Improving TIDES product risk assurance with NMR spectroscopy

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USP TIDES APRIL 2024

Agenda

01 Introduction

02 Peptides

03 Oligonucleotides

04 Conclusions

01 Introduction

We love NMR! Why??

Image courtesy of Marie – Helene Cullum (artist)

Source: © M-H Jeeves

- ✓ Primary quantification method (no response factor needed)
- ✓ Selectivity
- ✓ Resolution
- ✓ Structural information
- ✓ Diastereomers
- ✓ Non-destructive
- ✓ Online => real-time

01 Peptides

Therapeutic peptides ‘the sweet spot’ for NMR

High dispersion spectral data

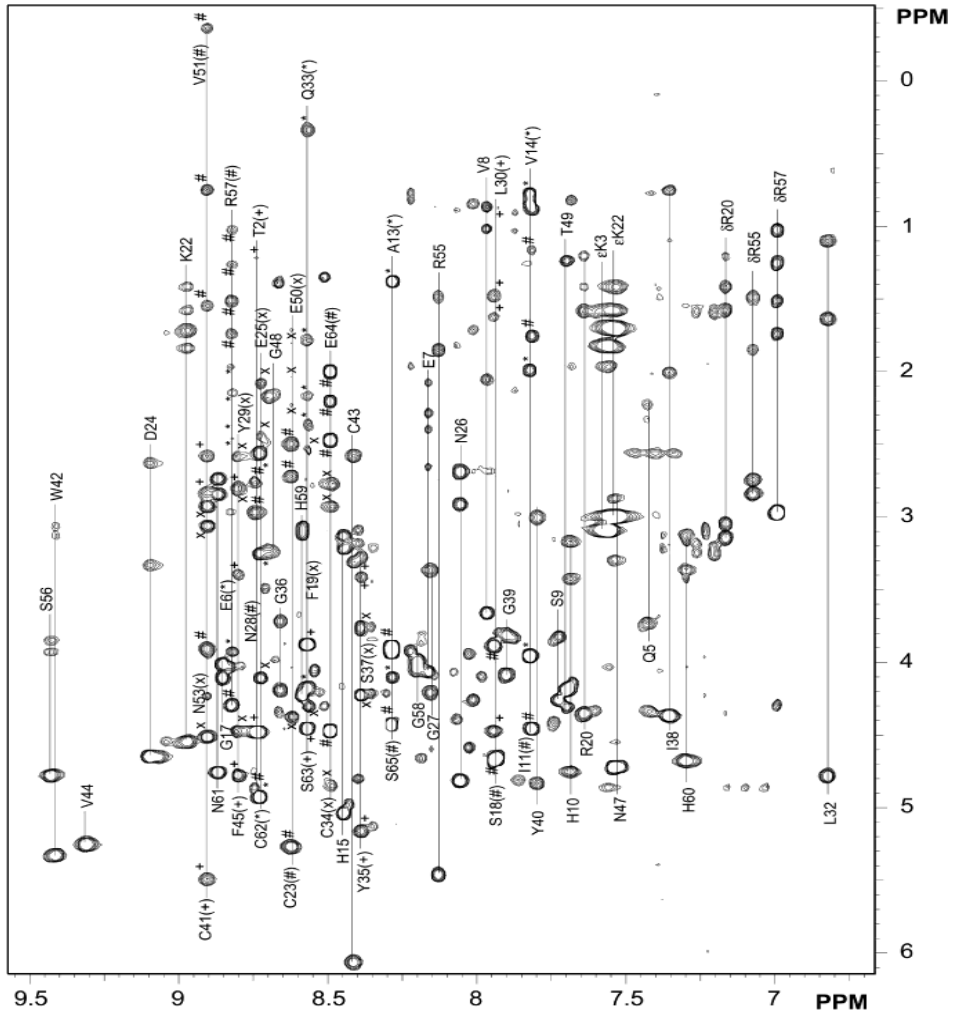
NMR applications

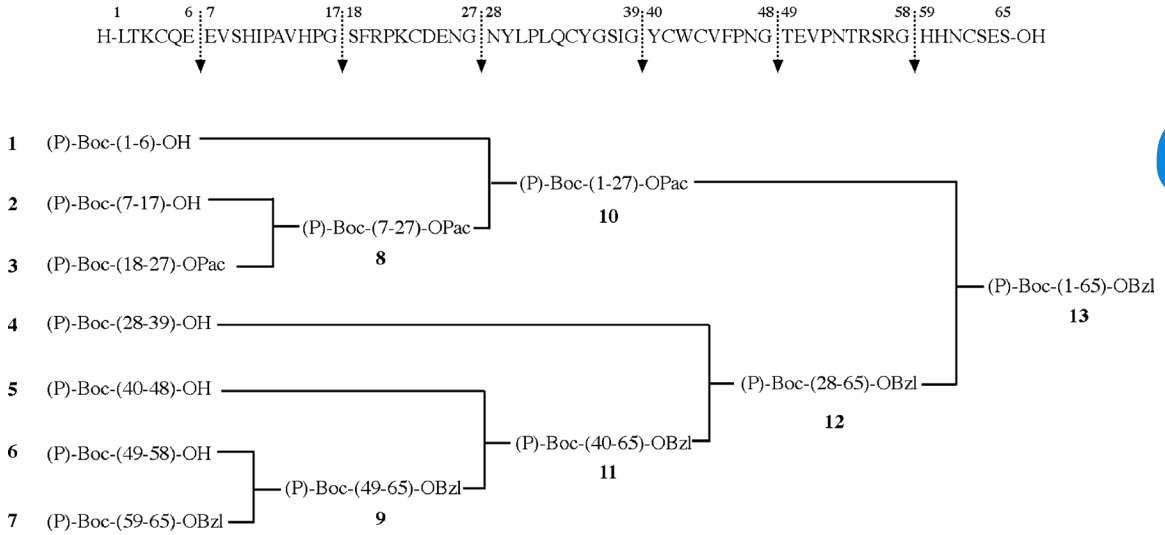
- Primary, secondary and tertiary structure [1, 2, 3]
- Dynamics
- Binding [1,2,3]
- Similarity assessment [4]
- Quantification (absolute and relative) [5]

1. *Chiva C, Barthe P, Codina A ... Sakakibara S, Albericio F, Giralt E, JACS, 2003;125:1508-1517*
2. *Codina A, Love JD, Li Y, Lazar MA, Neuhaus D, Schwabe JW, Proc Natl Acad Sci U S A. 2005;102(17):6009-6014.*
3. *Codina A, Benoit G, Gooch J T, Neuhaus D, Perlmann, Schwabe JWR, JBC, 2004:279, 53338*
4. *Haxhom GW, Bent O, Malmstrom J, J Pharm Sci, 2019;108: 3029 (2019)*
5. *Bradley SA, Jackson WC Jr, Mahoney PP, Anal Chem. 2019; 5,91(3):1962-1967*

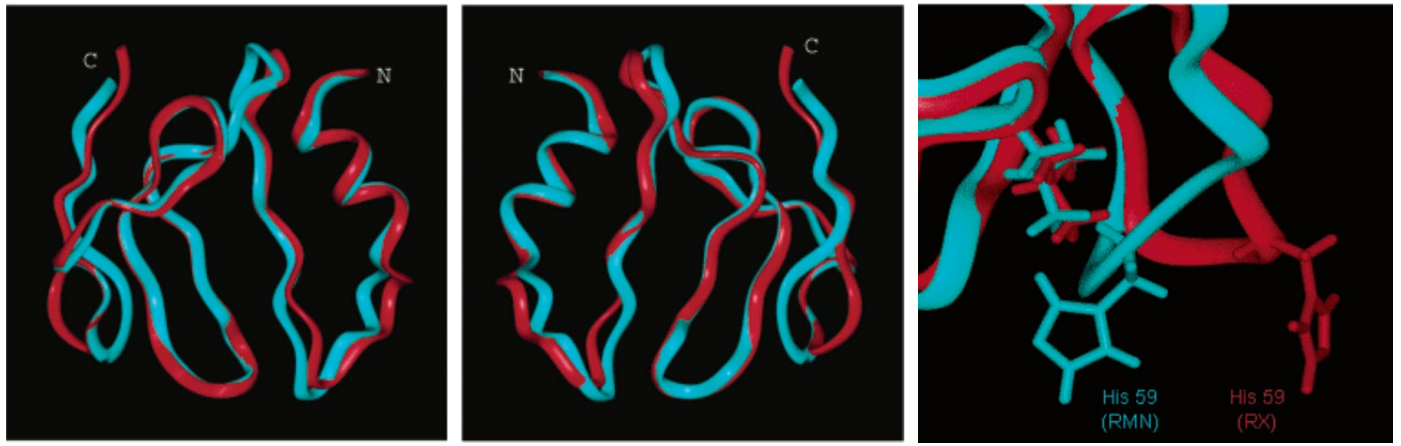
Total synthesis and structure determination of P41icf

a potent inhibitor of human cathepsin L





65 aa



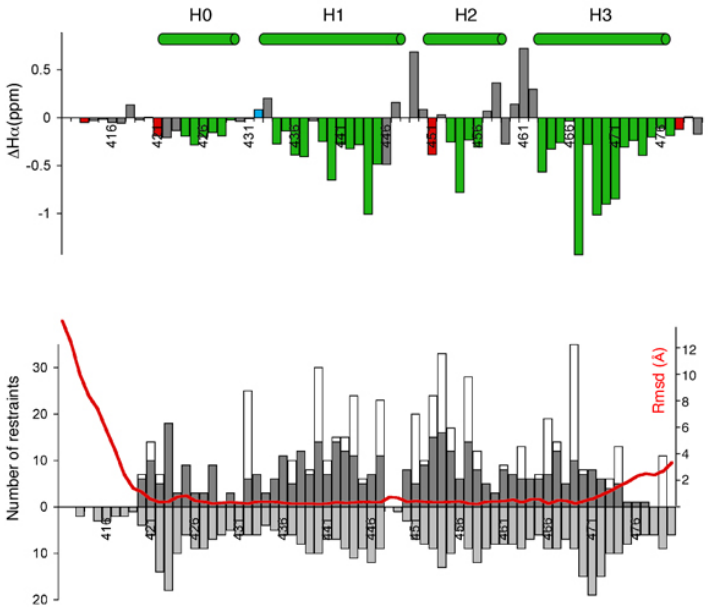
NMR solution structure (cyan) and X-ray structure (PDB code 1icf) of the protein complexed with cathepsin L (red).

