Peptide crystal simulations reveal hidden dynamics

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Supporting Information

Parameters for special residue Aib.

% RESIDUE AIB [13 atoms, −0.0000 net charge]

Atom Name	Charge	Type Name	LJ Sigma	LJ Epsilon
N	−0.415693	N	3.249999	0.170000
H	0.271895	H	1.069078	0.015700
CA	0.117598	CT	3.399670	0.109400
CB1	−0.182497	CT	3.399670	0.109400
HB11	0.060299	HC	2.649533	0.015700
HB12	0.060299	HC	2.649533	0.015700
HB13	0.060299	HC	2.649533	0.015700
CB2	−0.182497	CT	3.399670	0.109400
HB21	0.060299	HC	2.649533	0.015700
HB22	0.060299	HC	2.649533	0.015700
HB23	0.060299	HC	2.649533	0.015700
C	0.597290	C	3.399670	0.086000
O	−0.567890	O	2.959922	0.210000

Bond A:B	Stiffness	Eq. Length
CB2 :HB21	340.000000	1.090000
CB2 :HB22	340.000000	1.090000
CB2 :HB23	340.000000	1.090000
CB1 :HB11	340.000000	1.090000
CB1 :HB12	340.000000	1.090000
CB1 :HB13	340.000000	1.090000
N :H	434.000000	1.010000
C :O	570.000000	1.229000
CA :CB1	310.000000	1.526000
CA :CB2	310.000000	1.526000
CA :C	317.000000	1.522000
N :CA	337.000000	1.449000

Angle A:B:C	Stiffness	Eq. Angle
HB22:CB2 :HB23	35.000000	1.911136
HB21:CB2 :HB22	35.000000	1.911136
HB21:CB2 :HB23	35.000000	1.911136
HB12:CB1 :HB13	35.000000	1.911136
HB11:CB1 :HB12	35.000000	1.911136
HB11:CB1 :HB13	35.000000	1.911136
CA :CB1 :HB11	50.000000	1.911136
CA :CB1 :HB12	50.000000	1.911136
CA :CB1 :HB13	50.000000	1.911136
CA :CB2 :HB21	50.000000	1.911136
CA :CB2 :HB22	50.000000	1.911136
CA :CB2 :HB23	50.000000	1.911136
H :N :CA	50.000000	2.060188
CB2 :CA :C	63.000000	1.939062
CB1 :CA :CB2	40.000000	1.911136
CB1 :CA :C	63.000000	1.939062
CA :C :O	80.000000	2.101377
N :CA :CB1	80.000000	1.914627
N :CA :CB2	80.000000	1.914627
N :CA :C	63.000000	1.921608

Dihedral A:B:C:D	Penalty	Period	Phase
HB23:CB2 :CA :C	0.155556	3.000000	0.000000
HB22:CB2 :CA :C	0.155556	3.000000	0.000000
HB21:CB2 :CA :C	0.155556	3.000000	0.000000
HB13:CB1 :CA :CB2	0.160000	3.000000	0.000000

HB13	:CB1	:CA	:C	0.155556	3.000000	0.000000
HB12	:CB1	:CA	:CB2	0.160000	3.000000	0.000000
HB12	:CB1	:CA	:C	0.155556	3.000000	0.000000
HB11	:CB1	:CA	:CB2	0.160000	3.000000	0.000000
HB11	:CB1	:CA	:C	0.155556	3.000000	0.000000
CB1	:CA	:CB2	:HB21	0.160000	3.000000	0.000000
CB1	:CA	:CB2	:HB22	0.160000	3.000000	0.000000
CB1	:CA	:CB2	:HB23	0.160000	3.000000	0.000000
H	:N	:CA	:CB1	0.000000	2.000000	0.000000
H	:N	:CA	:CB2	0.000000	2.000000	0.000000
H	:N	:CA	:C	0.000000	2.000000	0.000000
N	:CA	:CB1	:HB11	0.155556	3.000000	0.000000
N	:CA	:CB1	:HB12	0.155556	3.000000	0.000000
N	:CA	:CB1	:HB13	0.155556	3.000000	0.000000
N	:CA	:CB2	:HB21	0.155556	3.000000	0.000000
N	:CA	:CB2	:HB22	0.155556	3.000000	0.000000
N	:CA	:CB2	:HB23	0.155556	3.000000	0.000000
CB2	:CA	:C	:O	0.000000	2.000000	0.000000
CB1	:CA	:C	:O	0.000000	2.000000	0.000000
N	:CA	:C	:O	0.000000	2.000000	0.000000

Parameters for special residue Boc.

% RESIDUE BOC [16 atoms, 0.0000 net charge]

Atom Name	Charge	Type Name	LJ Sigma	LJ Epsilon
C2	−0.360090	CT	3.399670	0.109400
H1	0.070719	HC	2.649533	0.015700
H2	0.070719	HC	2.649533	0.015700
H3	0.070719	HC	2.649533	0.015700
C1	0.586731	CT	3.399670	0.109400
C3	−0.360090	CT	3.399670	0.109400
H4	0.070719	HC	2.649533	0.015700
H5	0.070719	HC	2.649533	0.015700
H6	0.070719	HC	2.649533	0.015700
C4	−0.360090	CT	3.399670	0.109400
H7	0.070719	HC	2.649533	0.015700
H8	0.070719	HC	2.649533	0.015700
H9	0.070719	HC	2.649533	0.015700
O1	−0.383349	OS	3.000012	0.170000
C5	0.744134	C	3.399670	0.086000
O2	−0.503715	O	2.959922	0.210000

