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Aromatic-Proline Interactions: Electronically Tunable CH/π Interactions

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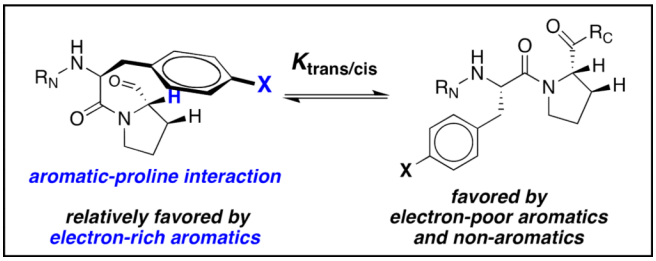
CONSPECTUS

Proline residues have unique roles in protein folding, structure, and function. Proline combined with the aromatic amino acids comprise the encoded cyclic protein residues. Aromatic protein side chains are defined by their negatively charged π faces, while the faces of the proline ring are partially positively charged. This polarity results from their two-point connection of the side chain to the electron-withdrawing protein backbone, and the lower electronegativity of hydrogen compared to carbon, nitrogen, and oxygen. The hydrogens adjacent to the carbonyl and amide nitrogen, Hα and Hδ, respectively, are the most partially positive. Proline's side chain is also conformationally restricted, allowing for interaction with aromatic residues with minimal entropic or steric penalty. Proline and aromatic residues can interact favorably with each other, due to both the hydrophobic effect and the interaction between the π aromatic face and the polarized C-H bonds, called a CH/π interaction. Aromatic-proline interactions can occur locally, for example to stabilize cis-amide bonds, and over larger distances, in the tertiary structures of proteins, and intermolecularly in protein-protein interactions. In peptides and proteins, aromatic-proline sequences more readily adopt cis-prolyl amide bonds, where the aromatic ring interacts with the proline ring in the cis conformation. In aromatic-proline sequences, Trp and Tyr are more likely to induce cis-amide bonds than Phe, suggesting an aromatic electronic effect. This result would be expected for a CH/π interaction, in which a more electron-rich aromatic would have a stronger (more cis-stabilizing) interaction with partial positive charges on prolyl hydrogens. In this Account, we describe our investigations into the nature of local aromatic-proline interactions, using peptide models. We synthesized a series of 26 peptides, TXPN, varying X from electron-rich to electron poor aromatic amino acids, and found that the population of cis-amide bond ($K_{trans/cis}$) is tunable by aromatic electronics. With 4-substituted phenylalanines, we observed a Hammett correlation between aromatic electronics and $K_{trans/cis}$, with cis-trans isomerism electronically controllable by 1.0 kcal/mol. All aromatic residues exhibit a higher cis population than Ala or cyclohexylalanine, with Trp showing the strongest aromatic-proline interaction. In addition, proline stereoelectronic effects can modulate cis-trans isomerism by an additional 1.0 kcal/mol. The aromatic-proline interaction is enthalpic, consistent with its description as a CH/π interaction. Proline-aromatic sequences can also promote cis-prolyl bonds, either through interactions of the aromatic ring with the preceding cis-proline, or with the Hα prior to cis-proline. Within proline-rich peptides, sequences commonly found in natively disordered proteins, aromatic residues promote multiple cis-amide bonds due to multiple favorable aromatic-proline interactions. Collectively, we found aromatic-proline interactions to be significantly CH/π in nature, tunable by aromatic electronics. We discuss these data in the context of aromatic-proline and aromatic-glycine interactions in local structure, in tertiary structure, in protein-protein interactions, and in protein assemblies.



Introduction

Proline residues have unique functions in proteins due to their conformational restriction, the absence of a hydrogen bond donor on the amide, and their special susceptibility to form a cis amide bond, owing primarily to the differential sterics of a secondary versus tertiary amide bond (Figure 1).[1–3] Cis-trans isomerism of prolyl amide bonds fundamentally changes local and long-range structure in proteins and is a rate-determining step in protein folding, with prolyl isomerases critical in protein folding in vivo.[4] In model peptides and in proteins in the PDB, proline-proline, aromatic-proline, and proline-aromatic sequences have the highest propensities to adopt a cis prolyl amide bond, suggestive of specific favorable interactions between aromatic residues and proline.[1,2,5–14] Local structural preferences inform on the relative strengths of long-range interactions, and thus data on local aromatic-proline interactions provide a basis for understanding these interactions in long-range intramolecular and intermolecular contexts. Consistent with this idea, aromatic-proline interactions appear to play a special role in stabilizing small proteins and in specific protein-protein interactions.[1,6,15–17]

In early studies on prolyl cis-trans isomerism by NMR, Wüthrich observed that phenylalanine-proline amide bonds had higher propensity for cis amide bonds compared to non-aromatic-prolyl amide bonds.[18] Subsequent analysis of the PDB and in peptides confirmed that aromatic-proline sequences were particularly prone to cis amide bond formation, and moreover suggested that Trp-Pro and Tyr-Pro sequences had a greater propensity for cis amide bond than Phe-Pro sequences.[2,5,9–14,19] Of particular note are studies by Dyson and Wright in SXPYDV peptides and Fischer in Ac-AXPAK-NH_2 peptides, who analyzed X=all canonical amino acids for their effects on X-Pro cis-trans isomerism. Fischer observed that Trp-Pro ~ Tyr(O^-)-Pro > Tyr-Pro > Phe-Pro > His-Pro in prolyl cis amide bond propensity, strongly suggesting an aryl electronics component to cis-trans isomerism.[12,13] Raleigh also found these preferences, both in water and in organic solvents.[12] Similarly, Pro-Aromatic sequences also exhibit an increased propensity for cis-Pro amide bonds, especially when preceded by an additional aromatic or proline residue.[7–11,20–23]

In view of these observations, plus geometries of aromatic-proline interactions in the PDB, where the Hα, Hβ, or Hδ of Pro are commonly under the aromatic ring, Pal and Chakrabarti suggested that aromatic-proline interactions are favored due to a CH/π interaction[19,24,25], in which the partial positive charge on a proline hydrogen(s) interacts favorably with the π face of the aromatic ring (Figure 2).[1,6,26,27] The partial positive charge would be greatest for Hα or Hδ protons due to adjacent carbonyl or amide electron-withdrawing groups. If the aromatic-proline interaction is well-described as a CH/π interaction, then more electron-rich aromatic residues should have a greater preference for a cis amide bond, while more electron-poor aromatics should have a reduced driving force for a CH/π interaction and thus relatively promote the sterically preferable trans amide bond. This hypothesis is well-supported by data on the canonical aromatic amino acids in all available protonation states.

However, because of limited natural aromatic residues, these differences could potentially be explained by differences in hydrophobicity (Trp>Tyr>His), heteroatoms, or aberrant effects of electrostatics (Tyr(O^-) versus Tyr, or His versus His(H^+)). In order to better understand the nature of aromatic-proline interactions, with general applications in protein folding and design of protein recognition interfaces, as well as specific application to understand cis-trans isomerism in sequences containing aromatic and proline residues, several years ago we initiated the systematic investigation of aromatic-proline interactions using a range of natural and non-natural amino acids within model peptides. This account summarizes our progress to date in understanding aromatic-proline interactions, within the context of other work on this interesting CH/π interaction.

Local Aromatic-Proline Interactions

Aromatic-*cis*-Pro Interactions

The effects of aromatic electronics on cis-trans isomerism of an aromatic-proline amide bond were examined within model Ac-TXPN-NH_2 (X=aromatic amino acid) tetrapeptides. These peptides contained an aromatic-proline core and flanking residues that favor cis amide bonds, as determined from experimental data and from propensities in type VI β-turns, which have an obligatory X-Pro cis amide bond between the *i*+1 and *i+2* residues.[7,9,28] Thr and Asn were the most favorable non-aromatic/non-proline residues at the *i* and *i*+3 positions, respectively, to promote cis amide bonds. Within this context, we synthesized peptides containing a series of electron-rich and electron-deficient aromatic amino acids at position X, including the canonical aromatic residues Phe, Tyr, and Trp. As controls, we synthesized peptides containing the non-aromatic residues Ala and cyclohexylalanine (Cha), which is hydrophobically similar to Phe but non-aromatic and thus incapable of a CH/π interaction.[13] Peptides were synthesized using a series of commercially available aromatic amino acids, ranging from electron-rich 4-amino-phenylalanine to electron-poor 4-pyridylalanine, 4-nitro-phenylalanine, and pentafluorophenylalanine.[29]

In view of the chemical versatility of aryl thiols and thiolates, including the multiple oxidation states accessible to cysteine, we also sought to examine peptides related to thiophenylalanine, the sulfur analogue of tyrosine and a functional hybrid of tyrosine and cysteine. To achieve this, we developed a novel, practical methodology for the synthesis of peptides containing thiophenylalanine derivatives, via copper-mediated cross-coupling reaction of thiolacetic acid and iodophenylalanine, conducted on solid phase on fully synthesized peptides (Scheme 1).[30] This reaction was compatible with all canonical amino acid functional groups, and was demonstrated on peptides up to 20 amino acids. The thioacetylated phenylalanine generated in this reaction was converted to thiophenylalanine in solution or on solid phase. Tyrosine-to-thiophenylalanine substitution in the trp cage resulted in miniprotein stability similar to the native trp cage.[15] In addition, thiophenylalanine-containing peptides were readily alkylated and/or oxidized to methyl, allyl, and 2-nitrobenzyl thioethers; sulfoxide; sulfone; activated disulfide; glutathione disulfide; and sulfonic acid derivatives (Scheme 2). The thiophenylalanine derivatives represent a range of aromatic electronic properties, from highly electron-rich (thiolate) to electron-deficient (sulfone, sulfoxide, sulfonate, thioester).

