Bridged beta(3)-Peptide Inhibitors of p53-hDM2 Complexation: Correlation between Affinity and Cell Permeability

Published in final edited form as:
J Am Chem Soc. 2010 March 10; 132(9): 2904–2906. doi:10.1021/ja910715u.

Bridged β³-Peptide Inhibitors of p53-hDM2 Complexation– Correlation Between Affinity and Cell Permeability

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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 Arg_8 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-peptides[1-4] 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 Arg_8 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-peptide[11] helix improves uptake without the addition of significant mass.[24,25] Similarly, Verdine, Walensky, and others[26-33] reported that insertion of a hydrocarbon bridge (a "staple") between the *i* and *i+4* positions of an α-helix[34] 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*.

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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 hydrocarbon[34] bridge. This analysis, supported by recent work of Perlmutter[42] and Seebach[44] 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) dichloride[34] 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 $hDM2_{1\text{-}188}$ (Figure 2B). β-peptides containing a diether or hydrocarbon bridge between positions 4 and 7 bound $hDM2_{1\text{-}188}$ 2-fold better (K_d = 53.9 ± 22.7 and 94.1 ± 18.4 nM, respectively) than the corresponding unbridged analogs (K_d = 114 ± 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 $hDM2_{1\text{-}188}$ between 4 and 8-fold worse (K_d = 548 ± 58 and 546 ± 96 nM, respectively) than unbridged analogs (K_d = 139 ± 13 and 68.1 ± 7.8 nM, respectively). *In silico* analysis suggests that the lower $hDM2_{1\text{-}188}$ 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 β53-12. 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 β53-12SB3, 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, Voyce 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]

