REUNION DES UTILISATEURS RMN BRUKER

From biologics characterisation to bioproduction analytics

Martial Piotto, Ph.D.

28 November 2023

Outlook

01 **Peptides**
Therapeutic peptides: where are we today?

02 **Oligonucleotides**
Therapeutic oligonucleotides – where can we help?

03 **Antibodies**
NMR analysis of biotherapeutics

04 **Bioproduction**
Understanding and Monitoring

05 **PIPAc** project
Intelligent production of Active Pharmaceutical Ingredient (API)

01 Peptides

Therapeutic peptides, the ‘sweet spot’ of NMR

High dispersion spectral data

NMR applications

- 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*

FDA encourages the use of state-of-the art technology

Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations

Guidance for Industry

DRAFT GUIDANCE

Despite improvements in analytical techniques, current analytical methodology may not be able to detect or characterize all relevant structural and functional differences between the two protein products. A thorough understanding of each analytical method's limitations will be critical to a sponsor's successful identification of residual uncertainties and, in turn, to the design of subsequent testing. In addition, there may be incomplete understanding of the relationship between a product's structural attributes and its clinical performance. FDA encourages the use of available state-of-the-art technology. Sponsors should use appropriate analytical methodologies that have adequate sensitivity and specificity to detect and characterize differences between the proposed product and the reference product.

https://www.fda.gov/media/125484/download

*https://www.casss.org/docs/default-source/hos/2021-hos-speaker-presentations/speaker-presentation-**chen-kang-cder-fda**-2021-.pdf?sfvrsn=8f7418ed_3*

- What we don't measure or control may lead to unintended consequences such as loss of potency or efficacy. Or even pathological action.
- High resolution/high sensitivity data => improved product risk assurance (product knowledge)
- MS and NMR are considered high sensitivity and high-resolution state-of-the-art (modern) techniques

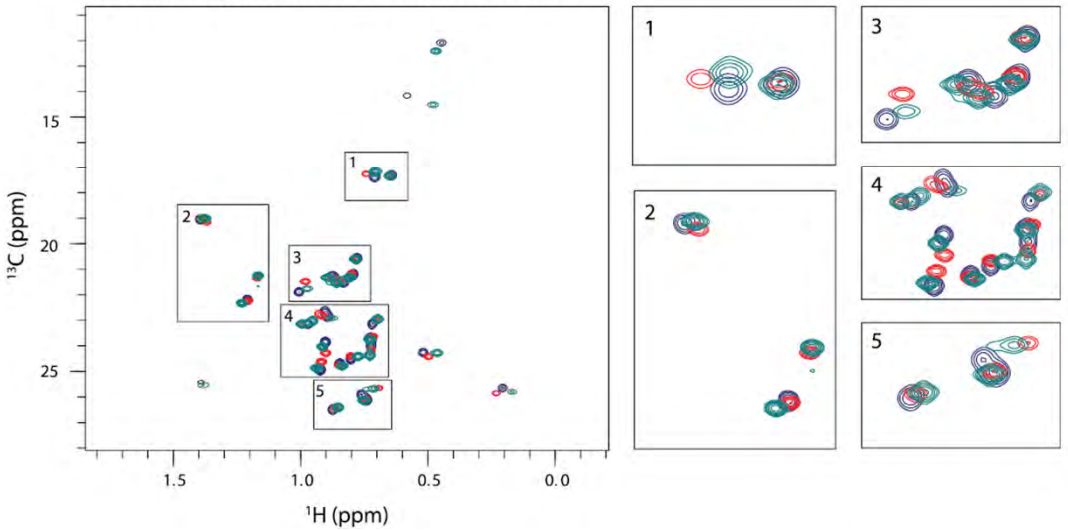
https://www.fda.gov/drugs/news-events-human-drugs/cder-scientists-use-modern-measurement-tools-quality-assurance-and-comparability-complex-drugs

HOS Characterization of Pharmaceutical Proteins: Insulin and Glucagon like peptide 1 (GLP-1)

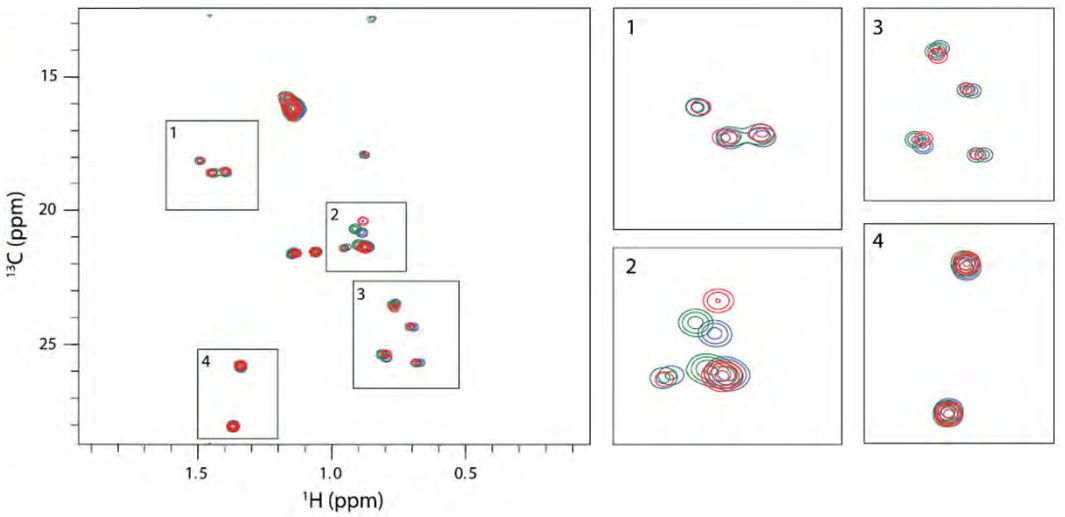
Higher-Order Structure Characterization of Pharmaceutical Proteins by 2D Nuclear Magnetic Resonance Methyl Fingerprinting

