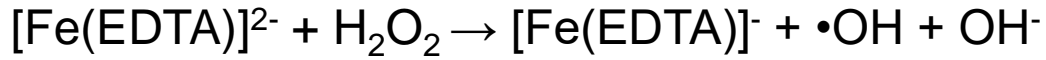
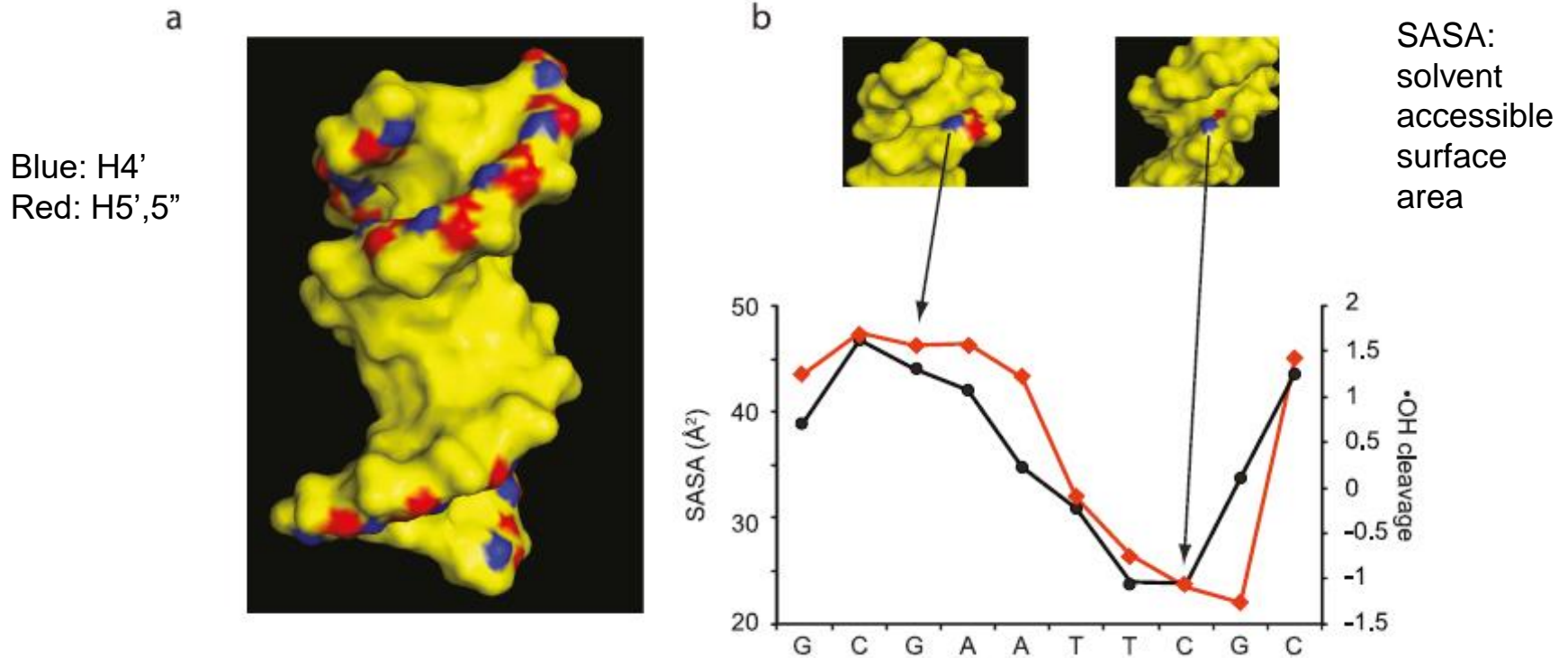


Folding of the Group I Intron

Guo F, Gooding AR, Cech TR. **Structure of the Tetrahymena ribozyme: base triple sandwich and metal ion at the active site.** Mol Cell. 2004 16(3):351-62

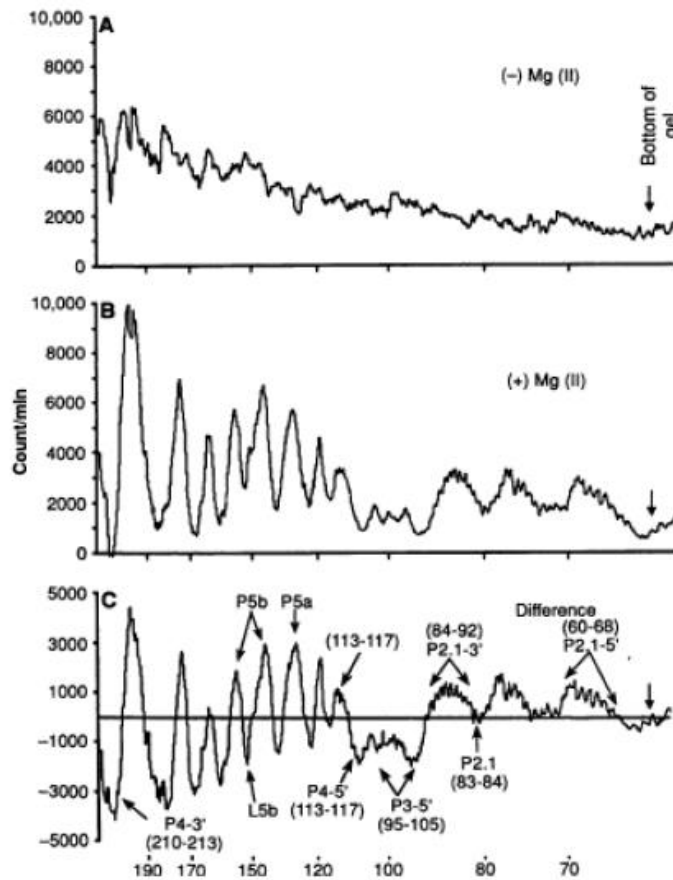
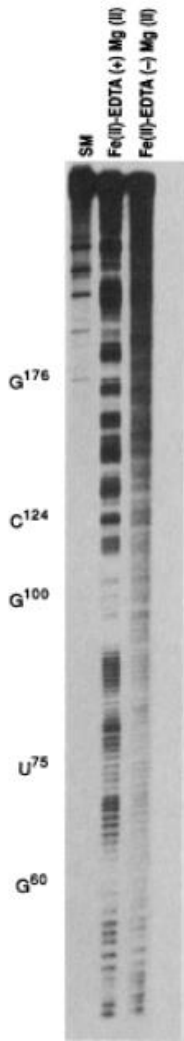


