Protein Physics 2016 Lecture 6, February 5

Secondary structure stability, beta-sheet formation & stability

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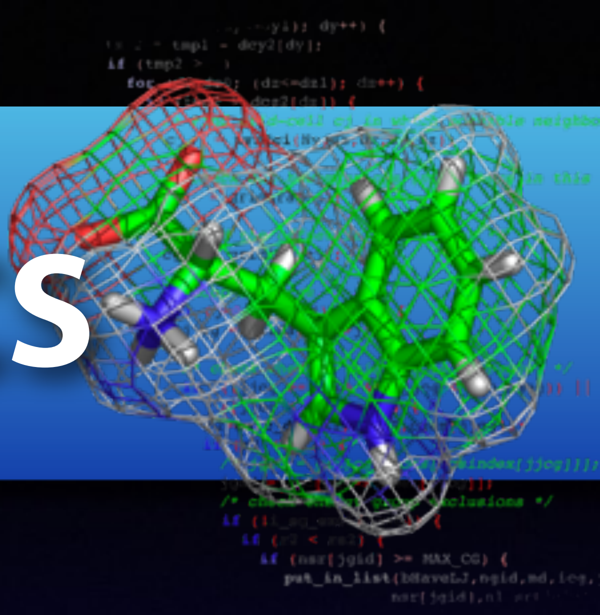
Theoretical & Computational Biophysics

Recap of statistics



- Energy - Entropy
- Entropy - microstates - volume & order
- Probability of being in a state i:

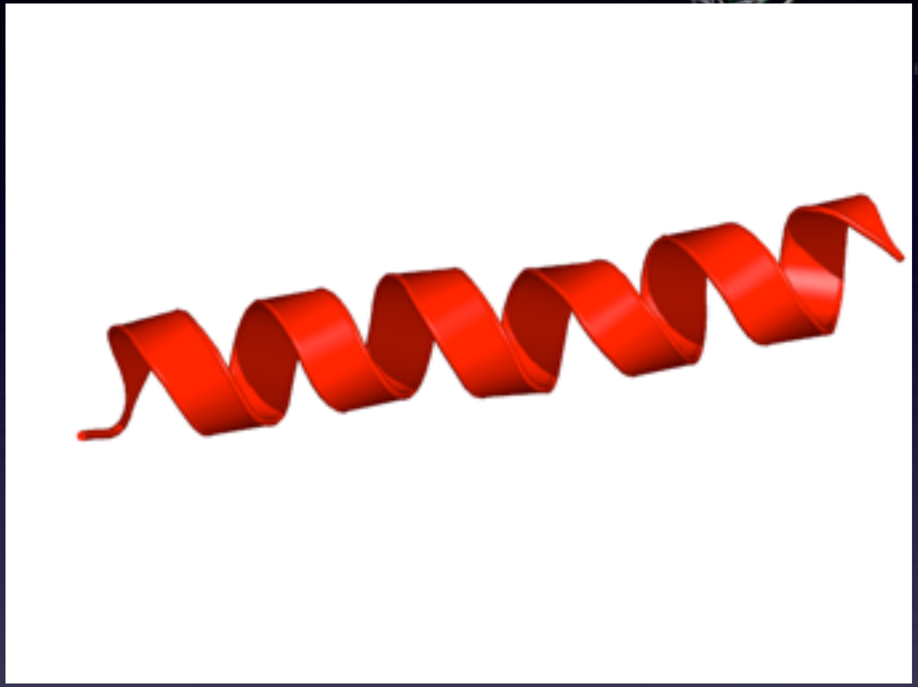
$$w_i(T) = \frac{\exp\left(-\epsilon_i/k_B T\right)}{Z(T)}$$

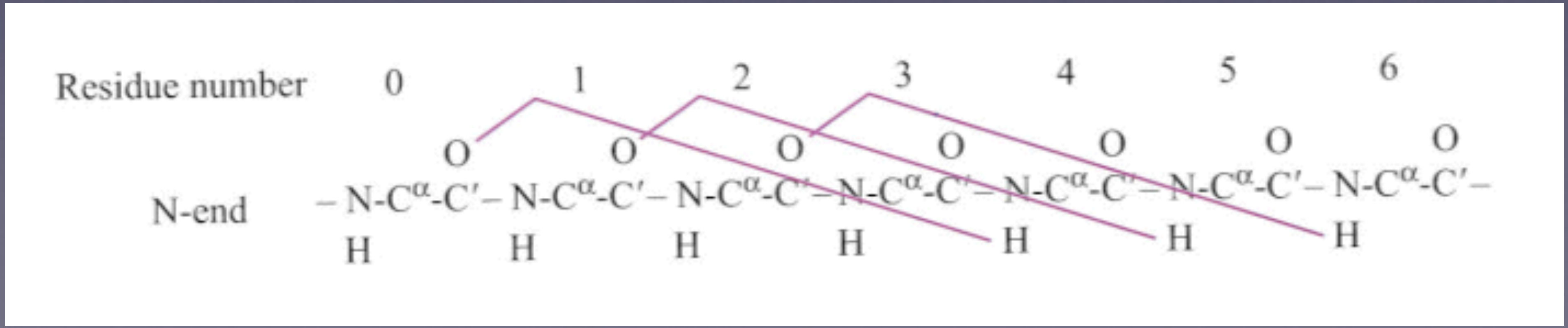
- Partition function:

$$Z(T) = \sum_i \exp\left(-\epsilon_i/k_B T\right)$$

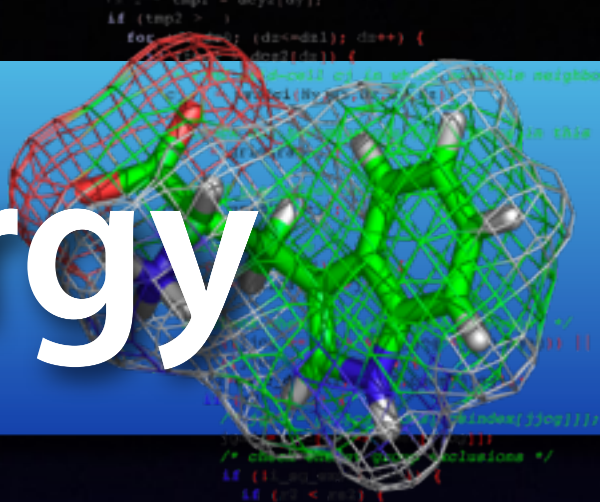
Alpha helix formation

- Hydrogen bonds: i to i+4
  - 0-4, 1-5, 2-6
- First hydrogen bond "locks" residues 1,2,3 in place
- Second stabilizes 2,3,4 (etc.)
- N residues stabilized by N-2 hydrogen bonds!





Alpha helix free energy



- Free energy of helix vs. “coil” states:

number of residues

H-bond free energy

Entropy loss of fixating one residue in helix

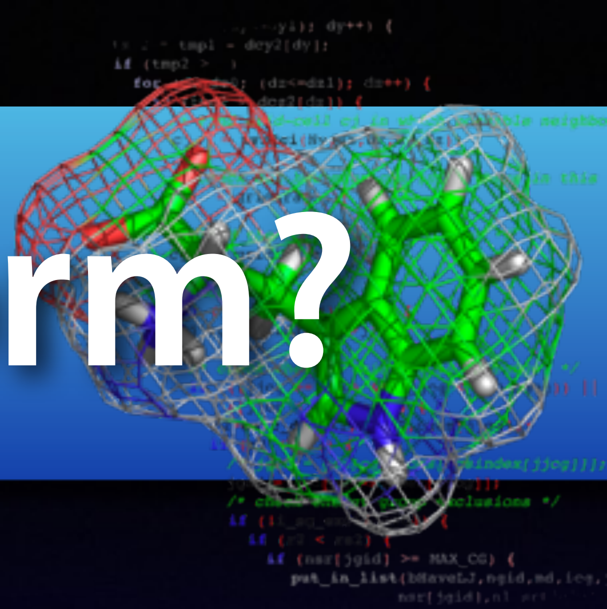
$$\begin{aligned}\Delta F_\alpha = F_\alpha - F_{\text{coil}} &= (n-2) f_{\text{H-bond}} - nTS_\alpha \\ &= -2 f_{\text{H-bond}} + n\left(f_{\text{H-bond}} - TS_\alpha\right)\end{aligned}$$

Helix initiation cost

Helix elongation cost

$$\Delta F_\alpha = f_{\text{INIT}} + n f_{\text{EL}}$$

How does a helix form?

