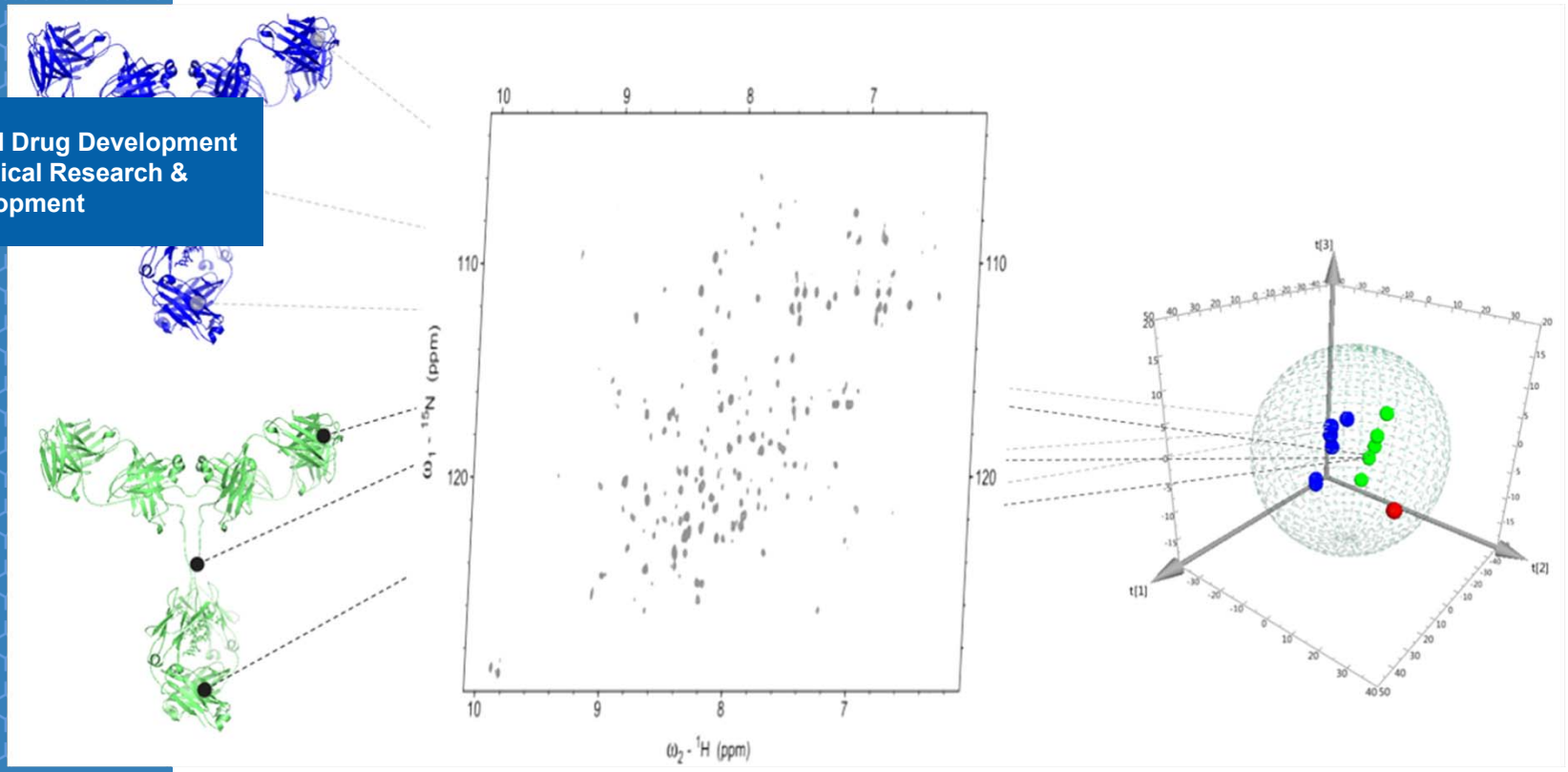
Global Drug Development
Technical Research & Development



Computational Methods for Comparison of NMR Spectra

B. Japelj, G.Ilc, M. Zidar, J. Marušič, J. Senčar, D. Kuzman, J. Plavec

HOS 2019, San Mateo

April 9th, 2019

NOVARTIS

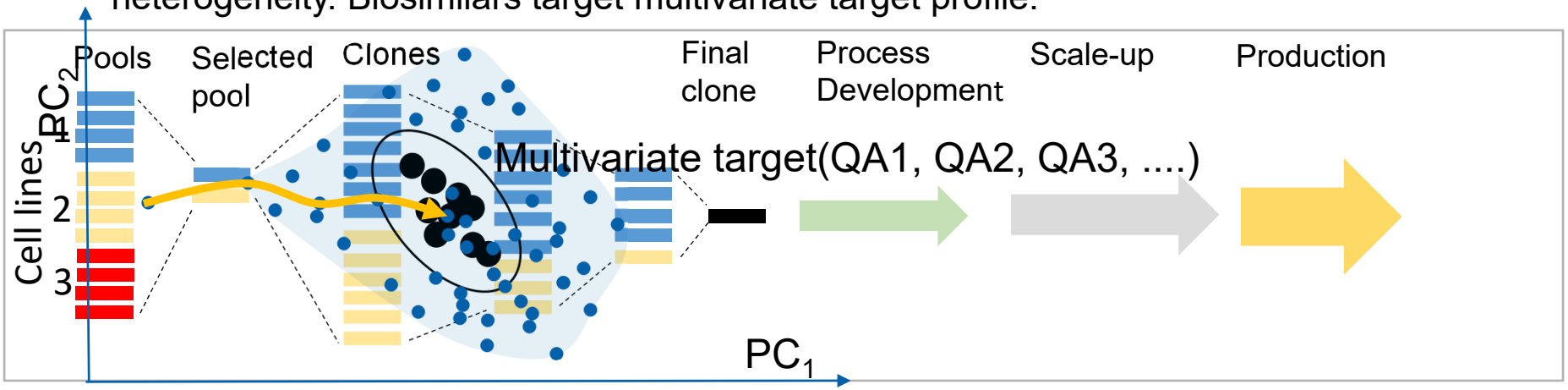
Agenda

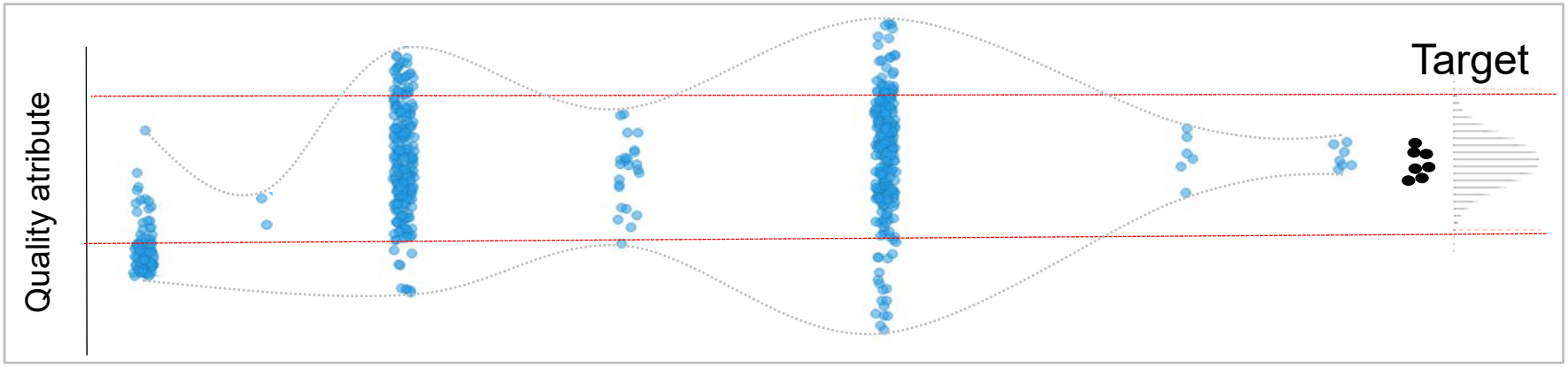
- Introduction
- NMR fingerprinting experiments
- Computational methods for spectral comparisson
- High troughput 1D NMR workflow for stability screening
- Conclusion

NOVARTIS

Introduction

Biological drugs are produced using a complex bioprocess which results in structural heterogeneity. Biosimilars target multivariate target profile.

