

## running HIFI

*NMRFAM workshop - 2006*

tonelli@nmrfam.wisc.edu

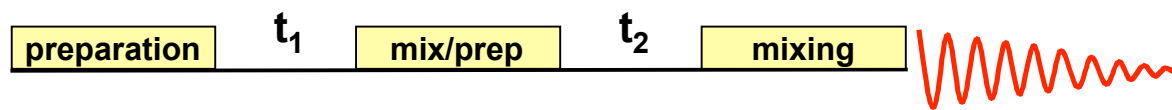
- pulse sequence
- collecting orthogonal and tilted planes
- processing tilted planes
- predicting the best angle for a tilted plane (**hifi.sh** program)
- extracting peak list (**generate\_peaklist.sh** program)
- automated HIFI (**mHIFI** macro)
- macro for collecting/processing orthogonal planes (**mORTHO0** macro)

macro mORTH00

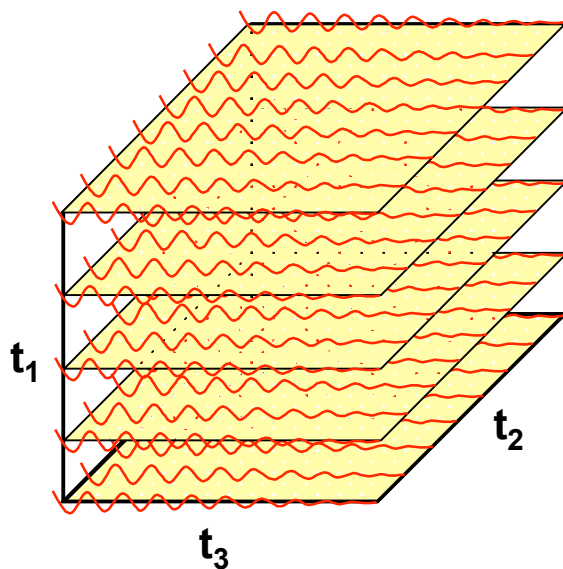


pulse sequence:

“in principle” any sequence can be modified to run for HIFI

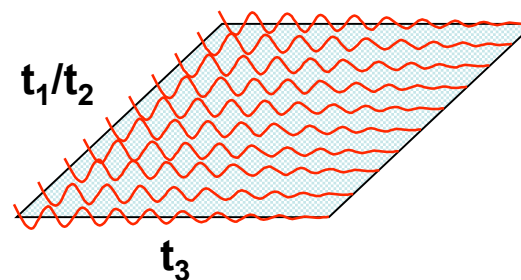


Conventional 3D spectrum

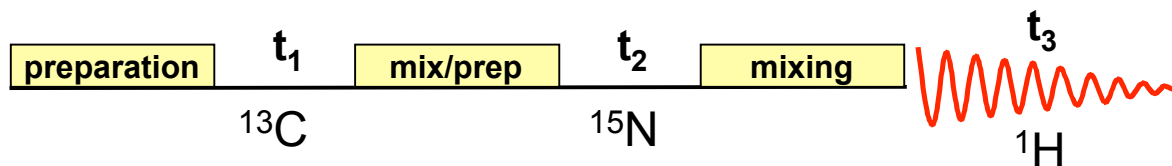


$t_1$  and  $t_2$  are incremented independently

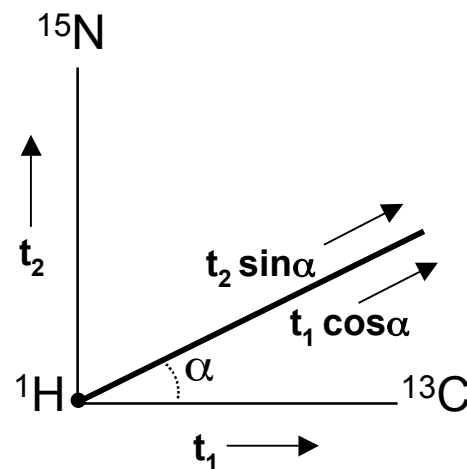
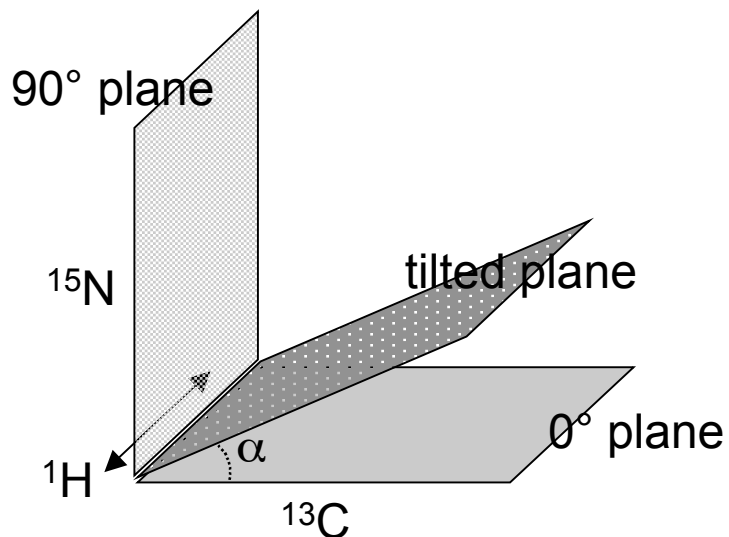
RD spectrum



