Coil-Helix Transformation of Polypeptide at Water-Lipid Interface

Gerhard Müller
Ganga Sharma
Yana Reshetnyak
Oleg Andreev
University of Rhode Island

Michael Karbach
Bergische Universität Wuppertal

pHLIP: pH - Low - Insertion Peptide

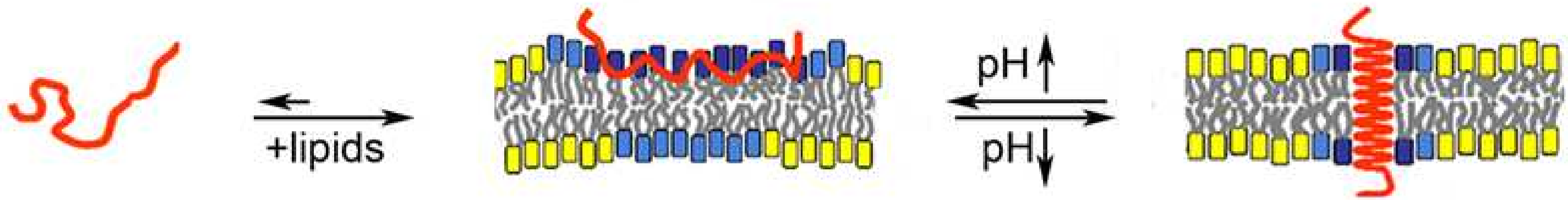
N terminus

+ (R), − (D), − (D), − (D), − (D), − (E)

A E Q N P I Y W A R Y A D W L F T T P L L L L D L A L L V D A D E G T

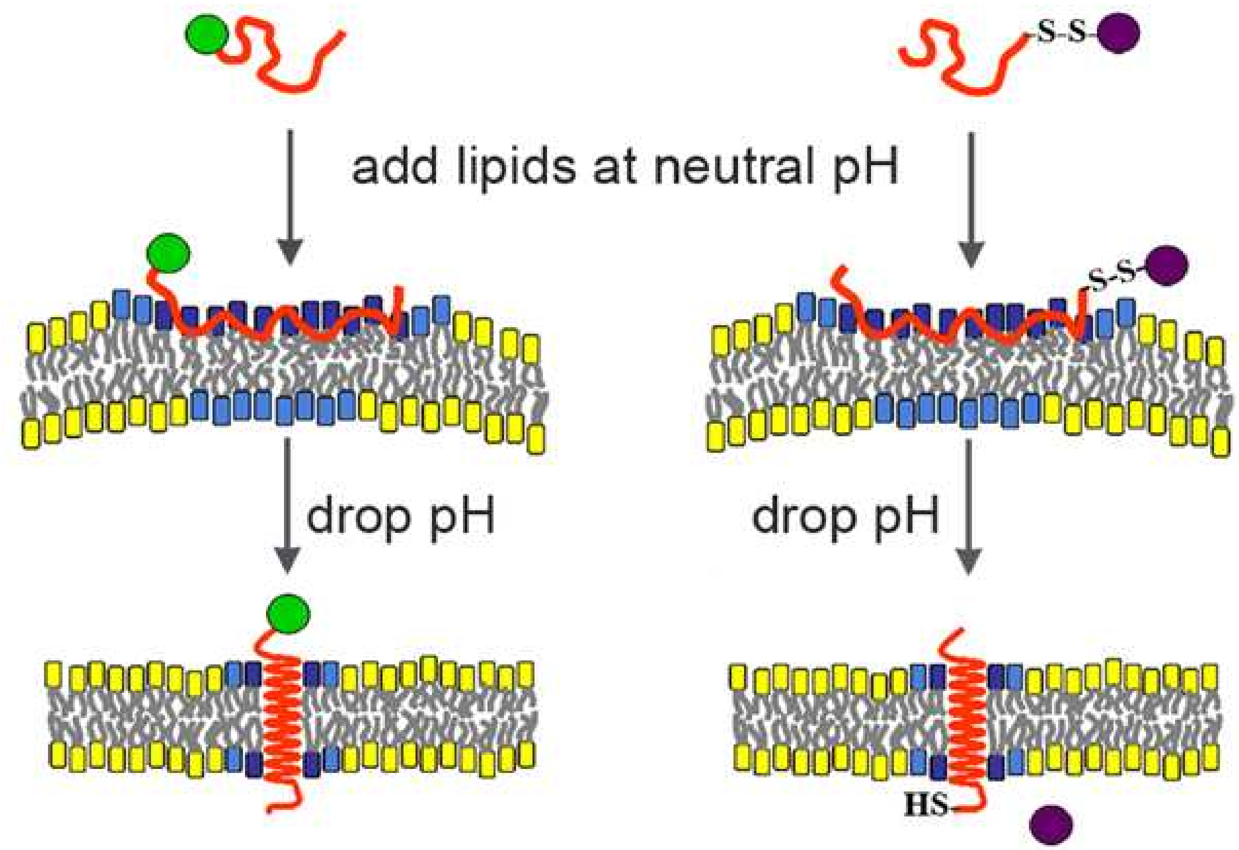
flanking | trans−membrane | flanking

C terminus



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

Medical Applications



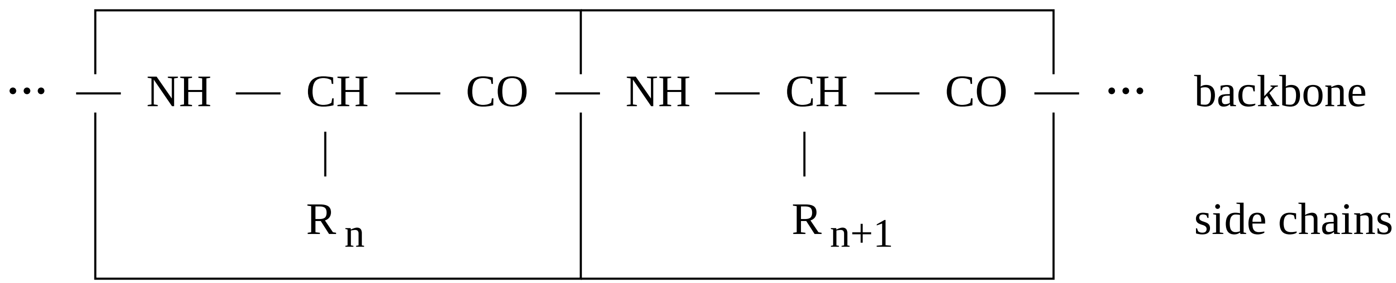
diagnostic agent

cargo $\longrightarrow$ marker

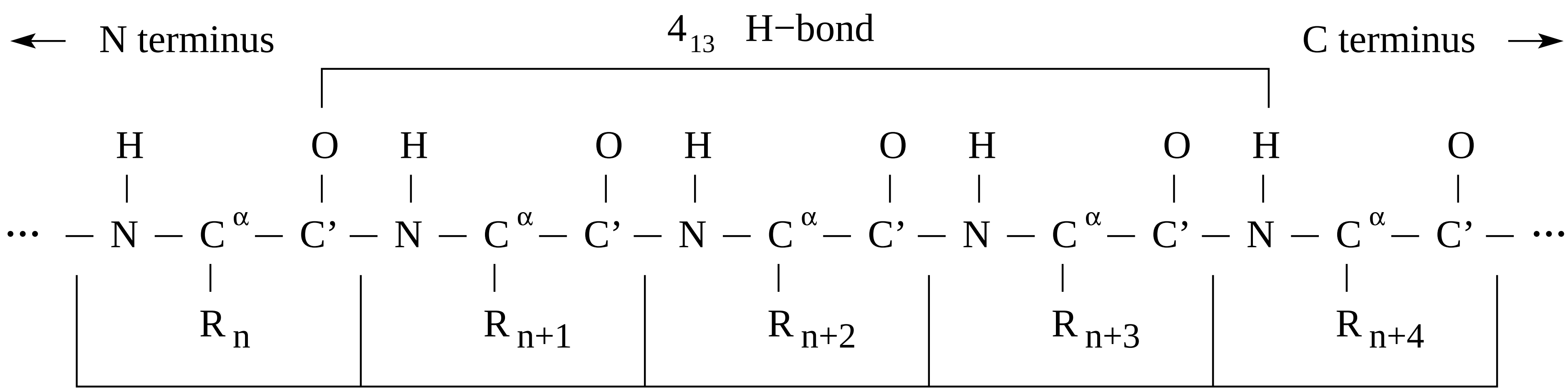
therapeutic agent

cargo $\longrightarrow$ drug

Amino acids linked into polymer.
Backbone $\longrightarrow$ periodic. Side chains $\longrightarrow$ aperiodic.
Residues n and $n+1$ coupled by peptide bond.

