Short Course:

Solution Structure Determination
In Organic Chemistry and Chemical Biology

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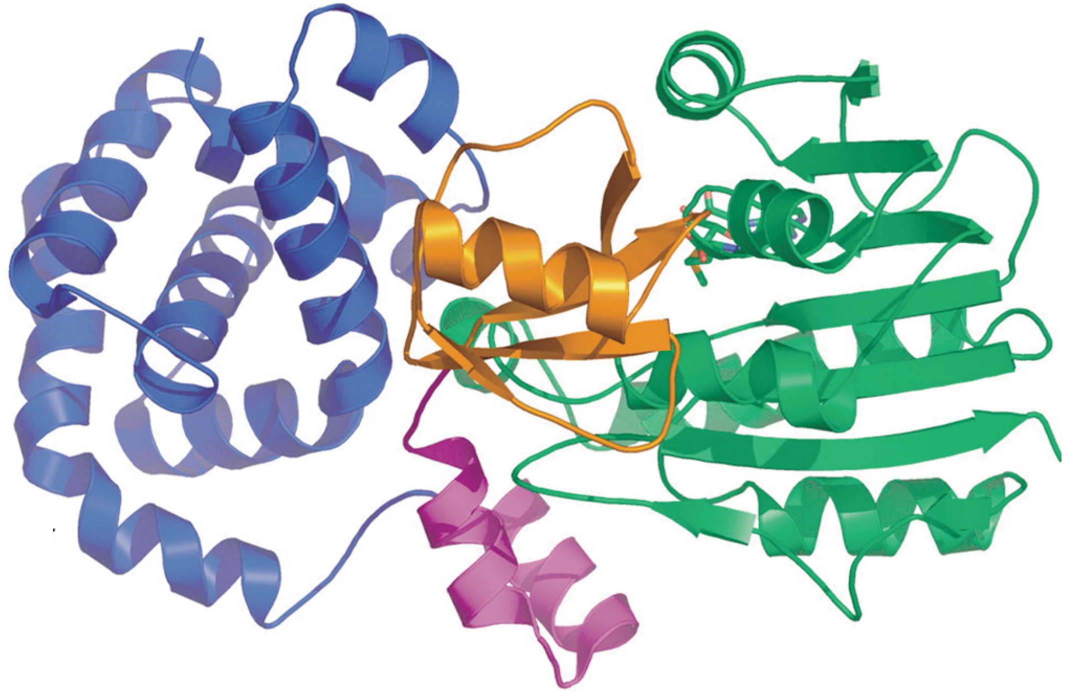
III. Autonomously folding protein secondary structure (β-sheet)

Folded Proteins: Many Tertiary Structures, But Only a Few Common Secondary Structures (Helix and Sheet)



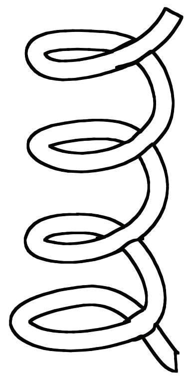
Fmu, an RNA m^5C methyltransferase

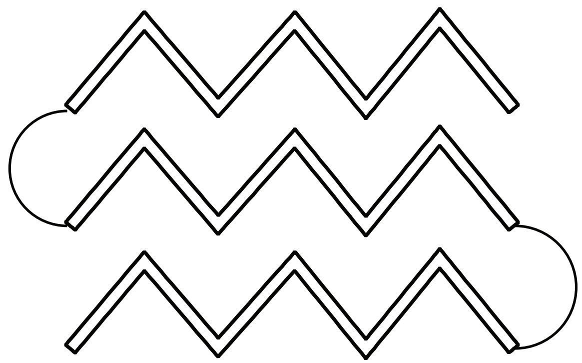
Foster et al. *Structure* **2003**, *11*, 1609.

Protein Tertiary Structures are More Stable Than Isolated Secondary Structures

→ **Autonomously folding secondary structures are required to study sequence-stability relationships.**

Design challenge: Helices form from contiguous segments, but sheets do not.

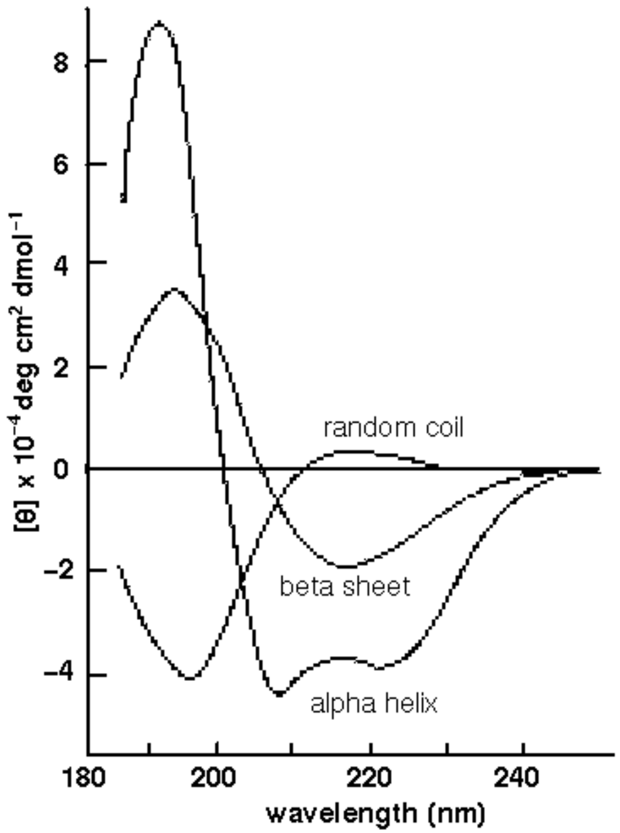




Circular Dichroism (CD) as a Tool for Polypeptide Structure Analysis in Solution (Low-Resolution Information)

Circular dichroism = difference in absorption of left and right circularly polarized light.

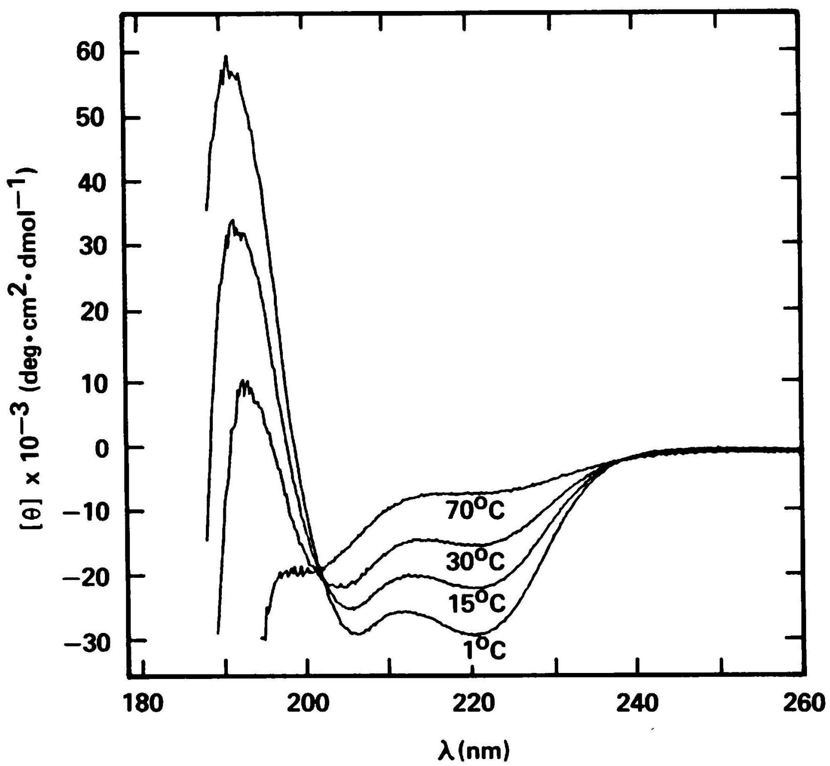
Far UV region: Absorption dominated by amide groups. Therefore, far-UV CD provides insight on backbone conformation, or 2º structure.



cryst.bbk.ac.uk

Autonomously Folding α-Helices Detection via Circular Dichroism

Ac - Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala - NH_2

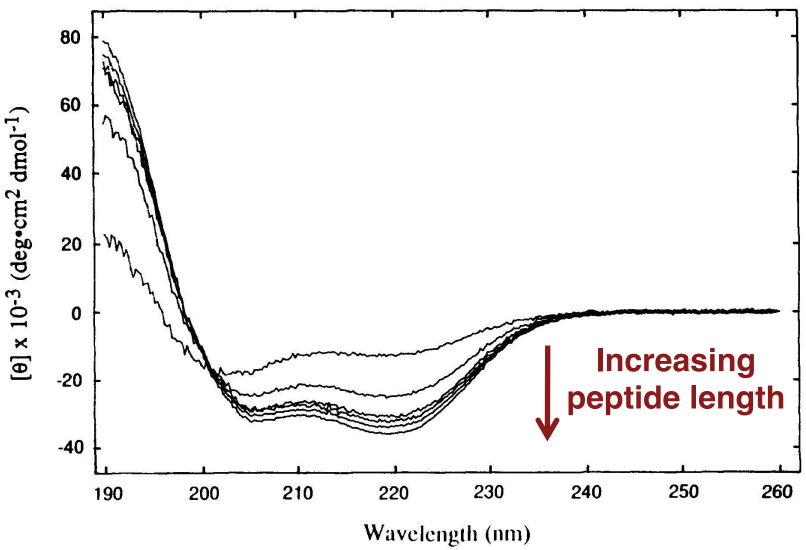


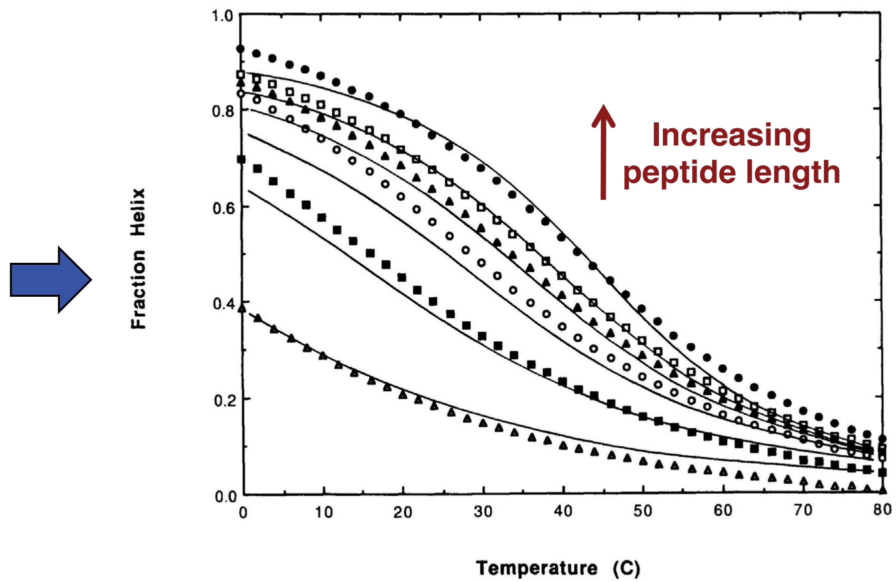
Thermally induced α-helix unfolding

Marqusee, Baldwin *Proc. Natl. Acad. Sci. USA* 84:8898 (1987)

Autonomously Folding α-Helices
Detection via Circular Dichroism

Ac - Tyr (Ala Glu Ala Ala Lys Ala)$_n$ Phe - NH_2



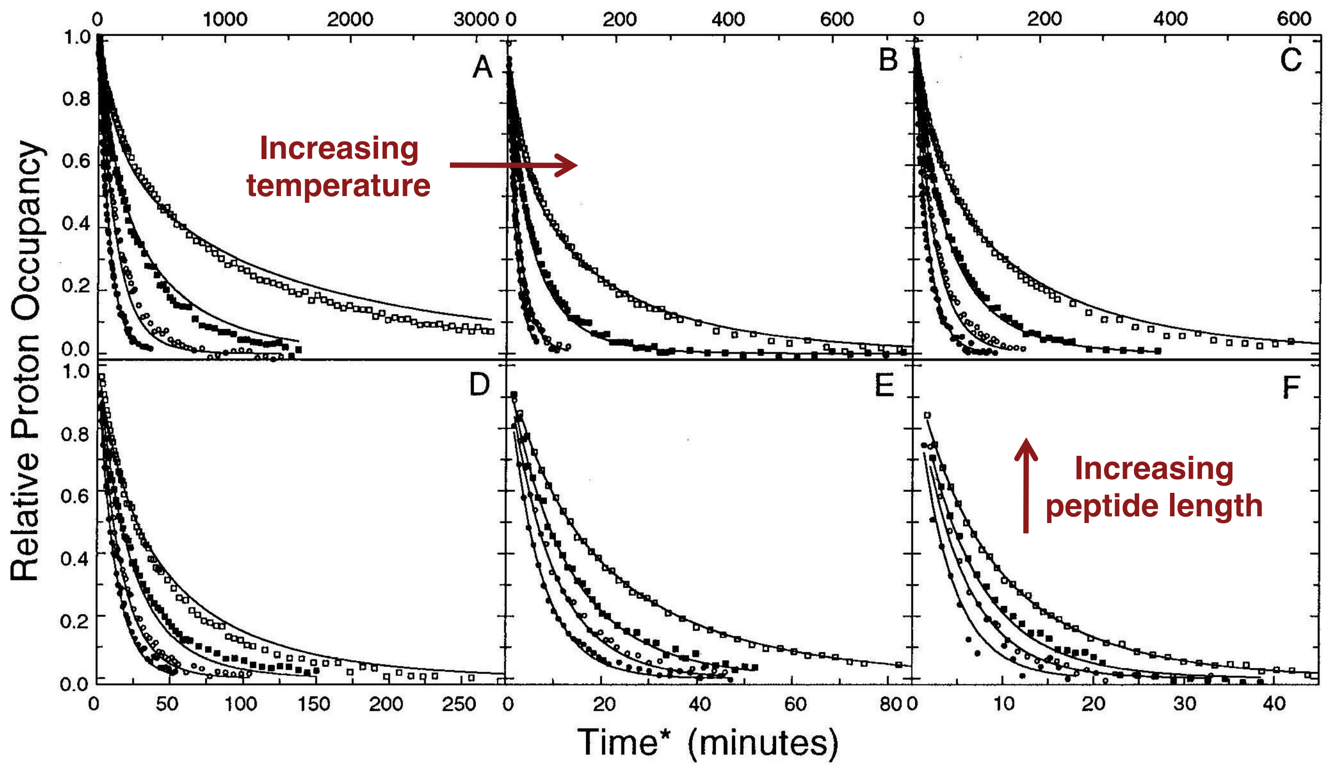


Scholtz, Qian, York, Stewart, Baldwin *Biopolymers* 31:1463 (1991)

Autonomously Folding α-Helices
Detection via Amide NH/ND Exchange

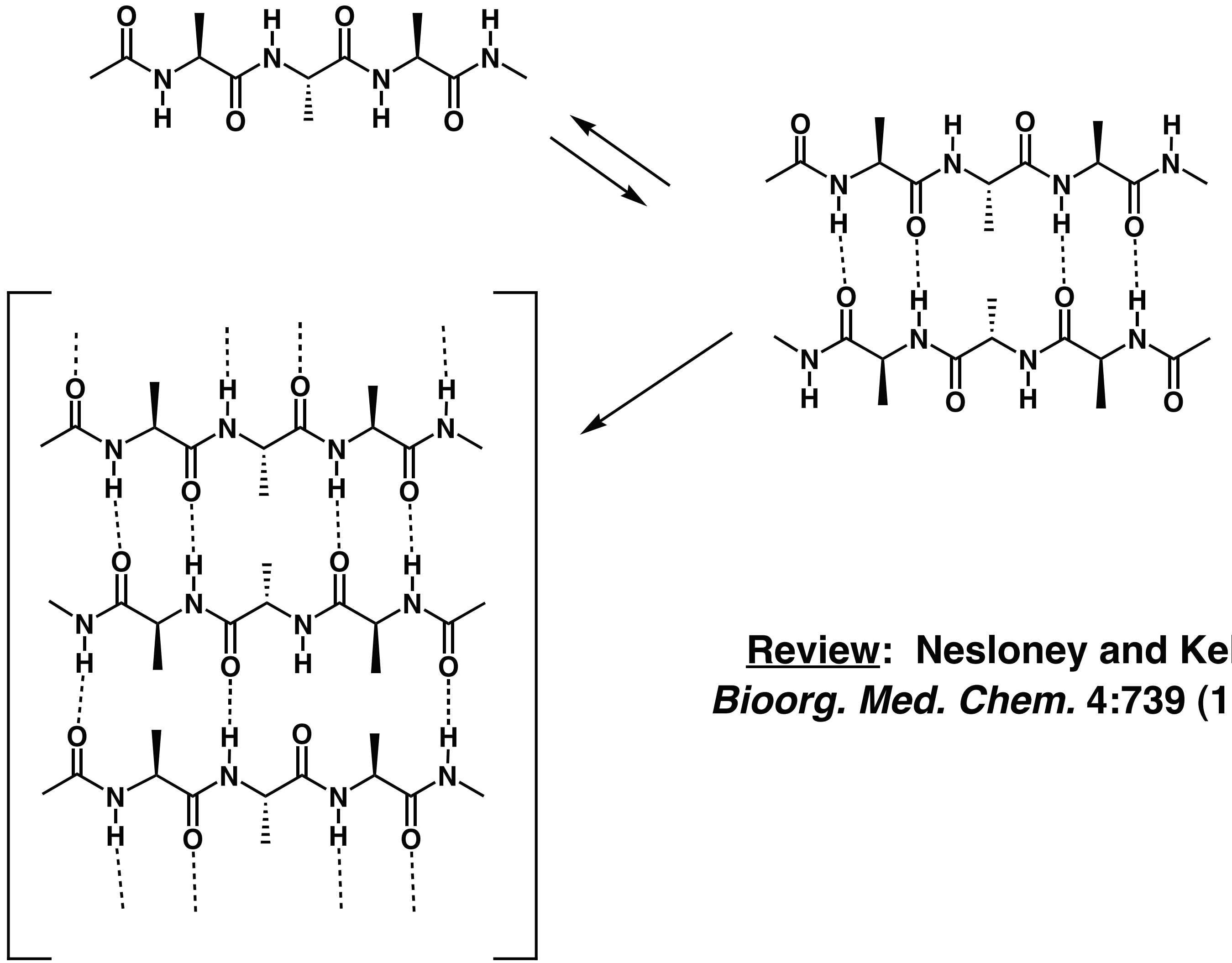
Intrahelical H-bonding inhibits NH/ND exchange.

Ac - (Ala Ala Lys Ala Ala)$_n$ Gly Tyr - NH$_2$



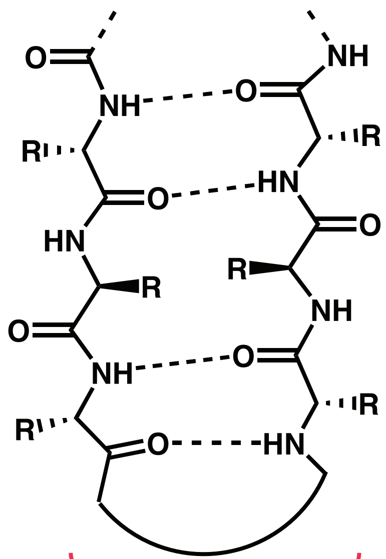
Rohl, Baldwin *Biochemistry* 36:8435 (1997)

β-Sheet Models: The Aggregation Problem

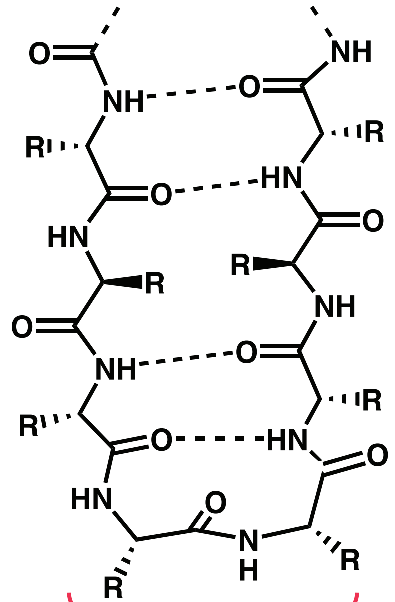


<u>Review</u>: Nesloney and Kelly
Bioorg. Med. Chem. **4:739 (1996)**

β-Hairpins: Prototype for Minimum-Sized Autonomously Folding β-Sheets



Peptide loop



Most common small loop
= β-turn (two residues)

➡ How do we specify the size and position of the loop in an autonomously foldling β-hairpin? (Two residues ideal.)

Importance of Type I' and Type II' β-turns:
Wilmot & Thornton *J. Mol. Biol.* **1988**, *203*, 221.

Design Rules forAutonomously Folding β-Hairpins (Minimal Antiparallel β-Sheets)

Blanco, Jiménez, Herranz, Rico, Santoro, Nieto *J. Am. Chem. Soc.* 115:5887 (1993)

First evidence of an autonomously folded β-hairpin in water (9 residues)

Tyr-Gln-Asn-Pro-Asp-Gly-Ser-Gln-Ala

Blanco, Rivas, Serrano *Nat. Struct. Biol.* 1:584 (1994)

First example of a β-hairpin found in a protein that folds autonomously in water (16 residues)

Gly-Glu-Trp-Thr-Tyr-Asp-Asp-Ala-Thr-Lys-Thr-Phe-Thr-Val-Thr-Glu

1995-2000: Establishment of design rules for β-hairpins that fold in water

Serrano *Adv. Prot. Chem.* 53:49 (2000)

Searle *J. Chem. Soc. Perk. Trans. 2* 1011 (2001)

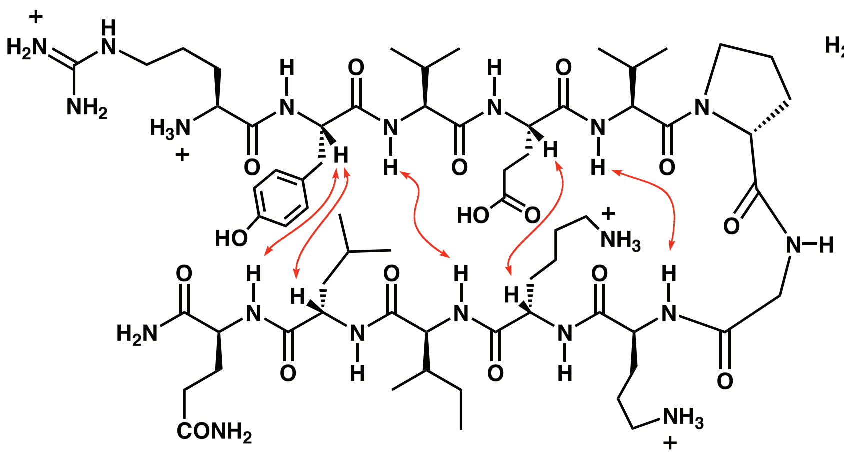
Santiveri, Santoro, Rico, Jiménez *J. Am. Chem. Soc.* 124:14903 (2002)

Gellman *Curr. Opin. Chem. Biol.* 2:717 (1998)

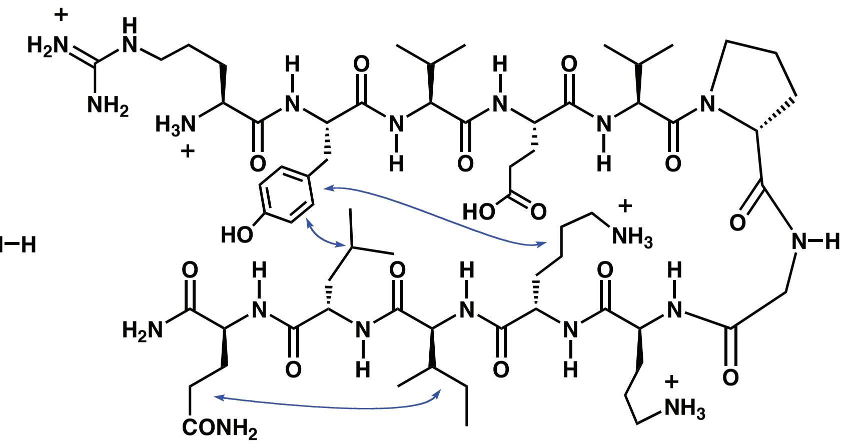
β-Hairpin Folding in Water -- Example

Arg-Tyr-Val-Glu-Val-DPro-Gly-Orn-Lys-Ile-Leu-Gln-NH_2

(ROESY, 9:1 H_2O:D_2O, pH 3.8, 4°C)



Backbone NOEs

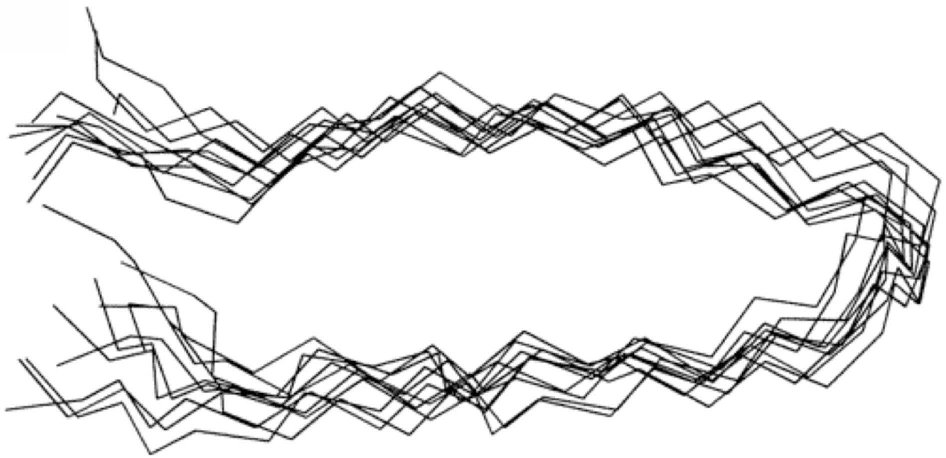


Sidechain NOEs

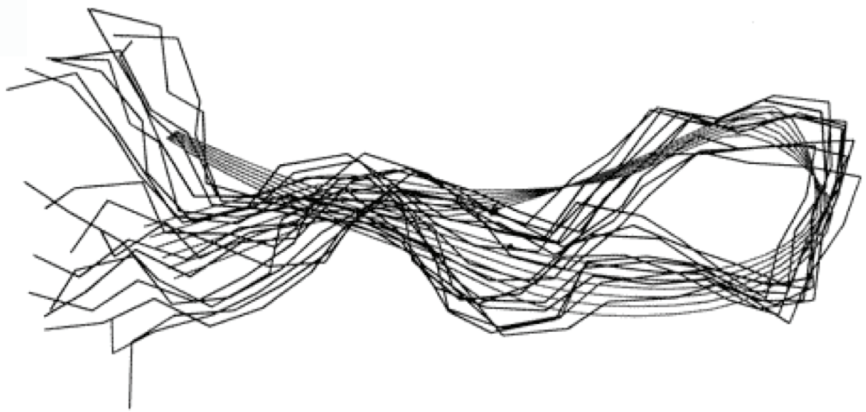
Stanger, Gellman *J. Am. Chem. Soc.* **1998**, *120*, 4236.
Syud, Stanger, Gellman *J. Am. Chem. Soc.* **2001**, *123*, 8667.

Haque et al., *J. Am. Chem. Soc.* **1994**, *116*, 4105; **1996**, *118*, 6975.
(Related: Balaram et al. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 137.)

