# Faster SEQUEST Searching for Peptide Identification from Tandem Mass Spectra

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| Journal: | *Journal of Proteome Research* |
| Manuscript ID: | Draft |
| Manuscript Type: | Article |
| Date Submitted by the Author: | n/a |
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# Faster SEQUEST Searching for Peptide Identification from Tandem Mass Spectra

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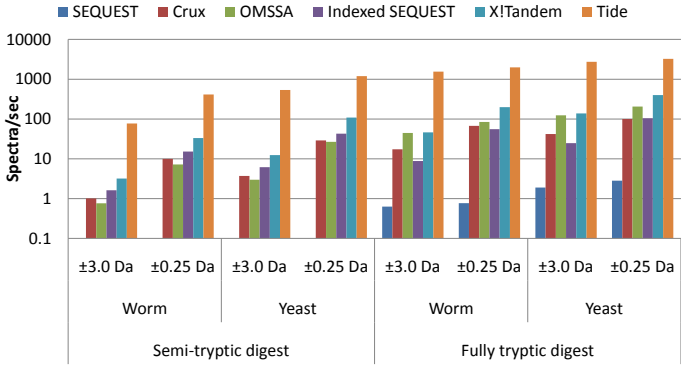
November 30, 2010

**Abstract**

Computational analysis of mass spectra remains the bottleneck in many proteomics experiments. SEQUEST was one of the earliest software packages to identify peptides from mass spectra by searching a database of known peptides. Though still popular, SEQUEST performs slowly. Crux and TurboSEQUEST have successfully sped up SEQUEST by adding a precomputed index to the search, but the demand for ever-faster peptide identification software continues to grow. Tide, introduced here, is a software program that implements the SEQUEST algorithm for peptide identification and that achieves a dramatic speedup over Crux and SEQUEST. The optimization strategies detailed here employ a combination of algorithmic and software engineering techniques to achieve speeds up to 170 times faster than a recent version of SEQUEST that uses indexing. For example, on a single Xeon CPU, Tide searches 10,000 spectra against a tryptic database of 27,499 *C. elegans* proteins at a rate of 1,550 spectra per second, which compares favorably with a rate of 8.8 spectra per second for a recent version of SEQUEST with index running on the same hardware.

Running title: Faster SEQUEST Searching
Keywords: shotgun proteomics, peptide identification

# Table of Contents Synopsis




Computational analysis of mass spectra remains the bottleneck in many proteomics experiments. We introduce Tide, which implements the SEQUEST algorithm for peptide identification while achieving a dramatic speedup over Crux and SEQUEST. For example, on a single CPU, Tide searches against a tryptic database of 27,499 proteins at a rate of 1,550 spectra/s, which compares favorably with a rate of 8.8 spectra/s for a recent version of SEQUEST with index.

# 1 Introduction

SEQUEST [1] pioneered the pure database search approach for analysis of tandem mass spectra from shotgun proteomics data. Despite the passage of some time, SEQUEST remains popular: a search on Google Scholar returns about 5,800 articles between 2006 and 2010 mentioning "SEQUEST" and "peptides".

Although SEQUEST enjoys considerable popularity, it runs slowly even on modern architectures. Performance varies significantly based on the size of the peptide database, and especially on the number of candidate peptides considered per spectrum, but analysis times are typically in the range of a second per spectrum identified. Consequently, efficient data analysis of an MS/MS experiment often requires significant computational resources, including dedicated computing clusters. Barriers of time and expense play a correspondingly restrictive role in experiment design. Conversely, faster identification broadens the possibilities for experimentation.

Many approaches exist for identifying peptides from tandem mass spectra (reviewed in reference [2]). These methods may be categorized broadly as *de novo* methods, which analyze spectra without reference to an external set of known peptides; database methods, which match spectra to the closest candidate peptide in a database of known peptides; and hybrid methods, which employ some combination of approaches.

Database search methods work by comparing each observed spectrum against a theoretically predicted spectrum for each peptide in a database, typically assigning a score to each database entry and reporting the highest-scoring result. The main scoring function of SEQUEST is $X_{Corr}$ [1, 3], which assigns a similarity score to any given pairing between an observed spectrum and the theoretical spectrum of a candidate peptide.

As originally implemented, $X_{Corr}$ is costly to compute, so several approaches have been used to improve the speed of the $X_{Corr}$ calculation. SEQUEST itself uses a faster preliminary score, $S_p$, expecting that the peptide with the highest $X_{Corr}$ will also have a sufficiently high $S_p$ to be included in a second scoring round. TurboSEQUEST and Crux [4] introduced to the SEQUEST method an index based on precursor mass to increase the efficiency of candidate peptide retrieval. Recently, a faster $X_{Corr}$ computation, performed as a dot product, was described in [3]. This method is included in Crux and more recent SEQUEST versions. However, despite such advances, the need for further speed improvements remains.

In parallel to these SEQUEST-specific developments, a host of competing database search methods have been described [5–14], along with a variety of methods for performing the searches efficiently. Many of these tools use a database index, usually indexing on precursor mass and, in one case, also by MS/MS fragment mass [15]. Other tools gain efficiency by reordering the spectra themselves [16]. A variety of algorithms use *de novo* analysis [12], filtering [17], two-pass searching [14], hashing [18] or metric space indexing [19] to efficiently reduce the effective size of the database. In general, such methods may perform very effectively at the cost of losing a few identifications, or slightly less efficiently in a lossless fashion. Finally, some tools are designed to exploit multi-core or multi-threaded CPUs [10], clusters of CPUs [20] or to make efficient use of the CPU cache [13, 21]. At least one vendor, SAGE, offers a combination hardware/software-based product.

