



# Solid State NMR Structure Determination of Hydrogen Bonding and Uniformity of Membrane Protein Helices

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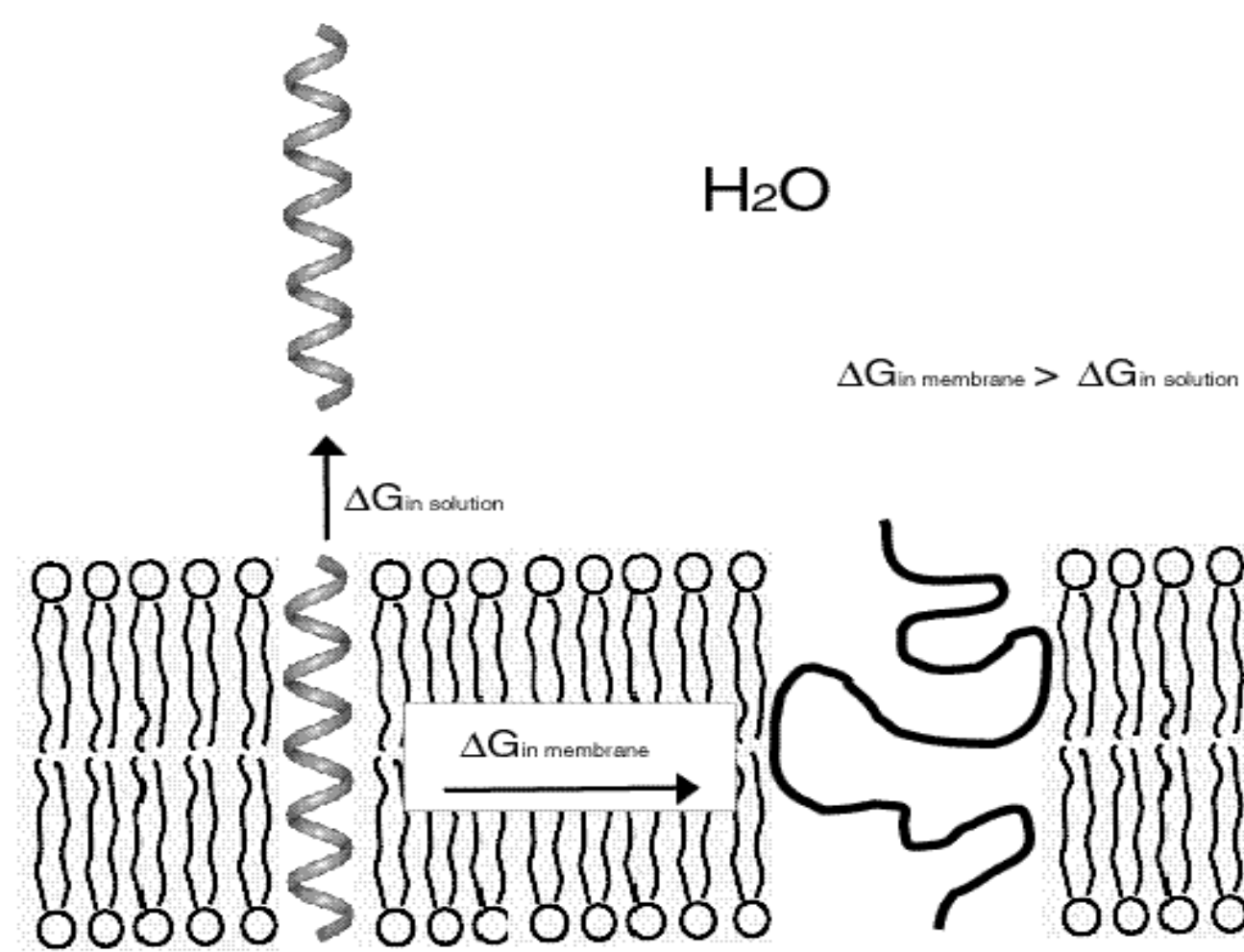
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## Abstract

