

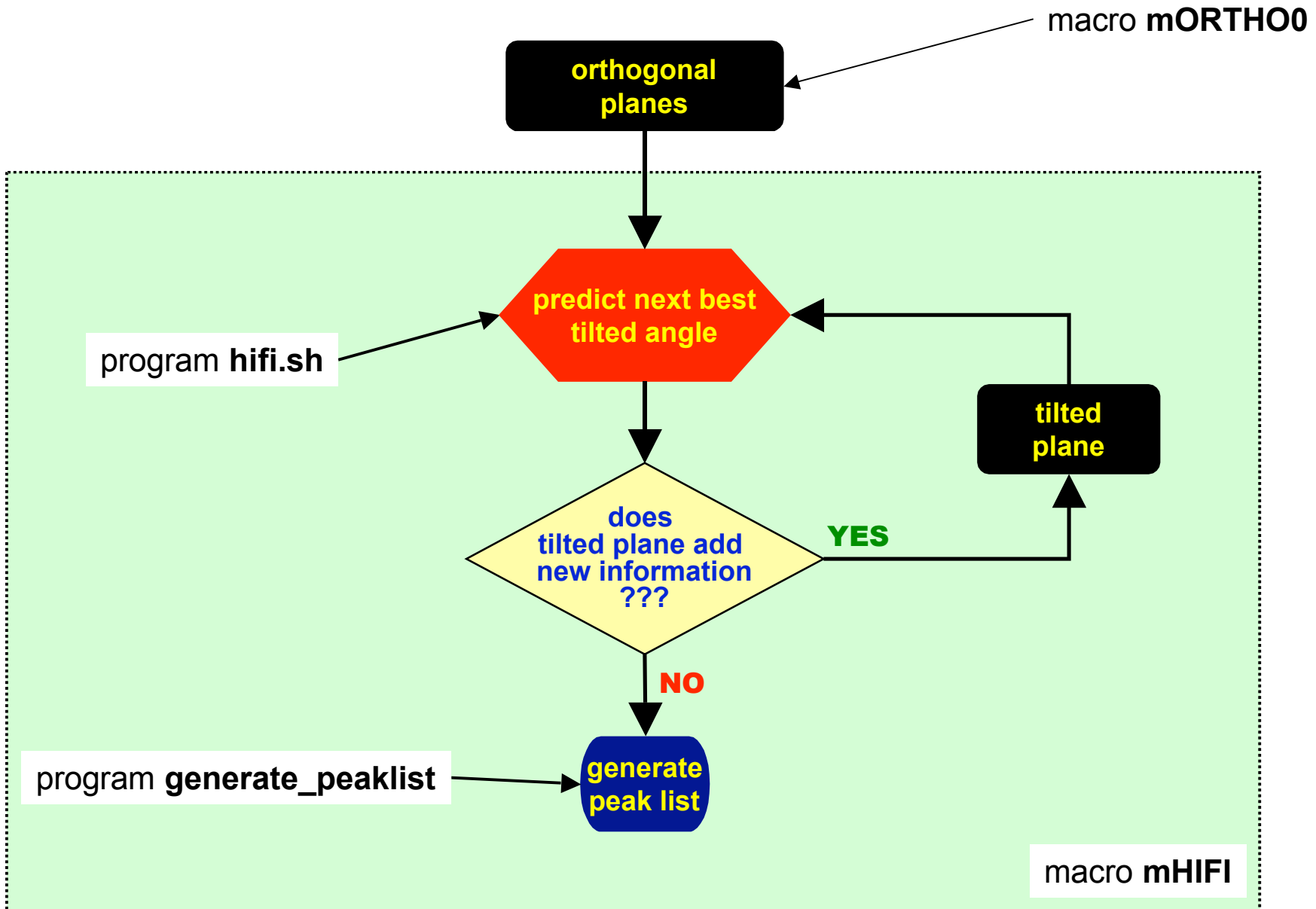
running HIFI

NMRFAM workshop - 2006

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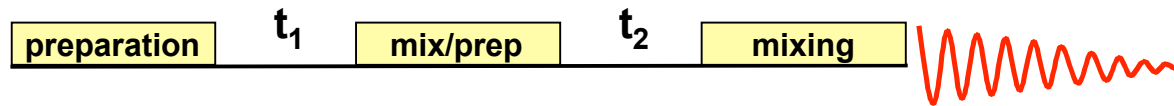
- pulse sequence
- collecting orthogonal and tilted planes
- processing tilted planes
- predicting the best angle for a tilted plane (**hifi.sh** program)
- extracting peak list (**generate_peaklist.sh** program)
- automated HIFI (**mHIFI** macro)
- macro for collecting/processing orthogonal planes (**mORTHO0**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

flow chart of HIFI

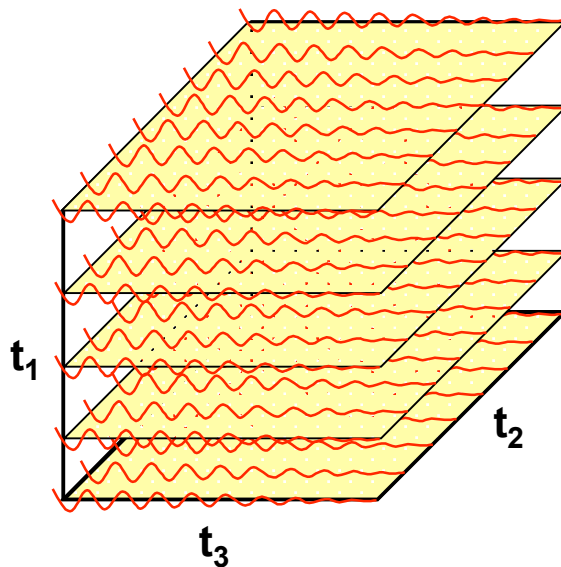


pulse sequence:

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

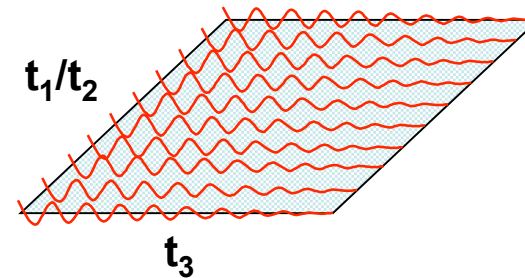


Conventional 3D spectrum

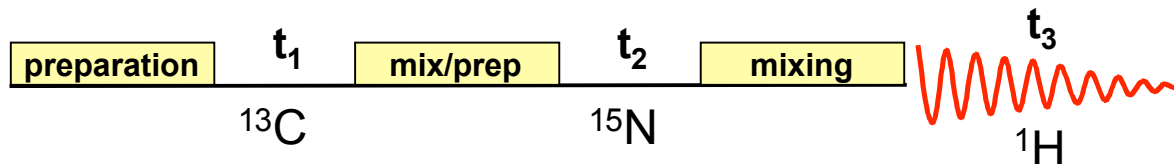


t_1 and t_2 are incremented independently

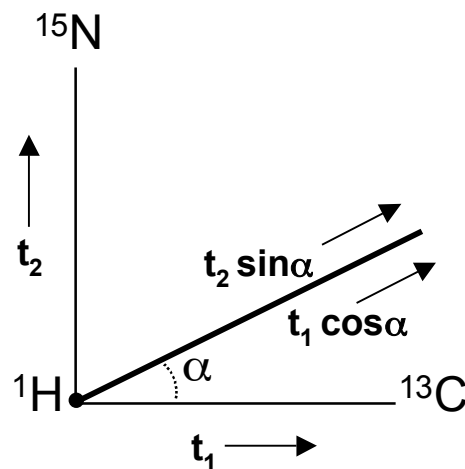
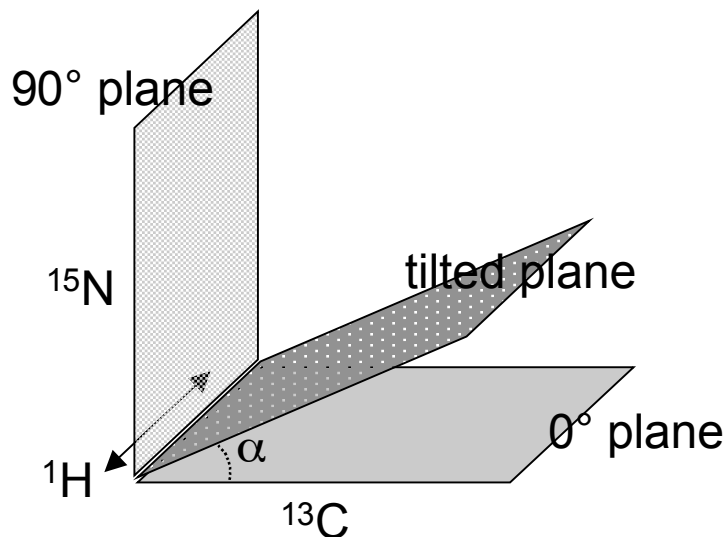
RD spectrum



t_1 and t_2 are incremented simultaneously point by point



orthogonal and tilted planes



¹³C dimension

sw1 ni
tau1 = i1 / sw1 i1 = 0 → (ni-1)

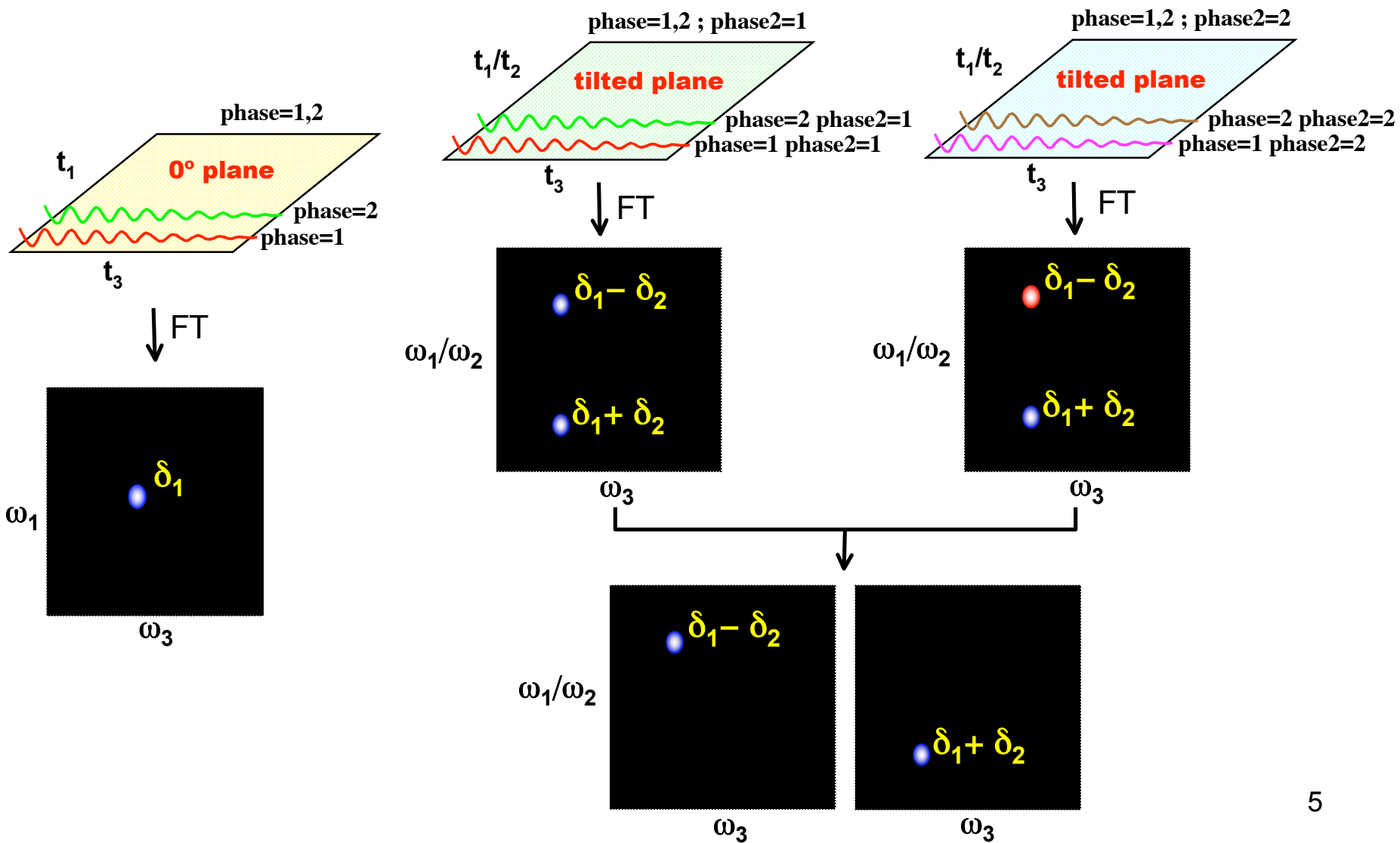
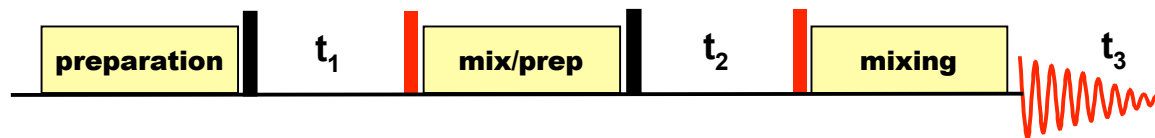
¹⁵N dimension

