Supplementary Material

The transient manifold structure of the p53 extreme C-terminal domain: Insight into disorder, recognition, and binding promiscuity by Molecular Dynamics simulations

E. Fadda and MG. Nixon

1. Supplementary Material for the Computational Method Section

Convergence of the cumulative 10 (2 μs) simulations has been determined in terms of average backbone RMSD relative to 2 distinct highly populated MoRFs, namely a asymmetric hairpin containing β sheet motifs (cluster 1 from MD 1) and a 3_{10} helical turn located at $_{376}$STS$_{378}$ (cluster 2 from MD 3).

Results for the backbone RMSD calculated relative to the hairpin structure are shown in Figure S.1, panel a), with the corresponding running average over the time interval. Results for the backbone RMSD calculated relative to the 3_{10} $_{376}$STS$_{378}$ helical turn are shown in panel b), with the corresponding running average over the time interval. Finally, the RMSD average correlation (RAC) values calculated relative to the asymmetric hairpin and to the 3_{10} $_{376}$STS$_{378}$ helical turn are shown in panel c). RAC values were calculated as,

$$RAC_t = \frac{\sum_{MD=1}^{10} RMSD_t}{t}$$

where t indicates the time interval from the start of the simulation up to 2 μs.

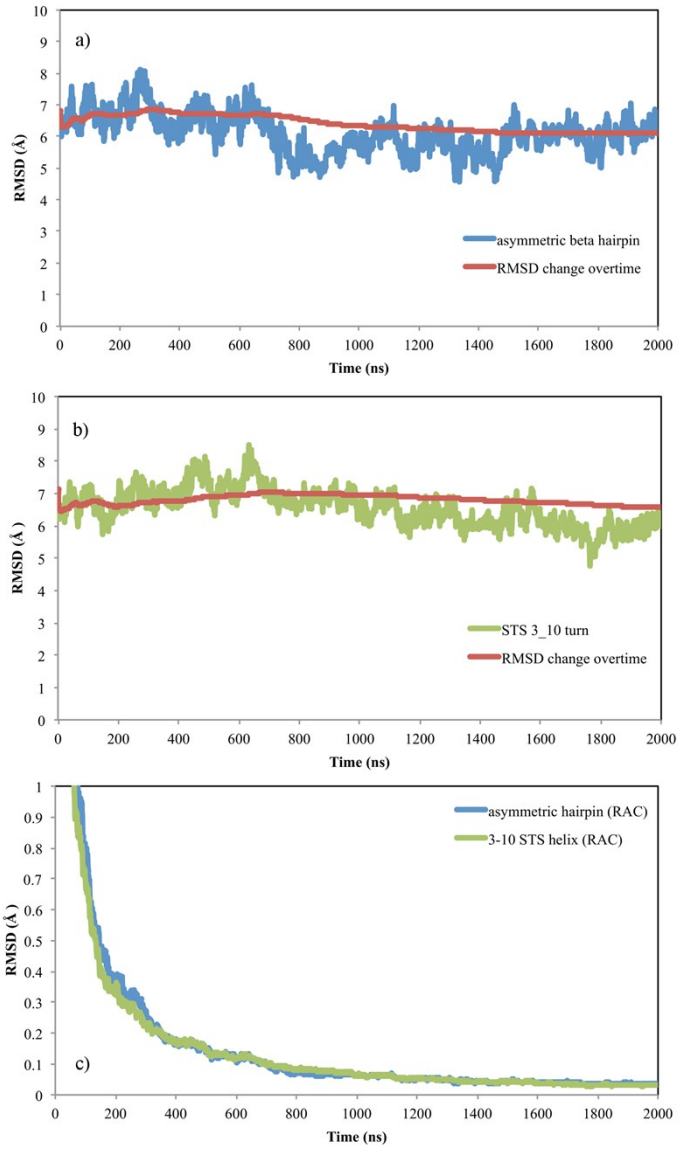

Figure S.1

The starting structures for the 10 MD simulations have been taken from a single trajectory of 100 ns, started from the peptide in a fully extended conformation, selected at 10 ns intervals. The correlation time calculated from the exponential

fit of the velocity autocorrelation function is 58.9 ps. The structural diversity of these starting structures has been evaluated in terms of backbone atoms RMSD values shown in **Table S.1**, where high structure similarity, below the 0.45 nm threshold chosen for the clustering analysis, is highlighted.

