

JOURNAL OF BIOMOLECULAR TECHNIQUES

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ABRF '99—Preliminary Agenda

Bioinformatics and Biomolecular Technologies:
Linking Genomes, Proteomes and Biochemistry.

March 19-22, 1999

Durham, North Carolina

PLENARY SESSIONS

Plenary Session 1: Genomes and Function.

Chair: Kenneth A. Walsh (University of Washington, Seattle, WA)

Richard K. Wilson (Washington University, St. Louis, MO) *C. elegans* Genome Project.

Michael Y. Galperin (National Center for Biotechnology Information, NLM, NIH, Bethesda, MD)
Hits and Misses in Functional Genomics: First Lessons from a Comparison of Bacterial and Archaeal Protein Sequences.

Plenary Session 2: Chromatin Structure and Gene Regulation.

Chair: David Landsman (National Center for Biotechnology Information, NLM, NIH, Bethesda, MD)

C. David Allis (University of Virginia, Charlottesville, Virginia) Linking Histone Modifications to Gene Activation.

Alan P. Wolffe (National Institute of Child Health and Human Development, NIH, Bethesda, Maryland)
Linking Histone Modifications to Gene Repression.

ABRF Award Presentation: **Marvin H. Caruthers** (University of Colorado, Boulder, CO)

Plenary Session 3: New and Emerging Techniques in Structural Biology.

Chair: Paul Matsudaira (MIT, Boston, MA)

David C. Schwartz (New York University, New York, NY)

Optical Mapping: A New Approach for Whole Genome Analysis.

Joachim Frank (Wadsworth Center, Albany, NY)

Ribosome Structure and Function, as Explored by Three-Dimensional Cryo-Electron Microscopy.

SCIENTIFIC SESSIONS

Twelve scientific sessions have been scheduled. Chairs/speakers include: **Andy Baxevanis, Warren Gish, Tara Maise, Francis Ouellette, John Quackenbush, John Stults, Kenneth A. Walsh, Karl Clauser, Gregg Fields.**

TUTORIALS

Nine tutorial sessions have been scheduled. Chairs/speakers include: **Chuck Staben, Clayton Naeve, Ken Mitchelhill, George Grills, Roland Annan, Duane Bartley.**

Mark Your Calendars!

JOURNAL OF BIOMOLECULAR TECHNIQUES

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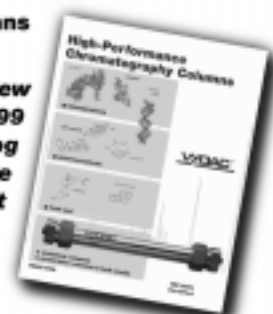
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Combined Use Of Proteases And Mass Spectrometry In Structural Biology

Richard W. Kriwacki¹ and Gary Siuzdak²

¹St. Jude Children's Research Hospital and ²The Scripps Research Institute

Proteolysis and mass spectrometry methods have been extended to the analysis of higher-order protein structure. Proteases have long been used as probes of native structure, and this approach has been rejuvenated and used in concert with various mass spectrometry techniques. We discuss the application of protease as probes of native structure, delineate the mass spectrometry methods that are appropriate in these studies, and offer several innovative case studies to illustrate key concepts in the combined use of proteolysis and mass spectrometry in studies of biomolecular assemblies.

Introduction

Biologic science is in the midst of an information and technical revolution because of the convergence of developments in many different areas, including genome and proteome sequencing, bioinformatics, and experimental and predictive structural biology. At the forefront of these developments are new analytic tools for meeting the demands of increased sequence data production. Prominent among these key technologies is biomolecular mass spectrometry (MS) because of the astounding sensitivity and accuracy of its protein mass determinations and the wealth of information about molecular composition inherent in a molecular mass. MS provides protein primary structure determination capabilities characterized by high accuracy and sensitivity and offers the potential for high throughput. New molecular and structural methods must be developed in order to remain astride the information revolution. The basis for these methods is the combined use of proteolytic digestion, mass analysis, and computer-based data analysis.

These methods can identify proteins, such as those from bands in acrylamide gels, using mostly automated protocols and subpicomole quantities. The target protein is completely digested using a sequence-specific protease such as trypsin (eg, within the gel matrix). The resulting fragments are extracted and prepared for mass analysis. MS analysis can yield information on fragment masses with accuracy approaching ± 5 ppm, or ± 0.005 Da for a 1000-Da peptide. The protease fragmentation pattern is compared with the patterns predicted for all proteins within a database, and matches are statistically evaluated. Because the occurrence of Arg and Lys residues in proteins is statistically high, trypsin cleavage (specific for Arg and Lys) usually produces a large number of fragments that have a reasonable prob-

ability of uniquely identifying the target protein. The success of this strategy relies on the existence of the protein sequence within the database, and with the sequences of whole genomes for several organisms complete (eg, *A. fulgidus*, *Bacillus subtilis*, *Caenorhabditis elegans*, *Escherichia coli*, *Saccharomyces cerevisiae*) and others well underway (eg, *Schizosaccharomyces pombe*, *Homo sapiens*), the likelihood for matches is reasonably high. Exact matches are readily identified, and homologous proteins are identified, albeit with lower statistical significance, placing a target protein within a particular family in the absence of an exact match.

Methods developed for primary sequence identification and elucidation using MS are particularly well suited for the analysis of higher-order, native protein structure. MS protocols used in primary structure analysis are directly transferable to the analysis of native structure, because they are used in the readout of information that is indirectly related to structure after the proteolysis reactions are performed. Analysis methods, however, must be modified to take into account the added spectral complexity resulting from incomplete proteolysis under limiting conditions. Proteases have long been used as probes of native structure, and this approach has been rejuvenated because powerful MS-based methods allow virtually complete identification of proteolysis reaction products.

We review the application of protease as probes of native structure and discuss the MS methods that are appropriate in these studies. We provide several innovative case studies that illustrate key concepts in the combined use of proteolysis and MS in studies of biomolecular assemblies.

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Limited Proteolysis as a Probe of Higher-Order Structure

Figure 1 illustrates how proteases can be employed as probes of secondary, tertiary, and quaternary protein structure under protease-limited reaction conditions. The kinetic accessibility of a site within a protein to a protease depends on several factors, including the physical compatibility of local chemical structure with the enzyme active site (ie, sequence specificity), the accessibility of the site to the protease, and the flexibility of the site (1,2). In the analysis of protein primary structure, physical compatibility governs the selectivity of cleavage. In most cases, a sequence-specific protease is used, reducing the number of fragments that are produced, improving the likelihood for statistically significant matches between observed and predicted fragment masses, and reducing the opportunities for spurious matches. However, site accessibility and flexibility must be overcome in the analysis of primary structure so that all possible sites are cleaved and fragment maps are complete. Discrimination between cleavage sites on the basis of structure-dependent variations in accessibility and flexibility is the foundation for the analysis of native higher-order protein structure with proteases.

