Pressure effects on folding/unfolding of proteins and Nucleic Acids

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Talk overview

- Introduction:
  - Folding of peptides and small proteins
  - Time scale limitations of molecular dynamics simulations
  - Replica exchange MD simulations

- All atom, explicit solvent, simulations of folding thermodynamics
  - Three helix bundle protein
  - A designed mini protein (the trp cage)
    - Pressure-Temperature Diagram
  - An RNA tetraloop

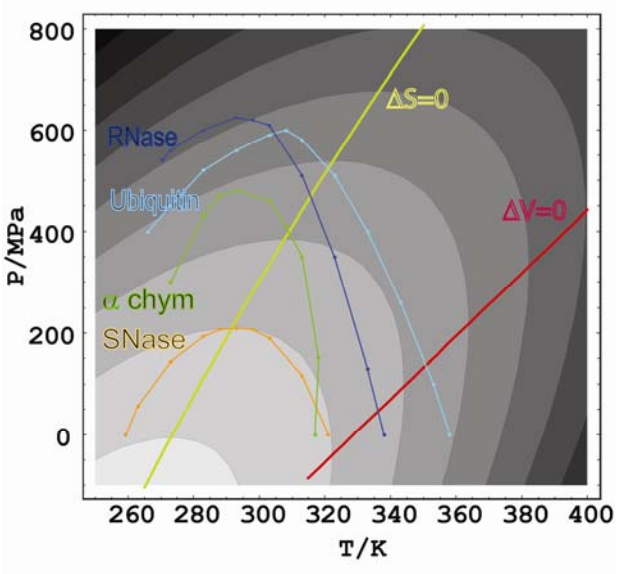
- Conclusions

History of Pressure unfolding of Proteins

- Bridgman, J Biol Chem. 1914- "The coagulation of albumen"

  "*The purpose of this note is to state a fact of possible biological interest which I have discovered incidentally in the course of other work. If the white of an egg is subjected to hydrostatic pressure at room temperature, it becomes coagulated, presenting an appearance much like that of a hard boiled egg*".

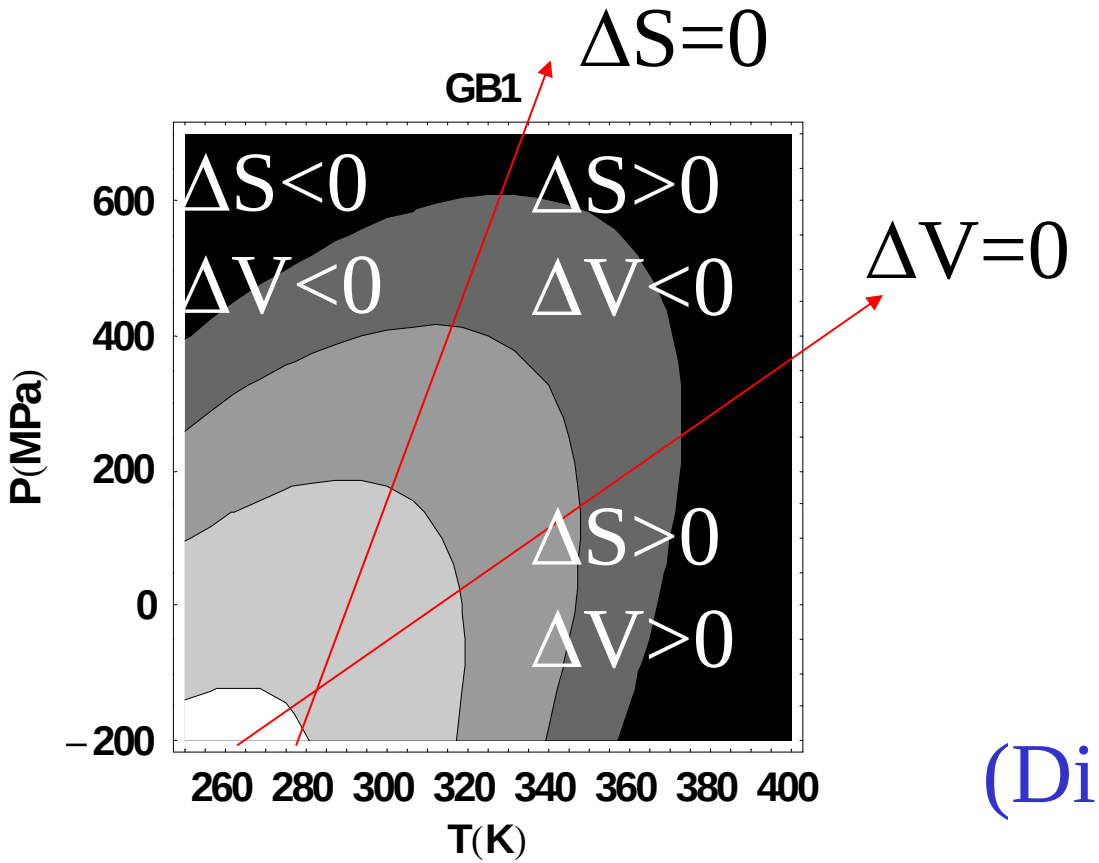
  "*The effect of temperature, which is not large, seems to be such that the ease of coagulation increases at low temperatures, contrary to what one might expect.*"



Gibbs free energy of unfolding (to second order in P)

$$\Delta G_U(P,T) = \Delta G_U^0 - \Delta S_U(T - T_0) + \Delta C_P(T - T_0) - \Delta C_P T \; Log(T/T_0) +$$

$$\Delta V_U(P - P_0) + \frac{1}{2}\Delta\beta(P - P_0)^2 + \Delta\alpha(P - P_0)(T - T_0)$$



ΔV = volume change (-100 ml/mol)

Δα = thermal expansivity (1.3 ml/mol.K)

Δβ = compressibility (0.02 ml/mol-bar)

Values between parenthesis measurements for SNase

(Diagram for GB1 hairpin)

Proteins under Pressure

- Proteins are compact structures with hydrophobic groups in the inside, polar group in the outside
  - What happens when you subject a protein to high pressure?
    - At high pressure the system becomes more compact
      - However, **proteins will unfold at high pressures (200-700 MPa).**

The Pressure Denatured Ensemble

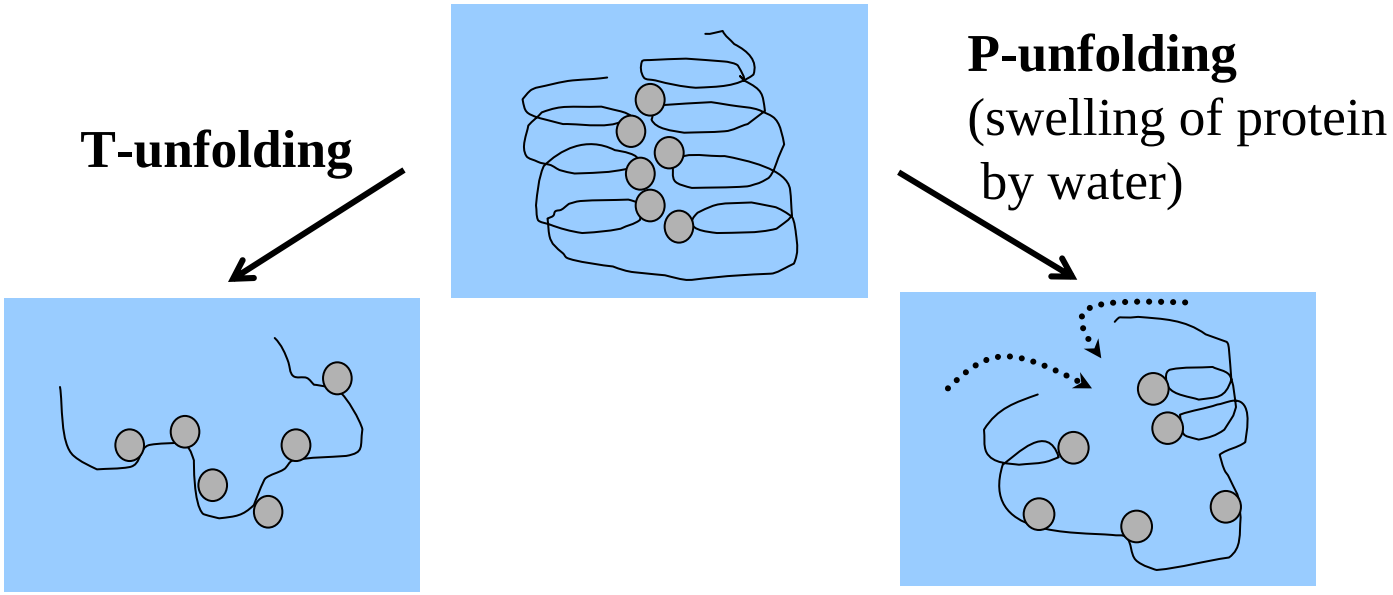
Experimental Results
(H-exchange, trp-fluorescence, SAXS):

- More compact compared to heat denatured proteins
- Elements of secondary structure retained
- Slow kinetics at elevated pressures.

Staphyloccocal Nuclease
Radius of Gyration $\mathbf{R_G}$

Folded	**17Å**
P-denatured	**36Å**
Heat denatured	**46Å**

(Panick et al, J. Mol. Biol**, 1998,** 329)



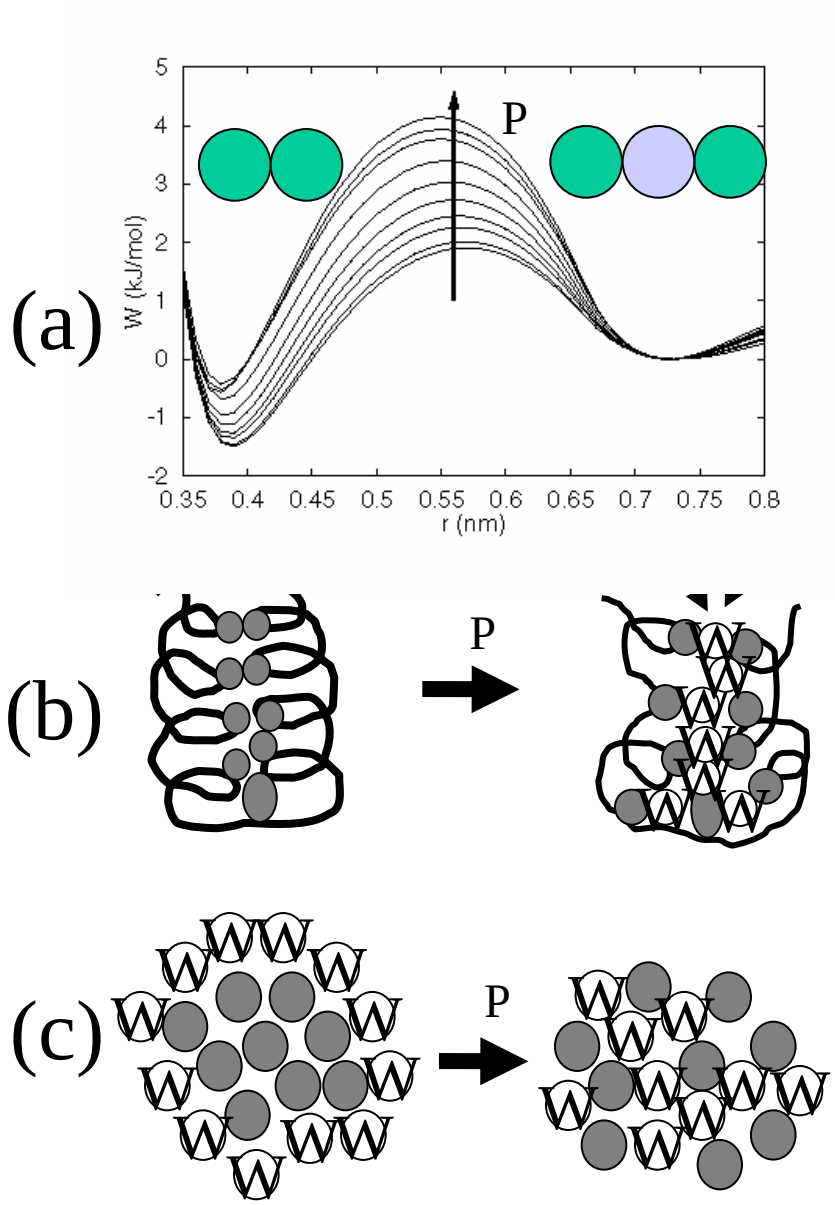
- Pressure changes the energy landscape without the use of denaturants or changes in temperature:

  For example:

  – Staph. Nuclease (SNase)
    - *shows multiple intermediates folding in ms at P ~ 0;*
    - *Shows two state folding/unfolding at 150 MPa*

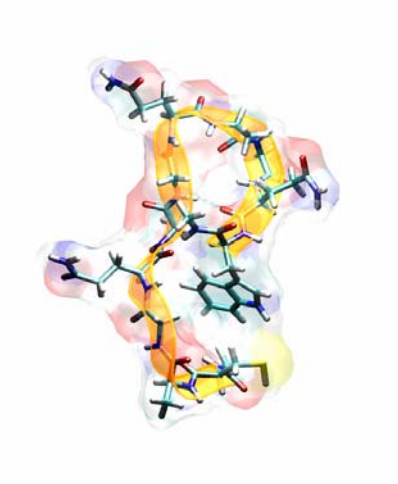
    (Panick, JMB 1998; Frye and Royer, 1997*)*
    - *Folding kinetic slows down*
- Pressure changes the balance between hydrophobic and polar interactions

Water Penetration Model for Pressure Induced Unfolding of Proteins



G. Hummer, S. Garde, A. E. Garcia, M. Paulaitis and L.R. Pratt, “The Pressure Dependence of Hydrophobic Interactions is Consistent with the Observed Pressure Denaturation of Proteins",Proc. Natl. Acad. Sci. 95:1552—1555 (1998).

