Instruction of Projeciton-Reconstruction NMR

By Wen-Jin Wu, 04-17-2007

This is the first modified version of the second prototype version for 3D Projection Reconstruction by Wolfgang Bermel from Bruker (“pr_readme”). It consists of:

- -

AU programs (-> $TOPSPINHOME/exp/stan/nmr/au/src)
pr_setup.be
pr_proc.be
pr_recos.be
pr_alpha.be

Pulse programs (-> $TOPSPINHOME/exp/stan/nmr/lists/pp)
pr_hncogp3d.t1.be
pr_hncogp3dsc.ww (^{15}N-semi constant time)
pr_hncagp3d.t1.be
pr_hncagp3dsc.ww **(^{15}N-semi constant time)**
pr_hncocagp3d.ww
pr_hncocagp3dsc.ww **(^{15}N-semi constant time)**
pr_hncacogp3d.ww
pr_hncacogp3dsc.ww **(^{15}N-semi constant time)**
pr_hncacbgp3d_dp9.ww
pr_hncacbgp3dsc_dp9.ww **(^{15}N-semi constant time)**
pr_hncbgp3d_dp9.ww
pr_hncbgp3dsc_dp9.ww **(^{15}N-semi constant time)**
pr_cbcaconhgp3dsc.ww **(^{15}N-semi constant time)**

- -

The files need to be copied into the appropriate directories.

(1). Create a 3D dataset and set all parameters as if you were to run a normal 3D. Then start **pr_setup.be**. The program will create new expnos for the 2D projections. Change to the first 2D and acquire the data (e.g. multizg).
(2). Go back to the 3D dataset and start **pr_proc.be**. This program will rearrange the 2Ds and store them under a set of new expnos. Go to these new expnos, transform and phase correct the spectra.
(3). Go back to the 3D dataset and start **pr_recos.be** to reconstruct the 3D cube.

- The program **pr_recos.be** reconstructs the 3D cube, and calculates an F1F2 projection and counts the peaks therein. This number can be used to check how the reconstruction improves as the number of peaks should converge towards the end. (This part may be replaced by a 3d peak picking at some stage).

- The program **pr_alpha.be** calculates an F1F3 projection from the reconstructed cube and counts the peaks therein. It will do this for angles between -80o and +80o and will search for a maximum. The idea is that the best suited projection angle is the one with the largest number of peaks. So the program will suggest the next projection angle to be recorded.

Additional note:
Under your 3D root directory you will find files:
“pr_setup”: this contains the number and the location of the 2D sub-spectra that you set up.

“pr_alpha”: this contains the prediction results from the macro pr_alpha.be

“pr_recos”: this contains the projection progress, number of cross peaks and tilted angles that have been used for reconstruction.

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Step-by-step tutorial on PR-NMR

Getting ready

(a). Place the pulse sequences in the directory of “pr_pulprog” into your topspin pulse sequence directory:
C:\Bruker\TOPSPIN\exp\stan\nmr\lists\pp

(b). Place the au programs in the directory of “pr_au” into the following directory:
(C:\Bruker\TOPSPIN\exp\stan\nmr\au\src
pr_setup.be
pr_proc.be
pr_recos.be
pr_alpha.be

(c). Copy the sample data set “workshop_hnco” into your topspin data directory:

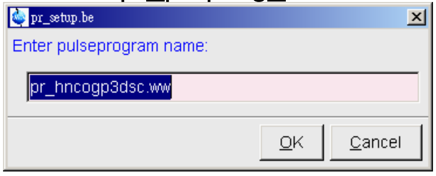
- C:\Bruker\TOPSPIN\data\your_name\nmr
- Or E:\data\your_name\nmr

(1). Setting up the experiments:

Under workshop_hnco 1/1, let’s practice setting up the experiments in PR-NMR mode. **(Note: for ^{15}N semi-constant time experiment, set l0=td2 (^{15}N)).**

- “pr_setup.be”↵
- Enter pulse sequence name:

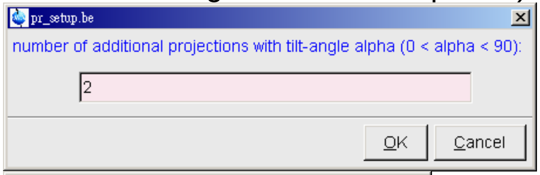
Format: pr_pulprog_name



Enter pulseprogram name:

