Histidine hydrogen-deuterium exchange (His-HDX) mass spectrometry for identifying protein-ligand interactions

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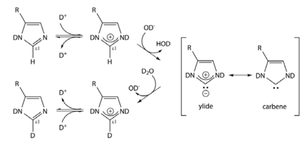
Introduction

Histidine Hydrogen-Deuterium Exchange Mass Spectrometry (His-HDX-MS) determines the HDX rates at the imidazole C2-hydrogen of histidine residues as it's HDX rate is significantly slower than the others. This property allows the HDX rate to evaluate the state of each histidine residue.
(e.g. Histidine folded in protein: not deuterium substituted.
Histidine exposed to heavy water: deuterium substituted)



Mechanism of the HDX reaction at the imidazole C2 hydrogen of histidine.

It is known that binding of a ligand stabilizes proteins against chemical or heat-induced denaturation. Based on this principle, several mass spectrometry-based proteomic approaches for identifying protein-ligand interactions have been developed, such as SPROX (stability of proteins from rates of oxidation) and TPP (thermal proteome profiling). Although these methods have shown encouraging results, identifying low abundant and multi-domain proteins is still challenging. We foresee that histidine hydrogen-deuterium exchange mass spectrometry (His-HDX-MS), which measures the slow HDX of histidine imidazole groups in proteins using mass spectrometry, has the potential to overcome these and evaluated the applicability of this platform to identify protein-ligand interadrawbacks. Here, we used His-HDX-MS as a platform to measure heat-induced unfolding of proteins and evaluate its applicability to the identification of protein-ligand interactions.

Methods – Experimental

Samples preparation

・Human MAPK 14 (p38a)
・Bovine serum albumin
(as a protective protein)

+

10 mM of Doramapimod (inhibitor)

↓

Incubation (40, 45, 50, 55, 60, 65, 75 ℃)
16 hr, 10mM HEPES (pH7.4)/ D_2O

↓

Denaturation
reduction, alkylation
Tryptic digestion

↓

LC-MS analysis

↓

Byos HDX workflow analysis

Byos

HDX workflow in Protein Metrics Byos®
Settings:
Precursor Mass Tolerance: 15 ppm
Fragment mass tolerance 1: 0.5 Da

Instrumentation:

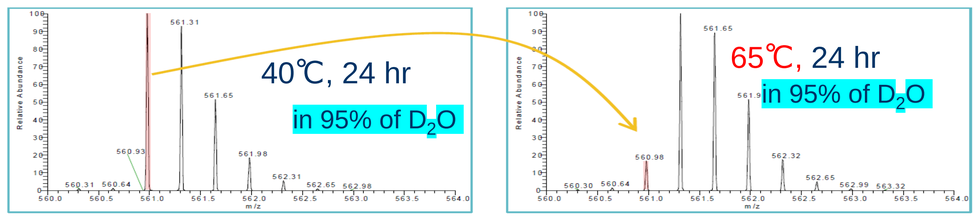
Nano LC :
ultimate 3000 RSLCnano
(Thermo Fisher Scientific)
Column: Monocap C18 Trap Column
(0.075 × 50 mm; GL Sciences Inc.)
C18 high resolution analytical column
(0.1 × 1,000 mm, GL Sciences Inc.)
A buffer: 0.1% trifluoroacetic acid/5% dimethyl-sulfoxide (v/v)
B buffer: acetonitrile in aqueous 0.1% acetic acid
Flow rate: 500 nL/min
Linear gradient of 10 to 35% over 60 min

Mass Spectrometer:

Q-Exactive
(Thermo Fisher Scientific)
Settings: Scan Range (*m/z*): 400-1,500
Resolution setting: 35,000 at *m/z* 200
Sheath gas: 0
In source-CID (V): 0
Normalized AGC Target : 3e6
RF Lens (%): 50
Microscans: 1

Results : Isotope distribution of thermally denatured peptide of MAPK 14

Fig. 1 Thermal denaturation of MAPK14 peptides containing a histidine residue changes their isotopic distribution in D_2O
MAPK14: HENVIGLLDVFTPAR, *m/z* 560.9738, z = 3 (Orbitrap XL, Resolution 30000)



The degree of deuterium incorporation into His-containing peptides can be calculated based on the shift of the center of m/z.

Fig. 2 Correlation coefficient between theoretical isotope distribution and D-substitution

Control

Number of D-substitutions	0.02868	0.10605	0.32546	0.63205	0.92236	1.27731	1.51282	1.66527
Correlation coefficient	0.99999	0.99998	0.99927	0.99254	0.96381	0.98579	0.9837	0.98527

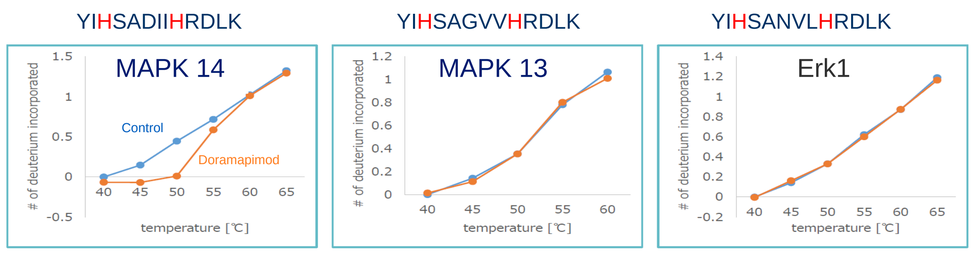
There is a high correlation between the deuterium substitution rate and the theoretical isotopic distribution for histidine containing peptides.