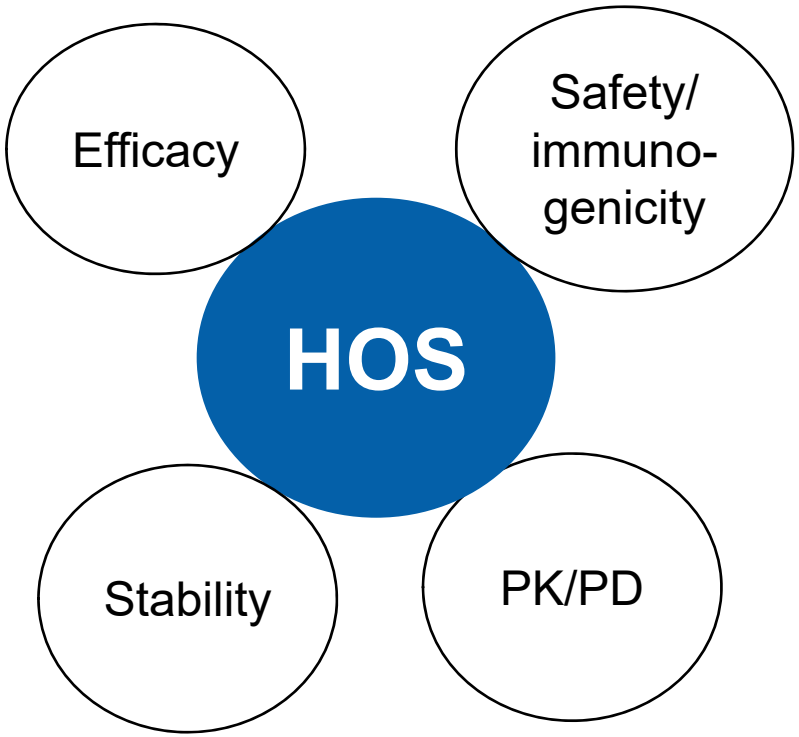




QA = f(raw materials, process parameters, formulation, conditions,...)

NOVARTIS

Higher order structure



	Biological drugs
Size	Large (mixture of related molecules)
	High molecular weight
Structure	Complex (heterogeneous), defined by the exact manufacturing process
Modification	Many options
Manufacturing	Produced in living cell culture
	Difficult to control from starting material to final API
	Impossible to ensure identical copy
Characterisation	Very difficult to completely characterize due to molecular composition and heterogenicity
Stability	Unstable, sensitive to external conditions
Immunogenicity	Could be immunogenic

Ref: http://www.gabionline.net/Biosimilars/Research/Small-molecule-versus-biological-drugs; Declerck PJ. GaBI J. 2012;1(1)

NOVARTIS

Higher order structure methods: Analytical characterization used during development of biosimilars

Typically 40 analytical methods, ≥ 100 product quality attributes

- X-ray crystallography
- Nuclear magnetic resonance (NMR)
- Cryoelectron microscopy
- Circular dichroism
- Fourier-transform infrared spectroscopy
- Mass spectrometry
- Hydrogen/deuterium exchange with mass spectrometry
- Dynamic light scattering (DLS)
- Microcalorimetry
- Biolayer interferometry
- Electron tomography
- Analytical ultracentrifugation
- Field-flow fractionation
- Free-electron laser scattering
- Raman spectroscopy
- Size-exclusion chromatography
- Static light scattering
- UV/fluorescence spectroscopy
- voltage electron microscopy
- Metal-enhanced fluorescence (MEF) or chemiluminescence (MEC)
- Anilinonaphthalene sulfonate (ANS) binding
- Chromatography using interactive resins
- Single-cell sensing
- Single-molecule fluorescence spectroscopy, ...

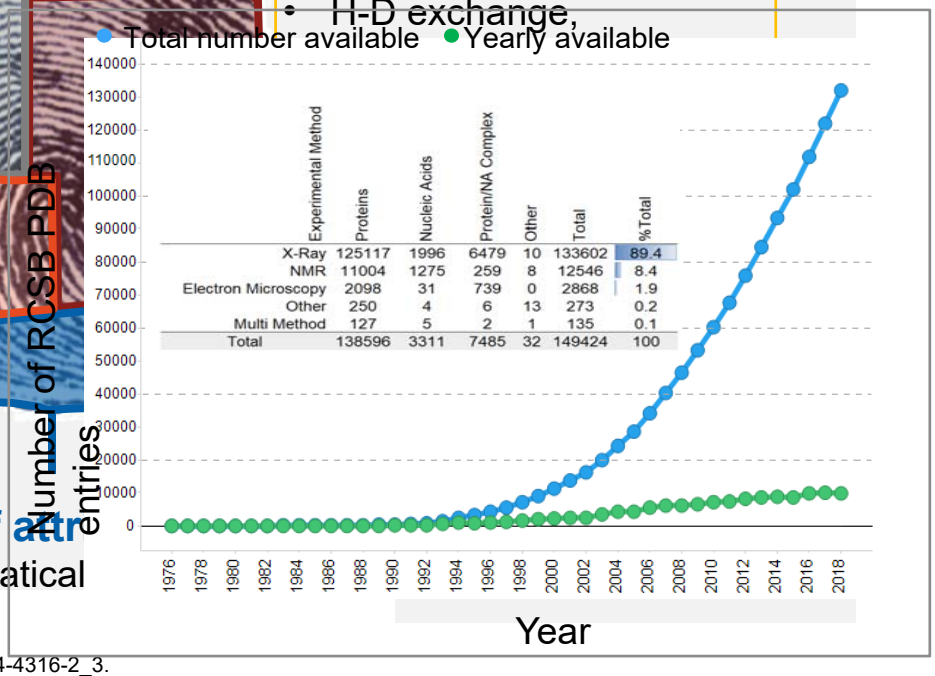
Primary structure:
- Intact MS
- Peptide mapping, ...

Impurities:
- CEX, cIEF acidic/basic variants
- LC glycation
- Peptide mapping: deamidation, oxidation, mutations, glycation
- SEC/FFF/AUC agregacija

Bioactivity (potency):
- Target binding
- Cytotoxicity (ADCC, ADCP, CDC)

Combination of attr
- MVDA, mathematical

Secondary/Tert./Quart. structure:
- NMR
- X-Ray crystallography
- CD
- FT-IR,...
- H-D exchange,



Ref.:
- DiRaola, M. BioPharm International. 30(8): 38–43
- Kranz et al, Techniques for Higher-Order Structure Determination. 10.1007/978-1-4614-4316-2_3.
- Scientific Considerations in Demonstrating Biosimilarity to a Reference Product. Guidance for Industry. FDA, 2015
- ICH Q6B, Specifications: test procedures and acceptance criteria for biotechnological/biological products Share,1999
- Ref.: Wei, Z. et al, BioProcess International (2011), 9(4): 58–66
- https://www.rcsb.org/stats/summary, March 1st, 2019

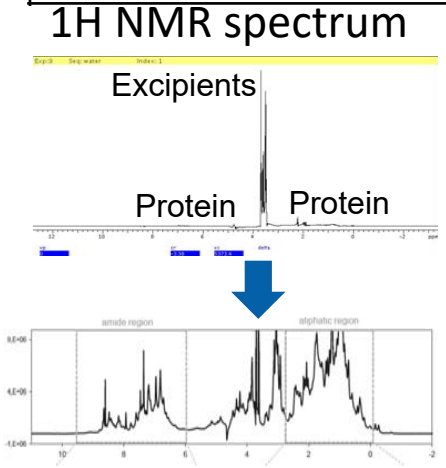
Ref. Wei Z. et al. BioProcess International (2011), 9(4): 58–66
https://www.rcsb.org/stats/summary, March 1st, 2019

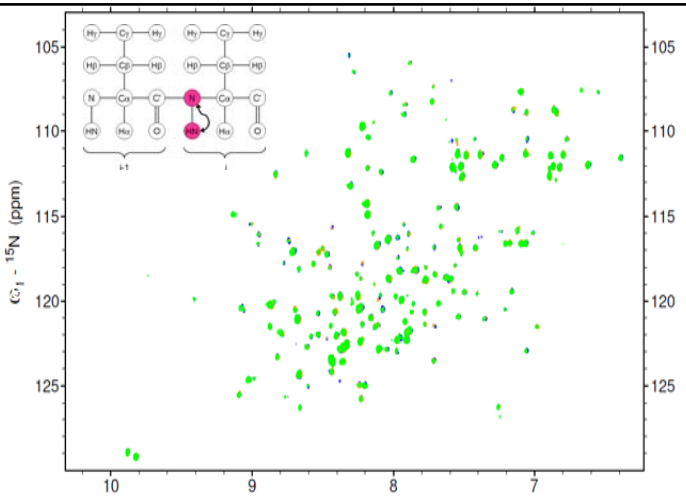
NOVARTIS

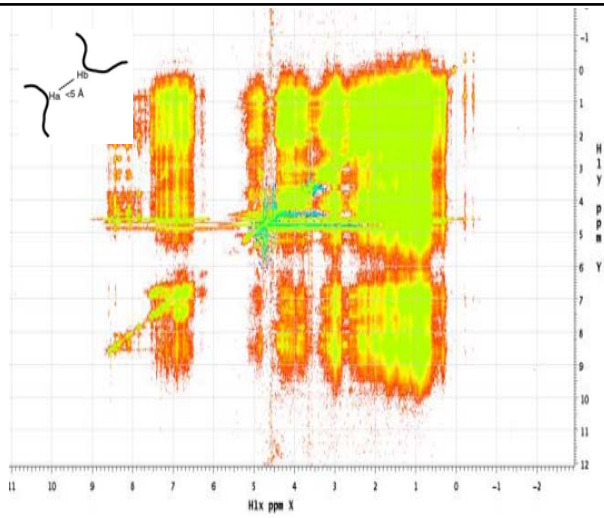
NMR fingerprinting experiments

NMR experiment	Advantages	Disadvantages
^{1}H spectra	Fast, simple	Overlapping signals, non-selective towards excipients
^{1}H-^{1}H NOESY	Higher resolution than 1D experiments, through space dipolar interaction	Overlapping signals, non-selective towards excipients, complex analysis
^{1}H-^{15}N gsHSQC (US/NUS)	Smaller number of signals / better dispersion than NOESY	Low sensitivity for non-labeled samples (0.37% nat. occuring ^{15}N isotope)
^{1}H-^{13}C gsHSQC (US/NUS)	Smaller number of signals / better dispersion than NOESY, works well for large proteins	Low sensitivity for non-labeled samples (1.11% nat. occuring ^{13}C isotope), only 6 residues have methyl groups
^{1}H-^{13}C-(sf)HMQC	Smaller number of signals / better dispersion than NOESY, works well for large proteins	Low sensitivity for non-labeled samples