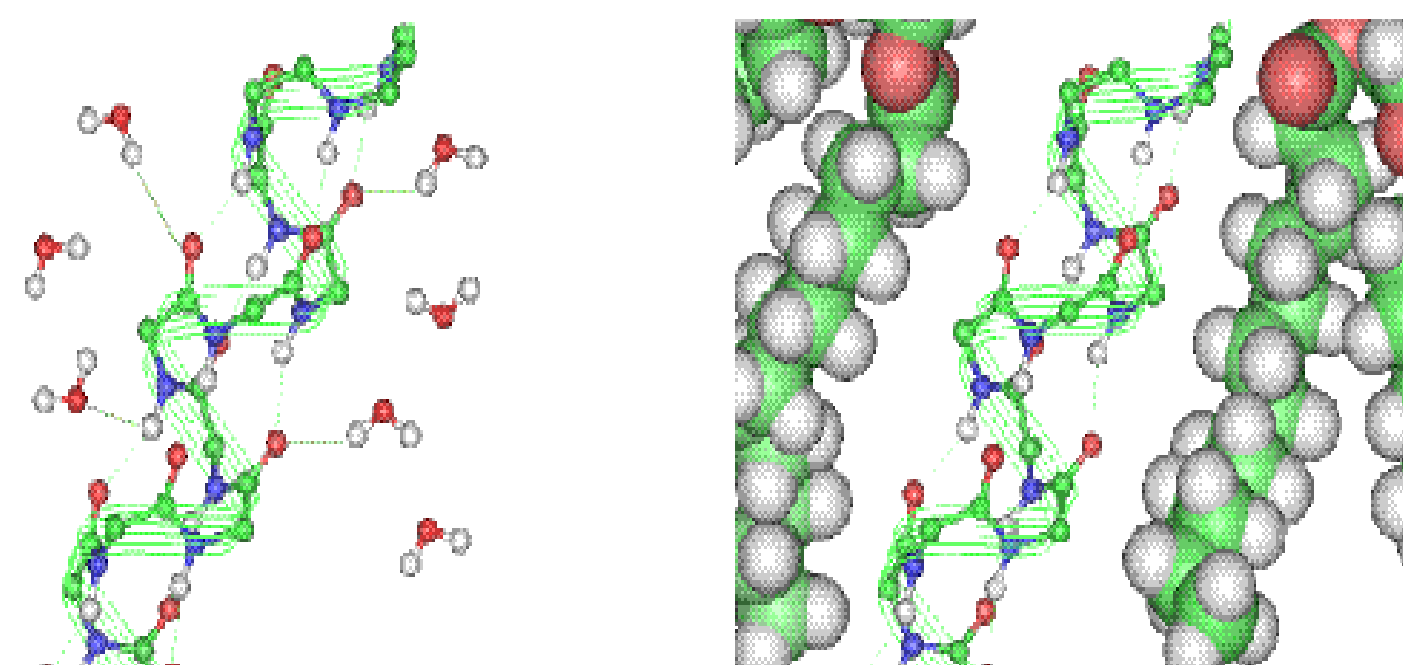
Backbone hydrogen bonds (H-bond) play a key role in stabilization of transmembrane helical protein structures. The H-bond strength is expected to be increased by the low dielectric and the scarcity of water in the membrane environment. It is demonstrated that a correlation exists between the PISA (Polar Index Slant Angles) wheels observed in PISEMA spectra and the H-bonding pattern in the transmembrane helices. The H-bonds and uniformity of membrane protein helices are compared with those of recently solved membrane protein X-ray crystal structures.

Anisotropic <sup>15</sup>N chemical shift and <sup>15</sup>N-<sup>1</sup>H dipolar interactions of the polypeptide backbone of the M2 transmembrane segment have been observed through 2D PISEMA solid-state NMR experiments. The orientational constraints strictly restrain the N-H and C=O orientations on the peptide plane and are sensitive to the backbone H-bonds (N-H...O=C). The H-bond length on the backbone of transmembrane helices has been optimized with respect to these orientational restraints on the picometer scale.

The helical stability of transmembrane polypeptides in low dielectric lipid environments is higher than that in aqueous environments. The large free energy cost of transferring an unsatisfied hydrogen-bond donor or acceptor from an aqueous to a nonpolar environment suggests that most hydrogen bonds must be satisfied when peptides are inserted into a membrane environment.



In a membrane environment, desolvation of the backbone NH and CO groups might be the source of  $\alpha$ -helical stability for transmembrane polypeptides. Water competes for the peptide backbone hydrogen bonds, thereby destabilizing them. But, in a membrane environment, scarcity of water and low dielectric conditions enhance the strength of hydrogen bonds and stabilize the helical structure.



In this work, to demonstrate the characterization of topology and the backbone hydrogen bonding of transmembrane helical structures, we have used the transmembrane peptide from M2 protein of Influenza A virus. This protein has a single

transmembrane helix and forms a pH activated H<sup>+</sup> channel in the viral coat.

Solid state NMR presents a unique opportunity to gain topological information on membrane proteins. The anisotropic spin interactions that form the axes of the PISEMA spectra relate the orientation of spin interaction tensors to the magnetic field axis fixed in the laboratory frame of reference. This unique observation in NMR spectroscopy and consequently, projection of  $\alpha$ -helices oriented with respect to the Z axis are imaged in the PISEMA spectrum. To demonstrate such an image, data from the transmembrane helix of M2 protein is presented in figure 1A and compared with a helical wheel in figure 1B.

