

Transition States in Protein Folding

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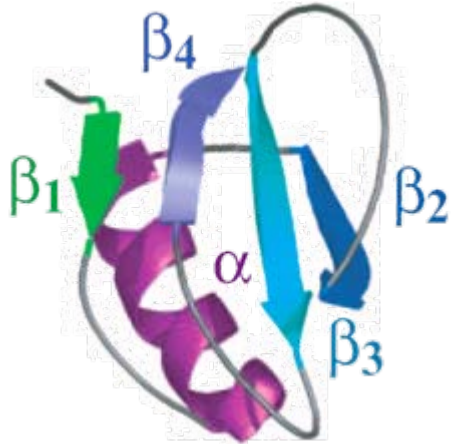


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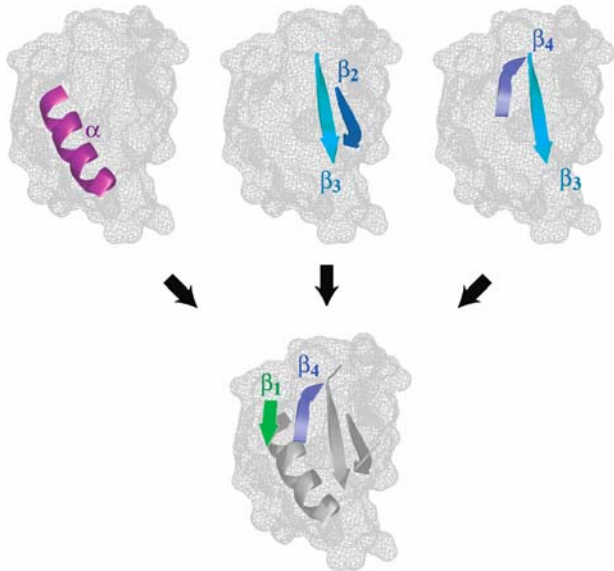
Overview

- Mutational Φ -value analysis of the folding kinetics
- Modeling Φ -values for α -helices
- Modeling Φ -values for small β -sheet proteins

Protein folding problems



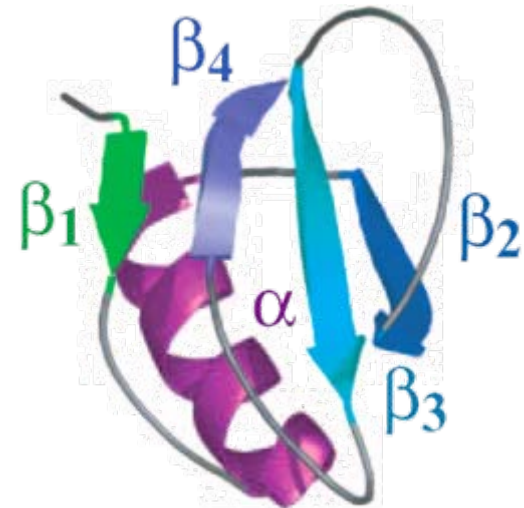
- **The structure problem:** In which native structure does a given sequence fold?



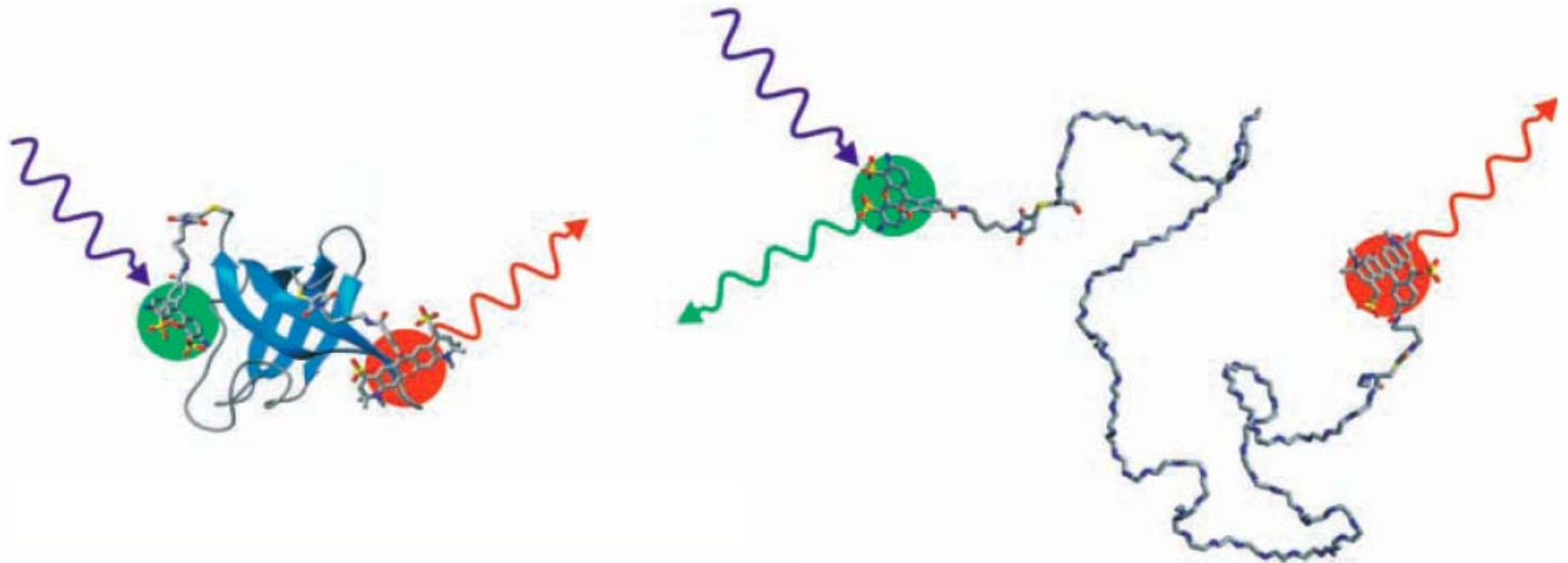
- **The kinetics problem:** How does a protein fold into its structure?

