Biophysics and structural bioinformatics II

Kinetics of protein folding

Kinetics of protein folding

‘Transition states
and the meaning of ϕ-values
in protein folding kinetics’

S. Banu Ozkan *et al*., **Transition states and the meaning of Φ-values in protein folding kinetics**
Nature Structural Biology, 2001, 8(9):765-769

- Introduction
- Model
- Landscape mapping
- Conclusions

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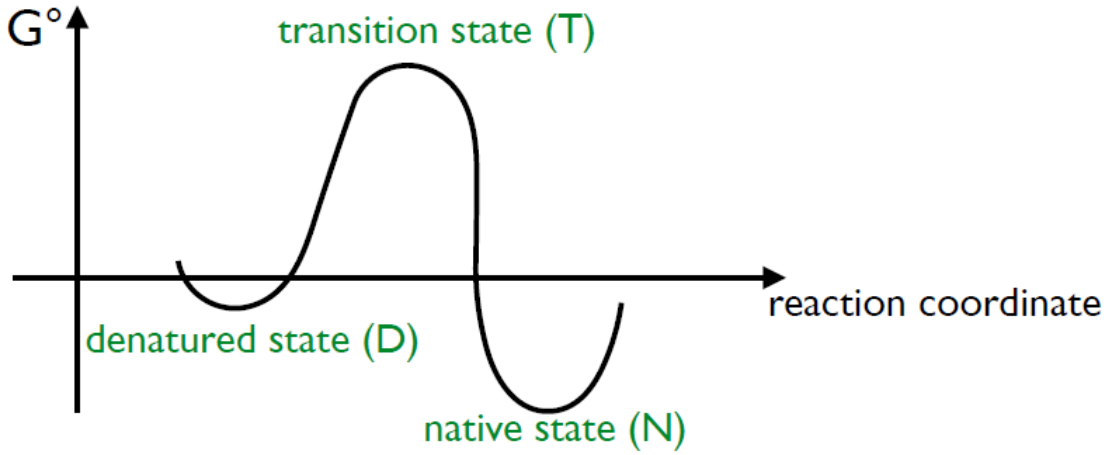
- Introduction : two-state protein folding
  - Rate processes that involves **single exponential relaxation** in both forward and reverse directions are interpretated in terms of **rate limiting steps** corresponding to **transition states** associated with an energy or entropy **barrier along a reaction coordinate**.



$$D \rightleftharpoons N$$

$$\frac{dD(t)}{dt} = -k_{DN} D(t) \rightarrow D(t) = D_0 \exp(-k_{DN} t)$$

$$\frac{dN(t)}{dt} = -k_{ND} N(t) \rightarrow N(t) = N_0 \exp(-k_{ND} t)$$

$$k_{DN} = \exp(-\Delta G_{DT} / RT)$$

$$k_{ND} = \exp(-\Delta G_{NT} / RT)$$

$$\Delta G = \Delta H - T\Delta S$$

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- Introduction : folding kinetics
  - Experimental identification of the chain conformations responsible for the observed relaxation rate → **ϕ-value analysis**:
    - A particular amino acid is mutated
    - The mutation changes the (thermodynamic) stability of the protein by an amount $\Delta_m \Delta G_{DN} = \Delta G_{DN_{mu\tan t}} - \Delta G_{DN_{wild-type}}$
    - The mutation affects the (kinetic) folding barrier by an amount $\Delta_m \Delta G_{DT} = \Delta G_{DT_{mu\tan t}} - \Delta G_{DT_{wild-type}}$
    - Then, $\phi = \Delta_m \Delta G_{DT} / \Delta_m \Delta G_{DN}$

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- Introduction : meaning of ϕ-values
  - Classical interpretation of ϕ
    - Hammond's postulate : *If two states occur consecutively during a reaction process and have nearly the same energy content, their interconversion will involve only a small reorganization of the molecular structures.*

      →**'Kinetic ruler' hypothesis** : ϕ indicates how native-like the conformation of the transition state is, and is thus monotonically (but not linearly) related to ξ
  - Implication
    - All protein conformations can be lined up along a **single axis of nativeness**, where are the transition state conformations responsible for the single exponential time constant
      →Nonclassical ϕ-values (<0, >1) would have nonsensical meaning

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- Introduction : meaning of ϕ-values
  - Problem
    - 10-20% of the hundreds of mesured ϕ-values for protein folding are **outside of this classical range**. ϕ-values as large as 8 have been measured. Negative ϕ-values have been observed in computer simulation.
  - Explanation
    - Nonclassical ϕ-values can arise from **parallel coupled flows of folding** such as in funnel-shaped energy landscape

      → Demonstration via modelling

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- Model : aim
  - Minimal requirements:
    - Single **exponential kinetics** (two-state folding)
    - Folding directed toward a unique native state by an **energy function based on conformation** and sequence-structure relationships (structural interpretation of folding kinetics)
    - **Sufficient simplicity** to avoid the use of assumptions about the transition state (direct obtention of the kinetics)