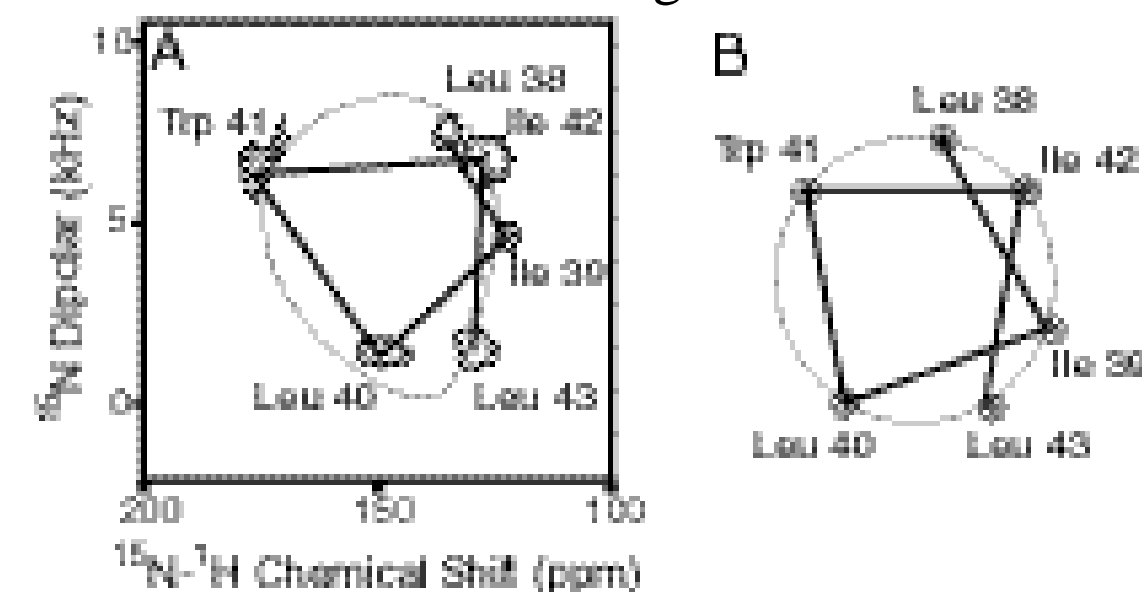


Figure 1. Comparison of PISA wheels and helical wheels. A) PISEMA spectra of single and multiple site labeled samples for residues 38 - 43 from M2-TMP have been superimposed. B) The helical wheel has been rotationally oriented to be in agreement with the PISA wheel.

