Foldamers: Structure and Function

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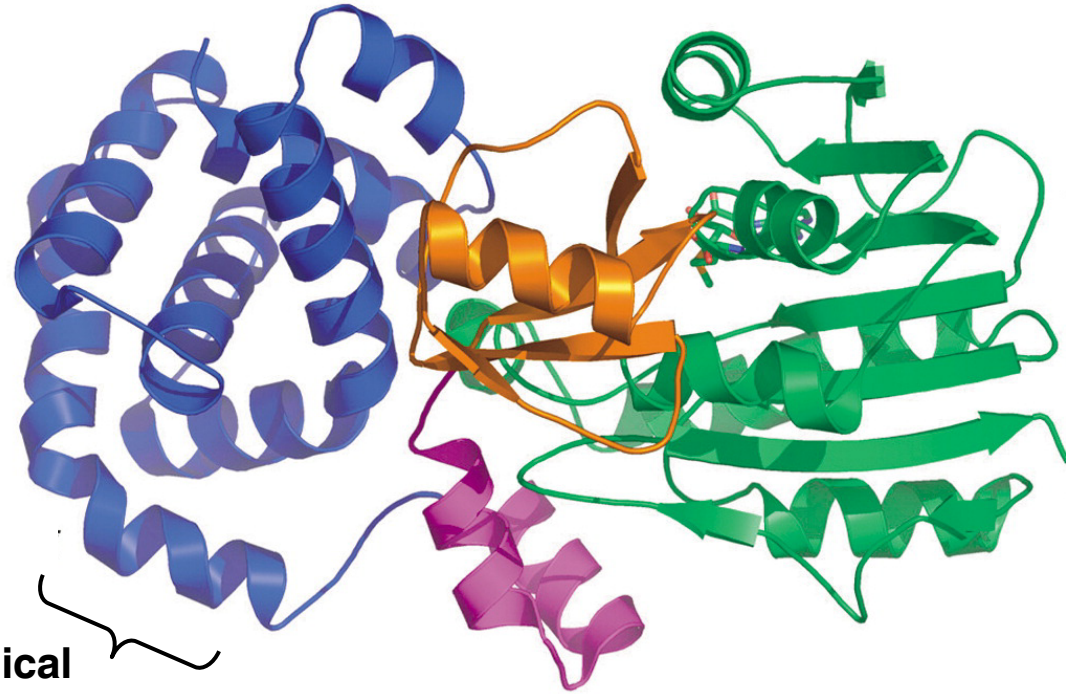
Folded Proteins: Secondary and Tertiary Structure

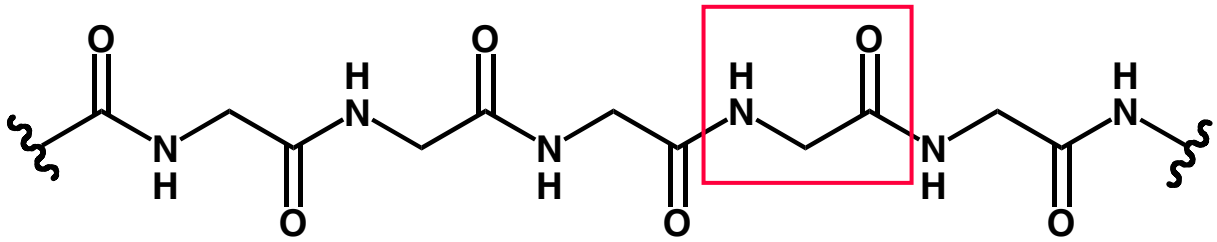
-- Folding usually required for function --

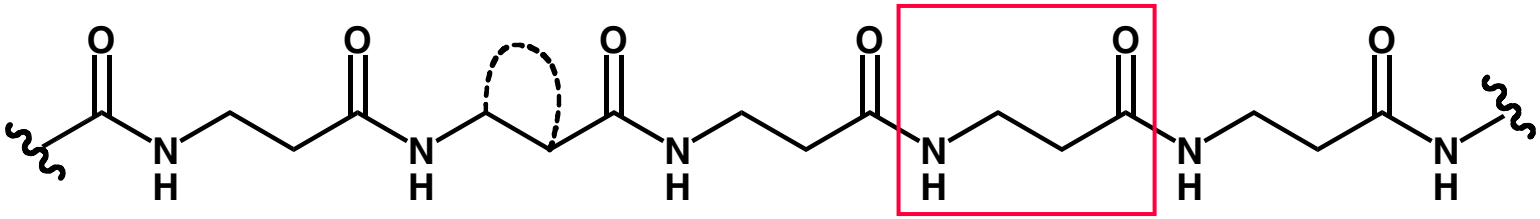


Fmu, an RNA m^5C methyltransferase

Foster et al. *Structure* **2003**, *11*, 1609.

FOLDAMERS -- Oligomers that adopt compact, predictable and well-defined conformations



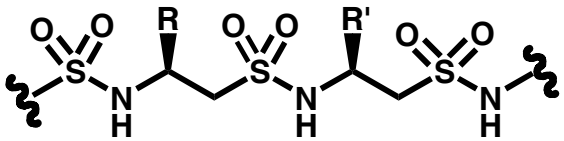


"β-Peptides"

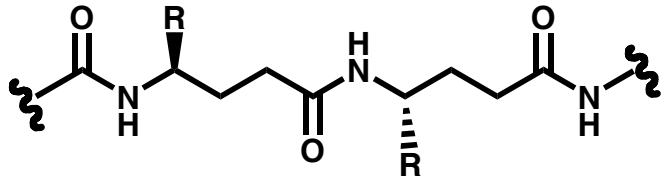
Seebach et al.; Gung et al.; Fleet et al.; DeGrado et al.; Diederichsen et al.; Ichikawa et al.; Winkler, Klein et al.; Fülöp et al.; Kessler et al.; Schepartz et al.; Berkessel et al.; Sharma, Kunwar et al., Chandrasekhar,Jagadeesh, Jagannadh et al.; Hofmann et al.; Wu et al.; Van Gunsteren et al.; Jorgensen et al.; …

Other Foldamers (Examples)

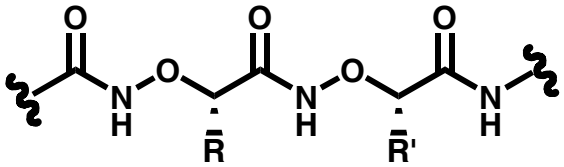
Gennari et al., Liskamp et al.



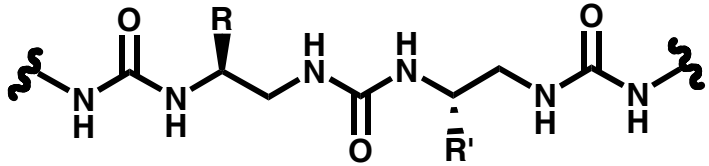
Hanessian et al., Seebach et al.



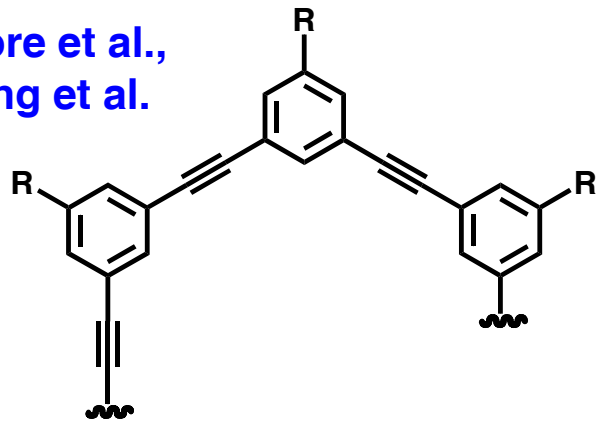
Yang et al.



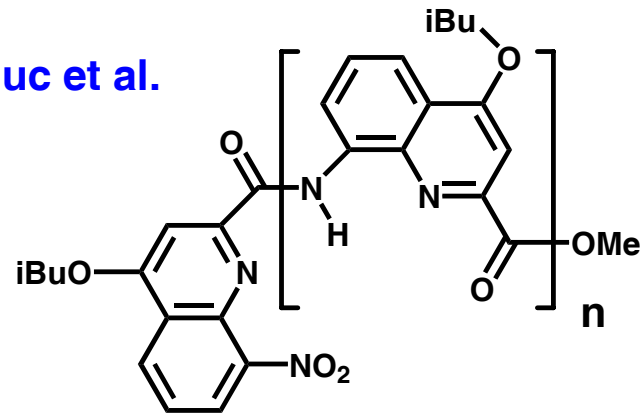
Guichard et al.



Moore et al., Gong et al.



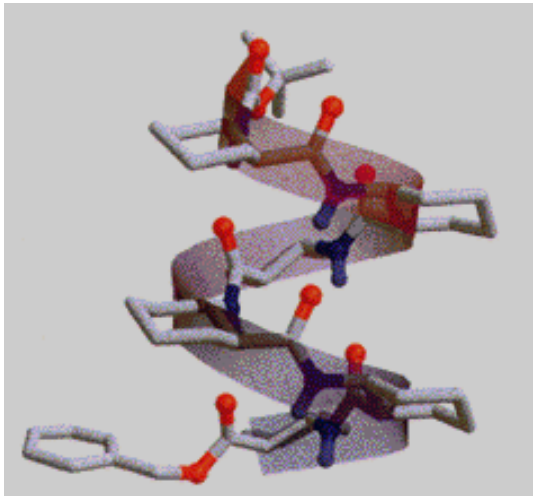
Huc et al.



β-Peptide Secondary Structure: Control over Helix Shape via Choice of β-Amino Acid Residues

14-Helix

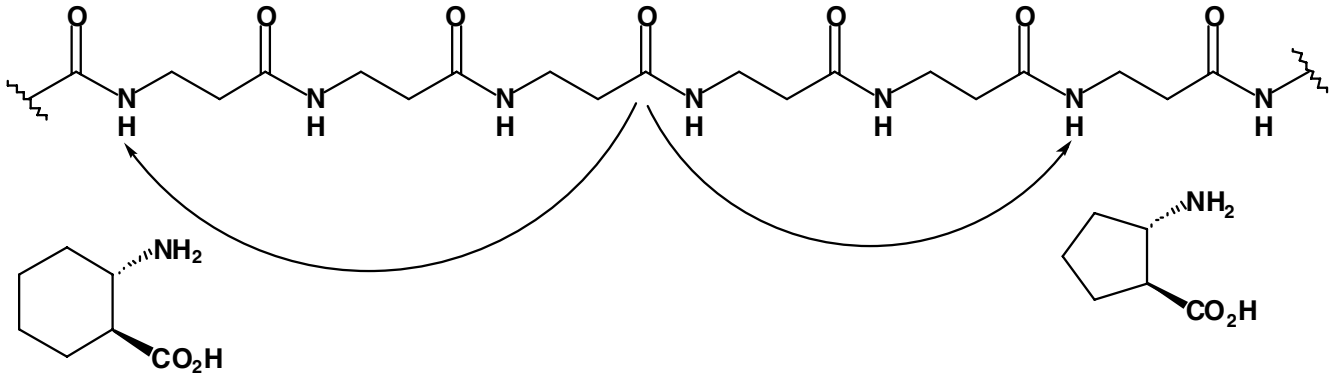
(I. L. Karle)



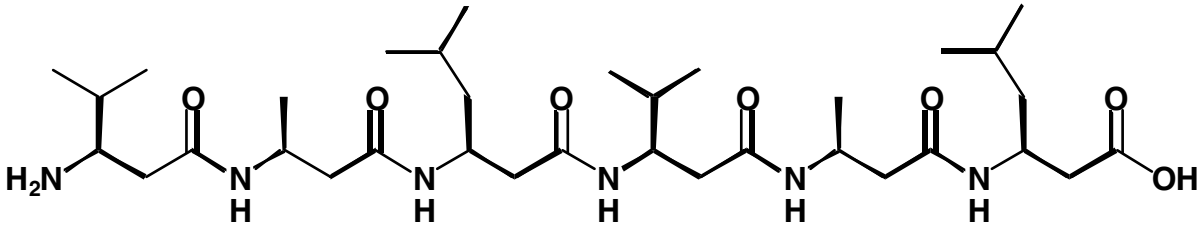
Appella, Christianson et al.
JACS **1996**, *118*, 13071.

12-Helix

Appella, Christianson et al.,
Nature **1997**, *387*, 381.

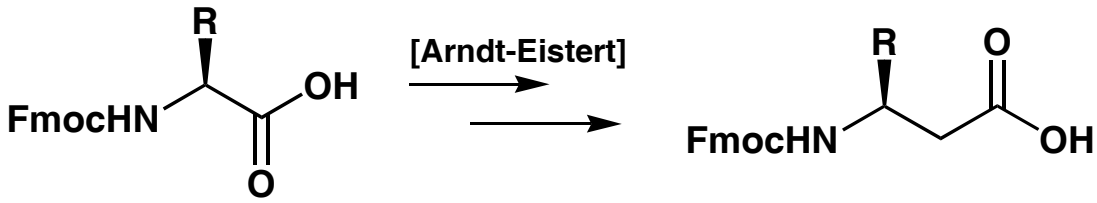


Seebach et al. --
β-Peptides with Acyclic Residues



14-helical in organic solvents

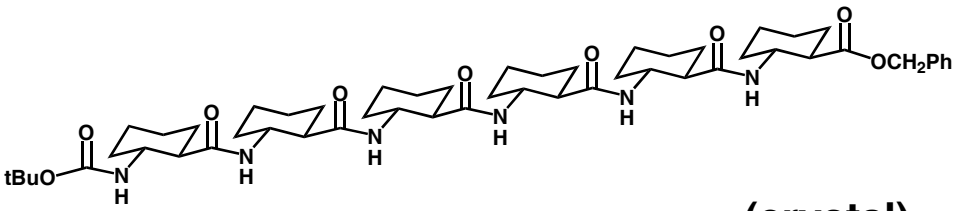
Seebach et al. *Helv. Chim. Acta* **1996**, *79*, 913 and 2043.
Seebach, Matthews *Chem. Commun.* **1997**, 2015.



Guichard, Abele, Seebach *Helv. Chim. Acta* **1998**, *81*, 187.

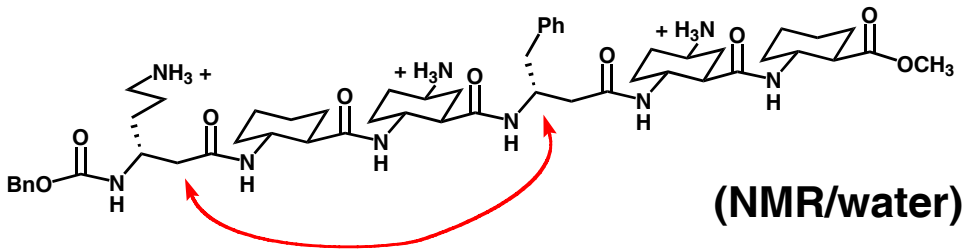
β-Peptides resist proteolytic degradation.

High Shape Stability: Helical Hexamer in Water



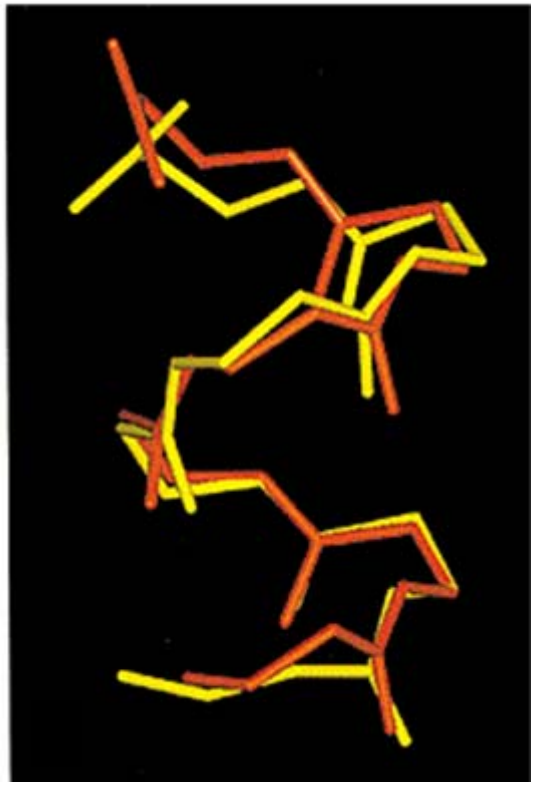
(crystal)

VS.