How does a protein fold?

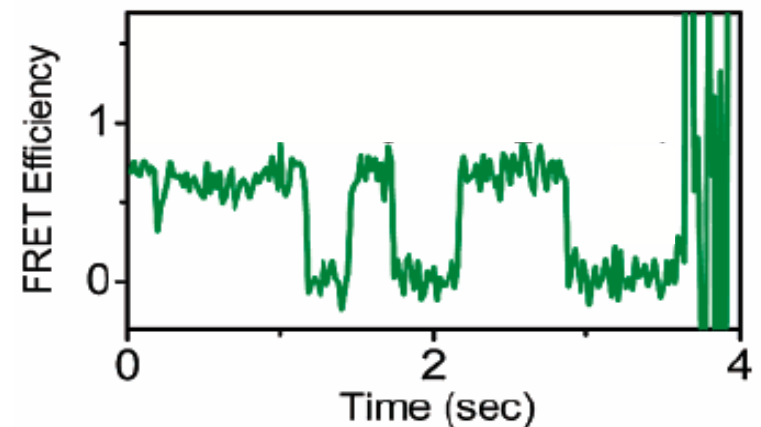
- The **Levinthal paradox**: How does a protein find its folded conformation as "needle in the haystack"?
- The "**old view**": Metastable folding intermediates guide a protein into its native structure
- The "**new view**": Many small proteins fold without detectable intermediates (**2-state proteins**)



2-state folding: Single molecules

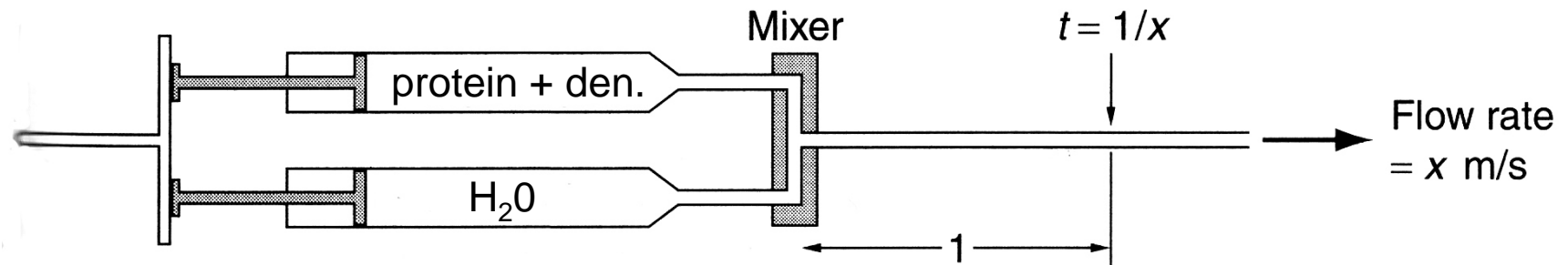


- Donor and acceptor dyes at chain ends
- State-dependent transfer efficiency

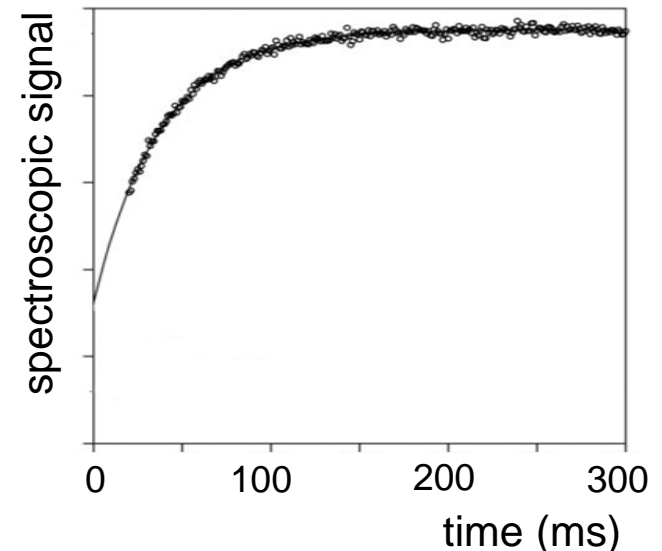
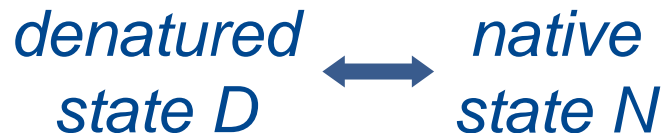


2-state folding: Protein ensemble

- **rapid mixing** to initiate folding



- **single-exponential relaxation** for 2-state process:



