Structural Bioinformatics

GENOME 541

Spring 2022

Lecture 2: Biomolecular Energy Functions

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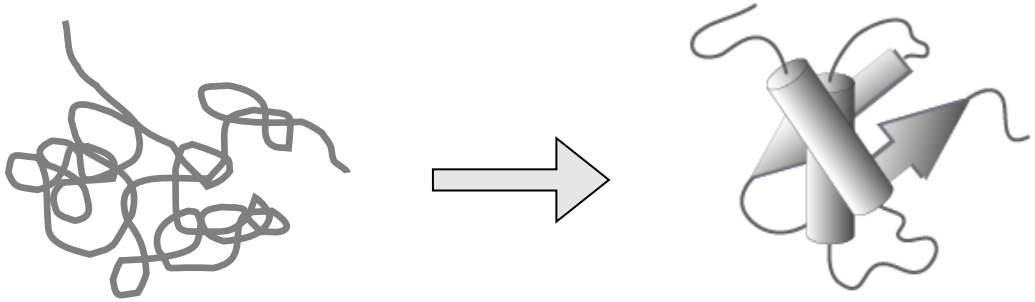
Protein Folding

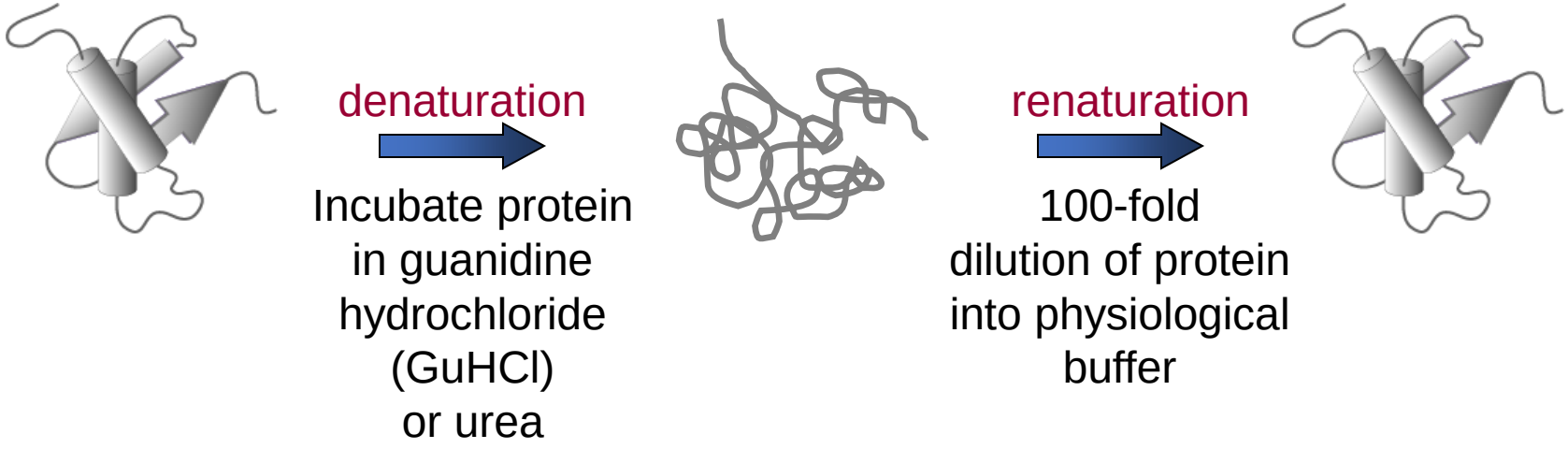


The process by which a protein goes from being an unfolded polymer with no activity to a uniquely structured and active protein.

Why do we care about protein folding?

- Understanding how protein's folds informs us of *sequence to structure* mapping
- Protein misfolding has been implicated in many human diseases (e.g. Alzheimer's, Parkinson's)

Protein folding *in vitro* is often reversible



- **the amino acid sequence of a polypeptide is sufficient to specify its three-dimensional conformation**

- protein folding is a spontaneous process that does not require the assistance of extraneous factors

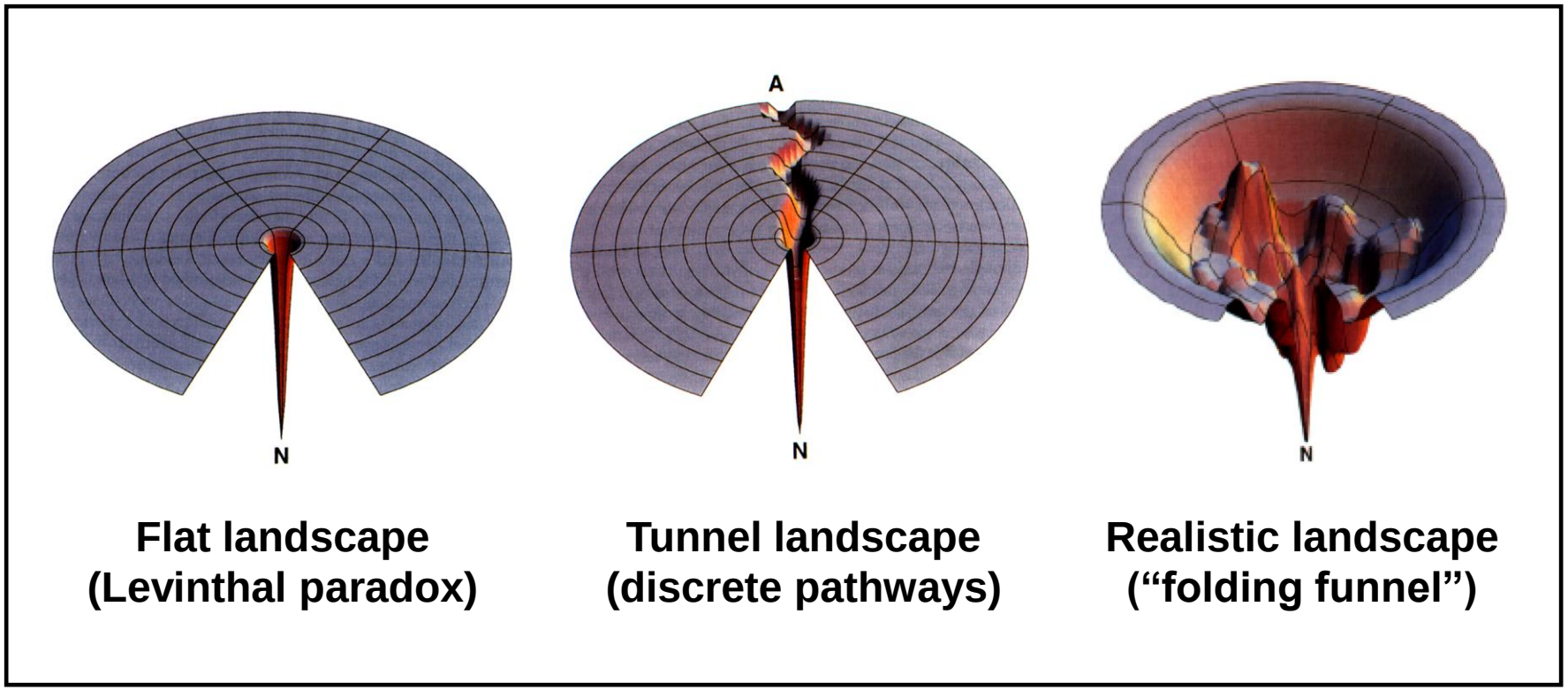
Anfinsen, CB (1973) Principles that govern the folding of protein chains. *Science* **181**, 223-230.

