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Interplay of Hydrogen Bonds and $n \rightarrow \pi^*$ Interactions in Proteins

Gail J. Bartlett[†], Robert W. Newberry[‡], Brett Van Veller[‡], Ronald T. Raines^{‡,§,*}, and Derek N. Woolfson^{*,†,||}

[†]School of Chemistry, University of Bristol, Bristol, United Kingdom

[‡]Department of Chemistry, University of Wisconsin–Madison, Madison, Wisconsin, United States

[§]Department of Biochemistry, University of Wisconsin–Madison, Madison, Wisconsin, United States

^{||}School of Biochemistry, University of Bristol, Bristol, United Kingdom

Abstract

Protein structures are stabilized by multiple weak interactions, including the hydrophobic effect, hydrogen bonds, electrostatic effects, and van der Waals' interactions. Among these interactions, the hydrogen bond is distinct in having its origins in electron delocalization. Recently, another type of electron delocalization, the $n \rightarrow \pi^*$ interaction between carbonyl groups, has been shown to play a role in stabilizing protein structure. Here, we examine the interplay between hydrogen bonding and $n \rightarrow \pi^*$ interactions. To address this issue, we used data available from high-resolution protein crystal structures to interrogate asparagine side-chain oxygen atoms that are both acceptors of a hydrogen bond and donors of an $n \rightarrow \pi^*$ interaction. Then, we employed Natural Bond Orbital analysis to determine the relative energetic contributions of the hydrogen bonds and $n \rightarrow \pi^*$ interactions in these systems. We find that an $n \rightarrow \pi^*$ interaction is worth ~5–25% of a hydrogen bond and that stronger hydrogen bonds tend to attenuate or obscure $n \rightarrow \pi^*$ interactions. Conversely, weaker hydrogen bonds correlate with stronger $n \rightarrow \pi^*$ interactions and the demixing of the orbitals occupied by the oxygen lone pairs. Thus, these two interactions conspire to stabilize local backbone–side-chain contacts, which argues for the inclusion of $n \rightarrow \pi^*$ interactions in the inventory of non-covalent forces that contribute to protein stability and, thus, forcefields for biomolecular modeling.

INTRODUCTION

Protein three-dimensional structures are the result of a fine balance of inter- and intramolecular forces, including the hydrophobic effect, van der Waals' interactions, dipole effects, and hydrogen bonds.^{1,2} Recently, it has been shown that the $n \rightarrow \pi^*$ interaction, an electronic delocalization effect analogous to the hydrogen bond, also plays a role in stabilizing protein structure.^{3–6} In the case of the hydrogen bond, electrons occupying the lone pair (n) orbital of the hydrogen-bond acceptor are delocalized into the empty antibonding σ^* orbital of the hydrogen-bond donor.⁷ Similarly, in an $n \rightarrow \pi^*$ interaction electrons from the n orbital of a carbonyl oxygen donor are delocalized into the antibonding π^* orbital of the carbonyl carbon acceptor, thereby drawing carbonyl groups closer together. The energy associated with an $n \rightarrow \pi^*$ interaction between amide bonds has been estimated to

*To whom correspondence should be addressed: rtraines@wisc.edu, d.n.woolfson@bristol.ac.uk.

SUPPORTING INFORMATION AVAILABLE

Additional figures describing secondary structure classification of local and non-local Asn groups, and selection of Asn examples for NBO calculations; archive files providing all input co-ordinates in Gaussian format for NBO calculations; data used for the analysis presented herein. This information is available free of charge via the internet at <http://pubs.acs.org>.

contribute at least 0.27 kcal/mol.⁸ Approximately 34% of residues in proteins are predicted to engage in $n \rightarrow \pi^*$ interactions between backbone carbonyl groups.⁶ It follows that $n \rightarrow \pi^*$ interactions could provide up to 9 kcal/mol of stabilizing energy to a 100-residue protein, which, given that the free energy difference between the folded and unfolded state has been estimated to be between 5 and 10 kcal/mol for an average protein of 100 residues,⁹ is a prodigious contribution.