Mutational analysis of 2-state folding

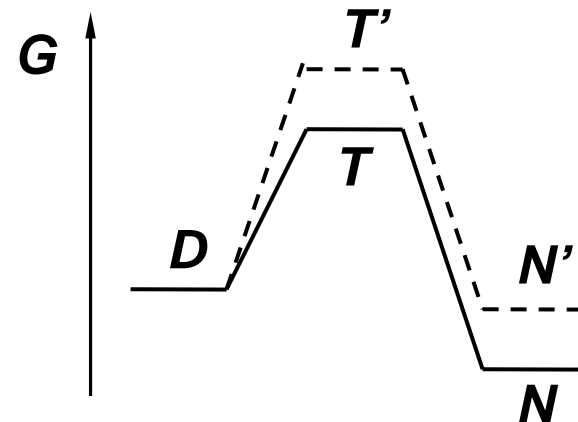
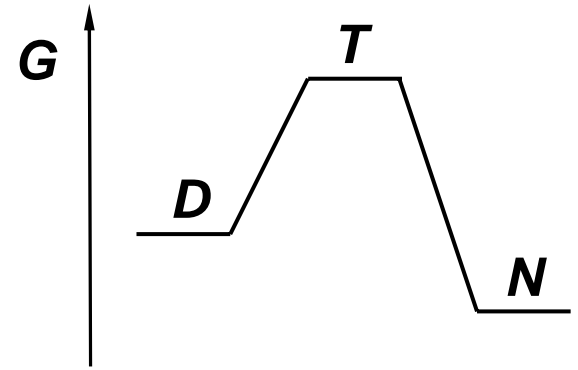
- **Transition state theory:**

$$k \propto \exp(-G_{T-D})$$

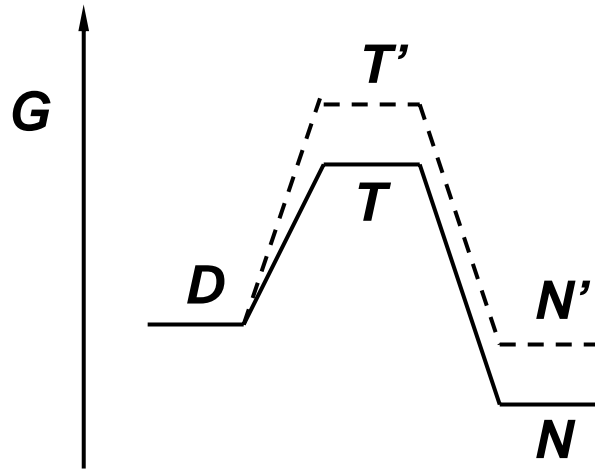
- **Mutations change the folding rate k and stability G_{N-D}**

- **Central quantities: Φ -values**

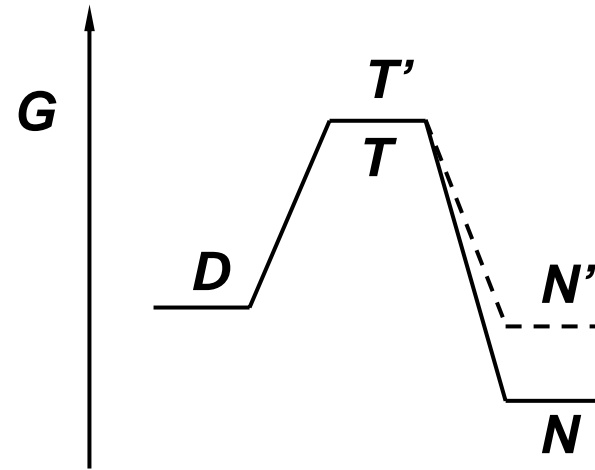
$$\Phi \equiv \frac{\Delta G_{T-D}}{\Delta G_{N-D}}$$



Traditional interpretation of Φ



$\Phi = 1$: mutated residue is native-like structured in T



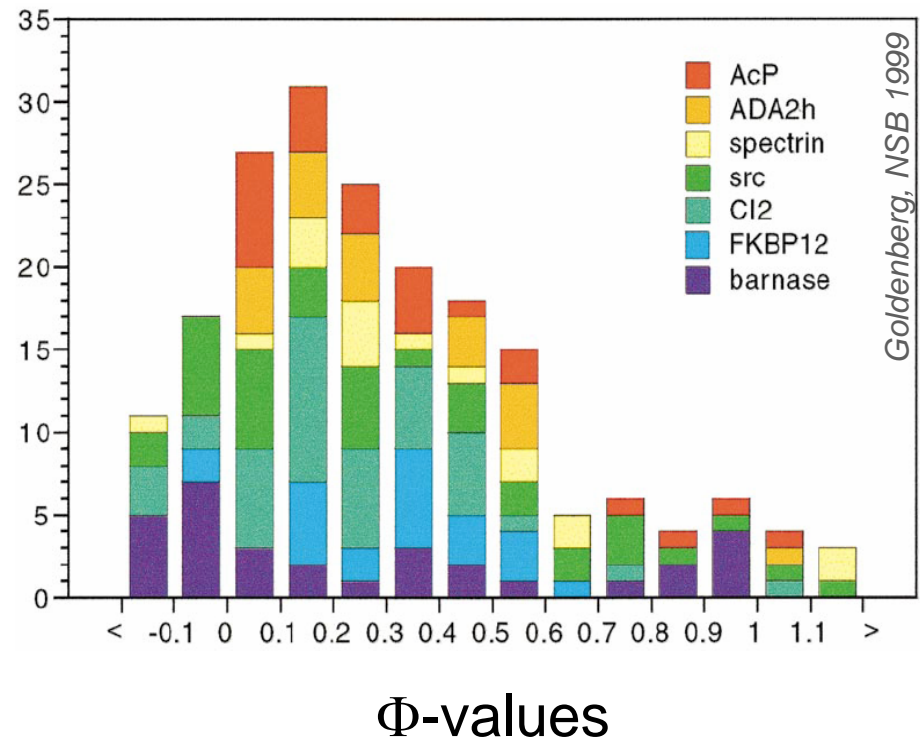
$\Phi = 0$: mutated residue is unstructured in T