## Calculation of PISA Wheels

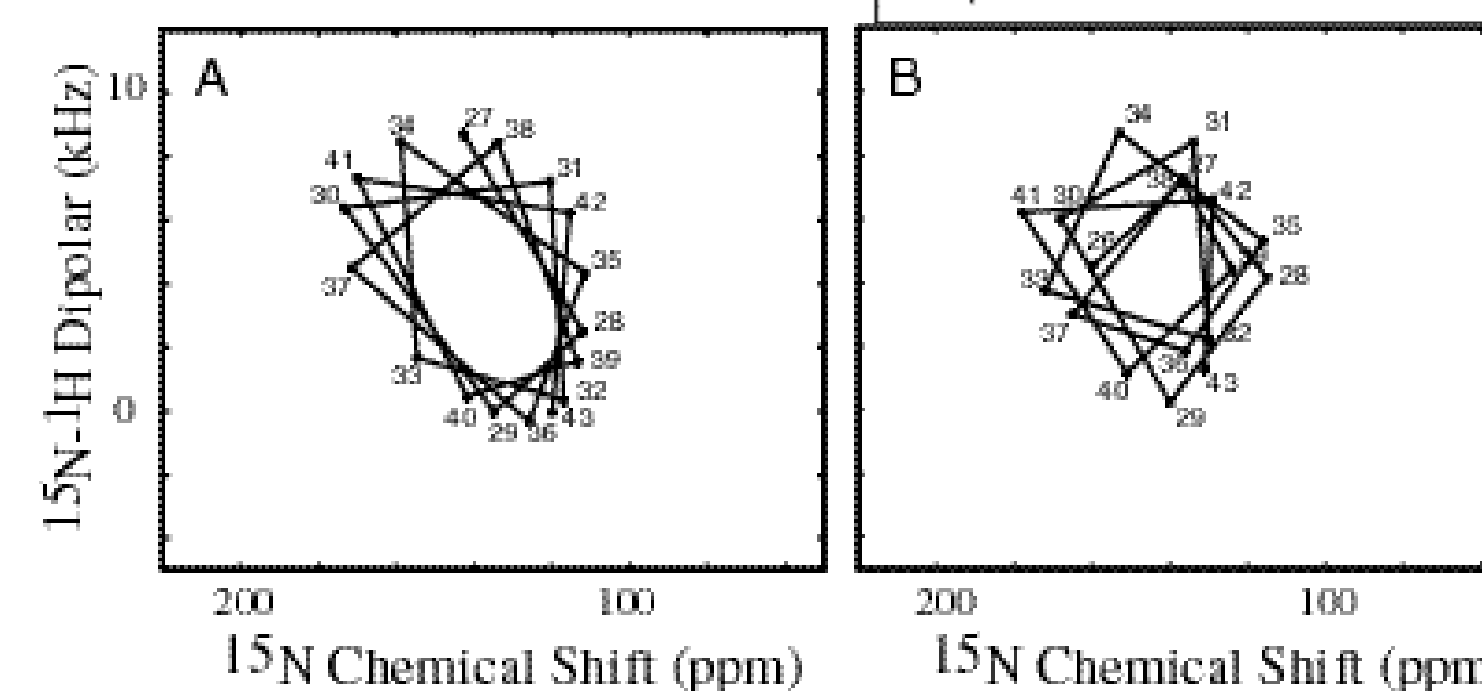
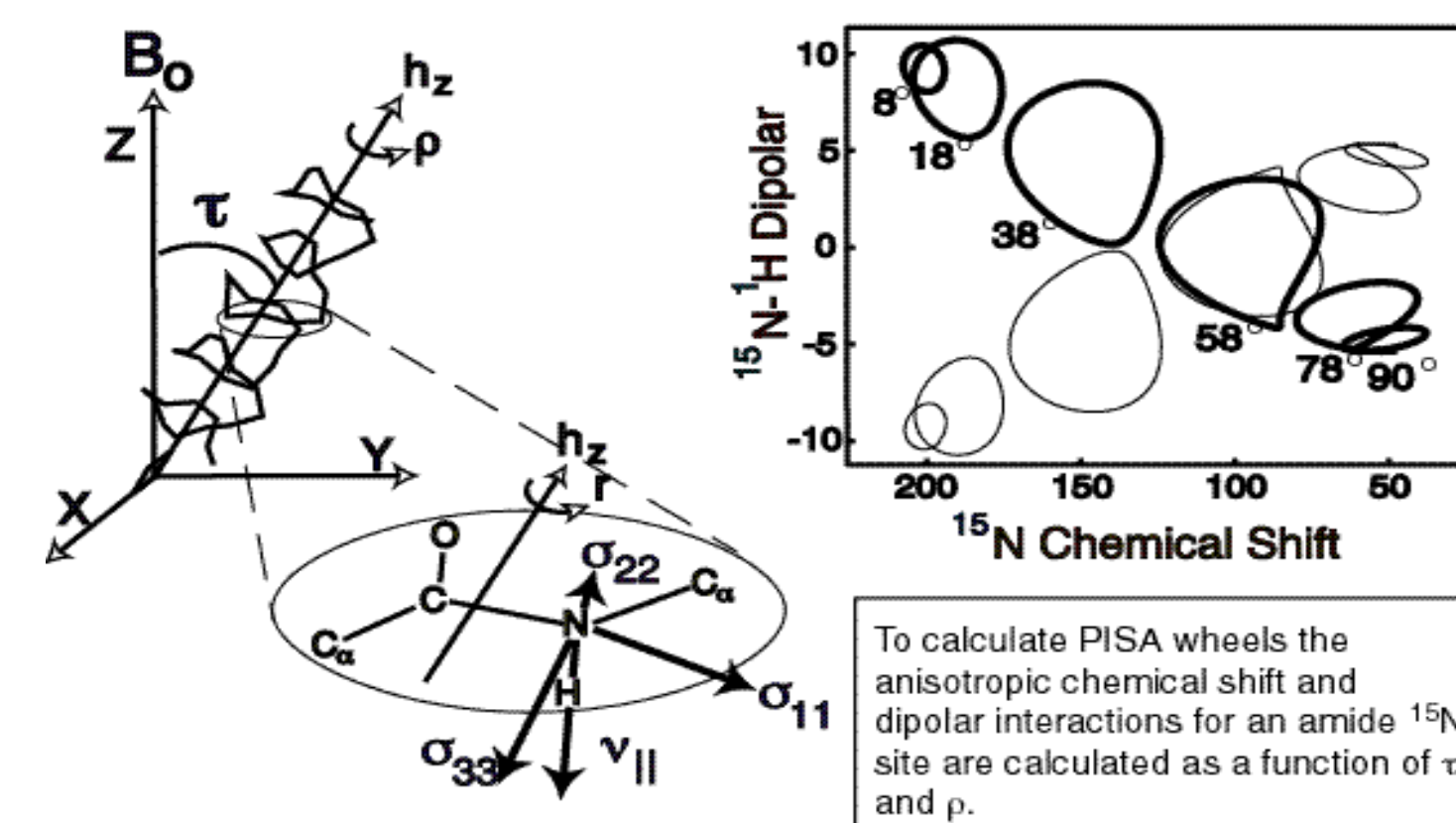


Figure 2. A theoretical PISA wheel is compared to experimental PISEMA 2D resonances. A) PISA wheel calculated from an ideal alpha helix using an averaged chemical shift tensor value. ( $\tau = 38$  and  $\rho = 5$  used for the calculation) B) experimental resonances from M2-TMP. Ideal values (A29, S31, G31, H37) were used for the missing experimental data.

