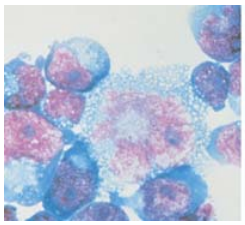


TECHNICAL NOTE

MIDAS™ Workflow Designer: Software for the Optimization of Targeted Peptide Experiments

Automating MRM Method Creation

In hypothesis-driven mass spectrometry based workflows such as post-translational modification (PTM) detection or biomarker verification, a suspected protein or modification is confirmed or quantified by performing experiments that specifically target peptides from the protein of interest. Multiple reaction monitoring (MRM) experiments on triple quadrupole instruments are generally recognized as the most sensitive and selective method for these types of targeted analyses. In an MRM experiment, the first quadrupole (Q1) is set to pass only the precursor ion, while the third quadrupole (Q3) is set to pass a specific fragment ion from that precursor. **M**RM **I**nitiated **D**etection **A**nd **S**equencing using the MIDAS™ Workflow (Figure 1) is an experimental strategy that is particularly powerful and practically indispensable for detecting low level components in a complex mixture and optimizing peptide MRM transitions. The MIDAS Workflow combines an MRM scan with a full MS/MS product ion scan to allow examination of **all** fragment ions in the same spectrum for sequence confirmation. As MRM is such a sensitive technique, the full scan MS/MS experiment must also be highly sensitive in order to acquire high quality MS/MS data from lower level precursors.

QTRAP® systems combine triple quadrupole MRM selectivity with linear ion trap MS/MS sensitivity and are ideally suited for the MIDAS Workflow. In fact, QTRAP systems are the **only** platforms that will stand-up to the rigorous requirements of experiments in complex matrices where detection of low level species is desired for proteomics and biomarker research.

MIDAS™ Workflow Designer

MIDAS™ Workflow Designer is a powerful software program that eliminates a tedious bottleneck in the development of MRM methods by automating the creation of MRM transitions and methods. MIDAS Workflow Designer accepts protein sequences, calculates theoretical peptides and corresponding MRM transitions, then builds the methods for acquisition.

Key Features of MIDAS™ Workflow Designer

- Takes advantage of the power of QTRAP® systems for generating high sensitivity MRM and MS/MS data – both are required for robust peptide detection and confirmation from real-world complex samples
- Builds MRM methods from protein or peptide sequences for biomarker verification or protein/PTM detection and confirmation
- Intelligently determines MRM transitions in silico, allows user curation
- Automatically considers modifications such as phosphorylation or ubiquitination
- Creates MRM transitions for unlabeled or labeled peptides, such as iTRAQ® or mTRAQ™ reagent labeled peptides
- Links directly to quantitation results

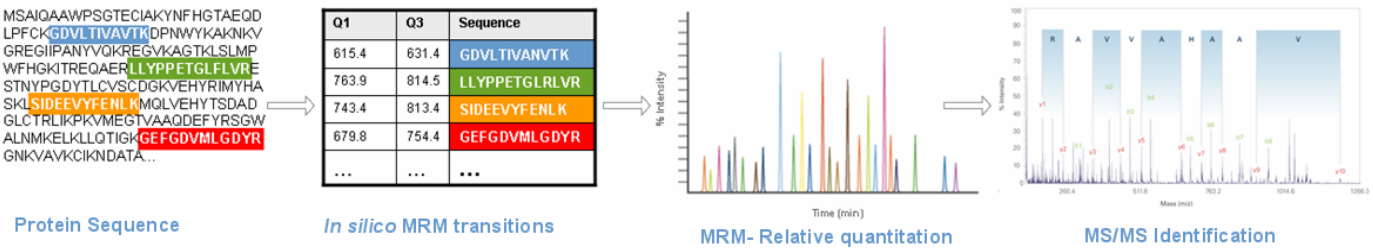


Figure 1. The MIDAS™ Workflow.

Loading Proteins and Specifying Sample and Acquisition Details

MIDAS™ Workflow Designer works by creating MRM methods from peptides generated by *in silico* digestion of a protein sequence. Thus, the first step in the process involves loading the protein sequence or list of endogenous peptides of interest into the software. Figure 2 shows the main window for MIDAS™ Workflow Designer software. Once a protein or peptide is added, the sequence is displayed in the *Protein Sequence* window. Multiple sequences are easily added into the software for creation of MRM transitions for many proteins simultaneously within one method or multiple methods.