In any database approach to peptide identification, the number of candidate peptides may be as much as quadratic in the size of the protein database, so all search methods must

manage space efficiently. The original SEQUEST did not include a peptide index at all, but rather scanned the database file repeatedly for each new peptide. This approach requires little memory and disk space, but it runs slowly. Modern desktop computers have far more capacious memories and disks than those from the time SEQUEST was first developed, but memories are still typically too small to accommodate a complete peptide list for many searches. Consequently, compression schemes such as [22] have been used to reduce memory bloat.

Here, we introduce Tide, a much faster implementation of the SEQUEST algorithm. Various versions of SEQUEST exist that differ in detail. Tide's analysis of MS/MS spectra follows that of Crux [4], an open source software package based on SEQUEST. Tide yields identical $X_{Corr}$ scores to those of Crux (version of 4/14/09), when Crux is compiled using double-precision floating point numbers. However, through a combination of algorithmic enhancements, improved system design, and better use of machine resources, Tide is dramatically faster than recent versions of SEQUEST and Crux, particularly when the database is created using fully enzymatic digestion. Tide approaches space limitations by curtailing its use of machine memory; however, Tide is not engineered toward low disk usage because disk is typically a far cheaper resource than memory or time. The Tide software, including source code, is freely available for academic and non-profit use as part of the Crux software toolkit `http://noble.gs.washington.edu/proj/crux`.

# 2 Materials and methods

Tide is written in standard C++ including the standard template libraries; all code is single-threaded. During development, timing and profiling experiments were done on a 2.4 GHz Dual Pentium processor with 4 GB of memory running Linux. Final timing measurements were performed on 2.33GHz Dual Xeon processor with 16GB memory running Linux, with all code compiled in 64-bit mode.

Two benchmark datasets were used for both development and final timing: a "yeast" set, and a larger "worm" set. The yeast set was acquired on an LTQ ion trap mass spectrometer from a tryptic digest of an unfractionated *S. cerevisiae* lysate and analyzed using a 4-h reverse-phase separation, yielding 37,641 spectra [23], from which 10,000 spectra were randomly sampled. These spectra were searched against a protein database consisting of the predicted open reading frames from *S. cerevisiae* (released 2004-04-02, 6298 proteins). The worm benchmark was derived from a 24-h MudPIT analysis of *C. elegans* proteins containing 207,804 spectra, from which 10,000 spectra were randomly sampled. These spectra were searched against a protein database consisting of the predicted open reading frames from *C. elegans* and common contaminants (Wormpep v160, 27,499 proteins). The spectra and databases comprising these benchmarks are available at `http://noble.gs.washington.edu/proj/tide`.

Peptide indexes were generated from each benchmark protein database for use with Tide and Crux. The indexes contained tryptic peptides of length 6–50 amino acids and mass 200.0–7200.0 Da. These same search parameters were applied to the SEQUEST searches. Except where noted, a precursor mass tolerance window of 3.0 Daltons and a fully tryptic peptide database were used in all experiments. Tide and Crux experiments were run with

full $X_{Corr}$ calculation on all candidate matches. SEQUEST experiments were run with the preliminary scoring pass $S_p$.

# 3 Results

## 3.1 The SEQUEST algorithm and $X_{Corr}$

The goal of peptide identification by database search is to label each experimentally observed spectrum from an MS/MS run with the peptide most likely to have generated the spectrum. Two sources of input are examined. The first is a collection of tandem mass spectra, each with an observed precursor mass and one or more possible charge states. The second is a collection of protein sequences, usually called the database, regardless of the storage mechanism. A successful identification of an observed spectrum as a match to a candidate peptide sequence requires reasonable correspondence between features of the observed spectrum and theoretically computed features of the candidate peptide. The SEQUEST algorithm approaches the task of peptide identification in four steps.

First, for each input spectrum to be identified, candidate peptides are retrieved from the database, based on the precursor mass associated with the input spectrum. The precursor mass of the observed spectrum must match the theoretical mass of the candidate within a user-specified tolerance, defaulting to 3.0 Daltons. If more than one possible charge state is given for the precursor ion, then candidate selection is repeated for each charge state. If the database is large, then many candidate peptides will be identified for each input spectrum. The pairing of a single input spectrum with a single candidate peptide is termed a *peptide-spectrum match* (PSM).

Next, each observed spectrum is preprocessed as follows. A set of bins, each of width 1.0005079 Daltons, is laid over the full range of the m/z values reported in the input file for the spectrum. Each input MS/MS peak is bucketed into the nearest bin, which retains only the highest intensity peak that fell into that bin. Each bin's intensity value is then replaced with its square root. The range of bins from lowest m/z to highest is then divided into ten equally spaced regions. Within each region, the intensity of each bin is normalized so that the most intense bin in every region has a value of 50. This completes the preprocessing step performed on each spectrum.