(NMR/water)

NOE

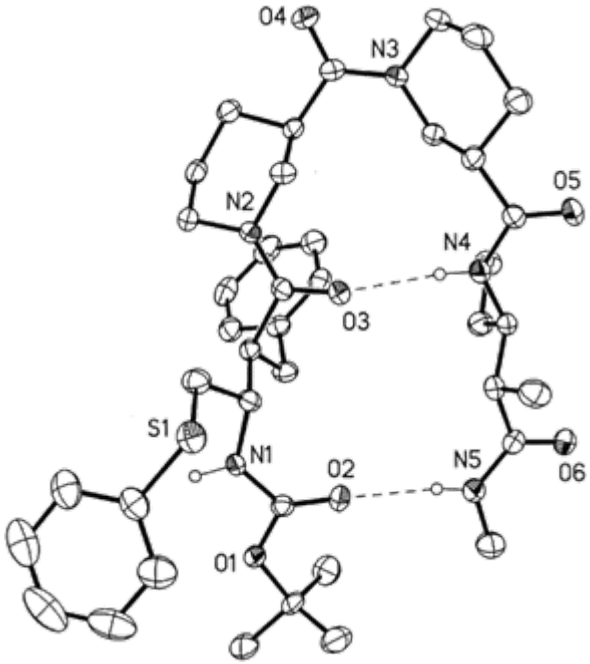


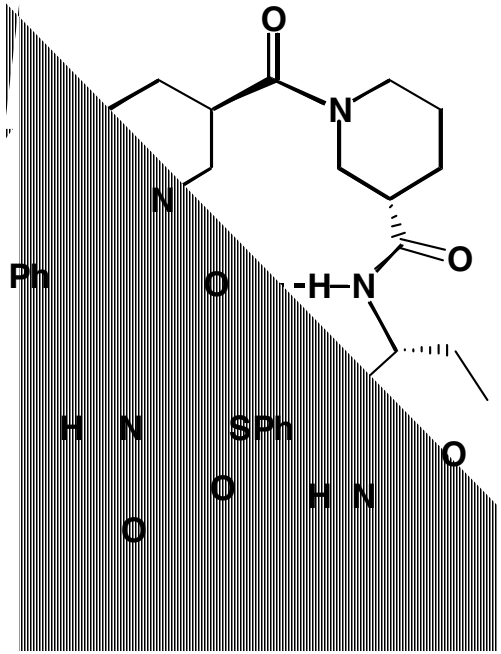
Backbone overlay: rmsd = 0.27 Å

Appella, Barchi, Durell, Gellman *J. Am. Chem. Soc.* **1999**, *121*, 2309.
Raguse, Lai, Gellman *J. Am. Chem. Soc.* **2003**, *125*, 5592.

12-Helix in water: Wang, Espinosa, Gellman *J. Am. Chem. Soc.* **2000**, *122*, 4821.
Lee, Syud, Wang, Gellman *J. Am. Chem. Soc.* **2001**, *123*, 7721.

A Minimal β-Peptide Hairpin: One Residue in Each Strand and a Reverse Turn

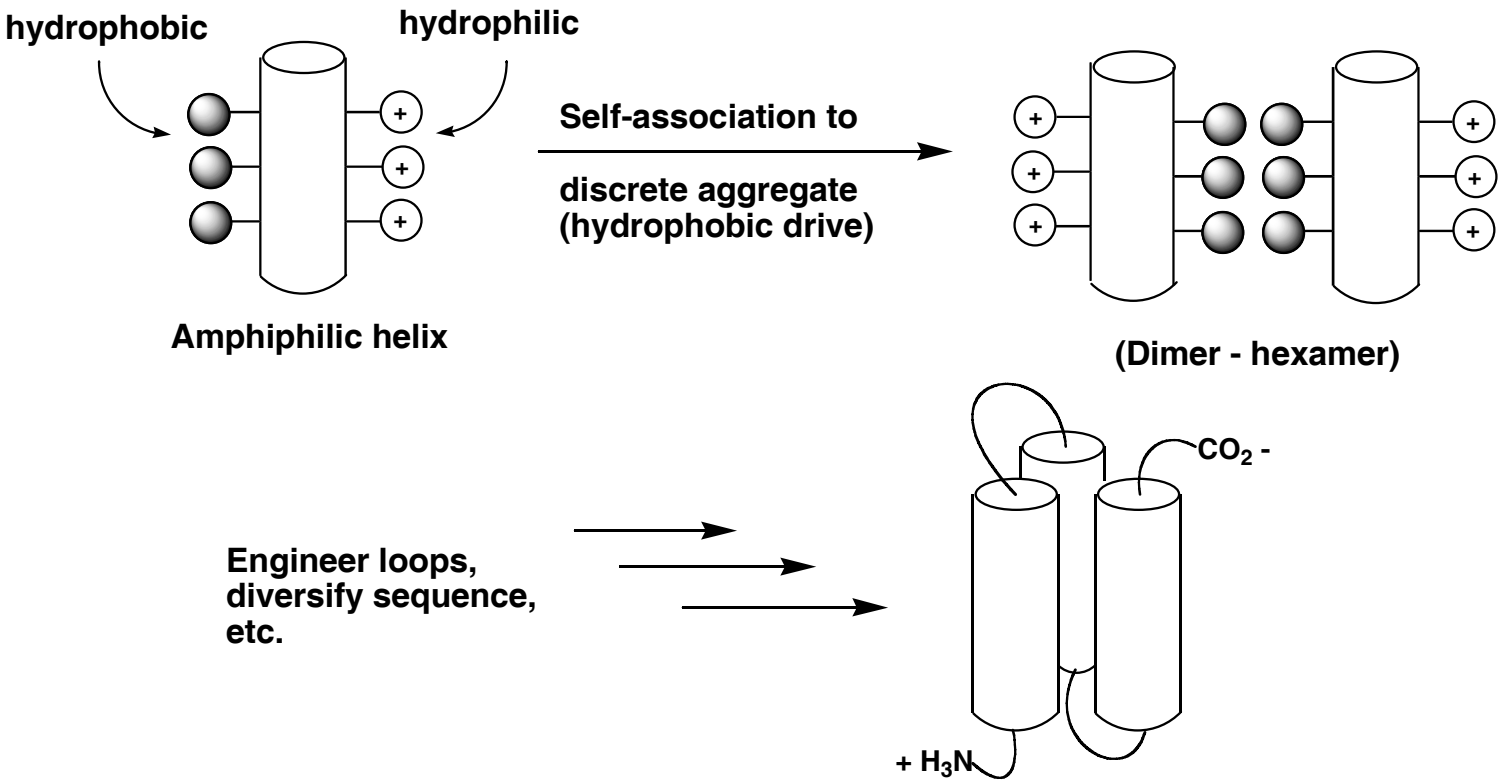




Chung, Christianson, Stanger, Powell, Gellman *J. Am. Chem. Soc.* **1998**, *120*, 10555.

[See also: Seebach, Abele, Gademann, Jaun *Angew. Chem. Int. Ed.* **1999**, *38*, 1595.]

Toward Helix-Bundle Tertiary Structure

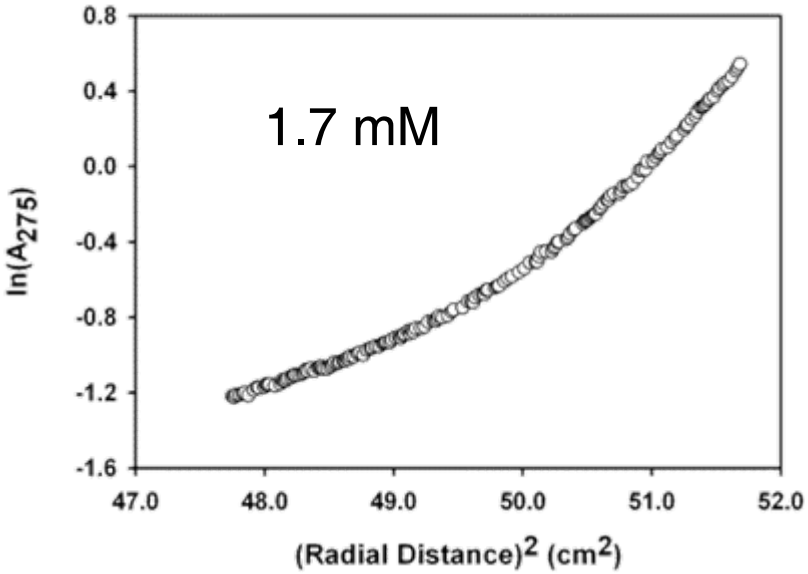


Protein Precedent: DeGrado et al. *Science* **1995**, *270*, 935.

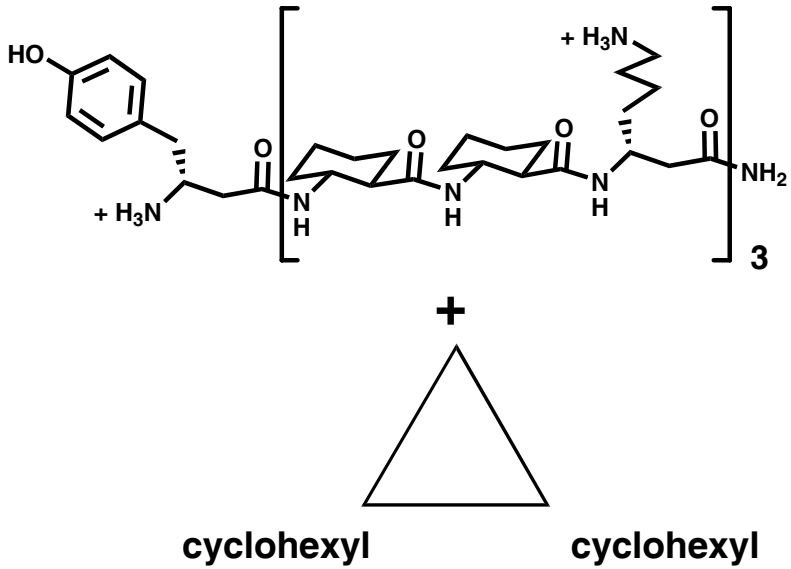
Raguse, Lai, LePlae, Gellman *Org. Lett.* **2001**, *3*, 3963.

Cheng, DeGrado *J. Am. Chem. Soc.* **2002**, *124*, 11564

Self-Association Evidence from Analytical Ultracentrifugation: Hexameric Bundle?

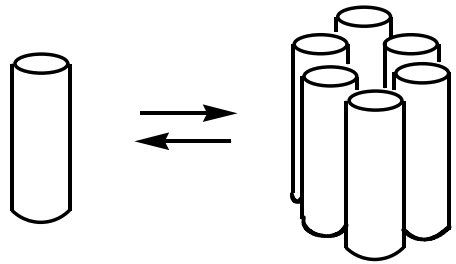


(60 krpm, 10 mM Tris, pH 8.0)



(14-Helix Wheel)

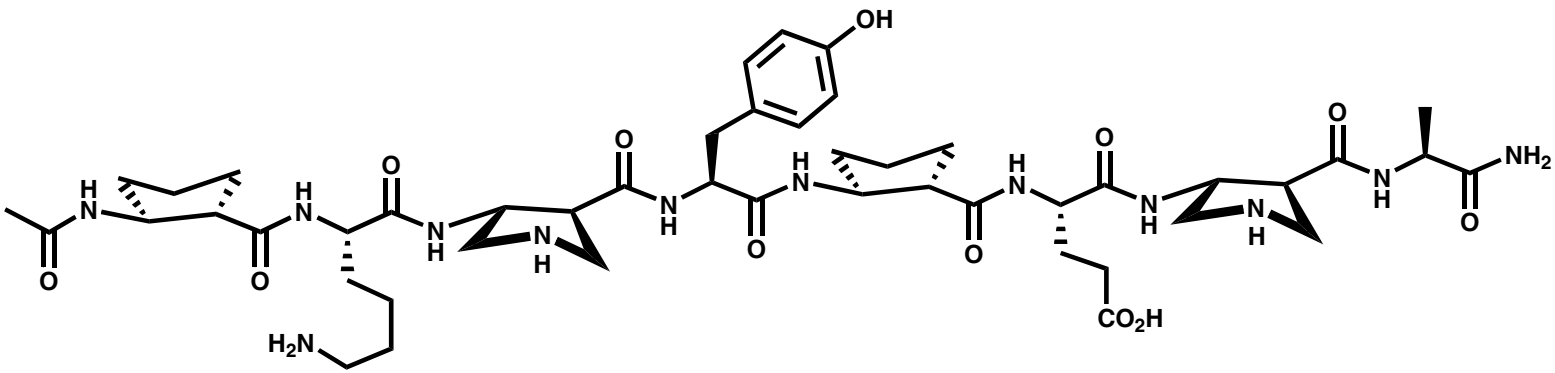
Data consistent with
Monomer-hexamer equilibrium



Raguse, Lai, LePlae, Gellman *Org. Lett.* **2001**, *3*, 3963.

[Similar work: Qiu, Petersson, Matthews, Schepartz *J. Am. Chem. Soc.* **2006**, *128*, 11338.]

α/β-Peptides: Foldamers with Heterogeneous Backbones

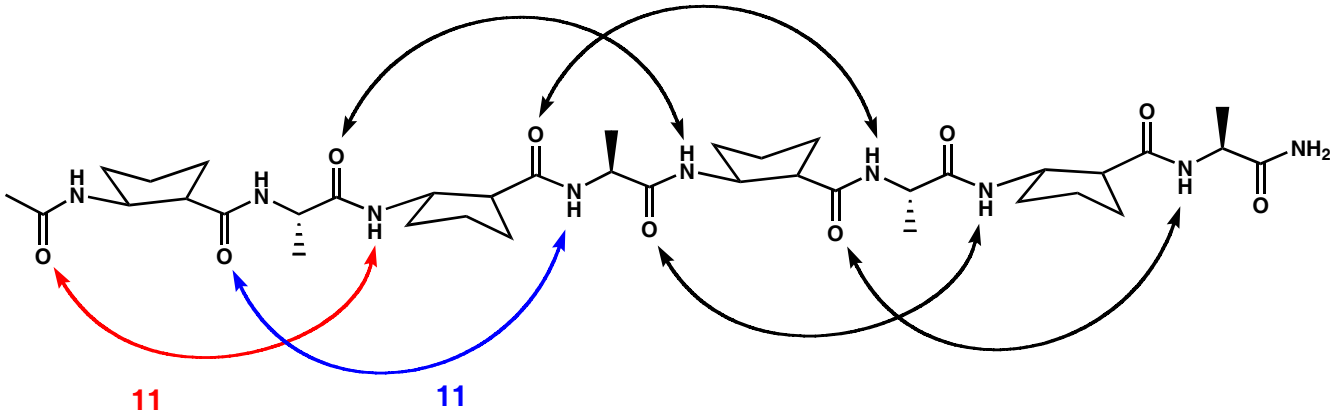


Numerous i --> i+2 and i --> i+3 NOEs (CD_3OH)

Hayen, Schmitt, Ngassa, Thomasson, Gellman *Angew. Chem. Int. Ed.* **2004**, *43*, 505.

