

# **SAXS and collapse in protein folding**

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**with Jaby Jacob, Bryan Krantz, & Shane Dothager**

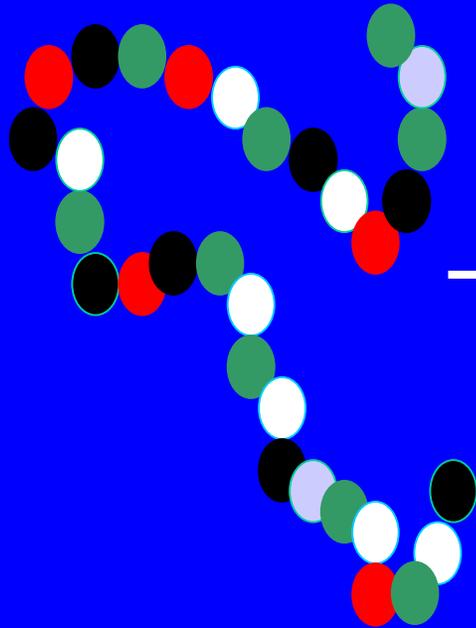
**And P. Thiyagarajan (ANL)**

**NIH and Packard Foundation for their generous support.**

# Protein folding

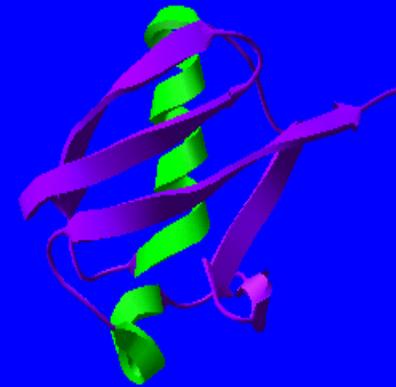
1D  
Sequence

Unique 3D  
Structure



local structure

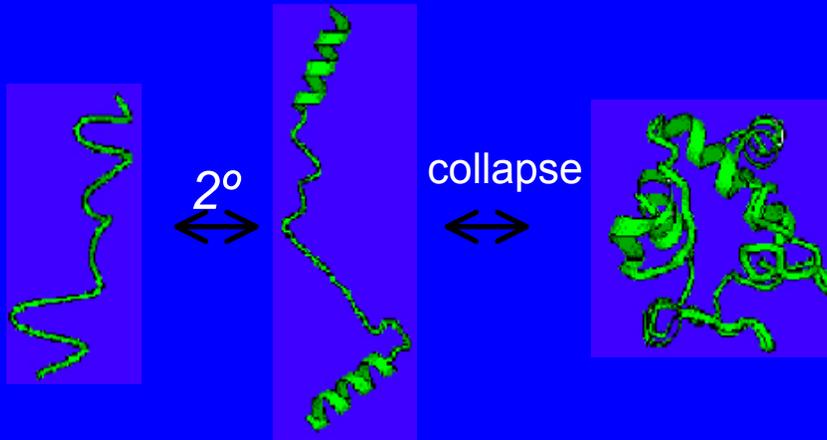
long-range structure



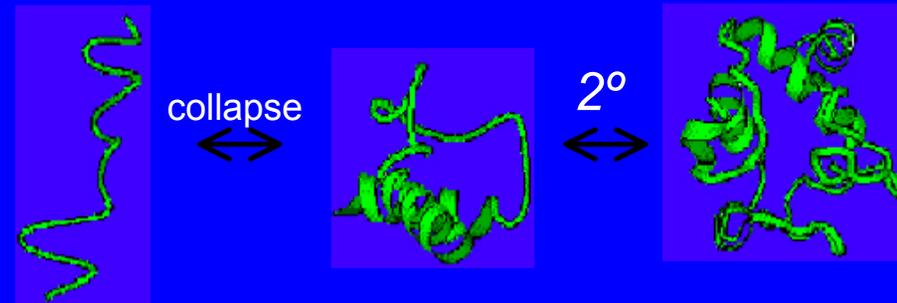
Vast conformational search over in milliseconds!

# How to reduce the Conformational Search?

Solve problem locally  
(e.g. Diffusion-collision model)

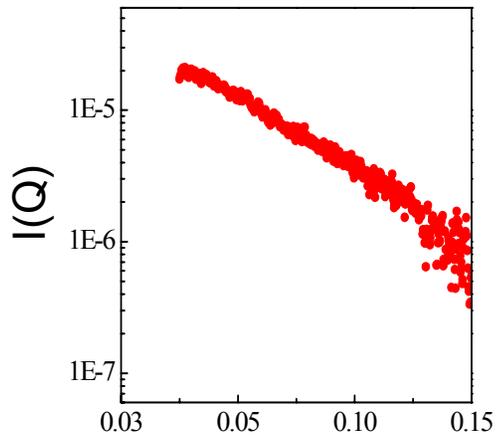
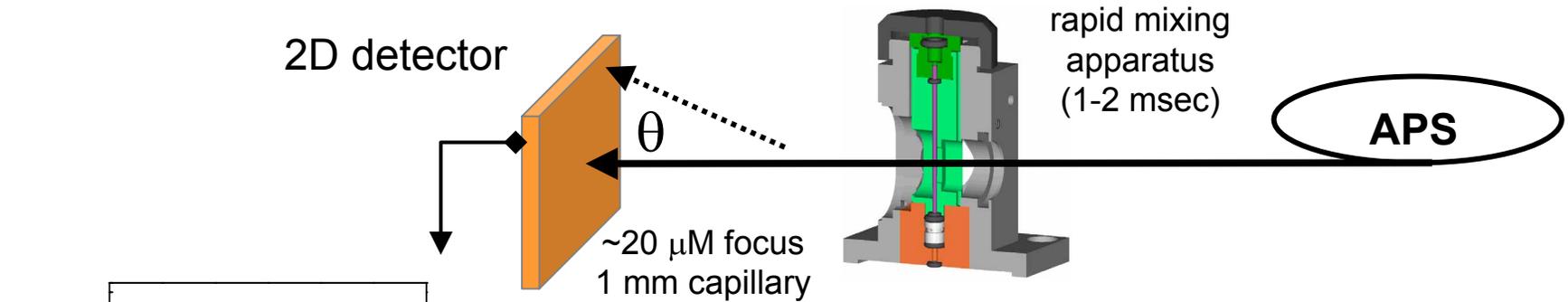


Early Collapse  
(e.g. most simulations)



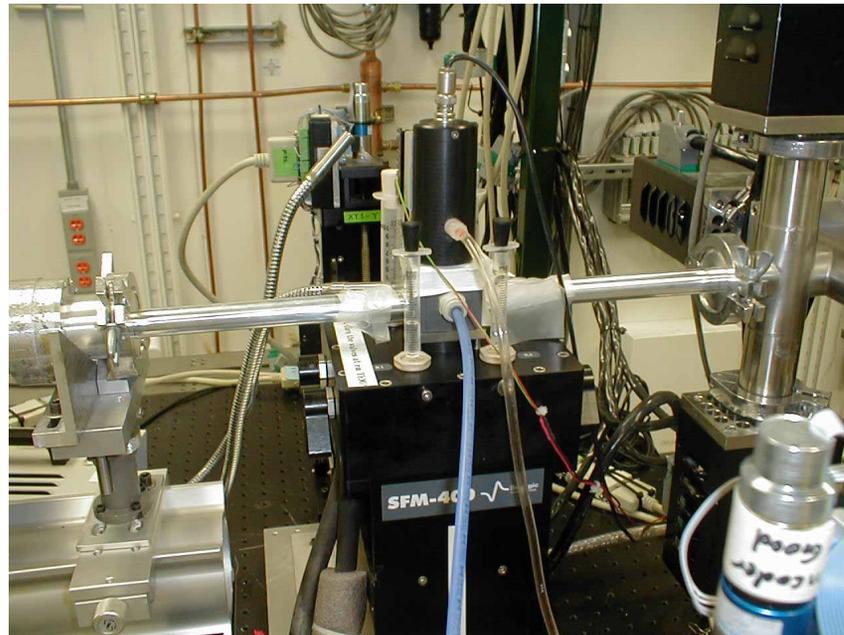
Knowledge of when collapse occurs delineates folding models.