Separately, a theoretical spectrum is computed for each candidate peptide. The amino acid sequence, of length $\ell$, of the candidate peptide is used to compute a theoretical mass for each of the $\ell - 1$ $b$- and $y$-ions corresponding to all left and right substrings of the amino acid sequence. The theoretical mass of each of these ions is then bucketed into bins of width 1.0005079 Daltons, just as for the observed spectrum. The intensity of each of these $b$- and $y$-ions is given a value of 50. Additionally, each of the following ions is computed and bucketed to complete the theoretical spectrum:

- the two bins flanking each of the $b$- and $y$-ions each with intensity 25,
- a peak with intensity 10 representing the neutral loss of ammonia from each $b$- and $y$-ion,
- a peak with intensity 10 representing the neutral loss of water from each $b$-ion, and

- each $a$-ion, with intensity 10.

For spectra with precursor charge of 3 or higher, doubly-charged versions of each of the above ions are included in the theoretical spectrum.

The last step in the SEQUEST algorithm is to compare the preprocessed observed spectrum and candidate theoretical spectrum for each peptide-spectrum match. Preprocessing of an observed spectrum or generating a theoretical spectrum for a candidate peptide yields a peak-intensity vector, any pair of which may be compared. After a spectrum is processed to obtain a length-$N$ vector $u$ and a candidate match's theoretical spectrum is computed to get another vector $v$, where $N$ is the number of bins, the following function is computed to obtain the $X_{Corr}$ as the score for the peptide-spectrum match:

$$X_{Corr}(u, v) = \langle u, v \rangle - \frac{1}{150} \sum_{\tau=-75}^{75} \sum_{i=1}^{N} v_i u_{i-\tau}$$

For each spectrum, the PSM with the highest $X_{Corr}$ scores is output to the user.

SEQUEST mitigates the slowness of computing $X_{Corr}$ for every PSM by computing an approximate preliminary score ($S_p$) for each peptide-spectrum match that it collects; only the 500 highest scoring candidates by $S_p$ are fully scored by $X_{Corr}$. Tide does not compute $S_p$ because it is able fully to compute $X_{Corr}$ extremely efficiently; computing $S_p$ as a preliminary score would not be expected to improve Tide's speed.

For most applications, the database of proteins, from which candidate peptides are derived, is considered to change infrequently. As a consequence, an arbitrary amount of precomputation may be performed on the peptide set before input spectra are to be analyzed. Several recently developed search tools, including TurboSEQUEST, Crux and Tide, take advantage of this opportunity by indexing the peptide set by precursor mass ahead of time.

Crux, Tide and various versions of SEQUEST all implement the algorithm above, but there are some differences among them. Crux's scoring is not identical to SEQUEST, though it is similar; see [4] for a comparison of Crux and an early version of SEQUEST. Through successive optimizations, a consistent aim of Tide was very precise fidelity to Crux's results and $X_{Corr}$ computation, though Crux, in turn, adhered more loosely to SEQUEST. Tide's scoring is identical to Crux's when Crux is compiled with double-precision floating-point arithmetic.

Tide supports searching databases with variable post-translational modifications (PTMs). At indexing time, the user may indicate a list of possible PTMs to include in the index. The user specifies each variable modification as a triple: a limit on the number of occurrences per peptide, a set of amino acids that are subject to the modification, and the corresponding mass. Multiple variable modification types may be specified. For example, the specification "2M+16.0, 5STY+79.97" indicates that up to two occurrences of methionine may be oxidized and up to five occurrences of any residues serine, threonine, or tyrosine may be phosphorylated. Peptides that are subject to the indicated variable modifications will appear in the database multiple times, reflecting the various modified forms.

In both SEQUEST and Tide, including PTMs increases the number of candidate peptides exponentially in the number of modifiable amino acids per peptide. As with SEQUEST and other database search engines, the user is encouraged to use variable modification search judiciously so as not to create large numbers of false positive matches or increase search

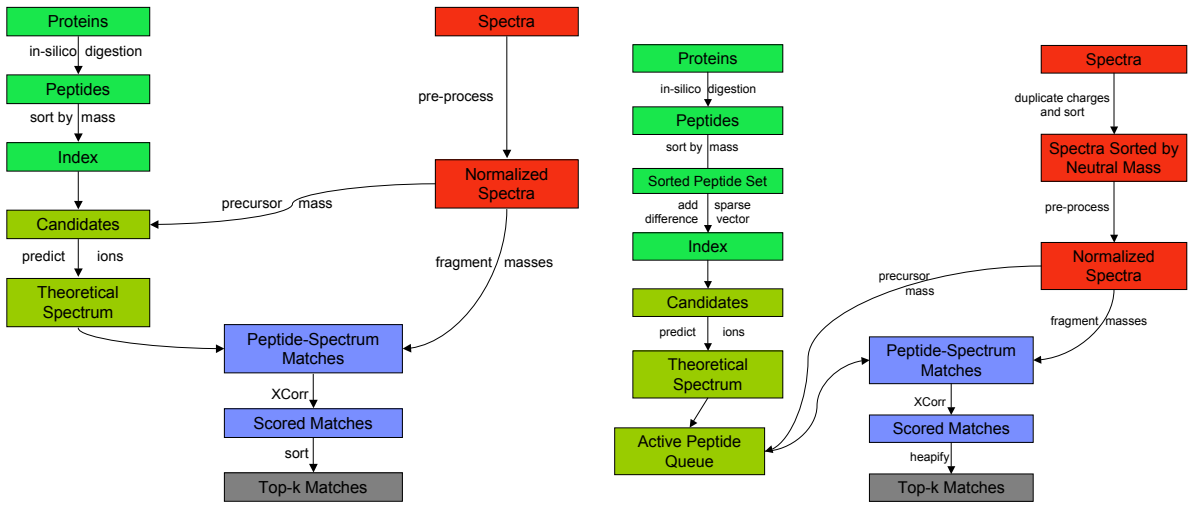



Figure 1: **Data flow in Tide before and after optimization.**

times exponentially. Tide scores a match with a modified peptide exactly as it would the unmodified peptide, except that it accounts for the change in mass of the modified residues.

