In silico improvement of β^3-peptide inhibitors of p53•hDM2 and p53•hDMX

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Computational methodologies

To construct a model of β-peptides interacting with hDM2 the structure of hDM2 in complex with an 8-mer p53 peptide analog was downloaded from the protein data bank (PDB code 2GV2, resolution 1.8 Å).[1] A conformation from the 14-helical NMR structure of a similar β-peptide in solution[2] was then overlaid on the 8-mer p53 peptide analog based on the hypothesis that the β-peptide residues of the recognition face Leucine3, Tryptophan6, Phenyalanine9 mimic the position of the residues Leu19, Tryptophan 23 and Phenylalanine 26 of peptides fragments of the p53 binding domain seen in other crystal structures.[3] The side chain torsions of Tryptophan6 and Phenylalanine9 were manually adjusted to avoid steric clashes and the 8-mer peptide was then deleted.

The resulting β-peptide hDM2 complex was parameterized using the OPLS-AA force field[4] and subjected to a conjugate gradient minimization with harmonic restraints on the Cα positions of hDM2 to reduce structural modifications. The complex was then equilibrated with a room temperature Monte Carlo simulation with MCPRO.[5] The final configuration was used as a starting point for substituents scans with the programs BOMB[6] and MCPRO to generate the β-peptides of interest. A similar procedure was used to construct the model of a β-peptide in complex with hDMX, starting from the structure of humanized zebrafish MDMX in complex with a peptide analog of p53 (PDB code 2Z5T, resolution 2.3 Å).[7]

Free energy calculations on the protein-ligand complexes were performed with MCPRO using Metropolis Monte Carlo sampling[8] and free energy perturbation theory.[9] In this approach the relative binding affinity of a pair of analogs A-B is calculated as the difference in the free energy change for the conversion of analog A into B bound to the protein, and for the conversion of analog A into B in solution (equation 1).

(1) $$\Delta\Delta G_{bind}(A \rightarrow B) = \Delta G_{bound}(A \rightarrow B) - \Delta G_{solution}(A \rightarrow B)$$

For each β-peptide, free energy changes for the bound β-peptides were computed using 10 evenly spaced windows and double wide sampling.[9] The simulations were initiated from an equilibrated TIP4P[10] water cap of 22 Å radius centered on the β-peptide in the complex and consisted of 5M solvent moves only, 10M moves of full equilibration, followed by 20M moves of averaging. A residue based non bonded cutoff of 10 Å was employed. The β-peptides residues were fully flexible, while only the side chain bond angles and torsions of selected hDM2 and hDMX residues in the vicinity of the β-peptide were sampled. The computed relative affinities to p53●hDM2 for all the analogs considered are listed in Table SI-1. Affinities to p53●hDMX were only computed for a subset of analogs. Additionally, the computations were repeated with concerted rotations Monte Carlo moves to allow backbone relaxation of the protein residues in hDM2 and hDMX for the β-peptides analogs discussed in the main text of the manuscript.[11] To maintain a satisfactory acceptance rate the default range of change of the torsional degrees of freedom of the β-peptides backbone atoms were reduced to 0.125 degrees (CB-N-C-CA) and 0.5 degrees (CA-CB-N-C, C-CA-CB-N and N-C-CA-CB).

The simulations of the β-peptides in solution used a similar protocol and a 14-helical conformation similar to a NMR solution conformation was assumed for all the analogs considered.[2]

In some instances it was unclear on to which face of a phenyl ring ortho or meta substituents would preferably orient, so both conformations were considered for the solution calculations and the simulations that gave the lowest free energy change were used in eq (1).