How Do Proteins Fold?

- Cyrus Levinthal tried to estimate how long it would take a protein to do a random search of conformational space for the native fold.
- Imagine a 100-residue protein with three possible conformations per residue. Thus, the number of possible folds = $3^{100} = 5 \times 10^{47}$.
- Let us assume that protein can explore new conformations at the same rate that bonds can reorient (10^{13} structures/second).
- Thus, the time to explore all of conformational space = $5 \times 10^{47}/10^{13} = 5 \times 10^{34}$ seconds = 1.6×10^{27} years >> age of universe
- This is known as the **Levinthal paradox.**

How do proteins fold?

Do proteins fold by a very discrete pathway?

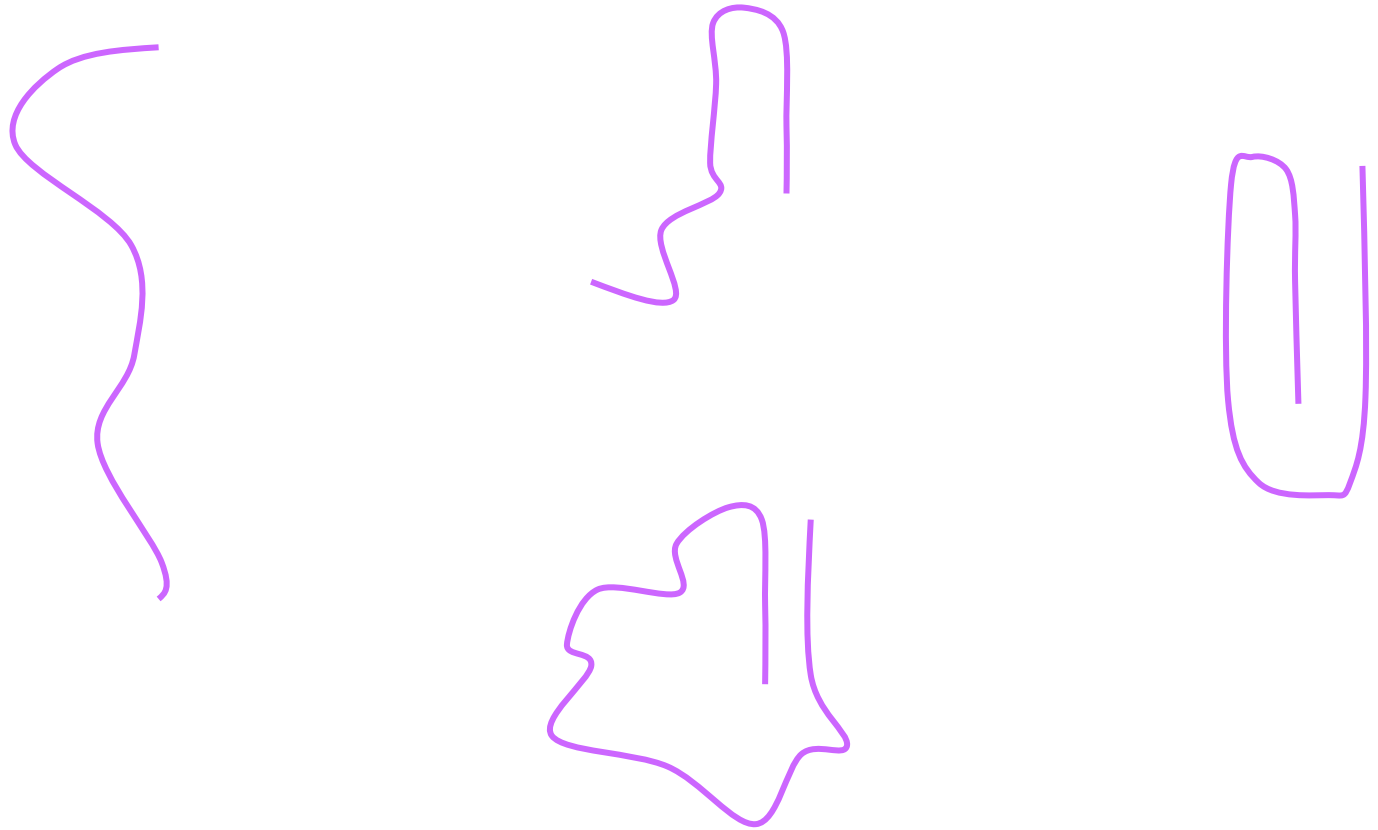


Flat landscape (Levinthal paradox)

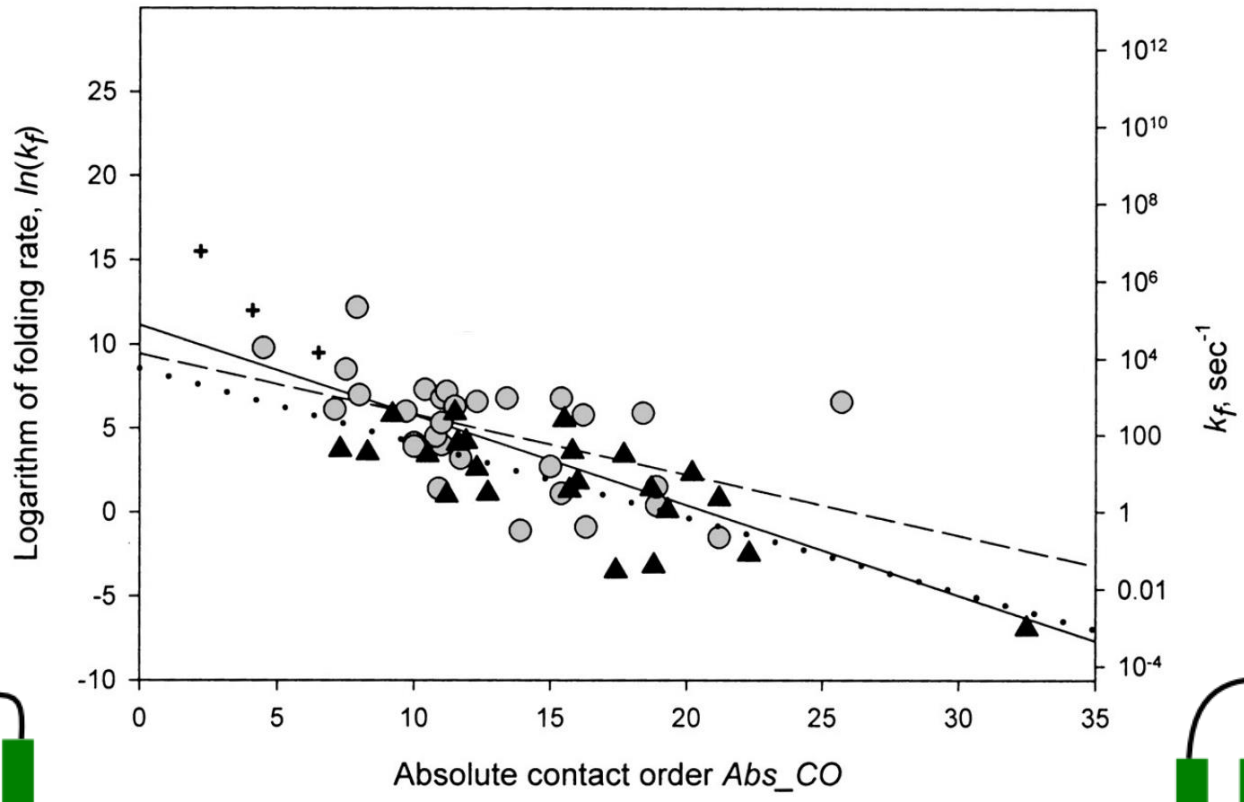
Tunnel landscape (discrete pathways)

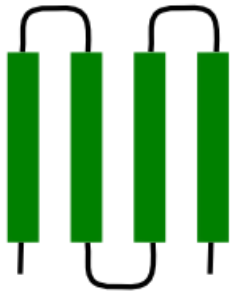
Realistic landscape (“folding funnel”)

Do certain portions of a protein fold first?



