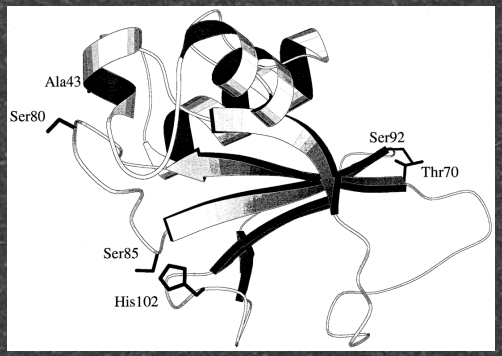
GeoFold: Topology-based protein unfolding pathways capture the effects of engineered disulfides on kinetic stability.

Vibin Ramakrishnan[1,2,5], Sai Praveen Srinivasan[1,4], Saeed M Salem[3,7], Suzanne J Matthews[3,6], Wilfredo Colón[1,4], Mohammed Zaki[3], Christopher Bystroff [1,2,3*],

Proteins, in review



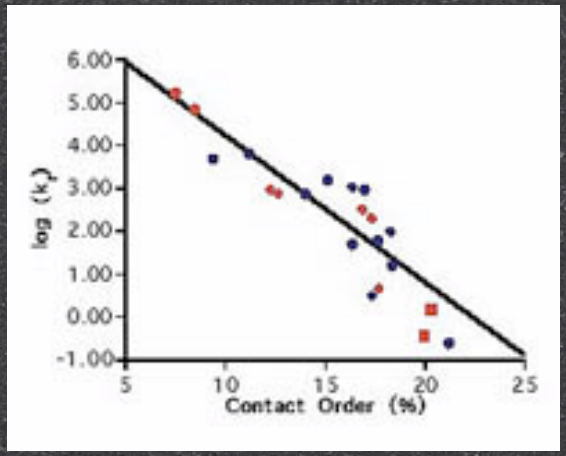
coordinates → unfolding pathway → k_u unfolding rate

k_f can be predicted from the structure.

$$U \underset{k_u}{\overset{k_f}{\rightleftharpoons}} F$$

$$K_{eq} = \frac{k_f}{k_u}$$

Stability is a function of both rates



Contact order:
CO = 100% x < |i-j| / L >

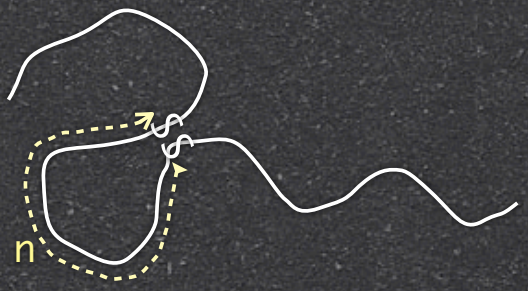
Plaxco, K. W., Simons, K. T., and Baker, D. (1998)

Flory's equation predicts k_f.

$$\Delta S = -2.1 - 3/2(\ln n)$$

Decreased entropy of the unfolded state due to a disulfide link between residues separated by n.

Flory, P., Statistical mechanics of chain molecules. *Carl Hanser Verlag, 1989* **1989**, 432

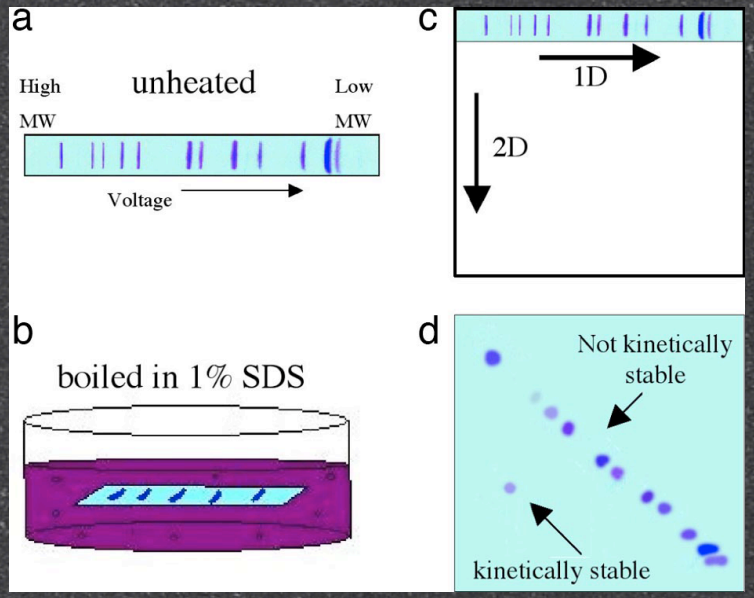


k_u is more difficult

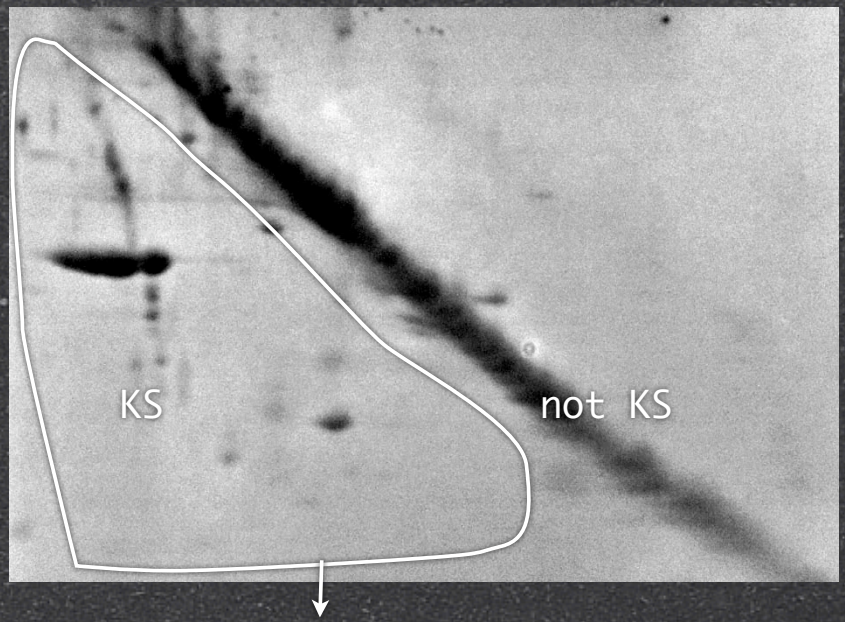
depends on...

- packing of core sidechains
- electrostatic potential
- number and arrangement of hydrogen bonds
- stiffness of the backbone
- topology

Can we learn something about unfolding kinetics from extremely stable proteins?



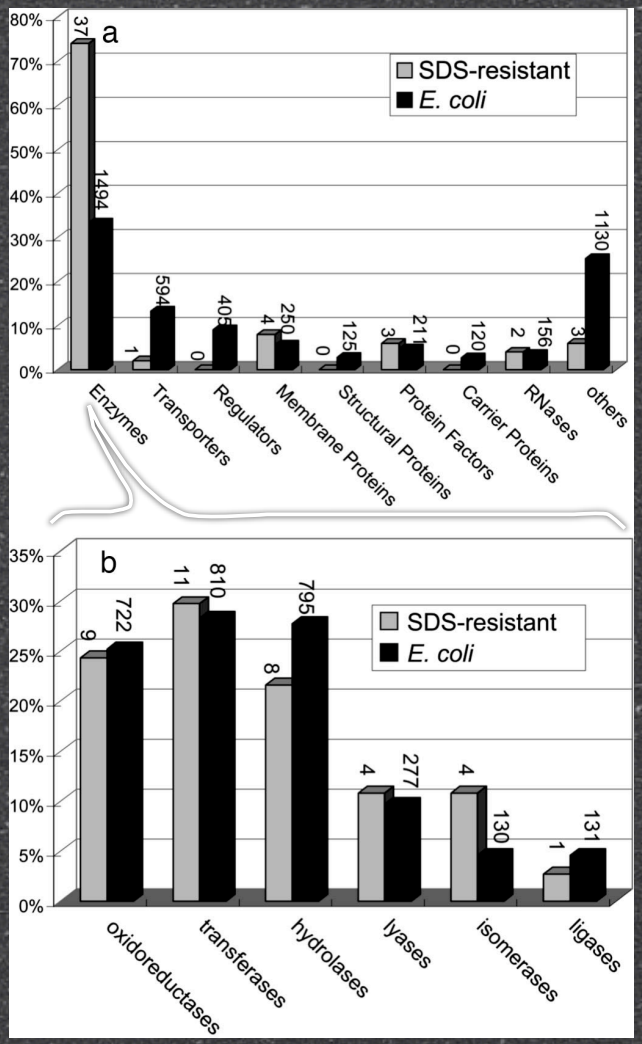
...providing a basis for 2D gel separation of KS/non-KS