Folding Time scales

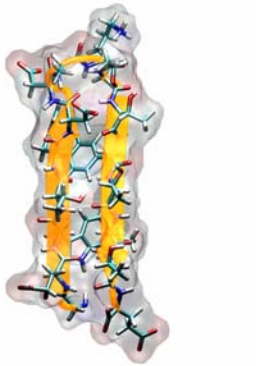


Loop closing in a random coil

~ 10 ns

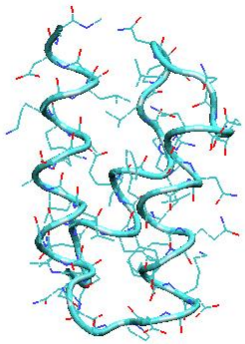
Alpha helix formation

~ 200 ns



beta hairpin

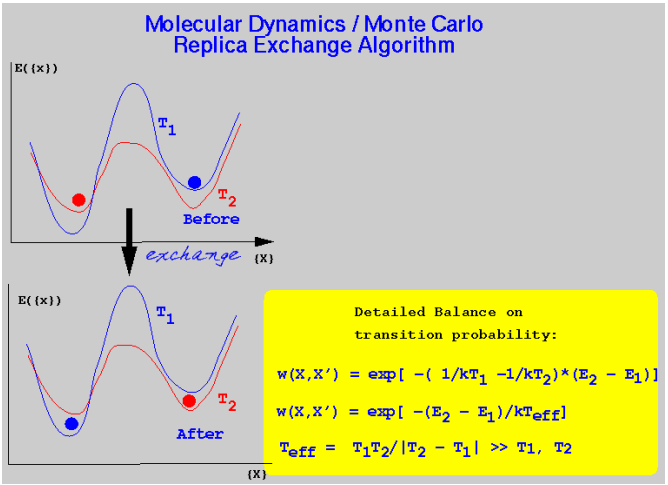
~ 1-10 μs

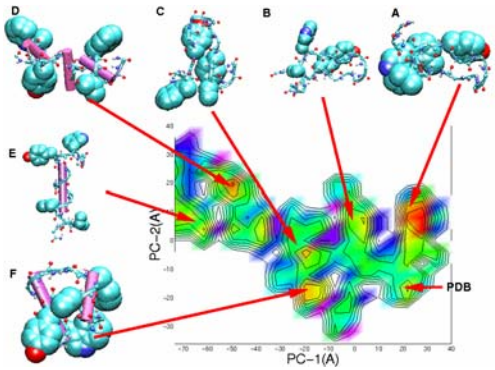


Folding of proteins:

1 μs – sec

Enhanced Sampling Methods

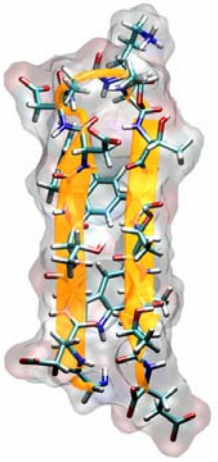




Hukushima K and Nemoto K, J. Phys. Soc. Japan 65: 1604-1608 (1996)
Sugita and Okamoto, Chem. Phys. Lett. 314: 141 (1999)

Garcia and Sanbonmatsu, Proteins 42:345-354 (2001)

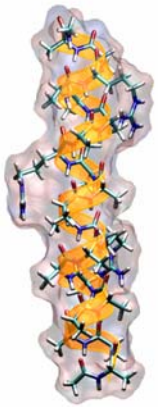
Pressure and Temperature folding/unfolding of peptides



- Two systems:

1. GB1: 41-56 -hairpin from the B1 domain of protein G
   - GEWTYDDATKTFTVT

2. AK peptide (alpha helix):
   - Ac-AA(AAKAA)3AAY-Nme



Volume-Temperature REMD

The simulations reported here were conducted using a grid of 253 & 360 different (V,T)-states, each state characterized by its volume V and temperature T.

Starting from the total partition function of the extended ensemble, we have derived the acceptance rule for state swapping moves between two states *i* and *j* as being according to the acceptance probability:

$$P_{acc} = \min\{1, \exp[\beta_i (U(\vec{s}_i^{\,N}; L_i) - U(\vec{s}_j^{\,N}; L_i)) + \beta_j (U(\vec{s}_j^{\,N}; L_j) - U(\vec{s}_i^{\,N}; L_j))]\}$$

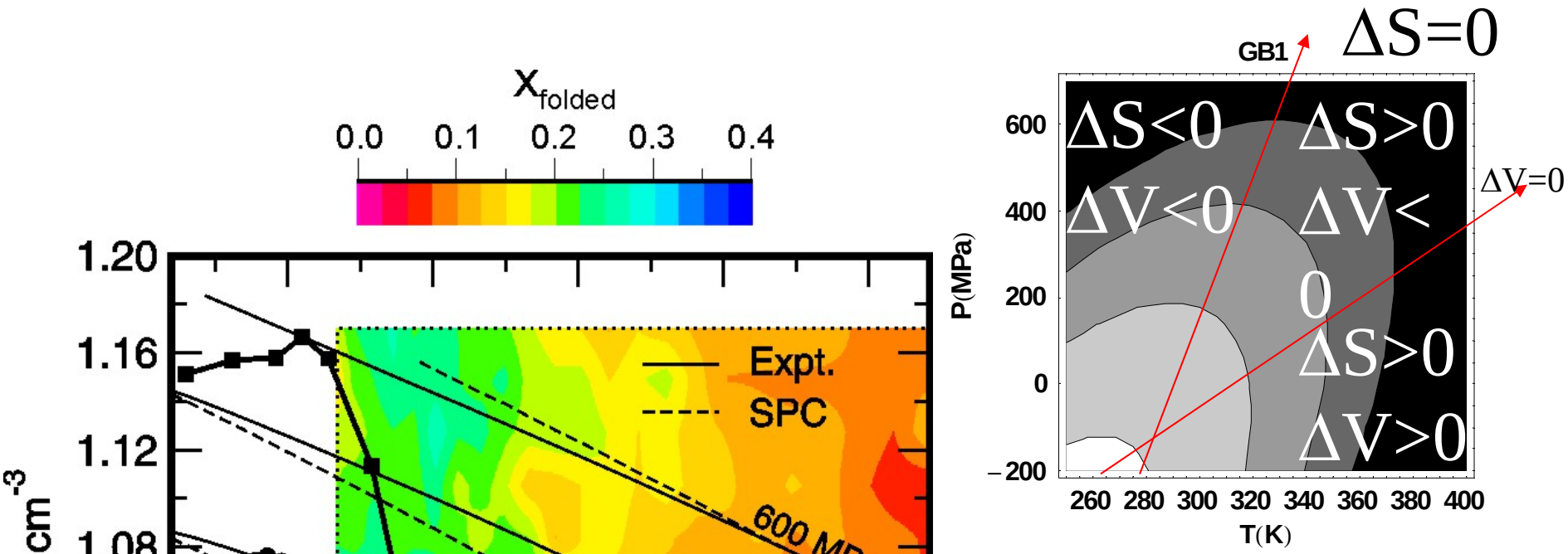
with $\beta_i = 1/k_B T_i$, $\vec{s}^{\,N} = L^{-1}\vec{r}^{\,N}$

$$U(\vec{s}_j^{\,N}; L_i) \approx U(\vec{s}_j^{\,N}; L_j) - (P_j - \frac{M}{\beta_j' V_j})\times(V_i - V_j)$$

$$U(\vec{s}_i^{\,N}; L_j) \approx U(\vec{s}_i^{\,N}; L_i) - (P_i - \frac{M}{\beta_i' V_i})\times(V_j - V_i)$$

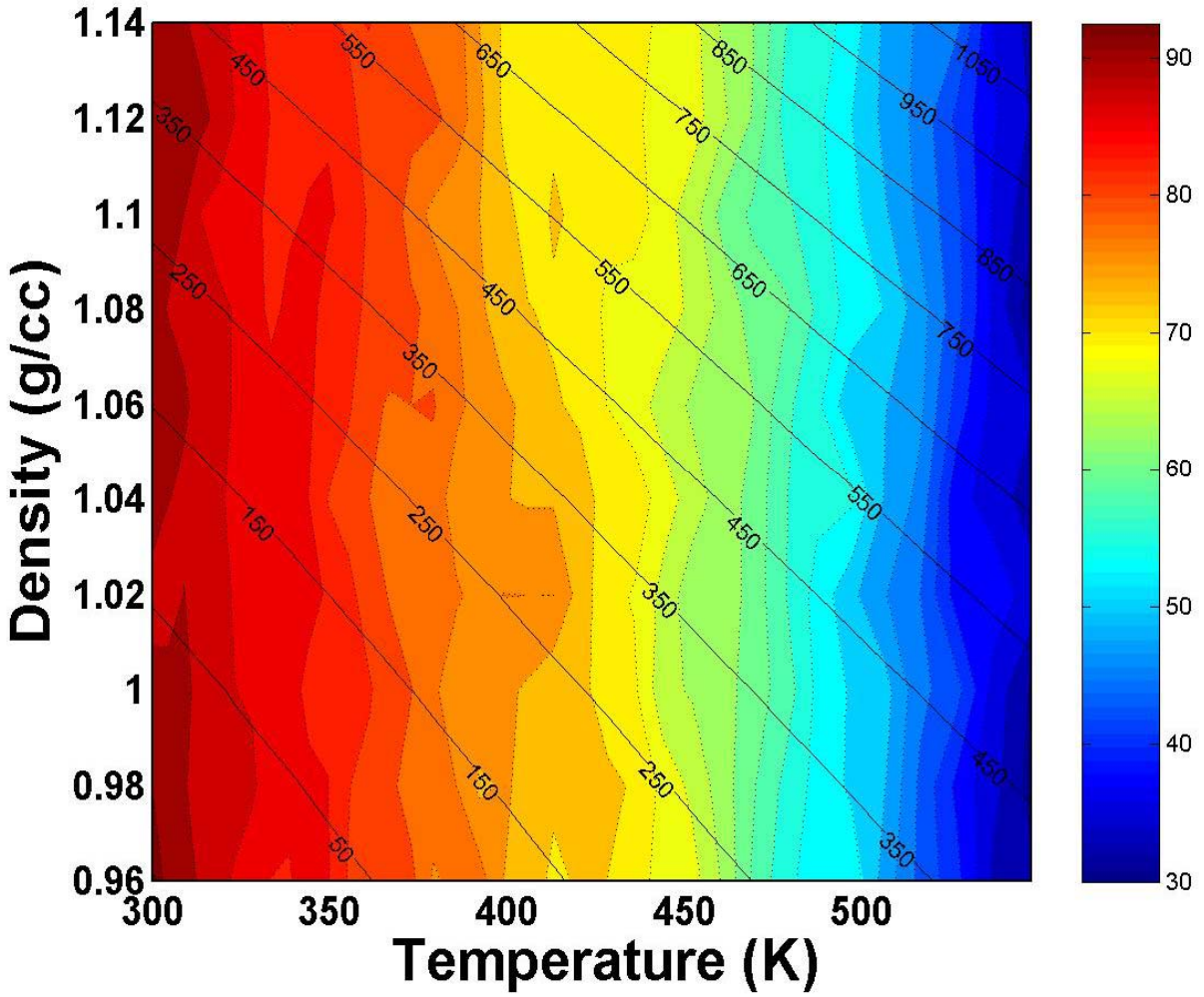
Approximation is valid for small volume changes

