Supporting information for "Molecular Simulations Identify Binding Poses and Approximate Affinities of Stapled α-helical Peptides to MDM2 and MDMX"

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Table S1: Peptides and their experimental activities as taken from Ref. 15 or Ref. 16 of the main text. These references are denoted here by superscripts "a" and "b" respectively. For ATSP-7342 binding to MDMX a value of 1000 nM was chosen to represent the affinity for the sake of comparison.

Peptide	Ki MDM2 (nM)	Ki MDMX (nM)
ATSP-3900	8.0[a]	12.7[a]
A17	3.0[a]	7.3[a]
A18	24.1[a]	105.2[a]
A19	228[a]	13224[a]
ATSP-4641	5.0[a]	34[a]
A22	20.8[a]	76.9[a]
A23	196[a]	8600[a]
A25	17.5[a]	12.5[a]
A26	550[a]	862[a]
A28	5.0[a]	11.2[a]
ATSP-6935	1.2[b]	8[b]
ATSP-7041	0.9[b]	6.8[b]
ATSP-7342	536[b]	>1000[b]

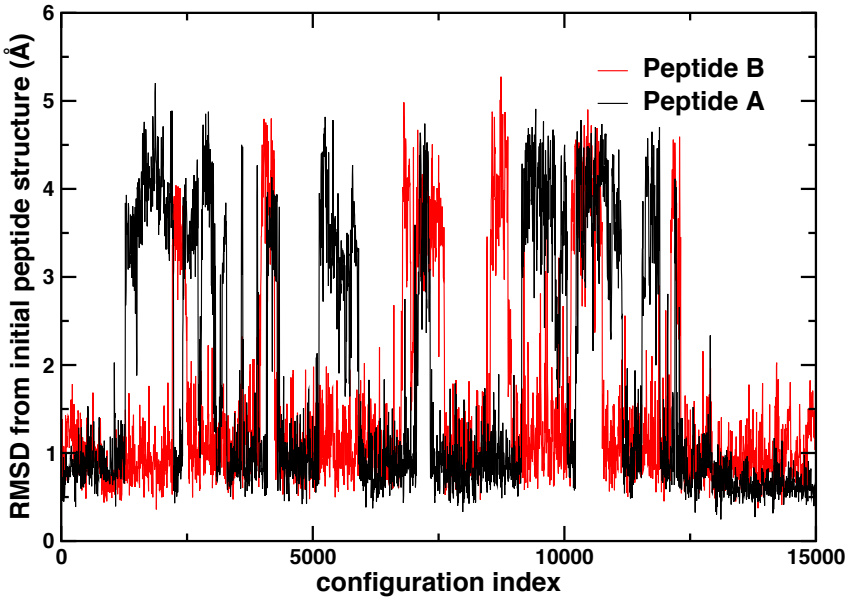


Figure S1: RMSD from the initial peptide conformation for Peptide A (black line) and Peptide B (red) line as they bind and unbind from the target protein. RMSD is measured as described in the main text and a low RMSD indicates a primarily helical structure. Results shown are for a selected trajectory corresponding to Movie S1.