Bond A:B	Stiffness	Eq. Length
C4 :H7	340.000000	1.090000
C4 :H8	340.000000	1.090000
C4 :H9	340.000000	1.090000
C3 :H4	340.000000	1.090000
C3 :H5	340.000000	1.090000
C3 :H6	340.000000	1.090000
C2 :H1	340.000000	1.090000
C2 :H2	340.000000	1.090000
C2 :H3	340.000000	1.090000
C5 :O2	570.000000	1.229000
O1 :C5	450.000000	1.323000
C1 :C3	310.000000	1.526000
C1 :C4	310.000000	1.526000
C1 :O1	320.000000	1.410000
C2 :C1	310.000000	1.526000

Angle A:B:C	Stiffness	Eq. Angle
H8 :C4 :H9	35.000000	1.911136
H7 :C4 :H8	35.000000	1.911136
H7 :C4 :H9	35.000000	1.911136
H5 :C3 :H6	35.000000	1.911136
H4 :C3 :H5	35.000000	1.911136
H4 :C3 :H6	35.000000	1.911136
C1 :C3 :H4	50.000000	1.911136
C1 :C3 :H5	50.000000	1.911136
C1 :C3 :H6	50.000000	1.911136
C1 :C4 :H7	50.000000	1.911136
C1 :C4 :H8	50.000000	1.911136
C1 :C4 :H9	50.000000	1.911136
H3 :C2 :C1	50.000000	1.911136
H2 :C2 :H3	35.000000	1.911136
H2 :C2 :C1	50.000000	1.911136
H1 :C2 :H2	35.000000	1.911136
H1 :C2 :H3	35.000000	1.911136
H1 :C2 :C1	50.000000	1.911136
O1 :C5 :O2	80.000000	2.181662
C4 :C1 :O1	50.000000	1.911136
C3 :C1 :C4	40.000000	1.911136
C3 :C1 :O1	50.000000	1.911136
C1 :O1 :C5	60.000000	2.042036
C2 :C1 :C3	40.000000	1.911136
C2 :C1 :C4	40.000000	1.911136
C2 :C1 :O1	50.000000	1.911136

Dihedral A:B:C:D				Penalty	Period	Phase
H9	:C4	:C1	:O1	0.250000	1.000000	0.000000
H9	:C4	:C1	:O1	0.000000	3.000000	0.000000
H8	:C4	:C1	:O1	0.250000	1.000000	0.000000
H8	:C4	:C1	:O1	0.000000	3.000000	0.000000
H7	:C4	:C1	:O1	0.250000	1.000000	0.000000
H7	:C4	:C1	:O1	0.000000	3.000000	0.000000
H6	:C3	:C1	:C4	0.160000	3.000000	0.000000
H6	:C3	:C1	:O1	0.250000	1.000000	0.000000
H6	:C3	:C1	:O1	0.000000	3.000000	0.000000
H5	:C3	:C1	:C4	0.160000	3.000000	0.000000
H5	:C3	:C1	:O1	0.250000	1.000000	0.000000
H5	:C3	:C1	:O1	0.000000	3.000000	0.000000
H4	:C3	:C1	:C4	0.160000	3.000000	0.000000
H4	:C3	:C1	:O1	0.250000	1.000000	0.000000
H4	:C3	:C1	:O1	0.000000	3.000000	0.000000
C3	:C1	:C4	:H7	0.160000	3.000000	0.000000
C3	:C1	:C4	:H8	0.160000	3.000000	0.000000
C3	:C1	:C4	:H9	0.160000	3.000000	0.000000
H3	:C2	:C1	:C3	0.160000	3.000000	0.000000
H3	:C2	:C1	:C4	0.160000	3.000000	0.000000
H3	:C2	:C1	:O1	0.250000	1.000000	0.000000
H3	:C2	:C1	:O1	0.000000	3.000000	0.000000
H2	:C2	:C1	:C3	0.160000	3.000000	0.000000
H2	:C2	:C1	:C4	0.160000	3.000000	0.000000
H2	:C2	:C1	:O1	0.250000	1.000000	0.000000
H2	:C2	:C1	:O1	0.000000	3.000000	0.000000
H1	:C2	:C1	:C3	0.160000	3.000000	0.000000
H1	:C2	:C1	:C4	0.160000	3.000000	0.000000
H1	:C2	:C1	:O1	0.250000	1.000000	0.000000
H1	:C2	:C1	:O1	0.000000	3.000000	0.000000
C2	:C1	:C3	:H4	0.160000	3.000000	0.000000
C2	:C1	:C3	:H5	0.160000	3.000000	0.000000
C2	:C1	:C3	:H6	0.160000	3.000000	0.000000
C2	:C1	:C4	:H7	0.160000	3.000000	0.000000
C2	:C1	:C4	:H8	0.160000	3.000000	0.000000
C2	:C1	:C4	:H9	0.160000	3.000000	0.000000
C4	:C1	:O1	:C5	0.800000	1.000000	3.141594
C4	:C1	:O1	:C5	0.383000	3.000000	0.000000
C3	:C1	:O1	:C5	0.800000	1.000000	3.141594
C3	:C1	:O1	:C5	0.383000	3.000000	0.000000
C1	:O1	:C5	:O2	1.400000	1.000000	3.141594
C1	:O1	:C5	:O2	2.700000	2.000000	3.141594
C2	:C1	:O1	:C5	0.800000	1.000000	3.141594
C2	:C1	:O1	:C5	0.383000	3.000000	0.000000

Parameters for special residue Ome.

