

## Looped PROjective Spectroscopy applied to HMQC-NOESY experiment

### Pulse Sequence **L-PROSY.NH\_wvm**

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Reference: M. Novakovic, S. F. Cousin, M. J. Jaroszewicz, R. Rosenzweig and L. Frydman, *J. Magn. Reson.*, 2018, **294**, 169–180.

Legend of important parameters:

**cnst4** – J coupling between Nitrogen and directly bound protons

**cnst54** – Excitation offset of shaped pulses

**cnst55** – Excitation bandwidth of shaped pulses

**d8** – NOESY mixing time

**l1** – Number of L-PROSY loops

Typical settings:

cnst54=9.0-8.0 ppm center of excitation (set >9 to minimize H<sub>2</sub>O excitation)

cnst55=3-4: excitation bandwidth

NOTE: values are in ppm of referenced chemical shift (transmitter offset at H<sub>2</sub>O)

PC5 90 degree and Reburp 180 degree shaped pulses utilized in the sequence

Depending on the amide-water chemical exchange, J delay should be optimized

In structured proteins J(NH) = cnst4 = 90-95 Hz

For IDPs and unstructured proteins try to increase cnst4 in the pulse sequence

Structured proteins:

NOESY mixing time d8 =130-200 ms optimal

Number of loops l1=4-10 optimal

Even though it is sample and field dependent, usually safe bet is 6x150 ms

Unstructured proteins:

NOESY mixing time 70-150 ms optimal

Number of loops l1=8-12 optimal

Even though it is sample and field dependent, usually safe bet is 10x80 ms

In general:  $l1 \cdot d8 \leq T_1$  (aliphatic protons) since  $T_1$  sets up the upper boundary for enhancements

For optimal enhancements: test the buildup – convert the sequence to 1D and popt d8/l1

1. Small proteins (bellow 8 kDa) – set mixing time to 150 ms and vary the number of loops 1-10 only for first  $t_1$  increment and monitor the buildup of aliphatic protons  
Then set the loop number that gave the highest cross-peak signal and vary the mixing time 50-200 ms in steps of 25 ms

2. Medium-size proteins (above 8 kDa) – set mixing time to 100 ms and vary the number of loops 1-14 only for first  $t_1$  increment and monitor the buildup of aliphatic protons  
Then set the loop number that gave the highest cross-peak signal and vary the mixing time 50-150 ms in steps of 25 ms
3. For very large proteins such as BSA and similar, NOE effect is already quite efficient, and L-PROSY can provide around 1.6x enhancement maximum when the theoretical maximum of cross-peak intensity is reached – usually 4-6x100 ms
4. For unstructured proteins and partially folded proteins – set mixing time to 100 ms and vary the number of loops 1-14 only for first  $t_1$  increment and monitor the buildup of aliphatic protons

Avoid using very short mixing time since that can cause looping artifacts such as higher harmonics artefacts and in some cases severe losses in amide signal upon looping (amide protons need sufficient time to recover for the following loop). Short mixing times only beneficial for amide protons that exchange fast with water.

d1 around 2-4s to let aliphatic protons recover.

### **Installation:**

- 1) Copy the pulse programs from the pp folder to your TopSpin#/exp/stan/nmr/lists/pp/user folder;
- 2) Copy the parameter folders from the par directory to your TopSpin#/exp/stan/nmr/par/user folder;
- 3) Optional: copy the contents of the par.catalog folder to your home/.topspin1/par.catalog folder; this ensures the new experiments are available from the Experiment Selector;

This completes the installation.