NOE-Restrained Dynamics (DYANA)



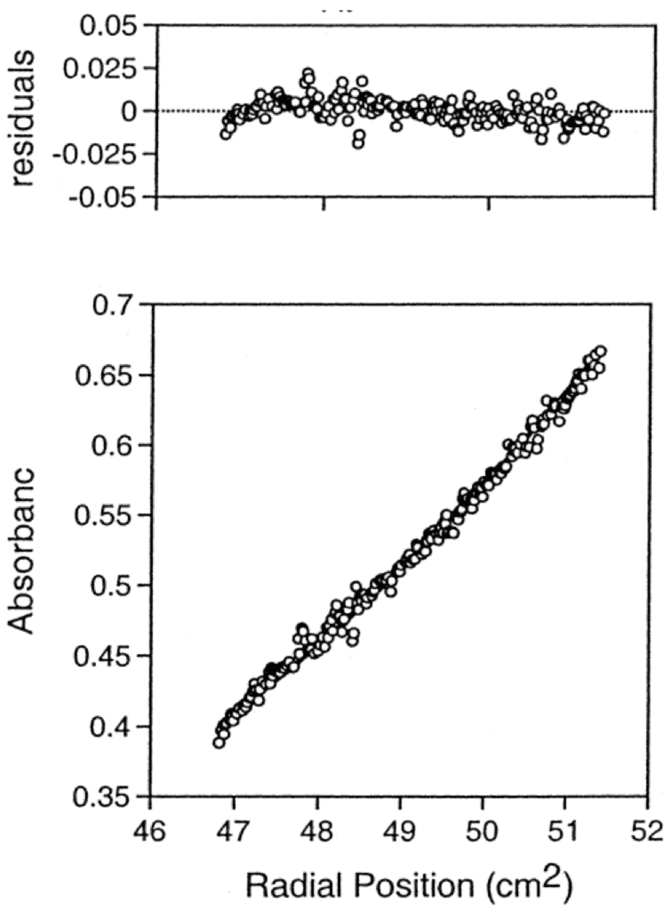
Top

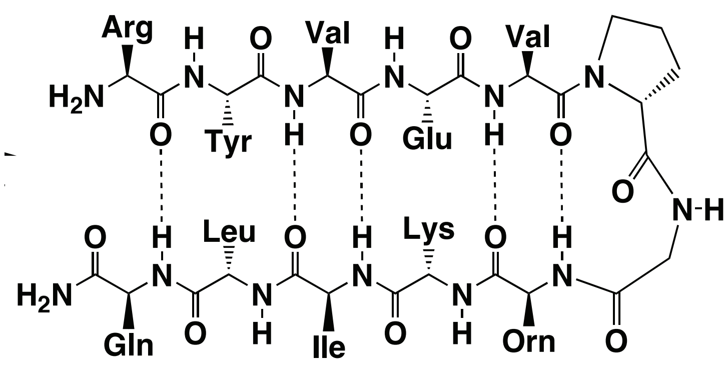


Side

Arg-Tyr-Val-Glu-Val-DPro-Gly-Orn-Lys-Ile-Leu-Gln-NH_2

Analytical Ultracentrifugation: Control for Aggregation





2 mM peptide, 100 mM
acetate buffer, pH 3.8, 4°C

Theoretical MW = 1416
Deduced MW = 1260
(Note: charge ≥ +3)

Analytical Ultracentrifugation: Analysis (Single Ideal Species)

$$\frac{d\ln(c)}{dr^2} = \frac{M_P(1-\bar{v}\rho)\omega^2}{2RT}$$

c = concentration of protein (mg/mL)

r = radial distance from center of rotation (cm^2)

M_p = mass of the anhydrous polypeptide

$\bar{v}$ = partial specific volume (mL/mg) *

ρ = solvent density (mg/mL)

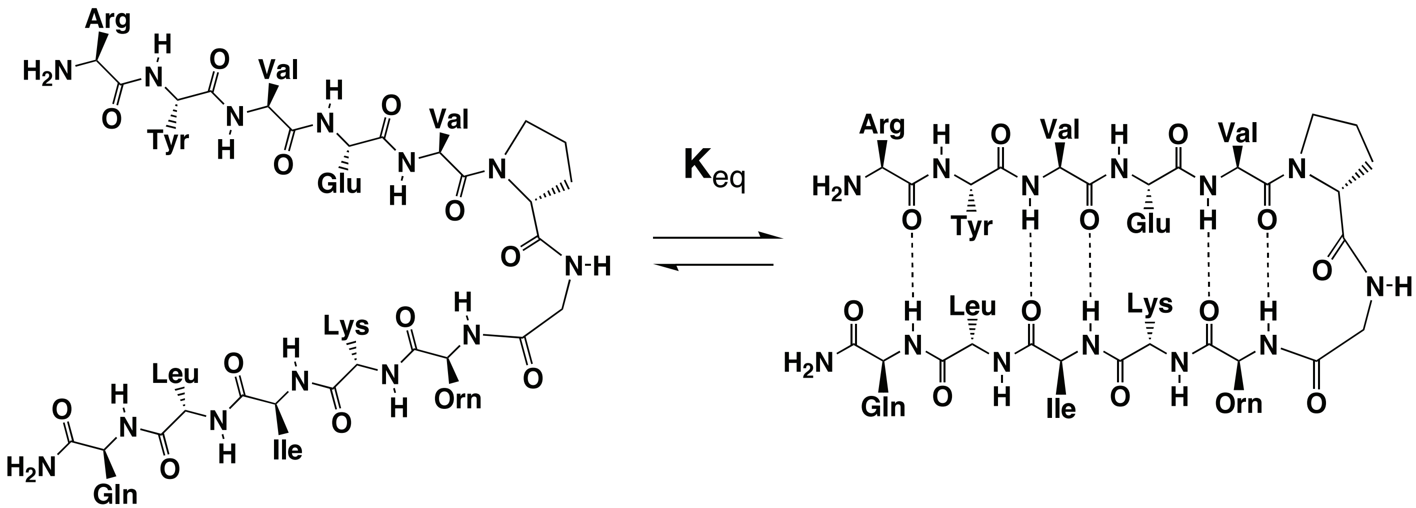
ω = radial velocity (revolutions/min)

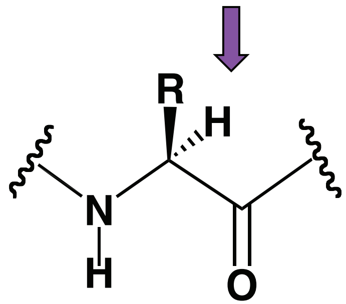
R = gas constant (erg/K/mol)

T = temperature (K)

* COMMENT: The partial specific volume can be measured, but is nearly always calculated using amino acid composition.

How to quantify the folding equilibrium?

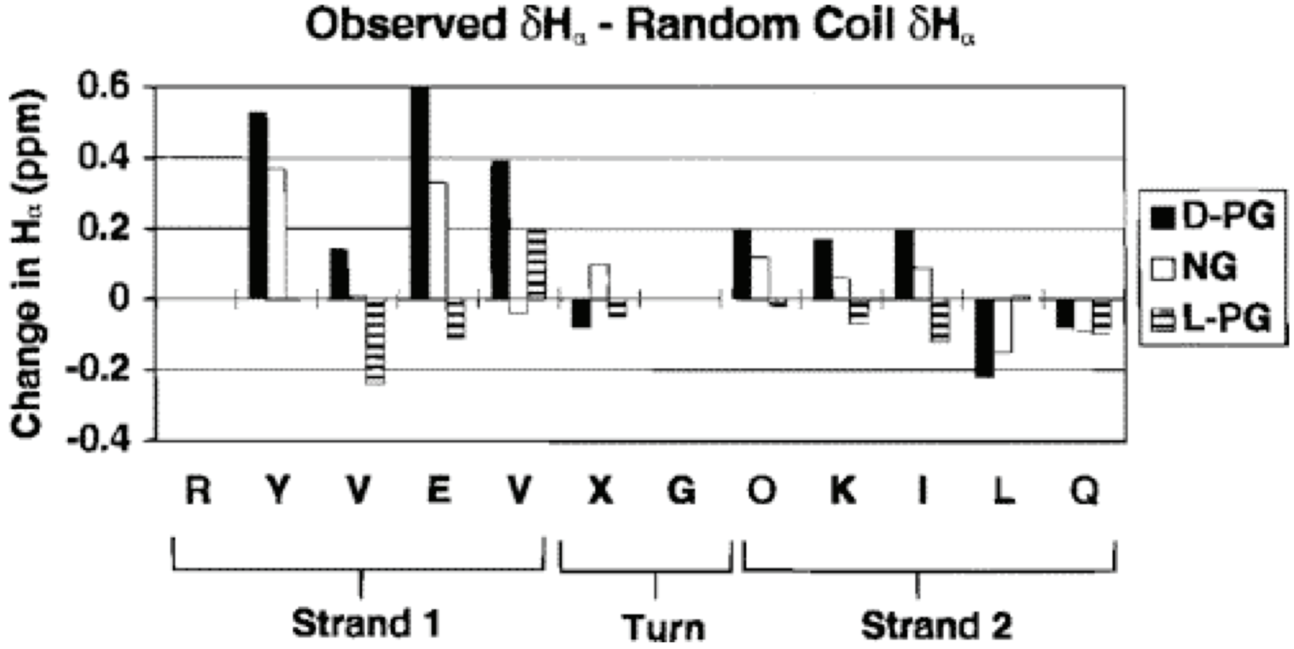




α-H chemical shifts are very sensitive to conformation:

Wishart et al.
Biochemistry **1992**, *31*, 1647.

Secondary Structure Indicated by Hα Chemical Shifts ("Chemical Shift Index")



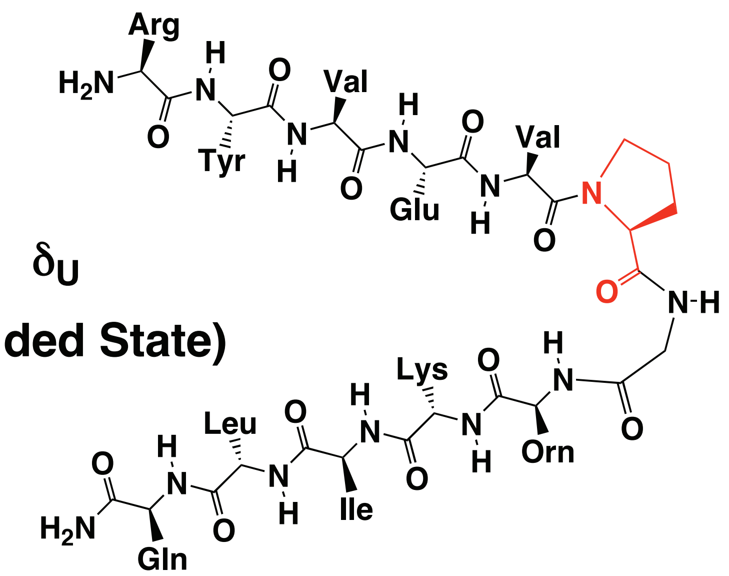
β-Hairpins with NG loops: Ramírez-Alvarado et al. *Nat. Struct. Biol.* 1996, *3*, 604.
de Alba et al. *J. Am. Chem. Soc.* 1997, *119*, 175.
Maynard, Searle *Chem. Commun.* 1997, 1297.

β-Hairpin Population Analysis

Reference Peptides for NMR-based β-Hairpin Population Determination

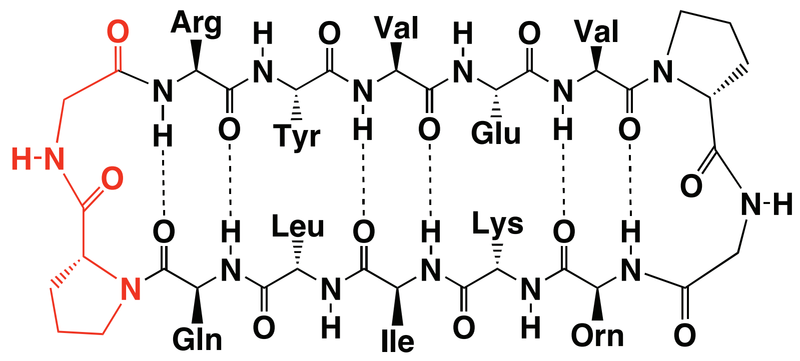
(Rapid equilibration on NMR time scale)

$$\beta\text{-Hairpin \%} = \frac{\delta_{obs} - \delta_U}{\delta_F - \delta_U}$$



δ_U

(Unfolded State)

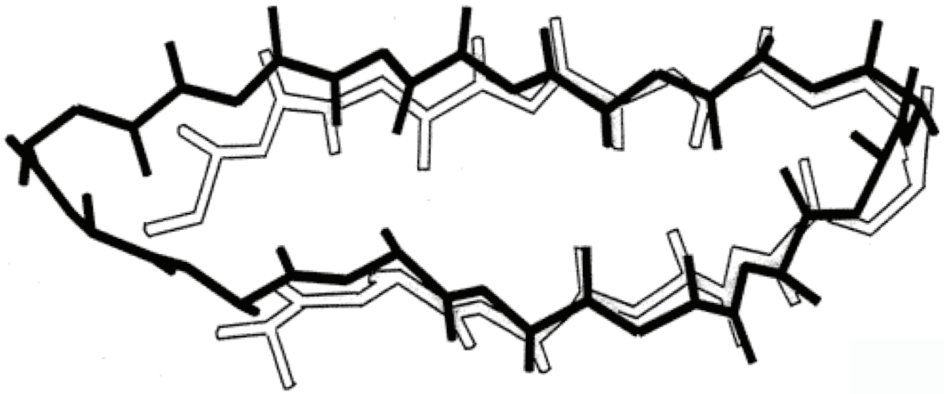


δ_F

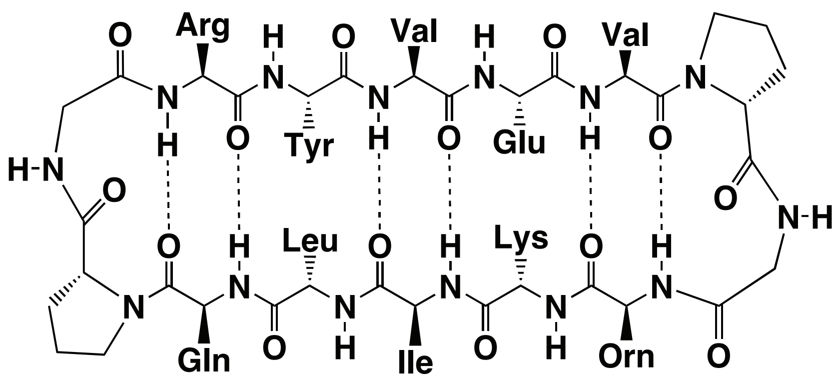
(Folded State)

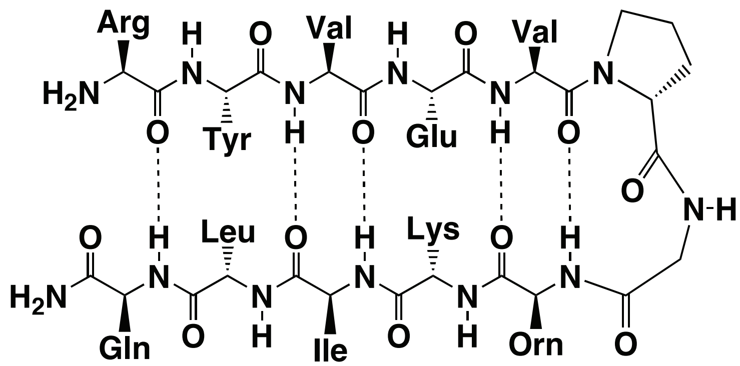
Syud, Espinosa, Gellman *J. Am. Chem. Soc.* **1999**, *121*, 11577.

Cyclic Peptide as Folded Reference



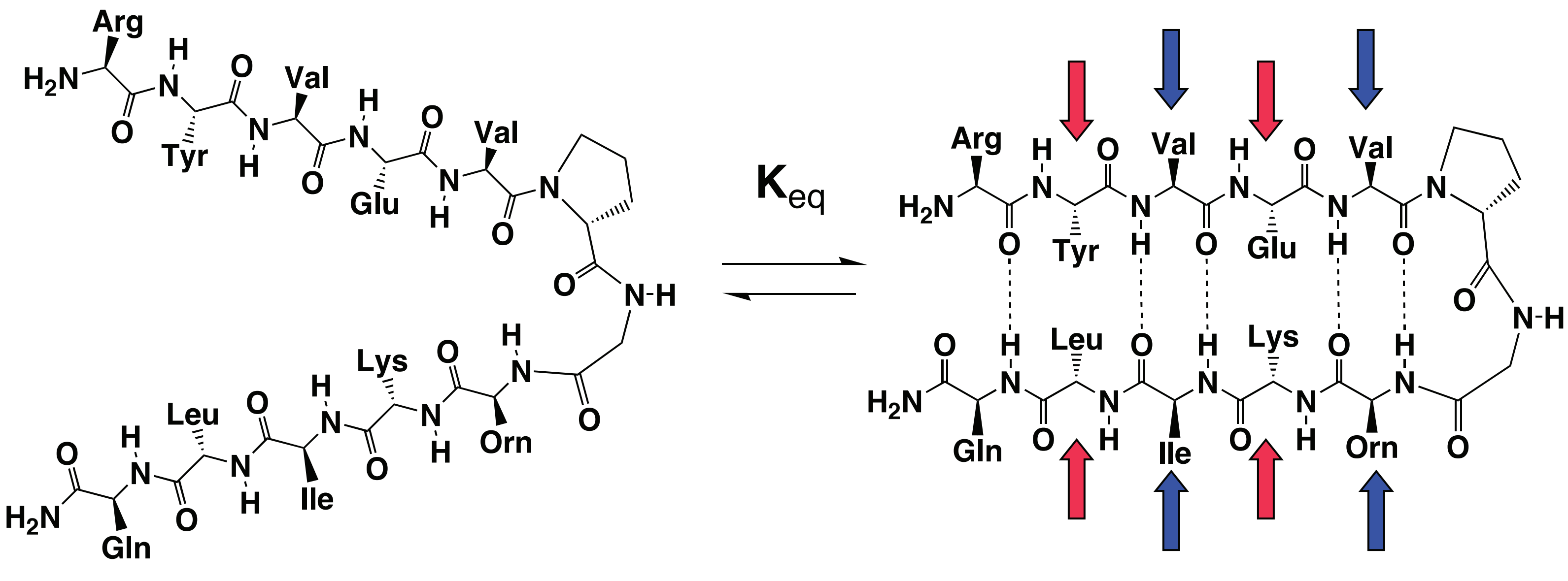
NOE-restrained dynamics-derived structure overlay





Syud, Stanger, Gellman *J. Am. Chem. Soc.* **2001**, *123*, 8667.

Residue Choice for Population Analysis?

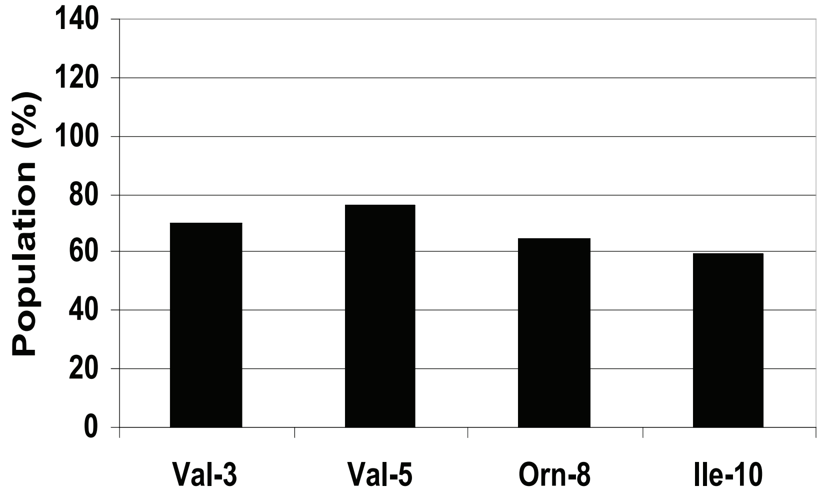


Non-Hydrogen Bonded Residues

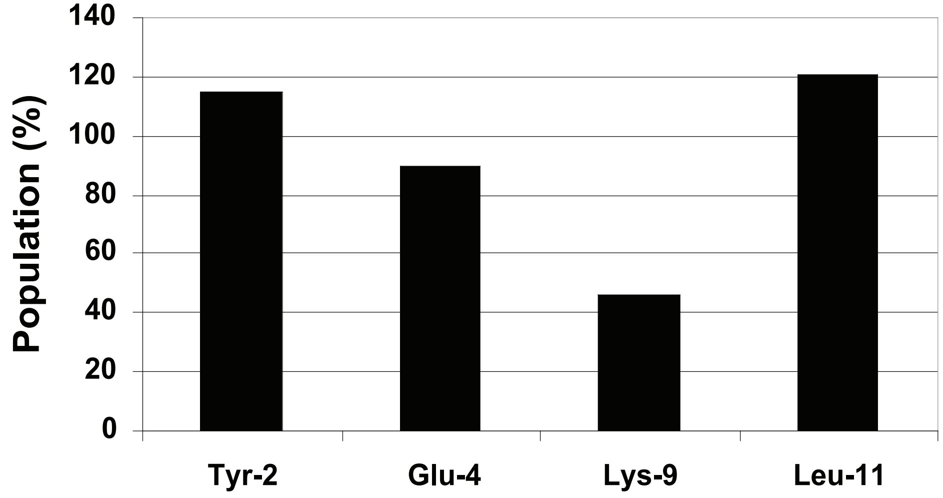
Hydrogen Bonded Residues

Population Analysis at Strand Residues

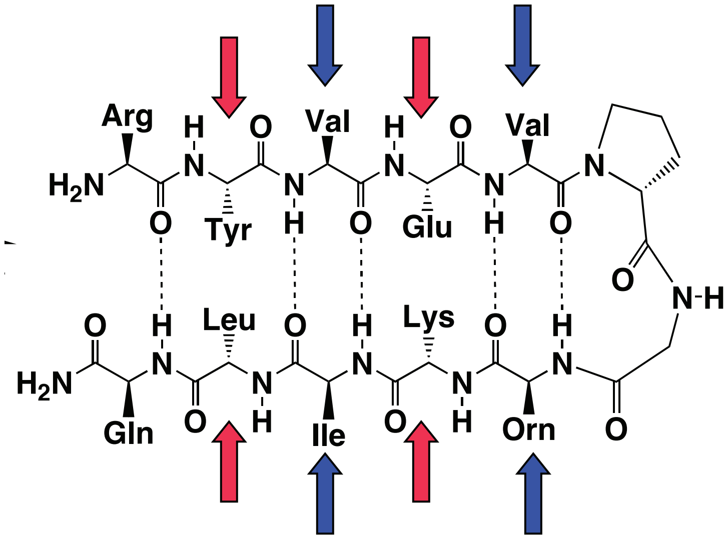
H-Bonded Residues



Non-H-Bonded Residues



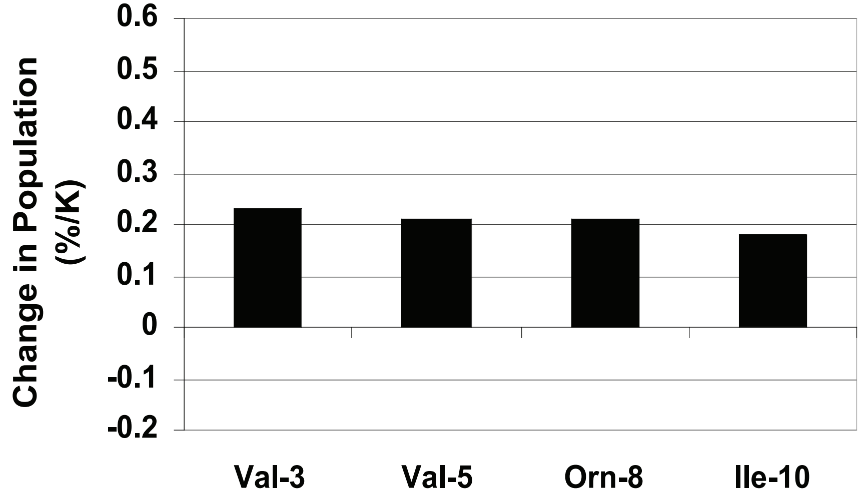
68 ± 7%

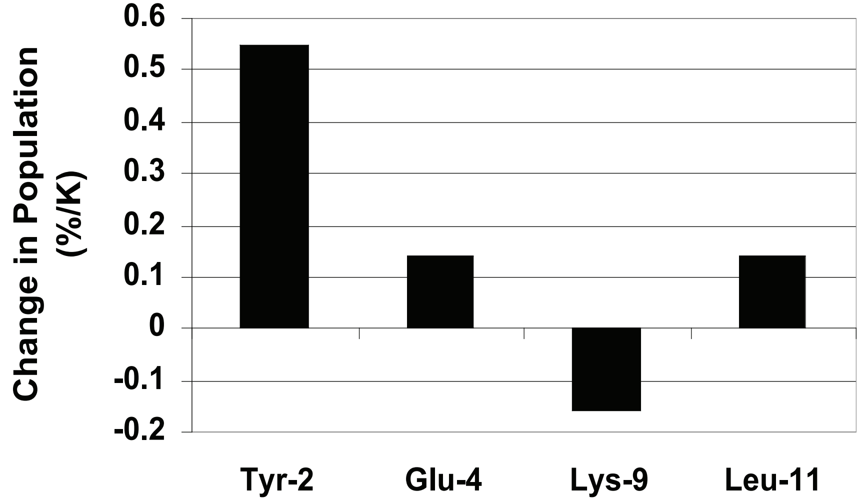


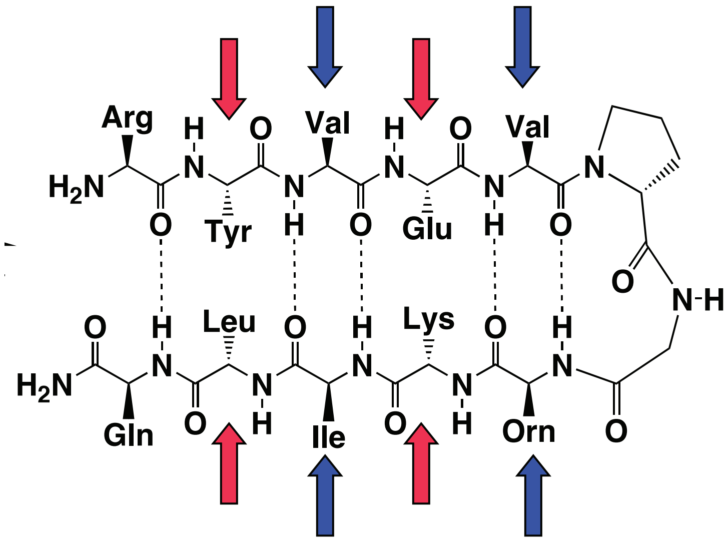
93 ± 34%

2 mM peptide,
100 mM acetate
buffer, pH 3.8, 4°C

Variable Temperature Studies: Evidence for Two-State Behavior





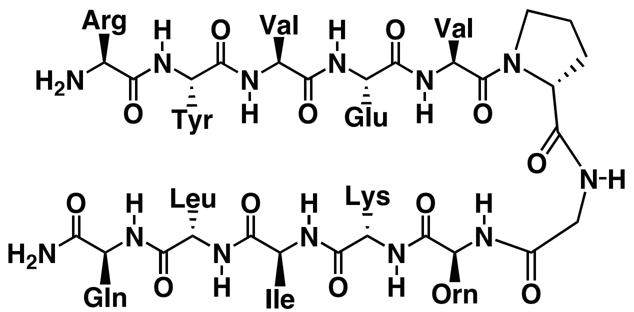


[Aromatic side chain effect?]

D-Pro-Gly vs. a Proteinogenic Sequence

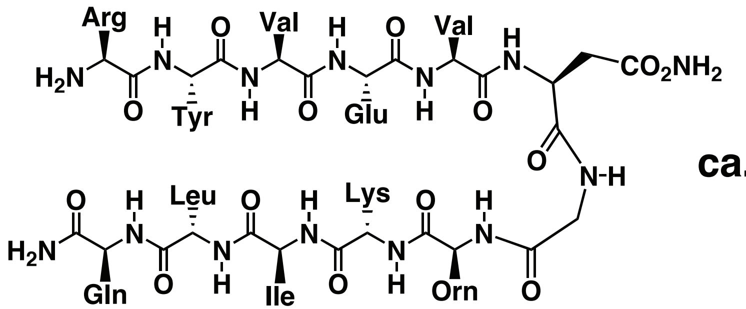
β-Hairpin Populations in Aqueous Solution (4°C)

D-Pro-Gly loop



ca. 68% β-Hairpin

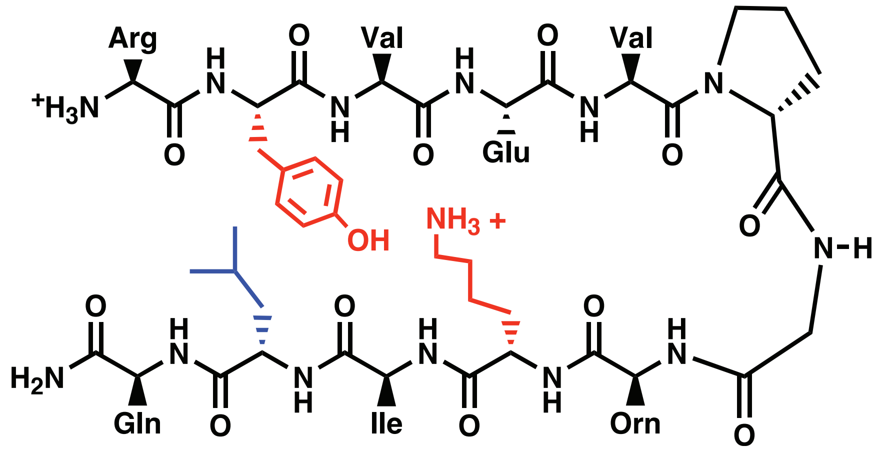
L-Asn-Gly loop



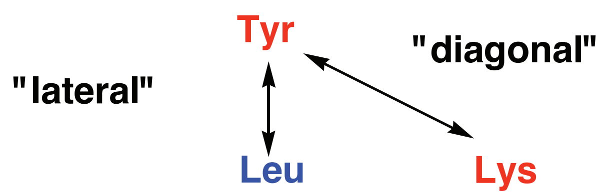
ca. 42% β-Hairpin

β-Hairpins with NG loops: **Ramírez-Alvarado et al. *Nat. Struct. Biol.* 1996, *3*, 604.**
de Alba et al. *J. Am. Chem. Soc.* 1997, *119*, 175.
Maynard, Searle *Chem. Commun.* 1997, 1297.

