

Crux: Rapid Open Source Protein Tandem Mass Spectrometry Analysis

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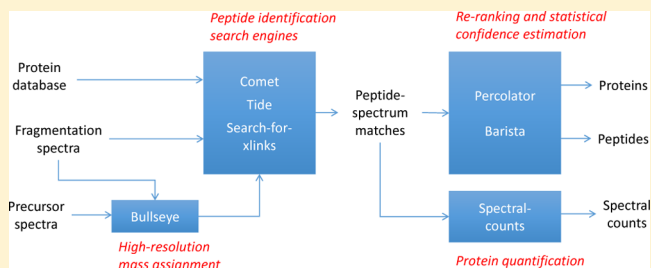
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S Supporting Information

ABSTRACT: Efficiently and accurately analyzing big protein tandem mass spectrometry data sets requires robust software that incorporates state-of-the-art computational, machine learning, and statistical methods. The Crux mass spectrometry analysis software toolkit (<http://cruxtoolkit.sourceforge.net>) is an open source project that aims to provide users with a cross-platform suite of analysis tools for interpreting protein mass spectrometry data.



Modern mass spectrometers produce massive amounts of data. For example, a Thermo Fusion mass spectrometer produces >24 GB of compressed data per day. Keeping pace with such a machine requires balancing three competing needs: analysis software must be *robust*, ensuring that the program executes successfully and that the results are valid, *efficient* to keep pace with the rapid rate of data acquisition, and *state-of-the-art*, glean-ing as much information as possible from the data by bringing to bear the latest algorithms and statistical methods.

To simultaneously address these three needs, we created an open source software toolkit called Crux (<http://cruxtoolkit.sourceforge.net>, Figure 1) that is capable of efficiently and accurately analyzing a variety of types of shotgun proteomics data. Originally, Crux consisted of a single search engine.¹ In Crux v2.0, the original search engine has been replaced by two search engines, Comet² and Tide,³ both of which implement SEQUEST-style searching.⁴ In addition, a specialized search engine provides the capability to identify cross-linked peptides.⁵ The Bullseye preprocessor assigns high-resolution masses to fragmentation spectra,⁶ and the Percolator⁷ and Barista⁸ post-processors use machine learning techniques to identify and assign statistical confidence estimates to spectra, peptides, and proteins. Peptide and protein quantification can be carried out using a spectral counting tool.⁹

Robust parsing of diverse file formats is an ongoing challenge in computational proteomics. Accordingly, we have adopted the open source ProteoWizard library,¹⁰ which enables Crux to parse a wide variety of file formats (Table 1). In particular, ProteoWizard allows the parsing of vendor-specific raw files when Crux runs under Windows. Furthermore, support for various open file formats allows interoperability between Crux and other search engines as well as toolkits such as the Trans-Proteomic Pipeline¹¹ and MSDaPI¹² that provide summarization and visualization functionality.

A variety of other mass spectrometry analysis toolkits have been produced, including commercial products (Scaffold, LabKey Server, Mascot tools) and academic software (pFind Studio,¹³ Bumbershoot,¹⁴ the Trans-Proteomic Pipeline,¹¹ MaxQuant,¹⁵ OpenMS,¹⁶ the Global Proteome Machine,¹⁷ and the Central Proteomics Facilities Pipeline¹⁸). Each of these toolkits offers distinct features (Table 2). Crux offers extensive confidence estimates, including false discovery rate and posterior error probability estimates at the spectrum, peptide, and protein levels and has recently added functionality (to the Tide

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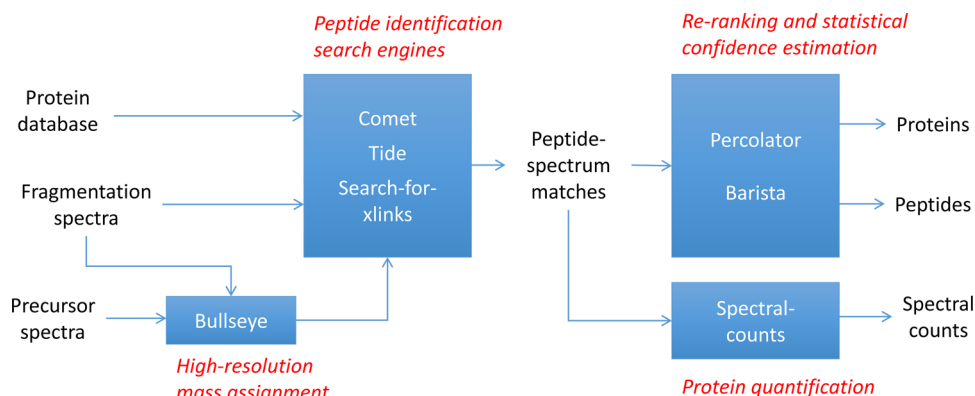


Figure 1. Crux analysis workflow and sample results. Crux provides tools for identifying spectra derived from single peptides or from cross-linked peptides as well as tools for postprocessing the resulting identifications to yield peptide- and protein-level identifications.