    → Chain lattice Go models

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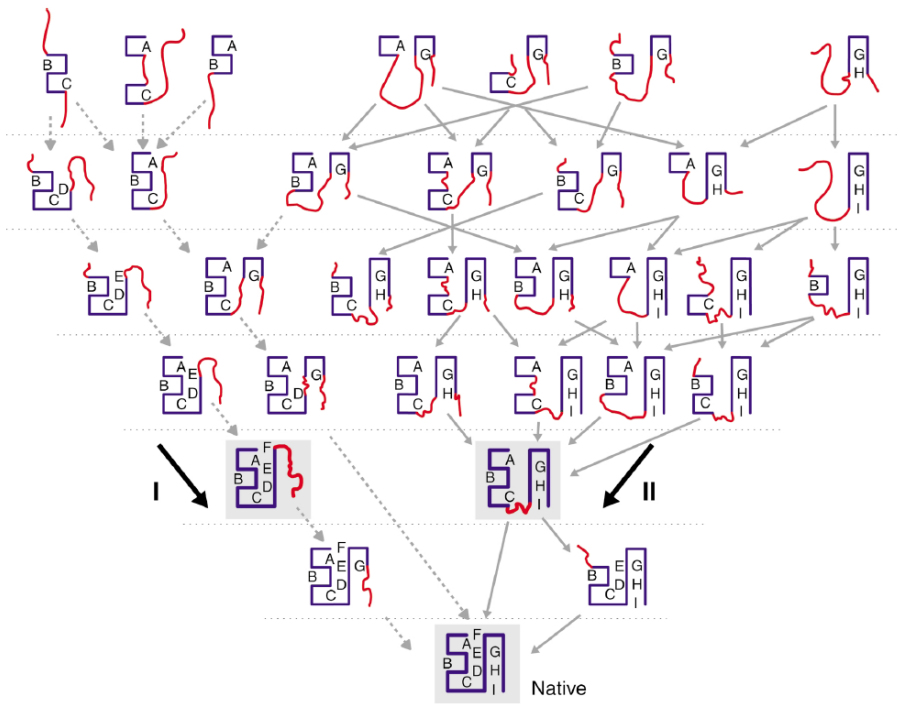
- Model : approach
  - Lattice Go model chain
    - Conformation structure
      →2D Lattice
    - Conformation energy
      →Native contacts
    - Folding
      →Conformation chain



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- Model : definition
  - Conformation transition rate

$$A_{ij} = \exp(-\Delta G_{ij} / RT)$$

$$\Delta G_{ij} = \exp[-\nu < (\Delta r_{ij})^2 >^{1/2}] * \exp[-(q_i - q_j)\varepsilon H_{(q_i, q_j)} / RT]$$

q_i : Number of native contacts

ε : Attractive potential of a native contact

$H_{(q_i, q_j)}$: Heaviside step function (= 0 if $q_i > q_j$)

$< (\Delta r_{ij})^2 >^{1/2}$: RMS deviation of residue coordinates after optimal superimposition of conformations i and j

ν : Scaling parameter penalizing/favoring the transitions between dissimilar/similar conformations

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- Model : kinetics
  - Time evolution of the conformational ensemble

$$\frac{dP(t)}{dt} = A * P(t)$$

$P(t)$: N-dimensional vector of the instantaneous probabilites of the N conformations

A : N x N transition rate matrix

  - Solutions

$$P(t) = P(0) * \exp(-At) = P(0) * \exp(-\Lambda t) * B^{-1}$$

$P(0)$: Vector of initial probabilities

Λ : Diagonal matrix of the eigenvalues of A

B : Matrix of eigenvectors

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- Model : study case
  - Native structure lattice model
    - Two-stranded antiparallel **β-sheet** adjecent to a **helix**
  - Main folding routes
    - Channel 1: **faster** (high flux)
    - Channel 2: **slower** (low flux)
  - Nonclassical ϕ-values
    - ϕ>1: a mutation that destabilizes the native state **speeds up folding**
    - ϕ<0: a mutation that destabilizes the native state **slows down folding**

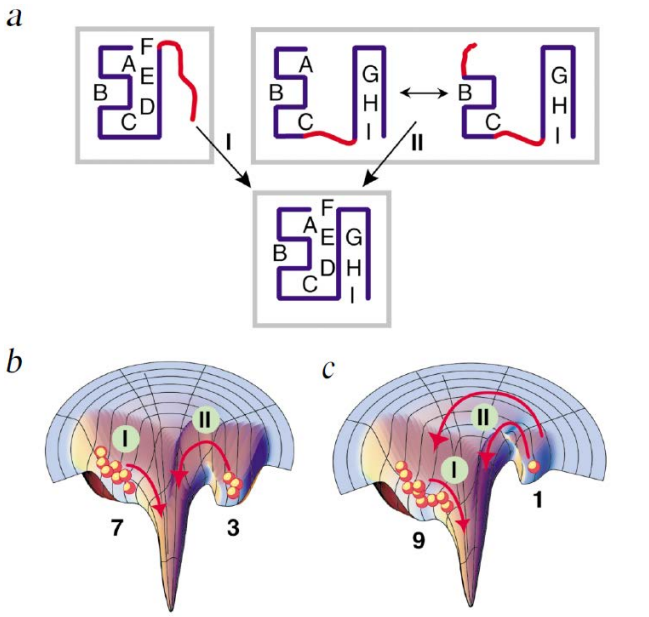

Table 1 Φ-values resulting from 30% destabilization of native contacts

Type of native contact	Φ-value
A	0.012
B	0.096
C	0.251
D	0.990
E	0.093
F	0.035
G	−0.357
H	−0.296
I	−0.085

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- Landscape mapping : motive
  - No single quantity ξ ('kinetic ruler') monotonically related to ϕ, that applies at the same time to all microscopic trajectories

  →Find **physical explanation of ϕ-values** by analyzing its correlation with all possible routes in the energy landscape

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- Landscape mapping : method
  - Map kinetic distances between conformations by **fixing molecular structures** into specific conformations, starting the reaction and **measuring the time to product**.
    - Fix m native contacts, giving an initial conformation
    - Measure the time τ required to reach the successive macroconformation n along a particular macroroute
    - The corresponding energy of activation is $E_{act,mn} = -RT \ln(1/\tau_{mn})$
    - Do so for every possible set of native contacts