% RESIDUE OME [5 atoms, 0.0000 net charge]

Atom Name	Charge	Type Name	LJ Sigma	LJ Epsilon
O	−0.192797	OS	3.000012	0.170000
C	−0.099998	CT	3.399670	0.109400
H1	0.097598	H1	2.471353	0.015700
H2	0.097598	H1	2.471353	0.015700
H3	0.097598	H1	2.471353	0.015700

Bond A:B		Stiffness	Eq. Length
C	:H1	340.000000	1.090000
C	:H2	340.000000	1.090000
C	:H3	340.000000	1.090000
O	:C	320.000000	1.410000

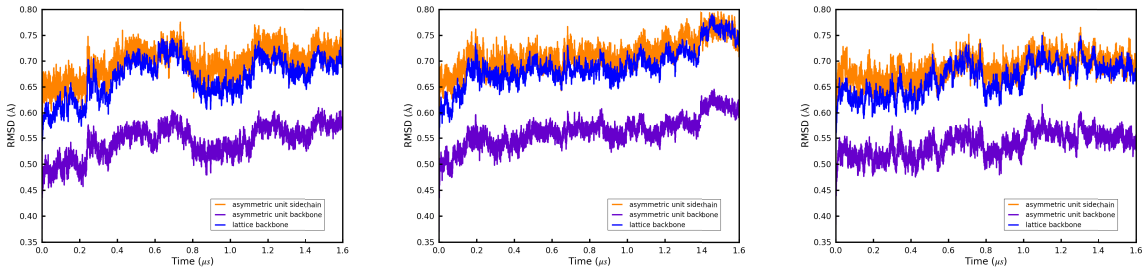
Angle A:B:C			Stiffness	Eq. Angle
H2	:C	:H3	35.000000	1.911136
H1	:C	:H2	35.000000	1.911136
H1	:C	:H3	35.000000	1.911136
O	:C	:H1	50.000000	1.911136
O	:C	:H2	50.000000	1.911136
O	:C	:H3	50.000000	1.911136

Parameters for Amber molecular dynamics.

General flags:
  imin = 0, nmropt = 0

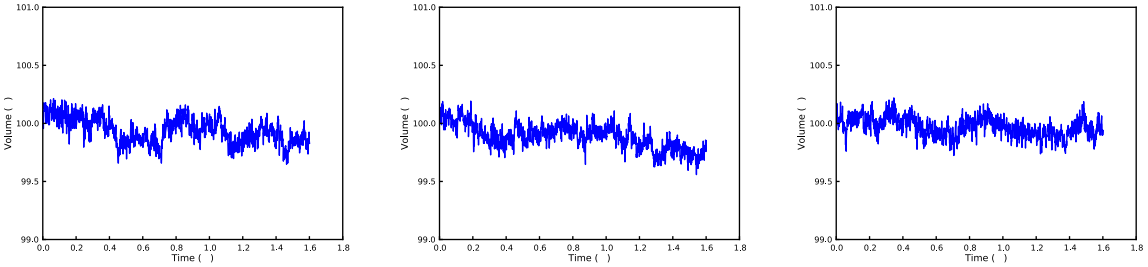
Nature and format of input:
  ntx = 5, irest = 1, ntrx = 1

```
Nature and format of output:
  ntxo = 1, ntpr = 10000, ntrx = 1, ntwr = 0
  iwrap = 0, ntwx = 10000, ntwv = 0, ntwe = 0
  ioutfm  = 1, ntwprt  = 0, idecomp = 0, rbornstat= 0

Potential function:
  ntf = 2, ntb = 2, igb = 0, nsnb = 25
  ipol = 0, gbsa = 0, iesp = 0
  dielc = 1.00000, cut = 9.00000, intdiel = 1.00000

Frozen or restrained atoms:
  ibelly = 0, ntr = 0

Molecular dynamics:
  nstlim =  20000000, nscm =  10000, nrespa = 1
  t = 0.00000, dt = 0.00200, vlimit =  -1.00000

Langevin dynamics temperature regulation:
  ig = 353685
  temp0 = 294.00000, tempi = 277.00000, gamma_ln = 1.00000

Pressure regulation:
  ntp = 1
  pres0 = 1.00000, comp =  44.60000, taup = 5.00000

SHAKE:
  ntc = 2, jfastw = 0
  tol = 0.00000

| Intermolecular bonds treatment:
|  no_intermolecular_bonds = 1

| Energy averages sample interval:
|  ene_avg_sampling = 10000

Ewald parameters:
  verbose = 0, ew_type = 0, nbflag = 1, use_pme = 1
  vdwmeth = 1, eedmeth = 1, netfrc = 1
  Box X = 43.171 Box Y = 49.041 Box Z = 53.513
  Alpha = 116.410 Beta = 95.530 Gamma = 93.160
  NFFT1 = 48 NFFT2 = 56 NFFT3 = 56
  Cutoff= 9.000 Tol =0.100E-04
  Ewald Coefficient =  0.30768
  Interpolation order = 4
  Setting ifbox to 3 for non-orthogonal unit cell
```



(a) (b) (c)

Figure S1: Positional RMSDs of heavy atoms relative to the X-ray structure for the three 1.6 s. Plot for the 2.4 s simulation is given in the main article. Details of each metric are given in the main text. Purple: asymmetric unit RMSD for backbone (N,CA,C) atoms. Orange: asymmetric unit RMSD for side-chain heavy atoms. Blue: lattice RMSD for backbone atoms.



(a) (b) (c)

Figure S2: Supercell volume presented as a percentage of experimental supercell volume over the course of the three 1.6 s trajectories. Volume of the supercell over the course of the 2.4 s simulation is presented in the main article. In all cases average volume is maintained to within 0.2% of experiment, although convergence is not clear in two cases. Instantaneous fluctuations of the volume have amplitudes of an additional 0.2%.

