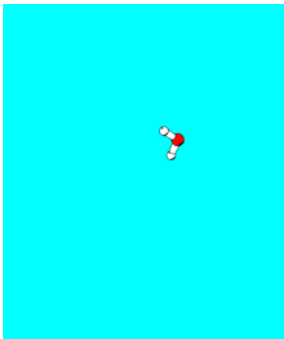


Helix folding pathways

Krzysztof Kuczera

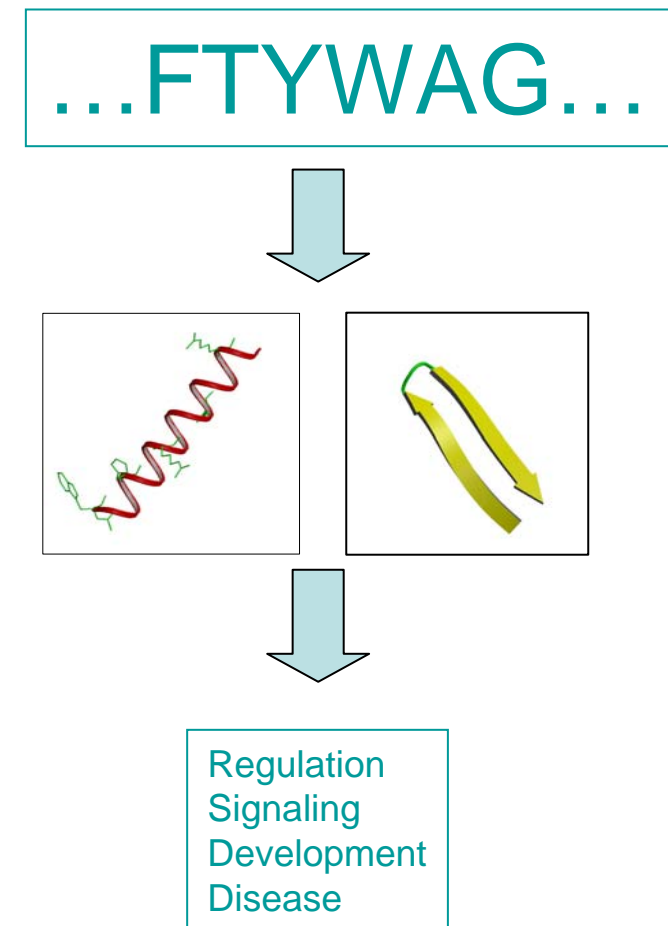
Departments of Chemistry and Molecular Biosciences, University of Kansas,
Lawrence, KS 66045



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 – μ s time scale experiment/simulation overlap
- Peptides = building blocks of proteins
→ understanding of fundamental biological processes



Peptide Folding Simulations

GOALS:

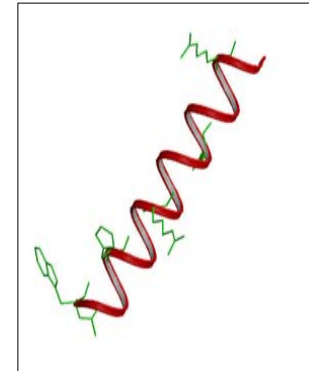
- Predict process: populations, rates, paths
- Verify methods: algorithms and force fields
- Complement experimental data
- Understanding → design materials, drugs

EXPERIMENTAL data: typically

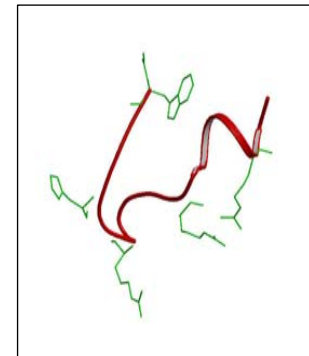
- structure and population of folded state
- folding and unfolding rates (T)
- rarely: “nucleation rate”

Unique ROLE for simulations: microscopic

- Information on pathways
- Information on unfolded state(s)
- Dynamics \perp to reaction coordinate



$\tau=300$ ns
50% α
@300 K



Folding Simulation Methods

Fast processes: ($\tau \approx 10\text{-}100\text{ ns}$)

Direct molecular dynamics (MD) gives complete description

Slow processes:

Populations:

Enhanced sampling methods

-e.g. replica-exchange MD

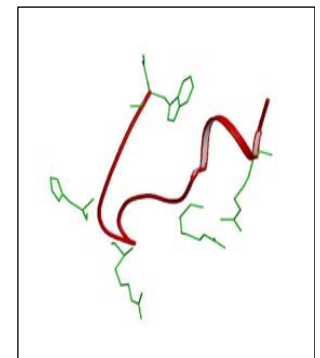
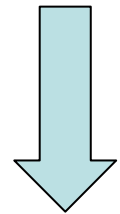
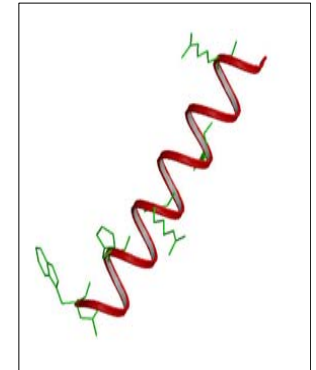
Kinetics:

Specialized algorithms

-e.g. MSM, PPTIS, Milestoning

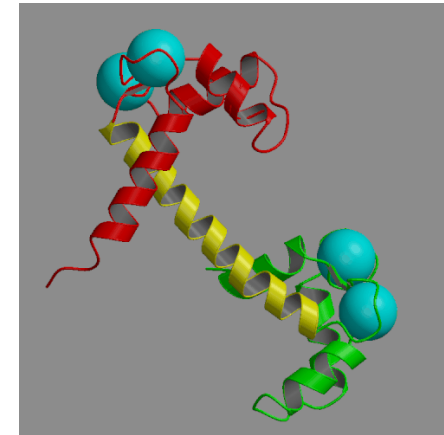
Limitations:

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



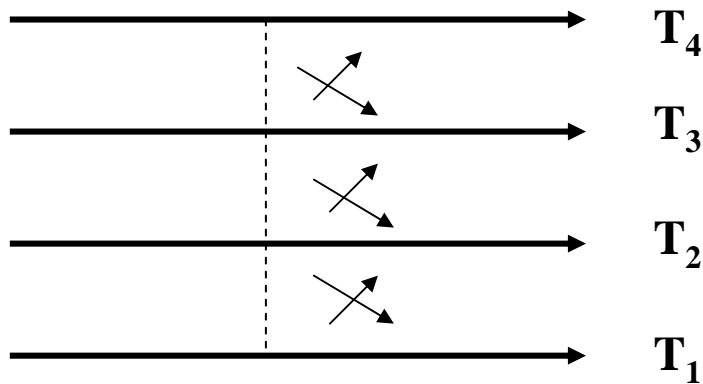
Newton's 2nd Law

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

Verlet algorithm

$$\begin{aligned} \mathbf{x}(t + \Delta t) &= 2\mathbf{x}(t) - \mathbf{x}(t - \Delta t) \\ \mathbf{v}(t) &= \frac{\mathbf{x}(t + \Delta t) - \mathbf{x}(t - \Delta t)}{2\Delta t} \end{aligned}$$

Replica-exchange molecular dynamics



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

Advantages:

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

$$w(i \rightarrow j) = 1 \quad \Delta \leq 0$$

$$w(i \rightarrow j) = e^{-\Delta} \quad \Delta > 0$$

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

$$\beta_i = \frac{1}{kT_i}$$

WH5: Fastest Folding α -helix

Experimental at 300 K:

CD spectroscopy:

% α = 20-25 %

Fluorescence T-jump:

Relaxation $\tau_1 = 5.3 \pm 1.9$ ns

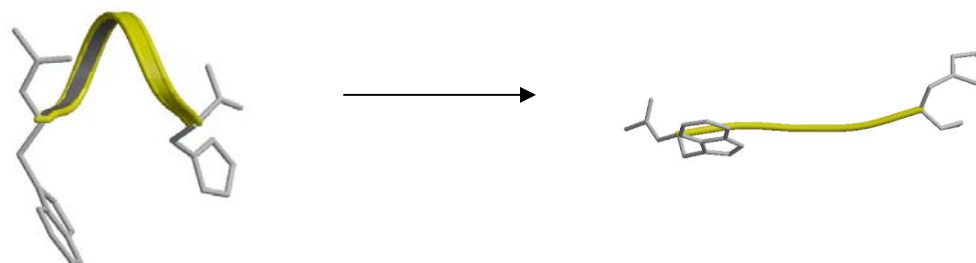
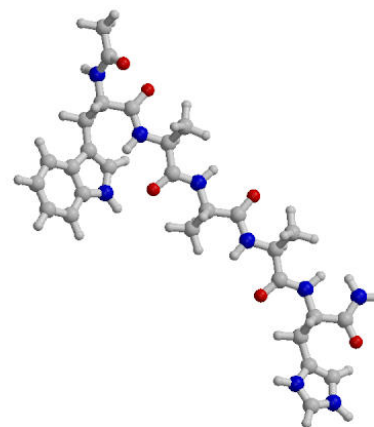
$\tau_2 = 0.85 \pm 0.3$ ns

Gouri S. Jas, Baylor University

Angewandte Chem. (2009) **48**:5628

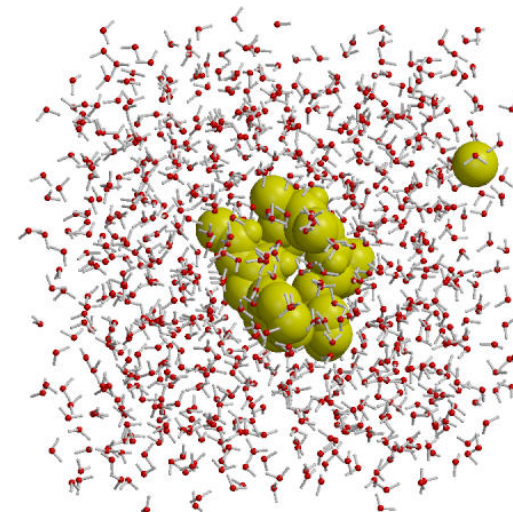
Sequence: 5 aa

Ac-Trp-Ala-Ala-Ala-His⁺-NH₂

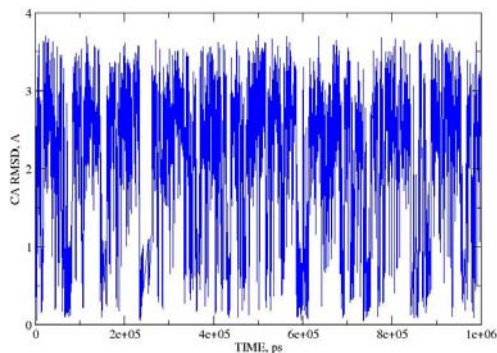


WH5 : Global MD

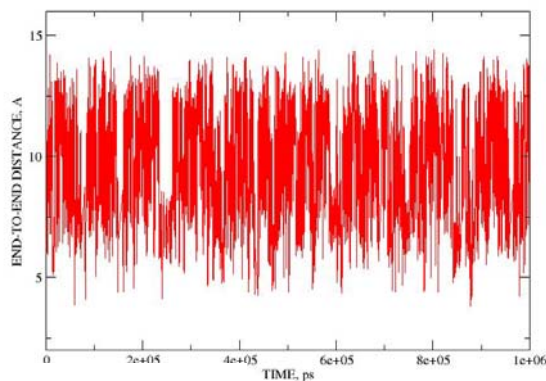
MD: 1,000 ns NPT at 300 K, 1 bar with GROMACS program and several protein force fields, ≈ 1000 waters, 1 Cl⁻
960 ns with CHARMM program and CHARMM ff



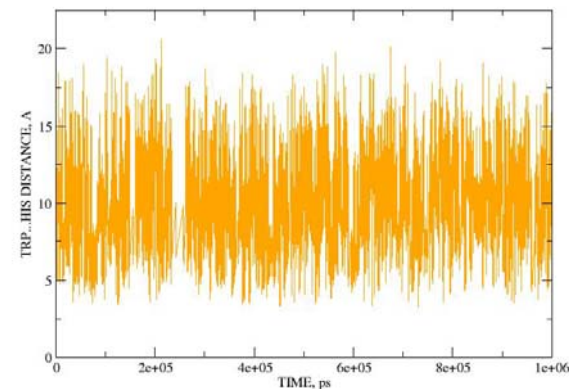
WH5 1,000 ns MD : 1 bar 300 K OPLS/AA TIP3P



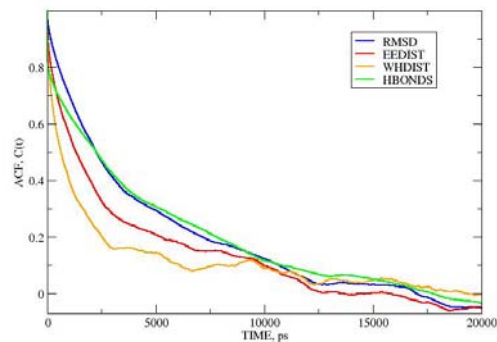
WH5 1,000 ns MD: 1 bar 300 K OPLSAA TIP3P



WH5 1,000 ns MD: 1 bar 300 K OPLSAA TIP3P

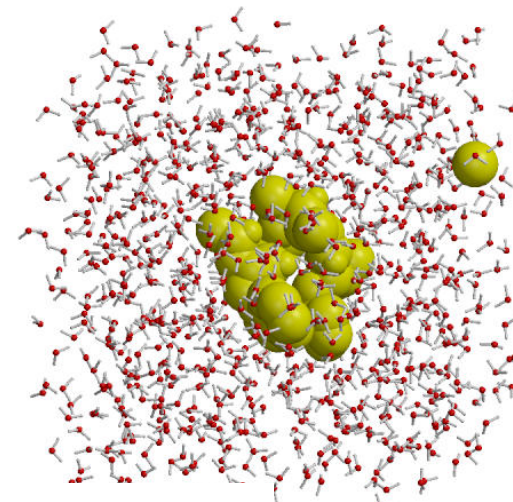


WH5 1,000 ns MD 1 bar 300 K OPLSAA TIP3P

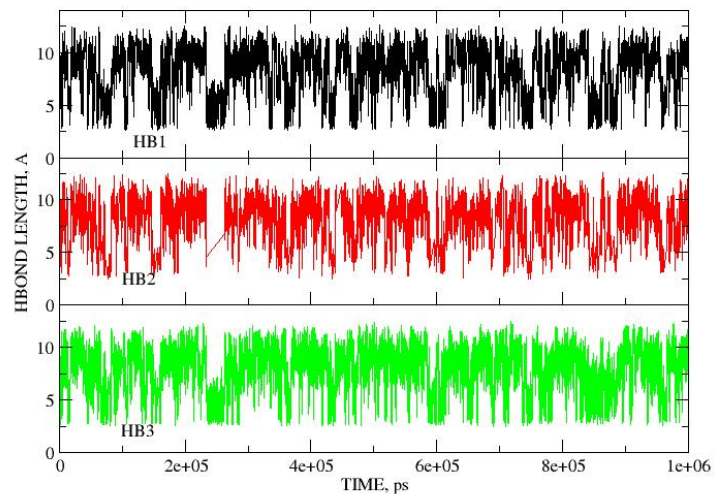


Sample OPLS/AA results

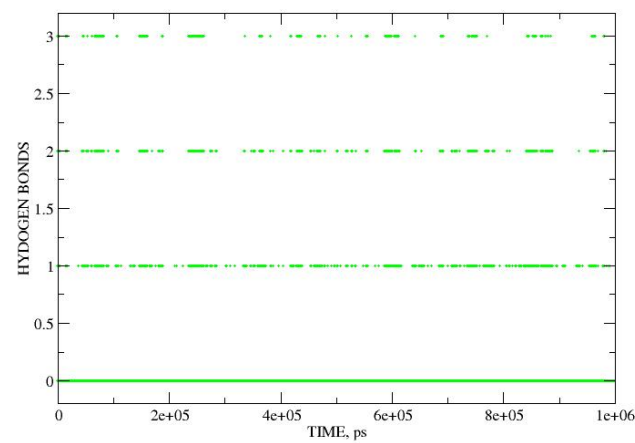
WH5 : Local MD



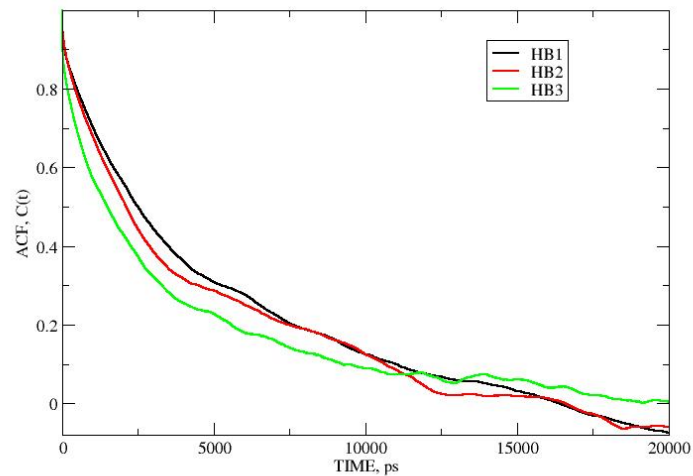
WH5 1,000 ns MD: 1 bar 300 K OPLSAA TIP3P



