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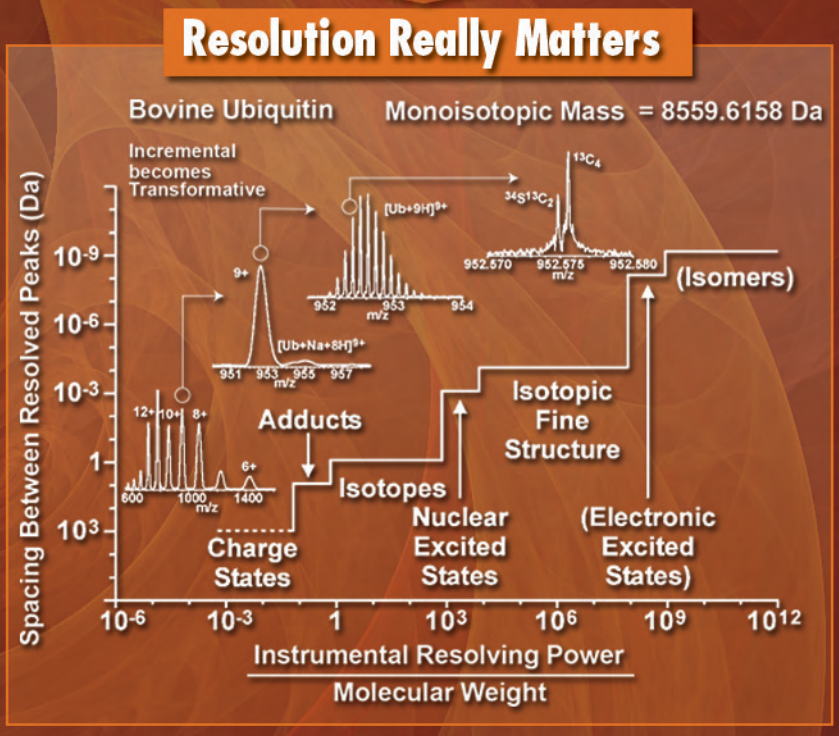
Science Challenges and Design Concepts

for the Next-Generation High-Performance FT-ICR Mass Spectrometer



Summary of a workshop held
January 16-17, 2008
at the National High Magnetic Field Laboratory
Organized by the NHMFL and the
Environmental Molecular Sciences Laboratory

About the cover figure:

Mass spectrometric resolution (defined here as the spacing between resolved peaks) does not increase monotonically with resolving power for electrosprayed biomolecules. Rather, mass spectral resolution progresses along a series of stairsteps, each revealing new molecular information. First, one must resolve different charge states. No additional peaks appear until adducts are resolved, then not again until isotopic peaks are resolved (unit mass resolution), and finally when isotopic "fine structure" (i.e., different elemental compositions of same nominal mass) is resolved. High mass resolving power greatly extends the onset of each "plateau" in the figure. The vertical axis is mass spectral resolution, scaled in Daltons. The horizontal axis is a mass-independent measure of resolving power.

Reference: Marshall A.G., C.L. Hendrickson, and S.D. Shi. 2002. "Scaling MS plateaus with high-resolution FT-ICR." *Analytical Chemistry* 74(9):252A-259A.

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Science Challenges and Design Concepts for the Next-Generation High-Performance FT-ICR Mass Spectrometer

Summary of a workshop held January 16-17, 2008
at the National High Magnetic Field Laboratory,
Tallahassee, Florida

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Organized by the
National High Magnetic Field Laboratory and
Environmental Molecular Sciences Laboratory

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Introduction

Mass spectrometry (MS) is among the most sensitive, molecule-specific, and generally applicable analytical techniques currently available. Although there are many important performance metrics, the mass resolving power of an analytical mass spectrometer is the ultimate limiting parameter for many of its most important applications. It has been demonstrated repeatedly that a whole new world of science opens up for exploration each time the resolving power of a mass spectrometer is increased (cover figure).

It is also generally recognized that High-Field Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is the technique of choice for reaching the highest mass resolving power and mass measurement accuracy (MMA). The unequaled resolution of FT-MS is supported by experts in the field, including Alexander Makarov, inventor of the Orbitrap mass spectrometer, who said at the European FT-MS Society in Moscow in September 2007, "FT-ICR is, and always will be, the highest-resolution mass analyzer." The fundamental reason the physical parameter can be measured with the highest accuracy and resolution based on modern electronics is the radio frequency (RF), and the cyclotron frequency of a charged species in a highly uniform magnetic field is the unique signature for the ion with a particular mass-to-charge ratio. This was demonstrated initially by an 11.5-Tesla (T) FT-ICR MS developed at the Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (PNNL) in the mid-1990s and, most recently, by the implementation of a custom-built FT-ICR spectrometer operated at nearly 15 T at the National High Magnetic Field Laboratory (NHMFL) at Florida State University.

> *FT-ICR is, and always will be, the highest-resolution mass analyzer.*
>
> *—Alexander Makarov, inventor of the Orbitrap mass spectrometer*

The nearest competing technology for the highest performance MS is the commercial Orbitrap instrument, an impressive new technology that uses electrostatic fields and precisely machined electrodes to constrain ion motion in a trap of special geometry. However, magnetic field technology, driven principally by nuclear magnetic resonance (NMR) spectroscopy requirements, has now advanced to the stage that magnetic field homogeneity substantially exceeds that of the electrostatic field in the Orbitrap FT mass spectrometer. That instrument operates near the limit of technology of laser machining, falls short of current FT-ICR performance, and far short of the anticipated resolution of a higher-field FT-ICR system.

However, even higher resolving power and accuracy is required to capture the full range of information for increasingly complex natural mixtures (e.g., fossil fuels and bio-fuels and their products/byproducts) and many other current analytical challenges (e.g., peptide and protein identification in the context of systems biology research). Because all key measures of FT-ICR MS performance improve linearly (mass resolving power, scan speed) or quadratically (broadband mass accuracy, dynamic range, highest non-coalesced mass) with increased magnetic field strength, a suitably constructed High-Field FT-ICR MS will provide greatly enhanced performance over any existing or contemplated MS technique. These efforts will, in turn, enable new measurements that will revolutionize basic biological and environmental research with broad impacts on our health and the health of our environment.

Workshop Background

A High Field FT-ICR MS will provide greatly enhanced performance over any existing or contemplated MS technique.

This workshop is rooted in two prior technical meetings. The first was a workshop in August 2006, organized by staff at EMSL to consider science challenges and technology opportunities for enabling breakthrough science at EMSL in the next decade. That workshop was specifically charged to identify advances in measurement science needed for the recapitalization of EMSL for its second decade of operation as a national user facility. Some 100 scientists from 40 institutions met in Richland, Wash., and developed a comprehensive set of priorities for new instrumentation for molecular identification. One of their conclusions was that magnet technology was now sufficiently advanced that EMSL should explore the acquisition of a 21-T FT-ICR system in its recapitalization program. A subsequent meeting by the European FT-MS Society in Moscow in September 2007 brought together 85 mass spectrometrists, including international experts in FT-ICR and Orbitrap techniques, who discussed recent accomplishments in highest performance MS and opportunities for further advancements in FT-based technologies.

Over the past 2 years, specialists at the NHMFL have further advanced magnet technology and the concept for a wide-bore horizontal magnet. This expertise in magnet technology is strongly complemented by the FT-ICR user facility at the NHMFL, especially their recent experience in deploying the highest-field FT-ICR technology currently available. This high level of resident expertise was the principal impetus for choosing the NHMFL as the venue for the most recent workshop that brought together FT-ICR specialists, magnet specialists, selected proteomics and general MS specialists from EMSL and NHMFL, and representatives from the user community. This workshop was held at the NHMFL on January 16-17, 2008. Names and affiliations of the 32 participants are listed in Appendix A.

Workshop Purpose and Organization

The primary purpose of the workshop, co-organized by the NHMFL and EMSL, was to review the state of the art of high-performance MS, evaluate whether newly developed magnet technology could deliver significantly enhanced performance, and describe the science drivers for developing next-generation MS instrumentation.

The workshop was opened by co-organizer Dr. Jean Futrell, Battelle Fellow and Chief Scientist for Fundamental and Computational Sciences at PNNL. He explained the background for organizing the workshop and charged the group to address the following questions:

1. What are the general and specific scientific drivers for higher-field FT-ICR MS capability development? What can we accomplish scientifically that we can only dream of doing with current capability?

2. What specific and unique instrumentation/hardware/software needs can be identified, addressed, and achieved? Magnetic field, sample introduction, ionization methods, ion storage/mass selection/transfer, ion simulations, excitation/detection, and data acquisition/storage/analysis are all open targets. What will the state-of-the-art FT-ICR MS system look like 5 years from now, and how do we address the technology gaps?

3. What use model is appropriate for addressing the scientific community's need for access to High-Field FT-ICR MS when it becomes available?

The workshop (agenda attached as Appendix B) began with plenary talks (summarized in the Workshop Proceedings section) highlighting current developments in superconducting magnet technology, FT-ICR instrumentation, large-scale simulations of ion motion, and FT-ICR applications. The talks were followed by breakout groups that discussed in-depth magnet design, instrumental design and optimization, and potential applications. Each breakout group participant was invited to present 1-5 slides to stimulate discussion of ideas that were generally organized around the three questions posed by Dr. Futrell. Breakout group reports are included in the Workshop Proceedings section.

The second day of the workshop began with summaries of breakout group discussions presented by each discussion leader, followed by open discussion of the entire group. The consensus views of the group are summarized here.