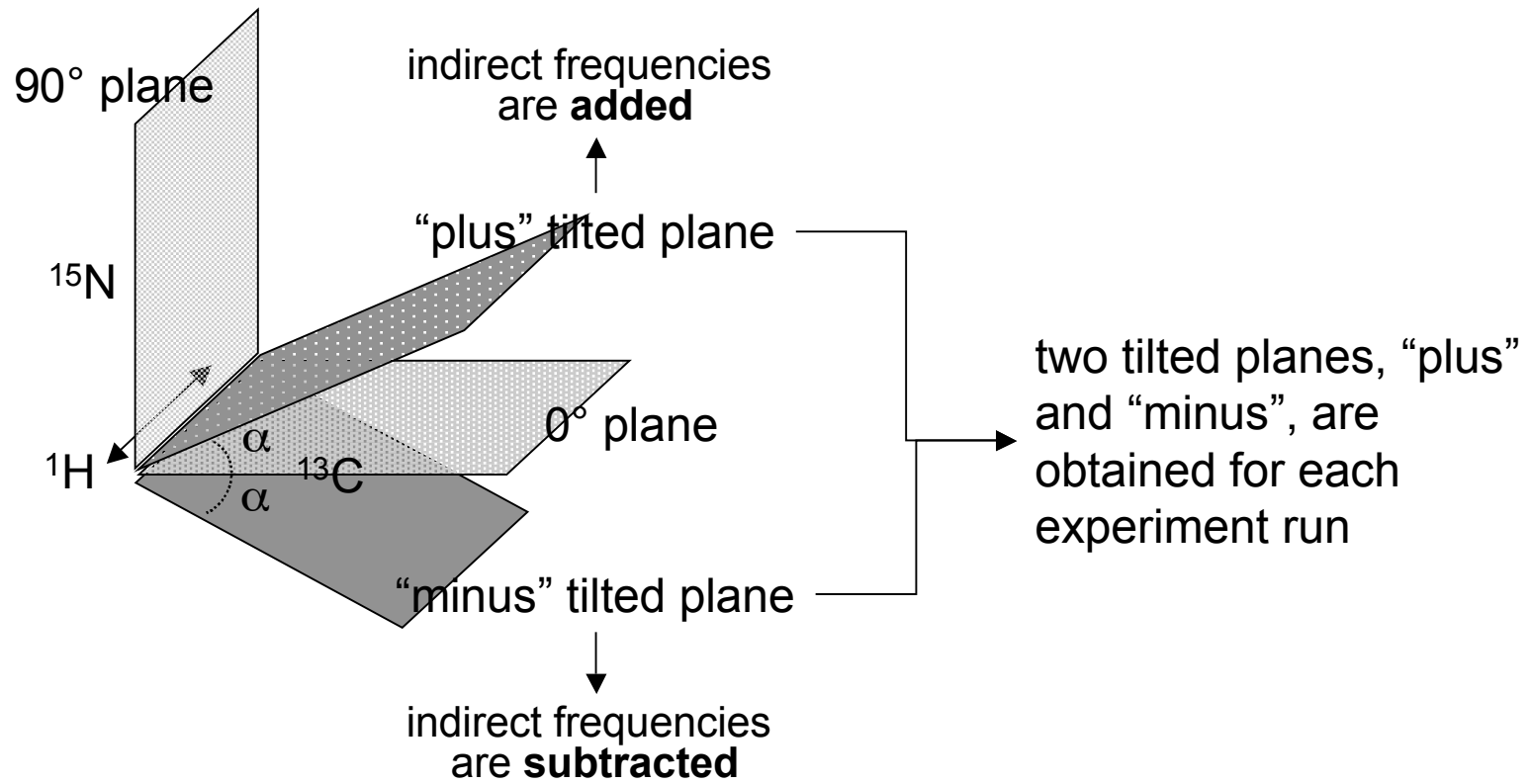
sw2 ni2
tau2 = i2 / sw2 i2 = 0 → (ni-2)

¹³C/¹⁵N co-evolved dimension

sw1 sw2 ni tilt
tau1 = (i1 / sw1) • cos(tilt) i1 = 0 → (ni-1)
tau2 = (i1 / sw2) • sin(tilt) i1 = 0 → (ni-1)

running HIFI...





