Heat Stabilization of the Tissue Proteome: A New Technology for Improved Proteomics

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After tissue or body fluid sampling, proteases and other protein-modifying enzymes can rapidly change composition of the proteome. As a direct consequence, analytical results will reflect a mix of in vivo proteome and ex vivo degradation products. Vital information about the presampling state may be destroyed or distorted, leading to variation between samples and incorrect conclusions. Sample stabilization and standardization of sample handling can reduce or eliminate this problem. Here, a novel tissue stabilization system which utilizes a combination of heat and pressure under vacuum was used to stop degradation in mouse brain tissue immediately after sampling. It was found by biochemical assays that enzymatic activity was reduced to background levels in stabilized samples. Western blot analysis confirmed that post-translational phosphorylations of analyzed proteins were stable and conserved for up to 2 h at room temperature and that peptide extracts were devoid of abundant protein degradation fragments. The combination of reduced complexity and proteolytic inactivation enabled mass spectrometric identification of several neuropeptides and endogenous peptides including modified species at higher levels compared to nonstabilized samples. The tissue stabilizing system ensures reproducible and rapid inactivation of enzymes. Therefore, the system provides a powerful improvement to proteomics by greatly reducing the complexity and dynamic range of the proteome in tissue samples and enables enhanced possibilities for discovery and analysis of clinically relevant protein/peptide biomarkers.

Keywords: proteomics • peptidomics • sample preparation • degradation • proteolysis • protease • phosphatase • enzymatic activity • sample handling • sample stability

Introduction

The dynamic nature of proteins presents a challenge in proteomics, yet the analysis and understanding of the proteome provide information which is not attainable from other sources, that is, DNA and RNA. In proteomics, diseased tissue samples or proximal fluids are often a more straightforward material for analysis to reach clinically relevant results due to higher analyte concentrations compared to plasma and serum,1,2 which, because of dilution and clearance, carry lower concentration of pertinent proteins. However, it has been shown that, following tissue sampling, substantial alterations of the proteome occurs.3–7 Removal of a sample from its natural sur-
Sample Stabilization

Table 1. Outline over the Comparative Experiments Carried out in This Study

<table>
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<tr>
<th>comparative experiments</th>
<th>Stabilizor treated</th>
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<tr>
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<td>7</td>
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The numbers in the Table refer to the Figures in the present paper for each individual experiment.

evident. In fact, in the low mass region, improperly handled samples are often of little practical use other than to visualize and study the degradation processes. The postsampling produced peptides affect the results in proteomic analyses either directly by modifying the protein from which they derive and/or indirectly by a serious contribution to sample complexity in bottom-up (shotgun) analyses. In the latter case, a dense proteolytic background in the mass spectra tend to mask the analytical signals from the in vivo proteome by ionic suppression or dynamic range limitations of the MS analysis. Hence, proteins in biological samples rapidly need to be stabilized in a standardized manner postsampling for reliable quantitative and identification analyses. In, for example, histological examinations, where the effects of postsampling degradation are apparent, protein stabilization is a fundamental part of sample preparation. In proteomics where sample disintegration and homogenization is an integral part of normal workflow, the need for protein stabilization is even greater. The freeze/thaw and homogenization actions assist proteolytic enzymes, which are normally kept separate from cellular proteins in vivo, to access substrates which they would otherwise not encounter. This facilitates and substantially accelerates degradation.

There are multiple strategies to hinder degradation of a sample, such as chemical enzyme inhibiting cocktails, freezing, heating, pressurizing, and chemical manipulations such as cross-linking or pH alterations. Many common techniques for sample stabilization are either of reversible character, unfavorable for general downstream analysis techniques, impractical or limited in some other way, such as toxic or expensive. For example, in vivo fixation through focused microwave irradiation to a mouse brain has previously been shown to successfully hinder postsampling variation in mouse brain samples. However, the application area for the focused microwave instrument is limited to the size of small rodents and optimized for the brain of living samples. In our laboratory, there has been a need for a tool capable of rapid stabilization of any type of tissue samples from all species. The possibility of attaining fresh samples is not always present and it is therefore important to be able to stabilize samples from a frozen state to avoid degradation during thawing, sample preparation and analysis.

