BESTSEL

Secondary Structure Estimation

qHOS

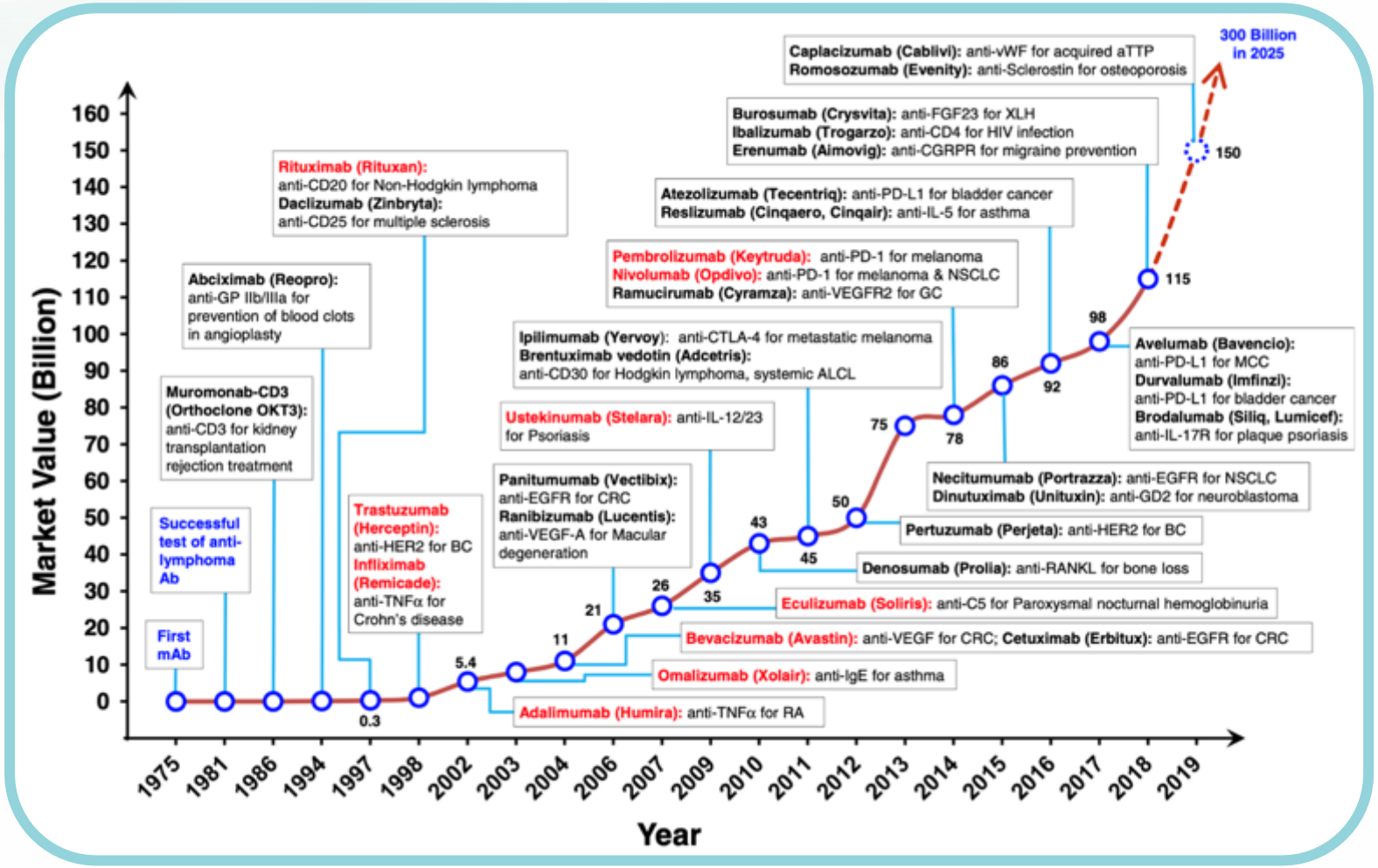
quality control of Higher-Order Structure

NEW DRUG MODALITIES PROJECT

8th April 2024

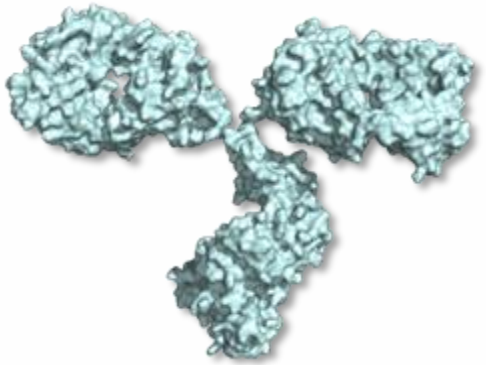
JASCO Corporation

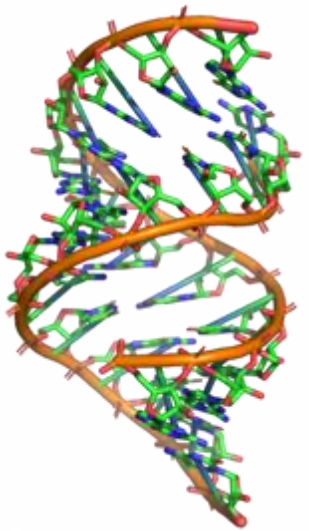
Antibody therapeutics market growth



Lu et al. *J. Biomed. Sci.* (2020) 27:1. https://doi.org/10.1186/s12929-019-0592-z

CGCR of biopharmaceutilcal is nearly 10% !!





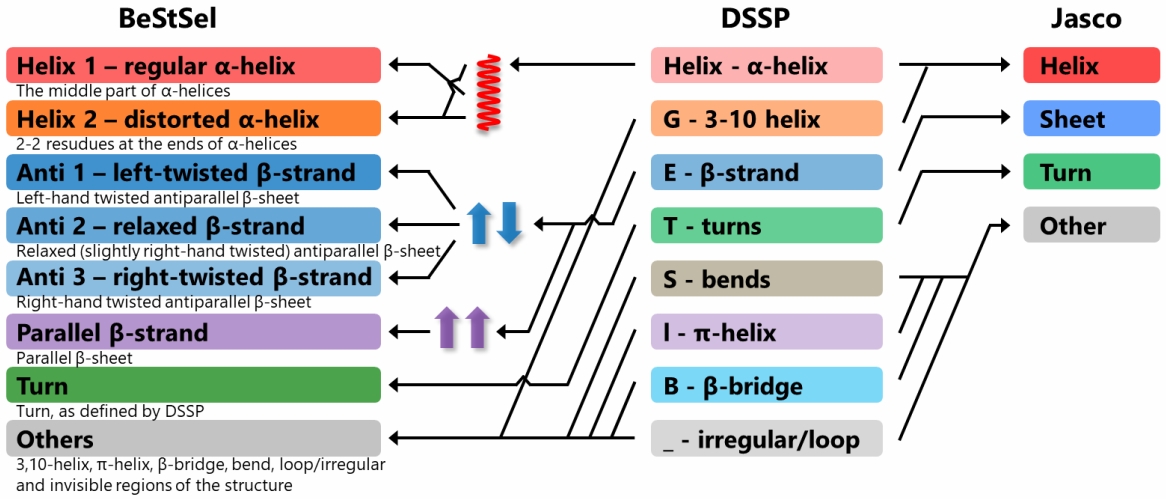
BESTSEL Secondary Structure Estimation

Advantage of BESTSEL?

