HDX-MS FOR ASSESSING COMPARABILITY BETWEEN INNOVATOR AND BIOSIMILAR BIOTHERAPEUTICS

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INTRODUCTION

- The expiration of patents and other intellectual property rights for originator biological medicines over the next decade opens up opportunities for biosimilars to enter the drug market and provides affordable biological medicines to patients.
- However, the development of biosimilars are quite challenging because of the size and complexity of the active substance and nature of the manufacturing process. As mentioned by FDA early this year on the guidance of biosimilar therapeutic products, "the three-dimensional conformation of a protein is an important factor in its biological function. ..... Any differences in higher order structure between a proposed product and a reference product should be evaluated in terms of a potential effect on protein function and stability..."
- Hydrogen deuterium exchange mass spectrometry (HDX-MS) can precisely define tertiary protein structure and, to varying extents, quaternary structure, which could further add to the body of information supporting biosimilarity.
- This study highlights the use of HDX-MS for assessing comparability between an innovator therapeutic mAb Remicade (Infliximab) and a commercial biosimilar product Inflectra.
- Three batches of innovator products during manufacturing process were analyzed at peptide levels in order to gain insight into the conformational difference. The HDX-MS experiments were conducted on an automated HDX platform and showed high reproducibility.

WATERS INTEGRATED HDX MS SOLUTION

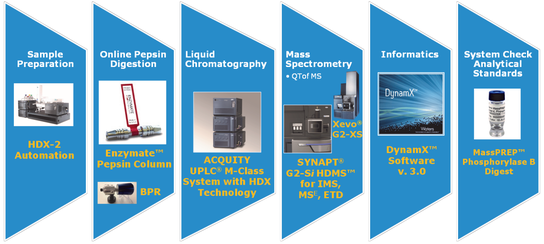

Figure 1: Waters integrated HDX-MS solution addresses each step of HDX-MS workflow for global (Intact), local (peptide) and residue (amino acid) levels analysis. The Enzymate along with back pressure regulator (BPR) enable high pressure pepsin digestion of proteins. DynamX 3.0: Industry leading HDX-MS informatics for automated processing of global (Intact), local (peptide), and residue (AA, ETD) levels of HDX MS data.

METHODS

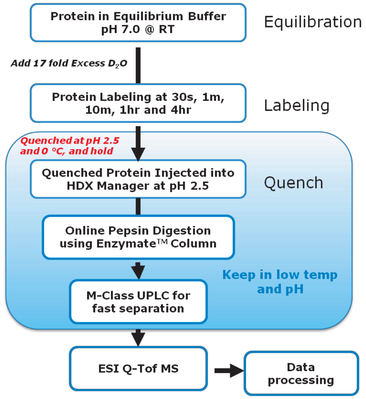

Figure 2. HDX workflow at peptide level.

Sample Preparation:

Both innovator (3 biological lots) and biosimilar (1 biological lot) were treated equally during all analysis. The labeling reaction was initialized by adding 15-fold of phosphate buffer (pH 6.8) to protein stock (~14 µM). After variable reaction times (30 sec, 1 min, 10 min, 60 min and 240 min), the labeling reaction was quenched by adding pre-chilled quenching buffer with TCEP and GdnHCl. All the sample preparation were operated by LEAP PAL3 system and timing was scheduled by Chronos (LEAP).

Chromatography:

Quenched solution flew through Enzymate™ column (Waters, 2.1 x 30 mm, 130 Å, 5 µm) to complete online digestion and then went to cold chamber inside of HDX manager for separation. Analytical column was ACQUITY UPLC BEH C18 column, 1.7 µm 1.0 x 100 mm. The trap column was an ACQUITY VanGuard column, BEH 18 1.7 µm 2.1 x 5 mm.

MS analysis:

Data were collected by Waters Synapt G2-S HDMS instrument.

Data processing:

Undeuterated control was processed using PLGS 3.0 for peptide identification. DynamX 3.0 was used to measure the deuterium uptake of each peptides and generate all visualization graphs.

RESULTS AND DISCUSSION

Figure 3. Sequence coverage of Infliximab. *(A) HC and (B) LC. Peptides displayed here were identified from triplicate runs for both innovator (2 batches) and biosimilar (1 batch) samples. 194 peptides were identified from HC and showed in blue bars and 88 peptides were identified from LC and showed in cyan bars. Average*