Cis and trans prolyl amide bonds are in slow exchange on the NMR timescale, and thus the relative populations of cis and trans conformations quantifiable. All TXPN peptides were analyzed by NMR spectroscopy and $K_{trans/cis}$ determined to identify the role of aromatic electronics in aromatic-proline cis-trans isomerism (Table 1). The data indicated that electron-rich aromatic amino acids relatively favored cis amide bonds, with the electron-rich aromatic residues Trp, Tyr(O^-), 4-NH_2-Phe, and 4-S^--Phe the most cis-favoring amino acids. In contrast, electron-poor aromatic amino acids more favored trans amide bonds, with

protonated 4-pyridylalanine, 4-SO_2Me-Phe, and 4-NO_2-Phe the most trans-favoring amino acids. The control non-aromatic peptides TAPN and TChaPN were more trans-favoring than all peptides containing aromatic amino acids, as expected if CH/π interactions promote cis amide bonds. Notably, in this context, Trp was the most electron-rich amino acid. Overall, compared to non-aromatic controls, cis-trans isomerism was tunable by 1.0 kcal/mol, providing an approximate value for the potential strength of an individual aromatic-proline interaction.

Hammett relationships are linear free energy relationships that are widely employed to understand aromatic electronic effects.[31] Hammett relationships describe the correlation of aromatic electronic effects on benzoic acid acidity (pK_a) with aromatic thermodynamic or kinetic phenomena, in the form log K_X/K_H=σρ, or log k_X/k_H=σρ, where σ=the effect (ΔpK_a) of a substituent Z on the acidity of 4-Z-benzoic acid relative to benzoic acid, and ρ = the relative sensitivity to aromatic electronics, with ρ=1 for benzoic acid acidity. While Hammett correlations are generally employed to understand through-bond aromatic electronic effects on reactivity and structure, Hammett relationships have also been observed in through-space interactions of aromatic rings. [32–36] Analysis of $K_{trans/cis}$ for all 4-substituted-Phe peptides indicated that a Hammett correlation was observed between prolyl amide cis-trans isomerism and the electronics of the aromatic residue preceding proline (Figure 3). The Hammett correlation of aromatic electronics with cis-trans isomerism is consistent with the aromatic-proline interaction being well-described as a CH/π interaction[24].

Proline has two limiting side chain conformations, the *Cγ-exo* and the *Cγ-endo* ring puckers (Figure 4). Proline ring pucker correlates with protein main chain conformation.[1,2,5,37] In cis amide bonds, the *endo* ring pucker is the predominant conformation. Within trans amide bonds, an *exo* ring pucker is more common when Pro is in compact conformations (α-helix (α_R), polyproline helix (PPII)), while the *endo* ring pucker is observed when Pro is in extended conformations. 4-Substituted proline residues can modulate cis-trans isomerism via stereoelectronic effects, with conformational preferences depending on the stereochemistry of substitution (*4R* favors *exo* ring pucker, while 4*S* favors *endo* ring pucker) and the electronics of the substituent, with more electron-withdrawing substituents inducing a stronger conformational preference. Thus, 4*R*–hydroxyproline (Hyp) and 4*R*–fluoroproline (Flp) promote an *exo* ring pucker and trans amide bond, while 4*S*–hydroxyproline (hyp) and 4*S*–fluoroproline (flp) promote an *endo* ring pucker and a relative preference for a cis amide bond. Fluoroprolines and the nitrobenzoates of hydroxyproline induce the strongest stereoelectronic effects due to the highly electron-withdrawing nature of these substituents.[38–42] Overall, within a TYProxN (Prox=Pro or 4-substituted proline), we observed that stereoelectronic effects allowed modulation of cis-trans isomerism by 1.0 kcal mol^{-1}, comparable in magnitude to aromatic electronic effects in aromatic-proline interactions (Table 2).[43]

Aromatic electronic effects and proline stereoelectronic effects may be combined to broadly tune cis-trans isomerism in peptides containing an aromatic-proline sequence. Combining an electron-poor aromatic and a strongly *exo*-promoting proline yields a strong preference for trans amide bond. In contrast, the combination of an electron-rich aromatic and a strongly *endo-favoring* proline derivative leads to an increased preference for a cis amide bond. Interestingly, aromatic effects on cis-trans isomerism appear to be stronger when an endo-favoring proline derivative is present (TYPN versus TWPN, $\Delta\Delta G_{trans/cis}$=0.24 kcal mol^{-1}; TYflpN versus TWflpN, $\Delta\Delta G_{trans/cis}$=0.49 kcal mol^{-1}), possibly due stereoelectronic effects restricting the proline conformation. The peptide TWflpN exhibits 60% cis amide bond in aqueous solution at room temperature. NMR data indicate a strong aromatic-proline interaction with the Pro Hα (δ=3.50 ppm) and Hβ (δ=0.66, 0.59 ppm) in the cis

conformation and adoption of a type VIa1 β-turn, based on a compact conformation at the Trp_{cis} ($^3J_{\alpha N}$=*4.2* Hz) and ROEs indicative of β-turn. The effects of aromatic electronic effects and proline stereoelectronic effects were approximately additive, with an overall 2.0 kcal mol^{-1} tunability in cis-trans isomerism in this peptide context.

The relative strength of aromatic-aromatic versus aromatic-proline interactions was examined within the model peptide context Ac-XYPN-NH_2, X=all 20 canonical residues (Table 3).[28] Here, Tyr can either interact with the subsequent Pro residue to favor cis amide bond, or alternatively may interact independently with the X residue, with no aromatic-proline interaction and thus an increased preference for trans amide bond. Here, where X is equivalent to the *i* position of type VI β-turns, non-aromatic residues had minimal effect on $K_{trans/cis}$ (=3.0–4.1). In contrast, aromatic residues disfavored cis amide bonds due to favorable aromatic-aromatic interactions, in a manner dependent on aromatic electronics (Trp>Tyr>Phe>His>$His(H_+)$). The electron-deficient protonated His was the most cis-favoring residue ($K_{trans/cis}$=2.9), whereas all other aromatic residues exhibited higher populations of trans amide bonds ($K_{trans/cis}$=4.2–8.0) than non-aromatic residues. Ac-WYPN-NH_2 had a similar $K_{trans/cis}$ as Ac-TChaPN-NH_2, indicating that local aromatic-aromatic and aromatic-proline interactions are energetically competitive. NMR data were consistent with aromatic-aromatic interactions stabilizing the trans conformation in these peptides: upfield chemical shifts in *i*+1 Tyr H_β and *i+2* H_δ Pro (Table 3) in the trans conformation correlate with aromatic electronics and $K_{trans/cis}$, suggesting trans-favoring aromatic-aromatic interactions and potentially aromatic-trans-Pro(*i*+2) interactions.

The thermodynamics of the aromatic-proline interactions were examined by temperature-dependent NMR to obtain the van't Hoff enthalpic and entropic contributions (Table 4). In X-Pro sequences, the trans conformation is normally enthalpically favored (e.g. Ac-GP-OMe, ΔG=−1.27 kcal mol^{-1}) [44].Previous work in random coil model peptides found that aromatic-proline sequences relatively increase the enthalpic preference for the cis conformation (though trans is still enthalpically favored).[13,45] We investigated four peptides (YYPN, AYPN, TWPN, and TWflpN) to identify the relative enthalpic contributions of aromatic-aromatic and aromatic-proline electronic effects and proline stereoelectronic effects (Figure 5, Table 4).[28,29] These data indicate (1) that aromatic-proline interactions are enthalpically favorable (up to 1.0–1.5 kcal mol^{-1} ΔH relatively favoring cis amide bond), consistent with a CH/π interaction and inconsistent with a classical hydrophobic effect being the primary basis of the aromatic-proline interaction;[24,25] (2) that aromatic-aromatic interactions (YYPN versus AYPN or Ac-GP-OMe) are similar in enthalpy to aromatic-proline interactions and can counteract aromatic-proline interactions, consistent with data that aromatic-aromatic-proline sequences were the most trans-favoring aromatic-proline sequences; and (3) that 4*S*–fluoroproline (flp) further stabilizes an aromatic-proline cis amide through enthalpically favorable stereoelectronic effects (up to 1.7 kcal mol^{-1} additional favorable ΔH). The peptide TWflpN, which stabilizes the cis amide bond via both aromatic-proline interactions and stereoelectronic effects, is particularly noteworthy because of its enthalpic preference for the cis amide bond, in contrast to the normal enthalpy-driven preference for a trans amide bond. TWPN also exhibited an enthalpic preference for the cis amide bond.

