New NMR methods for structural analysis of fluorinated systems

Coral Mycroft

NMR Methodology Group, The University of Manchester

EUROMAR

Utrecht, The Netherlands

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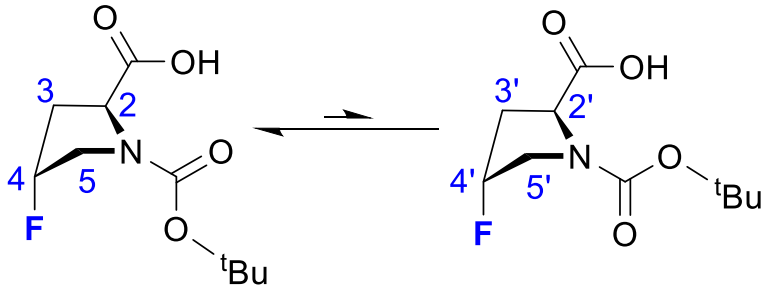
^{1}H NMR of fluorinated systems

The analysis of fluorine-containing systems is an ongoing challenge in ^{1}H NMR.

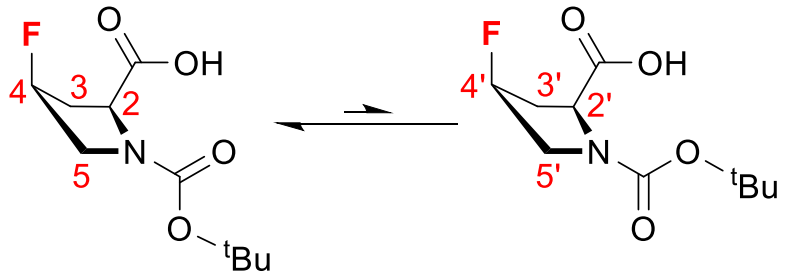
- ✓ Structural information from δ_H
- ✓ $|J_{HH}|$ couplings ranging from 0 - 25 Hz
- ✓ $|J_{HF}|$ couplings ranging from 0 - 60 Hz

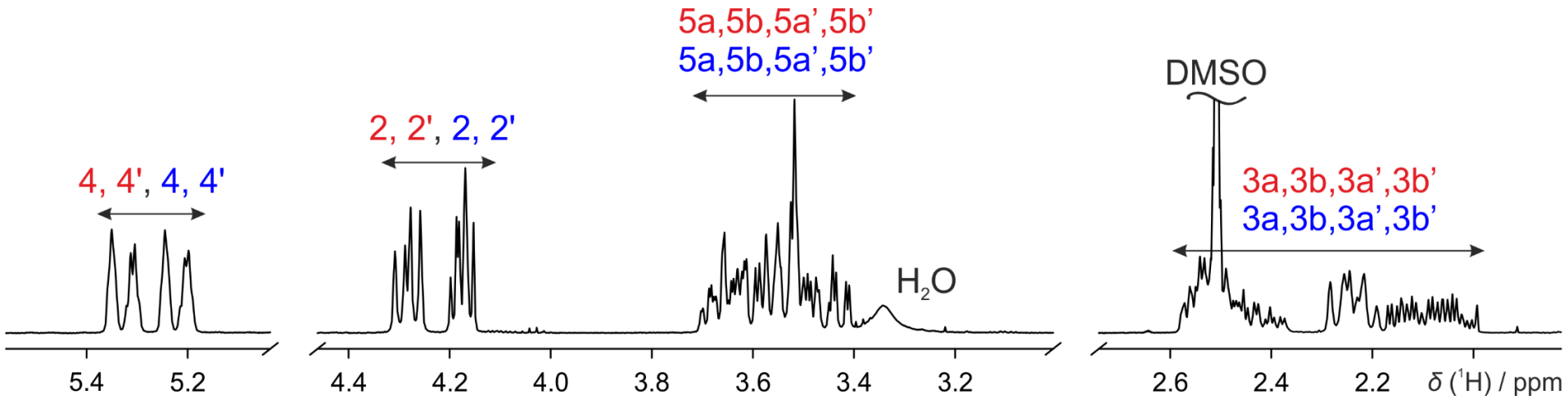
- ✗ Severe signal overlap
- ✗ Poor spectral resolution

(4*S*)-*N*-Boc-L-fluoroproline



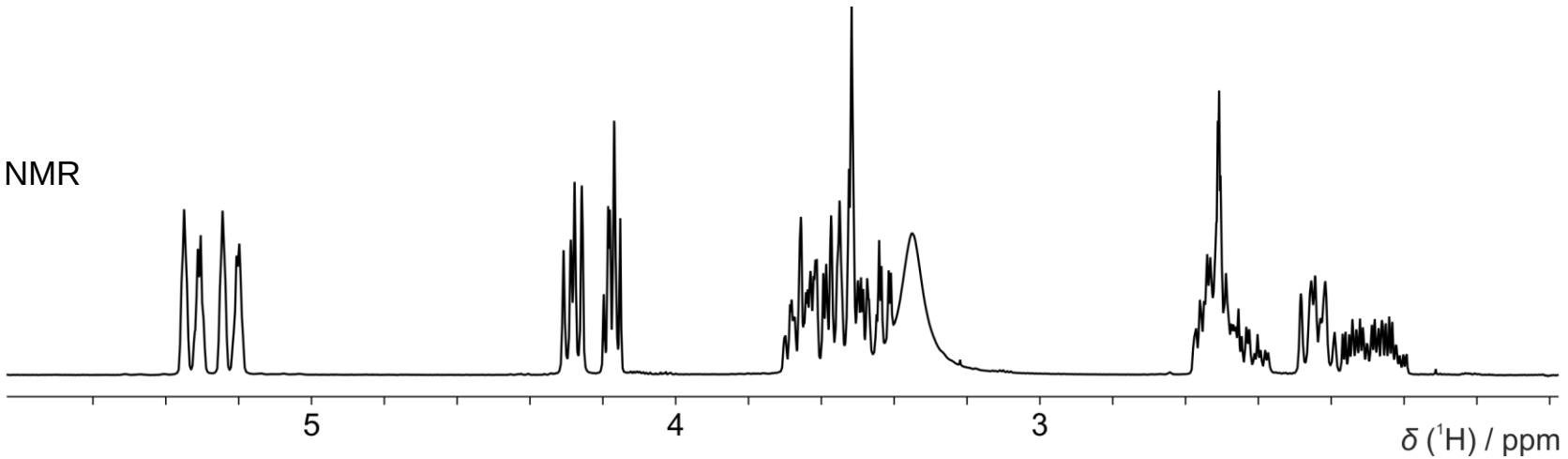
(4*R*)-*N*-Boc-L-fluoroproline





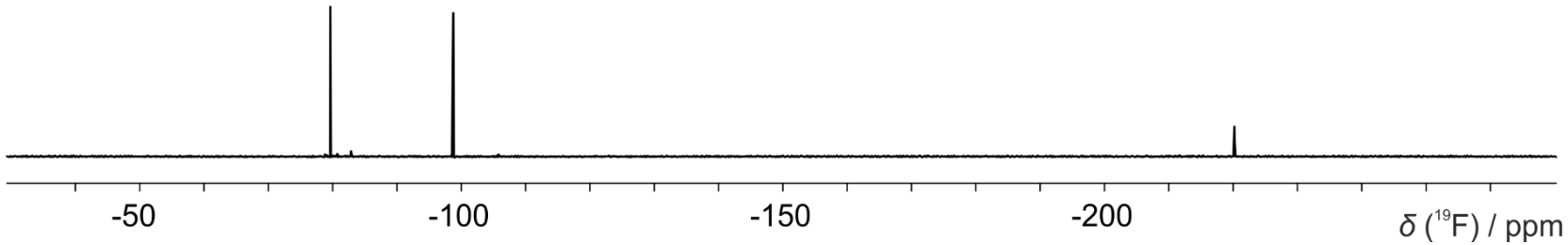
^{1}H NMR of fluorinated systems – ^{1}H vs ^{19}F NMR

^{1}H NMR



- ✓ More structural information
- ✗ Severe signal overlap
- ✗ Poor spectral resolution

^{19}F NMR

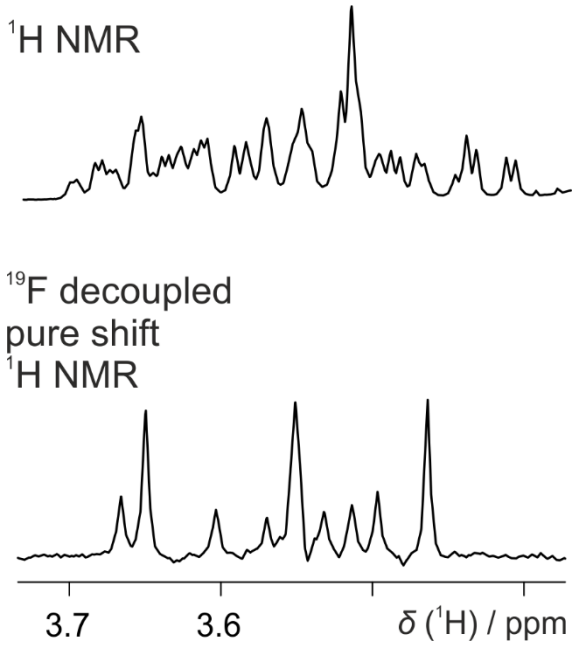


- ✗ Less structural information
- ✓ Less signal overlap
- ✓ Good spectral resolution

For ^{19}F-containing complex systems, we need more sophisticated and problem-specific methods

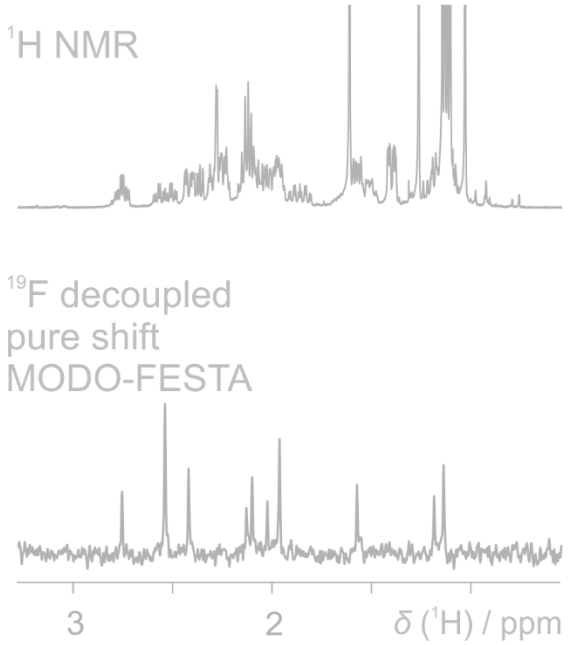
Overcoming signal overlap in ^{1}H NMR in ^{19}F containing systems