Gitte W. Haxholm, Bent O. Petersen, Joan Malmstrøm*

Novo Nordisk A/S, R&D, Novo Nordisk Park, Maaloev, Denmark

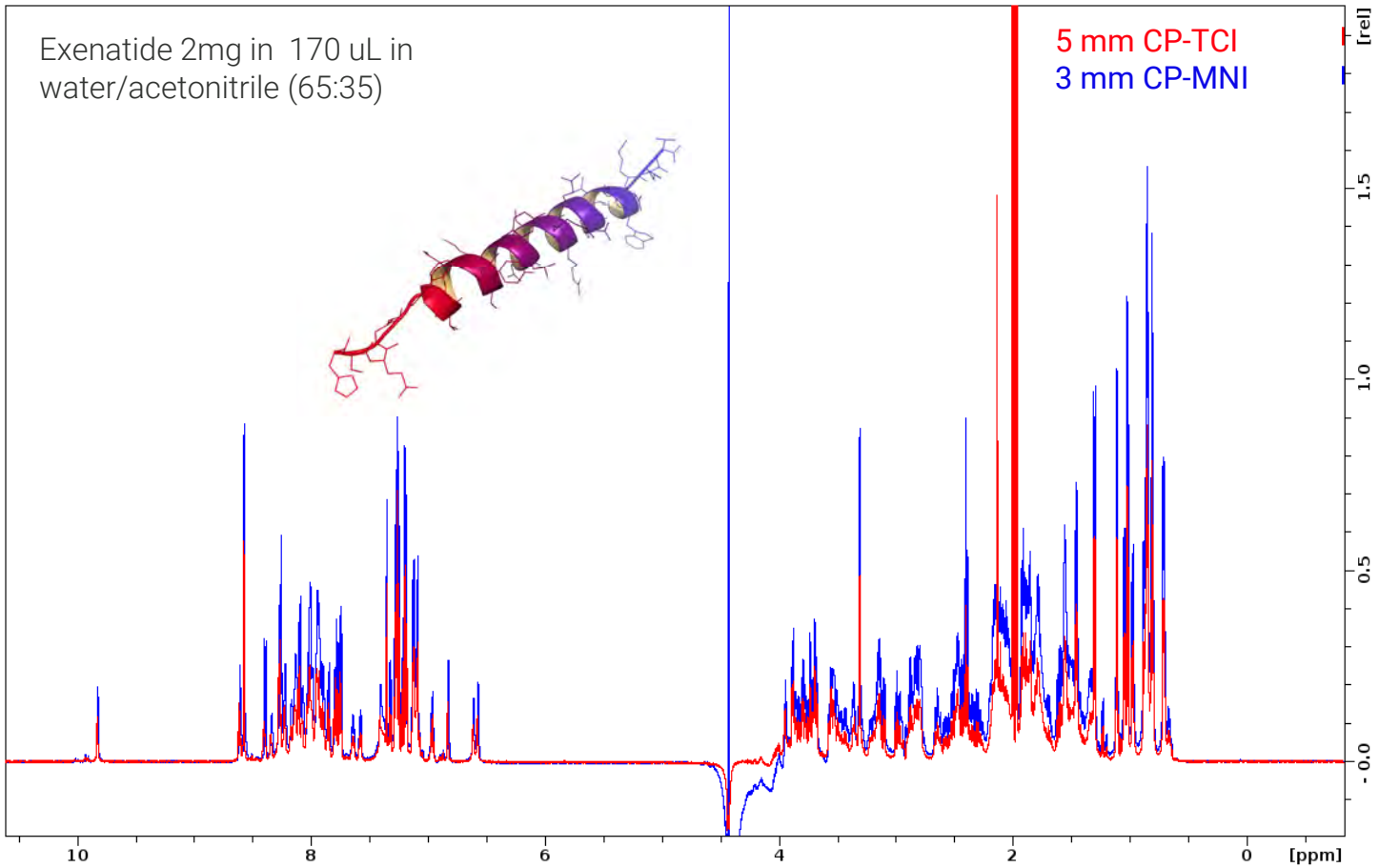


Reference insulin analog (teal) and 2 related insulin variants (red, blue) varying by exchange of only one amino acid in the backbone.



Reference GLP-1 analog (blue) with 40% a-helix and 2 synthetic stereoisomers with 25% (red) and 20% (green) a-helix

Glucagon like peptide (GLP) proton NMR spectra @ 600 MHz



- Insulin hyperglycaemia, stable in solution
- Glucagon hypoglycaemia, GLP and analogues treat type II diabetes, obesity
- 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

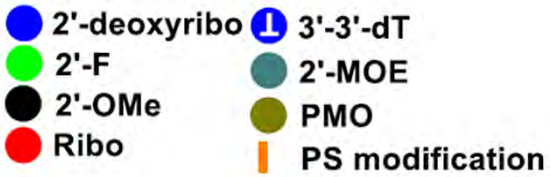
02 Oligonucleotides

Therapeutic Oligonucleotides
Eighteen nucleic acid therapeutics approved by the FDA as of 2023

- The challenge:
  - Characterization of modified therapeutic oligonucleotide fragments (15 to 30 nucleotides)
  - Quality control
  - Biosimilars
  - …

5	SPINRAZA® (nusinersen), 2016, spinal muscular atrophy (SMA)	SMN2, CNS	18-mer SSO, PS, 2'-O-MOE, splicing modification (exon inclusion)		12.5 mg once every 4 months, IT
6	ONPATTRO® (patisiran), 2018, hereditary ATTR (hATTR)	TTR, liver	21-mer/21-mer siRNA, 2'-OH/2'-OMe/2'-H, RISC Ago2		0.3 mg/kg once every 3 weeks, IV, LNP (DLin-MC3-DMA, PEG-DMG lipid, cholesterol, DSPC)
7	TEGSEDI® (inotersen), 2018, hereditary ATTR (hATTR)	TTR, liver	20-mer gapmer ASO, PS, 2'-O-MOE/2'-H, RNase H1		300 mg once weekly, SC
8	GIVLAARI® (givosiran), 2019, acute hepatic porphyria	ALAS1, liver	21-mer/23-mer siRNA, enhanced stabilization chemistry (ESC), PS, 2'-OMe/2'-F, RISC Ago2		2.5 mg/kg once a month, SC, GalNAc conjugate

Table 1. Summary of approved oligonucleotide-based therapeutics



Therapeutic oligonucleotides

NMR has a role to play:

- Characterize batches of therapeutic oligonucleotides containing mixtures of phosphorothioate diastereoisomers
- Composition is believed to affect the pharmaceutical activity

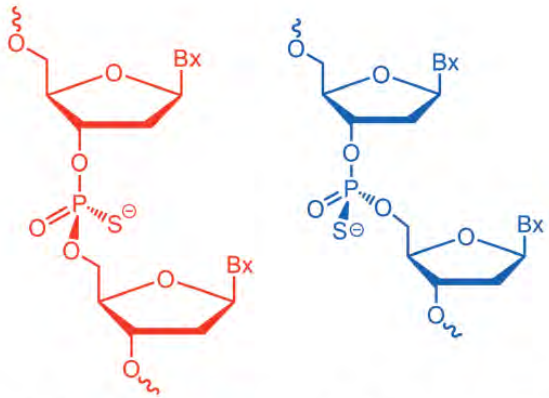


FIG. 1. Schematic representation of phosphorothioate-modified oligonucleotides with *Sp* (*red*, *left*) and *Rp* (*blue*, *right*) configuration at the phosphorous atom.

NUCLEIC ACID THERAPEUTICS
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Mary Ann Liebert, Inc.
DOI: 10.1089/nat.2021.0089

Open camera or QR reader and scan code to access this article and other resources online.