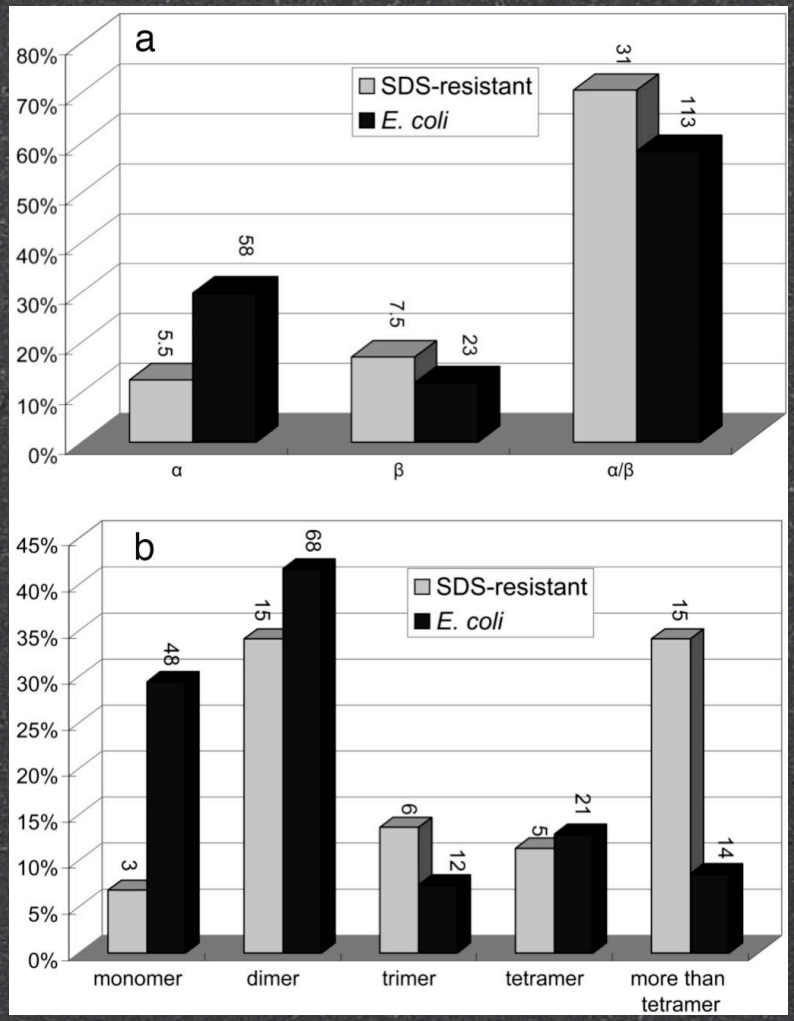
to mass spec sequencing

Xia, K., et al. (2007) Identifying the subproteome of kinetically stable proteins via diagonal 2D SDS/PAGE. *Proc Natl Acad Sci U S A*, 104(44): 17329-34.

Kinetically stable proteins tend to be ..

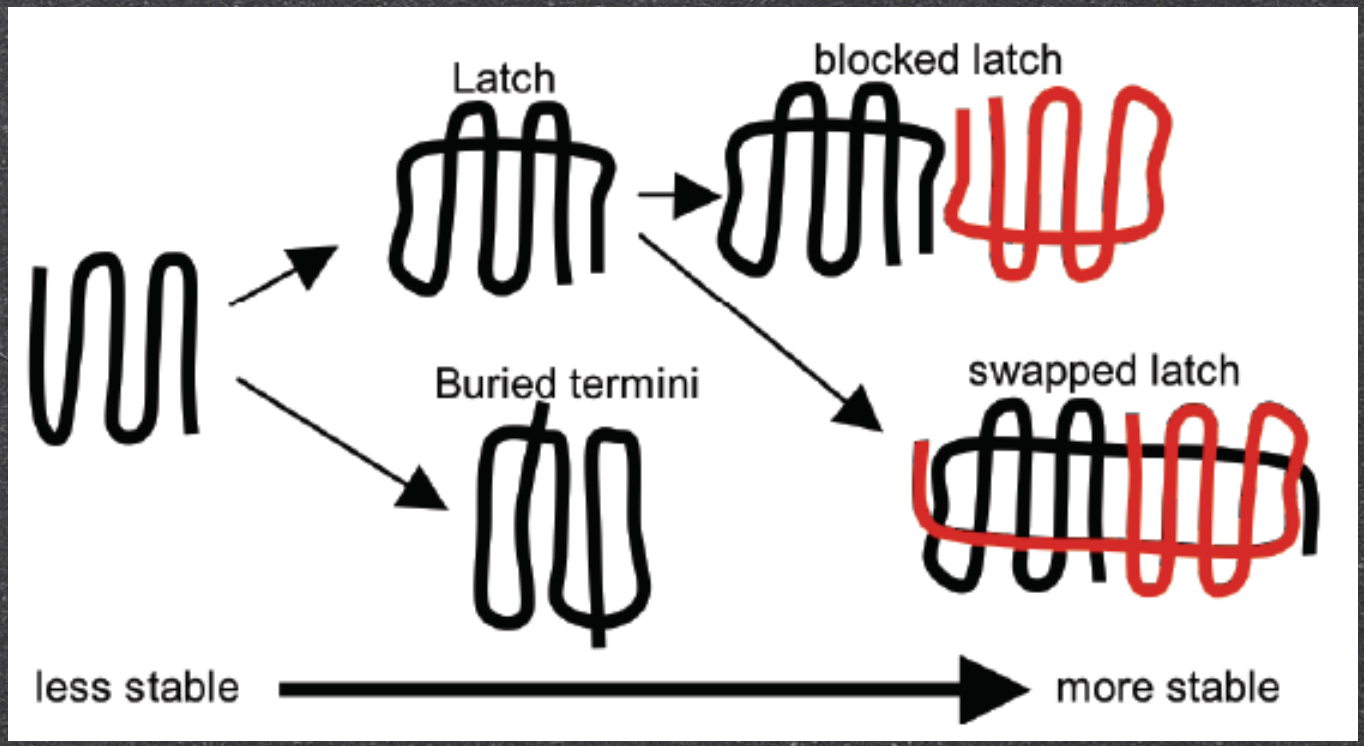


... enzymes, ...