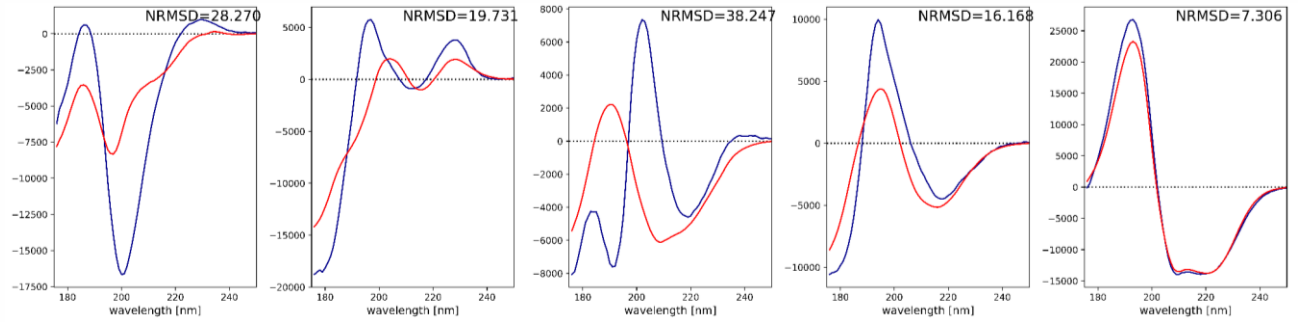
JASCO's PCR analyzed with **4 structures**
BeStSel analysis with **8 structures**

- High accuracy of secondary structure estimation
- High spectral agreement

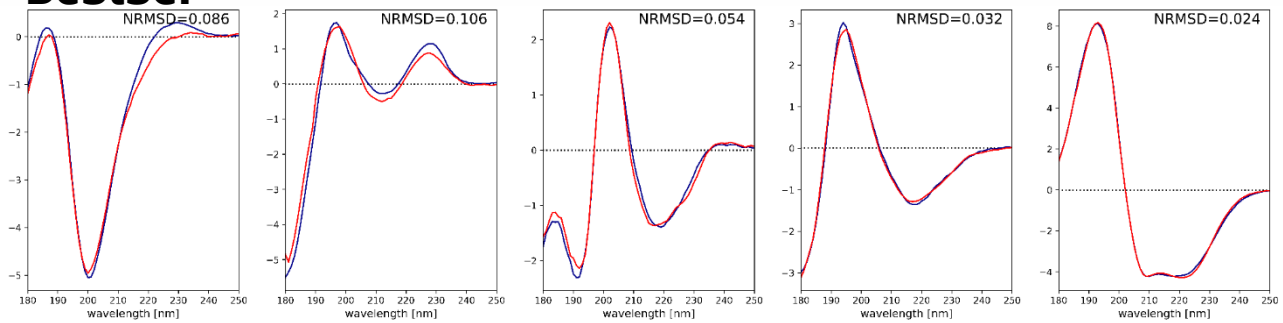
The basic elements of secondary structure



JASCO PCR



BeStSel

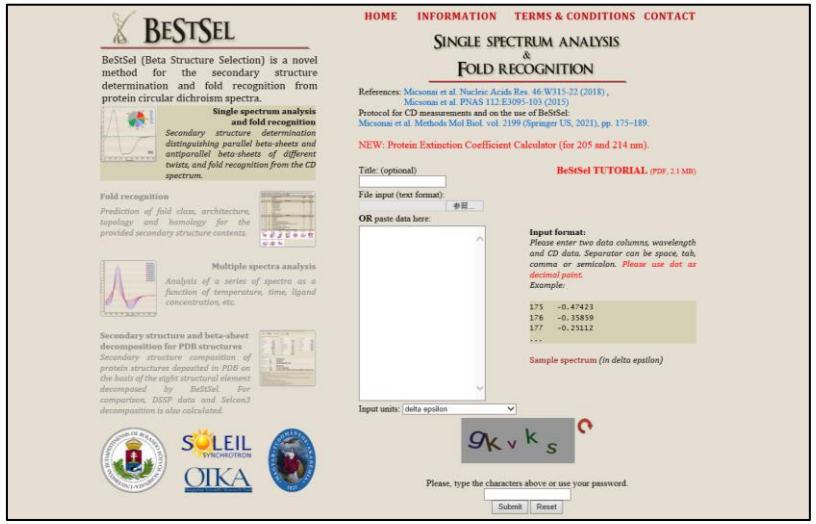


Especially it is suitable for β-sheet rich proteins as antibodies to get accurate structure analysis.

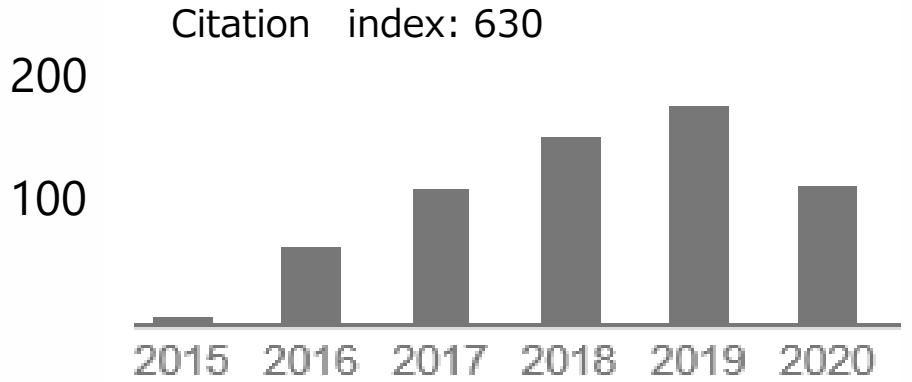
BESTSEL Secondary Structure Estimation

What is BESTSEL?

