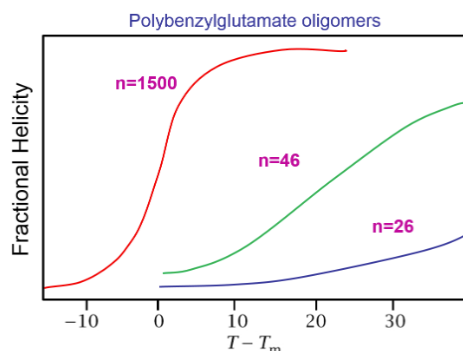


18.1: Helix–Coil Transition

Cooperativity plays an important role in the description of the helix–coil transition, which refers to the reversible transition of macromolecules between coil and extended helical structures. This phenomenon was observed by Paul Doty in the 1950s for the conversion of polypeptides between a coil and α -helical form,² and for the melting and hybridization of DNA.³ Bruno Zimm developed a statistical theory with J. Bragg that described the helix–coil transition, which forms the basis of our discussion.⁴

One of the observations that motivated this work is shown in the figure below. The fraction of helical structure observed in the polypeptide poly-benzylglutamate showed a temperature-dependent melting behavior in which the steepness of the transition increased with polymer chain length. This length dependence indicates a higher probability of forming helices when more residues are present, and that the linkages do not act independently. This suggests a two-step mechanism. The rate-limiting step of forming an α helix is the nucleation of a single hydrogen bonded residue $i \rightarrow i + 4$ loop. Once this occurs, the addition of further hydrogen bonds to extend this helix is much easier and occurs in rapid succession.



Adapted from B. H. Zimm, P. Doty and K. Iso, Proc. Natl. Acad. Sci. U. S. A. **45**, 1601-1607 (1959). Copyright 1959 PNAS.

To model this behavior, we imagine that the polypeptide consists of a chain of segments that can take on two configurations, H or C .

Symbol	Name	Effect
H	Helix	Decreases entropy but also lowers enthalpy
C	Coil	Raises entropy

To specify the state of a conformation through a sequence, i.e.,

... $HCHHHHCCCCHHH$...

Remember to not take this too literally, and be flexible in the interpretation of your model. Although this model was derived with an α -helix formation in polypeptides in mind, in a more general sense H and C do not necessarily refer explicitly to residues of a sequence, but just for independently interacting regions.

If there are n segments, these can be divided into n_H helical and n_C coil segments.

$$n_H + n_C = n$$

The segments need not correspond directly to amino acids, but structurally and energetically distinct regions. Our goal will be to calculate the fractional helicity of this system θ_H as a function of temperature, by calculating the conformational partition function, q_{conf} , by an explicit summation over i microstates, Boltzmann weighed by the microstate energy E_i :

$$q_{\text{conf}}(n) = \sum_{i \text{ config.}} e^{-E_i/k_B T} \quad (18.1.1)$$

Non-cooperative Model

We start our analysis by discussing a non-cooperative model. We assume:

- Each segment can switch conformation between H and C independently of the others.
- The formation of H from C lowers the configurational energy by $\Delta\epsilon$. $\Delta\epsilon = E_H - E_C$ is a free-energy change per residue, where $\Delta\epsilon < 0$. We will take the coil state to be the reference energy $E_C = 0$.

- Therefore the energy of the system is determined from the number of H residues present, not the specific sequence of H and C segments.

$$E_i = E(n_H) = n_H \Delta \varepsilon$$

Then, we can calculate q_{conf} using $g(n, n_H)$, the degeneracy of distinguishable states for a polymer of length n with n_H helical segments. The conformational partition function is obtained by

$$q_{\text{conf}}(n) = \sum_{n_H=0}^n g(n, n_H) e^{-n_H \Delta \varepsilon / k_B T} \quad (18.1.2)$$

