



*The Abdus Salam*  
**International Centre for Theoretical Physics**



**SMR/1845-2**

**Conference on Structure and Dynamics in Soft Matter and  
Biomolecules: From Single Molecules to Ensembles**

***4 - 8 June 2007***

**Cooperativity, Non-cooperativity and Barriers in Protein Folding**

Hue Sun CHAN

*University of Toronto  
Faculty of Medicine  
Department of Biochemistry  
1 King's College Circle  
Toronto M5S 1A8*

# **Cooperativity, Non-cooperativity and Barriers in Protein Folding**

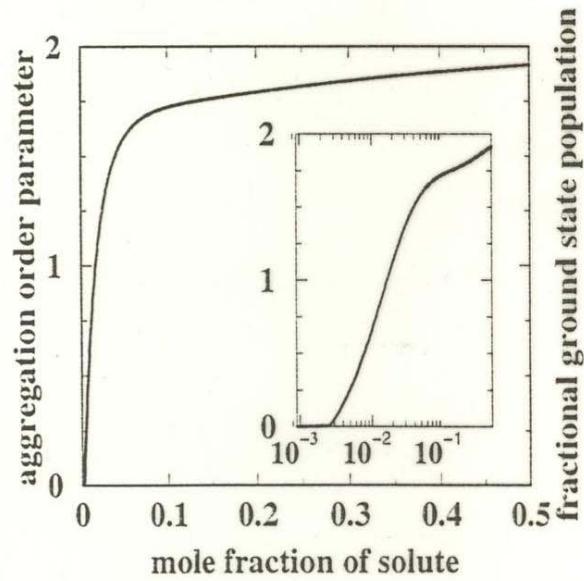
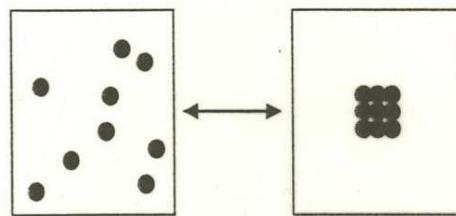
**Hue Sun CHAN**

**Departments of Biochemistry, and of Medical Genetics & Microbiology  
Faculty of Medicine, University of Toronto, Ontario M5S 1A8 Canada**

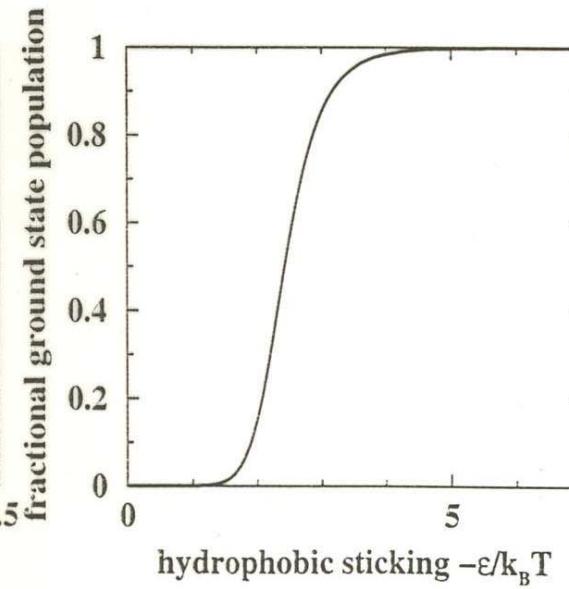
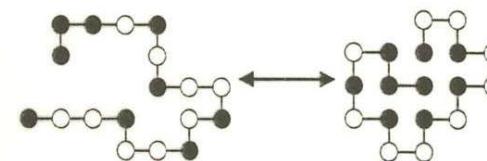
<http://biochemistry.utoronto.ca/chan/bch.html>

# "Cooperativity" as Sharpness of Configurational or Conformational Transitions

Solvophobic aggregation

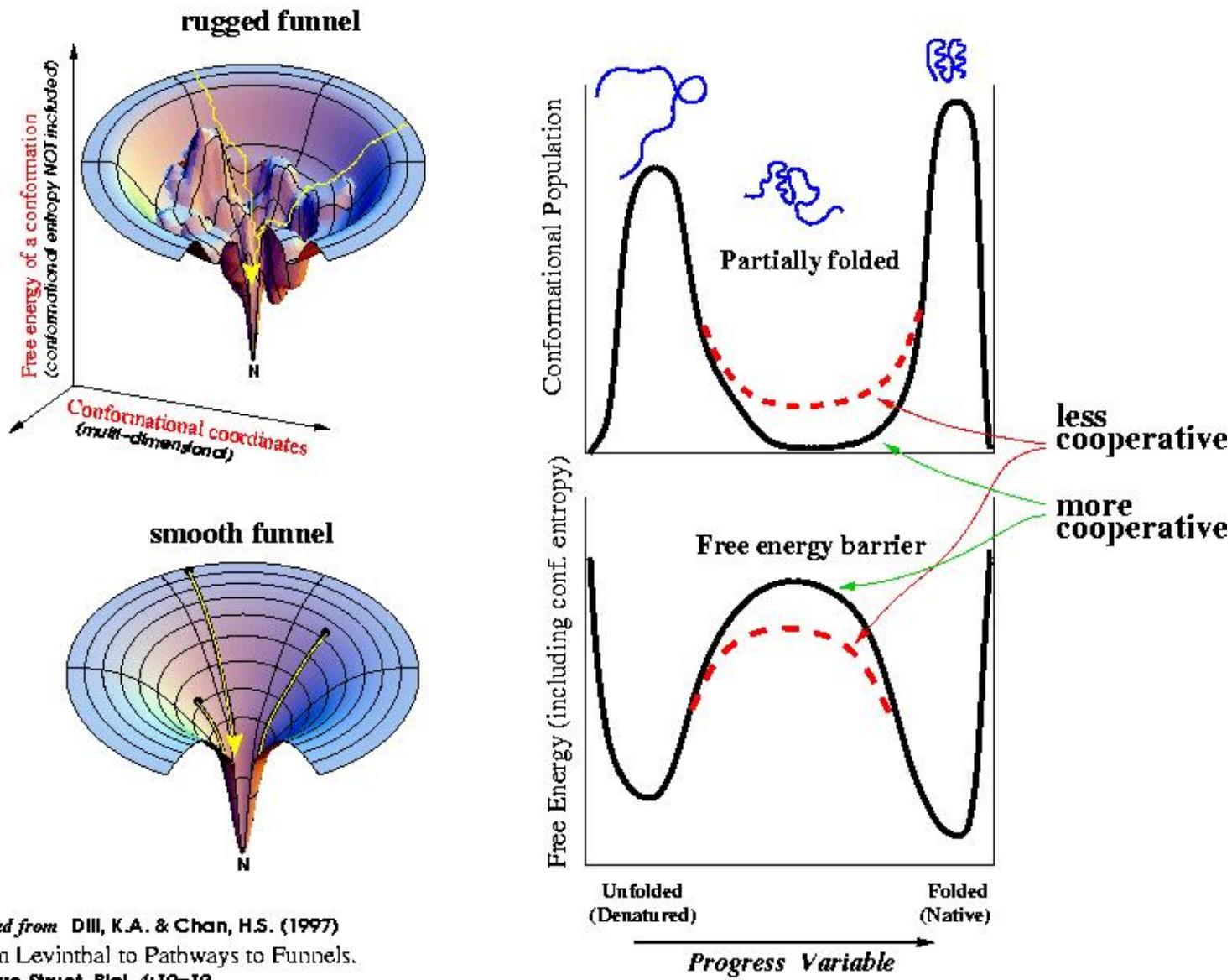


Coil-globule and folding transition



Chan, Shimizu, Kaya, *Methods Enzymol* (2004)

## More cooperative folding means fewer kinetic traps and less chance of misfolding



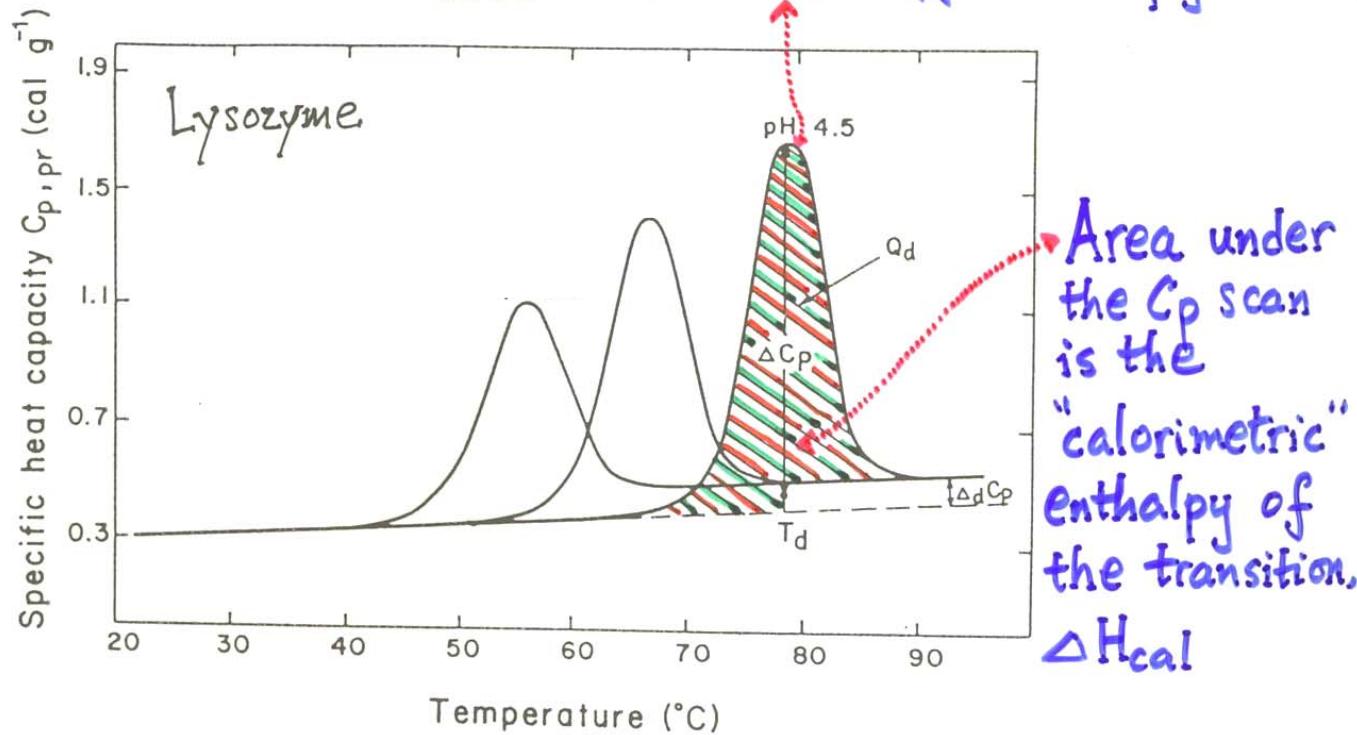
Adapted from: Dill, K.A. & Chan, H.S. (1997)  
From Levinthal to Pathways to Funnels.  
*Nature Struct. Biol.* 4:10–19

# What do we know from experiments?

## The calorimetric criterion for "two-state" transitions

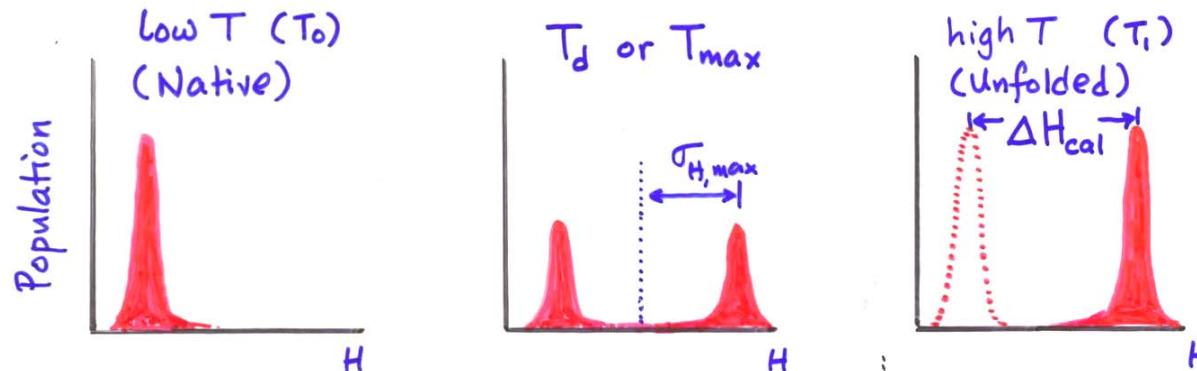
$$\boxed{\frac{\Delta H_{vH}}{\Delta H_{cal}} \approx 1}$$

Peak  $C_p$  value is used to determine "vant Hoff" enthalpy,  $\Delta H_{vH}$



Privalov, Adv. Protein Chem. 33: 167 (1979)

