String Matching and Online Retention Time Prediction for Real-Time Information-Driven Mass Spectrometry

By

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fulfillment of the requirements for the degree of

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Submitted by Robert James Peace
in partial fulfillment of the requirements for
the degree of Master of Applied Science in Biomedical Engineering

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Abstract

Tandem mass spectrometry (MS/MS) is a powerful analytical technique which is the current state of the art with respect to protein identification. MS/MS experiments are characterized by the generation of abundant spectral data; as a result, mass spectrometry data analysis techniques are extremely important for the correct identification of proteins. Analysis of MS/MS data is ultimately limited, however, by MS/MS data acquisition. Currently, most MS/MS data collection is performed in a naive abundance driven fashion which does not make optimal use of experimental hardware. Information-driven tandem mass spectrometry (idMS/MS) techniques seek to couple data analysis and data acquisition with the goal of guiding data acquisition in order to increase hardware efficiency and consequently the quality and quantity of protein identifications in an experiment. idMS/MS presents several challenges, primarily a real-time deadline on data analysis which is imposed by the mass spectrometry hardware. Commodity parallel hardware such as GPGPU and Cell B/E show promise for meeting the real-time requirements of idMS/MS. A computationally intensive step in the MS/MS data analysis pipeline – searching peptide sequence tag data over large proteomic or genomic databases – has been parallelized using these platforms in order to explore the platforms' suitability for idMS/MS. Results of these experiments are extremely promising, with a highly tailored searching algorithm producing throughputs of 2066.4 Gbit/s on a NVIDIA GeForce GTX 570 graphics card, 943.8 Gbit/s on a NVIDIA GeForce GTX 260 graphics card and 219.3 Gbit/s on a QS22 Cell blade server. These experiments represent typical peptide sequence tag search conditions. During idMS/MS data analysis, the idMS/MS system must also be aware of the experiment's current retention time. This presents a second challenge for the creation of an idMS/MS system. Currently, mapping of retention time to elution time is restricted to linear models which are a function of the conditions of the

experiment, and which require careful calibration of the instrument and precise knowledge of the LC gradient. We have leveraged our real-time access to spectral data in order to develop a novel probabilistic approach which maps currently observed survey ion spectrum data to a predicted retention time map of the experiment. Using this method, we have predicted the retention time during the elution of 9 confidently identified peptides during a MS/MS experiment with a mean squared error of 0.00627 relative to the retention time values of these peptides, using only data available to the mass spectrometer during the experiment, and without the requirement of prior knowledge of the instrument. This approach does not require characterization of the LC gradient, therefore the need for a parameterized linear gradient is removed. Additionally, as a step toward the creation of the retention time / experiment time mapping algorithm, we have developed a novel ANN network which has predicted the retention time of 1917 peptides with a R^2 value of 0.9483 using only 23 inputs and 10 internal nodes.

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List of Abbreviations

MS	Mass Spectrometry
MS/MS	Tandem mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
idMS/MS	Information-driven mass spectrometry
Cell B/E	Cell Broadband Engine
GPGPU	General purpose programming on graphics processing units
API	Application programming interface
NET	Normalized elution time
AMT	Accurate mass and time
ANN	Artificial neural network
SVM	Support vector machine
SVR	Support vector regression
QSRR	Quantitative structure-retention relationship
MSE	Mean squared error

1 Introduction

1.1 Introduction

This section provides relevant background information on the topics of mass spectrometry, including tandem mass spectrometry and liquid chromatography mass spectrometry, commodity parallel hardware, and string matching.

1.1.1 Proteomics

Proteomics is the study of the structure and function of proteins. A protein is a biochemical compound consisting of any number of amino acids connected via peptide bonds into a chain-like structure. Amino acids are molecules which contain an amine group, a carboxylic acid group, and a side chain; there are 20 distinct side chains, each of which corresponds to one of 20 possible amino acids. Proteins are produced in the cell nucleus through the process of transcription and translation, which generates amino acid chains from DNA sequences based on the genetic code.

Once a protein has been produced through transcription and translation, it folds into a complex three-dimensional structure which, in turn, largely determines the function of the protein. This structure is not relevant to this thesis, however. Only the primary structure of the protein – the amino acids present and the order in which they appear – will be examined. Thus, for our purposes, proteins can be encoded as character strings with each character representing an amino acid. For example, a protein that consists of the amino acids Alanine (A), Arginine (R), Asparagine (N), and Aspartic Acid (D) in that order is represented by the string **ARN D**.

1.1.2 Mass Spectrometry

Mass spectrometry is an analytical chemistry technique which is capable of determining the molecular composition of unknown sample molecules. Mass spectrometry is a widely used technique, common in fields such as water quality assessment, pharmaceuticals, proteomics, and lipidomics. For the purpose of this thesis, we will limit the scope of mass spectrometry data analysis to proteomics. Thus, we can assume that the input to any mass spectrometry experiment is a sample with an unknown mixture of proteins, and that the desired output of the experiment is the unique identification of as many proteins in the sample as possible or the determination of specific high-importance biomarker proteins.

The most common mass spectrometry pipeline is the tandem mass spectrometry pipeline, or MS/MS pipeline. The first step in the MS/MS pipeline is a digest of proteins into peptides – amino acid chains which are subsequences of an original protein sequence. Typically, protein digestion is performed by the enzyme trypsin, which cleaves proteins at specific peptide bonds – after any **R** or **K** amino acid which is not followed by a **P** amino acid. Thus, knowing the sequence of a protein, it is possible to predict all peptides which would be produced by a tryptic digestion.

Once a protein has been digested into peptides, these peptides are then ionized using either matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) techniques. For our purposes, we will assume that ESI ionization is used for all experiments. The ionized peptides are then propelled through a chamber using an electric or magnetic field which imparts kinetic energy onto each peptide relative to the peptide's charge. The resulting velocity of a peptide is proportional to the mass-to-charge (m/z) ratio of the peptide. Using a detector at the end of the

chamber, a mass-to-charge histogram of all peptide material in the mass spectrometer can be determined by measuring the time of flight of peptides or the amount of deflection in the path of the peptides. This histogram is represented by a m/z spectrum, in which peaks correspond to the m/z ratios of molecules which were abundant in the digested sample, i.e. peptides. Due to the presence of carbon in peptide molecules and the natural occurrence of C^{13} carbon isotopes, each peptide results in a cluster of peaks which are evenly spaced at a distance of $1/(\text{charge of peptide})$. Thus, by measuring the separation distance within a cluster of peaks, it is possible to determine the charge of a peptide, and therefore the mass of the peptide. This process is known as de-isotoping.

Figure 1-1 - A m/z spectrum resulting from mass spectrometry

The production of m/z spectra is the fundamental operation of mass spectrometry hardware. This operation is performed repeatedly throughout an experiment, at a rate of several spectra per second on a modern mass spectrometer. M/z spectra which contain peptide peaks are used as an input for tandem mass spectrometry, which is detailed in the following section.

1.1.3 Tandem Mass Spectrometry

Tandem mass spectrometry, or MS/MS, represents a second dimension of data collection in a mass spectrometry experiment. MS/MS extends the mass spectrometry pipeline by delving into specific peptide peaks of a m/z spectrum. A MS/MS experiment starts with the production of a standard m/z spectrum, known as a survey ion scan. Once a survey ion scan has been performed, peptide material with a specific m/z ratio is extracted from the mass spectrometer. For example, upon generating the spectrum seen in Figure 1-1, the mass spectrometer is likely to extract all material with a m/z ratio of 1480 as this m/z ratio relates to an abundant peak in the spectrum. Once extracted, the material with the specific m/z ratio – which ideally consists only of a single peptide – enters a collision chamber. Inside this chamber, the peptides are broken into smaller amino acid subsequences at random; breakage almost always occurs between amino acids because of the weak peptide bonds between amino acids. The resulting ions are sent through the mass spectrometer again, resulting in another m/z spectrum, known as a product ion spectrum. Because the product ion spectrum contains only information relating to a single peptide and the total mass of the peptide is known from the survey ion scan, it is often possible to ascertain the identity of this peptide using the product ion spectrum information. Figure 1-2 demonstrates an overview of the MS/MS pipeline as described in this section.

Figure 1-2 - Major steps in the MS/MS pipeline. Data is colour-coded by peptide; all four peptides appear in the survey ion scan, while the product ion scan is limited to a single peptide.

One commonly used technique for extracting peptide identification information from product ion spectra is *de novo* sequencing. In order to determine the identity of amino acids, *de novo* sequencing observes the distance between peaks in the product ion scan; because the peptide was fragmented at random, any inter-peak distance which corresponds to the mass of a single amino acid is likely to be caused by the presence of two subsequences of a peptide: one subsequence which contains the amino acid relating to the inter-peak distance, and another subsequence which is similar, but is missing the amino acid in question. That is to say, an inter-peak distance which matches an amino acid mass is evidence that that amino acid is present in the peptide. By finding concurrent inter-peak distances relating to amino acids, *de novo* sequencing develops a peptide sequence tag, a short sequence of amino acids which are known to be a part of the peptide. For example, in Figure 1-3, inter-peak distances corresponding to the sequence tag **LVLV** are seen in the

product ion spectrum. Thus, the peptide is likely to contain the sequence **LVLY**. Peptide sequence tags are prone to ambiguity due to the isobaric nature of certain amino acids, i.e. **GG** and **K**, and due to missing peaks in product ion spectra which result in pairs of amino acids whose order is uncertain.

Once a peptide sequence tag has been obtained, this tag can be searched against proteomic databases; all proteins which contain this peptide sequence tag are potentially present in the unknown protein sample. With the information gained from multiple peptide sequence tag searches, the identity of proteins in a sample can be determined. Proteins are not considered to be identified in a sample unless multiple peptide sequence tags confirm its identity – in mass spectrometry literature, protein identifications based on the strength of a single peptide sequence tag match are not considered to be well-founded.

Figure 1-3 - De novo sequencing producing LVLY from a product ion spectrum

A second technique which determines the identity of a peptide based on spectral data is spectral matching, which correlates an experimental spectrum with databases of predicted peptide spectra. The strength of the correlation is based on the number of peaks in the database spectrum which share a mass with peaks in the experimental spectrum. Spectral matching is not explored during this thesis.

1.1.4 Liquid Chromatography and Retention Time

While the MS/MS pipeline is capable of identifying proteins through the use of product ion spectra, complex samples produce incomprehensible survey ion spectra if all of the sample material enters the mass spectrometer at once. In order to reduce the complexity of survey ion scans, the MS/MS pipeline is commonly prepended with liquid chromatography, producing the LC-MS/MS pipeline. In this pipeline, peptides are not fed directly into the mass spectrometer after being digested, but are rather bound to a column which contains beads coated in a C18 resin. The column is immersed in a mixture of water and an organic liquid such as acetonitrile. This mixture flows from the column to the mass spectrometer continuously for the duration of a LC-MS/MS experiment. Initially, the mixture contains only water; the hydrophobicity of peptides prevents them from eluting from the column and into the mass spectrometer. Over time, however, an acetonitrile gradient is introduced into the mixture. acetonitrile gradients are typically linear, and thus can be defined by a start time at which acetonitrile begins to flow and a set rate of increase in acetonitrile over time. As a result of the acetonitrile gradient, peptides elute from the column and into the mass spectrometer in roughly the order of their hydrophobicity; weakly hydrophobic peptides elute early in the experiment, while strongly hydrophobic peptides elute toward the end of the experiment. Throughout the experiment, the mass spectrometer continually cycles between producing a survey ion spectrum and producing product ion spectra from the most abundant

peaks of the survey ion spectrum. Product ion spectra are aggregated for the duration of the experiment and stored in a flat file, which is analysed after the experiment in order to identify proteins from the initial sample.

LC-MS/MS as described above is the most widely used form of mass spectrometry experiment, and is the type of mass spectrometry experiment which we will focus on in this thesis. Figure 1-4 demonstrates an overview of the LC-MS/MS pipeline.

Figure 1-4 - Overview of the LC-MS/MS pipeline. Peptides travel from the liquid chromatography phase to the mass spectrometer, where they are ionized and then detected based on their mass/charge ratio. During a product ion scan, peptides pass through a collision chamber between ionization and detection.