Extraction of δ information



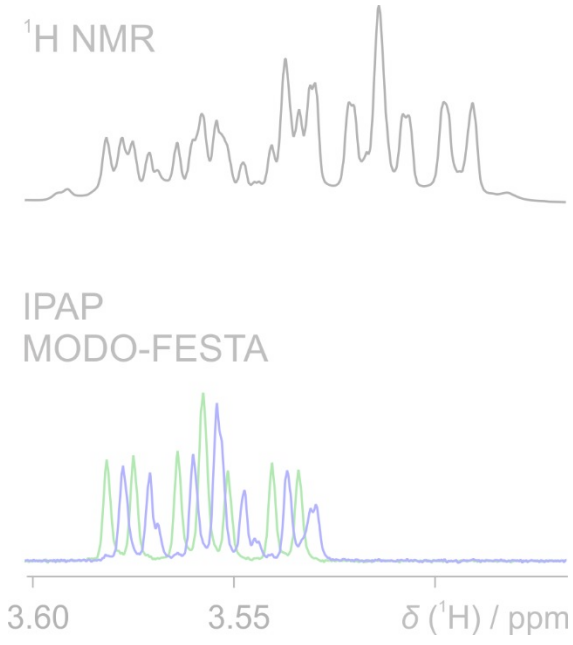
Heteronuclear pure shift ^{1}H NMR

Extraction of individual spin system δ information



Pure shift FESTA

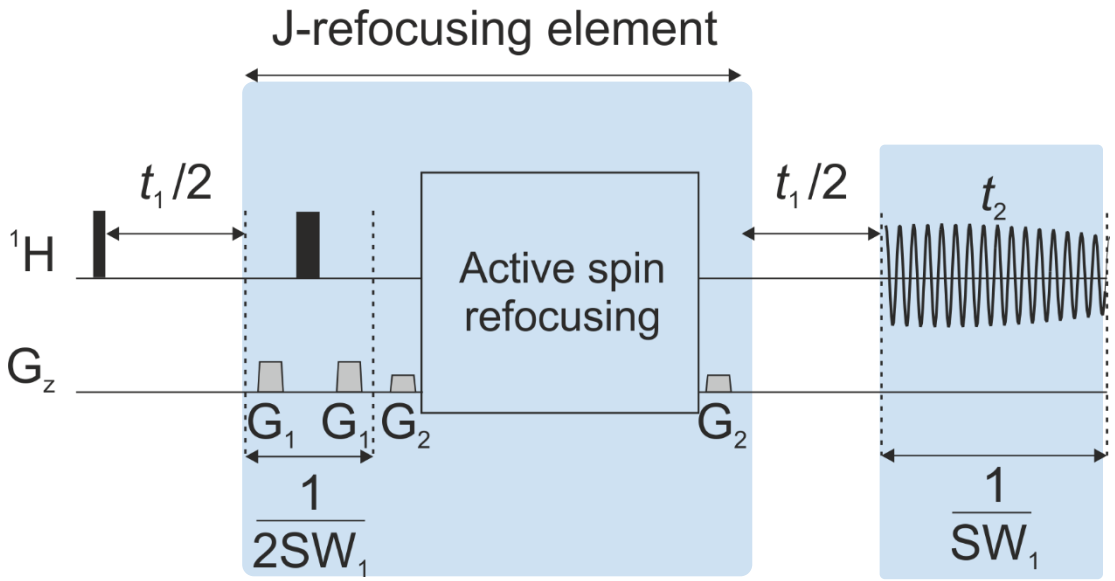
Extraction of the signs and magnitudes of J_{HF} couplings



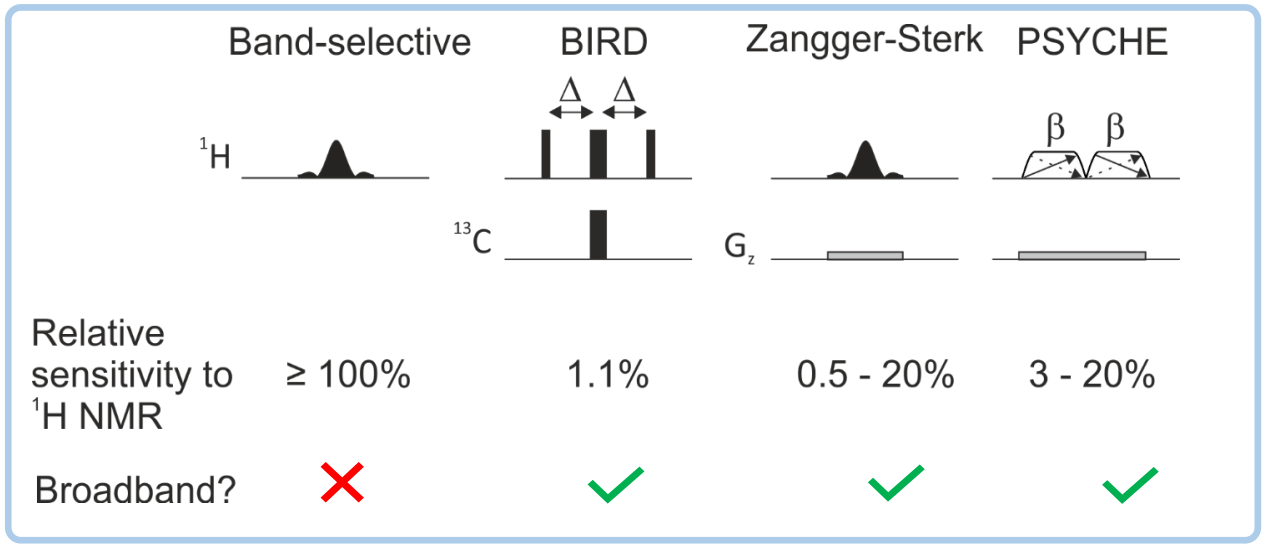
IPAP - FESTA

Pure shift ^{1}H NMR with interferogram acquisition

Pure shift methods collapse multiplicities originating from J_{HH} couplings
Chemical shift and J_{HF} coupling information can be extracted



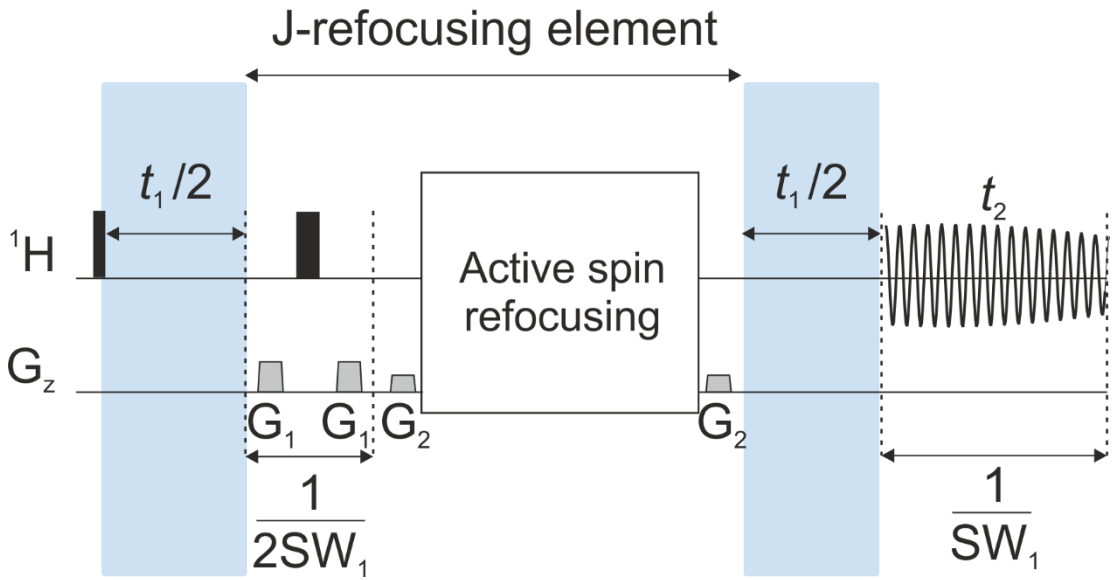
Active spin refocusing element:

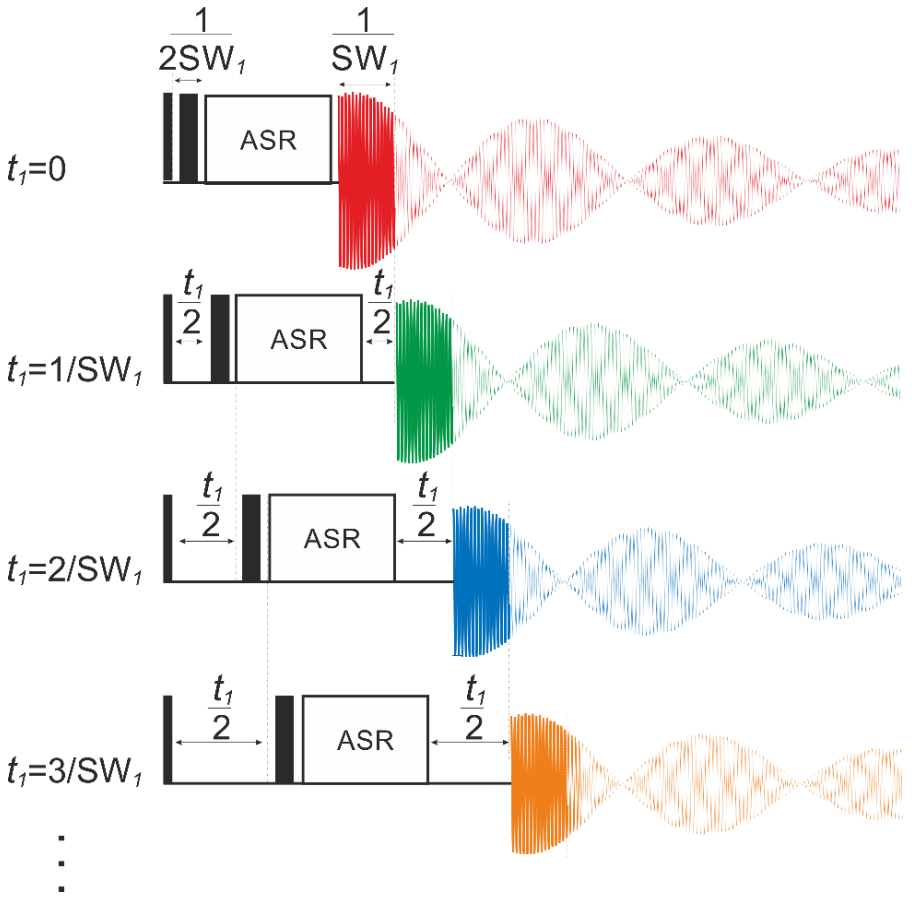


- J-refocusing element ensures J_{HH} coupling is refocused at $t_2 = 1/(2SW_1)$ whilst allowing δ to evolve
- $1/SW_1 << 1/J_{HH}$ to minimise J_{HH} evolution during the chunk

Pure shift review: *Magn. Reson. Chem.* **55**, 2017, 47-53.

