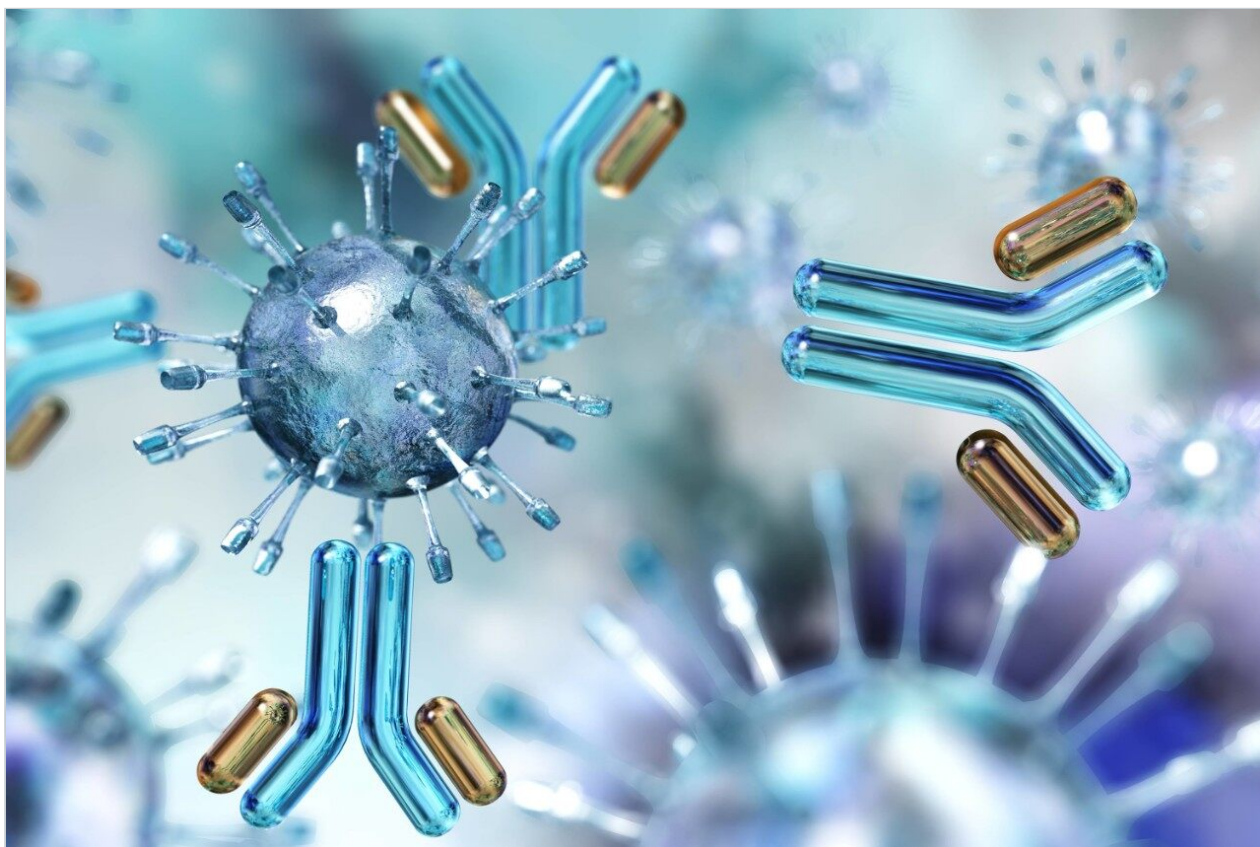




## Hydrogen Deuterium Exchange MS Analysis for Calmodulin Protein Conformation

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Waters Corporation



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

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## Abstract

To determine conformational changes between apo- and holo-calmodulin using hydrogen deuterium exchange combined with high resolution MS.

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## Introduction

The correct protein conformation is essential for biological function. A large number of highly diverse diseases are caused by inherited or acquired modifications in protein structure. Protein conformation is a major concern in the biopharmaceutical industry where it is important to assure lot-to-lot reproducibility of a protein product or its integrity following storage. Changes in protein conformation are also of interest to pharmaceutical researchers who study the effect of an active pharmaceutical ingredient (API) on its target protein.

The hydrogen deuterium exchange (HDX) MS analysis is used to measure changes in protein conformation. When HDX is combined with UPLC separations and high-resolution MS, it's possible to discover subtle changes in conformation. Well established techniques, such as x-ray crystallography and NMR, determine protein structure. However, these technologies require substantial amounts and are challenged by higher molecular weight and dynamic proteins. The UPLC-MS approach described here measures global or local changes in protein conformation using very small amounts of wide-ranging proteins.