The arrows in Figure 1 mark potential cleavage sites within a hypothetical protein; these sites are surface exposed and located in flexible loop regions. The distribution of amino acids in a protein guides the choice of protease to be used as a structural probe. Ideally, sites should be evenly distributed throughout the sequence and have a reasonable likelihood of being accessible and flexible. Because amino acids with hydrophilic side chains are found in greater abundance on the surface of proteins at the solvent interface, proteases that cleave at hydrophilic sites are preferred in structure analysis. Trypsin and V8 pro-

tease, which cleave basic (K, R) and acidic sites (D, E), respectively, are good choices.

Table 1 lists proteases commonly used in protein structure analysis (3); more extensive lists are available elsewhere (4,5). The non-sequence-specific proteases such as subtilisin Carlsberg often are used as structural probes. This approach allows protein structure to be probed in a non-sequence-biased manner. The potential for generating large numbers of related fragments is high, complicating the analysis of mass spectral data and the identification of peptide fragments on the basis of mass. Despite this potential problem, sequence-specific and non-sequence-specific proteases can be effectively used in parallel for structural analysis.

Limited proteolysis has been used to probe the structures of individual proteins and those of multicomponent macromolecular assemblies. The simplest application involves the use of proteases in domain mapping, which is extensively used in structural biology. The term *domain* refers to minimal structural elements within proteins that often are associated with protein function (6). For technical reasons beyond the scope of this review, protein samples for x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy ideally should possess highly ordered domain structures and be free of unstructured elements at the amino and carboxyl termini. Unstructured segments can spoil the formation of crystals, cause nonspecific aggregation, and give rise to resonance interference in NMR spectroscopy studies. Although not exclusively true, structured domains often are the functional elements within proteins, and determination of structure for these domains therefore provides insights into structure-function relationships. Because proteins often are composed of several individual domain building blocks connected by relatively unstructured linker domains, the

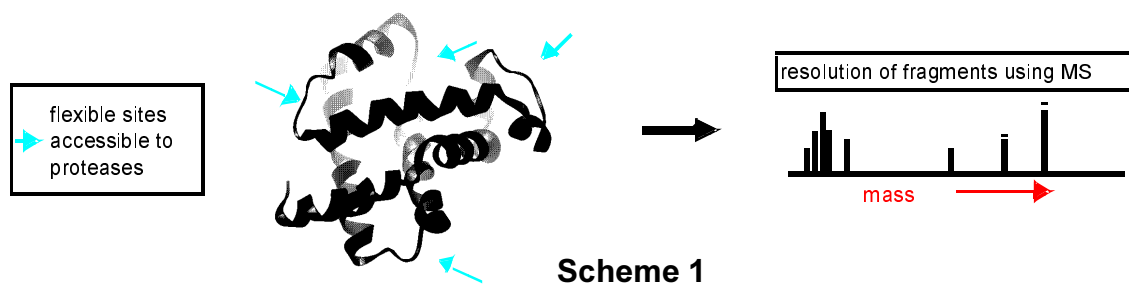


Figure 1. Schematic illustration of the use of proteolytic cleavage as a probe of protein structure. The arrows mark surface exposed and flexible sites that would be susceptible to proteolytic cleavage. If a sequence specific protease were used, the marked sites would also have to contain the protease recognition sequence to sustain cleavage. Mass analysis of all fragments together yield the cleavage ‘map’ that provides information on secondary, tertiary and, in multicomponent assemblies, quaternary structure.

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Table I. Proteases commonly used in structural analysis [after Konigsberg (3), Carrey (4) and Coligan (5)].

<i>Protease</i>	<i>Specificity^a</i>	<i>pH optima</i>	<i>Inhibitors^b</i>
Chymotrypsin	P ₁ = W, F, Y P' ₁ = nonspecific	7.5-8.5 Ca ²⁺ -activated	DFP, PMSF, TPCK
Elastase	P ₁ = A, V, I, L, G, S, T P' ₁ = nonspecific	7.5-8.5	DFP, PMSF
Endoproteinase Asp-N	P ₁ = nonspecific P' ₁ = D	6.0-8.0	EDTA, 1,10-phenanthroline
Endoproteinase Glu-C (V8 protease)	P ₁ = E (or E and D) P' ₁ = nonspecific	7.8 (4.0)	DFP
Endoproteinase Lys-C	P ₁ = K P' ₁ = nonspecific	8.5	DFP, TLCK
Pepsin	P ₁ = nonspecific P' ₁ = nonspecific, but cannot be V, A, G	2.0-4.0	DFP, PMSF, TPCK
Proteinase K	P ₁ = nonspecific P' ₁ = nonspecific	7.5-12.0	PMSF, DFP
Subtilisin Carlsberg	P ₁ = nonspecific P' ₁ = nonspecific	7.0-8.0	PMSF, DFP
Thermolysin	P ₁ = nonspecific P' ₁ = L, F, I, V, M, A	7.0-9.0	EDTA
Trypsin	P ₁ = K, R P' ₁ = nonspecific <u>but cannot be P</u>	8.5	DFP, PMSF, TLCK

cleavage sites



^a P₁ ——— P'₁ ; P₁ is on the amino-terminal side of the scissile bond and P'₁ on the carboxy-terminal side.

^b DFP, diisopropyl fluorophosphate; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; TLCK, tosyllysine chloromethyl ketone ;TPCK, tosylamido-2-phenylethyl chloromethyl ketone.

reduction of a multidomain protein to its individual domains through proteolysis provides a mechanism for functional characterization on a domain-by-domain basis.

Limited proteolysis was the original method for deletion analysis of protein structure-function relationships, but this approach has largely been replaced by DNA-based deletion analysis. Because proteases discriminate on the basis of structure, they still deserve a place as first-line tools in the analysis of protein structure, especially in combination with biomolecular MS (7). In domain mapping experiments, protease reaction kinetics must be controlled to yield only limited digestion (ie, single-hit kinetics), because extensive peptide bond scission reduces protein thermodynamic stability. Through local unfolding, multiple cleavages

expose secondary cleavage sites that provide information only indirectly related to native structure. Under limiting, or single-hit conditions, only the most kinetically accessible sites are cleaved, providing reliable information on three-dimensional protein structure.

