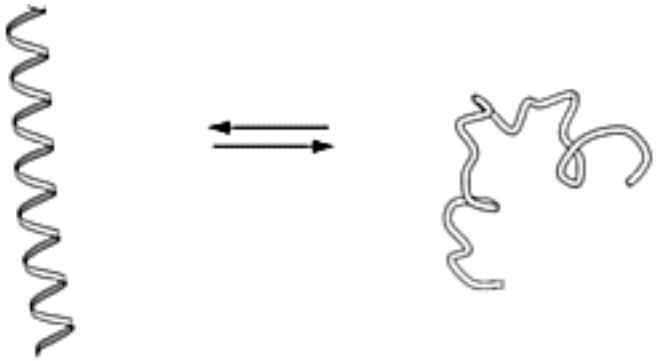
OAK RIDGE National Laboratory

Center for Molecular Biophysics

Theory of Helix-Coil Transition



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Aim

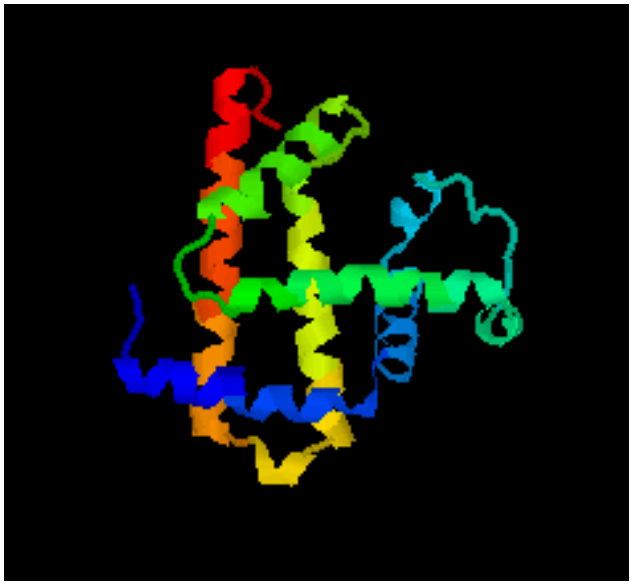
- Introduce simplest theory of helix-coil transition of short polypeptides
- Demonstrate one example of “pen and paper” theoretical physics

Outline

- State the problem
- Recap of alpha-helix and random coil
- Statistical Mechanics applied to this problem
- Derive the model, examine number of h-bonds
- Experimental investigation

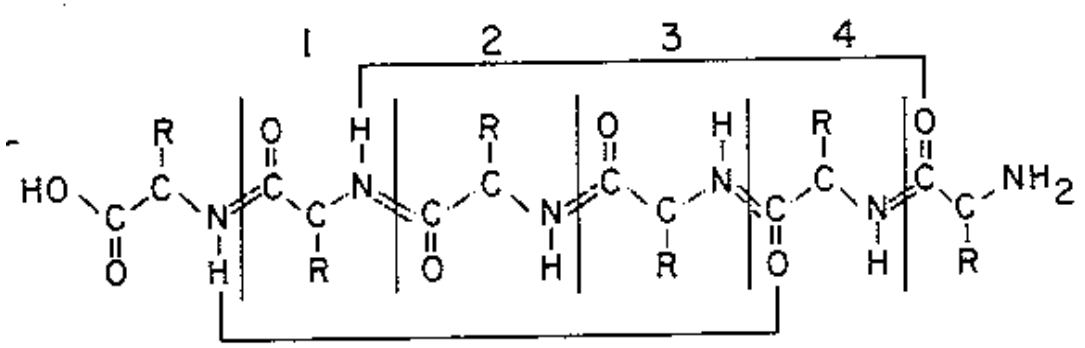
Statement of Problem

- N linked amino acids, called residues
- Each residue can exist in only two states:
- helical (h)
- coil (c)
- experiments:
  - degree of helicity varies between different polypeptides
  - Increase temperature (T): number of units in helical state decreases
- Theory predicts average number of units in h state
- Do it by counting h-bonds in the polypeptide backbone



Recap of helices and coils

α-helix (Pauling '51)

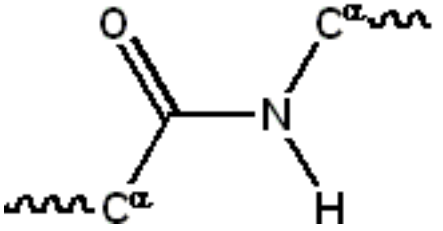


h-bond:

- N-H i^{th} residue
- C=O $(i+4)^{th}$ residue
- spans 3 residues
- restricts their conformations

amide group:

- rigid planar structure
- freedom of rotation of alpha-C



→ define helical residue:
dihedral angles of Cα characteristic of helix (Cα-N-C-Cα = 97)

→ define coil residue: dihedral angles not these of helix

N+2 amino acids
N residues: amino-acids that *can* be in h state (2 at end rotate freely, ignored)
maximum number of h-bonds possible = N-2

Thermodynamics of Transition

single residue transition from coil to helix
initial approach, to check if helix forms or not

use Gibb's free energy change ΔG: $\Delta G = \Delta H - T\Delta S$

free energy enthalpy entropy

$\Delta G < 0$ helix favoured
$\Delta G > 0$ helix <u>not</u> favoured

two competing factors:
enthalpy $\Delta H < 0$: strong h-bond forms favors helix
entropy $\Delta S < 0$: molecule loses configurational freedom does not favour helix

increase T: effect of entropy more significant
low T : enthalpy dominates (helix)
hight T: entropy dominates (coil)
there must be a transition with temperature

Statistical Mechanics

each residue can be in either of two states: h or c

→ represent peptide as a sequence describing the states of the residues

e.g. ccccchhhhhhhhccccccchhhhhcc...

System can exist in many conformations, or "microstates"

e.g. N=10 residues: cchhhhhhcc, cccchhhhhh, ccccccccc, hhhhhcchhh

energy of microstate G{h,c} depends on sequence of h's and c's

statistical mechanics:

examine ALL states

calculate average properties of system (energy, no h-bonds etc)

Partition Function

to obtain average quantities must compute partition function Z
(sum over all microstates)

$$Z = \sum_{\{h,c\}} e^{-G\{h,c\}/k_B T}$$

probability of a given microstate {h,c} $P\{h,c\} = \frac{e^{-G\{h,c\}/k_B T}}{Z}$

once we have partition function, can compute average quantities

interested in average number of h-bonds (indicator of degree of helicity)

$$\langle n \rangle = \sum_n n\, P(n)\, g_n$$

n: number of h-bonds
P(n): probability having n h-bonds
g_n: degeneracy

degeneracy: number of states that have same number of h-bonds

e.g. N=6 n=1 ccchhh, cchhhc, chhhcc, hhhccc has g=4

(1) what is energy G{h,c} of a sequence

(2) perform the summation over all states

$$Z = \sum_{\{h,c\}} e^{-G\{h,c\}/k_B T}$$

Approximations:

• one sequence approximation: only one sequence of helical residues is allowed

cchhhhhh allowed chhhchhh not allowed

• only length of sequence matters, not its position on the chain- residues do not interact

chhhhc same as cchhhh

statistical weight of each residue " $e^{-G/kT}$ "

1 if in coil state
σs if the first helical residue
s for all other helical residues

e.g. cchhh has statistical weight 1*1*σs*s*s

σ: formation of first h (nucleation):
difficult since it offsets large decrease in entropy σ << 1
experiments σ ≈ 0.0001

s: determines if helix is favoured: depends on temp
s>1 growth of helix is probable
s<1 growth of helix is not probable
experiments: s≈1.35

Temperature Dependance

energy change per monomer upon helix formation: $\Delta G = \Delta H - T\Delta S$

ΔH and $\Delta S < 0$

s related to probability of a residue being helical
depends on ΔG

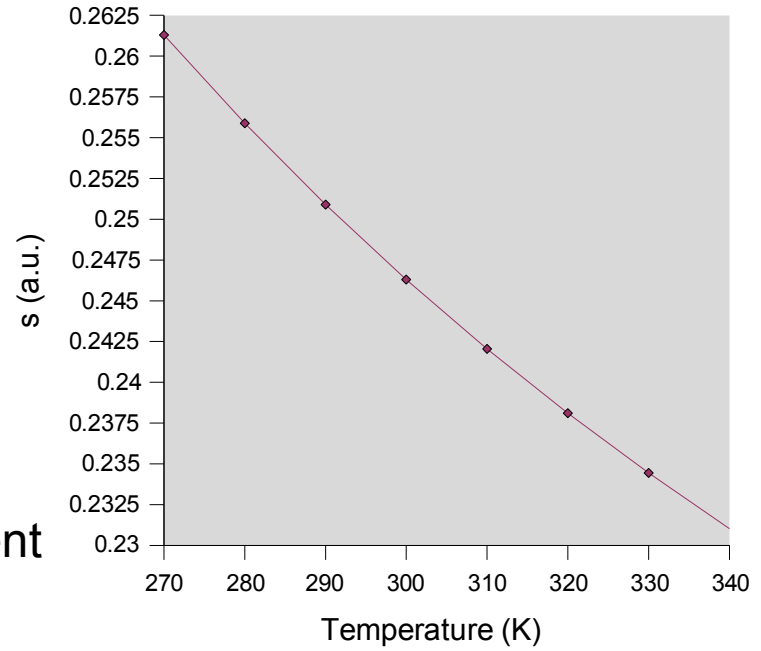
σ probability to form 1st helical residue
residues at end of chain do not form h-bonds(no enthalpy gain)
additional energy change for residue in interface helix/coil
$\Delta G_{interface} = -\Delta H$

