UNIVERSITY OF CAMBRIDGE
Department of Chemistry

Open-access experiments on Arran 700MHz cryoprobe

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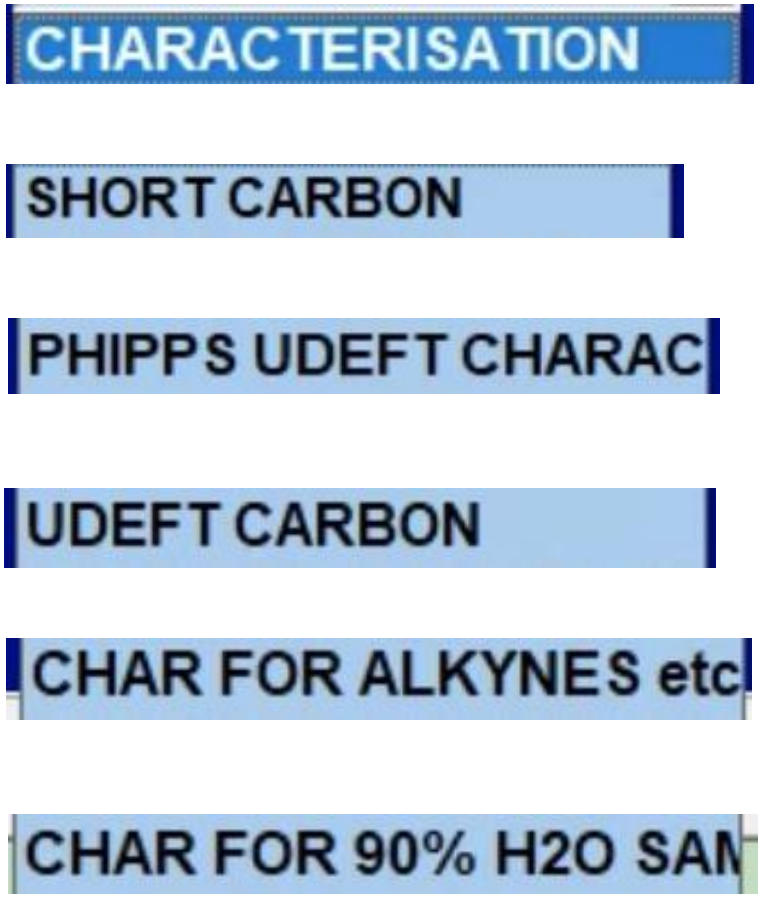
Rationale

- NMRKiosk will run spectra overnight (7pm-8am)
- Samples can be submitted any time to wait in the queue
- We want reasonable sample throughput
- But also better data than the current systems, particularly carbon
- Cryoprobe has ability to deal with lower concentration samples

- We are testing new methods

- Trained user access available in daytime with support from NMR team

Experiment summary – see later slides for detail



- Standard full char. for >5mM conc.
- 10 minute carbon
- Special char. for dilute/fluorinated samples
- Carbon for dilute/fluorinated samples
- For samples with odd C-H J coupling
- For samples with a lot of water

Fair use rules

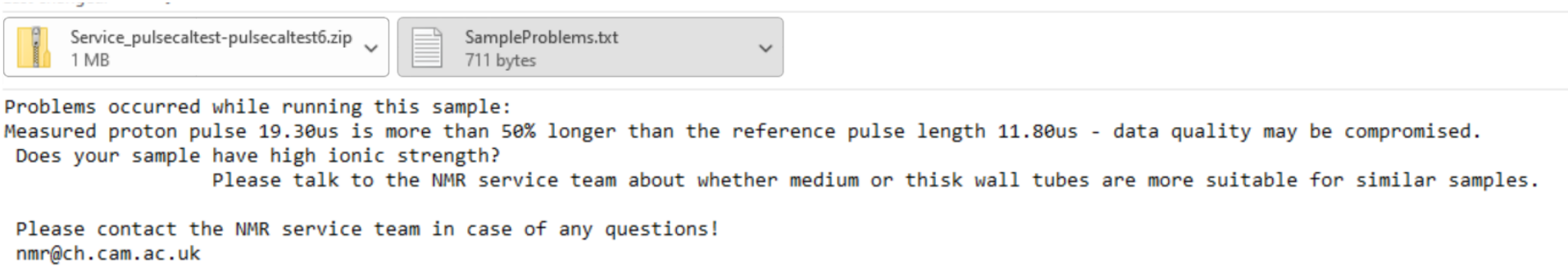
- We want to increase capacity for everyone
- New groups coming to the department
- Short carbon: **up to 4 per night**
- Standard char, UDEFT carbon: **up to 2 per night**
- Phipps char, H2O/D2O char, Alkynes char, **1 per night**
- Charging: 2 GBP for the short carbon, 4 GBP for others

2H spectrum

- For internal debugging purposes we acquire a quick 2H spectrum
- Last experiment in each case
- Mostly not interesting- just solvent peak
- But allows us to see how much deuterium is there
- If it fails to lock, might help us understand why
- Quality control of solvents can be useful…

IMPORTANT: Ionic / conductive samples

- The cryoprobe is much more sensitive to sample conductivity
- Potential tuning problems
- Potential loss of data quality due to pulse miscalibration
- If there is 50% increase in pulse length you will get a note with the data email (this will also indicate other problems that may have occurred):



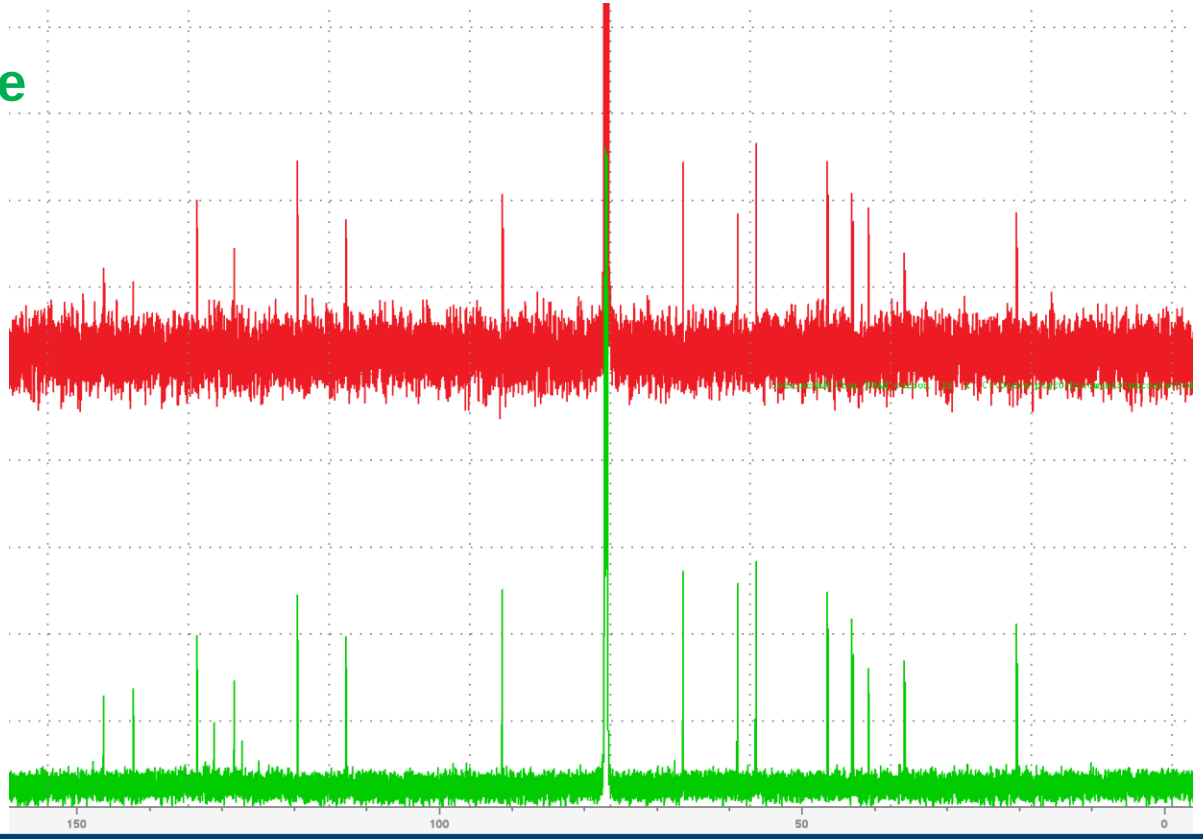
Carbon experiments

- Carbon S:N comparisons on standard ethylbenzene sample:
  - Arran, 700 MHzTXO cryoprobe: **2900:1**
  - Aberlour,service 500MHz DCH cryoprobe: 2900:1
  - Tobermory, 400MHz Prodigy cryoprobe **600:1**
  - Glenfairn, 500MHz broadband probe **360:1**