In addition to probing static protein structure, protease sensitivity can be used to monitor changes in structure caused by, for example, ligand binding (1,2) and the addition of denaturants (8). Limited proteolysis can be used to probe the structure of multicomponent assemblies, including peptide-protein complexes, protein-protein complexes, and protein-DNA complexes. A common feature of these applications is that the protease is used to provide contrast between the associated and unassociated states of

the system. The formation of an interface between a protein and another macromolecule excludes solvent molecules and macromolecules such as proteases and protects otherwise accessible sites from protease cleavage.

Mass Spectrometry Techniques Used In Protease Mapping Studies

MS has become an integral part of biologic research primarily because of the development of matrix-assisted laser desorption and ionization (MALDI) (9) and electrospray ionization (ESI) (10). MALDI and ESI have greatly advanced our ability to characterize large, thermally labile molecules by providing an efficient means of generating intact, gas-phase ions. These two techniques have been used to gain molecular weight information on biologic samples with unprecedented speed, accuracy, and sensitivity. Developments in instrumentation (11) coupled with newer sampling methods have enabled higher levels of sensitivity, increased mass range, and better mass accuracy and promoted an increasing number of MS-based applications in the study of covalent and noncovalent protein structure. Both approaches offer unique and complementary capabilities.

ESI and MALDI are fundamentally different ionization techniques, but they achieve essentially the same end result: nondestructive vaporization and ionization. With electrospray, ions are formed directly from solution (usually an aqueous or aqueous and organic solvent system) by creating a fine spray of highly charged droplets in the presence of a strong electric field. Vaporization of these charged droplets produces singly or multiply charged gaseous ions. ESI can be interfaced with liquid chromatography in the analysis of proteolytic digests directly from the solution phase or with tandem mass analyzers such as ion traps and triple quadrupoles to perform collision-induced dissociation experiments on peptides.

In nano-ESI (12), a variation of ESI used for protein identification, the spray needle is made very small (tip $\sim 5 \mu\text{m}$) and is positioned close to the entrance of the mass analyzer, resulting in greatly increased efficiency. For instance, the flow rates for nano-ESI sources are on the order of tens of nanoliters per minute, and the total amount of sample consumed typically is less than femtomoles. Another advantage is that the droplets formed are smaller than in normal ESI, making nano-ESI more tolerant of salts and other impurities.

MALDI mass analysis generates gas-phase ions by laser vaporization of a solid matrix-analyte mixture in which the matrix (usually a small crystalline organic compound) acts as a receptacle for energy deposition. The relatively low number of charge states generated with MALDI, along with its high sensitivity and ability to simultaneously generate ions from multicomponent mixtures, makes it especially well suited for complex biomolecular samples such as pro-

teolytic digests. Moreover, MALDI-MS offers a reliable way of analyzing proteins and peptides.

ESI and MALDI-MS commonly use quadrupole and time-of-flight (TOF) mass analyzers, respectively. ESI with quadrupole mass analyzers typically has accuracy on the order of 0.01%, and ESI with the quadrupole ion trap mass analysis offers the additional advantage of allowing collision-induced dissociation experiments to be performed without having multiple analyzers. ESI quadrupole ion traps are being used extensively in the analysis of tryptic peptide digests for accurate protein identification.

MALDI with TOF analyzers constitutes one of the simplest mass analyzing devices and has accuracy between 0.1% and of 0.005%. These systems operate by accelerating a set of MALDI-generated ions with the same amount of energy down a flight tube. Because the ions theoretically have the same energy, the ions with different m/z values reach the detector at different times. Although the TOF analyzer has limited resolving power with MALDI (typically <2000), the addition of the reflectron, which reduces the kinetic energy distribution of ions that reach the detector, has improved performance. TOF reflectron mass analyzers are capable of generating high-resolution and high-accuracy mass measurements (errors <50 ppm). ESI and MALDI also are being coupled to the ultrahigh-resolution ($>10^5$) Fourier transform mass analyzer with part-per-million (<10 ppm) accuracy. Higher accuracy is proving to be valuable in protein identification and protein mass mapping.

Analysis of Peptides and Proteins

The utility of ESI and MALDI for primary structural analysis lies in their ability to provide accurate molecular weight information on intact compounds, information that is extremely useful for protein identification. For example, an unknown protein often can be unambiguously identified by mass spectral analysis of its constituent peptides produced by chemical or enzymatic treatment of the sample. MALDI is especially well suited for such analyses, because complex mixtures of peptides are directly amenable to MALDI analysis. The molecular weights of individual peptides in a protein digest are easily determined by using a combination of liquid chromatography and ESI-MS.

Peptide and protein analysis can be facilitated by initiating fragmentation in the gas phase. Fragment ions generated inside ESI and MALDI mass spectrometers by collision-induced dissociation (CID) often yield information about the primary structure of a sample (13). Tandem mass analysis techniques such as ESI ion traps, Fourier transform MS (FTMS), or triple quadrupoles and MALDI-FTMS involve selecting an ion of interest with the mass analyzer and isolating it in a collision cell. Once in the collision cell, the selected ion undergoes collisions with an inert gas such as argon, creating fragments that can be mass analyzed to

provide information about their sequence. This multiple mass analysis approach is often referred to as tandem MS or MS². Because the CID behavior of peptides is already well characterized, tandem MS with CID can be used to acquire direct sequence information on small peptides (<3 kDa).

Two important advantages of MALDI-MS are its sensitivity and ability to analyze complex polypeptide mixtures. These features also are being used to sequence biopolymers. The protein ladder sequencing technique originated by Chait et al. (14) allows stepwise removal of each amino acid in a peptide, a process in which each residue is chemically or proteolytically removed from the amino-terminal end to produce sequence-defining peptide fragments. Alternatively, amino acids can be enzymatically removed from the carboxyl terminus (15). A MALDI mass spectral readout enables generation of the resulting protein sequencing ladder. This method, which allows each amino acid to be identified from the mass difference between successive peaks, can provide sequence information on peptides of more than 30 residues. Sequence data has been obtained from larger proteins by enzymatic cleavage combined with protein ladder sequencing (15-17).

Software Packages for Spectrographic Analysis of Proteolytic Digests

Several software packages have been developed to facilitate the analysis of MS data for proteins and have been especially useful in the analysis of multicomponent fragmentation patterns generated through proteolytic digestion. Two such software packages are described to illustrate some of the features that are available. These two packages are available free of charge to not-for-profit organizations over the World Wide Web.

