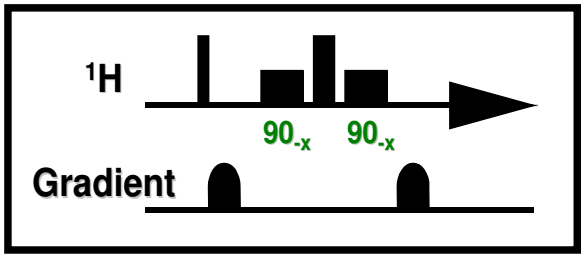
Bruker pulse programs and pulse programming 2004

Example: pulse program nomenclature

cbcaconhgp3d	S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993) with gradients
cbcaconhgpwg3d	S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993) watergate
cbcanhgp3d	S. Grzesiek & A. Bax, J. Magn. Reson. 99, 201-207 (1992)
cbcanhgpwg3d	S. Grzesiek & A. Bax, J. Magn. Reson. 99, 201-207 (1992)
ccaconhgp2h3d	S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993) 2H-decoupled
ccaconhgp3d	S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993)
ccaconhgp3d.2	S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993)
ccanhgp2h3d	(S. Grzesiek & A. Bax, J. Magn. Reson. 99, 201-207 (1992)) S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993)
ccanhgp3d	(S. Grzesiek & A. Bax, J. Magn. Reson. 99, 201-207 (1992))
ccanhgp3d	S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993)
ccanhgp3d.2	(S. Grzesiek & A. Bax, J. Magn. Reson. 99, 201-207 (1992)) S. Grzesiek & A. Bax, J. Biomol. NMR 3, 185-204 (1993)
ccconhgp2h3d	S. Grzesiek, J. Anglister & A. Bax, J. Magn. Reson. 101 B, 114-9 (1993)
ccconhgp3d	S. Grzesiek, J. Anglister & A. Bax, J. Magn. Reson. 101 B, 114-9 (1993)
clmlevphpr	A. Bax & D.G. Davis, J. Magn. Reson. 65, 355-360 (1985) presaturation
cosydclrqf	A. Bax & R. Freeman, J. Magn. Reson. 44, 542 (1981) magnitude mode
cosyjdqf	A. Bax & R. Freeman, J. Magn. Reson. 44, 542 (1981) homonuclear j-decoupled
cosylrqf	A. Bax & R. Freeman, J. Magn. Reson. 44, 542 (1981)
cosyqfr2	A. Bax & G. Drobny, J. Magn. Reson. 61, 306 (1985) two-step relay
cosyqfr3	A. Bax & G. Drobny, J. Magn. Reson. 61, 306 (1985)
cosyqfrl	A. Bax & G. Drobny, J. Magn. Reson. 61, 306 (1985)
hncogpia	M.Ottiger, F. Delaglio & A. Bax, J. Magn. Reson. 131, 373-378 (1998) IPAP
hncacbgpjc3d	J.-S. Hu & A. Bax, J. Biomol. NMR 11, 199-203 (1998) j-coupling

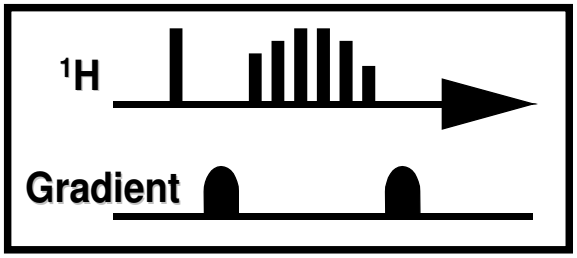
Pulse sequences with water suppression

Watergate



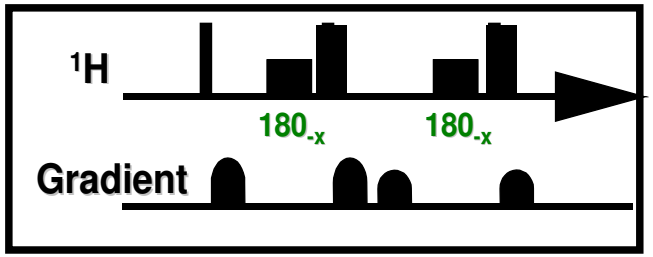
J.Biomol.NMR 2(1992)661

3-9-19



Sklenár JMR 102(1993)244

Excitation sculpting



Shaka JMR A 112(1995)275

1D-pulse programs

zggpwg	***p3919gp***	***zgesgp***
denoted by		
„wg“	***„19“ or „w5“***	***„es“***

for example ***cosydfesgpph***
dipsi2esgpph
mlevesgpph
noesyesgpph
roesyesgpph

The file Pulprog.info

- explains the two-character codes used in Bruker pulse program nomenclature

For a pulseprogram the first characters (usually up to 6, but sometimes more) specify the type of experiment, e.g. DEPT, COSY, NOESY etc.. Further properties of the pulseprogram are indicated by a two-character code, which is added to the name in alphabetical order. For 2D experiments the mode (absolute value, phase sensitive or echo-antischo) is always indicated. H- or X-decoupling is assumed to be default for heteronuclear experiments, but not for homonuclear ones (except inad).

ar experiment for aromatic residues
at adiabatic TOCSY
bi with bird pulse for homonuclear J-decoupling
bp using bipolar gradients
cc cross correlation experiment
cp with composite pulse
ct constant time
cw decoupling using cw command
dc decoupling using cpd command
df double quantum filter
di with DIPSI mixing sequence

etc.

Pulse Programs: where to find the files

Editing a pulse program:

edpul ***(list of all shown)***
edcpul ***(edit current pulse program)***

How to find information about pulse programs:

--> ***edpul *info***

Param.info ***parameters used for pulse programs***
Pulprog.info ***nomenclature of pulse program names***
Update.info ***information about changes in pulse program library***

The file Param.info

- contains the Bruker conventions for power levels, pulses, delays and loop counters throughout the pulse programs

;pl0 :
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl4 : f4 channel - power level for pulse (default)
;pl5 : f5 channel - power level for pulse (default)
;pl6 : f6 channel - power level for pulse (default)
;pl7 : f7 channel - power level for pulse (default)
;pl8 : f8 channel - power level for pulse (default)

;pl9 : f1 channel - power level for presaturation
;pl10: f1 channel - power level for TOCSY-spinlock
;pl11: f1 channel - power level for ROESY-spinlock

;pl12: f2 channel - power level for CPD/BB decoupling
;pl13: f2 channel - power level for second CPD/BB decoupling
;pl14: f2 channel - power level for cw decoupling
;pl15: f2 channel - power level for TOCSY-spinlock

;pl16: f3 channel - power level for CPD/BB decoupling

Identifiers for pulse duration and power

The observe channel is called the F1-channel

Default hard pulses on the observe (=transmitter) channel:

power level	**pl1**
90° pulse	**p1**
180° pulse	**p2**

Further important pulses applied via the transmitter channel:

- ***solvent presaturation***

power level	**pl9**

- ***Proton TOCSY spinlock***

powerlevel	**pl10**
90° pulse	**p6**

- ***ROESY spinlock***

power level	**pl11**
length of spinlock	**p15**

complete list in: /u/exp/stan/nmr/lists/pp/Param.info

Identifiers for pulse duration and power

F2-channel	power level	pl2
	90° pulse	p3
	180° pulse	p4
	decoupling program	cpdprg2
	90° dec. pulse	pcpd2
	dec. power level	pl12
F3-channel	power level	pl3
	90° pulse	p21
	180° pulse	p22
	decoupling program	cpdprg33
	90° dec. pulse	pcpd3
	dec. power level	pl16
F4-channel	decoupling program	cpdprg4
	90° dec. pulse	pcpd4
	dec. power level	pl17