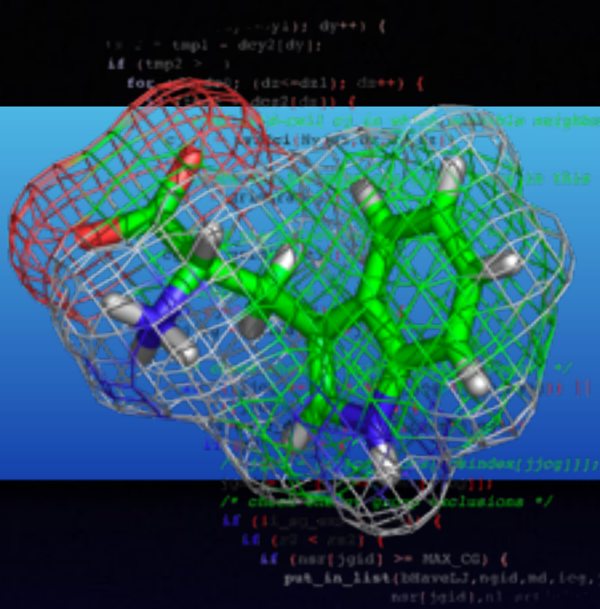


- Landau: Phases cannot co-exist in 3D
- First order phase transitions means either state can be stable, but not the mixture
- Think ice/water - either freezing or melting

$$n \propto V \propto r^3$$

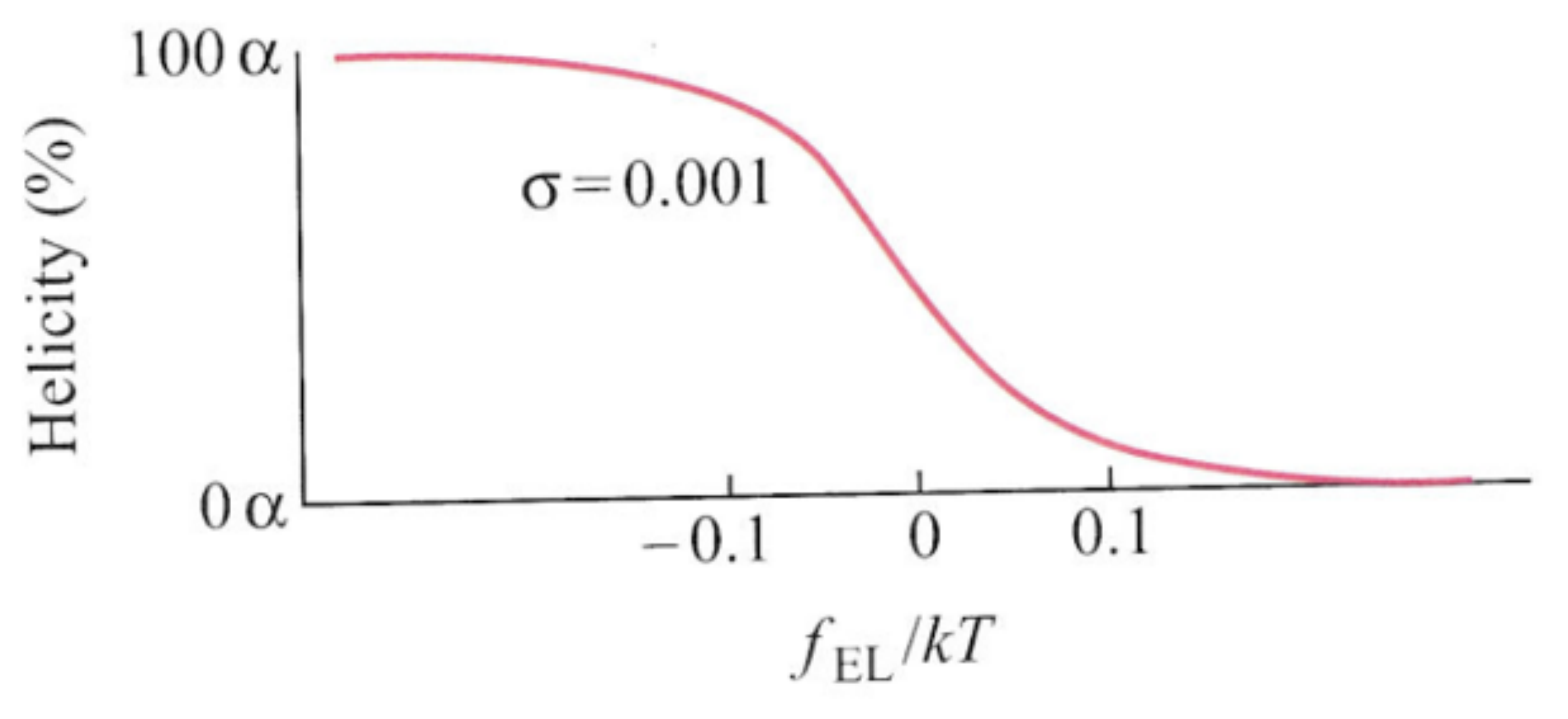
$$A \propto r^2 \propto n^{2/3}$$ Surface tension costly!

- But a helix-coil transition in a chain is 1D!
- Interface helix/coil does not depend on N!



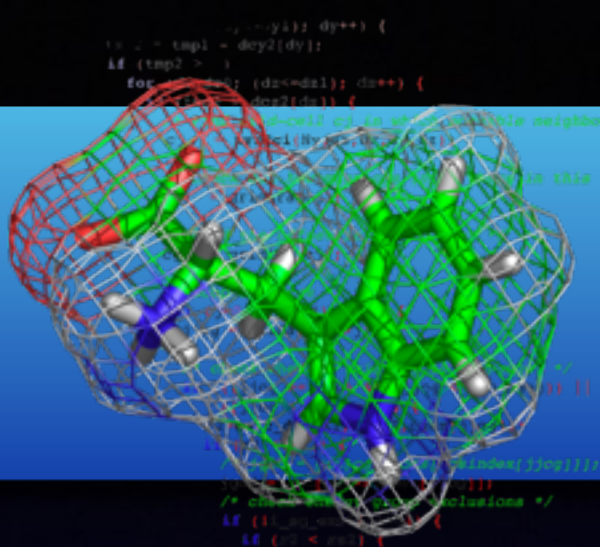
Helix stability

- Temperature dependence
- Elongation term dominant for large N
- Why? Since it is raised to the power of N!



Highly cooperative, but NOT a formal phase transition! (width does not go to zero)

Formation...



- Rate of formation at position 1:

τ: 1-residue elongation

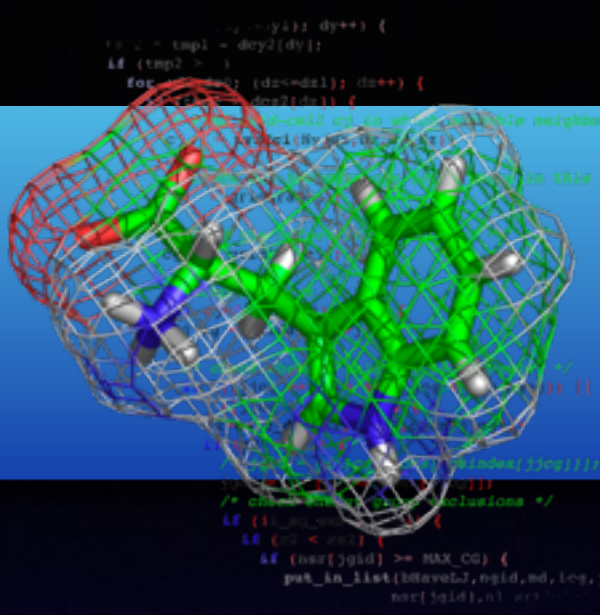
$$t_{\mathrm{INIT0}} = \tau \exp\left(f_{\mathrm{INIT}}/kT\right) = \tau/\sigma$$

