# HYDROGEN / DEUTERIUM EXCHANGE MASS SPECTROMETRY SYSTEM FOR INVESTIGATING CONFORMATIONAL CHANGES IN CALMODULIN PROTEIN UPON CALCIUM BINDING

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## OVERVIEW

This study is to investigate the conformational changes in calcium binding protein, calmodulin, utilizing a Waters nanoACQUITY UPLC® System with Hydrogen/Deuterium Exchange (HDX) Technology.

## INTRODUCTION

Hydrogen/deuterium exchange mass spectrometry (HDX MS) has proven to be a useful analytical method for the study of protein dynamics and changes to protein conformation. The applications in HDX MS require a system that can perform rapid chromatographic separations at 0 °C and accurate mass measurements of deuterium labeled proteins and peptides with small quantities of material. Recent improvements in LC-MS have made HDX MS an indispensible tool for discovery and development of protein drugs. In this study, a nanoACQUITY UPLC System with dedicated cooled module (HDX manager) was used as a robust HDX MS platform for protein conformational analysis. In this system, online pepsin digestion was coupled to highly reproducible separation performed at low temperature.[1] High sequence coverage, up to 94% was obtained by the confident peptide identification. Precursor and fragment ions were acquired in a data-independent manner by alternating the collision energy between a low and elevated energy state on a Q-Tof Mass spectrometer ($MS^E$). We report a recent HDX MS study of conformational changes in important intracellular protein, calmodulin with calcium bound (holo) and without calcium (apo).

## METHODS

**Waters nanoACQUITY UPLC® with HDX Technology**

- Binary Solvent Manager (BSM): Eluent A: 0.1 % formic acid in water, Eluent B: 0.1 % formic acid in acetonitrile
- Auxiliary Solvent Manger (ASM): Eluent A: 0.05 % formic acid in water
- HDX manager : online digestion/desalting and separation at 0 °C

**Waters Xevo® QTof MS**

ESI positive mode was used. Capillary/Cone: 3.0 kV/37 V, Source/ Desolvation temperature: 80 °C / 175 °C, Desolvation gas: 800 L/h

**Chromatography**

- **Peptides**: The analytical column was an ACQUITY UPLC® BEH C18 1.7 µm 1.0 x 100 mm. The trap column was an ACQUITY UPLC VanGuard® Pre-column, BEH C18, 1.7 µm 2.1 x 5 mm.
- **Intact Protein** : The analytical column was an ACQUITY UPLC® BEH C4 1.7 µm 2.1 x 50 mm. The desalting column was used a MassPREP Desalting cartridge column, 2.1 x 5 mm.

**On-line pepsin digestion** : 2.1 x 30 mm pepsin column from Applied Biosystems was used.

**Protein preparation**

Calmodulin protein (CaM) from Sigma was prepared with calcium (Holo) and without calcium bound (apo).[2] Labeling solution was prepared with 20 mM HEPES in $D_2O$ at pH 7.07 with CaCl2 for holo and without $CaCl_2$ for Apo. The labeled protein was quenched after the timed incubation with 33 mM HCl to pH 2.5.

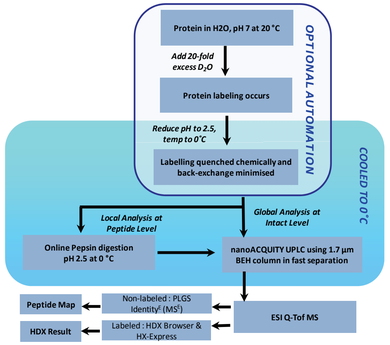



*Figure 1. Workflow of HDX MS analysis for peptide level. Protein labeling and injection can be automated using Leap HDX automation manager (blue line). Digestion and separation can be done at 0 °C inside of a HDX manager.*

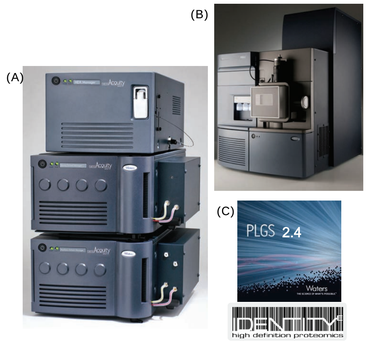



*Figure 2. (A) HDX analyses presented in this study utilized Waters nanoACQUITY UPLC system with HDX technology. The system consists of HDX manager (top unit), nano binary solvent manager (middle), and nano auxiliary solvent manager (bottom unit). (B) Xevo QTof MS ESI-MS (C) ProteinLynx Global Server™ (PLGS) ver.2.4 with Identity$^E$ for accurate peptide identification.*

## RESULTS & DISCUSSION

### 1. Intact HDX

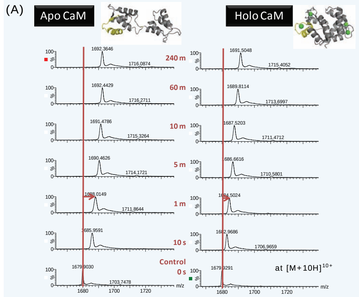



*Figure 3(A) Intact protein analysis is shown for global HDX MS. Intact spectra of apo and holo CaM showed the mass shift from the control to each time point at charge state 10+. Apo CaM showed greater mass shift than the holo (red arrows) due to the conformational change.*