WH5 1,000 ns MD: 1 bar 300 K OPLSAA TIP3P



WH5 1,000 ns MD: 1 bar 300 K OPLSAA TIP3P



Sample OPLS/AA results

WH5: helix populations and kinetics

Force Field	T_{fold} ns	T_{unf} ns	T_r ns	T_{nuc} ns	% α HB	% α PP
OPLS/AA	23.	4.1	3.6	0.6	13	11
CHARMM	20.	9.7	6.5	1.0	23	21
G43A1	87.	0.8	0.8	0.1	2	8
G53A6	500.	0.4	0.4	0.05	0.4	3
AMBER03	7.1	8.0	3.8	0.4	31	27
AMBER99P	0.4	9.3	0.4	0.1	64	49
AMBER99SB	44.	3.4	3.1	0.3	6	7
AMBERGS	3.5	233.	3.5	0.1	84	65

Experiment:
% α = 20-25%

Relaxations:
5.3 and 0.8 ns

Folding:
 $T_{\text{fold}} \approx 30$ ns
 $T_{\text{unf}} \approx 6$ ns

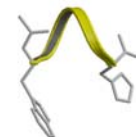
Amazing agreement:

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

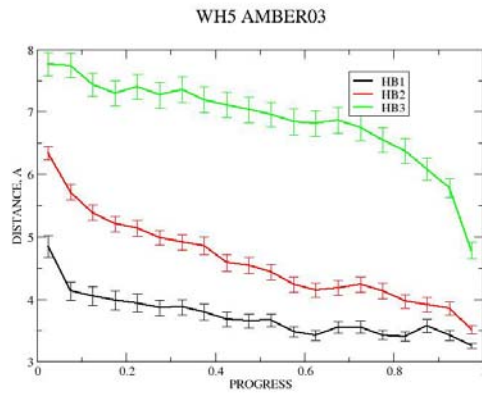
Corresponding $\Delta E \approx 1$ kcal/mol at 300 K

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

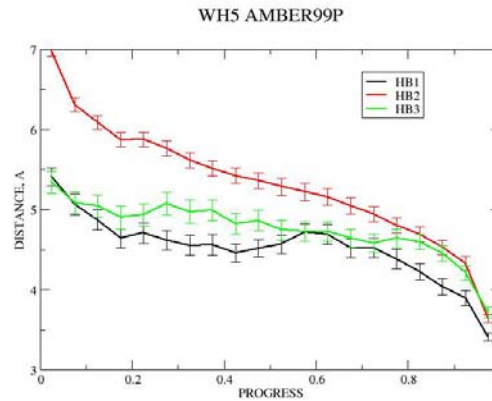
$$K = \frac{1-\alpha}{\alpha} = \frac{k_u}{k_f} = \frac{\tau_f}{\tau_u}$$



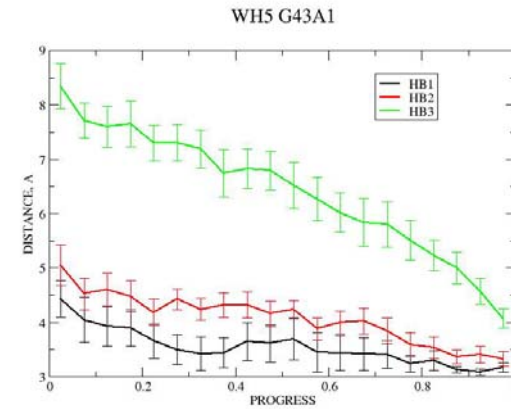
Folding of WH5: pathways



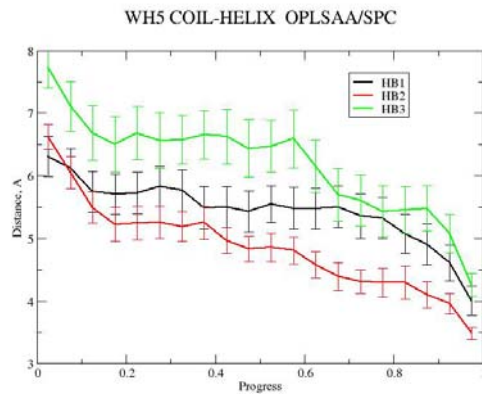
AMBER03, AMBERGS: 1-2-3



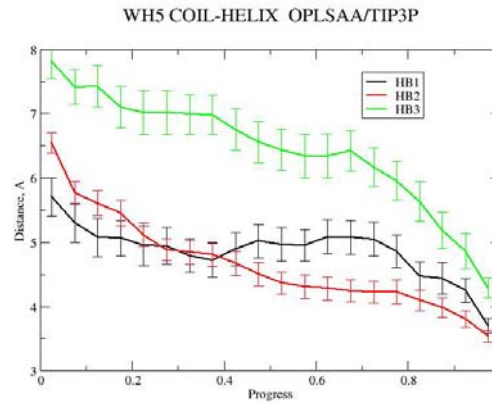
AMBER99P: (1+3)-2



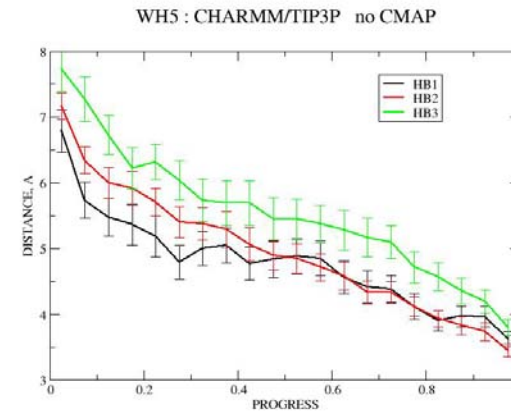
G43A1, AMBER99SB: (1+2)-3



OPLS/AA(SPC): 2-1-3

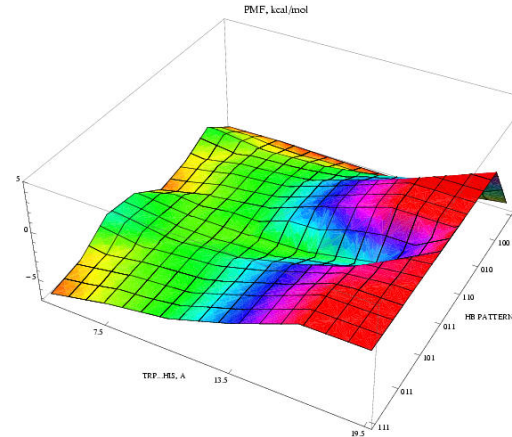
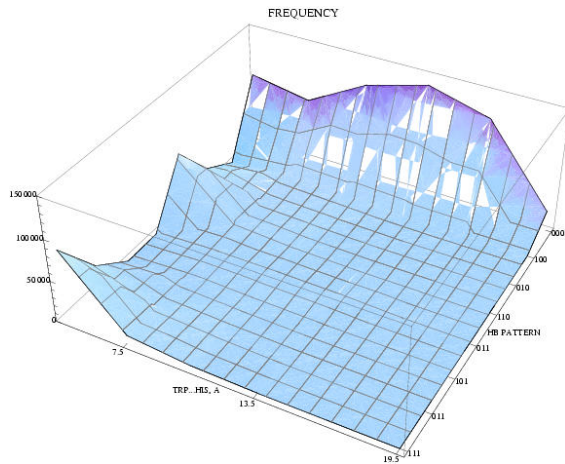


OPLS/AA(TIP3P): 2-1-3
or (1+2)-3



CHARMM: 1+2+3
or (1+2)-3

WH5: Trp...His distance (CHARMM)

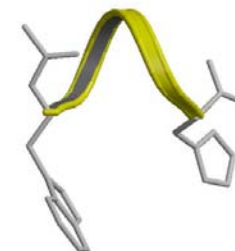
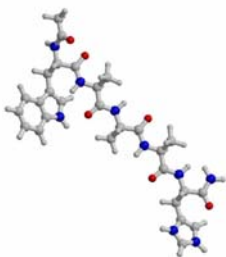


Correlations:

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

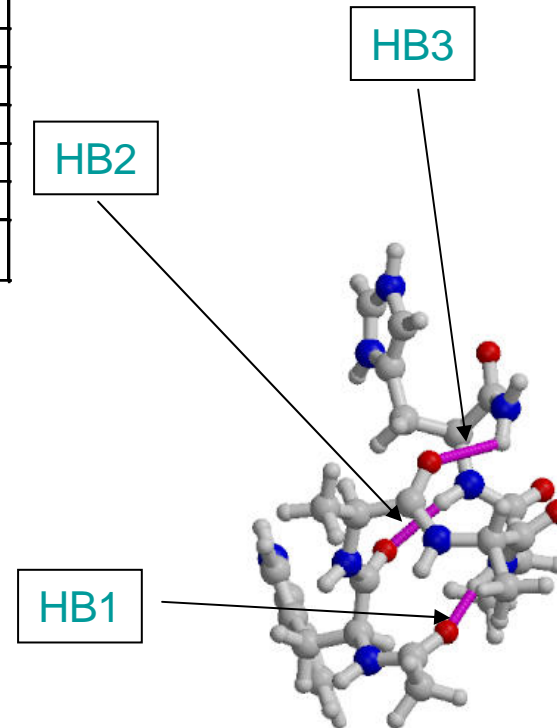
Force Field	HB1			HB2			HB3		
	τ_f	τ_u	τ_r	τ_f	τ_u	τ_r	τ_f	τ_u	τ_r
AMBER03 ^a	261	355	150	784	911	421	147	50	37
AMBER99P ^a	39	366	35	102	1066	93	36	228	31
AMBER99SB ^a	815	110	97	3258	351	317	637	53	49
AMBERGS ^a	24	853	23	148	30354	147	43	392	39
G43A1 ^a	2278	126	119	1384	88	83	3246	45	44
G53A6 ^a	2440	44	43	5460	45	45	4274	23	23
OPLS/AA ^a	1066	218	152	2768	623	508	840	148	126
OPLS/AA ^b	723	187	149	2699	762	594	496	110	90
CHARMM ^b	478	236	158	3160	1596	1060	218	122	78

^aWith SPC water ^bWith 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

Force Field	Total	Internal	Elec	vdW	PP	PS	PP:EI	PP:vdW	PS:EI	PS:vdW
AMBER03	1.8	-2.6	1.3	3.0	19.6	-29.0	16.2	6.0	-26.3	-2.7
AMBER99P	3.0	-1.7	1.2	3.4	9.3	-13.8	5.0	6.0	-11.5	-2.3
AMBER99SB	-0.9	-4.2	-0.8	4.1	20.5	-33.5	17.9	6.8	-30.3	-3.2
AMBERGS	5.0	1.6	1.5	1.9	16.4	-18.2	9.7	5.1	-15.5	-2.7
CHARMM27 ^a	6.2	0.9	4.1	1.2	23.8	-32.1	18.3	4.5	-29.9	-2.2
CHARMM22 ^b	2.9	-2.2	3.8	1.3	25.1	-39.6	22.4	4.9	-37.1	-2.5
CHARMM22 ^c	2.4	-2.3	2.7	2.0	29.2	-47.9	27.0	4.5	-45.5	-2.4
G43A1	0.3	-1.0	1.3	0.0	6.2	-12.6	5.7	1.5	-11.6	-1.0
G53A6	-12.2	-1.1	-20.8	9.7	8.6	-15.4	8.1	1.6	-14.5	-1.0
OPLS-AA ^d	1.9	-0.2	1.5	0.6	18.0	-31.1	14.5	3.7	-29.2	-1.9
OPLS-AA ^e	1.5	0.4	0.4	0.7	4.9	-7.5	3.5	1.1	-7.4	-0.1

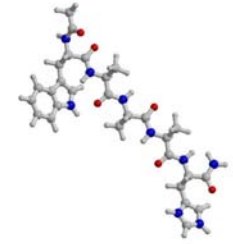
^a With CMAP, PME and GROMACS

^b Without CMAP, with PME and GROMACS

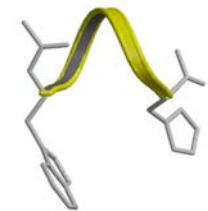
^c Without CMAP, cutoff electrostatics, with GROMACS

^d With SPC water ^e With TIP3P water

WH5: CONCLUSIONS



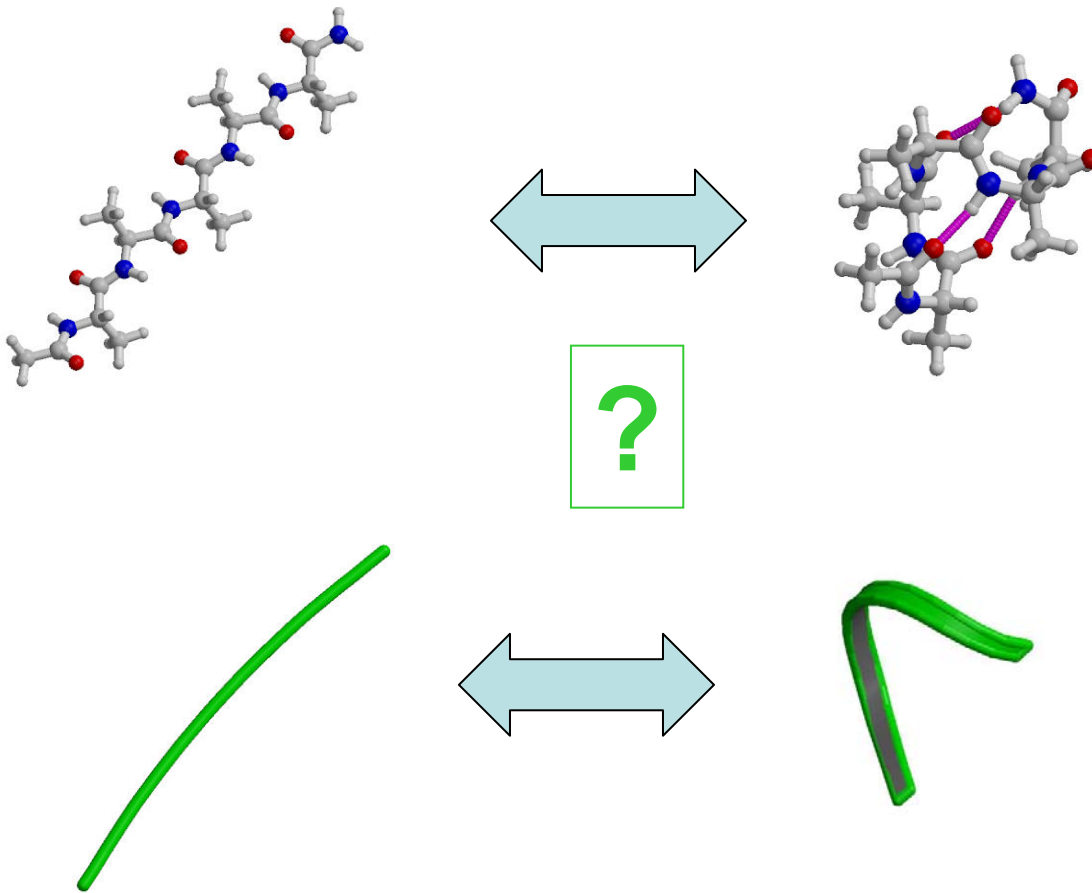
- Most popular force fields give reasonable predictions for WH5 helicity and kinetics
- Assignment of relaxations: 5 ns process → helix folding,
1 ns process → helix nucleation = formation of HB2 (or HB1+HB2)
- Force fields differ in details of predicted folding pathway; a majority suggest a “zipper” 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

