A troubleshooting guide:

Mass spec experts share their advice on handling key aspects of sample prep

Mass Spec Sample Prep Tech Guide

Q&A:

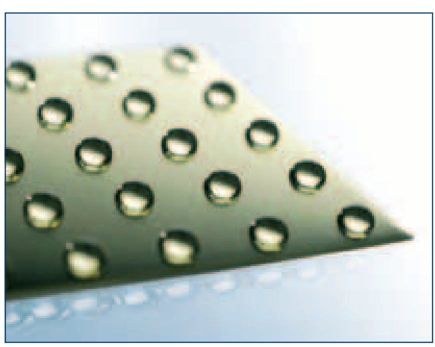
Genome Technology
Inside Integrated Biology

GenomeWeb
The GenomeWeb Intelligence Network

Advertisement

Increased sensitivity and throughput in MALDI-MS

In proteomics, high-quality data require high-quality sample preparation procedures. The latest additions to QIAGEN's proteomics product line are Mass·Spec Chips, which provide elegant solutions for MALDI-MS sample preparation and offer numerous advantages over conventional methods. Mass·Spec·Focus chips provide on-chip processing to minimize sample losses and maximize signals. Functionalized surfaces provide in situ concentration, desalting, cleanup, or purification of phosphopeptides. High-density Mass·Spec·Turbo chips are pre-spotted with matrix and offer superior sensitivity in high-throughput MALDI analysis.



Make the most of your MALDI-MS samples with:

- **Mass·Spec·Focus Chips** — zoom in on your target protein using on-chip sample preparation
- **Mass·Spec·Turbo Chips** — power through your sample with high-density pre-spotted matrix MALDI chips

Professor Mark Baker, Chief Executive Officer, Australian Proteome Analysis Facility (APAF), Sydney, Australia

We have utilized Mass·Spec·Focus Chips with our high-end ABI mass spectrometers and have achieved dramatically better limits of detection. The results have been fantastic. Also, we are very excited about our recent results with the IMAC surfaces.

Mass·Spec Chips complement QIAGEN's Qproteome range of proteomics sample prep kits. Advancements in the Qproteome and Mass·Spec product ranges will offer you more choice and flexibility, and keep you at the cutting edge of proteomics sample prep and MALDI-MS analysis.

Discover how to make the most of your MALDI samples at www.qiagen.com/maldiprep !

WWW.QIAGEN.COM

Table of contents

Letter from the Editor 4

Index of Experts 4

Q1: How do you **minimize the presence of salt, detergent, and other contaminants** in your sample? 6

Q2: Which techniques do you use to **deplete or partition high-abundance proteins**? 8

Q3: Which **fractionation methods** do you use after sample enrichment? 9

Q4: What strategies do you use to include **internal or external calibration standards**? 10

Q5: How do you **optimize protein extraction** from difficult or complex samples? 11

Q6: Under what **conditions do you label proteins, and when you do, which peptide-labeling reagents** do you use? . . .12

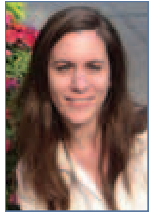
Five minutes a day keeps you ahead of the helix.

GenomeWeb

Visit 2, 3, 4 times a day for concise, authoritative news reports on the business and technology of genomics, updated continuously throughout the business day.

www.GenomeWeb.com

Letter from the editor



For this latest installment in *Genome Technology*'s tech guide series, we've assembled an impressive panel of experts on mass spec sample prep.

In the pages to come, you'll see what the contributors below have to say about handling key pre-experimental challenges such as extracting protein from complex sources, applying calibration controls, and more.

Many thanks are due to the experts in this guide, as well as to those who played a supporting role. We are grateful to Douglas Hinerfeld and John Leszyk for consulting on the responses submitted by Sunny Tam, and to Dave Sarracino for advising on MingMing Ning's contribution.