Using β-Hairpins to Examine Factors that Control β-Sheet Stability: Lateral vs. Diagonal Sidechain contacts



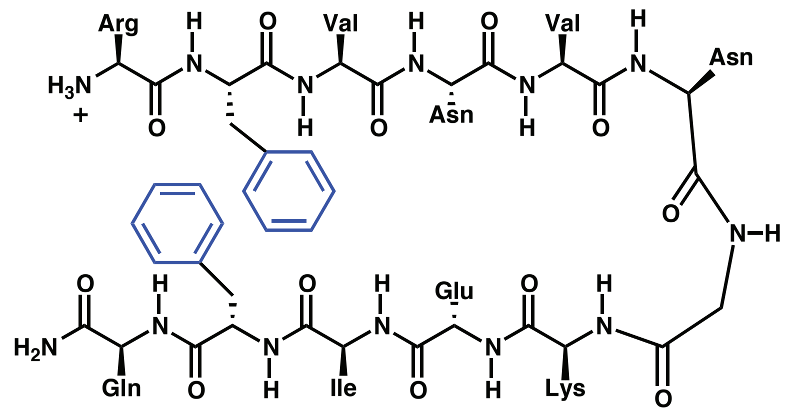
<u>Key interstand interactions?</u>

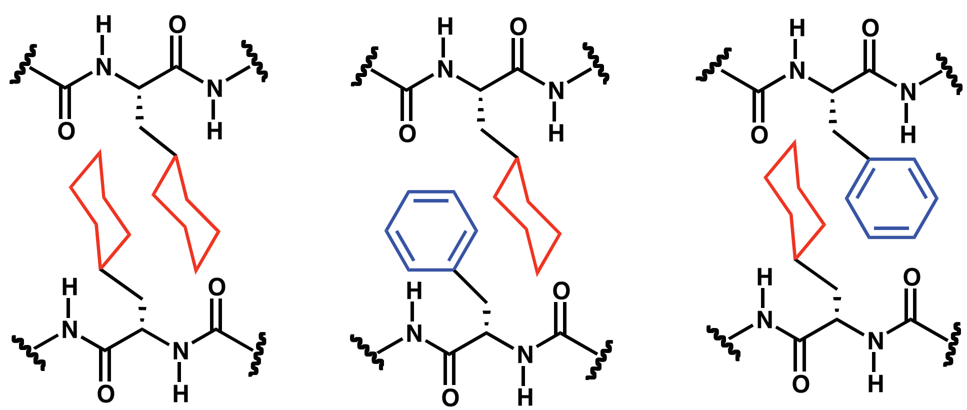


Syud, Stanger, Gellman *J. Am. Chem. Soc.* **2001**, *123*, 8667.

Statistical Survey: Cootes et al. *Proteins: Struct. Funct. Genet.* **1998**, *32*, 175.

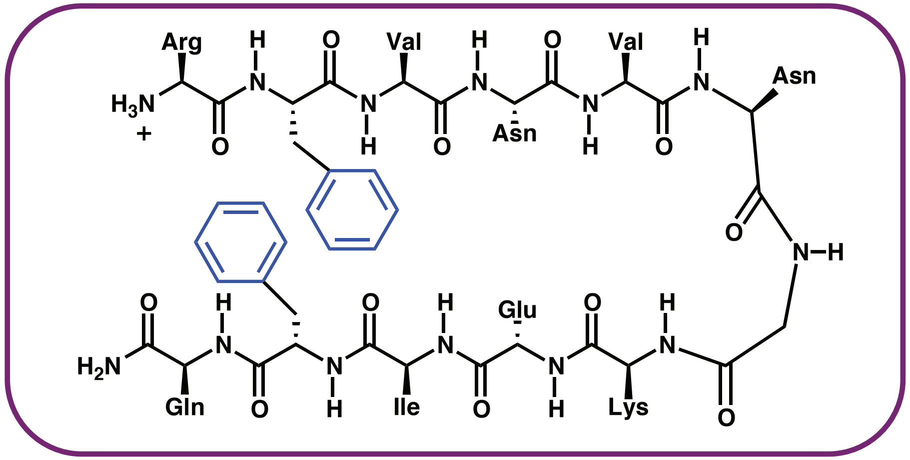
Using β-Hairpin as a Platform to Explore Non-Covalent Interactions in Water



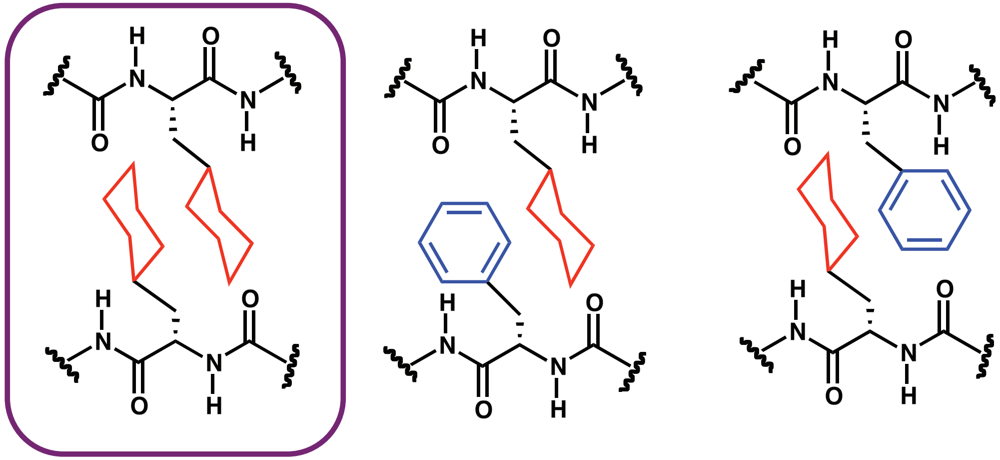


Tatko, Waters *J. Am. Chem. Soc.* 124:9372 (2002)

Using β-Hairpin as a Platform to Explore Non-Covalent Interactions in Water

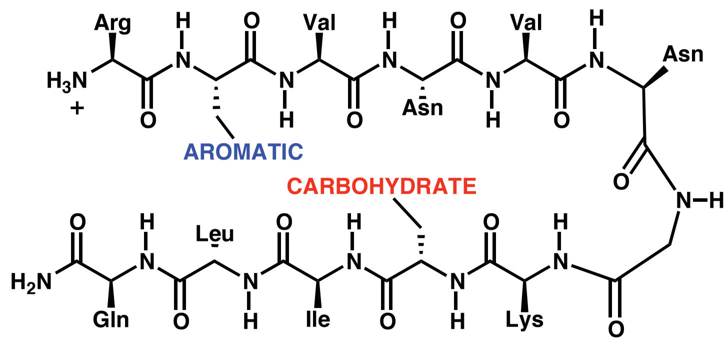


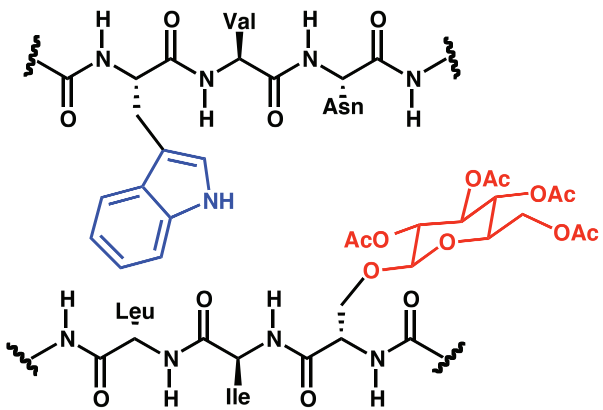
Aromatic-aromatic
or
Aliphatic-aliphatic
is superior to
Aromatic-aliphatic.



Tatko, Waters *J. Am. Chem. Soc.* <u>124</u>:9372 (2002)

Using β-Hairpin as a Platform to Explore Non-Covalent Interactions in Water: π-Carbohydrate

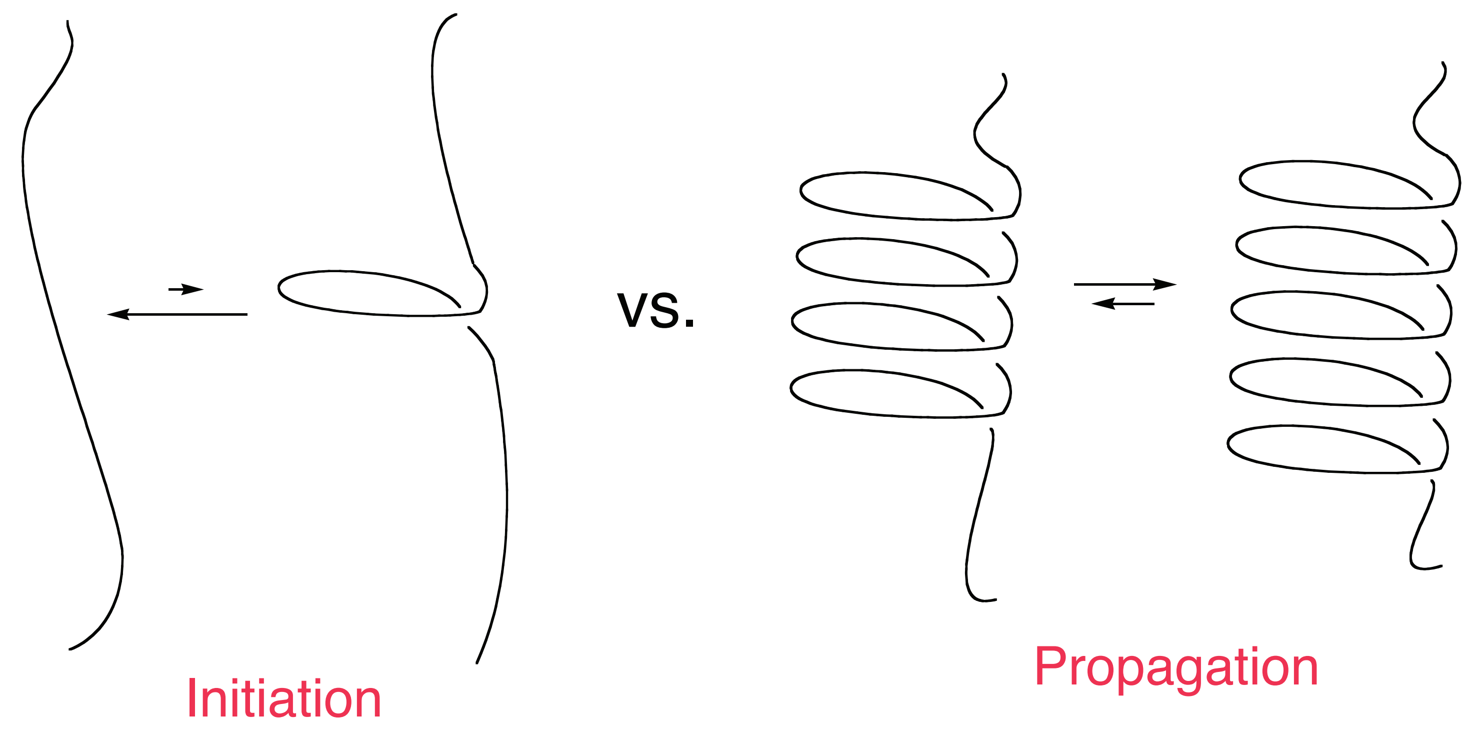




π-carbohydrate interaction stabilizes hairpin conformation by ~0.8 kcal/mol

Laughrey, Kiehna, Riemen, Waters *J. Am. Chem. Soc.* 130:14625 (2008)

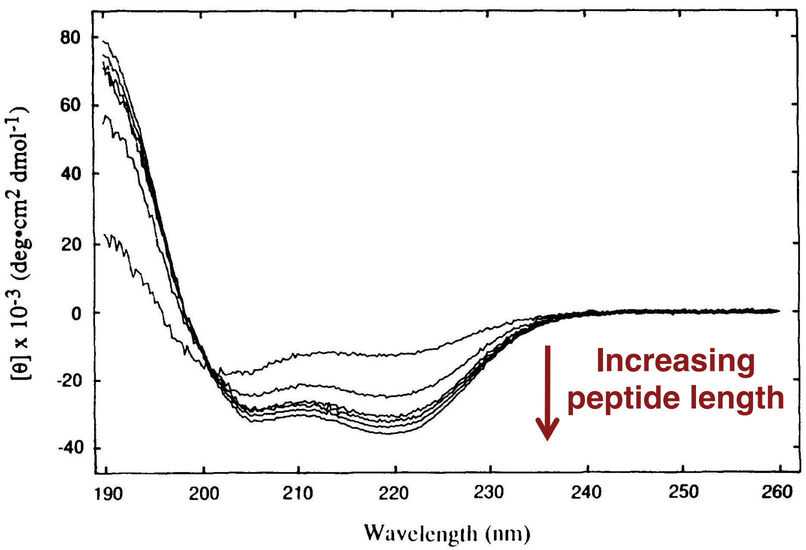
Length-Dependent Cooperativity in the α-Helix

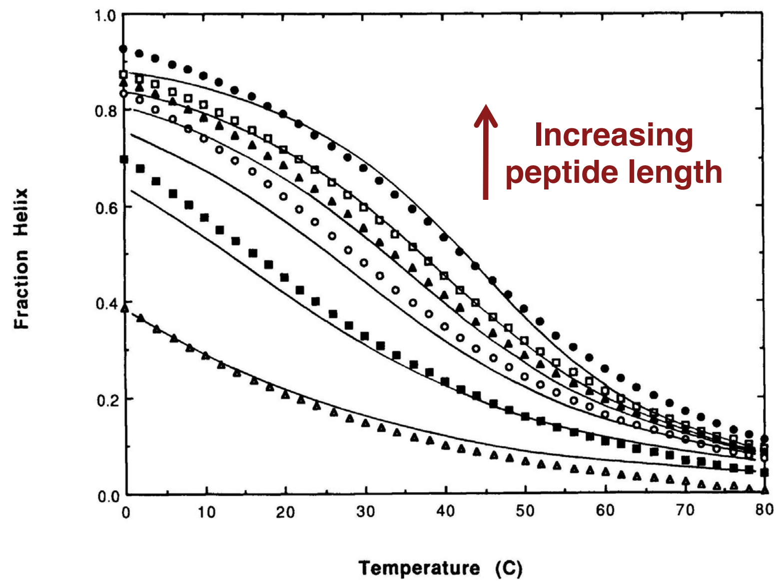


Longer helices are more stable; balance between entropic cost of helix initiation and enthalpic benefit of helix propagation.

Autonomously Folding α-Helices Detection via Circular Dichroism

Ac - Tyr (Ala Glu Ala Ala Lys Ala)$_n$ Phe - NH_2

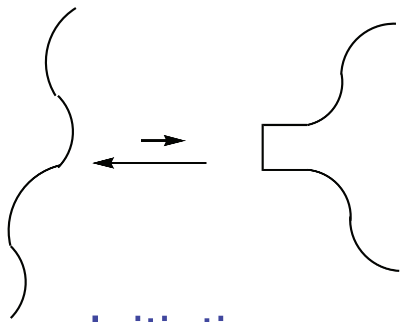




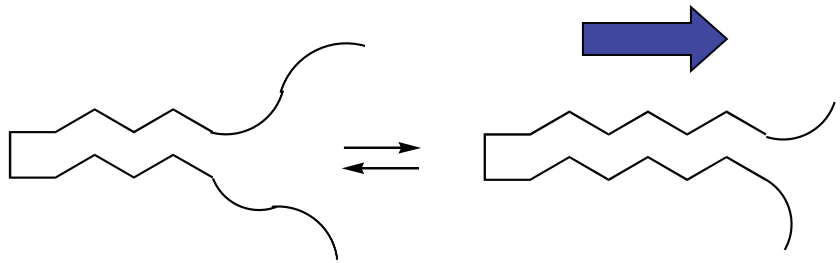
Scholtz, Qian, York, Stewart, Baldwin *Biopolymers* 31:1463 (1991)

Length-Dependent Cooperativity in β-Sheet?

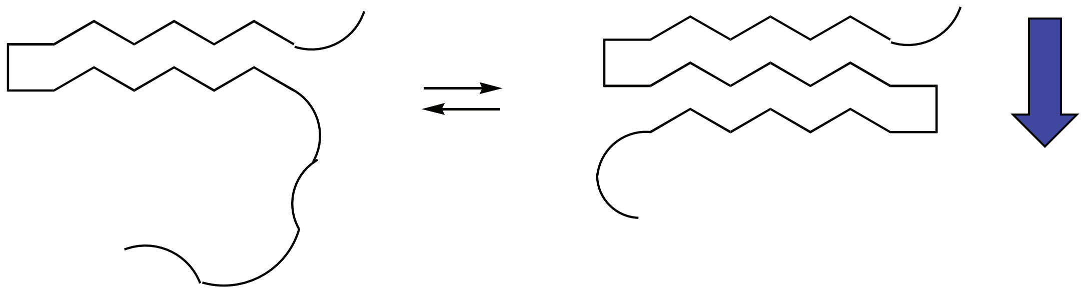
Two dimensions possible



Initiation

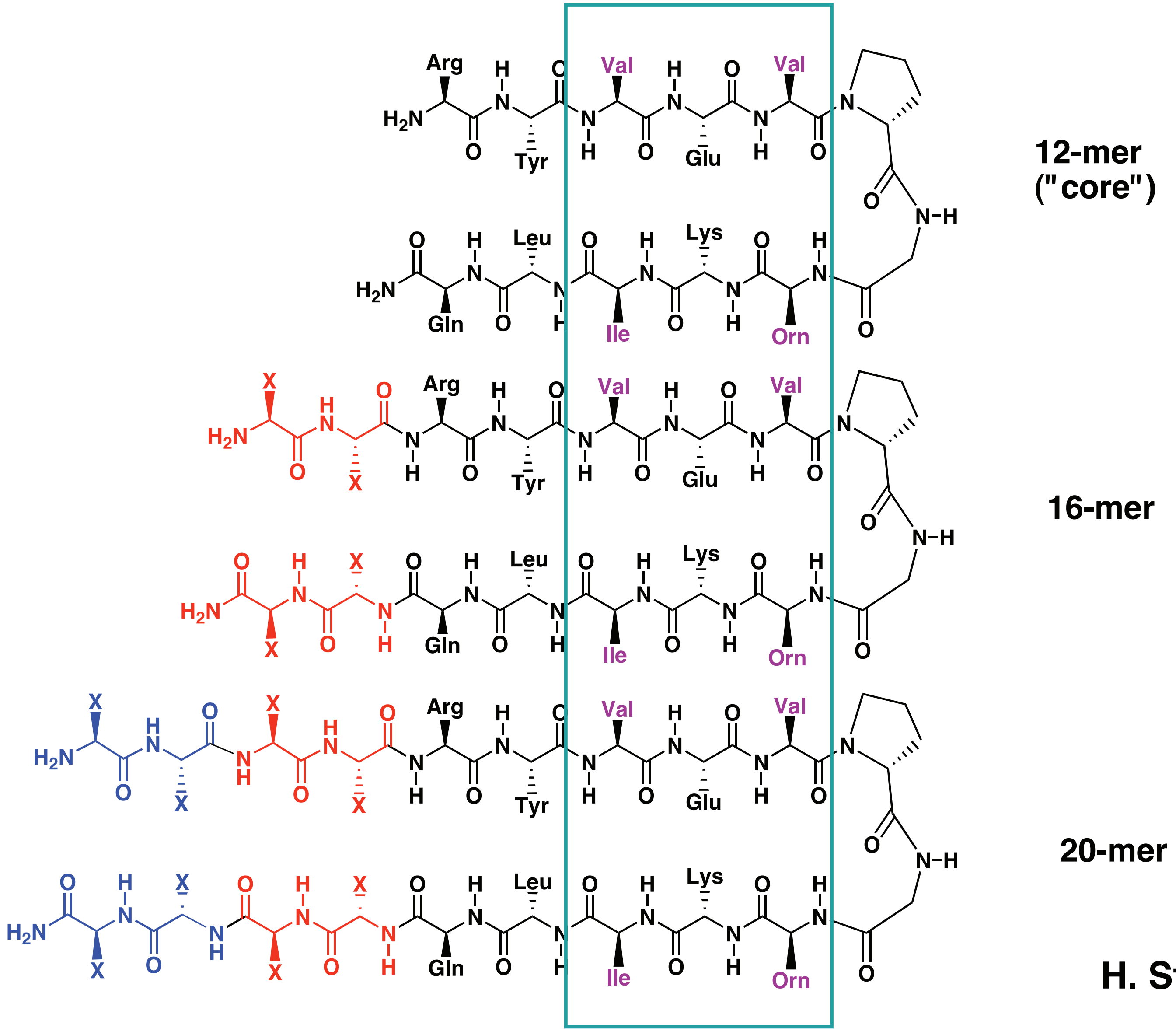


Propagation - along strand



Propagation - perpendicular to strand

EXPERIMENTAL DESIGN: Cooperativity Along the Strand Direction in Antiparallel β-sheet?



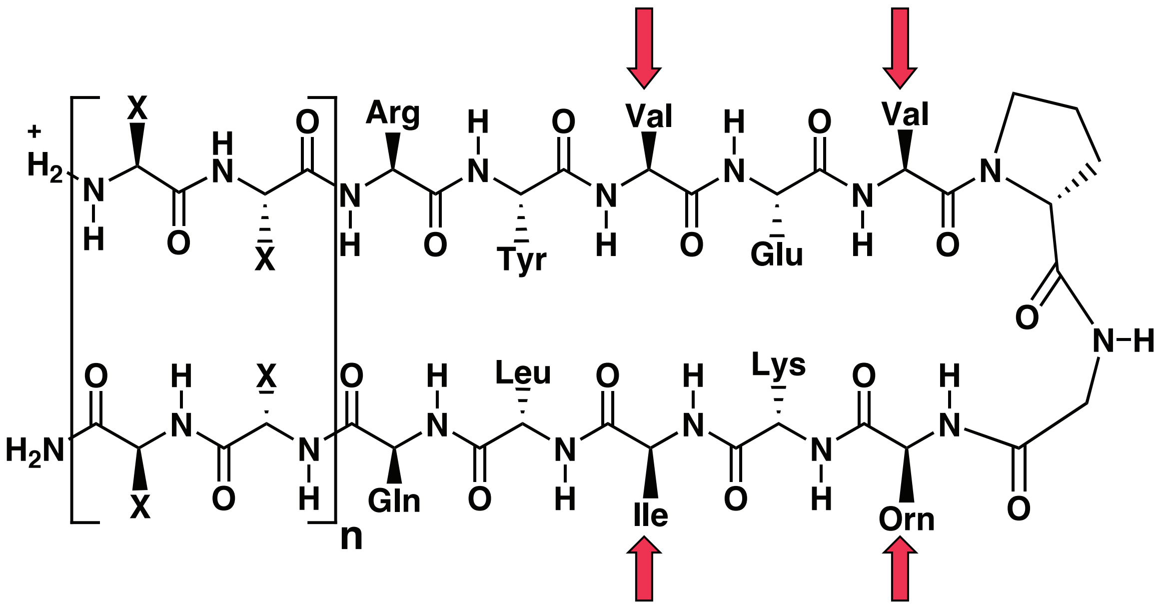
H. Stanger

Quantitative Evaluation of Cooperativity Along the Strand Direction in Antiparallel β-sheet (aqueous solution, 4°C)

12-mer, n = 0

16-mer, n = 1

20-mer, n = 2



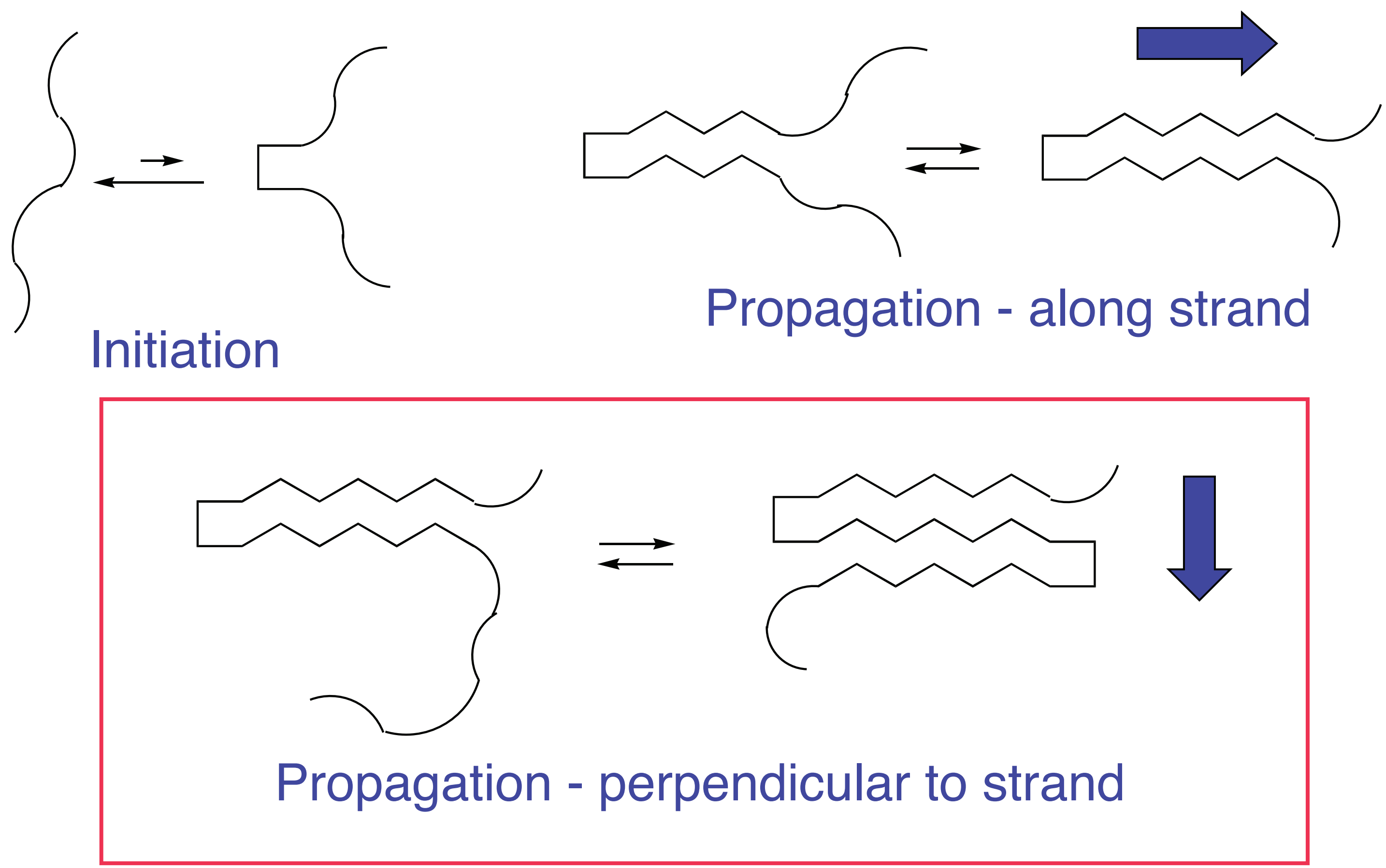
General Trend [ΔΔG (kcal/mol)]:

16mer vs. 12mer → Hairpin stabilization by 0.2 - 0.3 kcal/mol

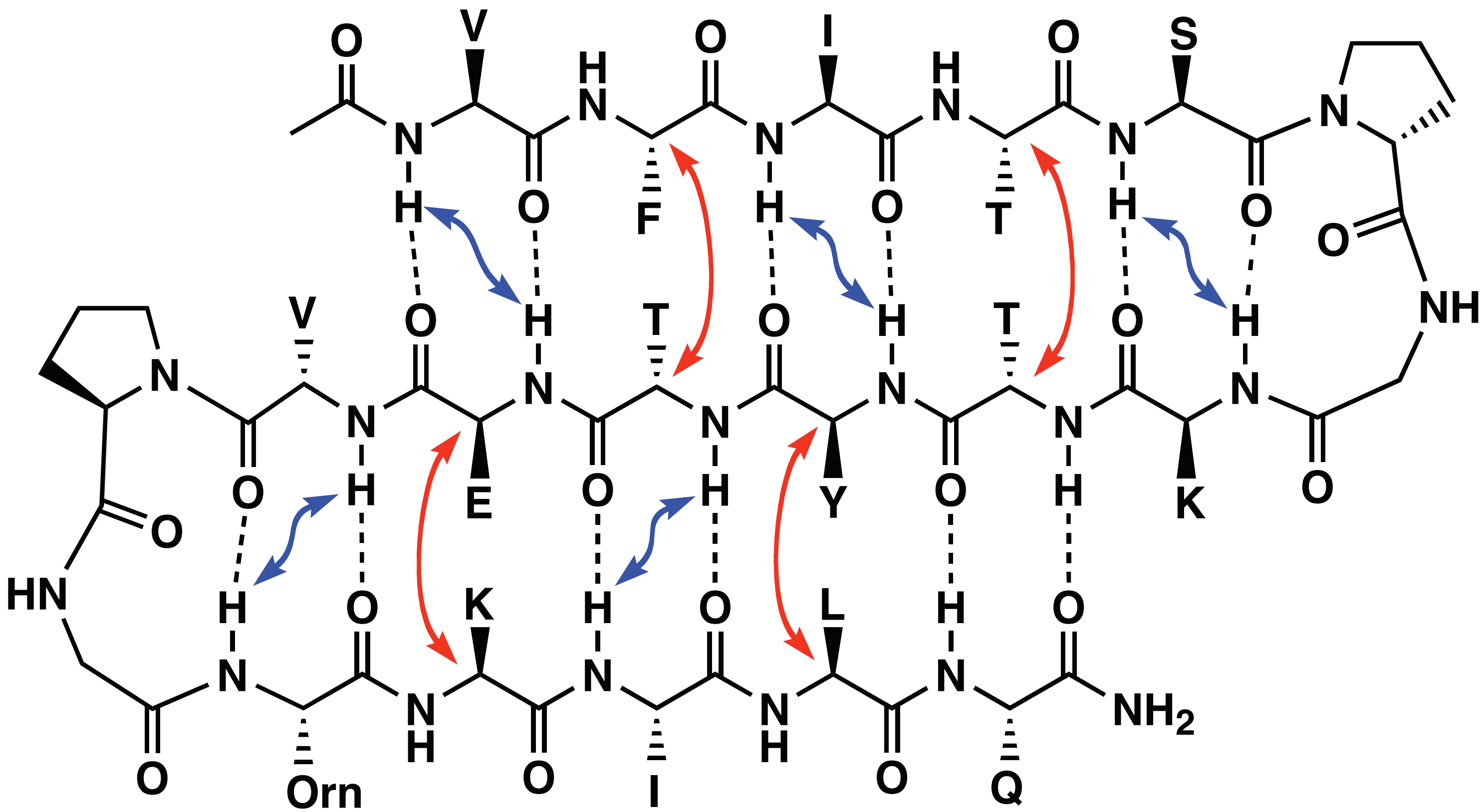
20mer vs. 16mer → No additional hairpin stabilization!