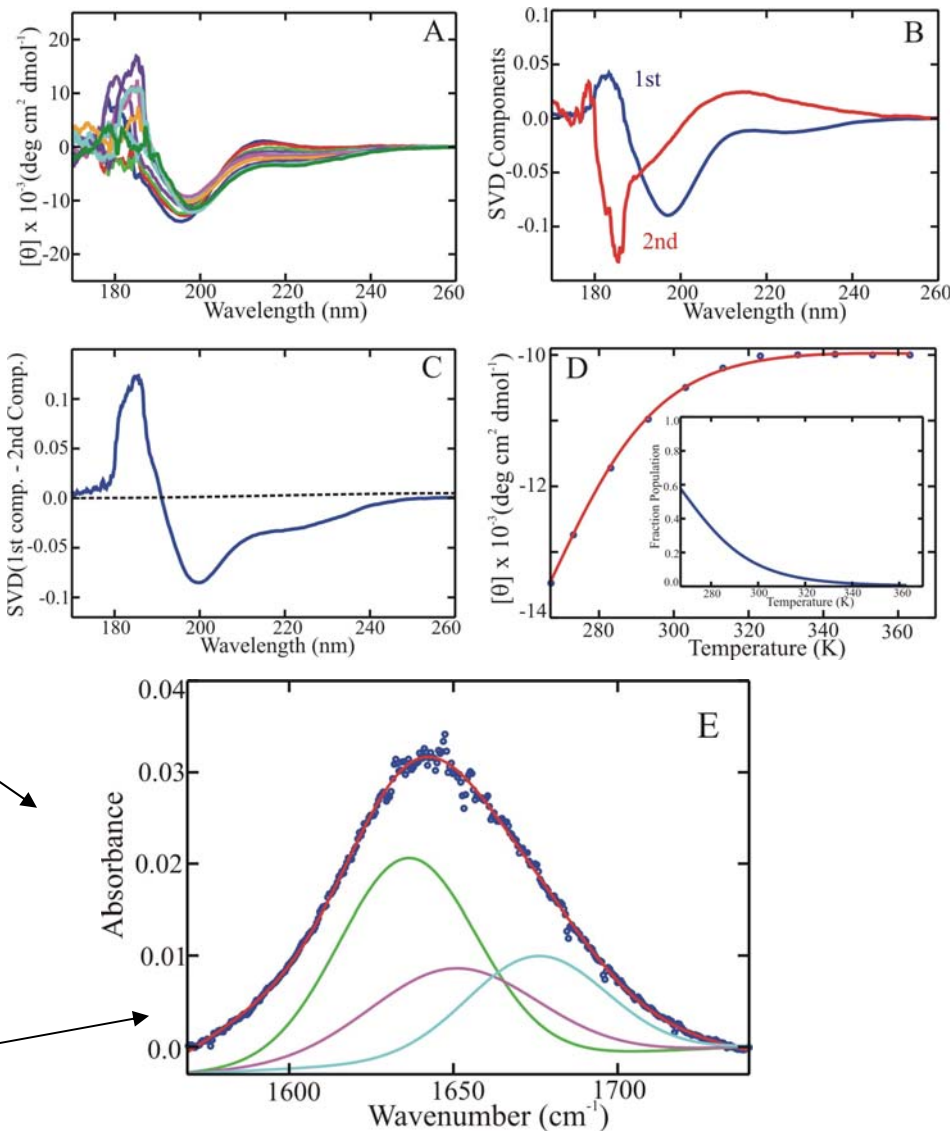


New experimental data on ac-Ala₅-NH₂

- CD of Ac-Ala₅-NH₂ over 266-363 K
→ melting transition with $T_m = 271$ K $\Delta H = 9.5$ kcal/mol
13 ± 2 % helix @300 K
- FTIR measurement of amide I peak:
26 ± 5 % helix @293 K
- New experimental data support population of α -helix @ low temperature

[Hegefeld, DeLeon, Kuczera & Jas (2010)
J.Phys.Chem.B 114:12391]

Green : α Magenta: β Cyan: turn



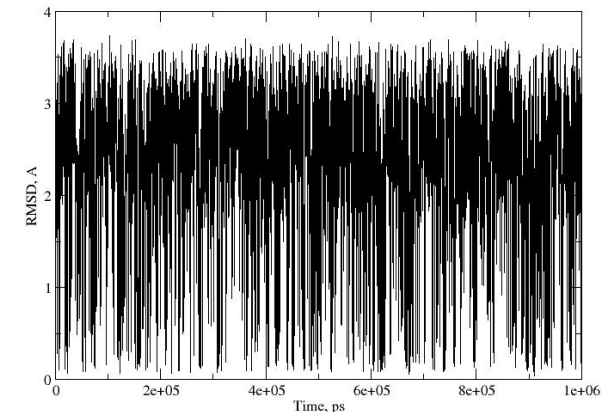
Folding of Ac-Ala₅-NH₂ : kinetics from MD

	T _{fold} ns	T _{unf} ns	T _r ns	T _{nuc} ns	% α HB	% α PP
OPLS/AA	7.2	0.6	0.6	0.1	5	9
CHARMM	6.1	5.5	2.9	0.2	37	40
G43A1	12.0	0.4	0.3	0.07	2	8
G53A6	170.	0.25	0.25	0.02	0.4	4
AMBER03	3.9	2.5	1.5	0.2	23	24
AMBER99P	0.2	16.2	0.3	0.04	39	42
AMBER99SB	4.4	0.5	0.5	0.1	2	4
AMBERGS	1.6	9.8	2.0	0.3	71	60

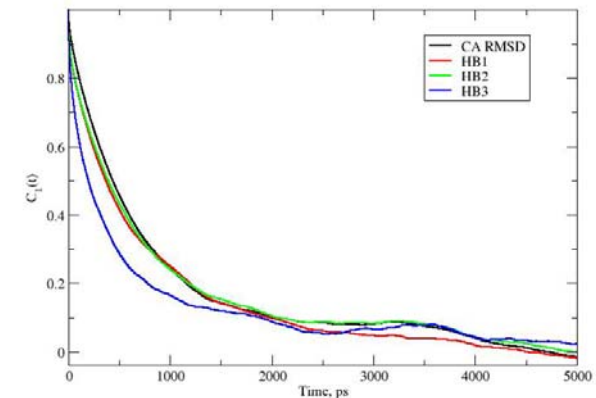
1. Predicted kinetic and equilibrium parameters span 2-3 orders of magnitude; helicities agree with exp. data
2. Helix content tends to be lower and kinetics faster compared to WH5 - consistent with W...H interaction.

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

ALA5 MD : CA RMSD FROM HELIX



ALA5 MD : TIME EVOLUTION (OPLS/AA)



Sample OPLS/AA results

Conclusions

- Helix content for most popular models is in good agreement with **new experimental data**
- Calculated folding, unfolding and nucleation rates of Ac-Ala5-NH₂ 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

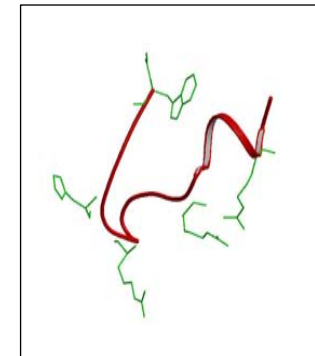
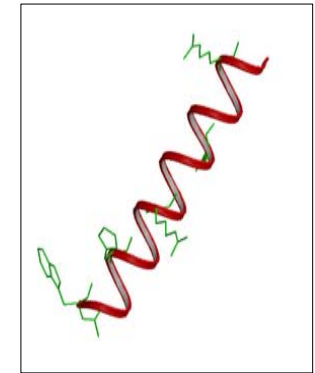
Sequence: 21 aa

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

Ac-Trp-Ala-Ala-Ala-His⁺-Ala-Ala-Ala-Arg-Ala-Ala-Ala-Ala-Arg-Ala-Ala-Ala-Ala-Arg-Ala-Ala-NH₂

Significantly more complex than WH5

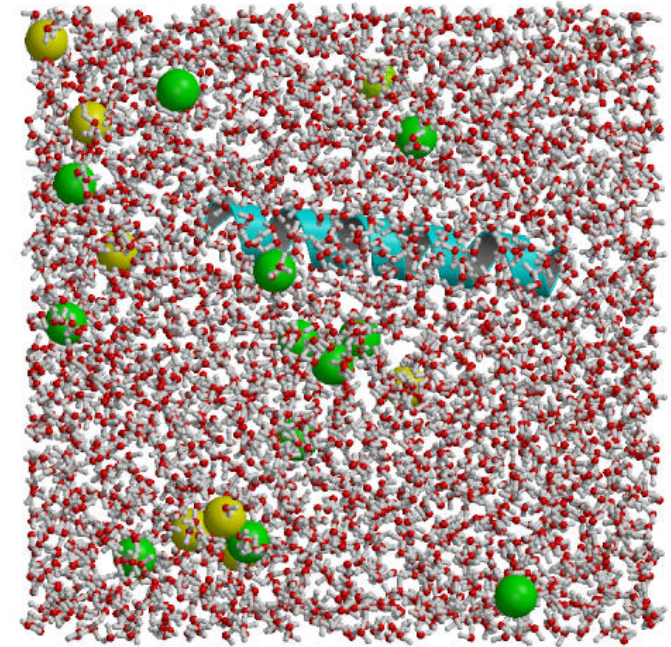
- 21 residues
- 19 hydrogen bonds
- EXPERIMENTAL:
% α = 45%, τ_r = 280 ns at 300 K



Thompson, Munoz, Jas, Henry, Eaton & Hofrichter (2000) *J.Phys.Chem.B* **104**:378
Jas & Kuczera (2004) *Biophys.J.* **87**:3786

WH21 SIMULATIONS

Potential	Type	Conditions	Length
OPLS-AA	REMD	NR=64, 280-500 K	1 μ s
	MD	NVT, 300 K, start= α	13 μ s
	MD	NVT, 300 K, start=ext	10 μ s
	MD	NPT, 300K/1bar, start=ext	8 μ s
	Milestoning	NVT, 300 K	3 paths
AMBER03	REMD	NR=50, 290-500 K	1 μ s
	MD	NVT, 320 K, start= α	17 μ s
	MD	NVT, 320 K, start=ext	5 μ s



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

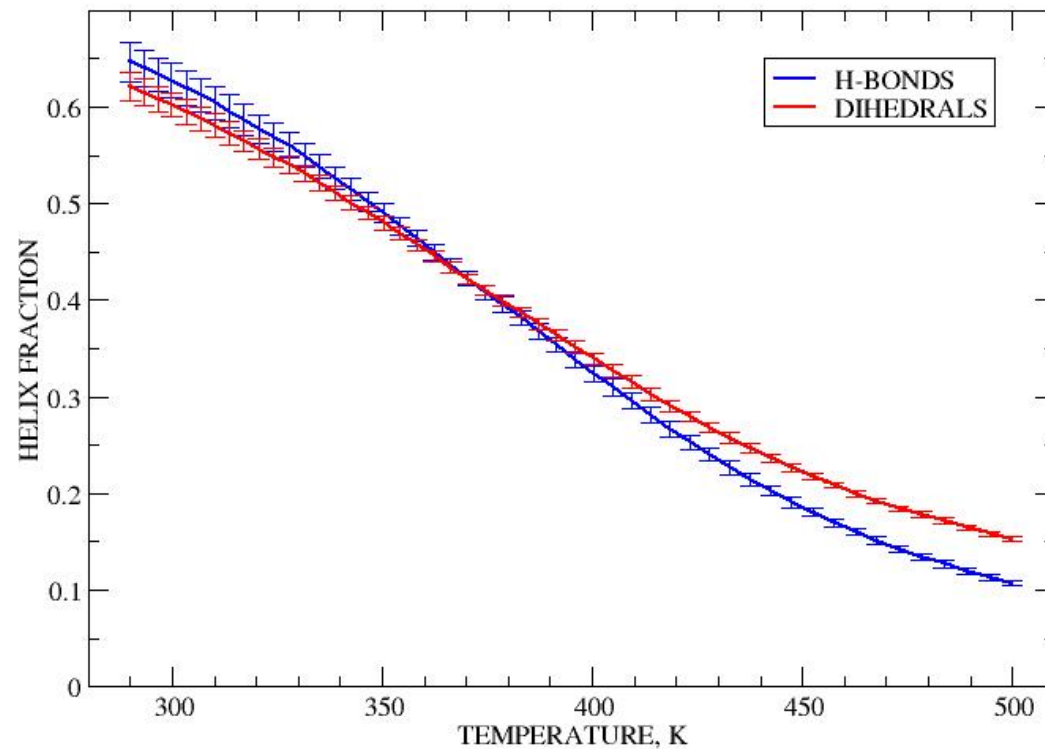
50 replicas, 290-500 K, 1 μ s
Convergence: \approx 100 ns

% α at 300 K = 63 %
 $T_m \approx$ 340 K
 $\Delta H = -3.5$ kcal/mol
 $\Delta S = -10$ cal/(mol K)

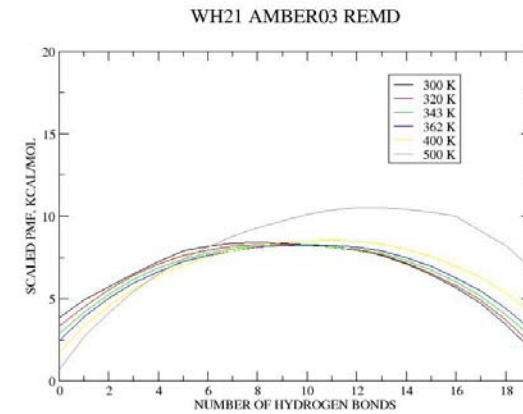
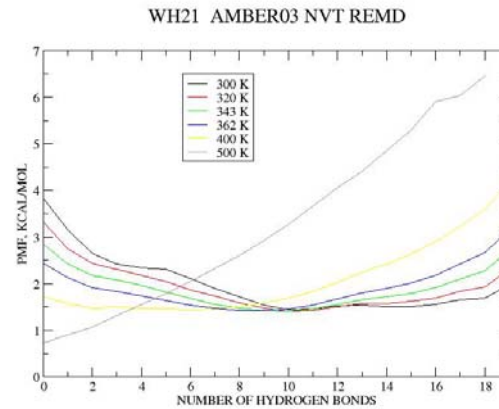
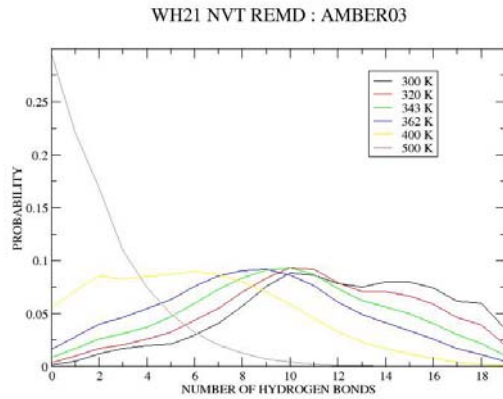
Experimental:
 $T_m = 296$ K
% α at 300 K \approx 45%
 $\Delta H = -12$ kcal/mol
 $\Delta S = -40$ cal/(mol K)

Jas & Kuczera (2004) *Biophys.J.* **87**:3786

AMBER03 Melting Curve



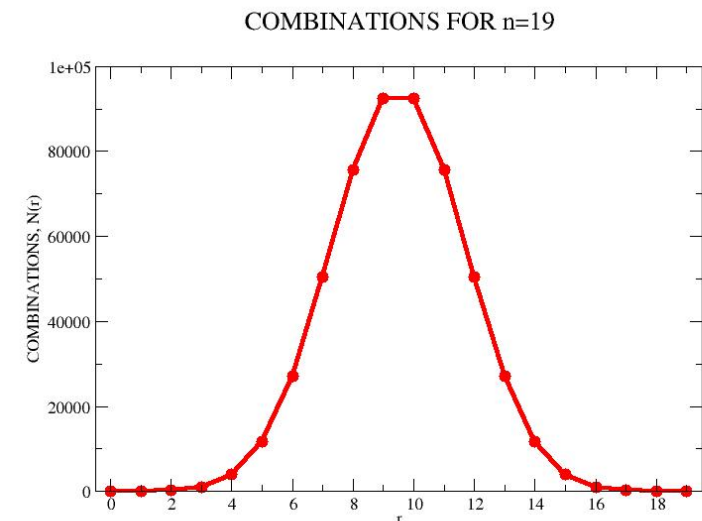
WH21: AMBER03 REMD



Microscopic insight: H-bond distributions

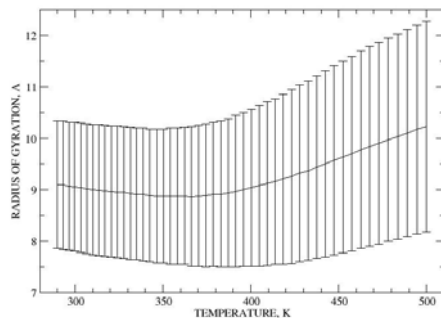
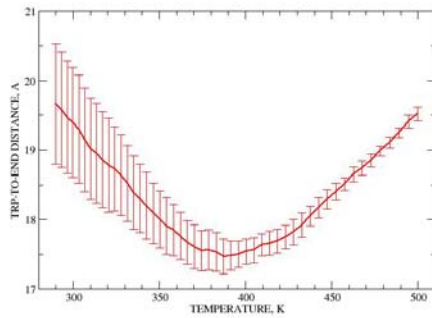
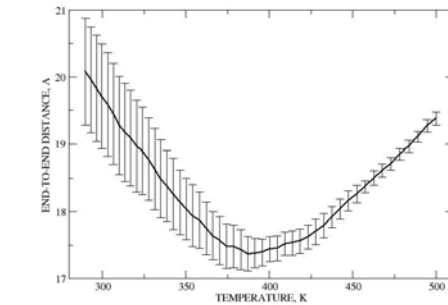
- little fully helical state NHB=19: pop 3.6 % at 300 K
- large populations of partially folded states, NHB= 6-12 are due to large number of combinations

$$N(r) = \frac{n!}{r!(n-r)!}$$

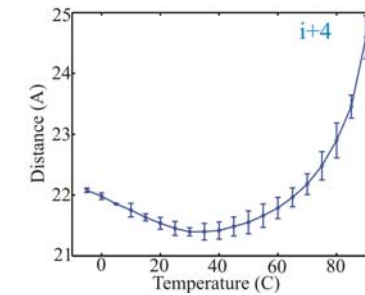
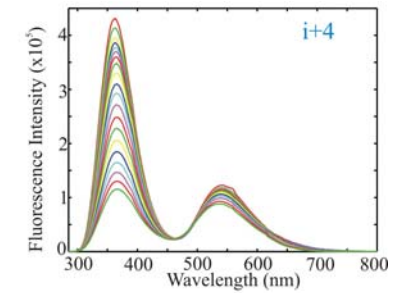
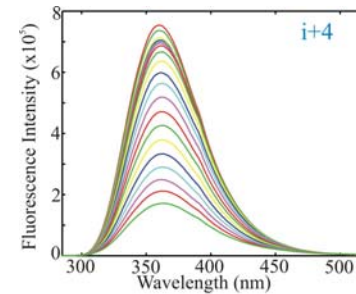