# Measure dimensions at early folding stages using SAXS BioCat beam-line, Advanced Photon Source, Argonne National Lab

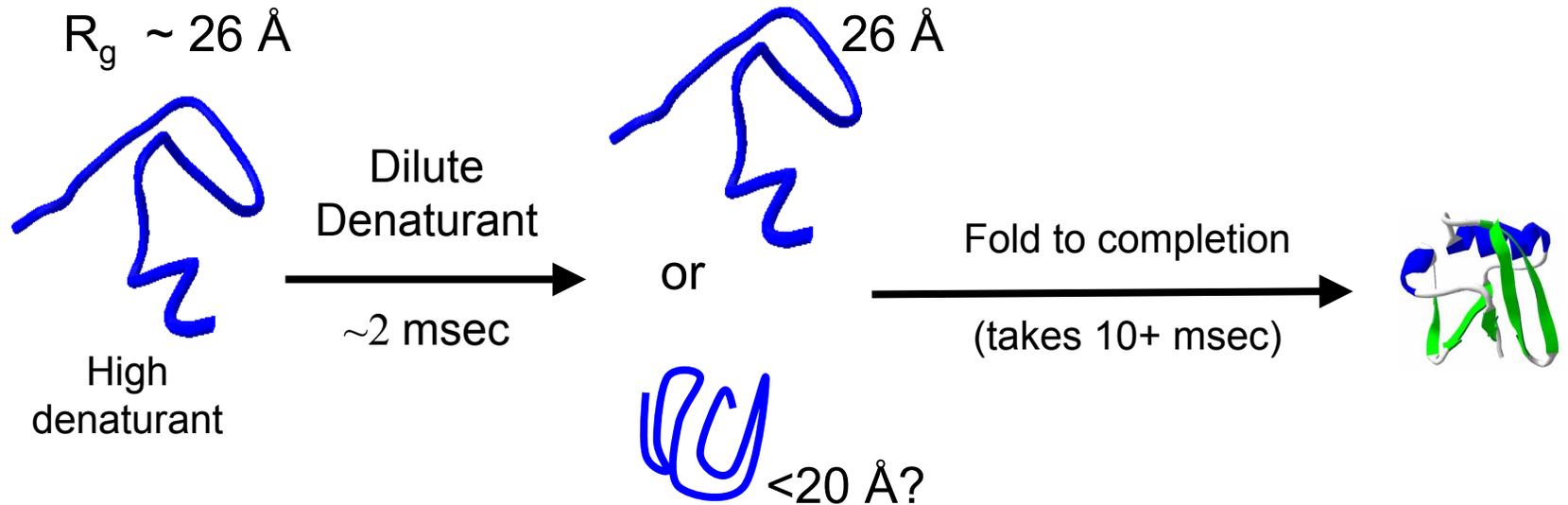


$$I(Q) = I_0 e^{-Q^2 R_g^2 / 3}$$

$$1/\text{width} \propto R_g$$



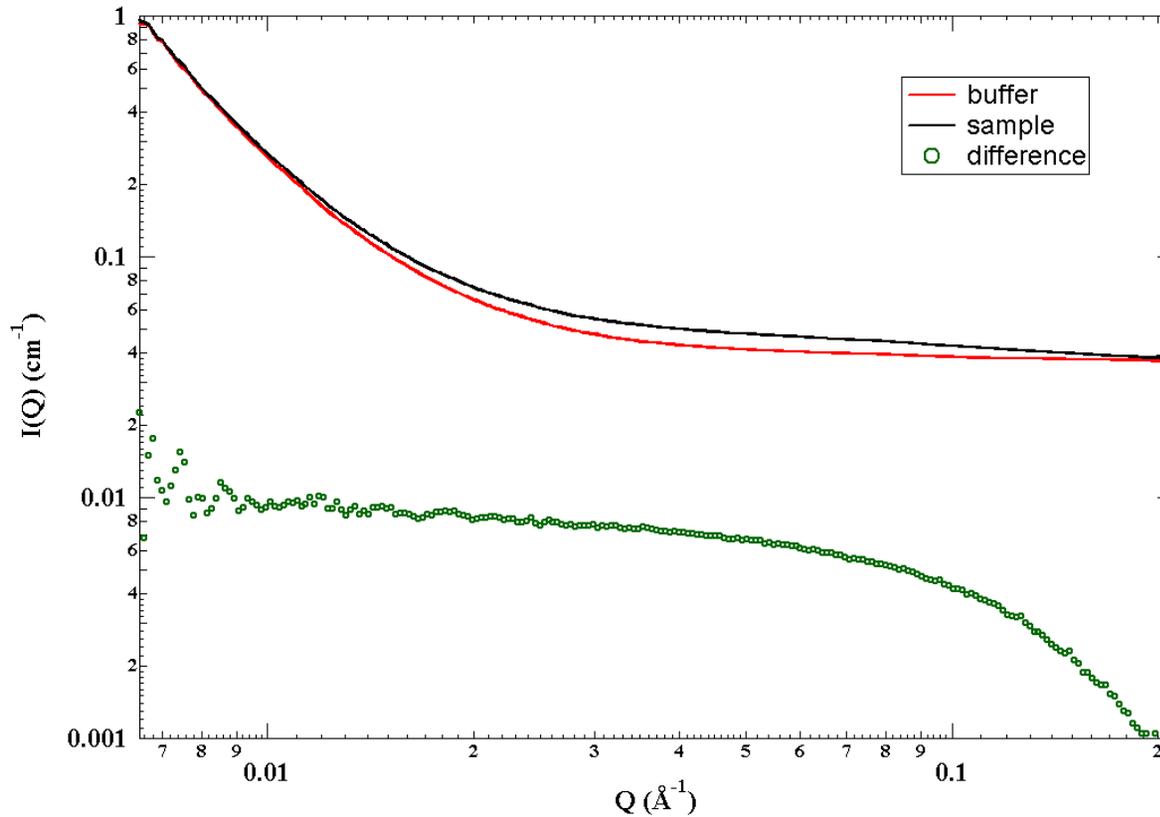
Does the polypeptide collapse upon denaturant dilution?



Experimental strategy:

Measure  $I(Q)$  for 0.4 s during continuous-flow period where protein has folded for  $\sim 2 \text{ msec}$ .

