Protein Physics 2016 Lecture 11, February 26

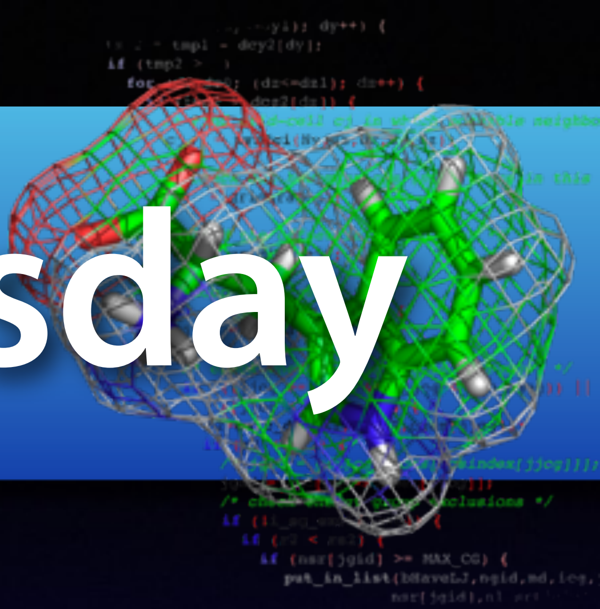
Kinetics of Protein Folding

Magnus Andersson

magnus.andersson@scilifelab.se

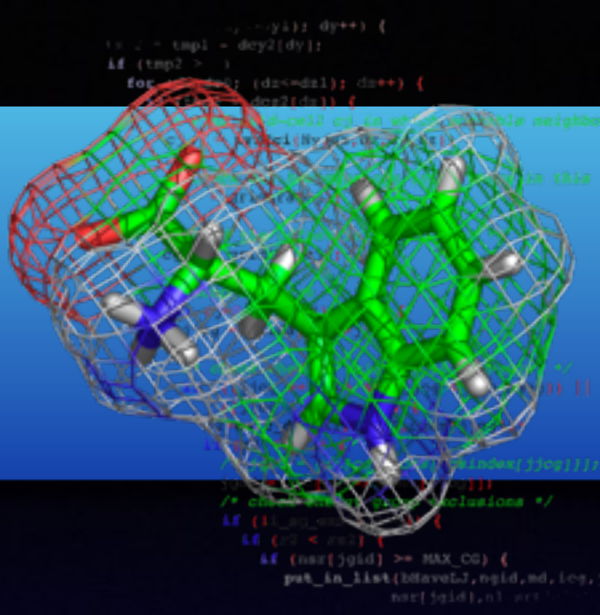
Theoretical & Computational Biophysics

Recap from Wednesday



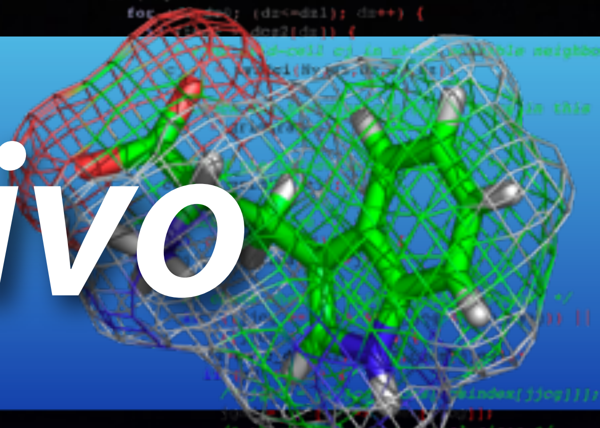
- Folding thermodynamics
- Cooperative *and* all-or-none transition
- Cold & hot denaturation
- Molten globule and coil conformations
- Barriers & energy gap stabilization
- Proteins are different from random chains!

Today

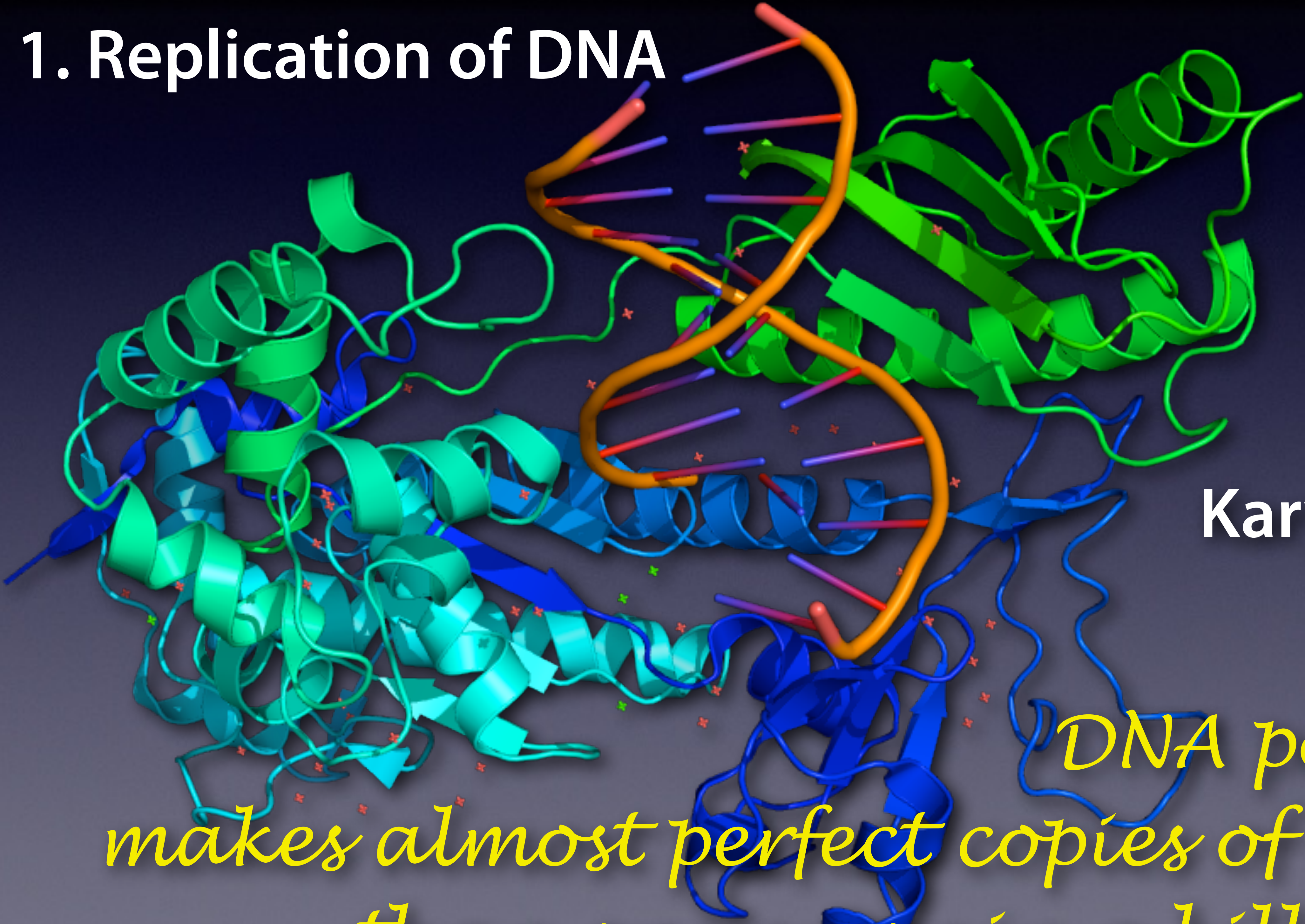


- Folding kinetics
- Folding vs. unfolding equilibrium
- How fast do proteins fold? Why?
- Chaperones, transition states
- Effective folding rates
- Kinetic vs. thermodynamic stability
- Folding nuclei, mutation studies
- Energy landscapes, folding pathways
- Solution to Levinthal’s Paradox

Protein synthesis *in vivo*



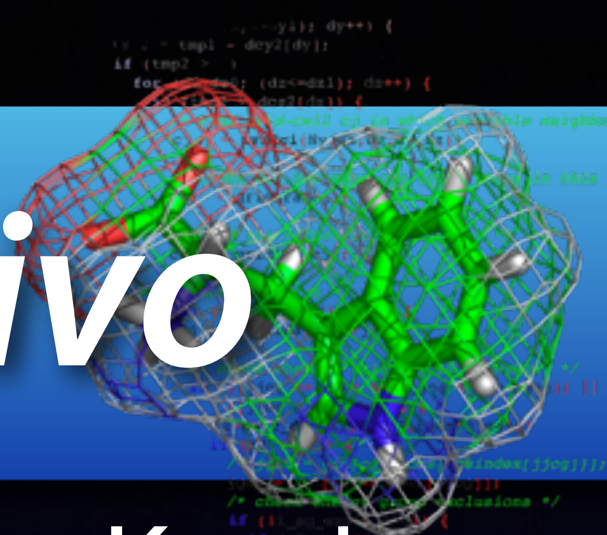
1. Replication of DNA



Kary Mullis, PCR:
Nobel 1993

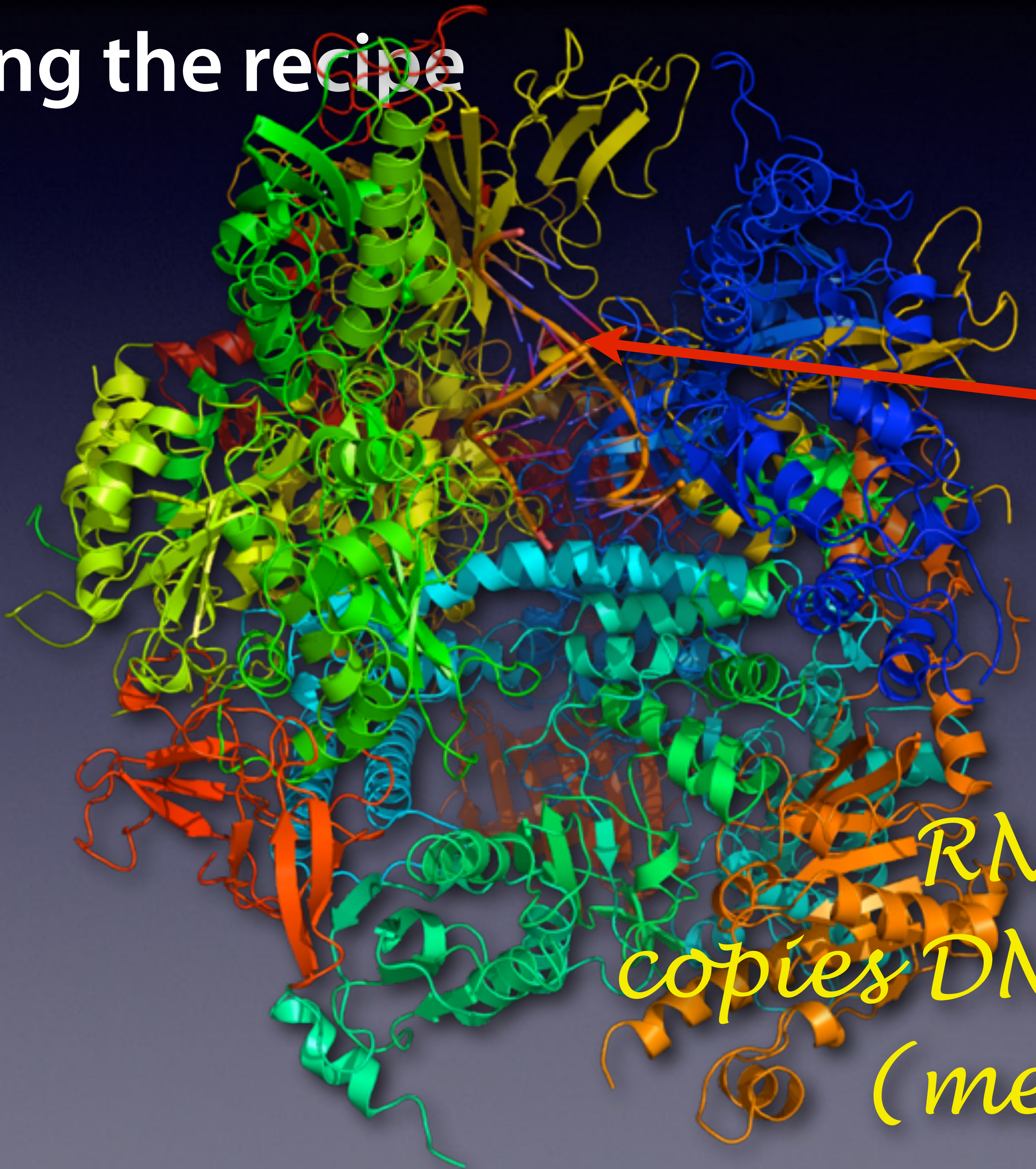
DNA polymerase makes almost perfect copies of DNA - less than one error in a billion bases!

Protein synthesis *in vivo*



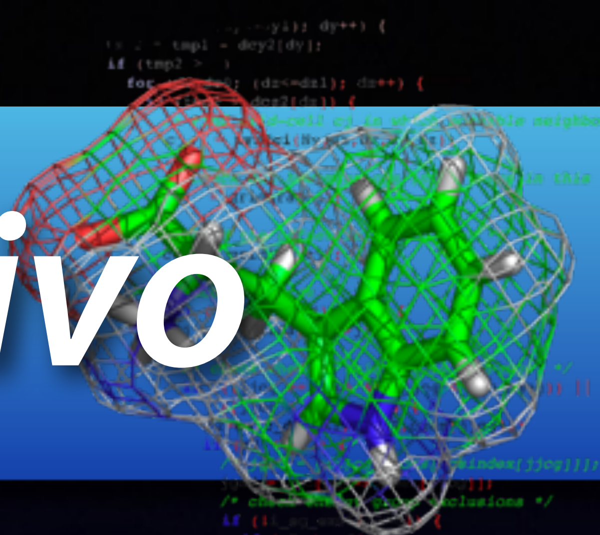
2. Reading the recipe

Roger Kornberg
Nobel Prize 2006

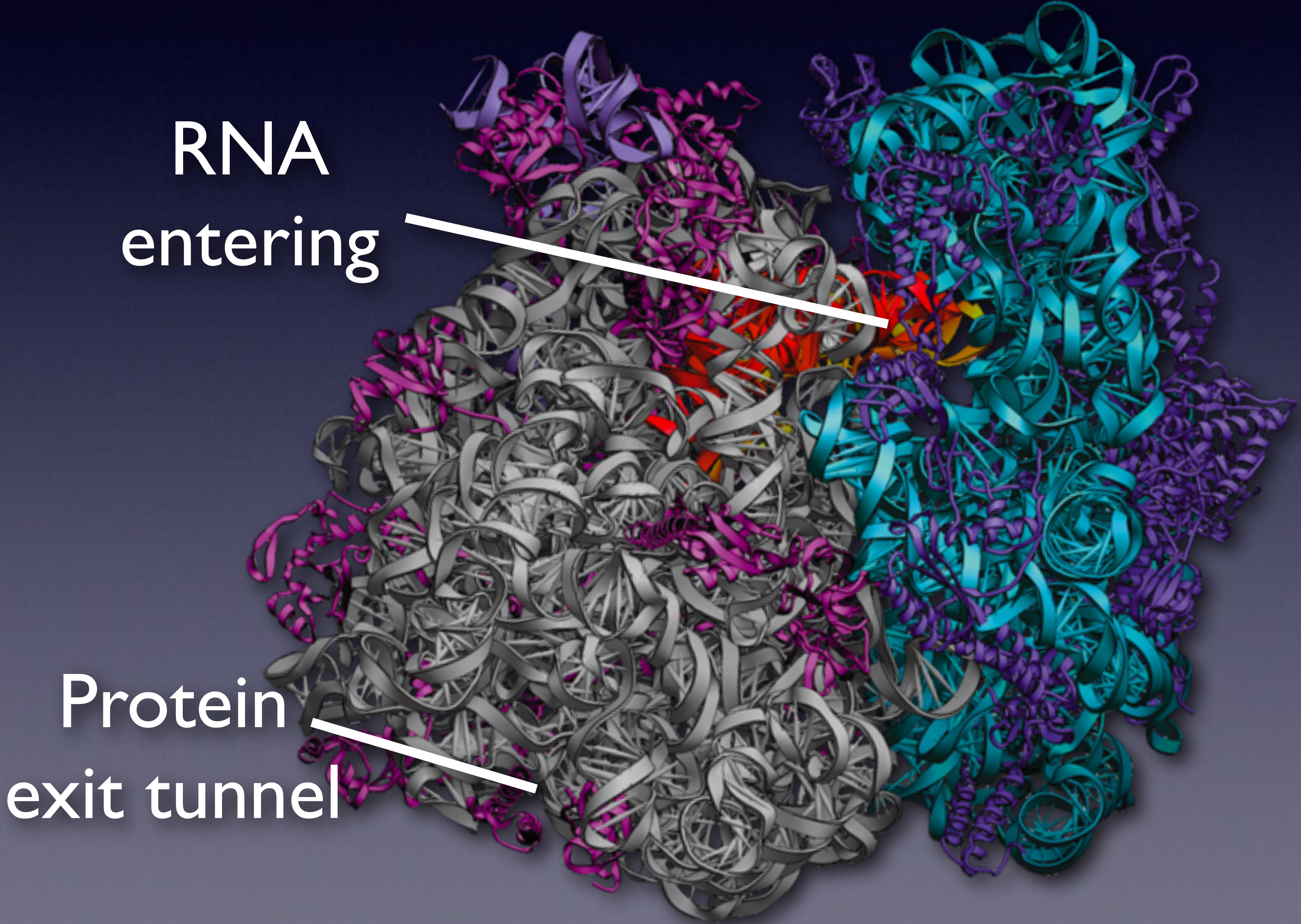


RNA polymerase copies DNA into mRNA (messenger RNA)

