Helix folding pathways

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Warszawa-Torun, maj-czerwiec 2011
Peptide dynamics: Significance

- Peptides = biologically active structure-forming molecules
- Peptides = small size allows study of sequence – structure – dynamics – function relations
- Peptides = flexible, dynamic systems motions on ps – μs time scale experiment/simulation overlap
- Peptides = building blocks of proteins → understanding of fundamental biological processes
Peptide Folding Simulations

GOALS:
- Predict process: populations, rates, paths
- Verify methods: algorithms and force fields
- Complement experimental data
- Understanding ➔ design materials, drugs

EXPERIMENTAL data: typically
- structure and population of folded state
- folding and unfolding rates (T)
- rarely: “nucleation rate”

Unique ROLE for simulations: microscopic
- Information on pathways
- Information on unfolded state(s)
- Dynamics ⊥ to reaction coordinate

\( \tau = 300 \text{ ns} \)
50% α
@300 K
Folding Simulation Methods

Fast processes: \( (t \approx 10-100 \text{ ns}) \)
- Direct molecular dynamics (MD) gives complete description

Slow processes:
- Populations:
  - Enhanced sampling methods
    - e.g. replica-exchange MD
- Kinetics:
  - Specialized algorithms
    - e.g. MSM, PPTIS, Milestoning

Limitations:
- Force field accuracy, system size
MOLECULAR DYNAMICS SIMULATIONS

- Model system of N atoms
- Introduce potential energy $U(x,y,z)$
- Calculate force acting on each atom
- Solve Newton’s equations of motion
- Generate a trajectory for each atom $x_i(t)$
- Analyze structure, motions and interactions
- Relate to experimental observations

\[ m_i \frac{d^2 \vec{r}_i}{dt^2} = \vec{F}_i = \nabla_i U \]

**Newton’s 2\textsuperscript{nd} Law**

**Verlet algorithm**

\[
x(t + \Delta t) = 2x(t) - x(t - \Delta t)
\]
\[
v(t) = \frac{x(t + \Delta t) - x(t - \Delta t)}{2\Delta t}
\]
Replica-exchange molecular dynamics

Propagate independent trajectories at temperatures $T_1 < T_2 < T_3 < ...$
Stop and compare energies
Exchange between neighbors

Advantages:
+ accelerated sampling @ low T
+ Boltzmann distributions @ all T
+ Minimal process communication
+ Property sampling as $f(T)$

$$w(i \rightarrow j) = \begin{cases} 1 & \Delta \leq 0 \\ e^{-\Delta} & \Delta > 0 \end{cases}$$

$$\Delta = (\beta_j - \beta_i)(E_i - E_j)$$

$$\beta_i = \frac{1}{kT_i}$$
WH5: Fastest Folding $\alpha$-helix

Experimental at 300 K:

CD spectroscopy:
% $\alpha$ = 20-25 %

Fluorescence T-jump:
Relaxation $\tau_1 = 5.3 \pm 1.9$ ns
$\tau_2 = 0.85 \pm 0.3$ ns

Sequence: 5 aa
Ac-Trp-Ala-Ala-Ala-His$^+$-NH$_2$

Gouri S. Jas, Baylor University
WH5 : Global MD

MD: 1,000 ns NPT at 300 K, 1 bar with GROMACS program and several protein force fields, ≈1000 waters, 1 Cl⁻ 960 ns with CHARMM program and CHARMM ff
WH5 : Local MD

Sample OPLS/AA results
**WH5: helix populations and kinetics**

<table>
<thead>
<tr>
<th>Force Field</th>
<th>$T_{\text{fold}}$ ns</th>
<th>$T_{\text{unf}}$ ns</th>
<th>$T_r$ ns</th>
<th>$T_{\text{nuc}}$ ns</th>
<th>% α HB</th>
<th>% α PP</th>
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<td>OPLS/AA</td>
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<td>3.6</td>
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<td>CHARMM</td>
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<td>21</td>
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<tr>
<td>AMBER99P</td>
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<td>65</td>
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**Experiment:**

%$\alpha = 20\text{-}25\%$

**Relaxations:**

5.3 and 0.8 ns

**Folding:**

$T_{\text{fold}} \approx 30$ ns

$T_{\text{unf}} \approx 6$ ns

$$\frac{1}{\tau_r} = \frac{1}{\tau_{\text{fold}}} + \frac{1}{\tau_{\text{unf}}}$$

$$K = \frac{1-\alpha}{\alpha} = \frac{k_u}{k_f} = \frac{\tau_f}{\tau_u}$$

**Amazing agreement:**

Most force field predictions are within a factor of 10 of experimental data!