Traditional interpretation of Φ

- Φ : degree of structure formation of a residue in T

- **Inconsistencies:**

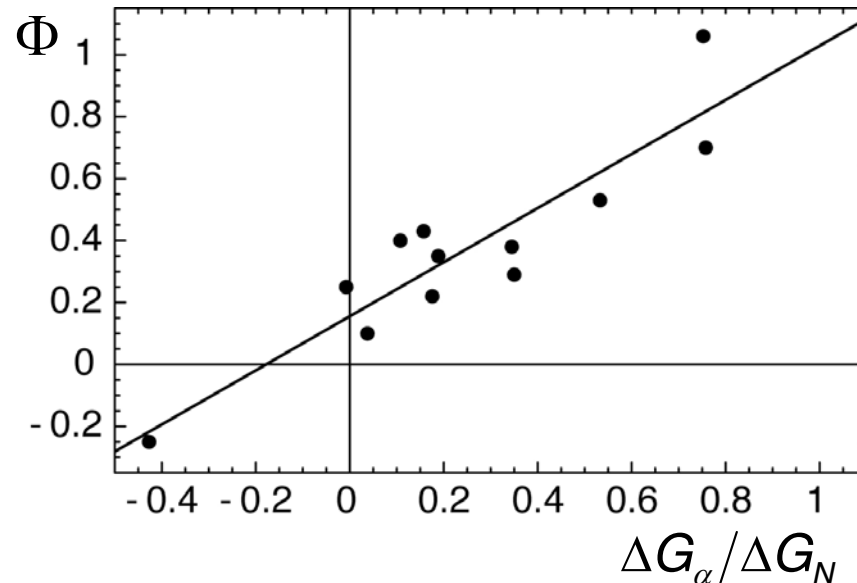
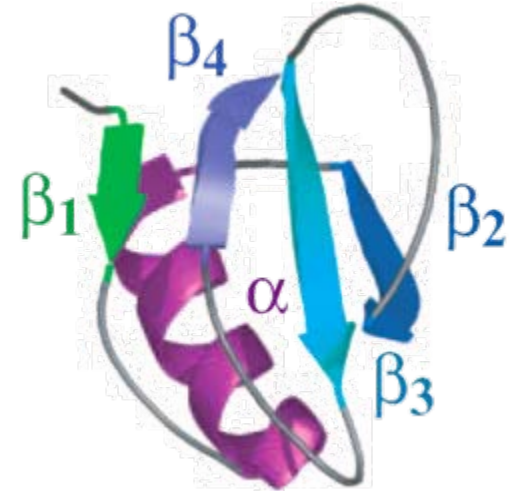
- some Φ 's are < 0 or > 1
- different mutations of the same residue can have different Φ -values



Example: α -helix of CI2

mutation	Φ
S12G	0.29
S12A	0.43
E15D	0.22
E15N	0.53
A16G	1.06
K17G	0.38
K18G	0.70
I20V	0.40
L21A	0.25
L21G	0.35
D23A	-0.25
K24G	0.10

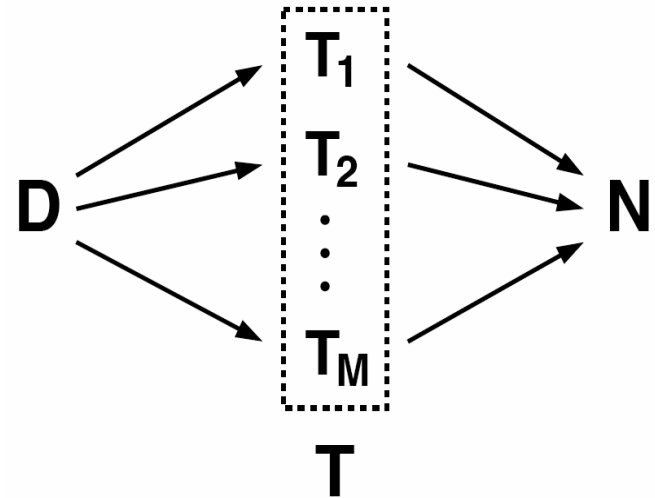
- Φ -values for mutations in the helix range from -0.25 to 1.06
- Our finding:



- Mutational Φ -value analysis of the folding kinetics
- **Modeling Φ -values for α -helices**
- Modeling Φ -values for small β -sheet proteins

Helix cooperativity

- we assume that a helix is either **fully formed or not formed** in transition-state conformation T_i



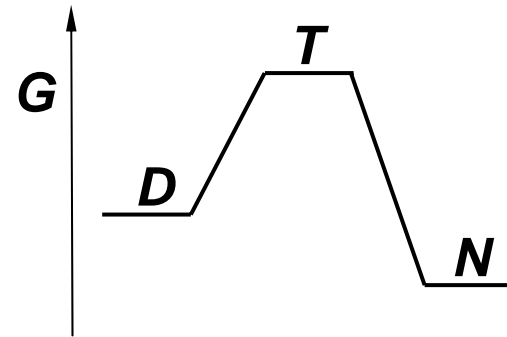
- we have **two structural parameters** per helix:
 - the **degree of secondary structure** χ_α in T
 - the **degree of tertiary structure** χ_t in T

