- 1P058 主成分解析を用いたNMR滴定実験のデータ解析
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Principal component analysis of the pH-dependent conformational transitions of bovine β -lactoglobulin monitored by heteronuclear NMR
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To clarify the pH-dependent conformational transitions of proteins, we propose a new approach in which structural changes monitored by HSQC spectroscopy were analyzed using a principal component analysis (PCA). We use bovine β -lactoglobulin (β LG), a protein widely employed in protein folding studies, as a target.

First, we measured HSQC spectra at various pHs, and subjected them to a PCA. The analysis revealed three apparent transitions with pK_as of 2.9, 4.9, and 6.8, consistent with previous reports using different methods. Next, Gdn-HCl-induced unfolding was examined by measuring tryptophan fluorescence at various pHs. Between pH 2 and 8, β LG exhibited a number of structural transitions as well as changes in stability represented by the free energy change of unfolding, ΔG_U .

By combining the NMR and fluorescence results, the change in ΔG_U was suggested to result from the decreased pK_a of some acidic residues. Notably, the native state at neutral pH is destabilized by deprotonation of Glu89, leading to an increase in the relative population of the intermediate. Thus, the PCA of pH-dependent HSQC spectra provides a more comprehensive understanding of the stability and function of proteins.

- 1P059 統合管理型 NMR データ解析ソフトウェア KUJIRA : グラフデータ構造の導入によるインテラクティブな解析
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Interactive and systematic NMR data analysis based on graph data structure on the integrated NMR data analysis platform, KUJIRA.
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As of this fiscal year, our research group has determined more than 1200 protein structures by NMR techniques for the Protein 3000 Project. Our software KUJIRA [1], a package of integrated modules for systematic and interactive analysis of NMR data, has contributed to almost all the structure studies. "Sync-Jump" and "Show Assign" functions are the most important functions of KUJIRA, which allow the user to manipulate a series of 2D spectrum strips extracted from 3D spectra belonging to the same sync-jump class. The functions assist the user to find and correct the problems involved in the assignments of signals readily. The data structure based on graph theory has been widely applied to describe the chemical structure system such as in the software for computer graphics of molecules, in the data base of chemical compounds and so on. We have developed a new function of KUJIRA to generate the data structure based on graph theory for the chemical structure of protein and the simplified magnetization transfer of the NMR experiments. Using the new function, the user can customize any desired type of "Sync-Jump" protocols as well as "Show Assign", which enable to validate the assignments of NMR signals interactively and/or automatically. [1] KUJIRA, a package of integrated modules for systematic and interactive analysis of NMR data directed to high-throughput NMR structure studies. Kobayashi, N, Iwahara, J., Koshiba, S., Tomizawa, T., Tochio, N., Guntert, P., Kigawa, T. and Yokoyama, S. J. Biomol NMR (2007) in press.

- 1P060 3 重共鳴 4 次元 NMR 測定への非線形サンプリングの応用
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Applications of nonlinear sampling scheme for four dimensional triple resonance NMR spectroscopy.
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Despite its potential advantages in analysis, long duration of measurement and limited digital resolution in indirectly observed dimensions prevent 4D NMR experiments from being used in protein NMR projects routinely. In this presentation, we demonstrate the benefits of the nonlinear sampling scheme and 3D maximum entropy processing in 4D triple-resonance experiments.

In the case of 4D HCC(CO)NH experiments on smaller proteins, we measured a data set of 512 (¹H^N) x 16 (¹H) x 10 (¹³C) x 8 (¹⁵N) complex points by employing a nonlinear sampling scheme for indirect dimensions, and succeeded in measuring spectra of equivalent quality with approximately 1/5 duration, when compared with employing a conventional sampling scheme (512 (¹H^N) x 32 (¹H) x 16 (¹³C) x 12 (¹⁵N) complex points). Nonlinear sampling scheme was also proved to be useful for 4D TROSY-HNCOCA experiments on larger proteins. We could obtain almost equivalent spectra only from 512 (¹H^N) complex points x 2048 random sampling points, when compared with conventionally obtained data (512 (¹H^N) x 16 (¹H) x 16 (¹³C) x 8 (¹⁵N) complex points). Further, 4D spectra with much higher resolution were obtained by extending the acquisition time for indirectly observed dimension in combination with nonlinear sampling scheme.

- 1P061 ポンプ・プローブテラヘルツ分光装置の開発と生体高分子の低周波振動と機能研究への応用
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Development of pump-probe THz spectroscopy system and its application to studies on low-frequency vibrations and functions of biomolecules
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It is believed that low-frequency vibrational motions of biomolecules play an important role on their functions. Terahertz (THz) spectroscopy is a suitable method to investigate such vibrational motions. Most of the studies using the THz time domain spectroscopy (THz-TDS) focus on systems in the thermal equilibrium state. However, in order to clarify the effect of the low-frequency motion on biomolecular functions, it is important to investigate the low-frequency motion in the middle of the functional process. The investigation is considered to be possible by pump-probe THz spectroscopy. That is, the low-frequency motion of a biomolecule is probed by the THz field pulse at an arbitrary time after its function is initiated by a laser pulse. Since the THz-TDS is not single-shot measurement, the pump-probe THz spectroscopy using the THz-TDS as a THz probe method is not suitable for samples with their relaxation time longer than the period of a laser pulse repetition. Thus, we have developed a single-shot THz spectroscopy system by using a chirped laser pulse and a multi-channel detector, which was proposed by Jiang et al. (Appl. Phys. Lett. 72 (1998) 1945), and achieved a frequency range and resolution comparable to those by the THz-TDS spectroscopy. Further, we have performed the THz spectroscopy for protein and DNA in order to explore which biomolecule is suited for the pump-probe THz spectroscopy and what the suitable condition of the sample is.