Short Course:

Solution Structure Determination
In Organic Chemistry and Chemical Biology

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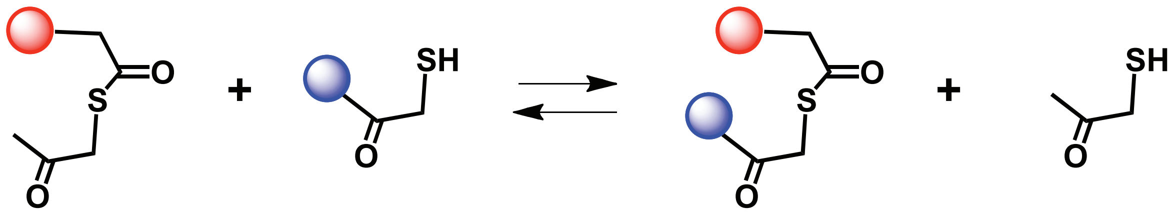
IV. Themodynamic analysis of minimal protein tertiary structural units

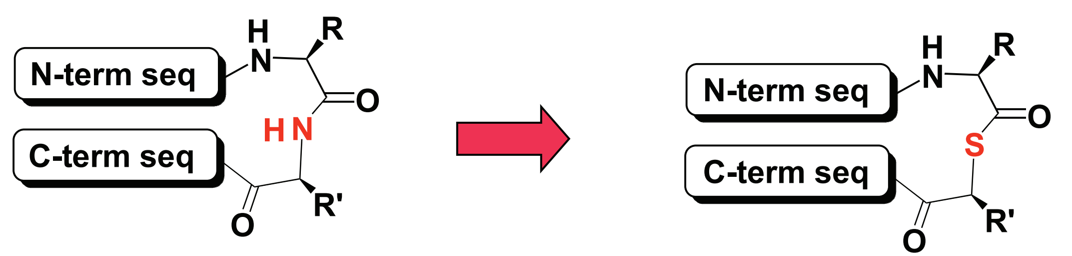
Solution Structure Analysis From a Reaction Equilibrium



Thioester Thiol Thioester' Thiol'

Thiol-Thioester Exchange

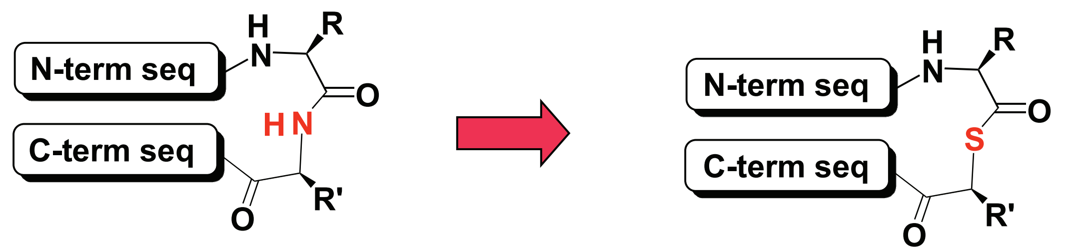
Backbone Thioester Exchange (BTE)



Protein of interest

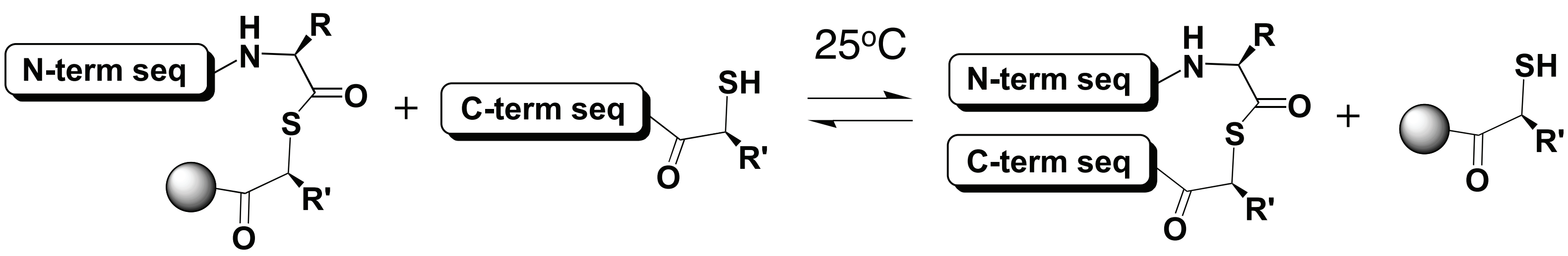
Thioester analogue

Backbone Thioester Exchange (BTE)

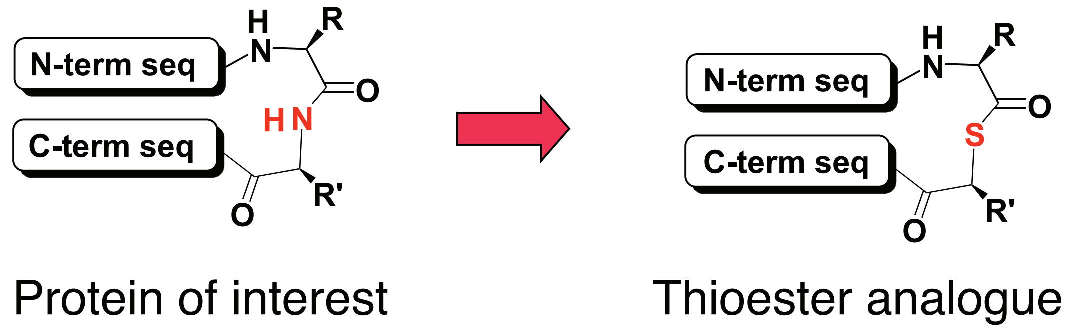


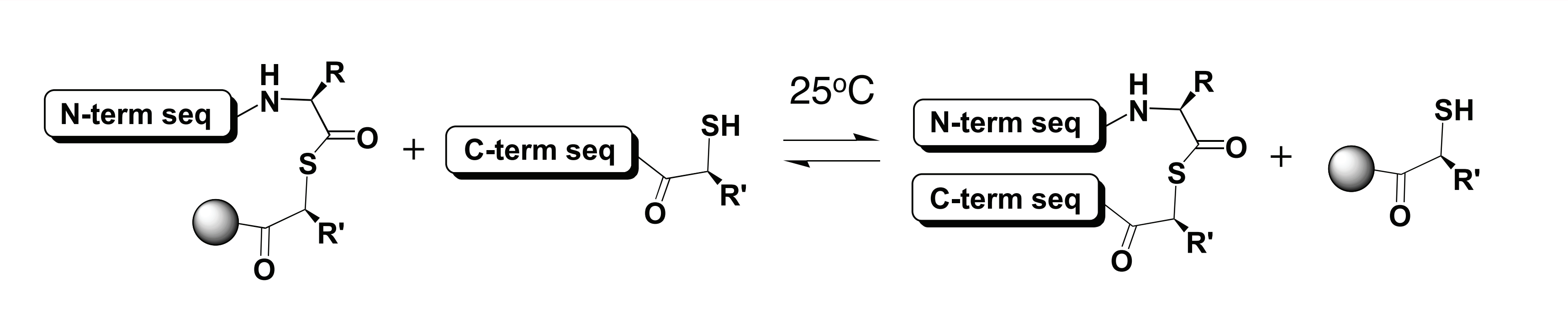
Protein of interest

Thioester analogue



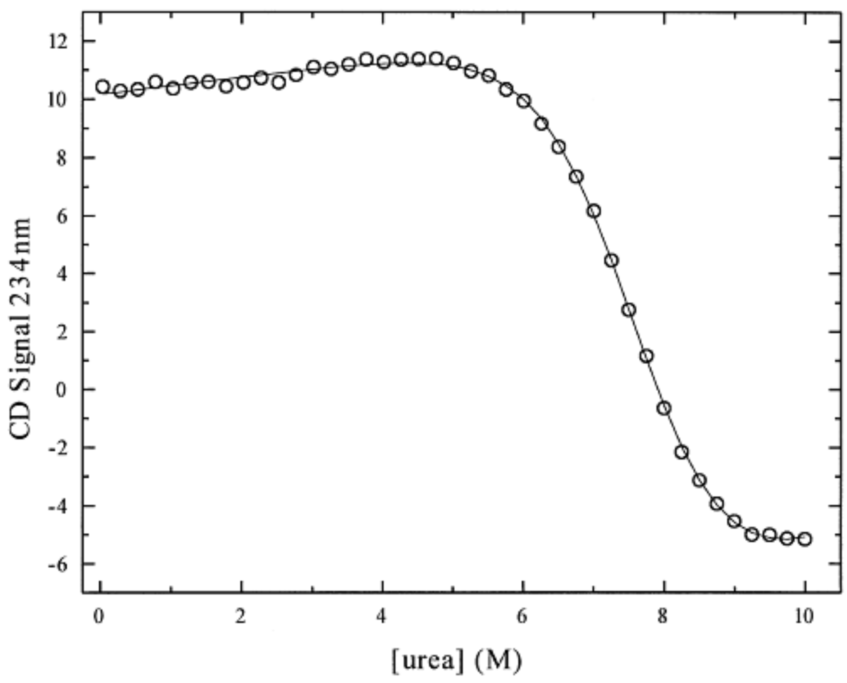
Backbone Thioester Exchange (BTE)

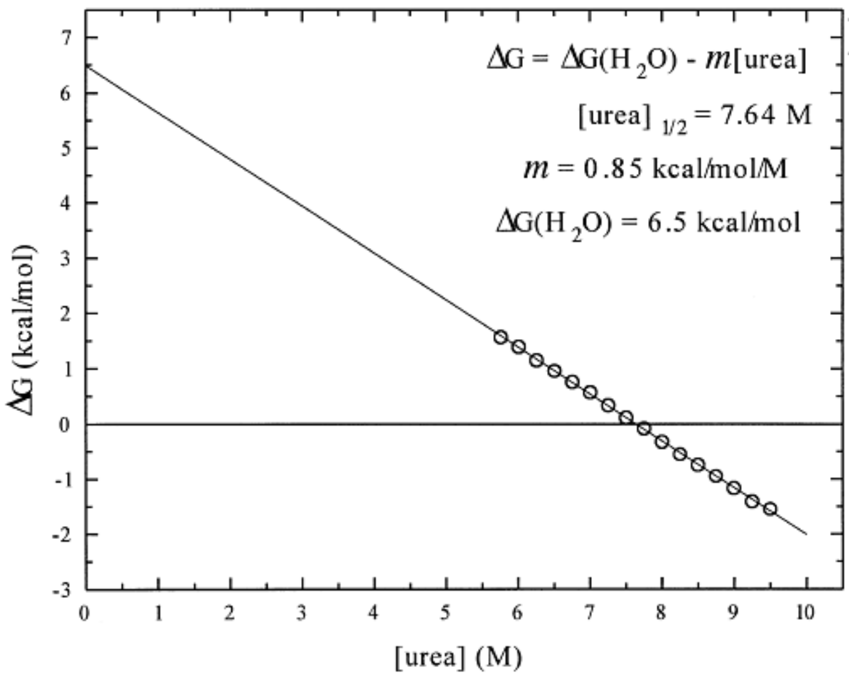




- Thiol/thioester exchange occurs rapidly under native conditions, without interference from other reactions (cf. NCL).
- Thioester and 2° amide are conformationally similar.
- <u>Limitation</u>: Replaced amide should not form internal H-bonds.

BTE Measurements Made Under Native Conditions.
Contrast: Chemical or Thermal Denaturation





Example: RNase Sa

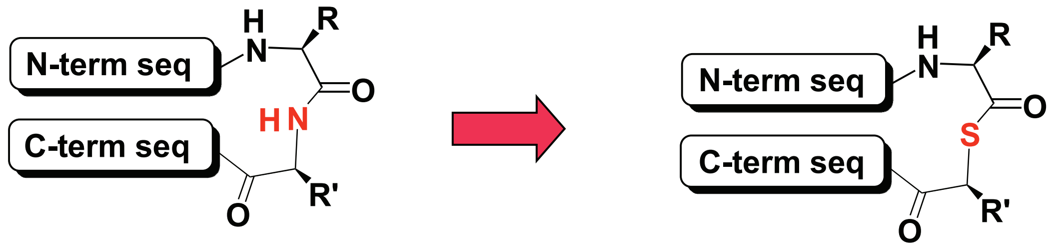
Pace & Shaw *Proteins Struct. Funct. Genet.* **2000**, *Suppl. 4*, 1.

Concerns

1) Extrapolations to native conditions (e.g., zero denaturant) may not be valid.

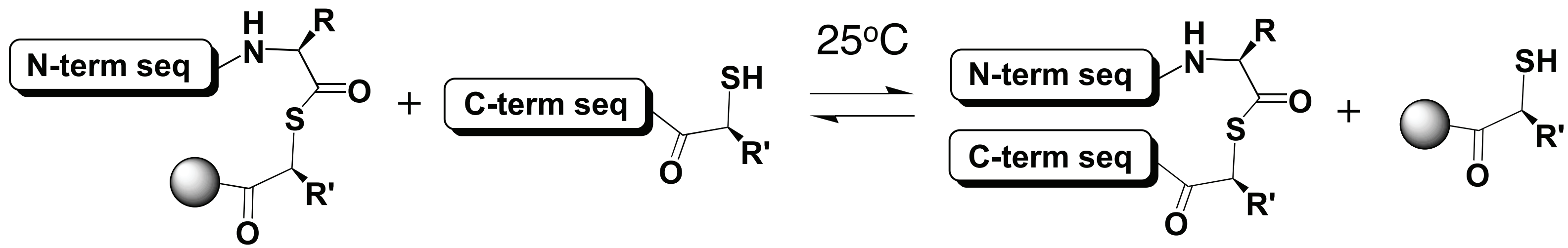
2) Comparisons among mutants can be problematic, particularly for small proteins, in which single mutations can cause dramatic loss of conformational stability.

Backbone Thioester Exchange (BTE)



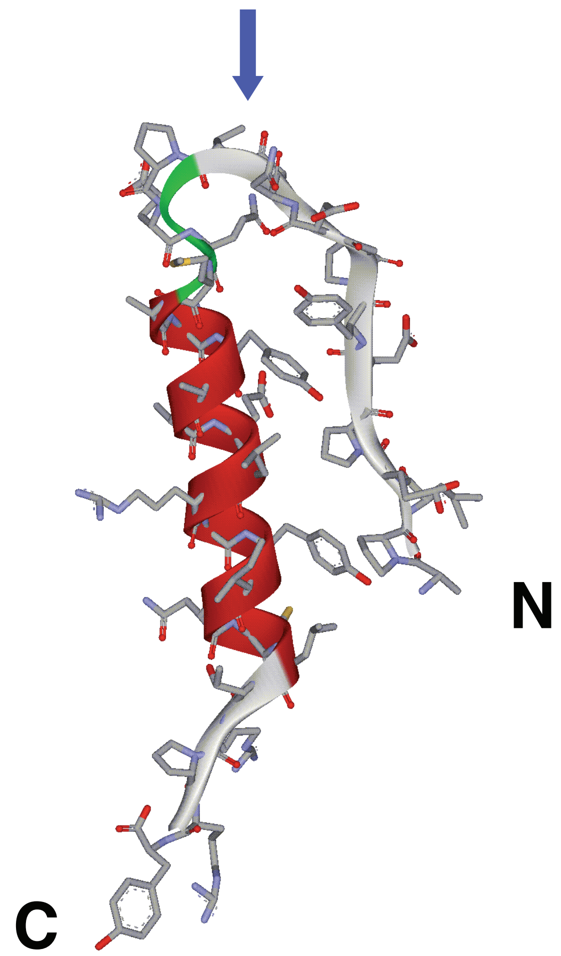
Protein of interest

Thioester analogue



- Thiol/thioester exchange occurs rapidly under native conditions, without interference from other reactions (cf. NCL).
- Thioester and 2° amide are conformationally similar.
- Limitation: Replaced amide should not form internal H-bonds.

First Application: Bovine Pancreatic Polypeptide (bPP)



NMR structure in aqueous solution:
Li et al. *Biochemistry* **1992**, *31*, 1245.
(PDB: 1BBA)

36 residues

Tertiary Structure:
α-Helix packed against a PPII helix.

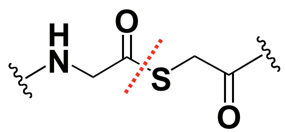
Quaternary Structure: Homodimer

Sequence Design

bPP	H_2N - **APLEP EYP GD NATPE QMAQY AAELR RYINM LTRPR Y** - $CONH_2$
Mutant (m-bPP)	H_2N - **APREP EYP GG NATPE QMAQY AAELR RYINM LTRPR Y** - $CONH_2$
Thioester (t-bPP)	H_2N - **APREP EYP GX NATPE QMAQY AAELR RYINM LTRPR Y** - $CONH_2$

("Pseudo-wild type")

...GX... =



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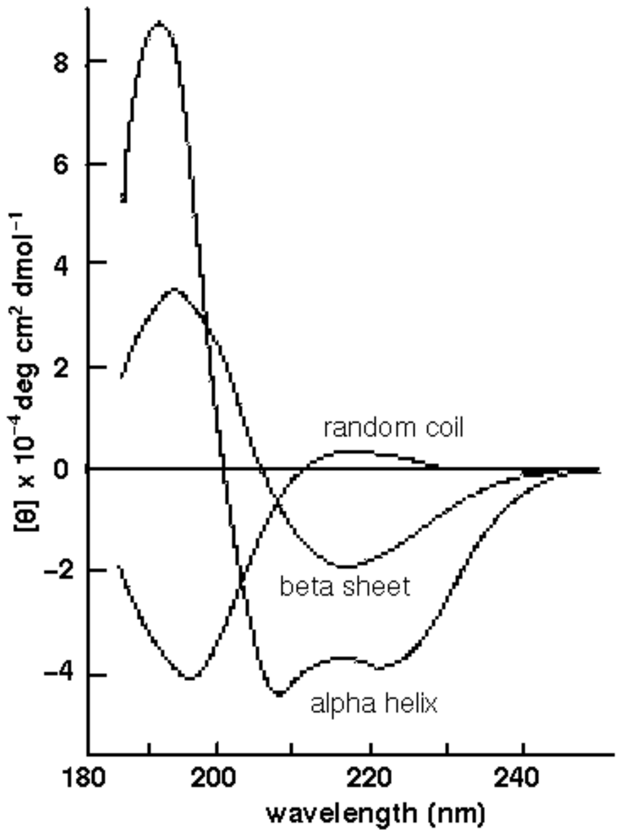
Circular Dichroism (CD) as a Tool for Polypeptide Structure Analysis in Solution

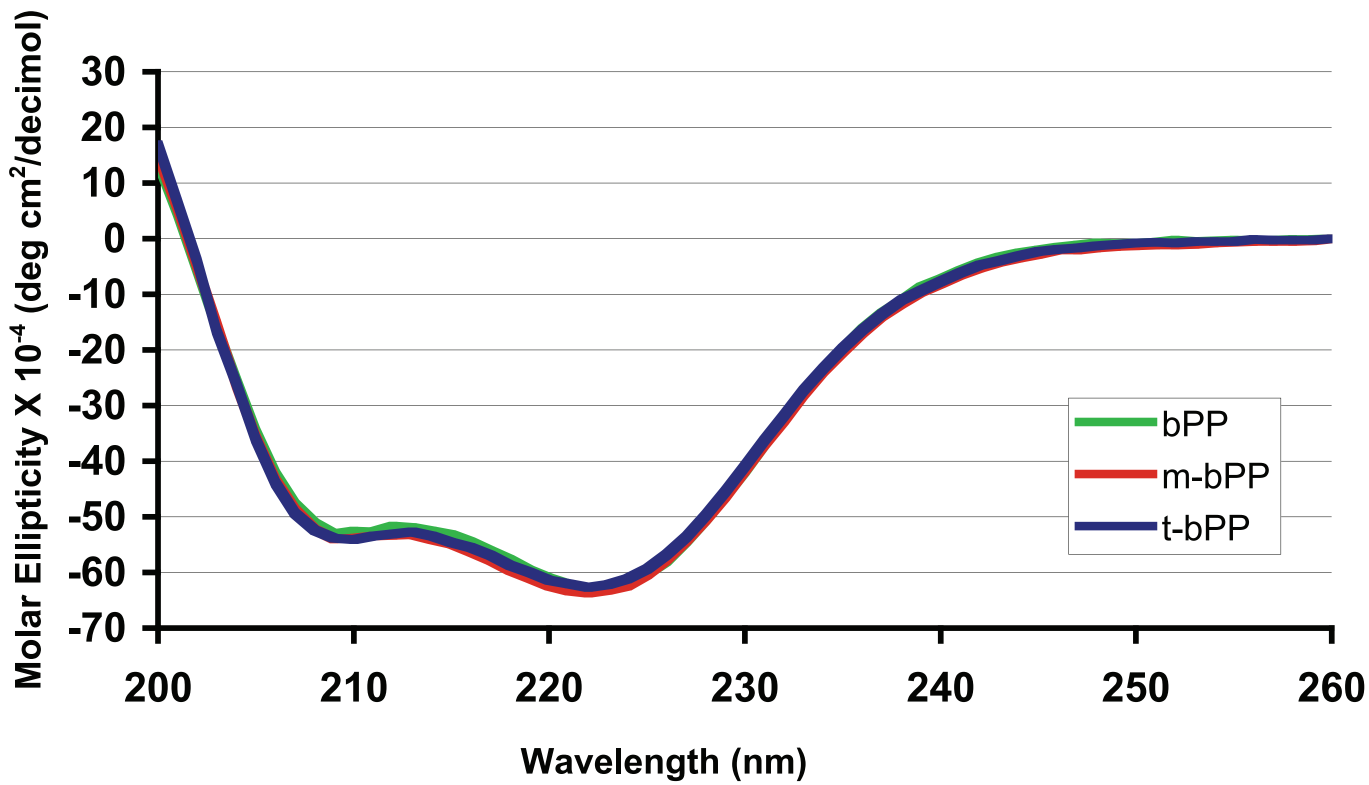
Circular dichroism = difference in absorption of left and right circularly polarized light.