cis-Pro-Aromatic Interactions

Aromatic residues also interact with proline residues preceding them to favor X-cis-Pro-aromatic amide bonds.[9–11,20,21,23,46] cis-Pro-Aromatic interactions can result from the aromatic ring interacting with the preceding proline ring, or from the aromatic ring interacting with Hα two residues prior to it (H_α(*i*−2)-cis-Pro-Aromatic(*i*) interactions), which involves a cis prolyl amide bond (Figure 2).[1,6–8,20] Studies by Creighton and Basu indicated that cis-Pro-Aromatic interactions, primarily with H_α (*i*−2) of Gly or Pro two

residues prior to Tyr/Trp/Phe, are enthalpically favorable and dependent on electronics of the aromatic residue.[10,11,20,22,23] Aromatic-Pro-aromatic sequences also exhibit a high propensity for cis amide bonds, with aromatic rings able to stack against both faces of the proline ring.[8] Interestingly, Dyson and Wright found that in SYPXDV peptides, the highest population of cis amide bonds was observed with Phe, rather than Tyr or Trp, at the X position. We also observed that TYPF was more cis-favoring than TYPW, and that both favored a type VIa1 conformation (ideal $^{3}J\alpha N$=4 Hz) relative to TYPN or TYPG (Table 5), as they also observed. While speculative, electron-rich aromatic residues both preceding and following Pro might have a stronger driving force for aromatic-aromatic interactions over aromatic-proline interactions. In this context, we also observed that Pro at the *i+3* position (TYPP) favored cis amide bond compared to non-aromatic residues. However, with Pro at the *i+3* position, the $^{3}J_{\alpha N}$ for Tyr_{cis} (8.7 Hz) indicated a more extended conformation, suggesting that Pro at the *i+3* position favors type VIa2 or VIb β-turns (ideal 9–10 Hz).

Aromatic-proline interactions in proline-rich sequences

Proline-rich domains are natively disordered protein sequences that are prominent in higher eukaryotes. Proline-rich domains are the most common domain in *Drosophila* and the third-most common domain in humans.[47] Proline-rich domains have numerous dynamic roles, including engagement in protein-protein interactions critical to intracellular signaling.[16,17] Because proline-rich domains are disordered, understanding their dynamic structure is particularly challenging, as crystallography is not normally possible. Studies on peptides thus have particular value in understanding structure in disordered regions of proteins. We have addressed the effects of aromatic residues on local structure of proline-rich sequences in model tetrapeptides and in a model proline-rich sequence, Ac-GPPXPPGY-NH_2.

In Ac-PYPN-NH_2, where both Aromatic-cis-Pro and cis-Pro-Aromatic interactions are possible, we observed cis-trans isomerism at both X-Pro amide bonds, with 45% of the total peptide population containing at least one cis amide bond [28]. The data indicated (a) that Pro at the *i* position most strongly favored Tyr-cis-Pro ($K_{trans/cis}$=2.5 for Tyr-Pro with Ac-trans-Pro, compared to 3.0–4.1 for other non-aromatic residues and 4.2–8.0 for aromatic residues (see above)); (b) that Ac-cis-Pro-Tyr, in which Tyr may interact with either Ac or cis-Pro to stabilize a cis amide bond, was energetically similar to Tyr-cis-Pro ($K_{trans/cis}$=3.7 for Ac-Pro in a Tyr-trans-Pro context); and (c) that the presence of one Xaa-Pro bond cis favors a second cis amide bond ($K_{trans/cis}$=1.9 for Ac-Pro with Tyr-cis-Pro, or =2.7 for Tyr-Pro with Ac-cis-Pro, versus above). These data suggest that when Tyr residues are near multiple proline residues, particularly high populations of cis amide bond might be expected. Indeed, in the peptide Ac-PYPP-NH_2, NMR indicated 7 of 8 possible combinations of cis and trans amide Xaa-Pro bonds present, with the major species representing only 35% of the total population and four other species each 8–21% of the population, indicating substantial conformational hetereogeneity in slow exchange.

We have also analyzed peptides in the proline-rich context Ac-GPPXPPGY-NH_2, X=all canonical amino acids, to identify the propensities of all amino acids for polyproline helix (PPII), the proposed major conformation in proline-rich domains.[46] We found that Pro, Leu, Ala, and long linear amino acids most favored PPII; that short polar (Ser, Thr, Cys) and β-branched amino acids disfavored PPII; and that aromatic residues particularly disfavored PPII in this proline-rich context. We observed by NMR that when X=non-aromatic residues, less than 10% cis amide bond was observed, despite possible cis-trans isomerism at four X-Pro bonds, suggesting that PPII significantly reduces X-Pro cis-trans isomerism. In contrast, in peptides with X=Phe/Tyr/Trp, 45–60+% cis amide bond was observed (Figure 6). These data indicate that the combination of possible Aromatic-cis-Pro and Pro-cis-Pro amide bonds renders aromatic residues particularly unfavorable in proline-rich sequences, due to severe

conformational heterogeneity. Proline-rich sequences combine the possibility of favorable Pro-cis-Pro-Aromatic and favorable Aromatic-cis-Pro interactions that have been previously analyzed independently (Figure 1). In addition, aromatic residues normally favor *i*+1 and *i* +2 trans amide bonds through Aromatic(*i*)-Xaa-Yaa(NH)(*i*+2) interactions, in which the aromatic-amide *i/i+2* interaction is enthalpically favorable and comparable in energy to H_α(*i* −2)-cis-Pro-Aromatic(*i*) interactions.[10,11,21,48] In proline-rich sequences, this trans-favoring aromatic-amide interaction is not possible due to the lack of a prolyl amide proton, thus yielding sequences with multiple interactions favoring cis amide bonds. Our data on the very high population of cis amide bonds when aromatic residues are in proline-rich sequences, due to multiple aromatic-proline interactions, suggests that nature should strongly select against aromatic residues in proline-rich domains. Indeed, analysis of the PDB and proteomics data revealed very low frequencies of aromatic residues in proline-rich domains. In one of only three PPFPP sequences found in the pdb, the structure exhibits a Pro-cis-Pro-Phe conformation, with a Pro_α(*i* −2)-cis-Pro-Aromatic(*i*) interaction between the *i−2* and *i* residues (Figure 6). Moreover, data on collagen $(Pro\text{-}Hyp\text{-}Gly)_n$ repeats indicate that replacement of Pro or Hyp with aromatic residues was remarkably destabilizing, and that nature selects against aromatic residues at these positions in collagen, presumably due to severe cis-trans isomerism and its kinetic and thermodynamic consequences.[50] Collectively, these data provide an explanation why aromatic residues are not commonly observed in the ligands of SH3 domains, despite potentially favorable aromatic-aromatic interactions: aromatic residues in proline-rich sequences prevent formation of the polyproline helix needed for recognition, due to cis-trans isomerism mediated by multiple aromatic-proline interactions, and compete intramolecularly for recognition of proline residues.

Non-local aromatic-proline and aromatic-glycine interactions: intramolecular and intermolecular CH/π interactions mediated by proline

Interactions between aromatic rings and proline rings are also observable in protein stability and protein-protein interactions.[6] This effect is most prominent in small proteins, where the hydrophobic effect makes a lesser contribution to protein stability. Most notably, in Andersen's 20 residue trp cage miniprotein, the defining structural feature is a short α-helix with two aromatic residues (Tyr3, Trp6) packed against a polyproline helix (Pro18, Pro19). These residues are critical for miniprotein stability, with Trp6 particularly important: significantly reduced stability was observed with the hydrophobically similar (but electronically different) naphthylalanine (Figure 7).[15,51]

Protein interaction domains are central to organismal complexity, with protein-protein interactions providing mechanisms for specialization and response to diverse stimuli. Proline-rich domains are among the most common domains in eukaryotes. Lim observed that organismal complexity correlates with the number of proteins with domains that bind other proteins via proline residues and polyproline helices (e.g. SH3, WW, EVH1, GYF, profilin, and UVE domains).[16] In humans, over 400 proteins have domains which bind their target via polyproline helices. Protein domains which bind their target as polyproline helices typically contain multiple aromatic residues in the binding cleft, and these residues are critical for target recognition and specificity. For example, the Abl SH3 domain (1abo) binds its proline-rich target primarily by the aromatic residues Tyr70, Phe72, Trp99, Trp110 and Tyr115, a pattern similarly observed in other SH3 domains and PPII-binding domains (Figure 7).[52–54]