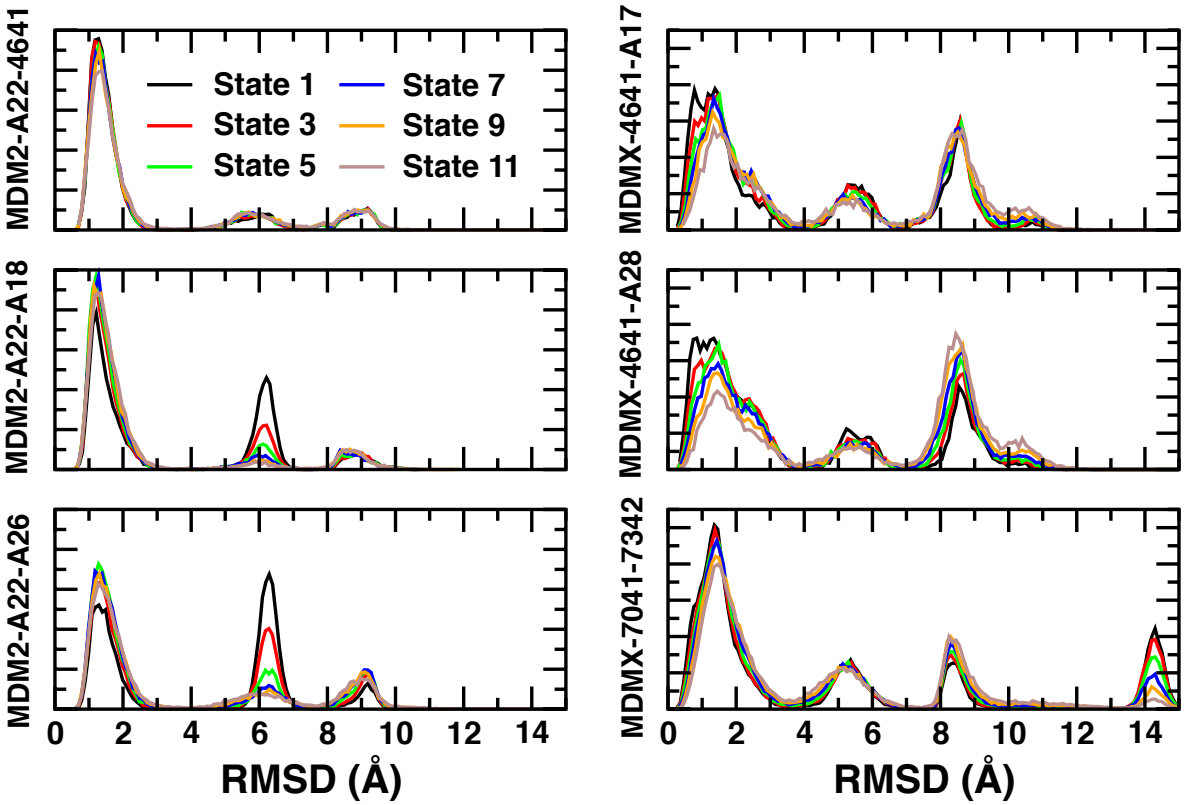


Figure S2: Peptide RMSD with respect to crystal pose for 6 selected states and 6 selected systems in the range from the lowest state (replica state 1) to replica state 11. Peptide RMSD is computed as described in the main text. Included peptide conformations are from states in the lower portion of the replica ladder where the binding MELD restraints are sufficiently imposed. For the purpose of this graph both peptide A and peptide B are combined in the same distribution. Although in some cases the peak heights may vary, all systems sample the same conformations as indicated by the peak position. Thus, replicas in the lower portion of the ladder mix well and are sampled consistently.