$$s = e^{-\Delta G/kT} = e^{-\Delta H/kT + \Delta S}$$

$$\sigma = e^{-2\Delta G_{interface}/kT} = e^{2\Delta H/kT}$$

both depend on temperature, σ is much smaller

experiments: σ considered to be temp independent



Ludwig Boltzmann

Gelatine

- polypeptides (300-4000 amino acids)
- extended left-handed proline helix
- forms gels below about 35°C
- dissolve at low temperature to give 'melt in the mouth' products

N=4 example

4 redidues, possible microstates

microstate	cccc	chhh	hhhc	hhhh
stat weight	1	σs^3	σs^3	σs^4
n	0	1	1	2

partition function: $Z = 1 + 2\sigma s^3 + \sigma s^4$

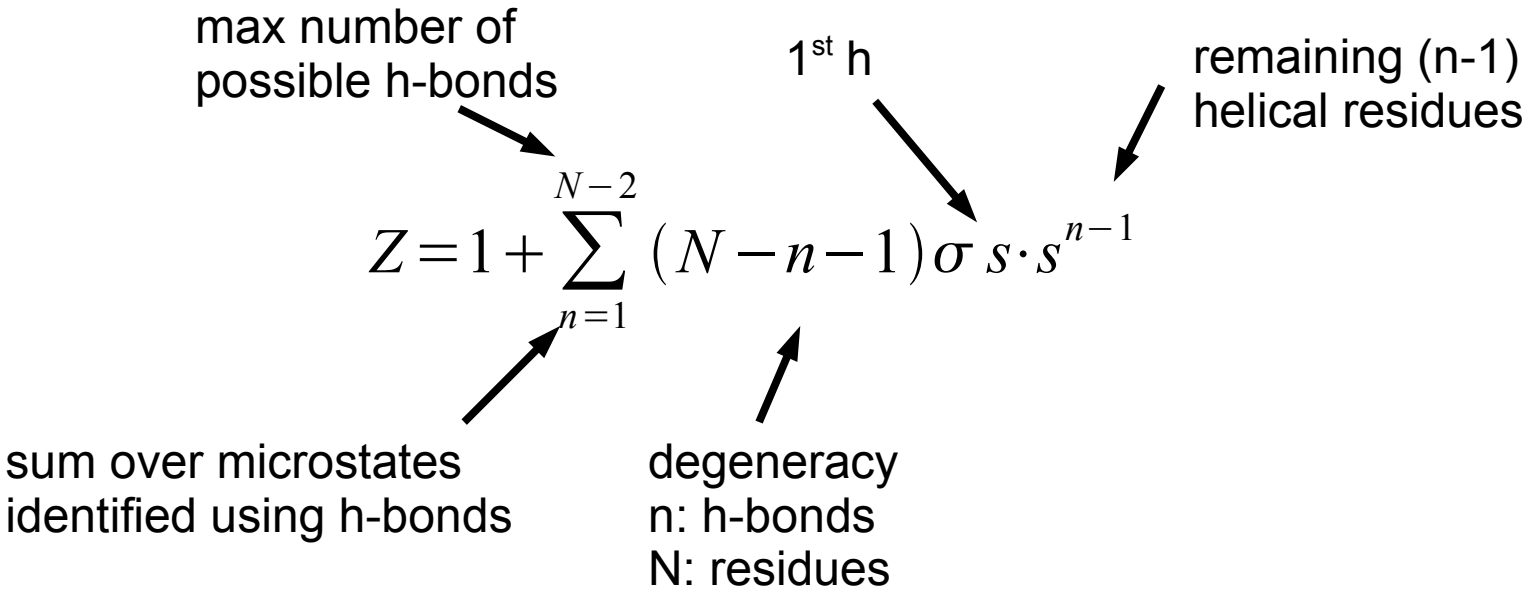
we are interested in average number of h-bonds