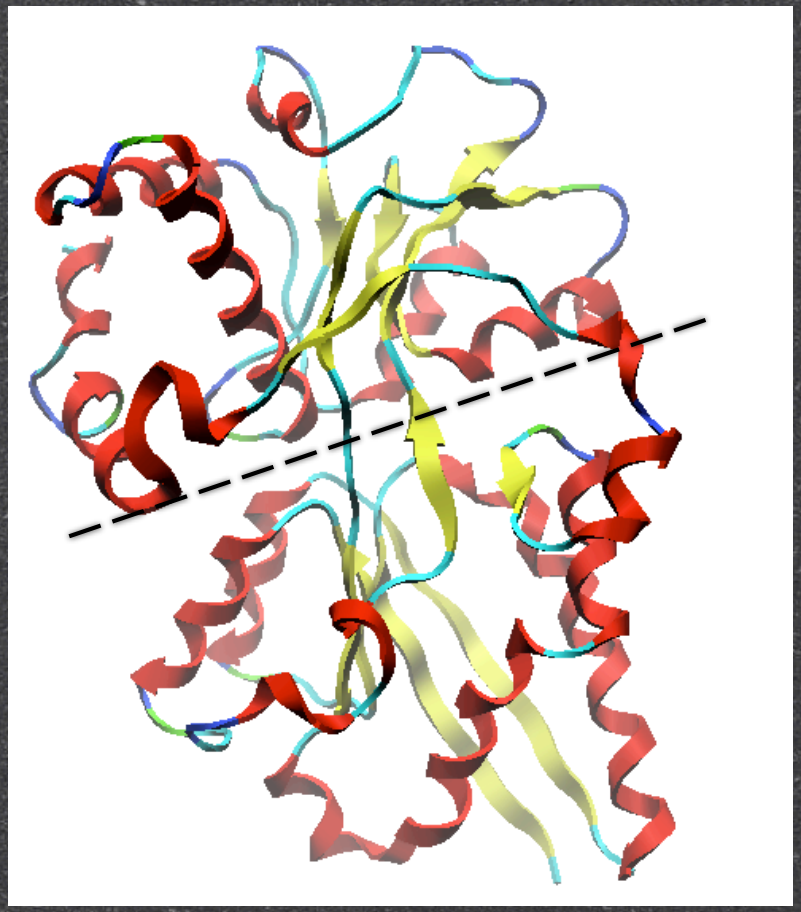


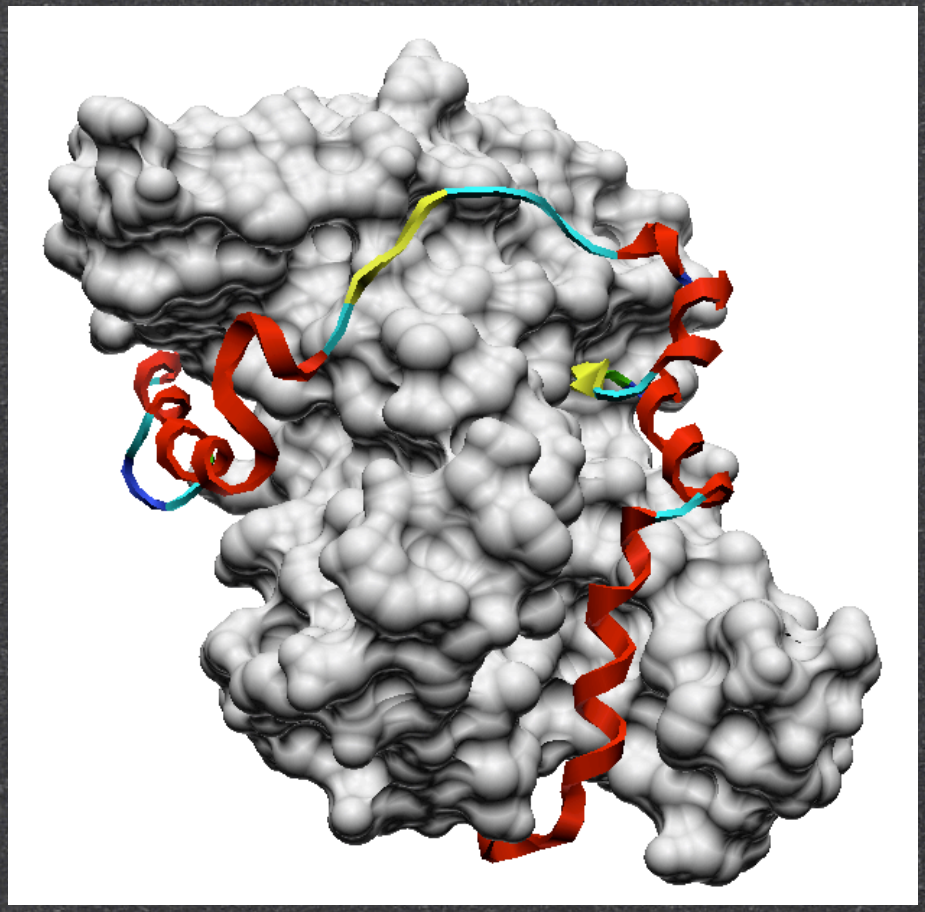
... multimeric, α/β

Kinetically stable proteins have characteristic topologies



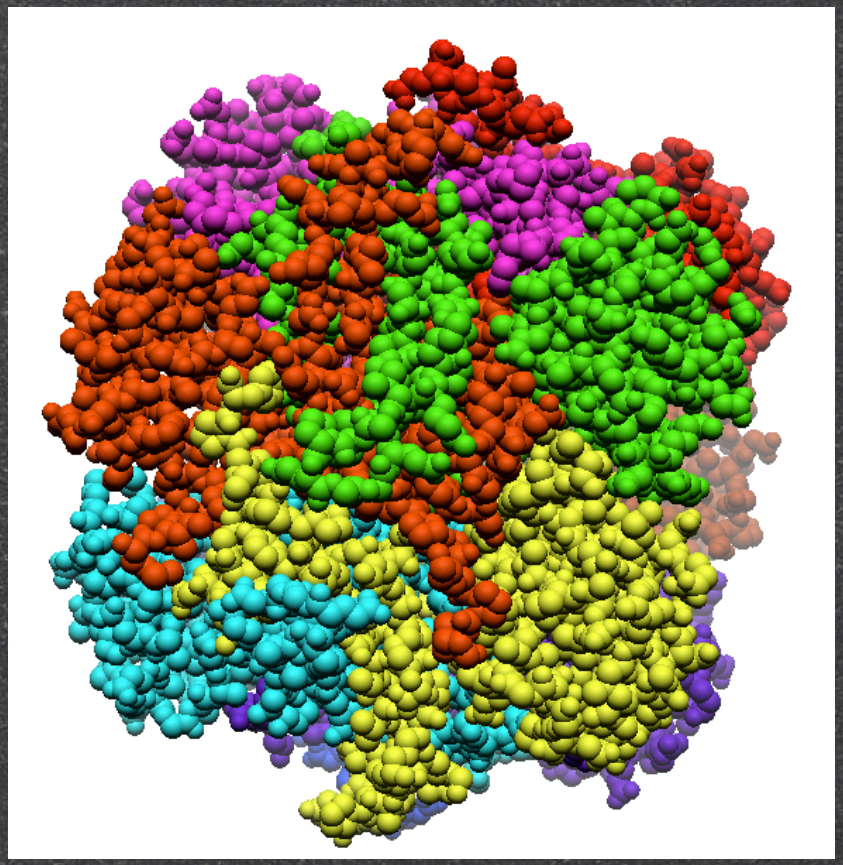
A “latch”

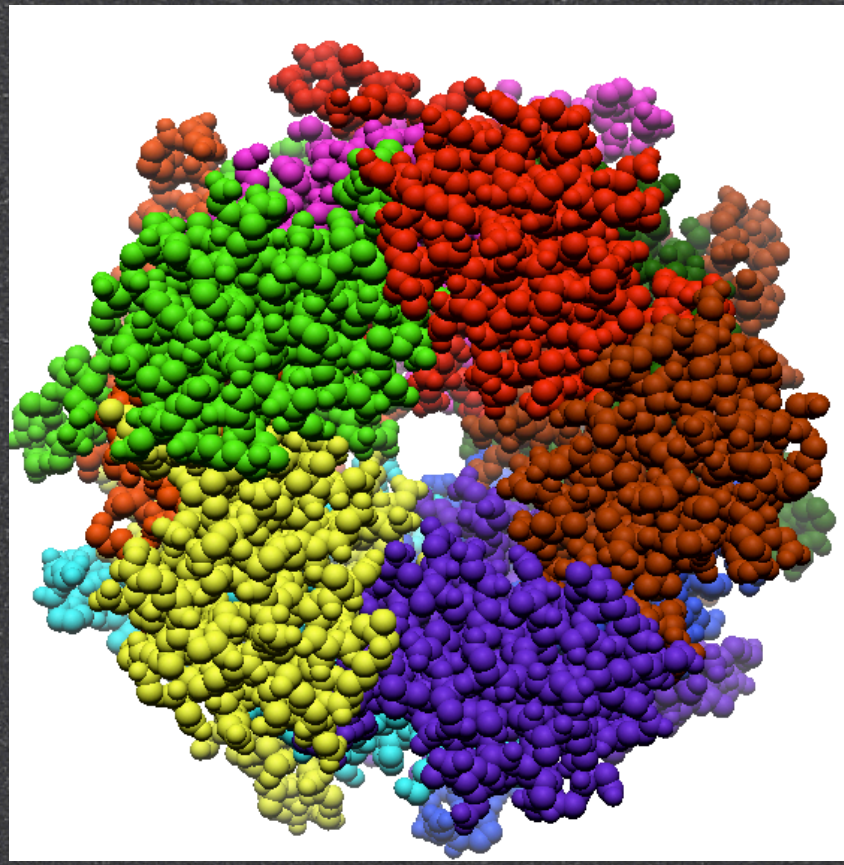




1sbp: sulfate-binding protein .