The online free software for protein secondary structure analysis using CD spectra, developed by Dr. Kardos of Eötvös Loránd University, is widely used around the world.



Number of paper citations for BESTSEL



Algorithm	Year of publication	Citation index	Average number of citations/year	Paper Title
BeStSel	2015	630	**105**	Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy
SELCON3	1999	765	**36**	Estimation of the number of helical and strand segments in proteins using CD spectroscopy
CONTIN	1981	2292	**57**	Estimation of globular protein secondary structure from circular dichroism
K2D2	2008	394	**30**	K2D2: Estimation of protein secondary structure from circular dichroism spectra
K2D3	2011	551	**55**	Prediction of protein secondary structure from circular dichroism using theoretically derived spectra
CDNN	1992	1142	**39**	Quantitative analysis of protein far UV circular dichroism spectra by neural networks

BESTSEL Secondary Structure Estimation

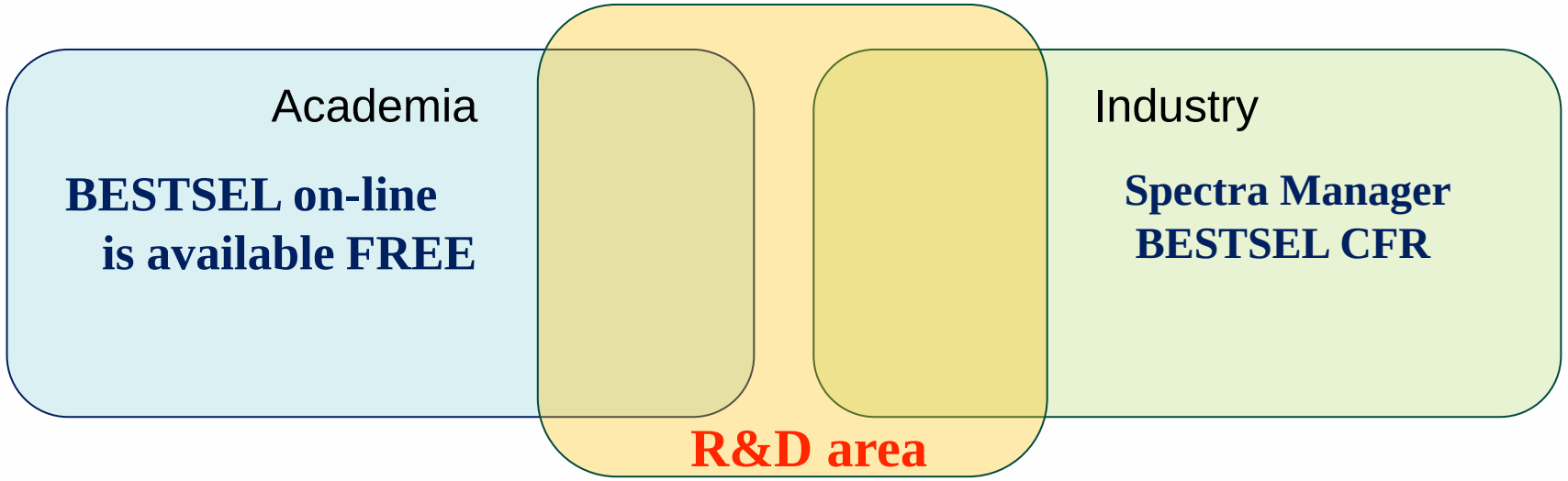
The protein secondary structure estimation algorithm BeStSel was developed by Dr. József Kardos and Dr. András Micsonai of Eötvös Loránd University (ELTE), and it has been widely used in protein research fields globally today. We, JASCO, formally signed up the agreement to collaborate with the research group led by Dr. Kardos for developing the off-line version software Spectra Manager BeStSel CFR.
On 29th June, we attended the official ceremony for this agreement at ELTE in Hungary.

Spectra Manager BESTSEL

And then, we finally agreed with ELTE and developed

Spectra Manager BESTSEL 2.5 as non-CFR.

This means,,,

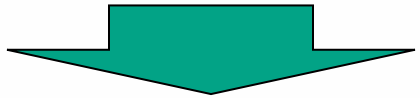


Spectra Manager BESTSEL 2.5 (Non-CFR) can be proposed to this area who wants to use it for R&D purpose under closed environment

We can propose customers with suitable package under their usage environment.

Ordering information 1

7000-J104AV	J-1100ST	J-1100ST CD Spectrometer with Spectra Manager 2.5	JWMVS multivariate SSE program is included as standard
7000-J105AV	J-1500-150ST	J-1500-150ST CD Spectrometer with Spectra Manager 2.5	JWMVS multivariate SSE program is included as standard
7000-J132AV	J-1700-150ST	J-1700-150ST CD Spectrometer with Spectra Manager 2.5	JWMVS multivariate SSE program is included as standard
7000-J106AV	J-1500-450ST	J-1500-450ST CD Spectrometer with Spectra Manager 2.5	JWMVS multivariate SSE program is included as standard
7000-J133AV	J-1700-450ST	J-1700-450ST CD Spectrometer with Spectra Manager 2.5	JWMVS multivariate SSE program is included as standard



New P/N will be registered as below by removing JWMVS multivariate SSE program.
It means that both P/N and price are available in parallel so that we can manage this change as smooth as we can.

7000-J204AV	J-1100ST	J-1100ST CD Spectrometer with Spectra Manager 2.5	
7000-J205AV	J-1500-150ST	J-1500-150ST CD Spectrometer with Spectra Manager 2.5	
7000-J232AV	J-1700-150ST	J-1700-150ST CD Spectrometer with Spectra Manager 2.5	
7000-J206AV	J-1500-450ST	J-1500-450ST CD Spectrometer with Spectra Manager 2.5	
7000-J233AV	J-1700-450ST	J-1700-450ST CD Spectrometer with Spectra Manager 2.5	

Pricing would not be changed on those new parts numbers.