In evaluating the partition functions in helix-coil transition models, it is particularly useful to define a “statistical weight” for the helical configuration. It describes the influence of having an H on the probability of observing a particular configuration at $k_B T$:

$$s = e^{-\Delta \varepsilon / k_B T} \quad (18.1.3)$$

For the present model, we can think of s as an equilibrium constant for the process of adding a helical residue to a sequence:

$$s = \frac{P(n_H + 1)}{P(n_H)}$$

This equilibrium constant is related to the free energy change for adding a helical residue to the growing chain. Then we can write eq. (18.1.2) as

$$q_{\text{conf}}(n) = \sum_{n_H=0}^n g(n, n_H) s^{n_H}$$

Since there are only two possible configurations (H and C), the degeneracy of configurations with n_H helical segments in a chain of length n is given by the binomial coefficients:

$$g(n, n_H) = \frac{n!}{n_H! n_C!} = \binom{n}{n_H} \quad (18.1.4)$$

since $n_C = n - n_H$. Then using the binomial theorem, we obtain

$$q_{\text{conf}}(n) = (1 + s)^n \quad (18.1.5)$$

Also, the probability of a chain with n segments having n_H helical linkages is

$$P(n, n_H) = \frac{g(n, n_H) e^{-E(n_H) / k_B T}}{q_{\text{conf}}} = \binom{n}{n_H} \frac{s^{n_H}}{(1 + s)^n} \quad (18.1.6)$$

✓ Example: $n = 4$

The conformations available are shown here.

q	No. of Helices	Sequences	Microstate Count
q_0	0 H	$CCCC$	1
q_1	1 H	$HCCC$ $CHCC$ $CCHC$ $CCCH$	4
q_2	2 H	$HHCC$ $CHHC$ $CCHH$ $HCHC$ $CHCH$ $HCCH$	6
q_3	3 H	$CHHH$ $HCHH$ $HHCH$ $HHHC$	4
q_4	4 H	$HHHH$	1

The molecular conformational partition function is

$$\begin{aligned}
 q_{\text{conf}} &= \overbrace{1}^{\text{all } C} + \overbrace{4e^{-\Delta\varepsilon/k_{BT}}}^{\text{one } H} + \overbrace{6e^{-2\Delta\varepsilon/k_{BT}}}^{\text{two } H} + 4e^{-3\Delta\varepsilon/k_{BT}} + e^{-4\Delta\varepsilon/k_{BT}} \\
 &= 1 + 4s + 6s^2 + 4s^3 + s^4 \\
 &= (1+s)^4
 \end{aligned}$$

The last step follows from Pascal's Rule for binomial coefficients. From eq. (18.1.6), the probability of having two helical residues in a four-residue sequence is:

$$P(4, 2) = \frac{6s^2}{(1+s)^4}$$

To relate this to an observable quantity, we define the fractional helicity, the average fraction of residues that are in the H form.

$$\theta_H = \frac{\langle n_H \rangle}{n} \quad (18.1.7)$$

$$\langle n_H \rangle = \sum_{n_H=0}^n n_H P(n, n_H) \quad (18.1.8)$$

Using this amazing little identity, which we derive below,

$$\langle n_H \rangle = \frac{s}{q} \frac{\partial q}{\partial s} \quad (18.1.9)$$

You can use eq. (18.1.5) to show:

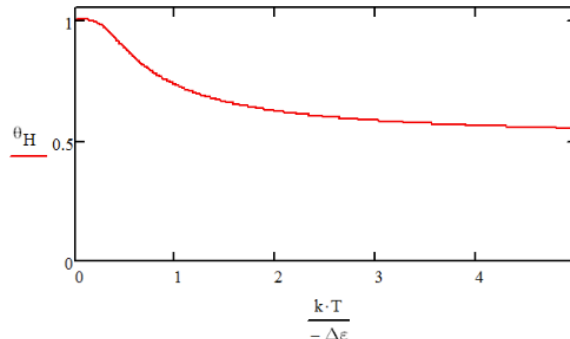
$$\langle n_H \rangle = \frac{ns}{1+s} \quad (18.1.10)$$