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}C and ^{15}N dimensions)

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

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

n_{i2} = 0 *
phase2 = 1 *
f2180 – ignored

HIFI = 'n' *
tilt - ignored

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

n_i = 0 *
phase = 1 *
f1180 – ignored

n_{i2} = # of increments
phase2 = 1,2 (States-TPPI)
f2180 = 'n' or 'y'

HIFI = 'n' *
tilt - ignored

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

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}C dimension)

phase2 = 1,2 (States-TPPI quadrature detection for ^{15}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

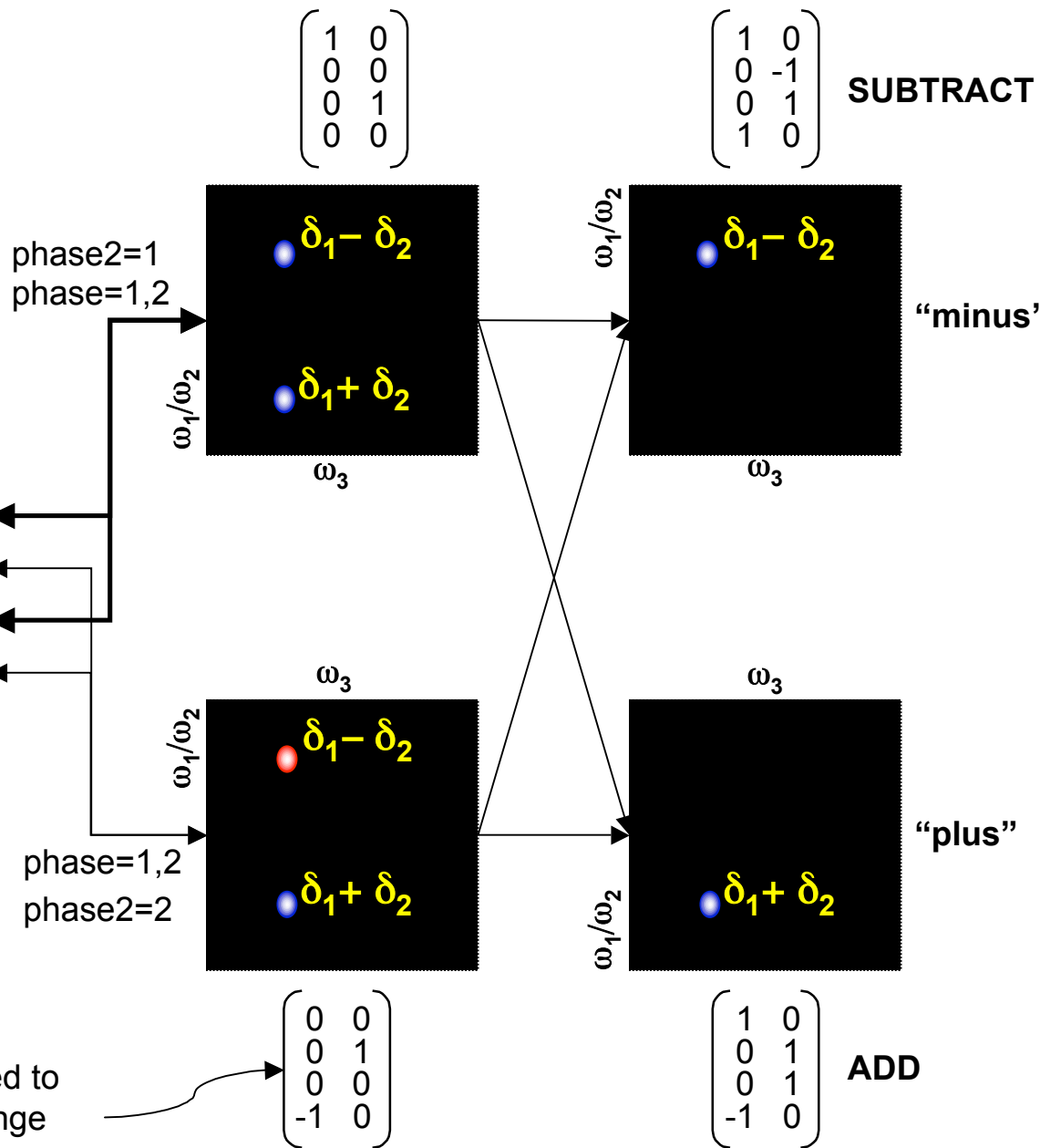
for each increment 4 FIDs are collected with all different combinations of phase and phase2:

array='phase,phase2'