1H NMR spectrum







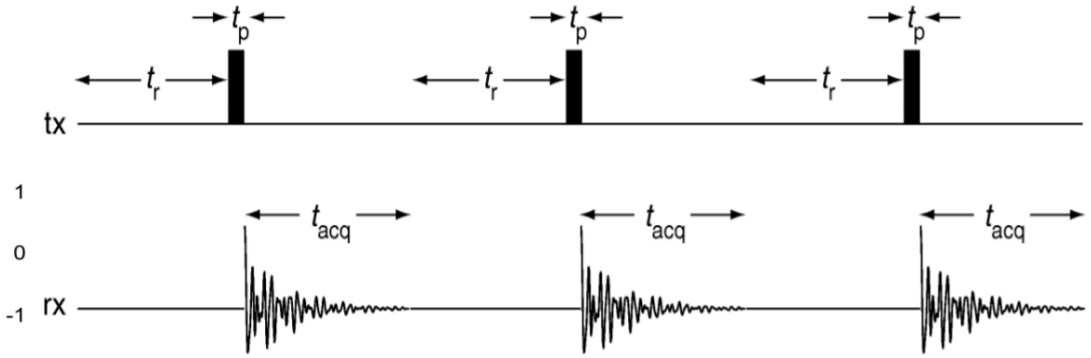
Bodenhausen, G. et al. Chem. Phys. Letters. 69 (1): 185–189 (1980)
http://www.cryst.bbk.ac.uk/PPS2/projects/schirra/html/2dnmr.htm
Keeler, J. (2010). Understanding NMR Spectroscopy (2nd ed.). Wiley. pp. 184–187.
https://www.chemie.uni-hamburg.de/nmr/insensitive/tutorial/en.lproj/

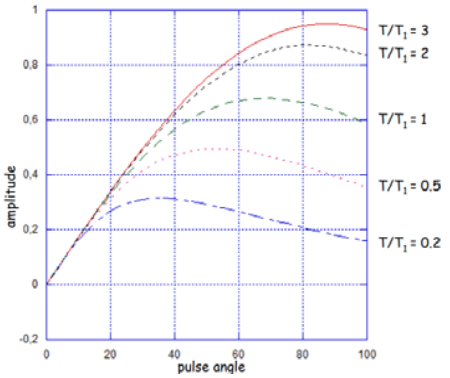
*Abbreviations:
US- uniform sampling
NUS- non-uniform sampling
sf-SOFAST

NOVARTIS

Tricks to increase S/N:

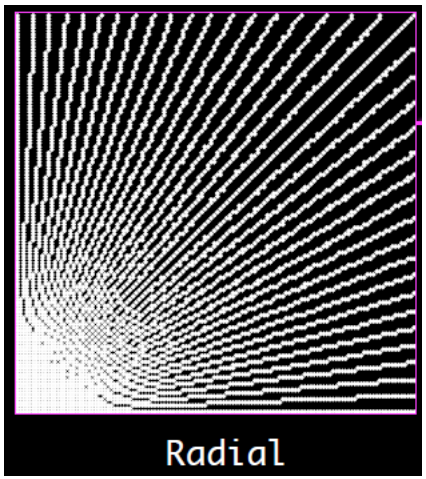
- Temperature (45-50°C)
- Optimize concentration
- Digestion
- CH_3 relaxation
  - 3-fold symetry
  - Fast rotation around the C-C bond
- Ernst Angle: SOFAST HMQC, BEST-HSQC

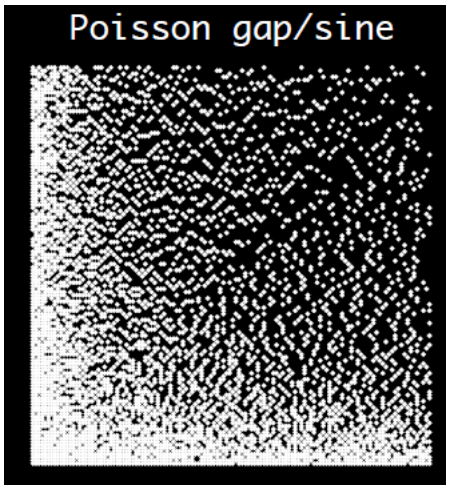




- non-uniform sampling

partial sampling of points in the indirect dimension(s) (triangular, spiral, Poisson Gap, and Burst sampling)

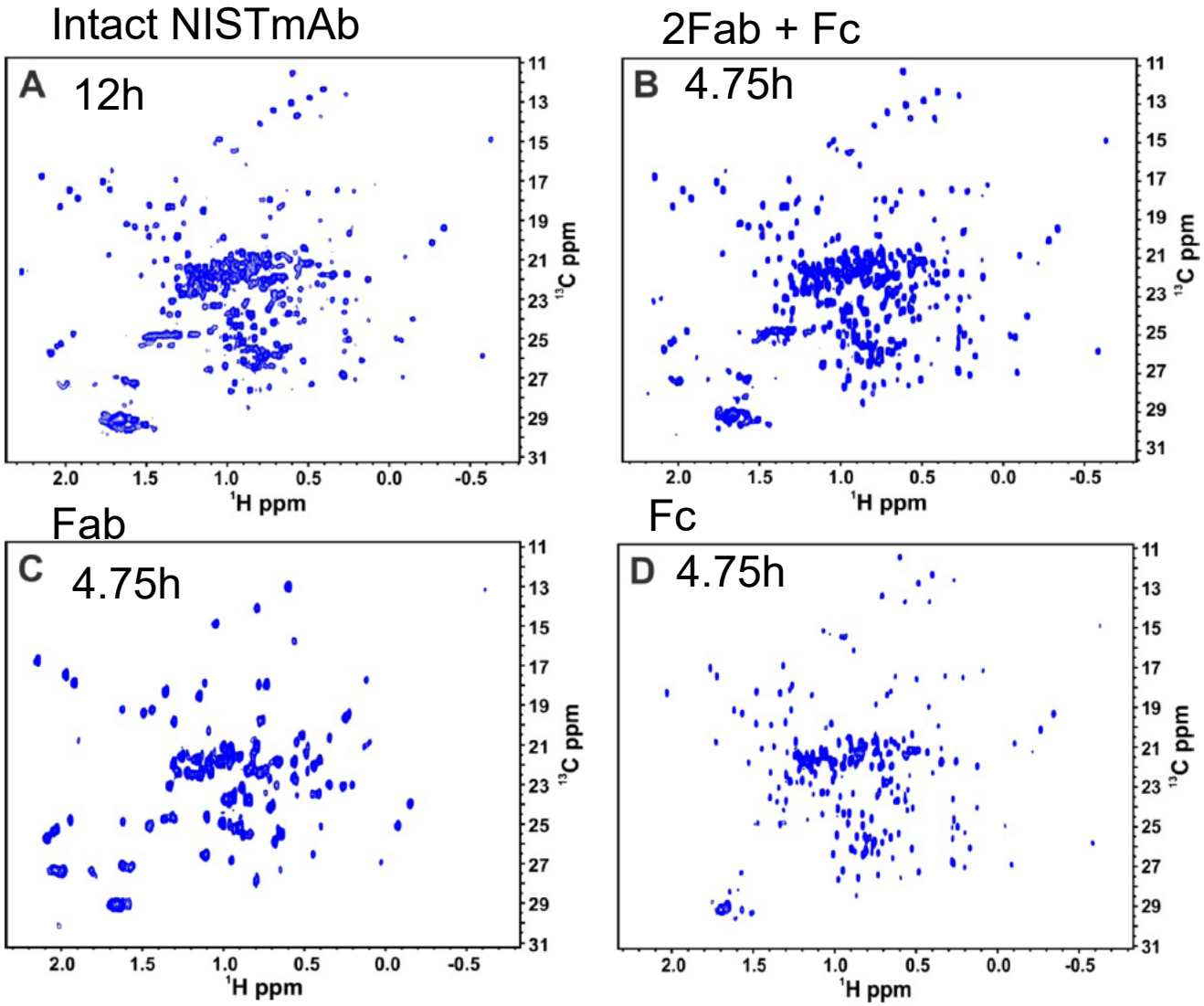




J. Hoch: The ABC of NUS
P.Schieder, 2009

SOFAST: band-Selective Optimized Flip-Angle Short-Transient
BEST: Band-selective Excitation Short-Transient

NMR methyl fingerprint method of an intact mAb and fragments at natural isotopic abundance



