



G-NMR: Establishing a Network of German NMR Centers

Projekt-Besprechung in Frauenchiemsee
12. September 2013

Agenda

PLENUM

- Betrieb und Wartung von NMR-Infrastrukturen
 - Beschuß über Nutzerordnung (12:30-13:00) (H. Schwalbe)
 - NMR-Workshops und *Summer Schools* (13:00-13:30) (B. Reif)

BREAK-OUT SESSIONS

- Einheitliche Standard-Proben mit *standard operation procedures* (13:00-14:00)
 - Liquid-State NMR (F. Löhr, G. Gemmecker)
 - Solid-State NMR (B. Reif, C. Glaubitz)
- IT-Netzwerk für Technologie-Transfer (14:00-15:00)
 - Datenspeicherung, Austausch Software / Pulssequenzen (S. Asami, J. Lopez, M. Betz)
 - Unterrichtsmaterialien, Lehrkonzepte (Richter, Gemmecker)

Pauschale Kosten

Routine-NMR (Stundensätze)

NMR Gerät	Feldstärke	NMR Gerät	Feldstärke
≤ 400 MHz		500 – 600 MHz	
5,00 € (15,00 €)		10,00 € (20,00 €)	

„Forschungs“-NMR (Tagessätze)

	Feldstärke 500 – 600 MHz	Feldstärke 700 – 800 MHz	Feldstärke ≥ 900 MHz (bzw. 850 MHz WB)
Nutzungsdauer an NMR-Geräten bis zu 7 Tagen:			
	960 €/Tag (40 €/h)*	1.440 €/Tag (60 €/h)*	1.920 €/Tag (80 €/h)*
Nutzungsdauer an NMR-Geräten zwischen 8 und 20 Tagen:			
Nutzung als wissenschaftliche Zusammenarbeit	800 €/Tag (33,34 €/h)*	1.200 €/Tag (50 €/h)*	1.600 €/Tag (66,67 €/h)*
Nutzungsdauer an NMR-Geräten von 21-31 Tagen (Festpreis)			
	16.000 € (21,51 €/h)**	24.000 € (32,86 €/h)**	32.000 € (43,02 €/h)**



G-NMR: Establishing a Network of German NMR Centers

Experimente und Standards für Lösungs-NMR

Arbeitsgruppen-Besprechung in
Frauenchiemsee
12. September 2013

DFG

Qualitätskontrolle der NMR Spektrometer

- Regelmäßige Durchführung und Dokumentation (z.B. halbjährlich)**
- Gewährleistung gleichbleibender Geräte Leistungsvermögen**
- Nachweis gegenüber externer Benutzer über die Eignung der Spektrometer**

Proben:

- 0.3 - 3% CHCl_3 in Acetone- d_6**
- 0.1% Ethylbenzene in CDCl_3**
- ASTM (60% C_6D_6 / 40% p-Dioxane)**
- 10% Ethylbenzene in CDCl_3**
- 2mM Sucrose in 10% H_2O / 90% D_2O**
- [$^{13}\text{C};^{15}\text{N}$]-Ubiquitin**
- ([$^2\text{H};^{13}\text{C};^{15}\text{N}$]-MBP), optional**



Qualitätskontrolle der NMR Spektrometer

Routine NMR-Spektrometer in der Automation

Verwendung von Bruker Standard Tests

- WS: Wasserunterdrückungstest an 2mM Sucrose, Shim + ^1H -Puls
- Lsrh: Lineshape mit Rotation, Shim
- Lsnh: Lineshape ohne Rotation, Shim + Störungen
- SNH: Protonempfindlichkeit
- SNA: Kohlenstoffempfindlichkeit ohne Entkopplung
- SNC: Kohlenstoffempfindlichkeit mit ^1H -Entkopplung

Messungen unter Verwendung von IconNMR

Auswertung und Dokumentation via Python-Skripte

Beispiel, AV500HD mit BBO-Prodigy:

```
2013-08-09 ws WaterHump: 6.7/21.9 Resolution: 9% S/N: 425:1 P1: 11.2us
2013-08-09 lsnh Hump: 4.7/13.9 Resolution: 0.44
2013-08-09 snh S/N(2ppm): 1278.8:1 S/N(200Hz): 1504.2:1 P1: 11.1us
2013-08-09 sna S/N: 822.3:1 P1: 10.2us
2013-08-09 snc S/N: 879.7:1 P1: 10.2us
```

20/09/2012

Akquisitionsparameter für den Wasserunterdrückungstest

Testprobe: 2mM Sucrose mit 0.5mM DSS, 2mM NaN_3 in 10% D_2O / 90% H_2O (Z10246)

- Temperatur: **298K** (kalibriert mit MeOH d4)
- Pulseprogramm: **zgpr**
- Akquisitionszeit (Aq): **1s**
- TD (^1H) = Abhängig vom Feld
- SW (^1H) = **12ppm**
- Relaxationszeit: d1 = **5s**
- NS = **8**, DS = **4**,
- cw Anregungsfeld: **max. 50Hz**

Prozessierung:

- nur FT, keine window function, kein bcmod, automatic Basislinie Korrektur
- Si = **16K** (wenn TD kleiner als 16k sonst 32k)
- Bestimmung von S/N: au-prog, **suppcal**

20/09/2012

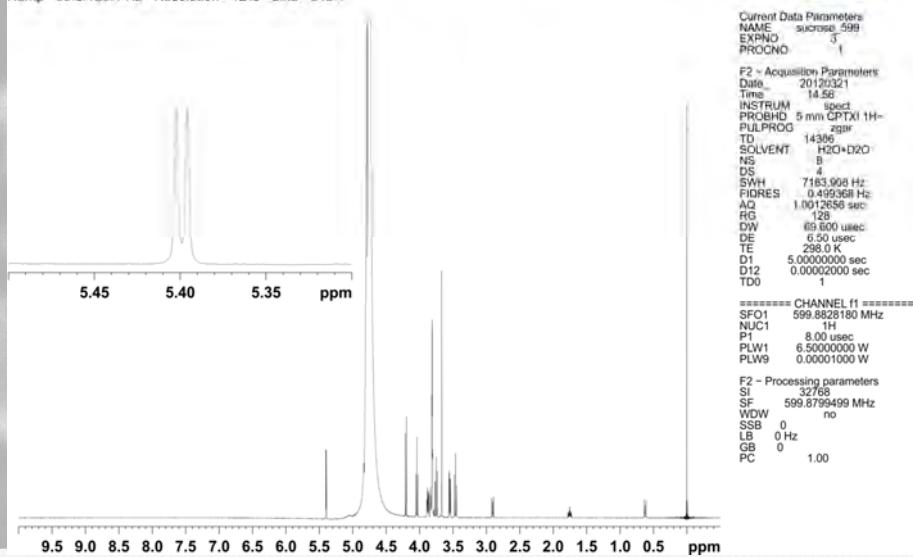
Sucrose: 1D presat. Experiment

(Standard Bruker Test mit 2mM Sucrose, 0.5mM DSS, 2mM NaN₃ in
10% D₂O / 90% H₂O)

