**2D Co-ANAFOR manual (ver. 201218)**

**What is Co-ANAFOR?**

Co-ANAFOR is a method for the reconstruction of multidimensional NMR from undersampled data. In Co-ANAFOR, chemical shifts of the signals determined from another high resolution spectra (usually 2D HSQC or TROSY) are utilized for the reconstruction. Therefore, peak height ratios can be accurately determined even for the crowded data like 2D spectra of proteins. See the reference for details of the theories and comparison with other methods.

**Required programs**

・Bruker Topspin®

・Anaconda (platform for scientific computing libraries on Python (Numpy, Scipy, etc.))

・NMR spectra analysis softwares (Sparky etc.)

・Scripts for the reconstruction with Co-ANAFOR

For python 3.X

2D spectra reconstruction：201218python3\_COANAFOR2D.py

3D spectra reconstruction (F2 axis only)：201220python3\_COANAFOR3D.py

For python 2.X

2D spectra reconstruction：201217python2\_COANAFOR2D.py

3D spectra reconstruction (F2 axis only)：201219python2\_COANAFOR3D.py

**1. Installation of required programs**

(1) Download & install Anaconda (Individual Edition, free). Both Python2.X and Python3.X can be used for Co-ANAFOR.

(2) Download the program for executing Co-ANAFOR.

**2. NMR Measurement**

**2-1. Target spectra measurement**

Measure the 2D spectra as usual, with setting the data points in indirect dimension (F1 td) to ~1/3-1/4 of those after the reconstruction.

**2-2 Guide-FID measurement**

(1) Measure the 2D spectra with chemical shifts and linewidth identical to those of 2-1 (typically 1H-15N HSQC or TROSY) as usual, with setting the data points in indirect dimension (F1 td) to those after the extrapolation,

(2) Perform phase correction, window function manipulation, Fourier transform, and baseline correction of the guide-FIDs. Do not apply linear prediction, because it causes subtle but significant chemical shift change.

(3) Perform peak picking of the guide-FIDs by Topspin. Pick all resonances, except for noises or artifacts.

**3. Reconstruction of the spectra**

 (1) Copy the peaklist.xml file in the (guide-FID filename)/(experimental number)/pdata/1 directory, which was created with the operation 2-2, to the (target data filename)/(experimental number)/pdata/1 directory, which was created with the operation 2-1.

(2) Copy the Co-ANAFOR executing file to the (target data filename)/(experimental number) directory.

(3) Open the Co-ANAFOR executing file with a text editor (vi, gedit, etc.) and edit the following parameters:

**window, SSB, LB, GB**

Set the parameters for F1 window function in the same manner as in Topspin.

(\*Do not remove the quotation marks in the window parameter.)

**numext:**

Set the ratio of the number of data points in F1 dimension before and after the reconstruction. Decimal is acceptable. We usually recommend to set to 3 or 4.

**lambda1**

Set the Coefficient of the Tikhonov regularization. We recommend to set to 0.01-0.1.

Increase this value if artifacts like Supplementary Fig. S1 in the original paper are　observed in the spectra after the reconstruction.

**decim:**

We recommend not to change this value. (The intensity values are divided by this value to avoid the data overflow.)

**UniformLWF1**

Set to ”True” unless experimentally determined F1 T2 values are used.

(Set to “False” if they are used.)

**DefaultLWF1, LWF1**

If this value is set to “True”, F1 T2 values of the extrapolated data are automatically set to 1/t1max.

If this value is set to “False”, F1 T2 values of the extrapolated data are manually set to pi\*LWF1.

(These values are ignored if UniformLWF1 is False.)

Set manually if the acquisition time after the extrapolation is markedly different from T2.

**UniformLWF2, LWF2**

These parameters are not used, but do not remove them.

**siglim**

Set the difference of the chemical shifts between the center and edge of signals in F2 dimension (ppm). We usually recommend to set to 0.03, and decrease and increase if the spectra are crowded and sparse, respectively.

(4) Open the target data with topspin and set the edp parameters of the target data as follows:

\*Window function and linear prediction in F1 dimension are set to OFF.

\*F2 window function parameters are set as usnal.

\*F1 SI is set to smaller than the number of data points after the extrapolation. (We recommend to set to the same value as F1 td.)

\*We can cut the right half of the spectra by setting F2 STSI = F2 SI \* 0.5.

Then, execute xfb, abs, and xht1 commands on Topspin.

(5) Run the Co-ANAFOR executing program on a PC equipped with Anaconda, with the following command:

*python 201219python3\_COANAFOR2D.py*

*(or python 201217python2\_COANAFOR2D.py)*

If the program runs successfully, ”writing as binary 2rr...”, “writing as binary 2ri...” are displayed in the terminal.

\* If ”… int too large..” error occurs, try increasing the “decim” value in the Co-ANAFOR executing program.

(6) In Topspin, open another spectra and return to the target spectra. Then, the spectrum after the extrapolation is displayed.

(7) Transfer the data to Sparky or other analyzing software as usual. Return to (3) if you want to perform extrapolation again. (The extrapolated 2rr and 2ri data, which are created by the Co-ANAFOR program, are overwritten if xfb is performed to the target data again.)

**4. Reconstruction of 3D spectra**

The script “201217python2\_CoANAFOR3D.py” or “201219python2\_CoANAFOR3D.py” can be utilized to reconstruct the F2 axis of 3D spectra, like HNCA, HN(CO)CA, HNCACB, 15N-edited NOESY, with using 2D HSQC or TROSY spectra as their Guide-FIDs.

Differences between the reconstruction of 2D and 3D spectra are mainly as follows:

**・In the operation 3-(1), copy the peaklist.xml file to the (target data filename)/(experimental number) directory, instead of (target data filename)/(experimental number)/pdata/1.**

・Utilization of experimentally determined transverse relaxation rates are not supported.(Section 3 must be skipped.)

・Use tht2, instead of xht2, for the Hilbert transform.

・In the case of constant time evolution in F2 dimension, the setting parameter “Constant” in the 3D Co-ANAFOR executing file is set to “True”. (Transverse relaxation rates are set to zero.)

・Large lambda1 value (~0.1) may be preferable, due to the limited number of the data points.

・The calculation may stop with error due to the lack of memory. In such cases, reduce the SI (particularly in F3 dimension) or use processors with large memories (or modify the program).