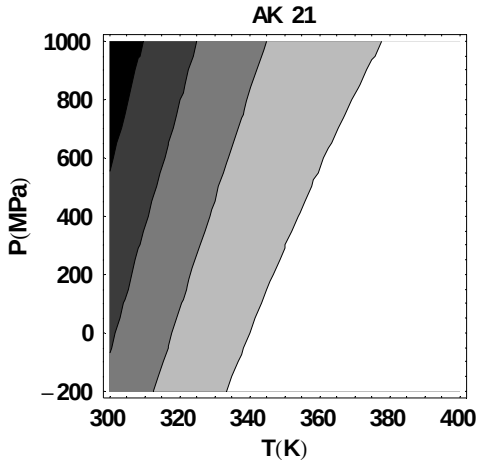
Where M: number of molecules, primed β implies instantaneous temperature



Filled Squares:
Folding/unfolding transition of Ubiquitin
Color Insert:
Fraction of folded states of the GB1 beta hairpin
Solid lines:
Density isobars for water (exp.)
Thin dashed lines:
Density isobars
obtained for SPC water

D. Paschek and A.E. Garcia, “Reversible temperature and pressure denaturation of a protein fragment: Replica exchange molecular dynamics simulation study.” Phys. Rev. Let. 93, 238105 (2004)





(Color) Insert: Fraction of alpha helical states x_{folded} of the AK peptide

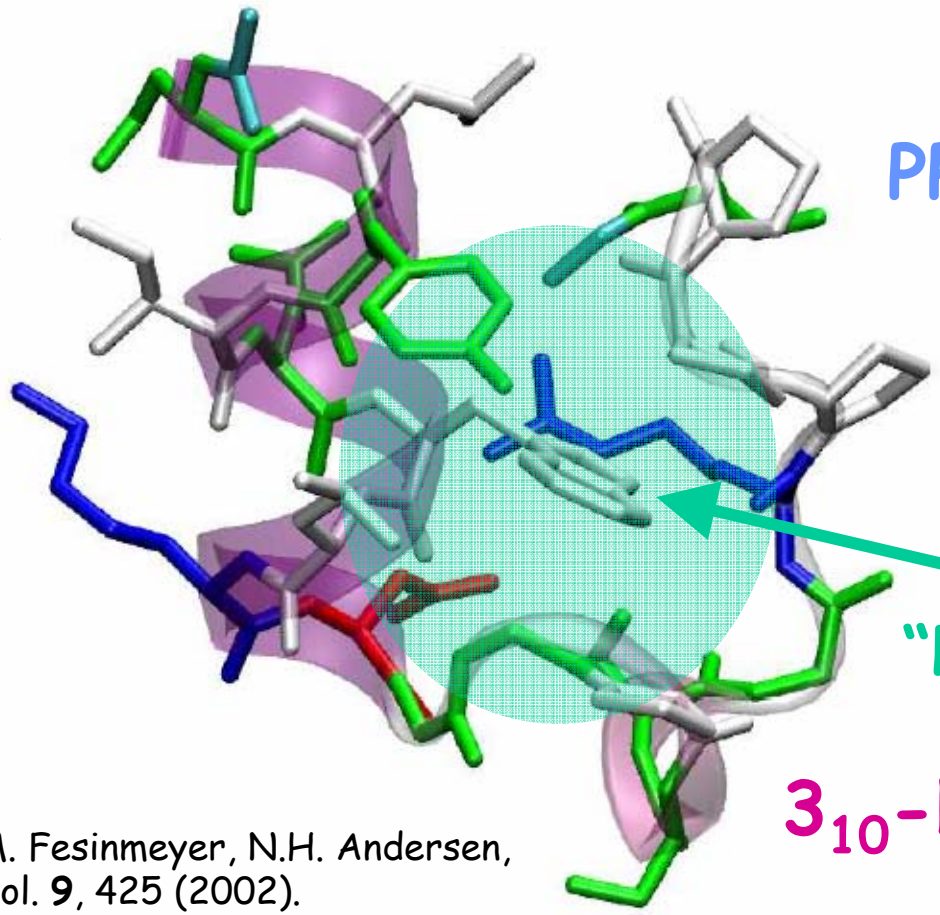
Solid lines: Density isobars for water (experimental data)

No pressure dependence of alpha helix stability (no burried waters)

Paschek, Gnanakaran & Garcia, PNAS 102, 6765-6770 (2005).

Trp-cage miniprotein Tc5b:

fastest folding
"protein": 4.1 μs



α-helix

PPII-helix

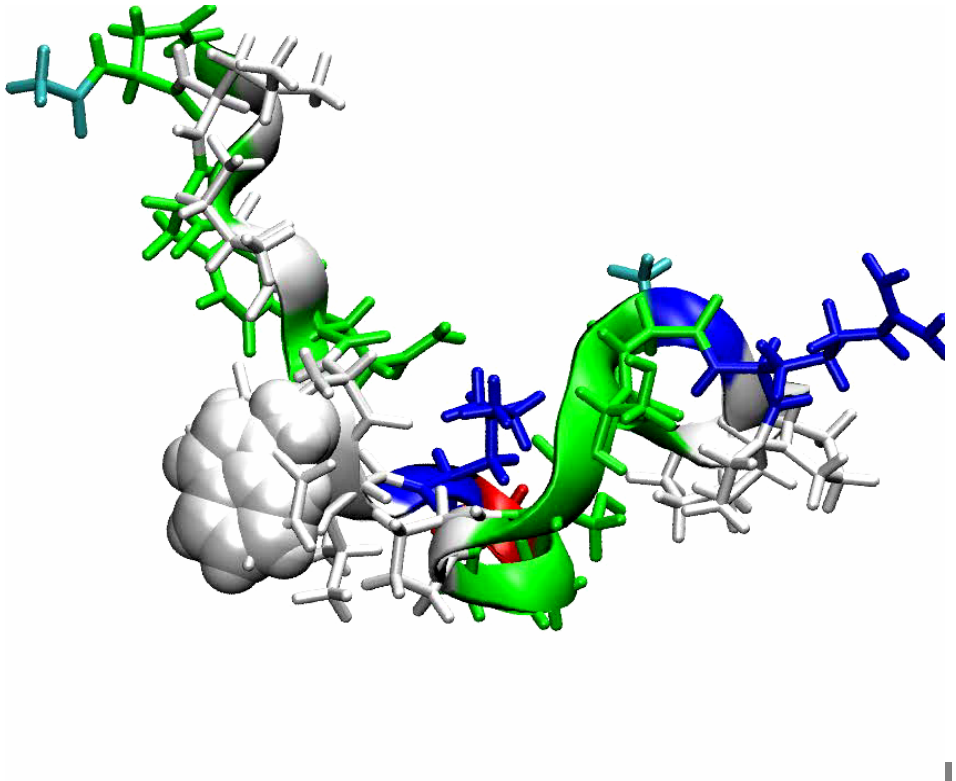
"Hydrophobic core"

3_{10}-helix

J.W. Neidigh, R.M. Fesinmeyer, N.H. Andersen,
Nature Struct. Biol. **9**, 425 (2002).

Ac-NLYIQ-WL DG-GPSSG- PPPS-NMe

Does the model Trp-cage fold ?



Replica ID # 02: 0…50 ns

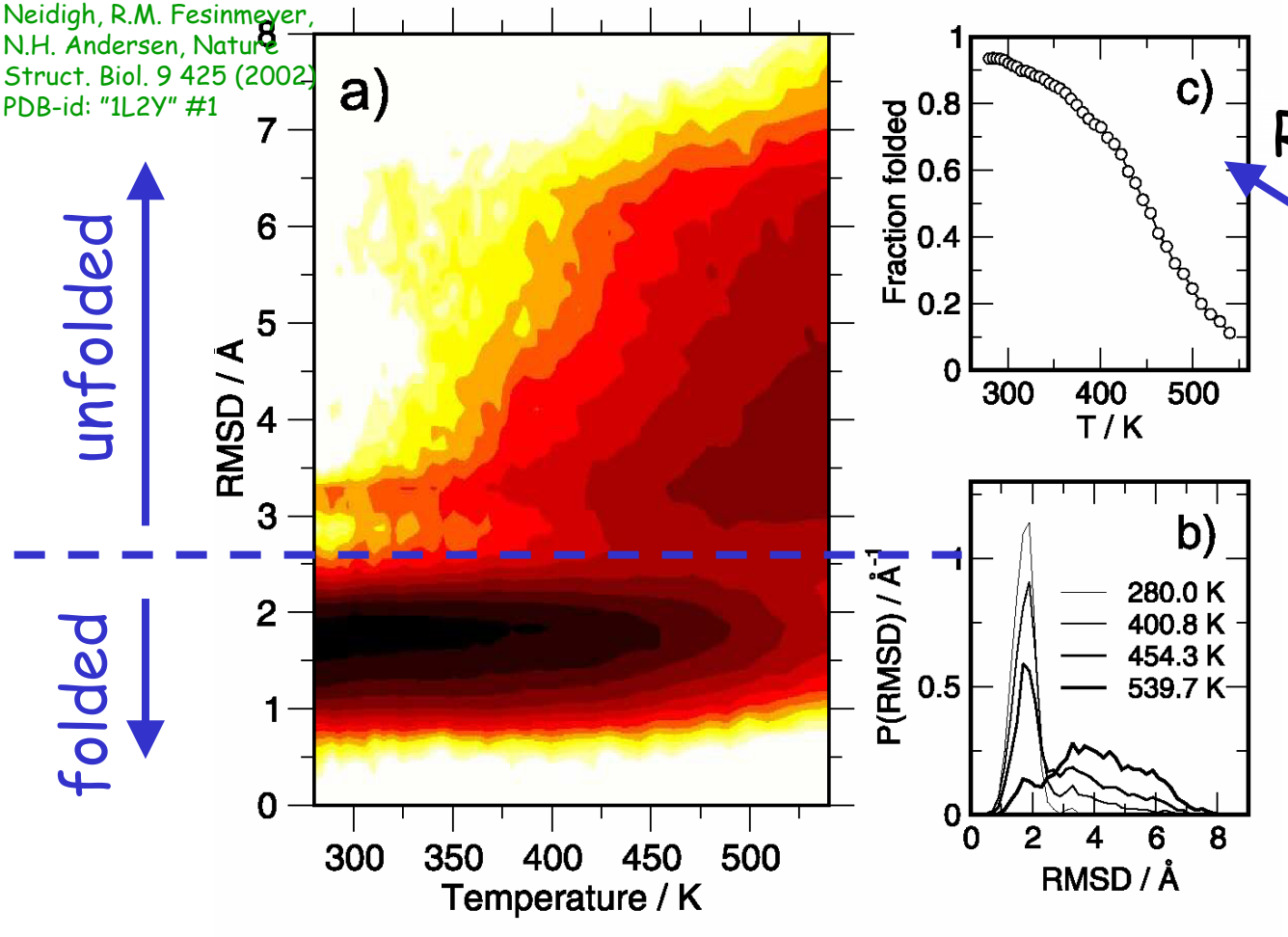
REMD-Simulation:

40 Replicas
280.0 K- 539.8K
AMBER94
2637 TIP3P
Ewald (PME)
100 ns/replica (4 μs accumulated)

Starting from an extended configuration

Folding: Free Energy Map of the Trp-cage

RMSD: NMR: J.W. Neidigh, R.M. Fesinmeyer, N.H. Andersen, Nature Struct. Biol. 9 425 (2002) PDB-id: "1L2Y" #1