## 3.2 Baseline version of Tide

An initial rewrite of the search method of Crux, called Tide-v0, was produced with the goal of precisely matching the $X_{Corr}$ scores produced by Crux, but with a greatly simplified code base, more easily amenable to human analysis, machine timing and profiling, and staged optimizations. Tide-v0 served as a starting point for the sequential introduction of a series of optimizations described in Section 3.3. The operation of Tide-v0 was as follows, schematized by the data flow in Figure 1(A).

The left side shows the progression from a protein set, supplied as a FASTA file, to a set of peptides, to a set of theoretical spectra. Each of these datasets is computed in turn from its parent dataset in the diagram. The computational digestion of the proteins and the ordering of the peptides are precomputed during an indexing phase that needs to be run only once for a given protein database. Because such databases are considered to change infrequently, the index may be reused for many spectrometry runs. Such an indexing phase is not unique to Tide: a similar index is generated for use with Crux, TurboSEQUEST, and some other database search systems. During the indexing phase, each protein in the input FASTA file is computationally digested into peptides according to user-specified parameters, which may specify enzyme and minimum and maximum peptide sizes.

The right side of the figure shows the set of observed spectra, including precursor $m/z$ and possible charge states, which are input at search time. As each spectrum is considered in turn, candidate peptides are identified, based on precursor mass, from the precomputed index, and a theoretical spectrum is calculated for each candidate. The bottom of the figure shows the observed spectra and theoretical spectra matched by precursor mass, then scored by $X_{Corr}$. For spectra with multiple possible charge states, Tide simply iterates over each such state and considers it in turn.

## 3.3 A series of optimizations

We now briefly describe the successive algorithmic optimizations and techniques incorporated in Tide, showing the course of development from Tide-v0 to the current version of Tide. The reader interested in more detailed descriptions of the individual optimizations should refer to the supplementary material.

- **Sparse representation of theoretical peaks** Each peptide's theoretical spectrum consists of ten peaks for each amino acid in the peptide, for each charge state, corresponding to the major ion types and the related neutral losses. Since there are roughly 1,000 mass/charge buckets (depending on machine settings), and since most peptides are short (under 20 amino acids), the theoretical spectrum is typically sparse, so Tide uses a sparse representation of the theoretical peaks. This change enabled another technique—making theoretical peaks five-fold sparser

- **Heapify to find top matches** As Crux finds candidate peptide-spectrum matches, it adds them to an array, which it sorts to find the best five matches. In place of this sort, Tide uses a heapify operation which requires linear time rather than the $O(n \log(n))$ time required by the sort to find the top matches.

- **Linearizing background subtraction** Tide linearizes the double loop that calculates $X_{Corr}$, as described in the supplement. At the stage it was introduced, this speedup reduced the total running time by about 47% (see line 5 in Table 1).

- **Caching multiplications** The $X_{Corr}$ calculation requires computing a dot product between the observed spectrum with each candidate theoretical spectrum. Tide exploits the fact that the theoretical peaks may have one of only three possible intensities: 10, 25 or 50. A simple caching scheme thereby allows for the elimination of multiplications during the dot product computation.

- **Join with rolling window** Before any matching begins, Tide reads the observed spectra into memory and sorts them by mass. In case a spectrum has multiple possible charge states it appears in the sorted array once for each charge state, as the join is performed on the neutral (uncharged) mass. After the spectra are sorted, Tide iterates in parallel over the spectra and the presorted candidate peptides. Iteration in this fashion creates a "rolling window," which occupies only as much memory as is required to store a window's worth of theoretical spectra. This strategy, which is illustrated in Figure 1(B), enables reuse of the computation of the theoretical spectra so that no theoretical spectrum need ever be computed more than once.

- **Making the theoretical peaks vector five-fold sparser** Theoretical spectra in the SEQUEST algorithm occur in groups corresponding to cleavage events, with somewhat predictable spacing among the peaks within a group. Tide takes advantage of such peak groupings to represent the complete set of theoretical peaks even more sparsely.

- **Fixed point arithmetic** Rather than compute the dot product in double-precision floating-point arithmetic, Tide uses fixed-point arithmetic. To do this, Tide multiplies

each entry in the spectrum by a large constant ($10^7$) and rounds to the nearest integer. The constraints imposed by the normalization procedure ensure against underflow or overflow, and the fact that the dot product is a simple summation assures numerical stability. We therefore achieve the same results as Crux does to at least five or six decimal places.

- **FIFO memory allocator** Profiling of a larger dataset showed that significant time was being spent in memory heap operations, many of which were tied to allocating and deallocating space for theoretical spectra and associated data. Therefore, Tide includes a specialized first-in-first-out (FIFO) memory allocator that performs well on data associated with a queue.