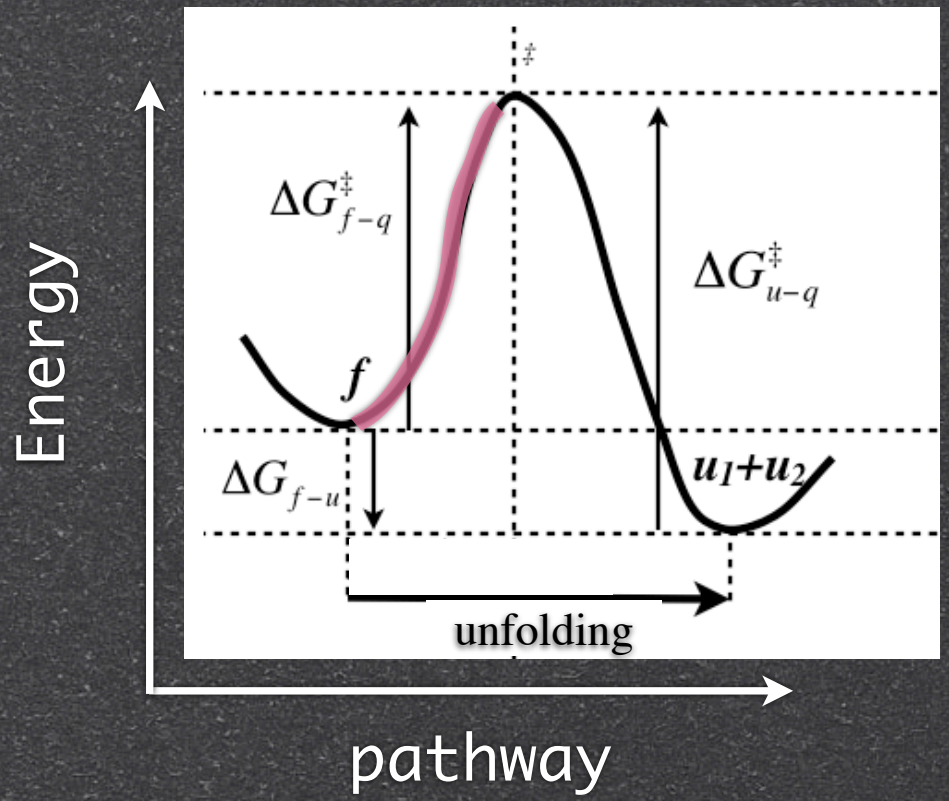
Domain-swapping





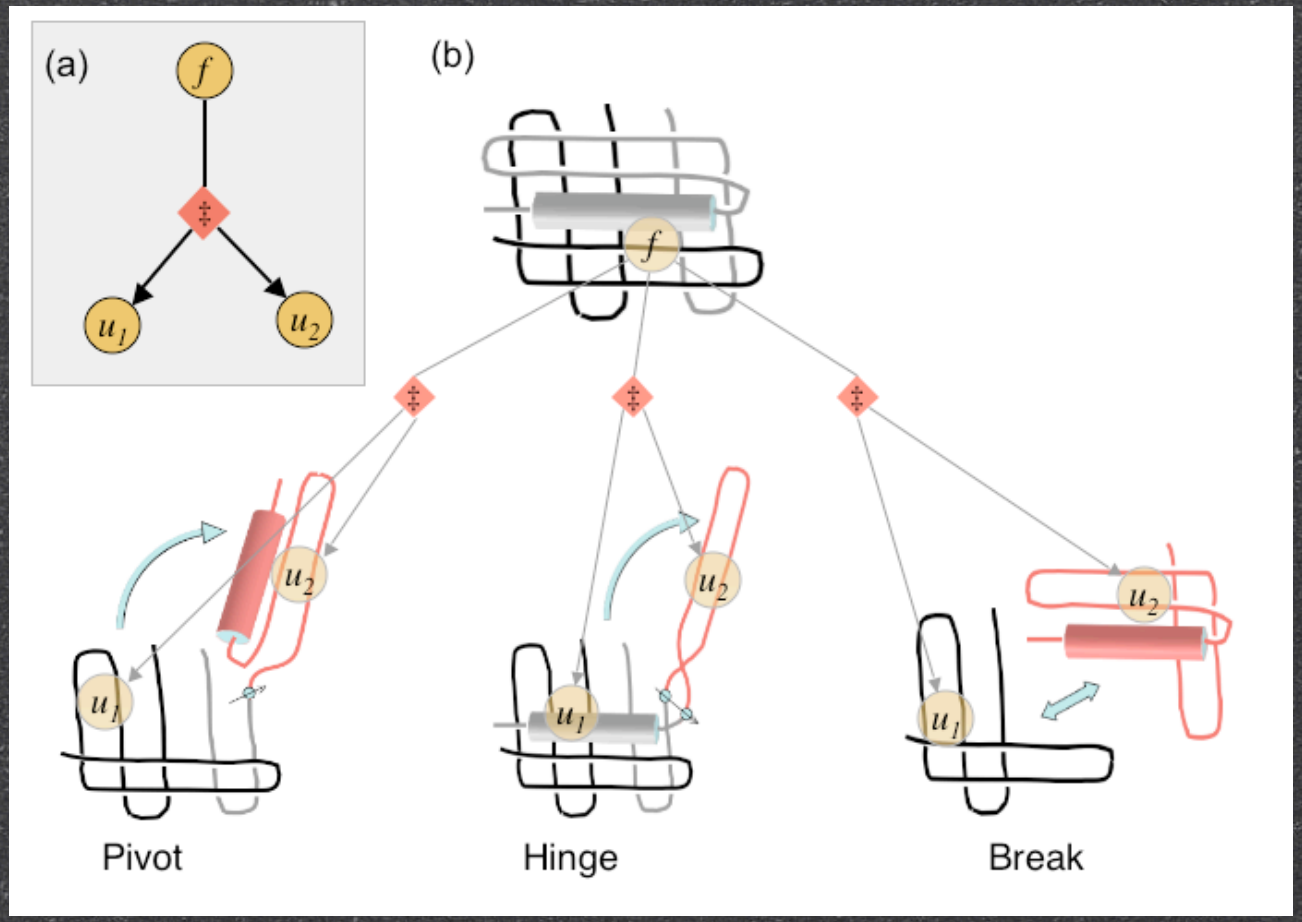
1dwk: Cyanase.

To predict kinetics, we need...



$$k = \zeta e^{-\frac{\Delta G^{\ddagger}_{f-q}}{RT}}$$

Finding a pathway of “cuts”

