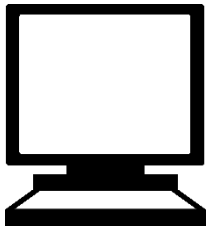
NMR Based Similarity Metrics for Higher Order Structure Assessment among U.S. Marketed Insulin Drug Products

Kang Chen

Division of Complex Drug Analysis (DCDA)
Office of Testing and Research (OTR)
Office of Pharmaceutical Quality (OPQ)
Center for Drug Evaluation and Research (CDER)
U.S. Food and Drug Administration (FDA)

Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.


FDA
Patients expect safe and effective medicine with every dose they take.
www.fda.gov

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.

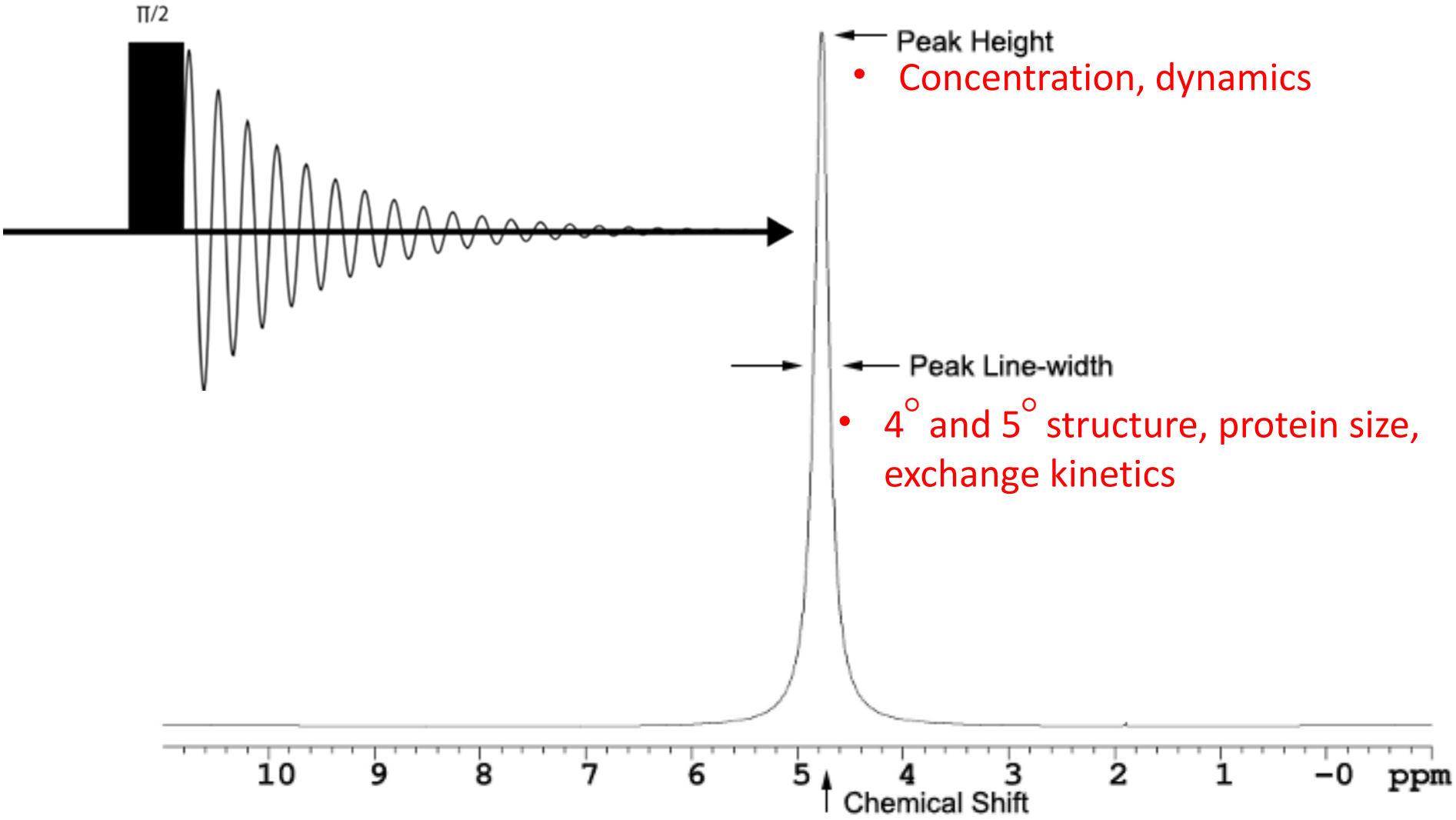

It is what gives patients confidence in their *next* dose of medicine.

Disclaimer

THIS PRESENTATION REFLECTS THE VIEWS OF THE AUTHOR AND SHOULD NOT BE CONSTRUCTED TO REPRESENT FDA'S VIEWS OR POLICES.

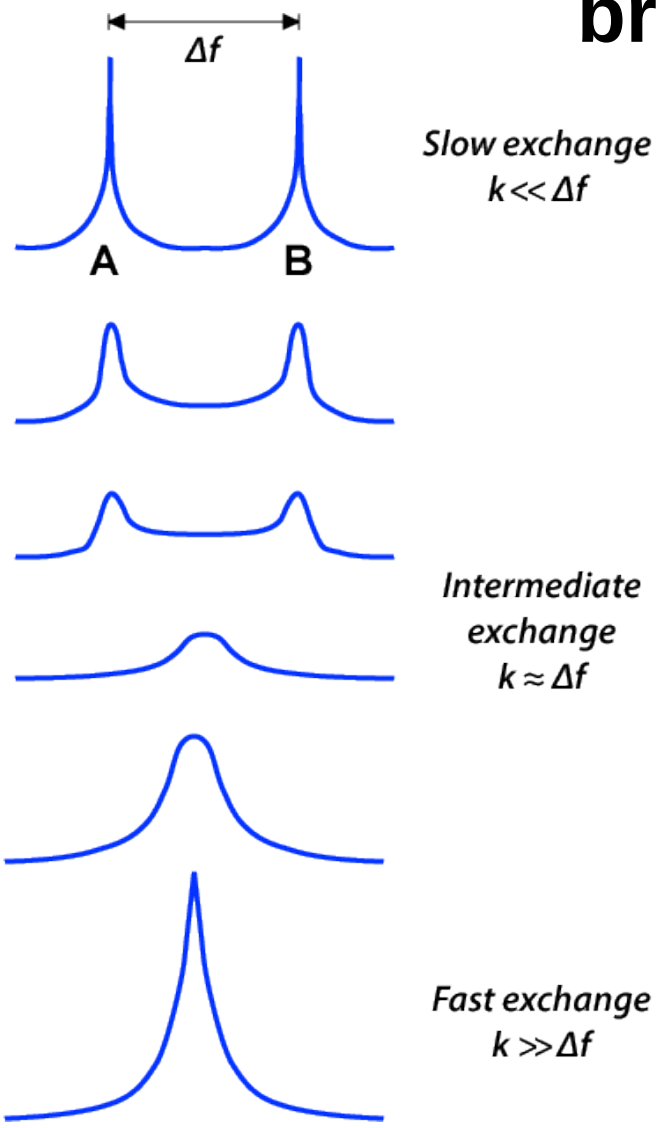
NMR Based Similarity Metrics for Higher Order Structure Assessment among U.S. Marketed Insulin Drug Products

What a protein/peptide NMR peak reveals



- Concentration, dynamics
- 4° and 5° structure, protein size, exchange kinetics
- Chemical identity and 2° and 3° structure

Exchange Kinetics and NMR Line-broadening



$$A \longleftrightarrow B$$

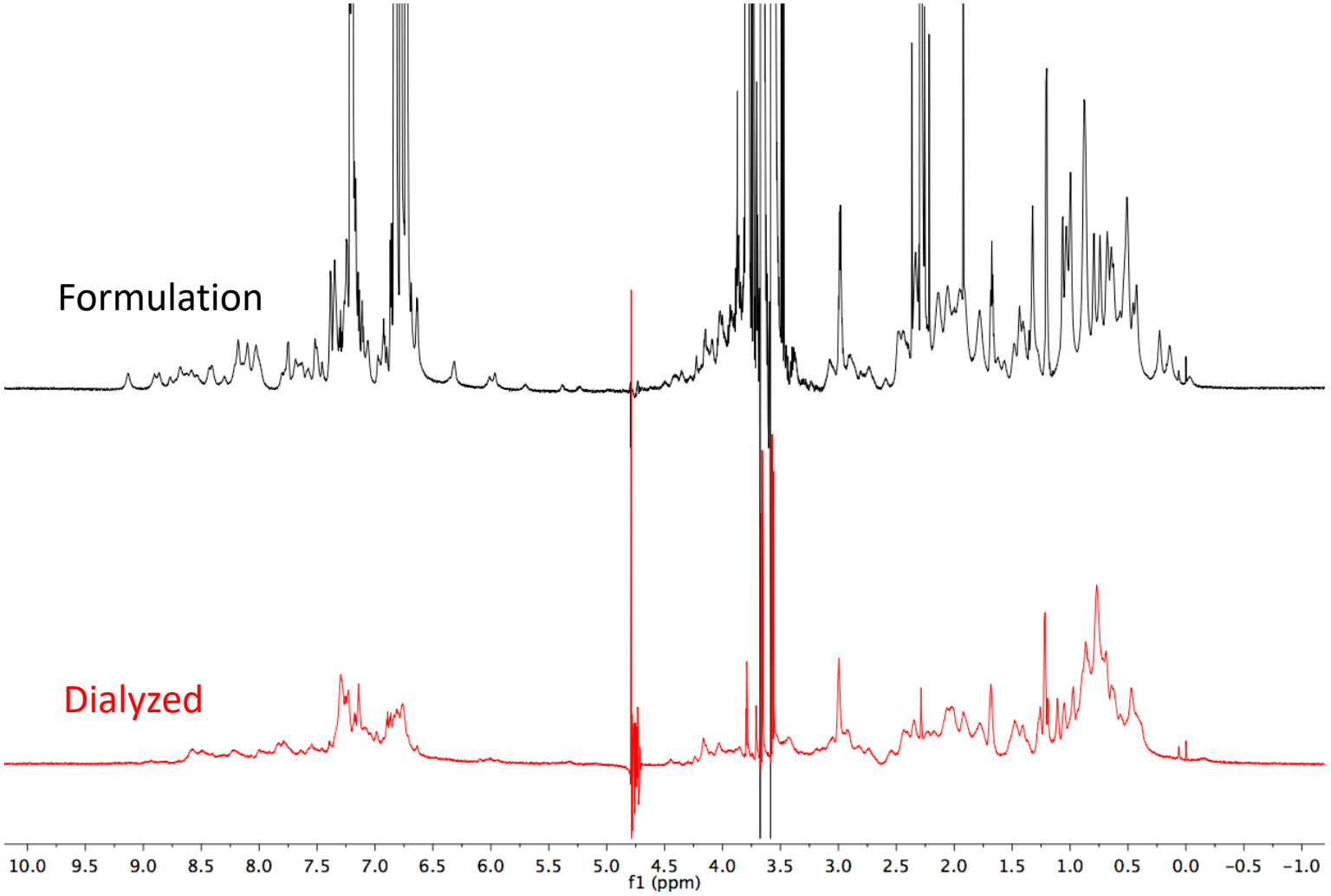
$$k=k_{AB}+k_{BA}, \quad \Delta f=|f_A-f_B|$$

Exchange: chemical or conformation;

Exchange/heterogeneity is common for any molecule in solution.

