Supplementary Information

Folding Simulations of a Nuclear Receptor Box-Containing Peptide Demonstrate the Structural Persistence of the LxxLL Motif Even in the Absence of Its Cognate Receptor

Triantafyllia Adamidou, Konstantina-Olympia Arvaniti
and Nicholas M. Glykos*

Department of Molecular Biology and Genetics, Democritus University of Thrace, University campus, 68100 Alexandroupolis, Greece, Tel +30-25510-30620, Fax +30-25510-30620, https://utopia.duth.gr/glykos/ , glykos@mbg.duth.gr

Figure S1

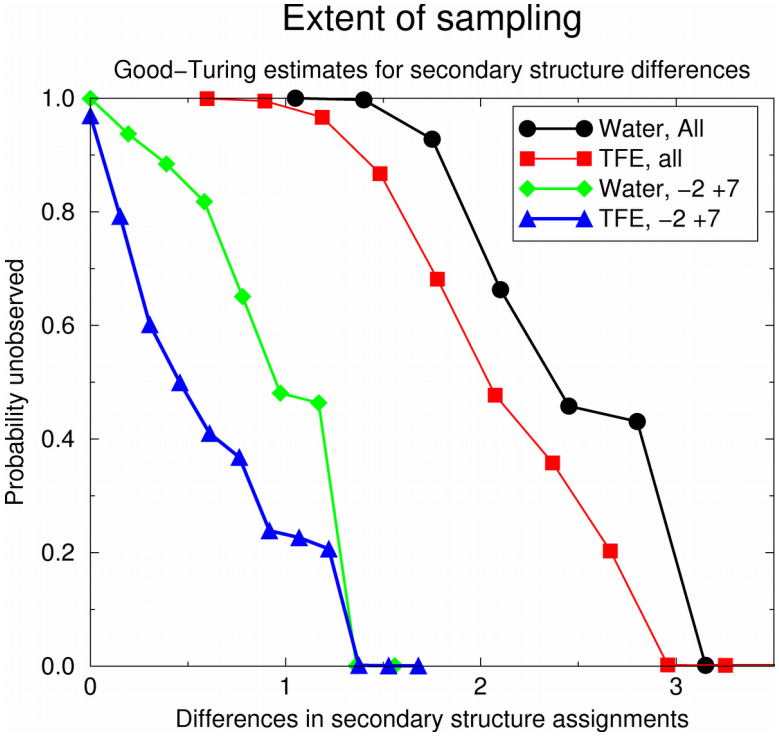

Figure S1 : Extent of sampling and statistical significance. Results from the application of Good-Turing statistics to estimate secondary structure uncertainties for the two trajectories (in water and TFE/water) and for all peptide residues (upper two curves) or for residues -2 to +7 (lower two curves). See section 2.4 for details.

