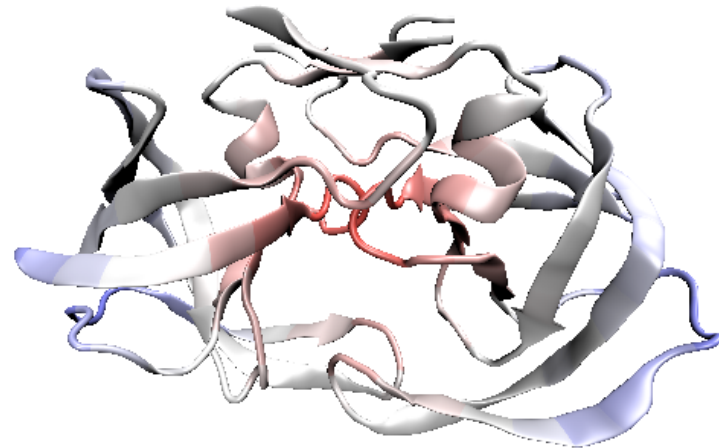
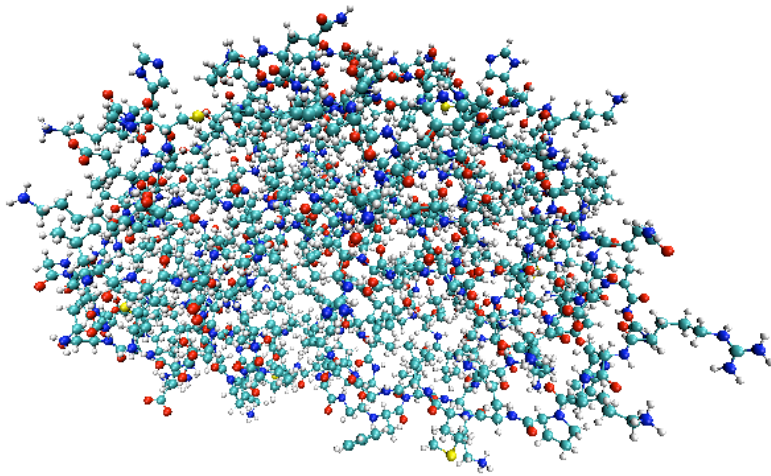


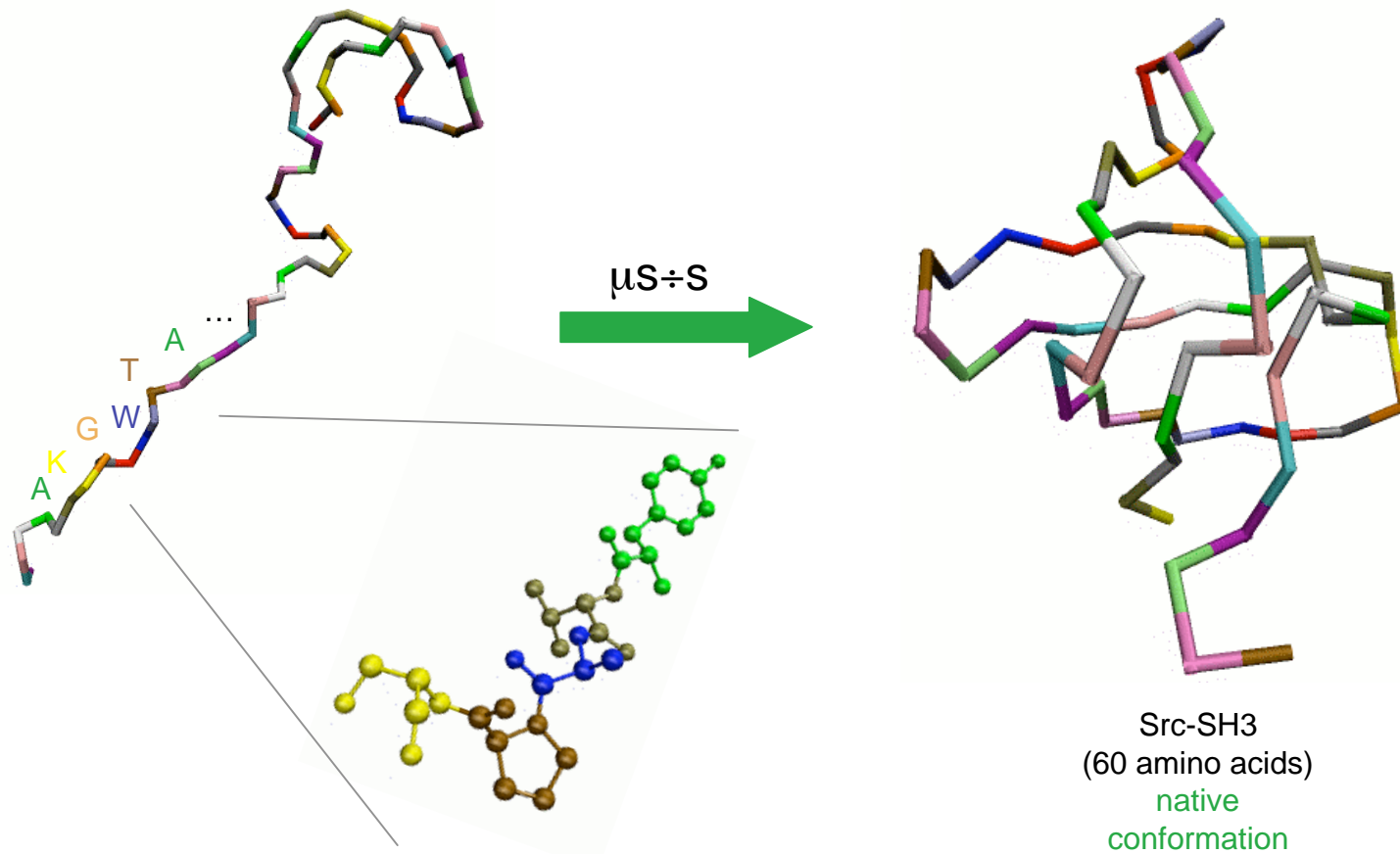
Protein folding: from simplified models to the design of folding inhibitors

