Ndukwe, I. E., Shchukina, A., Kazimierczuk, K., & Butts, C. P. (2016). Rapid and safe ASAP acquisition with EXACT NMR. *Chemical Communications*, *52*(86), 12769-12772. https://doi.org/10.1039/c6cc07140f

Journal Name

COMMUNICATION

Rapid and safe ASAP acquisition with EXACT NMR

I. E. Ndukwe,[a] A. Shchukina,[b,c] K. Kazimierczuk,[b] and C. P. Butts[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

The dangerously high power levels required for acquisition of the fast ASAP-HSQC and ASAP-HMQC experiments are mitigated by employing EXACT (Extended ACquisition Time) NMR. The utility of this technique is demonstrated by application of the EXACT ASAP-HSQC to chemical reaction monitoring, accelerating data acquisition by up to 2 orders of magnitude and providing evidence of it's value in fast quantitative NMR processes.

The acceleration of multi-dimensional NMR data acquisition has seen substantial steps forward in recent years. The single-scan 'ultra-fast' NMR techniques provide the most rapid approaches (~1 second for full 2D acquisitions)[1, 2] and lend themselves to studying time-limited phenomena, for example, reaction monitoring or chemical exchange processes. However, the quality of data obtained by these methods is often limited by a number of factors including sensitivity losses (occasioned by the need for highly selective and thus long pulses), lineshape distortion, molecular diffusion and/or the spectral widths to be accessed.[2-5] More conservative solutions, offering experiment times at minute time-scales, primarily focus on techniques to reduce or remove the traditional recovery delays (~1-2 seconds) where magnetisation returns to equilibrium between scans in the experiment *e.g* extended flip-back,[6] SOFAST,[7] BEST,[8] SMART[9] and ASAP.[10, 11] While the first three methods rely on the limited functionality and selective labelling specific to protein NMR spectroscopy, the latter two have more general applications to a range of molecular targets. SMART methods, however, require 3-dimensional field gradients which are not standard hardware on most current NMR spectrometers. In all cases, while significant experimental time savings are obtained by implementation of these methods, they do suffer from reduced sensitivity as equilibrium magnetisation is not recovered as efficiently as through traditional recovery delays. An often complementary solution to all of these methods is non-uniform sampling (NUS), whereby only a subset of time-increments in the indirect dimension are sampled,[12] reducing experiment times by up to an order of magnitude for each indirect dimension sampled, with the primary limitation being the ability of available algorithms to reconstruct the desired spectrum from the under-sampled datasets.

Of particular relevance to this report is the ASAP methodology, first applied to HMQC by Kupče and Freeman in 2007.[10] ASAP uses a homonuclear Hartmann-Hahn cross-polarisation sequence[13] between each scan to transfer equilibrium magnetisation from passive (donor) ^{12}C-attached protons which are not observed in the spectrum (and serve as magnetisation reservoirs), to the active (acceptor) ^{13}C-attached protons which are the observed spins. This allows the traditional relaxation delay between each scan to be replaced by a short cross-polarisation mixing period of <60ms, saving up to an order of magnitude in total experiment time, albeit with some loss of signal intensity. The ASAP concept was extended to the HSQC experiment,[11] demonstrating similar substantial time savings but with higher resolution potential in the indirect dimension.[14] In both ASAP experiments however, the absence of a relaxation delay means that there is near-continuous high-powered pulsing which puts dangerously high power demands on spectrometer hardware in addition to potential heating of the samples being studied. In practical terms this severely limits the length of the ^{13}C-decoupled signal acquisition and thus achievable resolution.

In view of these power handling and sample heating concerns, NMR spectroscopists have been very cautious about deploying ASAP experiments and at the time of writing only the original authors of the ASAP-HSQC sequence have reported its application – despite its substantial potential value to the wider scientific community. The ASAP methodology can be safely employed by shortening the FID acquisition to <60ms and/or increasing the recovery delay. The latter, however,

[a] School of Chemistry, University of Bristol. Cantocks Close, Bristol, BS8 1TS, UK.
[b] Centre of New Technologies, University of Warsaw. Banacha 2C, 02089, Warszawa, Poland.
[c] Institute for Spectroscopy, Russian Academy of Sciences. Fizicheskaya 5, 142190, Moscow, Troitsk, Russia.

† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

defeats the ASAP principle itself while the former limits the achievable resolution. Hence these experiments would clearly benefit from

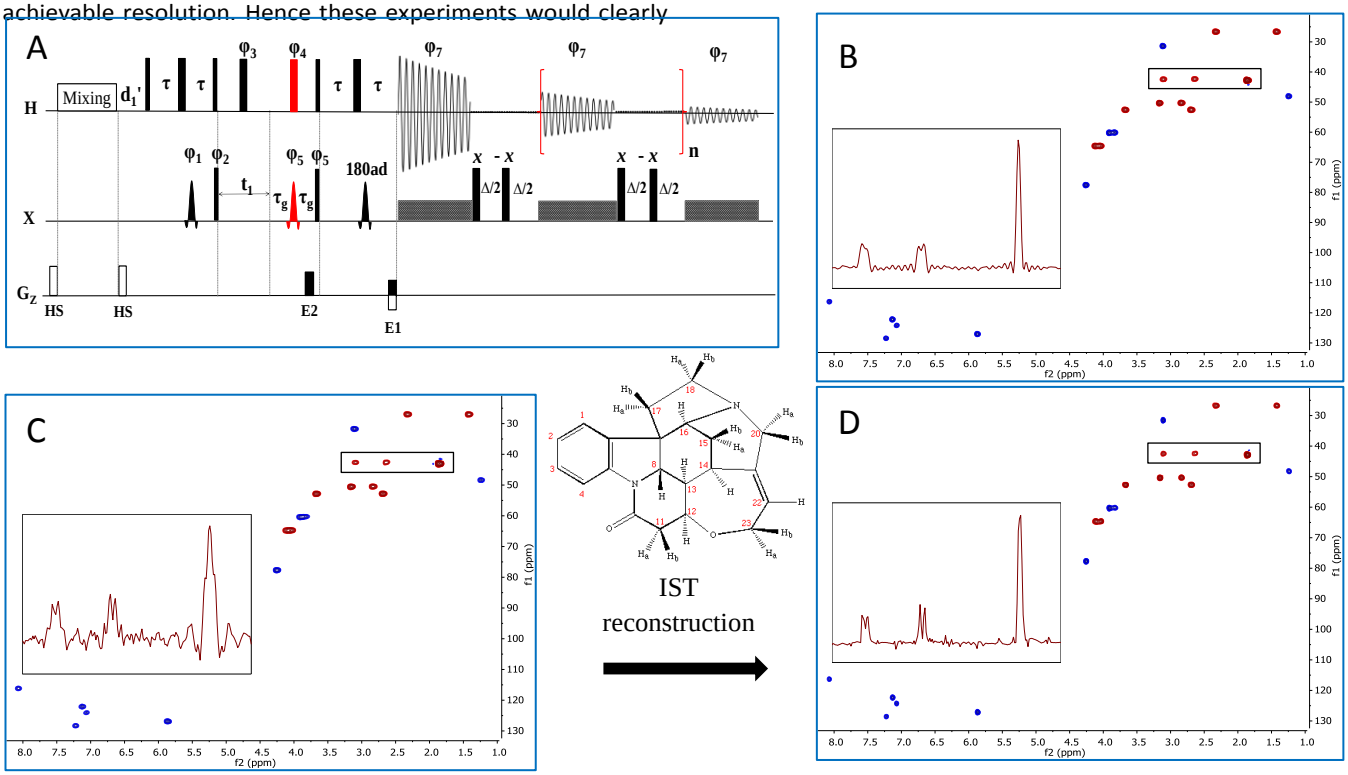

Figure 1. (a) Multiplicity edited EXACT ASAP-HSQC pulse sequence. Details are provided in the SI. Fourier transformed spectrum of strychnine (shown inset) acquired with (b) non-EXACT ASAPHSQC (42ms acquisition and delay d_1' of 94ms) and (c) EXACT-ASAPHSQC. (d) The IST reconstructed spectrum of the EXACT-ASAPHSQC dataset with the 1D trace (inset) revealing artefact suppression after reconstruction with iterative soft thresholding (IST). Window function not applied to the 1D traces shown.

a more practical solution to reducing their power-demands without unduly sacrificing resolution and we propose to achieve this by introducing delays into the acquisition period itself.