- **Compiled dot-product code** Following the above speed improvements, profiling revealed that most of the remaining time (about 60%) was spent in the dot product computation. Although this code had already been optimized twice—using cache lookups instead of multiplication operations, and using two such lookups rather than three—testing still showed that unrolling the loop and hard-coding specific values for the array of theoretical peaks was about twice as fast. To take advantage of this opportunity, Tide performs a run-time compilation for each theoretical spectrum to `x86` machine code to execute the sum with preset values. The appropriate code is generated in a buffer for each candidate peptide, and the program is instructed to jump to the buffer to run this peptide-specific dot-product code.

Table 1 shows timing results in the actual order these changes were introduced to Tide. Each line shows the performance change following the incorporation of perhaps a few changes at a time. Figure 2 shows the profile of major program components at various key points along the way.

The following features of the timing table bear some further exposition. The earliest working version of Tide shows a 45-fold improvement over the run-time of Crux. A substantial portion of this dramatic improvement likely reflects artifactual slowness of Crux that happened to be present at the time Tide got under way, and was since corrected in Crux. Newer versions of Crux, such as the one used for final timing measurements, are much faster.

Artifacts, however, do not completely account for the dramatic 45-fold speedup of Tide-v0 over Crux. The 3-Dalton mass window and full $X_{Corr}$ scoring for all candidates are settings for which Crux may not have been optimized, and they are time-consuming settings. Tide's code was also a lot more compact at this point (about 1,200 lines, compared to Crux's $\sim$32,000), and perhaps mere removal of some code complexity helped this initial number. The initial version of Tide also implemented heapify, described below; it used a compressed peptide file that holds pointers to all the proteins in which it is found; and it managed for these datasets to read all the compressed peptides into memory, eliminating most disk seeks. All these changes contributed to the immediate gains over Crux, but no separate measurements were made for each of these improvements. Versions of Tide from Table 1 line 6 onward do not require the index to fit into memory.

About midway through Tide's development, parsing of the input file was jettisoned, and the input spectra were represented by an uncompressed binary file. This was done because the particular input file format is incidental to the main search algorithms, there are many

| | Description | Worm | Yeast | % Change |
|---|---|---|---|---|
| 1 | Crux baseline (4/14/09) | 5:47:37.9 | 36:03.5 | |
| 2 | Rewrite including deduplication of peptides; heapify; compressed peptides file; and eliminating seeks. | 7:39.0 | 2:41.6 | 45.4-fold reduction |
| 3 | Sparse representation of theoretical peaks. [Note that input file parsing is introduced here, and removed later.] | | 1:50.5 | -31.6% |
| 4 | Fixed-capacity array for theoretical peaks; better memory management. | | 1:13.4 | -33.3% |
| 5 | Linearizing the background subtraction for $X_{Corr}$ computation. | | 0:38.9 | -47.2% |
| 6 | Active peptide queue and sorted spectra (rolling window join). | 0:38.8 | 0:13.8 | -64.5% |
| 7 | Eliminate input file parsing. [See text; and compare line 3 above.] | 0:25.6 | | -22.2% |
| 8 | Omit calculation with theoretical ions outside spectrometer's range. | 0:23.7 | | -7.4% |
| 9 | Sparse difference vector representation. | 0:24.7 | 0:09.0 | 4.2% |
| 10 | Array striping to eliminate one lookup during dot product calculation. | 0:20.4 | | -17.4% |
| 11 | Store the vector diffs to disk instead of calculating at runtime. | 0:15.5 | 0:05.9 | -24.0% |
| 12 | Fixed-point arithmetic. | 0:14.7 | 0:06.0 | -5.2% |
| 13 | FIFO memory allocator and run-time compiled dot-product code. | 0:8.6 | 0:4.4 | -34% |

Table 1: **Wall clock time after successive optimizations.** These measurements, taken during Tide's development, were done on a different machine than the one used for final timing measurements. See Section 2.