- measure of helicity of polypeptide
- can be measured experimentally

$$\langle n \rangle = \sum_n n\, g_n P(n) = \frac{0 + 1\times 2\times \sigma s^3 + 2\times 1\times \sigma s^4}{Z} = \frac{2\sigma s^3 + 2\sigma s^4}{1 + 2\sigma s^3 + \sigma s^4}$$

P(n) = probability to have n h-bonds (=stat weight / Z)
g_n = degeneracy

General Case: Partition function



$$Z = 1 + \sum_{n=1}^{N-2} (N - n - 1)\sigma s^{n}$$ evaluation of sums

$$Z = 1 + \sum_{n=1}^{N-2} (N-1)\sigma s^{n} - \sum_{n=1}^{N-2} n\sigma s^{n} = 1 + \frac{\sigma s}{(s-1)^{2}}\left[s^{N-1} - (N-1)s + N - 2\right]$$

General Case: h-bonds

$$\langle n \rangle = \sum_n n\, g_n P(n)$$

$$\langle n \rangle = \frac{\sum_{n=1}^{N-2} n \cdot (N-n-1) \cdot \sigma\, s^n}{1 + \sum_{n=1}^{N-2} (N-n-1)\, \sigma\, s^n}$$

evaluation of sums

$$\langle n \rangle = s \cdot \left(\frac{dZ}{ds} \right) \cdot \frac{1}{Z} = \frac{\frac{\sigma s}{(s-1)^3}\left[(N-2)s^N - N s^{N-1} + Ns - (N-2) \right]}{1 + \frac{\sigma s}{(s-1)^2}\left[s^{N-1} - (N-1)s + N - 2 \right]}$$

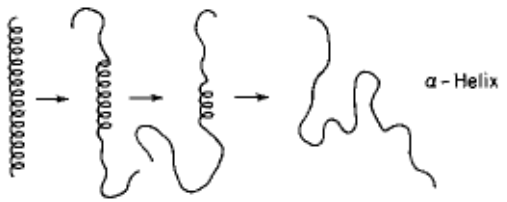
better (= experiments can measure easily) to get fractional helicity

$$\theta = \frac{\langle n \rangle}{(N-2)}$$

number between 0 and 1

max number of possible h-bonds

Approximations Revisited



- residue is either in h or c state
  proteins: coil or α-helix or β-sheet

- residues do not interact
  residues are the same, or very weak interaction of side groups

- one sequence approximation
  short peptide
  entropy penalty too costly to form two short helices

Experiments on helix-coil transition

aim:
- test previous theory
- derive values for parameters s and σ

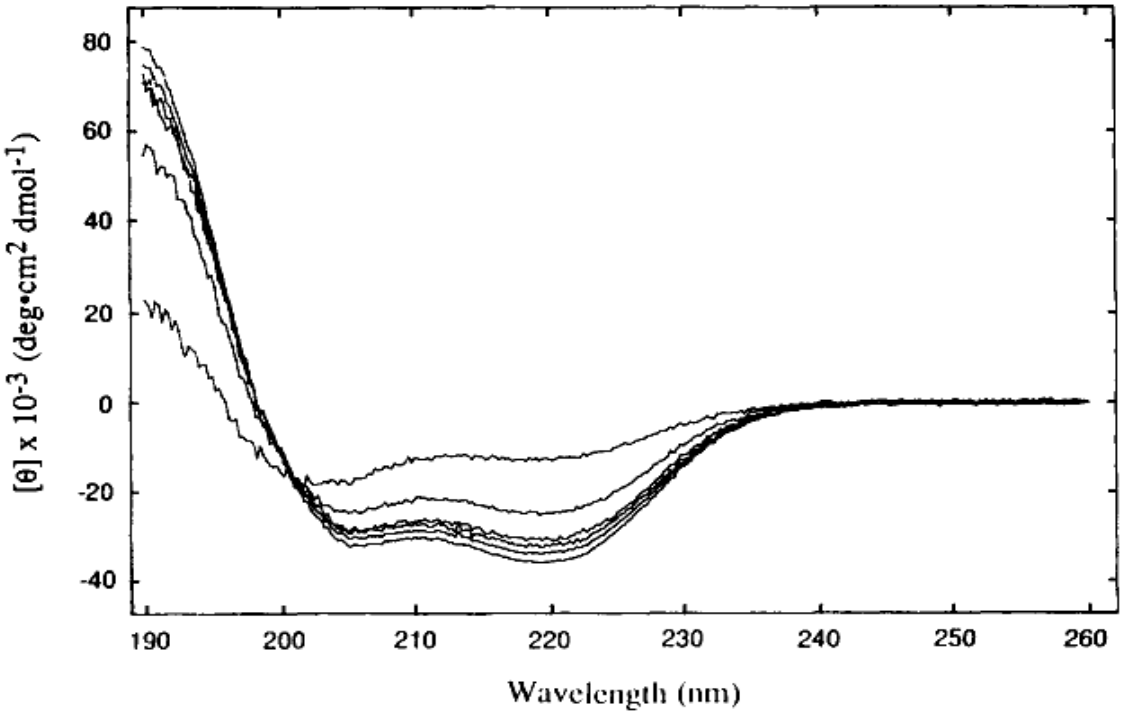
difficulty: helix-forming amino acids (Ala, Met) yield peptides that are not soluble in water