Stanger et al. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12015.

Length-Dependent Cooperativity in β-Sheet?



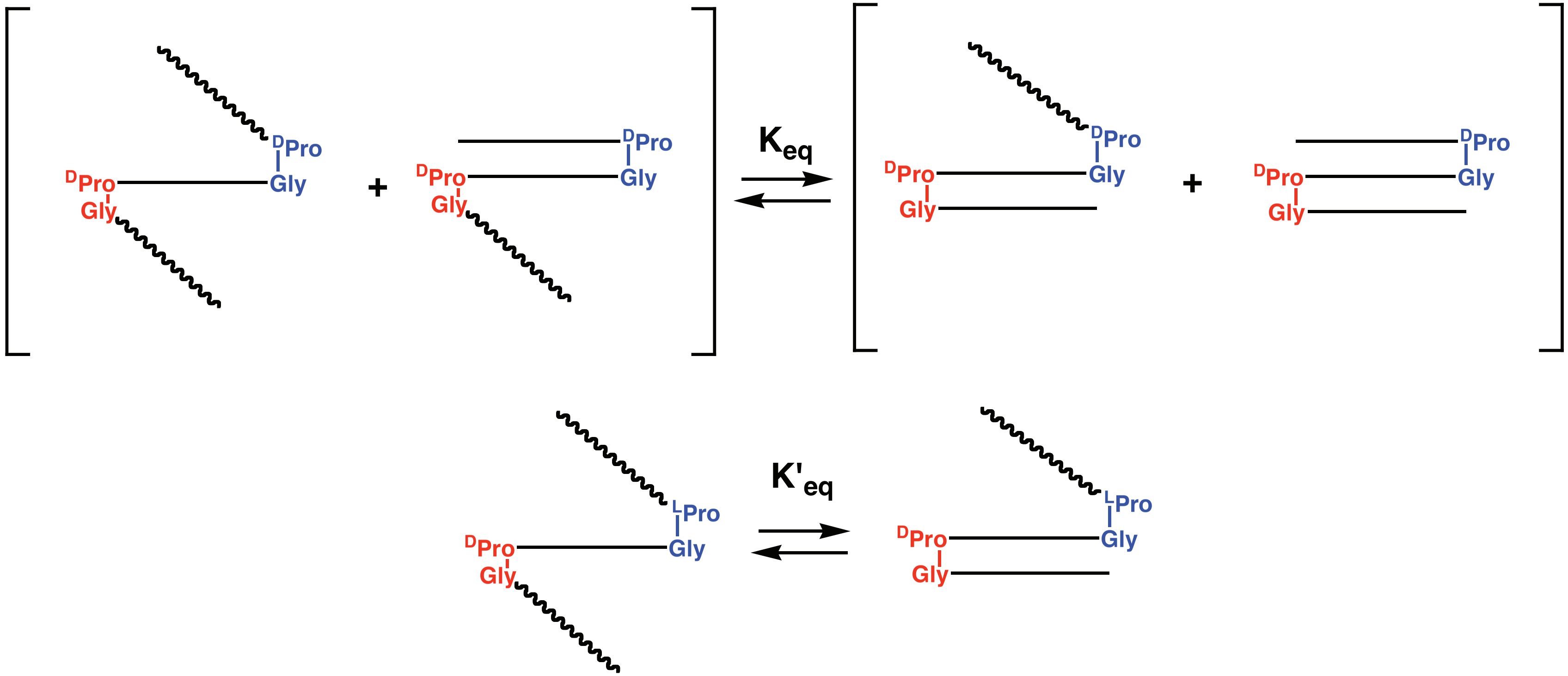
Design of a Triple-Stranded β-Sheet



Backbone NOEs: NH--NH and C_αH--CαH

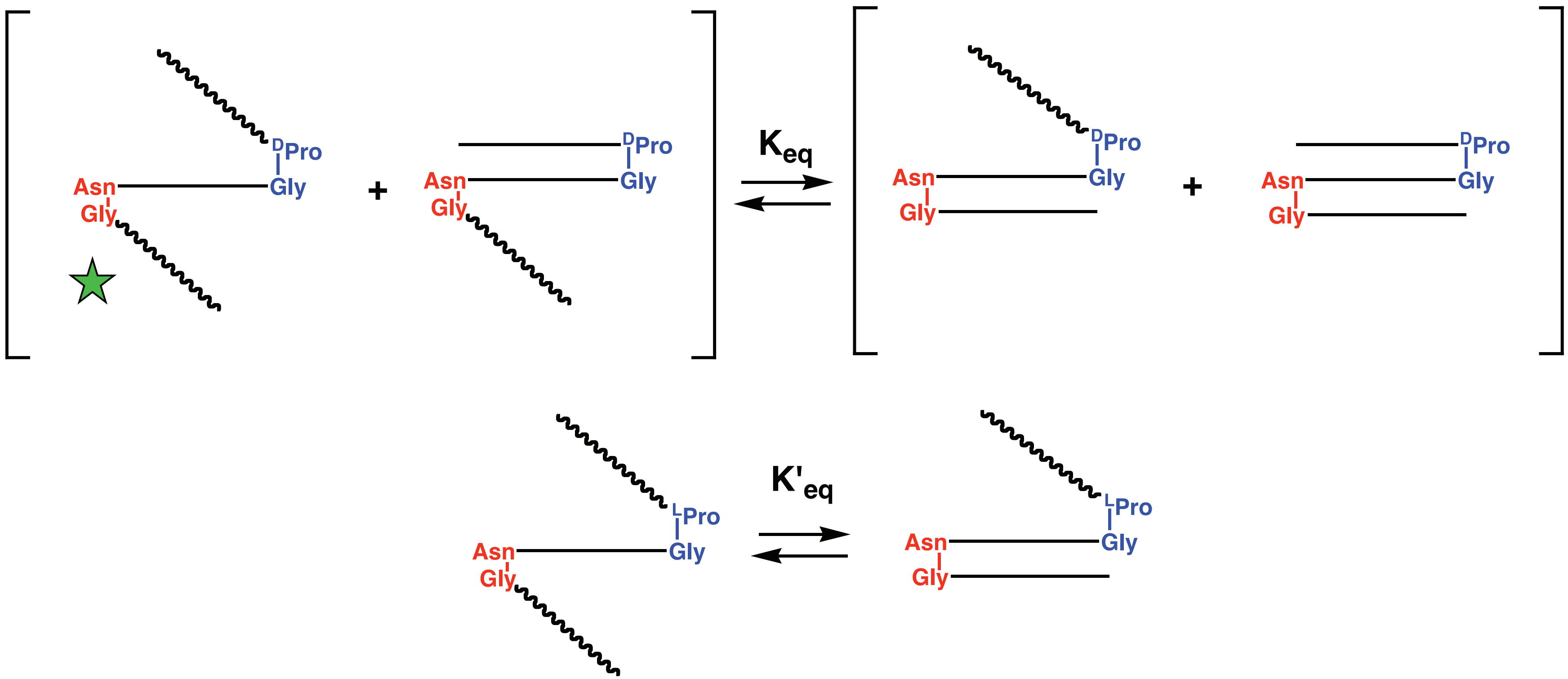
Schenck, Gellman *J. Am. Chem. Soc.* **1998**, *120*, 4869.

Experimental Design: Probing β-Sheet Cooperativity Perpendicular to the Strand Direction



$$\Delta\Delta G = - RT(\ln K_{eq} - \ln K'_{eq})$$

Experimental Design: Probing β-sheet Cooperativity Perpendicular to the Strand Direction - Part 2

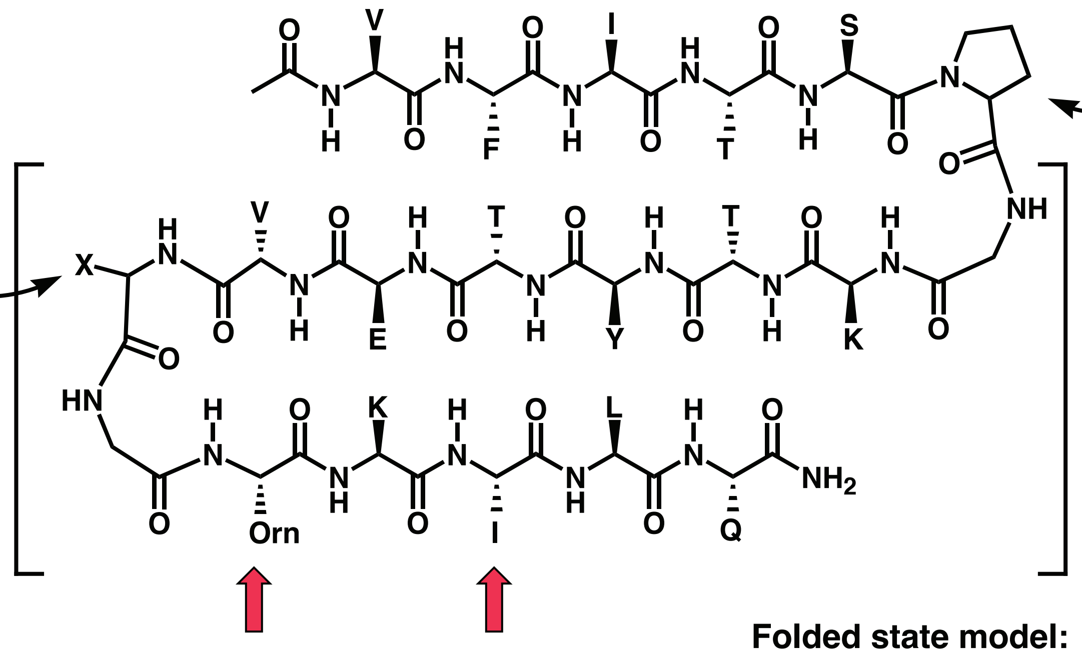


β-Hairpins with NG loops: Ramírez-Alvarado et al. *Nat. Struct. Biol.* **1996**, *3*, 604.
de Alba et al. *J. Am. Chem. Soc.* **1997**, *119*, 175.
Maynard, Searle *Chem. Commun.* **1997**, 1297.

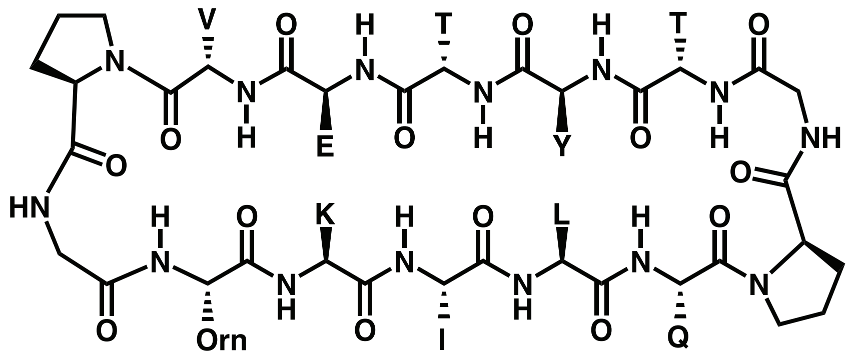
Quantifying β-Sheet Cooperativity Perpendicular to the Strand Direction

D-Pro or L-Asn

D-Pro vs. L-Pro



Folded state model:

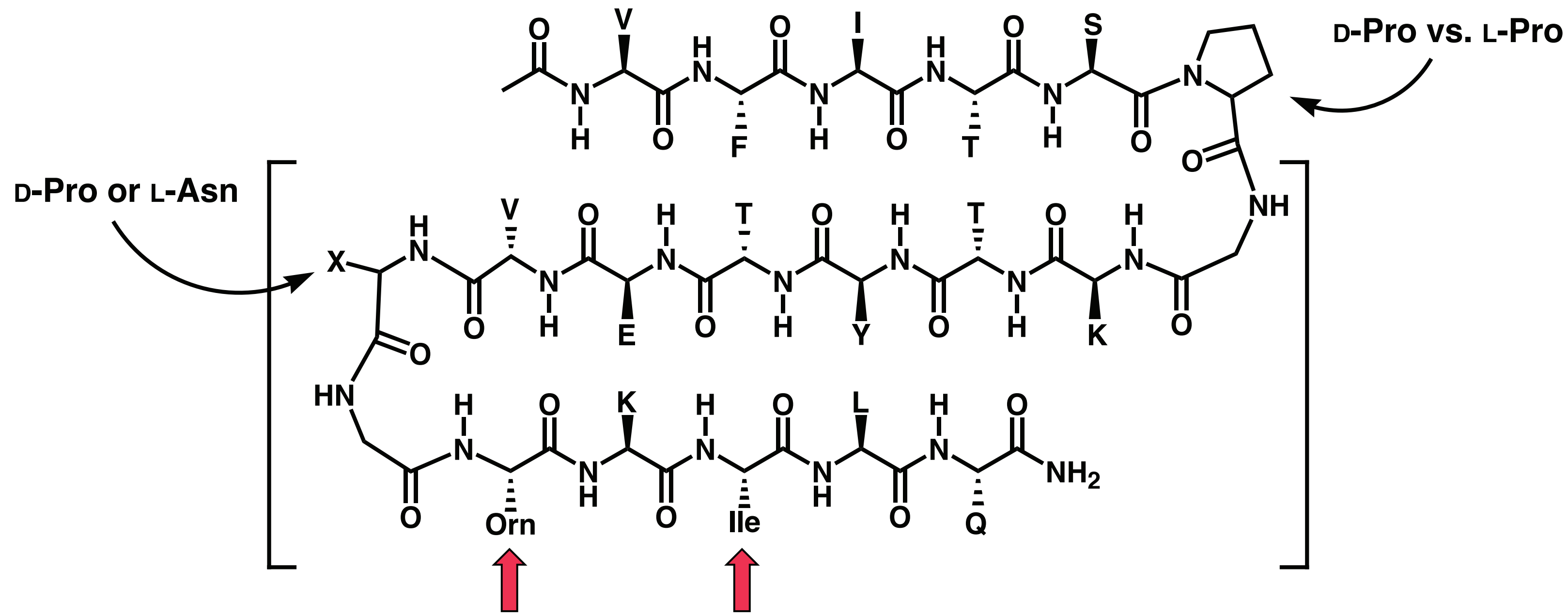


Random Coil model:

L-Pro, L-Pro

H. Stanger, F. Syud

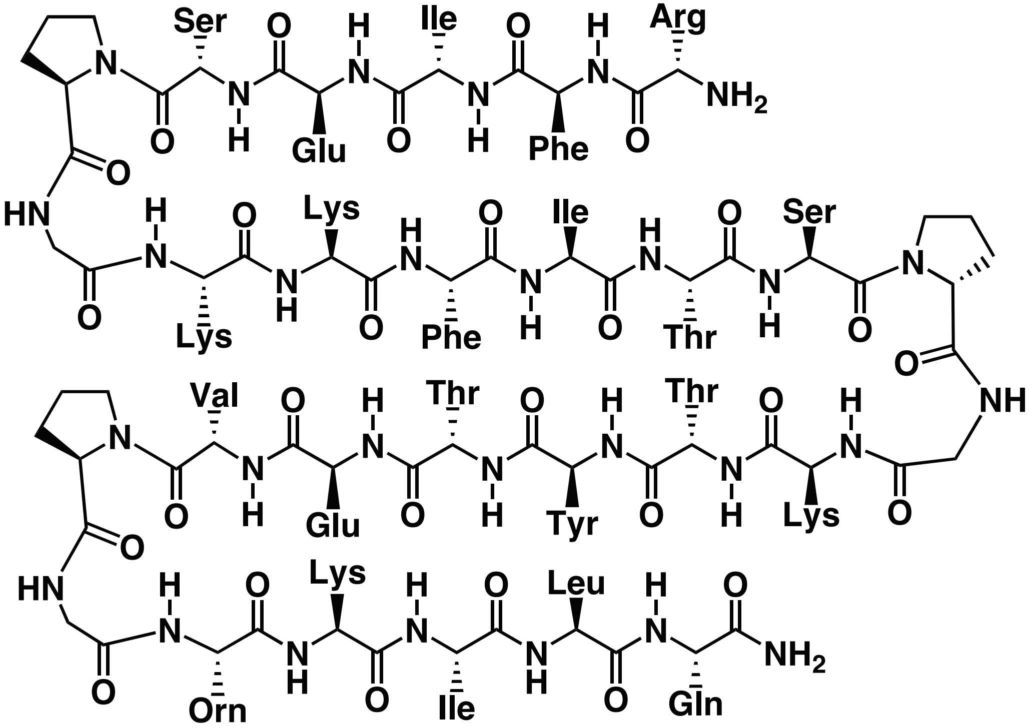
Quantification of β-sheet Cooperativity Perpendicular to the Strand Direction



ΔΔG (D-Pro - L-Pro)

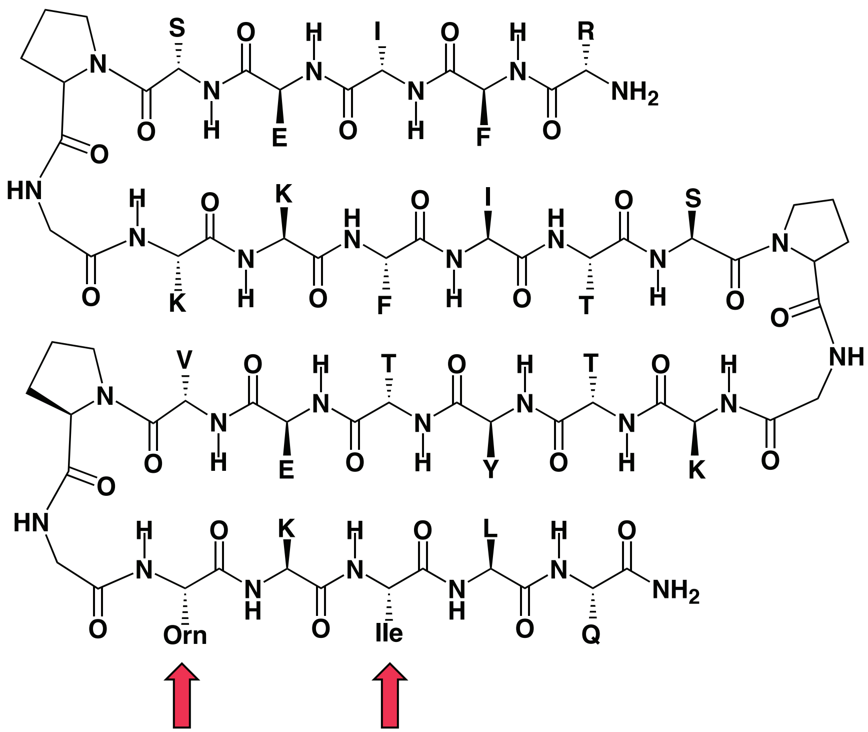
Lower Loop	Orn-16	Ile-18
D-Pro-Gly	- 0.40 kcal/mol	- 0.40 kcal/mol
L-Asn-Gly	- 0.45 kcal/mol	- 0.41 kcal/mol

4-Stranded Antiparallel β-Sheet Design



Syud, Stanger, Mortell, Espinosa, Fisk, Fry, Gellman *J. Mol. Biol.* 326:553 (2003)

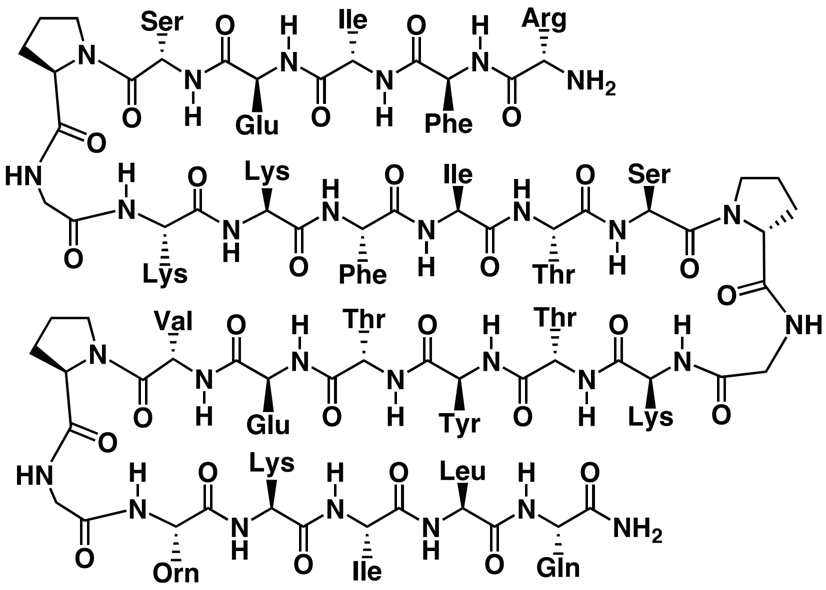
Quantification of β-Sheet Cooperativity Perpendicular to the Strand Direction



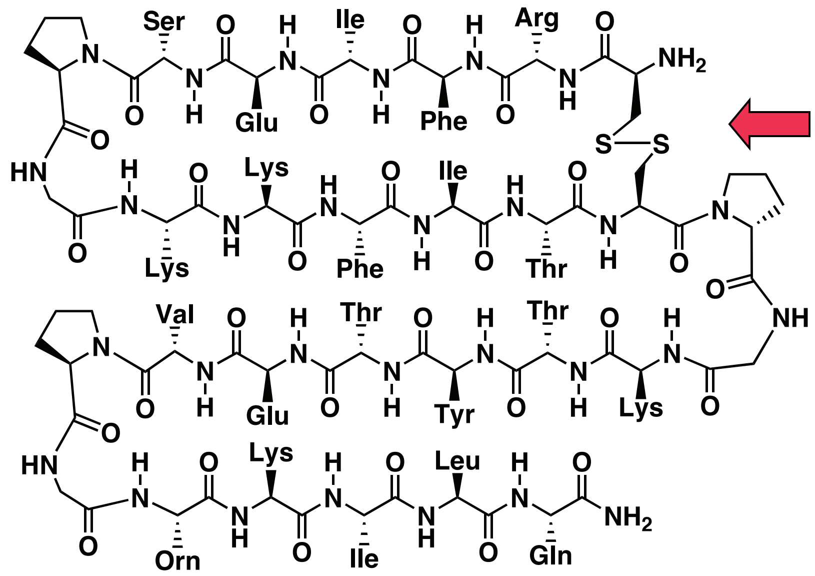
Peptide	ΔΔG (Peptide - LLD) Orn	ΔΔG (Peptide - LLD) Ile
LDD	- 0.32 kcal/mol	- 0.48 kcal/mol
DDD	- 0.45 kcal/mol	- 0.48 kcal/mol

Ambiguous result….

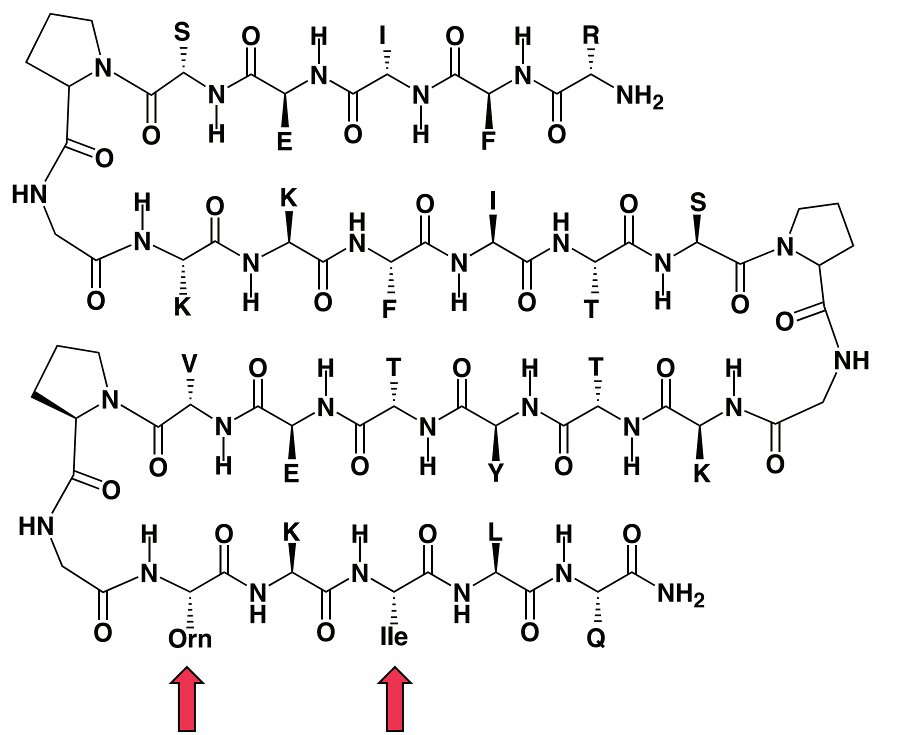
4-Stranded Antiparallel β-Sheet Design -- Locking in the N-Terminal β-Hairpin



vs.