— Jennifer Crebs

Index of experts

Philip Andrews
University of Michigan Medical School
Professor, Department of Biological Chemistry

Keith Ashman
Australian Proteome Analysis Facility
Director of Mass Spectrometry

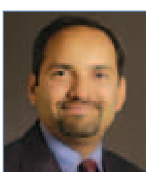
Pawel Ciborowski
University of Nebraska Medical Center
Director, Proteomics Program

Hediye Erdjument-Bromage
Memorial Sloan-Kettering Cancer Center
Associate Laboratory Member

David C. Muddiman
North Carolina State University
Professor, Department of Chemistry

MingMing Ning
Mass General Hospital
Harvard Medical School
Clinical Proteomic Research Center

Scott Patterson
Amgen
Senior Director, Medical Sciences



Alex J. Rai
Memorial Sloan-Kettering Cancer Center
Assistant Attending, Department of Clinical Laboratories

Gary Siuzdak
The Scripps Research Institute
Senior Director, Center for Mass Spectrometry

David W. Speicher
The Wistar Institute
Professor and Chair, Systems Biology Division

Sunny Tam
University of Massachusetts Medical School
Proteomic Consortium

Jinsam You
Indiana Center for Applied Protein Sciences
Senior Research Scientist

Are you trapped by organelle purification?

Automation Preparation Fractionation Accumulation Concentration Enrichment

Count on AWPT to improve your purification.

Alfa Wassermann Proteomic Technologies, LLC has developed a novel buoyant density accumulator technology permitting gentle isolation and enrichment of organelles.

The buoyant density accumulator preparative organellar separation system allows for the automated fractionation, concentration and enrichment of organelles by continuous-flow ultracentrifugation.

Visit us at ASMS Booth #143.

800.220.4488, ext. 178
www.awpt.us

How do you minimize the presence of salt, detergent, and other contaminants in your sample?

It depends on the nature of the sample. Precipitations of intact proteins with organic solvents (acetone or ethanol) are effective for many contaminants. Binding to solid supports (SCX, HILIC, RP) is effective for removing many contaminants from many peptides. Other methods are more desperate (washing MALDI samples with cold water, overspotting matrix, etc.). Larger amounts of protein can often be cleaned up by dialysis or size exclusion, but attention should be paid to minimizing proteolysis or other artifacts. The best solution, when possible, is to avoid introducing contaminants. Volatile buffers are helpful and reducing the introduction of detergents and polymers through contaminated glassware.

— Philip Andrews

> "It depends on the nature of the sample. Precipitations of intact proteins with organic solvents are effective for many contaminants."
>
> *— Philip Andrews*

- Consultation with clients to ensure suitable buffers are used (e.g. low salt, detergent-free)
- Buffer exchange
- Precipitation for intact protein samples; 1D or 2D gel separation prior to proteolysis
- SCX clean up after digestion
- SDS removal column
- ZipTips or similar methods
- Peptide trap columns

— Keith Ashman

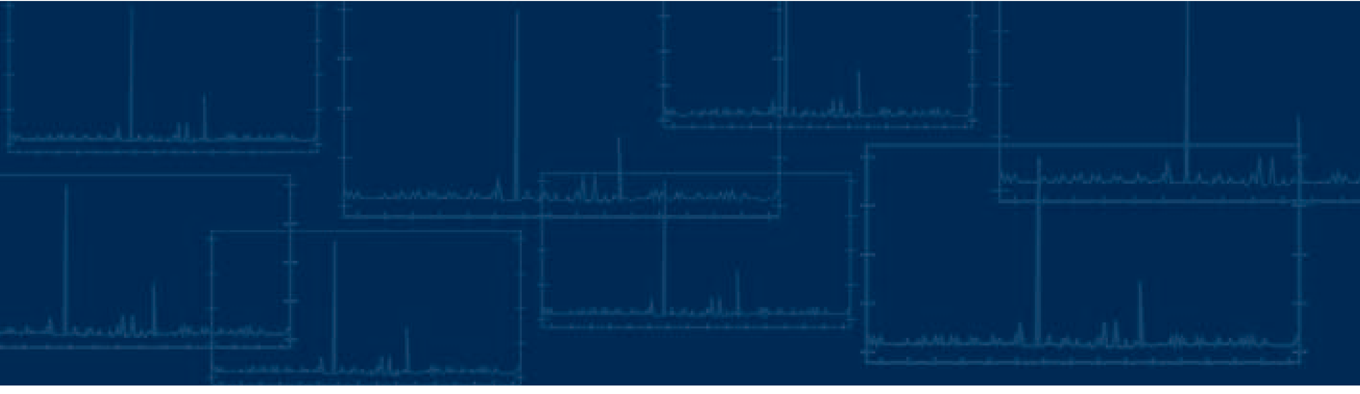
Culture supernatants of *in vitro* samples usually cause this problem because secreted proteins have to be concentrated and medium contain 0.14M of NaCl. For concentration we use membrane spin devices (Vivaspin filters from Vivascience) and will be using the de-salting RP-HPLC mRP-C18 column from Agilent. Other contaminants we remove using the 2D clean-up kit from Amersham/GE Healthcare for 2D SDS-PAGE. This technique removes also detergent and salts. For 1D SDS-PAGE we usually do not remove detergent, but keep the levels of lysis buffer as low as possible to begin with. Alternative techniques which we tested are acetone and chlorophorm:methanol precipitation. They are not used extensively since 2D clean-up kit works well. After tryptic digest we use ZipTips for sample cleaning.

