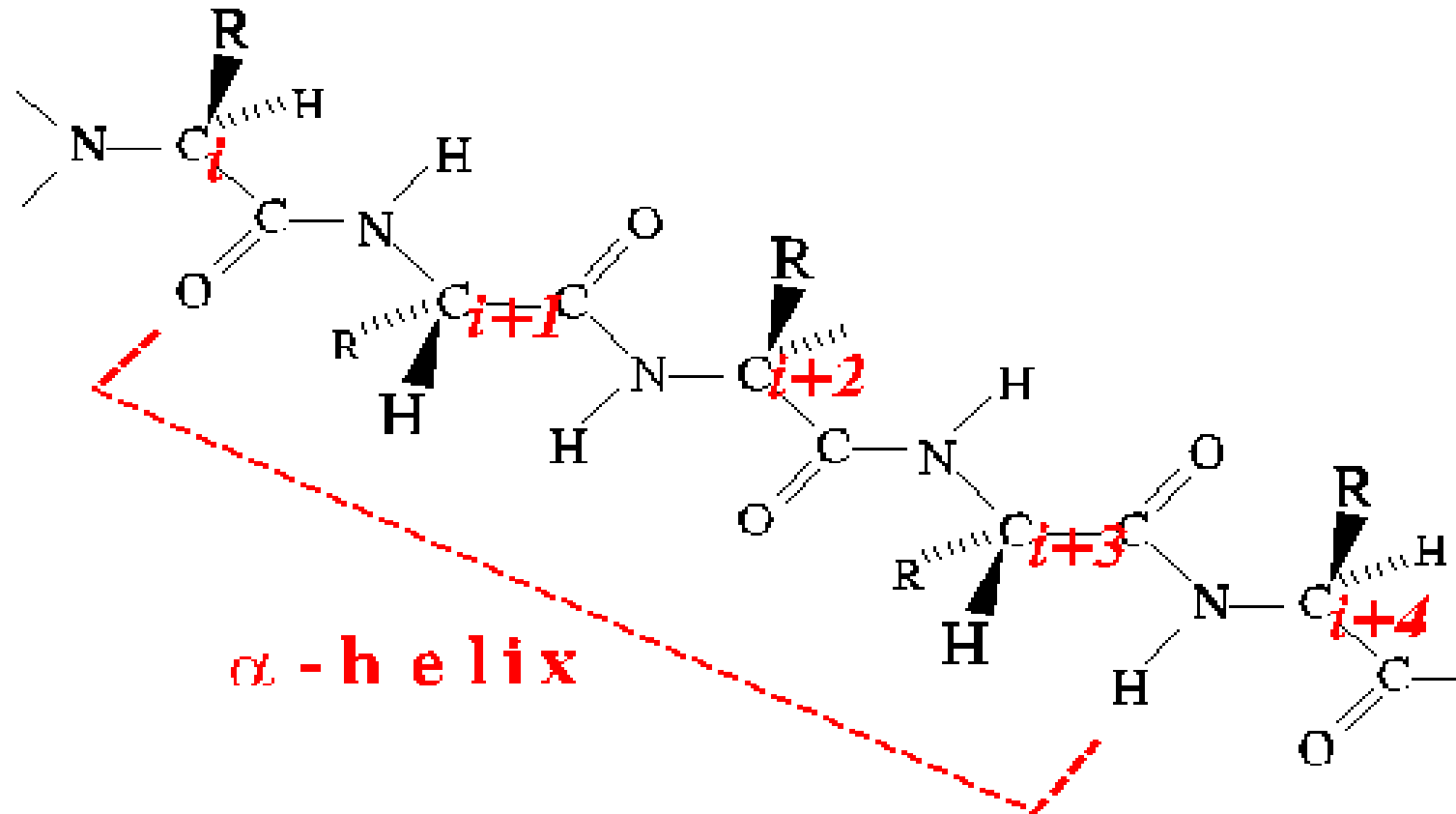


Lecture 9:
Stability & Kinetics of Secondary
Structure Formation

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α -Helix Definition: H-bond between i & $i+4$



**3 residues ($i+1, i+2, i+3$) fixed by 1 H-bond \rightarrow
n residues fixed by $n-2$ H-bonds**

**Free energy for formation of a helix from a coil
in homopolypeptides:**

→ f_H – free energy of formation of one H-bond
(energy of H-bond & entropy of H_2O mol.)