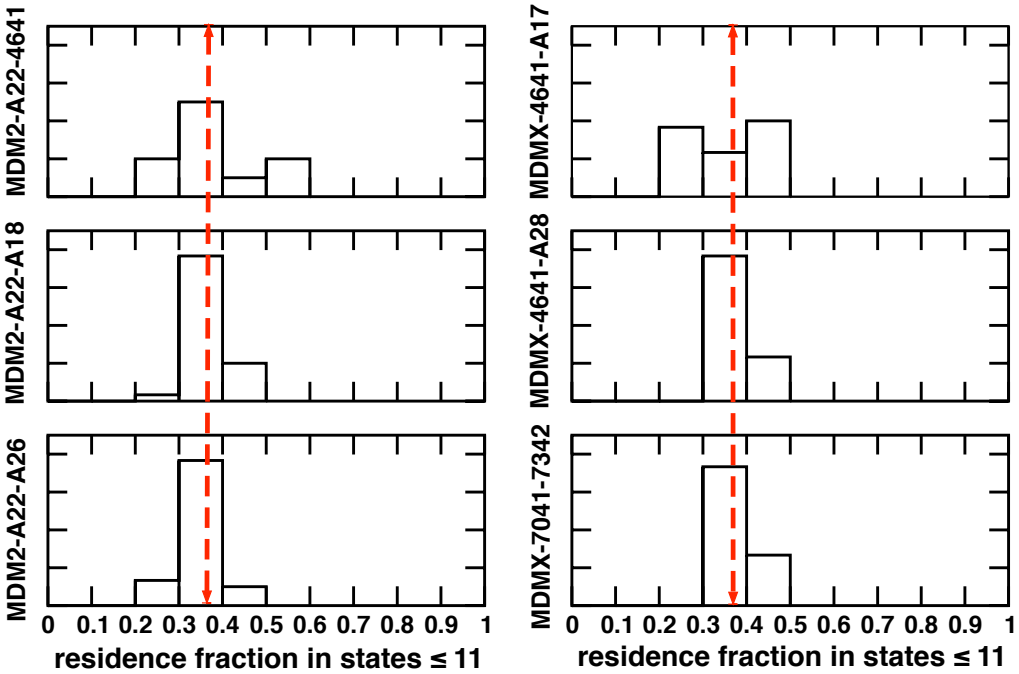


Figure S3: Mixing between states with index ≤ 11 and those with index > 11 can be characterized by the distribution of the fraction of time a replica spends in states with index ≤ 11. This distribution for the same 6 systems considered in Figure S3 is depicted above. Ideally this distribution should be sharply peaked at 11/30 or 0.367 (red, dashed line). Very poor mixing would correspond to a bimodal distribution with peaks at 0 and 1. The present results show that the mixing is always satisfactory and in most cases, quite good.

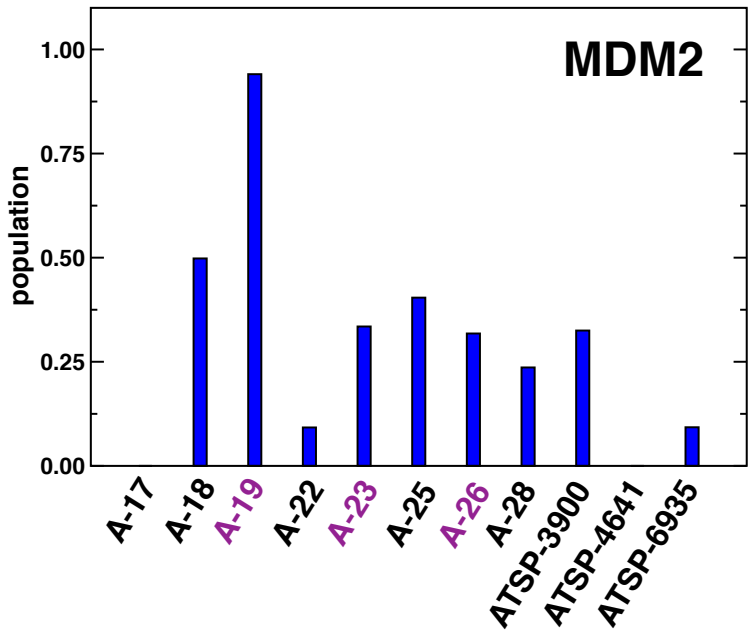


Figure S4: Population of the alternate binding mode of a given peptide to MDM2 that is shown in Fig. 3A of the main text. Within each run, clusters with a minimum size of 10% were considered in this analysis.

