OVERCOMING PEAK CAPACITY LIMITATIONS IMPOSED BY HYDROGEN EXCHANGE QUENCH CONDITIONS

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OUTLINE

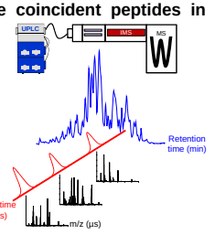
Objective
Incorporate ion mobility into the hydrogen exchange mass spectrometry workflow and investigate effects on sequence coverage and deuterium retention within complex samples

Methods
Hydrogen exchange (HX); ion mobility mass spectrometry (IMS); nanoACQUITY™ with HDX technology

Results
Ion mobility increased peptide coverage without sacrificing deuterium label or cycle time for complex samples

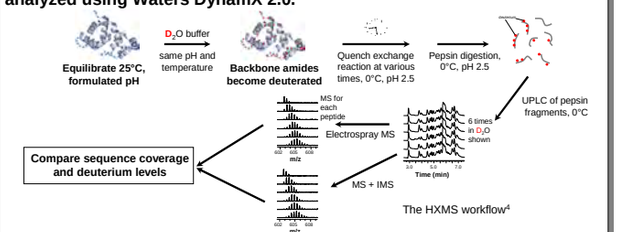
INTRODUCTION

HXMS is a powerful method for determining protein dynamics.[1] The need to analyze samples under quench conditions (low pH, low T) negatively affects LC peak capacity. Extending the LC gradient can improve peak capacity, however the increased separation time leads to lower deuterium recovery. IMS can separate coincident peptides in the gas-phase prior to MS detection. IMS increased the number of useful peptides in HXMS experiments on a 8.2 kDa protein by deconvolution of co-eluting species.[2] In this work, we examined the effects of IMS on peak capacity in a complex protein mixture. Further, HX was done on two large proteins. Peptide coverage and deuterium retention are compared in MS experiments with and without IMS.



METHODS

Continuous labeling hydrogen exchange experiments[1] were carried out at room temperature. Peptide exchange samples were analyzed using a Waters nanoACQUITY with HDX technology[3] coupled to a Waters SYNAPT G2 equipped with ESI source. Ion mobility separation was performed in the IMS cell pressurized with argon. All samples were digested online prior to RP-HPLC separation. Peptic peptides were identified using Waters PLGS 2.5, and deuterium incorporation data were analyzed using Waters DynamX 2.0.

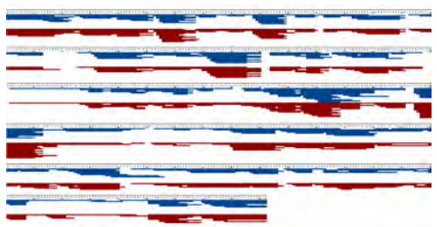


The HXMS workflow[4]

RESULTS AND DISCUSSION

MS with and without IMS

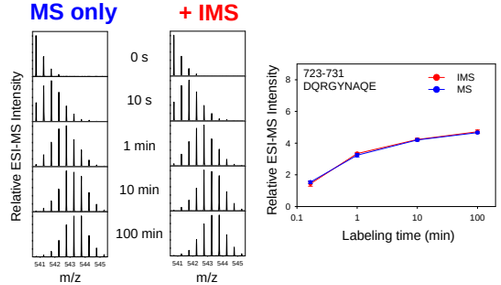
Similar sequence coverage is obtained with (IMS) or without (MS) ion mobility for peptic digest of 90 kDa phosphorylase b (peptide maps created using MSTools[5]).



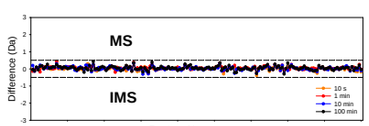
MS only
97%
271 peptides

+ IMS
98%
315 peptides

IMS does not affect deuterium recovery in HXMS experiments.

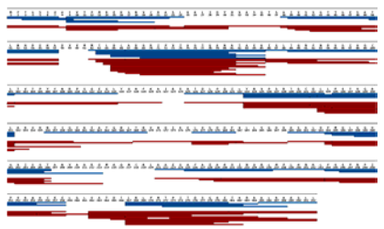


Plot depicting differences in deuterium retention for all peptic peptides of phosphorylase b at various labeling times measured with and without IMS. Dashed lines represent +/- 0.5 Da.



IMS Improves Peak Capacity in Complex Mixture

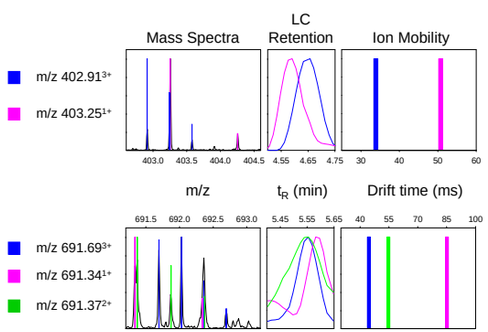
Peak capacity is greatly improved by IMS. In a 1:28 molar ratio mixture of 292 AA target protein and 645 AA of undesired protein, the 292 AA target peptide sequence is higher with IMS.



MS only
80%
43 peptides

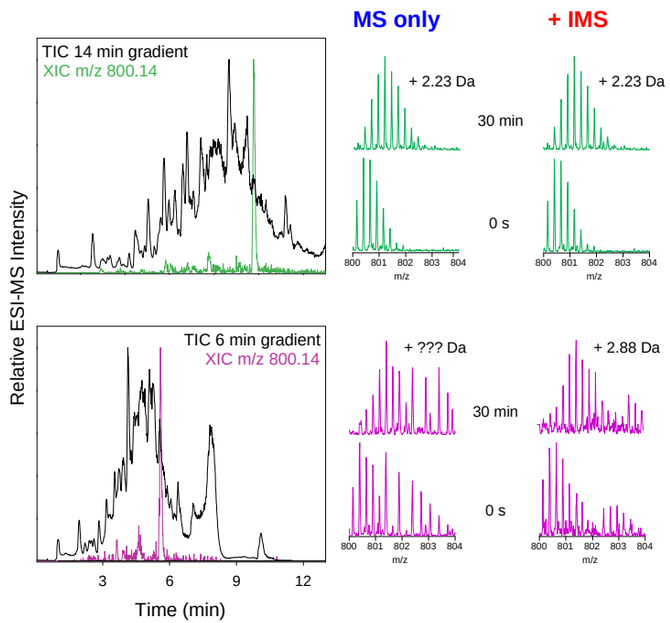
+ IMS
95%
65 peptides

IMS allows separation in mobility dimension of peptides not resolved by m/z or LC retention time.



IMS Saves Time and Label in Antibody Analysis

Short gradients facilitate deuterium recovery while long gradients allow for chromatographic separation. Additional mobility separation allows for the use of the shorter gradient, and thus increase deuterium recovery, even for a 150 kDa antibody.



IMS saves 0.65 Da with short LC gradient

REFERENCES

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ACKNOWLEDGEMENTS

We are grateful to the NIH (R01 GM101135) and Waters Corp. for funding.

CONCLUSIONS

- **No significant difference in deuterium uptake level was measured for peptides analyzed by MS or IMS**
- **Peak capacity was greatly increased for a complex protein mixture by enabling ion mobility**
- **Addition of IMS to the HXMS workflow allowed for the use of shorter LC gradients which lead to increased deuterium retention**