Knowledge of the geometry and energetics of these weak but abundant interactions is key to a full understanding of biomolecular systems, and for providing accurate forcefield parameters to model them. The current challenges in *de novo* structure prediction and protein design show that our understanding of these interactions is incomplete.^{10,11} In particular, since both hydrogen bonding and $n \rightarrow \pi^*$ interactions involve carbonyl oxygen lone pairs, we reasoned that the presence of a hydrogen bond could affect the geometry and energy of an $n \rightarrow \pi^*$ interaction, and *vice versa*. For example, it has been shown that a transannular $C'_{i-1}=O \cdots H-N/O$ hydrogen bond from a 4(*S*)-configured NH or OH donor on a substituted proline enhances the carbonyl as an acceptor of an $n \rightarrow \pi^*$ interaction.^{12–14} While hydrogen bond donation to the putative $n \rightarrow \pi^*$ acceptor should enhance the $n \rightarrow \pi^*$ interaction, the effect of hydrogen bond donation to the $n \rightarrow \pi^*$ donor is less clear. This issue is of importance for understanding the role of both hydrogen bonds and $n \rightarrow \pi^*$ interactions. Any cooperativity or interdependence between them is likely to have an impact on protein folding, engineering, and design; structure prediction and modeling; drug design; and other aspects of chemical and structural biology.

We sought to examine the interplay between hydrogen bonds and $n \rightarrow \pi^*$ interactions in protein structures by examining groups that could make both hydrogen bonds and $n \rightarrow \pi^*$ interactions. Many $n \rightarrow \pi^*$ interactions are found between sequential carbonyl groups in the protein backbone;⁶ however, these backbone atoms are under greater steric constraints than are protein side-chain atoms, which have greater conformational freedom. Hence, to study the intrinsic interplay between hydrogen bonds and the $n \rightarrow \pi^*$ interaction, we focused on protein side chains. Surveys of the Protein Data Bank (PDB)¹⁵ have identified self-contacting aspartate, asparagine, glutamate, and glutamine residues in protein structures,^{16,17} and propose that they provide significant stability. Similarly, quantum chemical calculations on self-contacting aspartic acid residues find evidence that these can interact *via* $n \rightarrow \pi^*$ interactions.¹⁸ A more recent study surveys the side-chain–backbone hydrogen-bonded motifs formed by asparagine and glutamine in protein structures.¹⁹ None of these previous studies consider hydrogen bonds and $n \rightarrow \pi^*$ interactions together.

For our analysis, we chose to consider asparagine residues. The asparagine side-chain oxygen atom is capable of accepting a hydrogen bond from a donor, and is additionally capable of donating an $n \rightarrow \pi^*$ interaction to its main-chain carbonyl. The same is true of glutamine, but there are far fewer examples of glutamine side-chain atoms contacting the backbone,¹⁷ presumably because of the entropic cost of tethering a longer methylene chain. By examining asparagine residues that make both one hydrogen bond and one $n \rightarrow \pi^*$ interaction, we have revealed how the hydrogen bond and $n \rightarrow \pi^*$ interaction interrelate.

RESULTS

Dataset Selection

First, we identified asparagine residues from a dataset of high-resolution protein structures that made single hydrogen bonds between their side-chain oxygen and the protein backbone (see: Methods). We focused exclusively on backbone NH hydrogen-bond donors because (a) they provided the greatest number of examples, and (b) there was less ambiguity about hydrogen placement, making the system more amenable to electronic structure calculations.

Upon inspection of this dataset, we observed two common motifs and therefore divided these residues into two categories: (1) those forming a single $\text{C}=\text{O}\cdots\text{H}-\text{N}$, $i \rightarrow i+2$ hydrogen bond, commonly found at turns in protein secondary structures (for secondary structure classification, see: Figure S1), and (2) those forming a single $\text{C}=\text{O}\cdots\text{H}-\text{N}$, $i \rightarrow i+n$ hydrogen bond, where $n > 5$ (Figures 1A and 1B). These were called “local” and “non-local” Asn groups, respectively. In addition, category (1) provided the largest number of $n \rightarrow \pi^*$ interactions made by an asparagine side-chain residue. The numbers of Asn residues making single $\text{C}=\text{O}\cdots\text{H}-\text{N}$, $i \rightarrow i+n$ hydrogen bonds where $n = 3 - 5$ were relatively small (334, 20 and 15 respectively), and the proportions of these that made $n \rightarrow \pi^*$ interactions were too small to draw any statistically significant conclusions about the interplay between the two types of interaction. Therefore, they were excluded from the analysis.