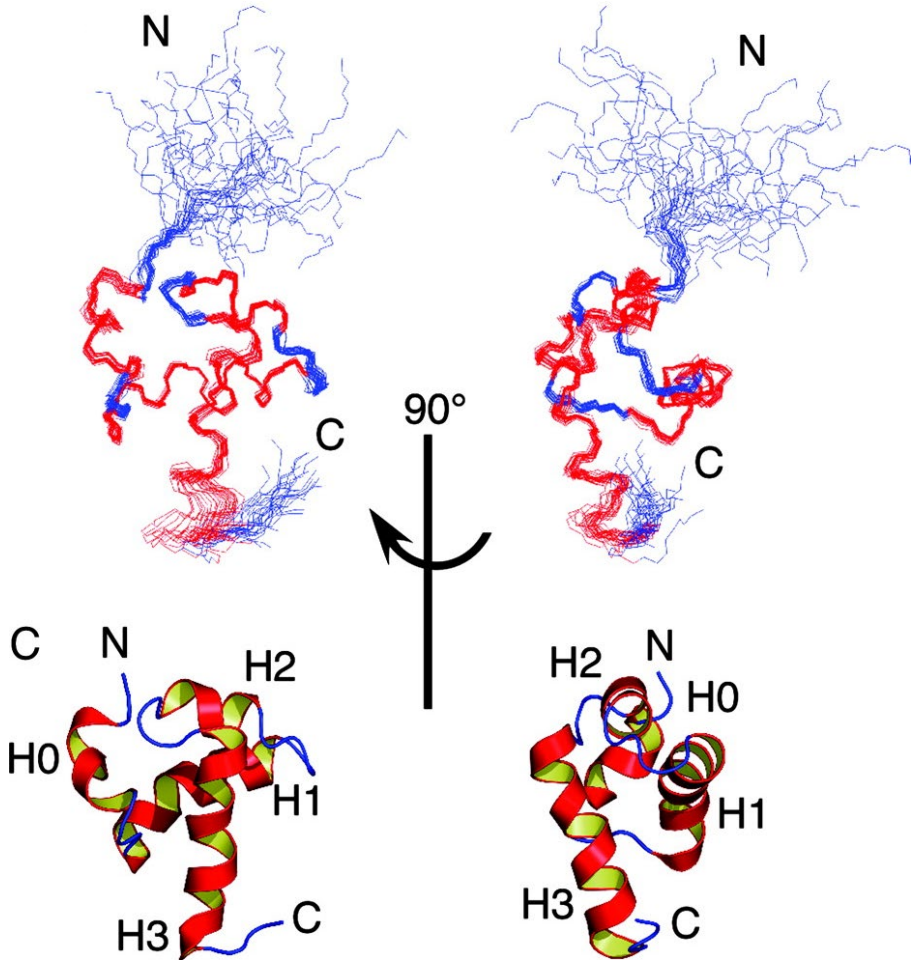
Chiva C, Barthe P, Codina A ... Sakakibara S, Albericio F, Giralt, E, 2003,125:1508-1517

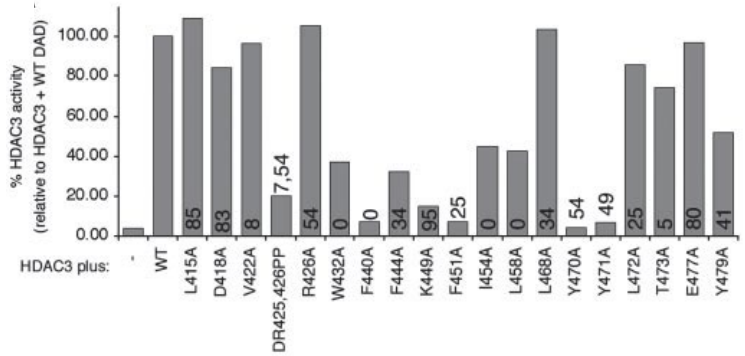
Secondary and tertiary structure determination

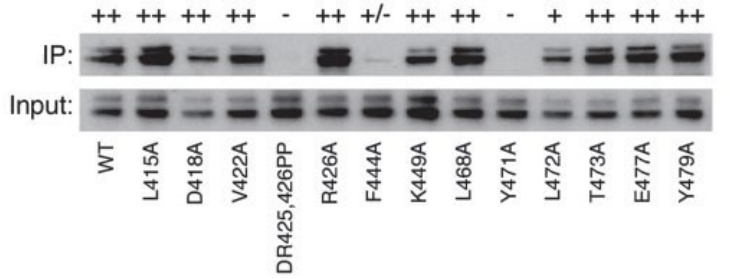
Interactions between between SMRT DAD and HDAC3

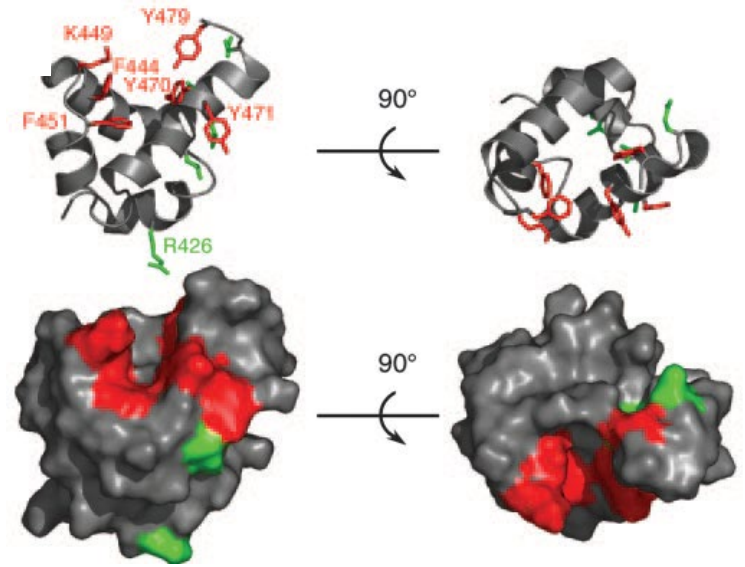
Structural insights into the interaction and activation of histone deacetylase 3 by nuclear receptor corepressors









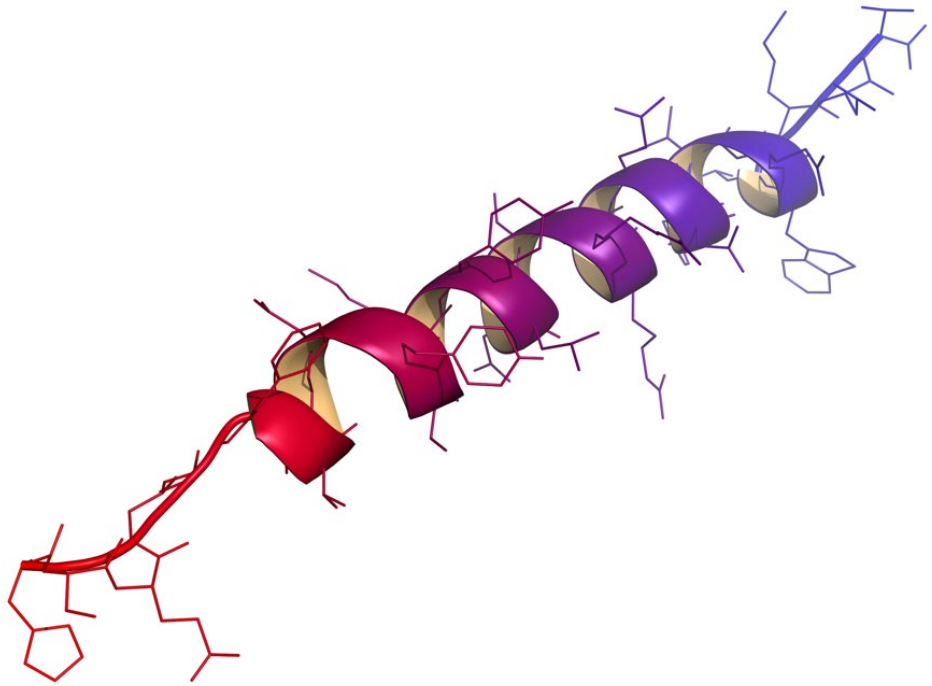


Codina A, Love JD, Li Y, Lazar MA, Neuhaus D, Schwabe JW. Proc Natl Acad Sci U S A. 2005;102(17):6009-6014.