Protein folding rates correspond with contact order

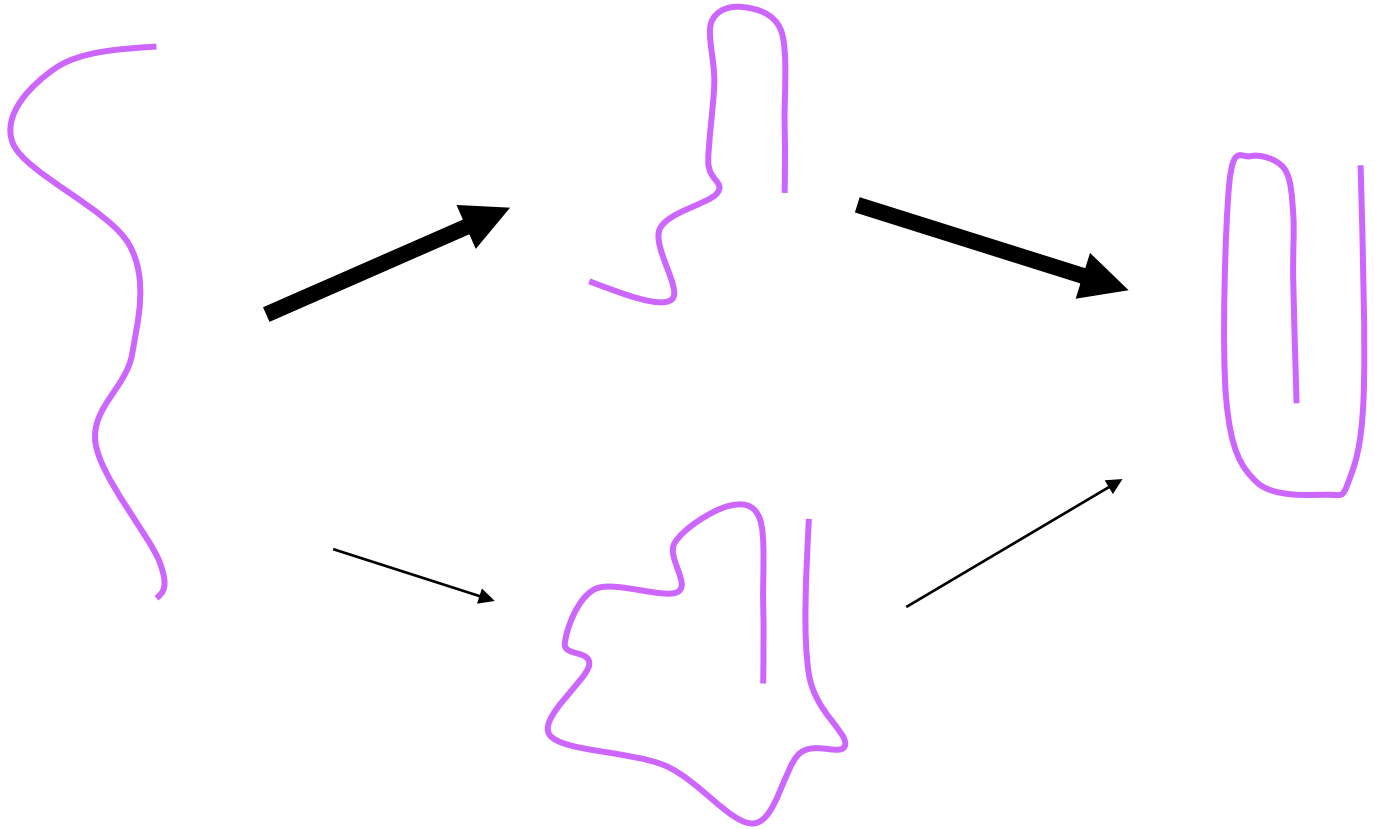




$$CO = \frac{1}{L \cdot N} \sum^{N} \Delta S_{i,j}$$

Do certain portions of a protein fold first?

Interactions between residues *close to each other along the polypeptide chain* are more likely to form early in folding.



Folding pathways and energy landscapes in protein folding

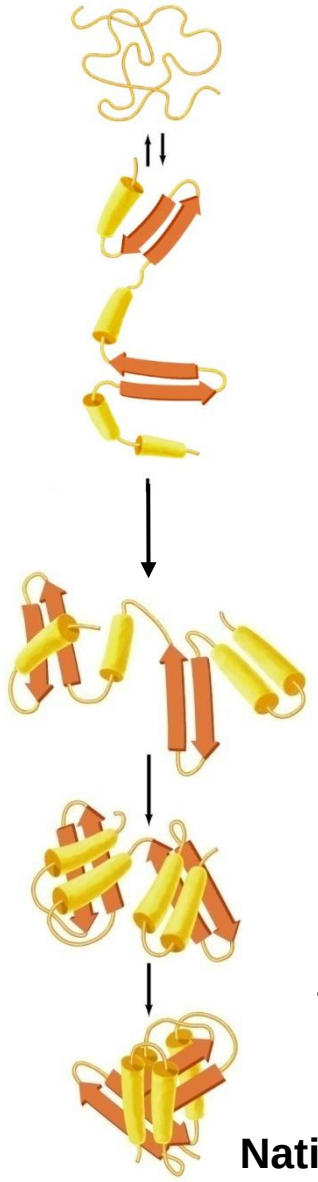


Figure 6-40 part 2 Fundamentals of Biochemistry, 2/e

Folding pathway (hypothetical, yet capturing current thinking):

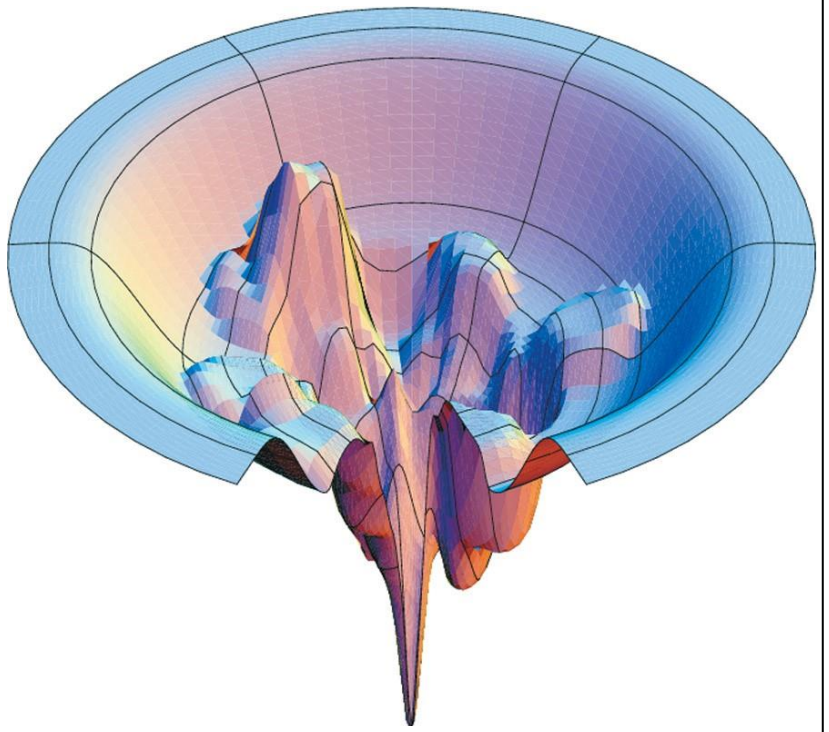
Proteins fold in a hierarchical manner. First, small local elements of secondary structure form.