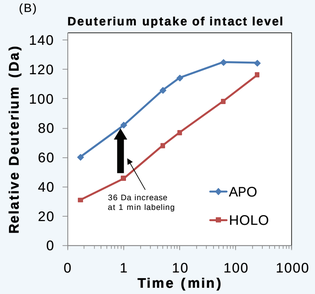
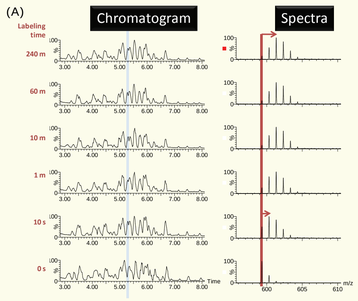
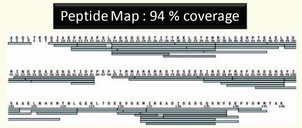



*Figure 3(B) The deuterium uptake curves in intact protein comparison is shown. The mass shift in y-axis was calculated from the deconvoluted intact mass. The global level conformational change was revealed by running fast intact HDX screening.*

### 2. Peptide HDX



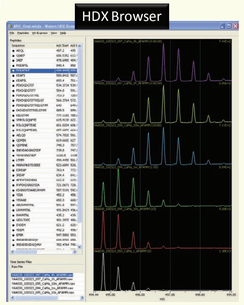




*Figure 4(A) Peptide analysis is shown for local HDX MS. The chromatograms of CaM peptides digested by online pepsin column was shown in left panel and The spectra for one of the peptides was shown in the right panel. The increased mass shift was seen from control (0s) to each time point (10s, 1m, 10m, 60m, and 240m) due to longer time exposure in deuterium labeling. (B) 94 % sequence coverage was achieved with many overlapping peptides. These peptides were identified by PLGS and the map was constructed [3] with common peptides exist both in apo and holo.*

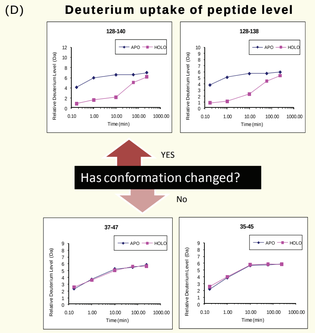



*Figure 4(C) Waters HDX Browser software was used to link the labeled raw data with peptide information and to display them in m/z and time. This software significantly reduced the data processing time. (D) The deuterium uptake curves for apo and holo comparison in peptide level. Top curves (peptide 128-140 and 128-138) illustrate that there are distinct difference in deuterium uptake between apo and holo. This means that there are obvious conformational changes in these regions. The bottom curves for peptides 37-47 and 35-45 showed no change in deuterium uptake. HX-Express was used to measure the deuterium uptake.[4]*

### 3. HDX results in 3D structure and Heat map

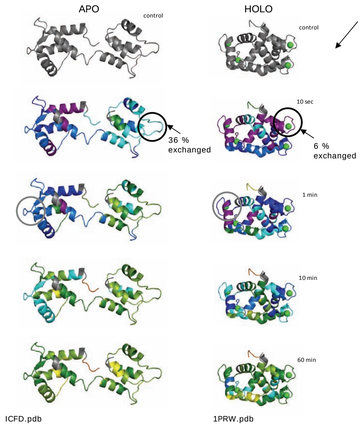



*Figure 5. Deuterium uptake information represented in CaM apo and holo 3D structures in time course at 10s, 1m, 10m, 60m, 240m labeling. At 10 s labeling, the same region of CaM were compared in black circles for apo and holo. Because of no calcium bound in apo, increased deuterium incorporation was found.*

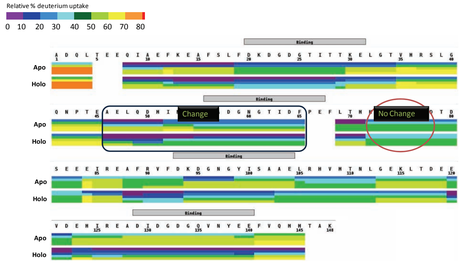



*Figure 6. Heat map of the entire CaM sequence for comparing HDX results. Two sets of colored bands of heat map represent the % deuterium uptake of CaM time course for apo CaM (top) and holo (bottom). Darker color represents less exchange. The highlighted region of the black box indicates the region with conformational change; different color trend was observed between apo and holo. The red circle represents no change.*

## CONCLUSIONS

- **The nanoACQUITY UPLC System with HDX Technology was very useful as a robust HDX MS platform for studying protein conformation.**
- **Global and local conformation of CaM were analyzed. The peptide-level comparison revealed the location of conformational changes. No visible change and minor change were observed upon various conditions for stability experiments.**
- **This HDX information is useful to better understand biophysical properties of the biomolecules.**

**References**

1. Wales, et al. (2008) Anal. Chem., 80, 6815-6820
2. Zhu, et al. (2003) Biochem., 42, 15388-15397
3. www.hxms.com/mstools/
4. www.hxms.com/HXExpress

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