— Pawel Ciborowski

The last step of purification is typically reversed-phase (RP) HPLC for polypeptides or SDS-PAGE for proteins. That will ensure samples are free of salts, detergents, and contaminants.

In case of protein identification and/or post-translational modification studies, SDS-PAGE-bound material is subjected to trypsinization; resulting peptides obtained from a single protein or 'gel-stacked' proteins (50-100 proteins stacked in a single gel slice) are then purified and concentrated using our homemade, RP-containing gel loading tips for manual, off-line, liquid chromatography. This clean-up step is always performed before both LC/MS/MS and MALDI-TOF analysis.

— Hediye Erdjument-Bromage



We carry out a lot of off-line 2D LC measurements, and after the strong-cation exchange step we desalt by extensively washing the peptides on a trap cartridge prior to reverse-phase HPLC.

— David Muddiman

We use HPLC and off-line disposable columns for clean up.

— MingMing Ning

Reversed-phase clean-up generally, often using Oasis cartridges and plates.

— Scott Patterson

The best way to minimize the presence of these contaminants is to pretreat your specimen in some manner. Possibilities include sample pre-fractionation (there are many types of chromatography for this purpose, including size exclusion to remove salts), dialysis to change the buffer system, protein precipitation using acetone or a similar procedure, and further sample enrichment using a more targeted approach, e.g. antibody-based targeting of an antigen of interest.

— Alex Rai

Since we're primarily performing LC/MS/MS experiments for protein identification or quantification, the presence of salts is typically not a big issue, though we do try to avoid detergents that can interfere in the LC/MS analysis. With MALDI, that becomes more of a problem because you have to remove the salts prior to MALDI analysis.

— Gary Siuzdak

> "Proteins that will be introduced directly into the mass spectrometer are often cleaned up on a reversed-phase column."
>
> *— David Speicher*

We use varied methods depending upon the type of sample and the processing steps prior to MS. For example, many samples are run on 1D gels followed by digestion in-gel. So any sample components compatible with SDS are fine. The trypsin digestion conditions are directly compatible with loading onto a reversed phase column connected on-line with the mass spectrometer. In other cases, if we need to clean up intact proteins, one option is TCA precipitation or ethanol precipitation. Alternatively, proteins that will be introduced directly into the mass spectrometer are often cleaned up on a reversed-phase column — either on-line or off-line.

— David Speicher

We normally use acetone precipitation, ultrafiltration, C18 or C4 ZipTip, or ion exchange column to eliminate salt or detergent.

— Sunny Tam

To minimize the presence of salt, detergent, or other contaminants, we use ether precipitation, filtration, and TCA/acetone precipitation.

— Jinsam You

Which techniques do you use to deplete or partition high-abundance proteins?

Predominantly immunodepletion columns, preparative electrophoresis (IEF, SDS), and organelle/membrane isolation. Other approaches are used as required in special cases.

—Philip Andrews

- Affinity columns are used to deplete high-abundance proteins
- Gel are used to separate high-abundance proteins away from proteins of interest

— Keith Ashman

To remove most abundant proteins from serum and CSF samples we use an HPLC column from Agilent. Soon we will be using columns to remove more than six [of the] most abundant proteins. Aurum BioRad spin minicolumns did not work well in our hands.