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



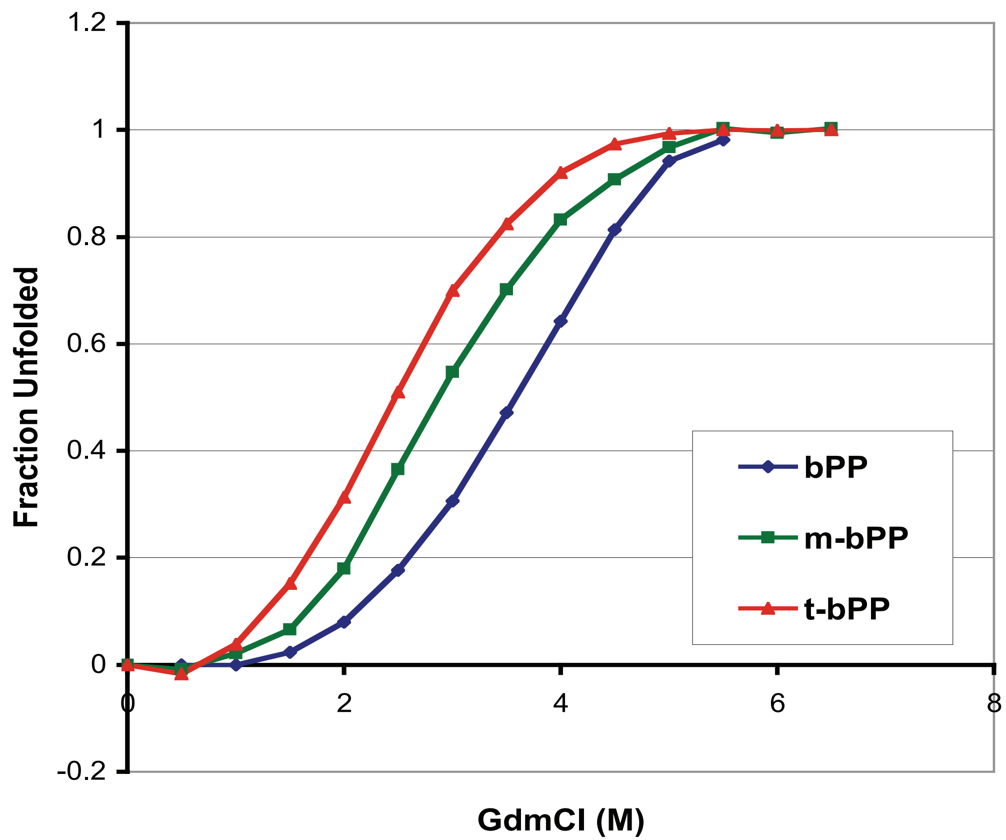
cryst.bbk.ac.uk

Circular Dichroism (CD) Comparison: bPP vs. double mutant (m-bPP) vs. thioester (t-bPP)



Room Temp, 50 mM Phosphate buffer, pH 7

Guanidinium Chloride Denaturation Data
(Derived from CD signal at 225 nm)



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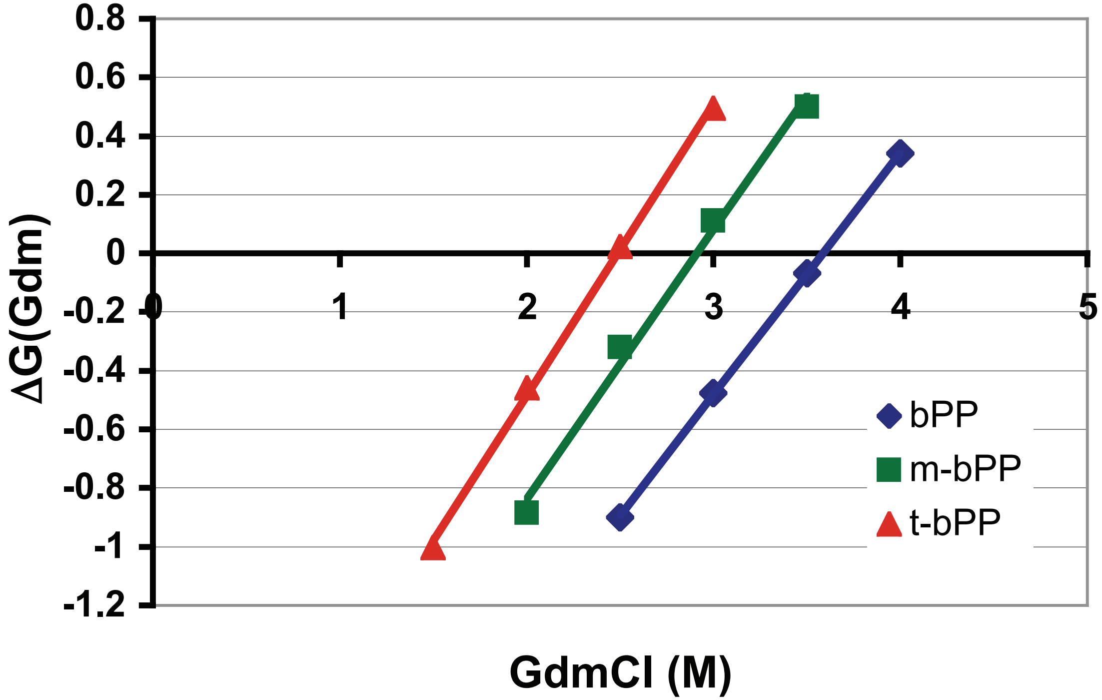
bPP

m-bPP (L3R,D10G)

t-bPP (L3R,D10thioGlyc)

Folding Free Energy ($\Delta G_{fold/Gdm}$)
(extrapolation to native conditions)

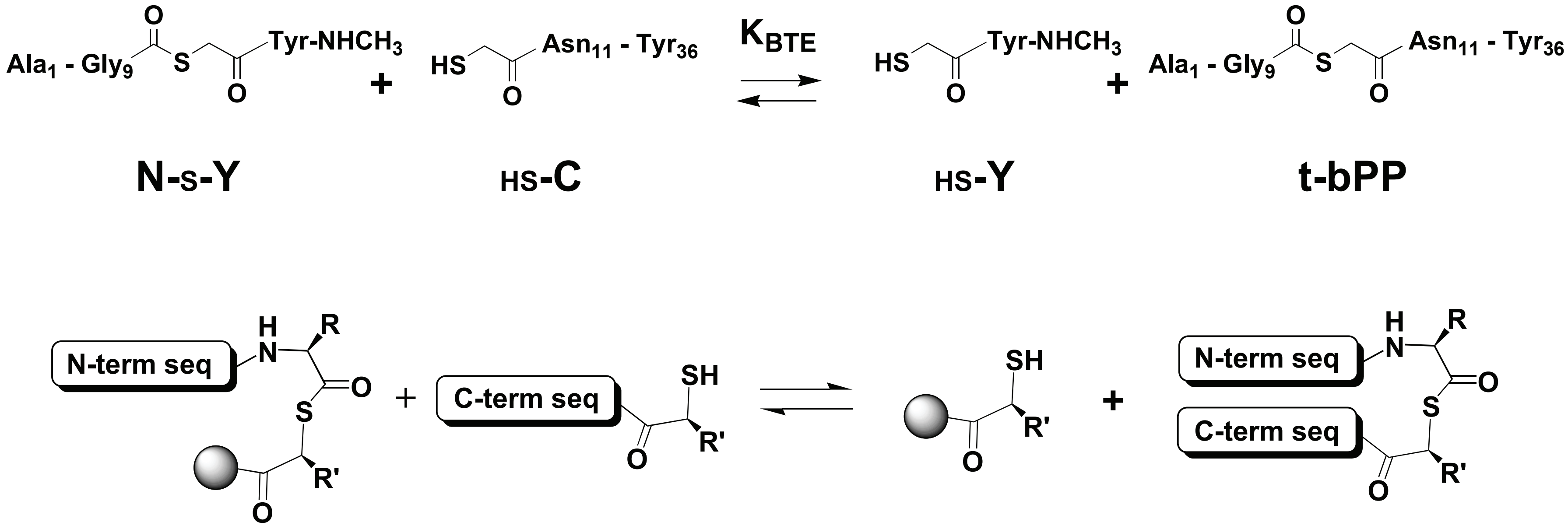


bPP: $\Delta G_{fold/Gdm}$ = -3.0 kcal/mol

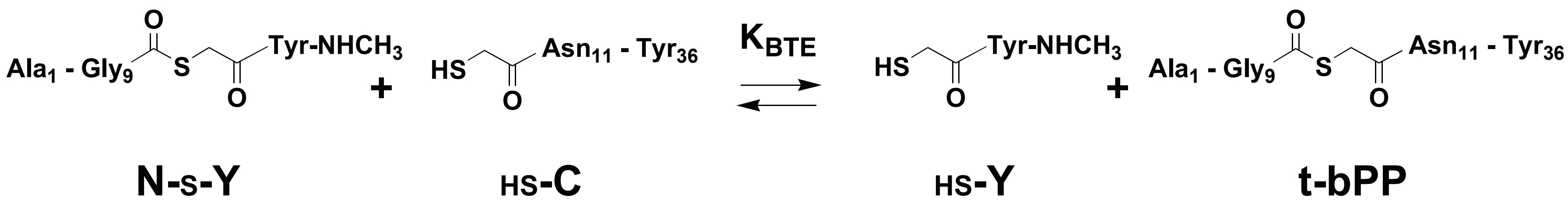
m-bPP (L3R,D10G): $\Delta G_{fold/Gdm}$ = -2.7 kcal/mol

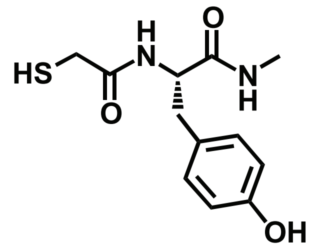
t-bPP (L3R,D10thioGlyc): $\Delta G_{fold/Gdm}$ = -2.5 kcal/mol

Measuring K_{BTE}: HPLC Analysis of Equilibrium Mixture



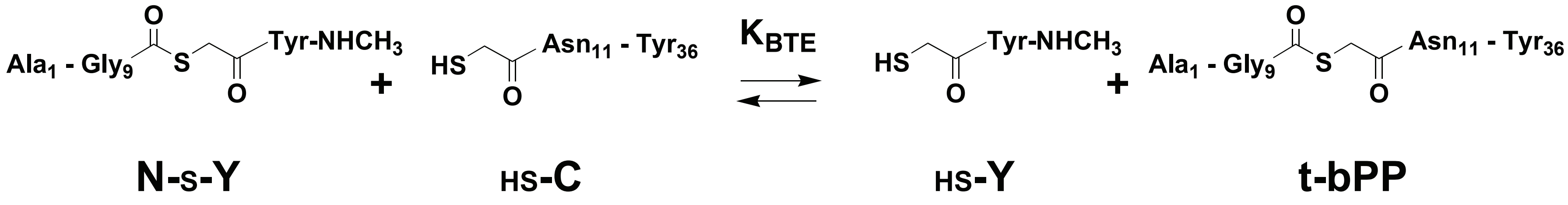
Measuring K_{BTE}: HPLC Analysis of Equilibrium Mixture

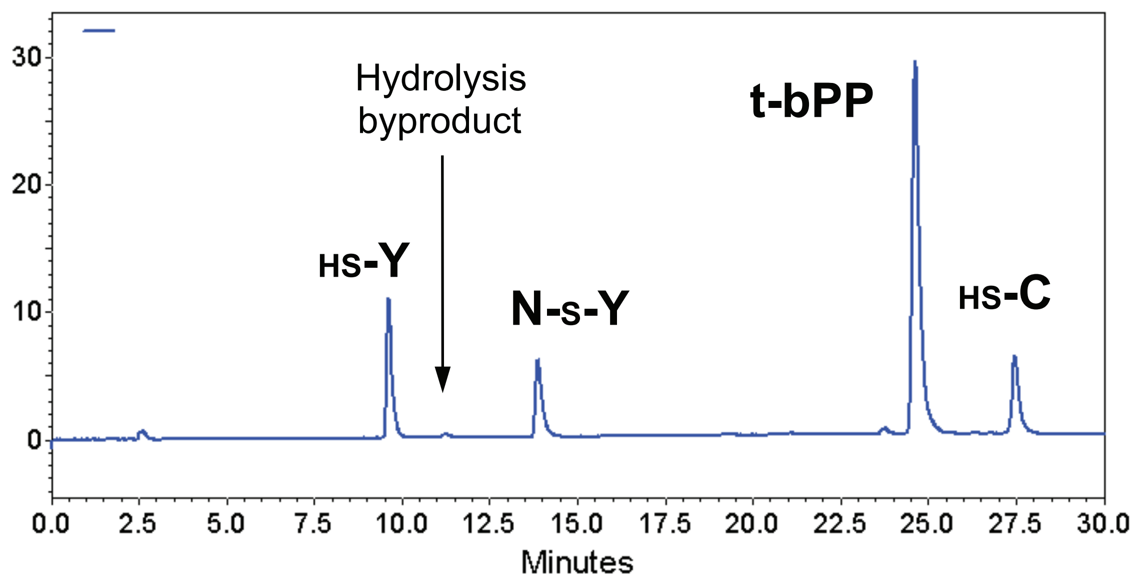




Note: Chromophore

Measuring K_{BTE}: HPLC Analysis of Equilibrium Mixture

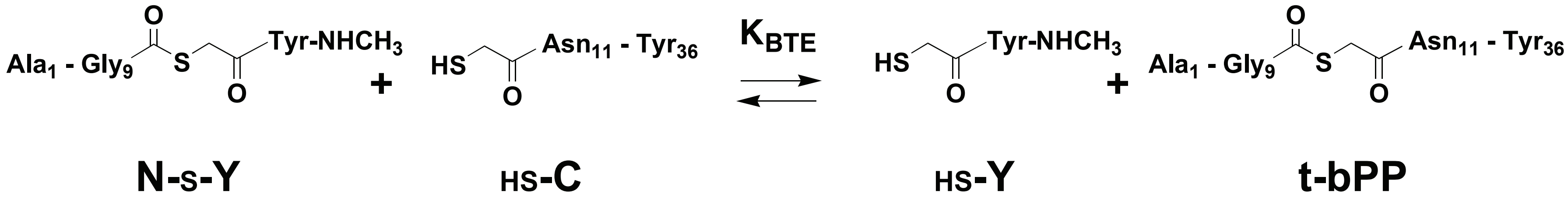


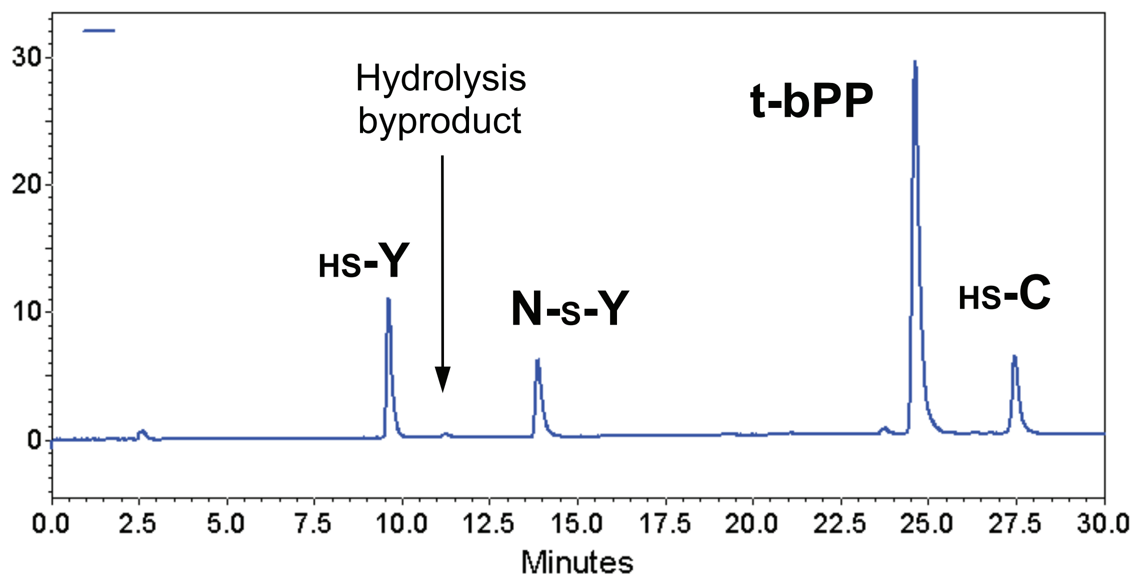


2.5 hr

[50 mM buffer, pH 7; 2 mM $P(CH_2CH_2CO_2^-)_3$]

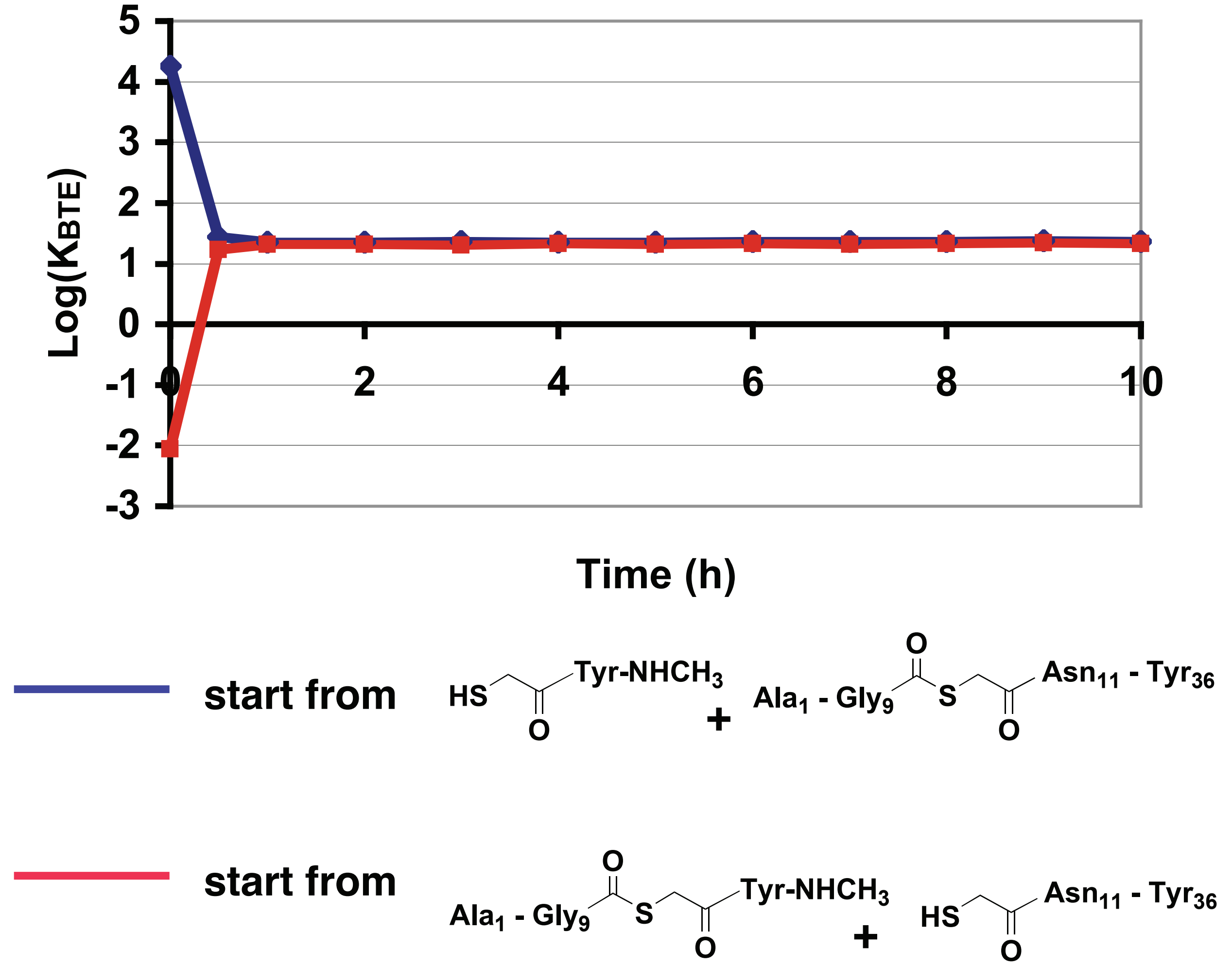
Measuring K_{BTE}: HPLC Analysis of Equilibrium Mixture





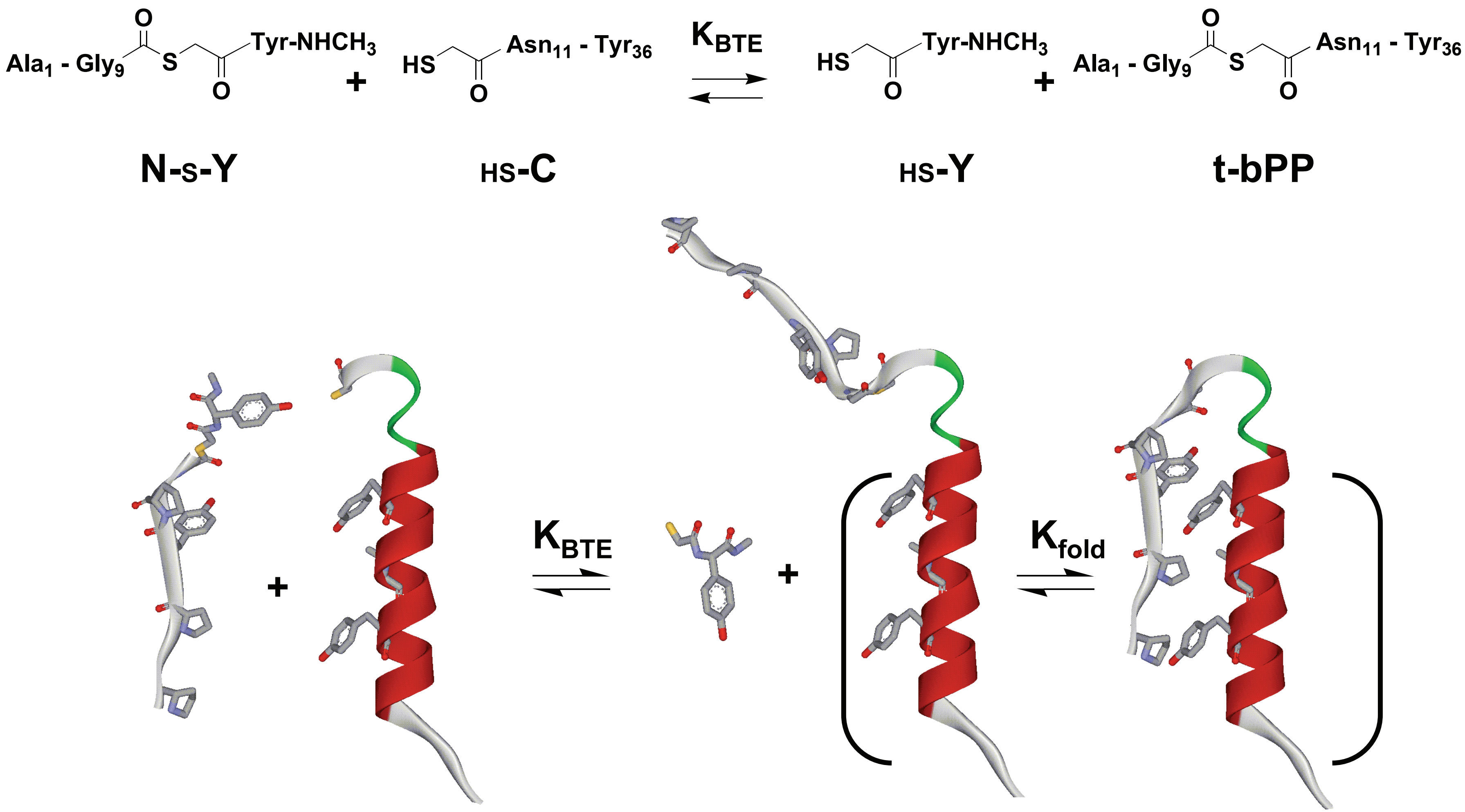
$$K_{BTE} = \frac{[\text{HS-Y}]\,[\text{t-bPP}]}{[\text{N-S-Y}]\,[\text{HS-C}]}$$

Control: Start from Alternative Thiol/Thioester Pairs

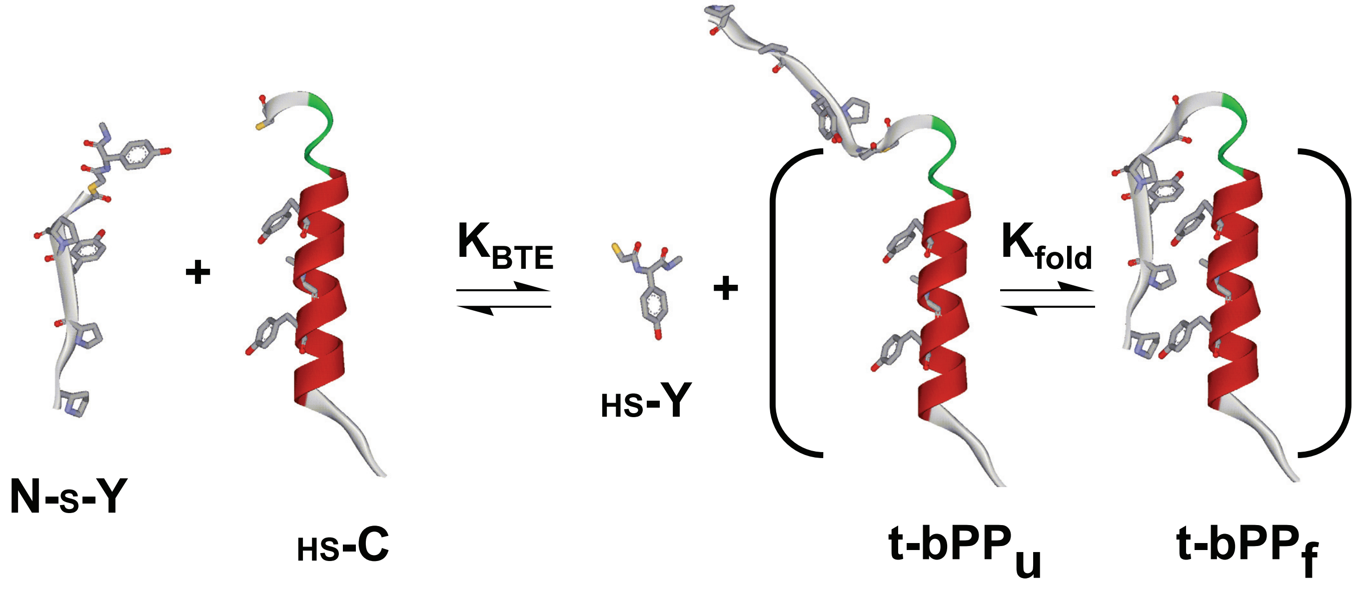


50 mM Phosphate buffer, pH 7; 2 mM $P(CH_2CH_2CO_2^-)_3$

Backbone Thioester Exchange: Embedded Folding Equilibrium



What is the relationship between K_{BTE} and K_{fold}?



$$K_{BTE} = \frac{[\text{HS-Y}]\{[\text{t-bPP}_u] + [\text{t-bPP}_f]\}}{[\text{N-S-Y}][\text{HS-C}]}$$

$$K_{fold} = \frac{\text{t-bPP}_f}{\text{t-bPP}_u}$$

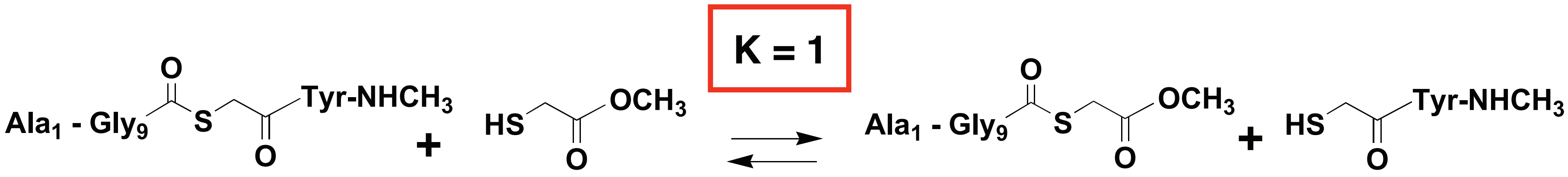
$$\therefore K_{BTE} = \frac{[\text{HS-Y}](K_{fold} + 1)[\text{t-bPP}_u]}{[\text{N-S-Y}][\text{HS-C}]}$$