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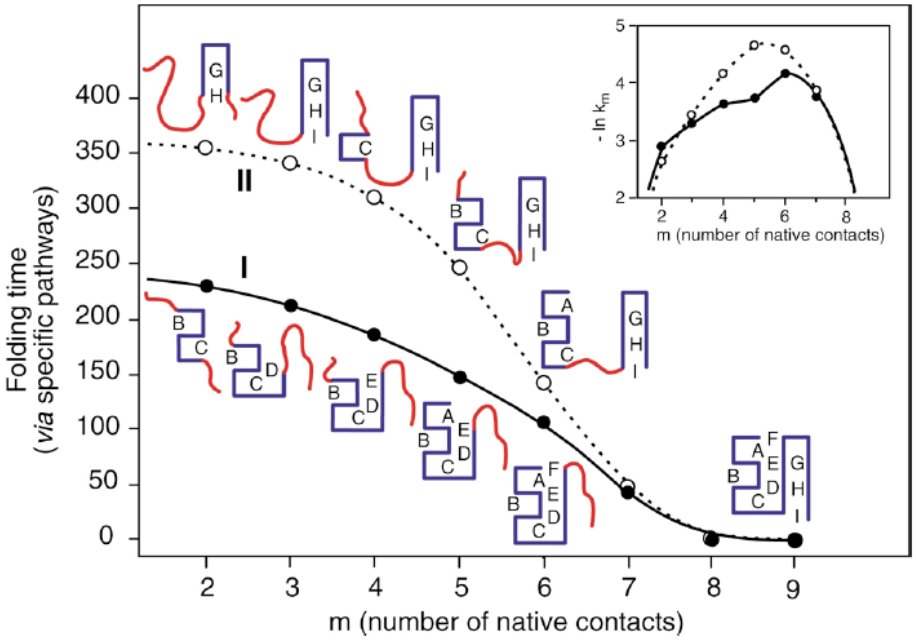
- Landscape mapping : conclusions
  - 'Macroroutes have barriers, microroutes do not'
    - The more the native contacts, the faster the conformational changes, but the less available the conformational changes
      → balance=energy barrier
    - The barrier is identified by the slowest macroroute and results from a property of the landscape and not of a microroute



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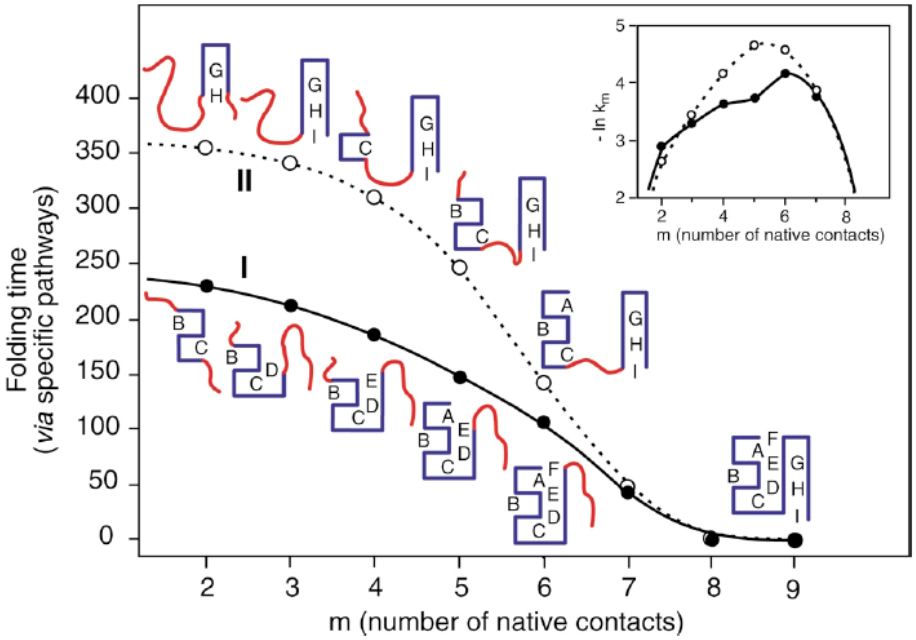
- Landscape mapping : conclusions
  - 'Folding involves nucleated zipping'
    - Native state is reached faster by starting from a particular set of native contacts
    - Because partially formed conformations are typically committed to a given folding route and must find uphill routes on the energy landscape in order to reach fast downhill folding routes



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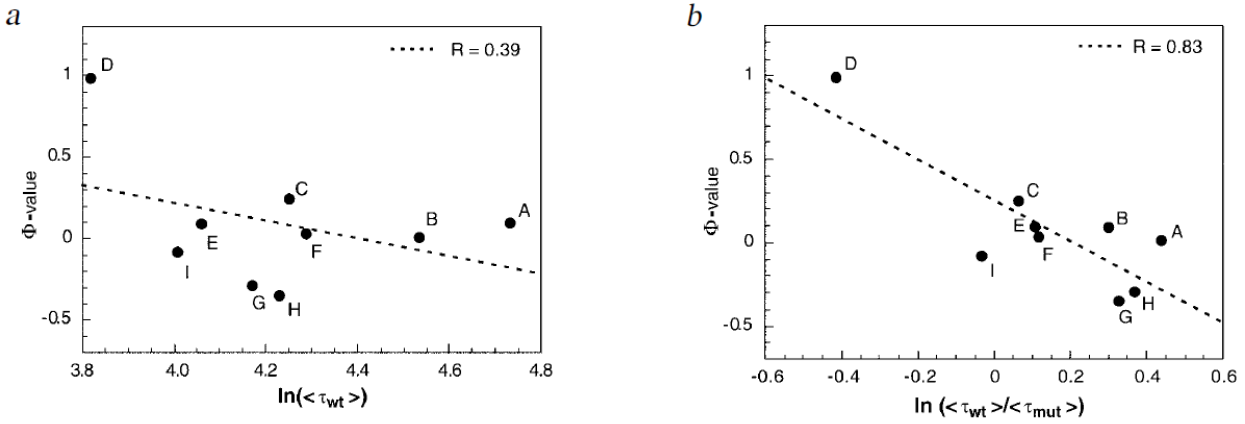
- Landscape mapping : relation to ϕ
  - ϕ correlation with τ_{mut} and τ_{wt}
    - ϕ correlates weakly with 'kinetic ruler' property $< \tau_{wt} >$
    - ϕ correlates more strongly with 'gatekeeper' property $<\tau_{mut}>/<\tau_{wt}>$



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- Conclusions : summary
  - Nonclassical ϕ-values have meaning when considering parallel coupled microscopic folding pathways
  - Folding barrier is due to the whole energy landscape and not to single conformational changes events
  - Some native contacts control the flow through this energy landscape state more then others
  - ϕ is not a ‘kinetic ruler’ of nativeness of the transition state but rather a ‘gatekeeper’ indicator of the degree to which a contact controls this flow , and thus the acceleration/deceleration of folding induced by mutation

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‘Transition state contact orders correlate with protein folding rates’

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J. Mol. Biol., 2005, 352:495-500

- Introduction
- Transition state ensembles determination
- Contact order calculation and analysis
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- Introduction
  - Interpretation of experimental ϕ-values in terms of the ratio of the number of native interactions in the transition state to that in the native state makes it possible to use the ϕ-values as restraints in simulations to determine the structures of the transition state ensemble
  - If sufficient sets of experimental ϕ-values of proteins are available → possibility of establishing a structural database of transition state ensembles that can be used to validate theoretical concepts in protein folding

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- Introduction
  - Study case : 10 proteins
    - Determination of the transition state ensembles based on experimental ϕ-values.
    - Calculation and analysis of the transition state contact order to validate and provide new insights into the observation that the native state contact order correlates with the logarithm of the rates of two-state folding.