In this study, a new instrument (Stabilizor T1, Denator AB) for stabilization of biological samples has been investigated. The instrument handles any type of tissue sample and is applicable on both fresh and frozen samples. The investigation, outlined in Table 1, includes a comparison between in vivo fixation through focused microwave irradiation, freezing combined with protease and phosphatase inhibitors and Stabilizor T1 treatment on fresh and frozen samples.

Materials and Methods

Stabilization Technology Principles. The Stabilizor T1 instrument enables efficient heat transfer through several features outlined in Figure 1. The heat conductive blocks are made out of aluminum and contain a vacuum seal which ensures that the sample is in contact with the heat block during the treatment. An upper heat block contacts the sample from above and presses the sample a pre determined percentage. Samples can be stabilized frozen or fresh and are heated for the time needed to reach above 90 °C in the whole sample without exceeding 95 °C in any part of the sample.

The sample is measured with a class 1 laser when it enters the treatment chamber in order to set the treatment time to a value where the inactivation temperature is reached. The laser measurement also enables complete automation of the process. For tracking reasons, log files with information from the sample inactivation such as treatment time, temperature, pressure, and sample ID are automatically collected from each run.

A sample container, Maintainer Tissue, has been designed to ensure sufficient contact with the heat source and is made out of thin polytetrafluoroethene (commonly known as Teflon) foils which allow efficient heat transfer. The foils also ensure that the tissue does not stick to the surface during the heating process. Once the sample is placed inside the cavity, air is evacuated and the upper flexible foil enfolds the sample. After treatment, the evacuated Maintainer may be stored in a freezer until further sample preparation.

Assessment of Phosphatase Activity. Brain tissue (occipital lobe cortex) from mice killed by decapitation were within 1 min following decapitation either heated at 95 °C in the Stabilizor T1 instrument (Denator AB, Sweden) at auto settings for fresh tissue (n = 5) or snap-frozen in liquid nitrogen and stabilized at auto settings for frozen tissue (n = 5). A third group of mice (n = 5) was killed by focused microwave irradiation (MW) to the brain for 1.4 s at 4.5–5 kW (Muromachi Kikai, Tokyo, Japan). The samples were homogenized by sonication (Vibra-Cell, Sonics & Materials) in 5.0 µL/PBS, and BSA, 100 µg. The homogenate was centrifuged at 20 000g for 30 min at +4 °C to pellet cell debris. The supernatant was collected and stored at −20 °C until activity measurement.

Phosphatase activity were colorimetrically assayed (Sensolyte pNPP Protein Phosphatase Assay Kit, AnaSpec, San Jose, CA) in a 96-well sample plate according to the supplied protocol. Briefly, a 50 µL sample of supernatant from extracts was mixed with an equal volume of pNPP reaction mixture and incubated for 45 min at +37 °C. No stop solution was used; instead, plates were scanned for absorbance directly after incubation at 405 nm. Blank, 1 x PBS, and BSA, 100 µg, samples were used as controls to establish a background baseline activity.

Assessment of Cytochrome C Oxidase Activity. Mouse brains were either stabilized (Stabilizor T1, Denator AB, Sweden) (n = 3) or snap-frozen within 1 min following decapitation (n = 3) and coronally sectioned in a microtome at −18 °C to 12 µm before they were thaw-mounted onto conductive glass.
slides. The sections were assayed in quadruplicates for cytochrome C oxidase activity using 3,3′-diaminobenzidine (DAB) substrate kit (Vector Laboratories, Burlingame, CA) in the following manner. Twenty-five milligrams of CytC type III (Sigma-Aldrich no. C2506) mixed with 4 g of sucrose was dissolved in 90 mL of 0.1 M 1× PBS, pH 7.4. The mixture was heated to +37 °C and then 66 drops of the DAB substrate were added for staining. The brain sections were then incubated for 1 h. Stained slides were washed twice in ddH2O and once in 80% EtOH.

Extraction Profile of Solubilized Protein (Recovery). Small pieces of mouse brain (cortex), liver, and heart were either snap-frozen (n = 5) or stabilized for 45 s at 95 °C (Stabilizor T1, Denator AB, Sweden) (n = 5) within 1 min following decapitation. The tissue samples were then pulverized with a ball mill (Retsch) while frozen with liquid N2 or with Tissue grinding kit (GE Healthcare). The ball mill was operated at 30 Hz for 45 s, whereas the tissue was ground for 2 min.