- ACCEPTANCE - System: AVII+600 OrderNo.: HH001806 Customer: Uni FRANKFURT Engineer: DGB
P/N Console: H03128/0027 Shim system: BOSS II
Probe: 5 mm CPTXI 1H-15N/13C/D Z-GRD Z44866/0030 Sample depth:21 Gas: O2 depleted air
Water suppression test: Sample: 2 mM Sucrose with 0.5 mM DSS, 2 mM NaN₃ in 10% D₂O and 90% H₂O (P/N: Z10246)
T=298K EU-NMR sample sucrose_599/3 21.3.2012
Hump= 60.9/129.1 Hz Resolution= 12% Sino= 845.1



- Wasserunterdrückung
- Auflösung
- s/n



20/09/2012

Protein Test-Experimente

(1 mM [¹³C/¹⁵N] Ubiquitin in 50mM HEPES pH=7, 10% D₂O)

- 2D [¹⁵N,¹H]-HSQC
- 2D [¹³C,¹H]-HSQC
- 2D [¹³C,¹H] plane of 3D HNCO
- 2D [¹H,¹H] plane of 3D NOESY-[¹³C,¹H]-HSQC
nur bei ≥ 700 MHz
- 2D [¹³C,¹H] plane of 3D (H)CC(CO)NH-TOCSY
nur bei ≤ 800 MHz
- optional: 2D ¹³C-detected CON
nur für entsprechende Probenköpfe

20/09/2012

Globale Parameter für Experimente mit der Ubiquitin-Probe

Temperatur: 298K

Frequenz:

- ^1H = **4.7 ppm** (optimiert)
- ^{13}C (Aliphatic) = **40 ppm**, ^{13}C (CO) = **176 ppm** (Referenziert auf DSS)
- ^{15}N = **118.5 ppm**

sweep widths:

- ^1H = **12ppm**
- ^{13}C (Aliphatic) = **70 ppm**, ^{13}C (CO) = **12 ppm** (**außer für CON**)
- ^{15}N = **40 ppm**

Datenpunkte:

Sollten in allen Dimensionen an die Akquisitionszeit der individuellen Experimente angepasst werden, in Abhängigkeit von der Feldstärke

Relaxationsintervall: d1 = **1s** (außer für **(H)CC(CO)NH-TOCSY**)

gradient shape: p16 = **1ms**, gpnam = **SMSQ10.100** (smoothed square amplitude)

Prozessierung:

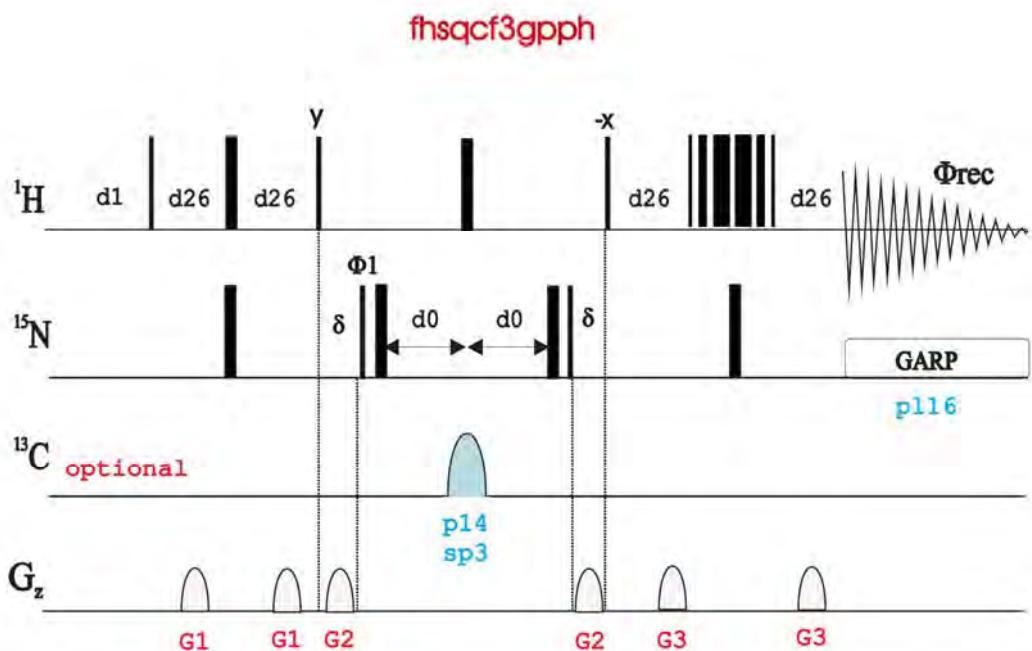
SI in F2 = **2048**, SI in F1 = **1024**

window function: squared sine (**qsine**) shifted by 90° (ssb=**2**)

keine extra Filterfunktion(**bcmode=no**), keine linear prediction,
Basislinie kann verwendet werden wenn nötig

20/09/2012

[$^{15}\text{N}, ^1\text{H}$]-FHSQC, Pulssequenz



20/09/2012

[¹⁵N,¹H]-FHSQC, Akquisitionsparameter



pulse program: **fhsqcf3gpph**

Ref.: S. Mori, C. Abeygunawardana, M. O'Neil-Johnson & P.C.M. van Zijl, J. Magn. Reson. B 108, 94-98 (1995)

