



Sameness Assessment of Peptide Therapeutics

Using 1D & 2D NMR Spectroscopy

Background & Regulatory Context

Peptide drugs, including GLP-1 analogues, are structurally complex and critically dependent on their **Higher Order Structure (HOS)**, a key quality attribute. Regulatory pathways (e.g. ANDA or biosimilar frameworks) require **demonstration of structural and functional sameness** to the reference product.



Objectives

Establish a robust analytical strategy for sameness assessment



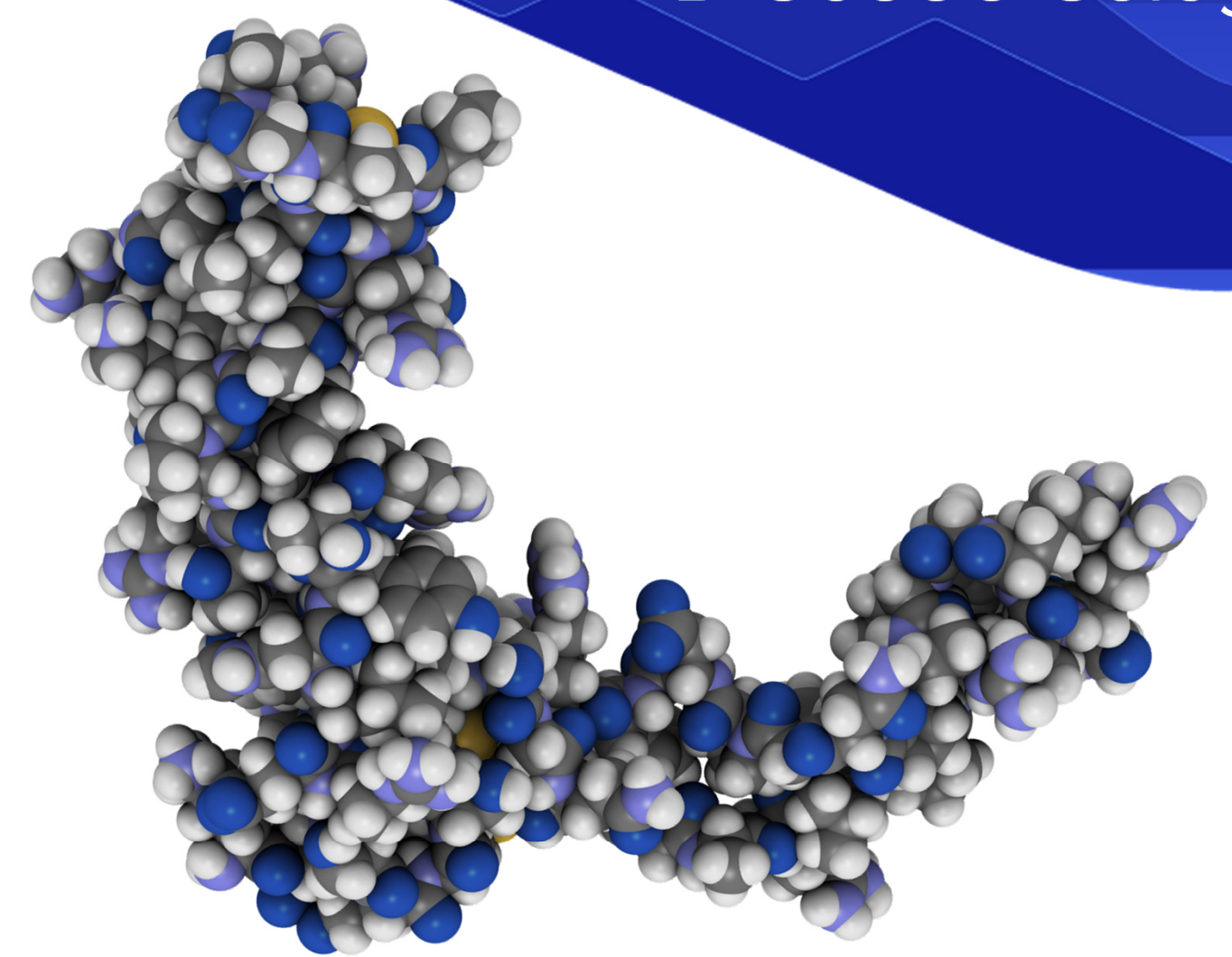
Enable regulatory-ready comparability



Use of orthogonal methods (1D and 2D NMR)