Table S. 1 Backbone RMSD values calculated for the 10 snapshots chosen as starting points for the 10 independent 2 μs MD simulations. The highlighted cells indicated values that are within the threshold chosen for the clustering analysis, i.e. 4.5 Å.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
S1	0.0									
S2	5.5	0.0								
S3	10.0	10.8	0.0							
S4	10.5	10.8	4.4	0.0						
S5	13.4	12.2	6.7	7.0	0.0					
S6	13.2	11.7	7.0	7.9	3.4	0.0				
S7	12.1	10.7	8.2	9.7	5.3	3.7	0.0			
S8	12.4	11.2	7.9	9.4	4.9	3.4	1.6	0.0		
S9	12.3	11.2	7.9	9.2	5.1	5.0	3.7	3.7	0.0	
S10	10.7	8.9	8.0	9.0	6.9	6.2	4.8	5.4	5.3	0.0

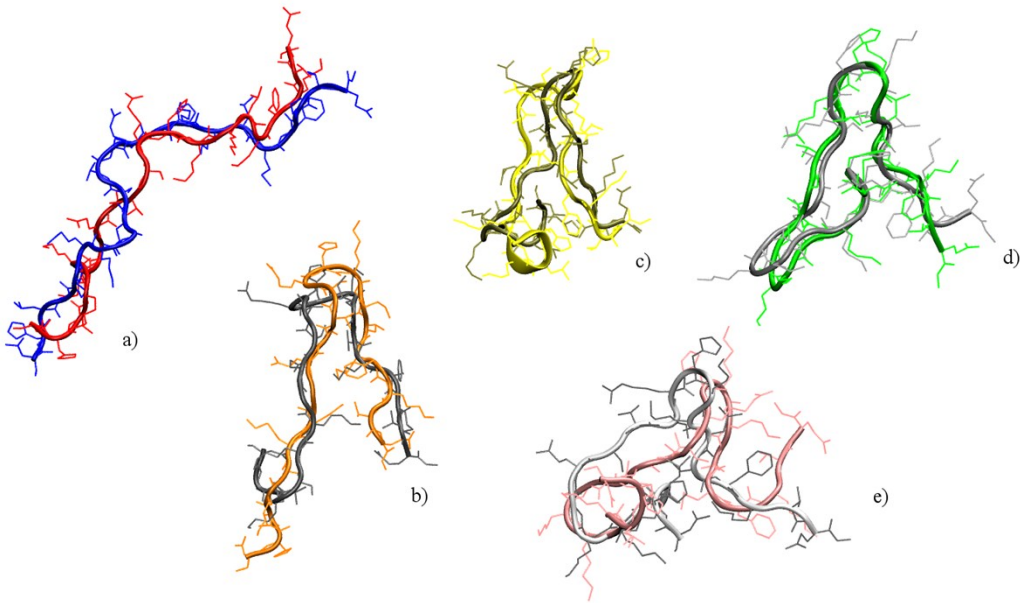

Figure S. 2. Structural alignment of the backbone atoms for the 10 snapshots selected at 10 ns intervals over 100 ns initial trajectory as starting points for the 10 independent 2 μs MD simulations. Structures are shown in pairs, namely s1 and s2 (panel a), s3 and s4 (panel b), s5 and s6 (panel c), s7 and s8 (panel d), and s9 and s10 (panel e).