# Ubiquitin in 1.5 M guanidine hydrochloride



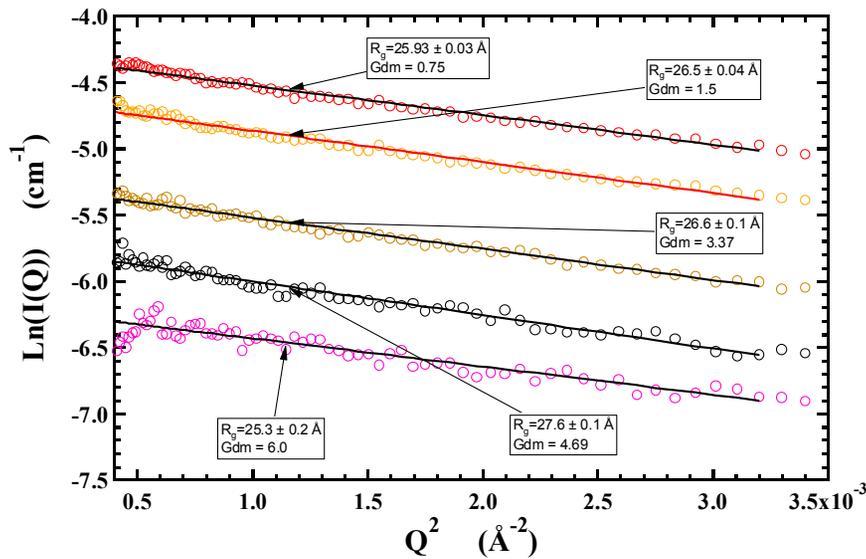
Sample concentration - 2 mg/ml

Exposure time - 360 msec

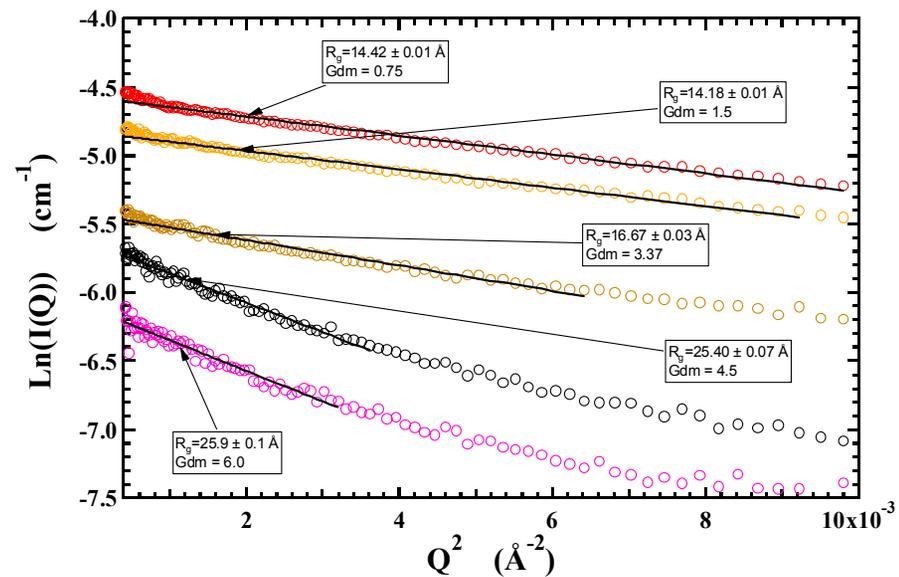
Number of exposures - 5 repeats for sample and buffer

# Guinier plots of *refolding* and *refolded* Ubiquitin

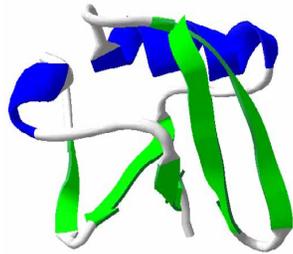
Right after  
denaturant dilution  
(~2 msec)



After folding has  
completed  
(10+ msec)

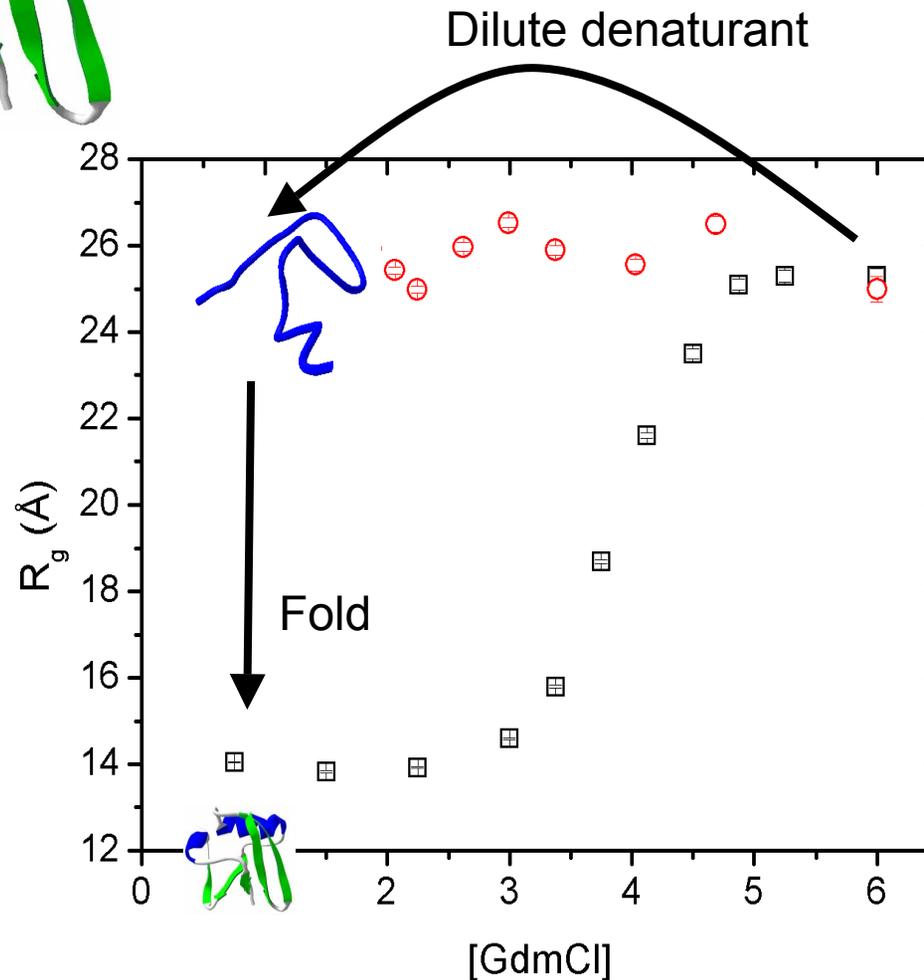


Ubiquitin:

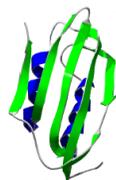


○ Size after denaturant dilution

□ Size after equilibration

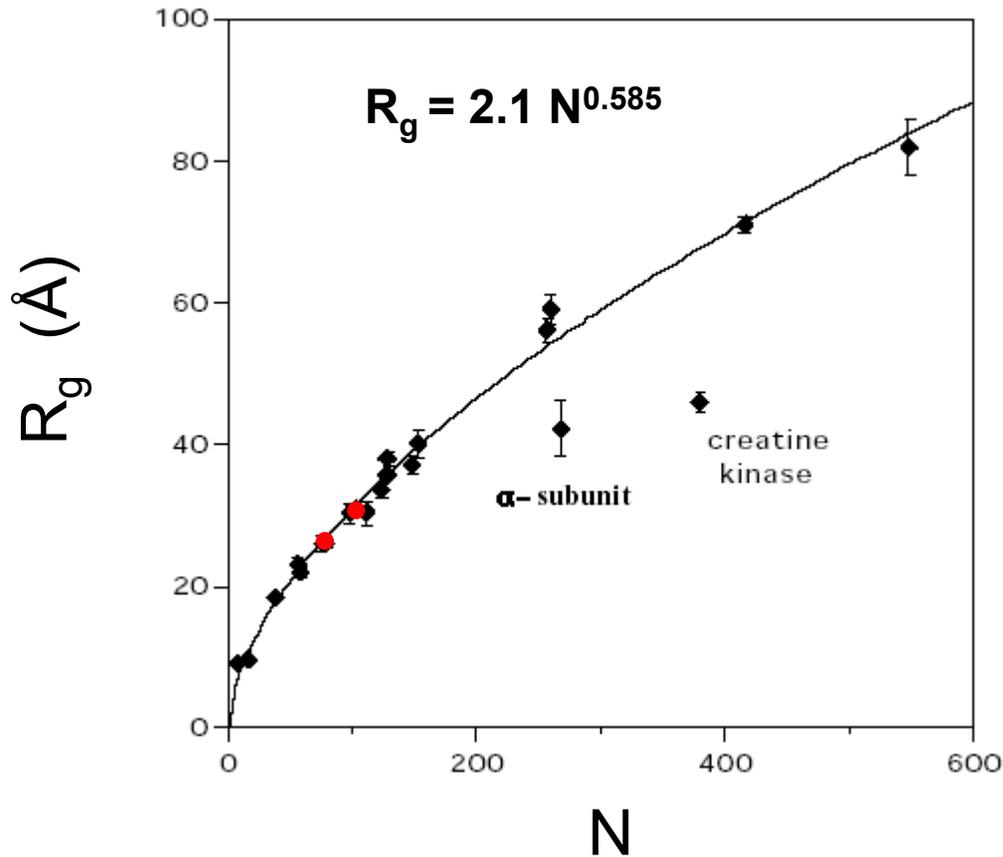


Jacob et al,  
JMB, 2004)



Ubiquitin (& ctAcp): Negligible collapse upon dilution of denaturant!