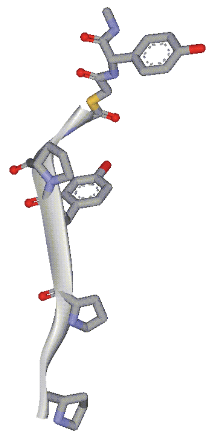
$$\frac{[\text{HS-Y}][\text{t-bPP}_u]}{[\text{N-S-Y}][\text{HS-C}]} = 1$$

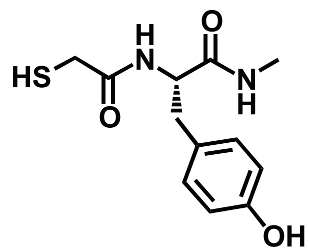
$$\therefore K_{BTE} = (K_{fold} + 1)$$

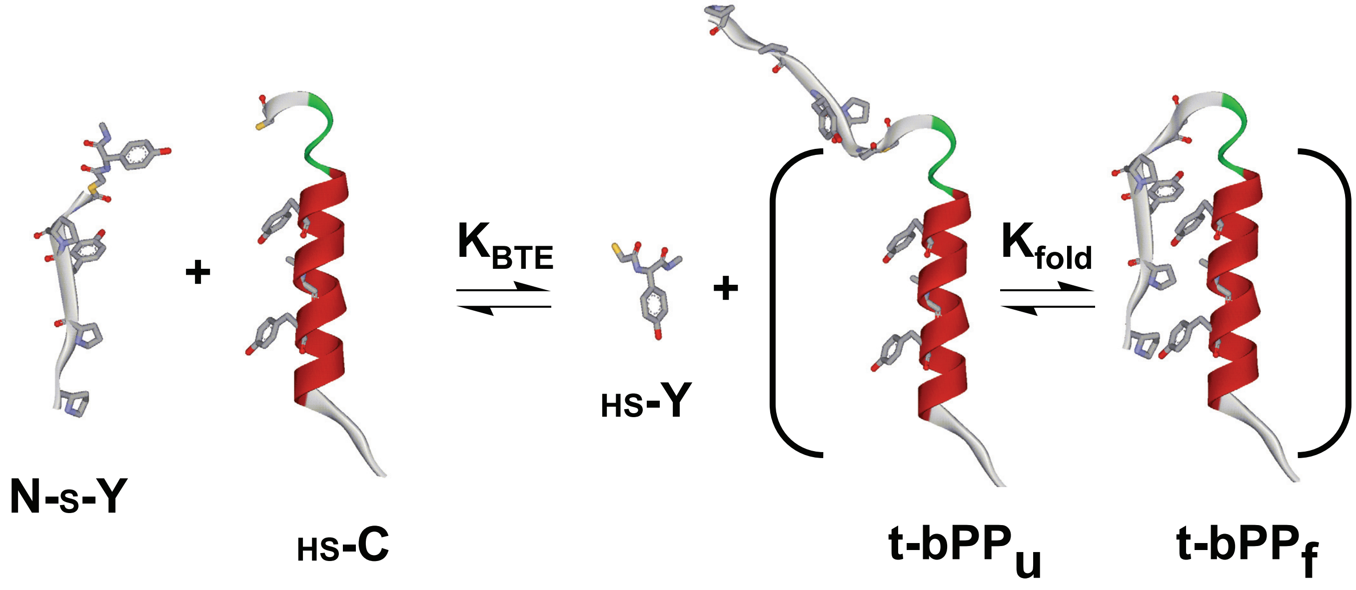
$$\Delta G_{Fold/BTE} = -RT\ln K_{fold}$$

Control: No Interaction Between Tyr Side Chain and N-Terminal Segment









$$K_{BTE} = \frac{[\text{HS-Y}]\{[\text{t-bPP}_u] + [\text{t-bPP}_f]\}}{[\text{N-S-Y}][\text{HS-C}]}$$

$$K_{fold} = \frac{\text{t-bPP}_f}{\text{t-bPP}_u}$$

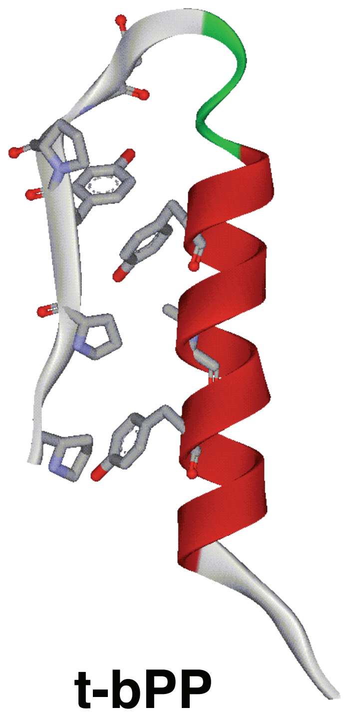
$$\therefore\ K_{BTE} = \frac{[\text{HS-Y}](K_{fold} + 1)[\text{t-bPP}_u]}{[\text{N-S-Y}][\text{HS-C}]}$$

$$\frac{[\text{HS-Y}][\text{t-bPP}_u]}{[\text{N-S-Y}][\text{HS-C}]} = 1$$

$$\therefore\ K_{BTE} = (K_{fold} + 1)$$

$$\Delta G_{Fold/BTE} = -RT\ln K_{fold}$$

$\Delta G_{Fold/BTE}$ vs. $\Delta G_{Fold/Gdm}$



t-bPP

$\Delta G_{Fold/BTE}$ = -1.4 kcal/mol

vs.

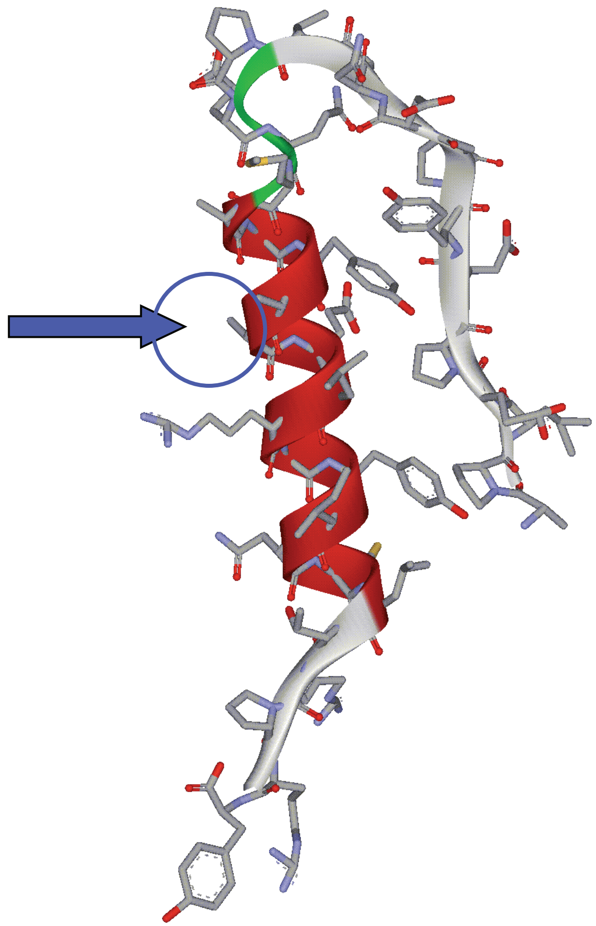
$\Delta G_{Fold/Gdm}$ = -2.5 kcal/mol

Why is $\Delta G_{Fold/BTE} < \Delta G_{Fold/Gdm}$?

<u>Hypothesis</u>: α-Helical segment disrupted by GdmCl but largely intact under native conditions (BTE)

Test Hypothesis: Ala-22 --> Gly Mutant of t-bPP

Ala 22 --> Gly



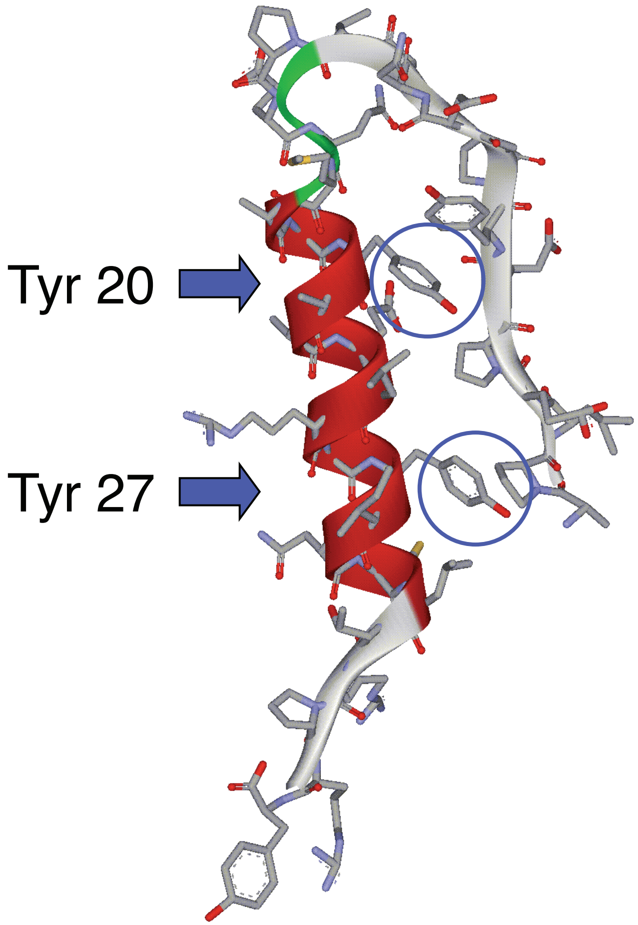
$\Delta G_{Fold/BTE}$ = -0.8 kcal/mol

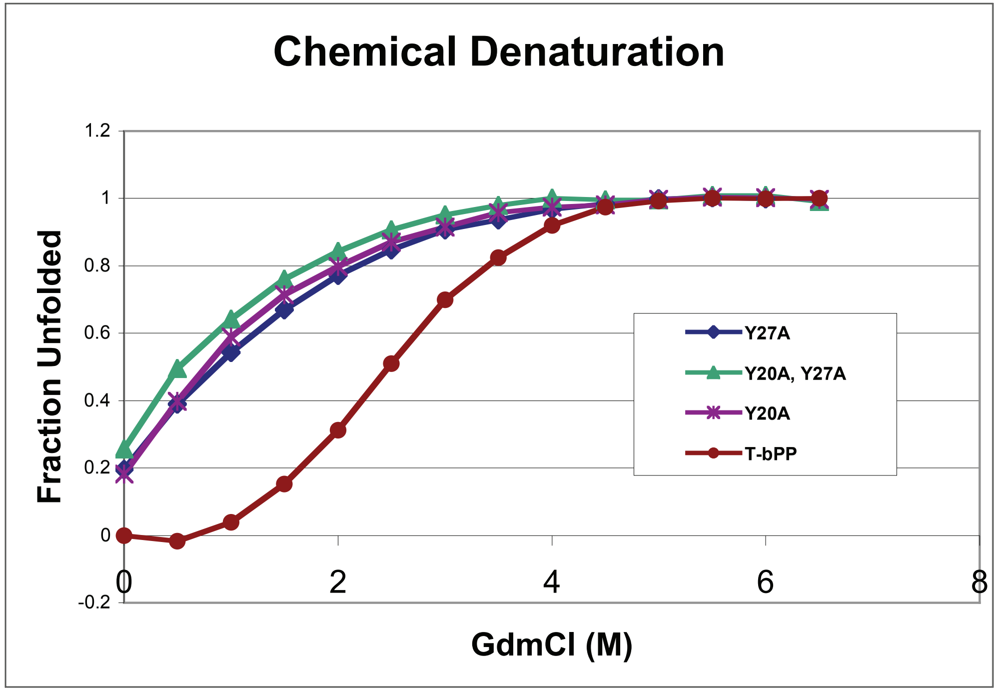
$\Delta G_{Fold/Gdm}$ ~ -0.7 kcal/mol

M. Woll

(Destabilize α-Helix without affecting tertiary or quaternary contact surfaces.)

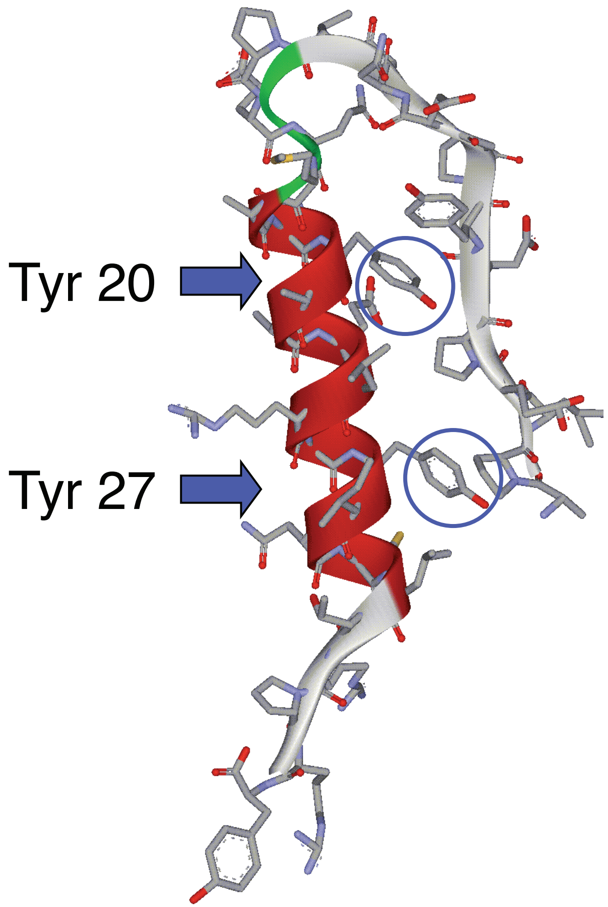
Probe Tertiary (and Perhaps Quaternary) Contact Surface: Tyr --> Ala Mutations

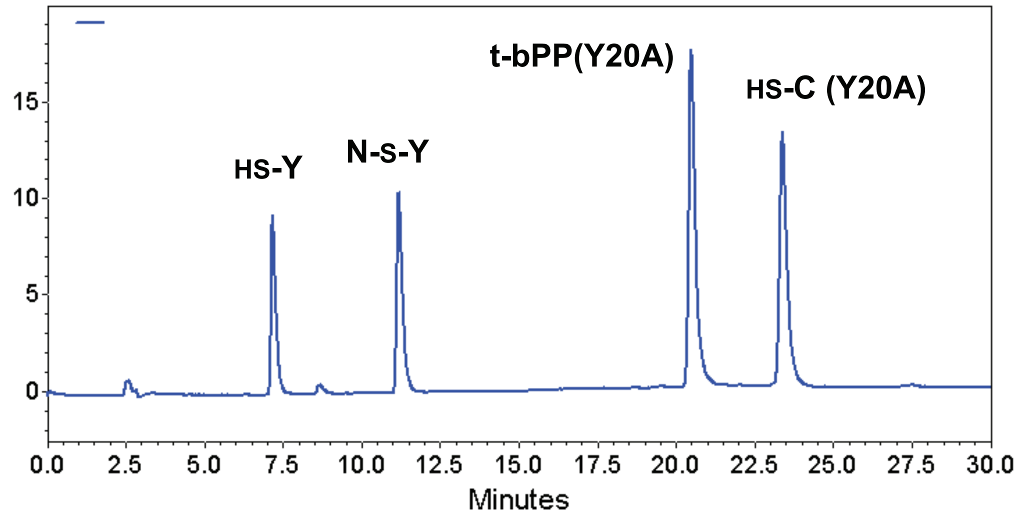




$\Delta G_{fold/Gdm}$ cannot be accurately determined because of partial folding.

The BTE Method Can Be Applied to Partially Folded Species





t-bPP: $\Delta G_{Fold/BTE}$ = -1.4 kcal/mol

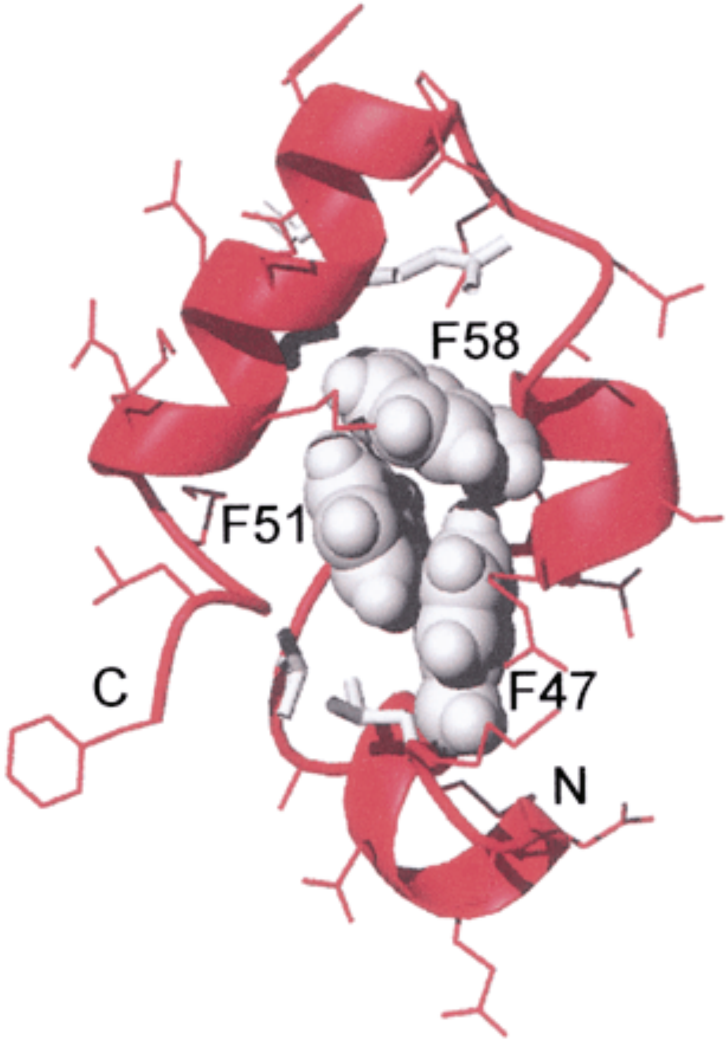
t-bPP(Y20A): $\Delta G_{Fold/BTE}$ = +0.8 kcal/mol

t-bPP(Y27A): $\Delta G_{Fold/BTE}$ = +0.1 kcal/mol

t-bPP(Y20,27A): $\Delta G_{Fold/BTE}$ = +1.1 kcal/mol

Woll & Gellman *J. Am. Chem. Soc.* 126:11172 (2004)

Second BTE Application:
Villin Headpiece Subdomain

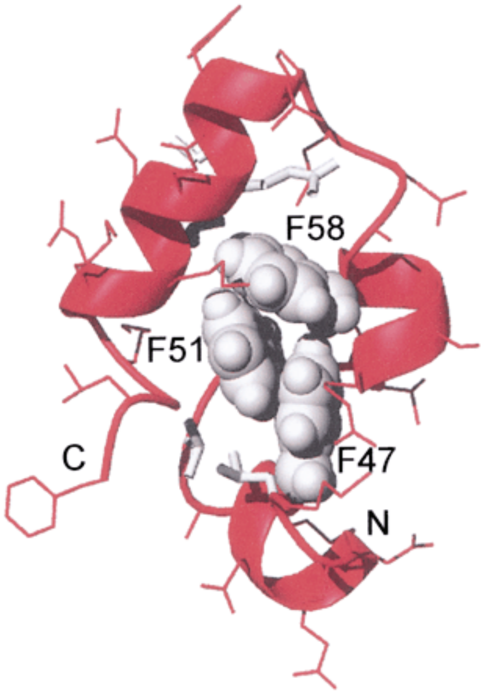


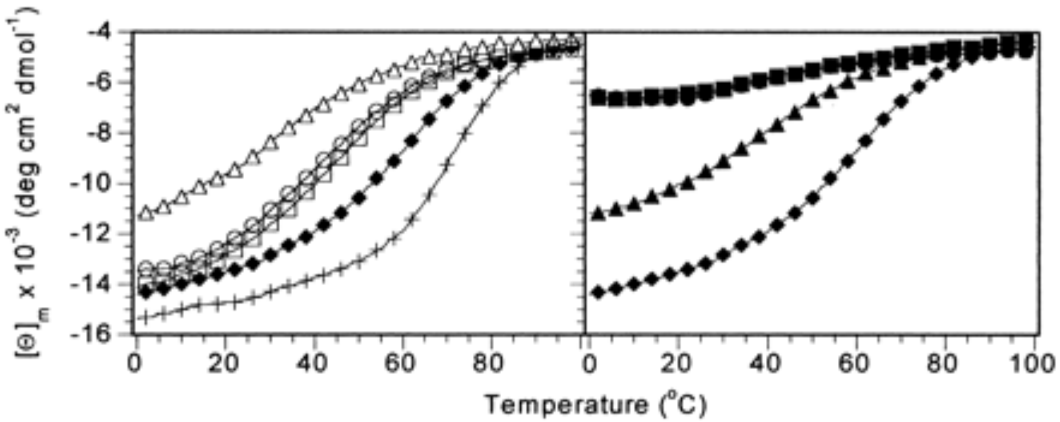
VHP = 35 Residues

McKnight, Matsudaira & Kim
Nat. Struct. Biol **1997**, *4*, 180.

Frank, Vardar, Buckley & McKnight
Protein Sci. **2002**, *11*, 680.

Villin Headpiece Subdomain: Phe --> Leu Mutations
(Fully Folded Endpoint Problem)





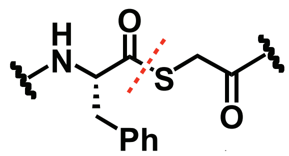
Single Mutants   Double Mutants

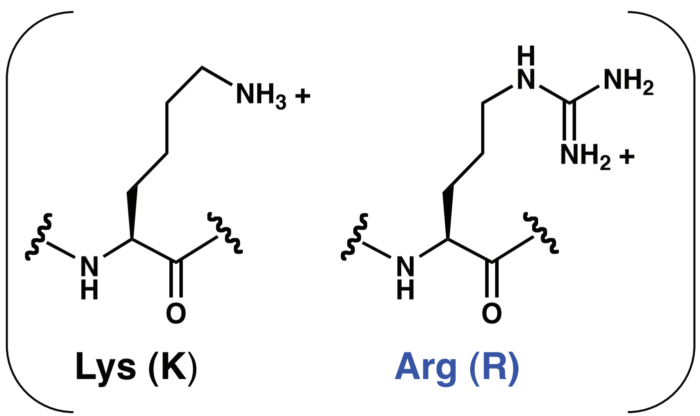
Frank, Vardar, Buckley & McKnight *Protein Sci.* **2002**, *11*, 680.