Pure shift ^{1}H NMR with interferogram acquisition





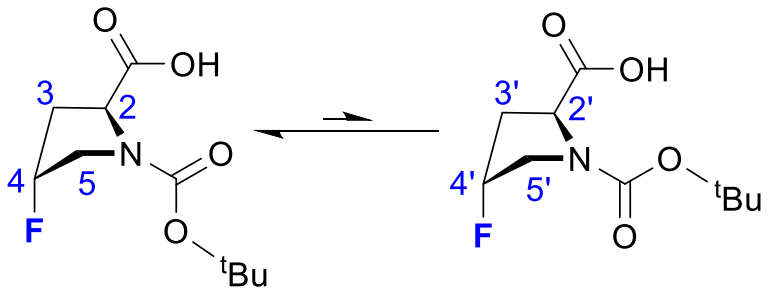
- J-refocusing element: ensures J_{HH} coupling is refocused at $t_2 = 1/(2SW_1)$ whilst allowing δ to evolve
- $1/SW_1 << 1/J_{HH}$ to minimise J_{HH} evolution during the chunk
- t_1: incremented delays as a multiple of $1/SW_1$

Pure shift review: *Magn. Reson. Chem.* **55**, 2017, 47-53.

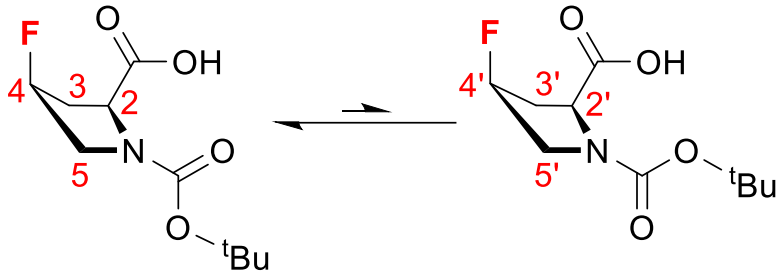
Pure shift ^{1}H NMR with ^{19}F containing systems

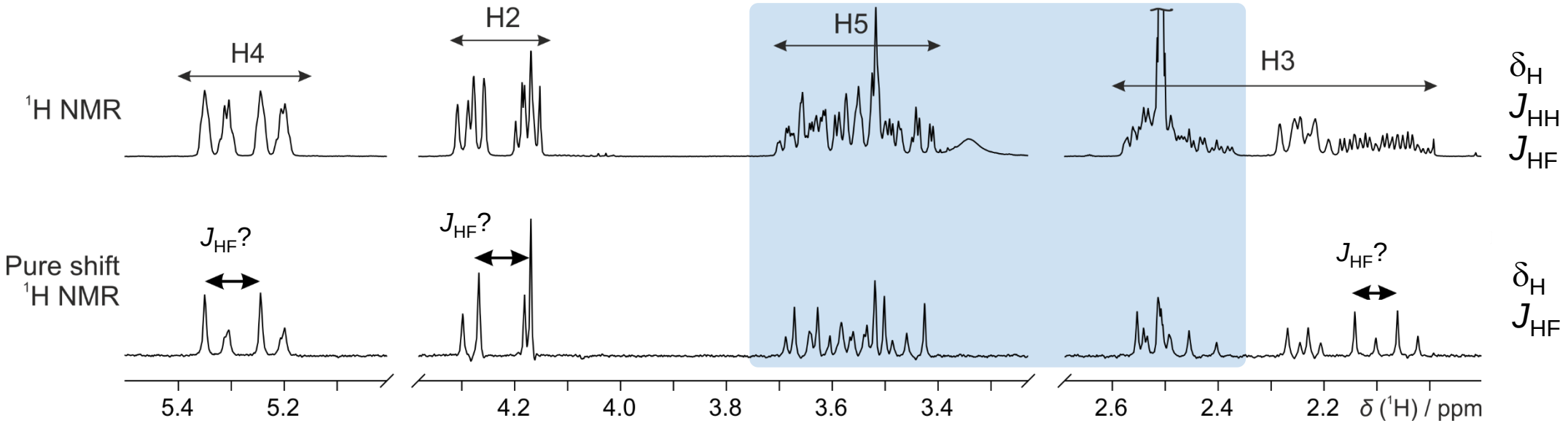
Pure shift methods partially simplify the spectra of ^{19}F containing systems

(4*S*)-*N*-Boc-L-fluoroproline



(4*R*)-*N*-Boc-L-fluoroproline



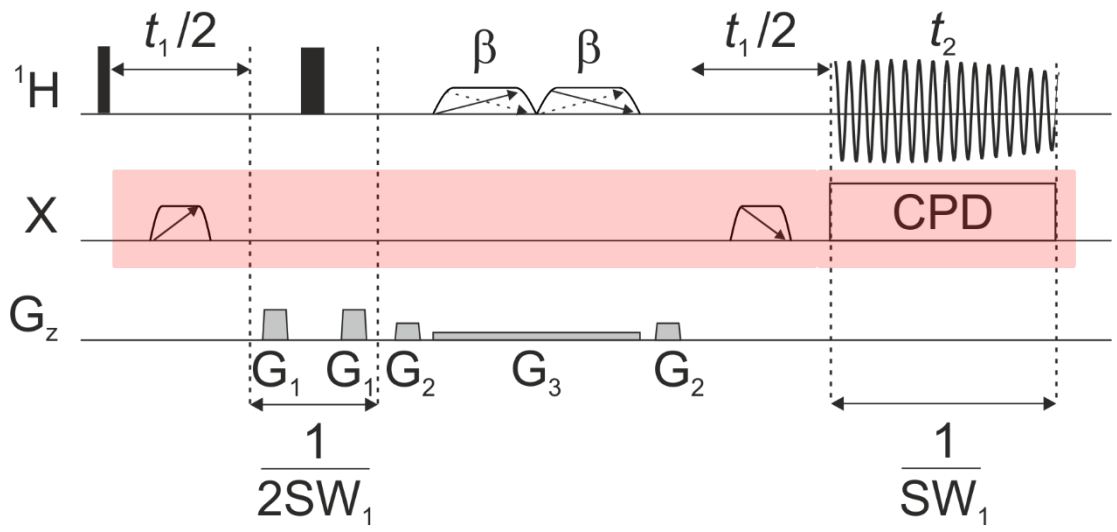


? Chemical shift information

? J_{HF} information

✕ Signal overlap due to J_{HF} couplings

Heteronuclear decoupled pure shift ^{1}H NMR with interferogram acquisition



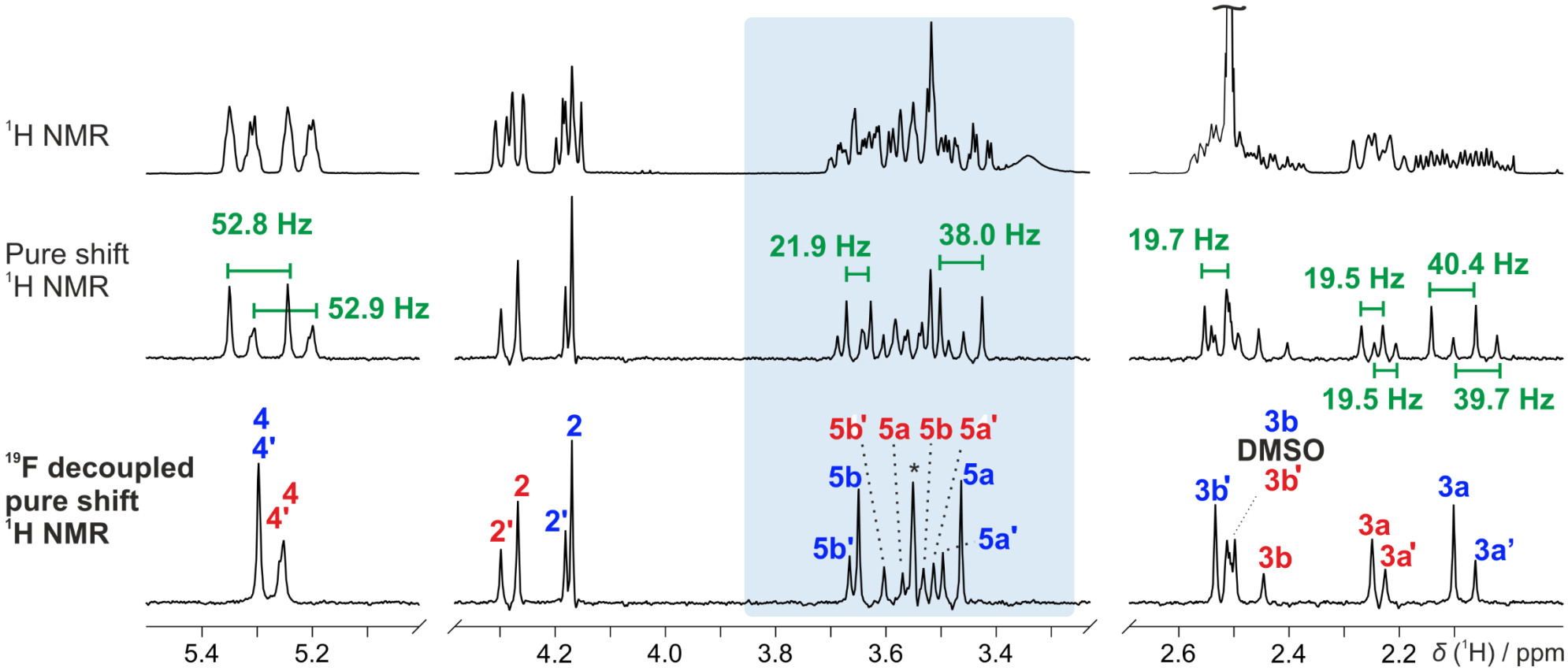
- Apply broadband adiabatic 180° pulses at the midpoints of the $t_1/2$ delays
  - Refocuses J_{HF} couplings at the beginning of the acquisition
  - Adiabatic pulses ensure all ^{19}F resonances are inverted
- Adiabatic heteronuclear decoupling during acquisition
  - J_{HF} couplings remain inactive during acquisition, independent of magnitude or ^{19}F chemical shift

