Literature update
March 17. 2008

Elsa Lundanes
• New techniques
  – on CHIPs
Polymer Microchips Integrating Solid-Phase Extraction and High-Performance Liquid Chromatography Using Reversed-Phase Polymethacrylate Monoliths

Jikun Liu,† Chien-Fu Chen,†† Chia-Wen Tsao,† Chien-Cheng Chang,‡ Chin-Chou Chu,‡ and Don L. DeVoe*†

Department of Mechanical Engineering, University of Maryland, College Park, Maryland, and Institute of Applied Mechanics, National Taiwan University, Taipei, Taiwan
Polymer microfluidic chips employing in situ photopolymerized polymethacrylate monoliths for high-performance liquid chromatography separations of peptides is described. The integrated chip design employs a 15 cm long separation column containing a reversed-phase polymethacrylate monolith as a stationary phase, with its front end seamlessly coupled to a 5 mm long methacrylate monolith which functions as a solid-phase extraction (SPE) element for sample cleanup and enrichment, serving to increase both detection sensitivity and separation performance. In addition to sample concentration and separation, solvent splitting is also performed on-chip, allowing the use of a conventional LC pump for the generation of on-chip nanoflow solvent gradients. The integrated platform takes advantage of solvent bonding and a novel high-pressure needle interface which together enable the polymer chips to withstand internal pressures above 20 MPa (~2900 psi) for efficient pressure-driven HPLC separations. Gradient reversed-phase separation of fluorescein-labeled model peptides and BSA tryptic digest are demonstrated using the microchip HPLC system. Online removal of free fluorescein and enrichment of labeled proteins are simultaneously achieved using the on-chip SPE column, resulting in a 150-fold improvement in sensitivity and a 10-fold reduction in peak width in the following microchip gradient LC separation.
Figure 1. (A) Chip design and (B) experimental system for HPLC separations employing dynamic sample injection, (C) chip design and (D) experimental system for online sample cleanup/enrichment-HPLC separations, with an integrated 5 mm long SPE trap column used for online sample cleanup and enrichment. The total length of the serpentine separation channels is 17 cm in both chip designs.
**Figure 5.** HPLC separation of FITC labeled BSA tryptic digest using different linear ACN gradients: (A) 8%, (B) 5%, and (C) 3.5% ACN/min. Injection time was 1 min, master flow rate was 1 mL/min, and column flow rate was 200 nL/min. With the use of the three labeled peptide peaks, an average retention time variance of 2.9% was determined for the 10 min gradient case.
Multidimensional Separation of Chiral Amino Acid Mixtures in a Multilayered Three-Dimensional Hybrid Microfluidic/Nanofluidic Device

Bo Young Kim,† Jing Yang,† Maojun Gong,† Bruce R. Flachsbart,‡ Mark A. Shannon,‡ Paul W. Bohn,§ and Jonathan V. Sweedler*,†

Department of Chemistry and Beckman Institute for Advanced Science and Technology, Department of Mechanical Science and Engineering, University of Illinois at Urbana–Champaign, Urbana, Illinois 61801, and Department of Chemical and Biomolecular Engineering and Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556
Microscale total analysis systems (μTAS) allow high-throughput analyses by integrating multiple processes, parallelization, and automation. Here we combine unit operations of μTAS to create a device that can perform multidimensional separations using a three-dimensional hybrid microfluidic/nanofluidic device composed of alternating layers of patterned poly(methyl methacrylate) and nanocapillary array membranes constructed from nuclear track-etched polycarbonate. Two consecutive electrophoretic separations are performed, the first being an achiral separation followed by a chiral separation of a selected analyte band. Separation conditions are optimized for a racemic mixture of fluorescein-isothiocyanate-labeled amino acids, serine and aspartic acid, chosen because there are endogenous D-forms of these amino acids in animals. The chiral separation is implemented using micellar electrokinetic chromatography using β-cyclodextrin as the chiral selector and sodium taurocholate as the micelle-forming agent. Analyte separation is monitored by dual-beam laser-induced fluorescence detection. After separation in the first electrophoretic channel, the preselected analyte is sampled by the second-stage separation using an automated collection sequence with a zero-crossing algorithm. The controlled fluidic environment inherent to the three-dimensional architecture enables a series of separations in varying fluidic environments and allows sample stacking via different background electrolyte pH conditions. The ability to interface sequential separations, selected analyte capture, and other fluidic manipulations in the third dimension significantly improves the functionality of multilayer microfluidic devices.
PMMA-poly(methyl methacrylate)
NCAM-nanocapillary array membranes

