*Journal of the Korean Magnetic Resonance Society* **2017**, *21,* 26-30
DOI 10.6564/JKMRS.2017.21.1.026

# Elucidating the Dynamic Properties of Globular Protein using Predicted Order Parameters and $^{15}$N NMR Relaxation

**Jong-Jae Yi[1,ǂ], Won-Je Kim[2,ǂ], Jin-Kyu Rhee[3], Jongsoo Lim[4], Bong-Jin Lee[2] and Woo Sung Son[1,*]**

[1]College of pharmacy, CHA University, 120 Haeyong-ro, Pocheon-si, Gyeonggi-do, 11160, Republic of Korea
[2]College of pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826, Republic of Korea
[3]Department of Food Science and Engineering, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul, 03760, Republic of Korea
[4]Discovery Technology Team, Dong-A ST Research Institute, 21 Geumhwa-ro 105beon-gil, Yongin-si, Gyeonggi-do, 17073, Republic of Korea

*Received Feb 12, 2017; Revised Feb 25, 2017; Accepted Mar 12, 2017*

**Abstract** Dynamic properties of proteins can present key information on protein-ligand and protein-protein interaction. Despite their usefulness, the properties of protein dynamics have not been obtained easily due to protein stability and short-term measurement. Here, it is shown that combined method for analysis of dynamical properties. It utilizes predicted order parameter and NMR relaxation data such as $T_1$, $T_2$, and heteronuclear NOE. The suggested method could be used to know the flexibility of protein roughly without precise dynamical parameters such as order parameters through model-free analysis.

**Keywords** Dynamics, Relaxation, Protein, Order Parameter, Structure, NMR, Pyrazinamidase

## Introduction

The proteins usually harbor unique three-dimensional structures determined by amino acid sequences. The three-dimensional structures of proteins are not strictly rigid, but flexible in solution involving conformational changes. The changes of structures have various scales in space and time, which have a relationship with biological functions of proteins such as allosteric interaction and catalysis.[1,2]

Nuclear magnetic resonance relaxation methods are useful tools for elucidating dynamical properties of protein. Dynamics of protein backbone can be measured using $^{15}$N NMR relaxation from the reorientation of N-H bond vector in protein. $^{13}$C relaxation with carbonyl groups also can be used for additional information but has difficulty in data analysis due to homonuclear couplings.[3]

Heteronuclear NMR relaxation of protein backbone is usually analyzed using model-free method. In the model-free method, the correlation between global and local motions is absent, allowing their separation.[4] Applications of the model-free method include additional, uncorrelated, local fast motions or extra relaxation effects due to chemical exchange. For model-free analysis, rates of relaxation of nuclei from NMR experiments are fitted to the proper model leading to extracted order parameters.[5]

Generalized order parameter, $S^2$, can explain the reorientational properties of N-H bond vector and describe temporal properties derived from an internal and an overall tumbling correlation time.[4] High quality of relaxation NMR data is necessary to obtain

* Correspondence to: **Woo Sung Son**, College of Pharmacy, CHA University, 120 Haeryong-ro, Pocheon-si, Gyeon ggi-do, 11160, Republic of Korea, Tel: +82-31-850-9398; Fax: +82-31-850-9398; E-mail: wson@cha.ac.kr
ǂ These authors contributed equally.

$S^2$ order parameters, which can be time consuming steps. Zhang and Bruschweiler have presented $S^2$ prediction method based on allowing easy and rapid estimation of the magnitude of fast time-scale backbone dynamics.[6] Combined analysis of experimental and predicted $S^2$ parameters can be used to obtain local contact aspects of the three-dimensional structure and to validate the state of the protein such multimeric state and ligand bound state. Here, it is shown that comparison of predicted $S^2$ order parameter and $^{15}N$ NMR relaxation data could speed up analysis of dynamical properties of pyrazinamidase (PncA) protein from *Mycobacterium tuberculosis*.[6]