The software package PAWS (Proteometrics, Rockefeller University, New York, NY, freeware; www.proteometrics.com) offers a user-friendly, intuitive interface that allows a wide variety of MS-related, protein-based operations to be performed. Protein sequences can be loaded from files or entered manually and the monoisotopic or average molecular mass calculated. The program allows specific amino acid sites to be chemically modified and the modified mass to be calculated. Theoretical cleavage reactions (enzymatic or chemical) can be performed and the resulting fragment masses calculated and presented in tabular and graphical form. Searching tools have been incorporated to aid the analysis of MS data from proteolytic or chemical cleavage reactions. A simple search tool identifies peptide sequences derived from a known protein sequence that match a particular target mass. Searches consider all possible peptide sequences or only sequences consistent with fragmentation resulting from a particular sequence-specific cleavage reaction. Lists of fragment masses can be entered manually or imported from files, and mul-

iple searches can be performed. Search results are presented in graphical and tabular forms. The PAWS package offers a powerful and easy-to-use set of tools to predict cleavage patterns and fragment masses and to search for and identify peptides derived from known protein sequences that match experimentally determined masses.

The Protein Prospector package (University of California at San Francisco Mass Spectrometry Facility, San Francisco, CA; Drs. Karl Clauser and Peter Baker; prospector.ucsf.edu) offers some of the same capabilities as PAWS, along with extensive database searching capabilities. The software, which can be used over the Internet or installed locally after appropriate licensing, is divided into several modules that perform specific functions. These include MS-Digest, which can be used to generate theoretical protein digests; MS-Product, which can predict fragmentation in the mass spectrometer caused by post-source decay and collision-induced dissociation; and MS-Comp, which can suggest amino acid compositions consistent with experimental MS data, including parent peptide mass and immonium ion fragmentation data. Database searching tools include MS-Fit, which compares an observed set of proteolytic fragment masses with those predicted for all proteins in a database; MS-Tag, which compares tandem MS peptide fragmentation patterns with predicted patterns; and MS-Edman, which compares short segments of a peptide sequence with segments in a protein database. The database searching tools in Protein Prospector have been developed to aid the identification of unknown proteins on the basis of MS data but are also well suited for the analysis of MS data from protease-based structure mapping experiments. The MS-Fit module can be used to match observed fragment masses against those predicted for the protein being studied. Instead of using the entire nonredundant protein database for comparison, the content of the database is limited to the protein under study. Because the protease cleavage reactions for mapping studies are performed under limiting conditions, the fragments that are produced may span one or more uncleaved protease sites, increasing the number of theoretical fragments that must be considered during the comparisons. However, MS-Fit accommodates this need with a settable parameter corresponding to the maximum number of missed cleavages. This capability is also included in MS-Digest, allowing tables of all possible peptide fragments to be generated.

Analysis of Protein-DNA Interactions

The first application of limited proteolysis and MALDI mass analysis to the study of a multicomponent biomolecular assembly was published in 1995 by Chait et al. (18). This combined approach was used in structural analysis of the transcription factor Max when free in solution and when bound to an oligonucleotide containing its

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specific DNA binding site. Max is a member of the basic helix-loop-helix (bHLH) family of DNA-binding proteins and was the target of crystallographic studies. An extensive series of limited proteolysis experiments were conducted using free Max. The products of digestion reactions were analyzed using MALDI-TOF MS, demonstrating the suitability of this MS technique for analysis of multicomponent biomolecular samples in the identification of fragments and in their relative quantitation. The results showed that Max usually is very susceptible to proteolytic cleavage. However, Max is less susceptible to digestion by a variety of proteases at high ionic strengths, suggesting that salt stabilizes Max's structure. Because cleavage requires accessibility and flexibility, this result suggested that Max's structure is more highly ordered in the presence of higher salt concentrations, with loop regions in less flexible states. These results, indicating that Max may be relatively flexible in the absence of DNA, are consistent with the inability to crystallize Max in the free state.

Much more dramatic stabilization of Max was observed in the presence specific DNA. In this case, cleavage rates were reduced 100-fold, indicating significant stabilization in the presence of DNA. This stabilization stems from the protection of potential cleavage sites in formation of the Max-DNA interface and from the added thermodynamic stability imparted to Max by association with DNA. The cleavage pattern within the Max-DNA complex revealed that the bHLH domain is the minimal requirement for DNA binding and that the leucine-zipper domain is dispensable for this activity. The Max-DNA interaction sites were identified (Figure 2). These results provided valuable insights about Max-DNA binding structure-function relationships that guided the successful crystallization and structure determination of the Max-DNA complex (19).

Analysis of Protein-Protein Interactions

The general approach outlined previously has been applied to the study of protein-protein interactions. The mapping of protein-protein complexes in situ, however, is complicated because peptide fragments are produced for all subunits within a complex. An MS-based approach to map protein-protein interactions that overcomes this complication was developed by Chait et al. (20), which maps the interaction of a protein growth factor with a monoclonal antibody. Basic fibroblast growth factor (bFGF) was digested with endoproteinase Asp-N, followed by immunoprecipitation of binding-competent peptides using a monoclonal IgG1 antibody (mAb). The multicomponent bFGF/mAb assemblies were subjected to MALDI-TOF MS analysis, allowing identification of the peptide segments of bFGF that constitute the binding epitope. Because the monoclonal antibody was raised against small bFGF-derived peptides that lack native structure, this method was successful in mapping the mAb-binding epitope. However, the method requires

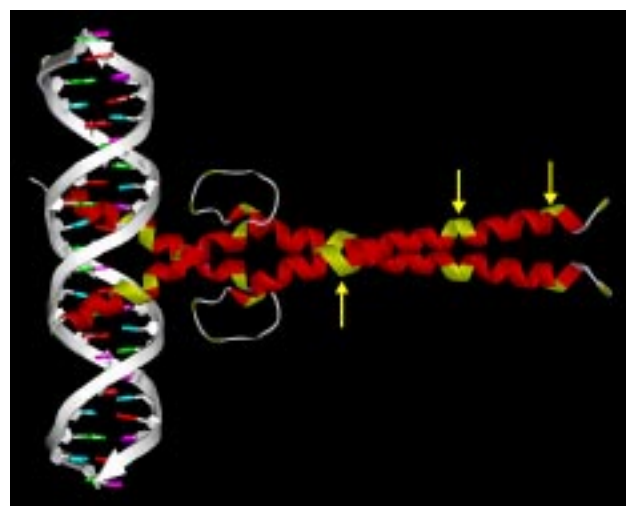
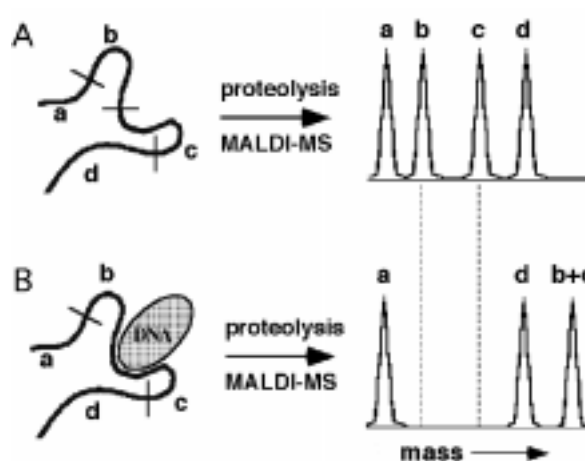