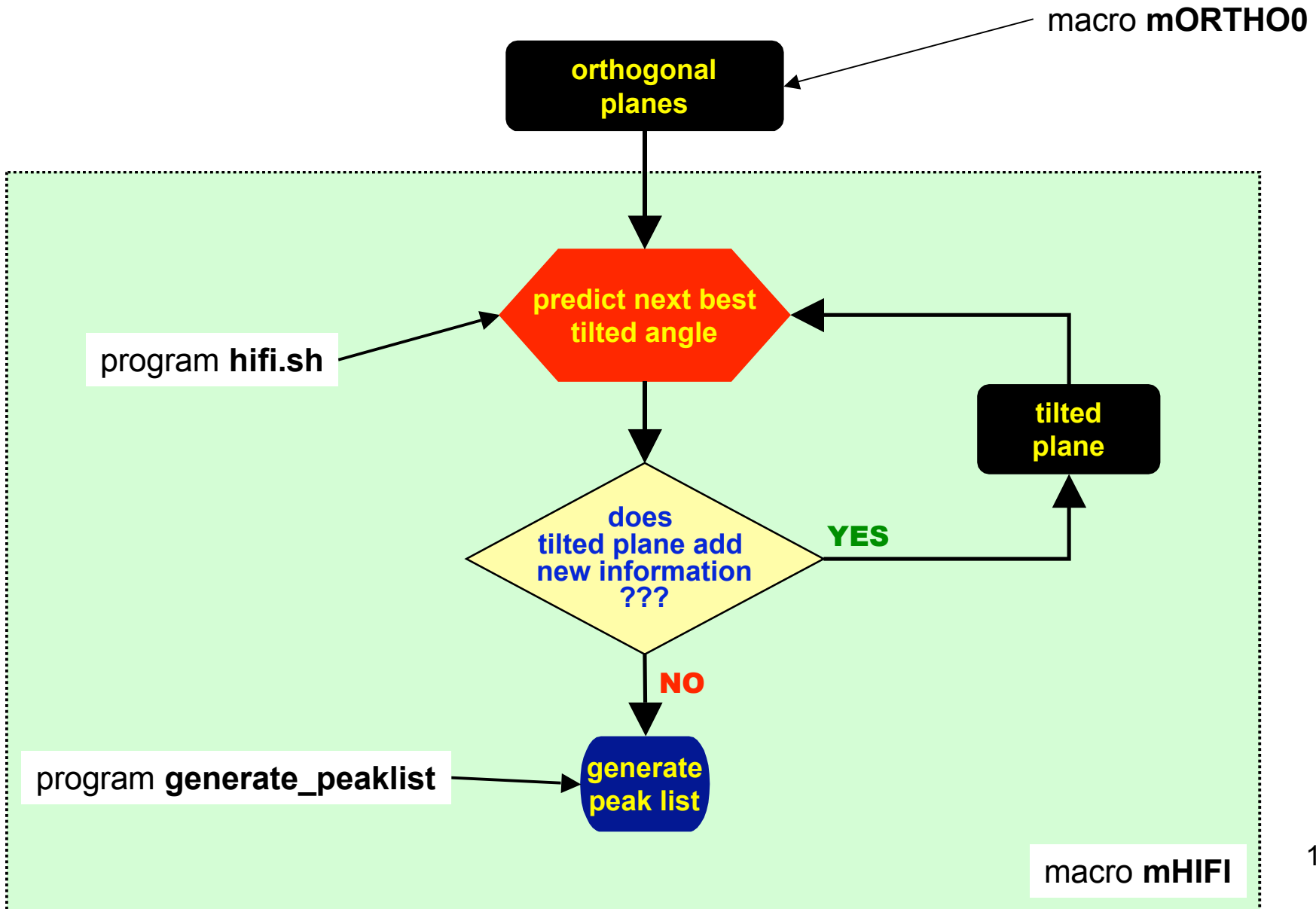
FID	phase	phase2
1	1	1
2	1	2
3	2	1
4	2	2

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

name of the plane in nmrPipe format, including path
 angle at which plane was collected

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

UNIX PROMPT	> 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	40
CONTENT	

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	path/hnco_40.ft2	40
CONTENT	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	33
CONTENT	

→ 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 ^1H , ^{13}C and ^{15}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	Value	Channel
sfrq	799.80866260	1 st channel – ¹ H
sw	12001.20012001	
dfrq	201.14575010	2 nd channel – ¹³ C
sw1	2000.00000000	
dfrq2	81.05318040	3 rd channel – ¹⁵ N
sw2	2199.97800022	

vnmr parameter name (points to the first column)
 absolute frequency in MHz and spectral window in Hz for each dimension (points to the second column)

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 (points to `path/generate_peaklist.sh`)
 name of file is fixed, but path can be specified if not in current directory (points to `path/workspace_hifi.mat`)
 name is flexible, path can be specified if needed (points to `path/frequency_list.txt`)
 prefix for output file (points to `hnco_`)

... 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

¹H ppm – direct dimension

¹³C ppm – 1st indirect dimension

¹⁵N ppm – 2nd indirect dimension


probability – 1=highest

Peak lists

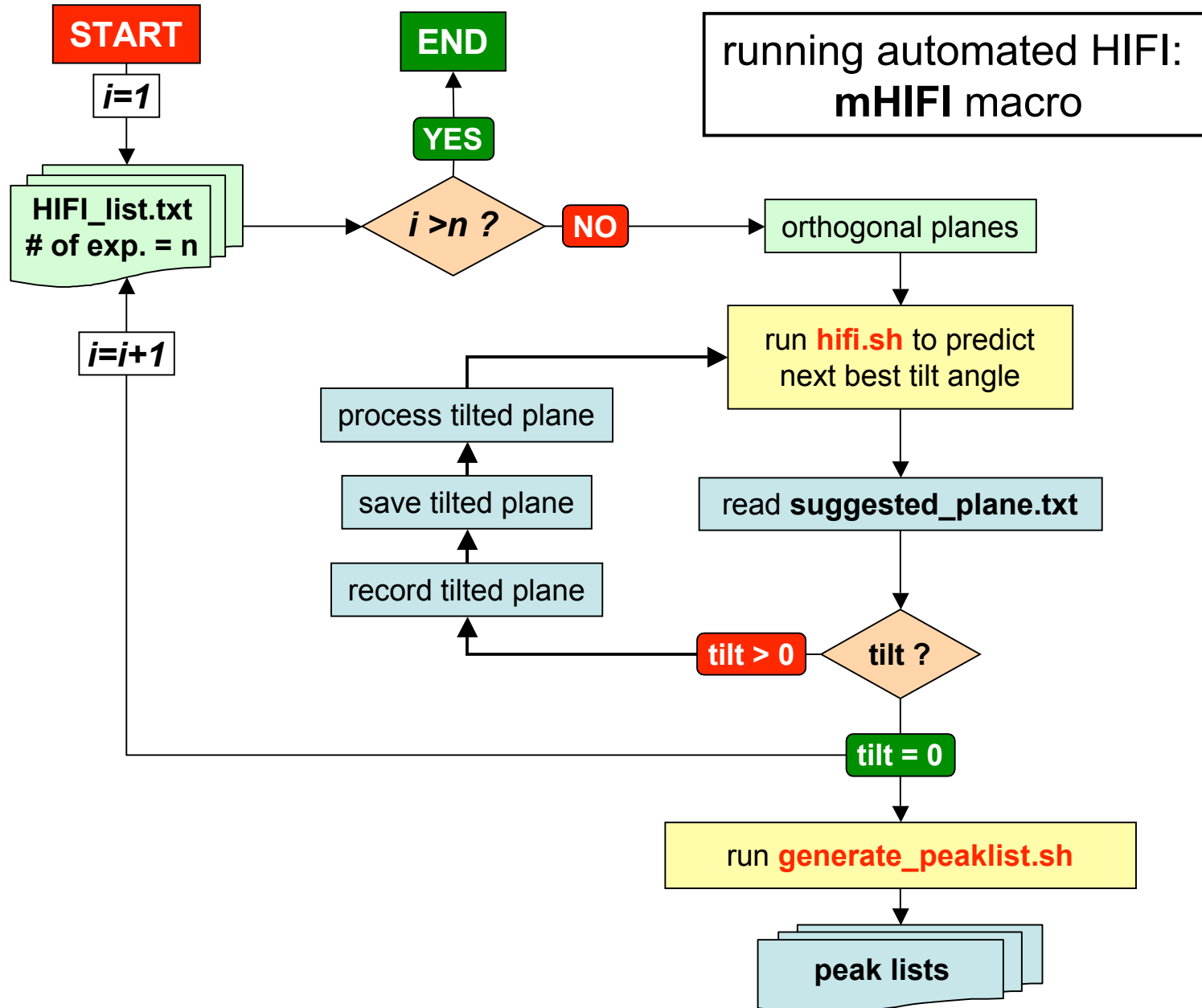
CBCA(CO)NH experiment for ubiquitin (76 residues)