$t_1$  and  $t_2$  are incremented simultaneously  
point by point



## orthogonal and tilted planes



### $^{13}\text{C}$ dimension

sw1 ni

$\tau_1 = i_1 / \text{sw1} \quad i_1 = 0 \rightarrow (n_1 - 1)$

### $^{15}\text{N}$ dimension

sw2 ni2

$\tau_2 = i_2 / \text{sw2} \quad i_2 = 0 \rightarrow (n_2 - 1)$

### $^{13}\text{C}/^{15}\text{N}$ co-evolved dimension

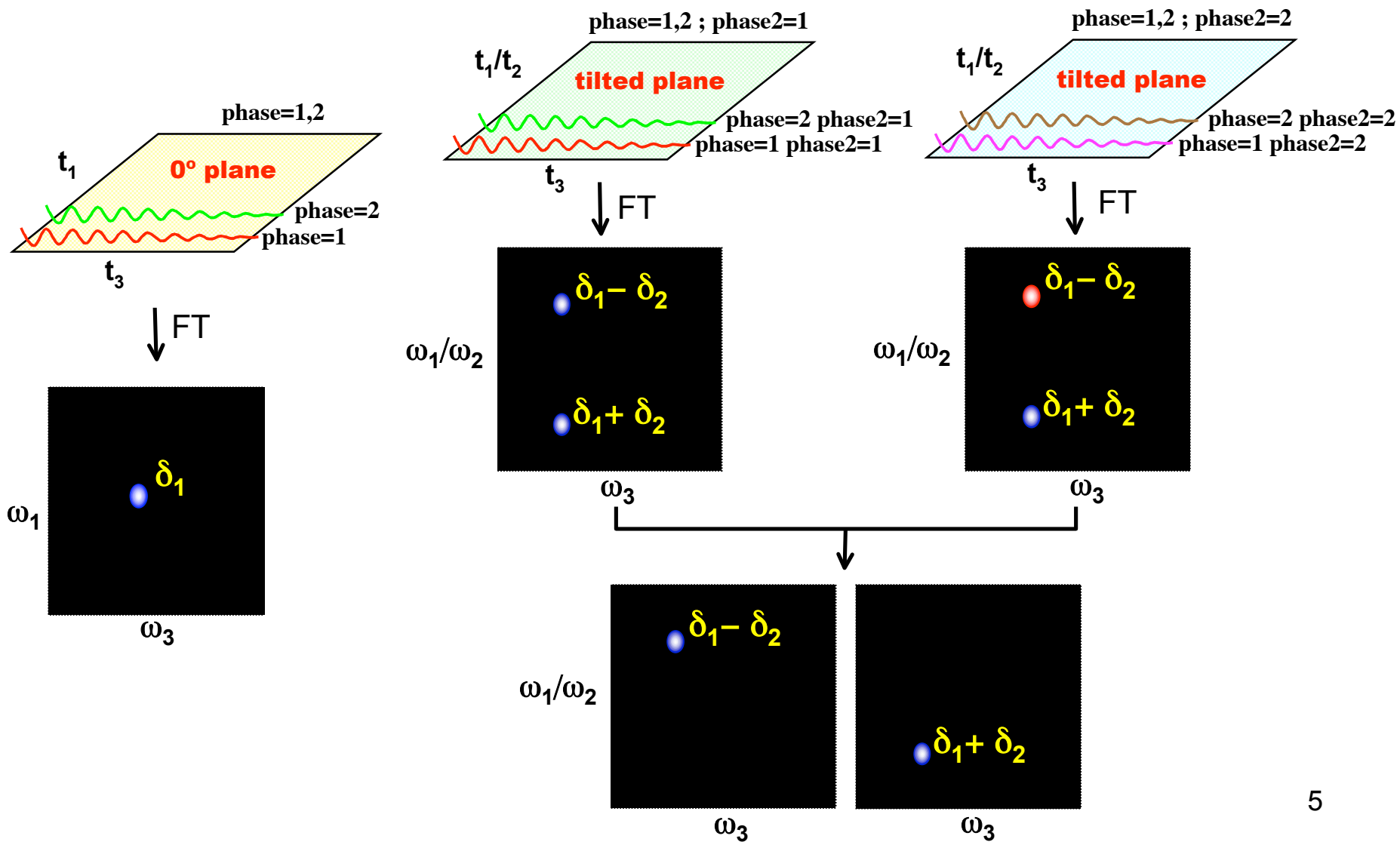
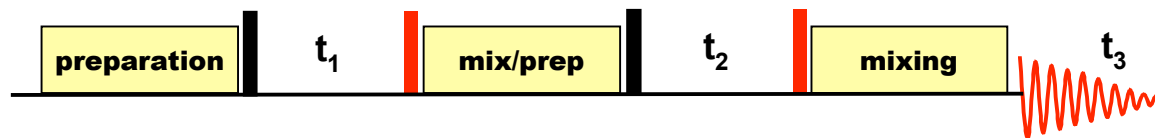
sw1 sw2 ni tilt

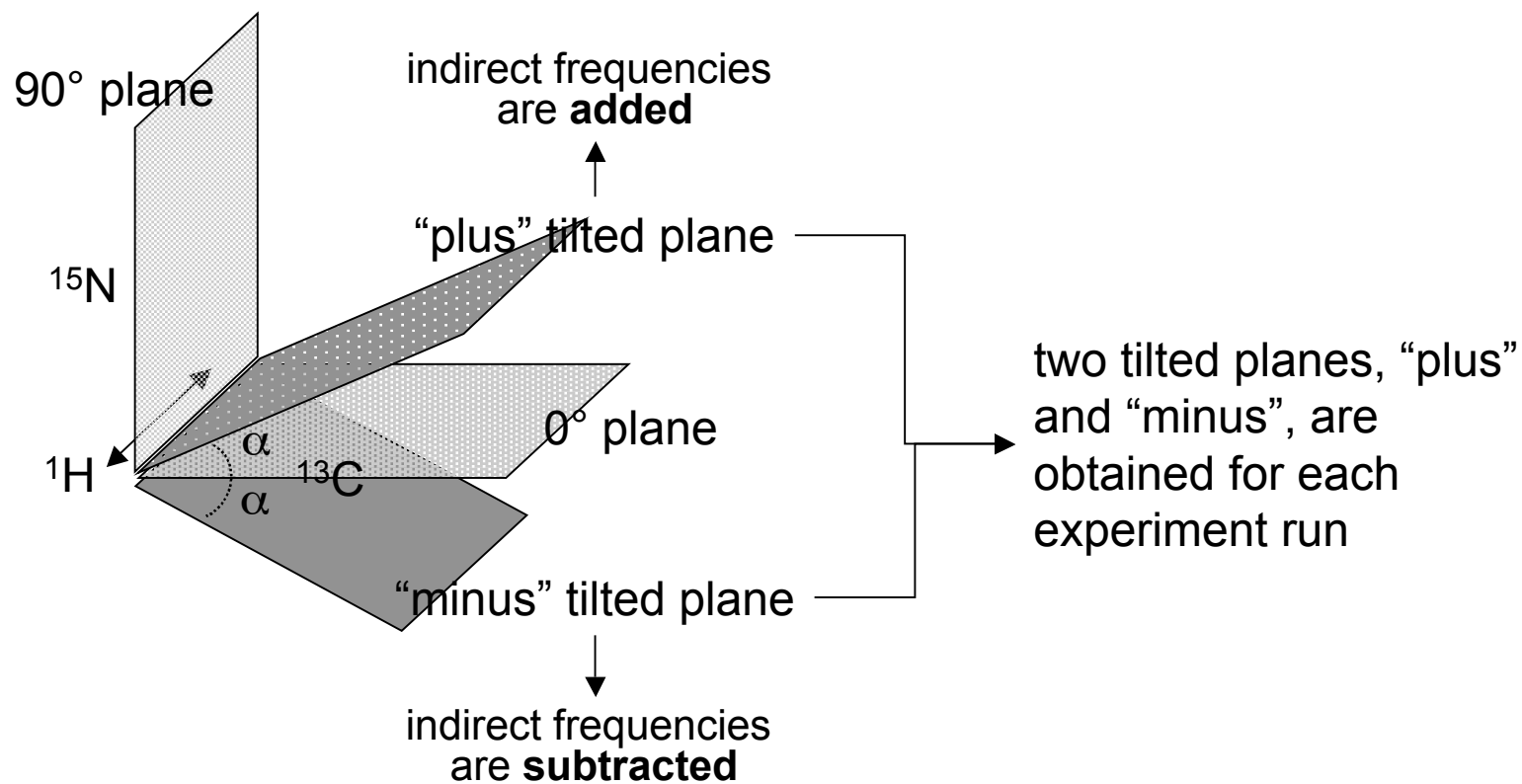
$\tau_1 = (i_1 / \text{sw1}) \cdot \cos(\text{tilt}) \quad i_1 = 0 \rightarrow (n_1 - 1)$

$\tau_2 = (i_1 / \text{sw2}) \cdot \sin(\text{tilt}) \quad i_1 = 0 \rightarrow (n_1 - 1)$

running HIFI...