Findings and Recommendations

High-Performance Mass Spectrometry is Uniquely Valuable for Understanding Complex Systems

When combined with breakthrough methods for chemical imaging (laser microscopy, laser probe microscopy, surface ionization MS, molecular SIMS), very-high-resolution MS has the potential to transform spatial, compositional, and temporal resolution of molecular structures and dynamics, thus enabling breakthrough discoveries in the understanding of complex systems. Analytical grand challenges in complex systems include achieving molecular-level understanding of biochemical pathways, cellular communication, microbial communities, and other interactions between biomolecules and their environments. Applications include human health, drug discovery, environmental impacts, environmental remediation, carbon sequestration, biomass conversion to biofuels, national security, and energy efficiency.

Currently available tools are largely inadequate to address these grand challenge analytical problems, and continued development of advanced tools and technologies is essential. While many scientific topics would benefit from the High-Field FT-ICR platform, a broad but manageable subset identified at the workshop is described here.

The increased sensitivity, resolving power, and accuracy of high-field MS will allow efficient characterization of proteins 2-4 times larger than can be resolved today. This major advance in top-down proteomics will make it possible, for the first time, to characterize key protein variants of more massive proteins with high impact across the entire range of biological applications of MS.

Proteomics and Other 'omics (lipidomics, metabolomics, glycomics, etc.). The "post-genomic era" provides us with information on a large number of genes and their splice variants (genomics) and an even larger number of possible proteins (proteomics), particularly if one counts post-translational modifications (proteomics). Comprehensive identification of other types of biomolecules—the lipidome, glycome, metabolome, etc.—is required to characterize multi-protein complexes; i.e., the interactome) and define cell biology at a molecular level.

Our ability to determine all the proteins (lipids, glycans, metabolites, etc.) in a cell and their variation over time and in response to stimuli is a major analytical challenge. From an analytical perspective, significant advances in sensitivity and dynamic range of detection are needed for these extremely complex samples (e.g., human or microbial community), extremely small samples (e.g., single cells), and highly dynamic range samples (e.g., blood plasma or microbial community) with sufficient speed to obtain a statistically meaningful number of analyses (e.g., to account for biological variability). High-Field FT-ICR MS is uniquely suited to address these challenges and dramatically impact biological, chemical, and physical sciences and our ability to address the most important questions in the health, energy, and environment areas.

Probing Intact Proteins and Protein Assemblies. The impact of high-field MS on the analysis of intact proteins and protein assemblies—one of the most important science challenges of the 21st century—will be even more dramatic. The increased sensitivity, resolving power, and accuracy of high-field MS will allow efficient characterization of proteins 2-4 times larger than can be resolved today (notably antibodies of ~150 kDa). This major advance in top-down proteomics will make it possible, for the first time, to characterize key protein variants of more massive proteins with high impact on biomedical understanding. As an example, the regulation and coordination of modifications at multiple sites within the same protein or complex (e.g., multi-site phosphorylation, histone modifications) is a key biological question that remains to be understood or even bounded. Because phosphorylation of any site on a given protein can affect its activity, degradation rate, localization, and ability to interact or bind with other proteins or divalent cations, the overall phosphorylation signature of the protein is critically important for deducing its biological function.

Understanding Microbial Communities. Microbial communities are another particularly important and grand challenge for current technology. Complete understanding of the dynamics in a microbial community requires the identification of all community members and protein players (proteome), together with oligo- and polysaccharides (glycome), lipid environment (lipidome) and metabolites (metabolome) involved in specific pathways. Huge diversity in these systems constitutes a scientific grand challenge and an opportunity for next-generation FT-ICR. Because microbial communities cross the boundaries between medicine, energy, and environment, this new capability could potentially enable practical applications of microbial biotechnology in all of these critically important areas.

Understanding Fossil Fuels. The analogous "omics" breakthrough in understanding fossil fuels is the rapidly developing field of **petroleomics**—the correlation and ultimately prediction of the properties and behavior of crude oil, kerogens, bitumens, tar sands, etc**.,** based on their complete chemical compositional characterization. Petroleomics originated from and has relied almost

The level of chemical detail provided by FT-ICR MS will enable rational processing of fuels, conversion into petrochemicals, and mitigation of environmental impact.

exclusively on FT-ICR MS and has transformed our understanding of the composition and physical and chemical properties of petroleum and petroleum fractions. This level of understanding provides a rational basis for the processing of fuels, their transportation, efficient conversion into petrochemicals, and mitigation of environmental impact. Progress in this field demands increased resolution, sensitivity, and dynamic range comparable to needs in the biological sciences. This capability will be even more important for the characterization of biomass and biofuels, complex mixtures that are both very different from petroleum in their composition and relevant properties. As a particularly relevant example, the August 6-8, 2007 DOE workshop *Basic Research Needs: Catalysis for Energy* points out that the chemical and structural information knowledge base for biofuels is very limited and that molecular-scale characterization is essential to understanding elementary processes of deconstruction of biomass and conversion to targeted fuel components.

Molecular-Level Characterization of Organic Aerosols. This area was identified as an additional exemplary 21st century scientific grand challenge. The origin, growth, and fate of these ubiquitous microparticles, originating from both natural and anthropogenic sources, are among the most poorly defined forcing functions on global warming and are of major environmental concern. Understanding their detailed chemical composition and the time evolution of this composition is key to understanding their physical and chemical properties. This understanding will be greatly advanced by applying techniques closely related to those used in petroleomics, and to which the same limitations of current techniques apply.

High-Resolution Chemical Imaging. A breakthrough category of the measurement sciences in this decade is the development of methods for high-resolution spatial characterization of complex materials with simultaneous information on chemical composition. At this workshop, research highlights achieved by the recent development of the laser ion microscope technique at the FOM Institute for Atomic/Molecular Physics (Amsterdam, The Netherlands) were summarized. This development bypasses the optical wavelength limitation of laser microprobe MS by the use of a diffuse laser beam to illuminate a large area of the surface while position-sensitive detectors in a multi-pass, time-of-flight (ToF) mass analyzer define both spatial and chemical composition. This combination improves the spatial definition by an order of magnitude with good mass resolution. A very recent advance couples normal rastering laser ionization with greatly improved mass spectral resolution available with a commercial FT-ICR. It was demonstrated that computer alignment of lower-resolution mass analysis (and more precise spatial resolution) with high-resolution mass analysis (with lower spatial resolution) preserves the advantages of both methods. By incorporating this approach in the proposed next-generation 21-T FT-MS development, we anticipate achieving unprecedented precision in spatial- and molecular-specific characterization of complex materials of biological and non-biological origin.

Next-Generation FT-ICR MS is Within Our Reach

Current developments in superconducting persistent magnet technology support the thesis that a 21-T High-Field FT-ICR MS system can be developed and deployed in a 3- to-5-year time frame. A full systems design approach is essential to achieve optimum performance.

A 21-T High-Field FT-ICR MS system can be developed and deployed in a 3- to-5-year time frame.

Existing scientific teams are well qualified to generate the design specifications for the horizontal bore superconducting magnet and oversee its fabrication. The necessary knowledge and skills also exist to undertake the system design and fabrication of ion sources, transfer and focusing elements, vacuum system, and data station. High-level modeling is required to define plasma effects in ion motion and perturbation of ion motion by ion-ion interaction and ion-conductor interactions in all trapping, ion preparation, and storage components, including the ICR cell itself. The latest generation of massively parallel supercomputers is now capable of carrying out the extensive modeling calculations in ICR cells of arbitrary geometry, ion source design, intermediate ion processing steps, and intermediate accumulation and storage stages.

The successful design and development of an integrated 21-T High-Field FT-ICR MS system is eminently feasible. However, to do so requires solutions to several major technical challenges. In particular, improvements in ion formation and transfer efficiencies, effective ion injection into intense magnetic fields (the magnetic bottle problem), advances in understanding ion motion, control of ion motion and trajectories, high-sensitivity detection, and high-precision digitization and processing of signals are all required. All of these challenges can be solved but will require coordinated efforts and teaming by an international ensemble of expert modelers and experimentalists.

In the context of wide-bore FT-ICR MS applications, 21 T is the limit attainable in the next 3-5 years, judged from the state of the art and current or projected developments in superconducting magnet technology. New concepts under discussion will likely make possible the fabrication of higher-field magnets in the longer (10-15 year) time frame. These concepts were discussed, but the workshop participants concluded that these as-yet-unproven technological approaches were too costly and risky to use in the next-generation FT-ICR MS.

The workshop consensus is that a concerted effort to design, build, and test a new, High-Field 21-T FT-ICR MS system is fully justified to advance the characterization and understanding of complex biological, chemical, and materials systems. The remainder of this report elaborates the rationale for this recommendation.

Technology Development Questions and Answers

Workshop co-organizer Dr. Alan Marshall, ICR Program Director at the NHMFL, summarized the historical development of high-performance FT-ICR MS over time, the advances that were made possible by each new increase in magnetic field strength, and some general conclusions about the next anticipated advance to 21-T field strength. Dr. Marshall posed several questions, the answers to which were elaborated in subsequent discussions and breakout sessions and are summarized here.

Why go to a higher magnetic field?

FT-ICR already offers the highest mass resolution and mass accuracy (by at least an order of magnitude) of any mass analyzer. Nevertheless, even higher resolution and accuracy is required to capture the full range of information for increasingly complex natural mixtures (e.g., fossil fuels and their products) and analytical problems (e.g., peptide identification for proteomics). Five different measures of FT-ICR MS performance improve linearly (mass resolving power; acquisition speed) or quadratically (broadband mass accuracy; dynamic range; highest non-coalesced mass) with increased magnetic field strength, B (Figure 1).

