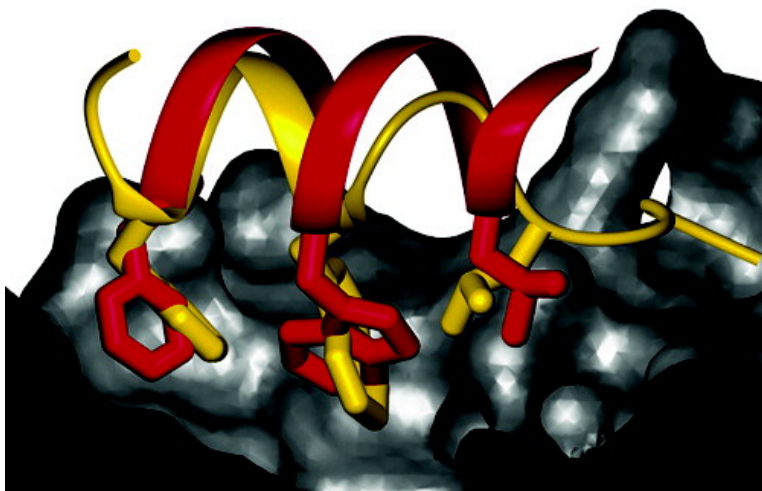


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Solution Structure of a β -Peptide Ligand for hDM2

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Recently, we described a β -peptide foldamer, β 53–1 (Figure 1A), that assembles into a 14-helix in aqueous solution, binds the oncoprotein hDM2 with submicromolar affinity, and inhibits the interaction of hDM2 with a peptide derived from the activation domain of p53 (p53AD).¹ The intact recognition epitope of β 53–1, including a high degree of helical structure, is required for selective inhibition of the p53AD·hDM2 interaction. Here, we present the solution structure of β 53–1 in methanol. The structure reveals details of a helix-stabilizing salt bridge on one helical face, novel “wedge into cleft” packing along another, and distortions in the β 53–1 14-helix that may maximize presentation of the p53AD recognition epitope. These details deepen our understanding of how β^3 -peptides fold and how they can be designed to form higher order structures^{2,3} and bind macromolecules.^{4,5}

Two-dimensional NMR spectroscopy was performed using 5 mM β 53–1 in CD₃OH at 10 °C. Previous circular dichroism and analytical ultracentrifugation experiments¹ and the NMR line widths observed herein are consistent with a monomeric, 14-helical structure for β 53–1 under these conditions. The proton resonances of β 53–1 were assigned unambiguously using TOCSY and natural abundance ¹H–¹³C HSQC spectra.⁶ ROESY experiments were then performed using mixing times of 200, 350, and 500 ms.⁷ The observed series of NH–C α H ROEs confirmed the sequential assignment by providing a backbone “ROE walk”. Three classes of medium-range ROEs characterize a 14-helical conformation: those between H_N(*i*) and H _{β} (*i*+2), H_N(*i*) and H _{β} (*i*+3), and H _{α} (*i*) and H _{β} (*i*+3).^{8,9} All 20 potential medium-range interactions of this type were observed in the ROESY spectra of β 53–1; in addition, 27 additional medium-range ROEs between side chains three positions apart were also observed.⁶ The large number of medium-range ROEs observed by NMR provides clear evidence for a high level of 14-helix structure in β 53–1; 449 ROEs quantified using a 350 ms mixing time were subsequently assigned and integrated using SPARKY.¹⁰ Peak volumes were converted to 151 upper-limit distance constraints⁶ and used to perform simulated annealing torsional dynamics on 100 random starting configurations of β 53–1 using DYANA.^{6,11} No constraint violations were reported among the resulting 20 lowest-energy structures, which are shown in Figure 1B.