Guido Tiana
Department of Physics, University of Milano



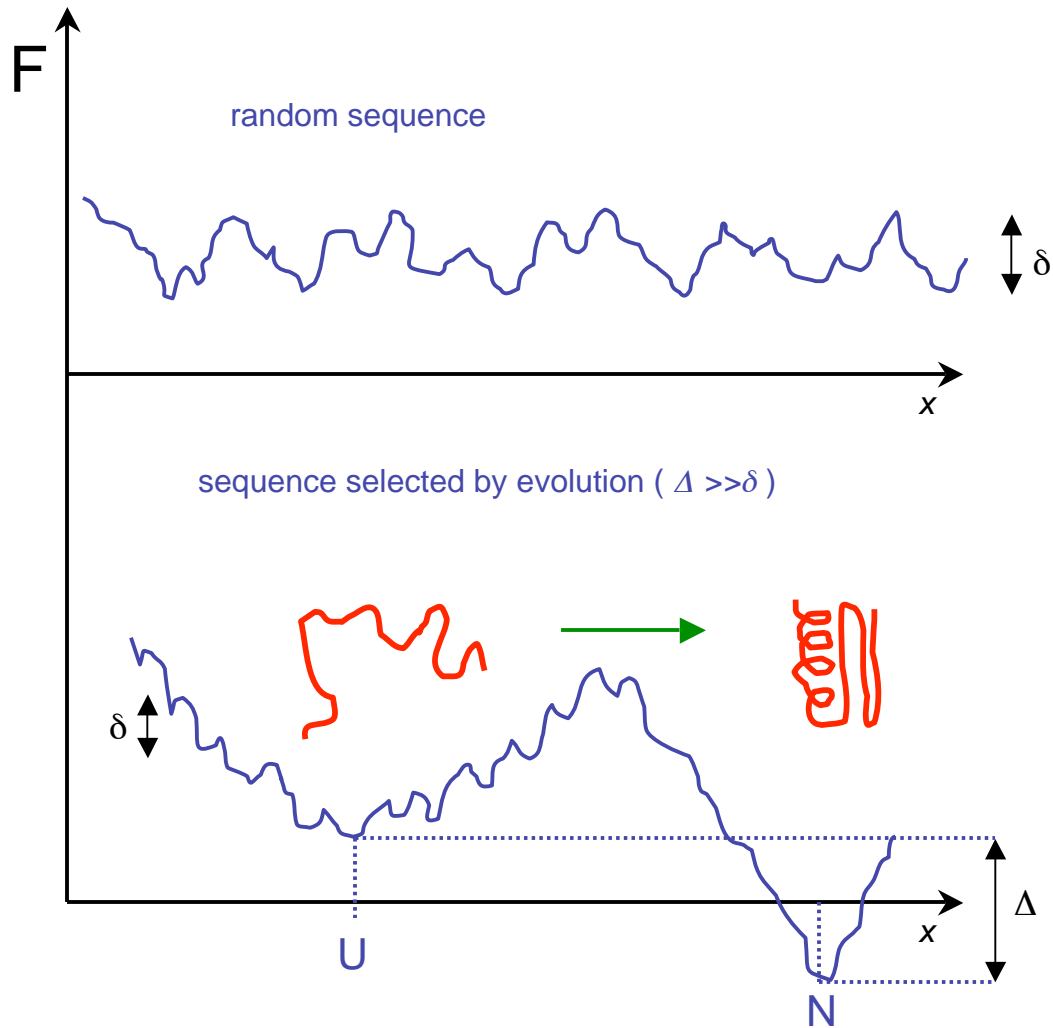
HIV-1 protease

Protein Folding



- questions:
- how can such a complicated system display a unique ($S \approx 0$) equilibrium state?
 - what is the native state of a given sequence?
 - how can folding be so fast?
 - what is the effect of mutation? are there other stable conformations? etc...

The source of all problems: the interactions within proteins are frustrated



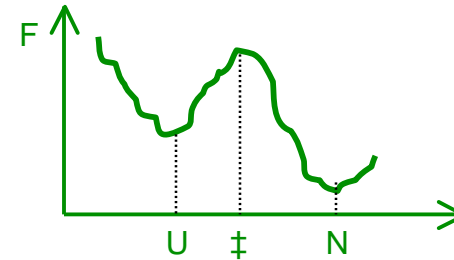
Residual frustration:

- still complicated to predict the N state
- protein marginally stable ($\Delta \approx 10 kT_{\text{room}}$)
- kinetics easily trapped

Ways to answer to the problems of protein folding:

• experiments

- x-ray and NMR to obtain the native structure
- calorimetry (C_v , ΔF_{NU})
- kinetics (fluorescence, CD, deadtime = ms/ μ s)
- mutations + kinetics to characterize the transition state
- recently, advanced NMR to describe the unfolded state



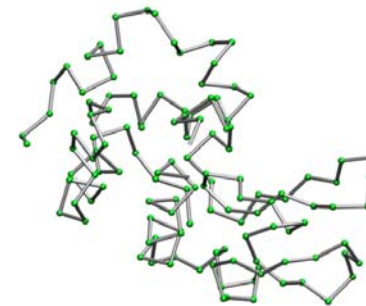
• analytical models

Theory of disordered systems to describe qualitatively the energy landscape.

$$[\log Z] = \lim_{n \rightarrow 0} \frac{[Z^n] - 1}{n}$$

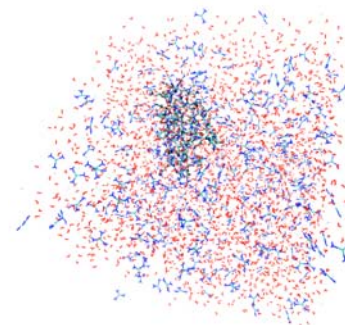
• very-simplified models

Basic features of protein folding (frustration, nucleation, etc.)
Test new ideas
Molecular evolution, protein aggregation, etc.



• simplified models

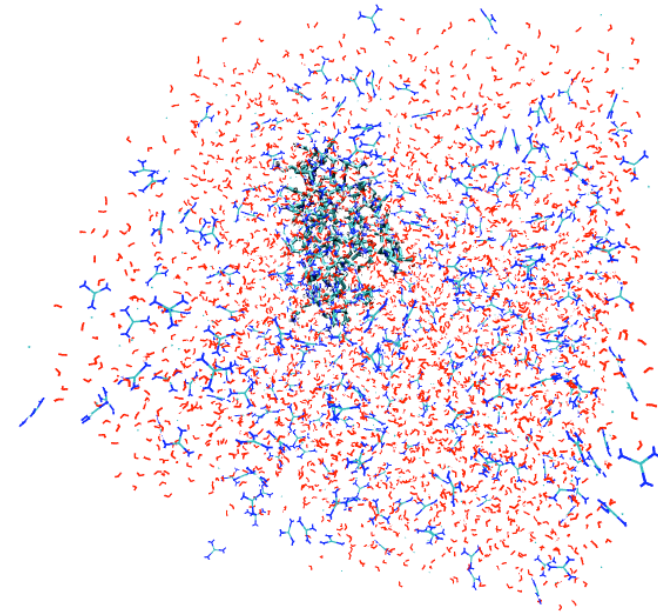
- Simulating conformational changes on ns/ μ s timescale of small proteins (folding/unfolding)
- Optimization algorithms in conformational space (see talk by Federico Fogolari)



