



Published in final edited form as:

J Am Chem Soc. 2010 March 10; 132(9): 2904–2906. doi:10.1021/ja910715u.

Bridged β^3 -Peptide Inhibitors of p53-hDM2 Complexation— Correlation Between Affinity and Cell Permeability

Arjel D. Bautista[#], Jacob S. Appelbaum[§], Cody J. Craig[#], Julien Michel[#], and Alanna Schepartz^{#,¶}

Alanna Schepartz: alanna.schepartz@yale.edu

[#]Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107

[§]Department of Cell Biology, and Molecular, Yale University, New Haven, Connecticut 06520-8107

[¶]Department of Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8107

Abstract

β -peptides possess several features that are desirable in peptidomimetics; they are easily synthesized, fold into stable secondary structures in physiologic buffers, and resist proteolysis. They can also bind to a diverse array of proteins to inhibit their interactions with α -helical ligands. β -peptides are not usually cell permeable, however, and this feature limits their utility as research tools and potential therapeutics. Appending an Arg₈ sequence to a β -peptide improves uptake but adds considerable mass. We reported that embedding a small cationic patch within a PPII, α - or β -peptide helix improves uptake without the addition of significant mass. In another mass-neutral strategy, Verdine, Walensky, and others have reported that insertion of a hydrocarbon bridge between the *i* and *i+4* positions of an α -helix also increases cell uptake. Here we describe a series of β -peptides containing diether and hydrocarbon bridges and compare them on the basis of cell uptake and localization, affinities for hDM2, and 14-helix structure. Our results highlight the relative merits of cationic patch and hydrophobic bridge strategies for improving β -peptide uptake and identify a surprising correlation between uptake efficiency and hDM2 affinity.

β -peptides¹⁻⁴ possess several features that are desirable in peptidomimetics;^{5,6} they are easily synthesized, fold into helices^{1-3,7} in physiologic buffers,⁸ and resist proteolysis.⁹ They also bind *in vitro* to proteins such as hDM2,¹⁰⁻¹⁴ hDMX,¹⁰ gp41,^{15,16} and others,¹⁷⁻¹⁹ and inhibit their interactions with α -helical ligands. β -peptides are not usually cell permeable, however, and this feature limits their utility as research tools and potential therapeutics. Appending an Arg₈ sequence to a β -peptide can improve uptake^{20,21} but adds considerable mass. We reported that embedding a small cationic patch within a PPII,²² α -²³ or β -peptide¹¹ helix improves uptake without the addition of significant mass.^{24,25} Similarly, Verdine, Walensky, and others²⁶⁻³³ reported that insertion of a hydrocarbon bridge (a “staple”) between the *i* and *i+4* positions of an α -helix³⁴ increases uptake.^{26,29,32,34-38} Here we describe a variety of β -peptides containing diether- and hydrocarbon bridges and compare them on the basis of cell uptake and localization, affinity for hDM2, and 14-helix structure. Our results highlight the relative merits of cationic patch and hydrophobic bridge strategies for improving β -peptide uptake and identify an unprecedented correlation between uptake efficiency and hDM2 affinity *in vitro*.

Correspondence to: Alanna Schepartz, alanna.schepartz@yale.edu.

Supporting Information **Available:** β -peptide synthesis, binding and cell uptake assays, and confocal microscopy images. This material is available free of charge on the Internet at <http://pubs.acs.org>.

Our studies began with an analysis of available x-ray^{39,40} and NMR structures^{13,41} of β -peptide 14-helices to identify those position pairs that would best tolerate an ether^{42,43} or hydrocarbon³⁴ bridge. This analysis, supported by recent work of Perlmutter⁴² and Seebach⁴⁴ suggested that a 21-atom bridge could be accommodated between most *i* and *i*+3 positions of a 14-helix. To test this prediction, we synthesized an analog of β -peptide **2**⁷ containing (O-allyl)- β^3 -L-Ser at positions 3 and 6 (**2(3-6)**, Figure 1), and subjected it to on-resin ring-closing metathesis using bis(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride³⁴ to generate **2(3-6)s**.⁴⁵ The circular dichroism (CD) spectra of **2**, **2(3-6)** and **2(3-6)s** were identical (Figure S1), indicating that this 21-atom diether bridge is accommodated between positions 3 and 6. Introduction of the diether bridge did not significantly increase or decrease the extent of 14-helix structure as judged by CD.