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- Transition state ensembles determination
  - All-atom molecular dynamics simulations restrained by experimental ϕ-values
    - Energy function of a given conformation C:

$$E_{tot}(C) = E_{MM}(C) + E_{\phi}(C)$$

E_{MM} : Molecular mechanics energy function

$$E_{\phi} = \alpha . \frac{1}{N_{\phi}} \sum_{i} (\phi_i^{calc}(t) - \phi_i^{\exp})^2$$

$$\rightarrow \mathrm{C}_{\min(E_{tot})} = [\mathrm{TSE}]$$

$\phi_i^{\exp}$ and ϕ_i^{calc} : experimental and calculated ϕ - values

N_{ϕ} : Number of available experimental ϕ - values

$\alpha = 10^7$: weight of the restraint in the energy function

    - Calculated ϕ-value of residue i:

$$\phi_i^{calc}(t) = N_i(t) / N_{i,nat}$$

$N_i(t)$: Number of native contacts made by each side - chain atom of residue i

$N_{i,nat}$: Number of native contacts in the native state

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- Transition state ensembles determination
  - No ensemble-averaged simulations
    - Experimental ϕ-values are measured as ensemble averages
    - « Ensemble-averaged simulations in which the experimental values are imposed as restraints simultaneously on M copies of the protein give the same results as the method that we used here in cases where parallel folding pathways are not present. »
    - No experimental evidence of parallel folding pathways for the proteins of this study
  - A model of the TSE of each protein is obtained by clustering all the structures and selecting representative ones

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- Transition state ensembles determination
  - Results

Table 1. Properties of the TSEs for the ten proteins considered here

PDBid	Common name	Fold type	N_{res}	$N_{\phi^{exp}}$	$\langle\phi_{exp;i}\rangle$	$\langle\phi_{calc;i}\rangle$	RMSD (Å)	k_f	C (NS)	C (TSE)
1aps	Acylphosphatase	α/β	98	22	0.30	0.26	6.4	0.23	0.211	0.156±0.013
1ten	Tenascin	β	89	25	0.28	0.23	7.1	2.9	0.170	0.103±0.017
1imq	Im9	α	89	19	0.29	0.23	8.2	1445	0.115	0.097±0.014
2ci2	CI2	α/β	65	35	0.23	0.20	8.5	48	0.151	0.081±0.016
1lmb4	λ Repressor	α	92	7	0.63	0.31	7.1	4900	0.075	0.063±0.013
2ptl	Protein L	α/β	64	42	0.32	0.28	5.7	60	0.173	0.106±0.014
1fmk	src SH3	β	57	25	0.36	0.28	8.5	57	0.197	0.117±0.019
1bk2	α-Spectrin SH3	β	57	16	0.35	0.27	5.6	76	0.202	0.130±0.024
1shf	Fyn SH3	β	59	12	0.42	0.32	9.7	94	0.184	0.104±0.008
1bf4	Sso7d	β	63	17	0.29	0.27	7.8	1043	0.124	0.094±0.014

N_{res} is the number of residues in the protein. $N(\phi^{exp})$ is the number of ϕ^{exp} values available for each protein. The average $\langle\phi_i^{exp}\rangle$ is computed using the $N(\phi^{exp})$ residues while the average $\langle\phi_i^{calc}\rangle$ is computed for the calculated TSE for all the residues that have native side-chain contacts, whether or not the ϕ values have been measured. The RMSD is relative to the native state structure in each case. The folding rate constants are in s^{-1}.

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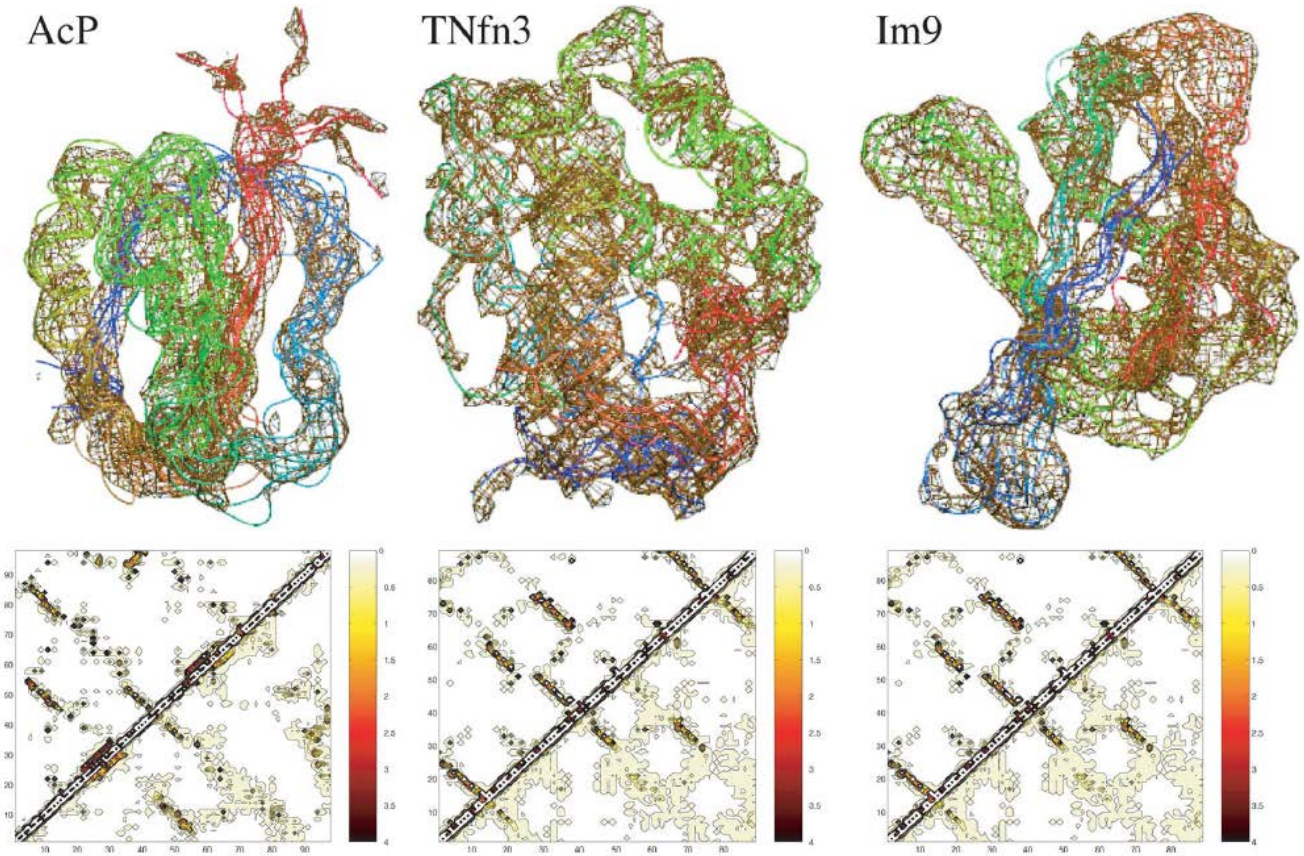
  - Results