Why are aromatic-proline interactions particularly favorable? As all amino acid H_α protons are electron-deficient (as seen in their downfield chemical shifts relative to other aliphatic protons), then aromatic residues should have favorable CH/π interactions with any protein alpha protons. The likely major reasons for the more ready observation of aromatic-proline

interactions are (1) the conformational restriction of the prolyl ring, which allows multiple side chain protons to interact with the aromatic ring, providing a greater hydrophobic driving force (ring-ring stacking interactions); (2) the presence of electron-deficient H_δ protons on the carbon bound to the prolyl nitrogen, which can also interact favorably (CH/π) with the aromatic ring;[1,6] and (3) due to (1), the absence of steric or conformational restriction (i.e. an entropic cost due to χ_1 restriction) for interaction with the aromatic ring. Indeed, a role of sterics in disfavoring non-aromatic-proline interactions should be identifiable via favorable CH/π interactions with glycine. In studies on peptides containing Xaa-Pro-Tyr sequences, Kemmink and Creighton found that Gly-Pro-Tyr sequences had a substantially greater cis preference than Ala-Pro-Tyr sequences ($\Delta\Delta G$=−0.33 kcal mol^{-1} stronger interaction for Gly-Pro-Tyr than Ala-Pro-Tyr), consistent with a steric clash or entropy reducing the strength of the Ala Hα(*i*)-cis-Pro-Aromatic(*i*+2) interaction.[11] This preference was also observed in the PDB, where Gly-Xaa-Aromatic CH/π interactions occur more frequently than Ala-Xaa-Aromatic.[19] Interestingly, Pro-Xaa-Xaa-Aromatic (*i*/*i*+3) CH/π interactions are observed as stabilizing elements at the N-terminus of α-helices, where trans amide bonds are present and the interaction promotes **a**-helical structure. Aromatic-proline interactions also lead to rapid collagen self-assembly, with aromatic residues from telopeptides interacting with prolines in the Pro-Hyp-Gly core collagen repeats to generate functional micron-scale collagen assemblies.[55] More broadly, in membrane proteins, glycine residues engage in favorable CH/π interactions with aromatic residues to promote helix stability and helix-helix assembly.[56] Notably, within membranes, the hydrophobic effect is of minimal energetic importance, so the CH/π interaction provides a critical enthalpic basis for stability and specificity in interactions between nonpolar residues.

In soluble proteins, a dramatic example of glycine CH/π interactions is the trp cage miniprotein.[15] In addition to favorable aromatic-aromatic and aromatic-proline interactions, an underappreciated interaction critical in trp cage stability is between Trp6 and Gly11 (Figure 7). Gly11 is part of a flexible loop (G**G$_{11}$**PSSG) that is a surprisingly high percent of the total sequence of a 20-residue miniprotein. NMR data indicate that one of the Gly11 H_α protons is particularly upfield-shifted (Gly Hα2 δ=0.96 ppm), consistent with an unusually tight interaction with Trp6. We have developed stabilized trp cage miniproteins (ΔT_m=up to +22°C) via stereoelectronic effects on 4-substituted prolines that restrict Pro12 to an *exo* ring pucker.[42] In these stabilized trp cage variants, Gly11 Ha2 is even further upfield-shifted (δ=0.40 ppm), a remarkable chemical shift for a protein H_α and consistent with a strong Gly-Trp CH/π interaction that is critical to stabilization of the trp cage.

Aromatic-proline interactions are also observed in protein-DNA, protein-RNA, and protein-small molecule interactions. Of particular note are dietary polyphenols, electron-rich polycyclic aromatic compounds that interact specifically with salivary proline-rich proteins.[17,57–60] These interactions, which directly affect the taste sensation of astringency, involve specific, enthalpically-favored recognition of the aromatic rings by the proline residues, with a preference for diproline sequences and the Hα face of proline. When you next drink a glass of red wine or sip on green tea, contemplate the electron-rich aromatics and proline residues engaging in CH/π interactions in your mouth, contributing to both the taste and absorption of these enjoyable antioxidants.

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Biography

Neal J. Zondlo is an Associate Professor of Chemistry and Biochemistry at the University of Delaware. A Green Bay, Wisconsin native, Neal received his B.A. in Biochemistry and Russian at Rice University in 1992. Neal received his Ph.D. in Organic Chemistry from Yale University in 1999, working in protein design with Alanna Schepartz. Neal completed postdoctoral research in asymmetric catalysis with Eric Jacobsen at Harvard University. Neal has been at the University of Delaware since 2001, working in the areas of protein design, electronic and stereoelectronic effects to control protein structure, natural and designed effects of protein post-translational modifications on protein structure, the development of peptide and protein modification chemistry, and the design of proteomimetics to inhibit protein-protein interactions.

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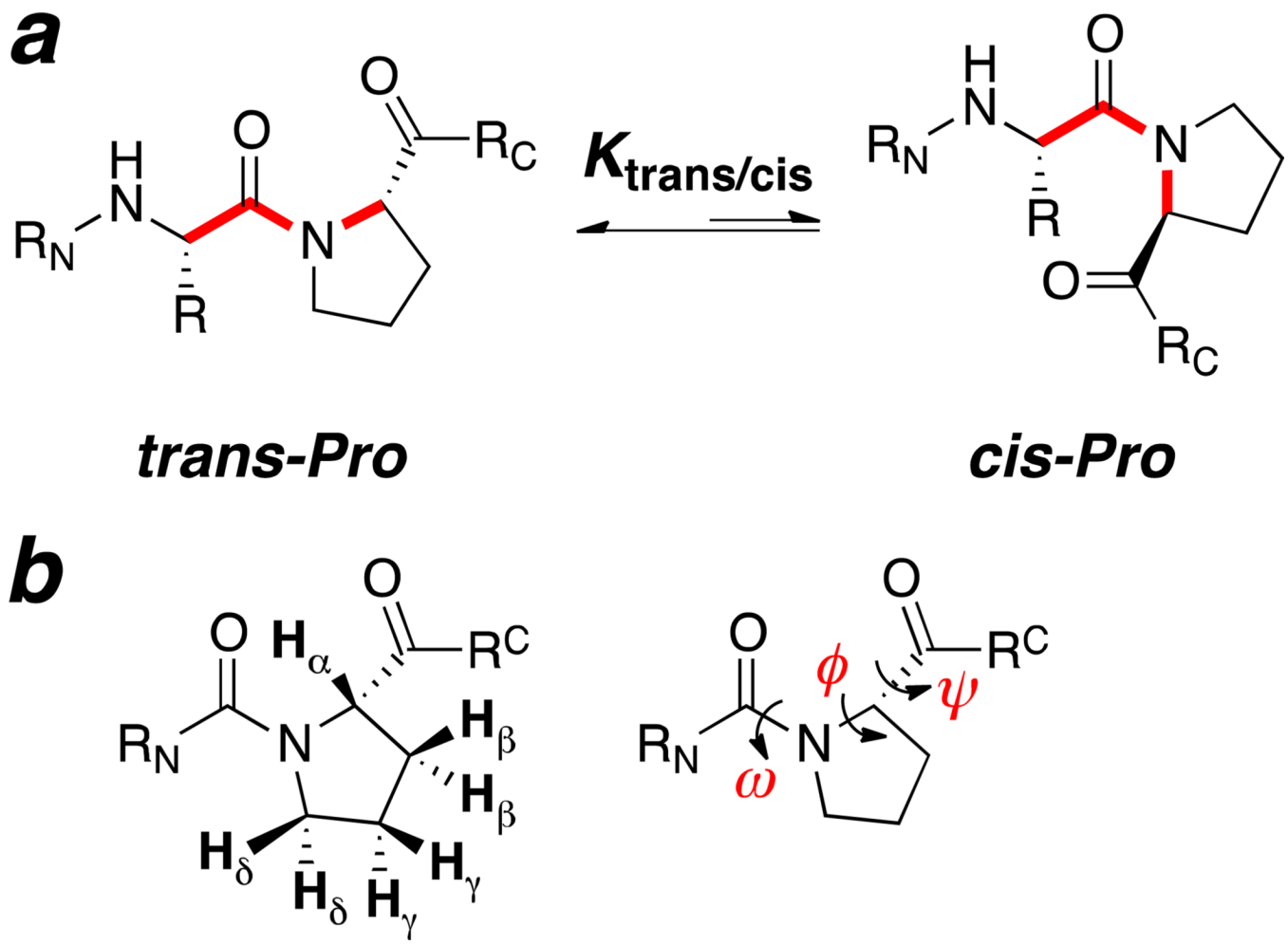


Figure 1.
(a) Cis-trans isomerism of an Xaa-Pro amide bond. (b) Proline nomenclature and main chain torsion angles (ϕ,ψ,ω).

