pHLIP: pH - Low - Insertion Peptide

- Tryptophan fluorescent spectroscopy
- Circular dichroism spectroscopy (CD, OCD)

![Diagram showing the sequence of amino acids with flanking and trans-membrane regions and the effects of pH on membrane interaction.](image-url)
Medical Applications

- Diagnostic agent
  - Cargo $\rightarrow$ Marker

- Therapeutic agent
  - Cargo $\rightarrow$ Drug

1. Add lipids at neutral pH
2. Drop pH
Amino acids linked into polymer. 
Backbone → periodic. Side chains → aperiodic. 
Residues $n$ and $n+1$ coupled by peptide bond.

$$\cdots \quad \text{backbone} \quad \cdots$$

$$\begin{array}{c}
\text{NH} \quad \text{CH} \quad \text{CO} \\
| \\
R_n \quad | \\
| \\
R_{n+1}
\end{array}$$

\(\alpha\)-helix stabilized by internal H-bonds (~ \(9k_B T\)).

$$\begin{array}{ccccccccccccccc}
N \quad & H \quad & O \quad & H \\
| \\
R_n \\
| \\
R_{n+1} \\
| \\
R_{n+2} \\
| \\
R_{n+3} \\
| \\
R_{n+4}
\end{array}$$
Amino Acid Residues

Length per residue: \( \ell_e \simeq 4\text{Å} \) (extended), \( \ell_h \simeq 1.5\text{Å} \) (helical).
Conformation with lower free energy is realized.

\[ \Delta G = G_{\text{coil}} - G_{\text{helix}} = \Delta H - T\Delta S. \]

\( \Delta H \): enthalpic contribution, \( T\Delta S \): entropic contribution.

non-polar environment: \( |\Delta H| \gg |T\Delta S| \)

polar environment: \( |\Delta H| \lesssim |T\Delta S| \)
**Generalized Pauli principle**  [Haldane 1991]

How is the number of states accessible to one particle of species $m$ affected if particles (of any species $m'$) are added?

$$\Delta d_m \doteq - \sum_{m'} g_{mm'} \Delta N_{m'} \quad \Rightarrow \quad d_m = A_m - \sum_{m'} g_{mm'} (N_{m'} - \delta_{mm'})$$

**Energy and multiplicity of many-body states**

$$E(\{N_m\}) = E_{pv} + \sum_{m=1}^{M} N_m \epsilon_m, \quad W(\{N_m\}) = \prod_{m=1}^{M} \frac{d_m + N_m - 1}{N_m} \frac{\Gamma(d_m + N_m)}{\Gamma(N_m + 1) \Gamma(d_m)}$$

- $E_{pv}$: energy of reference state
- $N_m$: number of particles from species $m$
- $\epsilon_m$: particle activation energies
- $g_{mm'}$: statistical interaction coefficients
- $A_m$: capacity constants
- $d_m$: number of open slots for a particle of species $m$
Thermodynamics with Statistical Interaction

System specifications:

- particle energies $\epsilon_m$
- statistical interaction coefficients $g_{mm'}$
- capacity constants $A_m$

Two tasks:

- combinatorial problem: $W(\{N_m\})$
- extremum problem: $\delta(U - TS - \mu N) = 0$

Partition function [Wu 1994]:

$$Z = \sum_{\{N_m\}} W(\{N_m\}) e^{-\beta E(\{N_m\})} = \prod_m \left( \frac{1 + w_m}{w_m} \right)^{A_m}$$

$$e^{\epsilon_m/k_BT} = (1 + w_m) \prod_{m'=1}^{M} \left( 1 + \frac{1}{w_{m'}} \right)^{-g_{m'm}}, \quad m = 1, \ldots, M.$$

Average number of particles:

$$w_m \langle N_m \rangle + \sum_{m'} g_{mm'} \langle N_{m'} \rangle = A_m, \quad m = 1, \ldots, M$$