Submitted

Heteronuclear decoupled pure shift ^{1}H NMR

(4*S*)-*N*-Boc-L-fluoroproline

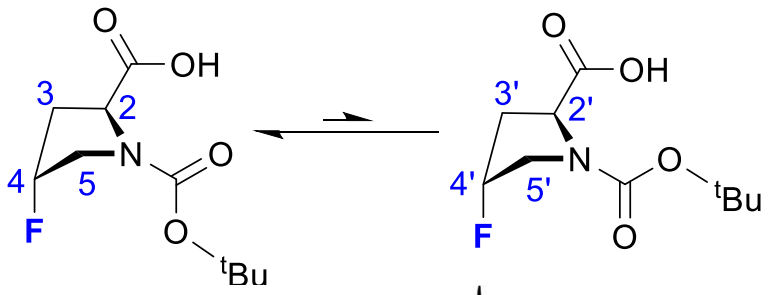
(4*R*)-*N*-Boc-L-fluoroproline



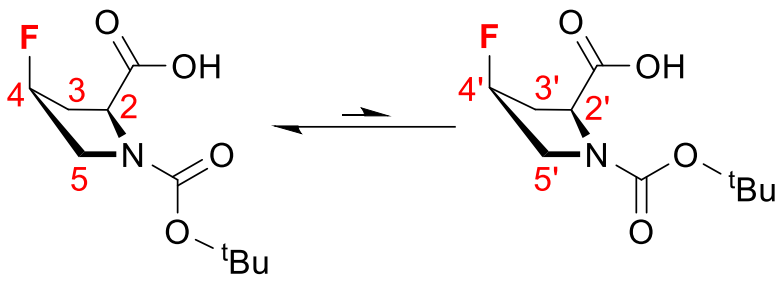
Submitted

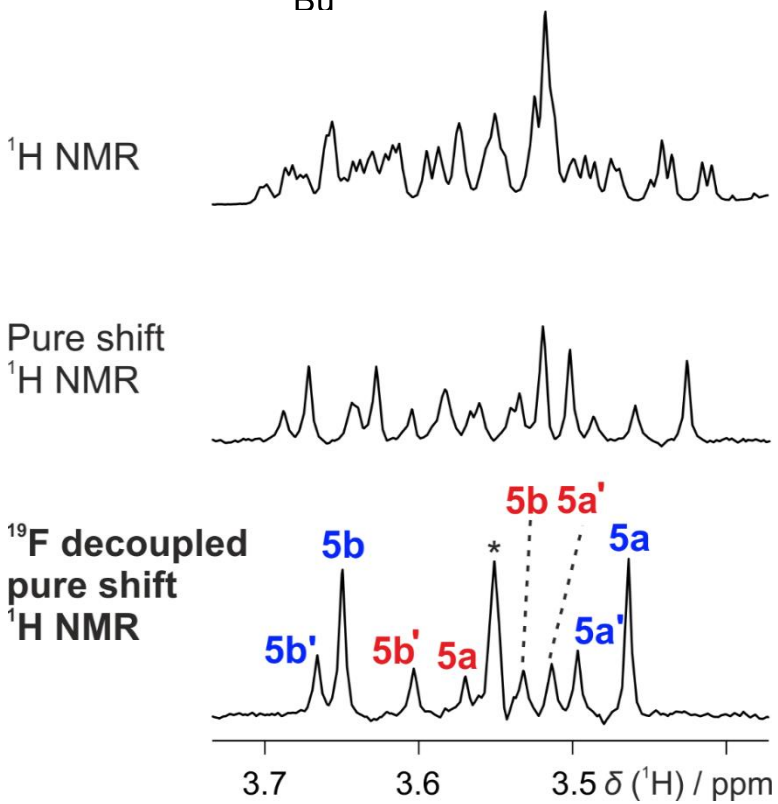
Heteronuclear decoupled pure shift ^{1}H NMR

(4*S*)-*N*-Boc-L-fluoroproline



(4*R*)-*N*-Boc-L-fluoroproline





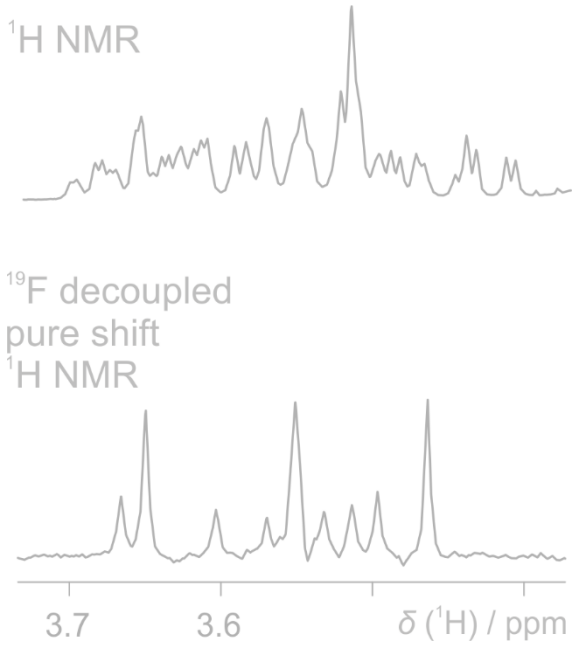
- One singlet per chemical environment
- Drastically reduced signal overlap
- Improved resolution
- No increase in experiment time compared to pure shift ^{1}H NMR

* Strong coupling artefact for **5a** and **5b**

Submitted

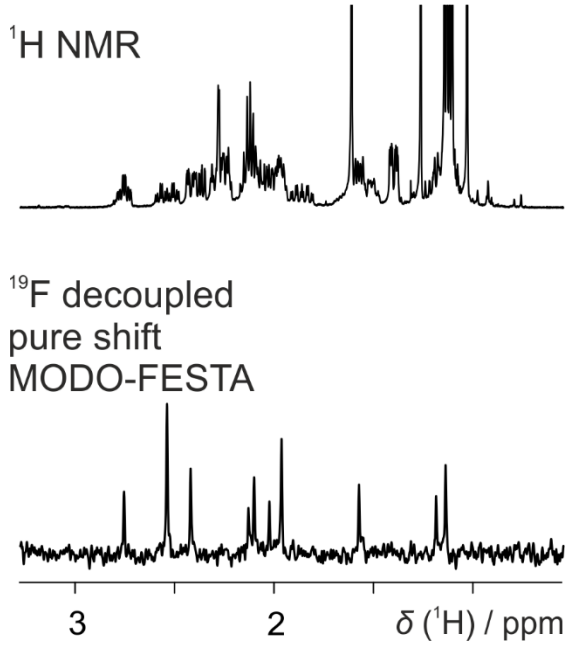
Overcoming signal overlap in ^{1}H NMR in ^{19}F containing systems

Extraction of δ information



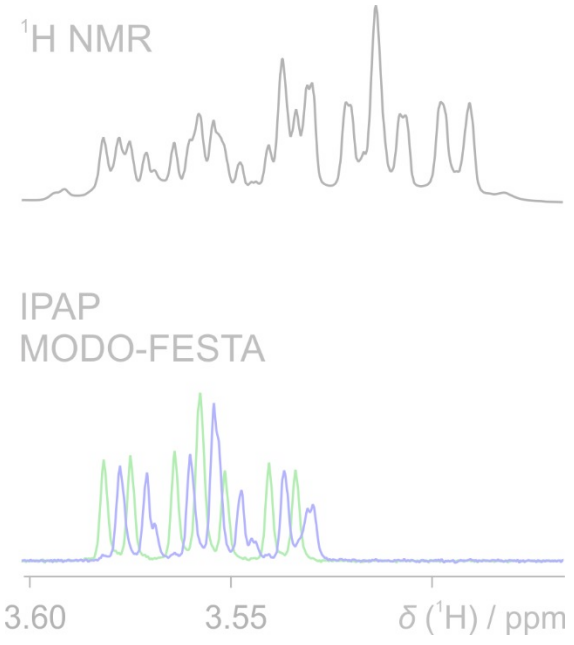
Heteronuclear pure shift ^{1}H NMR

Extraction of individual spin system δ information



Pure shift FESTA

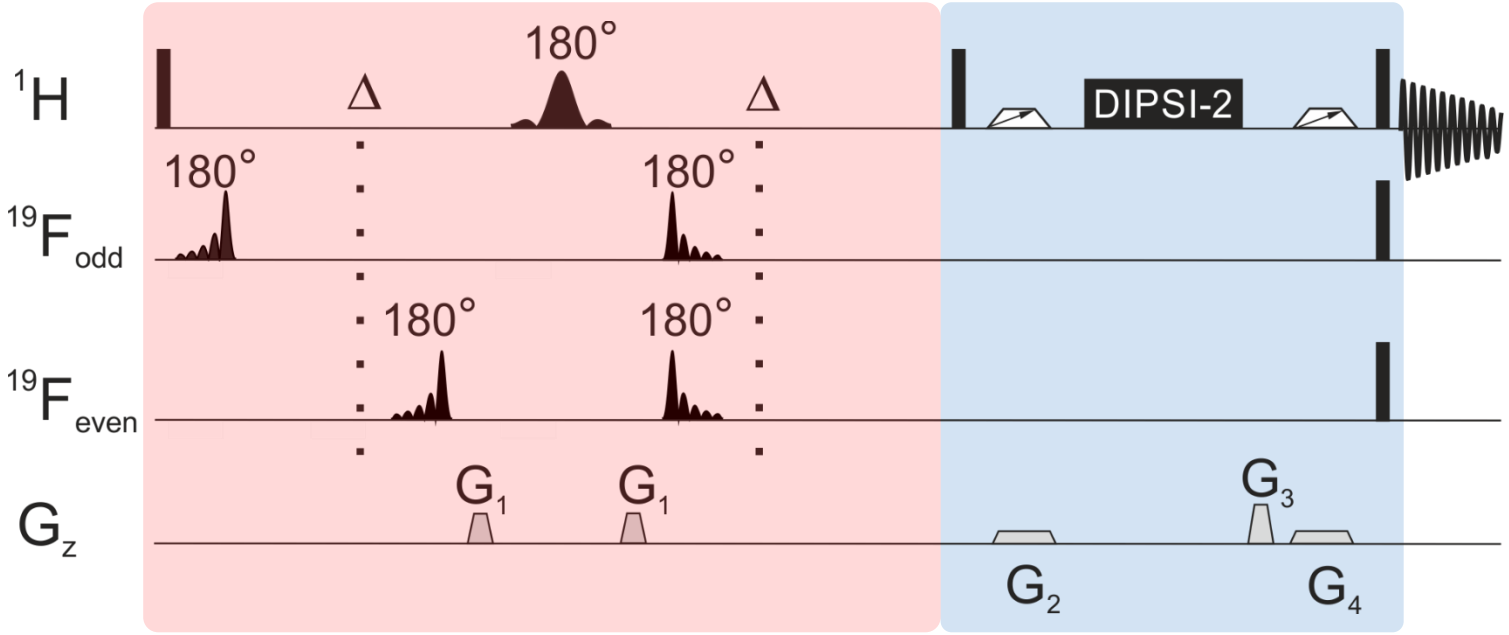
Extraction of the signs and magnitudes of J_{HF} couplings