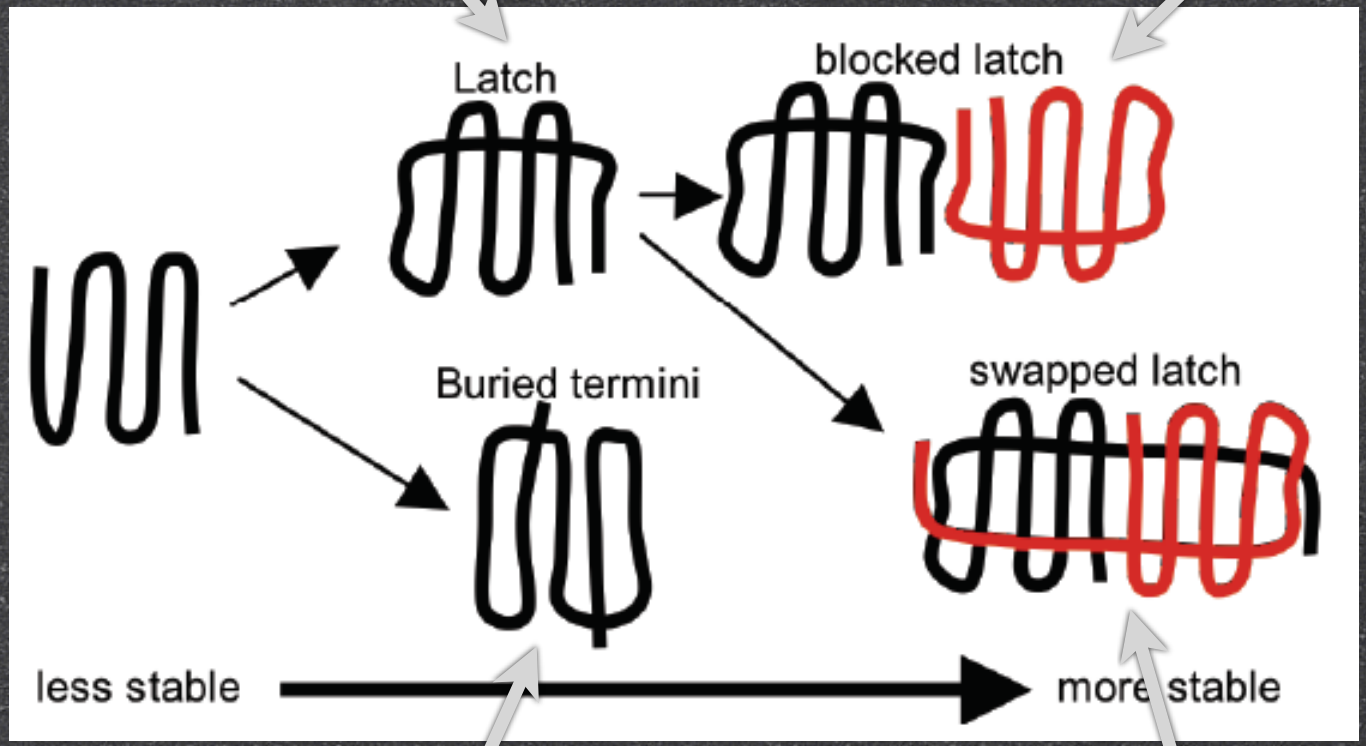


“cut” = {pivot | hinge | break}

How cuts capture topological features

fewer pivots

no pivots, only hinges,breaks



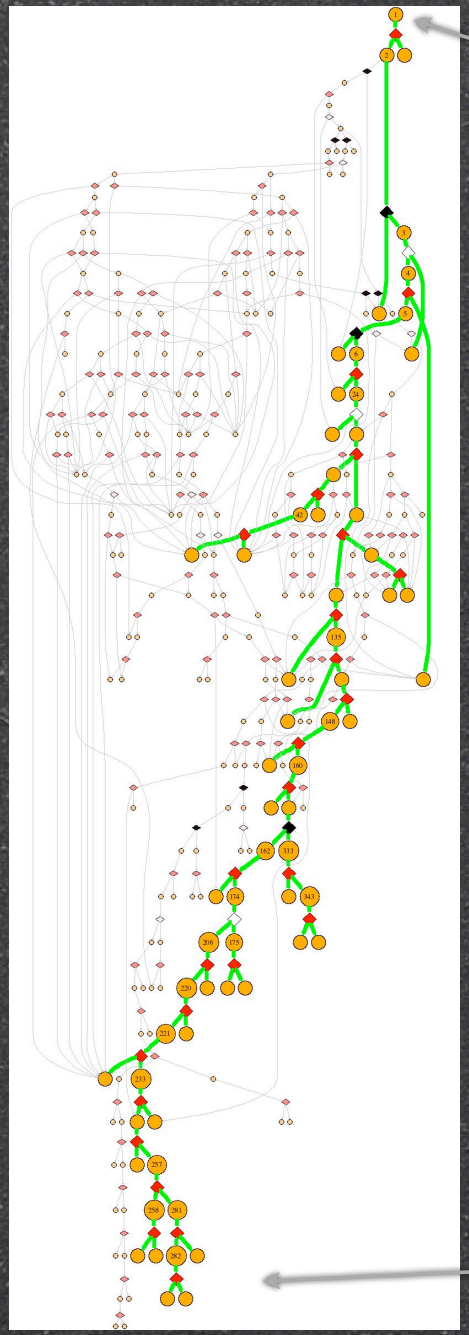
fewer pivots

no breaks, few pivots, hinges

```
// ------------Variables ------------
// f,u1,u2 = {coordinate sets}. (nodes)
// cut = {f,u1,u2,cuttype}. (edges)
// DAG = directed acyclic graph, {nodes, edges}.
// cuttype = (break|pivot|hinge|melt).
// kf(cut),ku(cut) = rate constants for folding, unfolding.
// C(f), dC(f) = concentration of f, rate of change in concentration of f.
// t, dt, F0 = time, time step, protein concentration
// ------------ Functions ------------
// GetBreak, GetPivot, GetHinge find new geometrically possible cuts,
//           returning False when there are no more.
// Split(f,cuttype) returns two subsets of f using cuttype.
//           Split(f, melt) returns {∅, ∅}
// Exists(f, DAG) returns True if f is already in DAG.
GeoFOLD:
1. f = entire protein
2. DAG = ∅
3. GetCuts(f, DAG)
4. UnfoldSim(DAG)

GetCuts(f, DAG):
5.  If (f == ∅) Return
6.  If Exists(f, DAG) Return
7.  while (cuttype ≠ melt) {
8.    If     (GetBreak(f)) {cuttype = break}
9.    Elseif (GetPivot(f)) {cuttype = pivot}
10.   Elseif (GetHinge(f)) {cuttype = hinge}
11.   Else                 {cuttype = melt}
12.   {u1, u2} = Split(f,cuttype)
13.   DAG = DAG ∪ {f, u1, u2, cuttype}
14.   GetCuts(u1, DAG)
15.   GetCuts(u2, DAG)
16. }

UnfoldSim(DAG):
17. C(1)=F0
18. For All cut ∈ DAG {calculate kf(cut) and ku(cut)}
19. While Not converged {
20.    t += dt
21.    For All f { dC(f) = 0 }
22.    For All cut ∈ DAG {
23.      dC(f)  = dC(f)  - C(f)*ku(cut) + C(u1)*C(u2)*kf(cut)
24.      dC(u1) = dC(u1) + C(f)*ku(cut) - C(u1)*C(u2)*kf(cut)
25.      dC(u2) = dC(u2) + C(f)*ku(cut) - C(u1)*C(u2)*kf(cut)
26.    }
27.    For All f { C(f) = C(f) + dC(f)*dt }
28.    Plot t, C
29.    If (All |dC| < VerySmall) Then converged
30. }
```

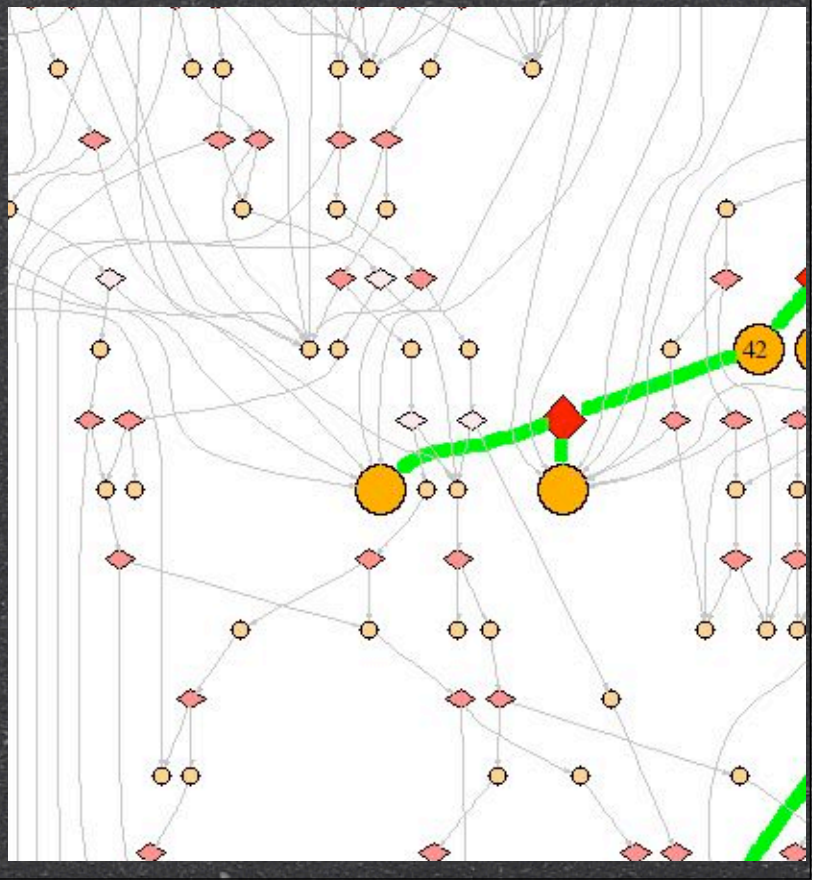


Native state

Intermediates

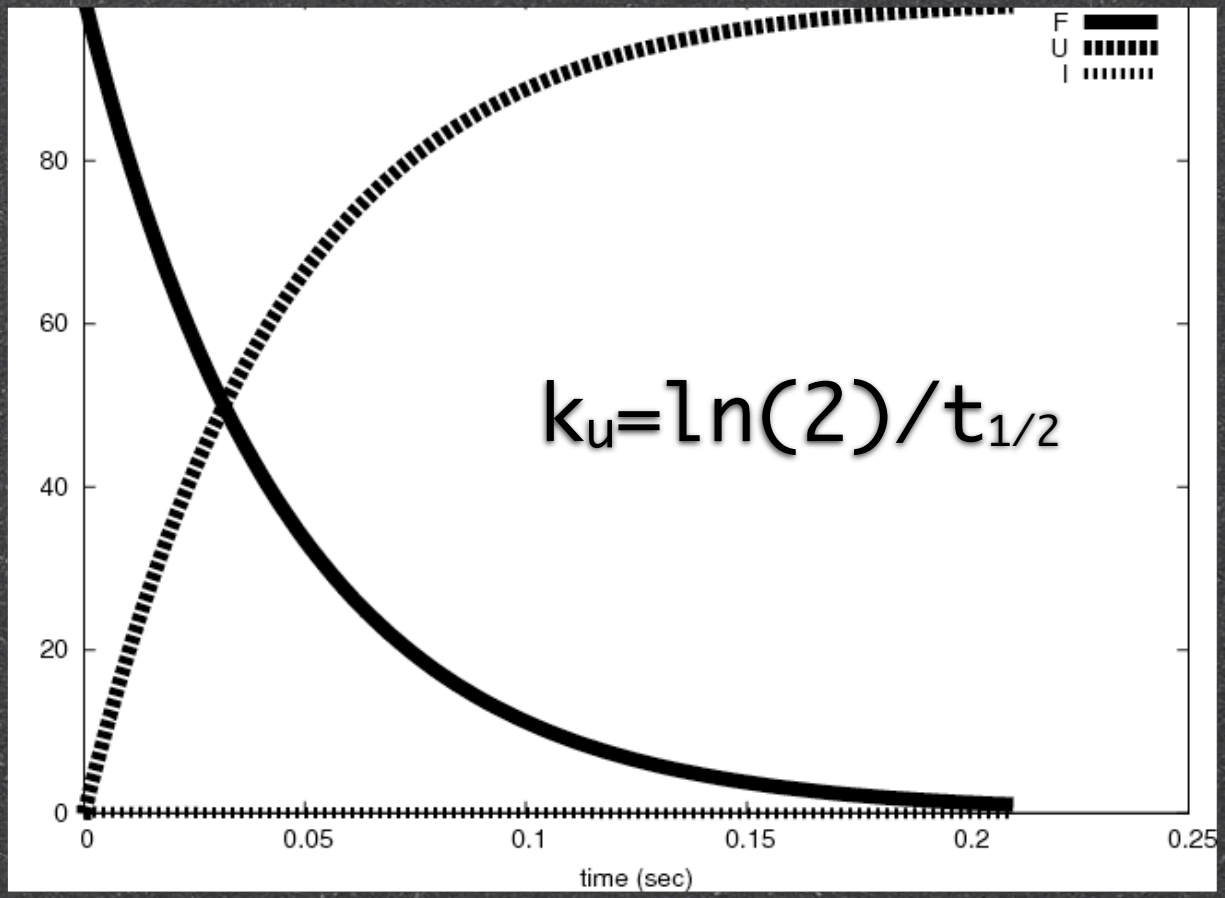
unfolded

An unfolding pathway = A directed acyclic graph with bifurcating edges.