Palmer, Kroenke, and Loria. (2001) Method Enzyml.

Insulin spectra under different buffer



Both chemical shift and line-width changed upon dialysis.

Higher Order Structure

- **Standard definition**: any non-covalent interaction (e.g., H-bond) stabilized secondary, tertiary and quaternary structures.

- **Broad definition**: quinary structure, oligomerization, aggregation, equilibrium and exchange kinetics among different structural forms, e.g., folding/unfolding, dimer/hexamer etc.

Agency's Guidance on HOS

A meaningful comparative analytical assessment depends on, among other things, the capabilities of available state-of-the-art analytical assays to assess, for example, the molecular weight of the protein, complexity of the protein (higher order structure and posttranslational modifications), degree of heterogeneity, functional properties, impurity profiles, and degradation profiles denoting stability.

Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations, *https://www.fda.gov/media/125484/download*

The sameness of active ingredient in a proposed generic synthetic peptide can be established through physicochemical characterization and biological evaluation. … the following properties and other properties, as appropriate: …Secondary structure; Oligomer/Aggregation states…

https://www.fda.gov/files/drugs/published/ANDAs-for-Certain-Highly-Purified-Synthetic-Peptide-Drug-Products-That-Refer-to-Listed-Drugs-of-rDNA-Origin-Guidance-for-Industry.pdf

Ideal NMR Approaches for HOS Comparison

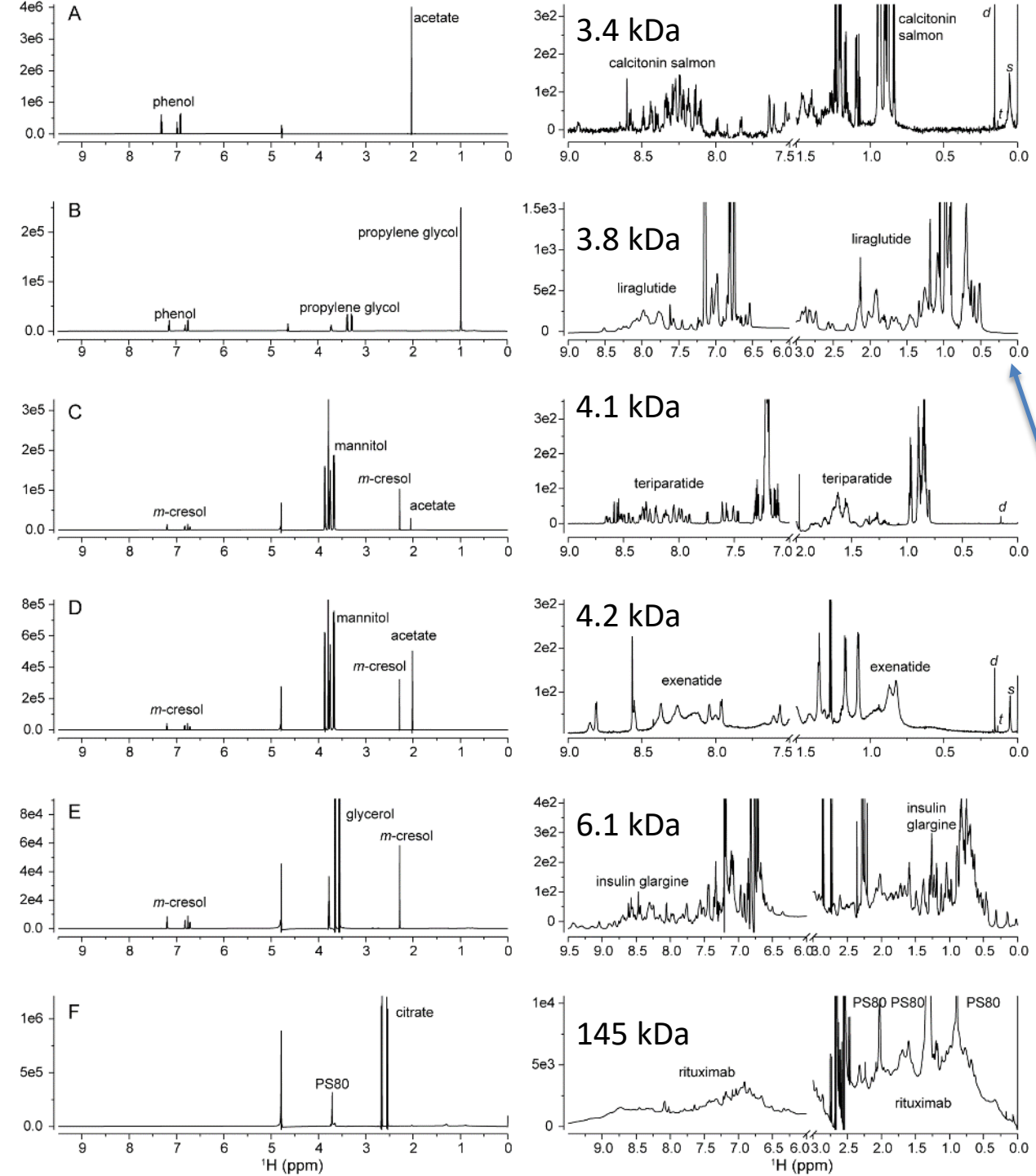
Direct testing on drug product (DP) whenever possible b/c,

- *HOS can change upon formulation difference;*
- *Better reproducibility;*
- *Generic/biosimilar sponsors may only have access to the originator DP.*

Problems for NMR in HOS Similarity

- The type of HOS properties reliably measured from DP using NMR was not entirely clear.
- Any similarity metrics for quantitative assessment?
- What level of similarity is realistically achievable?

1D NMR Spectra of protein DPs

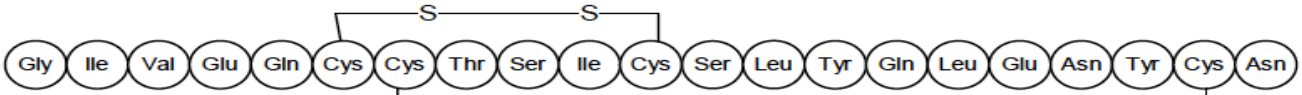


Protein aggregation and/or intermediate exchange in Saxenda® and Byetta®.

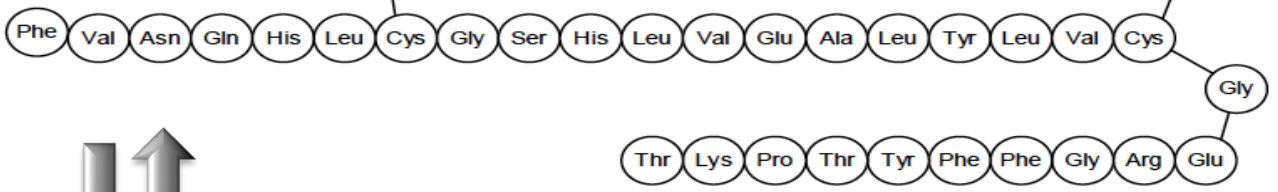
Well folded protein in Miacalcin®, Forteo®, Lantus®, Rituxan®.