BTE Sequence Design

VHP	H_2N - LSDED FKAV F G MTRS AFANL PLWKQ QNLKK EKGLF - CO_2H
m-VHP	H_2N - LSDED FRAV F G MTRS AFANL PLWRQ QNLRR ERGLF - CO_2H
t-VHP	H_2N - LSDED FRAV F X MTRS AFANL PLWRQ QNLRR ERGLF - CO_2H

...F X... =

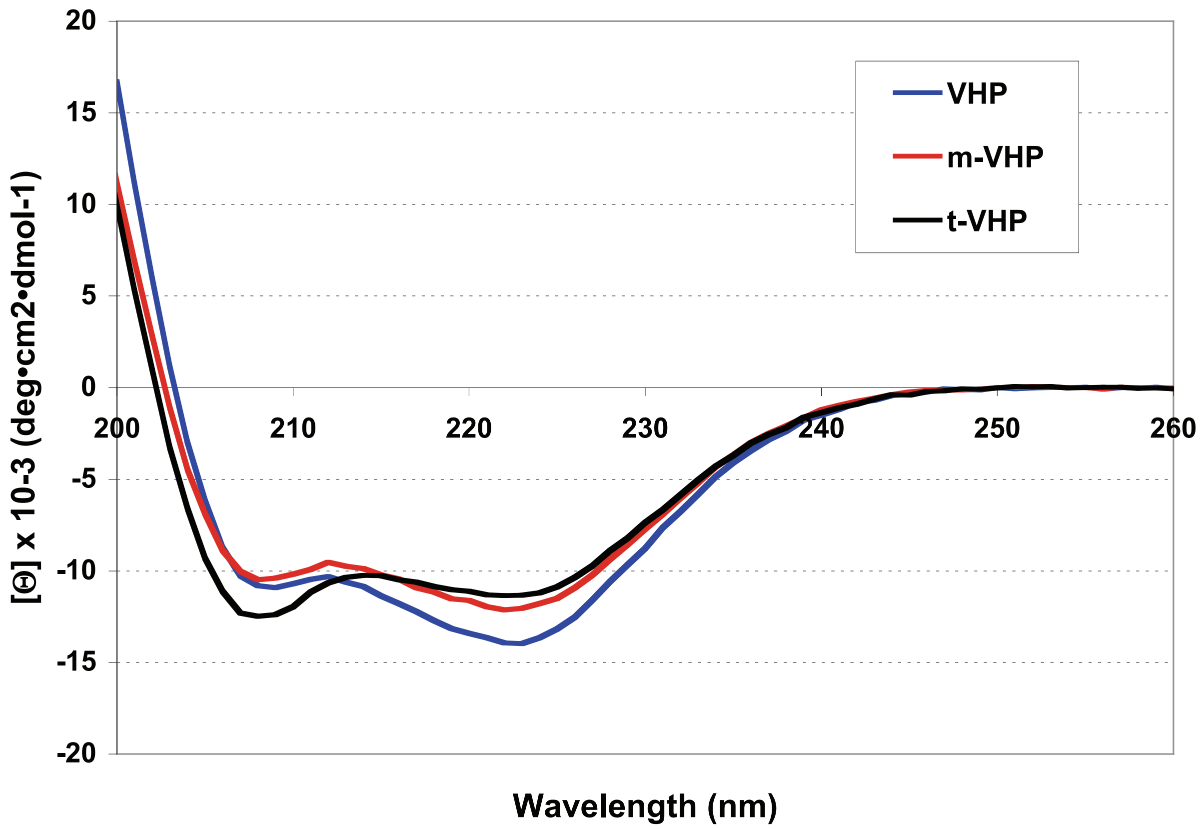




	$\Delta G_{fold/Gdm}$
VHP	-3.3 kcal/mol
m-VHP	-2.5 kcal/mol
t-VHP	-2.2 kcal/mol

$\Delta G_{fold/BTE}$ = -1.9 kcal/mol

CD Comparison

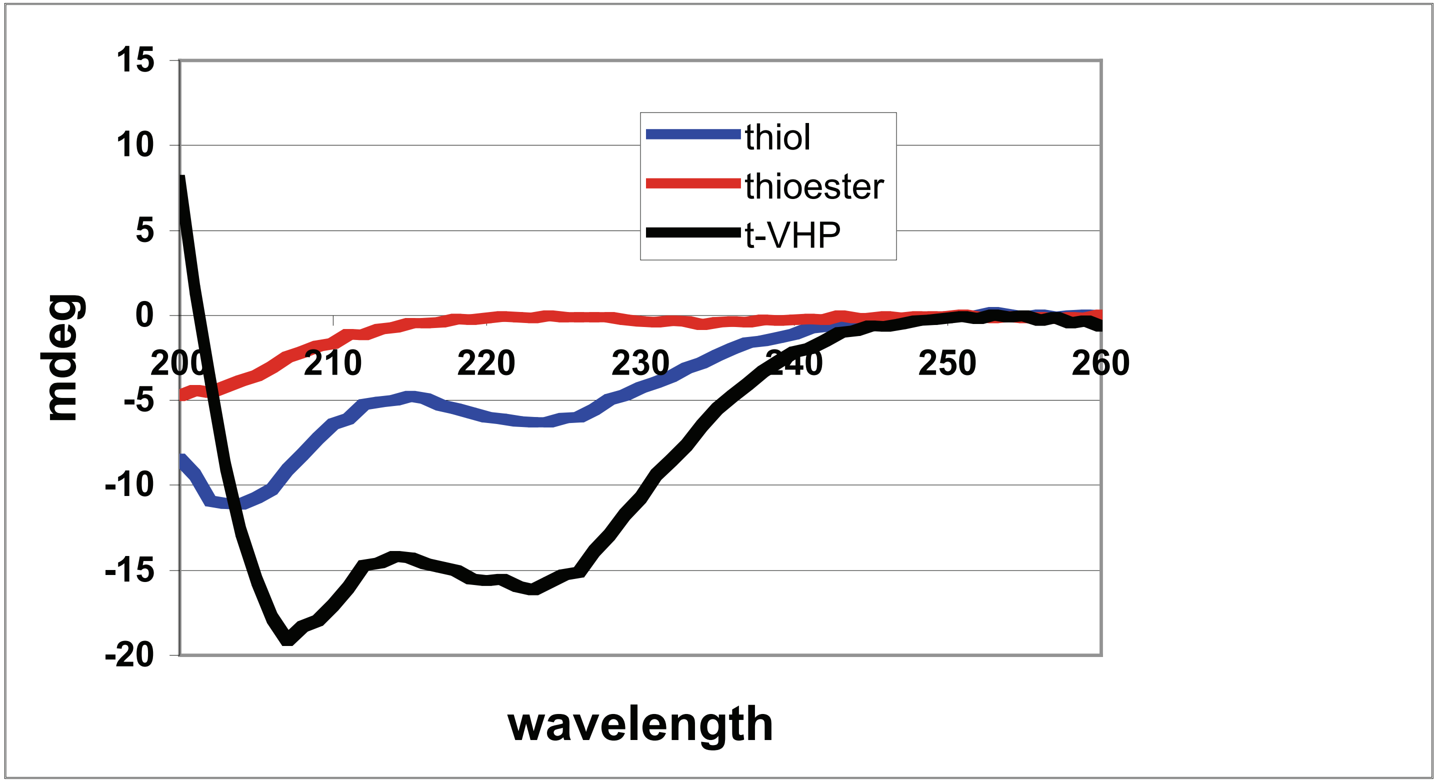


VHP H_2N - LSDED FKAV F G MTRS AFANL PLWKQ QNLKK EKGLF - CO_2H

m-VHP H_2N - LSDED FRAV F G MTRS AFANL PLWRQ QNLRR ERGLF - CO_2H

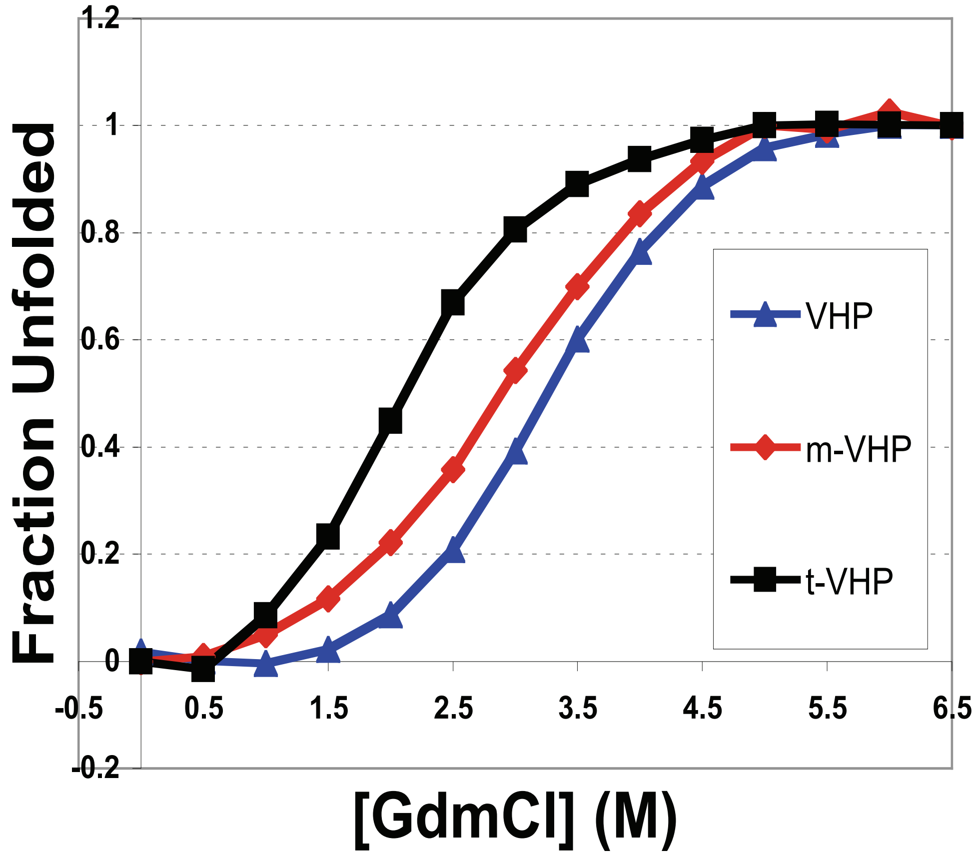
t-VHP H_2N - LSDED FRAV F X MTRS AFANL PLWRQ QNLRR ERGLF - CO_2H

CD Comparison: t-VHP vs. Fragments



t-VHP	AcNH - LSDED FRAVF XMTRS AFANL PLWRQ QNLRR ERGLF - CO_2H
Thiol	HS -XMTRS AFANL PLWRQ QNLRR ERGLF - CO_2H
Thioester	AcNH - LSDED FRAVF XY - $CONHCH_3$

Chemical Denaturation (CD at 225 nm)



	$\Delta G_{fold/Gdm}$
VHP	-3.3 kcal/mol
m-VHP	-2.5 kcal/mol
t-VHP	-2.2 kcal/mol

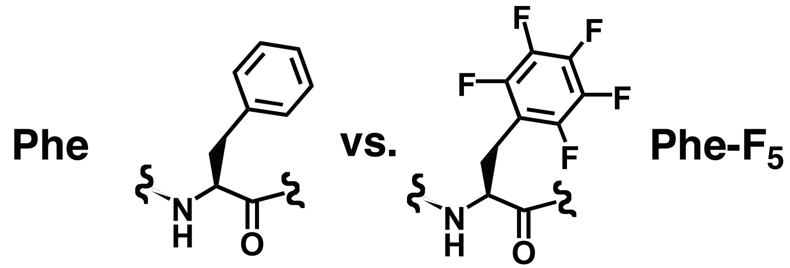
$\Delta G_{fold/BTE}$ = -1.9 kcal/mol

VHP H_2N - LSDED FKAV F G MTRS AFANL PLWKQ QNLKK EKGLF - CO_2H

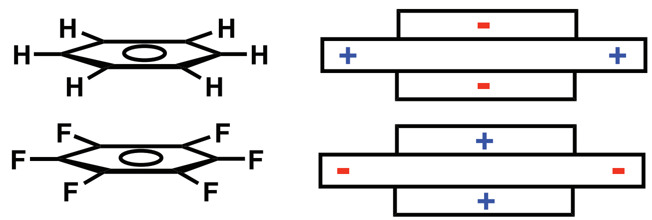
m-VHP H_2N - LSDED FRAV F G MTRS AFANL PLWRQ QNLRR ERGLF - CO_2H

t-VHP H_2N - LSDED FRAV F X MTRS AFANL PLWRQ QNLRR ERGLF - CO_2H

Phe vs. Phe-F_5 in the Core of VHP?



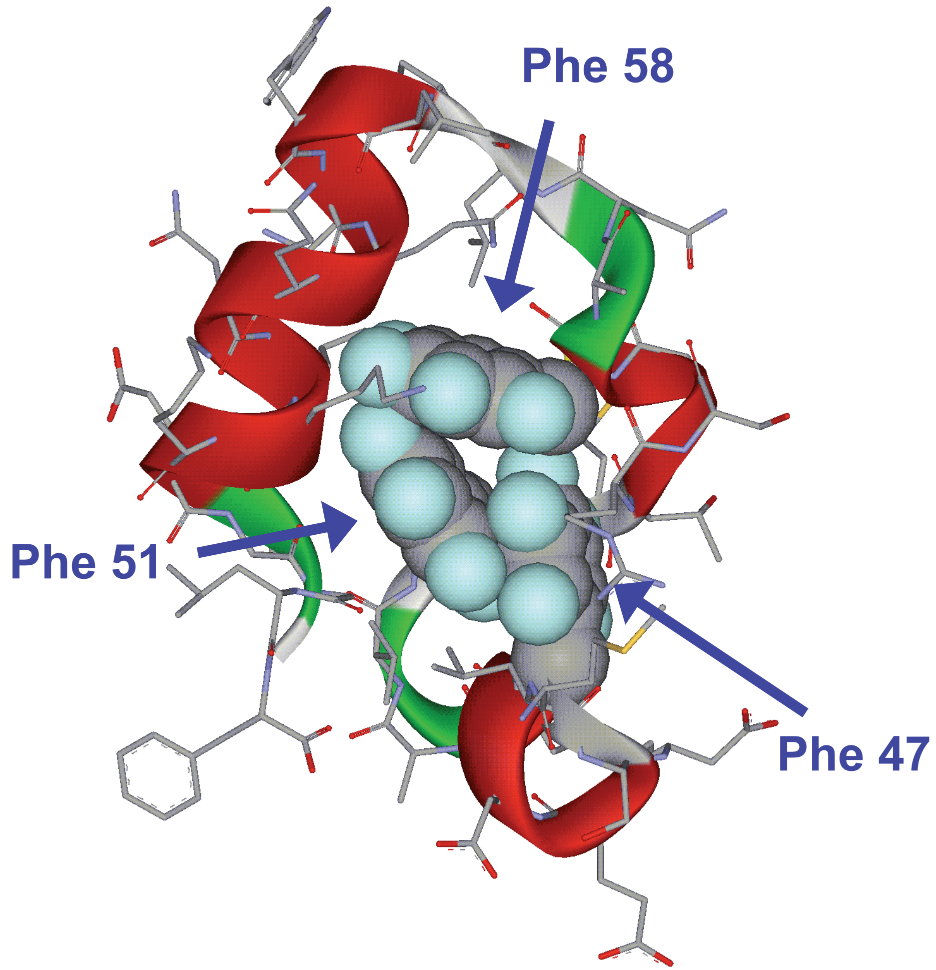
- **Benzene and hexafluorobenzene have complementary quadrupole moments.**
  Williams, *Acc. Chem. Res.* <u>26</u>:593 (1993);
  Dougherty et al. *J. Phys. Org. Chem.* <u>10</u>:347 (1997)



- **Aligned Phe/Phe and Phe/Phe-F_5 provide similar stabilization to an α-helix.**
  Waters et al. *J. Am. Chem. Soc.* <u>124</u>:9751 (2002)

- **Fluoro<u>alkyl</u> side chains are extremely hydrophobic and promote association of peptide surfaces.**
  Tirrell, DeGrado et al. *Biochemistry* <u>40</u>:2790 (2001)
  Kumar et al. *J. Am. Chem. Soc.* <u>123</u>:4393 (2001)

Phe --> F_5-Phe Mutations Evaluated by BTE



$\Delta G_{Fold/BTE}$

$F47f_5F$ = -1.2 kcal/mol

$F51f_5F$ = -2.5 kcal/mol ⟵

$F58f_5F$ = -1.5 kcal/mol

$F47,51f_5F$ = -1.7 kcal/mol

$F47,58f_5F$ = -1.2 kcal/mol

$F51,58f_5F$ = -1.2 kcal/mol

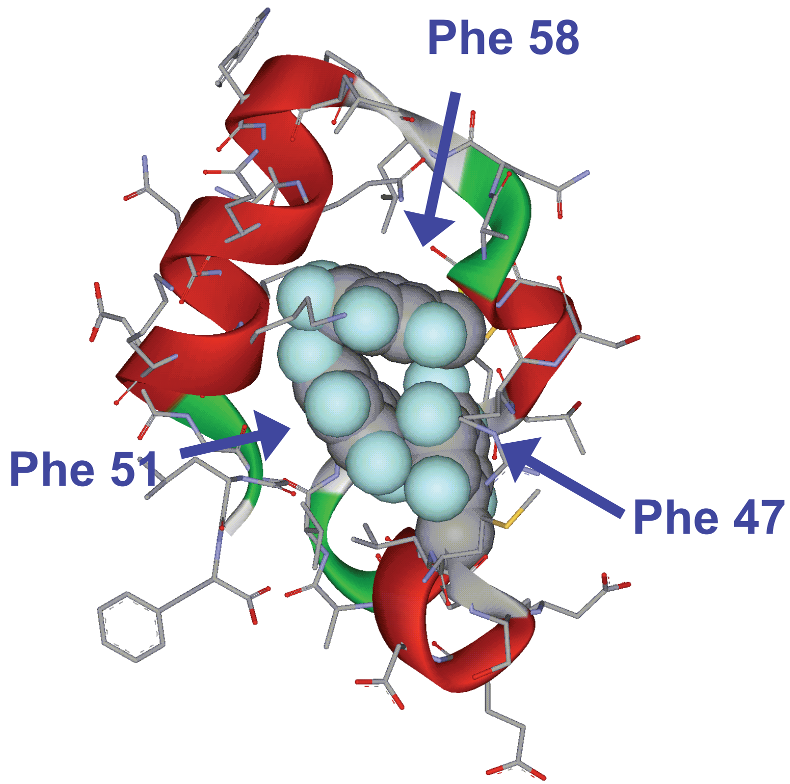
$F47,51,58f_5F$ = -1.1 kcal/mol

(t-VHP = -1.9 kcal/mol) ⟵

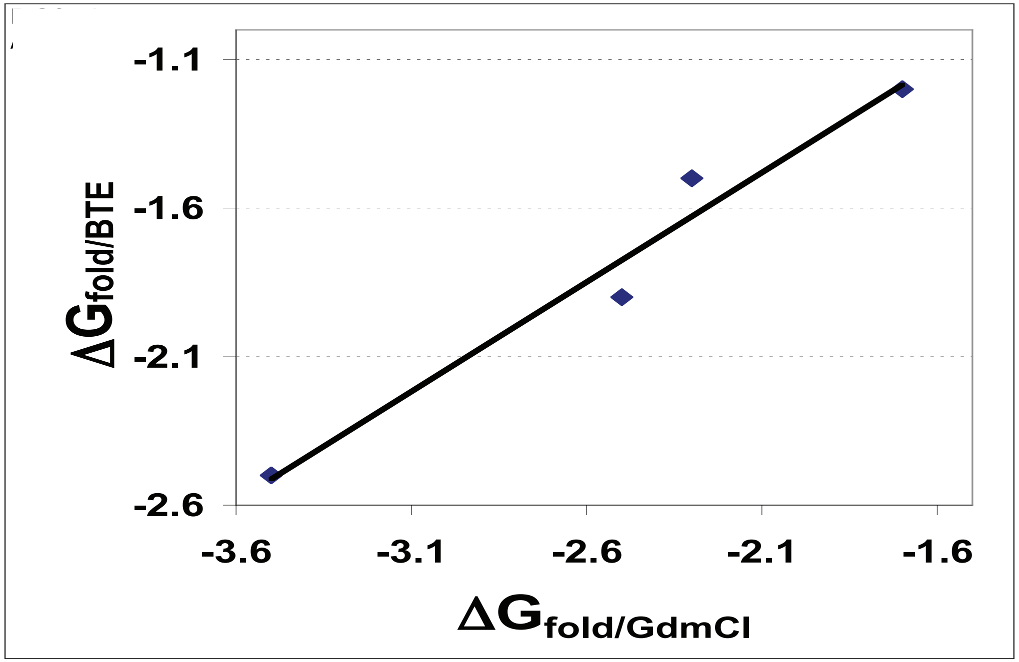
(Uncertainty ~ 0.1 kcal/mol)

Woll, Hadley, Mecozzi & Gellman *J. Am. Chem. Soc.* 128:15932 (2006)

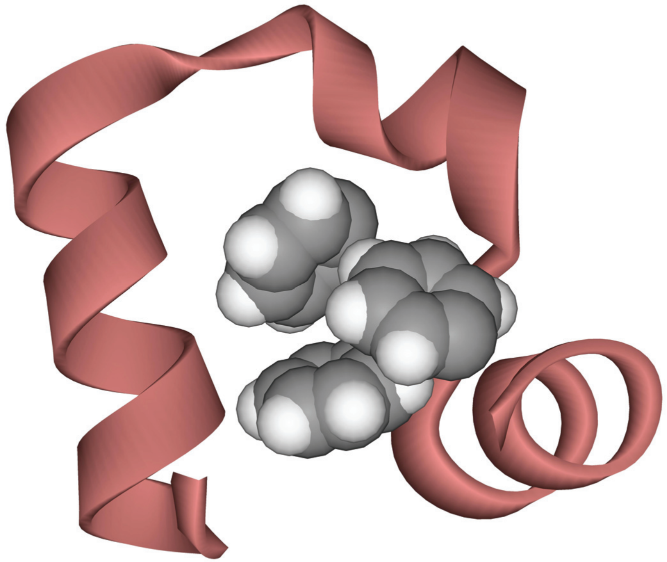
Control: Same Trends from BTE (thioester) vs. GdmCl (peptide)?



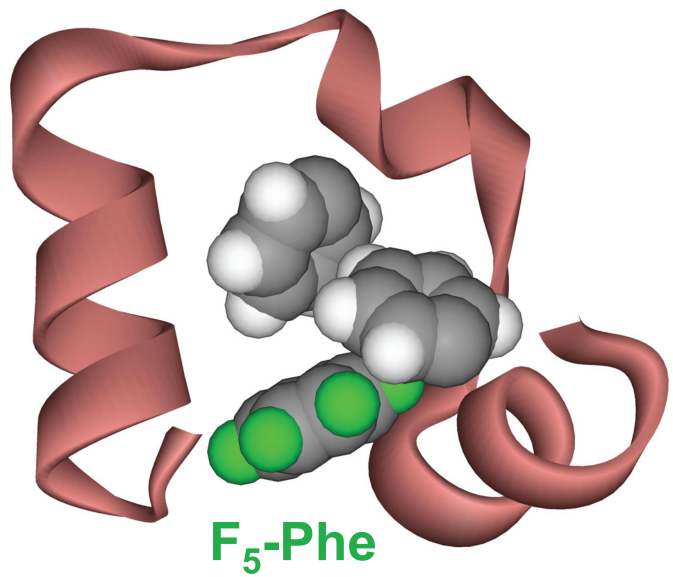
$\Delta G_{Fold/BTE}$	$\Delta G_{Fold/Gdm}$
$F47f_5F$ = -1.2 kcal/mol	$F47f_5F$ = -1.7 kcal/mol
$F51f_5F$ = -2.5 kcal/mol	$F51f_5F$ = -3.5 kcal/mol
$F58f_5F$ = -1.5 kcal/mol	$F58f_5F$ = -2.3 kcal/mol
(t-VHP = -1.9 kcal/mol)	(m-VHP = -2.5 kcal/mol)



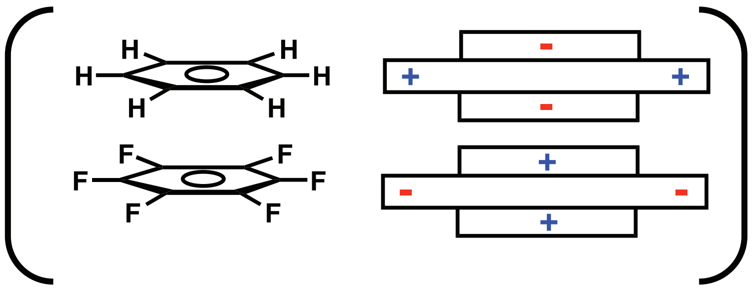
NMR Structure of Mutant Protein: Minimal Structural Response to F_5-Phe



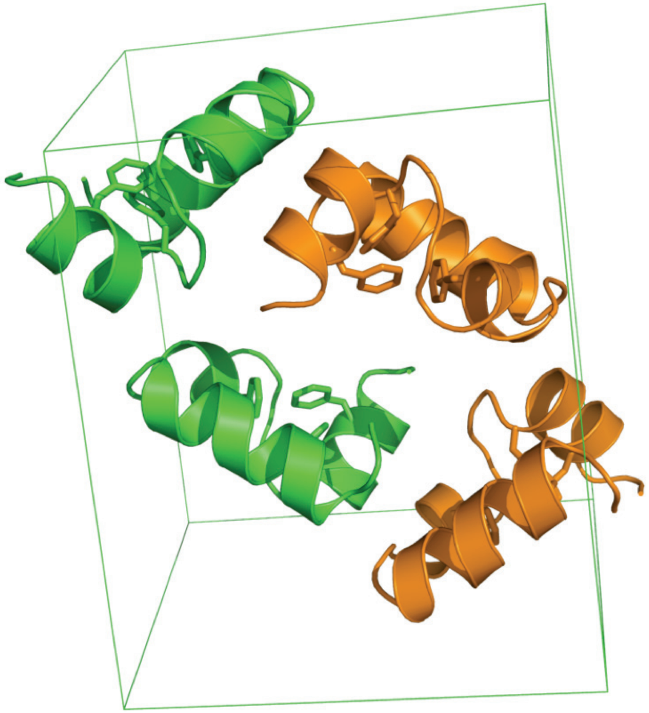
Chiu et al.
Proc. Natl. Acad. Sci. USA
102:7517 (2005)
(crystal structure)



Cornilescu, Hadley, Woll, Markley, Gellman & Cornilescu
Protein Sci. 16:14 (2007)



Racemic Protein Crystallization



Villin Headpiece Subdomain

L-VHP + D-VHP

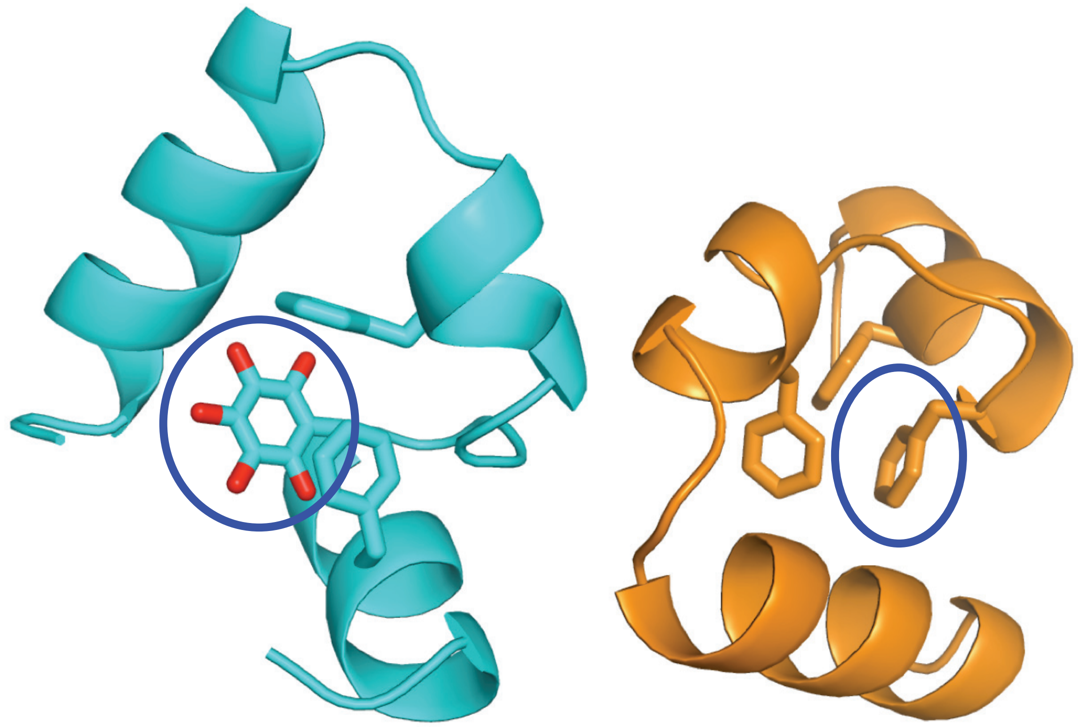
D. Mortenson

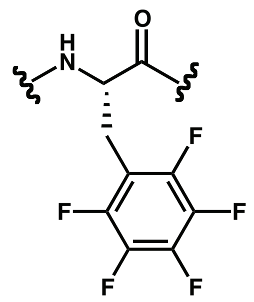
<u>**Racemic Proteins & Crystallization**</u>**:**