→ S_α – the entropy loss due to fixation of one residue
in the helix



$$\Delta F_\alpha = F_\alpha - F_{\text{coil}} = (n-2) f_H - n TS_\alpha = -2 f_H + n(f_H - TS_\alpha)$$



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

f_{INIT} – free energy of helix initialization

F_{ELONG} – free energy of helix elongation per one residue

Probability of a purely helical state relative to a purely coil state of an n-residue long peptide chain is:

$$\begin{aligned} p_{\text{HELIX}}/p_{\text{COIL}} &= \exp(-\Delta F_{\alpha} / k_B T) \\ &= \exp(-f_{\text{INIT}} / k_B T) \exp(-nf_{\text{ELONG}} / k_B T) \\ &= \exp(-f_{\text{INIT}} / k_B T) [\exp(-f_{\text{ELONG}} / k_B T)]^n \\ &= \sigma s^n \end{aligned}$$

**σ – probability of helix initiation
or helix initiation parameter**

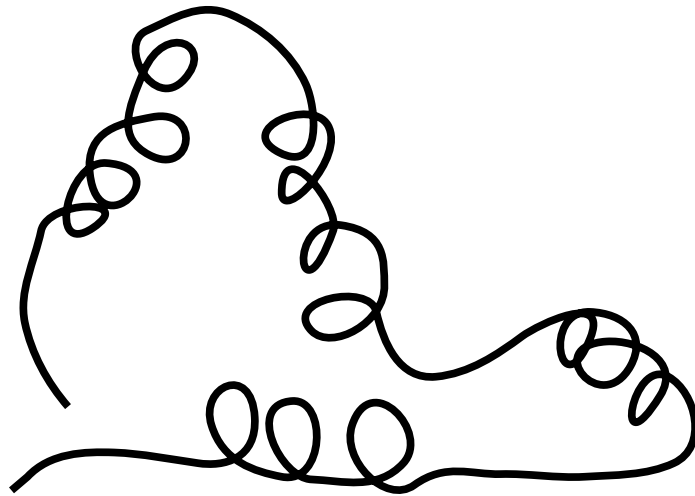
**s – probability of helix elongation by one residue
or helix elongation parameter**

σs^n – equilibrium constant for α to coil states

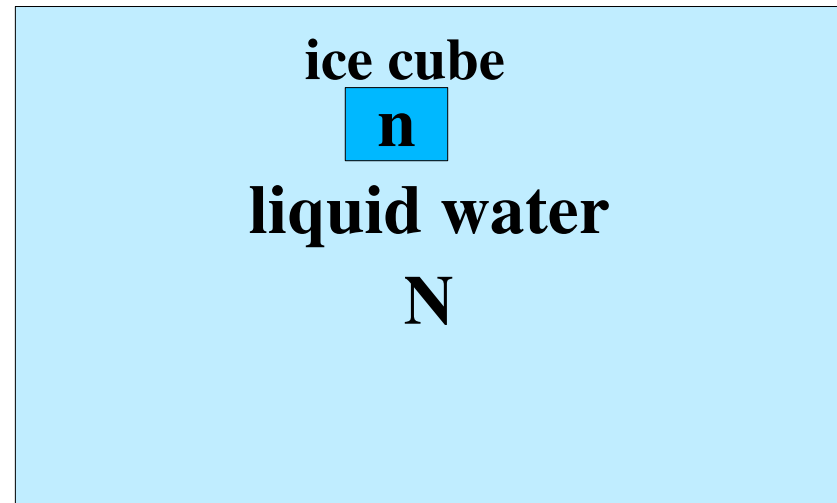
Varying temperature (or pH or solvent): Is there a “all-or-none” transition or just a gradual evolution of the helix from the coil state?