Experimental methodologies

Fmoc-protected α-amino acids, PYBOP®, HOBt, and Wang resin were purchased from Novabiochem (San Diego, CA). Dimethylformamide (DMF), trifluoroacetic acid (TFA), and piperidine were purchased from American Bioanalytical (Natick, MA). All other reagents were purchased from Sigma-Aldrich. Fmoc-(S)-3-amino-4-(3-trifluoromethylphenyl)butyric acid was purchased from AnaSpec, Inc. (San Jose, CA). The remaining Fmoc-β^3-(L)-amino acids were synthesized from enantiomerically pure α-amino acids via the Arndt-Eistert procedure[12] with the exception of Fmoc-(S)-3-amino-4-(6-

chloroindole)butyric acid, the enantiomeric resolution and homologation of which has been previously reported.[13] β^3-peptides were synthesized using a CEM MARS microwave reactor in a glass peptide-synthesis vessel with fritted glass at the top and bottom and a sidearm for addition of reagents. Peptides were synthesized on a 25 μmol scale using standard Fmoc chemistry with Wang resin and Fmoc-protected β^3-amino acid monomers as previously described.[13] The details of peptide labeling, protein expression and the fluorescence polarization direct binding assays have also been reported previously.[13]

Complete reference for reference 20

Shangary, S.; Qin, D. G.; McEachern, D.; Liu, M. L.; Miller, R. S.; Qiu, S.; Nikolovska-Coleska, Z.; Ding, K.; Wang, G. P.; Chen, J. Y.; Bernard, D.; Zhang, J.; Lu, Y. P.; Gu, Q. Y.; Shah, R. B.; Pienta, K. J.; Ling, X. L.; Kang, S. M.; Guo, M.; Sun, Y.; Yang, D. J.; Wang, S. M. *Proc Natl Acad Sci U S A* **2008**, *105*, 3933-3938.

Binding Motif	$\Delta\Delta G_{bind}$ hDM2	hDMX
L-W-F	0	0
L-W6F-F	-1.0 ± 0.1	
L-W6Cl-F	-2.8 ± 0.1	0.9 ± 0.2
L-W6Br-F	-2.4 ± 0.2	
L-W6I-F	-1.8 ± 0.4	
L-F-F	0	0
L-FoCH3-F	1.3 ± 0.1	
L-FmCH3-F	-2.0 ± 0.1	-2.5 ± 0.2
L-FpCH3-F	-1.0 ± 0.1	1.7 ± 0.1
L-FoCl-F	-2.5 ± 0.1	1.3 ± 0.1
L-FmCl-F	-3.9 ± 0.1	-4.0 ± 0.1
L-FpCl-F	-2.9 ± 0.1	-2.0 ± 0.1
L-FoCF3-F	-0.2 ± 0.2	
L-FmCF3-F	-5.2 ± 0.2	-3.9 ± 0.2
L-FpCF3-F	-1.1 ± 0.2	
L-FpClmCl-F	-3.7 ± 0.2	-3.9 ± 0.2
L-FpCloCl-F	-5.7 ± 0.2	-2.1 ± 0.2
L-2,5diClF-F	-4.4 ± 0.2	-2.4 ± 0.2
L-3,5diClF-F	-3.3 ± 0.2	
L-FpI-F	-2.4 ± 0.2	
L-FpCH2CH3-F	-3.4 ± 0.2	2.2 ± 0.1
L-FpSCH3-F	-3.6 ± 0.2	
L-FpOCH3-F	-2.0 ± 0.2	1.5 ± 0.3
L-FpOCF3-F	-3.0 ± 0.4	0.4 ± 0.4
L-FmCH2CH3-F	-3.8 ± 0.2	-2.4 ± 0.2
L-FmSCH3-F	-4.2 ± 0.3	-2.4 ± 0.3
L-FmOCH3-F	-3.5 ± 0.3	-3.0 ± 0.2
L-FmOCF3-F	-6.0 ± 0.4	-3.6 ± 0.4
L-FmCF3-F	0	0
l-FmCF3-F	-1.3 ± 0.3	0.4 ± 0.3
L-FmClpCL-F	0	0
L-FmClpCl-FpF	-0.6 ± 0.1	-0.4 ± 0.1

Table SI-1. Computed relative affinities for series of modeled β^3-peptides binding to hDM2 and hDMX. The predicted binding affinities in each section (separated by bold lines) are relative to the first compound of the section. The figures are in kcal•mol^{-1} and error intervals ($\pm 1\sigma$) are shown.