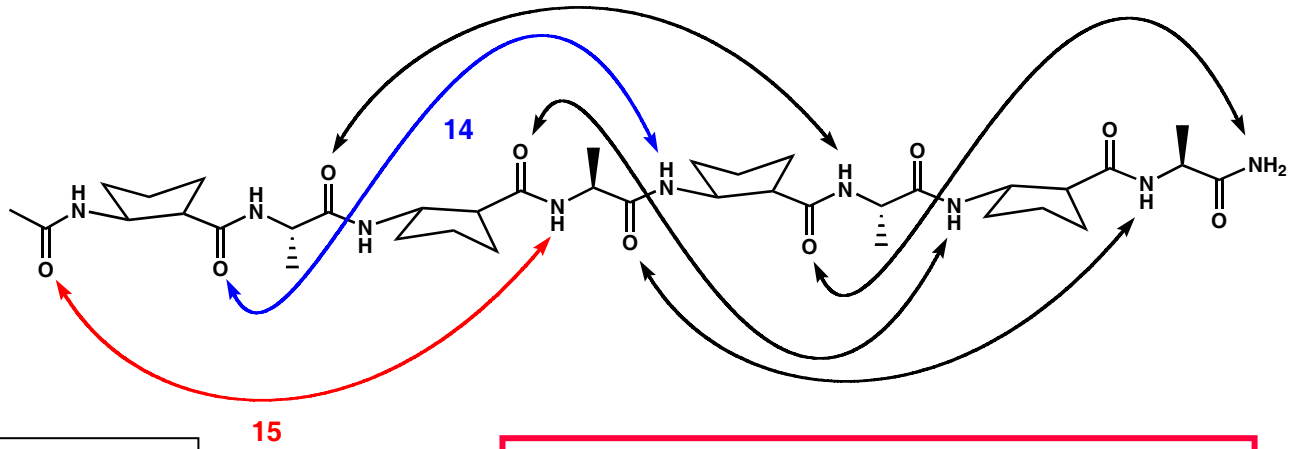
See Also: Reiser et al. *Angew. Chem. Int. Ed.* **2004**, *43*, 511.
Sharma et al. *Angew. Chem. Int. Ed.* **2005**, *44*, 5878.

α/β-Peptide Foldamers

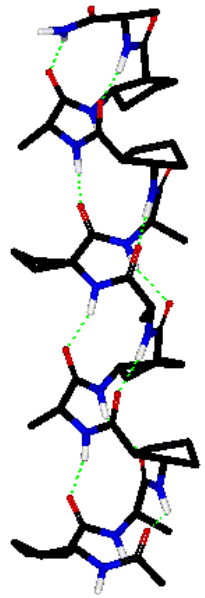


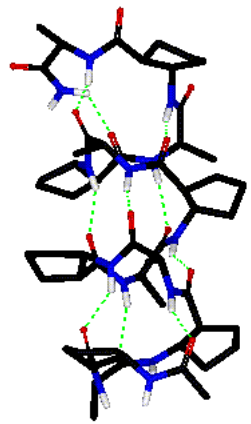
11-Helix

vs.



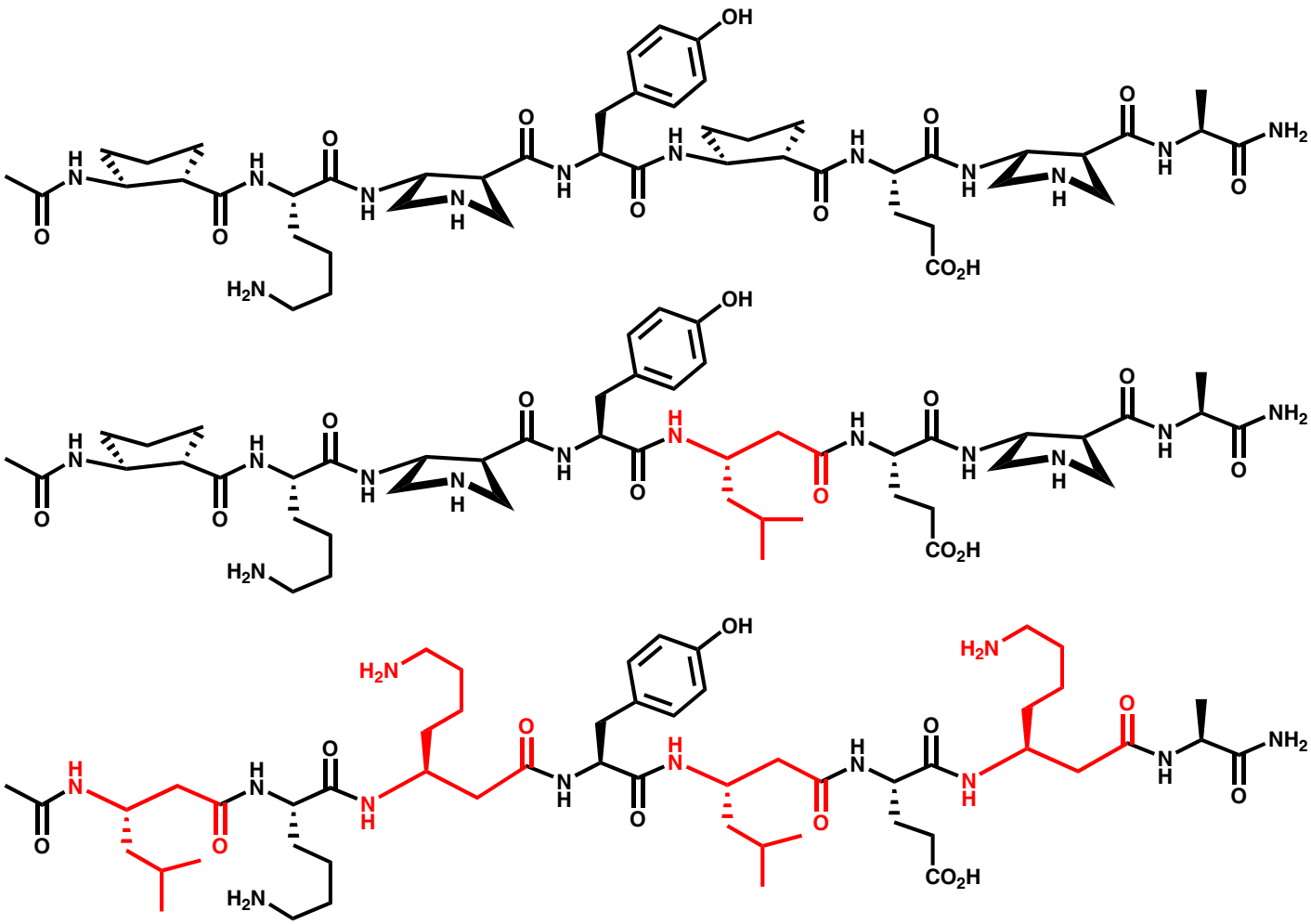
14/15-Helix





Compare: 3_{10}-Helix vs. α-Helix (i,i+3 vs. i,i+4 H-bonding)

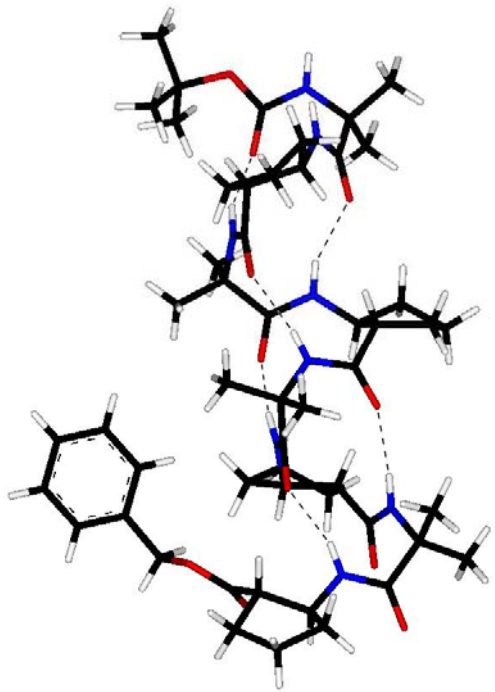
Cyclically Constrained β-Residues Necessary for α/β-Peptide Helix Formation

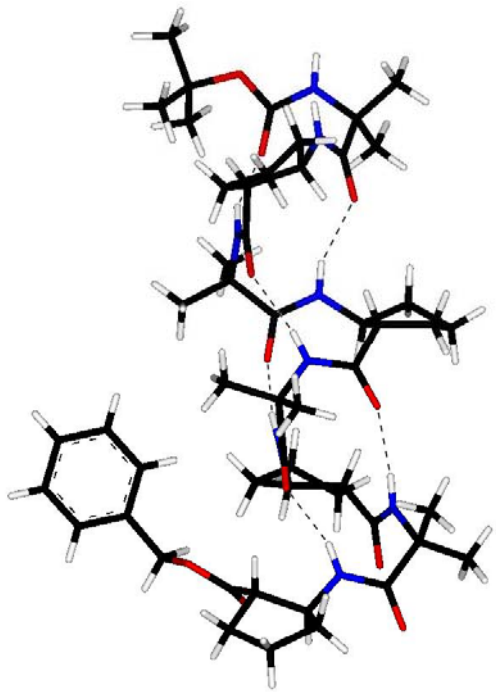


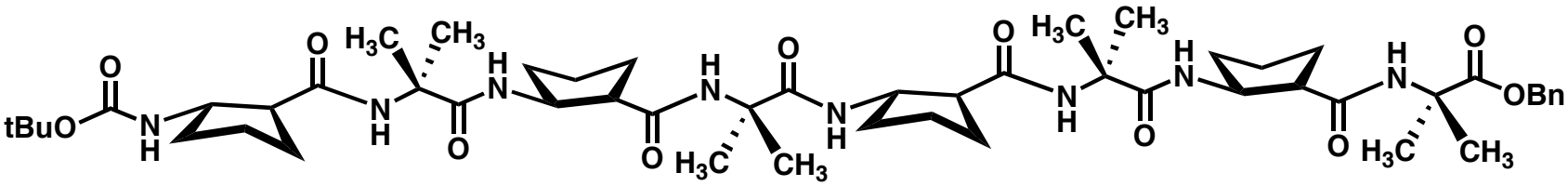
Medium-range NOEs in CD_3OH
Many
Many
None

Schmitt, Choi, Guzei, Gellman *J. Am. Chem. Soc.* 127:13130 (2005).

Crystal Structure: 11-Helical α/β-Peptide Octamer (Stereo)

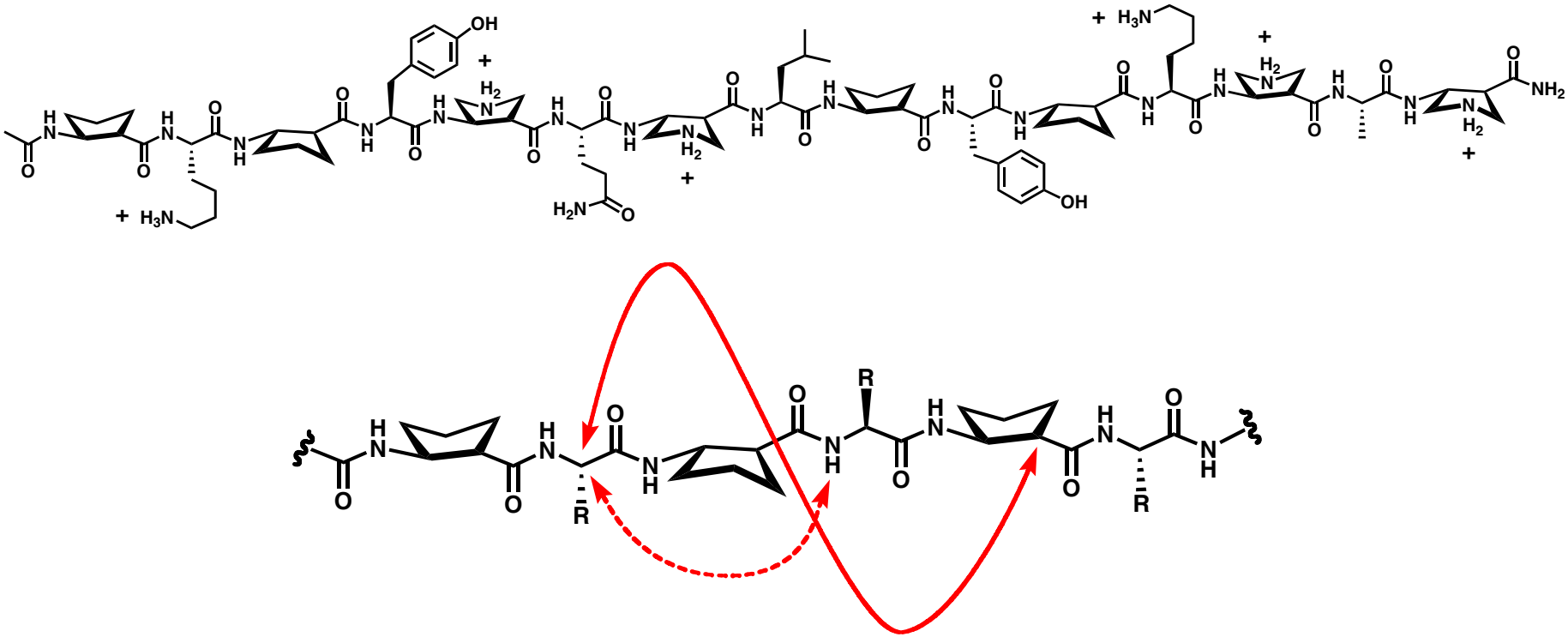






Schmitt, Choi, Guzei, Gellman *J. Am. Chem. Soc.* 127:13130 (2005).

Increasing α/β-Peptide Length Favors 14/15-Helix over 11-Helix (CD_3OH or H_2O/D_2O)



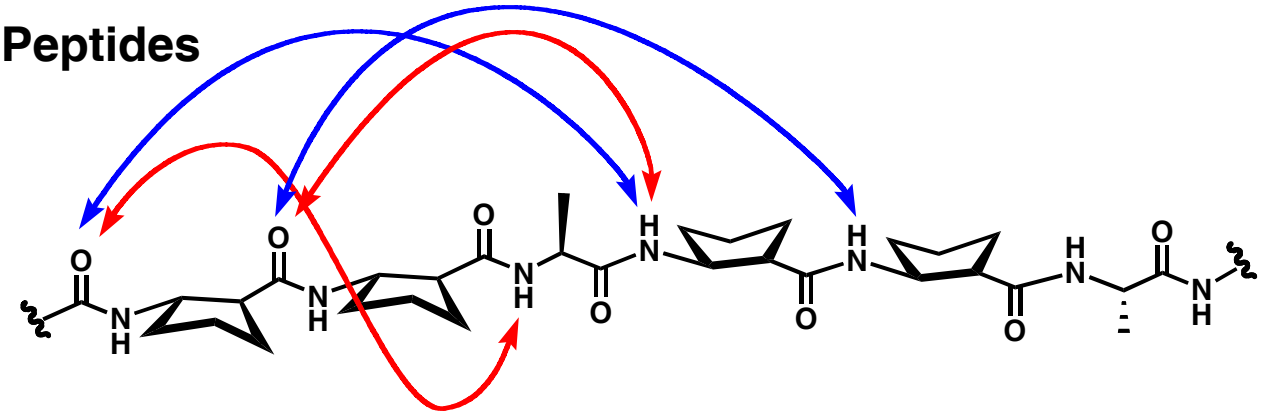
NOE pattern expected for 14/15-helix but not 11-helix [4 or 5 of 7 observed]

NOE pattern expected for 11-helix but not 14/15-helix [none observed]

Schmitt, Weisblum, Gellman *J. Am. Chem. Soc.* 126:6848 (2004).