Recently, we have reported the EXACT (EXtended ACquisition Time)[15] approach to acquisition in the directly detected dimensions of NMR experiments. EXACT NMR introduces time periods (delays) between short 'bursts' of NMR data point acquisition (data chunks) – all of which lie on the Nyquist grid – during which time the receiver is gated-off and no data points are acquired. The missing data points in the resulting burst-sampled[15] FIDs can then be reconstructed by algorithmic methods analogous to those used for existing NUS applications, such as Compressed Sensing (CS) or Maximum Entropy (MaxEnt) methods.[16-18] Key to the effectiveness of this technique, is that chemical shift evolution of the desired/observed spins continues unperturbed during these receiver-gated gaps in the FID. In the case of EXACT-HSQC it was shown that gating the continuous broadband heteronuclear decoupling allowed multi-second 13C-decoupled acquisition times in the direct dimension, well beyond the typical sub-500ms HSQC FID lengths.

The EXACT concept is here incorporated into the ASAP-HSQC sequence as shown in Figure 1a (see SI for EXACT ASAP-HMQC). Broadband heteronuclear decoupling is not used during the receiver-gated periods (Δ) of the FID and is instead replaced by a pair of 180° ^{13}C pulses separated by a variable time period, Δ/2. These 180° pulse pair refocuses heteronuclear coupling during the gap periods but with significantly lower power demands than the continuous decoupling it replaced. The first ^{13}C pulse is placed just after the gating-off of both the receiver and continuous ^{13}C-decoupling, with the second pulse applied at the midpoint of the gap, as shown in Figure 1a. Crucially, the ^{13}C pulse pair does not affect ^{1}H chemical shift or homonuclear evolution between data chunks, so the acquired ^{1}H data points in the EXACT experiments match those which would be acquired at the same points in the FIDs of the parent ASAP-HSQC or ASAP-HMQC.[10, 11] It should be noted that multiplicity editing is implemented here for the ASAP-HSQC sequence, enabling discrimination of CH/CH_3 and CH_2 carbon centres and also reducing t_1 noise due to more efficient coherence selection in a single scan, albeit with sensitivity losses ranging from 25 – 55% for each resonance compared to the non-edited version (see SI for pulse sequence).

The EXACT ASAP-HSQC (Figure 1a) was acquired and the resulting EXACT FID Fourier Transformed to give a spectrum (Figure 1c) which appear grossly correct, but the desired peaks are overlaid with NUS aliasing artefacts in F2 (clearly visible in the inset in Figure 1c). This potentially obscures weak peaks in the spectrum but reconstruction of the missing data points with Iterative Soft Thresholding (IST)[19-21] substantially reduces these aliasing artefacts (Figure 1d). As expected, sensitivity is lower than the standard HSQC method due to incomplete recovery of magnetisation between increments, although Schulze-Sünninghausen *et al*[11] reported that sensitivity per

unit time can be higher for the ASAPHSQC experiment when optimised and averaged across several scans. Crucially, the EXACT acquisition significantly reduces the high duty cycle imposed by the ASAP experiments on the spectrometer hardware/electronics – ^{13}C channel duty cycles are ~64% and ~26% respectively for the non-EXACT and EXACT ASAP-HSQC experiments with equivalent FID lengths (105ms) and experiment time (37s). Acquiring an ASAP-HSQC spectrum with comparable duty cycle to the EXACT version requires a very short (42ms) acquisition time (420 data points) and a longer relaxation delay (d_1') of 94ms (Figure 1(b)). Comparison with the reconstructed EXACT ASAP-HSQC spectrum (Figure 1d) demonstrates the loss in F2 resolution even after linear prediction of the truncated FID.

In addition to the ^{13}C decoupled acquisition period, the DIPSI sequence adds greatly to the power being put into the sample and further raises the risk of sample heating. To check for this, ^{1}H spectra were acquired immediately before and after an extended 16 scan EXACT ASAP-HSQC experiment (9.5 minutes total acquisition time) on a sample of CH_3OH in CD_3OD. The chemical shift difference ($\Delta\delta$) between the CH_3 and OH peaks is a very sensitive temperature probe, but showed no change before and after the experiment suggesting that no significant sample heating (<<0.1 °C) had occurred.