Splitting up free energies

- we split up mutation-induced free energy changes into **secondary** and **tertiary** components:

$$\Delta G_N = \Delta G_\alpha + \Delta G_t$$

$$\Delta G_T = \chi_\alpha \Delta G_\alpha + \chi_t \Delta G_t$$



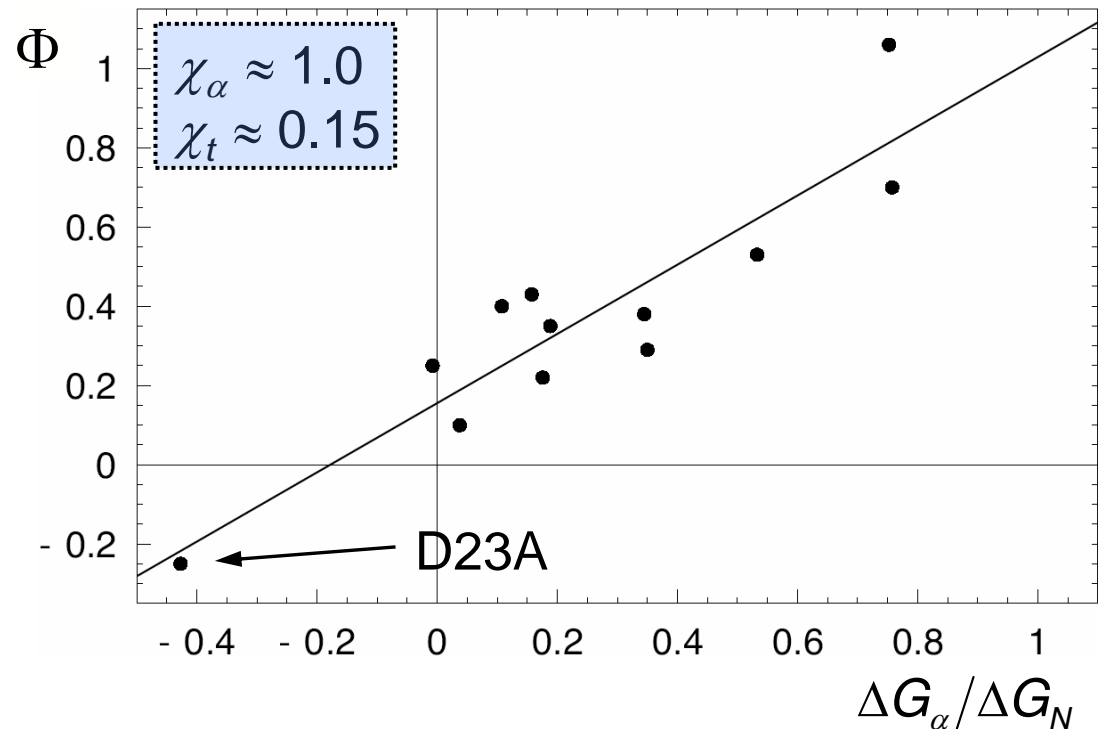
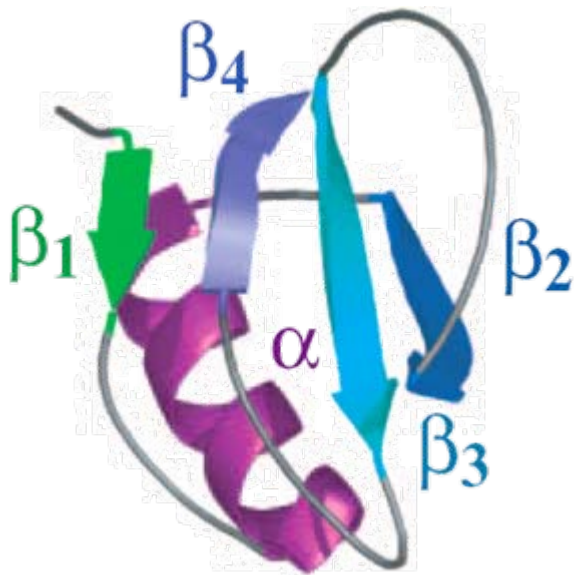
- general form of Φ -values for mutations in an α -helix:

$$\Phi \equiv \frac{\Delta G_T}{\Delta G_N} = \chi_t + (\chi_\alpha - \chi_t) \frac{\Delta G_\alpha}{\Delta G_N}$$

Φ -values for α -helix of CI2

general formula: $\Phi = \chi_t + (\chi_\alpha - \chi_t) \frac{\Delta G_\alpha}{\Delta G_N}$