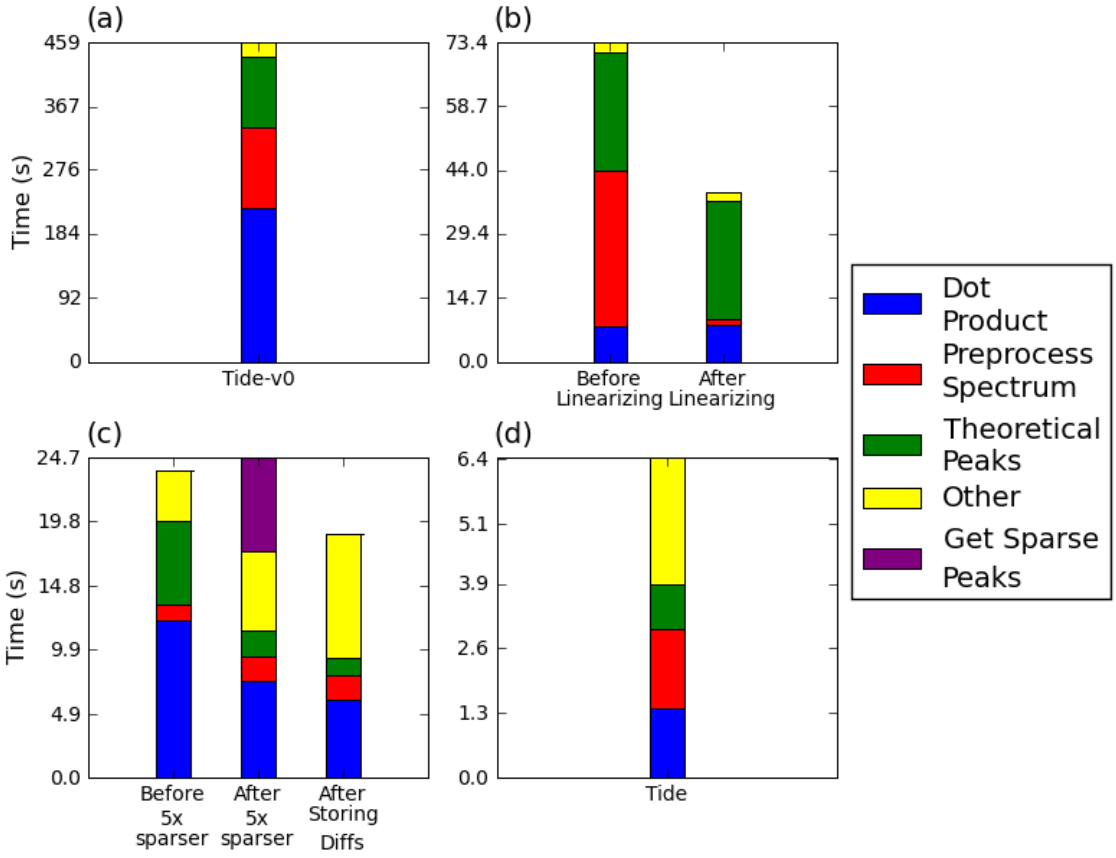
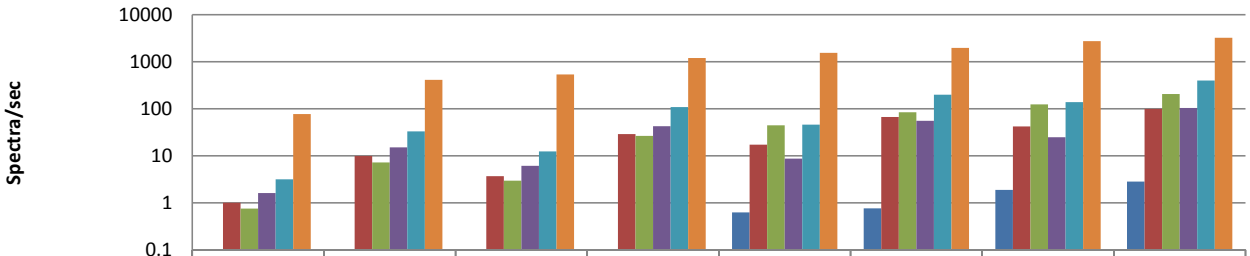



Figure 2: **Profile of various development stages of Tide** for the worm benchmark (10,000 spectra). Each profile shows how much computing time was spent in each of the major phases of Tide's operation at various points during development. Such profiles aided in deciding how best to proceed with optimization efforts. Profiles shown are (a) Tide-v0; (b) before and after linearizing background subtraction (Supplement Section 3); (c) before and after fivefold sparser representation, and after storing $d$ to disk (Supplement Section 6); and (d) the current version of Tide. For each plot, the (diminishing) total execution time is indicated via the y-axis scale.




| | Semi-tryptic digest | | | | Fully tryptic digest | | | |
|---|---|---|---|---|---|---|---|---|
| | Worm | | Yeast | | Worm | | Yeast | |
| | ±3.0 Da | ±0.25 Da | ±3.0 Da | ±0.25 Da | ±3.0 Da | ±0.25 Da | ±3.0 Da | ±0.25 Da |
| SEQUEST | | | | | 0.63 | 0.77 | 1.89 | 2.83 |
| Crux | 1.01 | 9.95 | 3.7 | 29 | 17.3 | 67.4 | 42 | 99.9 |
| OMSSA | 0.76 | 7.21 | 2.97 | 26.6 | 44.7 | 84.6 | 124 | 206 |
| Indexed SEQUEST | 1.62 | 15.2 | 6.16 | 42.8 | 8.77 | 55.4 | 24.8 | 104 |
| X!Tandem | 3.19 | 33.3 | 12.4 | 109 | 46.3 | 199 | 138 | 399 |
| Tide | 77.2 | 413 | 536 | 1198 | 1554 | 1980 | 2738 | 3244 |

Figure 3: Performance of Tide compared to SEQUEST, Crux, OMSSA, Indexed SEQUEST (11/2009), and X!Tandem. Performance was measured in eight settings, varying the percursor mass tolerance window, the digest (fully tryptic candidate peptides or semi-tryptic), and the dataset (*C. elegans*, "worm dataset" or *S. cerevisiae*, "yeast dataset"—see Methods). Bar heights in log scale show spectra processed per second, with numerical results given below. Each experiment was repeated at least 3 times with average timings shown, except for the X!Tandem experiments. All SEQUEST experiments, as well as Crux experiments using partial digestion, were performed with 100 randomly-selected spectra. The remaining experiments, including all Tide experiments, were performed using 10,000 benchmark spectra.

input file formats available, and optimizing the ms2 format in particular fell outside the scope of efforts on Tide. The timing numbers in two lines of the Table 1 reflect this decision: Line 3 includes the parsing for the first time (a binary file was used beforehand), and Line 7 removes it again.

