

Overview

- ▶ The PeptideAtlas contains consistently-analyzed results from many MS/MS experiments.
- ▶ SRM (MRM) assays allow sensitive and confident identification of target proteins.
- ▶ Recent work allows storage of validated SRM assays, i.e. Q1/Q3 pairs, with associated annotations.
- ▶ Providing interfaces to query and export such transitions in the context of the PeptideAtlas supports SRM-based workflows.
- ▶ The Yeast MRM Atlas is a very rich set of such transition data.

¹ SRM, Selected Reaction Monitoring, is the IUPAC-approved terminology⁴

Introduction

The PeptideAtlas¹ is a publicly accessible compendium of observed peptides and associated information, based on a large number of contributed MS/MS datasets. Data are processed through a consistent analysis pipeline, allowing consistent identifications and the computation of reliable false discovery rates. Herein we describe enhancements to the PeptideAtlas which support selected reaction monitoring (SRM/MRM) analysis of peptides. The addition of features for storing and retrieving sets of user-validated and other high quality precursor/fragment (Q1/Q3) ion pairs enhance the value of this resource for researchers conducting targeted proteomics experiments².

Methods

We have collected a considerable number of validated Q1/Q3 pairs from the literature⁴, our own lab³, and from various collaborators. These are stored in the PeptideAtlas database along with experimental parameters such as collision energy, retention time, and relative intensity. Consensus spectral libraries are made for each Atlas build using SpectraST (Spectra Search Tool)⁵. The consistently observed fragment ions in these consensus spectra can be used to select targets for observed peptides which do not have such user-validated Q1/Q3 ion pairs. Users are also able to upload validated SRM transitions to the PeptideAtlas via web forms or other software tools under development.

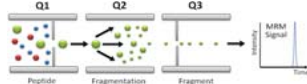


Figure 1. Graphical depiction of SRM assay in a triple quadrupole mass spectrometer. (Adapted from Schmidt et al.¹¹)

Improved support for targeted proteomics workflows in PeptideAtlas

David Campbell¹, Eric Deutsch¹, Henry Lam¹, Paola Picotti², and Ruedi Aebersold^{1,2,3}

1. Institute for Systems Biology, Seattle, WA 98103, USA
2. Institute of Molecular Systems Biology, ETH Zurich, CH-8093 Zurich, Switzerland
3. Faculty of Science, University of Zurich, CH-8006 Zurich, Switzerland

Results

SRM Workflow

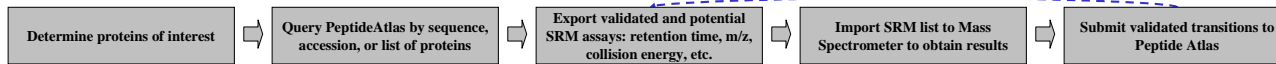


Figure 2. Greatly simplified schematic of targeted SRM-based workflow. Proteins of interest are used to query database, returning validated SRM assays and/or highly probable transition pairs to use for MS/MS analysis. Once data are acquired from MS instrument, information about validated transitions can be uploaded to the PeptideAtlas. An xml format for representing transitions, TraMIL⁶, under design by the HUPO Proteomics Standards Initiative is the recommended data format for these submissions, and is supported by SRM workflow software currently in development.

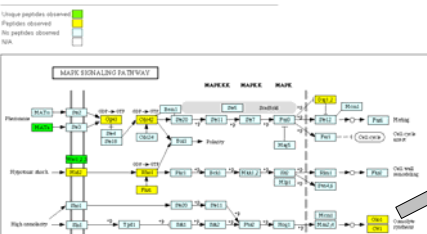
Peptide Atlas Tools

A Atlas Search

Search Select Build Browse Peptides Peptide Proteins Browse Proteins Submit as TSV



D KEGG Pathway



F SRM Table

Protein	Sequence	Chg #1	m/z	Intensity	SEC	BORGAC	Chg	Amid	Spectrum
YR12TW	ANGQSFVTVQGER	3	474.24	517.39	10000.00	45	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	685.31	3564.00	47	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	618.32	3562.30	46	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	692.25	2744.70	46	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	746.36	1936.70	47	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	765.39	1152.20	47	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	529.25	1031.00	46	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	803.39	433.00	46	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	517.40	10000.00	45	24.74	28.26	2 Best
YR12TW	ANGQSFVTVQGER	3	474.24	685.31	3564.00	47	24.74	28.26	2 Best
YR12TW	ASAGSGSPTRK	2	666.30	703.40	10000.00	37	30.42	30.62	8 Best
YR12TW	ASAGSGSPTRK	2	666.30	702.50	1468.30	36	30.42	30.62	8 Best
YR12TW	ASAGSGSPTRK	2	666.30	697.40	1468.30	36	30.42	30.62	8 Best
YR12TW	ASAGSGSPTRK	2	666.30	696.50	3966.90	36	30.42	30.62	8 Best
YR12TW	ASAGSGSPTRK	2	666.30	429.21	1120.90	36	30.42	30.62	8 Best
YR12TW	ASAGSGSPTRK	2	666.30	703.70	10000.00	37	30.42	30.62	8 Best
YR12TW	ETQVNLGEGK	2	639.23	909.40	10000.00	49	35.99	9.84	9 Best
YR12TW	ETQVNLGEGK	2	639.23	776.38	8833.00	47	35.99	35.99	9 Best

