Thermodynamic and Kinetic Characterization of Folding Mechanisms of CheY Circular Permutants

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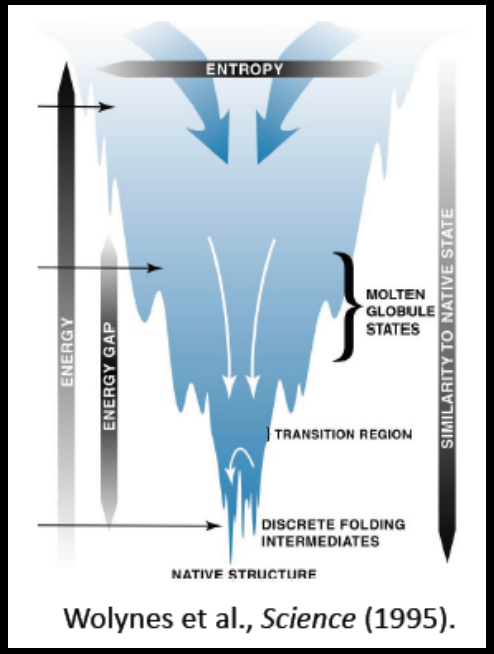
Sequence-dependent folding behavior

Free Energy Landscape Theory

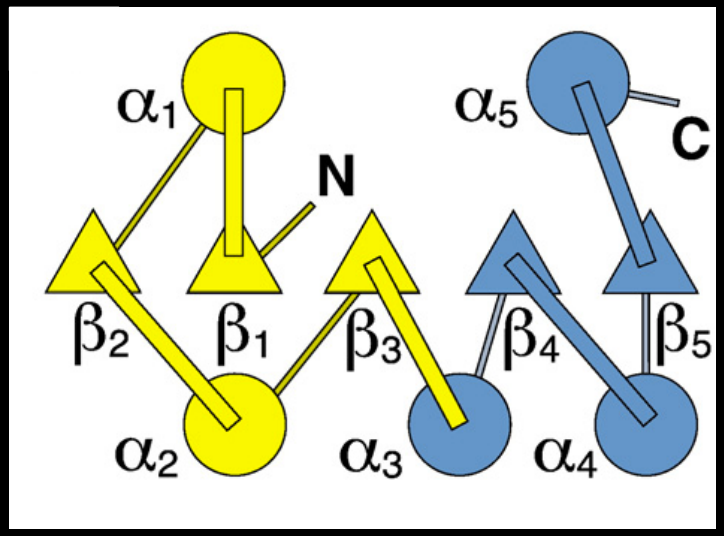
- Folding characterized by free energy minima
- Analyze at T_f to see basins of unfolded and native state



Wolynes et al., *Science* (1995).

CheY protein

- $(\alpha\beta)_5$
- How do cuts between secondary structures affect folding?
- Inform protein engineering