Protein synthesis *in vivo*



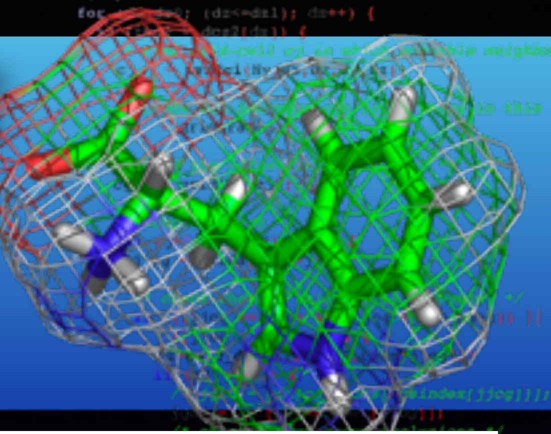
3. Making proteins from RNA



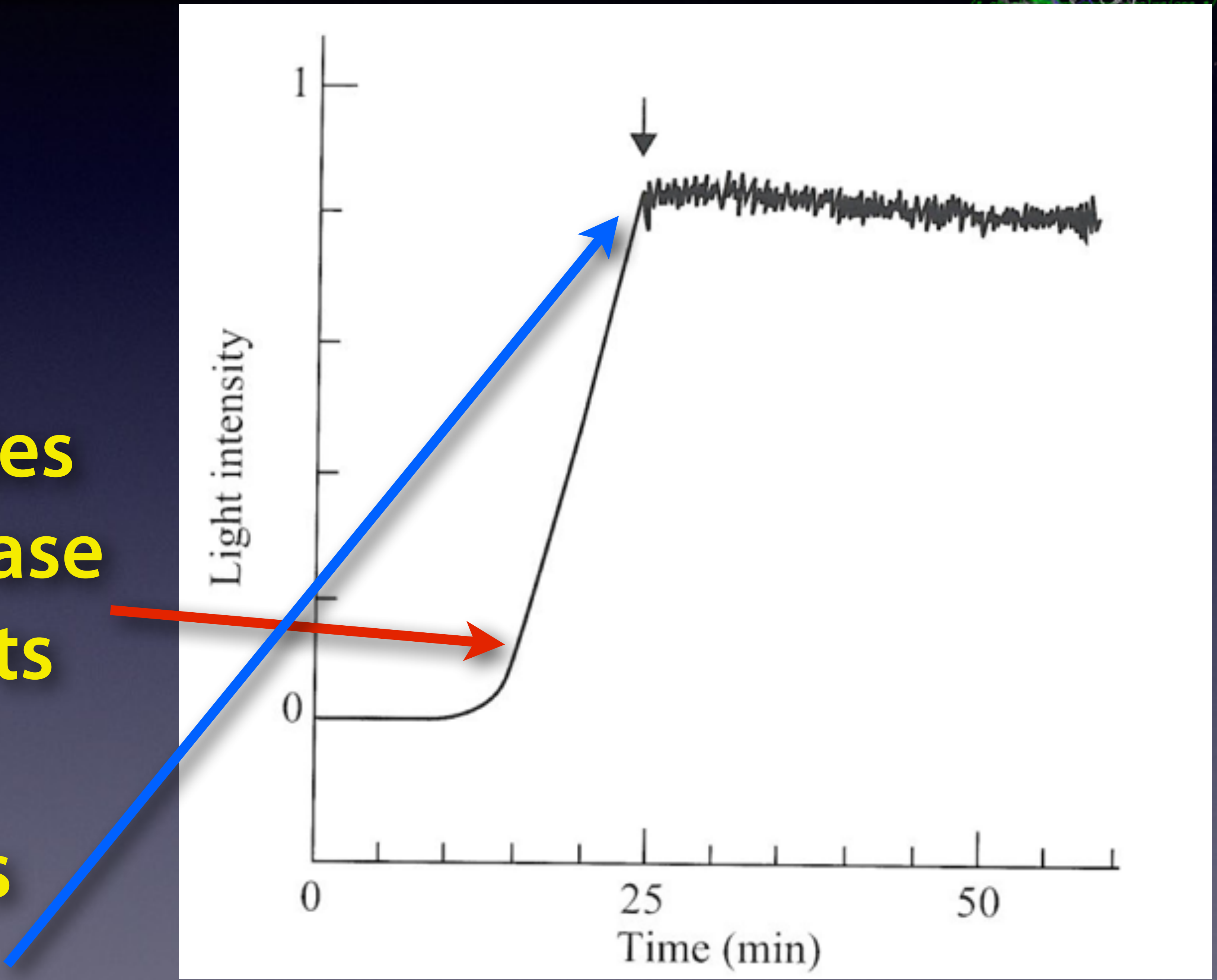
The 70S ribosome:
100,000 atoms
Tom Steitz 2001
(Nobel 2009)

in vivo folding takes seconds to minutes!

Experimental evidence of slow synthesis



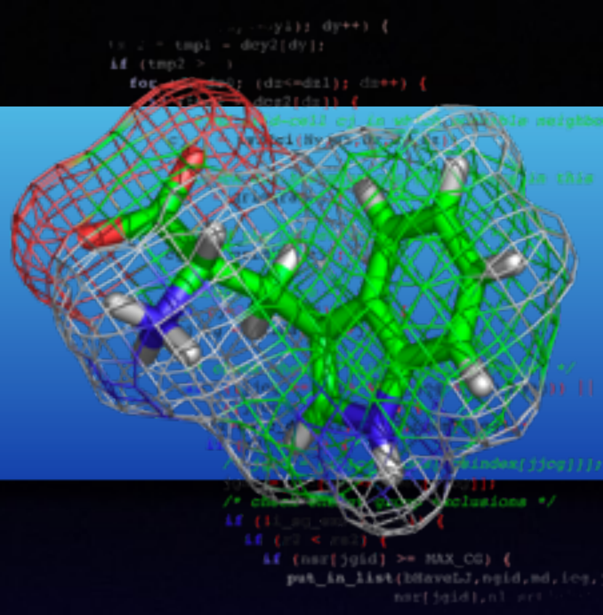
Light emission by luciferase protein



Takes several minutes for intensity to increase after synthesis starts

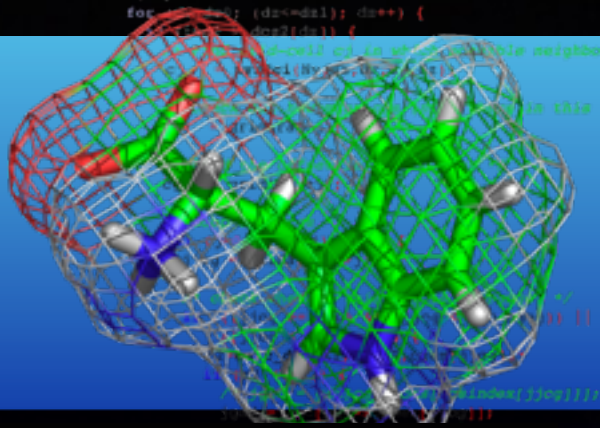
The increase stops instantly when synthesis is stopped

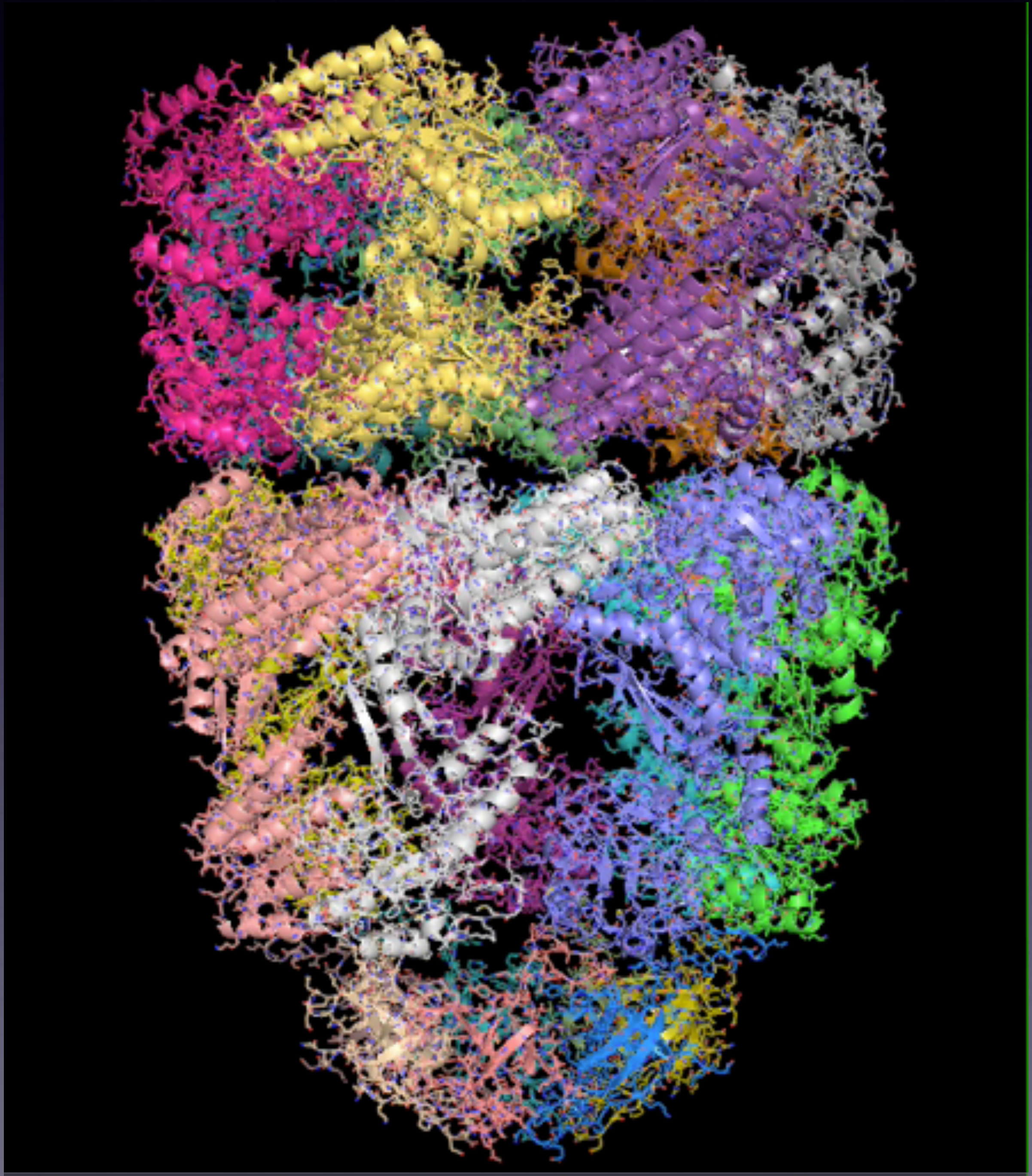
Folding



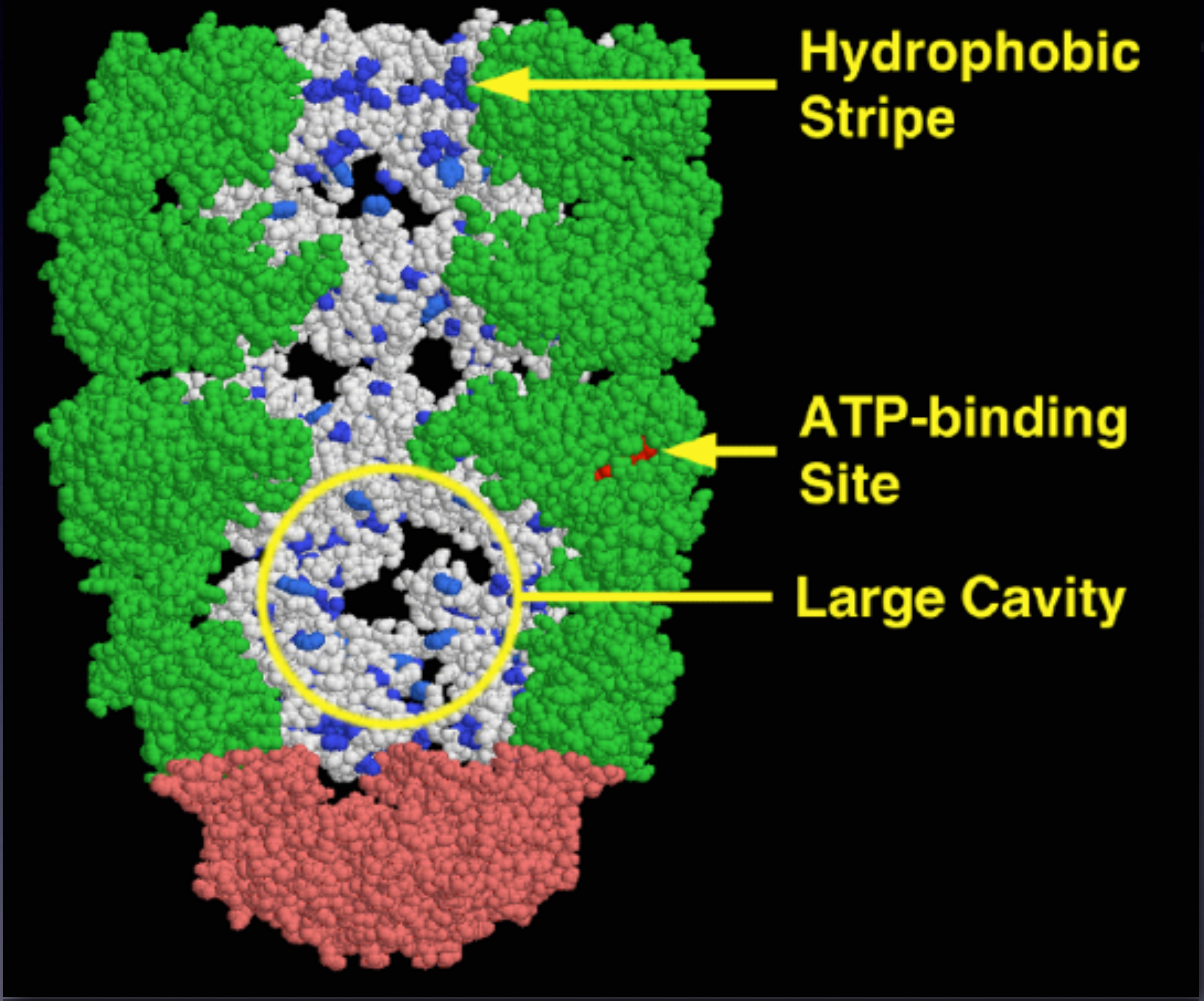
- Co-translational *in vivo* folding
- Anfinsen 1961: Globular proteins are capable of spontaneous refolding after denaturation *in vitro*
- Not true for all proteins
  - Enzymes improve folding rate
  - Some proteins collapse and stick together before folding (many hydrophobic parts)

Chaperones



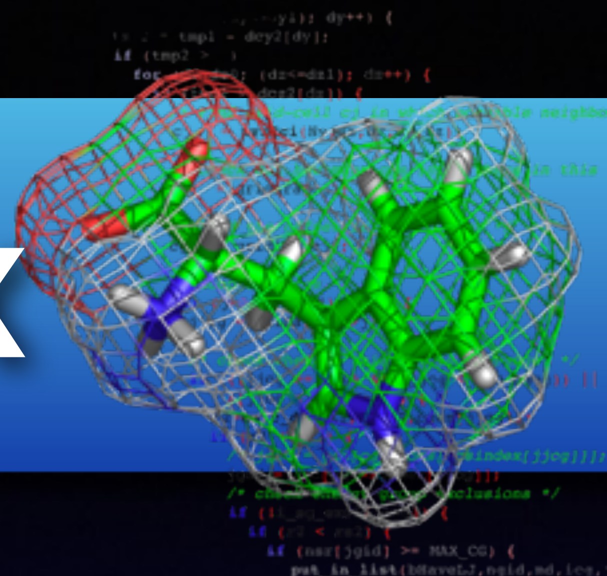


GroEL/ES



Helps correct protein misfolding?