In order to evaluate the relative uptake of bridged β -peptides in the context of a functional molecule of diverse sequence, we synthesized a series of variants of β **53-12**,¹⁰ an inhibitor of p53-hDM2 complexation (Figure 1). These variants contained either (O-allyl)- β^3 -L-Ser (to generate a diether bridge) or (*S*)-3-aminooct-7-enoic acid (to generate a hydrocarbon bridge) at *i* and *i*+3 positions 2 and 5 (**25.O-s** and **25.C-s**, respectively) or 4 and 7 (**47.O-s** and **47.C-s**, respectively). According to the CD spectra (Figure 2), all bridged β -peptides assumed a 14-helical structure and were modestly more helical than unbridged analogs (Figure S2).

As a prelude to evaluating cell uptake and localization, we employed a direct fluorescence polarization assay to compare hydrocarbon and diether bridged β -peptides on the basis of affinity for hDM2₁₋₁₈₈ (Figure 2B). β -peptides containing a diether or hydrocarbon bridge between positions 4 and 7 bound hDM2₁₋₁₈₈ 2-fold better ($K_d = 53.9 \pm 22.7$ and 94.1 ± 18.4 nM, respectively) than the corresponding unbridged analogs ($K_d = 114 \pm 28$ and 253 ± 75 nM, respectively), in line with analogous comparisons in an α -peptide context.³⁵ By contrast, β -peptides containing a diether or hydrocarbon bridge between positions 2 and 5 bound hDM2₁₋₁₈₈ between 4 and 8-fold worse ($K_d = 548 \pm 58$ and 546 ± 96 nM, respectively) than unbridged analogs ($K_d = 139 \pm 13$ and 68.1 ± 7.8 nM, respectively). *In silico* analysis suggests that the lower hDM2₁₋₁₈₈ affinity of β -peptides **25.C-s** and **25.O-s** results from steric hindrance between the hydrocarbon bridge and the hDM2 surface that is absent in the complex with peptides **47.C-s** and **47.O-s** (Figure 3, compare A and B).

We next set out to monitor the mammalian cell uptake and sub-cellular localization of diether- and hydrocarbon bridged β -peptides based on β 53-12. Uptake was monitored using flow cytometry, whereas sub-cellular localization was assessed using confocal microscopy (Figure 4). β -peptides containing diether or hydrocarbon bridges between positions 4 and 7 were taken up significantly more efficiently (MCF = 8.21 ± 0.45 and 8.63 ± 0.77 , respectively) than unbridged analogs (MCF = 3.23 ± 0.31 and 2.63 ± 0.32 , respectively), irrespective of bridge structure. By contrast, β -peptides containing diether or hydrocarbon bridges between positions 2 and 5 were taken up poorly, irrespective of bridge structure, and behaved much like the unbridged analogs. In all cases, as judged by flow cytometry, the greatest uptake was observed with β -peptide β 53-12SB3, which contains a cationic patch on one 14-helix face but no bridge of any kind (Figure 4AB).

