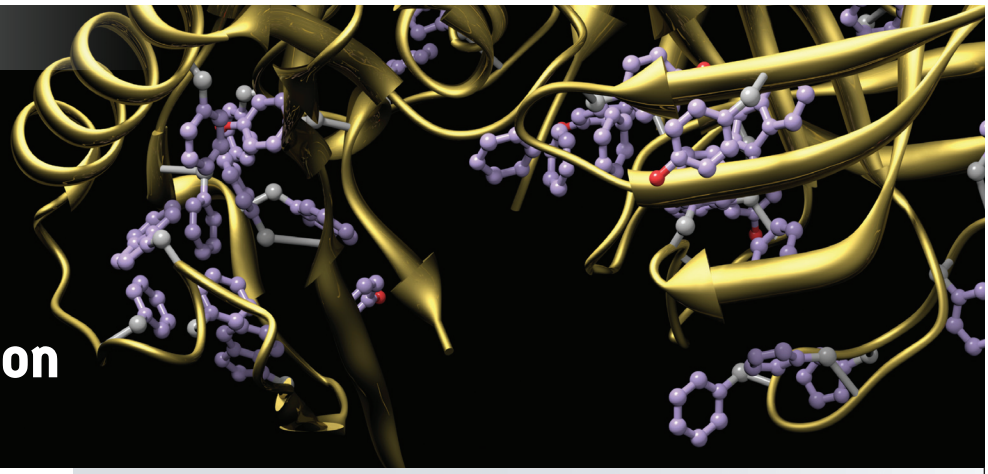
[TECHNOLOGY BRIEF]

Mobility Assisted Data Independent Protein Identification



GOAL

Quantitative analysis of complex tryptic peptide mixtures requires a protein identification strategy that is accurate, precise, and identifies species with high reproducibility. In addition, the depth to which a complex sample can be interrogated is crucial as this defines the lower abundance limit of proteins that maybe quantified. The SYNAPT® G2 HDMS™ System has substantial improvements in mobility resolution, mass resolution, dynamic range, and sensitivity; qualitative protein identification is presented here.

BACKGROUND

Protein identification is often challenged by the sensitivity and specificity required. For example, the presence of contaminating peptides within the collision cell during the collision induced dissociation (CID) process, leading to mixed fragment ion spectra is often ignored. This often unnoticed loss in acquisition specificity can readily result into incorrect peptide and protein identifications, which can lead to an increase in protein identification false discovery rate (FDR). However, protein identification experiments are conducted on increasingly complex samples. Instrumental technology advances should be accompanied by more selective acquisition methods, processing and deconvolution software that acknowledge chimericy and dedicated search algorithms.

Achieve greater than 85% identification reproducibility with 1D-LC-HDMS^E

THE SOLUTION

A tryptic digest of a *Saccharomyces cerevisiae* lysate was analyzed in replicate at various concentration levels, ranging from 50 ng to 1.6 µg loaded on-column. The peptides were separated and analyzed using a nanoACQUITY UPLC® System coupled to a SYNAPT G2 HDMS. The data were acquired in LC/HDMS^E mode – an unbiased mobility assisted TOF acquisition method – switching between low and elevated energy on alternate scans and correlating precursor and product ions by means of retention and drift time alignment; this principle is illustrated in Figure 1. Searches were conducted with ProteinLynx GlobalSERVER™ v2.5 using a species specific database; an example is shown in Figure 2.

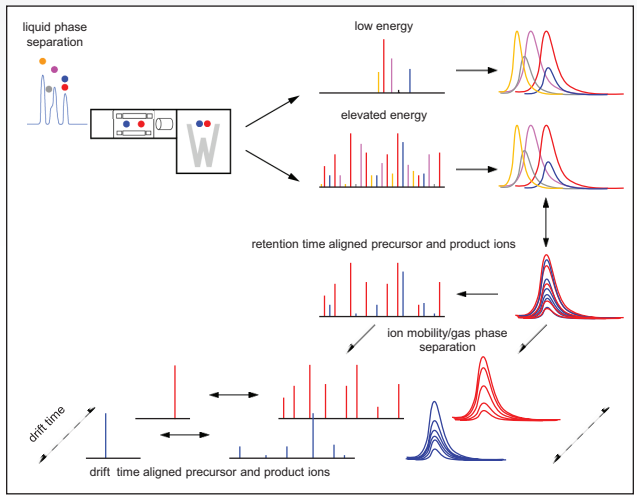


Figure 1. Principle of mobility assisted data independent acquisition (HDMS^E).