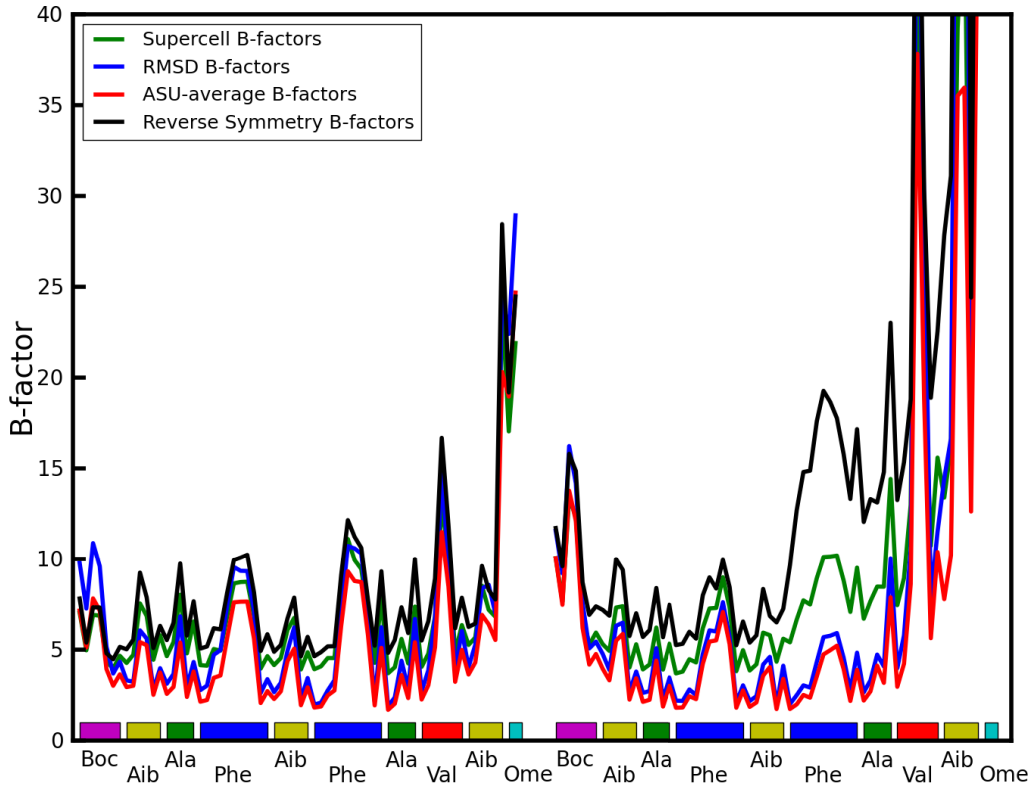


Figure S5: Comparison of four different approaches to calculating B-factors from a crystal simulation. "RMSD" and "Reverse Symmetry" are describe in the main text. "Supercell" approach consists in fitting each trajectory snapshot of the entire supercell to the reference supercell created from experimental coordinates, then calculating B-factors from positional variance for each atom in the supercell separately, then averaging over the 36 equivalent crystal copies of each atom (one in each unit cell) to obtain the final B-factor. This approach is rejected becuase of the unnatural way in which the structures are superimposed when trying to rmsd-fit multiple unit cells at once. The "ASU-average" method consists in rotationaly/translationaly fitting all trajectory snapshots and calculating B-factors for each unit cell (asymmetric unit in the P1 case) separately; then averaging the B-factors overal all 36 unit cells. This approach is rejected because it amounts to taking a mean of the same variances as the "RMSD" approach which will mathematically always underestimate the true variance.

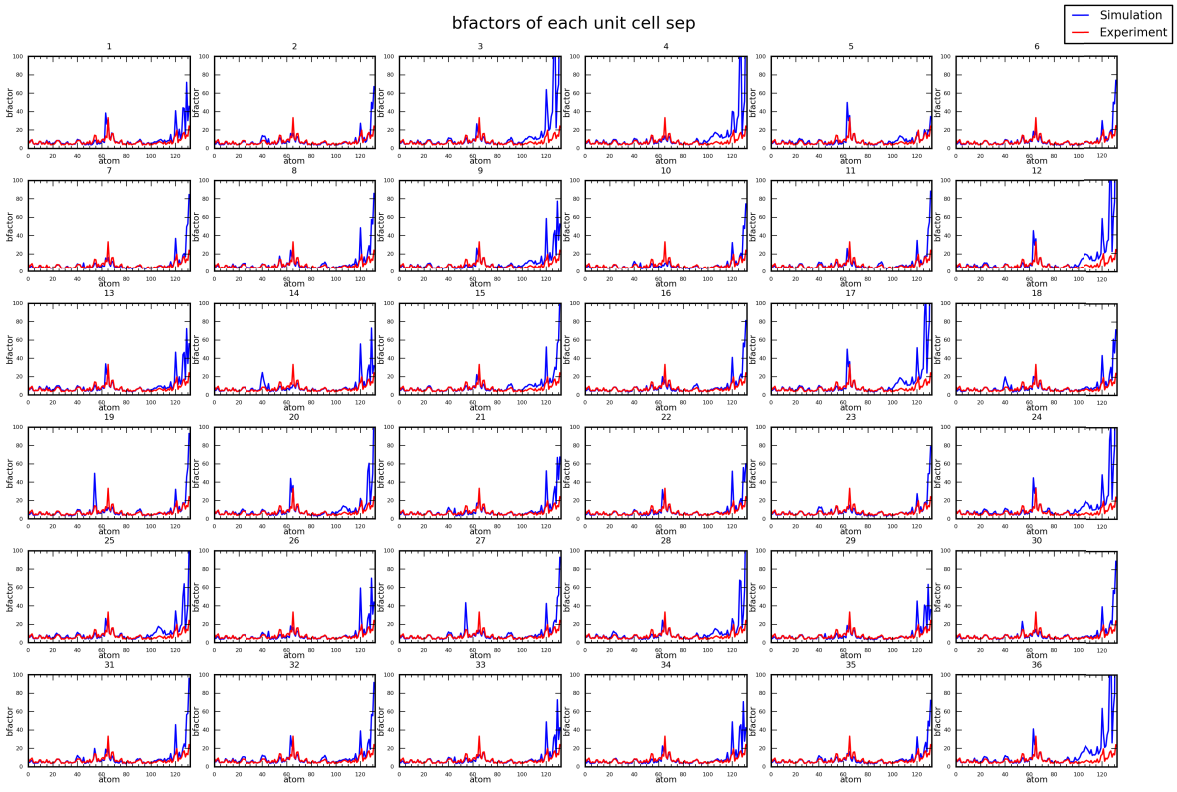


Figure S6: "RMSD" method B-factors (see main text for additional details) for the heavy atoms of each of the 36 unit cells separately. The B-factors correspond to the intra-molecular positional fluctuations in each of the unit cells.