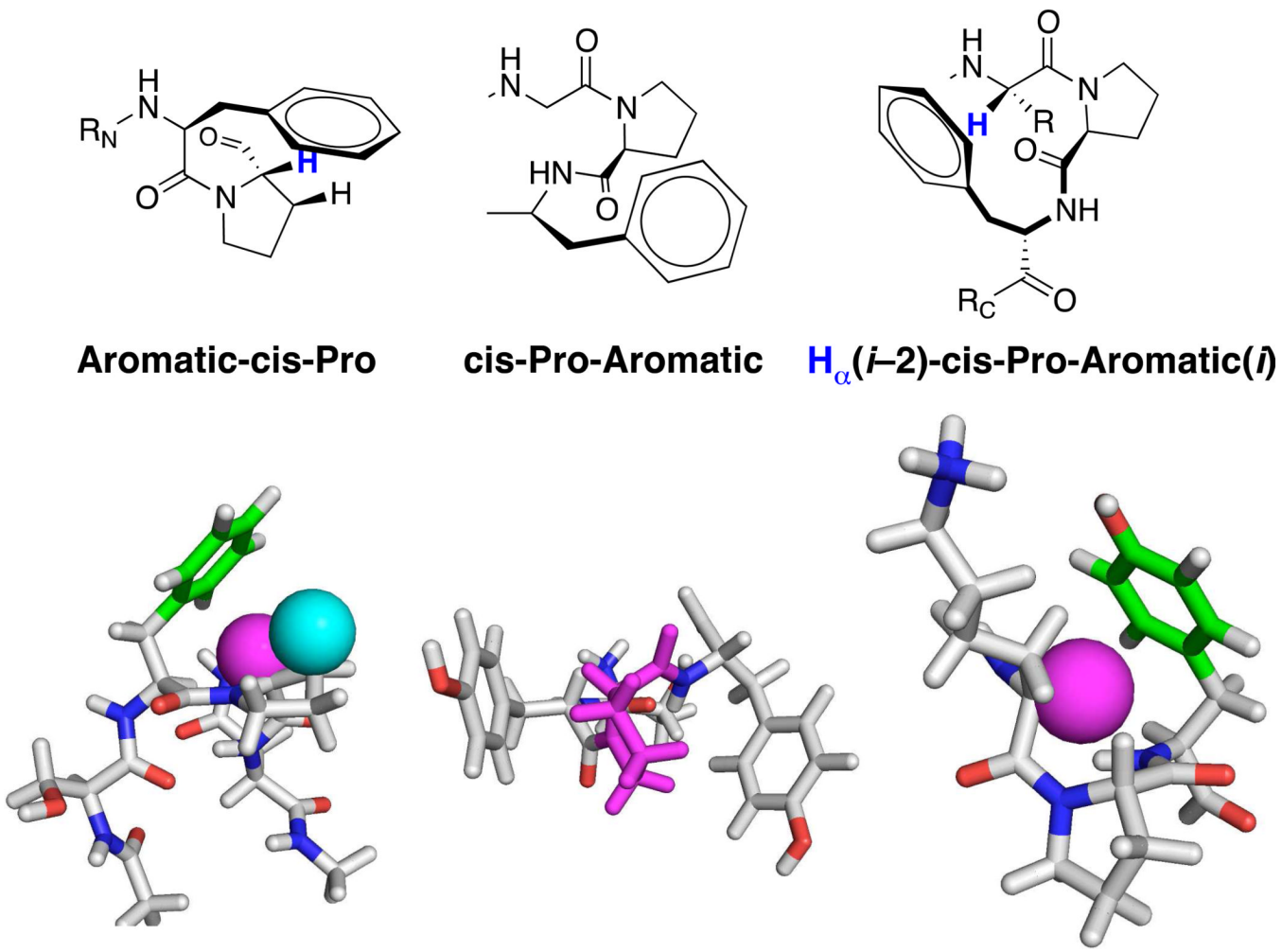


Figure 2.

Local aromatic-proline interactions. (top) Schematic representations of aromatic-cis-Pro, cis-Pro-Aromatic, and Hα(*i*−2)-cis-Pro-Aromatic(i) interactions that stabilize prolyl cis amide bonds. (bottom) (left) An aromatic-cis-Pro interaction (pdb 1h4i)[3] in the sequence SFPN with Hα (magenta) and Hβ (cyan) interactions with Phe shown (center) Proline with both aromatic-cis-Pro and cis-Pro-aromatic interactions in the sequence TYPY (pdb 1ade)[27]. (right) Hα(*i*−2)-cis-Pro-Aromatic(*i*) interaction (Hα magenta) in the sequence KPY (pdb 2dur)[26]. Hydrogens were added via Pymol.

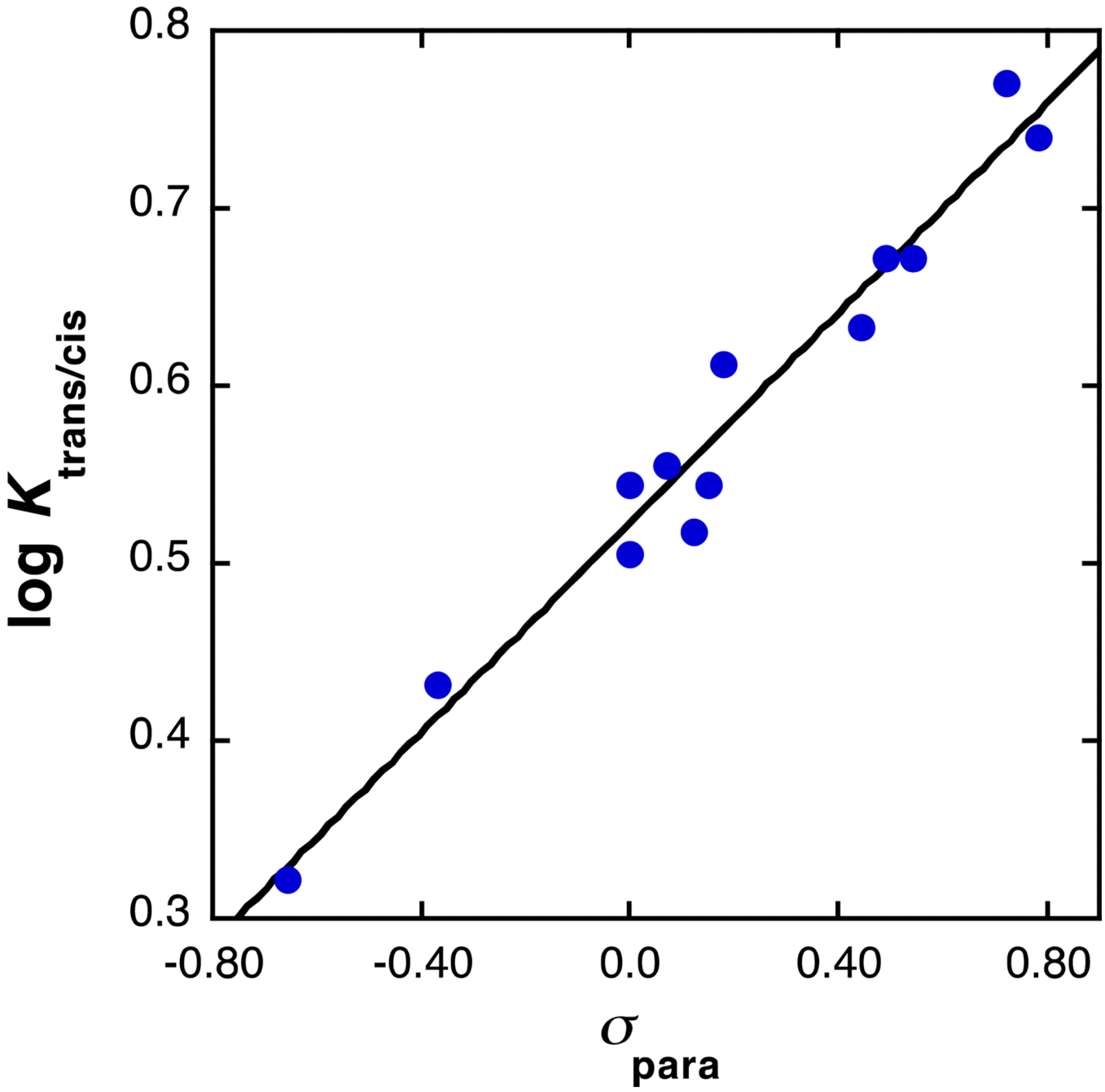


Figure 3.

Hammett plot correlating cis-trans isomerism of an aromatic-proline amide bond ($K_{trans/cis}$ of Ac-T(4-Z-Phe)PN-NH_2 peptides) with the Hammett σ_{para} constant[31] (σ_{para}= pK_a(PhCOOH) – pK_a(4-Z-Ph-COOH)) for the Z substituent (ρ = 0.295±0.017, R=0.98) for all 13 neutral Ac-T(4-Z-Phe)PN-NH_2 peptides with established σ_{para} for Z.[29,30,41]