Results : Differential interaction of doramapimod with three kinases in HDX

	Inhibition @ 1μM doramapimod
MAPK 14	97 %
MAPK 13	60 %
Erk 1	3 %

Fig. 3 Only MAPK14, through interaction with doramapimod, showed a slower conformational change due to thermal denaturation.

Kinase Profiling Inhibitor Database
https://www.kinase-screen.mrc.ac.uk/kinase-inhibitors



Parameter settings for His-HDX analysis

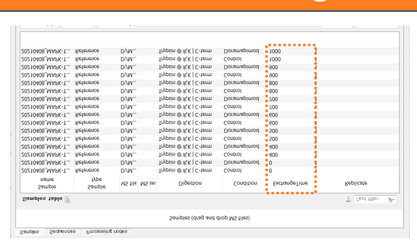
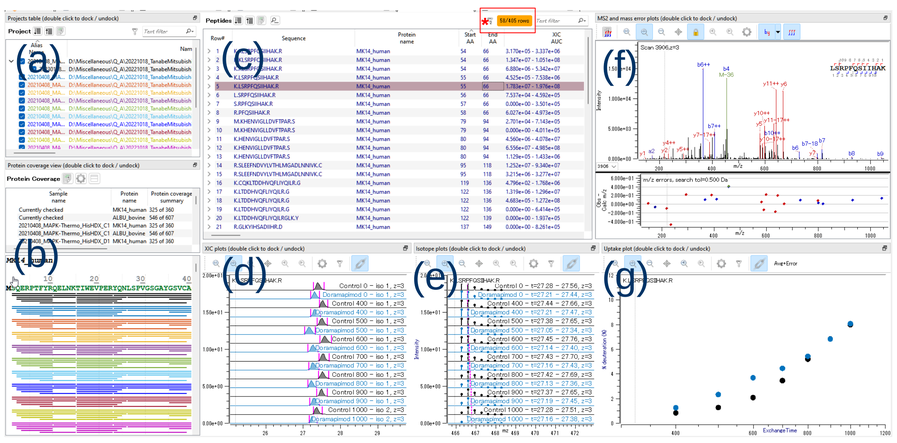


Fig. 4 Parameter for His-HDX in Byos
In a conventional HDX analysis, the reaction time must be entered in seconds in the Exchange time columns.

For the His-HDX analysis, we entered the alternative values for the incubation temperatures in the Exchange time column.

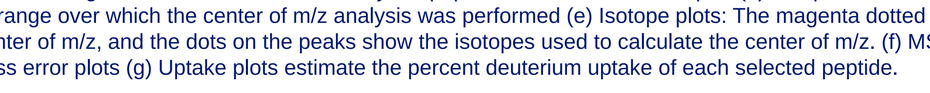
Results : HDX analysis of thermal denaturated MAPK 14 with doramapimod

Fig. 5 HDX project overview of Byos



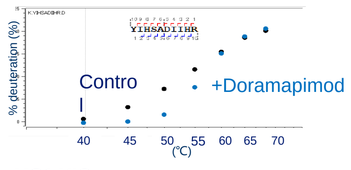
(a)Project table: Raw data info (b) Protein coverage of the peptides in a Peptide table (C) The peptide table allows users to filter peptides using the filter function to show only the peptides of interest in the report (d) XIC plot is showing the time range over which the center of m/z analysis was performed (e) Isotope plots: The magenta dotted lines show the center of m/z, and the dots on the peaks show the isotopes used to calculate the center of m/z. (f) MS2 spectrum and mass error plots (g) Uptake plots estimate the percent deuterium uptake of each selected peptide.

(h)



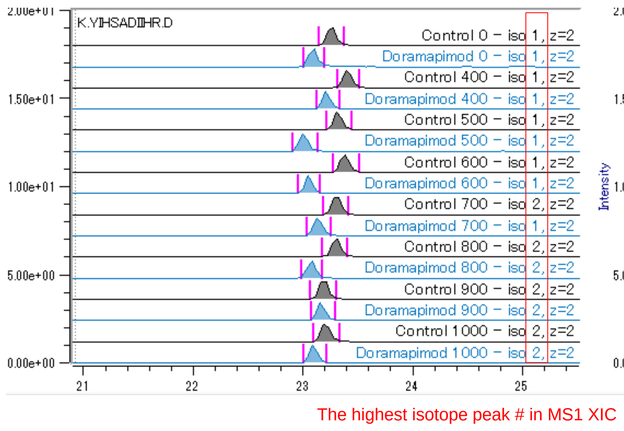
(h) Histidine-containing peptides of MAPK14 can be filtered using the funnel button on the peptide table.