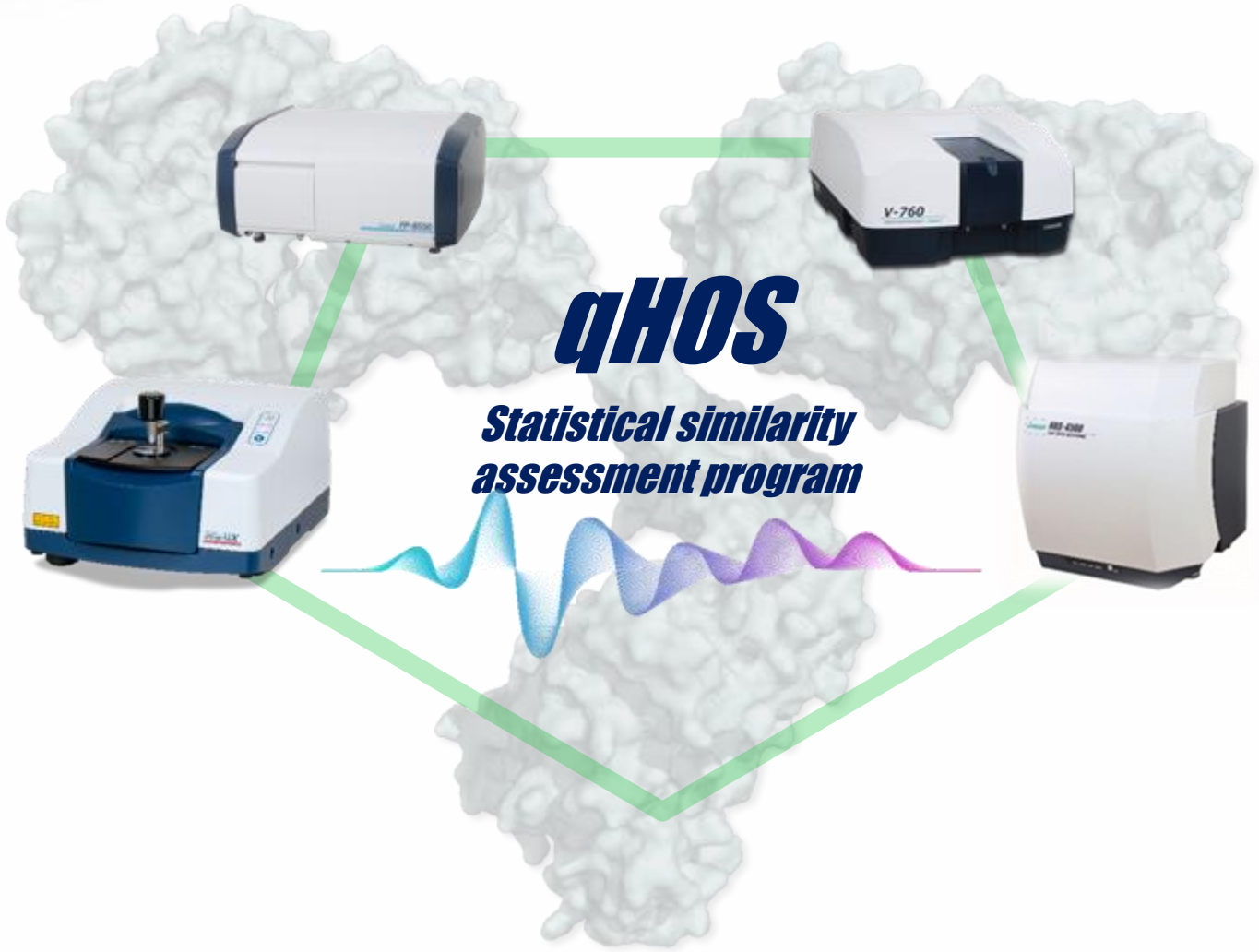
Spectra Manager BESTSEL *Ordering information 2*

JWMVS multivariate SSE has registered as separated optional program as well as JWSSE protein analysis program. Customer can choose SSE program from BESTSEL JWMVS and JWSSE.

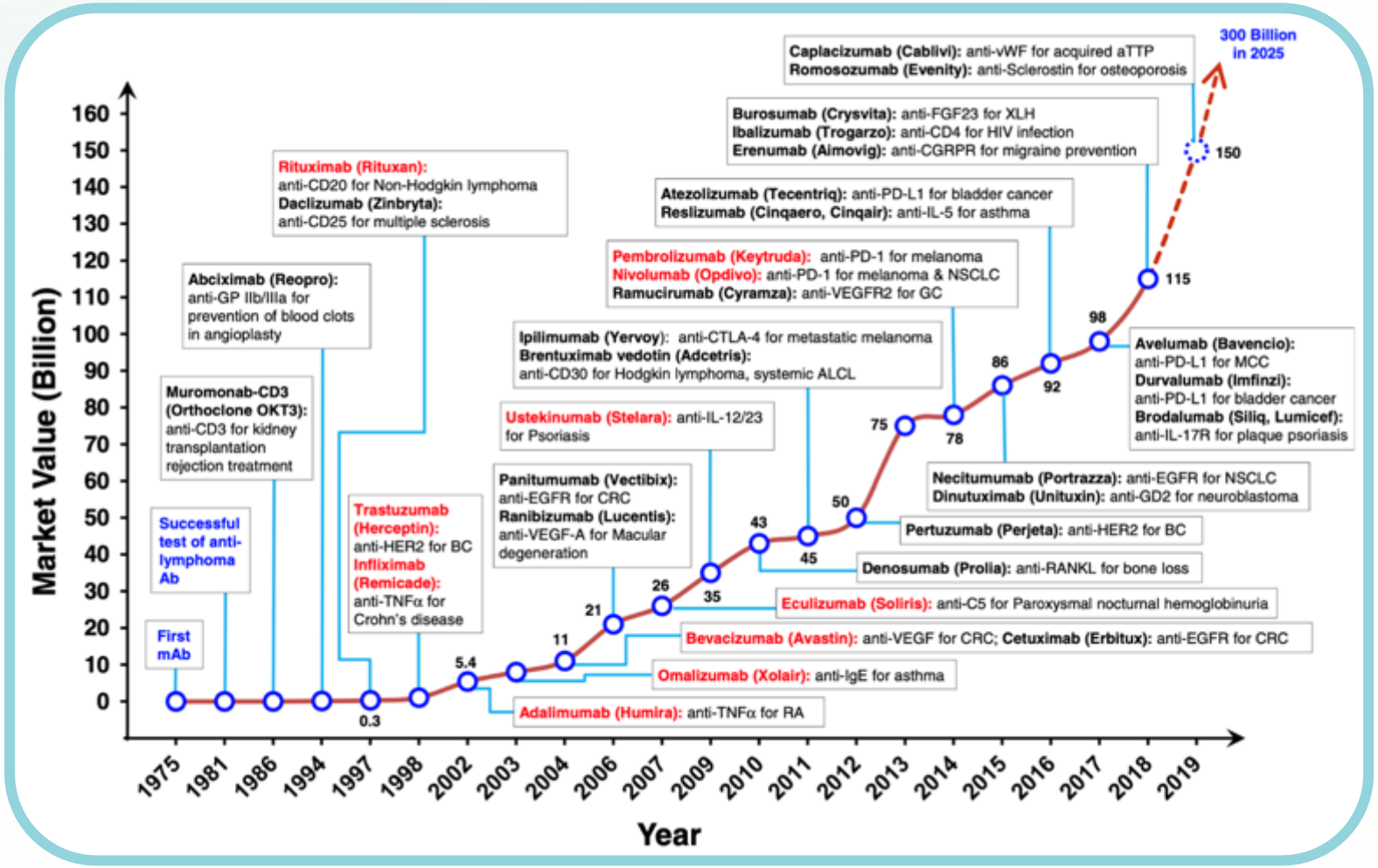
4880-J145A	JWMVS-529	JWMVS-529 CD Multivariate SSE analysis program	
4880-J150A	JWBeStSel-532	JWBeStSel-532 BeStSel program	
4880-J143A	JWSSE-513	JWSSE-513 Protein SSE program	