## Experimental Methods

*Protein Preparation-* PncA protein was expressed and purified with the protocols as published previously.[7]

*Nuclear magnetic resonance (NMR)-* NMR experiments for backbone assignment were conducted with the methods as reported previously .[7]

*$^{15}N$ $T_1$ relaxation measurement-* $^{15}N$ $T_1$ spectra were recorded at 313 K on Bruker DRX500 spectrometer equipped with a triple-resonance, pulsed field gradient probe with an actively shielded z-gradient and a gradient amplifier unit. $^{15}N$ $T_1$ values were measured from the spectra recorded with eight different durations of the delay T: T = 5, 65, 145, 246, 366, 527, 757, and 1148 ms. $T_1$ spectra were recorded with magnetization relaxing as $\exp(-T/T_1)$ and in such a way that the delay between scans affected only the sensitivity and not the extracted $T_1$ values. Relaxation delays of 1 s were employed in the measurement of $^{15}N$ $T_1$ values. To permit the estimation of noise levels, duplicate spectra were recorded for T = 246 ms.

*$^{15}N$ $T_2$ relaxation measurement-* The experiment is a series of heteronuclear single quantum coherence spectroscopy (HSQC) measurements modified so that the magnetization will remain on the nitrogen a different amount of time in each measurement. The delay time during which the $^{15}N$ relaxes is specified by the parameter L4, where be set differently for each determination in the series (T= 34.176, 51.264, 68.352, 85.439, 102.528, 119.616, 153.792, and 170.879 ms). The total time of this series of measurements were about 48 hours (for NS = 32 and 8 total determinations) at 313 K on Bruker DRX 500. The spectra were processed like normal HSQC spectra and then $T_2$ values were extracted by fitting the decline in signal strength over time to a decreasing exponential. To permit the estimation of noise levels, duplicate spectra were recorded for T = 102.528 ms.

*$^1H$-$^{15}N$ heteronuclear NOE measurement-* $^1H$-$^{15}N$ steady-state NOE values are obtained by recording spectra with (NOE experiment) and without (NONOE experiment) the use of $^1H$ saturation applied before the start of the experiment. The level of water suppression in spectra recorded without $^1H$ saturation is often significantly worse than in the $^1H$ saturation case and can lead to difficulties in obtaining accurate values for peak intensities. Moreover, any saturation of protons prior to the start of the NONOE experiment gives rise to a truncated small NOE effect. Therefore, suppression of the strong $H_2O$ resonance is achieved via the use of gradients to select for the $^{15}N \rightarrow {}^1H$ coherence transfer pathway and the application of a $^1H$ 90°pulse followed by a gradient pulse immediately prior to the start of the experiment to eliminate $^1H$ magnetization. The proton saturation period was 3 s. Elimination of all $^1H$ magnetization (including solvent) in this way could be achieved in 2 - 3 ms, which is short that $^1H$-$^{15}N$ cross-relaxation may safely be neglected during this interval.

*Relaxation Data Analysis-* Relaxation data was analyzed using NMRVIEW and TENSOR2. Dynamics parameters including order parameters and molecular rotational diffusion parameters were obtained.[8-12]

*Prediction of Order Parameters*- The estimation of NMR $S^2$ order parameters of N-H bond vectors of protein backbone was performed with s2predict.py python script. Three-dimensional structure of PncA was generated with homology modeling using MODELLER.[6,13,14]

## Results

The backbone dynamics of *M. tuberculosis* PncA have been determined through solution NMR measurements of relaxation parameters $T_1$, $T_2$, and the steady-state NOE of the amide resonances. Figure 1 represents the results of the relaxation measurements, showing the measured values plotted against residue number. Residues for which relaxation measurements could not be assigned included the N-terminal M1, P54, P62, P69, P70, P77, P83, P115 and overlapping residues. The values of the three sets of relaxation measurements (NOE, $T_1$, and $T_2$) are correlated with the mobility of each amide in the protein. Internal motions affect the rate at which an excited nucleus may sample the fluctuating fields around it to exchange energy and relax. Examination of the experimental data reveals that *M. tuberculosis* PncA is distinctly different motional regimes across the protein backbone.

