

Improving NMR methods to solve 3D molecular structure in solution.

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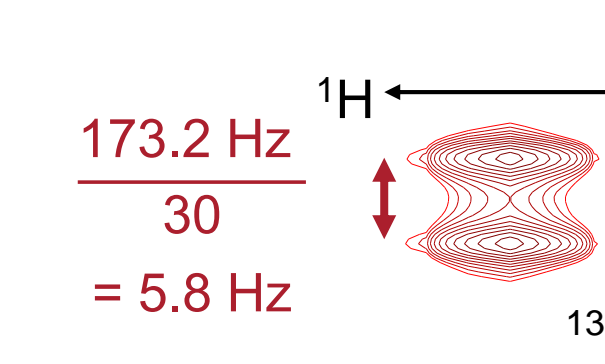
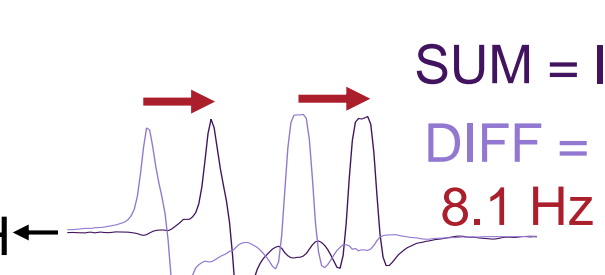
Introduction

The accuracy of heteronuclear (¹H-¹³C) scalar coupling constants (ⁿJ_{CH}) measured experimentally with several 2-dimensional methods was evaluated by comparison to coupled ¹³C data. ⁿJ_{CH} can be related to 3-dimensional structure either by using a computational model or in the case of ³J_{CH} by empirical methods. Therefore the accuracy of ⁿJ_{CH} calculated using Density Functional Theory (DFT) was also compared to coupled ¹³C data. With the accuracy of DFT calculation and experimental methods known it was possible to turn to the generation of empirical equations relating ³J_{CH} to structure. A parameterised empirical equation relating dihedral angle (Φ_{HC} and Ψ_{CX}) to ³J_{CH} (based on data generated by DFT) was identified. There are also several other important variables when determining ³J_{CH} including substituent pattern, the nature of the coupling pathway and bond angle which are discussed below.