and

$$\theta_H = \frac{s}{1+s} \quad (18.1.11)$$

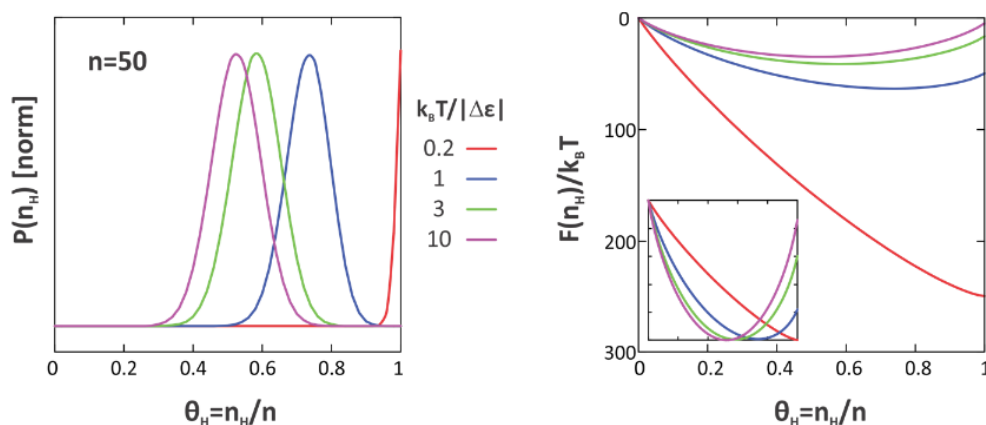
This takes the same form as one would expect for the simple chemical equilibrium of an $C \rightleftharpoons H$ molecular reaction. If we define the equilibrium constant $K_{HC} = [H]/[C]$, then the fraction of molecules in the H state is $\theta_H = [H]/([C] + [H]) = K_{HC}/(1 + K_{HC})$. In this limit $s = K_{HC}$.

Below we plot eq. (18.1.11), choosing $\Delta\epsilon$ to be independent of temperature. θ_H is a smooth and slowly varying function of T and does not show cooperative behavior. Its high temperature limit is $\theta_H = 0.5$, reflecting the fact that in the absence of barriers, the H and C configurations are equally probable for every residue.



We can look a bit deeper at what is happening with the structures present by plotting the probability distribution for finding n_H helical segments within a chain of length n , eq. (18.1.6), and the associated energy landscape (a potential of mean force):

$$F(n, n_H) = -Nk_B T \ln [P(n, n_H)] \approx -Nk_B T \ln [g(n, n_H) s^{n_H}]$$



The maximum probability and free-energy minimum is located at full helix content at the lowest temperature, and gradually shifts toward $n_H/n = 0.5$ with increasing temperature. The probability density appears Gaussian, and the corresponding free energy appears parabolic. Using similar methods to that described above, we can show that the variance in this distribution scales as $n^{-1/2}$. The presence of a single shifting minimum is referred to as a transition in a one-state system, rather than two-state behavior expected for phase transitions. Here n_H is the order parameter that characterizes the extent of folding of the helix.

Where does eq. (18.1.9) come from? For the moment, we will drop the “conf” and “H” subscripts, mainly to write things more compactly, but also to emphasize the generality of this method to all polynomial expansions. Using eq. (18.1.2), $q = \sum_n g s^n$, and recognizing that g is not a function of s :

$$\begin{aligned} \frac{\partial q}{\partial s} &= \sum_n n g s^{n-1} \\ &= \frac{1}{s} \sum_n n g s^n \end{aligned}$$

From eq. (18.1.6), $P_n = g s^n / q$, we can write this in terms of the helical segment probability

$$\frac{1}{q} \frac{\partial q}{\partial s} = \frac{1}{s} \sum_n n P_n \quad (18.1.12)$$

Comparing eq. (18.1.13) with eq. (18.1.12), $\langle n \rangle = \sum_n n P_n$, we see that

$$\frac{s}{q} \frac{\partial q}{\partial s} = \langle n \rangle \quad \text{or} \quad \frac{\partial \ln q}{\partial \ln s} = \langle n \rangle \quad (18.1.13)$$