NO “all-or-none” transition can exist!

Landau's theorem: No first—order transition in a system with two one-dimensional phases.



RC→ α



water liquid→solid

How does the surface (interface) between two phases scale with n ?

→ coil→helix: surface area independent of n

→ liquid water→ice: surface area and thus surface free

energy of ice scales as $\sim \xi n^{2/3}$

entropic part: $-k_B T \ln(N)$

e.g. $n=xN$ ($0 < x < 1$)

$$\Delta F = \xi (xN)^{2/3} - k_B T \ln(N)$$

N large: $\Delta F > 0$ (all-or-none: ice either completely melts or overtakes the liquid)

→ BUT, phase coexistence between coil & helix (1D system)

favorable:

$$T^* - \text{helix and coil the same } F \rightarrow f_{\text{ELONG}} = 0$$

→ positional entropy of n-residue long helix on N-residue peptide: $f_{\text{INIT}} = -k_B T \ln(N-n) < 0$ (dominant at large N)

→

In a 1D system transition DOES NOT occur, rather the phases tend to mix up!

What is the characteristic length of helix n_0 at the midpoint of the coil→helix evolution?

→ N-residue peptide at mid-transition temperature where

$$F(\text{coil}) = F(\text{helix}) \rightarrow f_{\text{ELONG}} = 0 \text{ (s=1)}$$

→ f_{INIT} – helix initiation free energy

→ $\sim N^2/2$ – number of possible realizations of a helix in the N-residue chain (helix incl. 3 or more residues)

- the free energy of insertion of helix is: $f_{\text{INIT}} - 2 T k_B \times \ln(N)$
- if $f_{\text{INIT}} - 2 T k_B \times \ln(N) > 0$, the insertion will happen
- characteristic length of the helix segment, n_0 , at the midpoint of coil→helix transition is:

$$n_0 = \exp (+f_{\text{INIT}}/2k_B T) = \sigma^{-1/2}$$

- experimentally: midpoint temperature corresponds to 50% helicity of a very long peptide (as measured by CD)
- for most amino acids:

$$n_0 = 30, f_{\text{INIT}} = 4 \text{ kcal/mol, \& } \sigma = 0.001$$

- Estimation of the H-bond free energy f_{H} and the entropy loss TS_{α} caused by fixation of one residue in the α -helix:

$$f_{\text{H}} = -f_{\text{INIT}}/2 = -2 \text{ kcal/mol \& } (f_{\text{ELONG}} = 0) TS_{\alpha} = f_{\text{H}} = -2 \text{ kcal/mol}$$

Which, f_{INIT} or f_{ELONG} , affects the helix stability the most?