## Hydrogen bond

A hydrogen bond occurs when two electronegative atoms compete for the same hydrogen atom:



The degree of linearity is measured by the angle  $\phi$ , the distance of hydrogen bond (H-O) is measured by  $d$ .

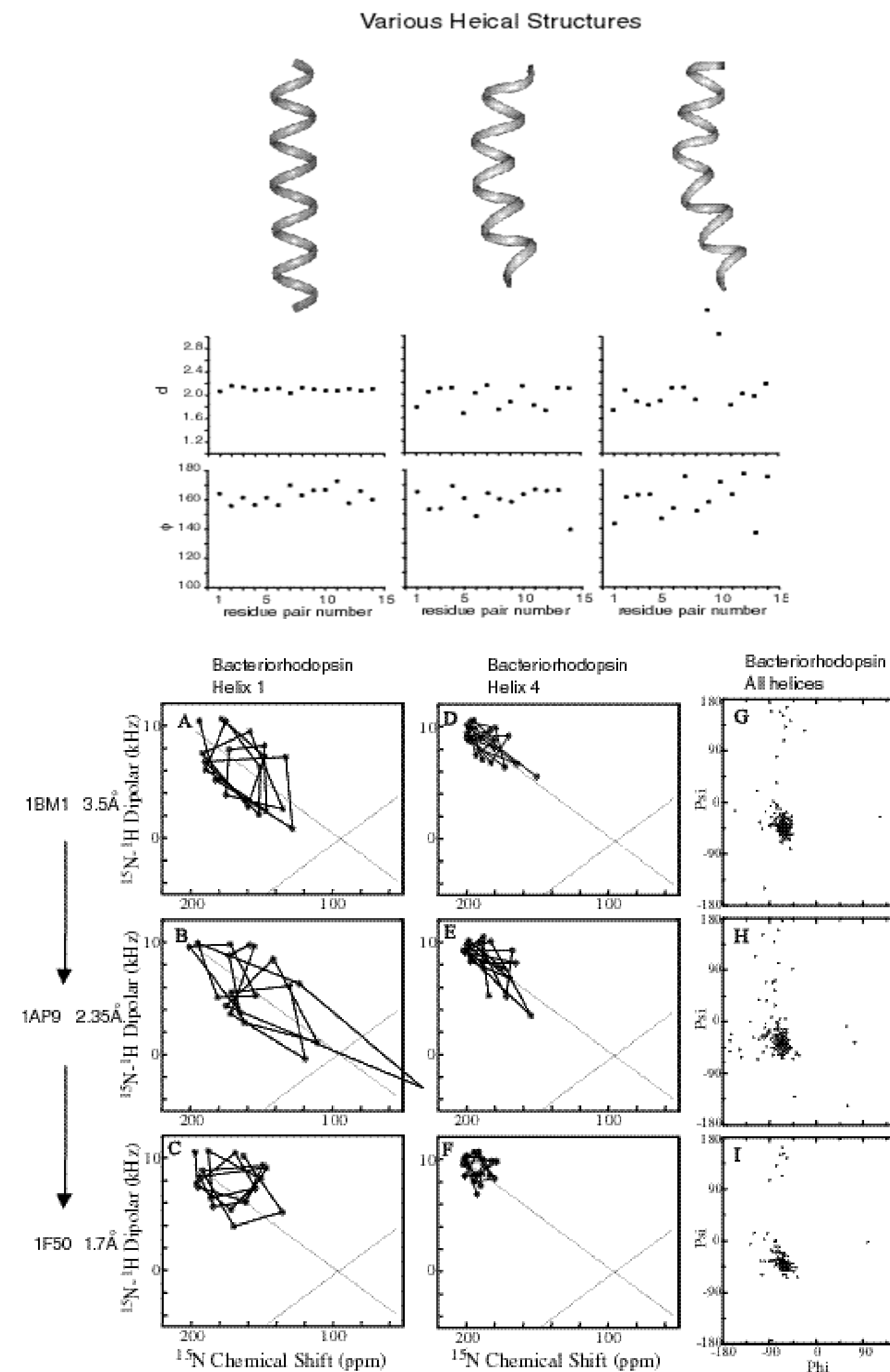
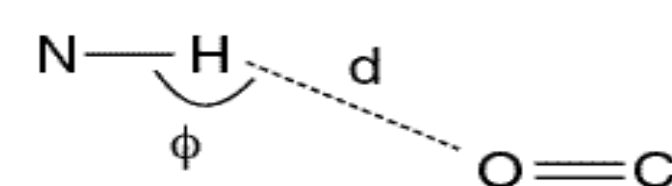