This method of obtaining averages from derivatives of a polynomial appears regularly in statistical mechanics.⁵

Cooperative Zimm–Bragg Model

Let's modify the model to add an element of cooperativity to the segments in the chain. In order to form a helix, you need to nucleate a helical turn and then adding adjacent helical segments is easier. The probability of forming a turn is relatively low, meaning the free energy barrier for nucleation of one H in a sequence of C is relatively high: $\Delta G_{nuc} > 0$. However the free-energy change per residue for forming H from C within a helical stretch, ΔG_{HC} , stabilizes the growing helix. Based on these free energies, we define statistical weights:

$$s = e^{-\Delta G_{HC}/k_B T}$$

$$\sigma = e^{-\Delta G_{nuc}/k_B T}$$

s and σ are also known as the Zimm–Bragg parameters. Here, s is the statistical weight to add one helical segment to an existing continuous sequence (or stretch) of H , which we interpret as an equilibrium constant:

$$s = \frac{[\dots CHHHHCC\dots]}{[\dots CHHHCCC\dots]} = \frac{P_H(n_H + 1)}{P_H(n_H)}$$

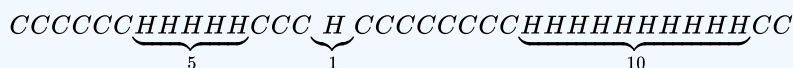
σ is the statistical weight for each stretch of H . This is purely to reflect the probability of forming a new helical segment within a stretch of C . The energy benefit of making the helical form is additional:

$$\sigma s = \frac{[\dots CCCHCC\dots]}{[\dots CCCCCC\dots]} = \frac{P_H(\nu_H + 1)}{P_H(\nu_H)}$$

ν_H is the number of helical stretch segments in a chain. Note that the formation of the first helical segment has a contribution from both the nucleation barrier (σ) and the formation of the first stabilizing interaction (s). The statistical weight for a particular microstate is then $e^{-E_i/k_B T} = s^{n_H} \sigma^{\nu_H}$. Since ΔG_{nuc} will be large and positive, $\sigma \ll 1$. Also, we take $s > 1$, and the presence of cooperativity will mainly hinge on $\sigma \ll s$.

✓ Example

A 35 segment chain has $2^{35} = 3.4 \times 10^{10}$ possible configurations. This particular microstate has fifteen helical segments ($n_H = 16$) partitioned into three helical stretches ($\nu_H = 3$):



We ignore all C 's since the C state is the ground state and their statistical weight is 1.

$$e^{-E_i/k_B T} = s^{n_H} \sigma^{\nu_H} = s^{16} \sigma^3$$

Now the partition function involves a sum over all possible helical segments and stretches:

$$q_{\text{conf}}(n) = \sum_{n_H=0}^n \sum_{\nu_H=0}^{\nu_{\text{max}}} g(n, n_H, \nu_H) s^{n_H} \sigma^{\nu_H} \quad (18.1.14)$$

Since the all-coil state ($n_H = 0$) is the reference state, it contributes a value of 1 to the partition function (the leading term in the summation). Therefore, the probability of observing the all-coil state is

$$P(n, n_H = 0) = q_{\text{conf}}^{-1} \quad (18.1.15)$$

From eq. (18.1.15), the mean number of helical residues is

$$\langle n_H \rangle = \frac{1}{q_{\text{conf}}} \sum_{n_H=0}^n \sum_{\nu_H=0}^{\nu_{\text{max}}} n_H g(n, n_H, \nu_H) s^{n_H} \sigma^{\nu_H}$$

In these equations, ν_{max} refers to the maximum number of helical stretches for a given n_H , $n_H/2$ for even n_H and $(n_H/2) + 1$ for odd n_H .