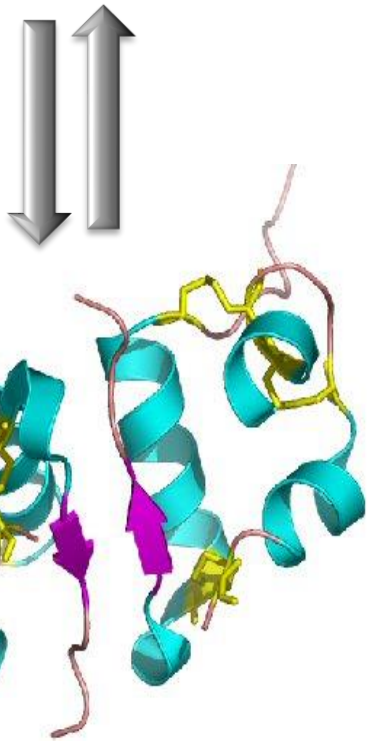
Insulin Structure and Equilibrium

A-chain, 21 aa

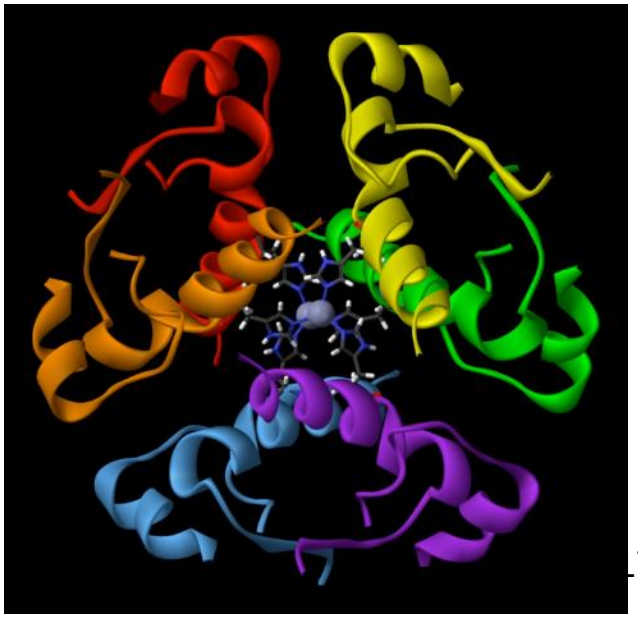


B-chain, 30 aa





Phenol
Zn^{++}



Pdb:1mso,
1ev6, 1lph

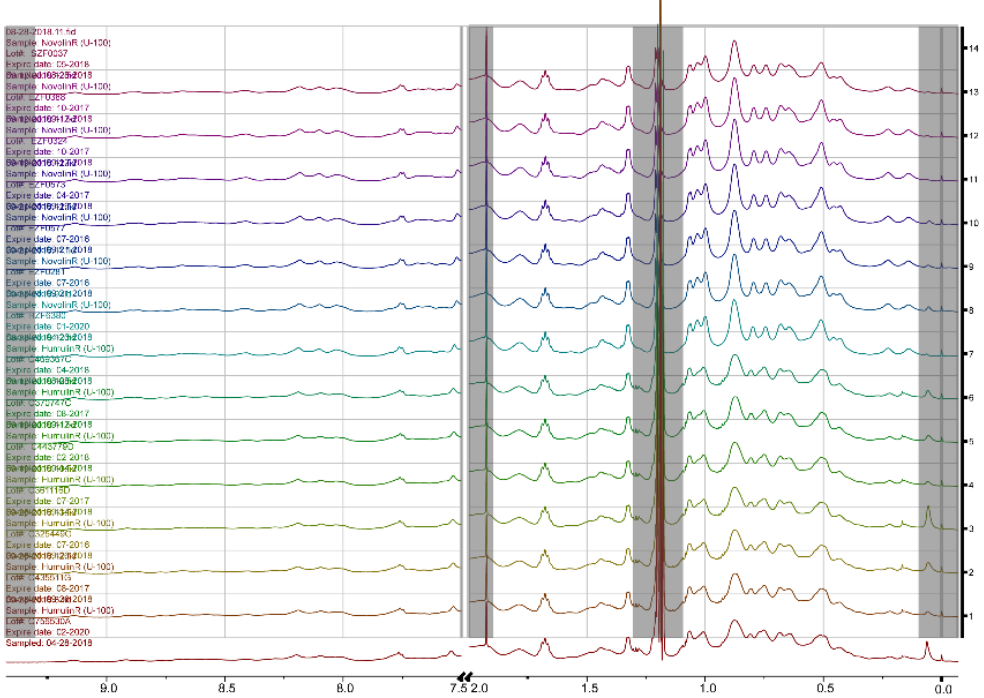
Journal of Molecular Biology [2002, 318(2):479-490]

Some of US Marketed Insulin Drug Products

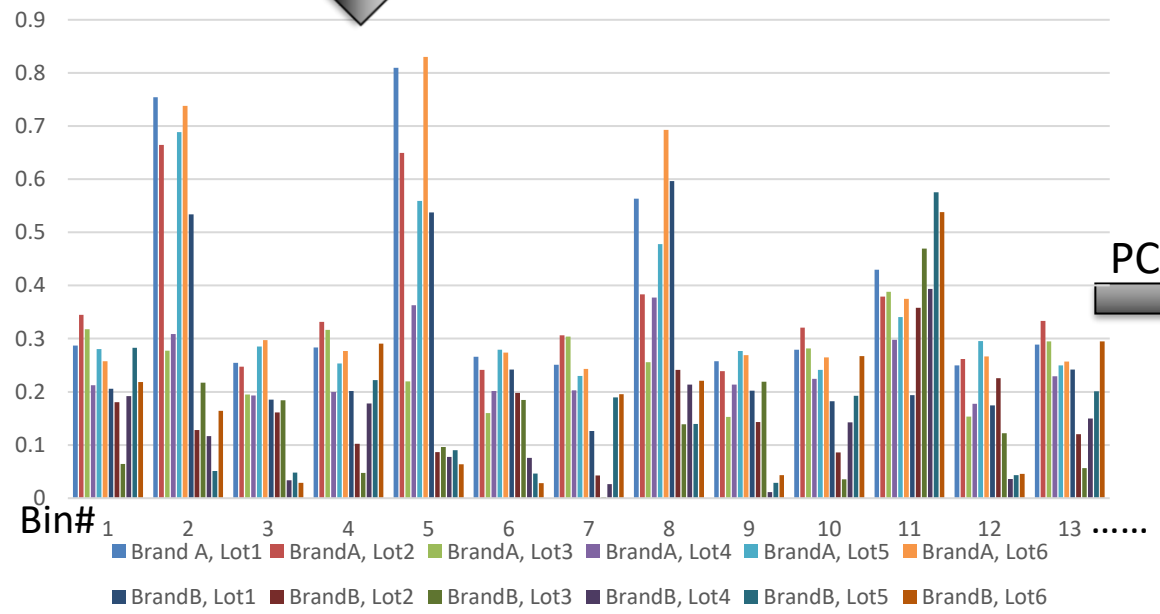
Insulin Type	Drug Substance	Drug Product	Approval Type	Year approved
Rapid acting	Insulin Lispro B28: P->K B29: K->P	Humalog®	New Drug	1996
		Admelog®	Follow-on 505(b)(2)	2017
Long acting	Insulin Glargine B3: N->K B29: K->E	Lantus®	New Drug	2000
		Basaglar®	Follow-on 505(b)(2)	2015
Short acting	Insulin Human	HumulinR®	New Drug	1982
		NovolinR®	New Drug	1991

Wang, D.; Park; Patil, S.; Smith, C.; Leaser, J.; Keire, D.; Chen, K., An NMR Based Similarity Metric for Higher Order Structure Quality Assessment among U.S. Marketed Insulin Therapeutics. J. Pharm. Sci, **2020**.