Then, these coalesce to yield larger supersecondary structure units.

These units coalesce with other units to form larger elements: domains and the complete folded chain.

Native state

Free energy

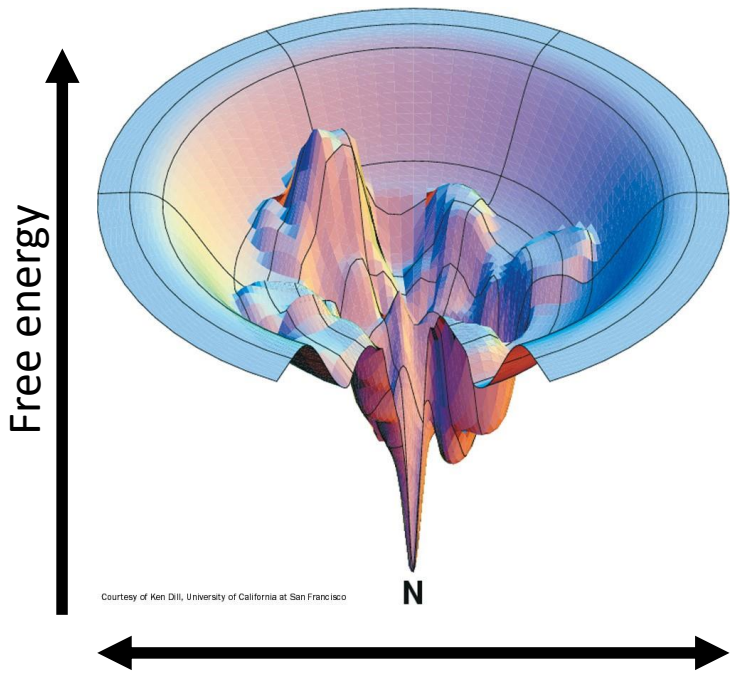


Courtesy of Ken Dill, University of California at San Francisco

Native state

Current thinking about the nature of the energy landscape during protein folding

Modeling the protein free energy landscape



- Under *Anfinsen's hypothesis*, the state of lowest free energy is the native state
- Represent the various enthalpic and entropic effects governing folding with *parameterized equations*
  - vdW interactions
  - electrostatic interactions
  - solvent entropy
  - etc.
- **Predicting protein structure** involves identifying the lowest-energy state of the protein

Factors stabilizing the native state of proteins

Keep in mind that one has to consider the folded versus the unfolded state IN WATER!

Conformational Entropy:

The protein has a much greater entropy in the unfolded than in the folded state!

Hydrophobic interactions:

Nonpolar sidechains come together in folded protein to minimize contact with water.

A major determinant of protein stability is the entropy gain of bulk water!

Hydrogen bonds:

Important to make H-bonds in in folded protein;
they are made *with water* in the unfolded state.

Native proteins almost never have unpaired donors/acceptors in the core!

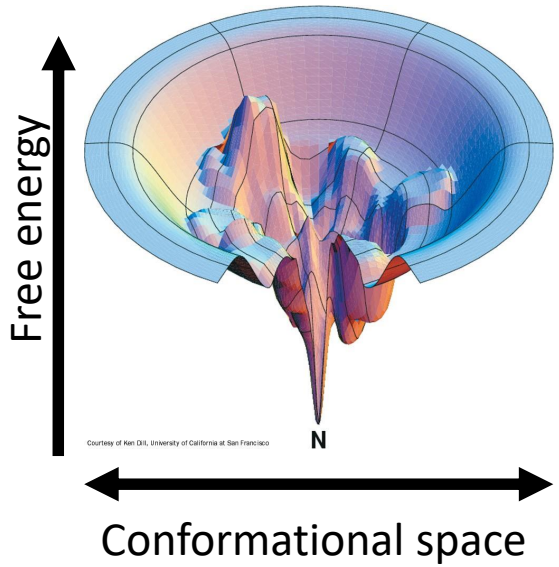
Electrostatic effects:

Salt bridges between opposite charges relatively weak due to electrostatic screening by water

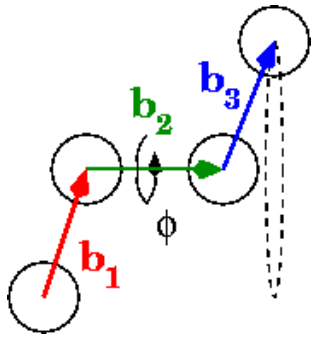
Van der Waals interactions:

Important to make these in the native state since they are made *with water* in the unfolded state

Modeling the protein free energy landscape

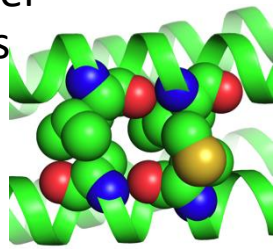


Bonded interactions

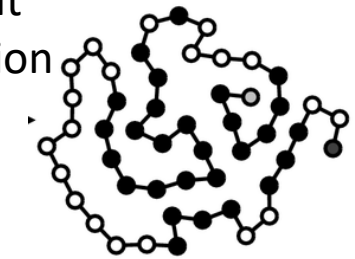


Non-bonded interactions

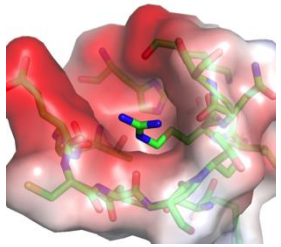
van der Waals



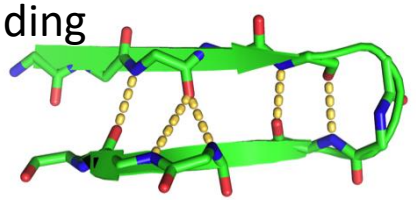
Implicit solvation



Electro-statics



Hydrogen bonding



Modeling covalent forces

Bond lengths

$$V_{bond} = K_b(b - b_0)^2$$

K_b = force constant

b_0 = equilibrium length

Chemical type	K_{bond}	b_o
C-C	100 kcal/mole/Å^2	1.5 Å
C=C	200 kcal/mole/Å^2	1.3 Å
C=-C	400 kcal/mole/Å^2	1.2 Å