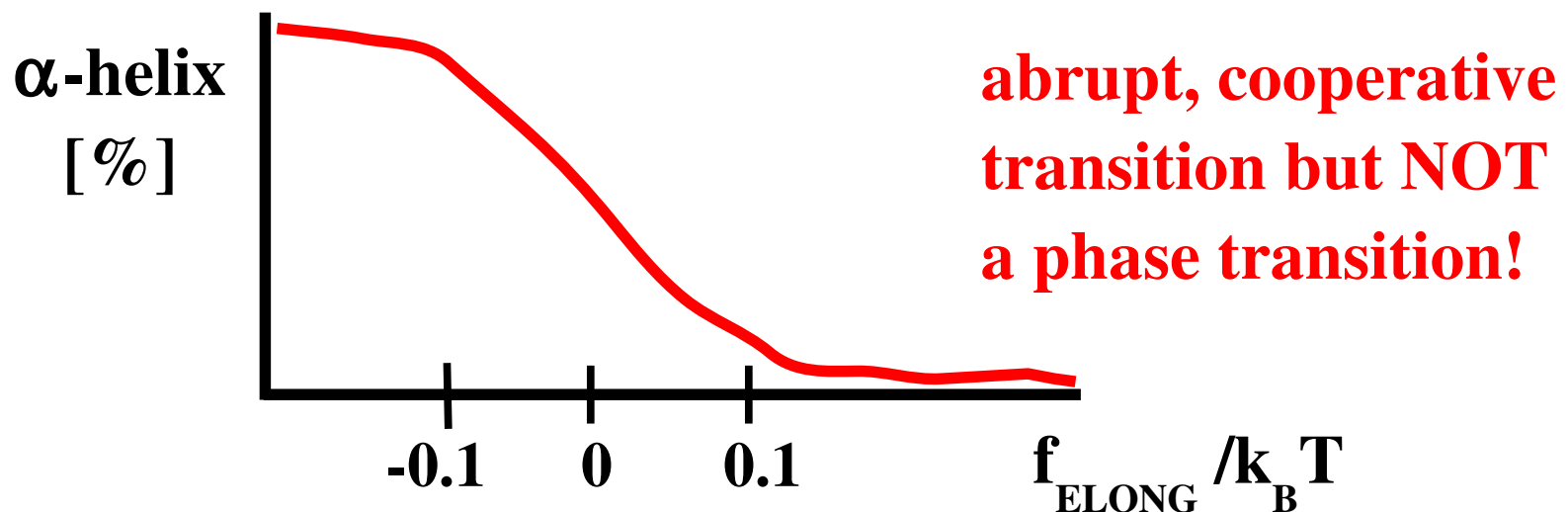
→ both f_{INIT} & f_{ELONG} are temperature-dependent

→ f_{ELONG} – multiplied by n (length of the helix)

→ at $n_0 f_{\text{ELONG}} / k_B T \sim 2$ helicity disappears (only coil conf.)

→ at $n_0 f_{\text{ELONG}} / k_B T \sim -2$ coil disappears (only helix conf.)

– $-2/n_0 < f_{\text{ELONG}} / k_B T < 2/n_0$ (coil and helix coexist!)



Amino acid specificity:

→ alanine (Ala): the most “helix-forming”

$$f_{\text{ELONG}} = -0.4 \text{ kcal/mol (s=2)}$$

→ glycine (GLY): the most “helix-breaking”

$$f_{\text{ELONG}} = +1 \text{ kcal/mol (s=0.2)}$$

(in proline NH group does not participate in H-bonding

→ $0.001 < s < 0.01$, not determined accurately yet)

Potentiometric titration, a method to measure the stability of the helical state (poly-Glu, poly-Lys, ...):

→ by uniformly charging the helix, we destroy it (in helix, the side-chain charges are closely packed → stronger repulsion)

→ dependence of the total charge & helicity on the pH of the medium

Rate of α -Helix Formation

→ α -helices formed rapidly: $\sim 0.1\mu\text{s}$ in 20-30 residue

→ the rate depends on the rates of two processes:

(1) helix initiation (the first “nuclei”)

(2) helix elongation

→ process (a) requires activation energy → rate limiting step

→ process (b) is faster (no activation free energy barrier)