Figure 2. Mapping a Protein/DNA interface using limited proteolysis and MALDI-MS [after Chait and co-workers(19)]. A) Schematic view of proteolysis of a protein alone followed by MALDI-MS analysis. Cleavage sites are identified on the basis of I) the observed fragment masses, II) the known primary sequence, and III) known protease sequence-specificity. B) Formation of the protein/DNA complex protects one site from cleavage, producing an altered protease cleavage 'map'. Comparison of the maps in A and B give information about amino acids at the protein/DNA interface. C) Experimental results for the Max/DNA complex shown in the context of the 3D crystal structure [Ferre-D'Amare, *et al.*(20)]. The sites marked in light grey are cleaved in the absence in DNA while only the sites marked by arrows are cleaved in the presence of DNA. The bHLH domain at the interface with DNA is protected.

that a target protein sustain binding activity after proteolysis, and it would have only limited utility in the studies of proteins that require native structure for protein binding activity.

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An alternative approach to mapping protein-protein interfaces that overcomes the complications described was demonstrated by Kriwacki and Siuzdak (8,21). The experimental scheme is illustrated in Figure 3. The method exploits the high mass accuracy, resolution, and sensitivity of MALDI-TOF MS, combined with the power of stable isotope labeling, and it offers access to isotope-filtered mass spectra of individual subunits within multiprotein assemblies. Figure 3 (panel 1), illustrates two concepts central to mapping interfaces within assemblies. First, proteolysis reactions are performed for one component before and after formation of a multiprotein assembly (panel 1, left and right, respectively). Second, proteolysis reactions for the complex are performed in duplicate, with one subunit prepared at natural isotopic abundance in one experiment and in an isotope-labeled form in a second (panel 1, right). Other proteins within the assembly are used at natural isotopic abundance in both experiments. Reaction products are analyzed using MALDI-TOF MS (panel 2). In panel 2 (right), a subset of peaks in the upper spectrum appear at shifted positions in the lower spectrum (black versus open bars); these shifts correspond to the mass differences between unlabeled and labeled fragments. The isotope-filtered mass spectrum is obtained by subtracting these two spectra (panel 3). After data analysis, regions within the target protein that are pro-

tected from proteolysis in the assembly are identified (panel 4, right).

This mapping strategy has been applied to the complex between p21, a cell cycle regulatory protein, and cyclin-dependent kinase 2 (Cdk2). Experimental data are shown in Figure 4 (left), with a histogram of the final results shown on the right. MALDI analysis of the tryptic fragments of p21-B (the kinase inhibitory domain of p21) in the presence and absence of Cdk2 revealed a segment of 24 amino acids in p21-B that is protected from trypsin cleavage, thereby identifying the segment as the Cdk2 binding site on p21-B. The top trace (Figure 4, panel 1, shows a region of the MALDI-TOF mass spectrum for p21 after proteolysis with trypsin in the absence of Cdk2. The middle and bottom traces show spectra after proteolysis of the p21/Cdk2 complex, with p21 at natural abundance (middle) and ^{15}N labeling (bottom). Cdk2 is unlabeled in both cases. Analysis of the peaks in the top trace allowed identification of 28 trypsin sites in p21. Analysis of peaks in the lower panels showed that 4 of the 28 sites were protected from cleavage in the presence of Cdk2. The dotted lines in the lower traces mark peaks that exhibit an m/z shift from one spectrum to the other and reveal fragments derived from p21. The utility of the isotope labeling strategy is illustrated in the first panel of Figure 4, near m/z 6000 (marked by ovals). In the

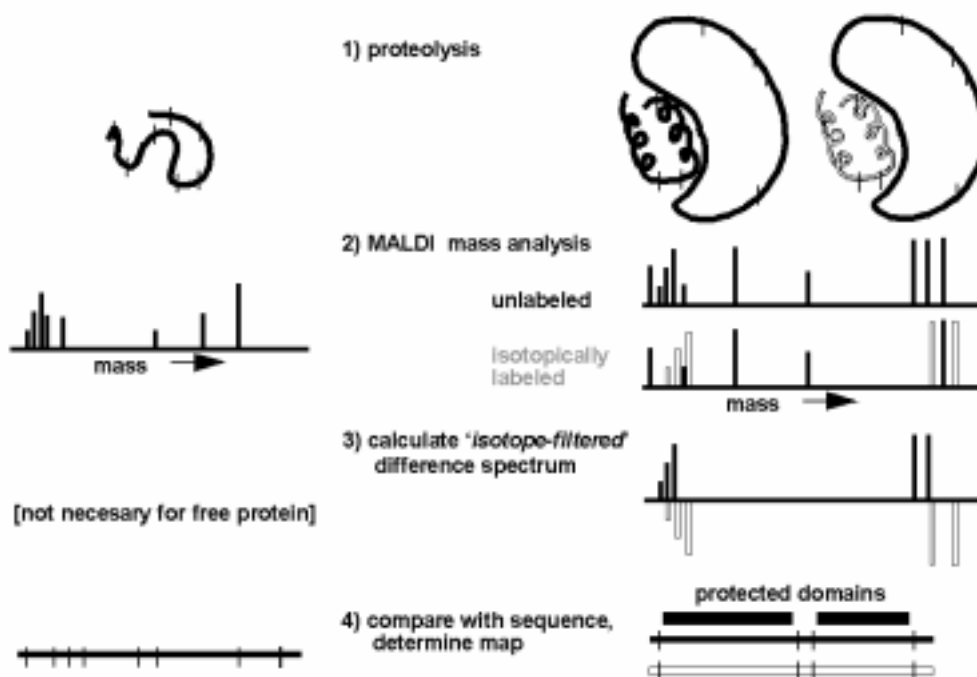


Figure 3. Schematic illustration of combined use of limited proteolysis, isotope labeling and MALDI-TOF mass analysis in protein-protein interface mapping. The left panel illustrates the analysis of p21 alone while the right panel illustrates the utility of isotope labeling one subunit within a multicomponent assembly in simplifying data analysis. At the top, right, regions within p21 (black lines and open lines) are protected from cleavage due to the formation of a complex with Cdk2 (kidney shape).