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

SUMMARY

The reproducibility of replicate analysis and a dilution series experiment of a *Saccharomyces cerevisiae* lysate by 1D-LC HDMS^E is demonstrated. As shown in Figure 3, protein identification reproducibility averages 80% between replicate experiments, whereas the intersection across increasing on-column loads is typically better than 85%.

Acknowledegement

Rob Beynon of University of Liverpool is kindly acknowledged for donating the *Saccharomyces cerevisiae* sample.

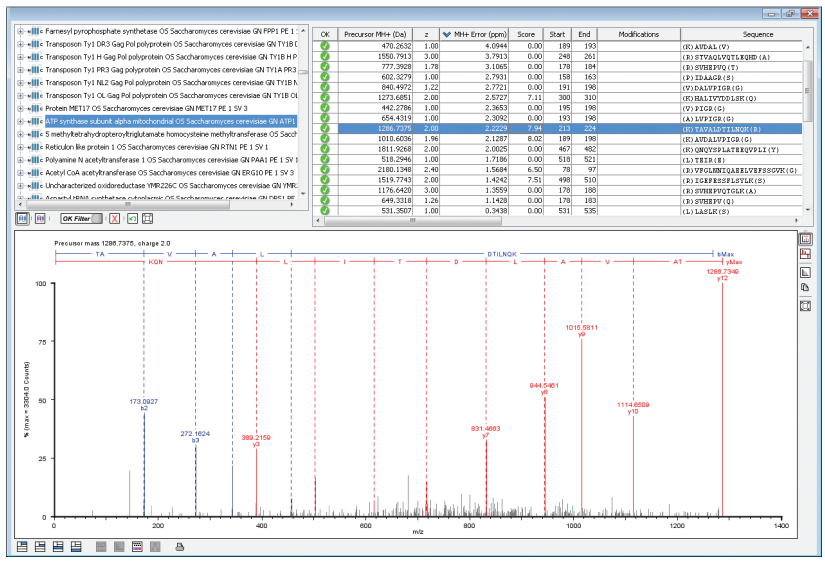


Figure 2. Medium abundant protein identification example from 1.6 µg of Saccharomyces cerevisiae loaded on-column and analyzed in 1D-LC/HDMS^E mode of acquisition.

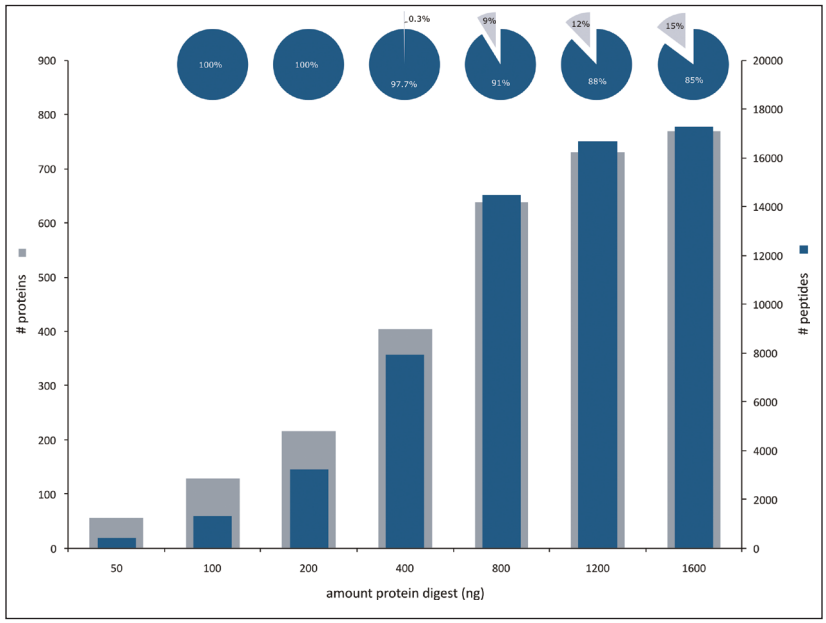


Figure 3. Reproducibility and cross sections non-redundant identifications from 50 ng to 1.6 µg loaded on-column.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com