The ensemble of calculated structures of β 53–1 (Figure 1B) shows a 14-helix with an average backbone atom RMSD from the mean structure of 0.17 ± 0.07 Å. The backbone torsions of individual structures deviate little from the mean, even at the termini (Figure 1C), illustrating the robustness of the β 53–1 14-helix in methanol. The helix is characterized by approximately 1.61 Å rise per residue and 3.0 residues per turn for residues 1–6, with a slight unwinding to approximately 1.49 Å rise per residue and 3.3 residues

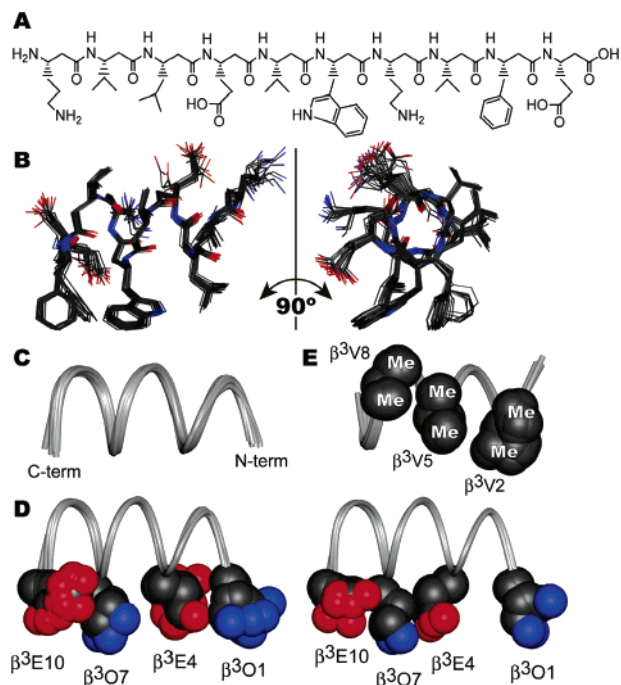


Figure 1. (A) Chemical structure of β 53–1, shown with N-terminus at left. (B) Solution structure of β 53–1 in CD₃OH at 10 °C, shown as a bundle of 20 lowest-energy structures, with C-terminus at left. (C) Ribbon representation of the backbones of 20 lowest-energy structures. (D) Two subpopulations of ion pairing configurations. Superposed at left are 17 structures in which β^3 O1 and β^3 E4 are proximal; superposed at right are three structures in which β^3 E4 and β^3 O7 are proximal. (E) Conformations of β^3 -homovaline residues illustrating the “wedge into cleft” packing found in all 20 lowest-energy structures.

per turn for residues 7–10. This unwinding appears to be unique to β 53–1, as it was not observed in NMR structures of unrelated β^3 -peptides with and without side chain ion pairing.^{8,9,12} Side chains are also well-defined among the lowest-energy structures, with an overall average heavy atom RMSD from the mean of 0.60 ± 0.10 Å.

β 53–1 contains four charged side chains arranged to favor formation of helix-stabilizing^{8,13} salt bridges on one 14-helix face.¹⁴ In all 20 low-energy structures, the terminal nitrogen of β^3 O7 and the nearest terminal oxygen of β^3 E10 are characterized by a consistent separation of 5.5 ± 0.6 Å. The relative positions of the remaining two ion pairs fall into two subpopulations (Figure 2D). In 17 structures, the terminal nitrogen of β^3 O1 and the nearest terminal oxygen of β^3 E4 are closer (5.4 ± 0.9 Å) than the equivalent atoms of β^3 E4 and β^3 O7 (6.8 ± 0.9 Å). By contrast, in the remaining three structures, the terminal nitrogen of β^3 O7 and the nearest terminal oxygen of β^3 E4 are closer (3.6 ± 0.4 Å) than the equivalent atoms of β^3 O1 and β^3 E4 (7.7 ± 1.3 Å). This interplay

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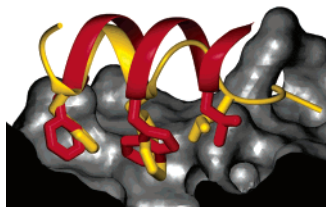


Figure 2. Overlay of the methanol solution structure of $\beta 53-1$ (red ribbon and side chains) with the crystal structure of a p53AD-derived peptide (gold ribbon and side chains) bound to hDM2 (gray surface).²⁴ Side chains of $\beta 53-1$ not implicated in recognition have been omitted, and part of the hDM2 surface has been cut away for clarity.

among potential ion pairs suggests that the central salt bridge is weaker than those near the termini and supports the hypothesis that multiple interconnected ion pairs play a key stabilizing role.^{8,13-15}

