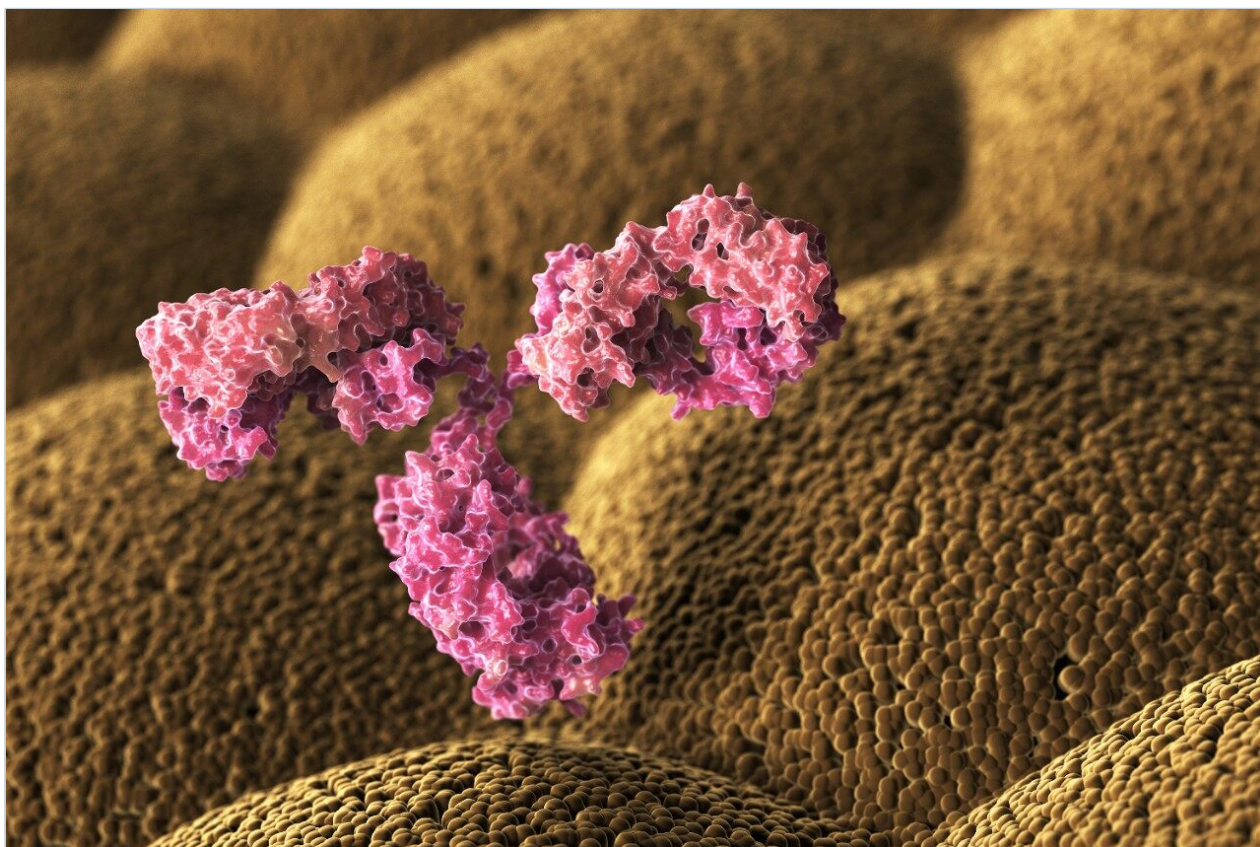


Note d'application

## Localized Conformation Analysis of Mutated Human IgG1 by HDX MS and nanoDSC

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Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

This application brief describes the process of probing mAb conformation change caused by amino acid mutation using both Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS) and nano Differential Scanning Calorimetry (DSC) technologies.

### Benefits

HDX MS provides insights in days that may be challenging to achieve in months with other techniques.

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## Introduction

Conformational changes in biotherapeutics may impact their safety and efficacy. Therefore it is important to regulators and manufacturers to understand what affects conformational changes. Guidelines also stipulate that manufacturers must produce a well-characterized biotherapeutic product (WCBP) including knowledge of higher order structure (HOS). The analytical tools described here help organizations more effectively discover and develop biotherapeutics in order to maintain their competitiveness.

A human immunoglobulin G (IgG) was developed with a site-specific mutation. When the higher order structures of non-mutated and mutated IgGs were compared using alternative methods, the localized conformational changes at the substitution sites were not easily detected. Two techniques were used in this study: nano Differential Scanning Calorimetry (DSC), and hydrogen deuterium exchange with mass spectrometry. Both techniques contribute to the understanding of the higher order structure of biomolecules and biotherapeutics.