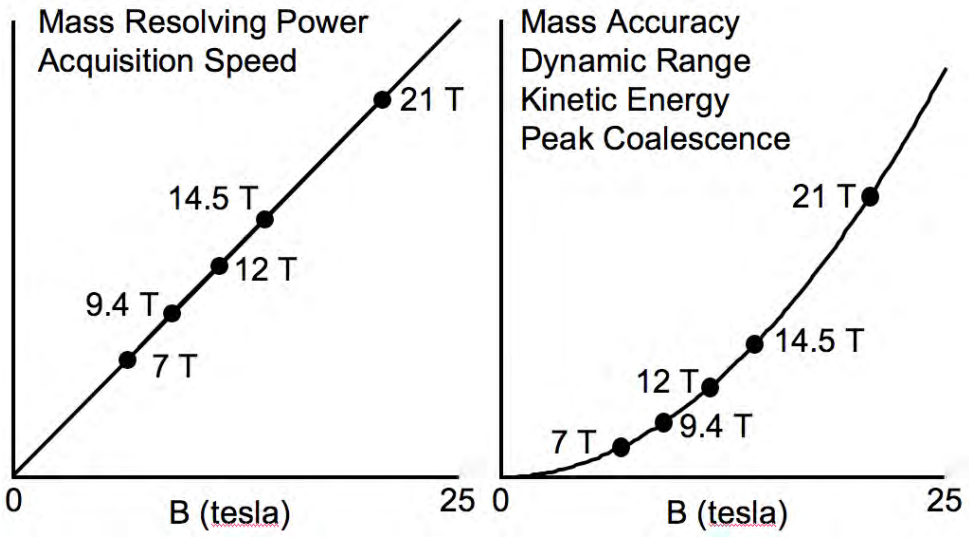

Figure 1. Improvement in FT-ICR performance measures with increased magnetic field strength.

Because all of these parameters taken together comprise the figure of merit for a mass analyzer, more than an order of magnitude of enhanced performance will be available in a suitably constructed 21-T system.

To illustrate this point we note that an increase by a factor of 2 in mass resolving power can be more than twice as useful: it can make an all-or-nothing difference (Figures 2 and 3). Moreover, because the advantages shown in Figure 1 are effectively independent, their effect is multiplicative, not parallel. For example, it is possible to detect more peaks (higher dynamic range), and even more peaks (higher mass, because the number of possible peaks increases exponentially with mass), and yet more peaks (higher resolution). *The net effect is transformational, not incremental.*

Another advantage of higher field is that (as in FT-NMR) experiments that can be performed only with heroic effort at low field become routine at high field. And experiments must be routine to gain widespread usage and have greater impact.

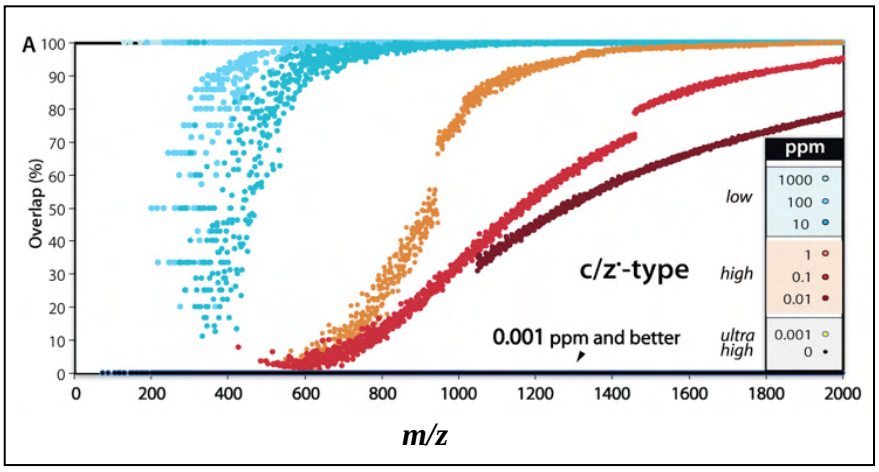

Figure 2. With sufficient mass accuracy major peptide fragment ions are chemically distinct, regardless of amino acid composition or the presence of common modifications, and can be can be uniquely identified.

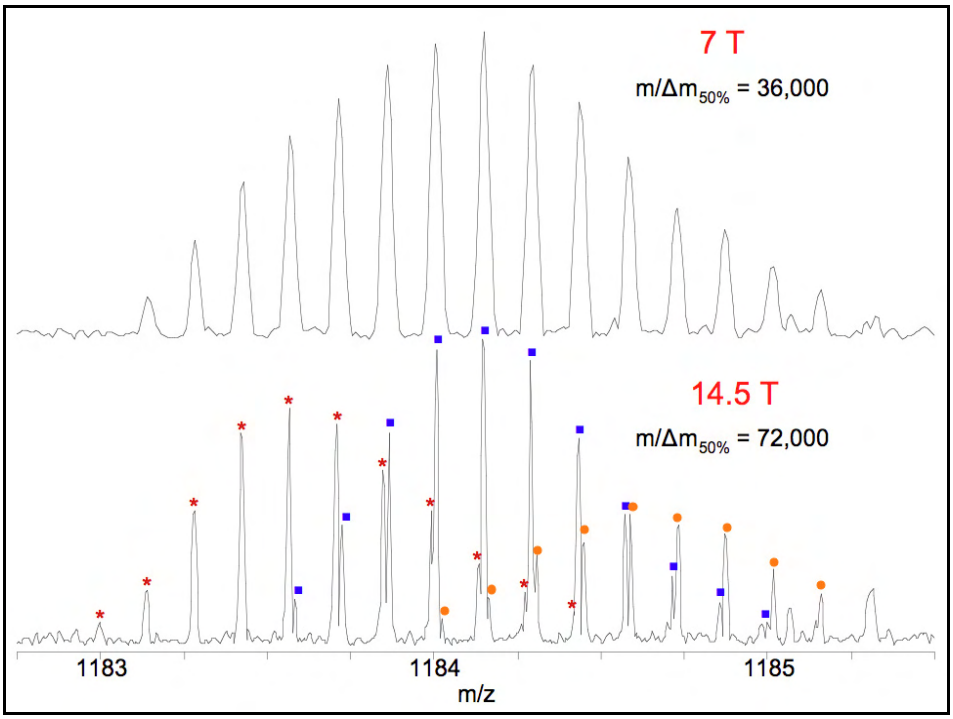

Figure 3. Comparison of neuropeptide spectra at two magnetic fields illustrates how the interfering peptides can be resolved, thus providing information unobtainable at lower field.

Why do we choose 21 Tesla?

As explained in the magnet breakout session report and in Dr. Roth's plenary summary, 21 T is the highest wide-bore magnetic field achievable with currently feasible superconducting wire that meets FT-ICR system requirements. Also, NHMFL has successfully built a 21-T wide-bore NMR vertical unshielded magnet, and that experience will translate directly to design of a horizontal, actively shielded, 21-T wide-bore ICR magnet. The choice of magnet vendor will depend on open competitive bids, based on specifications developed in consultation with NHMFL's magnet science and technology staff and the combined in-house and external expertise available from the NHMFL and EMSL staff and user base.

21 T is the highest magnetic field and bore diameter achievable with currently feasible superconducting wire.

Why not wait for a commercial instrument?

Historically, each increase in magnetic field has been introduced first for custom-built instruments, and then later commercially (Figure 4). Moreover, many of the major non-magnet improvements in FT-ICR MS were developed by academic and government labs well before they became available commercially. Such efforts serve to identify, gauge, and potentially resolve technical risks so that the private sector can manage them within the market environment. Thus, we propose to implement the next major increase in B-field at the two leading centers for FT-ICR MS instrument development: NHMFL and EMSL.

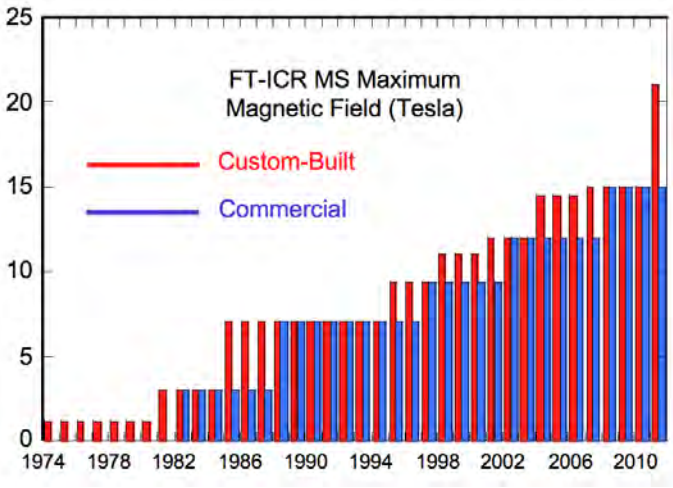

Figure 4. Comparisons between custom and commercial FT-ICR MS maximum magnetic fields projected through 2010.

The next-generation FT-ICR is much more than the magnet

As noted in the instrumentation breakout session report, taking full advantage of higher magnetic field requires that all other aspects of the FT-ICR MS experiment be optimized as well: sample introduction; multiple ionization modes; ion focusing, accumulation, and transmission to the ICR cell; ICR excitation and detection; mass calibration; data reduction; and automation of all of these processes. For example, the NHMFL has achieved a factor-of-2 improvement in mass resolution by "phasing" the spectrum to yield absorption-mode rather than magnitude-mode display (Figure 5). Continuing improvements in the efficiencies of sample introduction, ion focusing, and mechanical configuration are actively under way at both EMSL and NHMFL.

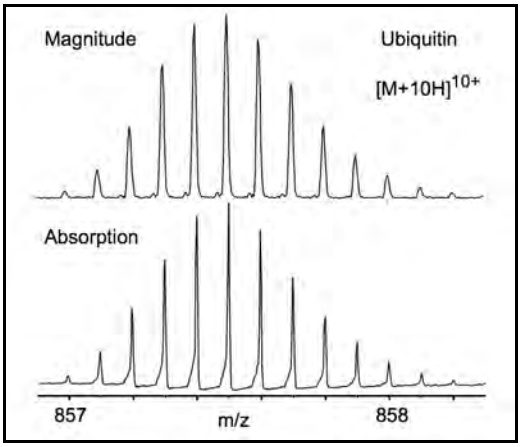

Figure 5. Magnitude and absorption spectra of electrospray-ionized ubiquitin, $[M + 10H]^{10+}$ (at 9.4 T), derived from the same time-domain data. The absorption-mode spectrum was obtained by Fourier deconvolution.