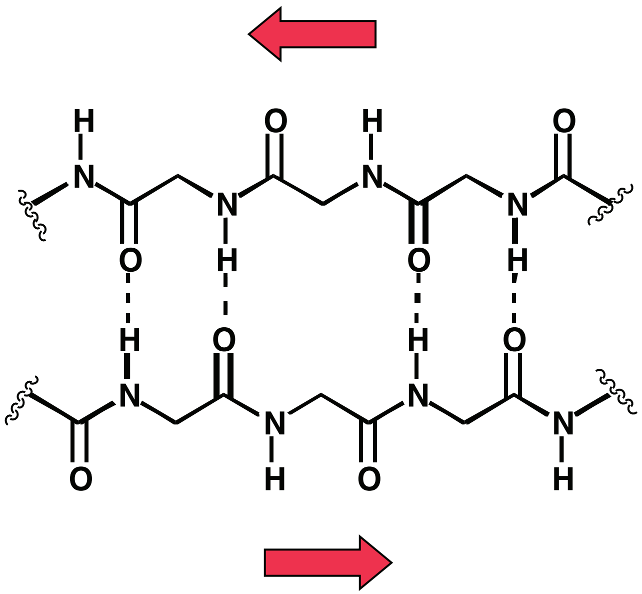
Quantification of β-sheet Cooperativity Perpendicular to the Strand Direction

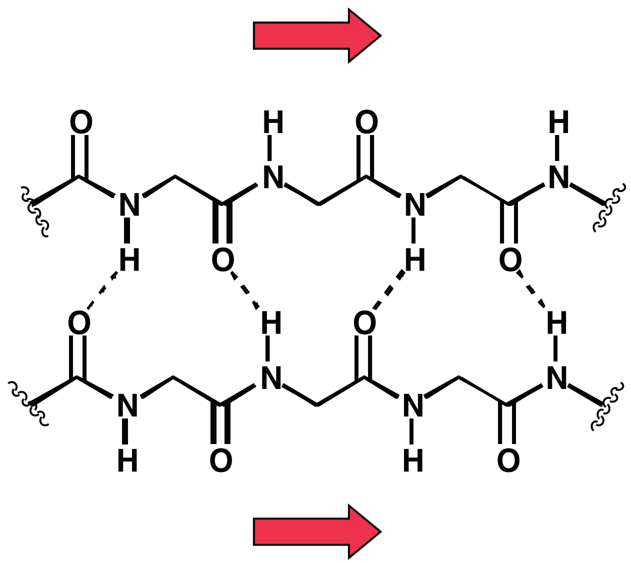


ΔΔG (Peptide - LLD)

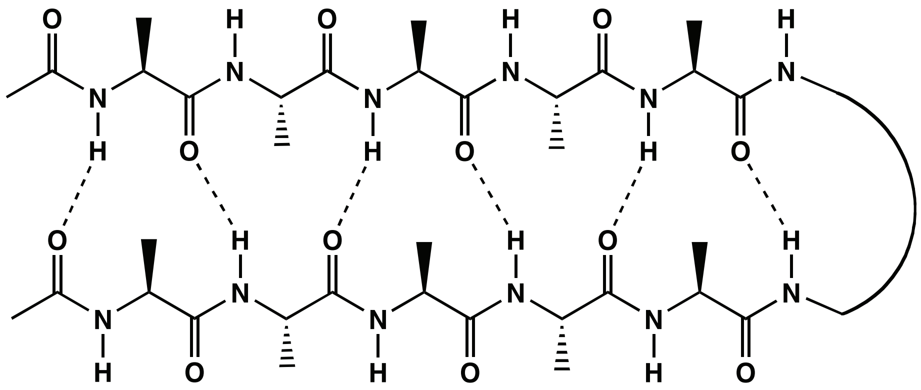
Peptide	Orn	Ile
LDD	- 0.32 kcal/mol	- 0.48 kcal/mol
DDD	- 0.45 kcal/mol	- 0.48 kcal/mol
DDDc	- 1.07 kcal/mol	- 0.86 kcal/mol

Antiparallel vs. Parallel β-Sheet

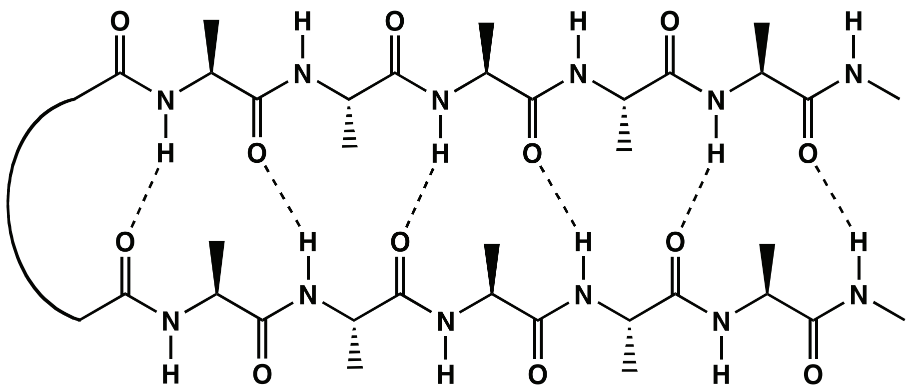




Minimal Model Systems for PARALLEL β-Sheet?



C-to-C linkage

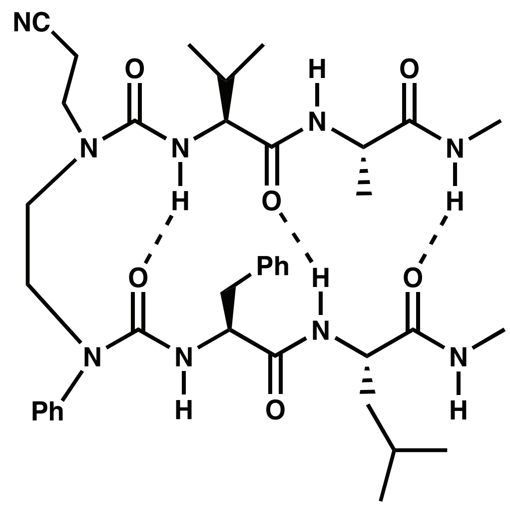


N-to-N linkage

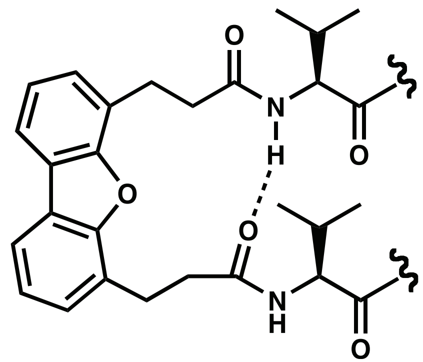
Parallel β-hairpin construction requires unnatural linkage strategies (C-terminal to C-terminal and/or N-terminal to N-terminal)

Early Work on Parallel Linkers
(N-to-N Connection)

Folding in organic solvents (H-bond driving force).

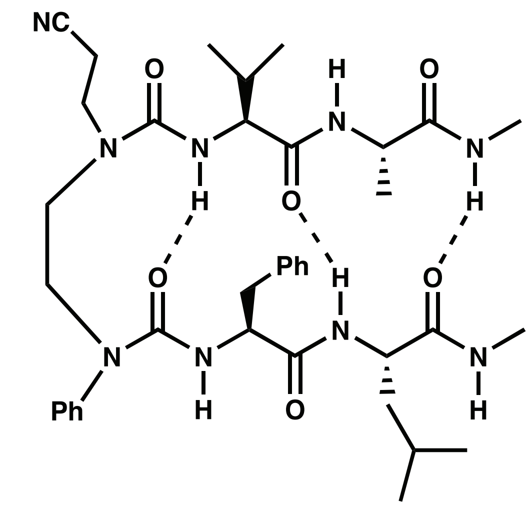


Nowick et al.
***J. Org.Chem.* 60:7368 (1995)**

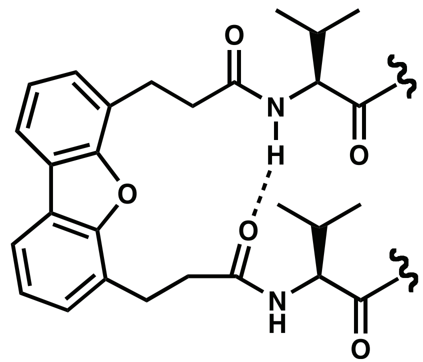


Kelly et al.
***Bioorg. Med. Chem.* 7:39 (1999)**

Early Work on Parallel Linkers
(N-to-N Connection)



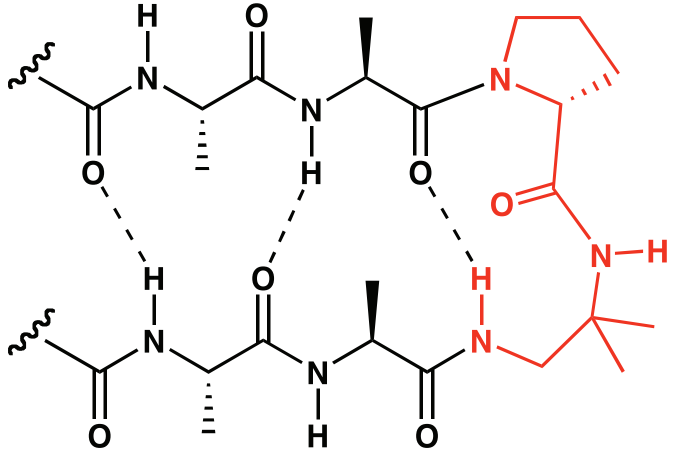
Nowick et al.
***J. Org.Chem.* 60:7368 (1995)**

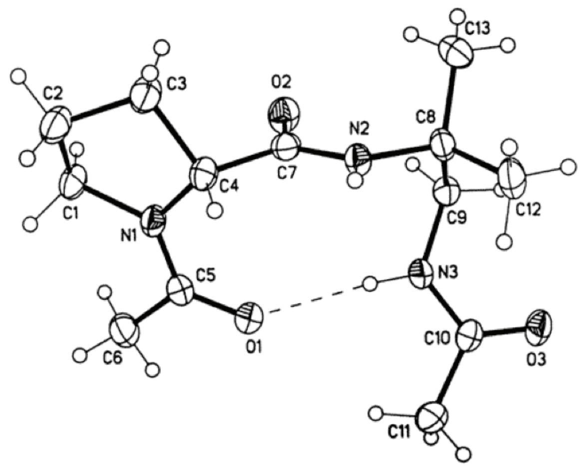


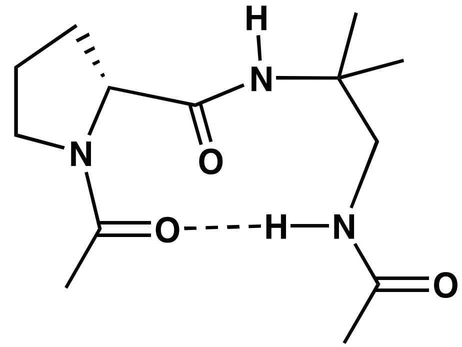
Kelly et al.
***Bioorg. Med. Chem.* 7:39 (1999)**

No cross-strand NOEs in water.

C-to-C Linker With a Twist: Proline-Based Diamine

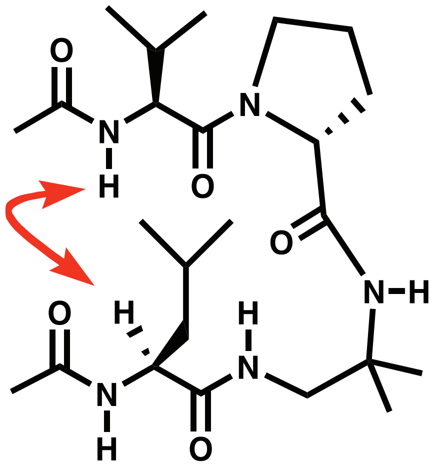


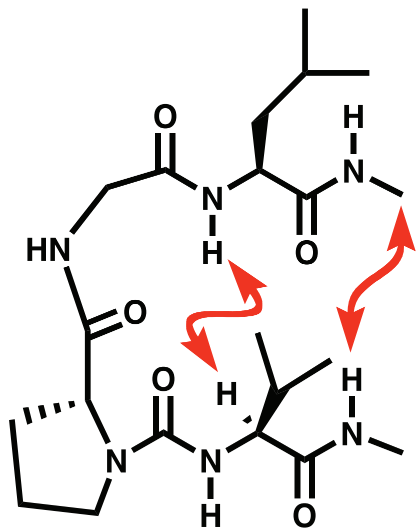




Fisk, Powell, Gellman *J. Am. Chem. Soc.* **2000**, *122*, 5443.

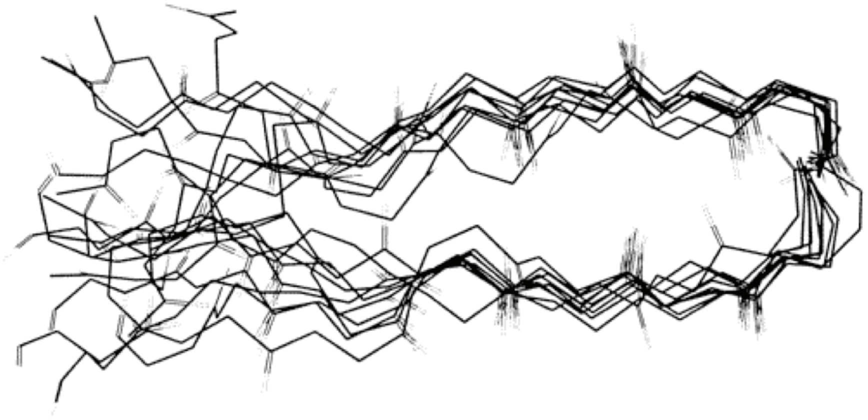
NOE Analysis: Parallel β-Sheet formation in Minimal Model Systems (Chloroform)



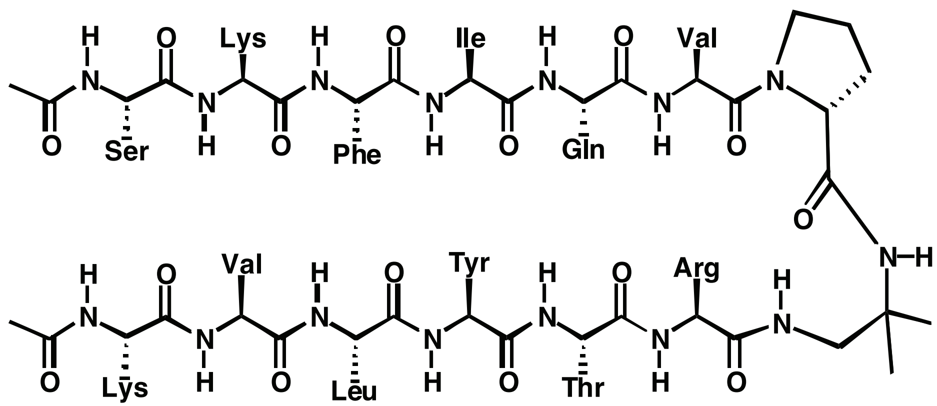


(No β-sheet NOEs in L-Pro diastereomers)

A Parallel β-Sheet Model System that Folds in Water

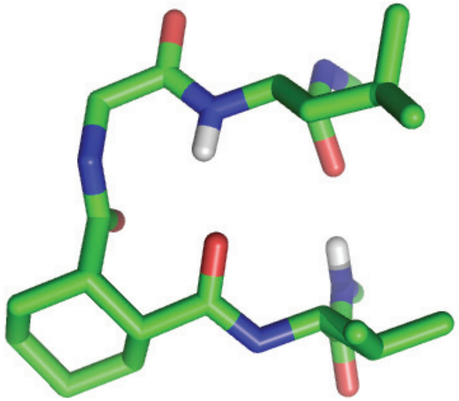


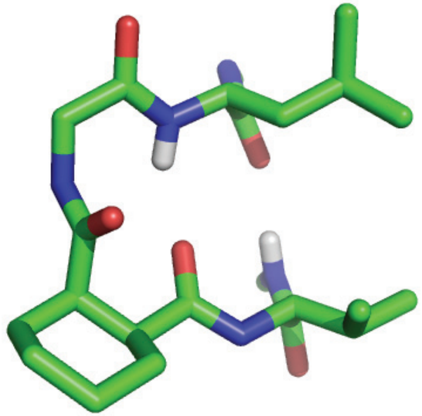
Family of structures from NOE-restrained dynamics



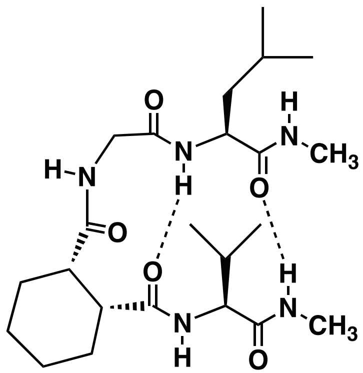
Fisk and Gellman *J. Am. Chem. Soc.* **2001**, *123*, 343.

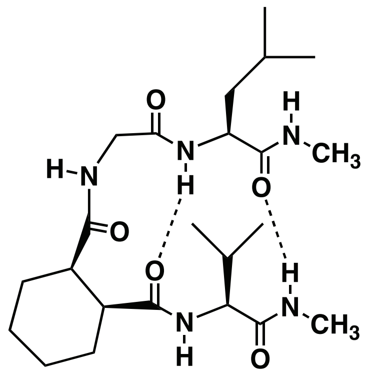
N-to-N Linker for Parallel Strands:
Both Configurations Promote Parallel β-Sheet Interactions





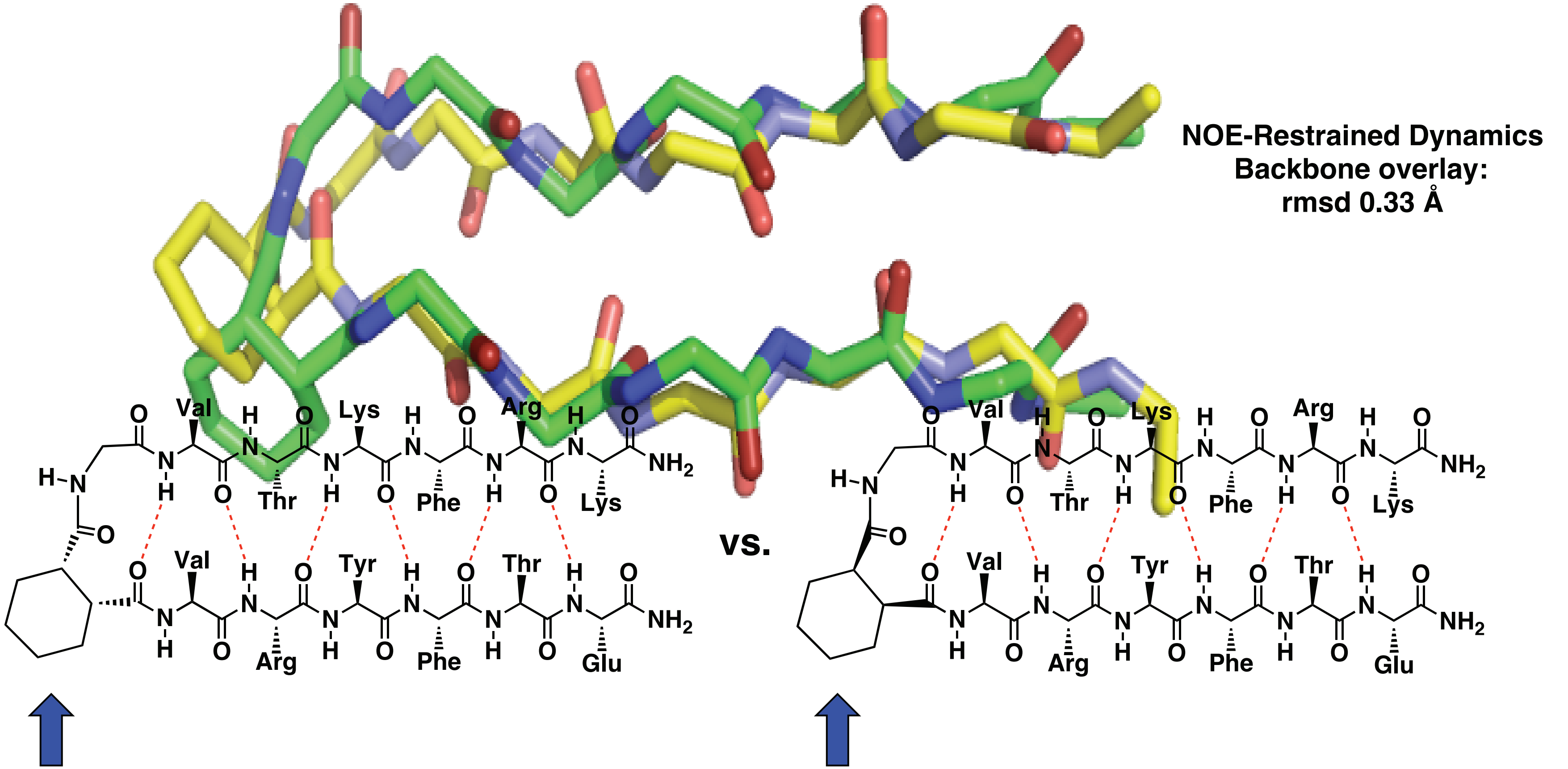
Crystal structures





Freire, Fisk, Peoples, Ivancic, Guzei, Gellman *J. Am. Chem. Soc.* 130:7839 (2008)

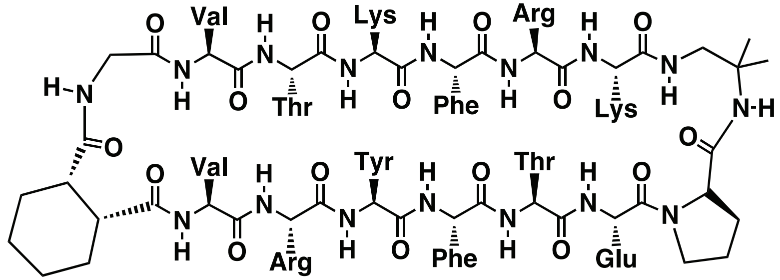
N-to-N Linker for Parallel Strands:
Parallel β-sheet Formation in Aqueous Solution

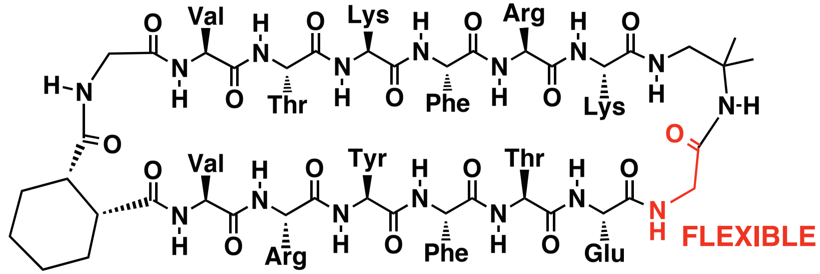


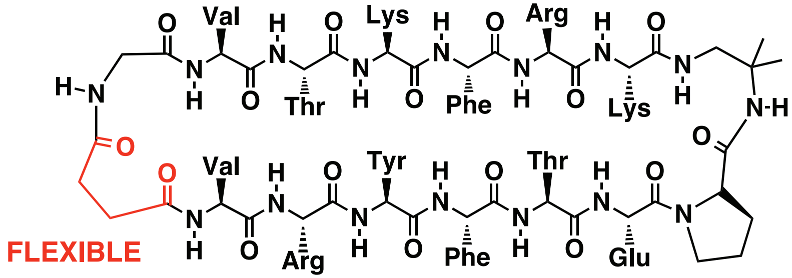
(2D NMR @ 600 MHz; 2.5 mM peptide in 100 mM acetate buffer, pH 3.8)

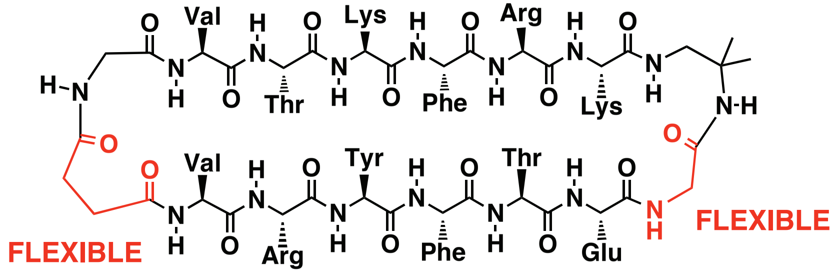
Freire, Fisk, Peoples, Ivancic, Guzei, Gellman *J. Am. Chem. Soc.* 130:7839 (2008)

Macrocyclic Designs: Preorganization of One Linker is Sufficient For Folding in Aqueous Solution

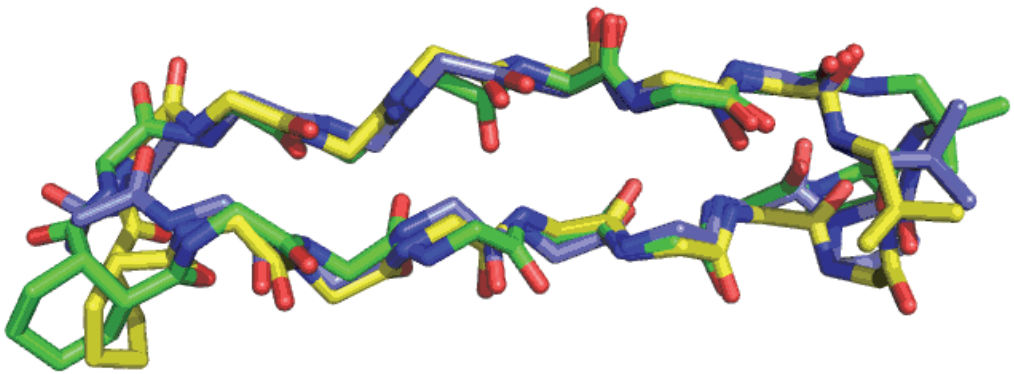








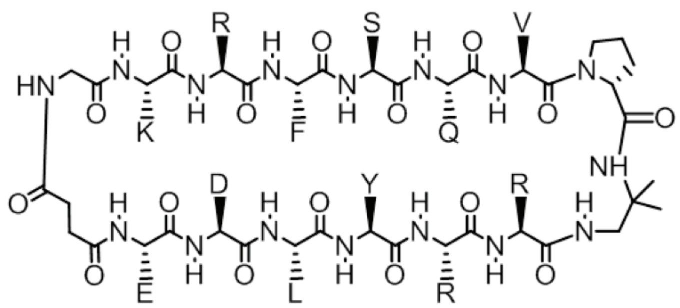
NO FOLDING

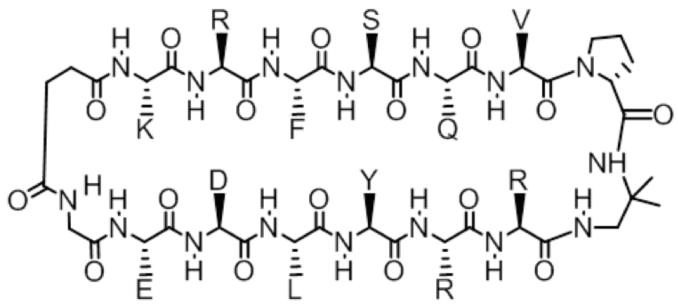


2D NMR:
Overlay of 3 backbones

Freire, Gellman
***J. Am. Chem. Soc.* 131:7970 (2009)**

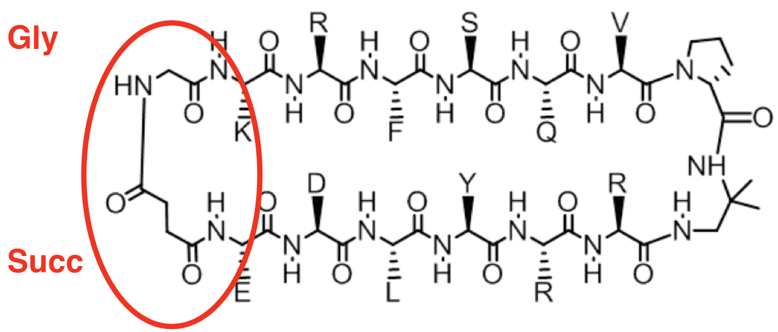
Linker Orientation Plays a Critical Role in Hairpin Stability

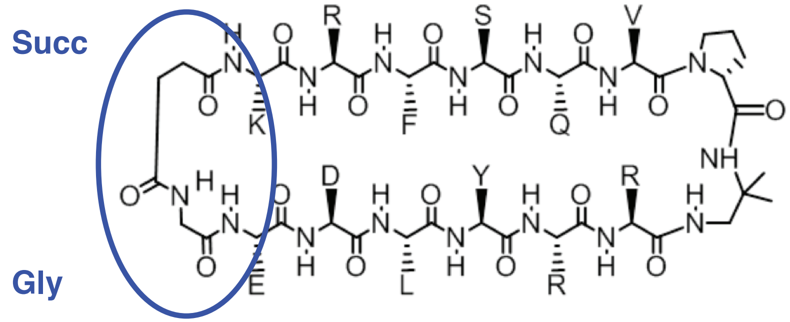




A. Almeida

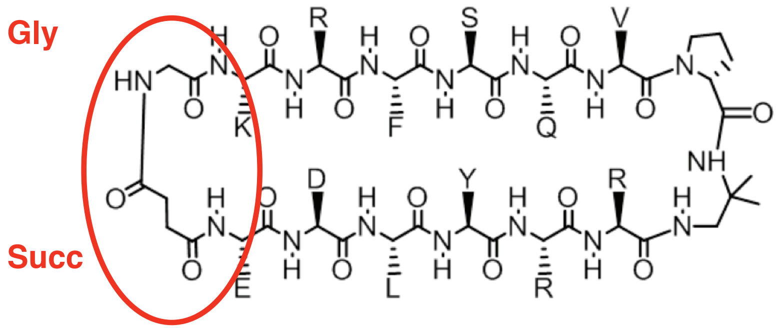
Linker Orientation Plays a Critical Role in Hairpin Stability



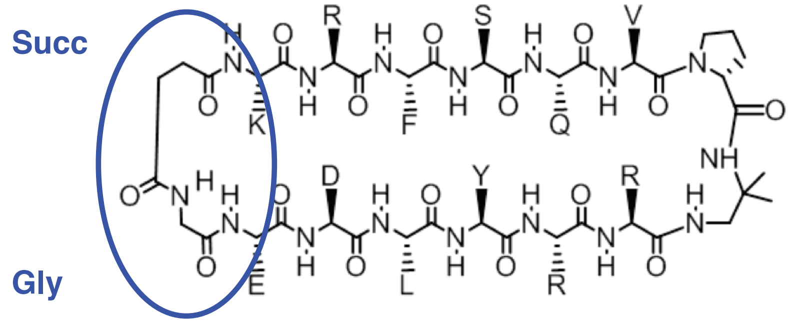


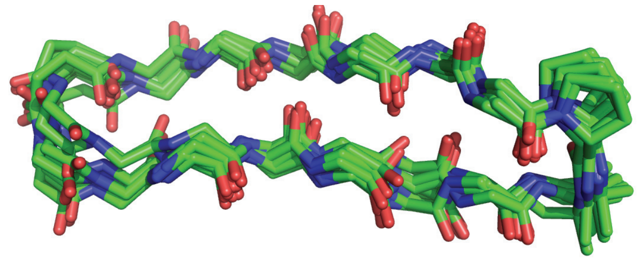
A. Almeida

Linker Orientation Plays a Critical Role in Hairpin Stability



Very few NOEs;
not well folded.