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- Contact order calculation and analysis
  - Definition
    - Native state contact order: $C(NS) = \frac{1}{LN} \sum_{j>i} C_{ij} S_{ij}$
    - Transition state contact order: $C(TSE) = < \frac{1}{LN} \sum_{j>i} C_{ij} S_{ij} >$

C_{ij} : contact map for the residues i and j

$S_{ij} = | i - j |$: separation between residues i and j along the chain

L : length of protein

N : number of contacts

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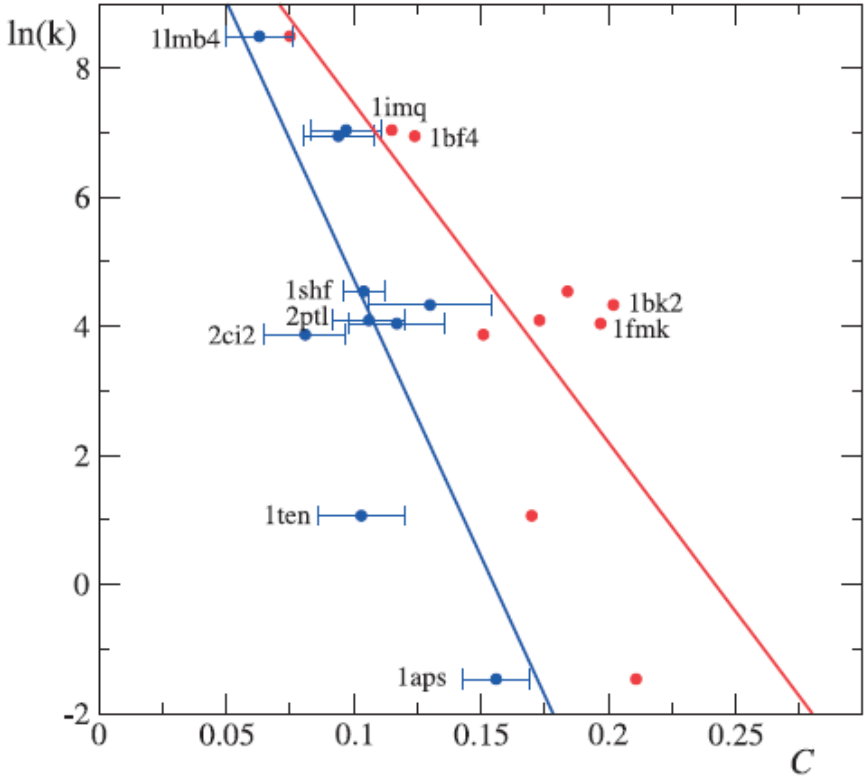
- Contact order calculation and analysis
  - Correlation between ln(k) and C



$$R_{NS} = -0.79\ (p < 0.01)$$

$$R_{TSE} = -0.76\ (p < 0.01)$$

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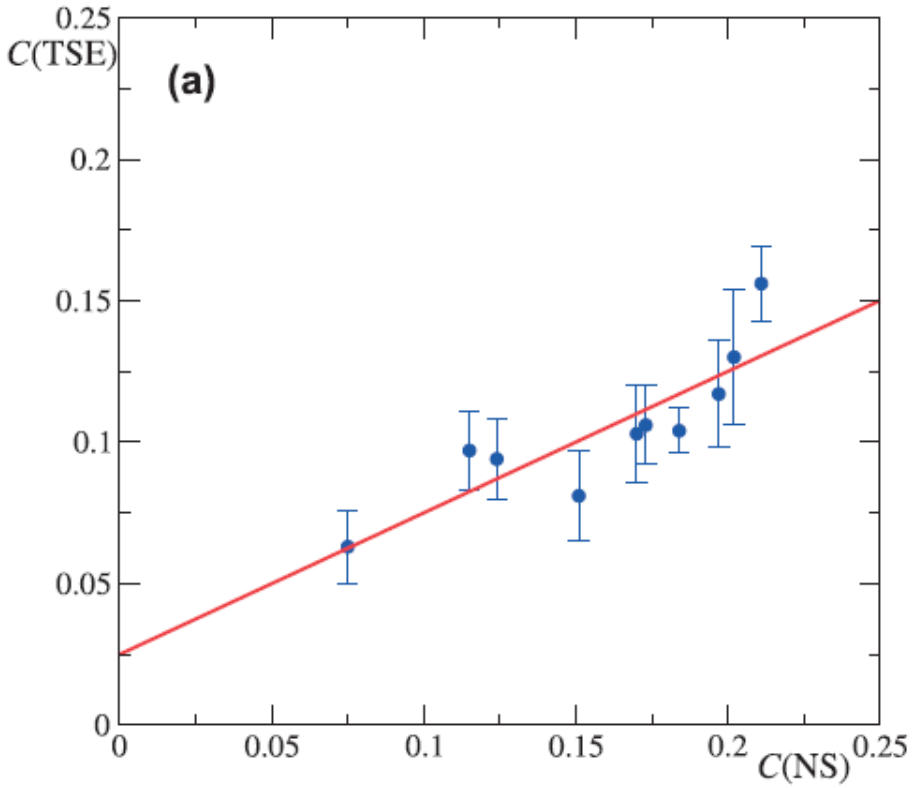
- Contact order calculation and analysis
  - Correlation between C(TSE) and C(NS)



