

SSSC Discovery Series

NMR3

Pulse Sequences and Programming

Topics:

1. Pulse sequences

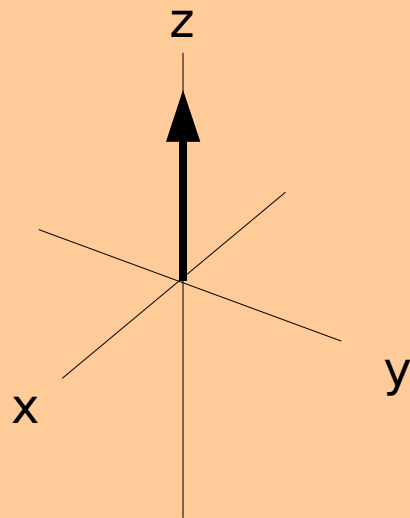
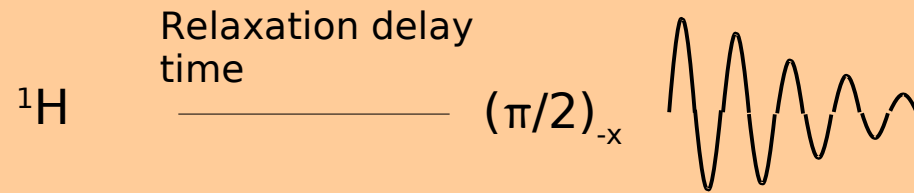
- simple ^1H 1D experiment
- ^{13}C experiment
- pulse building blocks: spin echo, bird, composite
- APT experiment
- Solvent suppression
- DEPT experiment
- COSY experiment
- HMQC experiment

2. Pulse programs

- Bruker pulse programming language and applications
- Simple phase programming
- Macros ... make the computer work for you!
- Bruker automation programming and ...
- **no** quantum mechanics!

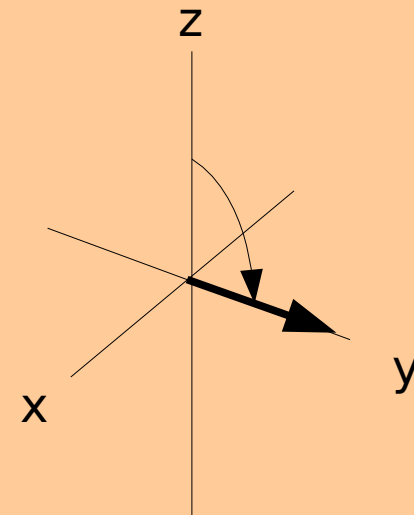


The Simple ^1H 1D Experiment

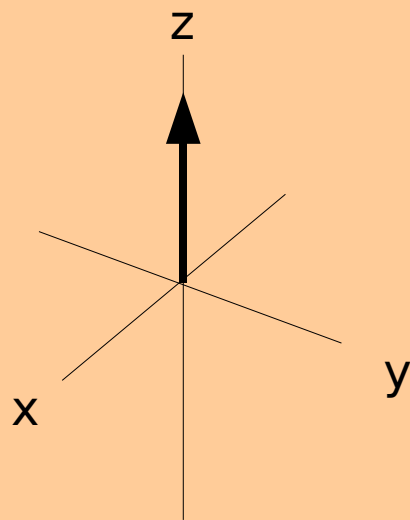
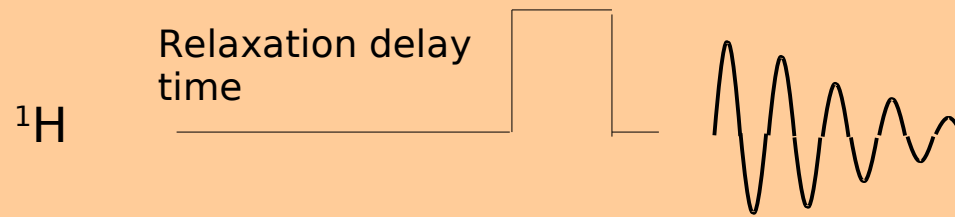


Equilibrium magnetisation

Pulse of angle $(\pi/2)_{-x}$
 $90^\circ(-x)$



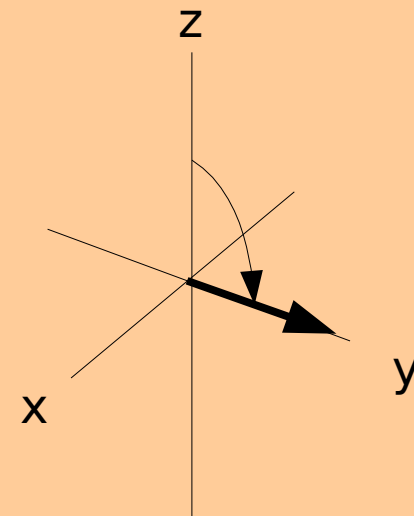
The Simple ^1H 1D Experiment



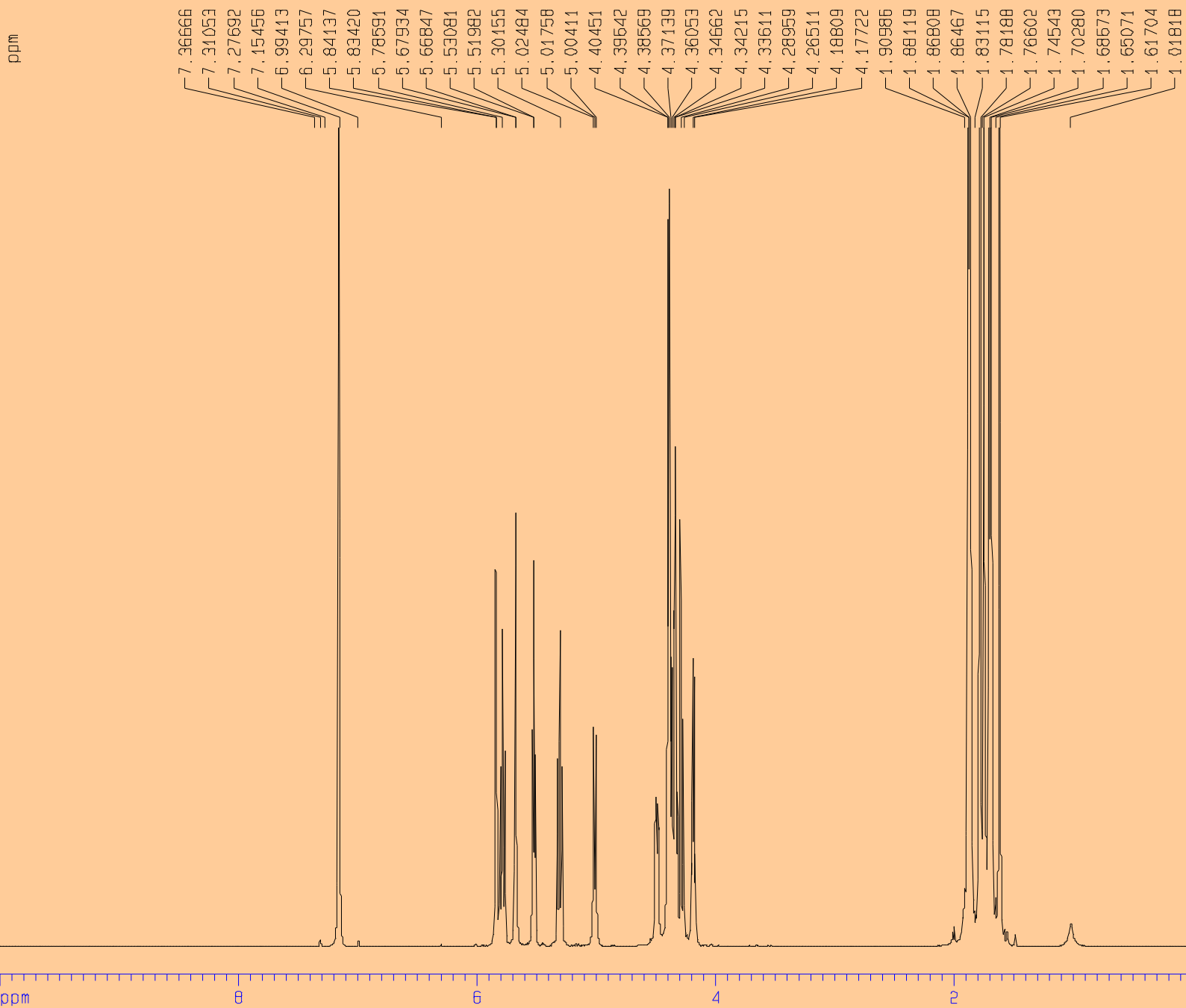
Equilibrium magnetisation

Pulse of angle
 $(\pi/2)_{-x}$

$90^\circ(-x)$



The Simple ^1H 1D Experiment



Current Data Parameters
NAME test
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20070309
Time 9.17
INSTRUM spect
PROBHD 5 mm TXI 13C Z
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 2
SWH 5000.000 Hz
FIDRES 0.162588 Hz
AQ 3.2768499 sec
RG 32
DM 100.000 usec
DE 8.00 usec
TE 297.8 K
D1 1.0000000 sec
MCREST 0.0000000 sec
MCWAK 0.0150000 sec

----- CHANNEL f1 -----
NUC1 1H
P1 11.00 usec
PL1 5.50 dB
SF01 500.1325139 MHz

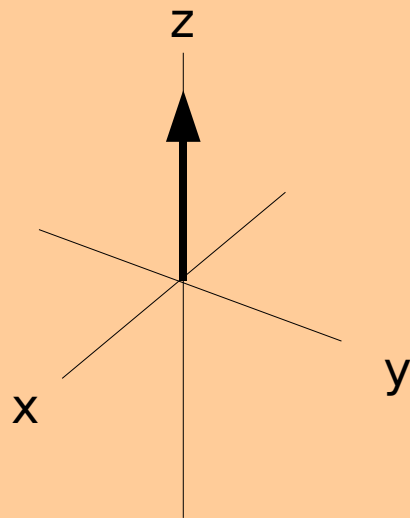
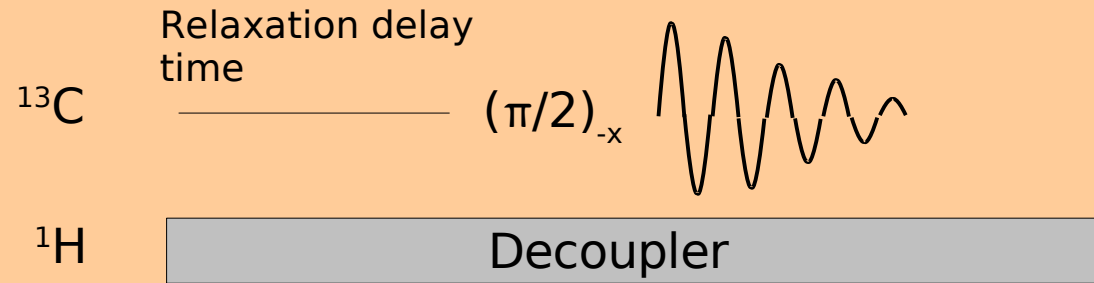
F1 - Acquisition parameters
NO 2
TD 256
SF01 500.1324 MHz
FIDRES 20.011526 Hz
SW 10.243 ppm
FRMQDE States