**Chemically denatured Proteins:  
 $R_g$  scales with length the same as a self-avoiding random walk**



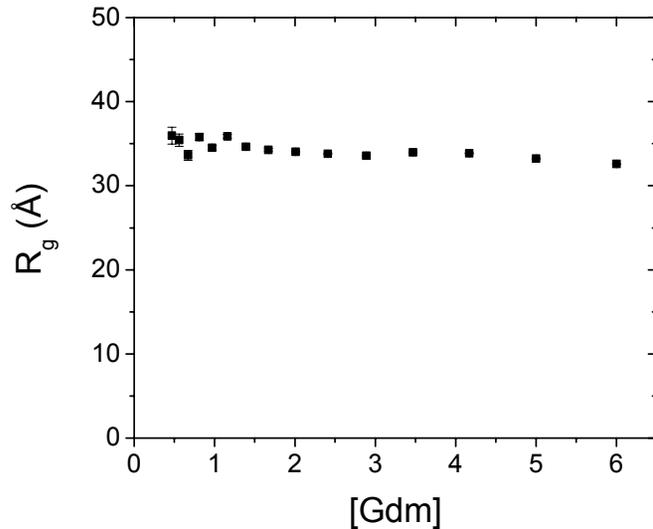
From K. Plaxco

Unfolded Ub & ctAcp fall on this line *even under aqueous conditions*  
(Ub: 26 versus 26.5 and ctAcp: 31 versus 30.7Å)

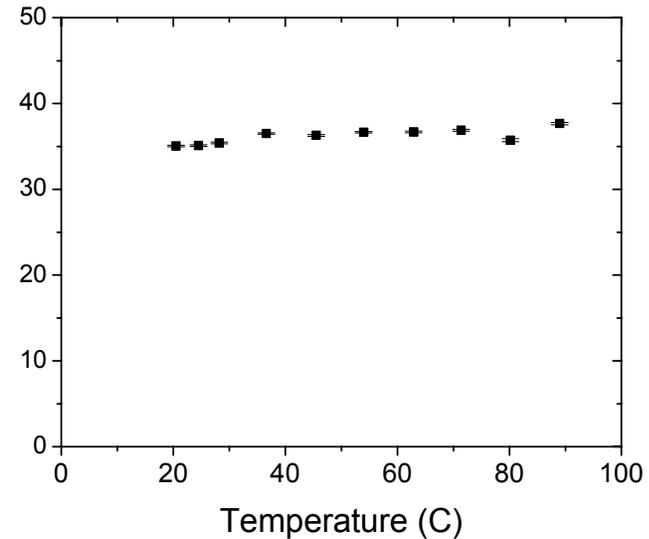
# Polypeptides can behave like random coils under aqueous conditions

No denaturant or Temperature dependence for  $R_g$

Equilibrium mode using non-folding polypeptide (RNase A with disulfide bonds broken)



Increased strength of protein-protein interactions at lower denaturant concentrations does not result in compaction (protein-water interactions are stronger).



Unfolded peptides are in the high temperature limit, where  $T\Delta S_{\text{config}} \gg \Delta H$

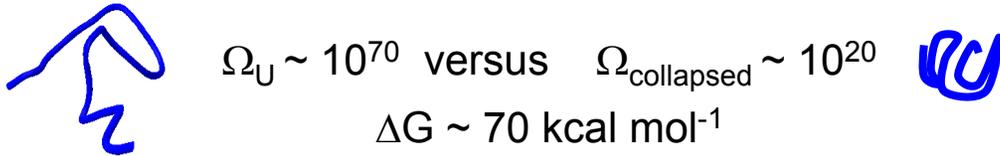
(shrinking or expanding reduces number of conformations, i.e. entropy)

# Why is water such a good solvent for polypeptides?

Hydrophobic interactions are strong  
(and will eventually drive folding to the native state for foldable sequences).

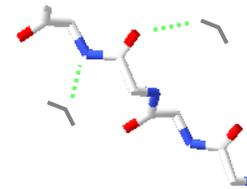
But...

1. Collapse is inhibited by the **loss of conformational entropy**:



2. **Removal of water from backbone** is very demanding – Must satisfy H-bonding requirements.

Each unsatisfied, buried amide and carbonyl costs several kcal mol<sup>-1</sup>



Net stability of a protein < 10 kcal mol<sup>-1</sup>

**Therefore, non-specific hydrophobic collapse is unfavorable**

## **Conclusions (for small proteins which fold cooperatively)**

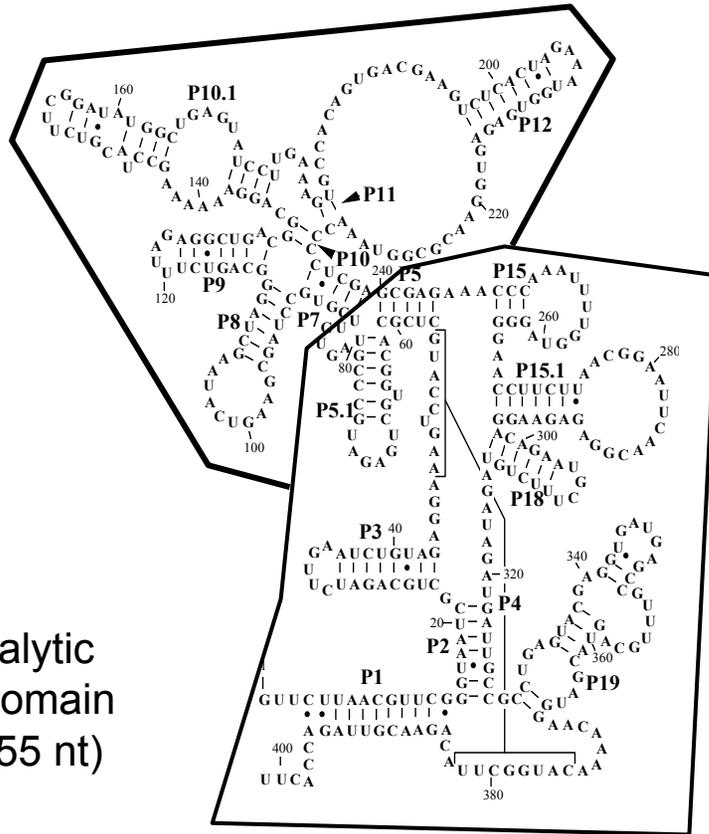
1. Early collapse is not an obligatory step in folding
2. Water is a good solvent for unfolded chains (prior to the major folding event).
3. Polypeptides can behave like random coils in water

**Exceptions occur for larger proteins, and those with disulfide bonds or prosthetic groups.**

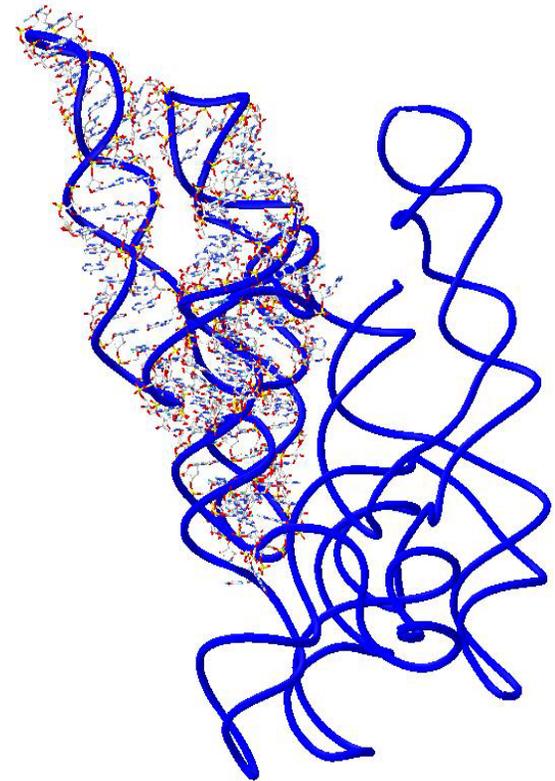
# New area: RNA folding

## Ribozyme P RNA

Specificity  
S-domain  
(~150 nt)



Catalytic  
C-domain  
(~255 nt)