Configurational entropy [Isakov 1994]:

$$S(\{N_m\}) = k_B \sum_{m=1}^{M} \left[ (N_m + Y_m) \ln (N_m + Y_m) - N_m \ln N_m - Y_m \ln Y_m \right]$$

$$Y_m \doteq A_m - \sum_{m'=1}^{M} g_{mm'} N_{m'}$$
### Specifications for combinatorics and energetics \((\mu = 3)\):

<table>
<thead>
<tr>
<th>motif</th>
<th>cat.</th>
<th>(m)</th>
<th>(\epsilon_m)</th>
<th>(A_m)</th>
<th>(g_{mm'})</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>h1h</td>
<td>host</td>
<td>1</td>
<td>(\epsilon_n)</td>
<td>(N - 2)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>121</td>
<td>hybrid</td>
<td>2</td>
<td>2(\epsilon_g)</td>
<td>0</td>
<td>2</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>232</td>
<td>hybrid</td>
<td>3</td>
<td>2(\epsilon_g)</td>
<td>0</td>
<td>3</td>
<td>-0</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>tag</td>
<td>4</td>
<td>(\epsilon_g)</td>
<td>0</td>
<td>4</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>tag</td>
<td>5</td>
<td>(\epsilon_g)</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>tag</td>
<td>6</td>
<td>(\epsilon_g)</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- \(W(\{N_m\}) = \prod_{m=1}^{2\mu} \left( \frac{d_m + N_m - 1}{N_m} \right)\), \(d_m = A_m - \sum_{m'=1}^{2\mu} g_{mm'}(N_{m'} - \delta_{mm'})\)

- \(E(\{N_m\}) = E_{pv} + \sum_{m=1}^{2\mu} N_m \epsilon_m\)
- Length of coil segment: \( N = 16 \)
- Particle content: \( N_1 = 1, \ N_2 = 2, \ N_3 = 2, \ N_4 = 1, \ N_5 = 3, \ N_6 = 1 \)
- Energy: \( E(1, 2, 2, 1, 3, 1) - E_{pv} = \epsilon_n + 13\epsilon_g \)
- Multiplicity: \( W(1, 2, 2, 1, 3, 1) = \begin{pmatrix} 1 \\ 1 \end{pmatrix} \begin{pmatrix} 3 \\ 2 \end{pmatrix} \begin{pmatrix} 3 \\ 2 \end{pmatrix} \begin{pmatrix} 3 \\ 1 \end{pmatrix} \begin{pmatrix} 6 \\ 3 \end{pmatrix} \begin{pmatrix} 2 \\ 1 \end{pmatrix} = 360 \)
Parameters and Processes

- Nucleation parameter: \( \tau = e^{(\epsilon_g - \epsilon_n)/k_B T} \) \( (0 \leq \tau \leq 1) \)
- Growth parameter: \( t = e^{\epsilon_g/k_B T} \) \( (0 \leq t < \infty) \)
- Range parameter: \( \mu = 1, 2, \ldots, \infty \)
Mathematical Model (4)

Polynomial equation for solution of order $\mu + 1$ for $w = w_{\mu+1}(t, \tau)$:

$$(1 + w_{\mu+1} - t)S_\mu(w_{\mu+1}) = t\tau S_\mu-1(w_{\mu+1})$$

$$w_1 = \frac{S_\mu(w)}{\tau S_\mu-1(w)} = \frac{t}{1 + w - t}, \quad w_m = \left\{ \begin{array}{ll}
\frac{S_{\mu-m+2}(w)}{S_{\mu-m}(w)} & : m = 2, \ldots, \mu \\
\frac{w}{w} & : m = \mu + 2, \ldots, 2\mu
\end{array} \right.$$  