— Pawel Ciborowski

Affinity purification of protein complexes usually overcomes the problem of high-level background; i.e., proteins non-specifically associating with any protein of interest. We encourage investigators to utilize FLAG-tagged or TAP-tagged baits to study protein-protein interactions. Pilot experiments must be done to further establish best ways of dissociating the interacting proteins from the bait. There is always a process of going back and forth to get optimum conditions until a useful and interesting complex is obtained in quasi-pure form.

— Hediye Erdjument-Bromage

We have been working with the MARS column and continue to do so. It is a very nice solution and we find it to be very robust. We are also working with a newer technology based on peptide libraries — interesting concept and our initial data looks promising.

— David Muddiman

For plasma, we have used albumin depletion. In cell culture, we have worked out a protocol with serum-free media so that there is little or no albumin or high-abundance protein present in the feeding media. This has enhanced our dynamic range in detecting low-abundance proteins in conditioned media.

— MingMing Ning

For partitioning of these samples, one can use various chromatographic or bulk fractionation procedures, such as ammonium sulfate fractionation or ion-exchange chromatography. These are the two most common methodologies. However, any procedure which can allow for selection of a subset of proteins based on chemical characteristics will suffice. For depletion methodologies, many are commercially available — Sigma and Pierce have kits available for this purpose. In addition, Beckman Coulter has specific chicken immunoglobulins (IgY antibodies to high-abundance proteins) available for targeting of specific antigens.

— Alex Rai

We're typically using the immunodepletion MARS [Multiple Affinity Removal System] columns that Agilent has — which gets rid of the top six proteins such as IgGs, albumin, transferrins — and that has worked extremely well for us.

— Gary Siuzdak

(continued on p.13)

Which fractionation methods do you use after sample enrichment?

Several, ranging from electrophoresis (SDS, IEF, blue native gels, etc.) to chromatography methods (SCX, reversed-phase, immunoenrichment).

— Philip Andrews

- 1D and 2D gels
- Chromatography, SCX fractionation, reversed-phase HPLC directly into the mass spectrometer

— Keith Ashman

Currently 1D and 2D SDS-PAGE, although we used liquid chromatography in the past.

— Pawel Ciborowski

For complex protein samples, we ask the investigator to prepare the proteins by using SDS-PAGE with a 4 percent stacking gel and 10 percent separating gel in the presence of pre-stained molecular weight markers. As soon as the proteins leave the stacking gel and enter the separating gel (as judged by the mobility of the standard marker stack), we ask that the stack is allowed to travel about 2 mm to 1 cm in the separating gel, depending on the estimated complexity of the mixture (as previously determined by running a very small fraction of the complex and silver-staining the displayed bands). Then, electrophoresis is stopped and the gel stained with Coomassie. Depending on the length of the stack, we may divide it into up to three (or more) gel slices, starting from the top of the stack all the way to the end, for tryptic digest, etc. The data obtained from the individual LC/MS/MS runs of each of the slices are eventually combined and searched to generate a single list of identified proteins.

— Hediye Erdjument-Bromage

At the peptide level, we carry out off-line 2D-LC — something we started doing about four years ago. At the protein level, we have found the mRP column from Agilent to be quite effective.

— David Muddiman

We have used both in-gel fractionation and chromatographic methods.

— MingMing Ning

It varies, sometimes affinity methods, other times ion-exchange.

— Scott Patterson

Actually, I prefer to perform bulk separation procedures, such as fractionation (or ammonium sulfate precipitation) first, and then enrich my sample to concentrate my protein target of interest. Fractionation methods include ion-exchange fractionation, either anion- or cation-based. I will then subsequently use a more targeted approach, such as immunoprecipitation with a specific antibody.

However, such an endeavor (target purification) depends on the endpoint of interest. There is a big difference between isolating a known protein of interest versus preparing to perform a screening procedure to identify a novel biomarker. With these two distinct endpoints, different strategies must be employed. In the former, you have the advantage of knowing and exploiting the biochemical properties of your target.