With the advent of LC-MS/MS experiments, peptide retention time – the percentage of acetonitrile in solution at which a specific peptide will elute from a column into a mass spectrometer – has become an important physical property of peptides. This value is often referred to as the normalized elution time (NET) of a peptide, and ranges from 0 to 1 where a higher number represents later elution during a typical experiment. LC-MS/MS experiments are defined by an acetonitrile gradient (LC gradient) which occurs throughout the experiment; therefore, a well-defined relationship exists between the retention time of a peptide and its elution during an experiment. This relationship is non-trivial, however, as LC gradients are not always

linear, retention time prediction is approximate, and LC gradients are not always known prior to an experiment. Because of the advent of the LC-MS/MS pipeline, techniques which aim to predict the retention time of a given peptide based on the peptide's sequence are now important elements of mass spectrometry data analysis. By performing an *in silico* digestion on a proteomic database, or by performing *in silico* transcription, translation, and digestion of a genomic database, then calculating the mass and predicting the retention time of each resulting peptide, it is theoretically possible to build an accurate mass/time (AMT) database which captures all of the expected survey ion spectrum peaks for a LC-MS/MS run. These databases can be viewed as a two-dimensional graph, as shown in Figure 1-5. We can picture a LC-MS/MS experiment as a 'scan' from left to right across this two-dimensional graph, where a subset of the peaks – corresponding to the peptides present in the sample – are uncovered by the scan.

Figure 1-5 - An accurate mass/time database

1.1.5 Commodity Parallel Hardware Architectures

Parallel computing has recently seen an increase in popularity, largely due to the advent of commodity parallel hardware architectures such as Sony/Toshiba/IBM's Cell Broadband Engine (Cell B/E) and general purpose programming on graphics processing units (GPGPU) using the Nvidia CUDA platform.

1.1.5.1 The Cell Broadband Engine

The Cell Broadband Engine (Chen, Raghavan, Dale, & Iwata, 2007), a heterogeneous multi-core processor, was released in 2006 by Sony, Toshiba and IBM. This architecture was originally designed to power the Playstation 3 console, however it has seen success for parallel scientific computing (Bader & Agarwal, 2007) (Petrini et al., 2007), powering parallel clusters such as the US Department of Energy Roadrunner cluster, which was the first computer to break the petaflop/s barrier and the world's fastest supercomputer from June 2008 through June 2009 (Los Alamos National Security, LLC for the U.S. Department of Energy's NNSA, 2010-2011).

The Cell B/E incorporates a single general purpose 64-bit Power Processing Element (PPE) and eight high-throughput Synergistic Processing Elements (SPEs) onto a single chip. These nine processors are connected with a ring bus, the Element Interconnect Bus (EIB).

Figure 1-6 - Overview of the Cell B/E architecture

Typically, the PPE is used only for task management, as the PPE is responsible for launching and coordinating SPE kernel threads. The bulk of computation is performed by the SPEs, which are highly tailored to data-intensive computing. Each SPE is capable of 128-bit-wide SIMD operations at 3.2GHz, and each SPE is outfitted with a memory management unit which handles data transfer independently of computation. All SPE data, including heap, stack, and instructions, are stored in a 256KB local store (LS) which is integrated into each SPE. Each SPE is capable of running kernels independently; the 8 SPEs can each perform a separate function at one time. The Element Interconnect Bus, which is responsible for all memory transfer within the Cell B/E chip, takes the form of four uni-directional ring buses which when fully utilized provide a theoretical peak bandwidth of 307.2GB/s.

1.1.5.2 The CUDA Platform

Nvidia's CUDA platform (http://www.nvidia.com/object/cuda_home_new.html) for GPGPU is the result of a recent trend toward the use of graphics processing units for parallel processing of scientific data. The CUDA platform allows graphics cards to be programmed directly, using a C-like application programming interface (API). Similarly to the Cell B/E architecture, the CUDA programming paradigm revolves around an all-purpose processor, the CPU, which launches and coordinates GPU threads (known as kernels) on multiple high-throughput processors on the GPU. However, the main difference between the Cell B/E and CUDA platforms is that a typical CUDA GPU has hundreds of simpler processors. The GPU architecture is dominated by a series of multiprocessors, each of which contains a single instruction unit, several SIMT (single instruction multiple thread) processors, and a number of caches. All of the multiprocessors are connected to the GPU main (device) memory, which is in turn connected to the CPU main memory through the PC's main bus.

1.2 Motivation

While MS/MS represents the state of the art for many important biochemical tests, most notably protein identification, current mass spectrometry techniques are fundamentally limited by a disconnect between data acquisition and data analysis. MS/MS data analysis techniques have seen great improvements in the past few years, making use of new technologies and techniques, however these techniques can only work with the data given to them, and MS/MS data acquisition makes use of relatively crude algorithms for survey peak selection. The most common form of survey peak selection algorithm for MS/MS is abundance-driven MS/MS, wherein precursor ions are chosen based only on their abundance in the mass spectrometer at the time of the latest survey scan. This method of survey peak selection is clearly sub-optimal, especially when low-abundance biomarkers are present in a sample which could be important to protein identification. Figure 1-7 demonstrates the abundance driven MS/MS pipeline.

An information-driven MS/MS pipeline, wherein data analysis is performed during the experiment and precursor ions are selected based on the likelihood that they will uniquely identify proteins, could improve the data acquisition stage of mass spectrometry experiments. Previous work has proven that state of the art MS/MS hardware only captures a fraction of possible precursor ions (Michalski, Cox, & Mann, 2011), demonstrating a need for intelligent precursor ion selection. Additionally, it has been proven that the move from abundance-driven to even a simple information-driven MS/MS pipeline would increase the number of unique protein identifications during an experiment (Zerck et al., 2009). This work, however, was done only on simulated experiments. A complete information-driven MS/MS data analysis pipeline has not been implemented at this time.

Figure 1-7 – Abundance-driven MS/MS pipeline. Product ion scans are repeated on high-abundance peptides for the entirety of the experiment.

The move toward online “in-the-loop” data analysis techniques introduces several major challenges which are not present in the current paradigm of MS/MS data analysis. Chief among these challenges is the real-time deadlines introduced by the mass spectrometry hardware; current mass spectrometry hardware produces multiple spectra every second, and each iteration of the idMS/MS data analysis loop must be performed between the production of spectra. Therefore, an entire cycle of MS/MS data analysis must be performed within a fraction of a second in order for idMS/MS to become possible.

Fortunately, recent developments in commodity parallel hardware allow for high-throughput data intensive computing which could potentially meet the real-time deadlines imposed by the mass spectrometer without the need for complex hardware setups or advanced technical knowledge. Platforms such as the Cell Broadband

Engine (Cell B/E) and general purpose programming on graphics processing units (GPGPU) using CUDA will facilitate idMS/MS once a full pipeline is implemented.

As can be seen in Figure 1-8, the idMS/MS pipeline contains several critical steps, all of which must be performed in series within the mass spectrometer's real-time deadline. Until the point where enough information has been gathered to perform information-driven MS/MS, product ion spectra are selected using an abundance-driven methodology (step 1). When a product ion spectrum is generated by the mass spectrometer, *de novo* sequencing is performed on the spectrum (step 2), producing a peptide sequence tag which can then be searched against a proteomic database (step 3). All protein matches for the peptide sequence tag are recorded, and the list of all proteins for which there is evidence of their existence is used to determine a score for each of the peaks of the latest survey ion mass list (step 4). When a survey ion spectrum is generated by the mass spectrometer, the peaks of the spectrum are converted into a survey ion mass list. Using the retention time / experiment time mapping module, the current best-guess retention time is determined based on this mass list (step 5). The current retention time is also used as a factor for determining the score of each peak. Once all of the peaks have been scored, high-scoring peaks are then chosen for future product ion scans (step 6).

Step 3 of the pipeline, searching genomic or proteomic databases for peptide sequence tags, clearly represents the challenge of parallelization of data-intensive analysis. Additionally, searching peptide sequence tags across proteomic or genomic databases is analogous to exact string matching; genomic or proteomic databases are stored as flat files on disk and moved into memory during searching, and are typically in the range of hundreds of megabits in size. Exact string matching, and by extension peptide sequence tag matching, is a problem which is prevalent in

computer science literature and which can be benchmarked in a straightforward manner. These factors make step 3 an ideal first step toward parallel MS/MS data analysis.

Figure 1-8 - Information-driven MS/MS pipeline. Hardware products are highlighted in red, while idMS/MS products are highlighted in blue.

Challenges inherent in moving MS/MS data analysis online cannot all be solved through parallelization, however. In developing an idMS/MS pipeline, it becomes apparent that knowing the current retention time of the experiment is essential to successful precursor ion selection – peptides which are currently being eluted into the mass spectrometer are more relevant than peptides which have already eluted and passed through the instrument. Prior MS/MS data analysis techniques did not require a knowledge of retention time until the experiment was completed and all data could be looked at as a whole, so a novel approach was required in order to map retention time to experiment time using data as it is produced by the mass spectrometer. This time mapping algorithm is also useful outside of the idMS/MS pipeline, as it would allow directed MS/MS experiments (whose goal is to find

evidence of specific proteins) to locate peptides along the time scale of an experiment without the need for input from mass spectrometry technicians. Current techniques for the prediction of the elution time of peptides, such as SSRCalc (<http://hs2.proteome.ca/SSRCalc/SSRCalcX.html>), require that instruments be calibrated precisely and that LC gradients be known prior to the experiment, two requirements which are not present for our time-mapping algorithm.

1.3 Statement of the Problem

Given idMS/MS' potential for improvement of MS/MS data analysis and the wide availability of commodity parallel hardware, we feel that the production of an idMS/MS pipeline is an ideal candidate for the advancement of protein identification. This thesis presents two major contributions which are essential to the production of the idMS/MS pipeline.

In order to gauge the suitability of the Cell B/E and CUDA platforms for data-intensive MS/MS analysis, while at the same time developing a critical element in the idMS/MS pipeline, we aim to implement a novel string matching algorithm which is highly tailored to the task of searching peptide sequence tags over large genomic or proteomic databases. This algorithm should be capable of searching six-frame genomic databases, and should be able to handle ambiguities which are commonly seen in peptide sequence tags. Once implemented on both the Cell B/E and CUDA platforms, the peptide sequence tag matching algorithm will provide evidence as to which parallel hardware platform is the ideal candidate for idMS/MS implementation.

In addition to the peptide sequence tag matching algorithm, we seek to develop a novel algorithm for the purpose of mapping an experiment's current wall time to the calculated retention time value of the experiment. This algorithm will calculate the

current retention time of an experiment in real time, using only data which is available from the mass spectrometer during the experiment. Because the amount of data available to the algorithm is limited, a non-parametric probability-based model is likely to be ideal for this problem. As a step toward the development of this algorithm, we aim to develop a real-time retention time prediction method which strikes a balance between prediction accuracy and computational intensity.

1.4 Contributions

Chapter 3 presents several contributions related to the problem of peptide sequence tag matching on the Cell B/E hardware. The primary contribution of this chapter is the development of the orthogonal Parabix algorithm for the Cell B/E. This novel extension to the Parabix algorithm (Cameron, Herdy, & Lin, 2008) is well-suited to the task of peptide sequence tag matching, and far outperforms all existing string matching algorithms for this task. The orthogonal Parabix algorithm as implemented on the Cell B/E sustains a search throughput of 215.4 Gbit/s. When compared to general purpose string searching methods, the orthogonal Parabix algorithm provides a speedup of approximately 124x. In comparing the Cell B/E implementation of the orthogonal Parabix algorithm to an equivalent serial implementation, we see speedups of over 10x.

Chapter 3 also presents the first implementations of established string searching algorithms on the Cell B/E, including the Boyer Moore and Rabin Karp algorithms. Note that initial development of the Cell B/E implementations of existing string searching algorithms was performed during a 4th year undergraduate project. This thesis did, however, improve on these implementations, increasing search throughputs. Chapter 3 forms the basis of the journal article, "Exact String Matching

for MS/MS Protein Identification using the Cell Broadband Engine” (Peace, Mahmoud, & Green, 2011).

The primary contribution of chapter 4 of this thesis is the implementation of the orthogonal Parabix algorithm on the CUDA platform. This implementation, running on a Nvidia GTX570 graphics card, achieves a sustained search throughput of 2066.4 Gbit/s - an order of magnitude greater than those seen on the Cell B/E platform. This implementation also breaks the 100x speedup barrier when compared to an equivalent serial implementation.