Levinthal's paradox

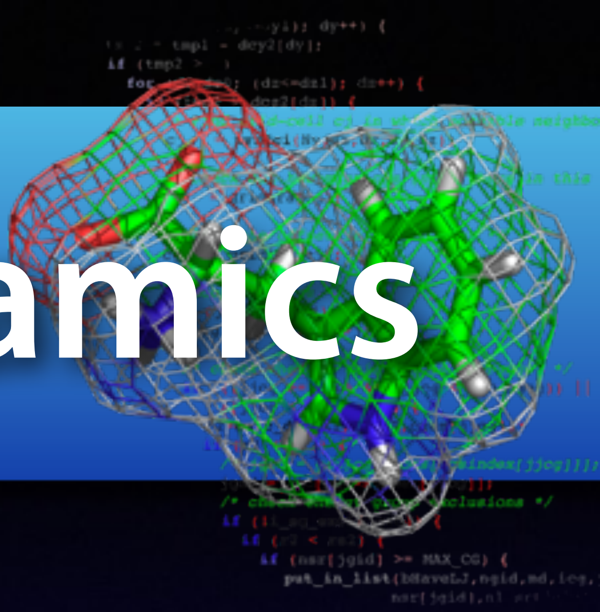


- How can a protein fold in realistic time?
- n=2-3 conformations per residue
- 100 residue chain
- n^{100} possible chain conformations
- Would take $>10^{10}$ years to sample!
- Paradox:
    - Native state should be most stable one
    - But no way to try them all!?

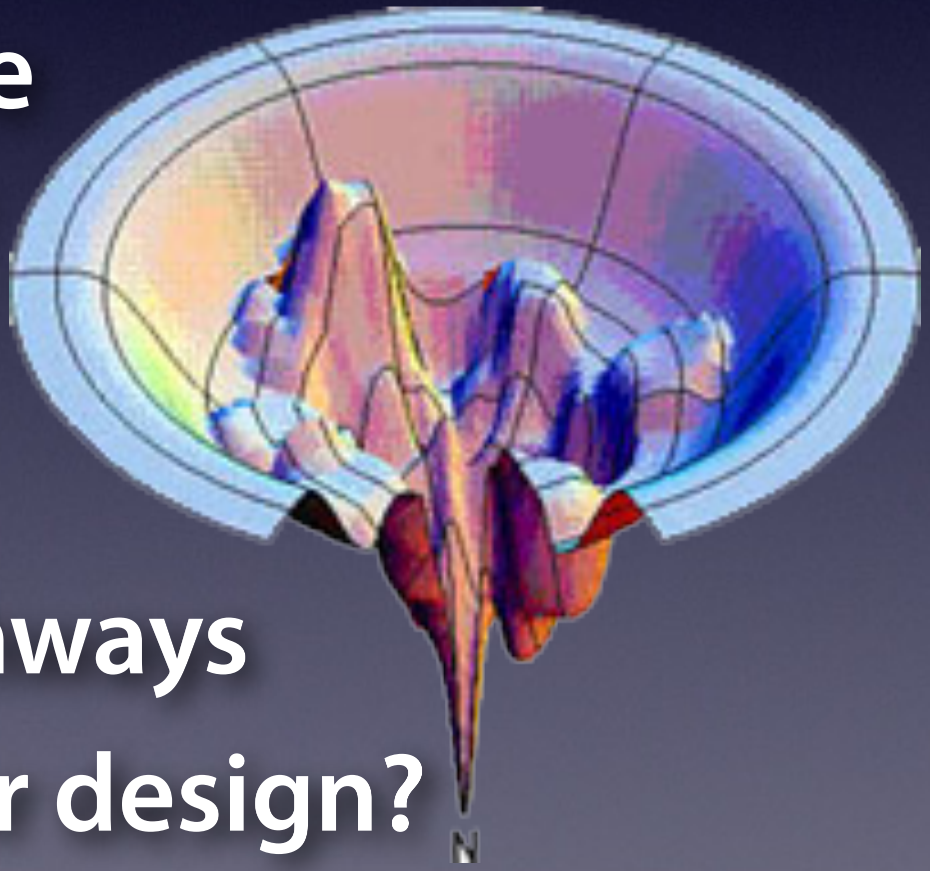
Not generally true...

Phillips: Could folding start around the N-terminal end of the chains?

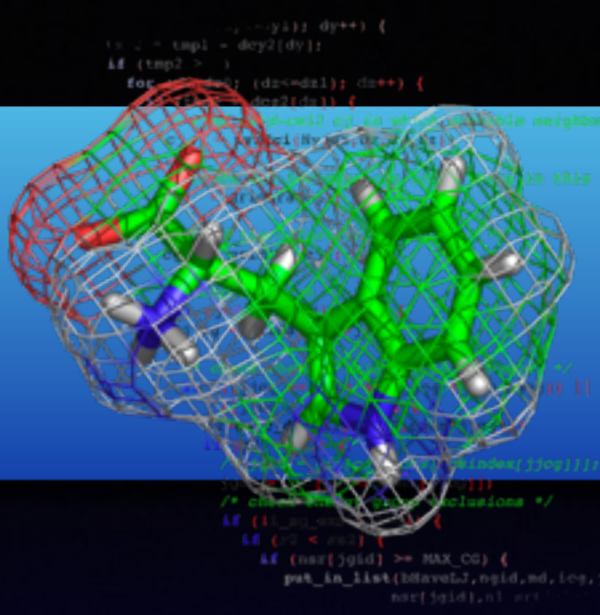
Kinetics vs. Thermodynamics



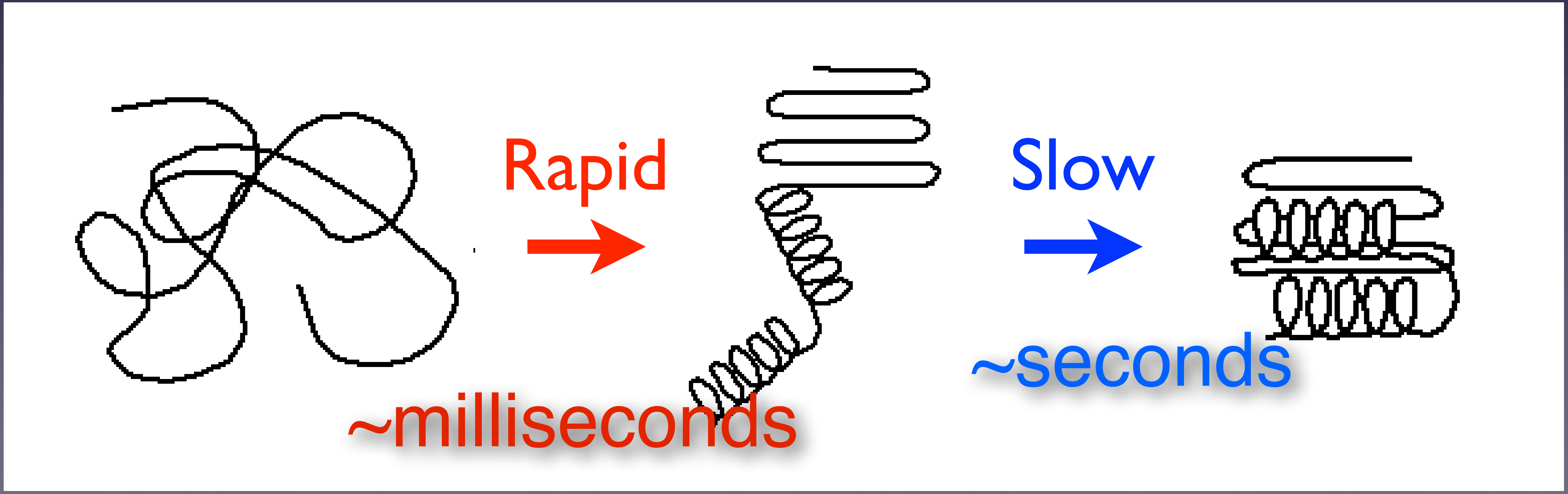
- Levinthal: Proteins are not under thermodynamic control but kinetic
- HUGE implications: Native state would be the *easiest accessible* free energy minimum
- Quite different from thermodynamics - folding pathways
- Is it true? What does it mean for design?
- We need to understand *how* proteins fold



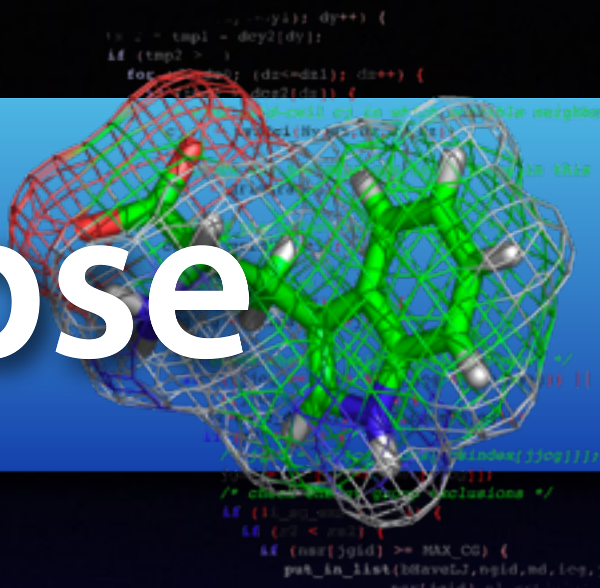
Diffusion-collision



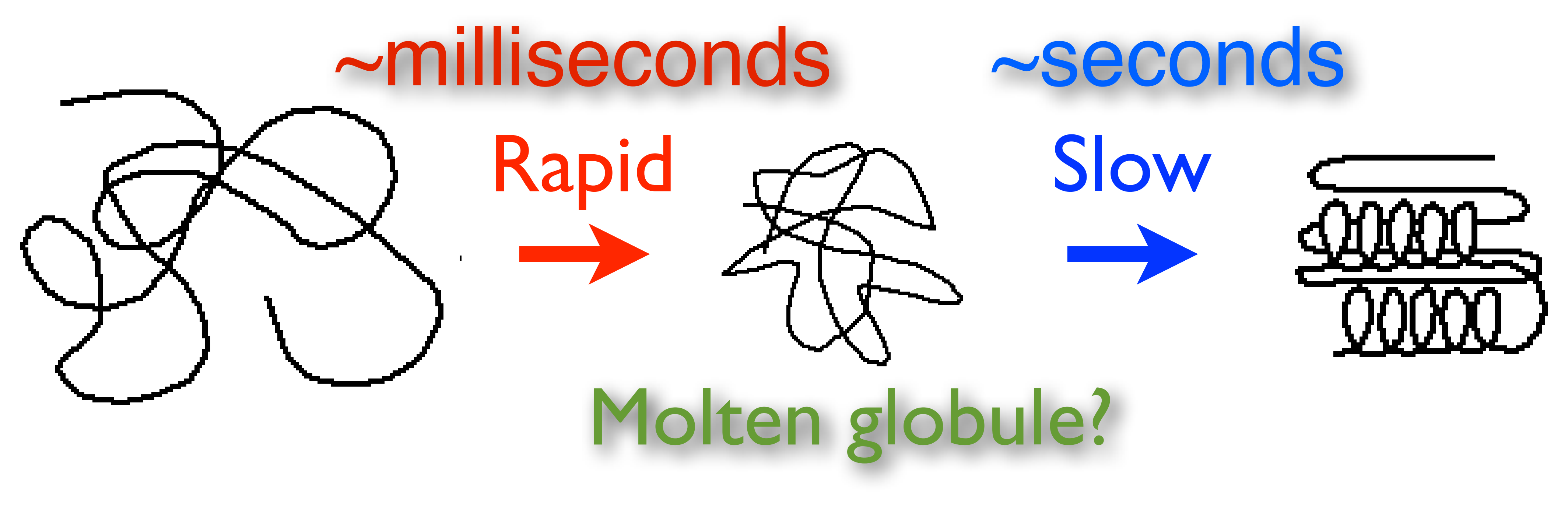
- a.k.a. “Framework model”
- Rapid initial formation of helices/sheets
- Assembly of secondary structure units



Hydrophobic collapse



- Initial hydrophobic collapse
- Gradual growth of secondary structure



~milliseconds

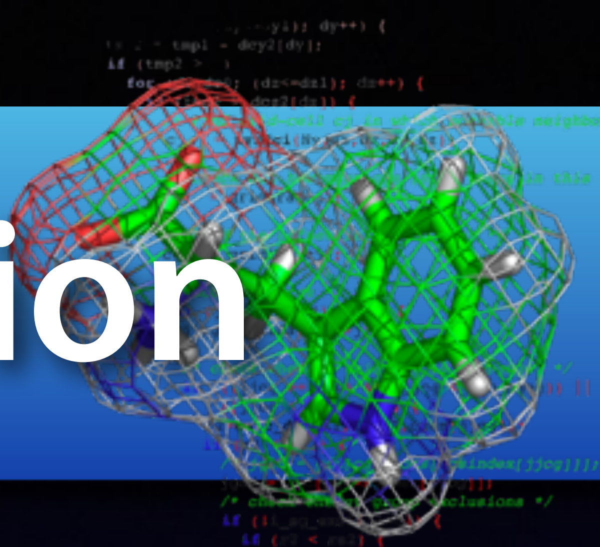
Rapid →

~seconds

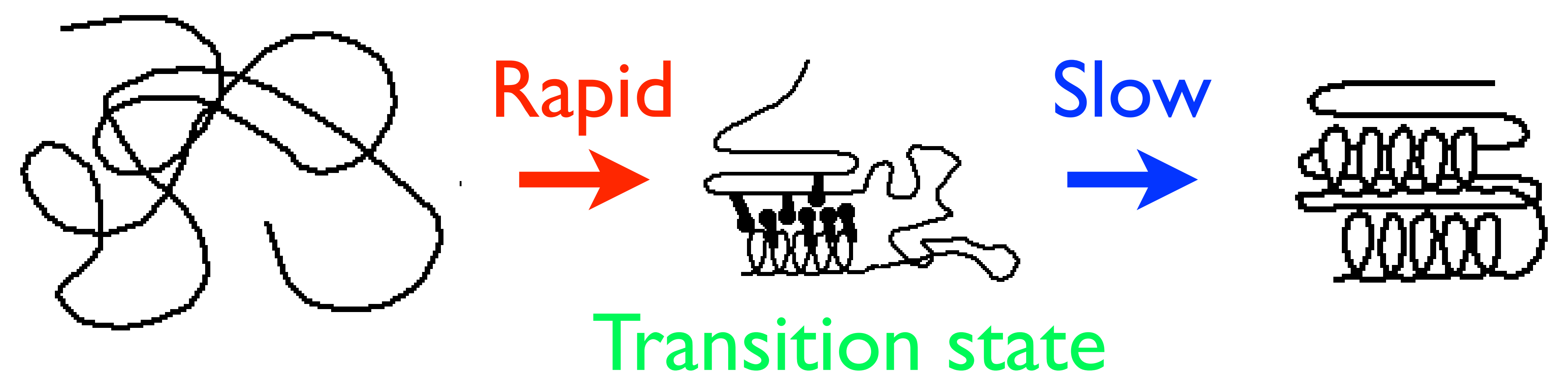
Slow →

Molten globule?

