2010 NMR User Training Course I
Advanced NMR Experiments
March 02nd, 2010

10:00-10:50
News and New Experiments in HFNMRC
by Dr. Chi-Fon Chang, HFNMRC, Academia Snica

11:00-12:00
Rapid Data Acquisition of 3D Triple Resonance Experiments by Projection-Reconstruction NMR
by Dr. Winston Wen-Jin Wu, HFNMRC, Academia Sinica

Hands-On 13:30-
Place : B1A Meeting RoomTopics : Projection-Reconstruction Data Processing
Instructors : Dr. Winston Wen-Jin Wu

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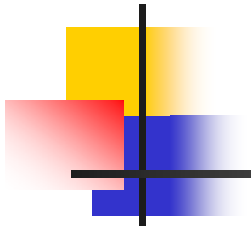
News and New Experiments in HFNMRC

Chi-Fon Chang
HFNMRC, Academia Snica



NEWS in HFNMRC

Part I: Moving 850MHz magnet into HFNMRC

Part II: Testing and Standard Experiments Setting

Part III: What's good about 850MHz?

NEW Experiments in HFNMRC

Part I: Dynamics Related Experiments

(1) Relaxation Dispersion Experiments
- **R1rho measurement**
- **CPMG type**

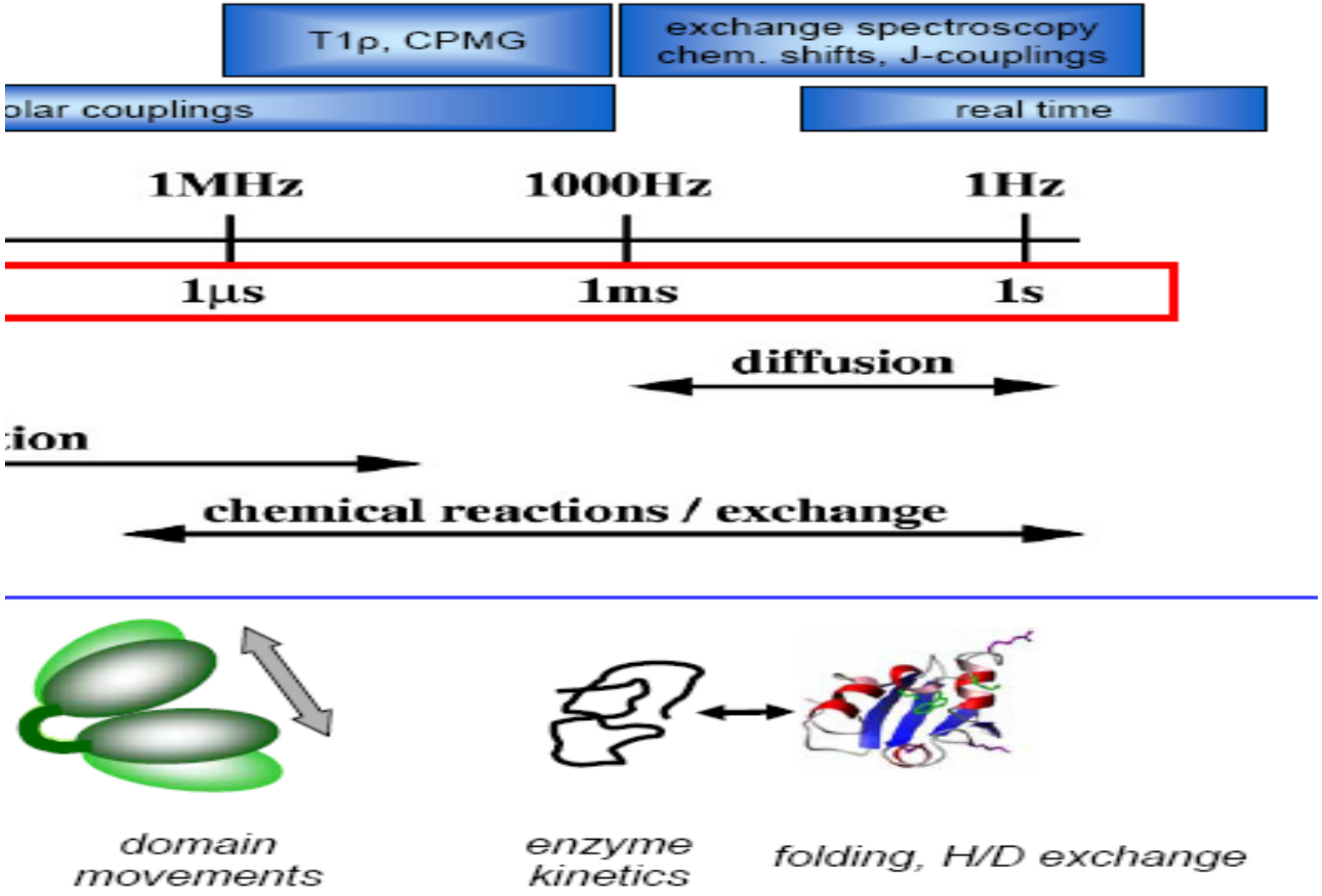
(2) Paramagnetic Relaxation Enhancement Measurement