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## Parameters involved with setting up HIFI experiments

### Already existing parameters:

- sw1/sw2    spectral windows in indirect dimensions
- ni/ni2    number of increments in indirect dimensions
- phase/phase2    used for States-TPPI quadrature of indirect dimension
- f1180/f2180    flag for starting indirect dimension at half dwell-time

### New parameters:

- HIFI    flag for turning on HIFI data collection
- tilt    angle of HIFI tilted plane

collect orthogonal plane – 0 and 90 degrees (e.g.  $^{13}\text{C}$  and  $^{15}\text{N}$  dimensions)

0 degrees ( $^1\text{H}$ - $^{13}\text{C}$  plane)

ni = # of increments  
phase = 1,2 (States-TPPI)  
f1180 = 'n' or 'y'

ni2 = 0 \*  
phase2 = 1 \*  
f2180 – ignored

HIFI = 'n' \*  
tilt - ignored

90 degrees ( $^1\text{H}$ - $^{15}\text{N}$  plane)

ni = 0 \*  
phase = 1 \*  
f1180 – ignored

ni2 = # of increments  
phase2 = 1,2 (States-TPPI)  
f2180 = 'n' or 'y'

HIFI = 'n' \*  
tilt - ignored



collect tilted plane – >0 and <90 degrees

HIFI = 't'

0 < tilt < 90

ni – number of increments

phase = 1,2 (States-TPPI quadrature detection for  $^{13}\text{C}$  dimension)

phase2 = 1,2 (States-TPPI quadrature detection for  $^{15}\text{N}$  dimension)

f1180 = 'n' or 'y' (flag for starting HIFI indirect dimension at half dwell time)

ni2 = 0\* (must be zero)

f2180 – ignored

example

VNMR  
COMMAND  
LINE

```
HIFI='t'  
tilt=40  
ni=64  
phase=1,2  
phase2=1,2  
f1180='y'  
ni2=0
```

collect a 40° HIFI tilted plane with 64 indirect complex points (x4 - given the two simultaneously evolving frequencies) and starting the indirect dimension at half dwell time

\* these parameters are NOT ignored and must be set to these values

## processing tilted planes

(the FIDs needs to be combined properly)

vnmr (or vnmrJ)

two macros (same that are used for processing PR spectra):

**wft2dx** – to obtain spectrum with added indirect frequencies**wft2dy** – to obtain spectrum with subtracted indirect frequencies

after setting up the window function for the indirect dimension (can use **ssb1** macro) simply type **wft2dx** or **wft2dy** to obtain the spectrum where the indirect frequencies are added or subtracted, respectively)

example (in the vnmr command line type)

VNMR  
COMMAND  
LINE**ssb ssb1** NMRFAM macros - set window function for direct and indirect dimension**wft2dx** or **wft2dy**

these macros simply define the proper coefficients for combining the FIDs:

**wft2dx** : wft2d(1,0,-1,0, 0,-1,0,-1, 0,-1,0, -1, -1,0,1,0)**wft2dy** : wft2d(1,0,-1,0, 0,1,0,1, 0,-1,0, -1, 1,0,-1,0)

## processing tilted planes

## nmrPipe

use the command **QMIX** to combine the FIDs according to a given matrix

script for processing “**plus**” spectrum

FILE CONTENT

```
#!/bin/csh -f
set MATRIX = (1 0 \
              0 1 \
              0 1 \
              -1 0 ) } matrix used by the QMIX command for
                      combining the FIDs

nmrPipe -in HIFI.fid
| nmrPipe -fn QMIX -ic 4 -oc 2 -cList $MATRIX -time //
| nmrPipe -fn POLY -time //
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 0.5 //
| nmrPipe -fn ZF -size 2048 //
| nmrPipe -fn FT //
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di //
| nmrPipe -fn TP //
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 1.0 //
| nmrPipe -fn ZF -size 1024 //
| nmrPipe -fn FT //
| nmrPipe -fn PS -p0 -90.0 -p1 180.0 -di //
-verb -ov -out HIFI_plus.ft2
```

script for processing “**minus**” spectrum

FILE CONTENT

```
#!/bin/csh -f
set MATRIX = (1 0 \
              0 -1 \
              0 1 \
              1 0 ) } matrix used by the QMIX command for
                      combining the FIDs

nmrPipe -in HIFI.fid
| nmrPipe -fn QMIX -ic 4 -oc 2 -cList $MATRIX -time //
| nmrPipe -fn POLY -time //
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 0.5 //
| nmrPipe -fn ZF -size 2048 //
| nmrPipe -fn FT //
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di //
| nmrPipe -fn TP //
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 1.0 //
| nmrPipe -fn ZF -size 1024 //
| nmrPipe -fn FT //
| nmrPipe -fn PS -p0 -90.0 -p1 180.0 -di //
-verb -ov -out HIFI_minus.ft2
```

## figuring out the matrices

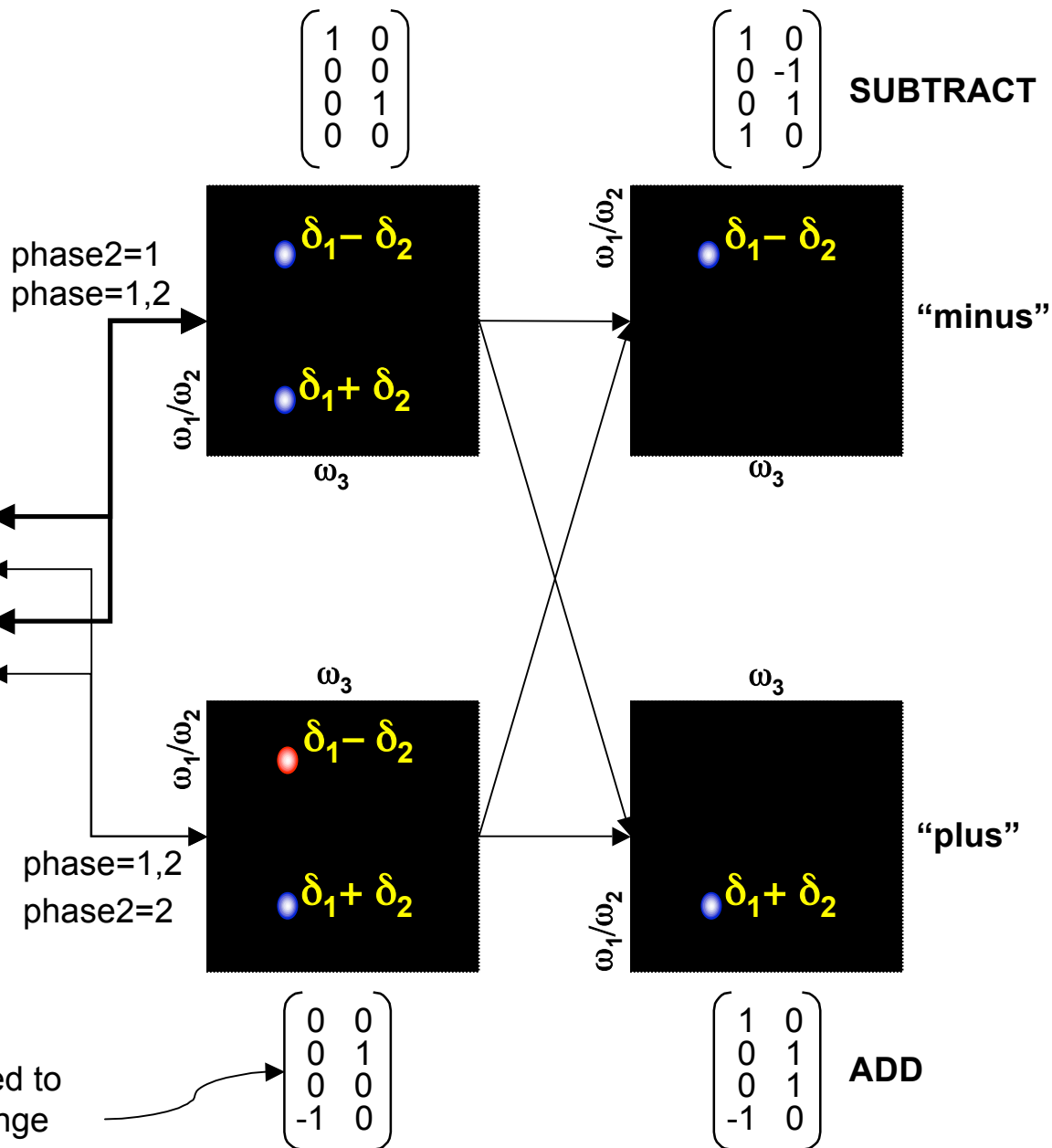
for each increment 4 FIDs  
are collected with all different  
combinations of phase and  
phase2:

array='phase,phase2'

