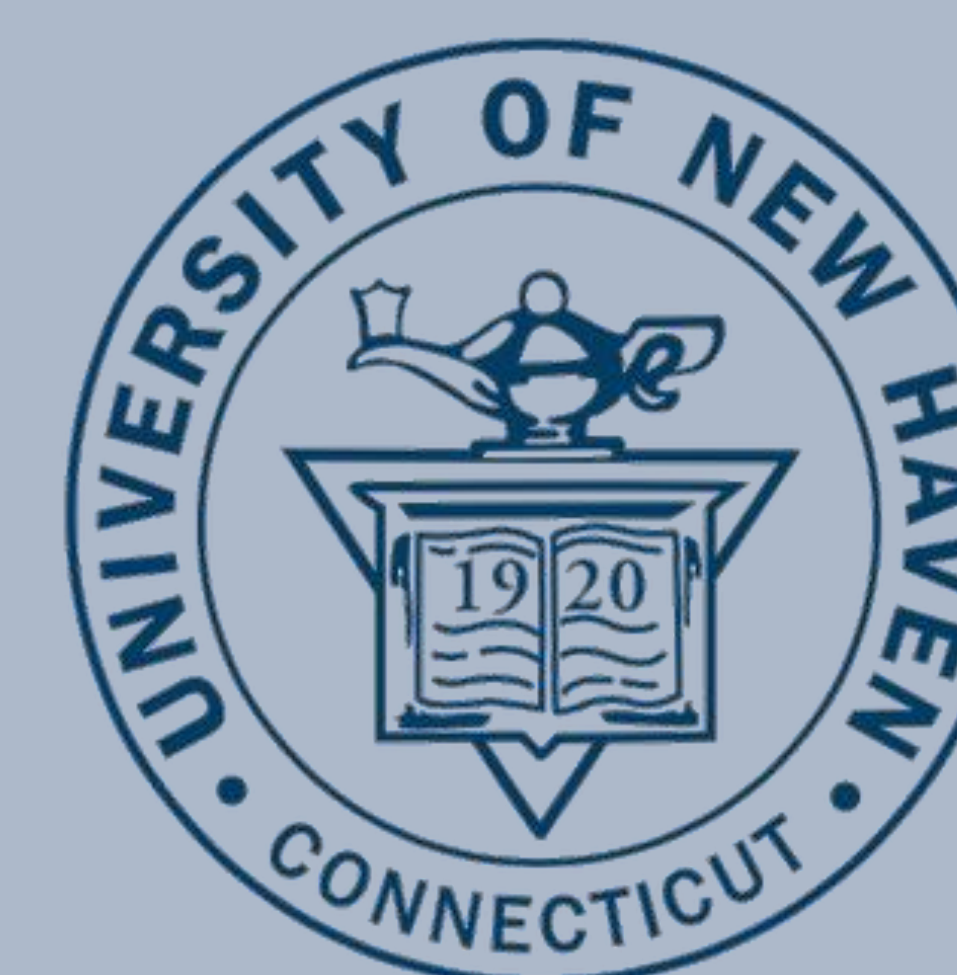


# STUDY OF THE INTERACTION BETWEEN TET PROTEIN AND VITAMIN C

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### Introduction

Ten Eleven Translocation (TET) enzymes oxidize 5mC in DNA to various derivatives, which is important to biological processes. Mutations in TET enzymes have been found to occur in myeloid disorders such as Acute Myeloid Leukemia. Ascorbic acid, which is commonly referred to as Vitamin C, interacts with TET enzymes by inducing demethylation of DNA in only the TET components.

A 2-D NOESY NMR experiment allowed for the exploration of the TET protein and ascorbic acid interaction. NOESY uses the dipolar interaction of nuclear spins to correlate protons in amino acid sequences. This is important for the determination of protein structures.

The goal of the project was to analyze specifically how ascorbic acid interacts with TET enzymes by using 2-D NMR, which could reveal information for AML drug design research.