**Figure 1.** Schematic illustration of the device structure and photograph. Panel A describes the individual layers of the multilayered device; (a) is the 1.5 mm thick polycarbonate cap; (b), (c), (e) (patterned, 100 μm (width) × 20 μm (depth)), and (f) are 20 μm thick PMMA layers, and (d) is a 10 μm thick NCAM. (B) Photo of a typical device (i) and a schematic diagram showing the microfluidic channel labeling scheme (ii). NCAM is omitted in panel B, part ii, for clarity.
Hybrid Capillary-Microfluidic Device for the Separation, Lysis, and Electrochemical Detection of Vesicles

Donna M. Omiatek, Michael F. Santillo, Michael L. Heien, and Andrew G. Ewing

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, and Department of Chemistry, Göteborg University, Kemivägen 10, SE-41296 Göteborg, Sweden
The primary method for neuronal communication involves the extracellular release of small molecules that are packaged in secretory vesicles. We have developed a platform to separate, lyse, and electrochemically measure the contents of single vesicles using a hybrid capillary-microfluidic device. This device incorporates a sheath-flow design at the outlet of the capillary for chemical lysis of vesicles and subsequent electrochemical detection. The effect of sheath-flow on analyte dispersion was characterized using confocal fluorescence microscopy and electrochemical detection. At increased flow rates, dispersion was minimized, leading to higher separation efficiencies but lower detected amounts. Large unilamellar vesicles (diameter ~ 200 nm), a model for secretory vesicles, were prepared by extrusion and loaded with an electroactive molecule. They were then separated and detected using the hybrid capillary-microfluidic device. Determination of size from internalized analyte concentration provides a method to characterize the liposomal suspension. These results were compared to an orthogonal size measurement using dynamic light scattering to validate the detection platform.
**Figure 1.** Three layer PDMS device for the end-column lysis and electrochemical detection of vesicles separated by capillary electrophoresis. (A) Schematic of device (not drawn to scale). Three 200 $\mu m \times 125 \mu m$ channels converge into a $650 \mu m \times 125 \mu m$ channel where contents exiting the capillary are lysed and detected at a carbon-fiber microelectrode. (B) Bright field and confocal fluorescence images of the device. A continuous injection 100 $\mu M$ rhodamine B solution (red) is flowed through the capillary using electroosmotic flow (166 V/cm), and 36 $\mu M$ FITC solution (green) is flowed through the lysis channels using hydrodynamic flow controlled by a syringe pump (0.5 $\mu L/min$, scale bar = 200 $\mu m$).
Microchip Atmospheric Pressure Photoionization for Analysis of Petroleum by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Markus Haapala,† Jeremiah M. Purcell,‡ Ville Saarela,‖ Sami Franssilä,‖ Ryan P. Rodgers,‡,§ Christopher L. Hendrickson,‡,§ Tapio Kotiaho,†,⊥ Alan G. Marshall,*,‡,§ and Risto Kostiainen*,†

Division of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland, Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306-4390, Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310-4005, Microfabrication Group, Department of Micro and Nanosciences, Helsinki University of Technology, P.O. Box 3500, FI-02015 TKK, Finland, and Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014 Helsinki, Finland
Atmospheric pressure photoionization (APPI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has significantly contributed to the molecular speciation of petroleum. However, a typical APPI source operates at 50 \( \mu \text{L/min} \) flow rate and thus causes a considerable mass load to the mass spectrometer. The recently introduced microchip APPI (\( \mu \text{APPI} \)) operates at much lower flow rates (0.05–10 \( \mu \text{L/min} \)) providing decreased mass load and therefore decreased contamination in analysis of petroleum by FT-ICR MS. In spite of the 25 times lower flow rate, the signal response with \( \mu \text{APPI} \) was only 40% lower than with a conventional APPI source. It was also shown that \( \mu \text{APPI} \) provides very efficient vaporization of higher molecular weight components in petroleum analysis.
**Figure 1.** Microfabricated heated nebulizer chip (25 mm length by 10 mm width).
Effect of capillary cross-section geometry and size on the separation of proteins in gradient mode using monolithic poly(butyl methacrylate-co-ethylene dimethacrylate) columns

Ivo Nischang\textsuperscript{a}, Frantisek Svec\textsuperscript{b}, Jean M.J. Fréchet\textsuperscript{a,b,*}

\textsuperscript{a} Department of Chemistry, University of California, Berkeley, CA 94720-1460, USA
\textsuperscript{b} The Molecular Foundry, L. O. Lawrence Berkeley National Laboratory, Berkeley, CA 94720-8139, USA