EXACT NMR applies to the direct (^{1}H) dimension of the HSQC and hence is compatible and entirely complementary to 'normal' non-uniform sampling of the indirect (^{13}C) dimension. EXACT ASAP-HSQC acquisition with 50%, 25% and 12.5% NUS t1 increments (that is 48, 24 and 12 real t1 increments respectively), allowed experiments to be completed in 19, 11 and 6 seconds respectively (see Figures 3a, 3b and 3c). IST reconstruction of the two dimensions successfully reproduced the expected HSQC spectra (Figures 3d, 3e and 3f respectively). The reconstructed spectra showed artefacts in line with what is observed in F_1 NUS spectra, such that the EXACT 50% NUS spectrum had negligible baseline reconstruction artefacts while the reconstructed 12.5% sampled spectrum began to show clear artefacts in the contour plots (highlighted with purple cycles in Figure 3) as the number of missing data points increases. The artefacts which arise from imperfect reconstruction are easily identified by a comparison of the reconstructed spectrum and the Fourier transformed spectrum – although this distinction is slightly more challenging in the 12.5% NUS spectrum due to the prominence of random and t_1 noise (compare Figures 3c and 3f). The speed of the 12.5% NUS EXACT ASAP-HSQC acquisitions is nearly comparable to those obtained with ultrafast 2D methods but without the concomitant bandwidth, peak shape[4] and resolution limitations.

It is important to note that the peaks of the EXACT ASAP-HSQC spectrum have comparable relative volumes to the standard HSQC spectrum of strychnine and thus have similar quantitative value, but can be employed for monitoring changes in substrate concentrations which occur on second-to-minute, rather than minute-to-hour timescales. This is demonstrated by monitoring the proto-deboronation of a 75mM solution of 2,6-difluorophenylboronic acid in 50:50 deuterated dioxane/D2O with 1.5 equivalents of KOH, at room temperature. Figures 4a and 4b show plots of 1H and EXACT ASAP-HSQC experiments of substrate concentration (mM) – 2,6-difluorophenylboronic acid – deduced from the absolute peak intensities vs time (seconds) of acquisition. 1H NMR (32s

