J|A|C|S COMMUNICATIONS

Published on Web 04/26/2010

$n \rightarrow \pi^*$ Interaction and $n)(\pi$ Pauli Repulsion Are Antagonistic for Protein Stability

Charles E. Jakobsche,[†] Amit Choudhary,[‡] Scott J. Miller,*[,†] and Ronald T. Raines*[,§]

Department of Chemistry, Yale University, New Haven, Connecticut 06520, and Graduate Program in Biophysics and Departments of Biochemistry and Chemistry, University of Wisconsin, Madison, Wisconsin 53706

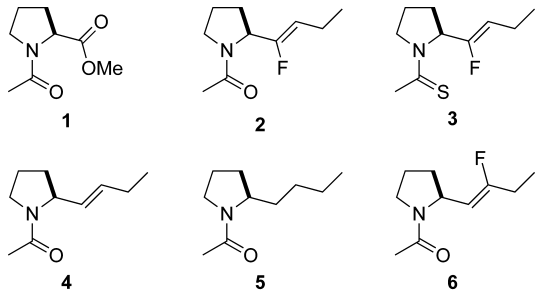
Received February 2, 2010; E-mail: scott.miller@yale.edu; rtraines@wisc.edu

The interplay between electronic effects and steric effects underlies molecular conformation. For example, the common C═O···H−N hydrogen bonds within protein main chains may be viewed as favored by the delocalization of an oxygen lone pair (n) into the antibonding orbital (σ^*) of the N−H bond but disfavored by Pauli repulsion[1] between n and the N−H bonding orbital (σ).[2] Here we report on a second example of this type of dichotomy within protein main chains.

In common elements of protein secondary structure, the oxygen (O_{i-1}) of a main-chain amide is proximal to the carbon (C_i') of the subsequent amide.[3] This short contact is promoted by $n \rightarrow \pi^*$ electronic delocalization, wherein an oxygen lone pair (n) overlaps with the C_i'═O_i antibonding orbital (π^*) of the subsequent peptide bond.[3−5] We suspected that, as in a hydrogen bond, this electronic effect is antagonized by a steric effect, here arising from Pauli repulsion between n and the C_i'═O_i bonding orbital (π).

To unveil any $n)(\pi$ Pauli repulsion, we sought a π system that is isosteric with a carbonyl group but provokes little $n \rightarrow \pi^*$ interaction. We suspected that alkenyl groups, which lack the polarity of carbonyl groups, could have this attribute. To enable quantitative comparisons, we chose the AcProOMe (**1**) model system,[6] in which n is directed toward π^* in the trans conformation but not in the cis conformation (Figure 1). The value of $K_{trans/cis}$ reports on the differential stability of the trans and cis conformations and can be measured by using NMR spectroscopy. We suspected that replacing the ester of **1** with an isosteric fluoroalkene[7] would attenuate the $n \rightarrow \pi^*$ interaction. Hence, we synthesized and analyzed **1** and its fluoroalkenyl isostere, **2**.

We found evidence that unfavorable Pauli repulsion can indeed antagonize a favorable $n \rightarrow \pi^*$ interaction. Replacing the carbonyl acceptor with a fluoroalkene switches the conformational preference of the amide bond from trans to cis (Table 1). We resorted to hybrid density functional theory and Natural Bond Orbital (NBO)[8] analyses to reveal the basis for this dramatic shift in conformational preference.



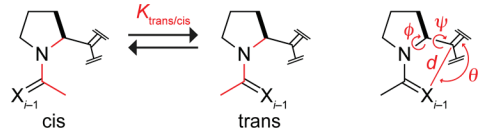


Figure 1. Definition of equilibrium constant $K_{trans/cis}$, distance d, planar angle θ, and dihedral angles ϕ and ψ. X = O in **1**, **2**, and **4**−**6**; X = S in **3**.