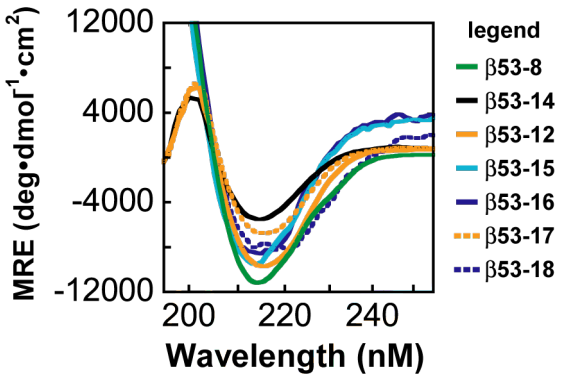

Figure SI-1. Circular dichroism spectra of 50 μM β^3-peptides in binding buffer (10 mM Tris, 100 mM NaCl, 0.01% Tween, pH 7.4).

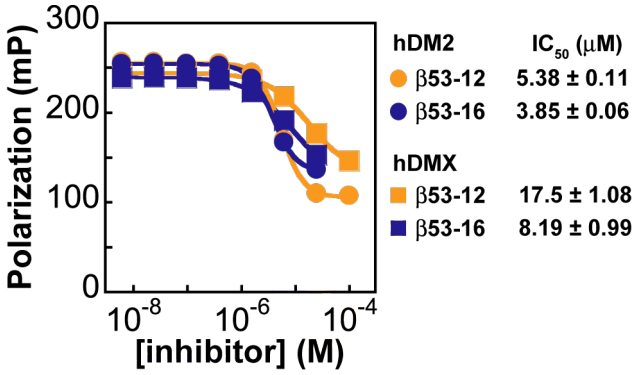

Figure SI-2. Competition fluorescence polarization assay: plot of the polarization of the **p53AD$_{15\text{-}31}^{flu}$**•hDM2$_{1\text{-}188}$ and the **p53 12/1^{flu}**•hDMX$_{1\text{-}200}$ complexes as a function of the concentration of unlabeled peptide shown. Details on the p53-based control molecules and this assay have been reported previously.[13] Briefly, unlabeled **β53-12** and **β53-16**, at concentrations ranging from 10 nM to 200 μM, competed with **p53AD$_{15\text{-}31}^{flu}$** or **p53 12/1^{flu}** for binding to hDM2$_{1\text{-}188}$ or hDM2$_{1\text{-}200}$, respectively. Each value shown represents the average of at least four independent determinations, each performed in triplicate and averaged; error bars represent the standard error.

Table SI-2. Theoretical and MALDI-TOF MS-observed molecular weights for reported β^3-peptides and controls.

β^3-peptide	Formula	Mass calcd ($M+H^+$)	Masses found
β53-8^{flu}	$C_{99}H_{136}N_{14}O_{22}S$	1907.3	1908.3 ($M + H^+$), 1930.3 ($M + Na^+$)
β53-13^{flu}	$C_{99}H_{135}ClN_{14}O_{22}S$	1940.7	1939.9 ($M + H^+$), 1960.8 ($M + Na^+$)
β53-14^{flu}	$C_{97}H_{135}N_{13}O_{22}S$	1869.1	1870.6 ($M + H^+$), 1892.6 ($M + Na^+$)
β53-12^{flu}	$C_{98}H_{134}F_3N_{13}O_{22}S$	1935.3	1937.9 ($M + H^+$), 1959.8 ($M + Na^+$)
β53-15^{flu}	$C_{97}H_{134}ClN_{13}O_{22}S$	1902.1	1903.8 ($M + H^+$), 1925.1 ($M + Na^+$)
β53-16^{flu}	$C_{97}H_{133}Cl_2N_{13}O_{22}S$	1936.1	1936.7 ($M + H^+$), 1960.0 ($M + Na^+$)
β53-17^{flu}	$C_{98}H_{134}F_3N_{13}O_{22}S$	1937.1	1936.6 ($M + H^+$), 1958.5 ($M + Na^+$)
β53-18^{flu}	$C_{97}H_{132}Cl_2FN_{13}O_{22}S$	1956.0	1958.9 ($M + H^+$), 1979.7 ($M + Na^+$)