The localization of bridged β -peptides upon cell uptake was explored in more detail using confocal microscopy. HeLa cells were treated with fluorescently labeled β -peptide (green) as well as Alexa Fluor® 647 labeled transferrin and Hoescht 33342 to visualize recycling endosomes^{46,47} (red) and nuclei (blue). β -peptides containing a diether or hydrocarbon bridge between positions 4 and 7 are distributed widely among Tf+ and Tf- endosomes, as well as nuclear and cytosolic compartments, whereas those containing the analogous bridge between positions 2 and 5 are not (Figure 3). Indeed, β -peptides containing a diether or hydrocarbon bridge between positions 2 and 5 are taken up more poorly than the unbridged analog (Figure

S4). These results highlight an intriguing correlation between hDM2 affinity and cell uptake; it is possible that the structural features that lower hDM2 affinity (Figure S3) also lower uptake efficiency. Indeed, it appears that for these β -peptides, an increase in 14-helix secondary structure does not necessarily confer increased cell uptake.²⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NIH (GM 74756), the National Foundation for Cancer Research, and a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme (J.M.). A.D.B. is grateful to Bristol-Myers Squibb for a graduate research fellowship.

References

1. Cheng RP, Gellman SH, DeGrado WF. *Chem Rev* 2001;101:3219–3232. [PubMed: 11710070]
2. DeGrado WF, Schneider JP, Hamuro Y. *J Pept Res* 1999;54:206–217. [PubMed: 10517158]
3. Gellman SH. *Acc Chem Res* 1998;31:173–180.
4. Seebach D, Overhand M, Kunhle FNM, Martinoni B, Oberer L, Hommel U, Widmer H. *Helv Chim Acta* 1996;79:913–941.
5. Bautista AD, Craig CJ, Harker EA, Schepartz A. *Curr Opin Chem Biol* 2007;11:685–592. [PubMed: 17988934]
6. Kritzer JA, Stephens OM, Guarracino DA, Reznik SK, Schepartz A. *Bioorg Med Chem* 2004;13:11–16. [PubMed: 15582447]
7. Kritzer JA, Tirado-Rives J, Hart SA, Lear JD, Jorgensen WL, Schepartz A. *J Am Chem Soc* 2005;127:167–178. [PubMed: 15631466]
8. Hart SA, Bahadoor ABF, Matthews EE, Qiu XJ, Schepartz A. *J Am Chem Soc* 2003;125:4022–4023. [PubMed: 12670203]
9. Frackenpohl J, Arvidsson PI, Schreiber JV, Seebach D. *ChemBioChem* 2001;2:445–455. [PubMed: 11828476]
10. Harker EA, Daniels DS, Guarracino DA, Schepartz A. *Bioorg Med Chem* 2009;17:2038–2046. [PubMed: 19211253]
11. Harker EA, Schepartz A. *ChemBioChem* 2009;10:990–993. [PubMed: 19266537]
12. Kritzer JA, Lear JD, Hodsdon ME, Schepartz A. *J Am Chem Soc* 2004;126:9468–9469. [PubMed: 15291512]
13. Kritzer JA, Luedtke NW, Harker EA, Schepartz A. *J Am Chem Soc* 2005;127:14584–14585. [PubMed: 16231906]
14. Murray JK, Gellman SH. *Pept Sci* 2007;88:657–686.
15. Bautista AD, Stephens OM, Wang L, Domaoal RA, Anderson KS, Schepartz A. *Bioorg Med Chem Lett* 2009;19:3736–3738. [PubMed: 19497744]
16. Stephens OM, Kim S, Welch BD, Hodsdon ME, Kay MS, Schepartz A. *J Am Chem Soc* 2005;127:13126–13127. [PubMed: 16173723]
17. English EP, Chumanov RS, Gellman SH, Compton T. *J Biol Chem* 2006;281:2661–2667. [PubMed: 16275647]
18. Lee EF, Sadowsky JD, Smith BJ, Czabotar PE, Peterson-Kaufman KJ, Colman PM, Gellman SH, Fairlie WD. *Angew Chem Int Ed* 2009;48:4318–4322.
19. Sadowsky JD, Fairlie WD, Hadley EB, Lee HS, Umezawa N, Nikolovska-Coleska Z, Wang S, Huang DCS, Tomita Y, Gellman SH. *J Am Chem Soc* 2007;129:139–154. [PubMed: 17199293]
20. Jones SW, Christison R, Bundell K, Joyce CJ, Brockbank SMV, Newham P, Lindsay MA. *Br J Pharmacol* 2005;145:1093–1102. [PubMed: 15937518]
21. Tung CH, Weissleder R. *Adv Drug Deliv Rev* 2003;55:281–294. [PubMed: 12564981]