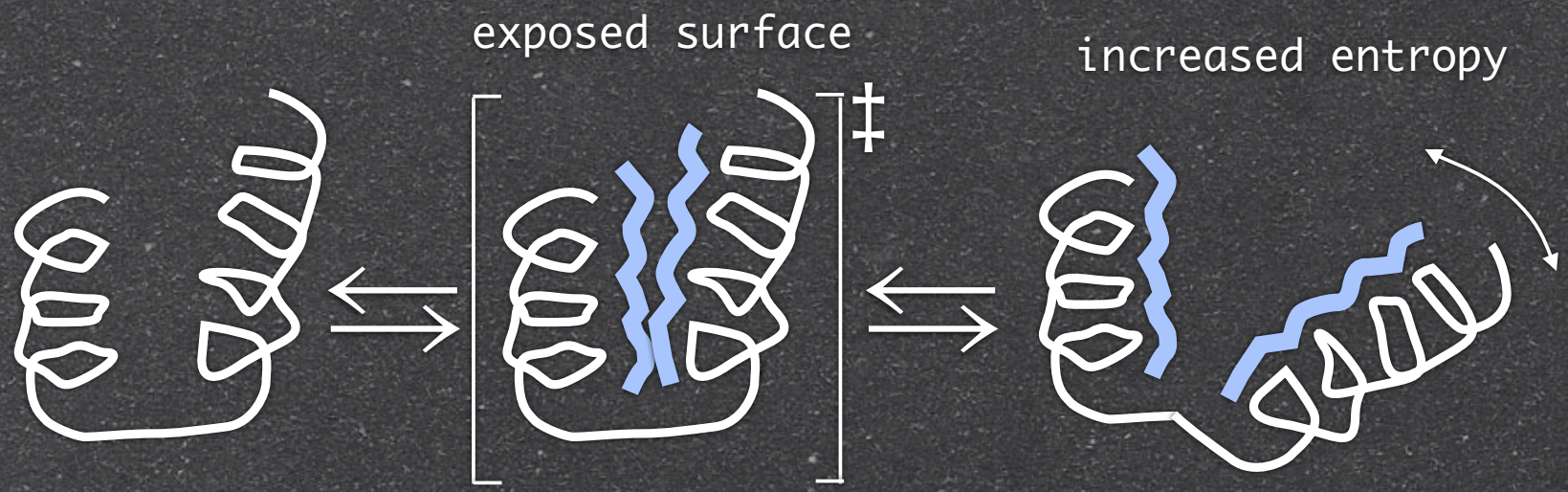
www.bioinfo.rpi.edu/geofold

concentration



time

Energy terms



$$\Delta G_{solv} = \omega \Delta SAS$$

surface tension

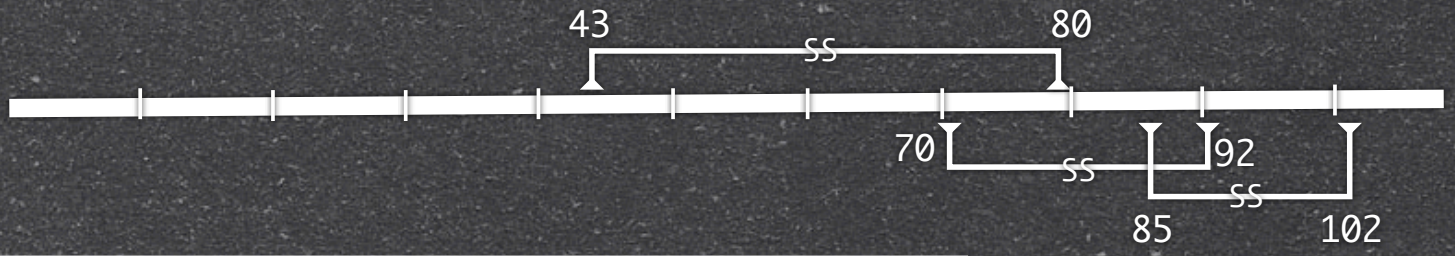
change in surface area

other terms

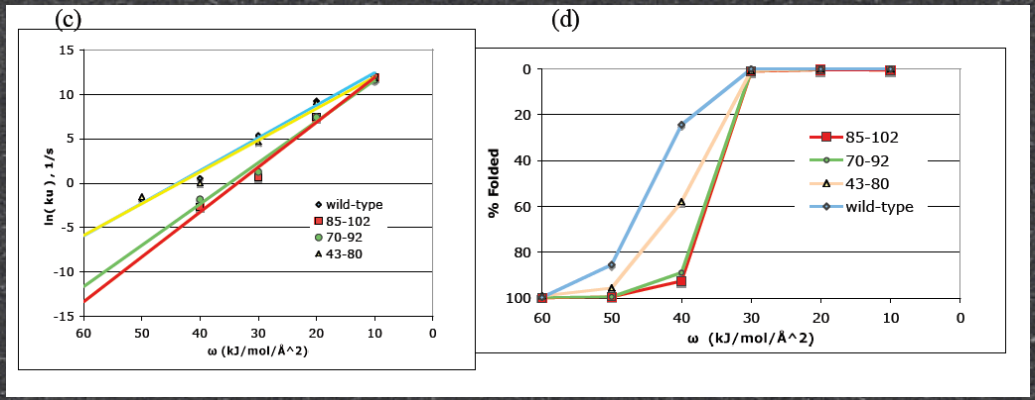
Hydrogen bonding	backbone entropy
Buried cavities	sidechain entropy
Electrostatic	

Barnase

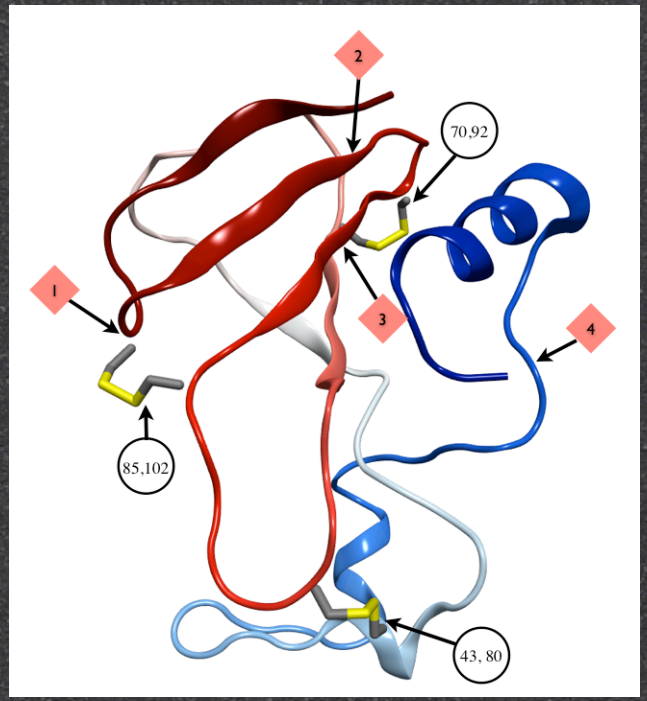
Clarke, J.; Fersht, A. R., Engineered disulfide bonds as probes of the folding pathway of barnase: Increasing the stability of proteins against the rate of denaturation. *Biochemistry* **1993,** *32* (16), 4322-4329.