As noted in Dr. Eugene Nikolaev's plenary summary, FT-ICR MS design has already reached the limit for single-ion numerical simulations. Further progress requires brute-force analysis of the collective motions of millions of Coulomb-coupled ions in static and time-varying magnetic and electric fields. Fortunately, such large-scale simulations are now available to guide FT-ICR MS instrumental design and optimization. It thus appears feasible to achieve significant improvements in overall mass spectrometer design, which will enhance sensitivity, mass range, resolution, and quantitation, during the 2-to-3-year period required for design and construction of the 21-T magnet.

Why should the U.S. lead this development?

Leadership in any high-tech field is precarious. In 2008, the United States offers the highest-performance FT-ICR MS facilities in the world, at NHMFL and EMSL. However, because the advantages of higher magnetic field are so large and numerous, continued leadership requires that the next major jump in magnetic field must take place here. We are poised with the expertise and infrastructure, but we need the next-generation magnet to hold the lead, and to capitalize on the lead by applying it to national strategic needs.

What could we do that we cannot do today?

A High-Field FT-ICR MS system will significantly enhance overall analytical performance—sensitivity, dynamic range, accuracy, resolution, and speed/throughput—and enable previously intractable applications, including the characterization of very complex mixtures (organisms' and community's -omes, natural and environmental mixtures). By providing an enhanced molecular-level understanding of complex systems, this revolutionary mass spectrometer holds promise to facilitate sound policy decisions affecting environmental remediation, waste processing, energy, and human health.

Optimizing the investment

Once the scientific case and technology challenges for 21-T FT-ICR MS were made (i.e., the first two charges to the workshop), the third charge to the workshop—where such instrument(s) should be sited to optimize successful implementation and usage—was discussed. Workshop participants unanimously supported the development and initial placement of such systems at national user facility laboratories (specifically, at NHMFL and EMSL) available to the broader scientific community for the following reasons:

- **Expertise.** Most important, NHMFL and EMSL together offer unparalleled technical expertise in design, development, and operation of new FT-ICR MS instrumentation. Users of those facilities are attracted as much for the in-house expertise as for the instrumental capabilities.
- **Cost.** Because much of the spectrometer (other than magnet) is custom-built in-house, the capital cost is lower by a factor of ~3. In addition, there is a major saving in the cost of (continuing) service contracts by using national user facilities. Because most of the spectrometer is custom-built, troubleshooting, maintenance, and repair are inherently available in-house. A typical spectrometer service contract is 10% of the purchase price per year, representing a major cost saving.
- **Continuous Upgrading.** Most MS user facilities are predicated on purchase of major new equipment in the first year, and then not again until the project is renewed (i.e., every 5 years, if successful). In contrast, NHMFL and EMSL provide *continuous* upgrading of their instruments so that performance remains at the leading edge.
- **Exceeding Commercial Instrument Performance.** Commercial instruments are limited to techniques for which they hold patents or closely held trade knowledge. National user facilities, on the other hand, implement best practices developed by their staff and collaborating scientists, including vendors (that typically compete with each other). In

addition, whether new developments are first conceived in-house or externally, EMSL and NHMFL implement them immediately, whereas others must wait (typically several years) until a vendor brings out a new model that includes such improvements. Thus, EMSL and NHMFL typically provide lead instrumental capabilities not available elsewhere for 1-3 years.

- **User Program Infrastructure.** Both NHMFL and EMSL are configured as external user facilities, with computerized logging, peer review, and selection of user proposals, on-site accommodation, and staff assigned to help users.
- **Range of Experiments.** Because 21-T FT-ICR instrumentation is a major capital investment, its capabilities should span the full range of important applications. No individual investigator program can cover that range. Because FT-ICR programs at EMSL and NHMFL are highly complementary, the combination of the two facilities covers the entire range of applications described here.
- **Cyber-Connectivity.** Both EMSL and NHMFL provide for extensive capability for remote data reduction (and even instrument operation—one of NHMFL's FT-ICR instruments has been operated from Birmingham, England, and several major EMSL instrument systems (various NMR systems) are also currently remotely accessible).
- **Outreach.** A major impact of the FT-ICR MS programs at NHMFL and EMSL is that they are providing pilot data for new entrants into the field. Those groups often go on to acquire their own instruments. Thus, unlike some other major user facilities (e.g., synchrotrons), which tend to attract mainly the same users from year to year, the *cumulative* number of FT-ICR MS external users increases steadily with time. Thus, national FT-ICR MS facilities not only serve the user base but also expand it.

  Such facilities also serve the broader educational outreach mission of cooperating institutions. For example, NHMFL hosts an annual one-day Open House (4,600 attendees in 2008) as well as tours (e.g., ~10,000 middle school students per year) to increase its public outreach. EMSL conducted approximately 150 tours in FY 2007, showcasing technical capabilities to over 850 participants.

Workshop Proceedings

Summaries of Plenary Lectures

Ion Simulations as a Guide to FT-ICR Performance – Dr. Eugene Nikolaev, Russian Academy of Sciences

Design of an FT-ICR instrument based on a 21-T superconducting magnet should be based on state-of-the-art knowledge accumulated by the MS community about constructing not only the FT-ICR part of the instrument but also all other parts responsible for ion formation transportation, accumulation, and ejection to the ICR cell. One of the main goals of this workshop is to design an instrument with the highest performance in resolution, mass accuracy, spectra acquisition rate, and dynamic range. All this is impossible to achieve without extensive and realistic computer simulation of ion behavior in all parts of the instrument.

The biggest challenge for these simulations is taking into account ion-ion interactions and ion interaction with electrodes producing electric fields (image charge interaction; see Figure 6). Taking into account that increasing the dynamic range of FT-ICR measurements forces us to deal with more than millions of ions, a new computational technique should be used based on parallel supercomputer codes. Such a technique was developed and implemented on supercomputers with common access memory. It is based on state-of-the-art digital methods for electric field simulations: particle in cell algorithm, particle-particle algorithm and surface charge algorithm, and ion dynamic simulation methods using the Boris algorithm.

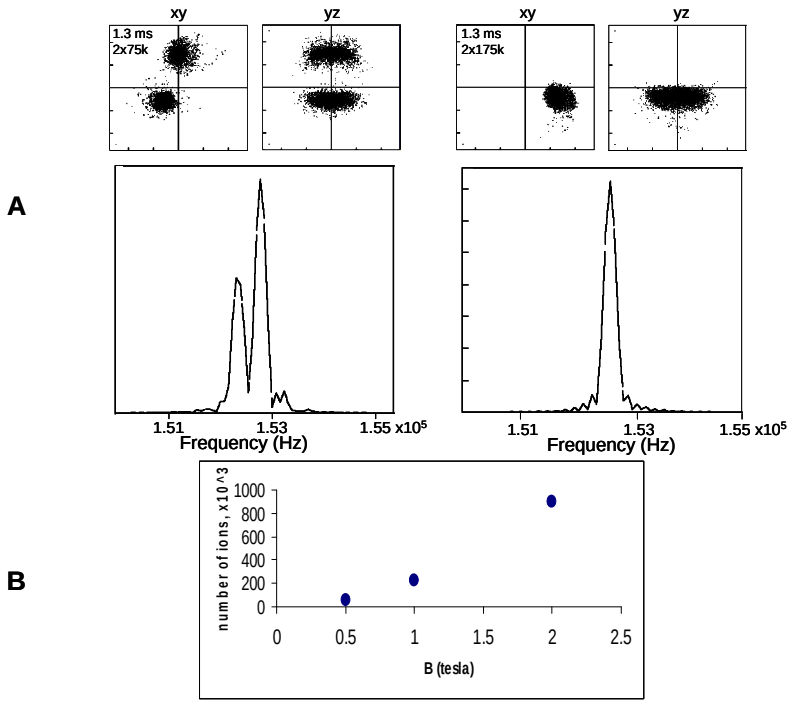

Figure 6. (A) Influence of charge density within an ICR cell on detected ions' frequencies. At higher number density, closely spaced ion clouds are moving synchronously and corresponding FT-ICR spectrum displays only one peak instead of two. (B) This peak coalescence shows nearly quadratic dependence on the magnetic field strength; hence, higher field minimizes above effect and offers superior dynamic range of measurements.

A supercomputer code was created that simulates dynamics of ion clouds incorporating up to 10 million ions in electric and magnetic fields of arbitrary configuration. The applicability of this code was demonstrated by modeling ion cloud motion in transfer and accumulation quadrupoles, Penning (FT-ICR) cells of different geometry, and Kingdon (Orbitrap) cells. Simulation results demonstrated for the first time different non-neutral plasma effects on ion motion dynamics in an FT-ICR cell: axial and cyclotron motion phase locking, axial structure formation, and cloud splitting.

High-Field ICR Magnet Design/Performance – Dr. Gerhard Roth, Bruker BioSpin

Over the past decade, Bruker has developed a very advanced ultra-high-field, high-resolution NMR and high-field horizontal bore magnet technology. The key technologies are a sub-cooling technology for highest fields, advanced wire technology, shielding technology and screening, high-field superconducting joints, and refrigeration technology.

These technologies led to production of high-field magnets, such as the 850-MHz wide-bore, 900-MHz, and 950-MHz actively shielded magnets, as well as the 15-T actively shielded and refrigerated FT-ICR MS magnets that are nitrogen-free and have a 1-year helium hold time. The technologies also led to the most compact systems in the field and allowed the design of an ultra-compact, shielded 800-MHz magnet. In addition, a large variety of lower-field magnets have also contributed to the long-term development of expertise and production experience. Combining sub-cooling technology with horizontal bore magnet technology allowed the design and construction of a non-shielded 750-MHz magnet with a 25-cm room temperature bore, which is currently being installed in Paris.