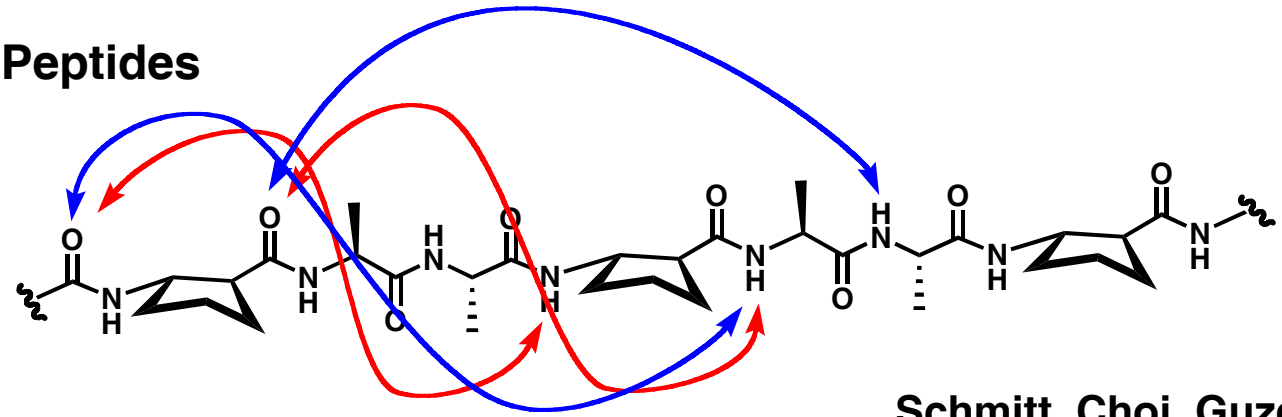
α/β-Peptides: Beyond 1:1 Sequence Alternation

α,β,β-Peptides



i,i+3 vs. i,i+4 C=O--H-N H-bond patterns

α,α,β-Peptides



Schmitt, Choi, Guzei, Gellman
J. Am. Chem. Soc. **128:4538 (2006).**

Can we use foldamers to disrupt specific protein-protein interactions?

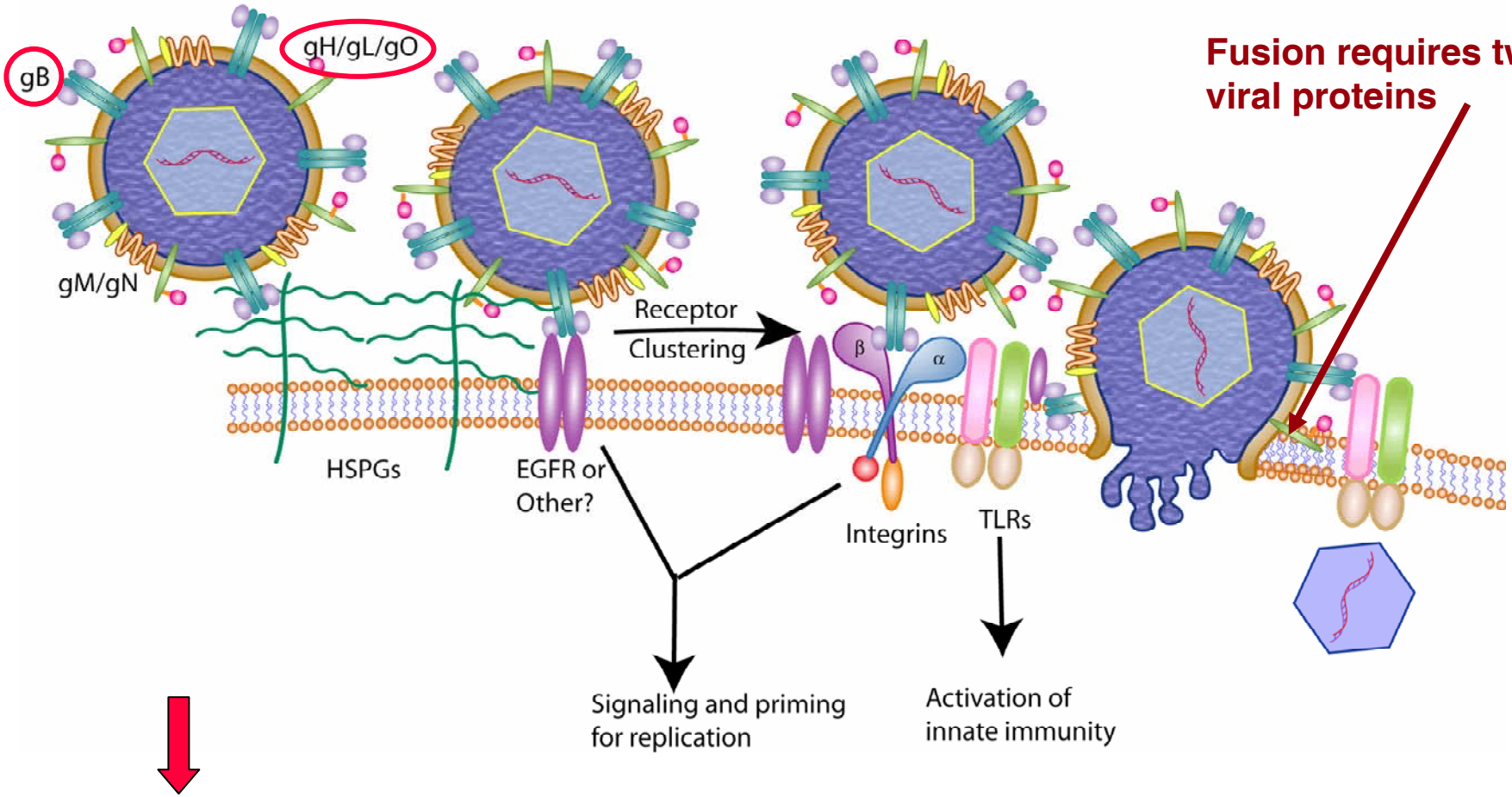
-- Important fundamental challenge in molecular recognition.

-- Critical biomedical need.

-- Small molecule-based approaches often ineffective.

-- Hypothesis: Foldamers will provide a general approach for rational development of protein-protein interaction antagonists.

β-Peptides to Inhibit Human Cytomegalovirus Entry?

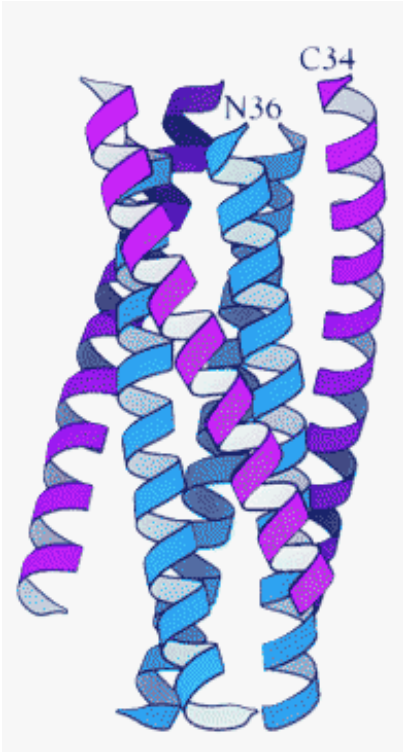


Compton, T. 2003. Trends in Cell Biology. 14:4-8.

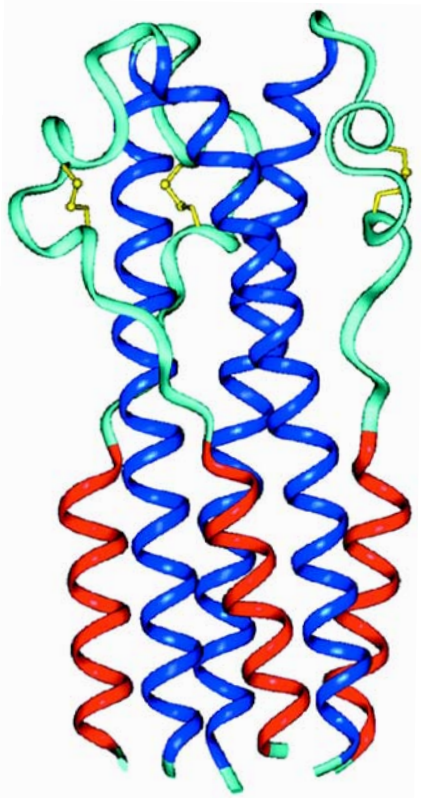
Helix-Helix Interactions Critical for Many Viral Entry Mechanisms (Class I)

SARS Spike Protein

HIV-1 gp41



Ebola Gp2



Chan et al. *Cell*, **1997**, *89*, 263.
Xu et al. *J. Biol. Chem.* **2004**, *279*, 49414.
Malashkevich et al. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 2662.

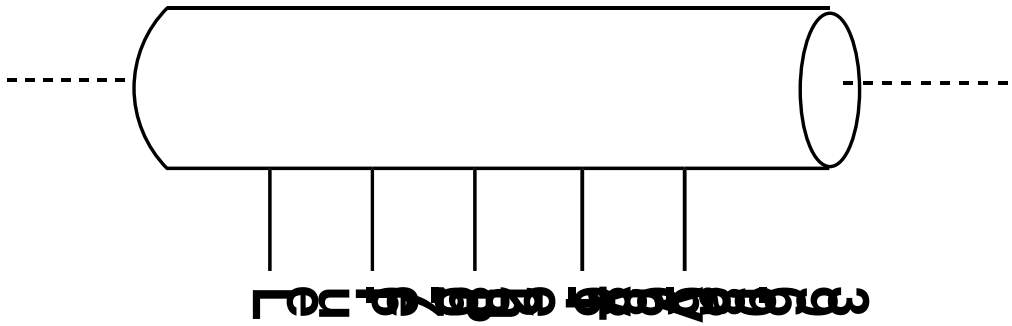
Proposed Amphiphilic α-Helix in hCMV Protein gB

675 703

Ac-NVFD**L**EE**I**MRE**F**NS**Y**KQR**V**KYVEDKVVDPC-NH_2

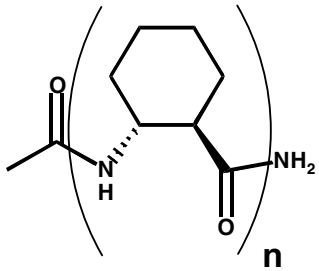
90% Inhibition of HCMV Entry at 500 μM (30-mer)

Lopper, M.; Compton, T. *J. Virol.* **2004**, *78*, 8333.

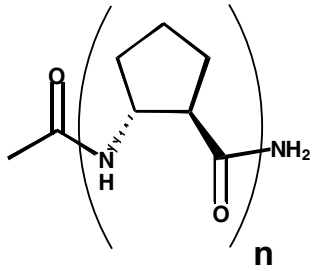


Replace α-helix backbone with a foldamer backbone?

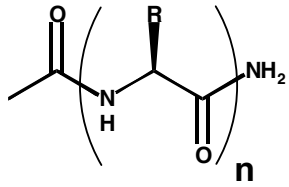
Which β-peptide scaffold is best to mimic an α-helix?



14-Helix



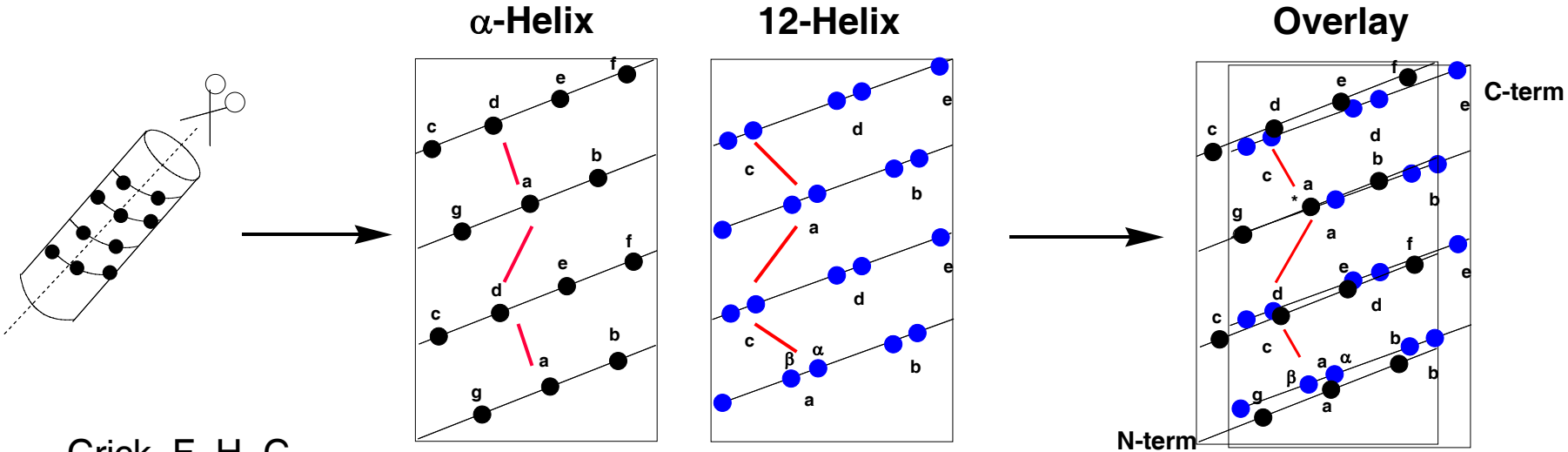
12-Helix



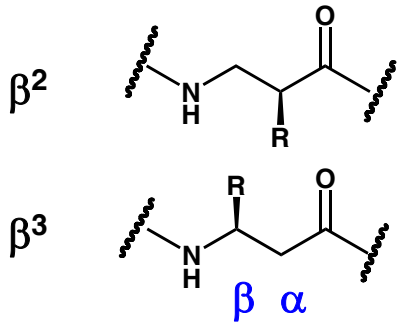
α-Helix

		14-Helix	12-Helix	α-Helix
	Rise per residue	1.6 Å	2.1 Å	1.5 Å
➡	Inner diameter	4.0 Å	3.1 Å	3.2 Å
	Residues per turn	3.2	2.6	3.6
➡	Rise per turn	5.1 Å	5.4 Å	5.4 Å
➡	Dipole orientation (+ --> -)	C to N	N to C ⬆	N to C

β-Peptide Design: Helical Net Approach



Crick, F. H. C.
Acta Cryst. **1953**, *6*, 689.