- Rate of formation anywhere (n0≈1/√σ):

$$t_{\mathrm{INIT}} = \tau/\sqrt{\sigma}$$

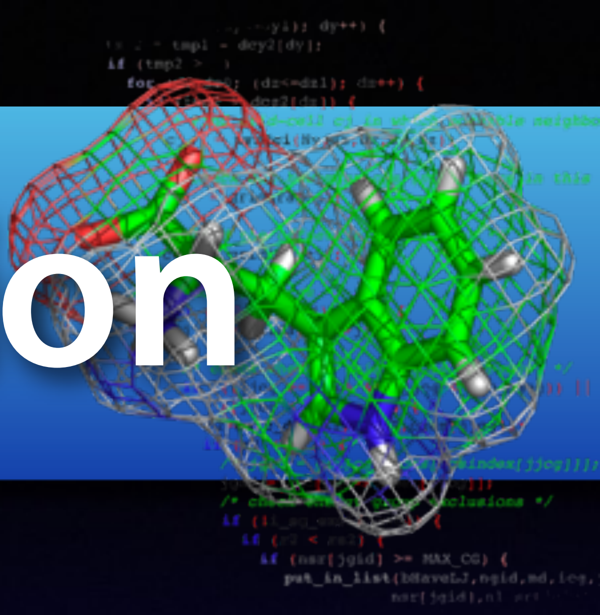
- Propagation to all residues: $tn_0 = \tau/\sqrt{\sigma}$
- Total time is ~2t_{INIT}, halftime thus ~t_{INIT}.
- Half time spent on initiation, half elongation!

Helix summary



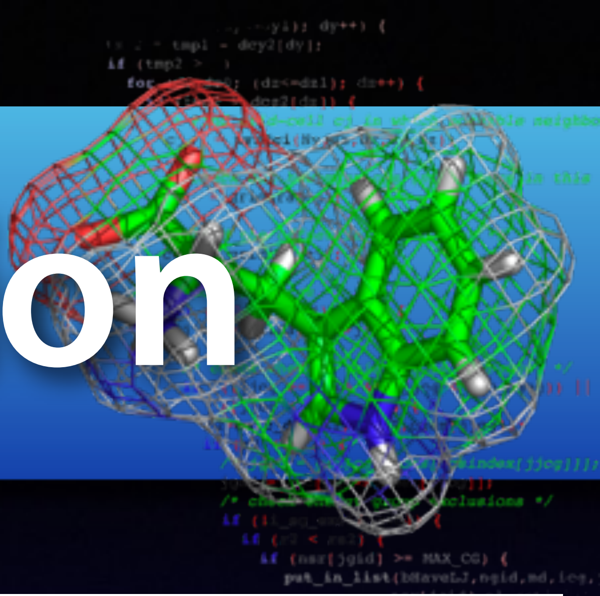
- Very fast formation
- Both initiation & elongation matters
- Quantitative values derived from CD-spectra
- Low free energy barriers, ~1kcal/mol
- Characteristic lengths 20-30 residues

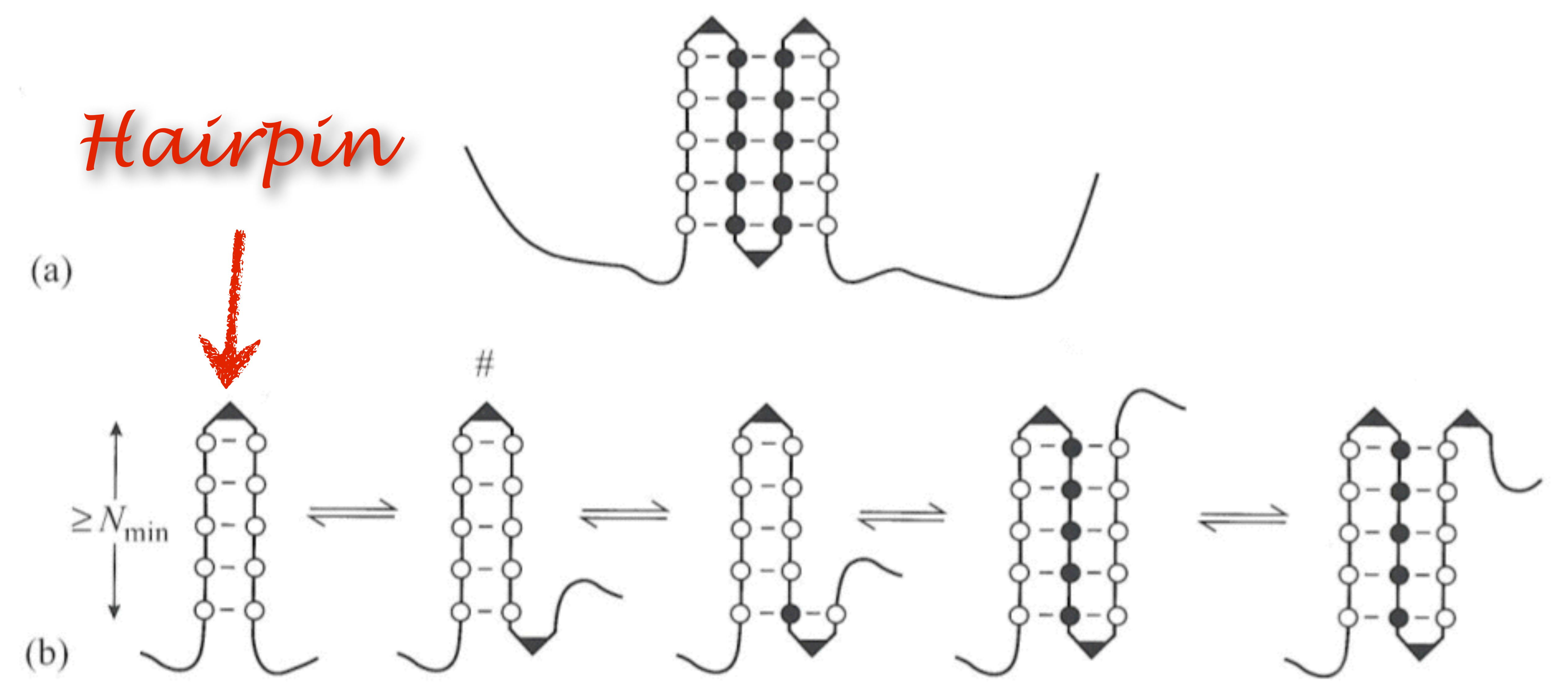
Beta sheet formation



- Experimentally: Can take hours to weeks!
- But sometimes just a millisecond. Why?
- Is it initiation- or elongation-limited?
- Beta sheet formation appears to be a typical first-order phase transition!