$$R = 0.85$$

$$slope = 0.5$$

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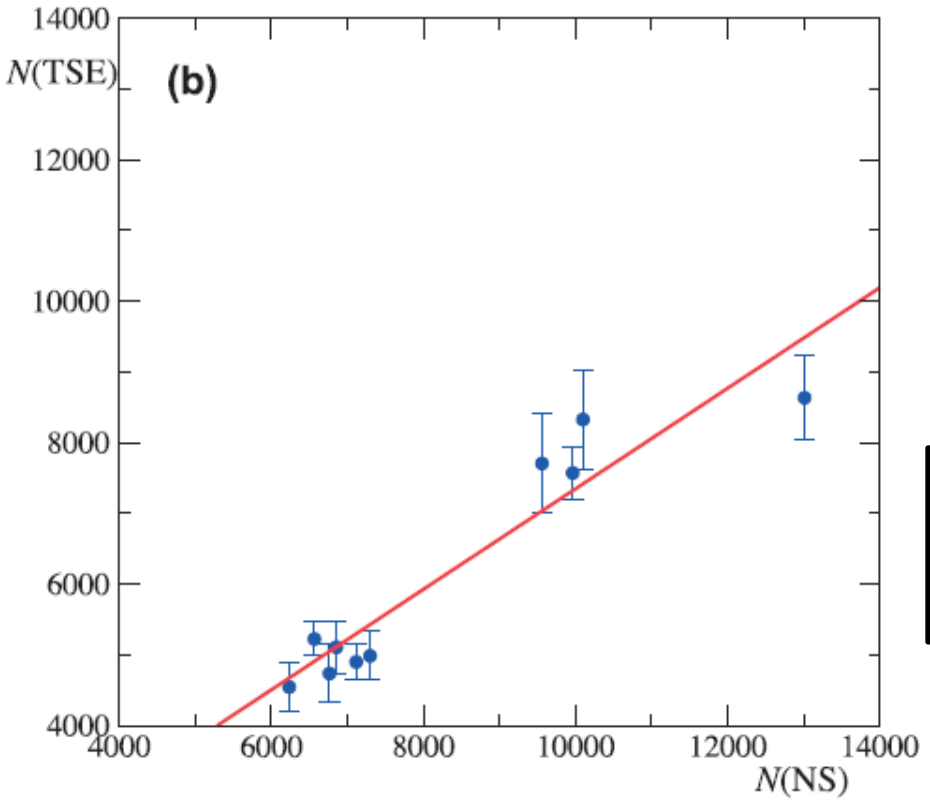
- Contact order calculation and analysis
  - Correlation between N(TSE) and N(NS)



$$R = 0.95$$

$$slope = 0.71$$

« C(NT) varies by less than 2%
if a random subset (of about 30%)
of contacts is removed »

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- Conclusions
  - C(TSE) is proportional to, but lower than C(NS)

    → the structures of the TSE are less compact/organized than those of the NS
  - C(TSE) and C(NS) correlate similarily to ln(k) eventhough N(TSE) is only about 70% of N(NS)

    → the key contacts of the native structure are already present in the TSE

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- Conclusions
  - The results **show how the contact order captures the loss in entropy** resulting from the formation of these key interactions in the transition state and leads to the observed inverse correlation with the folding rates.
  - In addition, they **illustrate the general value of using experimental restraints** in molecular dynamics simulations to determine ensembles of protein structures in atomic-level detail.

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- Introduction
  - The dominant folding pathways are the ones that maximize formation of native interactions and minimize the configurational entropy → altering the foldig mechanism may be possible by changing the stability of the native substructures
  - Promising candidates are proteins with structural symmetry as they may have several folding routes with energy barriers close in magnitude

S. Nauli *et al*., **Computer-based redesign of protein folding pathway**
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- Introduction
  - IgG binding domain of protein G and protein L is an α-helix packed against a four-stranded β-sheet formed by two symetrically opposed β-hairpins
  - The symmetry of the two proteins is broken during folding:
    - In protein G : the 2nd β-hairpin is formed and the 1st is disrupted at the rate-limiting step
    - In protein L : the 1st β-hairpin is formed and the 2nd is disrupted at the rate-limiting step

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- Introduction
  - Hypothesis: formation of the β-hairpin of lower free energy occurs at the rate limiting step
  - Test: switch the folding mechanism of protein G to that of protein L by increasing the stability of the 1st hairpin and decreasing that of the 2nd hairpin

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- Design
  - Goal 1: Increase the stability of the 1st hairpin by removing positive φ angles and including a more favorable type I′ or II′ β-turn in place of the native type I β-turn.
  - Procedure:
    - Identify alternative conformations of the hairpin by searching PDB for hairpin conformations with termini that superimpose with residues 7 and 16 (strands 1 and 2)
    - Graft the new β-turns → Creates 322 alternative hairpin conformations
    - Determine low free energy sequences for each alternative
    - Consider the alternative sequences only for residues 6-16 and determine the lowest energy sequence-structure combination

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- Design
  - Lowest energy sequence-structure combination
    - containing a type I′ turn : NuG2

$$E_{LJ} = -25.8\,\text{kcal/mol} \quad \text{VS} \quad E_{LJ}(WT) = -19.4\,\text{kcal/mol}$$

    - containing a type II′ turn : NuG1

$$E_{LJ} = -23.6\,\text{kcal/mol} \quad \text{VS} \quad E_{LJ}(WT) = -19.4\,\text{kcal/mol}$$

Table 1 Sequences of designed proteins[1]

WT	6-ILNG**KT**LKGET-16
NuG1	6-**FIVIGD**RVVVV-16
NuG2	6-VIVL**NG**TTFTY-16

[1]Residues 1–5 and 17–57 are identical to wild type protein G. Turn residues are shown in bold. All variants of protein G were made and purified using published methods[9]. The identity of the proteins were verified by mass spectrometry.