Fenton chemistry is the generation of a hydroxyl radical by Fe(EDTA) that is oxidized by peroxide. The neutral hydroxyl radical abstracts a hydrogen from deoxyribose or ribose, resulting in strand scission. Preferred sites are H5' > H4' > H3' ≈ H2' (deoxyribose).



Cleavage is determined by solvent accessibility, and for the famous Drew-Dickerson DNA dodecamer, cleavage efficiency reveals how sequence dependence in B-form structure exposes or protects the deoxyribose.

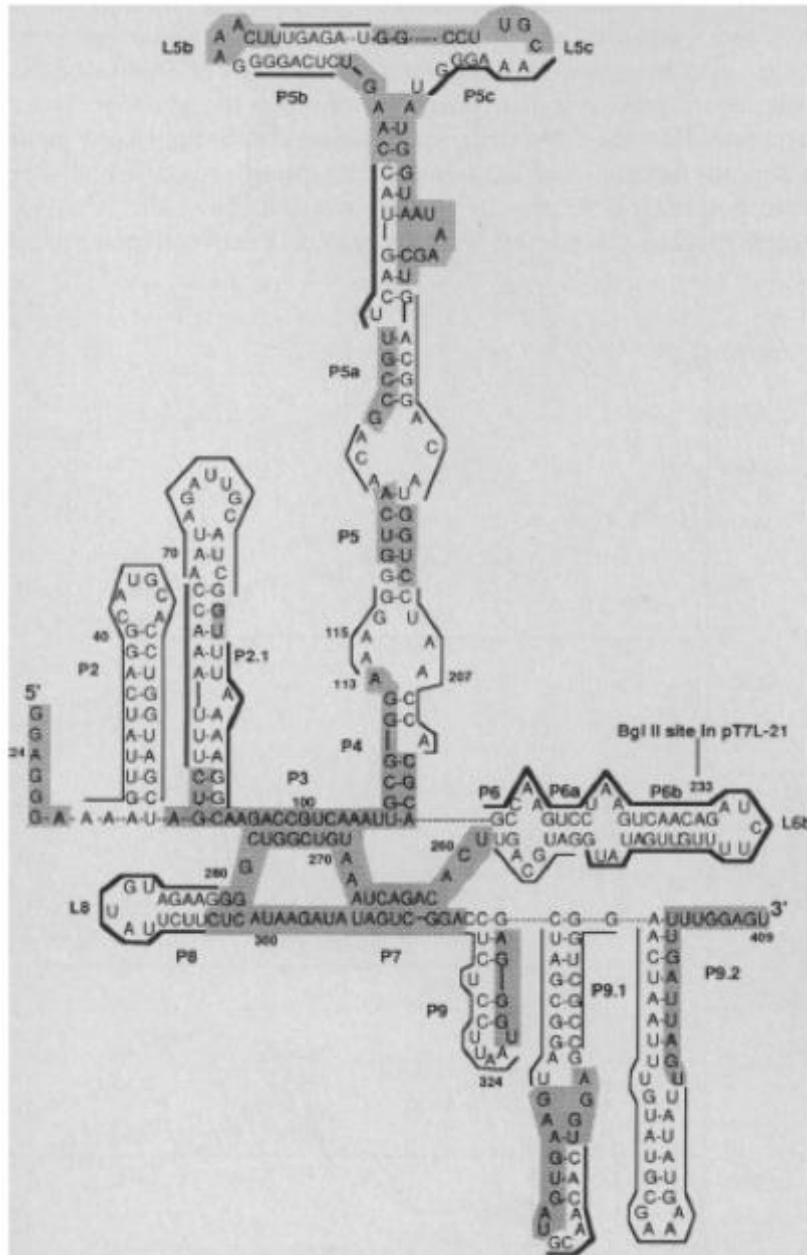
The 414 nucleotide Tetrahymena Group I intron: Does it have an inside and an outside?
 Proteins have solvent-accessible surfaces and internal cores; is this true for a large folded RNA?



Without Mg^{2+} there is uniform cleavage over the entire RNA.

With Mg^{2+} there are areas that are preferentially cleaved and some that were completely protected.

The difference between traces were mapped onto the secondary structure of the intron.

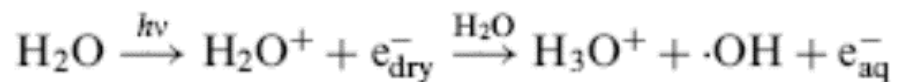


Protected sites are shaded.
Cleaved sites are outlined.

The authors made several points:

- ❑ Proteins have tightly packed interiors as a natural consequence of their nonpolar and hydrophobic amino acid side chains that avoid solvation.
- ❑ In contrast, the planar bases of a duplex are in the middle of the RNA helix and the anionic phosphates and polar sugars are on the outside of a duplex.
- ❑ How does this structure lend itself to compaction?
- ❑ The authors suggest that tertiary hydrogen bonding interactions between bases, sugars, and phosphates like those in tRNA will be present in the structure of the Group I intron. Stacking interactions also contribute to tertiary interactions.
- ❑ “Finally, magnesium ions, neutralizing the anionic phosphates and perhaps bridging helices, could allow the backbones of different helices to be packed close together”.