Disulfide mutant	Result predicted by Flory's equation. $\Delta S = -2.1 - 3/2(\ln n)$	Experi-mental result	GeoFold result
43-80	most stabilized	least	least
70-92	stabilized		
85-102	least stabilized	most	most



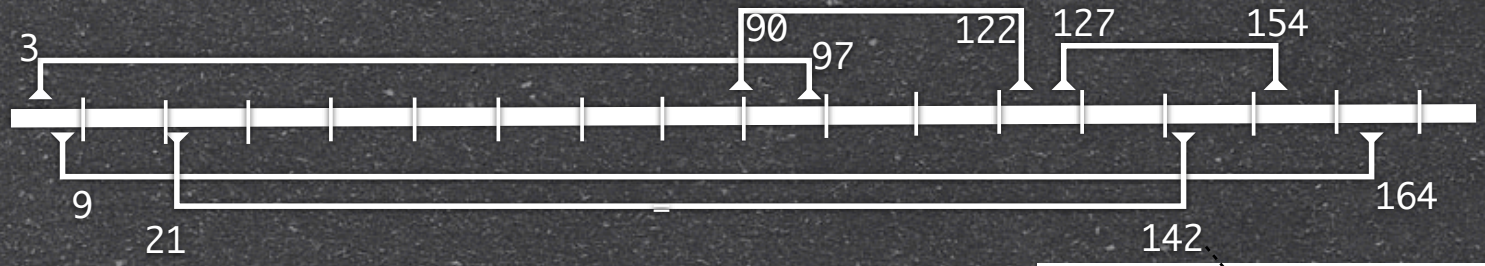
Simulated kinetics

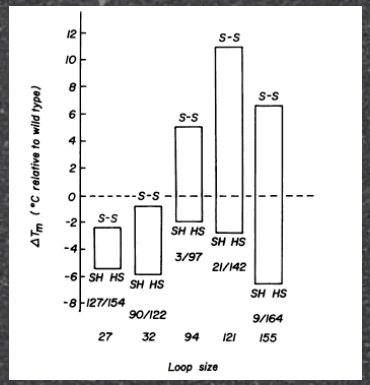


Simulated pathway: C-term first

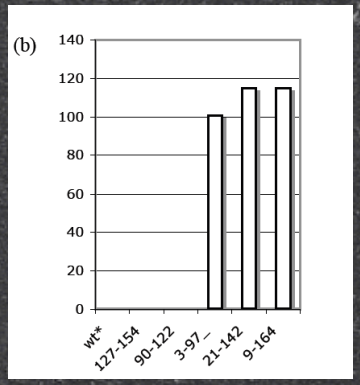
Lysozyme

Matsumura, M.; Becktel, W. J.; Levitt, M.; Matthews, B. W., Stabilization of phage T4 lysozyme by engineered disulfide bonds. *PNAS* **1989,** 86 (17), 6562.

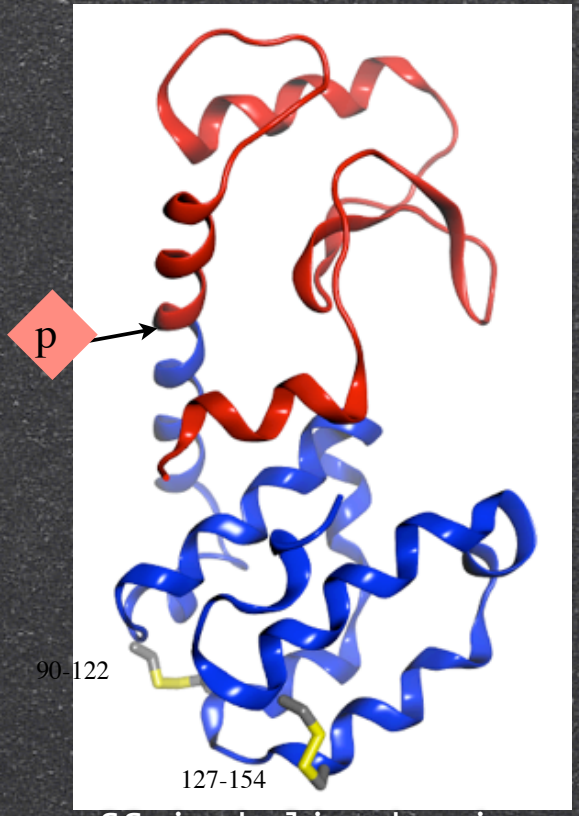




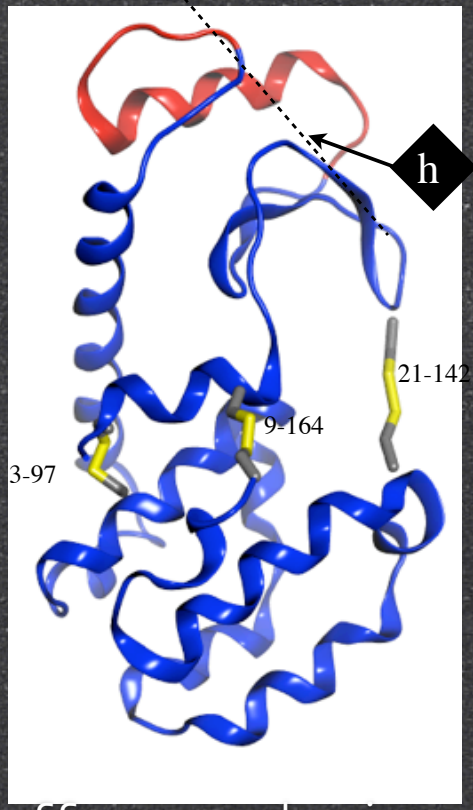
experimental



simulated



SS in helix domain
allow wt pathway



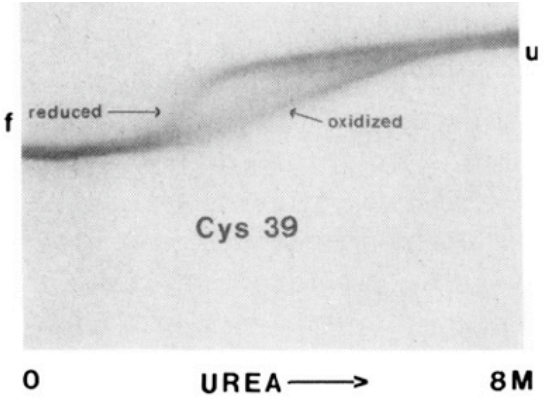
SS across domains
block wt pathway

DHFR

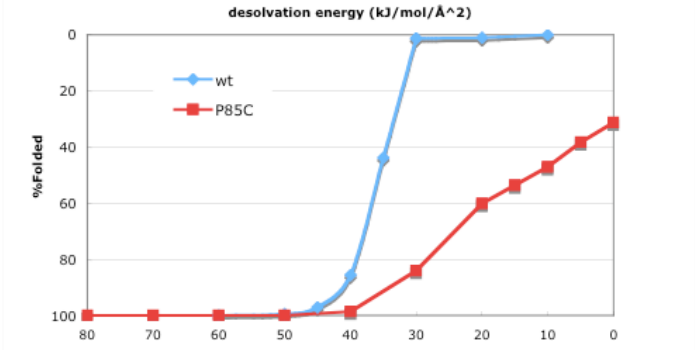
Villafranca, J. E.; Howell, E. E.; Oatley, S. J.; Xuong, N. H.; Kraut, J., An engineered disulfide bond in dihydrofolate reductase. *Biochemistry* **1987,** *26* (8), 2182-2189.

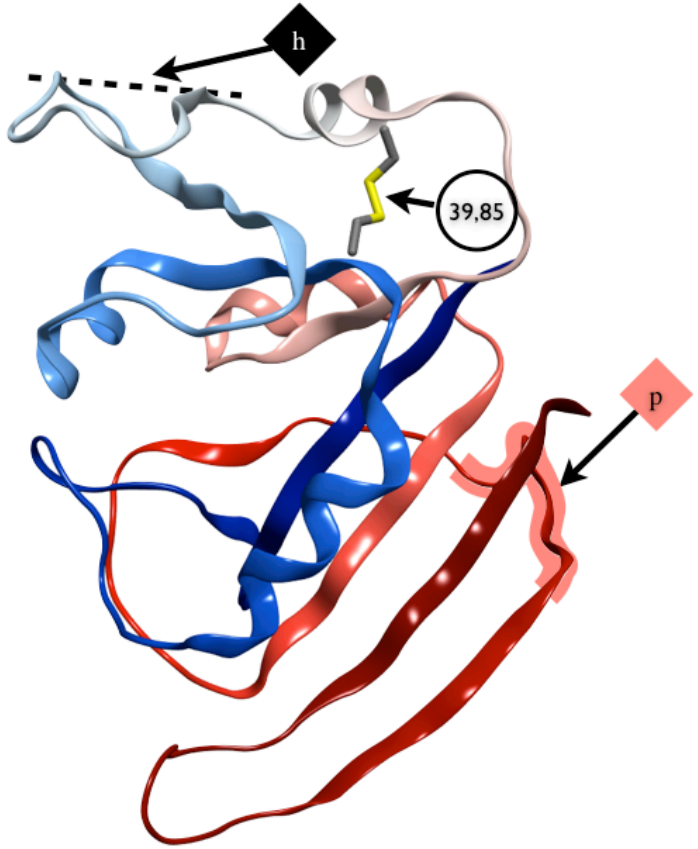
low denaturant pathway.
middle hinges first

Experimental equilibrium unfolding



Simulated equilibrium unfolding



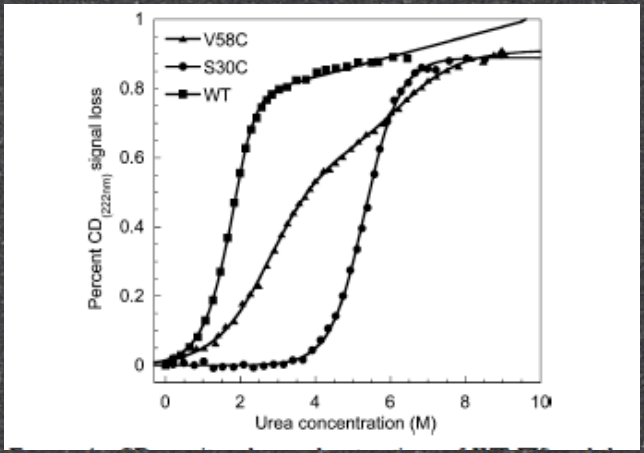


high denaturant pathway.
end pivots first.