Fig. 6 The deuterated % curves of the peptide at the inhibitor binding site were altered with and without doramapimod

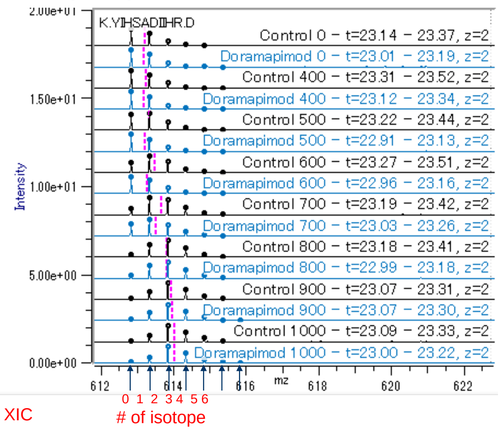


The values of % deuterateion were calculated from the shift of the center of m/z (the pink dashed lines) in the uptake plots, which were the averagine of isotopes with the dots.

MS1 XIC



Uptake plot

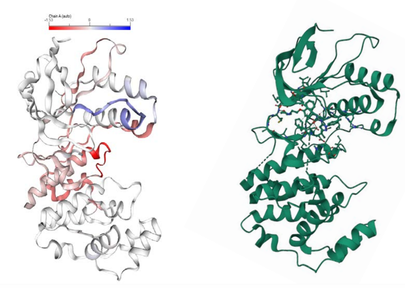


The highest isotope peak # in MS1 XIC

0 1 2 3 4 5 6
# of isotope

Results : Protein 3D of doramapimod and thermal denatured MAPK14

Fig. 7 The % deuterium curve shifts occurred other than at the inhibitor binding sites



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        10         20         30         40         50         60
MSQERPTFYR QELNKTIWEV PERYQNLSPV GSGAYGSVCA AFDTKTGLRV AVKKLSRPFQ
        70         80         90        100        110        120
SIIHAKRTYR ELRLLKHMKH ENVIGLLDVF TPARSLEEFN DVYLVTHLMG ADLNNIVKCQ
       130        140        150        160        170        180
KLTDDHVQFL IYQILRGLKY IHSADIIHRD LKPSNLAVNE DCELKILDFG LARHTDDEMT
       190        200        210        220        230        240
GYVATRWYRA PEIMLNWMHY NQTVDIWSVG CIMAELLTGR TLFPGTDHID QLKLILRLVG
       250        260        270        280        290        300
TPGAELLKKI SSESARNYIQ SLTQMPKMNF ANVFIGANPL AVDLLEKMLV LDSDKRITAA
       310        320        330        340        350        360
QALAHAYFAQ YHDPDDEPVA DPYDQSFESR DLLIDEWKSL TYDEVISFVP PPLDQEEMES
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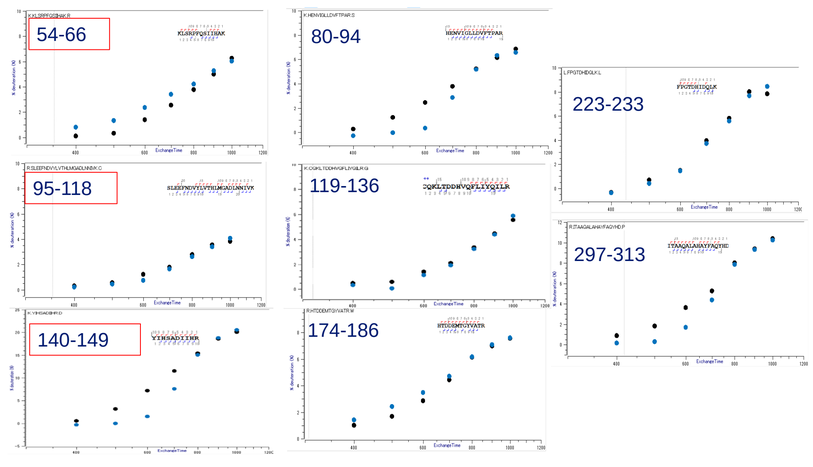
Inhibitor binding site



Conclusions

- Individual histidine residues in the protein have different stability depending on the position of the protein.
- The doramapimod - MAPK 14 interaction is likely to have a broad impact on MAPK 14 conformational changes.
- The Byos HDX workflow makes it easy to analyze complex deuterium ratio calculations and visualize the results, helping to reduce analysis time.

References:

- Lodowski et al., Methods Mol Biol, 2015;1271:123-132. Analysis of conformational changes in rhodopsin by histidine hydrogen-deuterium exchange.
- Miyagi et al., PLoS One, 2011. Histidine Hydrogen-Deuterium Exchange Mass Spectrometry for Probing the Microenvironment of Histidine Residues in Dihydrofolate Reductase, 6, 2, e17055.

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