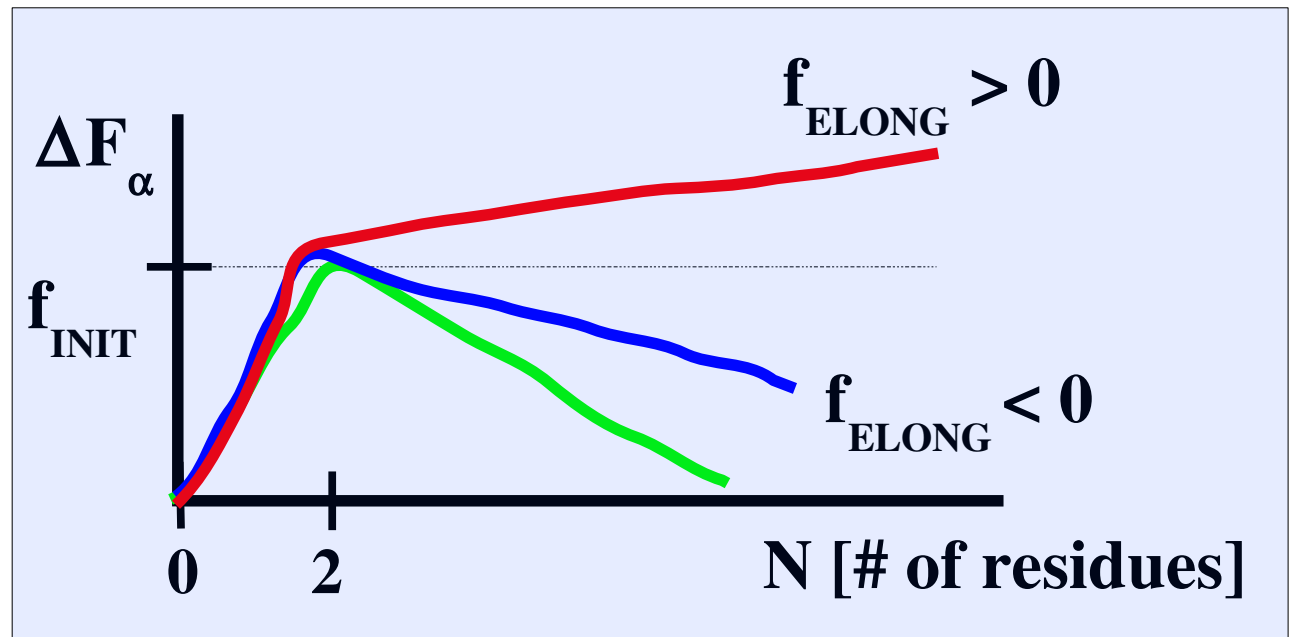
(a) $f_{\text{ELONG}} > 0$

(b) $f_{\text{ELONG}} < 0$

(a) NO helix

(b) activation

barrier ΔF_{α}



Following the transition state theory, helix initiation step (1):

$$t_{\text{INIT}\alpha} = \tau \exp(+f_{\text{INIT}}/k_B T),$$

where τ is time for helix elongation by one residue

$$t_{\text{INIT}\alpha} = \tau/\sigma$$

[by definition, $\sigma = \exp(-f_{\text{INIT}}/k_B T)$].

$t_{\text{INIT}\alpha}$ – time for helix initiation at each residue

helix on average spans $n_0 = \sigma^{-1/2}$

\Rightarrow

time for helix initiation at any of n_0 residues,

$$t_{\alpha} = t_{\text{INIT}\alpha} / n_0 = \tau/\sigma = \tau \exp(+f_{\text{INIT}}/2k_B T)$$

Half of the time is spent in helix initiation anywhere in the chain and half of the time is spent on helix elongation (~ns).

Kinetics of β -Structure Formation

- β -structure formation can take ms, hours, or weeks
- however, folding rate of proteins with β -structure not much lower than of proteins with α -helix

⇒

HOW CAN THIS BE EXPLAINED?

- two-dimensional nature of a β -sheet (as opposed to 1D helical structure) ALLOWS for the 1st order phase transition
- because the β -sheet edges have fewer contacts than internal residues (and form less H-bonds), edges have a higher F (like a drop of liquid water in vapor/piece of ice in liquid water)

⇒

- the boundary grows with the number of residues N in the β -sheet (1st order phase transition RC→ β -structure possible)

- the edge of the β -sheet consists of:
 - edge β -strands
 - edge loops/turn separating β -strands
- f_{β} – free energy of a residue in the center of a β -sheet
- Δf_{β} – free energy of a residue at the edge of the β -sheet
- U – free energy of a bend/loop/turn
- the edge free energies $> 0 \Rightarrow \Delta f_{\beta} > 0$ & $U > 0$
 (if not, β -sheet would fall into pieces)

Kinetics will be different for the two cases:

(a) $f_{\beta} + \Delta f_{\beta} < 0$

(b) $f_{\beta} + \Delta f_{\beta} > 0$

$f_{\beta} < 0$ (or else β -sheet will not form)

- (a) - long β -hairpin more stable than a RC**
 - only the turns contribute to the activation energy
(free energy barrier)**
 - ΔF very similar to ΔF_{α} (turn – α -helix)**
- (b) - β -hairpin on itself unstable β -sheet stabilized by association of many β -hairpins into the β -sheet**
 - formation of a “nucleus” determine the free energy barrier**
 - a nucleus is a β -sheet transition state, such that its growth, e.g. addition of β -strand(s), minimizes F**

Because of the barrier in scenario (b), β -sheet formation can be extremely slow process.