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- Design
  - Goal 2: reduce the stability of the 2nd hairpin by mutating Asp 46 to Ala 46
    - in NuG1 → NuG1/D46A
    - In NuG2 → NuG2/D46A

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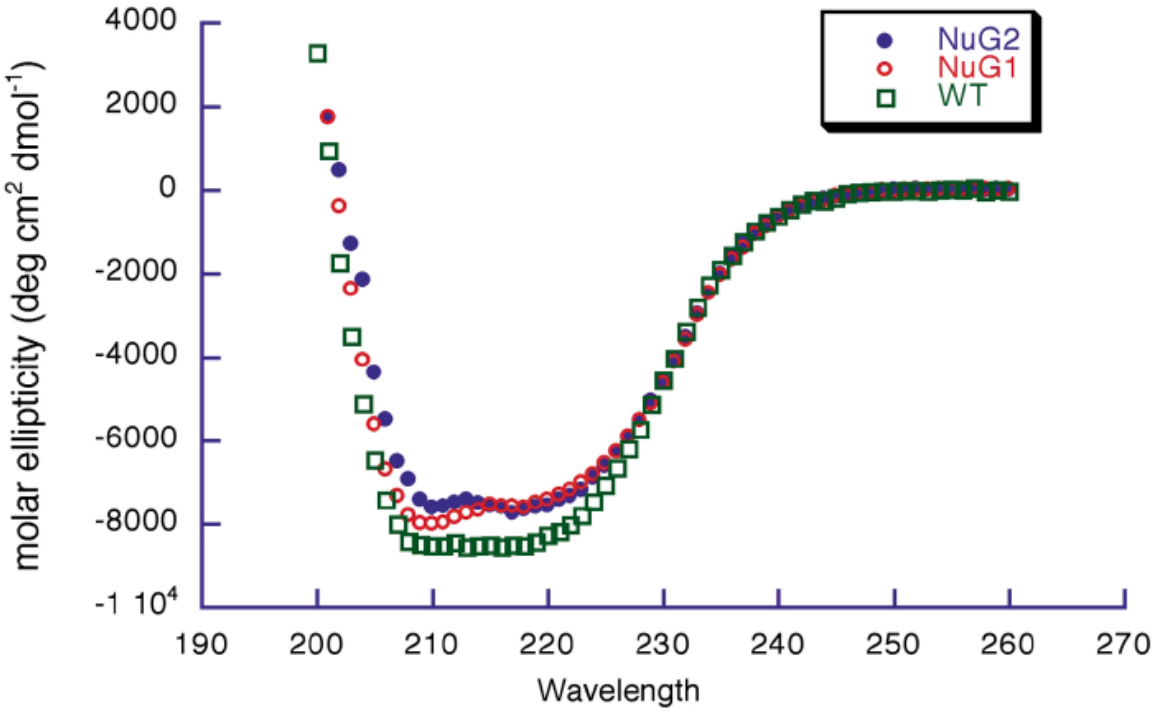
- Experimental study
  - Circular dichroism spectral signature of NuG1 and NuG2 are nearly identical to that of the wild-type



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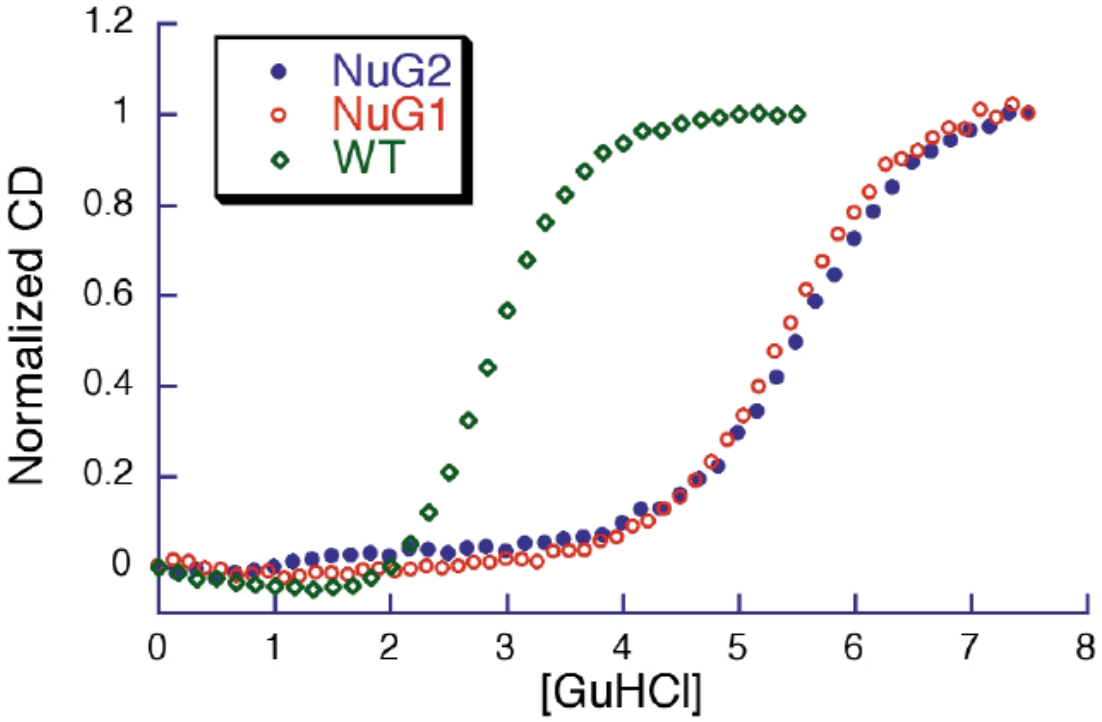
- Experimental study
  - NuG1 and NuG2 exhibits native-like cooperative unfolding and are dramatically stabilized