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## Results and Discussion

A typical workflow of an HDX reaction involves incubating a protein in solution with deuterium oxide ( $D_2O$ ) where hydrogen atoms on the protein backbone exchange with the deuterium in solution. The more accessible portions of the protein exchange rapidly compared to the less exposed parts which may be buried in the center of a protein or involved in hydrogen bonding. When the exchange reaction is complete, the pH is decreased to 2.5 to quench the reaction.

After pH reduction, the protein may be analyzed intact for global analysis, or may be exposed to an acidic

protease, such as pepsin, to digest the protein for local analysis. Another important requirement is to perform highly resolving separations rapidly at 0°C. Operating at 0 °C minimizes deuterium losses due to back exchange with the chromatographic eluents.

To meet these analytical challenges, the nanoACQUITY UPLC System with HDX Technology reproducibly performs peptic digestion and UPLC separation before MS analysis. The nanoACQUITY UPLC System with HDX Technology consists of an ACQUITY ULC Binary Solvent Manager, Auxiliary Solvent Manager, and HDX Manager. The HDX Manager is capable of online pepsin digestion, online desalting, and fast, highly resolving chromatographic separations at 0 °C. A Xevo QToF MS with ProteinLynx Global SERVER 2.4 (PLGS) Software provides secure identification of peptic peptides.

To test this technology, conformation and dynamics of calmodulin (CaM) upon the calcium binding was studied. CaM is a  $\text{Ca}^{+2}$  receptor that regulates biological activities of many target proteins. Once  $\text{Ca}^{+2}$  binds to CaM, several intracellular processes are activated. CaM was incubated with  $\text{Ca}^{+2}$  and exposed to  $\text{D}_2\text{O}$  to monitor changes in its conformation.

The nanoACQUITY UPLC System with HDX Technology was interfaced with a Xevo QToF MS via a standard electrospray source for these experiments. CaM was solubilized in physiological buffer and incubated in the absence (control) and presence of  $\text{Ca}^{+2}$  in deuterium labeling solution. Aliquots were taken at timed intervals from the control and  $\text{Ca}^{+2}$  labeled CaM solutions. In each case, the sample was reduced from pH 7.0 to pH 2.5 and immediately placed into 0 °C in the HDX Manager for peptic digestion and resolution by UPLC.

These data indicate that there was an increase in deuterium uptake into the calmodulin molecule as a function of exposure time to calcium and the conversion of apo-calmodulin to holo-calmodulin. This is represented in the binding diagrams below.

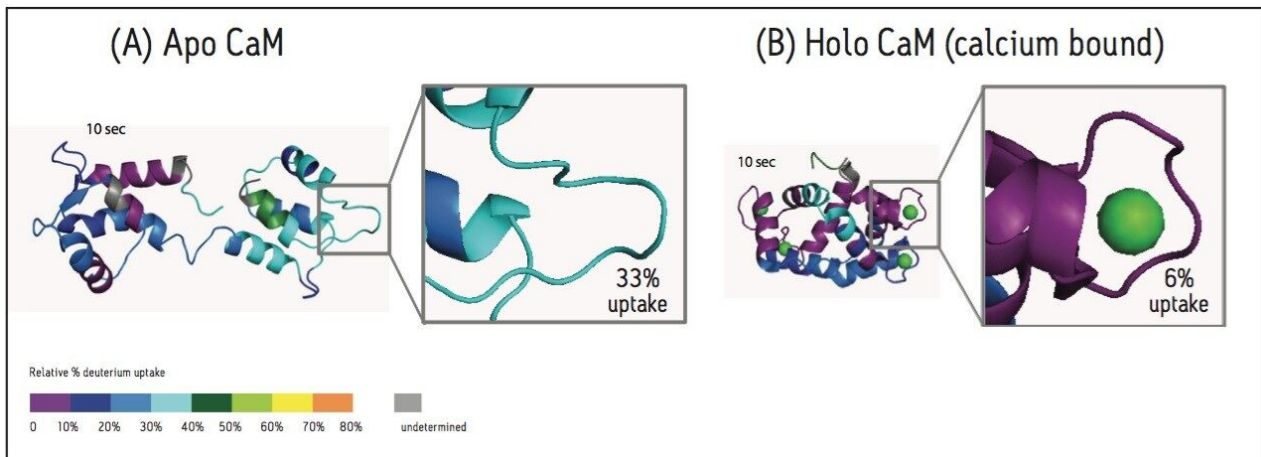


Figure 1. Reproducible chromatographic separations of calmodulin calmodulin peptides following online pepsin digestion. The peptides derived from online digestion were resolved on an ACQUITY BEH (1 mm x 10 cm) column at 0 °C in the HDX Manager. The typical separation profile is a linear gradient from 97% A to 60 % A at 40  $\mu$ L/min, where A= water/ 0.1% formic acid and B= acetonitrile/ 0.1% formic acid. The left-side panel shows the elution profile of the calmodulin peptic digest at times: 0, 10 s, 1 min, 10 min, 60 min, and 240 min following exposure to D<sub>2</sub>O. The panel on the right shows the resultant Xevo QTof spectra from the peptides at the same time points. Notice the increase in m/z of the isotopic distribution as a result of longer exposure to D<sub>2</sub>O.