# How does the calorimetric criterion work?



bimodal cooperative folding

$$\sigma_H = (\langle H^2 \rangle - \langle H \rangle^2)^{1/2} = \sqrt{k_B T^2 C_p}$$

$$\Delta H_{vH} = 2\sqrt{k_B T_{max}^2 C_{p,max}} = 2\sigma_{H,max}$$

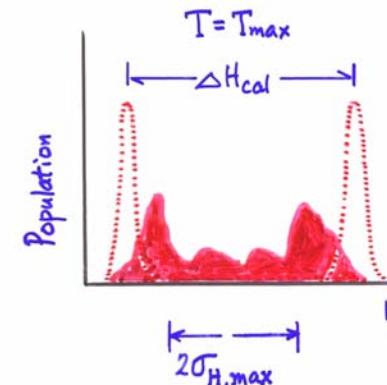
$\frac{\Delta H_{vH}}{\Delta H_{cal}} \approx 1 \Rightarrow$  Negligible intermediate-H population at  $T_{max}$   
 $\Rightarrow$  Two State Transition

$$C_p = \left( \frac{\partial H}{\partial T} \right)_p$$

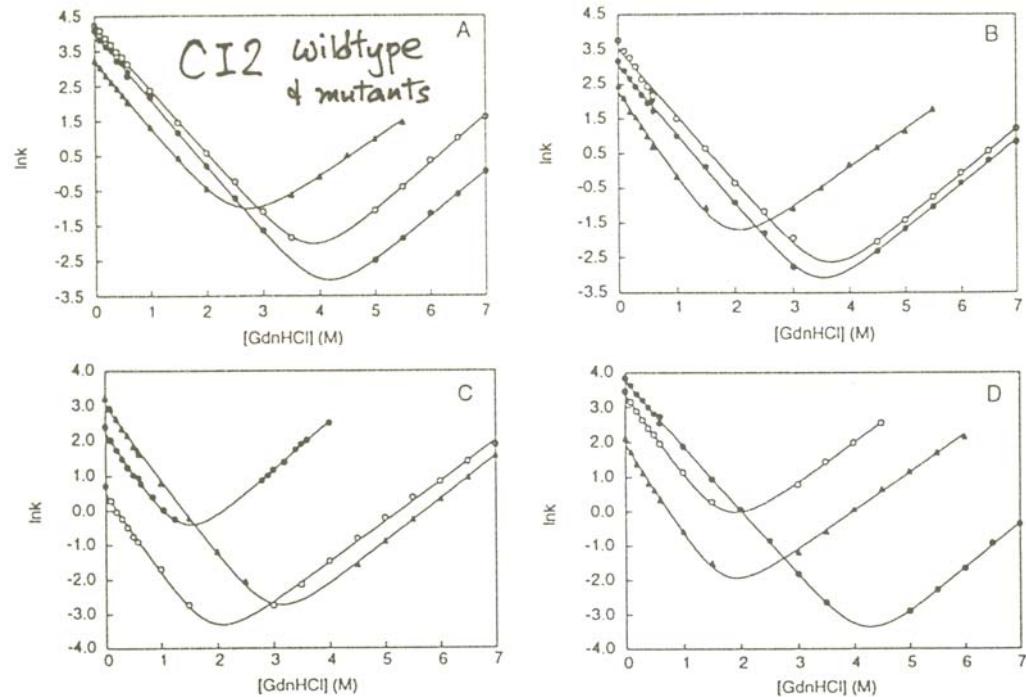
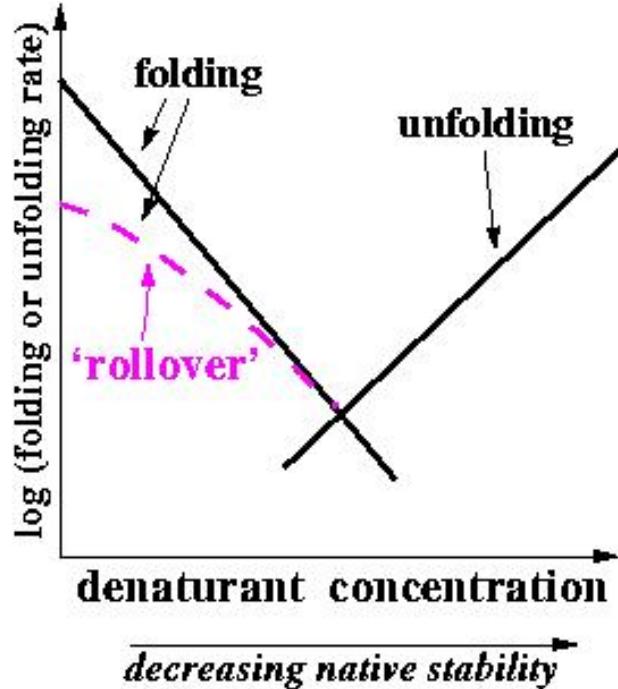
$$\Delta H_{cal} = \int_{T_0}^{T_1} dT C_p = H(T_1) - H(T_0)$$

A scenario in which  $\Delta H_{vH} < \Delta H_{cal}$   
(Not Two State)

unimodal noncooperative folding



## Kinetic manifestation of folding cooperativity: linear chevron plots



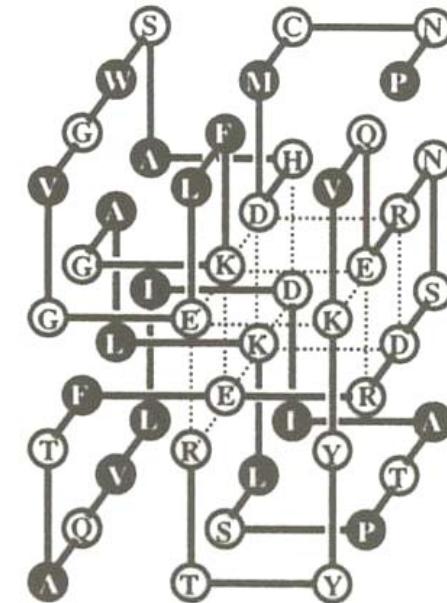
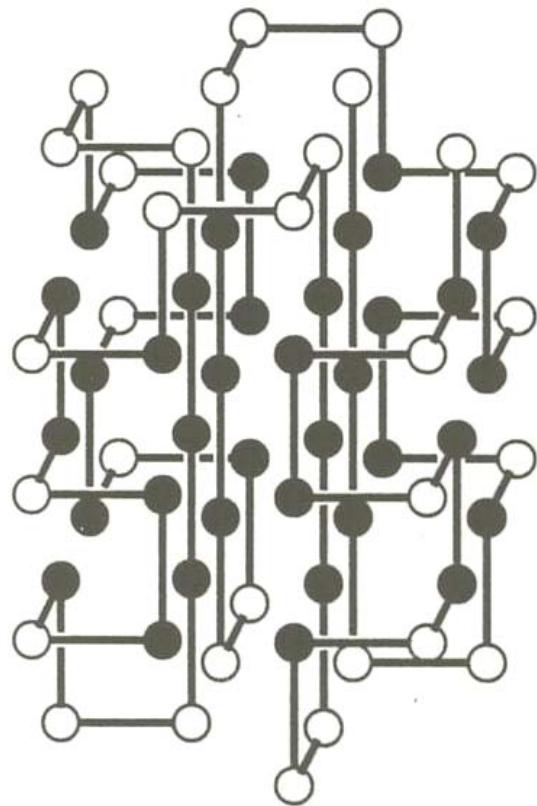
Data from Jackson et al., *Biochemistry* 32:11270 (1993)

Linear folding and unfolding arms imply a linear relationship between  $\log(\text{folding/unfolding rate})$  and equilibrium stability

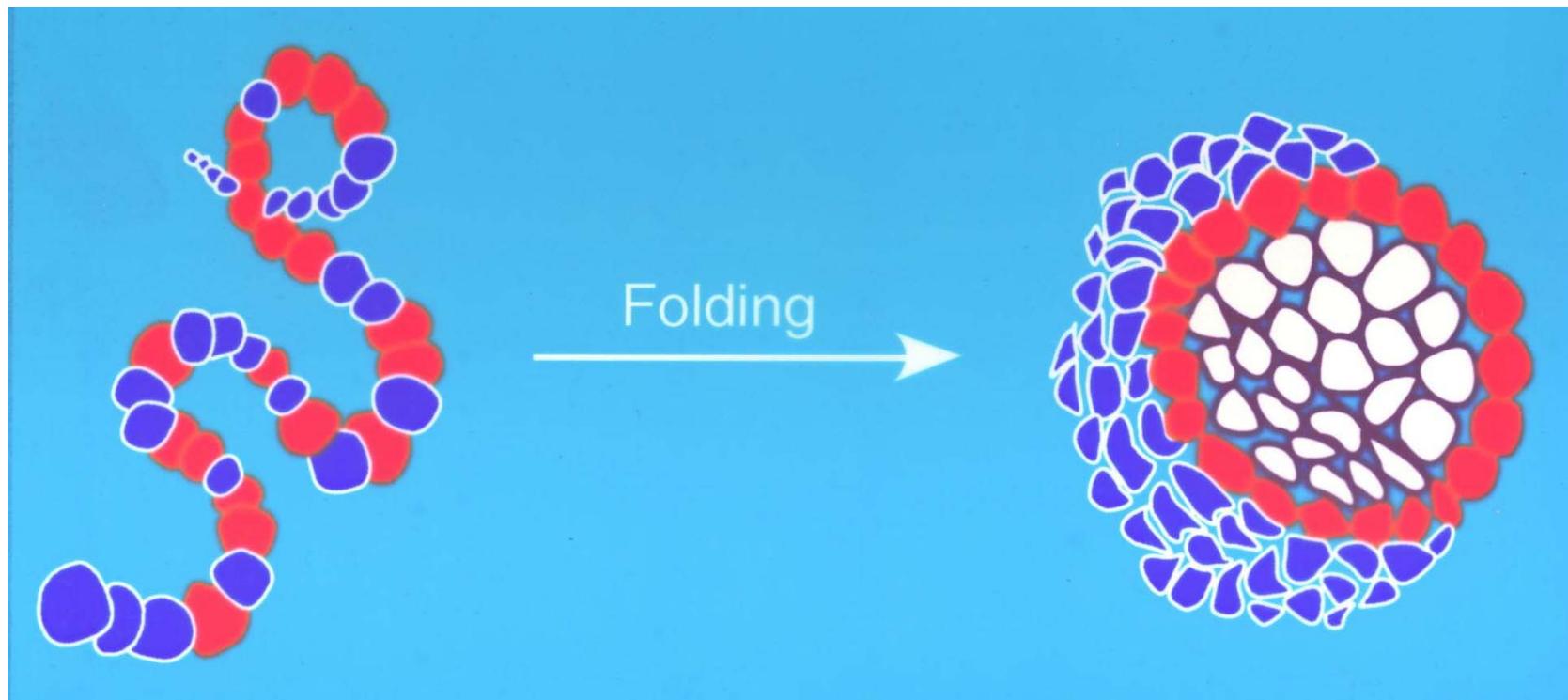
*chevron rollover is indicative of less cooperative or noncooperative folding*

*Do we understand the  
Physics of  
Generic Protein Properties?*

# Simple Heteropolymer Protein Models



# Models encapsulate our ideas about how driving forces in protein folding work

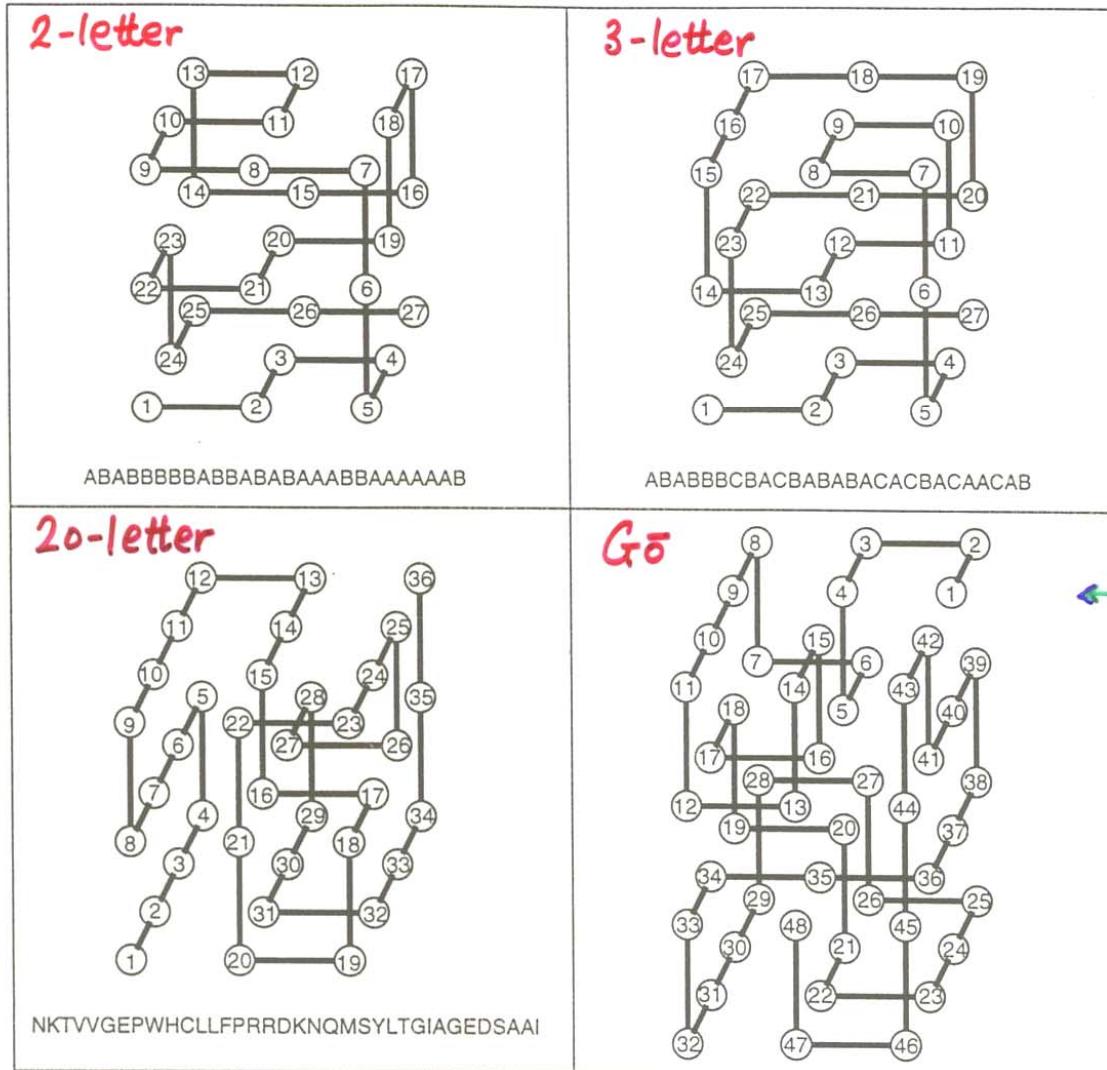


e.g., hydrophobic interactions

Chan & Dill, *Physics Today* (1993)

Generic protein properties are stringent constraints on modeling, providing important clues to the energetics underlying real protein behavior

