Unique Protein and Peptide Identifications Increase 20% with New Nano-ESI Source

Department of Systems Biology, Technical University of Denmark

Introduction

When testing a new mass spectrometry source in its 24/7/365 operation, scientists at the C-SIG (Cellular Signal Integration Group) lab observed a 20% increase in peptide and protein identification. The C-SIG lab was one of very few labs worldwide to test the new Thermo Scientific™ EASY-Spray™ nano-electrospray ion source prior to product launch. It was installed on their Thermo Scientific Q Exactive™ mass spectrometer.

"Installing the source is a very simple procedure—just attach it to the instrument and connect the column to the LC. When I had analyzed the data the day after it was installed, I realized we were getting about 20% more protein and peptide identifications compared to our old setup. That was a very exciting time."

Erwin M. Schoof, Ph.D. Student

Technical University of Denmark

Schoof conducts large-scale mammalian cell biology and cancer research at the Technical University of Denmark (DTU), Department of Systems Biology. The study of proteins phosphorylated by kinases in metastatic cancers is one of the department's main focuses. "Metastasis is responsible for more than 90% of cancer patient deaths, so we are looking for ways to prevent and stop it," Schoof said. "Kinases are known to play a major role in cancer. There are approximately 538 kinases in the human genome, and about 75% of global pharmaceutical R&D is aimed at targeting kinases to inhibit their phosphorylating activities. Cancer cells in general and metastatic cells in particular tend to be dys-regulated in their phosphorylation dynamics."

Instead of targeting single kinases, the laboratory's principal investigator, Professor Dr. Rune Linding, is searching for networks of kinases involved in the cell signaling of metastatic cancers. The goal is to devise combination therapies that target multiple kinases involved in metastasis within a given protein network state. Therapies targeting a single kinase often fail when cells develop resistance, by finding alternative ways to propagate a signal within the signaling network.

A lab with many types of analytical systems, a big part of their research is done using four Q Exactive mass spectrometers, making the lab's fleet one of the largest in the world. The EASY-Spray source was initially installed on one of their Q Exactive instruments.

Figure 1. The EASY-Spray source with an EASY-Spray column installed integrated all of the critically important nanobore tubing connections between the nanoLC and MS systems.

"I was very keen on trying it out," Schoof said. "I knew I would soon be running a set of precious samples that would require a month and a half for MS analysis. I didn't want to run into any practical issues. We need consistency of data and cannot have a column replacement or other hardware changes affect data reproducibility. Reproducibility is important because we run many replicates to ensure high quality of the data for biological relevance. One concern was the need to replace a column or emitter while we are running our samples. Could we install a new column and get the same spray efficiently set up? Would we get the same elution profiles? Would we have downtime that could delay the project and cause our samples to deteriorate? I was keen to try the source to see if it would generate stable, high-quality data."

Thermo SCIENTIFIC

EASY-Spray nano-Electrospray Ionization Device

Optimization and reproducibility are built in. The EASY-Spray (Figure 1) is a plug-and-spray nano-electrospray ionization (ESI) device that integrates, in a single unit, all the critically important nanobore tubing connections between your UHPLC and MS systems. The temperature-controlled column (which accommodates columns up to 50 cm), high-voltage electrode, and emitter are all integral to the EASY-Spray column. On one end a Thermo Scientific Dionex™ nanoViper™ fitting provides a quick and easy fingertight, leak-free connection up to 1000 bar to your nano UHPLC system such as the Thermo Scientific EASY-nLC 1000 or UltiMate™ 3000 RSLCnano systems. The other end is the emitter that delivers ionized sample to the MS – such as the Thermo Scientific Orbitrap Elite™, Q Exactive, Velos Pro™, and TSQ Vantage™ systems.

No Fiddling Required

"Installing the source is a very simple procedure—just attach it to the instrument and connect the column to the LC. When I analyzed the data the day after it was installed, I realized we were getting about 20% more protein and peptide identifications compared to our old setup. That was a very exciting time." Schoof said. (Figure 2)

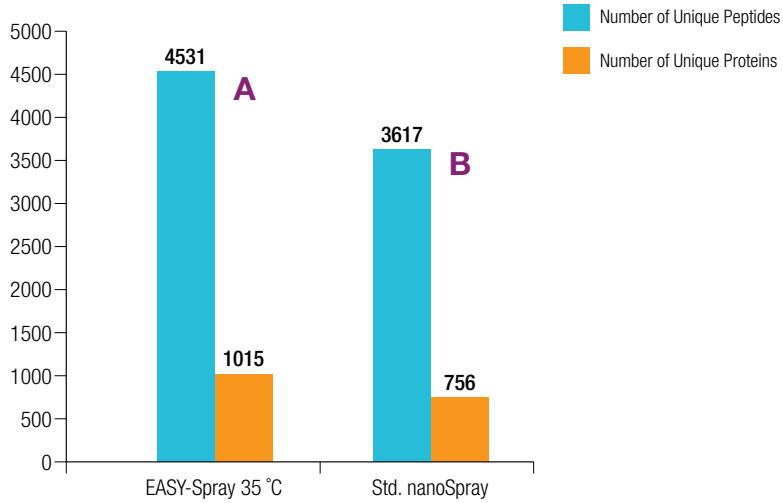


Figure 2. Unique proteins and peptides identified using: (A) the EASY-Spray source with a 15 cm C18 EASY-Spray column at 35 °C, and (B) the Thermo Scientific Nanospray Flex Ion Source with a 15 cm C18 column and coated glass emitter. Both setups were connected to a Thermo Scientific EASY-nLC 1000 system. These preliminary results showing an increase in identifiable proteins and peptides using the EASY-Spray source at a column temperature 35 °C were duplicated in Thermo Fisher Scientific laboratories.