Inhomogeneous Diastereomeric Composition of Mongersen Antisense Phosphorothioate Oligonucleotide Preparations and Related Pharmacological Activity Impairment

Lorenzo Arrico,[1,*] Carmine Stolfi,[2,*] Irene Marafini,[2] Giovanni Monteleone,[2] Salvatore Demartis,[3] Salvatore Bellinvia,[3] Francesca Viti,[3] Marie McNulty,[4] Irene Cabani,[5] Anita Falezza,[5] and Lorenzo Di Bari[1]

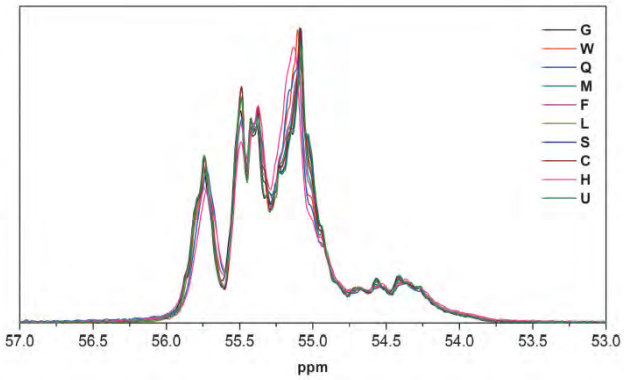
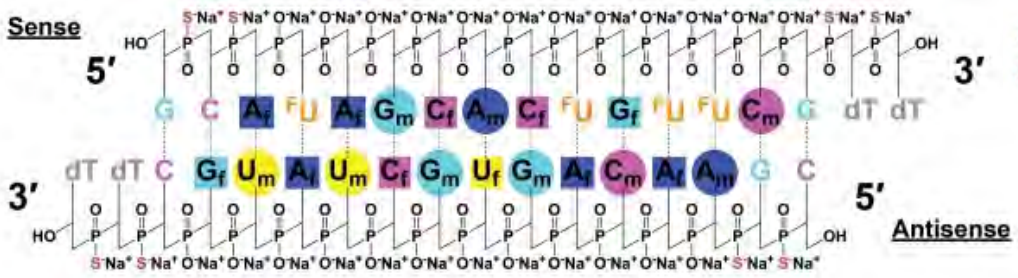
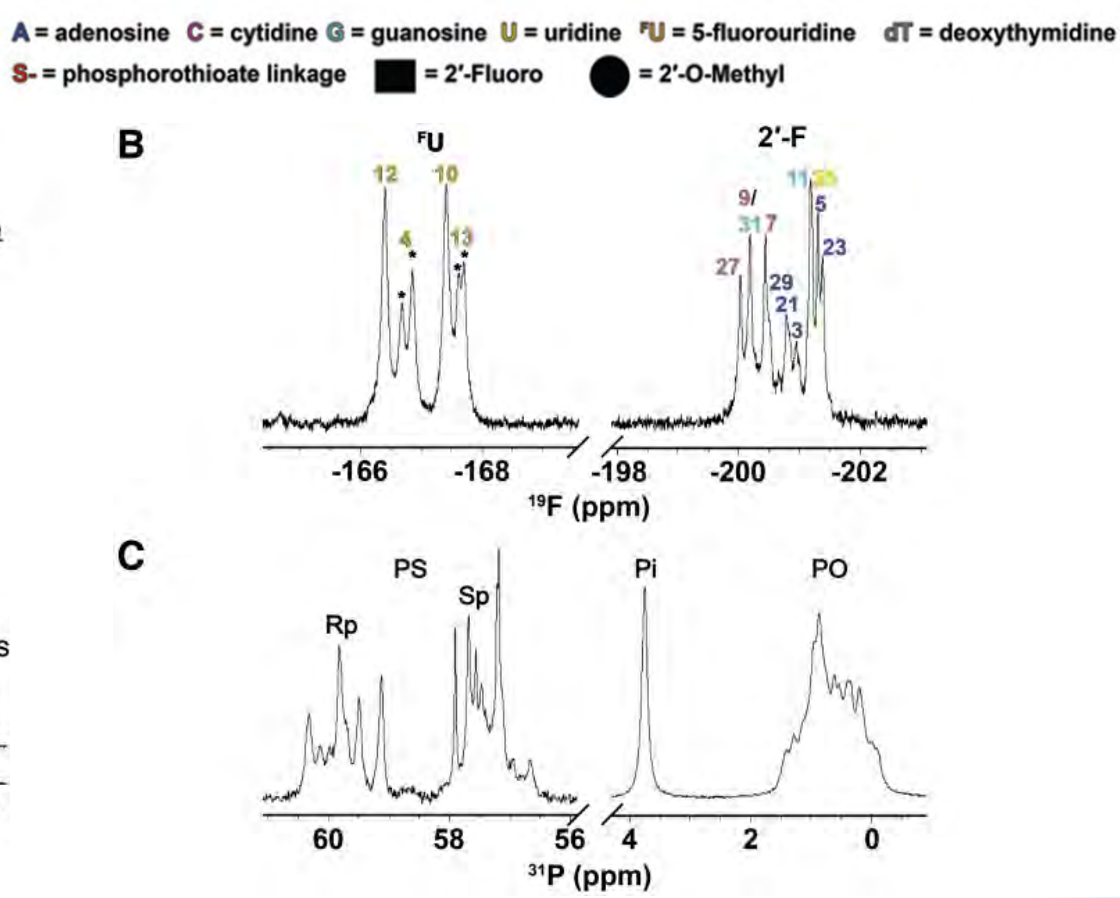


FIG. 3. The ^{31}P-NMR spectra of a subset of mongersen batches, revealing differences in the fine structures of the ^{31}P resonances.

OLIGONUCLEOTIDES

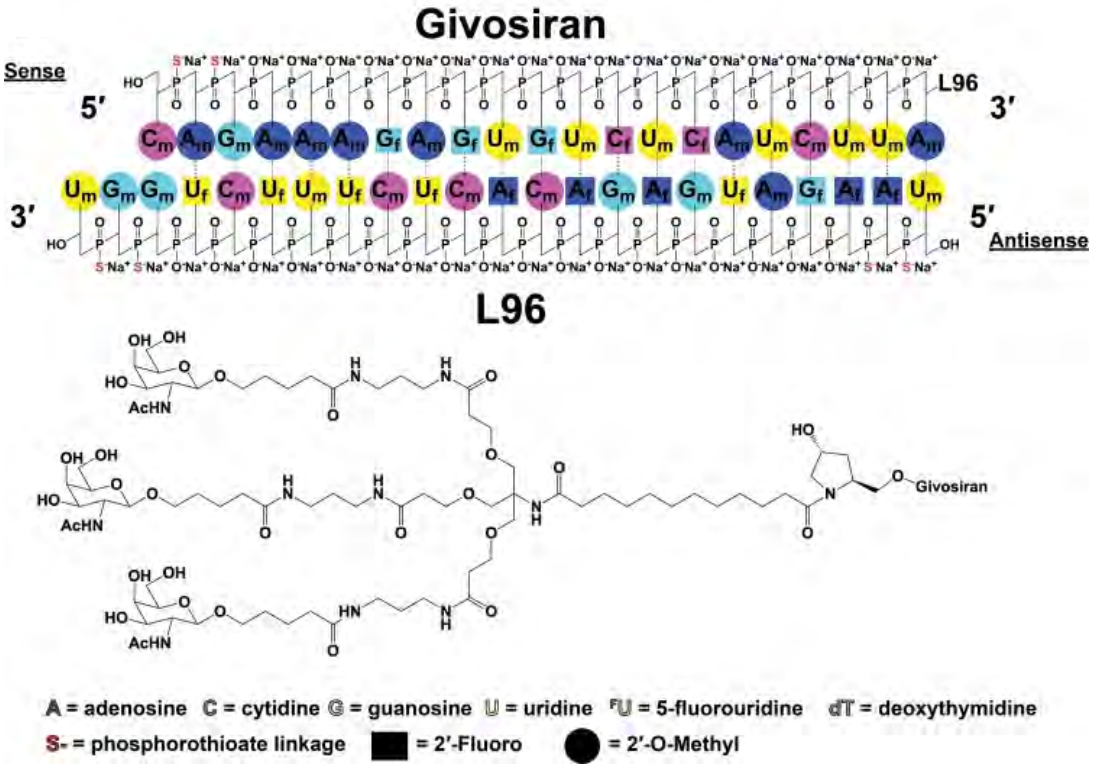
NMR fingerprinting to assess quality of model siRNA NIST study