Aq (¹H) = **71ms**, Aq (¹⁵N) = **105ms**

SW (¹H) = **12ppm**, SW (¹⁵N) = **40ppm**

NS = **2**, DS = **32**,

Δ (d26) = **2.631ms** (cnst4 = **95 Hz**)

¹H 3919-Watergate: d19 = 2 * DW (Dwell time), p27 = p0 = p1,
p18= p1

¹³C dec.: p14 = **500μs**, Adiabaticpuls = **Crp60,0.5.20.1 or Crp80,0.5.20.1** für hohe Felder

¹⁵N dec.: pcpd3 = **250μs** (@ 600 MHz, zu skalieren in Abhängigkeit von der Feldstärke) CPD_prog = **bi_garp_2pl**

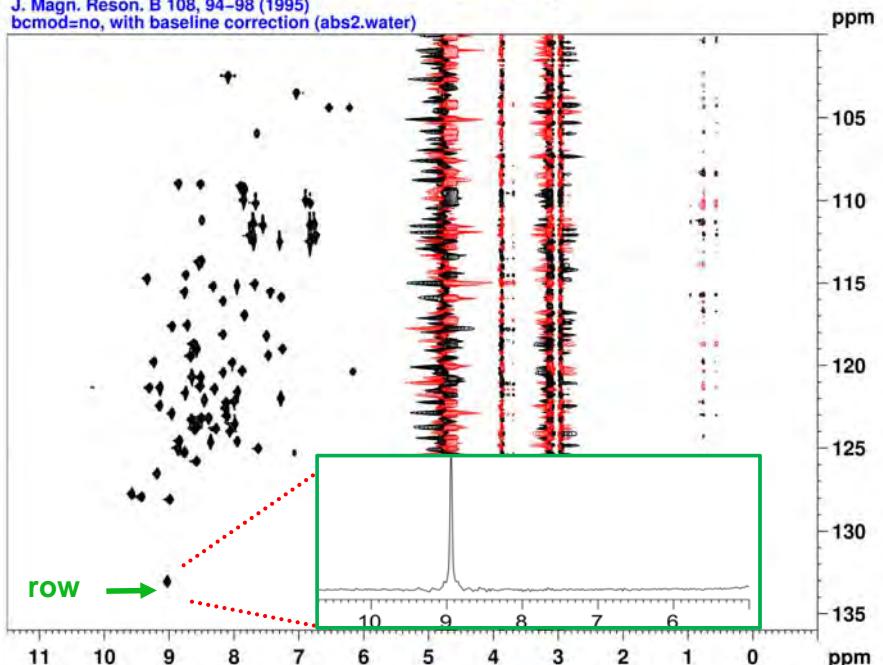
Gradients: G1 = **50%**, G2 = **80%**, G3 = **30%**

20/09/2012

[¹⁵N,¹H]-FHSQC, Spektrum von Ubiquitin

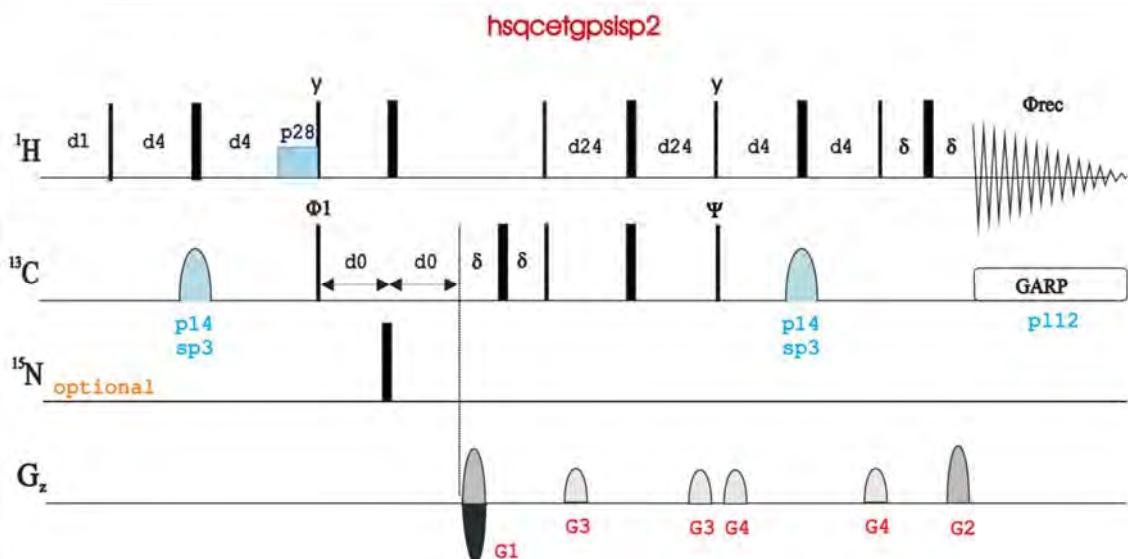


Ubiquitin [μ -¹³C/¹⁵N] ~1mM in 50mM HEPES pH=7 T=298K (sample 2)
600 MHz TCI-Cryoprobe
[¹⁵N,¹H]-HSQC NS=2 TD1=512 SW(F1)=40ppm Av600:ubi_test_eunmr/3 16.01.08
pp=fhsqcf3gpph
S. Mori, C. Abeygunawardana, M. O'Neil-Johnson & P.C.M. van Zijl,
J. Magn. Reson. B 108, 94-98 (1995)
bcmod=no, with baseline correction (abs2.water)



20/09/2012

[¹³C,¹H]-HSQC, Pulssequenz



20/09/2012

[¹³C,¹H]-HSQC, Akquisitionsparameter

pulse program: **hsqctgpsisp2**

Refs: A.G. Palmer III, J. Cavanagh, P.E. Wright & M. Rance, J. Magn. Reson. 93, 151-170 (1991)
L.E. Kay, P. Keifer & T. Saarinen, J. Am. Chem. Soc. 114, 10663-5 (1992)