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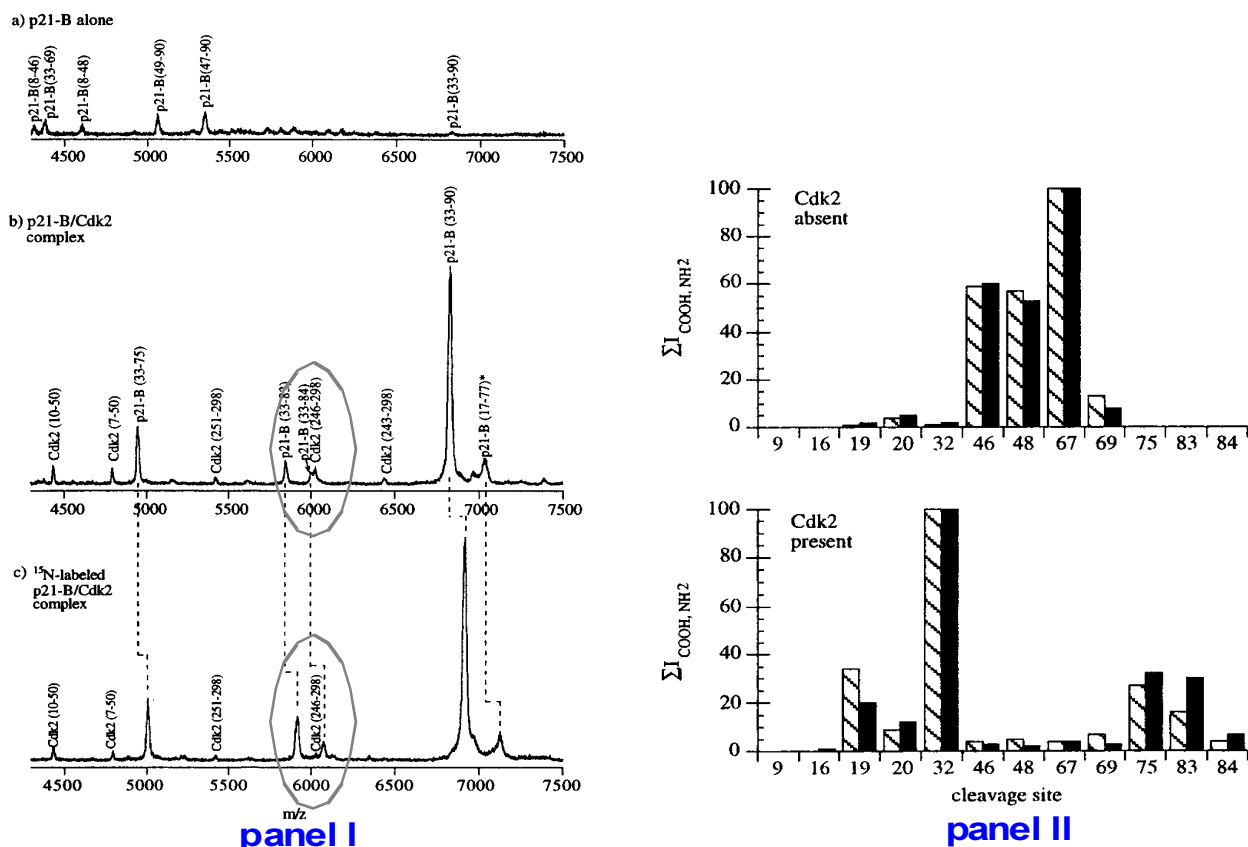


Figure 4. Interface mapping using limited proteolysis, isotope labeling and MALDI-TOF MS. Panel I, MALDI-TOF spectra for p21-B alone (top) and p21-B/Cdk2 complexes (middle and bottom panels). The dashed lines mark peaks that experience a mass increase in the lower spectrum with respect to the middle spectrum, allowing identification as p21-B-derived fragments. The ovals identify a region where the usefulness of isotope-labeling is particularly evident. Panel II, histograms showing trypsin accessibility versus position within p21-B amino acid sequence for p21-B alone (top) and p21-B/Cdk2 complex (bottom). The data represented by solid bars are derived from spectra for unlabeled samples and the hashed bars from spectra for ^{15}N -labeled samples.

middle trace, two nearly coincident peaks appear (p21 residues 33-84, Cdk2 residues 246-298); in the bottom trace, one of these peaks shifts to a new position. This result unambiguously identifies the left-hand peak in the middle trace as originating from p21 and the other as originating from Cdk2.

The mass accuracy of MALDI-MS instruments ($\pm 0.005\%$) allows reliable identification of most fragments from p21 and Cdk2 without resorting to isotope labeling. However, even this level of accuracy cannot allow identification in all cases because of the finite probability that fragments from the different subunits will have similar masses, as illustrated earlier. Figure 4 shows histograms of protease accessibility compared with position within the sequence for unlabeled and ^{15}N -labeled p21 (hashed versus solid bars) in the absence and presence of Cdk2. The four protected sites are clearly revealed in the bottom panel.

Other methods for examining protein-protein interactions rely on the chemical cross-linking of protein complexes before MALDI analysis. In one case, multimeric proteins are subjected to MALDI analysis after reaction with a cross-linking agent such as glutaraldehyde to determine their stoichiometry (22). Glutaraldehyde is used to covalently link the protein subunits in solution. Subsequent mass analysis of the covalently linked complex by MALDI permits an accurate assessment of the oligomeric state of the protein. In principle, the cross-linked species can be subjected to limited or complete proteolysis to reveal the amino acid residues involved in intermolecular covalent bonds.

