Supporting Information

Detecting the Afterglow of ^{13}C NMR in Proteins Using Multiple Receivers

Ēriks Kupče, Lewis E. Kay, and Ray Freeman*

Agilent Technologies, 6 Mead Rd., Yarnton, Oxford, OX5 1QU, UK; Departments of Molecular Genetics, Biochemisty and Chemistry, University of Toronto, Toronto, Ontario, M5S 1A8, Canada; Jesus College, Cambridge University, Cambridge, CB5 8BL, UK.

Sensitivity considerations

The overriding concern for the direct detection of the ^{13}C signal is signal-to-noise ratio, rather than resolution. Under these conditions we note the rather surprising general result that there is an optimum truncation point for a typical free induction signal at 1.26 times the time constant of the decay. The optimum signal-to-noise ratio is achieved by truncation at this point *irrespective* of the general level of the noise compared with the NMR signal. This counter-intuitive conclusion does not appear to have been reported elsewhere. Assuming that the signal decays exponentially (for example by spin-spin relaxation):

$$M(t) = M_0 \exp\left(-\frac{t}{T_2}\right) \qquad [1]$$

then the signal intensity for a typical resonance can be written:

$$S(t) = M_0 T_2 \left[1 - \exp\left(-\frac{t}{T_2}\right)\right] \qquad [2]$$

This is a function that starts at zero (when time t = 0) and rises to an asymptotic level when $t >> T_2$. The integral of random noise over the same interval is given by

$$\sigma(t) = \sigma_0 t^{1/2} \qquad [3]$$

Consequently the general expression for signal-to-noise ratio, S_n is:

$$S_n(t) = \frac{M_0}{\sigma_0} \frac{T_2\left[1-\exp\left(-\frac{t}{T_2}\right)\right]}{t^{1/2}} \qquad [4]$$

where the ratio M_0/σ_0 can be thought of as the inherent sensitivity of the experiment, Ξ while the rest of Eq. [4] contains a time dependent function that defines the optimum choice in acquisition time (point of truncation). This function starts at zero and passes through a maximum before falling back asymptotically to zero (Fig. 4S). The position of the maximum is found by setting the first derivative to zero:

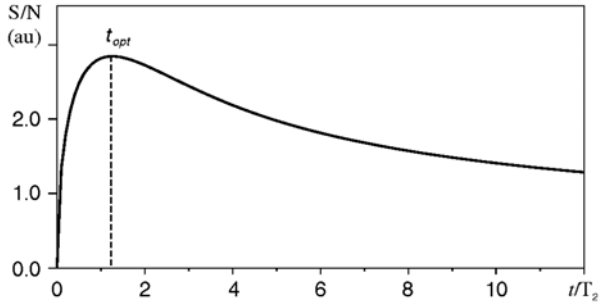

Fig. 4S. Calculated curve for the signal-to-noise ratio (S/N) of an exponentially decaying signal as a function of the truncation point (time t expressed in units of T_2). The vertical scale is in arbitrary units because any change in the relative noise level simply scales the curve without altering its shape. The maximum S/N ratio is achieved at $t = 1.256T_2$.

$$\frac{dS_n}{dt} = \Xi \frac{\exp\left(-\frac{t}{T_2}\right)\left[T_2 \exp\left(\frac{t}{T_2}\right) - 2t - T_2\right]}{-2t^{3/2}} = 0 \qquad [5]$$

This gives an expression for finding the optimum truncation point that yields the maximum signal-to-noise ratio:

$$\exp\left(\frac{t}{T_2}\right) = 1 + \frac{2t}{T_2} \qquad [6]$$

The optimum occurs when the free induction decay is truncated at t = 1.256 T_2, where the NMR signal intensity has fallen to 0.285 M_0.

The surprising conclusion is that the optimum truncation point does not depend on the level of noise compared with M_0 (the intrinsic sensitivity of the experiment, Ξ). Even at conditions of very low or very high noise the maximum of Eq. [4] remains at t = 1.256 T_2. Increased signal or noise simply changes the vertical scale of Fig. 4S without influencing the shape of the curve. At the extremes where S/N = 0 or ∞, the setting of t is of course irrelevant.