F2 - Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

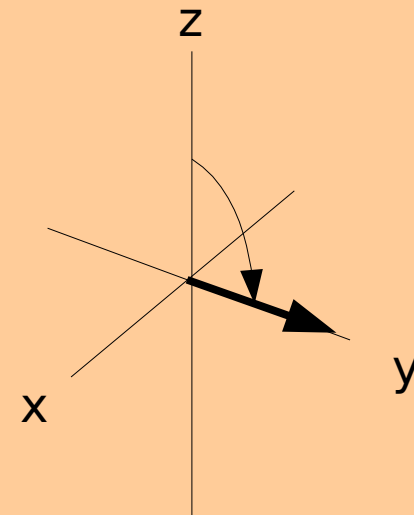
F1 - Processing parameters
SI 2048
MC2 TPPI
SF 500.1300000 MHz
WDW QBINE
SSB 2
LB 0.30 Hz
GB 0.1

1D NMR plot parameters
CX 20.00 cm
CY 50.00 cm
F1P 10.000 ppm
F1 5001.30 Hz
F2P 0.000 ppm
F2 0.00 Hz
PRMCH 0.50000 ppm/cm
HZCM 250.06502 Hz/cm

The Simple ^{13}C 1D Experiment

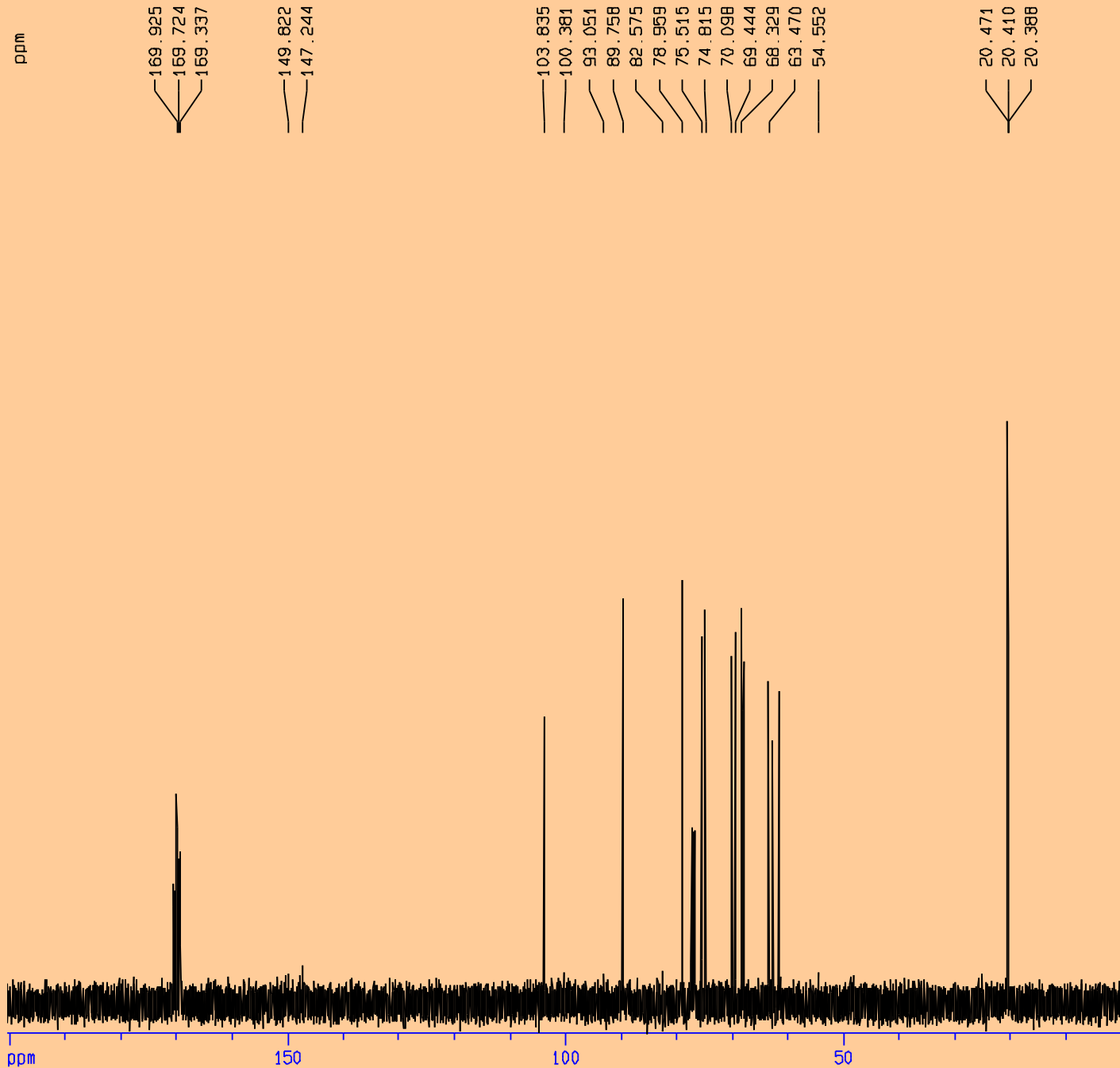


Pulse of angle $(\pi/2)_{-x}$



Equilibrium magnetisation

The Simple ^{13}C 1D Experiment



Current Data Parameters
 NAME 100mgSoAc
 EXPNO 2
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20020121
 Time 14.13
 INSTRUM spect
 PROBHD 5 mm PA8B0 8B-
 PULPROG zgpg
 TO 65536
 SOLVENT CDC13
 NS 8
 DS 4
 SWH 30030.029 Hz
 FIDRES 0.458222 Hz
 AQ 1.0912244 sec
 RG 1149.4
 DM 16.650 usec
 DE 6.00 usec
 TE 300.0 K
 D1 3.0000000 sec
 d11 0.0300000 sec
 d12 0.0000200 sec

===== CHANNEL f1 =====
 NUC1 ^{13}C
 P1 5.10 usec
 PL1 -1.00 dB
 SF01 125.7954618 MHz

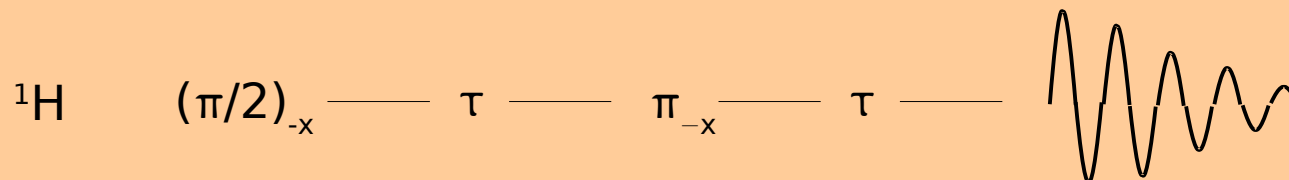
===== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 ^1H
 PCPD2 70.00 usec
 PL2 -1.00 dB
 PL12 14.00 dB
 PL13 14.00 dB
 SF02 500.2320009 MHz

F2 - Processing parameters
 SI 32768
 SF 125.7829854 MHz
 WDM EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

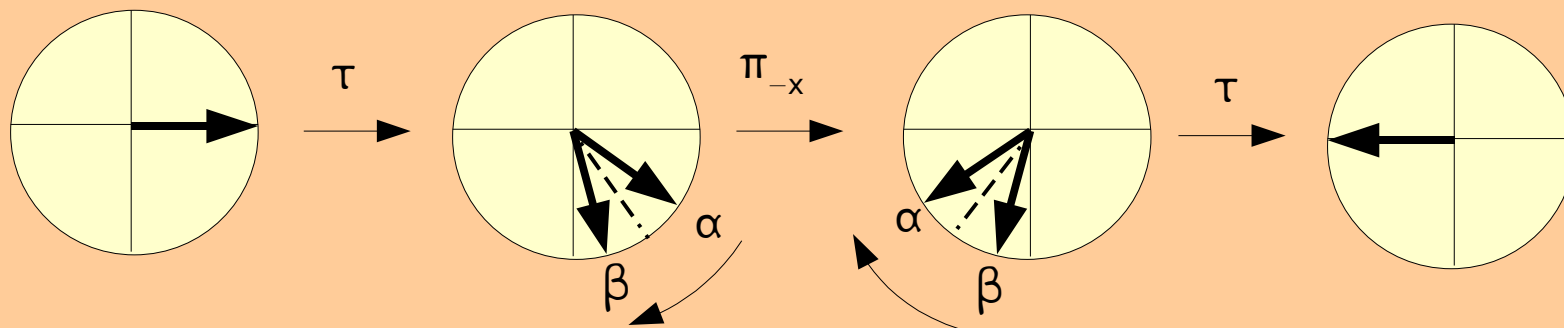
1D NMR plot parameters
 CX 20.00 cm
 CY 10.00 cm
 F1P 200.418 ppm
 F1 25209.17 Hz
 F2P -1.962 ppm
 F2 -246.75 Hz
 PPNM 10.11898 ppm/cm
 HZCM 1272.79602 Hz/cm

Pulse Clusters

Heteronuclear Coupled Spin Echo



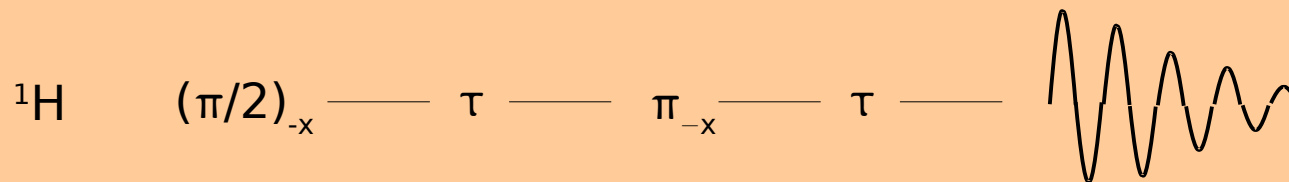
For an off-resonance pulse, ^1H coupled to ^{13}C (a doublet in this case):



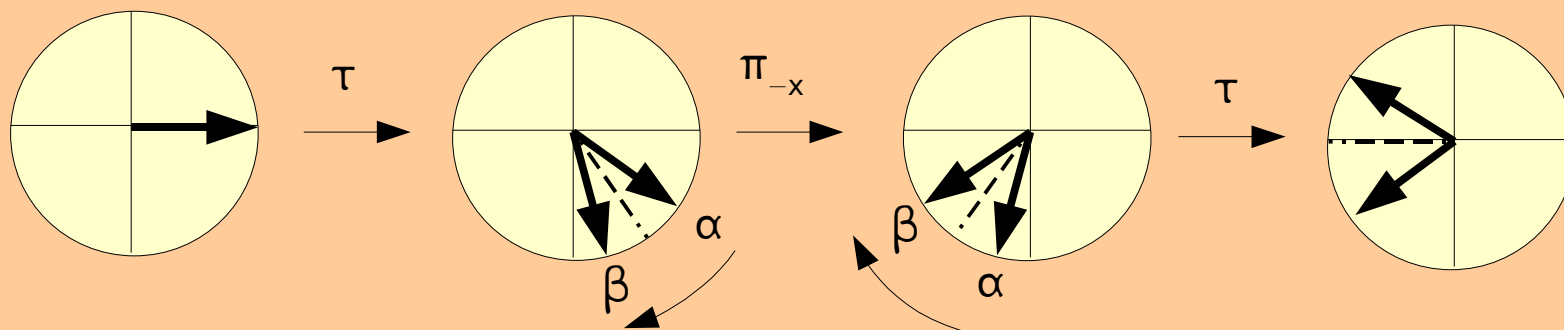
No matter what the chemical shift or the value of τ , the vector ends up in the **same spot**. This is a 'building block' of **many** nmr pulse sequences .. used to get rid of chemical shift effects. The two coupling vectors are said to '**refocus**' along the -y axis.