We performed geometry optimizations, frequency calculations, and NBO analyses at the B3LYP/6-311+G(2d,p) level of theory on eight conformations of **1** and **2** (see Tables S1 and S2 in the Supporting Information). We estimated the stabilization afforded by $n \rightarrow \pi^*$ electronic delocalization by using second-order perturbation theory, as implemented with NBO 5.0. In accord with our expectation, we found that fluoroalkene isostere **2** does not partake in an appreciable $n \rightarrow \pi^*$ interaction (Table 1). The π^* orbital of the carbonyl group in **1** is oriented properly for extensive $n \rightarrow \pi^*$ overlap, but that of the fluoroalkenyl group in **2** is not (Figure 2). Additionally, the energy difference between the n and π^* orbitals of **2** (33.2 kcal/mol) is ∼10-fold greater than that of **1** (3.5 kcal/mol). While the π^* orbital of the carbonyl is located primarily on the single carbonyl carbon, the π^* of the fluoroalkene isostere is distributed evenly between the two alkenyl carbons. Moreover, the distance between the donor oxygen (O_{i-1}) and acceptor carbon (C_i') is short in all low-energy conformations of **1** but long in **2** (Table S1). Finally, O_{i-1} in the low-energy conformations of **1** is along the Bürgi−Dunitz trajectory[9] ($\theta \approx 100°$), but O_{i-1} of **2** is off of that trajectory ($\theta \approx 125°$) (Table S1).

The conformational differences between **1** and **2** are evident in their computational energy landscapes (Figure 3A,B). As the value of d decreases, the interpenetration of the van der Waals surfaces of the donor and acceptor groups increases. That endows **1** but not **2** with conformational stability. In **1**, the $n)(\pi$ Pauli repulsion is offset by a strong $n \rightarrow \pi^*$ interaction; in **2**, the $n \rightarrow \pi^*$ interaction

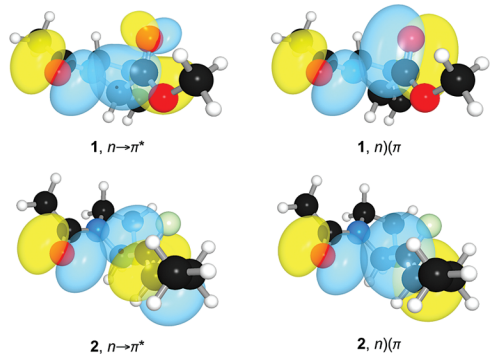


Figure 2. Overlaps between n and the π^* and π orbitals of **1** and **2** in their optimized geometries.

[†] Department of Chemistry, Yale University.
[‡] Graduate Program in Biophysics, University of Wisconsin.
[§] Departments of Biochemistry and Chemistry, University of Wisconsin.

Table 1. Conformational Properties of Compounds **1**−**6**

compound	$K_{trans/cis}$ [a]	chemical shift of C′$_i$ (ppm)	d (Å)[b]	θ (deg)[b]	ϕ (deg)[b]	ψ (deg)[b]	$n \rightarrow \pi^*$ (kcal/mol)[b]
1	3.7 : 1.0	ND	3.08	99.5	−71.12	152.67	0.40
2	1.0 : 1.7	156	3.28	124.9	−82.81	117.01	0.01
3	1.0 : 2.2	ND	3.59	126.3	−84.42	120.92	0.05
4	1.0 : 2.9	133	3.32	126.4	−84.02	116.56	0.02
5	1.4 : 1.0	ND	–	–	−78.89	167.16	–
6	1.0 : 4.0	105	3.25	104.1	−80.43	142.03	0.03

[a] Measured in $CDCl_3$ at 25 °C. [b] Computed in the optimized conformation (trans amide bond; C$^\gamma$-endo pyrrolidine ring pucker).