2. Supplementary Material for the Results Section

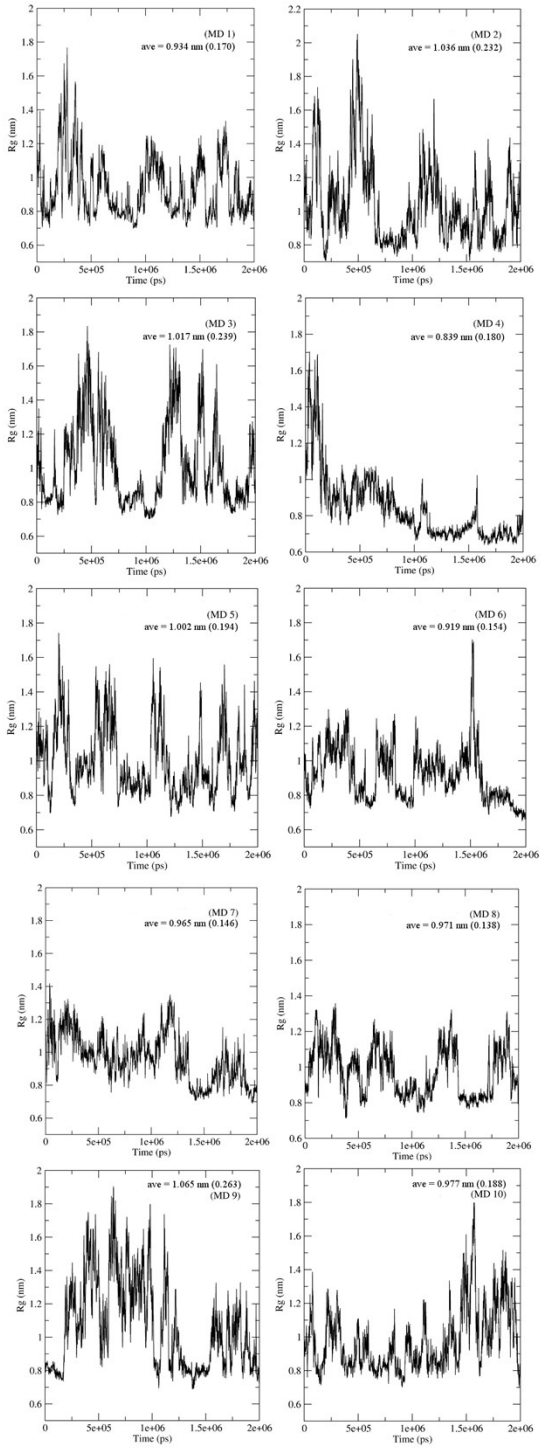

Figure S.3. Radius of gyration (Rg) values calculated over the 10 (2 μs) MD simulations, namely MD 1 to 10, of the 22 residue p53-CTD peptide unbound in solution. Standard deviations are indicated in brackets.

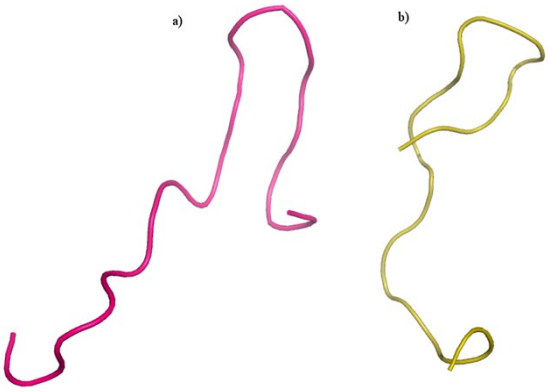

Figure S.4. Low populated β-sheet conformers identified through the MD simulation that slightly differ from the highest populated and most stable asymmetric fold. In panel a) the conformer corresponding to MD5 cluster 9, in panel b) the conformer corresponding to MD10 cluster 7.