Note also that running times were not always collected for both yeast and worm, as a profile of one or the other was often enough to discern where to focus effort; but at least one or the other time is always reported, as is the relative time improvement between versions. An average is shown in cases where both yeast and worm times were measured.

## 3.4 Final timing comparisons

Figure 3 shows the results of timing experiments for two SEQUEST versions, Crux, and Tide, performed on the benchmark datasets. For reference, timing comparisons are shown to X!Tandem and to OMSSA, although these software packages use different scoring methods than the SEQUEST method.

Compared to the earlier version of SEQUEST (version 2.8, 1999), Tide's speedup is over 1,000-fold in all cases, reaching as high as 2,500-fold for the worm benchmark with fully

tryptic digestion. The increase is more modest, but still dramatic, with respect to the recent SEQUEST version (November 2009), especially with respect to partial digestion—as little as 27-fold speedup for the worm set with 0.25 Da mass tolerance. The geometric average speedup for all datasets of Tide over the recent SEQUEST build is 54-fold. The earlier version of SEQUEST (1999) did not support partial enzyme digestion, and corresponding entries are blank in Figure 3.

Crux shows intermediate performance between that of SEQUEST and Tide. Tide ran at least 29-fold faster than Crux in all cases, and as high as 145-fold in the case of the yeast benchmark with partial digestion and a 3.0-Dalton precursor tolerance. With respect to the original SEQUEST version, Crux was as much as 87 times faster (worm benchmark, full digestion, 0.25-Dalton tolerance). In comparison to the recent indexed version of SEQUEST, Crux's performance was mixed, with Crux performing faster on three benchmarks and indexed SEQUEST performing faster in five. However, in no case was the performance difference between Crux and the recent SEQUEST more than a factor of two.

Although Tide performs very well in comparison to X!Tandem and OMSSA (a geometric average of 17 times faster than X!Tandem and 43 times faster than OMSSA over all the benchmark datasets), marked differences in scoring methods among these systems make fair comparisons difficult and are beyond the scope of this paper.

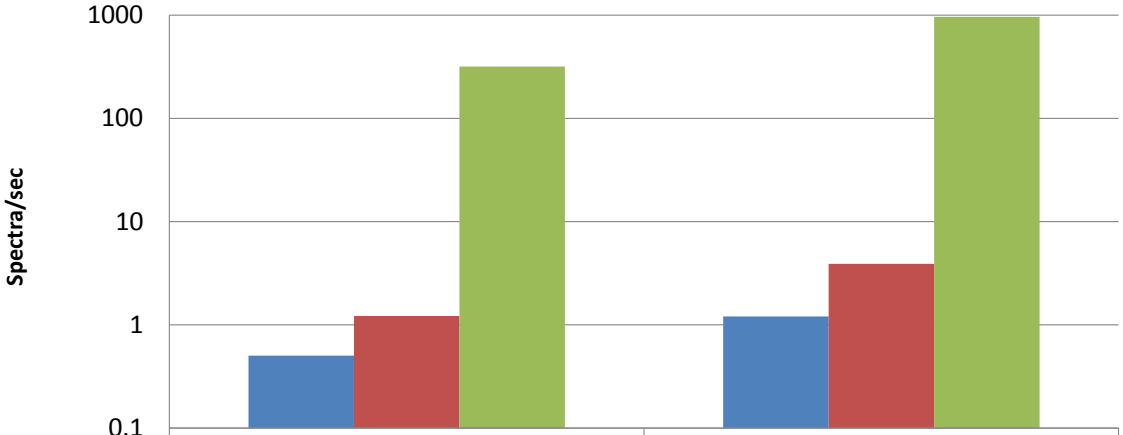
Four of the software packages discussed here make use of indexing to achieve fast execution. However, the relative speeds of three of these tools—indexed SEQUEST, Crux and X!Tandem—fall within a relatively narrow range of one another, spanning an average (geometric mean over all benchmarks) factor of 4 from the slowest to the fastest. Thus, Tide's achievement of a further 17-fold over the fastest of these methods is especially noteworthy.

Figure 4 shows the results of timing experiments comparing Tide to the same two versions of SEQUEST when modified peptides are included in the search. The benchmark sets the worm and yeast data sets, each with a 3.0 Da mass tolerance window including fully-tryptic peptides. Modified versions of each peptide were considered in this experiment, with up to two phosphorylations (+80 Da) per peptide at occurrences of serine, threonine, or tyrosine. In these experiments, Tide's relative speed is an average of over 708-fold over SEQUEST and 253-fold over the indexed version of SEQUEST.

# 4 Discussion

In this work, we have described a software implementation of the SEQUEST algorithm that searches at a rate of hundreds of spectra per second on a single CPU. This software thus represents more than a thousandfold improvement in speed relative to a recent single-CPU version of SEQUEST.