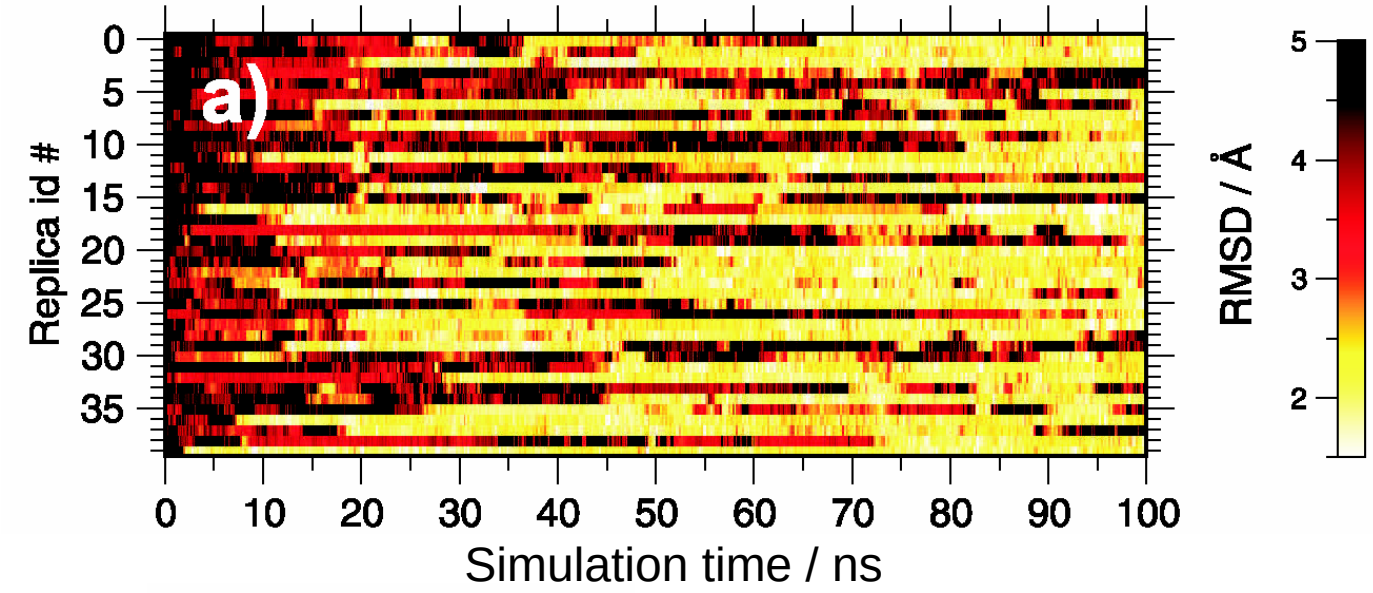
RMSD < 2.2 A

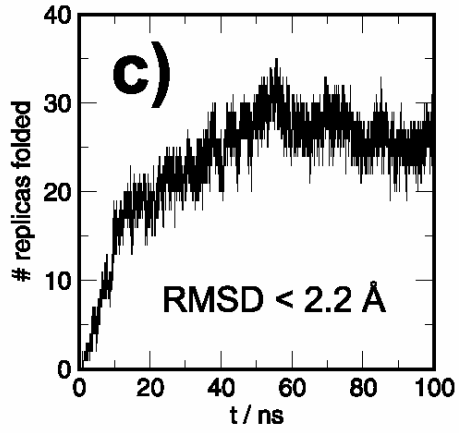
Transition Temperature: 440 K (Expt: 310 K)

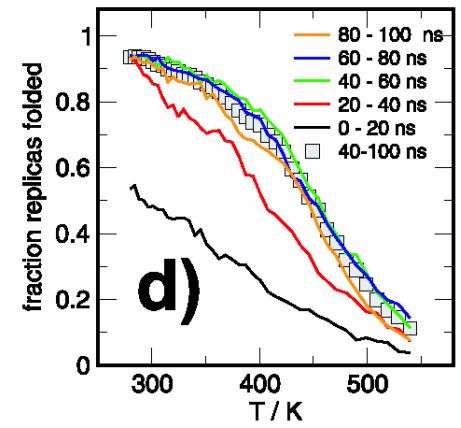
Two state folder: Qiu et al., *JACS* **124**, 12952 (2002)
Intermediate states: Neuweiler et al., PNAS **102**, 16650 (2005)

Paschek, Nymeyer, Garcia, JSB, 2007

Can we access the folding/unfolding equilibrium?

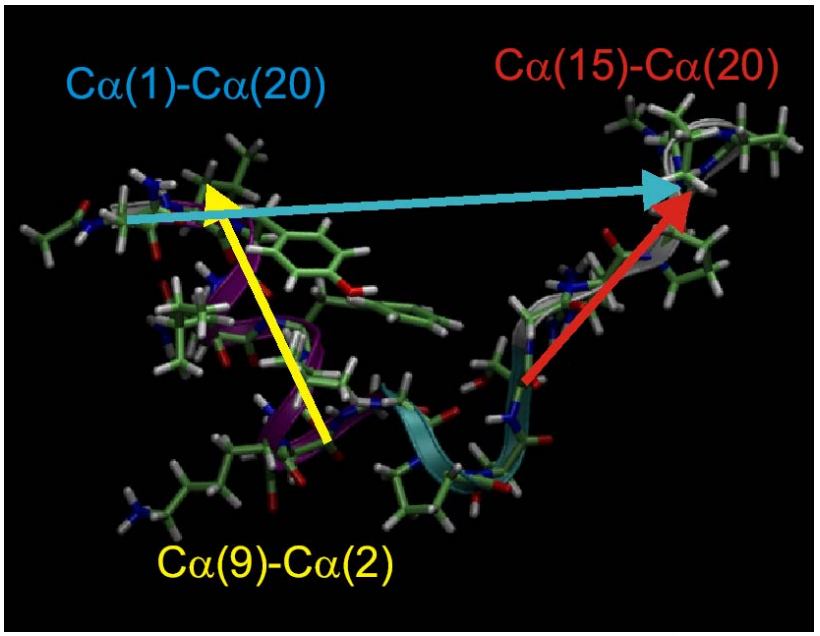






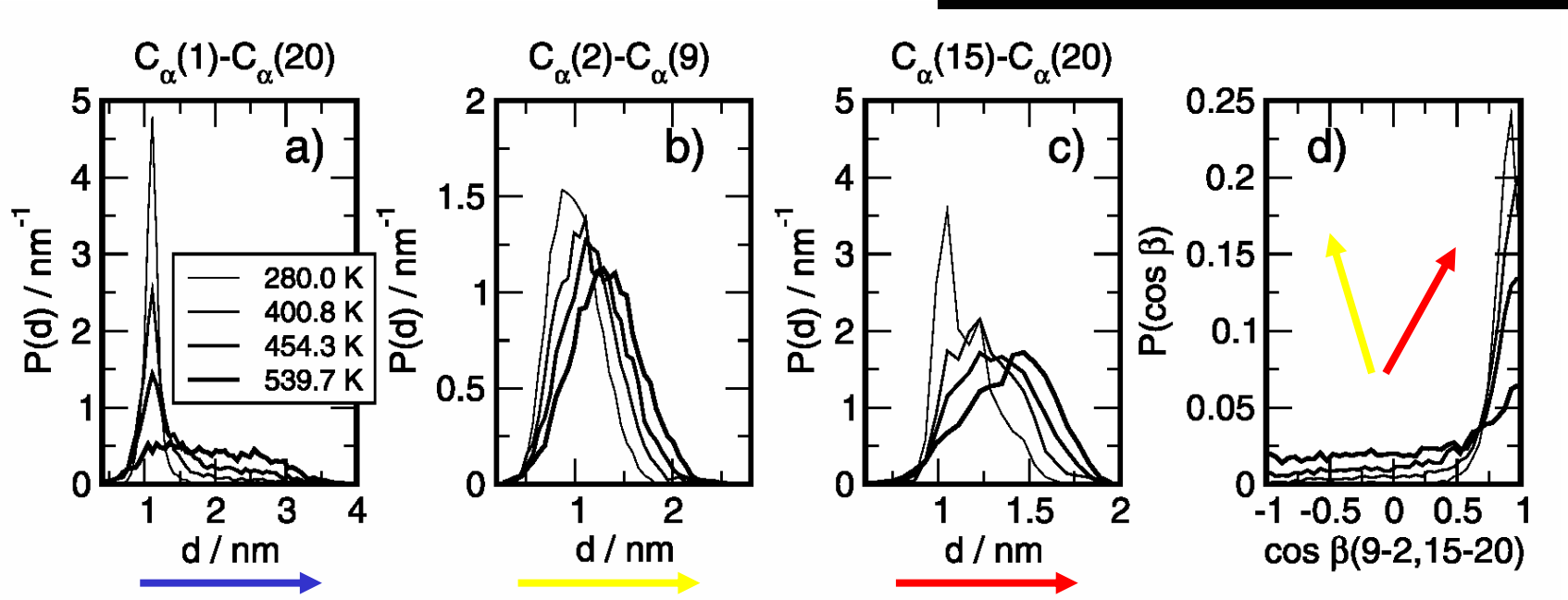
Average Lifetime of folded states in REMD-Ensemble: ~30 ns

Thermal Denaturation:

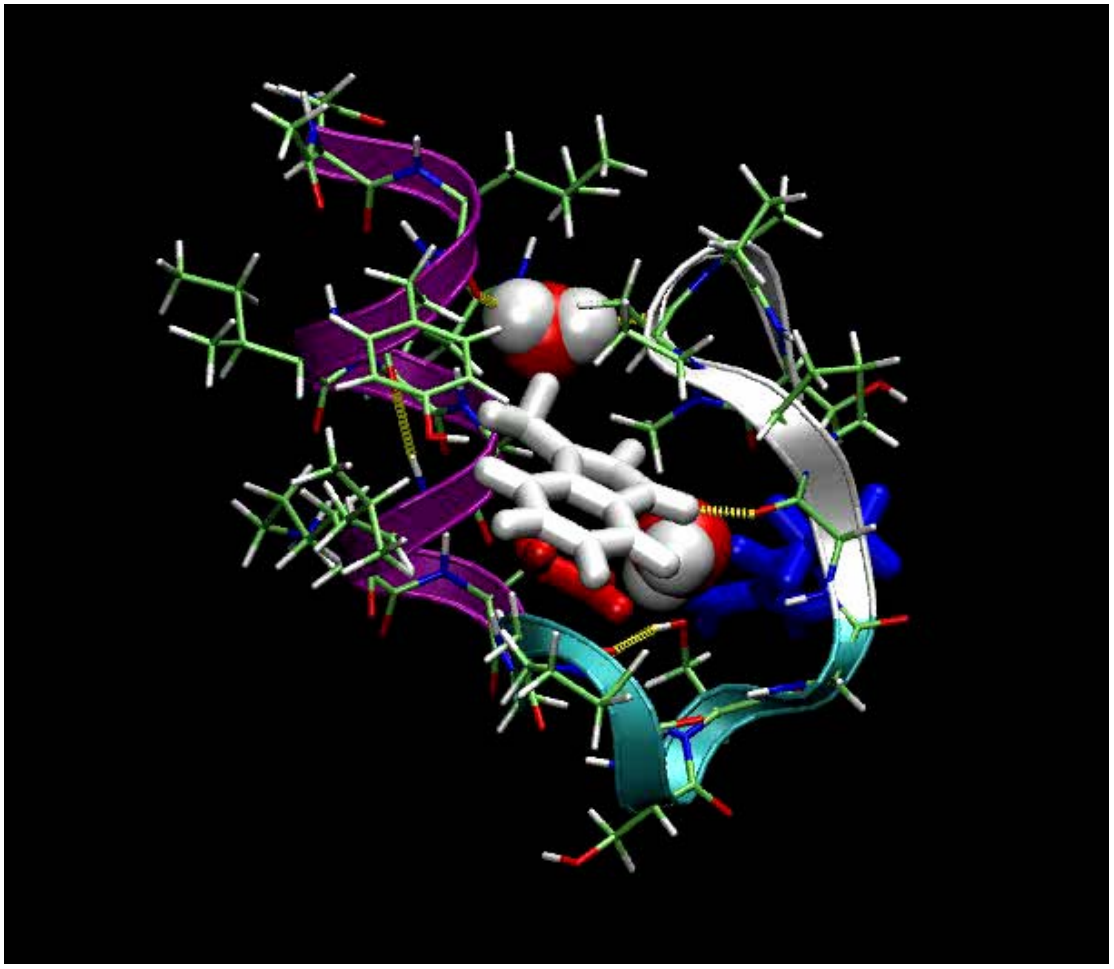


Fluorescence quenching of the Trp-sidechain due to water exposure: Neuweiler et al. PNAS **102**, 16650 (2005), Qiu et al. *JACS* **124**, 12952.