22. Daniels DS, Schepartz A. *J Am Chem Soc* 2007;129:14578–14579. [PubMed: 17983240]
23. Smith BA, Daniels DS, Coplin AE, Jordan GE, McGregor LM, Schepartz AJ. *Am Chem Soc* 2008;130:2948–2949.
24. Lawrence MS, Phillips KJ, Liu DR. *J Am Chem Soc* 2007;129:10110–10112. [PubMed: 17665911]
25. McNaughton BR, Cronican JJ, Thompson DB, Liu DR. *Proc Natl Acad Sci U S A* 2009;106:6111–6116. [PubMed: 19307578]
26. Kim YW, Verdine GL. *Bioorg Med Chem Lett* 2009;19:2533–2536. [PubMed: 19332370]
27. Kutchukian PS, Yang JS, Verdine GL, Shakhnovich EI. *J Am Chem Soc* 2009;131:4622–4627. [PubMed: 19334772]
28. Madden MM, Vera CIR, Song W, Lin Q. *Chem Commun* 2009:5588–5590.
29. Moellering RE, Cornejo M, Davis TN, Bianco CD, Aster JC, Blacklow SC, Kung AL, Gilliland DG, Verdine GL, Bradner JE. *Nature* 2009;462:182–188. [PubMed: 19907488]
30. Whelan J. *Drug Discov Today* 2004;9:907–907. [PubMed: 15501720]
31. Bhattacharya S, Zhang H, Debnath AK, Cowburn D. *J Biol Chem* 2008;283:16274–16278. [PubMed: 18417468]
32. Danial NN, et al. *Nat Med* 2008;14:144–153. [PubMed: 18223655]
33. Henchey LK, Jochim AL, Arora PS. *Curr Opin Chem Biol* 2008;12:692–697. [PubMed: 18793750]
34. Schafmeister CE, Po J, Verdine GL. *J Am Chem Soc* 2000;122:5891–5892.
35. Bernal F, Tyler AF, Korsmeyer SJ, Walensky LD, Verdine GL. *J Am Chem Soc* 2007;129:2456–2457. [PubMed: 17284038]
36. Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL, Korsmeyer SJ. *Science* 2004;305:1466–1470. [PubMed: 15353804]
37. Walensky LD, Pitter K, Morash J, Oh KJ, Barbuto S, Fisher J, Smith E, Verdine GL, Korsmeyer SJ. *Mol Cell* 2006;24:199–210. [PubMed: 17052454]
38. Zhang H, Zhao Q, Bhattacharya S, Waheed AA, Tong X, Hong A, Heck S, Curreli F, Goger M, Cowburn D, Freed EO, Debnath AK. *J Mol Biol* 2008;378:565–580. [PubMed: 18374356]
39. Daniels DS, Petersson EJ, Qiu JX, Schepartz A. *J Am Chem Soc* 2007;129:1532–1533. [PubMed: 17283998]
40. Goodman JL, Petersson EJ, Daniels DS, Qiu JX, Schepartz A. *J Am Chem Soc* 2007;129:14746–14751. [PubMed: 17985897]
41. Kritzer JA, Hodsdon ME, Schepartz A. *J Am Chem Soc* 2005;127:4118–4119. [PubMed: 15783163]
42. Bergman YE, Del Borgo MP, Gopalan RD, Jalal S, Unabia SE, Ciampini M, Clayton DJ, Fletcher JM, Mulder RJ, Wilce JA, Aguilar MI, Perlmutter P. *Org Lett* 2009;11:4438–4440. [PubMed: 19719157]
43. Blackwell HE, Grubbs RH. *Angew Chem Int Ed* 1998;37:3281–3284.
44. Ebert MO, Gardiner J, Ballet S, Abell AD, Seebach D. *Helv Chim Acta* 2009:2643–2658.
45. See Supporting Information for details.
46. Ghosh R, Gelman D, Maxfield F. *J Cell Sci* 1994;107:2177–2189. [PubMed: 7983176]
47. Hopkins C, Gibson A, Shipman M, Strickland D, Trowbridge I. *J Cell Biol* 1994;125:1265–1274. [PubMed: 7515888]