We have not directly compared Tide against the commercial indexed version of SEQUEST, TurboSEQUEST [24]. This is primarily because SEQUEST was implemented and validated on a Linux platform, whereas TurboSEQUEST is only available running under Windows. However, we have shown comparisons to an indexed version of SEQUEST that runs under Linux but is not widely available. Furthermore, Crux was previously compared to TurboSEQUEST, and the two programs were demonstrated to operate at approximately the same speed [4]. Thus, the results shown in Table 3 suggest that Tide, when ported to




| | Worm | Yeast |
|---|---|---|
| SEQUEST | 0.503 | 1.21 |
| Indexed SEQUEST | 1.22 | 3.91 |
| Tide | 318 | 960 |

Figure 4: Performance of Tide compared to SEQUEST and Indexed SEQUEST (11/2009) on benchmark datasets with variable modifications. Bar heights in log scale show the number of spectra processed per second. The same benchmark datasets were used as in Figure 3, but with up to two occurrences per peptide of phosphorylated residues serine, threonine, or tyrosine. Tests were run with a ±3.0 Dalton mass window and full tryptic digestion. SEQUEST experiments were run with 100 randomly-selected spectra. Tide experiments used 10,000 benchmark spectra.

Windows, will perform significantly faster than TurboSEQUEST.

Likewise, we have not compared Tide's speed with the speed of the full gamut of competing database search tools, except for X!Tandem and OMSSA as two illustrative examples. This is because the focus of this work is efficiency, subject to the constraint that Tide remains faithful to the SEQUEST method. X!Tandem, OMSSA and other database search tools do not follow the SEQUEST scoring method. To compare different search algorithms in a reasonable fashion would require jointly considering both efficiency and accuracy. Accurate identification requires not just a database search tool, but also one or more post-processing steps that integrate information across the entire mass spectrometry experiment, taking into account information about the spectra, peptides and proteins. Therefore, the most useful comparison of search speed and accuracy should be performed at the level of a complete identification pipeline. Such an evaluation is beyond the scope of the current study.

The speed improvements introduced in Tide are especially effective in common input cases but are less dramatic in some contexts. Shotgun proteomics experiments commonly require enzymatic digestion of the sample. In searching for enzymatic peptides, Tide performs up to thousands of times faster than SEQUEST. In the non-enzymatic case Tide is only about 7–8 times faster than the recent SEQUEST version as tested—a far more modest gain. However, Tide was not specifically optimized for this setting, and other opportunities for improving Tide's algorithm might exist.

Most of Tide's optimizations are most effective in cases where there are many candidate peptides per spectrum, because such cases provide opportunities to reuse the results of earlier computations. The number of candidates per spectrum will generally increase with the scale of the search problem: larger protein database, wider precursor tolerance, decreased enzyme specificity, and the inclusion of post-translational modifications in the database. With problems of greater scale, speed becomes increasingly important, and Tide's results are particularly encouraging in this context.

Tide's precise fidelity to Crux's scoring introduced constraints on the approach to optimization that would not have existed had Tide's output been allowed to vary slightly from Crux's. Conversely, because Crux's output is less faithful to SEQUEST's output, some of Tide's optimizations may only be possible because Crux and Tide differ from SEQUEST. We have not undertaken to investigate all of these differences, because they were shown [4] to have little overall effect on accuracy and because such differences arise even among various versions of SEQUEST (Supplementary Figure 1). Nevertheless, at this point we cannot be certain that all of Tide's speed would be preserved if we eliminated all scoring differences between Tide and a specific version of SEQUEST.

Perhaps the greatest operational difference between Tide and SEQUEST is that Tide does not compute SEQUEST's preliminary scoring function, $S_p$. The $S_p$ score was introduced into SEQUEST to speed up computation [1]. However, Tide is fast enough that it can efficiently compute the full $X_{Corr}$ calculation for all PSMs and does not require (nor is it likely to benefit greatly from) a preliminary scoring pass using $S_p$. Consequently, whereas SEQUEST may miss a candidate peptide with the highest $X_{Corr}$ because of the $S_p$ screen, Tide does not have this limitation. Note that, in some contexts [23, 25, 26], the $S_p$ value is needed for the top few PSMs as an additional scoring signal. Though $S_p$, which is computationally simpler than $X_{Corr}$, is not currently included in Tide, the cost of calculating $S_p$ for the top few PSMs should be marginal.

Most of Tide's improvements are highly optimized for $X_{Corr}$ only and are not likely to be applicable to scoring methods used in other peptide identification software programs. On the other hand, some optimizations included in Tide, such as the compact index, the rolling-window join, and storing exceptional cases to disk, will generalize to any type of database searching. But the specific methods for reducing the number of multiplications and memory lookup operations, caching partial results, grouping related theoretical peaks, on-the-fly compiling of the dot-product code, and the like, are highly specific to the $X_{Corr}$ method.

Because running SEQUEST is computationally intensive, Tide offers the possibility to run analyses that heretofore have been prohibitively expensive. Thus, Tide creates the potential for smaller laboratories to conduct more sophisticated experiments, to sidestep purchasing and managing large computing clusters, and to keep a spectrometer running full-time when analysis would otherwise be a bottleneck. Fast software also opens possibilities for further improvements of the identification methods themselves. By analyzing larger datasets, researchers can gather more data and, in turn, devise better analytical methods.

# Acknowledgments

The authors wish to thank Charles Grant, Barbara Frewen, Larry Ruzzo, Jimmy Eng, and Mike MacCoss for useful conversations, and Sean McIlwain for help in the design of the benchmarking scripts used to track running times of program runs. This work was supported by NIH awards R01 EB007057 and T32 HG00035.

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