Nucleation-condensation



- A few key residues start making native contacts and “lock-in” the structure
- Gradual growth & extension of structure

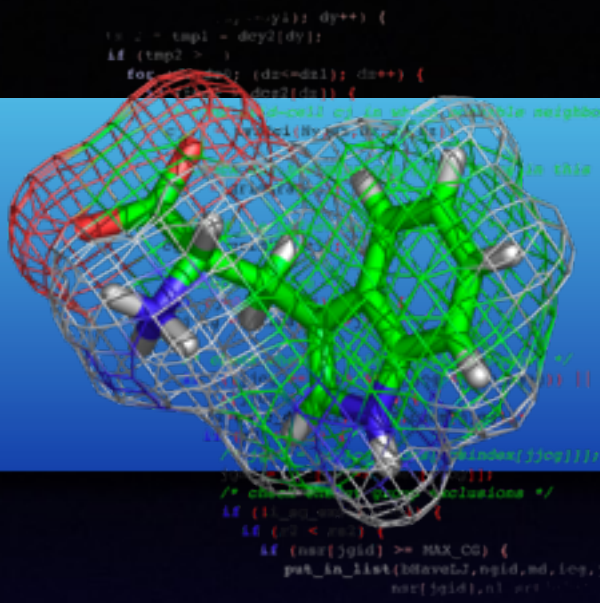


Rapid

Slow

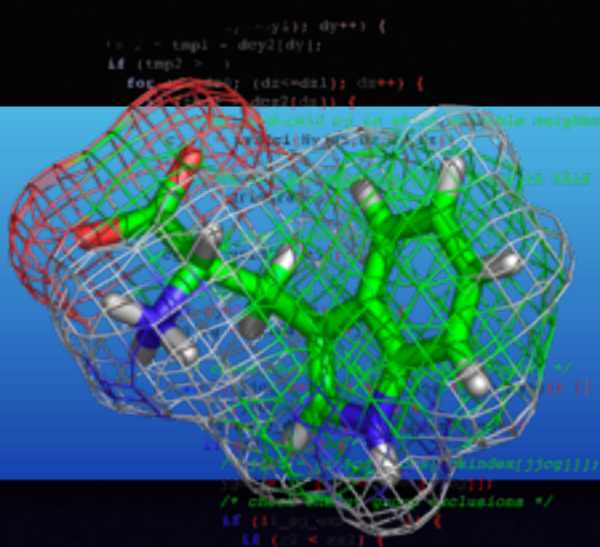
Transition state

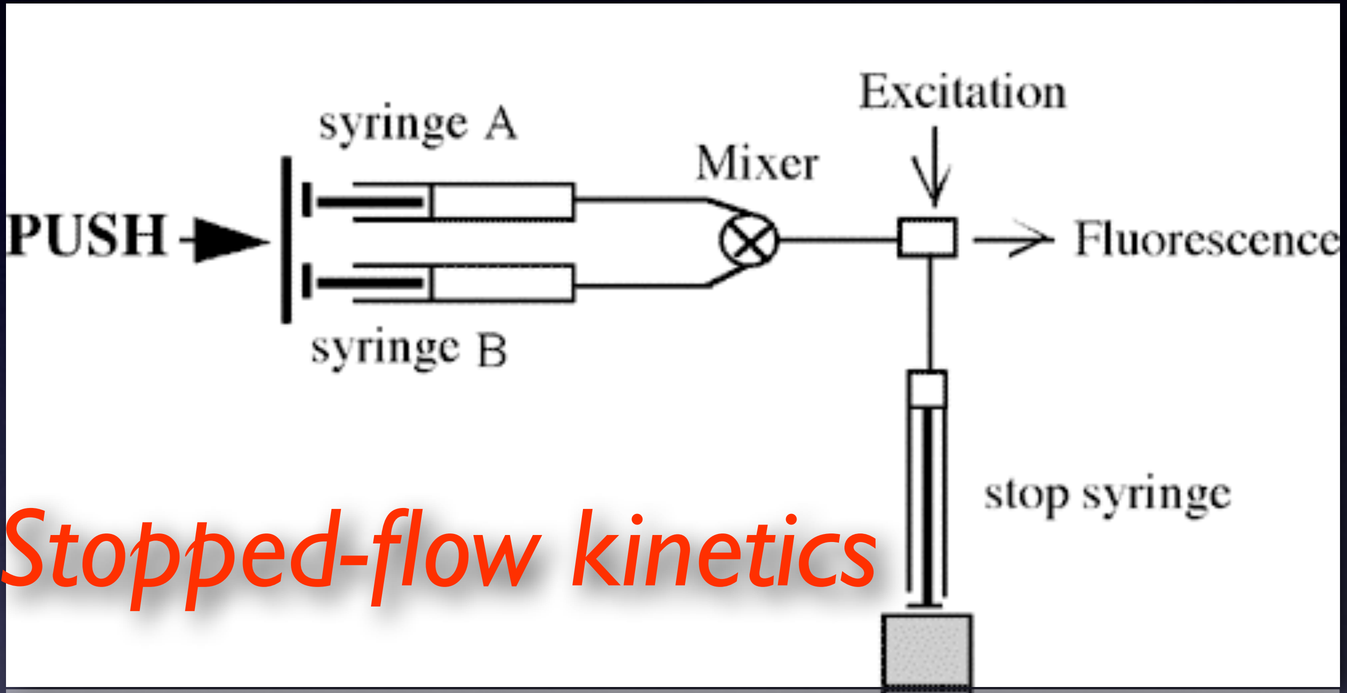
Intermediates & transition states



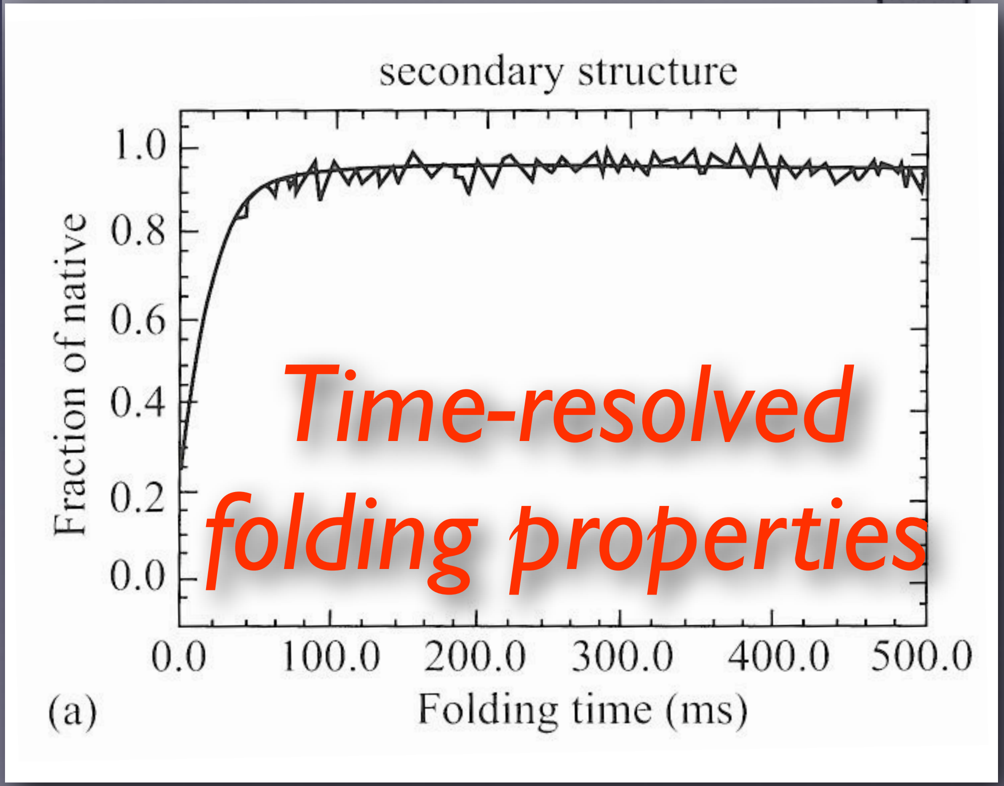
- A folding intermediate:
  - Stable structure along folding pathway
  - Local free energy minimum, observable
- A transition state:
  - Unstable bottleneck state we need to pass
  - Local free energy *maximum*
  - Cannot be observed directly - but determines the folding rate!

Experimental rates

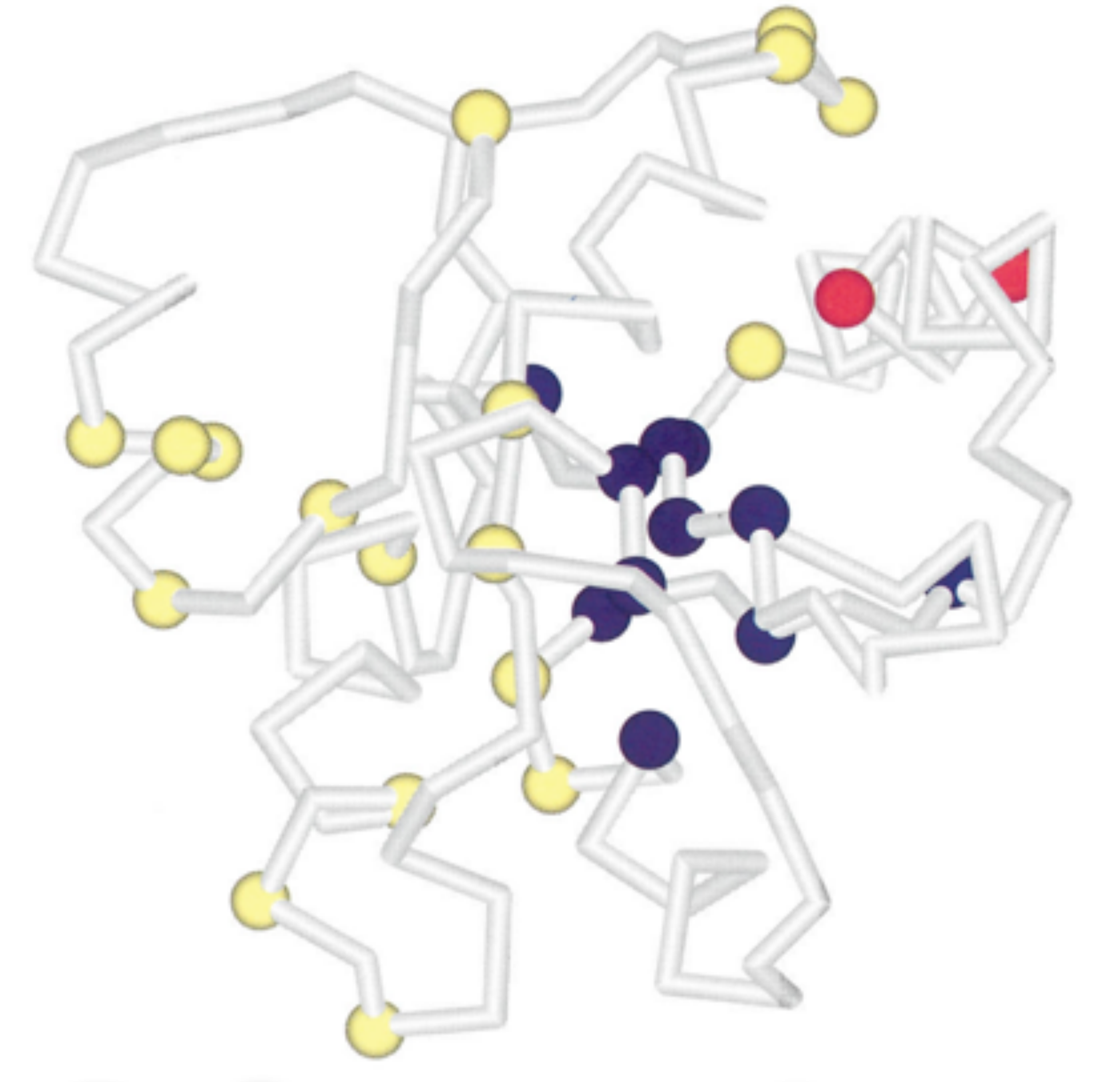




Stopped-flow kinetics

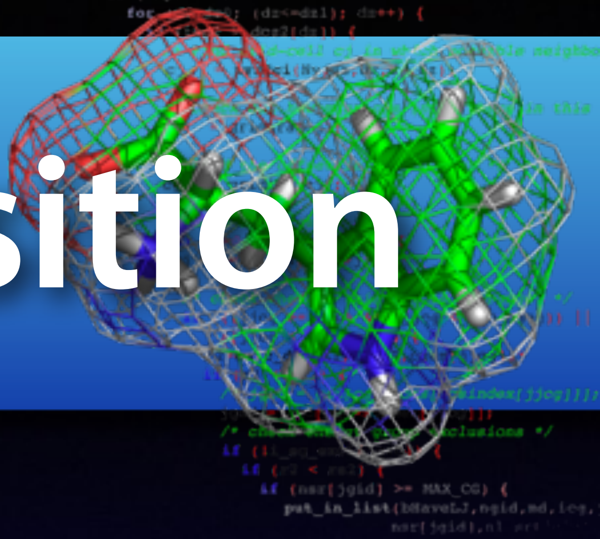


Time-resolved folding properties



Perform mutations to find residues involved in the transition state

Understanding the transition



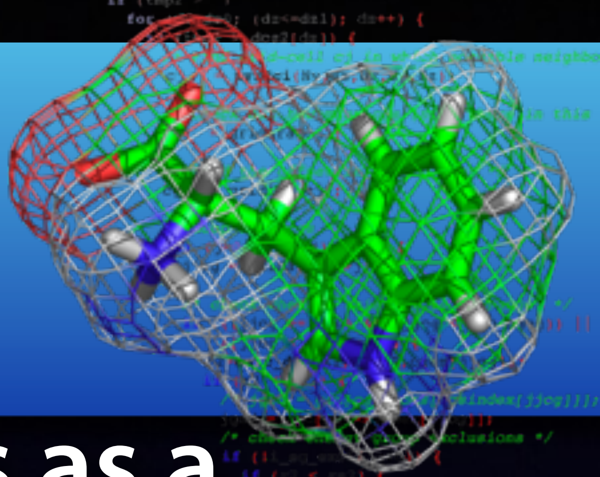
- Chemical transition-state theory
- Folding/unfolding rate equations

Determine k experimentally!

$$k_{A\rightarrow B} = k_0 \exp\left[-(F_{\#} - F_A)/RT\right]$$

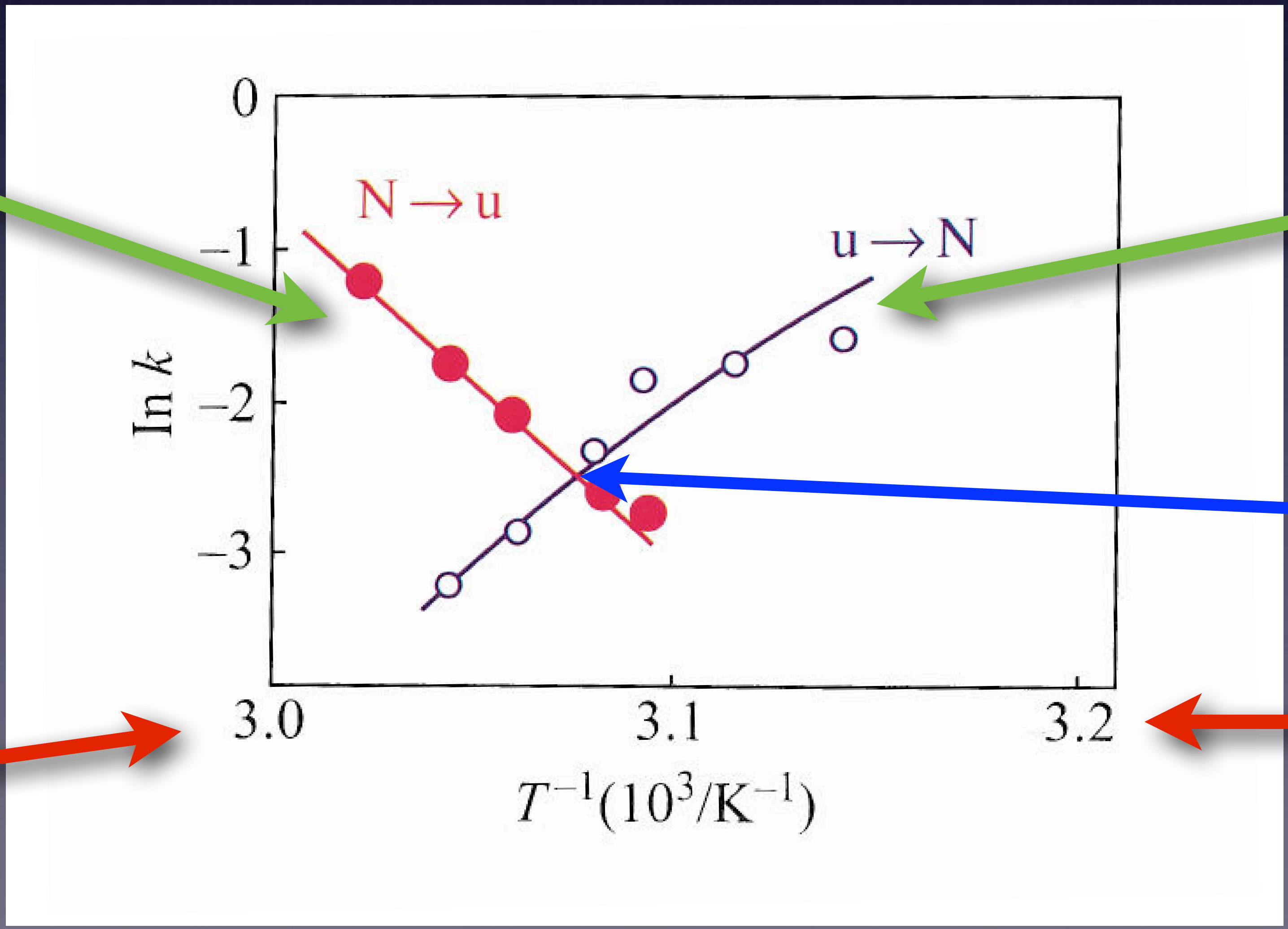
$$k_{B\rightarrow A} = k_0 \exp\left[-(F_{\#} - F_B)/RT\right]$$

Arrhenius plots



- Denaturation and renaturation rates as a function of reciprocal temperature (1/T)

Can be difficult to measure independently!