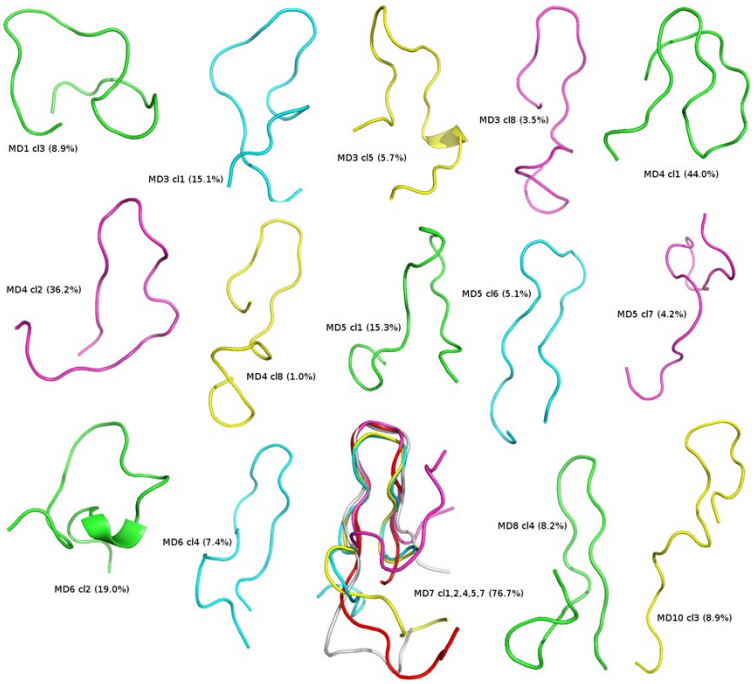

Figure S.5. Structures containing β-bridges visited during the 20 ms MD simulations. The label beside each structure indicates the MD run and the cluster number, 1 to 10, 1 being the highest populated. The relative population of the structure, or of the structures in case of MD7, over 2 μs is indicated in brackets. Structures are represented with the N-terminal tail on the left hand side of the image.

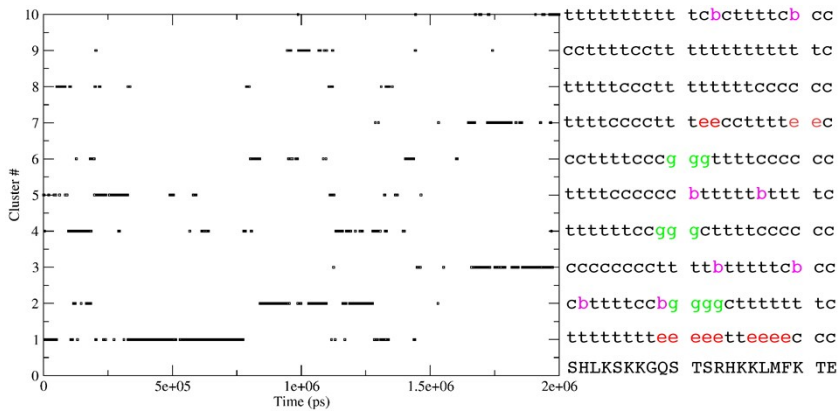

Figure S.6. Cluster ID overtime calculated for MD10. The secondary structure assignments (STRIDE) for the middle structure of each cluster is indicated on the right hand side, together with the peptide sequence. B-sheets, 310 helices, b-bridges, coils and turns are indicated with the letters, e (red), g (green), b (pink), c and t (black).

Table S 2. RMSD (Å) values matrix obtained by sequence alignment, followed by structural refinement, of all the β-sheet structural motifs identified during the 20 μs MD simulation. RMSD. The alignment was done with PyMol.