The simplest case of scenario (b) – β -hairpin NOT stable:

- β -hairpin consisting of 2 N-residue strands separated by

a turn: $F_1 = U + 2N (f_\beta + \Delta f_\beta)$

- association of 2 β -hairpins: $2F_1 - N f_\beta \dots$

- each addition of a β -hairpin adds to the total F

$$F \rightarrow F + N f_\beta + U$$

- for each addition to decrease the total F:

$$N_{\min} = U/(-f_\beta)$$

- the transition state (the most unstable state) is the β -hairpin (by our assumption) consisting of 2 strands of at least N_{\min} residues

- minimum $F^\#$ of initiating the hairpin & the next turn:

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

$$F^\# = 2 (U \Delta f_\beta) / (-f_\beta)$$

**The free energy of the transition state (free energy barrier)
for an unstable β -hairpin scenario**

→ $F^\#$ can be very large if $(-f_\beta)$ is close to zero

Transition states with much higher stability CANNOT exist:

**→ estimate of F_{\min} of a growing M-residue β -sheet with m
 β -strands:**

$$F(M,m) = Mf_\beta + 2(M/m) \Delta f_\beta + (m-1)U$$

→ find an optimal number of strands m at a given M:

$$dF/dm=0$$

$$- 2(M/m^2) \Delta f_\beta + U = 0 \rightarrow m_{\text{opt}} = M^{1/2} (2\Delta f_\beta / U)^{1/2}$$