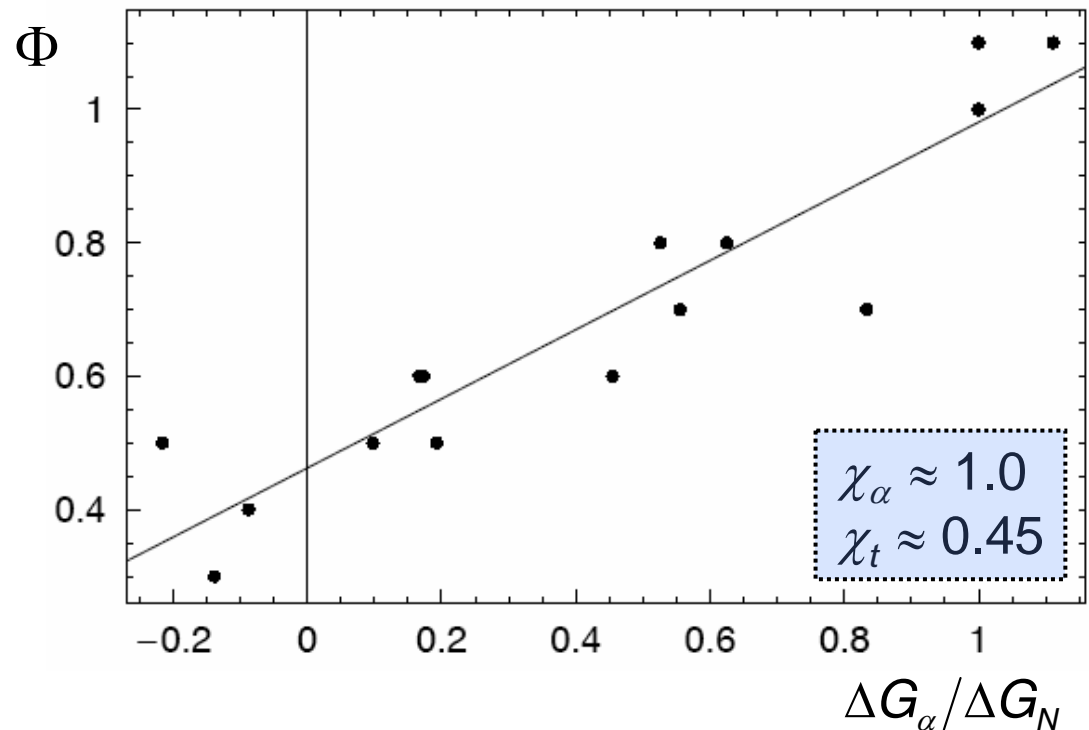
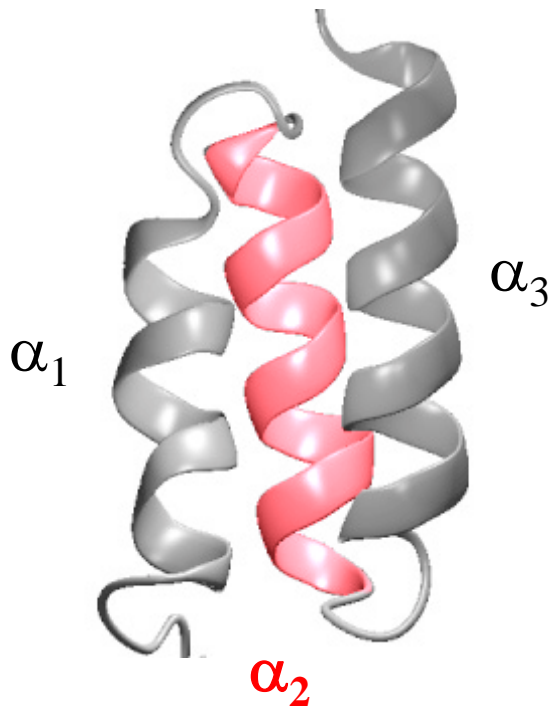
mutational data for CI2 helix:



Φ -values for helix 2 of protein A

general formula: $\Phi = \chi_t + (\chi_\alpha - \chi_t) \frac{\Delta G_\alpha}{\Delta G_N}$

mutational data for helix 2:



Summary

Consistent interpretation of Φ -values for helices:

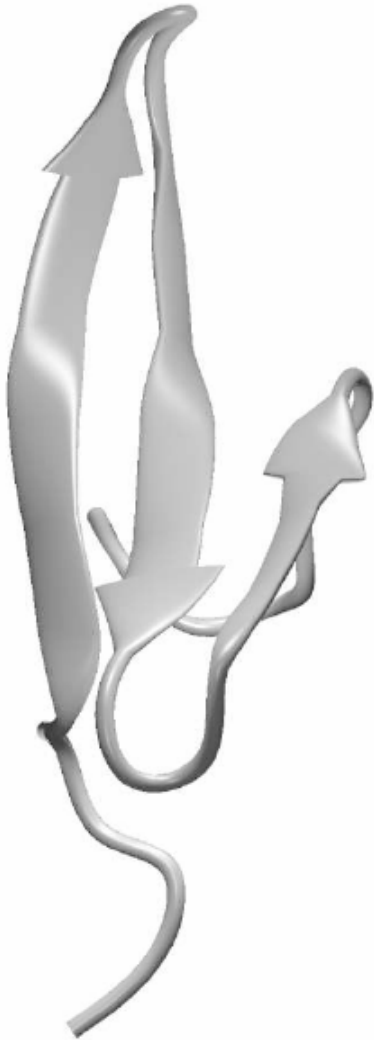
- with **two structural parameters**: the degrees of secondary and tertiary structure formation in T
- by **splitting up** mutation-induced **free energy changes** into secondary and tertiary components

C Merlo, KA Dill, TR Weikl, PNAS 2005

TR Weikl, KA Dill, JMB 2007

- Mutational Φ -value analysis of the folding kinetics
- Modeling Φ -values for α -helices
- **Modeling Φ -values for small β -sheet proteins**

Modeling 3-stranded β -proteins



- **WW domains** are 3-stranded β -proteins with **two β -hairpins**
- we assume that each **hairpin** is **fully formed or not formed** in the transition state

Evidence for hairpin cooperativity

QuickTime™ and a
decompressor
are needed to see this picture.