	b-sheet	MD1 cl1	MD1 cl2	MD1 cl4	MD5 cl9	MD6 cl1	MD6 cl3	MD6 cl5	MD6 cl8	MD7 cl3	MD7 cl6	MD7 cl9	MD8 cl1	MD8 cl2	MD8 cl3	MD8 cl5	MD8 cl10	MD10 cl1	MD10 cl7
MD1	cl1	**0.00**																	
MD1	cl2	**1.02**	**0.00**																
MD1	cl4	**0.56**	**0.90**	**0.00**															
MD5	cl9	**4.76**	**4.60**	**4.66**	**0.00**														
MD6	cl1	**2.24**	**2.23**	**2.08**	**3.87**	**0.00**													
MD6	cl3	**2.56**	**2.51**	**2.38**	**3.62**	**0.67**	**0.00**												
MD6	cl5	**2.75**	**2.79**	**2.62**	**4.04**	**1.63**	**1.62**	**0.00**											
MD6	cl8	**2.85**	**2.71**	**2.54**	**3.46**	**1.30**	**0.88**	**1.82**	**0.00**										
MD7	cl3	**2.30**	**2.33**	**2.13**	**4.09**	**1.40**	**0.89**	**1.69**	**1.79**	**0.00**									
MD7	cl6	**2.90**	**2.98**	**2.77**	**3.39**	**2.32**	**2.32**	**2.26**	**2.26**	**1.08**	**0.00**								
MD7	cl9	**3.47**	**3.32**	**3.39**	**3.23**	**2.72**	**2.89**	**3.52**	**2.61**	**3.11**	**2.91**	**0.00**							
MD8	cl1	**2.05**	**2.59**	**2.35**	**4.07**	**1.31**	**0.55**	**1.77**	**1.72**	**1.99**	**2.07**	**2.47**	**0.00**						
MD8	cl2	**2.25**	**2.09**	**2.02**	**4.08**	**1.69**	**1.99**	**2.71**	**1.55**	**1.34**	**2.06**	**2.84**	**1.84**	**0.00**					
MD8	cl3	**2.29**	**2.32**	**2.13**	**4.04**	**1.50**	**1.56**	**2.26**	**1.35**	**1.58**	**1.93**	**2.59**	**0.79**	**1.31**	**0.00**				
MD8	cl5	**2.39**	**2.42**	**2.25**	**3.75**	**1.33**	**1.36**	**1.85**	**1.47**	**0.52**	**2.65**	**2.56**	**0.73**	**1.31**	**0.72**	**0.00**			
MD8	cl10	**2.72**	**2.70**	**2.59**	**4.65**	**1.39**	**2.19**	**2.58**	**1.78**	**2.47**	**3.84**	**1.37**	**1.30**	**1.71**	**1.46**	**1.82**	**0.00**		
MD10	cl1	**2.31**	**2.12**	**2.33**	**4.59**	**2.22**	**3.23**	**3.45**	**3.57**	**3.05**	**3.55**	**4.07**	**3.34**	**2.87**	**3.12**	**3.22**	**4.43**	**0.00**	
MD10	cl7	**5.36**	**5.30**	**5.11**	**4.56**	**4.74**	**4.14**	**3.54**	**3.68**	**4.13**	**4.60**	**4.74**	**4.62**	**4.31**	**4.30**	**3.63**	**4.46**	**6.02**	**0.00**

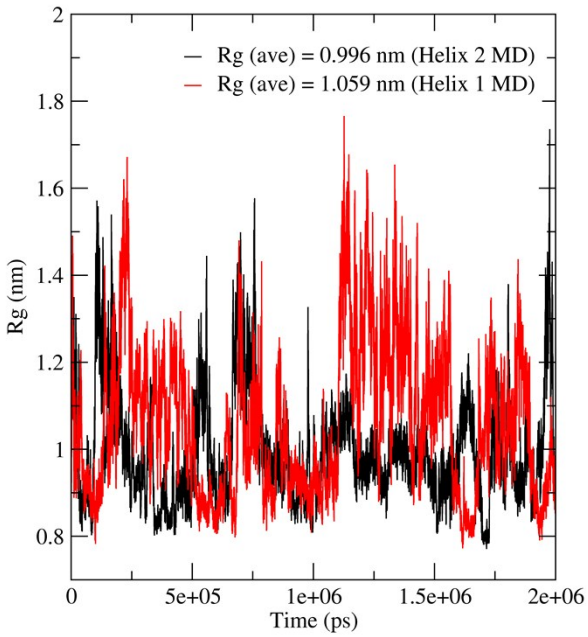

Figure S.7. Rg values (nm) from the 2 μs trajectories started from the helical conformation of the p53-CTD peptide when bound to the S100B(ββ) (PDBid 1dt7). The Helix 2 MD (black line) was started from structure 6 of the NMR ensemble, while Helix 1 MD (red line) was started from structure 3 of the NMR ensemble.