\section*{Abstract}

Porous polymer monoliths have been prepared in capillaries with circular or square cross-sections and lateral dimensions of 50, 75, 100 \textmu m as well as in a rectangular 38 \textmu m \times 95 \textmu m capillary. These capillaries have been used to determine the effect of the size and shape of their cross-section on the porous and hydrodynamic properties of poly(butyl methacrylate-co-ethylene dimethacrylate) monoliths. The capillaries were studied by scanning electron microscopy and evaluated for their permeability to flow and their performance in the liquid chromatographic separation of a protein mixture comprising ribonuclease A, cytochrome c, myoglobin, and ovalbumin using a linear gradient of acetonitrile in the mobile phase. No differences resulting from channel geometry were found for the various capillary columns. These results demonstrate that standard capillaries with circular geometry are a good and affordable alternative conduit for modeling the processes carried out in microfluidic chips with a variety of geometries.
Fig. 1. Experimental setup used in this study. Zero dead-volume connections were used for installation of the conduits.
• MS
RAPID COMMUNICATION

Performance of five different electrospray ionisation sources in conjunction with rapid monolithic column liquid chromatography and fast MS/MS scanning

Karl E. V. Burgess\(^2\), Alex Lainson\(^1\), Lisa Imrie\(^1\), Douglas Fraser-Pitt\(^1\), Raja Yaga\(^1\), David G. E. Smith\(^1,3\), Remco Swart\(^4\), Andrew R. Pitt\(^2\) and Neil F. Inglis\(^1\)

\(^1\) Moredun Proteomics Facility, Moredun Research Institute, Penicuik, Midlothian, UK
\(^2\) Integrative and Systems Biology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK
\(^3\) Institute for Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Glasgow, UK
\(^4\) Dionex Benelux B. V., Amsterdam, The Netherlands
The performances of five different ESI sources coupled to a polystyrene–divinylbenzene monolithic column were compared in a series of LC-ESI-MS/MS analyses of *Escherichia coli* outer membrane proteins. The sources selected for comparison included two different modifications of the standard electrospray source, a commercial low-flow sprayer, a stainless steel nanospray needle and a coated glass Picotip. Respective performances were judged on sensitivity and the number and reproducibility of significant protein identifications obtained through the analysis of multiple identical samples. Data quality varied between that of a ground silica capillary, with 160 total protein identifications, the lowest number of high quality peptide hits obtained (3012), and generally peaks of lower intensity; and a stainless steel nanospray needle, which resulted in increased precursor ion abundance, the highest-quality peptide fragmentation spectra (5414) and greatest number of total protein identifications (259) exhibiting the highest MASCOT scores (average increase in score of 27.5% per identified protein). The data presented show that, despite increased variability in comparative ion intensity, the stainless steel nanospray needle provides the highest overall sensitivity. However, the resulting data were less reproducible in terms of proteins identified in complex mixtures – arguably due to an increased number of high intensity precursor ion candidates.
etched and flame stripped tip. The two nanospray emitters yielded the highest number of high quality spectra, coupled with the highest ion intensities. Of these, it is clear that the higher sensitivity offered by the Proxeon stainless steel needle resulted ultimately in higher MOWSE scores, greater sequence coverage and higher quality MS/MS data. However, these gains were made at the expense of increased variability of ion intensity that, at least as applied to the bacterial outer membrane preparation studied here, resulted in reduced reproducibility of protein identification. It is likely that this observed decrease in reproduc-
Handheld Miniature Ion Trap Mass Spectrometers

Zheng Ouyang, Robert J. Noll, and R. Graham Cooks

Purdue University

For field applications, “miniature” and “rapid” have become almost synonymous, yet these small mass spectrometers are not useful if performance is too severely compromised. (To listen to a podcast about this feature, please go to the Analytical Chemistry website at pubs.acs.org/journal/ancham.)
Table 1. Handheld mass spectrometers