Predicted $S^2$ order parameters were calculated with equation (1) as published previously.[6] The equation relates $S^2$ of the N-H bond vector of amino acid *i* to close contacts experienced by the H atom and the carbonyl oxygen of the preceding amino acid *i*-1 with heavy atoms *k*.

$$S_i^2 = \mathrm{tahn}\left(0.8\sum_k \left(\exp(-r_{i-1,k}^{\mathrm{O}}/1\,\text{Å})\right) + 0.8\left(\exp(-r_{i,k}^{\mathrm{H}}/1\,\text{Å})\right)\right) + b \quad \cdots \ (1)$$

$r_{i-1,k}^{\mathrm{O}}$: the distance between the carbonyl oxygen of amino acid *i*-1 to heavy atom *k*.

$r_{i,k}^{\mathrm{H}}$: the distance between the amide proton H and heavy atom *k*.

*b* : correction factor (set to -0.1).

An analytical relationship is presented for the estimation of NMR $S^2$ order parameters of N-H

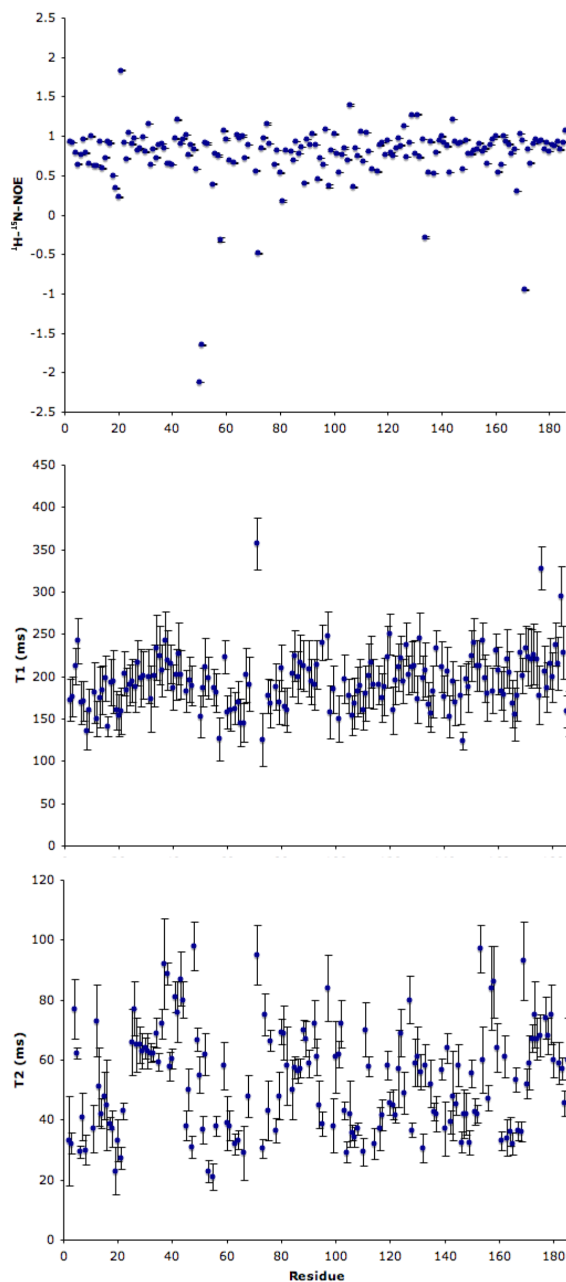


**Figure 1.** $^{15}$N NMR relaxation parameters of PncA. The values of proton-irradiated NOE, $T_1$, and $T_2$ for individual residues are shown as a function of residue number in the protein sequence. Errors in the measured relaxation parameters are also shown.