In addition to the increased throughput, the CUDA implementation of the orthogonal Parabix algorithm improves on the original implementation in several ways. The CUDA implementation is capable of searching six-frame genomic databases without affecting the search throughput, and handles ambiguous peptide sequence tags in such a way that false positive results are minimized while search throughput remains comparable to throughputs for unambiguous queries.

Chapter 4 presents a novel algorithm which applies probabilistic models to the problem of mapping wall time to retention time as a LC-MS/MS experiment is performed. This algorithm builds on the idea of the AMT database as a probability density space by employing Parzen window techniques and function maximization in order to estimate the current retention time which best matches the evidence provided by the mass spectrometer as the experiment is being performed. This approach was able to accurately predict the retention time of a set of 9 confidently identified peptides during a real LC-MS/MS experiment with a mean squared error of 0.00627.

As a step in the process of developing the retention time / wall time mapping algorithm, we have developed a novel ANN topology which balances prediction accuracy and computational intensity. This ANN is capable of predicting peptide retention with a high R^2 value of 0.9483 using only 23 inputs and 10 internal nodes. This ANN topology outperforms ANNs of similar size by a significant margin.

1.5 Organization of Thesis

This thesis consists of 6 chapters. Chapter 2 briefly discusses the current state of the art regarding mass spectrometry data analysis, parallel string searching, peptide retention time prediction, accurate mass/time databases for mass spectrometry, and commodity parallel hardware.

In chapter 3, we present peptide sequence tag searching on the Cell B/E, including implementations of established string matching algorithms on the Cell B/E and the novel orthogonal Parabix algorithm.

In chapter 4, we present peptide sequence tag matching efforts on the GPU, including improvements to the orthogonal Parabix algorithm in order to allow for searching of genomic databases and better handling of ambiguous peptide sequence tag queries.

Chapter 5 details the creation of the retention time / experiment time mapping algorithm, which is accomplished through novel use of statistical techniques in the domain of mass spectrometry.

Chapter 6 presents a summary of contributions and provides recommendations for future work.

2 Literature Review

This chapter details the state of the art in fields which are impacted by this thesis. Fields covered in this chapter include data analysis for mass spectrometry, accurate mass/time databases, retention time prediction, and exact string matching on commodity parallel hardware.

2.1 Computational Methods for Mass spectrometry

Mass spectrometry methods can be broadly separated into two categories: experimental methods, which aim to improve the spectral outputs of mass spectrometry hardware through the use of chemical and physical techniques; and computational methods, which aim to improve the interpretation of spectral data via data analysis techniques.

Recent efforts in computational methods for mass spectrometry have focused on steady improvement of spectral analysis of MS/MS product ion spectra, as can be seen in computational mass spectrometry reviews over past years: (Aebersold & Mann, 2003), (Nesvizhskii & Aebersold, 2004), (Sadygov, Cociorva, & Iii, 2004), (Johnson, Davis, Taylor, & Patterson, 2005), (Nesvizhskii, Vitek, & Aebersold, 2007), (Deutsch, Lam, & Aebersold, 2008). The major themes running through these reviews are computational techniques which improve the statistical identification of proteins based on a given set of spectra, and the presentation of data to mass spectrometry technicians. Quantitative analysis of spectral data is also explored during this time (Mueller, Brusniak, Mani, & Aebersold, 2008), however this type of analysis is not mature at present.

Accurate protein identification through comparison of spectra with MS/MS databases is a dominant driving force in computational mass spectrometry; many prominent computational mass spectrometry suites fill this role, including MASCOT, SEQUEST, X!Tandem. These packages are summarized by Kapp *et al.* (Kapp *et al.*, 2005). De novo sequencing combined with peptide sequence tag searches against proteomic databases (Seidler, Zinn, Boehm, & Lehmann, 2010) compete directly with MS/MS spectral database search engines; examples of de novo sequencing tools include Lutefisk (Johnson & Taylor, 2002), Pepnovo (Frank & Pevzner, 2005), and Peaks (Ma & Lajoie, 2009).

A particular focus in recent computational mass spectrometry efforts has been the creation of integrated, robust mass spectrometry software suites which incorporate several data analysis techniques into coherent toolsets that are designed for end-users of mass spectrometry hardware. One of the largest such initiatives has been the OpenMS project (Sturm *et al.*, 2008). This open source project was developed around TOPP, the open proteomics pipeline (Kohlbacher *et al.*, 2007), and TOPPView, a software tool for visualization of mass spectrometry data (Sturm & Kohlbacher, 2009). OpenMS is indicative of the recent emphasis on usability in computational mass spectrometry techniques.

Non-redundant mass spectrometry techniques, precursors to information-driven mass spectrometry, have been explored during recent years. These techniques use data analysis to guide product ion selection; however applications of these techniques are limited due to their lack of real-time data analysis. Scherl *et al.* developed a “non-redundant” mass spectrometry system (Scherl *et al.*, 2004) which works in conjunction with a MALDI MS/MS experiment for which the sample is separated prior to MS/MS analysis. During such a MALDI experiment, it is possible to examine any portion of the protein sample at any point in the experiment, so the

real-time requirements of popular ESI LC-MS/MS experiments are eliminated. This process was successful, however non-redundant MS/MS experiments are time consuming and MALDI is considered to be an outdated ionization technique compared to modern ESI. Zerck *et al.* have explored the effectiveness of a non-redundant LC-MS/MS experiment through simulation (Zerck et al., 2009). Although this experiment used a simple model for scoring the importance of selecting certain peptides, they still improved the average confidence of protein identifications and the number of identified proteins during an experiment. Parallelization of MS/MS data analysis for the purpose of idMS/MS has seen limited exposure, including parallelization of peptide sequence tag searching using FPGA hardware (Alex, Dumontier, Rose, & Hogue, 2005), parallelization of spectral processing using FPGA hardware (Bogdan, Coca, & Rivers, 2007), and the successful implementation of a spectral matching algorithm which could serve as an alternative to *de novo* sequencing and peptide sequence tag matching (Zhang, McQuillan, & Wu, 2011).

2.2 Accurate Mass/Time Databases

As an alternative to LC-MS/MS experiments, several groups have experimented with the use of AMT databases combined with high-accuracy survey scans in order to uniquely identify peptides directly from spectral data. Relative to spectral database searching techniques and *de novo* sequencing techniques for LC-MS/MS experiments, the use of AMT databases for high-resolution LC-MS experiments is not a mature technology; high-quality data analysis pipelines which utilize AMT databases for peptide identification are not available on the scale of spectral matching- or *de novo* sequencing-based pipelines. AMT LC-MS pipelines are not currently practical for all laboratories due to limitations in mass spectrometry hardware resolution as well as the accuracy of current retention time prediction modules; therefore, efforts in this field focus largely on the development of statistical models for peptide identification

via AMT database. The concept of accurate mass/time databases is based largely on previous efforts in the field of accurate mass tags, which is described by Conrads *et al.* (Conrads, G a Anderson, Veenstra, L Pasa-Tolić, & R D Smith, 2000).

A popular probabilistic method for AMT LC-MS identification confidence is the spatially localized confidence (SLiC) scoring method which treats the problem of identifying a peptide as a statistical classification problem, as developed by Anderson, Monroe, Daly, and Valafar (K. K. Anderson, Monroe, Daly, & Valafar, 2004). Norbeck *et al.* have explored the resolution and retention time accuracy required in order for AMT LC-MS to successfully identify peptides given the complexity of the proteome which is being searched (Norbeck *et al.*, 2005). This work, which is built on the SLiC method, estimates that a mass spectrometer with mass accuracy of 1ppm would be capable of identifying only 50% of the human proteome through AMT approaches; only top of the line Fourier transform ion cyclotron resonance (FC-ITR) mass spectrometry hardware is capable of 1ppm mass accuracy given proper external calibrated. Zimmer *et al.* provide a comprehensive review of AMT LC-MS techniques (Zimmer, Monroe, Qian, & Richard D Smith, 2006) which contains a more optimistic estimation of proteome coverage; they estimate that FC-ITR mass spectrometry hardware is capable of identifying approximately 70% of a typical mammalian proteome.

While AMT LC-MS is not yet applicable to databases which are as complex as the human proteome, the technique has been successfully applied to smaller databases with success. Anderson, Monroe, and Daly were capable of matching the identification accuracy of spectral database techniques for the identification of bovine serum albumin against a database which contained approximately 30 proteins (K. K.

Anderson, Monroe, & Daly, 2006), however this database is not on the scale of complete mammalian proteomes.

A recent improvement to the AMT LC-MS pipeline is the use of reference maps for enhanced retention time accuracy. This method incorporates strong cation exchange fractionation into a mass spectrometry pipeline in order to provide calibration runs prior to the experiment proper, thereby increasing the instrument's resolution. Kim et al. demonstrate that this enhanced AMT LC-MS pipeline is capable of identifying T47D breast cancer line proteins with a 98.3% correct identification rate (Kim et al., 2009). This study is representative of recent successes of AMT LC-MS pipelines for protein identification.

In addition to the development of AMT LC-MS pipelines which do not examine product ion spectra, AMT databases and associated techniques have been used in conjunction with traditional LC-MS/MS data analysis pipelines. AMT-enhanced LC-MS/MS techniques such as those suggested by May *et al.* (May et al., 2008) demonstrate the utility of AMT databases outside of specific AMT LC-MS experiments. This work also introduces a novel probabilistic scoring method for matching experimental results to AMT databases.

2.3 Retention Time Prediction

The retention time prediction problem is a regression problem for which the input is an arbitrary peptide sequence and the output is a value which represents the chromatographic conditions under which the peptide will elute from a column in a liquid chromatography experiment. Retention time prediction is an important factor of AMT workflows, as retention time measurements are limited by the ability to accurately predict retention times of database peptides. A recent review of retention

time prediction by Babushok and Zenkevich summarizes the major techniques for retention time prediction (Babushok & Zenkevich, 2010).

The most mature technique for peptide retention time prediction is the use of artificial neural networks (ANNs) for linear regression. These ANNs use as inputs peptide sequence information, and are trained on large data sets which represent the observed elution times for a wide range of peptides over multiple mass spectrometry experiments. The first such technique was proposed by Petritis *et al.*, and contained 20 inputs, each relating to the frequency of each amino acid over the length of the peptide sequence (Petritis *et al.*, 2003). This technique achieved an R^2 value of 0.87 on a test set of 1303 confidently identified peptides. Petritis *et al.* later improved on their model by receiving as ANN inputs an orthogonal encoding of the entire peptide and a small set of statistically derived features (Petritis *et al.*, 2006). This model produced an R^2 value of 0.967 on the same test set. The third major effort in using ANNs to predict peptide retention time is based on Petritis' 20-input model, however in this model four of the amino acid inputs are removed due to having near-neutral hydrophobicity values (Shinoda *et al.*, 2006). This model predicts peptide retention time with an R^2 value of 0.928. While performing slightly worse than Petritis' 2006 method, the reduced complexity of the network may lead to better generalization to new data.

Complementary to the ANN technique described above is the family of quantitative structure-retention relationship (QSRR) techniques. These techniques utilize statistically derived chromatographic parameters which are derived from peptide sequence information, as opposed to utilizing peptide sequence information directly. Early papers in the QSRR field focused on the identification of statistically relevant parameters (Put, Daszykowski, Baczek, & Vander Heyden, 2006), while more recent

papers have focused more on the development of models using parameters which are known to be relevant. An example of a state of the art QSRR model is the ELUDE model, which uses support vector regression (SVR) and an input vector of 60 statistically derived parameters (Moruz, Tomazela, & Ka, 2010). ELUDE was tested on several data sets, resulting in Pearson correlation coefficients between 0.92 and 0.97.