Aq (¹H) = **71ms**, Aq (¹³C) = **12.1ms**

SW (¹H) = **12ppm**, SW (¹³C) = **70ppm**

NS = **4**, DS = **64**,

D (d4) = **1.72ms** (cnst2 = **145Hz**), \rightarrow d24 = **0.86ms**

no ¹H trim pulse: p28 = **0 μ s**

¹³C: p14 = **500 μ s**, adiabatic pulse = **Crp60,0.5.20.1 or Crp80,0.5.20.1** für hohe Felder

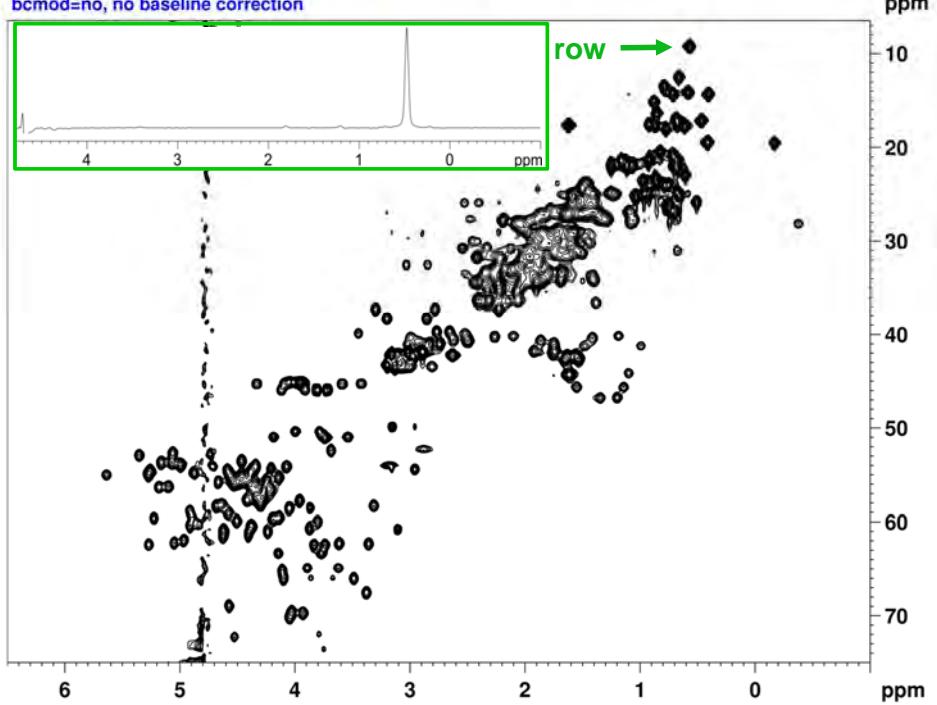
¹³C dec. : pcpd2 = **100 μ s**, (@ 600 MHz, zu skalieren in Abhängigkeit von der Feldstärke), CPD_prog = **bi_garp_4pl**

gradients: G1 = **80%**, G2 = **20.1%**, G3 = **11%**, G4 = **-5%**

20/09/2012

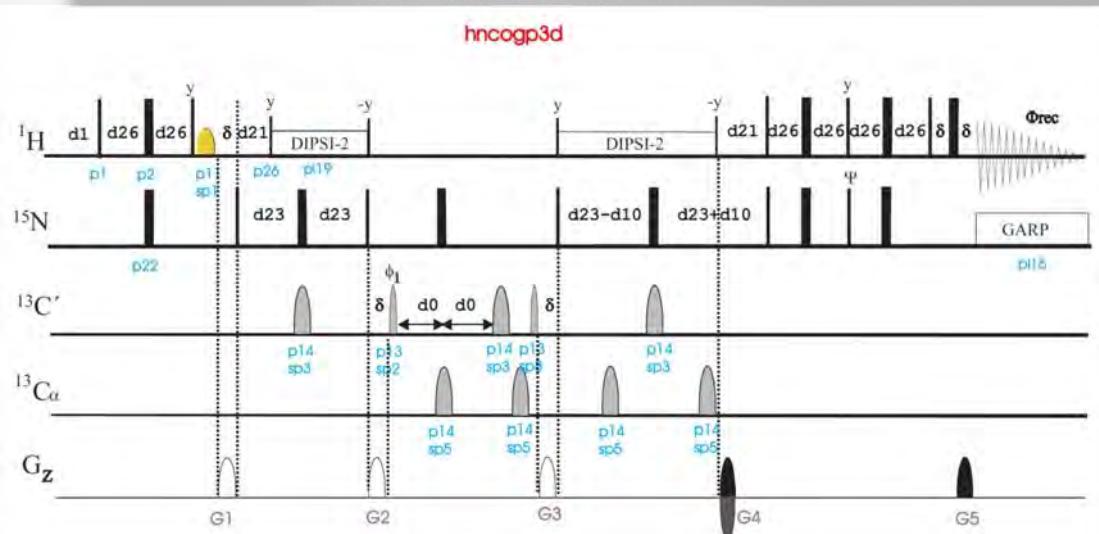
[¹³C,¹H]-HSQC, Spektrum von Ubiquitin

Ubiquitin [¹³C/15N] ~1mM in 50mM HEPES pH=7 T=298K (sample 2)
600 MHz TCI-Cryoprobe
[¹³C,¹H]-HSQC NS=4 TD1=256 SW(F1)=70ppm AV600:ubi_test_eunmr/4 16.01.08
pp=hsqcetgpsisp2
bcmod=no, no baseline correction



20/09/2012

3D HNCO Pulssequenz



20/09/2012

Akquisitionsparameter für 2D H(N)CO

pulse program: **hncogp3d**

Refs: S. Grzesiek & A. Bax, J. Magn. Reson. 96, 432 - 440 (1992)
J. Schleucher, M. Sattler & C. Griesinger, Angew. Chem. Int. Ed. 32, 1489-1491 (1993)
L.E. Kay, G.Y. Xu & T. Yamazaki, J. Magn. Reson. A109, 129-133 (1994)

Aq (¹H) = **71ms**, TD (¹⁵N) = **1**, Aq (¹³C) = **35.3ms**

NS = **4**, DS = **32**,

¹H flip-back: p11 = **1ms**, pulse (spnam1) = **Gaus.1000**

¹H dec. : pcpd = **70μs** (@ 600 MHz, zu skalieren in Abhängigkeit von der Feldstärke), CPD_prog = **dipsi2**