hDM2	K_d	ΔG_{bind}	$\Delta\Delta G_{bind}$ calc.	$\Delta\Delta G_{bind}$ exper.
β53-8	204 ± 17.7	–9.12	0	0
β53-13	30.1 ± 4.93	–10.25	–2.1 ± 0.1	–1.13
β53-14	816 ± 31.1	–8.30	0	0
β53-12	28.2 ± 4.79	–10.29	–4.8 ± 0.1	–1.99
β53-15	150 ± 15.9	–9.30	–2.5 ± 0.1	–1.00
β53-16	27.6 ± 5.88	–10.31	–4.4 ± 0.1	–2.01
β53-12	28.2 ± 4.79	–10.29	0	0
β53-17	39.6 ± 2.87	–10.09	–0.9 ± 0.3	–0.20
β53-16	27.6 ± 5.88	–10.31	0	0
β53-18	53.1 ± 0.65	–9.93	–0.4 ± 0.1	0.38

hDMX	K_d	ΔG_{bind}	$\Delta\Delta G_{bind}$ calc.	$\Delta\Delta G_{bind}$ exper.
β53-8	2130 ± 139	–7.73	0	0
β53-13	1600 ± 80.0	–7.90	–1.0 ± 0.2	–0.17
β53-14	>10,000	–6.82	0	0
β53-12	518 ± 41.3	–8.57	–4.6 ± 0.2	–1.75
β53-15	877 ± 166	–8.26	–3.4 ± 0.1	–1.44
β53-16	155 ± 30.0	–9.28	–5.4 ± 0.2	–2.46
β53-12	518 ± 41.3	–8.57	0	0
β53-17	971 ± 50.9	–8.20	–0.3 ± 0.3	–0.37
β53-16	155 ± 30.0	–9.28	0	0
β53-18	403 ± 96.0	–8.72	–0.9 ± 0.1	–0.56

Table SI-3. Detailed binding data for β^3-peptides studied. ΔG and ΔΔG values are given in kcal•mol^{-1} and K_d values are given in nM.

REFERENCES

(1) Sakurai, K.; Schubert, C.; Kahne, D. *J Am Chem Soc* **2006**, *128*, 11000-11001.
(2) Kritzer, J. A.; Hodsdon, M. E.; Schepartz, A. *J Am Chem Soc* **2005**, *127*, 4118-4119.
(3) Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N. P. *Science* **1996**, *274*, 948-953.
(4) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J Am Chem Soc* **1996**, *118*, 11225-11236.
(5) Jorgensen, W. L.; Tirado-Rives, J. *J. Comput. Chem.* **2005**, *26*, 1689-1700.
(6) Jorgensen, W. L. *Science* **2004**, *303*, 1813-1818.
(7) Popowicz, G. M.; Czarna, A.; Rothweiler, U.; Szwagierczak, A.; Krajewski, M.; Weber, L.; Holak, T. A. *Cell Cycle* **2007**, *6*, 2386-2392.
(8) Metropolis, N.; Rosenbluth, A. W.; Rosenbluth, M. N.; Teller, A. H.; Teller, E. *J. Chem. Phys.* **1953**, *21*, 1087-1092.
(9) Jorgensen, W. L.; Thomas, L. L. *J. Chem. Theory Comput.* **2008**, *4*, 869-876.
(10) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926-935.
(11) Ulmschneider, J. P.; Jorgensen, W. L. *J. Chem. Phys.* **2003**, *118*, 4261-4271.
(12) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913-941.
(13) Harker, E. A.; Daniels, D. S.; Guarracino, D. A.; Schepartz, A. *Bioorg. Med. Chem.* **2009**, *17*, 2038-2046.