Chebyshev polynomials of the 2\textsuperscript{nd} kind:

$$S_0(w) = 1, \quad S_1(w) = w, \quad S_{\mu+1}(w) = wS_\mu(w) - S_{\mu-1}(w)$$

Introduce $r_\mu(w) = \frac{S_\mu(w)}{S_{\mu-1}(w)} = \left\{ \begin{array}{ll}
\frac{1}{2} \left[w + \sqrt{4 - w^2}\right] \cot \left(\mu \arccos \frac{w}{2}\right) & : w < 2, \\
\frac{\mu + 1}{\mu} & : w = 2, \\
\frac{1}{2} \left[w + \sqrt{w^2 - 4}\right] \coth \left(\mu \text{Arcosh} \frac{w}{2}\right) & : w > 2
\end{array} \right.$$

Physical solution from $(w + 1 - t)r_\mu(w) - t\tau = 0$
Physical Quantities of Interest

- Free energy: \( \bar{G}(t, \tau) = -k_B T \ln (1 + w_1^{-1}) \)

- Entropy: \( \bar{S}(t, \tau) = -\left( \frac{\partial \bar{G}}{\partial T} \right)_{\epsilon_n, \epsilon_g} \)

- Enthalpy \( \bar{H}(t, \tau) = \bar{G} + T \bar{S} \)

- Helicity (order parameter): \( \bar{N}_{hl}(t, \tau) = 1 - \left( \frac{\partial \bar{G}}{\partial \epsilon_n} \right)_{T, \epsilon_g} - \left( \frac{\partial \bar{G}}{\partial \epsilon_g} \right)_{T, \epsilon_n} \)

- Density of (helix or coil) segments: \( \bar{N}_{seg}(t, \tau) = \left( \frac{\partial \bar{G}}{\partial \epsilon_n} \right)_{T, \epsilon_g} \)

- Average size of helix segments: \( L_{hs}(t, \tau) = \frac{\bar{N}_{hl}}{\bar{N}_{seg}} \)

- Average size of coil segments: \( L_{cs}(t, \tau) = \frac{1 - \bar{N}_{hl}}{\bar{N}_{seg}} \)
Structure of Solution (1)

Crossover: \( \tau > 0 \) and \( \mu < \infty \)

- \( w_0 = 2 \cos \left( \frac{\pi}{\mu + 1} \right) \), \( w_{as} = t + \tau - 1 \)
- \( w = \frac{1}{2} \left[ t - 1 + \sqrt{(t - 1)^2 + 4t\tau} \right] \) \( (\mu = 1) \)

First-order transition: \( \lim \tau \to 0 \) at \( \mu < \infty \)

- Transition point: \( t_0 = 1 + 2 \cos \left( \frac{\pi}{\mu + 1} \right) \)
- Solution: \( w = \begin{cases} t_0 - 1 & : t \leq t_0 \\ t - 1 & : t \geq t_0 \end{cases} \)
- Conformation:
  - pure coil at \( t < t_0 \)
  - pure helix at \( t > t_0 \)
Second-order transition: \( \mu \to \infty \) at \( \tau > 0 \)

- Transition point: \( t_c = \frac{3}{1 + \tau} \)
- Asymptotics for \( t \ll 1 \): \( w = t + \tau - 1 \)
- Solution: \( w = \begin{cases} 
2 & : 0 \leq t \leq t_c \\
\frac{t + \tau}{\lambda} & : t > t_c 
\end{cases} \)
  - \( \lambda = \frac{1}{2} \left[ t - 1 + \sqrt{(t + 1)(t - 3) + 4t\tau} \right] \)
- Conformation:
  - almost pure coil at \( t < t_c \)
  - mixed coil/helix at \( t > t_c \)
Helicity and Entropy (1)

Nucleation parameter: $\tau = 1, 0.25, 0.05, 0.0025, 0$
Helicity and Entropy (2)

Range parameter: $\mu = 2, 3, 4, 9, \infty$
Helix Segments and Coil Segments (1)

Average number

\[
\bar{N}_{\text{seg}} = \mu
\]