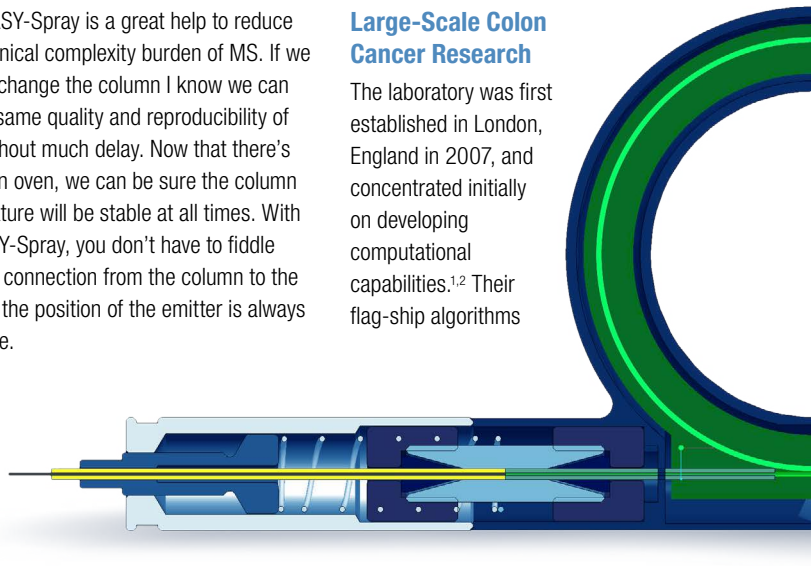
"The EASY-Spray is a great help to reduce the technical complexity burden of MS. If we have to change the column I know we can get the same quality and reproducibility of data without much delay. Now that there's a column oven, we can be sure the column temperature will be stable at all times. With the EASY-Spray, you don't have to fiddle with the connection from the column to the emitter; the position of the emitter is always the same.

"With the EASY-Spray, you don't have to fiddle with the connection from the column to the emitter; the position of the emitter is always the same. The total ion current variation that I get now is much lower than on the previous manual spray"

The total ion current variation that I get now is much lower than on the previous manual spray. I know that if I need to run a sample in three months I will get the same data out from the MS, at least from the technical aspect. That's a comforting thought and it lets us focus more on the biology, which is what we're primarily interested in. For me, that was the main reason to try it out. I'm happy to know if something goes wrong and I need to change a column, that with the EASY-Spray, I can simply run one or two test samples and continue with analyzing the important samples so that they don't deteriorate. This has been a really positive experience."

Large-Scale Colon Cancer Research

The laboratory was first established in London, England in 2007, and concentrated initially on developing computational capabilities.[1,2] Their flag-ship algorithms



NetworKIN and NetPhorest (http://NetworKIN.info and http://NetPhorest.info) correlate data from different sources to enable a deeper understanding of the function of kinases and other phospho-interacting domains. "Generation of data to get a long list of proteins is one thing", Schoof said, "but in order to determine the function of those proteins and answer fundamental biological questions, we needed better analytical tools. Our algorithms help answer those questions and analyze data from MS and integrate it with data from other sources such as high-throughput cell imaging."

The laboratory moved to Denmark in 2011 to be part of a new genome-scale biotechnology initiative, with a full suite of analytical instruments to conduct their large-scale network biology oriented basic and translational research.

Among many other projects under Professor Dr. Linding, the colon cancer metastasis research is very promising. Using cell lines and patient samples as an initial model system, they generate quantitative phospho-proteomic data on their Q Exactive systems. Using their algorithms, they can predict the kinases that are differentially active between different disease states. "Although we can accurately determine phosphorylation dynamics, due to the highly transient interaction between kinases and substrates, kinases causing observed phosphorylation events cannot be readily identified experimentally and directly on a large scale." said Schoof. Instead, they use a combination of experimentation and algorithms to predict the kinases involved and model quantitative signaling networks that may drive a given phenotype.

Subsequently, they test and refine the validity of the constructed network models by follow-up cellular and *in vivo* perturbation studies in order to functionally validate their role in the observed phenotype. In this way, they can gain a better understanding about the protein networks that are driving diseases such as cancer metastasis. By complementing the cell-line data with actual patient sample data, a greater clinical relevance can be obtained. This may give rise to further clinical research, including the development of therapeutic strategies. The lab plans to significantly expand their mass spectrometer fleet to accommodate the multitude of samples that need to be run for these experiments.

