Meet the family: NOE and Selective excitation experiments

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Introduction to NOE and selective excitation experiments

The use of selective excitation and Nuclear Overhauser Effect (NOE) based experiments allows the generation of unique structural information which may not be available from other experiments, e.g.: COSY, HSQC, HMBC etc.. NOEs also occur in a variety of other commonly used experiments, and their impact can range from a benefit to a nuisance depending on the situation.

xOESY: Basic principles

At their core, xOESY experiments rely on observation of scalar coupling (J) between spin systems through space, rather than coupling of adjacent components as observed in COSY. The basic principle of the phenomenon is that excitation "bleeds" over from the excited moiety to those in close proximity, resulting in a slight effect to the signal integrals of connected moieties.

The simplest usage of this effect is through 1D NOESY, which relies on selective excitation of a signal of interest to observe its correlations. The approach is similar to that used for classic solvent suppression experiments, where the unwanted peak is selectively excited prior to execution of the broadband excitation pulse to "crush" only that peak. 1D NOESY allows a quick, targeted approach to gain specific information about a molecule (Figure 1).

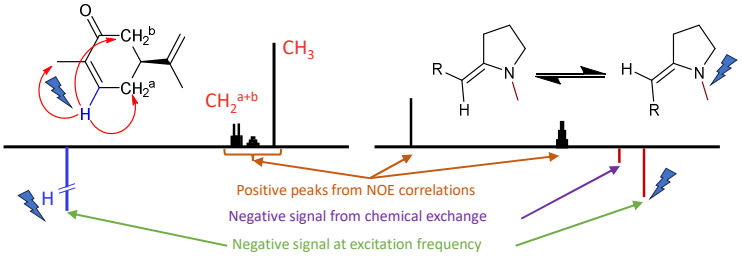

Figure 1: Examples of 1D NOESY spectra for NOE and chemical exchange data

Despite appearing like an abstract concept, NOEs play an indirect role in many common experiments, such as the ubiquitous power gated decoupling (zg**pg**) 1D $^{13}C\{^1H\}$ and $^{31}P\{^1H\}$ experiments. The buildup of NOEs yields increased signal intensity for H bound nuclei as these nuclei have positive gyromagnetic ratios. For nuclei with negative ratios it is possible for NOEs to completely negate signals, or yield strong negative signals if the NOE is sufficiently strong. A similar principle is used to generate HOESY spectra for these nuclei, which allows spatial correlation between heteronuclei.

2D NOE experiments follow the same principle as 1D, however require less configuration as excitation is performed across the entire range of the spectrum at the cost of more experiment time. The most common usage of NOE experiments includes the following:

- Confirmation of structural assignment generated using other experiments. Correlations will be observed between nuclei which are within proximity of one another. This allows bridging across typical "dead zones" in sequences, expanding assignment coverage (Figure 2).
- Confirmation of stereochemistry; this is not possible by other 2D experiments. Correlations between elements of unknown configuration can be used determine the correct structure (Figure 3).
- Confirmation of chemical exchange, i.e. rotamers, conformer, etc.. In these cases, correlation peaks can be observed between signals resulting from exchange (Figure 4).