Identifiers for pulse duration and power

Selective decoupling

decoupling program	mlevsp180
90° dec. pulse	pcpd2
dec. power level	pl12=sp15
selective pulse	spnam15
selective pulse offset	spoff15

Adiabatic decoupling

decoupling program	p5m4180
90° dec. pulse	pcpd2
dec. power level	pl12=sp15
selective pulse	spnam15

Identifiers for shaped pulses and gradients

Shaped pulse

power level	sp1	
shape	spnam1	
offset	spoff1	offset in Hz from middle of spectrum
phase	spoal1	zero order phase correction
		set to 0 for "pure phase" shapes

Note: - 2us before and after the shape are needed

- 3us is needed between back-to-back shapes (e.g. in cpd-programs)

- not needed for so called fastshapes (their duration per point is longer)

Gradient pulse

length	p16
strength	gpz1 (gpx1, gpy1)
shape	gpznam1

multiplier for gradient strength p16:gp1*EA <- igrad EA

(invert gradient sign - in echo-antiecho gradient selection)

Include files

Include files contain definitions / commands used by pulse programs,such as definitions of fixed delays or lengthy pulse program parts.

Where to find files under UNIX: (?)

/u/exp/stan/nmr/lists/pp

Which files do exist?

Avance.incl	**general definitions**
Delay.incl	**fixed delays as used in triple resonance**
Grad.incl	**definitions for GRASP**
Daz.incl	**definitions for DANTE-Z**
Solids.incl	**definitions for solids spectrometer**
System.incl	**configuration of 2H channel ('historical')**

Relation files

The relation files translate the pulse names in the PROSOL table to the pulse programming syntax used in the standard pulse program library.

Where to find the files:

/TOPSPINhome/conf/instr/spect/prosol/relations/
/TOPSPINhome/prog/tcl/libtix/prosol/lib/lists/paramDescr

Which files exist:

default	**general definitions**
triple	**syntax used in triple resonance**
lcnmr	**definitions for lcnmr**

Example of include and relation files

Programmer's File Editor

File Edit Options Template Execute Macro Window Help

invit2etf3gpsi

```
;invit2etf3gpsi
;avance-version (00/10/05)
;2D H-1/X correlation via double inept transfer
;   using sensitivity improvement
;for measuring N-15 T2 relaxation times
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition
;using f3 - channel
;using flip-back pulse

prosol relations=<triple>

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

"p2=p1*2"

"p22=p21*2"

"d0=3u"

"d11=30m"

"d12=20u"

"d24=1s/(cnst4*cnst11)"

"d25=1s/(cnst4*cnst12)"
```

pulse program

U:\XWIN-NMR\prog\tcl\libtix\prosol\lib\lists\triple

```
#../conf/instr/spect/prosol/relations/triple
#date: 23.10.2001 From Korea with.....
#
P[0]=P90[F1];                          # 90 deg pulse F1, 1H
P[1]=P90[F1];                          # 90 deg pulse F1, 1H
P[2]=P90[F1]*2;                        #180 deg pulse F1, 1H
P[3]=P90[F2];                          # 90 deg pulse F2, 13
P[4]=P90[F2]*2;                        #180 deg pulse F2, 13
P[5]=PTOC[F1]*0.66;                    # 60 deg pulse F1, 1H
P[6]=PTOC[F1];                         # 90 deg pulse F1, 1H
P[7]=PTOC[F1]*2;                       #180 deg pulse F1, 1H
P[8]=PSH3[F2];                         #adiabatic 180 F2, 13
P[9]=PTOC[F2];                         # 90 deg pulse F2, 13
P[10]=PTOC[F2]*2;                      #180 deg pulse F2, 13
P[11]=PSH8[F1];                        # flip-back pulse, F1
P[12]=PSH8[F1]*2;                      #180 deg sel. F1, 1H
P[13]=PSH4[F2];                        # Cali sel. 90 deg, F
P[14]=PSH6[F2];                        # Cali sel.180 deg, F
P[15]=TROE[F1];                        # ROESY
#
#
P[18]=PSH7[F1];                        # off-res presat, F1,
#
#P[20]=                                # trim pulse parset,
P[21]=P90[F3];                         # 90 deg pulse F3, 15
P[22]=P90[F3]*2;                       #180 deg pulse F3, 15
P[23]=PSH7[F2];                        # Calpha sel.  90 deg
P[24]=PSH9[F2];                        # Calpha sel. 180 deg
P[25]=PSH11[F2];                       # Calpha sel. 2 90deg
P[26]=PCPDP[F1];                       # 90 deg pulse F1, 1H
P[27]=P90[F1];                         # WATERGATE pulse, F1
#
P[29]=PSH9[F1];                        # flip back pulse 2
```

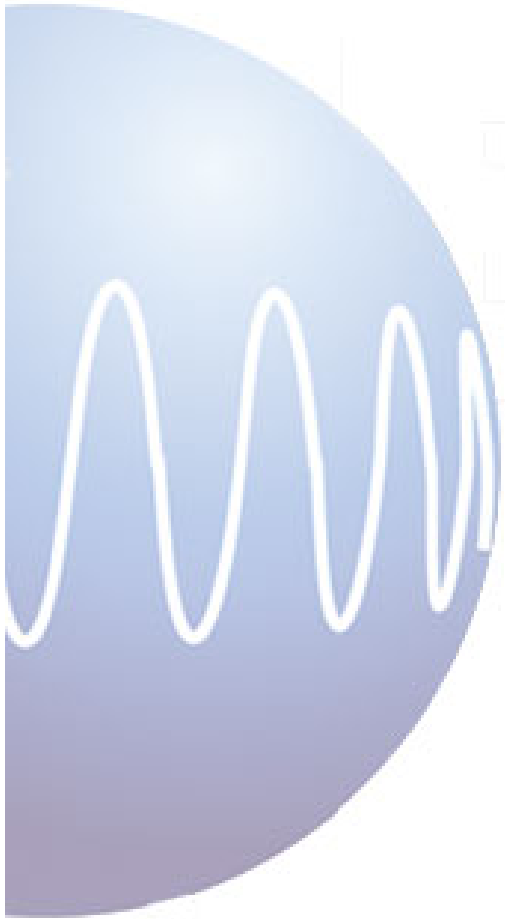
triple-relation file

Programmer's File Editor - [D:\Bruker\XWIN-NMR\prog\tcl\libtix\prosol\lib\lists\paramDescr]

File Edit Options Template Execute Macro Window Help

```
on};PLHD {homo decoupling};PLHC {band homo decoupling};PSH1 psh1;PSH2 psh2;PSH3 psh3;PSH4 psh4;PSH5 psh5;PSH6 psh6;PSH7 psh7;
;PLHD {homo decoupling};PLHC {band homo decoupling};PSH1 {90/270 excitation};PSH2 {180 refocussing};PSH3 psh3;PSH4 psh4;PSH5
LHD {homo decoupling};PLHC {band homo decoupling};PSH1 {90 excitation};PSH2 {180 adia refocuss};PSH3 {180 adia inversio};PSH4
LHD {homo decoupling};PLHC {band homo decoupling};PSH1 psh1;PSH2 psh2;PSH3 psh3;PSH4 psh4;PSH5 psh5;PSH6 psh6;PSH7 psh7;PSH8
LHD {homo decoupling};PLHC {band homo decoupling};PSH1 psh1;PSH2 psh2;PSH3 psh3;PSH4 psh4;PSH5 psh5;PSH6 psh6;PSH7 psh7;PSH8
```

paramDescr



Basics of pulse programming

Basics of pulse programming: comments

Syntax of pulse program line

Each pulse program starts with a short description of the experiment, literature references, predefined delays and other parameters