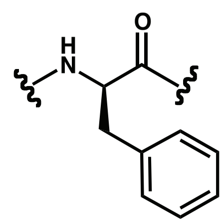
Zawadzke & Berg, *J. Am. Chem. Soc.* <u>114</u>:4002 (1992)
Wukovitz & Yeates, *Nat. Struct. Biol.* <u>2</u>:1062 (1995)
Kent et al. *J. Am. Chem. Soc.* <u>130</u>:9695 (2008)

<u>Quasi</u>racemic Crystallization of Proteins:

Direct Comparison of Native and Modified Forms



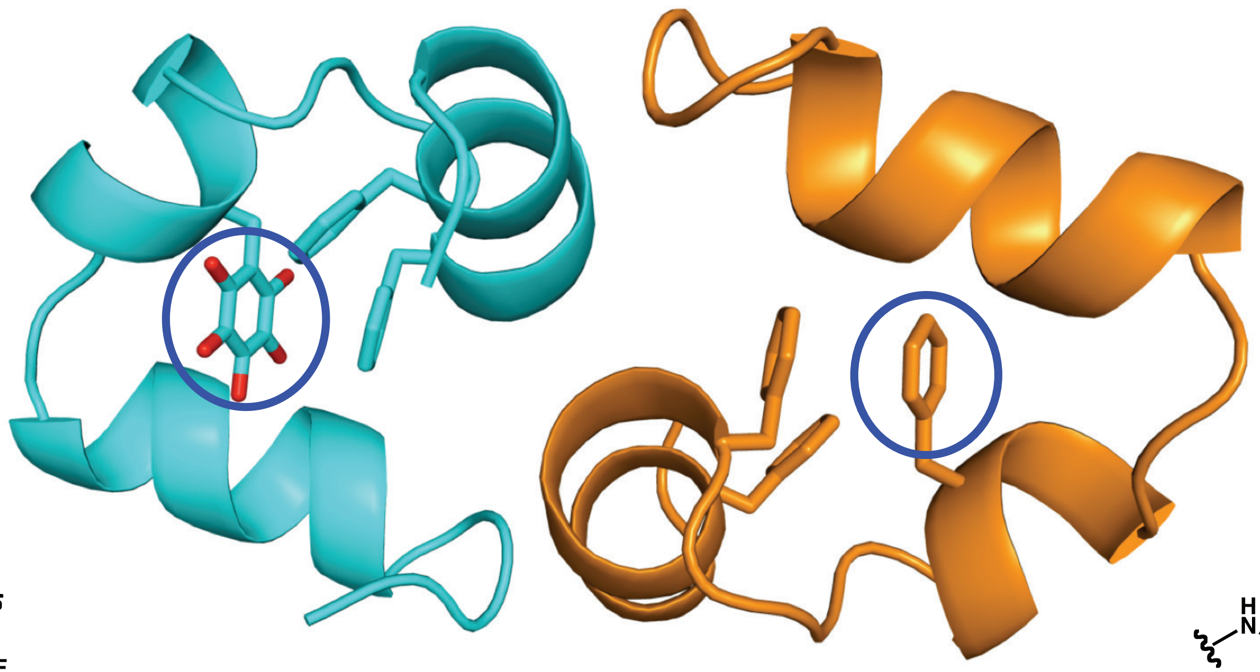


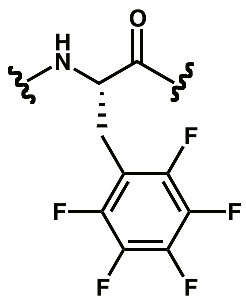


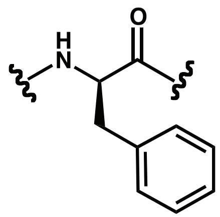
L-VHP(Phe51 → F_5Phe) + D-VHP

Mortenson, Satyshur, Guzei, Forest, Gellman *J. Am. Chem. Soc.* 134:2473 (2012)

Quasiracemic Crystallization of Proteins:
Direct Comparison of Native and Modified Forms





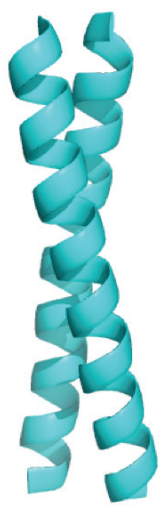


L-VHP(Phe58 → F_5Phe) + D-VHP

Mortenson, Satyshur, Guzei, Forest, Gellman *J. Am. Chem. Soc.* 134:2473 (2012)

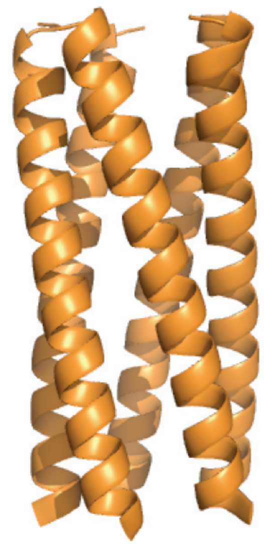
Third BTE Application: Coiled-Coil Dimers (Common Mode of α-Helix Association)

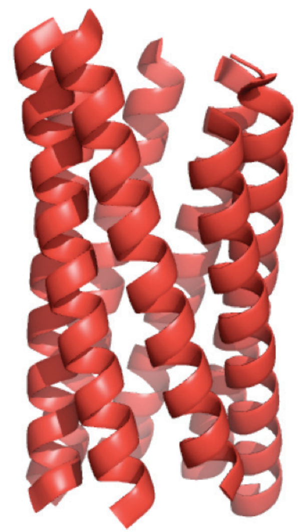
- 3^{o} structure (intramolecular) and 4^{o} structure (intermolecular)
- Many structural variations known
- Elucidate origins of stability and association preference?


1ZIK

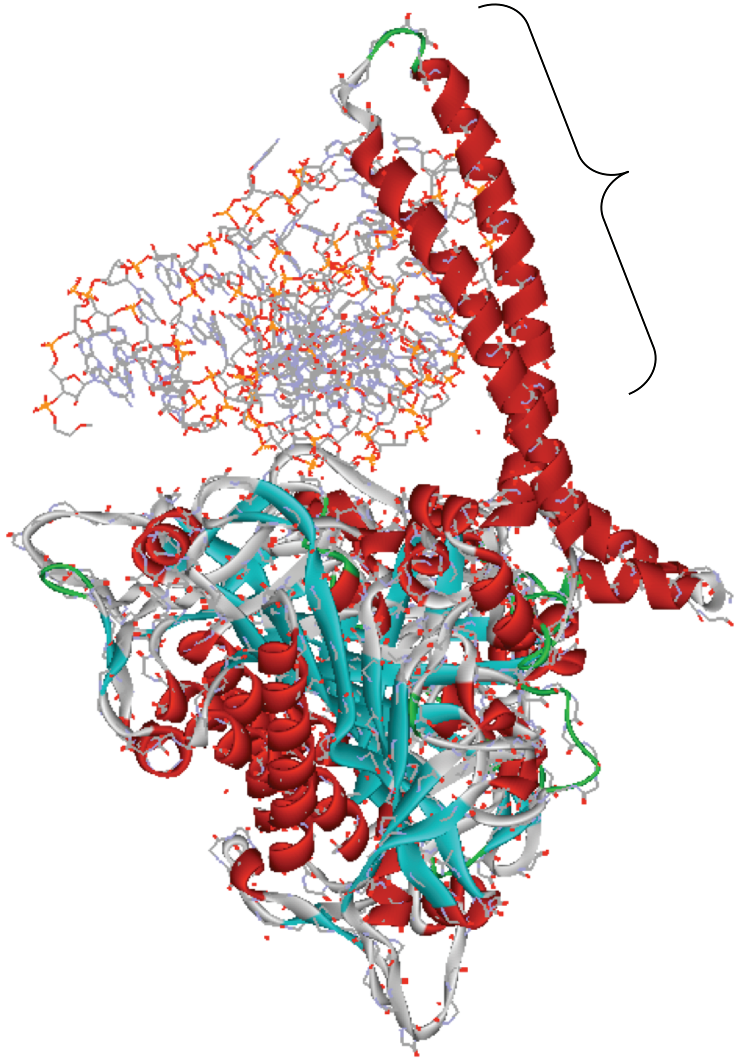

1ZIM


1GCL


2GUV


2HY6

Antiparallel Coiled-Coils:
A Common Tertiary Structural Motif in Proteins



Seryl t-RNA synthetase

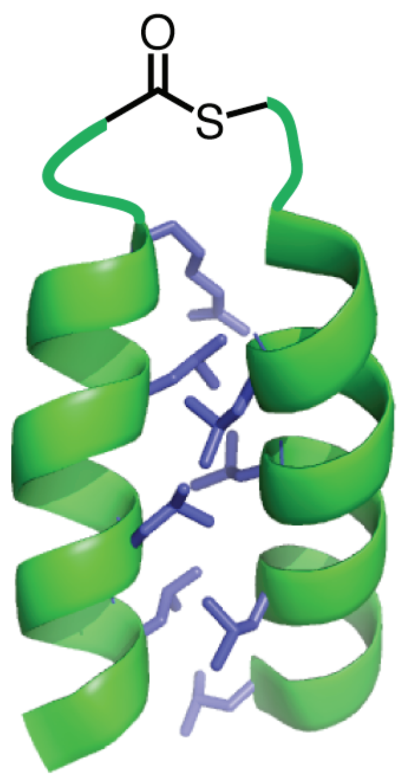
Biou et al. *Science* 263:1404 (1994)

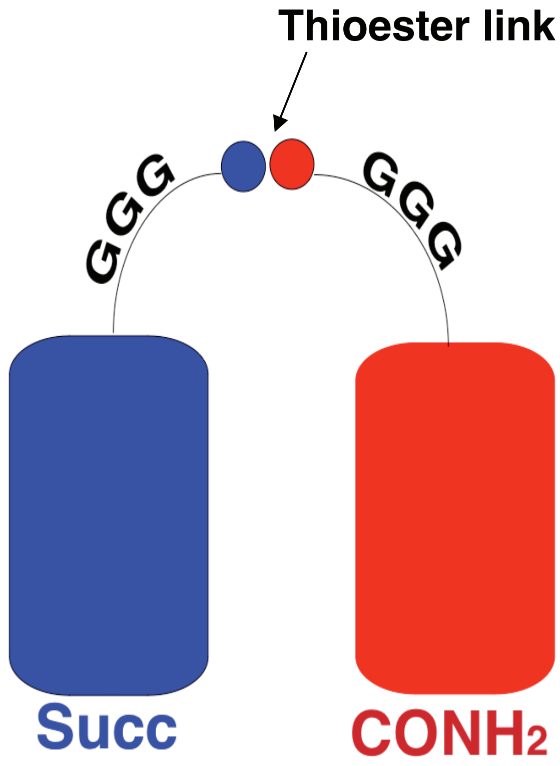
<u>Coiled-Coil Review</u>:

Woolfson
***Adv. Protein Chem.* (2005) 70:79.**

(Most work has focused on parallel coiled-coils.)

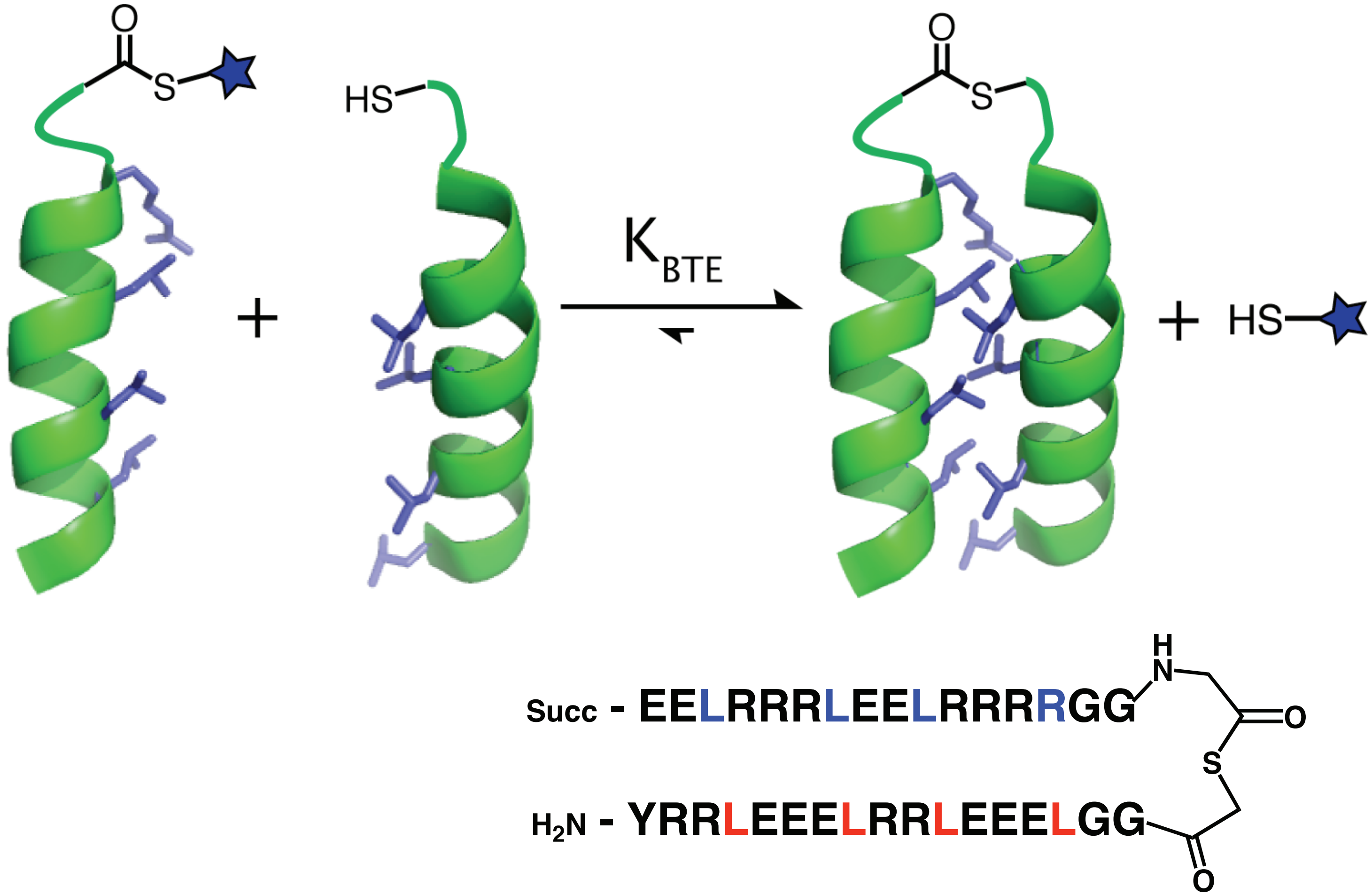
Design Goal: Minimum Length Intramolecular Antiparallel Coiled-Coil for BTE Analysis





E. Hadley

Design Goal: Minimum Length Intramolecular Antiparallel Coiled-Coil for BTE Analysis



Coiled-Coil Terminology: Heptad Repeat Pattern

1. **Seven α-amino acid residues (“heptad”) = 2 turns of α-helix.**

2. **For coiled coil-forming sequences, designate the positions in each heptad with letters: *a b c d e f g*.**

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3. **Sequences that form coiled coils feature a "heptad repeat" in which a hydrophobic residue (e.g., leucine = L) is found at positions *a* and *d*.**

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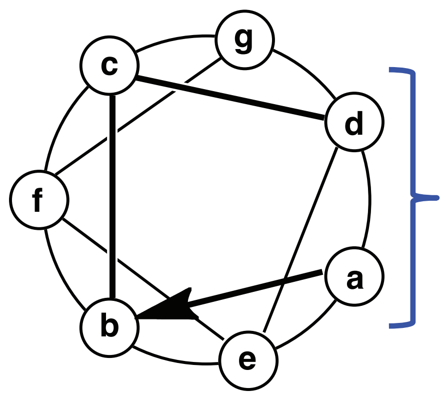
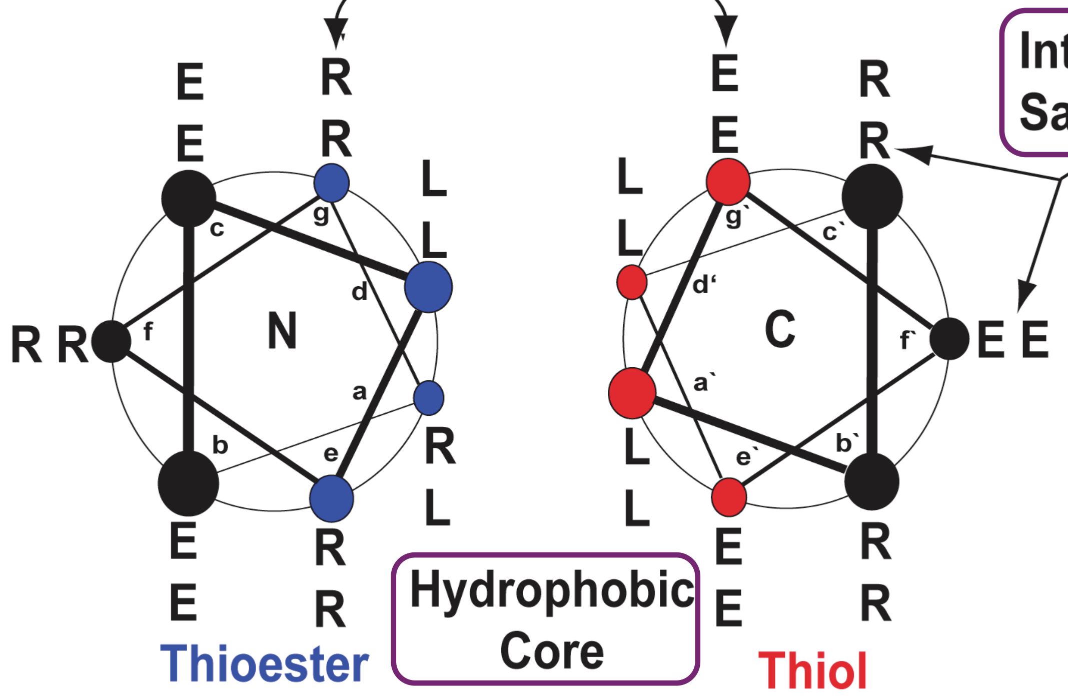
4. **"Helix-wheel" depiction of a heptad in an α-helical conformation (view along the helix axis.)**



Positions *a* and *d* align along one side of the α-helix, to create a hydrophobic "stripe."

Antiparallel Coiled-Coil Model System Design



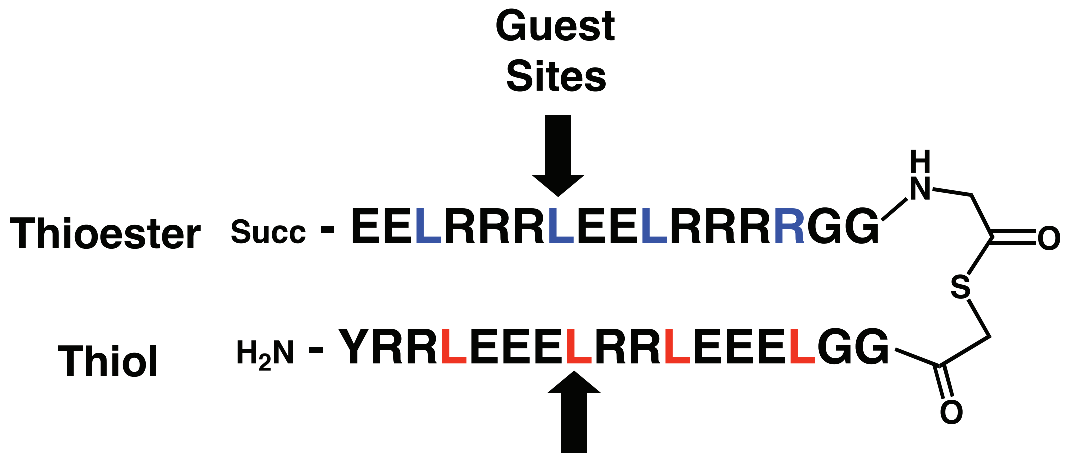
Design Precedent:

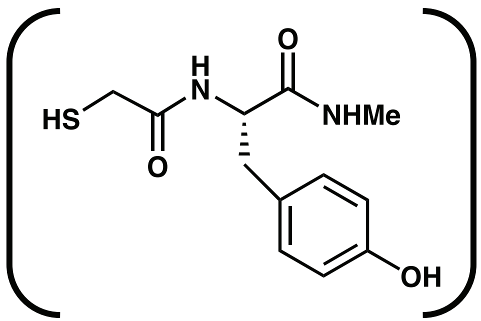
Burkhard et al.
J. Mol. Biol. 318:901 (2002)

(Short parallel/intermolecular coiled coils)

E. Hadley

Survey of Residue Pairing Propensities in Noncovalently Juxtaposed Sites

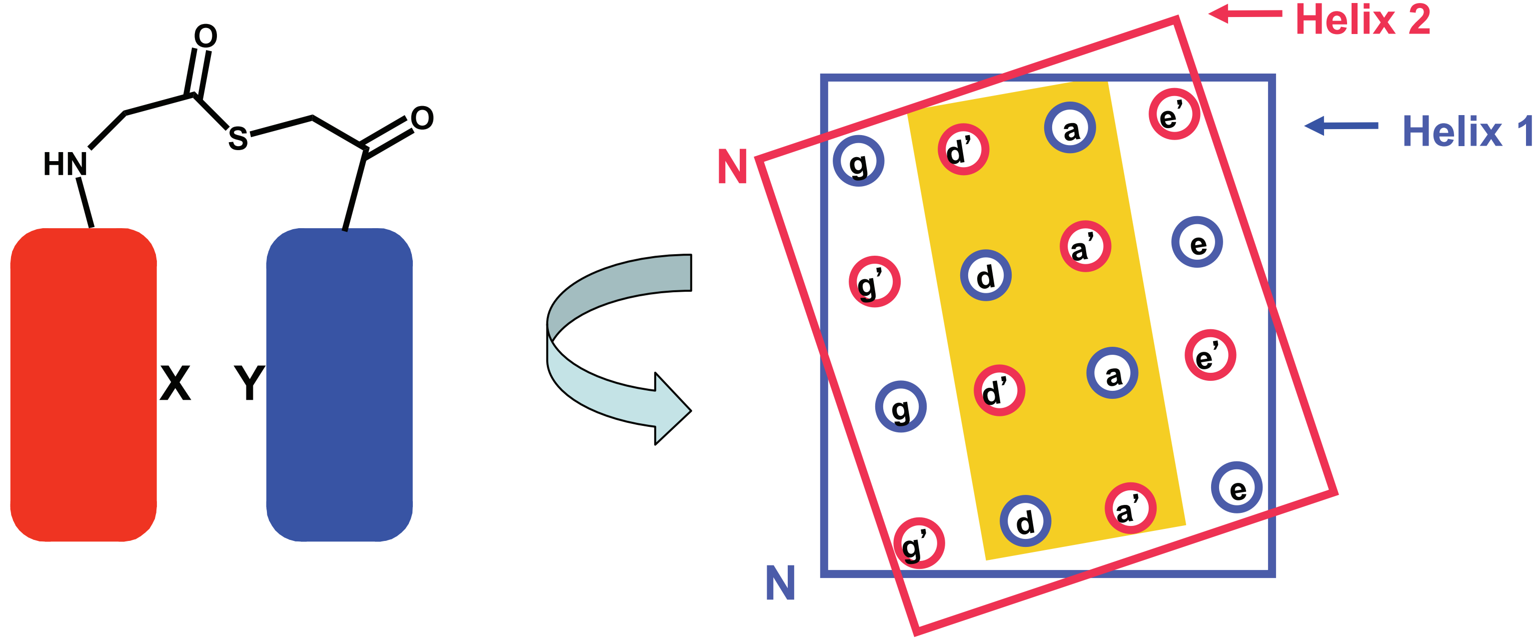




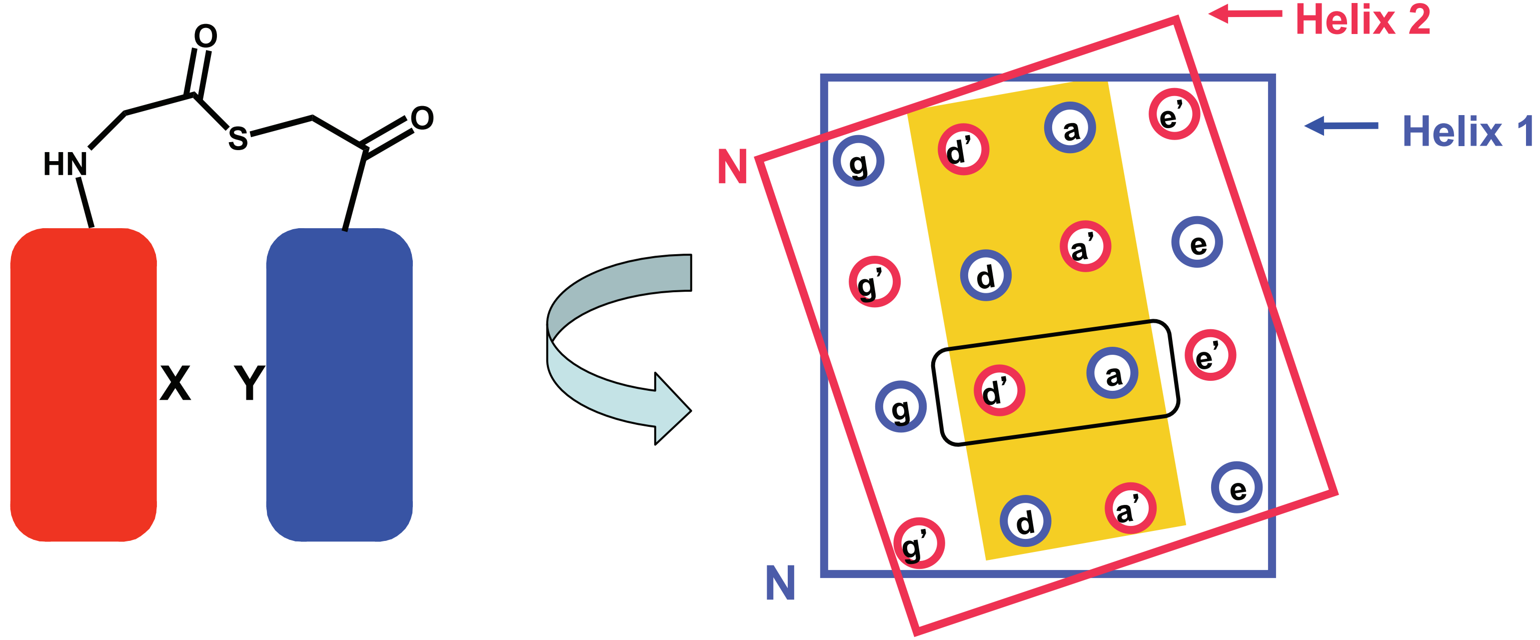
(Residue Pairing Propensities in <u>Parallel</u> Coiled-Coils: Vinson et al. *Biochemistry* 2002 & 2006)