— Alex Rai

We have developed a three-

(continued on p. 13)

What strategies do you use to include internal or external calibration standards?

Depends on the instrument and the sample. Internal standards can sometimes be used, but we are always somewhat concerned about ion suppression. It is important to scale the level of calibrants added as internal standards to the level of the analytes. Frequent running of external calibrants is necessary and monitoring long-term stability of instruments in LIMS or even a logbook is very helpful.

— Philip Andrews

For 2D SDS-PAGE DIGE we use Cy2 labeled internal standardization method following Amersham/GE Healthcare recommendations. Otherwise we use 2DQuant kit for protein determinations.

— Pawel Ciborowski

There is a tradition in our lab to emphasize mass accuracy. In case of peptide mass-fingerprint identifications, we use three added calibrants; when we analyze a known protein for post-translational modifications, we utilize peptides from the protein itself to calibrate the spectrum. In case of LC/MS/MS, the instrument is externally calibrated once a week. If, however, the standard peptide mixture that was run at the beginning of the day of the analysis was not within the mass accuracy we desire, the instrument is recalibrated for the LC/MS/MS experiment.

— Hediye Erdjument-Bromage

For mass measurement accuracy (the x-axis issue) we use a dual ESI source developed in my laboratory. It is robust and allows us to internally calibrate every mass spectrum. More recently, I have been working on the development of new calibration laws for FT-ICR mass spectrometry that further improve the results.

For quantification, we spike in internal standards and have developed some normalization strategies for our label-free proteomics approaches. When we use stable-isotope labeling, quantification is obviously more straightforward.

— David Muddiman

We have done ELISA or western quantification of known protein of interest within each sample as controls. This helps us to gauge sample-to-sample variability as well. We have also spiked the sample with known protein such as ovalbumin as internal control.

— MingMing Ning

For internal calibration purposes, I would include spiked proteins and peptides of defined/known molecular weight. You can either purchase individual proteins separately and combine them, or you can purchase a mixture of proteins. I would spike them into control buffer samples in addition to biological samples of a known matrix, such as serum, plasma, or other fluid. The purpose of the latter is to assess matrix effects, i.e., the impact of other proteins in your sample on the migration and detection of these known peptides and proteins.

For external calibration purposes, you can spot your protein/peptide mixture on a separate spot of the same chip, not in the presence of any of your experimental samples. This is usually done in the absence of matrix for the purpose of initial calibration of the instrument. It should be noted that mass spectrometry may not be quantitative under all conditions. Proteins are

(continued on p.13)

How do you optimize protein extraction from difficult or complex samples?

This is a very sample-dependent situation. Different sources of membranes may require varying ratios and types of detergents and other solubilizing agents (urea, organic solvents). Cytoskeletal proteins and large proteins may also have different requirements for solubilization.

Connective tissue, spore coat proteins, and fossil proteins may even require *in situ* proteolytic digestion in addition to the use of aggressive solubilizing conditions.

— Philip Andrews

We have a set of optimized SOPs, based on considerable experience. These are applied on a case-by-case basis. Essentially, acidic, basic, and organic solvent extractions using volatile buffers.

— Keith Ashman

"We do serial fractionation with chromatographic and/or in-gel methods to go deeper in increasing the dynamic range of detection."

— MingMing Ning

Currently we are studying secreted and cytosolic proteins which are relatively easy to extract. Therefore, we do not have much experience at this point in working with membrane, organelle, and other such types of proteins.

— Pawel Ciborowski

This is a case-by-case study and the conditions have to be optimized for each protein. If chaotropic agents or detergents are used, they will be removed before the sample can be processed for mass spectrometric analysis. If we are looking at dissociation of proteins interacting with a specific bait, as outlined in Q2, a thorough pilot study must be performed (with mass spectrometric identification) to establish the best conditions to extract/dissociate proteins from the bait protein.

— Hediye Erdjument-Bromage

Most of our effort to isolate a low-abundance protein from a complex biological matrix is by the use of a dual antibody approach. Given that we most often work with plasma, serum, and cerebrospinal fluid, protein extraction is rather straightforward.