Pulse Clusters

Homonuclear Coupled Spin Echo



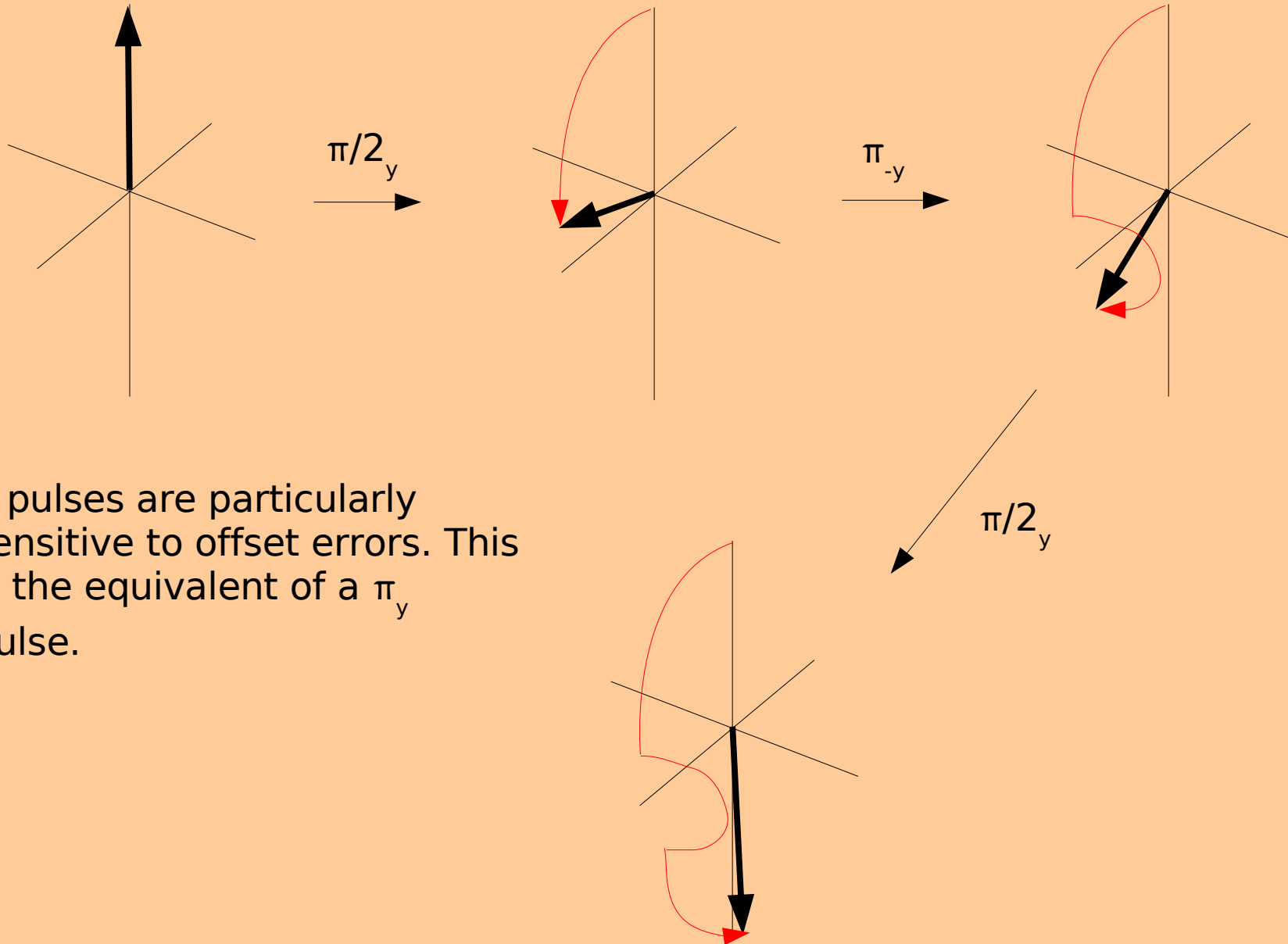
For an off-resonance pulse, ^1H coupled to ^1H (a doublet in this case):



In this case the spin labels are **swapped** after the π pulse. The chemical shift ends up along the $-y$ axis as in the heteronuclear spin echo case but the coupling vectors do not refocus.

Pulse Clusters

Composite pulses



π pulses are particularly sensitive to offset errors. This is the equivalent of a π_y pulse.

Pulse Clusters

Composite pulses

Composite 180° pulses to cancel offset effects:

$$90^\circ(x)180^\circ(y)90^\circ(x)$$

$$90^\circ(x)200^\circ(y)80^\circ(-y)200^\circ(y)90^\circ(x)$$

Composite pulses to remove phase distortion:

$$385^\circ(x)320^\circ(-x)25^\circ(x) \quad (\text{composite } 90^\circ \text{ pulse})$$

$$336^\circ(x)246^\circ(-x)10^\circ(y)74^\circ(-y)10^\circ(y)246^\circ(-x)360^\circ(x) \quad (\text{composite } 180^\circ \text{ pulse})$$

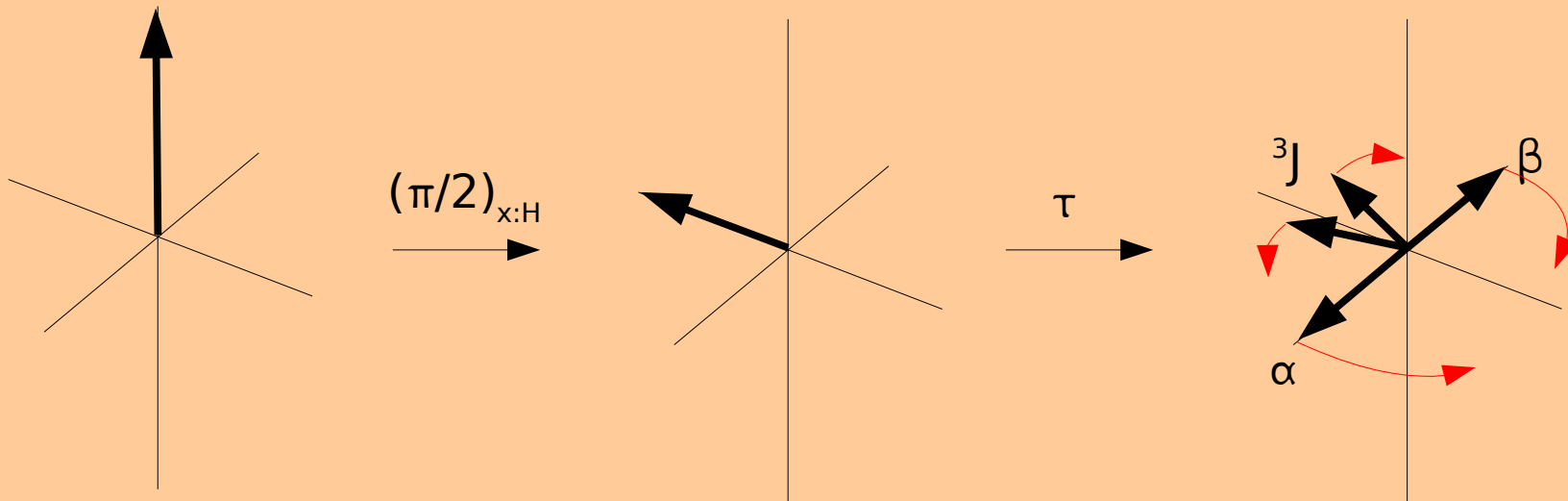
Pulse Clusters

BIRD pulses (Bilinear Rotation and Decoupling)