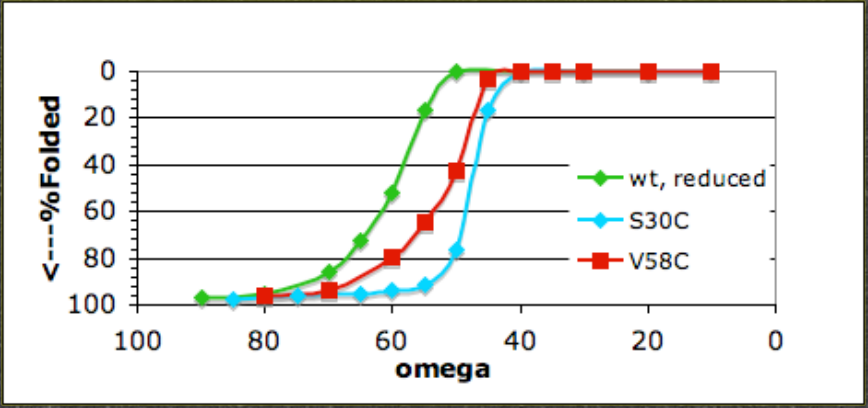
FIS

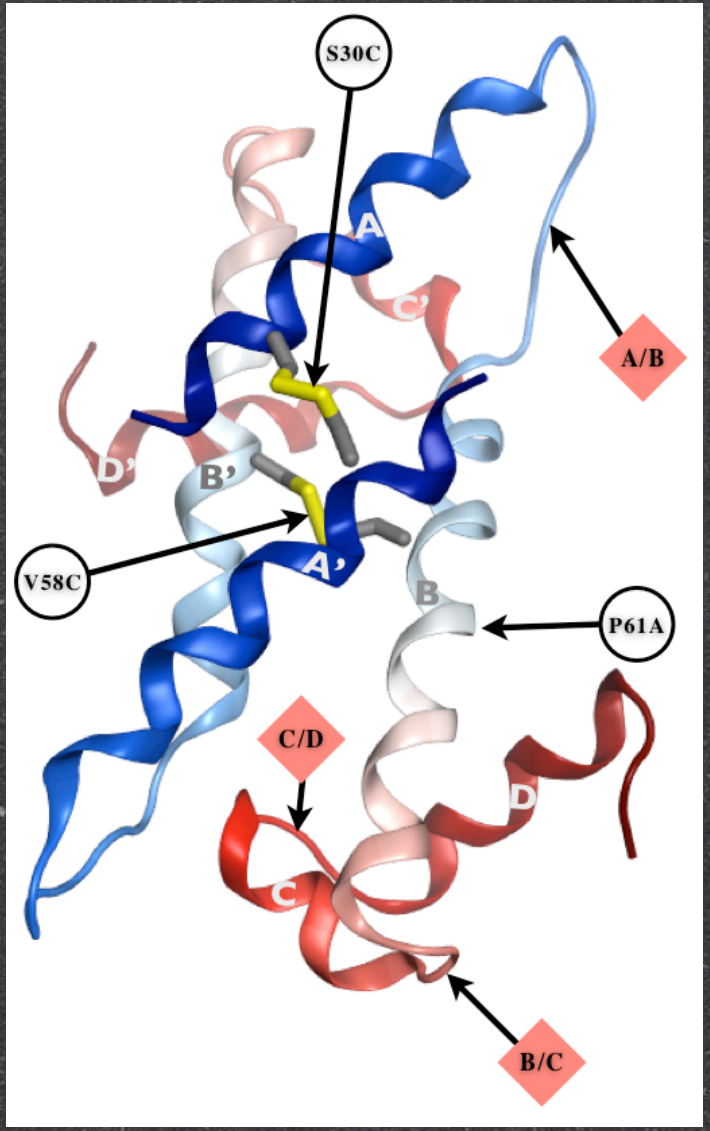
Meinhold, D.; Beach, M.; Shao, Y.; Osuna, R.; Colón, W., The location of an engineered inter-subunit disulfide bond in factor for inversion stimulation (FIS) affects the denaturation pathway and cooperativity. *Biochemistry* **2006,** *45* (32), 9767-9777.

Experimental equilibrium unfolding



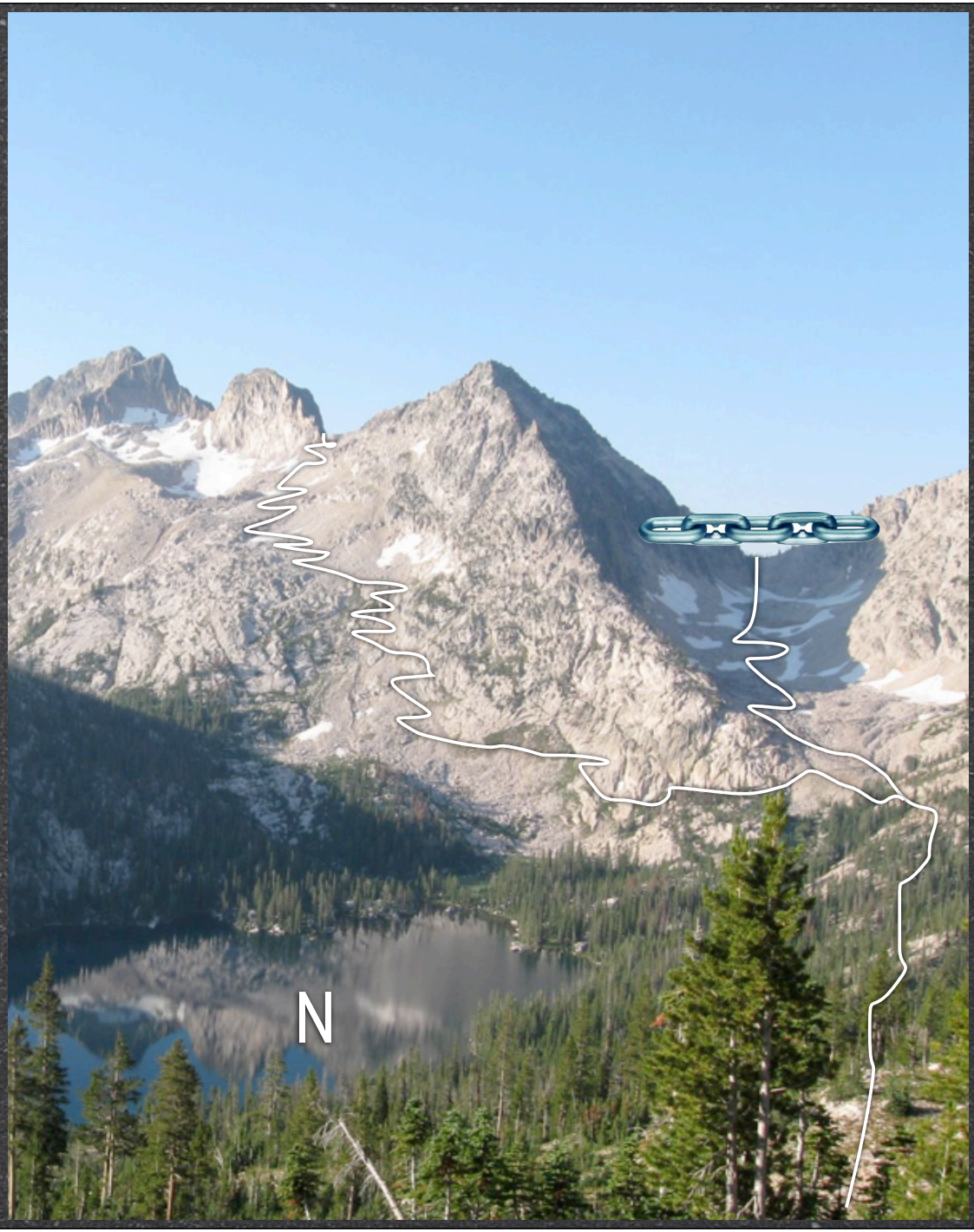
Simulated equilibrium unfolding





How to stabilize a protein using disulfides

- Find the transition state of the most energetically favorable unfolding pathway.
- Make a SS bond to block that pathway.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Rob Shaffer

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Vibin Ramakrishnan

Suzanne Matthews

Ke Xia

Yao-ming Huang

me

Think Globally,
Act Locally.

‡ Mohammed Zaki (RPI-Comp.Sci.)

ƒ Wilfredo Colón (RPI-Chemistry)

* Jon Dordick (RPI-Chem Eng.)