- Heptad repeat for α-helix:
  - **A**-b-c-**D**-e-f-g
  - **H**-x-x-**H**-x-x-x (coiled coil)
- Pentad repeat for 12-helix:
  - **A**-b-**C**-d-e
  - **H**-x-**H**-x-x

E. English

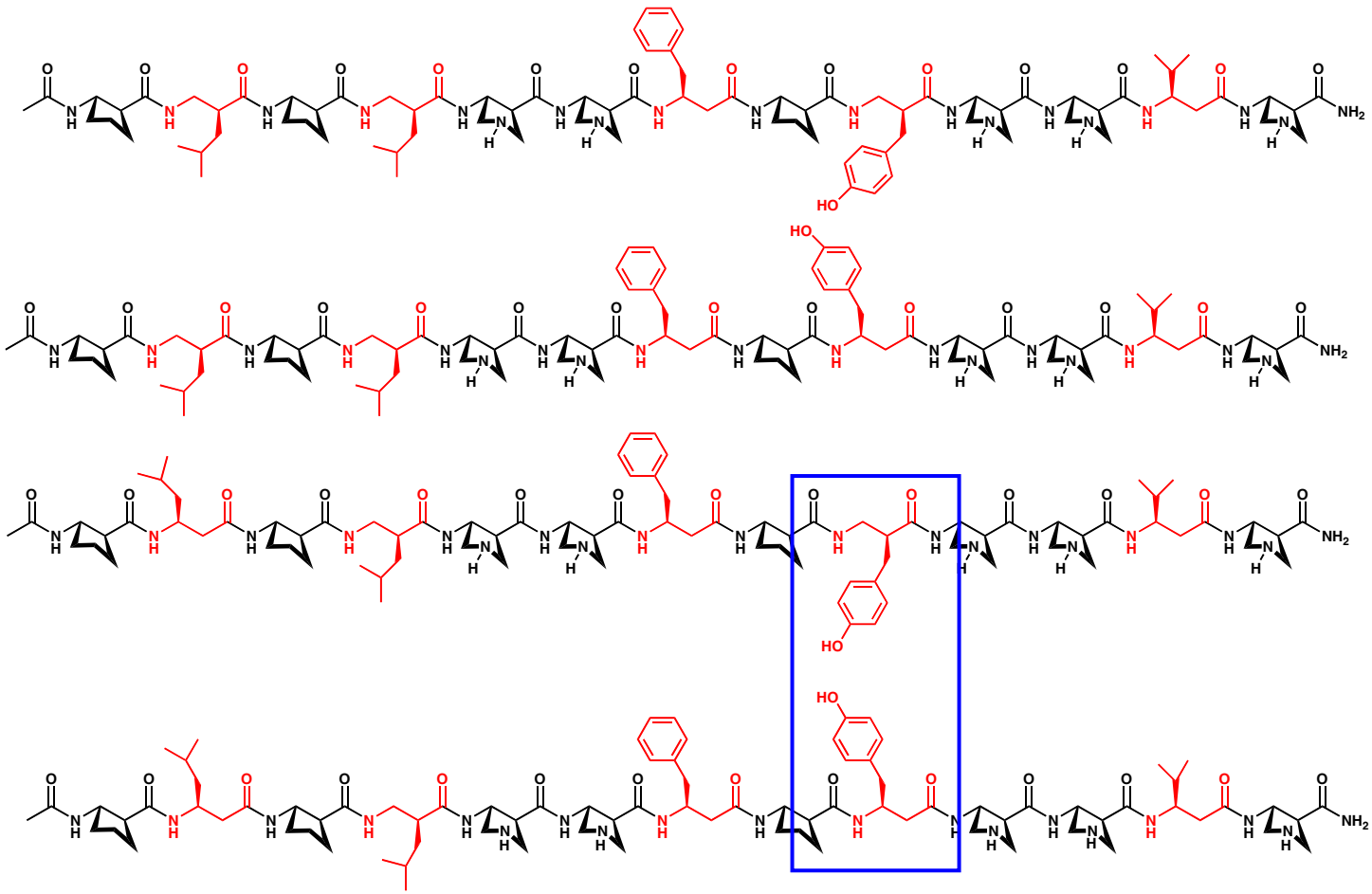
β-Peptide Mimics of Glycoprotein B

675 703

-NVFD**L**EE**I**MRE**F**NS**Y**KQR**V**KYVEDKVVDPC·

E. English,
R. Chumanov

% Inhibition
@ 500 μM



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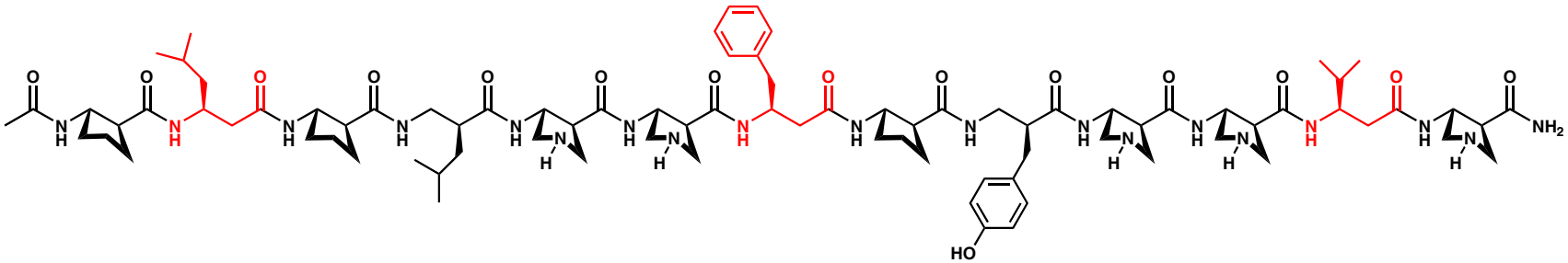
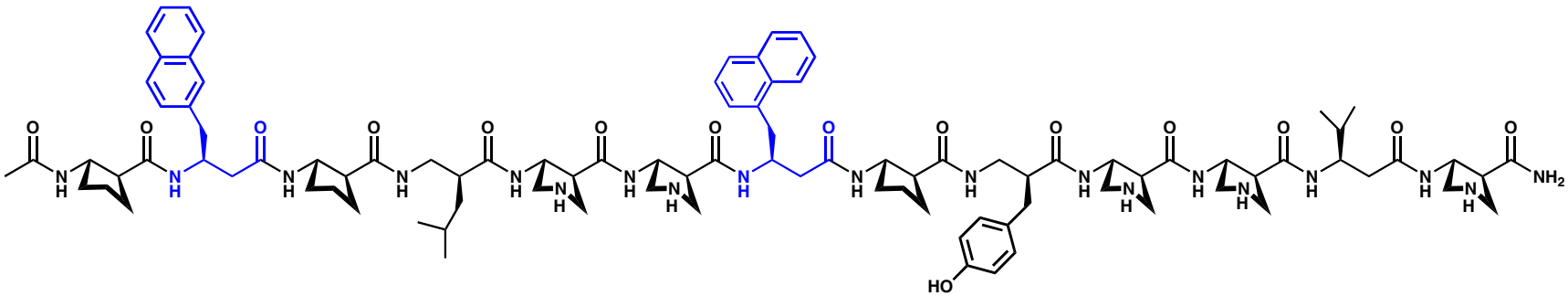
Second Generation Designs: Vary Non-Polar Side Chains at β^3-Positions





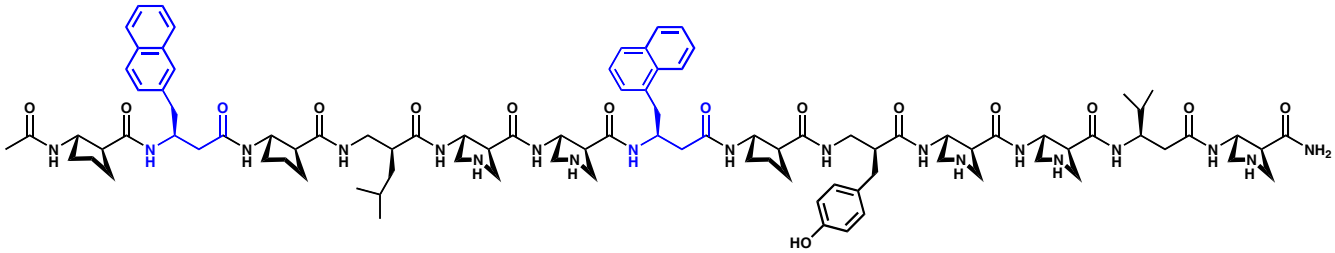
E. English, R. Chumanov

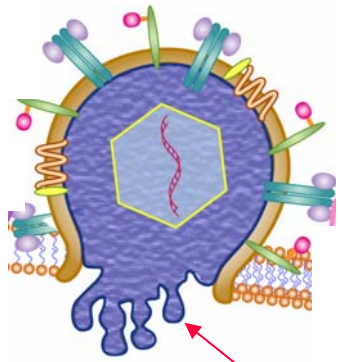
IC_{50} ~ 30 μM (cell-based assay)

(>10-fold better than gB α-peptide)

- **New tools for elucidating viral entry mechanism.**
- **New strategy for development of antiviral drugs?**

The β-Peptide Blocks Fusion of HCMV and Target Cell Membranes



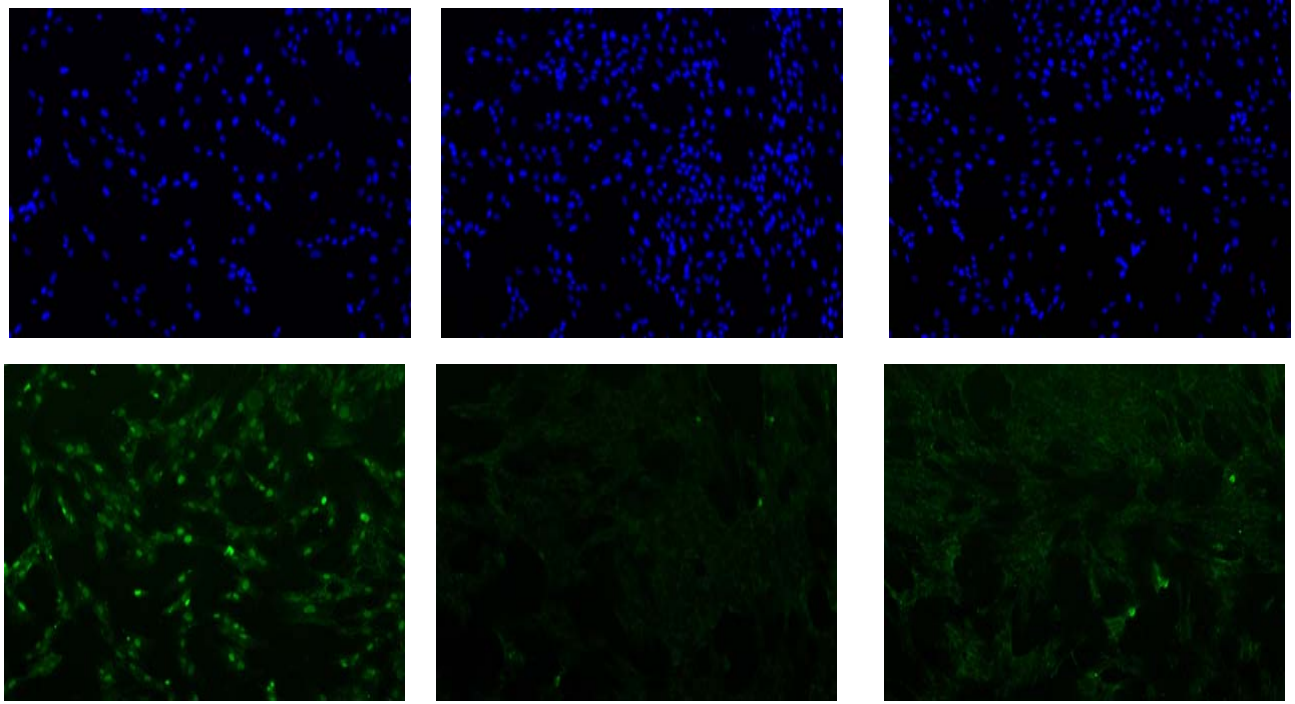


Viral protein pp65 enters cell and moves rapidly to nucleus after fusion.

Normal Infection

Negative Control (Heparin)

β-Peptide (100 μM)



DAPI stain (nuclei)

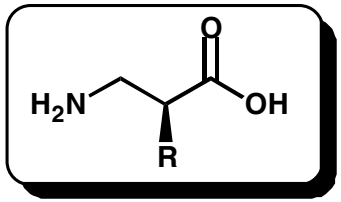
Cells fixed and stained 35 min after infection.

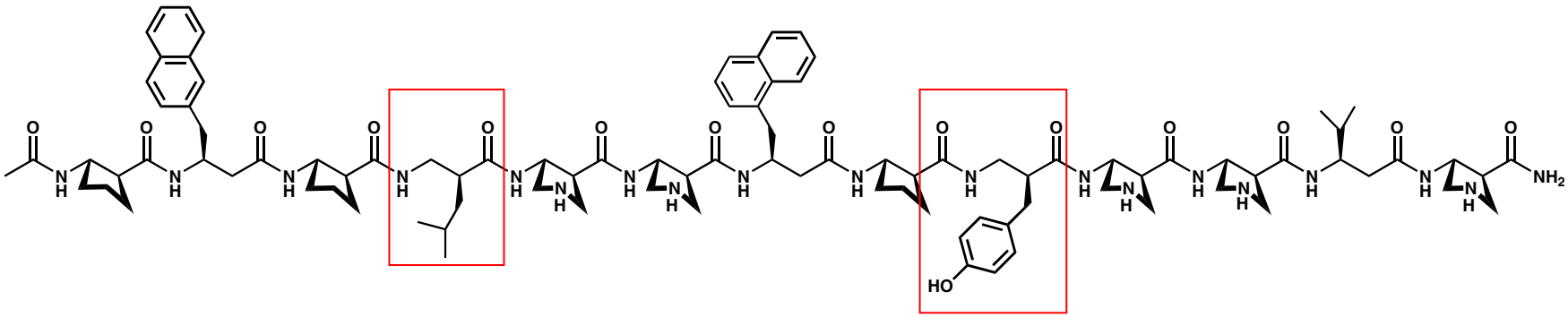
Antibody stain (pp65)

English, Chumanov, Gellman, Compton *J. Biol. Chem.* 281:2661 (2006).

Problem: Access to β^2-Residue Building Blocks

3 2 1

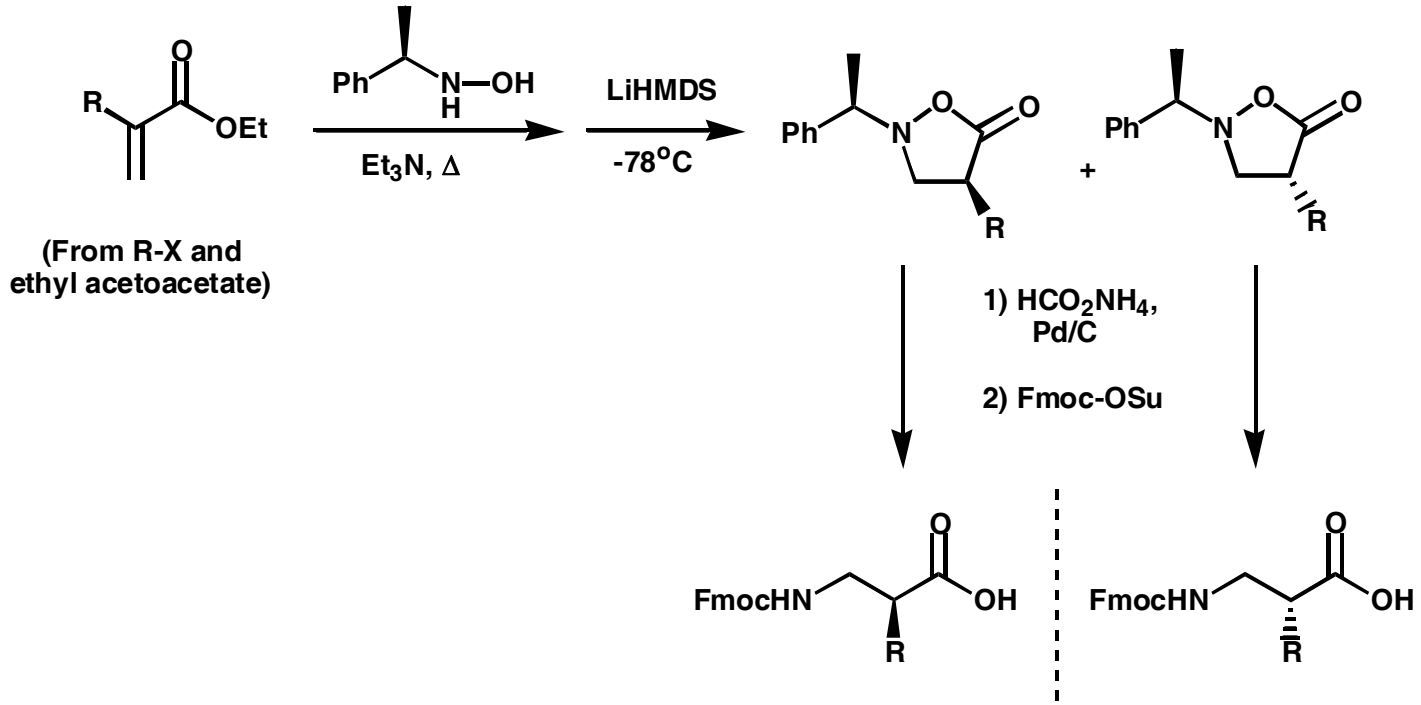




Review of β^2-Amino Acid Synthesis:

G. Lelais, D. Seebach *Biopolymers* 76:206 (2004)

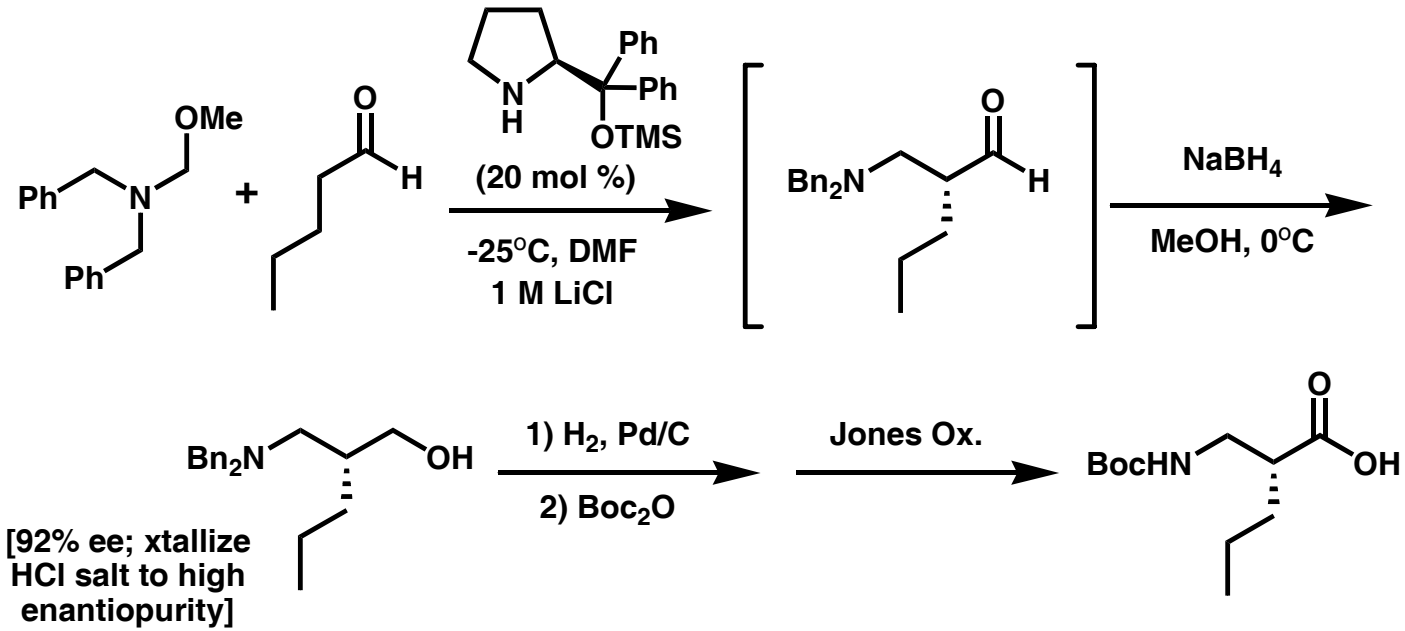
β²-Residue Building Blocks via Chiral Auxiliaries…



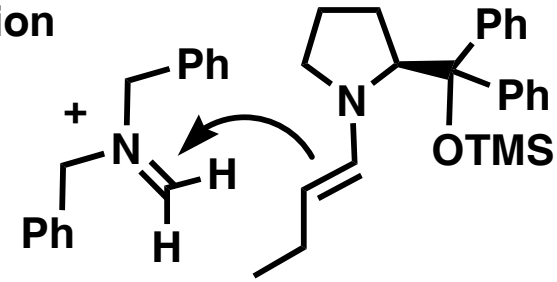
Lee, Park, Kim & Gellman *J. Org. Chem.* **2003**, *68*, 1575.

(cf. Baldwin and Aubé *Tet. Lett.* **1987**, *28*, 179.)

An Organocatalytic Route to β^2-Residue Building Blocks



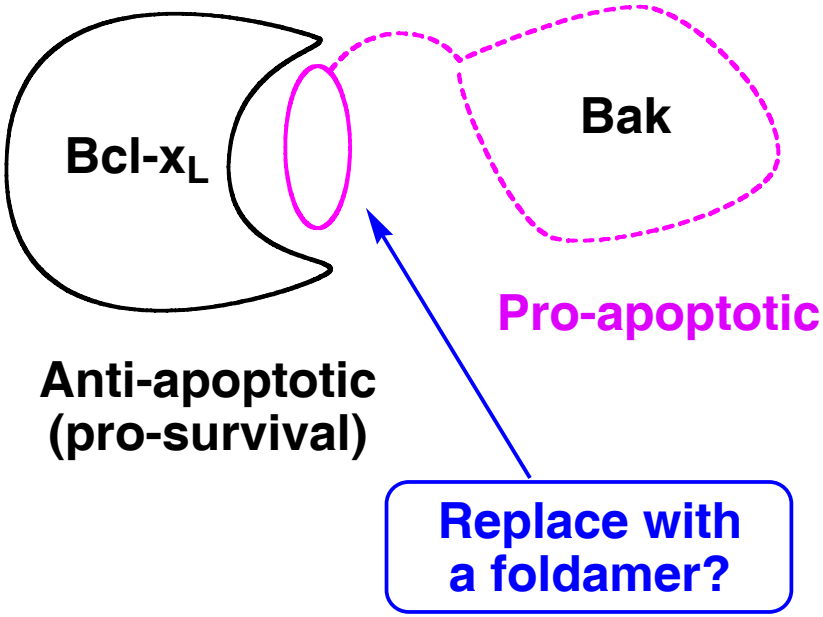
Key step = Mannich Reaction

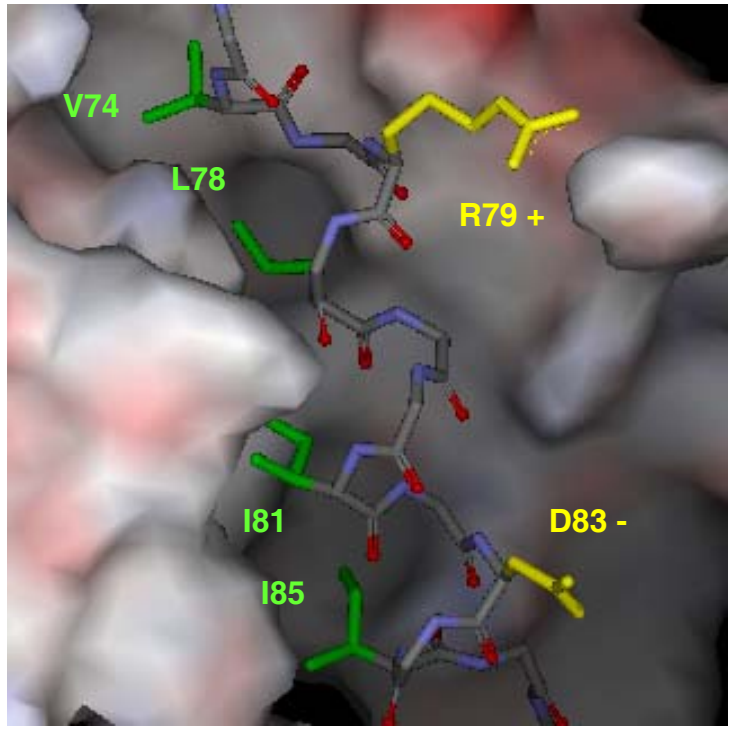


Catalyst precedent:
Hayashi, Jørgensen, Cordova

Chi & Gellman *J. Am. Chem.Soc.* 128:6804 (2006)

Inhibit Bcl-x_L/Bak Interaction?





Review:
Shangary and Johnson
Leukemia **2003**, *17*, 1470.

Bcl-x_L/Bak interface (NMR)

Fesik et al. *Science* **1997**, *275*, 983.

Ligands for the BH3-Recognition Cleft on Bcl-x_L/Bcl-2

Peptide Ligands

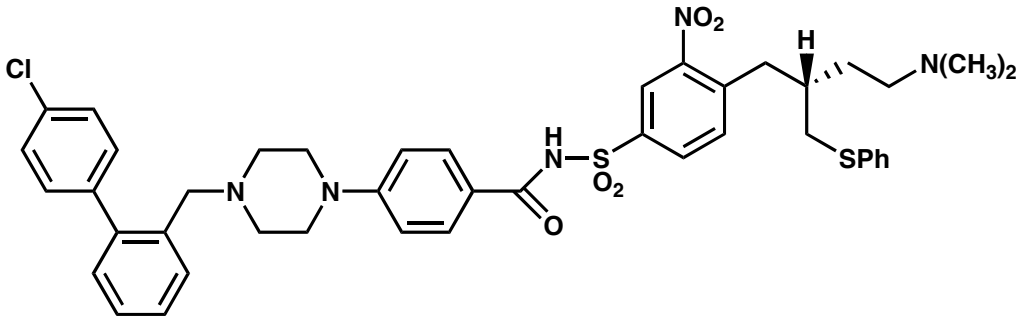
GQVGRQLAIIGDDINRR (Bak 16-mer)

NLWAAQRYGRELRRMSDEFVDSFKK (Bad 25-mer)

} **Fesik, 1997, 2000**

EDIIRNIARHLAXVGDX^{n}LDRSIW (X-Linked Bid 23-mer) **Korsmeyer, Verdine, 2004**

Small Molecule Ligand



Oltersdorf et al. *Nature* 435:677 (2005)

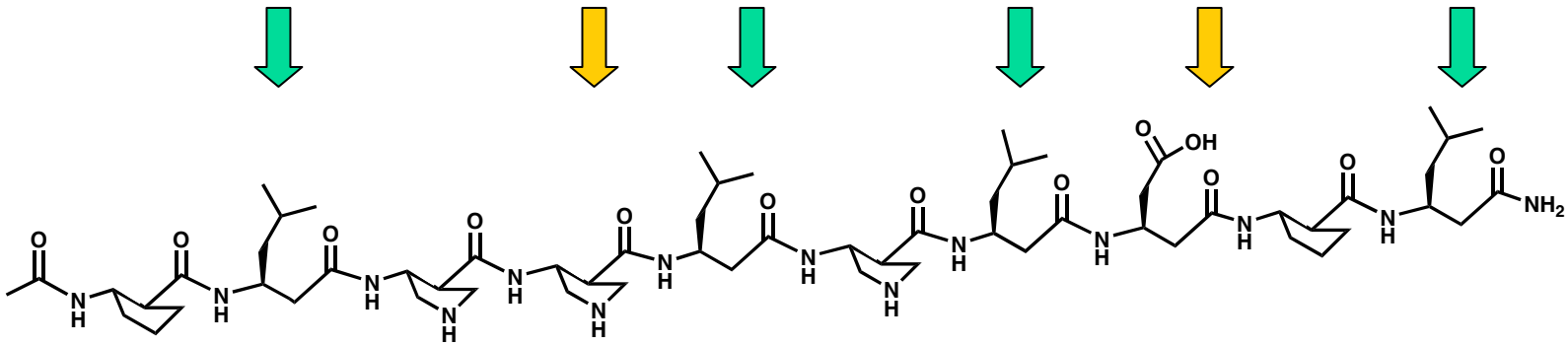
First Generation β-Peptide Design for Bcl-x_L/Bak Antagonism

Hydrophobic

Hydrophilic

Gly - Gln - Val - Gly - Arg - Gln - Leu - Ala - Ile - Ile - Gly - Asp - Asp - Ile - Asn - Arg

(Bak sequence; α-helix)

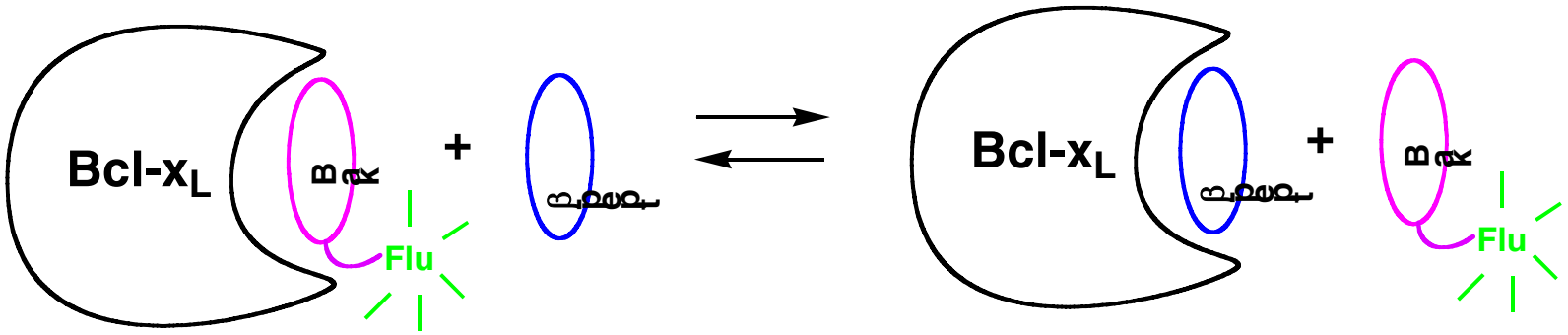


(β-peptide inhibitor candidate; 12-helix)

S. Wang et al., Univ. Michigan
Y. Tomita, Georgetown

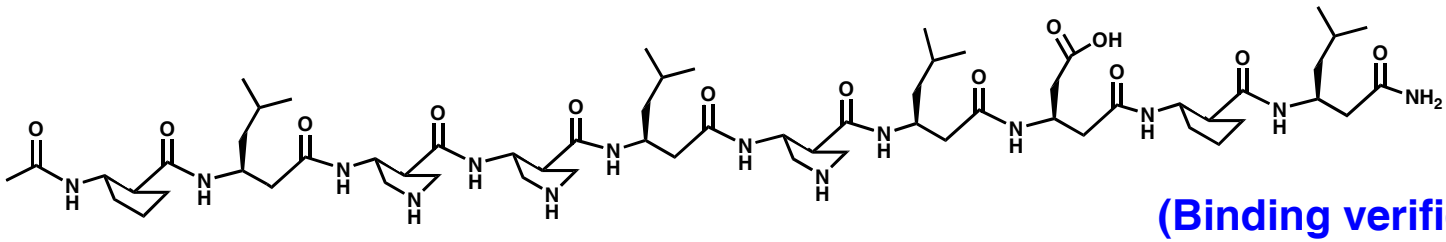
H.-S. Lee, N. Umezawa, **J. Sadowsky**

Screen for Binding to Bcl-x_L: Fluorescence Polarization Assay



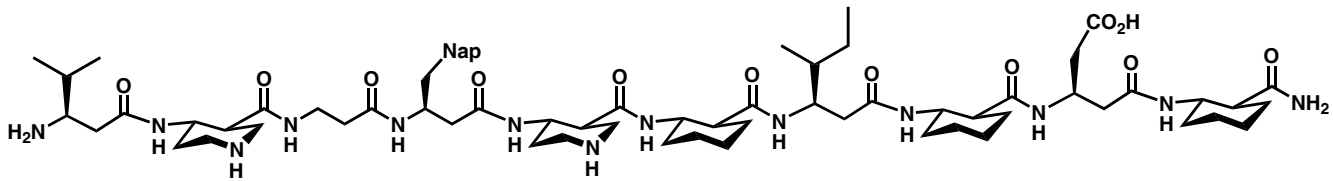
Zhang et al. *Anal. Biochem.* **2002**, *307*, 70.