Parameters Defining $n \rightarrow \pi^*$ Interactions within Asparagine residues

Two parameters are used to define $n \rightarrow \pi^*$ interactions between the asparagine side-chain and backbone carbonyl groups: the distance ($d_{n \rightarrow \pi^*}$) between the side-chain oxygen atom and the main-chain carbonyl carbon atom; and the angle (θ) between the side-chain oxygen, main-chain carbonyl carbon and main-chain oxygen atoms (Figure 1C). For the work presented herein, these parameters were used to give an operational definition of $n \rightarrow \pi^*$ interactions: $d_{n \rightarrow \pi^*} < 3.22 \text{ \AA}$, the sum of the van der Waals’ radii of oxygen and carbon; and $95^\circ < \theta < 125^\circ$, the approximate angle of the Bürgi–Dunitz trajectory for nucleophilic attack at a carbonyl carbon ($\sim 109^\circ$). In addition, the angle ε between the side-chain carbonyl carbon, side-chain carbonyl oxygen, and main-chain carbonyl carbon was recorded. This angle should approach 90° when complete demixing of the lone pair orbitals on the donor oxygen occurs.

Parameters Defining Hydrogen Bonds

The relevant hydrogen-bond parameters (Figure 1D) are $d_{n \rightarrow \sigma^*}$, between the side-chain oxygen atom and backbone nitrogen; and the angle ω , between the side-chain carbonyl carbon, side-chain carbonyl oxygen, and donor hydrogen atom. We also recorded the angle ρ between the hydrogen-bond donor and acceptor. This angle is a measure of hydrogen-bond ideality, which tends toward 180° for an optimal hydrogen bond. Inspection of hydrogen-bond parameters for both Asn groups (Figures 2B and 2D) showed that they are non-ideal, even in the non-local case, where steric restrictions should be at a minimum.

Hydrogen-Bond Geometry of Asparagine Residues

We found 823 Asn residues making a local Asn $\text{C}=\text{O}\cdots\text{H}-\text{N}$, $i \rightarrow i+2$ hydrogen bond in our dataset. The local Asn group was characterized by ω angles of $\sim 140^\circ$ and ρ angles of $\sim 165^\circ$ (Figure 2A). Those Asn residues making $n \rightarrow \pi^*$ interactions had a slightly higher average ω but a similar distribution of ρ . We found 972 Asn residues that made non-local Asn $\text{C}=\text{O}\cdots\text{H}-\text{N}$, $i \rightarrow i+n$ ($n > 5$) hydrogen bond in our dataset. This group had a slightly flatter distribution of ω with a mean of $\sim 125^\circ$, and the distribution of ρ was slightly shifted more towards a linear hydrogen bond (Figure 2B). In both groups, the distribution of ω values was shifted to higher values than those found previously,²⁰ with greater distortions observed in the local case. This shift could be because the previous study did not fully account for residues accepting multiple hydrogen bonds from different donors, whereas the present study does take this into consideration. This distinction could be significant—forcefields derived from previous studies²¹ have been used successfully in protein design,^{22–24} but two recent perspectives highlight difficulties with the treatment of the hydrogen bond.^{11,25} Specifically, the energy term for side-chain–backbone hydrogen bonds is derived from statistics for side-chain–side-chain hydrogen bonds; in our dataset, we observed significant deviation from this paradigm, suggesting that design efforts could be improved by better

accounting for side-chain–backbone hydrogen bond geometries, at least for asparagine residues.