The Nano DSC instrument from TA Instruments can detect changes in heat content down to the nanowatt range from 2 micrograms of protein. The heat signal on a DSC is used as a measure of the folding or unfolding process and can therefore provide insights into the overall structure. DSC is useful in providing rapid and robust measurements with little sample preparation.

HDX MS can be used to locate the region where the conformational change has taken place. HDX MS experiments provide extremely high detail and leverage the power of UPLC/QToF-MS<sup>E</sup> to maximize coverage of a protein. Here, a nanoACQUITY UPLC System with HDX Technology was used along with a Xevo G2 QToF MS. The local HDX workflow demonstrates the ability to detect differences in deuterium uptake at the

peptide level. The differences in deuterium uptake between different conformations allowed us to locate precisely where the mutated and non-mutated forms differed.

HDX data processing used to be tedious and time-consuming when performed manually. DynamX software was developed by Waters to automate data processing and provide efficiency that had not previously existed. DynamX automatically calculates deuterium uptake. Results are displayed in convenient comparative views: uptake curves, butterfly charts, and difference plots.<sup>1</sup> The processing time is thereby reduced from months of specialist interpretation to a day of reporting and curation. We report efficient HDX studies of a control and of two batches of mutated IgG1.

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## Results and Discussion

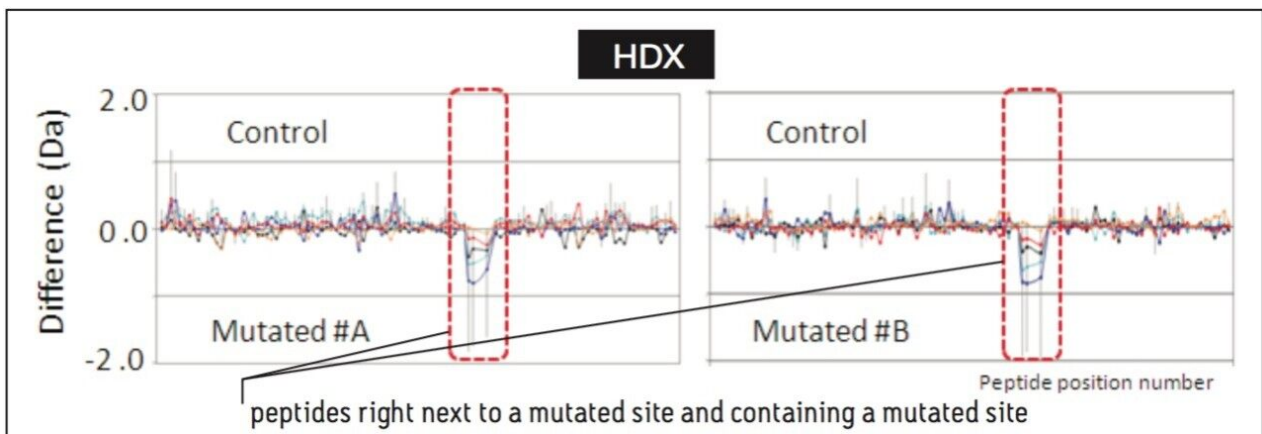


Figure 1. Background corrected DSC scan data of two mutated batches (green and blue traces) and control (red trace). Both mutated batches, #A and #B, had same amino acid mutation. The major difference is the low temperature transition indicating that the difference between the mutated samples and the control sample only affects a specific domain of the IgG. The shift to lower temperature (red arrow) of a portion of the trace indicates that the mutation has decreased the stability of this responding region of the IgG structure, i.e., that less energy is required to make it fold or unfold because the unfolding temperature is directly related to the free energy of the protein.