IPAP - FESTA

Heteronuclear spectral editing – MODO-FESTA

MODulated echO Fluorine-Edited Selective TOCSY Acquisition



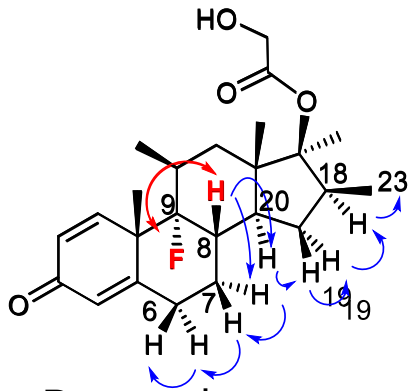
$\Delta = 1/(2\,J_{HF})$

Selective modulated echo
Selected ^{19}F coupled ^{1}H signal only (all other signals suppressed)

TOCSY
Transfers magnetisation throughout spin system

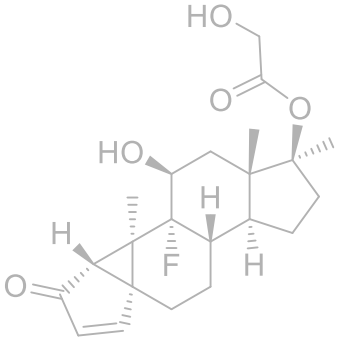
SRI-FESTA: *Anal. Chem.* 2018, **90**, 5445-5450.
MODO-FESTA: *Anal. Chem.* 2020, **92**, 2224-2228.

MODO-FESTA – Photodegradation study



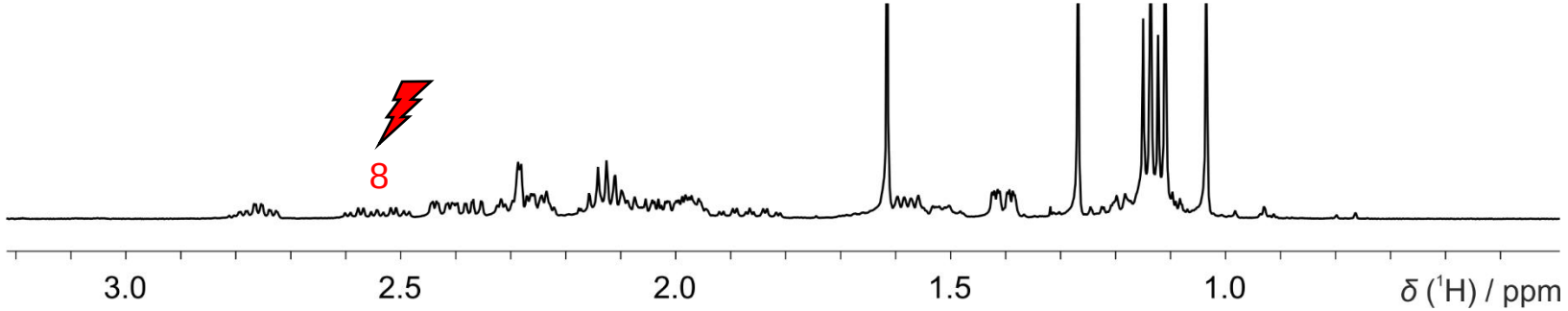
Betamethasone

UV light
(15 hr)

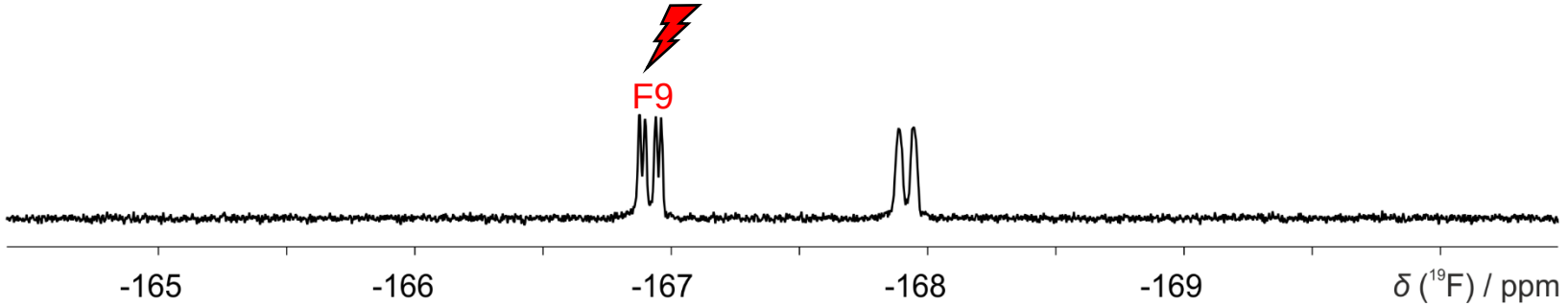


Lumibetamethasone

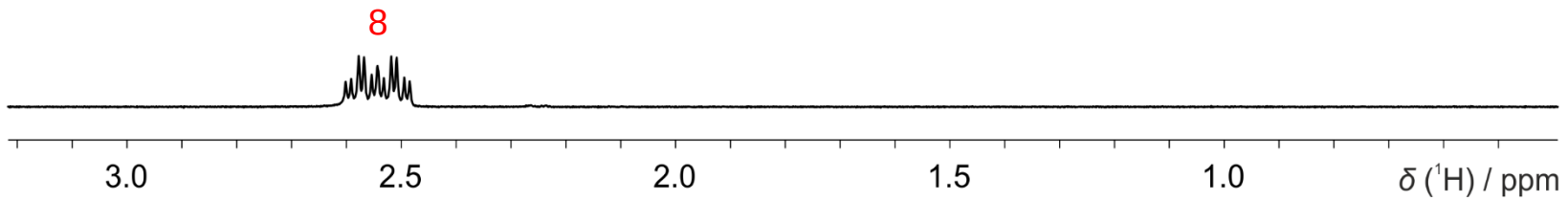
^{1}H NMR



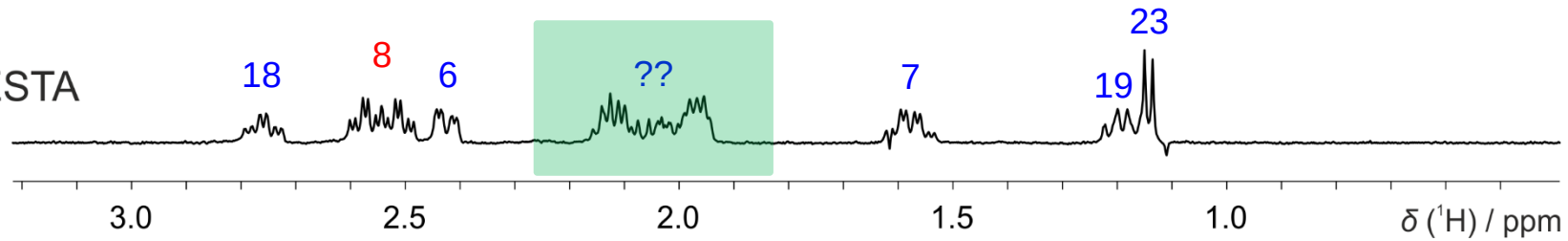
^{19}F NMR



MODO

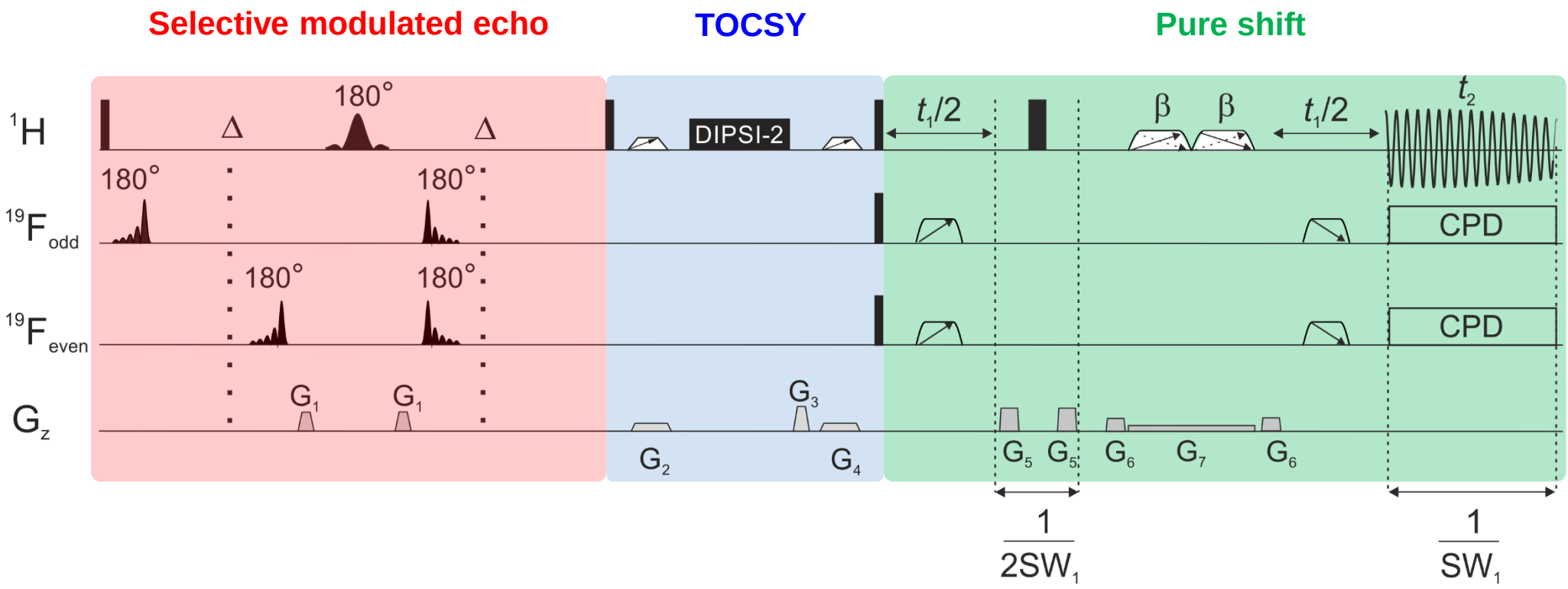


MODO-FESTA



Unpublished

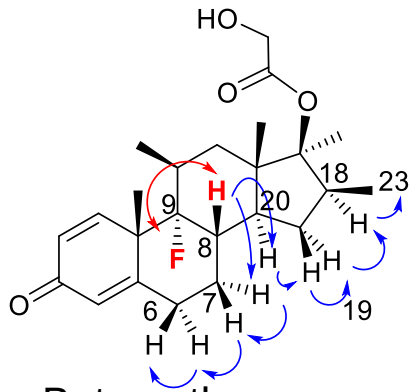
Pure shift MODO-FESTA



Selected ^{19}F coupled ^{1}H signal only → Transfers magnetisation throughout spin system → Suppression of the effects of J_{HF} and/or J_{HH} couplings