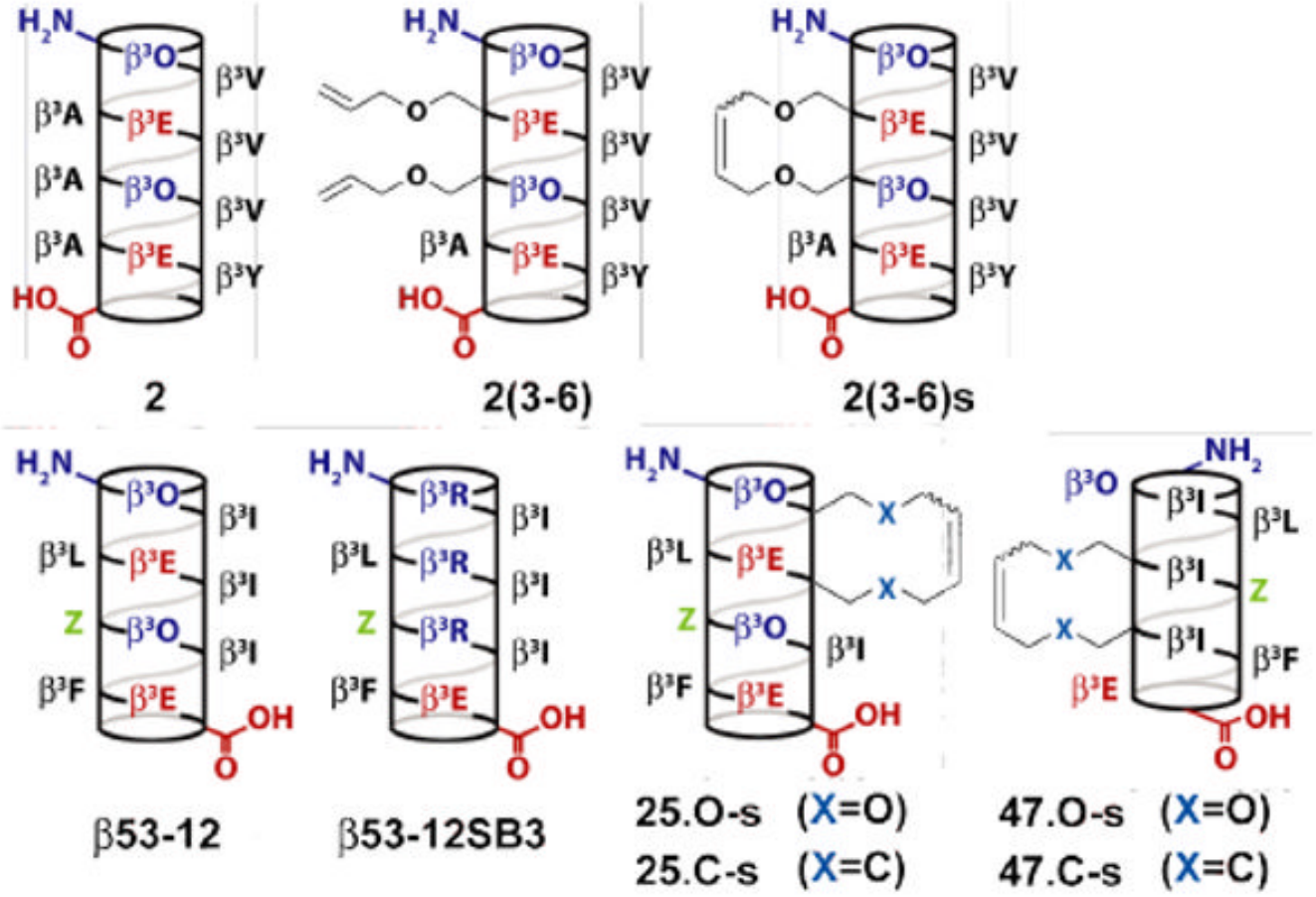

Figure 1.

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.