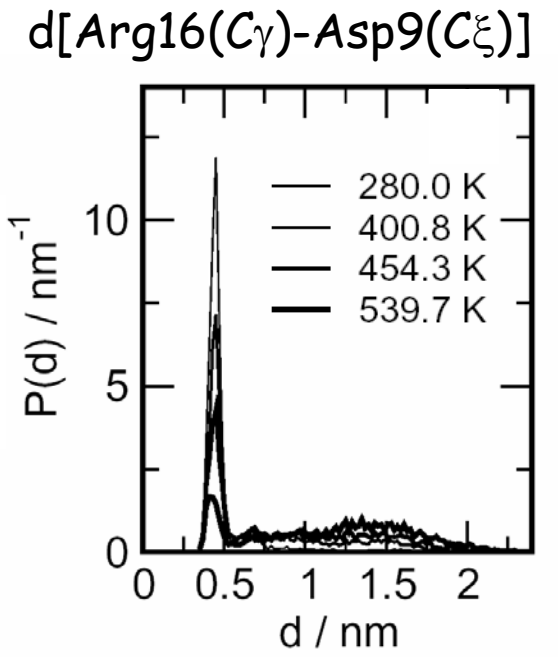
Opening of the Trp-cage



Sources of Stability:

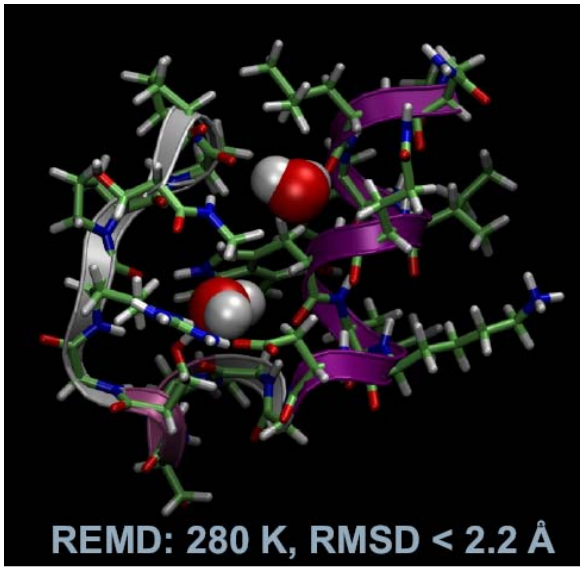


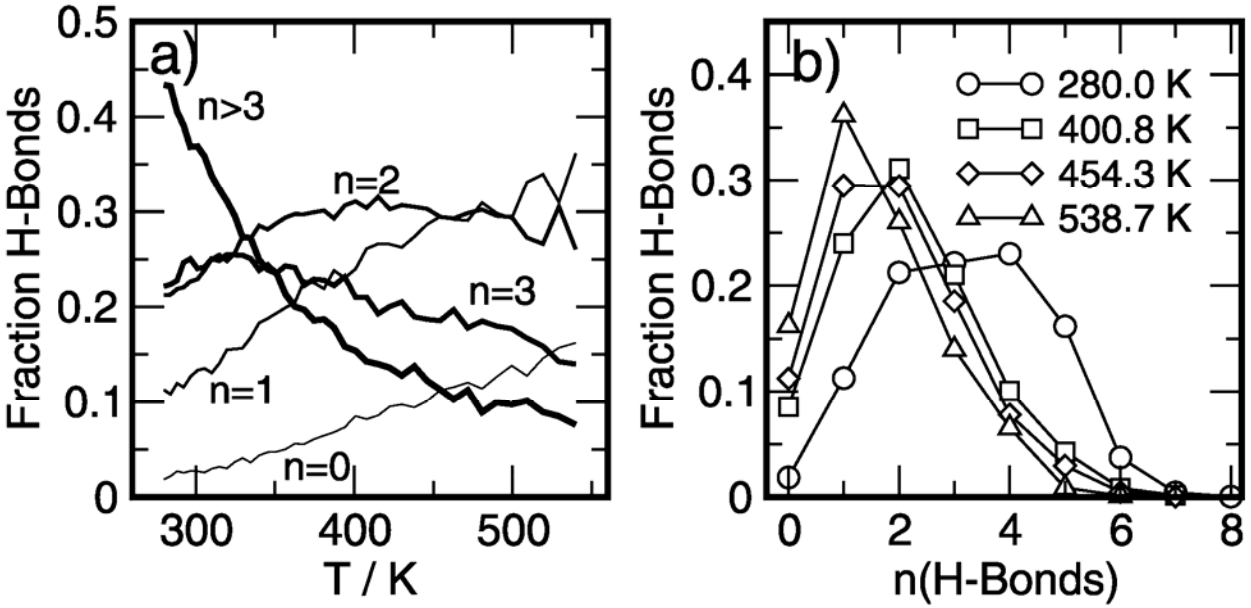
1. Salt-bridge: ***Asp9-Arg16***
2. Internal Water ?



“Internal Water”

Water-Trp-cage Hydrogen Bonds of the **two** internal water molecules





Calculated for **Folded** Configurations only !

Protein Thermodynamics

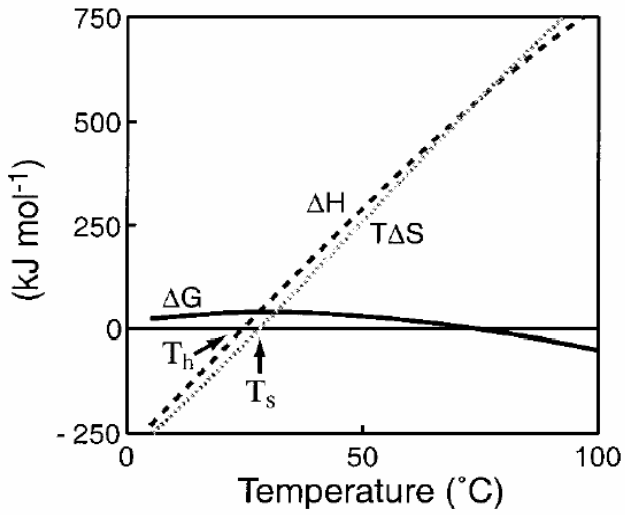
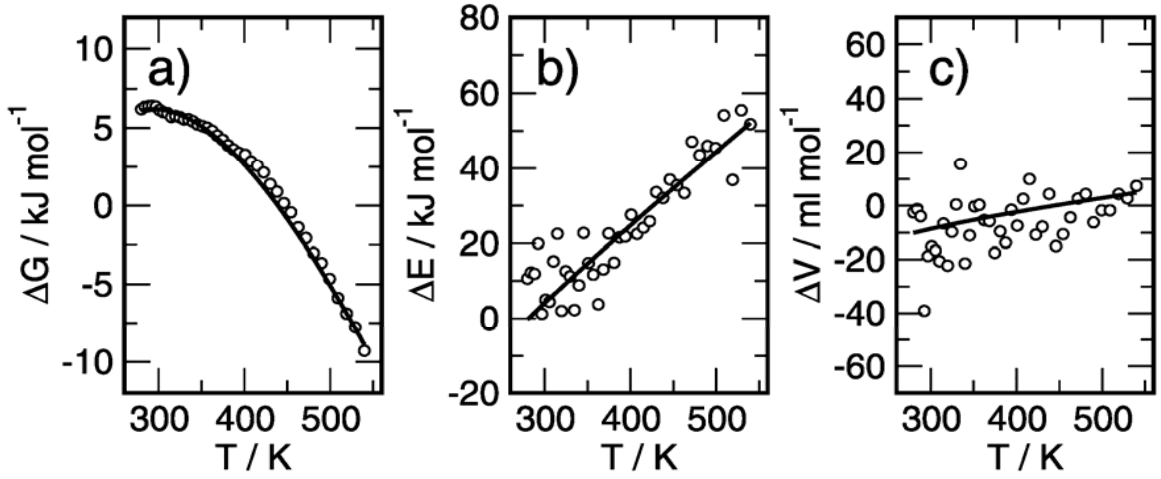


Figure 3. Protein unfolding free energy, $\Delta G = G_u - G_f$, entropy, ΔS, and enthalpy, ΔH, versus temperature. For proteins, $T_s \approx T_h$. Data on myoglobin from Makhatadze, G. I. and Privalov, P. L. *Biophys. Chem.* **1994**, *51*, 291.

1. Free Energy Difference $\Delta G = G_{unfold} - G_{fold}$: Parabolic Shape
2. Entropy/Energy compensation

Trp-cage Thermodynamics

$$\Delta G = G_{\text{unfold}} - G_{\text{fold}} = -RT \ln \frac{1 - x_{\text{fold}}}{x_{\text{fold}}}$$



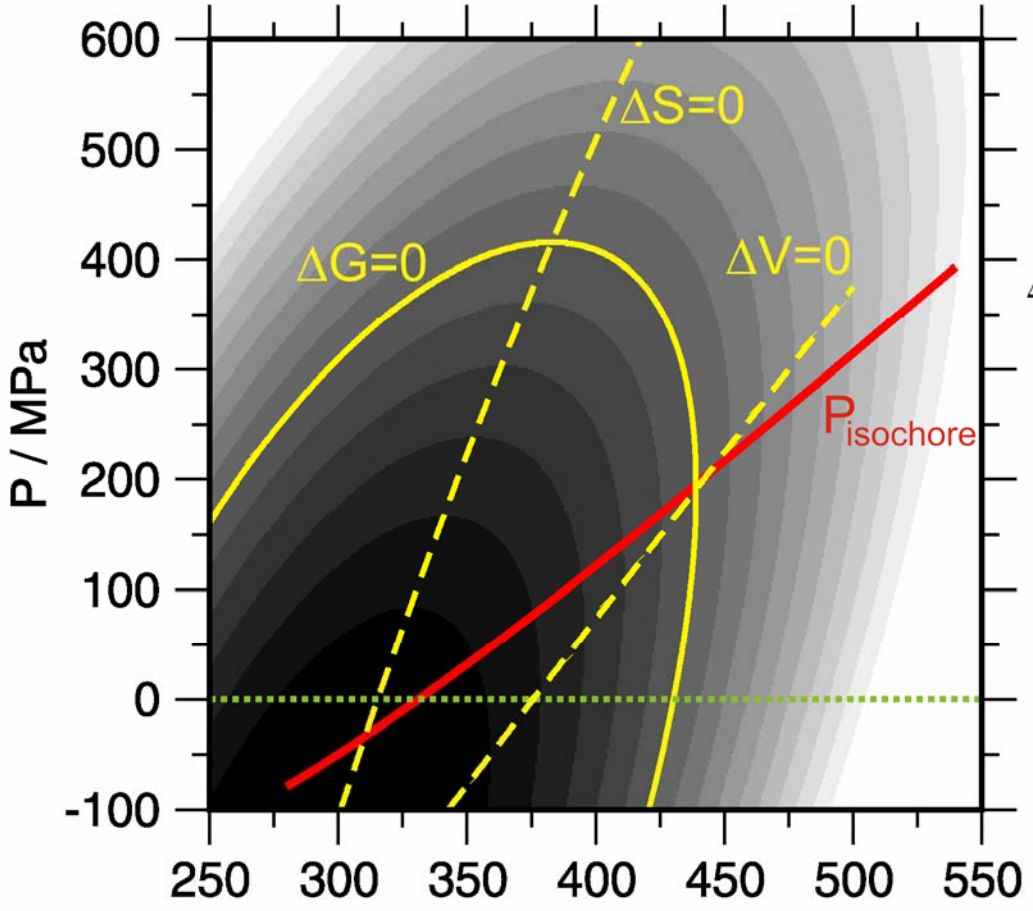
$$\begin{aligned}\Delta G &= \Delta\beta/2(P-P_0)^2 - \Delta C_P\left[T\left(\ln\frac{T}{T_0}-1\right)+T_0\right] \\ &+\Delta\alpha(P-P_0)(T-T_0) \\ &+\Delta V_0(P-P_0) - \Delta S_0(T-T_0) + \Delta G_0\end{aligned}$$