Table 1. File Formats in Crux

command	MS1 ^a	MS2	various ^{a,b}	FASTA	Tide index	TSV	pepXML	PIN	mzIdentML	SQT	Barista XML
Bullseye	in	in/out	in								
Tide index				in	out						
Tide search		in	in		in	out	out	out	out	out	
Comet		in	in	in		out	out	out	out	out	
Percolator						in/out	in/out	in	out	in	in/out
Barista		in	in			in/out	out			in	out
spectral counts		in	in			in/out	in		in	in	

^aAdditional vendor proprietary formats for MS1 and MS2 data are supported on Windows: Agilent MassHunter .d, Waters RAW, Thermo RAW, Applied Biosciences Wiff, and Bruker Compass .d/YEP/BAF/FID. ^bSupported open MS2 file formats include BMS2, CMS2, MGF, mzML, and mzXML.

Table 2. Comparison of Mass Spectrometry Analysis Toolkits^a

feature	TPP	MaxQuant	OpenMS	GPM	CPFP	Scaffold	LabKey Server	pFind Studio	Bumbershoot	Mascot tools	Crux
Tools											
high-res mass assignment	×	×	×	×		×		×		×	×
peptide database search	×	×	×	×	×	×	×	×	×	×	×
machine learning postprocessor	×	×			×	×	×		×	×	×
protein cross-link searching								×			×
RNA cross-link searching			×								
spectral counting		×		×		×	×			×	×
isobaric tag quantification	×	×	×			×		×		×	×
peak area quantification	×	×	×			×		×		×	
Statistical Confidence Estimates											
decoy-based estimates	×	×	×	×	×	×	×	×		×	×
parametric PSM <i>p</i> values				×		×		×			×
exact PSM <i>p</i> values											×
PSM <i>q</i> values	×		×		×	×	×	×	×	×	×
PSM PEPs	×	×	×			×				×	×
peptide <i>q</i> values	×				×	×	×	×	×		×
peptide PEPs	×	×								×	×
protein <i>q</i> values	×				×	×	×		×	×	×
protein PEPs	×	×				×					×
Input Spectrum File Formats											
Thermo.RAW	×	×	×				×	×	×	×	×
Waters.RAW	×		×				×		×	×	×
MDS/Sciex.wiff	×	×	×				×		×	×	×
Agilent.d	×		×				×		×	×	×
Bruker.d	×		×				×		×	×	×
MS1									×		×
MS2			×					×	×		×
mzML	×		×	×	×		×		×	×	×
mzXML	×	×	×	×			×		×	×	×
MGF			×	×	×			×	×	×	×

Table 2. continued

feature	TPP	MaxQuant	OpenMS	GPM	CPFP	Scaffold	LabKey Server	pFind Studio	Bumbershoot	Mascot tools	Crux
Input PSM File Formats											
PepXML	×		×				×				×
mzIdentML	×		×			×			×		
mzQuantML			×								
.dat (Mascot)	×					×					
.out (SEQUEST)	×					×					
.sqt (SEQUEST)	×					×					×
.srf (SEQUEST)						×					
other tool-specific formats						×					
Output File Formats											
tab-delimited	×	×	×	×		×	×	×	×	×	×
mzTab		×	×							×	
PepXML	×		×		×					×	×
ProtXML	×					×					
mzIdentML	×		×			×			×	×	×
mzQuantML			×								
Implementation											
free	×	×	×	×	×		×	×	×		×
source code available	×		×	×	×		×		×		×
open source license	×		×	×	×		×		×		×
Linux binaries			×			×	×	×	×	×	×
MacOS binaries			×			×	×				×
native Windows binaries	×	×	×			×	×	×	×	×	×
command line interface	×	×	×	×		×			×	×	×
graphical user interface	×	×	×	×	×	×		×	×	×	
application programming interface			×				×			×	

“Mascot tools” refers to Mascot Server and Mascot Distiller, which are licensed separately. GPM is Perl-based, so no binaries are needed. Scaffold parses tool-specific PSM formats produced by Proteome Discoverer, MS Amanda, Byonic, OMSSA, MaxQuant, SpectrumMill, X!Tandem, Waters Identity E, and Phenyx. Note that as of August 2014 CPFP is no longer actively maintained.