A common criticism toward ANN models for retention time prediction is the need for large training data sets which are produced through myriad experiments, all of which need to be temporally aligned. Thus, efforts have been made in order to produce models which are capable of accurately predicting retention time on a specific instrument given a small training set of experimental peptides which were recorded by the instrument in question. These efforts have seen some success through the use of SVR. Klammer *et al.* were able to predict retention time with an R^2 value of 0.908 using a SVR model with 63 peptide sequence inputs and a small training set produced by a linear trap quadrupole instrument (Klammer, Yi, Maccoss, & Noble, 2007). Pfeifer *et al.*'s oligo-border kernel method for SVR achieved an R^2 value of 0.88 for peptide retention time prediction using a limited training set derived from the large Petritis data set (Pfeifer, Leinenbach, Huber, & Kohlbacher, 2007). The oligo-border kernel method has been incorporated into the OpenMS software suite (Sturm *et al.*, 2008) as the RTPredict module. This module permits users to train a model to mimic their own instrument, however the collection of an instrument-specific training set is a barrier to adoption.

The SSRCalc retention time prediction algorithm (Krokhin, 2006) is a popular algorithm due to its simple web interface as seen at <http://hs2.proteome.ca/SSRCalc/SSRCalcX.html>. This interface represents a typical

retention time prediction interface; experiment-specific information is required and only linear LC gradients are accepted. The SSRCalc algorithm is in the QSRR family, and provides an R^2 value in the range of 0.95 to 0.97 for most test sets.

2.4 Exact String Searching on Parallel Architectures

Parallel hardware, including commodity parallel hardware such as the Cell B/E and GPU platforms, is commonly used for high-throughput exact string matching and regular expression matching. The driving force behind most high-throughput string matching algorithms is network intrusion detection (Vasiliadis, Antonatos, Polychronakis, Markatos, & S. Ioannidis, 2008) (Scarpazza, Villa, & Petrini, 2008b); large volumes of network traffic must be searched for expressions which are indicative of malicious traffic. Thus, nearly all parallel exact string matching and regular expression matching algorithms are variations of the Aho-Corasick algorithm (Aho & Corasick, 1975) which uses a discrete finite automaton to search streaming databases in constant time with respect to the number of search queries. A number of implementations of the Aho-Corasick algorithm are described below.

On the Cell B/E platform, the IBM T. J. Watson Research Center is responsible for almost all leading string matching implementations. This group focuses mostly on the exact matching subset of string matching problems as opposed to regular expression matching. Their initial efforts in this area (Scarpazza, Villa, & Petrini, 2007) produced an implementation of the Aho Corasick algorithm which had a theoretical limit of 40.88 Gbit/s on a single Cell B/E processor. The algorithm was not, however, tested in a real-world scenario. A subsequent effort by this group (Scarpazza, Villa, & Petrini, 2008b) focuses on real-world applications involving much larger dictionaries, ranging from 6,000 to 20,000 queries. This implementation of the Aho Corasick

algorithm achieves a worst-case throughput of 2.5 Gbit/s using two Cell B/E processors. This group has also demonstrated an implementation of the Aho Corasick algorithm which is capable of searching up to 200 queries simultaneously with a throughput of 40 Gbit/s, representing the highest achieved search throughput in Cell B/E string matching literature (Scarpazza, Villa, & Petrini, 2008a). In addition, this group has explored regular expression matching on the Cell B/E platform (Scarpazza & Russell, 2009), achieving peak throughputs between 8 Gbit/s and 14 Gbit/s.

Robert Cameron's Parallel Bitstreams for XML (Parabix) string matching algorithm offers a radically different approach relative to Aho Corasick algorithms (Cameron, Herdy, & Lin, 2008). This algorithm transposes queries and databases into bitstreams, which can then be searched using bitwise operations. The Parabix algorithm has been implemented on the Cell B/E (Cameron, 2008), however it is not benchmarked in any real-world applications, making comparisons between the Parabix approach and Aho Corasick implementations difficult.

Due to the recent popularization of the CUDA platform for scientific computing on graphics processing units, computer science literature contains several GPU implementations of string matching algorithms. As with string matching on the Cell B/E, network intrusion detection and computational biology are leading applications for these algorithms. As a precursor study in GPU string matching, Kouzinopoulos and Margaritis implemented several exact string matching algorithms, including the naive, Boyer-Moore-Horspool, Knuth-Morris-Pratt, and Quick Search algorithms (Kouzinopoulos & Margaritis, 2009). When searching databases of 20 queries, these algorithms achieve throughputs between 5 Gbit/s and 15 Gbit/s using a GTX 280 graphics card. Of these algorithms, the Boyer-Moore-Horspool algorithm achieved the highest throughput.

Vasiliadis *et al.* have developed GNORT, an Aho Corasick implementation for the GPU which is optimized for network intrusion detection (Vasiliadis, Antonatos, Polychronakis, Markatos, & S. Ioannidis, 2008). This implementation is capable of searching up to 4000 queries simultaneously with a throughput of 1.5 Gbit/s using an 8600GT card. Vasiliadis *et al.* have also implemented the Boyer-Moore and the Knuth-Morris-Pratt algorithms on the GPU, achieving throughputs of approximately 1 Gbit/s and 100 Mbit/s respectively with a 100-query dictionary. These experiments were also performed on a 8600GT graphics card. This group has also made efforts in the area of regular expression matching for network intrusion detection on GPU (Vasiliadis, Polychronakis, Antonatos, Markatos, & Sotiris Ioannidis, 2009) achieving throughputs of approximately 16 Gbit/s while searching a dictionary of 20 regular expression queries.

Schatz and Trapnell have produced an implementation of the Aho Corasick algorithm which is tailored to computational biology applications (Schatz & Trapnell, 2007). This GPU implementation has achieved a speedup of 35x relative to an equivalent CPU implementation, however no absolute measures of performance are offered by the authors.

3 Exact String Matching for MS/MS Protein Identification using the Cell Broadband Engine

3.1 Introduction

This chapter details the development of a string matching algorithm on the Cell B/E architecture for use in the idMS/MS data analysis pipeline. The algorithm has been designed and implemented with the intention of fulfilling step 3 of the idMS/MS pipeline, as seen in Figure 1-8 of section Section 1.2. Thus, the algorithm is tailored toward searching short peptide sequence tags against large static proteomic databases.

This chapter is based on the journal article, "Exact String Matching for MS/MS Protein Identification using the Cell Broadband Engine" (Peace et al., 2011).

3.2 Existing String Searching Algorithms

As a first step toward building a string matching module for a idMS/MS system, we have implemented and optimized several state of the art string matching algorithms on the Cell B/E in order to determine the suitability of these algorithms for idMS/MS. We have implemented the *shifting substring* (naive), Boyer-Moore (Boyer & J. S. Moore, 1977), Rabin-Karp (Karp & Rabin, 1987), and Parabix (Cameron, Herdy, & Lin, 2008) string matching algorithms, adapting them for the Cell B/E's multicore architecture and applying advanced optimization techniques such as loop unrolling, application of SIMD vector operations, data parallelism, and multi-buffering techniques. Our implementations of the Boyer-Moore and Rabin-Karp algorithms are the first Cell B/E implementations of these algorithms, to our knowledge. Initial prototyping and implementation of these algorithms on the Cell B/E was performed

as part of a fourth year undergraduate research project, with substantial additional optimization, recoding, and performance analysis performed during the master's research period.

3.3 The Orthogonal Parabix Algorithm

As described previously, the Parabix approach to string matching, as developed by Rob Cameron, is able to improve search times by encoding the search database as a set of bitstreams. While the bitwise encoding scheme of the Parabix algorithm offers a significant speedup relative to byte encoded alternatives, the Parabix algorithm was originally designed for XML parsing, a domain in which modifications to the database must be performed online. Any time spent transposing the database into bitstreams results in a decrease in online search performance, and the algorithm is designed around this restraint. Genomic and proteomic databases, on the other hand, are updated only a small number of times per year. Preprocessing of these databases may be done offline without affecting search performance. With this in mind, the concept of parallel bitstreams was adapted in order to maximize the amount of offline pre-processing done while minimizing online processing. The resulting algorithm, called the orthogonal Parabix algorithm, is theoretically capable of searching one word of a database using only $2n - 2$ bitwise operations, where n is the length of the query. On a modern computer architecture, a word contains 64 bits, allowing for 64 character locations to be searched in this time. Typical peptide query lengths are between 4 and 8 characters, therefore this algorithm is theoretically capable of searching over 10 database locations per bitwise operation under typical searching conditions.

Figure 3-1 - Orthogonal encoding of a database segment into bitstreams A, D, and F. Red arrows indicate the encoding of "A" amino acids by the first "A" bitstream

The algorithm pre-processes the database into 20 orthogonal bitstreams, each representing a single amino acid. For each bitstream, the i th bit is high if position i in the database is occupied by the amino acid represented by that bitstream. Figure 3-1 demonstrates this orthogonal encoding for amino acid bitstreams A, D and F.

An orthogonally encoded database requires 20 bits of storage per location in the database, a 250% increase relative to an equivalent database using a byte-wise encoding scheme. While significant, this increase in space usage is comparable to an efficient suffix array encoding (Ferreira, Oliveira, & Figueiredo, 2009).

For a given peptide sequence tag query, only the bitstreams which represent query characters are required to be in memory at runtime. When presented with a query, the orthogonal Parabix algorithm brings into memory the query bitstreams in the order that they appear in the query. Each query bitstream is shifted to the left by i characters, where i is the index of the bitstream character within the query. With the bitstreams shifted in this manner, the bitstreams will all contain the value 1 at a position n iff the complete query is present starting at position n . Therefore, ANDing

the shifted bitstreams will produce an output bitstream which contains a 1 at all

Figure 3-2 - Online Shift-AND procedure for query "ARND" in database containing "...QWVARNDA...". Bitstreams A, R, N and D are fetched into memory in the order that they appear in the query.

positions where the query is present in the database. Figure 3-2 demonstrates the online SHIFT and AND procedure for the query ARND.

The total number of SHIFT operations required during the online procedure is $n-1$, and the total number of AND operations is also $n-1$, where n is the length of the query. Thus, for each word in the database, $2n - 2$ bitwise operations are needed.

3.4 Results

In order to determine the optimal string matching algorithm for hdMS/MS, optimized Cell B/E implementations of all previously detailed string matching algorithms were applied to a sample proteomic database containing a subset of the human proteome and the resulting search times were compared. Each of the tests were performed on a QS22 Cell B/E BladeCenter server with two PowerXCell 8i processors and 32 GB of RAM. Only one processor, totalling 8 SPEs, was used during our experiments because all algorithms were designed and tested on a simulated Cell system which contained 8 SPEs. The naive string matching algorithm appears twice in these results – with

and without the use of SIMD vector operations – in order to demonstrate the increase in performance achieved through optimization on the Cell B/E.

Figure 3-3 demonstrates that each of the algorithms, as implemented, scale linearly with proteome size. In addition, Figure 3-3 demonstrates that the Orthogonal Parabix algorithm is extremely well-suited to the exact string matching problem which idMS/MS presents – its runtime line is indistinguishable from the x-axis for most file sizes. When searching a 256Mb input file for a query of length four, the orthogonal Parabix approach achieves a sustained throughput of 215.4Gbps, representing a speedup of 19.6x over the Parabix approach and a speedup of 124.0x over a naive string searching implementation.

Figure 3-3 - Search times under varying database size

Figure 3-4 demonstrates the speedup which is obtained when data parallelism is employed to distribute the database searching task concurrently among multiple SPEs (256Mb database, 4 character query). For all algorithms, the speedup for n SPEs is approximately linear. By extension, the Orthogonal Parabix algorithm retains its speedup over other algorithms regardless of the number of SPEs used.

Figure 3-4 - Search times under varying SPE usage

3.5 Conclusions

We have successfully implemented, optimized and benchmarked several single exact string matching algorithms on the Cell B/E hardware. While these implementations of existing string searches performed well on the hardware, the highly specialized searches required by idMS/MS present an opportunity for a more tailored approach to string searching. In response to this opportunity, we have created an extension of the Parabix approach – called the orthogonal Parabix approach – which is ideal for the string searching problem presented by hdMS/MS.

Running on a Cell B/E blade server, the Orthogonal Parabix string matching approach was able to achieve a sustained search throughput of 215.4 Gbps under typical peptide fragment search parameters – a speedup of 124x relative to general purpose string searching algorithms. Considering that the size of the human proteome is roughly 0.6Gb and a typical mass spectrometer obtains one sample per second, this throughput suggests that the orthogonal Parabix approach running on the Cell B/E hardware is capable of searching proteomic databases within the real-time deadlines imposed by idMS/MS. However, subsequent research (see the

proceeding chapter) has demonstrated that GPU hardware is even better suited to parallel data analysis tasks involving large proteomic or genomic databases; as a result, we have concluded that further parallelization of idMS/MS data analysis would be pursued on the GPU platform which produces yet another significant speedup relative to the Cell B/E platform.