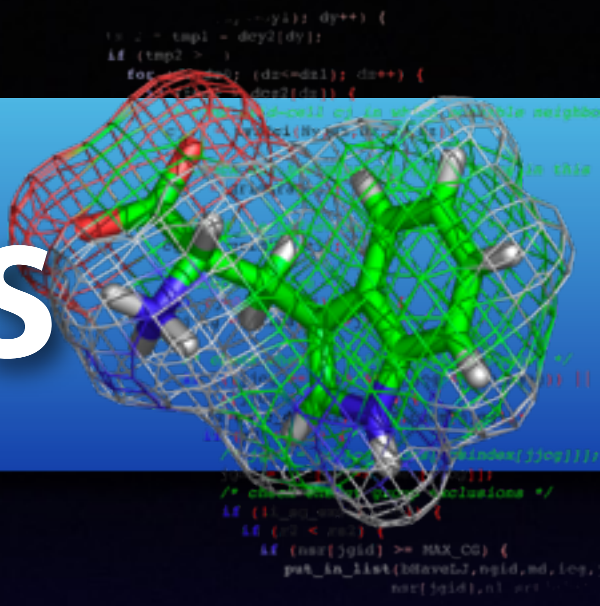
Beta sheet formation





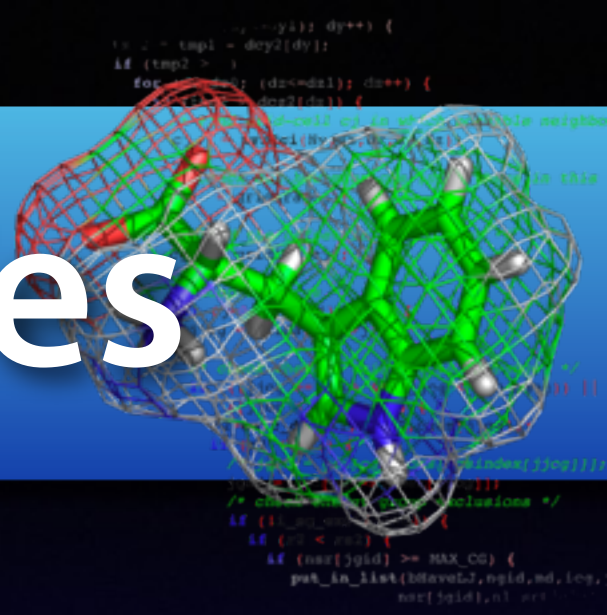
Hairpin

Sheets vs. Helices



- Beta sheets are two-dimensional
- Interface area grows with # residues
  - Phases cannot coexist and there will be a first-order phase transition
- Structure interface:
  - Sheet edges & bends/loops

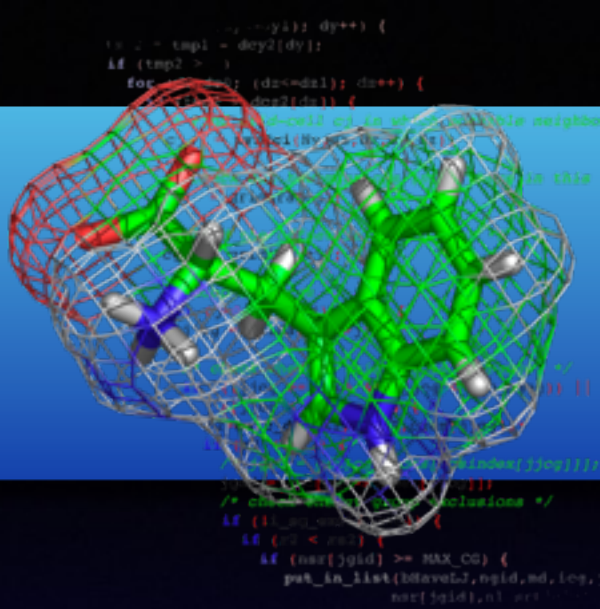
Beta sheet energies



- f_β: Free energy of residue inside a single beta hairpin, relative to the random coil
- Δf_β: Extra edge free energy
  - Total free energy at edge is $f_{\beta +} \Delta f_\beta$
- U: Free energy of bend/coil per residue
- Since sheets can form we must have $\Delta f_\beta > 0$ & $U > 0$!

Why?

Two Scenarios:



- $f_{\beta+} \Delta f_{\beta}<0$: A single long beta hairpin will be more stable than coil. Only a single turn required for formation
- $f_{\beta+} \Delta f_{\beta}>0$: Hairpins are only formed because of association with other residues into a beta sheet. Activation barrier is the formation of a sheet “nucleus”

Minimum strand length

- Consider the case when single hairpins are not stable.

$$\Delta F = U + 2N(f_\beta + \Delta f_\beta)$$

Turn

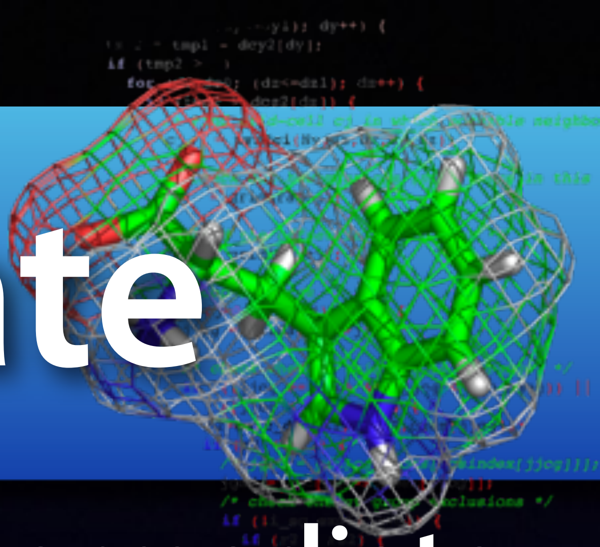
All residues face an edge

- Association with a new strand maintains edge, and gives us N new internal resides:

$$\Delta F' = -N f_\beta$$

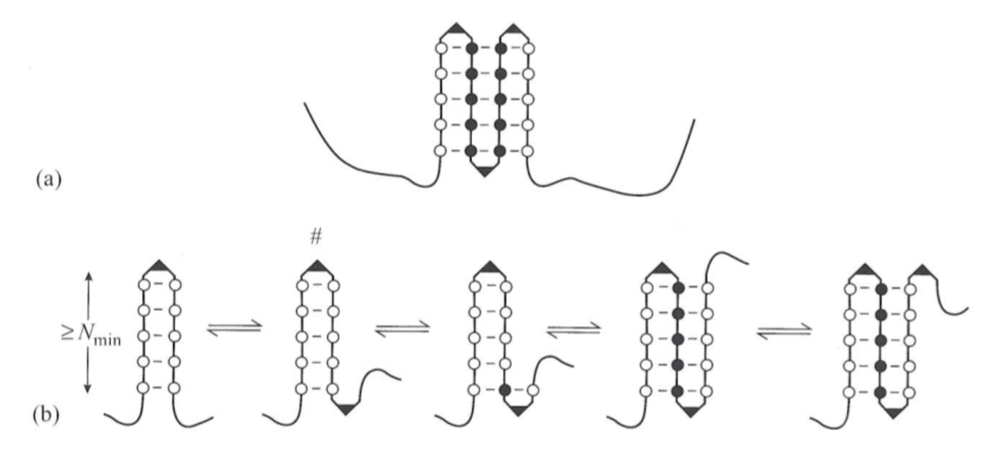
- Formation of next turn: $\Delta F'' = U$
- Minimum strand length: $N_{\min} = U/(-f_\beta)$

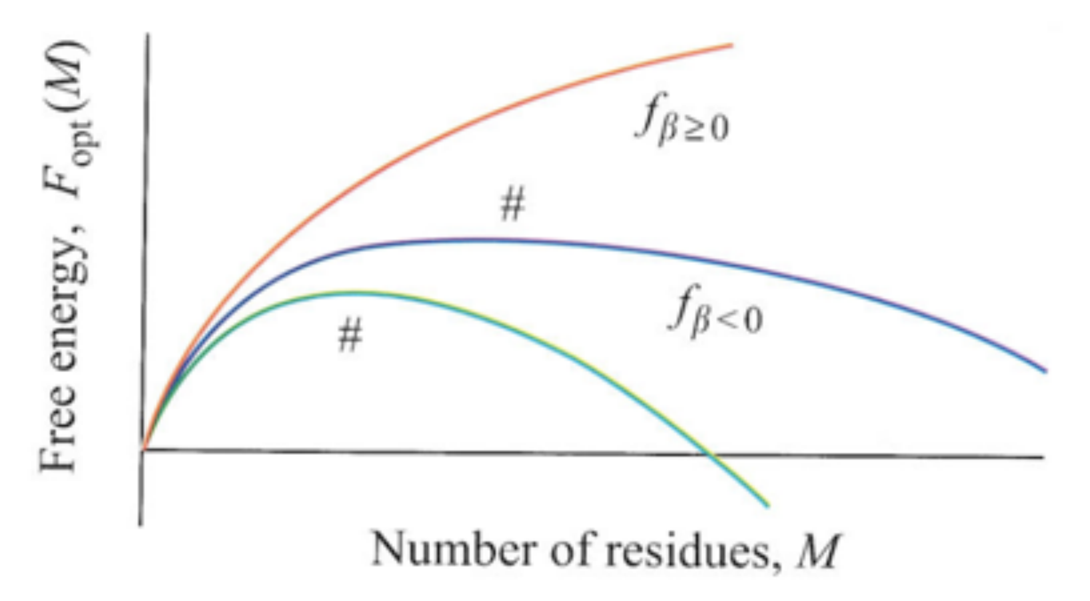
Beta transition state



- **Find the highest-free-energy intermediate: Single hairpin with a following turn**