E SRM Query

Atlas Build: Yeast_Transcriptome_P18_03_04
Consensus Library: YeastMRM_SP
Upload File of Proteins: VPLRCR1616VPLRCR1616VLR12TW
Peptide Sequence Constraint: [Empty]
Protein Name Constraint: [Empty]
Peptide Length: [Empty]
Best Probability Constraint: [Empty]
Number of Observations Constraint: [Empty]
Number of Observed Samples Constraint: [Empty]
Empirical Peptidomic Score Constraint: [Empty]
Number of Proteins Filtered by Constraint: [Empty]
Number of Consensus Libraries Filtered by Constraint: [Empty]
Number of Highest Intensity Fragments by: [Empty]
Number of Highest Intensity Fragments by: [Empty]
Include constraint validated in transition(s) available: [Empty]

QUERY REFRESH RESET

C Protein View



H Homologous sequence alignment



Figure 3. This figure depicts various search tools within the PeptideAtlas. A Shows the PeptideAtlas global search page, which allows users to search by sequence, accession, name, and various other annotations. Results link to B peptide information and C protein information pages. As shown, if there are user-validated SRM transitions available for a given peptide or protein, they are shown as a section in the page, available to download as a TSV file. D shows the KEGG pathway search feature, which allows users to see which proteins in a given pathway are observed in a given Atlas build. The green and yellow observed proteins link to the appropriate protein information pages. E shows the direct SRM query form, which returns preferentially validated transitions, and secondarily consensus derived transitions, up to the number requested by the user. F shows the results of such a query, and as in the peptide and protein views, information that would enable a researcher to replicate the assay. The peptide amino acid sequence and charge state of the observed precursor is provided, as are the Q1 and Q3 m/z values. The relative intensity of each fragment ion is also shown, scaled to 10000 units. The collision energy (CE) is provided for user-validated transitions, and an approximate value is given for consensus-derived Q1/Q3 pairs using a formula determined by Picotti et al.³ G Shows an alignment between a consensus spectrum and an experimentally derived mass spectrum, and H shows a sequence alignment with ClustalW⁷, in this case aligning homologous sequences from C. elegans, yeast, human, rat, and mouse.

The MRM Atlas³ is a database of validated SRM assays for ~1500 yeast proteins, and a specialized build of the PeptideAtlas. It was constructed by merging the results of more than 650 SRM-triggered MS2 analyses of S. cerevisiae protein digests, carried out on a QQQ instrument. 1324 proteins are represented by assays for at least one proteotypic peptide. The resource also contains assays for a small number of peptides common to a maximum of two proteins. All peptides were selected because they show intense signal response by electrospray ionization MS. Each identification was validated by collecting a full tandem mass spectrum of the peptide with the QQQ instrument used for SRM measurements

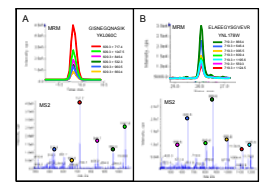


Figure 5. Taken from Picotti et al.³, this figure shows the similarity between the intensities of SRM traces and fragment ion relative intensities derived from MS2 spectra.

Acknowledgements

This work is supported in part by NHLBI contract No. N01-HV-28179.

References

1. Frank Desiere, Eric W. Deutsch, Ruedi Aebersold et al. "Integration of Peptide Sequences Obtained by High-Throughput Mass Spectrometry with the Human Genome". Genome Biology (2004)
2. Eric W. Deutsch, Henry Lam & Ruedi Aebersold "PeptideAtlas: a resource for target selection for emerging targeted proteomics workflows". EMBO reports (2008).
3. Paola Picotti, Henry Lam, Ruedi Aebersold et al. "A database of validated targeted proteomic assays for S. cerevisiae". Nature Methods 5, 913-914 (2008).
4. Leigh Anderson, and Christie L. Hunter. "Quantitative Mass Spectrometric Multiple Reaction Monitoring Assays for Major Plasma Proteins". Molecular and Cellular Proteomics (2006)
5. H. Lam, EW Deutsch, R Aebersold et al. "Development and validation of a spectral library searching method for peptide identification from MS/MS". (2007)
6. A. Schmidt, P. Picotti and R. Aebersold "Proteomeanalysis and systembiology BiSpektrum" (2008)
7. Murray et al. "Standard Definition of Terms Relating to Mass Spectrometry". IUPAC recommendations (2006).
8. TraMIL RFC: http://psdview.info/index.php?option=com_content
9. Larkin MA et al. "Clustal W and Clustal X version 2.0.0". Bioinformatics, 23, 2947-2948. (2007)



<http://www.peptideatlas.org>



<http://www.mrmatlas.org>