— David Muddiman

We do serial fractionation with chromatographic and/or in-gel methods to go deeper in increasing the dynamic range of detection. Although this sacrifices some of the "high-throughput" nature of the "omic" method, we are able to discover low-abundance, novel proteins of interest. We are working on standardizing procedures to increase throughput while widening detection limits.

— MingMing Ning

Try different solvent/buffer conditions — this hasn't often been a problem with the samples we deal with.

— Scott Patterson

This will depend largely on the type of sample that you are trying to extract proteins from — bacteria, yeast, plants, cell culture *(continued on p.14)*

Under what conditions do you label proteins, and when you do, which peptide-labeling reagents do you use?

We use chemical modification predominantly for quantification, although we also use it occasionally for modifying the MS/MS spectra (e.g., Keogh reagent) and verifying *de novo* assignments in difficult cases. The primary quantification reagents we use are iTRAQ and ICAT. We also use SILAC for quantification for tissue culture when appropriate.

— Philip Andrews

The only labeling technique we use at this time is for DIGE technology. We do not derivate peptides for quantitative mass spectrometry.

— Pawel Ciborowski

We rarely ever label proteins. However, we do receive them on occasion from investigators. For instance, we have studied tritium (H3) labeled histones to establish N-terminal tail of histone methylations (Lys and/or Arg methylation) by carrying out radio-isotope sequence analysis of the generated amino acids (by Edman degradation); released amino acids are then analyzed by scintillation. P32 is often used in case of phosphorylation site(s) mapping in a particular protein. The label simply helps us trace our steps during sample handling/enrichment. Recently, we have also looked at cysteine-rich proteins using differential alkylating agents which generate either Cys-N-Ethylmaleimide or Cys-iodoacetamide. Both of these reagents can be used before the protein is purified by SDS-PAGE. *In situ* generated, free Cys-containing peptides can further be derivatized by acrylamide as another reagent which also works well for this amino acid. We have not yet investigated metabolic labeling of proteins for quantitative analysis.

— Hediye Erdjument-Bromage

We use two main peptide labeling reagents, iTRAQ and $^{16}O/^{18}O$ labeling. We find these two methods to be quite effective at accomplishing our goals. We have evaluated other methods and even published some of them, but expense and/or poor protein coverage eliminated as a choice for future use. For cell culture experiments with immortalized cell lines, we use SILAC as our method of choice.

— David Muddiman

When binary comparisons are required, we have used ICAT, iTRAQ, and dimethyl labeling (with and without heavy isotopes).

— Scott Patterson

I have directly labeled proteins for two different purposes in recent years. The first thing you will need to start with is a relatively pure preparation of your protein/antibody of interest.

In the first case, I was interested in designing an assay for a particular antigen. I wanted to design an ELISA which required two different antibodies derived from different species (one antibody for capture and the other for detection), and in this case only rabbit antibodies [when] available. I was able to obtain reagents commercially (Sigma-Aldrich/Pierce Biotechnology, etc.) in order to label my antibodies directly to biotin. I then used this labeled reagent as a capture antibody, and coupled it to streptavidin conjugated secondary antibody as a universal reagent, in order to set up this procedure.

In a second instance, I was interested in performing double label immunofluorescence to assess co-localization of two different

(continued on p.14)

Q2: Which techniques do you use to deplete or partition high-abundance proteins?

(continued from p.8)

We use immunoaffinity resins. We have tested most of the current commercial products, and all of them work reasonably well. For human serum and plasma, we have extensively used the MARS top six depletion column from Agilent, and more recently have switched to top 20 depletion using a new column from Sigma, the Prot-20 column.

— David Speicher

To deplete high abundant proteins from human body fluids, we use antibody-based approaches, such as the ProteomeLab IgY-12 from Beckman-Coulter. We also use a dye-based approach such as the Montage Albumin depletion kit from Millipore. We also continue to evaluate new approaches as they become available.

— Jinsam You

Q3: Which fractionation methods do you use after sample enrichment?

(continued from p.9)

dimensional separation approach where we do the initial protein digestion, followed by reversed-phase fractionation, strong cation exchange separation, then finally reversed-phase LC/MS/MS.