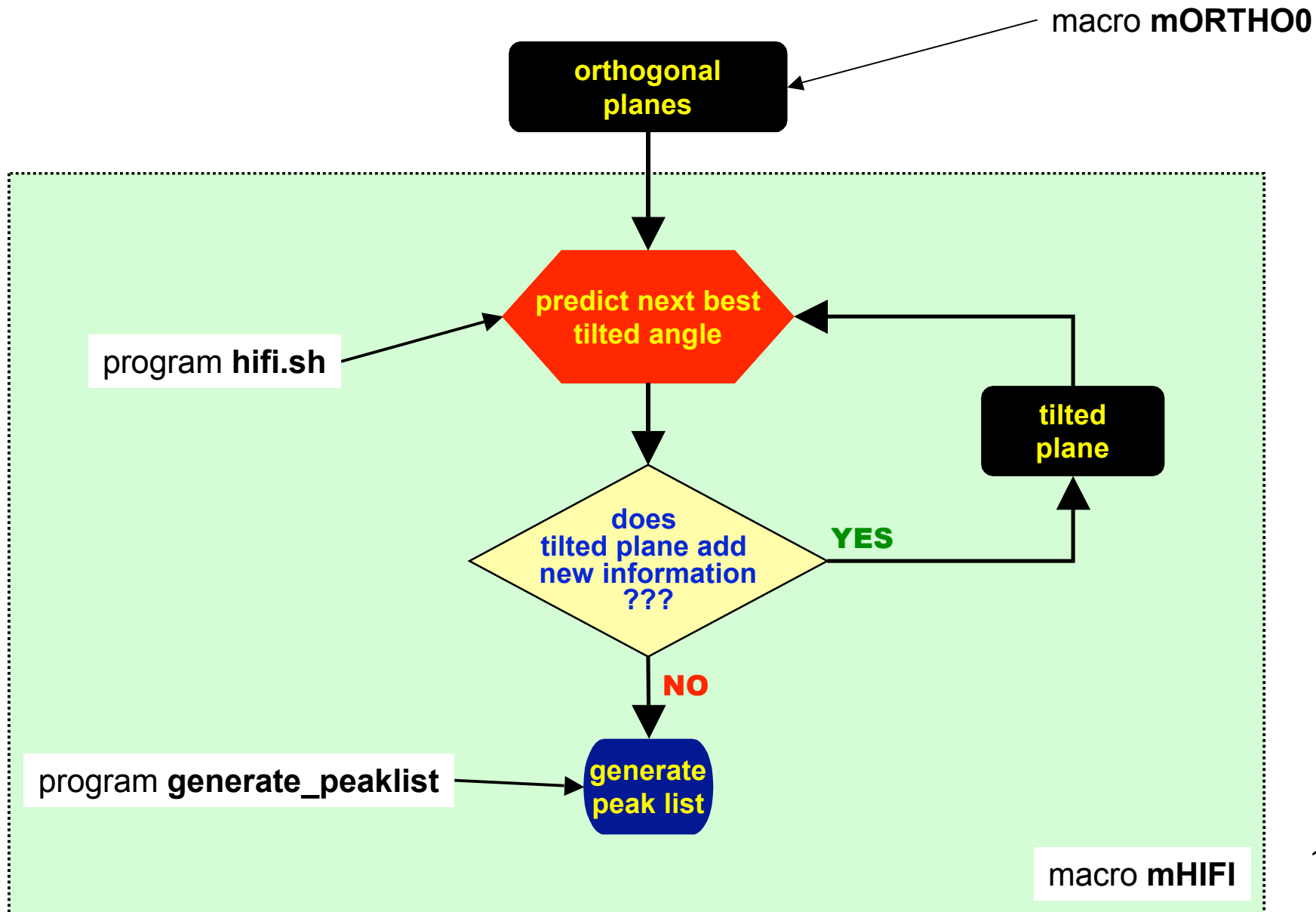
	phase	phase2
1	1	1
2	1	2
3	2	1
4	2	2

matrices are used to combine  
the FIDs properly

real and imaginary are swapped to  
account for the 90° phase change  
given by the cosine modulation



# flow chart of HIFI



## predicting the best angle for a tilted plane

program:

- hifi.sh** – standalone program, runs from the unix prompt
- predicts the tilt angle that yields the most information
- requires the number of residues in the protein as input

input files:

**orthogonal planes** or previously collected **tilted planes** – these must be processed using nmrPipe

**input\_hifi.txt** – contains names of orthogonal or tilted spectra in nmrPipe format

output files:

**workspace\_hifi.mat** – binary matrix file that contains all the results of the analysis of the orthogonal and tilted planes

**suggested\_plane.txt** – contains the predicted best angle for the next tilted plane. If this angle is 0, all information has been collected and no more tilted planes need to be recorded

example 1: run **hifi.sh** to predict first tilted plane

input files:

**hnco\_0.ft2, hnco\_90.ft2** – orthogonal planes processed with nmrPipe

**input hifi.txt**

FILE	CONTENT
	path/hnco_0.ft2 0
	path/hnco_90.ft2 90

angle at which plane was collected

name of the plane in nmrPipe format, including path

run **hifi.sh** program in a shell by typing at the unix prompt:

UNIX PROMPT	CONTENT
	> path/hifi.sh hnco 76

hifi.sh program with path

type of experiment ran

number of residues in the protein

output files:

**workspace\_hifi.mat**

**suggested plane.txt**

FILE	CONTENT
	40

suggested angle for collecting next tilted plane, e.g. 40

example 2: run **hifi** to predict another tilted plane after the first one

input files:

**hnco\_40.ft2, hnco\_220.ft2** – latest “plus” and “minus” tilted planes collected, processed using nmrPipe

**input hifi.txt**

FILE  
CONTENT

path/hnco_40.ft2	40
path/hnco_320.ft2	320

**workspace\_hifi.mat** – binary matrix with analysis of orthogonal planes and previously collected tilted planes

run **hifi.sh** program in a shell by typing at the unix prompt:

UNIX  
PROMPT

```
> path/hifi.sh      hnco      100
```

output files:

**workspace\_hifi.mat** – updated binary matrix

**suggested\_plane.txt**

FILE  
CONTENT

33
----

→ suggested angle for collecting next tilted plane, e.g. 33

if the suggested angle is 0, all tilted planes have been collected



## extracting the peak list

program:

**generate\_peaklist.sh** – standalone program, runs from the unix prompt  
– generates the list of peaks at the proper 3D  
frequencies in ppm, after all tilted planes are collected

input files:

**workspace\_hifi.mat** – binary matrix, generated by **hifi.sh**, containing the  
results from the analysis of the orthogonal and tilted planes

**frequency\_list.txt** – contains the absolute frequency in MHz (parameters  
sfrq, dof and dof2, for  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$ , respectively) and the spectral window in  
Hz for each of the three dimensions

output files:

**prefix\_full\_list.peaks, prefix\_1.peaks, prefix\_2.peaks, etc.** – files  
containing the list of 3D peaks in ppm classified according to probability  
(1=highest probability) ; **prefix** is input by the user (see example).