Bond angle

$$V_{angle} = K_q(q - q_0)^2$$

K_q = force constant

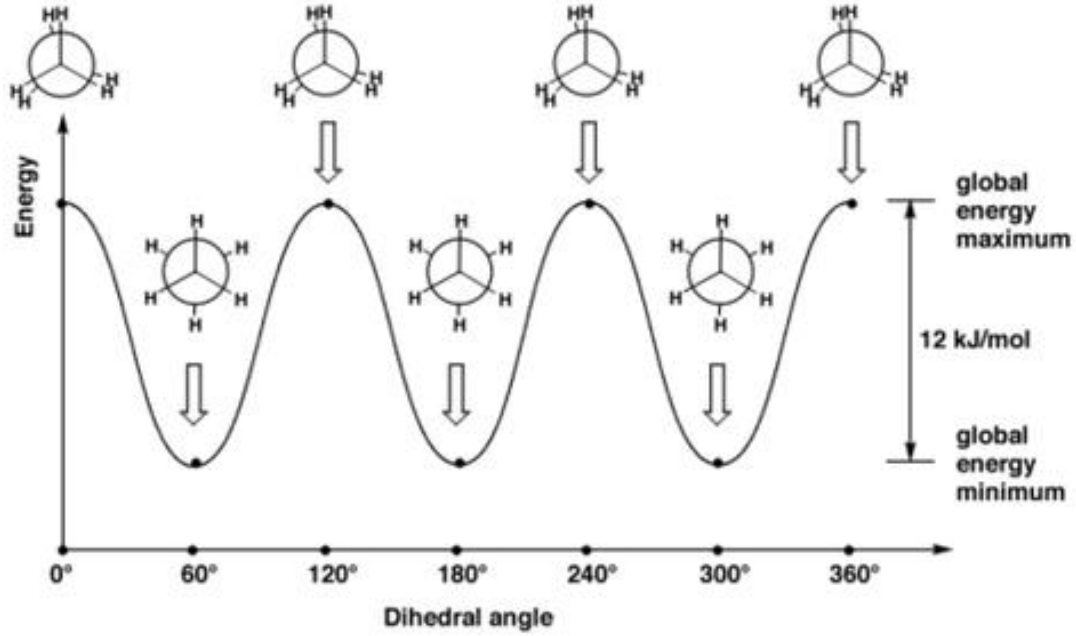
q_0 = equilibrium angle

Modeling covalent forces

Torsion angle

- Staggered conformations
  (angle +60, -60 or 180 are preferred).

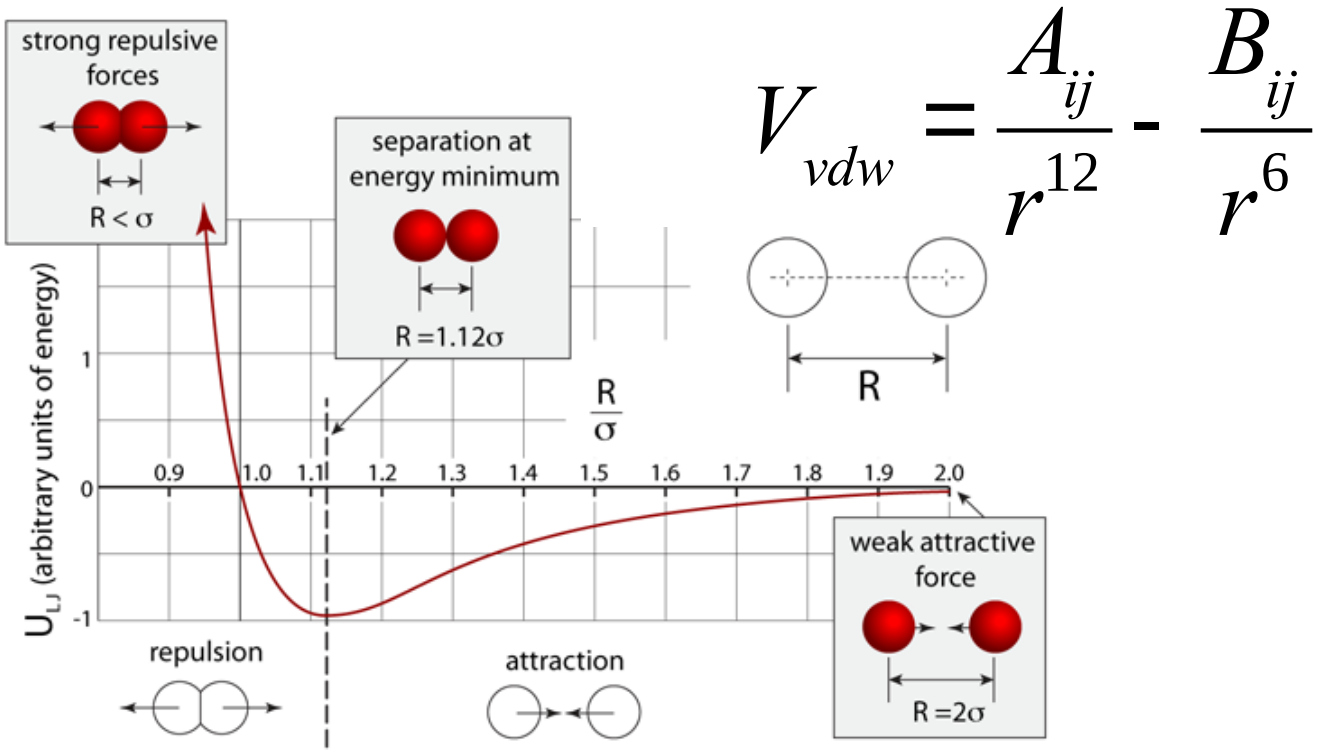
$$V_{torsion} = \sum_{n} k_n \cos(nf)$$



Nonbonded forces

Van der Waals forces

- Interactions between nonbonded atoms are expressed by the Lennard-Jones potential.
- Very high repulsive force if atoms closer than van der Waals radii; attractive force if distance greater



