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Using Pisa pies to resolve ambiguities in angular constraints from PISEMA spectra of aligned proteins

Francesca M. Marassi[a,*] & Stanley J. Opella[b]
[a]*The Burnham Institute, La Jolla, CA 92037, U.S.A.;* [b]*Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA 92093, U.S.A.*

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Abstract

The structures of proteins are mapped onto the patterns of resonances in NMR spectra of aligned samples. This is most clearly illustrated with Pisa wheels of helical membrane proteins, where the distinctive 'wheel-like' patterns of resonances reflect the tilt and rotation of the helices in the bilayers. These patterns contain both structural and assignment information. This Communication describes a simple way of using this information to resolve angular ambiguities inherent in orientational constraints derived from NMR data. This contributes to the use of solid-state NMR of aligned samples for protein structure determination.

Protein structures can be determined from angular constraints (Cross and Opella, 1983; Opella et al. 1987) derived from the orientationally dependent frequencies observed in solid-state NMR spectra of aligned samples. This approach is particularly attractive for the determination of the structures of membrane proteins in aligned lipid bilayers (Ketchem et al., 1993; Opella et al., 1999; Wang et al., 2001; Marassi and Opella, 2002). The ^{1}H-^{15}N dipolar coupling and ^{15}N chemical shift resonance frequencies measured in two-dimensional PISEMA (polarization inversion with spin exchange at the magic angle) spectra (Wu et al., 1994) of aligned ^{15}N-labeled proteins are the principal sources of angular constraints for backbone structure determination. The peptide plane orientations relative to the direction of the applied magnetic field are determined directly from the NMR frequencies, and protein structures are described as a series of linked peptide planes joined at their common C_α atoms (Opella et al., 1987; Quine and Cross, 2000). The degeneracies that complicate the interpretation of NMR frequencies as angular constraints can be resolved experimentally by measuring additional orientationally dependent frequencies involving sites that are not in the peptide plane. In this Communication, we demonstrate that these degeneracies can be resolved more simply by exploiting the symmetry properties of Pisa wheels, the characteristic wheel-like patterns of resonances observed in PISEMA spectra of aligned samples of ^{15}N labeled helical proteins (Marassi and Opella, 2000; Wang et al., 2000).

The ^{15}N chemical shift ($\nu_{\mathrm{N\ shift}}$) and ^{1}H-^{15}N dipolar coupling ($\nu_{\mathrm{NH\ cplg}}$) frequencies depend on the orientation of the molecular site with respect to the direction of the applied magnetic field, and on the magnitudes and orientations of the principal elements of the spin-interaction tensors. The frequencies are given by:

$$\nu_{\mathrm{N\ shift}} = \sigma_{11}\sin^2(\alpha-\chi)sin^2\beta+\sigma_{22}\cos^2\beta+\sigma_{33}\cos^2(\alpha-\chi)\sin^2\beta, \quad (1)$$

$$\nu_{\mathrm{NHcplg}} = \gamma_{\mathrm{H}}\gamma_{\mathrm{N}}h/r^3(3\sin^2\beta\cos^2\alpha-1), \quad (2)$$

where σ_{11}, σ_{22}, and σ_{33} are the principal elements of the ^{15}N chemical shift tensor, χ is the angle between σ_{33} and the NH bond, γ_{H} and γ_{N} are the gyromagnetic ratios of the ^{1}H and ^{15}N spins, h is Planck's constant, and r is the NH bond length. The polar angles α and β

*To whom correspondence should be addressed. E-mail: fruarassi@burnham.org

describe the peptide plane orientation in the magnetic field: α is the angle between the NH bond and the projection of the magnetic field direction on the peptide plane, and β is the angle between the normal to the peptide plane and the direction of the magnetic field (Tycko et al., 1986). The ^{15}N chemical shift tensor and the NH bond length are characterized reasonably well, for amide backbone sites of peptides, therefore, it is possible to extract α/β angular constraints from the resonance frequencies using equations [1] and [2]. Since the σ_{33} component of the ^{15}N chemical shift interaction tensor is not collinear with the NH bond, the ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling frequencies serve as independent angular constraints for peptide plane orientation. This feature is also responsible for the wheel-like patterns of the resonances in PISEMA spectra of aligned samples of helical proteins.

These spectral patterns can be directly analyzed for residues that are immobile on the relevant timescales (10^{-4} s). The symmetry properties of the spin interaction tensors give rise to degeneracies in the angular constraints. Each equation yields combinations of the angles α and β that are consistent with a frequency measurement, and that trace out a line on the α/β surface. The actual orientation of a peptide plane satisfies both Equations 1 and 2 and corresponds to a single α/β point. The orientation is determined for $0 < \alpha < 180$ and $0 < \beta < 90$; as a result, any one of four orientations **1**(α/β), **2**(α/180−β), **3**(180+α/β), and **4**(180+α/180−β) are consistent with the experimental data. In addition, since the PISEMA experiment does not distinguish between positive and negative ^{1}H-^{15}N dipolar couplings, resonances with couplings smaller than half maximal value have an additional pair of α/β solutions, Thus, there are up to eight possible symmetry-related peptide plane orientations consistent with the ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling frequencies.