01 Peptides example GLP-1

Glucagon maintenance of blood glucose in diabetic patients

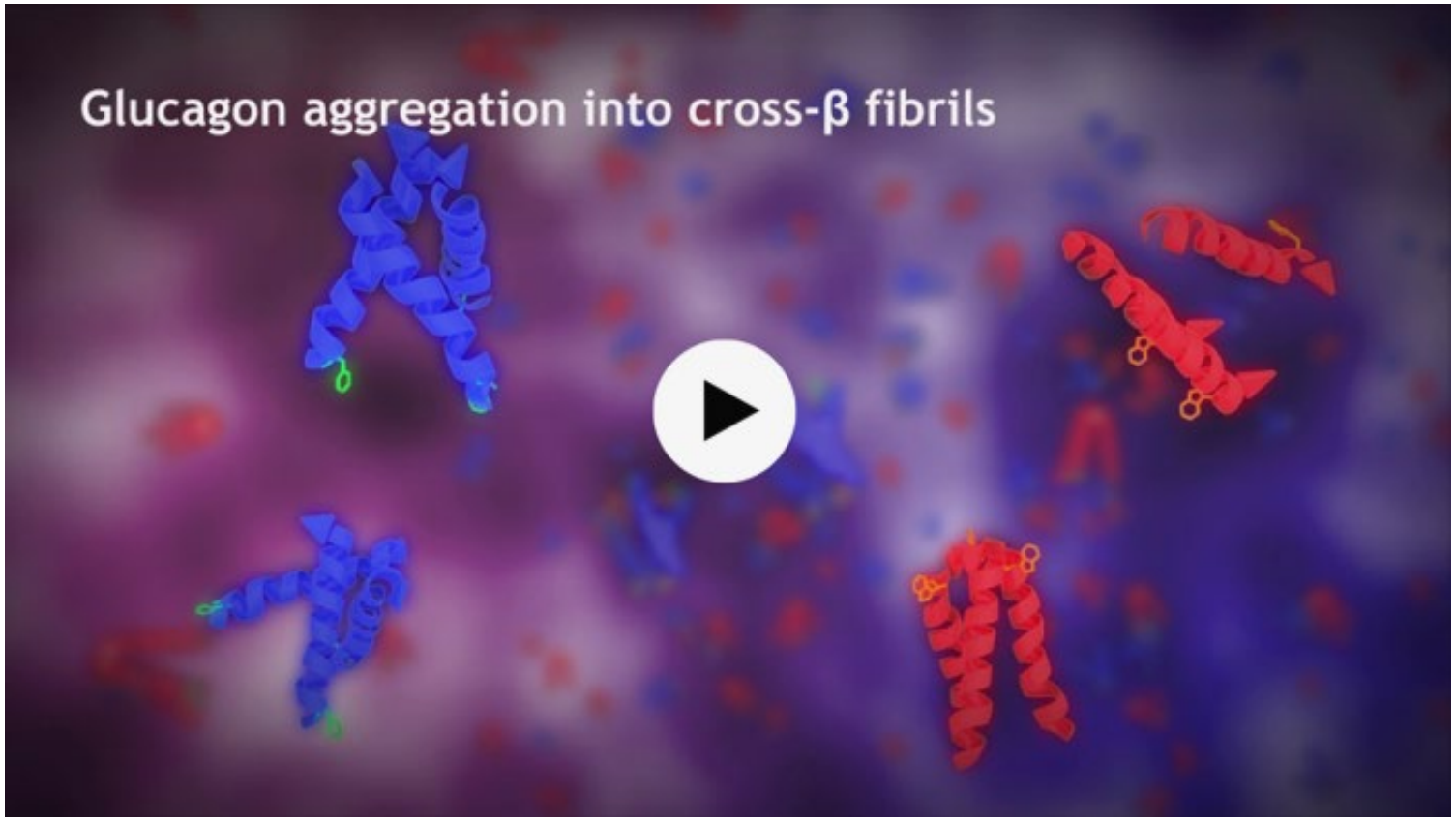
- Insulin hyperglycaemia, stable in solution
- Glucagon hypoglycaemia
- Glucagon fibrilizes rapidly at the acidic pH required for solubility => formulated as a lyophilized powder that is reconstituted in an acidic solution immediately before use



https://news.mit.edu/2019/structure-glucagon-fibrils-0624

Glucagon study by ssNMR leads to rational drug design (MSD & MIT)

- ssNMR determined of fibrils of synthetic human glucagon. It revealed an unexpected amyloid structure, formed by two antiparallel β-sheets. The glucagon gradual structural changes from intrinsically disordered to a-helix and from a-helix to β-sheets
- The study opens the path for the rational design of glucagon analogues that resist fibril formation and increase the therapeutic efficacy of the drug.



Yongchao Su et al., https://news.mit.edu/2019/structure-glucagon-fibrils-0624

Martin et al., Nature Structural & Molecular Biology, 26, 592 (2019)

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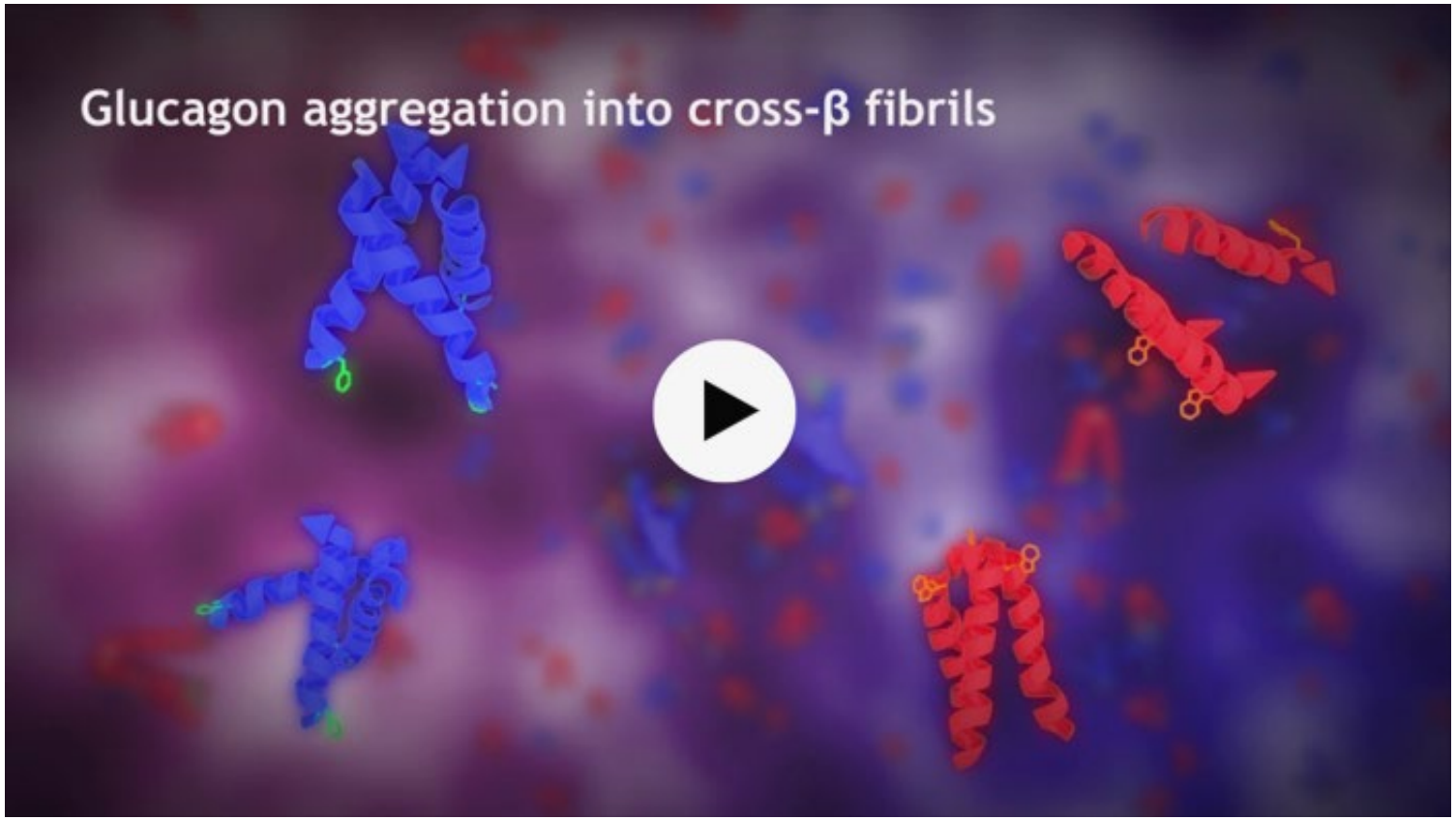


Yongchao Su et al., https://news.mit.edu/2019/structure-glucagon-fibrils-0624

Martin et al., Nature Structural & Molecular Biology, 26, 592 (2019)

Glucagon analogs 2D NMR fingerprinting

GLP-1 reference and isomers @ 600 MHz

J Pharm Sci. 2019 Sep;108(9):3029-3035. doi: 10.1016/j.xphs.2019.04.032. Epub 2019 May 10.

Higher-Order Structure Characterization of Pharmaceutical Proteins by 2D Nuclear Magnetic Resonance Methyl Fingerprinting

Gitte W Haxholm [1], Bent O Petersen [1], Joan Malmstrøm [2]