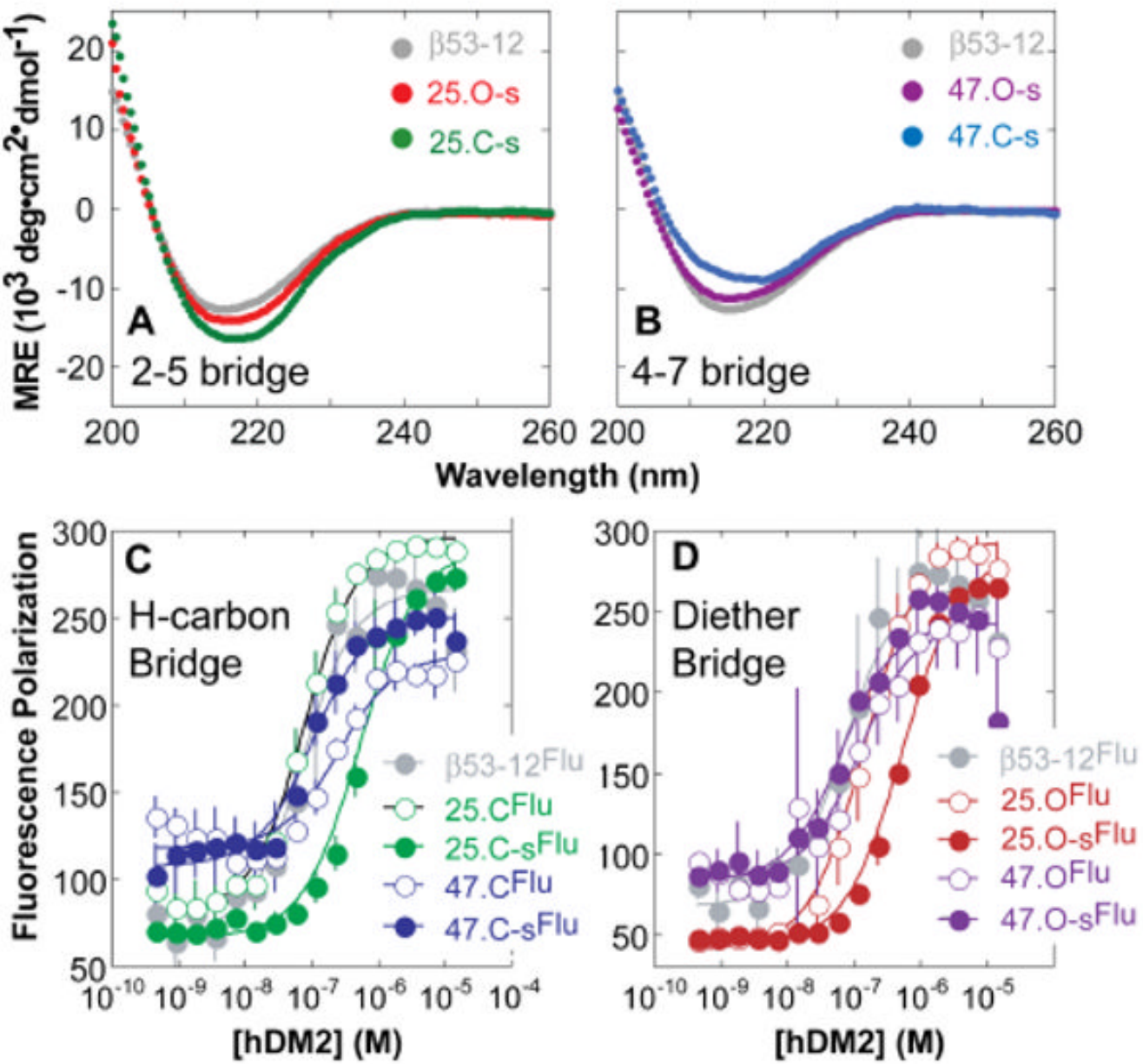

Figure 2.

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.

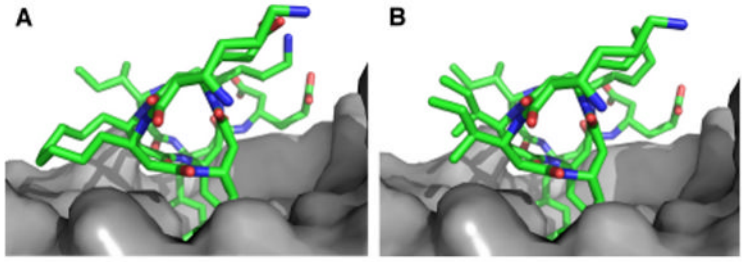

Figure 3.
Computational model of hDM2 (grey) in complex with (A) **25.C-s** or (B) **47.C-s**.[45]

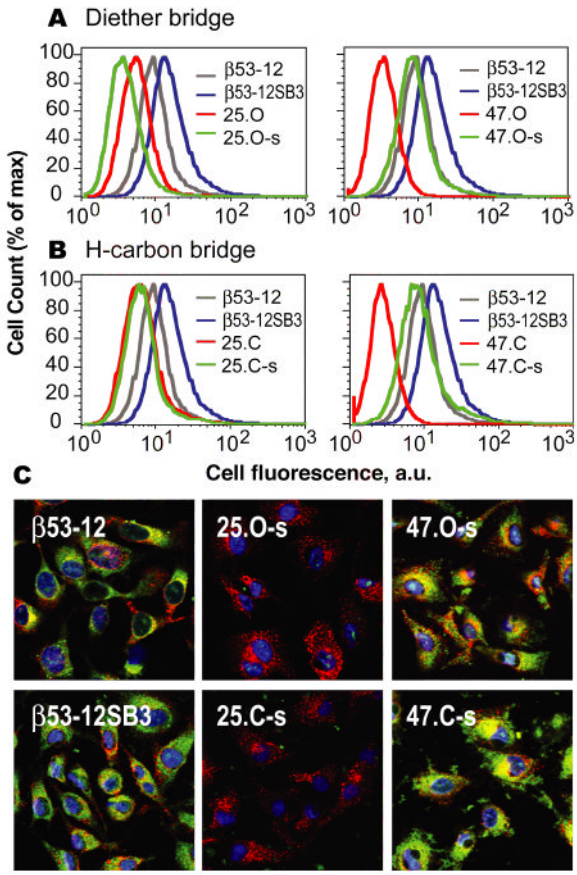

Figure 4.

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 mg•mL^{-1} Alexa Fluor 647-transferrin (red) and 150 nM Hoescht 33342 (blue).