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Weight (kg)</th>
<th>Power (W)</th>
<th>Mass analyzer</th>
<th>MS/MS?</th>
<th>Sample introduction or ionization mode</th>
<th>Mass range (m/z)/resolution (R)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini 10</td>
<td>10</td>
<td>70</td>
<td>Rectilinear ion trap</td>
<td>Yes</td>
<td>MIMS, direct leak, ESI, DESI</td>
<td>550/550</td>
<td>14</td>
</tr>
<tr>
<td>Mini 11</td>
<td>4</td>
<td>30</td>
<td>Rectilinear ion trap</td>
<td>Yes</td>
<td>MIMS, direct leak, ESI, DESI</td>
<td>2000/100</td>
<td>17</td>
</tr>
<tr>
<td>ChemCube/ChemPack</td>
<td>14</td>
<td>50</td>
<td>Quadrupole mass filter on chip</td>
<td>No</td>
<td>SPME, EI</td>
<td>400/100</td>
<td>13</td>
</tr>
<tr>
<td>Guardion-7</td>
<td>11</td>
<td>75</td>
<td>Miniature toroidal ion trap</td>
<td>Yes</td>
<td>SPME, EI</td>
<td>500/500</td>
<td>10, 24</td>
</tr>
<tr>
<td>Suitcase TOF</td>
<td>–</td>
<td>–</td>
<td>Miniature TOF</td>
<td>No</td>
<td>MALDI sample plate</td>
<td>50,000/30</td>
<td>25</td>
</tr>
<tr>
<td>Palm-Portable</td>
<td>1.5</td>
<td>5</td>
<td>Miniature CIT</td>
<td>No</td>
<td>Pulsed gas leak, EI</td>
<td>300/150</td>
<td>26</td>
</tr>
<tr>
<td>Griffin Analytical 600</td>
<td>25</td>
<td>–</td>
<td>Miniature CIT</td>
<td>Yes</td>
<td>MIMS, direct leak</td>
<td>425/400</td>
<td><a href="http://www.griffinanalytical.com">www.griffinanalytical.com</a></td>
</tr>
<tr>
<td>Portable GC/MS</td>
<td>6.4</td>
<td>42</td>
<td>Full-size QIT</td>
<td>No</td>
<td>Mini GC/preconcentration</td>
<td>100/220</td>
<td>27</td>
</tr>
<tr>
<td>O. I. Analytical</td>
<td>18</td>
<td>75</td>
<td>Mattauch–Herzog sector</td>
<td>No</td>
<td>Direct gas leak, EI</td>
<td>300/300</td>
<td>29</td>
</tr>
</tbody>
</table>

MIMS, membrane inlet MS; SPME, solid-phase microextraction; QIT, quadrupole ion trap; EI, electron impact.

Table 2. Characteristics of ion trap mass analyzers for use in miniature mass spectrometers

<table>
<thead>
<tr>
<th>Desired characteristic</th>
<th>Reason for the characteristic</th>
<th>Ion trap suitability</th>
</tr>
</thead>
<tbody>
<tr>
<td>High pressure tolerance</td>
<td>Pumping limitations of small instruments</td>
<td>Tolerates pressure best</td>
</tr>
<tr>
<td>Miniaturization</td>
<td>Minimize system size and weight</td>
<td>Easily miniaturized, optimized geometry</td>
</tr>
<tr>
<td>Tandem MS</td>
<td>Analyze complex samples</td>
<td>Single miniature ion trap can be used</td>
</tr>
<tr>
<td>High ion currents</td>
<td>Maximize signal strength</td>
<td>Arrays of small traps can be multiplexed to increase ion current</td>
</tr>
</tbody>
</table>
Figure 2. Cocaine on money. (a) Protonated cocaine molecule at m/z 304 shown on banknote by conventional geometry DESI. MS and (inset) MS/MS data provided by the Mini 10. (b) Mass spectrum from 10 ng cocaine on Teflon by using geometry-independent DESI. (Adapted from Ref. 19.)
Figure 3. The combination of ambient ionization by DESI with a miniature mass spectrometer by means of a suitable interface could result in an instrument for personal use. (Adapted from Ref. 28.)

if performance is too severely compromised. Analysis of complex mixtures must be possible—therefore, if chromatography is not an option (it is often too slow for on-line work), then tandem MS, ion mobility, or exact mass measurements must be available. Of these choices, only tandem MS is currently available on small systems.

personal mass spectrometer might become as useful and ubiquitous as the personal computer is today, however unlikely it may seem at present.
• Chromatography
Hydrophilic interaction liquid chromatography with alcohol as a weak eluent

Min Liu*, Judy Ostovic, Emily X. Chen, Nina Cauchon

Analytical Research & Development, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA

There has been a significant increase of interest in polar compound separation by hydrophilic interaction liquid chromatography (HILIC), in which acetonitrile is mostly used as a weak eluent. Although replacing acetonitrile with alcohols as organic modifiers has been previously reported, the separation mechanism was poorly understood. In this paper we explored the separation mechanism through the method development for the analysis of the trace amounts of polar and basic hydrazines, which were genotoxic in nature. Separation parameters such as the type and concentration of alcohol, acid modifier, and buffer in mobile phase as well as the choice of stationary phase and column temperature were studied. The data indicated that both electrostatic and hydrophilic interactions contributed to the retention and separation of the hydrazines. The results presented here provide insight into the adjustment of the retention and separation of analytes in HILIC mode with alcohol as a weak eluent. The optimized HILIC method coupled with chemiluminescent nitrogen detection (CLND) is simple and sensitive (reporting limit at 0.02%) and was applied to simultaneous analysis of hydrazine and 1,1-dimethylhydrazine in a pharmaceutical intermediate.
Fig. 3. Effect of the type of alcohols on the retention of hydrazines on a ZIC HILIC column. For each separation, isocratic run was performed in the mobile phase of TFA/water/alcohol (0.1/10/90, v/v/v), column temperature at 30°C, flow rate at 0.4 mL/min with a splitter and CLND. 1: 1,2-Dimethylhydrazine, 2: 1,1-dimethylhydrazine, 3: methylhydrazine and 4: hydrazine. Other conditions as in Fig. 2.
Fig. 4. Effect of the type of acid modifiers on the retention of hydrazines on a ZIC HILIC column. For each separation, isocratic elution was performed with acid/water/ethyl alcohol (0.1/30/70, v/v/v), as a mobile phase, column temperature at 30 °C, flow rate at 0.4 mL/min with a splitter and CLND. 1: 1,2-Dimethylhydrazine, 2: 1,1-dimethyldiazine, 3: methyldiazine and 4: hydrazine. Other conditions as in Fig. 2.
Fig. 5. The separation of hydrazines on different columns. Column temperature was at 30 °C; flow rate was 0.4 mL/min with a splitter and CLND. Mobile phase was formic acid/water/ethyl alcohol (0.5/20/80, v/v/v), 0.1% ACN (v/v) in ethyl alcohol was used as a void volume marker. 1: 1,2-Dimethylhydrazine, 2: 1,1-dimethylhydrazine, 3: methylhydrazine and 4: hydrazine. (a) Zorbax NH<sub>2</sub>, (b) Diol, (c) Amide-80 and (d) ZIC HILIC. Other conditions as in Fig. 2.
• Proteomics
Serum protein profiling by solid phase extraction and mass spectrometry: A future diagnostics tool?

Anne K. Callesen¹, ²*, Jonna S. Madsen³, Werner Vach⁴, Torben A. Kruse², Ole Mogensen⁵ and Ole N. Jensen¹

¹ Protein Research Group, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark
² Department of Biochemistry, Pharmacology and Genetics, Odense University Hospital, Odense C, Denmark
³ Department of Clinical Biochemistry, Vejle County Hospital, Vejle, Denmark
⁴ Department of Statistics, University of Southern Denmark, Odense M, Denmark
⁵ Department of Gynecology and Obstetrics, Odense University Hospital, Odense C, Denmark
Serum protein profiling by MS is a promising method for early detection of disease. Important characteristics for serum protein profiling are preanalytical factors, analytical reproducibility and high throughput. Problems related to preanalytical factors can be overcome by using standardized and rigorous sample collection and sample handling protocols. The sensitivity of the MS analysis relies on the quality of the sample; consequently, the blood sample preparation step is crucial to obtain pure and concentrated samples and enrichment of the proteins and peptides of interest. This review focuses on the serum sample preparation step prior to protein profiling by MALDI MS analysis, with particular focus on various SPE methods. The application of SPE techniques with different chromatographic properties such as RP, ion exchange, or affinity binding to isolate specific subsets of molecules (subproteomes) is advantageous for increasing resolution and sensitivity in the subsequent MS analysis. In addition, several of the SPE sample preparation methods are simple and scalable and have proven easy to automate for higher reproducibility and throughput, which is important in a clinical proteomics setting.
Sample preparation and fractionation for proteome analysis and cancer biomarker discovery by mass spectrometry

Sample preparation and fractionation technologies are one of the most crucial processes in proteomic analysis and biomarker discovery in solubilized samples. Chromatographic or electrophoretic proteomic technologies are also available for separation of cellular protein components. There are, however, considerable limitations in currently available proteomic technologies as none of them allows for the analysis of the entire proteome in a simple step because of the large number of peptides, and because of the wide concentration dynamic range of the proteome in clinical blood samples. The results of any undertaken experiment depend on the condition of the starting material. Therefore, proper experimental design and pertinent sample preparation is essential to obtain meaningful results, particularly in comparative clinical proteomics in which one is looking for minor differences between experimental (diseased) and control (nondiseased) samples. This review discusses problems associated with general and specialized strategies of sample preparation and fractionation, dealing with samples that are solution or suspension, in a frozen tissue state, or formalin-preserved tissue archival samples, and illustrates how sample processing might influence detection with mass spectrometric techniques. Strategies that dramatically improve the potential for cancer biomarker discovery in minimally invasive, blood-collected human samples are also presented.
Affinity prefractionation for MS-based plasma proteomics