**prefix\_full\_list.peaks** – complete list of 3D peaks

**prefix\_1.peaks** – list of 3D peaks with highest probability

example: run **generate\_peaklist.sh** to extract peak list at the end of HIFI run

input files:

**workspace\_hifi.mat** – binary matrix generated by **hifi.sh**

**frequency\_list.txt**

FILE CONTENT	sfrq	799.80866260	}	1 <sup>st</sup> channel – <sup>1</sup> H
	sw	12001.20012001		
	dfrq	201.14575010	}	2 <sup>nd</sup> channel – <sup>13</sup> C
	sw1	2000.00000000		
	dfrq2	81.05318040	}	3 <sup>rd</sup> channel – <sup>15</sup> N
	sw2	2199.97800022		

{ vnmr parameter name  
 { absolute frequency in MHz and spectral window in Hz for each dimension

run **generate\_peaklist.sh** program in a shell by typing at the unix prompt:

UNIX PROMPT **> path/generate\_peaklist.sh path/workspace\_hifi.mat path/frequency\_list.txt hnco\_**

{ generate\_peaklist program with path  
 { name of file is fixed, but path can be specified if not in current directory  
 { name is flexible, path can be specified if needed  
 { prefix for output file

... continue example

output files:

**hnco\_full\_list.peaks** – full list of 3D peaks in actual ppm

**hnco\_1.peaks**  
**hnco\_2.peaks**  
**hnco\_n.peaks** } partial lists of 3D peaks in actual ppm, divided  
according to probability (1=highest)

FILE  
CONTENT

1	7.735	177.685	117.999	1
2	8.197	177.394	120.517	1
3	8.226	176.753	121.816	1
.....				
44	8.080	175.126	124.332	1
45	8.029	176.138	121.559	1
46	7.948	174.025	121.362	1

peak number

$^1\text{H}$  ppm – direct dimension

$^{13}\text{C}$  ppm – 1<sup>st</sup> indirect dimension

$^{15}\text{N}$  ppm – 2<sup>nd</sup> indirect dimension

probability – 1=highest

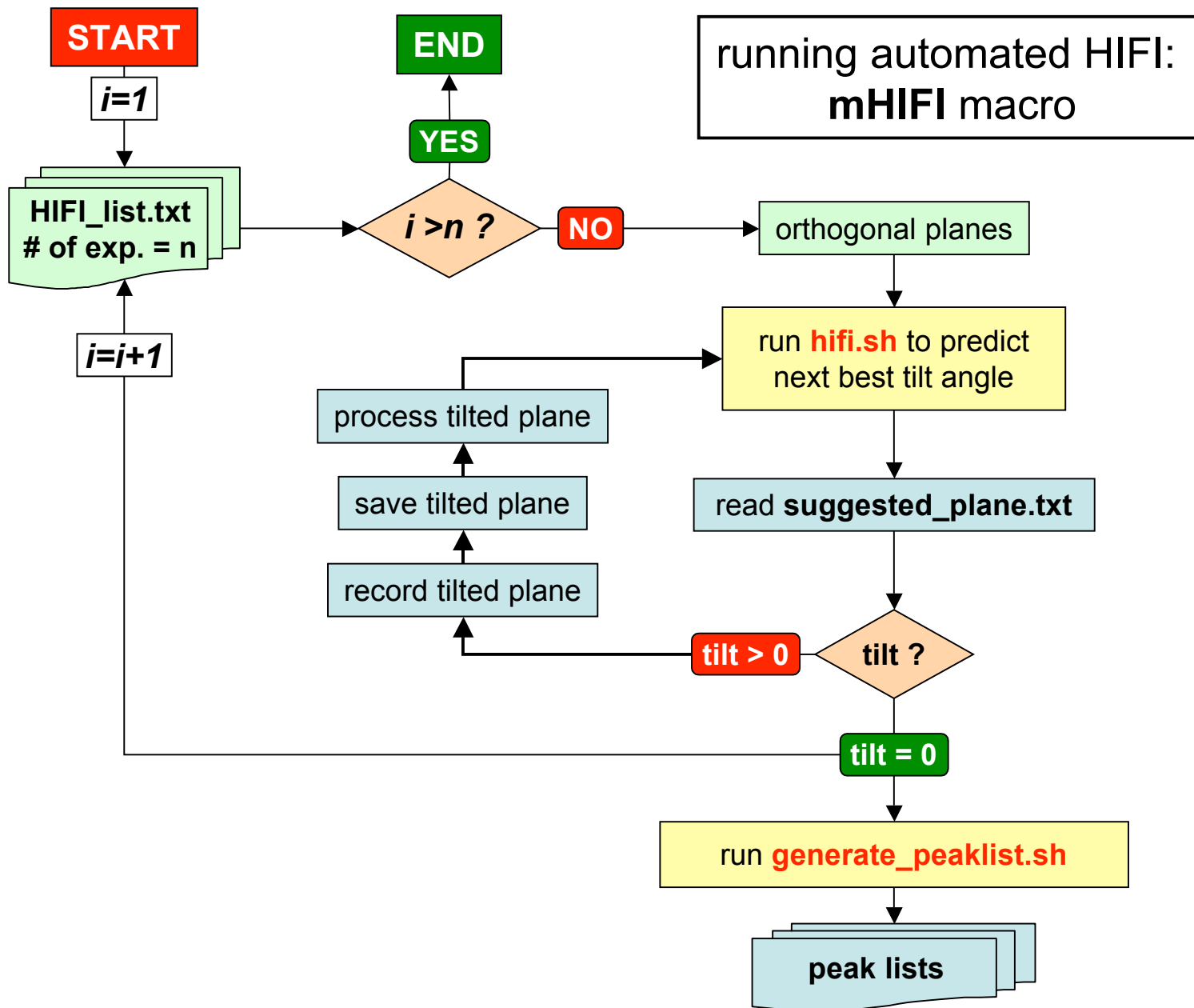
# Peak lists

CBCA(CO)NH experiment for ubiquitin (76 residues)

The number of peaks in each priority list depends on number of residues and probabilities of the peaks

Priority Index

First Priority List	Second Priority List	Third Priority List	Fourth Priority List
1 8.041 72.331 118.089 1 2 8.432 71.159 121.317 1 3 9.013 70.534 121.425 1 4 9.314 70.144 127.593 1 5 9.418 69.753 127.737 1 6 7.714 69.050 109.231 1 7 8.452 67.643 119.022 1 ... ... ... 156 7.440 37.281 111.314 1 157 7.440 37.383 111.258 1 158 7.917 27.541 119.879 1	1 8.628 69.597 125.155 2 2 8.641 64.908 117.444 2 3 8.445 63.736 120.420 2 4 8.210 55.610 124.222 2 5 8.537 54.282 123.899 2 6 7.140 54.126 118.914 2 7 8.047 39.983 116.081 2 ... ... ... 33 8.374 55.488 125.570 2 34 7.623 34.629 111.872 2 35 7.440 37.485 111.091 2	1 8.041 60.455 118.089 3 2 8.720 54.595 124.473 3 3 8.445 36.624 125.728 3 4 8.406 33.186 108.908 3 5 8.197 30.842 115.328 3 6 8.047 16.387 102.560 3 7 8.276 56.787 125.008 3 ... ... ... 26 8.693 29.835 120.465 3 27 8.269 45.237 115.331 3 28 7.440 37.587 111.035 3	1 8.432 59.517 121.317 4 2 8.419 56.079 123.039 4 3 8.720 54.907 108.836 4 4 8.197 30.842 124.222 4 5 8.106 55.619 124.574 4 6 9.359 45.693 121.196 4 7 9.359 45.868 121.123 4 ... ... ... 20 6.716 32.640 112.123 4 21 6.716 35.598 110.505 4 22 7.891 59.823 123.199 4



## running automated HIFI: **mHIFI** macro

vnmr/vnmrJ macro:

**mHIFI** – runs one or more automated HIFI experiments, including collecting and processing tilted planes, predicting next best tilt angle and generating peak lists

programs invoked by **mHIFI** macro:

**hifi.sh** – predicts the angle for the next best tilted plane

**generate\_peaklist.sh** – at the end of each HIFI experiment, it extracts the list of 3D peaks with proper frequencies in ppm

**nmrPipe** – used to process tilted planes

input files:

**orthogonal planes** – must be processed using nmrPipe

**HIFI\_input.txt** – text file with list of input parameters for running HIFI (see example)

output files:

**tilted planes** – including spectra processed with nmrPipe and processing scripts

**prefix \_ exp-directory** – for each completed HIFI experiment, a directory is created that contains, among other files (see example), the peak list files

example: run **mHIFI** macro to perform one or more automated HIFI experiments

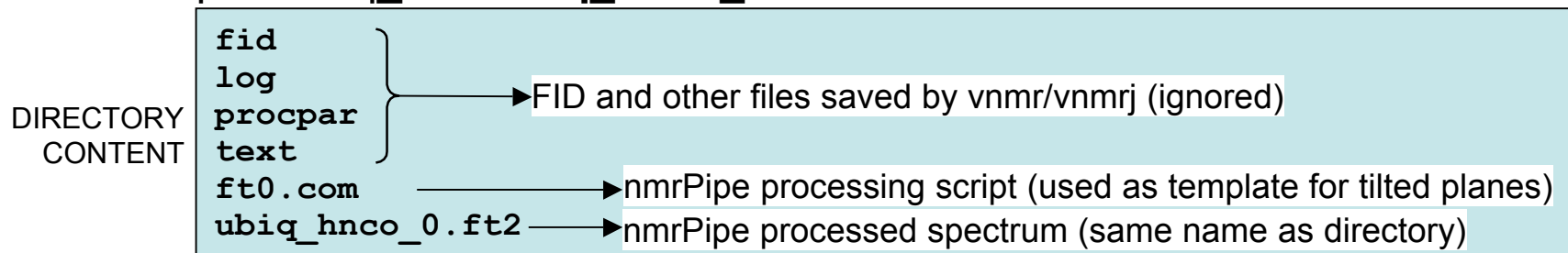
input files:

all input files must be present in the same directory, e.g. **path/ubiq\_HIFI**

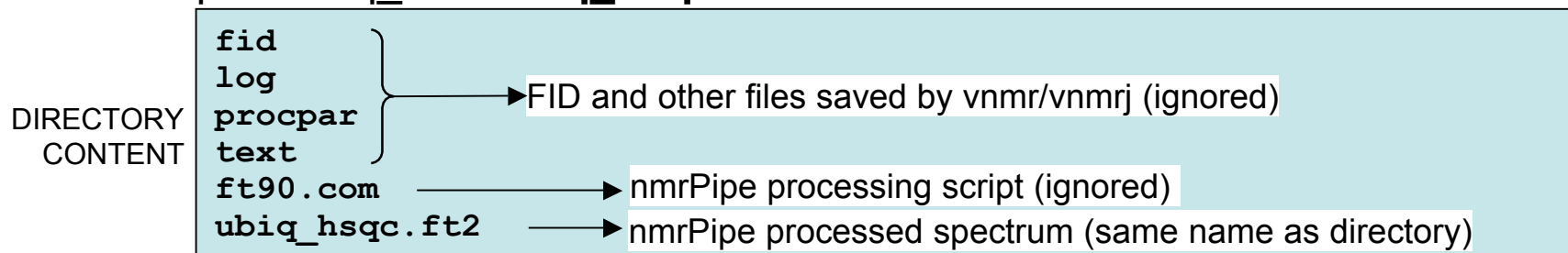
**orthogonal planes** – must be processed using nmrPipe:

- nmrPipe processed spectrum must have same name as saved FID, but with extension “.ft2”, and must be present within the same directory
- for 0° planes, nmrPipe processing script, **ft0.com**, must also be present within the fid directory (it will be used as a template for processing tilted planes)

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid** – 0° plane



**path/ubiq\_HIFI/ubiq\_hsqc.fid** – 90° plane (an HSQC is used for <sup>1</sup>H-<sup>15</sup>N plane)



example: run **mHIFI** macro to perform one or more automated HIFI experiments

input files:

**HIFI\_input.txt** – text file with list of input parameters for running HIFI:

- number of residues in the protein
- for each HIFI experiment
  - type of experiment
  - prefix
  - number of scans and number of increments to use for tilted planes
  - orthogonal planes

path/ubiq\_HIFI/ **HIFI\_input.txt**

FILE  
CONTENT

number of residues = 76				number of residues in the protein	
hnco	ubiq	2	48	ubiq_hnco_0	ubiq_hsqc
hncoca	ubiq	2	64	ubiq_hncoca_0	ubiq_hsqc
hnca	ubiq	4	64	ubiq_hnca_0	ubiq_hsqc
cbcaconh	ubiq	4	64	ubiq_cbcaconh_0	ubiq_hsqc
hncacb	ubiq	8	80	ubiq_hncacb_0	ubiq_hsqc
hncb	ubiq	8	80	ubiq_hncb_0	ubiq_hsqc

experiment type – all 7 experiments currently available are shown

prefix – combined with experiment type

number of increments for recording tilted planes

number of scans for recording tilted planes

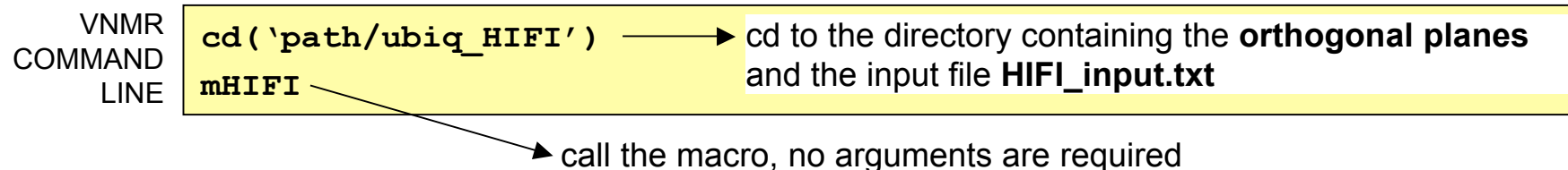
90° plane – same for all experiments – filename: **ubiq\_hsqc.fid/ubiq\_hsqc.ft2**

0° plane – filename: **ubiq\_hnco\_0.fid/ubiq\_hnco\_0.ft2**



example: run **mHIFI** macro to perform one or more automated HIFI experiments

run **mHIFI** macro by typing at the vnmr/vnmrj command line:

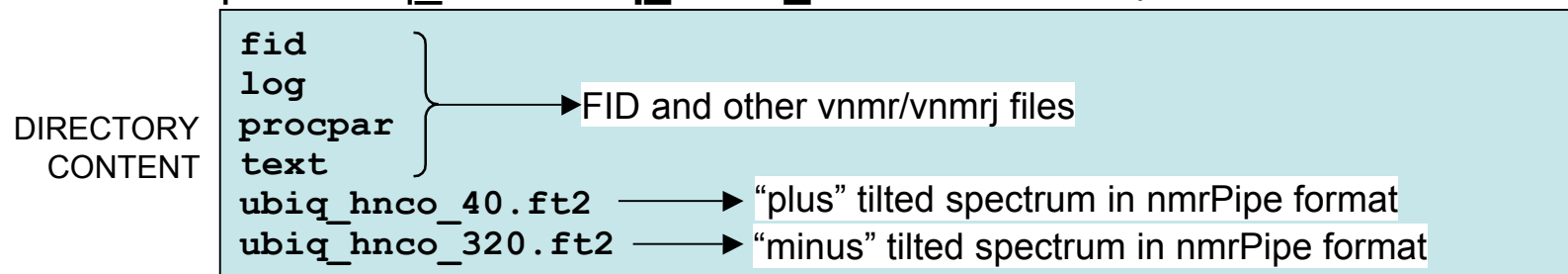


output files:

prefix for output filenames: **prefix\_experiment-type**, e.g. **ubiq\_hnco**

- tilted planes**
- all tilted planes ran are saved and processed with nmrPipe
  - tilted planes filenames: **prefix\_tilt-angle** , e.g. **ubiq\_hnco\_40**
  - each saved directory includes:
    - fid and other vnmr files
    - “**plus**” and “**minus**” nmrPipe processed tilted spectra