Validation

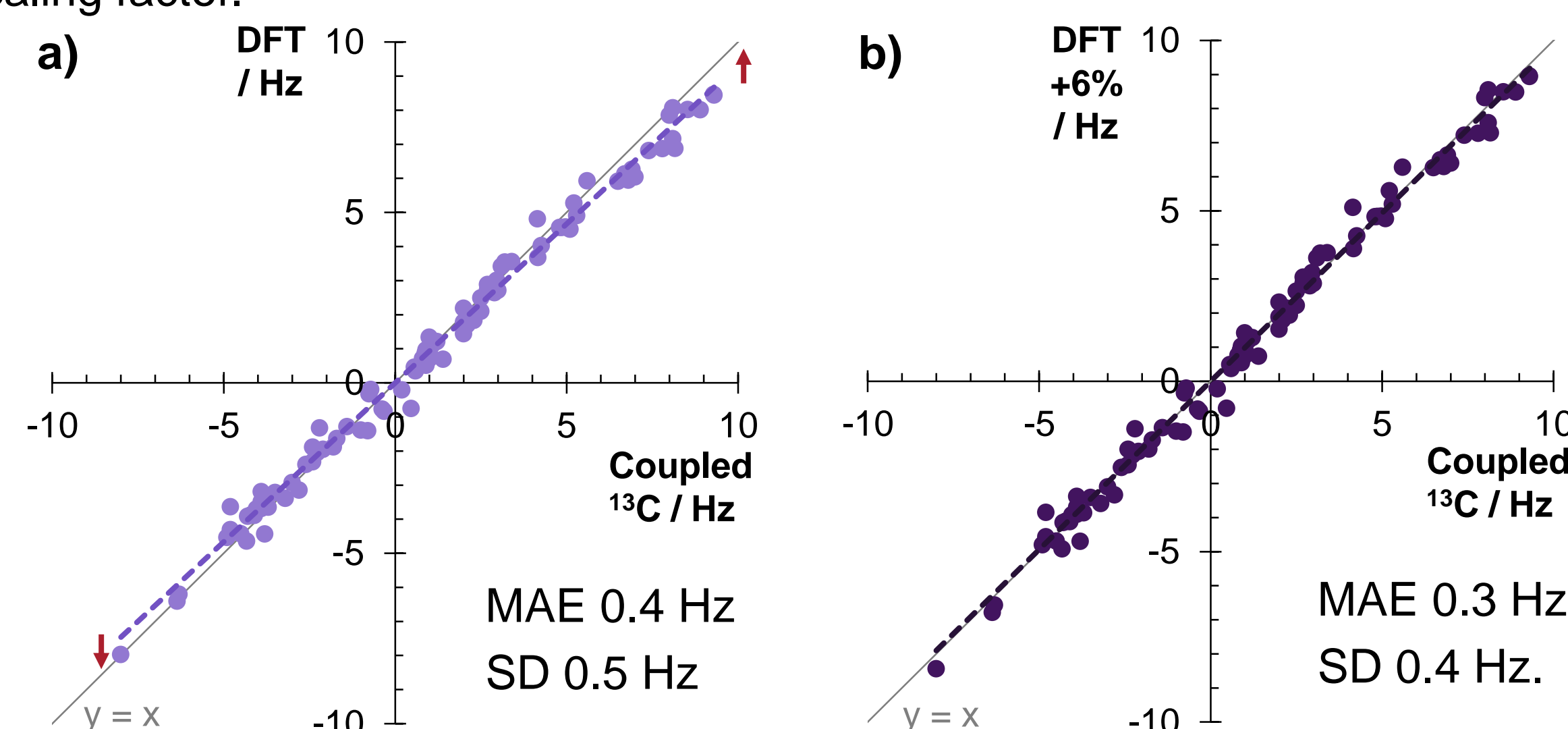
To generate empirical relationships between ³J_{CH} and structure it is first necessary to confirm the accuracy of the experimental data which will be used to test the relationship (left) and also the computational method (right) which will be used to calculate ³J_{CH} as a function of structure.

Experimental measurement of ⁿJ_{CH}

Method (applied to strychnine)	Analysis		Accuracy (MAE, SD) /Hz	Expt. time per ^{n>1} J _{CH}	No. of ^{n>1} J _{CH}
	Ease	Method			
Coupled ¹³C	Hard	Spin simulation Experimental	Used for comparison	34m	35
EXSIDE [1] <i>J_{LR} Matched</i>	Easy		0.19, 0.20	2h31m	52
EXSIDE (Corrected) <i>J_{LR} = 6 Hz</i>	EASY	SUM = IP + AP DIFF = IP - AP	0.20, 0.18	42m	46
HMBC IPAP [2] <i>J_{LR} = 6 Hz</i>	Medium		0.34, 0.43	3m	64

DFT computation of ^{n>1}J_{CH}

^{n>1}J_{CH} were calculated for strychnine, camphor and 3-methyl piperidine. a) 5-7% underestimation of ^{n>1}J_{CH} b) which can be corrected by applying a linear ~6% scaling factor.