Comments

- ***for part or a full single line use a semicolon***

```
p1 ph1          ;90deg. pulse on observe channel
```

- ***multiple lines can be commended with /* */***

```
/*              this is the start of the comment
p1 ph1
d2
p2 ph2
*/              this is the end of the comment
```

Labels and arithmetic expressions

Labels

Labels are jump addresses within the pulse program, mainly used for loops. The most common case is 'go=n', others are 'lo to *label* times m'.

```
1 ze
2 d1
.....
go=2
```

Arithmetic expressions

- ***Used to assign values to pulses, delays, loop counters. Note that not all acquisition parameters can be defined by those expressions (e.g. delay increments like 'in0').***
- ***Arithmetic expressions have to be placed at the beginning of the pulse program***
- ***The calculated values are NOT visible within eda, but only with ased.***

```
"d1=100m"   "d2=1s/(cnst2*2)"
```

Delays

Fixed delays

20u 20m 1s

Delay variables

[d0 ... d31] **(array)**

Delay modifier

d1*0.333

Variable delay

vd **(define via VDLIST)**

Random variation of delay

d8:r **(define % of variation via V9)**

Predefined delays

define delay DELAY1

"DELAY1=d2-p16-d16"

Special delays

de1 de2 **prescan delays as defined with 'edscon'**

aq **acquisition time**

Pulses

Fixed pulses

20up 20mp

Pulse variables

[p0 ... p31] (array)

Pulse modifier

*p1*0.333*

Pulses used for CPD

(pcpd1 ...pcpd8) (valid for CPD-program only!)

Predefined pulses

define pulse MYPULSE1

"MYPULSE1=d2-p16-d16"

Pulse phase

p1 ph1 (ph0 ... ph31 allowed)

fixed phase according to phase program

p1 ph1:r a constant phase *phcor1* is added to each entry of the phase program (phcor0 -phcor31 allowed)

Variable pulse

vp (define via VPLIST)

RF channel selection and incrementation

Pulse variables

p1:f1 ph1 or (p1 ph1):f1 new: (p1 ph1):f1 pl1

Shaped pulses

(p11:sp1 ph1):f1 or p11:sp1:f1 ph1

Increments for delays

Parameter array	***[IN0 - IN31]***	
Pulse program statement	***[id0 ... id31]***	***increment***
	[dd0 ... dd31]	***decrement***
	[rd0 ... rd31]	***reset to starting value***

Increments for pulses

Parameter array:	***[INP0 - INP31]***	
Pulse program statement	***[ipu0 ... ipu31]***	***increment***
	[dpu0 ... dpu31]	***decrement***
	[rpu0 ... rpu31]	***reset to starting value***

Decoupling modes and power levels

Power modes

cw:f1 cw:f2	***CW irradiation***
hd:f2	***homodecoupling mode***
cpd1:f1, cpd2:f2	***acquisition parameters***
cpds2:f2	***synchroneous decoupling mode***
cpdsng2:f2	***synchroneous decoupling mode, but amplifier blanking closed. No pulse unless the amplifier gate is opened***
do:f1 do:f3	***terminate CW or CPD decoupling***

Power levels

Hard pulses	
pl1:f1 pl2:f2	***[PL0 ... PL31] (for all channels)***
Shaped pulses	
p1:sp1	***[SP0 ... SP15] (for all channels).***

Loops and acquisition

go=n	*acquisition loop*
rcyc=n	*acquisition loop*
lo to 'label' times 20	*loop with fixed number of loops*
lo to 'label' times l31	*loop with loop counter [l0 ... l31]*
td, td1, td2, td, nbl, ns	*additional allowed loop counters*

Examples for the 'go' command

go=2 *go=2 ph31* *go=2 ph31 cpd2:f2* *go=2 ph 0 ph31 cpd2:f2*

Explicit programming of acquisition, the 'rcyc' command

d12 syrec	*;set ZF for acquisition, ZF=SFO1 + 22MHz*
2u adc ph31	*;start the analog-to-digital converter*
	;the receiver phase has to be defined here
aq	*;sample during the period AQ (acquisition time)*
rcyc=2	
sytra	**switch the observe channel back to SFO1. Can be used when pulses using the F1-channel are performed during the acquisition time**

Frequency switching and phase programs

Frequency switching

fq1:f1, fq2:f2	[fq1 ... fq8], according to frequency lists FQ1LIST ... FQ8LIST

Phase program and pulse phase

ph1=0 1 2 3	0^o 90^o 180^o 270^o
ph1=(360) 0 90 180 270	
ph1=('n') 'phase-value'	in general phase is: (360/n)*phase-value
ph1= (8) 1 7	360/8=45 => 45, -45

Increments for phases

[ip0 ... ip31]	increment by 90^o
[dp0 ... dp31]	decrement by 90^o
[rp0 ... rp31]	reset to value defined by phase program

Further allowed statements

ip1*3	performes a shift of the phase program by $3*90^o = 270^o$

Miscellaneous basic elements

ze	switch AD converter to replace-mode (allow acquisition memory to be overwritten)
zd	like 'ze', bit in addition dummy scans are set to zero

if/goto **replaced by** *if/else* *if "l1==1" else*

wr #0	write data to disc using current dataset definition
if #0	increment *disc file pointer* for SER 2D or 3D data
df #0	decrement the disc file pointer
rf #0	reset the disc file pointer

replaced by *the mc-command*

reset1:f1 reset1:f2	make RF channels 1 and 2 phase coherent
reset2:f1 reset2:f2	*(each SGU has three outputs)*
reset3:f1 reset3:f2	

aqsec	*312* or *321* - defines in which order delays *d0* and *d10* are incremented

New features in pulse programming since XWIN-NMR 3.5

Summary

- ***some pulse programs and parameter sets are renamed***

  INV4 → HMQC, HMBC

  INVI → HSQC

- ***mc command and parameter TD0 are introduced***
- ***new syntax for alignment of parallel pulses***
- ***phase correction in indirect dimension is redundant***
- ***Setting precompiler options are if-loops***
- ***frequencies and offsets are given as constants in ppm***
- ***new frequency lists in ppm***
- ***small indirect 1H dimension spectral widths can be used***

Advantages of the new mc command

- ***simplifies pulse programming***

- ***includes***
  - ***disk write (wr)***
  - ***file pointer incrementation (if)***
  - ***memory initialization (zd)***
  - ***expanded loop structure possible***

- ***no need to control actions required for phase sensitive 2D experiments (phase or delay incrementation) with delays***

- ***One pulse program can be used for different 2D phase modes***

FO	***phase sensitive 1D***
F1QF	***magnitude mode QF***
F1PH, F2PH	***QSEQ,phase sensitive TPPI, States or States-TPPI***
F1EA, F2EA	***phase sensitive echo-antiecho***

The mode for the phase sensitive acquisition has to be specified in the FnMODE parameter

The mc command in 1D sequences

Old, without mc:

```
1 ze
2 d1
   p1 ph1
   go=2 ph31
   wr #0
exit
```

New, with mc:

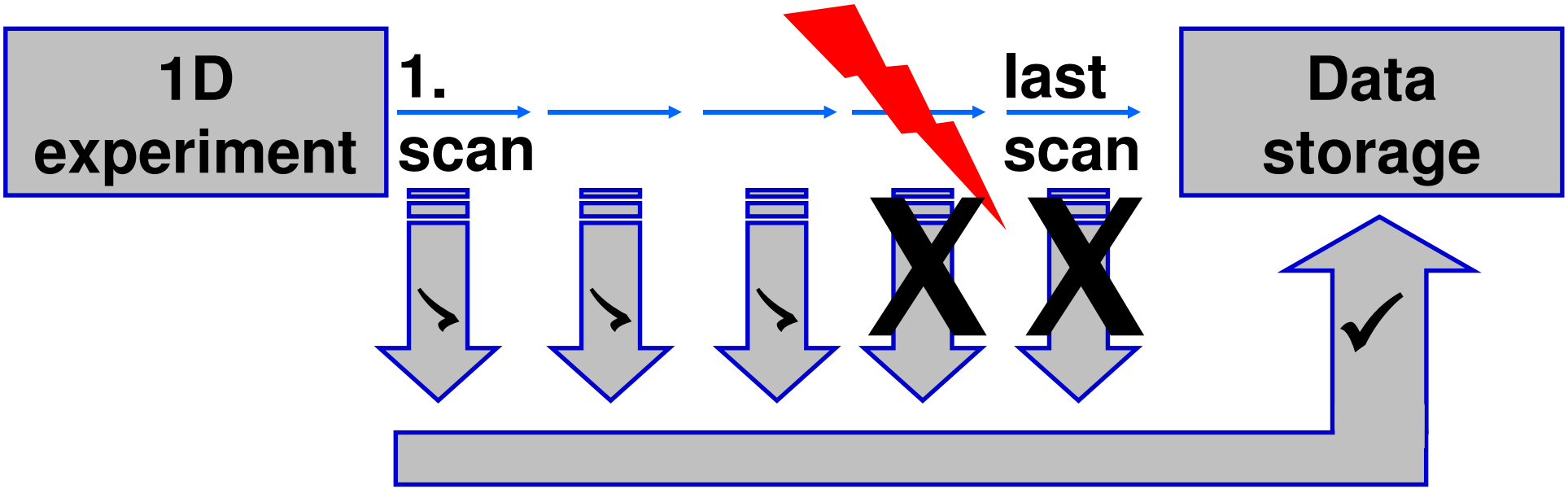
```
1 ze
2 30m
  d1
  p1 ph1
  go=2 ph31
  30m mc #0 to 2 F0(zd)
exit
```

New, expanded with mc:

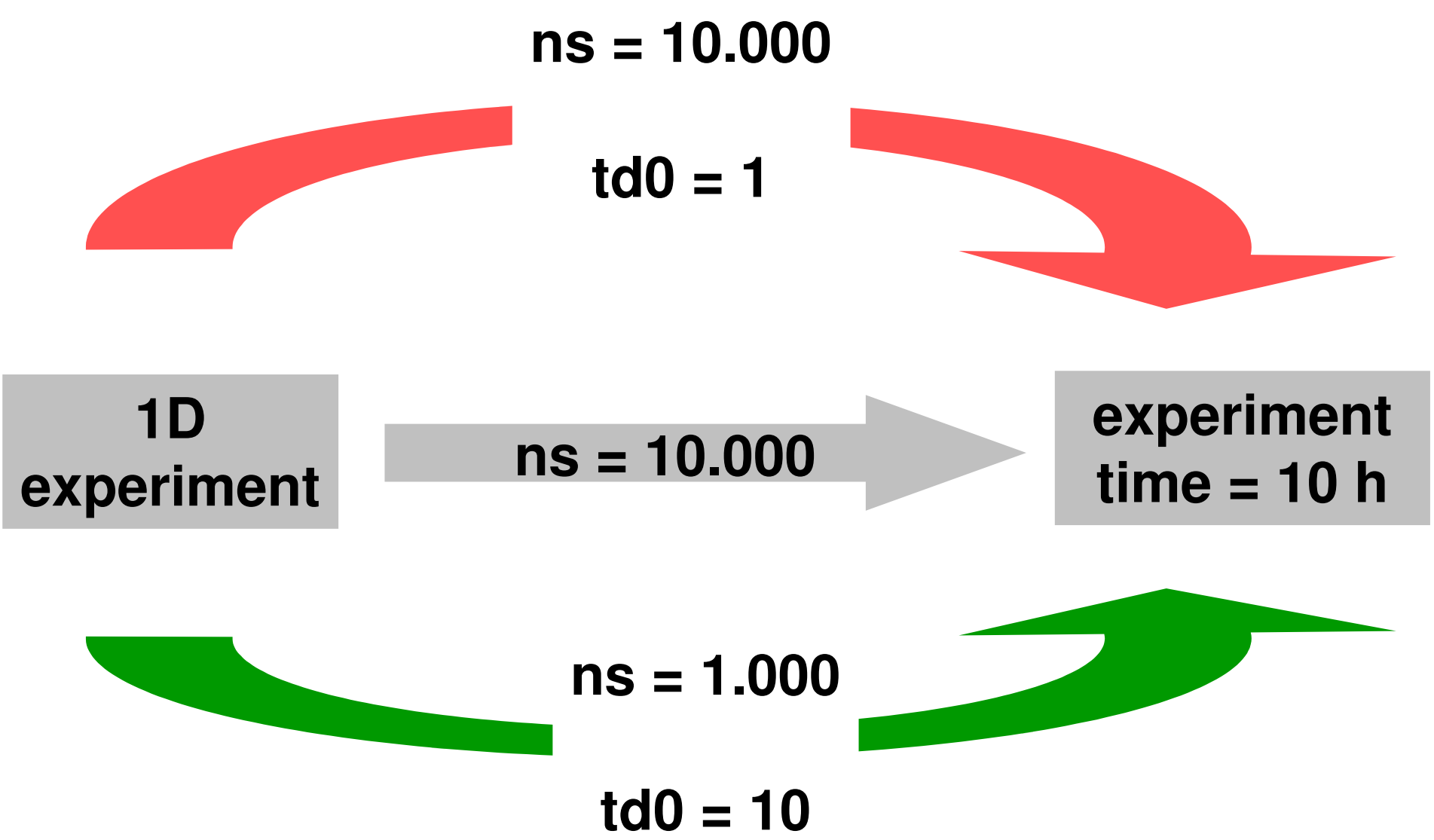
```
define delay MCWRK
define delay MCREST
"MCWRK=0.333333*30"
"MCREST=30m-30m"

1 ze
2 MCWRK*3
  LBLF0, MCREST
  d1
  p1 ph1
  go=2 ph31
  MCWRK wr #0
  MCWRK ze
  MCWRK zd
  lo to LBLF= times td0
exit
```

The mc command in 1D and td0

Data storage during acquisition !!