Dependence of $n \rightarrow \pi^*$ Interactions on Hydrogen-Bond Environment

725 $n \rightarrow \pi^*$ interactions were found where the Asn also makes a local hydrogen bond. Those interactions represent a much larger proportion than the 92 $n \rightarrow \pi^*$ interactions found where the hydrogen bond is a non-local interaction (88% of local versus 9.5% of non-local hydrogen bonds). Nonlocal hydrogen bonds likely ‘distract’ the Asn side chain from making a local $n \rightarrow \pi^*$ interaction, suggesting that hydrogen bonds form preferentially to $n \rightarrow \pi^*$ interactions, which we believe is consistent with their relative energies. In this case, hydrogen bonds can form with a more typical geometry (*i.e.*, $\omega \sim 120^\circ$) relative to the local case. Intrigued by the correlation of $n \rightarrow \pi^*$ prevalence and atypical hydrogen-bond geometry in local Asn contacts, we examined how the geometry of the hydrogen bond affects the geometry of the $n \rightarrow \pi^*$ interaction.

There is a relationship between ε and ω for the local Asn group (Figure 3A). As the hydrogen bond becomes more linear, *i.e.*, ω tends to 180° , the angle made by the donor C=O group with its acceptor carbonyl carbon, ε , tends to 90° . This trend, which is not observed in the non-local Asn group (Figure 3B), could indicate orbital demixing in the case of the local side-chain Asn residues making $n \rightarrow \pi^*$ interactions.

Computational Analyses

We probed the relationship between ε and ω further. Specifically, we carried out Natural Bond Orbital (NBO) calculations on a subset of local and non-local Asn residues and their hydrogen-bond donors so as to estimate the energy contributed to both the $n \rightarrow \pi^*$ interaction and the hydrogen bond ($n \rightarrow \sigma^*$ interaction) by the Asn side-chain carbonyl group. We restricted our NBO analysis to only the participating functional groups in vacuum, *i.e.* with a dielectric constant of 1 (see: Methods). Thus, we comment exclusively on relative energies associated with hydrogen bonds and $n \rightarrow \pi^*$ interactions and not their absolute magnitudes. We found, unsurprisingly, that hydrogen bonds were stronger than $n \rightarrow \pi^*$ interactions. In our calculations, an $n \rightarrow \pi^*$ interaction was worth anywhere between 6% and 23% of the competing hydrogen bond (Table 1). The hydrogen bond was also preferred to the $n \rightarrow \pi^*$ interaction—in the non-local hydrogen bonds, an average of $(92 \pm 8)\%$ of the total stabilization afforded to either the antibonding π^* or σ^* orbitals went to the antibonding σ^* . In the case of the local hydrogen bonds, this average favored the σ^* slightly less, at only $(78 \pm 11)\%$. We examined the relationship between hydrogen-bond parameters and $E_{n \rightarrow \sigma^*}$ as calculated with NBO analysis, and the $n \rightarrow \pi^*$ interaction parameters and $E_{n \rightarrow \pi^*}$ (Figure 4). As anticipated, in the local Asn group, as ω tended to 180° , $E_{n \rightarrow \sigma^*}$ increased, while as ε tended to 90° degrees, $E_{n \rightarrow \pi^*}$ increased, similar to the trend observed from the PDB in Figure 3A. This trend held for ε in non-local cases, but not for the hydrogen bond, which showed no preferred ω for high values of $E_{n \rightarrow \sigma^*}$. In both cases, the stabilization was at its greatest when the distances $d_{n \rightarrow \sigma^*}$ and $d_{n \rightarrow \pi^*}$ were smallest, that is, when the orbital overlap was greatest.

We noted some differences between the local and non-local Asn residues. Hydrogen bonds in the non-local Asn group were worth almost double the energy compared with the local Asn group, whereas the $n \rightarrow \pi^*$ interaction was worth about half the energy in the non-local set compared to the local set. The weakness of the hydrogen bonds observed in local contacts is consistent with the less-ideal geometry apparent in our survey of protein structures for these residues. In the presence of these weaker hydrogen bonds, however, there was a corresponding increase in the strength of the $n \rightarrow \pi^*$ interaction. To investigate the propensity of these weaker, less-ideal hydrogen bonds to enable $n \rightarrow \pi^*$ interactions, we

examined the contributions of individual carbonyl lone pairs to both hydrogen bonds and $n \rightarrow \pi^*$ interactions.