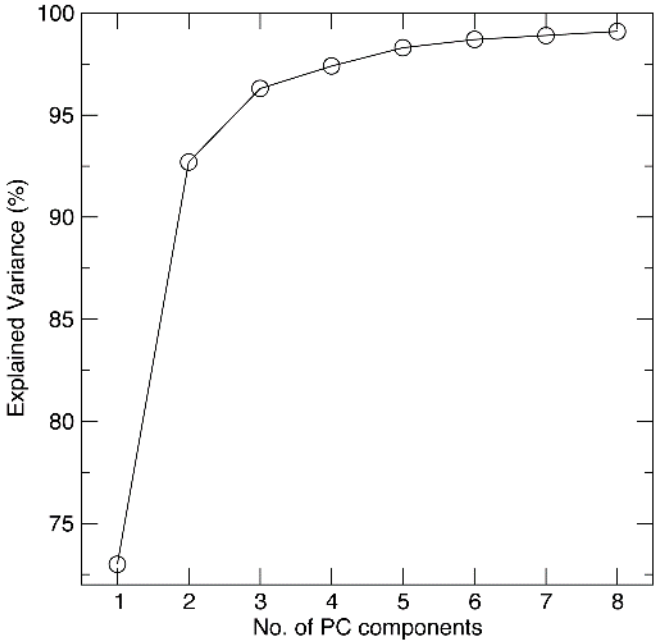
Principal Component Analysis



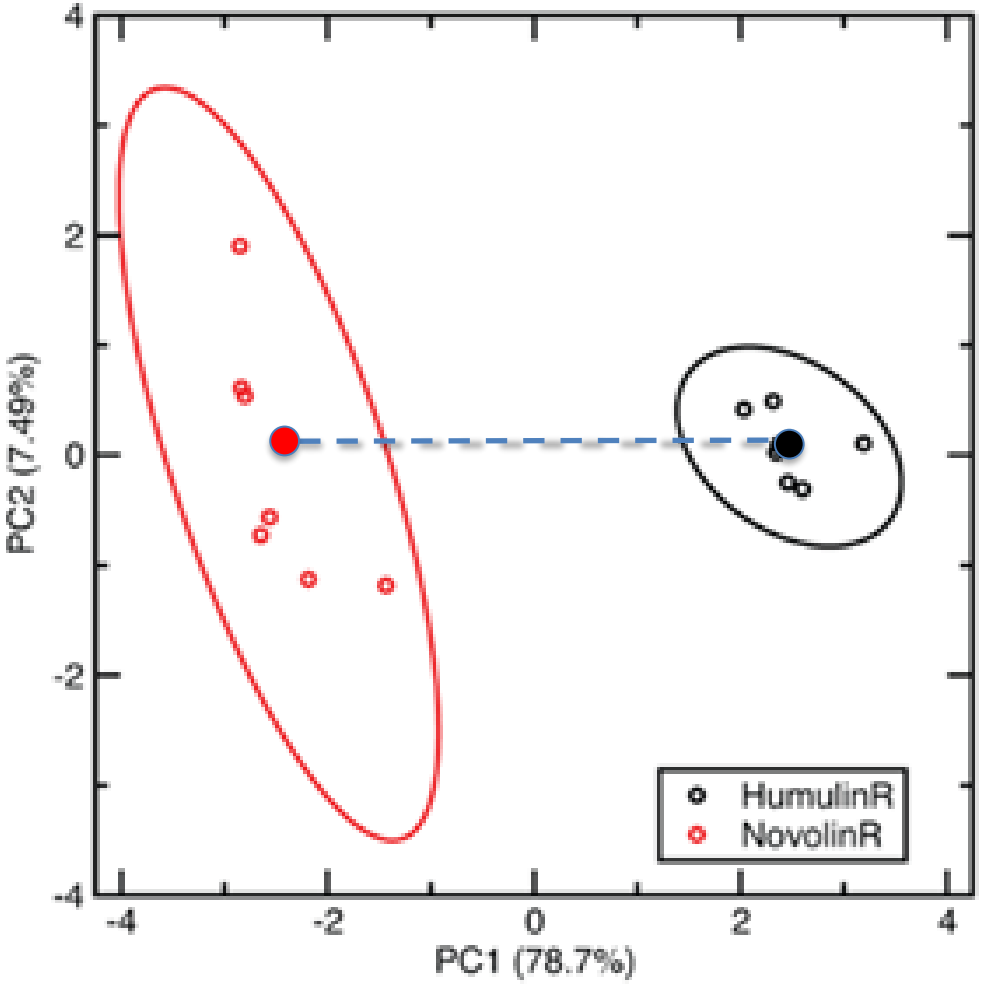
Digitization



PCA



Inter-brand Similarity



Mean vector of the HumulinR®

$$\bar{Z}_H = \left(\sum_{i=1}^{\mathrm{m}} Ha_i\right)/\mathrm{m}$$

Mean vector of the NovolinR®

$$\bar{Z}_N = \left(\sum_{i=1}^{\mathrm{n}} Na_i\right)/\mathrm{n}$$

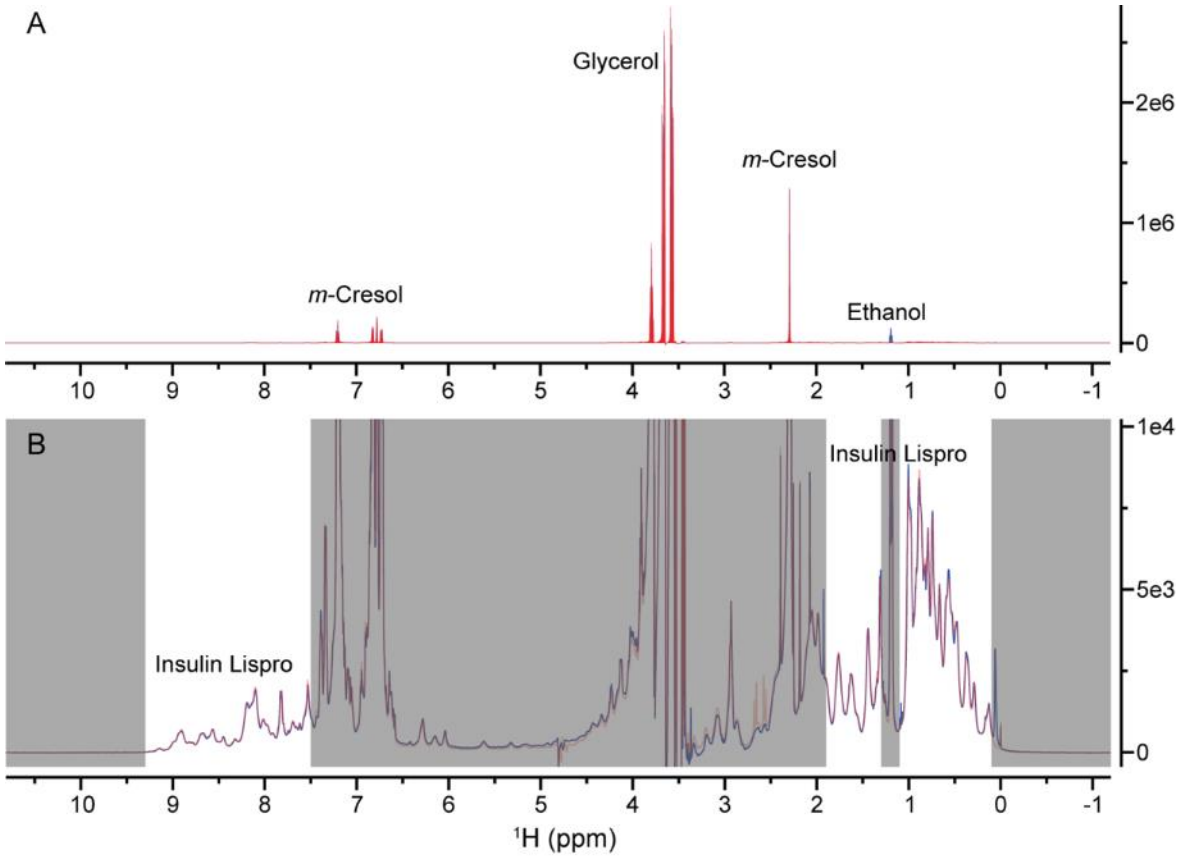
Covariance matrices

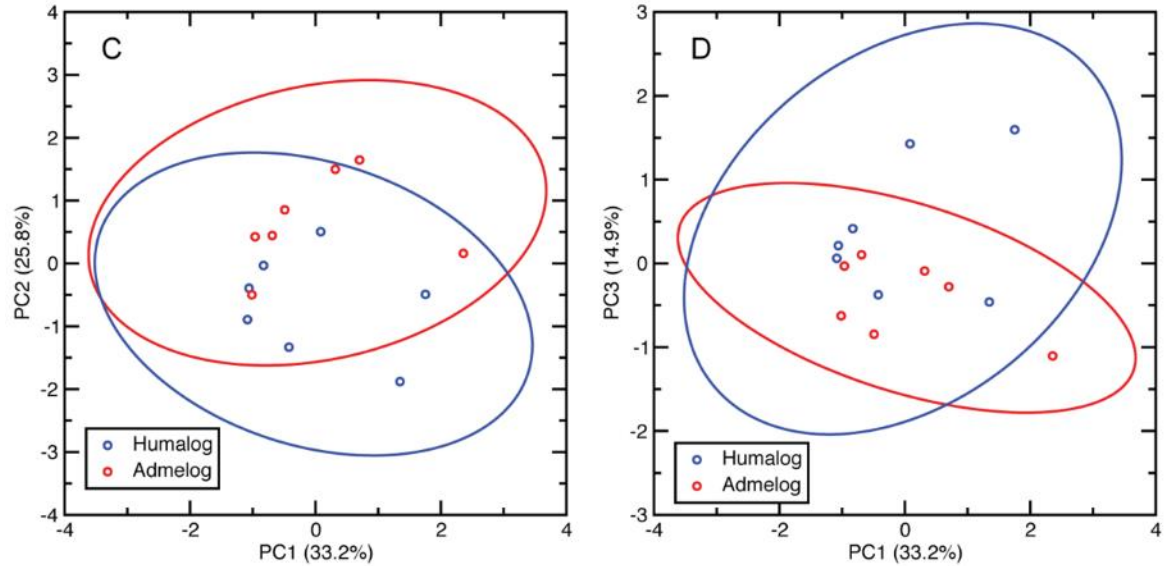
$$S = (mS_H + nS_N)/(m+n)$$

Mahalanobis distance (D_M)

$$D_M = \mathrm{sqrt}[(\bar{Z}_H - \bar{Z}_N)S^{-1}(\bar{Z}_H - \bar{Z}_N)']$$

Chen K, Park J, Li F, Patil SM, Keire DA. *AAPS PharmSciTech*, 2018, 19(3):1011-1019.