4 Six-Frame Genomic String Matching for Peptide Sequence Tags using the CUDA Platform

4.1 Introduction

This chapter describes the CUDA platform implementation of the orthogonal Parabix string searching algorithm. This algorithm fulfils the requirements of peptide sequence tag searching for idMS/MS, as highlighted in step 3 of Figure 1-8 in section 1.2. In addition to the implementation of the orthogonal Parabix algorithm on the GPU platform, the algorithm was improved significantly relative to the previous Cell B/E implementation. These improvements include the ability to search genomic in addition to proteomic databases, and improved handling of ambiguities which arise from *de novo* sequencing of product ion spectra. The throughput of the orthogonal Parabix algorithm is also improved by an order of magnitude relative to the Cell B/E implementation. Finally, the GPU platform will receive future support and new GPU hardware is developed regularly.

4.2 Adapting Orthogonal Parabix to Genomic Databases

In order to search a query peptide fragment against all proteins encoded by a species' genome, rather than the subset of proteins which have been previously discovered and annotated, it is imperative that our search algorithm be able to search DNA databases in all six reading frames (i.e. all 6 possible interpretations of the DNA sequence by the translation and transcription protein synthesis machinery within a cell).

The pre-processing steps of the orthogonal Parabix algorithm are capable of adapting from proteomic databases to six-frame genomic databases without impacting the online search throughput of the algorithm. The pre-processing of genomic databases is accomplished through a process which converts the genomic database into two sets of three interleaved proteomic databases in such a way that the three proteomic databases in each set can be searched using the SHIFT and AND procedure described above with no additional overhead.

The process begins by constructing the genetic complement of a given genomic database, then reversing this genetic complement database front to back. The original genomic database represents three of the reading frames for the genome, while the newly-built genetic reverse complement database represents the previously-unseen reading frames and is stored in a manner identical to the original genomic database.

Once the database representing the genetic reverse complement has been built, *in silico* translation and transcription are performed on all six frames of the genome.

Figure 4-1 - Converting a genomic database into a proteomic database

This *in silico* translation and transcription is performed in each location of the original and complemented databases, creating two proteomic databases for which the amino acid at location n of the proteomic database resulting from reading frame i is the result of the translation and transcription of the codon at the corresponding location in the corresponding genetic database. Figure 4-1 demonstrates the three steps used to convert a genomic database into proteomic databases. In this figure, each of the three colours red, blue and green correspond to the three reading frames present in the genomic database.

The proteomic databases are then converted into orthogonal bitstreams in the manner described in the previous section. These steps can all be performed during database pre-processing. At this point, the six reading frames could be separated into six databases, however this de-interleaving step is not necessary as the online orthogonal Parabix algorithm can search the interleaved database with no effect on performance relative to a database of proteomic origin which contains the same amount of amino acids. Thus, the database remains interleaved in order to reduce pre-processing requirements.

During the online portion of the algorithm, the amount which each bitstream is shifted is increased by a factor of three so that amino acids within the same reading frame are aligned with each other during the SHIFT and AND procedure. Increasing the shift length on each bitstream does not increase the computation time for a given query; the series of operations required for searching are identical for proteomic and genomic databases. No additional memory transfers are required relative to a database of the same size for which bitstreams are shifted by only one bit. Therefore, no searching overhead is introduced when bitstreams are built from genomic rather than proteomic databases.

4.3 GPU Implementation Details

In the GPU implementation of the orthogonal Parabix algorithm, an orthogonally encoded database is loaded from file into GPU global memory as an array of bitstreams so that all bitstreams are available in GPU memory prior to the determination of a query. In addition, an output bitstream of length equal to the database length is created in GPU global memory. All bits of the output bitstream are initialized to 0. GPU memory size represents a limitation on the size of the genomic database which can be searched at one time, however this limitation is not relevant when genomic databases are limited to genes which are protein-encoding. 1GB is a typical mid-range GPU memory size; using the previously-described encoding scheme, approximately 4×10^8 amino acids can be stored in GPU memory, which corresponds to a genomic database containing 2×10^8 base pairs. The human genome contains 2.91×10^9 base pairs, however only 1.1% of these base pairs could potentially encode proteins (Venter et al., 2001). All possible proteins encoded by the human genome, then, can be represented by 3.2×10^7 base pairs. This is well within the limits of any CUDA-capable GPU.

Once the host receives a query, this query is converted by the CPU into a query code - an array of integers, where the i th position of the array contains the bitstream index of the i th amino acid of the query sequence tag. That is, the integer at `querycode[i]` is the index of bitstream m in the array of bitstreams iff the query at i is represented by amino acid m . This query code is then transferred to the GPU global memory. This encoding of the query allows for direct indexing into the array of amino acid bitstreams, reducing overhead during searching.

Once data has been set up on the GPU's global memory, $\lceil n/w \rceil$ GPU kernel threads are launched by the host device, where n is the number of amino acid locations in each proteomic bitstream and w is the word size in bits (typically 64). Each of these kernel threads performs the SHIFT and AND series of operations on a single word of the bitstreams relating to the query. No data is moved explicitly into GPU shared memory by the kernel functions; optimal performance is achieved when the input and output bitstreams and the query are all stored in GPU global memory. In addition, using texture pipelines to access global memory does not increase search throughput. Figure 4-2 demonstrates the features of the CUDA memory hierarchy which were explored during implementation of the orthogonal Parabix algorithm.

Figure 4-2 - CUDA memory hierarchy. Performance is optimal when all data is stored in GPU global memory and the traditional memory pipeline is used.

Unfortunately, byte-addressable memory does not allow for the exact SHIFT and AND procedure as described previously. For query hits which straddle the boundaries of addressable memory, bitstreams for terminal amino acids in the query cannot be shifted leftward in order to align with the first amino acid in the query using the previously described SHIFT and AND procedure. For example, the query "ARND" which appears in consecutive words "VVVVVVAR" and "NDVVVVVV", the amino acids N and D cannot be aligned with the amino acids A and R using a simple leftward shift. Therefore, a more complex SHIFT and AND procedure was developed for the GPU kernel. For a query of length N , in order to search each position of word i of the

database, words i and $i+1$ are accessed in global memory for bitstreams relating to characters 2 through N of the query. The online SHIFT and AND procedure is then performed on the word tuples; when searching databases which are genetic in origin, word i of query character n is shifted to the left by $3n$, while word $i+1$ is shifted to the right by (word size - $3n$). When searching databases which are proteomic in origin, word i of query character n is shifted to the left by n while word $i+1$ is shifted to the right by (word size - n). All shifts are unsigned. Once shifted, the words at i and $i+1$ are then ORed together, producing a single word which can be ANDed with other bitstreams in the manner defined in the original SHIFT and AND procedure in order to produce a result bitstream. Figure 4-3 demonstrates the modification to the SHIFT and AND procedure which allows the GPU kernel to operate using word-

Figure 4-3 – Modification to SHIFT and AND procedure for handling byte-addressable memory on GPU

addressable memory. In this figure, 8-bit words are used for illustrative purposes, and the required word must be shifted to the left by 3 bits.

Initial implementations of the orthogonal Parabix algorithm on GPU wrote the output bitstream directly to global memory, then copied this bitstream from global memory to the CPU memory. This series of memory operations was costly, however, and profiling of this code showed that the real-time portion of the algorithm was memory-bound, as can be seen in Figure 4-4; 77% of the GPU run time of the

algorithm was spent transferring the output stream from GPU global memory to CPU memory, which appears in Figure 4-4 as memcpyDtoH (memory copy, device to host). For the purposes of peptide sequence tag searches, however, the level of precision provided by this complete output stream is not required. Knowing the location of the result to within one word is sufficient so long as this approximate location can be used to uniquely identify the protein in which the peptide sequence tag has been found. With this in mind, the output of the algorithm was modified such that the resulting bitstream was compressed by a factor of one word – each bit in the output bitstream represents the presence or absence of one or more search hits in one word of locations in the database. A second pass of the output bitstream can be performed on the host in order to determine the precise location of a match once all of the approximate matches have been returned. For a word size of 64 bits, then, the amount of data transferred from GPU global memory to CPU global memory is reduced by a factor of 64. This optimization is well suited to the GPU implementation of the orthogonal Parabix algorithm. Each kernel deals with one word of the database bitstreams, therefore each kernel is responsible for writing a single bit to the output stream as opposed to an entire word. In order to ensure that each result is uniquely mapped to a protein, the orthogonal bitstreams are built during pre-processing such that each protein is zero-padded to the nearest word boundary. For a typical database, the increase in database size caused by this zero padding is between 3% and 7%. With this optimization in place, the orthogonal Parabix algorithm becomes compute bound, with almost 93% of the GPU run time dedicated to computation. This optimization then has the dual effect of increasing the search throughput significantly and of allowing the algorithm to scale more successfully with GPU hardware performance as more processing power is introduced in future hardware releases. The move from memory-bound to compute-bound processing can be seen in Figure 4-4.

Figure 4-4 - Comparison of relative time spent on compute vs. memory operations before and after optimization of memory transfers. Green bars represent post-optimization numbers. "search" represents computation time, while "memcpyHtoD" and memcpyDtoH" represent time spent on memory transfers between host and device

4.4 Handling Ambiguity in Peptide Sequence Tags

Because of the nature of MS/MS spectra and the *de novo* process, peptide sequence tags often contain ambiguities which must be accounted for when these tags are searched against a database. The GPU implementation of the orthogonal Parabix algorithm has been adapted in order to handle typical forms of ambiguity with a minimum of false positives, expanding the number of valid queries which the algorithm can accept.

The first common form of ambiguous query is an *order-ambiguous query* in which two amino acids are known to be adjacent to one another at a certain position of the query but their relative positions are unknown – for example, a query could be ARSD or ASRD. This class of query is caused by the absence of a single peak in a spectrum; the resulting *de novo* sequencing determines that the distance between two peaks

corresponds to a unique pair of amino acids without knowing the amino acids' order within that pair.

This class of query can be represented by a series of bitwise operations which are based on the SHIFT and AND series of operations. Given an order-ambiguous query with characters R and S which occupy query positions i and $i+1$ in an unknown order, the modified search kernel performs the standard SHIFT and AND operations for all positions in the query except i and $i+1$. For positions i and $i+1$, the modified kernel implements the following bitwise procedure:

$$[(R \ll i) \& (S \ll i + 1)] \mid [(R \ll i + 1) \& (S \ll i)]$$

In this procedure, a bitstream for the sequence "RS" and a bitstream for the sequence "SR" are created independently. These bitstreams are then joined using an OR operation, resulting in a bitstream for which a 1 is present iff either "RS" or "SR" is present.

The second case of query ambiguity which is handled explicitly by a modification to the orthogonal Parabix algorithm is a query with an isobaric subsequence. Because the masses of certain amino acid sequences – such as GG and K – are indistinguishable when using most mass spectrometers, *de novo* sequencing often produces motifs in which two or more short sequences could be present at a specific location.

The orthogonal Parabix framework does not allow for a single query to represent both the GG and K motifs, due to the rigid structure of the SHIFT and AND procedure; any amino acids which occur downstream of the GG / K subsequence must be shifted to the left by a different amount depending on the presence of the GG or K

subsequence, and the SHIFT and AND procedure does not allow this flexibility. As a result, a query with GG / K ambiguity is launched as two separate queries, one of the form $aGgb$ and one of the form aKb . Launching two queries represents a significant decrease in performance relative to an unambiguous query – a slowdown of approximately 1.6x – however in this worst-case scenario the orthogonal Parabix algorithm is still preferable to alternative string searching methods.