Once proteins have been loaded into the software, the next step is to provide *Sample Details,* such as the *Enzyme* used for sample preparation (e.g., Trypsin), the maximum number of *Missed Cleavages* to consider, and any *Fixed* or *Variable Modifications*. The software can consider a wide range of different modifications as illustrated in the drop down menus in the right pane of Figure 2, or users have the capability to add new modifications if desired. *Acquisition Method Details* are specified in the lower section of the window. Here, a *Starter Method* is loaded from which the subsequent new MRM methods will be created and saved with the *New Method* name. The Starter Method is a QTRAP® system acquisition method and simply acts as a template from which the *New Method* containing the MRMs of interest can be built. A range of precursor *Charge States* to be considered for each peptide MRM is specified by selecting the appropriate values from the drop down menus.

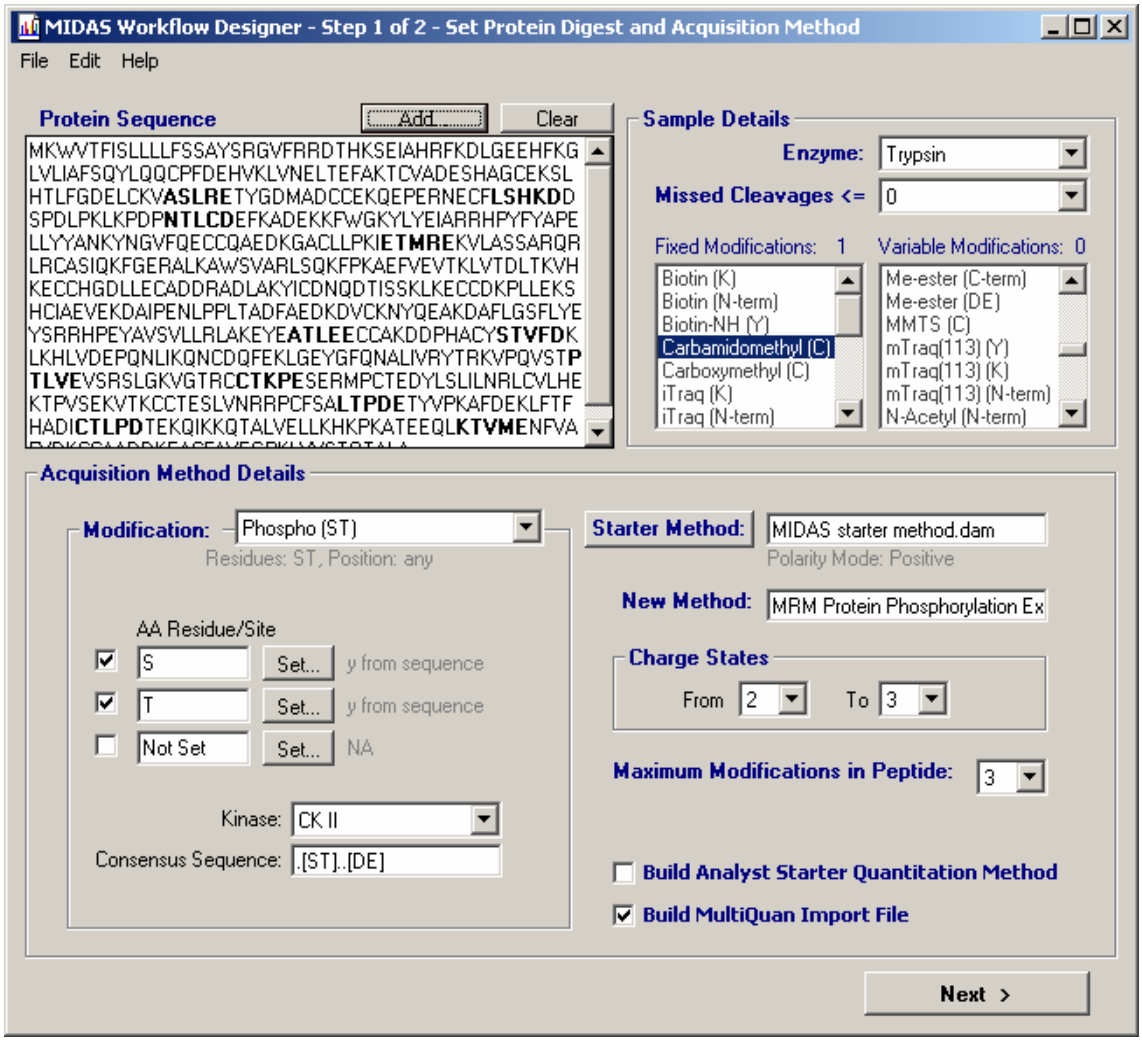


Figure 2. MIDAS™ Workflow Designer Main Window. Protein or peptide sequences are loaded, *Sample Details* are specified, and *Acquisition Method Details* are configured in this window.

MIDAS™ Workflow Designer software can be used for a variety of applications, targeting many types of peptides and modifications. For example, detection of low level phosphorylation sites[1,2] is a very common application of the MIDAS Workflow. Here, peptides which contain phosphorylation sites (either by residue or consensus sequence) can be specifically targeted by selecting Phospho (STY) as a *Modification* under the *Acquisition Method Details* (Figure 2*)*. In this example, an additional consensus criteria is considered, the method will only target those peptides that could be phosphorylated due to the *Kinase* CK II. After *Kinase* selection, all residues that abide by the CK II *Consensus Sequence* (.