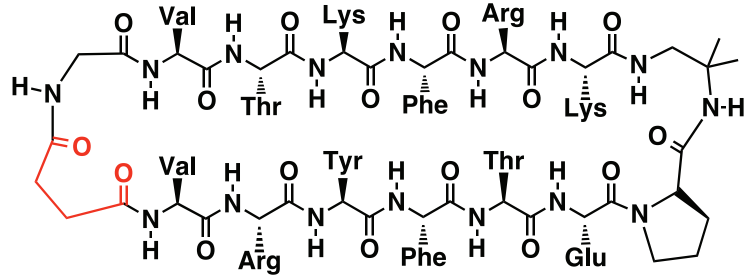


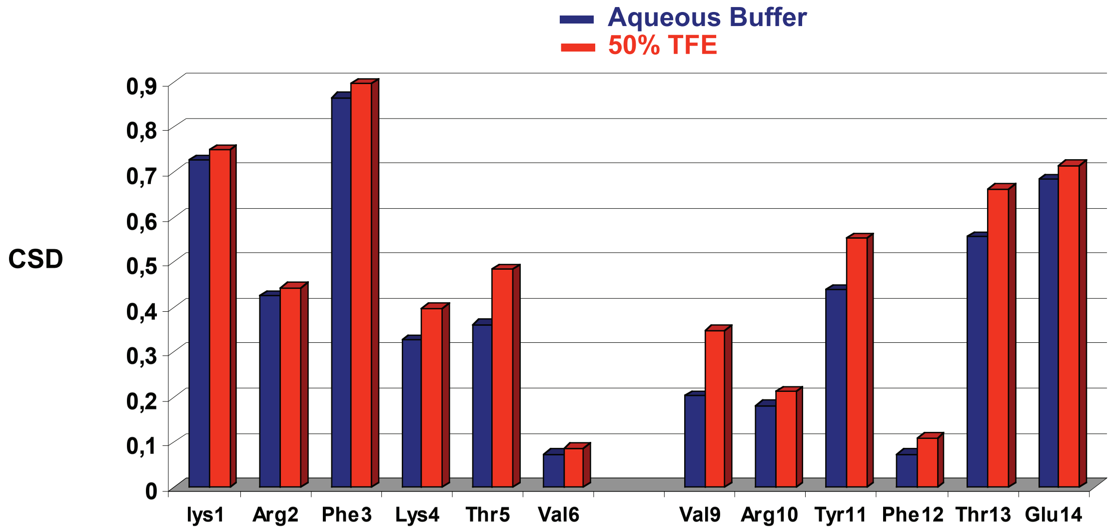
NOE-based structure;
backbone RMSD = 0.5 ± 0.2 Å

A. Almeida

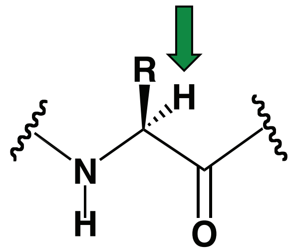
9:1 H_2O:D_2O, 100 mM D_3AcOH, pH 3.8, trace DSS

Macrocyclic Designs: Fully Folded State Reference



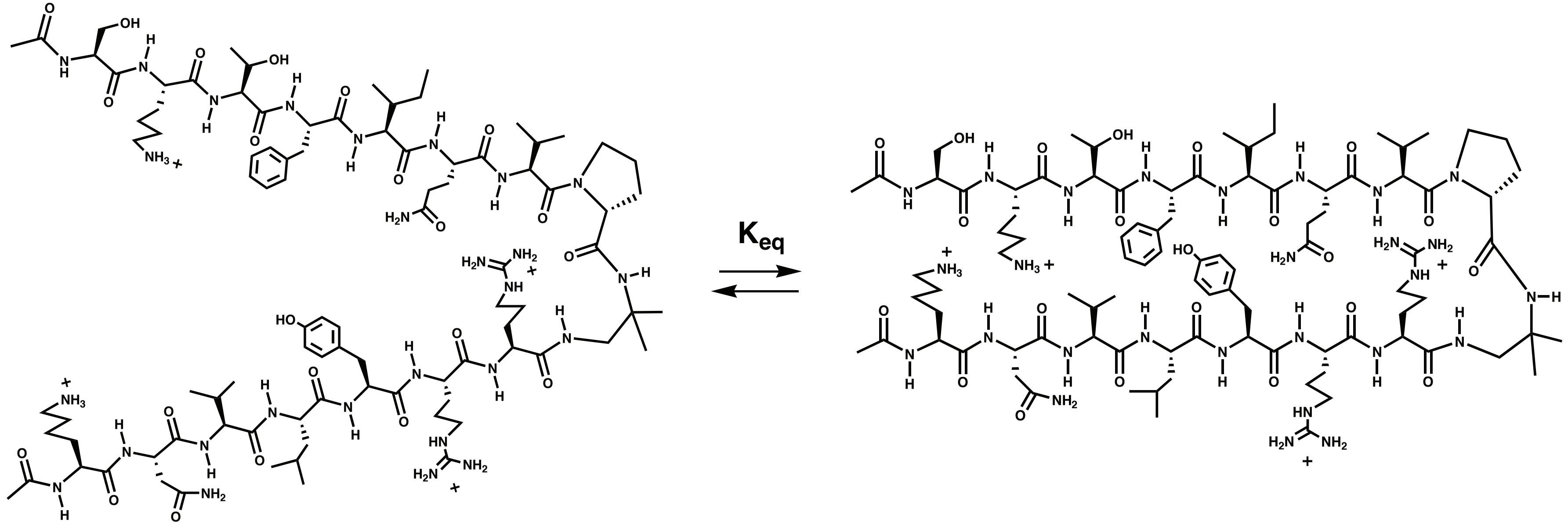


CSD = [$\delta_{C\alpha H}$(peptide) - $\delta_{C\alpha H}$(random coil)]

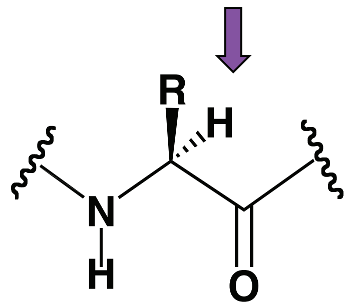


Freire, Gellman *J. Am. Chem. Soc.* 131:7970 (2009)

Population Analysis: Rapid Equilibration Between Folded and Unfolded States (NMR Timescale)

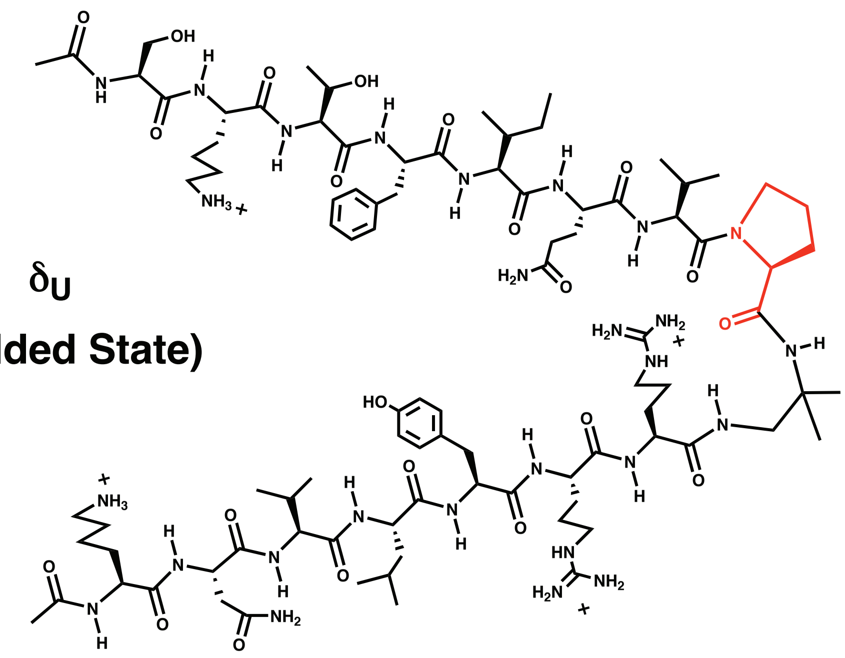


α-H chemical shifts are very sensitive to conformation:



Wishart et al.
Biochemistry **1992**, *31*, 1647.

Population Analysis: Reference Compounds for the Folded and Unfolded States



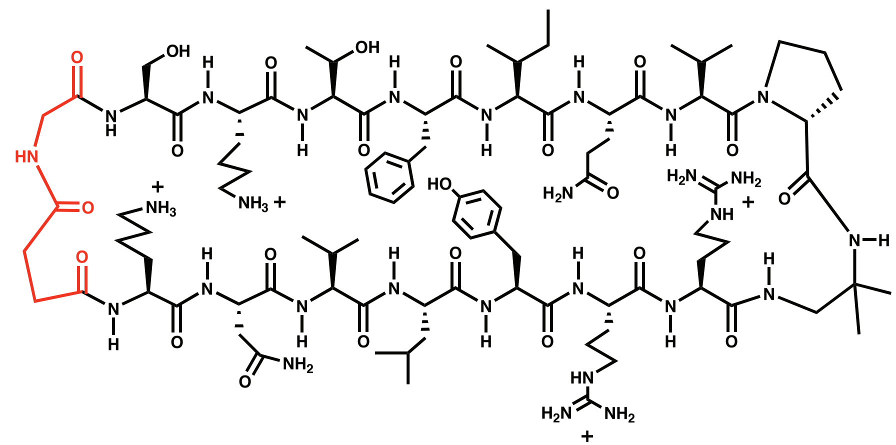
δ_U

(Unfolded State)

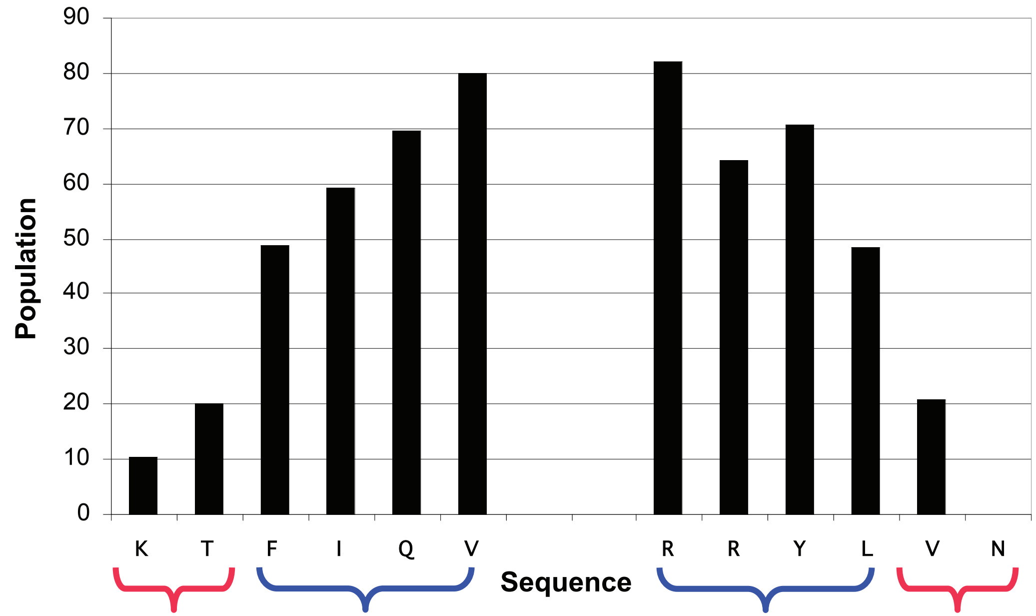
$$\beta\text{-Hairpin \%} = \frac{\delta_{obs} - \delta_U}{\delta_F - \delta_U}$$

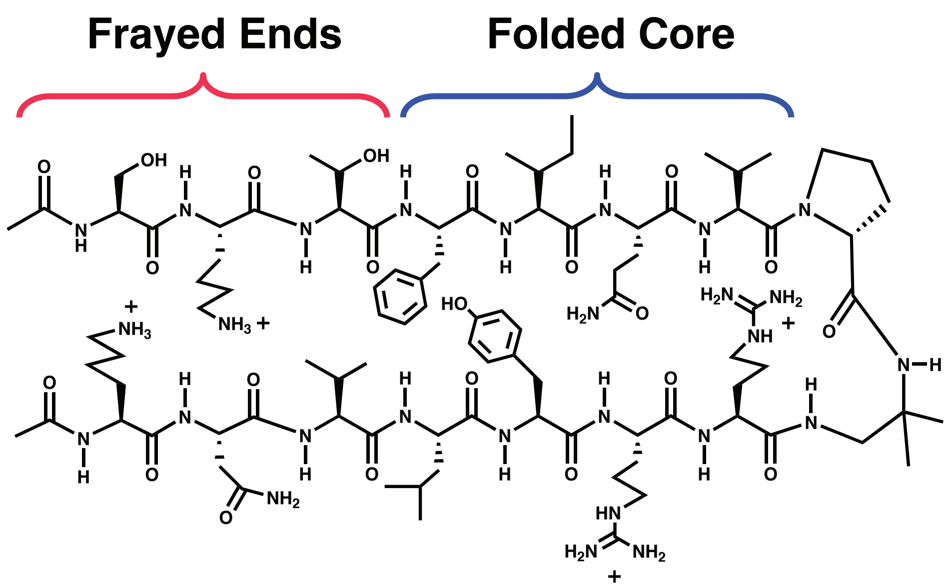
δ_F

(Folded State)



Population Analysis at Strand Residues

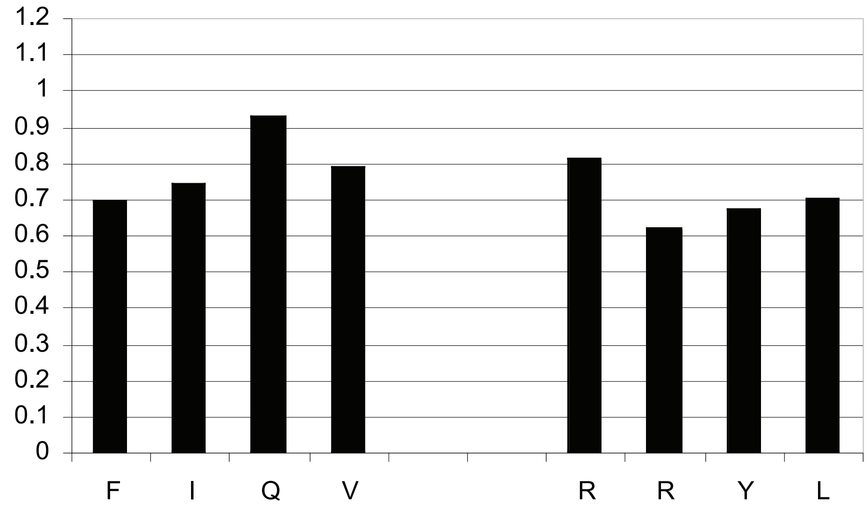




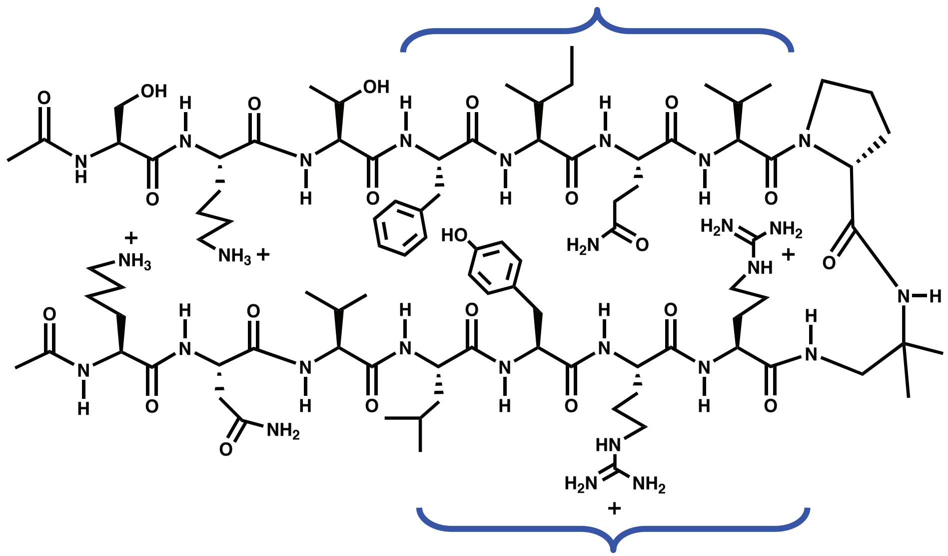
2 mM peptide, 100 mM acetate buffer, pH 3.8, 4°C

Temperature-Dependent Variations: Evidence for Two-State Behavior

Relative Population



$$\text{Relative Population} = \frac{\text{Pop. @ 64}^{o}\text{C}}{\text{Pop. @ 4}^{o}\text{C}}$$

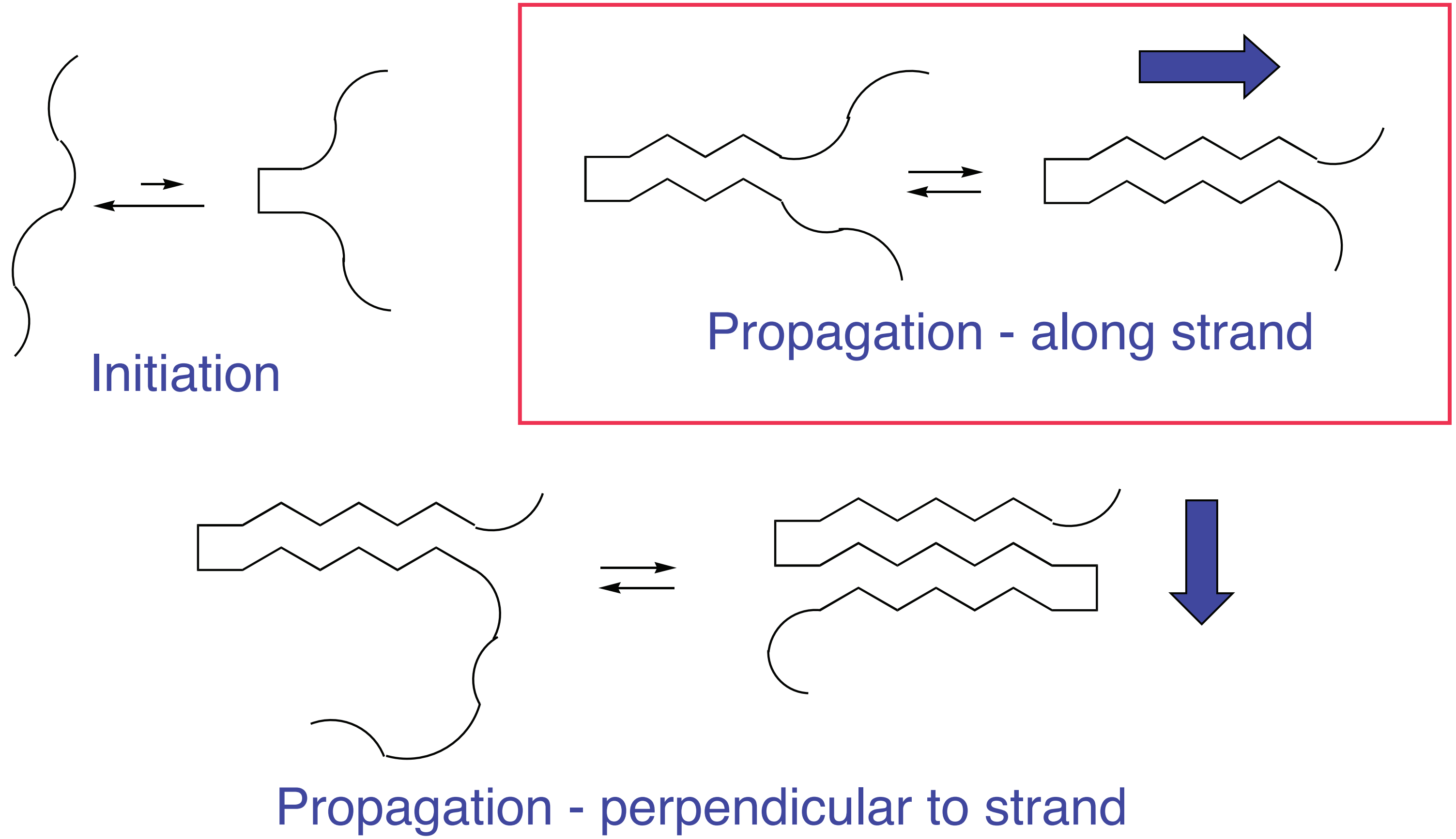


<u>van' t Hoff Analysis</u>:

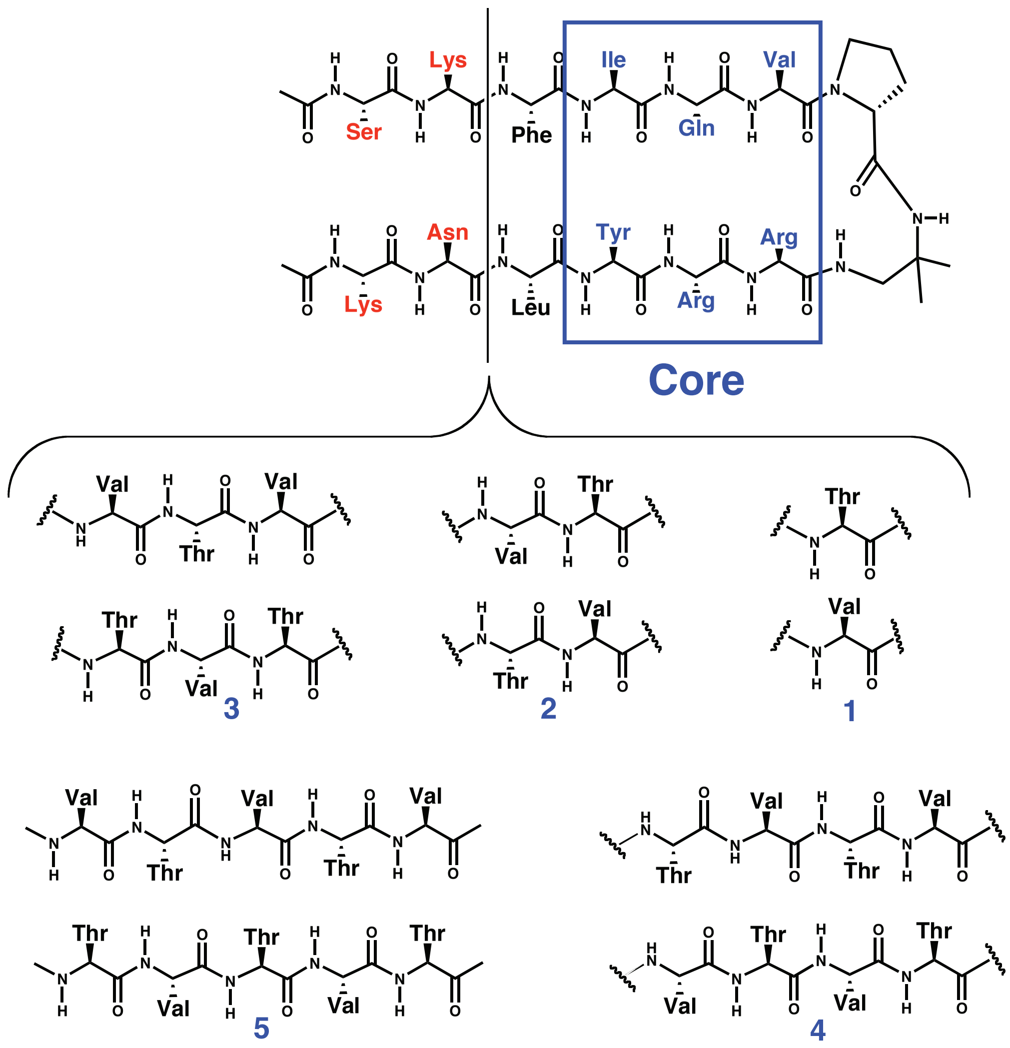
ΔH = -3.2 kcal/mol
ΔS = -10 cal/mol deg
ΔC_p = -100 cal/mol deg

Fisk, Schmitt, Gellman
J. Am. Chem. Soc. **<u>128</u>:7148 (2006)**

Length-Dependent Cooperativity in Parallel β-Sheet?

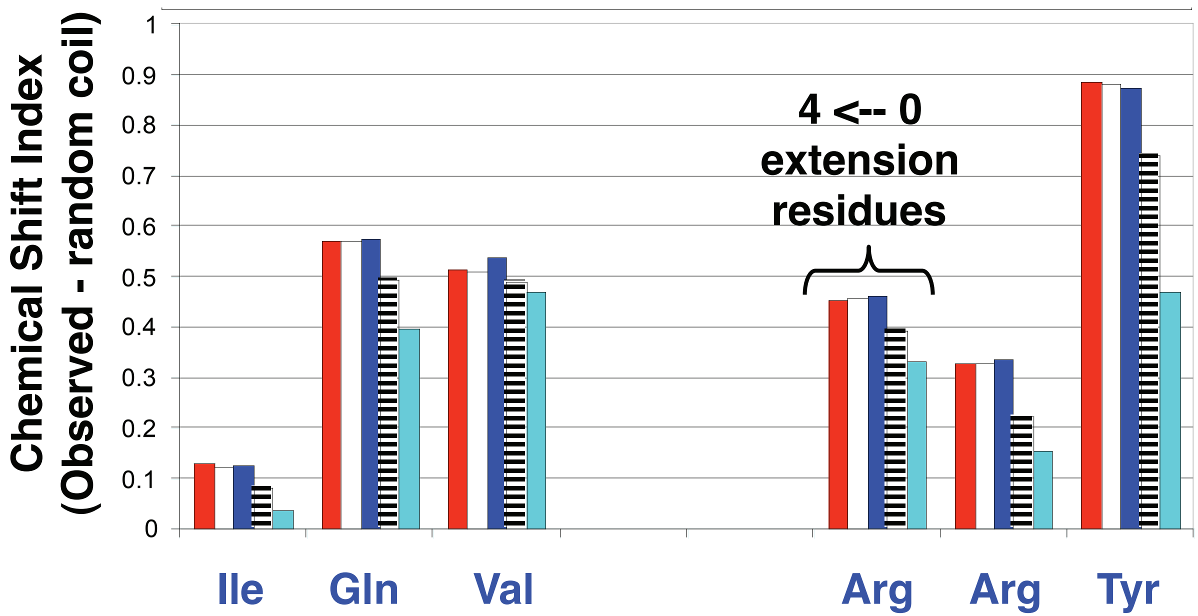


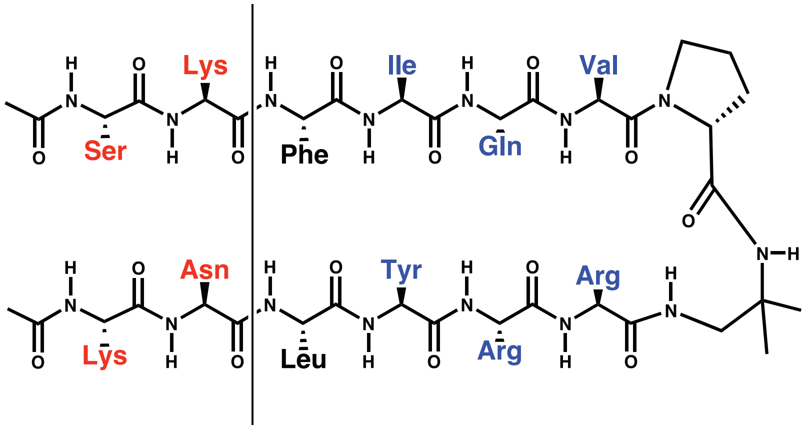
Length-Dependent Cooperativity Along the Strand Direction in Parallel β-Sheet?



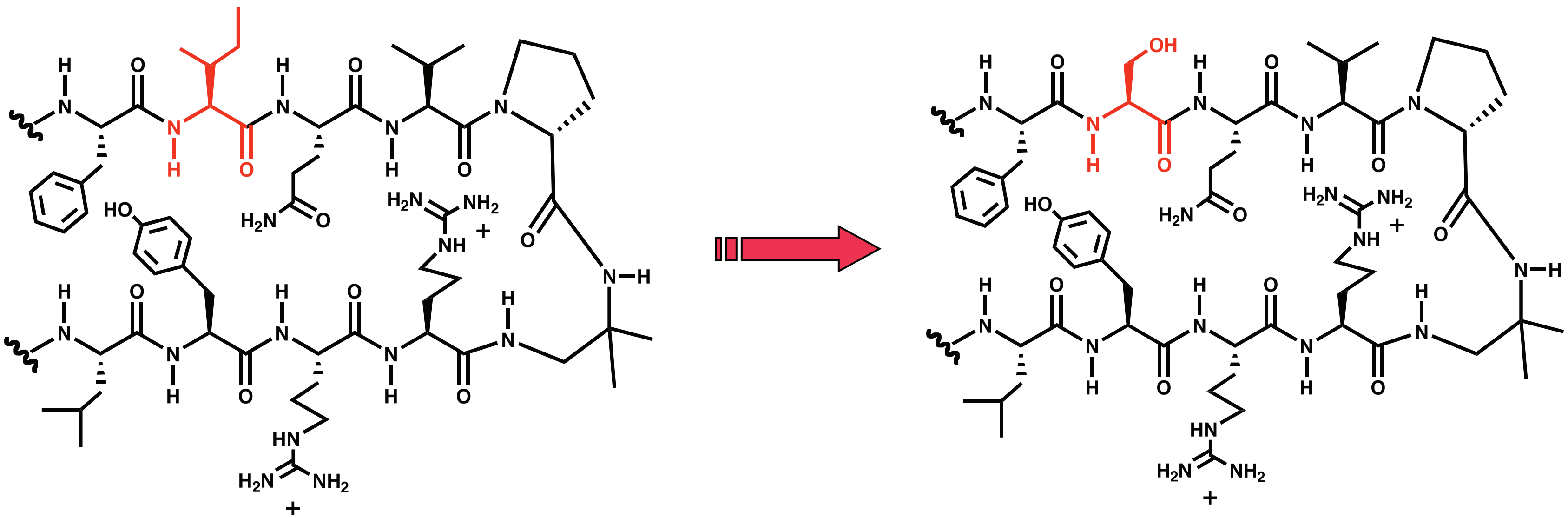
J. D. Fisk

Stability Plateau After Two Extensions: Intrinsic Strand Length Limit or Core too Stable?



