Shape of NMR signal

\[ S(t) = e^{i2\pi \nu_0 t} e^{-t/T_2} \]

- Frequency of the signal oscillation
- Rate of decay of the signal in xy plane (T_2)
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FID: Free-Induction Decay

Zero-filling

- Addition of zeros to end of data
  - NO additional information
  - Improve peak definition
- Typically double or quadruple collected points
  - Eg., Collect 1024 pts then zerofill to 2048 or 4096
Shape of NMR signal after FT

\[ S(\nu) = \frac{1/T_2}{(1/T_2)^2 + (\nu_0 - \nu)^2} + i \frac{(\nu_0 - \nu)}{(1/T_2)^2 + (\nu_0 - \nu)^2} \]

**Real** (Absorption)

**Imaginary** (Dispersion)

FWHM = 1/T_2

\[ S(\nu) = A(\nu - \nu_0) + iD(\nu - \nu_0) \]
Phase of receiver ‘imperfect’

- Phase of receiver of zero (x) is almost never zero

\[ S(\nu) = A(\nu - \nu_0) + iD((\nu - \nu_0)) \]

\[ S(\nu) = A(\nu - \nu_0) + iD((\nu - \nu_0)) \]

\[ S(\nu) = [A(\nu - \nu_0)\cos(\phi) - D(\nu - \nu_0)\sin(\phi)] + i[A((\nu - \nu_0)\sin(\phi) + D(\nu - \nu_0)\cos(\phi)] \]
Zeroth-order phase correction

Apply a Zero-order phase correction of $-\phi$

$$S_{ph0}(\nu) = S(\nu)e^{-i\phi}$$

First-order phase correction

- Delays and/or mistimings impart frequency dependent phase correction

Ideal

Real (Absorption)

Ph0 corrected

Imaginary (Dispersion)

Spectrum with large delay after correcting zero-order phase
First-order phase correction

Apply a Zero-order phase correction of $-\phi$
And a First-order phase correction of $-vt$

$$S_{ph1}(\nu) = S(\nu)e^{-i\phi}e^{-i2\pi\nu t}$$

Real

Imaginary

Phasing Spectra : Routine

1. Set the zeroth order phase for one signal (most intense)
2. Change first order phase
   This will affect the apparent zero-order phase
3. Sufficient first-order phase has been added when all signals appear to have the same zero-order correction
4. Re-adjust the zero-order phase
   • If all signals are adsorption great!
   • If not, loop through steps 2-4 until they are

   • NB: Spectra which require large first-order phase corrections may end up with a baseline which is ‘bowed’ about the centre
Phasing 2D spectra

- Many conventional 2D spectra are not acquired in a phase sensitive manner (magnitude mode)
  - COSY
  - HMQC
  - HMBC
- For spectra which are phase sensitive (e.g., HSQC) the idea is the same as for 1D
  - Adjust the zero-order phase of a signal at one frequency
  - Then adjust first-order, if necessary, on a peak at very different frequency
- NB: typically only the directly-detected (‘horizontal’) dimension will need phasing

Phasing Examples

- Sample: Tryptophan in D$_2$O
- Folder: Tryptophan_D2O and Tryptophan_2D

1. Open experiment 1
   1. Zero-fill and then Fourier Transform the data
   2. Phase the spectrum
      1. Can you phase all the signals with only ph0?
      2. What are the final phase constants (ph0 and ph1) used?
         1. NB: depending on software ph1 may be in time or degrees
   2. Repeat with data in experiment 2 and 3
      1. How different are the values of ph0 and ph1 from what you obtained with experiment 1?
   3. There is an HSQC in experiment 10, work out how to phase this dataset.
Apodization