(ST)..(DE)) are shown in bold font in the *Protein Sequence* window. Finally, if the goal of the MRM method creation is ultimately quantitation, quantitation methods can be automatically created.

Peptide MRM List Creation

After selecting the criteria for peptide MRM creation, the software automatically generates a list of peptide MRM transitions. As shown in Figure 3, the list contains every peptide that fits the criteria set in the previous window.

MRM *Selection Filters* are automatically applied to intelligently filter the MRM list for those transitions most likely to be detected. Users can adjust the filtering of the list by editing the *Selection Filters* in the right hand pane. Additionally, users can manually curate the MRM list using the check boxes to the far left.

As shown in Figure 3, peptides are listed that follow all of the criteria from the main window, namely: tryptic peptides that are phosphorylated on S or T and contain the CK II consensus sequence. Peptides can contain up to one missed cleavage. MRM transitions are created with Q1 set to the doubly or triply charged precursor and Q3 set to the first y-ion fragment above the m/z of the precursor. The collision energy is automatically set based upon the modifications being considered and the peptide properties. To further reduce the list of MRMs, a charge state filter can be applied. The filter, called *Determine z from Basic Residues*, determines the most likely charge state to be observed for a peptide based upon the number of basic residues in the peptide sequence and removes transitions from other charge states.