¹³C: sel. 90° pulse: p13 = **320us** (spnam2,8) = **Q5.1000**,
sel. 180° pulse: p14 = **256us** (spnam3,5) = **Q3.1000**

(@ 600 MHz, zu skalieren in Abhängigkeit von der Feldstärke)

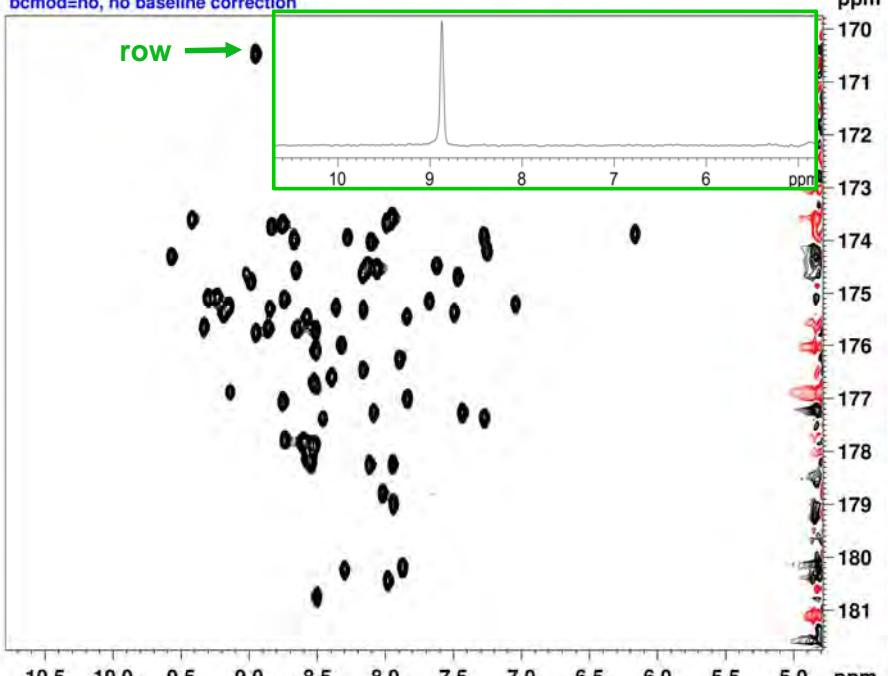
¹⁵N dec. : pcpd3 = **250μs** (@ 600 MHz, zu skalieren in Abhängigkeit von der Feldstärke), CPD_prog = **bi_garp_4pl**

Gradients: G1 = **60%**, G2 = **-40%**, G3 = **10%**, G4 = **80%**, G5 = **8.1%**

20/09/2012

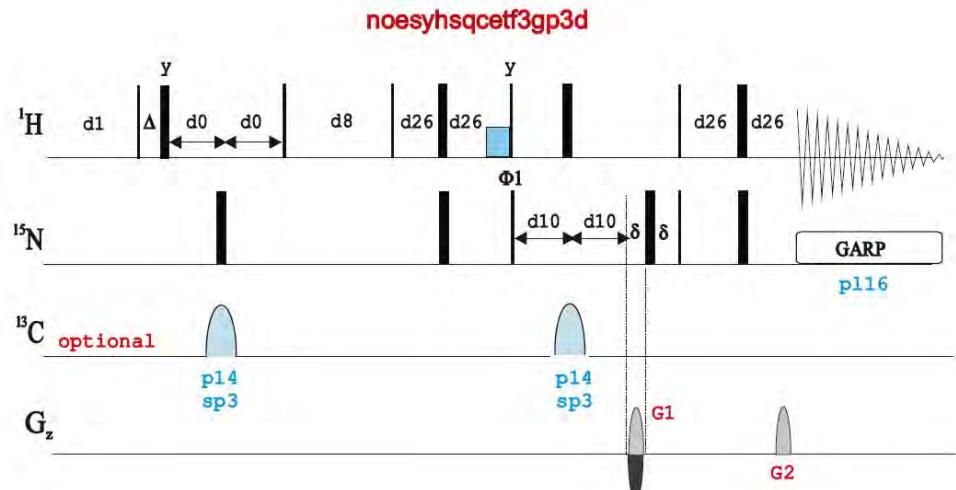
2D H(N)CO Spektrum von Ubiquitin

Ubiquitin [u-¹³C/¹⁵N] ~1mM in 50mM HEPES pH=7 T=298K (sample 2)
600 MHz TCI-Cryoprobe
[¹³C,¹H]-plane-HNCO NS=4 TD1=128 SW(F1)=12ppm AV600:ubi_test_eunmr/8 1
pp=hncogp3d
bcmod=no, no baseline correction



20/09/2012

3D NOESY-[¹³C,¹H]-HSQC Pulssequenz



20/09/2012

Akquisitionsparameter für eine ¹H-¹H plane eines NOESY-[¹³C,¹H]-HSQC

pulse program: noesyhsqctf3gp3d

mit gradient coherence selection, no sensitivity enhancement

Aq (¹H) = 71ms, TD (¹³C) = 1, Aq (¹H indirect) = 17.8ms

SW (¹H) = 12ppm in F1 and F3

NS = 8, DS = 32,

Δ (d4) = 1.72ms (cnst2 = 145Hz)