Affiliations + expand

PMID: 31082403 DOI: 10.1016/j.xphs.2019.04.032

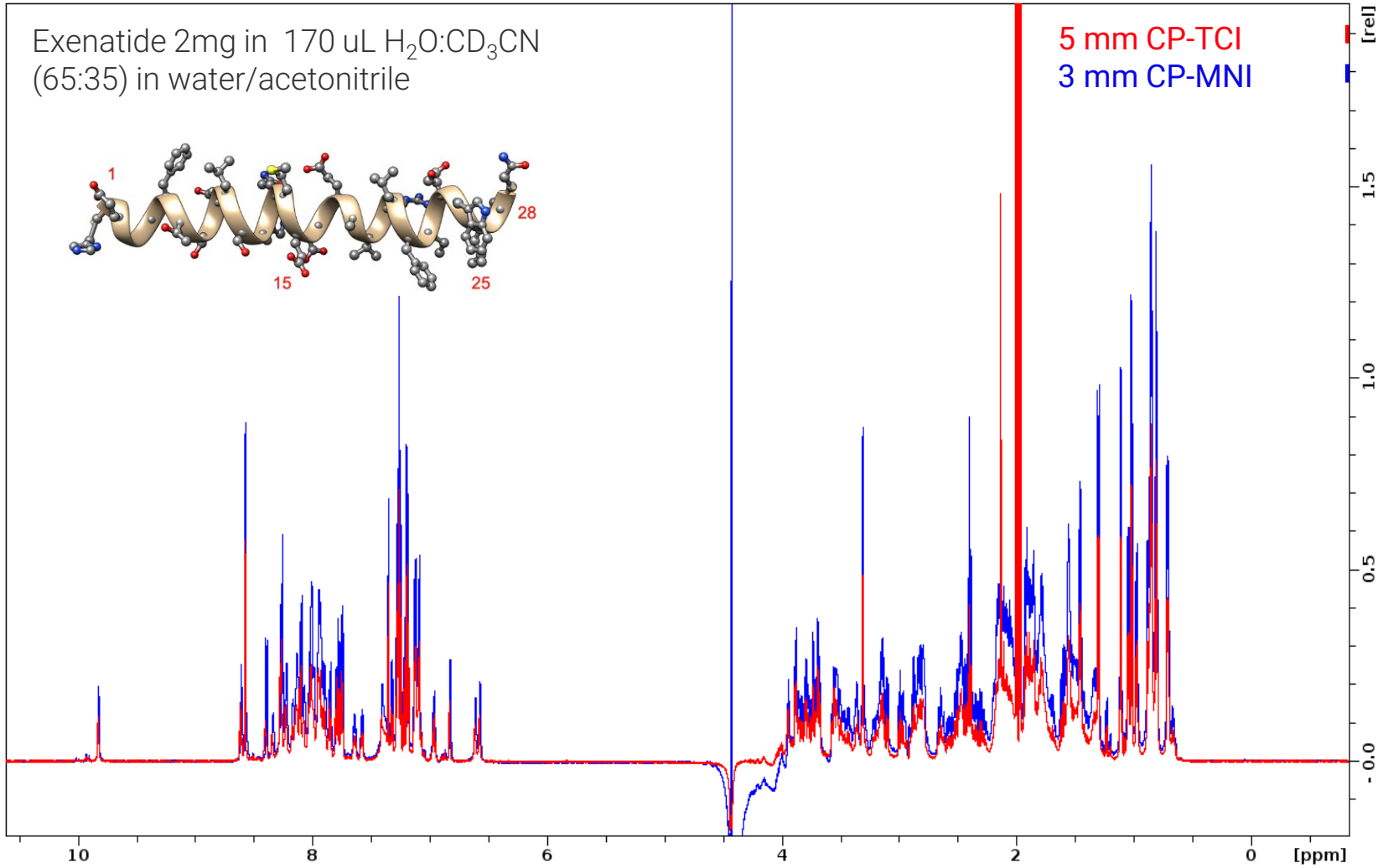
Abstract

A key challenge in the analytical assessment of therapeutic proteins is the comprehensive characterization of their higher-order structure (HOS). To directly assess HOS, a new type of assay is warranted. The most sensitive and detailed method for characterizing HOS is unquestionably nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy provides direct information about the HOS at an atomic level, and with modern NMR spectrometers and improved pulse sequences, this has become feasible even on unlabeled proteins. Hence, NMR spectroscopy could be a very powerful tool for control of HOS following, for example, process changes resulting in structural changes, oxidation, degradation, or chemical modifications. We present a method for characterizing the HOS of therapeutic proteins by monitoring their methyl groups using 2D H, C-correlated NMR. We use a statistical model that compares the NMR spectrum of a given sample to a reference and results in one output value describing how similar the HOS of the samples are. This makes the overall result easy to interpret even for non-NMR experts. We show that the method is applicable to proteins of varying size and complexity (here up to ~30 kDa) and that it is sufficiently sensitive for the detection of small changes in both primary and HOS.

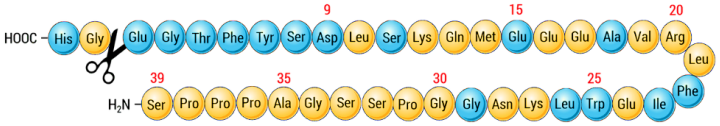
Keywords: GLP-1; biopharmaceutical characterization; biosimilar(s); insulin; nuclear magnetic resonance spectroscopy; physical characterization; protein folding; protein(s); spectroscopy; structure.

Haxhom GW, Bent O, Malmstrom J, J Pharm Sci, 108: 3029 (2019)

Solution NMR of glucagon-like-peptide Exenatide at 600 MHz



- Antidiabetic drug
- Injected subcutaneously
- 53% homology with human GLP-1
- Bind and activates GLP-1 receptor=>↑insulin↓glucagon, slows gastric emptying



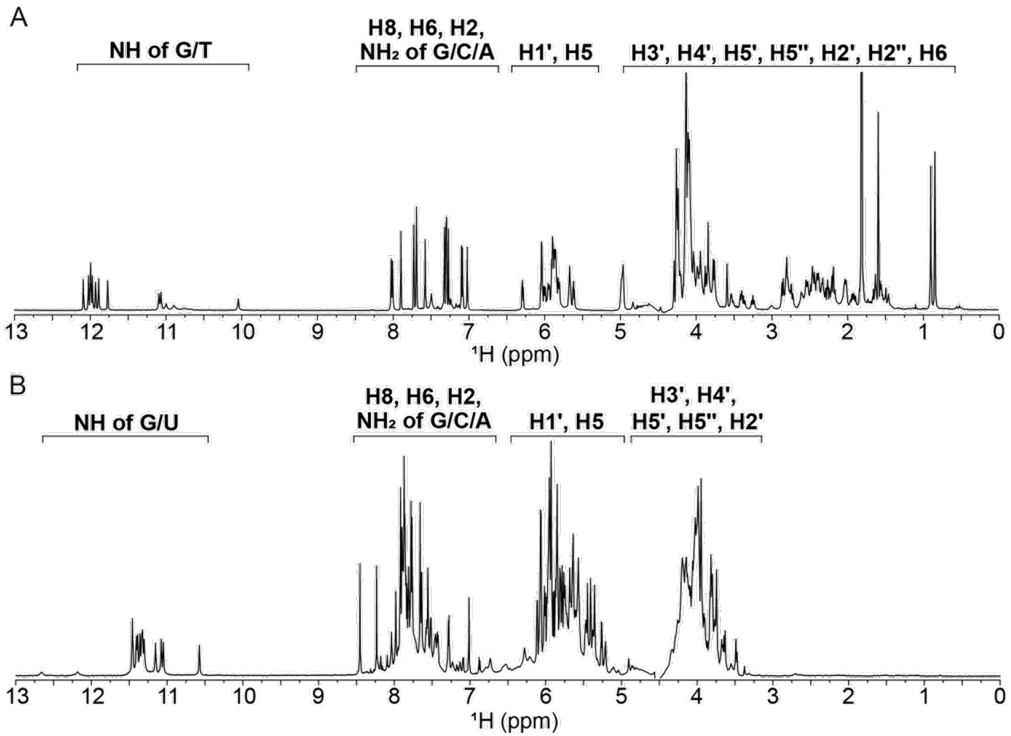
https://pdb101.rcsb.org/global-health/diabetes-mellitus/drugs/incretins/drug/exenatide/exenatide

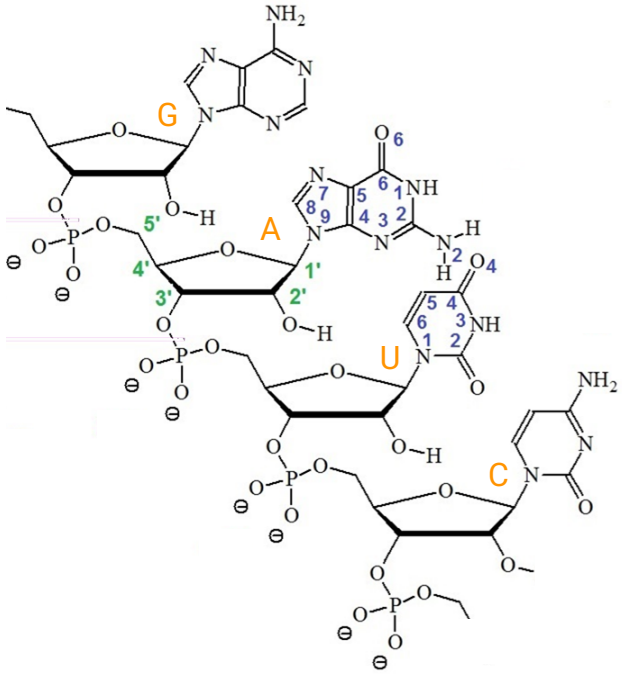
02 Oligonucleotides

NMR study of nucleic acids

- NMR has been used for many years to determine **structure** of nucleic acid, **dynamics** and **binding**
- Low proton density of nucleic => rapid detection and identification of **hydrogen bonds** => assessment of **folding** and **secondary structure**
- 3rd structure determination
- Unambiguously identify **hydrogen-bonding** in (non)Watson–Crick base pairs with imino to nitrogen hydrogen bonds
- Fingerprinting -> quality assessment
- Stereochemistry

^{1}H NMR at 800 MHz of 1 mM GGTTGGTGTGGTTGG (A)
CUCUGGGUCCGGGCUGGGUAUGGGAAC (B)



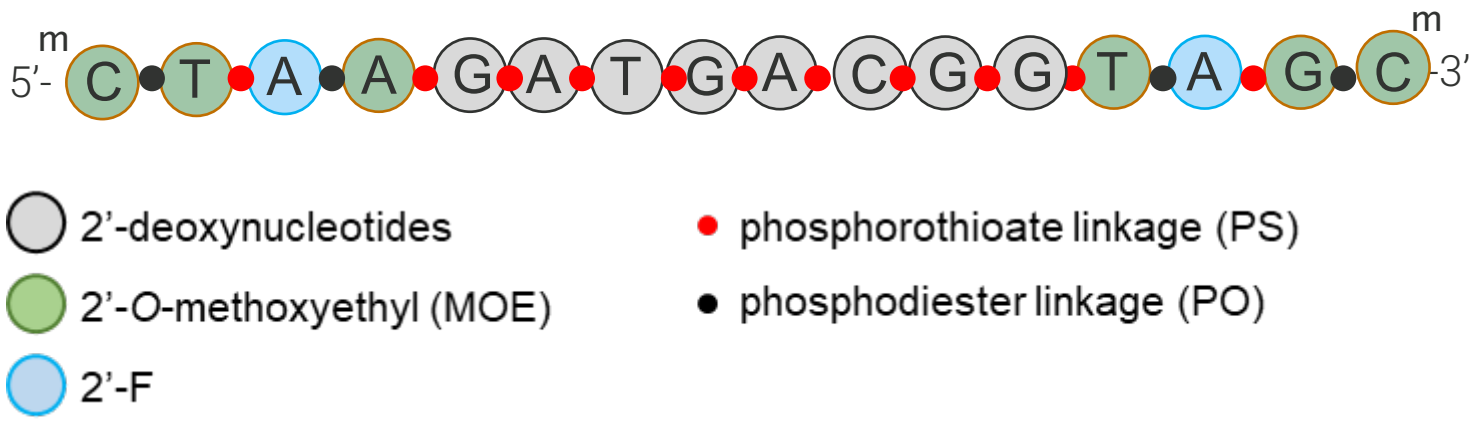


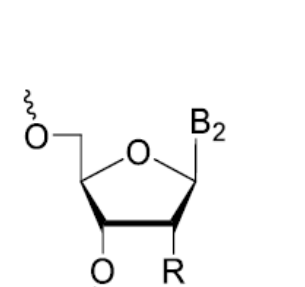
Plavec J. (2022). NMR Study on Nucleic Acids. In: Sugimoto, N. (eds) Handbook of Chemical Biology of Nucleic Acids. Springer, Singapore.