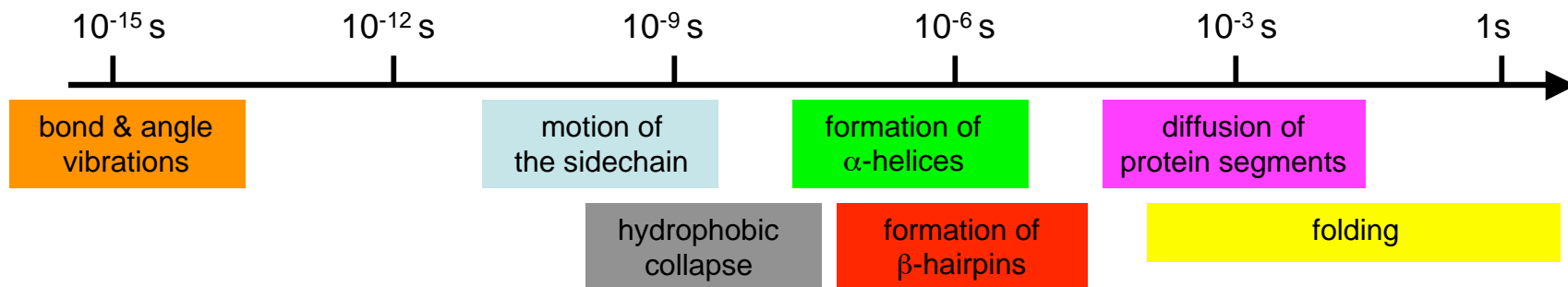
Simplified models

brute-force method: find the properties of a sequence by simulating its dynamics

- an all-atom model, usually with explicit solvent
- a potential (e.g., Gromos, Amber, Charmm)
- a simulation algorithm (e.g., molecular dynamics with heat bath)



problem...



Molecular Model-building by Computer

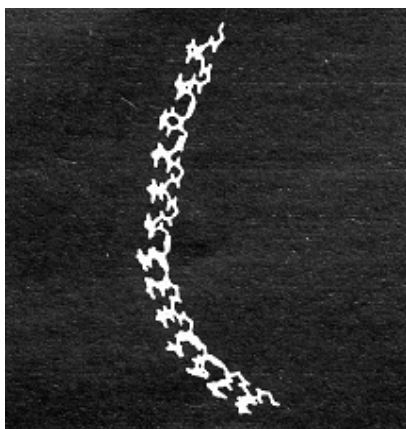
In which biochemists observe models of giant molecules as they are displayed on a screen by a computer and try to fold them into the shapes that they assume in nature

by Cyrus Levinthal

C. Levinthal, Scientific American 214, 42 (1966)



from online Museum of University of Massachusets

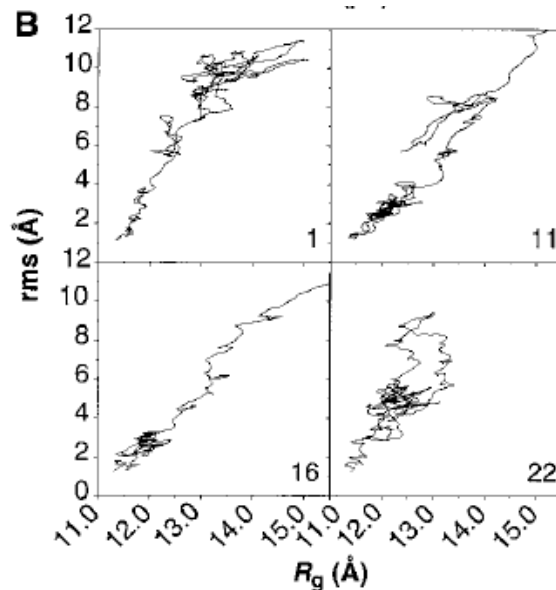
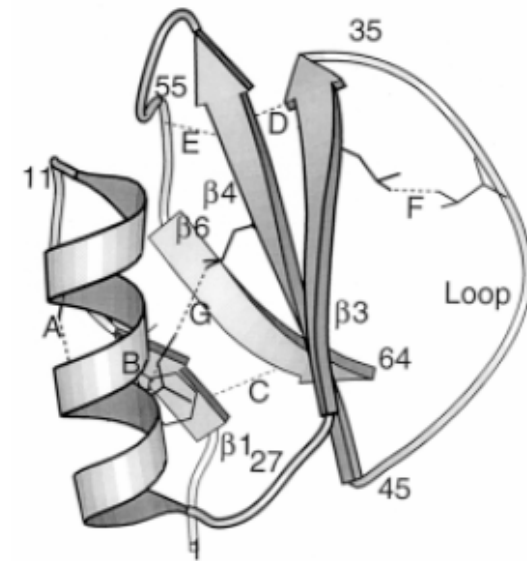
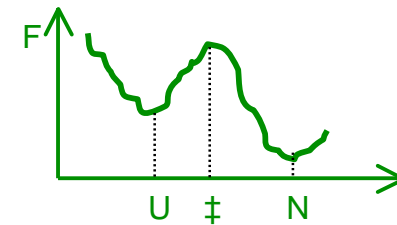


"the individual sections do not bend, but the rotation angles between sections are changed to close the chain into a circle"

“New View” of Protein Folding Reconciled with the Old Through Multiple Unfolding Simulations

Themis Lazaridis and Martin Karplus*

Twenty-four molecular dynamics trajectories of chymotrypsin inhibitor 2 provide a direct demonstration of the diversity of unfolding pathways. Comparison with experiments suggests that the transition state region for folding and unfolding occurs early with only 25 percent of the native contacts and that the root-mean-square deviations between contributing structures can be as large as 15 angstroms. Nevertheless, a statistically preferred unfolding pathway emerges from the simulations; disruption of tertiary interactions between the helix and a two-stranded portion of the β sheet is the primary unfolding event. The results suggest a synthesis of the “new” and the classical view of protein folding with a preferred pathway on a funnel-like average energy surface.