Amplifying the ^{13}C afterglow

In the practical case of detection of the ^{13}C afterglow (see the main text), the optimum truncation level calculated above attenuates the refocused ^{13}C magnetization rather severely (to 0.285 M_0). After refocusing and spin-spin relaxation for a further equal period, the remaining ^{13}C intensity is 0.08 M_0. This is the fraction transferred to the NH protons. In principle, unless the spectrometer probe has been designed to favor ^{13}C detection, the predicted enhancement attributable to magnetization transfer to protons is given by $(\gamma_H/\gamma_C)^{3/2} \approx 8$ (allowing for the unfavorable spin population ratio). For this reason, although the ^{13}C afterglow is quite weak, a reasonably strong proton signal corresponding to 0.64 M_0 (^{13}C) can be detected. This estimate is roughly corroborated in Fig. 5S which shows a trace from the ^{13}C direct detection experiment and the comparable proton trace.

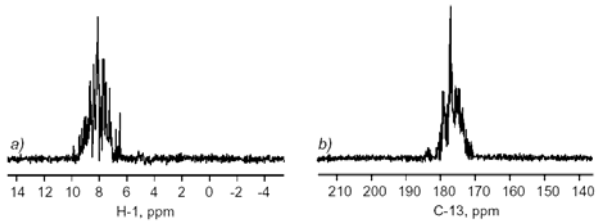

Fig. 5S. Signal intensities in the first traces of (a) ^1H- and (b) ^{13}C-detected experiments of Fig. 1 (see the main text). The sample is 1 mM CN-NuiA (nucelease A inhibitor) in H_2O/D_2O (9:1). The experimental details are equivalent to those in Fig. 2 of the main text, except the CO direct detection acquisition time was set to 0.024 seconds.

Note that the maximum in Fig. 4S is rather broad, which gives a comfortable room for optimizing the relative sensitivities of the ^1H and ^{13}C detected spectra simply by adjusting the corresponding acquisition times. The conclusion is that it is well worth taking the trouble to detect the ^{13}C afterglow by magnetization transfer to protons.

C-13 Chemical shift scaling in the carbon-carbon correlated spectra of the projection reconstruction experiment

As described in the main text, the ^{13}C-detected (HA)CACO 2D spectrum is recorded in parallel with the tilted plane of the 3D (HA)CA(CO)NNH projection reconstruction experiment. Note that the tilted plane is recorded by jointly incrementing (t_1,t_2) according to cos δ /sw_{tilt} and sin δ /sw_{tilt} where δ is the tilt angle and sw_{tilt} is the spectral width in the indirectly detected dimension. This scaling applies also to the F_1 domain of the 2D (HA)CACO spectrum and has to be accounted for at the processing stage. This is achieved simply by scaling sw_1 by a factor of 1/cosδ. The resulting spectrum (see Fig. 6S) perfectly matches that recorded in the conventional experiment (see Fig. 2a in the main text).

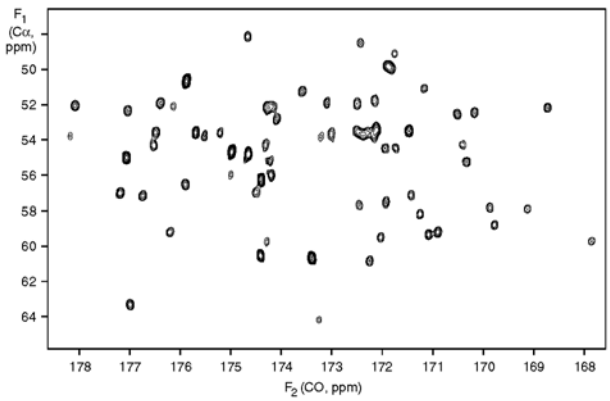

Fig. 6S. The 2D (HA)CACO spectrum recorded in parallel with the tilted plane of the 3D (HA)CA(CO)NNH experiment. For experimental details see the main text.

Applicability to larger protein systems

We have explored the applicability of the experiment of Fig 1 to larger proteins using the nuclease A inhibitor (NuiA, 143 residues) as a test system. 'Conventional' (HA)CACO/(HA)CA(CO)NNH data sets have been recorded in 3 hours/experiment at both 25 and 2°C with the results shown in Figure 7S. All of the acquisition parameters are identical to those used in recording spectra of the smaller GB1 protein with the exception of the CO direct detection acquisition time that was set to 24 ms to account for faster CO relaxation rates in this system. Analyses of the resultant spectra establish that essentially all of the cross-peaks observed at 25°C were also present in data recorded at 2°C, albeit with lower signal to noise (avg signal to noise of peaks 107:52 and 112:18 for the 2D and 3D data sets, respectively).[15]N relaxation experiments indicate that NuiA tumbles with an effective correlation time of 8.8±0.6 and 17.5±1.2 ns at 25 and 2°C; it is clear, therefore, that the present methodology will be applicable to many small and medium sized proteins, at least in studies at 14T (600 MHz ^1H frequency) or lower where CO CSA related line-broadening is not prohibitive.