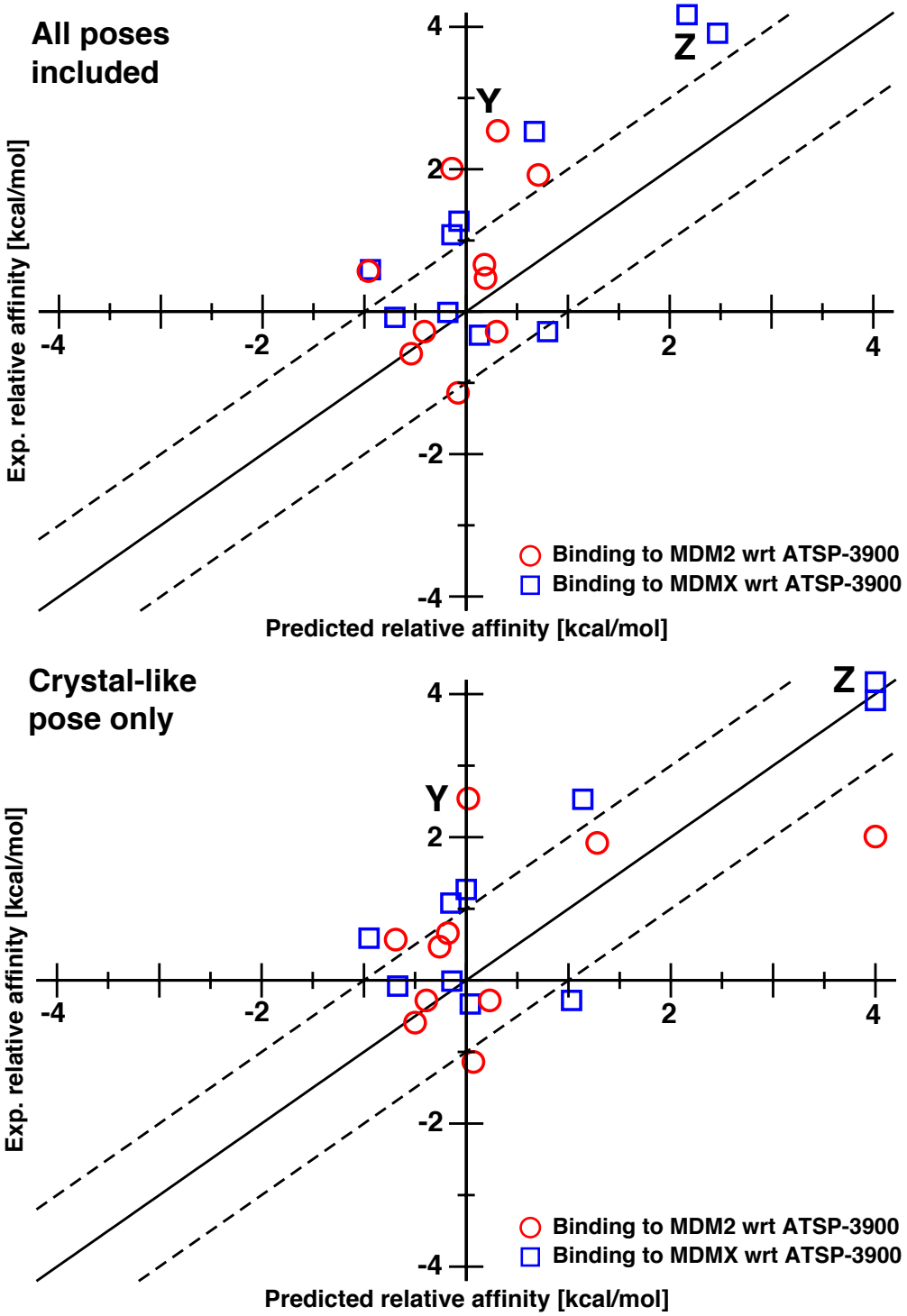


Figure S5: Same as Fig. 4 in the main text but with ATSP-3900 as the reference peptide.

