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研究種目：若手研究(B)

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研究課題名(和文)立体構造とアミノ酸判別に基づくNMRシグナル帰属法の開発

研究課題名(英文) A signal assignment method for protein NMR using amino-acid and structural information

研究代表者

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交付決定額(研究期間全体)：(直接経費) 3,300,000円

研究成果の概要(和文)：核磁気共鳴(NMR)法はタンパク質の構造・機能解析に役立つ手法であるが、高分子量タンパク質などではシグナル帰属の困難さが障害となる。本研究では、代表者らが以前開発した、アミノ酸の情報を持たせたタンパク質試料を作製する「符号化標識法」をもとに、立体構造が既知の場合にそれと組み合わせてシグナル帰属を行う方法、2種類の符号化標識法を組み合わせてシグナル帰属を行う方法、および難度の高いタンパク質の解析に資する技術の開発をおこなった。

研究成果の学術的意義や社会的意義








生命の様々な機能にとって重要な役割をもつタンパク質を解析することは、生命を理解しようとする基礎科学から、創薬などの応用まで、幅広い研究分野にとって重要である。本研究は、従来はNMR法による解析が困難であったり、不可能であったりしたタンパク質についても、迅速・簡便にシグナル帰属がおこなえる方法を提供することで、スピードアップをしたり、あるいは可能にしたりすることを目指すものである。

研究成果の概要(英文)：Nuclear magnetic resonance (NMR) is a useful method for structural and functional analysis of proteins. A signal assignment process is a major bottleneck for analysis of difficult targets such as large proteins. In this project, based on our previously reported amino-acid selective isotope labeling method, SiCode (Stable isotope encoding), I developed (1) a signal assignment method using known three-dimensional structures, (2) a signal assignment method using two sets of SiCode samples, and (3) some methods helpful for analysis of difficult target proteins.

研究分野：構造生物学

キーワード：蛋白質 NMR アミノ酸選択的安定同位体標識 符号化標識法 シグナル帰属

A>>7D>>73      >> \*73      A>>73      88

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 H00SQRB5[0      M[0  
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 S[0]     
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(2) 740[0]2bf[0]E[0]  
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 2b[0]  
 9x5[0]AcS3b[0]B[0]A[0]Z[0]0s  
 bT71b#E[0]      NMR M[0]      NMR c[0]pb2b[0]  
 4 N([0]b[0]2[0]      [c[0]b[0]2[0]  
 Q[0]b[0]4(9(52[0]E[0]O[0]pb[0]bg[0]  
 9(52b[0]      NMR b[0] [0] [0] )M[0]2c[0]      E[0]      [0]  
 b[0]b[0]Q[0]      S2[0]  
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 (1) 9(52[0]3Q[0] [0]      NOESY [0]  
 1253[0]b[0]M[0] (bgS4[0])S[0]  
 O[0]b[0]2b[0]M[0]E[0]      X[0])S4[0]  
 2gS4[0] b[0]S[0]      NMR E[0]  
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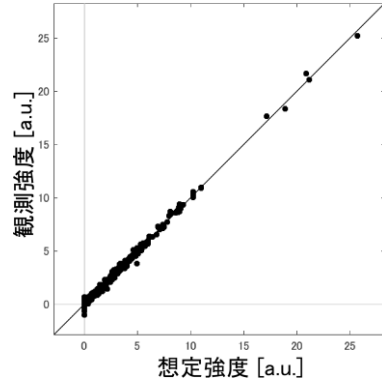
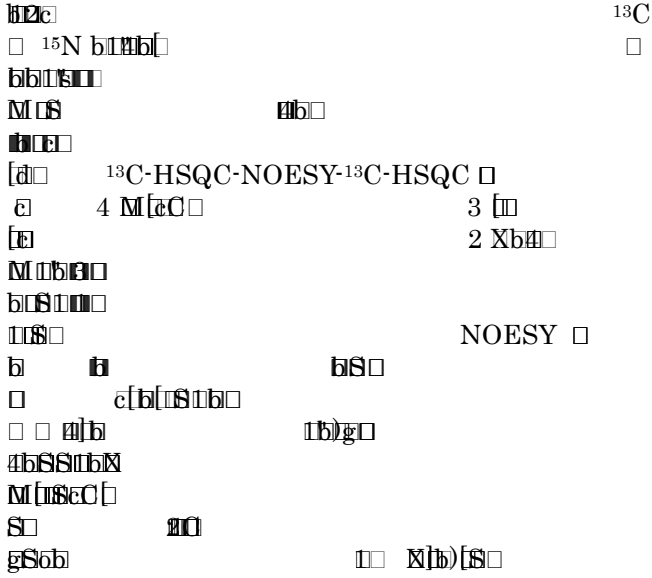
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 4gbSb[0]03[0]      [M[0]2b[0]M[0]c[0]b[0]