Hills and Brooks. *JMB (*2008) 382, 485-495

Three Circular Permutant Systems

CpB2: Cut between residues 35-36

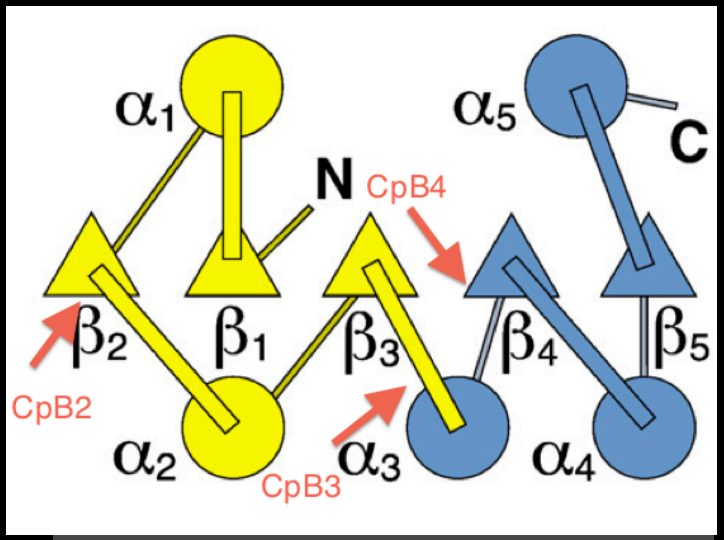
- End of β_2 in N-terminal

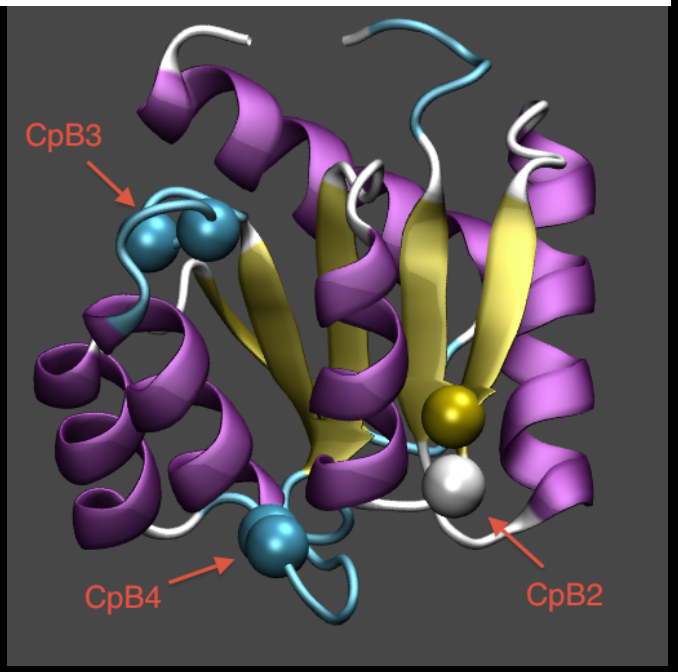
CpB3: cut between residues 62-63

- In turn between $\beta_3\alpha_3$; splits C-terminal and N-terminal

CpB4: cut between residues 77-78

- In turn between $\alpha_3\beta_4$ in C-terminal





The Go-Model

- Residues as C_α 'beads'
- Native contacts:
  - Side-chain side-chain interactions
  - Backbone H-bonds
- Lennard-Jones potential for *i - j > 2*

$$V_{ij} = \epsilon_{ij}[13(\frac{\sigma_{ij}}{r_{ij}})^{12} - 18(\frac{\sigma_{ij}}{r_{ij}})^{10} + 4(\frac{\sigma_{ij}}{r_{ij}})^{6}]$$

- Non-native repulsion

$$V_{ij} = \epsilon_{ij}[13(\frac{\sigma_{ij}}{r_{ij}})^{12}]$$

- This simple model has been successfully applied to investigate folding mechanisms of several proteins in the past