$$V_{vdw} = \frac{A_{ij}}{r^{12}} - \frac{B_{ij}}{r^{6}}$$

Nonbonded forces

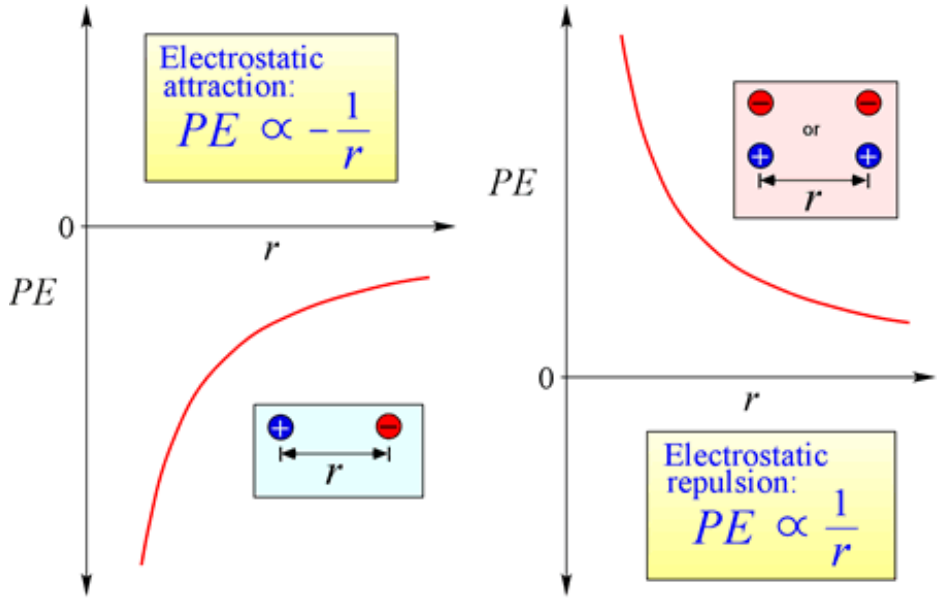
Electrostatic interactions

- Approximate dipoles by giving atoms a partial charge

$$V_{electrostatics} = k_e \frac{q_1 q_2}{\varepsilon r}$$

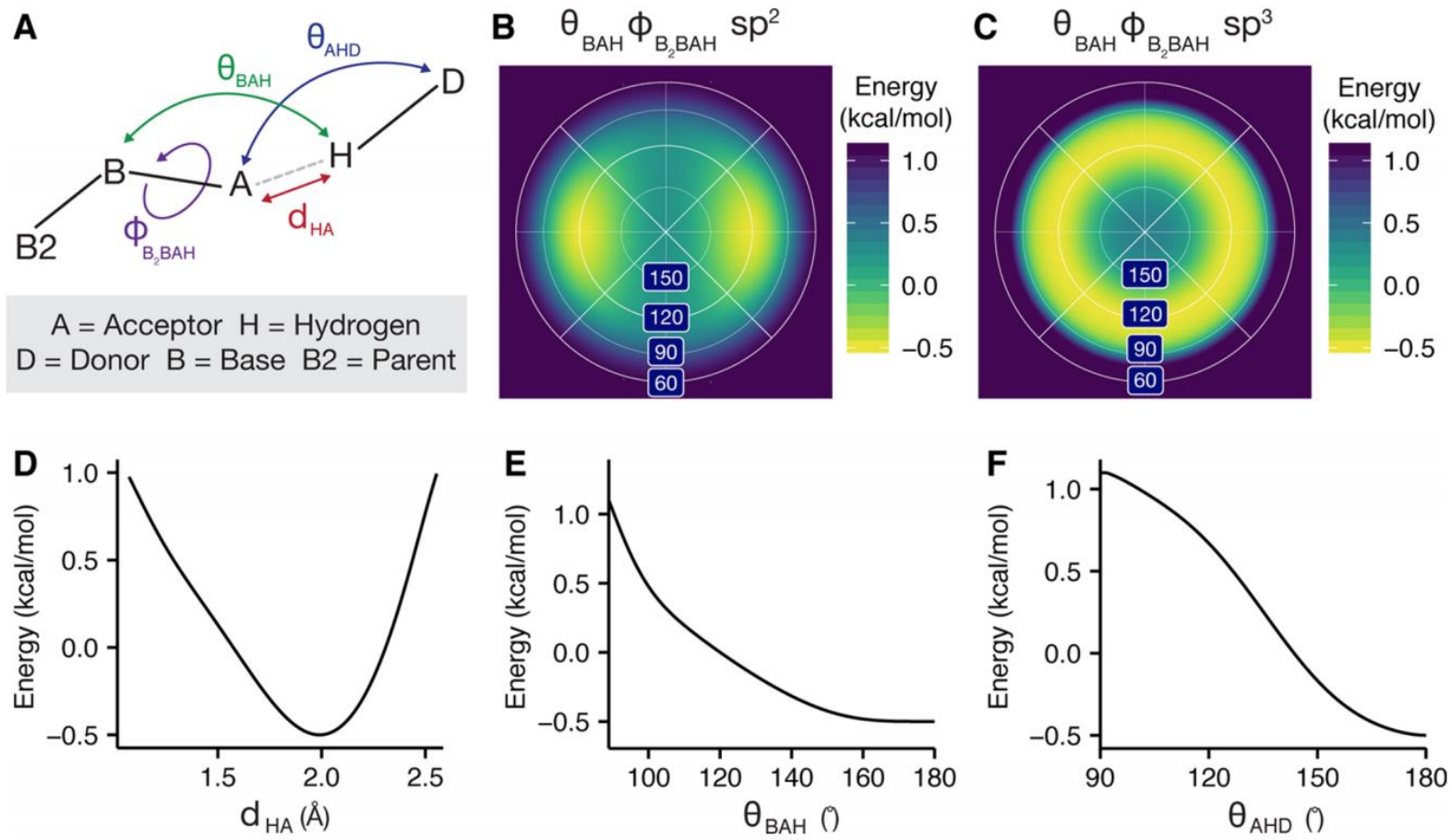
- Dielectic constant varies according to media: Ɛ=80 for water, and 4-6?? in the core of protein

- Electrostatic energy falls off much less quickly than for van der Waals interactions (chemically significant at ~15Å)

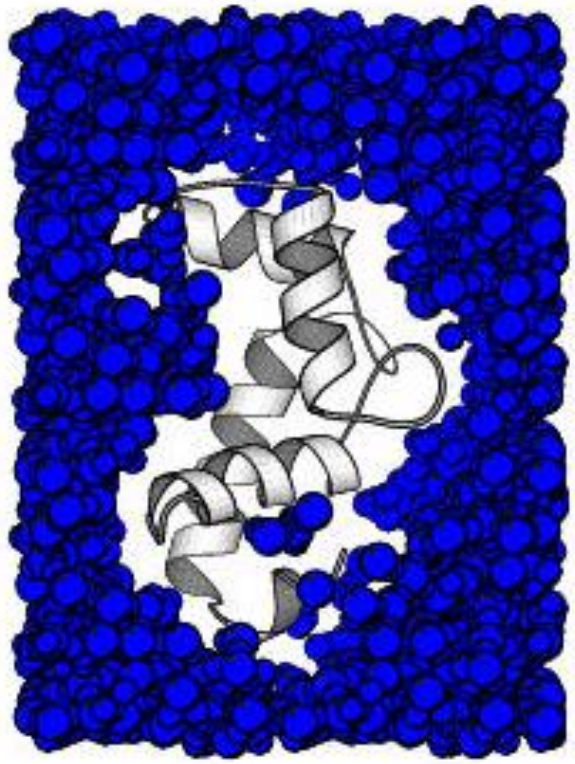


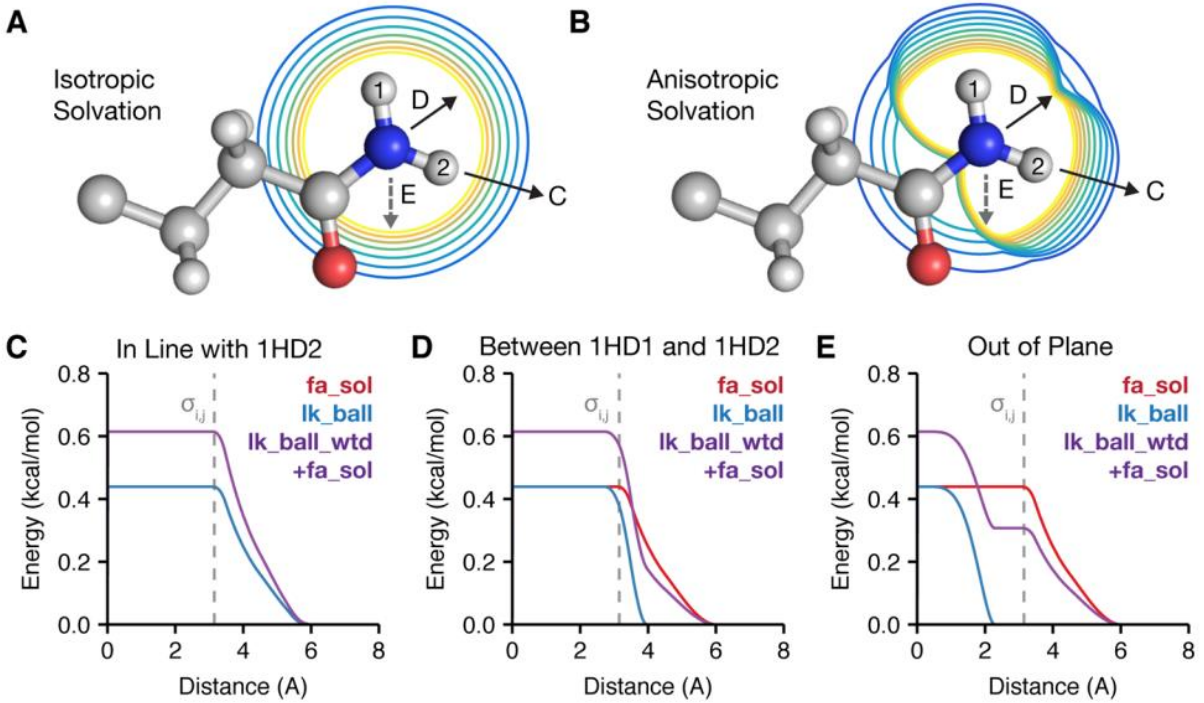
Nonbonded forces

Hydrogen bonding



Modeling the interactions of protein and solvent





Potential Energy

$$E_{pot} = \sum_{b} K_2 (b - b_0)^2 + \sum_{\theta} H_\theta (\theta - \theta_0)^2 + \sum_{\phi} \frac{V_n}{2} [1 + \cos(n\phi - \phi_0)]$$