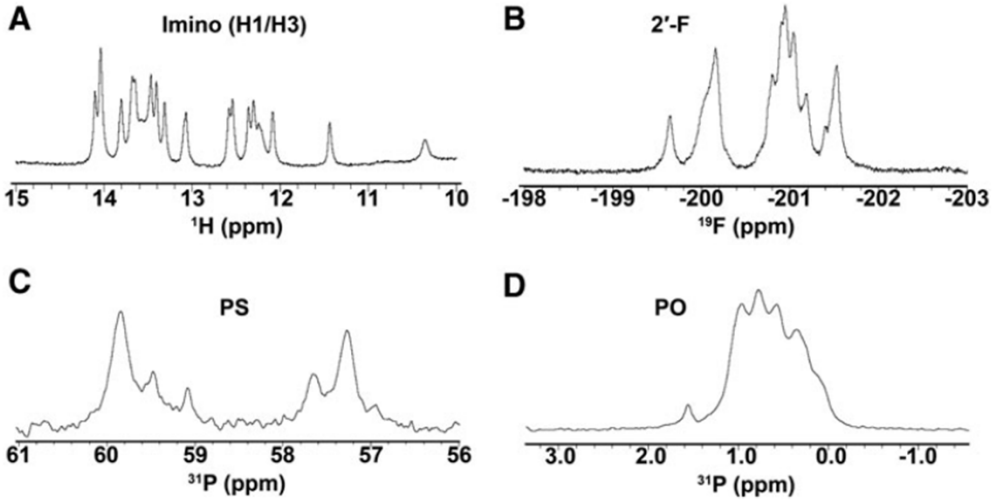




Becette OB, Tran A, Jones JW, Marino JP, Brinson RG. Nucleic Acid Ther. 2022;32(4):267-279

NMR fingerprinting to assess quality of simulated drug product Givosiran (Givlaari) NIST study





The authors anticipate **broad applicability of the NMR methods** to other **nucleic acid-based therapeutics** due to the generalized nature of the approach and ability to monitor **many quality attributes simultaneously.**

Becette OB, Tran A, Jones JW, Marino JP, Brinson RG. Nucleic Acid Ther. 2022;32(4):267-279

FDA guidance on Givosiran Sodium

Contains Nonbinding Recommendations

Draft – Not for Implementation

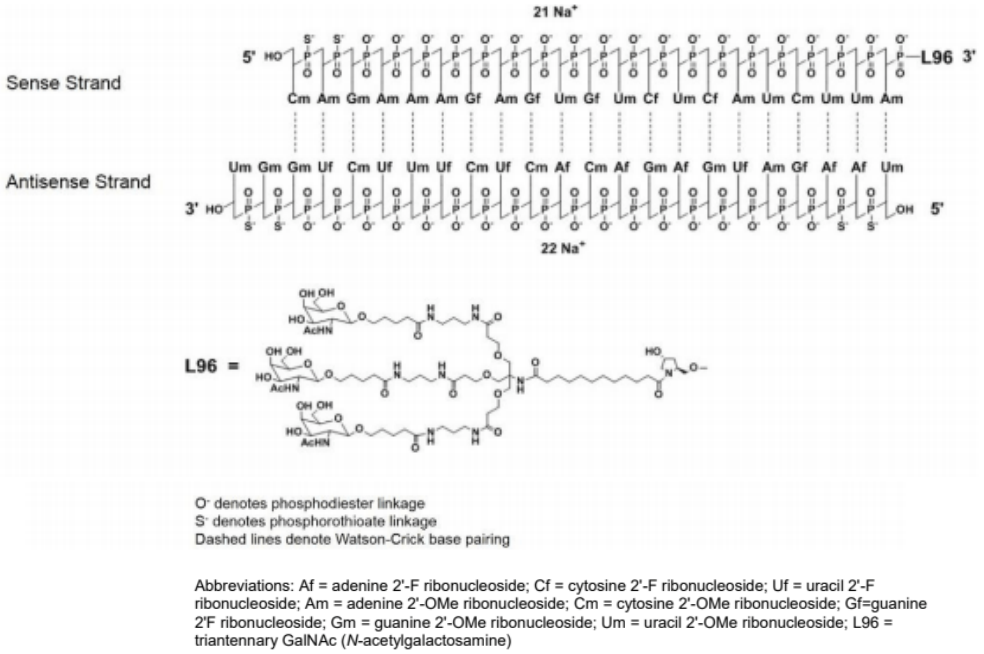
Draft Guidance on Givosiran Sodium

May 2023

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

The test API sequence, chemical structure and composition including strand composition, duplex vs residual single strands, diastereomeric composition, and P=S to P=O ratios should be compared to those of the API from the RLD using a broad range of orthogonal analytical methods with sufficient sensitivity, discriminating, and resolving power, that could include but are not limited to the following:

a. Mass spectrometry (MS), including tandem mass spectrometry (MS/MS)
b. Nuclear magnetic resonance (NMR) spectroscopy
c. Liquid chromatography (LC)
d. Flame atomic absorption spectroscopy (FAAS)
e. Duplex melting temperature (Tm)



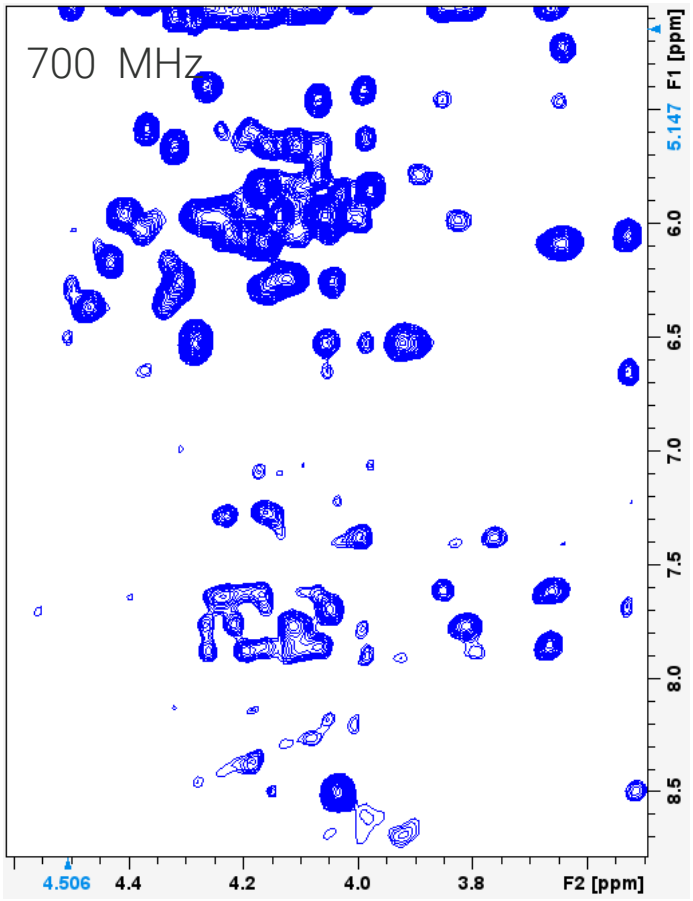
Abbreviations: Af = adenine 2'-F ribonucleoside; Cf = cytosine 2'-F ribonucleoside; Uf = uracil 2'-F ribonucleoside; Am = adenine 2'-OMe ribonucleoside; Cm = cytosine 2'-OMe ribonucleoside; Gf=guanine 2'F ribonucleoside; Gm = guanine 2'-OMe ribonucleoside; Um = uracil 2'-OMe ribonucleoside; L96 = triantennary GalNAc (*N*-acetylgalactosamine)