Zipper model

As a next step, we examine what happens with the simplifying assumption that one helical stretch is allowed. This is the single stretch approximation or the zipper model, in which conversion to a helix proceeds quickly once a single turn has been nucleated. This is reasonable for short chains in which two stretches are unlikely due to steric constraints. For the single stretch case, we only need to account for $\nu_H = 0$ and 1. For $\nu_H = 0$ the system is all coil ($n_H = 0$) and there is only one microstate to count, $g(n, 0, 0) = 1$. For a single helical stretch we need to account for the number of ways of positioning a single helical stretch of n_H residues on a chain of length n : $g(n, n_H, 1) = n - n_H + 1$. Then the partition function, eq. (18.1.15), is

$$q_{\text{zip}}(n) = 1 + \sigma \sum_{n_H=1}^n (n - n_H + 1) s^{n_H} \quad (18.1.16)$$

We can evaluate these sums using the relations

$$\begin{aligned} \sum_{n_H=1}^n &= \frac{s^{n+1} - s}{s - 1} \\ \sum_{n_H=1}^n n_H s^{n_H} &= \frac{s}{(s-1)^2} [ns^{n+1} - (n+1)s^n + 1] \end{aligned}$$

which leads to

$$q_{\text{zip}}(n) = 1 + \frac{\sigma s^2}{(s-1)^2} \left(s^n + \frac{n}{s} - (n+1) \right)$$

Following the general expression in eq. (18.1.6), and counting the degeneracy of ways to place a stretch of n_H segments, the probability distribution of helical segments is

$$P_H(n, n_H) = \frac{(n - n_H + 1) \sigma s^{n_H}}{q_{\text{conf}}} \quad 1 \leq n_H \leq n \quad (18.1.17)$$

This expression does not apply to the case $n_H = 0$, for which we turn to eq. (18.1.16). The helical fraction is obtained from $\theta_H = \frac{s}{n} (\partial \ln q_{\text{zip}} / \partial s)$:

$$\theta_H = \frac{\sigma s}{(s-1)^3} \left(\frac{ns^{n+2} - (n+2)s^{n+1} + (n+2)s - n}{n\{1 + (\sigma s / (s-1)^2)(s^{n+1} + n - (n+1)s)\}} \right)$$

Multiple stretches

Expressions for the full partition function of chains with length n , eq. (18.1.15), can be evaluated for one-dimensional models that account for nearest neighbor interactions (Ising model) using an approach based on a statistical weight matrix, \mathbf{M} . You can show that the Zimm–Bragg partition function can be written as a product of matrices of the form

$$\begin{aligned} q_{\text{conf}}(n) &= \begin{pmatrix} 1 & 0 \end{pmatrix} \mathbf{M}^n \begin{pmatrix} 1 \\ 1 \end{pmatrix} \\ \mathbf{M} &= \begin{pmatrix} 1 & \sigma s \\ 1 & s \end{pmatrix} \end{aligned}$$

Each matrix represents possible configurations of two adjoining partners, and \mathbf{M} raised to the n th power gives all configurations for a chain of length n . This form also indicates that we can obtain a closed form for q_{conf} from the eigenvalues of \mathbf{M} raised to the n th power. If \mathbf{T} is the transformation that diagonalizes \mathbf{M} , $\mathbf{A} = \mathbf{T}^{-1} \mathbf{M} \mathbf{T}$, then $\mathbf{M}^n = \mathbf{T} \mathbf{A}^n \mathbf{T}^{-1}$. This approach allows us to write

$$q_{\text{conf}} = \tilde{\lambda}^{-1} (\lambda_+^{n+1} (1 - \lambda_-) - \lambda_-^{n+1} (1 - \lambda_+))$$

with

$$\begin{aligned} \lambda_{\pm} &= \frac{1}{2} \left((1-s) \pm \tilde{\lambda} \right) \\ \tilde{\lambda} &= \lambda_+ - \lambda_- = ((1-s)^2 + 4\sigma s)^{-1/2} \end{aligned}$$

and the fractional helicity is obtained from

$$\theta_H = \frac{\langle n_H \rangle}{n} = \frac{s}{n} \frac{\partial \ln q_{\text{conf}}}{\partial s} \quad (18.1.18)$$