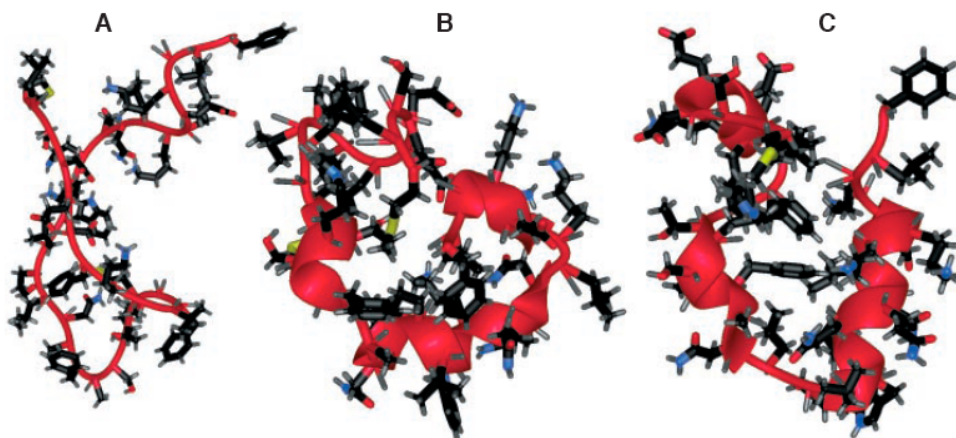
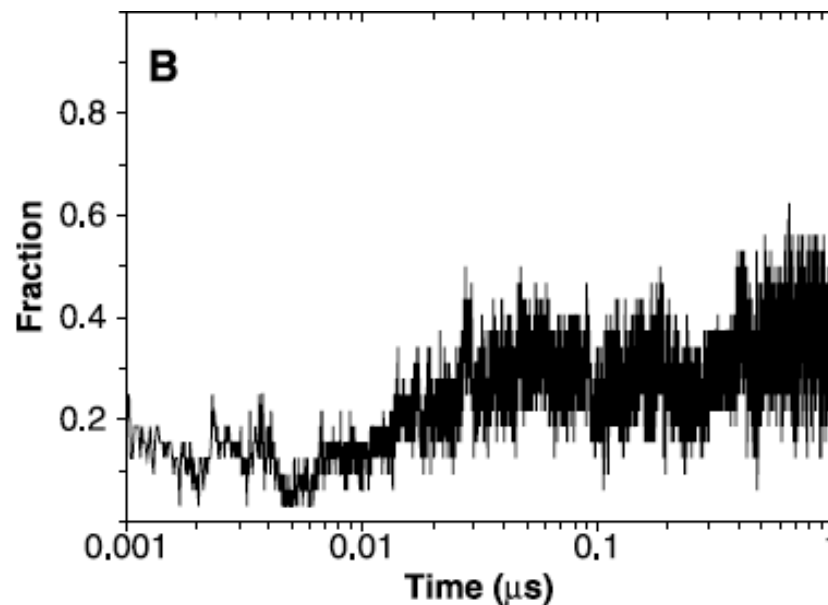
24 unfolding simulations of 200 ps at 500K (implicit solvent)

- stochasticity of unfolding trajectories
- the protein at the transition state (“folding nucleus”) displays well-defined contacts

Pathways to a Protein Folding Intermediate Observed in a 1-Microsecond Simulation in Aqueous Solution

Yong Duan and Peter A. Kollman*

An implementation of classical molecular dynamics on parallel computers of increased efficiency has enabled a simulation of protein folding with explicit representation of water for 1 microsecond, about two orders of magnitude longer than the longest simulation of a protein in water reported to date. Starting with an unfolded state of villin headpiece subdomain, hydrophobic collapse and helix formation occur in an initial phase, followed by conformational readjustments. A marginally stable state, which has a lifetime of about 150 nanoseconds, a favorable solvation free energy, and shows significant resemblance to the native structure, is observed; two pathways to this state have been found.



1 μs of villin headpiece
(36 amino acids & 3000 water molecules)

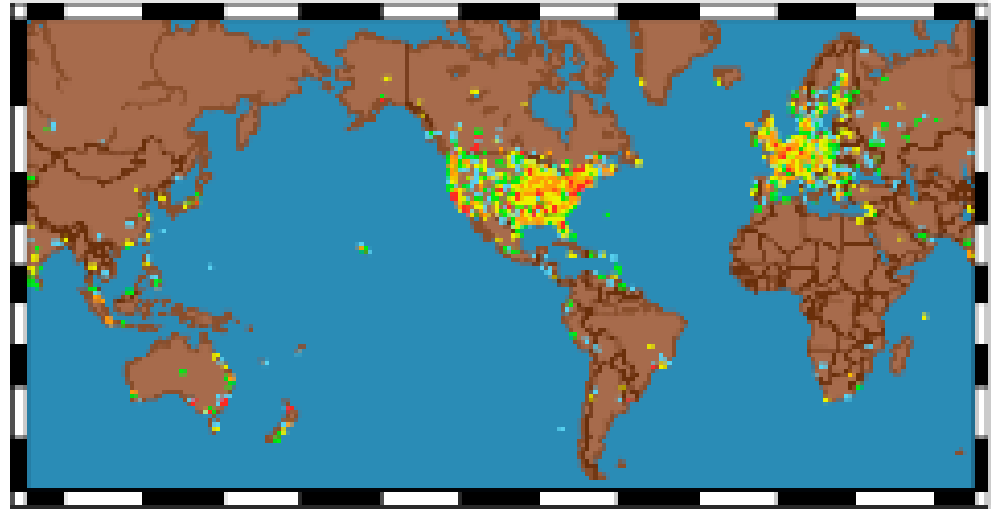
256 cpu on Cray T3E
it took 2 months

→ no sound kinetic information
(nor thermodynamic...)

↓
see talk by Giovanni Bussi

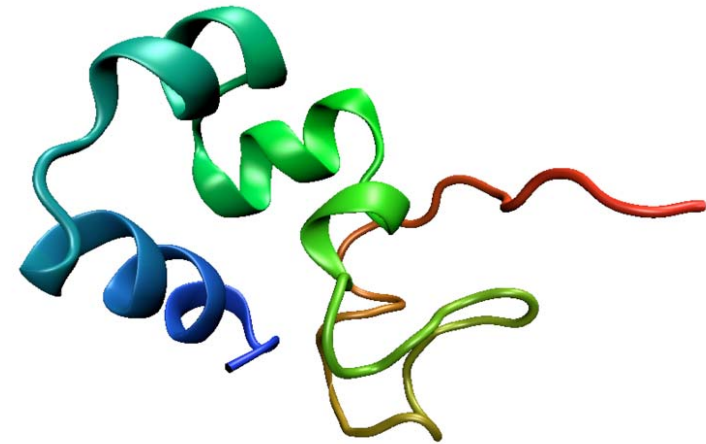
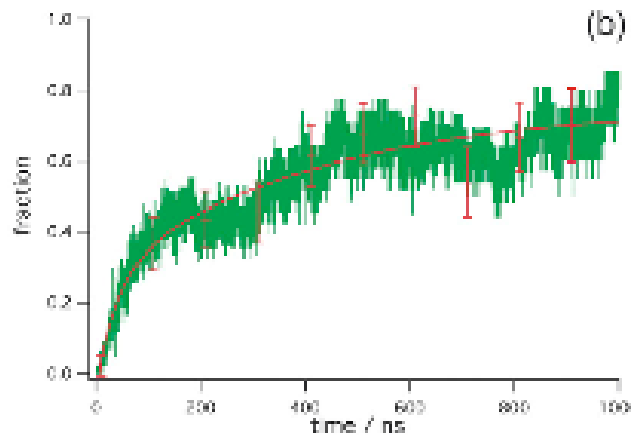
Folding@home

many independent folding events (parallelization
only on core-duo processors, MPI)



