding” to help restrict the conformational search in prediction 

What about folding cooperativity?
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