Karanicolas and Brooks. *Prot Sci (*2002) 11:2351

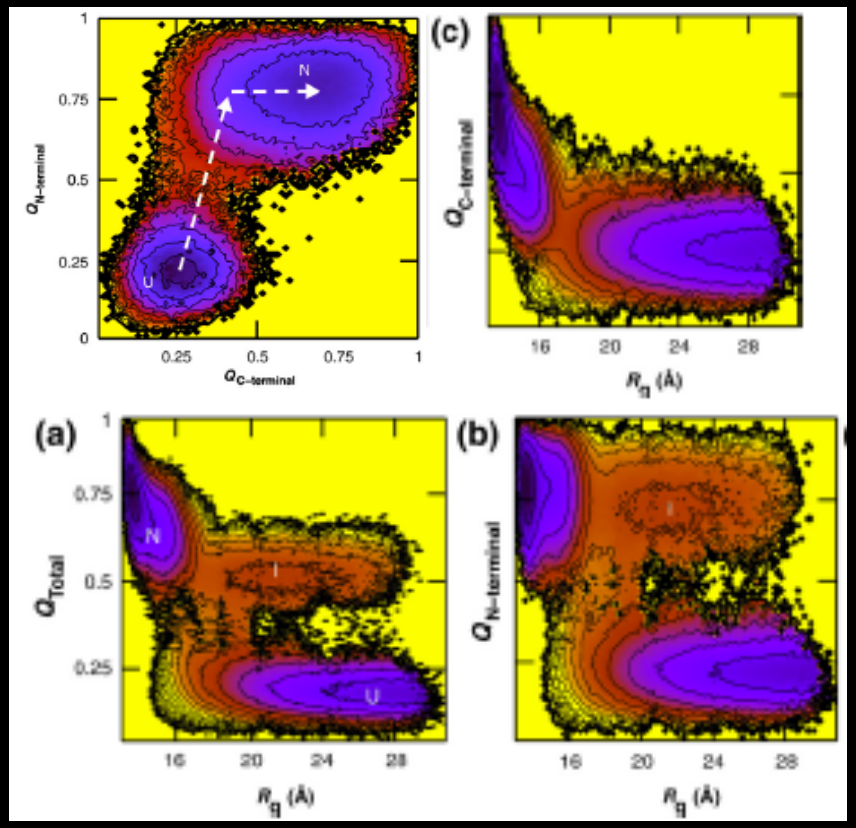
Simulation Details

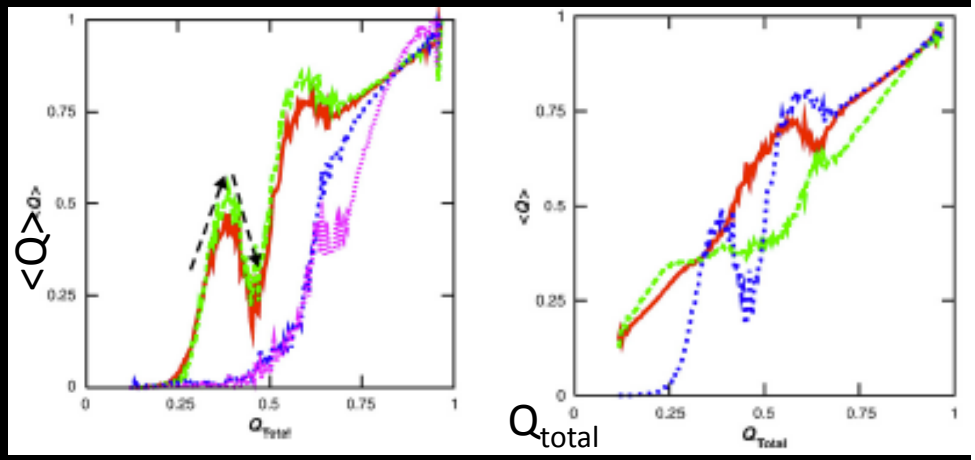
- Thermodynamic - Replica Exchange
  - 10,000 exchange cycles (exchange every 20,000 steps)
  - 4 temperature windows (.87 T_f, .97 T_f, 1.08 T_f, 1.20 T_f)
  - Umbrella bias: 8 rgyr biases spanning 1.0 $R_g^{\boldsymbol{0}}$ to 2.0 $R_g^{\boldsymbol{0}}$
  - Using *gorex.pl* from the MMTSB toolset
  - Unbiased data using WHAM

- Kinetic
  - 48 independent unbiased MD simulations at .87 T_f for 2 x 10^8 time steps
  - Unfolded state obtained from heating to 1.5 T_f
  - Time step is 22fs
  - Langevin Dynamics

Folding of CheY[1]

- N-terminus nucleation
- Frustration in $\alpha_2\beta_3\alpha_3\beta_4$ tetrad
- C-terminus folding is accompanied by loosening of entire structure
- Non-obligate intermediate
  - Structured N-terminus
  - Unstructured C-terminus
- Agrees with experimental results[2]





[1]Hills and Brooks. *JMB (*2008) 382, 485-495
[2]Kathuria, Day, Wallace & Matthews. *JMB* (2008) 382, 467-484