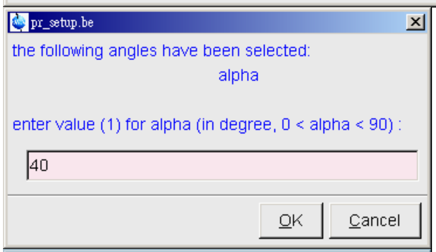
pr_hncogp3dsc.ww

- Key in the number of additional projections with tilted-angle alpha (0<alpha<90) (besides the 0- and 90-degree two basic spectra)



number of additional projections with tilt-angle alpha (0 < alpha < 90):

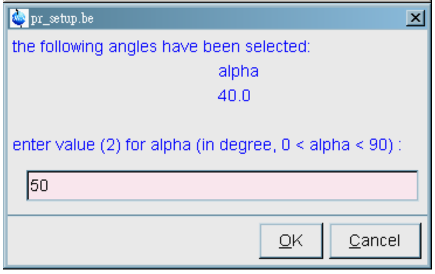
2



the following angles have been selected:
alpha

enter value (1) for alpha (in degree, 0 < alpha < 90) :

40



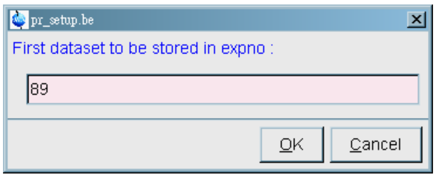
the following angles have been selected:
alpha
40.0

enter value (2) for alpha (in degree, 0 < alpha < 90) :

50

- Input the EXPNO for the first

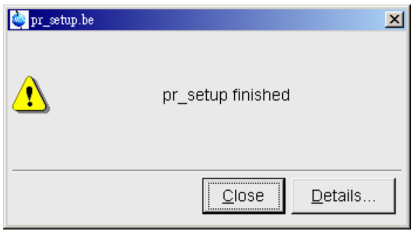
dataset to be stored in:



First dataset to be stored in expno :

89

- The PR-NMR set up is now finished



pr_setup finished

Open the “pr_setup” file under the current data directory
You will see the following:

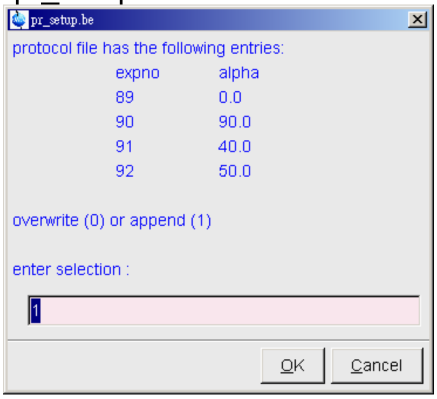
```
4
89   0.0
90   90.0
91   40.0
92   50.0
```

- multizg 89-92

- -

- (after analyzing the data, if you need to acquire more tilted angles, repeat “pr_setup.be”, and use “append” instead of “overwrite” to add more angles, and store the first data set in a new expno)

“pr_setup.be”



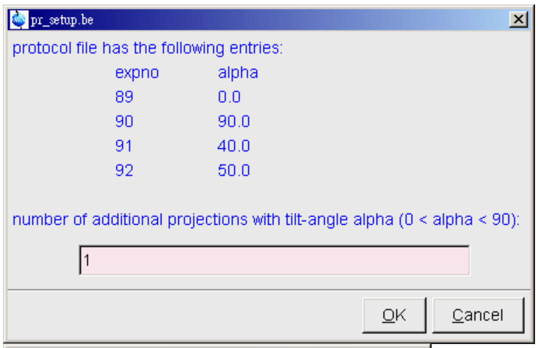
protocol file has the following entries:

expno	alpha
89	0.0
90	90.0
91	40.0
92	50.0

overwrite (0) or append (1)

enter selection :