Apply quantitative similarity metrics

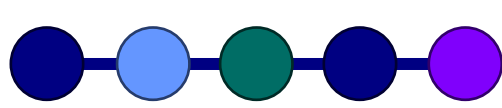


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Why “Sameness”?

Primary structure
sequence, modifications



HOS
secondary/tertiary structure or oligomerization

Impurities and process-related variants
No critical changes



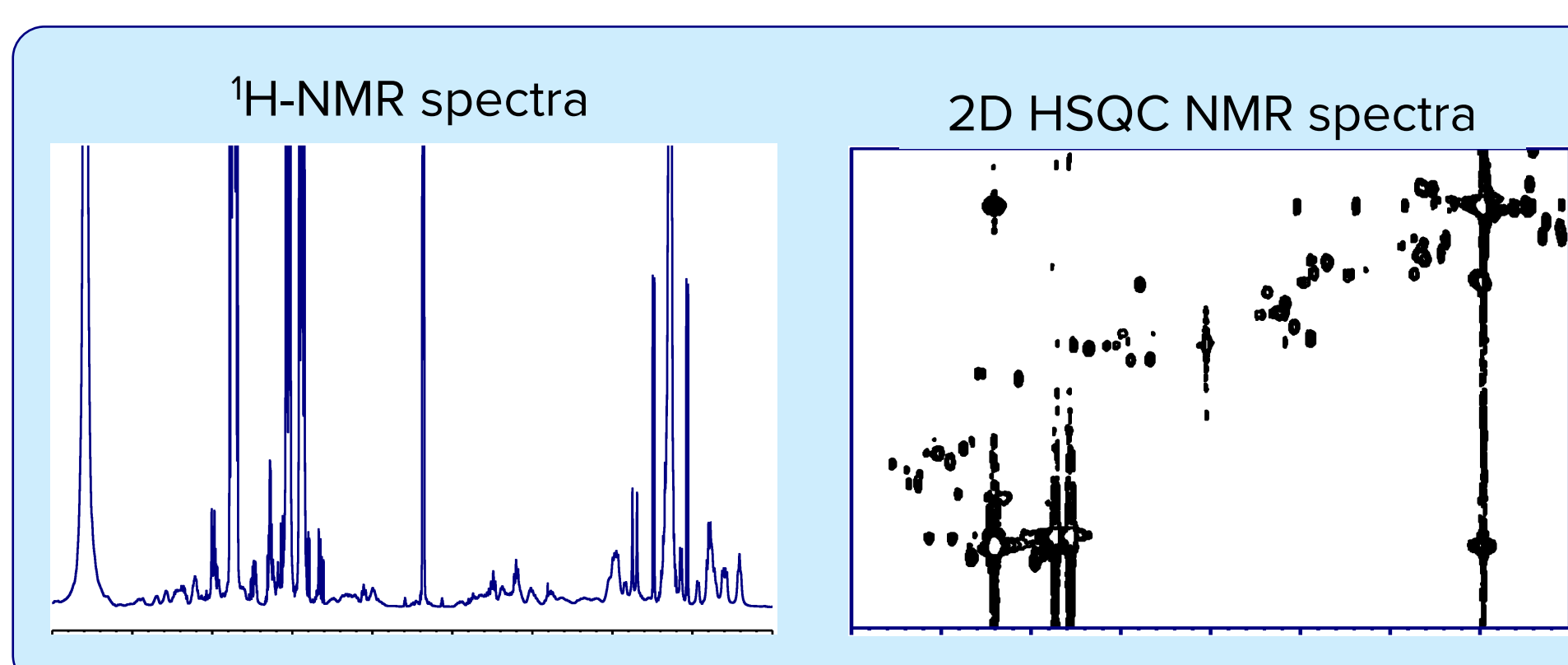
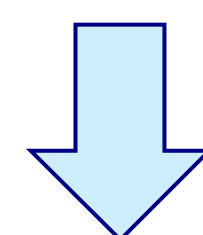
HOS is a critical quality attribute, as it can:

- influence efficacy
- affect safety
- drive immunogenicity

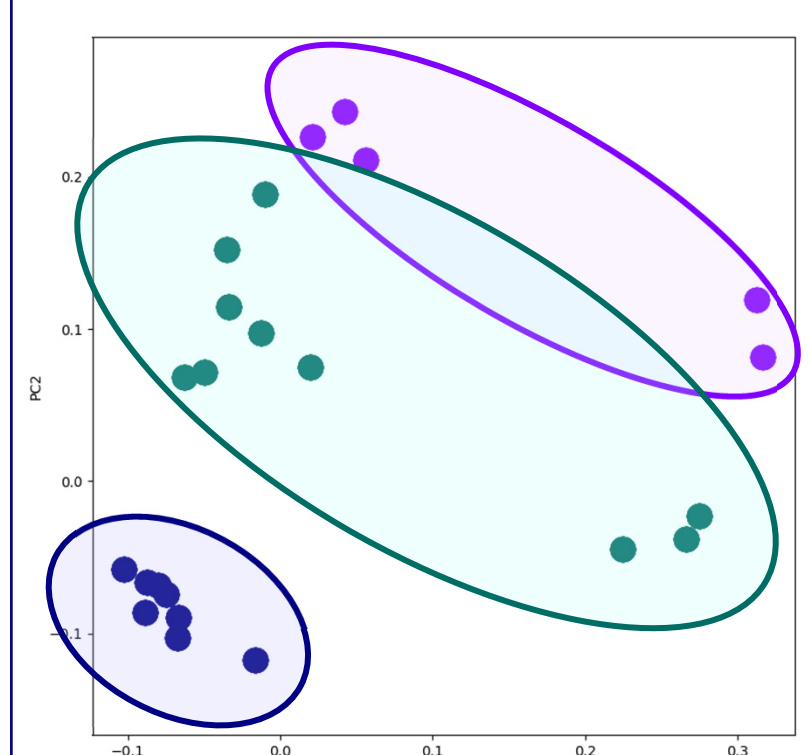
Analytical strategy



Reference listed drug (RLD) and test product are analyzed by 1D and 2D NMR

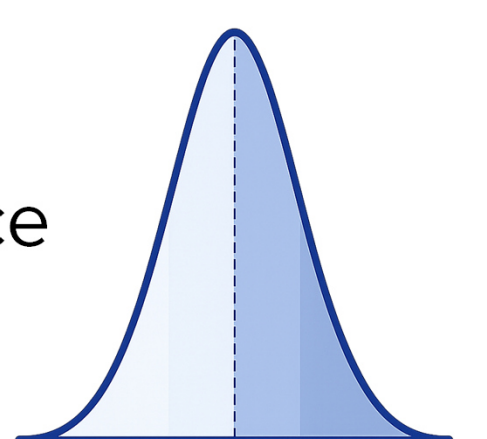


Quantitative sameness



PCA reduces complex spectral data to key variance patterns

Similarity is quantified using statistical distance metrics (e.g. Mahalanobis)