$T_0 = 331\,\text{K}$ $\quad P_0 = 0\,\text{MPa}$

$\Delta G_0 = 5.7\,\text{kJ mol}^{-1}$ $\quad \Delta S_0 = 15\,\text{J K}^{-1}\text{mol}^{-1}$ $\quad \Delta V_0 = -6.5\,\text{ml mol}^{-1}$

$\Delta Cp = 0.31\,\text{kJ K}^{-1}\,\text{mol}^{-1}$ $\quad \Delta\alpha = 14.5\,10^{-2}\,\text{ml K}^{-1}\,\text{mol}^{-1}$ $\quad \Delta\beta = -4.8\,10^{-5}\,\text{kJ mol}^{-1}\,\text{MPa}^{-2}$

Model Trp-cage: Plausible Stability Diagram



$$\Delta G = \Delta\beta/2(P-P_0)^2 - \Delta C_P\left[T\left(\ln\frac{T}{T_0}-1\right)+T_0\right] + \Delta\alpha(P-P_0)(T-T_0) + \Delta V_0(P-P_0) - \Delta S_0(T-T_0) + \Delta G_0$$

$T_0 = 331\,\mathrm{K}$ $P_0 = 0\,\mathrm{MPa}$

$\Delta G_0 = 5.7\,\mathrm{kJ\,mol^{-1}}$ $\Delta S_0 = 15\,\mathrm{J\,K^{-1}mol^{-1}}$ $\Delta V_0 = -6.5\,\mathrm{ml\,mol^{-1}}$

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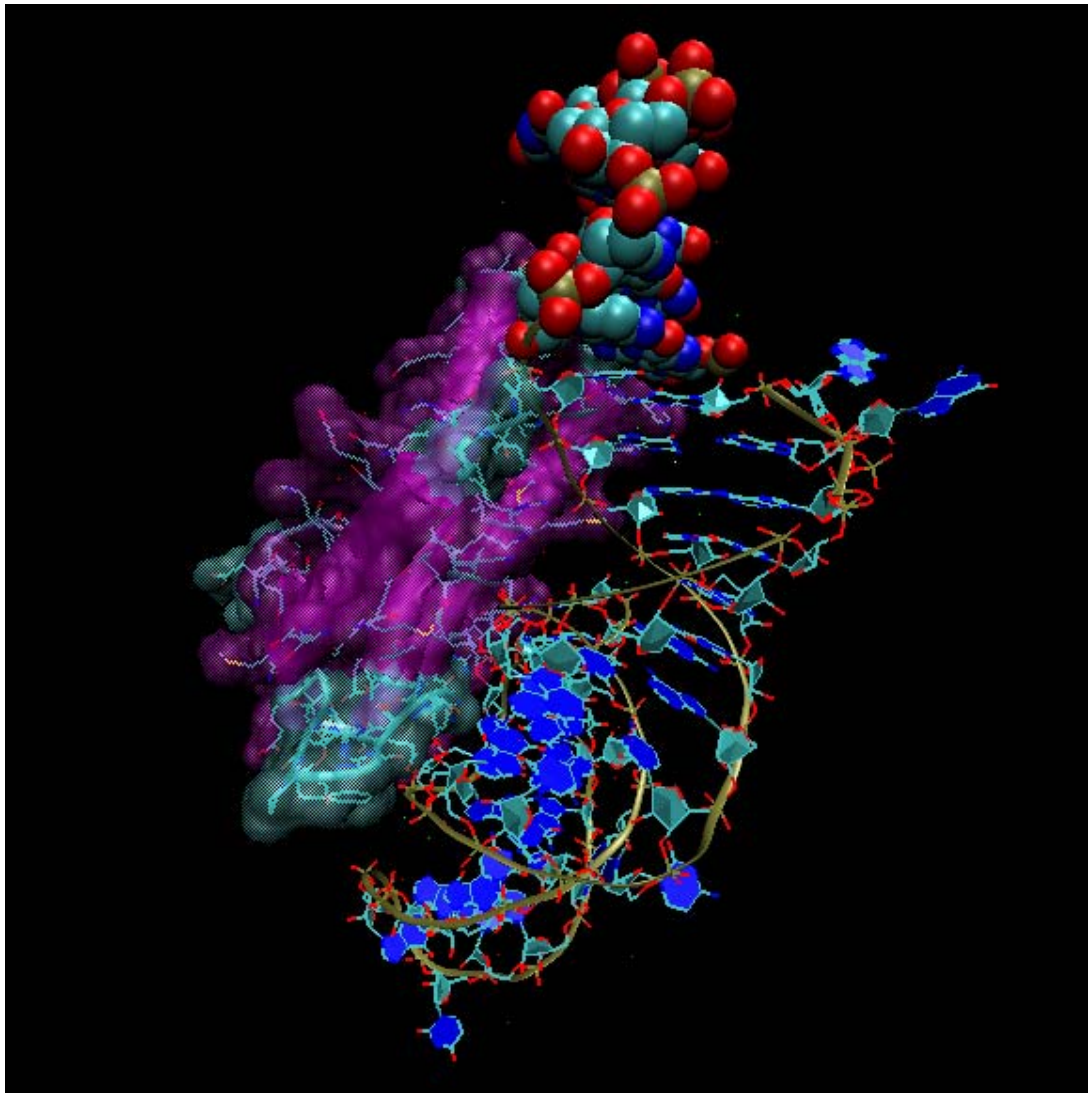
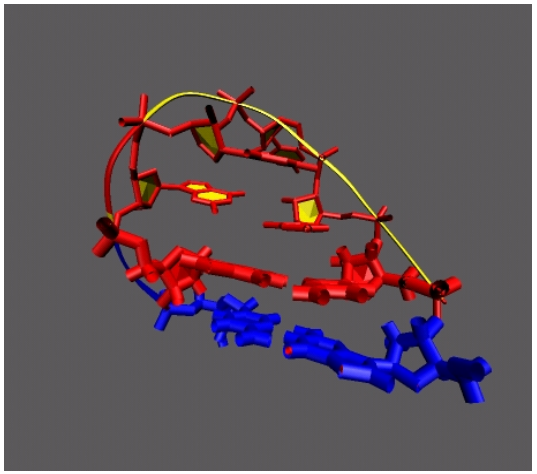
REMD are constant V calculations

Folding thermodynamics of an RNA tetraloop

30 S ribosomal protein S15
16 S ribosomal RNA fragment





^{31}gcUUCGg^{38}c Tetraloop

H Ma, Proctor,Kierzek, Kierzek, Bevilacqua and Grubele “Exploring th eenergy landscape of a small RNA Hairpin” JACS 2006

“Even a very fast-folding 8-mer RNA with an ideal tetraloop sequence has a rugged energy landscape, ideal for testing analytical and computational models”

Ennifar at al. J Mol. Bio. 304, 2000

H Ma, Proctor,Kierzek, Kierzek, Bevilacqua and Gruebele “Exploring the energy landscape of a small RNA Hairpin” JACS 2006

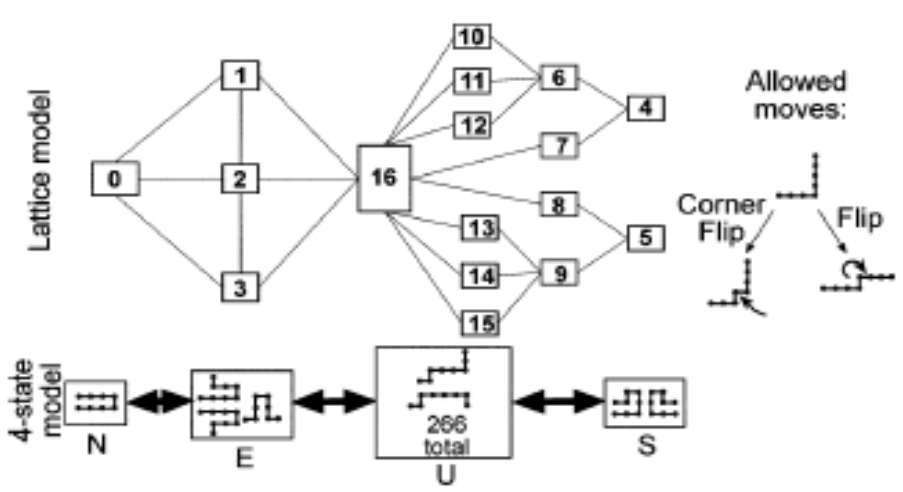
Four state model describes the kinetics:

S: Stem is formed; non native loops (off pathway)

U: Unfolded

E: Incomplete stem structure

N: Native



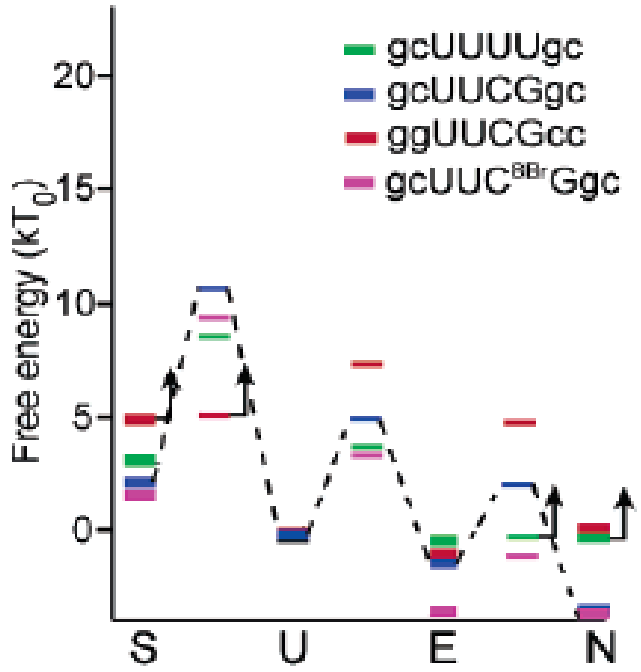
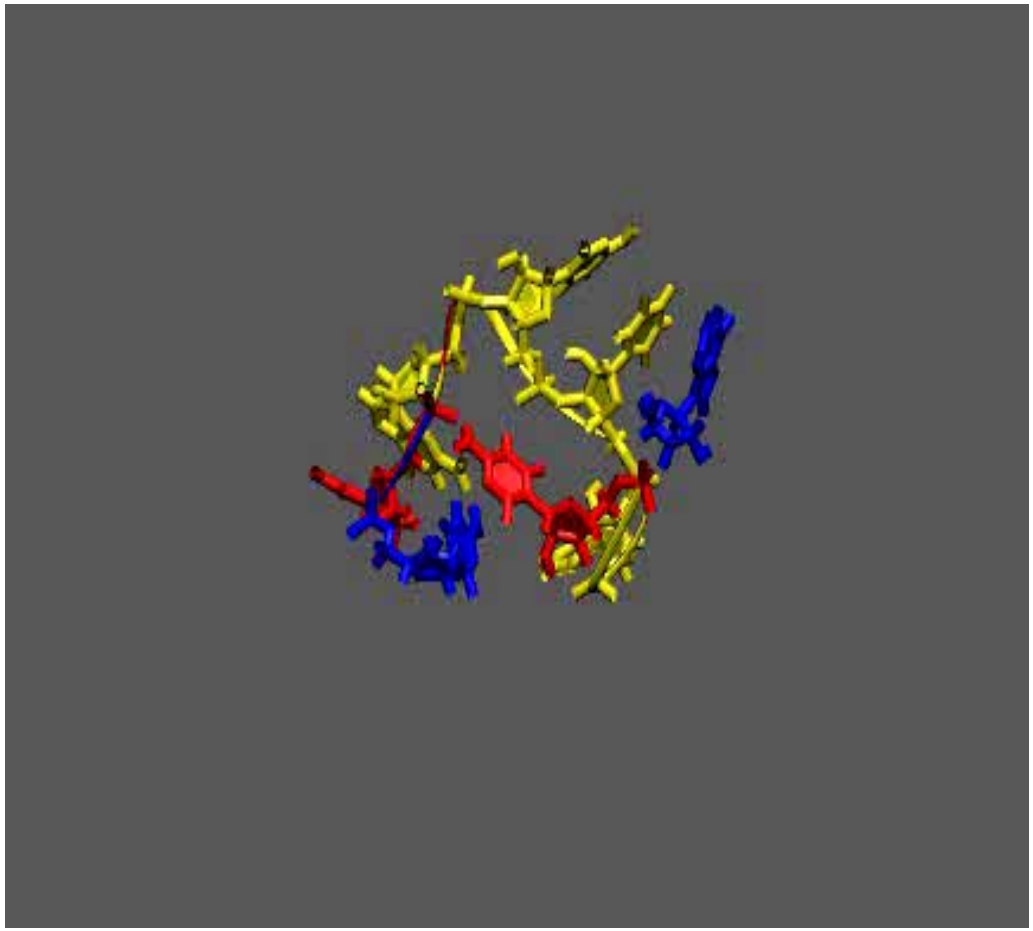
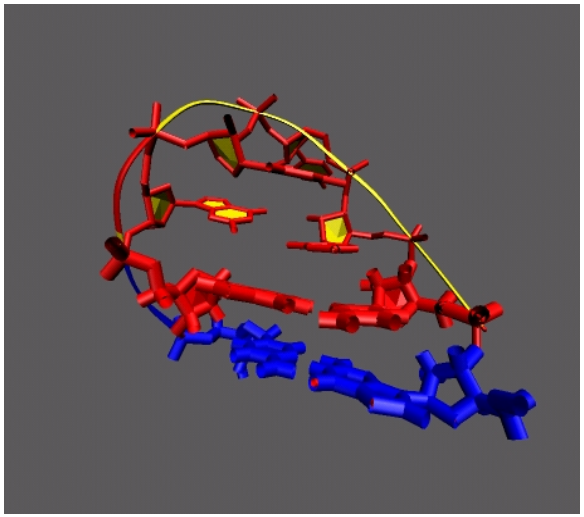