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

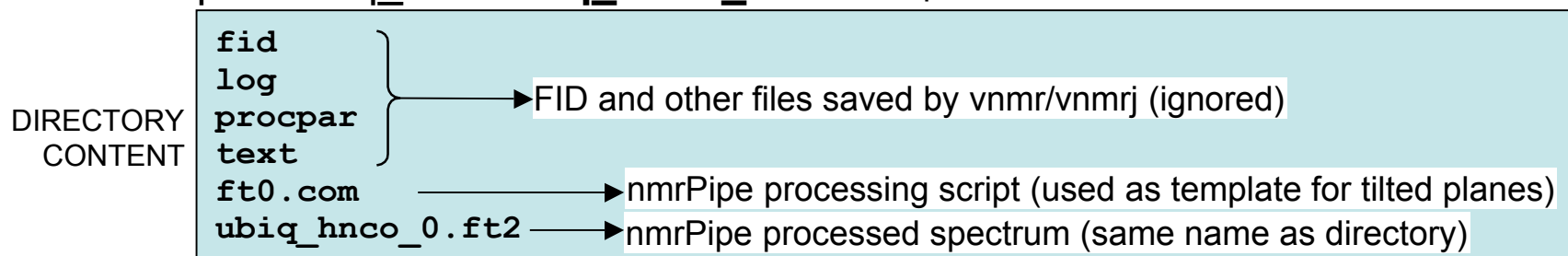
input files:

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

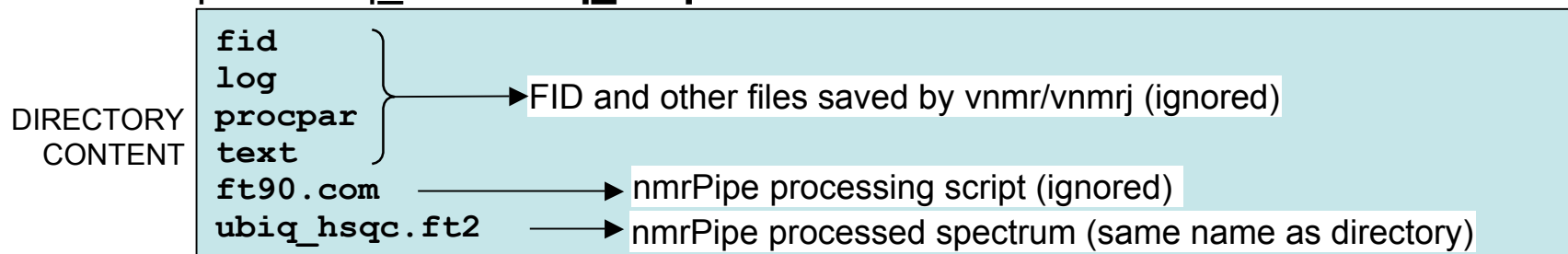
orthogonal planes – must be processed using nmrPipe:

- 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 ^1H - ^{15}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:

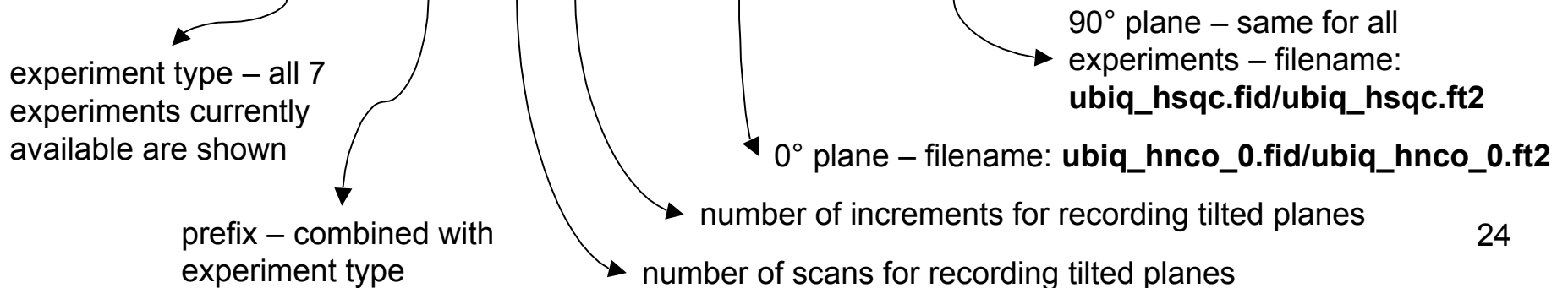
- number of residues in the protein
- for each HIFI experiment
 - type of experiment
 - prefix
 - number of scans and number of increments to use for tilted planes
 - orthogonal planes