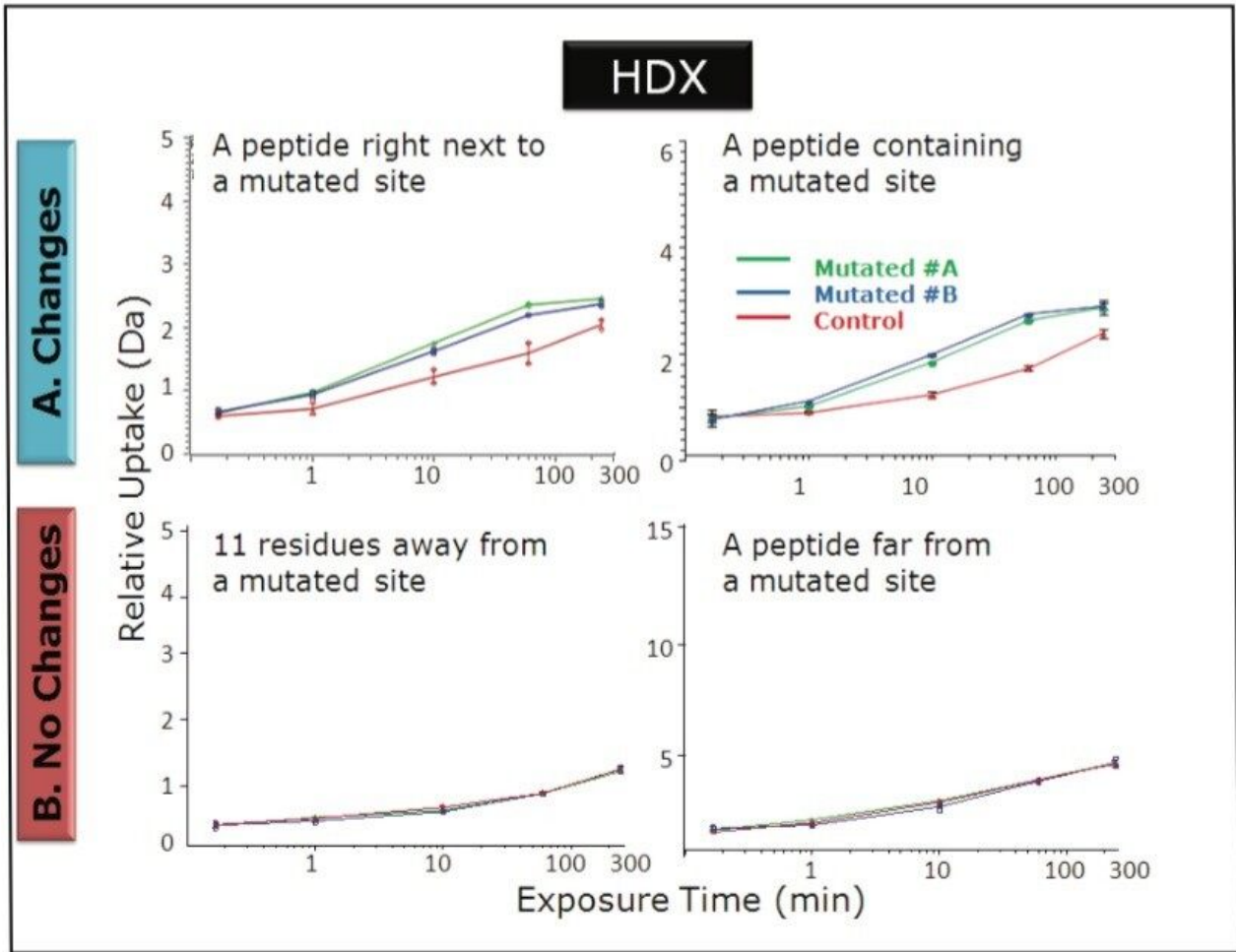


Figure 2. The deuterium uptake curves from the HDX MS study with (top two panels) and without changes (bottom two panels) comparing the control to the mutated samples for four selected peptides. The key finding in this figure is that HDX MS has located at the peptide level where the conformational changes have taken place in the mutant species. Each panel shows the corresponding peptides from the Control, Mutant A, and Mutant B batches. The deuterium uptakes of the peptides were measured at exposure times 0.17, 1, 10, 60, 240 min. (A) The top two panels show that the peptides from the mutated samples had faster exchange rates compared to the ones from the control. These peptides were found nearby or at the mutated site. (B) Conversely, two other peptides located far from the mutated site showed the same deuteration uptake as the control. This indicated that there were no conformational changes at those locations. In both the heavy and light chains only the mutation sites showed changes.