Corresponding $\Delta E \approx 1$ kcal/mol at 300 K
Folding of WH5: pathways

- AMBER03, AMBERGS: 1-2-3
- AMBER99P: (1+3)-2
- G43A1, AMBER99SB: (1+2)-3
- OPLS/AA(SPC): 2-1-3
- OPLS/AA(TIP3P): 2-1-3 or (1+2)-3
- CHARMM: 1+2+3 or (1+2)-3
WH5: Trp…His distance (CHARMM)

Correlations:

R(W…H) – RMSD from helix: \( r = 0.55 \)

R(W…H) - HB1, HB2, HB3: \( r = 0.43, 0.59, 0.35 \)

Close Trp…His contact is correlated with global RMSD from helix & HB2 formation
WH5 hydrogen bond dynamics

<table>
<thead>
<tr>
<th>Force Field</th>
<th>HB1 $\tau_r$</th>
<th>HB1 $\tau_u$</th>
<th>HB1 $\tau_f$</th>
<th>HB2 $\tau_r$</th>
<th>HB2 $\tau_u$</th>
<th>HB2 $\tau_f$</th>
<th>HB3 $\tau_r$</th>
<th>HB3 $\tau_u$</th>
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<td>355</td>
<td>150</td>
<td>784</td>
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<td>CHARMM$^b$</td>
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<td>1596</td>
<td>1060</td>
<td>218</td>
<td>122</td>
<td>78</td>
</tr>
</tbody>
</table>

$^a$With SPC water  $^b$With TIP3P water

H-bond dynamics time constants in ps.

Relaxation of central hydrogen bond HB2 is in the 0.1-1.0 ns range for most studied FF.

$$\frac{1}{\tau_r} = \frac{1}{\tau_u} + \frac{1}{\tau_f}$$
## WH5 MD: coil-helix energy components

<table>
<thead>
<tr>
<th>Force Field</th>
<th>Total</th>
<th>Internal</th>
<th>Elec</th>
<th>vDW</th>
<th>PP</th>
<th>PS</th>
<th>PP:El</th>
<th>PP:vdW</th>
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<td>-29.0</td>
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<td>1.1</td>
<td>-7.4</td>
<td>-0.1</td>
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</table>

\(\text{a}\) With CMAP, PME and GROMACS  
\(\text{b}\) Without CMAP, with PME and GROMACS  
\(\text{c}\) Without CMAP, cutoff electrostatics, with GROMACS  
\(\text{d}\) With SPC water  
\(\text{e}\) With TIP3P water
WH5: CONCLUSIONS

• Most popular force fields give reasonable predictions for WH5 helicity and kinetics

• Assignment of relaxations: 5 ns process $\rightarrow$ helix folding, 1 ns process $\rightarrow$ helix nucleation = formation of HB2 (or HB1+HB2)

• Force fields differ in details of predicted folding pathway; a majority suggest a “zipper” model, with folding initiated at the N-terminus and progressing consecutively to C-terminus

• Helix formation is cooperative, h-bond transitions are correlated

• Trp…His sidechain interactions stabilize helix

• Force field accuracy is the limiting factor for biomolecular simulations

• More detailed experimental data is needed for FF calibration
The story of Ala₅

Sequence: 5 aa
Ac-Ala-Ala-Ala-Ala-Ala-NH₂
New experimental data on ac-Ala$_5$-NH$_2$

- CD of Ac-Ala$_5$-NH$_2$ over 266-363 K
  - melting transition with $T_m = 271$ K $\Delta H = 9.5$ kcal/mol
  - $13 \pm 2$ % helix @300 K

- FTIR measurement of amide I peak:
  - $26 \pm 5$ % helix @293 K

- New experimental data support population of $\alpha$-helix @ low temperature


**Diagram**: Green: $\alpha$  Magenta: $\beta$  Cyan: turn
Folding of Ac-Ala$_5$-NH$_2$ : kinetics from MD

<table>
<thead>
<tr>
<th></th>
<th>$\tau_{\text{fold}}$ ns</th>
<th>$\tau_{\text{unf}}$ ns</th>
<th>$\tau_r$ ns</th>
<th>$\tau_{\text{nuc}}$ ns</th>
<th>$% \alpha$ HB</th>
<th>$% \alpha$ PP</th>
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<td>OPLS/AA</td>
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<td>4</td>
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<td>0.3</td>
<td>71</td>
<td>60</td>
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1. Predicted kinetic and equilibrium parameters span 2-3 orders of magnitude; helicities agree with exp. data
2. Helix content tends to be lower and kinetics faster compared to WH5 - consistent with W…H interaction.