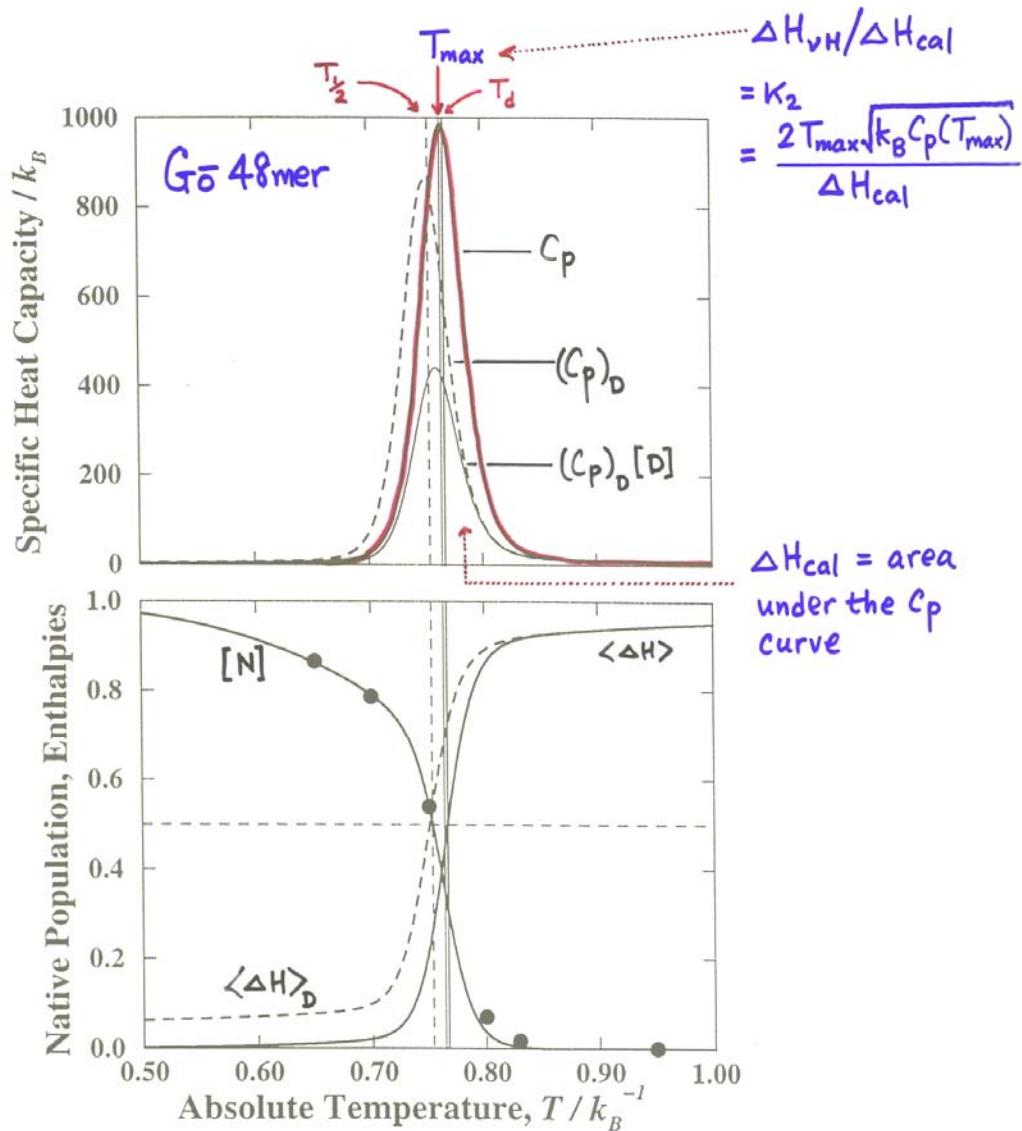
# Evaluating lattice protein models against the $\Delta H_{vH}/\Delta H_{cal} \approx 1$ calorimetric 2-state criterion



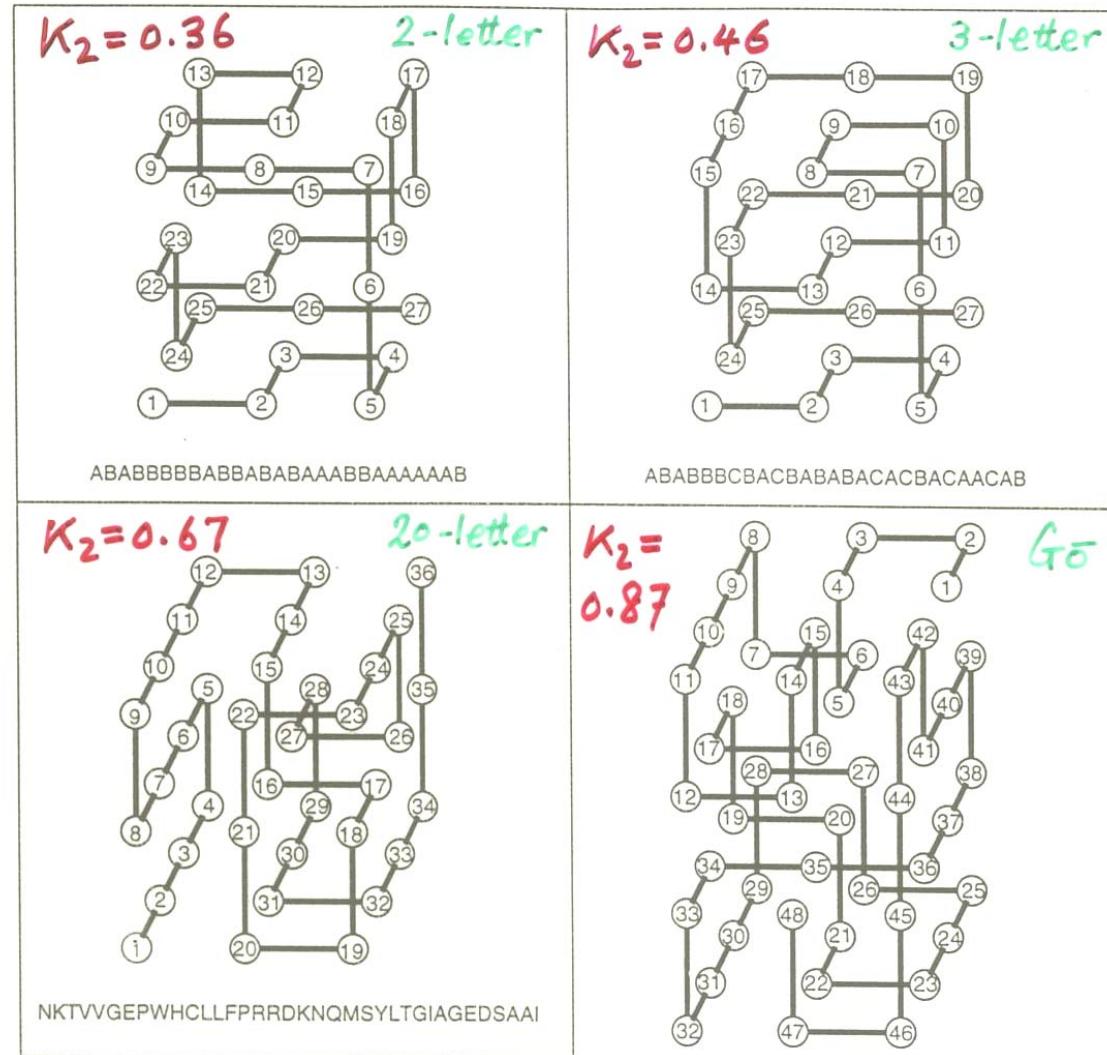
Kaya & Chan, *Proteins* (2000)

We performed computer experiments on these models to test whether they satisfy the calorimetric criterion of two-state-like folding cooperativity

$\Delta H_{vH}/\Delta H_{cal}$  determined by Monte Carlo histogram method



... and found  
that none of the  
models tested  
meets the  
calorimetric  
two-state  
standard,  
not even the  
native-centric  
*Gō model*

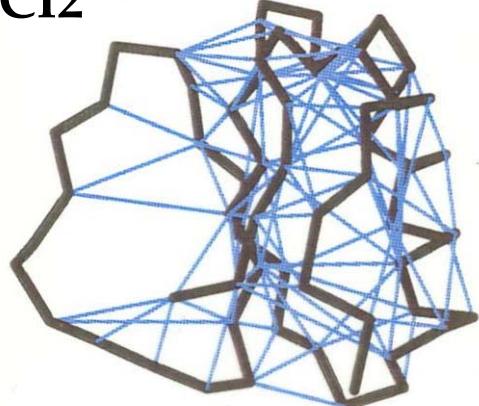


Kaya & Chan, *Proteins* (2000); *Phys Rev Lett* (2000)

# *Continuum C<sub>α</sub> Gσ Models*

$$\begin{aligned} V_{\text{total}} &= V_{\text{stretching}} + V_{\text{bending}} + V_{\text{torsion}} + V_{\text{non-bonded}} \\ &= \sum_{\text{bonds}}^{N-1} K_r(r - r_0)^2 + \sum_{\text{angles}}^{N-2} K_\theta(\theta - \theta_0)^2 \\ &\quad + \sum_{\text{dihedrals}}^{N-3} \{K_\phi^{(1)}[1 - \cos(\phi - \phi_0)] + K_\phi^{(3)}[1 - \cos 3(\phi - \phi_0)]\} \\ &\quad + \sum_{i < j-3}^{\text{native}} \epsilon \left[ 5 \left( \frac{r'_{ij}}{r_{ij}} \right)^{12} - 6 \left( \frac{r'_{ij}}{r_{ij}} \right)^{10} \right] + \sum_{i < j-3}^{\text{non-native}} \epsilon \left( \frac{r_{\text{rep}}}{r_{ij}} \right)^{12} \end{aligned}$$

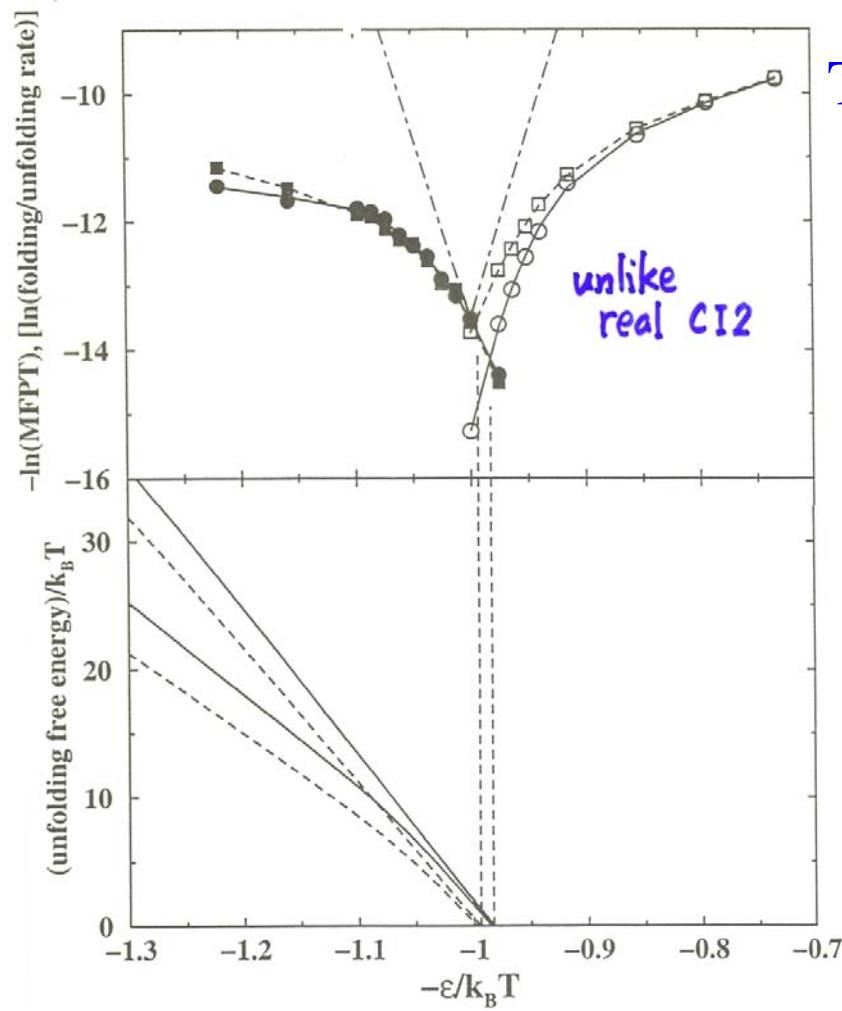
CI2



Clementi et al., *JMB* (2000); Koga & Takada, *JMB* (2001);  
Kaya & Chan, *JMB* (2003)

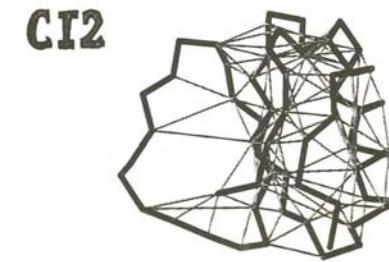
**Native-centric local and non-bonded interactions**  
**Langevin dynamics**  
**Thermodynamically quite cooperative**

*Go-model chevron plots have significant chevron rollovers, implying that in many cases Gō models are less cooperative than the real proteins they aim to mimick*

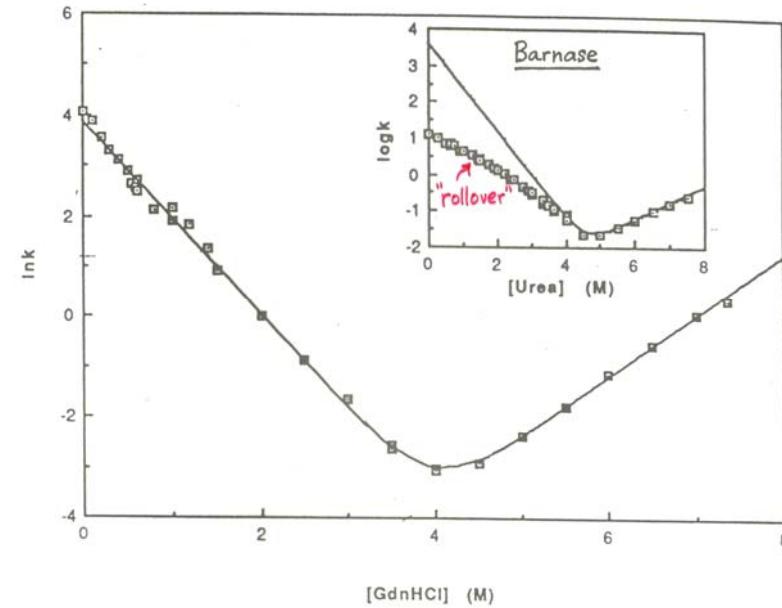


Kaya & Chan, *JMB* (2003)

Theory

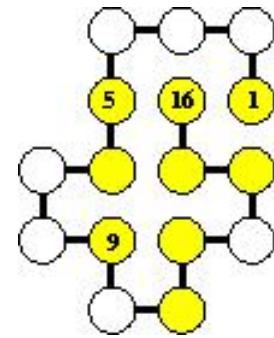


Experiment

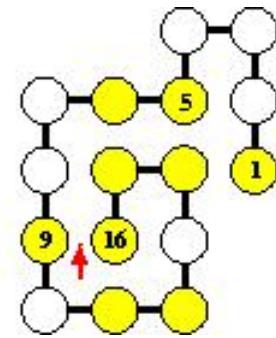


Jackson & Fersht, *Biochemistry* (1991)