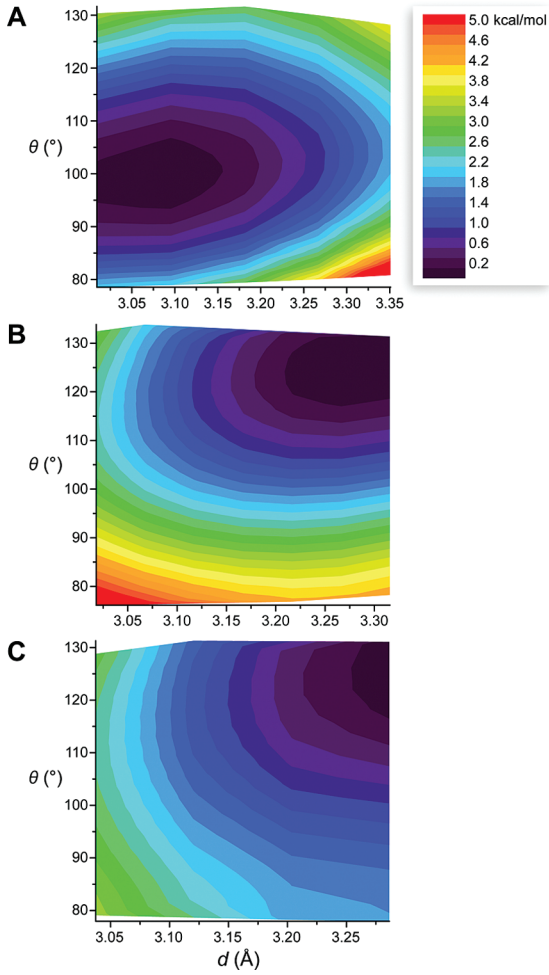


Figure 3. Conformational energy landscapes of (A) **1**, (B) **2**, and (C) **4**.

Figure 4. Overlap of the σ (C$^\alpha_i$−H) and σ^* (C′$_i$−F) orbitals of **2** in its optimized geometry.

does not overcome that repulsion. Natural Steric Analysis (NSA) supports the existence of the antagonistic Pauli repulsion in low-energy conformations (Table S1).

Fluoroalkene **2** lacks a favorable $n \rightarrow \pi^*$ interaction despite restricted rotation of its C$^\alpha_i$−C′$_i$ bond (ψ in Figure 1). The anti rotamer is stabilized by a hyperconjugative interaction between the C$_\alpha$−H bonding orbital (σ) and the C′$_i$−F antibonding orbital (σ^*) (Figure 4).[10] This rotamer gives rise to a larger value of $^3J_{H,F}$ for the trans conformation (16 Hz) than the cis conformation (8 Hz).

If n)(π Pauli repulsion destabilizes the trans conformation of **2**, then its amplification should further reduce the population of that conformation. Some of us had shown previously that the sulfur of a thioamide is a better $n \rightarrow \pi^*$ donor than is the oxygen of an amide.[4e] However, because sulfur is larger than oxygen and C=S bonds are longer than C=O bonds, sulfur should engender greater n)(π Pauli repulsion. To search for that manifestation, we replaced the donor oxygen (O$_{i-1}$) in amide **2** with sulfur. We found the value of $K_{trans/cis}$ for thioamide **3** to be less than that for amide **2** (Table 1). An origin in increased n)(π Pauli repulsion is supported by NSA (Table S1).

Likewise, we reasoned that attenuating any n)(π Pauli repulsion should stabilize the trans conformation. We suspected that a comparison of alkene **4** with alkane **5**, which lacks the acceptor π orbital, would allow us to test our reasoning. Again, we found evidence for n)(π Pauli repulsion, as the value of $K_{trans/cis}$ for alkane **5** is greater than that for alkene **4** (Table 1).