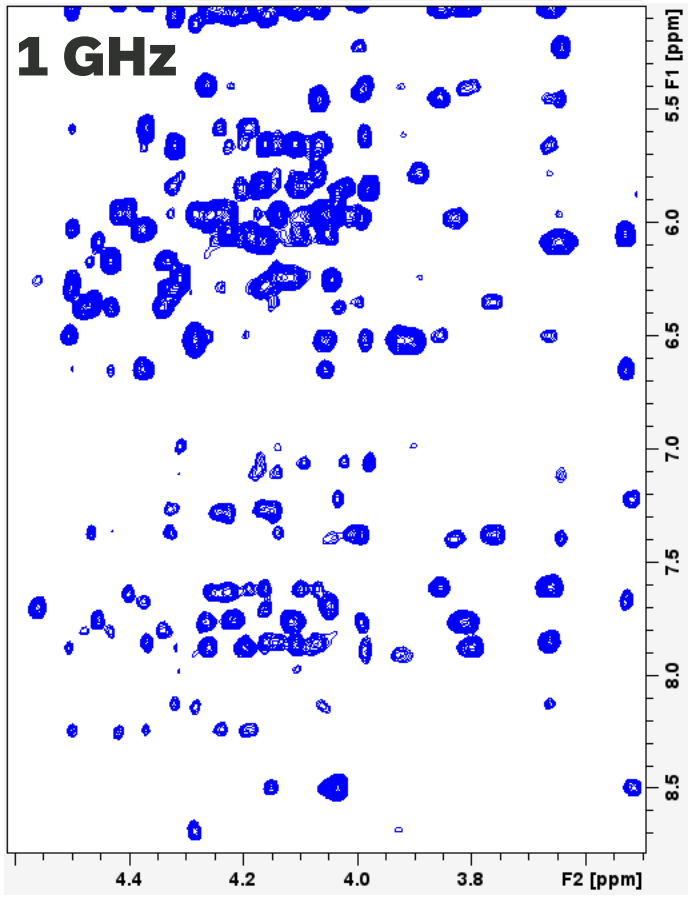
https://www.accessdata.fda.gov/drugsatfda_docs/psg/PSG_212194.pdf

https://www.bachem.com/news/galnac-delivering-promise-of-oligonucleotides/

30 nt DNA aptamer in interaction with testosterone
Ascend Evo 1.0 GHz

Data courtesy
Jose Martins (Ghent University)
Miquel Pons & Margarida Gairí
(University of Barcelona)





2D NOESY 100 ms mixing time 200 nmole of DNA (0.4 mM, 1.9 mg)

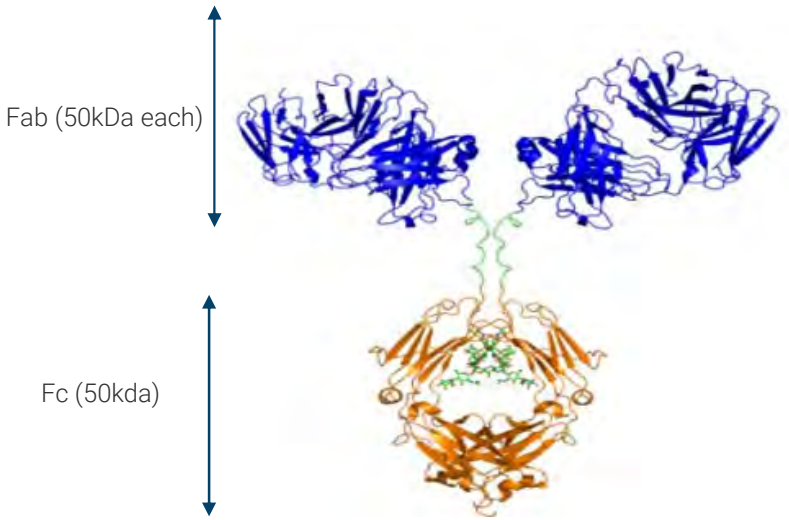
03 Antibodies

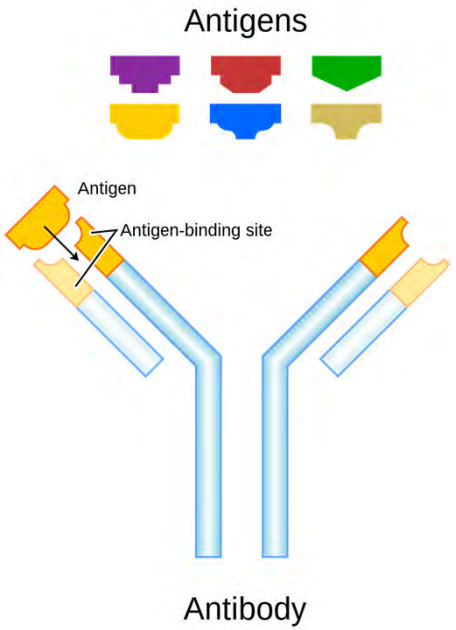
Biologics: mAbs (monoclonal antibodies)

Goal is to obtain an accurate fingerprint of the mAb (HOS) and analyze both drug substance and drug product

The challenge

- MW ca. 150 kDa for a full antibody
- Fast T2 relaxation
- Natural abundance only !
- Acquire mainly 1D ^{1}H spectra and 2D $^{1}H/^{13}C$ spectra of CH_3 groups