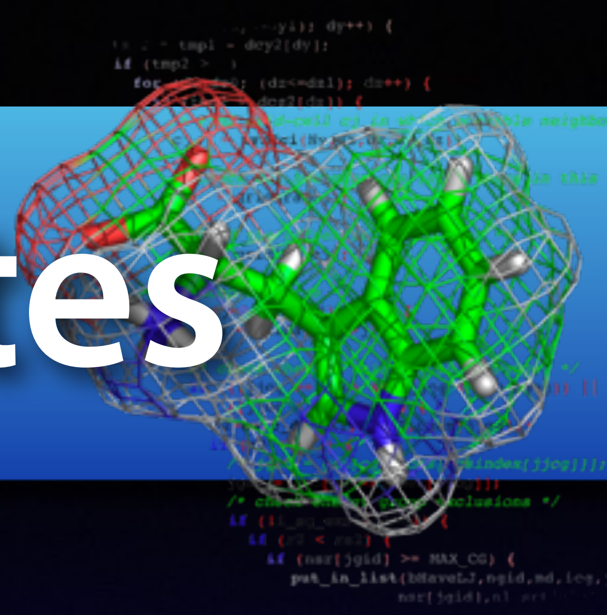
$$F^{\#} = U + 2N_{\mathrm{min}}(f_\beta + \Delta f_\beta) + U = 2(U\Delta f_\beta)/(-f_\beta)$$





The book goes into some detail to prove that this is the lowest possible transition state energy! Why is that important?

Beta formation rates



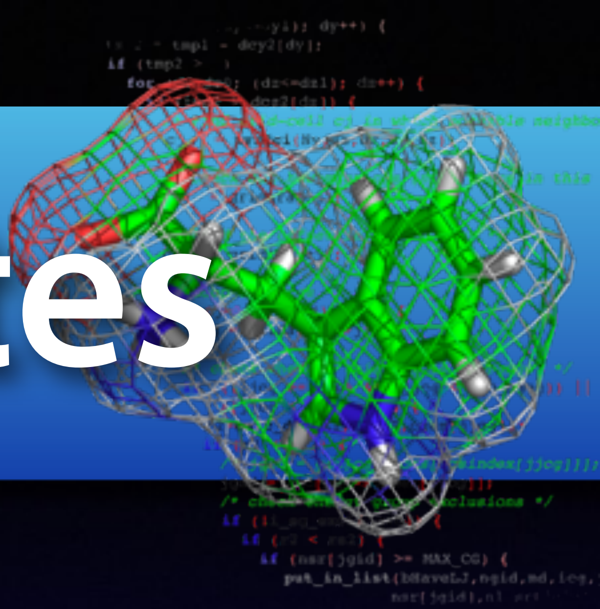
- **Initiation at a given point:**

$$t_{\mathrm{INIT0}} \approx \tau_\beta \exp\left(+F^{\#}/kT\right)$$

- **Initiation *somewhere*:** t_{INIT0}/N
- **Initiation is entirely time-limiting**
- **Total formation time:** $t \approx \tau_\beta \exp\left(F^{\#}/kT\right)/N$
- **And remember that we had:**

$$F^{\#} = U + 2N_{\mathrm{min}}(f_\beta + \Delta f_\beta) + U = 2(U\Delta f_\beta)/(-f_\beta)$$

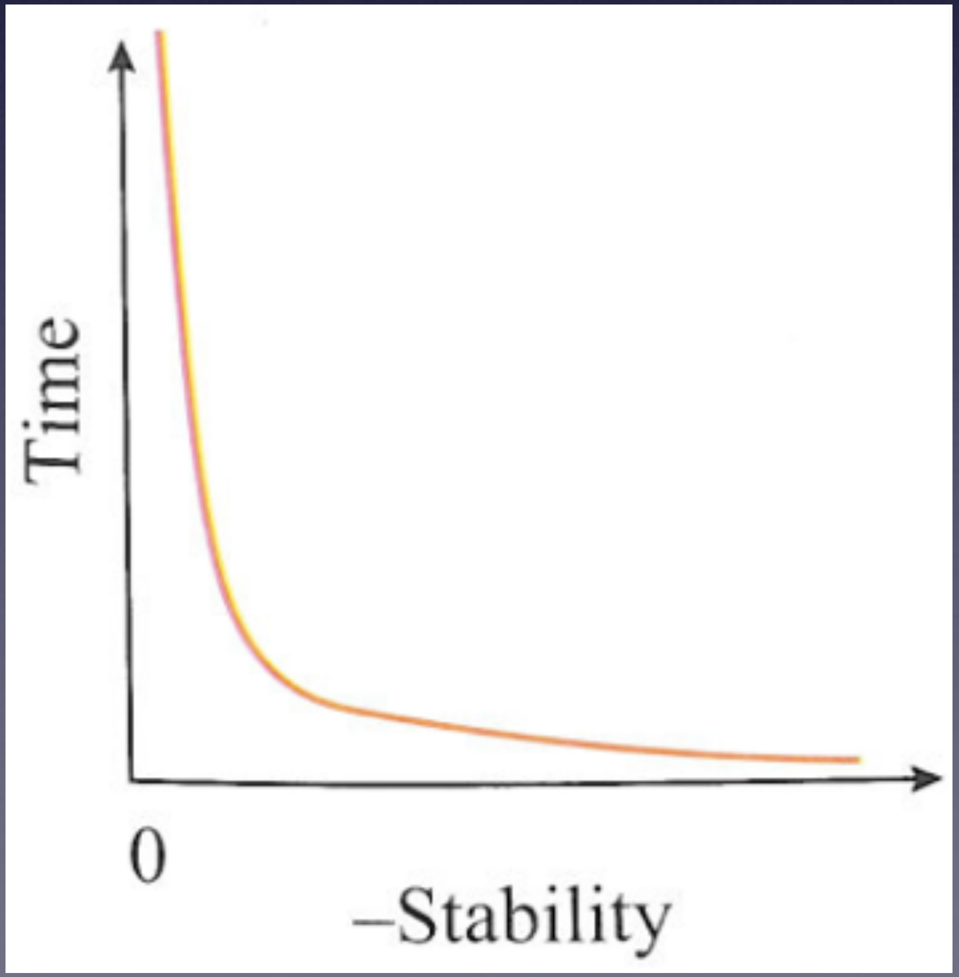
Beta formation rates



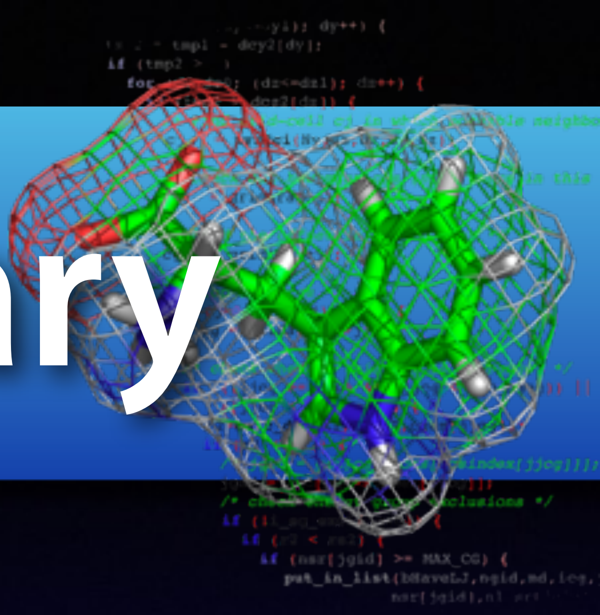
- Rate depends on β-structure stability:

$$t_\beta \approx \exp\left[A/(-f_\beta)\right]$$

- Exponential dependence on residue beta stability explains wide range of formation times observed in experiments!