Problem: Helical β-Peptides are weak inhibitors



12-Helical
IC_{50} ~ 800 μM

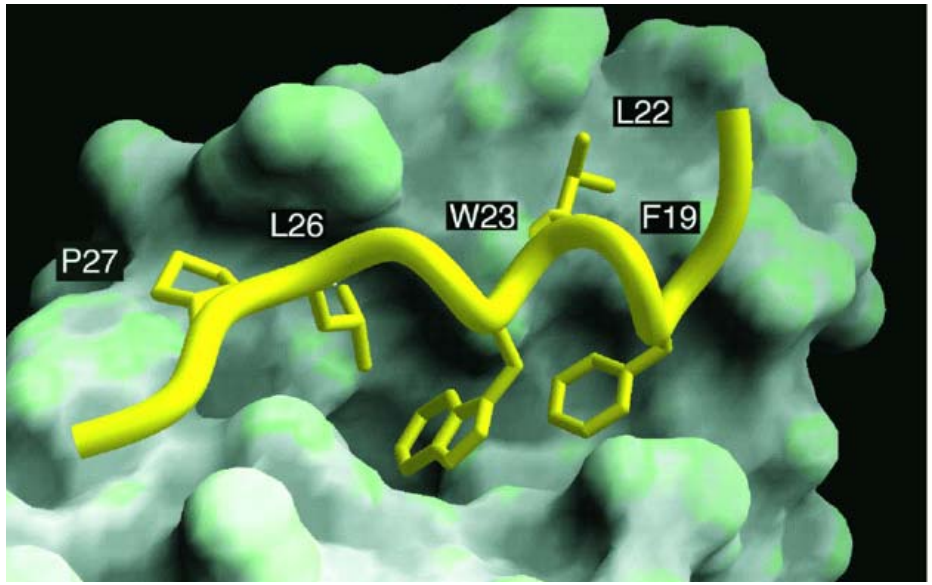
(Binding verified by HSQC)



14-Helical
IC_{50} ~ 600 μM

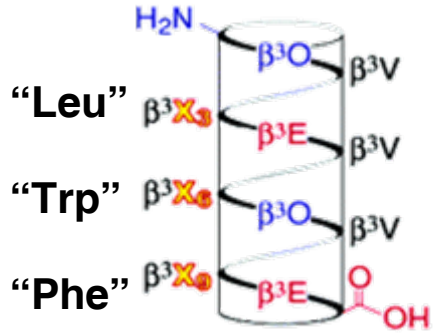
(Bak 16-mer IC_{50} ~ 0.2 μM)

A β-Peptide Mimic of p53 N-terminal α-helix Binds to MDM2



N-terminal segment of p53 bound to mDM2

(Kussie et al. *Science* 274:948 (1996))



14-Helical Scaffold

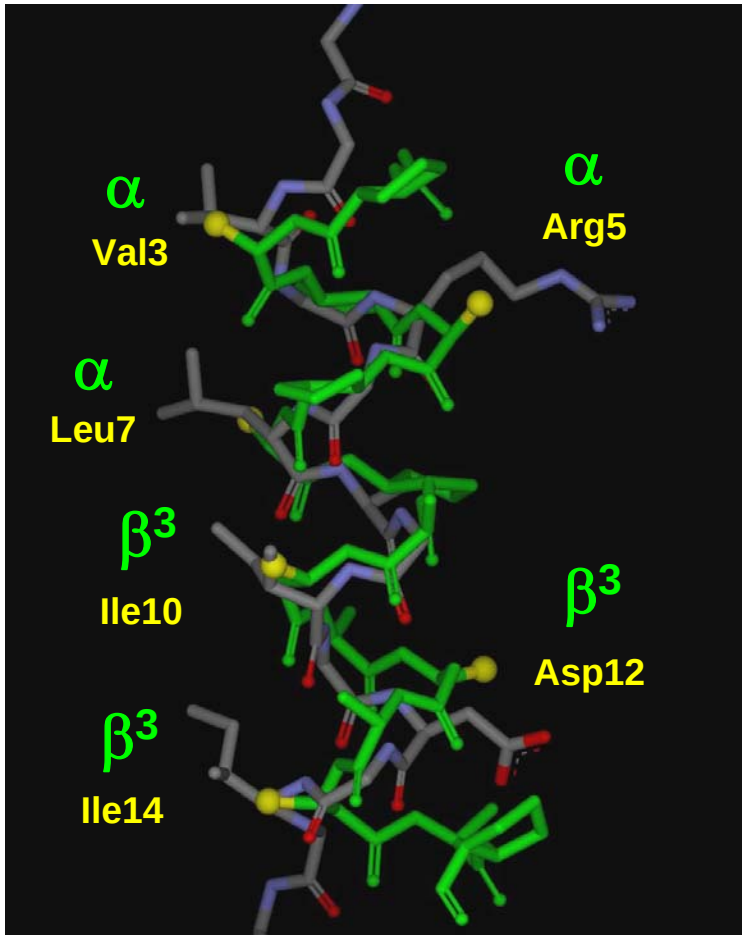
IC_{50} ~ 94 μM (FP assay)

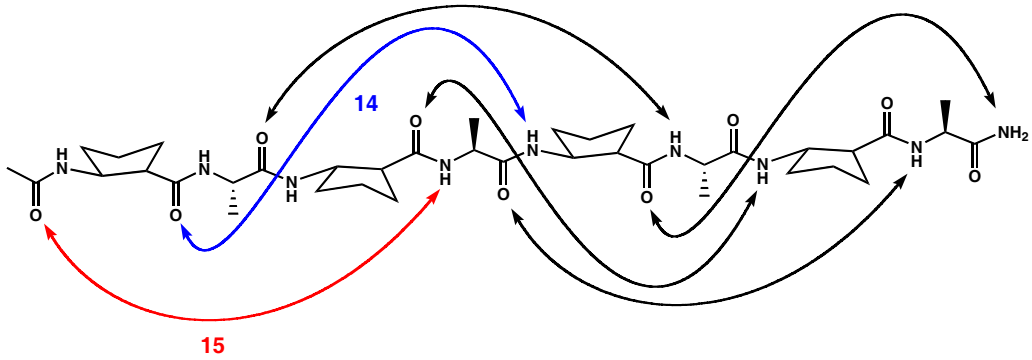
(p53 15-31: IC_{50} ~ 2 μM)

Schepartz et al.
***J. Am. Chem. Soc.* 126:9468 (2004)**

See also:
***J. Am. Chem. Soc.* 127:14584 (2005)**

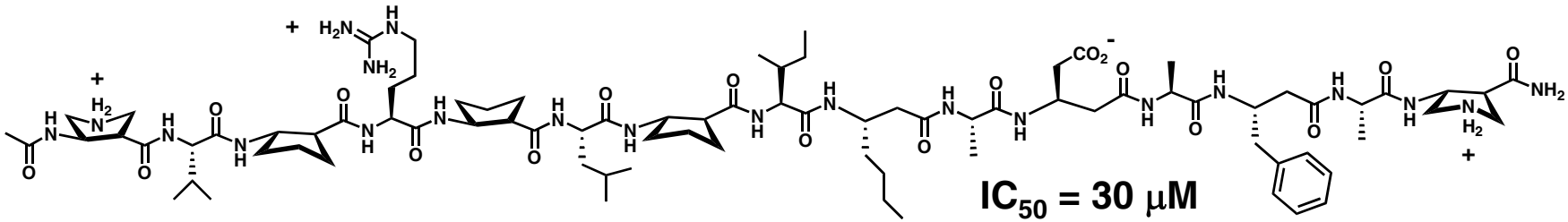
α/β-Peptide mimics of Bak 16-mer (α-Helix)?



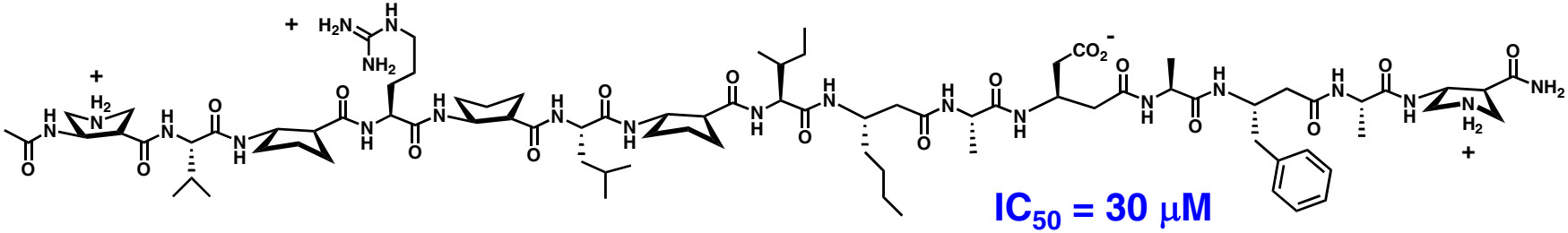


14/15-Helix

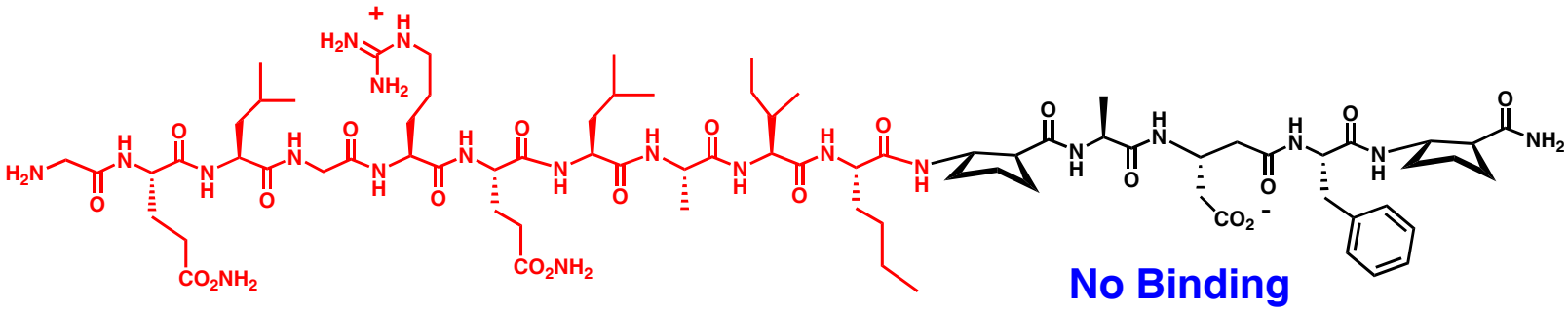
J. Sadowsky



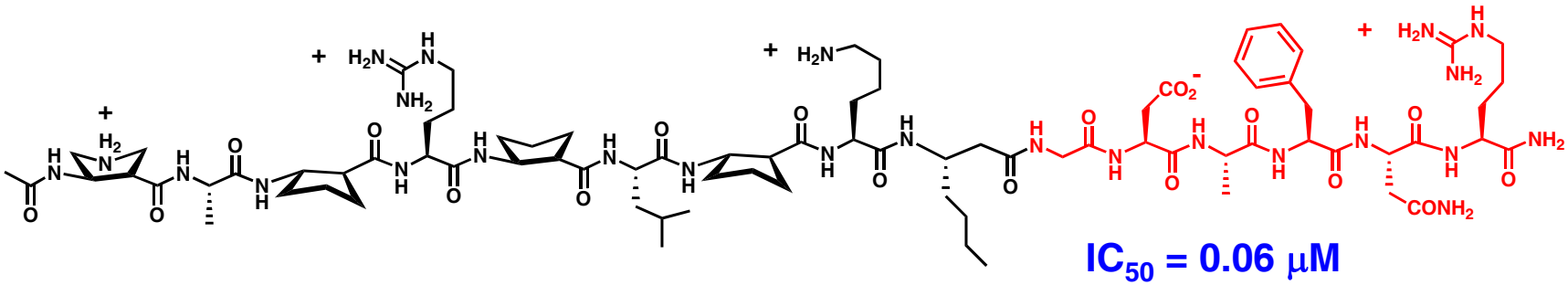
Chimeric ($\alpha/\beta + \alpha$) BH3-Mimics: Identifying a Region of Poor Mimicry



IC_{50} = 30 μM



No Binding

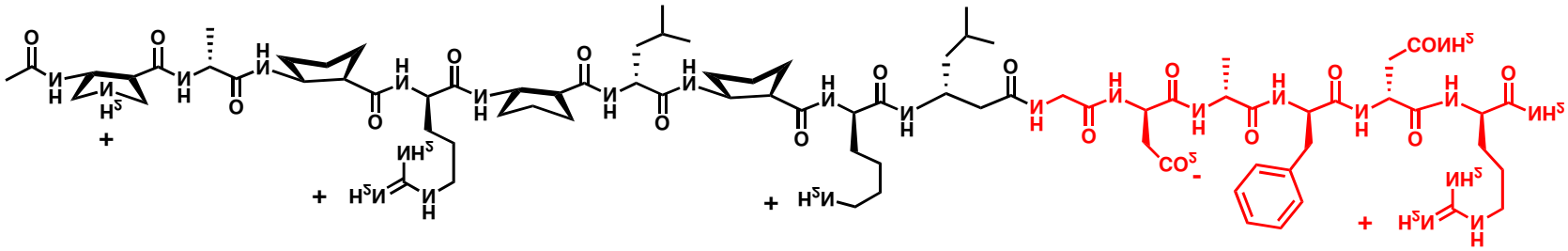


IC_{50} = 0.06 μM

(K_d BSA > 100 μM)

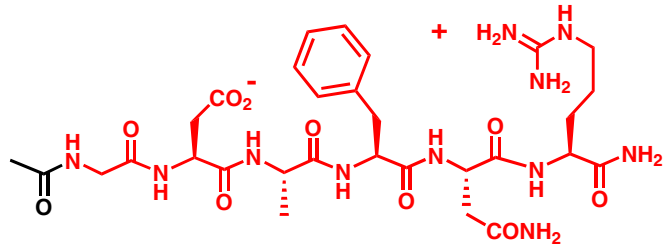
J. Sadowsky

Chimeric ($\alpha/\beta + \alpha$) BH3-Mimics: Key Controls

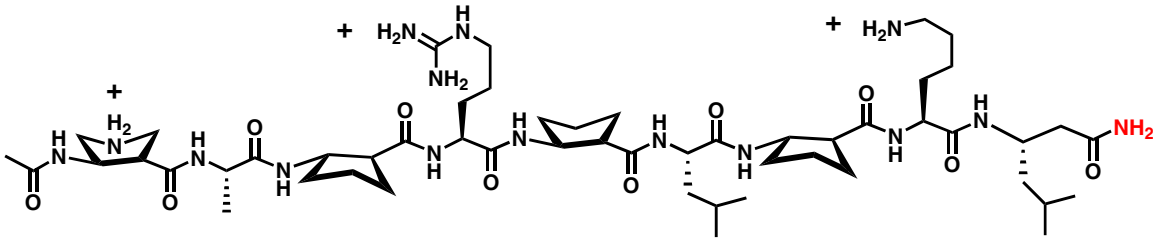


IC_{50} = 0.03 μM (K_i ~ 0.7 nM)