The modern version of this experiment uses synchrotron radiation to hydrolyze water, and the hydroxyl radical does the cleavage.

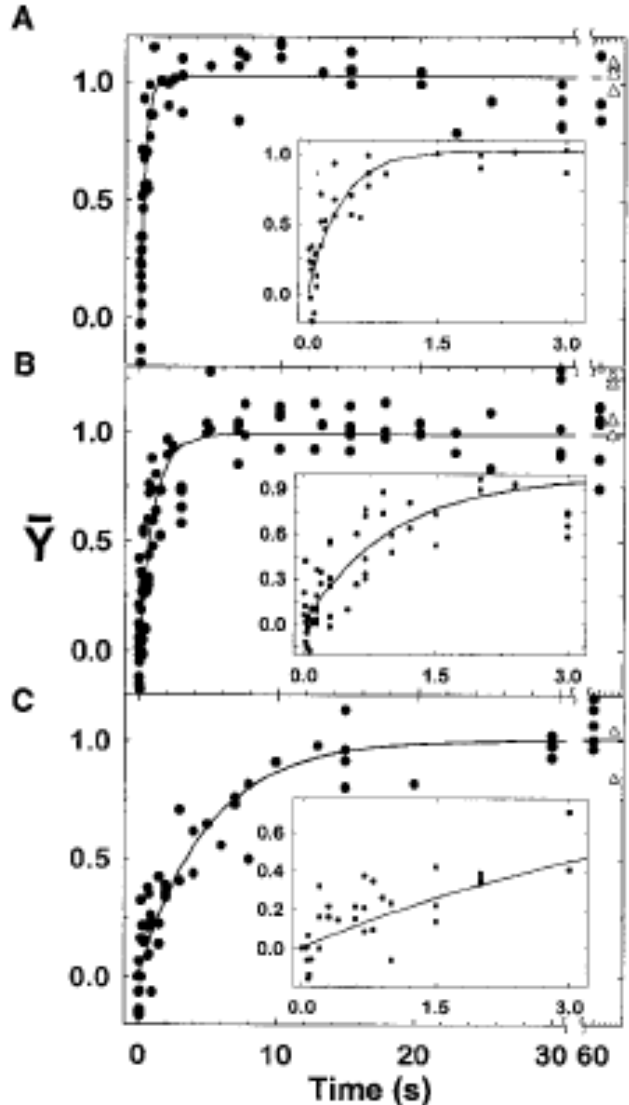


In an x-ray beam, water is hydrolyzed. Radiolysis of the water leads to hydroxyl radicals, and they interact with the ribose basis similar to the Fe(II)-EDTA chemistry. The oxidation of the riboses depends on solvent accessibility, but is independent of secondary structure or sequence.

Now, do these experiments as a function of time (stopped-flow quench). The speed of the reaction allows mapping of the folding process in real time.

(Sclavi et al., Science 279: 1940-1945.)

Experiment: prepare end-labeled RNA, mix it in the stopped-flow with Mg^{2+} to a final concentration of 10 mM. After mixing, start sampling by irradiating with the beam and collecting samples that are run on denaturing polyacrylamide gels to quantify the cleavage (protection) with time. The time resolution of the experiments was 10 ms.



Here are the data describing the time dependence of protection of sites.

\bar{Y} is the fractional saturation of single protected sites, determined from the power dependence of the beam $p = p_{\text{lower}} + (p_{\text{upper}} - p_{\text{lower}}) \bar{Y}$ and $\bar{Y} = 1 - e^{-kt}$. P is the saturation, p_{upper} , p_{lower} the upper and lower limits of the transition curve, k the first order rate constant, and t time in seconds.

Curve A is protection of nt 174-176: $k = 2.7 (-1.3, + 1.8) \text{ s}^{-1}$

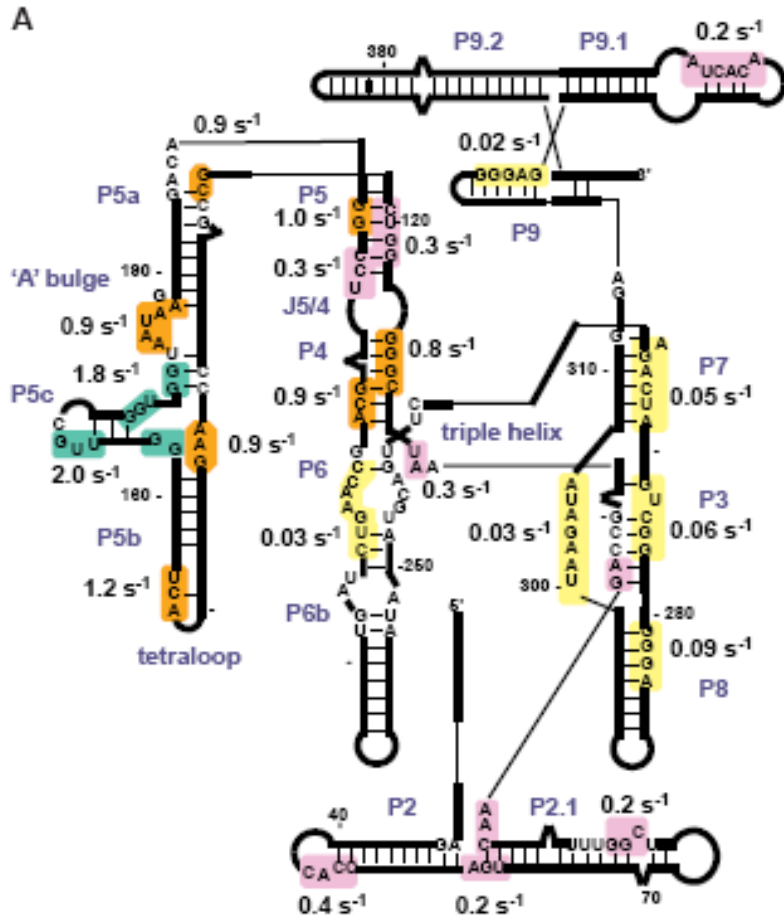
Curve B is nt 183-189. $k = 0.9 (\pm 0.3) \text{ s}^{-1}$