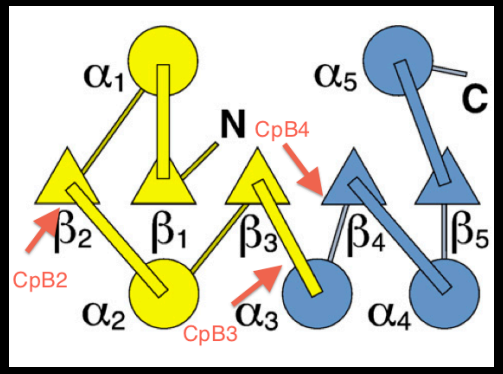
Change in T_f

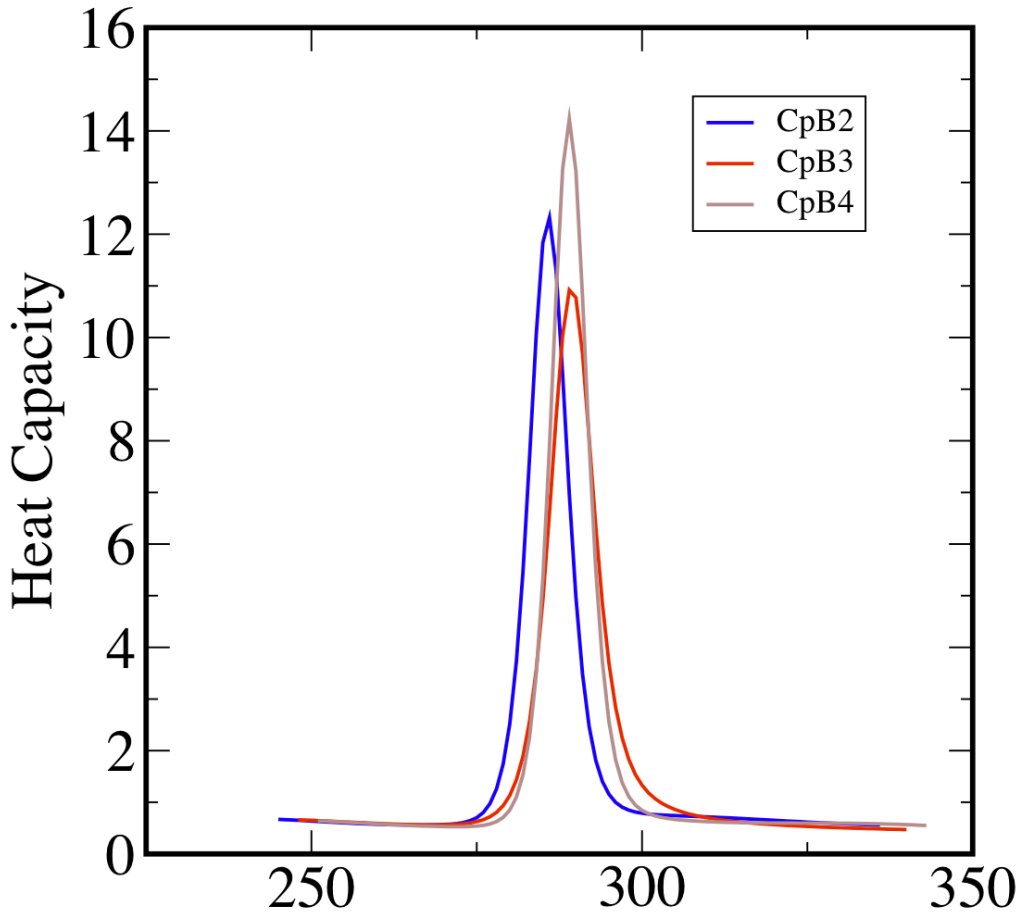
Native State Stability
- T_f at peak

T_f CpB2 = 280 K

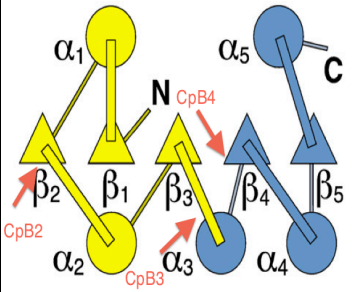
T_f CpB3 = 285 K

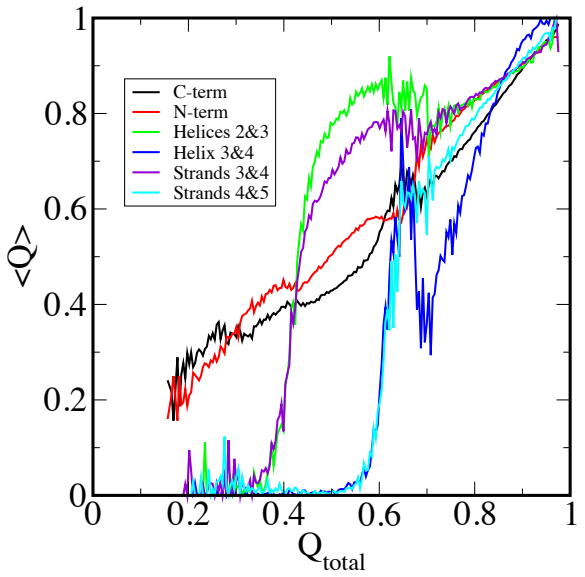
T_f CpB4 = 287 K