Enantiomer IC_{50} > 1000 μM



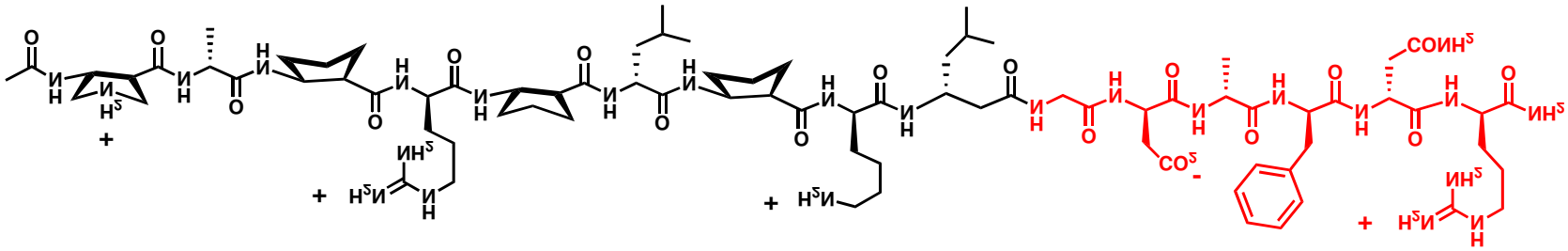
IC_{50} >> 500 μM



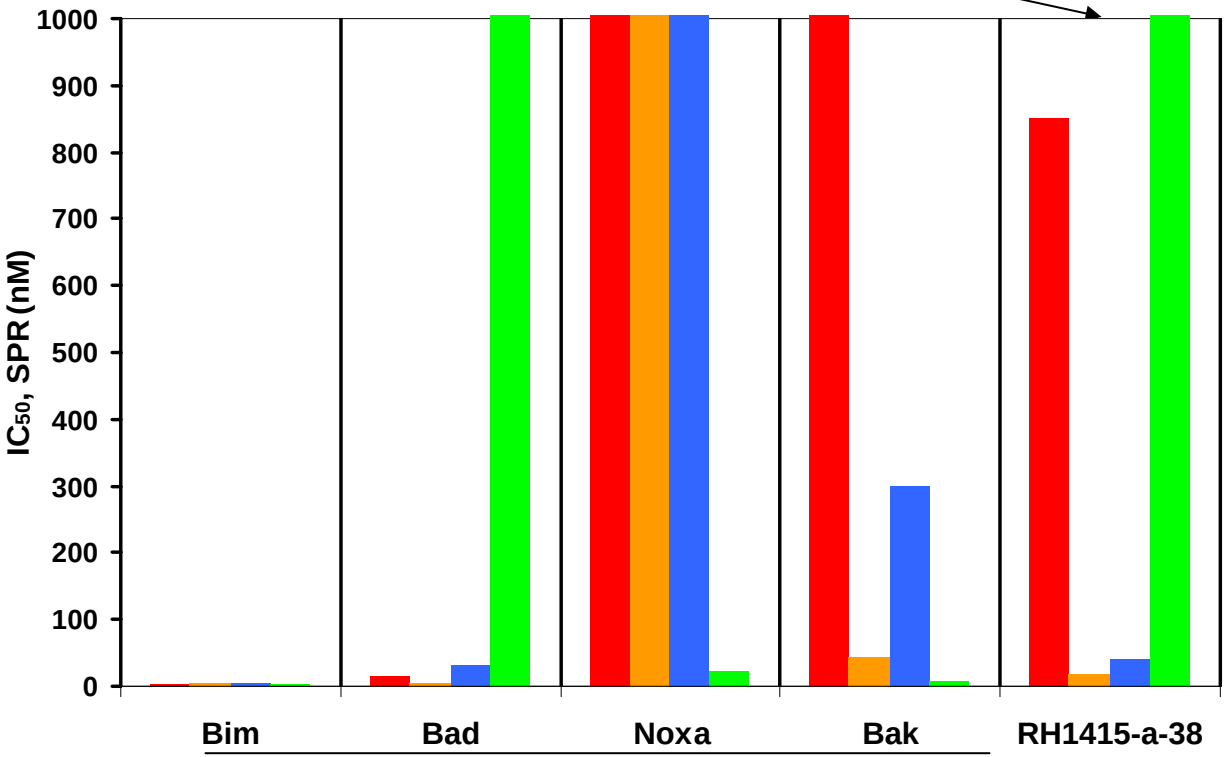
IC_{50} = 120 μM

Sadowsky et al. *J. Am. Chem. Soc.* 127:11966 (2005)

Bcl-2 Family Member Binding Selectivity (SPR Assay)



RH1415-a-38

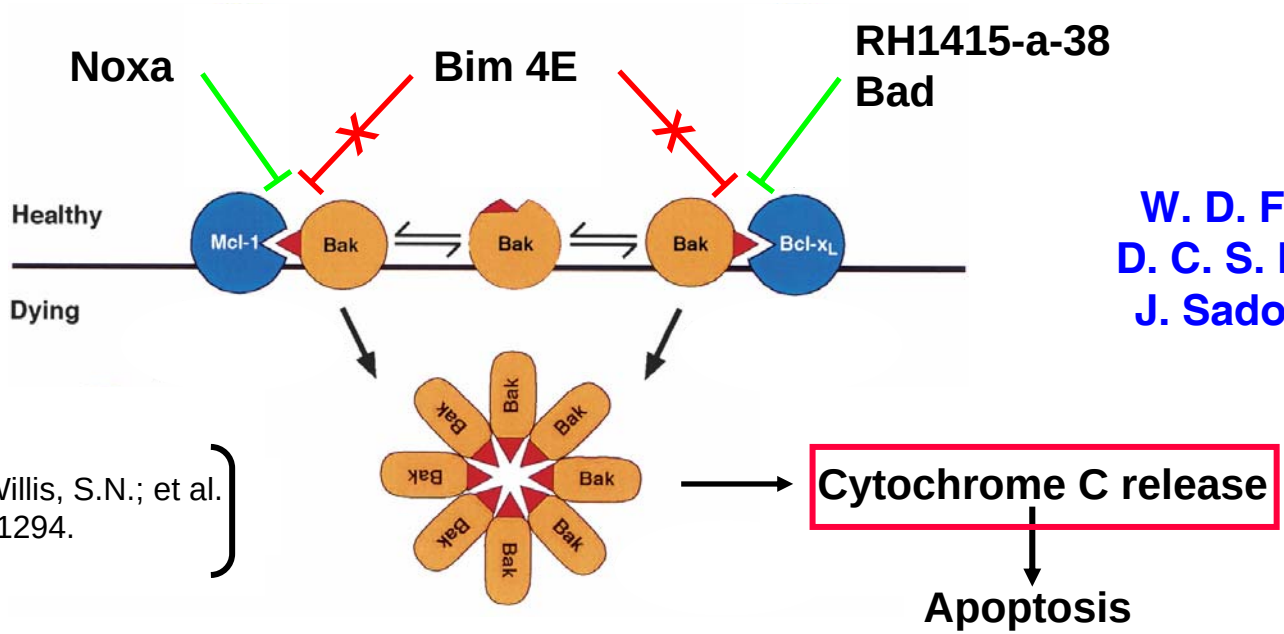


(Anti-apoptotic)

Natural BH3 Domain Peptides
(Pro-apoptotic)

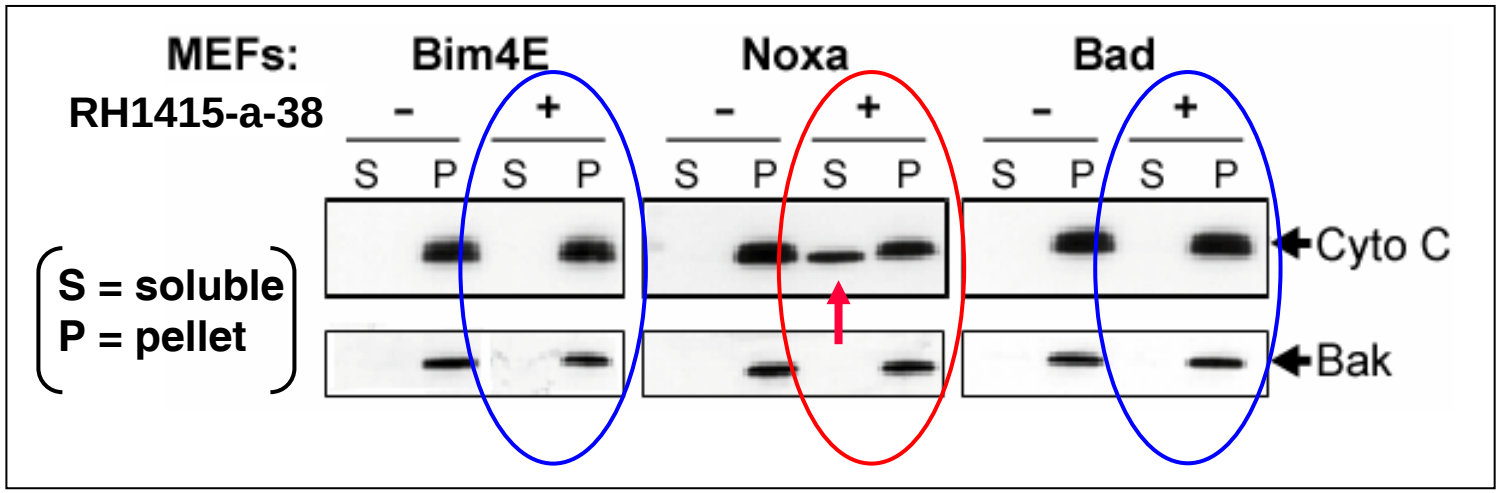
W. D. Fairlie, D. C. S. Huang
(WEH Institute, Australia)

Cytochrome C Release Assays (Cell Extracts)
(Engineered Mouse Embryonic Fibroblasts)



W. D. Fairlie
D. C. S. Huang
J. Sadowsky

Figure adapted from: Willis, S.N.; et al. *Genes Dev.* **2005**, *19*, 1294.

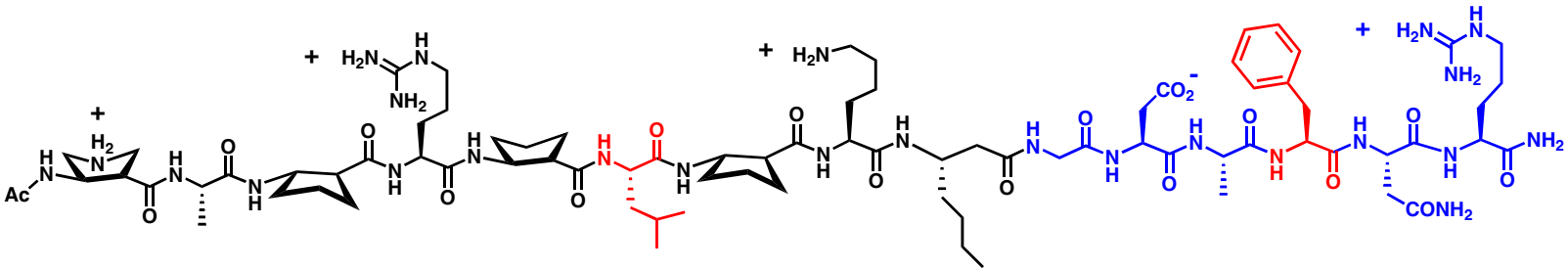


Activity in Cell Culture? (Kill Cancer Cells?)

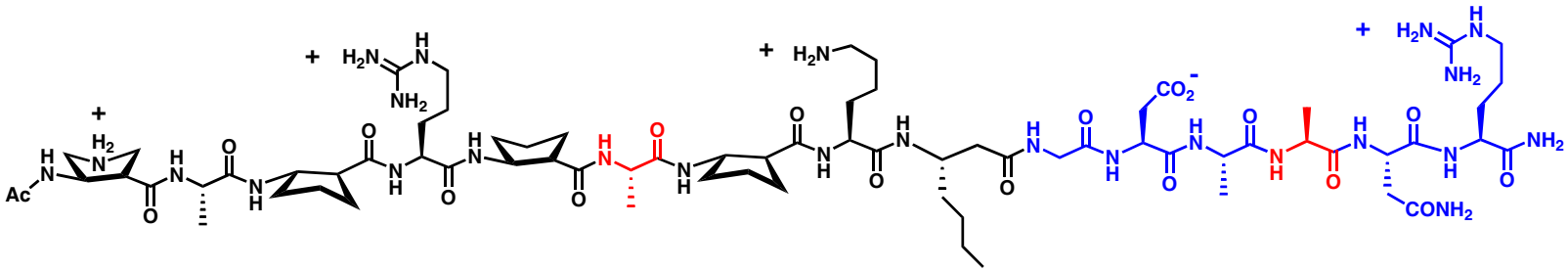
$$\text{-}(\alpha/\beta + \alpha)\text{---}\mathbf{Arg_8}$$

Bcl-x_L Ligand **Cell Entry**

IC_{50} (FP)

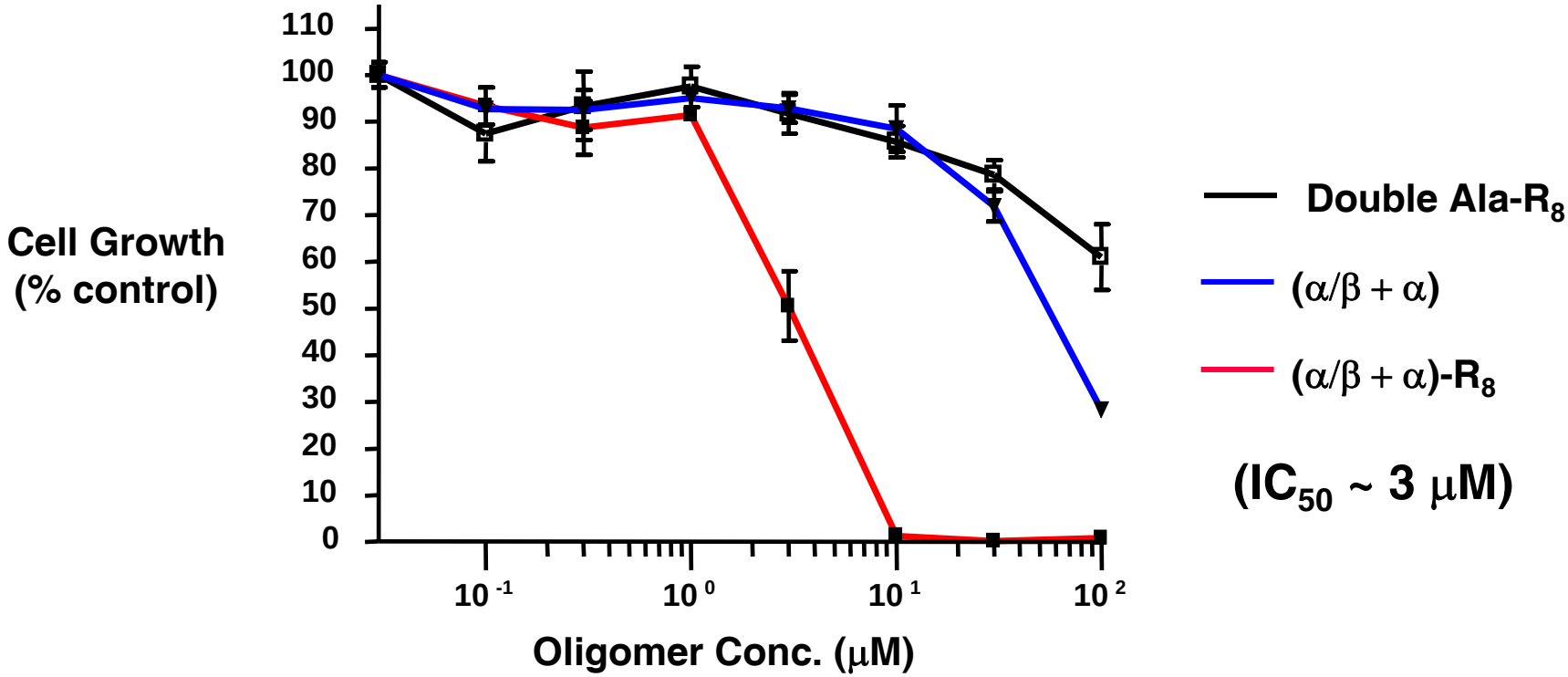


0.06 μM



150 μM

Inhibition of Growth of Human Breast Cancer Cells (MDA-MB-231)



J. Sadowsky
S. Wang (Michigan)

Conclusions

1. **Foldamers appear to be promising for platforms for the design of molecules with specific and useful functions.**

2. **Increasing the number of foldamer scaffolds with distinct shapes (i.e., distinct ways to arrange side chains in space) should enhance our ability to achieve specific molecular tasks, such as blocking specific protein-protein interactions.**

Foldamers @ Wisconsin

Past

Dan Appella
Laurie Christianson
Juan Espinosa
Nick Fisk
Ahlke Hayen
Jon Lai
Hee-Seung Lee
Paul LePlae
Justin Murray
Jin-Seong Park
Tim Peelen
Emilie Porter
Terra Potocky
Tami Raguse
Marina Schinnerl
Meg Schmitt
Faisal Syud
Naoki Umezawa
Xifang Wang

Present

Robin Chi
Soo Hyuk Choi
Emily English
Seth Horne
Kim Peterson
Will Pomerantz
Jack Sadowsky
Matt Windsor

Collaborators

Joe Barchi
Teresa Compton
Doug Fairlie
David Huang
Isabella Karle
York Tomita
Shaomeng Wang

$$$

National Science Foundation
National Institutes of Health