1

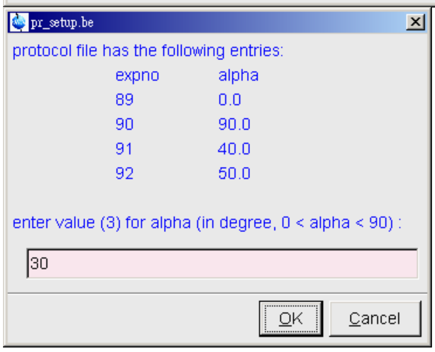


protocol file has the following entries:

expno	alpha
89	0.0
90	90.0
91	40.0
92	50.0

number of additional projections with tilt-angle alpha (0 < alpha < 90):

1

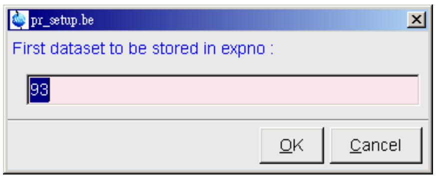


protocol file has the following entries:

expno	alpha
89	0.0
90	90.0
91	40.0
92	50.0

enter value (3) for alpha (in degree, 0 < alpha < 90) :

30



First dataset to be stored in expno :

93

- The “pr_setup” file is then updated

```
5
89   0.0
90   90.0
91   40.0
92   50.0
93   30.0
```

“zg” the new experiment of 93/1

- Alternatively, you can add the new tilted angle experiment to any new experiment number. For example in 130/1 instead of 93/1 as suggested (you may have other experiments queued up in 93/1 for acquisition).

The “pr_setup” file is then updated

```
5
89   0.0
90   90.0
91   40.0
92   50.0
130   30.0
```

(2). Processing the PR-NMR data

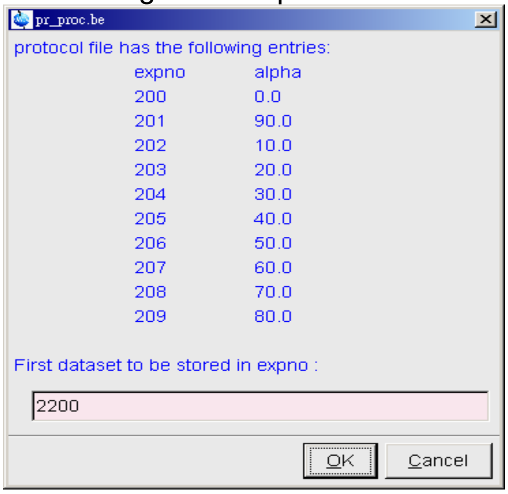
(2a). rearrange the 2D-projection spectra, and process them.

Sample data set: “workshop_hnco/31” is the processed PR-NMR 3D HNCO 32/1 is for you to practice.

(1). The 2D-projeciton spectra are from 200/1 to 209/1 (written to the file “pr_setup”).

(2). Under the root directory 32/1: change the file size under EDP. Use 1H=1K, 15N=256, 13C=256, use your usually processing parameter for all other parameters: i.e. ssb=3, linear prediction (LPFc, NCOEF=32 for a

(3). Type in the command “pr_proc.be” to rearrange the acquired 2D data.



protocol file has the following entries:

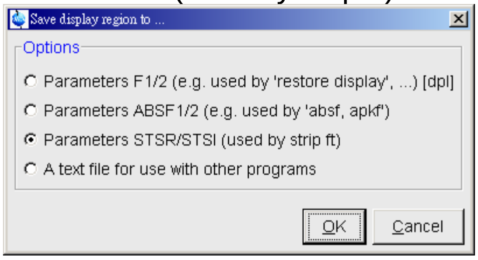
expno	alpha
200	0.0
201	90.0
202	10.0
203	20.0
204	30.0
205	40.0
206	50.0
207	60.0
208	70.0
209	80.0

First dataset to be stored in expno : 2200

(4). Open the file “pr_proc”, the rearranged data location is reported here.