The mc command in 1D and td0



The mc command in 2D

F1QF	**phase insensitive**	**QF**
F1PH	**phase sensitive**	**QSEQ, States, TPPI, States-TPPI**
F1EA	**Echo-Antiecho**	**Echo-Antiecho**

The acquisition and processing parameter FnMODE define the mode for F1 (and F2, for 3D-experiments) incrementation

For details, see XWINNMR help -> Other topics -> Writing pulse program

MC clause	t1 quadrature mode	action after	
		odd increment	even increment
F1PH(ip1, id0)	TPPI	ip1+ id0	ip1 + id0 again
F1PH(ip1, id0)	States-TPPI	ip1	id0
F1PH(rd10 & rd30 & ip4, id0)	TPPI	rd10+rd30+ip4+id0	
F1PH(rd10 & rd30 & ip4, id0)	States-TPPI	rd10+rd30+ip4	id0

The mc command in 2D

old

```
;noesytp

#include <Avance.incl>

"d0=3u"

1 ze
2 d1
3 p1 ph1
  d0
  p1 ph2
  d8
  p1 ph3
  go=2 ph31
  d1 wr #0 if #0 ip1 id0 zd
  lo to 3 times td1
exit
```

new

```
;noesyph

#include <Avance.incl>

"d0=3u"

1 ze
2 d1
3 p1 ph1
  d0
  p1 ph2
  d8
  p1 ph3
  go=2 ph31
  d1 mc #0 to 2 F1PH(ip1, id0)
exit
```

Syntax of parallel pulses

Parallel pulses can be written in different lines of the pulse program, if they are combined by an opening and closing bracket

old

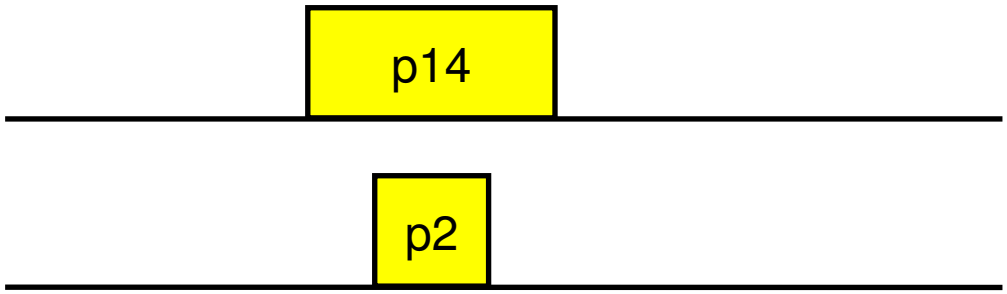
```
(p14:sp5 ph1) :f2 (d13 p22 ph1) :f3
```

new

```
(
   (p14:sp5 ph1) :f2
   (d13 p22 ph1) :f3
)
```

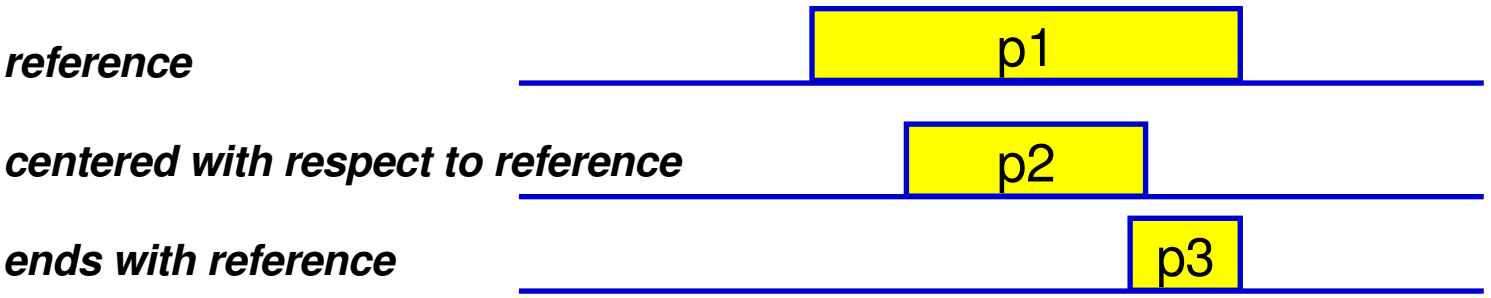
Alignment: relative orientation of parallel pulses
macros: center / ralign / lalign / reference

```
(center (p2 ph4) :f1 (p14:sp3 ph6) :f2)
```

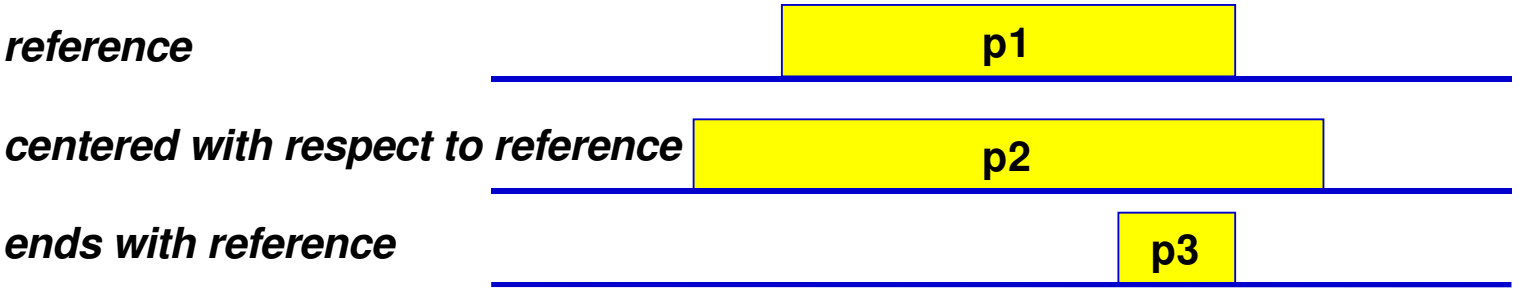


Alignment of parallel pulses

```
(
   reference (d0 p1 ph2 d0) :f1
   center (p2 ph3) :f2
   ralign (p3 ph3) :f3
)
```



p1 > p2
p1 > p3

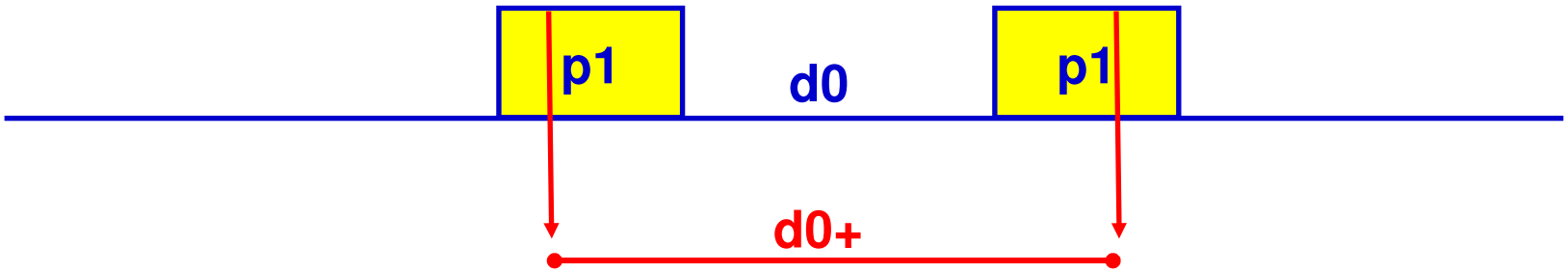


p1 < p2
p1 > p3

Exact phase correction values for F1

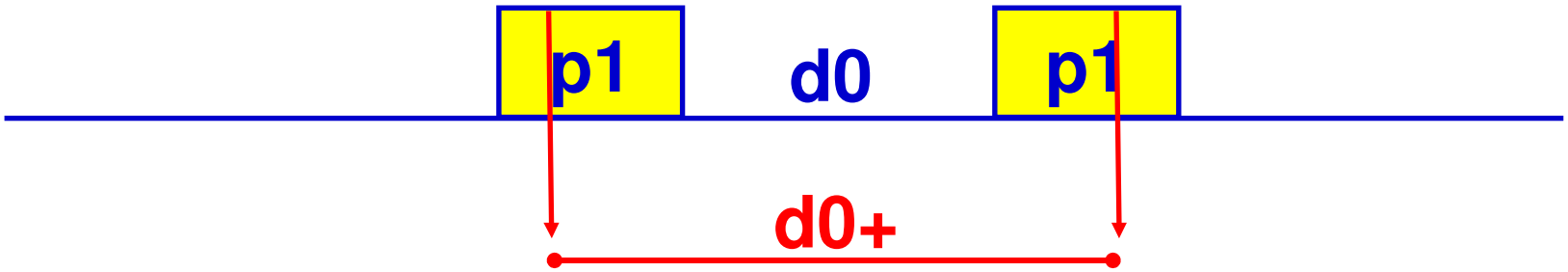
Trick: Account for the chemical shift evolution during the pulse!