Part II: Experiments for large proteins

Experiments for Ile, Leu and Val Methyl Assignments

Part I. Dynamics Related Experiments

(1) Relaxation Dispersion Experiments to measure us-ms motion



Why relaxation dispersion?

- Relaxation dispersion monitor dynamics on the range or ms to ms (10^{-6} to 10^{-3}).

- Relaxation dispersion can separate the contribution from exchange between different conformations (Rex) from total R2 relaxation

- Rex is a function of exchange rates, populations and chemical shifts of the different conformations. Thus, give information on the kinetics, thermodynamics, as well as structure of protein substrates. Folding intermediates can be detected as well.

- Parameters that characterize the kinetics of the chemical exchange process are obtained from the variation of **R1rho as a function of ω_e** (effective field in the rotation frame), called **relaxation dispersion**.

- CMPG relaxation experiments monitor the decay of transverse magnetization in a series of spin-echo pulse sequence elements. Chemical exchange is characterized from the variation in the transverse relaxation rate constant , **R2, as a function of the time delay τ_{cp}.** Where an effective field strength for the CPMG experimetn can be defined as $\omega_{cpmg}=12^{1/2}/\tau_{cp}$

What Experiments?

- **CPMG** and **R1rho** experiments have been applied for studying exchange processes that occur in the **microsecond to millisecond** time scale.

- The accessible range of effective magnetic field strengths determines the time scale of the process that can be studied by CPMG and R1rho techniques.

- The effective field strengths typically employed in **CPMG** relaxation experiments are on the order of 25-500 Hz; consequently, experiments are most often used to characterize slower, **millisecond time (ms) scale chemical exchange** processes.

- The effective field strengths typically employed in **R1rho** relaxation experiments are of the order of 1-6 kHz, although weaker fields can be utilized to provide overlap with the CPMG experiment; consequently, R1rho experiments are most often used for faster **microsecond (μs) time scale chemical exchange** processes.

$$R_{1\rho} = R_1 \cos^2\theta + R_2 \sin^2\theta$$ ⋯⋯▶ R2 can be calculated from R1rho and R1

$$R_2 = R_2^0 + R_{ex}$$ ⋯⋯▶ Rex can then be calculated, where R_2^0 is the relaxation rate other than exchange

$$R_{ex} = k_{ex}\Phi_{ex}/(k_{ex}^2 + \omega_e^2)$$

(JACS, 20004, 126, 2247-2256)

(1) Relaxation Dispersion Experiments to measure us-ms motion

(1-1) R1rho relaxation Dispersion Experiments

Reference: Arthur G. Palmer, III* and Francesca Massi, Chem. Rev. 2006, 106, 1700-1719

1700 *Chem. Rev.* **2006,** *106,* 1700–1719

Characterization of the Dynamics of Biomacromolecules Using Rotating-Frame Spin Relaxation NMR Spectroscopy

Arthur G. Palmer, III* and Francesca Massi

Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, New York 10032

Received May 16, 2005

Standard Experiment : std1* Bruker Pulseprogram Library

- Experiment Type: NH HSQC type pseudo3D
- Standard Parameter Set: std1_3D_15N-R1rho_hsqctretf3gpsi3d.2
- Pulse Program: hsqctretf3gpsi3d.2
- Reference: JACS, 124,10743 (2002)