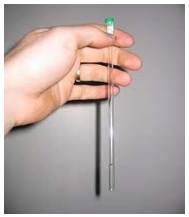
NIST,
900 MHz, 50oC
100% coverage of intact mAb in 12h

gChsqc

NOVARTIS

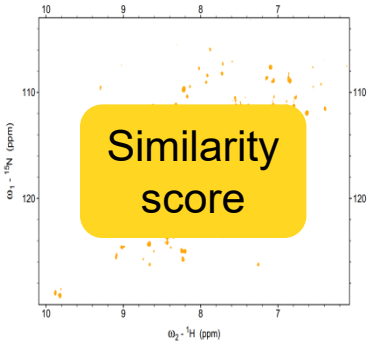
Arbogast, L.W. et al Anal. Chem. 2015, 87:3556-3561

NMR comparability

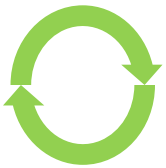


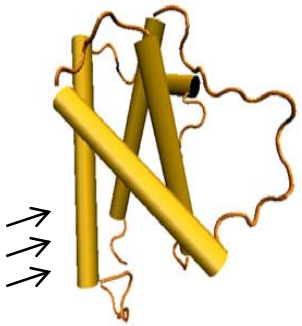
Reference sample (originator)

Biosimilar sample



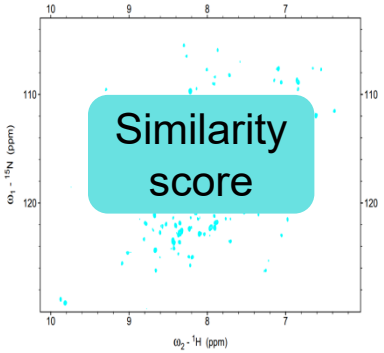
fingerprint – reference sample



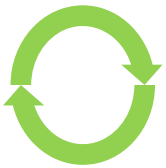


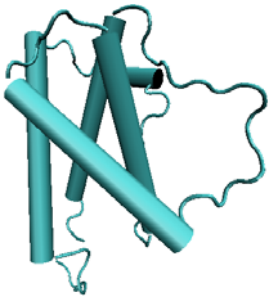
structure – ref.sample

II?



fingerprint –biosimilar sample





structure biosimilar sample

NOVARTIS

Computational methods for comparison of NMR fingerprints:

- 1D/amide/methyl fingerprint spectra overlays
- Principal component analysis
- CCSD (combined chemical shift difference)
- t-test analogue for chemical shift quantitation
- Normalized distances
- Peak shift difference- population analysis
- linear regression analysis of binned NMR spectra
- Image analys by spectral subtraction
- Graph invariant sequential nearest neigbours (SNN-GI)
- Tucker3
- Hiearchical clustering

Ref.:
Japelj, B. et al Sci Rep. 6, 32201 (2016)
Brinson RG, et al. MAbs. 11(1):94-105 (2019)
Ghasriani, H. *et al Nat. biotechnol.* 34**,** 139–141 (2016)
Amezcua, C. Et al. J. Pharm. Sci. 102, 1724–1733 (2013)
Chen, K. Et al. AAPS PharmSciTech, Vol. 19, No. 3, April 2018 (2017)
Župerl, Š et al J. Chem. Inf. Model. 47, 737–743 (2007).
Arbogast, L. Et al Anal Chem 89, 11839-11845 (2017)

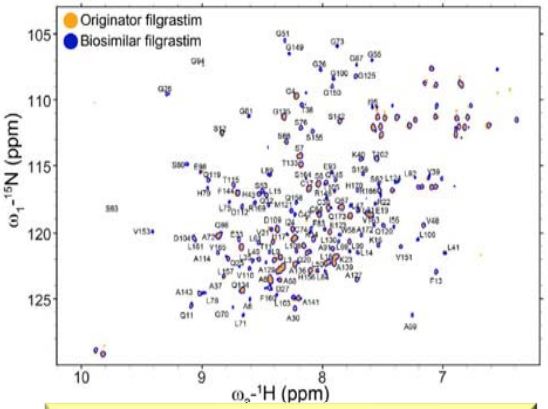
PCA, corr., Image analysis, norm. dist., HCA, t-test an.
CCSD, PCA
CCSD, PCA
Linear regression on binned NMR spectra – ECHOS-NMR
PCA, GI-SNN, Tucker3

GI-SNN Noesy spectra
Linear correlation, PCA

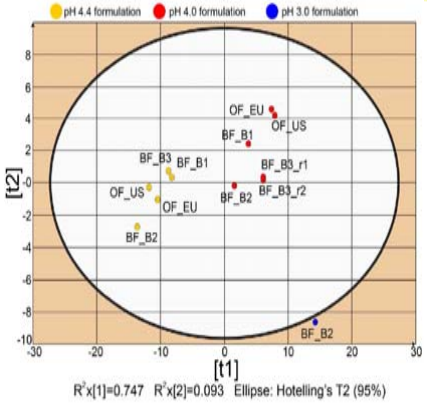
NOVARTIS

Similarity metrics overview

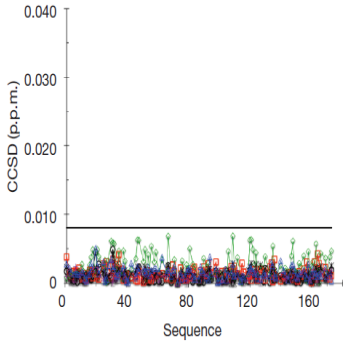
Spectral overlays



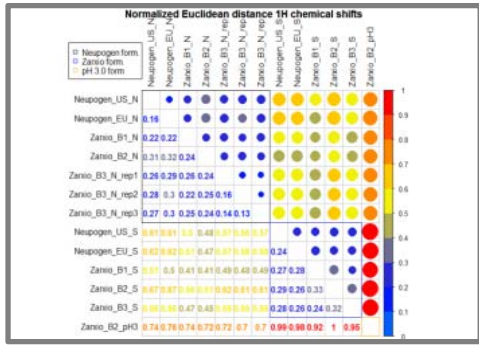
Principal components



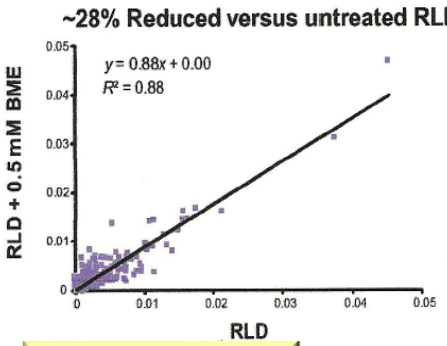
CCSD



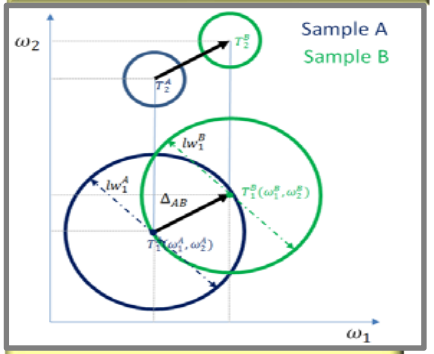
Normalized distances



Linear regression bined int.



t-test analogue



SNN-GI

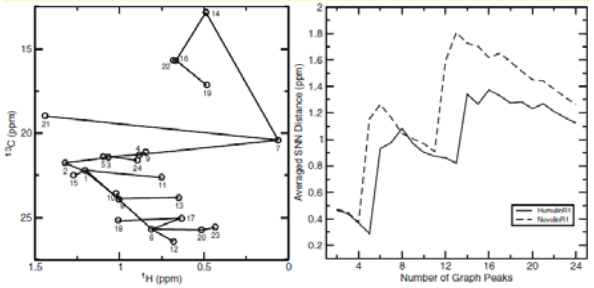
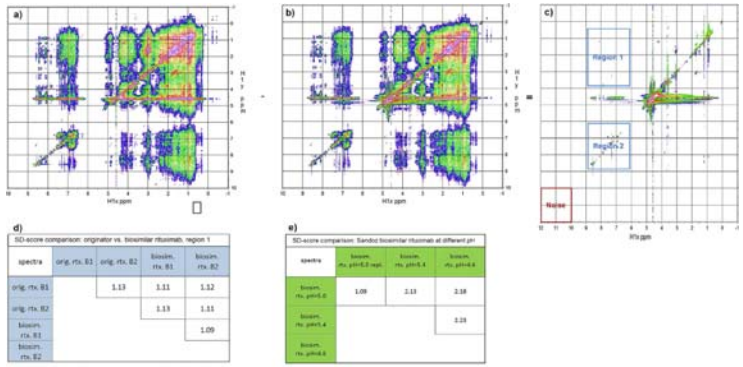
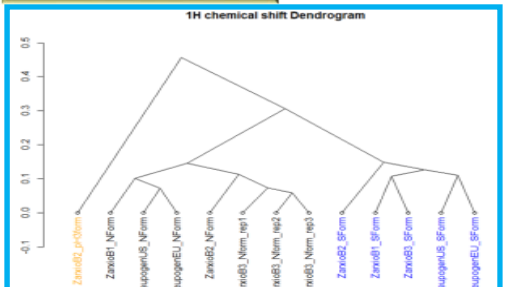