(1) (2) (3)

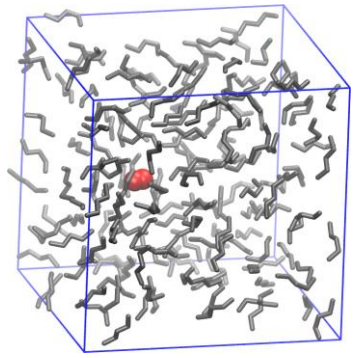
$$+ \sum \varepsilon [(r^*/r)^{12} - 2(r^*/r)^6] + \sum q_i q_j / \varepsilon_{ij} r_{ij} + \sum \left[\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} \right]$$

(4) (5) (6)

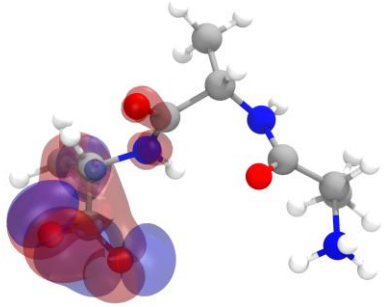
How is this useful?

- Compare relative energies of conformers of the same molecule
- Effect of substituents/mutations on energy
- Refining x-ray structures,
  determining structures from NMR data
- Structure prediction via simulations (next week!)

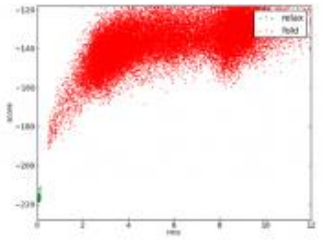
How are these functions parameterized?

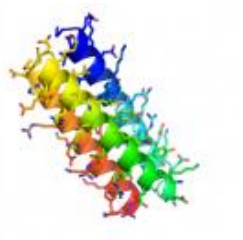


… to match biophysical experiments on small molecules



… to match “higher level theory” simulations on small systems





… to maximize the ability to recapitulate structures/properties from protein crystal structures

Monte Carlo

In molecular simulations, Monte Carlo is an importance sampling technique

1. Make a random move and produce a new conformation
2. Calculate the energy change delta *E* for the new conformation
3. Accept or reject the move based on the *Metropolis criterion*

$$P = \exp\left(-\frac{\Delta E}{kT}\right)$$ → Boltzmann factor

If delta *E*<0, then P>1, accept new conformation;
Otherwise:
if P>rand(0,1), accept,
else reject.

Simulated Annealing Monte Carlo

In **Simulated Annealing Monte Carlo**, we reduce the temperature as the simulation progresses:

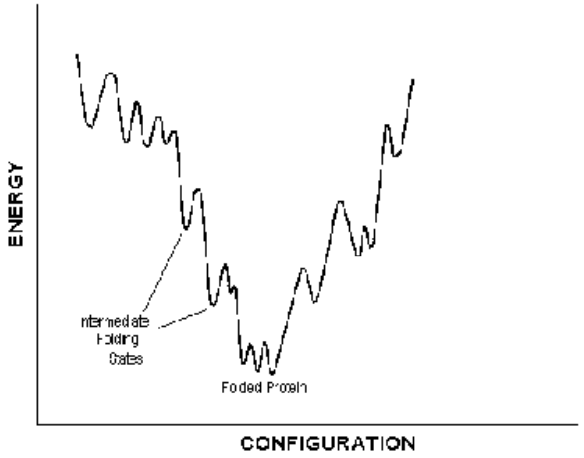
for i=0:i_{max}

$T_k=(T_{max}-T_{min})$ * $(i_{max}-i)/i_{max} + T_{min}$

Run k steps of Monte Carlo at temperature T_k

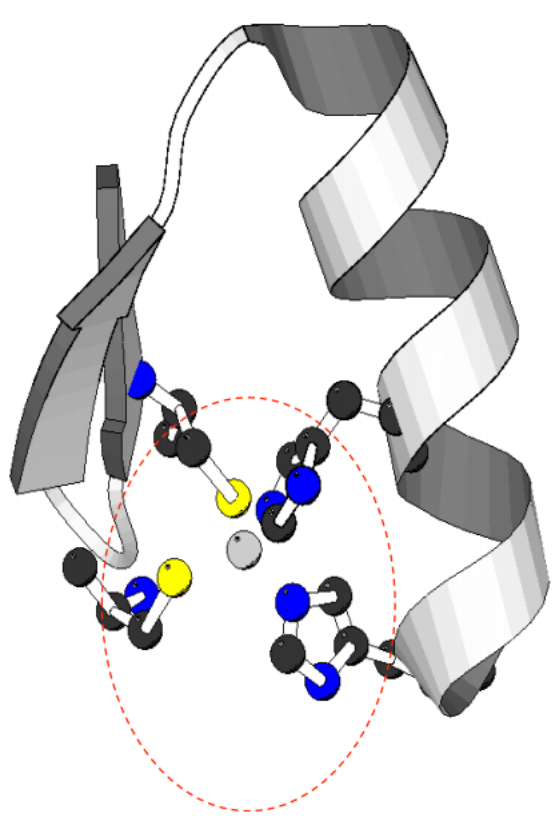
high T: accept almost all structures

low T: accept almost only better structures



Example:
Sidechain rotamer determination

- **Problem**: given the backbone coordinates of a protein, predict the coordinates of the sidechain atoms
- Each sidechain has a discrete number of states (“rotamers”)
- Monte Carlo moves:
  - replace sidechain with random rotamer



Molecular Dynamics

Algorithm

- For atom *i,* Newton's equation of motion is given by

$$F_i = m_i a_i \quad \Rightarrow \quad \mathbf{F}_i(t) = m_i \frac{\mathrm{d}^2 \mathbf{r}_i(t)}{\mathrm{d}t^2}$$

Here, $\mathbf{r}_i$ and m_i represent the position and mass of atom *i* and $\mathbf{F}_i(t)$ is the force on atom *i* at time *t*. $\mathbf{F}_i(t)$ can also be expressed as the gradient of the potential energy

$$\mathbf{F}_i = -\nabla_i V \quad \Rightarrow \quad -\nabla_i V = m_i \frac{\mathrm{d}^2 \mathbf{r}_i(t)}{\mathrm{d}t^2}$$

V is potential energy. Newton's equation of motion can then relate the derivative of the potential energy to the changes in position as a function of time.