Challenges for constructing a 21-T FT-ICR magnet include its field strength: +40% or +6 T above today's strongest FT-ICR field strength, corresponding to +250 MHz for proton NMR. This is a big step compared to current maximum field strength and the experience of how fast the maximum field could be increased. The magnet also requires active shielding and refrigeration to fulfill cryogenic specs. However, Bruker believes that, with implementation of the major key technologies and some additional developments, this field strength is within reach in a time scale of ~3 years.

Bruker has completed preliminary design of a 21-T magnet that meets the requirements for FT-ICR by offering a generous homogeneous volume of 84 mm length axially and >110 mm radially, or 108 mm length radially and 90 mm axially. The design is also optimized with respect to FT-ICR needs by offering an access length to the magnetic center of only 90 cm and with the 50 G point only 50 cm axially from the magnet vessel. It can be equipped with a newly developed 1.5-W pulse tube cooler (from CryoMech) allowing a 1-year helium hold time.

Bruker is also developing high-temperature superconductors; however, major basic developments in conductor and magnet technology are still needed to resolve issues such as superconducting jointing technology, conductor anisotropy, quenching behavior, etc. Only when all these issues are solved can a long-term stable magnet based on these materials be designed.

In summary, Bruker has developed advanced high-field magnet technology that led to the current highest-field shielded NMR, FT-ICR, and *in vivo* magnetic resonance imaging magnets. The

company has an excellent track record with >250 systems installed worldwide. A 21-T FT-ICR MS magnet is challenging, but within the scope of Bruker's technology. Higher-field magnets can also be built, once high-temperature superconductors become reliably available in sufficient quality and length, and the technological challenges associated with these conductors are solved.

New Horizons in High-Resolution Imaging Mass Spectrometry: The Need for Speed – Dr. Ron Heeren, Amolf FOM Institute for Atomic and Molecular Physics, The Netherlands

Imaging mass spectrometry (IMS) is an innovative new tool in modern proteomics, which combines microscopic visualization tools with the analytical capabilities of MS. Imaging MS can visualize many different classes of molecules directly on histopathological tissue sections without the use of molecular labels. A single IMS experiment thus provides different snapshots of the molecular landscape. This unique capability distinguishes IMS from the battery of microscopic imaging techniques used in the life sciences. Figure 7 is an example of very recent developments in atmospheric pressure ionization, a very promising technique for IMS.

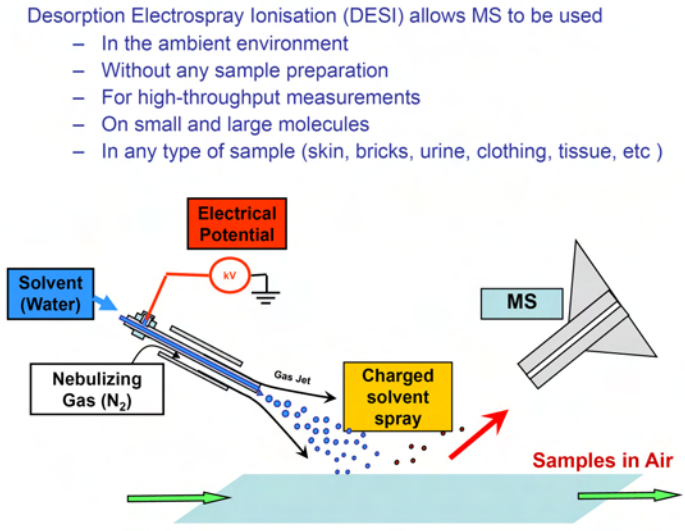

Figure 7. Example of atmospheric pressure DESI for environmental analysis with zero sample preparation.

MS itself is rapidly maturing as a powerful tool for proteomics applications as new sample-preparation approaches, instrumentation developments, and bioinformatics tools for analyzing data become increasingly available. Technology has evolved to the point where it enables high-resolution, MS-based microscale analysis of cells and tissues. IMS allows the development and application of different methodologies for local proteomics of cells and tissues.

The main application of biological IMS is foreseen to be in biomedical and pharmaceutical research. Targeted study molecules are predominantly peptides and proteins as well as small pharmaceutical components. Several studies have demonstrated the potential and pitfalls of IMS. Three main issues need to be addressed at the development stage of this biomolecular imaging approach: sensitivity, identification capabilities, and speed of analysis.

So far, most IMS studies have been performed with matrix-assisted laser desorption/ionization-time-of-flight (MALDI-ToF) mass spectrometers, which offer a wide mass range and the highest speed of analysis. The main drawback to this approach to date is the lack of adequate mass resolution and tandem MS (MS/MS) capabilities needed for more reliable identification of

detected species. FT-ICR MS can significantly advance IMS by adding high mass resolution and accuracy. It has already been demonstrated that the high mass resolution reveals new spatial features on biological tissue sections that would remain undiscovered by ToF MS (Figure 8). In addition, FT-ICR MS provides structural analysis through different types of MS/MS experiments.

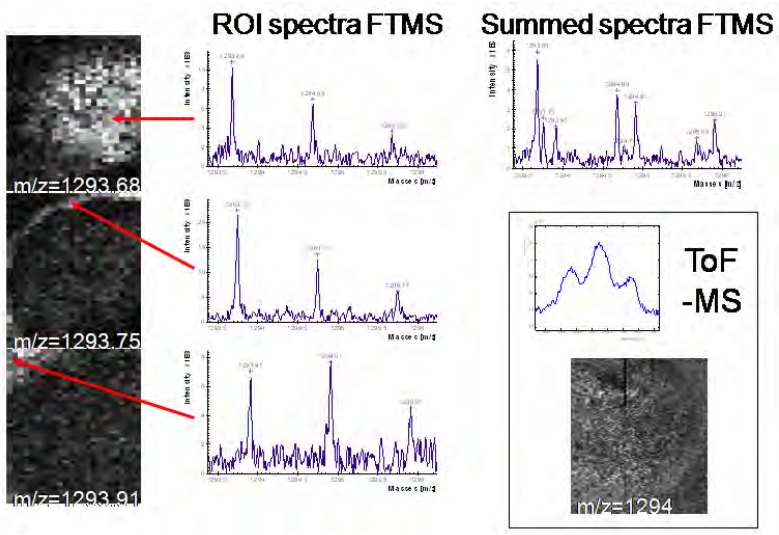

Figure 8. High-resolution MALDI-IMS capability provides the location specificity not achievable at lower resolution.

Unfortunately, FT-ICR-MS experiments take time, resulting in extremely lengthy experiments, especially when in-cell collision gas is used and when larger surface areas are studied. The analysis of larger areas can be accelerated by sacrificing resolution, multiplex analysis, and microscope mode analysis, but none of these methods is applicable to FT-ICR MS. In fact, the only solution is to move to higher-field magnets. High-field magnets can dramatically increase speed of analysis while preserving the same high mass resolution as obtained with lower-field magnets. Similarly, high-field magnets can significantly improve spatial resolution through increased spectral acquisition rate. Imaging with the High-Field FT-ICR will provide combined high quality spatial and spectral details and open a range of new research possibilities.

FT-ICR for Proteomics – Dr. Ljiljana Paša-Tolić, Pacific Northwest National Laboratory

The State of the Art. Technological advances and novel methodologies in the proteomics field are enabling exciting research in areas such as quantitative protein profiling to better understand cellular responses to environmental perturbations. An especially productive analysis approach has been from the "bottom up" whereby proteins are digested by proteases into polypeptides, which are then separated by liquid chromatography (LC), and then typically analyzed by tandem mass spectrometry (MS/MS). When coupled with strong cation exchange fractionation, LC-MS/MS technologies can now routinely identify thousands of proteins, but at the expense of analytical throughput and sensitivity. PNNL has developed the accurate mass and time tag approach, an LC-MS-based strategy that exploits both high MMA and LC elution time information to increase analytical throughput and overall sensitivity of measurements of a given biological system (Figure 9).

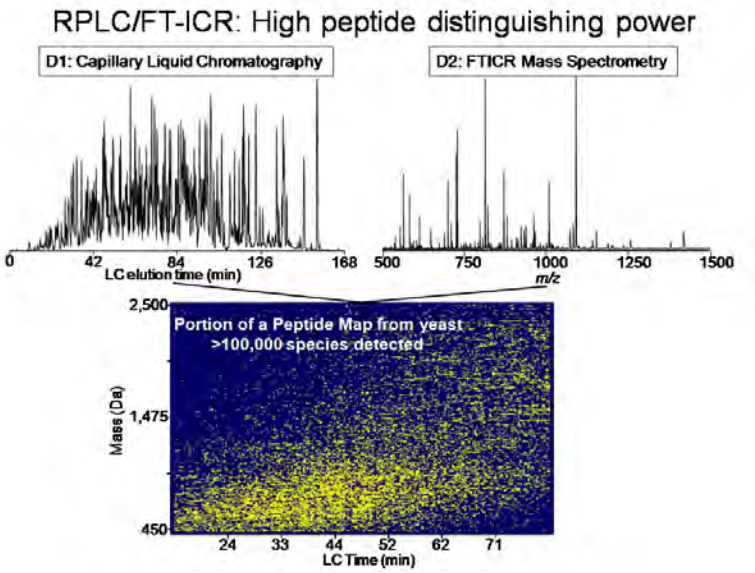

Figure 9. Successful combination of high-resolution separations with high-performance MS at PNNL to achieve high proteome coverage for comparative proteomics measurements.

Challenges and Opportunities. Despite significant advances in LC-MS-based technologies, challenges remain in implementing a proteomics platform for more advanced applications (i.e., complex systems, such as humans or microbial communities). A platform must have the robustness, sensitivity, and dynamic range of detection needed to effectively address extremely small samples (e.g., single cells), and high-dynamic-range samples (e.g., blood plasma or microbial communities). It must also provide sufficient throughput to enable a statistically meaningful number of analyses to account for biological variability and use effective quantitative approaches for high accuracy and reproducibility. By virtue of enhanced sensitivity, dynamic range coverage, and throughput, a high-magnetic field FT-ICR system designed specifically to target these challenges can be applied to generate robust quantitative measurements of complex systems.