WH21 AMBER03 REMD: DISTANCE DISTRIBUTIONS

Calculated:

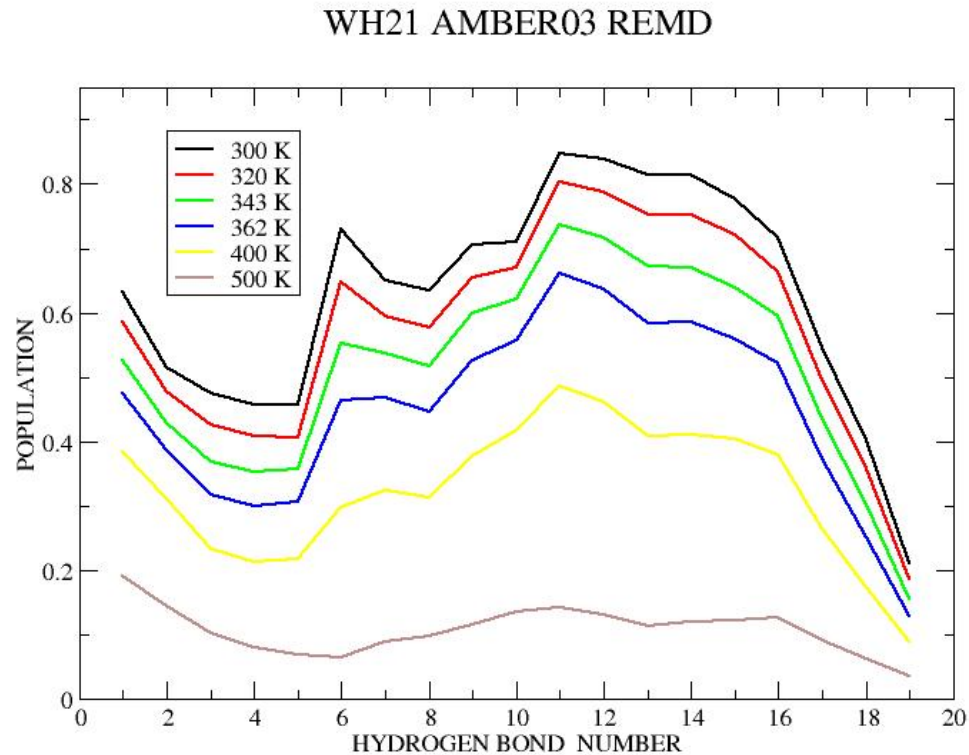


Observed: FRET experiments
by Gouri Jas & Carey Johnson



Conclusion:
Rgyr, end-to-end distance are not
good reaction coordinates for folding

WH21 AMBER03 REMD: MICROSCOPIC PICTURE OF MELTING



300 K:

Most stable:

HB1 - Trp...His effect?

HB6 - HB16 - center

Least stable:

Termini

Melting:

Roughly uniform along chain

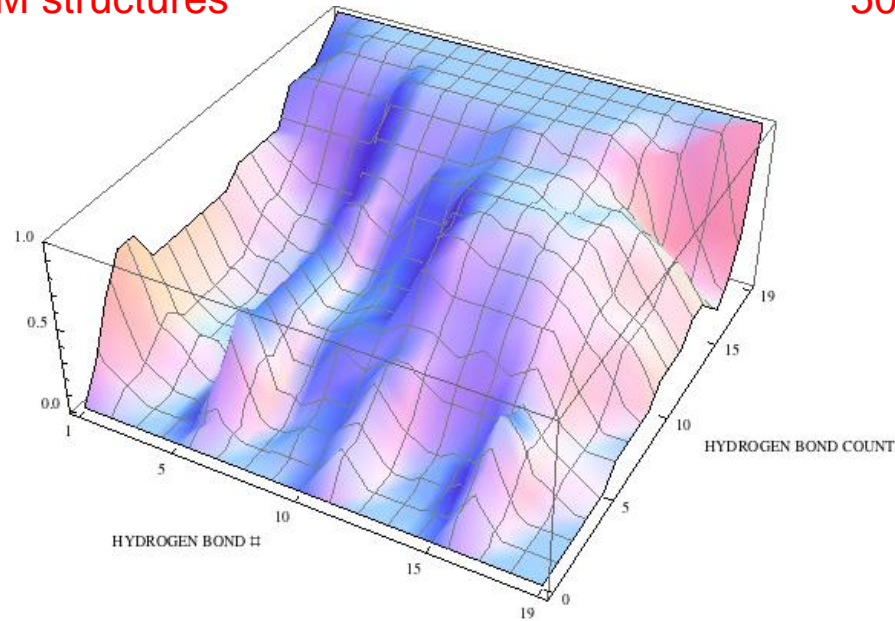
Persistence of

HB1 -- Trp...His

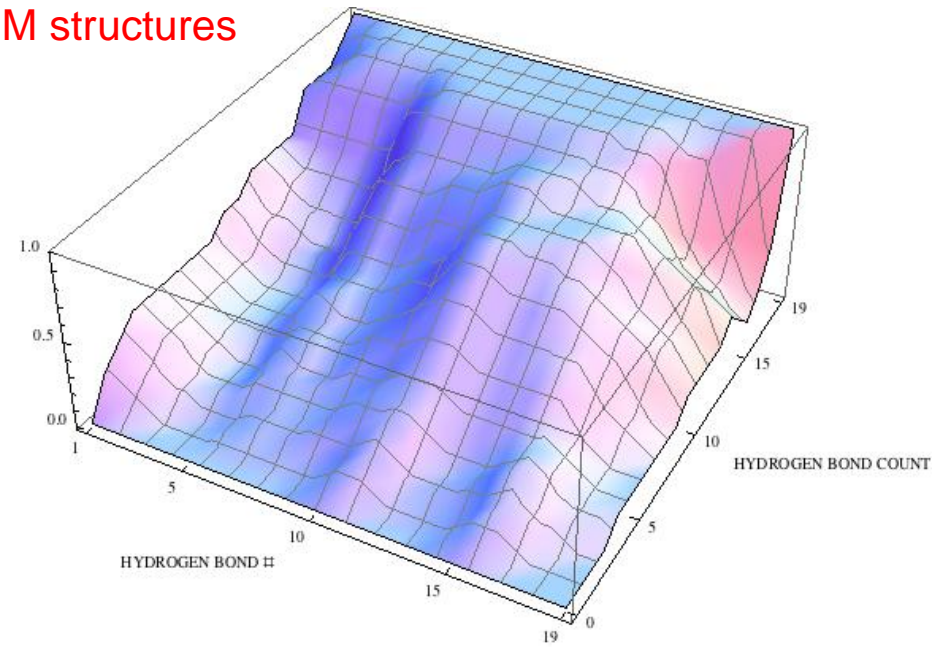
HB6, HB11, HB16 -- Arg

WH21 AMBER03 REMD: MICROSCOPIC PICTURE OF FOLDING

300 K replica:
1M structures



All data:
50 M structures



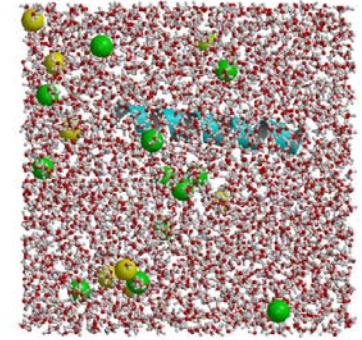
Folding:

- 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

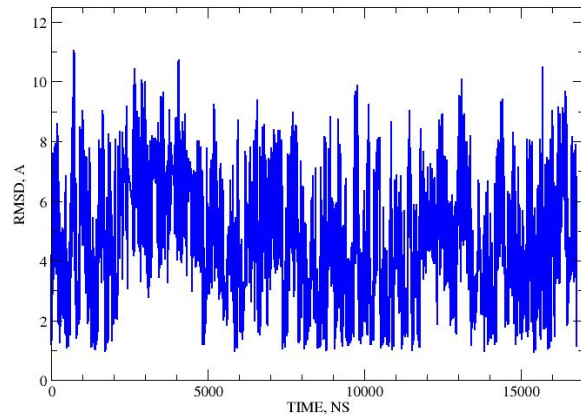
Unfolding:

- initiated at C-terminus
- passes through “island” intermediate
- persistent populations:
HB1 - Trp...His interaction ?
HB6, HB11, HB16 = [Arg - 3] ?

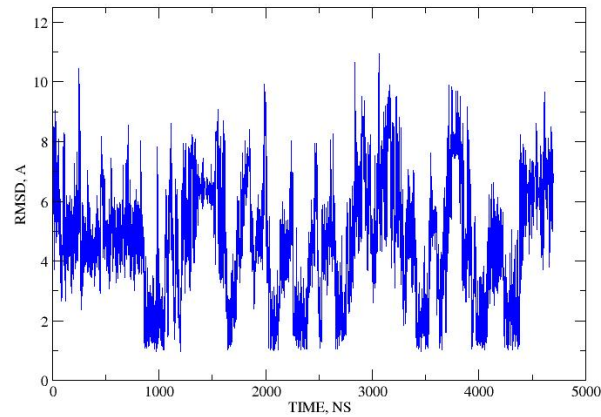
WH21 AMBER03 MD at T=320 K



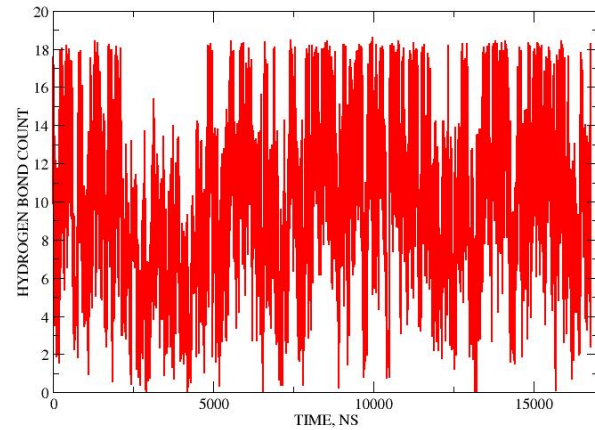
WH21 AMBER03 NVT MD T=320 K



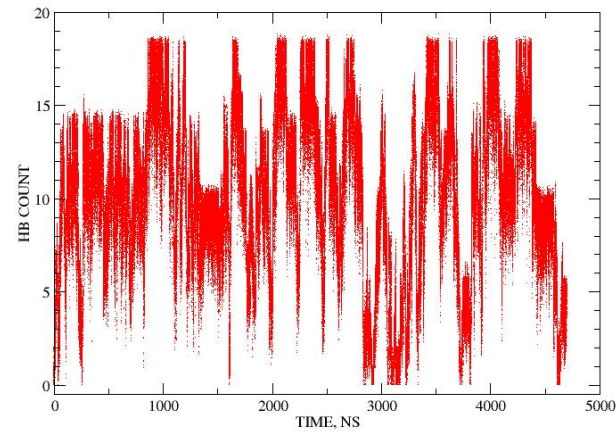
WH21 AMBER03 NVT MD, 320 K, START=EXT



WH21 AMBER03 NVT REMD T=320 K



WH21 AMBER03 NVT START=EXT



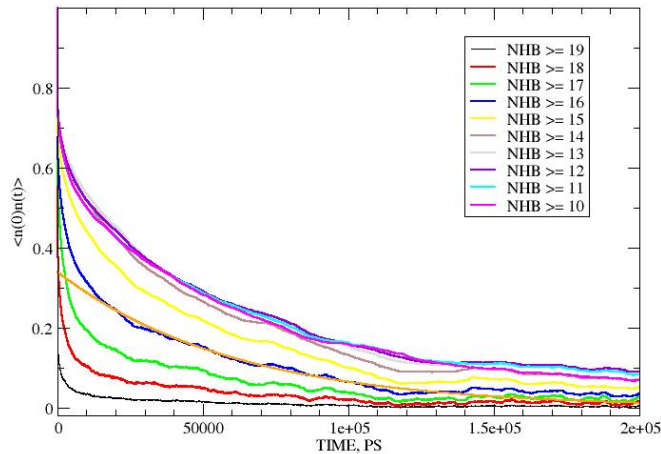
$\% \alpha = 55 \%$
Agrees with REMD

EXP: $\% \alpha = 20 \%$

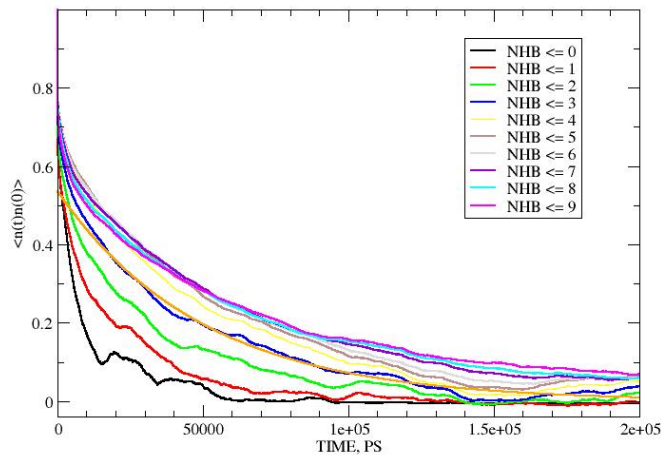
Multiple transitions

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

WH21 AMBER03 MD 320 K : SITE RELAXATIONS



WH21 AMBER03 MD 320 K : SITE CORRELATIONS



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 $\langle n(t)n(0) \rangle$

$$\tau_r \approx 60\text{-}80 \text{ ns}$$

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

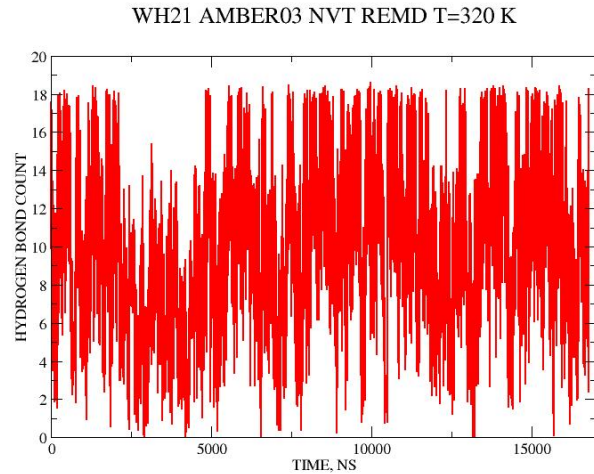
Experimental: at 320 K

$$\tau_r = 90 \text{ ns}$$

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

Gouri Jas, unpublished

WH21 AMBER03 MD at T=320 K: Folding kinetics II

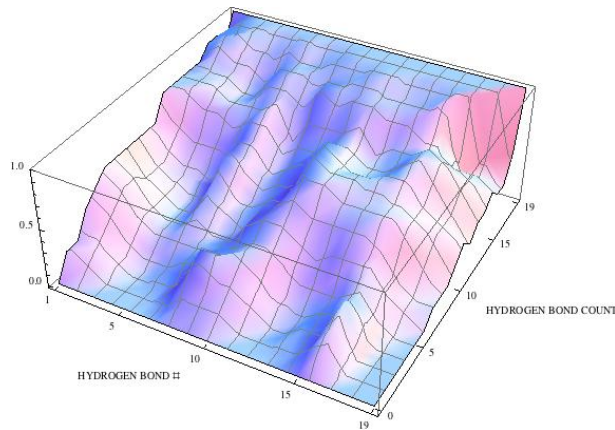


Folding/unfolding times from NHB(t)

result depends on definition of “helix” and “coil”

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

For helix = {NHB \geq 16} and coil = {NHB \leq 3}
 $\tau_f \approx 80$ ns ; $\tau_u \approx 90$ ns, [$\tau_r \approx 45$ ns]



