



# Strong cation exchange LC peptide retention time prediction and its application in proteomics

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## Overview

### Objectives

- Development of a strong cation exchange (SCX) liquid chromatography (LC) peptide retention time predictor by using artificial neural networks
- Use of the predictor in order to decrease the false discovery rates in proteomics experiments

### Introduction

Typical proteomic samples can contain thousands to millions of potentially detectable peptides, making it extremely difficult to identify them with high confidence. This shortcoming has led to the search for auxiliary metrics of confidence that can improve identification rates while concurrently reducing false discovery rates.

Peptide chromatographic retention times can be used as such a metric as it is readily available and contains information dependent on a peptide's physicochemical properties. We have previously [1-3] developed very accurate peptide retention time predictors for reversed phase liquid chromatography.

In this work, we describe the development of a peptide retention time predictor for strong cation exchange (SCX), a mode of chromatography commonly used for fractionating proteomic samples.

## Methods

### Liquid chromatography

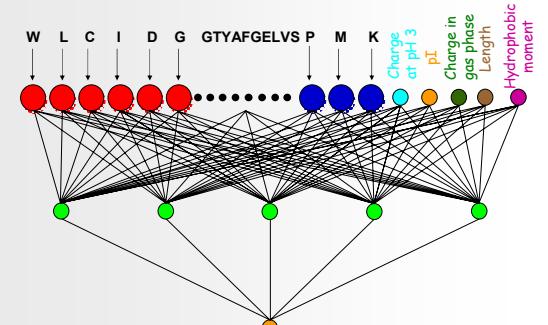
- Column: Polysulfopropyl Aspartamide (PolyLC), 200 x 2.1 mm, 300 Å
- Mobile phase A: 10 mM NH<sub>4</sub>COOH in 25:75 acetonitrile:water, pH = 3
- Mobile phase B: 500 mM NH<sub>4</sub>COOH in 25:75 acetonitrile:water
- Linear gradient, 2 slopes, 25 fractions

### Mass spectrometry

LTQ and LTQ-Orbitrap from Thermo

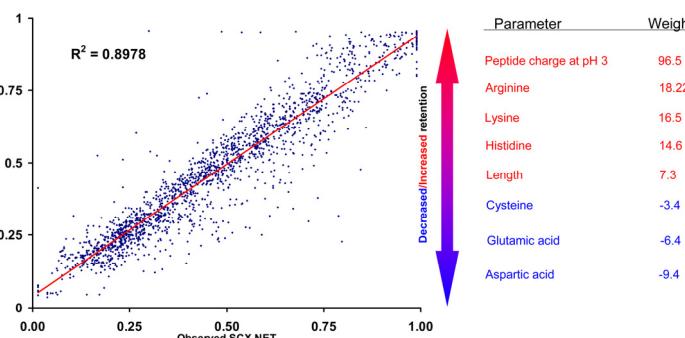
### Artificial neural network

- 191,000 confidently identified peptides from 2250 LC-MS/MS runs, 99% used for training, 1% for testing
- NeuroWindows version 4.5 with a standard back-propagation algorithm was used to develop and train an artificial neural network (ANN) predictor of SCX peptide retention times

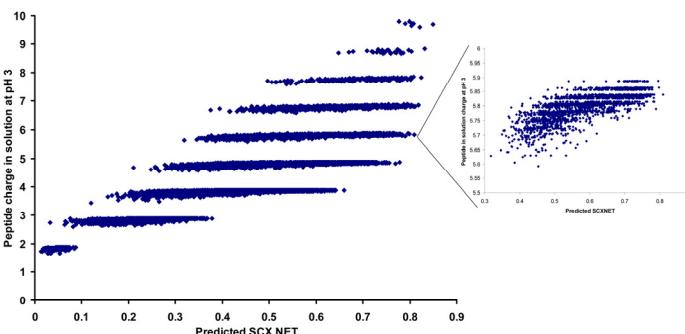


**Figure 1:** Schematic representation of the artificial neural network architecture used in this study (1055 input nodes, 5 hidden nodes and 1 output node). The large circles represent 21 length vectors while the smaller circles represent single length vectors. The black circles in the middle are used to show continuance.