- **Experiment time $\propto$ $(S:N)^2$ – at least 23x faster than other open access!**

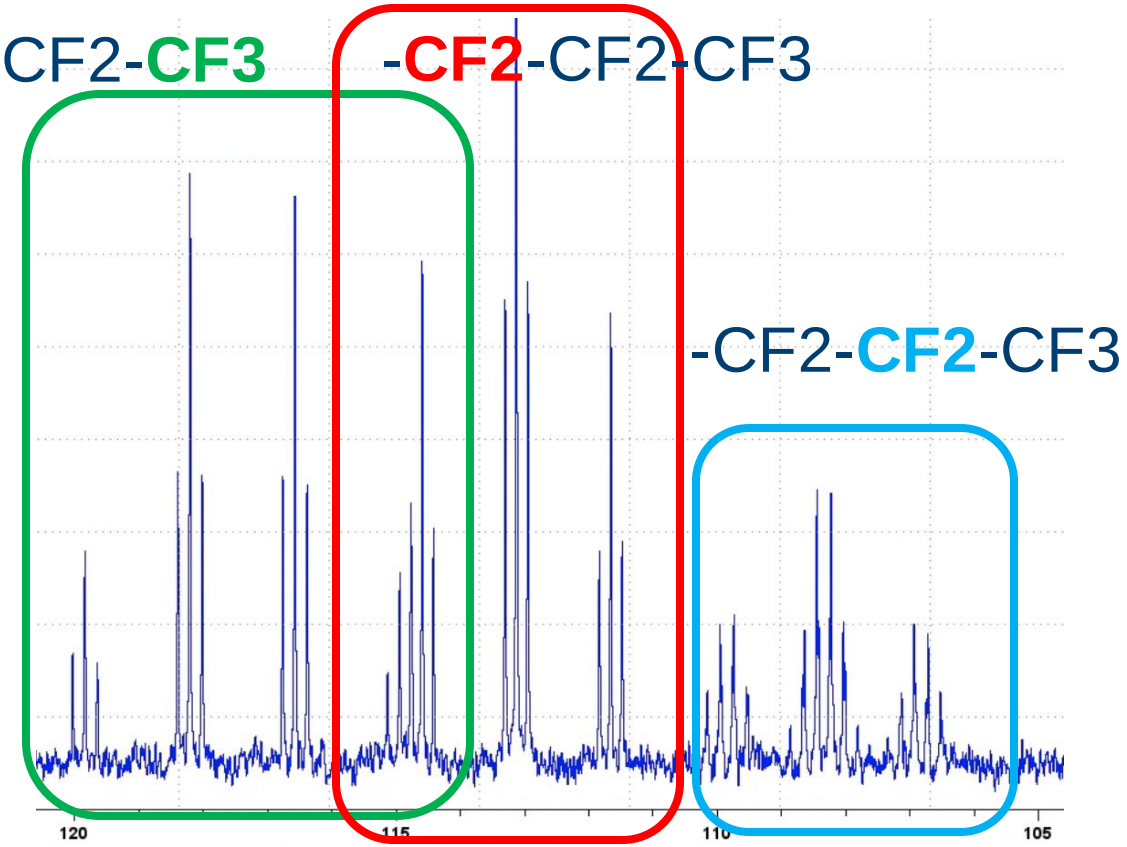
Carbon experiments

- Standard long carbon on Tobermory/Glenfairn: **100 minutes**
- New standard short carbon on Arran: **10 minutes!**
- **4mM sample**



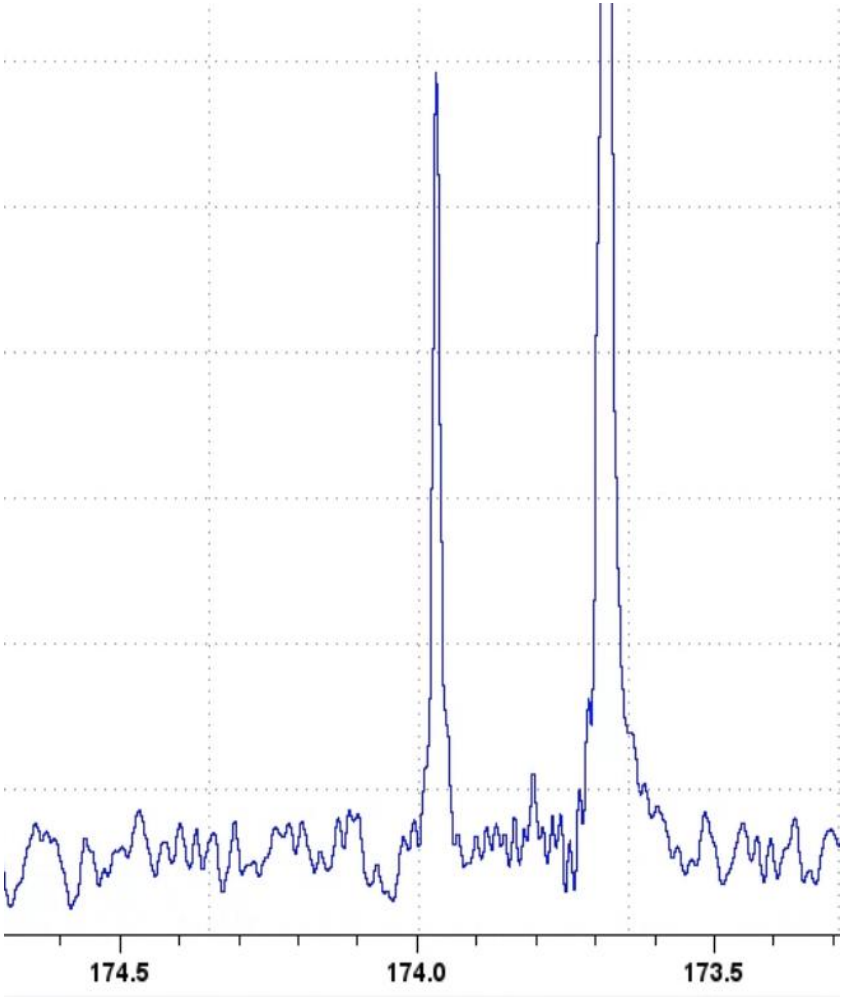
UDEFT carbon

- Example for –C3F8 moiety – 60mM concentration:
- 20mM OK for -C3F8
- Less is OK for –C2F5



UDEFT carbon: weak samples

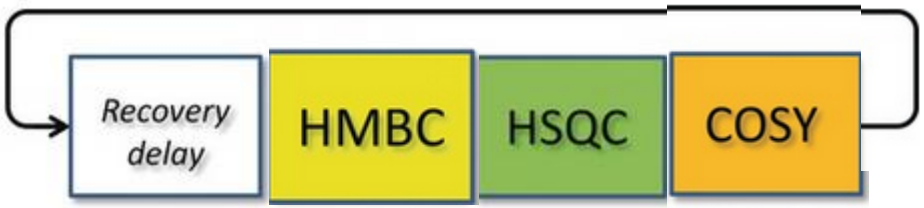
- Minor component concentration <4mM
- S:N for carbonyl ~20:1
- => **1mM** is possible



Characterisation experiments

- Speed plus sensitivity…
- Glenfairn takes **105** minutes/sample
- New char on Arran: >**30** minutes/sample
- Still, better sensitivity and resolution on Arran

Time saving by simultaneous experiments



- Time saving without losing sensitivity in HSQC/HMBC
- Generates a combined dataset (experiment 14) which is automatically split
- **Experiments 141/142/143 are HMBC/HSQC/COSY** respectively
- **Process as normal** – list of topspin processing commands in title