It's a convoluted extraction approach, but it's given us some nice data. Since we're dealing with a huge number of peptides, when you think about the fact you're starting out with 30,000 to 50,000 proteins and then you do a digest on them, you easily end up with a million peptides. So a one-dimensional approach is certainly not enough. A two-dimensional run is barely adequate. We ultimately need even higher-resolution separation, and that is why we developed this three-dimensional approach for our serum samples.

— Gary Siuzdak

The primary protein fractionation method we use is MicroSol IEF, which was developed in our lab, and is now available commercially as the Invitrogen ZOOM IEF fractionator. The other fractionation method we frequently use, either alone or after MicroSol IEF, is 1-D SDS PAGE. The entire gel lane is then cut into uniform slices followed by trypsin digestion and LC-MS/MS.

— David Speicher

After sample enrichment, sample can be further fractionated by liquid-phase isoelectric focusing, such as the Invitrogen ZOOM fractionator or Proteome Systems MCE apparatus. Following liquid phase pI fractionation, mixtures of proteins can then be resolved on IPG stips. Ion exchange chromatography or ultrafiltration by size are additional methods of partitioning proteins.

— Sunny Tam

Q4: What strategies do you use to include internal or external calibration standards?

(continued from p.10)

unique and exhibit different biochemical properties — even slight differences can result in differences in migration and/or detection, and equal amounts of two different proteins may give you different signal.

— Alex Rai

We have just published a paper in *Analytical Chemistry* (Smith et al., 2006) on metabolomics that we're also applying to proteomics. When we compare different data sets, we use the naturally occurring peptides as internal calibrants to align the retention time.

The approach was demonstrated for metabolomics, but it can be easily applied for proteomics, and that's essentially what we're doing right now. It's a non-linear approach to aligning the chromatograms, which allows you to get much more reliable comparisons between different data sets. I think this has just been a great thing — people have been struggling with this for a long time, and now they have a very nice solution that is a program that we wrote (primarily Colin Smith wrote it). It's an open source program [known as XCMS Analyte Profiling Software]. At least one manufacturer is implementing it into their programs.

— Gary Siuzdak

Q5: How do you optimize protein extraction from difficult or complex samples? (continued from p.11)

samples, tissues, etc. — all have different properties. Extraction from each of these will require different treatments; for example, the use of mortar and pestle, homogenizer, addition of specific buffers, detergents, or chaotropes, or a combination of these methods in a specific sequence for optimal protein extraction.

It should also be noted that if you know the biochemical characteristics of your protein target, you can employ particular methods to exploit the characteristics of your target in your extraction procedure. However, this may not always be possible — you may not know the characteristics of your protein target. If this is the case, you will need to extract as many proteins as possible. It may be easier to use general procedures, such as acetone precipitation, which has the general effect of precipitating all proteins.

— Alex Rai

We had a paper that just came out in *Analytical Chemistry* (Want et al., 2006) for protein extraction. This is primarily for metabolite analysis, but we performed Bradford assays for protein concentration determination. We were actually quite surprised, because acetonitrile is so commonly used for protein extraction or to remove proteins from samples so that you can look at xenobiotic metabolites. It turned out that a lot of protein was retained in the solution when you used acetonitrile — as much as six percent — so we prefer to use a methanol or a methanol-acetonitrile combination, because the protein amounts in those were much less — as low as two percent.

— Gary Siuzdak

This is a very challenging issue, and solutions are highly individualistic. I do not think there is any magic approach or a magic bullet. But in general, if we are preparing samples for SDS PAGE, we use SDS containing buffers or conventional SDS gel sample buffers. If we are going to perform IEF, we use buffers containing 9 M urea, or 8 M urea/2 M thiourea.

— David Speicher

Q6: Under what conditions do you label proteins, and when you do, which peptide-labeling reagents do you use?

(continued from p.12)

protein antigens in cell culture. I needed to obtain an antibody to each of my antigens, and the two antibodies to be derived from two different animal species, because of cross-reactivity with secondary reagents. Both of my antibodies were rabbit polyclonals, which was a problem. To get around this, I purchased fluorophore (FITC- and rhodamine-) coupling reagents from Molecular Probes, which has a good selection of reagents for this purpose. This allowed me to directly link each of the antibodies to different fluorophores and use them in conjunction in one experiment.