$$F_{\text{opt}}(M) = M f_{\beta} - U + 2M^{1/2}(2\Delta f_{\beta} U)^{1/2}$$

→ finding a maximum of $F_{\text{opt}}(M)$ will give us the barrier $F^{\#}$

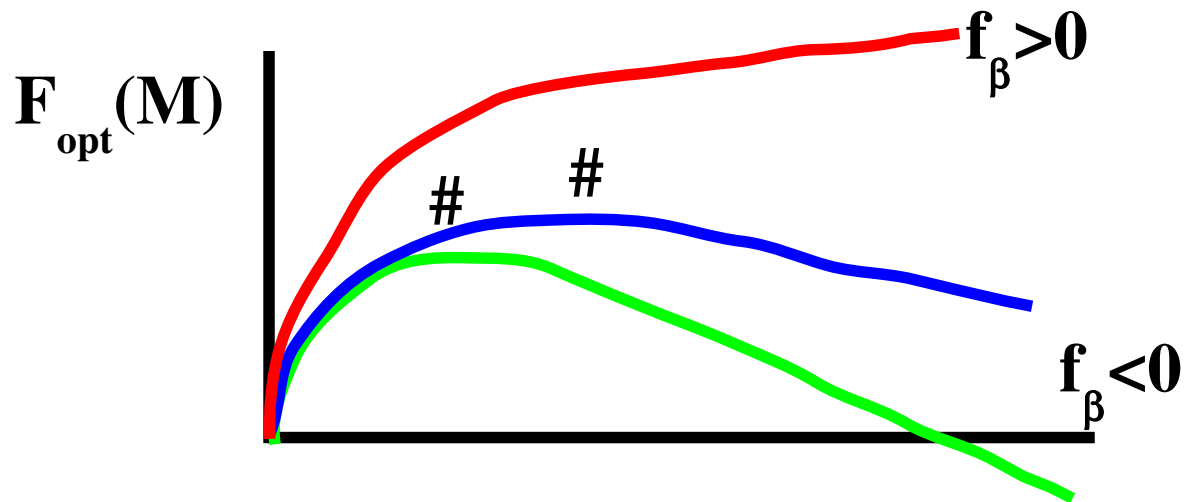
$$dF_{\text{opt}}(M)/dM = 0$$

⇒

$$M^* = 2(\Delta f_{\beta} U)/(-f_{\beta})^2$$

$$F^* = F_{\text{opt}}(M^*) = 2(U \Delta f_{\beta})/(-f_{\beta}) - U$$

→ free E barrier $F^{\#}/F^*$ strongly depends on the stability of β -structure $(-f_{\beta})$



Time of the β -structure initiation process:

$$t_{\text{INIT}\beta} \sim \tau_{\beta} \exp(+F^{\#}/k_B T)$$

τ_{β} – the time of an elementary step, β -sheet elongation

$t_{\text{INIT}\beta}$ – time to initiate β -structure in one given point of

the chain $\rightarrow t_{\beta} = t_{\text{INIT}\beta} / M$

t_{β} – time to initiate β -structure *anywhere* on the chain

$M\tau_{\beta}$ – time for expansion of the β -structure over M
residues

Initiation time $t_{\beta} = M^{-1} \tau_{\beta} \exp(+F^{\#}/k_B T)$ is the rate limiting
process in the β -structure formation:

$$t_{\beta} \sim \exp[A/(-f_{\beta})]$$

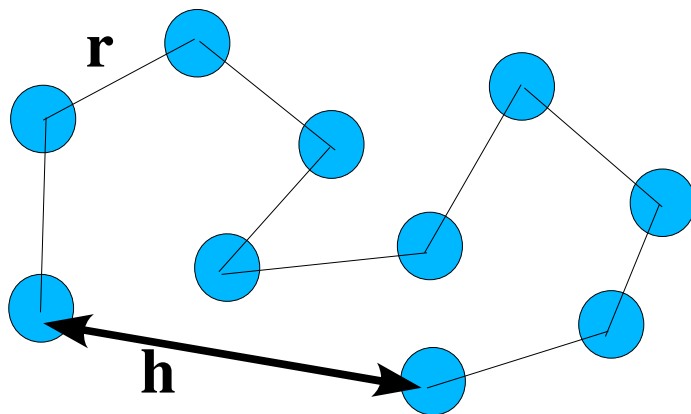
Explanation for extremely slow rates of β -structure formation!

What is a random coil (RC)?

- a peptide chain without any distinct structure
- no long-range order in the chain
- some weak short-range order possible (short lived)
- Experiments (light & X-scattering & hydrodynamic

Measurements):

- RC has extremely low density
- peculiar dependence of r & V on the chain length



“loose joint chain” model:

- each of M links of equal length
- r can include several residues
- each “stick” can freely move on the joint

- the end-to-end vector (the vector that joins the 1st and the last residue): $\mathbf{h} = \sum_{i=1}^M \mathbf{r}_i$
- \mathbf{r}_i are of the same length but different, random, directions

How does the length of \mathbf{h} scale with M ?

$$\rightarrow \mathbf{h}^2 = \left(\sum_{i=1}^M \mathbf{r}_i \right)^2 = \sum_{i=1}^M \mathbf{r}_i^2 + \sum_{i \neq j} \mathbf{r}_i \mathbf{r}_j$$

→ average over the above expression:

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

How does \mathbf{h} scale with M ?

$$\langle \mathbf{h}^2 \rangle^{1/2} = r M^{1/2}$$

Linear dimensions scale as $M^{1/2} \Rightarrow V \sim M^{3/2}$

The volume of a RC is proportional to $M^{3/2}$!

The loose joint model can be generalized as:

$$\langle h^2 \rangle = Lr,$$

where $L=Mr$ is a full contour length of the peptide chain & r is the characteristic length (length of the Kuhn segment), after which the chain “forgets” its direction:

- **Kuhn's length: 30-40Å (~10 residues)**
- **Rotational isomeric model of the RC:**

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