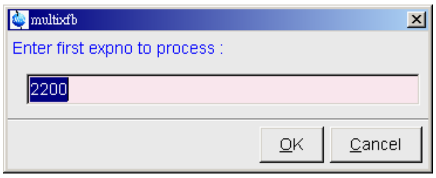
```
18
2200      0.0
2201      90.0
2202      10.0
2203      -10.0
2204      20.0
2205      -20.0
2206      30.0
2207      -30.0
2208      40.0
2209      -40.0
2210      50.0
2211      -50.0
2212      60.0
2213      -60.0
2214      70.0
2215      -70.0
2216      80.0
2217      -80.0
```

(5). Go to 2200/1, use “xfb” to FT this first 2D spectrum, phase the spectrum, and expand only the region that contains peaks (left-bottom to drag the defined region (exclude water), and use the right-bottom to save the display region to “parameters STSR/STSI (used by strip ft).

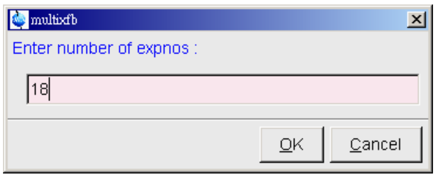


○ Parameters F1/2 (e.g. used by 'restore display', ...) [dpl]
○ Parameters ABSF1/2 (e.g. used by 'absf, apkf')
● Parameters STSR/STSI (used by strip ft)
○ A text file for use with other programs

(6). Now use the command “multixfb” to phase all of the spectra



Enter first expno to process : 2200



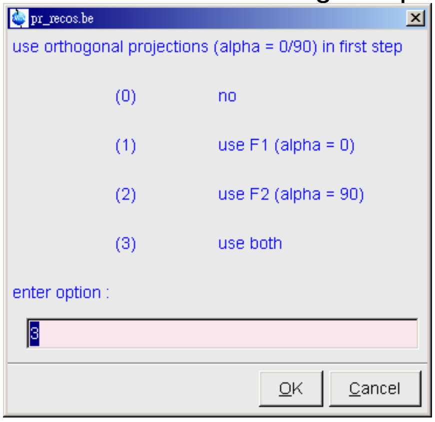
Enter number of expnos :

18

Now we are ready to reconstruct the 3D spectrum

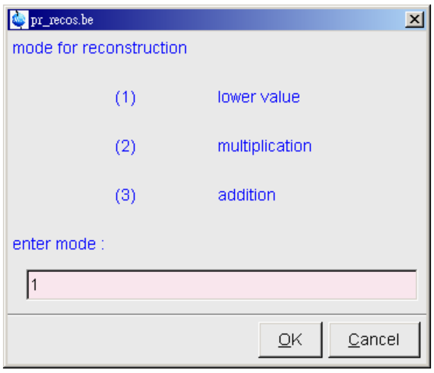
(2b). Reconstruction of the 3D spectrum

(7). Go back to the 3D root directory 32/1, key in the command "pr_recos.be", and the following window will pop up.
Choose "(3) use both" for using both of the C-H and N-H orthogonal planes



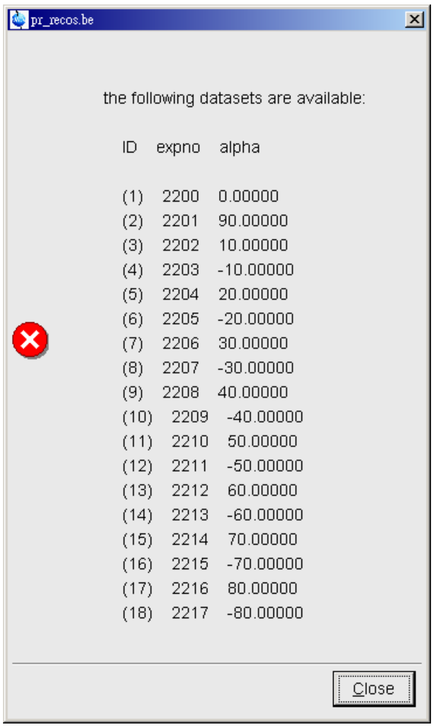
```
use orthogonal projections (alpha = 0/90) in first step

      (0)          no
      (1)          use F1 (alpha = 0)
      (2)          use F2 (alpha = 90)
      (3)          use both

enter option :
3
```