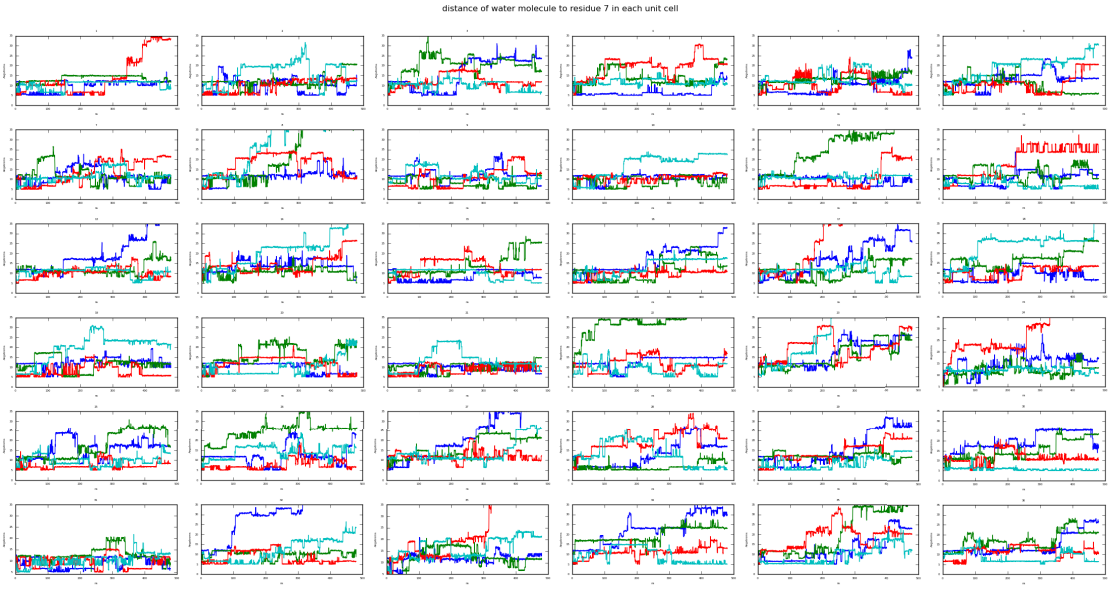


Figure S7: Diffusion of individual water molecules in each unit cell. One panel is plotted for each of the 36 unit cells, each of which initially contained four water molecules. The plots show the distance of each water molecule from the CA atom of residue 7 (chosen as a common point of reference) over the course of the first 500ns of the 2.4 s trajectory. In each plot, the corresponding water molecules related by crystal vector translations are plotted with the same color. All four of the crystallographic water positions are involved in the observed crystal dynamics: no crystal water position is special in this regard.

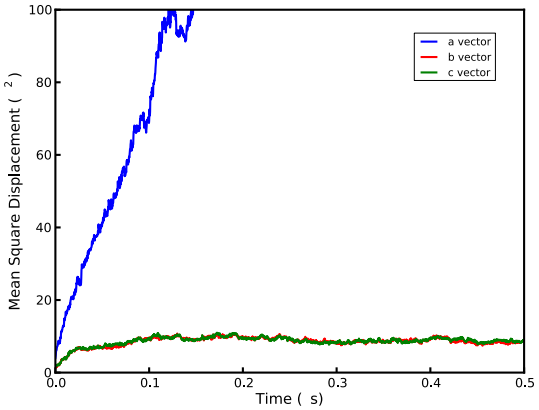


Figure S8: Mean square displacements (msd) of water molecules over the first 500 ns of the 2.4 s trajectory along each of the three crystal vectors. Water is highly mobile along the channels (blue, channels are co-linear with the crystal a-vector) but constrained by them (green and red). Water channels are estimated to be about 3-4 Å wide based on an msd of about 12 $Å^2$ in the b and c directions.