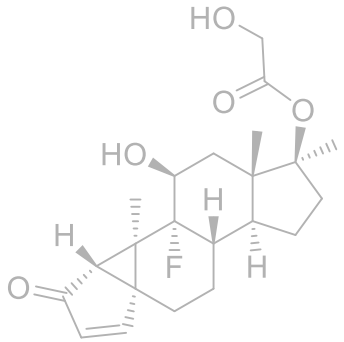
Unpublished

Pure shift MODO-FESTA – Photodegradation study



Betamethasone

UV light
(15 hr)



Lumibetamethasone

^{1}H NMR

MODO-FESTA

Pure shift
MODO-FESTA

29.6 Hz

^{19}F decoupled
pure shift
MODO-FESTA

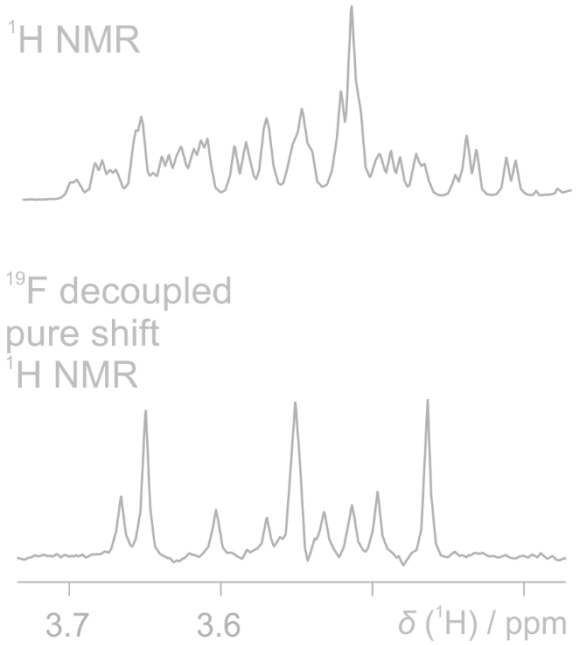
18
8
6
20
6'
7'
19
7
19
23

3.0
2.5
2.0
1.5
1.0
δ (^{1}H) / ppm

Unpublished

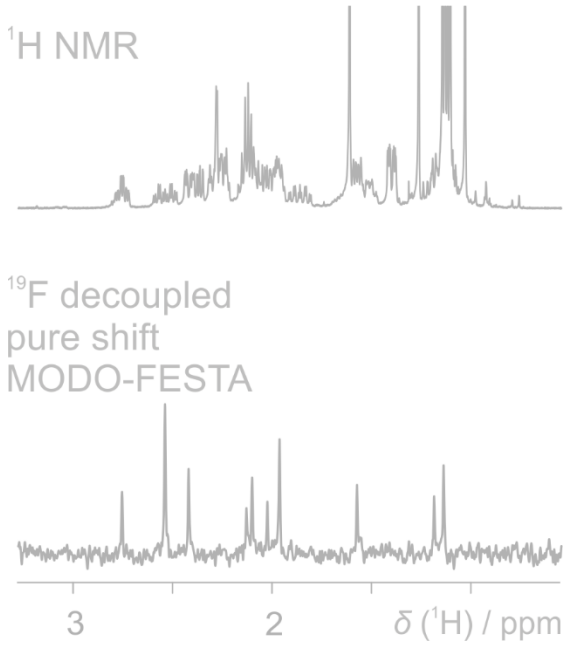
Overcoming signal overlap in ^{1}H NMR in ^{19}F containing systems

Extraction of δ information



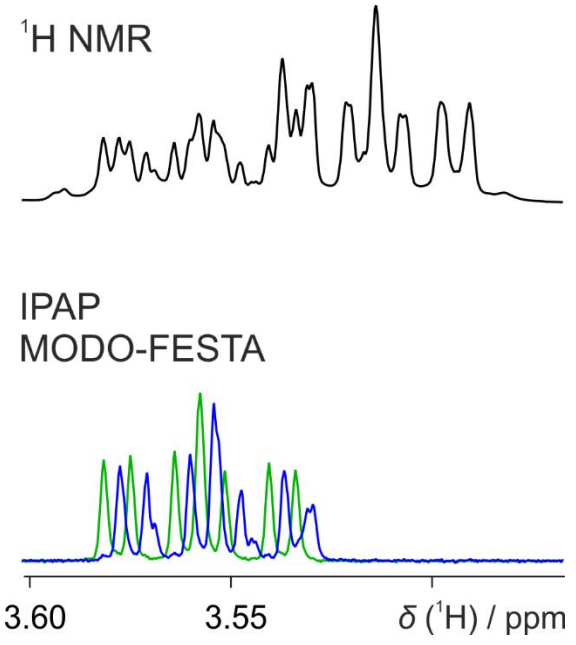
Heteronuclear pure shift ^{1}H NMR

Extraction of individual spin system δ information



Pure shift FESTA

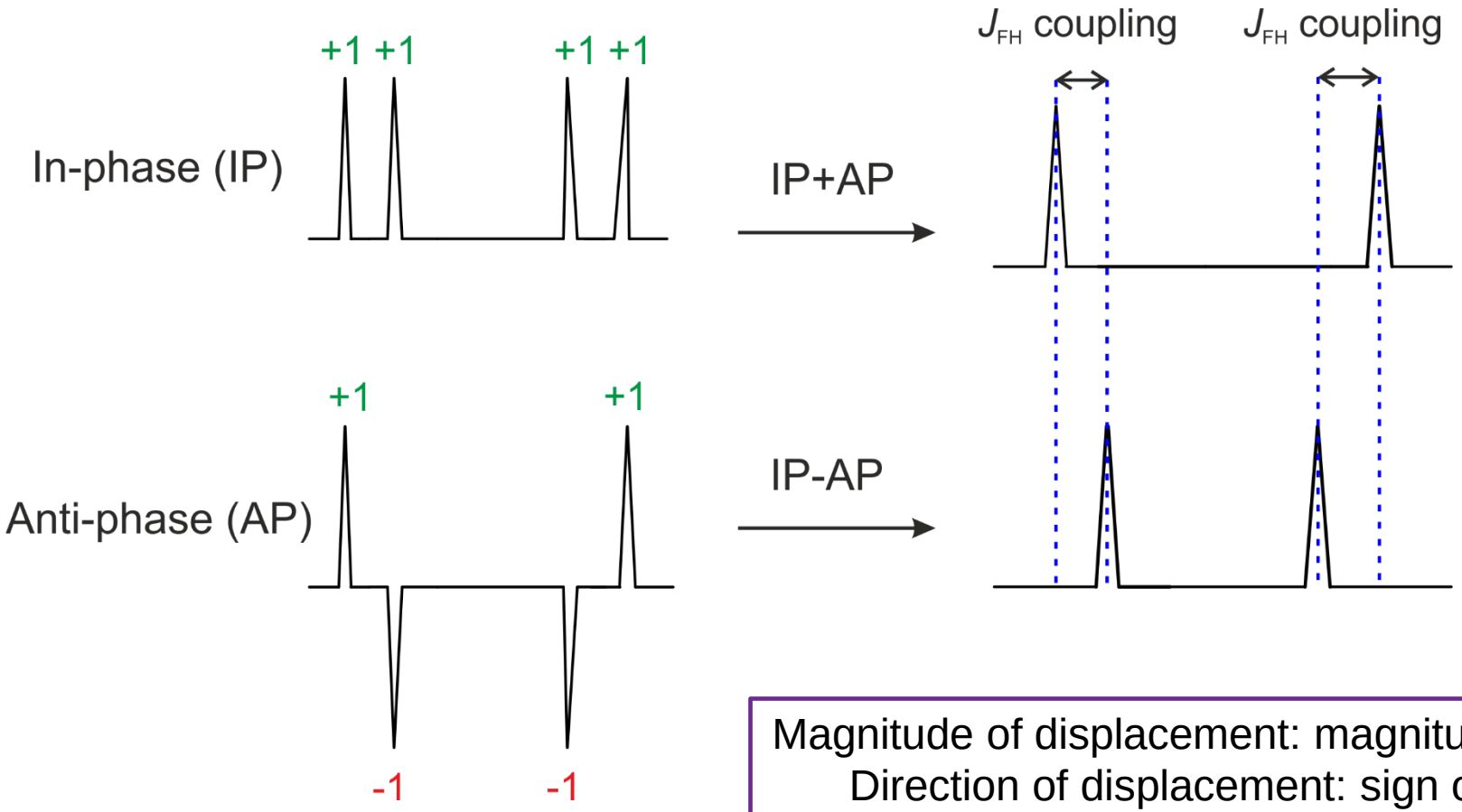
Extraction of the signs and magnitudes of J_{HF} couplings