An additional set of degeneracies arises from the existence of two ways of linking two peptide planes, both of which are consistent with the requirement of tetrahedral geometry and L-amino acid chirality at C_α. Because each dipeptide combination yields two possible pairs of ϕ/ψ dihedral angles, and each peptide plane has up to eight possible orientations, as described above, this results in 64 possible ϕ/ψ sets. Of these, only 32 are unique since the positive and negative magnetic field directions are not differentiated in the NMR experiment. Although this ambiguity can be resolved experimentally, in practice the actual ϕ/ψ dihedral angles are generally selected from the set of 32, based on the identification of chemically and energetically sensible solutions. In this Communication, we describe how the correct ϕ/ψ sets can be determined by taking advantage of the symmetry properties of Pisa wheels.

The observation of Pisa wheel patterns in the PISEMA spectra of aligned samples of ^{15}N-labeled proteins enables the qualitative determination of the protein secondary structure and orientation, since the wheel-like patterns for α-helices (Marassi and Opella, 2000; Wang et al., 2000) are distinctly different from the loop patterns associated with β-strands (Marassi, 2001). No resonance assignments are needed to determine the tilt of a helix, and a single assignment or other partial assignments data are sufficient to determine the helix rotation. Thus, Pisa wheels enable valuable structural information to emerge from the spectroscopic studies prior to complete three-dimensional structure determination. They also play crucial roles in short-circuiting the lengthy traditional sequential assignment and structure determination process.

The spectra in Figure 1 were calculated for a uniform α-helix (ϕ/ψ = −65°/−40°) (Marassi and Opella, 2000). While it is not possible to determine the sign of dipolar couplings with smaller than the half maximal values (< 5 kHz as displayed) from PISEMA spectra, the Pisa wheels provide a framework for determining the sign of dipolar couplings from the resonance frequencies. The dipolar couplings for helices with tilts less than 40° are positive (Figures 1A–C), whereas those for helices with tilt angles near 90° are negative (Figure 1G). The spectra of helices with intermediate tilts between about 40° and 90° (Figures 1D–F) display both positive and negative dipolar couplings. However, the signs of individual dipolar couplings can be determined from their positions in a Pisa wheel. This reduces the angular constraint ambiguities from eight to four symmetry-related α/β orientations for each peptide plane, and from 32 to 16 sets of ϕ/ψ dihedral angles for each dipeptide combination.

Figure 1 also shows that it is possible to select a single peptide plane orientation out of the symmetry-related set of four from the position of its resonance in the Pisa wheel. This is because the orientation of each peptide plane in an α-helix can be predicted from the helix tilt and rotation angles. In Figure 1 the pie shaped sections labeled **1**, **2**, **3**, and **4** in the helical (Shiffer and Edmunson, 1967) and Pisa wheels correspond, respectively, to the four peptide

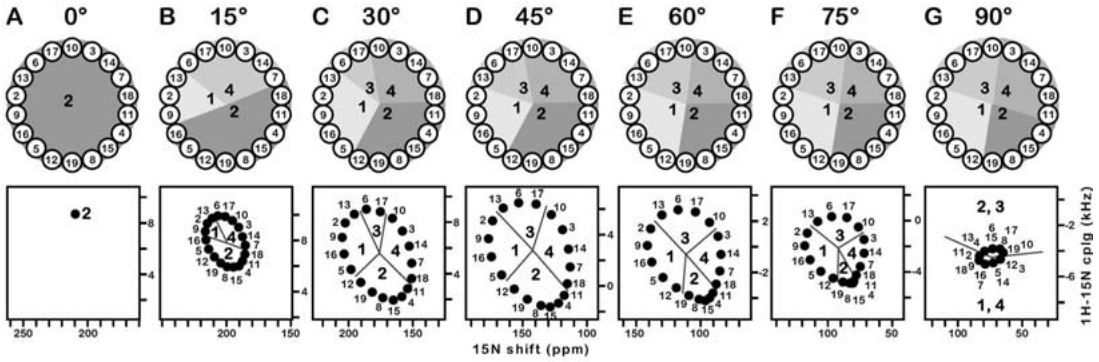

Figure 1. Helical wheel representations and PISEMA spectra calculated for a uniform α-helix ($\phi/\psi = -65/-40$) at varying helix tilts. The principal values and molecular orientation of the amide spin interaction tensors were taken from ref. 1. The four pie sections are labeled.

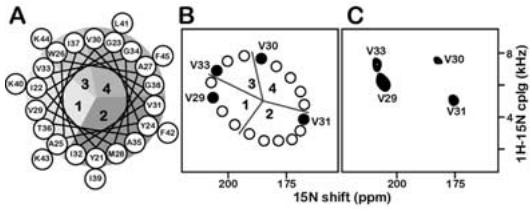

Figure 2. (A) Helical wheel representation of TM-pVIII. (B) Calculated PISEMA spectrum for a uniform α-helix ($\phi/\psi = -65/-40$) with a helix tilt of 26°. (C) Experimental PISEMA spectrum from ^{15}N-Val labeled pVIII in oriented lipid bilayers.

