A Top-Down Driven Approach to *De Novo* Sequencing of Proteins

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Overview

In this work, we introduce the concept of a *T*-Bruijn graph and propose a fast and reliable approach, named *Twister*, to *de novo* sequencing of proteins from combined sets of top-down and bottom-up mass spectra using such graphs.

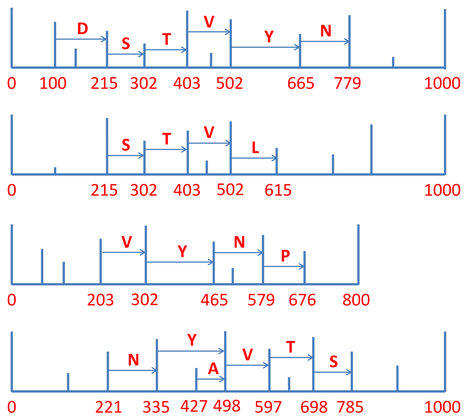
Introduction

Mass spectrometry is the most powerful technology for protein identification and characterization. The bottom-up strategy, which analyses peptides resulting from enzymatic digestion, and the recently emerged top-down strategy, which analyses intact proteins, are often viewed as complementary; however, when they are applied together, top-down techniques usually play a secondary role of a validation tool.

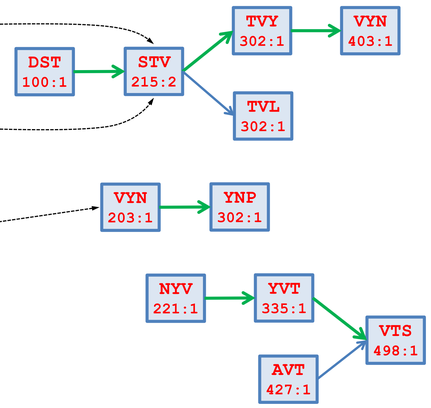
We describe a novel approach to *de novo* sequencing of proteins from combined sets of top-down and bottom-up tandem mass spectra. Thereby we grant the top-down data a leading role, and retrieve from it a number of seed fragments of the protein sequence, which then iteratively get extended, corrected, and whenever possible, merged with the aid of the information derived from the bottom-up dataset. In addition, the bottom-up spectra are handled in much the same way as the top-down ones. Finally, we point out that in case of contaminated samples or protein mixtures, our method outputs sequence fragments for various compounds, thus providing extra opportunities for analyzing a sample even in the absence of *a priori* knowledge on its composition. Our algorithms are implemented in a software tool *Twister*.

Methods

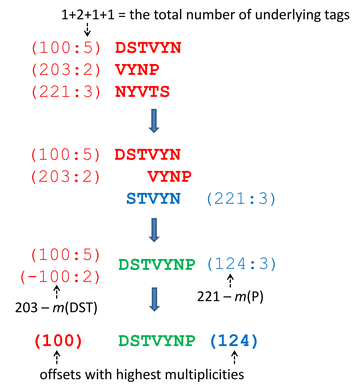
1) Tag generation



2) *T*-Bruijn graph construction and optimal paths extraction



3) Aggregated paths formation



$PM_{DSTVYNP}$ = **100** + *m*(**DSTVYNP**) + **124** = 1000: precursor mass estimate

When processing bottom-up mass spectra, we only compute optimal paths in a *T*-Bruijn graph and derive the amino acid strings they spell out. Those strings are subsequently used to validate, correct and extend the aggregated paths generated from the top-down data, to which end the former are appropriately aligned against the latter.

Results

Benchmarking: we tested *Twister* on a combined dataset for the alemtuzumab light chain.

Top-down experiments: a mixture of two antibodies, alemtuzumab (MabCampath) and adalimumab (Humira), was analyzed using an RPLC system coupled to an LTQ Orbitrap Velos with ETD and a Q-Exactive with CID or HCD, and 1523 ETD, 1282 CID and 1720 HCD spectra were acquired. Next, we applied the tool MS-Align+ to find the best protein-spectrum-match for each spectrum and the heavy and light chain of either antibody, and separated the 1,840 spectra assigned to alemtuzumab light chain into the top-down dataset.

Bottom-up experiments: alemtuzumab was digested with trypsin, chymotrypsin, proteinase K or pepsin, and analyzed using a nanoLC system coupled to an LTQ Orbitrap Velos with HCD; in total, 98,787 mass spectra were generated from both chains, and collected without separation into the bottom-up dataset.

Aggregated paths: obtained **41** in total, **16** of which passed the validation procedure.

Precursor mass estimate: the largest group of close values of PM estimates induced by the 41 aggregated paths comprised 7 values ranging from **23,553.837Da** to **23,554.911Da**. To obtain the final PM estimate for alemtuzumab light chain, we needed to subtract from their average of **23,554.455Da** the mass of **-2.016Da** corresponding to a disulphide bond formation, since most often, complementary ions correspond to a pair of polypeptides together containing precisely one disulphide bond. The resulting value of **23,556.471Da** differs from the theoretical mass of **23,566.703Da** of the alemtuzumab light chain by **0.232Da**, which is within **10ppm** of the latter.

Contaminants: the 9th top scoring aggregated path (which did not pass the validation procedure) had the amino acid sequence DFLQKKVAVLEDALEQ. A BLAST search for its reversed copy QELADELVAVKKQLFD against the non-redundant database reported a single sequence without mismatches (up to substituting L-7 with I) – and namely, *50S ribosomal protein L29 [Synechococcus sp. PCC 7002]*. The PM estimate of **7,858.347Da** induced by the respective aggregated path matches its theoretical mass upon truncation of the N-terminal methionine, being **7,859.344Da**, with a difference of **0.997Da**, which can be attributed to ±1Da errors often introduced in mass spectra at time of deconvolution. This protein must have appeared in the sample either as a carry-over protein from previous runs on *Synechococcus* on the same instrument or as a contaminant on the column.

Running time: on a 24-processor (Intel Xeon X5675, 3.07GHz) server with 190Gb RAM, *Twister* correctly retrieved the entire sequence of the alemtuzumab light chain in **47.85s**, being launched on the preprocessed mass spectra. The preprocessing stage required about 15min.

		top-down	bottom-up	
k		**4**	**4**	**3**
k-tags	aa sequences	921	14,127	4,839
	with offsets	4,005	57,549	100,212
T-Bruijn graph	vertices	2,794	30,361	49,214
	components	800	9,651	14,989

The 16 aggregated paths after validation and correction (red means correct):

LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS		
VFLFP	ERKLEVKTGQGFTR	SLTFTFDT
VSASLSSPSQTMQ	VSASLSSPSQTMQ	SLHQL
PVGTQLNNTNYLLL	KVDNAL	YQQK
PVTKS	CVVS	SGSGSGTD
KLQEP	QQYWN	NLDK

Conclusion

We have presented a fast and highly accurate method for *de novo* reconstruction of a protein sequence solely from MS data. Future research direction comprise automated determination of inner parameter values of the algorithm based on the input.

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