- Mode for reconstruction: lower value is recommended



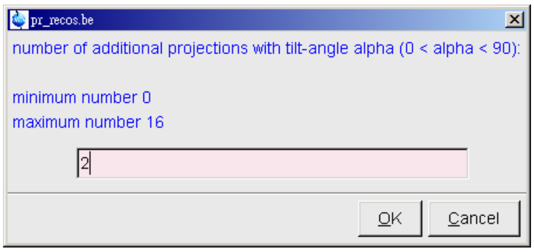
```
mode for reconstruction

      (1)          lower value
      (2)          multiplication
      (3)          addition

enter mode :
1
```

- Available 2D-tilted experiments are listed:

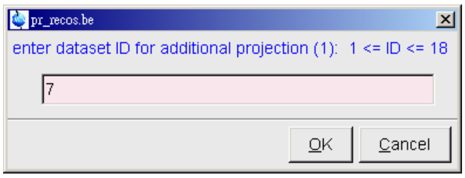


```
the following datasets are available:

 ID    expno    alpha

 (1)   2200    0.00000
 (2)   2201    90.00000
 (3)   2202    10.00000
 (4)   2203    -10.00000
 (5)   2204    20.00000
 (6)   2205    -20.00000
 (7)   2206    30.00000
 (8)   2207    -30.00000
 (9)   2208    40.00000
 (10)   2209    -40.00000
 (11)   2210    50.00000
 (12)   2211    -50.00000
 (13)   2212    60.00000
 (14)   2213    -60.00000
 (15)   2214    70.00000
 (16)   2215    -70.00000
 (17)   2216    80.00000
 (18)   2217    -80.00000
```

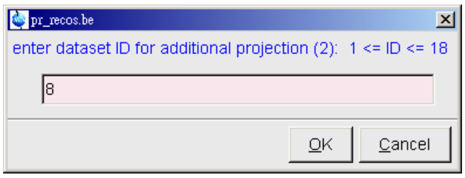
(8). Input the number of additional projections with tilted-angle alpha (0<alpha<90):
Let's use two additional projections for now.



```
number of additional projections with tilt-angle alpha (0 < alpha < 90):

minimum number 0
maximum number 16

2
```



```
enter dataset ID for additional projection (1):  1 <= ID <= 18
7
```



```
enter dataset ID for additional projection (2):  1 <= ID <= 18
8
```

It will then perform the 3D

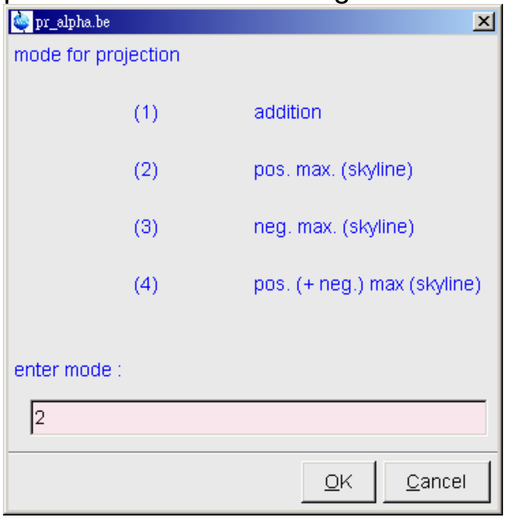
reconstruction

pr_recos finished

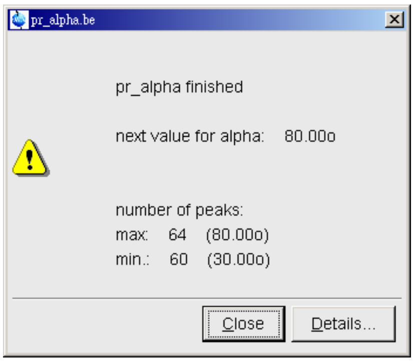
(9). When it is done, under the current directory, open the file “pr_recos” to view the reconstruction result. You can also look at the 3D cube now.