Figure 7. Free energy level diagram from the four-state fit at low temperature (T_0 = 298 K for gcUUCGgc, 291 K for ggUUCGcc, 301 K for gcUUC^{8Br}Ggc and 286 K for gcUUUUgc, to compare at similar relative temperatures below T_m). The free energies have been shifted to make U the reference state. The variants are color coded as in Figures 3 and 6. The arrows indicate states for which the fit provides only a limit on the free energy.

Folding of an RNA molecule

r(gcUUCGgc)





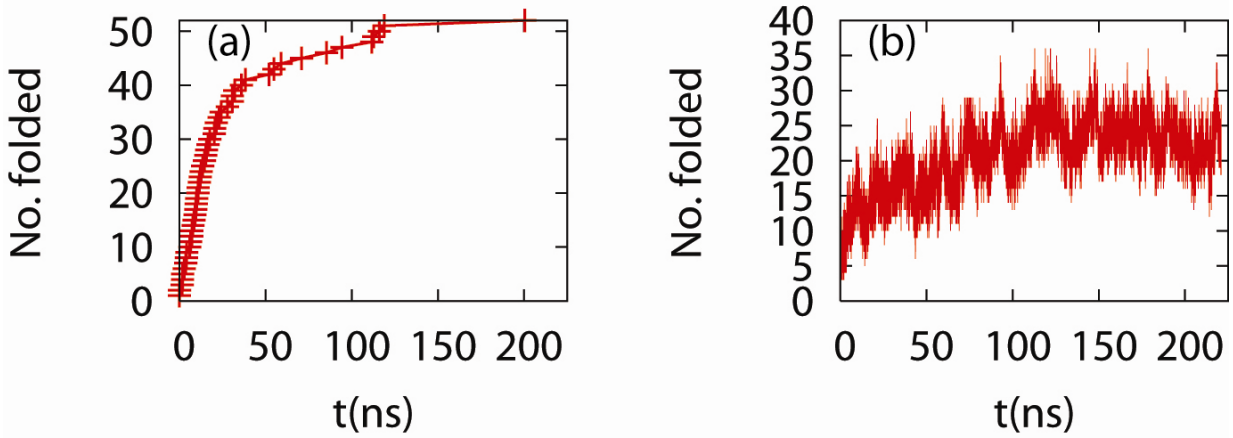
14 Na+, 7 Cl-
(150 mM excess salt)
2557 TIP3P waters
52 replicas (270-601 K)

225 ns/replica; 52 replicas
> 11 microsecond simulation

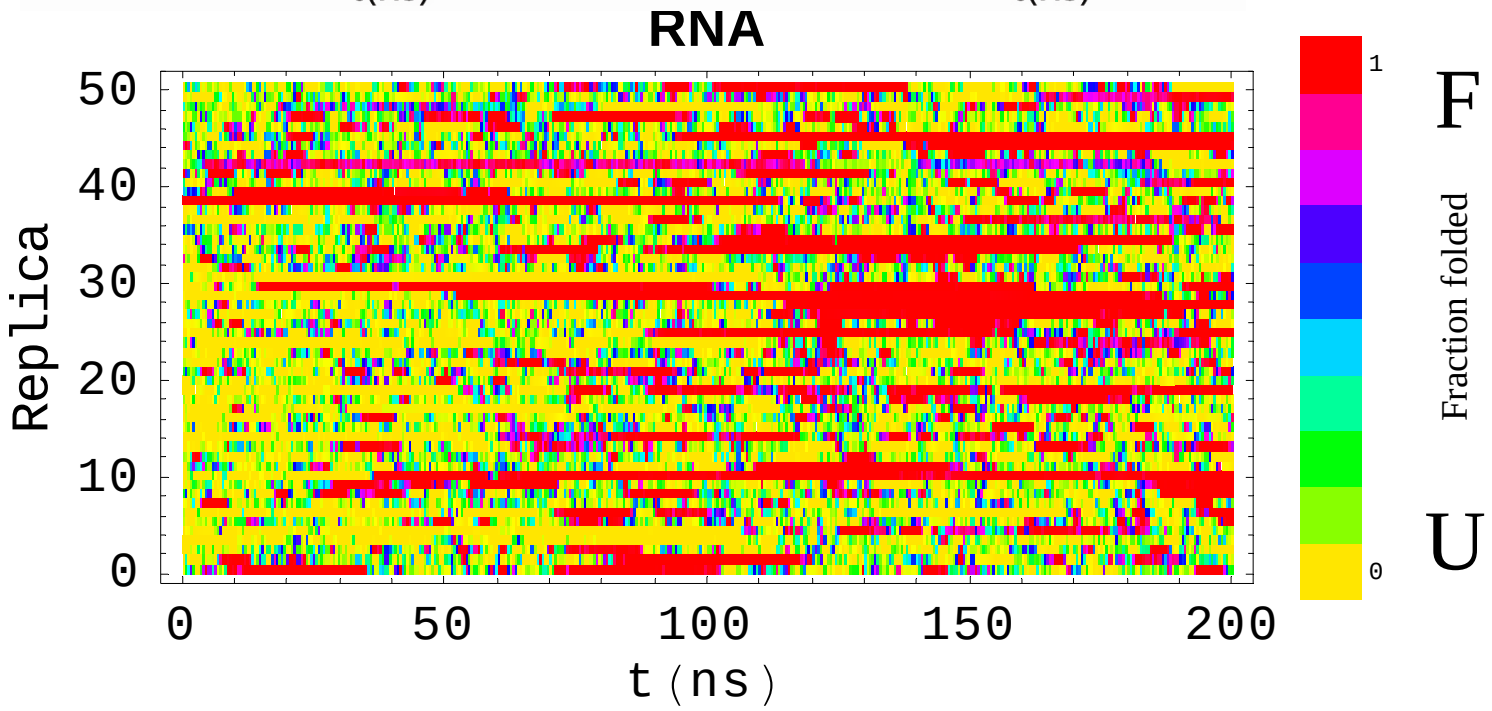
Parm99 force Field (Amber)
Starting from single helix, unstacked

Folds within 2.2 A rmsd
(2 stem base pairs + loop backbone)

Reaching Thermodynamic Equilibrium in Replica Calculations



(rmsd <.4 nm)

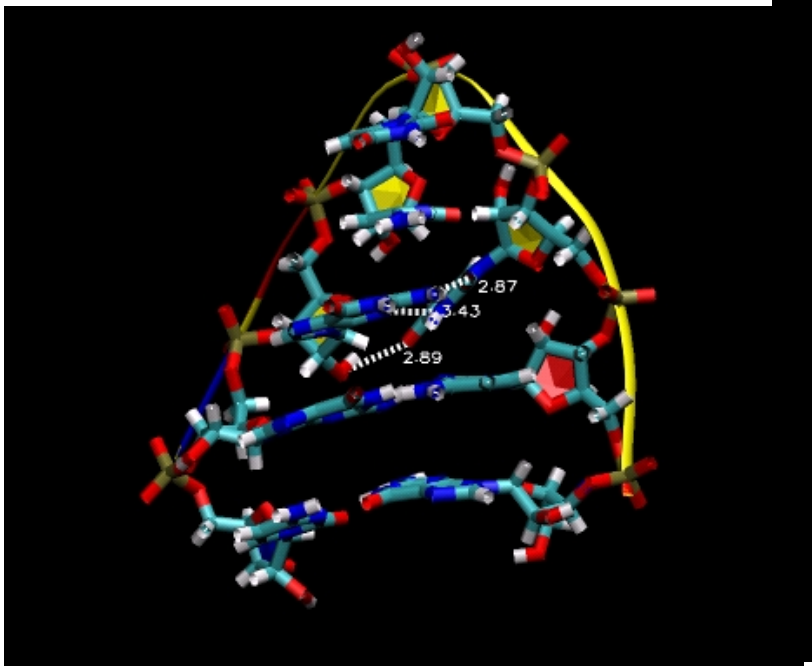


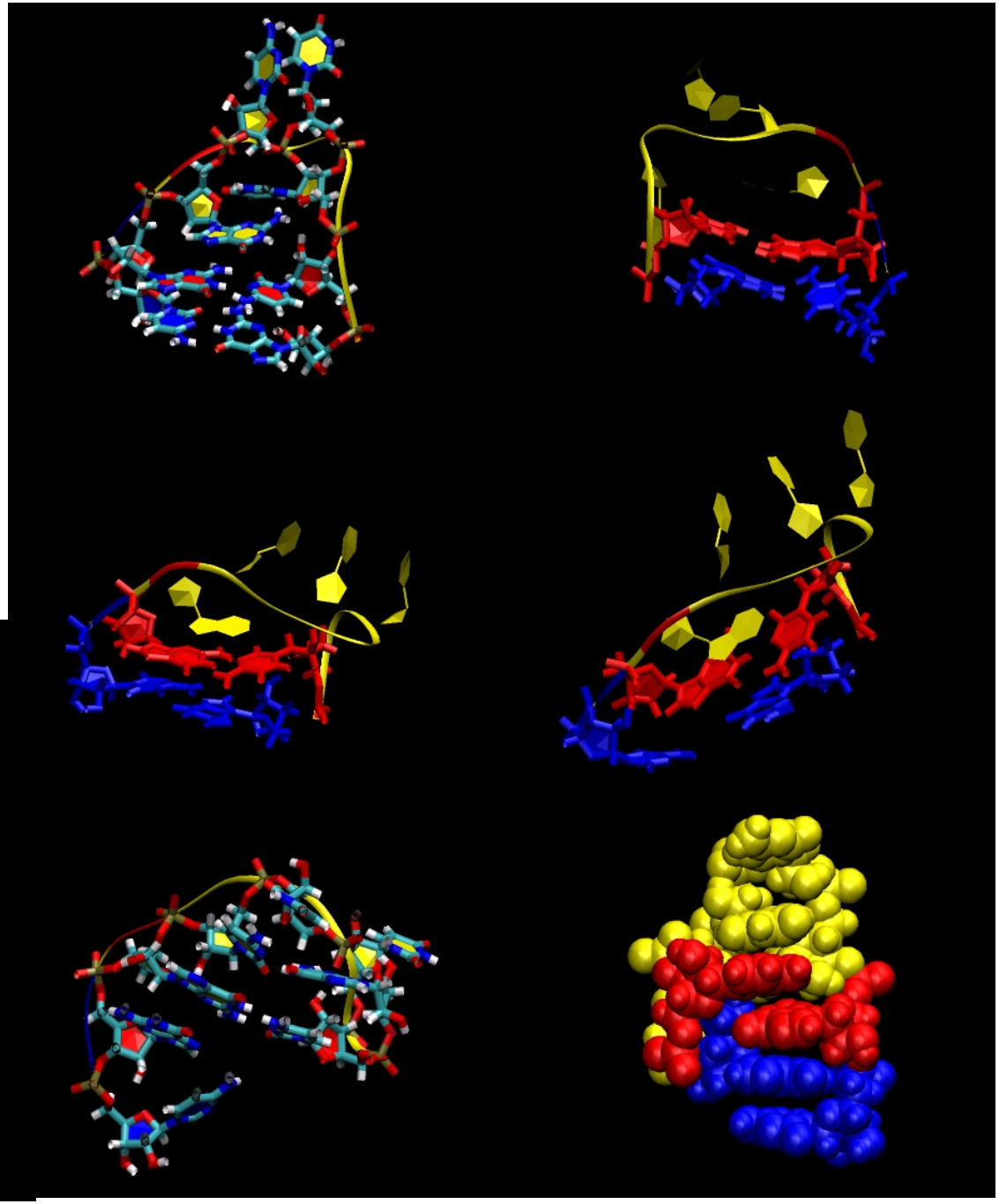
r(gcUUCGgc)