It contains several projects, the most ambitious is long-time folding:

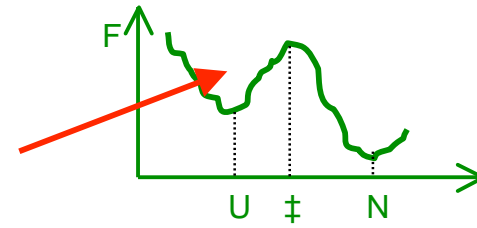
villin headpiece (36 amino acids), again;
410 trajectories of average length 863 ns.



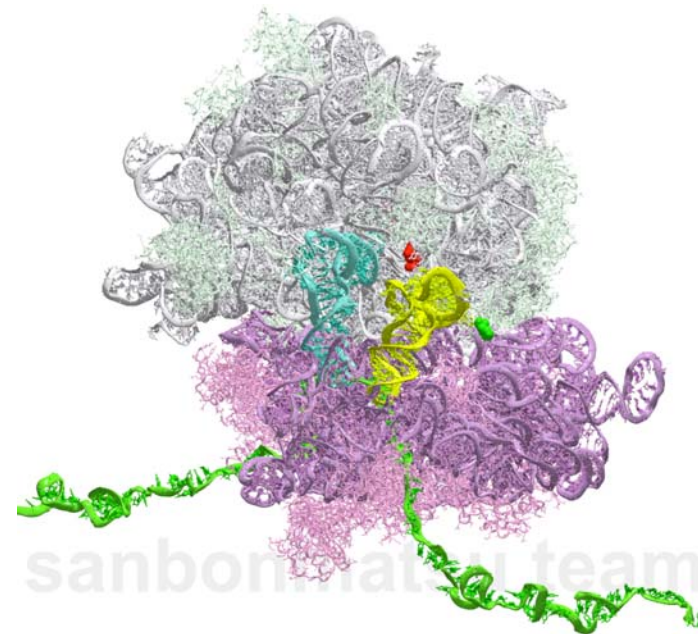
Simulations display non-two-state behaviour
which are not resolved in experiments.

The frontier

- the denatured state



- large proteins and complexes



- unstructured proteins



Another philosophy: very simple models

- simplified degrees of freedom
- potential tuned to reproduce some experimental data

$$U = \sum_{i < j} B_{ij} \left[\left(\frac{r_0}{r_{ij}} \right)^{12} - 2 \left(\frac{r_0}{r_{ij}} \right)^6 \right]$$

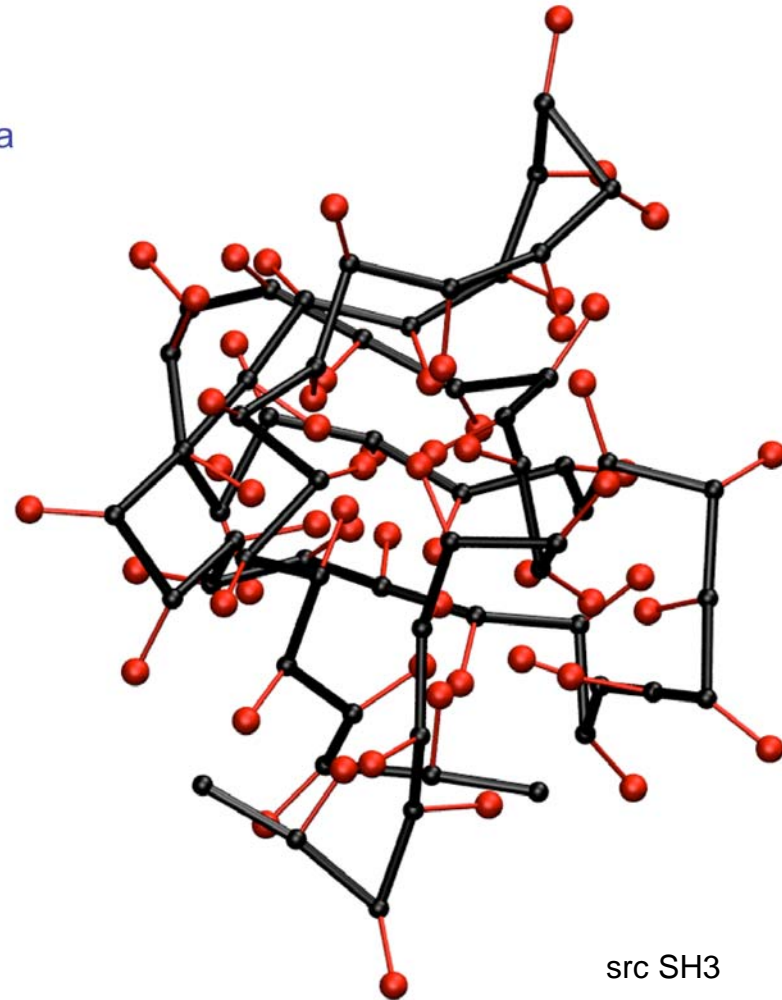
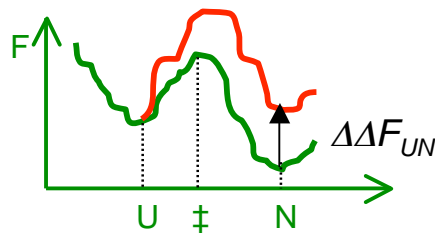
- native state as ground state

→ $B_{ij} > 0$ only if i and j are close in the native conformation.

(automatically cooperative transition)

- reproduce effects of mutations on the stability of the native state ($\Delta\Delta F_{UN}$)

→ obtain a set of B_{ij} from the $\Delta\Delta F_{UN}$ tabulated for each site.