path/ubiq\_HIFI/ubiq\_hnco\_40.fid – 40° tilted plane



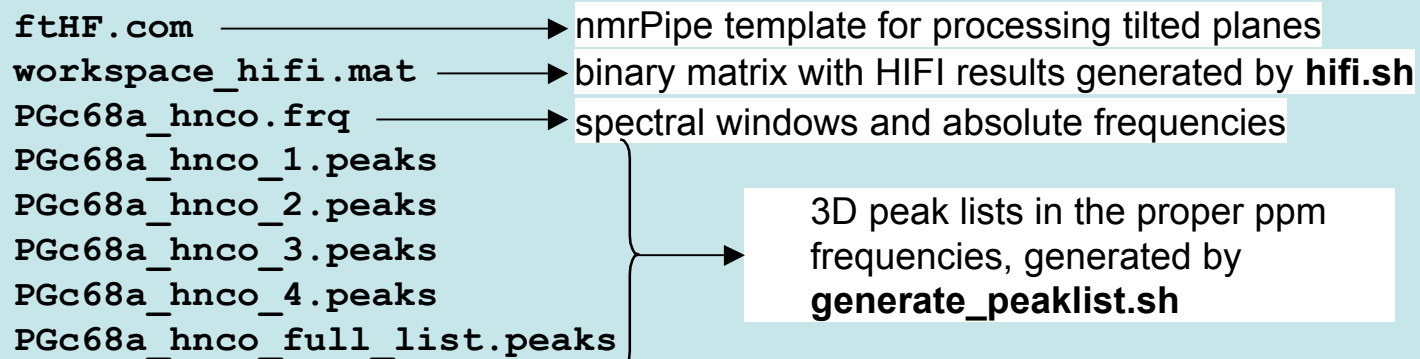
example: run **mHIFI** macro to perform one or more automated HIFI experiments

output files:

- prefix \_ exp-directory** – for each completed HIFI experiment, a directory is created with name: **prefix**, e.g. **ubiq\_hnco**  
– this directory contains, among other files (see example below), the peak list files

path/ubiq\_HIFI/ubiq\_hnco

DIRECTORY  
CONTENT



## Notes about running **mHIFI** macro

- all input files, including **orthogonal spectra** and **HIFI\_list.txt** file, must be within the same directory, e.g.: **path/myHIFI\_dir**
- make sure that orthogonal planes are processed and phased correctly:  
 phase in the indirect dimension should be either 0,0 or -90, 180  
 phase for the direct dimension should be the same for all backbone experiments
- make sure that processed orthogonal spectra are named properly, e.g. if FID is named:  
**path/HIFI\_directory/myprotein\_hnco\_0.fid**  
 the spectrum processed with nmrPipe should be named:  
**path/HIFI\_directory/myprotein\_hnco\_0.fid/myprotein\_hnco\_0.ft2**
- make sure that the nmrPipe processing script is named **ft0.com** and is in the same directory as the processed spectrum, e.g.:  
**path/HIFI\_directory/myprotein\_hnco\_0.fid/ft0.com**
- to record tilted planes, the **mHIFI** macro will load the corresponding 0° orthogonal plane and set the parameter tilt to the predicted best tilt angle
- the spectral window and offset for the 2<sup>nd</sup> indirect dimension (<sup>15</sup>N), **sw2** and **dof2**, are read from the 90° orthogonal plane, all other parameters (except **tilt** angle that is predicted) come from the 0° orthogonal plane
- the same 90° orthogonal plane can be used for all backbone assignment experiments, we usually choose a <sup>1</sup>H-<sup>15</sup>N HSQC

## macros to help collecting/processing orthogonal planes

vnmr/vnmrJ macro:

**mORTHO0** – acquire and process one or more 0° orthogonal planes, or process 0° orthogonal spectra that have already been recorded

input files:

**HIFI\_input.txt** – text file with list of input parameters (see example)

**orthogonal planes** – FID saved with vnmr

**ref.com** – nmrPipe processing script to be used as template for processing orthogonal planes

output files:

**orthogonal planes** –FID and other vnmr files, nmrPipe processed spectra and processing scripts

example 1: run **mORTHOO** macro to record and process 0° orthogonal planes

input files:

all input files must be present in the same directory, e.g. **path/ubiq\_HIFI**

**HIFI\_input.txt** – text file with list of input parameters for recording the 0° orthogonal planes

- same file used as input for running HIFI experiments with the **mHIFI** macro
- two optional extra columns for specifying the **offset (dof)** and **spectral window (sw1)** for the 1<sup>st</sup> indirect dimension (<sup>13</sup>C)
- if these columns are absent, the **dof** and **sw1** values loaded by default with the pulse sequence are used

path/ubiq\_HIFI/ **HIFI\_input.txt**

FILE  
CONTENT

number of residues = 76				→ ignored			
hnco	ubiq	2	48	ubiq_hnco_0	ubiq_hsqc	16711	2000
hncoca	ubiq	2	64	ubiq_hncoca_0	ubiq_hsqc	-7315	5500
hnca	ubiq	4	64	ubiq_hnca_0	ubiq_hsqc	-7315	5500
cbcaconh	ubiq	4	64	ubiq_cbcaconh_0	ubiq_hsqc	-9660	13025
hncacb	ubiq	8	80	ubiq_hncacb_0	ubiq_hsqc	-9660	13025
hncb	ubiq	8	80	ubiq_hncb_0	ubiq_hsqc	-9660	13025

experiment type – all 7 experiments currently available are shown

ignored

ignored

offset and spectral window for the 1<sup>st</sup> indirect dimension (optional)

0° plane – filename: **ubiq\_hnco\_0.fid/ubiq\_hnco\_0.ft2**

number of increments for recording 0° planes

29

number of scans for recording 0° planes

example 1: run **mORTHO0** macro to record and process 0° orthogonal planes

input files:

- ref.com**
- nmrPipe processing script to be used as template for processing the orthogonal planes
  - must be saved in main HIFI directory, e.g.: **path/ubiq\_HIFI**
  - the only parameters read from **ref.com** are:
    - phasing parameters for direct  $^1\text{H}$  dimension
    - extracting parameters for direct  $^1\text{H}$  dimension
  - **NOTE:** if the **ref.com** file is not found:
    - the zero and first order phase corrections are set to zero
    - no extraction is done.
  - phasing for the indirect dimension ( $^{13}\text{C}$ ) is either 0, 0 or -90, 180 depending on the value of **f1180** ('n' or 'y', respectively)
  - other parameters are set automatically within the **VNMR2PIPE** macro that is called by the **mORTHO0** macro

### ref.com

FILE CONTENT

```
#!/bin/csh -f

nmrPipe -in test.fid
| nmrPipe -fn POLY -time
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 1.0
| nmrPipe -fn ZF -size 2048
| nmrPipe -fn FT
| nmrPipe -fn PS -p0 44.0 -p1 180.0 -di
| nmrPipe -fn EXT -x1 8.0ppm -xn 6.0ppm -sw
| nmrPipe -fn TP
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 1.0
| nmrPipe -fn ZF -size 1024
| nmrPipe -fn FT
| nmrPipe -fn PS -p0 -90.0 -p1 180.0 -di
-verb -ov -out test.ft2
```

only these parameters are used

example 1: run **mORTHO0** macro to record and process 0° orthogonal planes

run **mORTHO0** macro by typing at the vnmr/vnmrj command line:

VNMR  
COMMAND  
LINE

`cd('path/ubiq_HIFI')` → join the directory containing **HIFI\_input.txt** and **ref.com** (optional) input files  
`mORTHO0` → call the macro, no arguments are required

output files:

**orthogonal planes** – spectra are recorded and saved with the name specified in the **HIFI\_list.txt** input file, e.g.:

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid**

– FID is processed with nmrPipe and saved within the FID directory with the same name but extension “.ft2”, e.g.:

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid/ubiq\_hnco\_0.ft2**

– the nmrPipe script used for processing is saved in the same directory with name **ft0.com**

– to reprocess the spectra from outside vnmr (vnmrJ), e.g. for adjusting the phase, use the script named **run\_ft0** which is created inside the fid directory, e.g.:

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid/run\_ft0**

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid** – 0° plane

DIRECTORY  
CONTENT

`fid`  
`log`  
`procpa`  
`text`  
`ft0.com`  
`ubiq_hnco_0.ft2`

→ FID and other files saved by vnmr/vnmrj

→ nmrPipe processing script

→ nmrPipe processed spectrum (same name as directory)

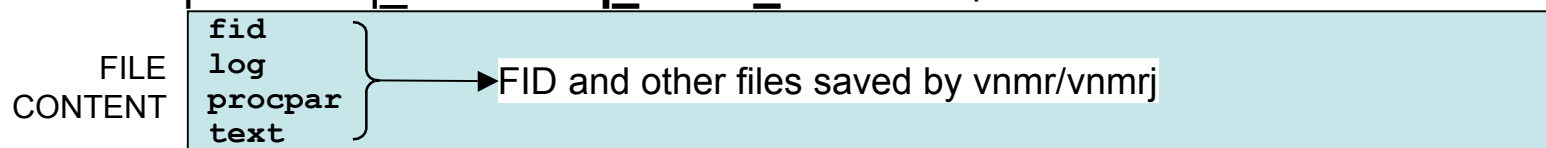
example 2: run **mORTHO0** macro to process already existing 0° orthogonal planes

input files:

all input files must be present in the same directory, **path/ubiq\_HIFI**

**0° orthogonal planes**– FID saved by vnmr/vnmrJ and to be processed by nmrPipe

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid** – 0° plane



**HIFI\_input.txt** – text file with list of parameters

– only the filename is used, e.g. first orthogonal fid to process is:

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid/fid**

**path/ubiq\_HIFI/ HIFI\_input.txt**

FILE CONTENT

number of residues = 76 → ignored							
hnco	ubiq	2	48	ubiq_hnco_0	ubiq_hsqc	16711	2000
hncoca	ubiq	2	64	ubiq_hncoca_0	ubiq_hsqc	-7315	5500
hnca	ubiq	4	64	ubiq_hnca_0	ubiq_hsqc	-7315	5500
cbcaconh	ubiq	4	64	ubiq_cbcaconh_0	ubiq_hsqc	-9660	13025
hncacb	ubiq	8	80	ubiq_hncacb_0	ubiq_hsqc	-9660	13025
hncb	ubiq	8	80	ubiq_hncb_0	ubiq_hsqc	-9660	13025

ignored ignored ignored ignored ignored ignored ignored

0° plane to be processed – filename:  
**ubiq\_hnco\_0.fid/ubiq\_hnco\_0.ft2**



example 2: run **mORTH00** macro to process already existing 0° orthogonal planes

input files:

- ref.com** – nmrPipe processing script to be used as template for processing the orthogonal planes
- must be saved in main HIFI directory, e.g.: **path/ubiq\_HIFI**
  - the only parameters read from **ref.com** are:
    - phasing parameters for direct  $^1\text{H}$  dimension
    - extracting parameters for direct  $^1\text{H}$  dimension
  - phasing for the indirect dimension ( $^{13}\text{C}$ ) is either 0, 0 or -90, 180 depending on the value of f1180 ('n' or 'y', respectively)
  - other parameters are set within the **VNMR2PIPE** macro that is called by the **mORTH00** macro

### ref.com

FILE CONTENT

```
#!/bin/csh -f

nmrPipe -in test.fid
| nmrPipe -fn POLY -time
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 1.0
| nmrPipe -fn ZF -size 2048
| nmrPipe -fn FT
| nmrPipe -fn PS -p0 44.0 -p1 180.0 -di
| nmrPipe -fn EXT -x1 8.0ppm -xn 6.0ppm -sw
| nmrPipe -fn TP
| nmrPipe -fn SP -off 0.45 -end 0.98 -pow 2 -c 1.0
| nmrPipe -fn ZF -size 1024
| nmrPipe -fn FT
| nmrPipe -fn PS -p0 -90.0 -p1 180.0 -di
-verb -ov -out test.ft2
```

only these parameters are used

**NOTE** – if the nmrPipe script **ft0.com** is already present in the FID directory, the **ref.com** is ignored and **ft0.com** is used as is to process the spectrum

example 2: run **mORTHO0** macro to process already existing 0° orthogonal planes

run **mORTHO0** macro by typing at the vnmr/vnmrj command line:

VNMR  
COMMAND  
LINE

`cd('path/ubiq_HIFI')` → join the directory containing **HIFI\_input.txt** and **ref.com** input files  
`mORTHO0` → call the macro, no arguments are required

output files:

**orthogonal planes** – FID is processed with nmrPipe and saved within the FID directory with the same name but extension “.ft2”, e.g.:

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid/ubiq\_hnco\_0.ft2**

– the nmrPipe script used for processing is saved in the same directory with name **ft0.com**

– to reprocess the spectra from outside vnmr (vnmrJ), e.g. for adjusting the phase, use the script named **run\_ft0** which is created inside the fid directory, e.g.:

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid/run\_ft0**

**path/ubiq\_HIFI/ubiq\_hnco\_0.fid** – 0° plane

DIRECTORY  
CONTENT

<code>fid</code>	}	→ FID and other files saved by vnmr/vnmrj
<code>log</code>		
<code>procpar</code>		
<code>text</code>		
<code>ft0.com</code>	→ nmrPipe processing script	
<code>ubiq_hnco_0.ft2</code>	→ nmrPipe processed spectrum (same name as directory)	

## Notes about running **mORTHO0** macro

→ a possible strategy for recording the orthogonal planes could be:

1. run **mORTHO0** to record and process all 0° orthogonal planes using the default values for **sw1** and **dof** (no value needs to be specified in **HIFI\_list.txt**, this is the only input file required at this point)
2. from within **vnmr** adjust the <sup>13</sup>C offset and spectral window in the indirect dimension (**sw1** and **dof**) for each experiment and enter these values in columns 7 and 8 of **HIFI\_list.txt** (do not worry about the **nmrPipe** processed spectra at this point)
3. re-acquire the spectra with the updated **HIFI\_list.txt**, i.e. with the optimized 13C window. You need to delete, rename or move the above spectra first, since **mORTHO0** will not overwrite any existing spectrum)
4. using **nmrDraw**, phase and set the correct parameters for extracting the direct dimension:
  - enter the correct values into the **ft0.com** files
  - to reprocess the spectra you can use the **run\_ft0** script which is found within each **\*.fid** directory
5. the same 90° orthogonal plane can be used for all experiments, we recommend using an <sup>1</sup>H-<sup>15</sup>N HSQC
  - make sure that the <sup>1</sup>H dimension acquisition parameters are identical for both the 0° and 90° planes
  - make sure that the processing parameters, such as zerofilling and extraction are also the same

→ phase for the indirect dimension should be either 0,0 or -90, 180, depending on the value of **f1180** ('n' or 'y', respectively). These phase parameters are set automatically by the **mORTHO0** macro.

→ phase for the direct dimension should be the same for all backbone experiments (occasionally HNCO has a different phase than all other experiments, I am not sure why)

→ most parameters used for recording the 0° orthogonal planes are those loaded by default with the pulse sequence (including **pw** and **tof**), make sure that the probefile is updated and has the correct values

→ only the number of scans, **nt**, the number of increments, **ni**, and possibly offset and spectral window for the <sup>13</sup>C dimension, **dof** and **sw1**, are set in the **HIFI\_list.txt** file

flow chart for **mORTHOO** macro