In the non-local Asn group, both lone pairs made a large contribution to the antibonding σ^* orbital, but a small one to the antibonding π^* orbital, pointing towards orbital hybridization on the carbonyl oxygen (Table 2; Figure 5). By contrast, in the local Asn group, it was apparent that orbital demixing took place—electrons from the first lone pair (LP1, which occupies a predominantly s -type orbital) provided the most stabilization to the hydrogen bond and hardly any to the $n \rightarrow \pi^*$ interaction. The second lone pair (LP2), which occupies a predominantly p -type orbital, provided about half its energy to the $n \rightarrow \pi^*$ interaction and half to the hydrogen bond. A clear separation between lone-pair contributions can be seen for the local Asn group (Figure 6A), but by contrast, there was no segregation in lone-pair contributions for the non-local Asn group. Again, this distinction indicates orbital hybridization in the non-local group, and orbital demixing for the local group.

These calculations were carried out in the gas phase. One might expect water to be the most forbidding solvent for both hydrogen bonds and $n \rightarrow \pi^*$ interactions because of its ability to compete for relevant orbitals. However, while hydrogen bonding is sensitive to the dielectric constant of the medium, the strength of an $n \rightarrow \pi^*$ interaction has been found to change only slightly in different solvents.^{3,26} Taken together, these points suggest that the interplay we observe from our calculations would likely be amplified in water, *i.e.* hydrogen bonds may be weaker and the overall contribution from $n \rightarrow \pi^*$ interactions may be increased.

DISCUSSION

The canonical angle at which lone pairs of electrons protrude from a carbonyl oxygen to make a hydrogen bond is 120° .²⁷ This angle can arise from two sp^2 hybridized orbitals sitting like ‘rabbit ears’ on the carbonyl oxygen. In their analysis of the PDB, Baker and co-workers show that the preferred angle for hydrogen-bonded side-chain carbonyl groups is in fact $\sim 120^\circ$.²⁰ Indeed, in the absence of local constraints, we have observed a tendency of Asn side-chain carbonyl groups to form strong, typical (*i.e.*, $\omega \sim 120^\circ$) hydrogen bonds with the peptide main chain. These hydrogen bonds reduce the prevalence of $n \rightarrow \pi^*$ interactions involving these residues and attenuate the resulting energy of observed $n \rightarrow \pi^*$ interactions. In these cases, the hydrogen bond tends to obscure the $n \rightarrow \pi^*$ interaction, and we can therefore expect the effects of $n \rightarrow \pi^*$ interactions to be relatively low.

In the presence of local constraints imposed by the global folding of the peptide chain, however, the typical geometry of the hydrogen bond is compromised significantly with concomitant reduction in hydrogen-bond energy. The geometry of these hydrogen bonds is consistent with orbital demixing, and, by extension, allows enhanced $n \rightarrow \pi^*$ interactions. As a result, $n \rightarrow \pi^*$ interactions in the presence of local hydrogen-bond constraints are both more prevalent and of significantly higher energy. These stronger $n \rightarrow \pi^*$ interactions seem to compensate for the weaker hydrogen bonds, allowing for side-chain–backbone contacts that might not be observed otherwise. Moreover, an extreme hydrogen-bonding angle occurs in the backbone of the α -helix, where the $C=O \cdots H-N$, $i \rightarrow i+4$ hydrogen bond is found at $\sim 160^\circ$. We argue that this angle is associated with orbital demixing, where the hydrogen bond draws on electrons in the n_s -type orbital⁴ that protrudes out from the carbonyl oxygen atom along the direction of the $C=O$ bond (Figure 5). The ensuing liberation of the electrons in an n_p -type orbital⁴ enables the $n \rightarrow \pi^*$ interaction and thereby provides additional stability to the α -helix.