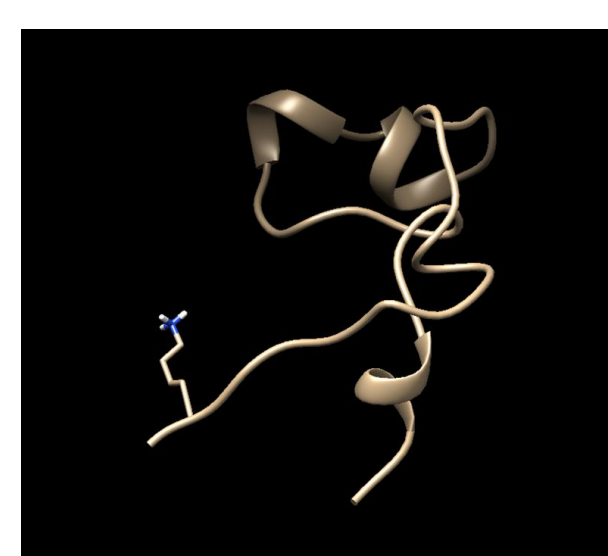
### Sample Preparation

Approximately 0.5 ml of TET1 protein was extracted using a pipette and placed in a NMR tube. Next, 1 ml of deuterated water was added to the NMR tube using a new pipette, and the tube was labeled "Tet1". Another 0.5 ml of TET1 was extracted and put into a new NMR tube using a pipette, then approximately 0.5 ml of Ascorbic acid was pipetted into this NMR tube. This was followed by another ml of deuterated water, and the tube was labeled "Tet1AA". The process of preparing these two samples was repeated using TET3. A total of four NMR samples were prepared.

### Materials

#### TET1

Molecular weight:  
110 kDa  
2136 Amino Acids long  
3 Alpha helices  
1 Beta strand  
2 Turns