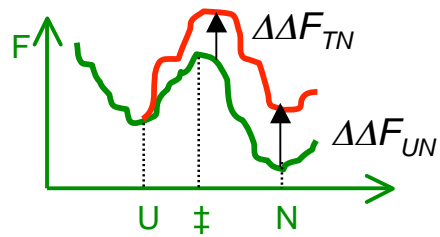


src SH3

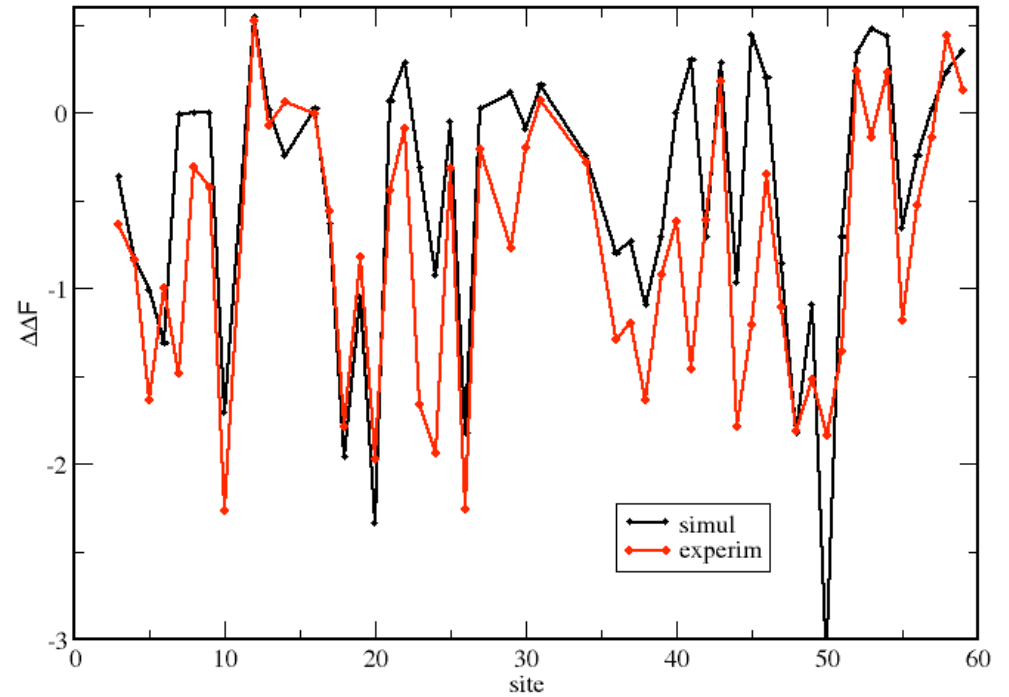
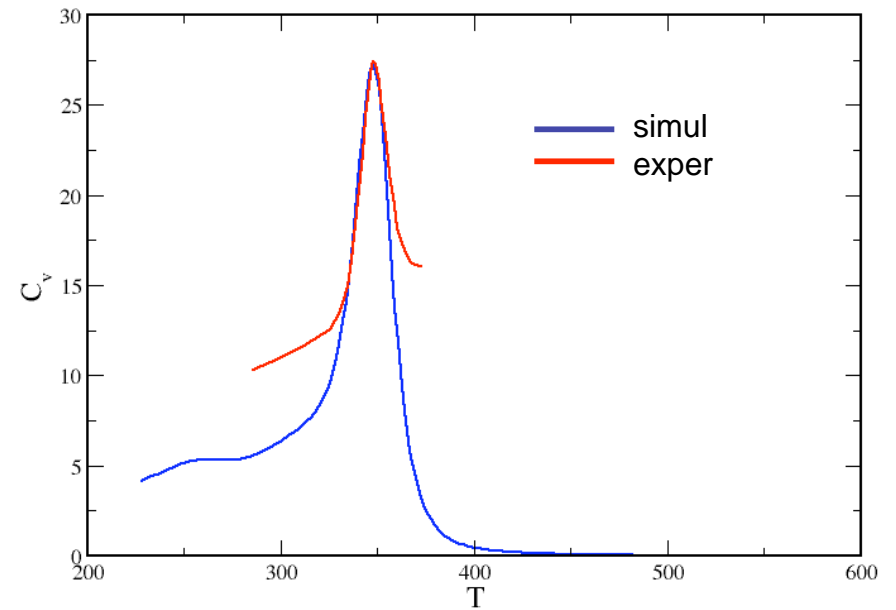
Simple results from the model