Viral Studies

MS is useful for many higher-order structural studies, particularly the analysis of capsid quaternary protein structure (protein-protein interactions) of nonenveloped vi-

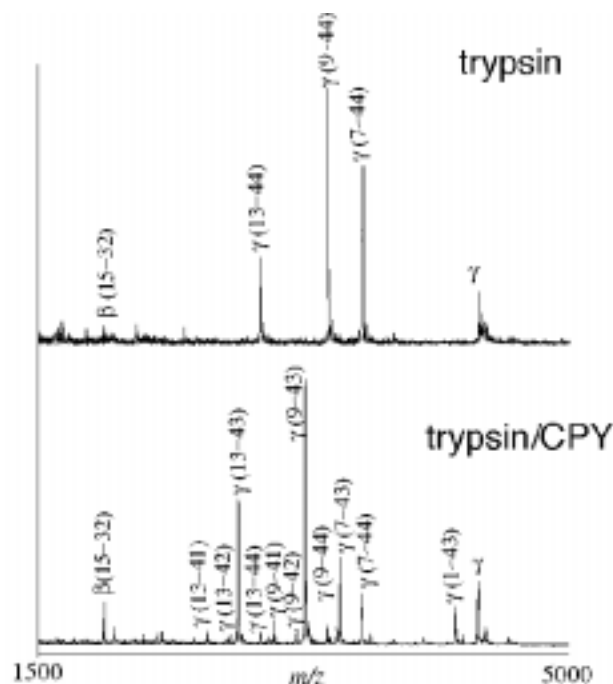


Figure 5. Digestion of Flock House viral capsid proteins performed directly on the virus. (Top) MALDI-MS data generated from the trypsin digest. (Bottom) Data generated from the carboxypeptidase Y digest of the trypsin digest (or endo/exo sequential digestion) of the viral capsid protein. β and γ denote the β -protein and γ -peptide segments of the α -protein, respectively, as discussed in the text, while the numbers in parenthesis are the N-terminal and C-terminal amino acids of the proteolytic fragments.

ruses (23). Capsid protein subunits, which make up the protective outer shell of the virus, provide structural stability and play a major role in infectivity. Although such protein-protein interactions typically have been mapped through x-ray crystallography, protein mass mapping is gaining more recognition as an effective technique. Conventional protein mapping has been used for probing the primary structure (ie, amino acid sequence) of individual proteins by incorporating chromatography, gel electrophoresis, or both techniques. Although proteolytic cleavage can provide indirect information about the domain structure of proteins, the method has not been routinely applied to protein-protein complexes because of the limitations in resolving and identifying the multiple fragments produced with conventional methods. Protein mass mapping shows great promise because MS is well suited to the analysis of complex mixtures of biomolecules and viral proteins, offering high sensitivity, resolving power, and accuracy.

Limited proteolysis and MALDI-MS experiments (24) were performed on Flock House virus, a nonenveloped, icosahedral RNA animal virus with dimensions (~300 Å)

similar to those of rhinovirus and poliovirus. Its protein coat or capsid is composed of 180 copies of a single gene product, α -protein, which is autocatalytically cleaved to peptides, β -protein and γ -peptide, during maturation. The autocatalytic cleavage products are easily observed through MALDI-MS. In using time-resolved proteolysis followed by MALDI-MS analysis, it was expected that the reactivities of virus particles to different proteases would reflect the surface-accessible regions of the viral capsid and offer a new way of mapping the viral surface. When these experiments were performed, cleavages on the surface-accessible regions were observed, but cleavages internal to the viral capsids (based on the crystal structure) were also generated. Observation of such cleavages was a surprising and initially perplexing result. In these studies, identification of the viral capsid protein fragments was facilitated by sequential digestion in which proteins were first digested by an endoprotease such as trypsin, followed by exposure to an exoprotease such as carboxypeptidase Y (Figure 5) (24). These results, along with the x-ray data, indicate that portions of the β -protein and γ -peptide are transiently exposed on the surface of the virus (Figure 6). These viral portions are implicated in RNA release and delivery.

Studies have been extended to the common cold virus (human rhinovirus), revealing previously undocumented viral structural dynamics and the inhibition of such dynamics by an antiviral agent (16). Results indicate that binding of the antiviral agent causes local conformational changes in the drug-binding pocket and stabilizes the entire viral capsid.

Conclusions and Trends

The examples presented illustrate the utility of combining proteolysis and MS analysis in structural studies of proteins and multicomponent proteinaceous assemblies. The experimental techniques that these approaches rely on are simple, efficient, and readily accessible. Moreover, as biomolecular MS continues to expand as the technique of choice in bioanalytic studies, the necessary MS instrumentation is becoming a standard feature of biotechnology core facilities. Biologic scientists are in a position to obtain information on molecular interactions without the need for site-directed mutagenesis and free of the caveats associated with this technique. The MS component of the experiments still requires expert operation of increasingly complex instrumentation and will remain in the realm of support facilities. Because computer analysis of mapping data requires expertise and chemical knowledge of the underlying cleavage and modification reactions, it may be best provided as support services. These methods are within reach of the entire biologic community. The important task is to educate the biologic community about the utility of these structure mapping methods and to expand their implementation.

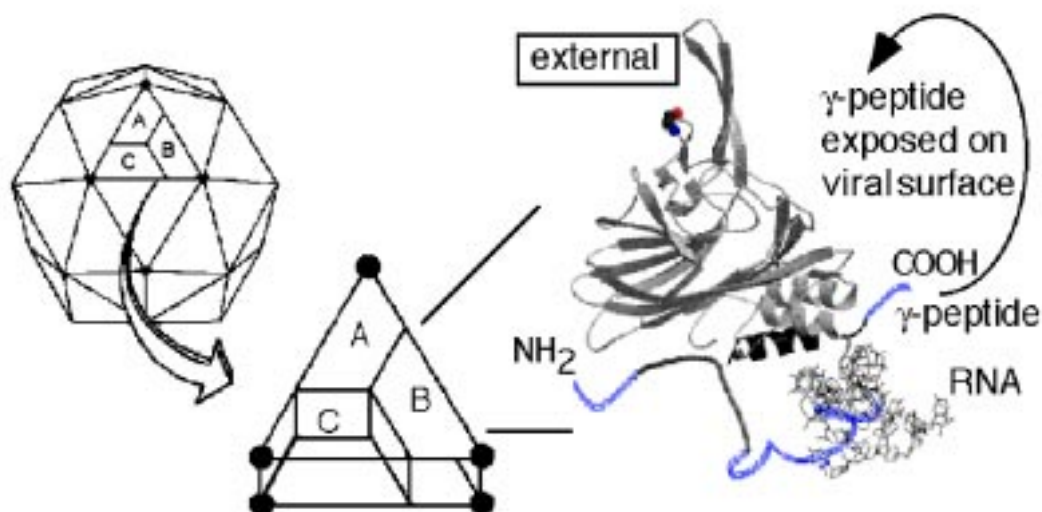


Figure 6. Crystal structure of Flock House virus shows that the γ -peptide and the N- and C-terminus of the β -protein are localized internal to the virus. Yet, proteolytic time-course experiments demonstrated that these domains are transiently exposed on the viral surface.