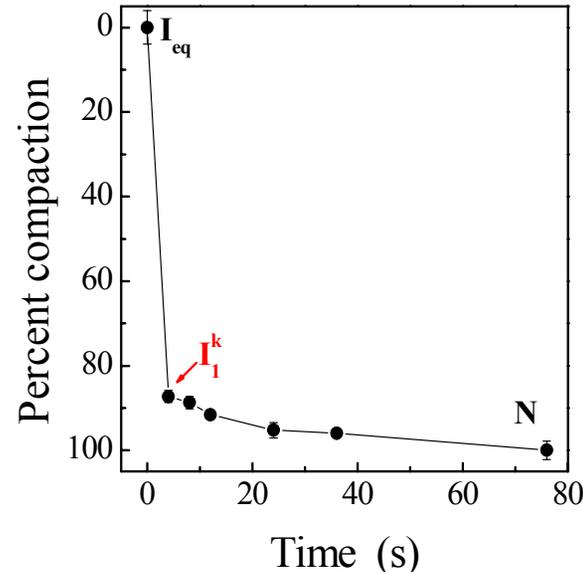
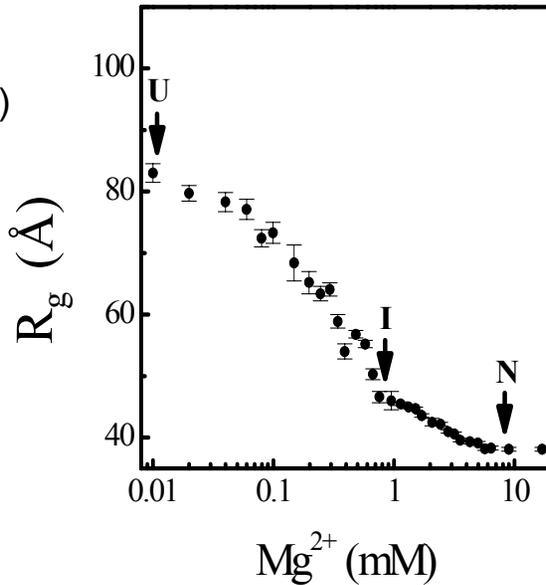


Model by Westhof & Co.  
JMB 1998

# RNA Folding Rules are different

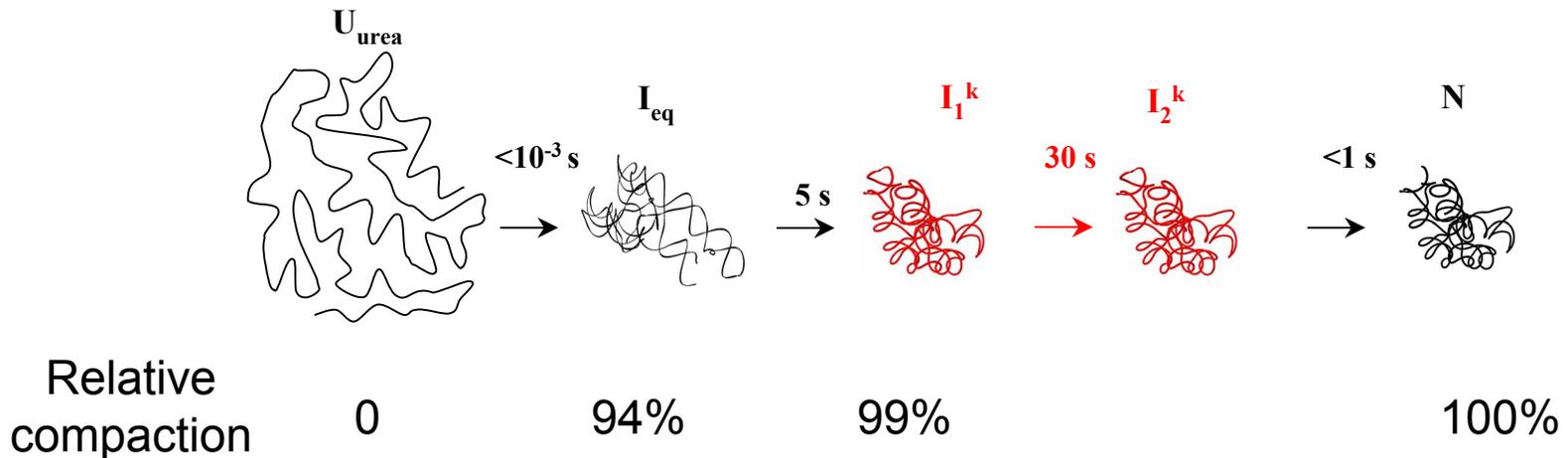
## Many highly collapsed intermediates

C-domain  
(255 nucleotide  
80kD, 0.3 mg/ml)



w/ Prof. Tao Pan  
Univ. of Chicago

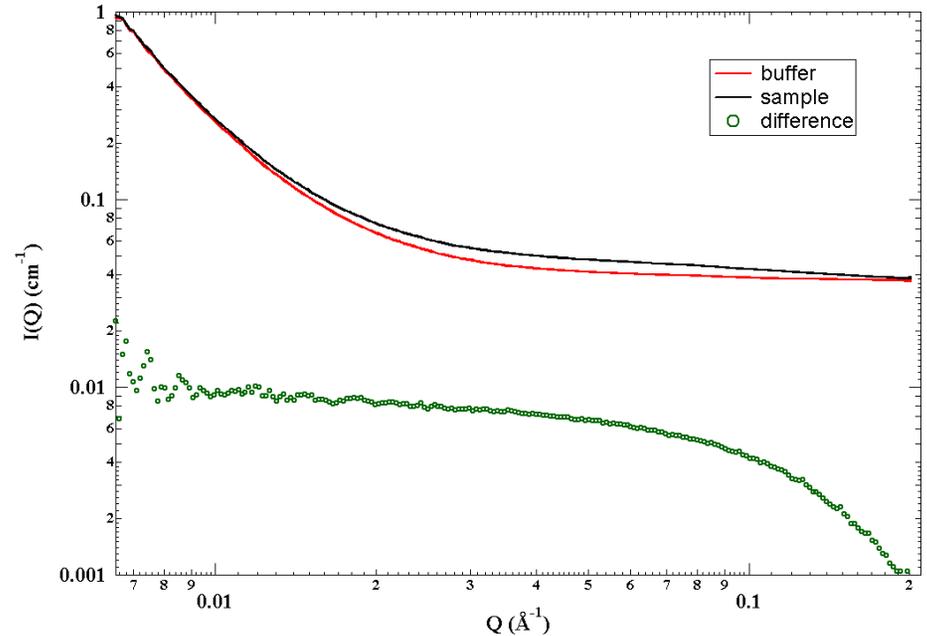
From Fang et al,  
*Biochemistry*, 2000  
*PNAS* 2002



## Technical Issues:

### 1. Lower backgrounds

- i) reliably access lower  $Q$
- ii) less sample
- iii) less potential aggreg'n
- iv) less sensitive to background subtraction



### 2. Detectors that can do time-resolved studies at high fluxes

Often CCD's take seconds to read-out.

Hence, no “on-the-fly” studies on the subsecond time-scale  
(current experiments were conducted in continuous-flow mode)

### 3. Reproducibility – invariably need to take multiple acquisitions:

Detector issues, beam movement?