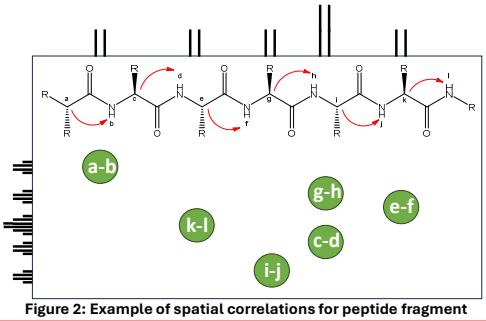

Figure 2: Example of spatial correlations for peptide fragment

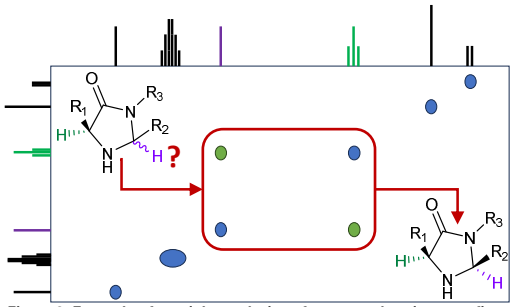

Figure 3: Example of spatial correlations for stereochemistry confirmation

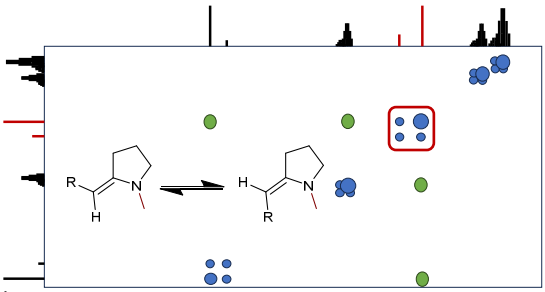

Figure 4: Example of chemical exchange correlations

NOESY & ROESY: Same but different

NOESY and ROESY represent the most widely used variants of the basic 2D NOE experiment within Almac Sciences; both offer similar information depending on use case/conditions. The key difference between the experiments is their performance for small (MW < 600Da), medium (MW = 600 - 2000Da) and large (MW ≥ 2000Da) molecules.

The maximum NOE intensity (NOE_{max}) is a function of both the Larmour Frequency ($\boldsymbol{W_0}$) and Correlation Time ($\boldsymbol{\tau_c}$) for both experiments. τ_c is affected by a myriad of factors, most importantly diluent viscosity, MW and temperature. Under fixed conditions, the relationship can be simplified to τ_c increasing with MW. The differences in polarisation transfer schemes for the two experiments yield different NOE_{max} profiles (Figure 5). NOESY will transition from a positive NOE_{max} (0.5) to negative NOE_{max} (-1.0), passing through a null point where no NOEs will evolve. ROESY maintains a positive NOE_{max}, increasing from 0.38 to 0.65 with increasing correlation time. In essence both experiments will approach optimal sensitivity with increasing MW.

This translates to a change of phase for NOESY correlations relative to the diagonal correlations, potentially occluding resolution of NOE correlations from chemical exchange and spurious correlations, hindering interpretation (Figure 6).

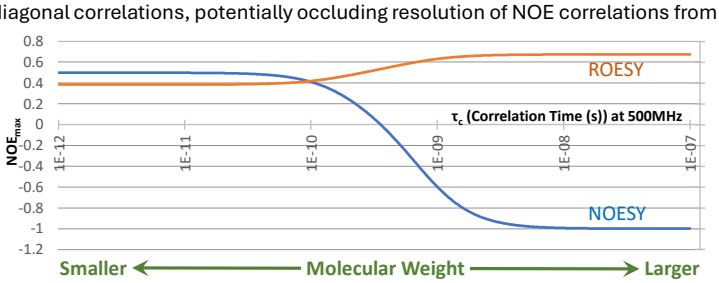

Figure 5: Plot of maximum theoretical NOE intensity (NOE_{max}) vs τ_c (Correlation time) at 500MHz

Both experiments can yield misleading correlations under certain conditions. NOESY spectra can include COSY correlations for smaller molecules. These bands can be readily identified by comparison to a COSY spectrum. ROESY can yield long range correlations akin to those produced by TOCSY in the opposite phase to NOEs; as it is possible for these to be identified they can be discarded. Evolutions of both experiments are available which reduce occurrence of these artifacts. Evolution of NOEs for NOESY and ROESY can be optimised by adjusting mixing time (**d8** and **d15** respectively); increasing leads to more intense correlations but reduces intensity differences over distance. Adjustments should be carefully considered as changes in mixing time will affect visibility of chemical exchange, and can result in sensitivity losses due to T_2 relaxation.

In conclusion, both experiments offer a variety of use cases to further understanding of a molecule, however careful selection and interrogation of data must be performed to ensure that accurate conclusions are drawn. It is recommended that ROESY is used for all but large molecules.

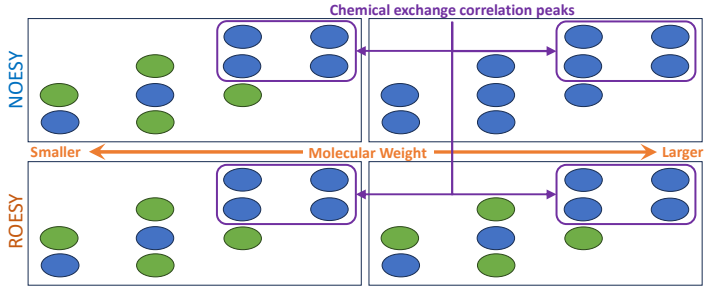

Figure 6: Phase of correlation peaks in NOESY and ROESY experiments vs Molecular Weight

Key: NOESY: Nuclear Overhauser Effect Spectroscopy | ROESY: Rotating-Frame (Nuclear) Overhauser Effect Spectroscopy | HOESY: Heteronuclear Overhauser Effect Spectroscopy