Mischzeit: d8 = 100ms

no ¹H trim pulse: p28 = 0 μ s

¹³C: p14 = 500 μ s, adiabatic pulse = Crp60,0.5.20.1 or

Crp80,0.5.20.1 für hohe Feldstärken

¹³C dec. : pcpd2 = 100 μ s (@ 600 MHz, zu skalieren mit der

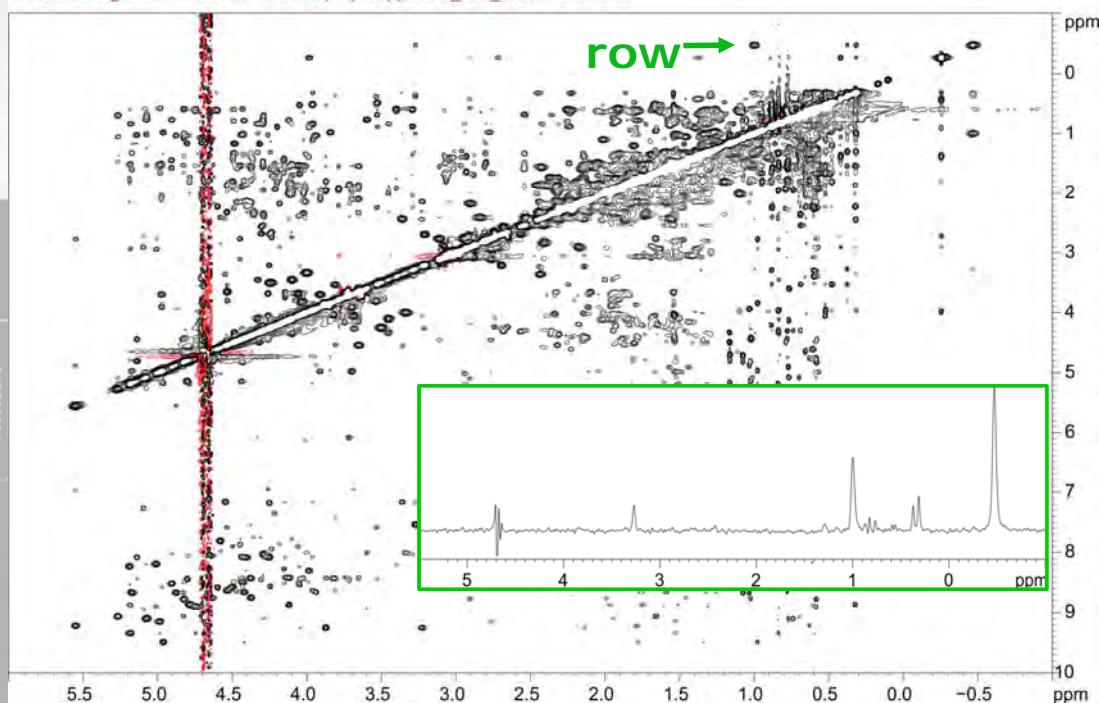
Feldstärke), CPD_prog = bi_garp_4pl

gradients: G1 = 80%, G2 = 20.1%

20/09/2012

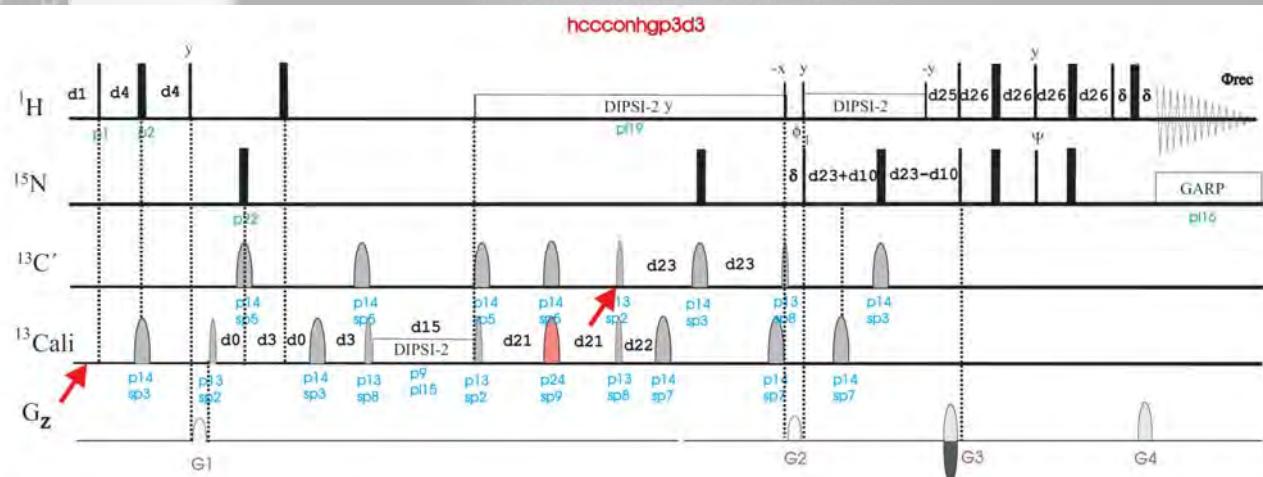
^1H - ^1H plane eines NOESY-[^{13}C , ^1H]-HSQC von Ubiquitin

Ubiquitin [$\text{u}-^{13}\text{C}/^{15}\text{N}$] 1mM in 50mM HEPES pH=7 T=298K
 AV III 950° TCI-HCN Cryoprobe
 2D NOESY-[$^{13}\text{C},\text{H}$]-HSQC (1H-1H plane) gradient-selected, non-sensitivity enhanced version
 100ms mixing time NS=8 TD1=406 SW(F1)=12ppm ubi_test_950/2 14.5.2012



20/09/2012

3D (H)CC(CO)NH-TOCSY Pulssequenz



20/09/2012

Akquisitionsparameter für ein 2D (H)CC(CON)H-TOCSY

pulse program: **hccconhgp3d3**

Refs: G.T. Montelione, B.A. Lyons, S.D. Emerson & M. Tashiro, J. Am. Chem. Soc. 114, 10974-75 (1992)

S. Grzesiek, J. Anglister & A. Bax, J. Magn. Reson. 101 B, 114-9 (1993)

B.A. Lyons & G.T. Montelione, J. Magn. Reson. 101 B, 206-9 (1993)

T.M. Logan, E.T. Olejniczak, R.X. Xu & S.W. Fesik, J. Biomol. NMR 3, 225-31 (1993)

R.T. Clowes, W. Boucher, C.H. Hardman, P.J. Domaille & E.D. Laue, J. Biomol. NMR 3, 349-354 (1993)

Aq (¹H) = **71ms**, TD (¹⁵N) = **1**, Aq (¹³C) = **6ms**

relaxation delay (d1) = **2s**, NS = **8**, DS = **64**,

CC-TOCSY: mixing time: d15 = **21ms**, pulse length: p6 = **32μs**
(@ 600 MHz, should be scaled according to the field)