Empirical prediction of ³J_{CH}

$${}^3J_{CH} = \sum_{i=0}^n \sum_{j=0}^n C_{ij} \cos(i\Phi) \cos(j\Psi) + \sum_{i=0}^n \sum_{j=0}^n S_{ij} \sin(i\Phi) \sin(j\Psi) + \sum_{i=0}^n \sum_{j=0}^n T_{ij} \cos(i\Phi) \sin(j\Psi) + \sum_{i=0}^n \sum_{j=0}^n U_{ij} \sin(i\Phi) \cos(j\Psi)$$

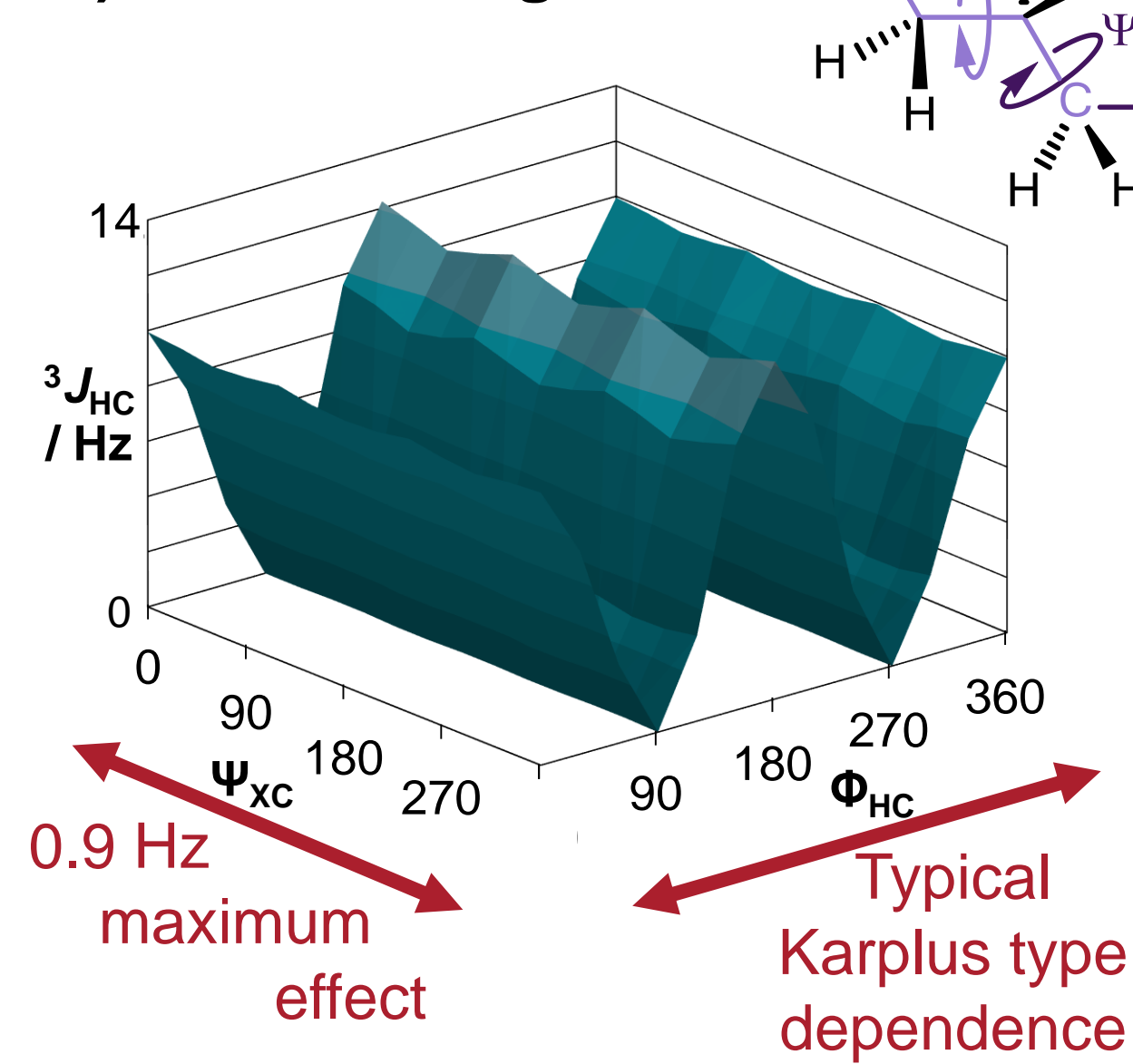
Contains typical Φ dependant Karplus terms Improved estimation accounting for coupling pathway and substituent effects [3]