Molecular Dynamics

Numeric integration by using the **Verlet algorithm**

- Given initial velocity 0 and position x_i, numerically integrate to get position at time $t+\delta t$
- Taylor expansions to 3rd order for i

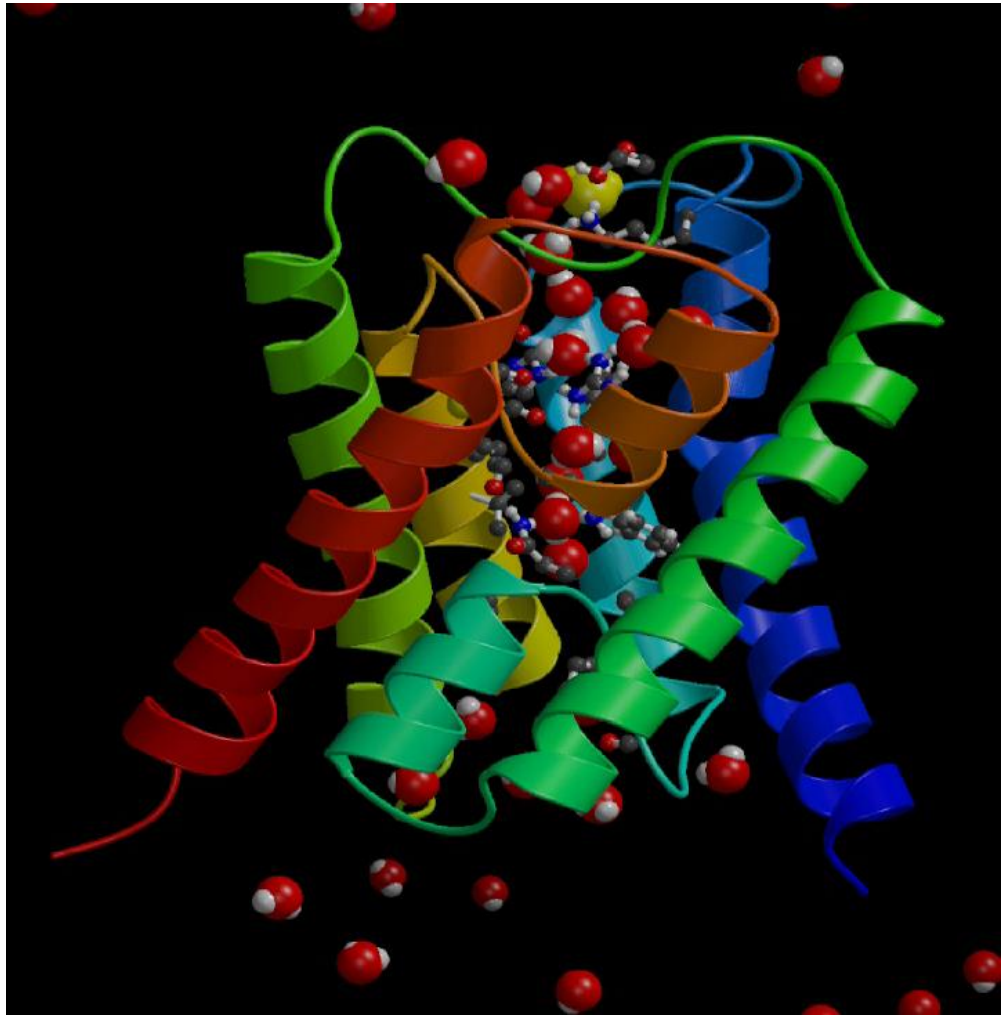
$$\mathbf{r}(t+\delta t) = \mathbf{r}(t) + (\delta t)\mathbf{v}(t) + \frac{1}{2}(\delta t)^2\mathbf{a}(t) + \frac{1}{6}(\delta t)^3\mathbf{b}(t) + ...$$

$$\mathbf{r}(t-\delta t) = \mathbf{r}(t) - (\delta t)\mathbf{v}(t) + \frac{1}{2}(\delta t)^2\mathbf{a}(t) - \frac{1}{6}(\delta t)^3\mathbf{b}(t) + ...$$

- Adding these equations gives [up to order $(\delta t)^4$]:

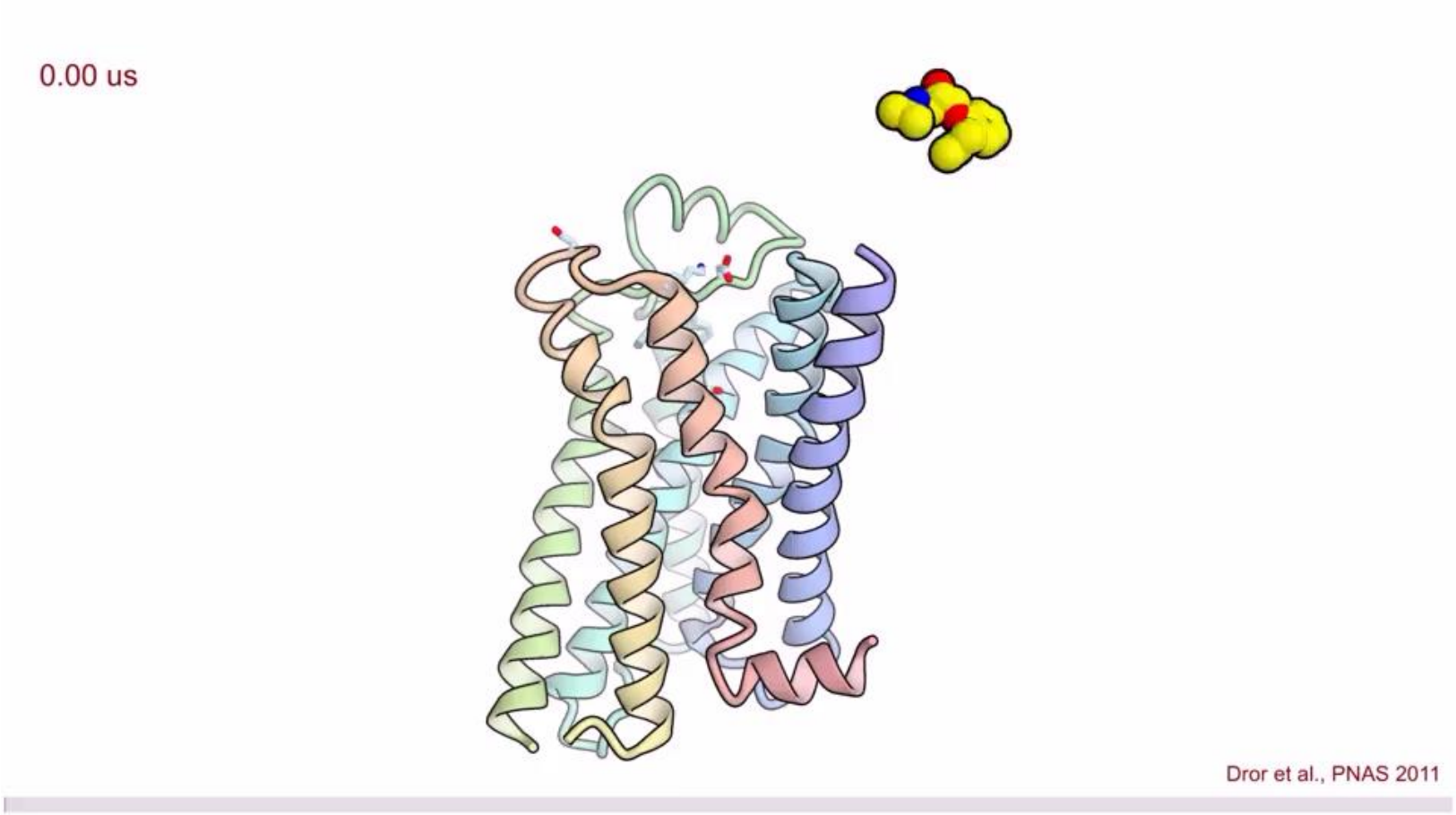
$$\mathbf{r}(t+\delta t) = 2\mathbf{r}(t) - \mathbf{r}(t-\delta t) + (\delta t)^2\mathbf{a}(t) + O[(\delta t)^4]$$

Aquaporin-1



(B.L. de Groot and H. Grubmüller: Science 294, 2353-2357 (2001))

Drug binding to GPCRs



Dror *et al. PNAS 2011*