# Chevron rollovers: kinetic traps lead to slowdown



Native,  
7HH  
contacts

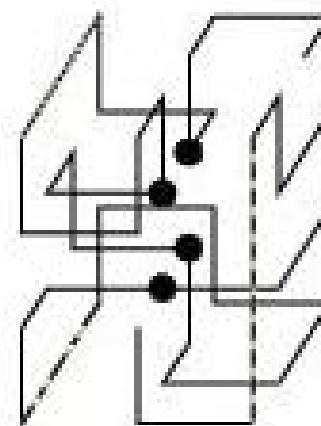


Misfolded  
*with*  
*nonnative*  
*contacts*,  
5HH contacts

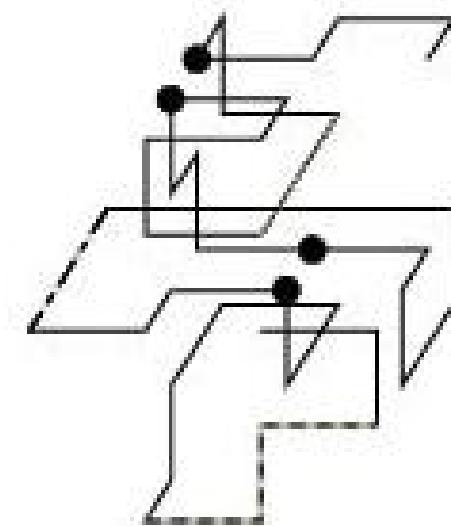
*HP Model*

- mild kinetic traps can exist in  $G\bar{o}$  models

*$G\bar{o}$  Model*



Native



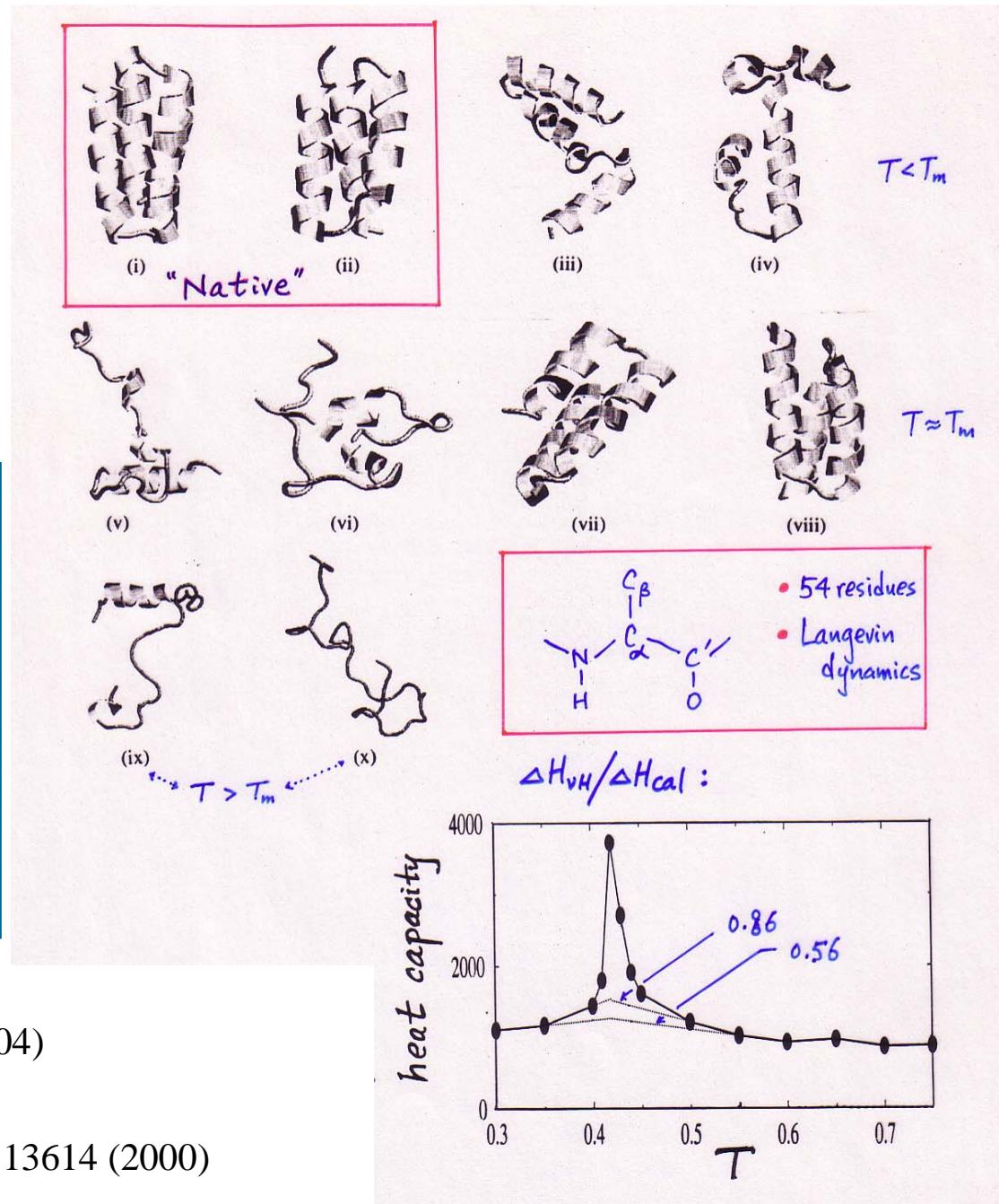
native contacts,  
nonnative topology

Kaya & Chan, *Phys Rev Lett* (2003)

# *A simplified atomic model of 3-helix protein*

## Lesson:

Folding cooperativity is  
*not* a corollary of a  
sequence's ability to fold  
to an essentially unique  
structure

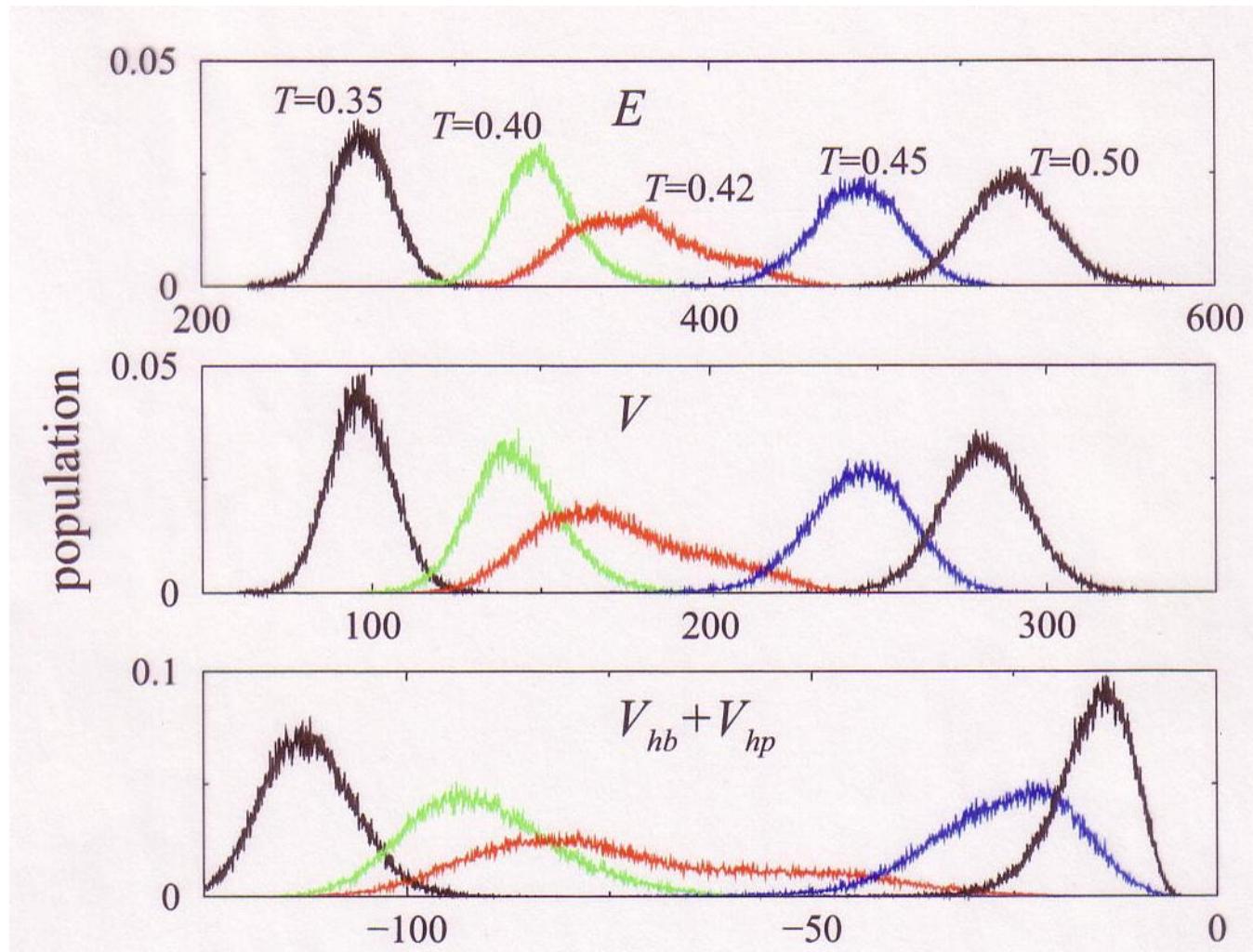


Knott & Chan, *Chem Phys* **307**:187 (2004)

Model adapted from:

Irback, Sjunnesson & Wallin, *PNAS* **97**:13614 (2000)

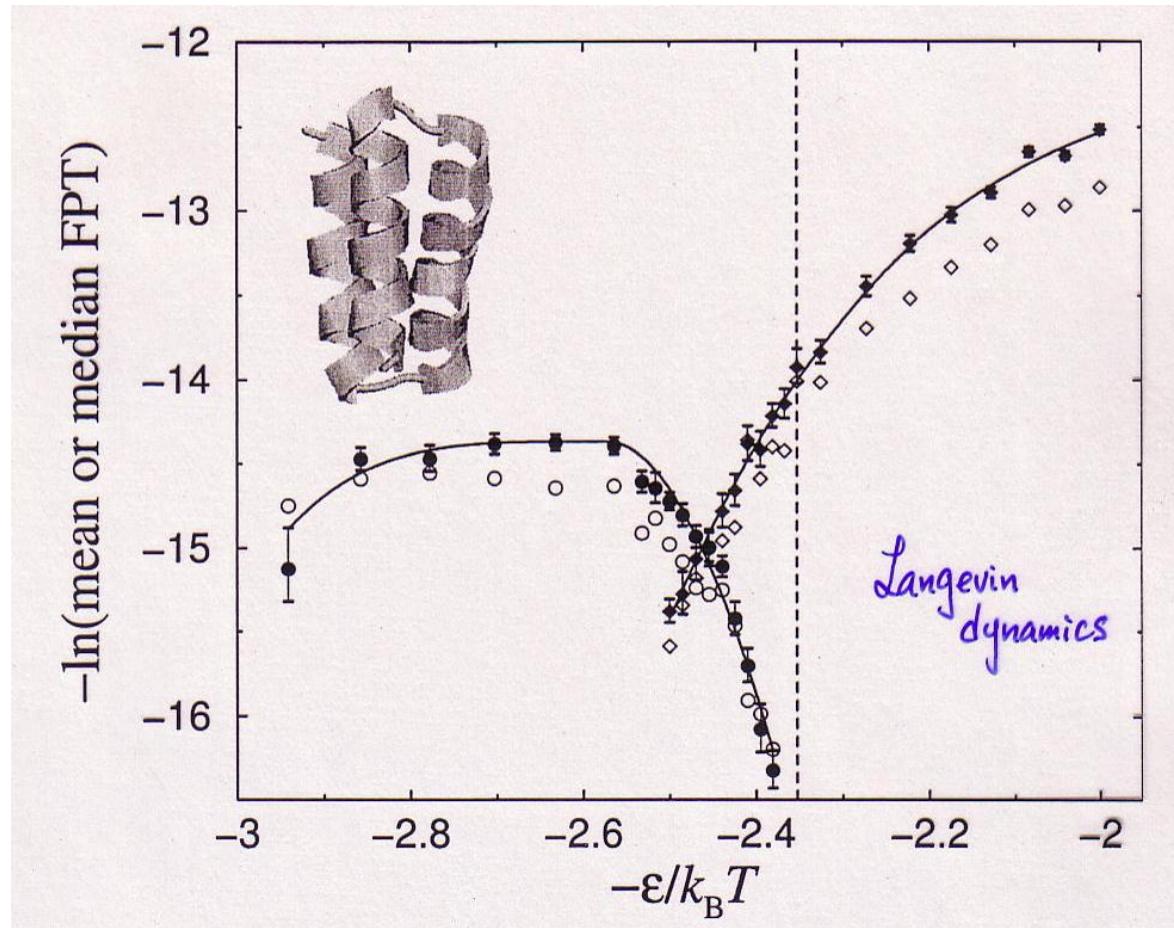
Energetic basis for noncooperative folding:  
**A unimodal energy (enthalpy) distribution**



Knott & Chan, *Chem Phys* (2004)

Folding cooperativity is *not* a corollary of a sequence's ability to fold to an essentially unique structure:

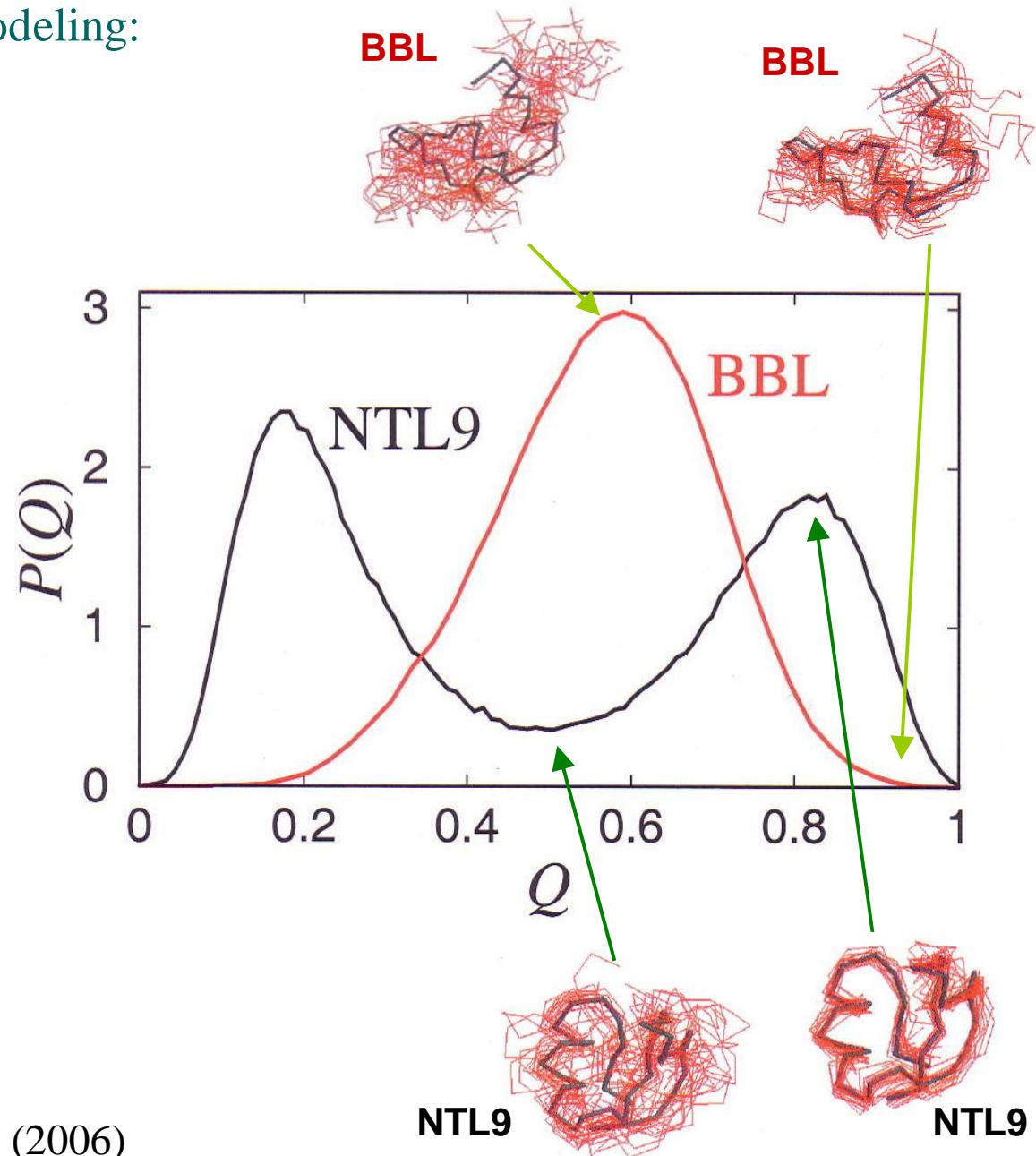
## Significant chevron rollover in a simplified atomic model with a general potential



Knott & Chan, *Proteins* (2006)

Simple native-centric Gō modeling:

# A topological basis for possible unimodal “downhill” folding?

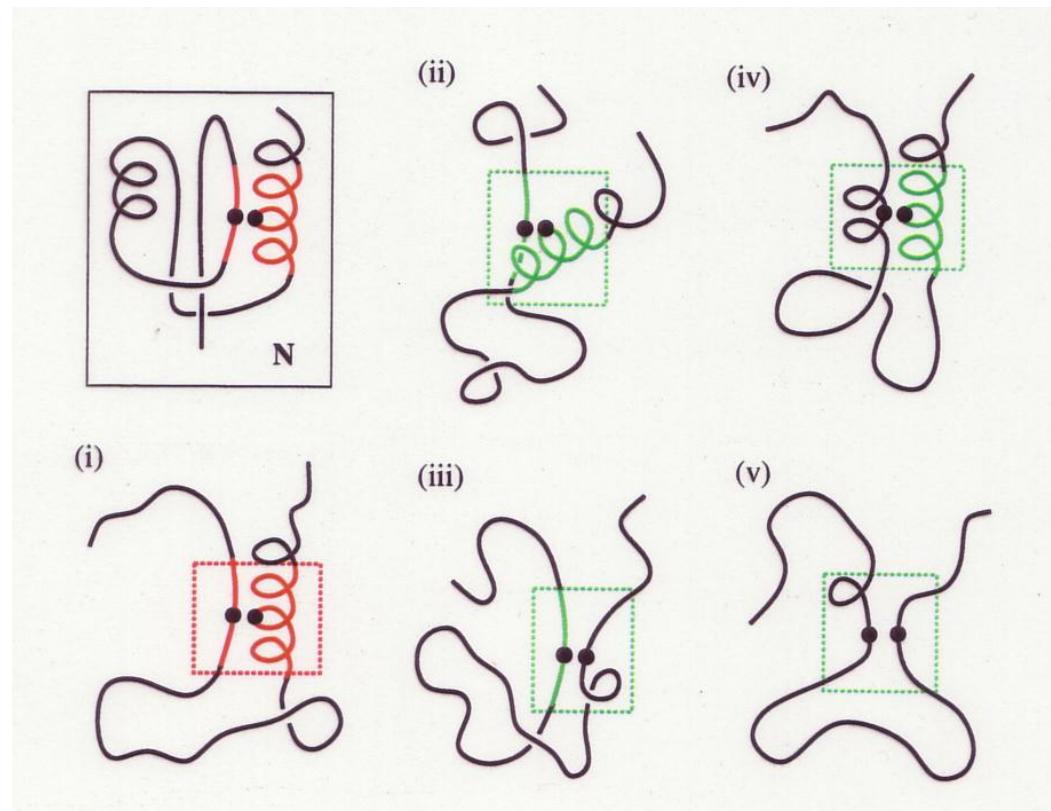


*Even with native biases, pairwise additive interactions do not account for cooperative folding*

Better Mesoscopic Principles?

# ‘Many-body’ interactions needed to account for folding cooperativity

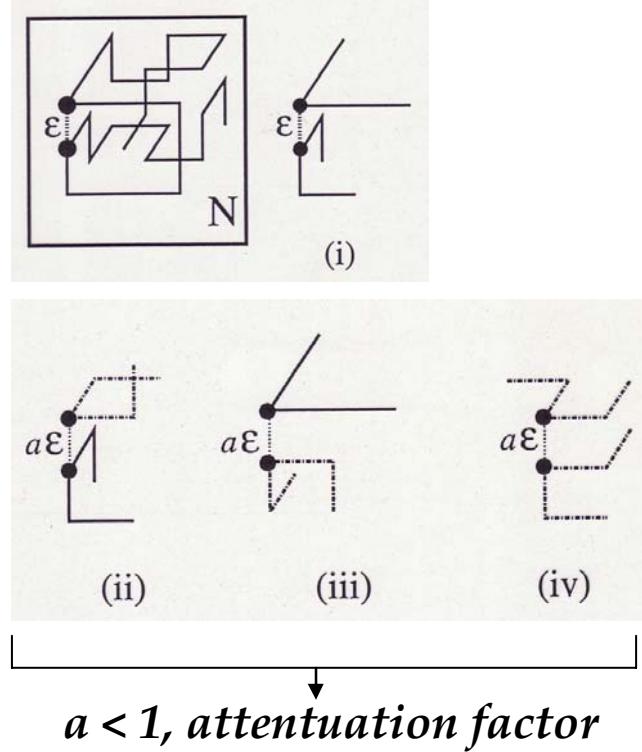
*A hypothesized cooperative interplay between favorable nonlocal interactions and local conformational preferences*



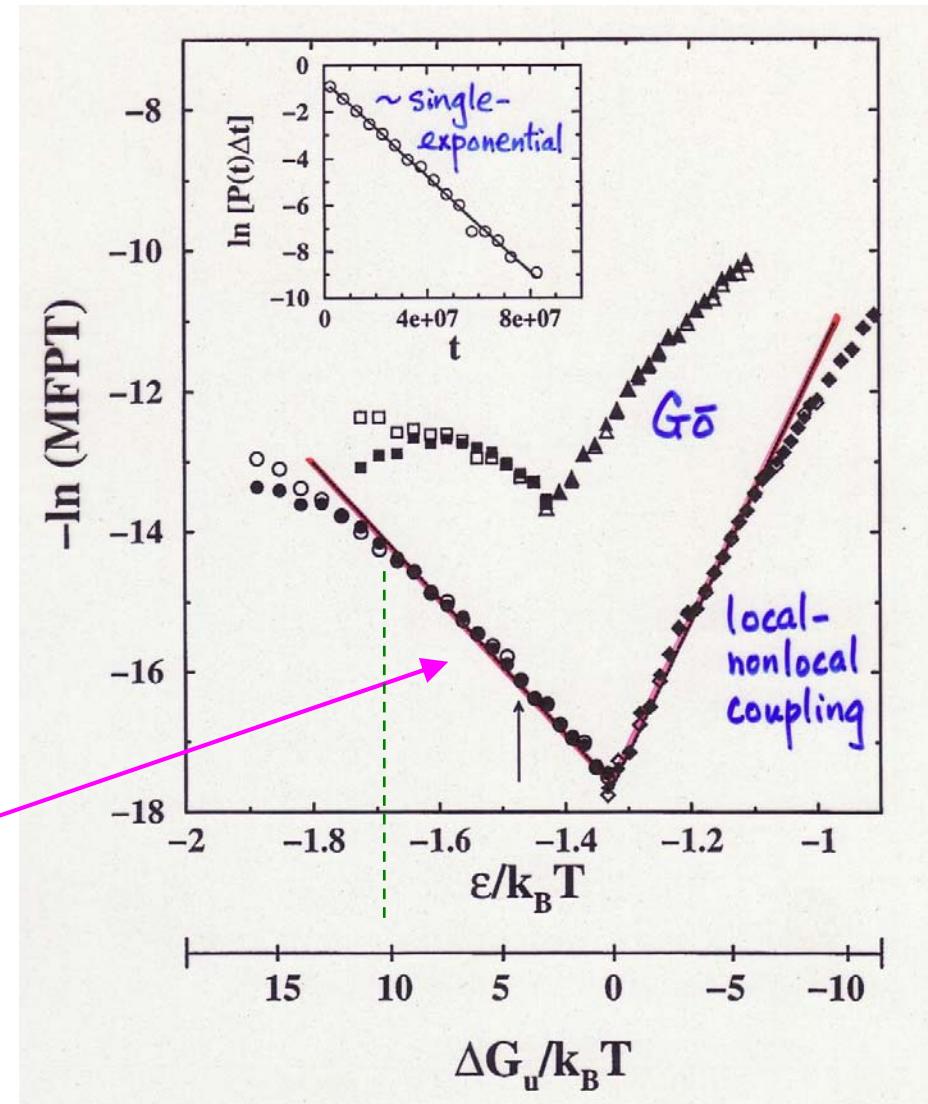
*(local-nonlocal coupling)*

Kaya & Chan, *Proteins* (2003)

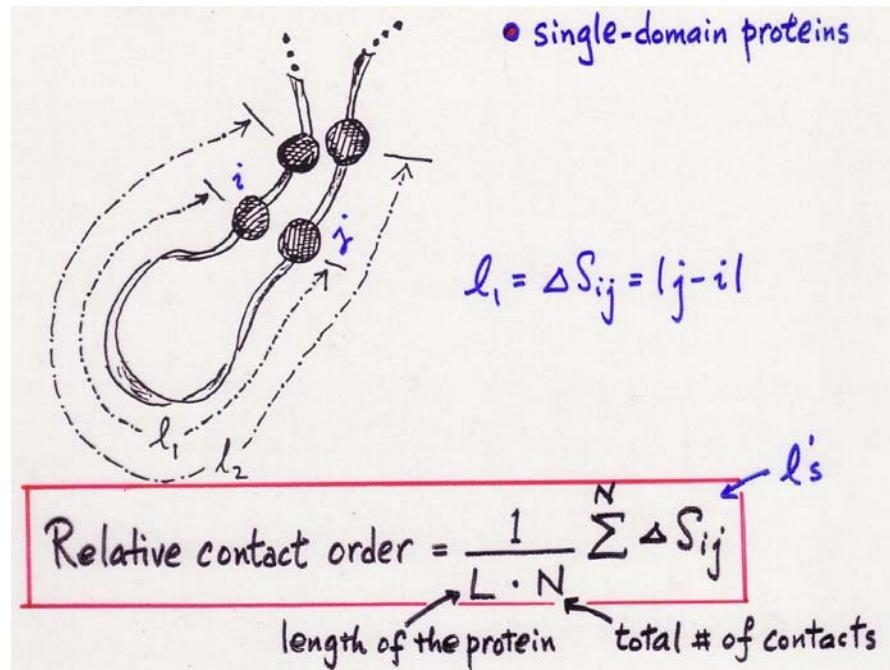
## Testing the local-nonlocal coupling idea by lattice models



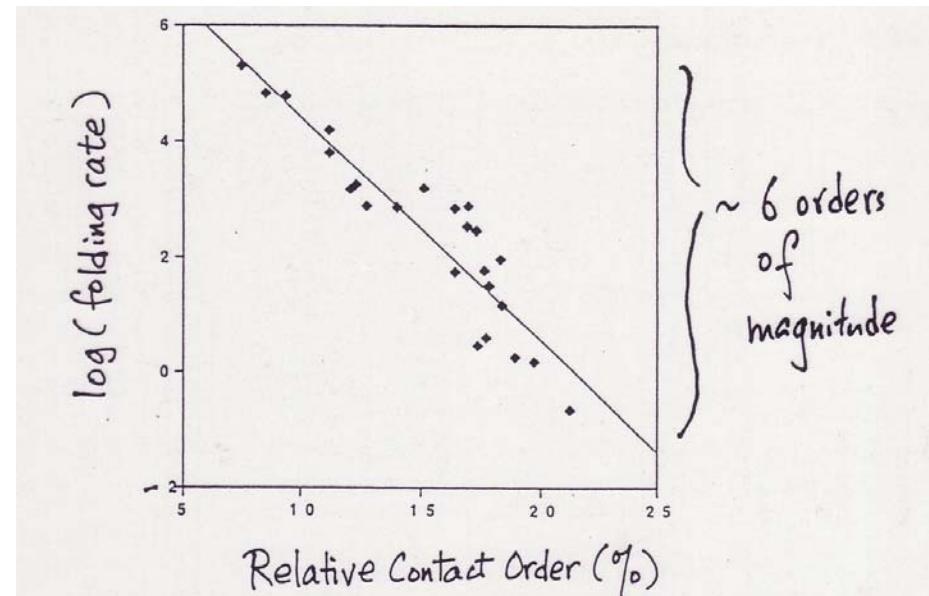
Apparent Two-State Kinetics



## Native Topology (Contact Pattern) Dependent Folding Rates



Plaxco, Simons & Baker, *JMB* (1998)