qHOS quality control of Higher-Order Structure

- Statistical similarity evaluation
- Robust evaluation using noise weighting method
- Student, Welch, TOST t-test implementation
- Auto concentration correction
- Orthogonal similarity assessment
- Regulatory compliance with Spectra Manager CFR™



Antibody therapeutics market growth



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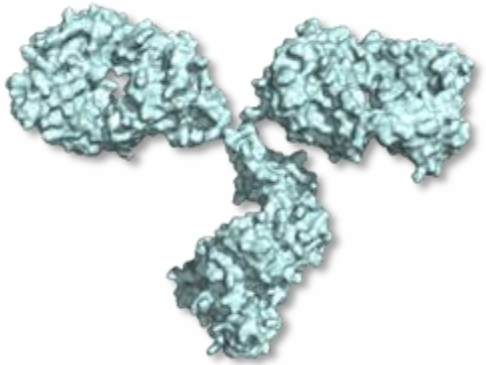
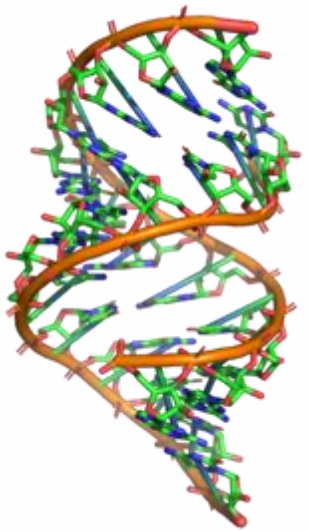




Necessity of objective assessment

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

COMPARABILITY OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

Q5E

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

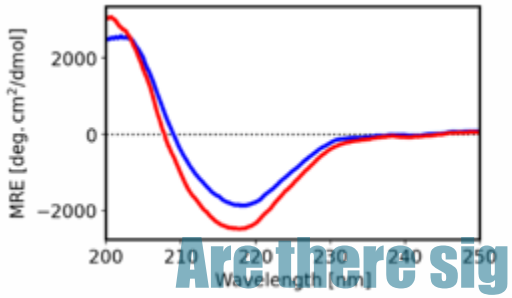
SPECIFICATIONS: TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Q6B

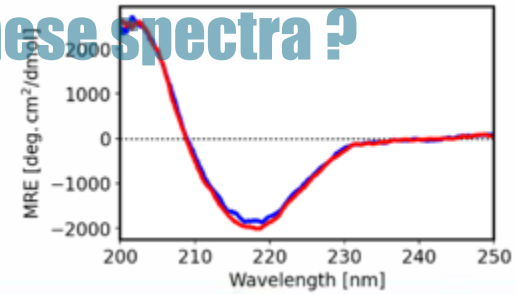
Statistical Approaches to Evaluate Analytical Similarity

Guidance for Industry

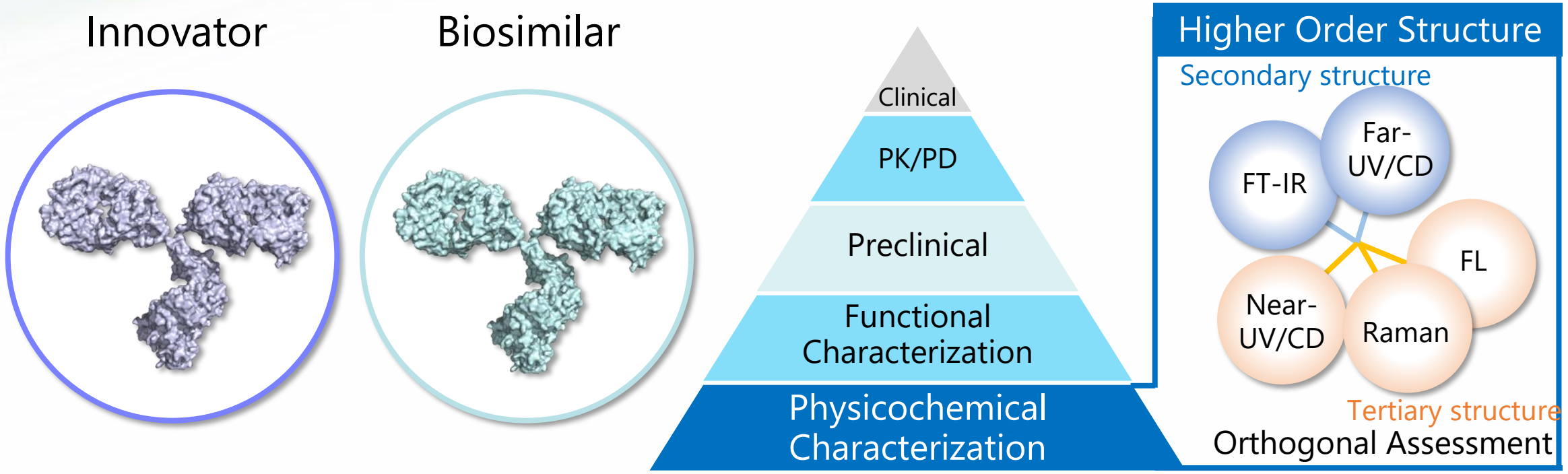
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)



Are there significant differences in these spectra ?



HOS similarity assessment for biosimilar



Increasing the importance of analytical characterization of biosimilars.
Regulatory authorities recommend orthogonal assessment of biosimilar quality attributes using multiple instruments based on different principles.