Proteins from stabilized and nontreated brain, liver, and heart samples were homogenized and extracted in 5× the weight of the sample. One percent hot SDS solution was added at 5× the weight of the sample. The steel balls were removed and the pulverized tissue was homogenized further by 10 × 2 s microtip ultrasound bursts at 40 W (Vibra-Cell, Sonic & Materials). Cell debris was pelleted by centrifugation at 20 000g before protein concentrations were measured using 2D Quant Kit (GE Healthcare, Sweden). SDS-PAGE was run using an Invitrogen XCell SureLock System with precast 1 mm NuPAGE Novex 12% Bis-Tris gels with the manufacturer’s recommended buffers. Twenty micrograms of protein was loaded per lane. Proteins were visualized using GelCode Blue Stain Reagent (Pierce).

Assessment of Protein Solubility. Small pieces of brain (cortex), liver, heart, or skeletal muscle from one mouse were either snap-frozen (n = 5) or stabilized at 95 °C (Stabilizor T1, Denator AB, Sweden) (n = 5) within 1 min following decapitation. The tissue samples were then pulverized with a ball mill (Retsch) while frozen with liquid N2 or with Tissue grinding kit (GE Healthcare). The ball mill was operated at 30 Hz for 45 s, whereas the tissue was ground for 2 min.

Proteins from stabilized and nontreated brain, liver, and heart samples were homogenized and extracted in 5× the weight in either 1% hot SDS, or 15 times the weight in standard proteomics IEF buffer (8 M Urea, 4% CHAPS, 50 mM Tris-base) using 10 × 2 s microtip ultrasound bursts at 40 W (Vibra-Cell,
Each sample was analyzed during a 40 min gradient from Germany) was used as spray emitter and analytical column. LC system (Ettan MDLC, GE Healthcare, Uppsala, Sweden) at in 0.25% acetic acid. The eluate was infused using a nanoflow SPECTROMETRY. The peptide extract (5 isolate the peptide content. 10 kDa centrifugal cutoff filter (Microcon YM-10, Millipore) to desalted on a precolumn (PepMapT, LC Packings, Amsterdam, Amsterdam, The Netherlands) at a flow rate of 10 µL/min of 0.25% acetic acid in water. A 15 cm, 75 µm i.d. fused silica needle (Proxeon Biosystems, Odense, Denmark) packed with Reprosil-Pur C18-AQ 3 µm resin (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) was used as spray emitter and analytical column. Each sample was analyzed during a 40 min gradient from 3–60% of azetropic acetonitrile (ACN)/water mixture (84%) in 0.25% acetic acid. The eluate was infused using a nanoflow LC system (Ettan MDLC, GE Healthcare, Uppsala, Sweden) at a flow rate of approximately 150 nL/min either into a Q-tof II mass spectrometer (Waters, Manchester, U.K.) for semiquan-
titative peptide analyses, or an LTQ ion trap mass spectrometer (Thermo Electron, San Jose, CA) for peptide identifications. The data acquisition from the mass spectrometer was performed in continuous mode at a frequency of 3.6 GHz and integrated into a single spectrum each second. Mass spectra were col-
clected in the m/z range of 300–1000. The data was exported to DeCyder MS 2.0 (GE Healthcare) for relative quantitation of peptide peak intensities integrated over time. Peptides were identified by amino acid sequence analysis of LTQ collision induced dissociation (CID) tandem MS (MS/MS) data. Four MS/MS spectra of the most intense peaks were obtained following each full-scan mass spectrum. The dynamic exclusion feature was enabled to obtain MS/MS spectra of coeluting peptides. Raw LTQ data were converted to dta files in Xcalibur (v. 1.4 SR1) and put together by an in-house developed script to Mascot generic files. Peptide identities were obtained by correlating the MS/MS spectra to the Mus musculus subdatabase of the Swiss-Prot database (10 431 sequences, release 48.8) using Mascot 2.1. The search parameters were as follows: partial oxidation of methionine (+16 Da), precursor ion mass tolerance of 1.5 Da, peptide mass tolerance of 1.5 Da, and fragment ions tolerance of 0.7 Da. No enzyme was specified to be used. The criteria for positive identification of a peptide was a Mascot score >50 (p < 0.05). The identification of phospho-
ylated peptides was done from MS/MS spectra where one of the three most abundant peaks in each spectrum corresponded to a neural loss of a phosphate group. These spectra were searched against SwePep precursor18 allowing for phosphory-
lations of serine, threonine and tyrosine and all hits were manually verified.