Hadley & Gellman *J. Am. Chem. Soc.* <u>128</u>:16444 (2006)

Close-up: Helix-Helix Interface

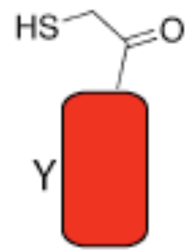


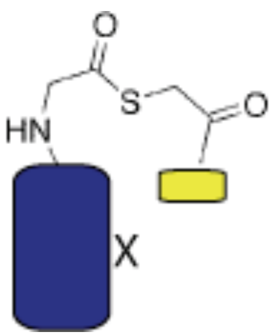
Close-up: Helix-Helix Interface



Side Chain Pairing Energetics (ΔG_{BTE})

Thiol





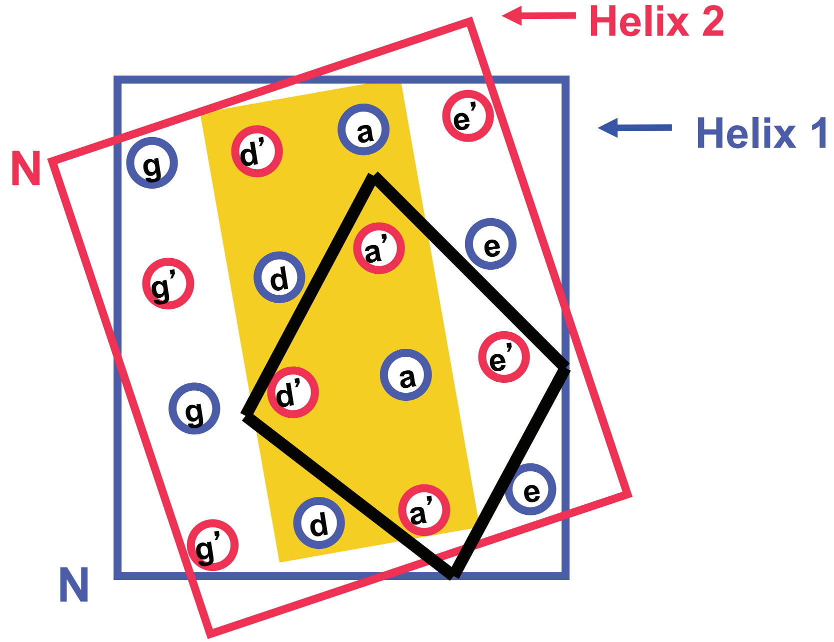
Thioester

X ↓ / Y →	L	I	V	N	A
L	-1.4	-1.3	-0.9	+0.2	-0.4
I	-1.7	-1.0	-0.8	-0.1	-0.9
V	-1.4	-0.8	-0.6	0.0	-0.9
N	+0.3	+0.2	+0.4	+0.5	+0.8
A	-0.7	-0.7	-0.5	+0.4	0.0

ΔG uncertainty ≈ 0.1 kcal/mol

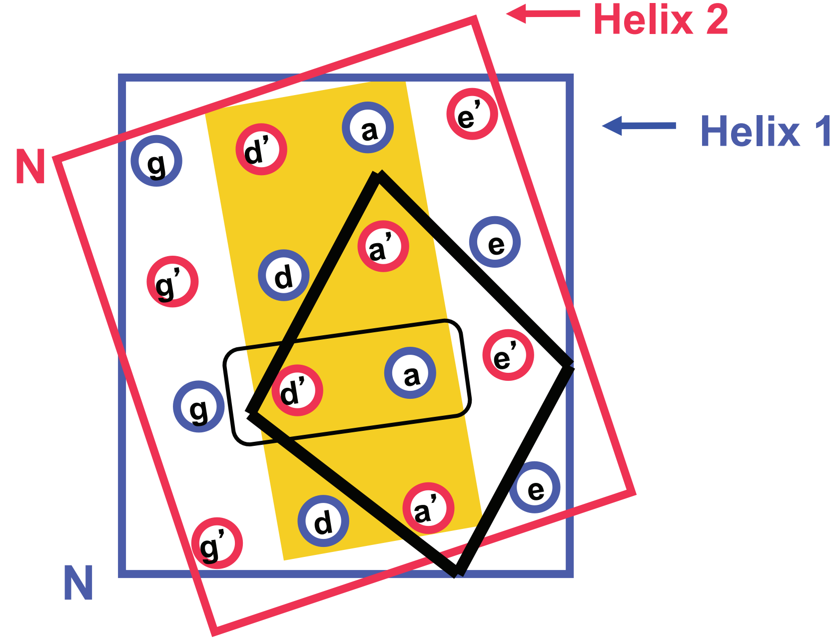
25 ΔG_{BTE} values from 10 short peptides

“Knobs into Holes”



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

“Knobs into Holes”
Lateral vs. Vertical Packing Partners



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

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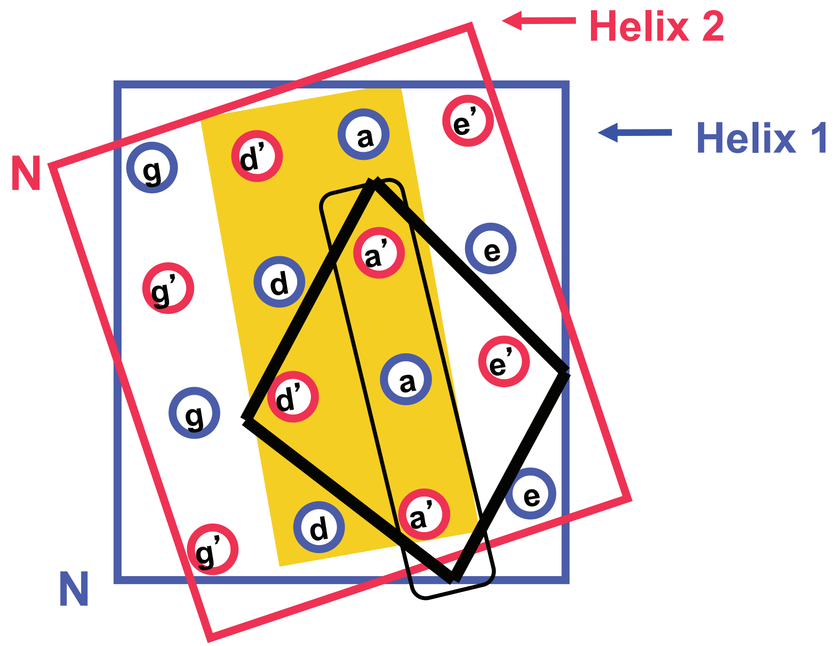
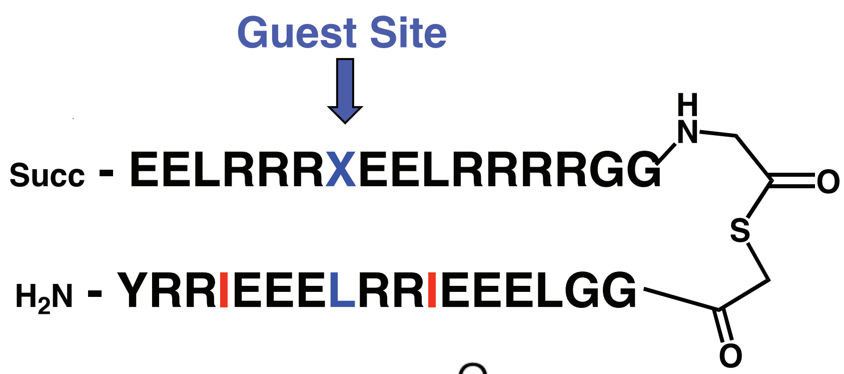
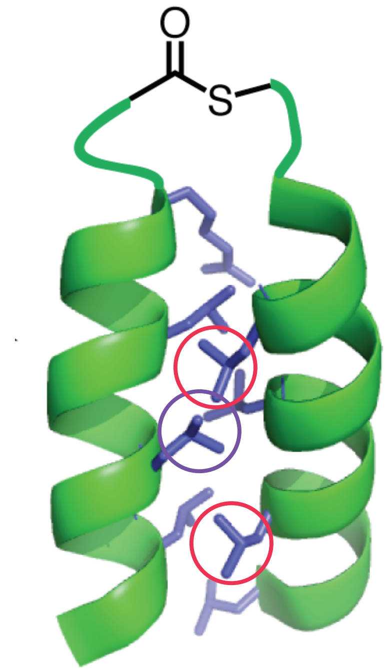


F. H. C. Crick *Acta Cryst.* **1953**, *6*, 689.

Probing "Vertical" Interactions in Antiparallel Coiled-Coil Interactions



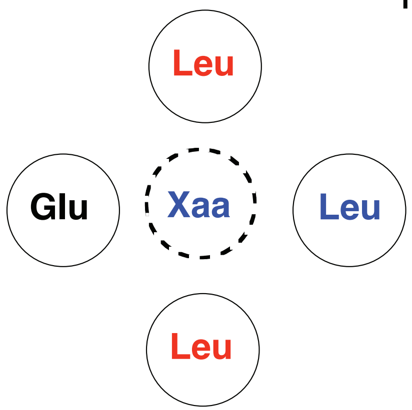


"Knob into hole"

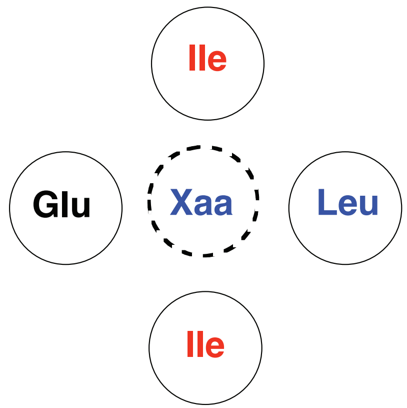
(Xaa = Knob)

Significant Context Effects from Vertical Packing Partners (Leu vs. Ile)

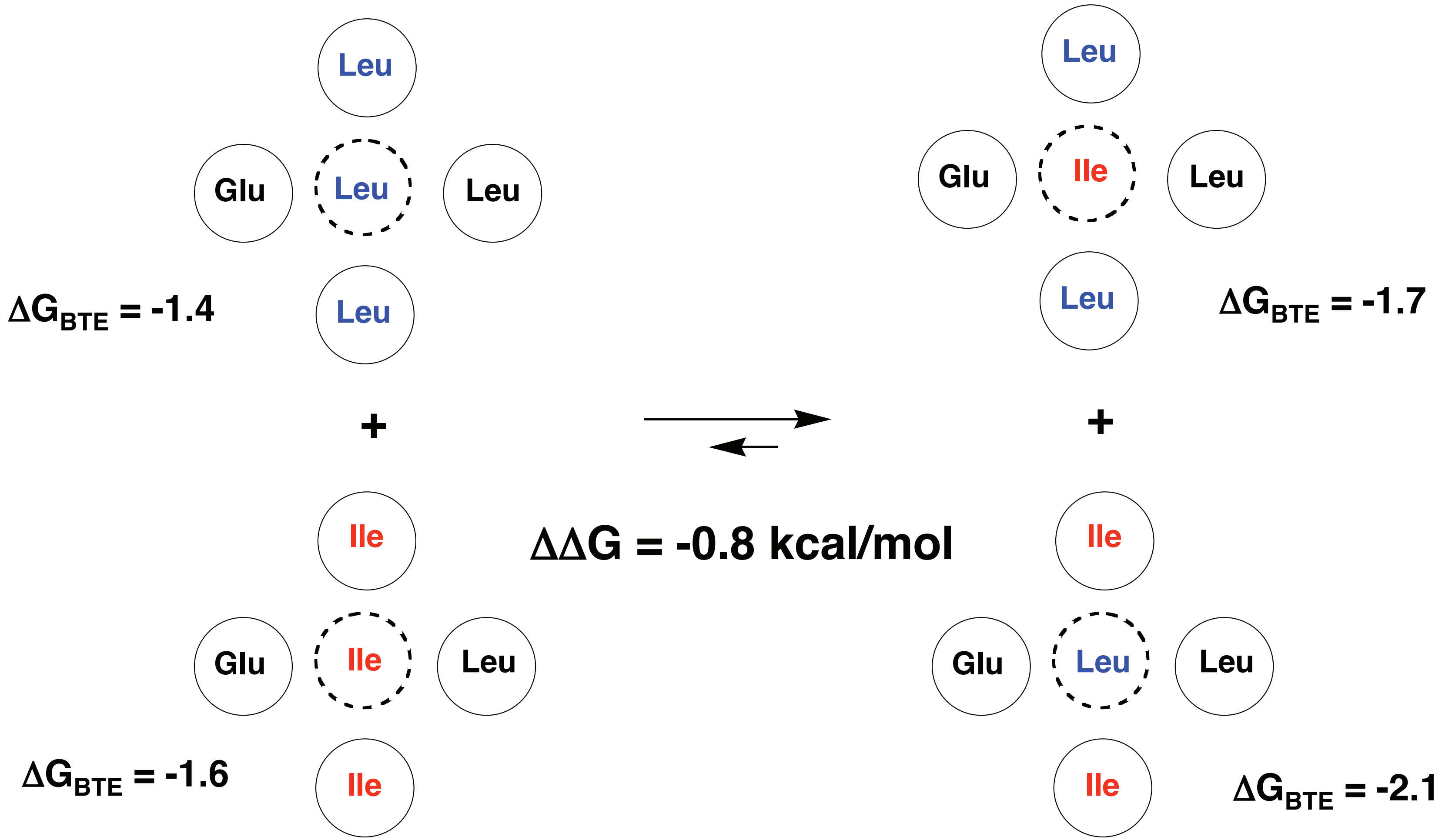
Xaa	ΔG_{BTE} (kcal/mol) Leu	Ile
Leu	-1.4	-2.1
Ile	-1.7	-1.6



vs.

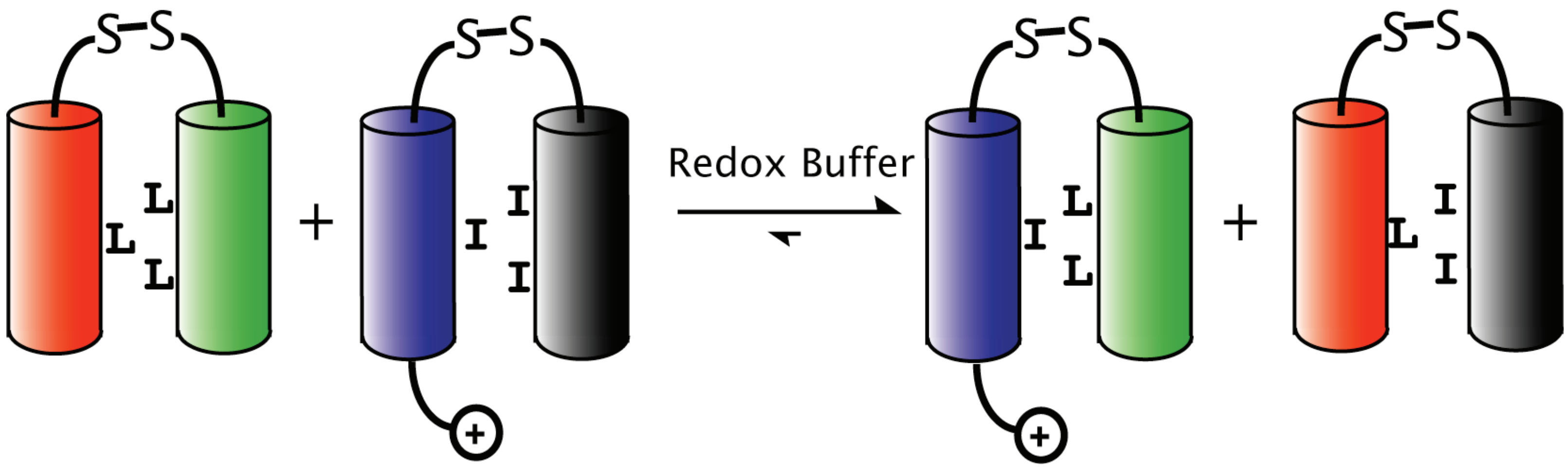


Discrimination Factor: Impact of Vertical Context on Antiparallel Coiled-Coil Partner Preference



Test BTE-based Conclusion: Discrimination Factor in a Different Model System

Design Basis: Oakley et al. *J. Am. Chem. Soc.* 123:3153 (2001)



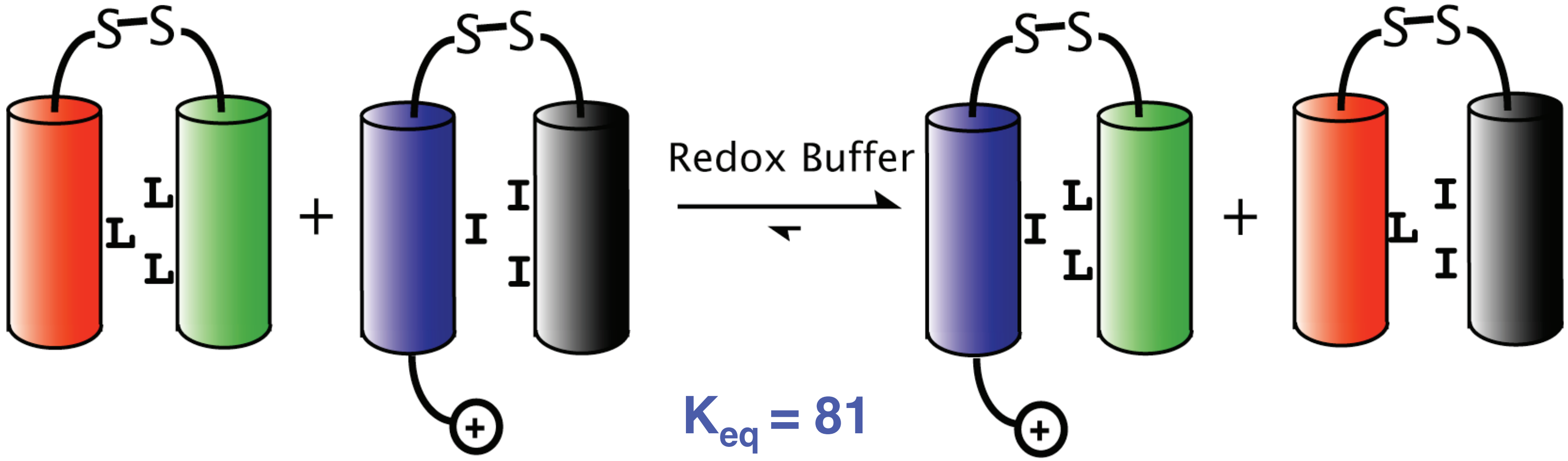
Ac-CGGAQLEKELQALEKKLAQLEWENQALEKELAQ-NH$_2$ [Red]

Ac-AQLKKKLQANKKELAQLKWKLQALKKKLAQGGC-NH$_2$ [Green]

Ac-CGGAQLEKELQALEKKIAQLEWENQALEKELAQGGK-NH$_2$ [Blue]

Ac-AQLKKKLQANKKEIAQLKWKIQALKKKLAQGGC-NH$_2$ [Gray]

Test BTE-based Conclusion:
Discrimination Factor in a Different Model System



$K_{eq} = 81$

(ΔG = -2.6 kcal/mol)

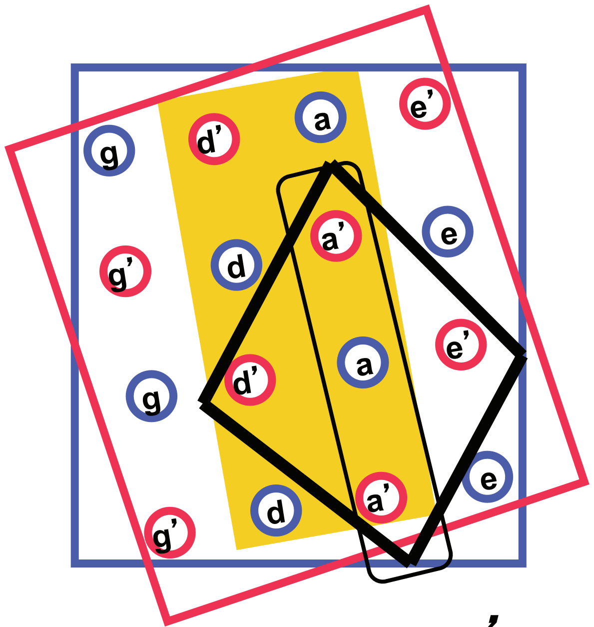
Ac-CGGAQLEKELQALEKKLAQLEWENQALEKELAQ-NH_2 [Red]

Ac-AQLKKKLQANKKELAQLKWKLQALKKKLAQGGC-NH_2 [Green]

Ac-CGGAQLEKELQALEKKIAQLEWENQALEKELAQGGK-NH_2 [Blue]

Ac-AQLKKKLQANKKEIAQLKWKIQALKKKLAQGGC-NH_2 [Gray]

Bioinformatic Analysis of Natural Antiparallel Coiled Coils



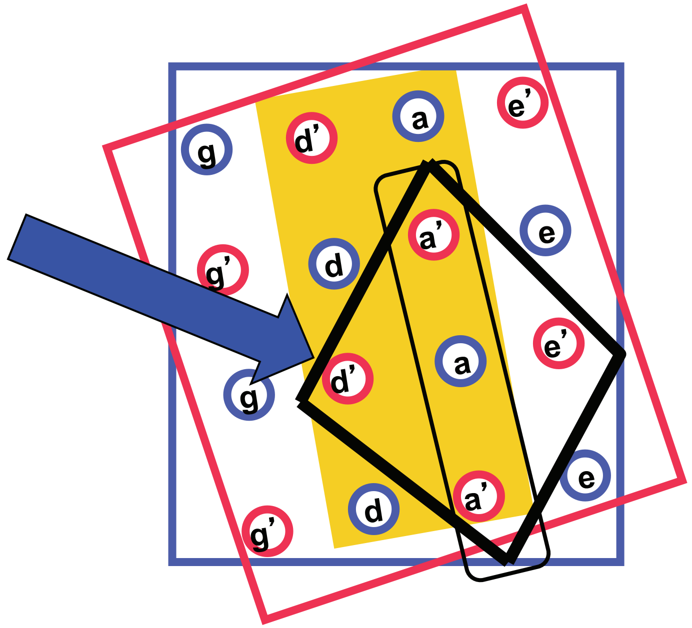
Antiparallel 2-helix coiled-coils in the PDB
With ≤70% sequence identity
(2072 knob-into-hole interactions):
Focus on *a*-*a'*-*a* vertical constellations.