- **β 3s** is a **designed 3-stranded β -protein** with 20 residues
- **transition state** rigorously determined from **folding-unfolding MD simulations**
- result: either **hairpin 1** or **hairpin 2** structured in T

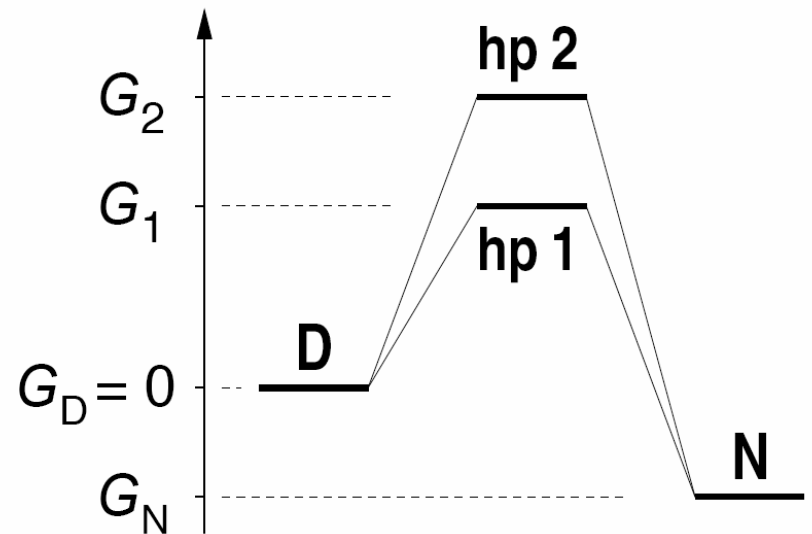
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A simple model for WW domains

- we have **two transition-state conformations** with a single hairpin formed

- the **folding rate** is:

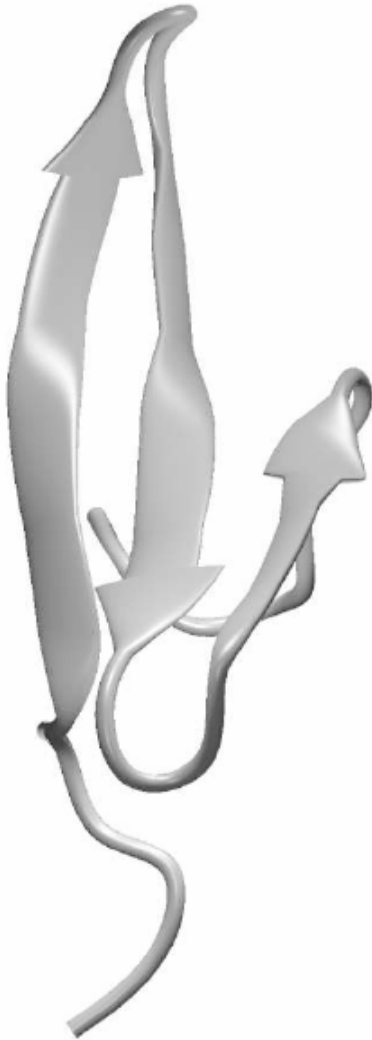
$$k \approx \frac{1}{2} \left(e^{-G_1/RT} + e^{-G_2/RT} \right)$$



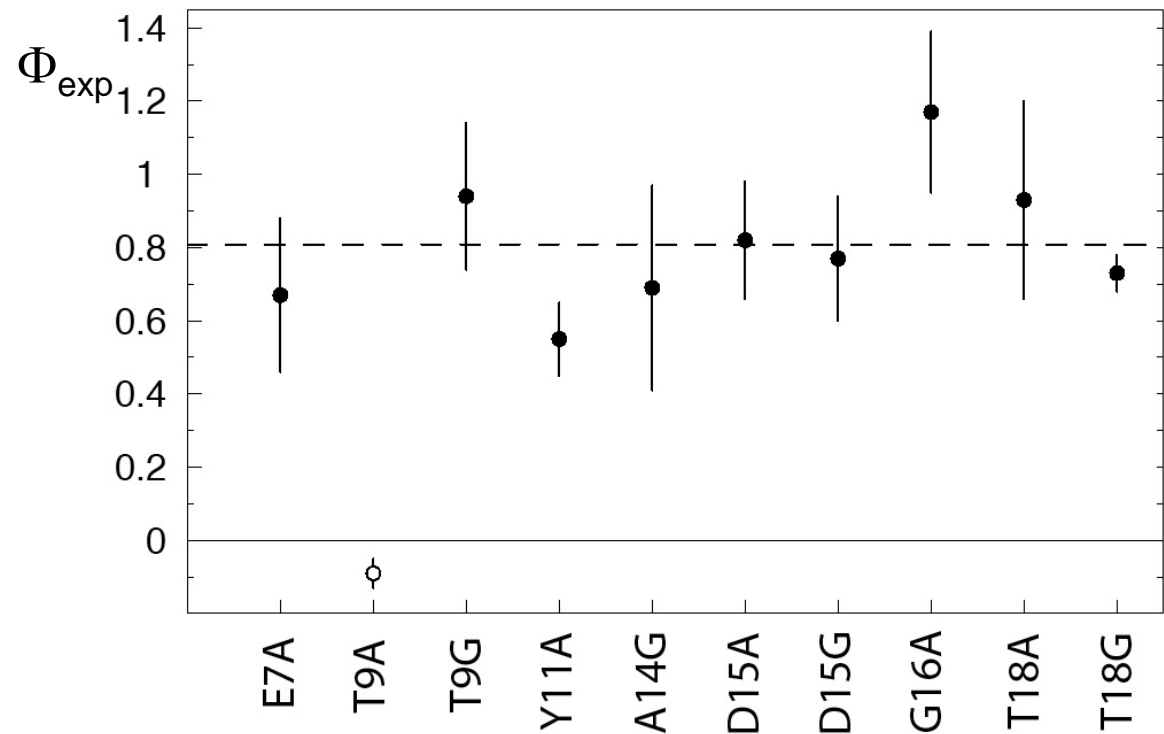
- **Φ-values** have the general form:

$$\Phi \equiv \frac{-RT \Delta \log k}{\Delta G_N} = \frac{\chi_1 \Delta G_1 + \chi_2 \Delta G_2}{\Delta G_N}$$