^1H $(\pi/2)_x$ — τ — π_x — τ — $(\pi/2)_x$

^{13}C ————— π_x —————

$$\tau = 1/(2^1J)_{\text{CH}}$$

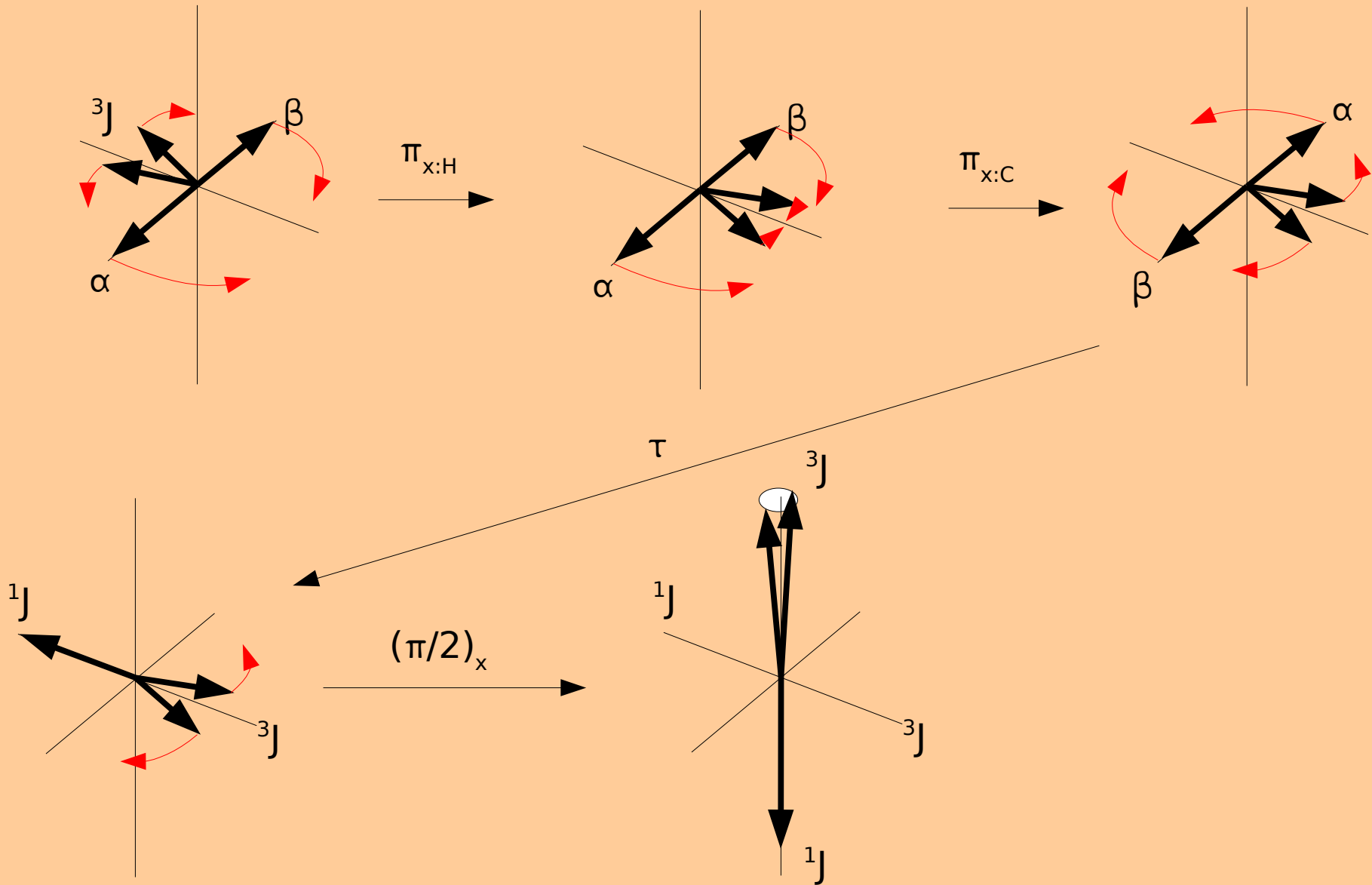


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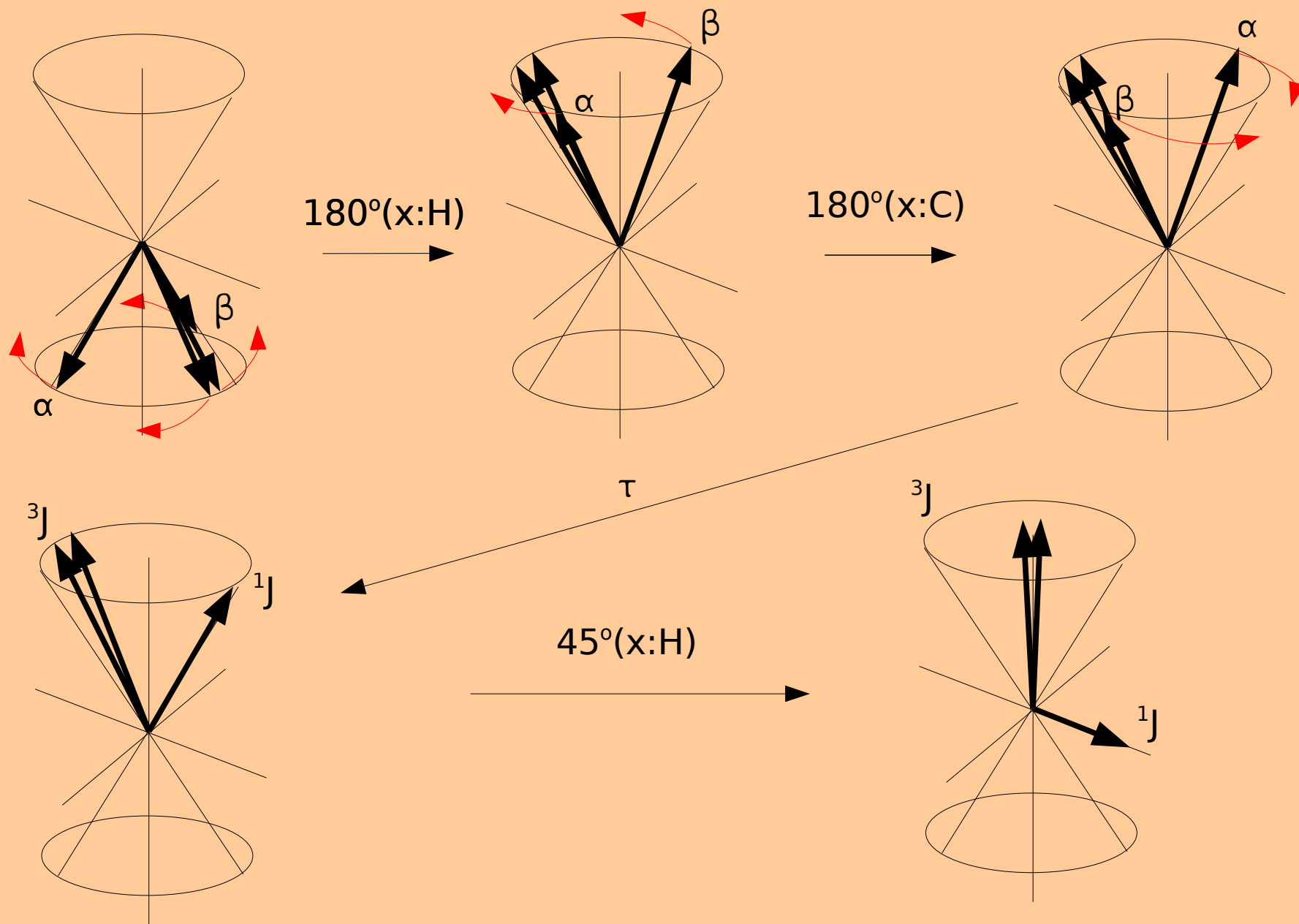
Pulse Clusters

BIRD pulses

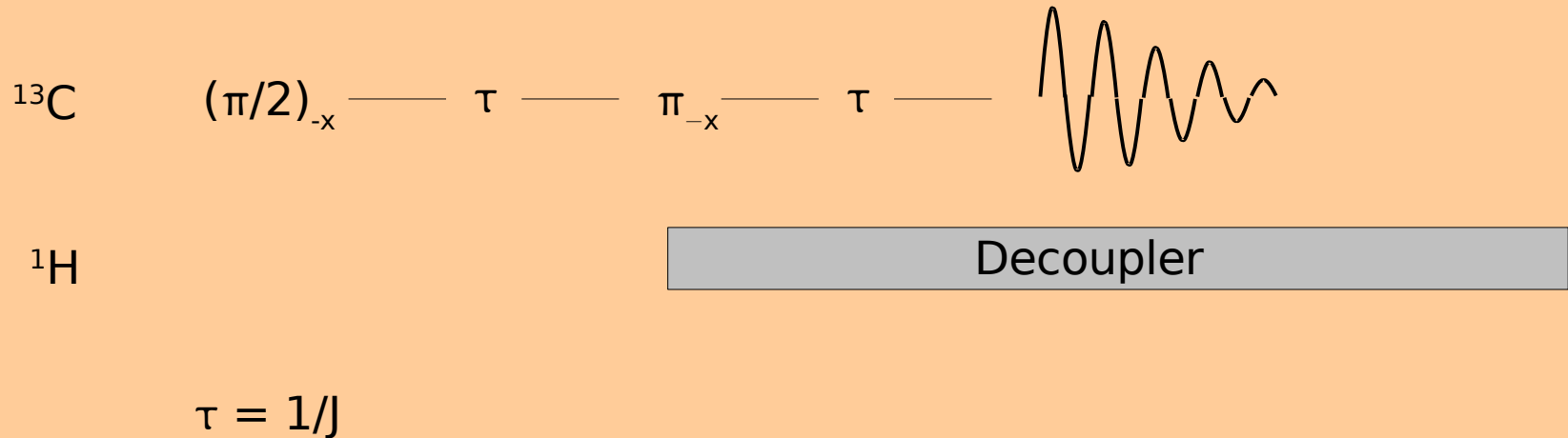
(**B**ilinear **R**otation and **D**ecoupling)



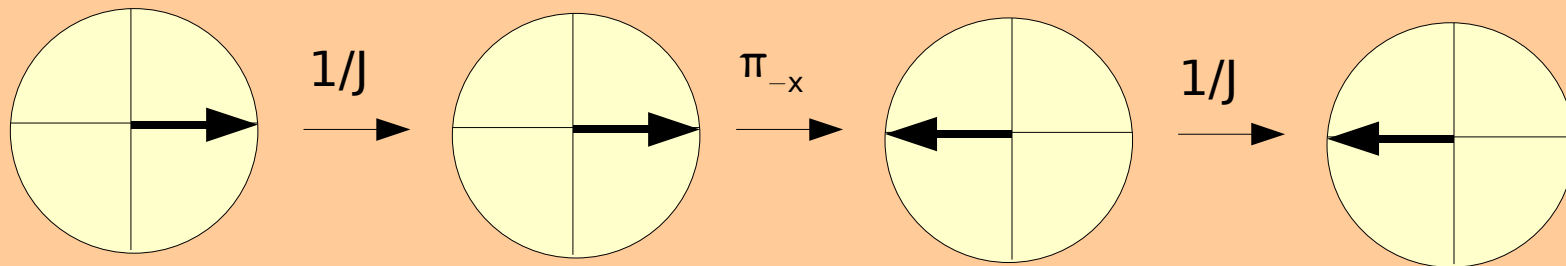
Pulse Clusters TANGO pulses



The APT or Jmodulation Experiment

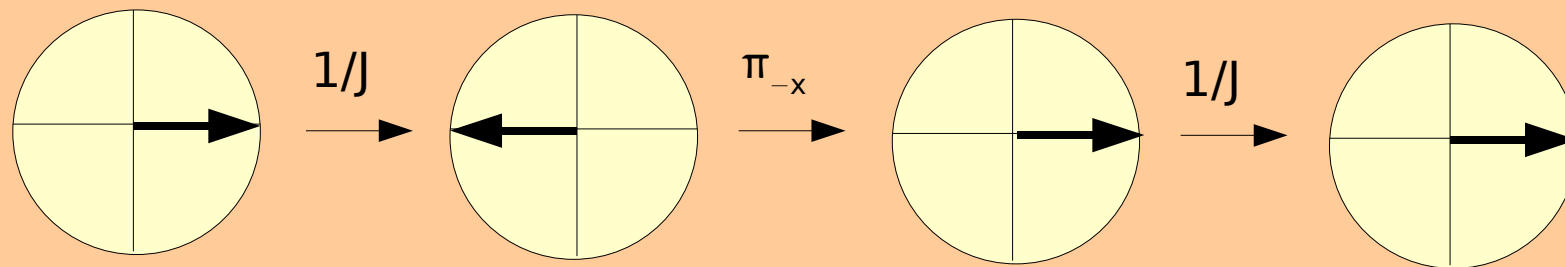


For a singlet (quaternary carbon) assuming an on-resonance pulse:

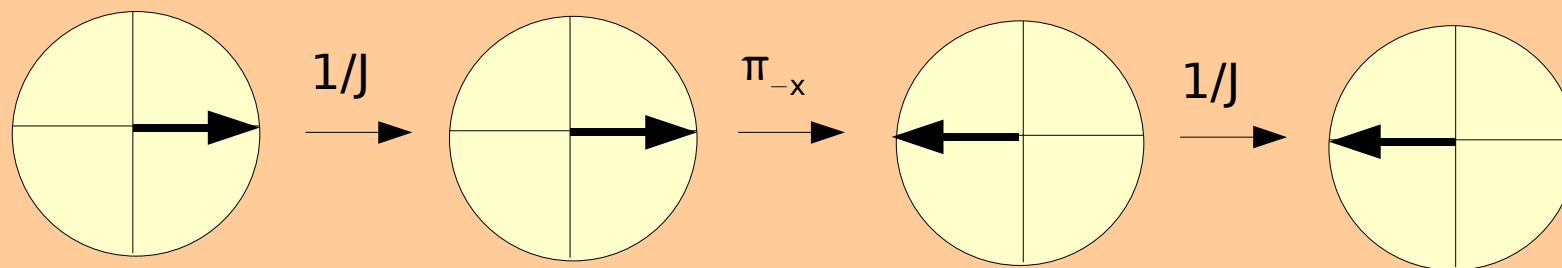


The APT or Jmodulation Experiment

For a doublet (CH), on-resonance pulse:

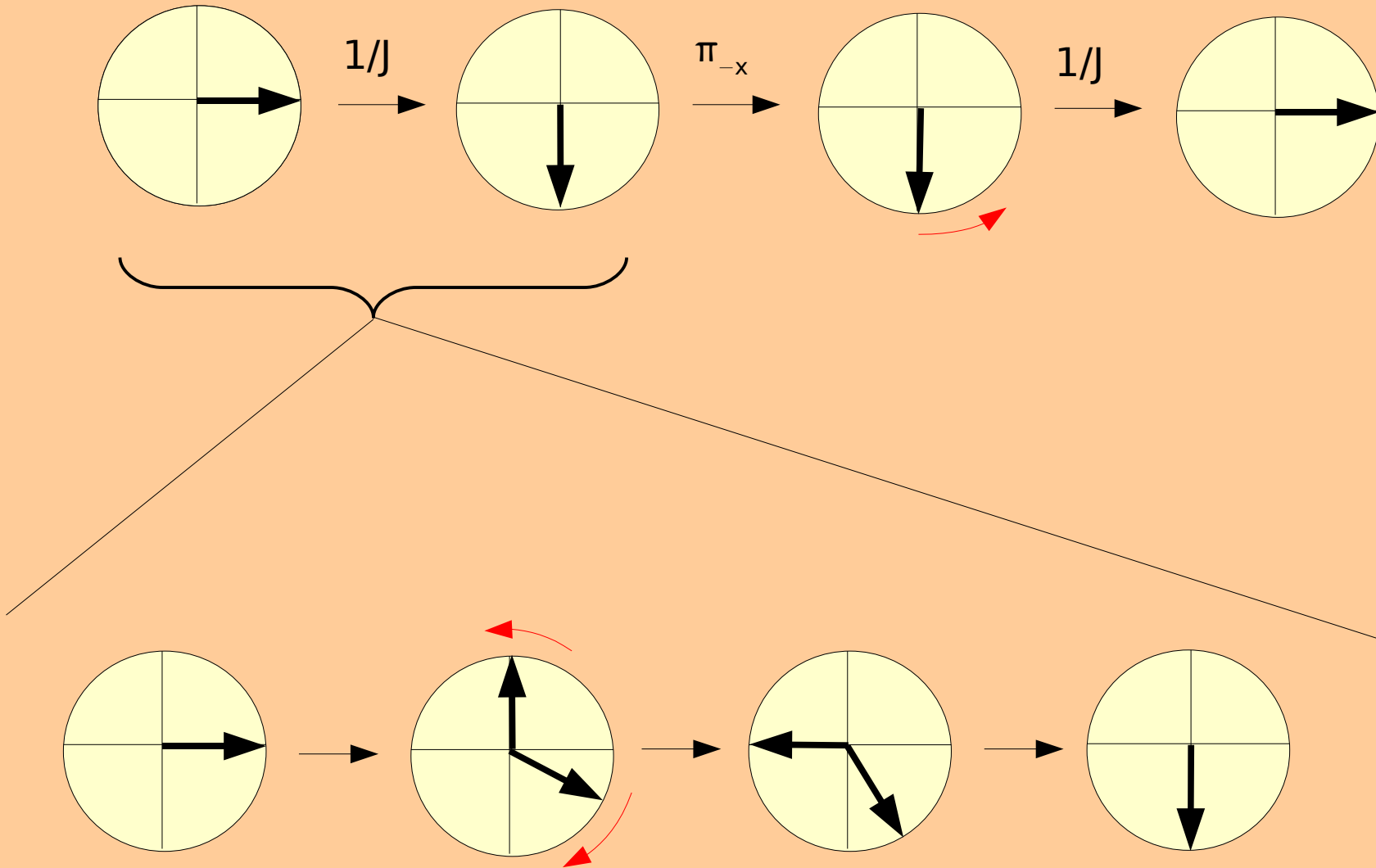


For a triplet (CH_2), on-resonance pulse:

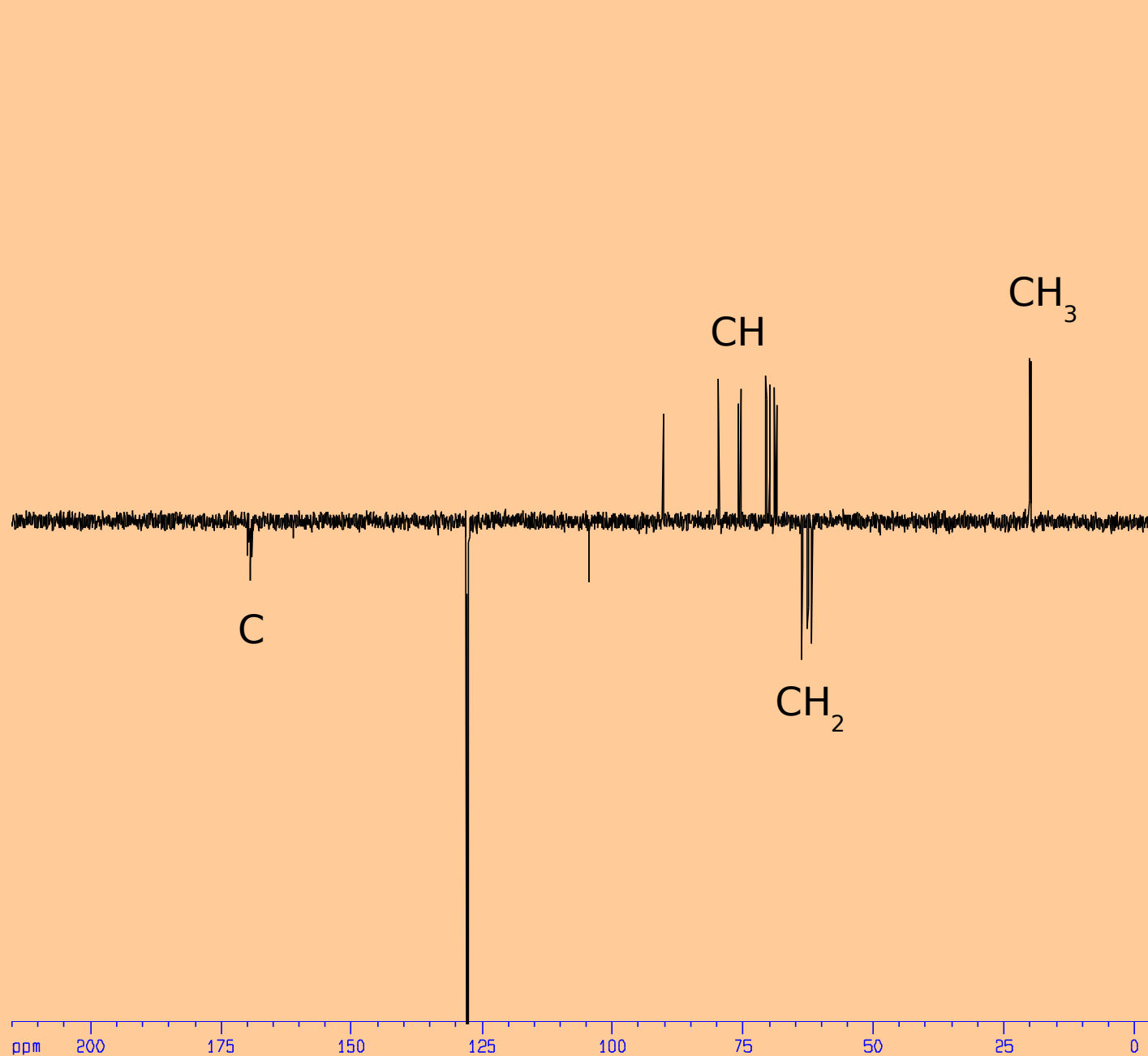


The APT or Jmodulation Experiment

For a doublet (CH), off-resonance pulse:



The APT or Jmodulation Experiment



Current Data Parameters
NAME test
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20070220
Time 9.32
INSTRUM spect
PROBHD 5 mm TXI 1H-13
PULPROG jmod
TD 65536
SOLVENT CDCl3
NS 74
DS 4
SWH 30030.029 Hz
FIDRES 0.458222 Hz
AQ 1.0912410 sec
RG 16384
DN 16.650 usec
DE 6.00 usec
TE 295.8 K
CNS12 145.000000
CNS111 1.000000
D1 2.0000000 sec
D20 0.00889655 sec
DELTA 0.00001783 sec
MCREST 0.0000000 sec
MCRMK 0.01500000 sec

----- CHANNEL f1 -----
NUC1 13C
P1 14.00 usec
p2 28.00 usec
PL1 -3.00 dB
SFO1 125.8206594 MHz

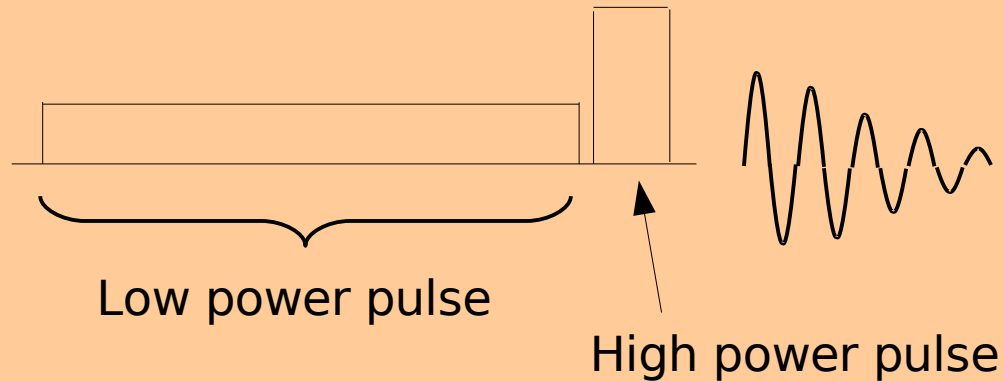
===== CHANNEL f2 =====
CROPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.50 dB
PL12 18.14 dB
SFO2 500.3320013 MHz

F2 - Processing parameters
SI 32768
SF 125.8080790 MHz
MDM EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
CY 12.50 cm
F1P 215.000 ppm
F1 27048.74 Hz
F2P -3.000 ppm
F2 -629.04 Hz
PPNDM 11.00000 ppm/cm
HZCM 1383.88892 Hz/cm

Solvent Suppression

Presaturation:

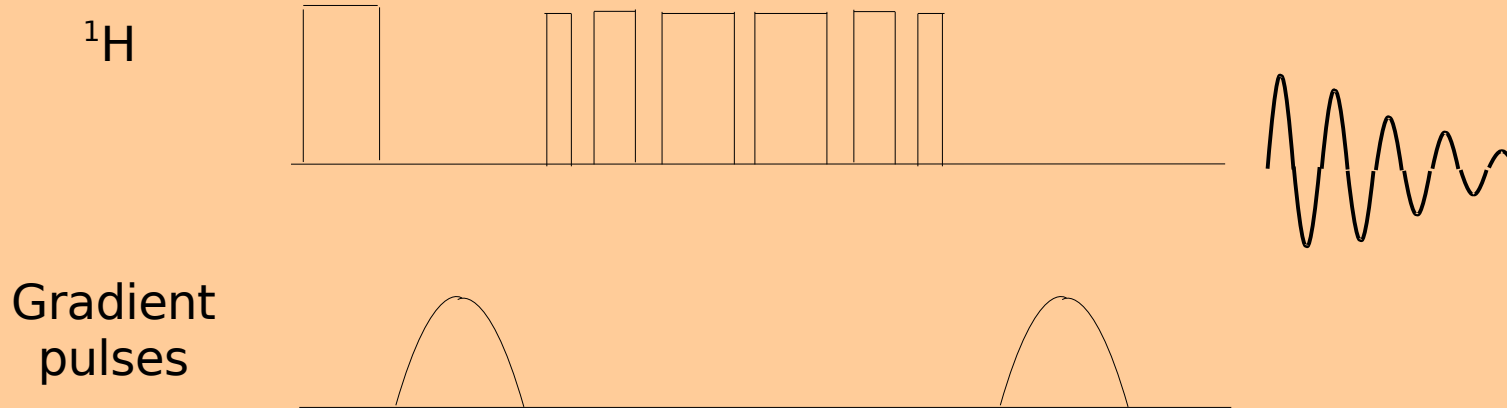


During the relaxation delay a long, low-power pulse is issued at the solvent frequency causing saturation of the solvent resonance and considerably reducing its signal amplitude. Since the low power pulse is relatively long its excitation bandwidth is very narrow and excites only the solvent resonance.