Simplifying these expressions for the limit of long chains ($n \rightarrow \infty, \lambda_+^{n+1} \gg \lambda_-^{n+1}$), one finds

$$q_{\text{conf}} \approx \left(\frac{1 + s + \lambda}{2} \right)^n$$

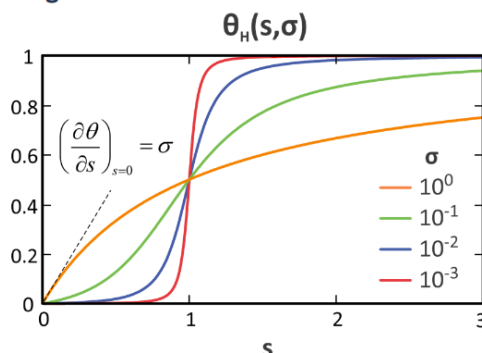
and

$$\theta_H = s \left(\frac{1 + \frac{1}{\lambda}(s - 1 + 2\sigma)}{1 + s + \lambda} \right) \quad (18.1.19)$$

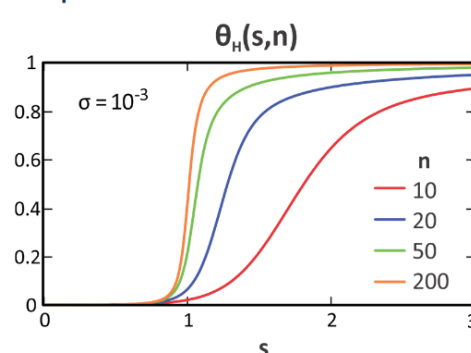
Note that when you set $\sigma = 1$, you recover the noncooperative expression, eq. (18.1.11). When $s \rightarrow 1$, $\theta_H \rightarrow 0.5$.

Below, we examine the transition behavior in the large n limit from eq. (18.1.20) as a function of the cooperativity parameter σ . We note that a sharp transition between an ensemble that is mostly coil to one that is mostly helix occurs near $s = 1$, the point where these states exist with equal probability. When the $C \rightleftharpoons H$ equilibrium shifts slightly to favor H (s slightly greater than 1), most of the sample quickly converts to helical form. When the equilibrium shifts slightly toward C , most of the sample follows. As σ decreases, the steepness of this transition grows as $(d\theta/ds)_{s=1} = 1/4\sigma^{1/2}$. Therefore, we conclude that highly cooperative transitions will have $s \approx 1$ and $\sigma \ll s$. In practice for polypeptides, we find that σ/s lies between 5×10^{-3} and 5×10^{-5} .

Large n limit:



n dependence:



Next, we explore the chain-length dependence for finite chains. We find that the cooperativity of this transition, observed through the steepness of the curve at $\theta_H = 0.5$ increases with n . We also observe that the observed midpoint ($\theta_H = 0.5$) lies at $s > 1$, where the single linkage equilibrium favors the H form. This reflects the constraints on the length of helical stretches available a given chain.

Temperature Dependence

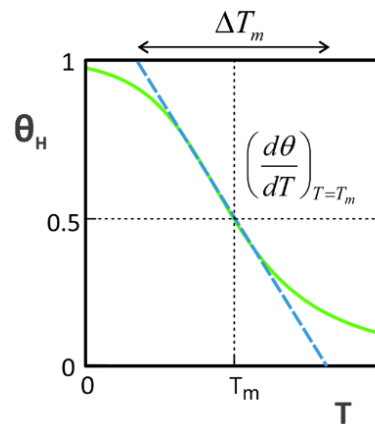
Now let's describe the temperature dependence of the cooperative model. The helix-coil transition shows a cooperative melting transition, where heating the sample a few degrees causes a dramatic change from a sample that is primarily in the C form to one that is primarily H . Multiple temperature-dependent factors make this a bit difficult to deal with analytically, therefore we focus on the behavior at the melting temperature T_m , which we define as the point where $\theta_H(T_m) = 0.5$.