IPAP - FESTA

IPAP methodology - Extracting J_{HF} coupling constants

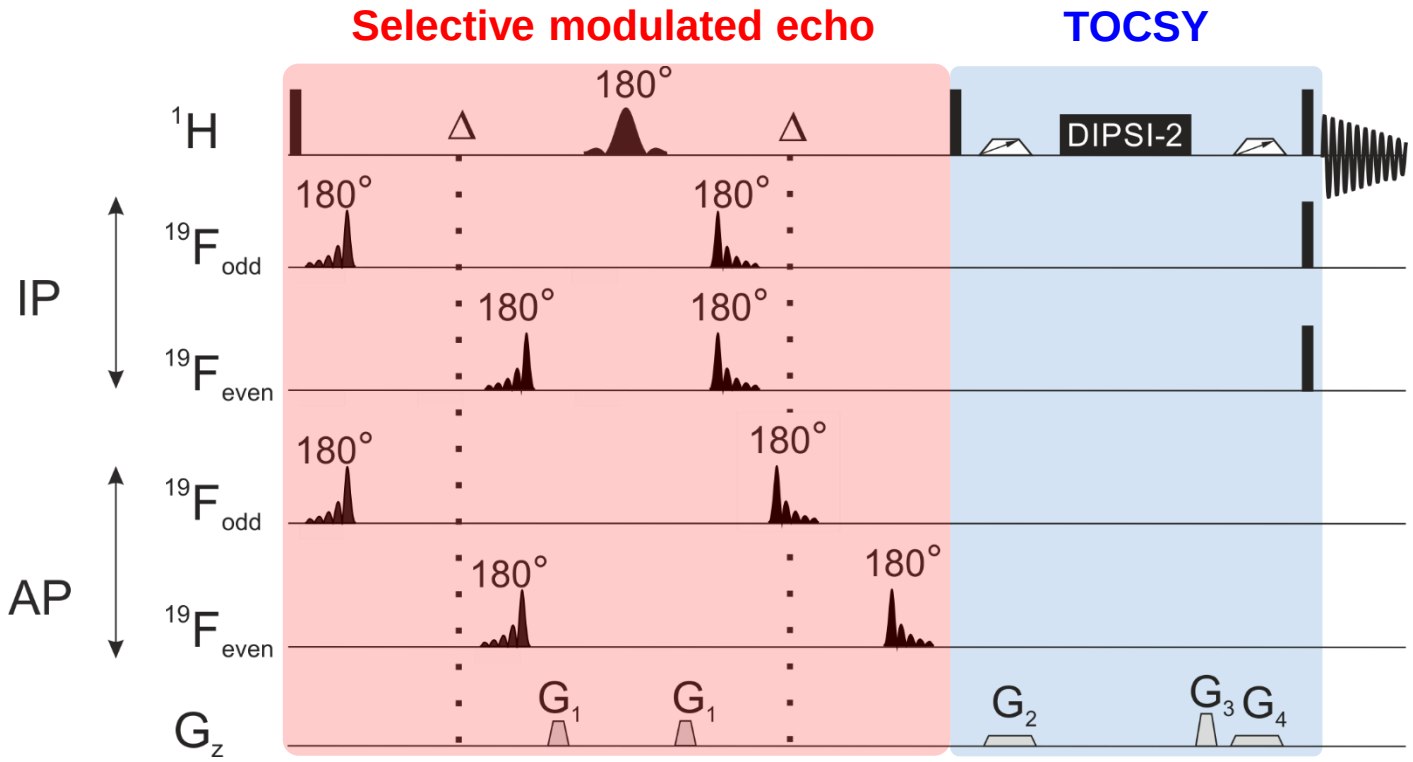
IPAP: **I**n-**P**hase **A**nti-**P**hase
Allows the $^{19}F = \alpha$ and $^{19}F = \beta$ components to be obtained in separate spectra
Fast and easy determination of the signs and magnitudes of J_{HF} couplings



Magnitude of displacement: magnitude of J_{HF}
Direction of displacement: sign of J_{HF}

Angew. Chem., 2012, **124**, 3985-3988

IPAP-FESTA - Extracting J_{HF} coupling constants

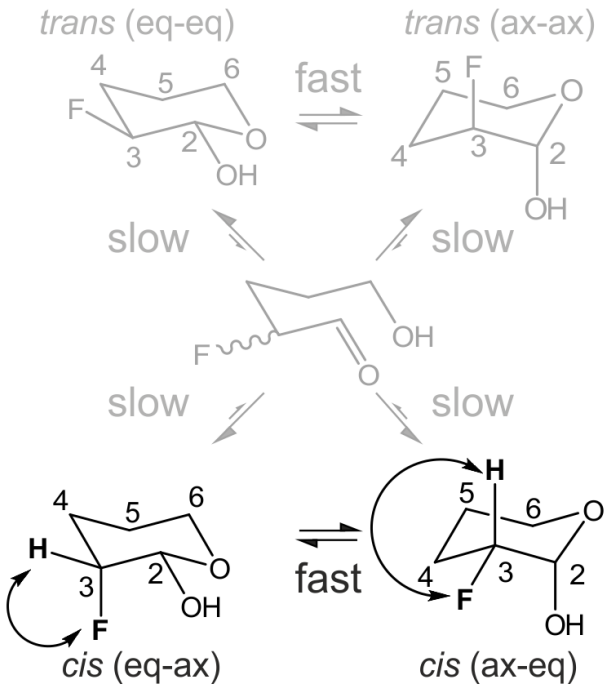


- 4 variations of the modulated echo
  - Differing ^{19}F pulse timings in the modulated echo
- 2 scans: IP-MODO-FESTA
- 2 scans: AP-MODO-FESTA

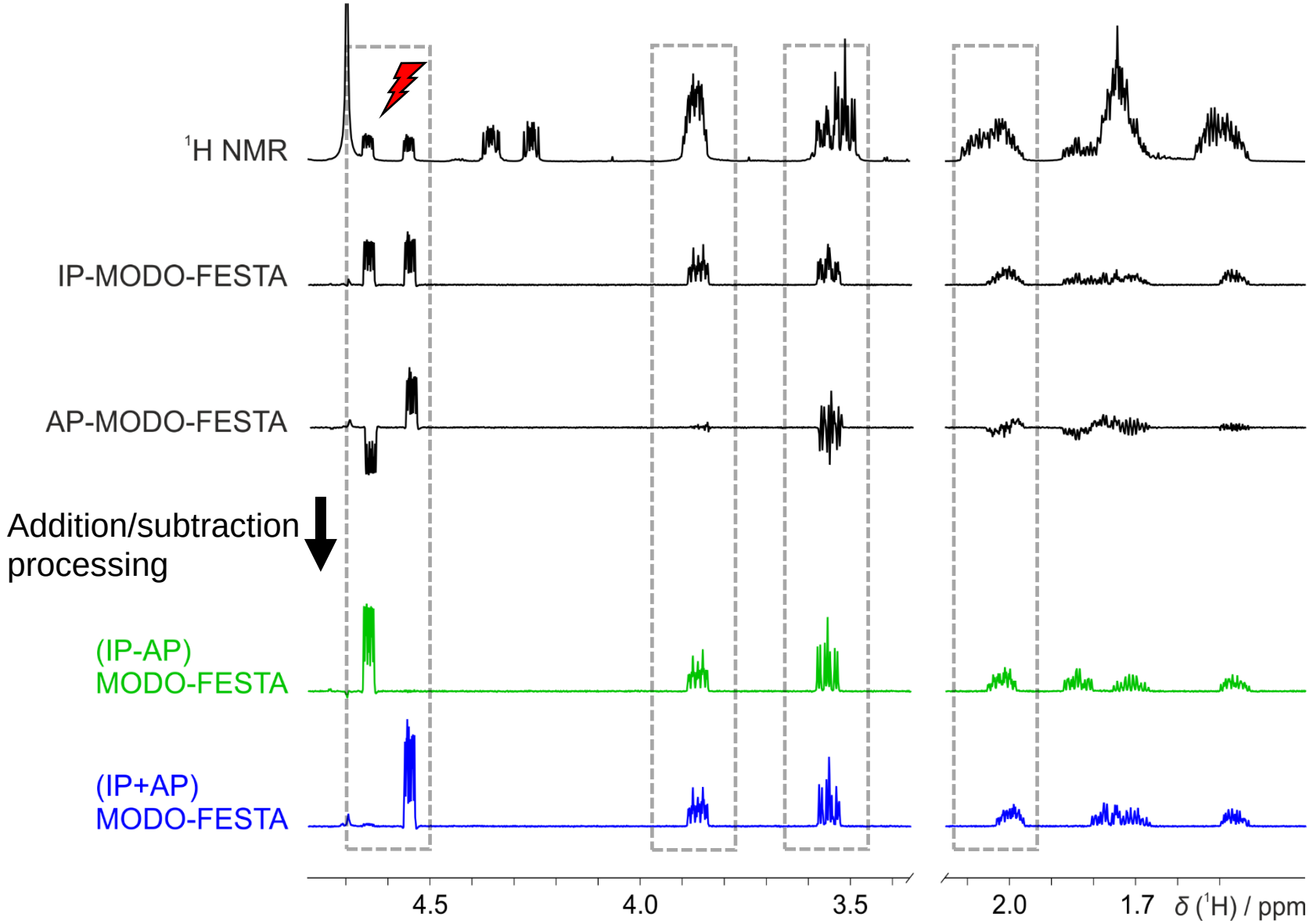
Unpublished

IPAP-FESTA - Extracting J_{HF} coupling constants

Fluorohydrin mixture



Fast/slow rates – on the chemical shift timescale



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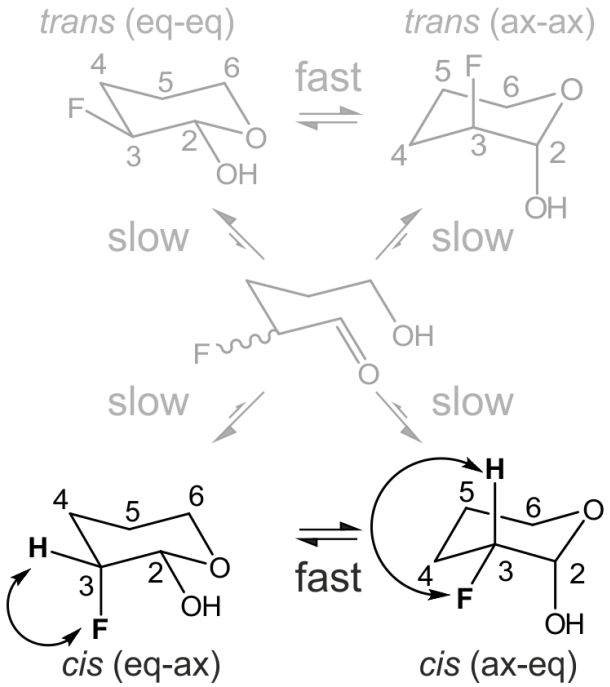
IPAP-FESTA - Extracting J_{HF} coupling constants