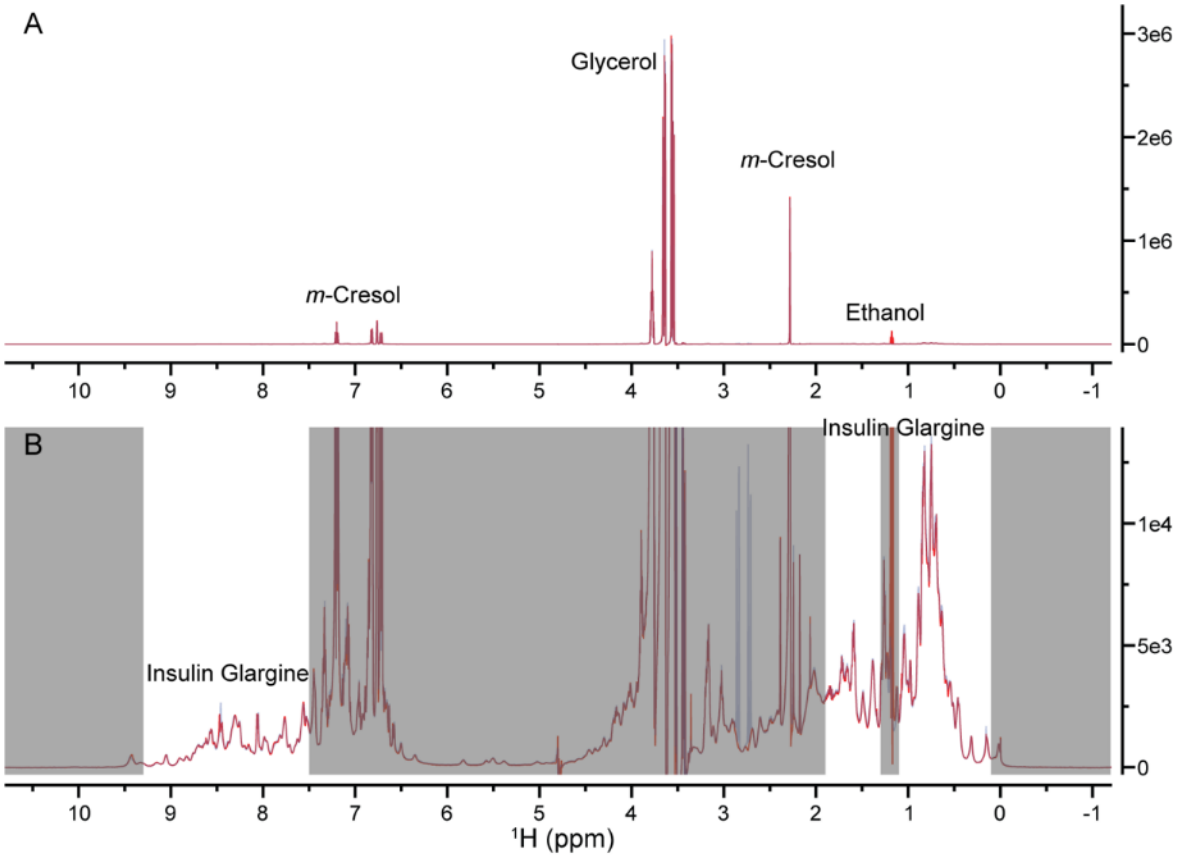
Humalog®

vs

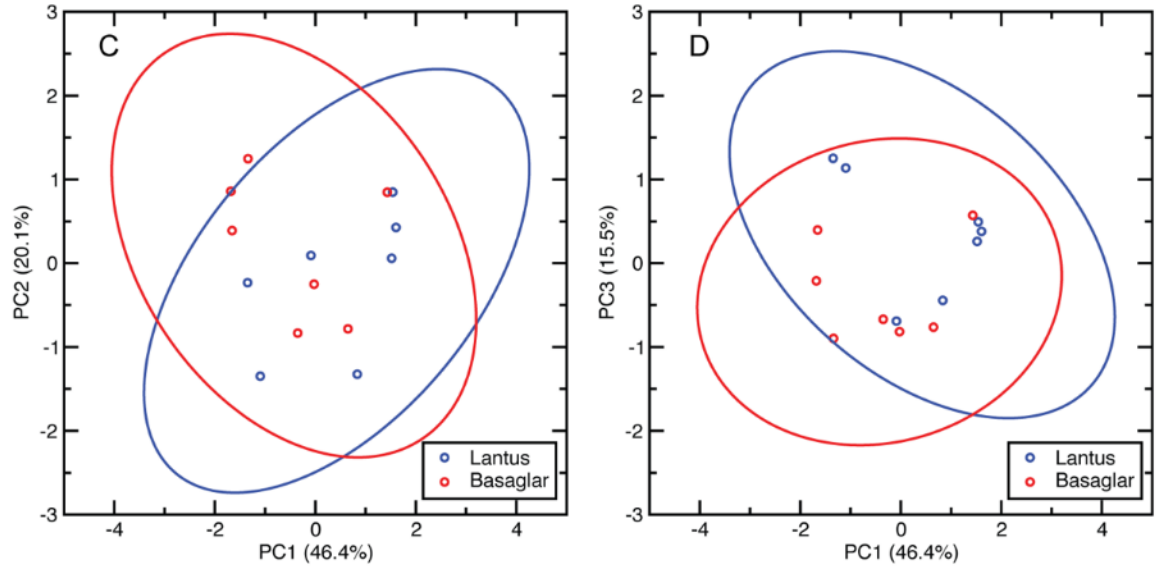
Admelog®

NMR sample: DP + 5% D_2O

$D_M = 1.58$

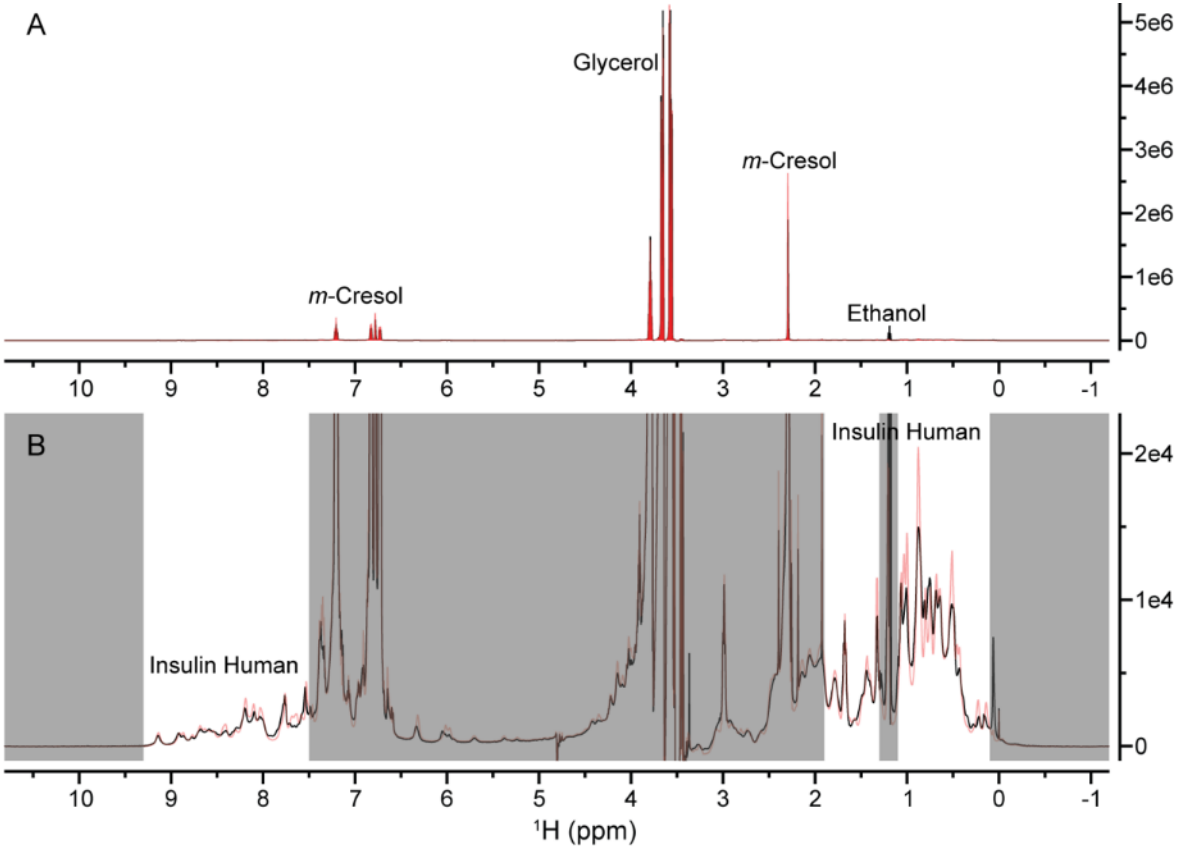


Lantus® and Basaglar®

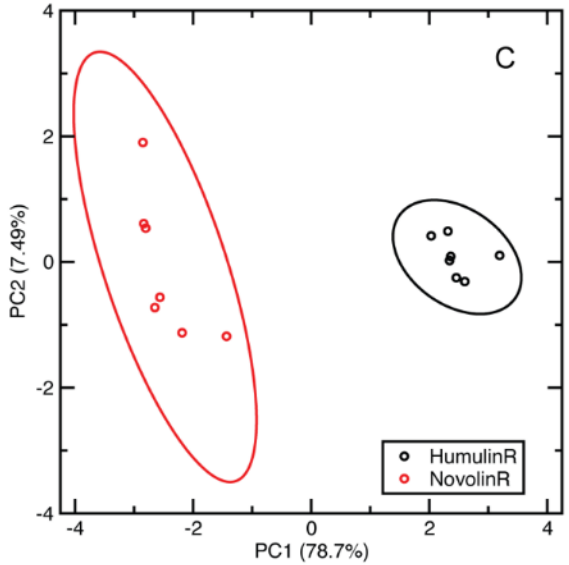


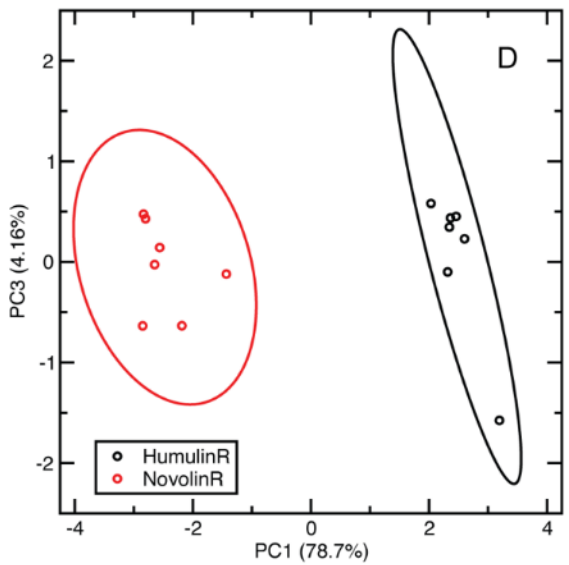
NMR sample: DP + 5% D_2O

$D_M = 3.29$



HumulinR® and NovolinR®





NMR sample: DP + 5% D_2O

$D_M = 20.5$

Achievable Similarity Metrics

Insulin Type	Drug Substance	Drug Product	Approval Type	Year approved	Inter-brand D_M
Rapid acting	Insulin Lispro	Humalog®	New Drug	1996	3.29
		Admelog®	Follow-on 505(b)(2)	2017	
Long acting	Insulin Glargine	Lantus®	New Drug	2000	1.58
		Basaglar®	Follow-on 505(b)(2)	2015	
Short acting	Insulin Human	HumulinR®	New Drug	1982	20.5
		NovolinR®	New Drug	1991	

Wang, D.; Park; Patil, S.; Smith, C.; Leaser, J.; Keire, D.; Chen, K., An NMR Based Similarity Metric for Higher Order Structure Quality Assessment among U.S. Marketed Insulin Therapeutics. J. Pharm. Sci, **2020**.

D_M in Metabolomics

Table 1
Summary of Mahalanobis distances for cluster separations and Hotellings T^2 and F-test statistics for various datasets and pretreatment conditions.

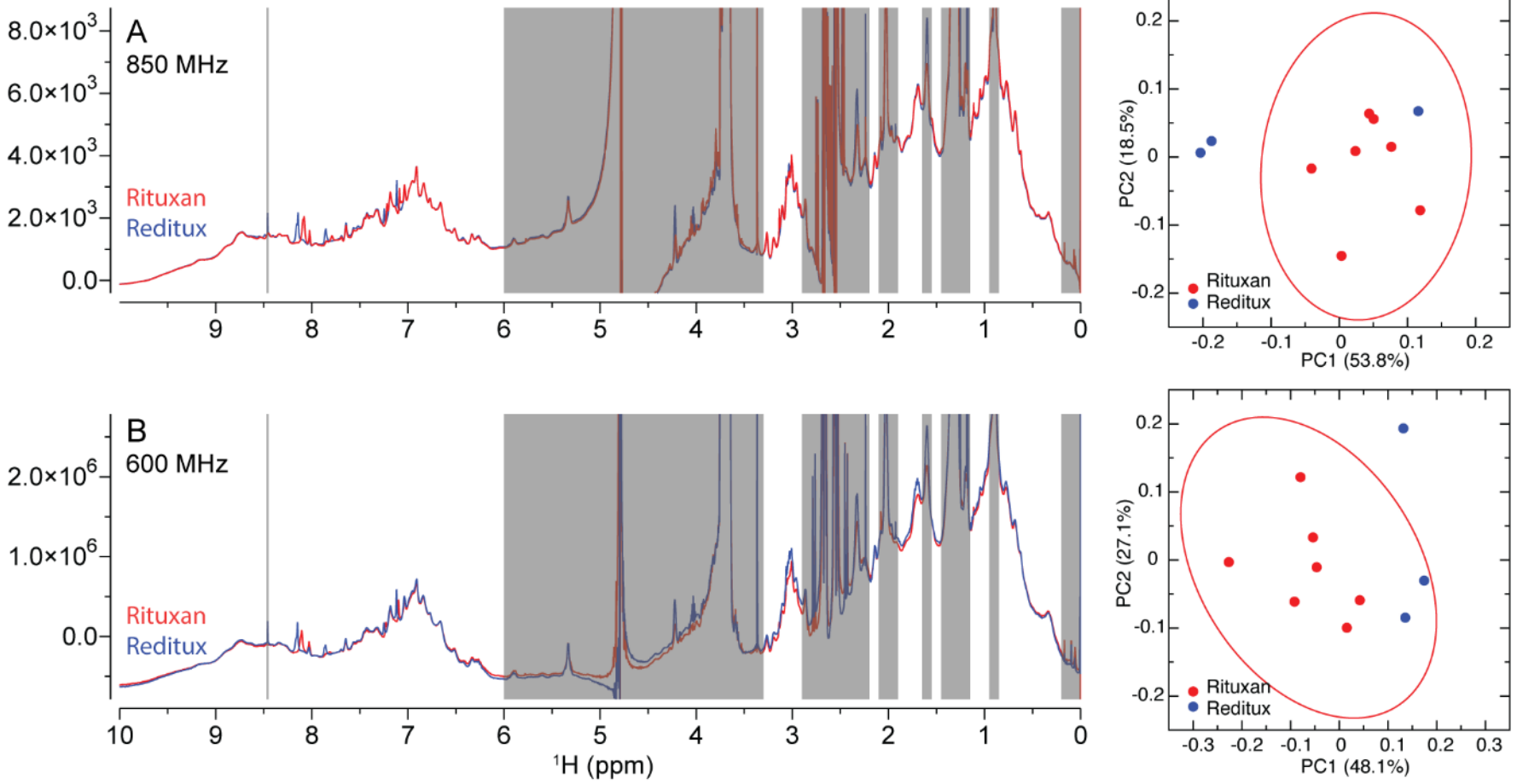
	Mahalanobis distance	Two-sample T^2 statistic	F-value	Critical F-value	Significant?
No scaling					
Total separation	7.65	582.21	283.64	3.24	Yes
Partial separation #1	0.93	6.97	3.37	3.32	Yes
Partial separation #2	1.38	15.57	7.53	3.32	Yes
No separation	0.21	0.50	0.24	3.21	No