Not a good choice when exchangeable protons are to be observed.

Solvent Suppression

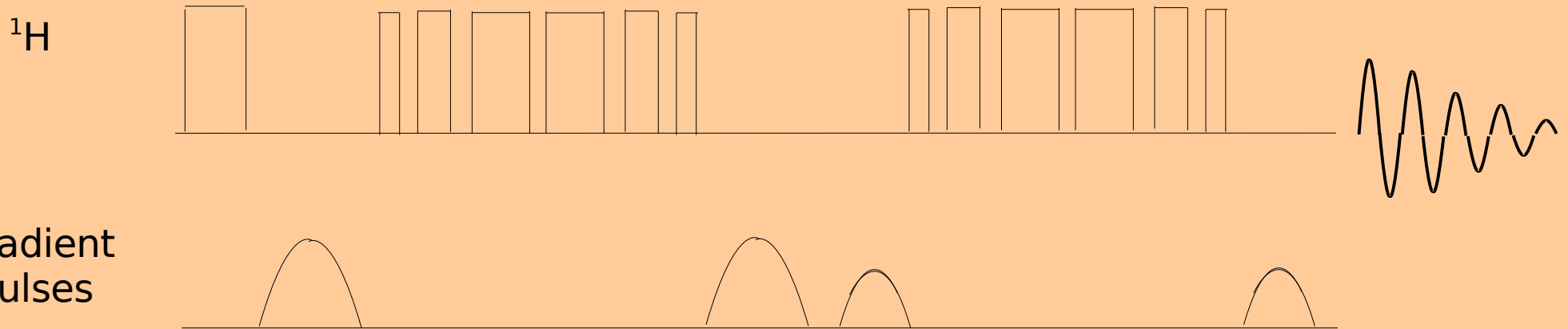
Watergate (WATER suppression by GrAdient Tailored Excitation):



An example of a class of pulse clusters called binomials ... in this case
3,9,19,19,9,3

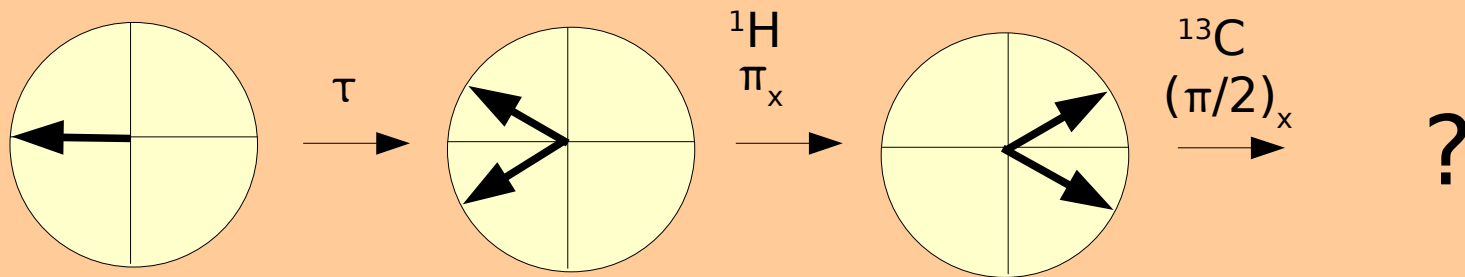
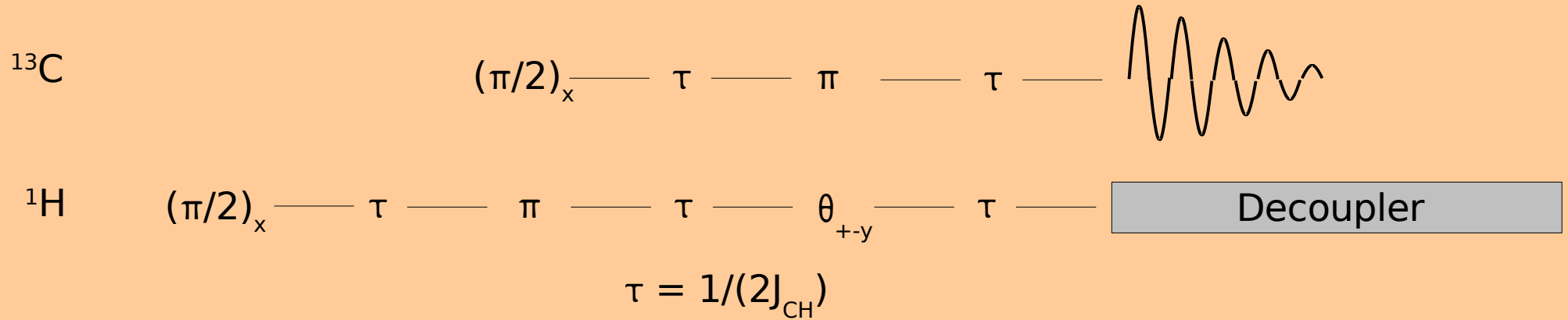
Solvent Suppression

Excitation sculpting:



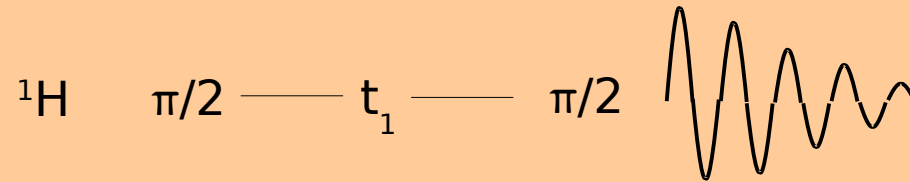
This is simply two Watergates in a row. The advantage of doing this experiment is that baseline distortion is much reduced.

The DEPT Experiment (Distortionless Enhancement by Polarization Transfer)



Must use quantum mechanically derived *product operators* to analyse this pulse sequence.

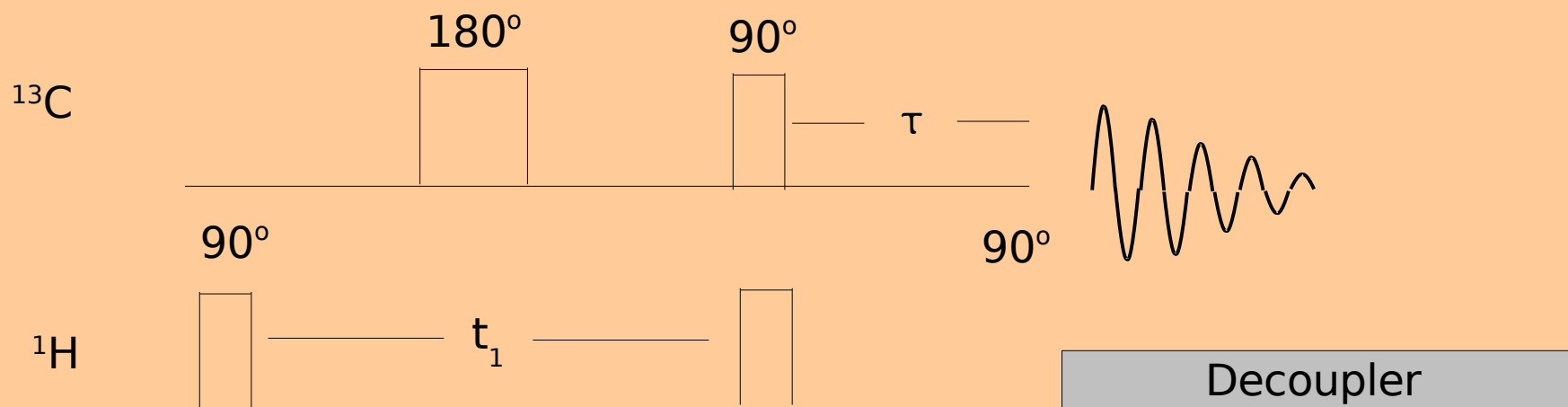
The COSY Experiment (Correlation Spectroscopy)



This deceptively simple pulse sequence cannot be analysed using the vector model ... quantum mechanical methods must be used.

t_1 is a **variable** time interval. Multiple experiments are done, acquiring data that differ only by the variable time interval, t_1 .

The HETCOR Experiment (Heteronuclear Correlation Spectroscopy)

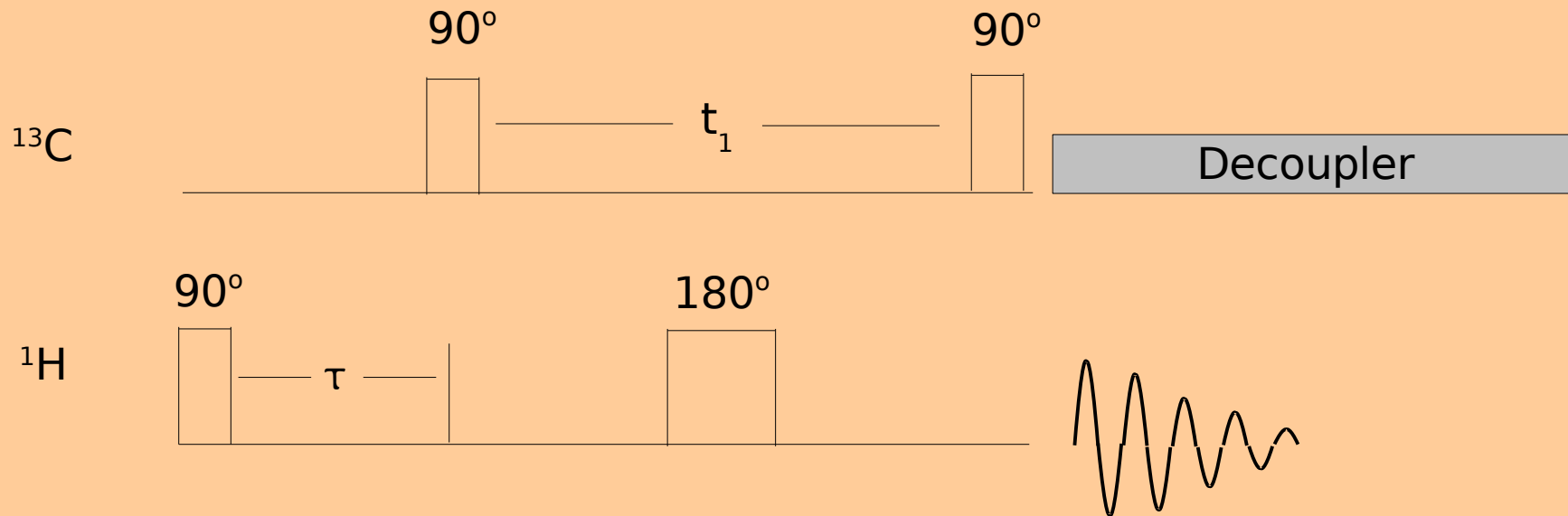


t_1 - variable time delay

$t = 1/(2J_{\text{CH}})$

^{13}C detected .. not used any more .. HMQC or HSQC more sensitive.