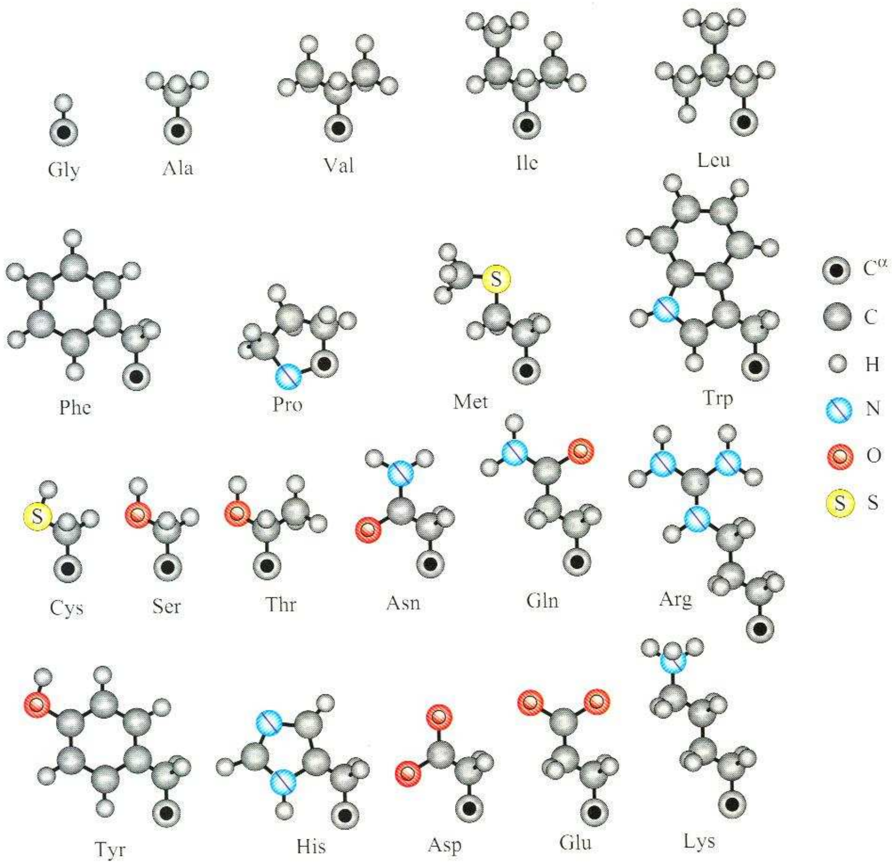


α-helix stabilized by internal H-bonds ($\sim 9k_\mathrm{B}T$).

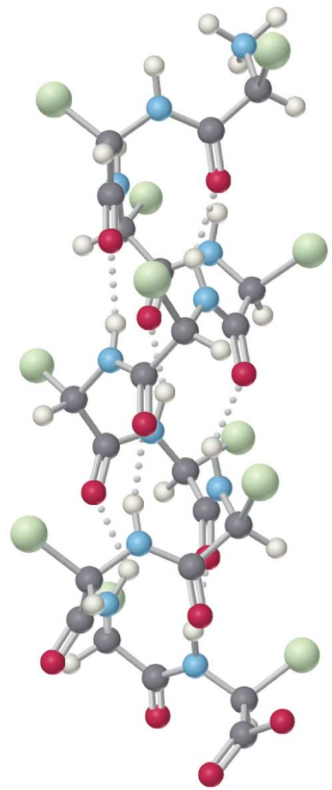


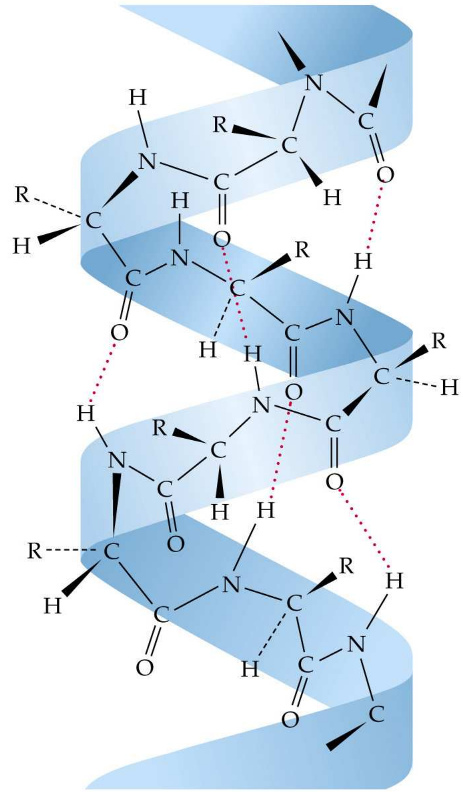
Amino Acid Residues

side chains



backbone





Length per residue: $\ell_e \simeq 4$Å (extended), $\ell_h \simeq 1.5$Å (helical).

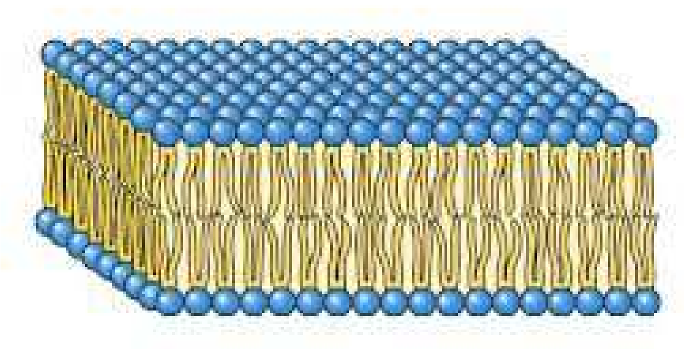
Conformational Change: Coil vs Helix

Conformation with lower free energy is realized.

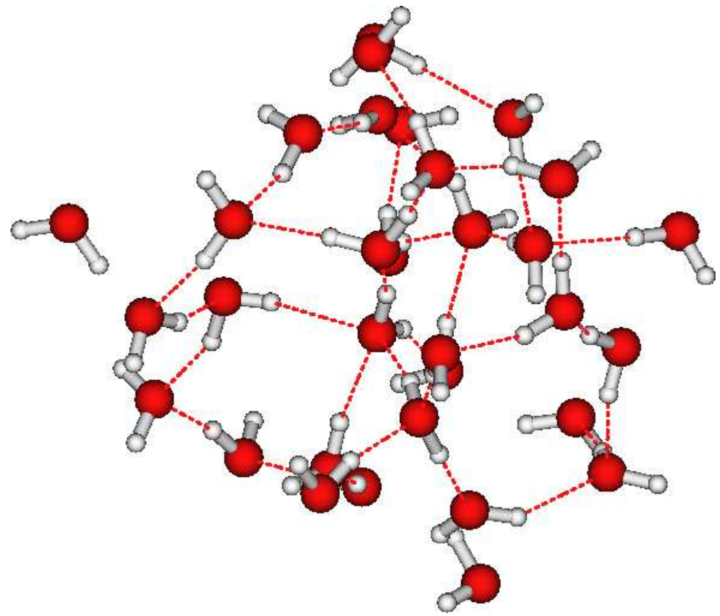
$$\Delta G \doteq G_{\mathrm{coil}} - G_{\mathrm{helix}} = \Delta H - T\Delta S.$$

ΔH: enthalpic contribution, $T\Delta S$: entropic contribution.

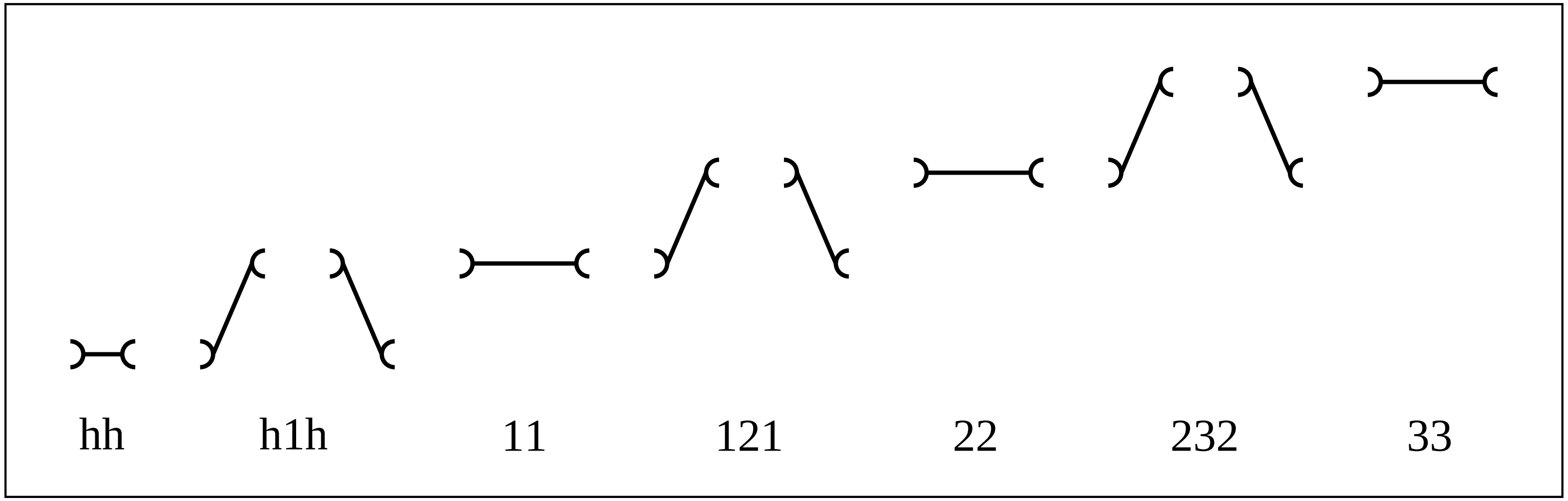
non-polar environment: $|\Delta H| \gg |T\Delta S|$