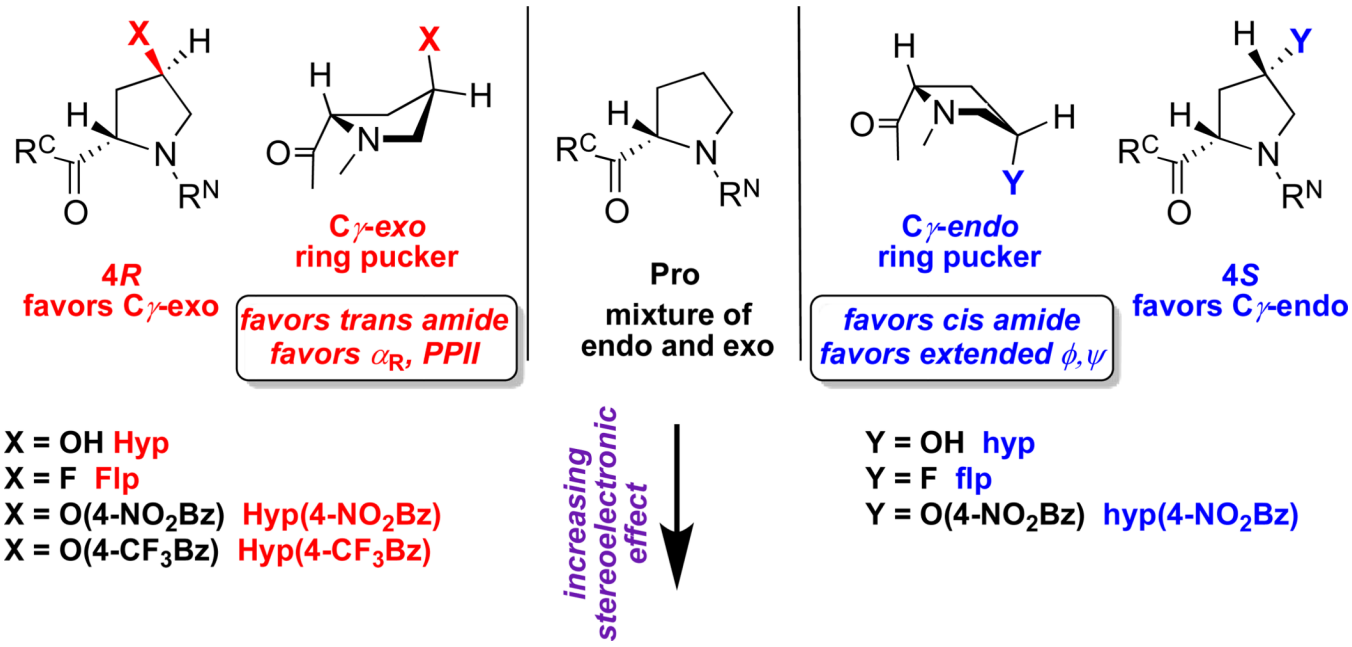


Figure 4.
4-Substituted proline residues, proline *exo* and *endo* ring puckers, and the effects of proline substitution on ring pucker and protein main chain conformational preferences.

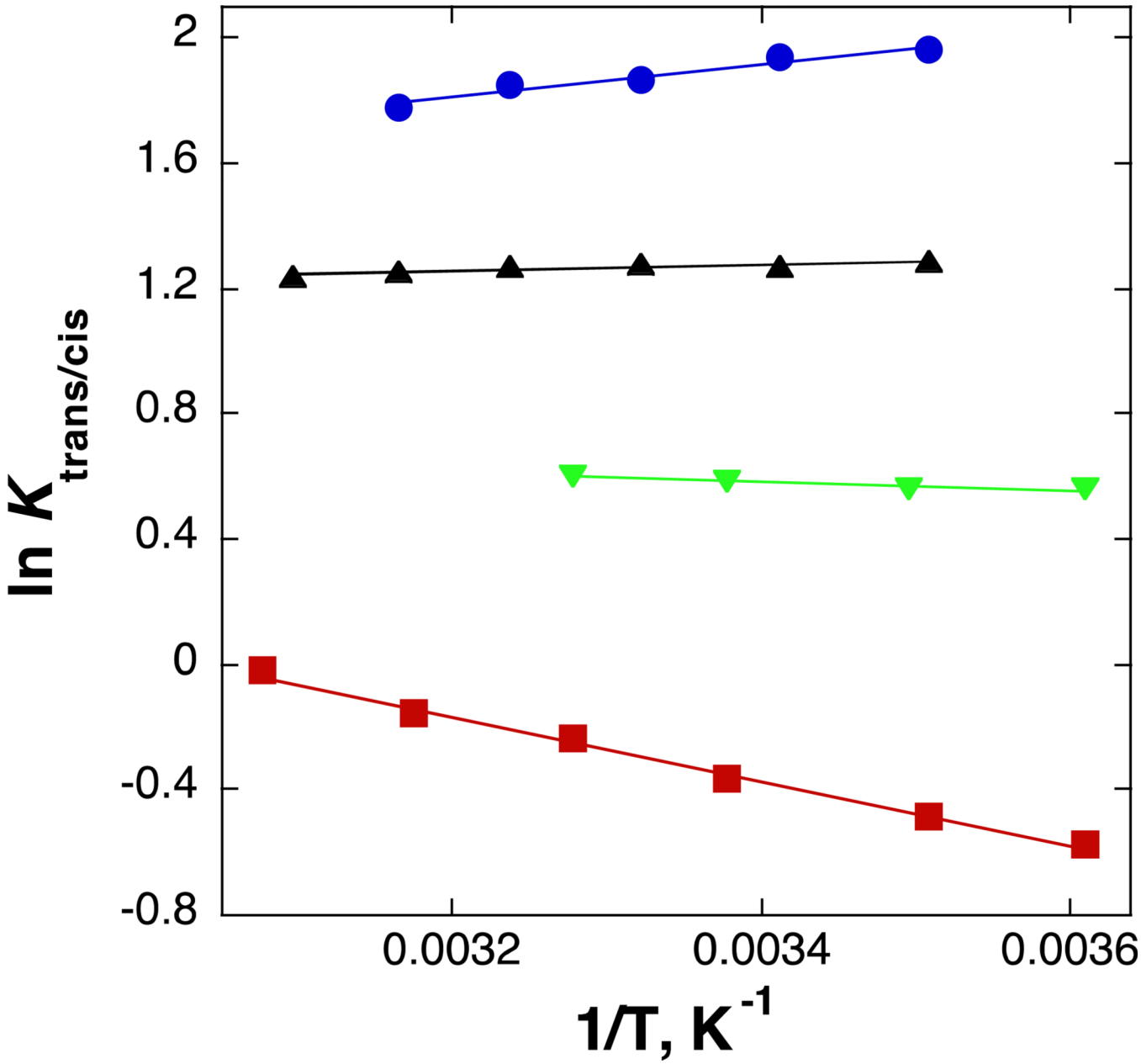


Figure 5.
Van't Hoff plot of the temperature dependence of cis-trans isomerism on the identity of *i* and/or *i*+1 residues.[28,29] Blue circles, YYPN; black triangles, AYPN; green inverted triangles, TWPN; red squares, TWflpN.

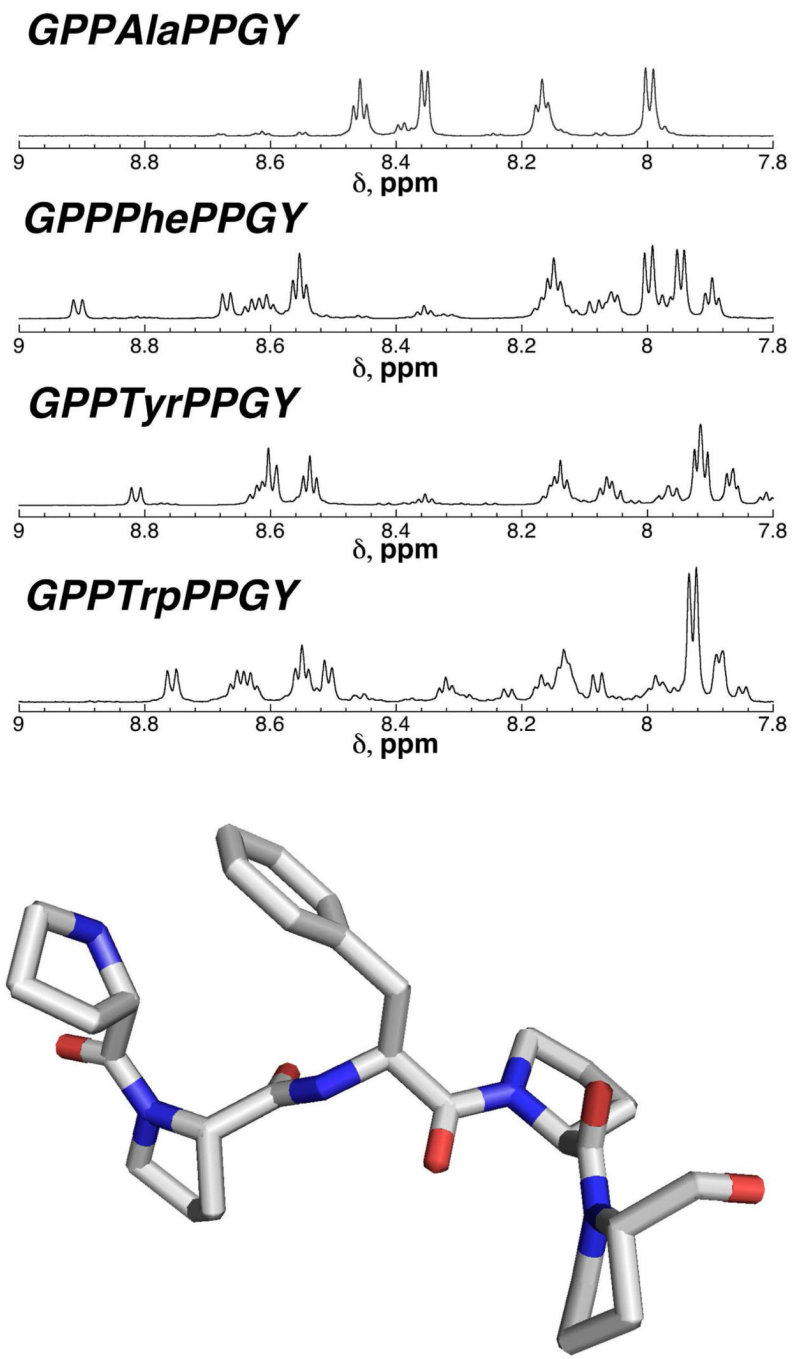


Figure 6.