System for comprehensive orthogonal assessment

JASCO products

qHOS
Higher order structure similarity evaluation software

- Statistical significant testing
- Support for multiple instruments

HTCD Plus
Circular Dichroism

Secondary and Tertiary

- Automated measurement
- High throughput and high sensitivity

FT/IR-4X
Infrared spectrometer

Secondary

- High S/N and resolution with small body
- One drop ATR measurement

NRS-4500
Raman microscope

Tertiary

- Microanalysis
- Chemical imaging

Multiple spectroscopic techniques provide orthogonal similarity assessments of secondary and tertiary structures, and data can be statistically analyzed using the single qHOS platform.

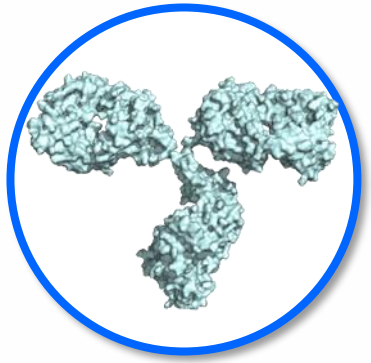
Materials and Methods

Materials

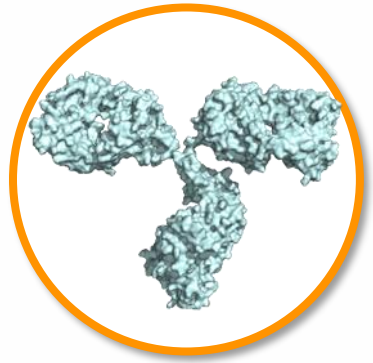
Rituximab

MabThera® (Innovator)
RIABNI™ (Biosimilar)
Anti CD20 monoclonal antibodies
Both samples were prepared to 10 mg/mL
Additive : Sodium citrate dihydrate 7.4 mg/mL,
Sodium chloride 9.0 mg/mL, Sodium hydroxide 9.0 mg/mL,
Polysorbate 80 0.7 mg/mL

MabThera®



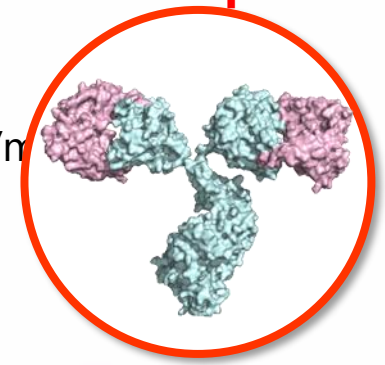
RIABNI™



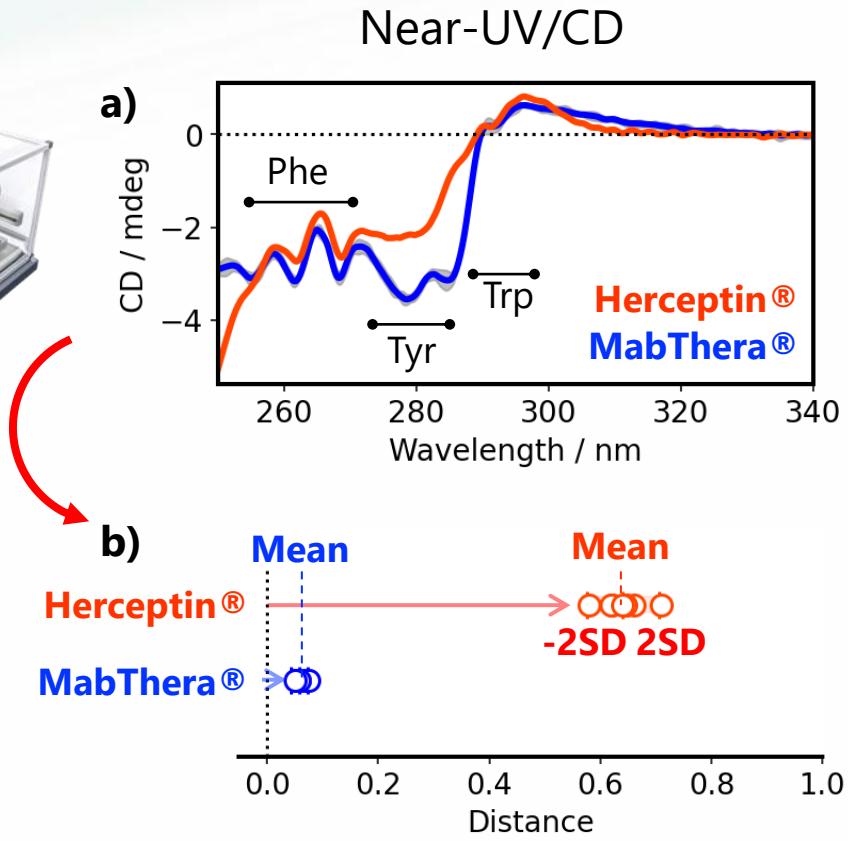
Trastuzumab

Herceptin® (Innovator)
Anti HER2 monoclonal antibody
The powder was dissolved in H_2O to a concentration of 10 mg/m
Additives: trehalose hydrate 4.7 mg/mL,
L-histidine hydrochloride hydrate 0.11 mg/mL,
L-histidine 7.4 x 10^{-2} mg/mL, polysorbate 2.1 x 10^{-2} mg/mL

Herceptin®



Similarity of tertiary structure for different antibody drugs



Result of t-test
p-value = 0.0
Significant difference

Figure 1. Similarity assessment of the tertiary structure of MabThera® and Herceptin®, antibody drugs with different targeting and formulation conditions.

The shapes of the near-UV/CD spectra of MabThera® and Herceptin® differ significantly (Fig. 1a). Similarly, the distribution of distances between MabThera® and Herceptin calculated from the CD spectra show a significant difference (Fig. 1b). The p-value obtained from the t-test is below the significance level of 0.05, indicating that Herceptin® has a different tertiary structure to MabThera®.