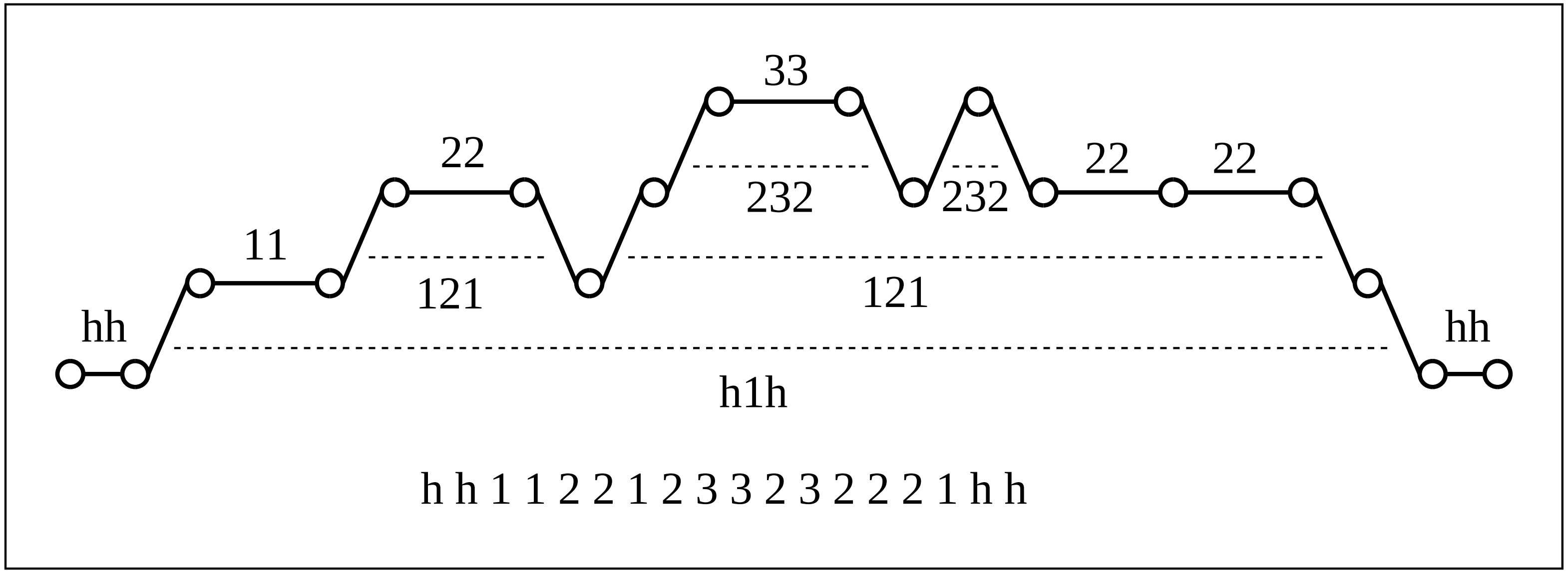


polar environment: $|\Delta H| \lesssim |T\Delta S|$



Mathematical Model (1)





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?

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

Energy and multiplicity of many-body states

$$E(\{N_m\}) = E_{\rm pv} + \sum_{m=1}^{M} N_m \epsilon_m, \qquad W(\{N_m\}) = \prod_{m=1}^{M} \underbrace{\binom{d_m + N_m - 1}{N_m}}_{\dfrac{\Gamma(d_m + N_m)}{\Gamma(N_m + 1)\Gamma(d_m)}}$$

- $E_{\rm pv}$: energy of reference state
- N_m: number of particles from species m
- ϵ_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 ϵ_m
- statistical interaction coefficients $g_{mm'}$
- capacity constants A_m

Two tasks:

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

Partition function [Wu 1994]: $Z = \sum_{\{N_m\}} W(\{N_m\}) e^{-\beta E(\{N_m\})} = \prod_m \left(\frac{1+w_m}{w_m}\right)^{A_m}$

$$e^{\epsilon_m/k_\mathrm{B}T} = (1+w_m) \prod_{m'=1}^{M} \left(1+w_{m'}^{-1}\right)^{-g_{m'm}}, \quad m = 1, \ldots, M.$$

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]:

$$S(\{N_m\}) = k_\mathrm{B} \sum_{m=1}^{M} \Big[(N_m + Y_m) \ln (N_m + Y_m) - N_m \ln N_m - Y_m \ln Y_m \Big]$$

$$Y_m \doteq A_m - \sum_{m'=1}^{M} g_{mm'} N_{m'}$$

Mathematical Model (2)

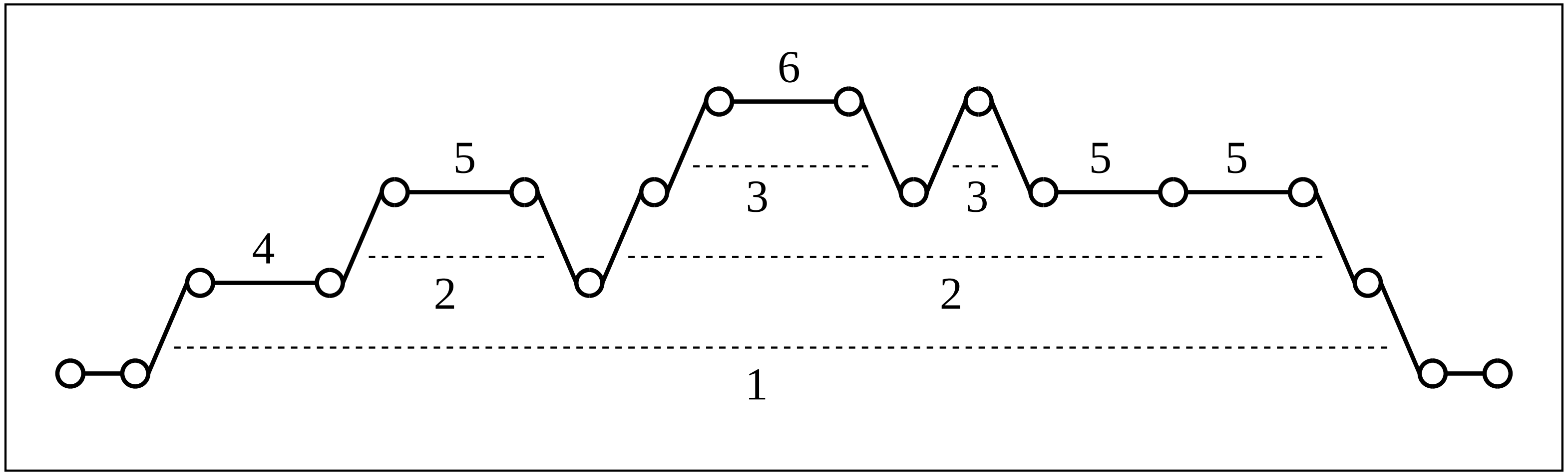
Specifications for combinatorics and energetics $(\mu = 3)$:

motif	cat.	m	ϵ_m	A_m
h1h	host	1	$\epsilon_{\rm n}$	$N-2$
121	hybrid	2	$2\epsilon_{\rm g}$	0
232	hybrid	3	$2\epsilon_{\rm g}$	0
11	tag	4	$\epsilon_{\rm g}$	0
22	tag	5	$\epsilon_{\rm g}$	0
33	tag	6	$\epsilon_{\rm g}$	0

$g_{mm'}$	1	2	3	4	5	6
1	2	2	2	1	1	1
2	-1	0	0	0	0	0
3	-0	-1	0	0	0	0
4	-1	-1	0	0	0	0
5	0	-1	-1	0	0	0
6	0	0	-1	0	0	0

- $W(\{N_m\}) = \prod_{m=1}^{2\mu} \begin{pmatrix} d_m + N_m - 1 \\ N_m \end{pmatrix}, \quad d_m = A_m - \sum_{m'=1}^{2\mu} g_{mm'}(N_{m'} - \delta_{mm'})$
- $E(\{N_m\}) = E_{\rm pv} + \sum_{m=1}^{2\mu} N_m \epsilon_m$