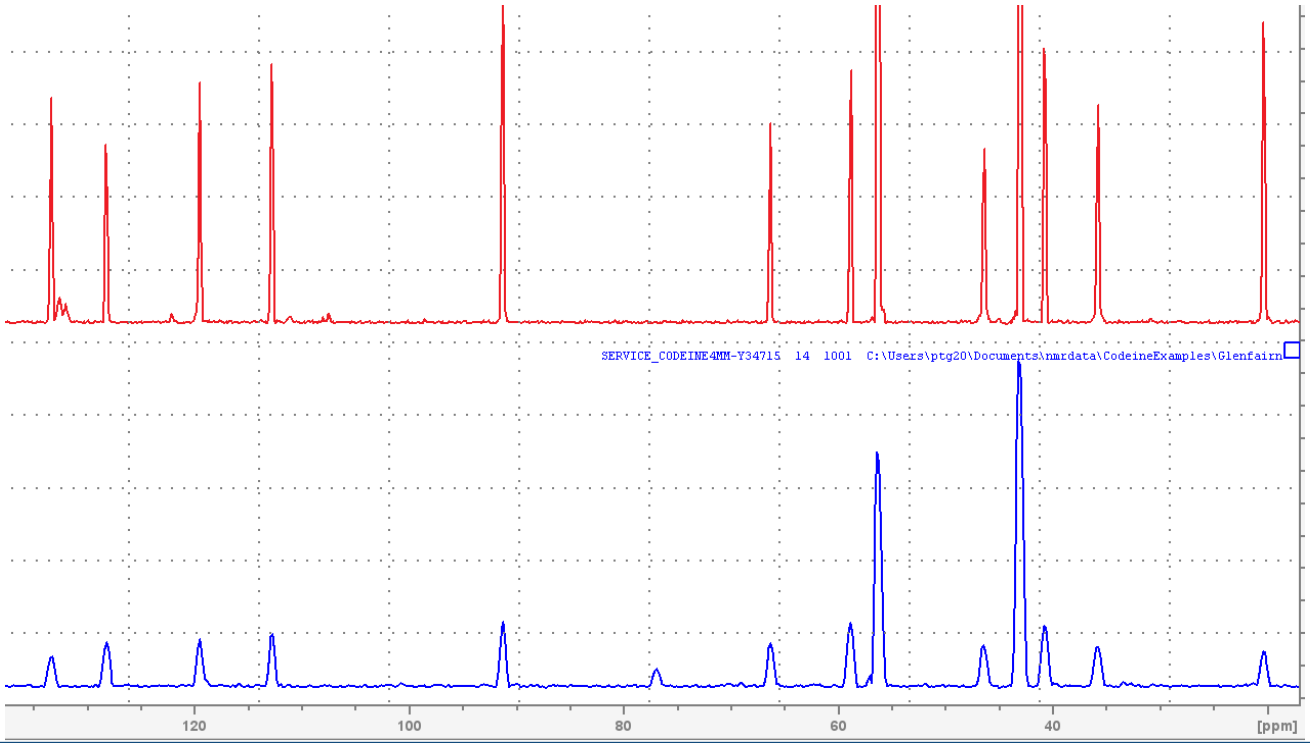
NOAH: NMR Supersequences for Small Molecule Analysis and Structure Elucidation

Dr. Ēriks Kupče ✉, Prof. Tim D. W. Claridge ✉

- https://doi.org/10.1002/anie.201705506

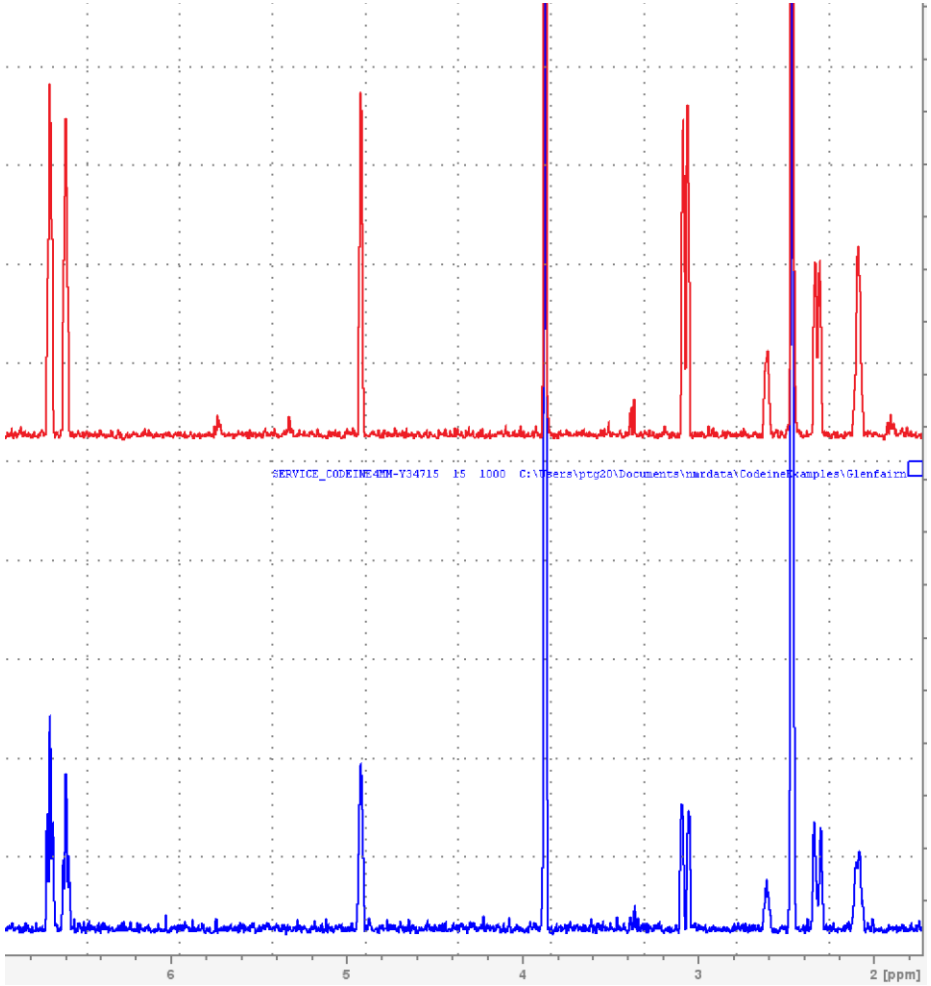
HSQC: Better sensitivity and resolution

- **Arran** vs **Glenfairn**
- Positive projection of HSQC, scaled to same noise level
- Carbon range on Arran also covers aldehydes!



HMBC: more sensitivity

- **Arran** vs **Glenfairn**
- Positive projection of HMBC
- Scaled to same noise level

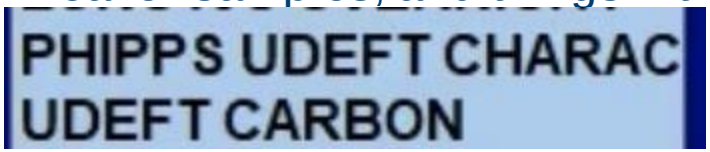


Challenging samples

- What about things you would previously submit to service?

- Concentration limits? Example sample above is **4mM**
- **For samples >5mM concentration short carbon is probably OK**
- **Likewise standard characterization**