Unfolding faster

Folding faster

Mid-transition 325K

Higher T

Lower T

Temperature dependence

The temperature dependence of the reaction rate makes it possible to estimate the transition energy barrier:

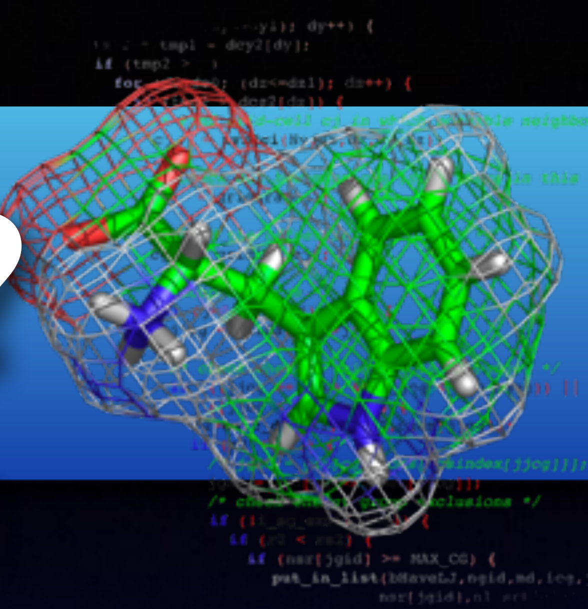
$$\frac{d\left[\ln(k_{A\rightarrow B})\right]}{d(1/T)} = \frac{d\left[\ln(k_0) - (F_\# - F_A)/RT\right]}{-(1/T^2 dT)} \approx \frac{E_\# - E_A}{R}$$

Not very T-dependent

$$\frac{d(F/T)}{dT} = -E/T^2$$

Derived a couple of lectures ago

What does it mean?



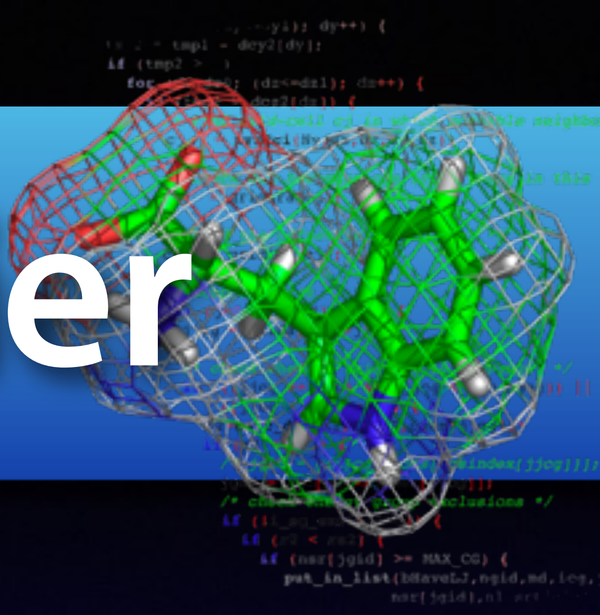
- Unfolding rate grows with T (normal)
- Folding rate drops with T (very abnormal!)
  - Folding gets faster at lower T!
- Using

$$\frac{d\left[\ln(k_{A\to B})\right]}{d(1/T)} \approx \frac{E_\# - E_A}{R}$$

- We get: $E_\# > E_{Native}$ (quite reasonable)
- But also: $E_{unfolded} > E_\#$!?

$E_{unfolded} > E_\# > E_{Native}$

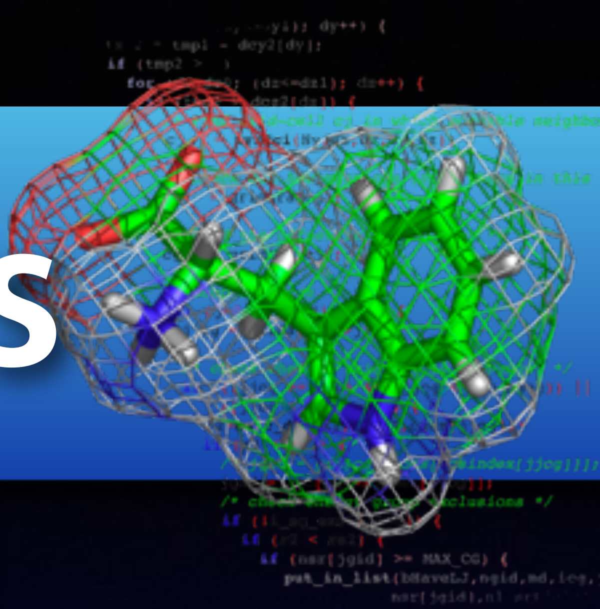
The transition barrier

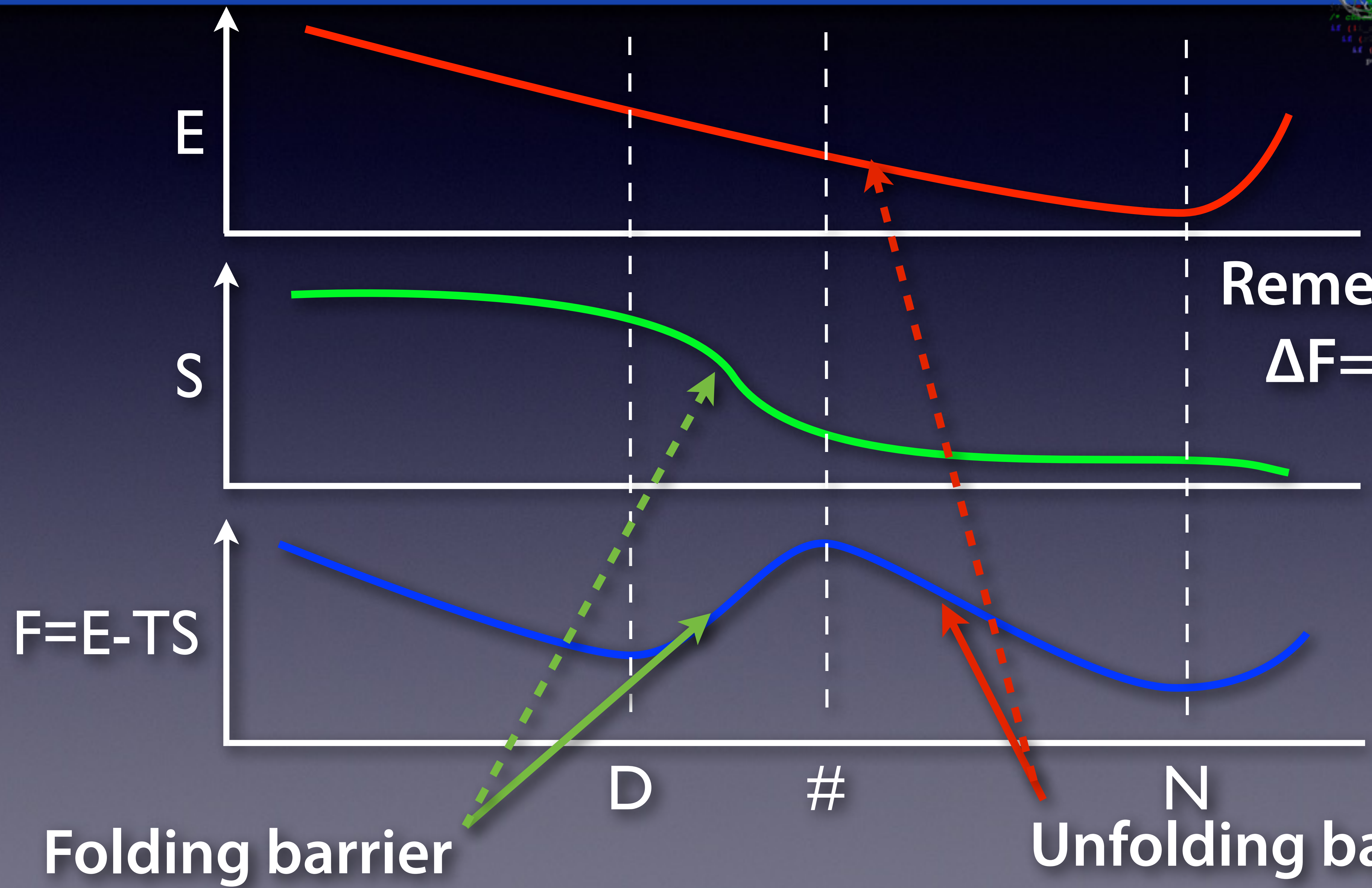


- Similarly, it is possible to calculate entropy S from Arrhenius plots, and from that we get $S_{unfolded} > S_{\#} > S_{Native}$
- Native to denatured: barrier is energy!
- Denatured to native: barrier is entropy!

No longer handwaving - proved from experiments!

Barrier components



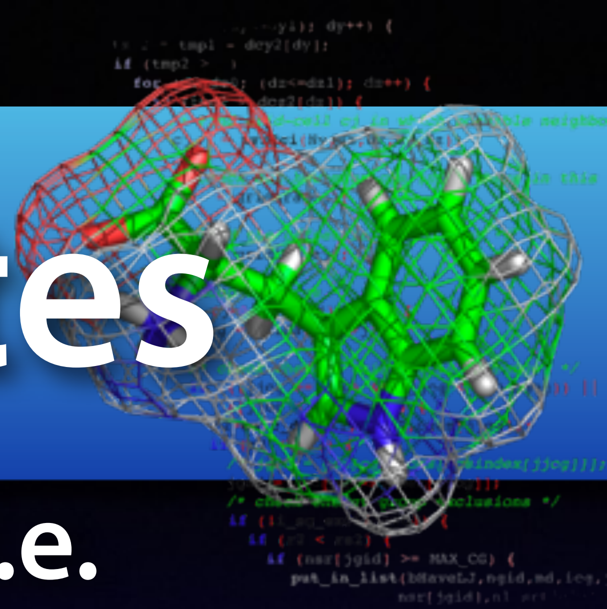


Folding barrier

Unfolding barrier

Remember:
ΔF=E-TS

Apparent folding rates



- **What is the equilibrium constant, i.e. rate of the entire process?**

$$K_{B:A} = \frac{k_{A\to B}}{k_{B\to A}} = \exp\left[-(F_B - F_A)/RT\right] = \frac{n_B^\infty}{n_A^\infty}$$

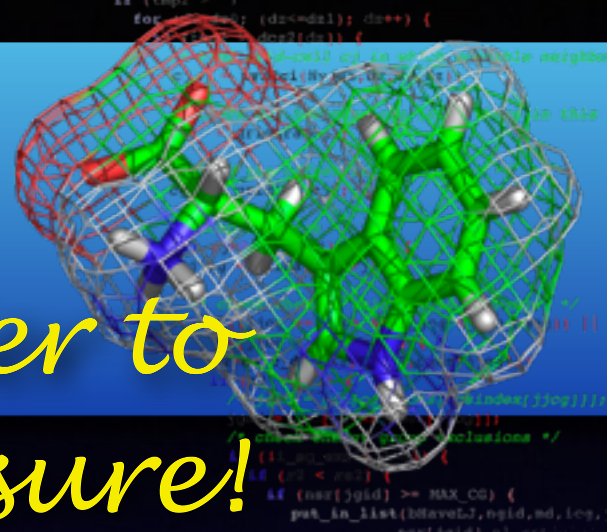
$$\frac{dn_A(t)}{dt} = -k_{A\to B}n_A(t) + k_{B\to A}n_B(t)$$

$$\frac{dn_A(t)}{dt} = -(k_{A\to B} + k_{B\to A})\left[n_A(t) - n_A^\infty\right]$$

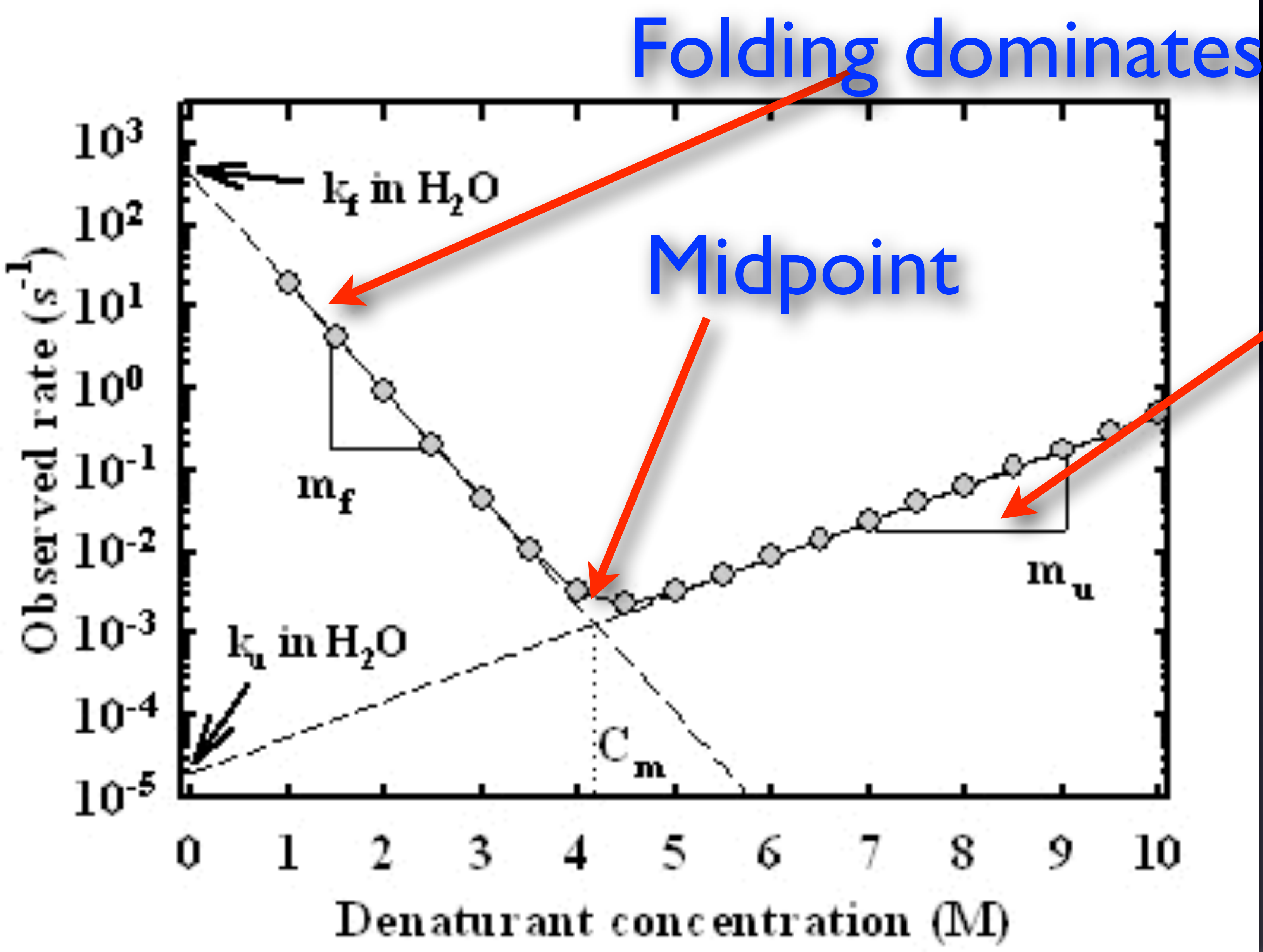
$$n_A(t) = \left[n_A(t=0) - n_A^\infty\right] \times \exp\left[-(k_{A\to B} + k_{B\to A})t\right] + n_A^\infty$$

Apparent rate: $k_{\text{app}} = k_{A\to B} + k_{B\to A}$

Chevron plots



Easier to measure!