C_{ij}, S_{ij}, T_{ij}, U_{ij} are constants for a given fragment

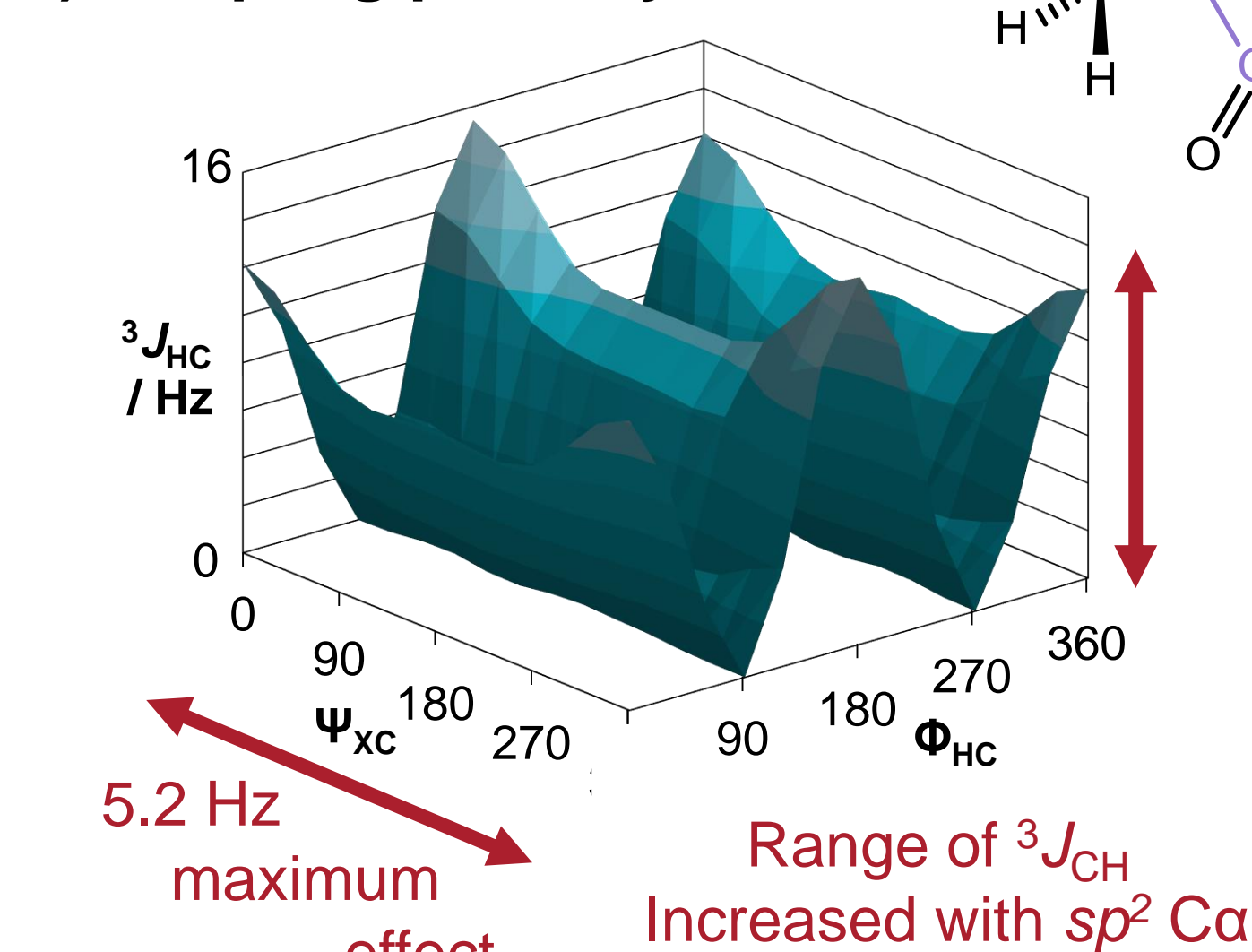
Important factors

Geometry optimisation and ³J_{CH} calculations (DFT +6%) were performed for 36 fragments with different