

New material for enriching phosphorylated proteins

- ▶ Simultaneous enrichment of phosphorylated proteins or peptides with single or multiple phosphorylation sites
- ▶ Trapping all types of phosphorylated proteins or peptides
- ▶ Scalable for lab-on-a-chip

Interest in phosphorylated proteins

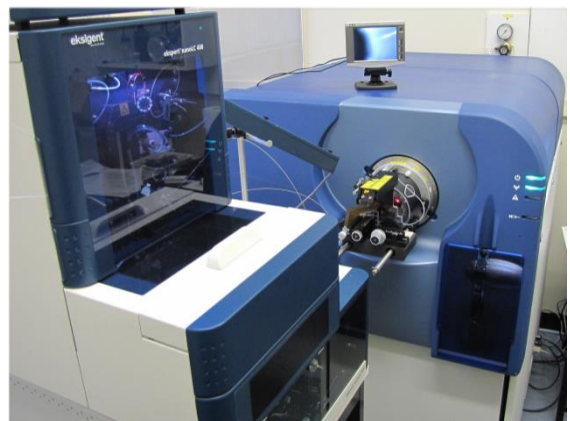
Proteins are responsible for carrying out and regulating all of the processes that occur within our bodies; such as regulating our metabolism, cell-division and DNA replication. Some proteins are modified after their synthesis to carry out specialized functions; phosphorylation is a key protein modification. Approximately 75% of human proteins are phosphorylated which highlights the importance of phosphorylation. Given the large proportion of phosphorylated proteins and their role in cellular functions it is no wonder that malfunctions in phosphorylation have been linked with a variety of diseases including virtually all forms of cancer, diabetes and kidney disease to name a few.

Given the burden that these diseases place on society worldwide it is socially important that we develop effective tools to understand how phosphorylated proteins play a role in these diseases. This knowledge could not only yield new biomarkers to improve our diagnostic abilities but potentially provide drug targets/therapeutic strategies that can effectively treat these conditions. This is why understanding phosphorylated proteins is of value to society. The Biomolecular Systems Analytics group at the University of Amsterdam is highly interested in phosphorylation of proteins.

Challenge

Problems in studying phosphorylation in particular are:

- Phosphorylated peptides are present in very low abundance compared to other proteins. Therefore, it is crucial to concentrate them by extracting them from non-phosphorylated proteins.
- Low ionization efficiency, which affects their ability to be detected by the current state-of-the-art technology in mass spectrometry.
- Multiple phosphorylation sites are possible (in multiple combinations) per protein; yet most techniques suitable for studying phosphorylation are either suitable for proteins with a single phosphorylation site or suffer from non-specific binding. That is, non-phosphorylated proteins are captured as well.



The invention

At the university, a porous nitrogen-doped carbon material has been developed, with an open porous network of micropores, mesopores and macropores. This hierarchical structure combines the advantages of a large surface area with excellent accessibility. The nitrogen doping creates local charges on the carbon surface, enhancing the retention of the phosphorylated proteins or peptides.

The advantage of using this column material is:

- High surface area (1000 m²/g)
- High permeability
- Accessible network of micropores, mesopores and macropores
- Shape selectivity
- Retains polar and non-polar compounds
- Excellent chemical and thermal stability

Compared to the current state-of-the-art, the new material and methods allows enrichment of phosphorylated peptides that are beyond the scope of current state-of-the-art technologies or methods. The solution which has been developed is scalable, and in principal should allow for lab-on-a-chip solutions.

R&D status

A first working prototype has been built, and measurements have confirmed that the method can efficiently accumulate material and works significantly better and easier than conventional method. A proof of concept has been achieved. The prototype is assembled using standard commercially components (ie. Eskigent LC with 5600+ QTOF MS Sciex, 2-way trapping valve, columns) The nitrogen doped porous carbon material has been produced in-house.

Intellectual property

The inventors of the method are Dr. Michelle Camenzuli, Prof. Gadi Rothenberg, and Stan Koot of the Van 't Hoff Institute for Molecular Sciences - HIMS University of Amsterdam, the Netherlands. A European patent application has been filed on 2017-05-24.

Within the Biomolecular Systems Analytics group, the primary application areas fall within the life sciences. However the tools and methods developed can be applied in numerous applications where powerful separations are required. The interest of the researcher and her group is in developing new equipment add-ons or instruments, so we are looking for a partner who is willing to develop this into a commercial instrument. There is still room for optimization, and the research team can support the further development.



Contact

If you are interested in the technology or have questions please contact Dr. Ir. Peter van der Donk – Business Developer at the Technology Transfer Office of the University of Amsterdam
+31 20 5258553 / +31 6 21526979 – P.J.T.vanderDonk@uva.nl – www.ix.nl