Curve C is nt 57-59; $k = 0.20 (\pm 0.05) \text{ s}^{-1}$

Open symbols are controls of pre-equilibrated RNA with Mg^{2+} .

Map these data onto the secondary structure:

Regions with similar folding rates are colored-coded.



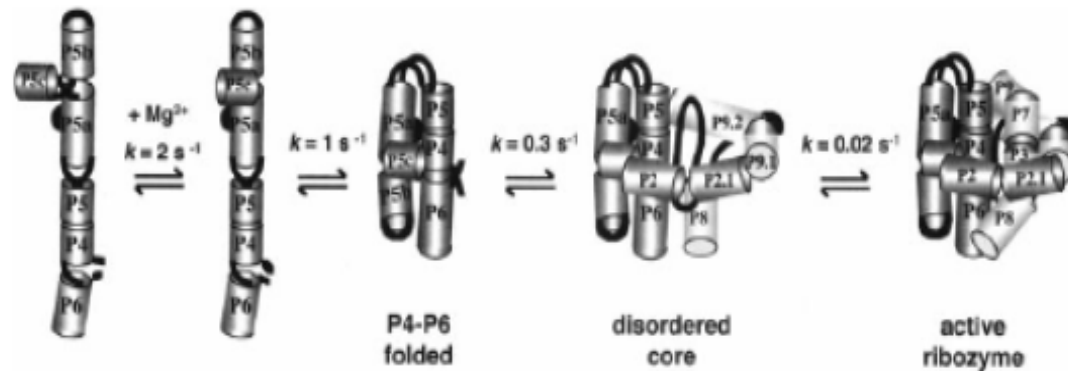
Green is fast: 2 s^{-1} .

Orange has a tetraloop/receptor and a fast folding rate of $\sim 1 \text{ s}^{-1}$.

Pink folds slower: 0.2 s^{-1}

Yellow folds slowest: 0.06 s^{-1}

The authors proposed the folding pathway shown, where P4-P6 folds first, followed by a disordered core, and final rearrangement into the active form.