Figure S2

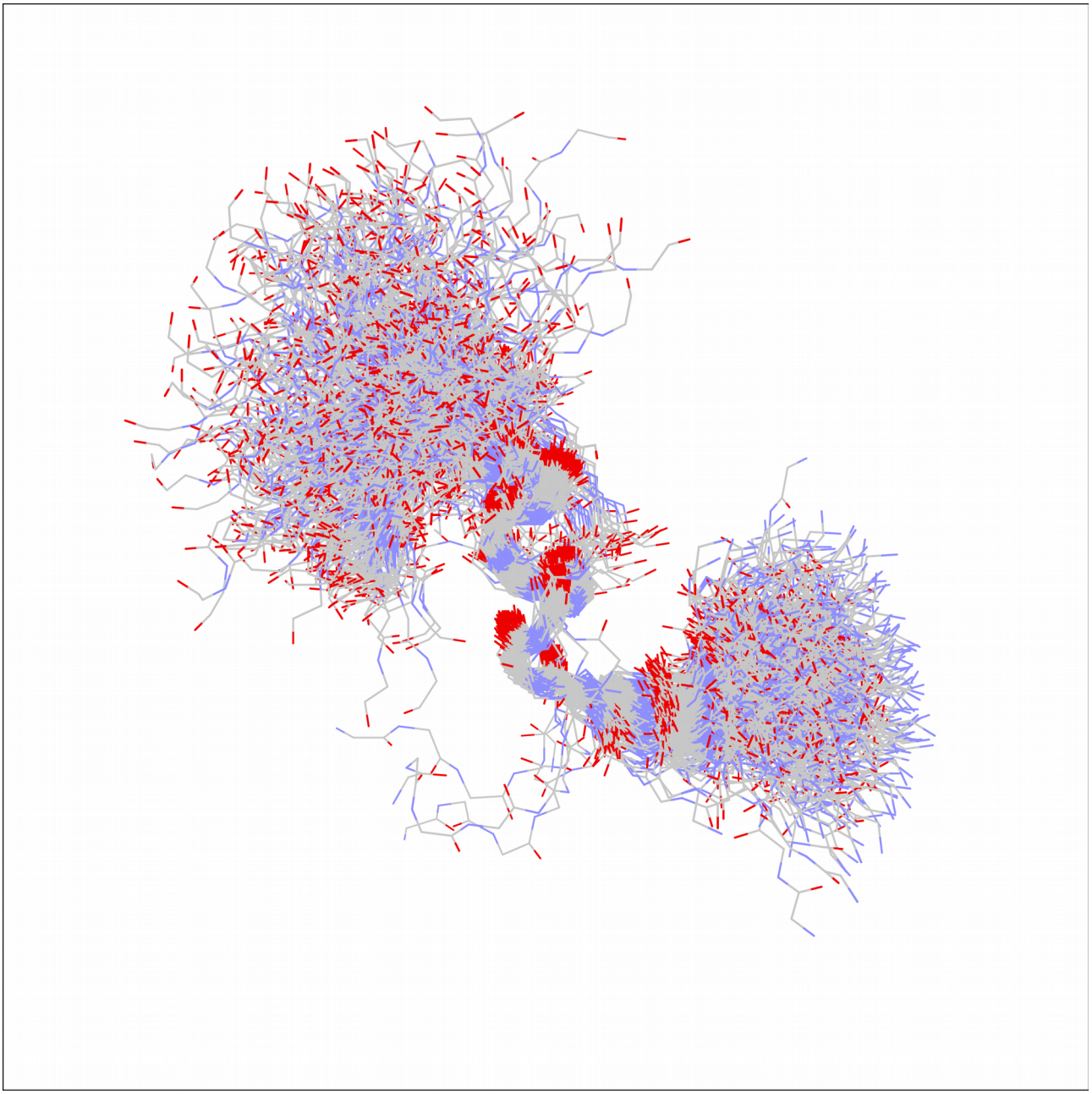

Figure S2 : Superposition of peptide structures that are members of the same dPCA-derived cluster. Superposition of 500 structures (backbone atoms only) that belong to same cluster as obtained from dPCA analysis. This corresponds to the smallest (T3) cluster shown for TFE/water in Figure 4 of the main text.

Table S1

NOE upper bound violations, Water simulation

NOE number	Proton Pairs	Residues & NOE Upper Bounds		Simulation $<r^{-6}>$
1	dαN (i, i+1)	-7V ↔ -6E	5.0	2.241
2		-6E ↔ -5E	3.3	2.256
3		-5E ↔ -4R	3.3	2.960
4		-3P ↔ -2S	3.3	2.300
5		-2S ↔ -1T	2.7	3.378
6		-1T ↔ +1L	2.7	3.218
7		+1L ↔ +2R	5.0	3.169
8		+2R ↔ +3A	**2.7**	**2.818**
9		+3A ↔ +4L	3.3	2.859
10		+4L ↔ +5L	5.0	2.704
11		+5L ↔ +6T	5.0	2.463
12		+6T ↔ +7N	5.0	2.590
13		+8P ↔ +9V	2.7	2.689
14		+9V ↔ +10K	3.3	2.719
15		+10K ↔ +11K	2.7	2.387
16		+11K ↔ +12L	2.7	2.316
17	dNN(i, i+2)	+9V ↔ +11K	5.0	4.427
18	dNN(i, i+1)	-5E ↔ -4R	5.0	2.072
19		-2S ↔ -1T	5.0	2.909
20		-1T ↔ +1L	5.0	2.589
21		+1L ↔ +2R	5.0	2.245
22		+2R ↔ +3A	5.0	2.780
23		+3A ↔ +4L	5.0	2.476
24		+4L ↔ +5L	5.0	2.448
25		+5L ↔ +6T	3.3	2.796
26		+6T ↔ +7N	3.3	2.504
27		+9V ↔ +10K	5.0	2.298