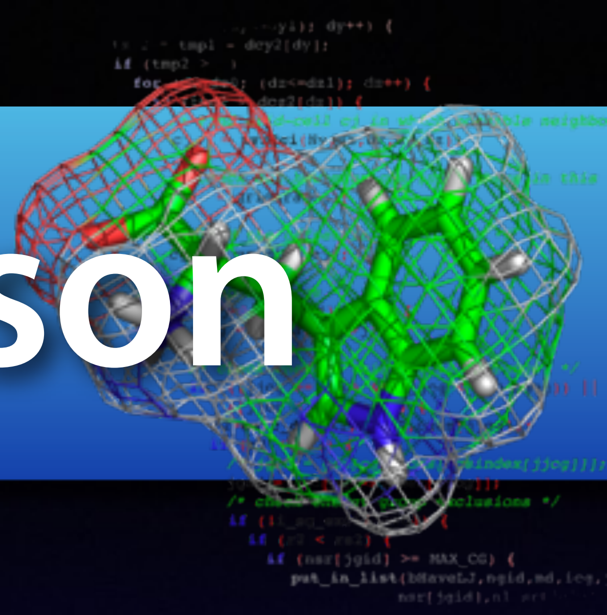


Beta sheet summary



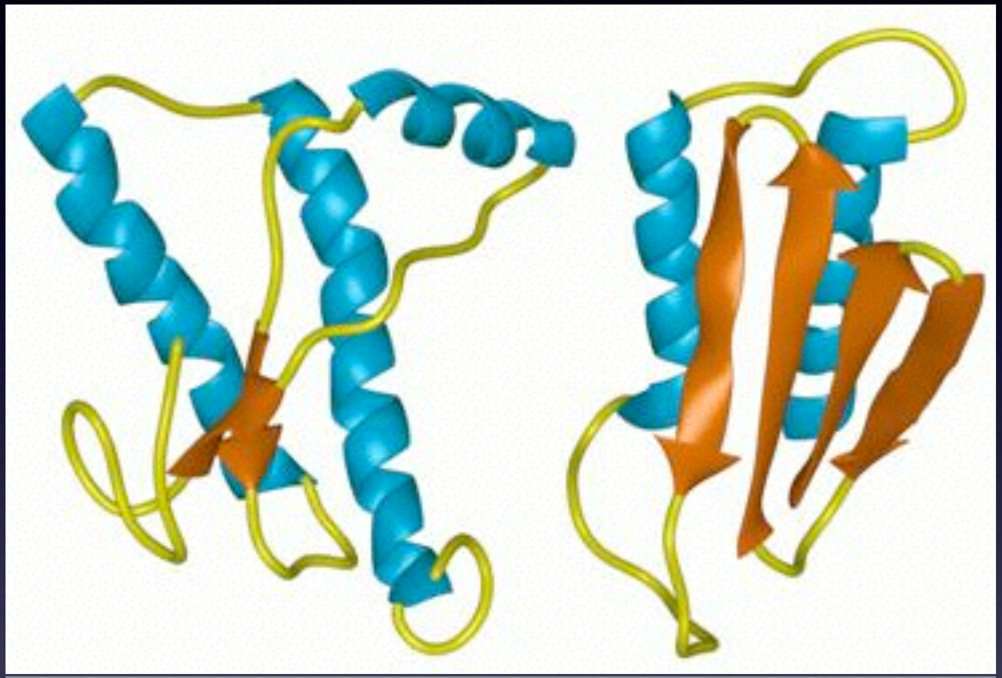
- Unstable sheets are extremely slow to form (hours to weeks)
- Stable sheets can form in milliseconds
- Significant free energy barrier
- Beta sheet folding is a first-order phase transition

Helix-sheet comparison



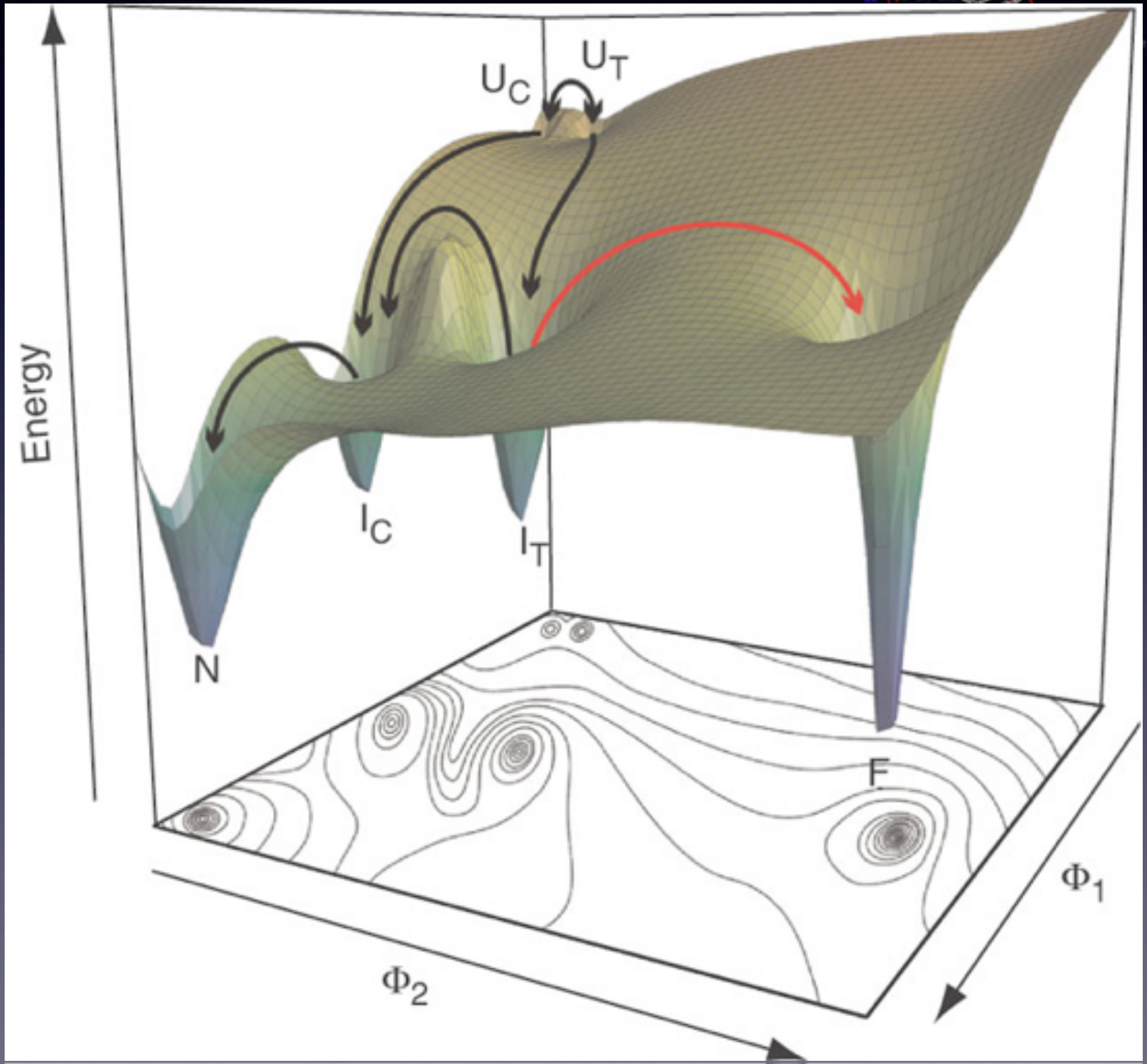
- The alpha helix “avoids” the phase transition - the boundary area does not increase with helix size
- Leads to much lower barriers, which can be overcome in microseconds
- The high free energy barrier of sheets is likely one of the explanations to prion/ amyloid protein misfolding diseases