Figure 3. PISEMA spectra simulation of A-C) helix 1 (residue 12-30) and D-F) helix 4 (residue 104-125) and ramachandran map of G-I) all helix region from X-ray structure of bacteriorhodopsin.

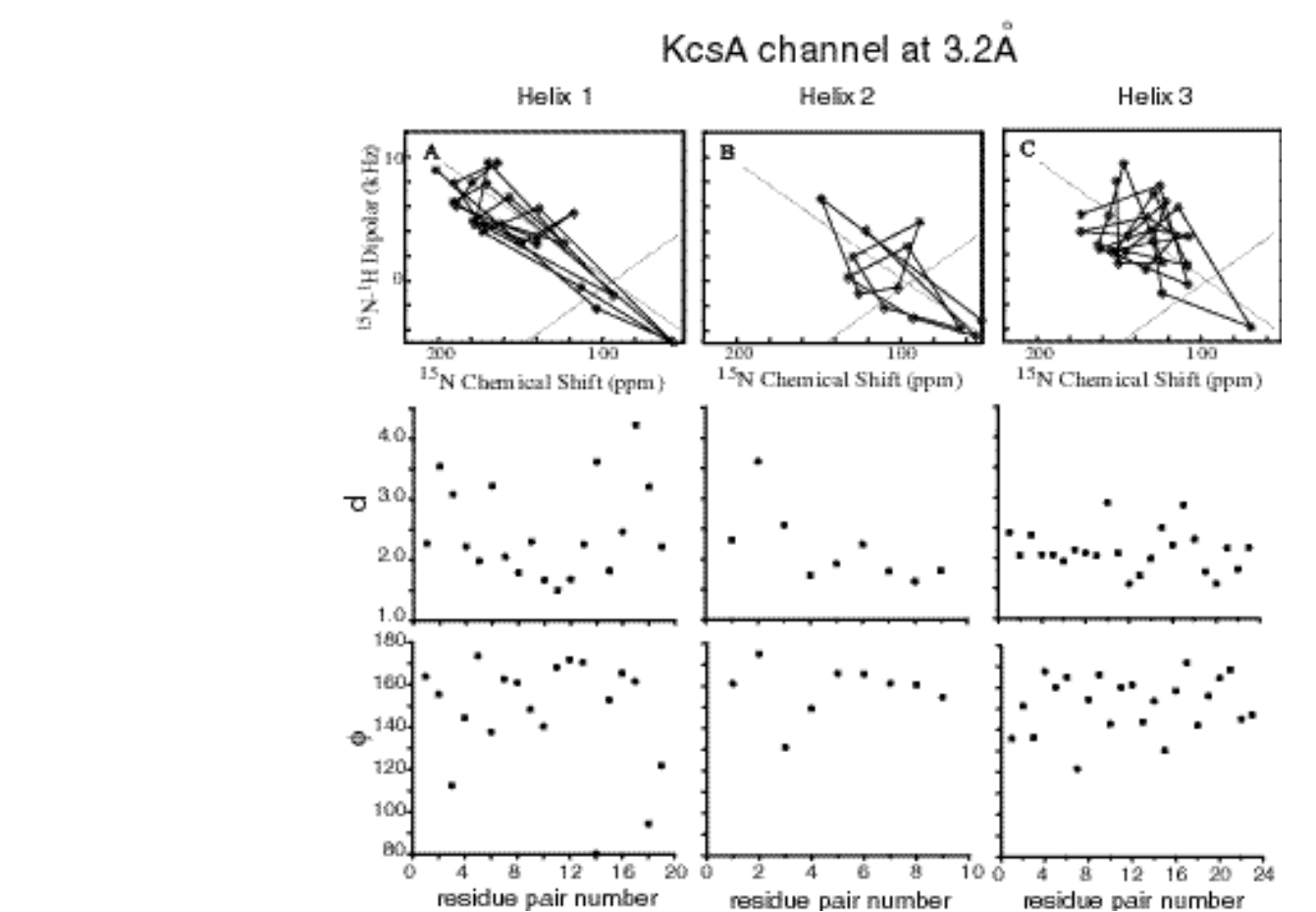
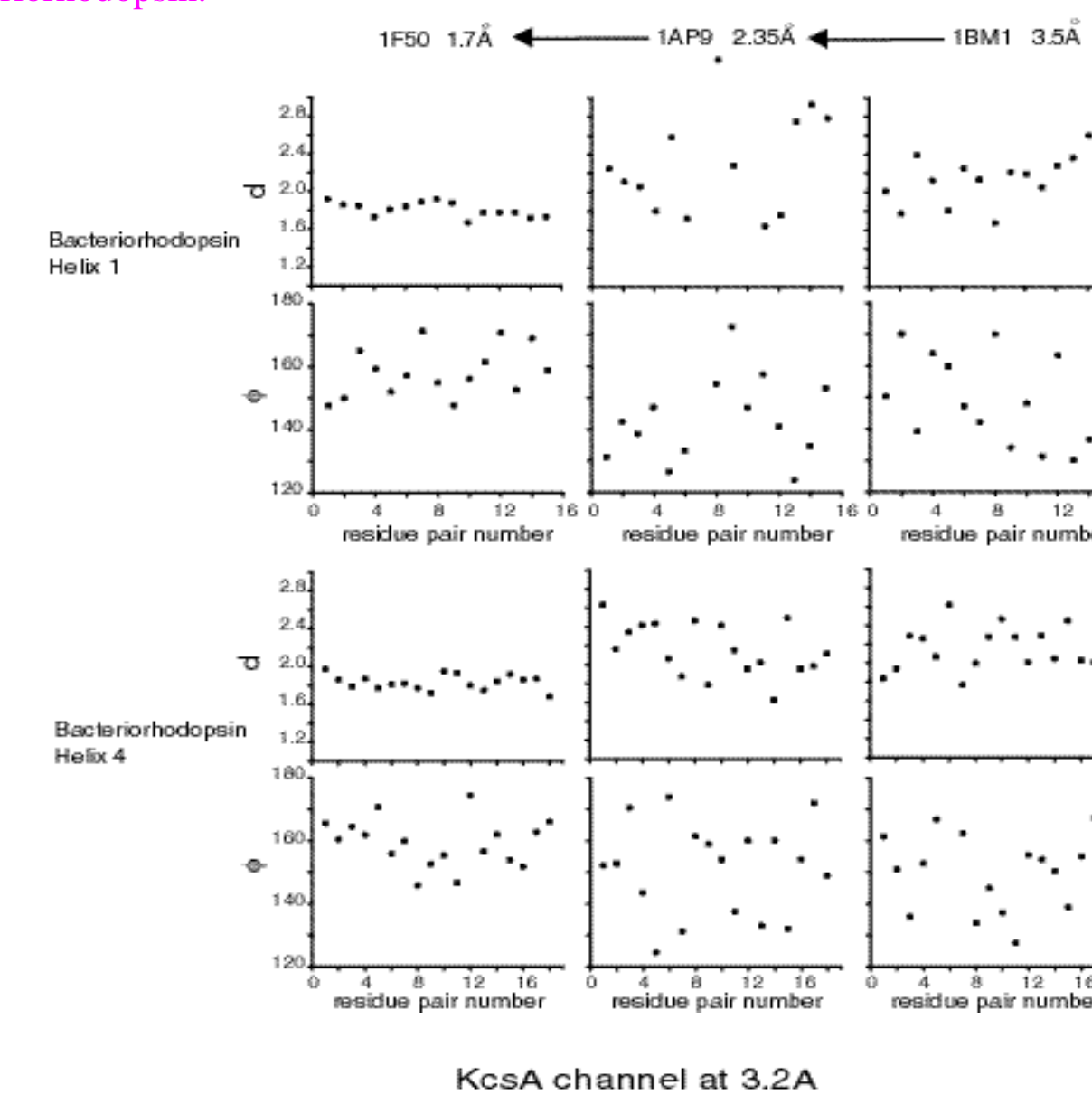