Maria Pernemalm, Rolf Lewensohn and Janne Lehtio

Karolinska Biomics Center (KBC), Dep. Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

The plasma proteome has proven to be one of the most challenging proteomes to profile using currently available proteomics technologies. A plethora of methodologies have been used to profile human plasma in order to discover potential biomarkers for disease and for therapy optimization. Affinity-based prefractionation coupled to MS has been shown to be one of the most successful ways to dig deeper into the plasma proteome. Depletion of high abundant plasma proteins is becoming an initial method of choice in any plasma profiling project. However, several other affinity-based enrichment methods have been published in recent years. Here we review both protein and peptide affinity prefractionation methods coupled with MS-based proteomics. Analysis of the proportion of cellular and extracellular annotated proteins of publicly available MS plasma proteomics data is performed to estimate the analytical depth of various prefractionation methods.
Short communication

Trypsin immobilization on an ethylenediamine-based monolithic minidisk for rapid on-line peptide mass fingerprinting studies

R. Nicoli\textsuperscript{a}, S. Rudaz\textsuperscript{a}, C. Stella\textsuperscript{b}, J.-L. Veuthey\textsuperscript{a,*}

\textsuperscript{a} Laboratory of Pharmaceutical Analytical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 20 Boulevard d’Yvoy, 1211 Geneva 4, Switzerland
\textsuperscript{b} Laboratory of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30 Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland
The aim of this work was to develop a trypsin-based micro-immobilized enzyme reactor prepared on a monolithic ethylenediamine BIA Separations CIM (convective interaction media) minidisk. The micro-immobilized enzyme reactor (IMER) was integrated in a liquid chromatography system hyphenated to electrospray ionization tandem mass spectrometry to carry out on-line protein digestion and identification. The performance of this IMER was compared with that obtained using a previously developed bioreactor prepared on a conventional CIM ethylenediamine disk and with that of the commercially available Poroszyme immobilized trypsin cartridge. In this work, we showed how different proteins were identified with good recoveries using a digestion time of 10 min only.

Fig. 2. IMERs-LC-ESI-MS/MS set-up for on-line protein digestion. Closed lines indicate position 1 (1–2) and dotted lines indicate position 2 (1–6). P1: isocratic pump delivering the mobile phase at 400 µl/min, 100 µl/min and 50 µl/min for IMERs based on CIM EDA disk, minidisk and Poroszyme, respectively; Injector: 50 µl of samples was loaded onto the IMERs; P2: quaternary pump delivering the acetonitrile gradient at 200 µl/min. For detailed conditions, see text.
Acid-Catalyzed Oxygen-18 Labeling of Peptides

Richard Niles,† H. Ewa Witkowska,†,‖ Simon Allen,† Steven C. Hall,†,‖ Susan J. Fisher,†,‡,§,∥,‖,⊥ and Markus Hardt*,†,#

Departments of Cell and Tissue Biology, Anatomy, and Pharmaceutical Chemistry, and UCSF Mass Spectrometry Core Facility, University of California—San Francisco, San Francisco, California 94143, and Lawrence Berkeley National Laboratory, Berkeley, California 94720
In enzymatic $^{18}$O-labeling strategies for quantitative proteomics, the exchange of carboxyl oxygens at low pH is a common, undesired side reaction. We asked if acid-catalyzed back exchange could interfere with quantitation and whether the reaction itself could be used as method for introducing $^{18}$O label into peptides. Several synthetic peptides were dissolved in dilute acid containing 50% (v/v) H$_2^{18}$O and incubated at room temperature. Aliquots were removed over a period of 3 weeks and analyzed by tandem mass spectrometry (MS/MS). $^{18}$O-incorporation ratios were determined by linear regression analysis that allowed for multiple stable-isotope incorporations. At low pH, peptides exchanged their carboxyl oxygen atoms with the aqueous solvent. The isotope patterns gradually shifted to higher masses until they reached the expected binomial distribution at equilibrium after ~11 days. Reaction rates were residue- and sequence-specific. Due to its slow nature, the acid-catalyzed back exchange is expected to minimally interfere with enzymatic $^{18}$O-labeling studies provided that storage and analysis conditions minimize low-pH exposure times. On its own, acid-catalyzed $^{18}$O labeling is a general tagging strategy that is an alternative to the chemical, metabolic, and enzymatic isotope-labeling schemes currently used in quantitative proteomics.
Quantitative proteomics is a rapidly expanding field, in particular, the application to clinical biomarker studies for diagnosis or prognosis of diseases, and the systematic analysis of protein functions in biological systems. **Isolation of a class of peptides or a subproteome enables reduction of sample complexity**, which is essential to perform sensitive, quantitative analyses over a wider dynamic range of protein concentrations. **Glycosylation is one of the most frequent PTMs**, and glycans have unique chemical properties that can be leveraged to selectively enrich for a subset of peptides, and thus facilitate the downstream analysis. The isolation of glycopeptides and its benefits for mass spectrometric measurements is discussed.
Phosphoproteomics: Miles To Go Before It’s Routine