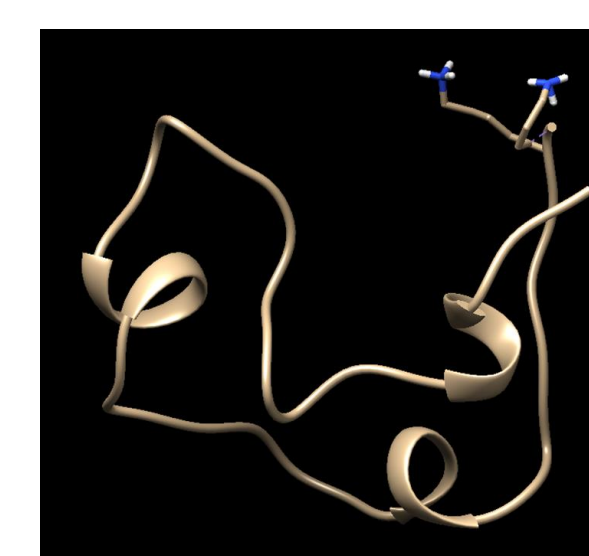


Interaction with DNA:  
Positions: 1580-1593  
Amino Acids:  
SWSMYFNGCKFGRS

Substrate Binding:  
Positions: 2049- 2051  
Amino Acids: YQH