vectors of the protein backbone from high-resolution protein structures. The relationship solely depends on close contacts of the peptide plane to the rest of the protein. Application of the relationship to a number of proteins with high-resolution X-ray and NMR structures yields $S^2$ values that are in good agreement with the ones determined from experimental relaxation data. Because there is no high-resolution structure of wildtype PncA from M. tuberculosis, NMR $S^2$ order parameter of N-HN vectors of the PncA protein backbone was estimated (Figure 2) from modeled structure using analytical relationship previously reported.[6,13,14]

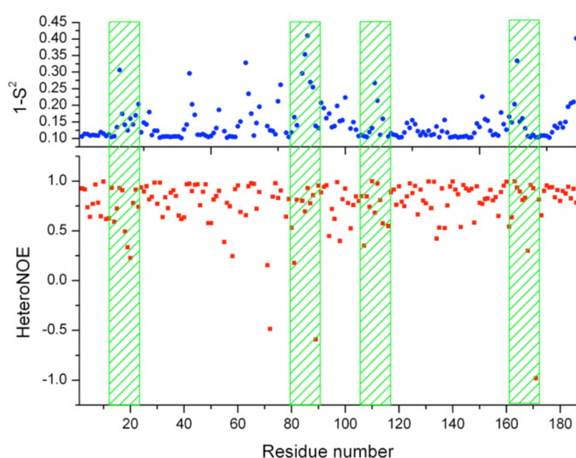


**Figure 2.** Predicted $S^2$ order parameters and $^{15}N$ heteronuclear NOE data of PncA. The regions of high mobility were shown as green-colored box.

Three-dimensional structure of PncA from *M. tuberculosis* was modeled using crystal structure of *Pyrococcus horikoshii* PncA having high sequence identity (37%). Overall global shape of two structures was similar. Secondary structure motif pattern was also similar. Conserved region has high sequence identity and possible active site was well conserved. Possible active site of PncA from *M. tuberculosis* seems to be at almost identical position with that of *P. horikoshii*. Also, catalytic triad and $Zn^{2+}$ binding site of PncA are highly conserved region. The volume of active site was almost the same with two PncA proteins. Compared with PncA from *P. horikoshii*, it is likely that PncA from *M. tuberculosis* could have more flexible residues were existed around active site (Figure 3).

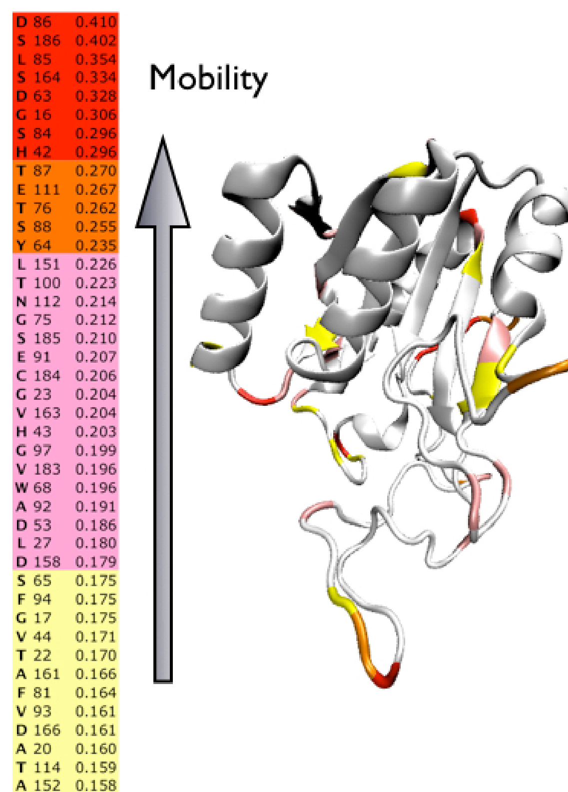


**Figure 3.** The mobility of PncA. The high mobility region was colored as red in box and protein structure. Columns in box represent type of amino acid, residue number, and predicted $1\text{-}S^2$ order parameters, respectively.