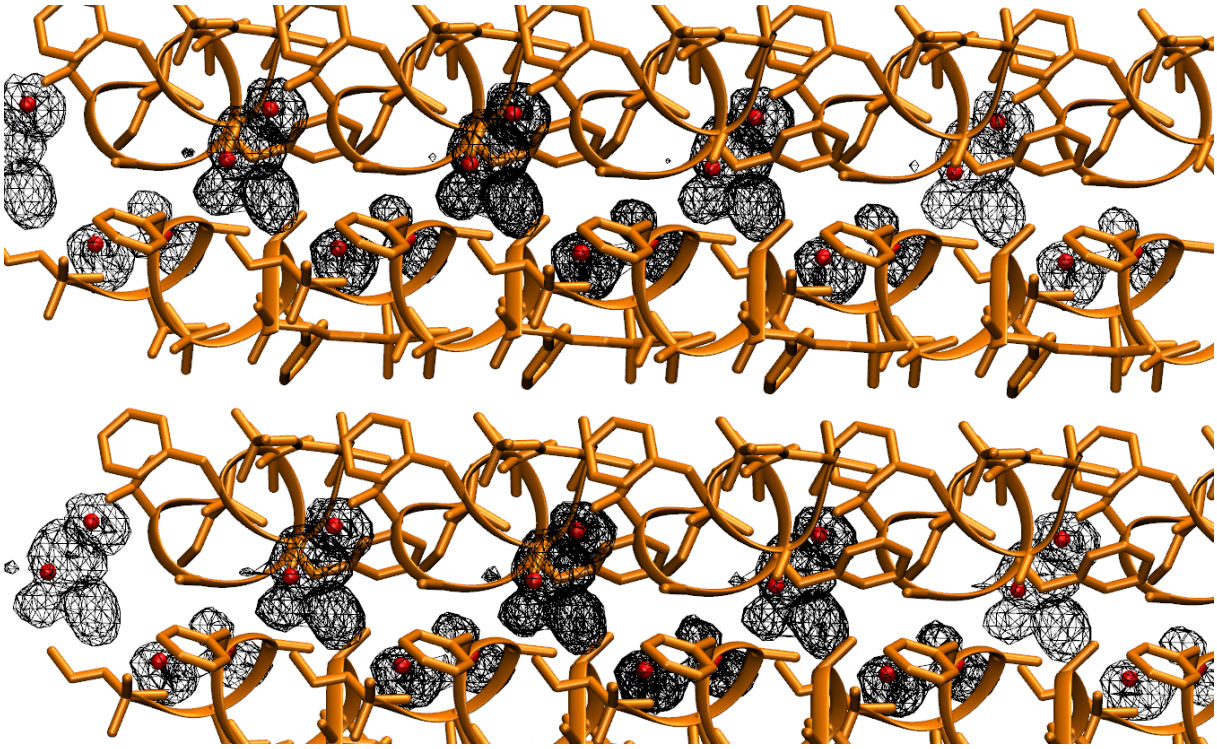


Figure S9: Water density observed in the 2.4 μs simulation. The time-averaged density of water was obtained by using crystal symmetry operations to superimpose all simulated waters onto a single unit cell. The mesh encloses 90% of the water density. Crystallographic peptide is shown in orange and crystallographic water oxygens as red spheres. The image depicts a periodic system to illustrate the relationships between water positions and neighboring unit cells.

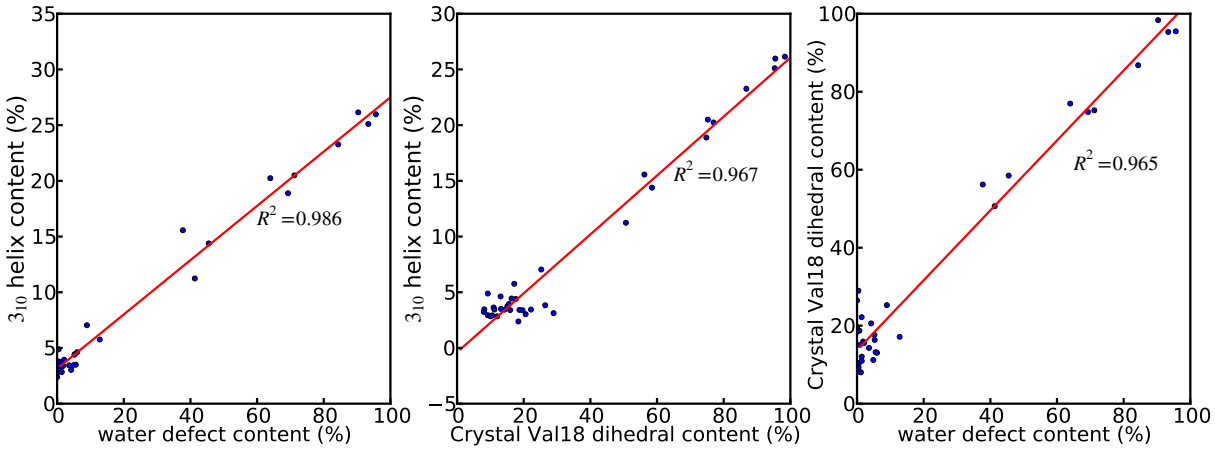


Figure S10: Scatter plot showing the correlation between each pair of the following three structural characteristics: Valine B8 rotamer in crystal conformation (χ_1 torsion is in gauche(-)), water "defective" unit cell content (unit cell contains only one or two water molecules) and 3_{10} helical propensity. Each point corresponds to one unit cell and plots the percentage of simulation time (2.4 μs trajectory) that a given unit cell exhibited the given structural characteristic. Best linear fits are shown with a red line together with their coefficient of determination.