- Easy Set Up Steps:

(1) rpar std1_3D_15N-R1rho_hsqctretf3gpsi3d.2

(2) getprosol 1H (us) (db)

(3) edit vd-list : delay in sec, ex: std_t1rho

(4) NBL: number of delays in vd-list

(5) td1: number of delays in vd-list (QF)

(6) 13C/15N sample: ZGOPTINS -DLABEL_CN

(8) d1

- Note for process:

xau splitpseudo.cf

(1) Relaxation Dispersion Experiments to measure us-ms motion

(1-2) CPMG relaxation Dispersion Experiments

Reference: Dong Long, Maili Liu, and Daiwen Yang, JACS 2008, 130, 2432-2433

J|A|C|S
COMMUNICATIONS

Published on Web 02/05/2008

Accurately Probing Slow Motions on Millisecond Timescales with a Robust NMR Relaxation Experiment

Dong Long,† Maili Liu,‡ and Daiwen Yang*,†

Department of Biological Sciences, 14 Science Drive 4, National University of Singapore, Singapore 117543, and Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan, China, 430071

Received November 20, 2007; E-mail: dbsydw@nus.edu.sg

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: NH TROSY type pseudo3D
- Standard Parameter Set: std2_3D_15N-T2Rex_ trrexetf3gpsi3d_3.cf
- Pulse Program: trrexetf3gpsi3d_3.cf
- Reference: JACS 130, 2432-3 (2008)

- Easy Set Up Steps:

(1) rpar std2_3D_15N-T2Rex_ trrexetf3gpsi3d_3.cf

(2) getprosol 1H (us) (db)

(3) edit vd-list : field strength in Hz, ex: std_Rex

(4) NBL: number of delays in vd-list

(5) td1: number of delays in vd-list (QF)

(6) decide d21 value: length of mixing time , ex: 25ms

(7) 13C/15N sample: ZGOPTINS -DLABEL_CN

(8) d1=2.5sec

- Note for process:

xau splitpseudo.cf

Part I. Dynamics Related Experiments

(2) Paramagnetic Relaxation Enhancement

Reference: G. Marius Clore and Junji Iwahara, Chem Rev. 2009, 019, 4108-4139

4108 *Chem. Rev.* **2009**, *109*, 4108–4139

Theory, Practice, and Applications of Paramagnetic Relaxation Enhancement for the Characterization of Transient Low-Population States of Biological Macromolecules and Their Complexes

G. Marius Clore*,† and Junji Iwahara*,‡

Laboratory of Chemical Physics, Building 5, National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Bethesda, Maryland 20892-0520, and Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas 77555-0647

Received January 26, 2009

Why Paramagnetic Relaxation Enhancement (PRE)?

- Conventional NMR observables are intrinsically short-range nature between "nuclei" and "nuclei". (chemical shifts, scalar coupling, dipolar coupling). The dipolar effect from "electrons" to "nuclei" are much stronger, thus can be detected over much larger distances.

- Paramagnetic relaxation enhancement (PRE) which causes faster relaxation (line broadening) and depends on electron-nucleus distance as $1/r^6$.

- The pseudocontact shift (PCS) which cause chemical shift depends on $1/r^3$.

- The long-range nature of paramagnetic effects permits the determination of large molecular complex structures.

- PRE can also as a probe of large amplitude motions and lowly populated transient intermediates in macromolecualr association.

- Unpaired electrons are introduced into the protein (metal binding protein) by paramagnetic lanthanide metals (鑭系元素), such as Dysprosium (鏑) or Terbium(鋱).

- For non-metal binding protein, paramagnetic are introduced as tags vis disulphide bond to a free CYS (ex: nitroside spin label MTSL or EDTE_Mn2+ or LCTs)

What Experiments?