The HMQC Experiment (Heteronuclear Multiple Quantum Coherence)



This is a so-called 'inverse detected' experiment. Instead of recording the signal from the ^{13}C nuclei, the ^1H signal is acquired. Maximum sensitivity achieved using an inverse probe.

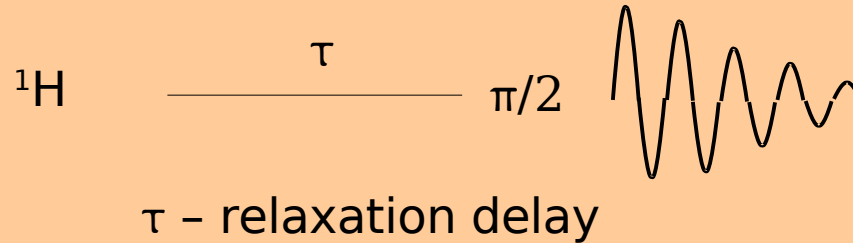
Can only use quantum mechanics to understand this pulse sequence.

Bruker Pulse Programs

Bruker spectrometers run pulse programs in the **pulse programmer** located in the console. The pulse programmer is actually a specialized computer which is built to allow very high speed switching of power levels and accurate timing of pulse lengths and delays.

The source code (that is, the human readable code) for the program is located on the workstation and can be edited there, if need be. Once the program is edited it is compiled on the workstation and the binary code is sent to the pulse programmer for execution. So, when an experiment is being run the pulse program is not actually executing on the workstation but rather in the console.

Bruker Pulse Programs



```
;zg  
;avance-version (02/05/31)  
;1D sequence
```

```
#include <Avance.incl>
```

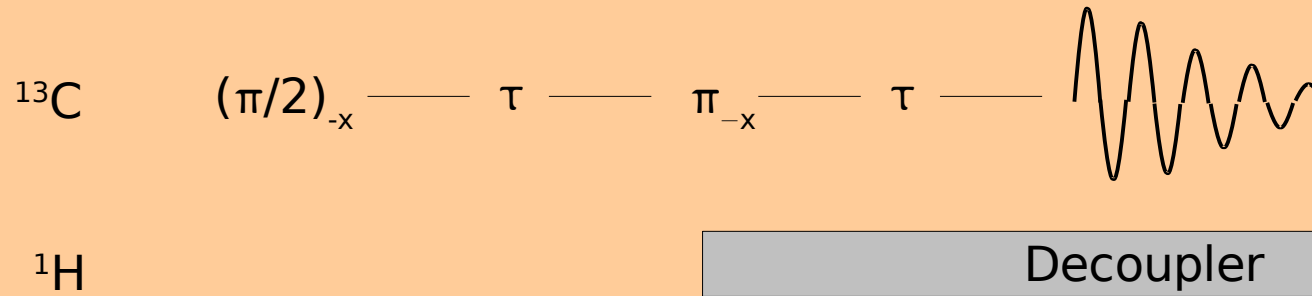
```
1 ze                   ;zero the memory  
2 d1                   ;relaxation delay time  
  p1 ph1               ;pulse, phase from ph1 list  
  go=2 ph31            ;acquire data, phase from list  
  30m mc #0 to 2 F0(zd) ;write to disk  
exit
```

```
ph1=0 2 2 0 1 3 3 1       ;pulse phase program  
ph31=0 2 2 0 1 3 3 1      ;receiver phase program
```

```
;p11 : f1 channel - power level for pulse (default)  
;p1 : f1 channel - high power pulse  
;d1 : relaxation delay; 1-5 * T1  
;NS: 1 * n, total number of scans: NS * TD0
```

This part gives some information about how to set up the experiment

Bruker Pulse Programs



```

;jmod
;avance-version (02/05/31)
;j-modulated spin-echo for X-nuclei coupled to H-1 to determine
;  number of attached protons
    
```

```

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

```
"p2=p1*2"
```

```
"d20=1s/(cnst2*cnst11)"
```

```

1 ze
  30m pl12:f2           ;set power level for decoupler
2 d1
  4u do:f2             ;decoupler off
  p1 ph1              ;carbon 90° pulse phase ph1
  d20                 ;1/2J delay time
  (p2 ph2):f1         ;carbon 180° pulse on transmitter (f1) phase 2
  d20 cpd2:f2         ;1/2J delay time, turn on 1H decoupler
  go=2 ph31           ;acquire data phase 31, loop back to 2
  30m mc #0 to 2 F0(zd) ;save data to disk
  d20 do:f2           ;decoupler off
exit                  ;finish
    
```

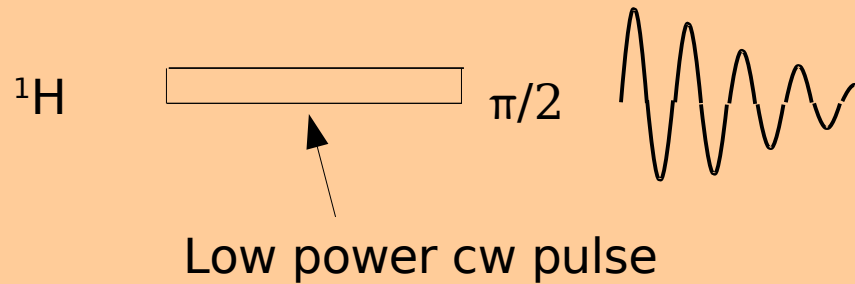
```

ph1=0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3
ph2=0 2 1 3 1 3 2 0 1 3 2 0 2 0 1 3
ph31=0 0 2 2 1 1 3 3
    
```

Bruker Pulse Programs

```
;pl1 : f1 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;d1 : relaxation delay; 1-5 * T1
;d20: 1/(J(XH)) X, XH2 positive, XH, XH3 negative
;    1/(2J(XH)) X only
;cnst2: = J(XH)
;cnst11: = 1 X, XH2 positive, XH, XH3 negative
;        2 X only
;NS: 4 * n, total number of scans: NS * TD0
;DS: 4
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence
```

Bruker Pulse Programs



```
;zgpr  
;avance-version (02/05/31)  
;1D sequence with f1 presaturation
```

```
#include <Avance.incl>
```

```
"d12=20u"
```

```
1 ze  
2 d12 pl9:f1 ;short delay, set transmitter power level to pl9  
  d1 cw:f1 ph29 ;relaxation delay, cw decouple pulse on transmitter channel  
  4u do:f1 ;short delay, turn off decouple pulses  
  d12 pl1:f1 ;short delay, set transmitter power level  
  p1 ph1 ;high power transmitter pulse  
  go=2 ph31 ;acquire data  
  30m mc #0 to 2 F0(zd) ;save data  
exit
```

```
ph1=0 2 2 0 1 3 3 1  
ph29=0  
ph31=0 2 2 0 1 3 3 1
```

```
;p1 : f1 channel - power level for pulse (default)  
;p9 : f1 channel - power level for presaturation  
;p1 : f1 channel - 90 degree high power pulse  
;d1 : relaxation delay; 1-5 * T1  
;d12: delay for power switching [20 usec]  
;NS: 1 * n, total number of scans: NS * TD0
```

Bruker Pulse Programs

```
;hmbcgpndqf
;HMBC
```

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

```
"p2=p1*2"
```

```
"d0=3u"
```

```
"d6=1s/(cnst13*2)"
```

```
1 ze
```

```
2 d1
```

```
3 p1 ph1
```

```
d6
```

```
p3:f2 ph3
```

```
d0
```

```
50u UNBLKGRAD
```

```
p16:gp1
```

```
d16
```

```
p2 ph2
```

```
50u
```

```
p16:gp2
```

```
d16
```

```
d0
```

```
p3:f2 ph4
```

```
4u
```

```
p16:gp3
```

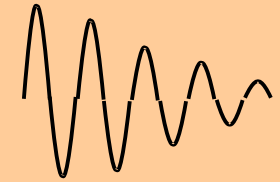
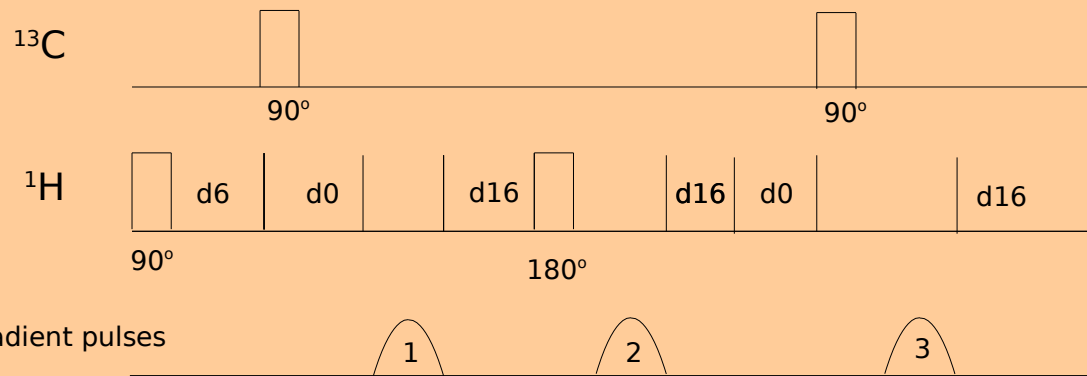
```
d16
```

```
4u BLKGRAD
```

```
go=2 ph31
```

```
d1 mc #0 to 2 F1QF(id0)
```

```
exit
```



```
;relaxation delay
;90° f1 channel high power pulse
;delay for evolution of long-range couplings
;90° f2 channel high power pulse
;variable delay
;turn on gradient amplifier
;gradient pulse 1
;delay for recovery from grad pulse
;180° f1 channel pulse
;50 usec delay
;gradient pulse 2
;delay for recovery from grad pulse
;variable delay
;90° f2 channel high power pulse
;4 usec delay
;gradient pulse 3
;delay for recovery from grad pulse
;4 usec delay, turn off gradient amplifier
;data acquisition
;store data
```

Bruker Pulse Programs

```
ph1=0
ph2=0 0 2 2
ph3=0 2
ph4=0 0 0 0 2 2 2 2
ph31=0 2 0 2 2 0 2 0
```

```
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel - 90 degree high power pulse
;p16: homospoil/gradient pulse
;d0 : incremented delay (2D)           [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d6 : delay for evolution of long range couplings
;d16: delay for homospoil/gradient recovery
;cnst13: = J(XH) long range
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 2 * n
;DS: 16
;td1: number of experiments
;FnMODE: QF
```

```
;use gradient ratio:   gp 1 : gp 2 : gp 3
;                       50 : 30 : 40.1  for C-13
;                       70 : 30 : 50.1  for N-15
```