Table S1 (continued)

NOE number	Proton Pairs	Residues & NOE Upper Bounds		Simulation $\langle r^{-6} \rangle$
28	dαN(i, i+2)	+2R ↔ +4L	**3.3**	**4.054**
29		+3A ↔ +5L	**3.3**	**4.339**
30		+7N ↔ +9V	5.0	4.380
31		+8P ↔ +10K	5.0	4.389
32	dαN(i, i+3)	-1T ↔ +3A	5.0	4.281
33		+1L ↔ +4L	5.0	3.602
34		+2R ↔ +5L	5.0	4.923
35		+4L ↔ +7N	5.0	4.441
36		+8P ↔ +11K	**5.0**	**5.292**
37	dβN(i, i+1)	-5E ↔ -4R	5.0	3.606
38		-2S ↔ -1T	5.0	3.448
39		+2R ↔ +3A	5.0	3.136
40		+3A ↔ +4L	**3.3**	**3.398**
41		+5L ↔ +6T	5.0	3.249
42		+6T ↔ +7N	3.3	2.950
43		+9V ↔ +10K	5.0	3.122
46		+11K ↔ +12L	5.0	2.996

Table S1 : Experiment vs Simulation, NOE violations, Water simulation. Direct comparison between the observed NOE upper bounds and the simulation-derived $\langle r^{-6} \rangle$ averages. The violations (5 in total) are marked with a bold typeface. The simulation averages were obtained for all peptide structures whose corresponding adaptive tempering temperature was less than 300K. The experimental upper bounds are as given by Yun *et al.*[9] The average upper bound NOE violation is 0.050 Å [this calculation is using all recorded NOEs, even those for which no violation has been recorded (which enter the calculation with an assigned violation of zero[53])].

Table S2

NOE upper bound violations, TFE/Water simulation

NOE number	Proton Pairs	Residues & NOE Upper Bounds		Simulation $\langle r^{-6} \rangle$
1	dαN(i,i+1)	-6E ↔ -5E	5.0	2.411
2		-5E ↔ -4R	3.3	2.690
3		-3P ↔ -2S	3.3	2.415
4		-2S ↔ -1T	5.0	2.972
5		-1T ↔ +1L	5.0	3.360
6		+1L ↔ +2R	3.3	3.172
7		+2R ↔ +3A	3.3	3.006
8		+3A ↔ +4L	3.3	2.960
9		+4L ↔ +5L	3.3	2.938
10		+5L ↔ +6T	5.0	2.837
11		+6T ↔ +7N	5.0	2.674
12		+8P ↔ +9V	**2.7**	**2.779**
13		+9V ↔ +10K	3.3	2.722
14		+10K ↔ +11K	2.7	2.385
15		+11K ↔ +12L	2.7	2.322
16	dNN(i,i+1)	-5E ↔ -4R	5.0	2.285
17		-1T ↔ +1L	5.0	2.619
18		+1L ↔ +2R	3.3	2.488
19		+2R ↔ +3A	3.3	2.693
20		+3A ↔ +4L	3.3	2.709
21		+4L ↔ +5L	3.3	2.584
22		+5L ↔ +6T	3.3	2.661
23		+9V ↔ +10K	3.3	2.284
24		+10K ↔ +11K	3.3	2.853
25	dαN(i,i+2)	-2S ↔ +1L	5.0	4.102
26		+7N ↔ +9V	**3.3**	**4.241**
27		+8P ↔ +10K	**3.3**	**4.429**
28		+9V ↔ +11K	**3.3**	**4.752**

Table S2 (continued)