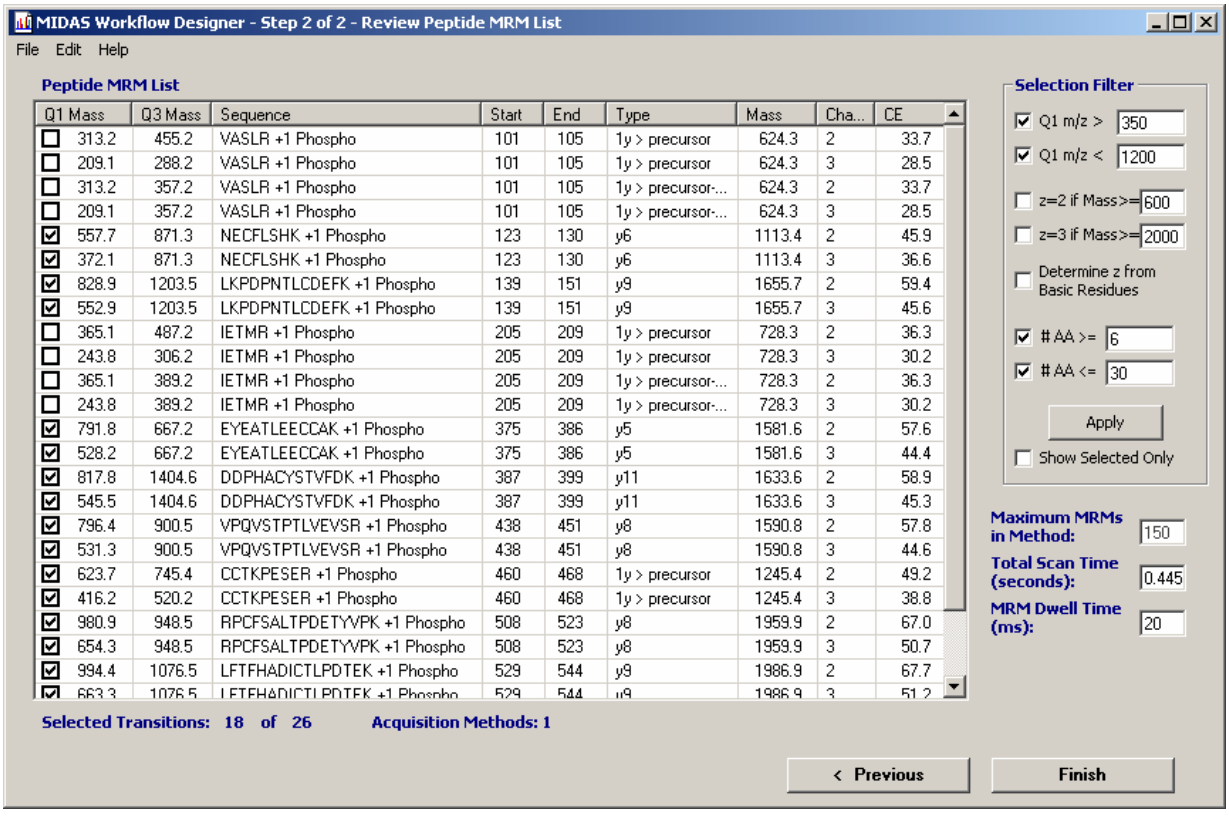
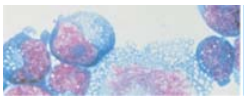


Figure 3. Peptide MRM List. Peptide MRM transitions are automatically created and displayed in the table for all peptides that match the criteria specified in the main window. Users can further filter the peptide list by adjusting the parameters in the *Selection Filter* pane or manually curating the MRM transition list.



The last step before MRM Method creation is to optimize the MIDAS™ workflow method by adjusting either the *Total Scan Time (seconds)* or the *MRM Dwell Time (ms)*. In Figure 3, an MRM dwell time of 20 ms will be used for every MRM transition. This results in a 0.45 second cycle time to perform all 18 transitions. Clicking the *Finish* button creates the MRM acquisition method, and the quantitation method if desired.

The automatic construction of quantitation methods enables an easy transition between the creation of MIDAS Workflow acquisition methods and the quantitation of the detected peptides. Methods can be automatically created for Analyst® Software quantitation or for MultiQuant™ Software.

Conclusions

MIDAS™ Workflow Designer is a powerful software program that greatly facilitates the creation of MIDAS™ Workflow Methods for targeted MS workflows. It takes advantage of the power of the QTRAP® systems that combine highly sensitive and selective MRM capabilities with highly sensitive MS/MS capabilities. This unique combination allows quantitative MRM and high sensitivity MS/MS to be obtained for peptides at extremely low amounts on column (down to tens of amols on nanoLC columns). Without highly sensitive MS/MS capabilities to match the MRM sensitivity, peptide detection and confirmation in serum, plasma, or other complex real-world samples is extremely difficult.

Hypothesis driven experiments enabled by the MIDAS™ Workflow on 4000 QTRAP system include:

Targeting low level phosphorylation sites on proteins of known sequence to identify or confirm modification location[1,2]

Validating weak protein identifications by specifically obtaining more MS/MS on additional peptides for that protein, increasing confidence in protein identification

Detection of low level proteins in complex mixtures

Quantitation of proteins/peptides with accompanying MS/MS for identity confirmation

Acknowledgements

*AB would like to extend a special thank you to Professor Anthony Whetton and his group (Patterson Institute for Cancer Research, Manchester, UK) for naming the MIDAS™ Workflow.[1]

1. Unwin *et al.*, (2005) Mol. Cell Prot. 4 (8), 1134-1144.
2. Cox *et al.*, (2005) J. Biomol. Tech. 16 (2), 83-90.

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