alaline-based short peptides: Ac-Y-(A-E-A-A-K-A)$_n$-F-NH$_2$ n=2-8

avoid side-chain interactions

use circular dichroism
to identify helix :
min at 222 and 208 nm
max at 190

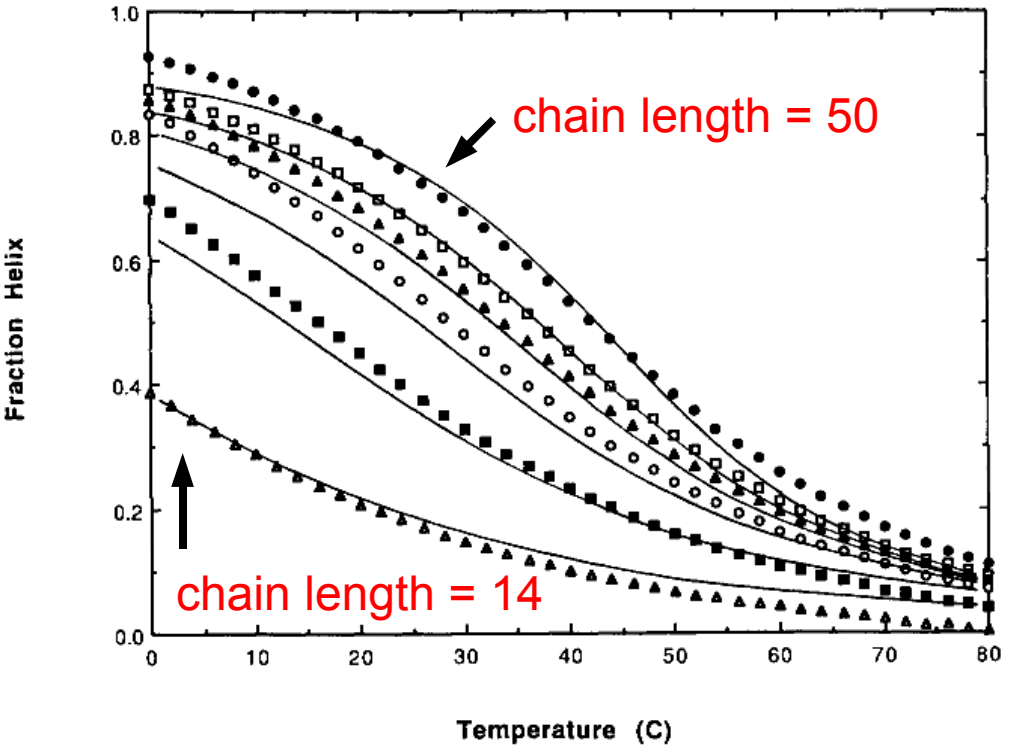
examine how min at 222
varies with T



Transition

fit parameters s,σ and
ΔH from calorimetry

x^a	$s(0°C)$	σ	ΔH^0 (cal/res)	Error[b]
0	1.33	0.0029	−930	0.87
1	1.35	0.0028	−955	0.30
2.5	1.35	0.0033	−955	0.51
3	1.37	0.0027	−985	0.91
Mean	1.35 ± 0.02	0.0029 ± 0.0003	−960 ± 20	



assume

σ temperature independent

s: -kT ln s = ΔG

Recap

Statistical Mechanics theory of helix-coil
- residue either h or c
- no interaction between residues
- one sequence approximation
- nucleation and growth approach

Evaluate
- partition function
- average number of h-bonds

Experiments
- CD to find helical fraction
- good agreement with theory
- fit data to obtain parameters σ and s