Peptide Analysis Using Matrix Assisted Laser Desorp-
tion/Ionization (MALDI) MS. Peptide extracts from snap-frozen and stabilized mouse brain tissue (striatum), prepared as previously described, were desalted, purified and enriched using C18 µ ZipTips (Millipore) according to the manufacturer’s recommendations. A 5 µL aliquot was acidified with an equal volume of 0.2% TFA. Peptides were eluted in 2 µL of 50% ACN/ 0.1% TFA and mixed 1:1 with α-cyano-4-hydroxycinnamic acid, 10 mg/mL in 50% ACN/0.1% TFA, and a dried droplet was spotted onto the MALDI target prior to MALDI analysis.
Spectra were collected in reflectron mode and analyzed with FlexAnalysis, ver. 3.0 (Bruker).

**Western Blot.** Mouse brain tissue (cortex) groups \( n = 5 \) were either stabilized within 1 min after decapitation and then homogenized, stabilized within 1 min after decapitation and left at room temperature for 2 h before homogenization, or stabilized 10 min after decapitation and then homogenized.

Quantitation of protein phosphorylation was carried out as previously described.\(^\text{19}\) Frozen brain tissue samples were suspended in 5× the volume of hot 1% SDS and homogenized by microtip sonication (Vibra-Cell, Sonics & Materials). Small aliquots of the homogenate were retained for protein deter-

(Autoflex III, Bruker, Bremen, Germany). Spectra were collected in reflectron mode and analyzed with FlexAnalysis, ver. 3.0 (Bruker).

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mination with a bicinchoninic acid kit (BCA1, Sigma, St. Louis, MO), using bovine serum albumin as a standard. Twenty micrograms of protein was loaded onto 4−20% TGI acrylamide gels (Invitrogen, Sweden) and the proteins were separated by SDS-PAGE and transferred to 0.2 µm nitrocellulose membranes (Immobilon-PSQ, Millipore, Sweden). The membranes were immunoblotted using commercially available phosphospecific antibodies recognizing MAPK (pThr202/pTyr205), CREB (pSer-133) and GSK 3β/beta two (pSer9) as well as antibodies recognizing total MAPK, CREB, and GSK 3β/beta two (Abcam plc, Cambridge, U.K.). Antibody binding was revealed by incubation with goat anti-rabbit horseradish peroxidase-linked IgG (Pierce Europe, Oud Beijerland, The Netherlands) and the ECL immunoblotting detection system (GE Healthcare, Uppsala, Sweden). Chemiluminescence was detected by autoradiography using DuPont NEN autoradiography film (Sigma, Sweden) and levels were quantified by densitometry using National Institutes of Health IMAGE 1.61 software.

**Results**

A method and an instrument for rapid protein inactivation was evaluated in an effort to improve analyses of fresh and
frozen tissue samples regarding proteins, endogenous peptides, and PTMs. The capability of this method was tested on mouse tissue using MS, immunoassays, colorimetric assays and electrophoresis.

**Enzyme Inactivation.** Any remaining phosphatase activity in the tissue samples following stabilizing treatment was assessed from colorimetric measurements of pNPP (Figure 2). There was a clear inactivating effect in the in vivo fixed group and the stabilized group compared to the snap-frozen group even when phosphatase and protease inhibitors were added to the extraction buffer (Student’s t test, \( p = 1.4 \times 10^{-6} \)). The inhibiting effect of added inhibitors could only be observed on snap-frozen tissue (\( p < 2.5 \times 10^{-4} \)).

The inactivation of cytochrome C oxidase was displayed by incubating snap-frozen cryosections of mouse brains, stabilized or not, with cytochrome C type III and DAB. After 1 h, a strong brown staining developed of the untreated brain section only, indicating oxidase activity (Figure 3).

**Protein Solubility.** The ability to solubilize proteins after or during sample treatment is paramount. Protein solubilization from denatured tissue is very dependent on solubilization buffer. The protein expression profiles displayed by SDS-PAGE were similar for snap-frozen and stabilized tissue when comparing brain, heart, and liver tissue (Figure 4A). Correspondingly, no significant differences in extraction levels were found between snap-frozen and stabilized tissues in both standard proteomics extraction IEF buffer (8 M urea, 2.5% CHAPS and 50 mM Tris-base) and hot 1% SDS buffer (Figure 4B). In 1× PBS and similar aqueous buffers, protein solubility was lower in stabilized tissue compared to native tissue (data not shown).