Experimental: at 320 K

$\tau_r = 90$ ns

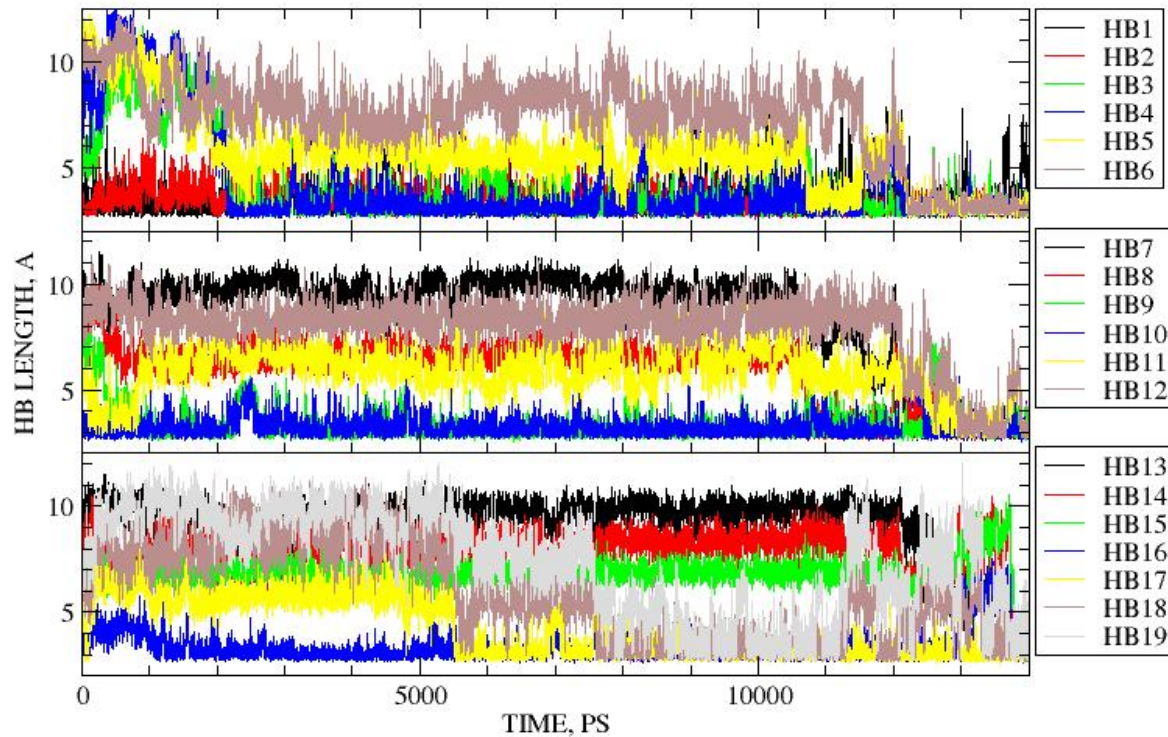
[$\tau_f = 120$ ns, $\tau_u = 450$ ns]

Gouri Jas, unpublished

AMBER03 MD: Transition patterns

WH21 AMBER03 MD at T=320 K: Folding pathways

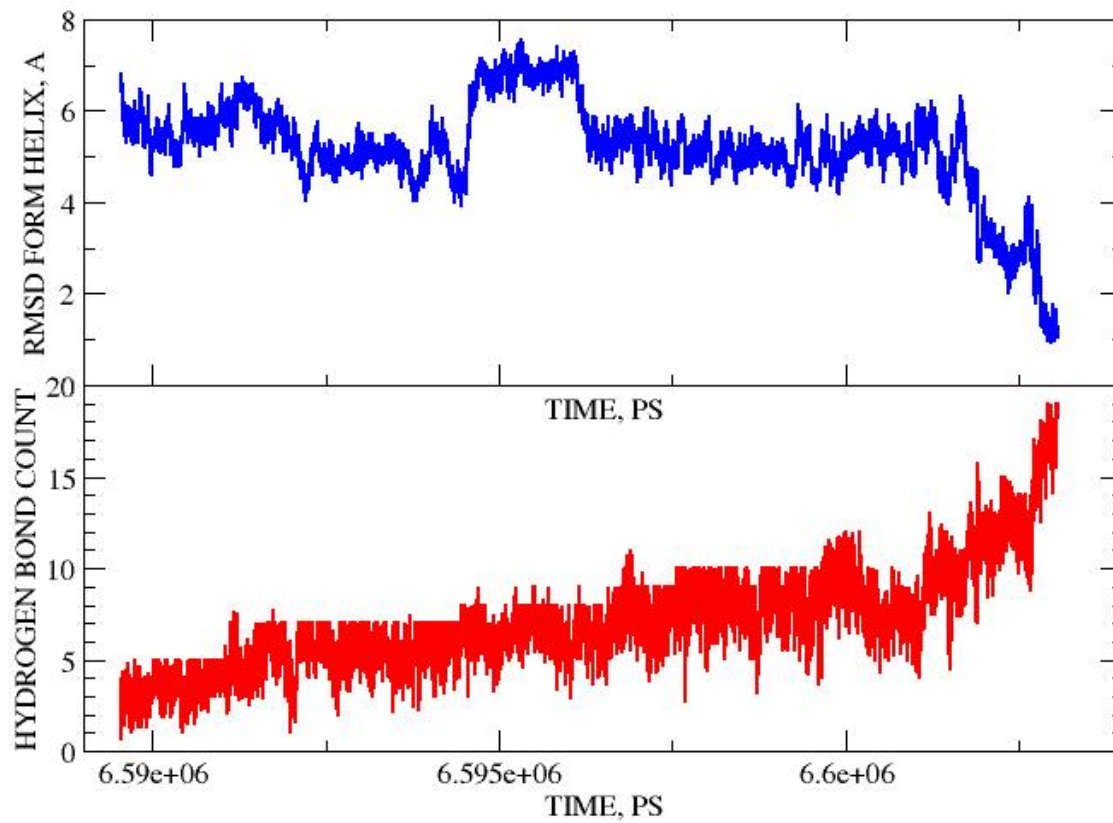
WH21 AMBER03 MD 320 K CH TRANSITION #6



- ≈ 1 “full” fold + 1 “full” unfold event per 1μ 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



WH21 AMBER03 MD at T=320 K: Folding pathways

Helix-Coil transition #2

HC	2	1059124.0	0	000000000000000000
HC	2	1053953.0	1	010000000000000000
HC	2	1053952.0	2	110000000000000000
HC	2	1053950.0	3	110000000000001000
HC	2	1053949.0	4	110000001000001000
HC	2	1053899.0	5	110000001100001000
HC	2	1053890.0	6	110000001110001000
HC	2	1053837.0	7	110000001110001001
HC	2	1052870.0	8	110000001100001111
HC	2	1052839.0	9	110000001110001111
HC	2	1050948.0	10	111000001110001111
HC	2	1050929.0	11	111100001110001111
HC	2	1001195.0	12	111000000011111111
HC	2	996471.0	13	111100000011111111
HC	2	951294.0	14	111111100010011111
HC	2	949322.0	15	111111110010011111
HC	2	925649.0	16	111111110001111111
HC	2	916900.0	17	111111110011111111
HC	2	916890.0	18	111111111011111111
HC	2	875022.0	19	111111111111111111

Helix-Coil transition #4

HC	4	2285609.0	0	000000000000000000
HC	4	2285607.0	1	010000000000000000
HC	4	2285605.0	2	010000000010000000
HC	4	2285592.0	3	110000000010000000
HC	4	2285397.0	4	111000000010000000
HC	4	2285365.0	5	111100000010000000
HC	4	2285356.0	6	111110000010000000
HC	4	2285315.0	7	111110000010001000
HC	4	2285270.0	8	111111000010001000
HC	4	2285264.0	9	111111100010001000
HC	4	2278612.0	10	110000000011111110
HC	4	2278477.0	11	110000000011111111
HC	4	2277818.0	12	110001000011111111
HC	4	2134670.0	13	111110001111111100
HC	4	2134655.0	14	111110001111111100
HC	4	2133817.0	15	011111111111111100
HC	4	2133780.0	16	111111111111111100
HC	4	2132998.0	17	111111111111111100
HC	4	2131534.0	18	111111111111111110
HC	4	2131533.0	19	111111111111111111

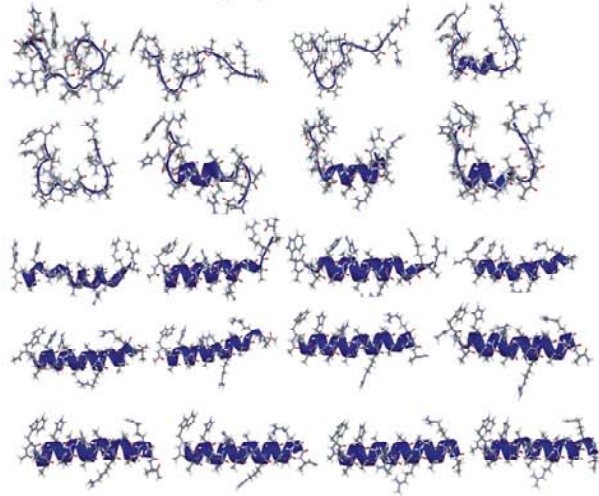
Helix-Coil transition #6

HC	6	6511886.0	0	000000000000000000
HC	6	6511885.0	1	100000000000000000
HC	6	6511879.0	2	110000000000000000
HC	6	6511876.0	3	111000000000000000
HC	6	6511854.0	4	101110000000000000
HC	6	6511853.0	5	111110000000000000
HC	6	6511848.0	6	111111000000000000
HC	6	6508281.0	7	111111000000001000
HC	6	6508241.0	8	111111000000001100
HC	6	6505314.0	9	111111110000000000
HC	6	6504490.0	10	111111111000000000
HC	6	6503388.0	11	111111111100000000
HC	6	6500026.0	12	111111111100010000
HC	6	6499580.0	13	111111111110001100
HC	6	6496756.0	14	111111111111001100
HC	6	6496381.0	15	111111111111011100
HC	6	6496320.0	16	111111111111111100
HC	6	6495087.0	17	111111111111111100
HC	6	6495024.0	18	111111111111111110
HC	6	6494990.0	19	111111111111111111

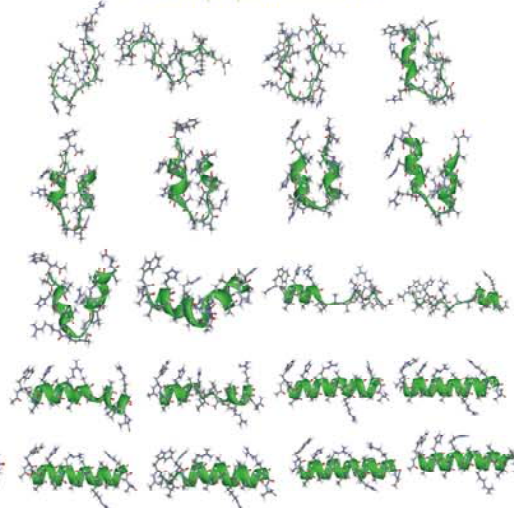
Following the time of last vist

Structural Transitions (Amber03)

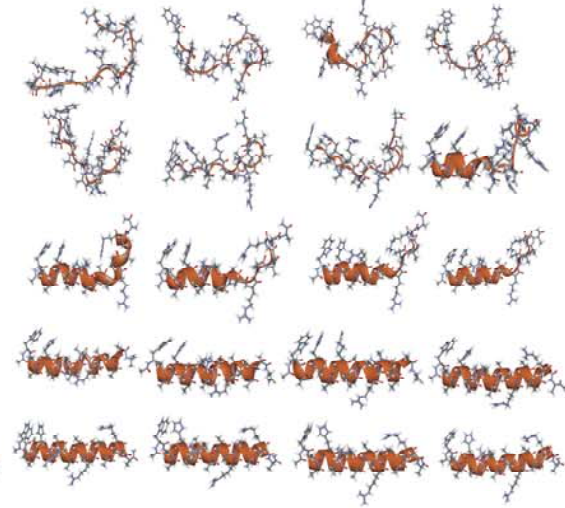
Amber03 (ch2): Coil \rightleftharpoons Helix



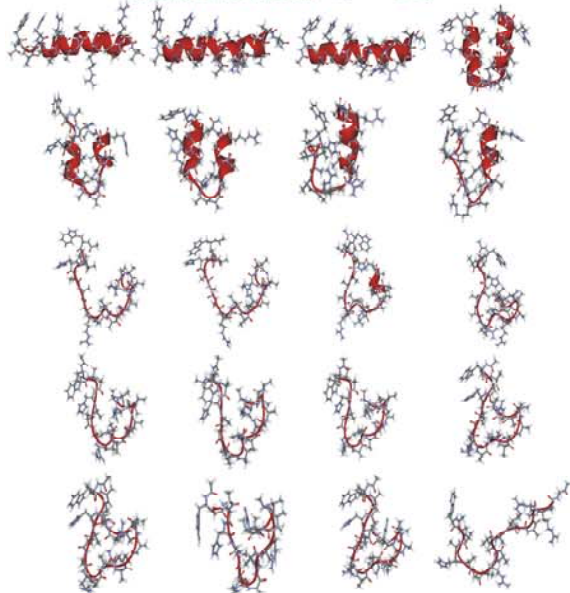
Amber03 (ch4): Coil \rightleftharpoons Helix



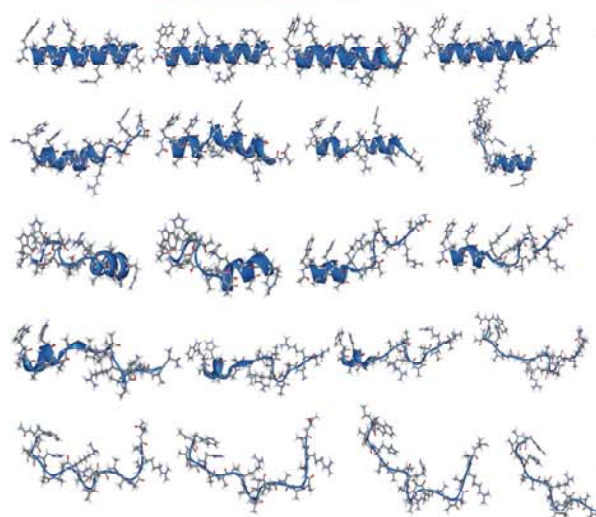
Amber03 (ch6): Coil \rightleftharpoons Helix



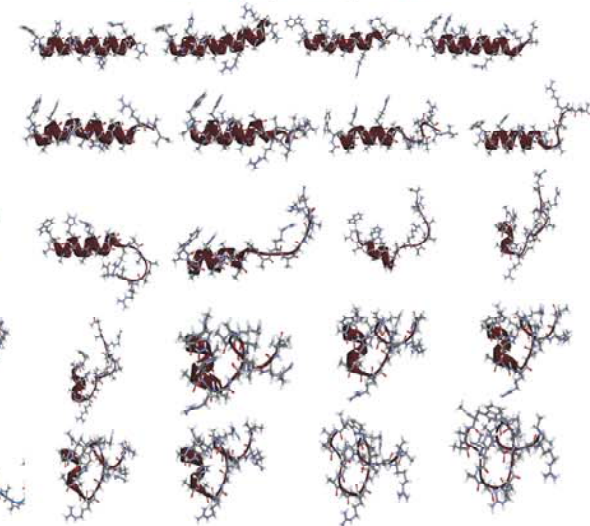
Amber03 (hc2): Helix \rightleftharpoons Coil



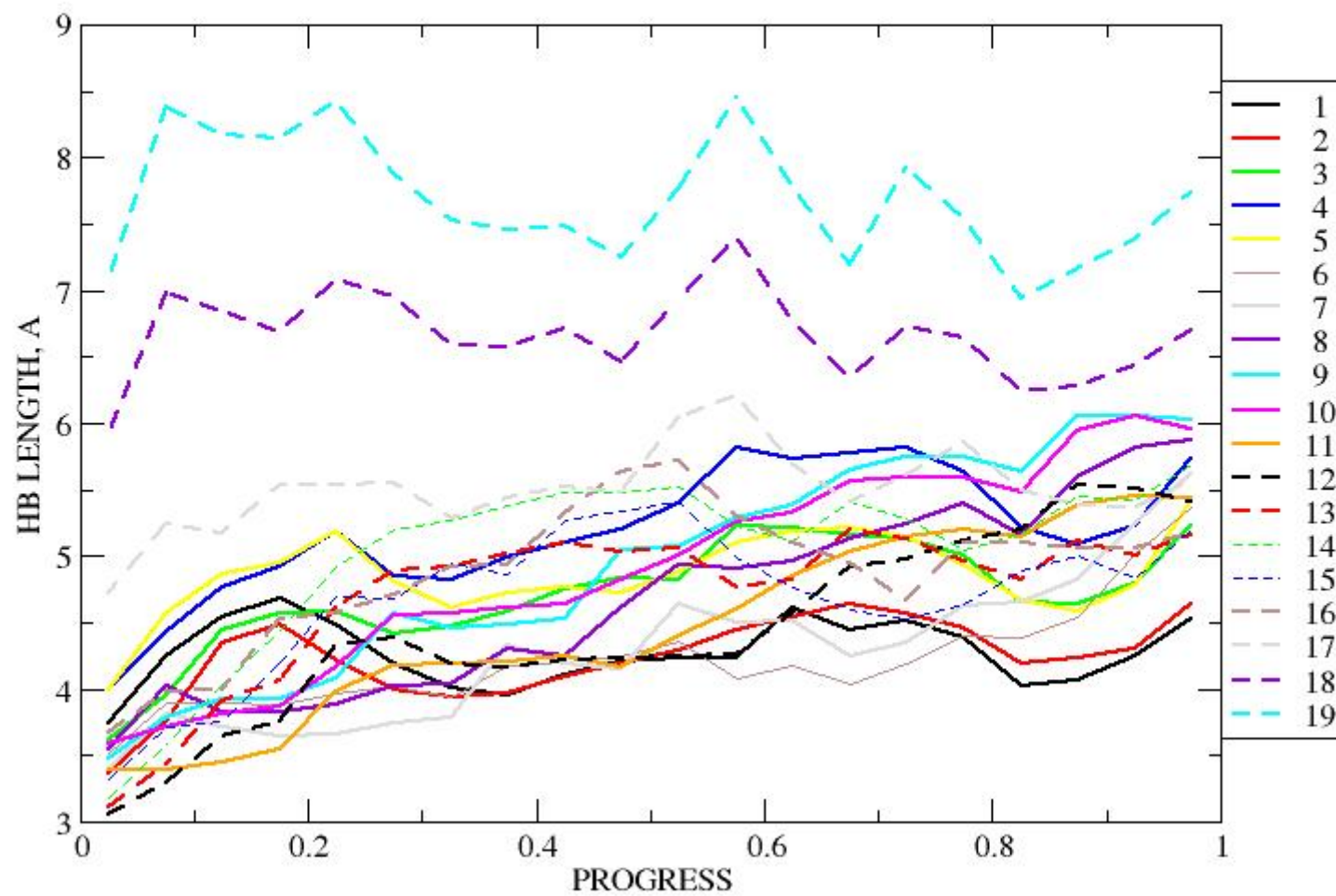
Amber03 (hc4): Helix \rightleftharpoons Coil