(c). Predict the next tilted plane to acquire

(10). Use the macro “pr_alpha.be” to predict the next tilted angle

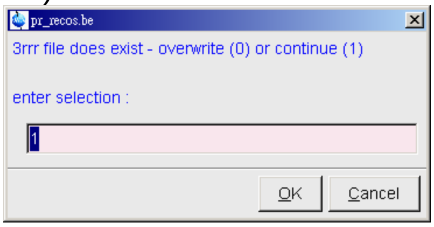


```
mode for projection

        (1)        addition
        (2)        pos. max. (skyline)
        (3)        neg. max. (skyline)
        (4)        pos. (+ neg.) max (skyline)

enter mode :
2
```

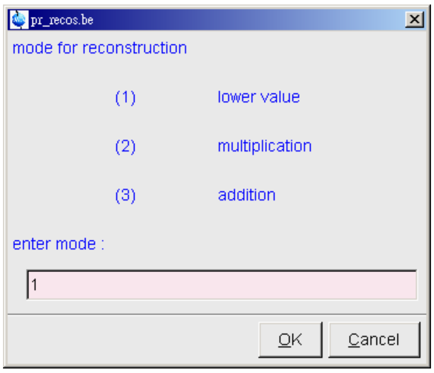


```
pr_alpha finished

next value for alpha:   80.00o

number of peaks:
max:   64   (80.00o)
min.:  60   (30.00o)
```

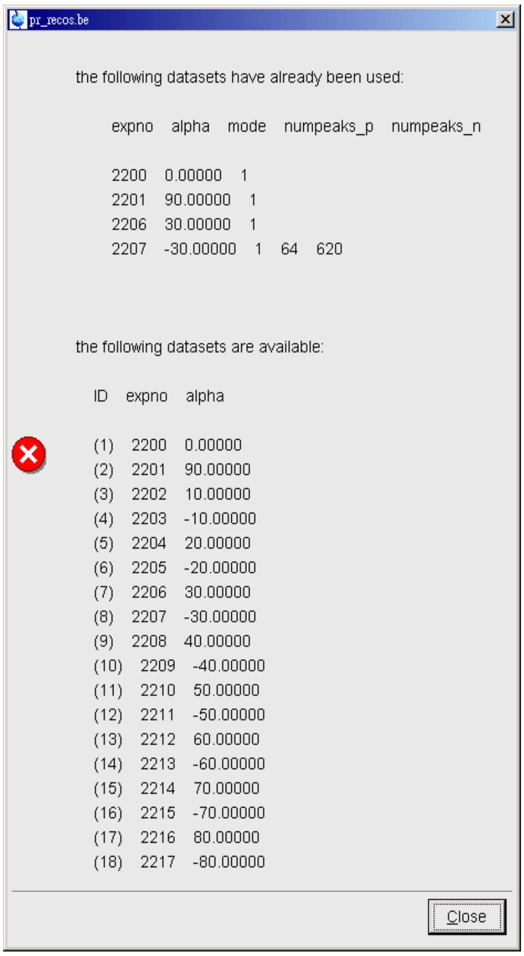
(11). continue to reconstruct the 3D spectrum by adding the 80 and -80 tilted spectra (not to overwrite the 3rrr file)



```
3rrr file does exist - overwrite (0) or continue (1)

enter selection :
1
```



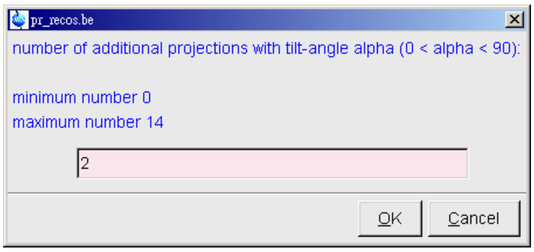
```
mode for reconstruction

        (1)        lower value
        (2)        multiplication
        (3)        addition

enter mode :
1
```



```
the following datasets have already been used:

    expno   alpha   mode   numpeaks_p   numpeaks_n

    2200   0.00000    1
    2201   90.00000   1
    2206   30.00000   1
    2207   -30.00000   1   64   620


the following datasets are available:

   ID   expno   alpha

   (1)   2200   0.00000
   (2)   2201   90.00000
   (3)   2202   10.00000
   (4)   2203   -10.00000
   (5)   2204   20.00000
   (6)   2205   -20.00000
   (7)   2206   30.00000
   (8)   2207   -30.00000
   (9)   2208   40.00000
   (10)   2209   -40.00000
   (11)   2210   50.00000
   (12)   2211   -50.00000
   (13)   2212   60.00000
   (14)   2213   -60.00000
   (15)   2214   70.00000
   (16)   2215   -70.00000
   (17)   2216   80.00000
   (18)   2217   -80.00000
```