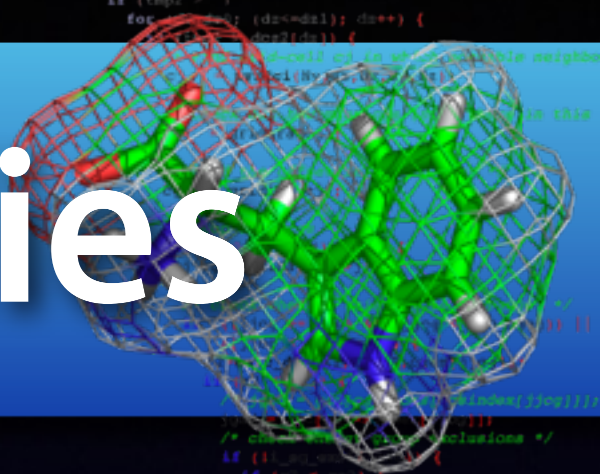
Folding dominates

Midpoint

Unfolding dominates

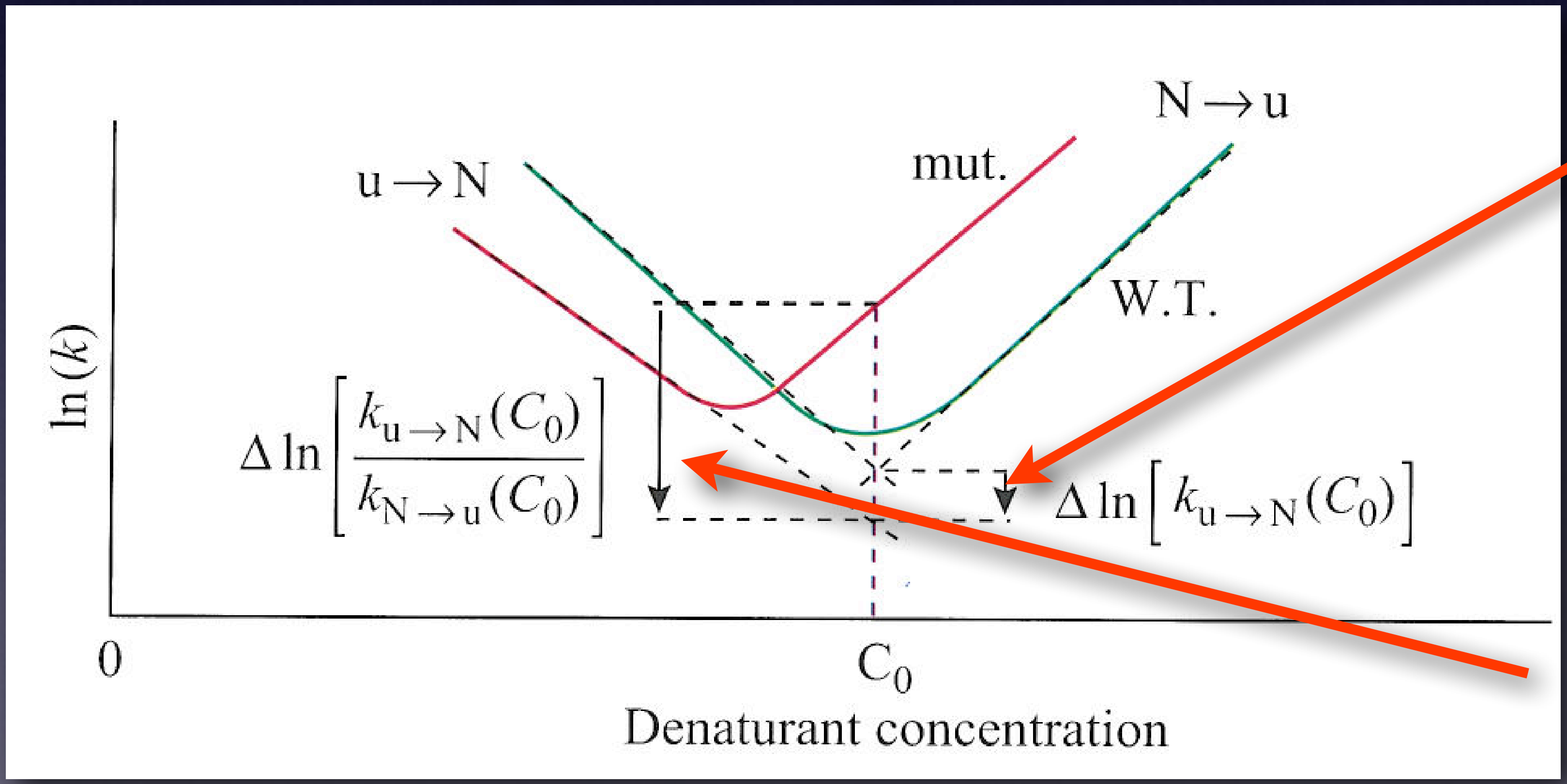
Chevrons

Transition state studies



- Can we find out which residues are forming the nucleus/transition state?
- What happens if you do mutations?

$k = \exp\{-\Delta F/RT\}$



Change in barrier unfolded-to-native

$\Delta(F_\#-F_u)=-RT\Delta\ln(k_{u\text{->}N})$

Change in stability of folded state: $\Delta(F_N-F_u)= -RT\Delta\ln(k_{u\text{-}N})+RT\Delta\ln(k_{N\text{-}u}) =-RT\Delta\ln(k_{u\text{-}N}/k_{N\text{-}u})$

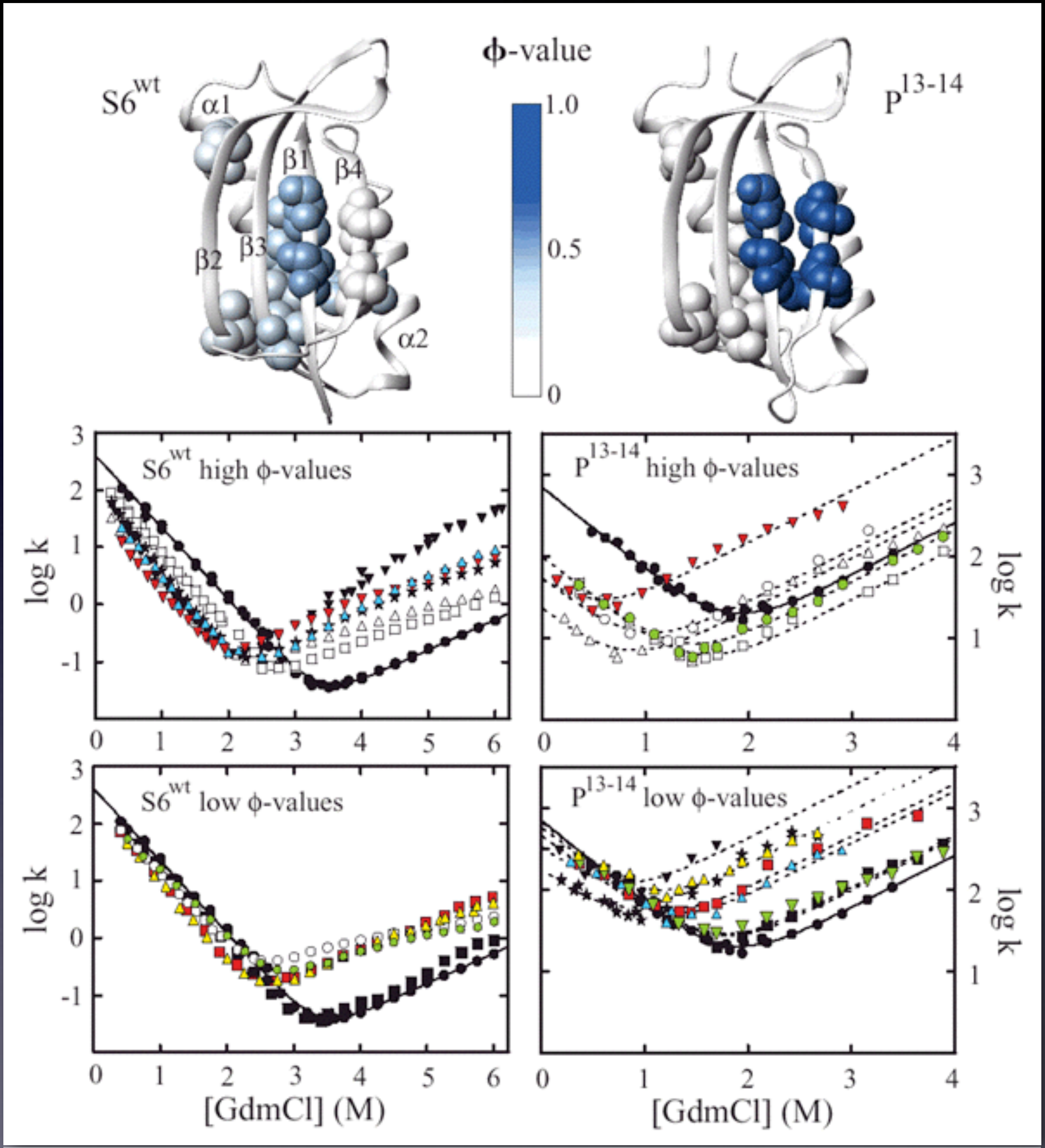
$\Phi_f= \Delta(F_\#-F_u)/\Delta(F_N-F_u)$ (Alan Fersht)

$\Phi_f=1$: Affects transition & final states equally

$\Phi_f=0$: Only affects final, not transition state

Note: Plot uses Ln(k):

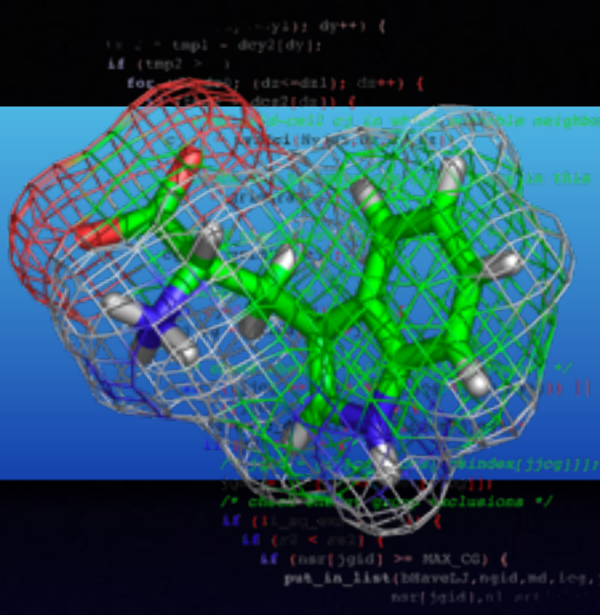
$\ln(a/b) = \ln(a)-\ln(b)$

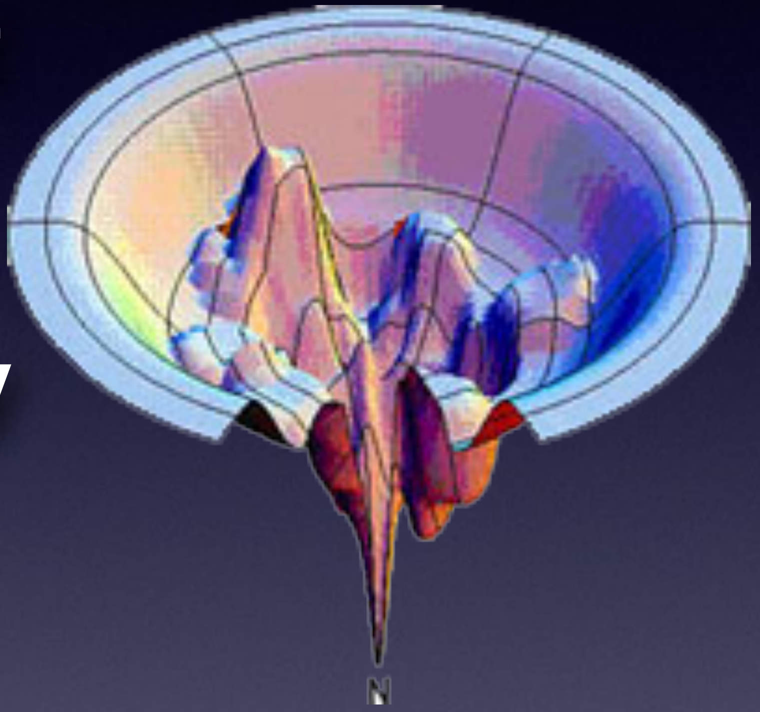


**Mikael Oliveberg (SU):
Change of transition
state with circular
permutation
of sequence**

**High Φ-values means
a residue participates
in the transition state**

Levinthal revisited



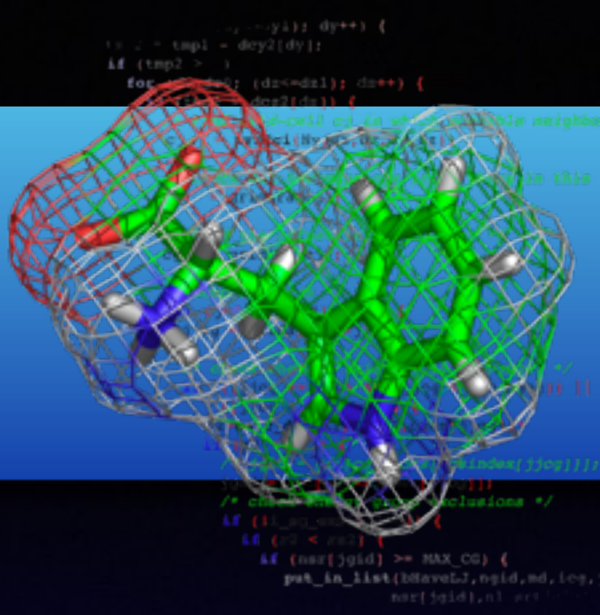


- So, how can we apply what we just deduced about folding kinetics?
  - Native state = lowest free energy or...
  - Native state = fastest folding

Or, could it be both?

Will stable structures automatically lead to rapid pathways?

Solving Levinthal



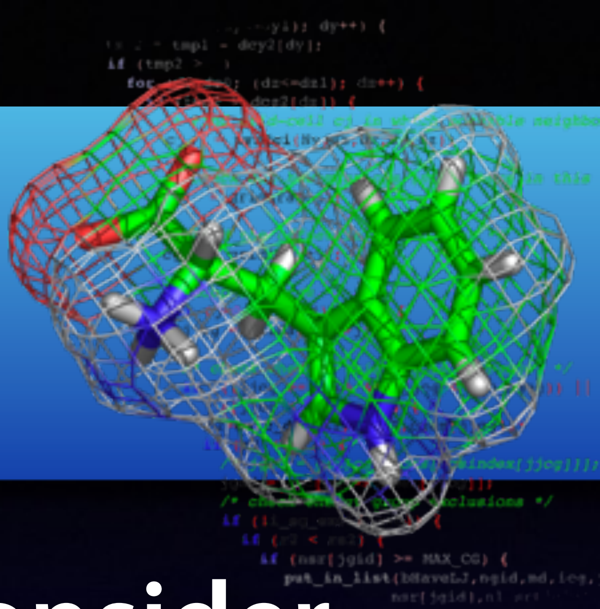
- Folding kinetics determined by barriers

$$t = t_0 \exp\left[\Delta F_{\#}/RT\right]$$

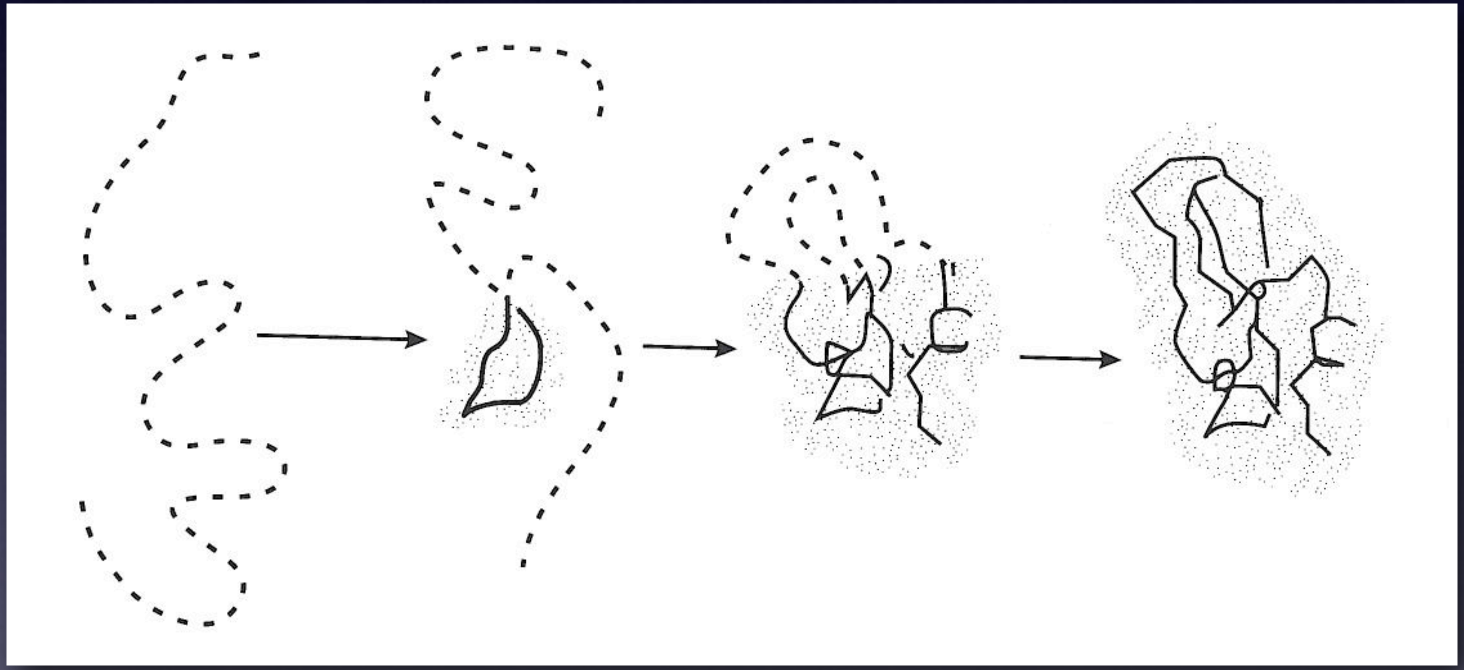
- t0 is single step, ~1-10ns (helix elongation)
- Both energy & entropy drops during folding
  - What happens first?
- If chain collapses too much before contacts are formed, we would get a huge ΔF barrier