Compound **4** offers another opportunity to probe for n)(π Pauli repulsion. The pendant fluoro group that is present in **2** but absent in **4** polarizes the π orbital, reducing the electron density on the acceptor carbon (C′$_i$). The net effect is to diminish n)(π Pauli repulsion, as evidenced by a larger value of $K_{trans/cis}$ for **2** than **4** (Table 1). Accordingly, we reasoned that polarizing the π bond in the opposite direction could *increase* the electron density on the acceptor carbon, thereby increasing any n)(π Pauli repulsion. Indeed, the value of $K_{trans/cis}$ for **6** is less than those for **2** and **4**. The correlation between the value of $K_{trans/cis}$ for compounds **2**, **4**, and **6** and the ^{13}C NMR chemical shift of each acceptor carbon (Table 1), which reports on its electron density, provides additional validation for our conclusions.

Some of us have argued[4e] that intimate carbonyl−carbonyl interactions, which are ubiquitous in many protein secondary structures,[3] involve $n \rightarrow \pi^*$ interactions and cannot be interpreted in terms of classical electrostatic models, such as dipole−dipole[11] or charge−charge interactions.[12] The results herein support this argument. First, if the interaction between adjacent carbonyl groups were manifested as a classical dipole−dipole interaction, replacing the C=O group with a C(sp^2)−F group would not elicit a reversal in the conformational preference from trans to cis. Second, the value of $K_{trans/cis}$ for **3** is less than that for **2**, despite the dipole moment of C=S being greater than that of C=O.[13] Third, the ϕ and ψ dihedral angles and the conformational energy landscapes of **2** and **4** (the latter of which lacks a dipole) are similar to each other, yet distinct from those of **1** (Table 1; Figure 3C).

The O$_{i-1}$···C′$_i$=O$_i$ distance is especially small in α-helices.[3] These short contacts position distal C=O and H−N groups in the main chain to form the canonical $i \rightarrow i + 4$ hydrogen bond (Figure 5). Our data indicate that n)(π Pauli repulsion deters such short contacts and would, unless counteracted by an $n \rightarrow \pi^*$ interaction, impair α-helix formation. Indeed, others have shown that replacing a single amide bond with an alkene or a fluoroalkene isostere severely disrupts α-helical structure.[14] Moreover, we put forth n)(π Pauli repulsion as the basis for the

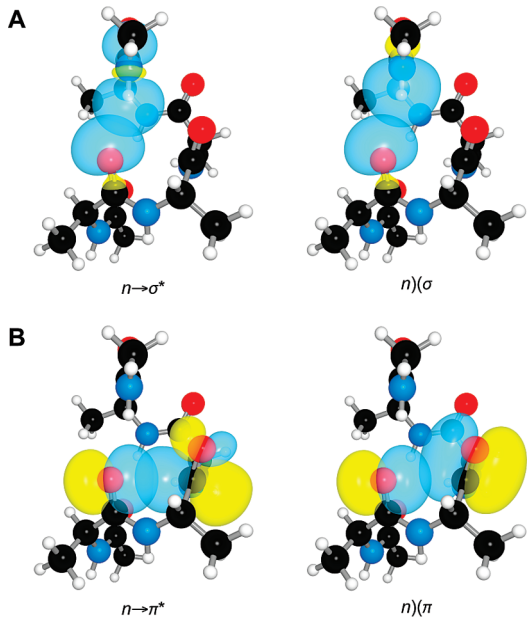


Figure 5. Orbital overlaps that stabilize (left) and destabilize (right) the α-helical conformation of an $AcAla_4NHMe$ model system: (A) $i \rightarrow i + 4$ hydrogen bond; (B) $n \rightarrow \pi^*$ interaction.

anomalous polarization of the $C'_i{=}O_i$ π bond toward O_i that has been observed in α-helices.[15] Analogous repulsion has been observed directly by atomic force microscopy at much larger donor−acceptor distances.[16]

Finally, we note the effect of $n)(\pi$ Pauli repulsion on the conformation of other molecules. The collagen triple helix has an $n \rightarrow \pi^*$ interaction between adjacent residues.[17] Each peptide bond in the triplet repeat of collagen strands has been replaced with an alkene isostere, and each substitution greatly diminishes the triple-helix stability.[18] Likewise, an altered conformational energy landscape could be responsible for the diminished biological activity of some small-molecule ligands containing an alkene or fluoroalkene isostere.[19] These isosteres appear to be excellent mimics only for amides and esters that are not engaged in $n \rightarrow \pi^*$ interactions. Implications for structural perturbations within more global elements of protein secondary structure remain an important avenue for further study.

