Time resolved small angle x-ray scattering studies of macromolecular dynamics

Lois Pollack

Applied and Engineering Physics

Cornell University

Plus many others (to be acknowledged at the end of the talk)

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Motivation for this work

- Identify problems in molecular biology that could benefit from the application of new technology

  (e.g. time scales too short to be easily accessible, signals too weak, background too high, sample consumption is prohibitive)

- Carry out experiments to gain new insight into mechanisms

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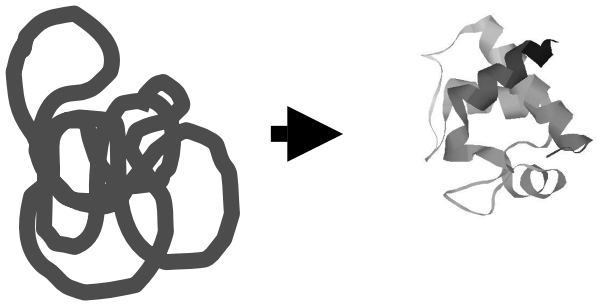
The ‘folding’ problem

- Relevant to proteins, RNA, DNA:
- How do you build a three-dimensional, biologically active structure from a one-dimensional polymer?



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RNA folding

- Much insight into protein function has come from understanding the interactions involved in folding

- The discovery of functional RNAs underscores the need to elucidate its folding mechanisms

- Recent, general reviews of RNA folding

P.B. Moore in ‘The RNA World’ (1999)

T.R. Sosnick & T. Pan, Curr Op Str Bio. (2003)

D. Thirumalai et al., Ann. Rev. Phys. Chem. (2001)

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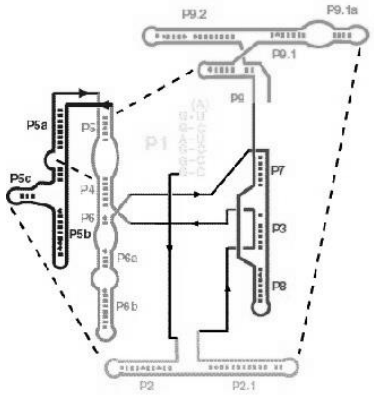
RNA is a biopolymer

Three levels of structure:

- primary (1 dimensional) –A–C–C–G–U–A–G–C–U–G–
- secondary (base pairing)



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- Tertiary structure (3 dimensional): the addition of small positively charged ions triggers tertiary structure formation.

- RNA folding=tertiary structure formation

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Prior work on the Tetrahymena ribozyme

Many groups have studied folding of this ribozyme using a variety of different techniques.

recent review: Treiber & Williamson, Curr. Op. Struct Biol. **11** (2001).

First specific/local contacts form in about 1 second (Sclavi et al, Science **279** 1998)—is this the first step in folding?

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To carry out the experiment

- Trigger and monitor folding rapidly (have to add Mg ions to unfolded RNA)

- Acquire good quality SAXS profiles at short times

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Our approach….

micro-fabricated flow cell:

- Fast mixing by diffusion
- Easy to calibrate
- Low sample consumption

Knight JB, Vishwanath A, Brody JP, Austin RH:
Phys Rev Lett 1998, 80: 3863–3866.

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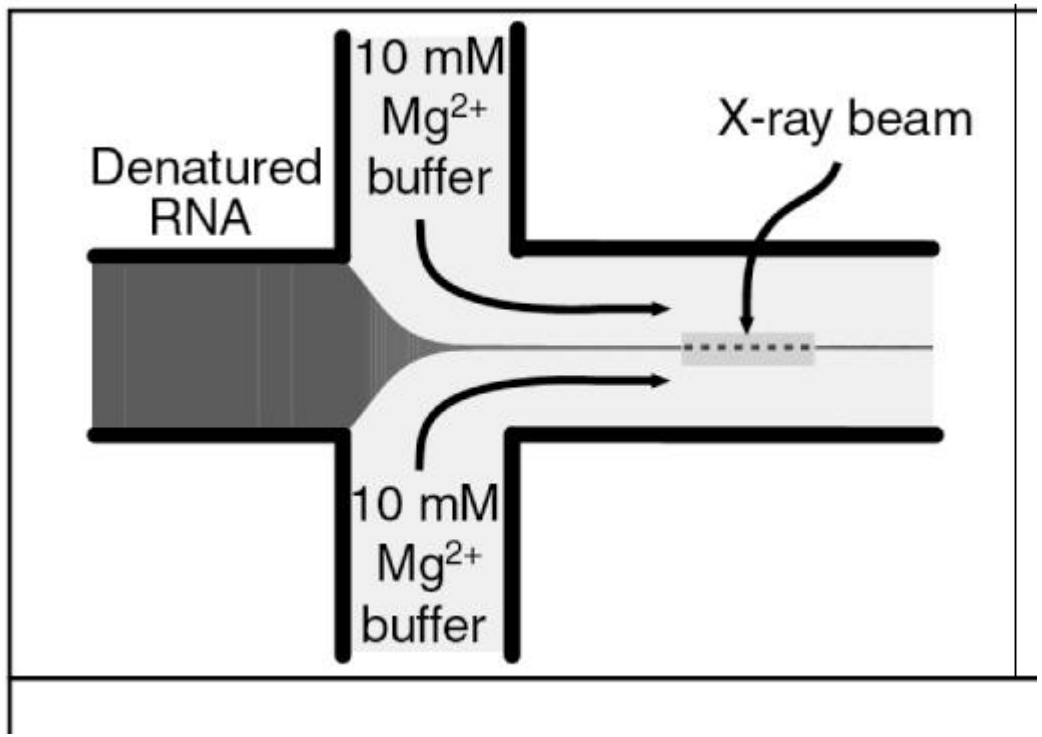
The folding experiment:



RNA: 2 mg/ml

X-rays: 10^{10} photons/sec, beam 10μ x 40 μ, 0.5 ms/point

Small angle x-ray scattering

- At the smallest scattering angles we get information about the largest spatial dimension in the problem (radius of gyration)

- at larger scattering angles we get information about smaller length scales, can be related to degree of compaction of the molecule (Kratky plots)

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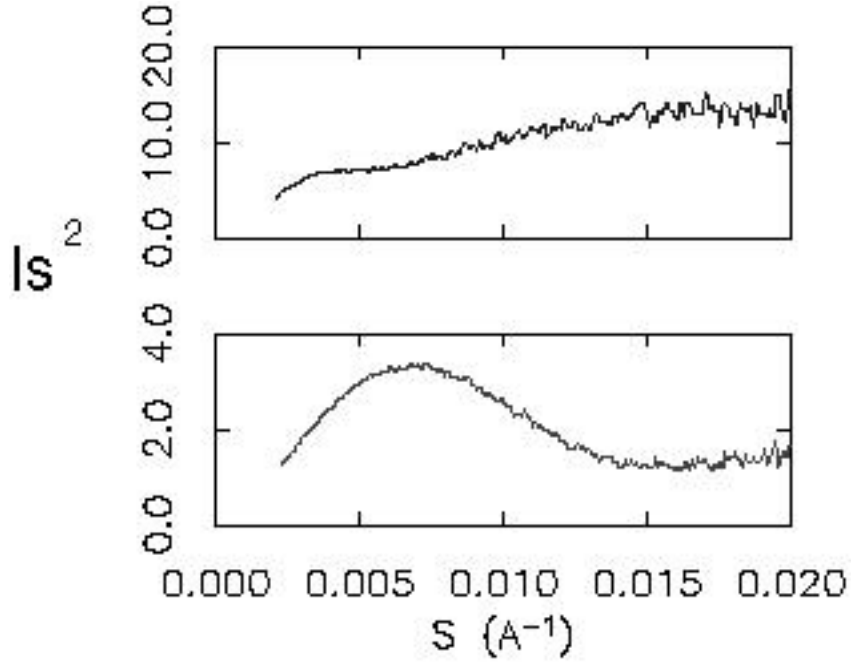
X-ray scattering profiles of unfolded (top) and folded (bottom) RNA:



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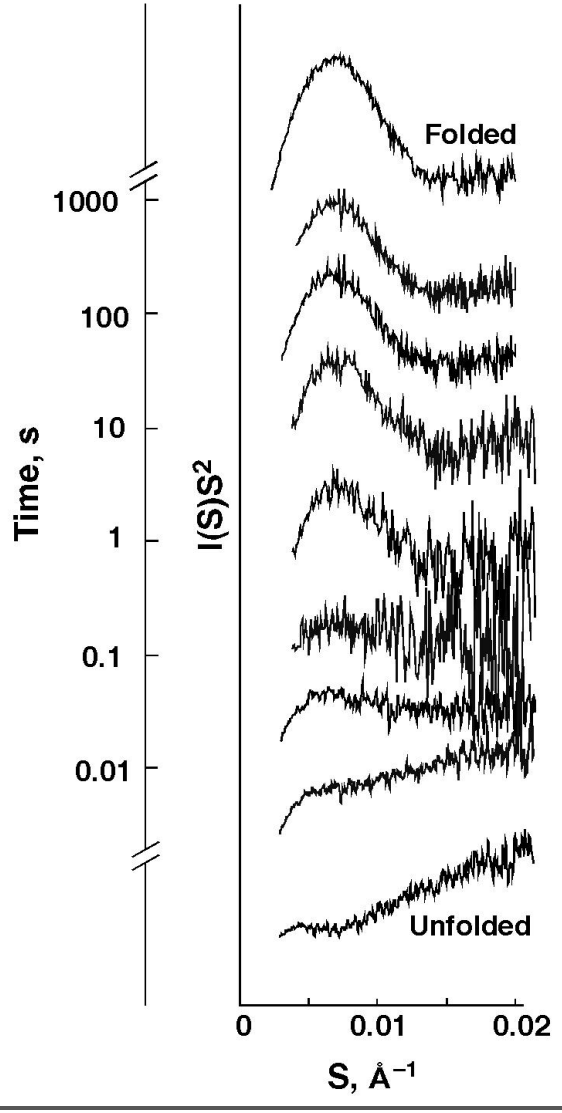
RNA folding observed by SAXS