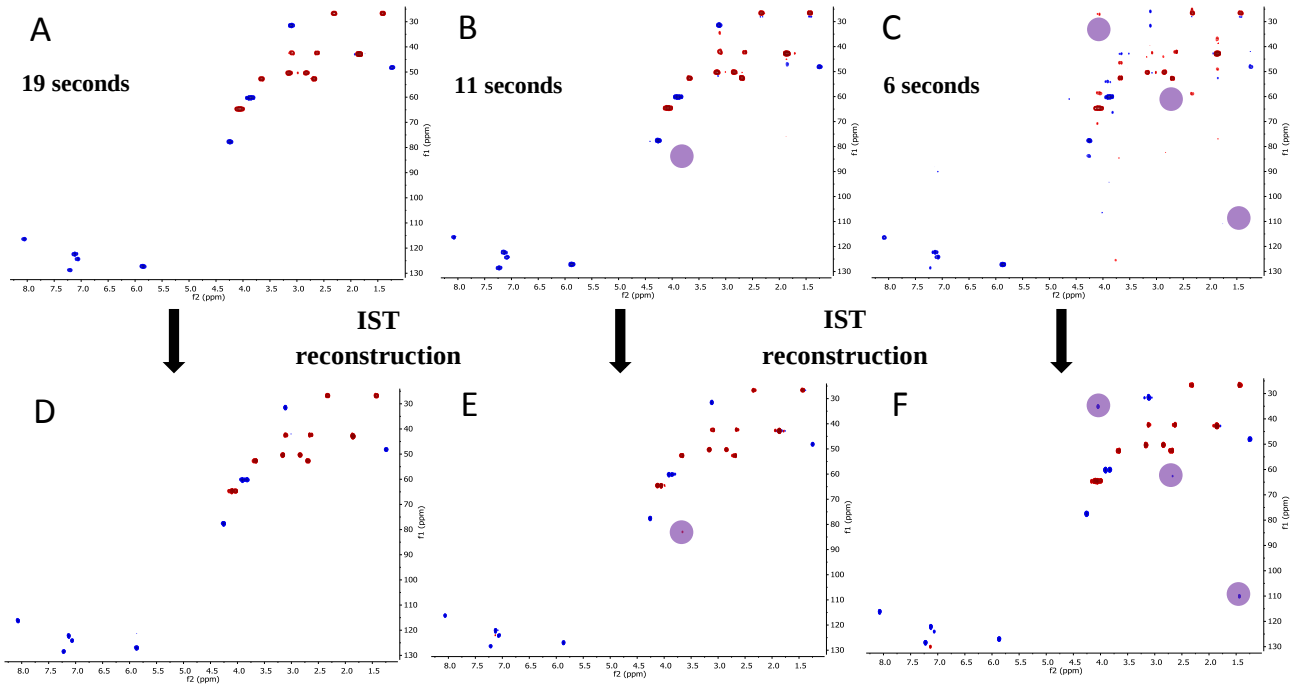

Figure 3. Strychnine spectra acquired with the sequence reported in this work resulting from the Fourier transformation of the zero-augmented NUS dataset acquired (a) and IST reconstructed spectra (b with application of 'standard' random NUS in the indirect dimension (at 12.5 % t1 increment). Peaks highlighted with purple cycles in the IST spectra are reconstruction artefacts which are not present in the FT spectrum (also highlighted with purple cycles)

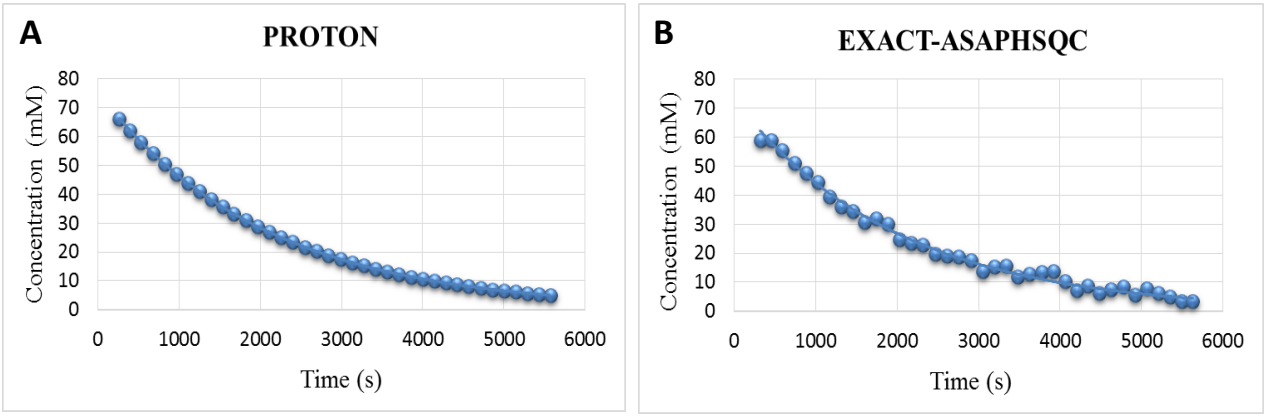

Figure 4. Plot of concentration (mM) deduced from absolute peak intensities from (a) ^{1}HNMR and (b) EXACT-ASAPHSQC (non-edited version) *vs* time for the proto-deboronation of 2,6-difluorophenylboronic acid (298K, 50:50 deuterated dioxane/D_2O)

experiment time) were acquired alternately with the EXACT ASAP-HSQC experiment (non-edited variant, 37s experiment time) and the reaction was monitored for ~1.5 hours.This reaction shows clear first order kinetics with comparable t½ (~23 minutes) and 1st order rate constant (~5.0 x 10^{-4} s^{-1}) values by both ^{1}H and EXACT ASAP-HSQC techniques, and is in accord with data recently reported elsewhere.[22] Up to 20 EXACT ASAP-HSQC datasets could be acquired within the first half-life of the reaction if desired (8 were acquired here, interleaved with the 1H NMR spectra) in comparison to 2 datasets which could have been collected using standard HSQC methods, thus providing much more time-resolution in the kinetic data during this crucial early stages of reactions. These were collected with no danger to the spectrometer hardware and electronics or appreciable sample heating.