**Sample OPLS/AA results**

**MD:** 1,000 ns NPT MD at 1 atm, 300 K with GROMACS
Conclusions

• Helix content for most popular models is in good agreement with new experimental data

• Calculated folding, unfolding and nucleation rates of Ac-Ala5-NH$_2$ tend to be faster than those for WH5

• Most ff predict that helical hydrogen bond formation is cooperative

• Helix-coil transition paths vary with model; most studied models predict a zipper-like mechanism, with unfolding initiated at C-terminus and folding initiated at N-terminus.

• We have achieved full sampling of conformations and dynamics for modest size systems; results are now primarily limited by force field accuracy

• More and better experimental data are also needed to calibrate molecular models
The “real” helix: WH21

Sequence: 21 aa

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Significantly more complex than WH5

- 21 residues
- 19 hydrogen bonds
- EXPERIMENTAL:
  \[\%\alpha = 45\%, \tau_r = 280 \text{ ns at 300 K}\]

### WH21 SIMULATIONS

<table>
<thead>
<tr>
<th>Potential</th>
<th>Type</th>
<th>Conditions</th>
<th>Length</th>
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<tbody>
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<td>REMD</td>
<td>NR=64, 280-500 K</td>
<td>1 μs</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>NVT, 300 K, start=α</td>
<td>13 μs</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>NVT, 300 K, start=ext</td>
<td>10 μs</td>
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<td></td>
<td>MD</td>
<td>NPT, 300K/1bar,start=ext</td>
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<td></td>
<td>Milestoning</td>
<td>NVT, 300 K</td>
<td>3 paths</td>
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<tr>
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<td>REMD</td>
<td>NR=50, 290-500 K</td>
<td>1 μs</td>
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<tr>
<td></td>
<td>MD</td>
<td>NVT, 320 K, start=ext</td>
<td>5 μs</td>
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</table>

MD production: 100 ns/day with GROMACS on 36 CPUs

**OPLS-AA**: 4 Cl⁻, 3563 SPC waters, 48.2 Å box at 300 K

**AMBER03**: 8Na⁺, 12 Cl⁻, 3587 TIP3P waters, 48.7 Å box at 320 K
WH21: AMBER03 REMD

50 replicas, 290-500 K, 1 μs
Convergence: ≈100 ns

%α at 300 K = 63%

\[ T_m \approx 340 \text{ K} \]
\[ \Delta H = -3.5 \text{ kcal/mol} \]
\[ \Delta S = -10 \text{ cal/(mol K)} \]

Experimental:

\[ T_m = 296 \text{ K} \]
\[ %\alpha \text{ at 300 K} \approx 45\% \]
\[ \Delta H = -12 \text{ kcal/mol} \]
\[ \Delta S = -40 \text{ cal/(mol K)} \]

Microscopic insight: H-bond distributions

- little fully helical state NHB=19: pop 3.6 % at 300 K
- large populations of partially folded states, NHB= 6-12 are due to large number of combinations

\[ N(r) = \frac{n!}{r!(n-r)!} \]
WH21 AMBER03 REMD: DISTANCE DISTRIBUTIONS

Calculated:

Observed: FRET experiments by Gouri Jas & Carey Johnson

Conclusion:
Rgyr, end-to-end distance are not good reaction coordinates for folding
300 K:
Most stable:
  HB1 - Trp…His effect?
  HB6 - HB16 - center
Least stable:
  Termini

Melting:
  Roughly uniform along chain
  Persistence of
    HB1 -- Trp…His
    HB6, HB11, HB16 -- Arg
WH21 AMBER03 REMD: MICROSCOPIC PICTURE OF FOLDING

300 K replica:
1M structures

All data:
50 M structures

Folding:
• initiated at specific sites: HB11 & HB16
• “island of stability” formed for HB10 - HB16
• island fluctuates and expands to HB6 - HB16 and the partly labile N-terminus
• C-terminus folds