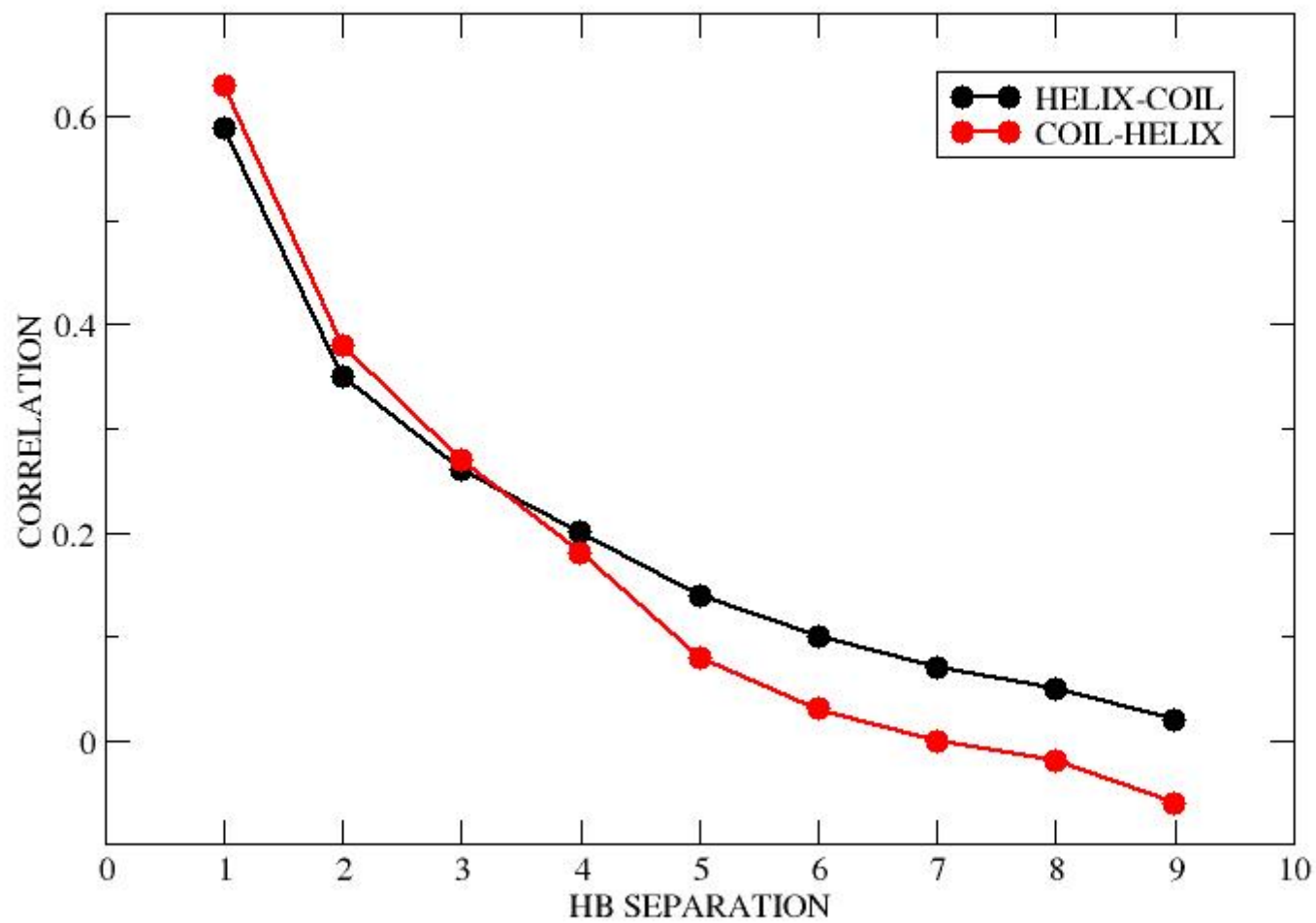
Amber03 (hc6): Helix \rightleftharpoons Coil



WH21 AMBER03 MD AT 320 K

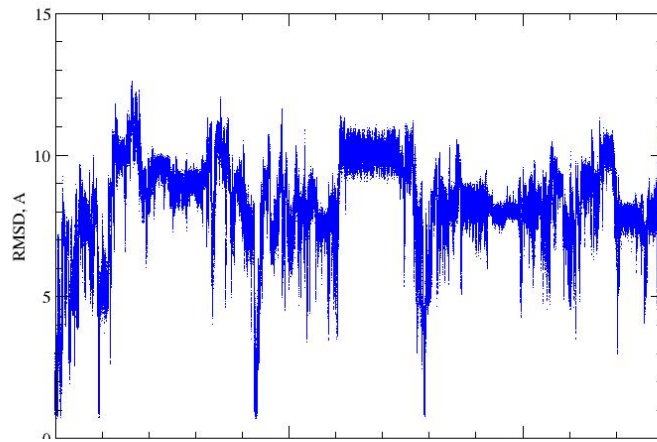


WH21 AMBER03 MD at 320 K: TRANSITION CORRELATIONS



WH21 OPLSAA MD at T=300 K

WH21 OPLS/AA MD 300 K START=HELIX



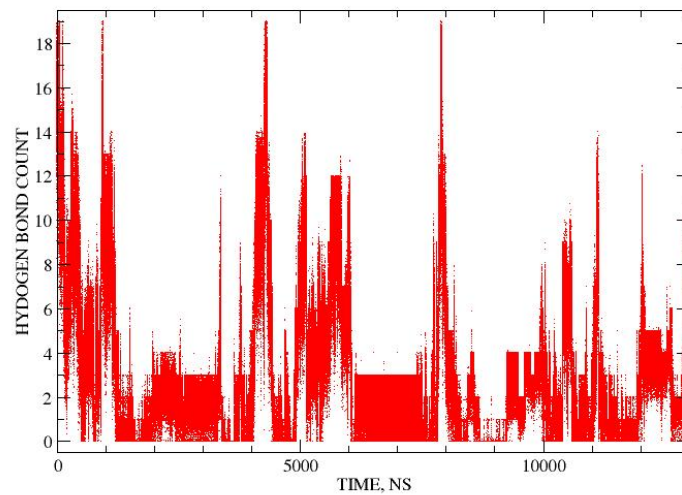
MD:

% α = 15%

Exp:

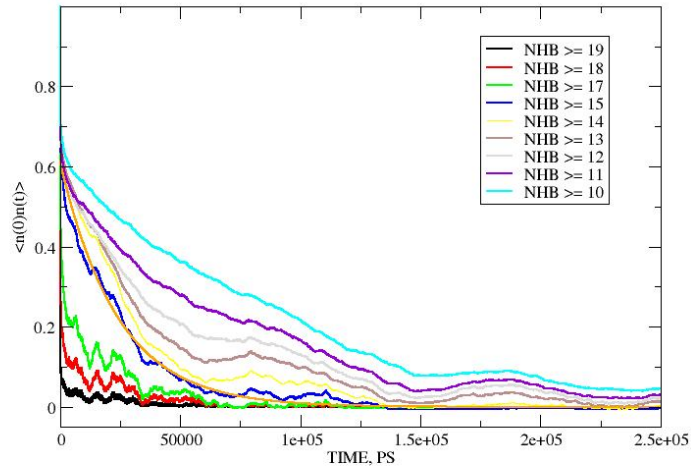
% α = 45%

WH21 OPLS/AA MD 300 K, START=HELIX



WH21 OPLSAA MD at T=300 K: Folding kinetics I

WH21 OPLSAA MD 300 K : SITE RELAXATION

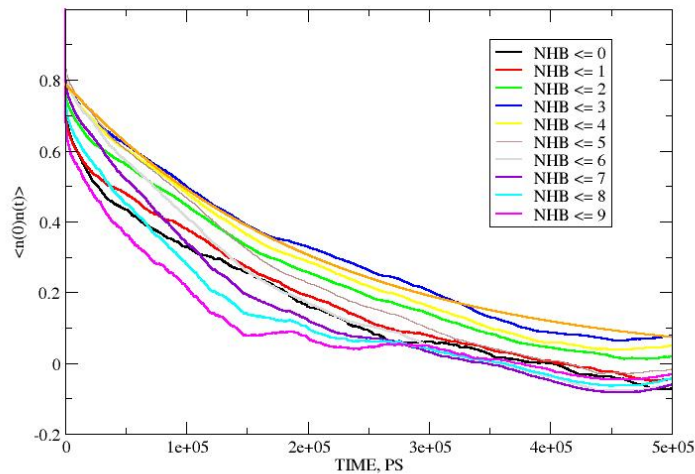


Autocorrelation functions of global variables:

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

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

WH21 OPLSAA MD 300 K : SITE CORRELATIONS



Site-site correlations $\langle n(t)n(0) \rangle$

$$\tau_r \approx 50\text{-}90 \text{ ns}$$

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

Experimental: at 300 K

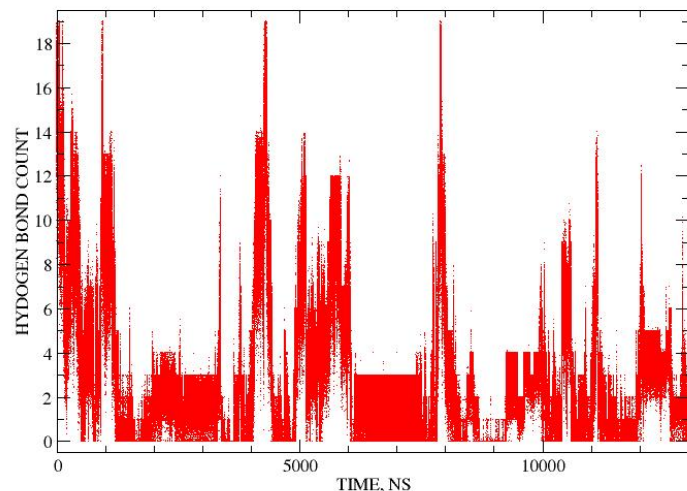
$$\tau_r = 280 \text{ ns}$$

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

Gouri Jas

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

WH21 OPLS/AA MD 300 K, START=HELIX



Folding/unfolding times from NHB(t)

result depends on definition of “helix” and “coil”

For helix = {NHB=19} and coil = {NHB=0}

$\tau_f \approx 200\text{-}300\text{ ns}$; $\tau_u \approx 3.1\text{ }\mu\text{s}$, [$\tau_r \approx 200\text{ ns}$]

For helix = {NHB \geq 16} and coil = {NHB \leq 3}

$\tau_f \approx 60\text{-}80\text{ ns}$; $\tau_u \approx 2.0\text{ }\mu\text{s}$, [$\tau_r \approx 70\text{ ns}$]

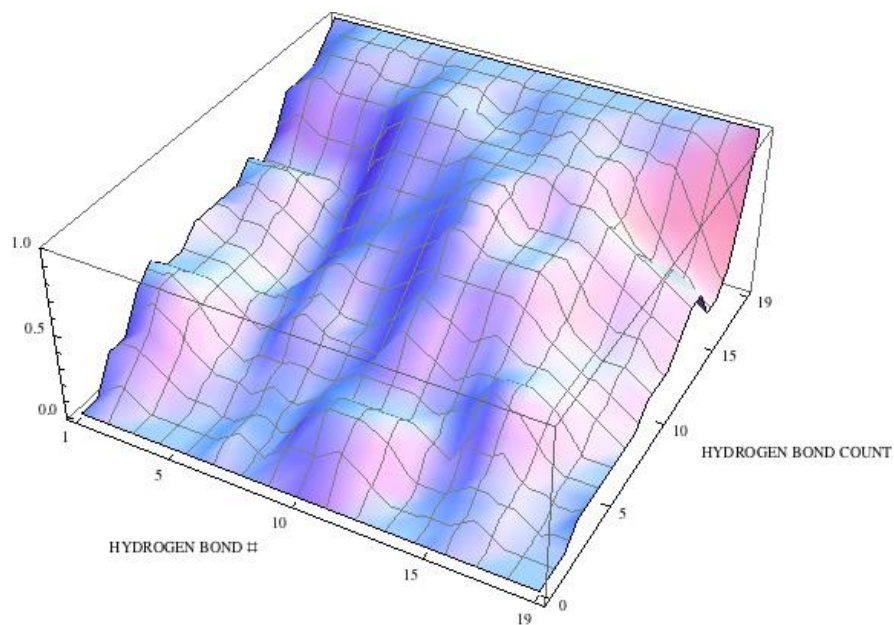
Experimental: at 300 K

$\tau_r = 280\text{ ns}$

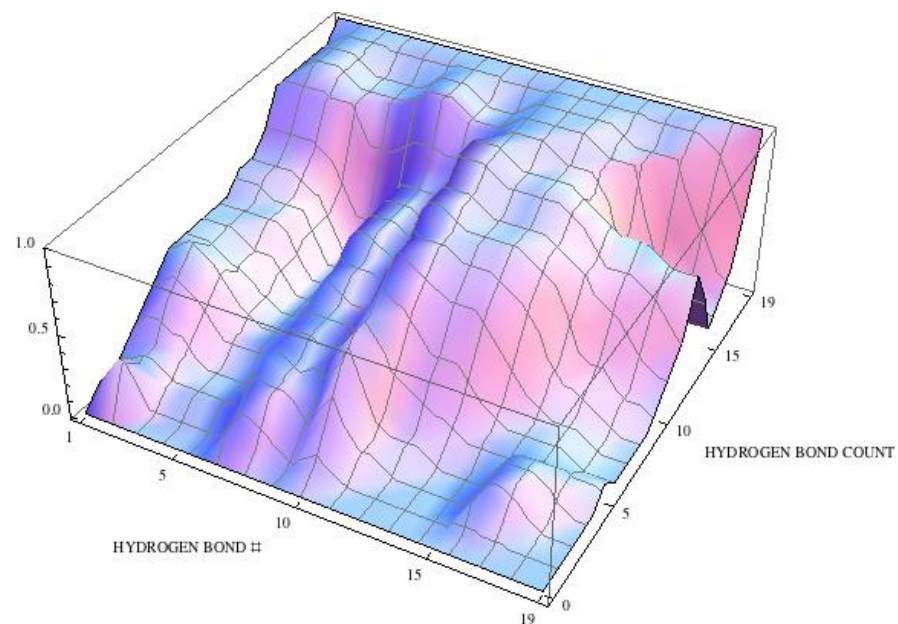
[$\tau_f = 560\text{ ns}$, $\tau_u = 560\text{ ns}$]

Gouri Jas

WH21 OPLSAA MD at 300 K



All MD patterns



Transition patterns

WH21 OPLSAA MD at T=300 K: Folding pathways

HC	1	203954.0	0	00000000000000000000
HC	1	203953.0	1	00000000011000000000
HC	1	203951.0	2	00000000010000010000
HC	1	203940.0	3	00000100010000010000
HC	1	203825.0	4	00000111010000000000
HC	1	203821.0	5	00000111110000000000
HC	1	203818.0	6	00000111111000000000
HC	1	203810.0	7	00000111111000010000
HC	1	201195.0	8	00000111111100000000
HC	1	175428.0	9	10000111111100000000
HC	1	172548.0	10	11000111111100010000
HC	1	154920.0	11	10000111111111000000
HC	1	154885.0	12	11000111111111000000
HC	1	154208.0	13	11000111111111100000
HC	1	148233.0	14	11111111111110000000
HC	1	142521.0	15	11111111111111000000
HC	1	136534.0	16	11111111111111010000
HC	1	136499.0	17	11111111111111100000
HC	1	117868.0	18	11111111111111101011
HC	1	117847.0	19	11111111111111111111

Helix-Coil transition #1

HC	2	1209577.0	0	00000000000000000000
HC	2	1209565.0	1	10000000000000000000
HC	2	1209562.0	2	10100000000000000000
HC	2	1209561.0	3	11100000000000000000
HC	2	1209560.0	4	11100000100000000000
HC	2	1209444.0	5	11100000100100000000
HC	2	1209110.0	6	1110000010010000011
HC	2	1209058.0	7	1110000010010000011
HC	2	1200413.0	8	1110000011111000000
HC	2	1198870.0	9	1110000011111000001
HC	2	1192819.0	10	1110000011111100000
HC	2	1189725.0	11	1110000011111110000
HC	2	1189570.0	12	111000001111111001
HC	2	1188597.0	13	111000001111111011
HC	2	1188528.0	14	1110000011111111111
HC	2	959176.0	15	01100011111111111111
HC	2	952774.0	16	1111111111111111100
HC	2	952757.0	17	1111111111111111100
HC	2	952646.0	18	111111111111110111
HC	2	952645.0	19	11111111111111111111