(lower backgrounds would help)



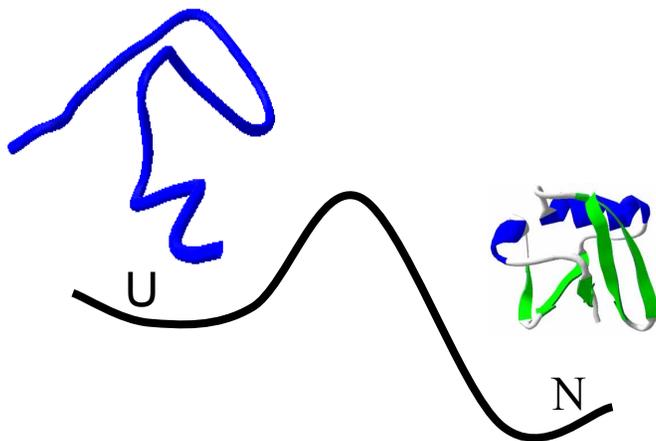
“Many small, monomeric proteins fold with simple **two-state kinetics** and show wide variation in folding rates, from **microseconds to seconds.**”

From S. Jackson *Folding & Design* 1998

*How do small single-domain proteins fold?*

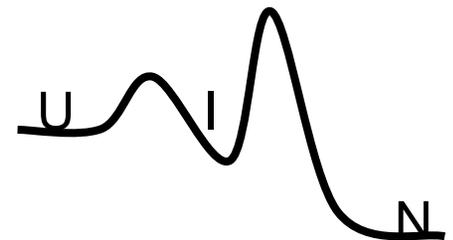
(see also Sosnick et al, *NSB* 1994, Sosnick et al, *Proteins* 1996, Krantz & Sosnick, *Biochemistry* 1999, Krantz et al. *JMB* 2002)

Two-state folding  $\equiv$  only the U and N states populate

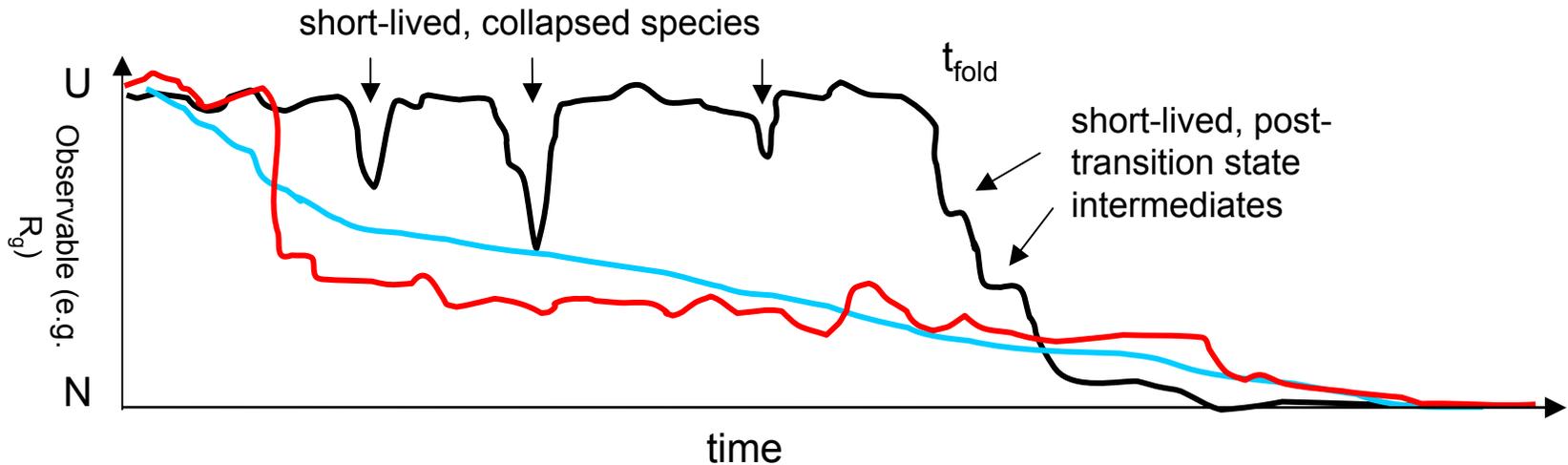


All the energy and surface burial occur in the sole barrier crossing.

Precludes formation of early collapsed intermediate.



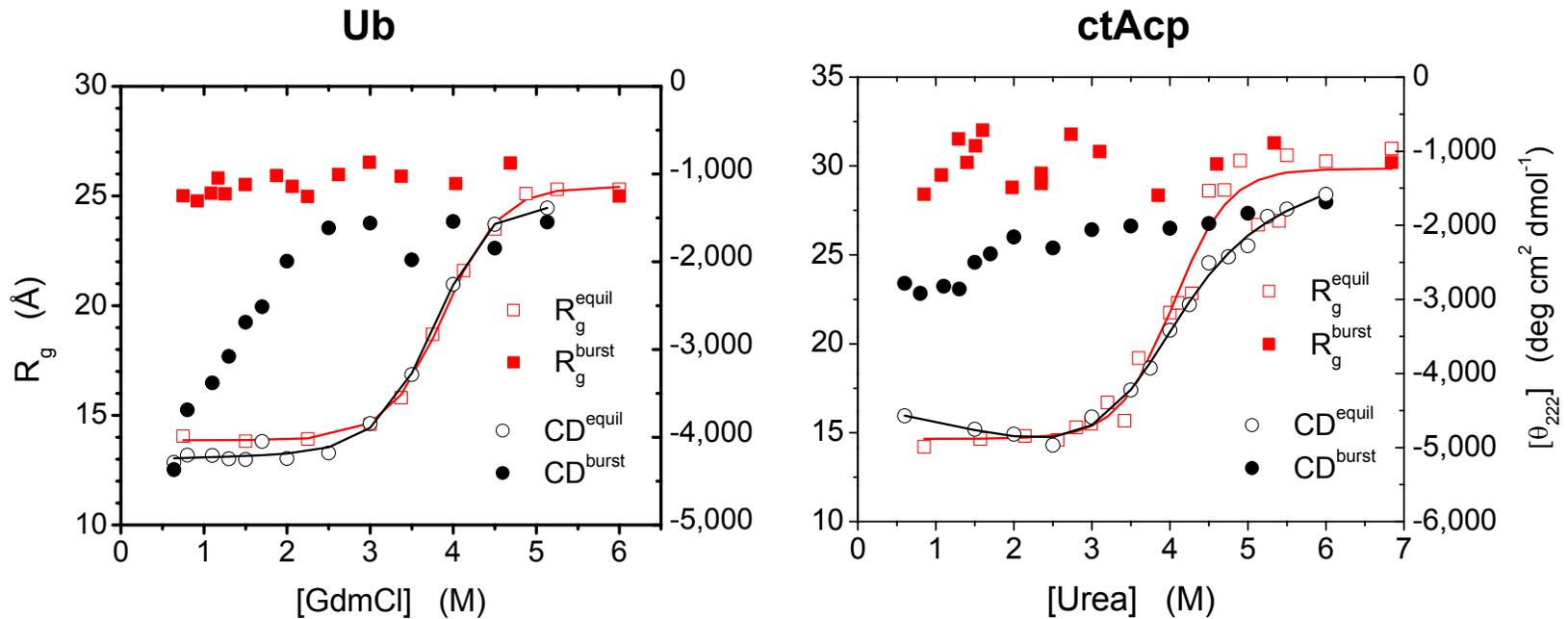
# Lack of an early collapse phase places constraints on computer simulations.



The black illustrative trace is consistent with ensemble behavior, as the observable probe has either the value of the unfolded state or native state for the majority of the trajectory.

The blue and red trajectories are inconsistent with ensemble two-state folding behavior and the present SAXS data, as collapsed intermediates populate.