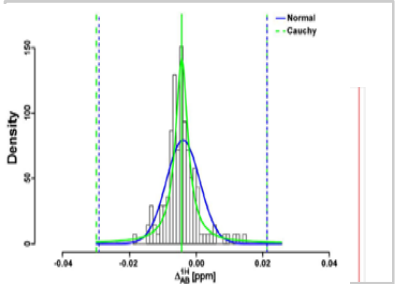
Image difference analysis



HCA



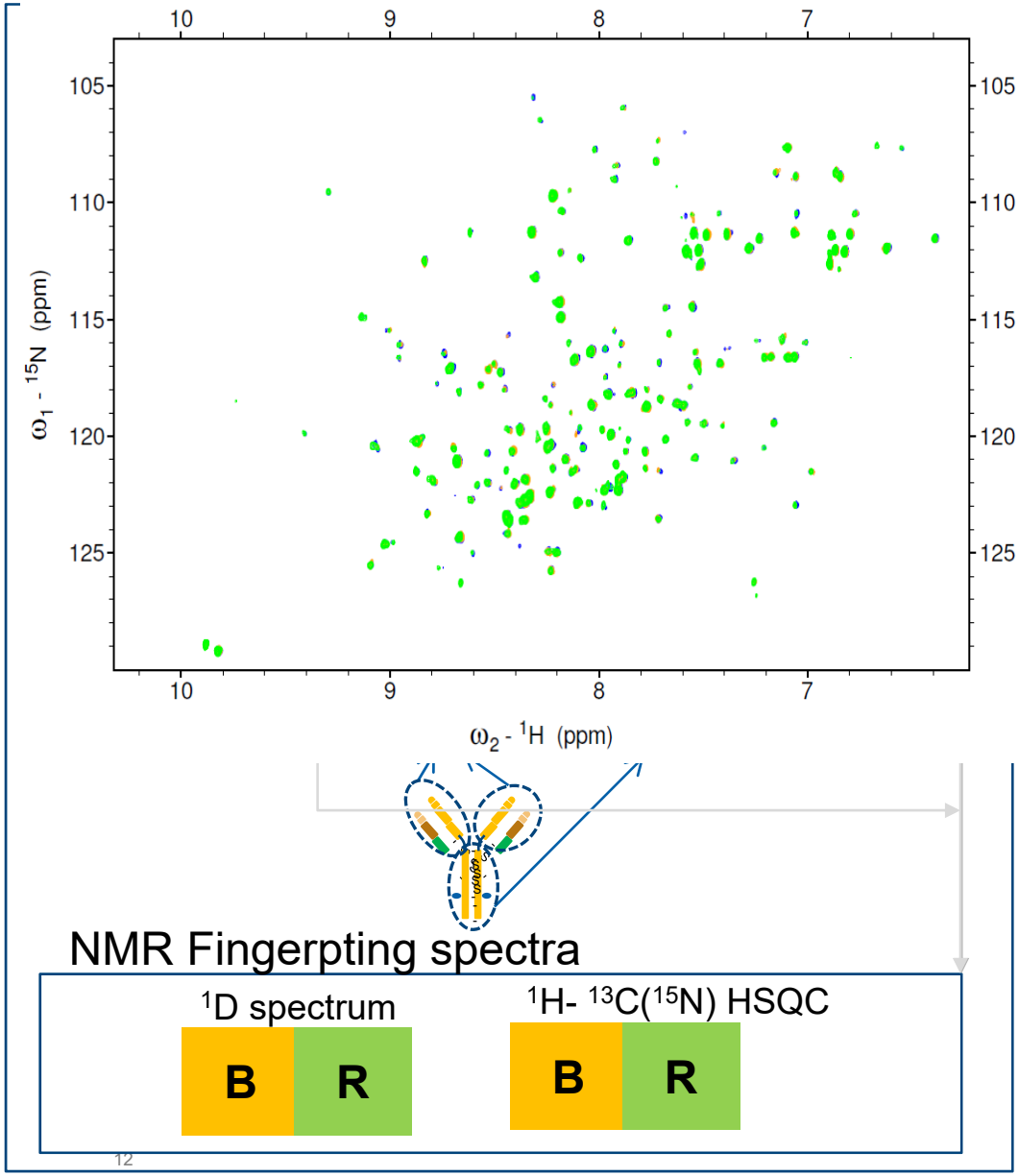
Peak shift differences



Ref.:
Japelj, B. et al Sci Rep. 6, 32201 (2016), Amezcua, C. Et al. J. Pharm. Sci. 102, 1724–1733 (2013)
Ghasriani, H. *et al Nat. biotechnol.* 34**,** 139–141 (2016), Chen, K. AAPS PharmSciTech, Vol. 19, No. 3, April 2018 (2017)
Župerl, Š et al J. Chem. Inf. Model. 47, 737–743 (2007), Arbogast, L. Et al Anal Chem 89, 11839-11845 (2017)

NOVARTIS

Comparison should be performed in the same environment (buffer)

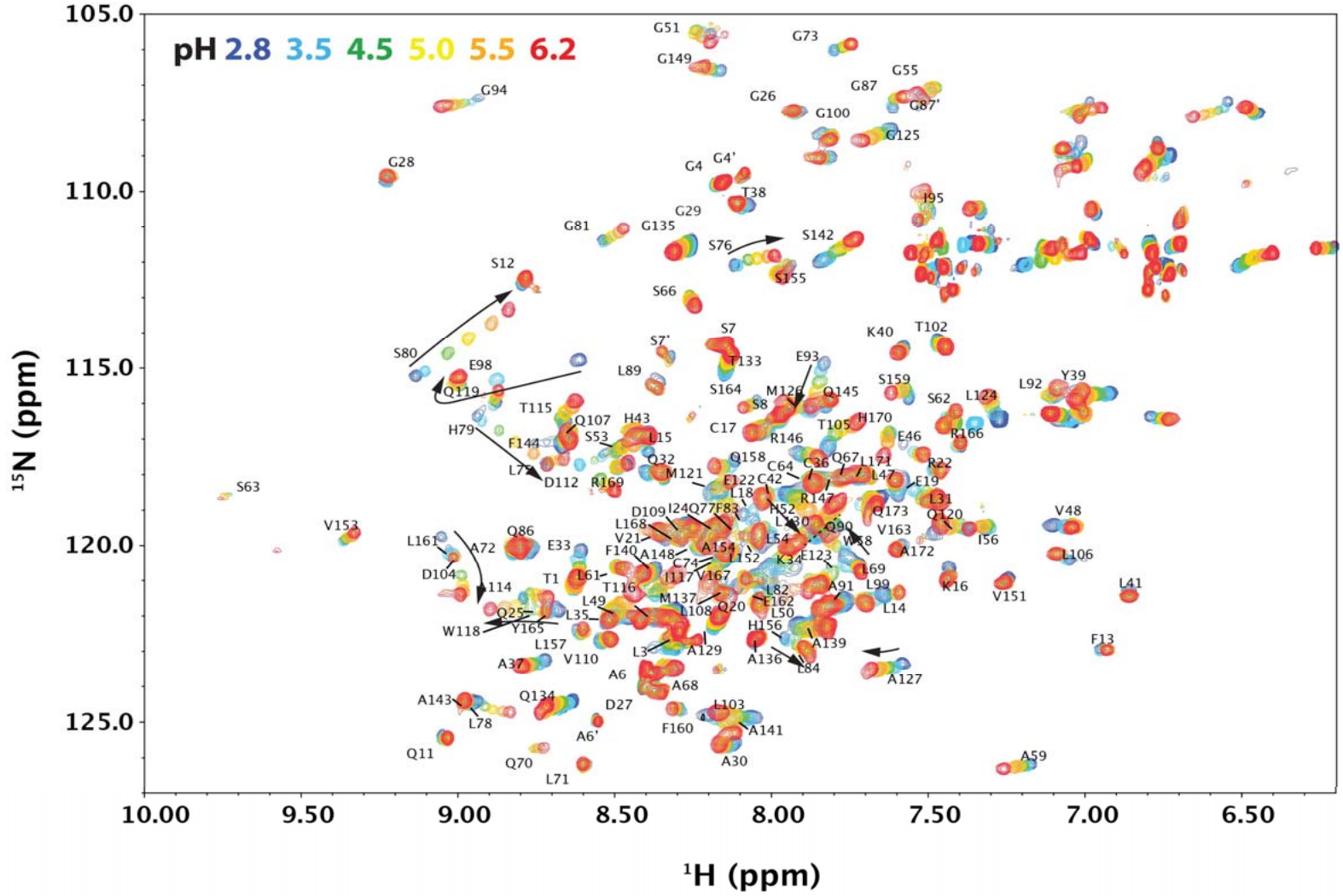


Legend:

color	Protein	Formulation
	Zarxio(R) Biosimilar filgrastim B1	Originator filgrastim
	Originator filgr. US	Originator filgrastim
	Originator filgr. EU	Originator filgrastim