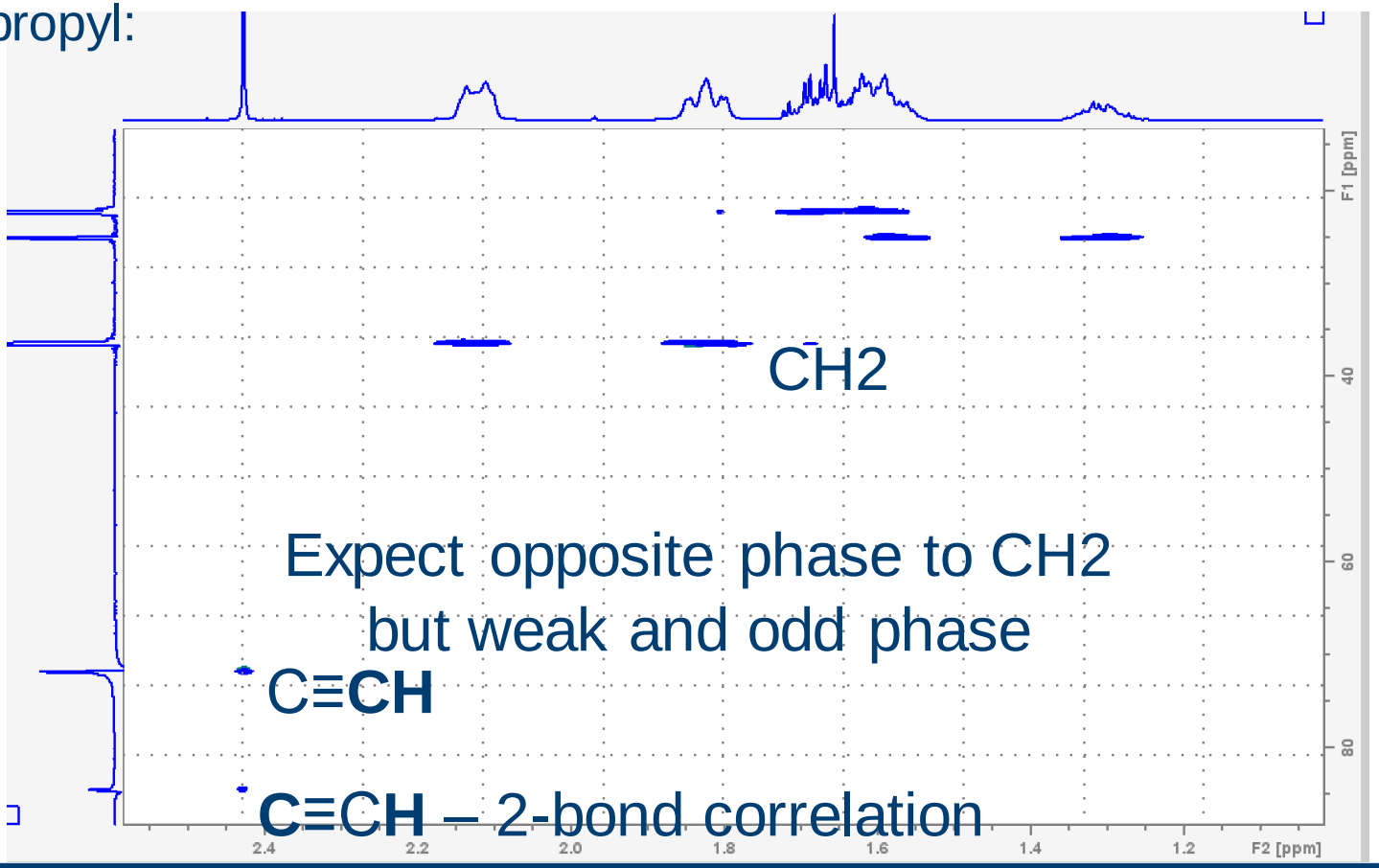
- For weaker samples, and things with fluorine couplings in:



- Slightly lower resolution carbon but better sensitivity – about **1mM concentration is possible**

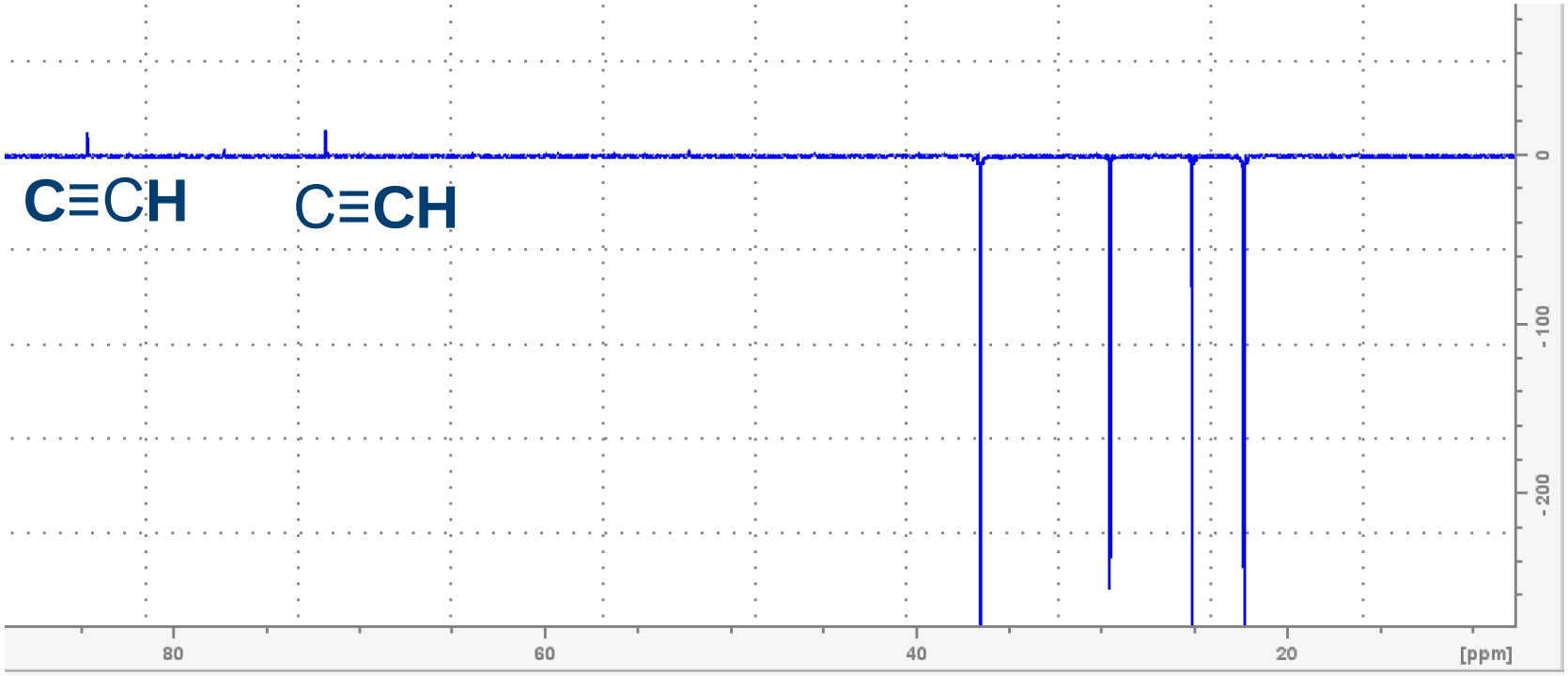
Special cases

- The way our standard HSQC/DEPT work we get odd/missing peaks for alkynes/cyclopropyl:



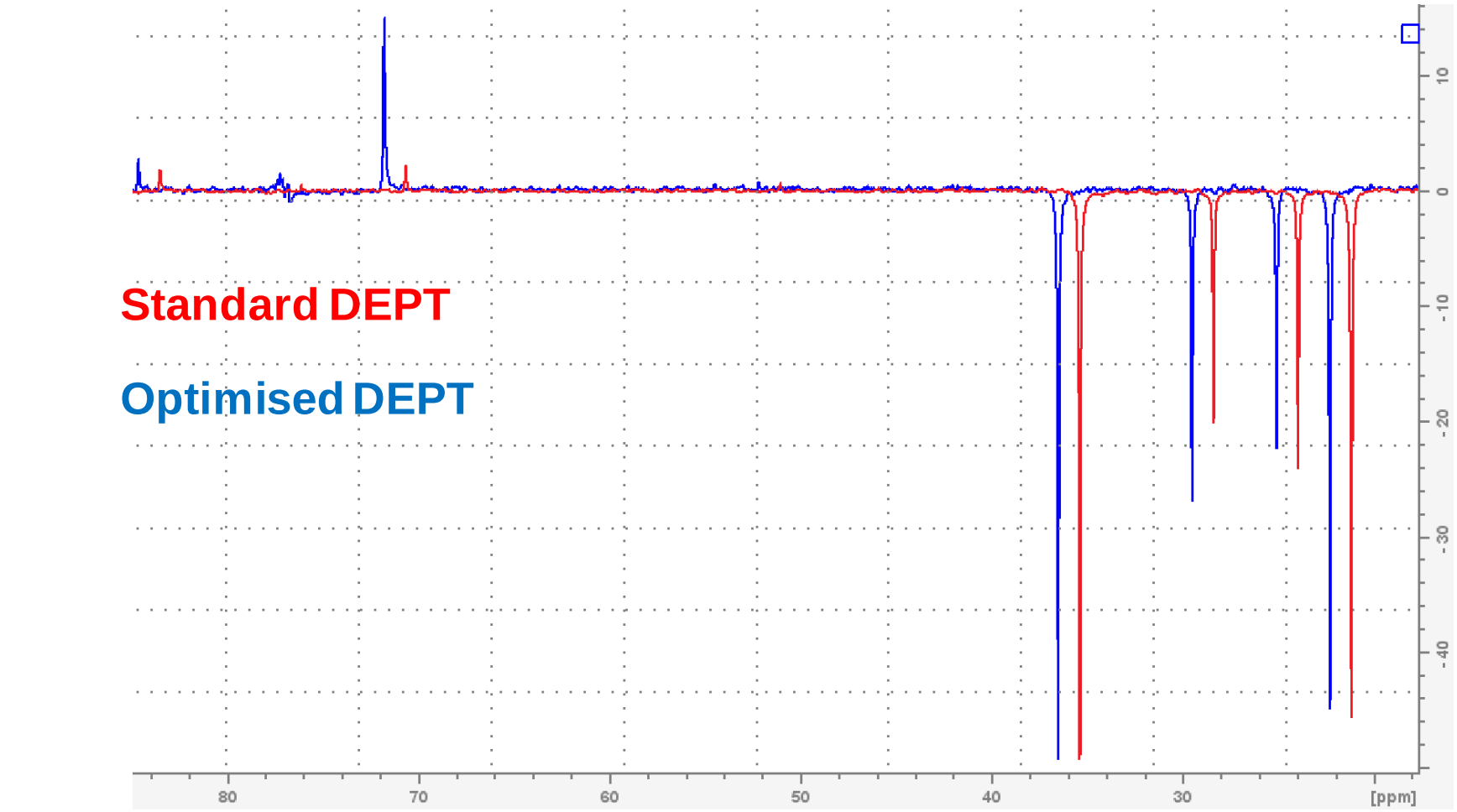
Special cases

- In DEPT, terminal and quaternary alkyne peaks show up with similar low intensity:



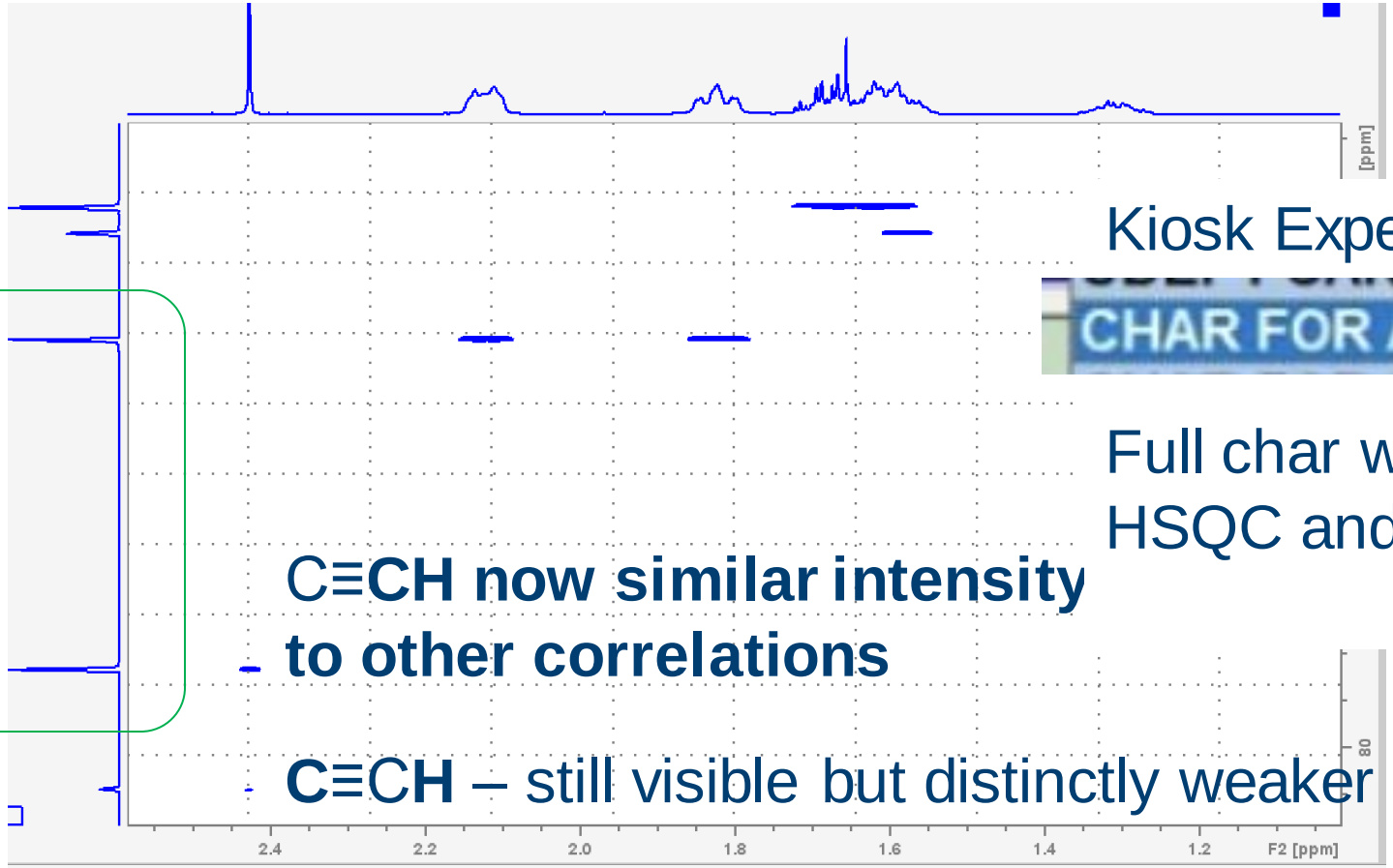
J-insensitive experiments: DEPT

ACCORD-DEPT https://doi.org/10.1002/mrc.2701



J-insensitive experiments – HSQC
COB-HSQC https://doi.org/10.1002/mrc.3846

- HSQC, no multiplicity editing, for J=120-250Hz:



Kiosk Experiment:

Full char with optimised HSQC and DEPT

C≡CH now similar intensity to other correlations

C≡CH – still visible but distinctly weaker

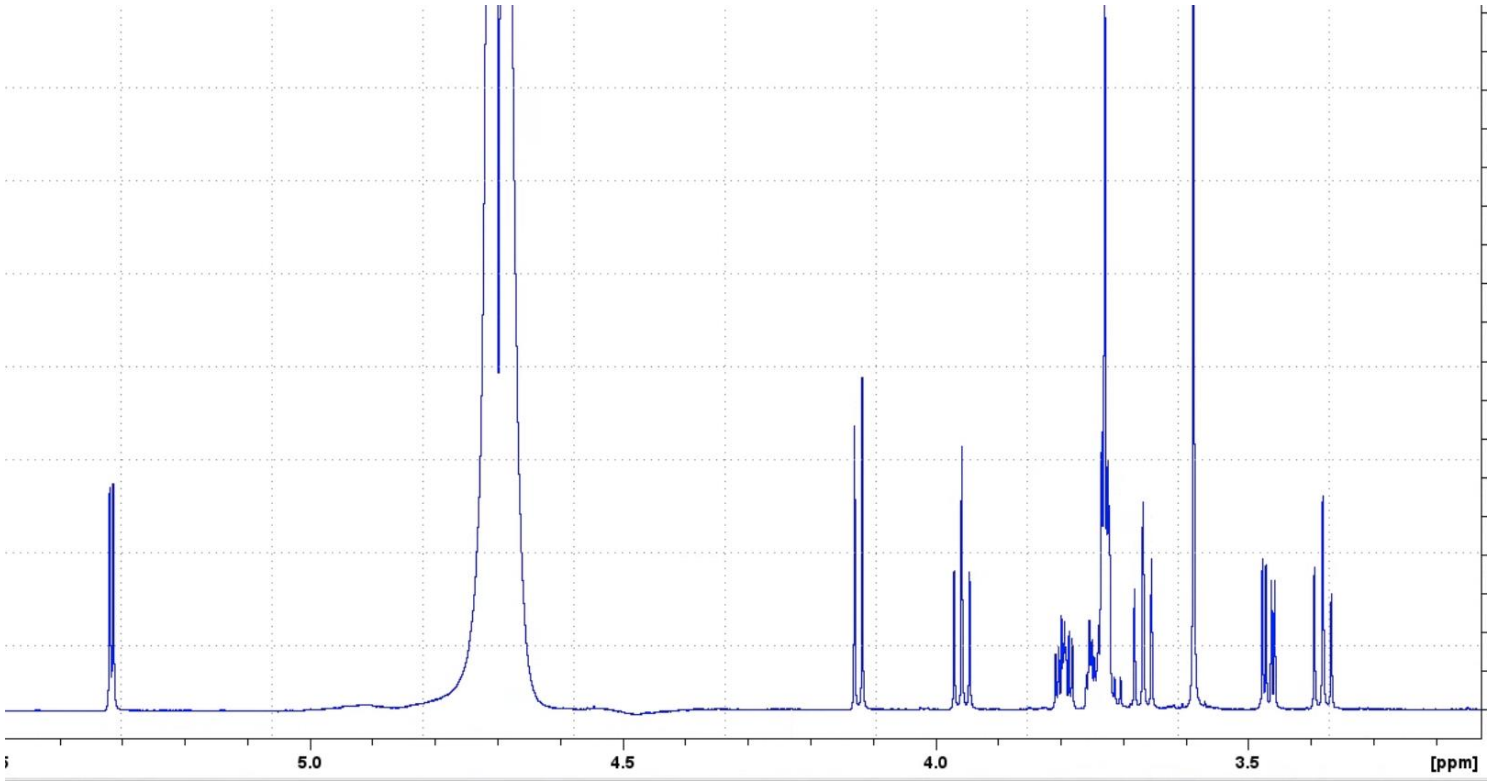
Samples in (mostly) protonated solvent

- H2O+D2O solvent available - some D2O needed for lock (>5%)
- Standard parameters don't do a very good job of getting rid of water signal
- So let's try a modified experiment
- Pulse lengths will be calibrated, but if the proton pulse is too long the experiment will fail. If your sample is very ionic, may need medium wall tubes (Wilmad 524-PP)

- Also suitable for samples with a lot of excess water

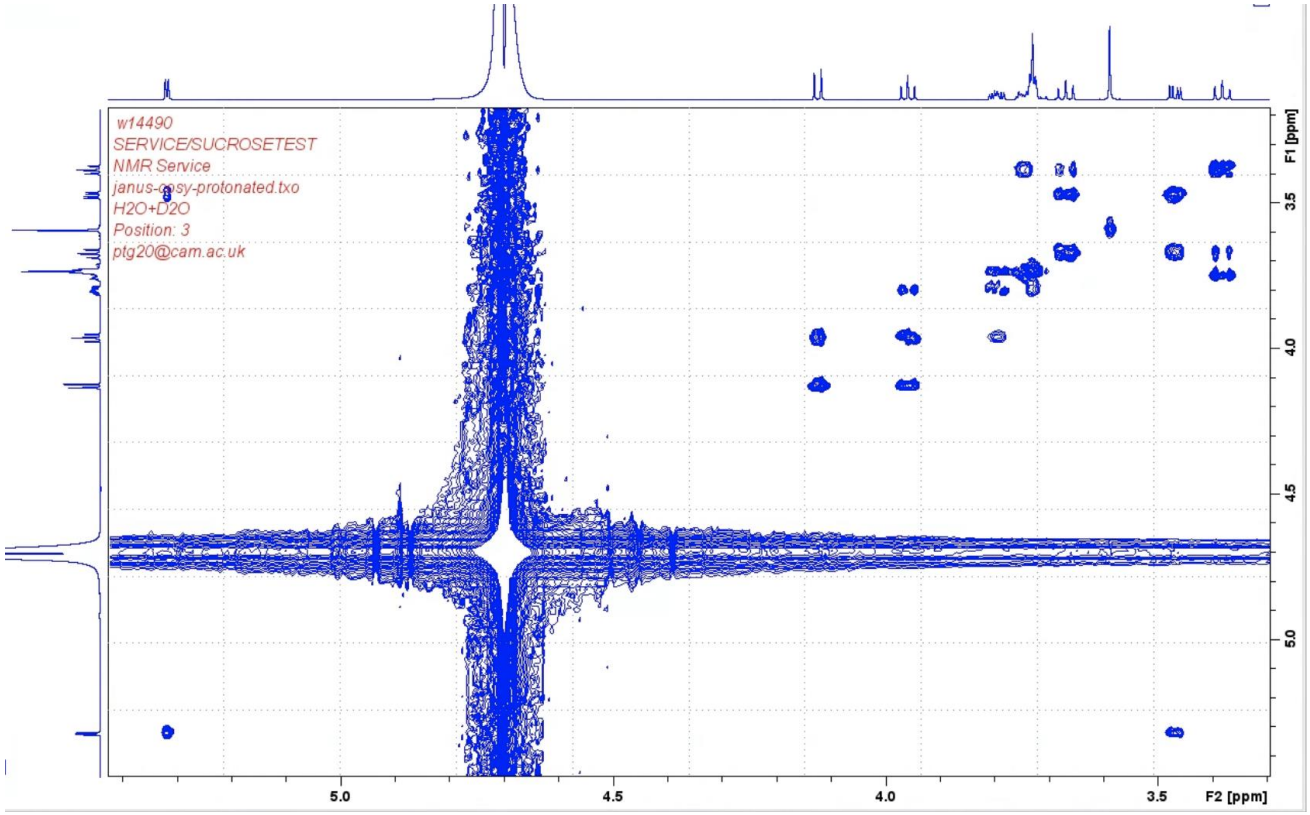
90% H2O results, 2mM sucrose sample

- Automated solvent suppression (experiment 11):



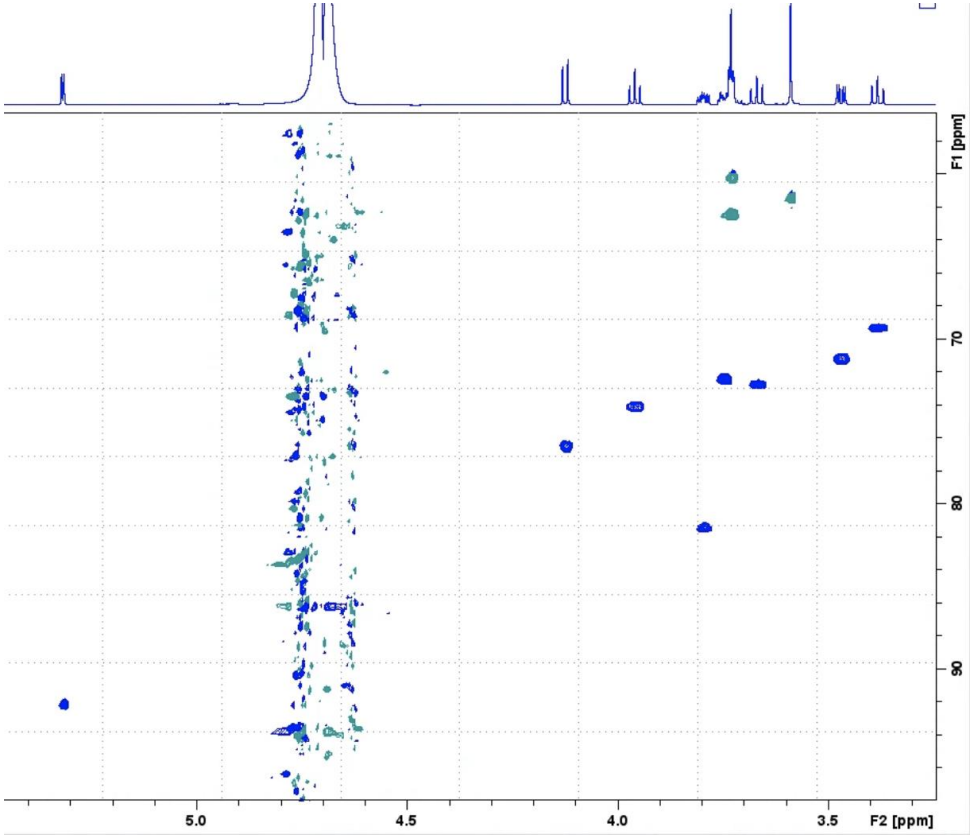
90% H2O results, 2mM sucrose sample

- COSY, solvent suppression with only double quantum filter:



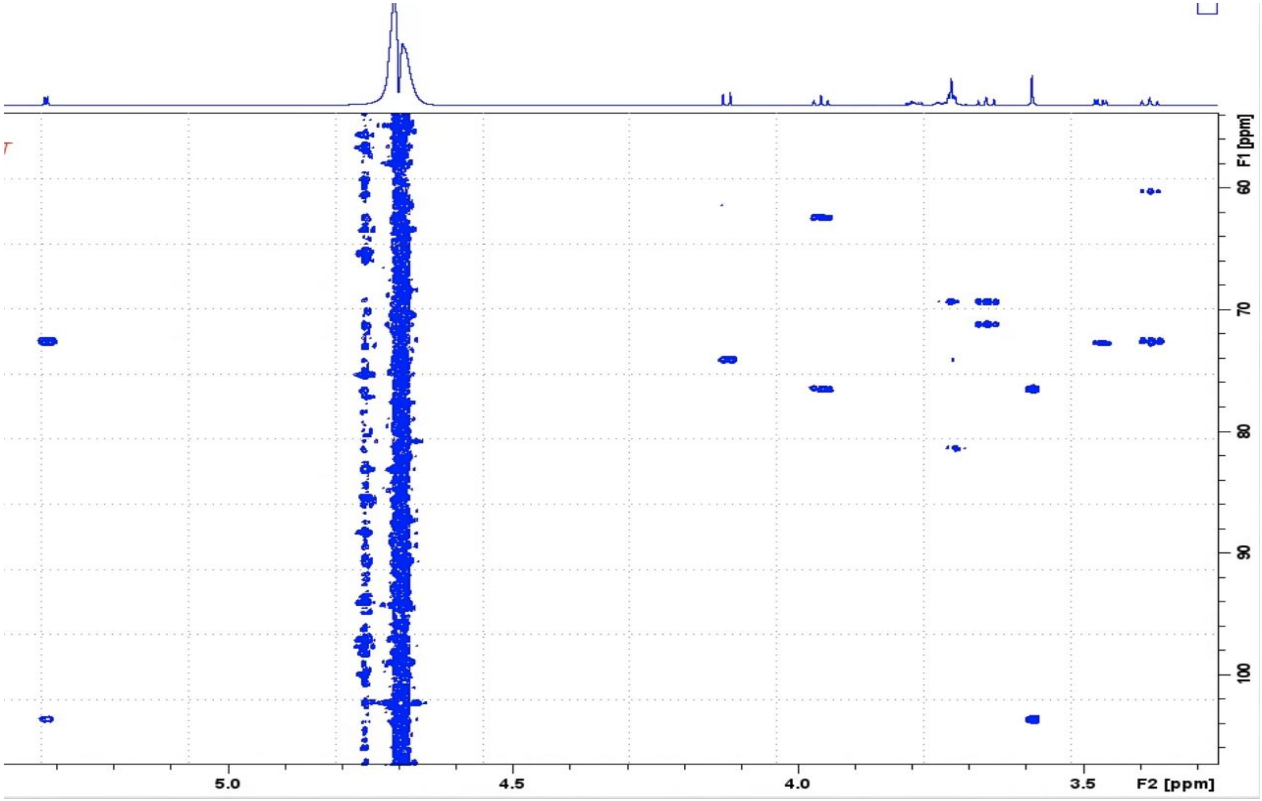
90% H2O results, 2mM sucrose sample

- HSQC, solvent suppression by selection of only CHn signals:



90% H2O results, 2mM sucrose sample

- HMBC, solvent suppression by selection of only C---Hn signals:

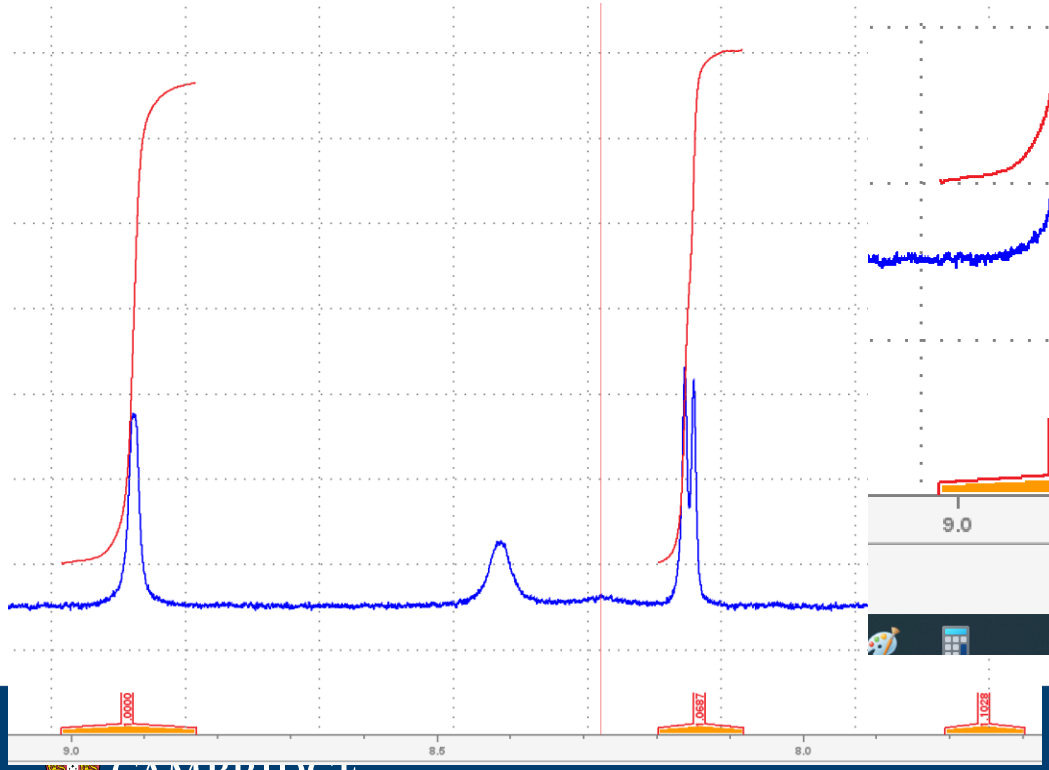


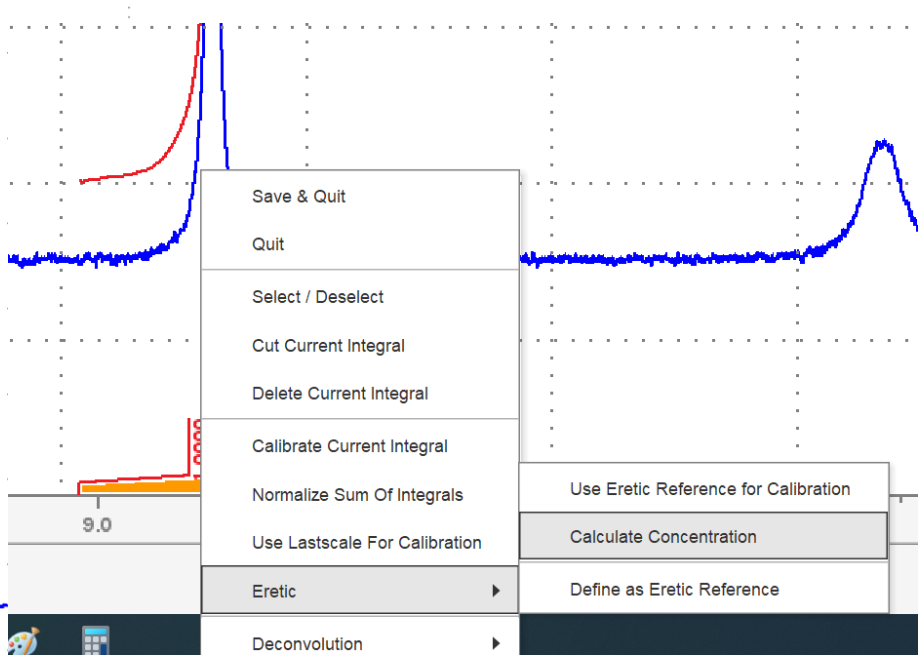
Quantitative proton

- Standard proton in experiment 10 is quantitative!
- Needs external reference data to get numbers out
- Get the zipfile from here:
- www.ch.cam.ac.uk/analytical/nmr/files/Arran_QuantRef_0.1EB_2022_09_26_CPTXO.zip
- Unzip that onto your computer somewhere useful