- Acquire 15N-HSQC of the paramagnetic protein ("oxidized"), and diamagnetic protein ("reduced")

- The peak height in the directly detected proton dimension of the HSQC could be used to determine distance constraint

$$I_{ox} \approx \frac{1}{R_2^*} \qquad I_{red} \approx \frac{1}{R_2} \qquad R_2^* = R_2 + \Delta R_2 \qquad \Delta R2 \text{ :spin label contribution}$$

$$\frac{I_{ox}}{I_{red}} = \frac{R_2 \exp(-\Delta R_2 t)}{R_2 + \Delta R_2} \qquad \Delta R_2 = \Delta(\frac{1}{T_2}) = \frac{K}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_H^2\tau_c^2}\right)$$

Battiste and Wagner, Biochemistry 39, 5355 (2000)

- However, intensity depends not only on the PRE 1H-Γ2 but also 1H-Γ1. For quantitative PRE investigations for macromolecules, measurement actual PRE 1H transverse relaxation rates (1H-Γ_2) rates is required.

$$\Gamma_2 = R_{2,\text{para}} - R_{2,\text{dia}} = \frac{1}{T_\text{b} - T_\text{a}} \ln \frac{I_\text{dia}(T_\text{b})\, I_\text{para}(T_\text{a})}{I_\text{dia}(T_\text{a})\, I_\text{para}(T_\text{b})}$$

T : time interval for 1H-Γ2 measurement

Clore and Iwahara, Chem Rev. 019, 4108-4139 (2009)

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: NH HSQC type
- Standard Parameter Set: std2_2D_15N-T2HnHSQC
- Pulse Program: PRE_HSQC_T2h.cf
- Reference: J. Mag. Res. 184,185-195 (2007)

- Easy Set Up Steps:

(1) rpar std2_2D_15N-T2HnHSQC

(2) getprosol 1H (us) (db)

(3) decide in11: corresponds to deltaT/4 for T2, (ex: 3.5ms)

(4) decide l8: number of different T-delays (ex: 2 for Two points)

(5) Notice that TD for F1 (1TD) is the total TD for all points. For example, TD=256 for 2 T-delays points, then TD=128 for each delay

(6) 13C/15N sample: ZGOPTINS –DLABEL_CN

- Note for process:

xau split (if two points, split → 2)

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: NH TROSY type
- Standard Parameter Set: std2_2D_15N-T2HnTROSY
- Pulse Program: PRE_TROSY_T2h.cf
- Reference: J. Mag. Res. 184,185-195 (2007)

- Easy Set Up Steps:

(1)rpar std2_2D_15N-T2HnTROSY

(2)getprosol 1H (us) (db)

(3)decide vdlist: deltaT/4 for T2 in sec, ex: std_PRE.cf (4us, 3.5ms)

(4)decide l8: number of different T-delays (ex: 2 for Two points)

(5)Notice that TD for F1 (1TD) is the total TD for all points. For example,
TD=256 for 2 T-delays points, then TD=128 for each delay

(6) 13C/15N sample: ZGOPTINS –DLABEL_CN

- Note for process:

xau split (if two points, split → 2)

Part II. Experimetns for large protein

Experiments for Ile, Leu and Val Methyl Assignments

Reference: Vitali Tugarinov and Lewis E. Kay, JACS, 2003, (125),13868-13878

JACS
ARTICLES
Published on Web 10/16/2003

Ile, Leu, and Val Methyl Assignments of the 723-Residue Malate Synthase G Using a New Labeling Strategy and Novel NMR Methods