coupling pathways and methyl substitution patterns. Dihedral angles (Φ and Ψ), bond angles (θ₁₋₃), substituent effects and coupling pathway effects were examined.

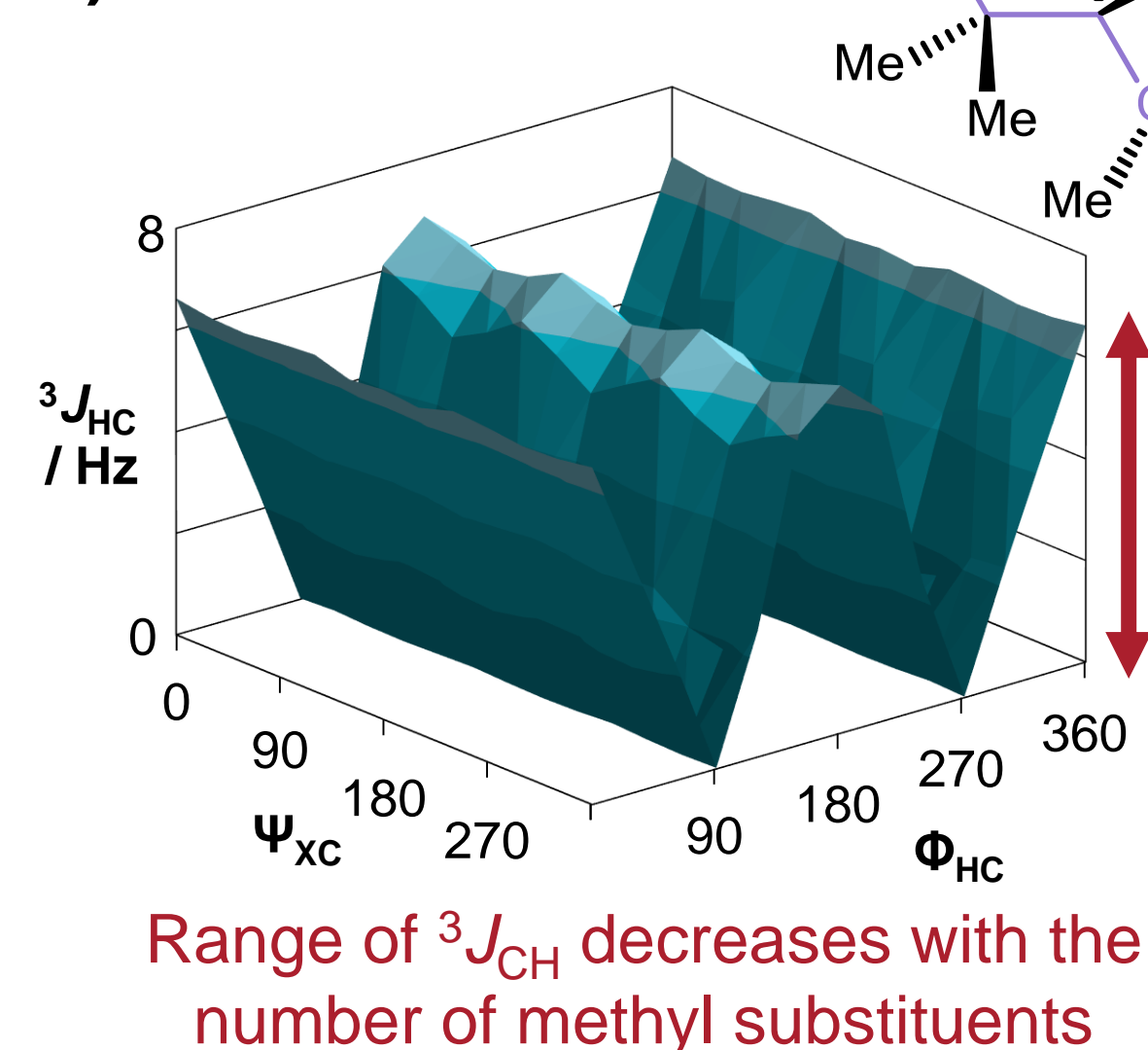
1) Dihedral Angles:



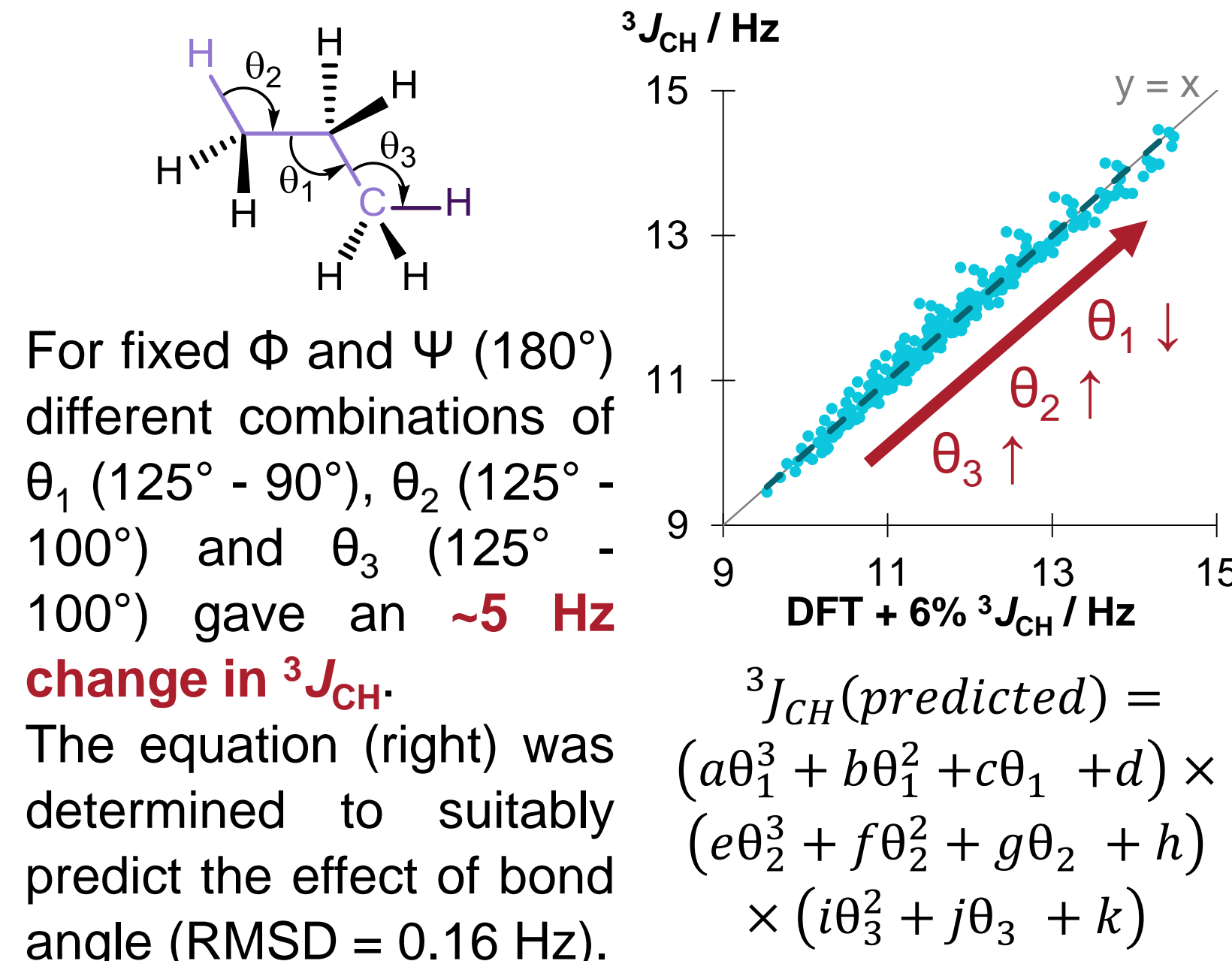
3) Coupling pathway:



2) Substituent Effects:

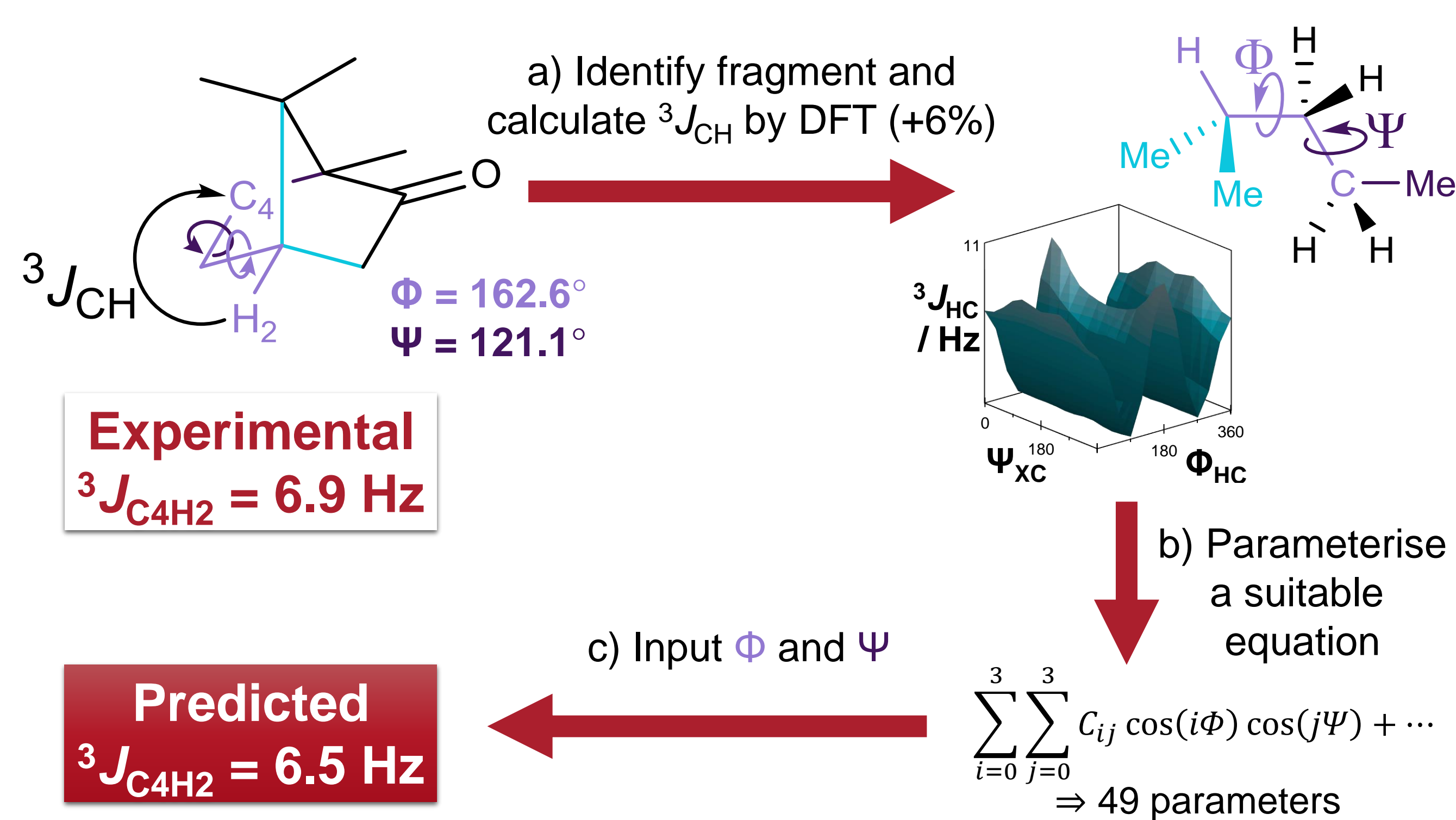


4) Bond Angle:



Application to camphor

The ³J_{CH} calculated by DFT can then be used to parameterise the equation above, relating ³J_{CH} to the dihedral angles Φ and Ψ. The suitability of these equations was then tested as described below using ³J_{C4H2} in camphor as an example:



This fragment based approach performed well when compared to ³J_{CH} determined by matched EXSIDE. However it is clear that methyl groups may not be a suitable proxy for non-sp³ C substituents. The effect of bond angle has also not been accounted for.

	Literature equations		This work	
	Aydin et al. [4]	Palermo et al. [5]	Including all C substituents	Excluding sp ² C substituents
MAE /Hz	1.0	1.0	0.8	0.6
SD /Hz	1.4	1.4	1.3	0.8
No. of values	16	13	16	8

Experimental

Spectra were run on a Varian VNMRs 500 MHz Direct Drive Spectrometer with Agilent OneNMR probe at 25°C or Bruker AVANCE III HD 500MHz NMR Spectrometer with 5mm DCH 13C-1H/D Cryo Probe at 25°C.

Geometry optimisation and NMR calculations were performed (Gaussian 09, DFT, mPW1PW91/6-311g (d,p), IEFPCM(Chloroform), GIAO method) to obtain total scalar coupling constants for strychnine, camphor and 3-methyl piperidine. This method (without a solvent model) was used to calculate ³J_{CH} for 36 fragments freezing structural parameters with the modredundant functionality. Empirical equations were parameterised by minimising the RMSD.