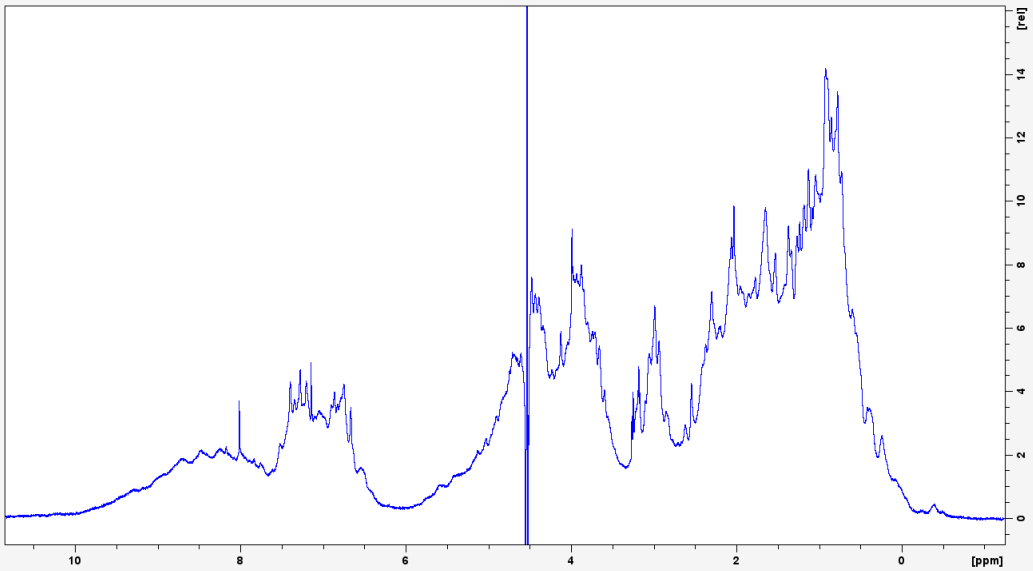
https://en.wikipedia.org/wiki/Antibody

mAb fingerprinting by NMR at very high field 1GHz data acquired with a TCI cryoprobe

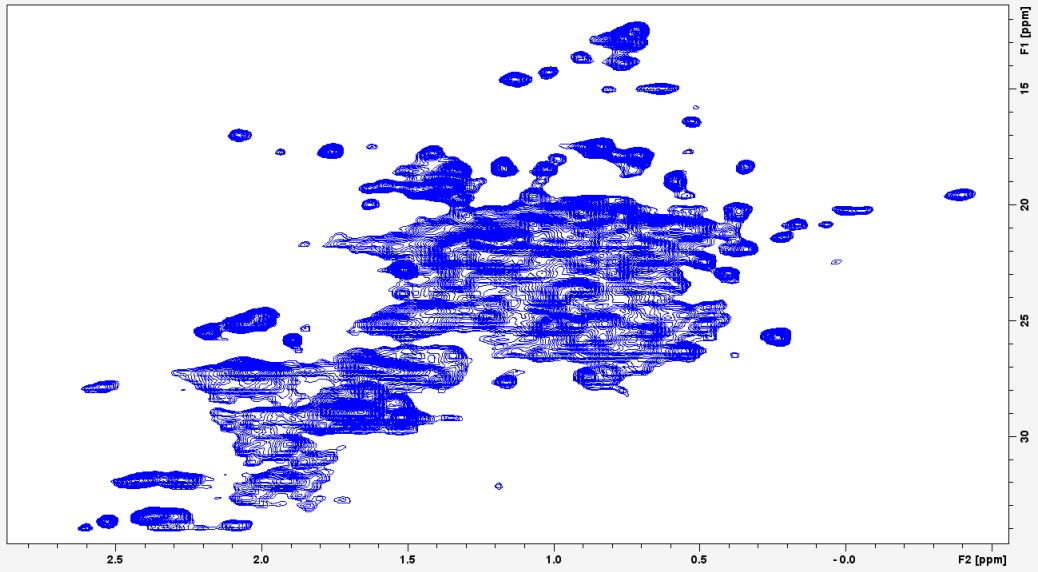
Sheep IgG

- IgG1 sample MW 150 KDa
- 286 µM in NIST Buffer
- Temperature 323 K
- Natural abundance

Data courtesy
Miquel Pons & Margarida Gairí
(University of Barcelona)



1D Spectrum showing all the 1H of the mAb
Experiment time 5 min



$^{1}H/^{13}C$ correlation: Each peak represents a different methyl group of the mAb (Amino acids observed: Met, Ile, Thr, Ala, Val, Leu)
Experiment time 7 hours

Higher Order Structure (HOS) analysis for mAbs studies Guidelines & acquisition parameters

Mestrelab Research chemistry software solutions

BRUKER

Parameters guidelines documents: sample preparation, data acquisition and data analysis

1D & 2D (HOS*) Topspin acquisition parameter sets (from TS 4.1.4)

Filter by file names: HOS* Exclude: Clear

Class = Any Dim = Any Show Recommended

Type = Any SubType = Any SubTypeB = Any Reset Filters

HOS_1DNOESY	HOS_1DSTE	HOS_AFHMQC	HOS_CPMGES1D	HOS_HSQC
HOS_T1RES1D	HOS_T1RESPE1D	HOS_XLAFHMQC	HOS_ZGESGP	HOS_ZGESGPPE

Analysis by BioHOS package (Mestrelab Research)
1D & 2D spectra analysis, link between NMR spectra and statistical tools (including bucketing)

BioHOS NOVA

1D Analysis (PCA, PLS, SIMCA & PROFILE)
2D Analysis (ECHOS, CCSD, PCA, PLS & SIMCA)

Contents lists available at ScienceDirect
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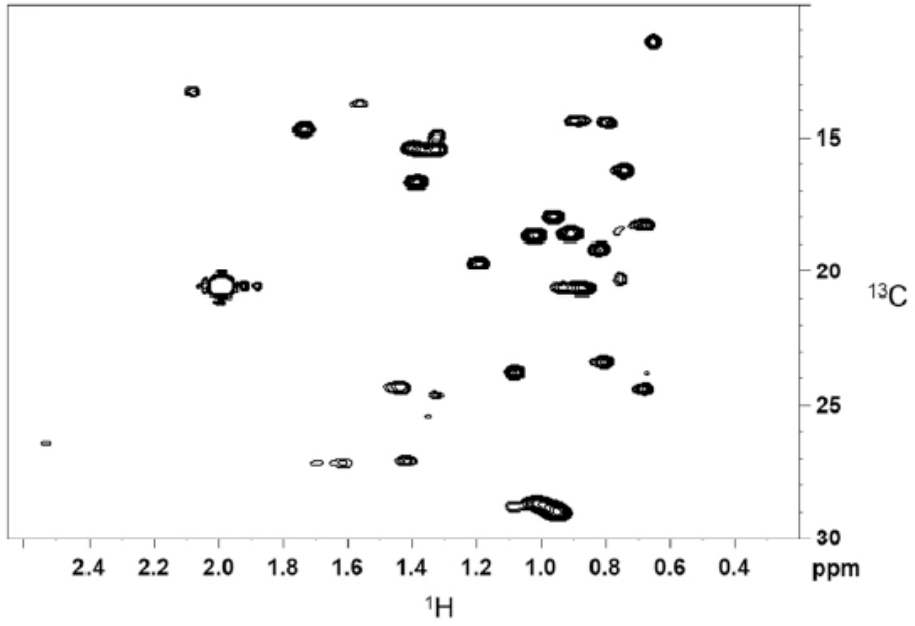
journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

NMR spectroscopy as a characterization tool enabling biologics formulation development

Junhe Ma [a,*], Charles Pathirana [a], David Q. Liu [b], Scott A. Miller [a]

[a] Chemical Process Development, Bristol Myers Squibb Company, 1 Squibb Drive, New Brunswick, NJ 08903, United States
[b] Vertex Pharmaceuticals, 50 Northern Avenue, Boston, MA 02210, United States



Mueller, L., J. Biomol. NMR, 2008, 42, 129-137; Rossler, P., Mathieu, D., Gossert, A.D. Angew. Chem. Int. Ed., 2020, 59, 19329-19337