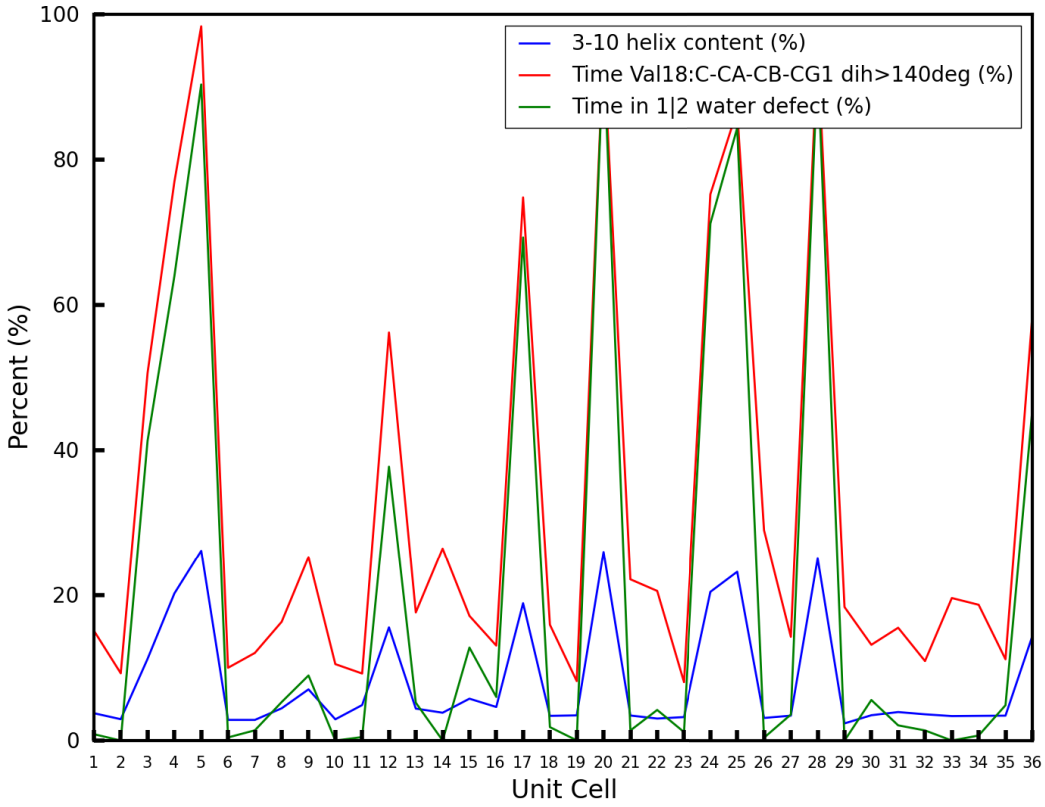


Figure S11: Per unit cell simulation time percentage that the given unit cell exhibits propensity for chain B to form a 3_{10} helix, Val B8 to adapt a gauche(-) rotamer and water defects to occur in the simulated unit cells.

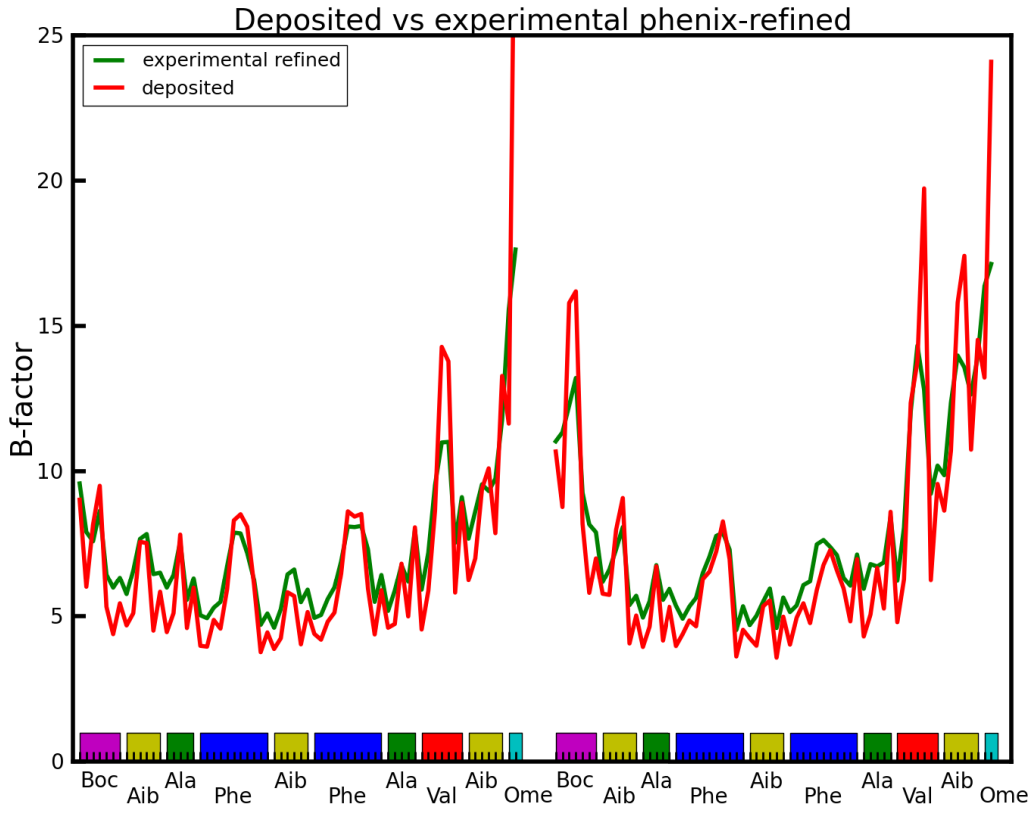


Figure S12: Comparison of the B-factors originally calculated by Aravinda *et al.* and the B-factors obtained in our re-refinement against the experimental density. For consistency of methods when analyzing the results of the simulation density refinement, we have compared against the results of our experimental density refinement throughout this paper.