two-state thermodynamics

effect of mutations



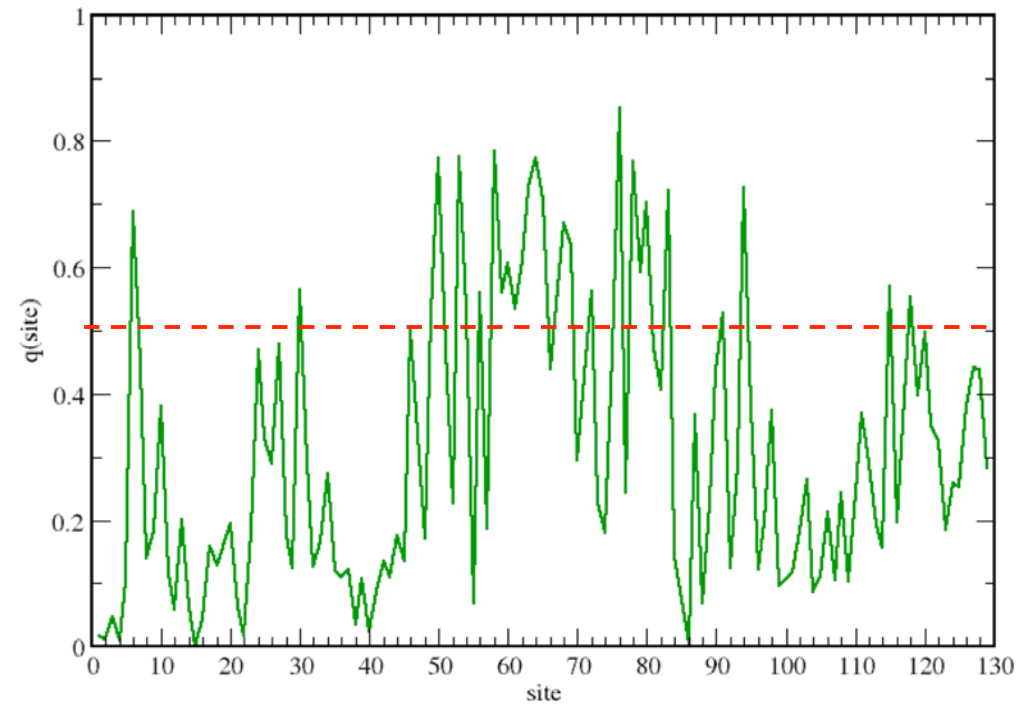
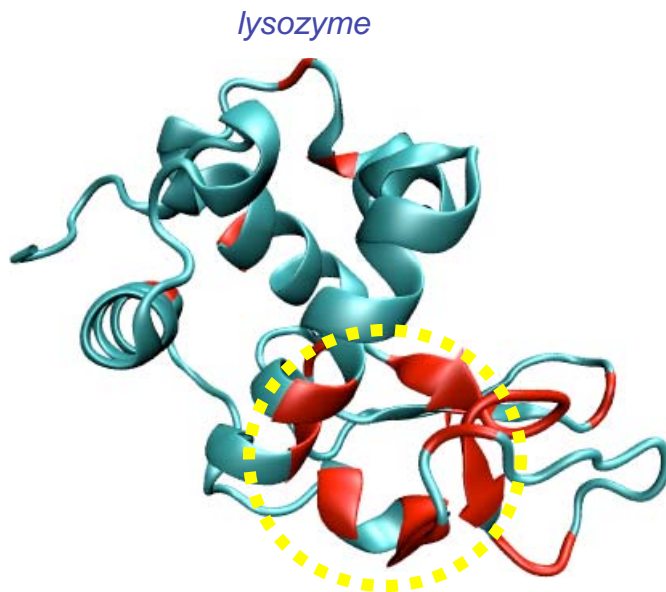
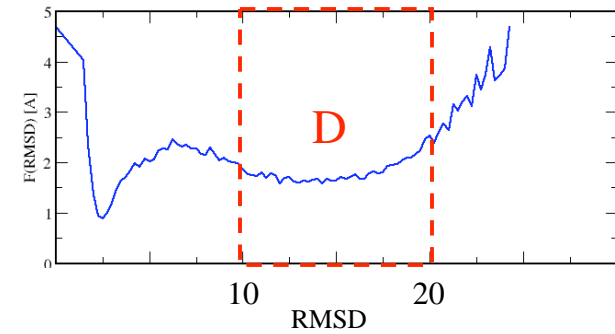
$\Delta\Delta F_{UN}$ $r=0.76$



Limelight on the denatured state

Is the denatured state a random coil?

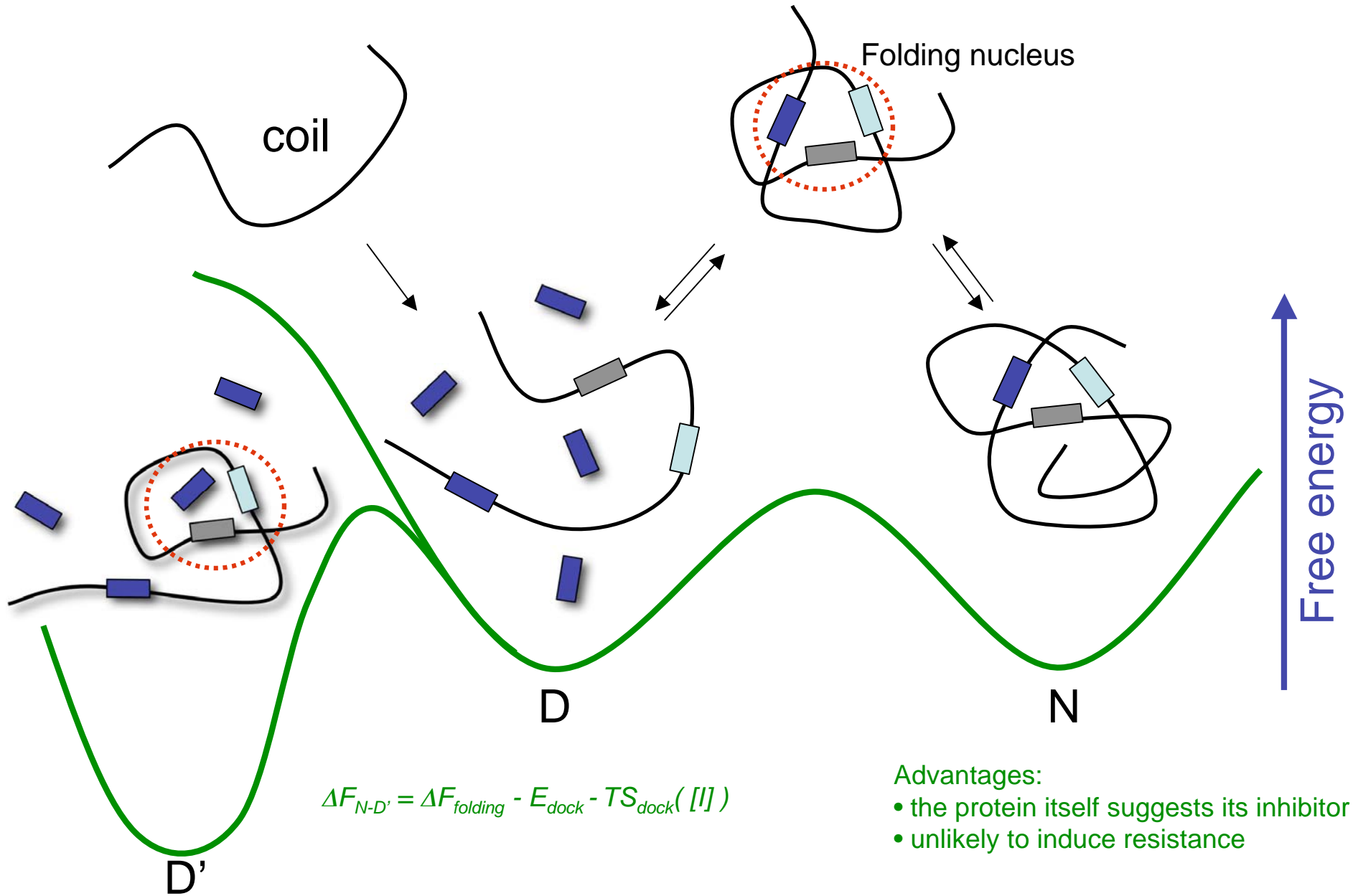
calculate the “nativeness” q_i of the sites in the denatured state