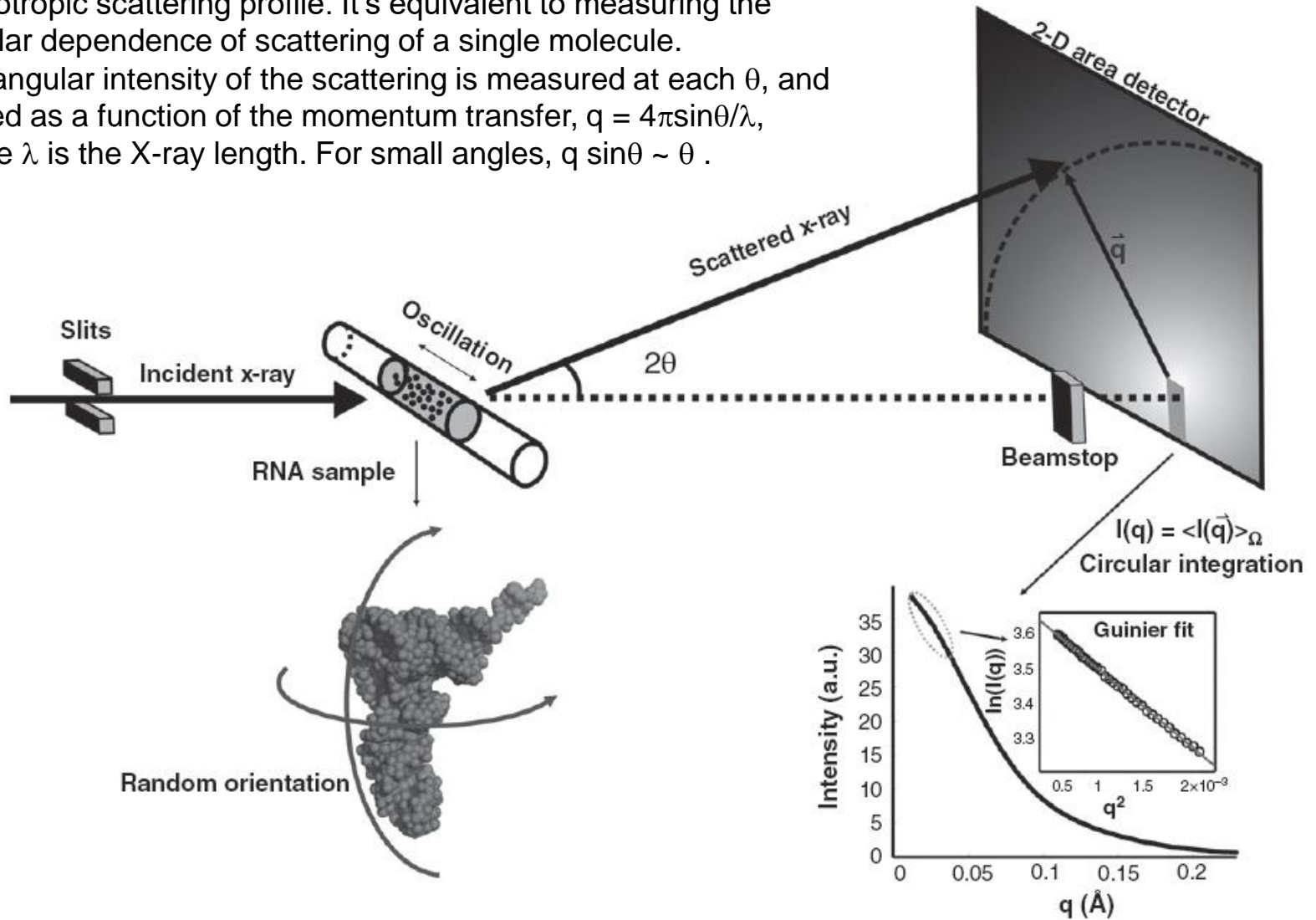


The authors also point out that slow folding could be the result of misfolding into kinetic traps. Turns out they were right: the group I intron does fold into a long-lived inactive form.

SAXS (small angle X-ray scattering).

Many molecules are randomly tumbling in the X-ray beam, giving an isotropic scattering profile. It's equivalent to measuring the angular dependence of scattering of a single molecule.

The angular intensity of the scattering is measured at each θ , and plotted as a function of the momentum transfer, $q = 4\pi\sin\theta/\lambda$, where λ is the X-ray length. For small angles, $q \sin\theta \sim \theta$.



From SAXS experiments, the **radius of gyration** of a molecule is most readily calculated.

Solution must be sufficiently dilute that intermolecular associations are avoided.

The molecule must be monodisperse (no dimers, aggregates).

Separate problems are conformational changes on the timescale of data collection (i.e. there is a conformational ensemble).

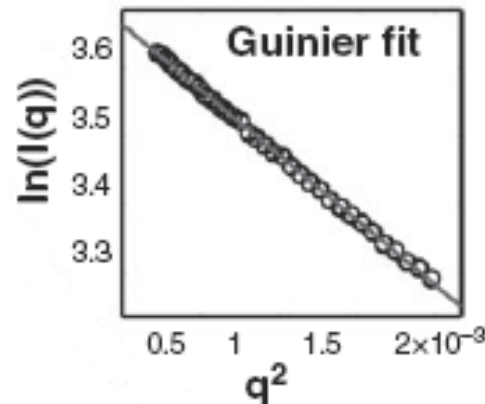
The radius of gyration (R_g) is calculated from the Guinier formula for small q .

$$I(q) = I(0)e^{-\frac{q^2 R_g^2}{3}}$$

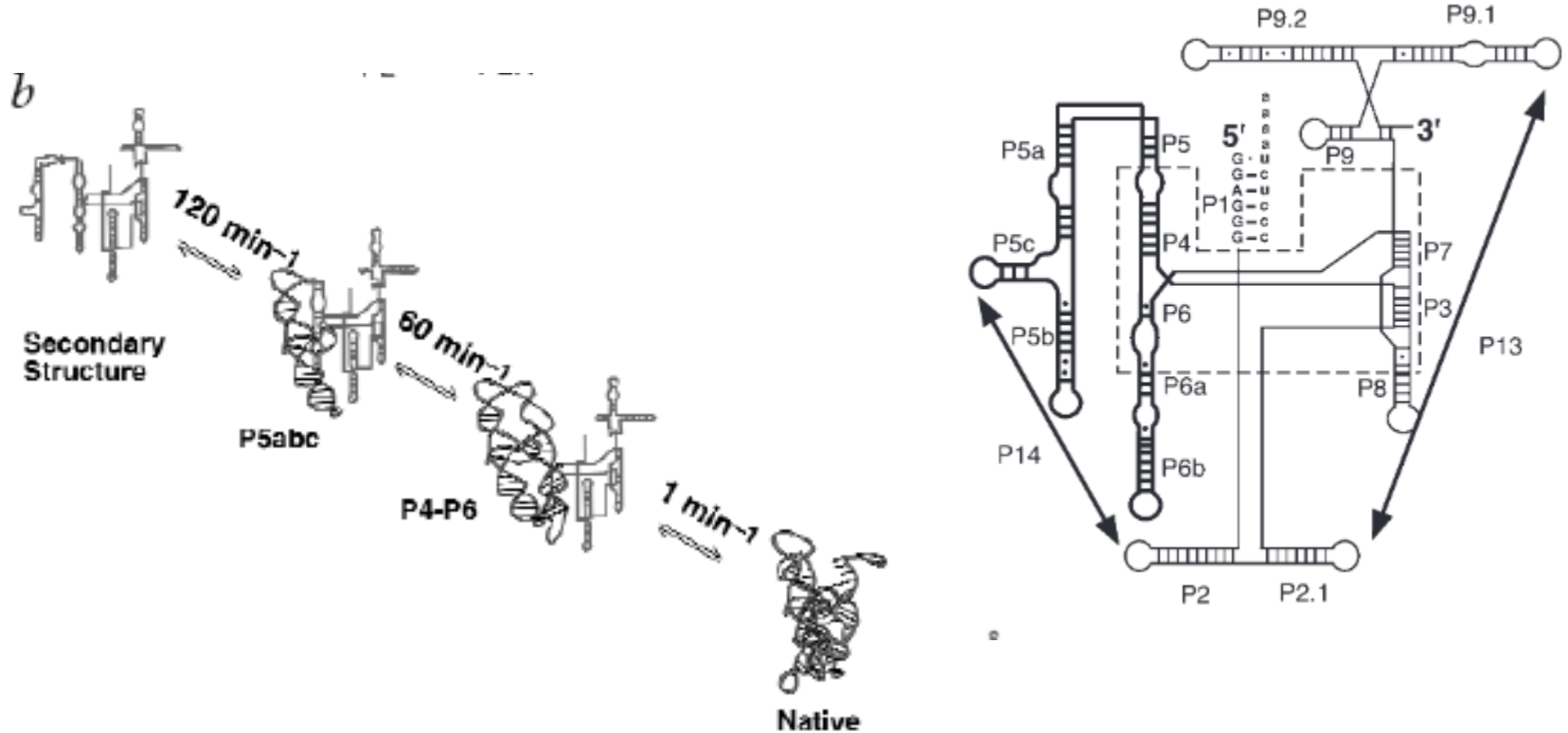
Plot $\ln(I(q))$ vs q^2 , and find the slope and intercept. The intercept $I(0)$ is related to the molecular weight