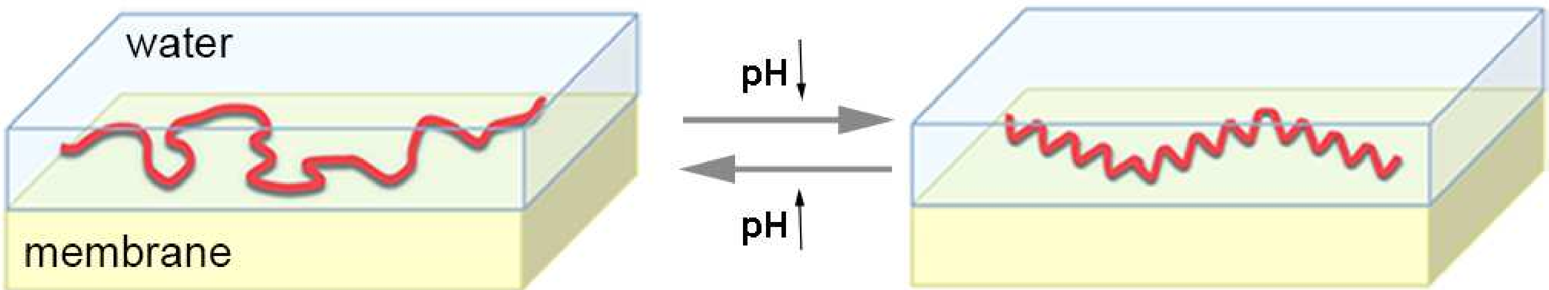
Mathematical Model (3)



- 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: $E(1,2,2,1,3,1) - E_{\mathrm{pv}} = \epsilon_{\mathrm{n}} + 13\epsilon_{\mathrm{g}}$
- Multiplicity: $W(1,2,2,1,3,1) = \binom{1}{1}\binom{3}{2}\binom{3}{2}\binom{3}{1}\binom{6}{3}\binom{2}{1} = 360$

Parameters and Processes

- Nucleation parameter: $\tau \doteq e^{(\epsilon_{\rm g}-\epsilon_{\rm n})/k_{\rm B}T} \quad (0 \leq \tau \leq 1)$
- Growth parameter: $t \doteq e^{\epsilon_{\rm g}/k_{\rm B}T} \quad (0 \leq t < \infty)$
- Range parameter: $\mu = 1, 2, \ldots, \infty$



Mathematical Model (4)

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

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

$$w_1 = \frac{S_\mu(w)}{\tau S_{\mu-1}(w)} = \frac{t}{1 + w - t}, \quad w_m = \begin{cases} \dfrac{S_{\mu-m+2}(w)}{S_{\mu-m}(w)} & : m = 2, \ldots, \mu \\ w & : m = \mu + 2, \ldots, 2\mu \end{cases}$$

Chebyshev polynomials of the 2^{nd} kind:

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

Introduce $r_\mu(w) \doteq \dfrac{S_\mu(w)}{S_{\mu-1}(w)} = \begin{cases} \dfrac{1}{2}\left[w + \sqrt{4 - w^2}\right] \cot\left(\mu \arccos \dfrac{w}{2}\right) & : w < 2, \\ \dfrac{\mu + 1}{\mu} & : w = 2, \\ \dfrac{1}{2}\left[w + \sqrt{w^2 - 4}\right] \coth\left(\mu \,\mathrm{Arcosh} \dfrac{w}{2}\right) & : w > 2 \end{cases}$

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

Physical Quantities of Interest

- Free energy: $\bar{G}(t,\tau) = -k_\mathrm{B}T \ln\left(1 + w_1^{-1}\right)$
- Entropy: $\bar{S}(t,\tau) = -\left(\dfrac{\partial \bar{G}}{\partial T}\right)_{\epsilon_\mathrm{n},\epsilon_\mathrm{g}}$
- Enthalpy $\bar{H}(t,\tau) = \bar{G} + T\bar{S}$
- Helicity (order parameter): $\bar{N}_\mathrm{hl}(t,\tau) = 1 - \left(\dfrac{\partial \bar{G}}{\partial \epsilon_\mathrm{n}}\right)_{T,\epsilon_\mathrm{g}} - \left(\dfrac{\partial \bar{G}}{\partial \epsilon_\mathrm{g}}\right)_{T,\epsilon_\mathrm{n}}$
- Density of (helix or coil) segments: $\bar{N}_\mathrm{seg}(t,\tau) = \left(\dfrac{\partial \bar{G}}{\partial \epsilon_\mathrm{n}}\right)_{T,\epsilon_\mathrm{g}}$
- Average size of helix segments: $L_\mathrm{hs}(t,\tau) = \dfrac{\bar{N}_\mathrm{hl}}{\bar{N}_\mathrm{seg}}$
- Average size of coil segments: $L_\mathrm{cs}(t,\tau) = \dfrac{1 - \bar{N}_\mathrm{hl}}{\bar{N}_\mathrm{seg}}$

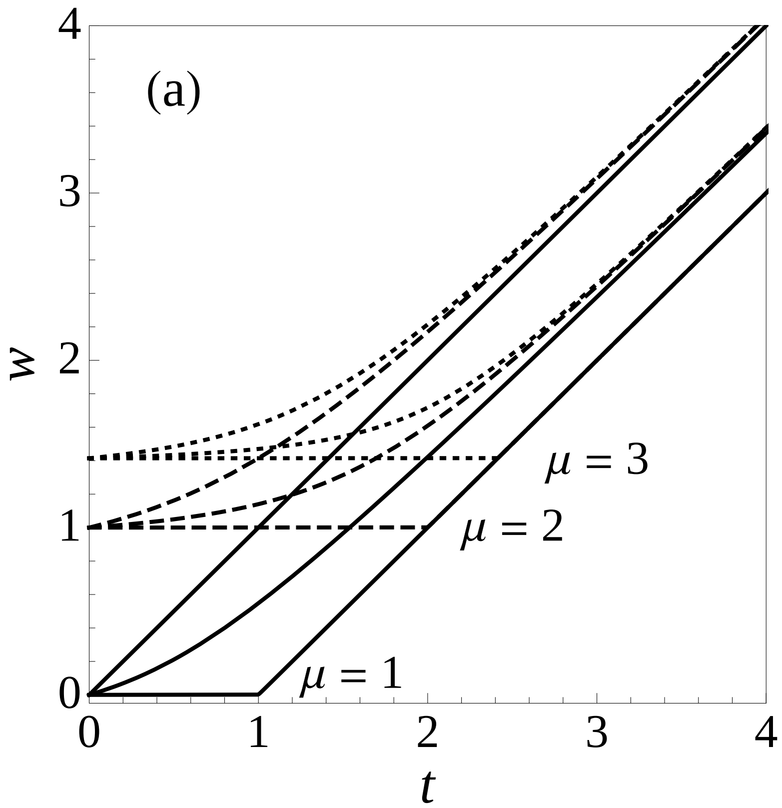
Structure of Solution (1)

Crossover: $\tau > 0$ and $\mu < \infty$

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

First-order transition: limit $\tau \to 0$ at $\mu < \infty$

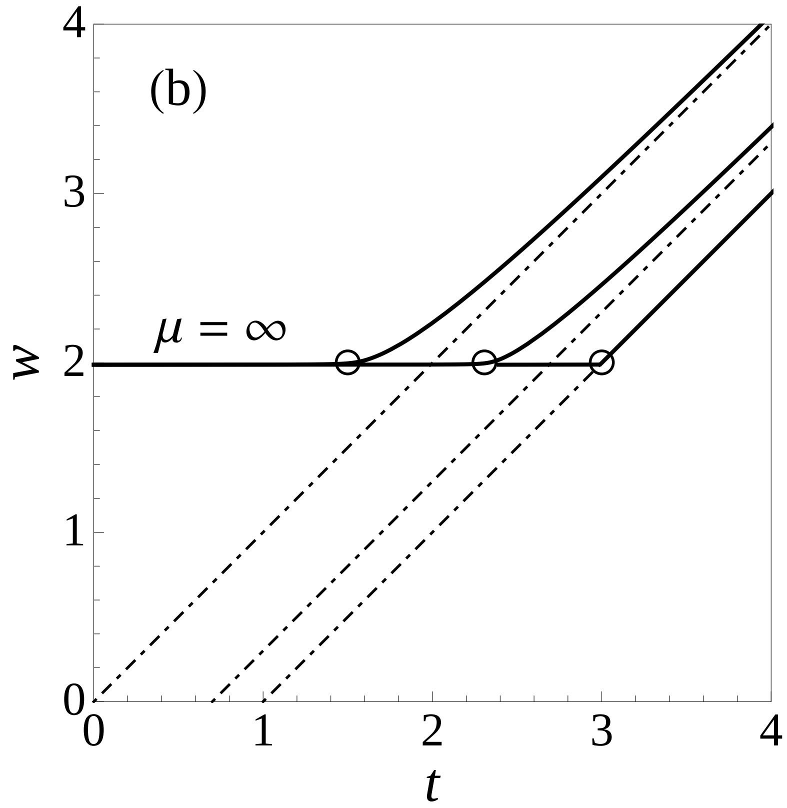
- Transition point: $t_0 = 1 + 2\cos\left(\frac{\pi}{\mu+1}\right)$
- Solution: $w = \begin{cases} t_0 - 1 & : t \leq t_0 \\ t - 1 & : t \geq t_0 \end{cases}$
- Conformation:
  - pure coil at $t < t_0$
  - pure helix at $t > t_0$