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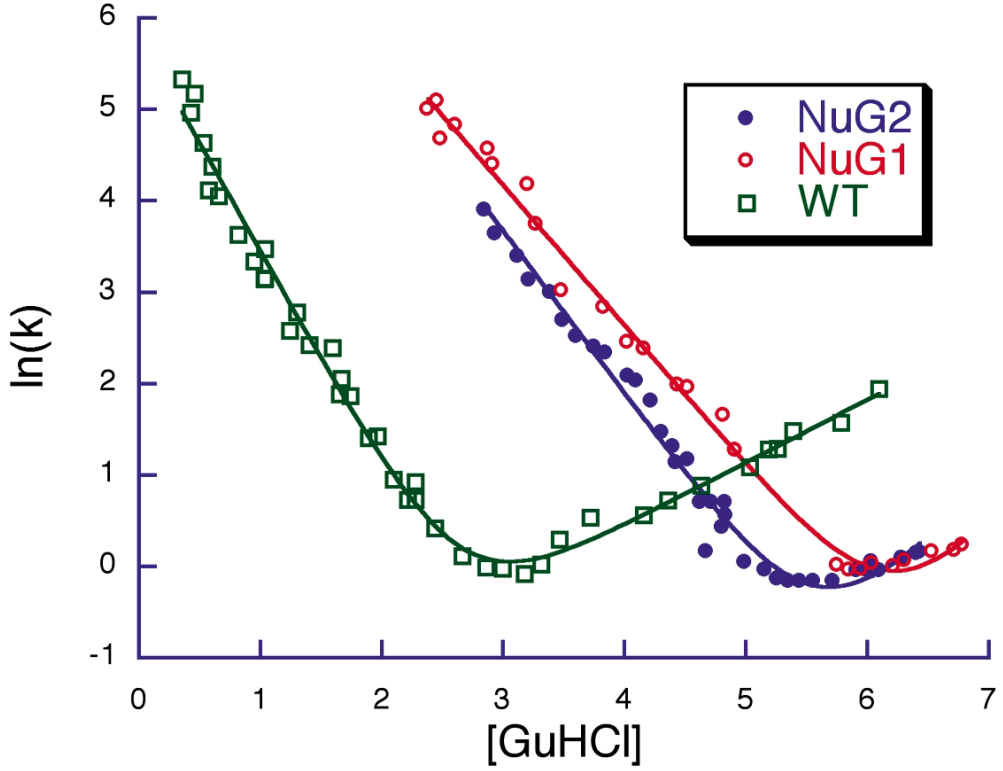
- Experimental study
  - NuG1 and NuG2 fold 100-fold faster than wild-type



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- Experimental study
  - Mutations in the two β-turns have opposite effects on the folding and unfolding rates of NuG1/D46A compared to the wild-type

ϕ-values of wild type and redesigned proteins

	$k_f^{1.5M}$	$k_f^{2.5M}$	k_u^{6M}	$\phi^{1.5M}$	$\phi^{2.5M}$
WT	9.6	0.89	6.2	–	–
T11A/WT	12.5	1.4	19	–0.30	–0.58
T49A/WT	3.0	0.22	5.4	1.1	1.1
NuG1/D46A	200	30	4.5	–	–
NuG1/D46A/G10A	52	10	7.4	0.73	0.68
NuG1/D46A/D11A	71	13	5.1	0.89	0.86
NuG1/D46A/T49A	86	21	24	0.33	0.18
NuG2/D46A	110	19	2.3	–	–
NuG2/D46A/N10A	57	9.2	1.6	2.1	2.0
NuG2/D46A/G11A	17	2.5	1.4	1.4	1.4
NuG2/D46A/T49A	55	12	8.6	0.35	0.26

[1]Thermodynamic and kinetic parameters were calculated as detailed in the Methods section.
[2]In kcal mol^{-1}.

T49 : mutation in the 2nd hairpin
G10A : mutation in the 1st hairpin

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- Experimental study
  - Mutations in NuG2/D46A also indicate a switched folding mechanism with the difference that mutations in the first turn slow the unfolding rate

ϕ-values of wild type and redesigned proteins

	$k_f^{1.5M}$	$k_f^{2.5M}$	k_u^{6M}	$\phi^{1.5M}$	$\phi^{2.5M}$
WT	9.6	0.89	6.2	–	–
T11A/WT	12.5	1.4	19	–0.30	–0.58
T49A/WT	3.0	0.22	5.4	1.1	1.1
NuG1/D46A	200	30	4.5	–	–
NuG1/D46A/G10A	52	10	7.4	0.73	0.68
NuG1/D46A/D11A	71	13	5.1	0.89	0.86
NuG1/D46A/T49A	86	21	24	0.33	0.18
NuG2/D46A	110	19	2.3	–	–
NuG2/D46A/N10A	57	9.2	1.6	2.1	2.0
NuG2/D46A/G11A	17	2.5	1.4	1.4	1.4
NuG2/D46A/T49A	55	12	8.6	0.35	0.26

[1]Thermodynamic and kinetic parameters were calculated as detailed in the Methods section.
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T49 : mutation in the 2nd hairpin
G10A : mutation in the 1st hairpin

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- Introduction
- Design
- Experimental study
- Conclusions

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- Conclusions
  - Substantial increases in protein stability can be achieved by optimizing the backbone conformation and sequence using computational protein design approach
  - Protein folding pathways can be switched by changing the stability of local structural elements

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- Conclusions
  - As the topology of the native state is determinant of protein folding rates and mechanisms,

    If seveal different folding pathways are equally consistent with the native state topology, the route involving formation of the substructures with the most favorable interactions is selected