Acknowledgment. We are grateful to W. L. Jorgensen and M. D. Shoulders for contributive discussions. This work was supported by NIH Grants R01 GM068649 (S.J.M.) and R01 AR044276 (R.T.R.).

Supporting Information Available: Synthesis and analysis procedures and computational data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Pauli, W. *Z. Phys.* **1925**, *31*, 373. (b) Pauli, W. *Z. Phys.* **1925**, *31*, 765. (c) Massimi, M. *Pauli's Exclusion Principle: The Origin and Validation of a Scientific Principle*; Cambridge University Press: Cambridge, U.K., 2005.
(2) (a) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899. (b) Weinhold, F.; Landis, C. R. *Valency and Bonding: A Natural Bond Orbital Donor−Acceptor Perspective*; Cambridge University Press: Cambridge, U.K., 2005. For another perspective, see: (c) Khaliullin, R. Z.; Bell, A. T.; Head-Gordon, M. *Chem.−Eur. J.* **2009**, *15*, 851, and references therein.
(3) Hinderaker, M. P.; Raines, R. T. *Protein Sci.* **2003**, *12*, 1188.
(4) (a) Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777. (b) DeRider, M. L.; Wilkens, S. J.; Waddell, M. J.; Bretscher, L. E.; Weinhold, F.; Raines, R. T.; Markley, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 2497. (c) Horng, J.-C.; Raines, R. T. *Protein Sci.* **2006**, *15*, 74. (d) Hodges, J. A.; Raines, R. T. *Org. Lett.* **2006**, *8*, 4695. (e) Choudhary, A.; Gandla, D.; Krow, G. R.; Raines, R. T. *J. Am. Chem. Soc.* **2009**, *131*, 7244.
(5) (a) Sonntag, L.-S.; Schweizer, S.; Ochsenfeld, C.; Wennemers, H. *J. Am. Chem. Soc.* **2006**, *128*, 14697. (b) Gao, J.; Kelly, J. W. *Protein Sci.* **2008**, *17*, 1096.
(6) As a comparator, we chose a methyl ester rather than an amide to avoid the complications of γ-turn formation, as has been observed in AcProN-HMe. See: Liang, G.-B.; Rito, C. J.; Gellman, S. H. *Biopolymers* **1992**, *32*, 293, and references therein.
(7) (a) Abraham, R. J.; Ellison, S. L. R.; Schonholzer, P.; Thomas, W. A. *Tetrahedron* **1986**, *42*, 2101. (b) Boros, L. G.; Corte, B. D.; Gimi, R. H.; Welch, J. T.; Wu, Y.; Handschumacher, R. E. *Tetrahedron Lett.* **1994**, *35*, 6033. (c) Bartlett, P. A.; Otake, A. *J. Org. Chem.* **1995**, *60*, 3107. (d) Wipf, P.; Henninger, T. C.; Geib, S. J. *J. Org. Chem.* **1998**, *63*, 6088. (e) Jakobsche, C. E.; Peris, G.; Miller, S. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 6707.
(8) (a) Weinhold, F. In *Encyclopedia of Computational Chemistry*; Schleyer, P. v. R., Allinger, N. L., Clark, T., Gasteiger, J., Kollman, P. A., Schaefer, H. F., III, Schreiner, P. R., Eds.; Wiley: Chichester, U.K., 1998; Vol. 3, pp 1792−1811. (b) Glendening, E. D.; Badenhoop, J. K.; Reed, A. E.; Carpenter, J. E.; Bohmann, J. A.; Morales, C. M.; Weinhold, F. *NBO 5.0*; 2001.