Ex. homonuclear 2D experiment



Real evolution time of the spins is d0+

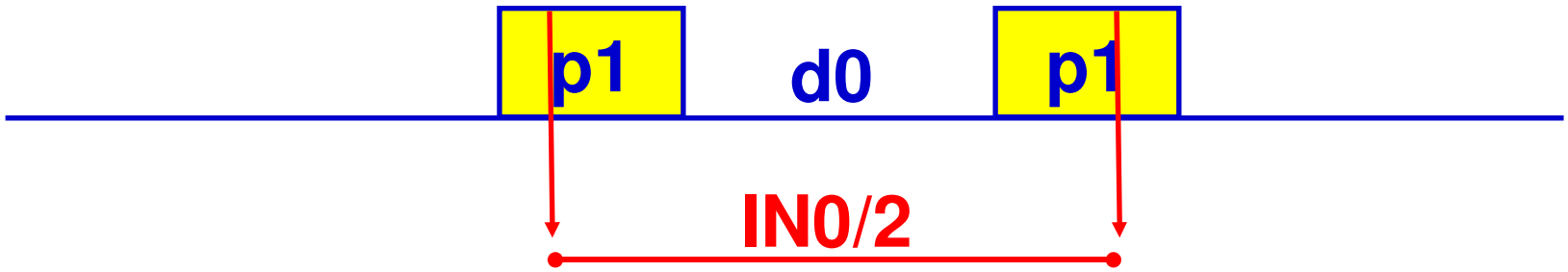
Exact phase correction values for F1



If the real evolution time d0+
is the same as half of the increment
d0+ = IN0/2

Then the necessary phase correction parameters
in the F1 dimension can be set exactly to the values
PHC0 = 90° ***PHC1 = -180°***
prior to the experiment!

Exact phase correction values for F1



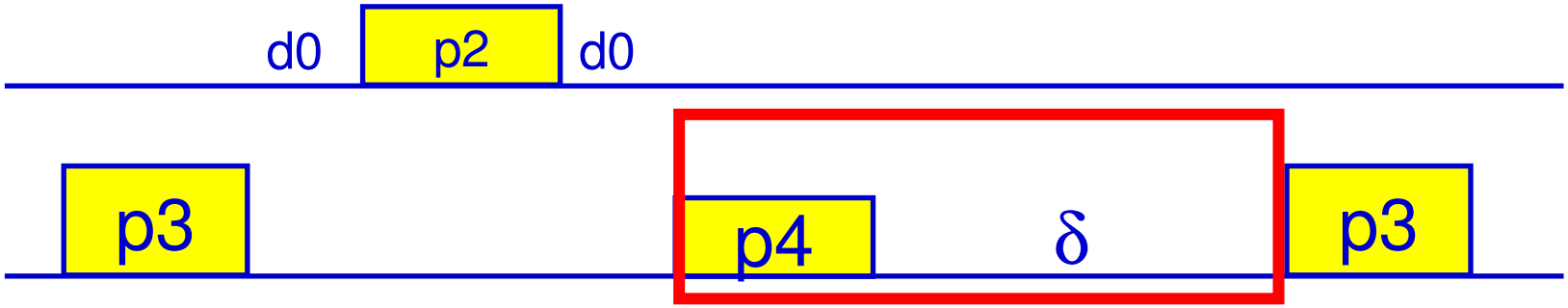
This is made possible by an on-the-fly correction of d0 within the pulse program

d0 = IN0/2 – p1*4/π

PHC0 = 90° **PHC1 = -180°**

Exact phase correction values for F1

Ex. heteronuclear 2D experiment



Accounting for the chemical shift evolution in heteronuclear 2D experiments is possible by an 180° refocussing puls (p4)
The delay δ is defined as:
δ = 2*d0+p2

The resulting values for phase correction in F1 are:
PHC0 = 0° **PHC1 = 0°**

Advantages of exact phase correction for F1

All Bruker parameter sets in the NMR-SUITE 3.5 contain the correct phase values in the F1 dimension

I.	baseline distortions are reduced to a minimum
II.	manual phase correction in F1 dimension is not necessary anymore
III.	automation does not need any phase correction AU programs

Setting precompiler options

Precompiler options allow parts of the pulse program to be used or to be neglected, depending on precompiler options

This allows to have one pulse program, which can be used for solvent suppression either by presaturation or by WATERGATE, e.g.

Options can be

- ***defined in the pulse program***
  - ***disadvantage: pulse program has to be modified when the option is changed***
- ***Acquisition parameter ZGOPTNS***
  - ***several option flags can be set together***

zg options - what is it (good for)?