No significant difference in the ability to extract proteins was found between ball mill (Retsch) homogenization and Tissue grinding (GE Healthcare) techniques (data not shown).

**Relative Quantitation of Phosphorylated Peptides.** The levels of three phosphorylated peptides from the Secretogranin precursor (scg1) were monitored by nanoLC-ESI MS and compared between the separate treatment groups (Figure 5). Two of the peptides, scg1 186–199 phosphorylated at Ser190 and scg1 313–330 phosphorylated at Ser318, showed significantly higher levels in stabilized samples (fresh and frozen) compared to snap-frozen (Student’s t test, \( p < 1.6 \times 10^{-6} \) and \( p < 1.3 \times 10^{-8} \), respectively). One peptide, scg1 375–383 phosphorylated at Ser378, was detected atunaltered levels in all four treatment groups, demonstrating a stable phosphorylation.

**Relative Quantitation of Phosphorylated Proteins.** In a Western blot analysis of phosphorylated CREB, GSK, and MAPK, it was shown that the stabilized samples conserved the phosphorylations of the analyzed proteins up to 2 h in room temperature. When the same proteins were analyzed after only 10 min postmortem in snap-frozen samples, they were detected at significantly lower levels for GSK (Student’s t test, \( p = 6.5 \times 10^{-6} \) and \( p < 1.3 \times 10^{-8} \), respectively). One peptide, scg1 375–383 phosphorylated at Ser378, was detected atunaltered levels in all four treatment groups, demonstrating a stable phosphorylation.

**Number of Peptide Fragments Derived from Degraded Proteins.** Proteolytic activity carried out by enzymes was inhibited in the stabilized and in vivo fixed samples. This is illustrated by a significantly larger number of detected peptides in the snap-frozen samples compared to the stabilized fresh (Student’s t test, \( p = 1.2 \times 10^{-2} \)) and frozen samples (\( p = 9.0 \times 10^{-3} \)), and in vivo fixed samples (\( p = 1.7 \times 10^{-3} \)) (Figure 7). Consequently, most of the identified peptides in snap-frozen tissue derived from ex vivo produced proteolysis of highly abundant proteins such as hemoglobin, tubulins, dynamin, heat shock proteins, and actins (Supplementary Table in Supporting Information). The recurrent appearance of many of these proteins in several differential proteomics studies suggests, as recently reported by Petrak and colleagues, they represent common cellular stress responses or reflect technical limitations of the technique rather than being specific markers.
of, for example, an investigated disease state. The detected ion intensity of a degradation fragment from the protein stathmin (stathmin 2–20) concurred with previously reported results where it was reported as an indicator for the general sample integrity.7

Relative Quantitation of Selected Endogenous Peptides. The postsampling degradation effect on endogenous peptides in nondenatured samples is evident in the MALDI MS analysis of brain extracts. All but one identified endogenous peptide was undetectable in untreated samples as compared to stabilized samples (Figure 8). For example, the enkephalin neuropeptides (met-enk-RF and met-enk-RSL) were clearly detected in the stabilized samples and almost undetectable in the snap-frozen samples. Interestingly, the isotopically labeled neuropeptides added as internal standards were only detected in stabilized tissue, aside from the labeled protein fragment stathmin (2–20) which was detected independent of sample treatment, indicating ex vivo proteolytic activity and the relative stability of the stathmin (2–20) fragment.

Discussion

To analyze tissue samples with sensitive and information rich methods in a diagnostic manner, it is essential that the prepared sample reflects the in vivo state of the tissue. Active enzymes in native samples degrade proteins and endogenous peptides rapidly after sampling which not only is reflected on the protein and peptide integrity and their levels, but also on the composition and level of the analytical background from ex vivo produced protein fragments and peptides of abundant proteins. Measures to remove these abundant proteins, such as depletion by affinity chromatography, are generally redundant and introduced too late in the sample handling process because of the quick degradation onset after tissue homogenization. Moreover, the extent of PTMs of proteins and endogenous peptides, for example, phosphorylations, are altered equally rapidly during handling in an active tissue sample.

One of several methods to inactivate a protein is by means of heat, at temperatures high enough to break the intramolecular hydrogen bonds, polar and van der Waals interactions. This causes the proteins in the sample to shift in secondary and tertiary structural arrangements and become denatured with a resulting loss of original functions.21 Consequently, enzymes of degradation, for example, proteases, are irreversibly inactivated along with other proteins.