Unfolding:
• initiated at C-terminus
• passes through “island” intermediate
• persistent populations:
  HB1 - Trp…His interaction ?
  HB6, HB11, HB16 = [Arg - 3] ?
WH21 AMBER03 MD at T=320 K

\%\alpha = 55 \%
Agrees with REMD

EXP: \%\alpha = 20 \%

Multiple transitions
WH21 AMBER03 MD at T=320 K: Folding kinetics I

**Autocorrelation functions of global variables:**

\[ \tau_r \approx 50 \text{ ns}, \ [\tau_f \approx 90 \text{ ns} ; \tau_u \approx 120 \text{ ns}] \]

\[ \tau_f = \tau_r / (1 - \alpha) \ ; \tau_u = \tau_r / \alpha \]

**Site-site correlations \(<n(t)n(0)>\)**

\[ \tau_r \approx 60-80 \text{ ns} \]

[\[\tau_f \approx 80-140 \text{ ns} ; \tau_u \approx 170-280 \text{ ns}]\]

**Experimental: at 320 K**

\[ \tau_r = 90 \text{ ns} \]

[\[\tau_f = 120 \text{ ns}, \tau_u = 450 \text{ ns}]\]

Gouri Jas, unpublished
Folding/unfolding times from NHB(t)
result depends on definition of “helix” and “coil”

For helix = \{NHB=19\} and coil = \{NHB=0\}
\(\tau_f \approx 320\) ns ; \(\tau_u \approx 540\) ns, \([\tau_r \approx 200\) ns\]

For helix = \{NHB\geq16\} and coil = \{NHB\leq3\}
\(\tau_f \approx 80\) ns ; \(\tau_u \approx 90\) ns, \([\tau_r \approx 45\) ns\]

Experimental: at 320 K
\(\tau_r = 90\) ns
\([\tau_f = 120\) ns, \(\tau_u = 450\) ns\]

Gouri Jas, unpublished
WH21 AMBER03 MD at T=320 K: Folding pathways

- \approx 1 \text{ "full" fold } + 1 \text{ "full" unfold event per } 1 \mu \text{s of MD}
- Waiting times 60 – 2600 ns
- Complex path details
- Transitions take up ca. 10% of the trajectory
WH21 AMBER03 MD at T=320 K: Folding pathways

WH21 AMBER03 MD 320 K CH TRANSITION #6

[Graph showing RMSD and hydrogen bond count over time]
WH21 AMBER03 MD at T=320 K: Folding pathways

Helix-Coil transition #2

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WH21 AMBER03 MD at T=320 K: Folding pathways

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Average last visit pattern – over 19 folding events
Structural Transitions (Amber03)
WH21 AMBER03 MD at 320 K: TRANSITION CORRELATIONS

![Graph showing correlation vs. HB separation with two lines: one for HELIX-COIL and one for COIL-HELIX.](image-url)
WH21 OPLSAA MD at T=300 K

MD:
%α = 15%

Exp:
%α = 45%
WH21 OPLSAA MD at T=300 K: Folding kinetics I

Autocorrelation functions of global variables:

\[ \tau_r \approx 70 \text{ ns}, \quad [\tau_f \approx 70 \text{ ns} ; \quad \tau_u \approx 500 \text{ ns}] \]

\[ \tau_f = \tau_r / (1-\alpha) \quad ; \quad \tau_u = \tau_r / \alpha \]

Site-site correlations \( <n(t)n(0)> \)

\[ \tau_r \approx 50-90 \text{ ns} \]

\[ [\tau_f \approx 50-90 \text{ ns} ; \quad \tau_u \approx 1.5-2.6 \mu \text{s}] \]

Experimental: at 300 K

\[ \tau_r = 280 \text{ ns} \]

\[ [\tau_f = 560 \text{ ns}, \tau_u = 560 \text{ ns}] \]

Gouri Jas
WH21 OPLSAA MD at T=300 K: Folding kinetics II

Folding/unfolding times from NHB(t)
result depends on definition of “helix” and “coil”

For helix = \{NHB=19\} and coil = \{NHB=0\}
\[\tau_f \approx 200-300 \text{ ns} ; \quad \tau_u \approx 3.1 \mu\text{s}, [\tau_r \approx 200 \text{ ns}]\]