Another feature incorporated into the design of $\beta 53-1$ was the inclusion of β^3 -homovaline (β^3V) residues at positions 2, 5, and 8. It was long surmised^{14,16-18} and recently proven¹⁵ that β^3 -amino acids branched at the first side chain carbon stabilize 14-helices, in stark contrast to the effects of such side chains on α -helices.¹⁹ The $\beta 53-1$ structure provides a clear rationale for these observations. All 20 low-energy structures contain a unique arrangement of β^3 -homovaline side chains in which one methyl group of a β^3V side chain nestles into a cleft formed by the two methyl groups of another β^3V side chain (Figure 2E). These interactions are especially noticeable between the side chains of β^3V5 and β^3V8 , which are in VDW contact²⁰ in 19 of 20 structures. Overall, interactions among the three β^3V side chains bury $155 \pm 13 \text{ \AA}^2$ of hydrophobic surface area from water (24% of the surfaces of these side chains). These packing interactions may explain why these and other branched residues stabilize 14-helices^{15,21,22} and suggest new avenues for the design of 14-helix bundles.²³

The remaining 14-helix face consists of residues that comprise the hDM2-binding epitope, namely, β^3 -homoleucine (β^3L3), β^3 -homotryptophan (β^3W6), and β^3 -homophenylalanine (β^3F9). We originally hypothesized that the side chains of these residues would form an extended hydrophobic surface that might mimic that of p53AD.¹ Interestingly, the β^3F9 side chain can access two specific conformations within the constraints used; the fact that this variability has been observed in another 14-helix structure⁹ implies that the side chain may indeed preferentially populate these rotamers within a 14-helix. The side chains of β^3W6 and β^3L3 are in VDW contact in all 20 structures, while the side chains of β^3W6 and β^3F9 are in VDW contact in the context of only one of β^3F9 's two preferred conformations (present in 6 of 20 low-energy structures). Overall, on average, the side chains of β^3L3 , β^3W6 , and β^3F9 comprise a continuous, solvent-exposed hydrophobic surface area of 520 \AA^2 . This value is comparable to the contact areas measured at the interfaces of transient homo- and heterodimeric protein complexes.²³

As a consequence of the unexpected unwinding near the C-terminus of $\beta 53-1$, the β^3F9 side chain is not aligned perfectly with the side chains of β^3L3 and β^3W6 along the helix axis (see Figure 1B). This subtle distortion may avoid steric repulsions between the large side chains of β^3F9 and β^3W6 . In fact, it is unclear whether the unwinding near the C-terminus, which is unique to $\beta 53-1$, is due to more favorable ion pairing, more favorable β^3V nesting interactions, or the need to avoid steric clashes on the recognition face containing large hydrophobic residues. As structures of other short, stable 14-helices are determined, it will be interesting to note what factors lead to similar distortions in the "ideal" 14-helix geometry.

Importantly, this subtle distortion allows the side chains comprising the $\beta 53-1$ recognition face to better mimic those on the p53AD α -helix. Overlays between $\beta 53-1$ in an idealized 14-helical conformation and p53AD bound to hDM2²⁴ revealed an imperfect alignment between the two ligands; while the β^3L3 , β^3W6 , and β^3F9 side chains of $\beta 53-1$ could superimpose with their counterparts on p53AD, the 14-helix backbone could not completely fit within hDM2's binding groove.¹ The comparable overlay with the solution structure of $\beta 53-1$ (Figure 2) shows no such conflict. In its solution conformation, $\beta 53-1$ can access all three of hDM2's hydrophobic pockets while occupying the same binding groove as p53AD with no steric clashes. This fit demands subtle unwinding near the $\beta 53-1$ C-terminus that staggers the side chains, producing a β^3 -peptide that is uniquely suited for α -helix mimicry. The solution structure of $\beta 53-1$ suggests that the extended, highly variable surface presented by a 14-helical β -peptide oligomer could be used as a platform to design small, metabolically stable inhibitors of protein interfaces containing one or more α -helices.²⁵

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Supporting Information Available: Assignment tables, ROE-derived upper-distance limits. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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