Average length

\[
L_{cs, lhs} = \mu
\]

Nucleation parameter: \( \tau = 1, 0.25, 0.05, [0.0025, 0] \)
Helix Segments and Coil Segments (2)

Average number

\[
\bar{N}_{\text{seg}}(t) = a \tau^t \quad \text{for } \tau = 1.0
\]

\[
\bar{N}_{\text{seg}}(t) = b \tau^t \quad \text{for } \tau = 0.2
\]

Average length

\[
L_{\text{ss}}, L_{\text{hs}}(t) = a \tau^t \quad \text{for } \tau = 1.0
\]

\[
L_{\text{ss}}, L_{\text{hs}}(t) = b \tau^t \quad \text{for } \tau = 0.2
\]

Range parameter: \( \mu = 2, 3, 4, 9, \infty \)
Heat Capacity and Latent Heat

Heat capacity:

\[ \tau = 0.05, 0.025, 0.01, 0.005 \]

Latent heat:

- in the limit \( \tau \to 0 \): \( T \Delta \bar{S} = \varepsilon_g \)
Outlook (1): Working Hypothesis

pHLIP variants with single permutations:

\[
\begin{align*}
\text{Tryptophan} & : \quad \text{ADNNPF} \text{IWYARYADLTT} \text{FPPLLDDD} \\
\text{Proline} & : \quad \text{ADNNPF} \text{IPYARYADLTT} \text{WILLDDD} \\
\text{Arginine} & : \quad \text{ADNNPF} \text{IYAYRADLTTFPLLWD} \\
\end{align*}
\]
Outlook (2): Membrane Environment

A

[MacCallum et al. 2008]

B

28/11/2014 [philip47 – 23/26]
Outlook (3): Profiles and Landscapes

\[ N_{th}(x) \]

(a) helicity

\[ \overline{G}_H(x)/k_BT \]

(b) free energy

\[ N_{hl}(\rho), \overline{G}_H(\rho) \]

(c) $N_R = 47$

\[ N_{hl}(\rho), \overline{G}_H(\rho) \]

$N_R = 35$

\[ N_{hl}(\rho), \overline{G}_H(\rho) \]

$N_R = 23$

28/11/2014 [phlip48 – 24/26]
Possible scenario for initiation of insertion:

- at pH 8 the two forces are balanced (on average) with $F_{\text{in}} + F_{\text{out}}$ acting as restoring force,
- drop to pH 4 enhances probability of protonation of Asp residues,
- force imbalance causes peptide to move toward membrane interior (first time scale),
- movement slows down rates of protonation and deprotonation (second time scale),
- comparison of time scales suggest instability that initiates insertion.
Outlook (5): Experiment and Theory in Tandem

Flow chart relating forthcoming experimental evidence to features of theoretical modeling

**Experiment**
- Apparent rates; Activation energies
  - Fluorescence/CD kinetics with single-Trp pHLIP variants at different temp.
- Structural intermediates: Pro-kink
  - Fluorescence kinetics of insertion and exit with single-Trp pHLIP variants
- Role of Arg on stability and exit
  - Fluorescence, CD, OCD steady-state 3 states, fluorescence titration with pHLIP-W30-R11 and pHLIP-W30-R15. Fluorescence kinetic exit
- Protonation of Asp; Role of H2O
  - Fluorescence kinetics with single-Trp pHLIP variants at low and high lipid:peptide ratios
- Lipid bilayer properties
  - SAXS kinetics studies for low and high lipid:peptide ratios and in the absence and presence of cholesterol
- Fluorescence/CD kinetics

**Theory**
- Rates of Asp protonation/deprotonation
- Balance of forces: $F_{in}$ and $F_{out}$
- Insertion/folding; exit/unfolding
- Landscapes of free energy
- Profiles of free energy, enthalpy, entropy, helicity
- Membrane: $\rho(x), \ell(x)$
- Coil-helix transition: $\ell, \tau, \mu$