Structure of Solution (2)

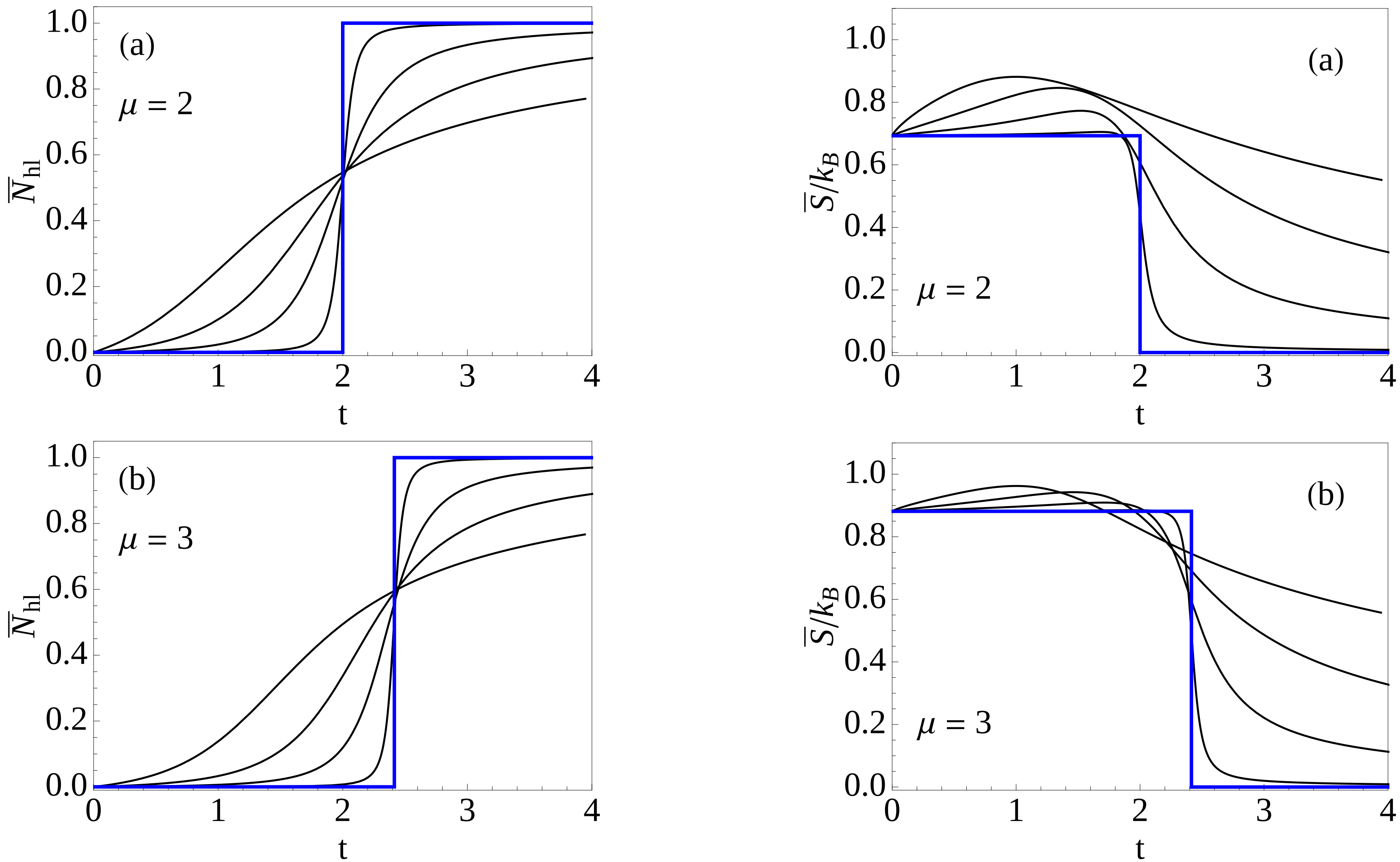
Second-order transition: limit $\mu \to \infty$ at $\tau > 0$

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



Helicity and Entropy (1)

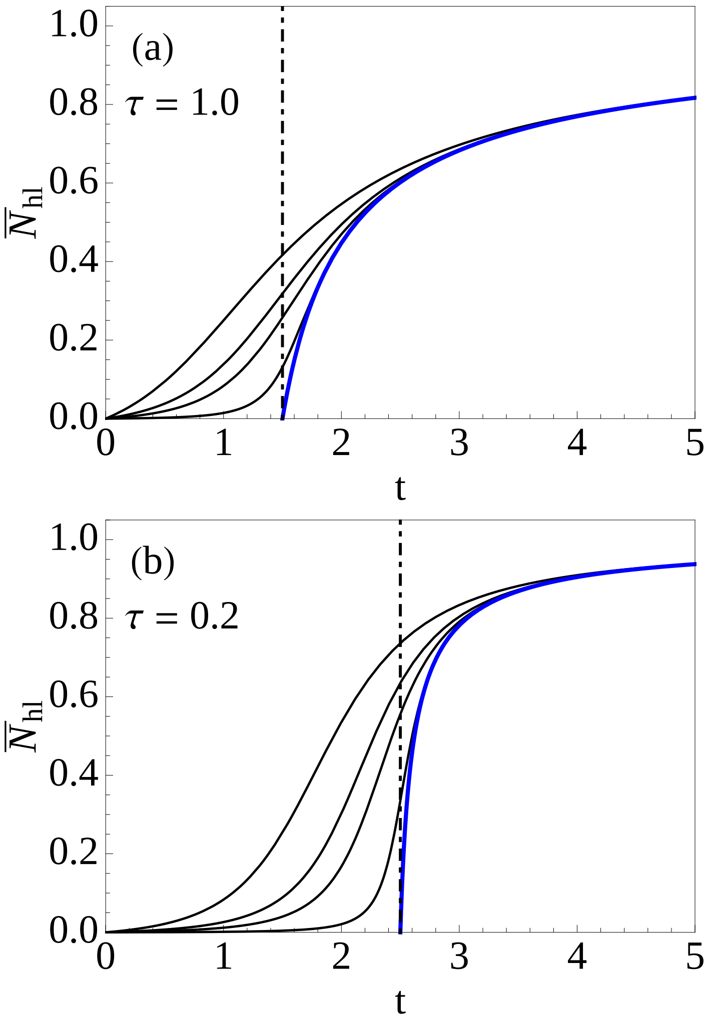
Helicity | Entropy



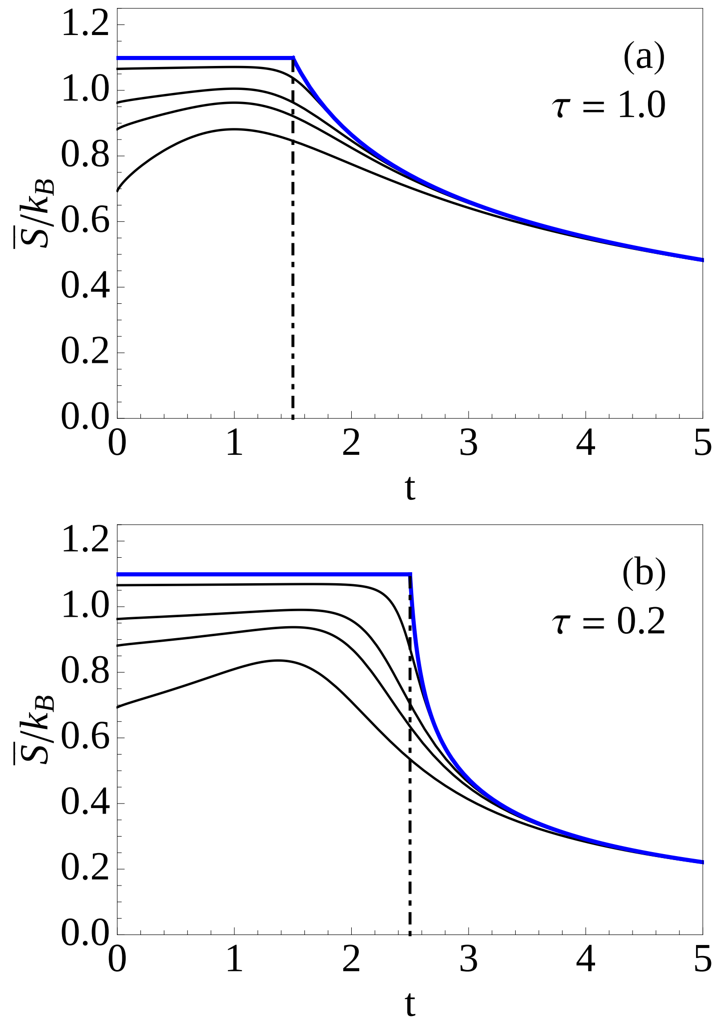
Nucleation parameter: $\tau = 1,\ 0.25,\ 0.05,\ 0.0025,\ 0$