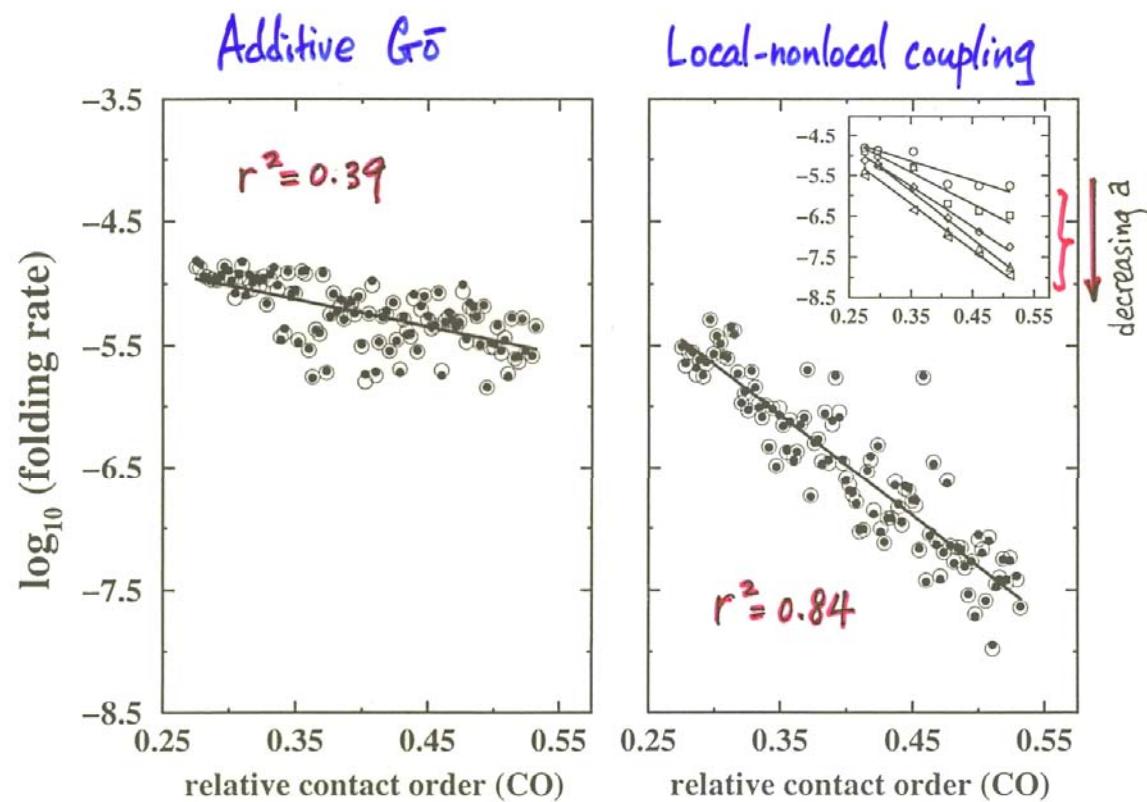
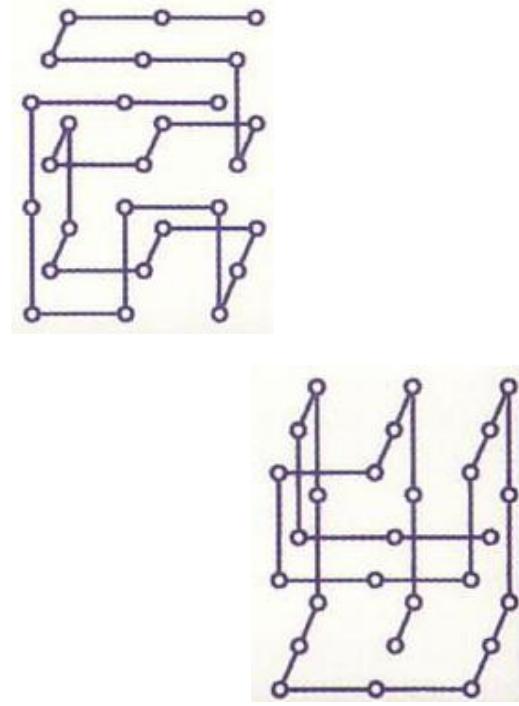


Plot from Plaxco et al. *Biochemistry* (2000)

*Can protein chain models capture this trend?*

# *Many-body interactions in the form of local-nonlocal coupling rationalize topology-dependent folding rates*

Folding rates of 97 lattice 27mers with  
different contact orders



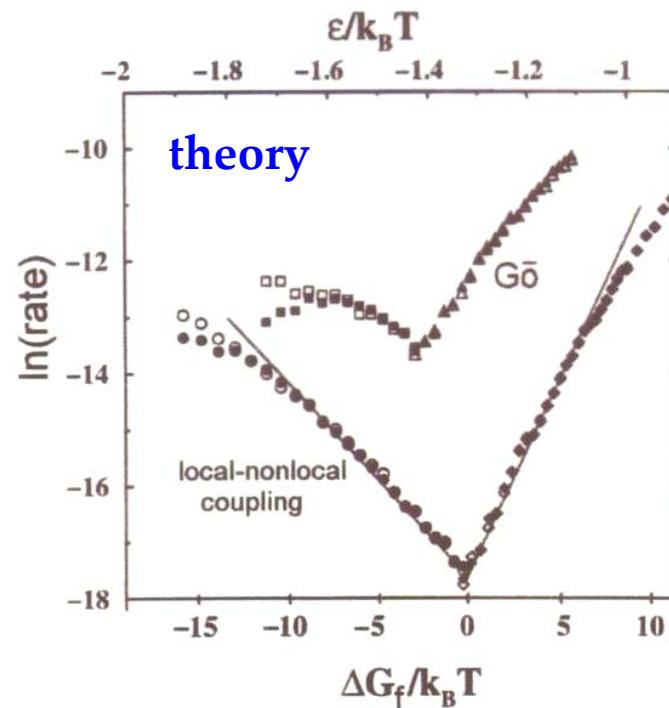
Kaya & Chan, *Proteins* (2003); Chan et al., *Methods Enzymol* (2004)

**Our theoretical perspective was corroborated by experiments:**

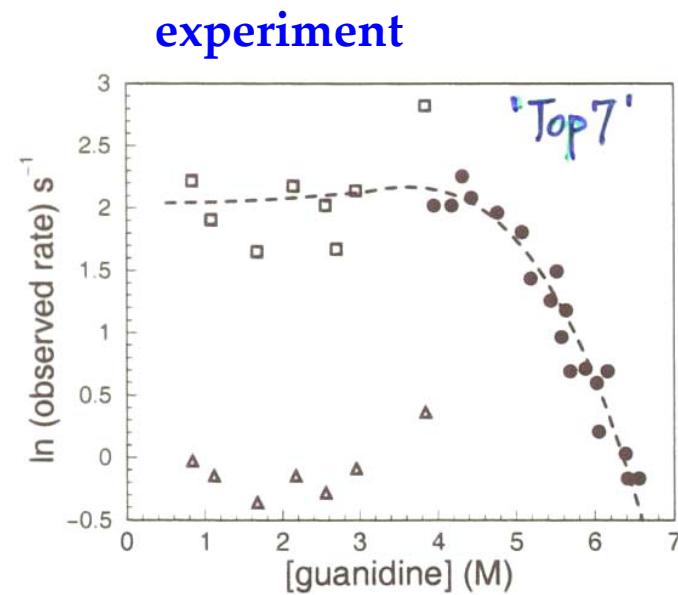
**Cooperativity is likely an evolutionarily selected trait; cooperativity is *not* a corollary of a protein's ability to fold**



**Top7, 1qys (93aa)**  
Kuhlman et al.,  
*Science* (2003)



Kaya & Chan, *Proteins* (2003);  
Dobson, *TIBS* (1999)



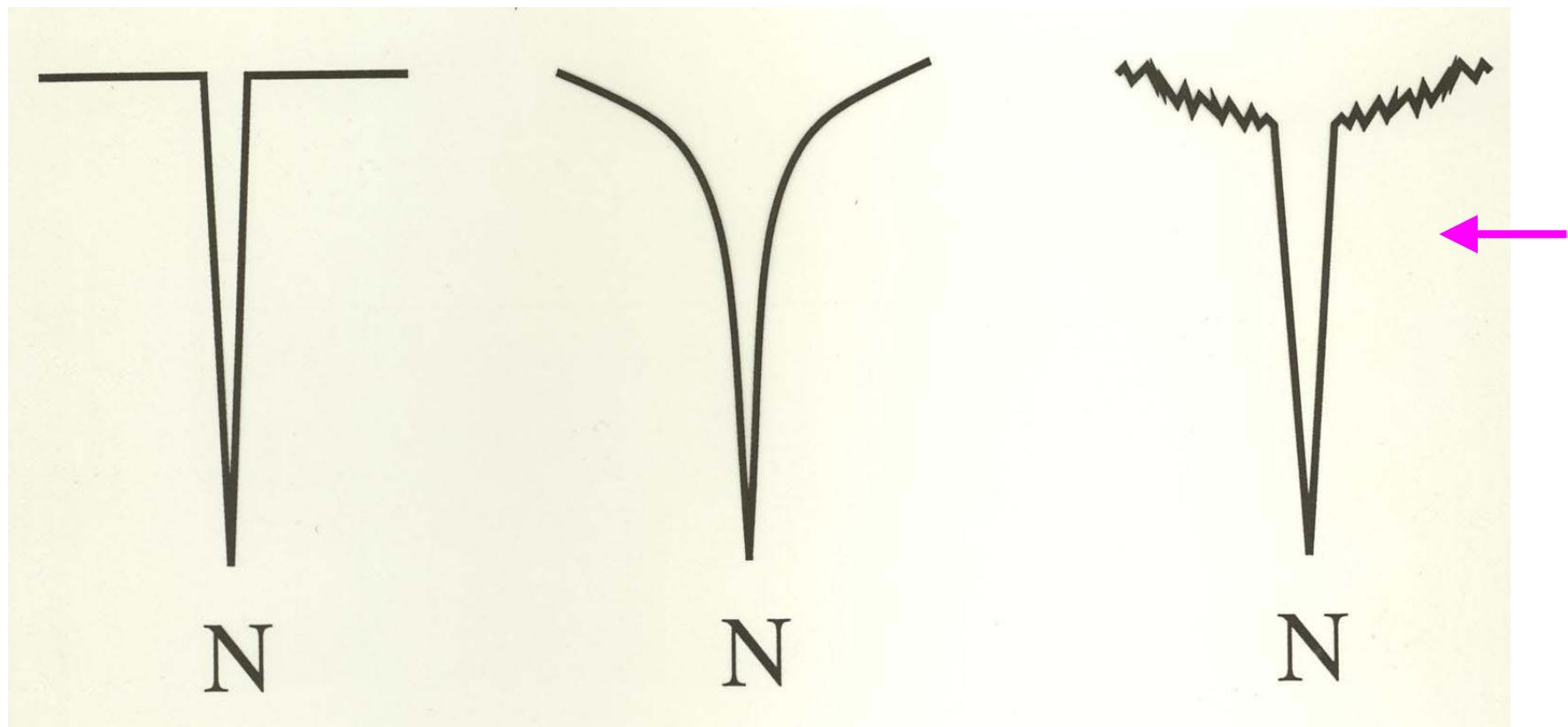
Scalley-Kim & Baker, *JMB* (2004);  
Watters et al., *Cell* (2007)

# Cooperativity Principles in Protein Folding

- Thermodynamic And Kinetic Cooperativities of small single-domain proteins should be used as stringent modeling constraints to gain insight into real protein energetics.
- Pairwise additive interactions appear to be insufficient, many-body interactions needed.
- Local-nonlocal coupling emerges as a plausible mechanism for apparent simple two-state cooperativity.
- Inasmuch as "consistency principle" and "principle of minimal frustration" are stipulated to be embodied in common Gō models, they should be superseded by cooperativity principles.
- Proteins that are less cooperative (e.g. chevron rollover, "downhill folding") can be addressed in the same framework.

Our model study and evaluation suggest strongly that...

Simple Two-State Protein Folding Kinetics Requires  
"Near-Levinthal" Thermodynamic Cooperativity

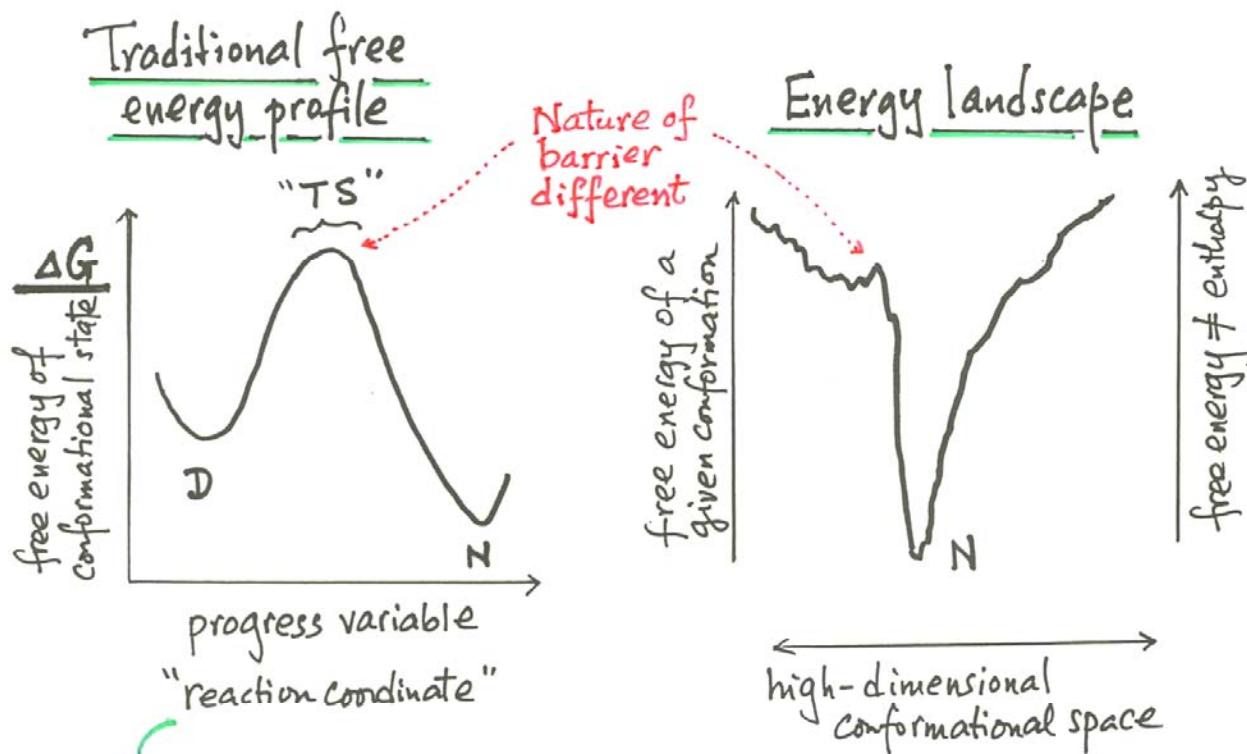


Kaya & Chan, *Proteins* (2003)