4.5 Results

All GPU and serial experiments detailed in this section were performed on the following hardware:

- **Serial System:** Intel Core i7 920 processor at 2.67 GHz
 - 12GB triple channel DDR3 memory at 523 MHz
- **GTX260 System:** NVIDIA GeForce GTX 260 graphics card
 - 192 CUDA cores
 - 896 MB global memory
 - 576 MHz core frequency
 - 999 MHz memory frequency
- **GTX570 System:** NVIDIA GeForce GTX 570 graphics card
 - 480 CUDA cores
 - 1280 MB global memory
 - 742 MHz core frequency
 - 1900 MHz memory frequency

Each of the experiments wherein the database size is fixed was performed with a database size of 512Mb. This database size is comparable to the subsection of the human genome which is capable of encoding proteins, which is itself approximately 512Mb.

Typical peptide sequence tag lengths are in the range of 4 to 6 characters, with peptide sequence tags of length 7 to 8 characters occurring infrequently. Therefore, all experiments focussed on queries of length 4 through 8.

For each data point, the GPU search kernel was launched 1000 times using a single pre-determined query, and the average search time of the 1000 runs was recorded. Similarly, for serial implementation data points, the online portion of the algorithm was run 1000 times and the average search time was recorded. Orthogonal Parabix performance is independent of the query composition, that is, any query of length n with ambiguities in the same locations will produce the same results regardless of the amino acids present in the query.

Timing for kernel code was performed using the `cudaEvent` data type, and timing for serial code was performed using the `time()` command. The resolution of the `cudaEvent` method of timing is equivalent to the period of the GPU. The `time()` command's resolution is one second, however this low-resolution timing method combined with the number of runs performed is sufficient in order to provide a baseline by which the speedup of the parallel algorithm can be measured. Both the `cudaEvent` timing model and the `time()` command record wall times, therefore the OS or other processes may have affected the results of these experiments. All GPU and serial code was compiled using Microsoft v90 compiler and the Nvidia CUDA Toolkit version 3.2 and run on the Windows 7 platform.

Figure 4-5 - Effect of query size on search times on GPUs. Serial search times are not present in order to highlight the comparison between GPU data points.

Figure 4-6 - Effect of ambiguous queries on search times. Experiment performed on GTX 570 graphics card.

Figure 4-5 and Figure 4-6 demonstrate the effect of query length and complexity on search times for the orthogonal Parabix algorithm. Increase in query size causes a linear increase in search times. Order ambiguity, which is handled using a modification to the online search algorithm, causes a small fixed increase in search times. This matches the expectations for this modification to the search algorithm. Isobaric ambiguity increases search times by a fixed percentage which is approximately 60% of an unambiguous query search. Total search time is not doubled in the case of the GG/K ambiguity because only one result is transferred

from GPU global memory to CPU memory. In the worst case scenario – a query of length 8 with a GG/K class of ambiguity – the GTX 570 sustains a throughput of 911.4 Gbps, which is well within reasonable bounds for the purposes of information-driven MS/MS and preferable to a similar unambiguous search throughputs using other searching algorithms.

Figure 4-7 - Comparison of GPU and CPU search times over varying database sizes.

Figure 4-7 demonstrates the speedup achieved when comparing the GPU implementation of the orthogonal Parabix approach with an equivalent serial implementation which takes advantage of the memory output optimization described in section 4.3. When searching a genomic database of length 512Mb, the serial implementation of the orthogonal Parabix algorithm produces a search throughput of 19.7 Gbit/s. The GPU implementation on a GTX 260 graphics card produces a search throughput of 943.8 Gbit/s, providing a 48x speedup over the serial implementation. The GTX 570 graphics card, meanwhile, produces a search throughput of 2066.4 Gbit/s – a speedup of 104x over the serial implementation. When compared to

general purpose string matching algorithms as implemented on GPU, the orthogonal Parabix produces a 40x speedup over both Boyer-Moore and Aho-Corasick based approaches as demonstrated by (Vasiliadis, Antonatos, Polychronakis, Markatos, & S. Ioannidis, 2008); this result takes into account the relative compute performance of the graphics cards used, as determined by Nvidia.

4.6 Conclusions

The results presented in the previous section demonstrate that the GPU platform is well-suited to the development of a information-driven MS/MS system. This platform provides a vastly superior peptide search throughput relative to equivalent Cell B/E and Intel Core i7 implementations. When searching a genome of comparable length to the human genome under typical peptide search parameters, the GPGPU implementation of the orthogonal Parabix algorithm was able to achieve a sustained throughput of 2066.4 Gbit/s on a NVIDIA GeForce GTX 570 graphics card and 943.8 Gbit/s on a NVIDIA GeForce GTX 260 graphics card. Under comparable circumstances, our Cell B/E implementation sustains 219.3 Gbit/s and a serial implementation sustains only 19.7 Gbit/s on the Intel Core i7 processor. Relative to general string searching methods implemented on the GPU, the orthogonal Parabix approach produces a speedup of approximately 40x (Vasiliadis, Antonatos, Polychronakis, Markatos, & S. Ioannidis, 2008). Taking these results into account, we believe that the GPU platform is the ideal candidate for the parallelization of information-driven MS/MS analysis, and that the orthogonal Parabix string matching algorithm, as implemented, is an ideal string matching algorithm for the purposes of information-driven MS/MS.

5 On-line Retention Time / Experiment Time Mapping for Experimental Mass Spectrometry Data

5.1 Introduction

This chapter details the creation of an algorithm which maps the current experiment wall time of a LC-MS/MS experiment to the expected peptide retention time of the experiment. This algorithm is capable of performing this mapping in real-time, and thus estimates the current retention time of an experiment as the experiment proceeds. The algorithm uses data which is available to any real-time MS/MS data analysis system via the mass spectrometer. This retention time / experiment time mapping algorithm is a vital part of the idMS/MS pipeline, as it informs the idMS/MS system of which peptides are likely to elute during current and upcoming survey scans, increasing the idMS/MS system's ability to determine the likelihood that a survey scan peak matches a peptide which has been deemed 'important' and the system's ability to select peptides based on the knowledge of which peptides will still be available to the mass spectrometer in future iterations. This algorithm is also useful for directed LC-MS/MS experiments which are interested in specific peptides, as the algorithm allows these experiments to locate peptides temporally without *a priori* knowledge of the experiment LC gradient. Because no LC gradient information is required as input to this algorithm, the algorithm is resilient to errors which could arise from miscalibration or loss of calibration of LC instruments.

As a step toward the creation of the retention time / experiment time mapping algorithm, we have developed a novel ANN architecture which is able to accurately predict the retention time of peptides using a limited set of peptide sequence inputs.

5.2 Overview of Online Retention Time / Experiment Time Mapping Module

Figure 5-1 demonstrates the process flow diagram surrounding the online retention time / experiment time mapping module, detailing its interaction with the system as a whole. Note that the inputs and outputs of this module do not relate to the peptide sequence tag matching module described in chapters 3 and 4, as can be seen in section 1.2 of this thesis.

Prior to the experiment, the module requires the input of a proteome database file containing the amino acid sequence of all proteins which could possibly be present in the sample. Optionally, a genomic database file can be used instead; this DNA sequence file would require *in silico* 6-frame translation as described in the previous chapter. The sequence database file is parsed offline; all proteins undergo *in silico* tryptic digestion in order to produce a list of expected peptides for the experiment. The tryptic digestion step accounts for the possibility of a single missed cleavage at all cleavage sites. This is labelled as step 1 in Figure 5-1. The mass and predicted retention time are calculated for each peptide, as seen in step 2 of Figure 5-1, resulting in a database of mass/time points which each correspond to a unique peptide from the fasta database file. This AMT database is queried by the retention time / experiment time mapping module during the experiment at each iteration of the algorithm.

The module receives two inputs during its real-time operation: a list of masses which are currently inside the mass spectrometer with accompanying intensity values and charge states, and the experiment time (in seconds) at which these masses were recorded. Low intensity masses are removed from the mass list because they are

likely to be statistical noise, and masses with related charges which are not 2+ or 3+ are removed from the mass list because ions outside of this charge range are unlikely to be peptides.

The output of the module is a single real value in the range of 0 to 1. This value represents the retention time which best matches the experimental data which has been recorded by the instrument. This value will be used by the idMS/MS system in order to predict the presence of important peptides in the mass spectrometer, and as a factor in the peptide-importance scoring module.

Figure 5-1 - Process flow diagram surrounding the retention time / experiment time mapping module

5.3 Peptide Retention Time Prediction

Retention time prediction is a machine learning problem which aims to predict the chromatographic conditions at which a specific peptide will elute during a LC-MS/MS experiment. All inputs to the retention time prediction model must be derived from the sequence of the protein as provided by the fasta database file.

5.3.1 Choosing a Linear Regression Technique for Retention Time Prediction

As seen in section 2.3, retention time prediction literature defines two major approaches to retention time prediction: support vector regression techniques using inputs based on quantitative structure-retention relationship, and artificial neural network techniques using peptide structure information as inputs. The two techniques differ largely with respect to the training sets which they employ; SVR-based retention time prediction techniques have been optimized for training on small, instrument-specific training sets while ANN-based retention time prediction techniques have been optimized for training on large data sets which are built from many experimental data points across varying instruments. The retention time prediction model used for the retention time / experiment time mapping module must be applicable for a variety of instruments. In addition, a retention time prediction module which is not instrument-specific removes the requirement that the system be trained on an instrument-specific data set, removing the need for user input; the ability of the system to perform its function without user input is one of the key features of the retention time / experiment mapping module. Petritis *et al.* have made available a data set of over 383,000 peptide sequences with retention

time values for the purpose of training and testing retention time prediction approaches, making large-scale non-specific retention time prediction a more attractive option. For these reasons, we have decided to implement an ANN model using peptide sequence information.

5.3.2 Implementation of existing ANN models

The current state of the art for retention time prediction using ANNs is a structure developed by Petritis *et al.* using 1052 inputs, 1050 of which orthogonally encode the sequence of the peptide. Details of the orthogonal encoding can be found at (Petritis *et al.*, 2006). The two remaining inputs represent the sequence length and the hydrophobic moment (defined below) of the peptide. Because Petritis *et al.* do not provide a trained ANN model, we have attempted to produce a model using the popular Fast Artificial Neural Network (<http://leenissen.dk/fann/wp/>) and Annie (annie.sourceforge.net/) libraries. However, because of the large number of inputs combined with the large number of training instances, these libraries were not able to successfully train a model under MS Windows memory limitations. Attempts were made to train the 1052-input model using recompiled versions of these libraries which circumvent MS Windows memory limitations; however these models failed to generalize to non-training points. Thus, we have decided to develop a novel ANN model using a lower number of inputs.

5.3.3 Producing a novel ANN architecture

In order to produce an optimal ANN architecture using a small number of inputs, we have tested the inclusion of several popular ANN inputs into a novel ANN model. All models described in this section were trained on a set of 381539 peptides, and tested on a set of 1917 peptides – every 200th peptide was chosen for the test set,

after the peptides were ordered alphabetically, in order to produce a test set which is representative of a range of peptides. Computational requirements prevented the use of more in depth testing methods such as cross validation. The metric used to determine the success of an ANN model was test set mean squared error (MSE). Both hidden and output layers of the neural network employ sigmoidal activation functions, and all test ANNs were trained for 1000 epochs using the resilient backpropagation training algorithm, an adaptive variation of the backpropagation algorithm which sets the learning rate parameter at each epoch based on current learning success (Riedmiller, 1994).

Amino acid frequencies

A proven alternative to the orthogonal encoding used by the 1052-input model is a 20-input model which encodes the frequency of each of the 20 amino acids present in the peptide. This encoding of the peptide structure is the basis for our ANN model. The training set provided to us makes a distinction between alkylated cysteine and unmodified cysteine. Our model, therefore, makes the same distinction, increasing the number of inputs to 21. This 21-input model produces a MSE of 0.001553.

Shinoda et al. have suggested a variation of this model which eliminates four inputs, relating to four amino acids which have a negligible effect on peptide hydrophobicity (Shinoda et al., 2006). However, our testing has shown that a 17-input model which removes hydrophobically neutral amino acids produces a MSE of 0.001741, higher than the 21-input model. Therefore, we have chosen the 21-input peptide encoding scheme as the basis for our ANN model.