Acknowledgment. The sample of NuiA was kindly provided by Dr. Robert E. London of National Institute of Environmental Health Sciences.

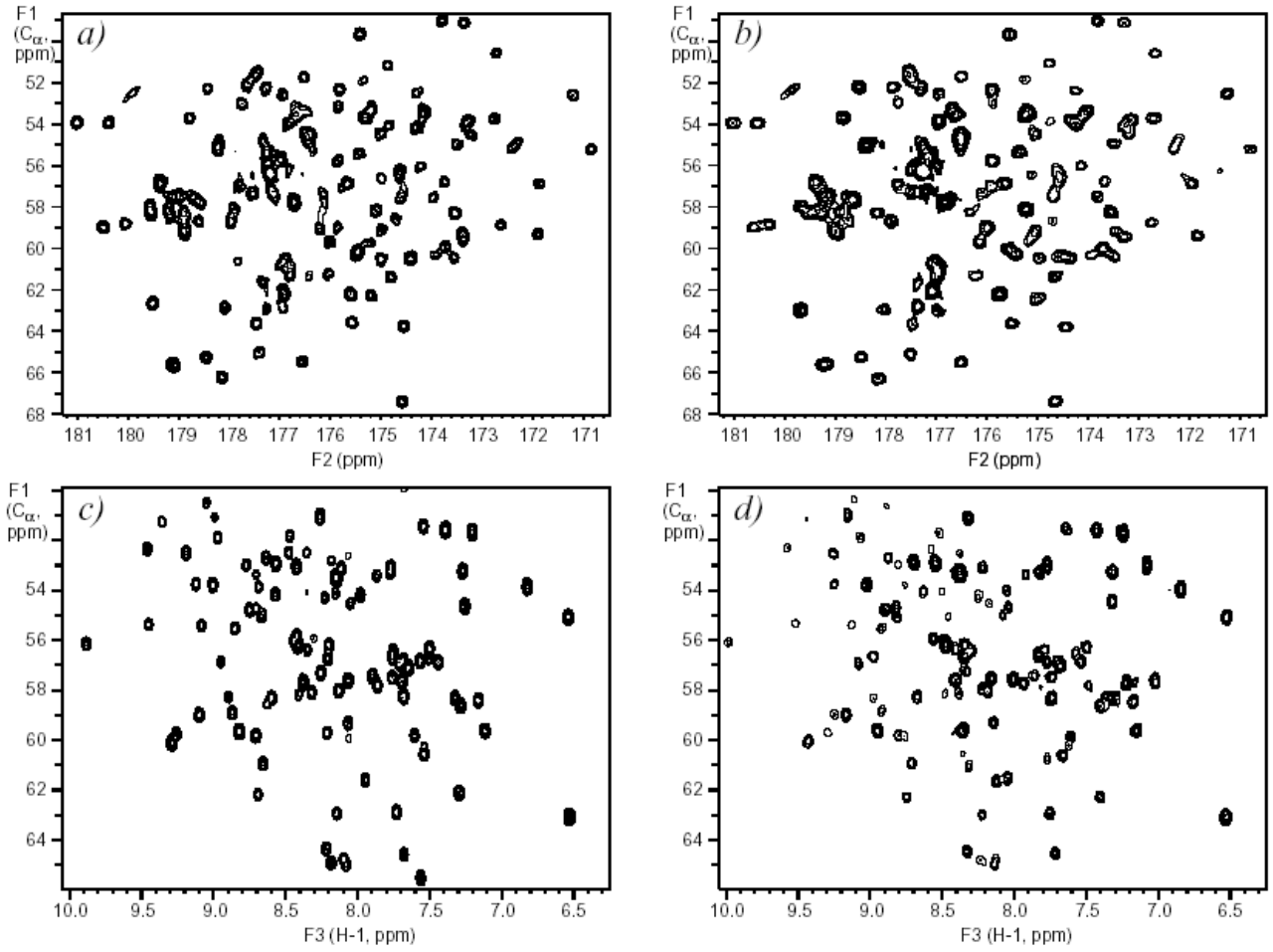

Fig. 7S. 2D (HA)CACO spectra (a) and (b) recorded in parallel with the 3D (HA)CA(CO)NNH experiment (c) and (d). The spectra shown in (a) and (c) were recorded at 25° C while spectra shown in (b) and (d) were recorded at 2° C. The sample is 1 mM CN-NuiA (nuclease A inhibitor) in H_2O/D_2O (9:1). The experimental details are equivalent to those in Fig. 2 of the main text, except that the CO direct detect acquisition time was set to 0.024 seconds.