Fluorohydrin mixture



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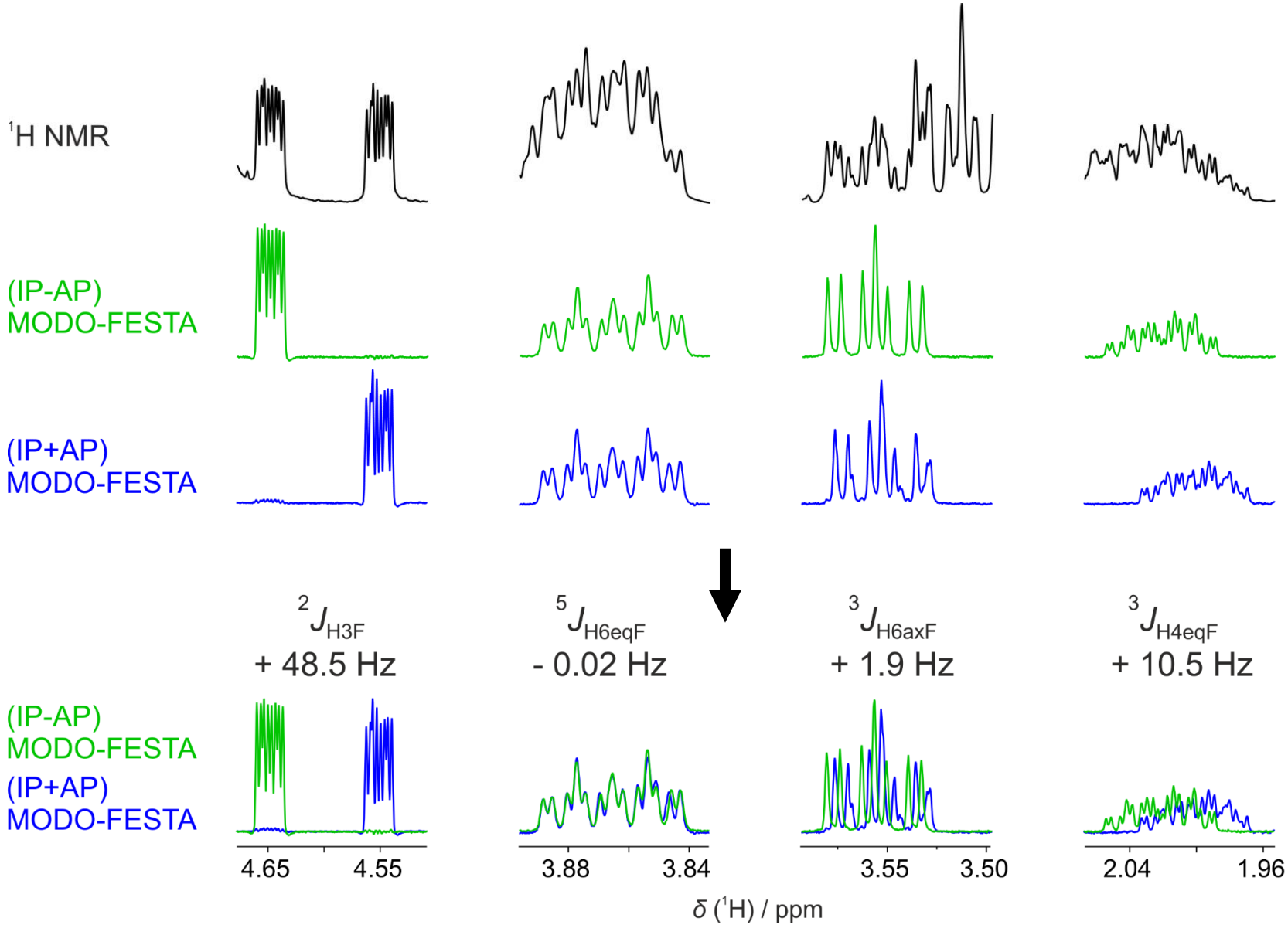
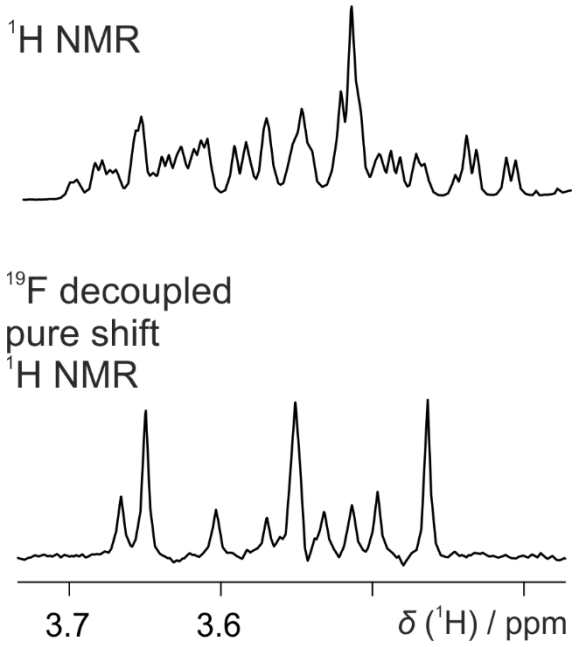


Unpublished

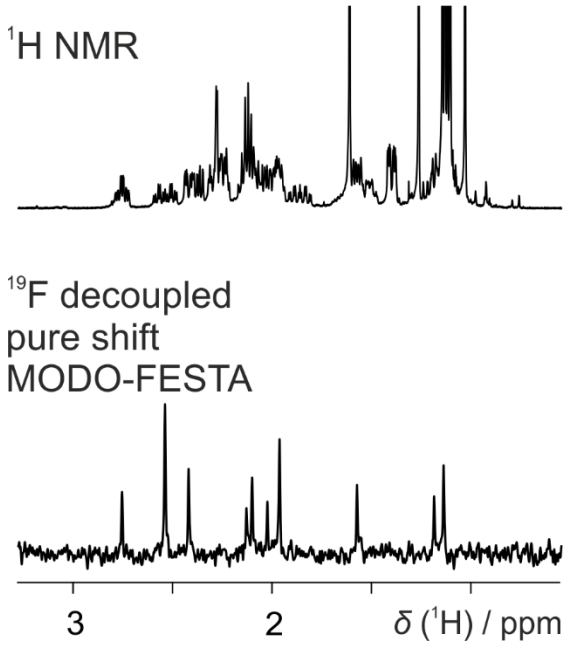
Summary: New NMR methods for structural analysis of fluorinated systems

Extraction of δ information



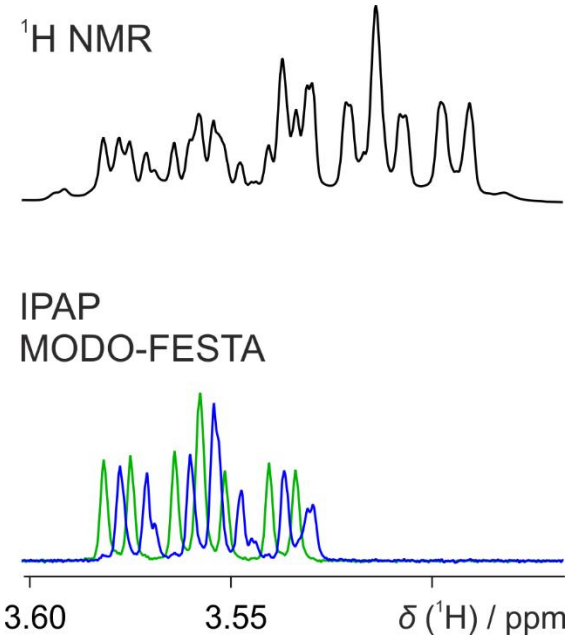
Heteronuclear pure shift ^{1}H NMR

Extraction of individual spin system δ information



Pure shift FESTA

Extraction of the signs and magnitudes of J_{HF} couplings



IPAP - FESTA

These methods are also applicable to other NMR active nuclei such as ^{31}P

Acknowledgments

University of Manchester

Dr. Laura Castañar Acedo

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Prof. Mathias Nilsson

Collaborators

Dr. Guilherme Dal Poggetto (MSD)

Dr. Thaís M. Barbosa (Nanalysis)

Dr. Cláudio F. Tormena (University of Campinas, Brazil)

Many thanks to the University of Manchester for the PhD studentship

Manchester NMR Methodology Group at EUROMAR

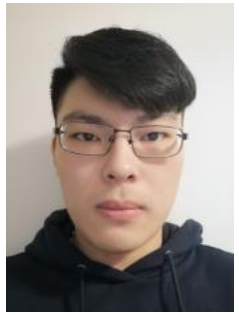
Emma Gates

GEMSTONE: ultra-selective NMR methods for complex spectra

13th July, 16:15
Room: Wit

Daniel Taylor
PO171
SABRE-enhanced real-time pure shift NMR spectroscopy

Howard Foster
PO316
Quantitative band-selective pure shift NMR



Runchao Li
PO330
Relaxational signal attenuation during selective refocusing pulses

Marshall Smith
PO344
SCALPEL NMR: performing surgery on spectra of complex mixtures

Thank you for listening!

Any questions?

coral.mycroft@manchester.ac.uk

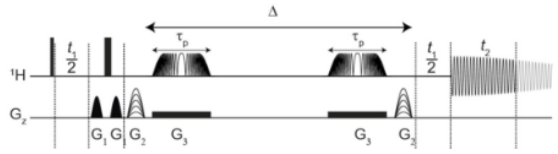
@coralmycroft @mancNMR

https://nmr.chemistry.manchester.ac.uk

Pulse Sequences

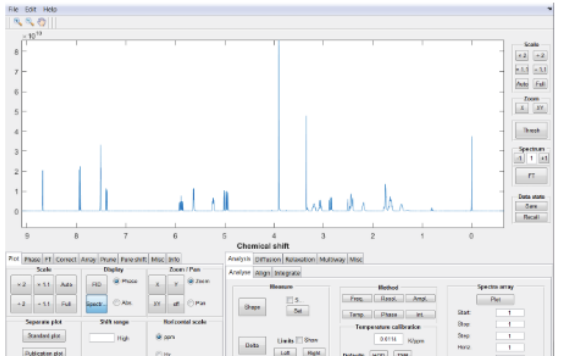
We are currently preparing many of our pulse sequences, parameter sets, example datasets and processing macros for the website. Some are available here but if you would like to use any of the other the sequences, as described in the publications section, please email us. The majority of sequences are available for Varian systems and we are gradually writing the Bruker variants.

The pulse sequences and any macros required for data conversion can be accessed from this part of the website.



Software

Software produced in-house, including The GNAT (General NMR Analysis Toolbox), the legacy DOSY Toolbox, and diffusion estimation.



Workshops and presentations

The slides from some of the workshops and presentations given by group members are available from this part of the website. There is a pure shift NMR package available for download as part of our 2017 workshop on pure shift NMR.