Hydrophobic Moment

The hydrophobic moment of a peptide is a function of the secondary structure of a peptide, resulting from helical structures which contain hydrophobic or hydrophilic amino acids on their surfaces. This property was discovered by Eisenberg *et al.* (Eisenberg, Schwarz, Komaromy, & Wall, 1984), who define hydrophobic moment as follows:

$$\mu_H = \left\{ \left[\sum_{n=1}^N H_n \sin(2n\pi/3.6) \right]^2 + \left[\sum_{n=1}^N H_n \cos(2n\pi/3.6) \right]^2 \right\}^{1/2}$$

Where n indexes the amino acids in the peptide, and H_n is a constant which has been derived experimentally for each amino acid. Petritis *et al.* utilize the hydrophobic moment in their 1052-input model.

When added to the 17-input model, the hydrophobic moment reduces MSE on the test set from 0.001741 to 0.001450. Because the inclusion of hydrophobic moment reduces MSE on the test set, hydrophobic moment has been included in our final ANN model.

Peptide Length and Mass

In addition to peptide encoding and hydrophobic moment, Petritis *et al.* have incorporated peptide length into the state of the art 1052-input model. Thus, we have tested a model using both hydrophobic moment and length in addition to the 21-input peptide encoding described previously. This model produced a test set MSE of 0.001361. As an alternative to peptide length, we have also explored the use of peptide mass, as peptide mass is a more accurate indicator of peptide size. A model containing the 21-input peptide encoding, hydrophobic moment and peptide mass

produced a test set MSE of 0.001338. Therefore, we have chosen to use peptide mass as an ANN input instead of the peptide length as used in previous papers.

Orthogonal Encoding of Terminal Amino Acids

Peptide retention time depends largely on the identity of amino acids at the N- and C- terminal locations of the peptide. Therefore, while it may not be feasible to orthogonally encode all amino acids within the entire peptides, it could be useful to orthogonally encode the terminal amino acids. We have explored this option, first by encoding the peptide at the C-terminus. Note that because the peptide was the product of tryptic digestion, only amino acids arginine or lysine can be present in this location, so only two inputs were used to encode this information. Encoding the C-terminal amino acid did not, however, improve the test set MSE of the model; the MSE using these inputs was 0.001355. Similarly, encoding the N-terminal amino acid produced a test set MSE of 0.001349, which is not an improvement over a model that does not orthogonally encode any terminal amino acids. Therefore, we have chosen not to include these inputs in our model.

Number of Hidden Nodes

Two of the defining features of any ANN model are the number of hidden layers in the architecture and the number of hidden nodes per layer. Because all previous successful ANN implementations for retention time prediction have used a single hidden layer (Shinoda et al., 2006) (Petritis et al., 2006) (Petritis et al., 2003), we have decided to use a single layer in our model. We have tested a range of hidden node numbers using the optimal 23-input configuration, and found that 10 hidden nodes appears to be suitable, as can be seen in Figure 5-2; beyond 10 hidden nodes, it appears as though any deviation in test set MSE is the result of statistical noise. This number of hidden nodes is significantly higher than previous successful ANN

architectures with similar numbers of inputs. Repetition of this experiment would eliminate the effects of noise in the test results and produce a more statistically robust answer to the question of the optimal number of hidden nodes in the architecture, however, training a single ANN model on a large dataset requires several hours of computation. Time constraints prevent repetitions of experiments from being performed.

Figure 5-2 - Test set MSE for varying numbers of hidden nodes in ANN architecture. MSE is optimal for 14 hidden nodes, however there is no clear downward trend past 10 nodes.

Number of Training Epochs

As a final step in optimizing the accuracy of our ANN architecture, we have varied the number of epochs for which the ANN is trained; an ideal number of epochs used during training balances under- and over-fitting to the training set data. Figure 5-3 demonstrates that 900 epochs is a suitable training length for our model; increasing the number of epochs beyond 900 does not have a consistently positive effect. As with the optimization of number of hidden nodes, computational requirements and time constraints prevent repetition of experiments.

Figure 5-3 - Test set MSE over varying training lengths. Training beyond 900 epochs does not appear to produce beneficial results

Results of detailed ANN model testing can be seen in section 5.5.1.

5.4 The Retention Time / Experiment Time Mapping Algorithm

As mentioned previously, the objective of the retention time / experiment time mapping module is to produce a retention time value which represents the most likely current retention time value given the evidence that we have seen from the mass spectrometer up to this point. Evidence is provided by matches between the masses of AMT database points and the masses of peptides which are currently eluting from the liquid chromatography column, as measured by the most recent MS survey scan.

5.4.1 Producing a Probability Density Function

We have employed a Parzen Window technique, which aims to estimate a random variable based on a sum of kernel functions, in order to produce a probability density function in the space of the AMT database. If we observe the AMT mass/time plot as a 2-dimensional probability space, then a sum of kernel functions, each of which is centered on a mass/time pairing from the AMT database, produces such a probability

density function (PDF). Given a mass which has been produced by the mass spectrometer and an arbitrary time value, this PDF estimates the likelihood that this observed mass/time pair relates to any known peptide in the AMT database. In other words, for a given experimental mass, we can determine the extent to which this mass supports the hypothesis "We are currently at retention time t " for all times in the range $0 \leq t \leq 1$. The amount of support for each time that this experimental mass provides can be pictured as a one-dimensional PDF, as seen in Figure 5-4.

Figure 5-4 - Creation of kernel function from 2D AMT database. A peptide mass m , represented by the blue line, was observed in the mass spectrometer. The resulting probability function is the result of kernel functions centered around the three AMT database points which have a mass close to m .

We have chosen as the kernel function K_p a Gaussian function of the form.

$$K_p(m, t) = e^{-\frac{(m-m_p)^2}{2\sigma_m^2} - \frac{(t-t_p)^2}{2\sigma_t^2}}$$

Where:

- m is the observed experimental mass
- m_p is the AMT database mass of the peptide at the center of the kernel
- σ_m is a mass standard deviation, which is determined by the accuracy of the mass spectrometry hardware
- σ_t is the time standard deviation, which is a function of the accuracy of the retention time prediction model
- t is the experimental time
- t_p is the AMT database time of the peptide at the center of the kernel

The Gaussian function accurately models a mass spectrometer which produces an unbiased estimate of mass, and for which m and t are independent. Because this is not the case for all mass spectrometers, alternate kernel functions may be required in order to produce correct results on all hardware. All hardware used for testing fall into the former category, therefore testing of alternate kernel functions for hardware which produces biased mass results was not performed. This kernel function will perform adequately for almost all mass spectrometry hardware, and the algorithm described in this section can be adapted to alternate kernel functions trivially.

With a 2-dimensional probability density function established, it is possible to produce a value which, for a list of experimental mass values, represents the most likely retention time value for the currently observed data.

For each retention time value t , $0 \leq t \leq 1$, the amount of support S for t being the correct retention time value, given a database with P known mass-time pairs,

and a list of L currently observed experimental masses, can be calculated using the following formula:

$$S(t) = \sum_{l=1}^L \sum_{p=1}^P K_p(m_l, t)$$

The retention time value which corresponds to the maximum of this support function is the retention time value which is most likely to correspond to the current experimental time.

Figure 5-5 – Production of a support function from multiple recorded masses. Masses m and n , was observed in the mass spectrometer. The resulting support function, seen in purple, is the sum of the blue and red functions. A maximum of the function appears where evidence is provided by both recorded masses.

Because the support function relies only on AMT database information which is known prior to an experiment, the support function could be pre-computed in a fine-grained mesh, removing the need to repeatedly compute exponential functions during real-time operation. Given this optimization, the retention time / experiment time mapping algorithm is not expected to require a significant portion of the processing time available between real-time deadlines.

5.4.2 Finding the Global Maximum of the Support Function

The preceding section of this thesis presents a function whose maximum represents the most likely retention time value relating to the current time in a mass spectrometry experiment. Calculating this maximum, however, is non-trivial. There is no computational method which can directly calculate the global maximum of a polymodal function, and function estimation methods are computationally expensive. Because of these factors, we have explored several techniques for finding the maximum of the support function, $S(t)$.

5.4.2.1 Naive Scanning Approach

As a baseline approach for finding the maximum of the support function, we have implemented a naive scanning algorithm for the function. This algorithm calculates the value of the support function for all t values in a finite set, then returns the maximum value among those calculated. Our implementation of this algorithm uses the set

$$\{t \mid t = 0.0001n, 0 \leq t \leq 1, n \in \mathbb{I}\}$$

While this algorithm returns a value which is only an estimation of the true location of the maximum of the support function, this approach is attractive because it is highly parallelizable. The result at each time t is completely independent of all other

results, so each of these results can be calculated in parallel, resulting in significant speedup.

5.4.2.2 Golden Section Search

The golden section search (Kiefer, 1953) is an iterative method which is capable of estimating the maximum or minimum of a unimodal function by narrowing the range in which this maximum can be found by a set percentage at each iteration. The range in which the maximum could exist is defined by three points along the function, $f(t_1)$, $f(t_2)$ and $f(t_3)$. $f(t_1)$ and $f(t_3)$ represent the boundary points of this range of the function, while $f(t_2)$ is a point which is in between $f(t_1)$ and $f(t_3)$. At each iteration of the algorithm, a point $f(t_4)$ is chosen in between $f(t_2)$ and either $f(t_1)$ or $f(t_3)$ – whichever is farther from $f(t_2)$. If the function is higher at $f(t_4)$ than at $f(t_2)$, then the new range in which the maximum resides is defined by points $f(t_2)$, $f(t_4)$, and whichever of $f(t_1)$ and $f(t_3)$ are farther from $f(t_2)$. If the function at point $f(t_4)$ is lower than $f(t_2)$, then the maximum resides in a range which is defined by points $f(t_2)$, $f(t_4)$, and whichever of $f(t_1)$ and $f(t_3)$ are closer to $f(t_2)$ along the time axis. By choosing point $f(t_4)$ such that $t_4 = t_1 - t_2 + t_3$, the algorithm is guaranteed to reduce the range by a factor of $1 - 1/1.618033$ or approximately 38%. Given the total range of $0 \leq t \leq 1$, this algorithm is capable of approximating t to a precision of ± 0.001 within 14 iterations.

Because the support function, $S(t)$, is expected to be polymodal, it is necessary to provide initial boundaries for the golden section search which divide $S(t)$ into a series of sections which are unimodal, or unimodal for the majority of the time range. In order to provide the algorithm with near-unimodal sections of the support function, the support function has been split into initial ranges which are defined on one side by the time of an AMT database point, and on the other side by the midpoint

between the time of the AMT database point and the time of the previous or next AMT database point, assuming all AMT database points are ordered by time. The sum of two Gaussian curves, such as those produced by adjacent AMT database points, will have at most one local minimum. If the two Gaussian curves are approximately equal in terms of height, the minimum will occur between the two centers of the Gaussians as seen in Figure 5-5 and the golden section search algorithm will perform well for both ranges.

Figure 5-5 - Golden section search ranges (pictured as vertical lines) relating to the sum of two gaussian curves of equal magnitude. The support function, in blue, would be seen as unimodal by the golden section search algorithm for each of the two sections of interest.

If one Gaussian is significantly larger than the other, then the local minimum may occur in a position which causes one golden section search to produce an erroneous result. However, if this is the case, then the maximum within the non-unimodal range will be dwarfed by the maximum in the adjacent unimodal range, so the algorithm as a whole (which aims to produce a global maximum of the function) will not produce an erroneous result. A demonstration of this effect can be seen in Figure 5-6.

Figure 5-6 - Golden section search over Gaussians of different amplitudes. The left region is clearly polymodal, and the golden section search may fail in this region, however the right section contains the global maximum and the golden section search will succeed in this region. Boundaries for the search are marked with vertical lines, and the support function is drawn in blue.

By performing several golden section searches over the ranges previously specified, we can be assured that the algorithm will produce an optimal result.

While theoretically more accurate than the naive scanning algorithm, the golden section search algorithm is not as parallelizable. For a golden section search with N iterations, the support function must be calculated in serial at $N+2$ points. Golden section searches over different sections of $S(t)$ are independent of each other, so parallelization can occur across searches, however the ideal implementation of a golden section search for our purposes will result in a $N+2$ -times slow down relative to an ideal implementation of the naive scanning algorithm. For our purposes, this represents an order of magnitude slowdown relative to the scanning approach.