These results also have implications for the parameterization of hydrogen bonds in different local environments in protein structure. Previously, side-chain–backbone hydrogen-bond

energies have been derived from the statistics for side-chain–side-chain hydrogen bonds.²⁰ The different angle preferences for the hydrogen bonds accepted by Asn in different environments is indicative of the need for a more environment-sensitive hydrogen-bond potential in molecular modeling. Additionally, we urge inclusion of $n \rightarrow \pi^*$ interactions in molecular-modeling forcefields. We have shown previously that $n \rightarrow \pi^*$ interactions are prevalent between sequential backbone carbonyl groups in a protein sequence, particularly in α -helices.⁶ In the Rosetta forcefield, for example, if a backbone–backbone hydrogen bond is made, neither the donor nor acceptor residue is allowed to participate in a backbone–side-chain hydrogen bond.²⁸ Addition of an $n \rightarrow \pi^*$ contribution here, especially in an α -helix, should improve computational accuracy. For example, a twenty-residue helix could contain 16 backbone hydrogen bonds, and 19 sequential backbone carbonyl $n \rightarrow \pi^*$ interactions. If each $n \rightarrow \pi^*$ interaction were worth 15% of a hydrogen bond, inclusion of an $n \rightarrow \pi^*$ interaction term would provide helix stabilization equivalent to approximately three extra hydrogen bonds. Thus, although the contribution of the $n \rightarrow \pi^*$ interaction is small in energetic terms (~5–25% of a hydrogen bond), the sheer quantity of them in protein structures suggests that they are of importance.

METHODS

Structural Dataset

A dataset of 2540 protein X-ray crystal structures was culled from the Protein Data Bank using the PISCES server²⁹ (resolution 1.6 Å, R factor < 0.3, each comprising a protein chain of 40 residues, with 40% sequence identity between any two structures). Hydrogen bonds were assigned using HBPlus,³⁰ with standard settings, except that asparagine side chains were allowed to flip according to hydrogen-bond assignment. Hydrogen bonds were assigned between defined donor (D) and acceptor (carbonyl oxygen (O)) atoms meeting the following distance and angle criteria: angle D···H···O 90°, angle H···O···C 90°, angle D···O···C 90°, distance D···O 3.9 Å, and distance H···O 2.5 Å, with at least 3 covalent bonds between donor and acceptor.³¹ Scripts were written in Perl to identify a set of 2839 asparagine residues accepting exactly one hydrogen bond. Residues making any hydrogen bonds to water were ignored. Secondary structure parameters were calculated using the modified Kabsch and Sander method³² as implemented in Promotif.³³

NBO Calculations

A subset of the asparagine residues and their hydrogen-bond donors identified from PDB were chosen for NBO calculations (Figure S2).³⁴ For the local Asn group, a set was chosen that had parameters of ε and ω on the line of least-squares-fit shown in Figure 3A. For the non-local group, a set was chosen manually that sampled a wide spread of ω values. The coordinates of the asparagine residue, hydrogen-bond donor residue and one residue on either side were extracted from the PDB file. All other residues were truncated to alanine, and the N and C termini were acetylated and amidated, respectively. We employed density functional theory (DFT) calculations at the B3LYP/6-311++G(2d,p) level of theory with Natural Bond Orbital (NBO) analysis using second-order perturbation theory to estimate the contribution of the lone pairs on the asparagine side-chain oxygen to the $n \rightarrow \pi^*$ interaction ($E_{n \rightarrow \pi^*}$) and $n \rightarrow \sigma^*$ interaction (hydrogen bond, $E_{n \rightarrow \sigma^*}$). DFT calculations were carried out with NBO 3.1³⁵ as implemented in Gaussian09.³⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Definition of (A) local and (B) nonlocal asparagine side-chain hydrogen bonds. Parameters defining (C) $n \rightarrow \pi^*$ interactions and (D) hydrogen bonds in an asparagine side-chain system.

Figure 2.