References

- [1] Krishnamurthy, V. V. J. Magn. Reson. Ser. A 1996, 121 (1), 33.
- [2] Sauri, J.; Parella, T. Magn. Reson. Chem. 2013, 51 (9), 509.
- [3] van Beuzekom, A. A.; de Leeuw, F. A. A. M.; Altona, C. Magn. Reson. Chem. 1990, 28 (1), 68.
- [4] Aydin, R.; Günther, H. Magn. Reson. Chem. 1990, 28 (5), 448.
- [5] Palermo, G.; Riccio, R.; Bifulco, G. J. Org. Chem. 2010, 75 (6), 1982.

Conclusions

- EXSIDE was the most accurate, easiest to analyse but also slowest 2-dimensional method for ^{n>1}J_{CH} measurement. HMBC was slightly less accurate but was ×10 faster per coupling constant measured.
- DFT showed a significant underestimation of ^{n>1}J_{CH}, which could be corrected by the use of a linear scaling factor (6%).
- A fragment based approach to empirically predicting ³J_{CH} showed that a 49 parameter equation dependant on two dihedral angles was reasonably effective. Therefore work will be put into generalising this equation and obtaining a large body of experimentally determined ³J_{CH} in structurally diverse molecules for validation.
- Empirical equations can rapidly relate ³J_{CH} to structure, however the more in depth DFT calculations currently still estimated ^{n>1}J_{CH} with greater accuracy. Therefore our target will be to compete with DFT in terms of accurate estimation of ³J_{CH}.

Acknowledgements

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