5.4.2.3 Gradient Ascent Search

The gradient ascent search algorithm is similar to the golden section search algorithm, in that it is capable of finding an approximation to the maximum or minimum of any unimodal function. Each gradient ascent search is initialized with a

time value which serves as a best estimate of the maximum of the function. The slope of the function is then calculated at this time value, and this slope multiplied by a small constant γ (i.e. step size or learning rate) is added to the initial best-estimate time. This process is repeated for a finite number of iterations, eventually settling on a local maximum where the slope is near-zero and thus no change in the estimate occurs.

As with golden section searches, gradient ascent searches are only able to find the maximums of unimodal functions. Therefore, multiple gradient ascent searches must be launched in order to assure that the global maximum of the polymodal support function is found. In performing gradient ascent searches on the support function, we have chosen as initial values the time values of each of the AMT database points. Given the interactions of Gaussians when summed as described in the previous section, these starting points ensure that the global maximum of the function will be determined.

As with the golden section search, the gradient ascent search requires sequential calculations of support function values for each gradient ascent, however multiple gradient ascents are independent and parallelizable. For the parameters used in our experiments – $\gamma=0.0001$ and 1000 iterations – each gradient ascent would require $1000+1$ sequential support function calculations and would therefore be slower than the naive scanning algorithm by a factor of up to 1001. Early stopping conditions for the algorithm can mitigate this high number of serial calculations, however the worst case scenario is still significantly slower than the naive scanning algorithm for a parallel system.

5.4.3 Experimental Setup

The retention time / experiment time mapping algorithm has been tested on a LC-MS/MS run of a "protmix" sample containing 7 proteins. The LC for the experiment has been set for a linear gradient which extends for 30 minutes, using a linear interpolation between the following points: 2% at 0 min, 5% at 0.5 min, 18% at 5 min, 30% at 16 min, 70% at 25 min, 100% at 25.1 min, 100% at 28 min, 2% at 28.1 min, 2% at 30 min. The retention times of the 9 most confidently identified peptides were predicted using our ANN model. Confidence of peptide identification was determined by the Mascot data analysis suite (<http://www.matrixscience.com/>). The predicted retention time values for these 9 peptides were then compared against the estimated retention times for survey scans during which the peptides were present in the mass spectrometer. Thus, the algorithm was tested on its ability to correctly predict the elution of important peptides. The average mean squared error between these calculated and predicted values is used as a measure of success for the retention time / experiment time mapping algorithm.

5.5 Results and Discussion

5.5.1 ANN Retention Time Estimation Model Accuracy

In order to compare our ANN retention time estimation model to existing models, we have examined the R^2 value of the model outputs to corresponding retention times of the 1917 peptides in the test set described above. Using our 23-input model, we have achieved a R^2 value of 0.9483. This value is below the value achieved by the 1052-input model proposed by Petritis *et al.* (0.967), but is significantly higher than both the 16-input model proposed by Shinoda *et al.* (0.928) and the 20-input model proposed by Petritis *et al.* (0.87) upon which this model is based. Figure 5-7 demonstrates the plot upon which this value has been derived. The increase in

accuracy of this model is largely due to the inclusion of the hydrophobic moment ANN input.

A summary of mean squared error values for ANN input configurations described in this paper can be seen in the proceeding table:

Table 5-1 - Test MSE values for ANN input configurations considered during development of final ANN model

ANN input configuration	MSE
21 AA frequencies	0.001553
17 AA frequencies	0.001741
17 AA frequencies + hydrophobic moment	0.001450
21 AA frequencies + hydrophobic moment + peptide length	0.001361
21 AA frequencies + hydrophobic moment + peptide mass	0.001338

Note that different test and training sets have been used for each of the previously reported experiments and this current experiment. The results of these experiments are not a direct comparison, however the increase in accuracy over existing low-input models is significant regardless of differences in experimental methods.

Figure 5-7 - Plot of predicted retention time of ANN model vs. actual retention time

5.5.2 Retention Time / Experiment time Mapping Accuracy

Using the scanning method of calculating the maximum of the support function, we have predicted the elution times of 9 high-confidence peptides with a mean squared error of 0.00627. All three of the methods used for determining the maximum of the function produced a MSE which is within 0.00001 of this value, which indicates that the three methods produce the same results to within a small margin of error. Tables 5-2 through 5-4, below, display the results of these tests. When calculating the retention time value of the experiment based on the elution time and the LC gradient information provided in section 5.4.3, we see a MSE of 0.00671. Therefore, our methods more accurately predict the retention time of the experiment during elution of these 9 peptides.

Because each of the three methods performed extremely similarly during our tests, the high simplicity and parallelizability of the naive scanning methods makes it an ideal candidate for determining the maximum of the support function.

Table 5-2 - Test results for the naive scanning method for retention time / experiment time prediction

Peptide Sequence	Actual retention time	Estimated retention time	Error ²
LLGLFPDAN	0.38823	0.372487	0.000247842
VGLSASTGLYK	0.440501	0.410982	0.000871371
DLILQGDATTGTDGNLELTR	0.28547	0.416382	0.017137952
SPDSHPADGIAFFISNIDSSIPSGSTGR	0.315807	0.470375	0.023891267
LGEYGFQNAILVR	0.36881	0.328293	0.001641627
GLSDGEWQQVLNVWGK	0.35261	0.407583	0.003022031
VNVDEVGGEALGR	0.258118	0.300397	0.001787514
DILNQITKPNDVYSFSLASR	0.434742	0.347091	0.007682703
QTALVELLK	0.44668	0.432879	0.000190468
			MSE:
			0.00627475

Table 5-3 - Test results for the golden section search method for retention time / experiment time prediction

Peptide	Actual retention time	Estimated retention time	Error ²
LLGLFPDAN	0.38823	0.372503	0.000247339
VGLSASTGLYK	0.440501	0.411055	0.000867067
DLILQGDATTGTDGNLELTR	0.28547	0.416406	0.017144236
SPDSHPADGIAFFISNIDSSIPSGSTGR	0.315807	0.470408	0.023901469
LGEYGFQNAILVR	0.36881	0.328223	0.001647305
GLSDGEWQQVLNVWGK	0.35261	0.407653	0.003029732
VNVDEVGGEALGR	0.258118	0.300481	0.001794624
DILNQITKPNDVYSFSLASR	0.434742	0.347123	0.007677094
QTALVELLK	0.44668	0.432887	0.000190247
			MSE: 0.00627767

Table 5-4 - Test results for the gradient ascent search method for retention time / experiment time prediction

Peptide	Actual retention time	Estimated retention time	Error ²
LLGLFPDAN	0.38823	0.372474	0.000248252
VGLSASTGLYK	0.440501	0.411023	0.000868952
DLILQGDAITGTDGNLELTR	0.28547	0.416427	0.017149736
SPDSHPADGIAFFISNIDSSIPSGSTGR	0.315807	0.470373	0.023890648
LGEYGFQNAILVR	0.36881	0.328294	0.001641546
GLSDGEWQQVLNVWGK	0.35261	0.407624	0.00302654
VNVDEVGGEALGR	0.258118	0.300534	0.001799117
DILNQITKPNDVYSFSLASR	0.434742	0.347133	0.007675342
QTALVELLK	0.44668	0.432883	0.000190357
			MSE: 0.00627672

6 Thesis Summary and Future Recommendations

6.1 Conclusions

In exploring the problem of peptide sequence tag matching on parallel hardware, we have developed the orthogonal Parabix algorithm, a novel extension of the Parabix algorithm which is extremely well-suited to searching peptide sequence tags against large proteomic or genomic databases. This algorithm, as implemented on both the Cell B/E and GPU platforms, outperforms all existing string matching algorithms under typical searches required by idMS/MS. The orthogonal Parabix algorithm has sustained search throughputs of 2066 Gbit/s on a Nvidia GTX 570 graphics card, 943 Gbit/s on a Nvidia GTX 260 graphics card, and 216 Gbit/s on a Cell B/E QS22 blade server. The orthogonal Parabix algorithm is capable of searching both six-frame genomic databases and proteomic databases at these throughputs, and is capable of handling specific query ambiguities which arise from *de novo* sequencing of product ion spectra. Given the success of implementing the orthogonal Parabix algorithm on commodity parallel hardware, we believe that these platforms are suitable for meeting the deadlines of real-time MS/MS data analysis; the GPU platform in particular appears to be well suited to this type of data-intensive computation.

We have also developed an algorithm which maps experiment wall time to retention time as the experiment occurs through the novel use of statistical methods. This algorithm is the first of its kind, as prior to the inception of idMS/MS, data analysis was performed strictly offline after the experiment. This algorithm does not require input from the user prior to or during the experiment and can map experiment time to arbitrary elution curves, which opens up the possibility of directed MS/MS studies without prior knowledge or calibration of instrument elution curves. As a step in the

creation of the retention time / experiment time mapping algorithm, we have developed a novel ANN topology which predicts retention times of peptides with a R^2 value of 0.9483 using only 23 input nodes and 10 hidden nodes. The retention time / experiment time mapping algorithm was able to successfully predict the retention time of an experiment during the elution of nine confidently identified peptides during a real LC-MS/MS experiment, resulting in a MSE of 0.00627 between predicted current retention time and retention time of identified peptides. The algorithm used only data available to the mass spectrometer during an experiment.

6.2 Summary of contributions

In this thesis, we have thoroughly explored the problem of peptide sequence tag matching against large genomic and proteomic databases. We demonstrated the first implementation of several well-established string matching algorithms on the Cell B/E, and introduced the orthogonal Parabix algorithm, a string matching algorithm which is highly tailored to mass spectrometry data analysis. This algorithm outperforms all competing algorithms by an order of magnitude when searches are performed under typical peptide sequence tag search conditions. The orthogonal Parabix algorithm was implemented on the Cell B/E and GPU platforms, is capable of searching six-frame genomic databases without any effect on search throughput, and accurately models query ambiguities which arise from *de novo* sequencing of product ion spectra.

We have also developed an algorithm which maps experiment wall time to retention time as the experiment is performed. This algorithm extends the idea of accurate mass/time databases as probability spaces by employing parzen window techniques combined with function maximization in order to determine the retention time which has the highest probability of occurring based on the precursor ion masses which are

currently being seen in the mass spectrometer. The algorithm also makes use of a novel ANN topology which produces accurate retention time predictions for peptides with a relatively small number of nodes. Our retention time / experiment time mapping algorithm is the first algorithm which is capable of mapping retention time to wall time as the experiment proceeds, which increases the capabilities of directed MS/MS experiments while enabling the idMS/MS system.

6.3 Recommendations for future work

As this thesis represents the first steps toward a complete idMS/MS system, there is a number of future steps which must be taken in order to implement the idMS/MS pipeline in its entirety. First and foremost, an interface must be developed which allows the idMS/MS system to communicate with mass spectrometry hardware. This interface will most likely be developed using modifications to existing middleware applications such as Applied Biosystems' AnalystQS software. The creation of this interface would define the information which we receive and transmit to the mass spectrometry hardware during the experiment, and as such would determine the functional and non-functional requirements of any idMS/MS system.

Another key step in the creation of the idMS/MS pipeline is the development of a parallel *de novo* sequencing algorithm. *De novo* sequencing is a computationally intensive but highly parallelizable task which is a critical step in the pipeline and must be performed within real-time deadlines. As a possible alternative to *de novo* sequencing, spectral database searching could be used in order to determine peptide information present in a product ion spectrum.

A final and important element of the idMS/MS pipeline which must be implemented is a module which scores precursor ions based on the likelihood that these ions will be

important to the experiment. One possible implementation of this module is an information theoretic framework; this framework would receive as inputs lists of ambiguously identified and confidently identified proteins, peak masses and intensities from the most recent MS survey scan, current retention time, and peptide retention times. Using these factors, the framework would score all peaks from the most recent MS survey scan based on the amount which they would reduce entropy within the probability space of unidentified proteins.

Within the retention time / experiment time mapping algorithm, there are several logical next steps which could be performed. Iterations of the algorithm must be performed within the real-time deadlines of mass spectrometry hardware, therefore elements of the algorithm must be parallelized. Obvious candidates for parallelization are the three algorithms which determine the global maximum of the support function. It is also possible that interpolation or smoothing techniques which incorporate data from several survey scans into a single retention time prediction value would improve the accuracy of retention time predictions. For example, simple linear regression of the most recent n points would likely produce a result that is much more resilient to distortion caused by a single data point which is not representative of the retention time trend.

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