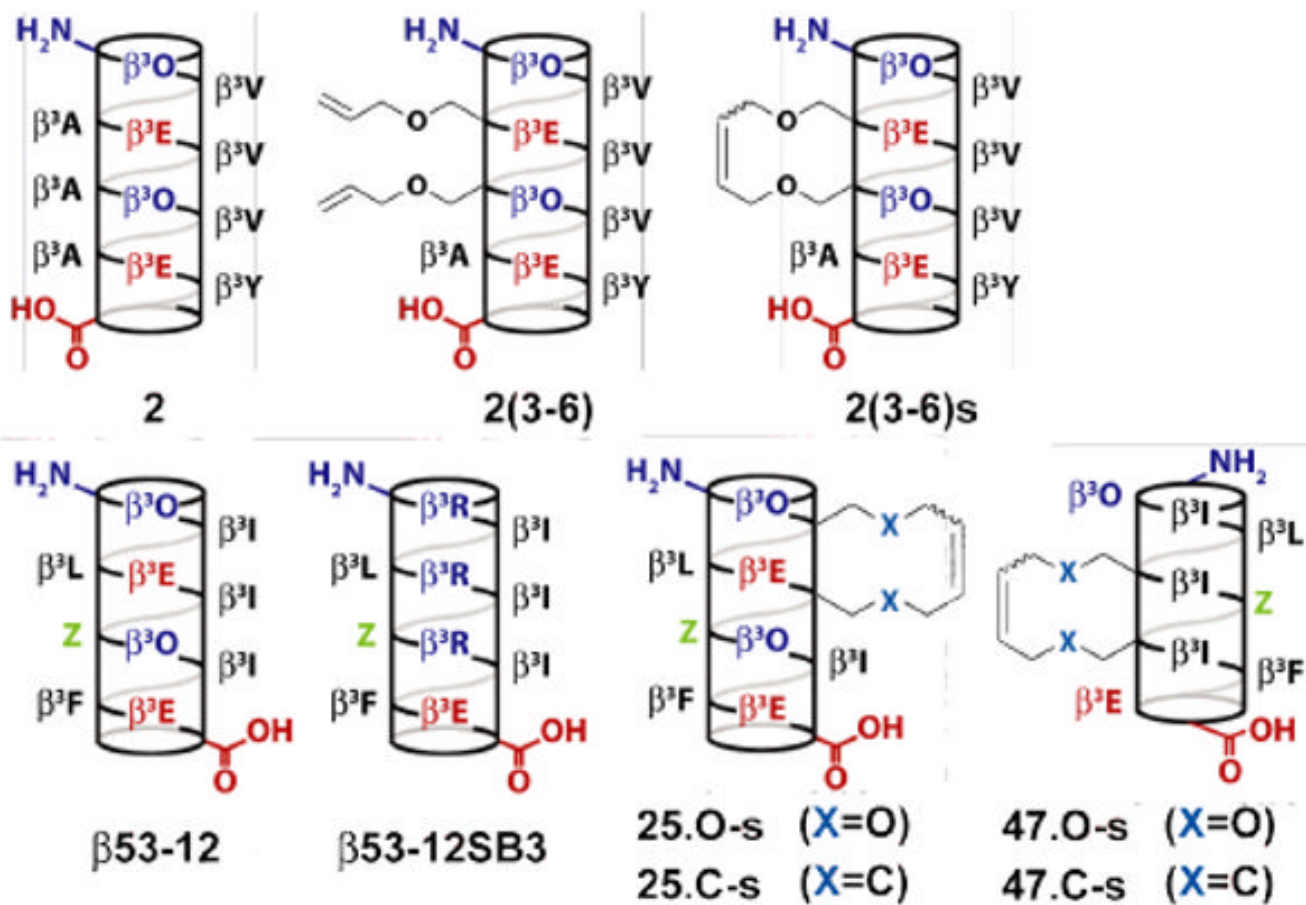


Figure 1. Helical net representation of β -peptides studied herein. β^3 -homoamino acids are identified by the single-letter code used for the corresponding α -amino acid. Orn represents ornithine. Z represents 3-(S)-3-amino-4-(2-trifluoromethylphenyl)-butyric acid.