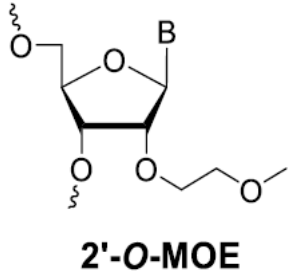
Model Therapeutic Oligonucleotide (16 chemically modified bases)

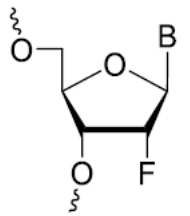
- 11x PS => 2^{11} = 2048 diastereoisomers
- 6 MOEs moieties
- 2 2'-F ribose
- 2 Met-C (^{m}C)
- 1C, 0U, 3T, 5A, 5G

- 144 nmol in 400 ul 25 mM sodium phosphate buffer in 100% D_2O (pH 7.1)
- 2x samples 200 uL (72 nmol, **0.4 mg, 0.36 mM**) in 3 mm tubes
- Natural abundance
- Temperature **50°C**

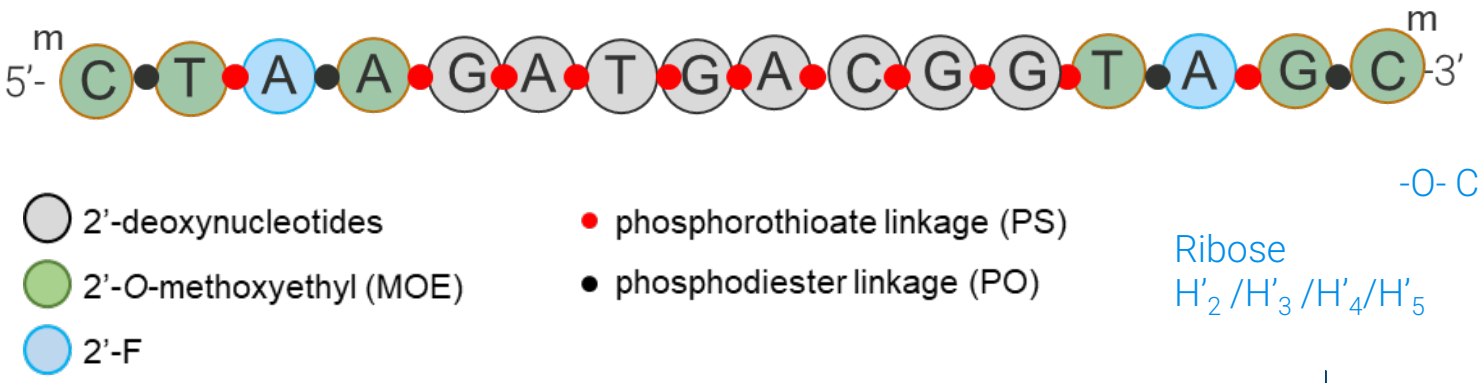








Model Therapeutic Oligonucleotide at 1 GHz



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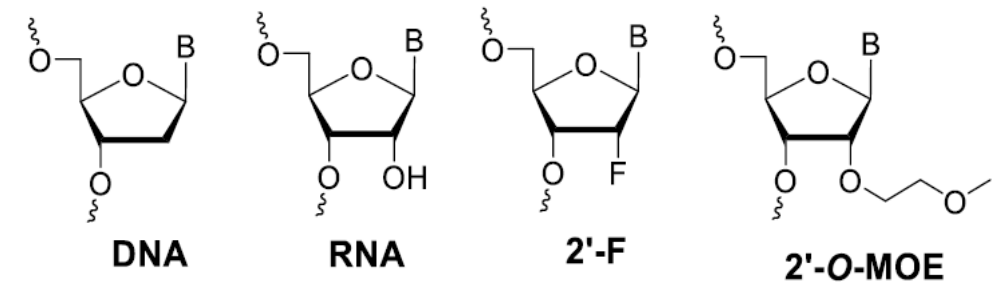
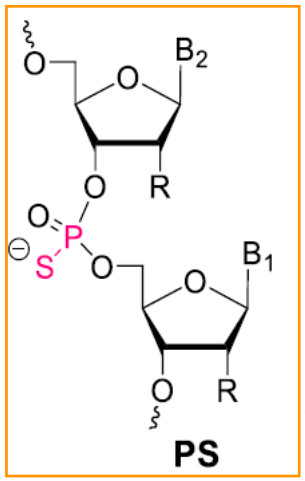
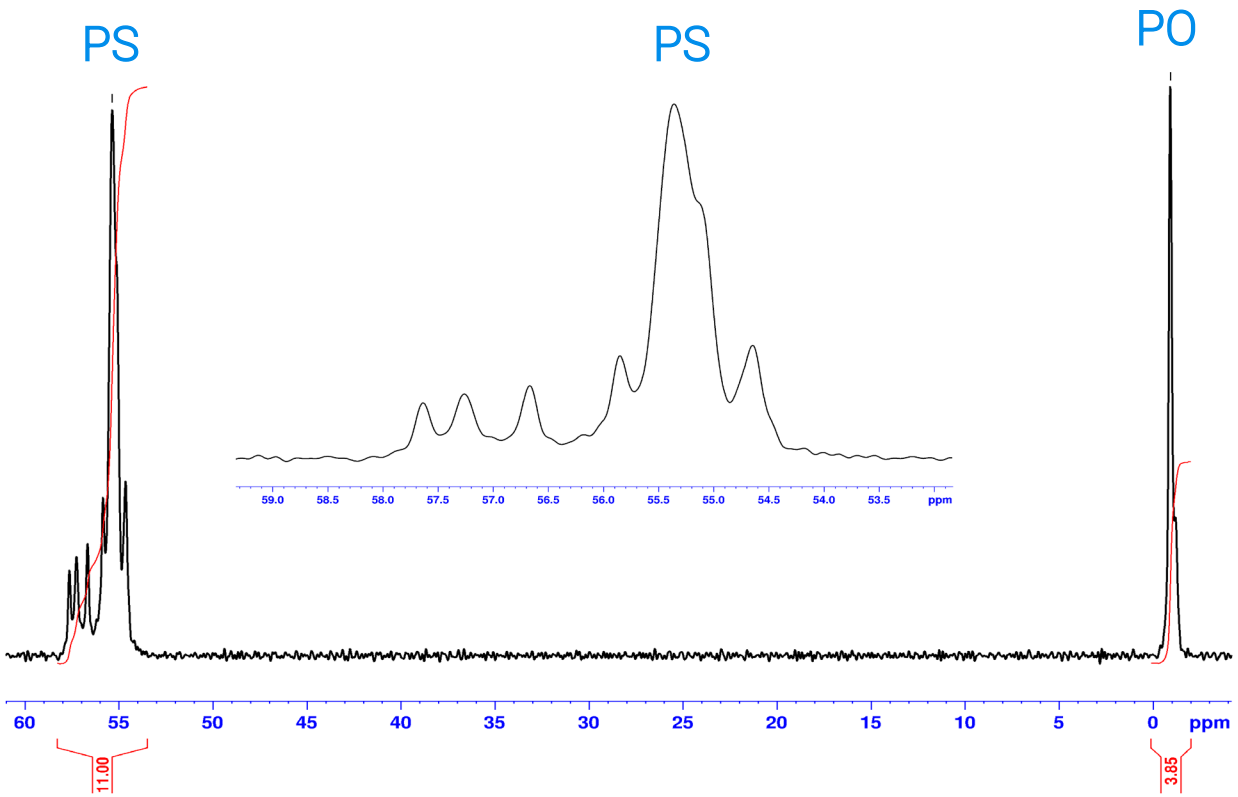




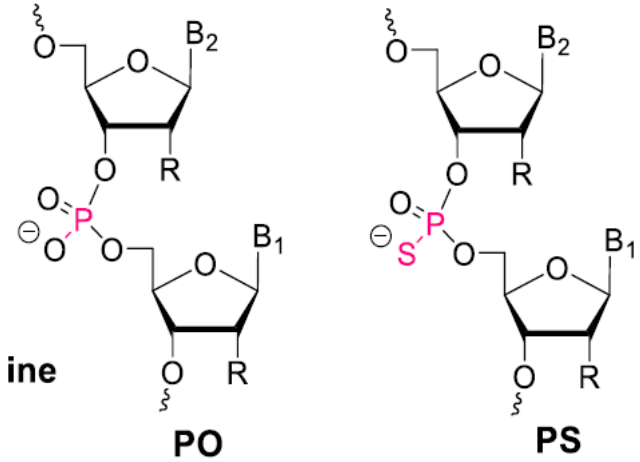
Model Therapeutic Oligonucleotide at 600 MHz