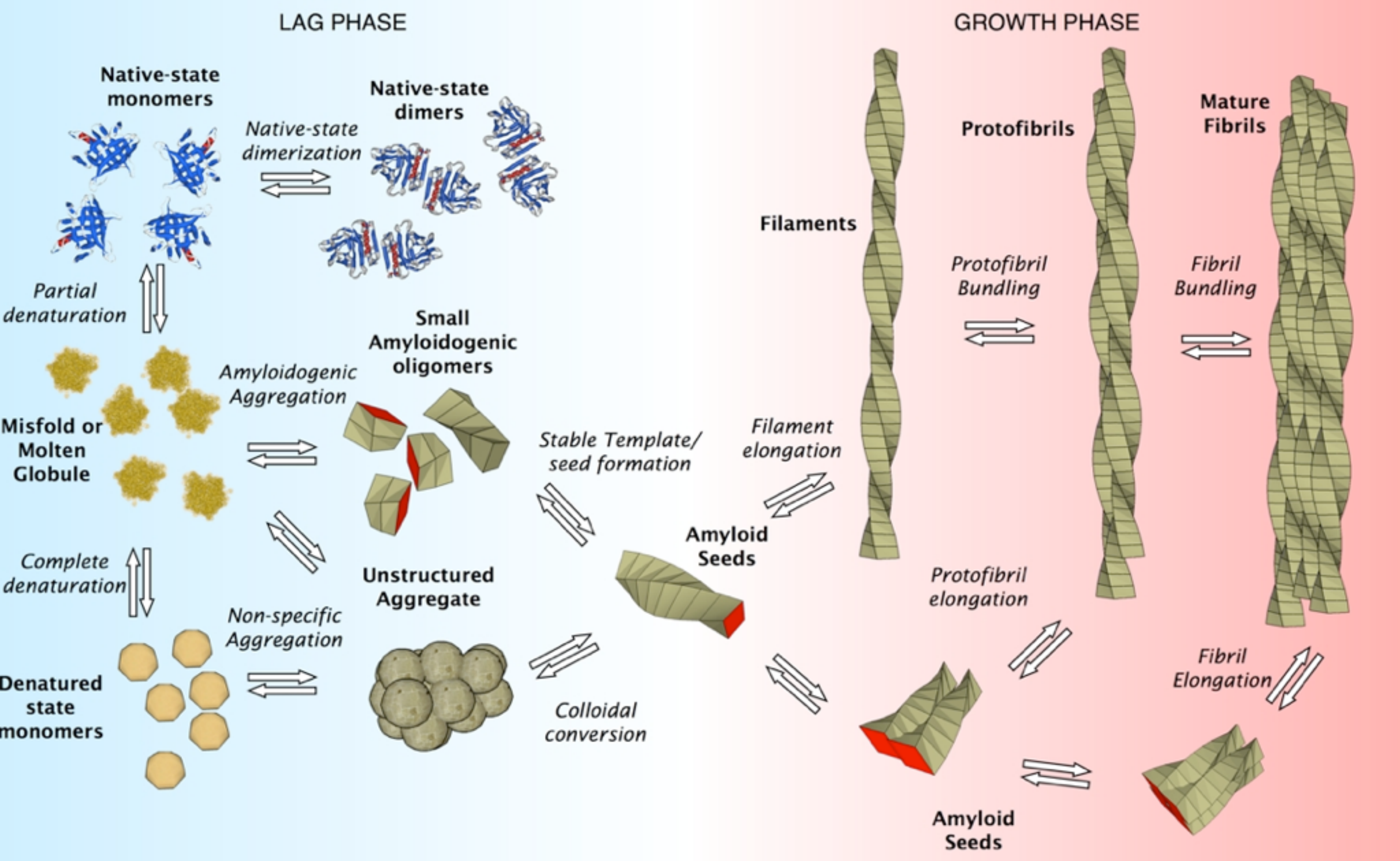
Misfolding - Prions



Misfolded form (right) is protease resistant

The *in vivo* state is only the second best here - but the free energy barrier to the lowest is very high!





LAG PHASE

GROWTH PHASE

Native-state monomers

Native-state dimerization

Native-state dimers

Partial denaturation

Misfold or Molten Globule

Amyloidogenic Aggregation

Small Amyloidogenic oligomers

Complete denaturation

Denatured state monomers

Non-specific Aggregation

Unstructured Aggregate

Colloidal conversion

Stable Template/ seed formation

Amyloid Seeds

Filament elongation

Filaments

Protofibril Bundling

Protofibrils

Fibril Bundling

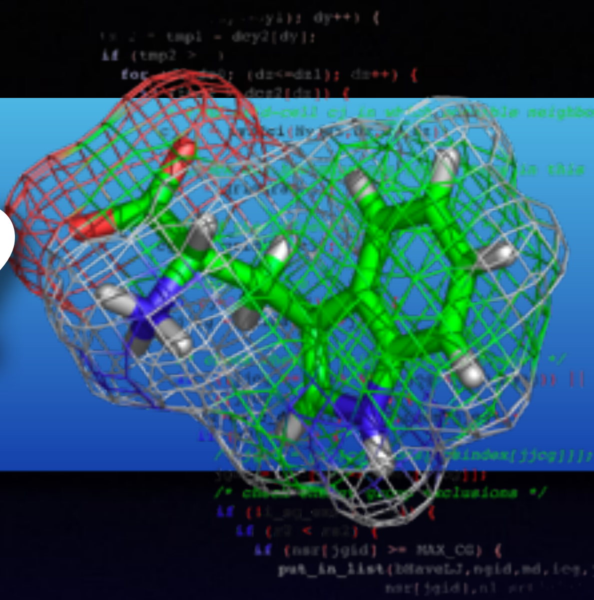
Mature Fibrils

Protofibril elongation

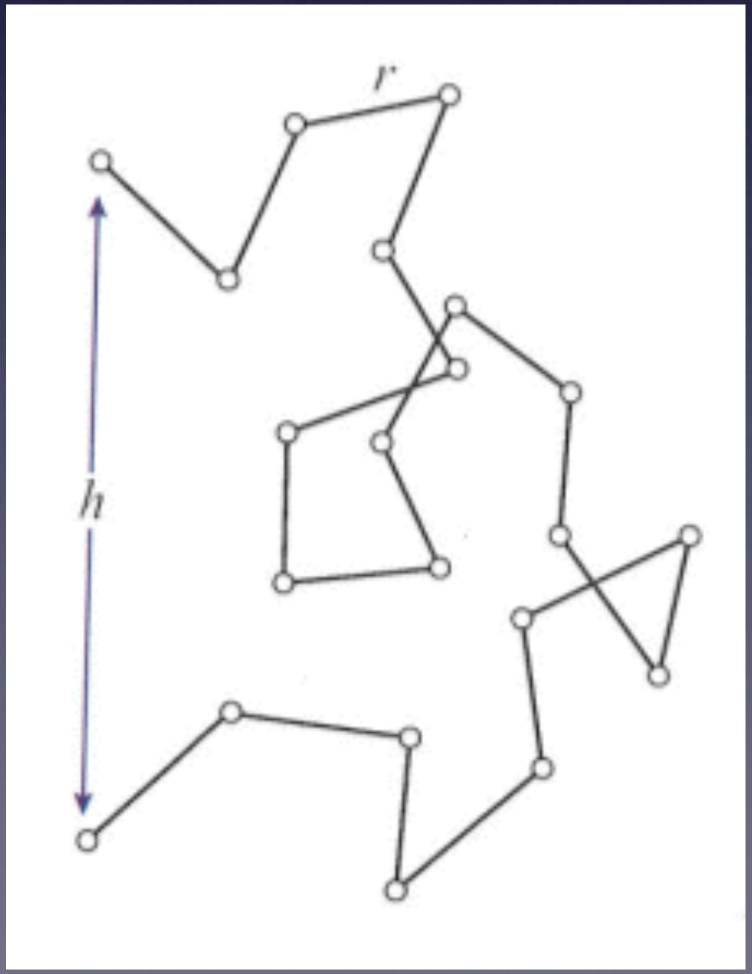
Fibril Elongation

Amyloid Seeds

What is the Coil?



- Less well-defined state than native
- It is NOT a stretched out linear chain!