Christine Piggee

Researchers see major technological advances but still have significant challenges to overcome.
Protein phosphorylation is a key regulator of cellular signaling pathways. It is involved in most cellular events in which the complex interplay between protein kinases and protein phosphatases strictly controls biological processes such as proliferation, differentiation, and apoptosis. Defective or altered signaling pathways often result in abnormalities leading to various diseases, emphasizing the importance of understanding protein phosphorylation. Phosphorylation is a transient modification, and phosphoproteins are often very low abundant. Consequently, phosphoproteome analysis requires highly sensitive and specific strategies. Today, most phosphoproteomic studies are conducted by mass spectrometric strategies in combination with phospho-specific enrichment methods. This review presents an overview of different analytical strategies for the characterization of phosphoproteins. Emphasis will be on the affinity methods utilized specifically for phosphoprotein and phosphopeptide enrichment prior to MS analysis, and on recent applications of these methods in cell biological applications.
Figure 1. Strategies for phospho-specific enrichment. Most commonly used strategies for immunoprecipitation, affinity chromatography or chemical modification applied for enrichment of phosphoproteins and phosphopeptides are illustrated.
Application of open tubular capillary columns coated with zirconium phosphonate for enrichment of phosphopeptides

Yanfeng Xue a,b,1, Junying Wei c,1, Huanhuan Han a, Liyan Zhao a, Dong Cao a, Jinglan Wang a, Xiaoming Yang a, Yangjun Zhang a,*, Xiaohong Qian a,**

a State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, No. 33 Life Science Park Road, Changping District, Beijing 102206, PR China
b School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China
c Department of Life Science and Technology, University of Beijing Institute of Technology, 5 South Street, Zhongguancun, Beijing 100081, PR China
ABSTRACT

A new approach utilizing open tubular capillary columns coated with zirconium phosphonate (ZrP-OTCC) for enrichment of phosphopeptides is described. The experimental conditions: interior diameter, length of capillary and flow rate was optimized using tryptic digest of α-casein (a phosphoprotein) as a model sample. The ZrP-OTCC was demonstrated to tolerate urea, sodium dodecyl sulphate (SDS), and NaCl. Further experimental results show that the ZrP-OTCC can trap the phosphopeptides even at the concentration of α-casein as low as $10^{-8}$ M. This column has also been successfully coupled online with nano-liquid chromatography for enrichment and then separation of phosphopeptides from a complex sample, and finally analyzed the phosphopeptides by mass spectrometry (MS).

Column size:
ID: 50 µm
L: 150 cm
Fig. 1. Model for preparation of the ZrP-OTCC and the procedure of enrichment of phosphopeptides. Detailed description for each step: (1) coating a thin layer of aminopropyl onto the interior surfaces of the OTCC; (2) transforming the aminopropyl-terminated OTCC into the phosphate-terminated OTCC; (3) loading zirconium ions into the phosphate-terminated OTCC to form the ZrP-OTCC; (4) capturing the phosphopeptides from the tryptic digest; (5) eluting the captured phosphopeptides from the ZrP-OTCC.
Automated metal-free multiple-column nanoLC for improved phosphopeptide analysis sensitivity and throughput

Rui Zhao¹, Shi-Jian Ding¹, Yufeng Shen, David G. Camp II, Eric A. Livesay, Harold Udseth, Richard D. Smith¹