Quantification and statistical significance analysis of group separation in NMR-based metabonomics studies

Aaron M. Goodpaster, Michael A. Kennedy *

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA

Rituxan® vs Reditux®



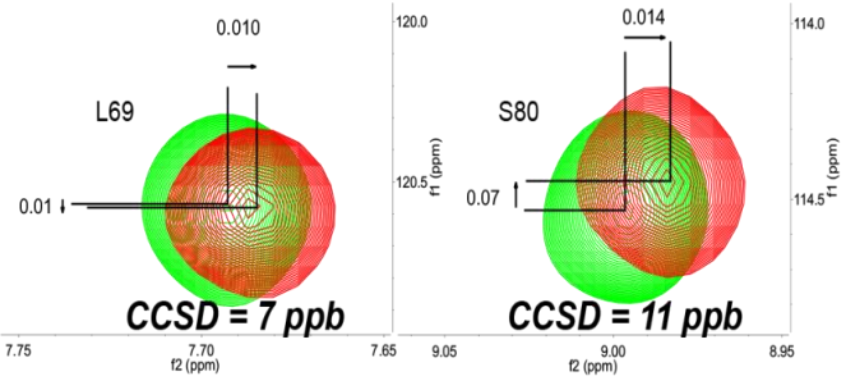
D_M = 1.95 (850) and 3.15 (600)

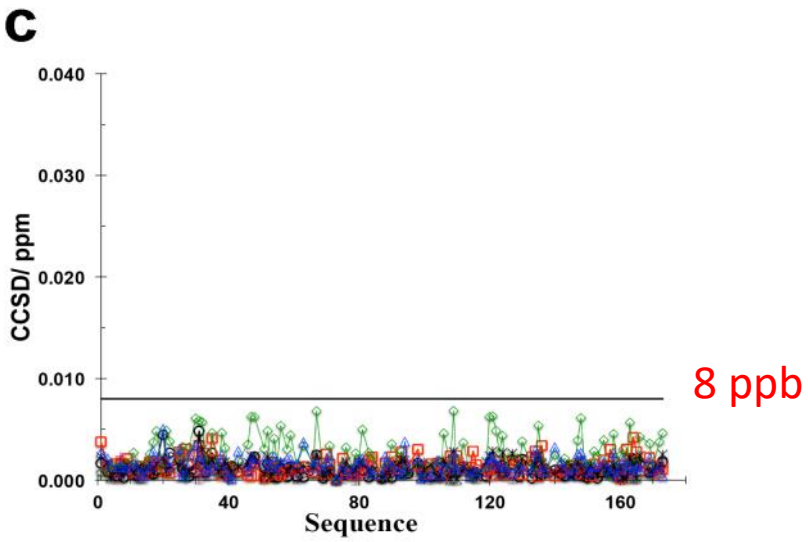
unpublished

Summary from 1D ^{1}H NMR

- Highly sensitive to protein HOS of folding, oligomerization and exchange kinetics.
- Realistically achievable metrics of PCA-D_M(< 3.3) has been verified on insulin and rituximab.
- How about 2D spectral similarity?

Quantifying Chemical Shift Difference





8 ppb

CCSD=

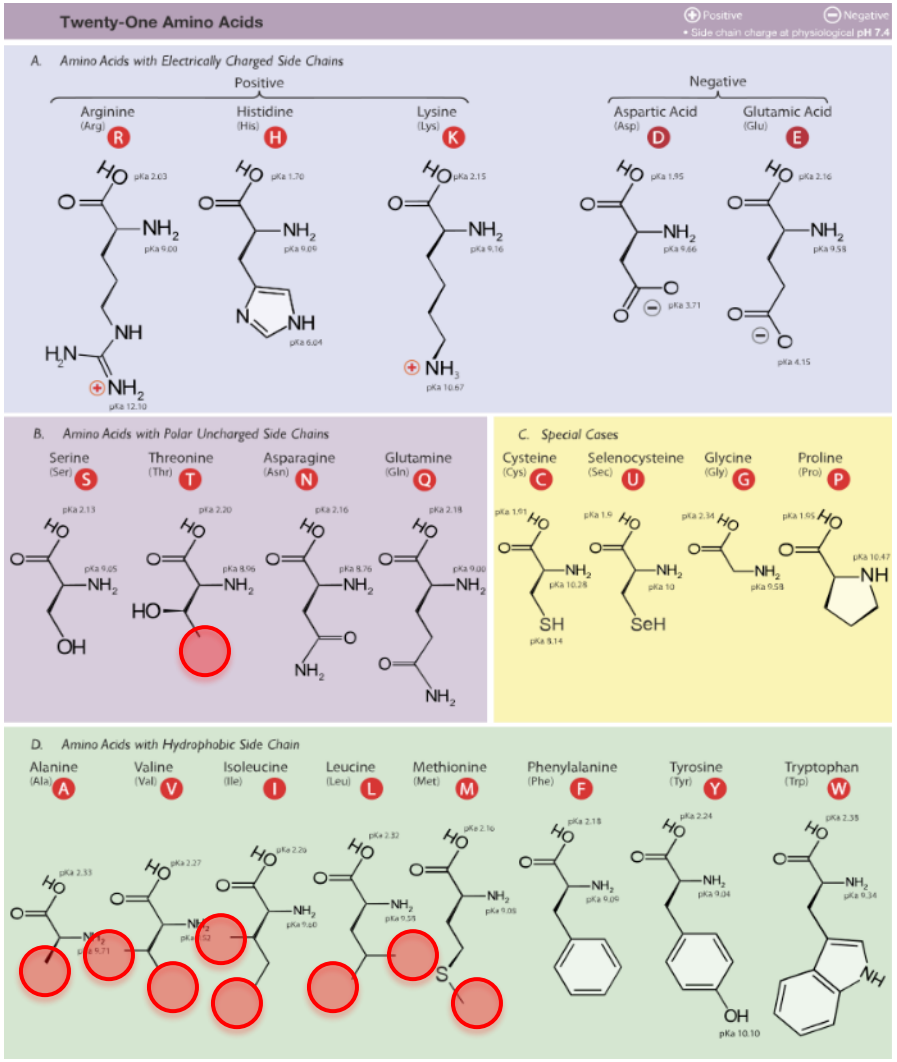
"combined chemical shift difference"

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

- Sensitive;
- Need peak picking;
- No count on peak intensity;
- PCA was also performed but technically challenging.

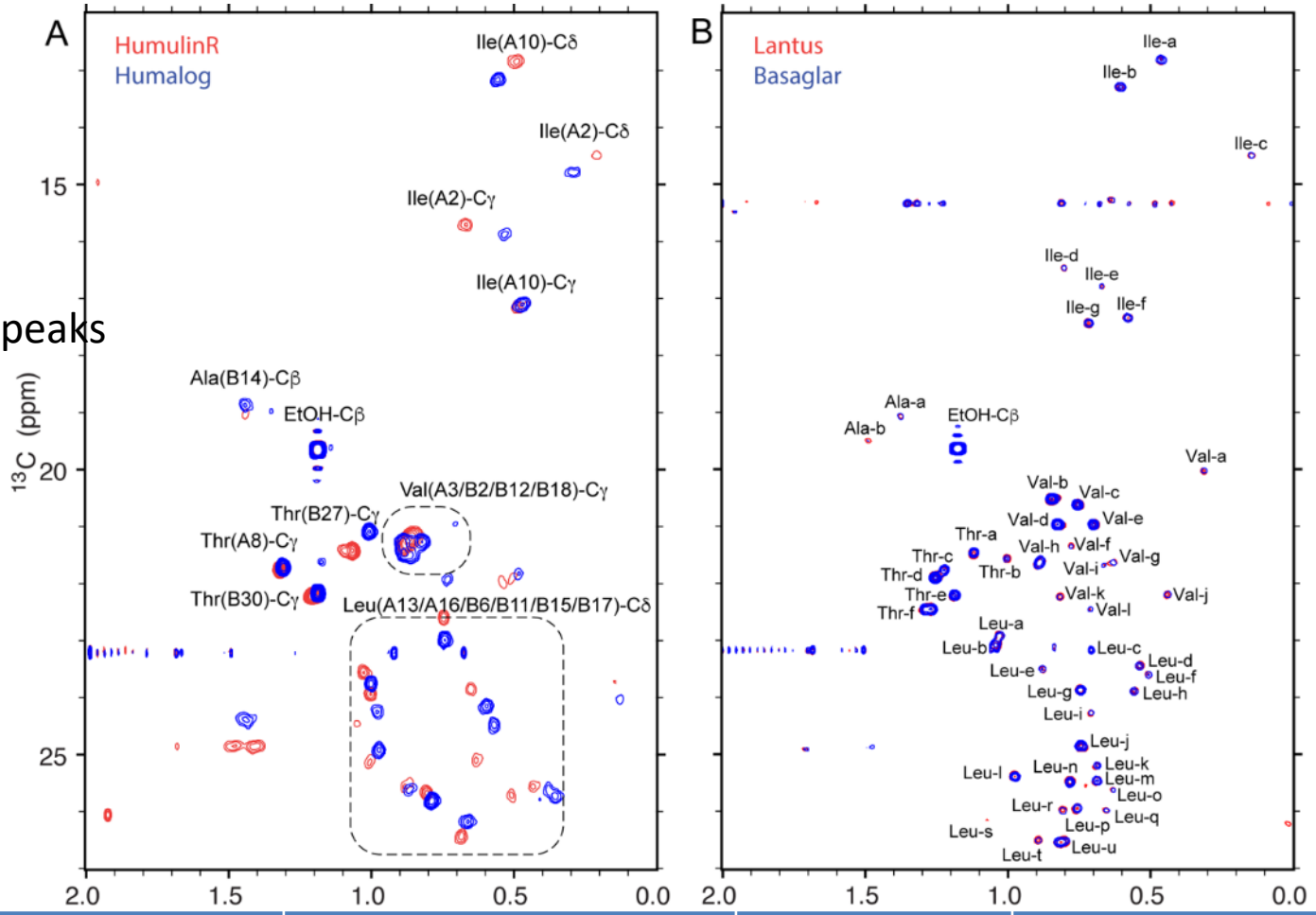
Ghasriani, Hodgson, Brinson, McEwen, Buhse, Kozlowski, Marino, Aubin & Keire 2016 Nature Biotechnology