— Alex Rai

Actually, because of this program [XCMS Analyte Profiling Software] that we've developed and its delivery of such good quantitative data, we've not been using labeling approaches. So we've been focusing on and have had success with an ICAT-like approach, as well as O^{16} and O^{18} labeling.

— Gary Siuzdak

We have used SILAC, cICAT, O^{18} labeling, iTRAC, and label-free methods. Each approach has pros and cons. There is no ideal label at present, although SILAC is reasonably robust for those applications where its use is feasible.

— David Speicher

For quantitative proteomics using MS, protein labeling is useful. The peptide labeling reagents can include ICAT, iTRAQ, SILAC or O^{18} labeling.

— Sunny Tam

Protein labeling in proteomics experiments can serve different purposes. For instance, to improve ionization efficiency in a MALDI experiment, we use O-methyl urea. For absolute quantification, we use isotopically labeled peptide. For relative quantification, we can use iTRAQ reagents from ABI.

— Jinsam You

Discover!

INNOVATION @ WORK

With Sigma, more unique protein data can be elucidated

Biomarker discovery is at the forefront of modern day proteomics, and human plasma has become the sample source of choice. Until now, discovering proteins that can be used as biomarkers from such a wide, dynamic range of human plasma, has felt like searching for a needle in a haystack.

Now Sigma is leading the way towards better biomarker discovery through removal of twenty high abundance proteins from human plasma. The segment of the plasma proteome that researchers can now observe is shifting dramatically towards the lower abundance proteins believed to be the frontier of discovery. The ProteoPrep 20 Plasma Immunoaffinity Kit removes 97% of the overall protein load, leading to the visualization of proteins previously not seen.

Through Sigma innovation, biomarker discovery has reached a new level. Researchers are now delving deeper into the plasma proteome and discovering more unique data.

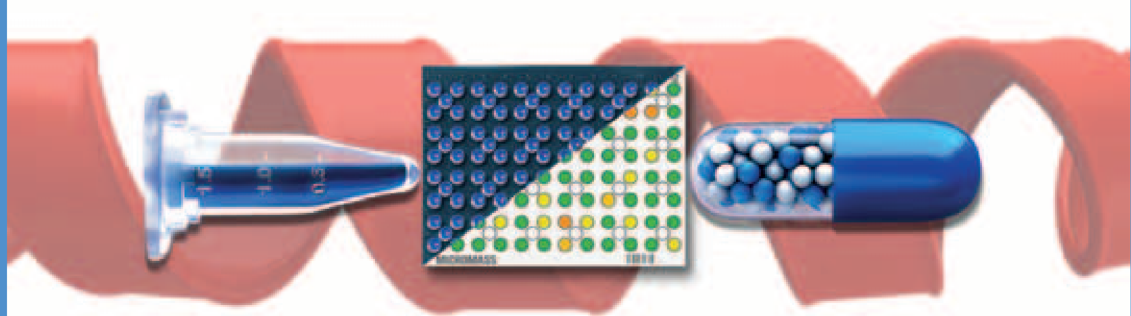
Find out how you can discover more unique proteins by visiting us at:

sigma-aldrich.com/prot20new

Accelerating Customers' Success through **Leadership** in **Life Science, High Technology** and **Service**
SIGMA-ALDRICH CORPORATION • BOX 14508 • ST. LOUIS • MISSOURI 63178 • USA

SIGMA®

Waters



For Proteomics ∴ Confidence

No other company brings more critical mass to proteomics from start to finish than Waters. From protein isolation and characterization to biomarker discovery and new therapy development, our exact mass approach delivers results you can believe in. That's because we can seamlessly integrate automated bio-informatics, industry-leading HPLC, Ultra Performance LC™ and MS/MS instrumentation, chemistries and services. As a result, we enable you to achieve the highest confidence, coverage and speed in your research program. To learn more about Waters' critical mass in proteomics, visit **www.waters.com/p1**

© 2006 Waters Corporation. Waters and Ultra Performance LC are trademarks of Waters Corporation.

View publication stats