Similarity assessment for secondary structure of biosimilar

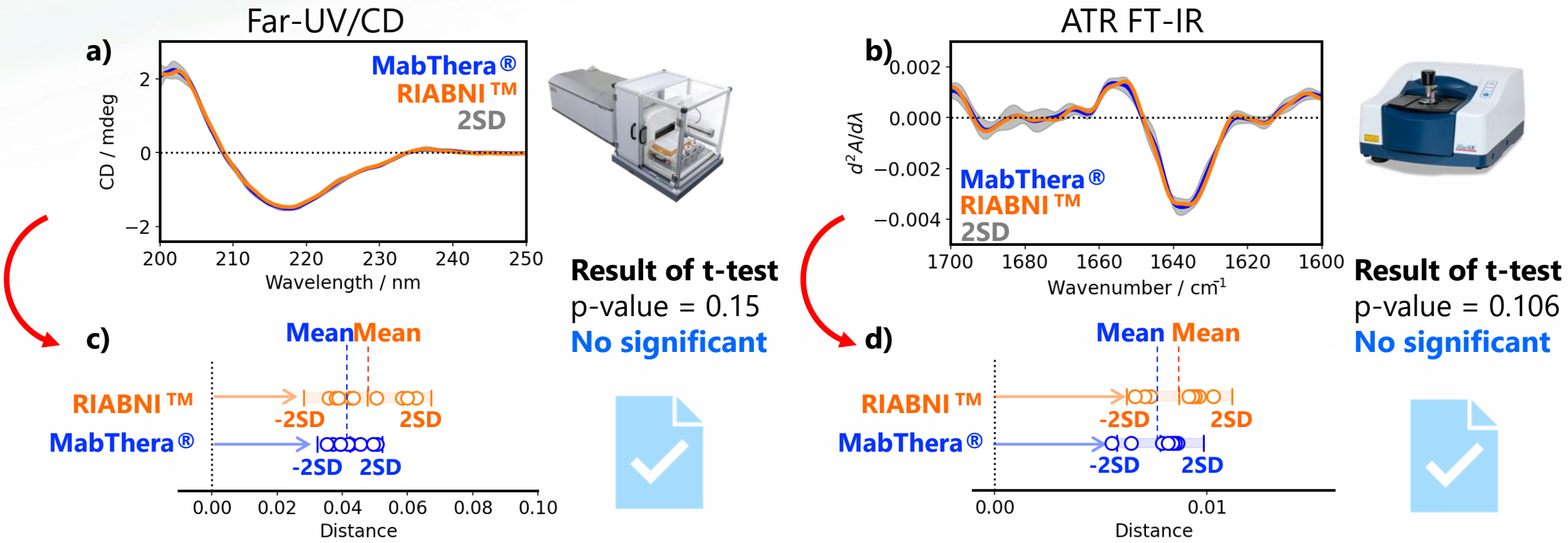


Figure 2. Orthogonal similarity assessment for the secondary structure of MabThera® and RIABNI™

The far-UV/CD and FTIR spectra of the biosimilar RIABNI™ are in excellent agreement with those of the innovator MabThera® (Figs. 2a and 2b), and the distributions of the distances between MabThera® and RIABNI™ are close to each other (Figs. 2c and 2d). The p-value is larger than the significance level of 0.05.

Similarity assessment for tertiary structure of biosimilar

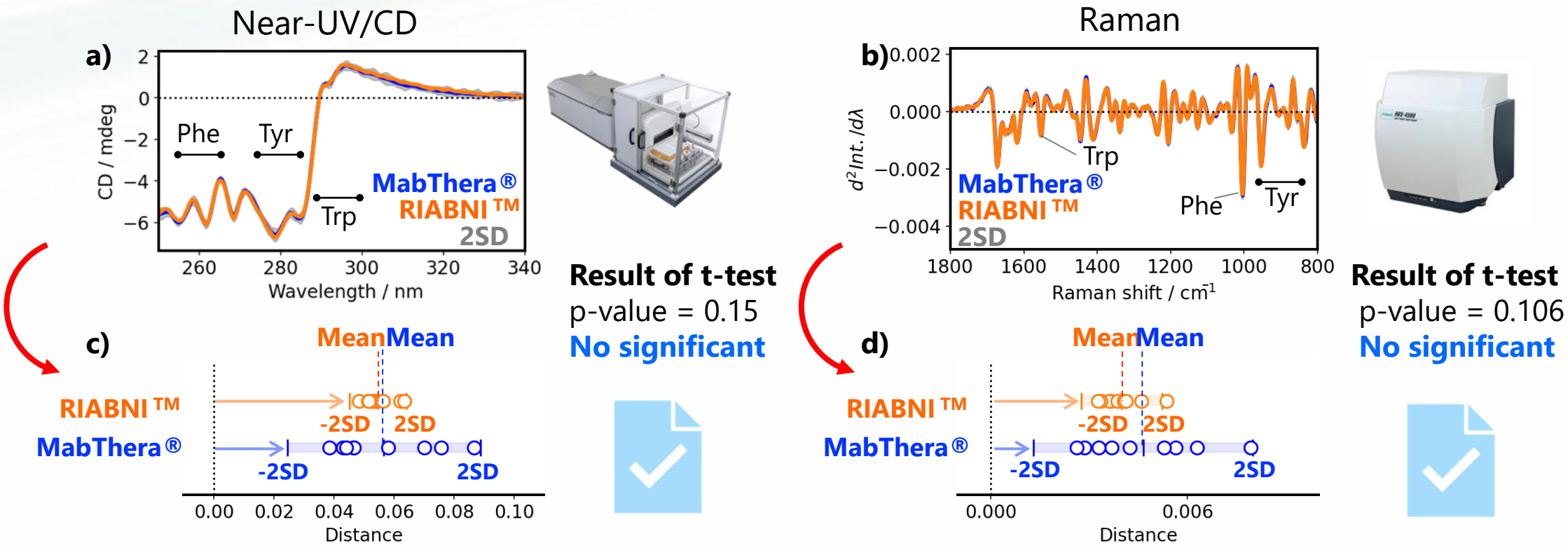


Figure 3. Orthogonal similarity assessment for the tertiary structure of MabThera® and RIABNI™

Similar to the secondary structure, the tertiary structure of MabThera® and RIABNI™ show excellent agreement in the near-UV/CD and Raman spectra (Figs. 3a and 3b), and the distribution of the distances between MabThera® and RIABNI™ are close to each other (Figs. 3c and 3d). The p-value is larger than the significance level of 0.05.