Amino Acids with Side-Chain Methyl



2D ^{1}H-^{13}C methyl spectra of insulin DPs

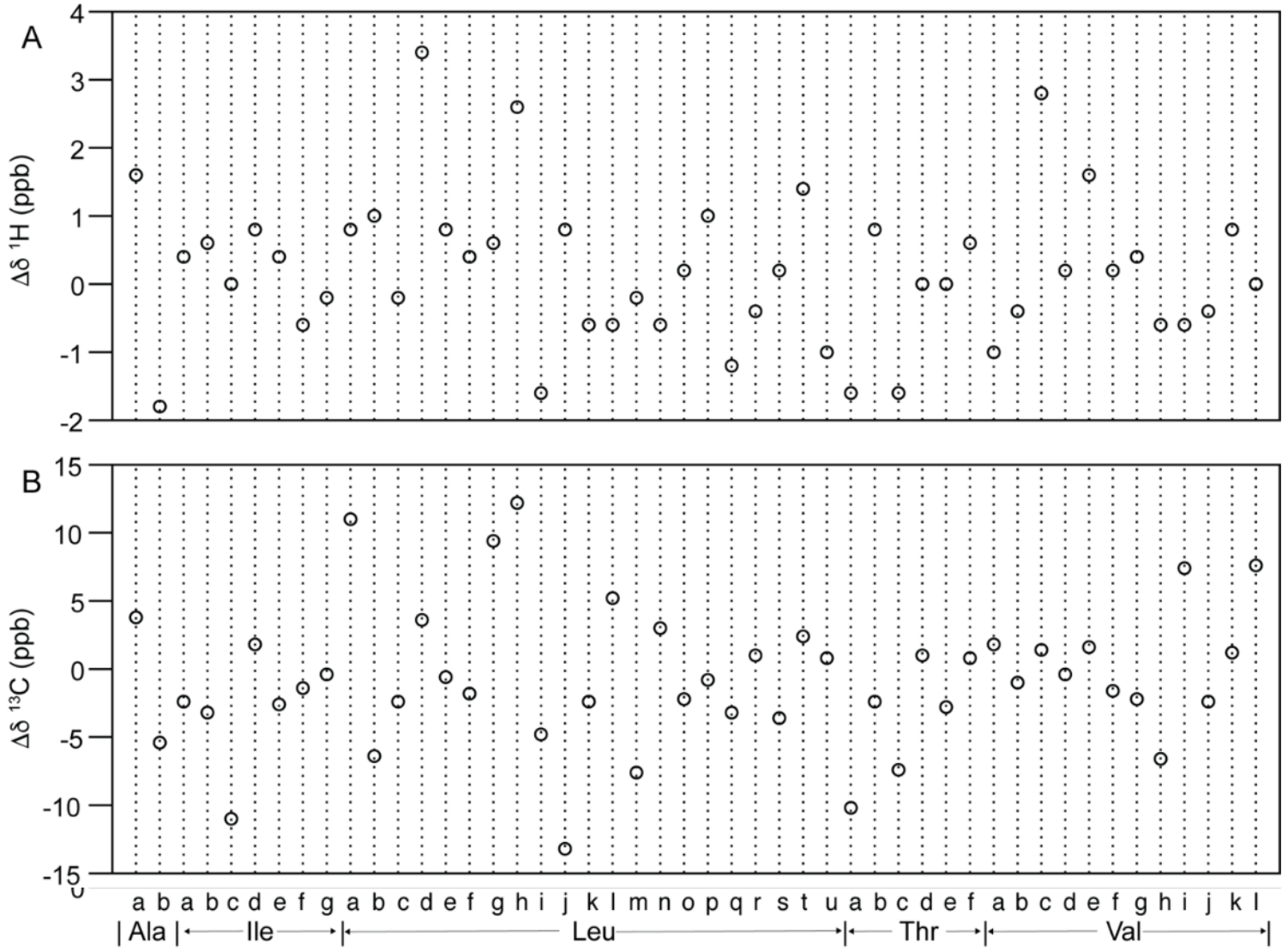
2 Ile
4 Val
3 Thr
6 Leu
1 Ala
= 28 methyl peaks



48 peaks
(slow EX)

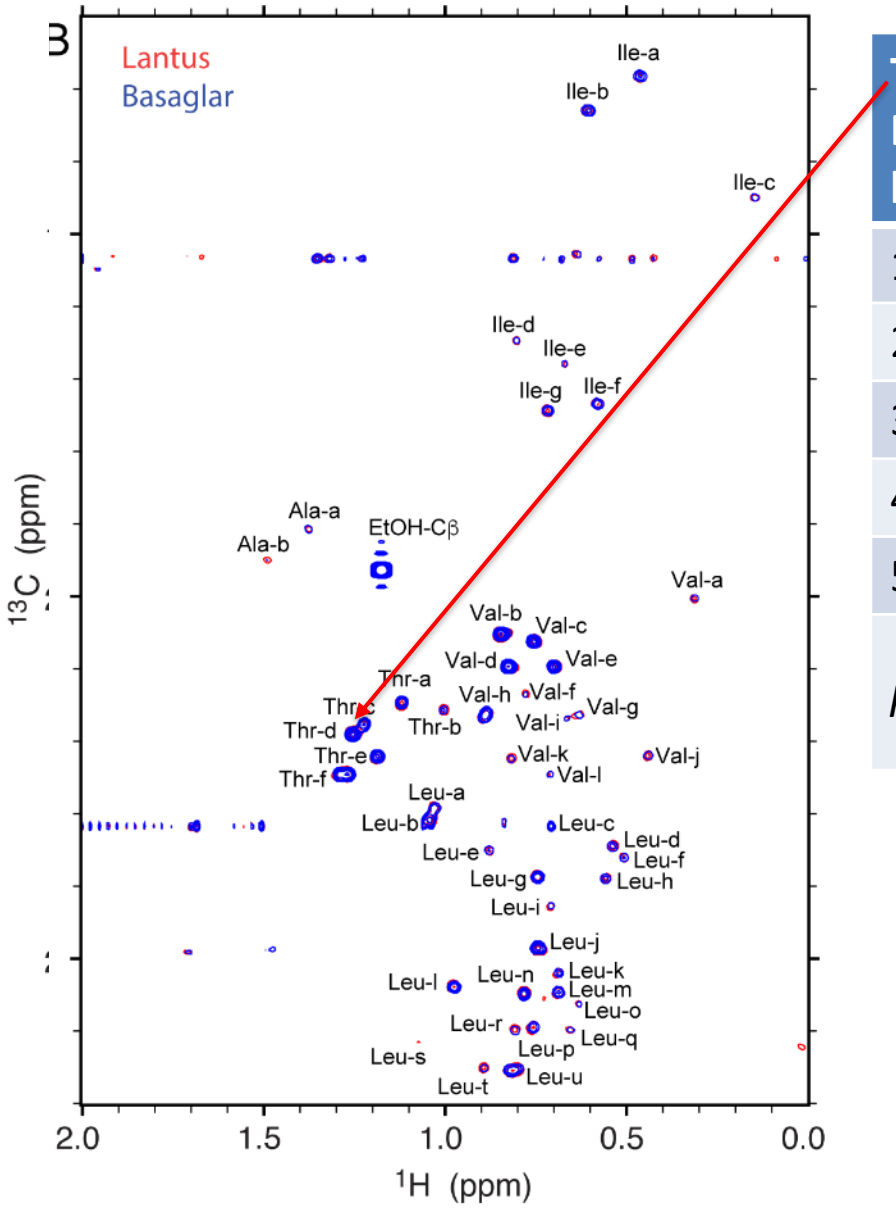
DP	DS	MW	Sequence
HumulinR®	Insulin human	5808	native
Humalog®	Insulin lispro	5808	P(B28)K, K(B29)P
Lantus®/Basaglar®	Insulin glargine	6063	N(A21)G, R(B31), R(B32)

Lantus® vs. Basaglar® in δ



5 lots averaged chemical shift (δ) from each brand; 2º and 3º HOS

Lantus® vs. Basaglar® in Peak Int.



Thr-d Peak height	Lantus® Technical repeat	Lantus® Inter-lot	Basaglar® Inter-lot
1	4.48e6	4.39e6	4.08e6
2	4.45e6	4.25e6	4.15e6
3	4.39e6	4.47e6	4.24e6
4	4.35e6	4.38e6	4.26e6
5	4.36e6	4.32e6	4.16e6
p value	0.35		
		0.0061 (< 0.05)	

Absolute peak height can't be used.