¹H dec. : pcpd = **70μs** (@ 600 MHz, should be scaled according to the field), CPD_prog = **dipsi2**

¹³C: sel. 90° pulse: p13 = **320us** (spnam2,8) = **Q5.1000**,
sel. 180° pulse: p14 = **256us** (spnam3,5) = **Q3.1000**
sel 180°(CA) pulse p24 = **320μs** (spnam9) = **Q3.1000**
(@ 600 MHz, should be scaled according to the field)

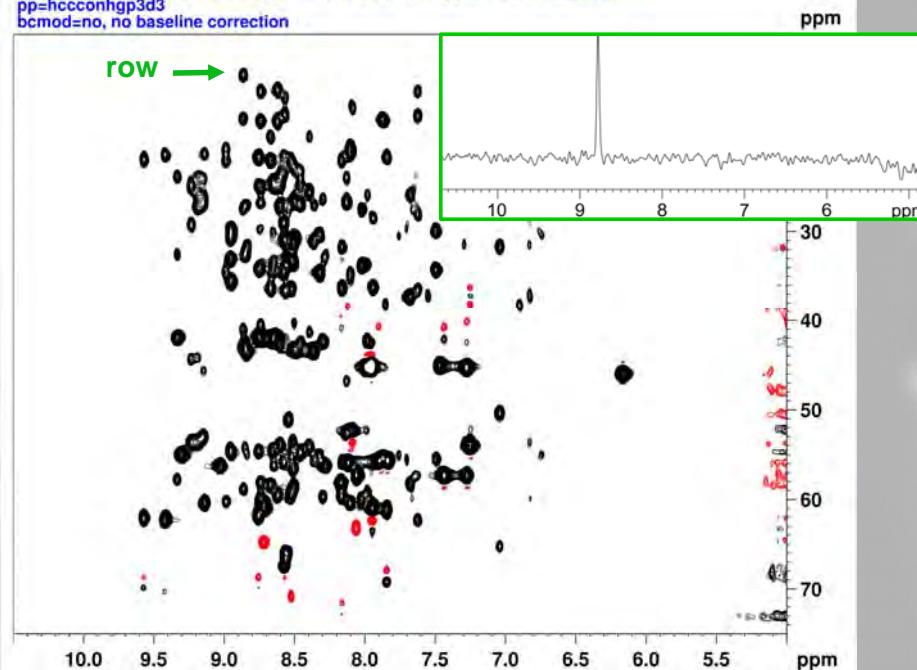
¹⁵N dec. : pcpd3 = **250μs** (@ 600 MHz, should be scaled according to the field), CPD_prog = **bi_garp_4pl**

gradients: G1 = **50%**, G2 = **-30%**, G3 = **80%**, G4 = **8.1%**

20/09/2012

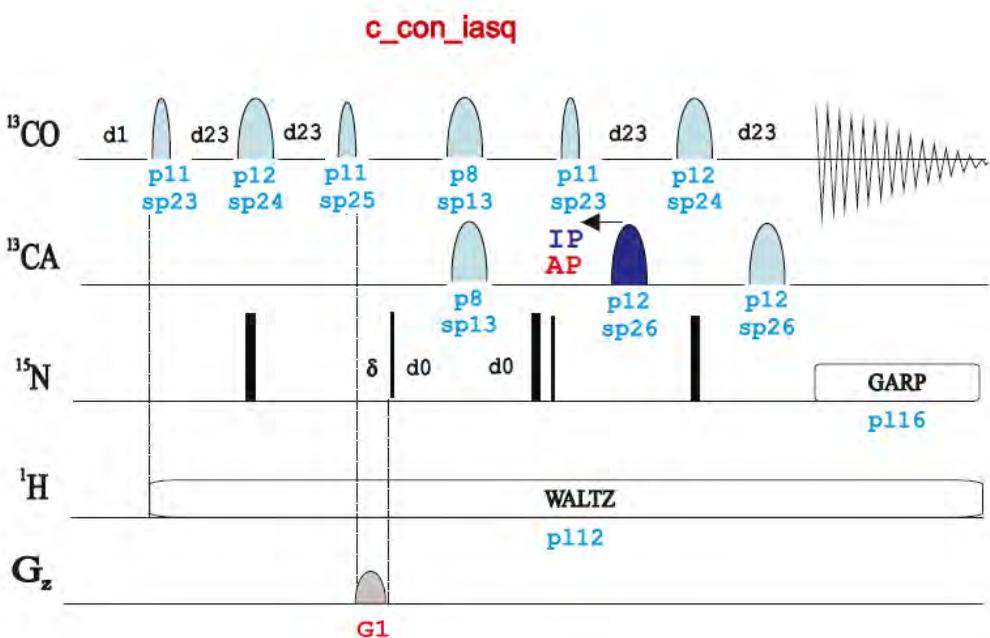
2D (H)CC(CON)H-TOCSY plane von Ubiquitin

Ubiquitin [¹³C/¹⁵N] ~1mM in 50mM HEPES pH=7 T=298K (sample 2)
600 MHz TCI-Cryoprobe
[¹³C,¹H]-plane-HCCCONH NS=8 TD1=128 SW(F1)=70ppm AV600:ubi_test_eunm
pp=hccconhgp3d3
bcmod=no, no baseline correction



20/09/2012

¹³C-Detektion: 2D CON Pulssequenz (optionales Experiment)



20/09/2012

¹³C-Detektion: Akquisitionsparameter

pulse program: **c_con_iasq** (uses IPAP to “decouple” ¹³Ca in F₂)

Refs: W. Bermel, I. Bertini, L. Duma, I.C. Felli, L. Emsley, R. Pierattelli & P.R. Vasos, Angew. Chem. Int. Ed. 44, 3089-3092 (2005)
L. Duma, S. Hediger, A. Lesage & L. Emsley, J. Magn. Reson. 164, 187-195 (2003)

SW (¹³C) = **20ppm**, SW(¹⁵N) = **40ppm**

Aq (¹³C) = **106ms**, Aq (¹⁵N) = **52.5ms** (TD1=**256*2**, IP and AP recorded interleaved, use au-program splitcomb for processing)

NS = **8**, DS = **64**,

¹H dec. : pcpd = **70μs** (@ 600 MHz, should be scaled according to the field), CPD_prog = **waltz65**