Aromatic residues in proline-rich sequences strongly favor cis amide bonds via cis-Pro-Aromatic and Aromatic-cis-Pro interactions. (top) NMR spectra of the amide regions of Ac-GPPXPPGY-NH_2 peptides.[46] Four resonances (one per amide NH) are expected for peptides with all trans amide bonds (e.g. X=Ala). Peptides with X=Phe,Tyr,Trp exhibit multiple species with cis-trans isomerism in slow exchange, with combinations of Pro-cis-Pro-aromatic and aromatic-cis-Pro species observed. For X=Trp, 8 of the 16 (2^4 combinations of cis and trans amide bonds at the X-Pro bonds) possible species were observed. (bottom) Structure of P-cis-PFPP sequence (pdb 2cul)[49], with an Hα(*i*−2)-aromatic(*i*) CH/π interaction stabilizing the Pro-cis-Pro amide bond.

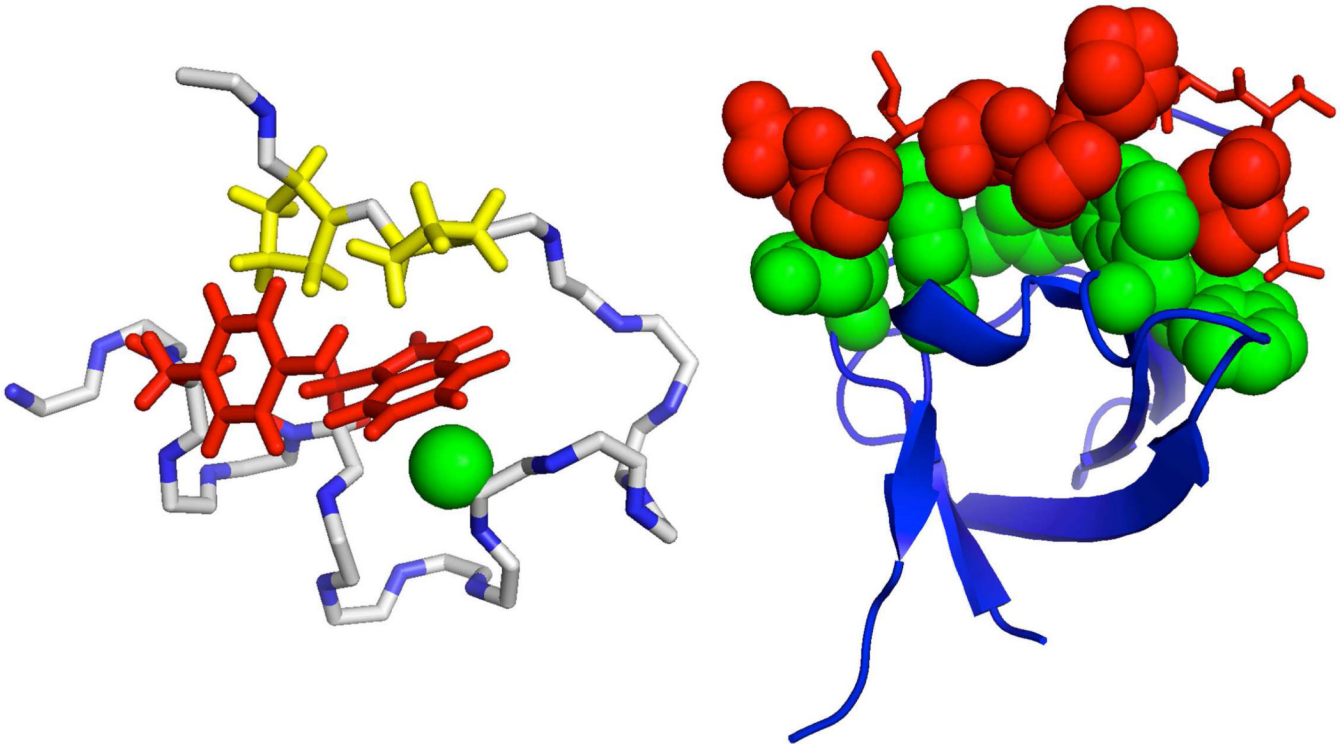
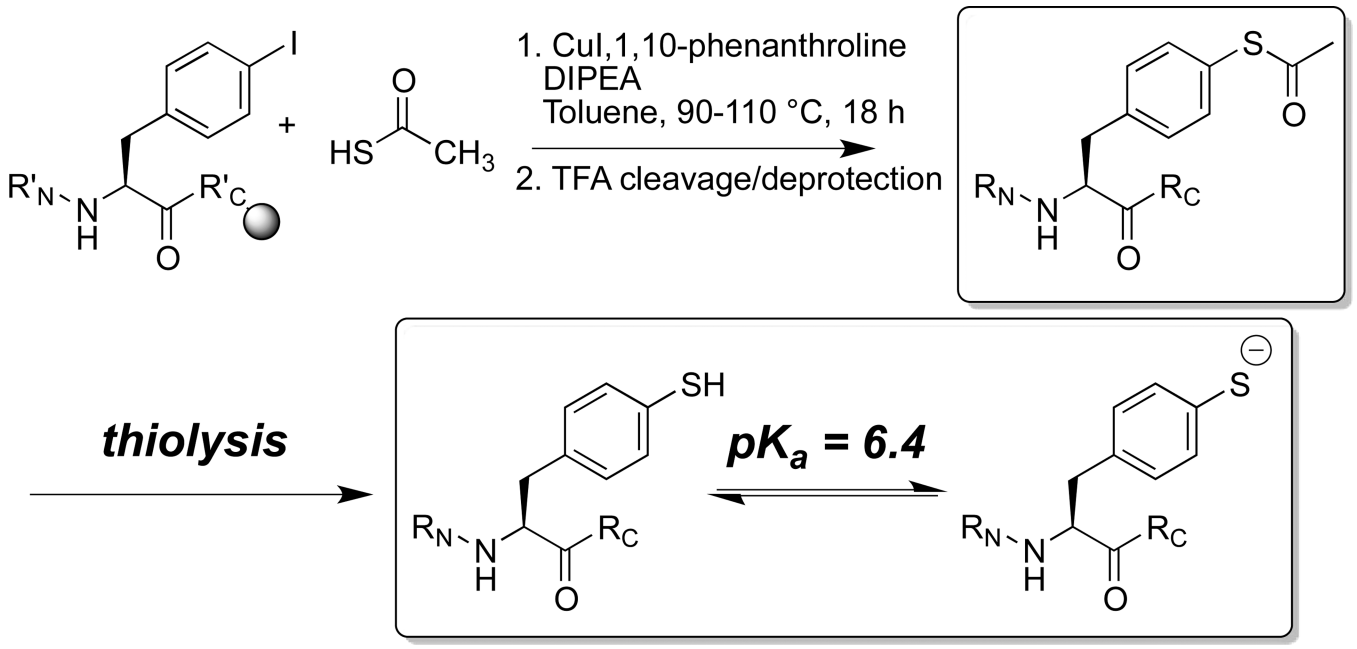
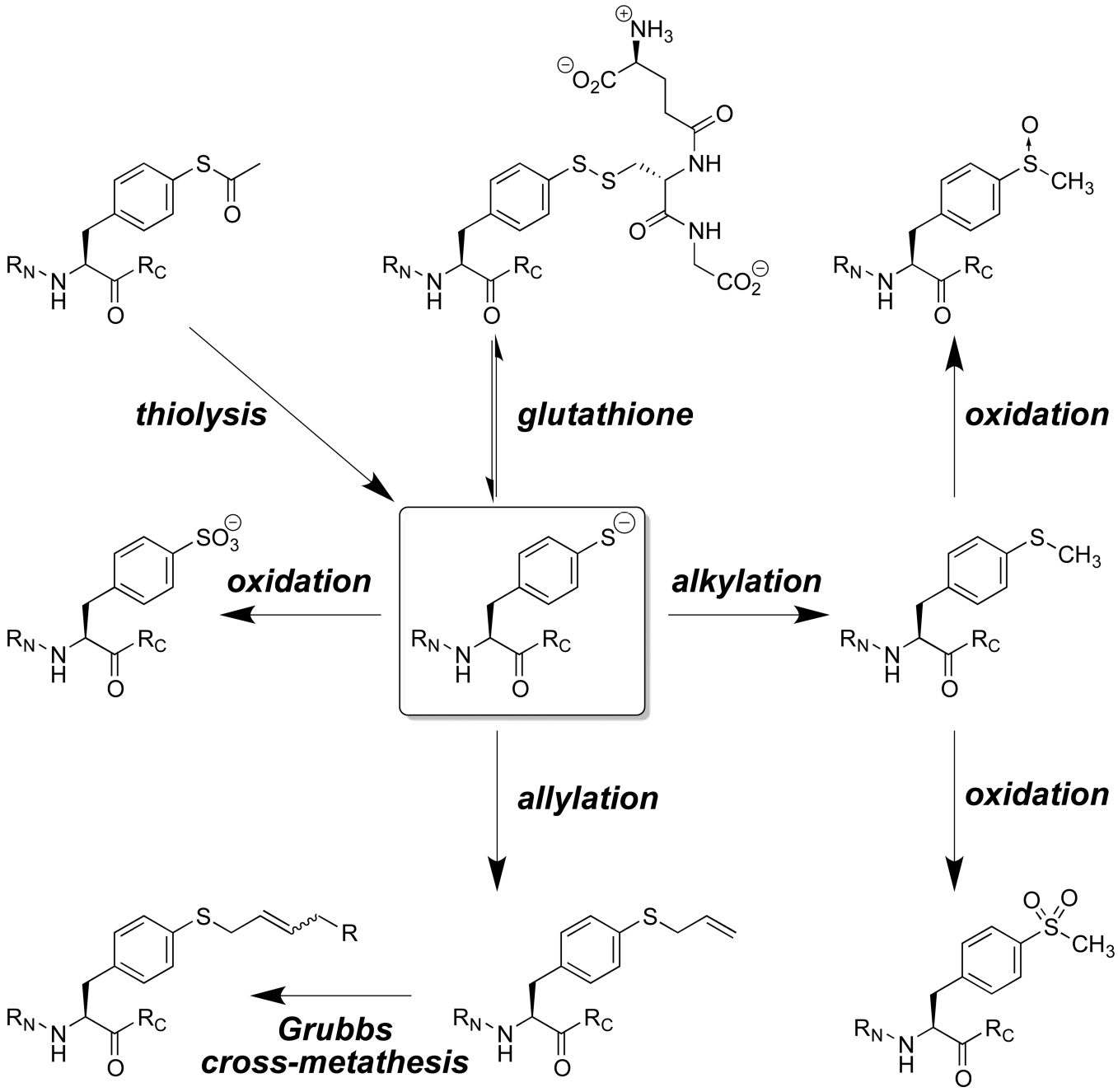