For helix = \{NHB\geq 16\} and coil = \{NHB\leq 3\}
\[\tau_f \approx 60-80 \text{ ns} ; \quad \tau_u \approx 2.0 \mu\text{s}, [\tau_r \approx 70 \text{ ns}]\]

Experimental: at 300 K
\[\tau_r = 280 \text{ ns}\]
\[\tau_f = 560 \text{ ns}, \tau_u = 560 \text{ ns}\]

Gouri Jas
WH21 OPLSAA MD at 300 K

All MD patterns

Transition patterns
WH21 OPLSAA MD at T=300 K: Folding pathways

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WH21 OPLSAA MD at T=300 K: Folding pathways

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Average state populations – from 5 helix-coil transitions
WH21: Helix unfolding kinetics

130 milestones
13,000 trajectories
≈1μs total simulation time


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CONCLUSIONS

• Most popular force-fields give realistic predictions of helix-coil equilibria and kinetics. Best: AMBER03, CHARMM (w/o CMAP) and OPLSAA

• For the pentapeptides we find that folding pathways are force-field dependent; this could be the effect of lack of significant central core

• For WH21 folding paths are similar between AMBER03 and OPLSAA:
  - unfolding initiated at 3-4 C-terminal residues
  - also 1-2 residues at N-terminal unfold early
  - central core of residues 11-16 remains highly helical through midpoint
  - persistent hydrogen bonds occur - specific sequence effects

• MD simulation time scales have reached a biologically interesting range but special methods are still needed for slow processes

• Experimental data needed for reference
Acknowledgments

Experiments:
Gouri Jas, Baylor University.

Computer time and technical assistance:
Academic and Research Computing Services,
Baylor University

Funding:
Baylor University internal funds
Big XII Fellowship from University of Kansas
J.T. Olden Fellowship. University of Texas, Austin
WH5 Figures

[Various graphs and charts depicting data analysis results related to WH5 1,000 ns MD at 1 bar 300 K OPLSAA TIP3P]
Folding of Ac-Ala$_5$-NH$_2$ : pathways

Transitions vary in
- duration time
- path details
Folding of Ac-Ala$_5$-NH$_2$ : patterns

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- Most FF : 000 dominant, very little 111, positive h-bond cooperativity
- Populated intermediates: involve h-bonds #1 and #2
- Unusual: AMBERGS
WH5 in OPLS/AA: conformations
WH5: conformational energy (CHARMM)
• At 300 K REMD=MD
• OPLS/AA, AMBER03, AMBER99P and CHARMM22 give excellent helicity predictions at 300 K
• Helix persistence exaggerated
• AMBER99SB – anti-melting

REMD simulations: 32 replicas, 280-450 K, 30 Å cubic box with ca. 1000 waters, 100 ns NPT trajectory with GROMACS
Folding of Ac-Ala$_5$-NH$_2$ : REMD

- G43A1, G53A6 and AMBER99SB underestimate helicity
- OPLS/AA & AMBER03 closest to new data @ room T
- AMBER99P, AMBERGS, CHARMM22/CMAP over-stabilize helix
- REMD: melting not modeled well by most of the studied potentials
- Deviations from experiment $\approx$2-3 kcal/mol energy @300 K for all studied force fields

**REMDD simulations:** 32 replicas, 280-450 K, 30 Å cubic box with ca. 1000 waters, 100 ns NPT trajectory with GROMACS, for all except CHARMM potential
**CHARMM REMD:** 40 ns in 37 Å bcc cell.
Ac-Ala5-NH$_2$ MD

**MD:** 1,000 ns NPT MD at 1 atm, 300 K with GROMACS
several popular force fields, ca. 1000 waters

400 ns NPT MD at 1 atm and 300 K with CHARMM/CMAP
Alanine-based peptide folding simulations

• **Replica exchange** simulations by Garcia et al. showed exaggerated helix stability in AMBER99
  ➔ modified potential AMBER99GS

• MD **simulation** of α-helix folding kinetics by Pande also suggested the need for modified (φ,ψ) potential
  ➔ modified potential AMBER99P

• Hummer proposed that most **popular force fields over-stabilize** the α–helix structure in short Ala-based peptides
  [Best et al. *Biophys.J.* **95:**L07 (2008)]

• Based on **NMR measurements of J couplings** in Ala\(_n\)