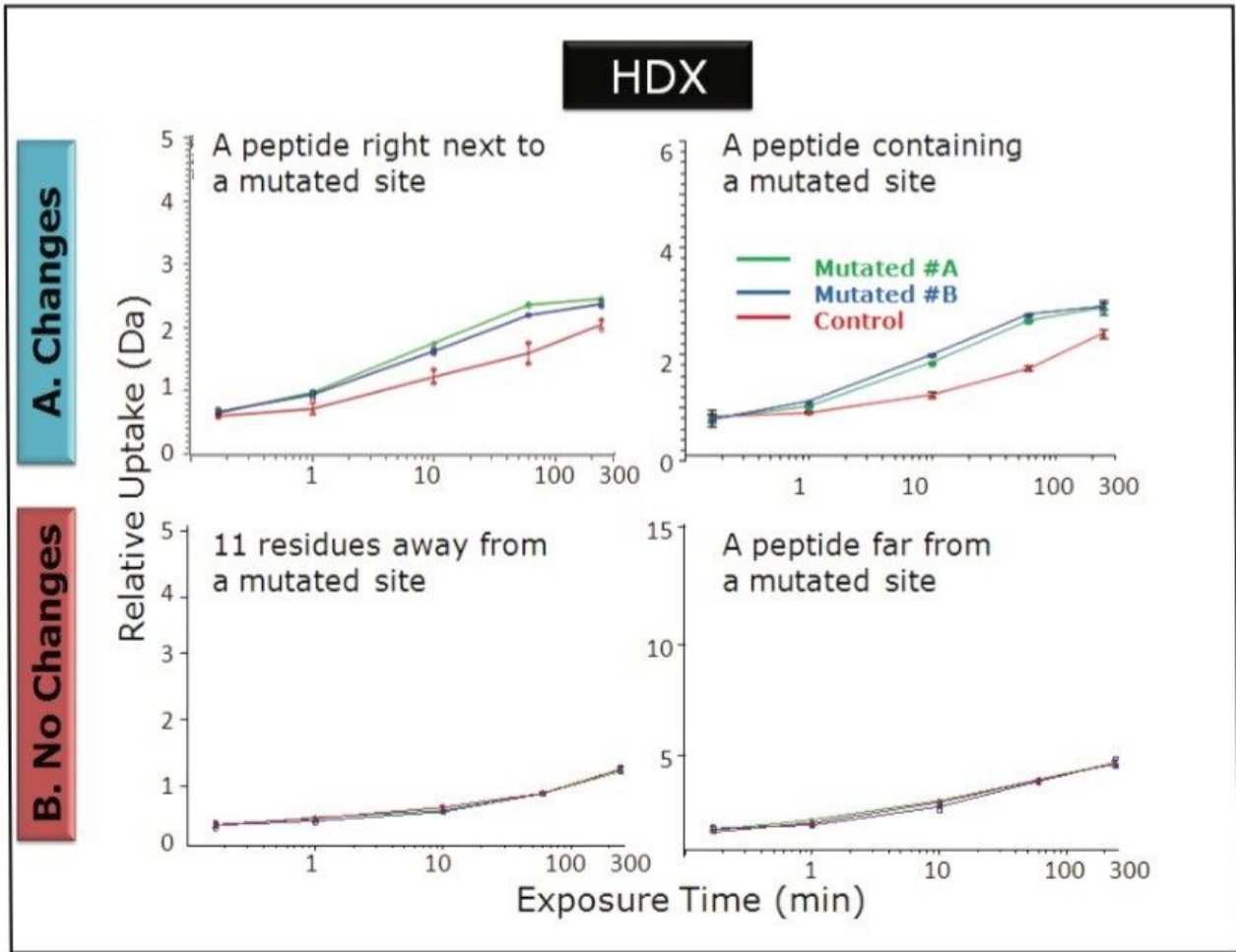


Figure 3. Comparison of deuterium difference between peptides from the IgG heavy chains. DynamX "Difference" charts comparing the mutated IgG #A and mutated IgG #B to the control in the left and right panels, respectively. This visualization helps to locate which peptides contain different uptakes in a comparative view. The red boxes highlight the peptides near and at a mutated site, showing where the largest changes were detected. Each data-point indicates the difference in uptake between the control and a mutated batch. The vertical bar represents the sum of the uptake differences across the time-points; the longer the vertical bar, the larger difference found between the control and mutated IgGs. The values of vertical bars in red boxes were significantly different compared to the other regions, indicating this region is responsible for the local conformational effect.

## Conclusion

Waters' tools for high order structure of protein allow the determination of conformational changes for all sectors of a biopharmaceutical company. Benchtop scanning calorimetry provides a robust and rapid measure of the change in folding across different temperatures. Rapid comparisons can be made for different states, to be used in discovery or development. HDX MS provides highly detailed information to locate where conformational changes occur.

HDX MS provides insights in days that may be challenging to achieve in months with other techniques. Both tools contribute to efficiency in an industry that requires robust, rigorous, and rapid information. Using these tools will help a pharmaceutical organization manage their pipeline effectively, satisfy regulators, and protect better against competition.

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## References

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2. DSC technical note (MCAPN-2010-04): Advantages of Using a Nano DSC when Studying Proteins that Aggregate and Precipitate when Denatured High-throughput DSC.
3. HDX application note (720003864en): Conformational Characterization of Calmodulin by Hydrogen Deuterium Exchange Mass Spectrometry.
4. Localized conformation analysis of mutated human IgG1 by hydrogen deuterium exchange mass spectrometry (HDX MS) and differential scanning calorimetry. Waters Corporation, PSTR134660310, presented at WCBP 2012.

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## Featured Products

[ACQUITY UPLC M-Class System with HDX Technology <https://www.waters.com/134778571>](https://www.waters.com/134778571)

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