path/ubiq_HIFI/ **HIFI_input.txt**

FILE
CONTENT

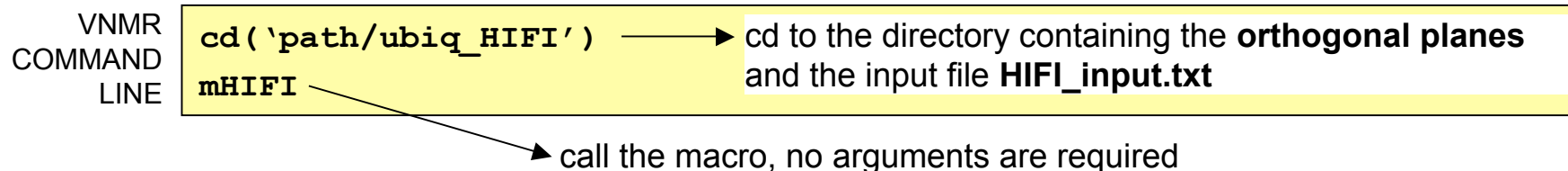
```

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
    
```



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

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

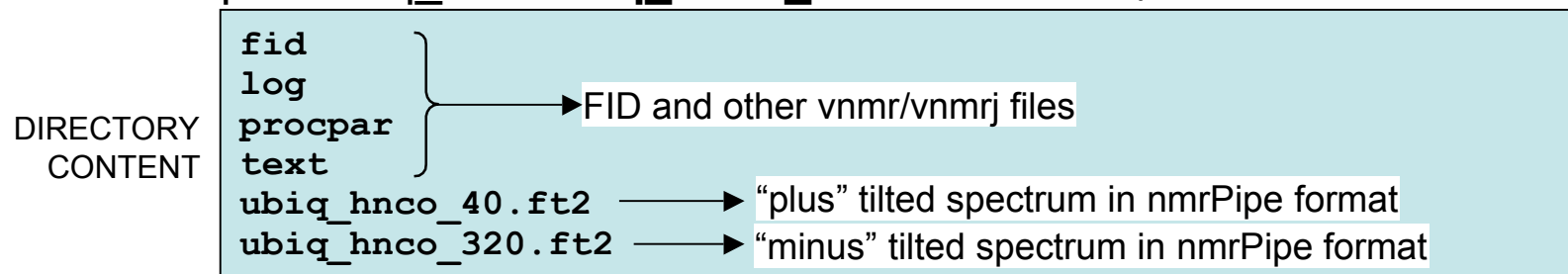


output files:

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



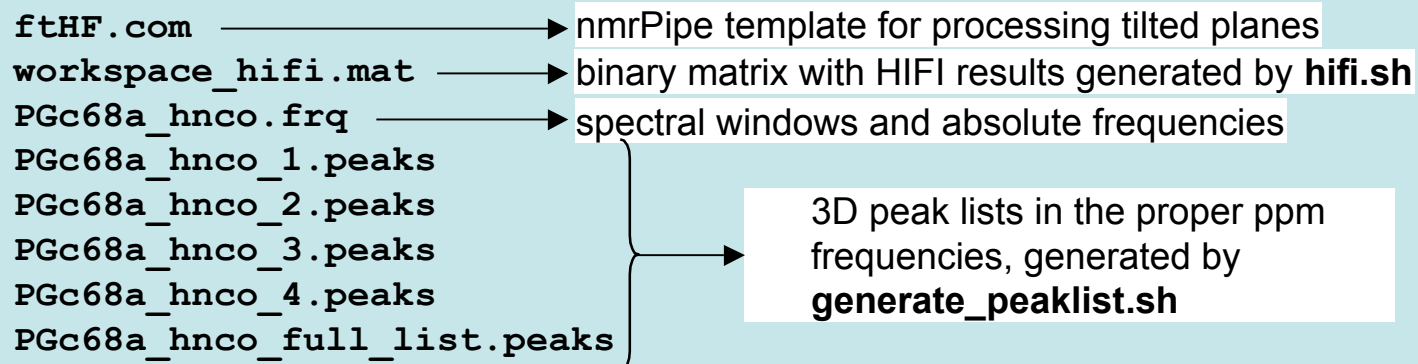
example: run **mHIFI** macro to perform one or more automated HIFI experiments