1D ^{31}P Multi Nuclei Inverse (MNI) probe



Determination of PS/PO ratio by integration

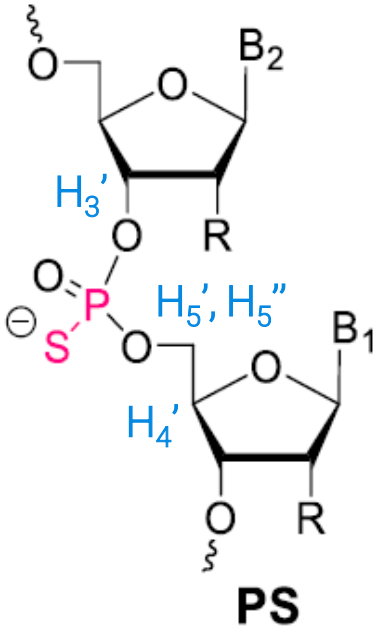
11/3.85 vs Theoretical 11/4

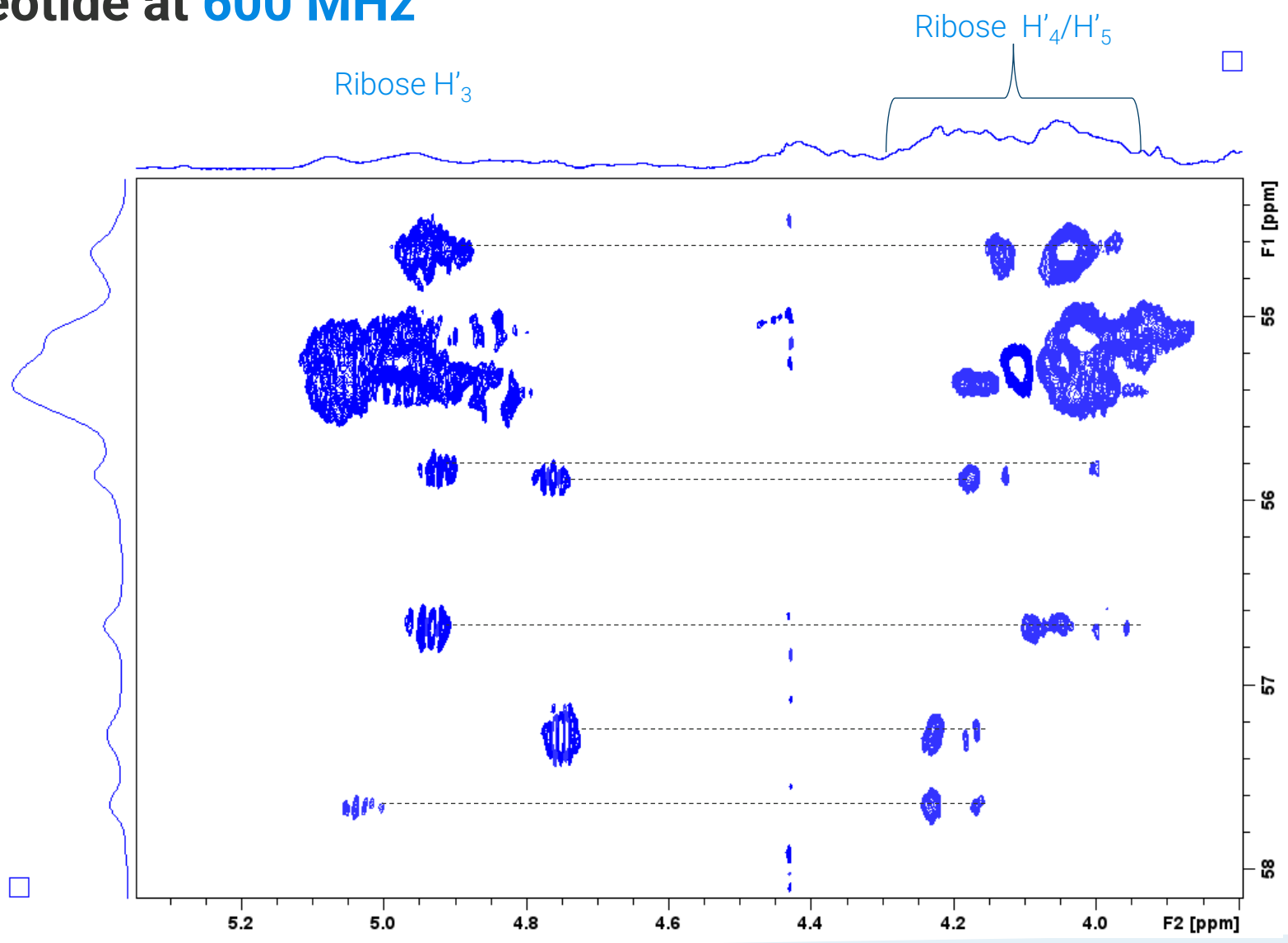


Model Therapeutic Oligonucleotide at 600 MHz

2D ^{1}H-^{31}P correlation MNI probe Fingerprint PS region

- Distinctive pattern for ^{31}P to H_3' / H_4' / H_5' correlations
- Accurate fingerprint
- Sequential assignment partially possible