```
;for z-only gradients:
;gpz1: 50% for C-13, 70% for N-15
;gpz2: 30%
;gpz3: 40.1% for C-13, 50.1% for N-15
```

```
;use gradient files:
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
```

Phase Programming

When using quadrature detection, two receivers are used. If they do not provide **identical** signals then there will be artifacts introduced into the spectrum. A dc offset will result in a 'peak' at the center of the spectrum and if the detectors are other than exactly 90° out of phase this will result in 'ghost' peaks mirrored through the center of the spectrum.

Use of a phase program will help to get rid of these unwanted artifacts. Actually, phase programming is much more general than this .. it is used to **select** wanted peaks and suppress unwanted peaks for a particular pulse sequence. The peak selection rules are, for the most part, complex and require at least a rudimentary understanding of the quantum mechanics of nmr spectroscopy so we will look only at the very simplest of phase programs here.

The CYCLOPS phase program is $x, y, -x, -y$ for pulses and $x, y, -x, -y$ for the receiver phase. The opposite phases, say x and $-x$, will act to cancel any dc offset effects and receiver phase effects. If you watch the acquisition of fid's you can see the effect of the phase program as sequential fid's are accumulated. The fid's move up and down ... this is the dc offsets being cancelled.

Macro Programming

Macros are basically simple shortcuts for doing repetitive tasks withing Xwin-nmr. If there is a series of commands that you type regularly you can put them into a macro and simply type the name of the macro. For example, one often types 'ef', 'apk', 'abs' in that order to Fourier transform, phase correct and baseline correct the spectrum. These can easily be put into a macro called 'proc' (or anything that you want) and then simply typing 'proc' will execute the commands.

To edit a macro type 'edmac' and either select an existing macro to edit or give a new name for a new macro. Be a bit careful that you don't give the macro the name of an already existing command. For example, you would not want to call your new macro 'ef' since this is the internal Fourier transform command.

The creative programmer can use these macros to advantage and make the computer work for her.

Bruker Automation Programming

Pulse programs are compiled and run in the **pulse programmer** which directly controls the low level operation of the nmr hardware.

Automation programs are compiled and run in the **workstation** and are capable of higher level functions such as opening windows on the workstation screen and interacting with the operator. These are very powerful programs capable of automating many tasks. If you can think of something that you want to automate .. it can probably be done. Examples of automation programs:

- **multizg**: for running a series of two or more experiments unattended
- **multi_zgvt**: for running a series of experiments at different temperatures
- **setproj**: for setting reasonable projection files for 2D experiments rather than using edg
- **paropt**: for optimizing an acquisition parameter
- **multiefp**: for performing the **same** fourier transform and phase correction on a series of experiments

Bruker Automation Programming

```
/** ^A _*_C+_+_*_ *****/
/*      multizg          18.05.2000          */
/*****/
/*      Short Description :          */
/*      Performs multiple acquisitions on increasing expnos.          */
/*****/
/*      Keywords :          */
/*      serial acquisitions          */
/*****/
/*      Description/Usage :          */
/*      This AU program performs multiple acquisitions on          */
/*      increasing expnos. If datasets do not yet exist, the          */
/*      current dataset and its parameters are copied. If the          */
/*      data sets already exist, then the experiments are          */
/*      executed as they are.          */
/*      The total experiment time is estimated and printed out.          */
/*      The number of experiments to be performed can be          */
/*      specified on the command line :          */
/*      xau multizg <no of experiments>          */
/*****/
/*      Author(s) :          */
/*      Name      : Rainer Kerssebaum          */
/*      Organisation : Bruker Analytik          */
/*      Email     : rainer.kerssebaum@bruker.de          */
/*****/
/*      Name      Date      Modification:          */
/*      rke      941207      created          */
/*      eng      000327      switch ZGSAFETY off if on          */
/*      eng      000518      PathSystemTemp instead of /tmp          */
/*****/
/*
$Id: multizg,v 1.10 2000/07/12 11:39:50 gsc Exp $
*/
```

```
char *envpnt, tmpfile[PATH_MAX];
int startExpno;
int expTime, zgsafety;
static void PrintExpTime();
```

```
GETCURDATA;
```

Bruker Automation Programming

```
startExpno = expno;
if (strlen(cmd) == 0)
{
    i1=10;
    GETINT("Enter number of experiments : ",i1);
}
else
{
    if (1 != sscanf(cmd, "%d", &i1))
    {
        STOPMSG("illegal input");
    }
}
expTime = 0;
TIMES(i1)
    SETCURDATA;
    expTime += CalcExpTime() + 4;
    IEXPNO;
END
DEXPNO;
PrintExpTime(expTime, i1);

(void) sprintf (tmpfile,"%s/expt",PathSystemTemp());
(void) remove (tmpfile);

/* Turn zg safety off if on. Turn it back on at the end. */
envpnt = getenv("UXNMR_SAFETY");
zgsafety = 0;
if(envpnt != NULL)
{
    if(strcmp(envpnt, "on") == 0)
    {
        zgsafety = 1;
        CPR_exec("env set UXNMR_SAFETY=off", WAIT_TERM);
    }
}

expno = startExpno;
SETCURDATA;
```

Bruker Automation Programming

```
TIMES(i1)
  (void) sprintf(text,"running experiment # %d",loopcount1+1);
  Show_status(text);
  sleep(4);
  ZG;
  IEXPNO;
END
DEXPNO;

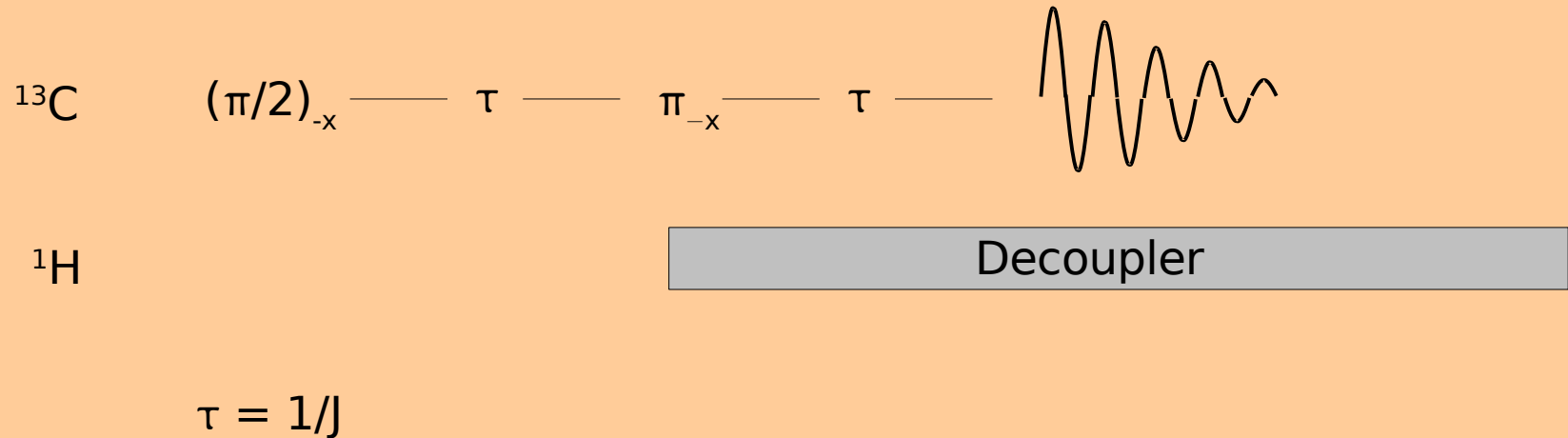
if(zgsafety == 1)
  CPR_exec("env set UXNMR_SAFETY=on", WAIT_TERM);

QUITMSG("--- multizg finished ---");

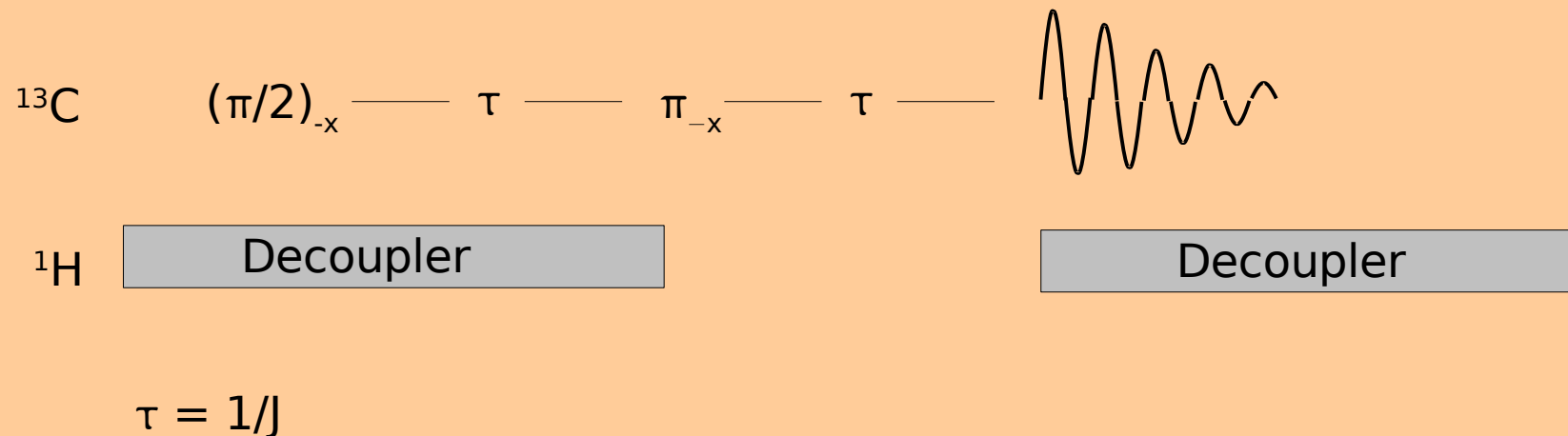
#include <inc/exptUtil> /* utilities for printing and calculation of
                        experiment time */
```

Projects

The Jmodulation experiment is usually done this way:



We will alter the pulse program to do it this way to get exactly the same result.



Projects

The HMBC pulse program as supplied by Bruker will not do solvent suppression. We want to do an HMBC experiment on sucrose dissolved in D₂O. If we use the 'canned' pulse program supplied by Bruker there will be a large solvent ridge in the 2D spectrum and our sucrose peaks will be very small. Solvent suppression should reduce the size of the solvent peak and allow us to increase the receiver gain which will give much better cross peaks in the spectrum.

The technique is to cut-and-paste the presaturation portion of a regular 1D presaturation pulse program into the HMBC pulse program and run the experiment.

References

(all available in the Natural Sciences library except *)

Introductory with little math:

*Bruker, *BASH programming manuals*

Sanders and Hunter, *Modern NMR Spectroscopy: A Guide for Chemists.*

Derome, *Modern NMR Techniques for chemistry research.*

Intermediate with math:

Levitt, *Spin Dynamics.*

Keeler, *Understanding NMR Spectroscopy.*

Advanced .. lots of math:

Slichter, *Principles of Magnetic Resonance.*

Ernst, Bodenhausen, Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions.*

Practical information:

*Braun, Kalinowski, Berger, *200 and More Basic NMR Experiments.*

Fukushima, Roeder, *Experimental Pulse NMR; A Nuts and Bolts Approach.*

My Website:

http://chem4823.usask.ca/nmr/practical_nmr.html