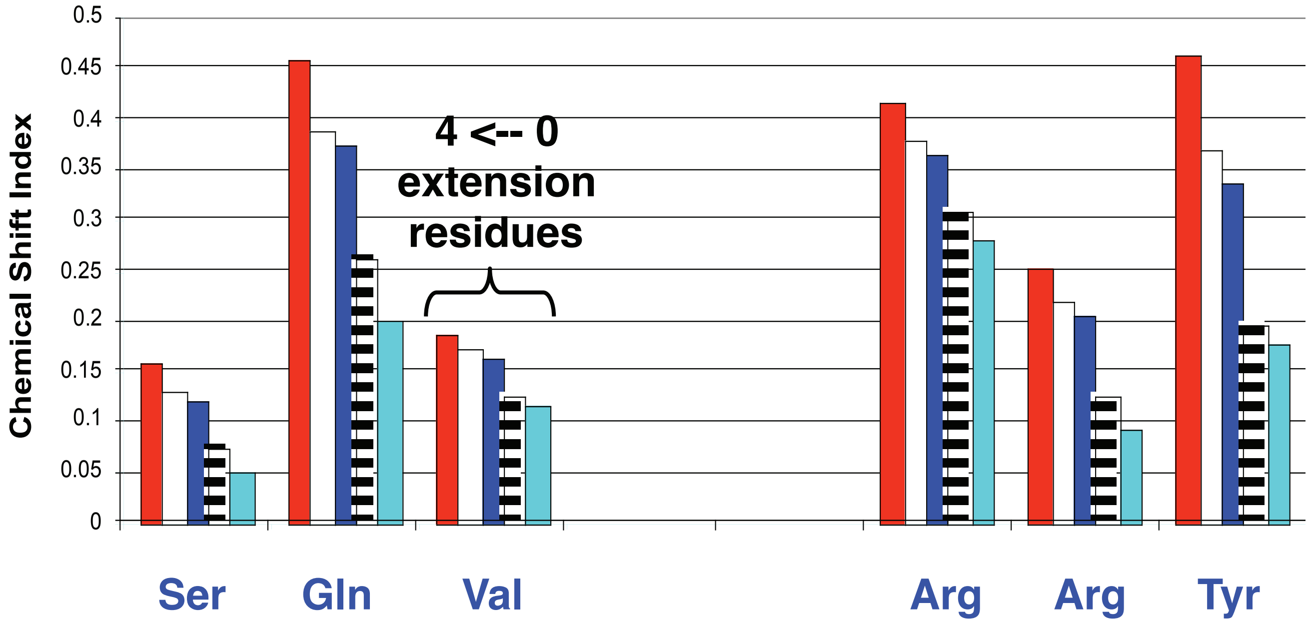


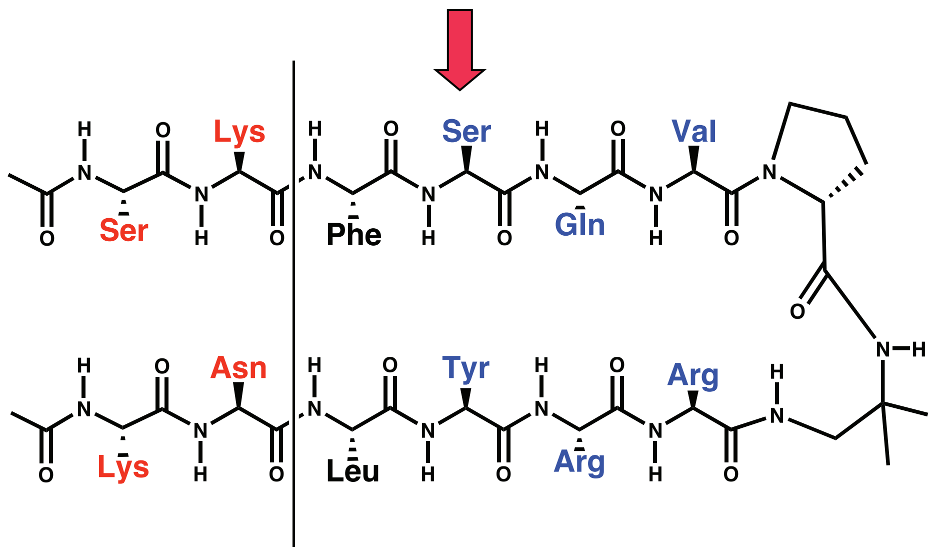
Design of Second Series: Destablize the Core (Ile --> Ser)



J. D. Fisk

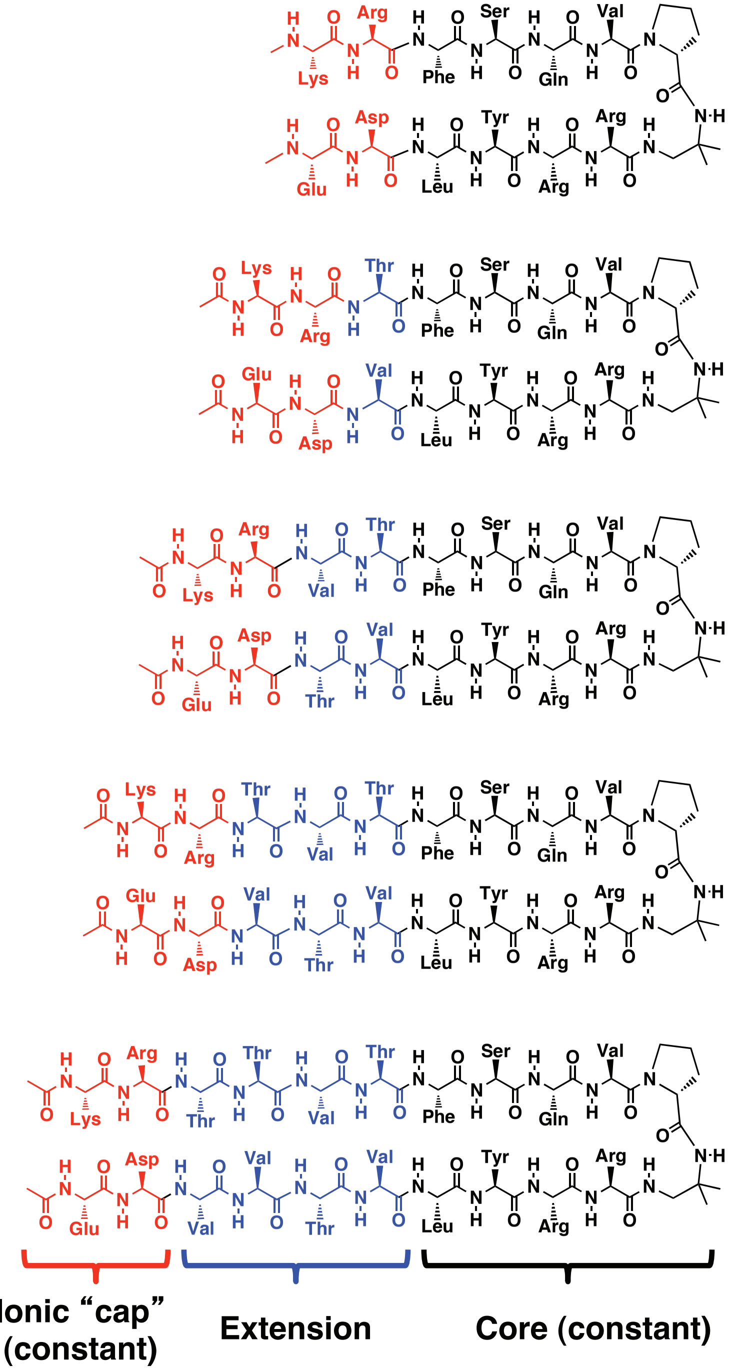
Extension Series, Destabilized Core: Parallel β-Sheet Becomes Steadily More Stable as Strands Lengthen



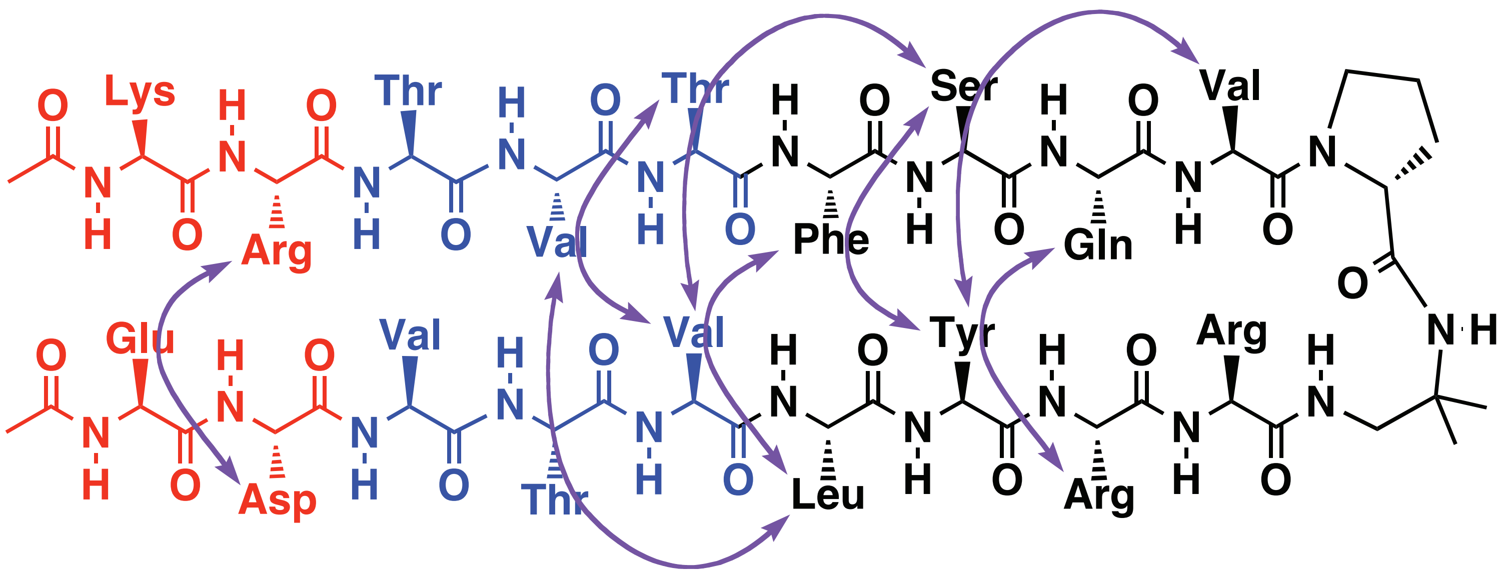


Experimental Design: Does Strand Length Affect Parallel β-Sheet Stability?

Freire, Almeida, Fisk, Steinkruger, Gellman
Angew. Chem. Int. Ed. 50:8735 (2011)



Parallel β-Sheet Folding Maintained Upon Strand Lengthening?

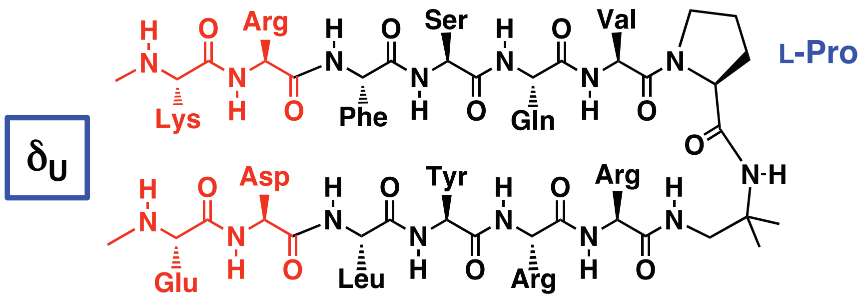


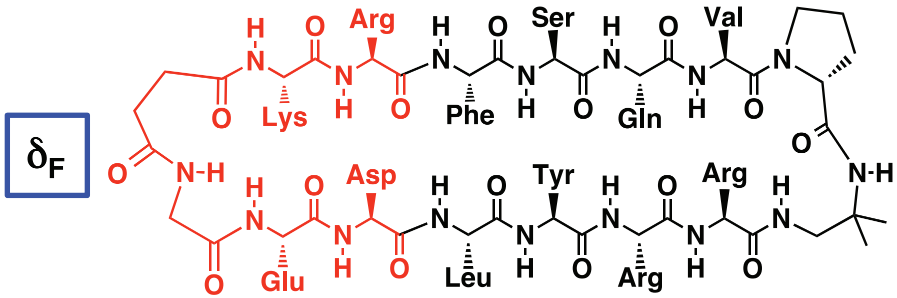
Cross-strand nOe’ s (inter-residue)

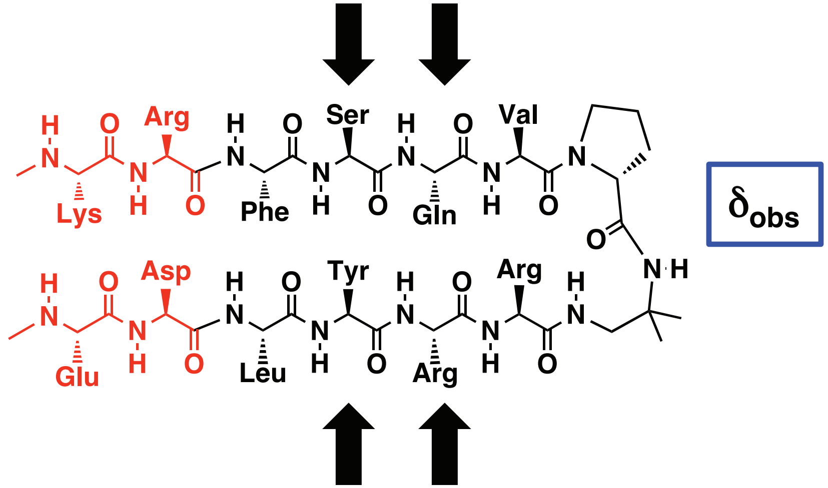
600 MHz, 4°C, 2.5 mM peptide,
9:1 H_2O:D_2O, 100 mM acetate buffer, pH 3.8

A. Almeida, F. Freire

Population Analysis (C_αH Chemical Shift Data)





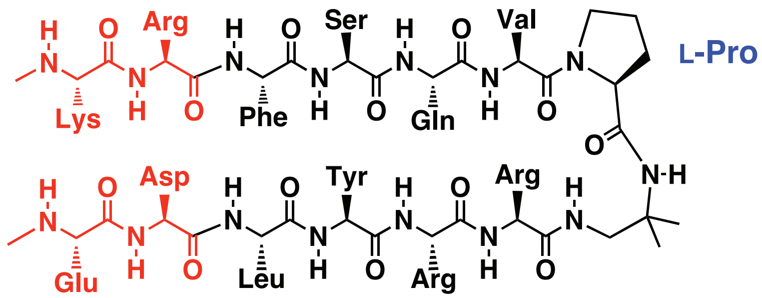


Residues for Population analysis

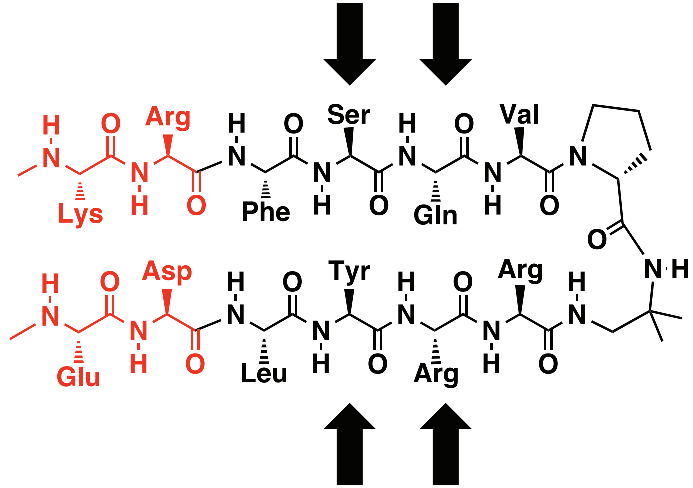
$$\textbf{Population (\%)} = \frac{\delta_{obs} - \delta_U}{\delta_F - \delta_U}$$

Population Analysis ($C_\alpha H$ Chemical Shift Data)

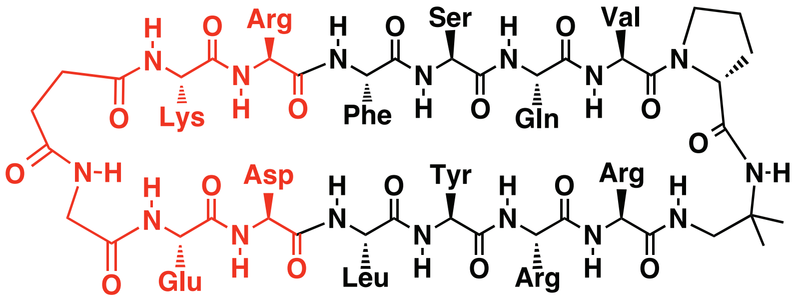
δ_U



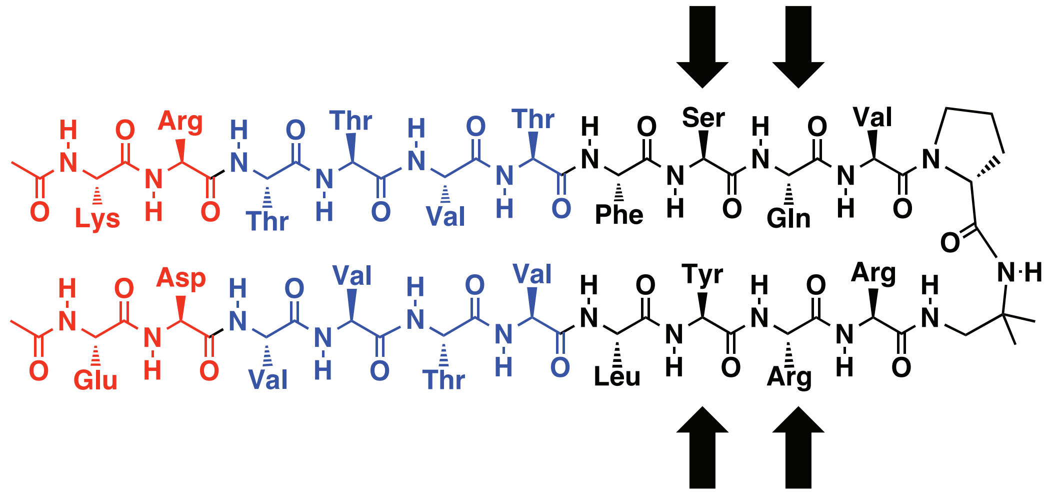
δ_{obs}



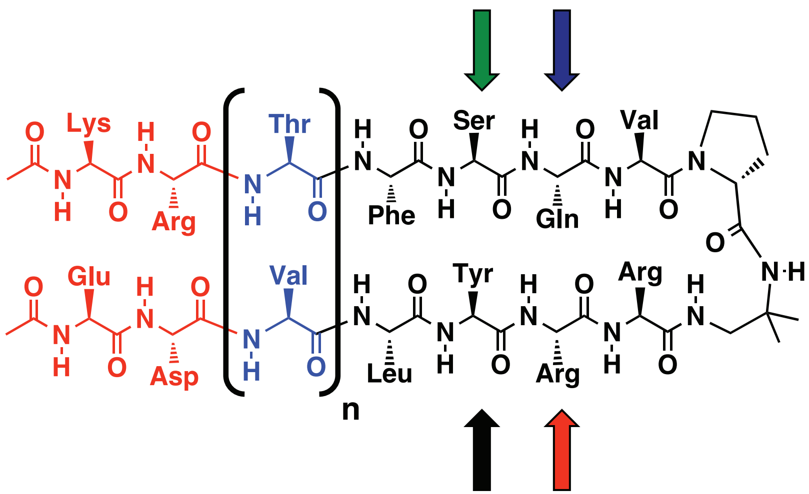
δ_F

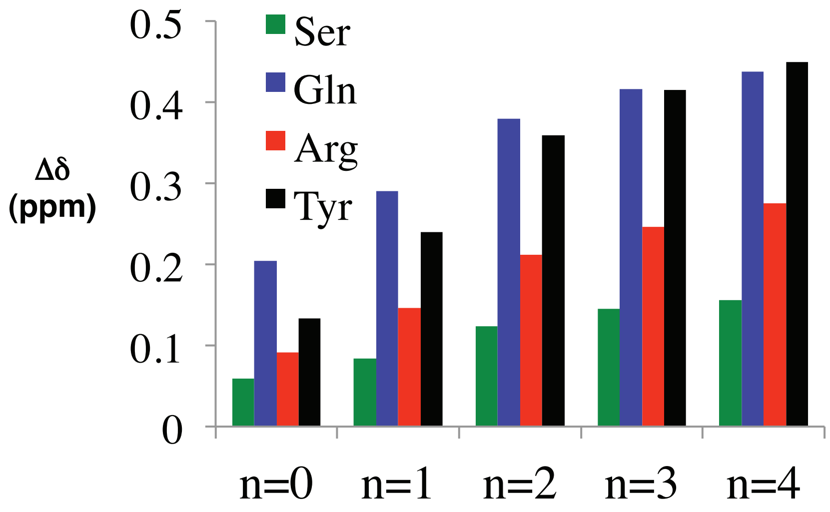


$$\text{Population (\%)} = \frac{\delta_{obs} - \delta_U}{\delta_F - \delta_U}$$

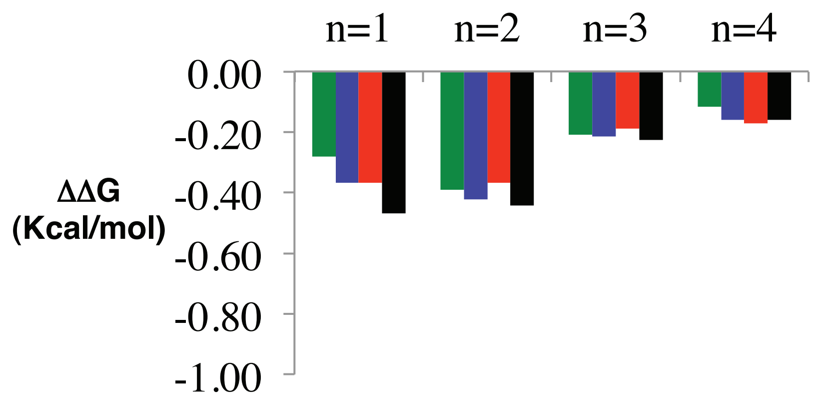


Parallel β-Sheet Becomes More Stable as Strands Grow Longer

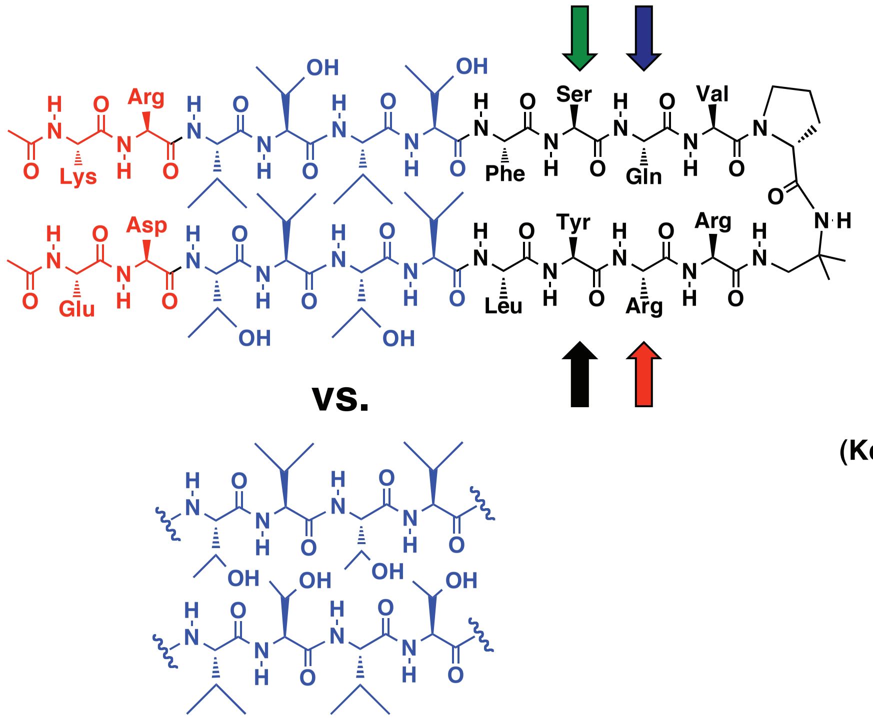




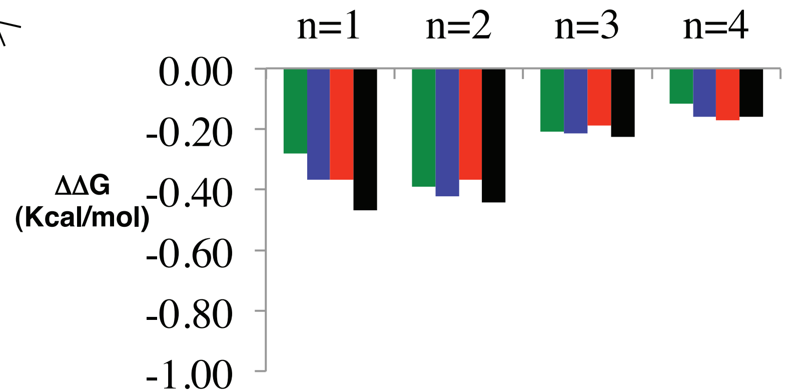
Increment per extension (n → n+1)

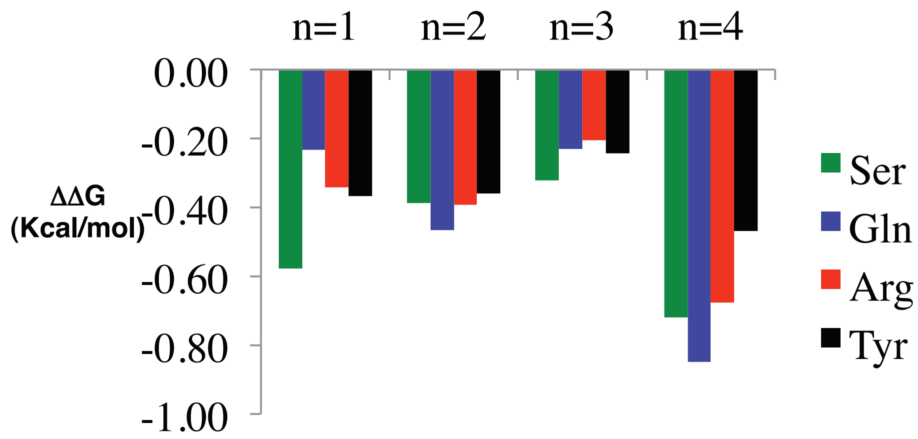


Parallel β-Sheet Becomes More Stable as Strands Grow Longer

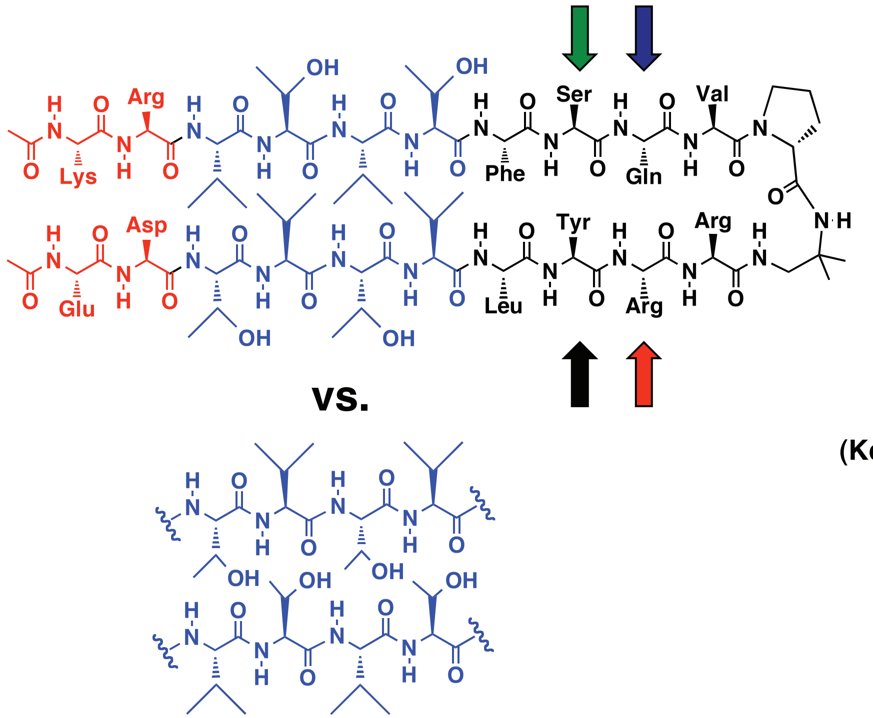


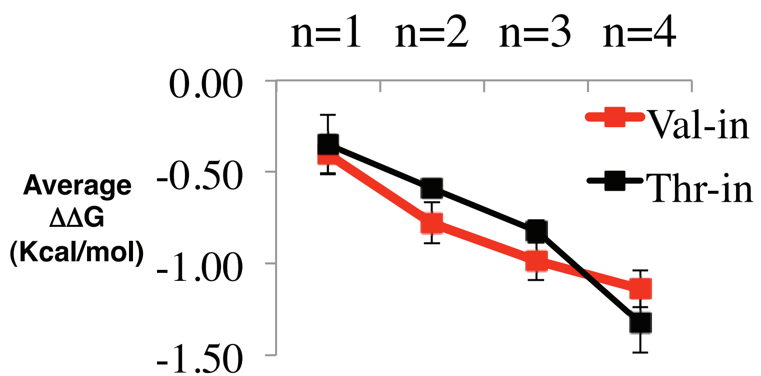
Increment per extension (n → n+1)