The *bacterial community* is such a complex system of interest because of its ability to radically affect human health and the health of our environment. In microbial communities, thousands of proteins with high sequence similarity can coexist, but such related proteins often have acquired new function or play roles in fine-tuning biological responses. Sensitive and accurate detection and quantification of specific targets in complex pools of similar sequences is needed to reveal the functional significance of such diversity. Similarly, complete understanding of microbial system and its dynamics will require the identification of protein players involved in specific pathways. Our ability to do this depends on the specificity and sensitivity of MS measurements, both of which generally increase with improved resolution, accuracy, dynamic range and sensitivity. A High-Field FT-ICR MS system will provide a level of performance that will enable us to eventually tackle complex natural microbial communities and ultimately determine the pathways important for e.g., bioenergy, carbon cycling, and bioremediation.

Another area that would significantly benefit from the High-Field FT-ICR MS is *top-down proteomics* (characterization of whole, intact proteins and modifications of such proteins). *Nature Methods* recently listed top-down proteomics among "methods to watch" as an emerging method essential for characterizing various protein variants (with potentially high impact in

biomedical research), as well as for detecting and associating multiple modification sites within the same protein (e.g., multi-site phosphorylation, histone methylation/acetylation). Because of its high resolving power and accuracy, coupled with demonstrated high sensitivity, FT-ICR is clearly the most powerful mass analyzer for top-down proteomics. Yet, slow acquisition rate of the FT-ICR limits its applicability for large-scale intact protein analyses. Because the acquisition rate scales linearly with the magnetic field strength (e.g., the high mass limit for on-line LC-MS operation scales from ~20 kDa at 7 T to ~ 70 kDa at 12 T), High-Field FT-ICR promises to significantly advance intact protein analysis.

Summary. Although proteomics has made rapid progress in recent years, improved technological approaches are needed before the field can realize its full potential, particularly for complex systems. As all attributes of FT-ICR performance—mass resolving power, acquisition speed (LC-MS), highest non-coalesced mass, number of ions, dynamic range, upper mass limit, etc.—scale linearly or quadratically with the magnetic field strength, a High-Field FT-ICR mass spectrometer will most certainly produce significant improvements in all aspects of proteomics.

Breakout Group Reports

Magnet Design Discussion Summary

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Gerhard Roth, Bruker Magnets
Alan Marshall, NHMFL
Mark Bird, NHMFL
Peter O'Connor, Boston University
Dave Koppenaal, EMSL, PNNL
Len Spicer, Duke University

This group of specialists in superconducting magnet design addressed the question of the highest practical field that can be achieved in the next 5 years. This field would be the principal feature of an actively shielded, wide-bore, highly homogeneous, horizontal superconducting magnet for ICR experiments. They were also asked to identify principal technical issues and any gaps in technology that must be over come to construct this kind of magnet.

The group agreed that for high-field superconducting magnet systems, 21 T is the technological limit of low-temperature niobium-based superconductors (LTS) as shown in Figure 10. Fields of 21 T have been achieved with LTS in similar NMR superconducting magnet systems in 52-mm vertical bores as a standard commercial product and in the vertical 105-mm bore of the one-of-a-kind Ultra-Wide Bore NMR magnet system at the NHMFL.

Based on the success of the NHMFL 21.1 T 900-MHz superconducting magnet system (with similar field and bore size specifications as those required by an FT-ICR system, but with different ancillary requirements, as shown in Table 1), the NHMFL Magnet Science and Technology team completed a benchmark conceptual design for a 21 T FT-ICR system and offered it as an option in an (abandoned) solicitation for proposals from the commercial sector. The result was an enthusiastic response from three of the top commercial suppliers of high-field superconducting magnet systems who promised delivery of a 21 T FT-ICR system, through challenging but achievable extensions of their existing technology, in 31 to 44 months. A summary of some of the existing, successfully implemented high-field superconducting magnet technology of the three participating vendors is listed in Table 2, illustrating the technical basis on which a 21 T FT-ICR system is founded.

For fields greater than 21 T, Figure 10 shows the superconducting performance of various LTS and high-temperature superconductors (HTS). The superconducting current density of Nb_3Sn, the highest performing LTS, decreases sharply as the magnetic field approaches 24 T. For typical engineering margins, ~21 T is the highest level safely achievable with Nb_3Sn, although some 52-mm bore systems have been produced that approach 22 T. As can also be seen in Figure 10, the current densities of the Bi-2212 and YBCO (when extrapolated) HTS conductors are at sufficiently high levels even up to 45 T to produce superconducting magnets. These HTS conductors are promising, but the system technology is not yet sufficiently mature. The highest fields achieved to date in developmental HTS test coils are 27 T to 29 T. It will be a minimum of 8 to 10 years before the first fully functioning HTS system with fields higher than 21 T are available (and 10-15 years more likely).

Table 1. Comparison of NHMFL 21 T FT-ICR Conceptual Design Requirements with Existing NHMFL 900-MHz Wide-Bore NMR System.

Requirement	21 T FT-ICR Conceptual Design	NHMFL 900-MHz NMR
Central Field (T)	21.0	21.1
Warm Bore (mm)	110	105
Stored Energy (MJ)	29	38
Maximum Inhomogeneity and Volume	10^{-5} peak to peak over a 50-mm-diameter by 80-mm-long cylinder	10^{-9} over a 40-mm-diameter spherical volume
Maximum Drift (T/h)	1.0×10^{-6}	2.5×10^{-7}
Maximum Axial Distance to Field Center from warm edge of cryostat (m)	~1.0	None
Maximum Fringe Field (T)	~0.01 at 1.5 m on axis from field center	None
Magnet Axis Orientation	Horizontal	Vertical
Cryogenic Environment	1.8 K subcooled helium II	1.8 K subcooled helium II

Table 2. Highest Performance Technology of Participating Suppliers

Salient Technology Feature	Value
Existing Available Central Field, Bore Size and Shielding for Vertical Orientation	22.3 T, 54-mm bore, actively shielded
Existing Available Central Field, Bore Size and Shielding for Horizontal Orientation	15 T, 110-mm bore with ferroshims installed, actively shielded
Nb_3Sn High-Field Wire	OST and EAS with round and rectangular cross-sections
Shimming Type	Superconducting and Ferro
Operating Temperature and Pressure	2.0 K to 2.2 K at 1 bar
Operating Current	250 A to 300 A
Stored Energy	12 MJ to 40 MJ
Persistent Joints	Persistent Nb_3Sn to Nb_3Sn joints in high fields. Persistent joints using NbTi require lower fields.
Quench Protection	Active and Passive
Refrigeration	Pulse Tube Cryocooler from Cryomech with no LN_2 reservoir required

Principal challenges associated with HTS are:

- **Anisotropic performance** – A typical characteristic of HTS is decreased current density as a function of the field direction across the conductor, which limits the critical current at the ends of coils where the radial field component increases.
- **Conductor cost** – The dominating cost of LTS systems is the cost of the superconductor. The present cost of HTS is much higher (and completely unbounded at present) than for LTS conductors.
- **Quench detection and protection** – HTS conductors are highly stable because of the associated higher temperatures at which they can superconduct. Therefore, it is more difficult to evenly distribute quench energy throughout the coil winding. Quench detection and protection remains to be demonstrated in a full-size HTS system.

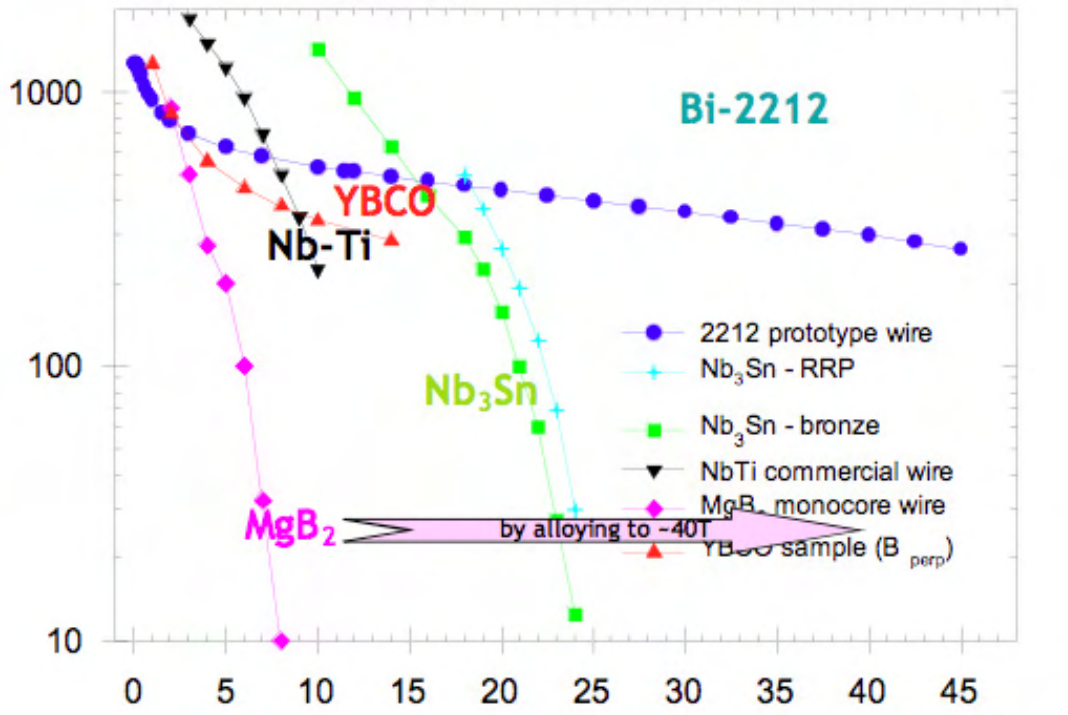

Figure 10. Superconducting current density performance as a function of magnetic field. This graph shows that low-temperature niobium-based conductors (the highest performing of which is Nb3Sn) are limited to fields of ~21 T in high-field magnet systems (with standard engineering margins applied) because of their sharp decrease in superconducting current density as the field approaches 24 T. HTS such as Bi-2212 and YBCO hold the promise of higher-field magnet systems to 30 T and higher because of their essentially linear current density performance as a function of fields even up to 45 T. However, the superconducting magnet system technology is not yet sufficiently mature to produce a high-field system with HTS conductors. It is expected to take at least 8 to 10 years to produce a usable high-field superconducting HTS magnet system higher than 21 T.