#### TET3

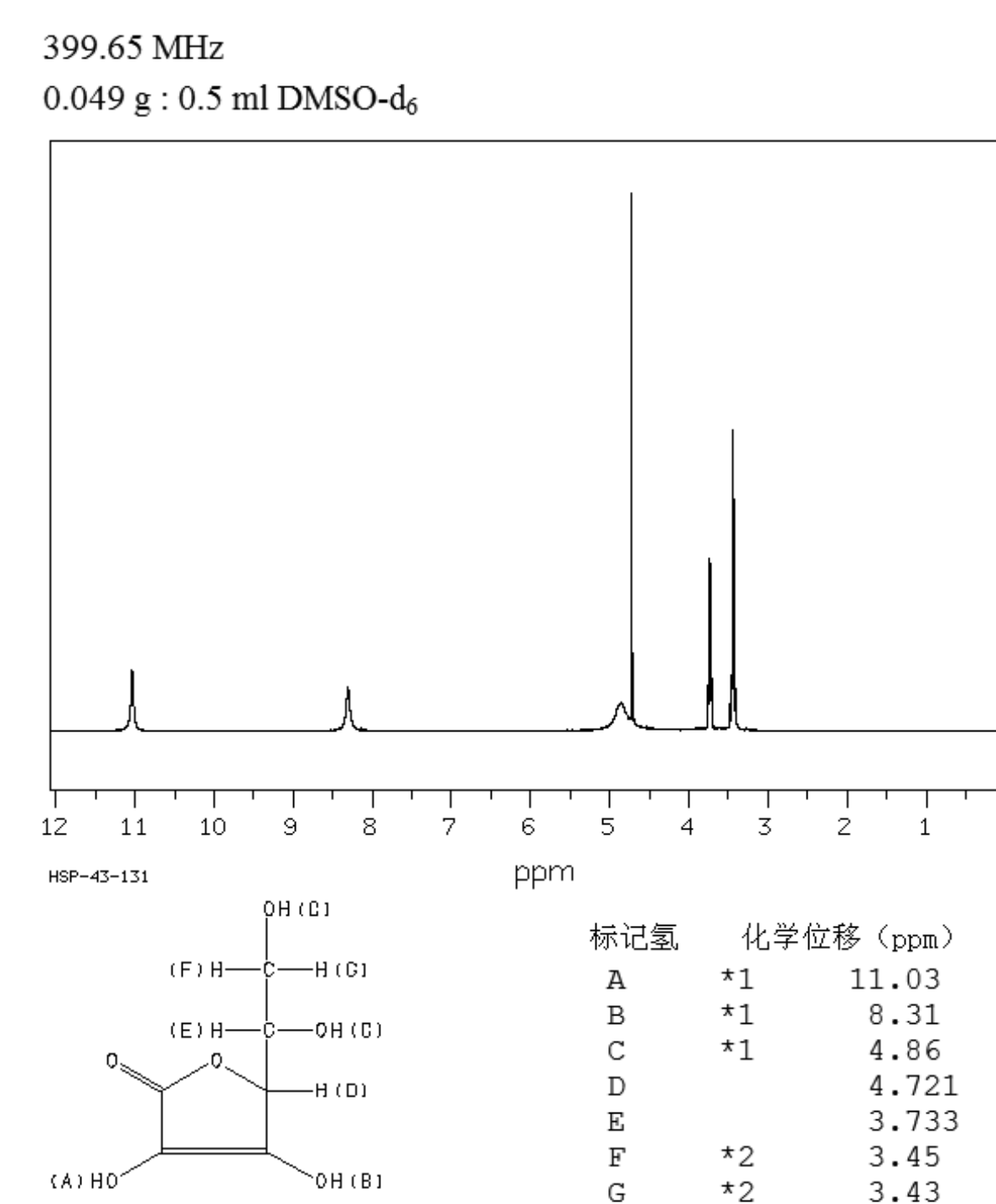
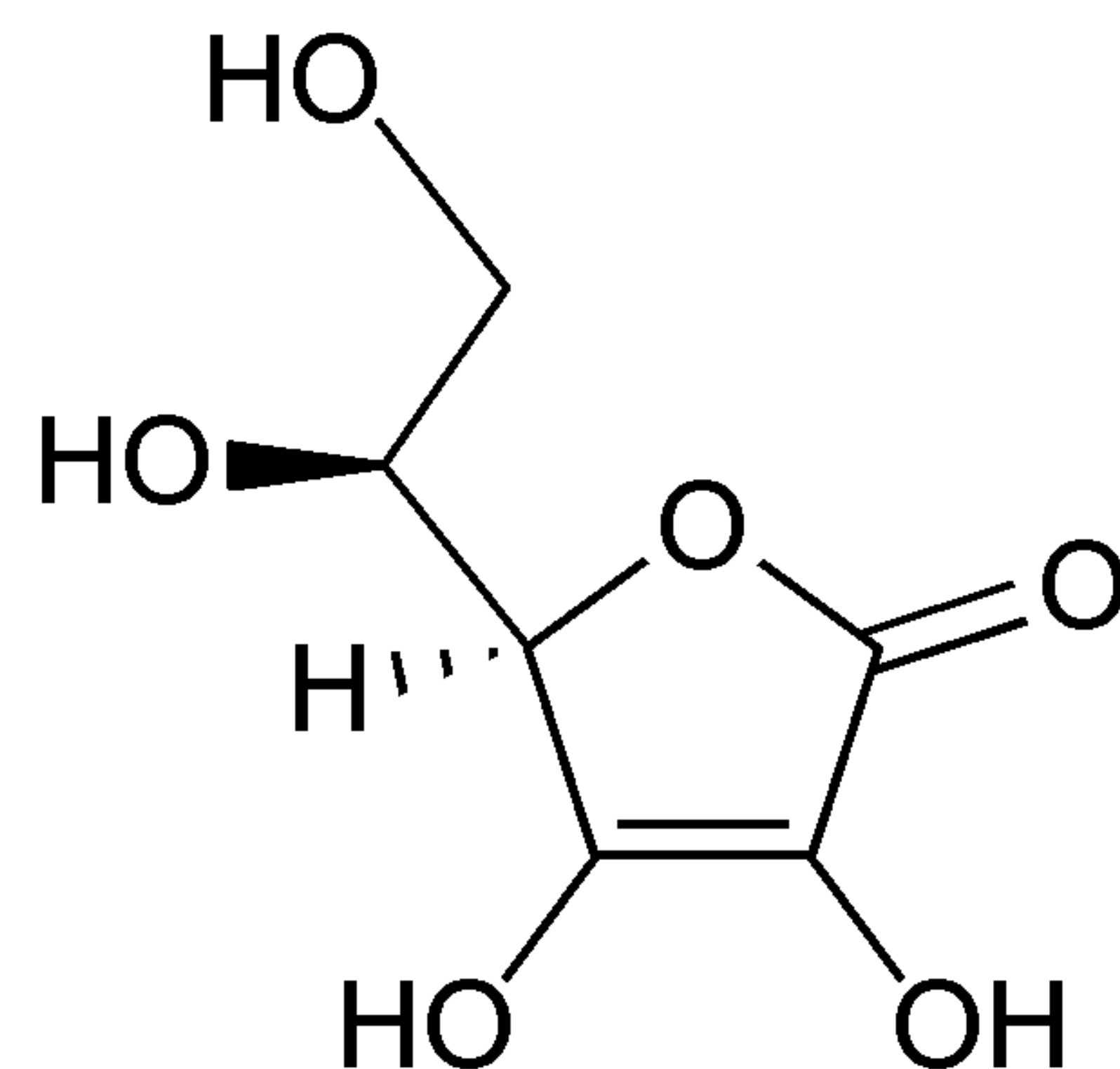
Molecular weight:  
140 kDa  
1660 Amino Acids long  
1 Alpha Helix  
1 Beta Strand  
2 Turns