NOVARTIS

NMR method sensitivity: pH effect on protein conformation

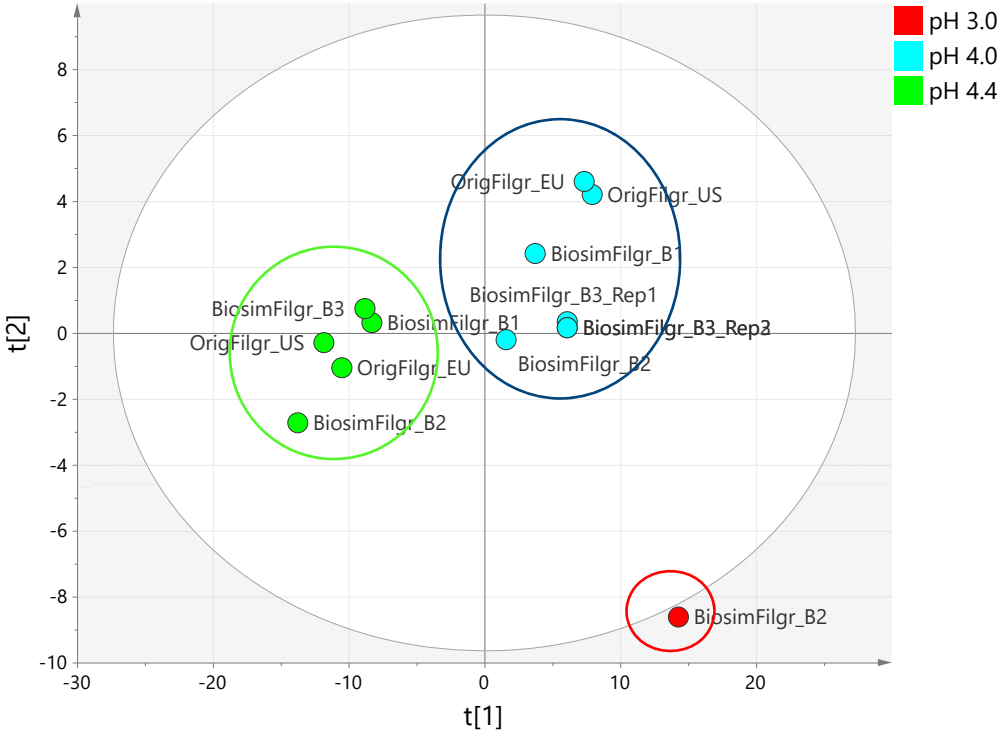


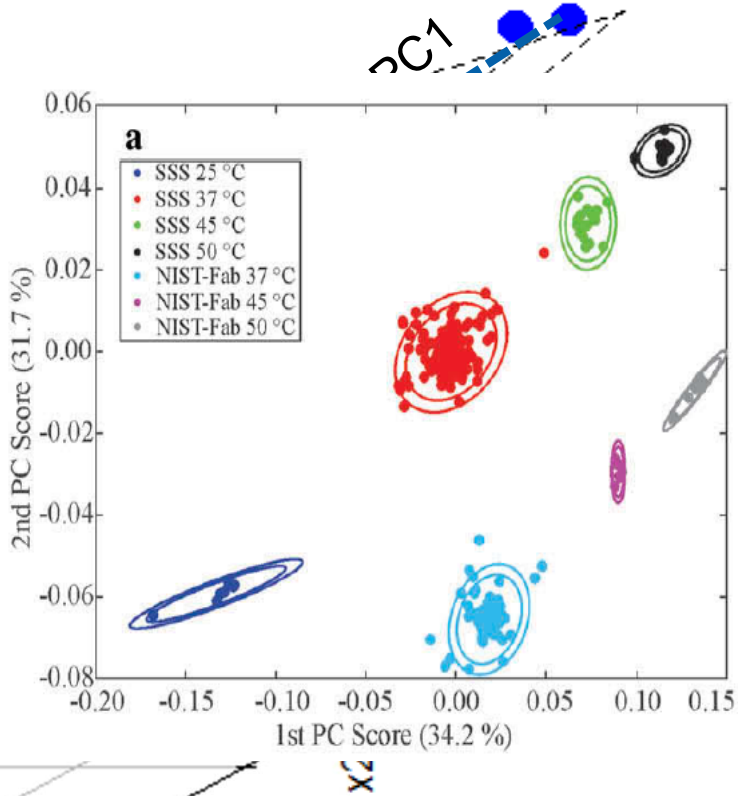
Aubin, Y., Hodgson, D.J., Thach, W.B. et al. Pharm Res (2015) 32: 3365, Suppl. Fig4

Japelj, B. et al Sci Rep. 6, 32201 (2016), Suppl. Fig2

NOVARTIS

Principal component analysis





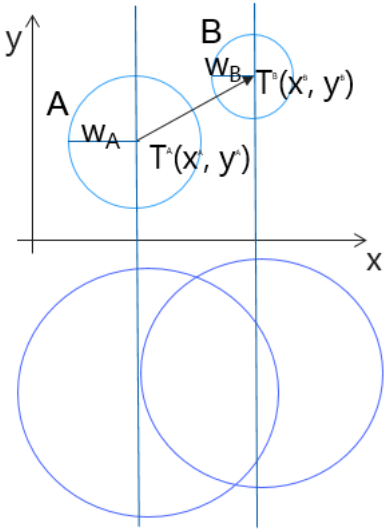
NMR method is sensitive enough to detect small pH induced conformational changes

Clustered PCA scatter plots of all peak lists from 354 ^{1}H-^{13}C spectra-26 laboratories

Brinson RG, et al. MAbs. 11(1):94-105 (2019)

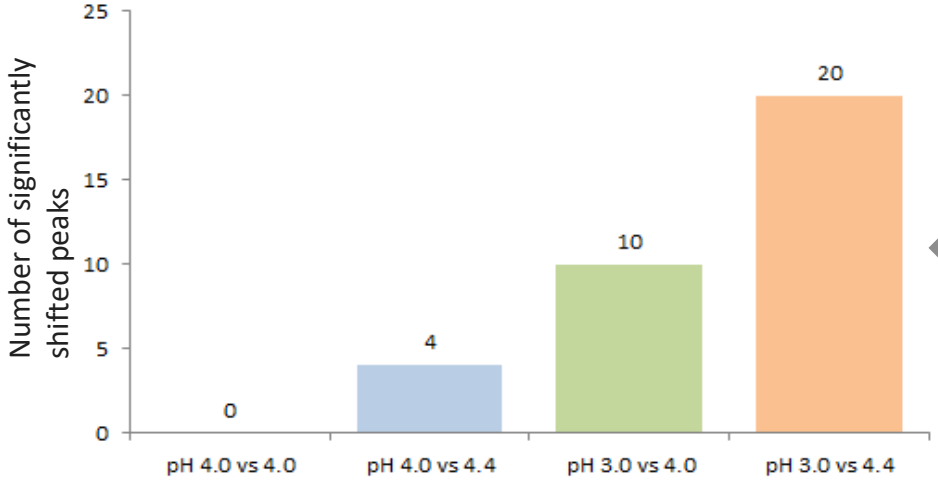
NOVARTIS

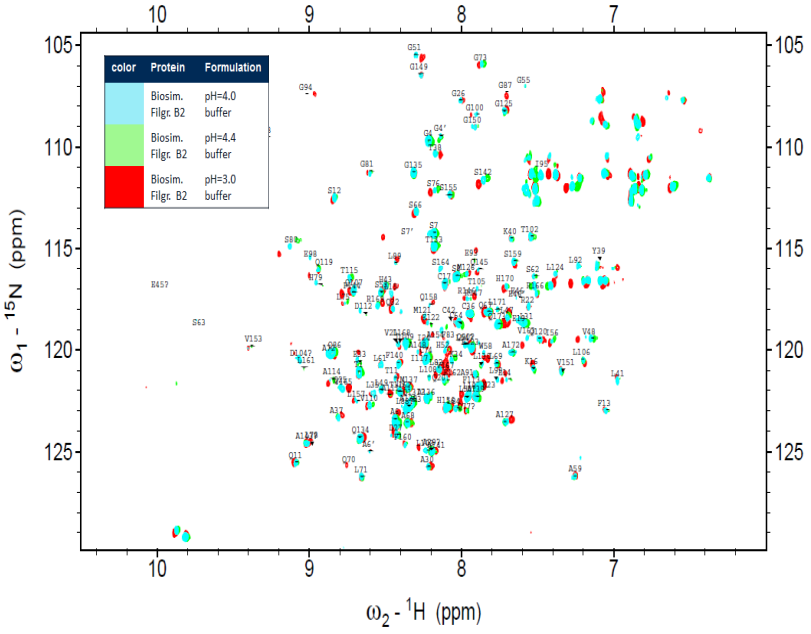
t-test analogue for chemical shift quantitation



$$d(T^A, T^B) = \sqrt{(x^A - x^B)^2 + \alpha^2 (y^A - y^B)^2}$$

$$t \sim \frac{d(T^A, T^B)}{\sqrt{w_A^2 + w_B^2}}$$



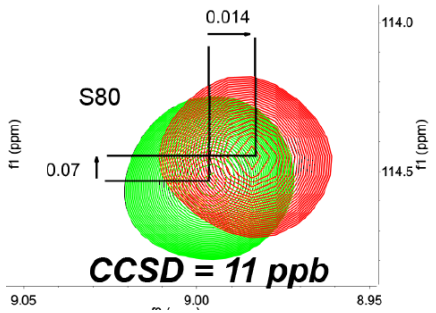


NOVARTIS

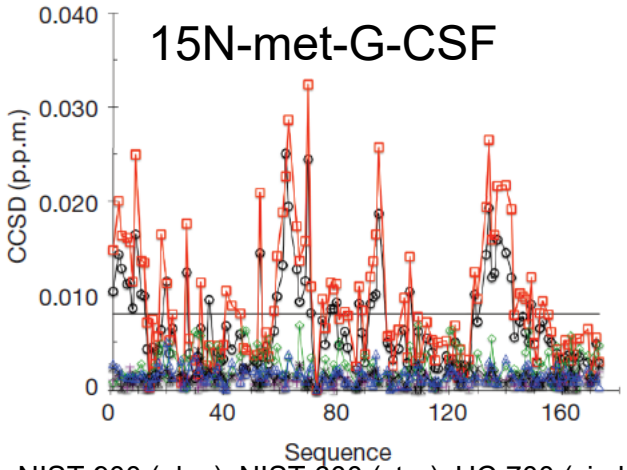
Combined chemical shift difference (CCSD)