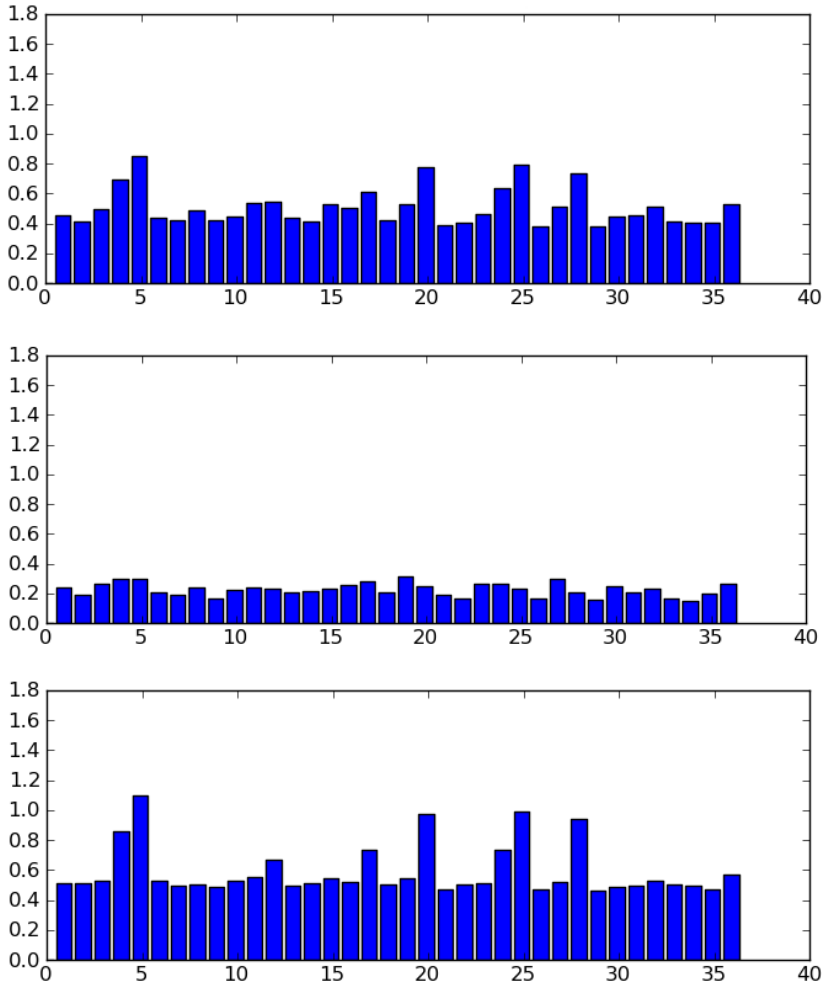


Figure S3: RMSD of individual unit cells to crystal. Top image shows rmsd of entire unit cell (both peptide chains). Middle image shows rmsd of chain A and bottom image shows rmsd of chain B. The average rmsd for monomer A is 0.23 Å while for monomer B it is 0.58 Å. Taken together these results indicate that the simulation converges very quickly to a structure that is generally in good agreement with the X-ray data but presenting some deviations near the C-terminus of monomer B. A subpopulation of unit cells with much higher monomer B rmsd is evident (unit cells 4, 5, 12, 17, 20, 24, 25, 28 and 36). The average monomer B rmsd of these cells is 0.84 Å while the average for the remaining cells is 0.51Å. Furthermore, a residue by residue analysis revealed that in the majority of the unit cells, Val18 and Aib19 contributed most significantly to the elevated rmsd (0.80Å and 0.25Å respectively while all remaining residues had rmsd's lower than 0.09Å). A calculation of monomer B rmsd for these 27 cells without Val18 and Aib19 gives a mean of 0.23Å (compared to 0.51Å if including Val18 and Aib19). On the other hand for the other 9 unit cells all residues contribute about equally to the rmsd and rmsd calcualted without Val18 and Aib19 is 0.63 (compared to .84).

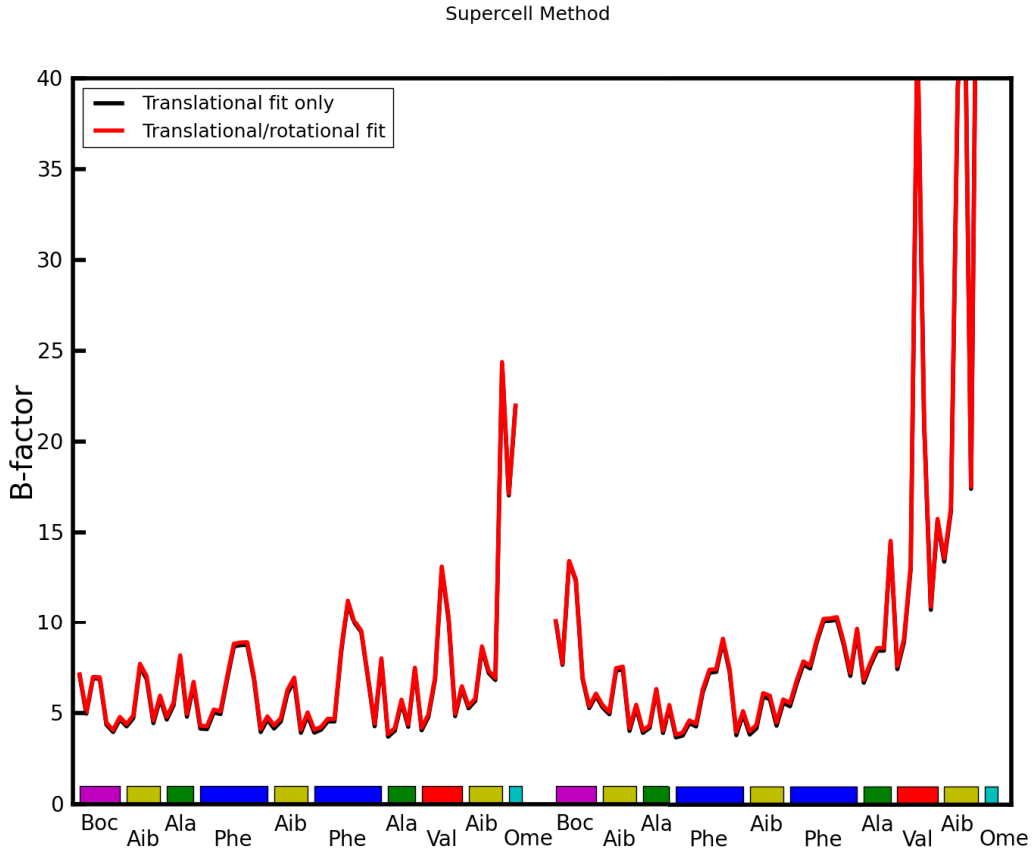


Figure S4: Comparison of "RMSD" B-factors obtained with only translational vs. translational and rotational fitting of supercell frames. The "RMSD" approach to calculating B-factors is described in the main text. It is mainly characterized by a fitting of each snapshot of each asymmetric unit to the experimental structure before calculating positional variance. Here fitting was carried out either as only a translational fit (centers of mass aligned) or a translational/rotational fit (centers of mass aligned and RMSD minimized). The result shows almost no impact on B-factors indicating that very little rigid body rotational motion of the asymmetric units relative to each other is present in the simulation.