04 Bio-Production

From bioprocess understanding to control with the Fourier 80

Brings the detector to the (bio)reactor. Monitors ‘where it happens as it happens’

Online monitoring by NMR

- Quantitative by definition
- Calibrates other techniques
- On-the-fly quantitative build-up curves
- Increased selectivity, resolution and sensitivity compared to vibrational spectroscopy
- Reduced risk - direct knowledge transfer from mid/high-field NMR to low-field benchtop

InsightMR for Fourier 80

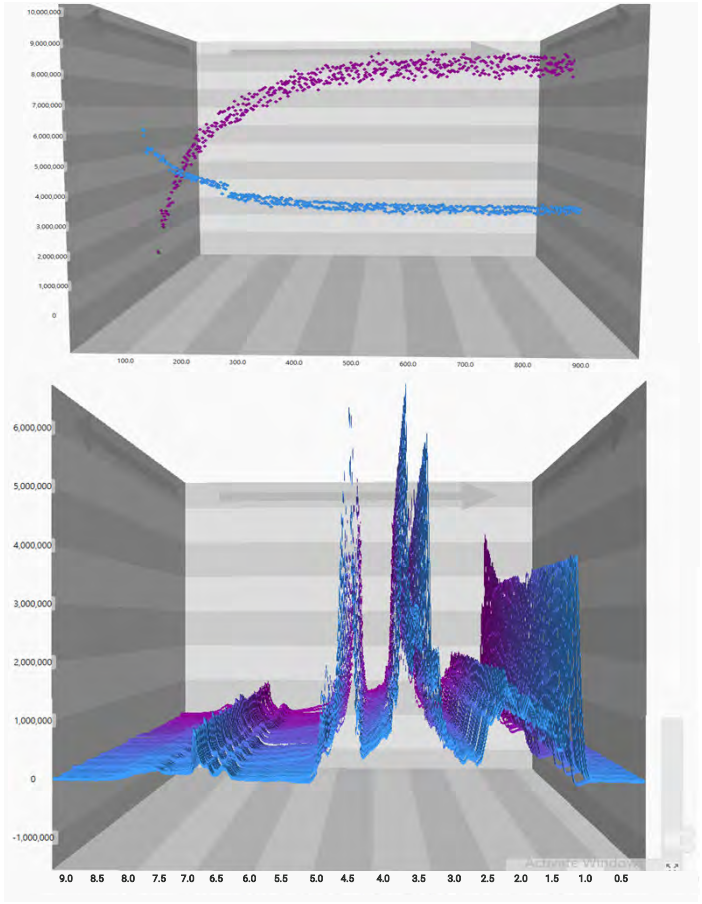
Control

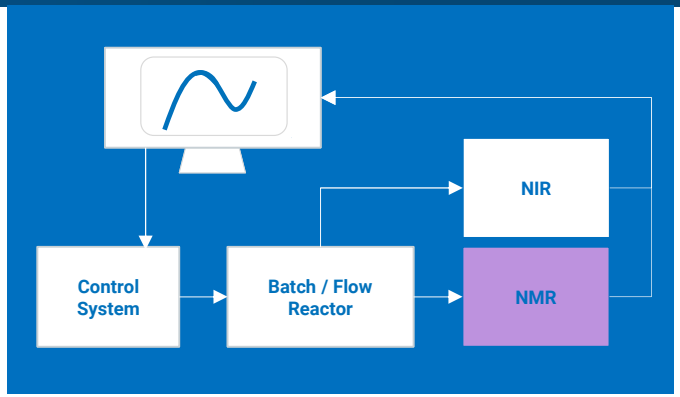
InsightMR for mid/high field NMR

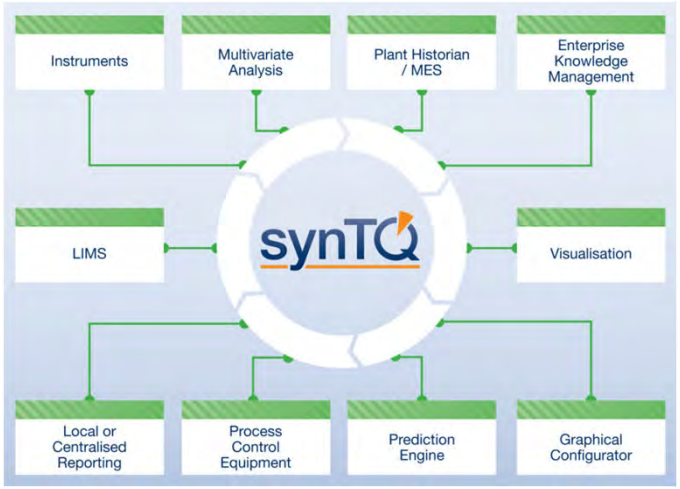
Understanding

Integrating NMR into Process Analytical Technology with synTQ

- Enabling simultaneous acquisition of data from different instruments with on-the-fly data visualisation
- Data driven results for increase understanding, increase quality, decrease risk and cost
- Integration with system controller (e.g., Temp. and pH in bioreactors) facilitating feedback control
- Complete data journey integrating with MVA models, historian, MES, EKM, LIMS

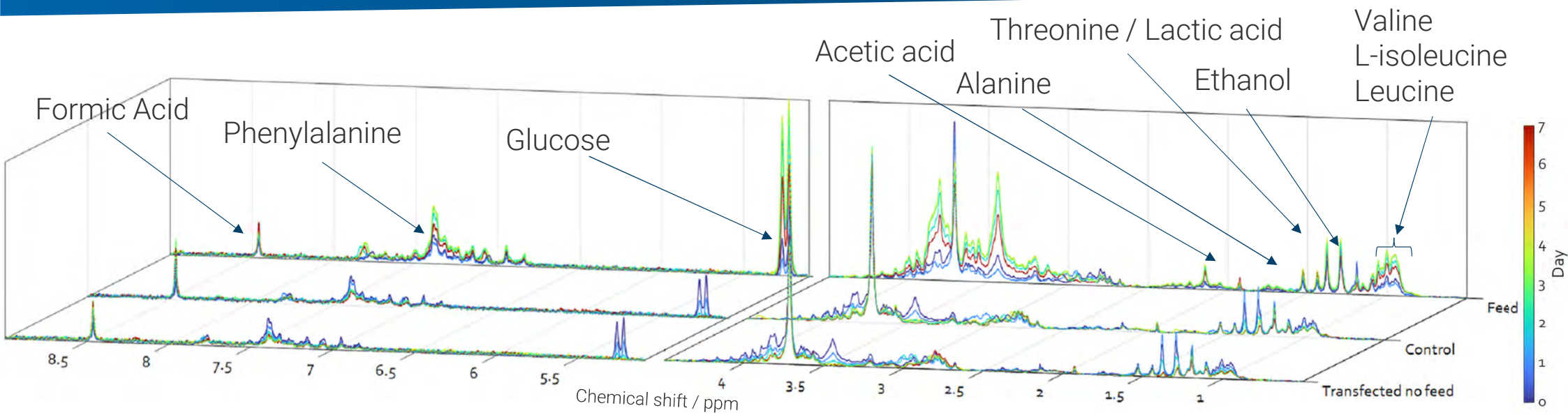






PEAXACT Automating data analysis with hard modelling

- Implementation of PEAXACT module in synTQ software for data analysis
- 7 days bioproduction of the IgG-like bispecific antibody
- Transfected mammalian cell using Expi CHO Expression system
- 3 different sample line
  - Control (no transfection); Transfected; Transfected with supplemented nutrients (from day 2)