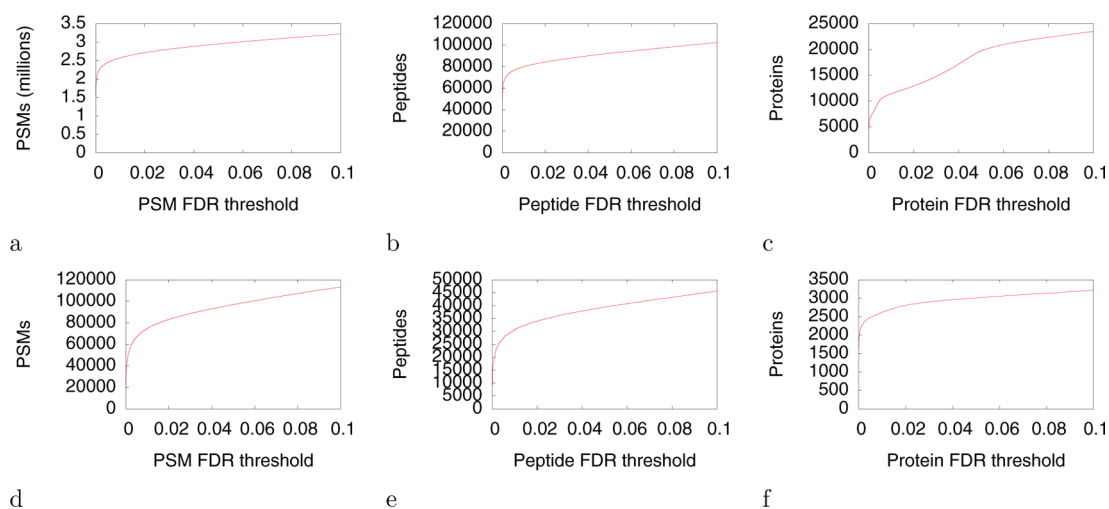


Figure 2. (a–c) We used Tide+Percolator to analyze 9 092 380 fragmentation spectra from 95 different human samples. The figure plots the number of spectra, peptides and proteins identified as a function of false discovery rate threshold. (d–f) Each panel plots, from Comet+Percolator analysis of 348 157 *Plasmodium falciparum* fragmentation spectra, the number of (respectively) spectra, peptides and proteins identified as a function of false discovery rate threshold. Total analysis time was 61.2 m (34.4 m for Comet and 26.8 m for Percolator). The number of proteins identified at 1% FDR (2618) by Comet+Percolator compares favorably with the published analysis (2767 proteins).

search engine) to compute exact p values using a dynamic programming approach.¹⁹

Crux supports a variety of workflows, providing users with flexibility to tailor their analysis to their experimental goals. The choice of search engine—Comet versus Tide—is a matter of personal preference and processing considerations and is not likely to substantially affect the final results. Tide is faster on a

single thread, but, unlike Comet, does not yet operate in multithreaded mode. Exact p values, which are only available in Tide, provide significantly improved statistical power at the expense of some computational overhead (roughly 0.2 s per spectrum). The two primary postprocessors, Percolator and Barista, offer more substantial differences. Both use a target-decoy machine learning approach. However, Percolator first

learns to rerank peptide-spectrum matches (PSMs) and then performs a probabilistic protein-level inference,²⁰ whereas Barista formulates both tasks jointly in a single discriminative learning procedure. Which approach performs better in practice is an open question that deserves further exploration.

To demonstrate the efficiency and accuracy of our software, we downloaded 224 GB of compressed data from a recent study of genetic control of protein abundance in humans²¹ (details in the Supporting Information). Searching these >9 million fragmentation spectra using Tide against a human protein database containing ~90,000 proteins and a matched set of decoys required 20.2 h of CPU time on a single thread, for a rate of 121 spectra/s. Postprocessing with Percolator required an additional 20.5 min. At 1% false discovery rate (FDR) thresholds for PSMs, peptides, and proteins, respectively, this analysis identified 2 576 283 PSMs, 79 976 peptides, and 11 432 proteins (Figure 2a–c). These results are comparable to the published analysis, which reported 2 726 242 PSMs corresponding to 71 800 distinct peptides at a 1% peptide-level FDR threshold. We also used Comet and Percolator to analyze a collection of 348 157 high-resolution spectra from the erythrocytic cycle of the malaria parasite *Plasmodium falciparum*,²² identifying at 1% FDR 74 974 PSMs, 30 640 peptides, and 2618 proteins (Figure 2d–e).

Crux is a command line tool, written in C++ and distributed as a single binary executable supporting a variety of commands. Users wishing to compile their own version of Crux can download the source code, which is covered by an Apache license. All Crux code undergoes code review and revisions to reflect our documented coding standards, and the software is automatically tested using a continuous integration system, which compiles Crux on three operating systems—Windows, MacOS and Linux—thereby providing up-to-date binary executables. Crux is under active development, with several important improvements and additions planned for the near future. In addition, we encourage community members to contribute to the toolkit.

■ ASSOCIATED CONTENT

📄 Supporting Information

Sample analyses. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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