- And add #17 (80) and #18 (-80)

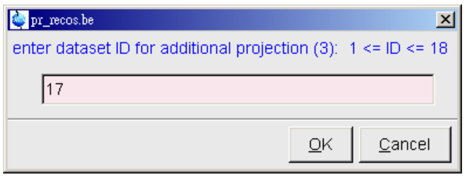
for reconstruction.



number of additional projections with tilt-angle alpha (0 < alpha < 90):

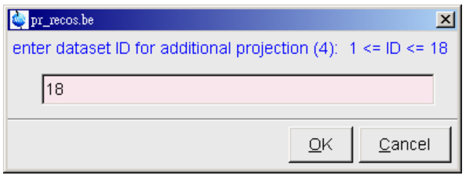
minimum number 0
maximum number 14

2



enter dataset ID for additional projection (3): 1 <= ID <= 18

17



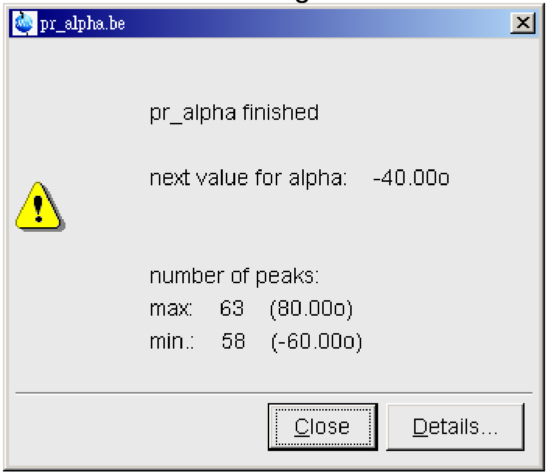
enter dataset ID for additional projection (4): 1 <= ID <= 18

18

- Exam the “pr_recos” file for status

```
6
2200    0.0     1
2201    90.0    1
2206    30.0    1
2207    -30.0   1    64   620
2216    80.0    1
2217    -80.0   1    61   501
```

- Again use “pr_alpha.be” to predict the next tilted angle



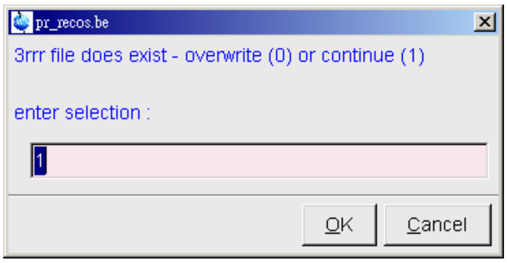
pr_alpha finished

next value for alpha: -40.00o

number of peaks:
max: 63 (80.00o)
min.: 58 (-60.00o)

(Pretend that we have continue to acquire the 40-degree angle data)

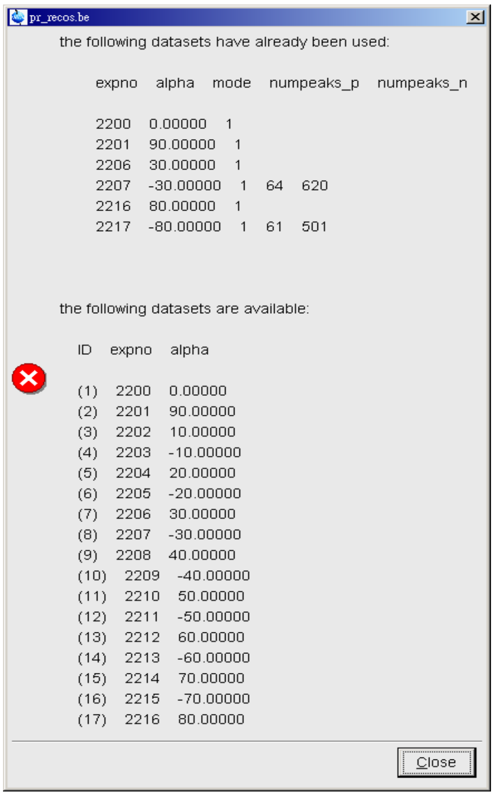
Now reconstruct the spectrum by adding the data of 40 and -40-degree data