Courtesy of Simon Kern, S-PACT

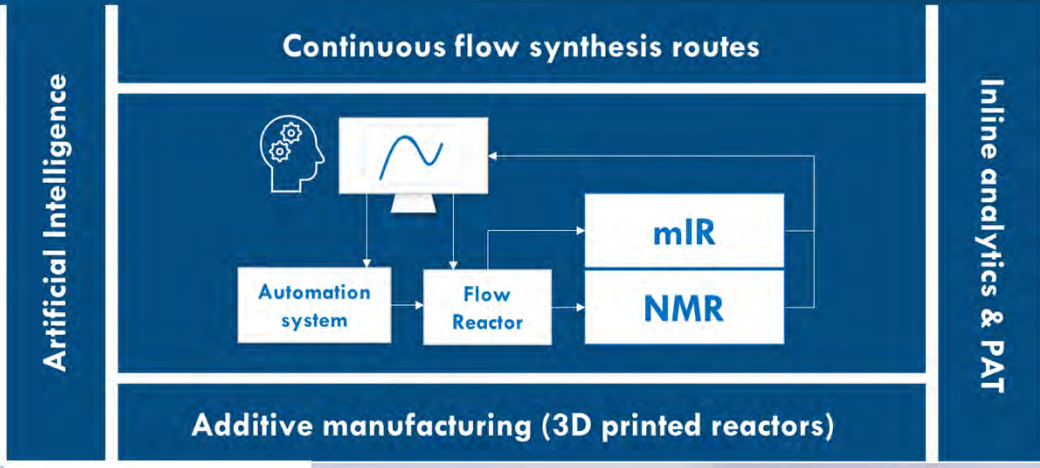
05

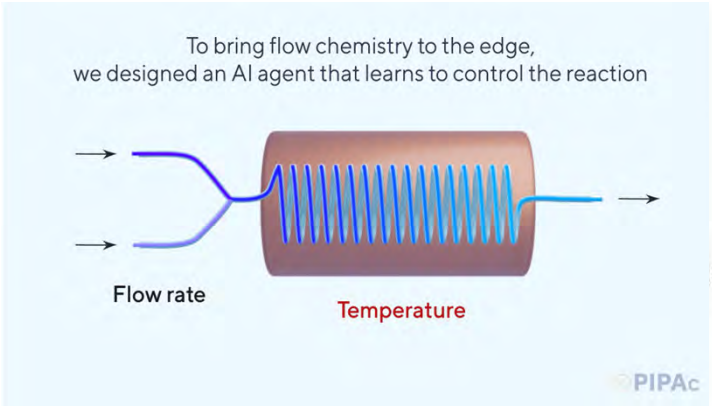
PIPAc:
Production Intelligente de Principes Actifs

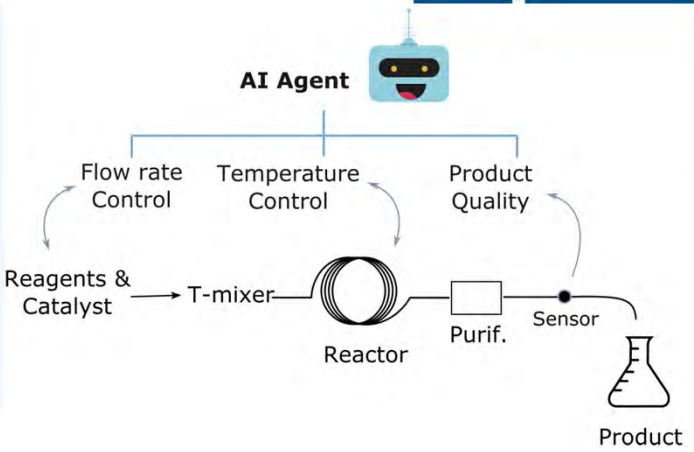
Fourier enabling API Production on Demand (PoD)

PIPAc Production Intelligente de Principes Actifs

- Intelligent production of API via creation of "**compact, mobile, autonomous and frugal production units**"
- **flow chemistry** with **artificial intelligence** enabled process control using NMR and mIR as detectors





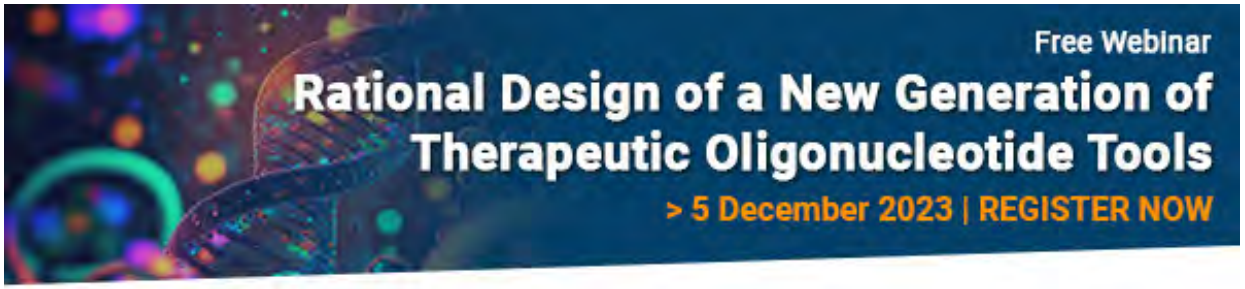


Acknowledgements

- BPIfrance: PIPAc Project
- UCB Pharma: Emmanuel Cornet and Cédric Schaefer
- Bruker BioSpin colleagues: Pharma Team, Software, Applications and R&D

PHARMA WEBINAR

Webinar “Rational Design of a New Generation of Therapeutic Oligonucleotide Tools” – 5 December, 17:00 CET

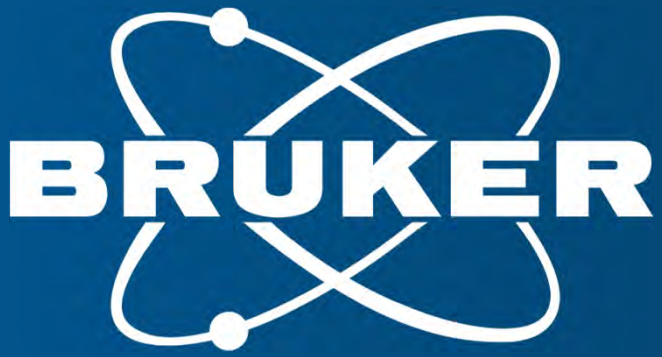


Presenting new chemically modified oligonucleotide tools with the potential to tackle multifactorial disorders (among them, cancer and related drug resistance issues), while overcoming the limitations of oligonucleotide therapeutics.

Dr. Montserrat Terrazas

Associate Professor Department of Inorganic and Organic Chemistry (Organic Chemistry Section), Faculty of Chemistry, University of Barcelona, Barcelona, Spain

SCAN ME!



Innovation with Integrity