We report on the development and characterization of automated metal-free multiple-column nanoLC instrumentation for sensitive and high-throughput analysis of phosphopeptides with mass spectrometry. The system employs a multiple-column capillary LC fluidic design developed for high-throughput analysis of peptides (Anal. Chem. 2001, 73, 3011–3021), incorporating modifications to achieve broad and sensitive analysis of phosphopeptides. The integrated nanoLC columns (50 μm i.d. × 30 cm containing 5 μm C18 particles) and the on-line solid phase extraction columns (150 μm i.d. × 4 cm containing 5 μm C18 particles) were connected to automatic switching valves with non-metal chromatographic accessories, and other modifications to avoid the exposure of the analyte to any metal surfaces during handling, separation, and electrospray ionization. The nanoLC developed provided a separation peak capacity of ~250 for phosphopeptides (and ~400 for normal peptides). A detection limit of 0.4 fmol was obtained when a linear ion trap tandem mass spectrometer (Finnegan LTQ) was coupled to a 50-μm i.d. column of the nanoLC. The separation power and sensitivity provided by the nanoLC–LTQ enabled identification of ~4600 phosphopeptide candidates from ~60 μg COS-7 cell tryptic digest followed by IMAC enrichment and ~520 tyrosine phosphopeptides from ~2 mg of human T cells digests followed by phosphotyrosine peptide immunoprecipitation.
Schematic diagram of the automated metal-free multiple-column nanoLC system. A: A photo of the automated metal–free multiple-column nanoLC system coupled with tandem mass spectrometry through electrospray ionization. B: The basic fluidic design of the nanoLC system; all valves used in this system were peek valves except refill and mobile phase valves; 4-cm long dual frits in-line SPE columns were used for the sample trapping with “back Flash” arrangement. C: To make the dual frits microSPE columns: (a): cut the packed capillary into 4cm for the microSPE column; (b): empty 1mm from both sides of the microSPE column; (c): Generate frits at the microSPE column both inlet and outlet (details are given in Section 2).
• YMSE
The correspondence problem for metabonomics datasets

K. Magnus Åberg • Erik Alm • Ralf J. O. Torgrip

Abstract In metabonomics it is difficult to tell which peak is which in datasets with many samples. This is known as the correspondence problem. Data from different samples are not synchronised, i.e., the peak from one metabolite does not appear in exactly the same place in all samples. For datasets with many samples, this problem is nontrivial, because each sample contains hundreds to thousands of peaks that shift and are identified ambiguously. Statistical analysis of the data assumes that peaks from one metabolite are found in one column of a data table. For every error in the data table, the statistical analysis loses power and the risk of missing a biomarker increases. It is therefore important to solve the correspondence problem by synchronising samples and there is no method that solves it once and for all. In this review, we analyse the correspondence problem, discuss current state-of-the-art methods for synchronising samples, and predict the properties of future methods.
Tuneable Microsecond-Pulsed Glow Discharge Design for the Simultaneous Acquisition of Elemental and Molecular Chemical Information Using a Time-of-Flight Mass Spectrometer

Auristela Solà-Vázquez, Antonio Martín, José M. Costa-Fernández, Rosario Pereiro, and Alfredo Sanz-Medel*

Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, c/Julián Clavería, 8, 33006 Oviedo, Spain
A microsecond-pulsed direct current glow discharge (GD) was interfaced and synchronized to a time-of-flight mass spectrometer MS(ToF) for time-gated generation and detection of elemental, structural, and molecular ions. In this way, sequential collection of the mass spectra at different temporal regimes occurring during the GD pulse cycle is allowed. The capabilities of this setup were explored using bromochloromethane as model analyte. A simple GD chamber, developed in our laboratory and characterized by a low plasma volume minimizing dilution of the sample but showing great robustness to the entrance of organic compounds in the microsecond-pulsed plasma, has been used. An exhaustive analytical characterization of the GD-MS(ToF) prototype has been performed. Calibration curves for bromochloromethane observed at the different time regimes of the GD pulse cycle (that is, for elemental, fragment, and molecular ions from the analyte) showed very good linearity for the measurement of the different involved ions, with precisions in the range of 7–13% (relative standard deviation). Actual detection limits obtained for bromochloromethane were in the range of 1–3 μg/L for elements monitoring in the GD pulse “prepeak”, in the range of 11–13 μg/L when monitoring analyte fragments in the plateau, and about 238 μg/L when measuring the molecular peak in the afterpeak regime.
Figure 5. Mass spectra obtained with micropulsed dc-GD-MS(ToF) after injection of bromochloromethane/hexane: (a) standard NIST mass spectra obtained via electron impact ionization; (b) mass spectra obtained in the prepeak time regime; (c) mass spectra obtained in the plateau time regime; (d) mass spectra obtained in the afterpeak time regime.
I sekken til påskefjellet?

© Springer-Verlag 2009

John Emsley: Molecules of murder. Criminal molecules and classic cases

D. Thorburn Burns

Bibliography
Molecules of murder. Criminal molecules and classic cases
John Emsley
RSC Publishing
Hardcover, 276 pages,
August 2008, £14.95