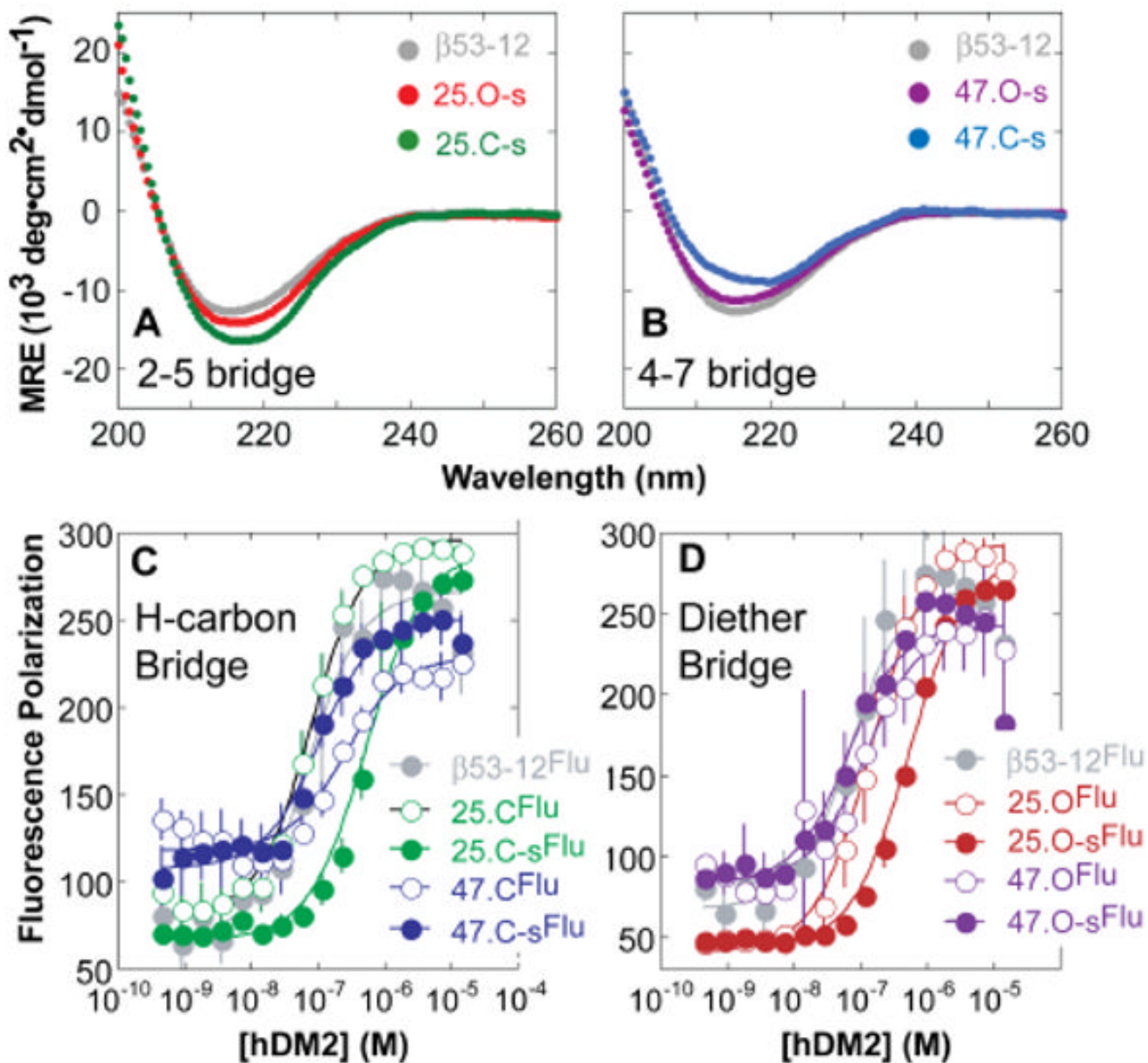


Figure 2. CD analysis of β -peptides containing hydrocarbon or diether bridges between residues (A) 2 and 5 or (B) 4 and 7. Fluorescence polarization (FP) analysis of hDM2 binding by β -peptides containing (C) hydrocarbon or (D) diether bridges.

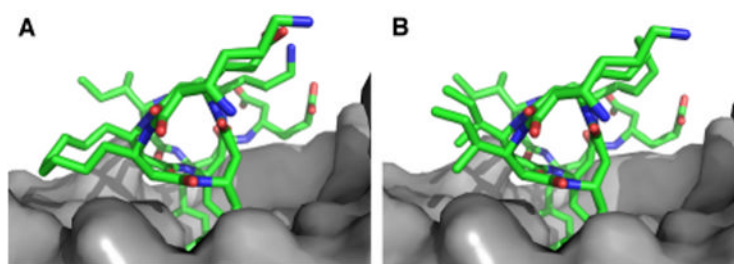


Figure 3. Computational model of hDM2 (grey) in complex with (A) 25.C-s or (B) 47.C-s.⁴⁵

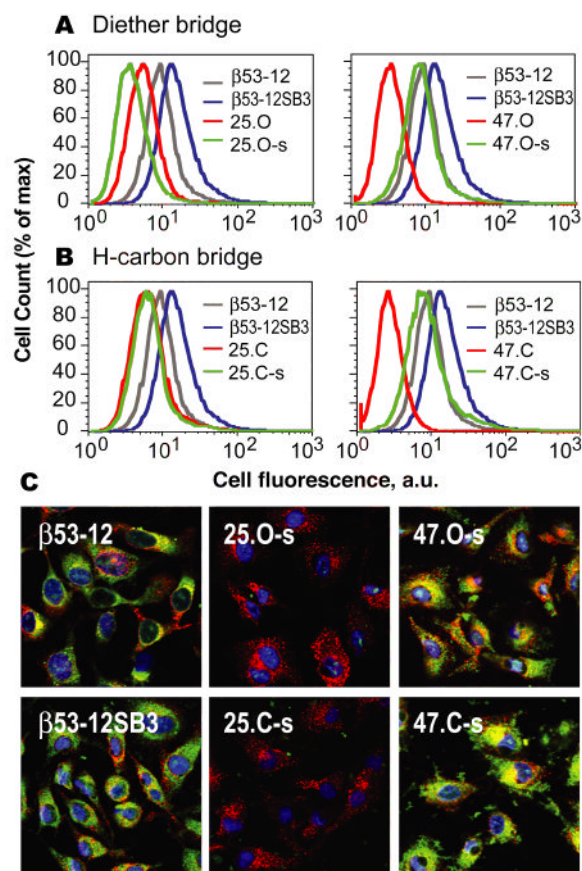


Figure 4.

HeLa cell uptake and localization of Flu-labeled β -peptides. (A,B) HeLa cells were incubated with 2 μM β -peptide for 4 h, treated with 0.25% trypsin for 10 min, washed with cold DMEM and PBS, and analyzed using flow cytometry. (C) Confocal microscopy of HeLa cells treated with 20 μM of the indicated β -peptide (green), 5 $\text{mg}\cdot\text{mL}^{-1}$ Alexa Fluor 647-transferrin (red) and 150 nM Hoescht 33342 (blue).