Forced degradation studies for MabThera®

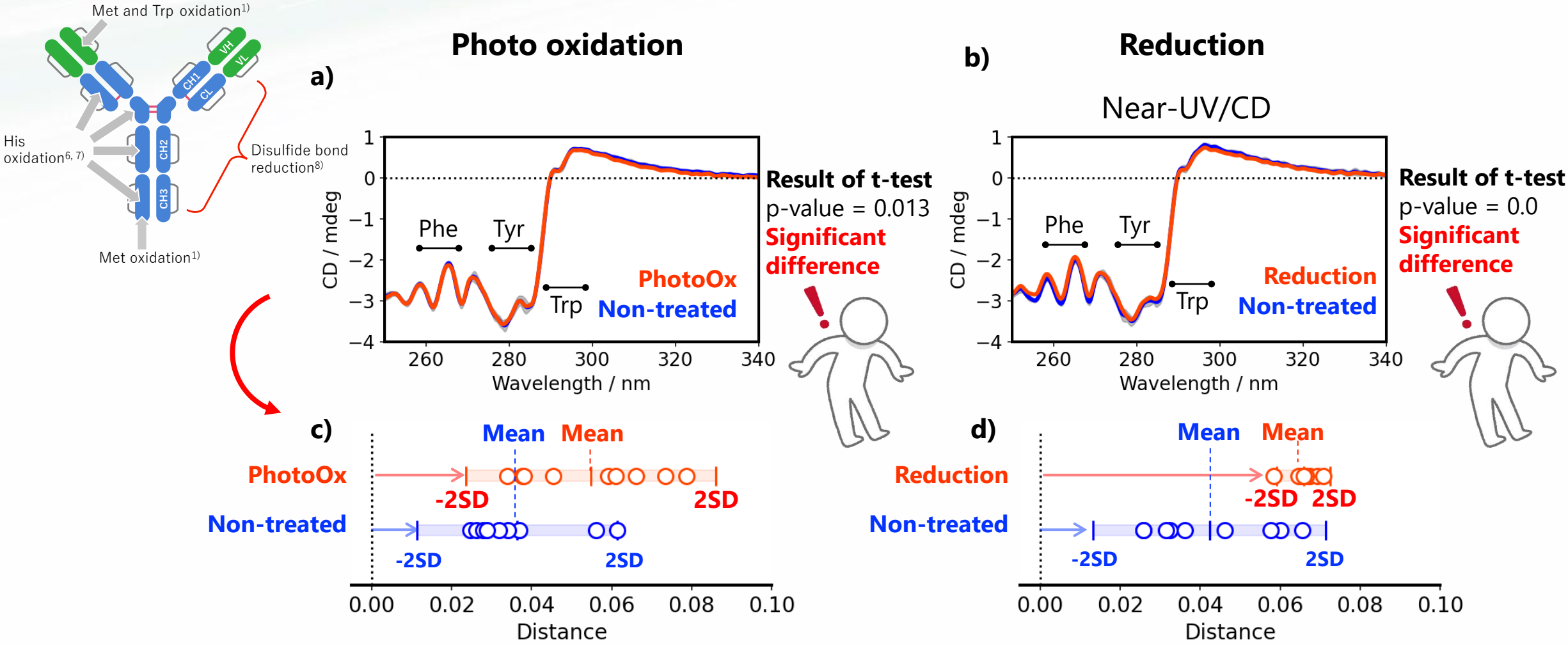
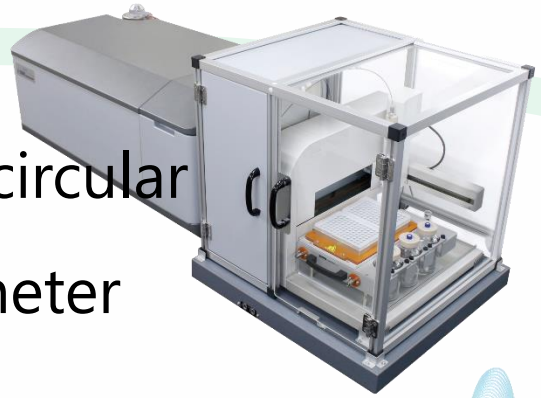


Figure 4. Similarity assessment for the tertiary structure of MabThera® and treated with stimuli

Summery

HTCD Plus

: High-throughput circular dichroism spectrometer



BeStSel

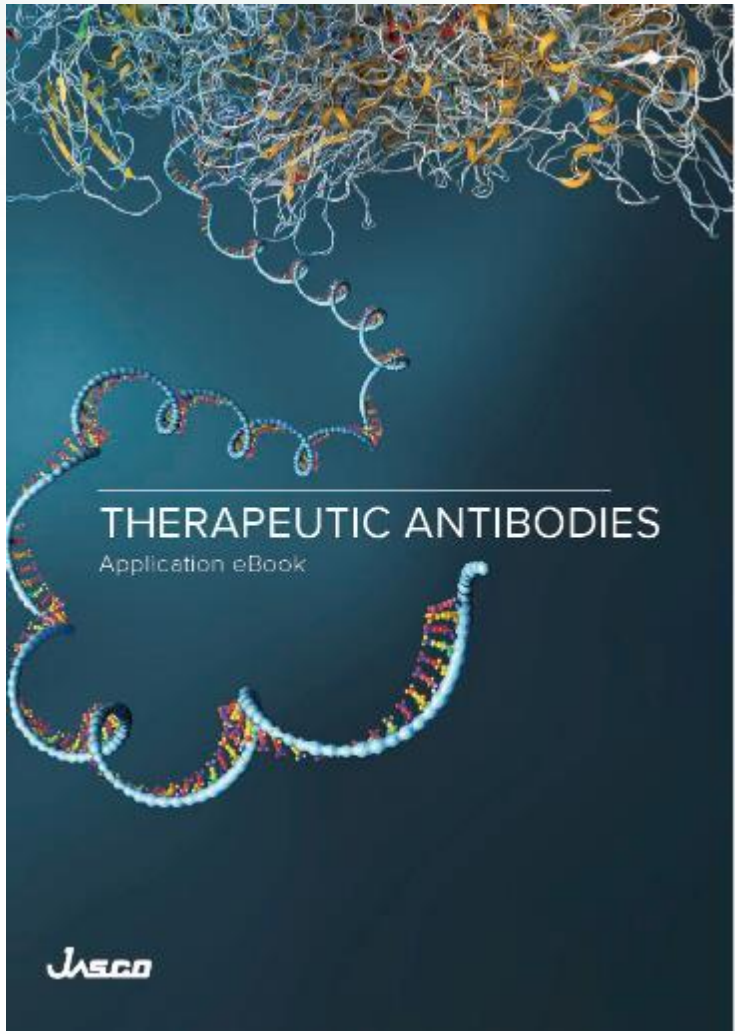
: Accurate secondary structure estimation

qHOS

: Statistical Evaluation of higher-order Structure

Accelerate CD spectroscopy to industry

More Applications in Therapeutic antibodies



CD/IR/Raman Applications in Therapeutic Antibodies

THERAPEUTIC ANTIBODIES Application eBook