$$MW = \frac{I(0)}{I(0)_{\text{standard}}} \cdot \frac{MaC_{\text{standard}}}{MaC} \cdot MW_{\text{standard}}$$

And the slope is proportional to R_g .



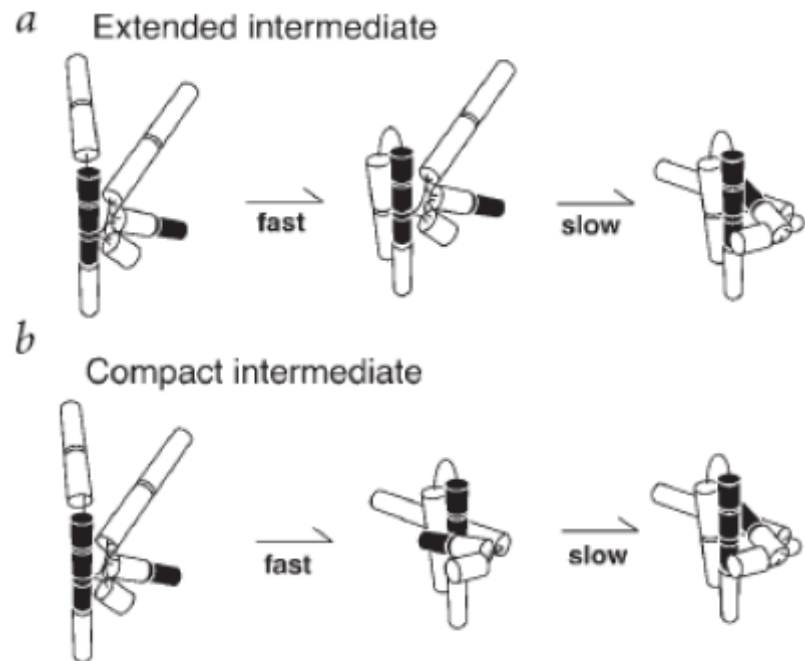
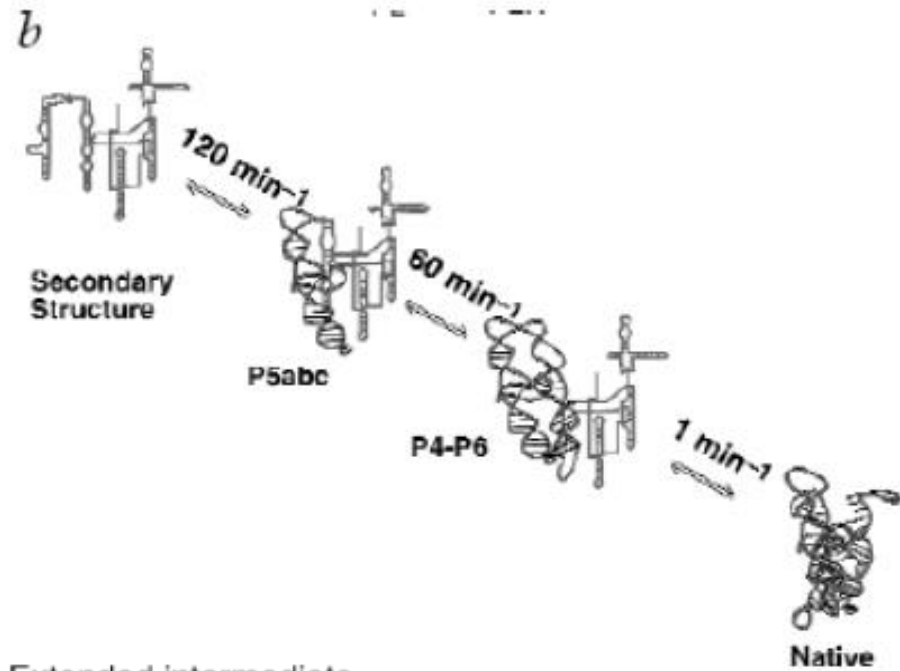
Russell et al. ((2000) Nat Str Biol 7:367-370) characterized Tetrahymena Group I folding using SAXS.



They found that when the intron folds in the presence of Mg^{2+} , it is compact. Moreover, the compaction happened fast, but the native structure wasn't completely formed until later. This led them to **propose “electrostatic collapse”** for the RNA.

Russell et al., reasoned that if the slow folding steps involved a search of conformational space by those parts of the RNA that had to associate via tertiary interactions, then by using SAXS to measure the radius of gyration as a function of folding, there should be a significantly smaller structure formed after those folding events were complete.

In contrast, if electrostatic collapse happened early, then the radius of gyration should be constant during subsequent rearrangements.



The experiment:

With NO Mg^{2+} , the RNA should be extended.

Result: $R_g = 74 \text{ \AA}^*$

Add 15 mM Mg^{2+} , incubate 30 min, 50° C , (conditions for optimal ribozyme activity), and remeasure.