3 4g5gM(0b[

(3) Qb9(52e)0beM  
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2>28Y

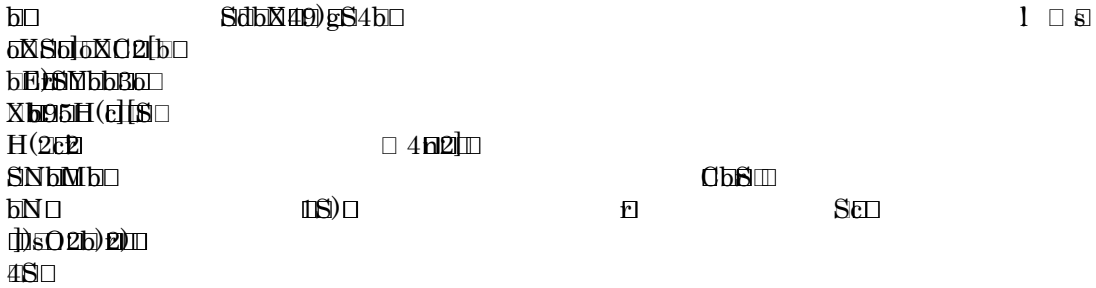
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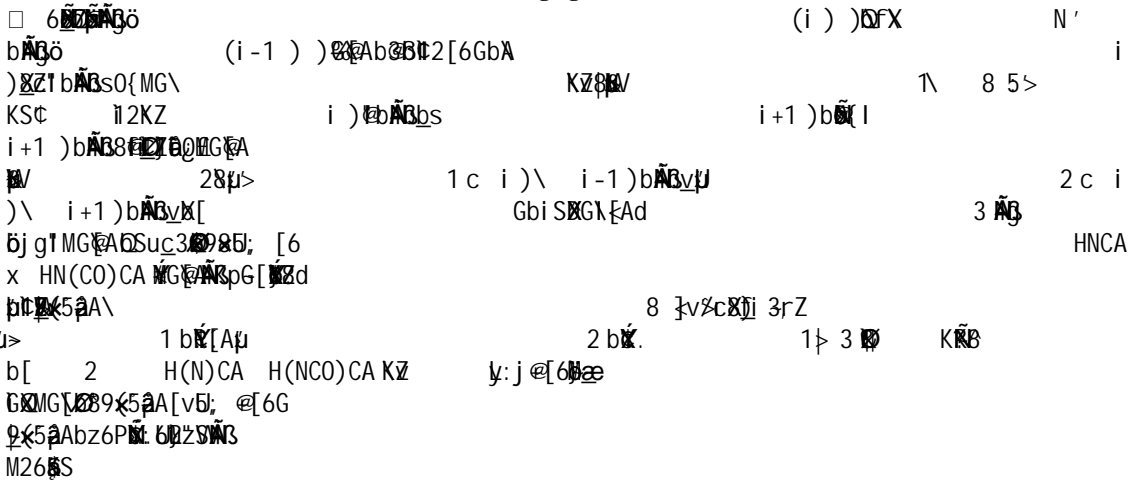
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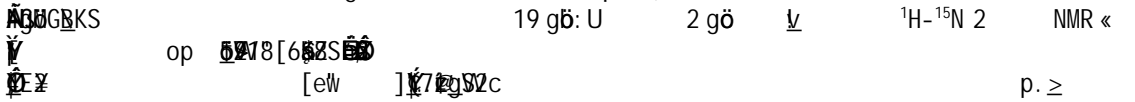
r) S10)hgS4b[



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(3)  $\text{b}007\text{b}0\_ 2(\text{M}/\text{p}6$   
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r S3b8K5AxB0Y8ZMAA ö  
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s Gb2c9K5AxB0Y8ZMAA ö NMR  
 b4: YVZCGXg\*

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 q T. Yamazaki et al., 1991, Biochemistry, 30: 6036-6047.  
 r M. Kainosho, T. Tsuji, 1982, Biochemistry, 21: 6273-6279.  
 s T. Yabuki et al., 1998, J. Biomol. NMR, 11: 295-306.  
 t G. Jaipuria et al., 2012, Adv. Exp. Med. Biol., 992: 95-118.  
 u B. Hoffmann et al., 2018, Prog. Nucl. Magn. Reson. Spectrosc., 105: 1-22.  
 v T. Kasai et al., 2015, J. Biomol. NMR, 63: 213-221.  
 w T. Kasai et al., 2016, J. Phys. Conf. Ser. 699: 012003.

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□ Takuma Kasai, Takanori Kigawa Selective isotope labeling strategy and computational interpretation of spectra for protein NMR analyses Journal of Physics: Conference Series 1w vol. 1036 2018 p.012007 DOI: 10.1088/1742-6596/1036/1/012007

r Kasai T, Kigawa T NMR 00  
 01w vol. 57 2017 pp. 153-156 DOI: 10.2142/biophys.57.153

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u Takuma Kasai, Shunsuke Ono, Toshiyuki Tanaka, Shiro Ikeda, Takanori Kigawa Simultaneous assignment and characterization of proteins by integrated analysis of time-domain NMR data 28th International Conference on Magnetic Resonance in Biological Systems 2018 "

v Kasai T, Kigawa T NMR 00 2018 73B

w Kasai T, Kigawa T in-cell NMR ↑T79(0)RS  
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x Takuma Kasai, Kae Higuchi, Kohsuke Inomata, Takanori Kigawa How to cope with noisy NMR spectra; application of a novel isotope labeling strategy to in-cell NMR □ 55 G 2017 "

- Takuma Kasai, Takanori Kigawa Selective isotope labeling strategy and computational interpretation of spectra for protein NMR analyses International Meeting on High-Dimensional Data Driven Science 2017 "
- z Takuma Kasai, Kae Higuchi, Kohsuke Inomata, Takanori Kigawa Stable isotope labeling strategy based on information science applied to in-cell NMR 20th Meeting of the International Society of Magnetic Resonance 2017 "
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- | Takuma Kasai, Kae Higuchi, Kohsuke Inomata, Takanori Kigawa Signal assignment strategy for protein NMR under challenging conditions ~~██████████~~ 54 G ~~██████████~~ 2016 "
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- ~ Takuma Kasai, Kenji Nagata, Masato Okada, Takanori Kigawa Model Fitting Method for Accurate Analyses of NMR Spectra 27th International Conference on Magnetic Resonance in Biological Systems 2016 "

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