NOE number	Proton Pairs	Residues & NOE Upper Bounds		Simulation $<r^{-6}>$
29	dαN(i,i+3)	+2R ↔ +5L	**3.3**	**3.600**
30		+3A ↔ +6T	**3.3**	**3.784**
31		+4L ↔ +7N	5.0	4.001
32		+8P ↔ +11K	**5.0**	**5.338**
33	dαN(i,i+4)	-3P ↔ +2R	**3.3**	**4.670**
34		-2S ↔ +3A	5.0	4.391
35		+1L ↔ +5L	5.0	4.280
36		+2R ↔ +6T	5.0	4.437
37		+3A ↔ +7N	5.0	4.291
38	dαβ(i,i+3)	-3P ↔ +1L	5.0	3.424
39		-2S ↔ +2R	3.3	2.947
40		-1T ↔ +3A	5.0	3.296
41		+1L ↔ +4L	5.0	3.060
42		+2R ↔ +5L	5.0	3.288
43		+4L ↔ +7N	5.0	3.585
44	dβ,N(i,i+1)	-5E ↔ -4R	5.0	3.529
45		-3P ↔ -2S	5.0	3.244
46		-2S ↔ -1T	5.0	3.377
47		-1T ↔ +1L	5.0	3.107
48		+1L ↔ +2R	5.0	3.123
49		+2R ↔ +3A	5.0	3.107
50		+3A ↔ +4L	5.0	3.172
51		+4L ↔ +5L	5.0	3.009
52		+5L ↔ +6T	5.0	3.292
53		+6T ↔ +7N	5.0	3.190
54		+8P ↔ +9V	**3.3**	**3.555**
55		+9V ↔ +10K	3.3	3.151
56		+11K ↔ +12L	5.0	2.954

Table S2 : Experiment vs Simulation, NOE violations, TFE/Water simulation. Direct comparison between the observed NOE upper bounds and the simulation-derived $<r^{-6}>$ averages. The violations (9 in total) are marked with a bold typeface. The simulation averages were obtained for all peptide structures whose corresponding adaptive tempering temperature was less than 300K.

Figure S3

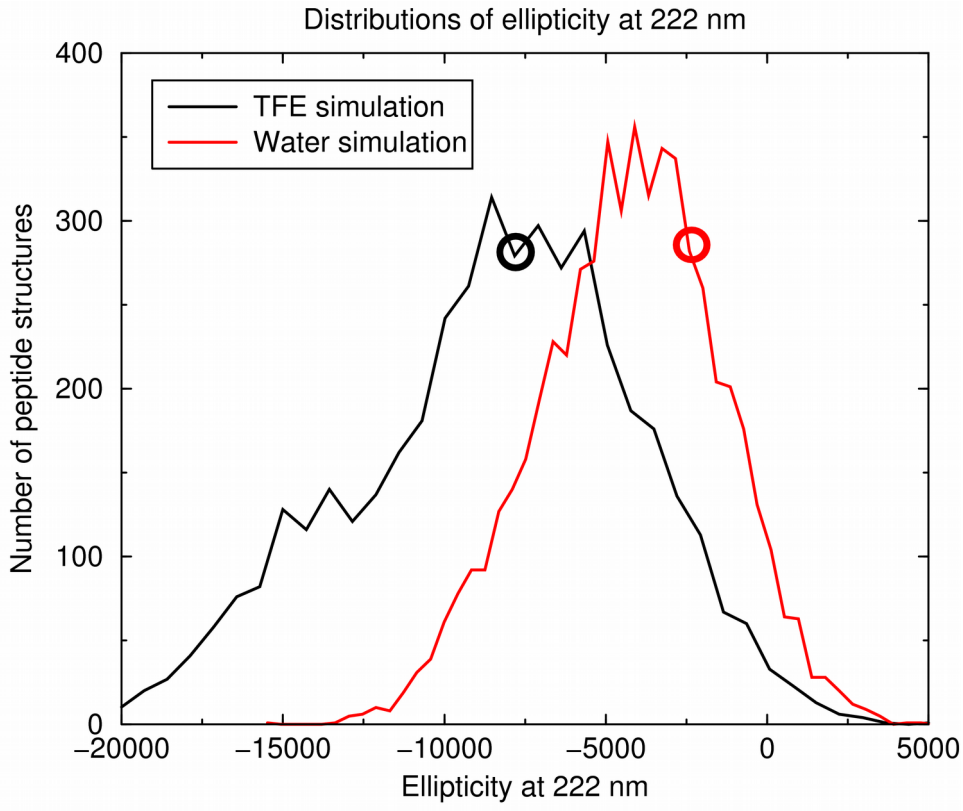

Figure S3 : Comparison with circular dichroism data. The two solid curves depict the distribution of ellipticities at 222 nm calculated (using the DichroCalc server[55]) from a total of approximately 12000 peptide structures recorded from the water (red curve) and the TFE/water (black curve) simulations. The black and red circles that are superimposed on these curves indicate the values of the experimentally obtained ellipticities at 222 nm.