Result: $R_g = 47 \text{ \AA}^*$ and its shape was more globular.

*using Guinier approximation
 $I(q) \approx I(0)\exp(-q^2R_g^2/3)$.
 Plot $\ln(I(q))$ vs q^2 to get slope.

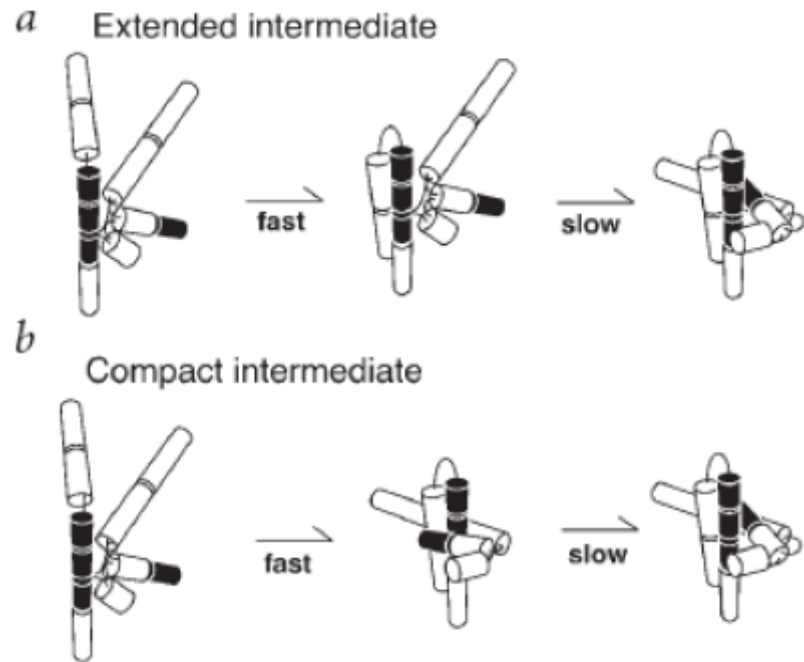
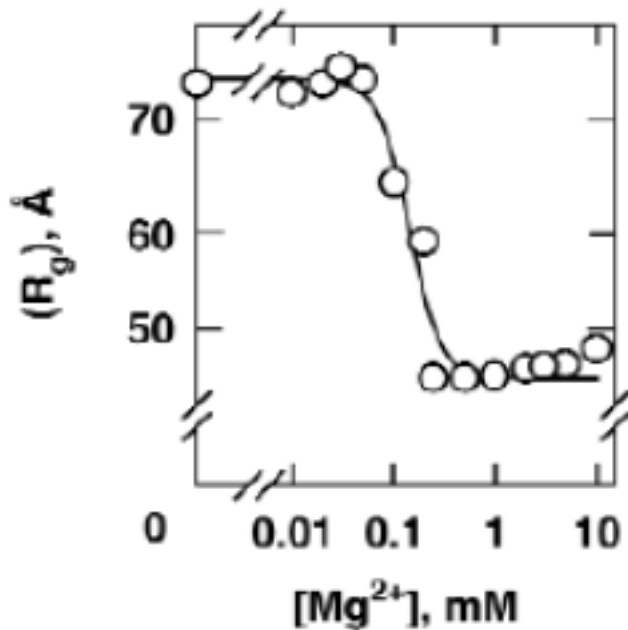


Table 1 SAXS characterization of ribozyme folding

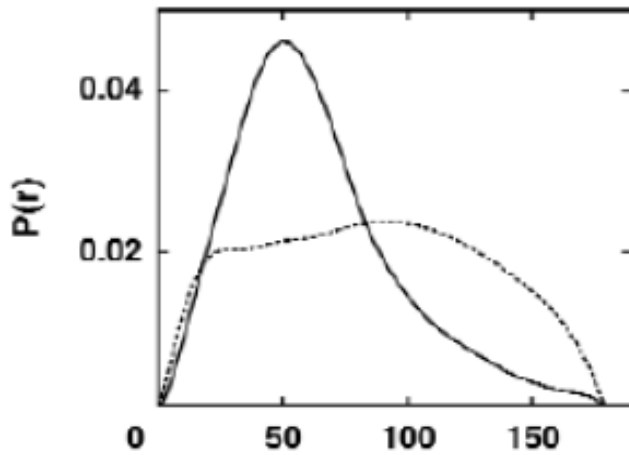
| Conditions ¹ | Ribozyme form ² | Radius of gyration (R_g ; \AA) ³ |
|-------------------------|----------------------------|--|
| No Mg^{2+} | Unfolded | 74 ± 4 |
| 15 mM Mg^{2+} | Native | 47.5 ± 0.4 |
| 50 mM Mg^{2+} | Native | 48.2 ± 0.3 |
| 20 mM Ca^{2+} | Native | 52 ± 2 |
| 100 mM Na^+ | Unfolded | 68 ± 5 |
| 1 M Na^+ | Unfolded | 68 ± 5 |



Plot the calculated R_g as a function of added Mg^{2+} from steady-state SAXS measurements.