Example: ^{15}N HSQC with/without 13C decoupling

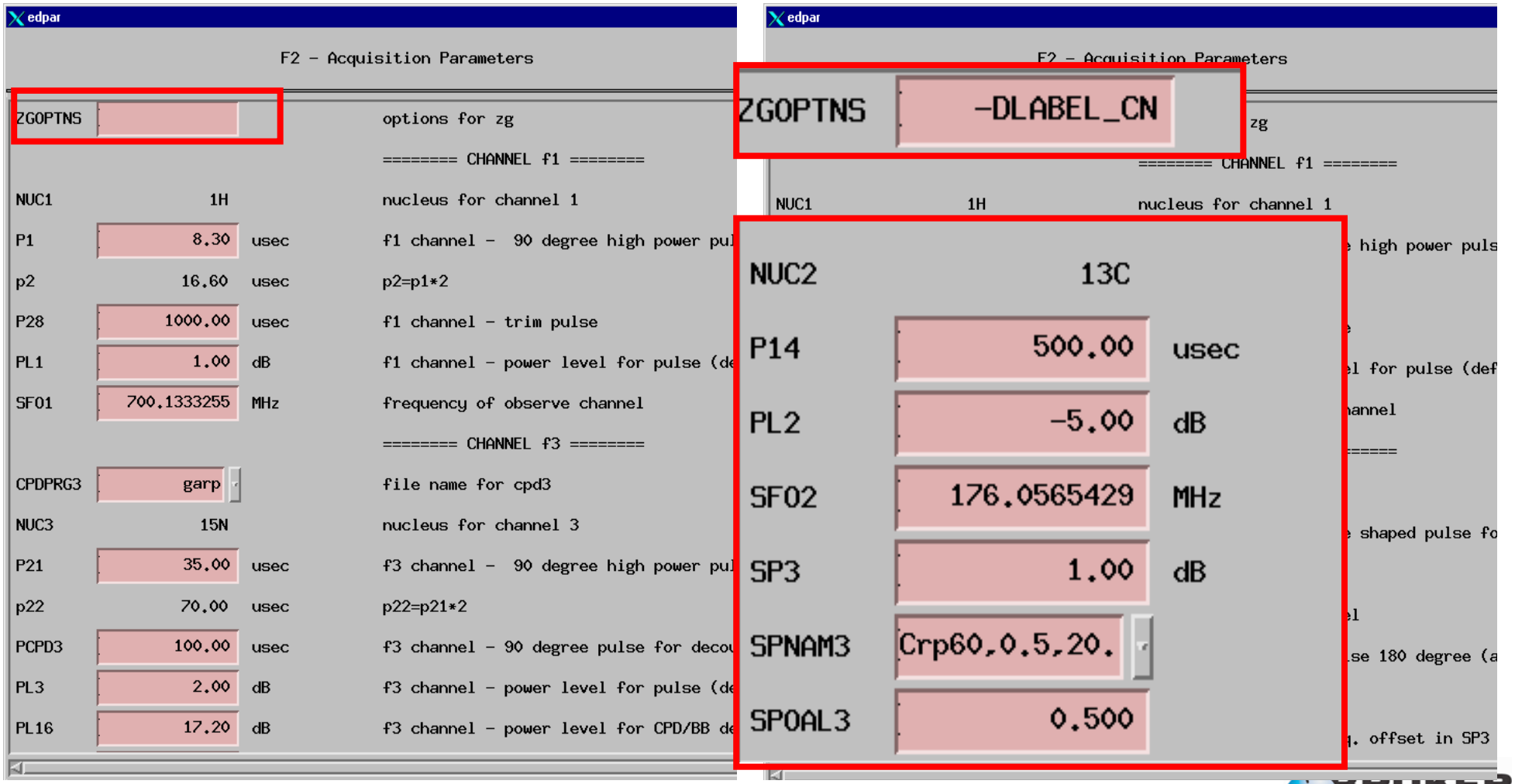
```
:hsqcetf3gp

#   ifdef LABEL_CN
"DELTA=p16+d16+larger(p2,p14)+d0*2"
#   else
"DELTA=p16+d16+p2+d0*2"
#   endif /*LABEL_CN*/

1 ze
  d11 pl16:f3
2 d1 do:f3
3 (p1 ph1)
  d26 pl3:f3
  (center (p2 ph1) (p22 ph6):f3 )
  d26 UNBLKGRAD
  p28 ph1
  d13
  (p1 ph2)
  3u
  p16:gp1
  d16
  (p21 ph3):f3
  d0
#   ifdef LABEL_CN
  (center (p2 ph5) (p14:sp3 ph1):f2 )
#   else
  (p2 ph5)
#   endif /*LABEL_CN*/

  d0
  p16:gp2*EA
  d16
  (p22 ph4):f3
  DELTA
  (ralign (p1 ph1) (p21 ph4):f3 )
  d26
  (center (p2 ph1) (p22 ph1):f3 )
  d13
  p16:gp3
  DELTA1 pl16:f3
  4u BLKGRAD
  go=2 ph31 cpd3:f3
  d1 do:f3 mc #0 to 2
   F1EA(igrad EA, id0 & ip3*2 & ip6*2 & ip31*2)
exit
```

zg options - how does it work?

For double labeled samples set ZGOPTNS to -DLABEL_CN

no zg-options,
no ^{13}C decoupling

zg-options set to $^{13}C/^{15}N$ labeled,
^{13}C-channel activated for decoupling



Example of setting precompiler options

A) ZGOPTNS ***-DPRESAT***

B) ZGOPTNS ***-DWATERGATE***

C) ZGOPTNS ***-DPRESAT -DWATERGATE***

```
#ifdef PRESAT
  d12 pl9:f1
  d1 cw:f1
  d13 do:f1
  d12 pl1:f1
#endif PRESAT
#ifdef WATERGATE
  d1 pl1:f1
#endif WATERGATE
```

Define frequencies via constants in ppm

frequencies within a pulse program without the need of external frequency lists

```
...
        d11 fq=cnst23(bf ppm):f2
...
```

The program takes BF2,
*adds a frequency cnst23*BF2*10e-6,*
*and sets SFO2= BF2 + cnst23*BF2*10e-6*
here cnst23 is the C-alpha chemical shift

If frequency lists are used,
they can be given in ppm

spoff calculation

Offsets for shaped pulses are defined directly in a pulse program

```
;hbhaconhgp3d
;avance version (02/05/31)
;HBHACONH

"spoffs2=0"
"spoffs3=0"
"spoffs5=bf2*((cnst21-cnst23/1000000)"

;cnst21: CO chemical shift (offset, in ppm)
;cnst23: Caliphatic chemical shift (offset, in ppm)
```

New definitions for frequency constants

For more information on the (new) parameter definitions see Param.info

```
;cnst18: H2O chemical shift (offset, in ppm)
;cnst19: H(N) chemical shift (offset, in ppm)
;cnst20: Haliphatic chemical shift (offset, in ppm)
;cnst21: CO chemical shift (offset, in ppm)
;cnst22: Calpha chemical shift (offset, in ppm)
;cnst23: Caliphatic chemical shift (offset, in ppm)
;cnst24: Caromatic chemical shift (offset, in ppm)
;cnst25: flag for cross peak / reference experiments
;cnst26: Call chemical shift (offset, in ppm)
;cnst27: ( Cgamma chemical shift (offset, in ppm) )
;cnst28: Haromatic chemical shift (offset, in ppm)
```

Indirect ^{1}H evolution in triple resonance

The ^{1}H spectral window in the indirect dimesion can be set to a small value, for instance 7-8 ppm

```
;hbhaconhgp3d
;avance-version (03/01/17)
;HBHACONH


p16:gp3
  d16 pl16:f3
  4u BLKGRAD
  go=2 ph31 cpd3:f3
  d11 do:f3 mc #0 to 2
     F1PH(rd10 & rd29 & rd30 & ip3, id0 & id20 & dd28 & dp3)
     F2EA(igrad EA & ip5*2, id10 & id29 & dd30)
exit

;Processing
;SR(F1): 1/4 SWH(F1)
```

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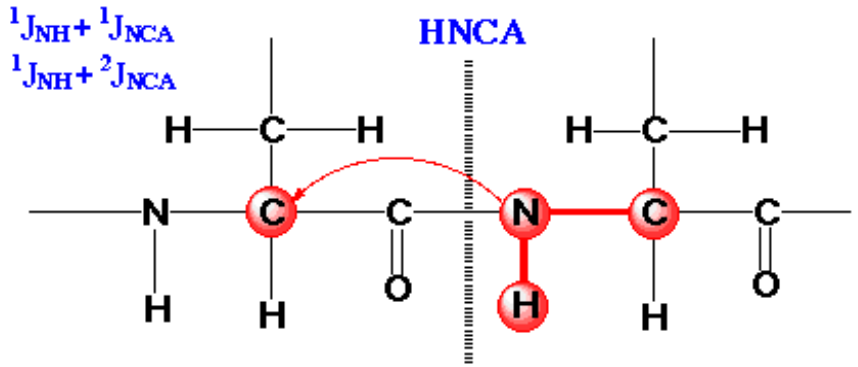
3D Triple-Resonance

3D HNCA

ESCRIPTION

The **3D HNCA experiment** is specifically designed to correlate ^{15}N and NH chemical shifts with the intra- and interresidue ^{13}CA carbon shifts by means of the ^{1}J(NH) and ^{1,2}J(N,CA) coupling constants. Intraresidue correlations can exclusively be extracted from a 3D HN(CO)CA experiment.