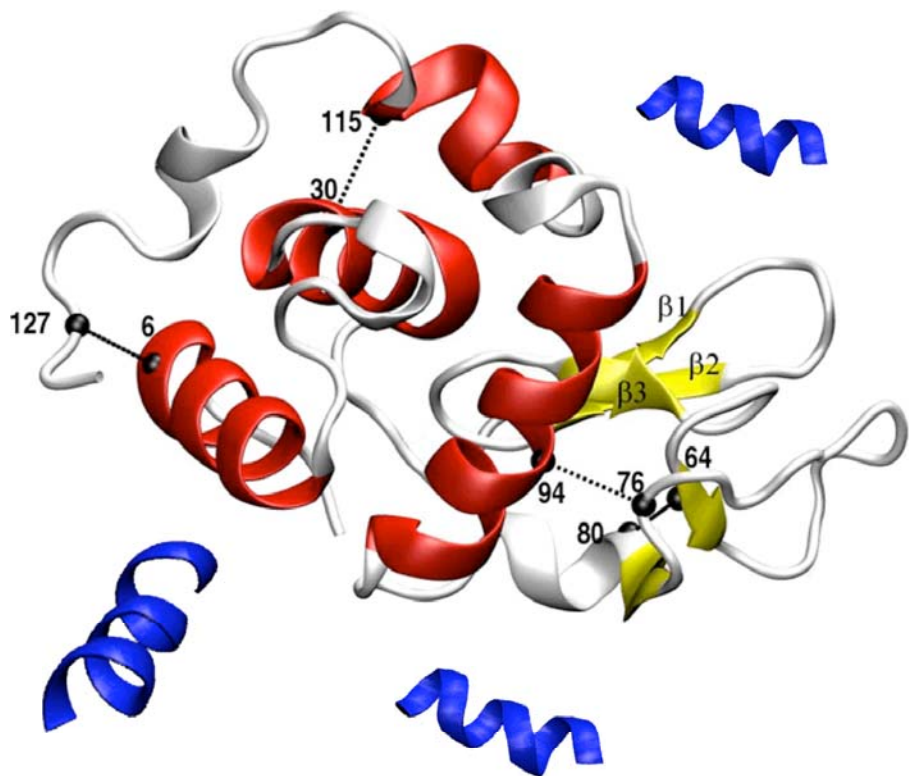


there is a lot of structure in the denatured state, as shown by recent NMR studies

(Klein-Seetharaman et al. Science 2002)

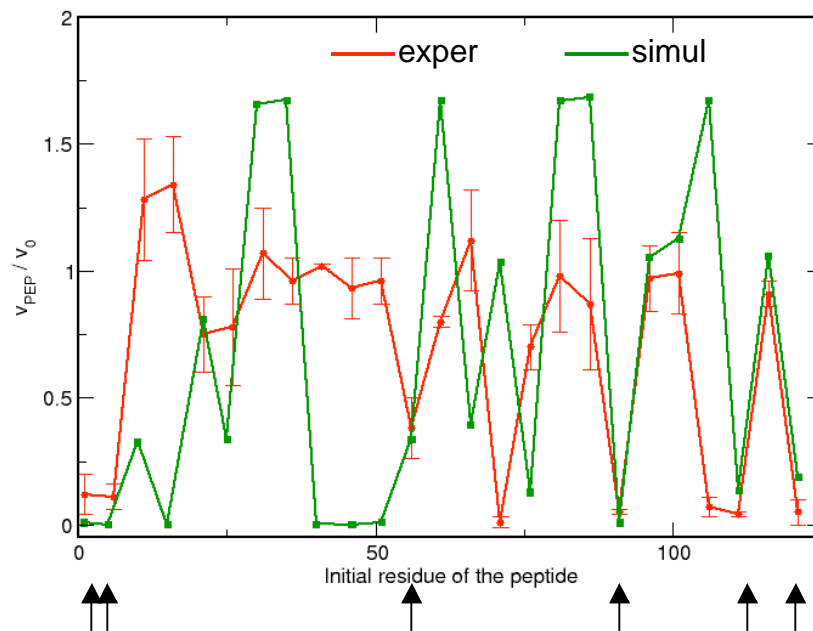
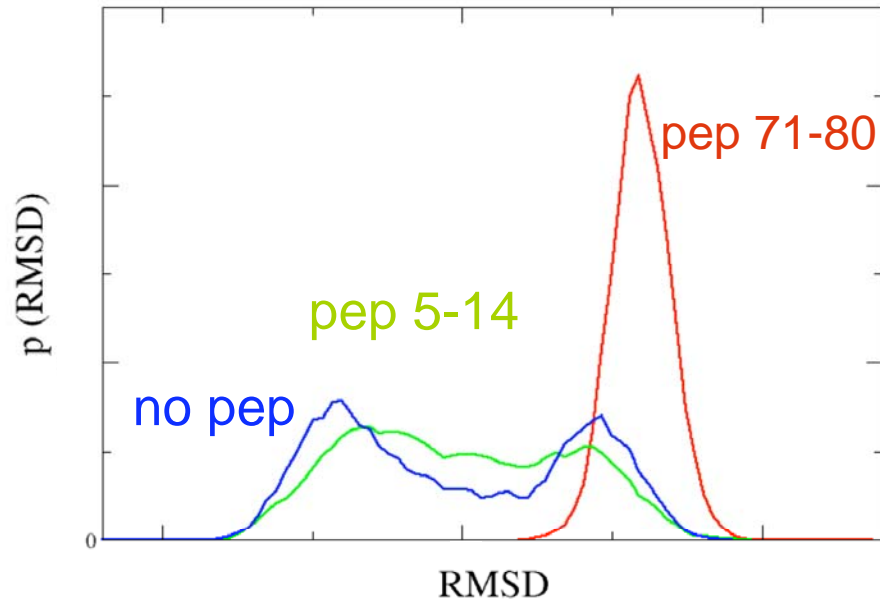
Idea from simple models! ...to inhibit a protein by destabilizing its native state
 use a peptide with the same sequence as such critical segments to block the formation of the folding nucleus.





The protein + 3 peptides
are simulated in a box at $T=T_f$

$$p_N = \int_0^8 dRMSD P(RMSD)$$



Conclusions~~s~~

(just one...)

Computation is important, but thinking is more important.

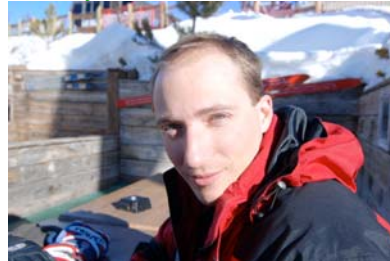
The thinkers....

experiment



Martina Caldarini
Francesca Vasile

theory



Ludovico Sutto



Carlo Camilloni



Davide Provasi

Ricardo A. Broglia
GT