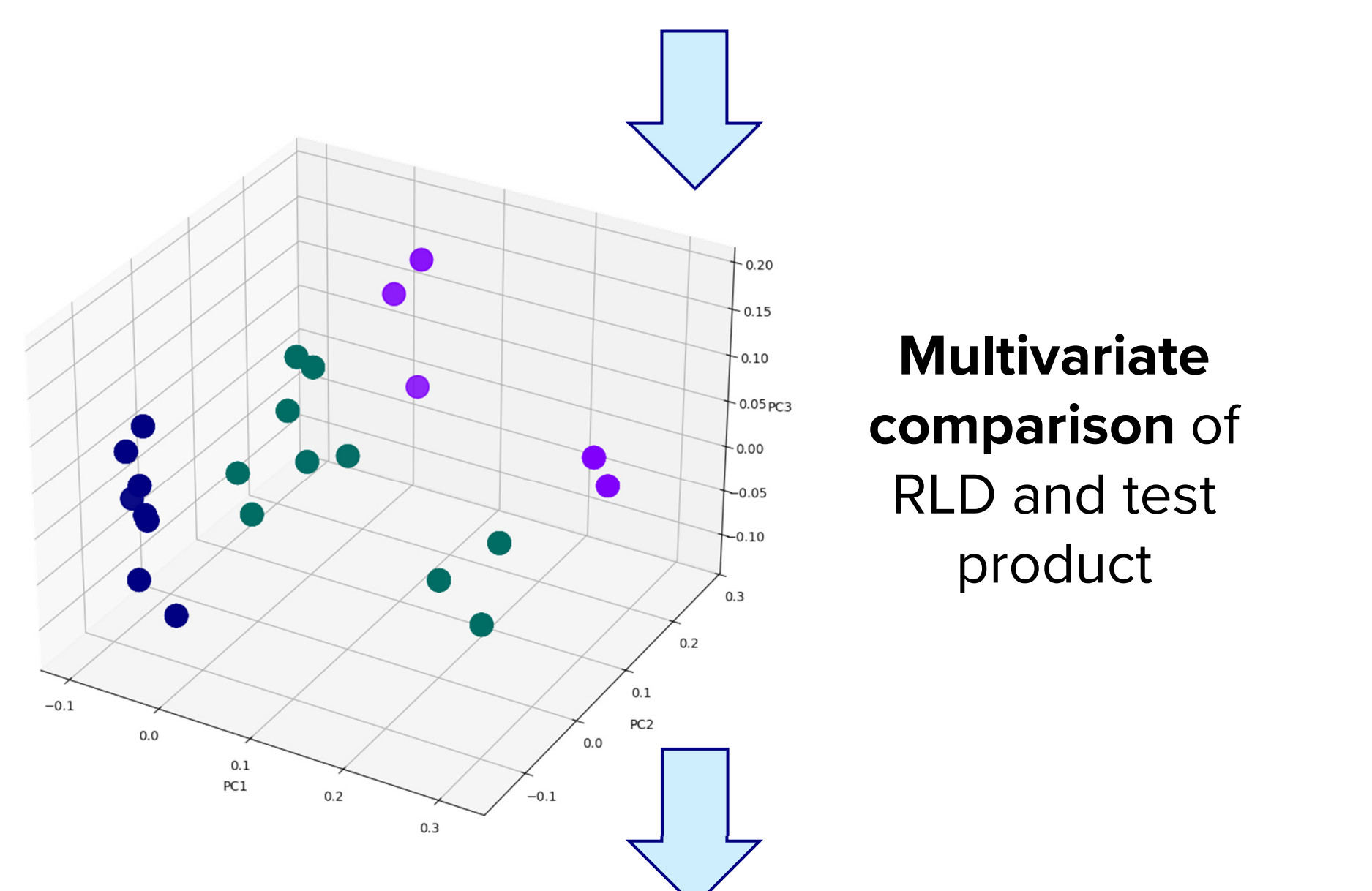
Key Challenges

Sources of apparent non-structural differences:



- pH-dependent chemical shift variations
- Formulation effects (excipients)
- dynamic (reversible) effects
- sample preparation artifacts
- measurements-related effects

Access to **representative samples** across **multiple batches and expiration dates** remains a major practical challenge.



Multivariate comparison of RLD and test product



Sameness evaluation based on predefined specifications and acceptance criteria

Take Home Message

Enables robust and quantitative discrimination between **true structural differences** and analytical variability

- Direct, structure-based **comparability using NMR**
- Integration of orthogonal methods and **chemometrics**
- Quantitative and **regulatory-relevant assessment**

Strong potential for **generics and biosimilars**.