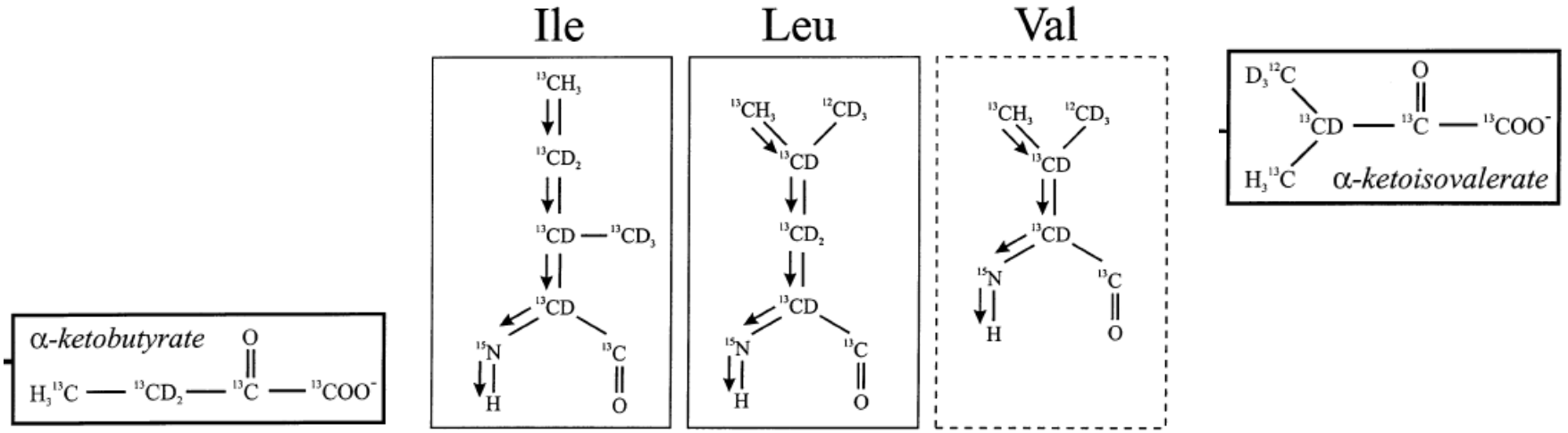
Vitali Tugarinov and Lewis E. Kay*

Contribution from the Protein Engineering Network Centres of Excellence and the Departments of Medical Genetics, Biochemistry, and Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Received June 4, 2003; Revised Manuscript Received July 31, 2003; E-mail: kay@pound.med.utoronto.ca

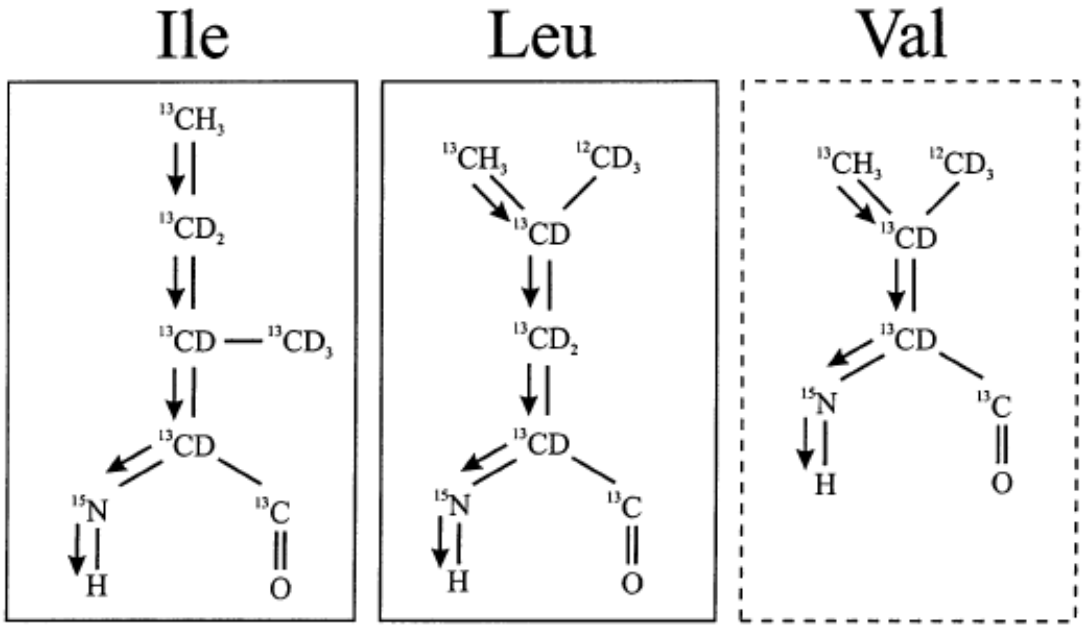
Why Methyl groups?

- Methyl groups give rise to intense correlations and have favorable relaxation properites.
- Methyls are most often localized to hydrophobic cores of proteins so that Methyl constraints from NOESY provide valuable information for structure determination
- Methyls are excellent reporters of dynamics in proteins
- Ile (δ1), Leu, Val –methyl protonated , highly detuerated 15N-13C labeled proteins are available using a pair of precursors to the growth medium, [3-2H], 13C α-ketoisovalerate and [3,3-2H], 13C α-ketobutyrate.



What kind of experiments?