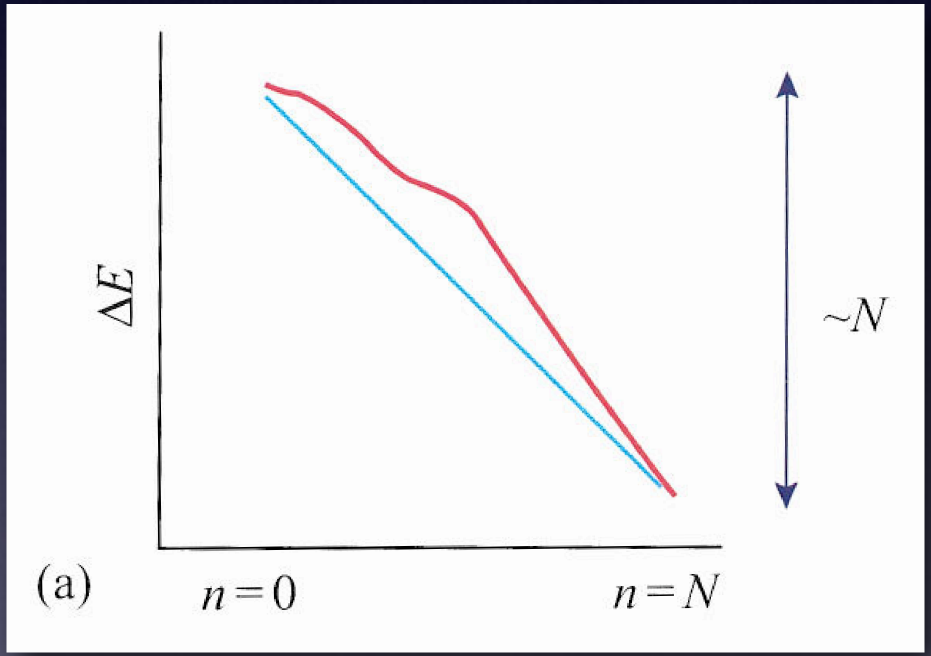
'Folding funnels' idea: Pathways of gradual energy decrease

Solving Levinthal



- Sufficient to find one good pathway; consider e.g. nucleation-condensation

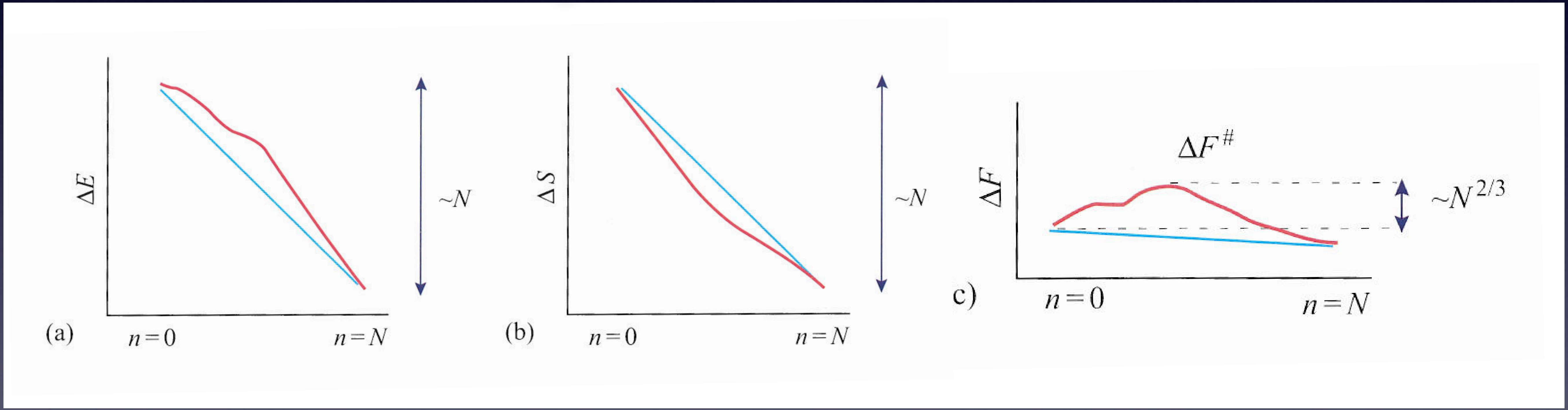




- Sequential: #interactions $\propto N_{native} \propto$ volume $\propto r^3$
- Fewer contacts initially: #interactions $\propto$ area $\propto r^2$
- Energy $= C_1 N_{native} + C_2 N_{native}^{2/3}$

It’s no longer a paradox!

- Entropy drops as N_{native}
- Faster initially, extra $N_{native}^{2/3}$ term here too



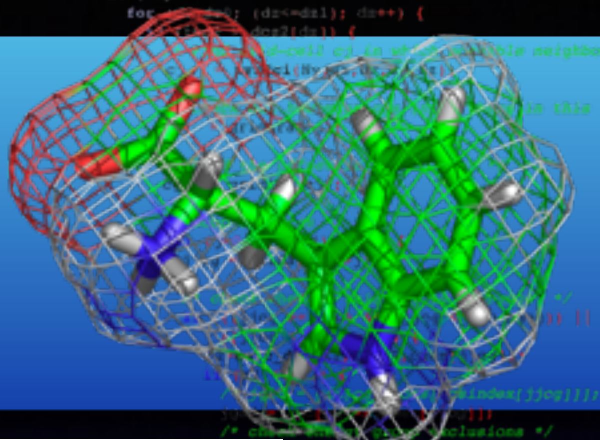
But what difference does it make?

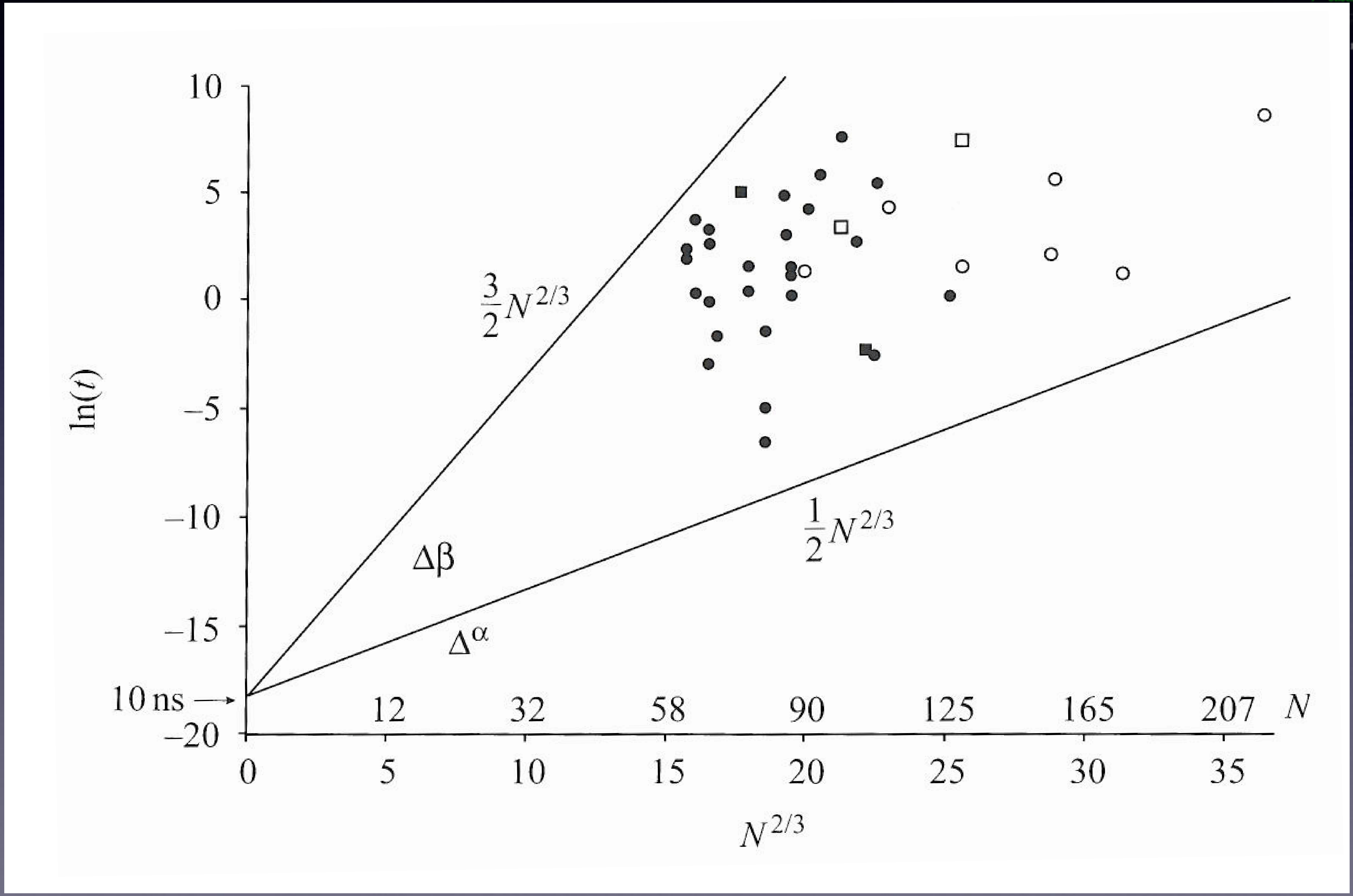
$$t = t_0 \exp\left[(1 \pm 0.5) N^{2/3}\right]$$

Seconds to minutes instead of 10^{10} years.

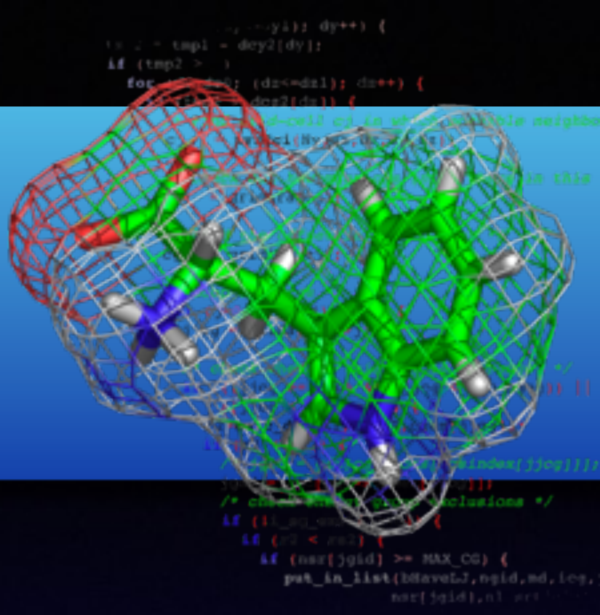
This is the solution to Levinthal’s paradox!

Experimental tests



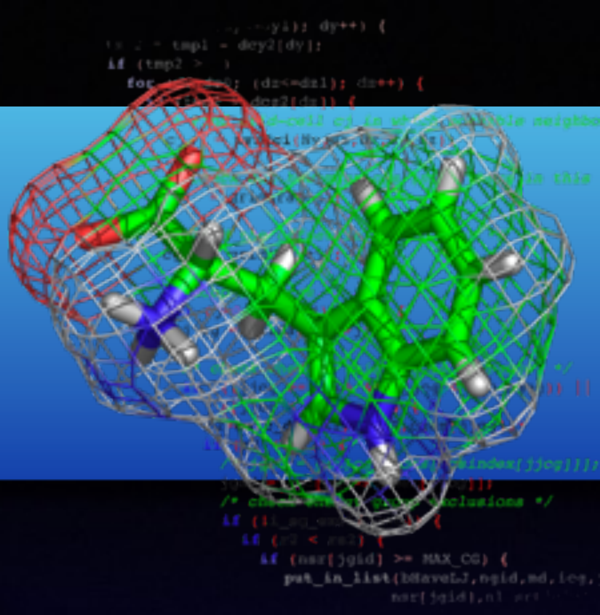


More on kinetics

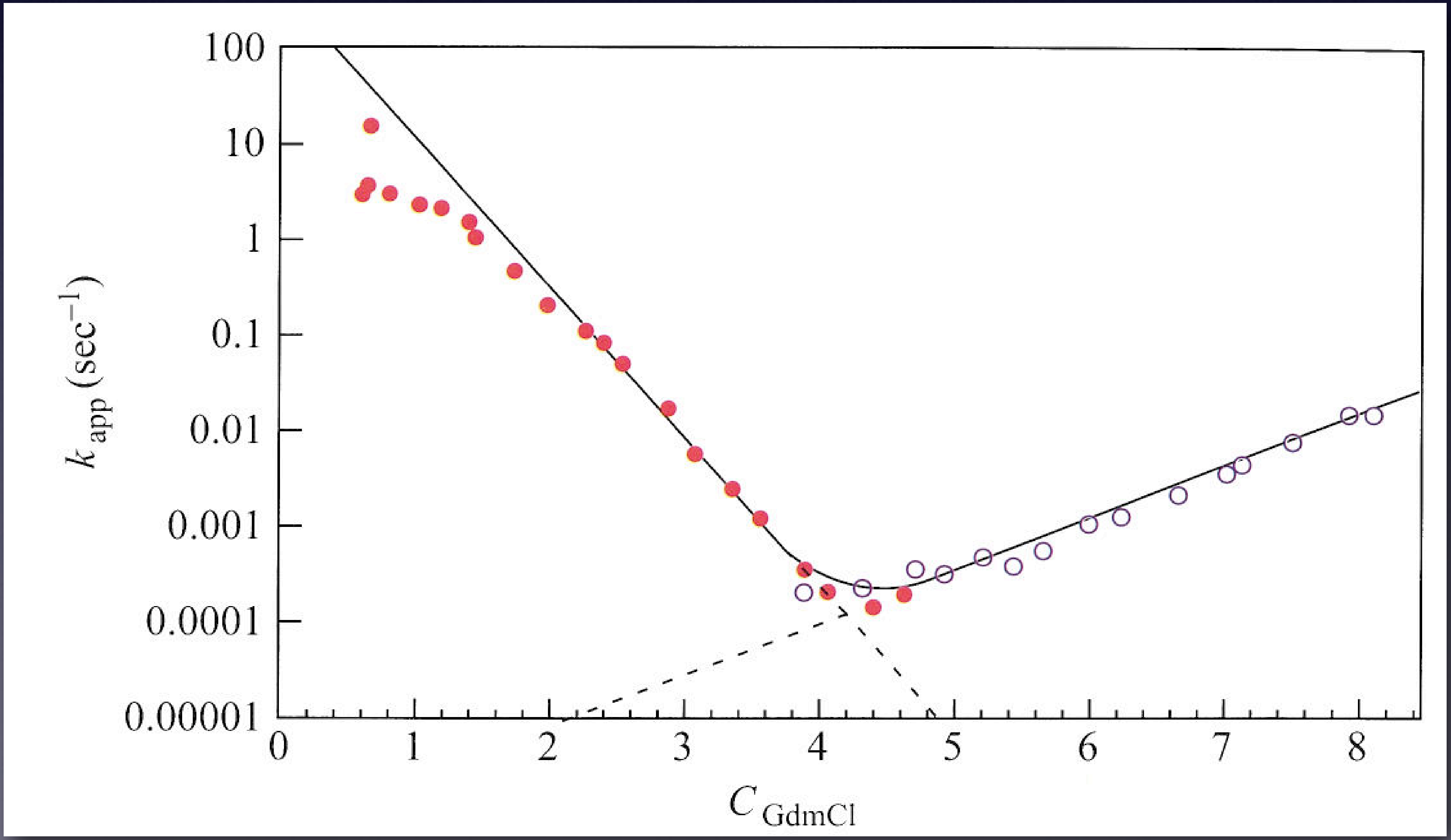


- Based on experimental folding rates, we can derive that the size of transition state must be approximately half the protein
  - Limits the size of folding units (domains)!
- Rate determined at equilibrium/midpoint
  - Real folding could only be faster
- Folding is a driven, not random, search

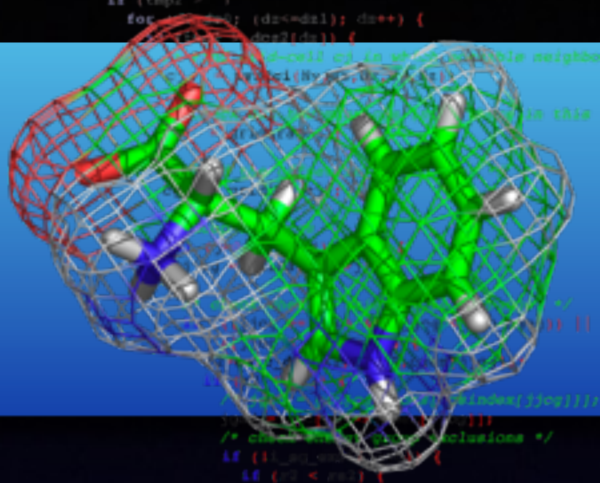
Stability vs. rates

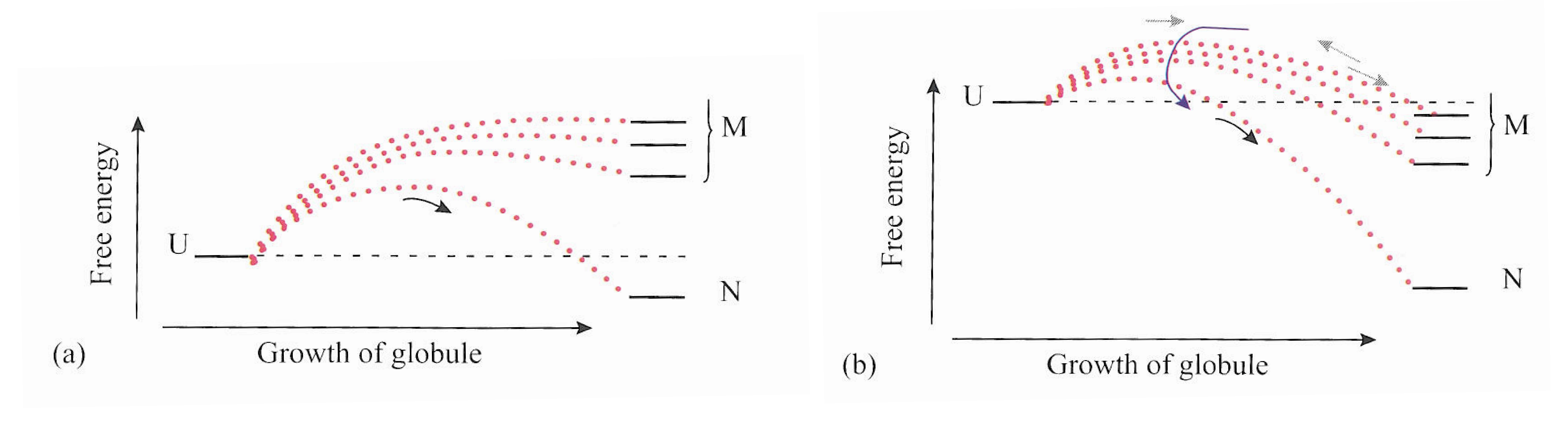


What happens as the native state free energy gradually gets more stable?