Histograms of hydrogen-bond geometry parameters in (A,B) local and (C,D) non-local Asn groups. The frequency in each bin has been corrected by $1/\sin(\omega)$ or $1/\sin(\rho)$, and has then been normalized so local and non-local groups can be compared. Red indicates residues testing positive for an $n \rightarrow \pi^*$ interaction according to the operational definition in Figure 1C. The height of the red bars indicates the corrected, normalized proportion of each bin making an $n \rightarrow \pi^*$ interaction with the backbone carbonyl group. The mean and standard deviation of the underlying distributions are as follows: (A) $n \rightarrow \pi^*$ -positive, 139.2 (12.0), $n \rightarrow \pi^*$ -negative, 133.2 (12.0); (B) $n \rightarrow \pi^*$ -positive, 156.7 (10.7), $n \rightarrow \pi^*$ -negative, 149.2 (14.1); (C) $n \rightarrow \pi^*$ -positive, 131.4 (13.5), $n \rightarrow \pi^*$ -negative, 133.9 (13.5); (D) $n \rightarrow \pi^*$ -positive, 158.0 (9.7), $n \rightarrow \pi^*$ -negative 158.7 (10.9).

Figure 3. Scatterplot of hydrogen-bond angle ω versus $n \rightarrow \pi^*$ interaction angle ϵ for $n \rightarrow \pi^*$ positive residues in (A) local and (B) non-local Asn groups. The black lines are from a linear least-squares fit (local $R^2 = 0.32$; non-local $R^2 = 0.03$).

Figure 4.

3D plots of (A,C) hydrogen-bond energy and (B, D) $n \rightarrow \pi^*$ interaction energy for (A, B) local Asn groups and (C, D) non-local Asn groups. Parameters are defined in Figures 1C and 1D. Energies were calculated from second-order perturbation theory analysis (as implemented in NBO 3.1) of 26 examples for the local group and 47 examples for the non-local group. The surface was generated as a grid with dimensions of the scattered data and an arbitrarily chosen set of rows and columns that were equally spaced across the grid. The z -value of the grid was computed as a weighted average of the z values of the scattered points. In addition, raw data points are plotted on top of the surface.

Figure 5.

Lone-pair orbitals on the asparagine side-chain oxygen for an asparagine making a local hydrogen bond (PDB 3KS3, residues 61–63) and an asparagine making a non-local hydrogen bond (PDB 2CXA, residues 24, 62). Values for $E_{n \rightarrow \pi^*}$ and $E_{n \rightarrow \sigma^*}$ as calculated with NBO analysis: A) $E_{n \rightarrow \pi^*}$, 2.7 kcal/mol (5% LP1, 51% LP2); $E_{n \rightarrow \sigma^*}$, 10.3 kcal/mol (95% LP1, 49% LP2); B) $E_{n \rightarrow \pi^*}$, 2.3 kcal/mol (9% LP1, 16% LP2); $E_{n \rightarrow \sigma^*}$, 14.6 kcal/mol (91% LP1, 84% LP2). Orbital images were generated with Chemcraft.

Figure 6. Scatterplots of $E_{n \rightarrow \pi^*}$ versus $E_{n \rightarrow \sigma^*}$ for individual lone pairs (LP1 = blue; LP2 = red) as assigned by using NBO analysis for (A) local Asn groups and (B) non-local Asn groups.

Table 1

Average energies (SD) of the hydrogen bond ($E_{n \rightarrow \sigma^*}$) and $n \rightarrow \pi^*$ interaction ($E_{n \rightarrow \pi^*}$) in the local and non-local Asn groups^a

	Local (<i>n</i> = 26)	Non-Local (<i>n</i> = 47)
$E_{n \rightarrow \sigma^*}$ (kcal/mol)	5.37 (3.51)	10.27 (4.71)
$E_{n \rightarrow \pi^*}$ (kcal/mol)	1.23 (0.49)	0.71 (0.55)

^aDifferences are statistically significant ($p < 0.05$).

Table 2

Average proportion of energy stabilization afforded to the hydrogen bond ($P_{n \rightarrow \sigma^*}$) and $n \rightarrow \pi^*$ interaction ($P_{n \rightarrow \pi^*}$) by each lone pair for local and non-local Asn groups

	Local		Non-local	
	$P_{n \rightarrow \sigma^*}$	$P_{n \rightarrow \pi^*}$	$P_{n \rightarrow \sigma^*}$	$P_{n \rightarrow \pi^*}$
Lone pair 1 (LP1)	0.930	0.070	0.959	0.040
Lone pair 2 (LP2)	0.439	0.561	0.822	0.178