EQUIREMENTS

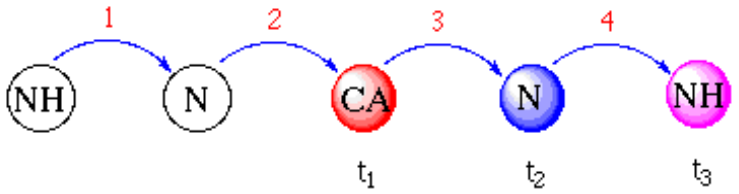
Implementation on AVANCE spectrometers equipped with a third channel. Improved versions using pulsed field gradients (PFGs) are also available and, therefore, in such cases gradient technology is required.

The experiment is applied on ^{15}N,^{13}C-labeled proteins. Because the amide (NH) protons are involved, the HNCA experiment must be recorded in

Pulse program information - NMR Guide

VERSIONS

The 3D HNCA pulse sequence **92JB195** and **94JACS6464** , **93ANG1489** , **94JMRB203-103** , and **94JMRA129-109**) is closely analog to the 3D HNCO experiment and consisted of the following out-and-back steps:



1. Initial transfer from ^{1}HN to ^{15}N via **^{1}J(NH)** using an INEPT pulse sequence.
2. Fixed evolution delay to achieve antiphase ^{15}N magnetization with respect to ^{13}CA via **^{1}J(N,CA)** and refocusing of ^{1}J(NH).
3. ^{13}CA chemical shift evolution during the variable evolution t_1 period in an HSQC-type way followed by ^{15}N chemical shift evolution during a constant-time evolution t_2 period with evolution of ^{1}J(NH) and refocusing of ^{1}J(N,CA).
4. Magnetization is finally transferred back to the NH protons by applying a retro-INEPT scheme and proton acquisition is recorded under ^{15}N decoupling.

Several improved versions have been proposed incorporating the following modifications:

- The original sequence used a different pathway in which ^{15}N chemical shift evolution takes place before the ^{13}CA chemical shift evolution delay (**90JMR496-89**).
- Constant-time period (CT) in the F_1(^{15}N) dimension, HSQC-like transfer in F_2(^{13}CA) dimension and optional ^{1}H decoupling instead of 180° ^{1}H pulses was first described in **92JMR432-96** .
- A refocused and optimized version (**92JB195**).
- Incorporation of PFGs (**92JB395**).

Pulse program information - NMR Guide

- Constant-time period (CT) in the F_1(^{15}N) dimension, HSQC-like transfer in F_2(^{13}CA) dimension and optional ^{1}H decoupling instead of 180° ^{1}H pulses was first described in **92JMR432-96** .
- A refocused and optimized version (**92JB195**).
- Incorporation of PFGs (**92JB395**).
- Improved sensitivity incorporating the PEP methodology in phase-cycled (**92JMR431-100**) and gradient-enhanced versions (**94JACS6464**) as described for the HNCO experiment (**93ANG1489** , **94JMRB203-103** , and **94JMRA129-109**).

3D HNCA pulse sequence | 3D HNCA using WATERGATE

- Selective CB-CO decoupling

3D CB/CO-decoupled HNCA

- Improved sensitivity by ^{2}H decoupling during the Constant-Time CA evolution period in ^{15}N,^{13}C,^{2}H-labeled proteins (**94JACS6464** and **94JACS11655**).

3D 2H-decoupled HNCA

The use of selective ^{13}CB decoupling during this period has also been proposed (**96JMRB91-113**).

- Improved sensitivity and resolution using the TROSY approach (**98PNAS13585** and **99JB85** and **99JB181-15**).

3D TROSY-HNCA | 3D 2H-decoupled TROSY-HNCA

- Improved sensitivity by simultaneous acquisition of two coherence pathways in the so-called HNCA+ experiment (**00JMR223-143**).
- For small and medium-size proteins: 2D H[NCA] (**94JB325**).
- The same connectivities can be traced out from a 3D (HA)CANH (**90JMR183-87** , **96JB98** , **97JB105** , and **95JB25**) and 3D(HA)CA(CO)NH (**97JACS9576** and **97JB105**) experiments.

Pulse program information - NMR Guide

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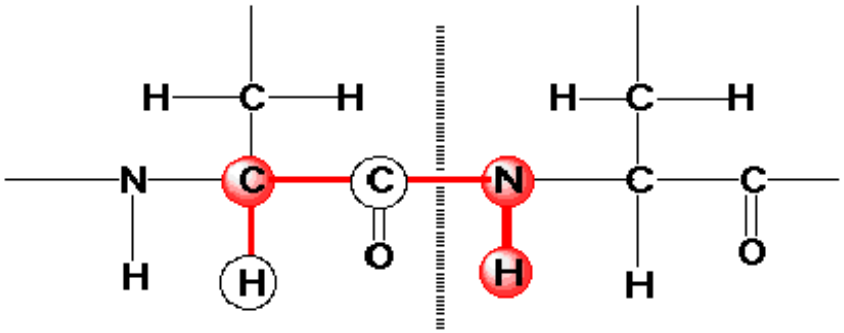
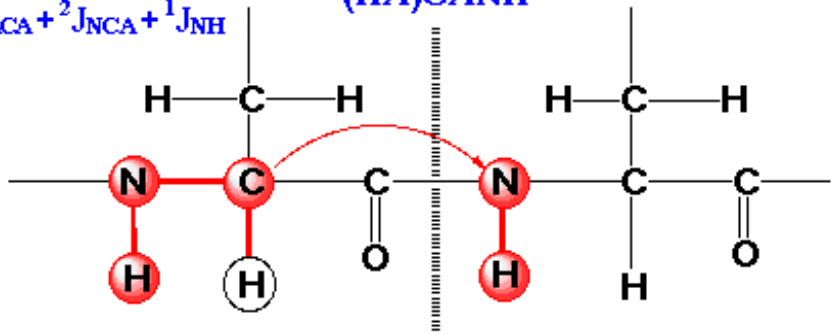
$^1J_{HACA} + {}^1J_{CACO} + {}^1J_{NCO} + {}^1J_{NH}$ (HA)CA(CO)NH



$^1J_{HACA} + {}^1J_{NCA} + {}^1J_{NH}$
$^1J_{HACA} + {}^2J_{NCA} + {}^1J_{NH}$ (HA)CANH



EXPERIMENTAL DETAILS

The HNCA experiment can be recorded in automation mode. More details on practical implementation of the 3D HNCA experiment on AVANCE spectrometers can be found in the corresponding **Tutorial 3D HNCA experiment**

Pulse program information - NMR Guide

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3D Triple-Resonance

3D HNCA

DESCRIPTION

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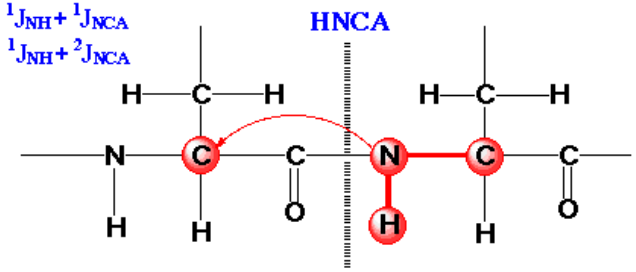

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