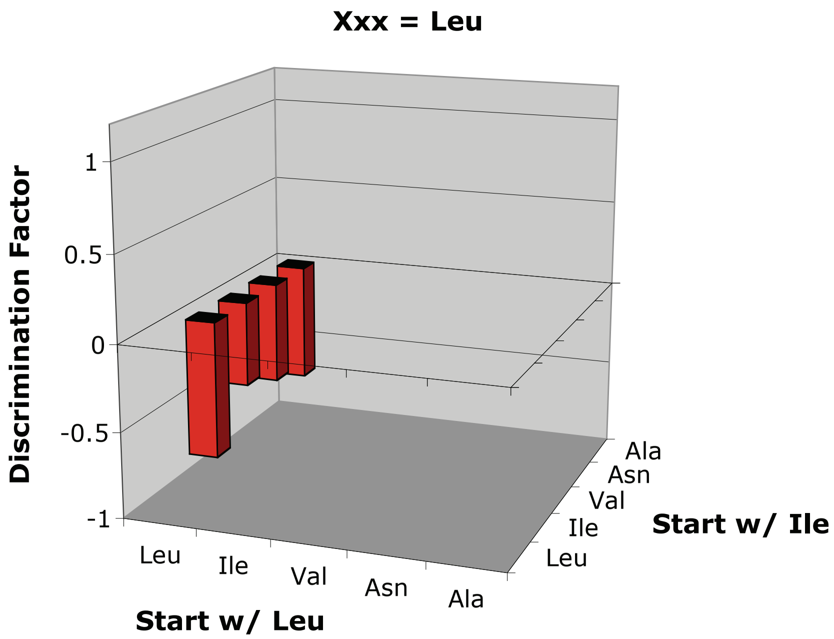
a	***a' = a' = Ile*** Obsd	Expect	O/E	***a' = a' = Leu*** Obsd	Expect	O/E
Leu	**6**	**9.4**	**0.6**	**26**	**16.5**	**1.6**
Ile	**19**	**14.2**	**1.3**	**21**	**24.7**	**0.9**

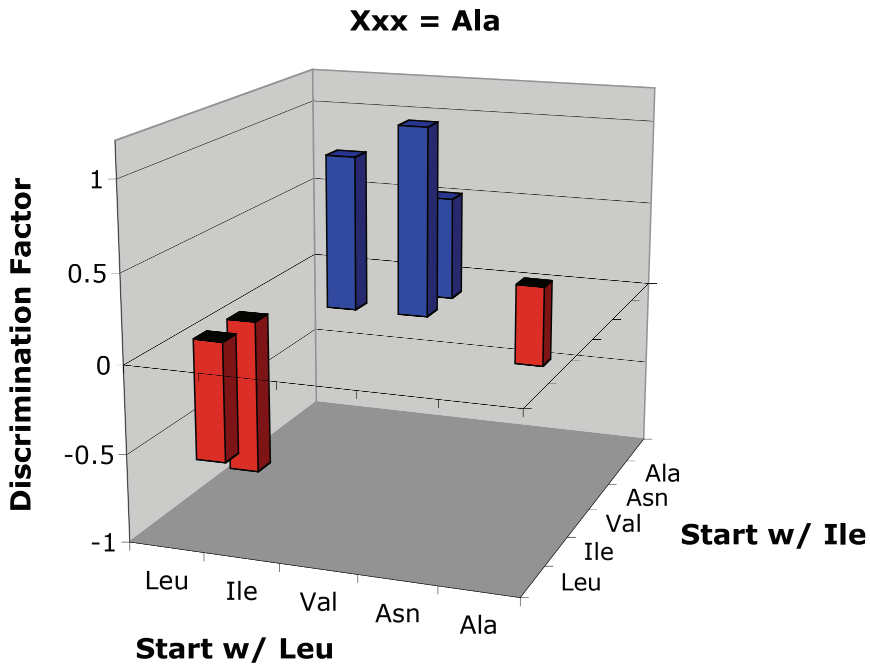
Theoretical ΔG = -0.8 kcal/mol (heterotriads favored over homotriads)

Hadley, Testa, Woolfson, Gellman *PNAS* 105:530 (2008)

Interplay Between Vertical And Lateral Interactions…

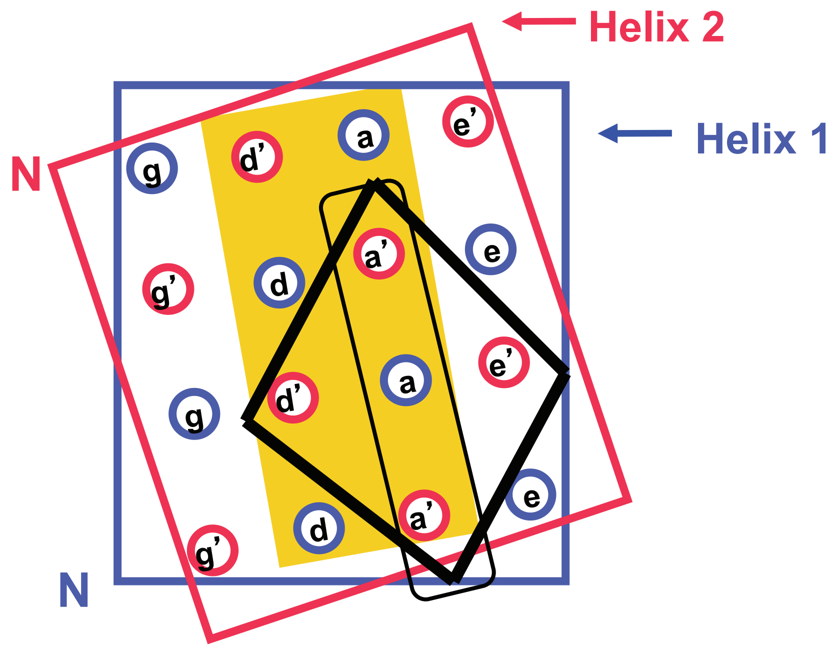






“Knobs into Holes”
Lateral vs. Vertical Packing Partners

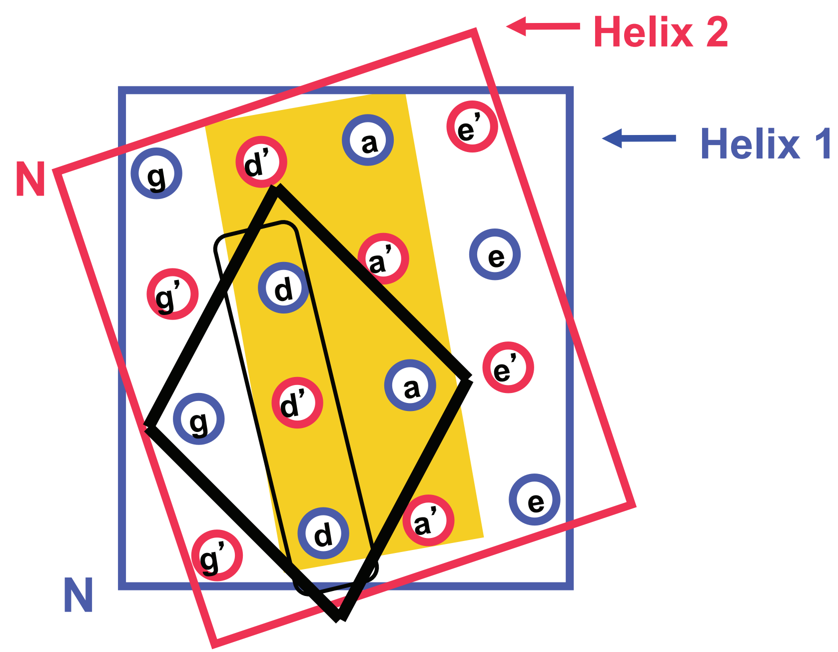
a’ -- a -- a’ vertical triads



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

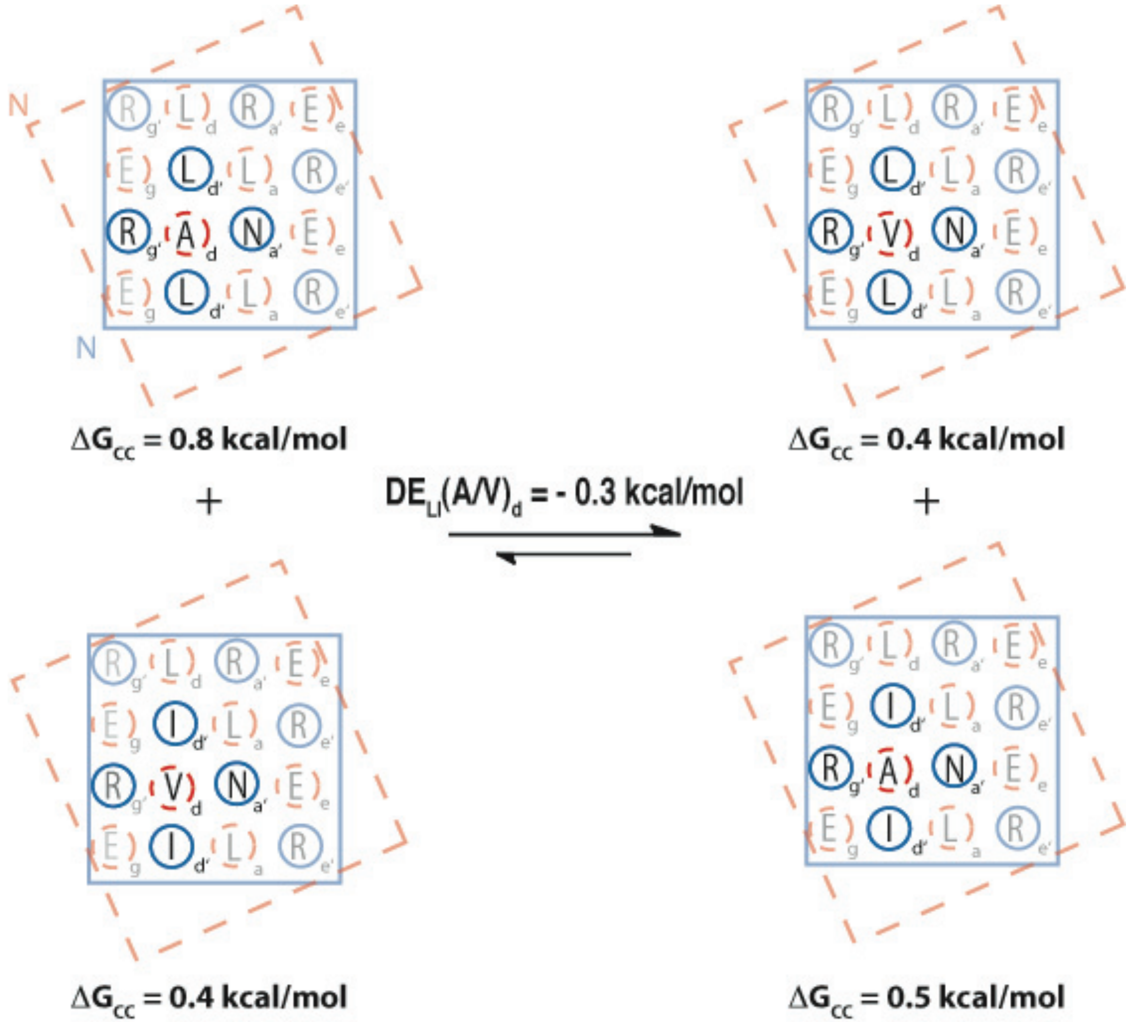
“Knobs into Holes”
Lateral vs. Vertical Packing Partners

d’ -- d -- d’ vertical triads?



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

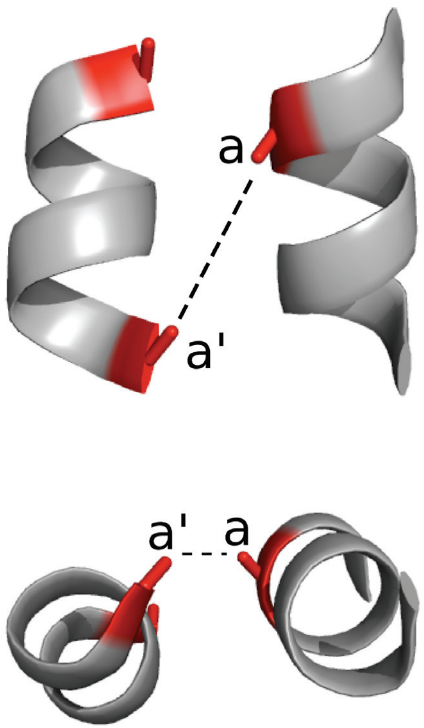
Smaller Vertical Context Impact at *d* positions



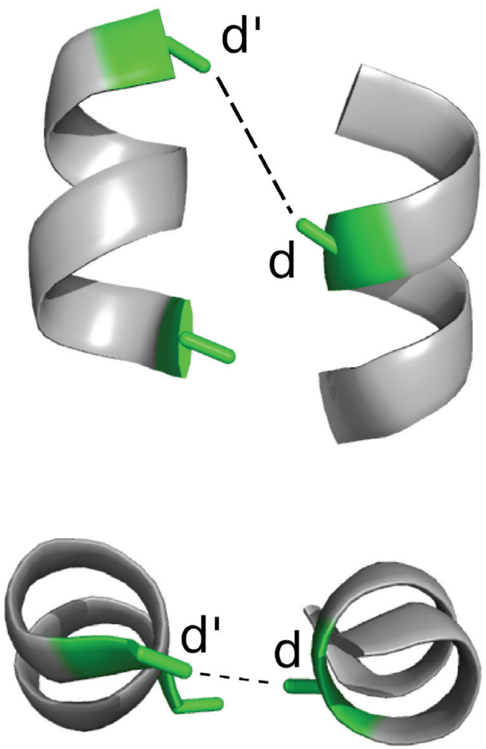
Only 18% of calculated $DE_{LI}(X/Y)_d$ values significant (vs. 42% in *a* position triads)

Origin of difference between *a* and *d* vertical triads?

a'--a--a'



d'--d--d'

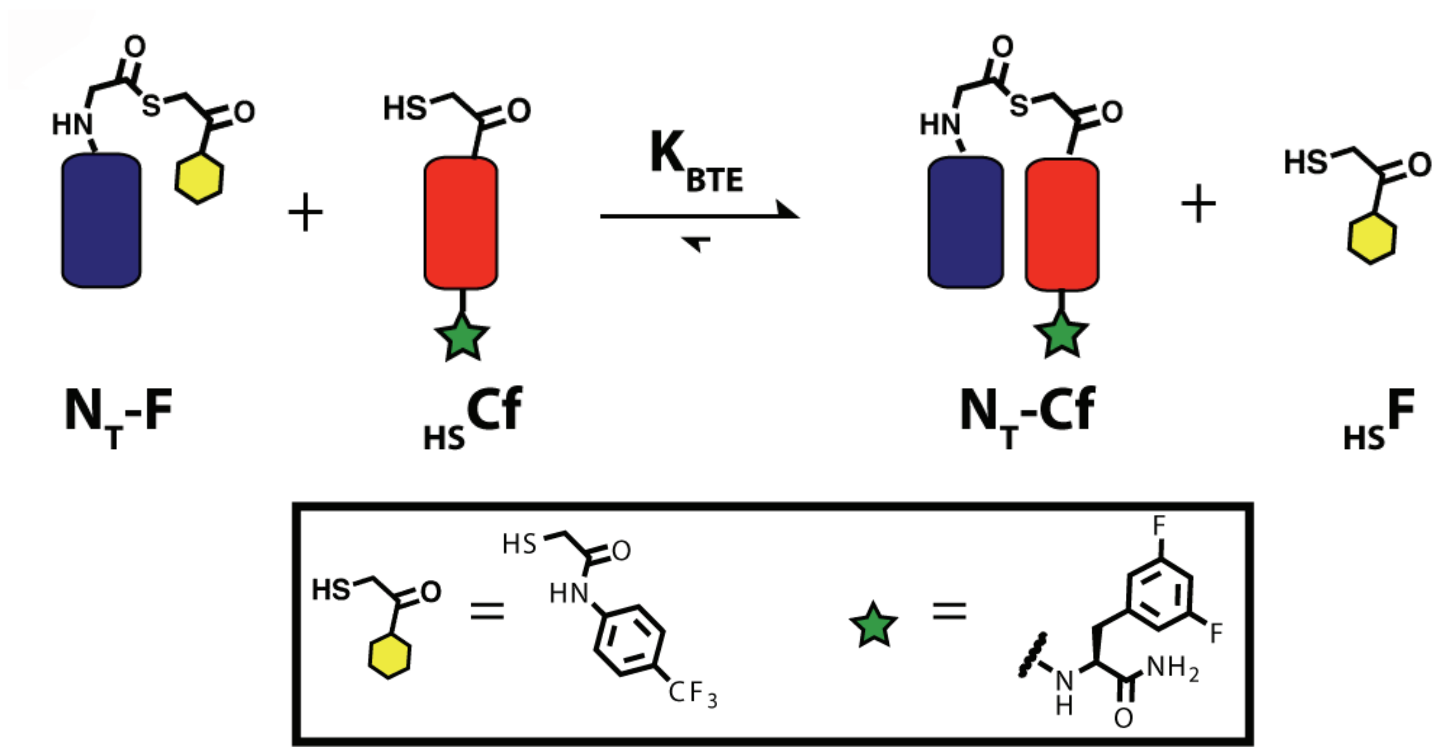


Cα-Cβ vectors from
PDB 2NOV
(antiparallel coiled-coil)

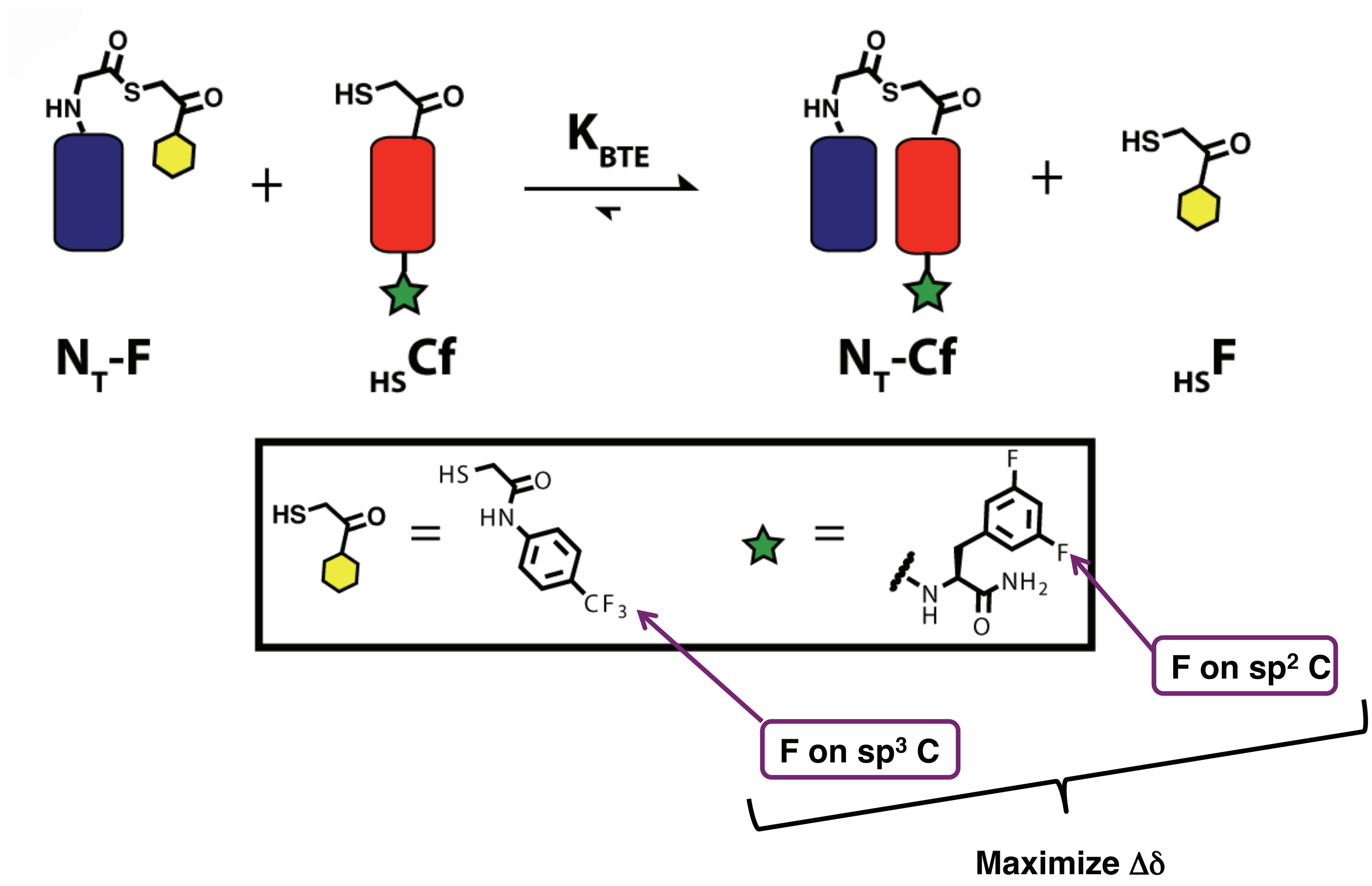
Geometry differences lead to greater opportunity for *a – a'* steric repulsions than *d – d'* steric repulsions.

Steinkruger, Bartlett, Hadley, Fay, Woolfson, Gellman *J. Am. Chem. Soc.* 134:2473 (2012)

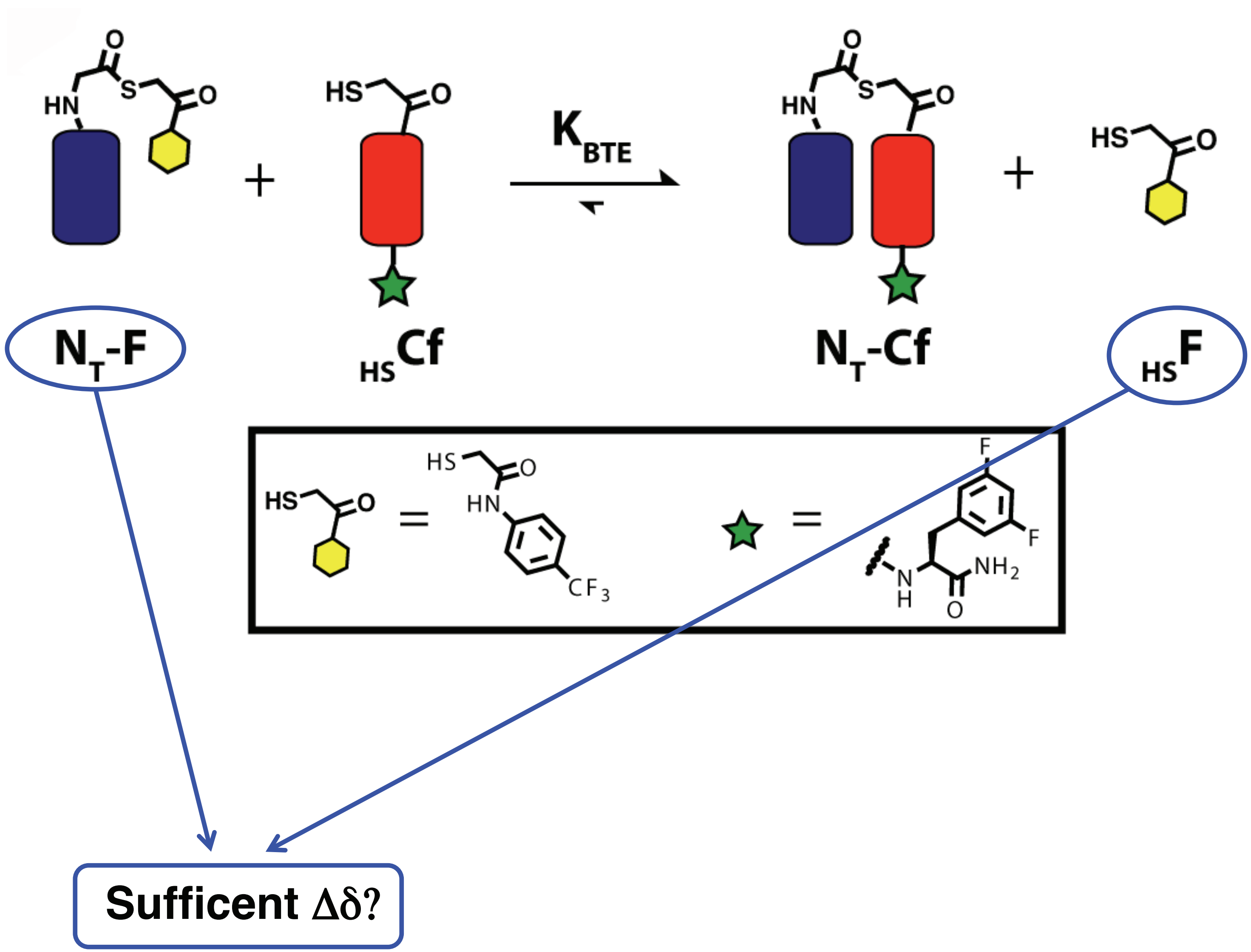
Monitor BTE by ^{19}F NMR?



Monitor BTE by ^{19}F NMR?



Monitor BTE by ^{19}F NMR?