*The nanoACQUITY UPLC System with HDX Technology.*

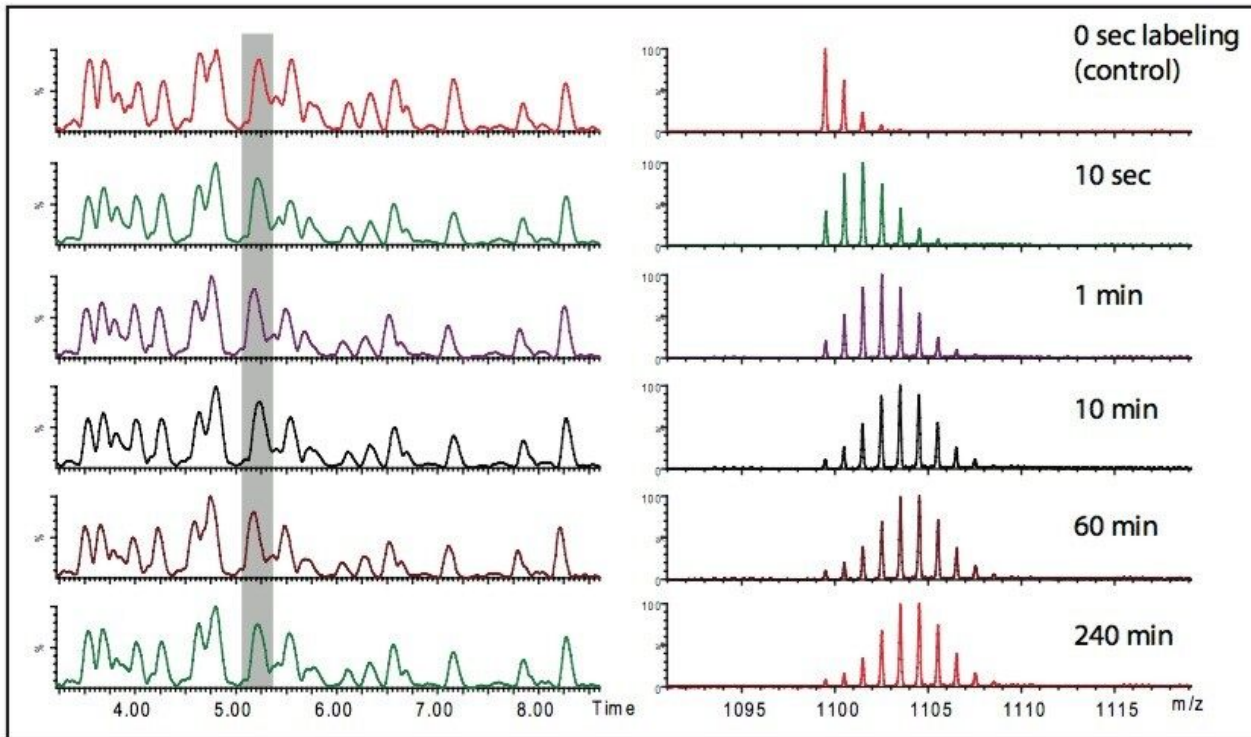


Figure 2. Crystal structure of CaM color coded to represent deuterium uptake. (A) Apo CaM and (B) holo CaM at 10 s deuterium labeling were compared. A significant difference in deuterium uptake was observed in the highlighted region where the conformation has been changed due to the calcium binding.

## Conclusion

The nanoACQUITY UPLC System with HDX Technology when combined with high resolution MS can effectively be used to determine small changes in protein conformation. This innovative approach to structural conformation analyses can augment data obtained from x-ray crystallography and NMR by providing in-depth knowledge about minor, local changes in protein structure solution that may be used to study function.

## Featured Products

ProteinLynx Global SERVER (PLGS) <<https://www.waters.com/513821>>

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

Hydrogen Deuterium Exchange with Mass Spec (HDX-MS) <<https://www.waters.com/10185909>>

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