hydrogen bond acceptor (by a similar rotation around the C^{β}-C^{γ} bond in concert with movement of the side chain toward the dimer interface).

A rapid equilibrium between these alternate conformations would explain why, on average, the Asn side chains appear to be symmetric based solely on NMR chemical shift information. However, the crucial point to be noted is that the conformations shown in Fig. 1, *A* and *B*, are actually chemically equivalent—the conformations are related by a simple 180° rotation about an axis normal to the page. Thus, we do not believe a specific conformer has been trapped in the crystal form because the crystal structure is equivalent to each of the two interconverting, but chemically equivalent, conformations shown in Fig. 1.

This situation represents an excellent example of the often mooted, but seldom demonstrated, complementarity of NMR and x-ray structural data. The exact nature of the hydrogen bonding pattern could not be ascertained from the NMR data alone but neither could the exchange phenomenon outlined in Fig. 1 be visualized using x-ray crystallography. The driving force for the exchange phenomenon remains to be determined; we have proposed that it might provide some entropic compensation for the enthalpically unfavorable desolvation that occurs when the Asn residues are buried at the hydrophobic dimer interface (Mackay et al., 1996).

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Response to G. F. King

In his Letter to the Editor, ("NMR Spectroscopy and X-Ray Crystallography Provide Complementary Information on the Structure and Dynamics of Leucine Zippers"), Glenn F. King commented on our recent paper published in *Biophysical Journal* (Shen et al., 1996). We welcome his contribution and the opportunity to enter into a deeper discussion of conformational variability and dynamics of peptide structures in the crystal and in solution.

As King reminds us, NMR spectroscopy in solution shows single resonances for the intrahelical GCN4 Asn 16 residues (Saudek et al., 1991) and their Jun equivalents (Junius et al., 1995). This observation implies a symmetrical arrangement of side chain protons in the Asn-Asn contact atoms. King suggests a structurally plausible "flip-flop" rotation of the mutually hydrogen-bonded Asn amide groups, by 180°, around the Cβ—Cγ bond. The Asn-Asn side chain CO-NH_2 hydrogen bond is asymmetrical, but a rapid Cβ—Cγ flip-flopping would establish, on the average, a symmetrical condition with the Asn 16 NH_2 protons from the two α-helices of the GCN4 dimer equally engaged

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in the side chain-side chain hydrogen bonds and producing identical NMR signals.

We agree with King that the flip-flop hypothesis is a highly probable and, indeed, a necessary conjecture called for by the degeneracy of the Asn 16 NMR signals. However, the crystallographic interpretation of the two different GCN4 crystal forms (O'Shea et al., 1991; Ellenberger et al., 1992), suggests that the Asn 16 side chains do adopt different and asymmetrical conformations. Table 1 below lists the Asn 16 χ_1 and χ_2 torsional angles in the two α-helices of the GCN4 dimer as observed in the two x-ray structures (cf. also Table 8 in Shen et al., 1996).

TABLE 1 Side chain torsions of Asn 16

Torsions (Asn 16)	O'Shea et al., 1991		Ellenberger et al., 1992	
	A helix	B helix	C helix	D helix
χ_1	−70°	−175°	−151°	−63°
χ_2	−17°	−5°	118°	−41°

It seems clear that 1) not only the two Asn 16 side chains within both the O'Shea et al. and the Ellenberger et al. dimer differ from each other, but also 2) the Asn 16 conformations of the two crystal structures are significantly different. In our manuscript, we expressed this by stating that ". . .in the two different crystals, these side chains adopted strikingly different conformations (Fig. 1). . ." and ". . . crystal conditions impose more asymmetric. . .conditions. . . thereby "'locking in"' certain conformers."

A detailed CONGEN analysis of the four Asn 16 side chains, reported here but not included in our original paper, supplies additional stereochemical and energetic details. In the O'Shea et al. structure, the Asn 16 ND2 atom of the A chain forms a strong hydrogen bond (−1.9 kcal) with the carbonyl oxygen of the Leu A12, whereas the Asn 16 ND2 atom of the B chain is only poorly engaged with the amide OD1 atom of the Asn A16. The unfavorable hydrogen-bonding energy of this interaction, +0.7 kcal, is due to a suboptimal H-bond geometry (donor-acceptor distance 2.6 Å, N-H-O angle 45°). Similarly, in the Ellenberger et al. structure, the Asn 16 ND2 atom of the C chain forms a good hydrogen bond (−1.4 kcal) with the carbonyl oxygen of the Leu C12, and the Asn 16 ND2 atom of the D chain makes only a poor H-bond with the Asn C16 OD1 atom (0.0 kcal; donor-acceptor distance 2.6 Å, N-H-O angle 76°). It is conceivable that the strong backbone-side chain hydrogen bond, in one of the Asn 16 residues in each of the dimers (the A chain; the C chain) is the main stabilizing factor of the (asymmetric) Asn-Asn pair, rather than the weaker A-B and C-D side chain-side chain bonds.

We also modeled the flip-flopped amide groups in both the above x-ray structures by adjusting the χ_2 torsions in the Asn-Asn pair and energy-minimizing the resulting models. In support of the King hypothesis, we obtained configurations symmetrical to those found in the starting x-ray structures, e.g., the Asn B16 ND2 atom (as opposed to the Asn A16 ND2) hydrogen-bonding the carbonyl O of Leu B12, and the Asn A16 ND2 atom (as opposed to the Asn B16 ND2) engaged in the weak side chain-side chain H-bond. However, the χ_2 rotations needed to achieve these flip-flops were not exactly equal to 180°.

In summary, we believe that side-chain torsional asymmetry and conformational interchange are both real phenomena that may or may not coexist in any particular GCN4 system under study. Torsional asymmetry, in particular, seems to be stabilized by favorable polar (electrostatic, hydrogen-bonding) interactions whose energetic content appears to be higher than the thermal noise at room temperature (~0.6 kcal per degree of freedom). This asymmetry is readily observable by eye in the different x-ray crystallographic structures.

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