Look at the slope of θ at T_m . From chain rule:

$$\frac{d\theta}{dT} = \frac{d\theta}{ds} \cdot \frac{ds}{dT} = \frac{d\theta}{ds} \cdot s \frac{d \ln s}{dT}$$

Since we interpret s as an equilibrium constant for the addition of one helical residue to a stretch, we can write a van't Hoff relation

$$\frac{d \ln s}{dT} = \frac{\Delta H_{HC}^0}{k_B T^2}$$



Note that this relation assumes that ΔH_0 is independent of temperature, which generally is a concern, but we will not worry too much since we are just evaluating this at T_M . Next we focus our discussion on the high n limit. From the Zimm–Bragg model:

$$\left(\frac{d\theta}{ds}\right)_{s=1} = \frac{1}{4\sigma^{1/2}}$$

Then, we set $s(T_m) = 1$, and combine these results to give the slope of the melting curve at T_m :

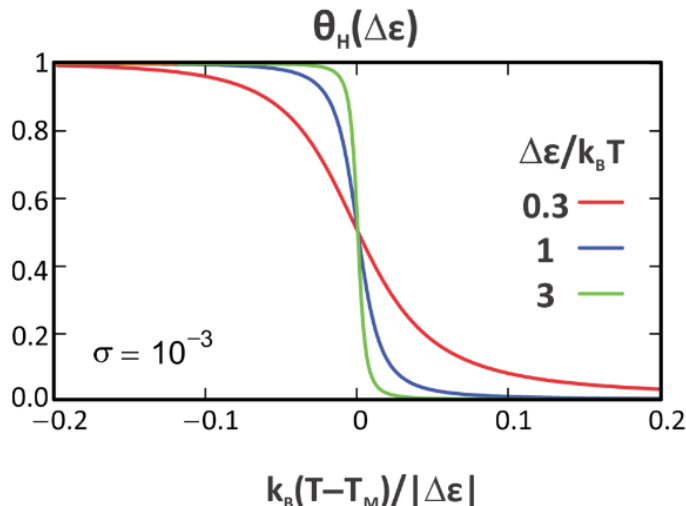
$$\left(\frac{d\theta}{dT}\right)_{T=T_m} = \frac{\Delta H_{HC}^0}{4\sigma^{1/2}k_B T_m^2}$$

The slope of θ at T_m has units of inverse temperature, so we can also express this as a transition width: $\Delta T_m = (d\theta/dT)_{T_m}^{-1}$.

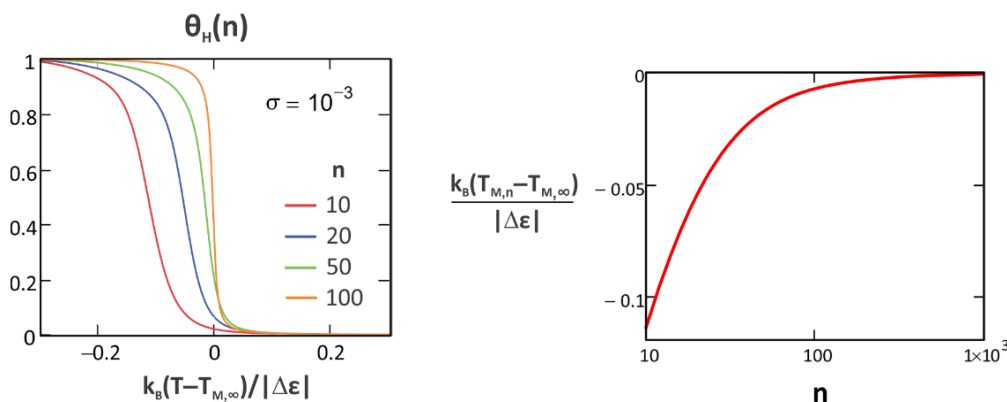
Keep in mind this van't Hoff analysis comes with some real limitations when applied to experimental data. It does not account for the finite size of the system, which we have seen shifts $s(T_m)$ to be > 1 , and the knowledge of parameters at T_m does not necessarily translate to other temperatures. To the extent that you can apply the assumptions, the van't Hoff expression can also be used to predict the helical fraction as a function of temperature in the vicinity of T_M using

$$\ln s = \frac{\Delta H_{HC}^0}{k_B} \left(\frac{1}{T_M} - \frac{1}{T} \right) \quad (18.1.20)$$

and assuming that σ is independent of temperature.