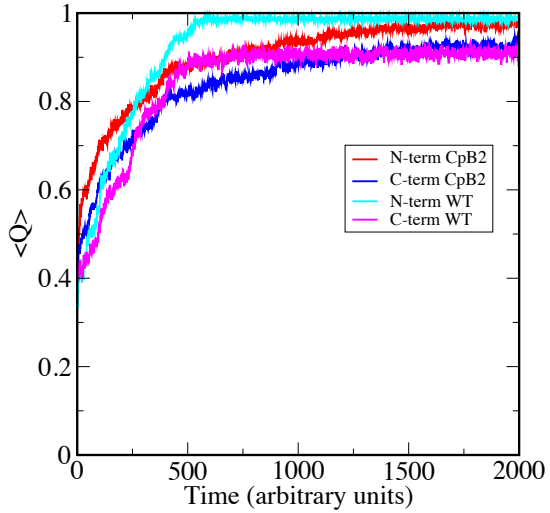


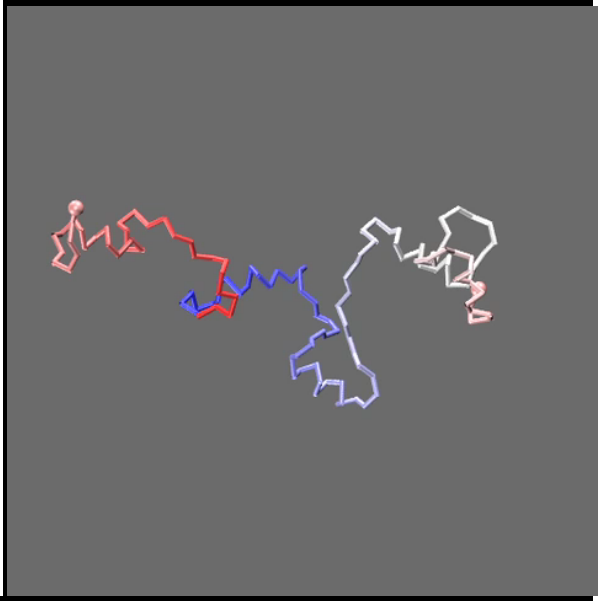


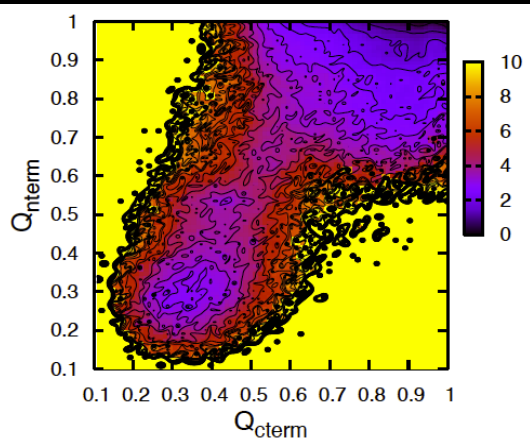
CpB2-Energy Landscape and Folding

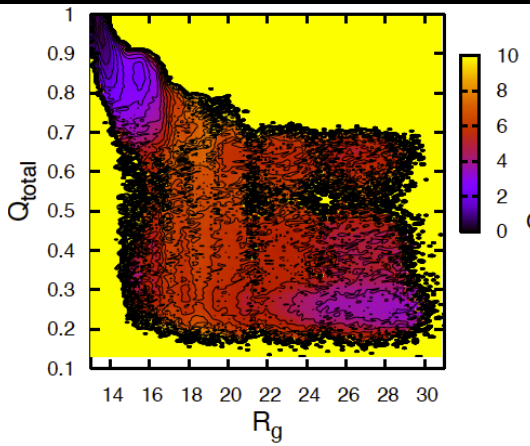


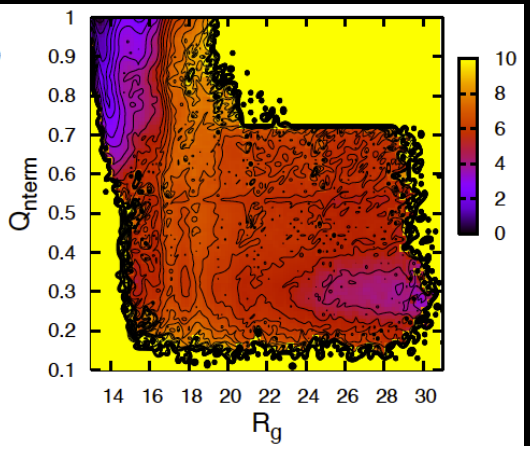




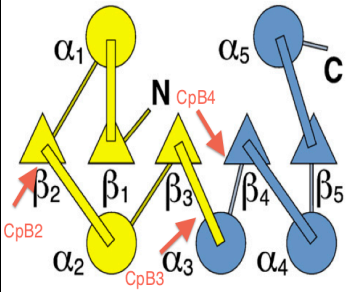