Figure 7.
Long range CH/π interactions. (left) Intramolecular aromatic-proline and aromatic-glycine interactions stabilize the trp cage. Tyr3 and Trp6 (red) interact favorably with each other and with Gly11 Hα2 (green sphere), Pro18, and Pro19 (yellow) (pdb 1l2y).[15] (b) Interdigitation of SH3 domain aromatic (green) and ligand proline (red) residues in the Abl SH3 domain (blue ribbon, green spheres) binding to a proline-rich ligand (red) (pdb 1abo).[54]



Scheme 1.
Synthesis of peptides containing 4-thiophenylalanine. R_N and R_C indicate the rest of the peptide sequence. The thiolysis may be conducted in solution or on solid phase.[30]



Scheme 2.
Solution interconversion of thiophenylalanine to functionalized and electronically modified aromatic derivatives.[30]

Table 1

$K_{trans/cis}$ for Ac-T-X-Proline-N-NH_2 peptides. Data were measured in 5 mM phosphate, 25 mM NaCl at 296 K.[29,30] ΔG=–RT ln $K_{trans/cis}$.

X =	$K_{trans/cis}$	σ_{para}	ΔGkcal mol^{-1}
4-(H^+)Pyridyl-Ala	7.6		−1.20
4-SO_2Me-Phe	5.9	0.72	−1.05
4-Pyridyl-Ala	5.7		−1.03
4-NO_2-Phe	5.5	0.78	−1.01
4-CF_3-Phe	4.7	0.54	−0.92
4-S(O)Me-Phe	4.7	0.49	−0.92
4-SBz-Phe	4.5		−0.89
F_5-Phe	4.4		−0.88
4-$^+NH_3$-Phe	4.4	0.60	−0.88
4-SO_3^--Phe	4.4	0.35	−0.88
4-S(2-nitrobenzyl)-Phe	4.4		−0.88
4-SAc-Phe	4.3	0.44	−0.86
4-I-Phe	4.1	0.18	−0.84
4-SSGlutathione-Phe	3.7		−0.77
4-SPh-Phe	3.6	0.07	−0.76
4-SH-Phe	3.5	0.15	−0.74
4-SMe-Phe	3.5	0.00	−0.74
4-$SCH_2CH=CHCH_2OH$-Phe	3.4		−0.72
4-S-Allyl-Phe	3.3	0.12	−0.71
Phe	3.2	0.00	−0.69
4-SSPy-Phe	2.9		−0.63
Tyr	2.7	−0.37	−0.59
4-S^--Phe	2.2	−1.21	−0.47
4-NH_2-Phe	2.1	−0.66	−0.44
Trp	1.8		−0.35
4-O^--Phe	1.7	−0.81	−0.31
Ala	10.7		−1.40
Cha	8.0		−1.23

Table 2

Combined electronic and stereoelectonic effects on cis-trans isomerism of an aromatic-proline sequence.[29,41,42] Data were collected at 296 K in 5 mM phosphate, 25 mM NaCl. n.d.=not determined due to spectral overlap. $^{3}J_{\alpha N}$=coupling constant between HN and Hα, which correlates with the ϕ torsion angle.[43]

	$K_{trans/cis}$	ΔG, kcal mol^{-1}	$^{3}J_{\alpha N}$, Ar$_{cis}$
T(4-H^{+}pyridylAla)FlpN	20.1	−1.76	8.3
T(4-NO$_2$-Phe)HypN	9.5	−1.32	8.1
TYHyp(4-CF$_3$Bz)N	8.2	−1.24	7.8
TYHyp(4-NO$_2$Bz)N	8.2	−1.24	n.d.
TYFlpN	7.0	−1.14	7.7
TYHypN	5.6	−1.01	7.5
TFPN	3.2	−0.68	6.9
TYPN	2.7	−0.59	6.1
TYhyp(4-NO$_2$Bz)N	1.8	−0.35	5.3
TYflpN	1.5	−0.24	4.9
TWflpN	0.65	0.25	4.2

Table 3

i residue aromatic effects on prolyl cis-trans isomerism in aromatic$_{i+1}$-proline$_{i+2}$ sequences.[a]

Peptide	$K_{trans/cis}$	ΔG, kcal/mol^{-1}	δ, $H_{\beta A}$ Y_{trans}	δ, $H_{\beta B}$ Y_{trans}	δ, $H_{\delta B}$ P_{trans}
H(H^+)YPN	2.9	−0.63	3.14	2.83	3.65
TYPN	3.0	−0.65	3.12	2.86	3.62
GYPN	3.3	−0.70	3.11	2.86	3.63
AYPN	3.7	−0.76	3.11	2.87	3.60
HYPN	4.2	−0.84	3.06	2.82	3.58
FYPN	5.7	−1.02	3.05	2.78	3.46
YYPN	6.7	−1.12	3.02	2.75	3.32
WYPN	8.0	−1.22	2.94	2.66	3.19

[a]Data were measured by NMR at 296 K at pH 4 (except HYPN, pH 8.5) in 5 mM phosphate, 250 mM NaCl.[28] All peptides were acetylated on the N-terminus and contained C-terminal amides.

Table 4

Van't Hoff-derived thermodynamic parameters for aromatic-proline sequences. Peptides have acetylated N-termini and are C-terminal amides unless otherwise indicated. AYPN and TYPN peptides have similar ΔH and ΔS (C. R. Forbes, unpublished data). Negative ΔH indicates that enthalpy favors the trans conformation, positive ΔH indicates that enthalpy favors the cis conformation.

peptide	ΔH, kcal mol^{-1}	ΔS, cal mol^{-1} K^{-1}	reference
YYPN	–1.01	+0.4	28
AYPN	–0.22	+1.8	28
TWPN	+0.28	+2.1	29
TWflpN	+2.0	+6.2	29
Ac-GP-OMe	–1.27		44
Ac-P-OMe	–1.04		38
Ac-Hyp-OMe	–1.87		38
Ac-flp-OMe	–0.73		40
Ac-FP-OMe	–0.25		45
GYPG	–0.65		13

Table 5

i+3 residue aromatic effects on amide *i*+1/*i*+2 cis-trans isomerism in Ac-TYPZ-NH_2 peptides.[28] Dyson and Wright analyzed a complete set of *i*+3 effects on cis-trans isomerism inSYPXDV.[7,9]

peptide, Z =	$K_{trans/cis}$	ΔG, kcal mol^{-1}	Y_{cis} $^3J_{\alpha N}$
Phe	2.0	−0.41	5.7
Trp	2.6	−0.56	5.5
Pro	2.6	−0.56	8.7
Asn	3.0	−0.65	6.3
Gly	4.3	−0.86	7.1
D-Ala	5.3	−0.98	6.6