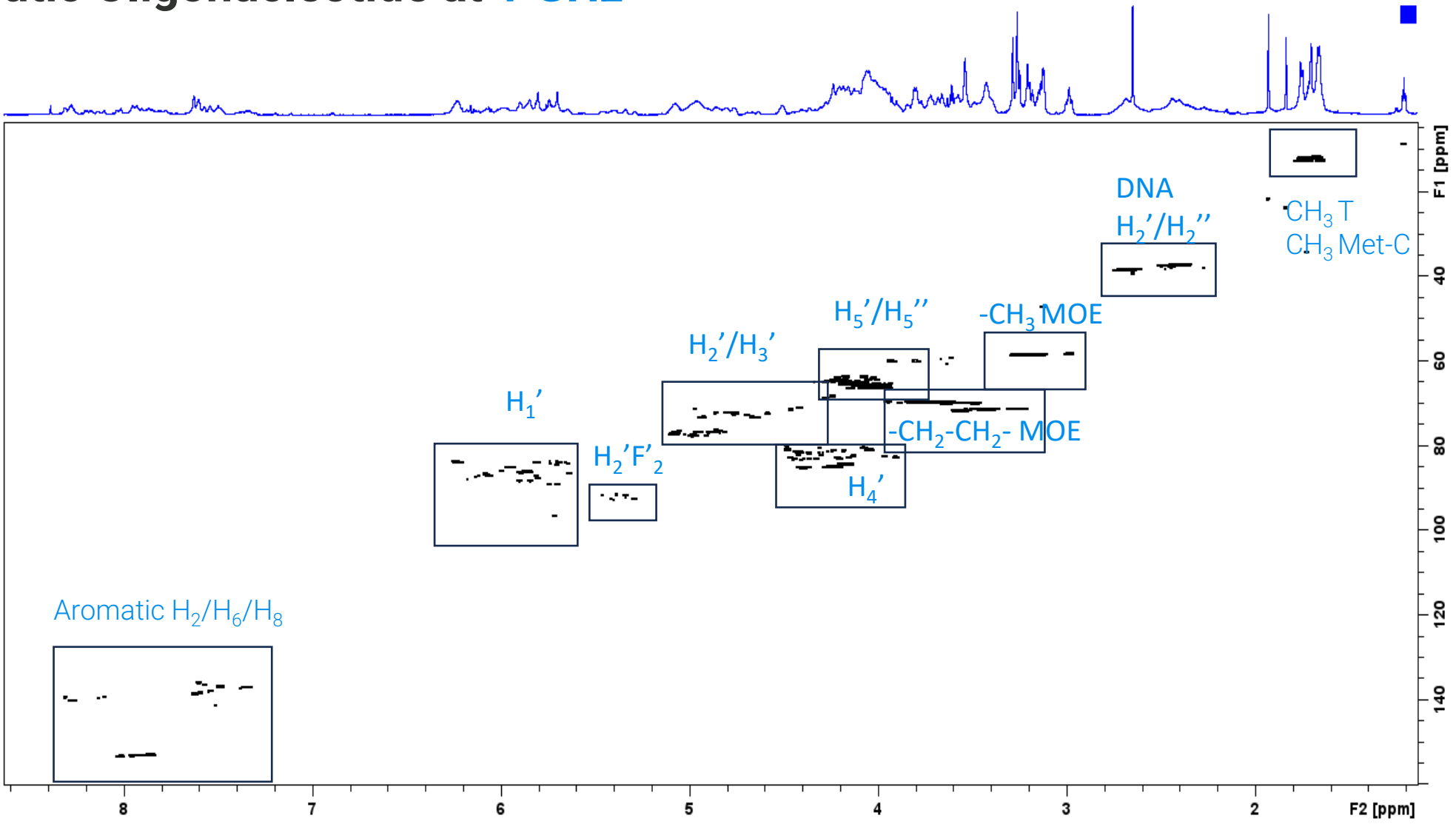


Model Therapeutic Oligonucleotide at 1 GHz

2D $^{1}H/^{13}C$ HSQC

Distinctive pattern for different regions of the oligonucleotide

Accurate fingerprint

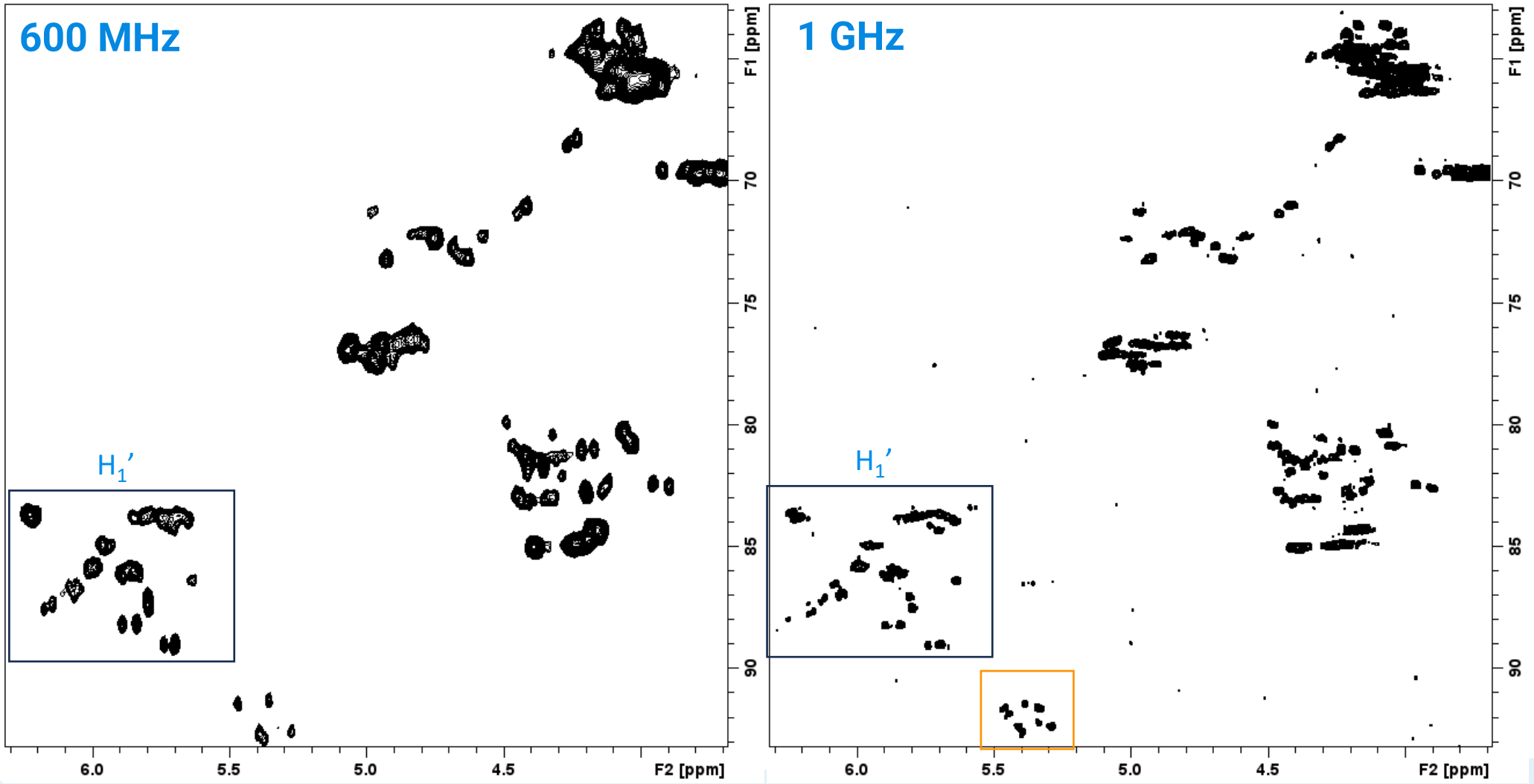


Model Therapeutic Oligonucleotide 600 MHz vs 1 GHz

2D $^{1}H/^{13}C$ HSQC

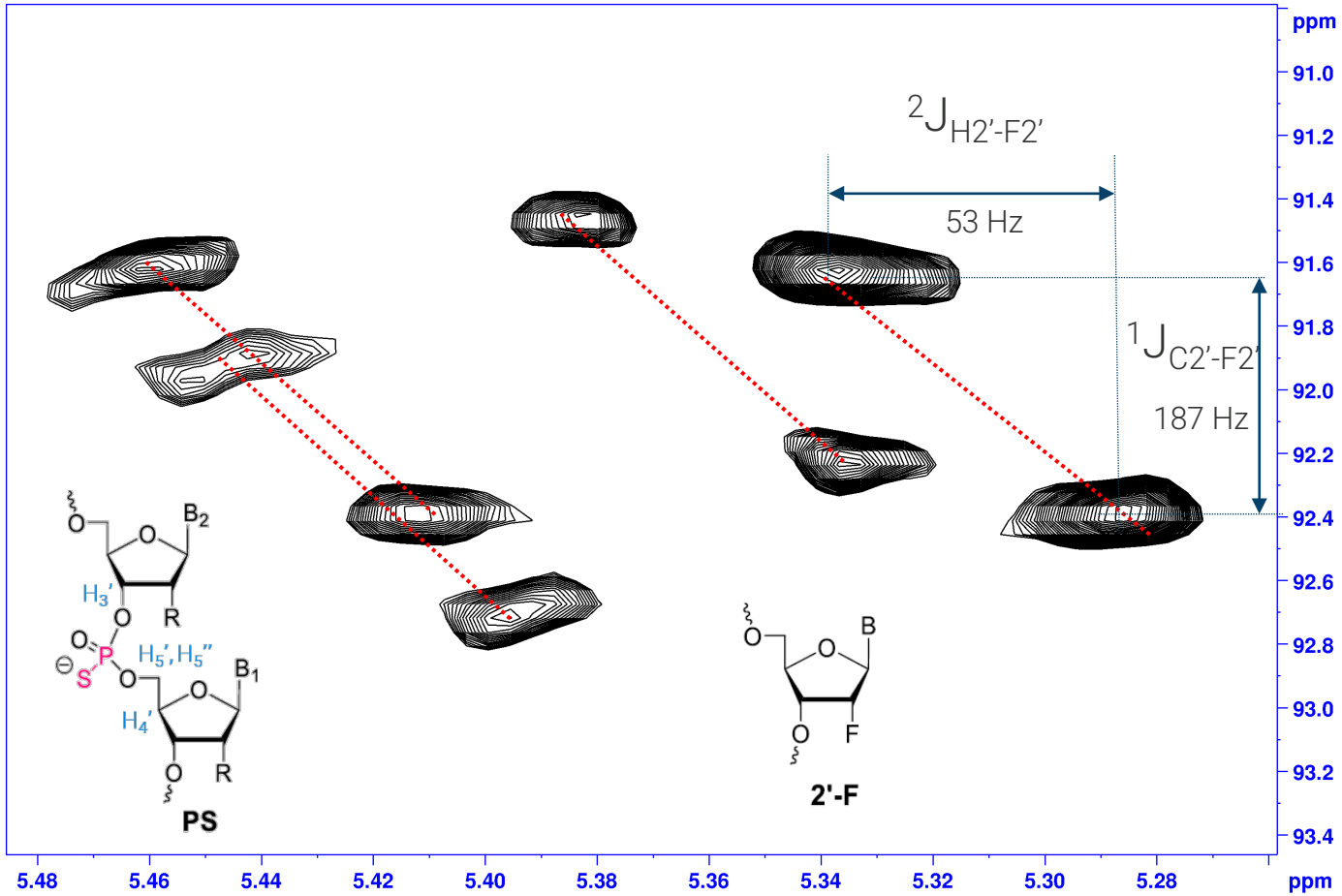
Significantly higher resolution obtained at 1 GHz

More accurate fingerprint



Model Therapeutic Oligonucleotide at 1 GHz

2D $^{1}H/^{13}C$ HSQC - Fluororibose region

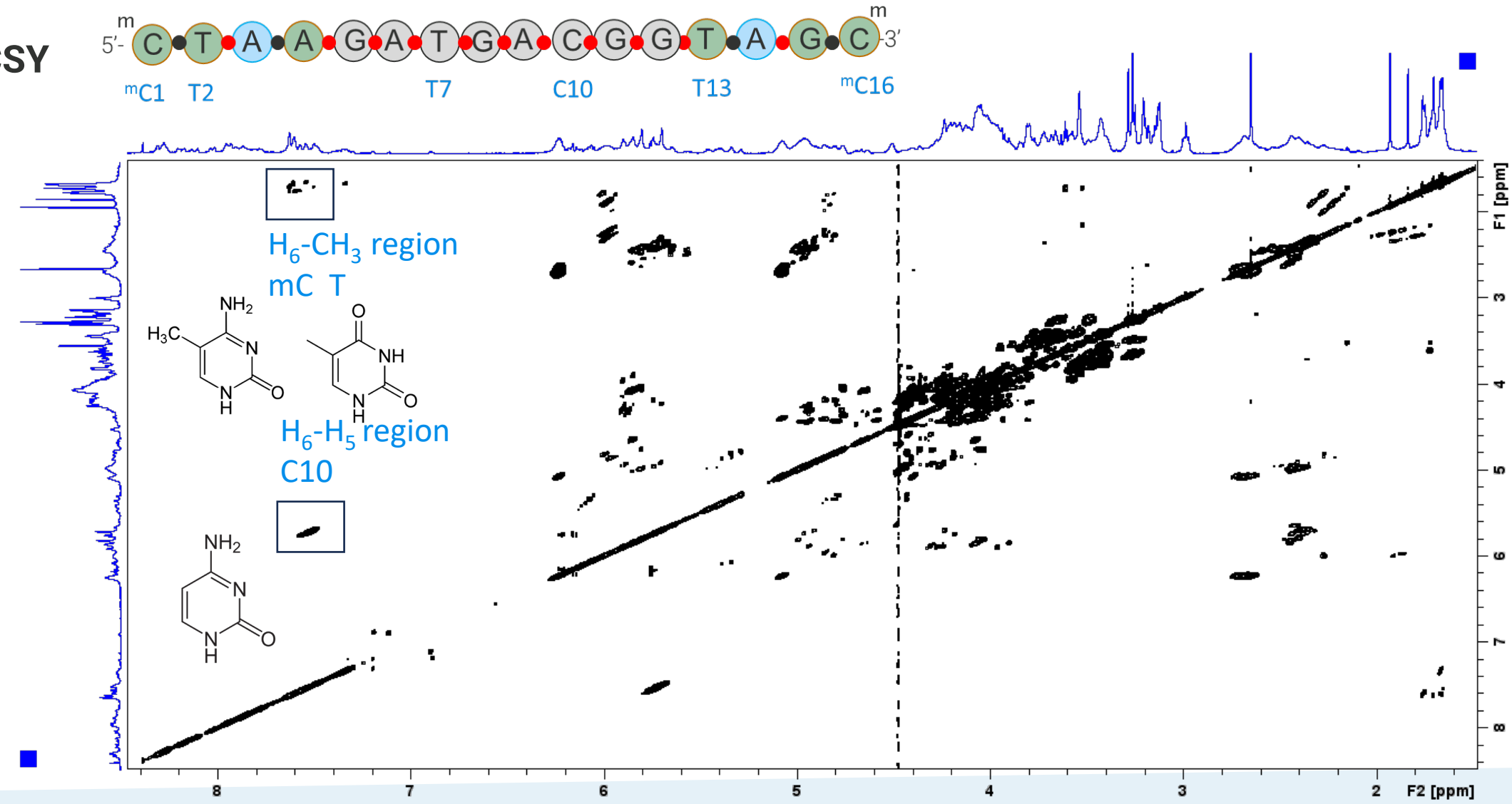


- 2 x 2'-Fluororibose rings present, but 4 distinct ^{19}F signals detected
- 2 x species detected at 50C
- PS group located next to the 2'-Fluoro ribose ring, one in the 3' position and the other in the 5' position
- The presence of R/S isomers gives rise to two signals for each H2' or F2' nucleus.
- Signals are well separated when PS is at the 3' position and less well separated when PS is at the 5´ position