Helix-Coil transition #2

HC	3	4369882.0	0	00000000000000000000
HC	3	4369881.0	1	00000001000000000000
HC	3	4369874.0	2	00010000100000000000
HC	3	4369872.0	3	00010001100000000000
HC	3	4369868.0	4	00010001110000000000
HC	3	4369841.0	5	00010011110000000000
HC	3	4369813.0	6	00010011111000000000
HC	3	4369487.0	7	00011011111000000000
HC	3	4369479.0	8	00011111111000000000
HC	3	4369453.0	9	00011111111100000000
HC	3	4369112.0	10	00011011111111000000
HC	3	4368996.0	11	00011111111111000000
HC	3	4347009.0	12	00111111111111000000
HC	3	4340956.0	13	0011111111111100100
HC	3	4340919.0	14	0011111111111110000
HC	3	4325820.0	15	00011111111111110000
HC	3	4325804.0	16	10011111111111111000
HC	3	4325252.0	17	10011111111111111111
HC	3	4322149.0	18	11011111111111111111
HC	3	4322139.0	19	11111111111111111111

Helix-Coil transition #3

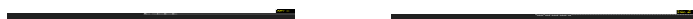
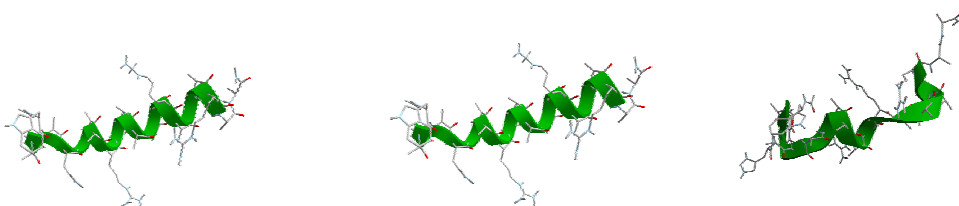
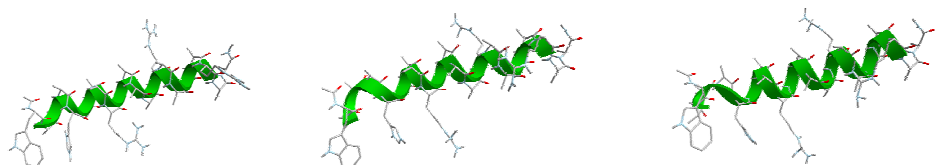
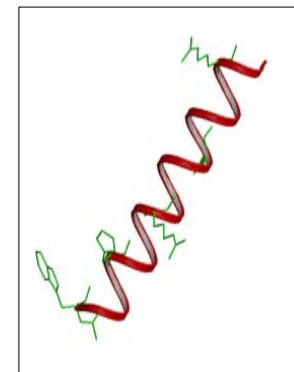
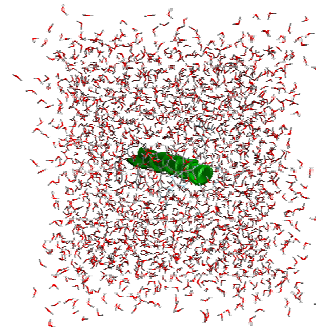
WH21 OPLSAA MD at T=300 K: Folding pathways

0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.1	0.1	0.0	0.0	0.0	0.4	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.1	0.1	0.1	0.0	0.0	0.1	0.7	0.5	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.1	0.1	0.1	0.0	0.0	0.1	0.7	0.6	0.5	0.3	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.2	0.2	0.1	0.0	0.0	0.2	0.7	0.6	0.6	0.6	0.3	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
5	0.3	0.3	0.2	0.1	0.0	0.3	0.7	0.6	0.6	0.7	0.5	0.4	0.2	0.1	0.1	0.0	0.0	0.0	0.0
6	0.3	0.3	0.2	0.1	0.1	0.2	0.7	0.6	0.5	0.8	0.7	0.6	0.4	0.2	0.1	0.0	0.0	0.0	0.0
7	0.3	0.3	0.2	0.2	0.1	0.2	0.7	0.7	0.6	0.9	0.8	0.7	0.6	0.4	0.2	0.1	0.0	0.0	0.0
8	0.2	0.2	0.1	0.2	0.1	0.2	0.8	0.8	0.7	0.9	0.9	0.8	0.8	0.7	0.4	0.1	0.1	0.0	0.0
9	0.2	0.1	0.1	0.2	0.2	0.2	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.8	0.7	0.2	0.1	0.1	0.0
10	0.2	0.2	0.1	0.2	0.2	0.2	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.8	0.7	0.5	0.3	0.1
11	0.2	0.2	0.2	0.2	0.2	0.2	0.9	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.8	0.7	0.6	0.3	0.2
12	0.3	0.3	0.2	0.3	0.3	0.3	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.7	0.6	0.5	0.3
13	0.4	0.4	0.4	0.4	0.3	0.4	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.8	0.7	0.6	0.6	0.5
14	0.9	0.9	0.8	0.8	0.8	0.8	1.0	1.0	1.0	0.9	1.0	1.0	0.9	0.9	0.7	0.3	0.2	0.2	0.1
15	0.9	1.0	0.9	0.9	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.3	0.2	0.2	0.1
16	0.9	0.9	0.9	0.8	0.8	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.8	0.5	0.4	0.3
17	0.9	1.0	1.0	0.9	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.9	0.6	0.4
18	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.7
19	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Average state populations – from 5 helix-coil transitions

WH21 : Helix unfolding kinetics

130 milestones
 13,000 trajectories
 $\approx 1\mu\text{s}$ total simulation time



WH21 Milestoning

Mean first passage time

Unfolding Elementary step

Path 1	280	ns	455	ps
Path 2	7	μs	1.6	ns
Path 3	86	μs	8.9	ns

Kuczera, Jas & Elber, *J. Phys.Chem. A* **113**:7461-7473 (2009)

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.

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Baylor University

Funding:

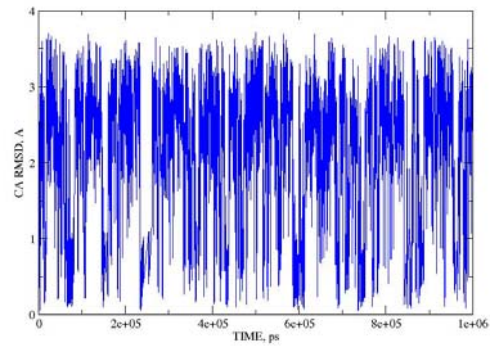
Baylor University internal funds

Big XII Fellowship from University of Kansas

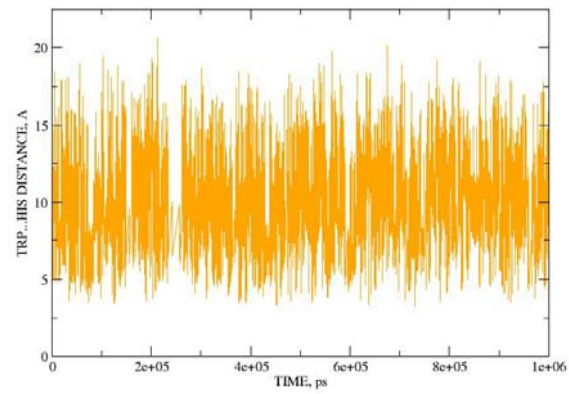
J.T. Olden Fellowship. University of Texas, Austin

WH5 FIGURES

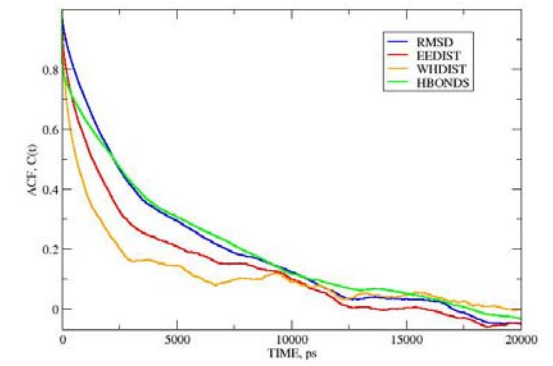
WH5 1,000 ns MD : 1 bar 300 K OPLS/AA TIP3P



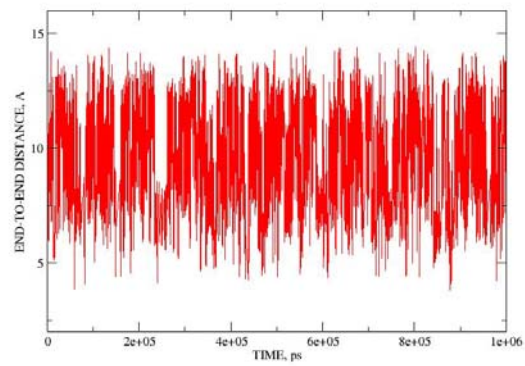
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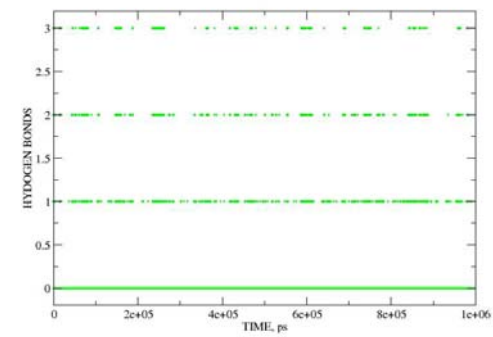
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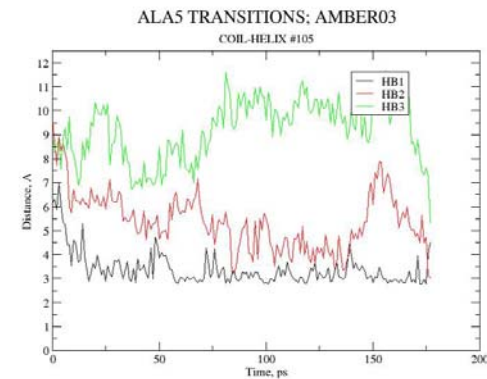
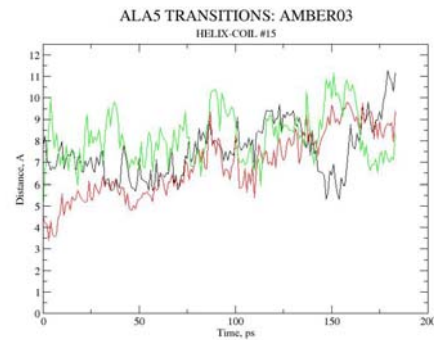
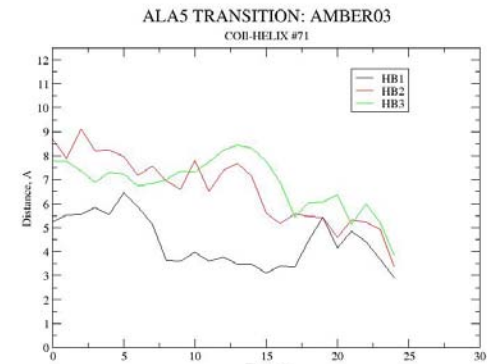
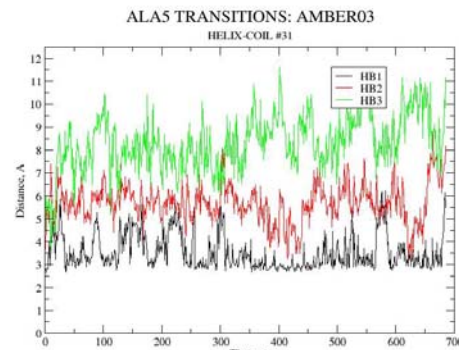
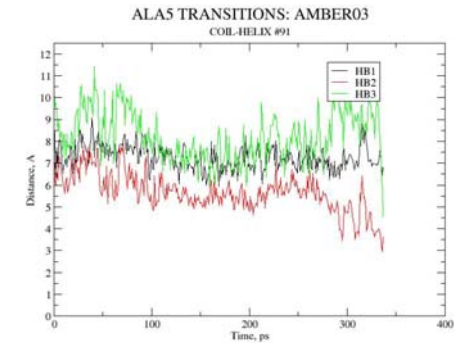
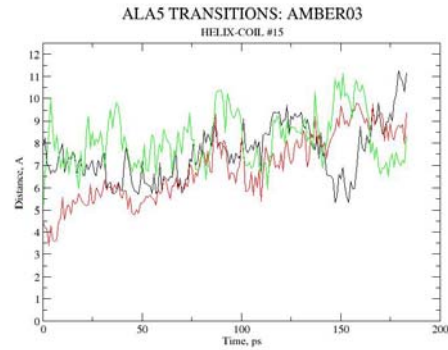
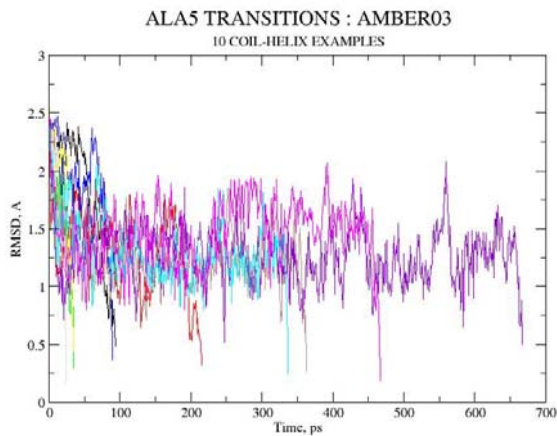
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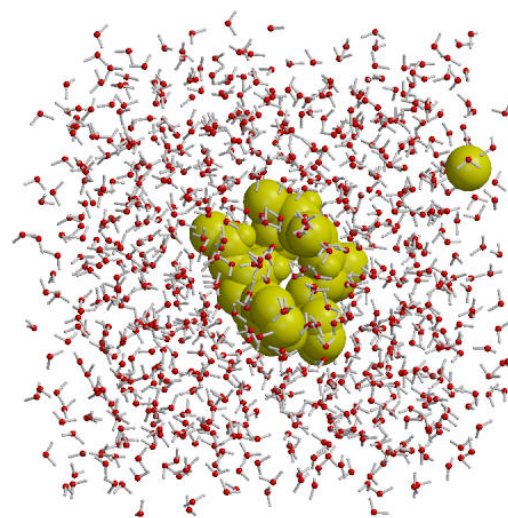
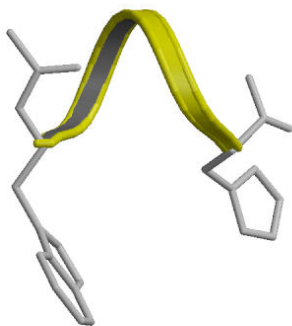
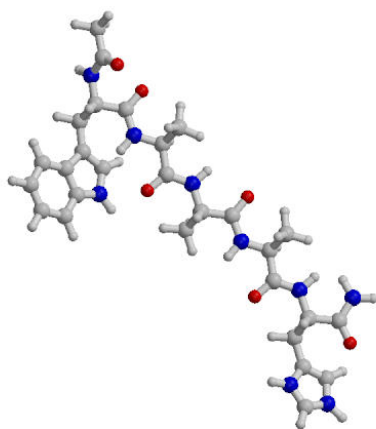
WH5 1,000 ns MD: 1 bar 300 K OPLSAA TIP3P



Folding of Ac-Ala₅-NH₂ : pathways



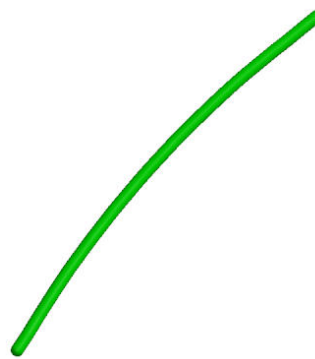
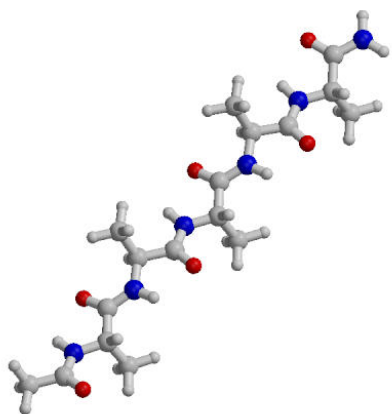
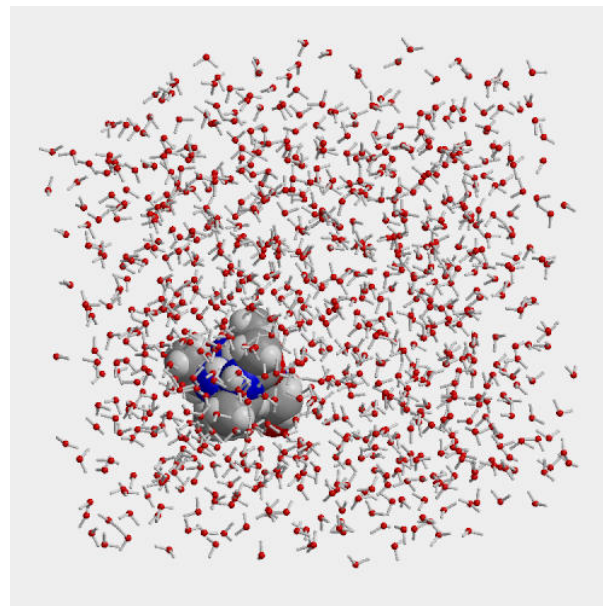
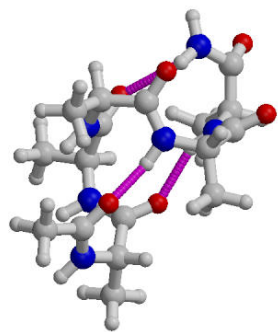
Transitions vary in
- duration time
- path details