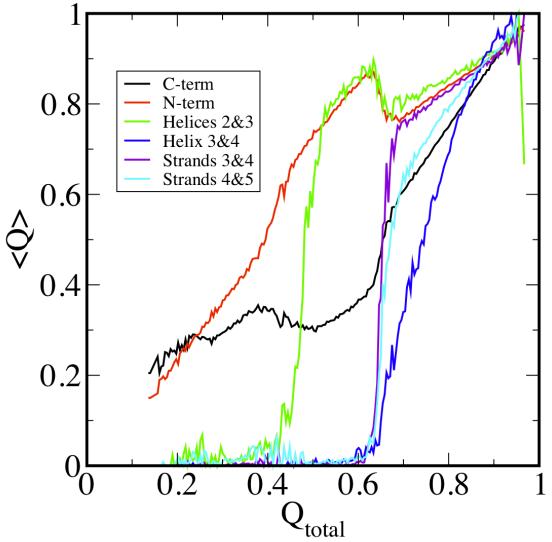


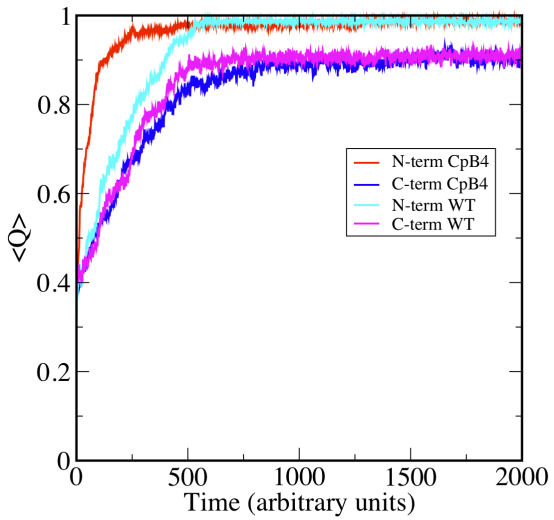


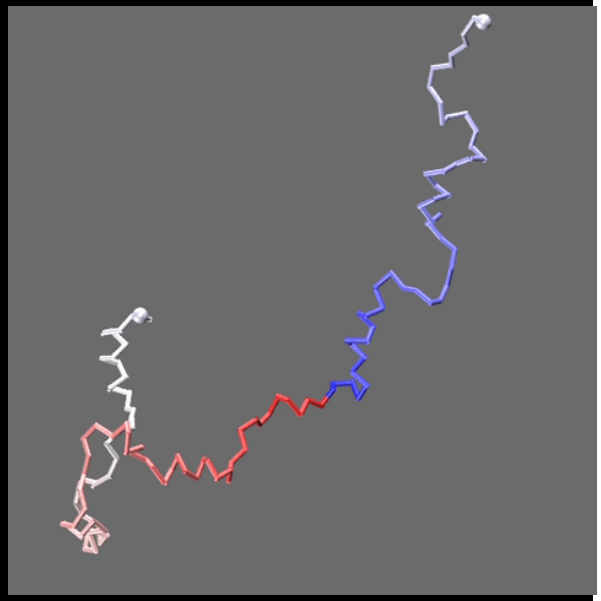


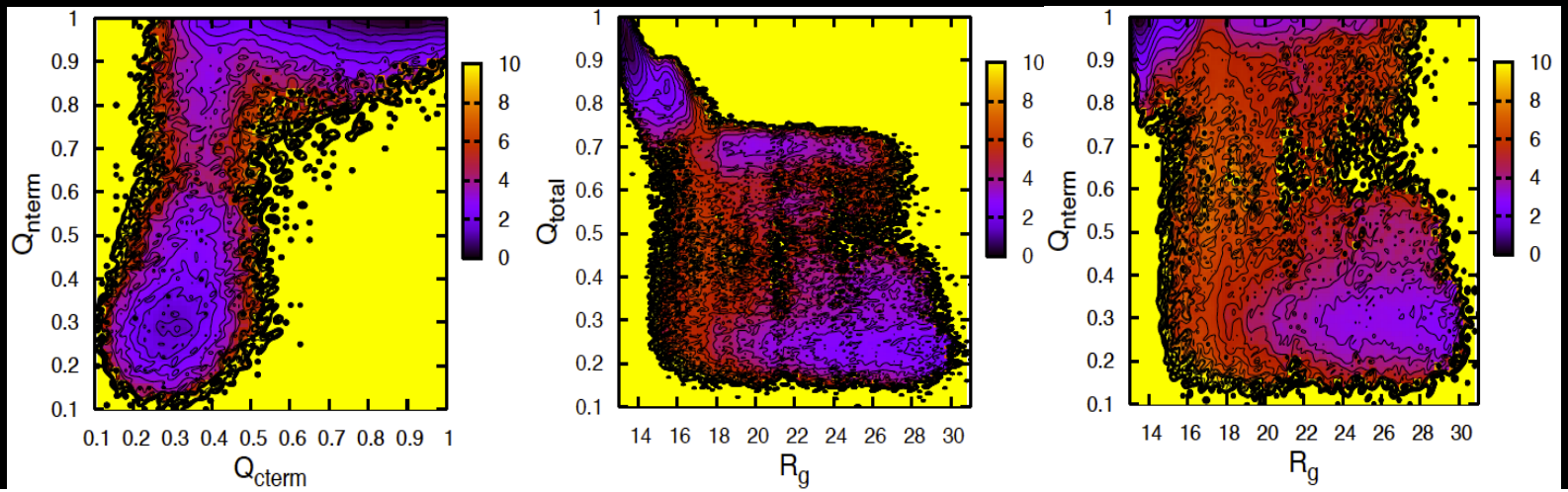
CpB4-Energy Landscape and Folding



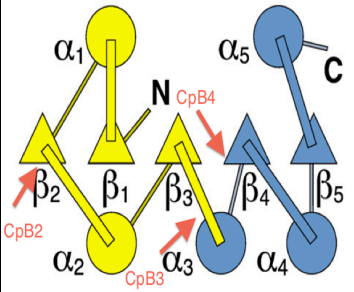