There are a number of available methods for heating of biological samples where the main methods are microwave irradiation and conductive heat transfer. Microwave irradiation overcomes the limitation of low heat conductivity in tissue and rapid heating can be achieved even in large samples. There are however several disadvantages with microwave irradiation, where the most severe is that homogeneous heating is difficult to achieve and hot spots are generated. This means that for the purpose of heat inactivation of tissue samples certain parts of the sample risk boiling before other parts of the sample have reached inactivation temperature. The subsequent analysis can be distorted due to nonhomogeneous conditions in the heating process. Highly specialized microwave applicators such as the Muromachi system achieves a higher degree of homogeneity due to the optimized applicator; however, in our hand, there are still samples that retain enzyme activity, probably due to cold spots during the heating process. Another limitation of the microwave technology at 2450 MHz is the low dissipation in frozen samples. It is exemplified by the difference in power dissipation between water at 25 °C and ice, where water is theoretically heated 4300× faster. There is always some thawing in a tissue sample and those parts are rapidly brought up to boiling point, whereas frozen parts are hardly affected at all. Samples of limited size can efficiently be heated with optimized conductive heat where the sample is brought into contact with a material with high heat conductivity and where sufficient amount of energy is stored to avoid temperature decrease in the transferring body. A key parameter is to ensure full contact with the sample since air is an efficient insulator of heat transfer (exemplified by the difference in thermal conductivity of air which is approximately 0.025 W/(m K) compared to Aluminum 237 W/(m K). An additional important factor for efficient heat transfer is that the sample container itself allows contact with the heat conducting surface.

The addition of protease and phosphatase inhibitors into the extraction buffer of snap-frozen samples is inadequate to eliminate the activity as shown in the present study. Remaining enzyme activity produces sufficient ex vivo protein fragments to skew the analysis by altering the levels of the analyte or by disguising it behind a substantial wall of proteolytic peptides. Thus, the addition of protease and phosphatase inhibitors merely introduces exogenous substances that may interfere with a downstream analysis further without offering complete protection from ex vivo enzyme activity.

The method and instrument we outline in this manuscript describe the use of thermal denaturing to inactivate the proteins after sampling. The system, consisting of the Stabilizor T1 instrument and the associated Maintainor Tissue card, uses a combination of heat, pressure, and vacuum to raise the temperature of the sample tissue fast, homogeneously and reproducibly. As a consequence, proteolytic enzymes denature and their activity is efficiently eliminated with a resulting stabilized and conveniently packed sample. The different analyses showed comparable results between in vivo fixed samples and ex vivo stabilized samples, both fresh and frozen. Of course, the results from ex vivo samples are also influenced by the speed of sample dissection.

It has been noted that both stabilized and in vivo fixed samples demand more rigorous homogenization methods for efficient solubilization. Concern has to be taken to maximize area exposition of the sample and to solubilize in buffer volumes sometimes exceeding 10× of the weight of the sample. With this in mind, representative protein recovery from the sample can be obtained in standard proteomic IEF buffer. However, the sample recovery experiments, in terms of buffers and homogenization methods, carried out in this study are not to be considered as complete but should be evaluated for optimized compatibility with the chosen subsequent application.

Conclusions

Proteome deterioration postsampling is a major problem facing proteomic research in general and temporal proteomics in particular. The tissue stabilizing technology presented in this paper effectively preserves the proteome of the sample by means of rapid and reproducible heat denaturation. In contrast to in vivo fixation by focused microwave inactivation, the presented tissue stabilization technology can be applied with similar results to a large range of tissues, both fresh and frozen. Heat stabilization is compatible with major proteomic workflows and has been shown to be beneficial compared to snap freezing methods even when chemical enzyme inhibitors were included. Compared to traditional protein stabilization ap-
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proaches, the use of heat stabilization avoids introduction of additives that can interfere with downstream analyses and protects proteins during thawing and subsequent preanalytical steps.

The effect of Stabilizor treatment on protein integrity of other tissues is currently in progress and being evaluated using 2D-DIGE, MS based proteomics, protein arrays, and MALDI-imaging.

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Supporting Information Available: Table listing the protein fragments identified in the snap-frozen group. This material is available free of charge via the Internet at http://pubs.acs.org.

References

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