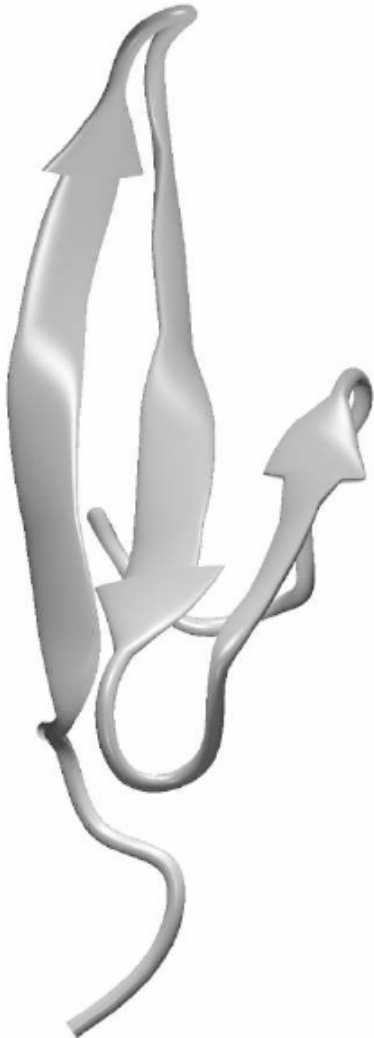
Φ -values for FBP WW domain



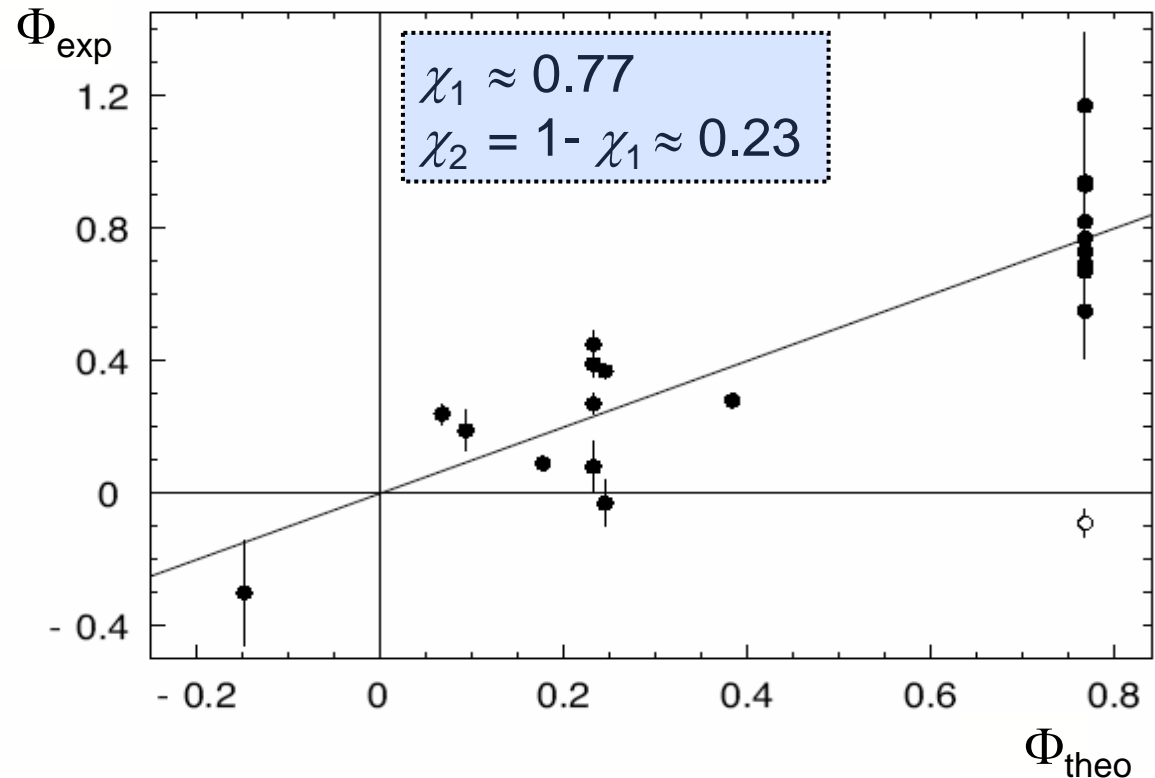
- general formula: $\Phi_{\text{theo}} = \frac{\chi_1 \Delta G_1 + \chi_2 \Delta G_2}{\Delta G_N}$
- a first test: Φ 's for mutations affecting only hairpin 1 should have value χ_1



Φ -values for FBP WW domain



- general formula: $\Phi_{\text{theo}} = \frac{\chi_1 \Delta G_1 + \chi_2 \Delta G_2}{\Delta G_N}$
- single-parameter fit:



Summary

Reconstruction of transition states from mutational Φ -values based on:

- **substructural cooperativity** of helices and hairpins
- **splitting up** mutation-induced **free energy changes**

C Merlo, KA Dill, TR Weikl, PNAS 2005

TR Weikl, KA Dill, J Mol Biol 2007

TR Weikl, Biophys J 2008