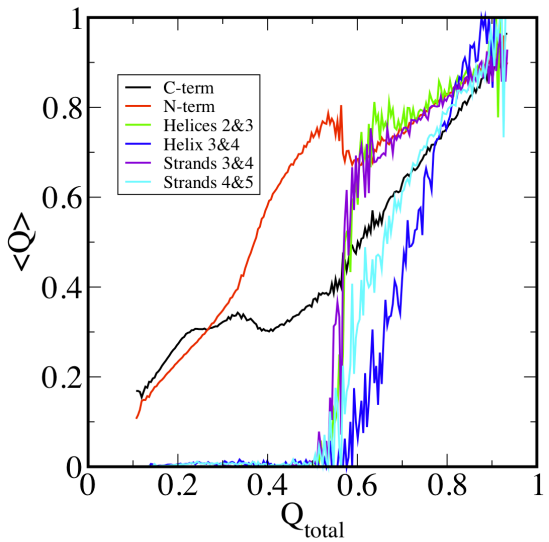


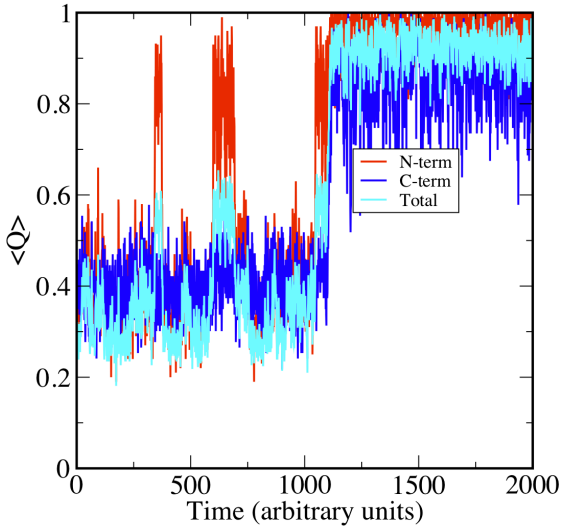


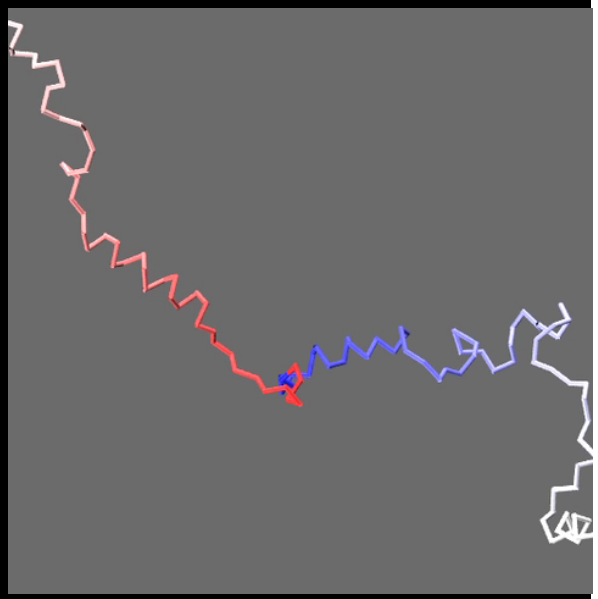


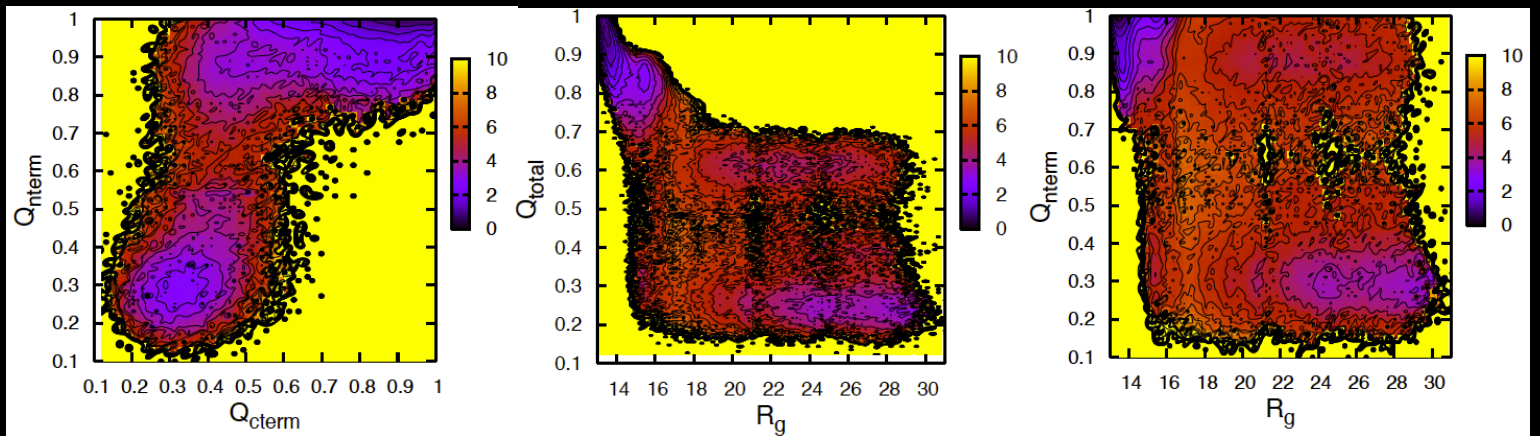
CpB3 – Energy Landscape and Folding (Major Error in Kinetics)











Conclusions

- Simulations predict cut-dependent folding for a constant amino acid sequence
- Cuts change stability of native state
- Location of intermediate on folding pathway may vary (CpB3)
- Suggests possibility of tuning protein folding pathways

Acknowledgements

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