The mobile regions from predicted order parameters and $^{15}N$ NMR relaxation data were very similar in four regions in amino residues of PncA including residues 17 - 22, 80 – 90, 105 - 115, and 160 – 171 (Figure 2). The predicted $1\text{-}S^2$ values range from 0.296 to 0.410 in highest mobile region of PncA. Especially, the mobile resides seem to be almost the loop region on the surface of protein (Figure 3). Also, phenylalanine 94, which is the aromatic acid in the active site of PncA, has moderate mobility (0.175 predicted $1\text{-}S^2$ value).

## Discussion

Considering the backbone mobility in the perspective

of the known biological activity of protein is very useful to determine molecular mechanism of protein function. The dynamic properties estimated in this study appear to be consistent with dynamics expected of the amino acids residues such loop region and active site. Because Protein-protein and protein-ligand interaction usually make a decrease in segmental flexibility, suggested method in this study could be used as fast protocol to confirm binding function of protein.

From this perspective, the analysis of key residue of PncA could be possible. H57 in *M. tuberculosis* PncA may play another key role in hydrolysis because H57 was very close to predicted active site and had lower flexibility than other aromatic residues around active site. With aromaticity, H57 may be able to increase orientation effect and stability of transient state. Moreover, H57 has active imidazole ring where there is unpaired electron harboring nucleophilic attack to substrate carbonyl carbon. More accurate high-resolution three-dimensional structure of PncA from *M. tuberculosis* is needed for confirming role of H57 and other aromatic residues around active site.

In conclusion, dynamical properties of PncA from *M. tuberculosis* obtained predicted order parameter and $^{15}$N NMR relaxation, presented the clue to the features of specific enzymatic function and binding information on active site of protein. Also, it is suggested that predicted $S^2$ order parameters could be used to determine mobility of globular protein with ease and fast.

**Acknowledgements**

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A1043288). This study also supported by the Lab. for Structure Based Drug Discovery, College of Pharmacy, Seoul National University.

**References**

1. Z. Bu, and D. J. Callaway, *Adv. Protein Chem. Str.* **83**, 163 (2011)
2. J. S. Fraser, M. M. Clarkson M. W, S. C. Degnan, R. Erion, D. Kern, and T. Alber, *Nature* **462**, 669 (2009)
3. J. G. Torre, P. Bernado, and M. Pons, *Methods Enzymol*. **394**, 419 (2005)
4. G. Lipari, and A. Szabo, *J. Am. Chem. Soc.* **104**, 4546 (1982)
5. A. G. Palmer, J. Williams, and A. McDermott, *J. Am. Chem. Soc.* **100**, 13293 (1996)
6. F. Zhang, and R. Bruschweiler, *J. Am. Chem. Soc*. **124**, 12654 (2002)
7. J. Yi, J. K. Yoo, J. K. Kim, and W. S. Son, *J. Kor. Magn. Reson. Soc.* **17**, 30 (2013)
8. W. Kim, J. Rhee, J. Yi, B. Lee, and W. S. Son, *J. Kor. Magn. Reson. Soc*. **18**, 36 (2014)
9. P. Dosset, J. Hus, M. Blackledge, and D. Marion, *J. Biomol. NMR* **16**, 23 (2000)
10. F. Cordier, M. Caffrey, B. Brutscher, M. A. Cusanovich, D. Marion, and M. Blackledge, *J. Mol. Biol.* **281**, 341 (1998)
11. P. Tsan, J. Hus, M. Caffrey, D. Marion, and M. Blackledge, *J. Am. Chem. Soc.* **122**, 5603 (2000)
12. M. Blackledge, F. Cordier, P. Dosset, and D. Marion, *J. Am. Chem. Soc.* **120**, 4538 (1998)
13. J. L. Goodman, M. D. Pagel, and M. J. Stone, *J. Mol. Biol.* **295**, 963 (2000)
14. A. Sali, and T. L. Blundell, *J. Mol. Biol.* **234**, 779 (1993)