## Burst $R_g$ and secondary structure formation (CD)

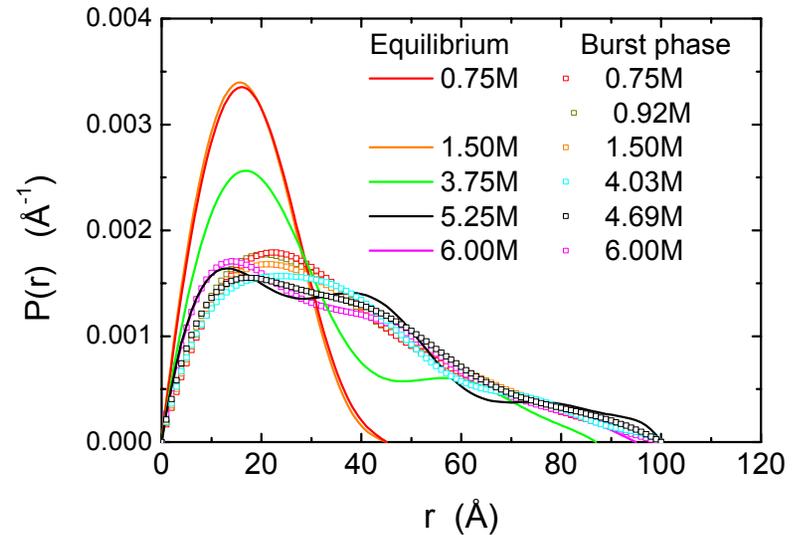
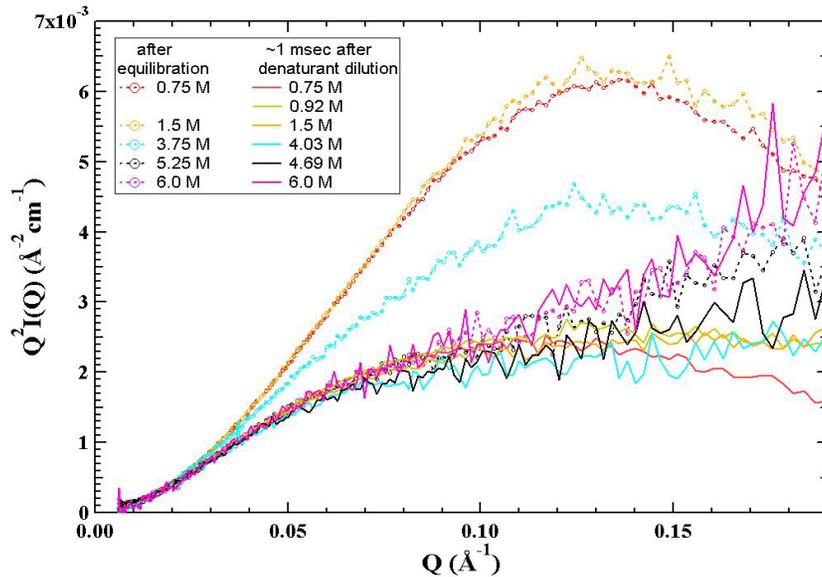


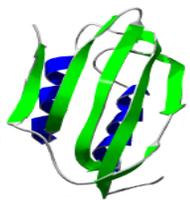
### Burst phase CD signals:

The removal of denaturant subtly alters the distribution of backbone dihedral  $\phi, \psi$  angles, most likely resulting in a shift from the polyproline II region to the helical region of the Ramachandran map.

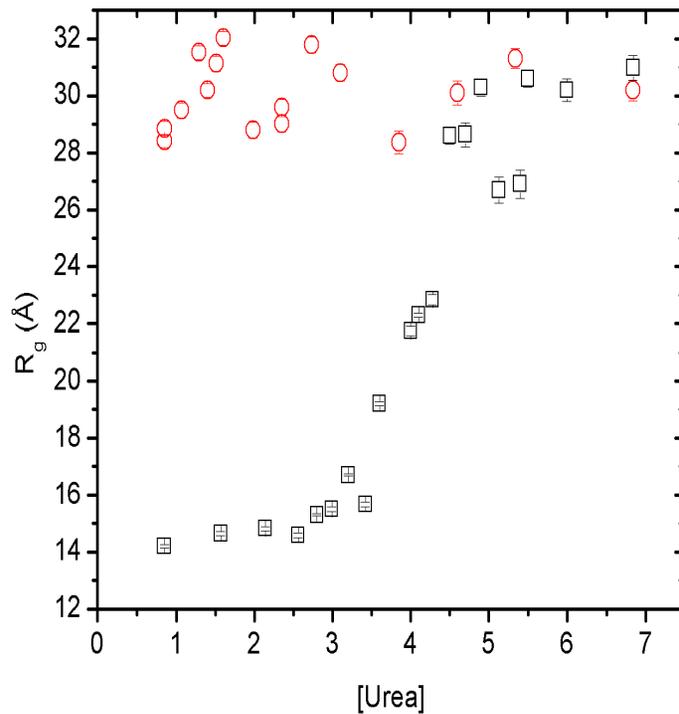
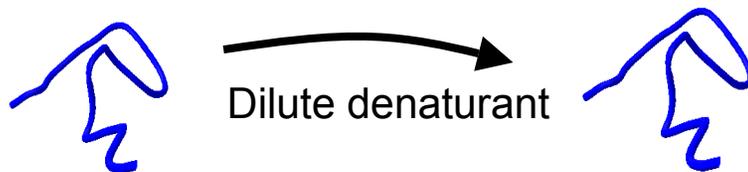
# Kratky and P(r) plots

Qualitatively demonstrates absence of any significant collapse from the random coil conformation in the “burst phase” upon dilution to low denaturant





Same result with CtACP:  
Negligible collapse upon dilution of denaturant



○ Size after denaturant dilution

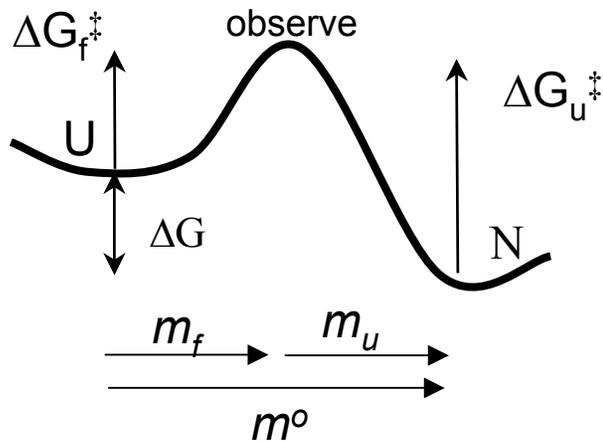
□ Size after equilibration

Folding times are msec to seconds – maybe we're not looking fast enough?

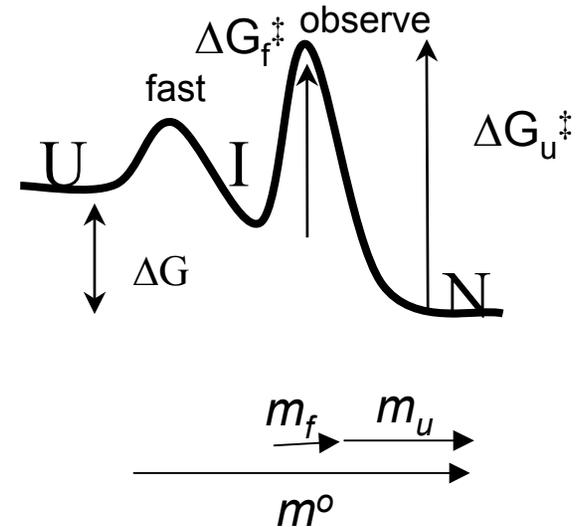
**No**

Energy conservation:  $\Delta G_{u^\ddagger} - \Delta G_{f^\ddagger} = \Delta G$

Surface burial:  $m^o = m_u + m_f$



Assume there is an early stable intermediate

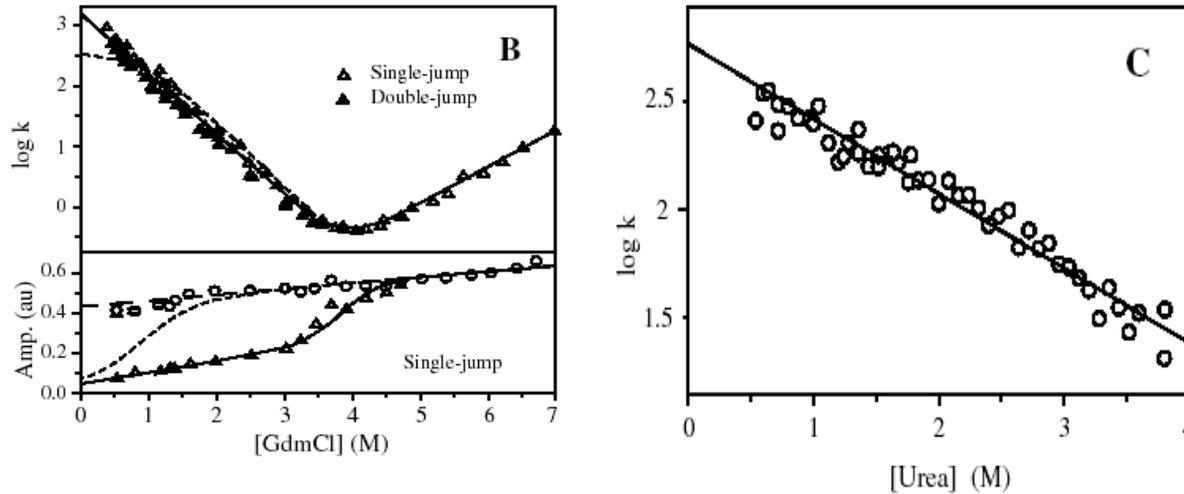
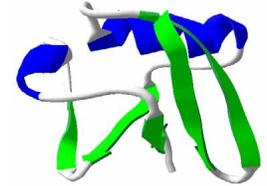