# Folding Barriers

entropic &  
enthalpic  
components

## Imageries of Protein Folding



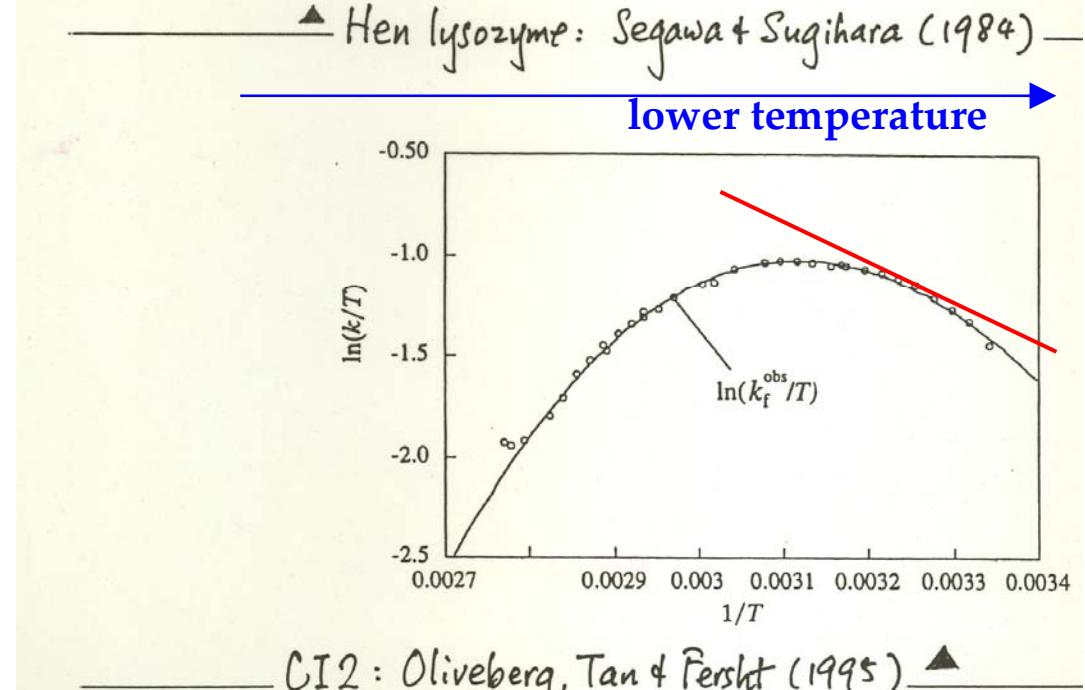
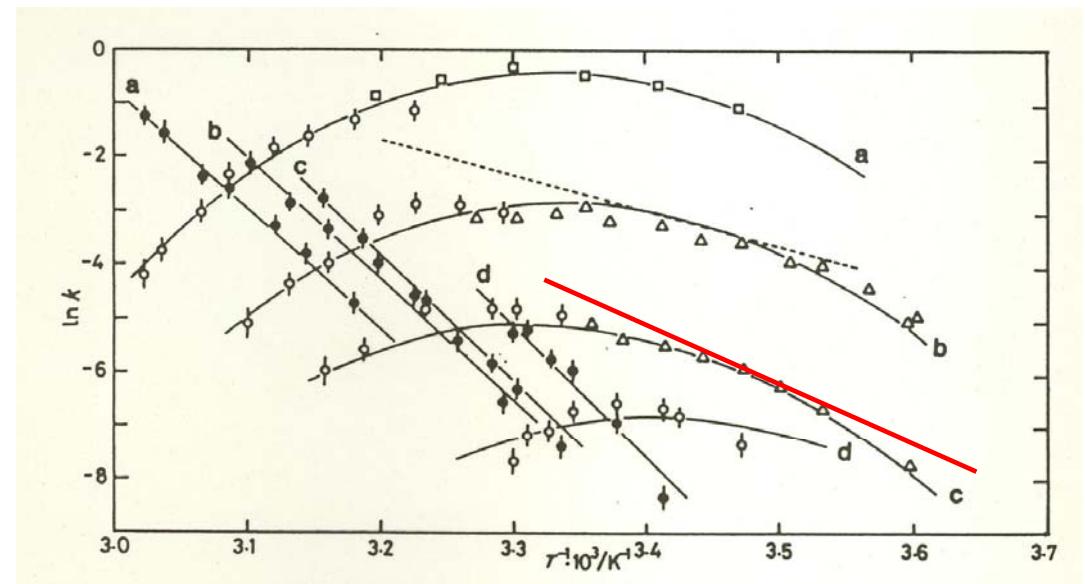
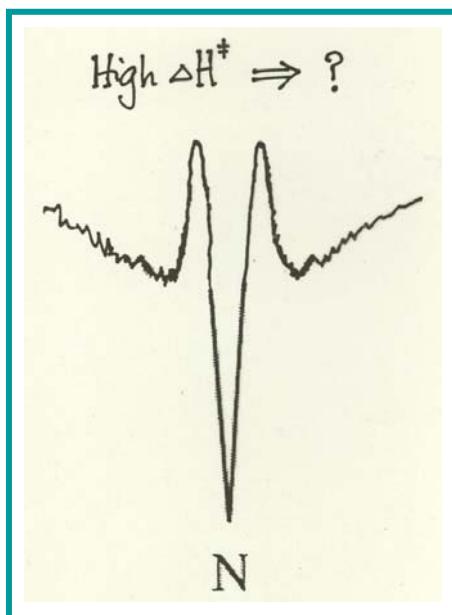
- Each "coordinate" can represent collectively many different conformations.

- = Potential of mean force (PMF), with solvent degrees of freedom pre-averaged.

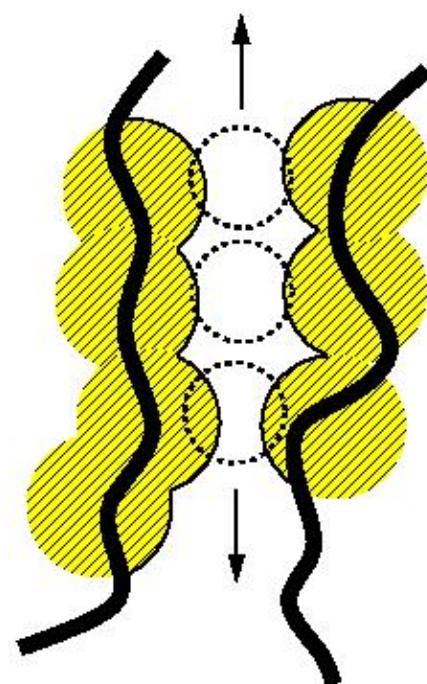
## Enthalpic barriers:

non-Arrhenius folding rates,  
positive unfolded-to-transition  
state enthalpy changes at  
some temperatures

*Does this mean that  
the folding landscape  
is not funnel-like?*



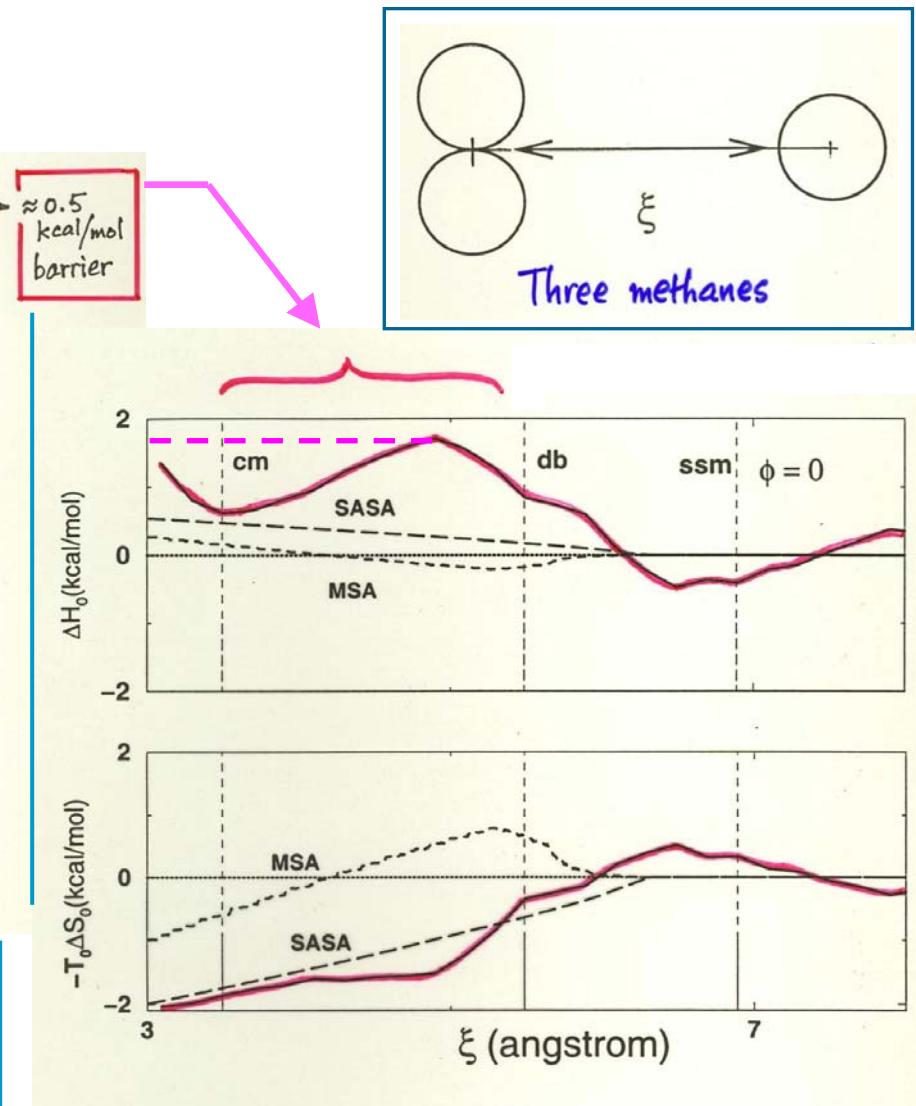
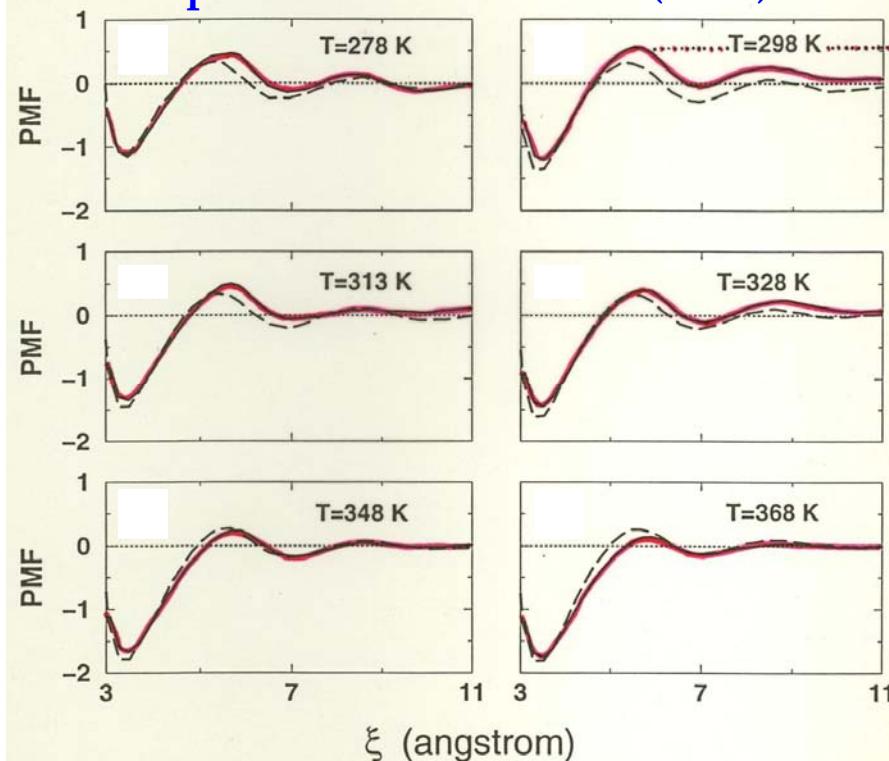
Desolvation is a likely origin of robust enthalpic barriers to protein folding



Liu & Chan, *JMB* (2005)

# Enthalpy-Entropy Compensation at Desolvation

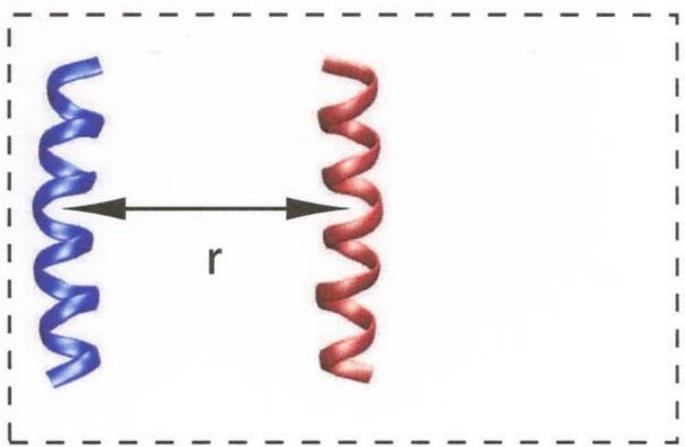
temperature dependence of the potential of mean force (PMF)



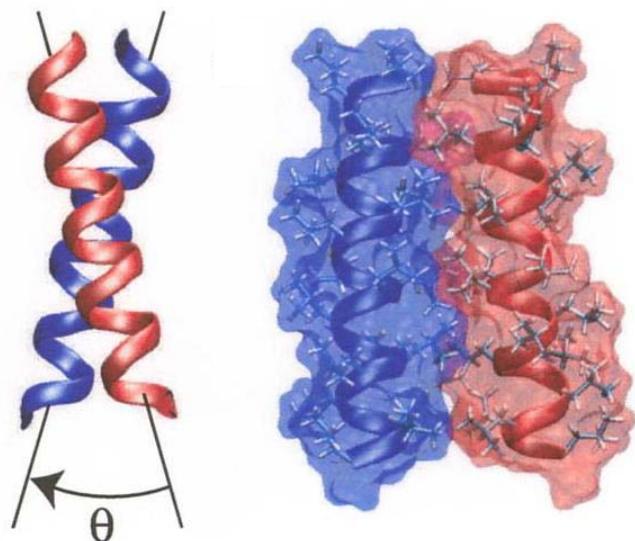
Enthalpic barrier can be significantly higher than the desolvation free energy barrier

Moghaddam, Shimizu & Chan, JACS (2005);  
Liu & Chan, JMB (2005)