Helicity and Entropy (2)

Helicity



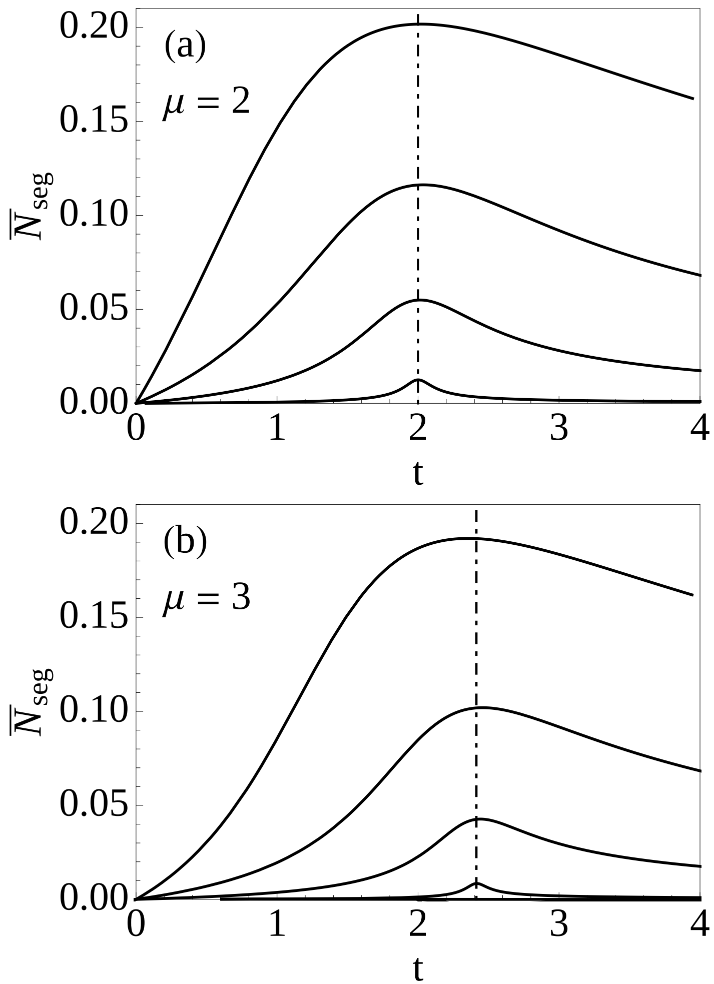
Entropy



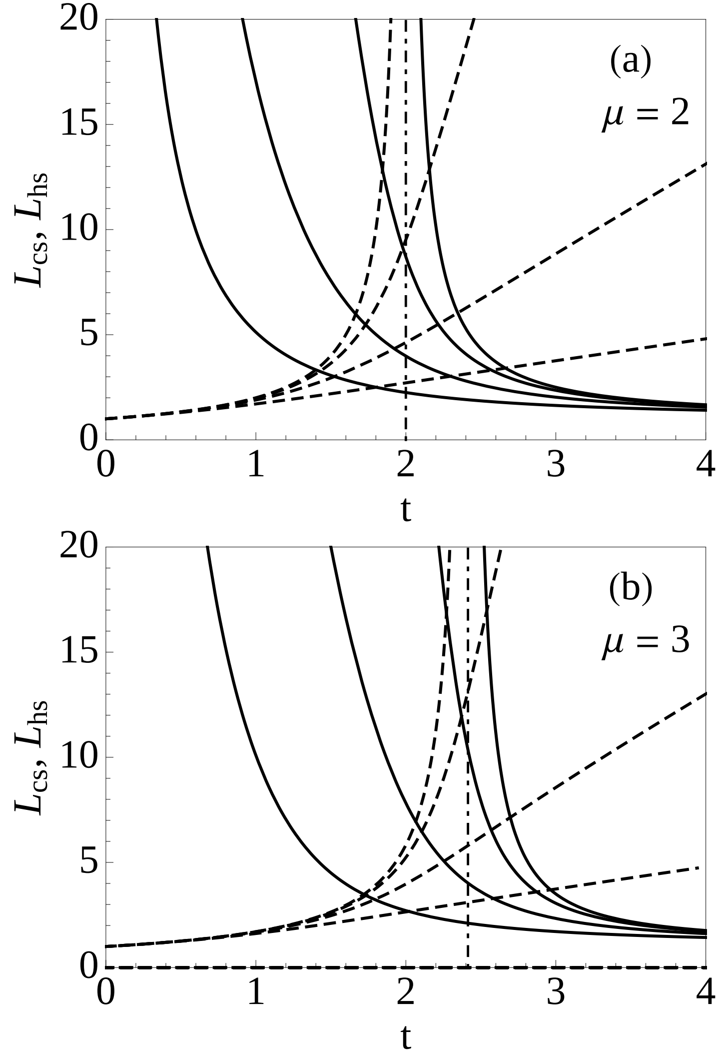
Range parameter: $\mu = 2,\ 3,\ 4,\ 9,\ \infty$

Helix Segments and Coil Segments (1)

Average number



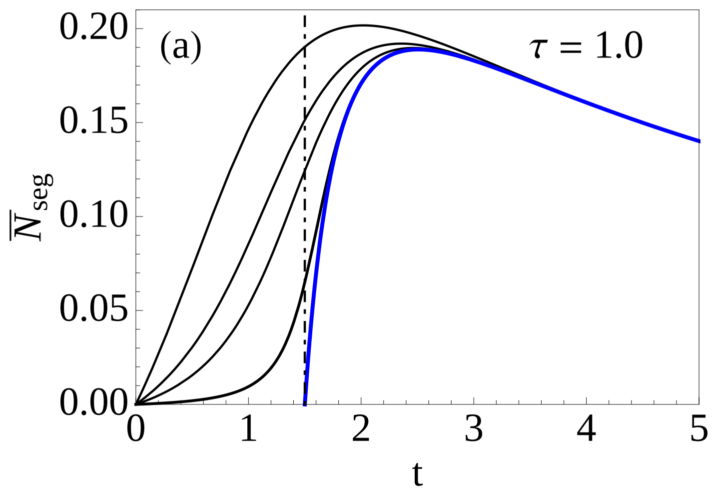
Average length

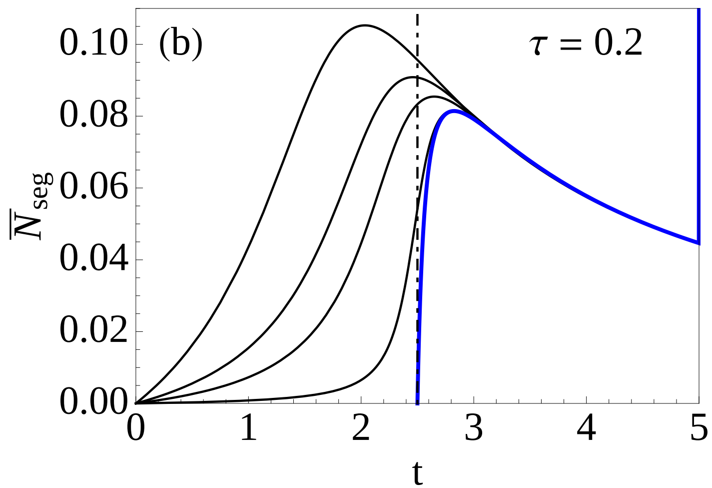


Nucleation parameter: $\tau = 1,\ 0.25,\ 0.05,\ [0.0025,\ 0]$

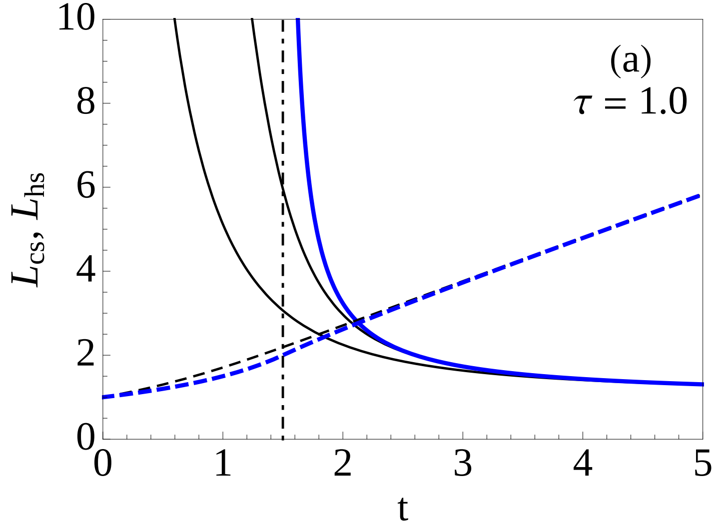
Helix Segments and Coil Segments (2)