Underestimate Energy :  $\Delta G_{u^\ddagger} - \Delta G_{f^\ddagger} < \Delta G$

And surface burial:  $m^o > m_u + m_f$

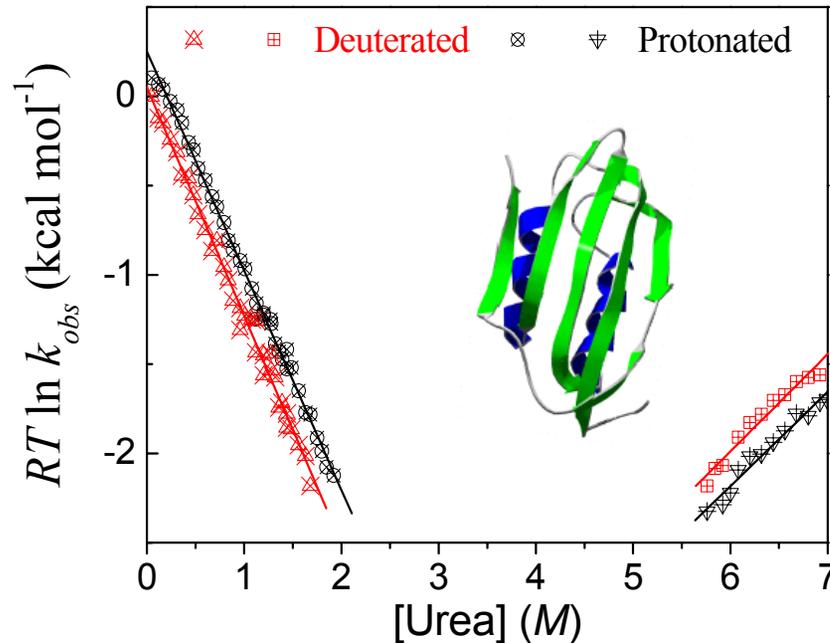
No missing energy, no missing surface burial -  
Therefore, the no early formation of a stable species which buries surface!

# Two-state Ubiquitin folding



- A) Chevron and amplitude data at 25 C, showing no rollover or missing amplitude.
- B) Folding rates down to low urea (0.4 M Na<sub>2</sub>SO<sub>4</sub>, pH 5.0, 10 C) show no rollover because the 1 msec dead time and other precautions avoided factoring in the slower phases. These and other results at a variety of conditions obey the 2-state chevron criteria.

# ctAcp satisfies the Chevron criteria for two-state folding.

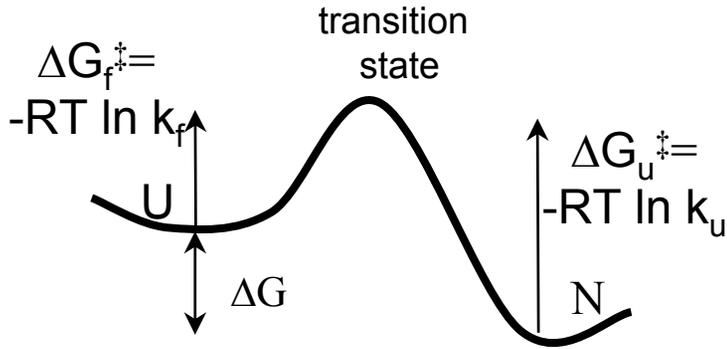


All the  $\Delta G$  and surface burial in the  $U \leftrightarrow N$  reaction are fully accounted for in the observed kinetic phase.

Inconsistent with the accumulation of an early intermediate.

**Time-resolved small-angle X-ray scattering studies are uniquely capable of measuring the collapse of the entire protein during the refolding process. We have measured the dimensions of two proteins within milliseconds of denaturant dilution using synchrotron-based, stopped-flow SAXS. Even upon a jump to strongly native conditions, neither ubiquitin nor common-type acylphosphatase contract prior to the major folding event. Thus, for these two denatured states, collapse is not energetically downhill processes even under aqueous, low-denaturant conditions. In addition, water appears to be as good a solvent as that with high concentrations of denaturant, when considering the over-all dimensions of the denatured state. Experimental considerations of conducting such experiments will be discussed.**

# Demonstrating 2-state behavior: Chevron Analysis

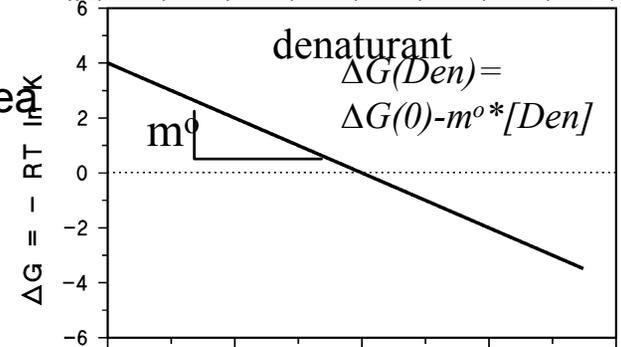
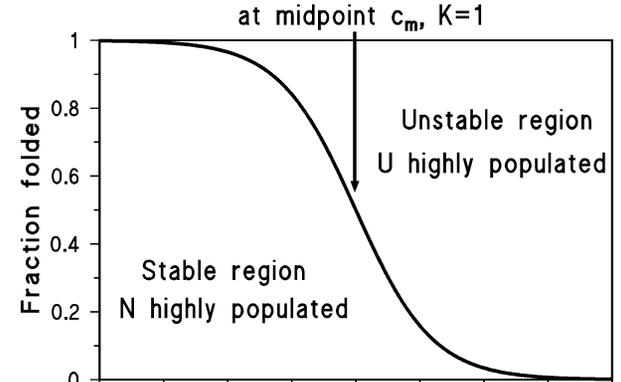


$$K = \frac{[U]}{[N]} = \frac{k_u}{k_f}$$



## Equilibrium

$m^\infty$  surface area exposed on unfolding



Agreement between equil. and kinetics implies 2-state model is adequate:

## Kinetics

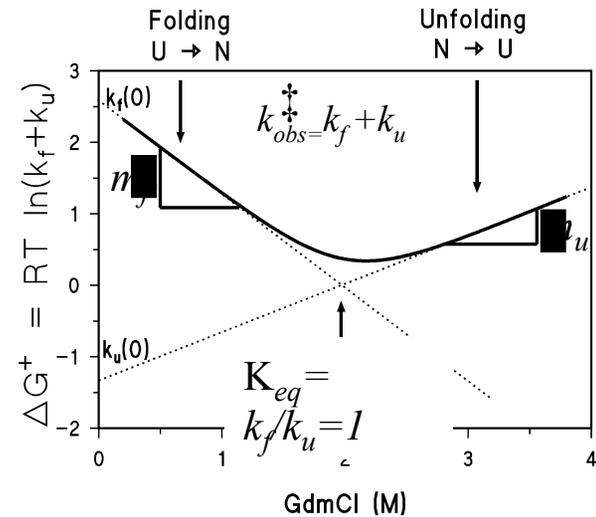
Energy:  $K = k_f/k_u$

(i.e.  $\Delta G_{kin} = \Delta G_u^\ddagger - \Delta G_f^\ddagger = \Delta G_{eq}$ )

Surface burial:  $m_{kin}^\phi = m_u + m_f = m_{kin}^\phi$

$m_f, m_u \propto$  surface area exposed going from starting state to transition state

All the  $\Delta G$  and surface burial is fully accounted for in the observed kinetic phase.



$\therefore$  no early stable intermediates – **2-state folding!**