# $\alpha$ -Helix association in water as a model for rate-limiting events in protein folding



A pair of 20-residue  
*poly-alanine or*  
*poly-leucine helices*



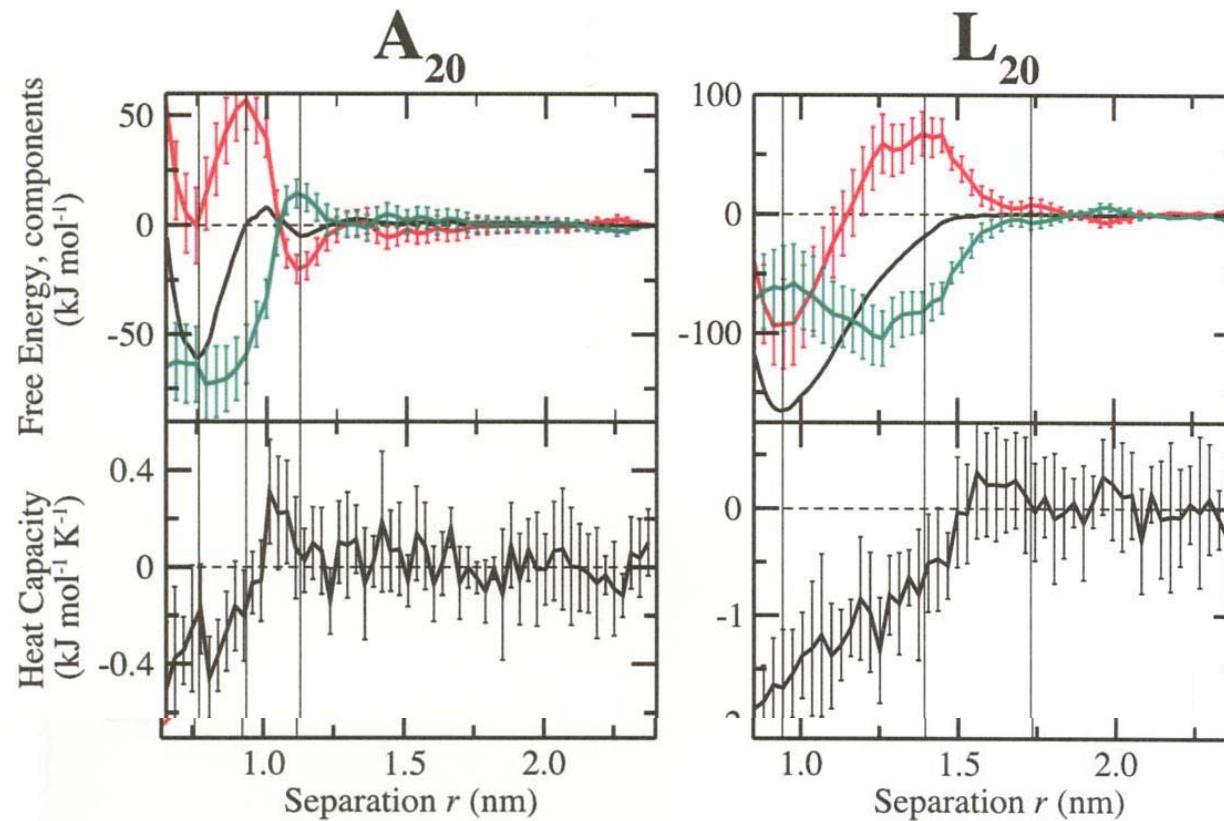
~3,800 water molecules

Simulated constant-pressure free energy of association (potential of mean force, PMF) at five temperatures

MacCallum, Moghaddam, Chan & Tieleman, *Proc Natl Acad Sci USA* (2007)

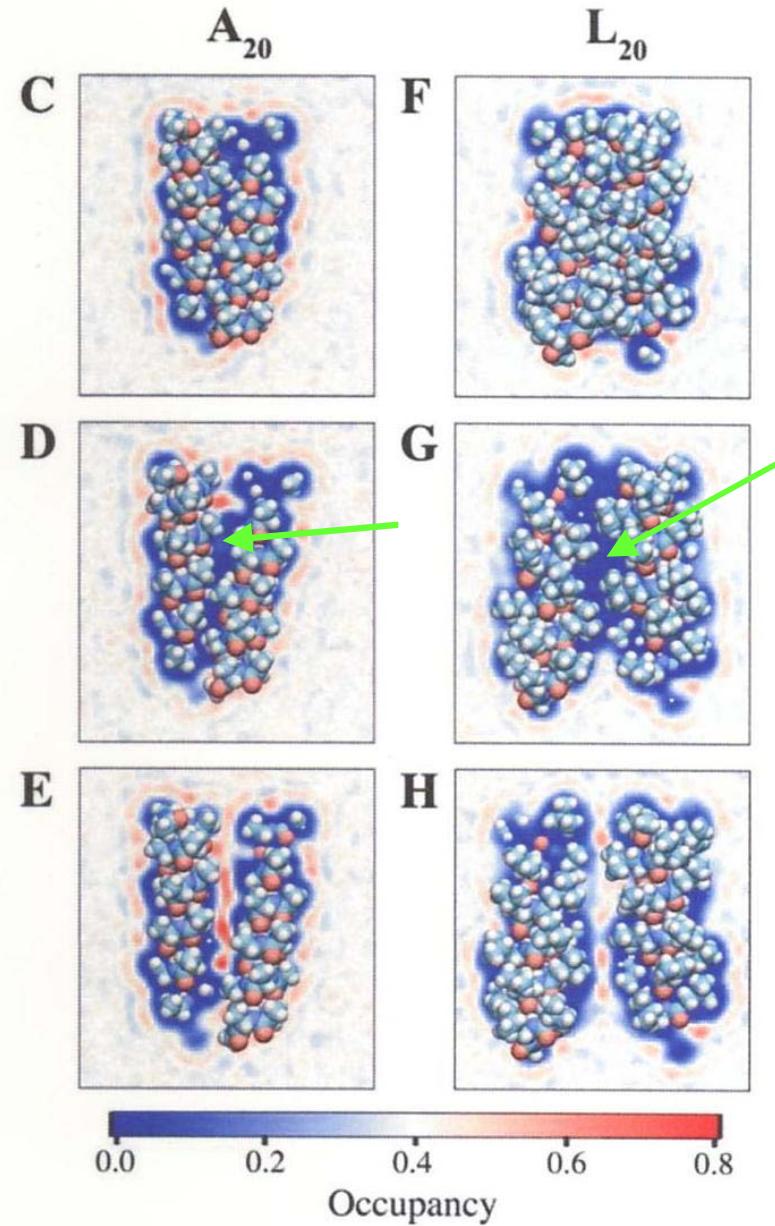
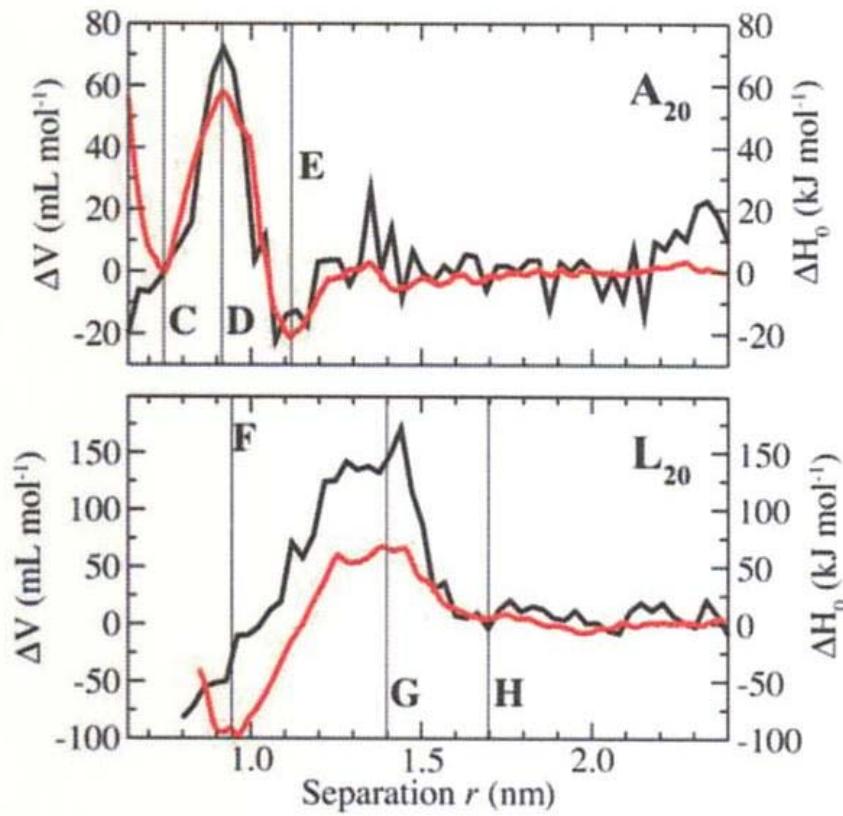
# Enthalpic desolvation barriers of ~ 50 kJ/mol comparable to that of protein folding

*Dramatic enthalpy-entropy compensation at the desolvation step leading to low or non-existence free energy barriers*



At 25 deg C,  
Enthalpic folding  
barrier height for  
**Cl2 is ~ 30kJ/mol**  
(Oliveberg et al., 1995)  
**CspB is ~ 32kJ/mol**  
(Schindler & Schmid, 1996)

# Enthalpic barriers caused by steric de-wetting

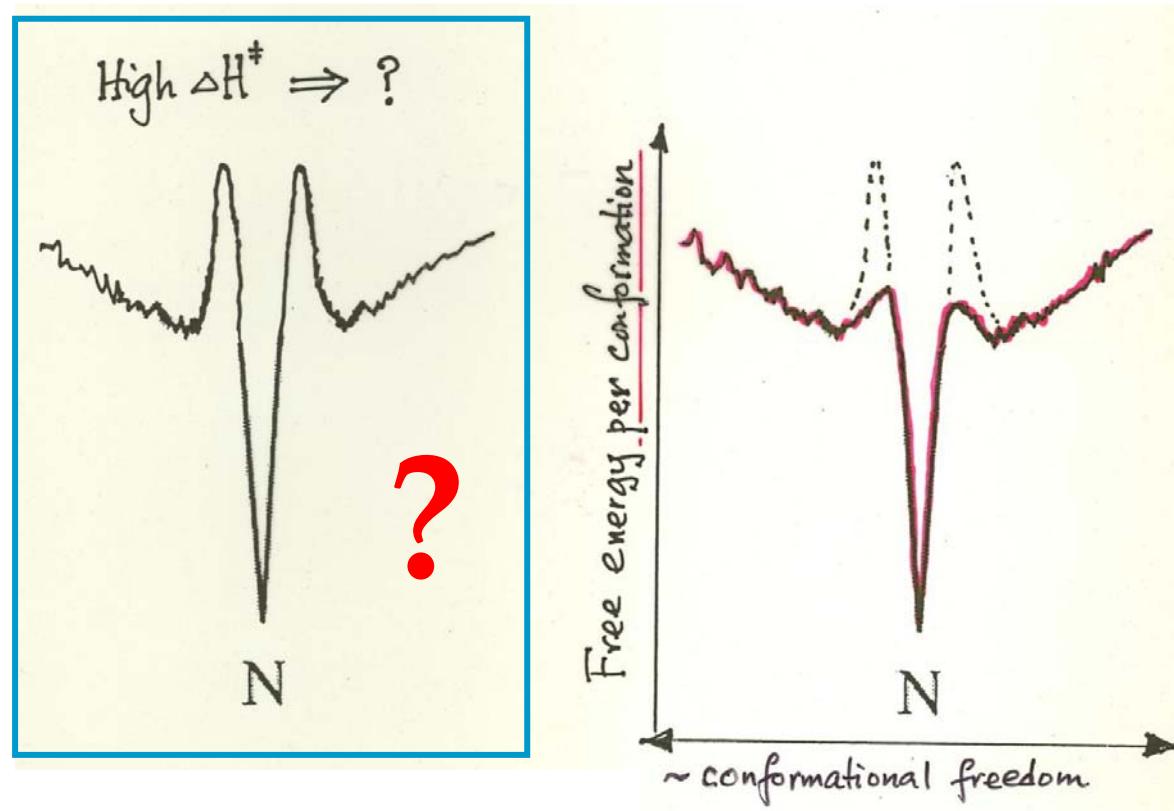
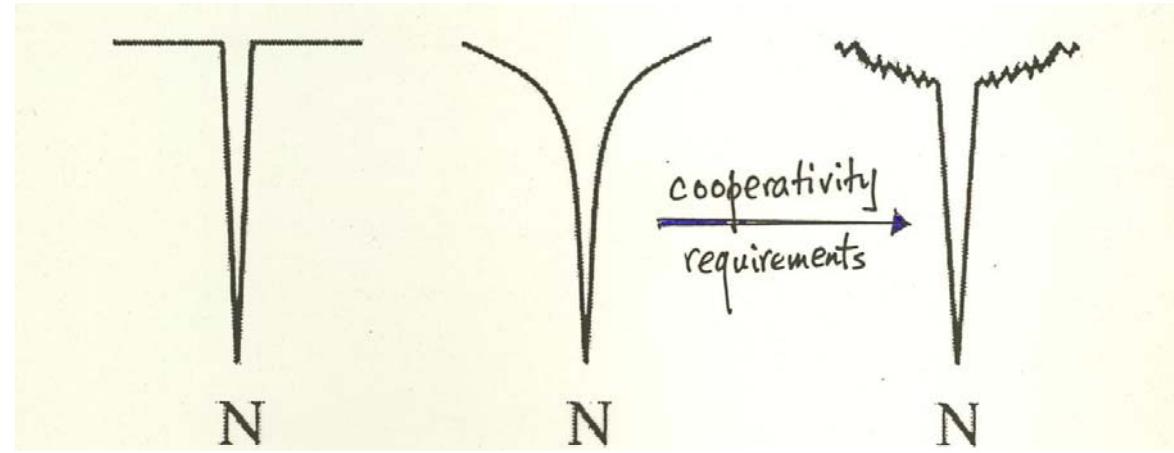


MacCallum, Moghaddam, Chan & Tieleman, *Proc Natl Acad Sci USA* (2007)

*Recap.:*

Folding cooperativity implies 'near-Levinthal' funnels

Enthalpic barriers can be consistent with funnel-like folding landscapes



# Co-workers

## University of Toronto



Artem Badasyan

Mikael Borg

Allison Ferguson

Zhirong Liu

Huseyin Kaya

Michael Knott

Maria Sabaye Moghaddam

Seishi Shimizu

Stefan Wallin

## University of Toronto

Prof. Julie Forman-Kay

Prof. Regis Pomes

## University of Calgary

Prof. Peter Tieleman

Justin MacCallum

## University of Muenster

Prof. Erich Bornberg-Bauer

David Vernazobres

Richard Wroe