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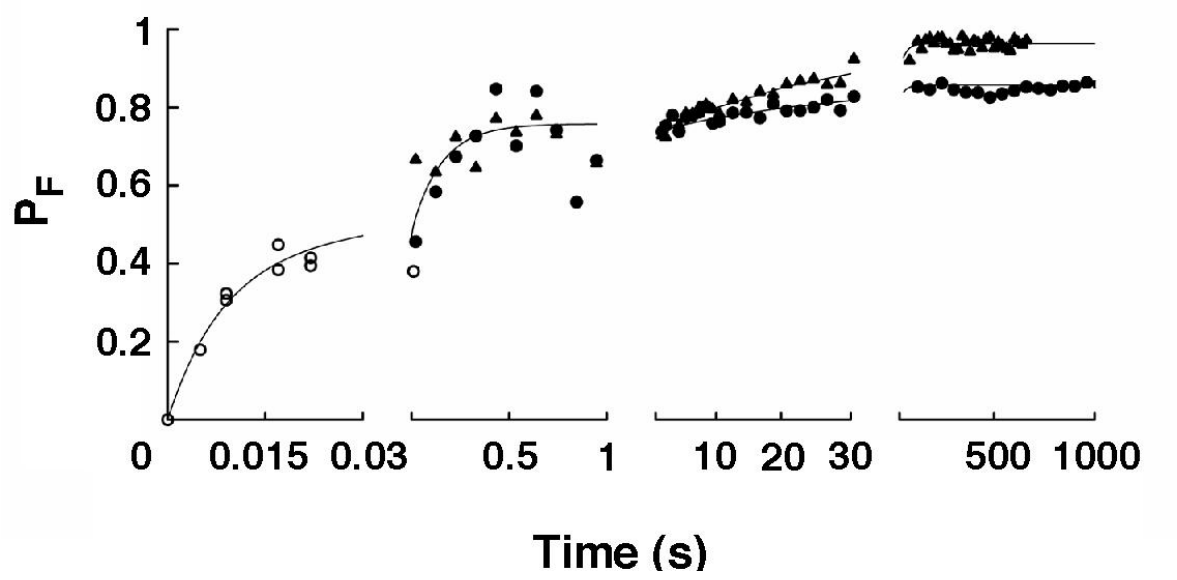
From Russell et al., PNAS **99** (2002)

Data interpretation through SVD



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From Russell et al., PNAS **99** (2002)

SVD revealed TWO fast phases

First phase has time constant of 7 +/- 1 ms

Second phase has time constant of 140 +/- 30 ms

Both phases are faster than required for stable tertiary structure formation.

What happens during each phase?

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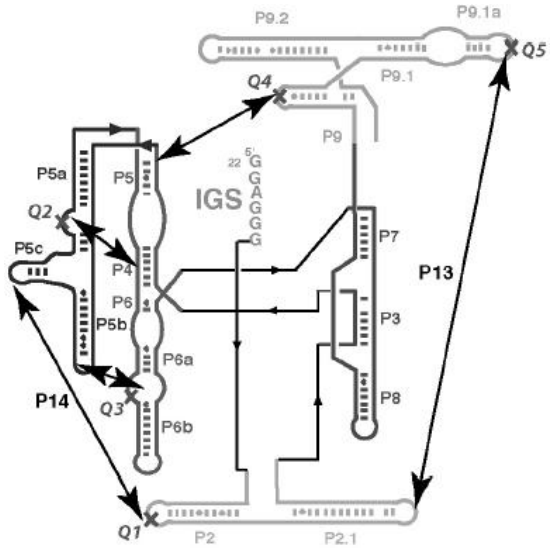
More recently: experiments on ‘mutants’: remove tertiary contacts



first phase present, second phase absent

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Interpretation

Fastest phase: electrostatic relaxation

Second phase: flickering tertiary contacts

Das et al., JMB (2003)

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Focus on physics of rapid electrostatic relaxation

- Is this a general observation for all RNA?
- Compare with proteins
- Compare with DNA
- Fundamental physics of electrostatic relaxation?

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My collaborators:
At Cornell
Pollack group (AEP):
L. Kwok, K. Andresen, J. Lamb, H.Y. Park (T. Mills, G. Maskel
H. Smith)
Sol Gruner's group (Physics):
M. Tate, G. Toombes
CNF staff

At APS:
8-ID-I
S. Mochrie, L. Lurio, S. Narayanan, A. Sandy

At Stanford:
Dan Herschlag's group (Biochem): R. Russell, R. Das
Seb Doniach's group (Phys/Appl. Phys): I. Millett, & at 12-ID:
J. Jacob, S. Siefert, P. Thiyagarajan

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