- **Coil winding technology requires development** – Currently, the most viable HTS conductors are available in tape form, which raises issues of homogeneity for pancake coils. The HTS current densities are also strain-sensitive resulting in a decrease in performance as the strain increases. Coil-to-coil, layer-to-layer, pancake-to-pancake, lead-in, lead-out transitions**,** and reinforcement must be developed and demonstrated with these conductors in a system with increasingly higher Lorentz forces as the field levels increase.
- **Reliability –** The long production lengths of HTS conductors and consistent performance as function of length required for a superconducting magnet system have not yet been demonstrated.
- **Persistent joints** – The persistent HTS electrical joints required for an FT-ICR (or NMR) system have not yet been achieved. Options for accommodating resistive (non-persistent) electrical joints and the lower n-values of HTS are to use an NMR field lock or other field compensation as the magnetic field decays over time. These systems are possible with some effort. Field lock is available for lower drift rates. Field compensation with Z_0 coil or current injection is possible for higher drift rates; however, there will be increased cryogenic heat load caused by joule heating of the joints.

Detailed Instrumentation Design Discussion Summary

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Alex Tolmachev, PNNL

The instrumentation subgroup agreed that high magnetic field provides manifold and multiplicative improvements (better mass resolving power, acquisition speed, mass accuracy, dynamic range, kinetic energy, and resistance to peak coalescence (as seen previously in Figure 11) that have transformed methodology in a wide range of scientific disciplines. However, as magnetic field strength increases the magnet also grows in size, stray field, and magnetic field gradient, and it is critical that the mass spectrometer is designed *as a complete system* so that the potential of the magnet is realized. The *system* must be flexible (e.g., to accommodate any ionization source or front-end separation technique). The best technology from industry and academia should be fully integrated to optimally perform a variety of experiments, including MS^n with collisional, photon, and electron induced dissociation, all on a timescale compatible with the best available analytical separation techniques.

Best success requires that the mass spectrometer design is integrated with the magnet design *prior to* the final magnet design review and that magnet siting is considered early enough to avoid issues after the magnet arrives. We expect magnet design and construction to require 2-3 years, so there is plenty of time to organize a mass spectrometer design team, identify potential issues (e.g., to what extent will the spectrometer warp the magnetic field?), and complete calculations, simulations, and experiments that provide the best *system* performance.

Consideration of current limitations in FT-ICR MS and further complications caused by the unique size and magnetic field strength of the proposed magnet led to discussion of three critical issues that limit performance today of all FT-ICR systems and must be addressed in the current system.

Electric Field. Ideal FT-ICR MS takes place in a perfectly homogeneous magnetic field, perfectly linear electrostatic field imposed by a static quadrupolar trapping potential, and perfectly uniform RF electric field (readily achieved with capacitively coupled electrodes) to generate a coherent ICR signal. Recent experiments by workshop participants Bruce, Kim, and Tolmachev and others and simulations by Nikolaev show that FT-ICR performance is often limited by nonlinearity in the electrostatic field caused by finite and imperfectly shaped ICR cell electrodes and ion-ion interactions. FT-ICR performance can be improved by appropriate shimming (with added trapping electrodes or an electron beam) that linearizes the trapping electric field (Figure 11). A shimmed cell improves resolving power, mass accuracy, signal-to-noise ratio, and dynamic range *simultaneously*, and we expect to shim the cell used in this high-field system. Proper calculation of the cell electrode geometry and voltages is complicated by the addition of the space charge electric field, but recent simulations can accurately account for ion-ion interactions, so that cell optimization can be done with realistic magnetic field inhomogeneity, space charge, collisions, and trap electrode geometry.

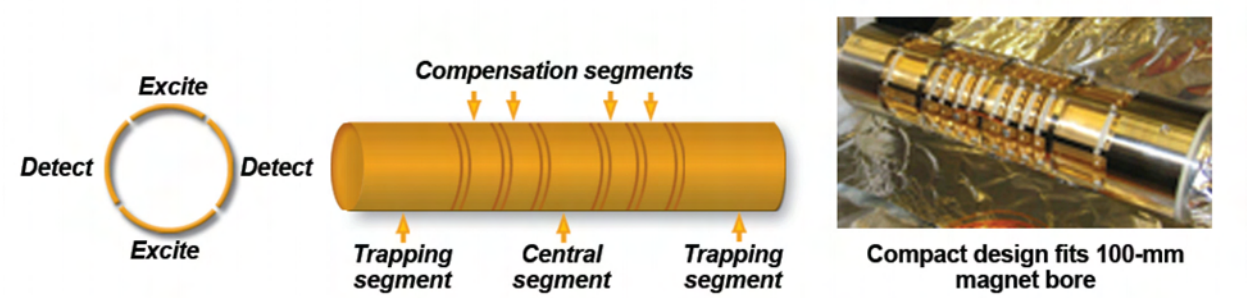

Figure 11. New harmonized FT-ICR cell: open, cylindrical, with four compensation segments.

Ion injection. Most FT-ICR systems ionize and trap analyte ions outside the magnet and then inject them into the FT-ICR cell in pulses, providing high duty cycle, scan rate, dynamic range, and optimized MS/MS. However, the pulsed ions travel to the FT-ICR cell with *m/z*-dependent velocity (the distance between the external and FT-ICR traps acts as a crude ToF mass spectrometer) that limits the *m/z* range of ions that can be trapped in the cell in a single experiment. This problem is exacerbated by a large magnet if it requires that the distance from the external ion trap to the FT-ICR cell be increased. The subgroup noted that advances in superconducting wire led to smaller magnets, and preliminary calculations show that a conservative 21-T design should be no larger than the current 14.5-T magnet installed at NHMFL.

As the ions travel from the external trap to the FT-ICR cell, they interact with a magnetic field gradient and must be confined near the magnet axis to minimize the repulsive "magnetic mirror" force and arrive at the FT-ICR cell. There are currently two methods used to focus the ions through the magnetic field. Most FT-ICR mass spectrometers use RF multipole (typically quadrupole, hexapole, or octopole) ion injection, which benefits from easy alignment and continuous ion trajectory correction. However, recent work at NHMFL by Beu shows that multipole ion transmission efficiency is reduced if, during transit through the magnetic field gradient, the ion cyclotron motion comes into resonance with the RF multipole drive frequency. Only ions below a low *m/z* threshold are affected, but the threshold increases linearly with magnetic field strength, so a greater *m/z* range will be impacted at higher magnetic field. The effect can be minimized by increasing the RF drive frequency and by minimizing the radial kinetic energy of the injected ions.

Electrostatic ion injection is a feasible alternative, and has been successfully implemented by a number of research groups, such as those at PNNL, and by industry. Optimal electrostatic injection requires detailed knowledge of the magnetic field gradient and careful alignment of the injection optics, but technology is available to accurately simulate and optimize ion injection. Both methods will be developed further and the best option will be selected after careful simulation and experiments.

Pressure. Ion radial kinetic energy scales with the square of the magnetic field strength, and can be used to affect high-energy collisional dissociation. However, undesirable collisional dissociation and/or redistribution between radial and axial kinetic energy during detection can limit FT-ICR performance, particularly for low *m/z* ions (ion kinetic energy and collision rate scale inversely with *m/z*). Recent results at 14.5 T show that the optimal pressure for detection of low *m/z* ions (i.e., below *m/z* 500) is ~1 x 10^{-10} torr (i.e., significantly lower than that provided by commercial instruments at lower magnetic field). The optimal pressure at 21 T may be even

lower, but careful choice of FT-ICR cell materials and good ultra-high vacuum practices should result in base pressure below 1 x 10^{-10} torr.

Summary. A fully integrated 21-T High-Field FT-ICR *system* will provide the highest resolving power and mass accuracy ever achieved, with high measurement rate and dynamic range, auguring for enormous impact across a wide range of science. Several issues require careful attention, including efficient ion injection, minimization of *m/z* discrimination, optimal FT-ICR cell geometry, and low base pressure. However, none of these issues is intractable, and recent advances have been made in each of these areas. Further, a critical mass of expertise stands ready to create new solutions that will make this system the world leader in high-performance MS.

Applications Discussion Summary

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This group, which represented industry, academia, DOE, PNNL, and the NHMFL, had the most diversity among its participants. The group discussed several key applications that would experience a significant or even transformational benefit from a High-Field FT-ICR system. The participants approached their discussion from the premise that FT-ICR is widely recognized as the leading MS platform in terms of MMA and resolving power, undeniably the two most important attributes for analysis of vastly complex mixtures (such as a proteome or heavy petroleum) as well as large biomolecules (such as intact proteins and protein assemblies).

Because all critical attributes of FT-ICR performance scale linearly or quadratically with the magnetic field strength (see Figure 1), and the overall improvement in performance is essentially multiplicative, a high-field FT-ICR system has the potential to revolutionize our understanding of living systems and their interaction/impact on the environment. The key advantages offered by a high-magnetic-field FT-ICR system are: improved dynamic range of measurements, MMA and quantitation ability (critical for characterization of complex mixtures); enhanced sensitivity (potentially enabling single-cell measurements); and increased spectral acquisition rate, i.e., throughput (critical for ‘omics research and MS-based imaging). Although a multitude of scientific topics would benefit from the High-Field FT-ICR platform, a broad but manageable subset emerged at the workshop. These are touched on here.