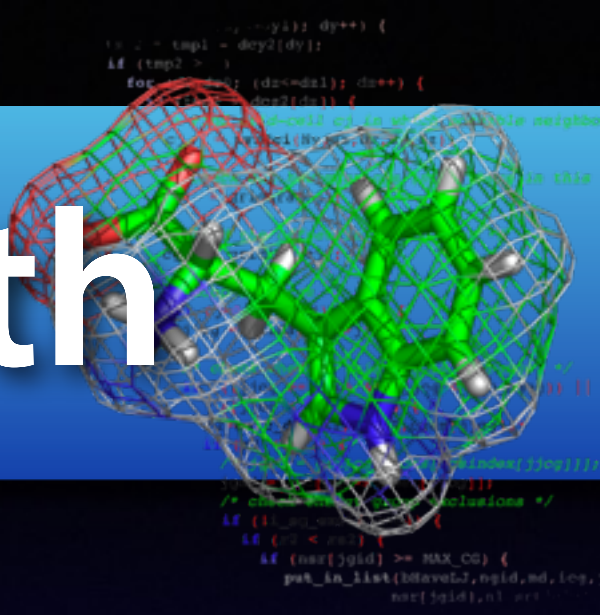


What is the average chain end-to-end distance?

$$\mathbf{h} = \sum_{i}^{N} \mathbf{r}_i$$

$$\begin{aligned} \mathbf{h}^2 &= \left(\sum_{i=1}^{N} \mathbf{r}_i \right)^2 \\ &= \sum_{i=1}^{N} \mathbf{r}_i^2 + \sum_{i=1}^{N} \sum_{j \neq i}^{N} \mathbf{r}_i \mathbf{r}_j \end{aligned}$$

Average coil length

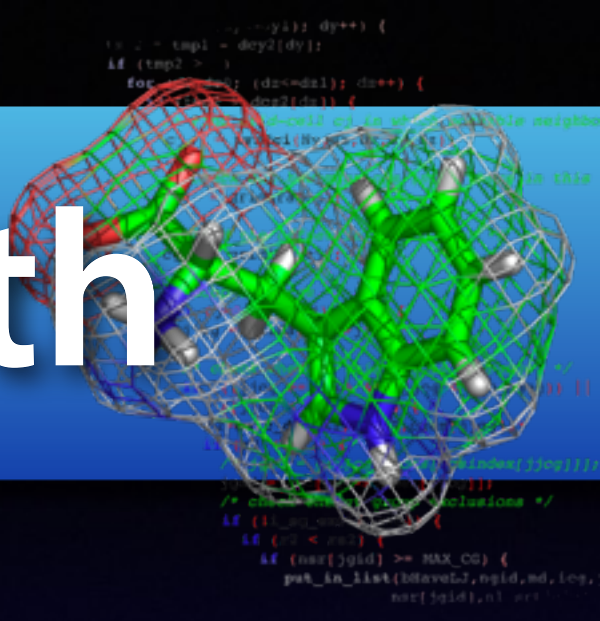


$$\begin{aligned}\langle \mathbf{h}^2 \rangle &= \left\langle \left(\sum_{i=1}^{N} \mathbf{r}_i \right)^2 \right\rangle \\ &= \left\langle \sum_{i=1}^{N} \mathbf{r}_i^2 + \sum_{i=1}^{N} \sum_{j \neq i}^{N} \mathbf{r}_i \mathbf{r}_j \right\rangle \\ &= \sum_{i=1}^{N} \langle \mathbf{r}_i^2 \rangle + \sum_{i=1}^{N} \sum_{j \neq i}^{N} \langle \mathbf{r}_i \mathbf{r}_j \rangle = N r^2\end{aligned}$$

Average length increases as $\sqrt{N}$

Average volume increases as $N^{3/2}$

Average coil length



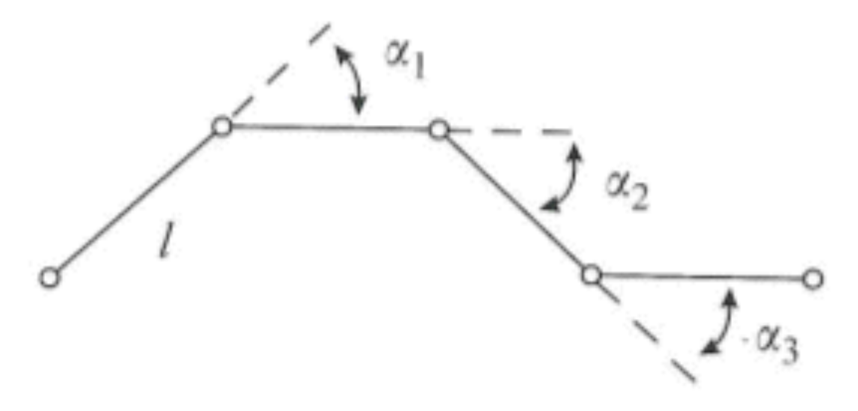
- Some problems:
  - Segments cannot have any orientation
  - Angle potentials
- Generalized expression: $\langle \mathrm{h}^2 \rangle = Nr^2 = Lr$

Chain contour length

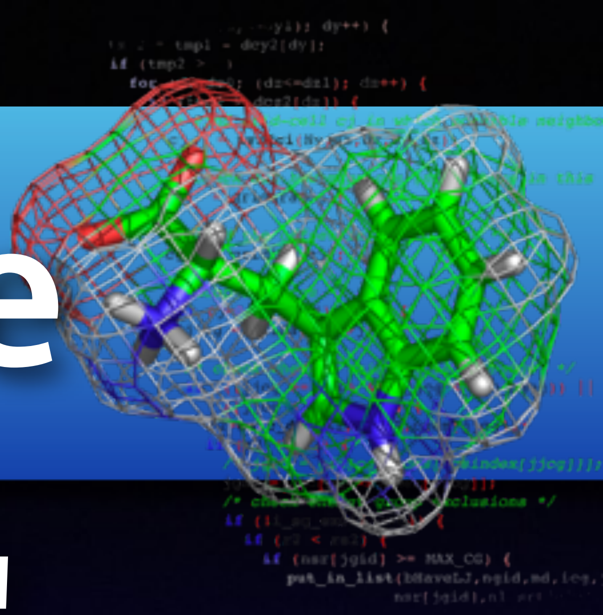
Segment size

- Rotational model:

$$r = l\,(1 + \langle \cos\alpha \rangle)\,/\,(1 - \langle \cos\alpha \rangle)$$



Excluded volume



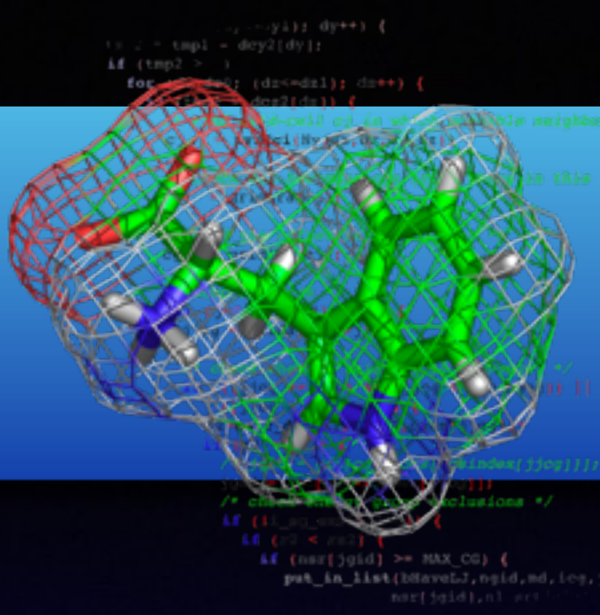
- Real chains cannot cross themselves!
- Segment i can never overlap with j, even if they are very far apart
- Excluded volume effects!

$$\sqrt{\langle \mathbf{h}^2 \rangle} \approx N^{0.588} r$$

Paul Flory

Nobel Prize 1974

Summary



- Alpha helix & beta sheets form in very different ways, that give them different properties!
- All determined by free energy barriers!
- There are natural sizes of helices/sheets
- Folding rates can be predicted with very simple qualitative arguments
- You should understand both how & *why* the are different (i.e. be able to explain)!