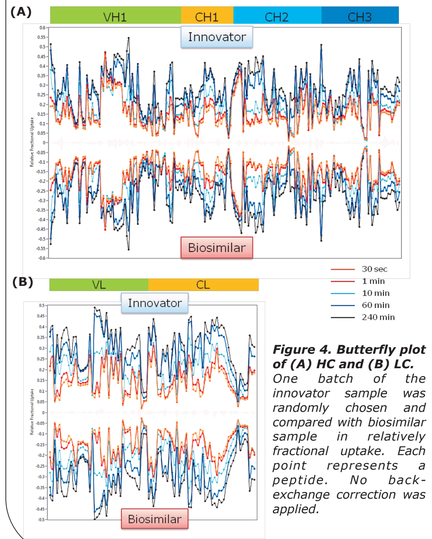

Figure 4. Butterfly plot of (A) HC and (B) LC. *One batch of the innovator sample was randomly chosen and compared with biosimilar sample in relatively fractional uptake. Each point represents a peptide. No back-exchange correction was applied.*

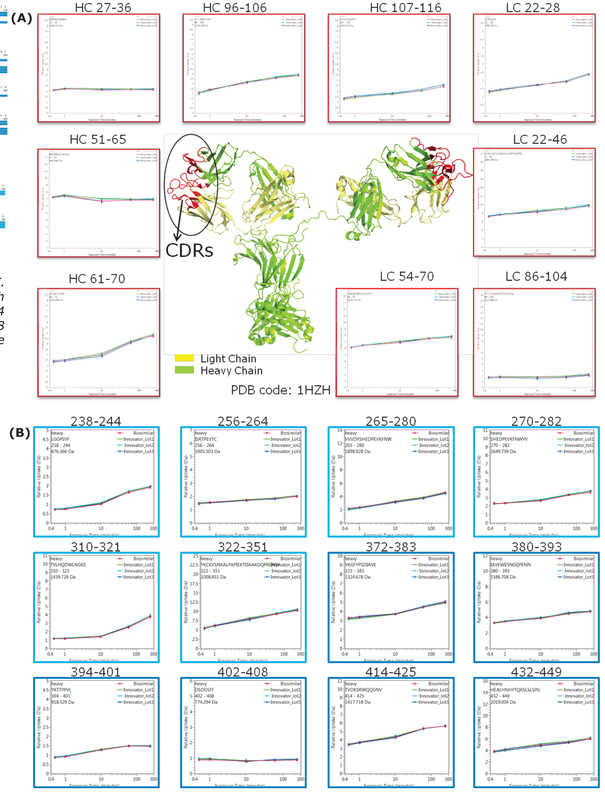

Figure 5. The relative deuterium level of peptides from (A) CDR region and (B) CH2, CH3 domains. *The level of deuteration was monitored at 0.17, 1, 10, 60, and 240 min for three batches of innovator and one batch of biosimilar sample. These traces are an average of three replicates with error bars at each time point ranging from ± 0.02 to 0.05 Da. The CDR region is highlighted in red on the insert antibody structure. LC is showed in yellow and HC is showed in green. (B) The uptake curves of example peptides from CH2 domain are highlighted in cyan and the ones from CH3 domain are highlighted in blue.*

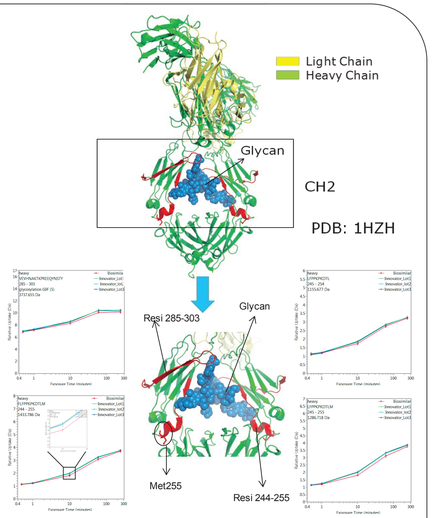

Figure 6. Comparison of deuterium incorporation of innovator and biosimilar samples. Representative deuterium incorporation profiles of regions (residues 244-255, 245-254, 245-255, 285-303) in CH2 domains show minute difference. The red line represents the data from biosimilar product, the green, cyan and blue lines represent the data from the three batches of innovator samples. The experiments have been repeated in triplicate runs. The location of the region displayed minor difference among biosimilar and innovator sample is colored in red in the model structure of IgG1 (PDB: 1HZH). Glycosylation is shown in blue. Met255 is circled and shown in stick.

CONCLUSION

- **Higher order structure of a protein is an important factor for its biological function.**
- **HDX-MS is reproducible and reliable method, and is able to localize the conformational changes.**
- **HDX-MS study showed that the conformation for the biosimilar product is highly comparable with the reference product overall. No distinct difference was observed in the CDR regions.**
- **Minute differences were observed in CH2 domain close to glycosylation area and attributed to minor differences in glycosylation between biosimilar and reference product.**
- **Our study shows great promise in adapting HDX-MS into biosimilar drug development process.**

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