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 2nd indirect dimension (¹⁵N), **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 ¹H-¹⁵N 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 **mORTHO0** 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 1st indirect dimension (¹³C)
- 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 1st 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 ¹H dimension
 - extracting parameters for direct ¹H dimension
 - phasing for the indirect dimension (¹³C) 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

```

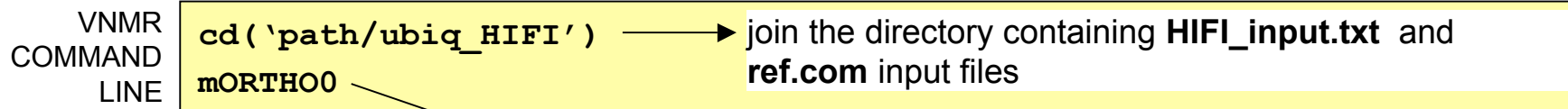
FILE CONTENT
#!/bin/csh -f

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:



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.:

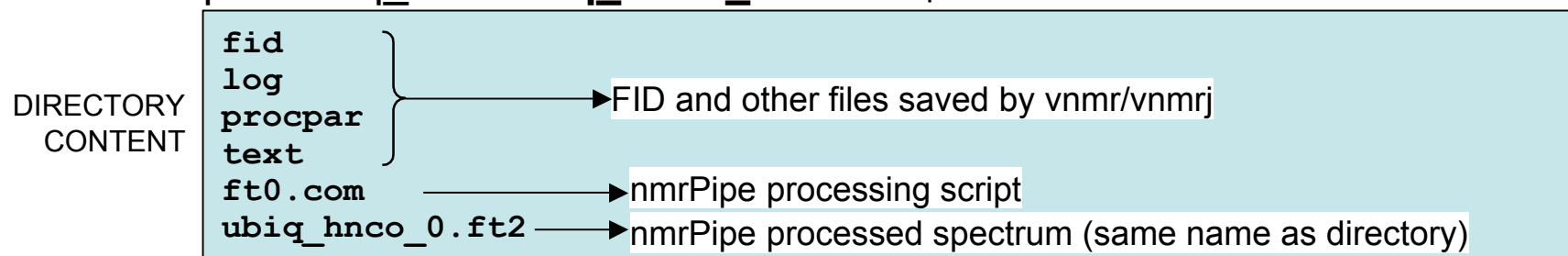
path/ubiq_HIFI/ubiq_hnco_0.fid/ubiq_hnco_0.ft2

– 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



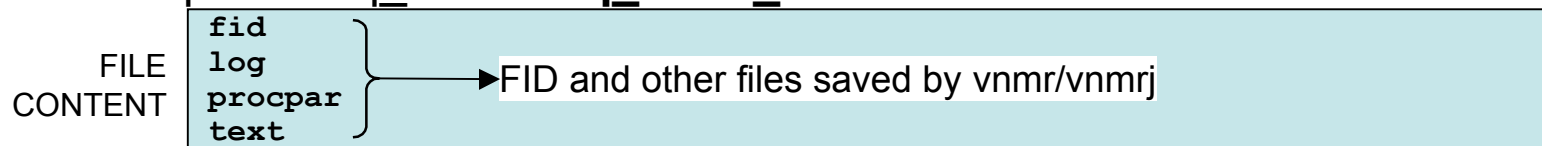
example 2: run **mORTHO0** macro to process already existing 0° orthogonal planes

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

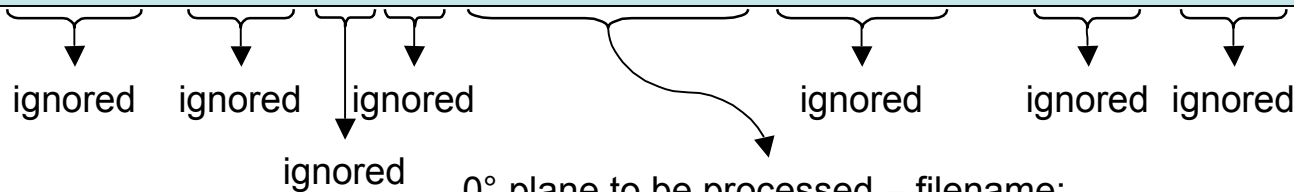
– only the filename is used, e.g. first orthogonal fid to process is:

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	



0° plane to be processed – filename:
ubiq_hnco_0.fid/ubiq_hnco_0.ft2

example 2: run **mORTHO0** 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 ¹H dimension
 - extracting parameters for direct ¹H dimension
 - phasing for the indirect dimension (¹³C) 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 **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

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	<pre>cd('path/ubiq_HIFI') mORTHO0</pre>	<p>—————> join the directory containing HIFI_input.txt and ref.com input files</p>
-------------------------	---	---

▲ 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.:

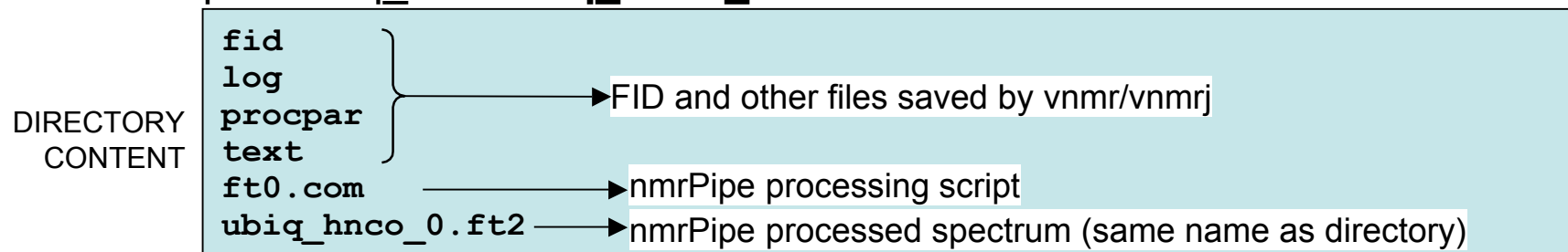
path/ubiq_HIFI/ubiq_hnco_0.fid/ubiq_hnco_0.ft2

– 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



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**)
2. phase and set the correct parameters for extracting the direct dimension, adjust the offset and spectral window in the indirect dimension (**sw1** and **dof**); input these values in **ref.com** and **HIFI_list.txt**, respectively
3. run **mORTHO0** to record and process the 0° orthogonal planes using the correct values
4. the same 90° orthogonal plane can be used for all experiments, we recommend using an ¹H-¹⁵N HSQC

→ phase for the indirect dimension should be either 0,0 or -90, 180, depending on the value of **f1180** ('n' or 'y', respectively)

→ phase for the direct dimension should be the same for all backbone experiments (occasionally HNC0 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 ¹³C dimension, **dof** and **sw1**, are set in the **HIFI_list.txt** file

algorithm for **mORTHO0** macro