Energy gaps

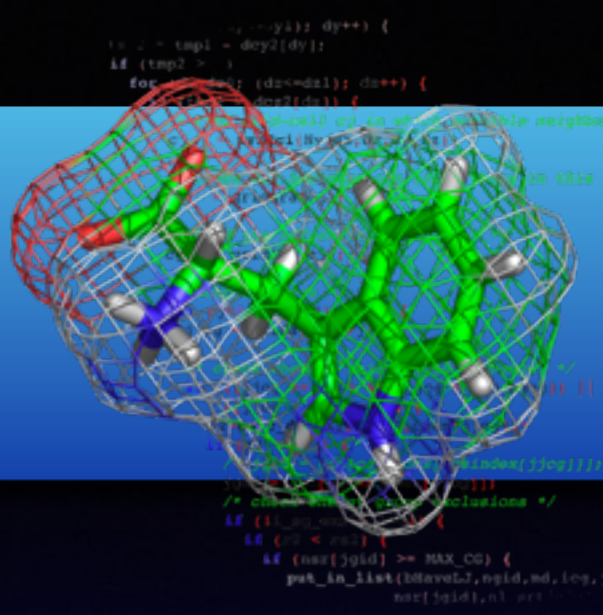


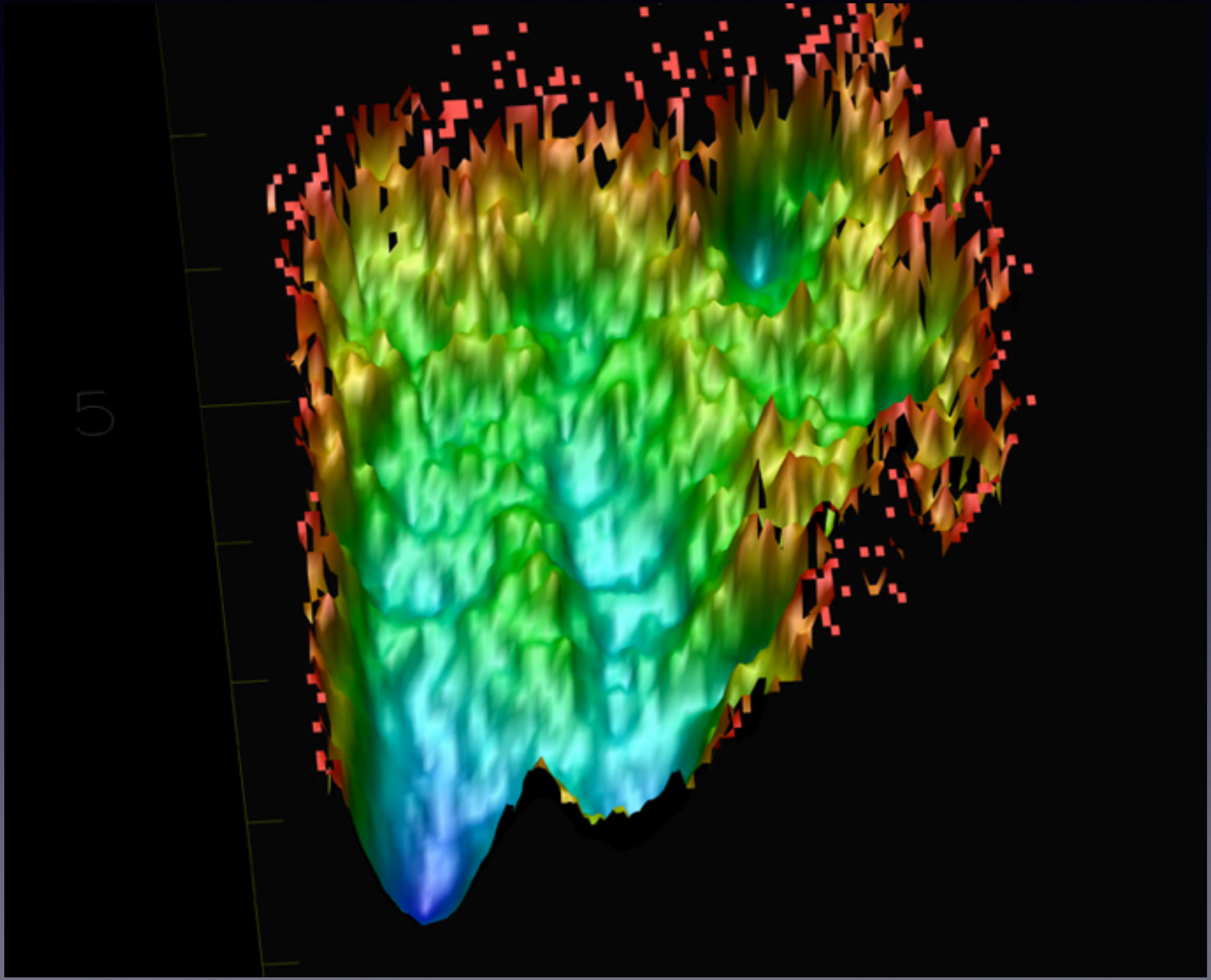


Very rapid folding

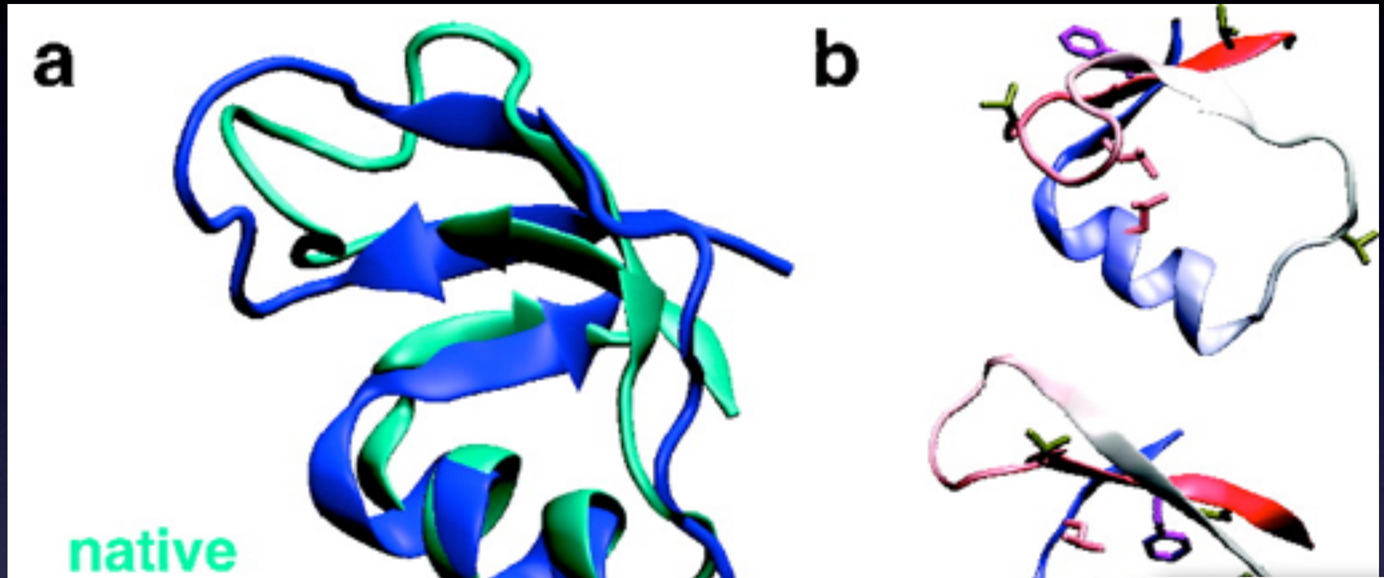
Still folds, but slower

Energy landscapes





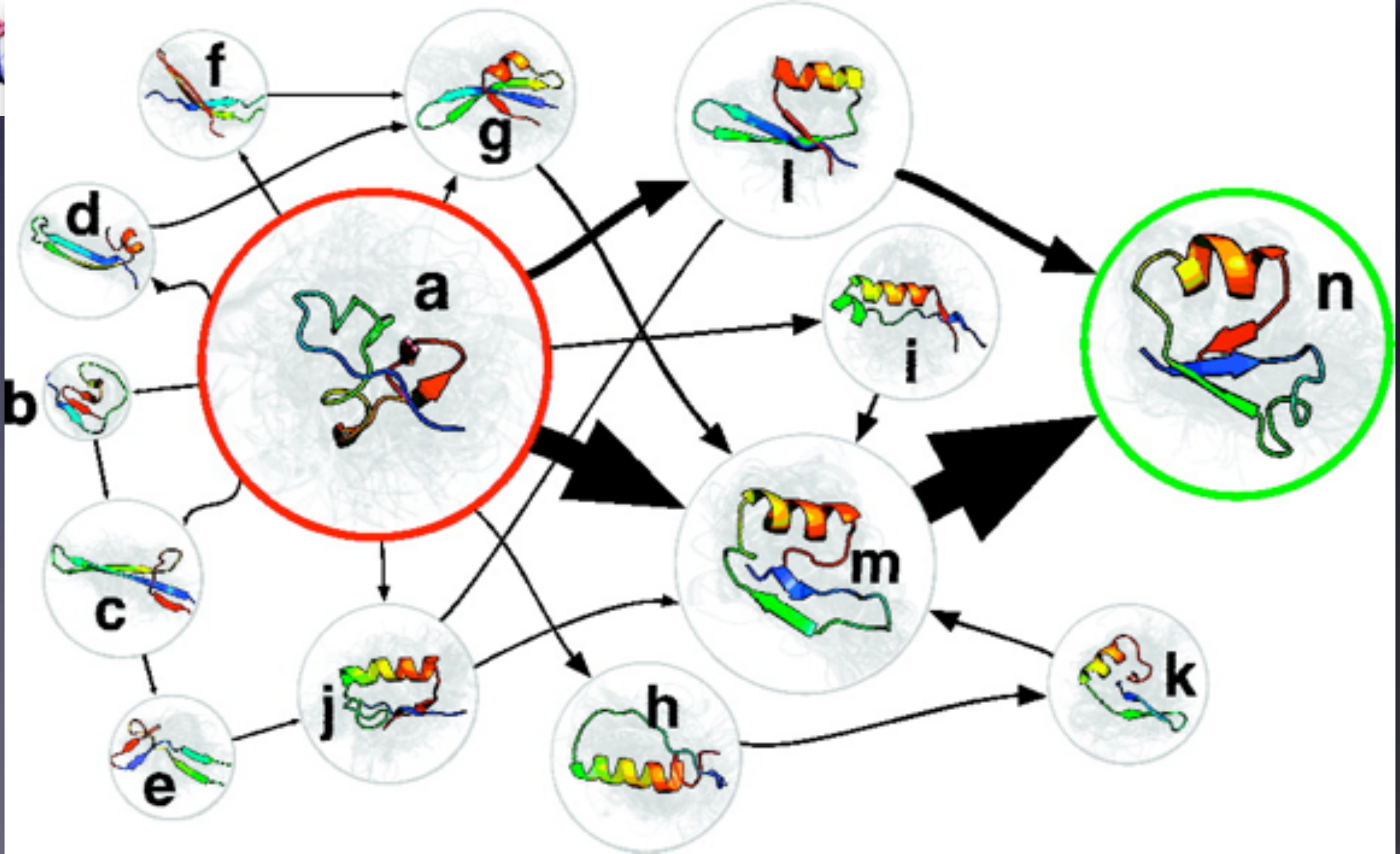
Real Recent Example



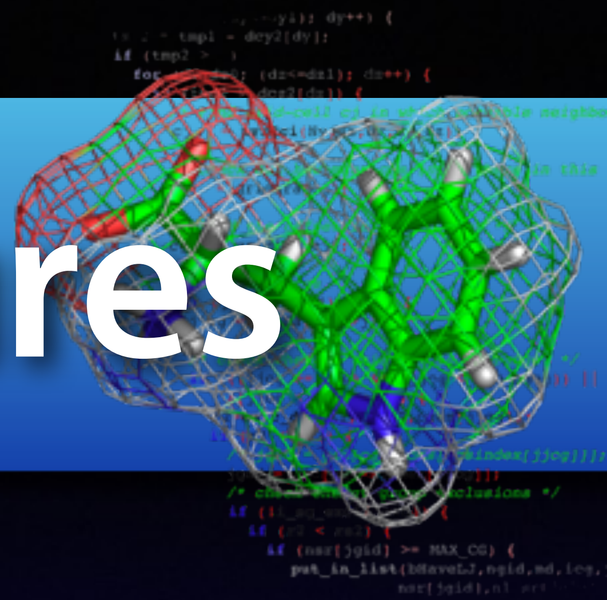
NTL9 - millisecond folding

Vince Voelz, 2009

Complex energy landscape with several intermediates, but two dominant pathways

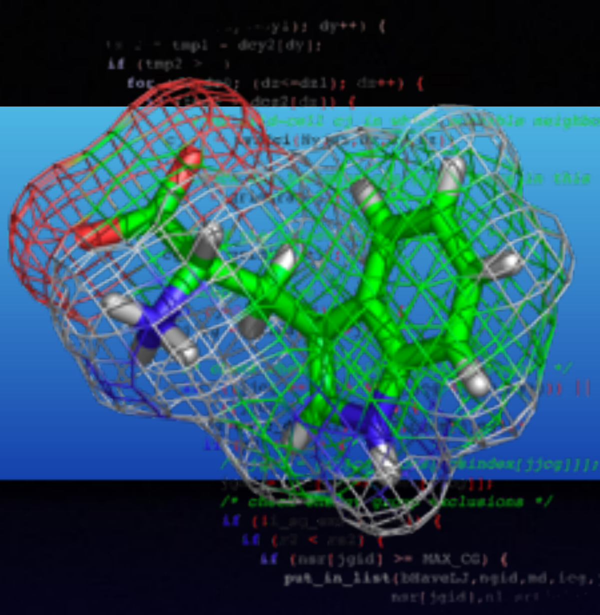


Folding temperatures



- Room (body) temperature:
  - Energetic attraction outweighs entropic restraints to folding
- High temperature:
  - Entropic resistance too large
- Low temperature:
  - Entropic resistance too small - structure will get trapped in closest local minimum

Summary



- Kinetic & thermodynamic stability can, and must be, unified in real proteins
- Folding rate studies, apparent rates
- Transition states explain folding
- Mutation stability, Φ_f , folding nuclei
- Solution to Levinthal's paradox
- Book chapters 19-21