¹³C: sel. 90° pulse: p11 = **320us** spnam23,25 = **Q5.1000, Q5tr.1000**
sel. 180° pulse: p12 = **250us** spnam24,26 = **Q3.1000**
(@ 600 MHz, should be scaled according to the field)
broadband (¹³C^a + ¹³CO) decoupling in F1: p8 = **500us**
(adiabatic pulse) spnam13 = **Crp60,0.5,20.1**

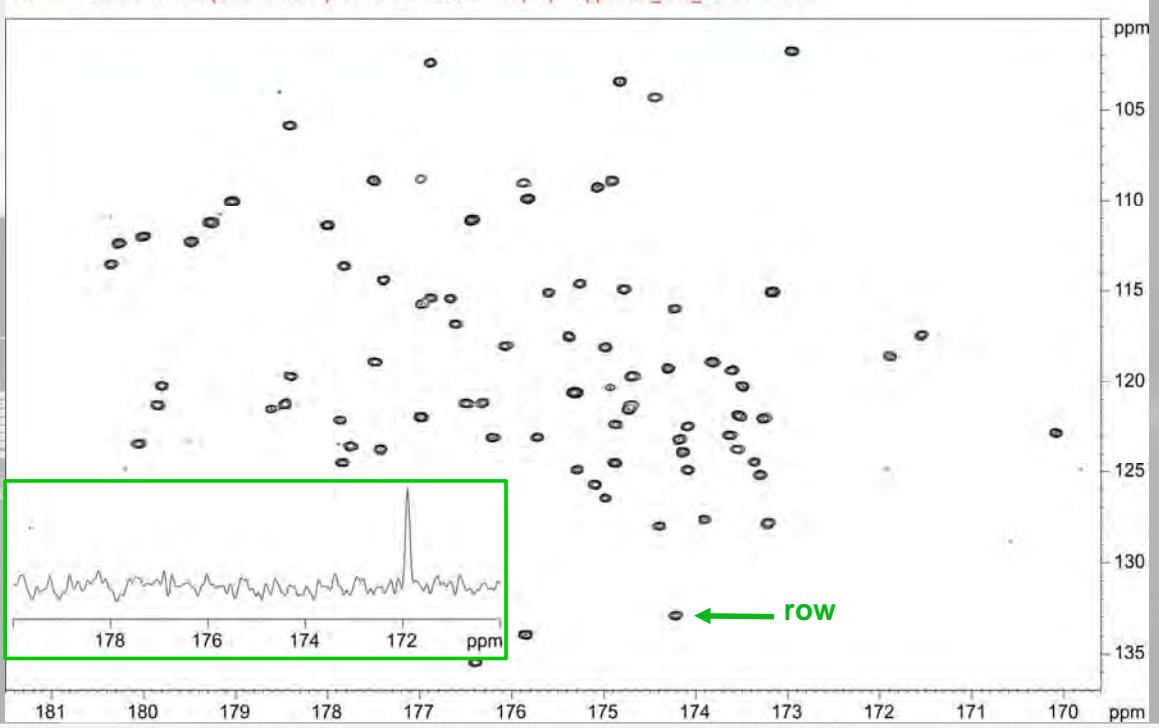
¹⁵N dec. : pcpd3 = **300μs** (@ 600 MHz, should be scaled according to the field), CPD_prog = **garp4**

gradient: G1 = **50%**

20/09/2012

¹³C-Detektion: 2D CON Spektrum von Ubiquitin

Ubiquitin [$\text{u-}^{13}\text{C}/^{15}\text{N}$] 1mM in 50mM HEPES pH=7 T=298K
AV II 600 TCI-HCN Cryoprobe
2D ¹³C-detected CON (Ca-CO IPAP) NS=8 TD1=256+2 SW(F1)=40ppm ubi_test_600/2 2.4.2012



20/09/2012

Workshops Biological NMR spectroscopy

Solution-State NMR

Bi-Annual EMBO Summer Schools

Theoretical Basis: Oschkinat/Griesinger/Boelens (Il Ciocco/Berlin), since

Hands-On: Sattler/Grzesiek (Basel/Garching), since 1999

Solid-State NMR

European School on Solid-State NMR, Advanced Level – Biological Solids

(Oschkinat/Nielsen/de Groot/Meier/Reif)

29.05.-02.06.2006, Brückentinsee

01.07.-06.07. 2008, Aarhus

28.05.-02.06.2010, Leiden

07.-12.10.2012, Brno

2014, Garching

Strategy for National (Bio-) NMR Workshops

Fundamental Principles

Product Operators

Dipolar Coupling / CSA Hamiltonian

Relaxation

NUS, Selective Pulses, Opt Contr

Advanced Methods:

Solution-State NMR

RDCs, TROSY, CPMG

Assignment Strategies

Advanced Methods:

Solid-State NMR

MAS, R2, R3, CP, Decoupling

REDOR, C7, FSLG

Assignment Strategies

Hands-On:

Solution-State NMR

Preparation of aligned samples,

Implementation of 3D and 4D pulse schemes

Hands-On:

Solid-State NMR

SIMPSON, Spinevolution, Shapes, CRAMPS

DNP (Theory+Praxis)

Hands-On

NMRpipe, Sparky, CCPN -> Assignment,

Structure Calculation (XPLOR-CNS, DYANA)

Potential German Partners (Solid-State NMR)

Solid-State NMR

Clemens Glaubitz, Frankfurt
Bernd Reif, Garching
Hartmut Oschkinat, Berlin
Adam Lange, Göttingen
Henrike Heise, Jülich
Gerd Buntkowski, Darmstadt
Daniel Huster, Leipzig
Anne Ulrich, Karlsruhe

Solution-State NMR

...

Jörg Matysik, Leipzig
Jörn Schmedt auf der Günne, Gießen
Ulrich Scheler, Dresden
Eike Brunner, Dresden
Hellmut Eckert, Münster

Kay Saalwächter, Halle

Potential Standard Samples for (bio)-solid-state NMR

- 1) alpha-glycine
- 2) U-13C,15N MLF (to be synthesized by China-Peptide)
- 3) N-Ac-Val.Leu-OH