Interaction with DNA:  
Positions: 850-863  
Amino Acids:  
SWSMYFGCKYARS

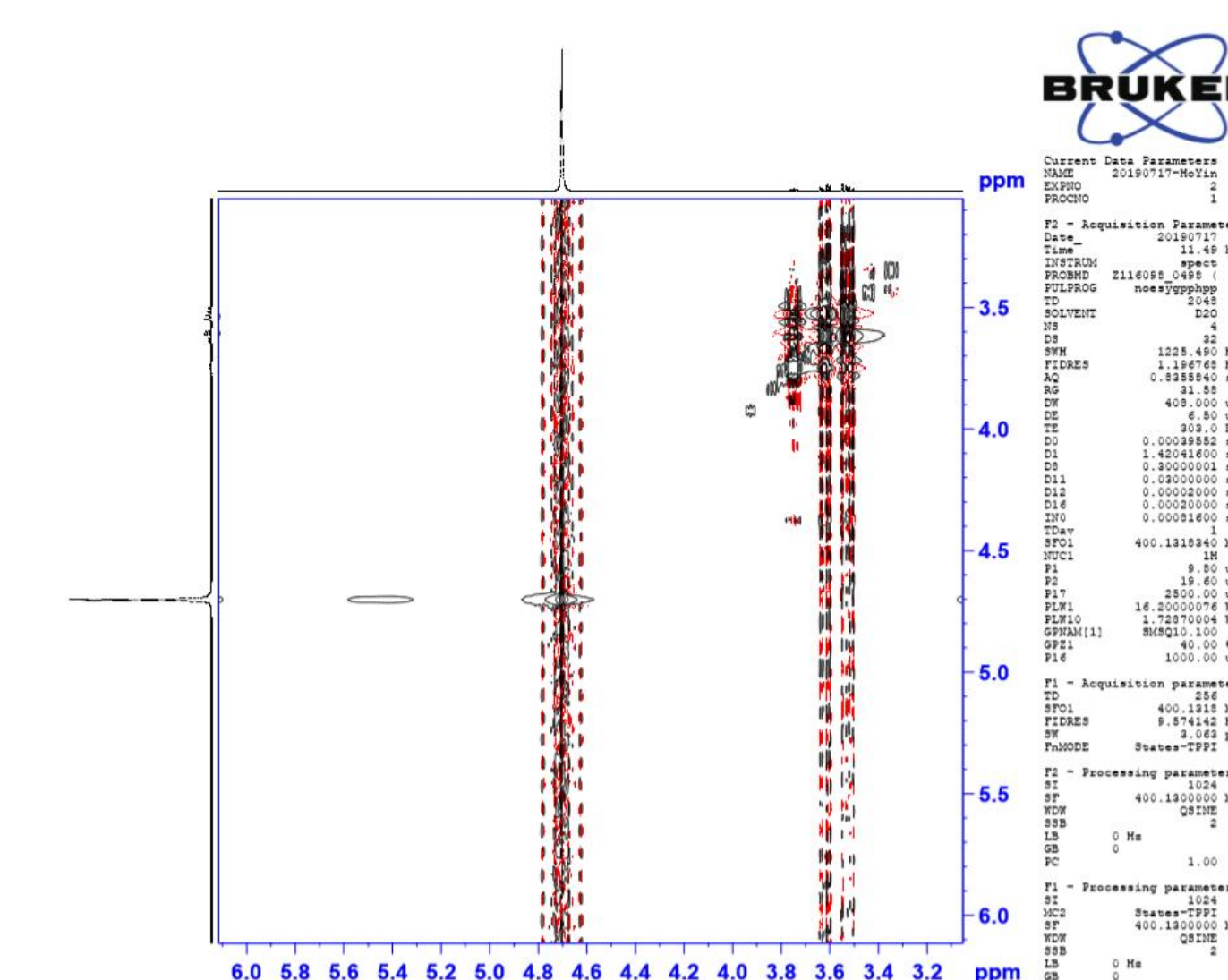
Substrate Binding:  
Positions: 1559-1561  
Amino Acids: YQH