(9) (a) Bürgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065. (b) Bürgi, H. B.; Dunitz, J. D.; Lehn, J. M.; Wipff, G. *Tetrahedron* **1974**, *30*, 1563. (c) Bürgi, H. B.; Lehn, J. M.; Wipff, G. *J. Am. Chem. Soc.* **1974**, *96*, 1965. (d) Bürgi, H. B.; Dunitz, J. D. *Acc. Chem. Res.* **1983**, *16*, 153. (e) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; Wiley-Interscience: New York, 1996. (f) Kirby, A. J. *Stereoelectronic Effects*; Oxford University Press: New York, 1996. (g) Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*; Oxford University Press: New York, 2000. (h) Anslyn, E. V.; Dougherty, D. A. *Modern Physical Organic Chemistry*; University Science Books: Sausalito, CA, 2006.
(10) Pophristic, V.; Goodman, L. *Nature* **2001**, *411*, 565.
(11) (a) Paulini, R.; Müller, K.; Diederich, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 1788. (b) Fischer, F. R.; Wood, P. A.; Allen, F. H.; Diederich, F. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17290.
(12) (a) Maccallum, P. H.; Poet, R.; Milner-White, E. J. *J. Mol. Biol.* **1995**, *248*, 361. (b) Maccallum, P. H.; Poet, R.; Milner-White, E. J. *J. Mol. Biol.* **1995**, *248*, 374. (c) Allen, F. H.; Baalham, C. A.; Lommerse, J. P. A.; Raithby, P. R. *Acta Crystallogr.* **1998**, *B54*, 320. (d) Deane, C. A.; Allen, F. H.; Taylor, R.; Blundell, T. L. *Protein Eng.* **1999**, *12*, 1025.
(13) Wiberg, K. B.; Rush, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 2038.
(14) Oishi, S.; Kamitani, H.; Kodera, Y.; Watanabe, K.; Kobayashi, K.; Narumi, T.; Tomita, K.; Ohno, H.; Naito, T.; Kodama, E.; Matsuoka, M.; Fujii, N. *Org. Biomol. Chem.* **2009**, *7*, 2872.
(15) Lario, P. I.; Vrielink, A. *J. Am. Chem. Soc.* **2003**, *125*, 12787.
(16) Gross, L.; Mohn, F.; Moll, N.; Liljeroth, P.; Meyer, G. *Science* **2009**, *325*, 1110.
(17) Shoulders, M. D.; Raines, R. T. *Annu. Rev. Biochem.* **2009**, *78*, 929.
(18) (a) Jenkins, C. L.; Vasbinder, M. M.; Miller, S. J.; Raines, R. T. *Org. Lett.* **2005**, *7*, 2619. (b) Dai, N.; Wang, X. J.; Etzkorn, F. A. *J. Am. Chem. Soc.* **2008**, *130*, 5396. (c) Dai, N.; Etzkorn, F. A. *J. Am. Chem. Soc.* **2009**, *131*, 13728.
(19) (a) Kaltenbronn, J. S.; Hudspeth, J. P.; Lunney, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Stier, M. A.; Tinney, F. J.; Woo, P. K. W.; Essenburg, A. D. *J. Med. Chem.* **1990**, *33*, 838. (b) Fincham, C. I.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; O'Toole, J. C.; Ratcliffe, J. C.; Rees, D. C.; Roberts, E. *J. Med. Chem.* **1992**, *35*, 1472. (c) Wai, J. S.; Bamberger, D. L.; Fisher, T. E.; Graham, S. L.; Smith, R. L.; Gibbs, J. B.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Kohl, N. E. *Bioorg. Med. Chem.* **1994**, *2*, 939. (d) Venkatesan, N.; Kim, B. H. *Curr. Med. Chem.* **2002**, *9*, 2243. (e) Welch, J. T. *Fluorine and Health*; Elsevier: Amsterdam, 2008.

JA100931Y