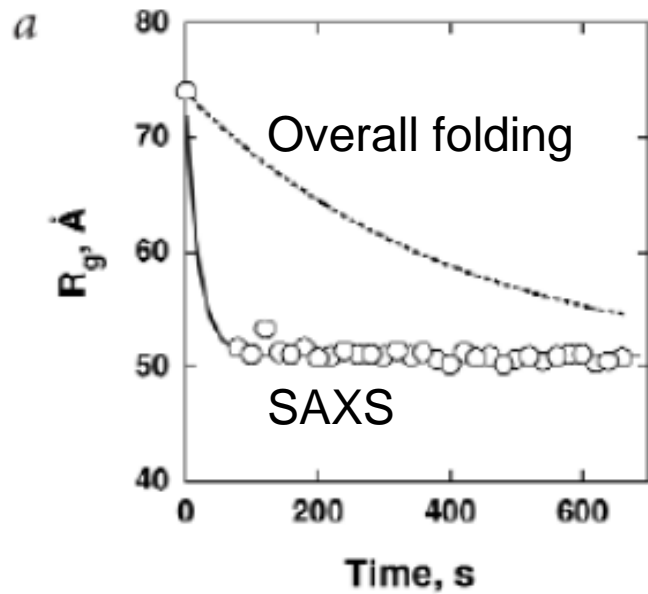
The transition is sharp and cooperative.

BUT: Look at the SAXS profile to estimate the multiplicity of states.



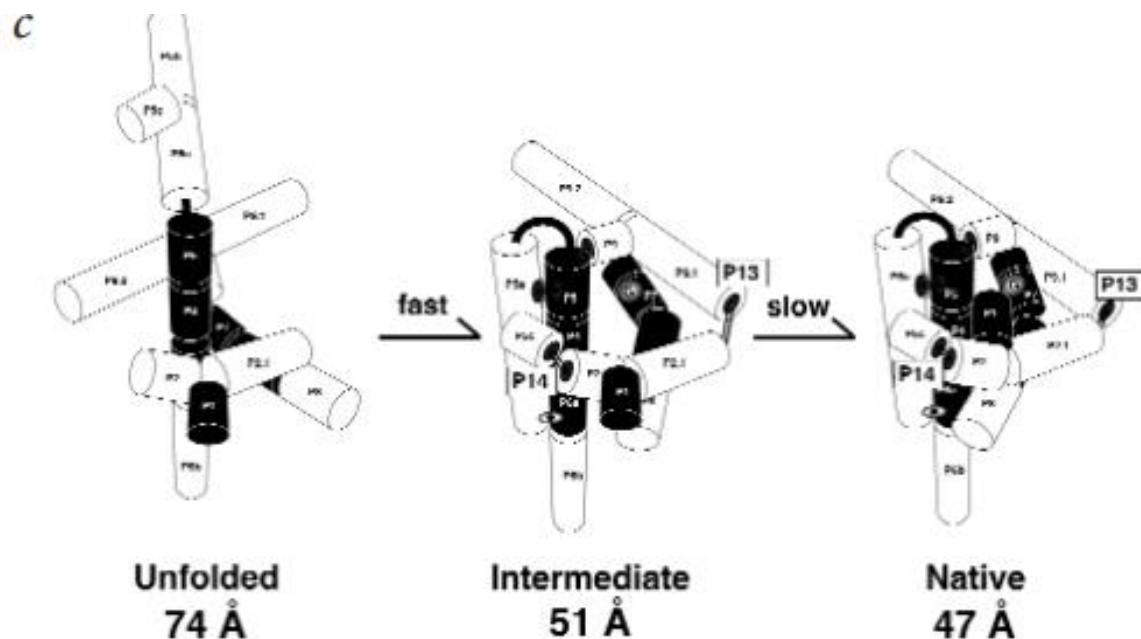
$P(r)$ is the distance distribution function.no Mg^{2+} , — 15 mM Mg^{2+} . The peak is indicative of a globular shape.

What is the timecourse of the folding?



Repeat the experiment, but now start data collection asap after the addition of Mg^{2+} to 15 mM. The result was that the rapid compaction occurred within the 1 min deadtime of the experiment.

They state that 95% of the enzyme is misfolded under these conditions. If the temperature were raised, it would rapidly convert into the active form, but under these conditions, the misfolded state is stable for hours.



They hypothesize that rearrangements occur within the core itself, and not the arms of the structure.

How do these misfoldings occur?

General principles:

RNA folding often requires Mg^{2+} ions.

RNA folding is hierarchical.

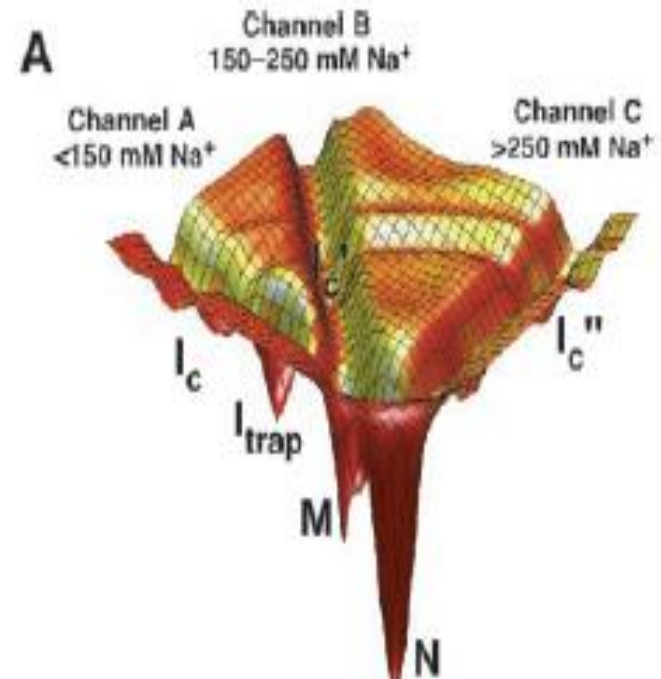
RNA molecules can misfold.

Concept: An RNA **folding funnel**.

M is Misfolded, and N is correct Native fold.

Intermediates are also shown.

Paths depend on ions, temperature,
mutations, starting structures.



How do you think an RNA folds during transcription?

Proteins also have folding funnels – how might they differ from the RNA funnels?