Quantitative proton

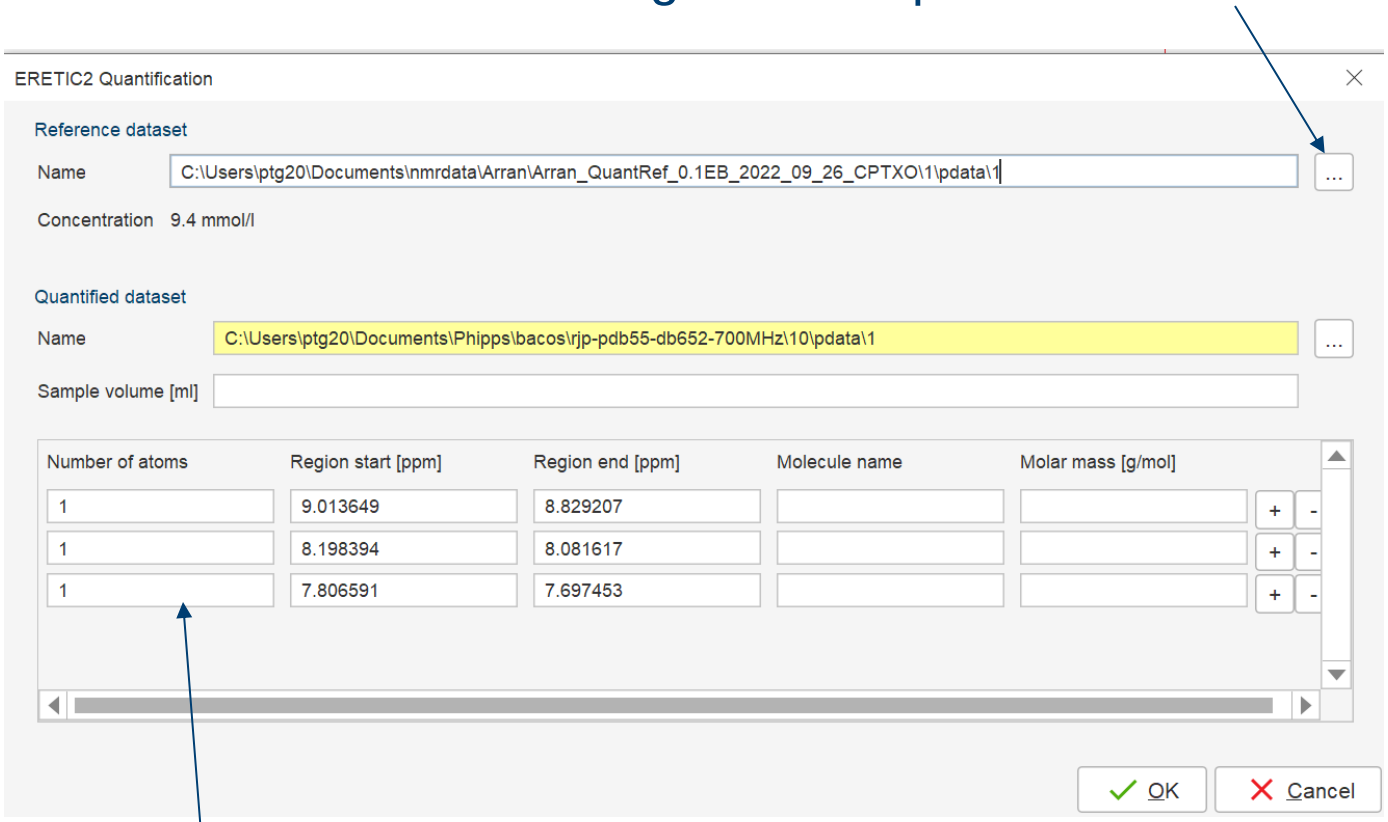
- Integrate peaks in your spectrum, and click to select integral of interest
  - Ctrl-click to select multiple
- Right click integral and select Eretic->calculate concentration





Quantitative proton

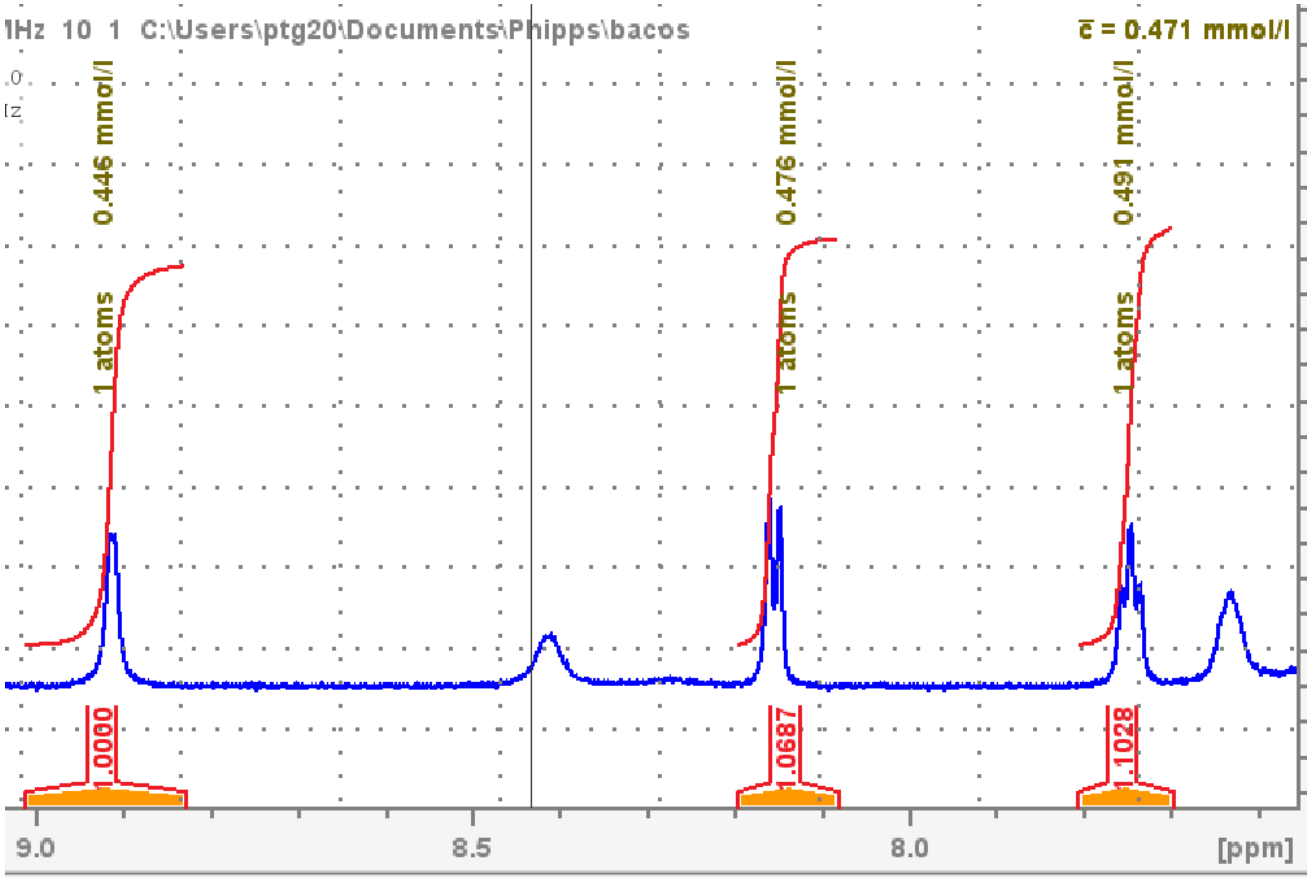
- Browse to the reference dataset- go into the pdata/1 folder



- Set number of protons for each integral region, and click OK

Quantitative proton

- Concentration will be displayed for each integral, and an average:



Quantitative proton- limitations!

- We don't have good data on the reproducibility of the result
  - Estimate something like 10% error
  - If you want real quantification talk to us!
- Affected by ionic strength of samples:
  - Indicated concentrations will be lower than actual
  - We can work out the answer in principle...
  - This applies to any sample where the proton pulse ends up longer than normal