- Cm(i)-N(i)-HN(i) or/and Cm(i)-N(i+1)-HN(i+1)
- Hm(i)-N(i)-HN(i) or/and Hm(i)-N(i+1)-HN(i+1)
- Caliph(i)-Cm(i)-Hm(i)
- CO(i)-Cm(i)-Hm(i).



Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: (Hme)Cme([C]CA)NH
- Standard Parameter Set: std2_3D_CmeNH
- Pulse Program: hmcmcbcanhgpwg3d.cf
- Reference: JACS. 125,13868-13878 (2003)

- Easy Set Up Steps:

(1)rpar std2_3D_CmeNH

(2)getprosol 1H (us) (db)

(3)ZGOPTINS :

No option: all methyl groups

-DLABEL_ALA : methyl groups in Cb position only (Ala)

-DLABEL_VAL : Cb & Cg position (Ala, Val, Ile Cg, Thr Cg)

-DLABEL_CG2: Ile Cd1 only

- Note for process:

SR(F1): (1/4 +n) * SWH(F1), n = number of folding (Cm)

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: (Hme)Cme([C]CACO)NH
- Standard Parameter Set: std2_3D_Cme(CO)NH
- Pulse Program: hmcmcbcaconhgpwg3d.cf
- Reference: JACS. 125,13868-13878 (2003)

- Easy Set Up Steps:

(1)rpar std2_3D_ Cme(CO)NH

(2)getprosol 1H (us) (db)

(3)ZGOPTINS :

No option: all methyl groups

-DLABEL_ALA : methyl groups in Cb position only (Ala)

-DLABEL_VAL : Cb & Cg position (Ala, Val, Ile Cg, Thr Cg)

-DLABEL_CG2: Ile Cd1 only

- Note for process:

SR(F1): (1/4 + n) * SWH(F1), n = number of folding (Cm)

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: Hme(Cme[C]CA)NH
- Standard Parameter Set: std2_3D_HmeNH
- Pulse Program: hmcmcbcanhgpwg3d2.cf
- Reference: JACS. 125,13868-13878 (2003)

- Easy Set Up Steps:

(1) rpar std2_3D_HmeNH

(2) getprosol 1H (us) (db)

(3) ZGOPTINS :

No option: all methyl groups

-DLABEL_ALA: : methyl groups in Cb position only (Ala)

-DLABEL_VAL: : Cb & Cg position (Ala, Val, Ile Cg, Thr Cg)

-DLABEL_CG2: Ile Cd1 only

- Note for process:

SR(F1): (1/4 + n) * SWH(F1), n = number of folding (Hm)

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: (Hme)Cme([C])CA
- Standard Parameter Set: std2_3D_HmeCmeCA
- Pulse Program: hmcmcbcagpwg3d.cf
- Reference: JACS. 125,13868-13878 (2003)

- Easy Set Up Steps:

(1)rpar std2_3D_ HmeCmeCA

(2)getprosol 1H (us) (db)

(3)ZGOPTINS :

No option: all methyl groups

-DLABEL_ALA: methyl groups in Cb position only (Ala)

-DLABEL_VAL: Cb & Cg position (Ala, Val, Ile Cg, Thr Cg)

-DLABEL_CG2: Ile Cd1 only

- Note for process:

SR(F1): (1/4) * SWH(F1) (Ca)

SR(F2): (1/4 + n) * SWH(F2), n = number of folding (Cm)

Standard Experiment : std2* HFNMRC pulseprogram library

- Experiment Type: (Hme)Cme([C]CA)CO
- Standard Parameter Set: std2_3D_HmeCmeCO
- Pulse Program: hmcmcbcacogpwg3d.cf
- Reference: JACS. 125,13868-13878 (2003)

- Easy Set Up Steps:

(1) rpar std2_3D_HmeCmeCO

(2) getprosol 1H (us) (db)

(3) ZGOPTINS :

No option: all methyl groups

-DLABEL_ALA : methyl groups in Cb position only (Ala)

-DLABEL_VAL : Cb & Cg position (Ala, Val, Ile Cg, Thr Cg)

-DLABEL_CG2: Ile Cd1 only

- Note for process:

SR(F2): (1/4 + n) * SWH(F2), n = number of folding (Cm)