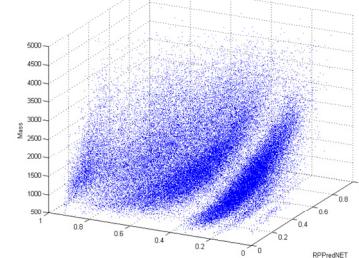
## Results



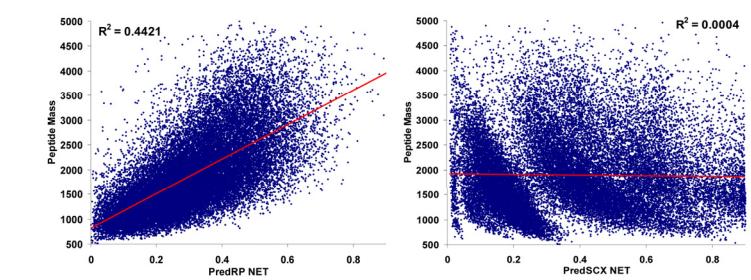
**Figure 2:** The graph depicts predicted vs. observed strong cation exchange peptide normalized elution time correlations for the ~1900 confidently peptides identifications of the testing set. On the right the main parameters affecting the strong cation exchange retention and their weighted contribution. The main contributor by far is the peptide charge in solution at pH 3.



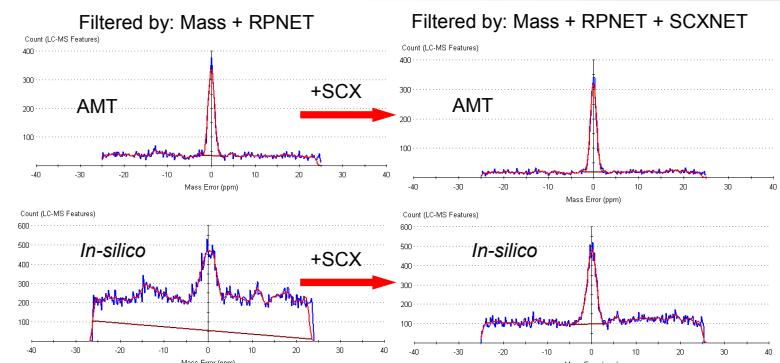
**Figure 3:** The graph shows the dependency between strong cation exchange peptide normalized elution time and the peptide charge in solution at pH 3.



**Figure 4:** Peptide spatial distribution according their mass, reversed phase and strong cation exchange normalized elution time. It is now possible to use three different metrics to assign peptide identifications in an accurate mass and time (AMT) tag like approach [4].



**Figure 5:** Unlike reversed phase liquid chromatography, peptide elution time in strong cation exchange liquid chromatography is orthogonal to the peptide mass making it a better additional metric for peptide assignment purposes in an AMT tag like approach.



Database		AMT tag <i>E. coli</i>		In-silico <i>E. coli</i>	
Filtering criteria		RP + Mass	RP+ SCX + Mass	RP + Mass	RP+ SCX + Mass
E. coli Fraction 19	# of Peptide ID	2391	2279	2520	2389
	FDR	11%	6%	54%	34%

**Figure 6:** Number of peptides identified and corresponding false discovery rates of an *E. coli* strong cation exchange peptide fraction when matched against an AMT tag *E. coli* database (~39,000 of peptides) and an *in-silico* *E. coli* database (182,000 of peptides) with and without the use of the strong cation exchange information. The use of SCX peptide elution time information decreases the FDRs by 30-50 % without compromising # of peptide identifications.

## Conclusions

- Strong cation exchange LC peptide retention time can be predicted with good accuracy. The peptide charge in solution at the pH of the mobile phase is the parameter that mainly affects peptide retention time in SCX.
- The peptide elution time in SCX can be used as an additional metric in an AMT tag like approach in order to further increase the confidence of peptide identifications.
- Future work will involve the development of the appropriate algorithms that will allow the integration of SCX information in our AMT tag platform.

## Acknowledgements

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