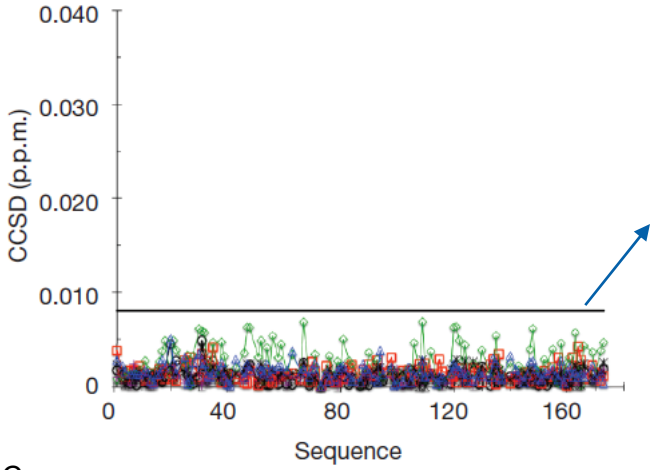
$$CCSD = \sqrt{(0.5 * [(\delta_H)^2 + (\alpha * \delta_N)^2])}$$

α=0.1



Temperature calibration





8 p.p.b.

NIST 900 (plus), NIST 600 (star), HC 700 (circle), HC 600 (square), FDA 500 (diamond) and MPA 600 (triangle)

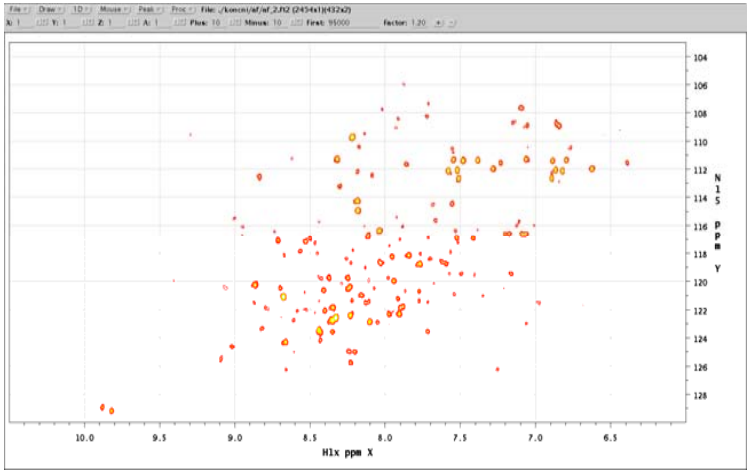
Ghasriani, H. *et al Nat. biotechnol.* 34, 139–141 (2016)

NOVARTIS

Image difference analysis

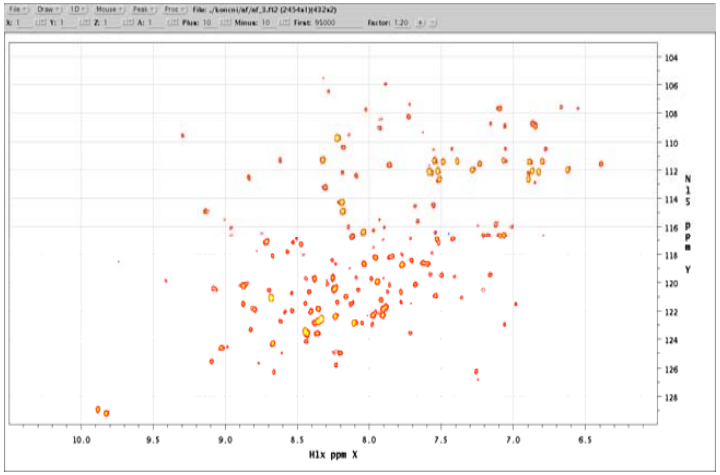
Subtraction of G-CSF ^{1}H-^{15}N HSQC spectra

Originator filgrastim EU



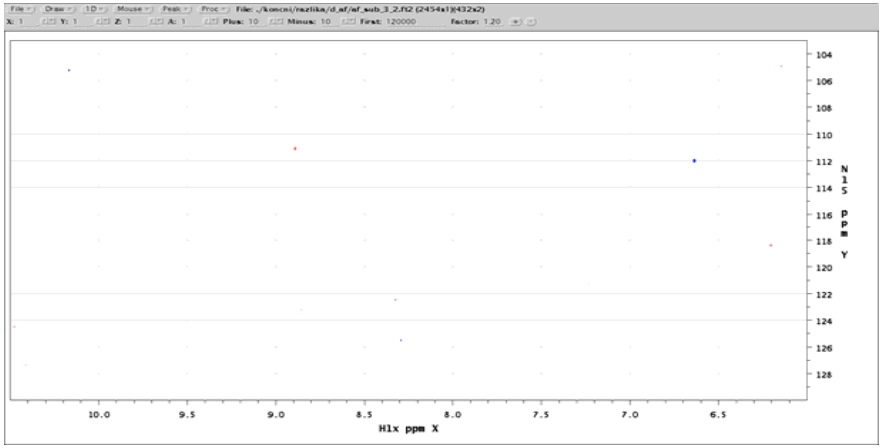
−

Biosimilar filgrastim B1



=

Difference spectrum

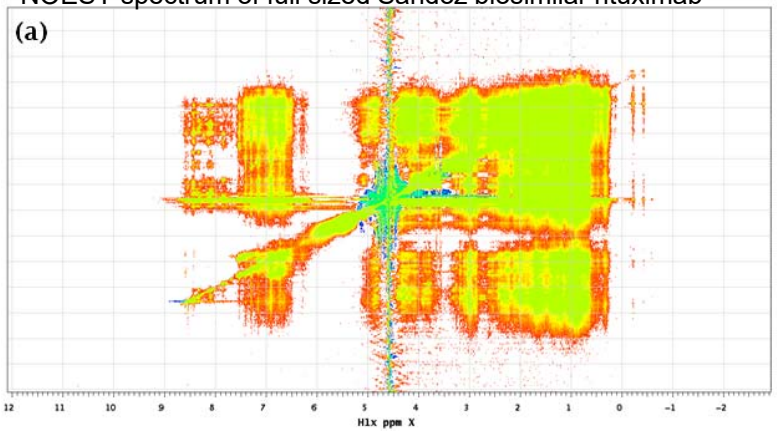


NOVARTIS

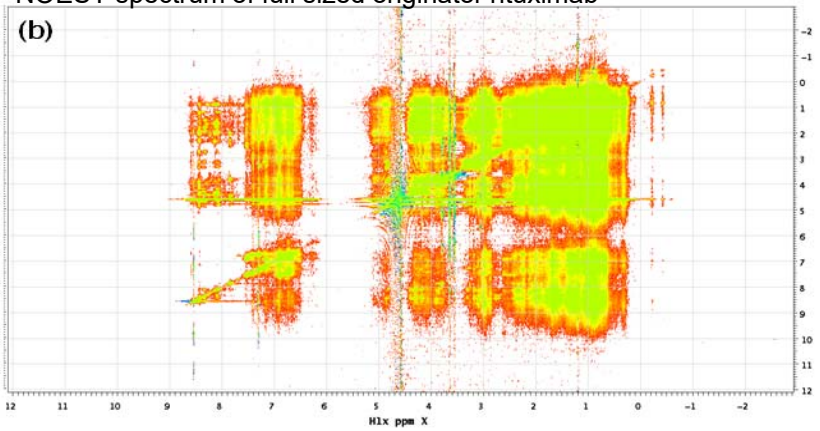
Image difference analysis

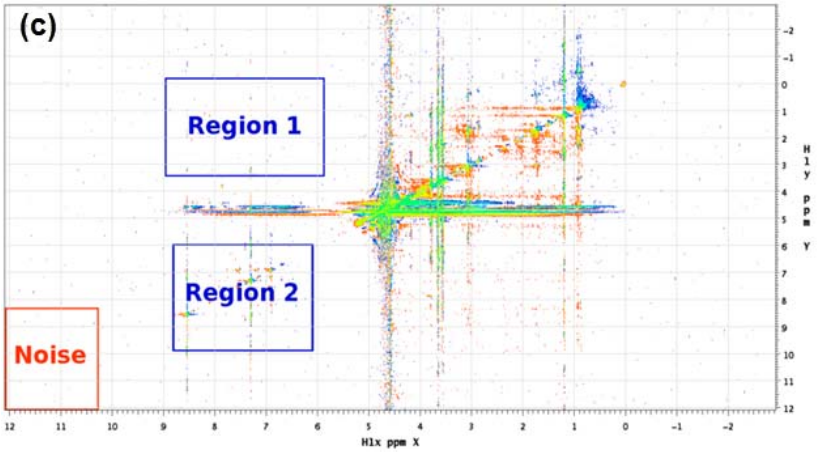
Subtraction of rituximab NOESY spectra

NOESY spectrum of full sized Sandoz biosimilar rituximab



NOESY spectrum of full sized originator rituximab





In house software

Raw data (FID)