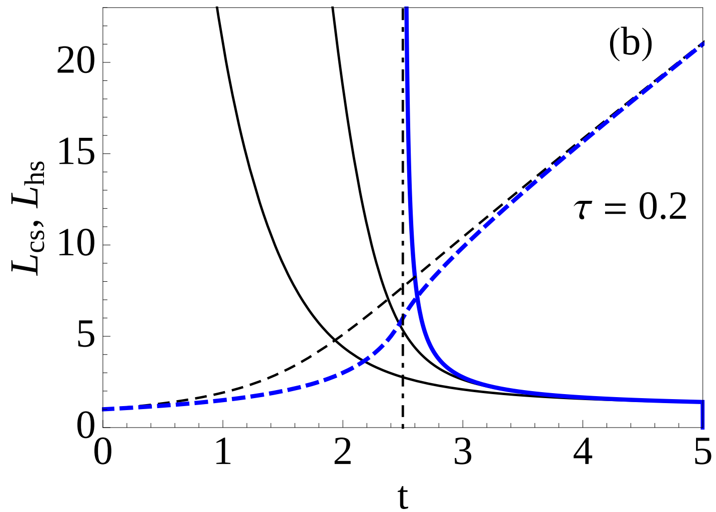
Average number





Average length

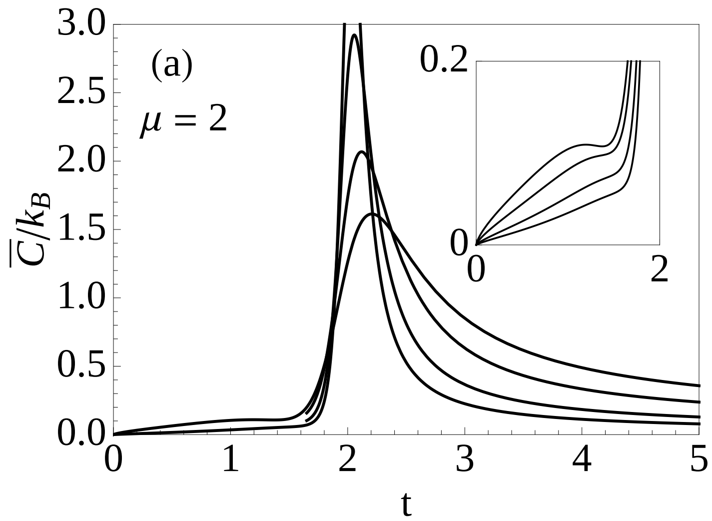




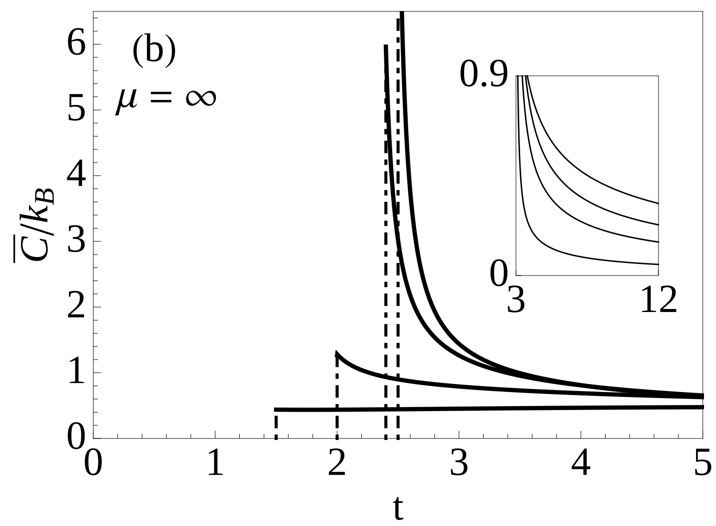
Range parameter: $\mu = 2,\ [3],\ 4,\ [9],\ \infty$

Heat Capacity and Latent Heat

Heat capacity:



$\tau = 0.05,\ 0.025,\ 0.01,\ 0.005$



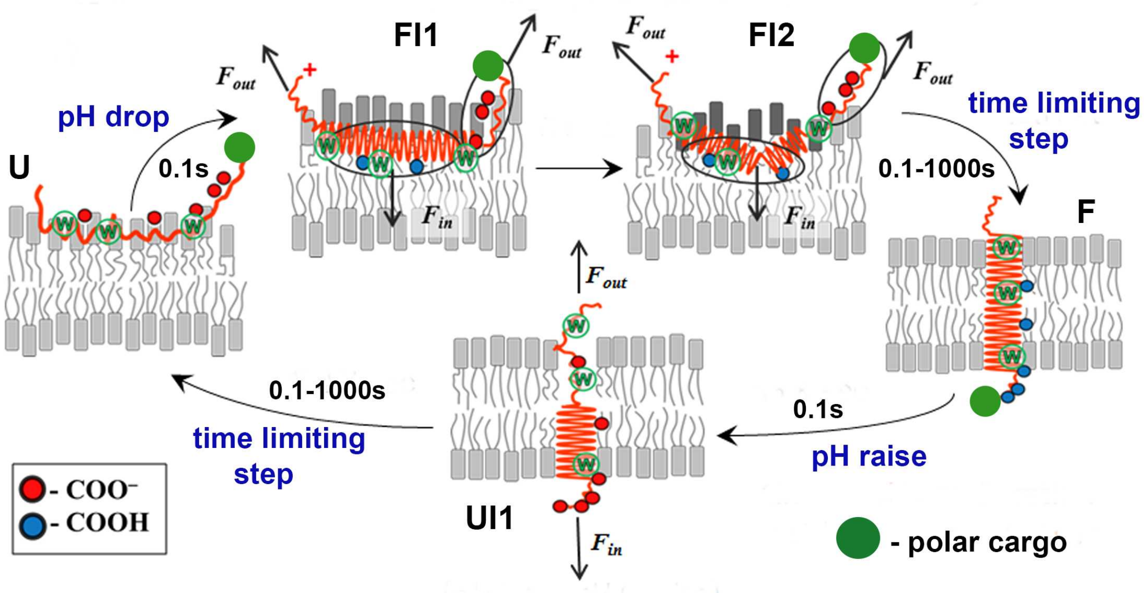
main plot: $\tau = 1.0,\ 0.5,\ 0.25,\ 0.25$

inset: $\tau = 0.2,\ 0.1,\ 0.05,\ 0.01$

Latent heat:

- in the limit $\tau \longrightarrow 0$: $T\Delta\bar{S} = \epsilon_{\rm g}$

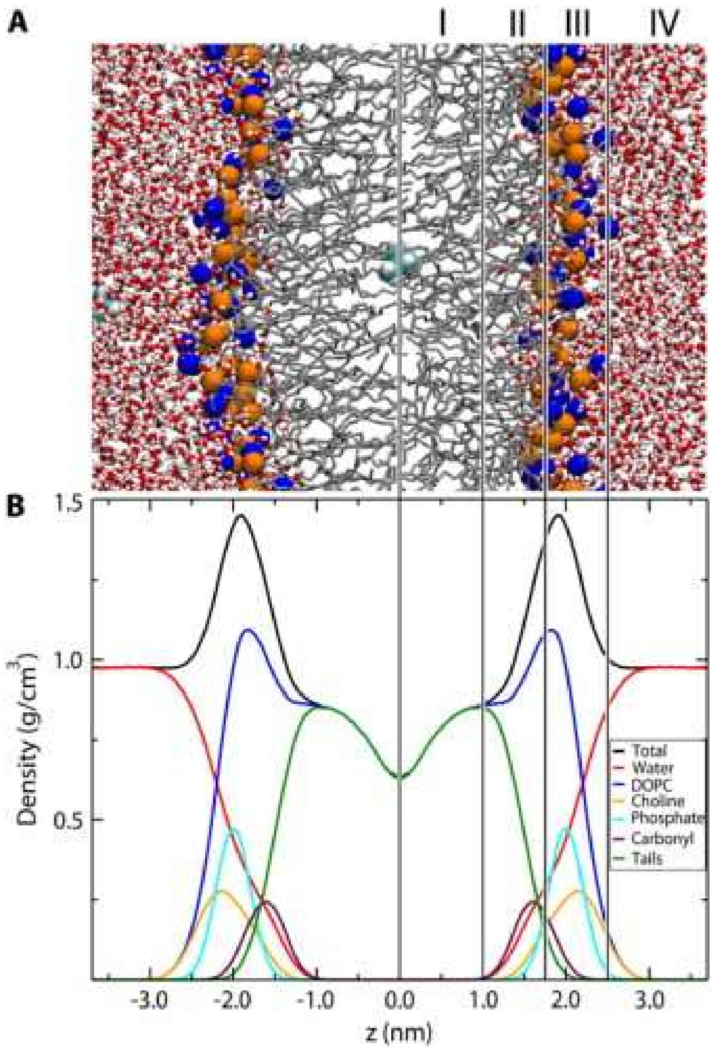
Outlook (1): Working Hypothesis



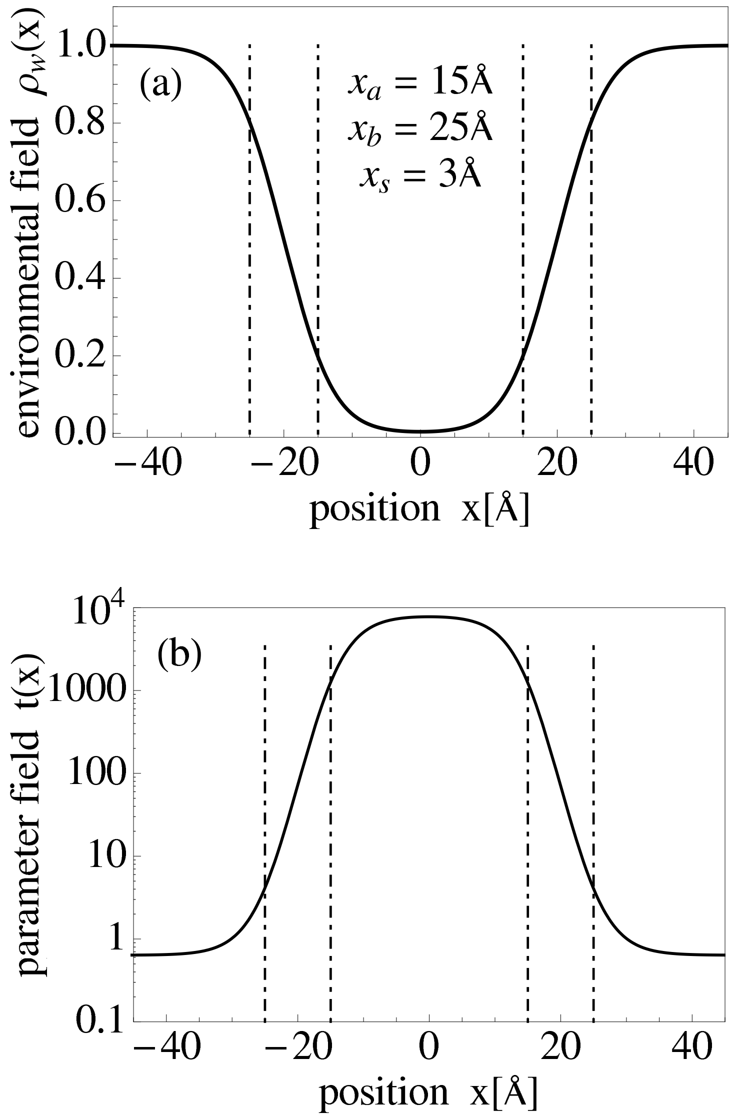
pHLIP variants with single permutations:

A D N N P W I Y A R Y A D L T T F P L L L L D L A L L V D F D D A D N N P F I Y A R Y A D L T T W P L L L L D L A L L V D F D D A D N N P F I Y A R Y A D L T T F P L L L L D L A L L V D W D D	Tryptophan
A D N N P F P Y A R Y A D L T T W I L L L L D L A L L V D F D D	Proline
A D N N P F I Y A Y R A D L T T F P L L L L D L A L L V D W D D A D N N P F I Y A T Y A D L R T F P L L L L D L A L L V D W D D	Arginine

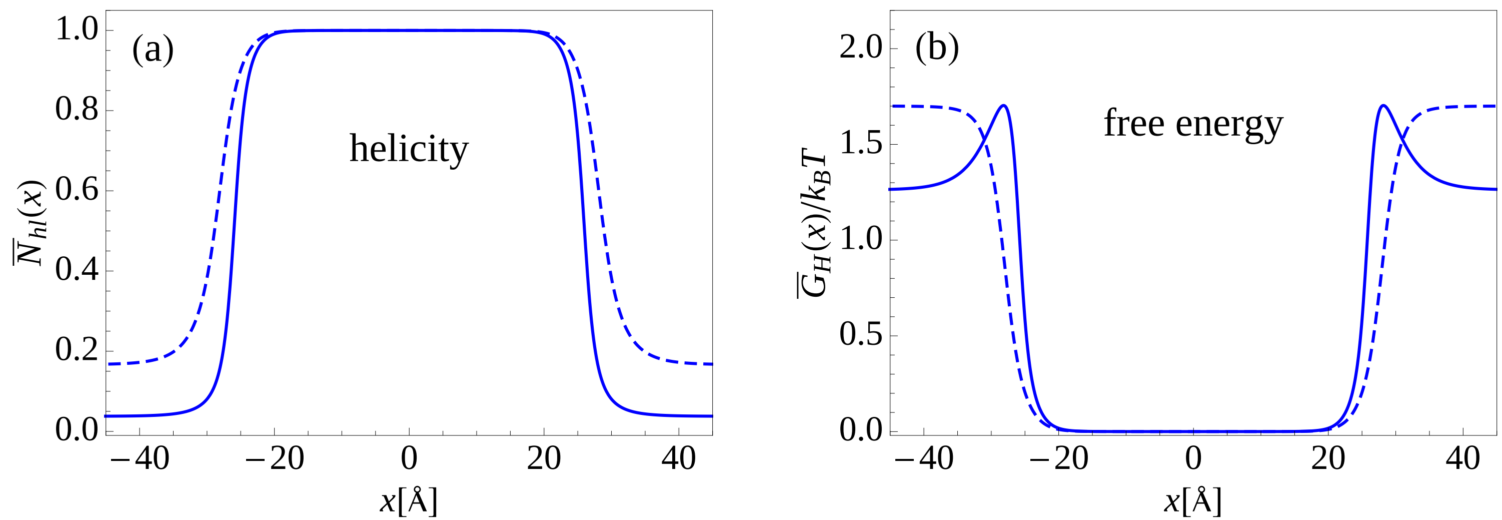
Outlook (2): Membrane Environment

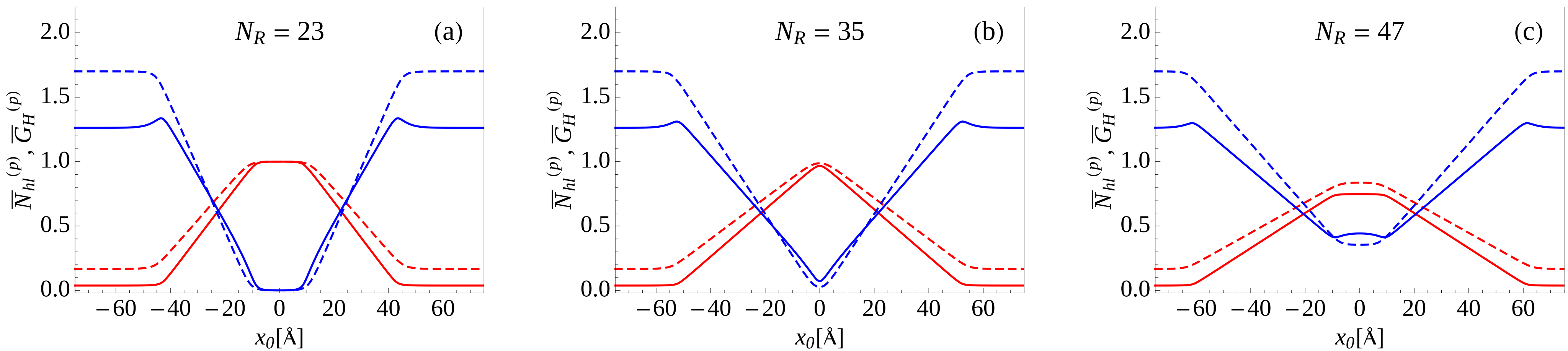


[MacCallum et al. 2008]

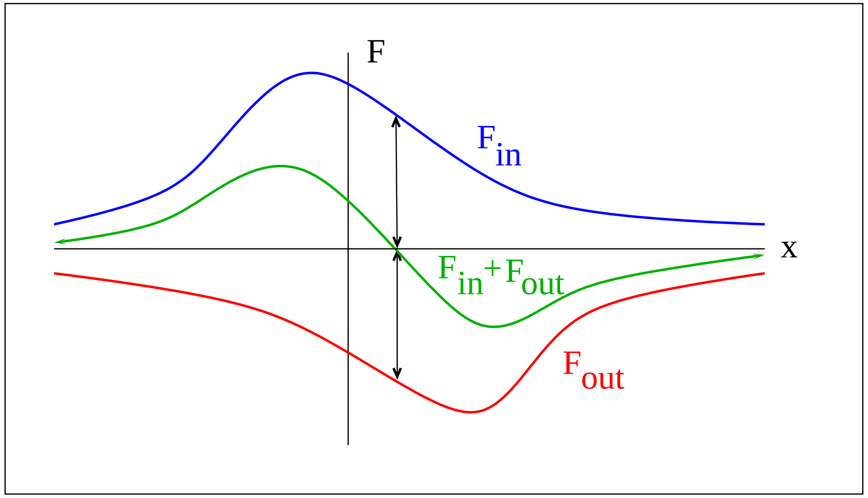


Outlook (3): Profiles and Landscapes





Outlook (4): Kinetics



F_{in}: hydrophobic force

F_{out}: electrostatic force

Possible scenario for initiation of insertion:

- at pH 8 the two forces are balanced (on average) with $F_{\mathrm{in}} + F_{\mathrm{out}}$ 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