		Val30 peptide plane							
		1 (α / β)		2 (α / 180-β)		3 (180+α / β)		4 (180+α / 180-β)	
Val29 peptide plane	1 (α / β)	159.6	-132.2	159.6	-151.3	105.6	48.5	105.6	67.5
		-108.5	151.3	-108.5	132.2	-54.5	-67.5	-54.5	-48.5
	2 (α / 180-β)	-159.6	151.3	-159.6	132.2	-105.6	-67.5	-105.6	-48.5
		108.5	-132.2	108.5	-151.3	54.5	48.5	54.5	67.5
	3 (180+α / β)	-105.6	-48.5	-105.6	-67.5	-159.6	132.2	-159.6	151.3
		54.5	67.5	54.5	48.5	108.5	-151.3	108.5	-132.2
	4 (180+α / 180-β)	105.6	67.5	105.6	48.5	159.6	-151.3	159.6	-132.2
		-54.5	-48.5	-54.5	-67.5	-108.5	132.2	-108.5	151.3

Figure 3. Dihedral angle solutions from the combination of four possible α/β constraints for the Val29-Val30 dipeptide link. Since positive and negative magnetic field directions are indistinguishable, there are only 16 unique solutions (shaded area). The actual orientations of the individual peptide planes are determined from the Pisa wheel spectrum in Figure 2 to be **1**(α/β) for Val29 and **4**(180+α/180−β) for Val30. This enables the correct pair of ϕ/ψ dihedrals to be selected, $\phi/\psi = -54.5/-48.5$ (box).

plane orientations **1**(α/β), **2**(α/180−β), **3**(180+α/β), and **4**(180+α/180−β). For example, in a helix tilted by 30° (Figure 1C) the peptide plane for residue 2 has an orientation defined by **1**(α/β). It is connected to residue 3, with a peptide plane orientation defined by **4**(180+α/180−β), which, in turn, connects to residue 4, with orientation **2**(α/180−β). Therefore, it is possible to select a single peptide plane orientation for each residue, and hence a single set of ϕ/ψ dihedral angles connecting two peptide planes, based solely on which piece of a Pisa pie that it occupies.

This Pisa wheel restriction analysis was used in the calculation of the complete structure of the transmembrane domain (residues 20 to 45) of pVIII, the major coat protein of fd bacteriophage as part of the structure determination of this protein in lipid bilayers (Marassi and Opella, 2002). The TM domain of the membrane-bound form of pVIII is an α-helix, which crosses the lipid bilayers with a tilt angle of 26°. Its helical wheel representation is shown in Figure 2A (Schiffer and Edmunson, 1967). The corresponding Pisa wheel spectrum, calculated for a uniform α-helix ($\phi/\psi = -65°/-40°$) with 26° tilt, is shown in Figures 2B. The Val resonances are labeled to show that rotations of both the helical and Pisa wheels match the resonance pattern in the experimental PISEMA spectrum of the ^{15}N-Val labeled protein shown in Figure 2C. The ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling frequencies for each resonance in the experimental PISEMA spectrum provided the input to Equations 1 and 2 used to calculate the α/β orientations of their corresponding peptide planes. The structure of the protein was then determined by calculating ϕ/ψ dihedral angles for contiguous peptide planes, and applying the Pisa pie angular constraint restriction analysis described here.

The results obtained for the Val29-Val30 dipeptide link are shown in Figure 3. The combination of four possible α/β constraints for each residue leads to 32 ϕ/ψ dihedral angle solutions for a linked peptide pair. Since positive and negative magnetic field directions are indistinguishable, there are only 16 unique solutions shown in the shaded area of Figure 3. For example, the link between peptide plane orientations

4(180+α/180−β) for Val29 and **2**(α/180−β) for Val30 has dihedral angle solutions $\phi/\psi = 105.6°/48.5°$ or $-54.5°/-67.5°$, identical to those obtained for the link with orientation **1**(α/β) for Val29 and orientation **3**(180+α/β) for Val30.

The actual orientations of the individual peptide planes determined from the Pisa wheel spectrum in Figure 2B are **1**(α/β) for Val29 and **4**(180+α/180−β) for Val30. This, in turn, enables the correct pair of ϕ/ψ dihedral angles to be selected from Figure 3. This peptide link pair includes the correct solution ($\phi/\psi = -54.5°/-48.5°$) as well as an energetically unfavorable solution ($\phi/\psi = 105.6°/67.5°$) which is discarded.

The ability to calculate a unique correct backbone structure solely from ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling constraints paves the way for rapid automated methods of protein structure determination from solid-state NMR data, where a structural model of the protein is generated from a combination of Pisa wheels observed in the PISEMA spectrum, and the model is used to back-calculate and assign the experimental spectrum as a means of structure refinement.

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