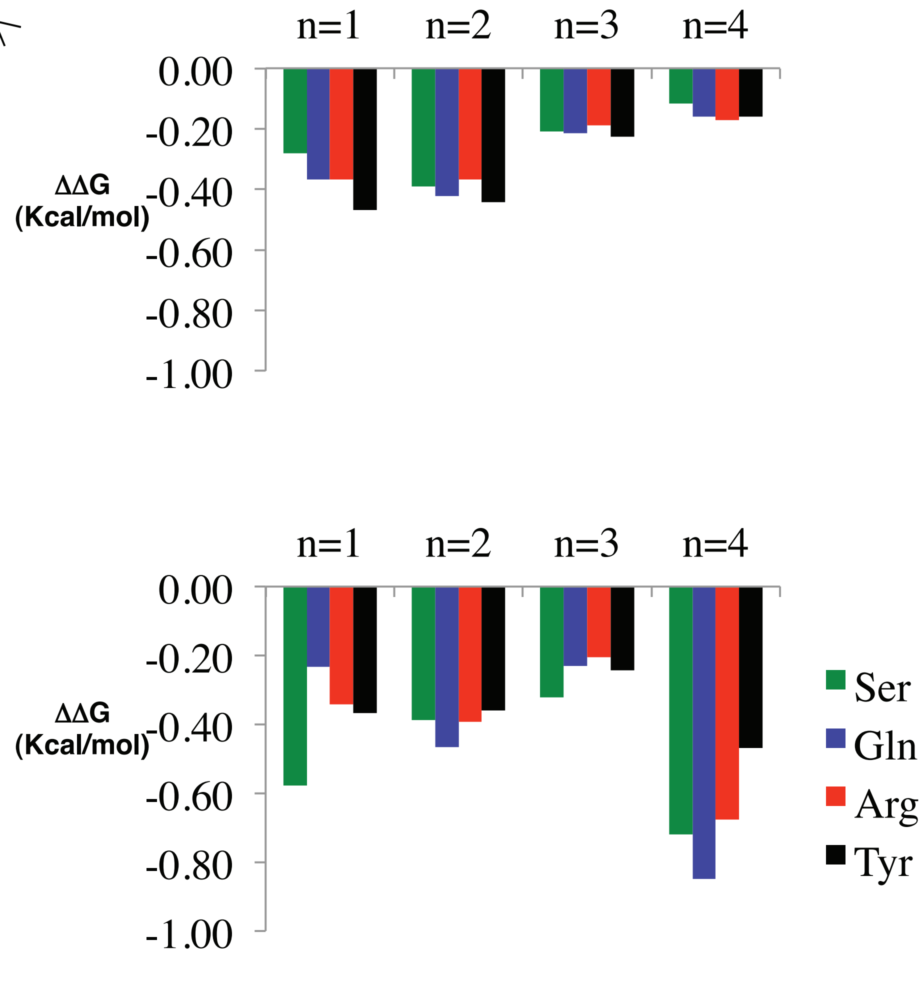


Parallel β-Sheet Becomes More Stable as Strands Grow Longer

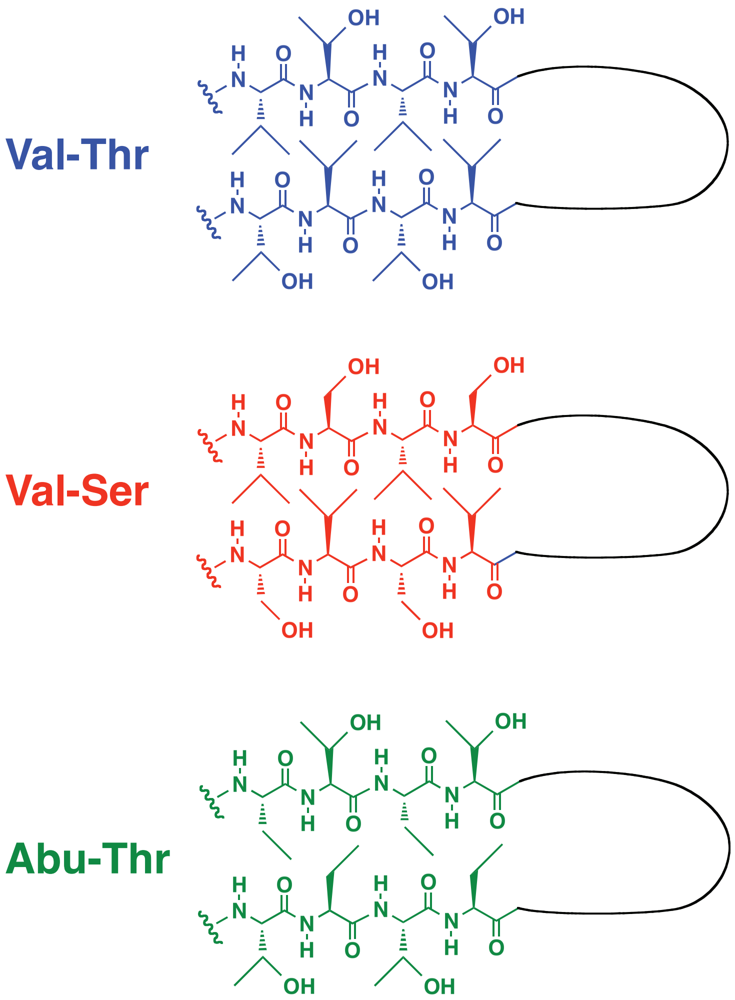


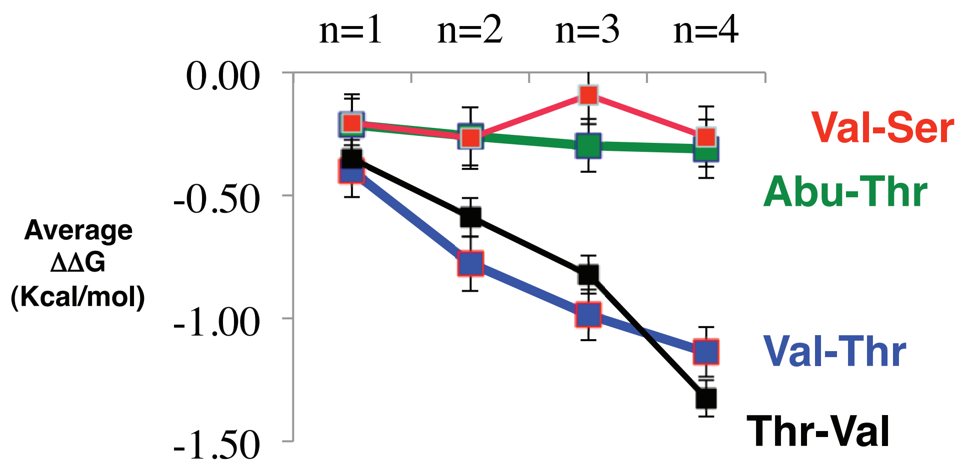


Increment per extension (n → n+1)

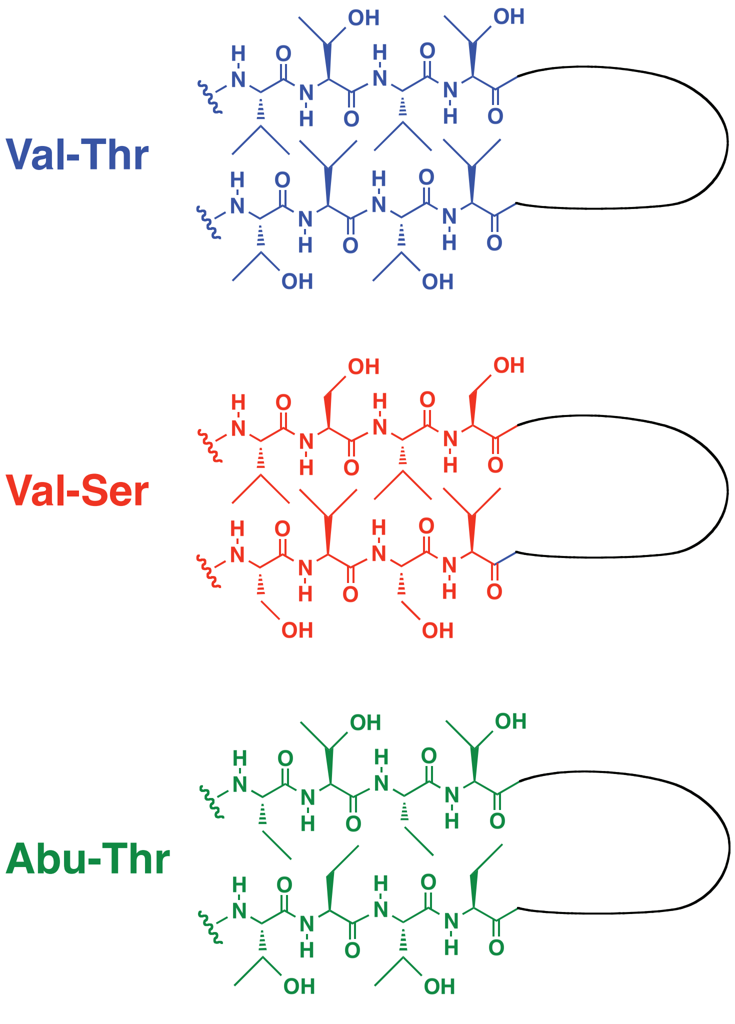


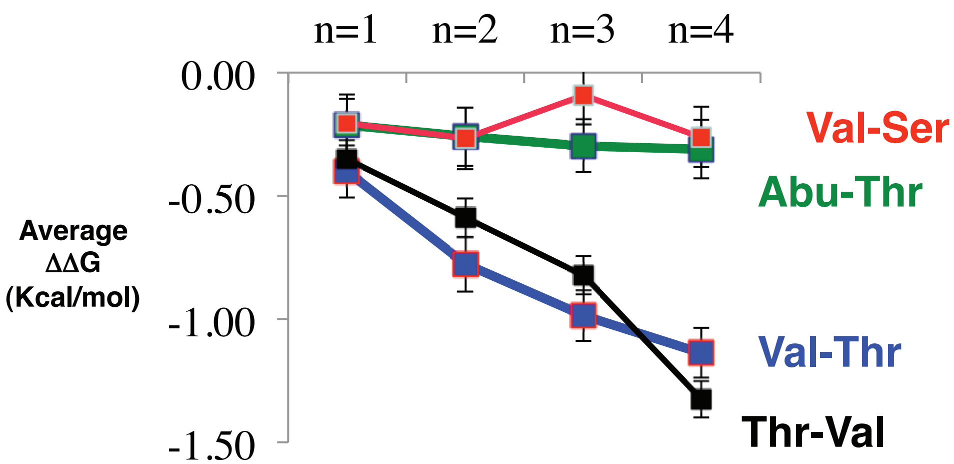
Side Chain Branching Required for Length-Dependent Stabilization of Parallel β-Sheet





Side Chain Branching Required for Length-Dependent Stabilization of Parallel β-Sheet

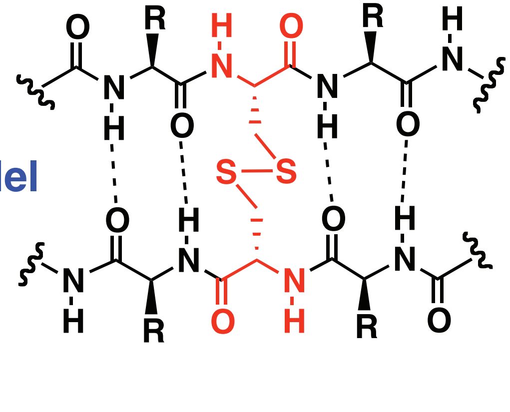




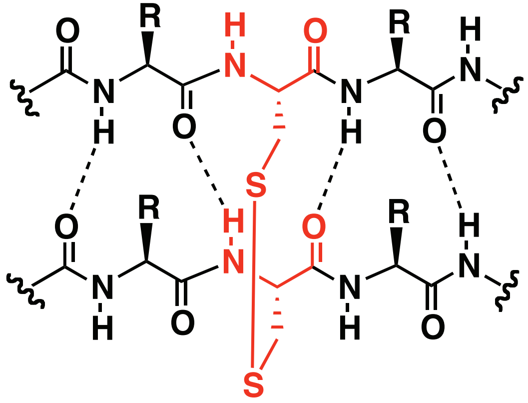
Contrast with antiparallel β-sheet:
No evidence of an intrinsic limit on strand length in parallel β-sheet.

Are Inter-Strand Disulfides Incompatible with Parallel β-Sheet?

Antiparallel



vs.

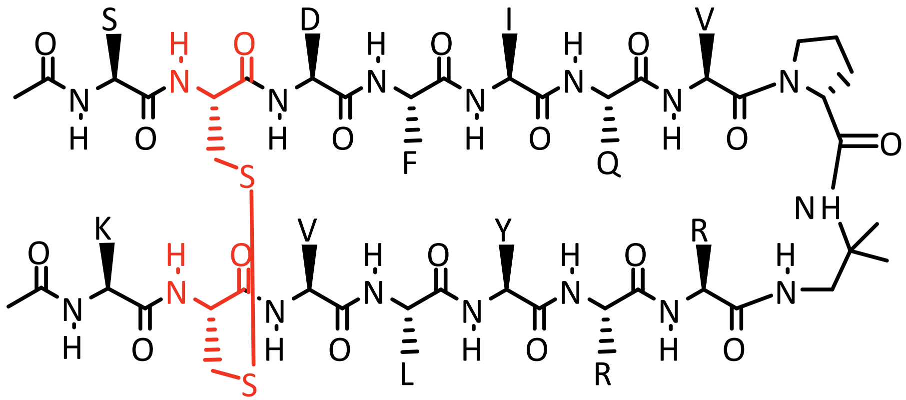


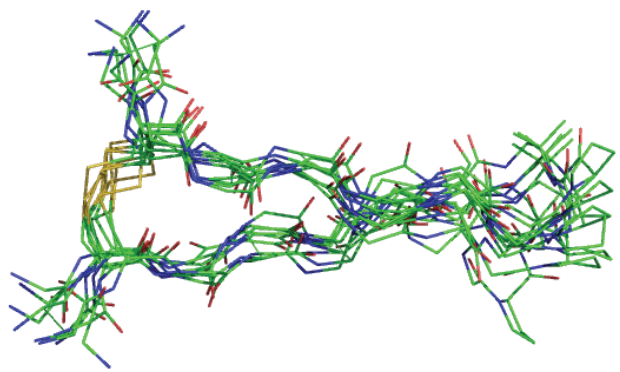
Parallel

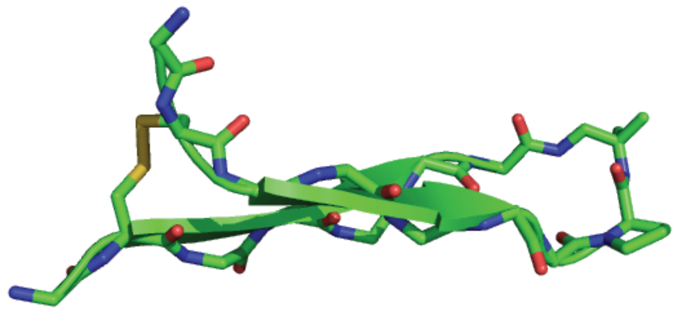
Common in Proteins
&
Known to Stabilize β-Hairpins

Rare in Proteins

Disulfide-Containing Parallel β-Sheet in Aqueous Solution: Structural Analysis Indicates Termination of the Sheet







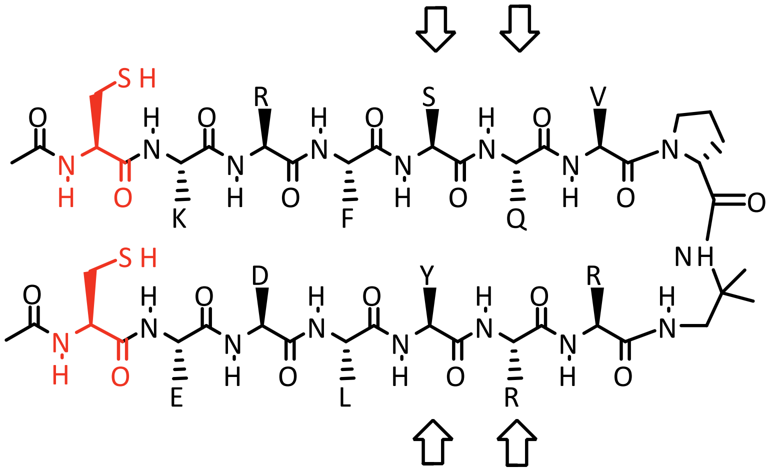
10 Best Structures from 2D NMR, pH 3.8, 4°C, NOE-restrained dynamics (CNS)

Average Structure

Almeida, Li, Gellman *J. Am. Chem. Soc.* 134:75 (2012)

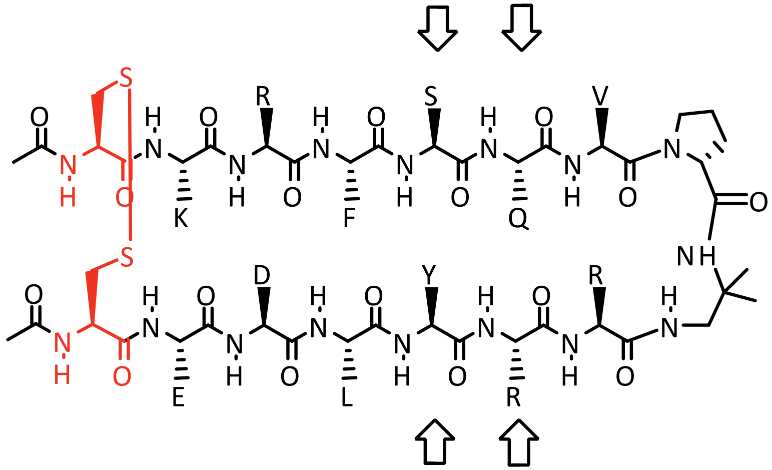
Quantifying Disulfide Effect on Parallel β-Sheet Stability

Peptide SH

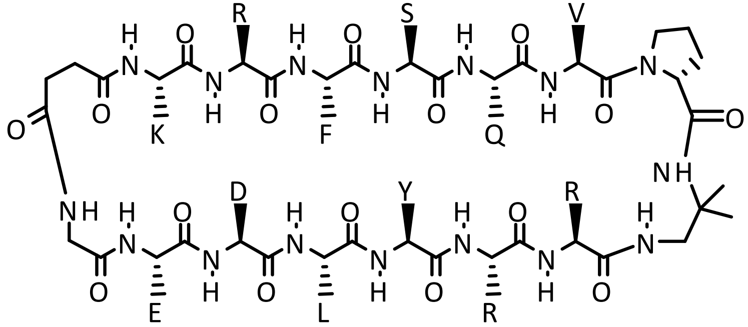


vs.

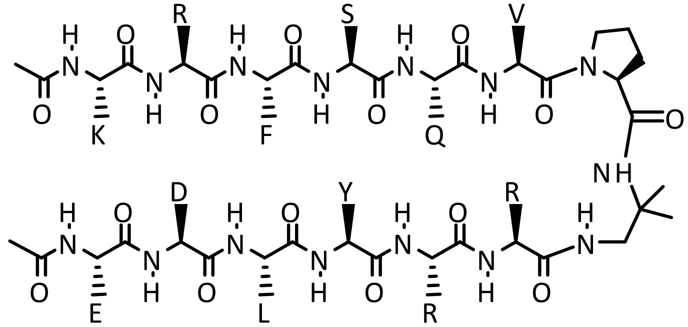
Peptide SS

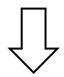


Highly Folded Control



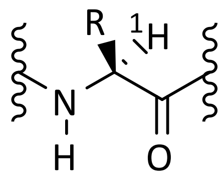
Unfolded Control

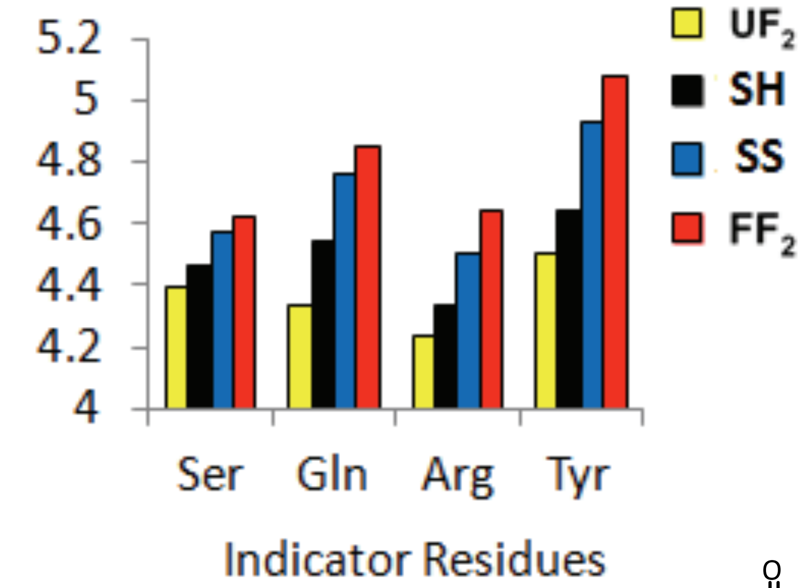




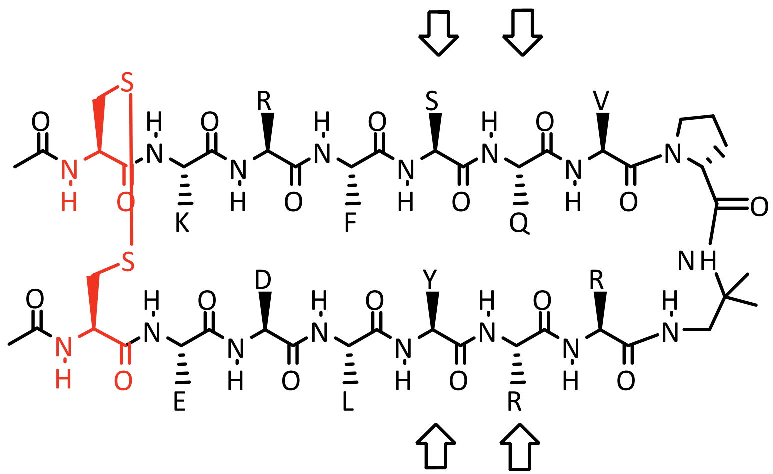
= site of population analysis ($\Delta\delta C_\alpha H$)

Chemical Shift Comparisons ($\delta C_{\alpha}H$)

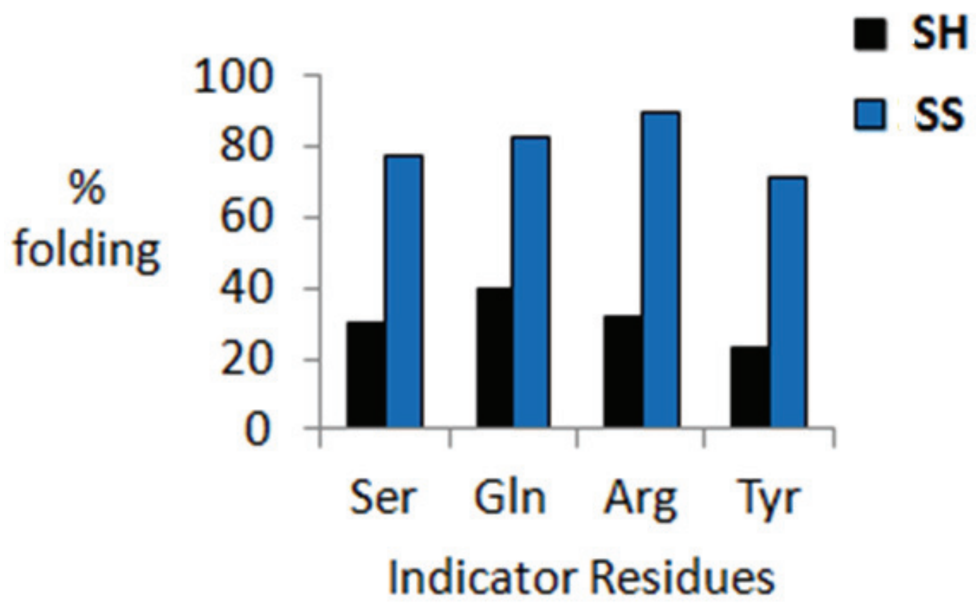




SS

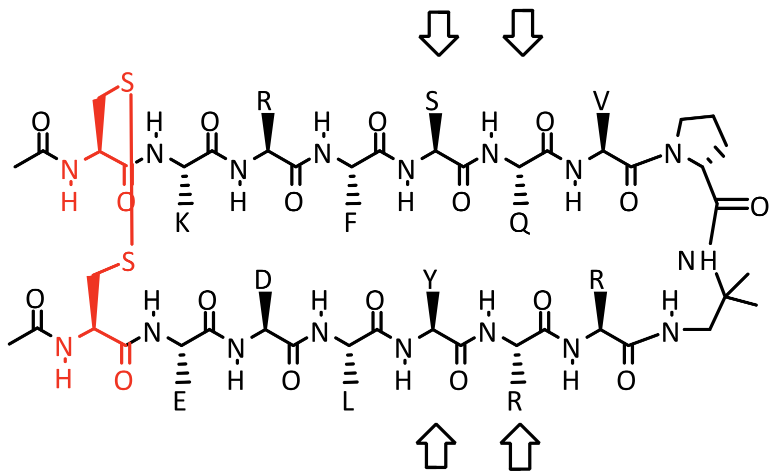


β-Sheet Population Analysis: Impact of the Disulfide



$$\beta\ sheet\ population\ (\%) = \frac{\delta_{obs} - \delta_U}{\delta_F - \delta_U}$$

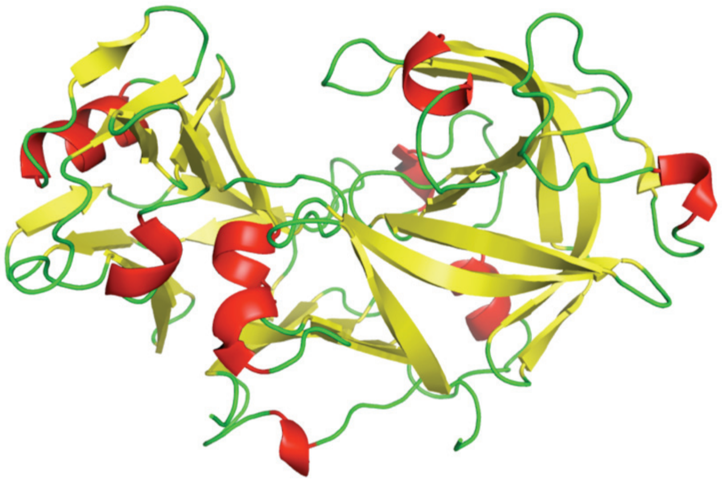
SS



Parallel β-Sheet Stabilization by disulfide ≈ 1.1 ± 0.1 kcal/mole

Stabilization & Termination

Conclusion: Autonomously Folding Secondary Structures Enable Evaluation of Sequence-Stability Relationships that would be Difficult to Assess in Proteins (Tertiary Structure Context)



Pepsin (PDB 6APR)

vs.