### Ascorbic Acid

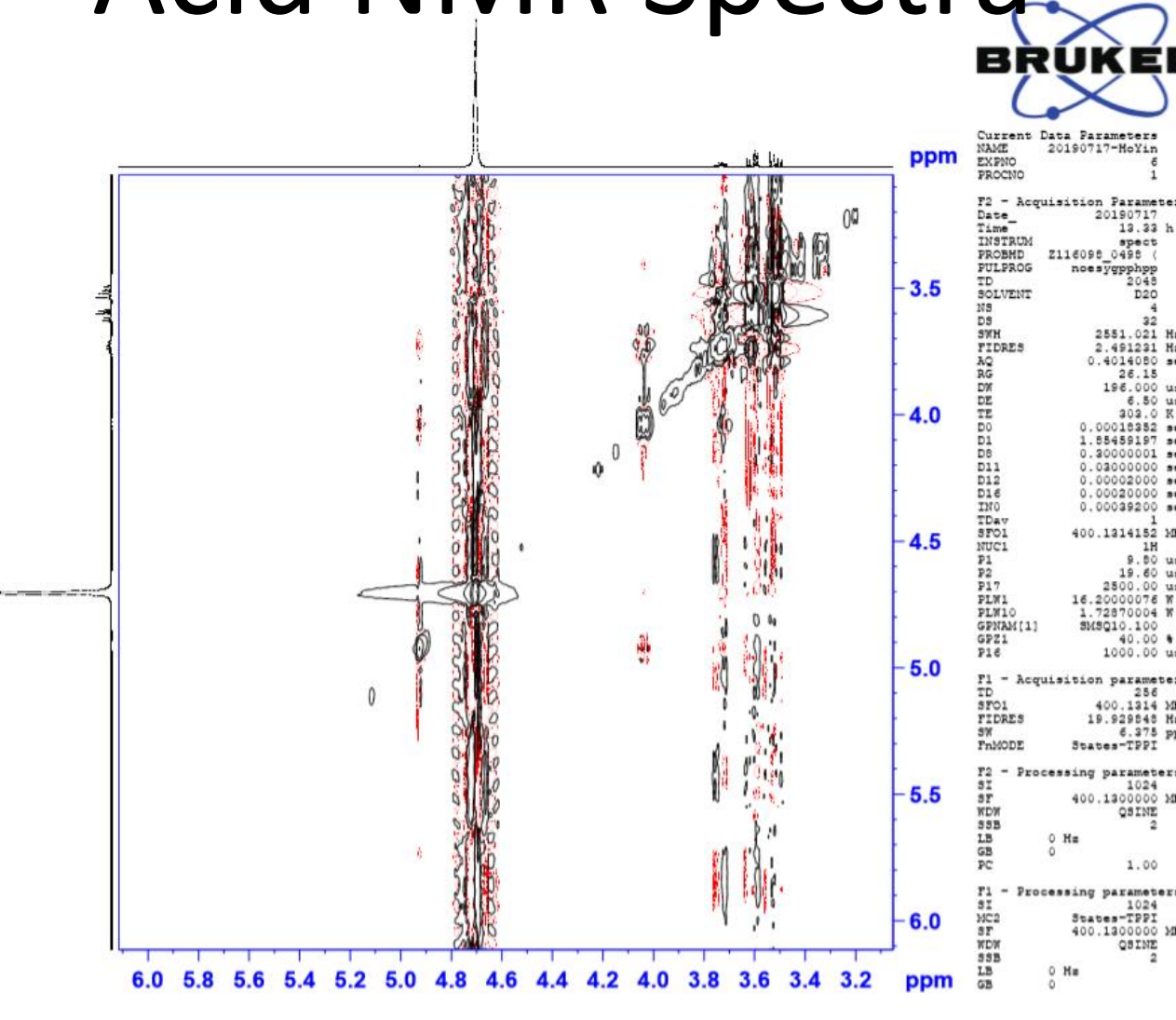


### 2-D NMR Results

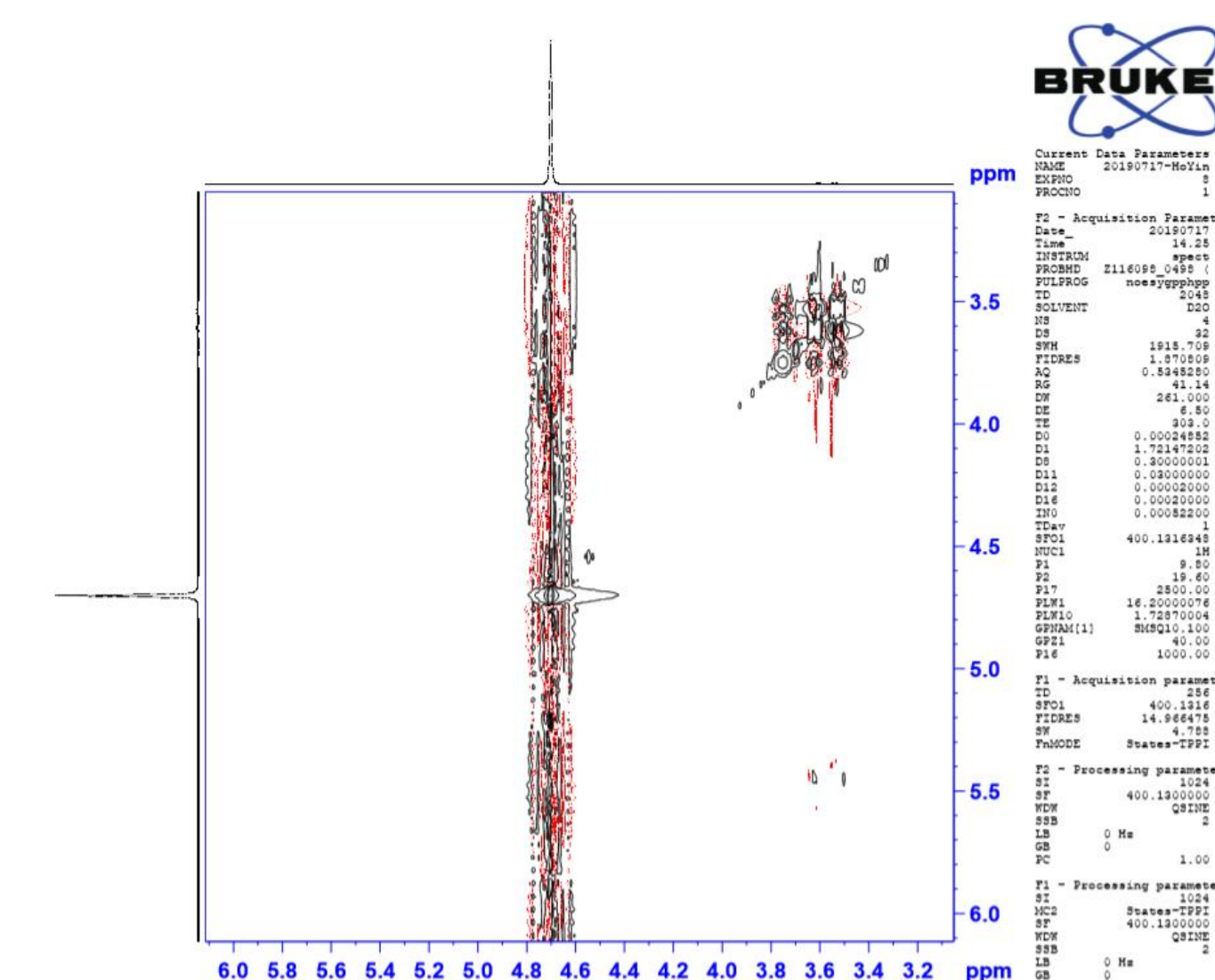
#### Pure TET1



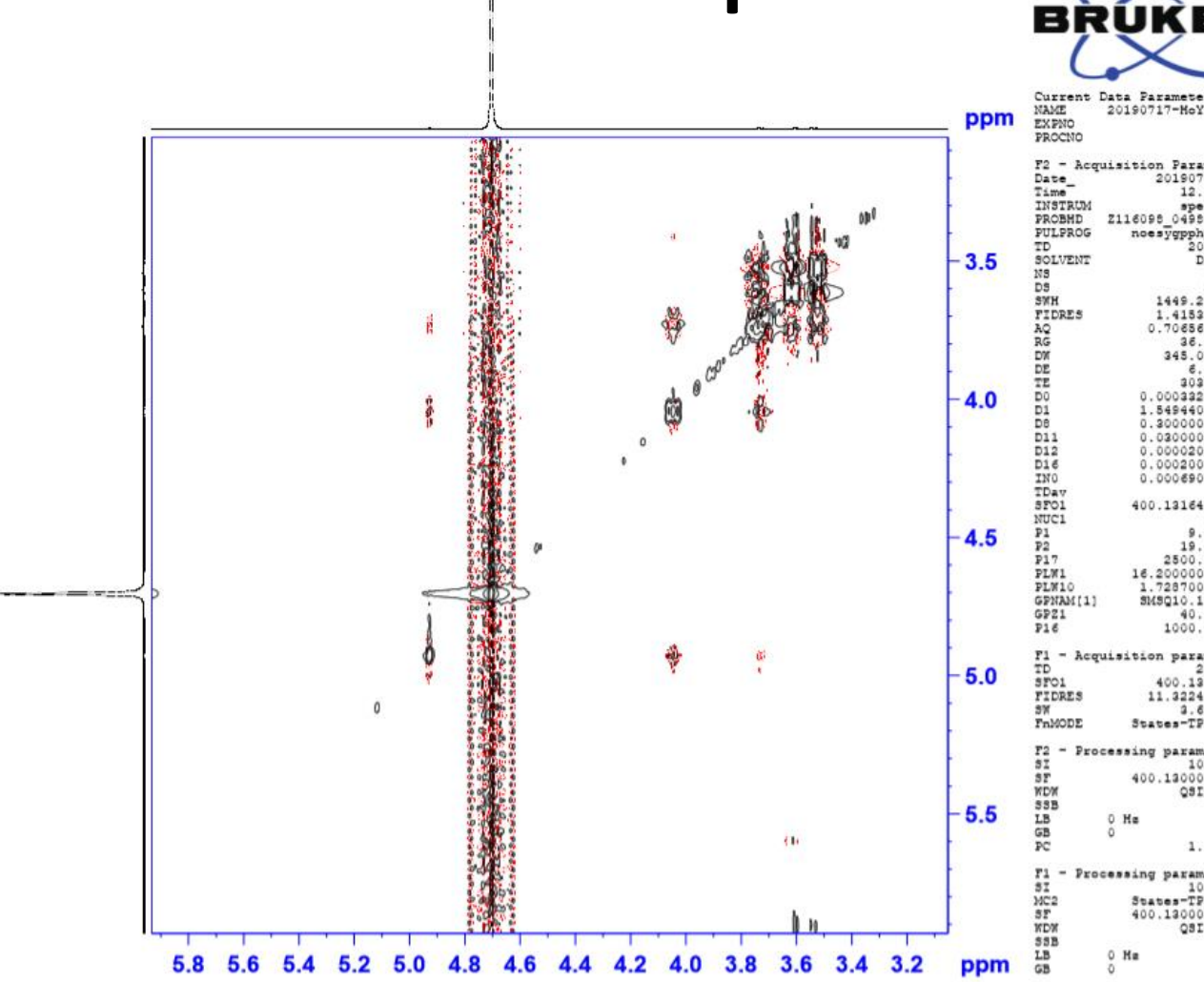
#### TET1 and Ascorbic Acid NMR Spectra



#### Pure TET3



#### TET3 and Ascorbic Acid NMR Spectra



### References and Acknowledgements

- Schirra, Joachim. *Two Dimensional NMR Spectroscopy*, 26 Nov. 1996
- UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics. "Methylcytosine Dioxygenase TET1." *UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics*, 31 July 2019
- UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics. "Methylcytosine Dioxygenase TET3." *UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics*, 31 July 2019
- Dr. Ho Yin Lo, Synovel Laboratory LLC
- Yale University West Campus, Chemical Biology Institute