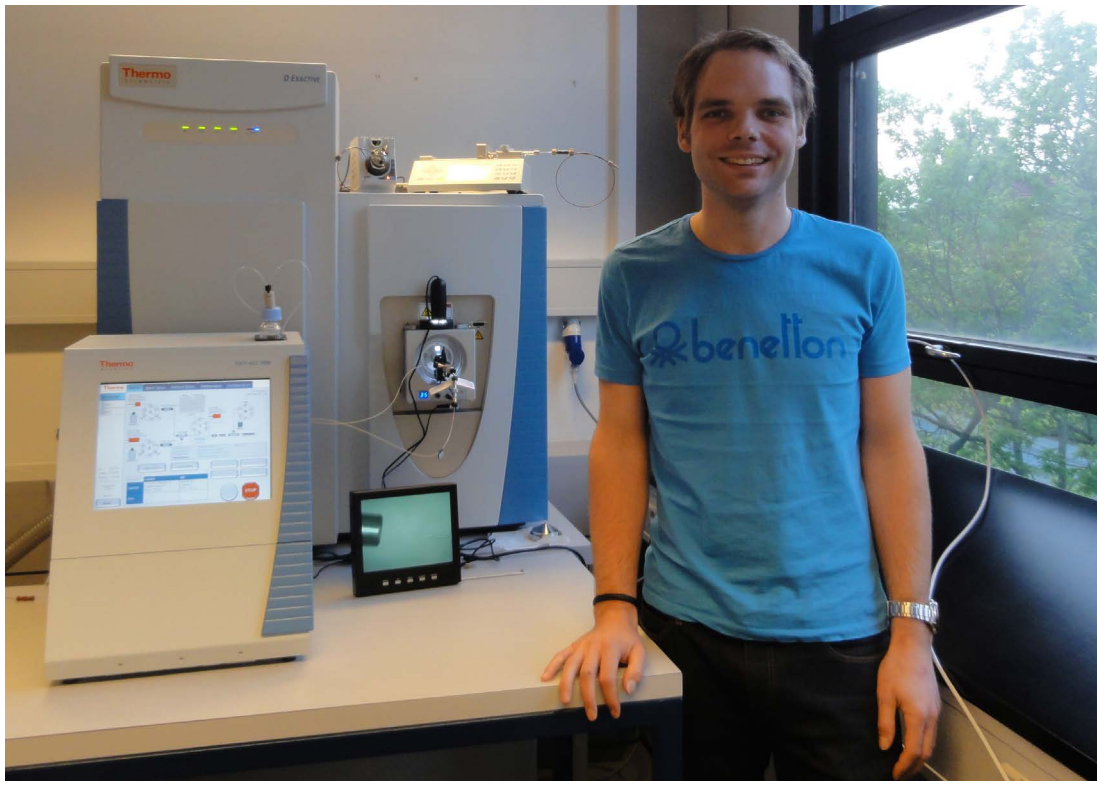
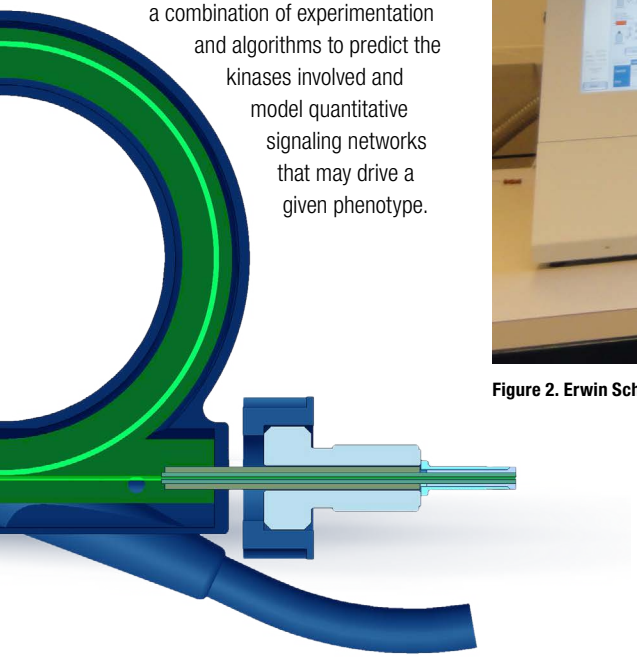


Figure 2. Erwin Schoof used the EASY-nLC 1000 interfaced with the Q Exactive mass spectrometer through the EASY-Spray source.



The EASY-Spray columns are high-tech assemblies that are carefully manufactured to the highest standards in nano-flow chromatography

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References

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2. Erler, J., Linding, R., Network Medicine Strikes a Blow Against Breast Cancer. *Cell.* **2012,** May 11; 149(4), 731-733.

3. Creixell, P., Schoof, E.M., Erler, J.T. and Linding R. Navigating Cancer Network Attractors for Tumor-Specific Therapy. *Nat. Biotechnol.* **2012,** 30, 842-48.

Figure 3. The EASY-Spray source easily connects to any Thermo Scientific mass spectrometer.

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