Below we show the length dependence of the melting temperature. As the length of the chain approaches infinite, the helix/coil transition becomes a step function in temperature. This trend matches the expectations for a phase transition: in the thermodynamic limit, the infinite system, will show discontinuous behavior. For finite lengths, the melting temperature T_m is lower than that for the infinite chain (T_m, ∞), but approaches this value for $n > 300$.



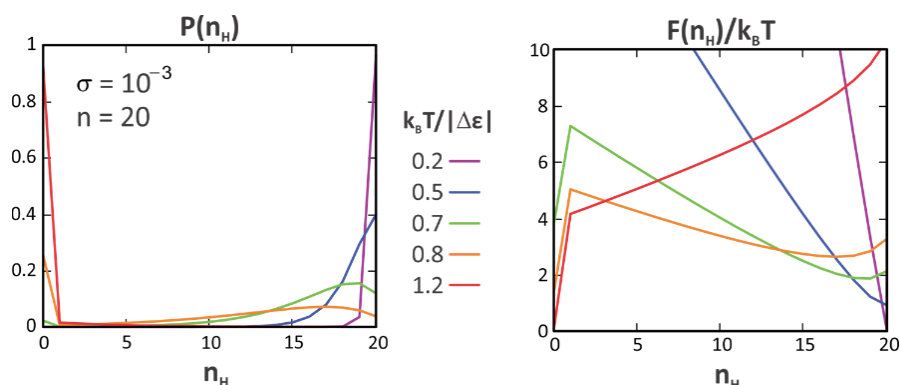
Calorimetric parameters for polypeptide chains

Side-chain only has a small effect on the helix–coil propagation parameter:

Sample	ΔH_{HC}^0 (mol^{-1} residue $^{-1}$)	σ	Other
Alanine-rich peptides, Ac-Y(AEAAKA)8F-NH ₂ , Ac-(AAKAA)kY-NH ₂	−0.95 to −1.3 kcal	0.002	
Poly(L-lysine), Poly(L-glutamate)	−1.1 kcal	0.0025	
Poly-alanine	−0.95 kcal	0.003	$s(0^\circ\text{C}) = 1.35$
Alanine oligomers	−0.85 kcal		$\Delta S^0 = 3 \text{ cal mol}^{-1} \text{ K}^{-1} \text{ residue}^{-1}$
Various homopolypeptides	$\sim 4 \text{ kJ}$		$\Delta C_P = -32 \text{ J mol}^{-1} \text{ K}^{-1} \text{ residue}^{-1}$

Free-Energy Landscape

Finally, we investigate the free-energy landscape for the Zimm–Bragg model of the helix–coil transition. The figure below shows the helical probability distribution and corresponding energy landscape for different values of the reduced temperature $k_B T / \Delta \epsilon$ for a chain length of $n = 40$ and $\sigma = 10^{-3}$. Note that $P(n_H)$ is calculated from eq. (18.1.18) for all but the all-coil state, which comes from eq. (18.1.16).



The cooperative model shows two-state behavior. At low temperature and high temperature, the system is almost entirely in the all-helix or all-coil configuration, respectively; however, at intermediate temperatures, the distribution of helical configurations can be very broad. The least probable configuration is a chain with only one helical segment.

This behavior looks much closer to the two-state behavior expected from phase-transition behavior. The free energy has minima for $n_H = 0$ and for $n_H > 1$, and the free energy difference between these states shifts with temperature to favor one or the other minimum.

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