Relative Peak Intensity

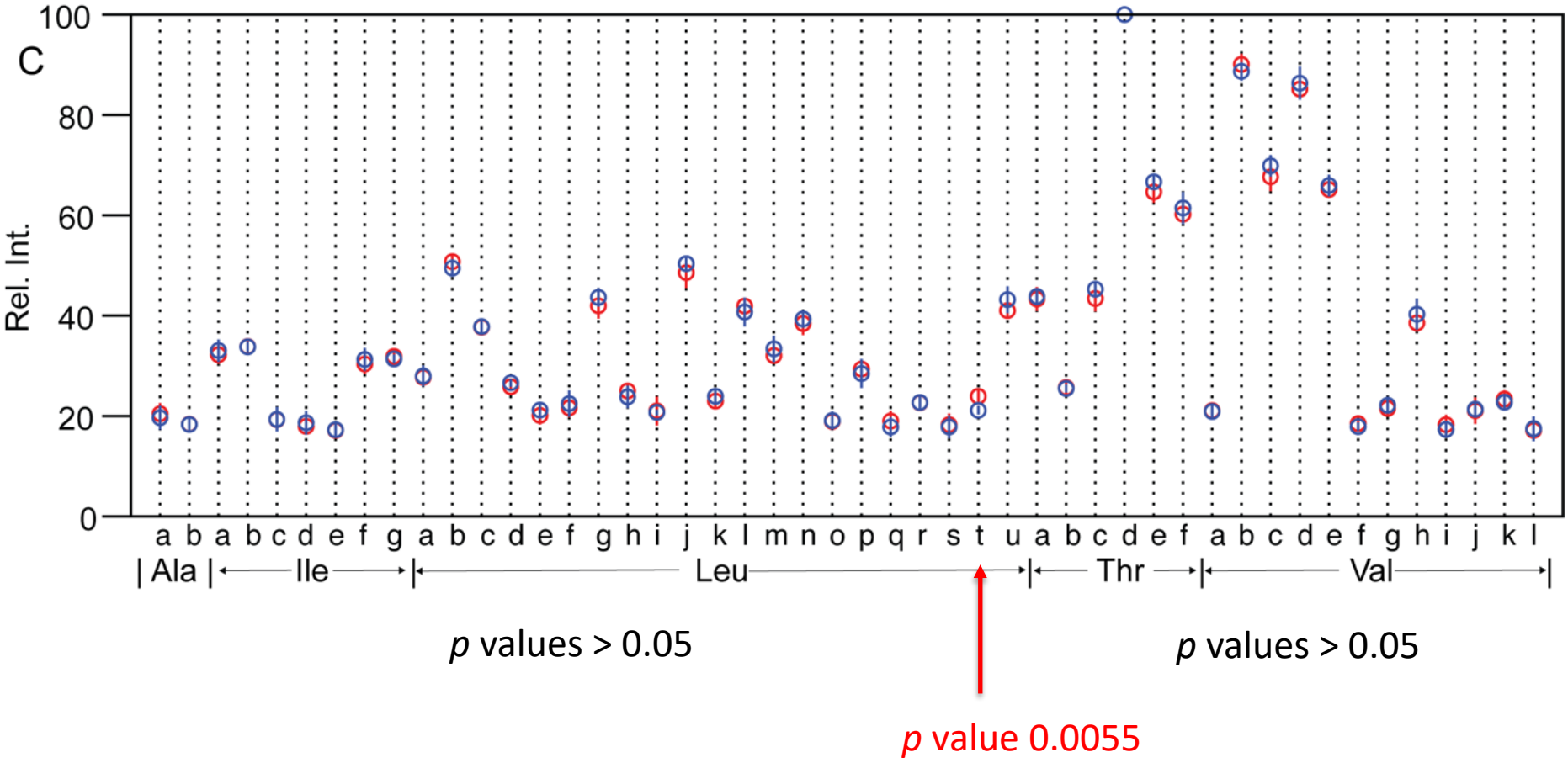
Peak	Lantus®					Basaglar®					p value
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	
Ala-a	17.5	20.7	19.8	20.8	23.4	20.2	17.0	22.7	20.8	17.6	0.61
Ala-b	18.0	19.0	17.4	18.1	19.5	16.3	18.8	17.3	18.7	20.7	0.96
Ile-a	30.2	34.7	32.7	30.6	32.8	34.5	33.2	29.6	35.5	32.4	0.54
Ile-b	35.3	34.5	33.6	32.5	33.3	34.9	34.3	32.2	34.1	33.3	0.92
Ile-c	20.1	21.9	19.2	17.8	17.4	20.9	21.1	15.5	18.3	21.0	0.96
Ile-d	20.0	16.8	17.8	16.9	18.0	19.8	20.8	17.2	15.2	20.2	0.57
Ile-e	16.9	15.9	16.4	17.3	19.2	19.3	15.7	16.5	16.7	17.9	0.93
Ile-f	29.8	32.3	27.4	30.4	31.9	30.6	31.4	35.0	28.7	30.7	0.52
Ile-g	32.0	31.4	30.6	30.9	34.6	31.0	32.6	31.8	31.0	30.4	0.54
Leu-a	27.6	26.4	26.7	27.2	30.7	29.1	27.9	25.4	26.6	30.8	0.84
Leu-b	52.8	52.0	48.8	49.4	50.7	50.9	49.3	50.3	46.5	50.3	0.28
Leu-c	36.9	37.6	36.3	39.3	38.4	39.9	35.9	38.0	38.3	37.2	0.88
Leu-d	25.7	25.9	26.7	24.8	25.8	26.6	25.3	27.0	27.0	27.2	0.11
Leu-e	22.0	17.7	18.8	20.9	21.1	23.4	20.9	22.3	19.4	20.0	0.35
Leu-f	22.5	18.8	21.6	21.2	24.0	23.6	25.7	22.1	18.5	22.3	0.58
Leu-g	41.1	41.1	39.6	41.7	46.2	44.7	43.6	43.5	40.8	45.7	0.26
Leu-h	23.3	26.6	23.6	25.6	25.8	25.9	24.6	21.2	21.6	25.7	0.35
Leu-i	20.1	22.2	16.9	21.1	24.8	21.3	21.0	21.1	20.2	19.9	0.83
Leu-j	46.4	50.1	44.5	49.9	52.0	51.8	50.8	48.3	49.6	51.1	0.28
Leu-k	21.7	25.4	21.7	23.1	23.0	26.2	22.1	23.7	24.9	22.6	0.38
Leu-l	42.4	41.8	41.6	41.3	42.3	43.9	41.4	37.5	38.0	42.9	0.43
Leu-m	31.2	31.0	32.7	31.2	33.9	34.2	34.6	29.1	32.9	36.0	0.34
Leu-n	36.2	38.2	37.2	38.4	42.3	40.8	37.6	37.3	40.5	40.5	0.52
Leu-o	18.1	17.6	18.8	21.4	18.5	18.8	17.3	20.2	21.6	17.7	0.85
Leu-p	30.6	30.6	29.4	28.0	28.1	31.7	28.7	24.7	26.4	30.8	0.57
Leu-q	19.1	21.8	19.7	16.1	18.3	18.6	19.9	15.1	16.7	18.7	0.38
Leu-r	24.2	22.7	23.0	21.9	21.5	22.6	24.7	23.5	20.5	22.2	0.96
Leu-s	15.3	19.1	18.4	17.3	21.4	15.7	17.9	15.6	18.9	21.0	0.75
Leu-t	23.0	25.3	23.7	25.3	22.4	21.7	21.5	21.6	19.6	21.3	0.0055
Leu-u	42.5	38.8	40.1	41.6	42.2	43.2	40.2	42.2	42.8	47.5	0.17
Thr-a	45.8	45.8	41.2	40.9	42.8	43.8	41.9	44.8	42.4	46.0	0.73
Thr-b	27.2	27.0	24.0	25.0	25.0	24.1	25.8	26.1	25.9	25.5	0.83
Thr-c	39.4	45.2	42.5	43.8	46.1	45.9	44.2	47.3	44.7	44.3	0.21
Thr-d	100	100	100	100	100	100	100	100	100	100	n/a
Thr-e	64.0	65.5	61.5	64.8	67.4	68.5	68.0	64.5	65.5	66.9	0.13
Thr-f	60.2	61.1	59.0	58.8	61.9	65.1	64.5	58.8	58.1	60.7	0.46
Val-a	22.5	21.6	22.1	19.1	20.0	22.3	21.7	18.7	20.6	21.3	0.85
Val-b	92.1	91.1	87.1	88.6	91.4	91.2	86.6	87.4	88.4	89.6	0.29
Val-c	67.2	65.7	64.6	68.9	71.8	73.0	70.7	67.1	69.2	69.6	0.2
Val-d	84.8	84.8	82.9	87.1	86.3	89.3	90.4	83.1	85.1	83.9	0.5
Val-e	64.2	65.2	63.6	67.1	65.6	68.1	67.2	62.5	66.4	65.3	0.52
Val-f	20.0	17.8	19.3	18.0	17.4	18.4	17.2	17.3	19.2	17.2	0.34
Val-g	20.9	20.2	21.8	21.3	23.5	20.9	21.6	21.1	22.1	25.1	0.52
Val-h	40.0	37.7	37.8	37.6	39.6	43.3	36.4	40.0	38.3	43.3	0.29
Val-i	15.9	20.7	17.5	17.3	20.0	18.1	19.3	16.4	15.4	17.4	0.42
Val-j	24.0	20.8	21.9	16.8	21.7	22.9	23.2	20.9	20.3	19.4	0.83
Val-k	24.2	21.1	25.7	22.8	23.0	21.5	21.9	24.0	24.3	22.0	0.55
Val-l	15.8	19.9	14.5	17.9	17.7	20.2	13.5	17.1	18.6	18.0	0.84

$$Rel.Int._x = 100 \times I_x / I_{Thr-d}$$

← $p < 0.05$

← Reference peak

Lantus® vs. Basaglar®



Peak height profile mapping.
Only 1/48 (2%) peaks had significant *p* value.
4º , oligomerization and HOS exchange.

Summary from 2D ^{1}H-^{13}C NMR

- Highly specific to protein sequence and HOS heterogeneity.

- No need to blind out non-DS peaks for comparison.

- Realistically achievable metrics:
  - $\Delta\delta$ < 4 ppb for ^{1}H;
  - $\Delta\delta$ < 15 ppb for ^{13}C;
  - 98% comparable peak heights.

CONCLUSIONS

a. NMR to fingerprint peptide/protein HOS in DP for folding, exchange and aggregation.

b. Unsupervised approaches on inter-brand NMR spectra comparison yielded similarity metrics of Mahalanobis distance (D_M), chemical shift difference ($\Delta\delta$) and peak height profile mapping.

c. These metrics were derived from spectra of marketed insulin DPs, therefore, realistic and achievable.

d. The approach would be helpful for drug manufacture and development.

Acknowledgement

FDA: Deyun Wang, You Zhuo, Mike Karfunkle, Cameron Smith and David Keire.

University of Maryland: Junyong Park

Discussions: Muthukumar Ramaswamy, Maria Gutierrez Lugo

Management: Cynthia Sommers, Jason Rodriguez

Support: CDER Critical Path “Chemometrics for 2D NMR spectra similarity assessment of complex drug products”