Model Therapeutic Oligonucleotide at 1 GHz

2D ^{1}H-^{1}H TOCSY

Different lineshapes observed for some cross-peaks



Model Therapeutic Oligonucleotide at 1 GHz

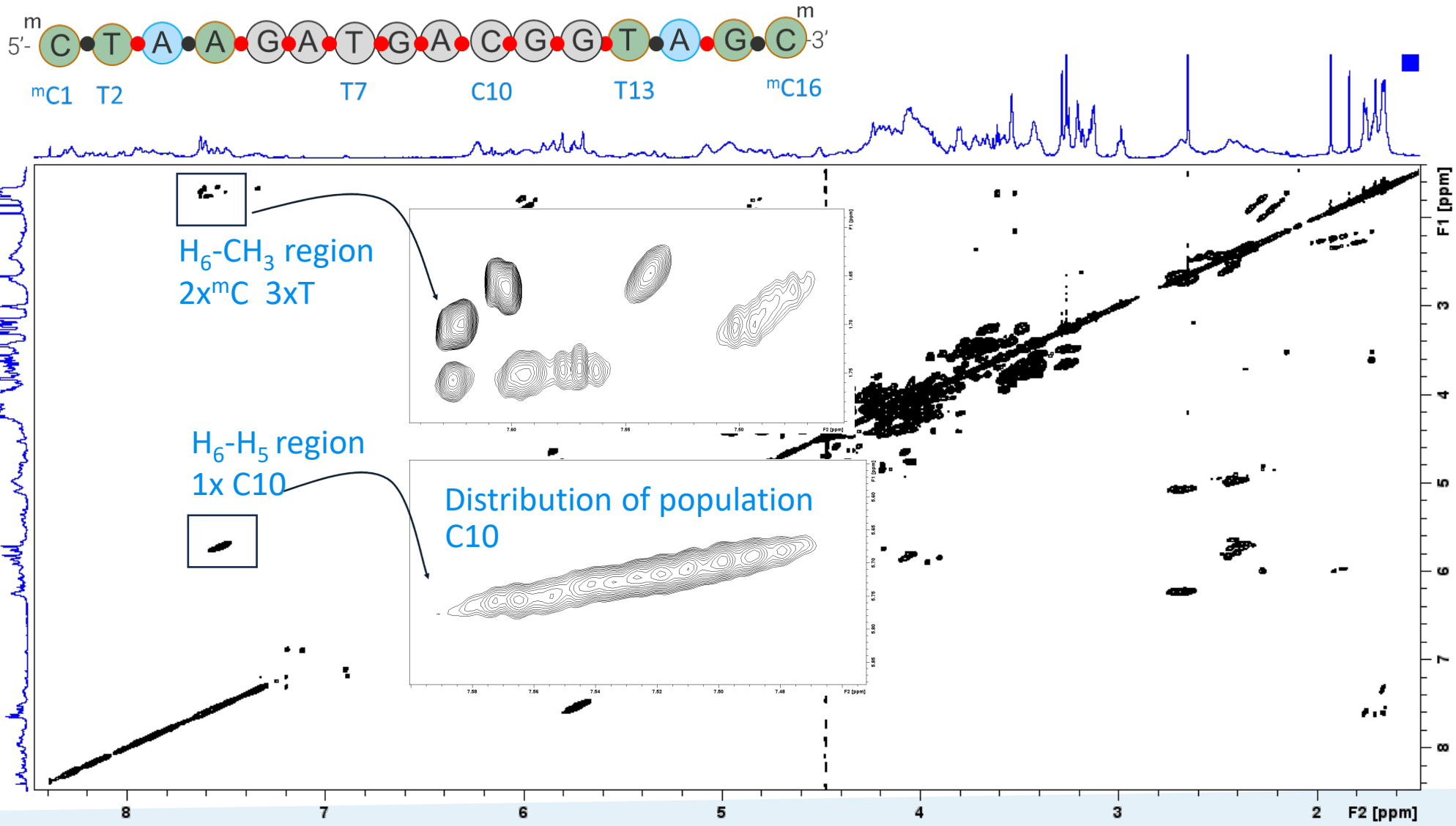
2D 1H-1H TOCSY

Distribution of diastereoisomers observed for C10

Methyl groups show a more homogeneous behavior but 5 peaks expected

and 8 or 9 signals observed

C10 experiences more strongly the effect of the PS diastereoisomers



04 Conclusions

Conclusions

- NMR is well established in industry for **traditional small molecules** medicinal and analytical chemistry support
- NMR is also well established in academia for **structural biology**, including structure characterisation of peptides and oligonucleotics
- Being a primary quantification, highly selective and with high resolution, makes it very powerful for the study of **therapeutic peptides and oligonucleotides**
- Example shown of **GLP-1 peptides** and how NMR data aids the rational design of more effective analogues
- Example shown of **therapeutic oligonucleotide model**: Identification of all chemical groups at 1 GHz, Accurate fingerprinting of different regions using different nuclei (^{1}H, ^{13}C, ^{31}P, ^{19}F, ^{15}N), partial assignment possible,
- Objective is to enable the development of **methodology** that **improves TIDES product risk assurance**, according to the **regulatory guidance**

The Barcelona team

- Miquel Pons
- Montserrat Terrazas
- Teresa Gonzalez
- Margarida Gairi

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GOBIERNO DE ESPAÑA
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Infraestructuras Científicas y Técnicas Singulares
R-LRB Red de Laboratorios de RMN de Biomoléculas
CCiTUB Centres Científics i Tecnològics UNIVERSITAT DE BARCELONA
UNIVERSITAT DE BARCELONA

Thank you!

Martial Piotto, Margarida Gairi, Teresa Gonzalez, Maksim Mayzel, Montserrat Terrazas, Miquel Pons

WEBINAR
ON DEMAND

OLIGONUCLEOTIDE-BASED THERAPETUCIS: NMR THECHNOLOGIES FOR CHARACTERISATION

CHI CELESTINE
AstraZeneca
THE VITAL ROLE OF NMR IN ANALYZING NUCLEIC ACIDS' STRUCTURE AND DYNAMICS FOR SMALL MOLECULE DRUG DISCOVERY ADVANCEMENTS

ROBERT BRINSON
IBBR, NIST
MULTI- ATTRIBUTE ASSESSMENT OF ANTI-SENSE THERAPEUTICS

MARTIAL PIOTTO
Bruker BioSpin
NMR OF RNA AND THERAPEUTIC OLIGONUCLEOTIDES

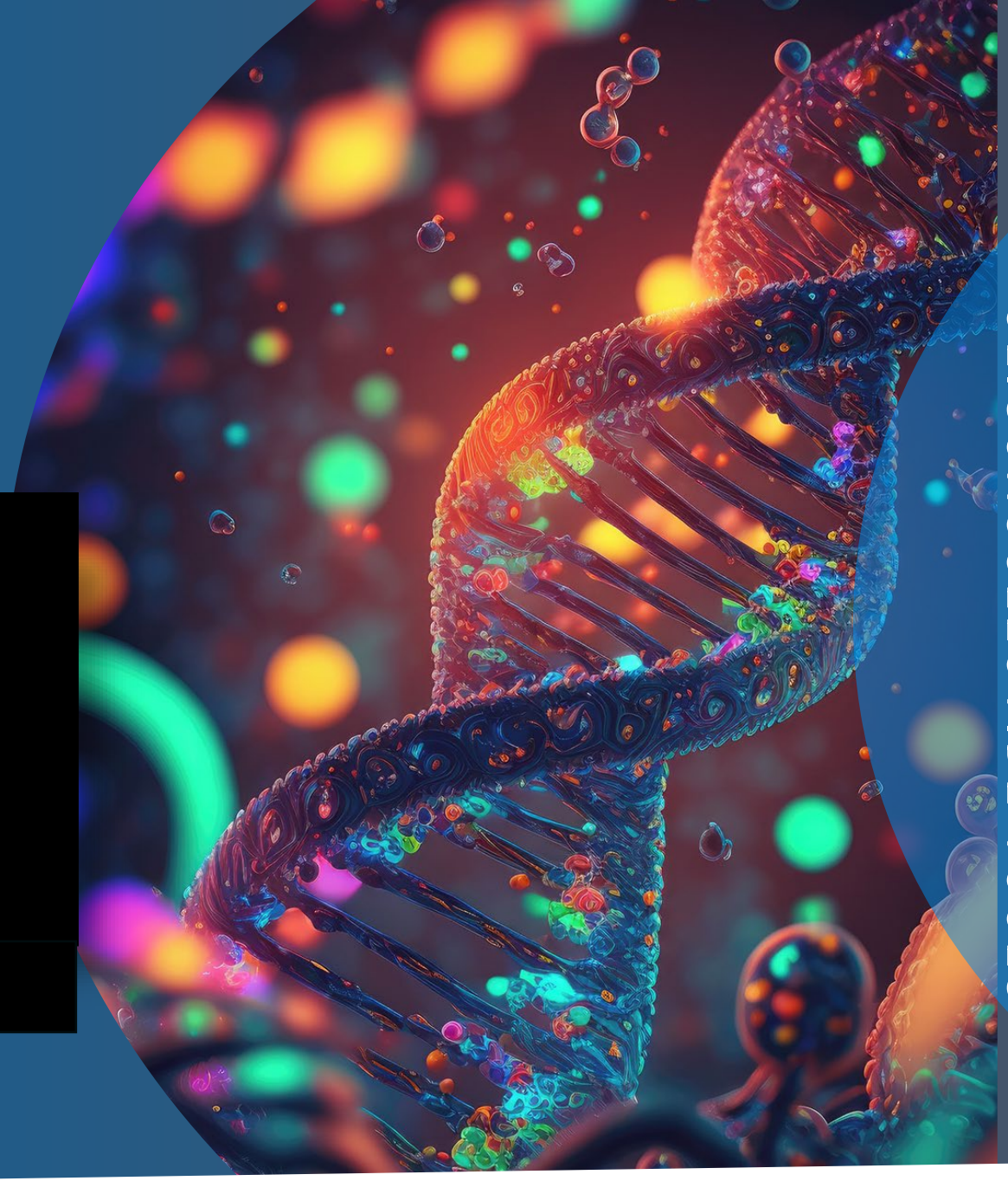
IGOR DIKIY
Regeneron Pharmaceuticals
MEASURING BASE-PAIRING BY NMR: APPLICATION TO MODEL SHORT DNAS

https://www.bruker.com/en/news-and-events/webinars/2024/oligonucleotide-based-therapeutics-nmr-techniques-for-characterization.html

WEBINAR
ON DEMAND

Montserrat Terrazas,
Uni. Barcelona

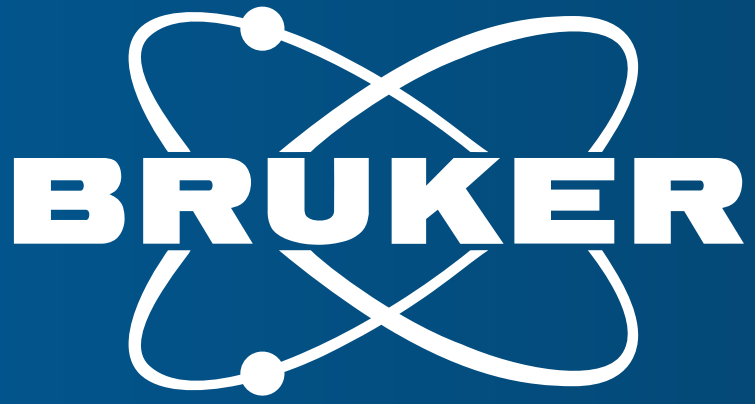
Martial Piotto,
Bruker BioSpin



BRUKER

RATIONAL DESIGN OF A NEW GENERATION OF THERAPEUTIC OLIGONUCLEOTIDE TOOLS

https://www.bruker.com/webinars



Innovation with Integrity