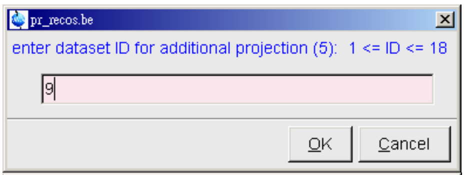
3rrr file does exist - overwrite (0) or continue (1)

enter selection :

1

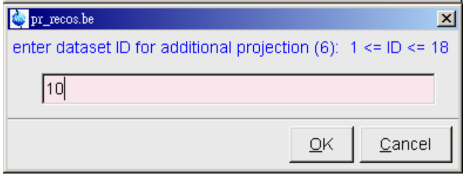


```
the following datasets have already been used:

    expno   alpha     mode   numpeaks_p   numpeaks_n

    2200    0.00000    1
    2201    90.00000   1
    2206    30.00000   1
    2207    -30.00000  1    64    620
    2216    80.00000   1
    2217    -80.00000  1    61    501


the following datasets are available:

    ID    expno   alpha

    (1)   2200    0.00000
    (2)   2201    90.00000
    (3)   2202    10.00000
    (4)   2203    -10.00000
    (5)   2204    20.00000
    (6)   2205    -20.00000
    (7)   2206    30.00000
    (8)   2207    -30.00000
    (9)   2208    40.00000
    (10)  2209    -40.00000
    (11)  2210    50.00000
    (12)  2211    -50.00000
    (13)  2212    60.00000
    (14)  2213    -60.00000
    (15)  2214    70.00000
    (16)  2215    -70.00000
    (17)  2216    80.00000
```



enter dataset ID for additional projection (5): 1 <= ID <= 18

9

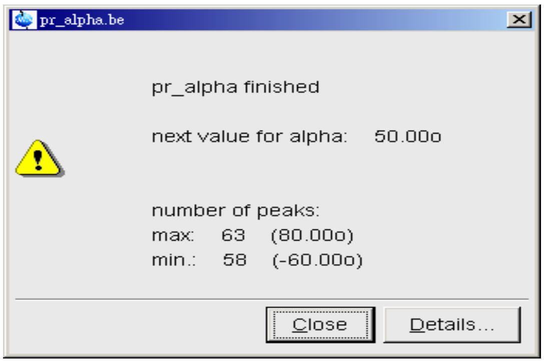


enter dataset ID for additional projection (6): 1 <= ID <= 18

10

- Now, let’s check the “pr_recos” again.

```
8
2200    0.0     1
2201    90.0    1
2206    30.0    1
2207    -30.0   1    64   620
2216    80.01
2217    -80.0   1    61   501
2208    40.01
2209    -40.0   1    61   541
```



pr_alpha finished

next value for alpha: 50.00o

number of peaks:
max: 63   (80.00o)
min.:   58   (-60.00o)

- Add 50 and -50 (#11 and #12) for reconstruction

……. Check the the “pr_recos” file again.

```
10
2200    0.0     1
2201    90.0    1
2206    30.0    1
2207    -30.0   1    64   620
2216    80.01
2217    -80.0   1    61   501
2208    40.01
2209    -40.0   1    61   541
2210    50.01
2211    -50.0   1    60   580
```

- Eventually, with using all 8 angle, it shows that only one-extra tilted angle (30-dgree) is sufficient for reconstructing a 3D HNCO for the 62 a.a. protein since no improvement in the number of cross peaks can be achieved with more tilted angles.

“pr_recos” using all tilted angles

```
18
1200    0.0     1
1201    90.0    1
1206    30.0    1
1207    -30.0   1    64   600
1216    80.01
1217    -80.0   1    61   517
1208    40.01
1209    -40.0   1    61   541
1210    50.01
1211    -50.0   1    60   594
1204    20.01
1205    -20.0   1    60   586
1214    70.01
1215    -70.0   1    60   547
1212    60.01
1213    -60.0   1    60
1202    10.01
1203    -10.0   1    60
```