Fourier transformation

Automatic phase correction

Automatic baseline correction

SRB vs Orig. Ritux. US vs EU reference product

SD-score

SD-scores, region 1	SBR B1, Orig. Rituxan® B1 replicate	SBR B1 Orig. Rituxan® B2	SBR B1 Orig. Rituxan® B1	SBR B1 Orig. Rituxan® B2	SBR B1 Sandoz biosim. ritux. B1
		pH=5.4	pH=5.0	pH=5.4	pH=4.6
SBR B1 Orig. Rituxan® B1 pH=5.0	1.09	1.13	1.18	1.18	
SBR B1 Orig. Rituxan® B2 pH=5.4				1.23	
SBR B1 Sandoz biosim. ritux. B1 pH=4.6					

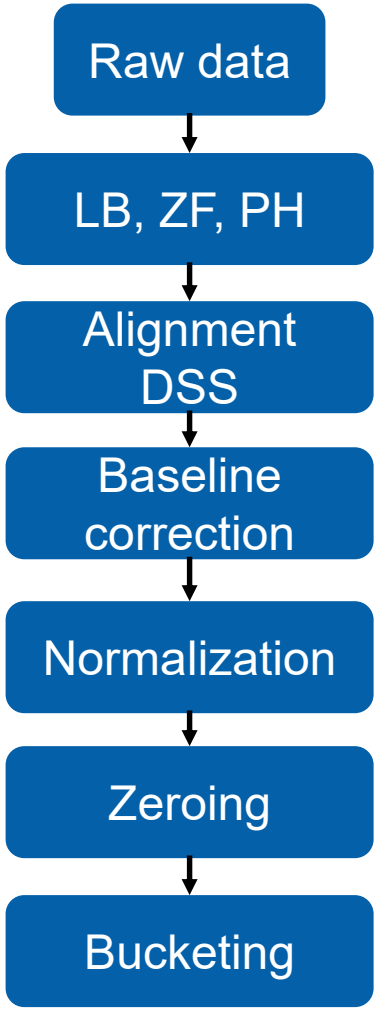
pH change of 0.4 units increases the SD-score by ~2-fold

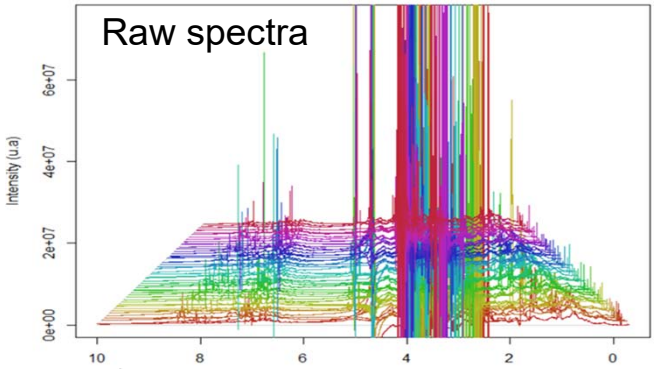
$$SD\ score = \sqrt{signal_{Region\ 1,2}^{\ 2} \Big/ signal_{Noise}^{\ 2}}$$

J.J. Helmus, C.P. Jaroniec, J. Biomol. NMR 2013, 55, 355-367

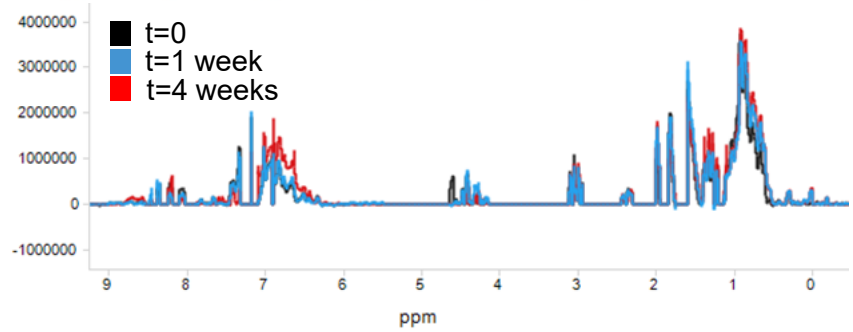
High troughput 1D NMR workflow for stability screening

Formulation screening: pH, NaCl, Stabilizers, Buffers

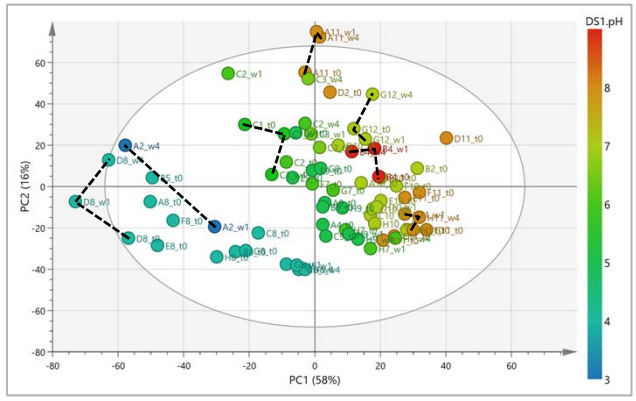




NMR spectra,
73 samples
14 stability samples
t ∈{0, 1 week, 1month}

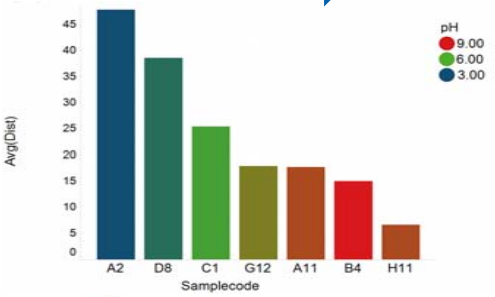


Normalization



Stability ranking:

unstable → stable



Jacob, D., et al NMRProcFlow, Metabolomics 13:36 (2017)

NOVARTIS

Conclusions

- NMR is a powerful method to compare protein HOS
- can detect conformational changes, changes in formulation, stability changes (PTMs), sequence variants, glycosylation
- methods available for small and large proteins: ^{1}H-^{13}C gsHSQC (methyl), ^{1}H-^{15}N HSQC (amide) fingerprints + rapid pulsing + NUS
- chemometrics/computational methods available to compare and evaluate differences between NMR spectra for global and peak-to-peak comparison

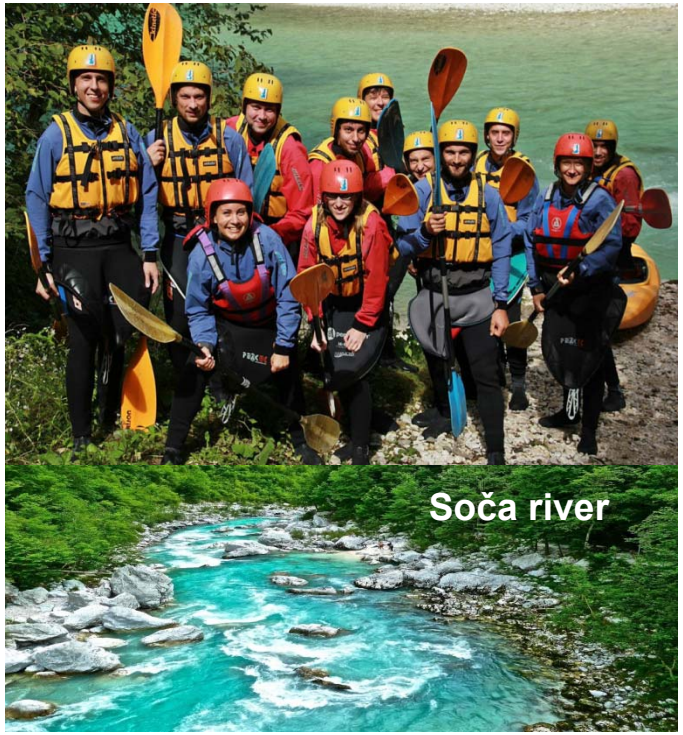
Acknowledgement

EN-FIST centre of excelence,

Ljubljana (SLO):

Gregor Ilc

Prof. Janez Plavec



Novartis Global Drug Development / Technical Research & Development:

Jaka Marušič

Drago Kuzman

Jure Senčar

Mitja Zidar

Mateja Salobir

Matej Horvat

Stefan Prasch

Isabel Feuerstein

Johann Holzmann

Prof. Uroš Urleb

NOVARTIS