Figure 4. PISEMA spectral simulation of A) helix 1 (residue 27-51), B) helix 2 (residue 62-74), C) helix 3 (residue 86-112) and hydrogen bond analysis from the X-ray structure of the KcsA channel at 3.2 A resolution, PDB 1BL8.

## Hydrogen bonding in globular proteins

(from Baker and Hubbard, *Prog. Biophys. Mole. Biol.* 1984)

Mean H-bond geometry in  $\alpha$ -helices (from 577 H-bonds)

2.06 A, (H-O distance), 2.99 (N-O distance)

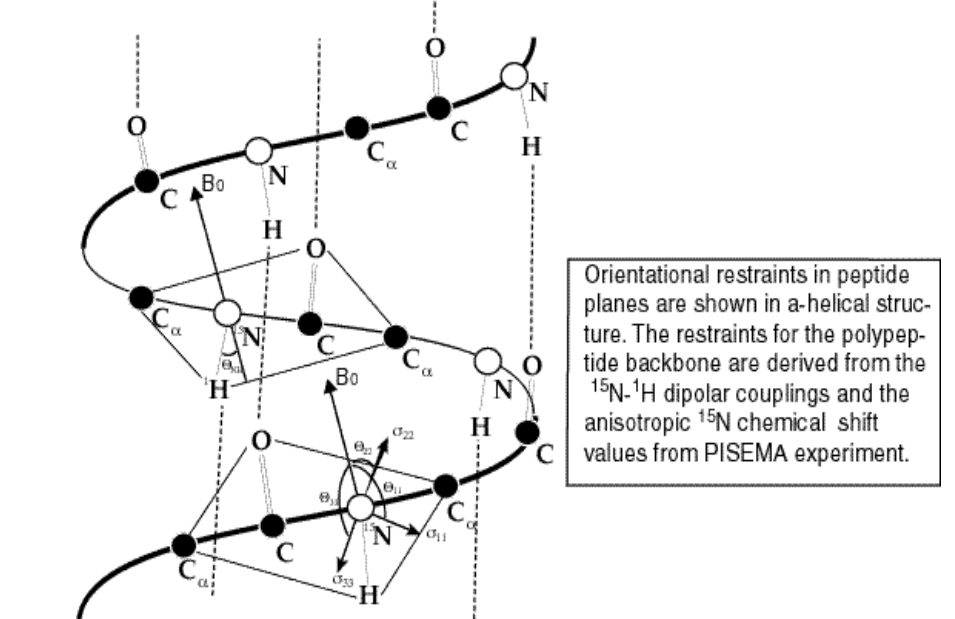
Mean H-bond geometry in  $\beta$ -sheets (from 516 H-bonds)

1.96 A, (H-O distance), 2.91 (N-O distance)

## Hydrogen bonding in Bacteriorhodopsin $\alpha$ -helices (H-O)

PDB	1F50	1QHJ	1AP9	IBM1
Resolution	1.7A	1.9A	2.35A	3.5A
Mean	1.92	2.02	2.12	2.12
Min	1.54	1.71	1.59	1.68
Max	2.48	2.48	2.50	2.49

## Oriental constraints



## Hydrogen bond distance optimization

We found that hydrogen bond distances in refined models are always shorter than the distance of target hydrogen bonds from Baker and Hubbard's work (hydrogen bonding in globular proteins). Then, we searched the optimum distance of backbone hydrogen bonding in the M2-TMP varied with the target distance. During the calculation, the orientational restraints from the PISEMA experiment were used to refine the backbone geometry.

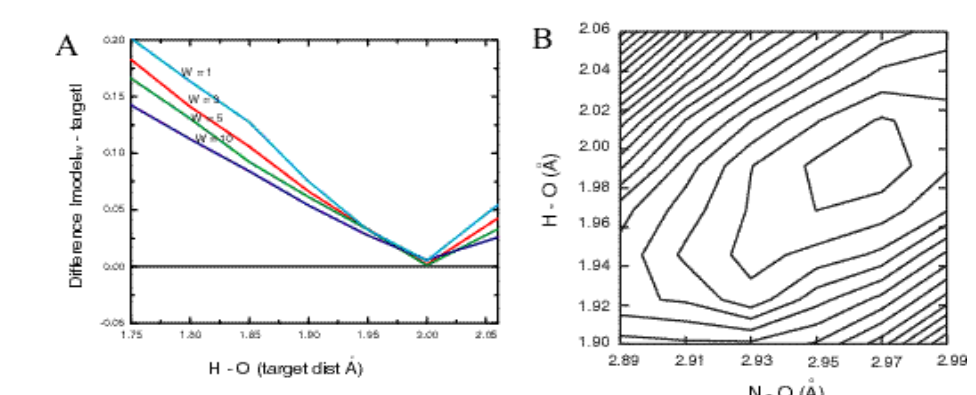


Figure 5. A) The penalty  $[av(model)-target]$  value for hydrogen bonds in a refinement run of M2-TMP structure is shown as a function of the target hydrogen bond distances. The energy of hydrogen bond is expressed as  $E_{\text{bond}} = w \cdot (d_{\text{model}} - d_{\text{target}})$  in the energy calculation of model system. The optimum target distance of hydrogen bonds are around 2.00 A regardless of weighting. B) The penalty of hydrogen bond energy ( $E_{\text{bond}}$ ) surface for a M2-TMP backbone structure. The N - O and H - O target distance were searched to find the optimum of the penalty<sub>hydrogen</sub> function. The minimum value of hydrogen bond distances were found at 1.98 A (H - O) and 2.97 A (N - O) distance with respect to the solid state NMR orientational restraints.