Clustering of structures

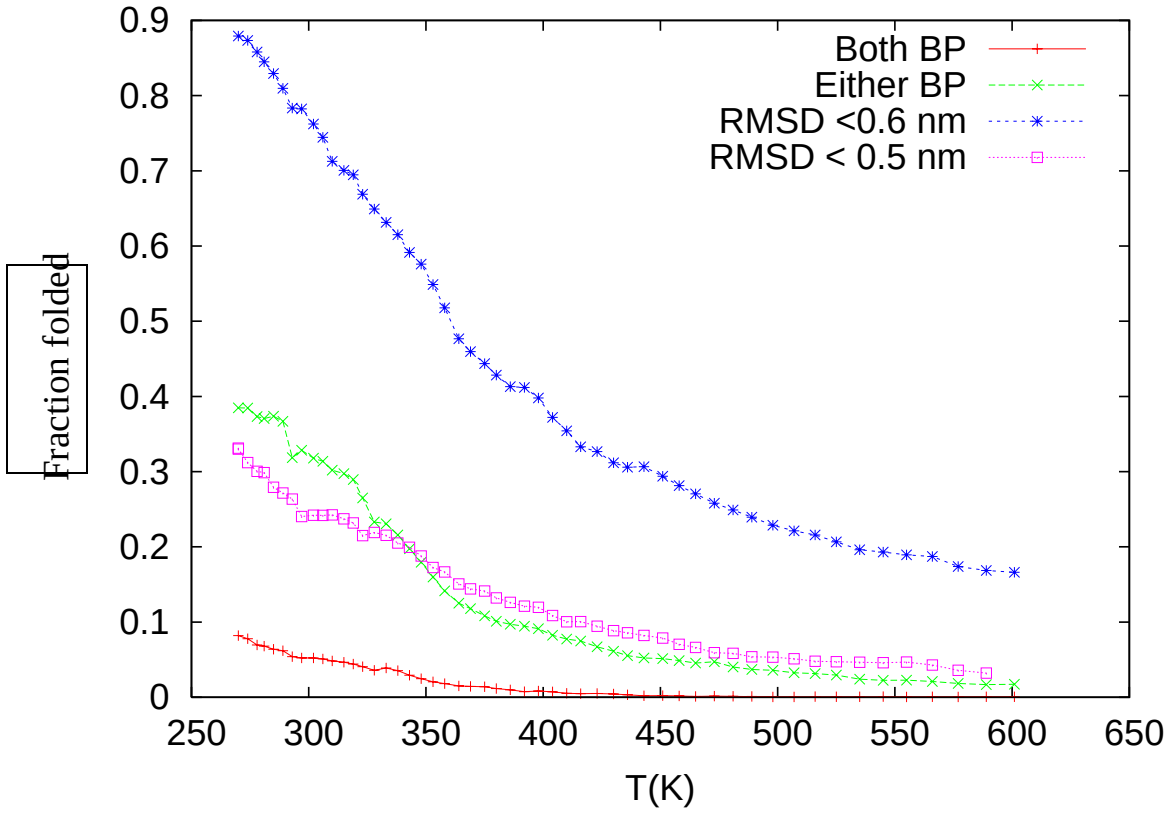
(with rmsd < 6 A)

T=270 K

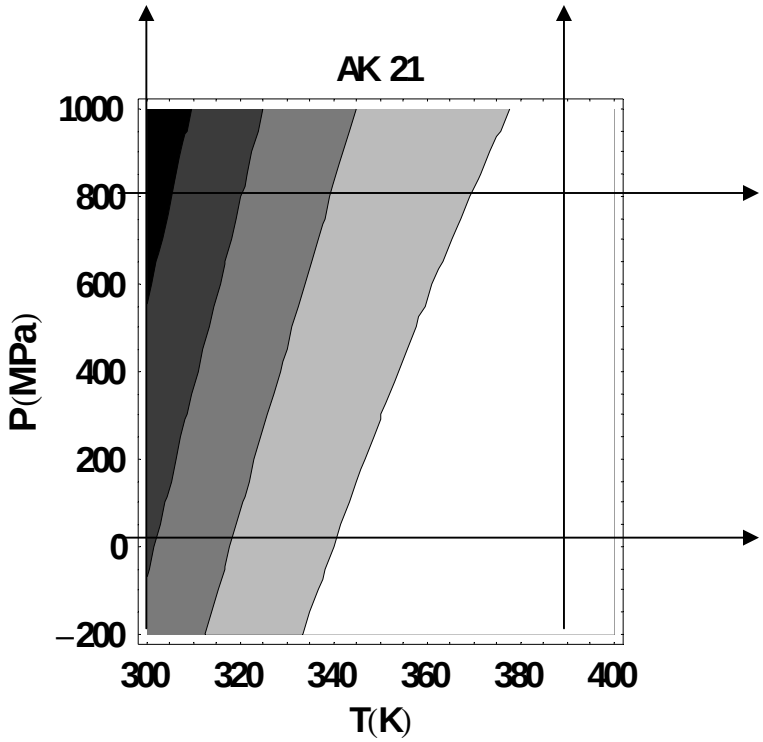




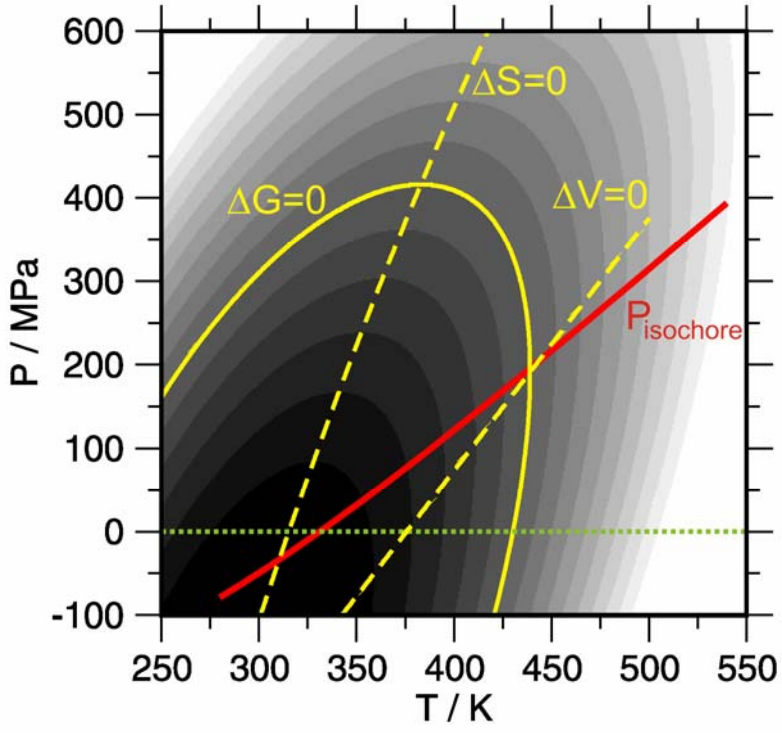
R[gcUUCGgc] Thermodynamics



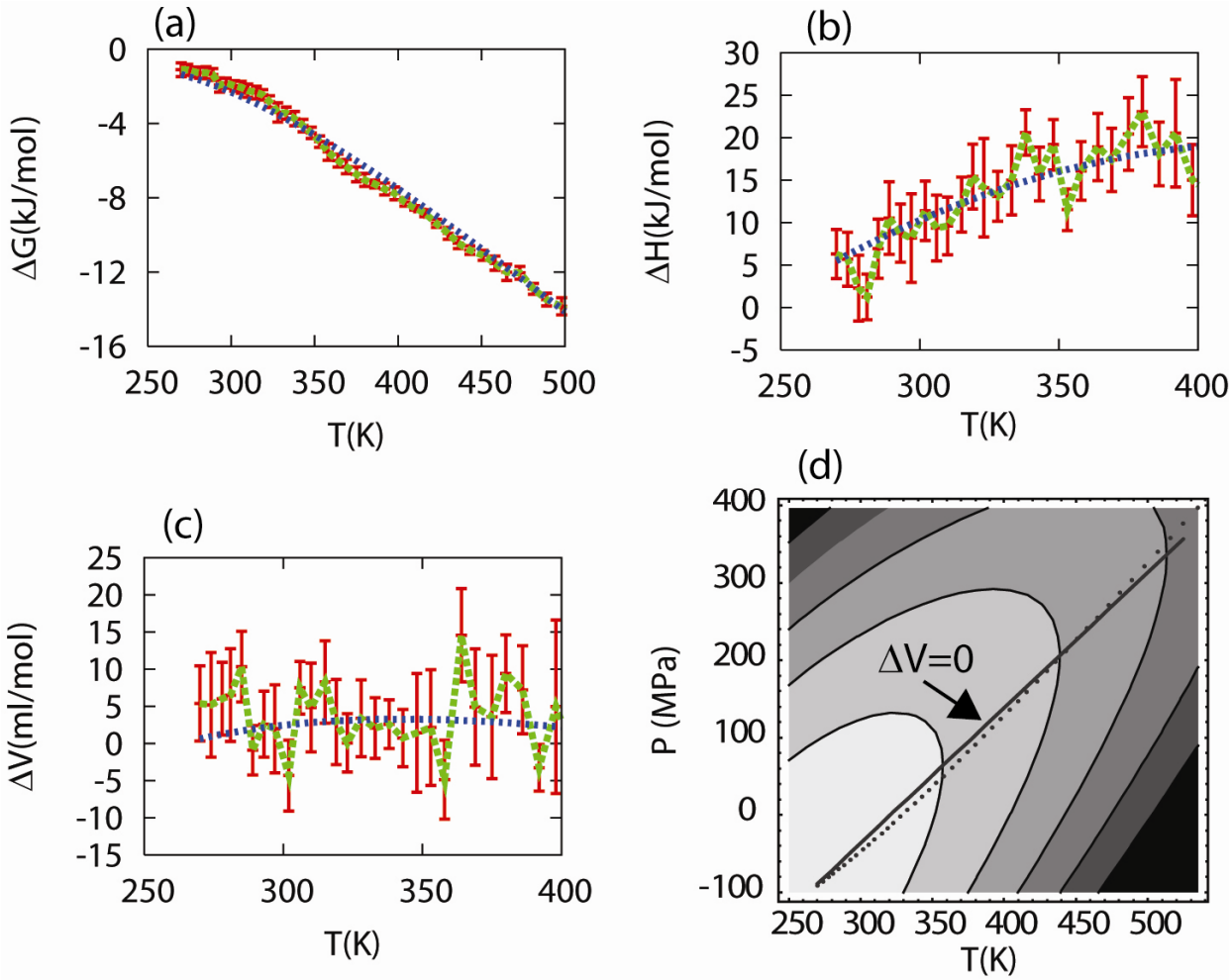
P-T diagram


AK 21
P(MPa)
1000
800
600
400
200
0
−200
300
320
340
360
380
400
T(K)

?


ΔS=0
ΔG=0
ΔV=0
P isochore
P / MPa
600
500
400
300
200
100
0
-100
250
300
350
400
450
500
550
T / K

P-T stability diagram for an RNA Tetraloop



Experiments?

Conclusions

I. We can study the folding/unfolding equilibrium, without any bias, using molecular simulations:

1. **Folding thermodynamics of the trp cage:**
   P-T Diagram predicted
   - Cold denaturation predicted
   - Pressure denaturation predicted

2. **RNA oligomer tetraloop**
   - Pressure induced unfolding predicted

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