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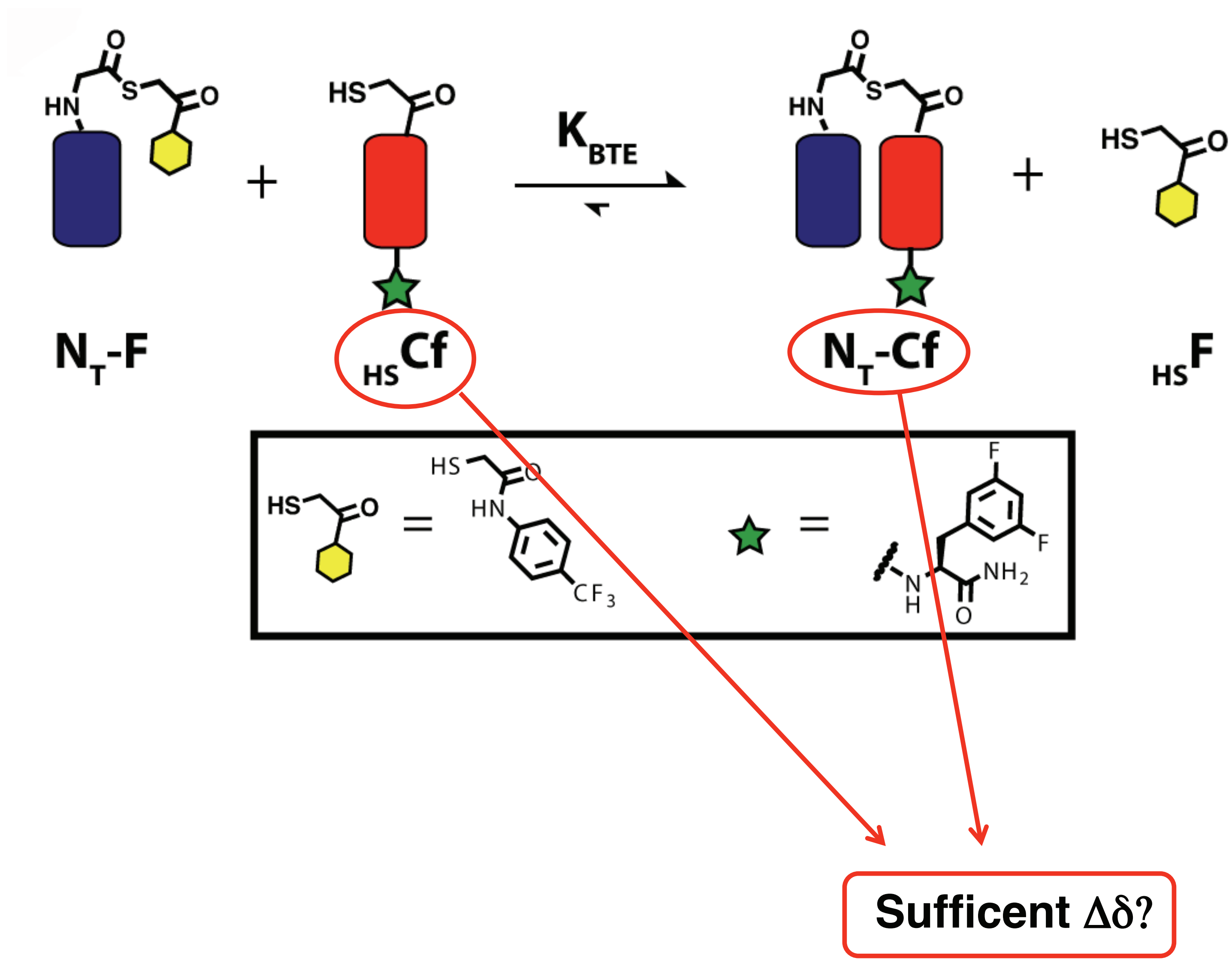
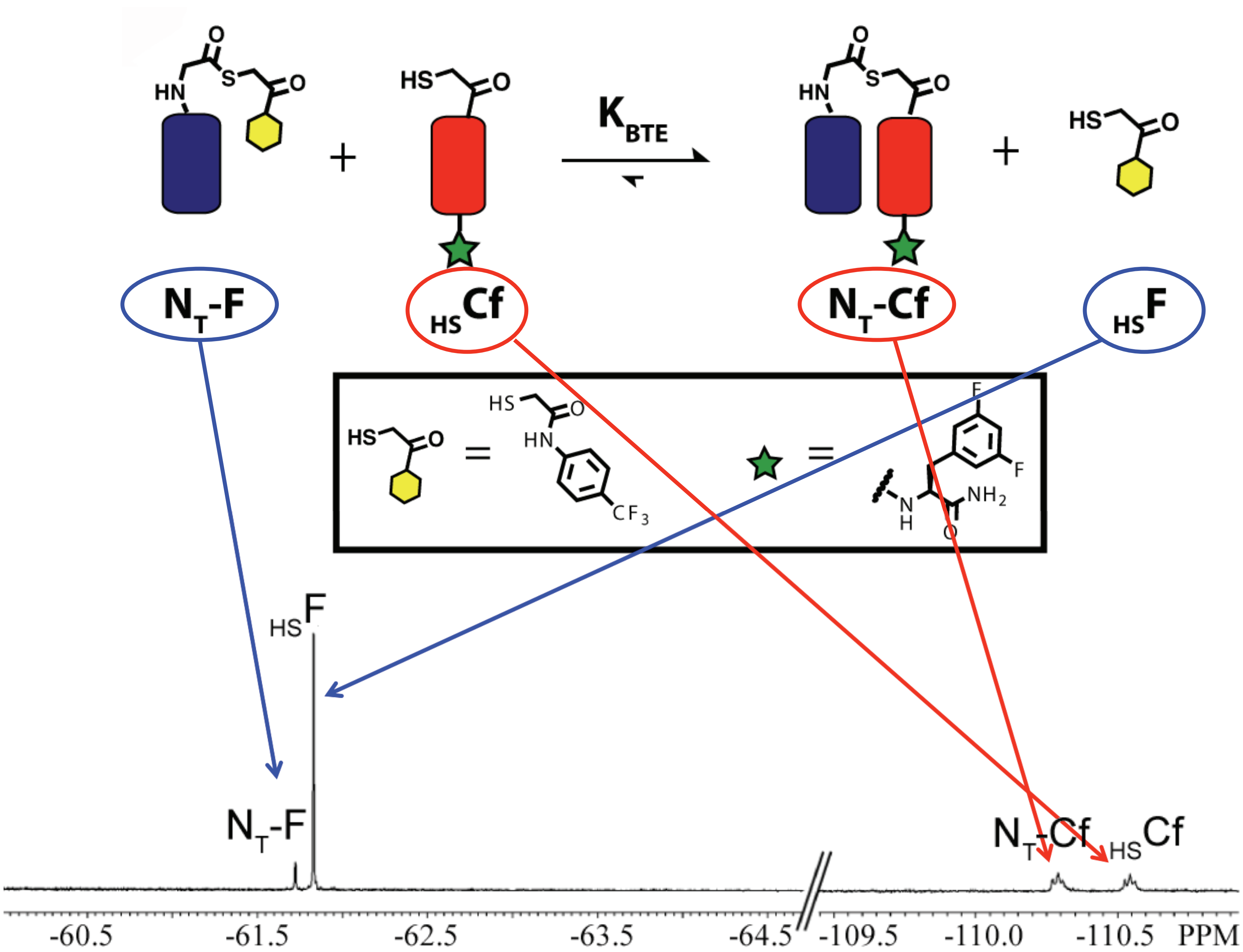
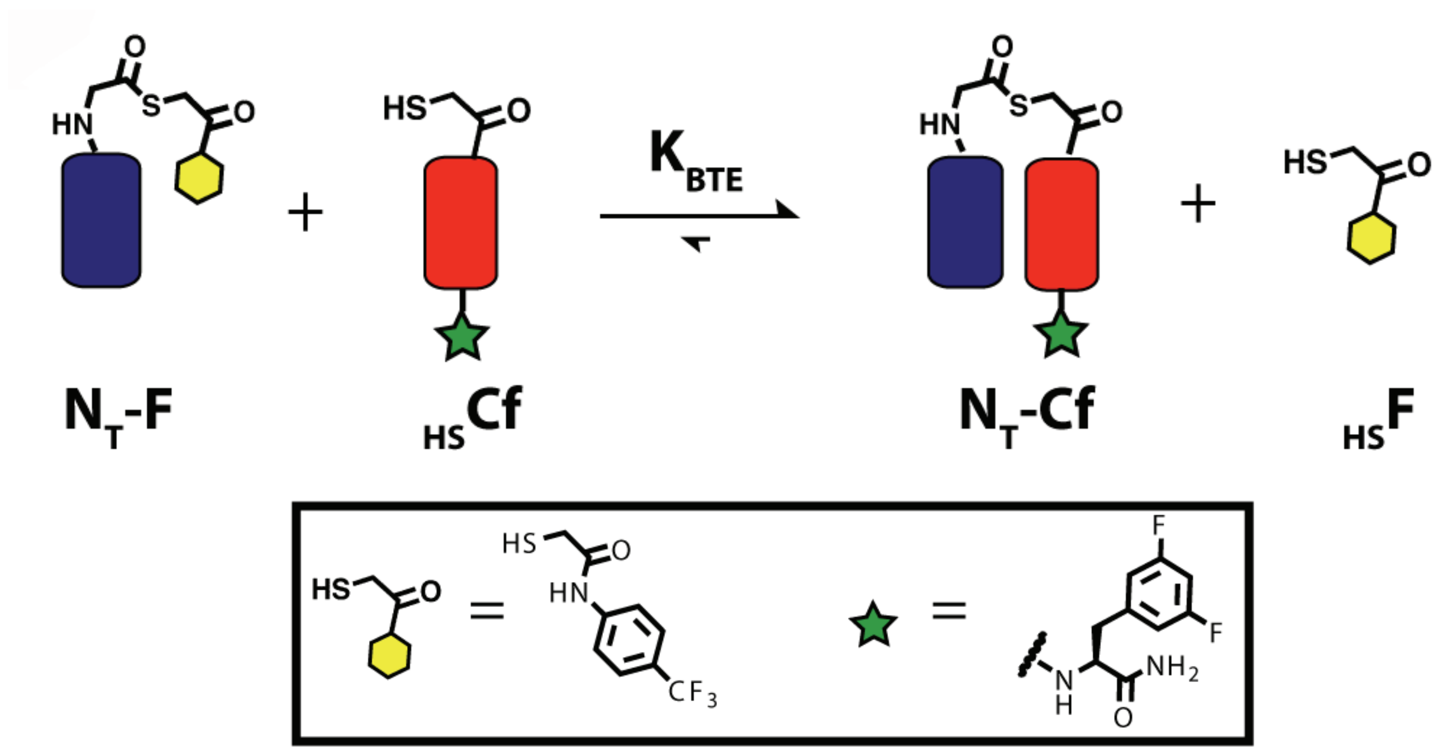


Monitor BTE by ^{19}F NMR?



Quantitative BTE Comparison: ^{19}F NMR vs. HPLC



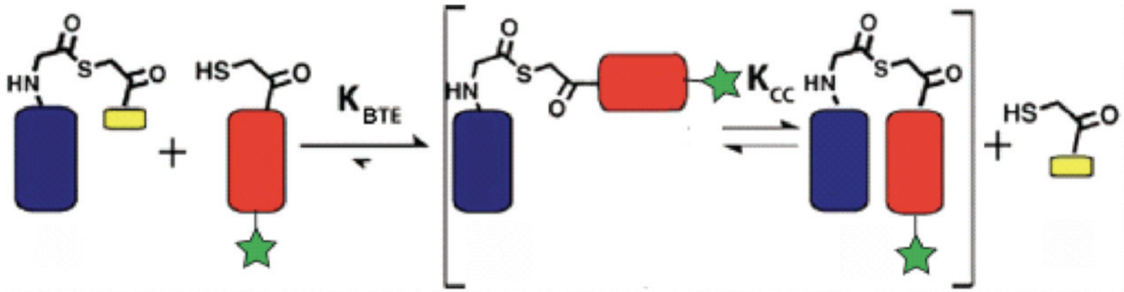
^{19}F NMR integration: ΔG_{Fold} = -1.4 kcal/mol

vs.

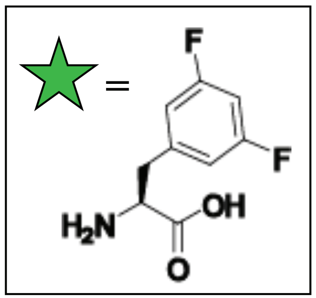
HPLC*: ΔG_{Fold} = -1.2 kcal/mol

*Peptides contain Tyr in place of fluoroaromatics

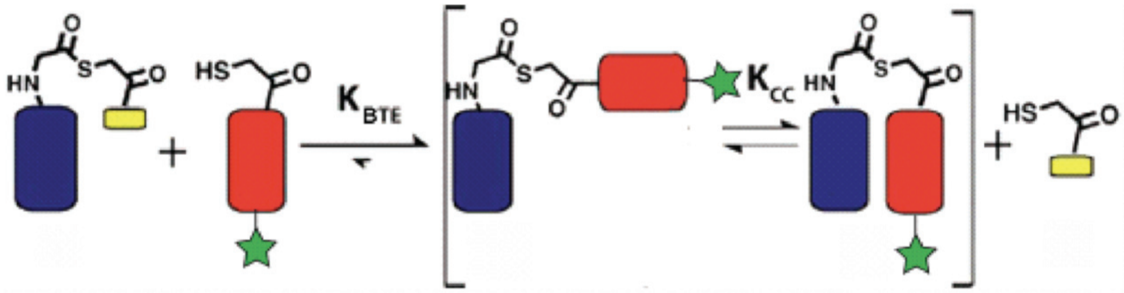
Correlate Folding-Induced ^{19}F Chemical Shift Perturbation ($\Delta\delta$) with Coiled-Coil Stability?



$_{HS}Cf$ N_T-Cf

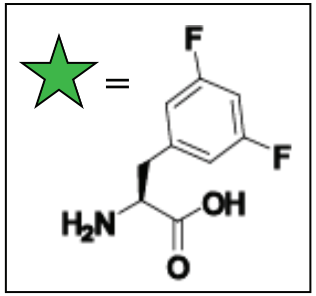


Correlate Folding-Induced ^{19}F Chemical Shift Perturbation ($\Delta\delta$) with Coiled-Coil Stability?



$_{HS}$Cf

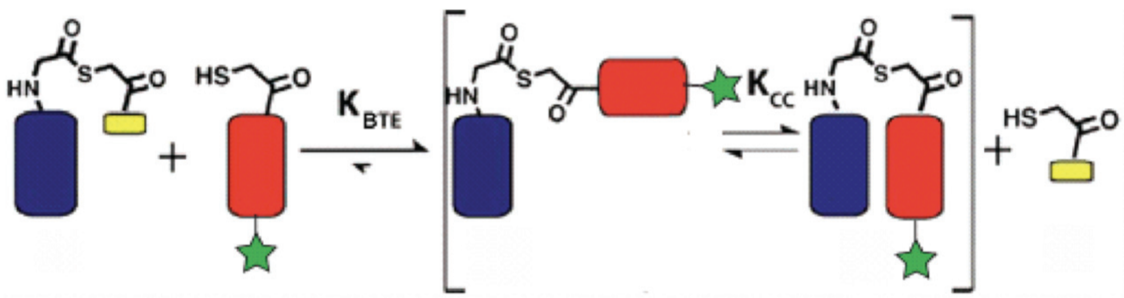
N_T-Cf



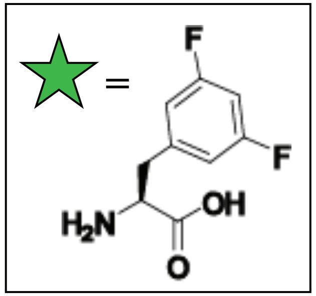
$$\Delta\delta = \delta(N_T\text{-Cf}) - \delta({}_{HS}\text{Cf})$$

Since $\delta(N_T$-Cf) is a population-weighted average, and $\delta({}_{HS}$Cf) should be relatively insensitive to sequence changes, perhaps $\Delta\delta$ provides a direct indication of ΔG_{Fold}?

Correlate Folding-Induced ^{19}F Chemical Shift Perturbation ($\Delta\delta$) with Coiled-Coil Stability?



$_{HS}Cf$ N_T-Cf



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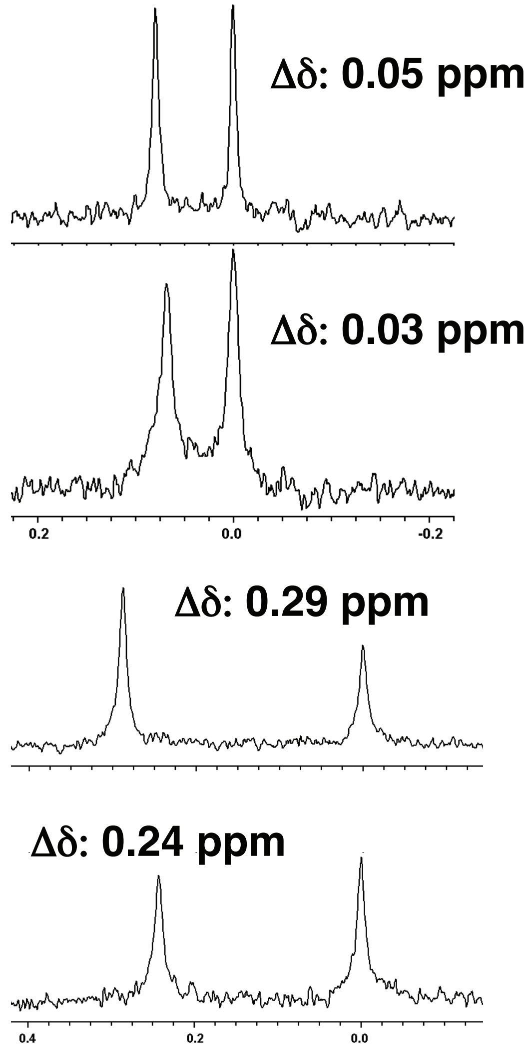
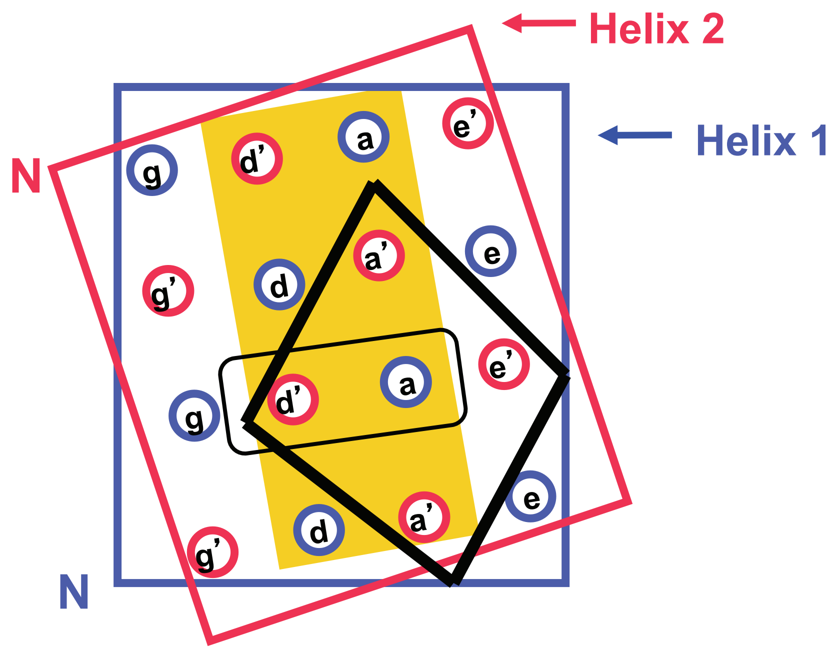
Selected sequence variants ($^{19}F\{^1H\}$ data):



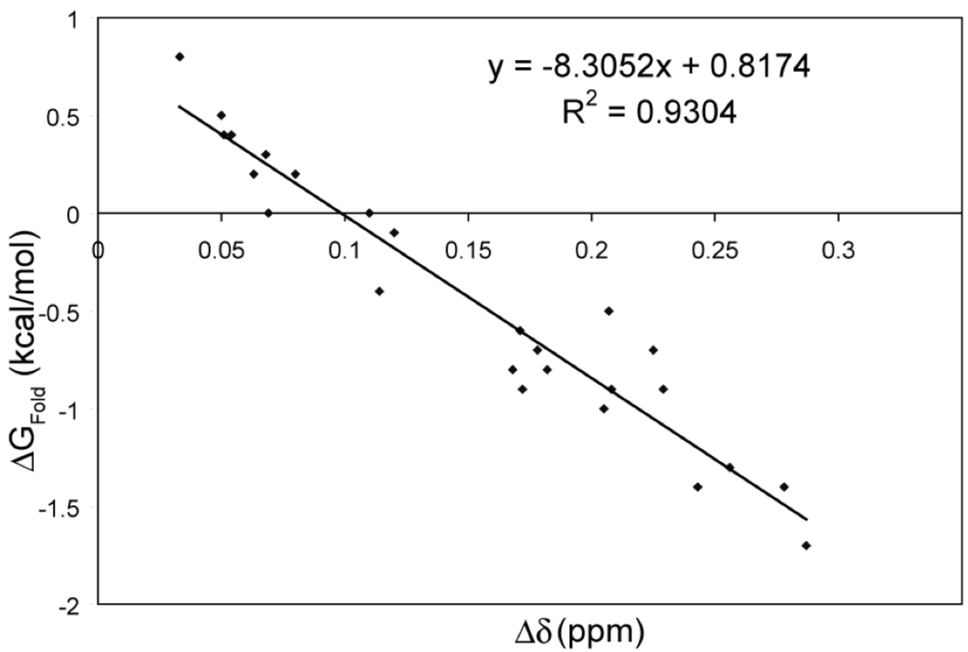
Knobs into Holes: Lateral Packing Partners



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

Correlating Δδ with ΔG_{Fold} (HPLC)

HPLC

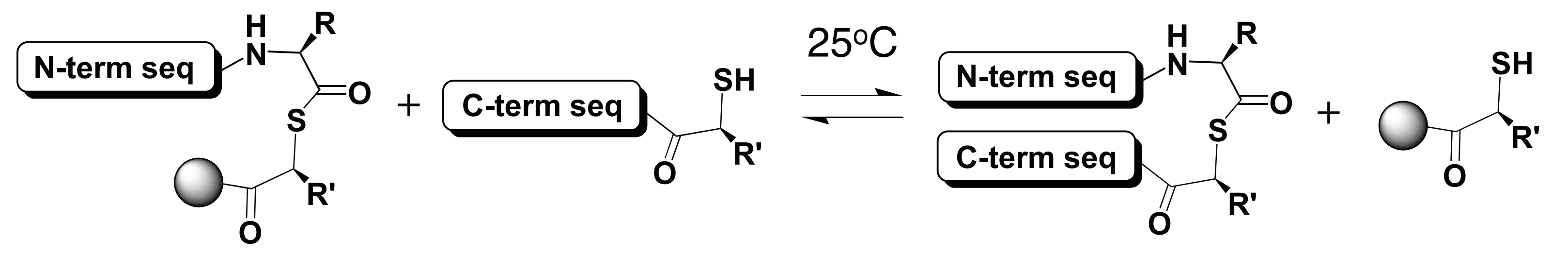


^{19}F NMR

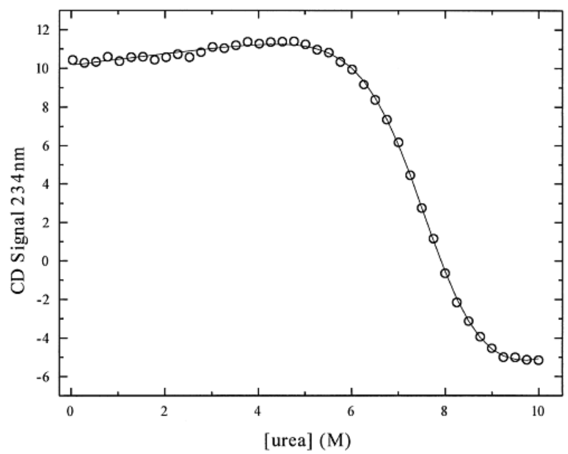
d' = lateral partner of a

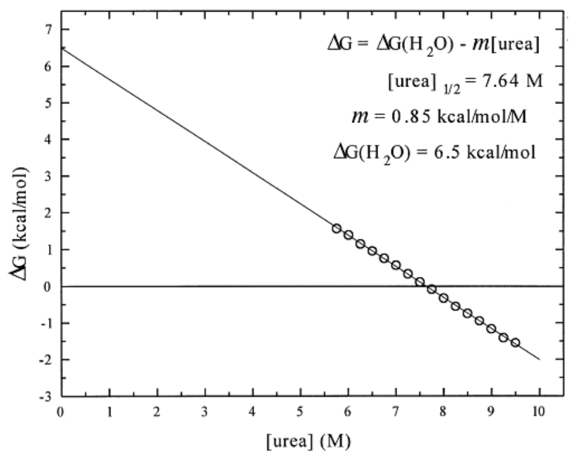
Pomerantz, Hadley, Fry, Gellman *ChemBioChem* 10:2177 (2009)

Conclusion: Backbone Thioester Exchange Offers a Useful Alternative Method for Assessing Tertiary Structure Stability Under Native Conditions



vs.





Pace & Shaw *Proteins Struct. Funct. Genet.* **2000**, *Suppl. 4*, 1.