In summary, the substantial risks of the fast ASAP experiments (ASAP-HSQC and ASAP-HMQC) can be completely mitigated by using EXACT NMR and the corresponding spectrum recovered by algorithmic reconstruction of the missing data points in the resulting FIDs. This provides opportunities for fast qualitative or quantitative NMR methodologies including analyses of multi-second transient events or processes, such as chemical reactions, where 2D spectra can be obtained in as little as 6 seconds, making these techniques competitive with the ultra-fast single-scan methods.

Acknowledgements

The authors acknowledge Abia State University, Uturu and Tertiary Education Trust Fund, Nigeria (sponsorship for IN) and the National Science Centre of Poland for supporting KK/AS (SONATA BIS 2012/07/E/ST4/01386 and OPUS 2015/17/B/ST4/04221 grants).

Notes and references

1. L. Frydman, T. Scherf and A. Lupulescu, *Proceedings of the National Academy of Sciences of the United States of America*, 2002, **99**, 15858-15862.
2. P. Giraudeau and L. Frydman, *Annual Review of Analytical Chemistry*, 2014, **7**, 129-161.
3. P. Pelupessy, L. Duma and G. Bodenhausen, *Journal of Magnetic Resonance*, 2008, **194**, 169-174.
4. P. Giraudeau and S. Akoka, *Journal of Magnetic Resonance*, 2008, **190**, 339-345.
5. P. Giraudeau and S. Akoka, *Magnetic Resonance in Chemistry*, 2011, **49**, 307-313.
6. T. Diercks, M. Daniels and R. Kaptein, *J Biomol NMR*, **33**, 243-259.
7. P. Schanda and B. Brutscher, *Journal of the American Chemical Society*, 2005, **127**, 8014-8015.
8. P. Schanda, H. Van Melckebeke and B. Brutscher, *Journal of the American Chemical Society*, 2006, **128**, 9042-9043.
9. B. Vitorge, G. Bodenhausen and P. Pelupessy, *Journal of Magnetic Resonance*, 2010, **207**, 149-152.
10. E. Kupče and R. Freeman, *Magnetic Resonance in Chemistry*, 2007, **45**, 2-4.
11. D. Schulze-Sünninghausen, J. Becker and B. Luy, *Journal of the American Chemical Society*, 2014, **136**, 1242-1245.
12. M. Mobli and J. C. Hoch, *Progress in Nuclear Magnetic Resonance Spectroscopy*, 2014, **83**, 21-41.
13. S. R. Hartmann and E. L. Hahn, *Physical Review*, 1962, **128**, 2042-2053.
14. A. Bax, M. Ikura, L. E. Kay, D. A. Torchia and R. Tschudin, *Journal of Magnetic Resonance (1969)*, 1990, **86**, 304-318.
15. I. E. Ndukwe, A. Shchukina, K. Kazimierczuk, C. Cobas and C. P. Butts, *ChemPhysChem*, 2016, DOI: 10.1002/cphc.201600541, n/a-n/a.
16. E. J. Candes and M. B. Wakin, *IEEE Signal Processing Magazine*, 2008, **25**, 21-30.
17. J. F. Martin, *Journal of Magnetic Resonance (1969)*, 1985, **65**, 291-297.
18. S. Sibisi, J. Skilling, R. G. Brereton, E. D. Laue and J. Staunton, *Nature*, 1984, **311**, 446-447.
19. S. G. Hyberts, A. G. Milbradt, A. B. Wagner, H. Arthanari and G. Wagner, *J Biomol NMR*, 2012, **52**, 315-327.
20. K. Kazimierczuk and V. Y. Orekhov, *Angewandte Chemie International Edition*, 2011, **50**, 5556-5559.
21. A. Papoulis, *IEEE Transactions on Circuits and Systems*, 1975, **22**, 735-742.
22. P. A. Cox, M. Reid, A. G. Leach, A. D. Campbell, E. G. King and G. C. Lloyd-Jones, unpublished work.