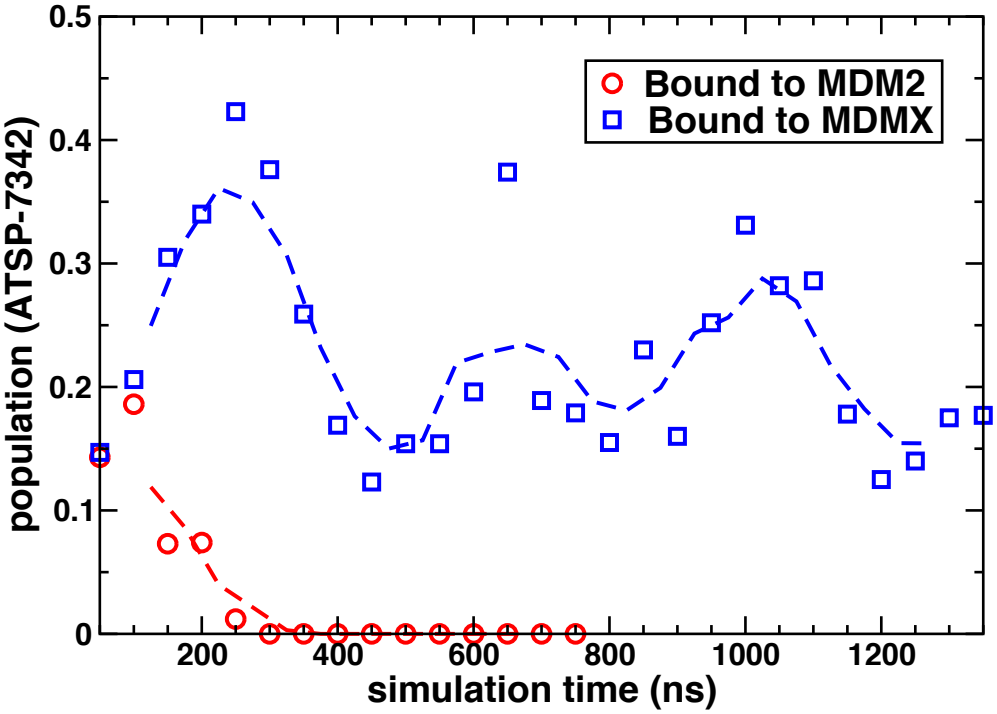
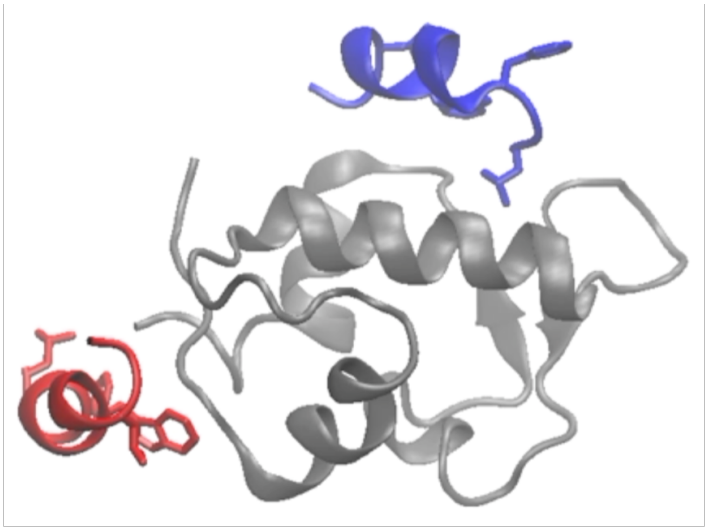


Figure S6: Population of ATSP-7342 bound to MDM2/X over the course of the MELD simulation as monitored from the lowest temperature replica. Each point is the population extracted from blocks of 1000 configurations. The dashed lines represent running averages over neighboring points.



Movie S1: Snapshot from a movie illustrating peptide binding simulations. Peptides A (blue) and B (red) are simulated with target protein MDM2 (gray). In the case of peptides, Phe, Trp, and Leu residues are shown explicitly. All others are represented by their secondary structure elements as rendered by VMD. This movie illustrates the binding and unbinding process that occurs in the course of the MELD simulation as one follows a trajectory up and down the replica ladder. It is provided in the Supporting Information.