- Multiplication of acquired (FID: free-induction decay) by a function
- Done for various reasons
  - Accentuate regions of the data which are more important (e.g., higher signal-to-noise)
  - Correct for artifacts
- Exponential (Lorentzian) multiplication most common in 1D
  - Optimum when set similar to ‘natural’ linewidth of peaks
- Lorentz-Gaussian multiplication (resolution enhancement) useful for improving resolution
  - Require high SNR
  - Care must be taken not to go too far
- Sine bell and sine-squared bell useful for multi-D data
  - Useful for truncated (incomplete) FIDs

\[
\begin{align*}
\text{Exponential} & : e^{-\alpha L B t} \\
\text{Lorentz-Gaussian} \quad \text{(Gaussian bell)} & : \\
& \quad e^{\left(-\alpha L B t - \frac{\alpha L B t^2}{2G B t_{\text{final}}} \right)} \\
\text{Sine bell} & : \sin\left(\pi - \frac{\pi}{s s b} \left(t / t_{\text{final}}\right) + \frac{\pi}{s s b}\right)
\end{align*}
\]
Apodization Examples

- Samples: HOD in D2O and styrene in CDCl3
- Folders: Apodization_D2O and Apodization_stryrene

1. Open experiment 1 in ‘Apodization_styrene’
   1. Zero-fill, FT, phase
   2. Set the exponential multiplication to 0.3, 1 and 5 Hz
   3. What happens to the spectrum?
   4. Try Lorentz-Gaussian
      1. Exponential value should be negative (comparable to peakwidths)
      2. Gaussian value should be set to where fractional point (0-1) where FID goes to zero
      3. What happens to the spectrum?
Apodization Examples (cont’d)

1. Open experiment 1 in ‘Apodization_D2O’
   1. Zero-fill, FT, phase
   2. Set the exponential multiplication to 0.3, 1 and 5 Hz
   3. What happens to the spectrum?

2. Open experiment 3 in ‘Apodization_D2O’
   1. Compare this FID to that of experiment 1
   2. What is different?
   3. Zero-fill, FT, phase
   4. Set the exponential multiplication to 0.3, 1 and 5 Hz
   5. What happens to the spectrum?
   6. Try apodizing this data with a sine bell (or sine-squared bell)
      1. How does the resulting spectrum compare to before
   7. Add a shift to the sine bell

Apodization Examples (extra)

Sample: Tryptophan
Folder: Tryptophan_2D

1. Process the 2D spectra in this folder with different apodization filters in the directly-detected dimension
   1. Are there changes in lineshape? Peak intensity?
2. Do the same in the indirect dimension
   1. Which filter works best
3. Do the same filters yield the best results for both the HSQC and COSY spectra?

NB: The truncation artefacts in the indirect dimension are only masked by apodization. Linear Prediction (see later) in the indirect dimension can improve resolution/lineshape
Baseline Correction

- Accurate integration requires good definition of zero intensity (i.e., baseline)
- Automatic Routines (polynomial or spline) work well for most small distortions
  - Try integrating exp. 2 in 'Tryptophan_D2O' with and without baseline correction

Portion of signal below zero contributes a negative value to sum (integral)

Linear Prediction

- Time-domain (FID) fitting routines to replace missing data
- Existing points used as a basis set
- Forward prediction generates points at end of FID
  - Useful when FID truncated (e.g., multi-D data)
- Backwards linear prediction generates point at beginning
  - Useful if long delay before acquisition (alternative to very large ph1)
Linear Prediction

Samples: Tryptophan and NaF
Folder: Tryptophan_2D and NaF_LP

Experiment 1 of ‘Tryptophan_2D’ is a high-resolution 2D COSY, experiment 2 is lower resolution (indirect dimension)
1. Process these spectra.
2. How do they compare?
Experiment 2 has fewer points in indirect dimension
1. Process with linear prediction in this dimension
2. What happens to the spectrum?
3. How does this ‘new 2’ compare to before LP?
Experiments 10 and 11 are analogous HSQCs (high and low-res)
Make the same comparisons as with the COSY data

NB: You could also try forward LP to correct the HOD spectra in Apodization_D2O which were truncated

Linear Prediction

• Experiment 1 of ‘NaF_LP’ is a 1D $^{19}$F spectrum
  • Process the spectrum and look at the baseline.
• Fluorinated polymers (e.g., PTFE, Kel-F) are commonly used in the construction of NMR probes leading to additional background signals
  • Baseline correction can occasionally fix this.
• A better approach is to ‘remove’ the offending data points and apply linear prediction
  • NB: The duration of the FID for a given signal is inversely proportional to its linewidth in frequency domain.
  • If there is a broad background signal where in the FID would you look for the ‘offending’ data points?
  • Can you find them?