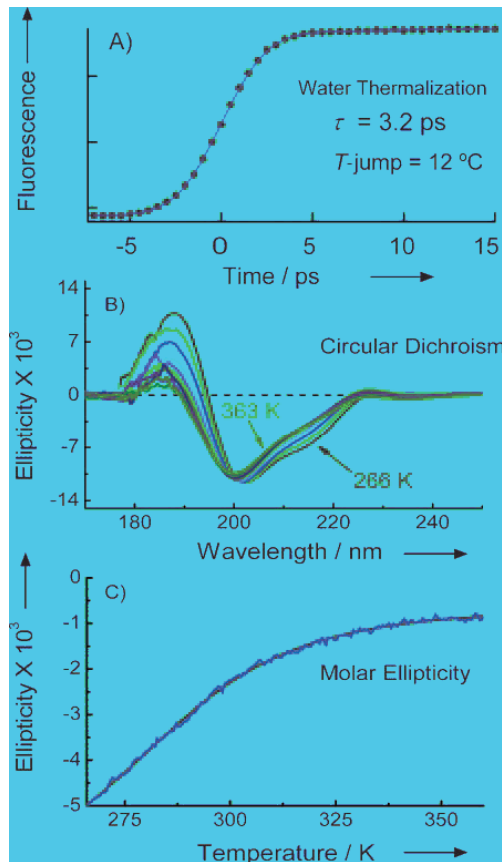


Folding of Ac-Ala₅-NH₂ : patterns

State	OPLS/AA		G43A1		AMBER03		AMBER99P		AMBER99SB		AMBERGS	
	Frac	Coop	Frac	Coop	Frac	Coop	Frac	Coop	Frac	Coop	Frac	Coop
000	0.869	1.2	0.954	1.0	0.592	1.3	0.302	1.4	0.958	1.0	0.155	6.3
100	0.042	0.8	0.021	0.7	0.098	0.6	0.151	0.8	0.018	0.8	0.040	0.7
010	0.018	0.4	0.011	0.5	0.057	0.4	0.095	0.6	0.013	0.7	0.028	0.4
110	0.013	4.2	0.009	14.	0.096	1.5	0.160	1.1	0.003	7.5	0.132	0.7
001	0.032	0.7	0.003	0.6	0.029	1.4	0.082	0.9	0.005	0.6	0.015	0.3
101	0.004	1.3	0.000	0.7	0.014	0.4	0.030	0.4	0.000	1.3	0.024	0.2
011	0.010	4.2	0.001	9.5	0.037	1.2	0.065	0.9	0.002	13.	0.094	1.6
111	0.013	67.	0.001	342.	0.078	6.5	0.112	2.0	0.001	310.	0.512	1.5

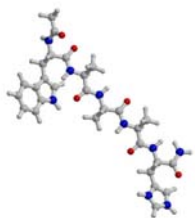
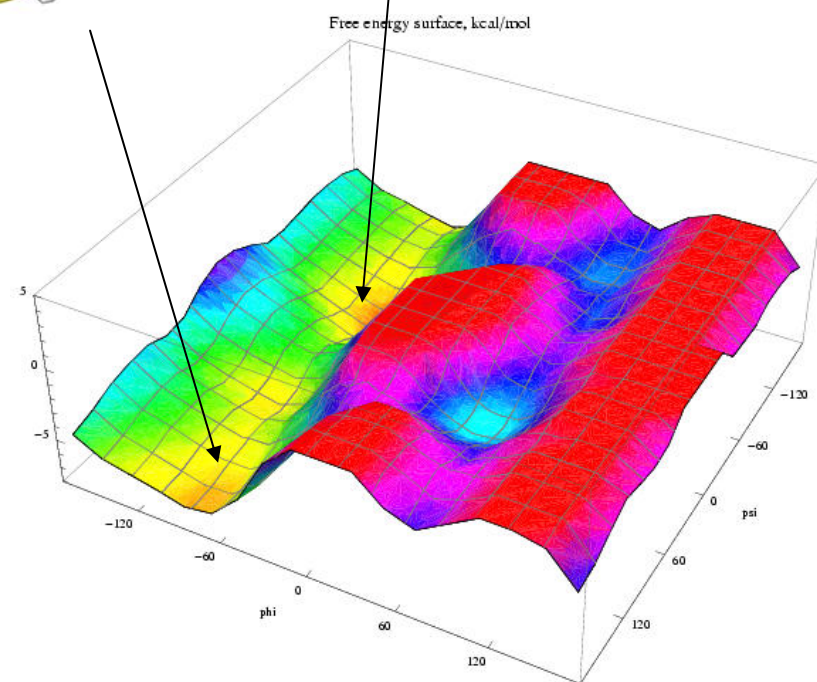
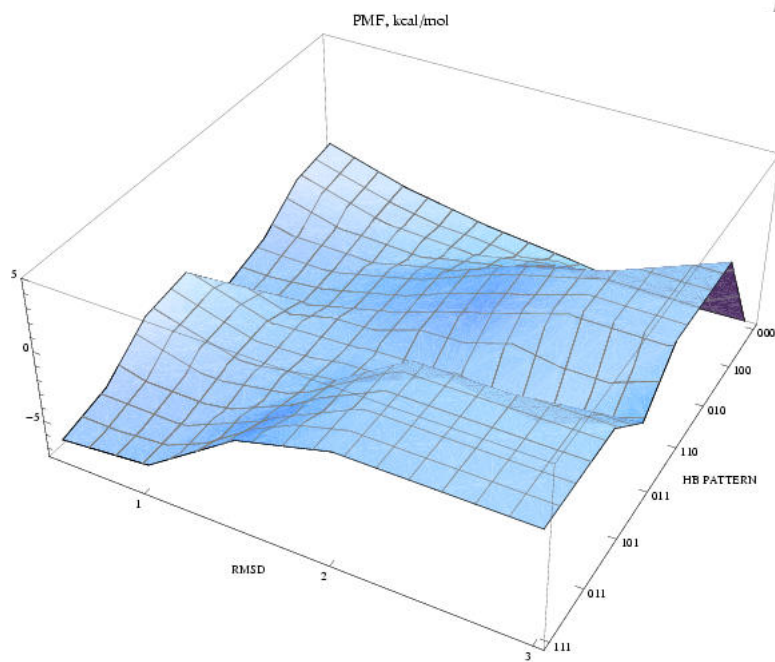
- Most FF : 000 dominant, very little 111, positive h-bond cooperativity
- Populated intermediates: involve h-bonds #1 and #2
- Unusual: AMBERGS



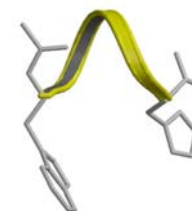
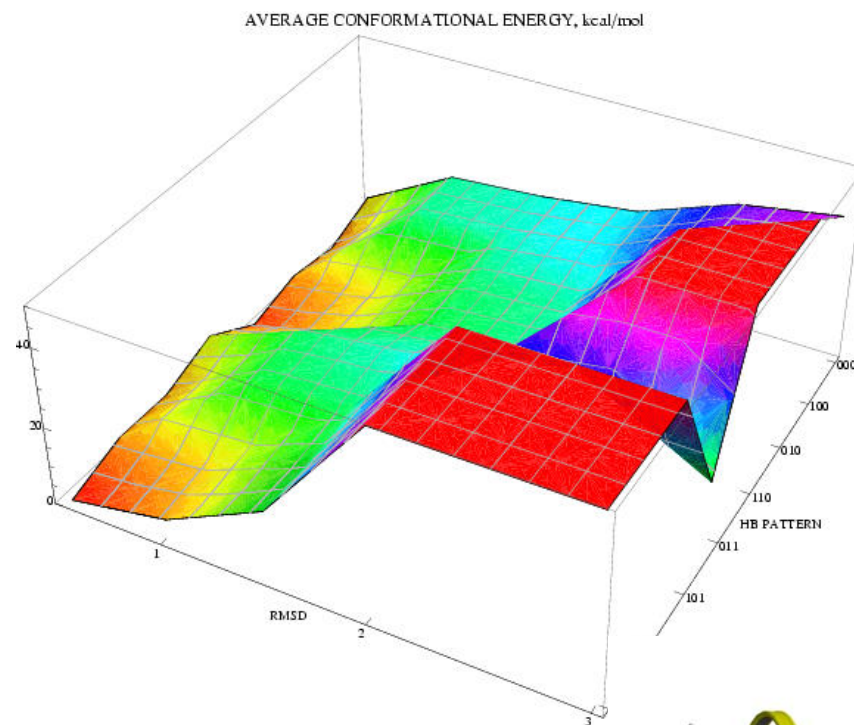
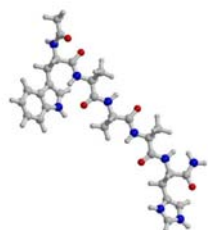
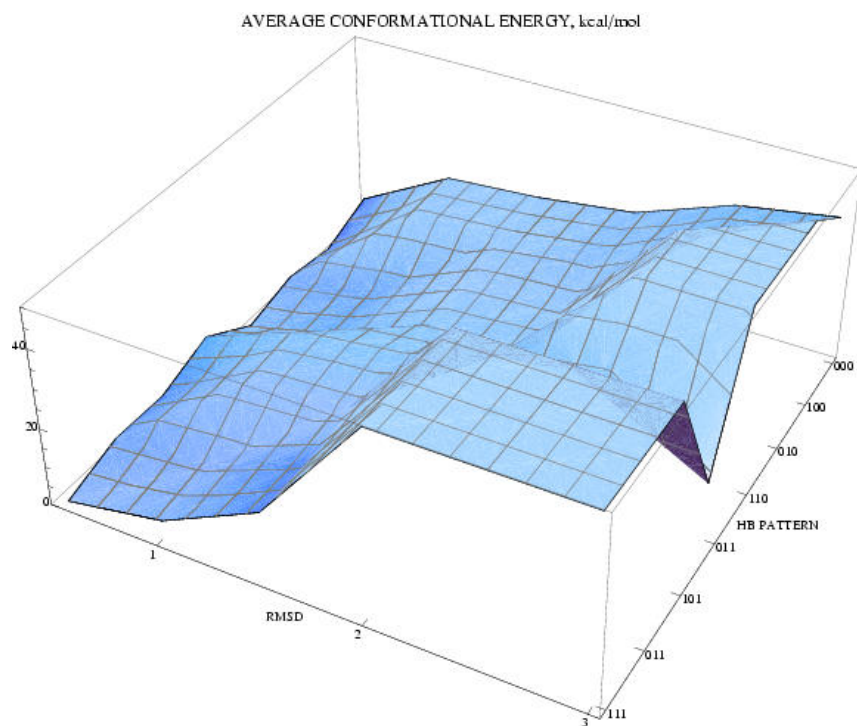


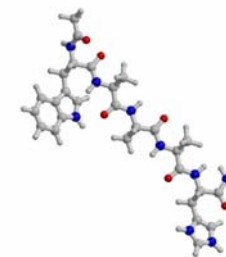
WH5 figs

WH5 in OPLS/AA: conformations



WH5: conformational energy (CHARMM)

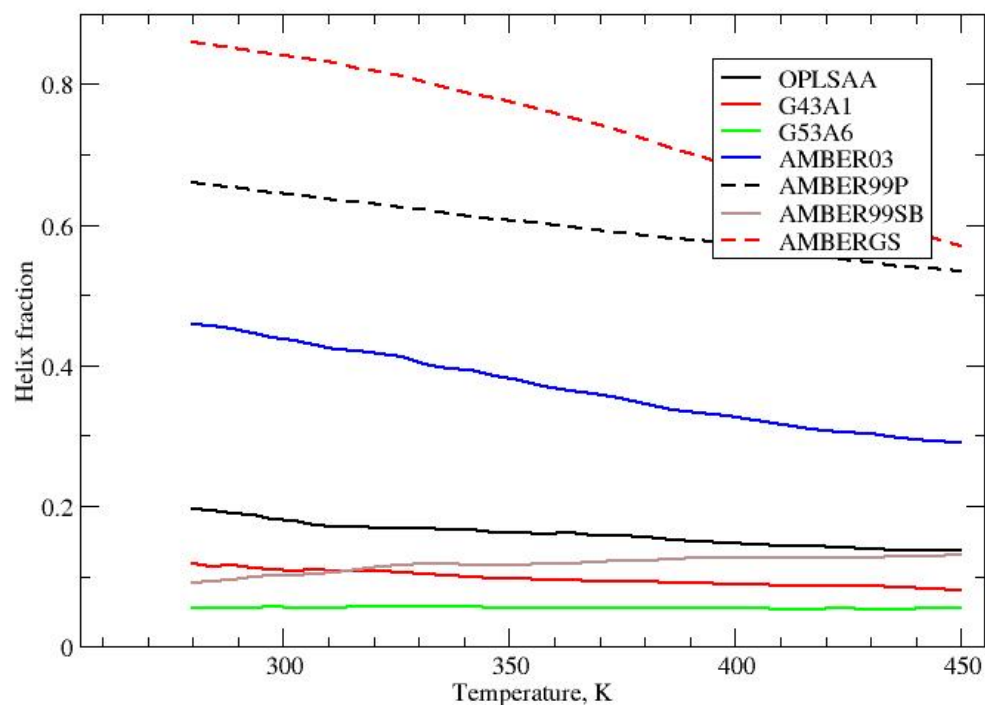




REMD of WH5

WH5 REMD : PP MELTING CURVES

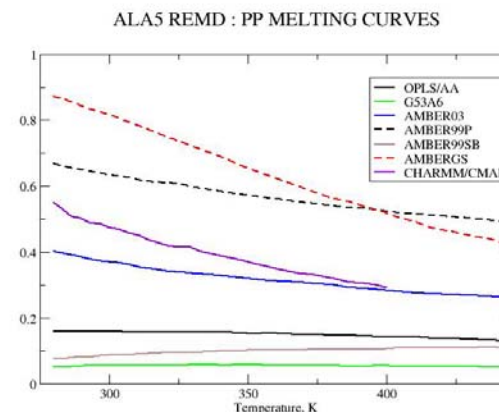
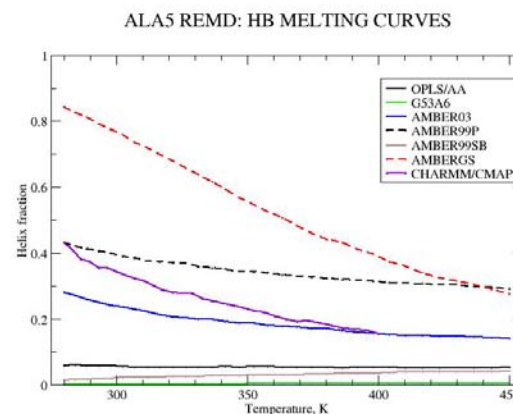
- At 300 K REMD=MD
- OPLS/AA, AMBER03, AMBER99P and CHARMM22 give excellent helicity predictions at 300 K
- Helix persistence exaggerated
- AMBER99SB – anti-melting



REMD simulations: 32 replicas, 280-450 K, 30 Å cubic box with ca. 1000 waters, 100 ns NPT trajectory with GROMACS

Folding of Ac-Ala₅-NH₂ : 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**

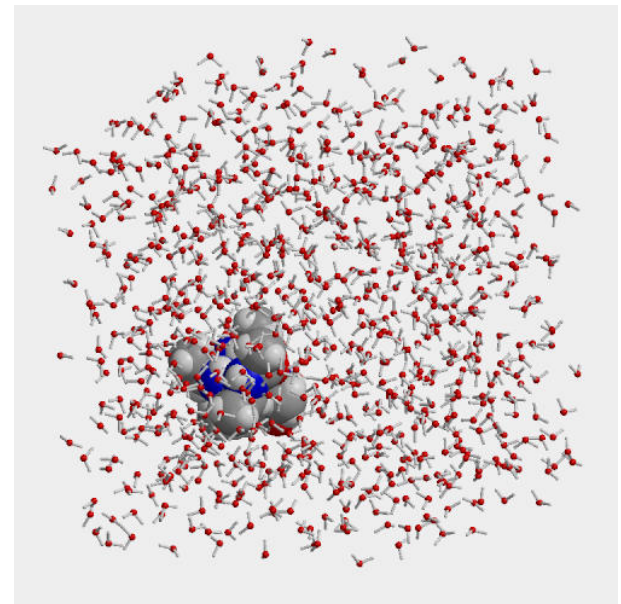


REMD simulations: 32 replicas, 280-450 K, 30 Å cubic box with ca. 1000 waters, 100 ns NPT trajectory with GROMACS, for all except CHARMM potential
CHARMM REMD: 40 ns in 37 Å bcc cell.

Ac-Ala5-NH₂ 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 [Graf et al., *J.Am.Chem. Soc.* **129**:1179 (2007)]