Characterization of Complex Mixtures. This applies to an organism’s proteome, lipidome, metabolome, glycome (and other ‘omes); environmental mixtures (e.g., aerosols, oil spills), petroleome, natural products, etc.

Proteomics. Our ability to determine all the proteins in a cell and their variation over time and in response to stimuli has vastly progressed over the last decade. However, many challenges remain. An effective proteomics platform must have the sensitivity and dynamic range to effectively address extremely small and complex samples (with thousands of proteins varying in abundance by ten orders of magnitude or more), while being robust enough to ensure the throughput needed to enable a statistically meaningful number of analyses. Similarly, we need better tools to characterize lipids/glycolipids, glycans/glycoproteins, and complex mixtures of metabolites. By virtue of enhanced sensitivity, dynamic range coverage, and throughput, a High-Field FT-ICR platform would enable a quantum leap toward understanding the machinery of life itself, opening up exciting new frontiers (e.g., enabling tailored/individualized medicine).

Petroleomics. The full molecular characterization of petroleum and, by extension, bio-based fuels, is an equally daunting challenge. Adding to the challenge is the evolution of world petroleum reserves toward heavy ends. The decrease in light "sweet" crude oils led to the use of heavier, more heteroatom-rich feedstocks for the production of petroleum products. Although heavy oil represents a substantial reserve of recoverable crude oil, the high viscosity and high heteroatom content present significant production challenges and result in reduced market price. Detailed characterization of recoverable crude oil reserves enabled by a High-Field FT-ICR platform would have significant impact on national security, energy efficiency, environmental disposal, and economy.

Aerosols. Aerosols are key elements in many environmental issues ranging from climate change to public health. Safe production and use of energy will require dramatically improved understanding of the origin, fate, chemical, and physical properties of fine atmospheric particles. Detailed characterization of the chemical composition of aerosols during their life cycle in the atmosphere—an area of greatest uncertainty in the atmospheric and aerosol science—is one of the major challenges in chemical analysis. Atmospheric aerosols are typically a diverse collection of particles that contain a complex mixture of compounds with a wide range of molecular weights, structures, physical properties, and chemical reactivities. The high resolution and accuracy offered by a High-Field FT-ICR platform will enable unambiguous identification of aerosol constituents, thus addressing yet another 21st century scientific grand challenge.

Large Molecules. The impact of High-Field MS on the analysis of intact proteins and protein assemblies may be even more dramatic. The increased sensitivity, resolving power, and accuracy of the high-field MS will allow efficient characterization of proteins 2-4 times heavier than the proteins whose primary structure and post-translational modifications can be resolved today. This top-down approach is essential for characterizing various protein variants (potentially high impact in biomedical studies), as well as the coordination of modified sites within the same protein (e.g., multi-site phosphorylation, histone code). A High-Field FT-ICR MS system promises to uncover the overall modification state of a protein, which is a key to understanding its biological function(s).

Macromolecular Assemblies. It is now believed that ~80% of proteins exist in multi-protein complexes rather than individual enzymes or receptors. Hydrogen/deuterium exchange coupled with MS analysis can identify contact surfaces in such complexes for systems that are not accessible to x-ray crystallography or NMR. However, the MS experiments require resolution of

dozens of overlapping isotopic distributions for partially deuterated peptides (much as in the example of Figure 2), and will thus greatly benefit from the higher mass resolution at 21 T.

Similarly, defining and mapping protein complex networks are crucial for fundamental understanding of biological processes. The ability to measure protein-protein interactions in biological systems has undergone significant advances in the past decade due to emergence and growth of numerous new molecular biology and MS-based technologies. For instance, chemical cross-linking approaches, carried out by linking the interacting proteins through covalent bonds followed by LC-MS for identification of the cross-linked proteins, have been employed for mapping topology of protein complex *in vitro* and determining the protein interaction partners *in vivo*. However, the identification of the sites of interaction or cross-linker labeling sites are highly challenging due to the complexity inherent with cross-linking approaches. While at present this has been only achieved for purified protein complex systems available in relatively large quantities, the improved FT-ICR performance at high magnetic field holds promise to significantly improve our ability to efficiently identify cross-linked products at a much larger scale and with higher throughput.

Cellular Communities (microbial, organelles, or tissue). Microbial communities are of particular interest to scientists because of their ability to radically affect human and environmental health and hence have significant impact in areas such as evolution, disease, corrosion, degradation, bioremediation, global cycling, etc.

The application of LC-MS to probe the metaproteome of a bacterial community is currently limited by the dynamic range and sensitivity of mass measurements, leading to under-sampling of the proteomic content of the community and limiting the specificity of assigning spectral profiles or identified peptide sequences to a community member. Additionally, it is likely that many unique identifiers might be associated with proteins present at or close to instrument detection limits. As a result, the challenge associated with sensitivity pertains to the large dynamic range of protein abundances from a single cell, which is further amplified by the large dynamic range of community member cell counts.

The improved dynamic range and sensitivity offered by a High-Field FT-ICR system will significantly enhance our ability to characterize protein expression patterns for the members of the community, thus furthering our understanding of how these microbes interact with their environment. This will allow us to determine, for example, the pathways important for bioenergy production in cultured and in as-yet-undiscovered or uncultured organisms. These efforts will open the potential for the increase in production of biofuels and the mitigation of the use of these fuels in the environment.

Imaging. IMS is an emerging technology for the analysis of protein expression in mammalian tissues based on the high molecular specificity and sensitivity provided by MS. The main application of biological imaging MS is foreseen to be in biomedical and pharmaceutical research, where the target molecules are peptides and proteins as well as small-molecule pharmaceutical components. The growing interest in this technique will most definitely inspire new technology developments. Because all key challenges at the present stage of biomolecular imaging approaches—sensitivity, identification capabilities, and speed of analysis—can be

effectively addressed by High-Field FT-ICR MS, this platform is well positioned to become a new enabling tool in biomolecular imaging sciences.

Fundamental Problems in Chemistry and Physics (e.g., re-measure the periodic table) Determining the *m/z* of the major elements of the periodic table with unprecedented accuracy yields—through Einstein's equation—the fundamental binding energies of subatomic particles. In effect, the thermodynamic stability of the elements is determined by their mass and increased precision and accuracy attainable by the system envisioned in this workshop will provide valuable information on nuclear stability.

Acronyms

AMT	accurate mass and time
DESI	desorption electrospray ionization
EMSL	Environmental Molecular Sciences Laboratory
FT-ICR MS	Fourier transform ion cyclotron resonance mass spectrometry
FT-MS	Fourier transform mass spectrometry (abbreviated name for above)
IMS	imaging mass spectrometry
LC	liquid chromatography
MALDI-ToF	matrix-assisted laser desorption ionization-time-of-flight
MMA	mass measurement accuracy
MS	mass spectrometry
MS/MS	mass spectrometry – mass spectrometry, or tandem mass spectrometry
NHMFL	National High Magnetic Field Laboratory
NMR	nuclear magnetic resonance
PNNL	Pacific Northwest National Laboratory
RF	radio frequency
SIMS	surface ionization mass spectrometry
T	Tesla (unit of magnetic strength)
ToF	time-of-flight

Appendix A – Workshop Participants

Workshop Participants		
Name	**Affiliation**	**E-mail Address**
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Appendix B – Workshop Agenda

Science Challenges and Design Concepts for Deploying a High-Magnetic-Field High-Performance FT-ICR Mass Spectrometer System January 16–17, 2008

Wednesday, 16 January 2008 (Sessions in B101 unless noted)

8:00 a.m.	Breakfast at Hotel
8:45 a.m.	Attendees will be picked up at hotel and driven to NHMFL
9:00 a.m.	Welcome and Charge (Jean Futrell, Pacific Northwest National Laboratory)
9:10 a.m.	Overview (Alan Marshall, National High Magnetic Field Laboratory)
9:35 a.m.	Ion Simulations as a Guide to FT-ICR Performance (Eugene Nikolaev, Russian Academy of Sciences)
10:05 a.m.	Break
10:30 a.m.	B210: High-Field ICR Magnet Design/Performance (Gerhard Roth, Bruker)
11:00 a.m.	A235: Novel FT-ICR Instrumentation (Ron Heeren, FOM-AMOLF)
11:30 a.m.	B101: FT-ICR for Proteomics (Ljiljana Paša-Tolić, PNNL)
12:00 p.m.	Lunch (on site; Discussion Group Leaders will confer with Julie Wiley)
1:30 p.m.	Discussion Groups: A235 Conf Room: Magnet Design (Chaired by Tom Painter, NHMFL) B210: Instrumentation (Chaired by Chris Hendrickson, NHMFL) B101: Applications (Chaired by Ljiljana Paša-Tolić, PNNL)
5:00 p.m.	Refreshments in Corridor adjoining the High-Field ICR Bay
6:40 p.m.	Depart for the University Center Club
7:00 p.m.	Dinner at the University Center Club; return to hotel afterwards

Thursday, 17 January 2008

8:00 a.m.	Breakfast at Hotel
8:45 a.m.	Attendees will be picked up at hotel and driven to NHMFL
9:00 a.m.	Summaries (15-20 min each) by Discussion Group Leaders
10:00 a.m.	Break
10:30 a.m.	Discussion: Develop List of Science Drivers and Magnet Specifications
12:00 p.m.	Box lunches provided (Attendees depart at leisure)
1:00 p.m.	PNNL and NHMFL representatives confer and prepare summary

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EMSL
Environmental Molecular Sciences Laboratory

Pacific Northwest
National Laboratory
Operated by Battelle for the
U.S. Department of Energy