What are the future directions for MS-based proteolytic mapping methods? Although current MS instrumentation is limited by low resolution and accuracy at the upper mass limits, indirect mass analysis (ie, only the products of probing reactions are flown through the mass spectrometer) makes the “window of opportunity” quite broad. We have discussed applications to biomolecular assemblies that have and have not been supported by high-resolution structural data. In the early stages of structural studies, the MS-based probing methods are particularly well suited to provide rapid access to low-resolution maps that are used to guide high-resolution studies. However, this stage may be an end point in some investigations in which the identification of interacting residues is the desired information. As a complement to high-resolution structural information from x-ray crystallography or NMR spectroscopy, probing studies have already been shown to provide valuable and even startling insights into protein dynamics and structural rearrangements. These investigations mark the take-off point for studies that seek to quantitate the molecular kinetics and thermodynamics of the underlying dynamic phenomena. We posit that MS methods will assume a position similar to that of gel electrophoresis as a primary research tool in coming years because of its superior sensitivity, precision, accuracy, and throughput.

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Optimization of In-Gel Digestion for HPLC and Mass Spectroscopy

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Abstract

A variety of approaches are used to link the unparalleled resolution of SDS PAGE with biochemical methods appropriate for protein structural analysis. Efforts in most recent years have been relatively successful in identifying proteins from 1D and 2D gels following enzyme digestion from nitrocellulose, PVDF and polyacrylamide. Selection of these supports is dictated by the type of analyses, throughput and data quality. Minimal sample loss is avoided with gel digestion, however background contaminants can obscure high sensitivity analysis in the low pmol range. Routine sample work-up has been reported with gel digestion protocols in the low pmol range with approximately 5-15% recovery using detection by either Coomassie Blue or silver staining. In order to continue refinement of this procedure and increase overall recovery, preparation of excised gel for subsequent digestion has been modified to increase both adsorption of enzyme into gel and substrate diffusion. The requirement to concentrate sample free of salt or detergent for obtaining high quality HPLC and mass spectrum is addressed.

Introduction

The necessity to obtain samples in high concentration and free of contaminants has become the rate-limiting step in protein structural characterization (1,2). Although the use of gel electrophoresis to isolate and characterize low-abundant proteins has become the method of choice, sample recovery is reported from 15 to 20%. Gel recovery of oligonucleotides has been shown to be more efficient with recoveries in the range of 50-70%. In the case of proteins or peptides, high background noise compromise data quality and interpretation while DNA samples contain salts which interfere with downstream procedures. Sample contaminants that contribute to background noise include most buffers (i.e. tris, phosphates, glycine) or surfactants (i.e. detergents, urea, guanidine) (3,4,5). A preparative membrane can be used to prepare in-gel samples salt-free in high yields that would permit harsh digestion and extraction conditions. An ideal adsorptive membrane should exhibit efficient binding for all analytes with high capacity and quantitative elution while removing most contaminants. To meet these requirements, a styrene sulfonate cation-exchange membrane has been incorporated into the Amicon Microcon® centrifugal device. A styrene sulfonate functional group offers a broad range of selectivity for the adsorption of polypeptides and other biomolecules.

A quantitative review of membrane performance shows rapid kinetics for adsorption with excellent analyte binding. The Microcon-SCX format offers a wide range of applications for concentrating complex peptide or oligonucleotide mixtures. Examples of in-gel digestion of polyacrylamide-containing-proteins are presented with recoveries in the range of 40 to 60%. In-gel recovery of oligonucleotides for DNA cloning is greater than 70% and salt free. Isolated oligo samples are also shown to be functional by polymerase extension. The flow characteristic of the membrane permits binding and desorption during 15 to 30 second centrifugations in a table-top centrifuge to accommodate 0.1-250 µg of sample. Applications include analyses by sequencing, HPLC, CE, mass spectroscopy, pulsed amperometric detection and preparation of gel-containing samples.

Methods

A. Use of Microcon-SCX

The membrane is wetted by adding methanol to the device, emptying and repeating with DIW. Samples are applied in volumes <500 µl at a pH below the pI or pKa of the analyte, with optimal binding at salt concentrations <0.1 M. For unknown samples, the pH is routinely lowered to about 2 with 5-10 µl of glacial acetic acid or diluted in 20 mM HCl to protonate free amines of peptide or analytes in sample. After loading sample into Microcon-SCX (Figure 1), binding occurs during a 30 second centrifugation. Sample adsorbs to membrane when protonated amines selectively exchange with [H⁺] of sulfonic acid. An optional wash step is performed by using 500 µl of 10 mM HCl and 10% MeOH.

B. Sample Elution

Following analyte binding and washing, a clean vial is placed into the unit, with the addition of 25-50 µl of desorption reagent to sample bound membrane, spun at 14,000 x g for 15 seconds and repeated with another 25-50 µl of desorption reagent. The salt-free eluted sample can be either used directly for analyses, neutralized or speedvac dried. As described in the following section, selection of desorbant will depend on both the application and the analysis.

C. Sample recovery from gel with Microcon-SCX

In-gel Protein Digestion:

1. For reduction-alkylation before electrophoresis, prepare sample in "cracking buffer", add 10 mM DTT, boil 3 minutes, cool to RT, add 20 mM iodoacetamide and incubate at room temperature for 20 min.

2. Run gel, stain/destain and excise bands containing the protein of interest which are cut into 1 mm slices and placed into the supplied Eppendorf tube. Wash in 50% acetonitrile (ACN), 200 mM NH₄HCO₃ to remove residual SDS and Coomassie Blue, save wash and completely dry gel sample in speedvac (9).

3. The gels are swollen in either trypsin (Sequencing grade modified Trypsin, Promega) or lysyl endopeptidase (Achromobacter Protease I, Wako Chemicals, USA) for 30 minutes in an appro-

priate volume at a concentration of 10 ng/ml in 0.1 M Tris-HCl, 5 mM CaCl₂, pH 8 (10) with or without 0.1% C18 Zwittergent (Calbiochem).

4. The supernatant is removed and replaced with 100-200 ml of 0.1 M Tris-HCl, 5 mM CaCl₂, pH 8. The gel slices are homogenized at 3,000-4,000 RPM to a slurry with the Eppendorf fitting pestle-homogenizer (KT749520-0000, VWR pestle), using a high speed mixer or drill as shown in Figure 1.

5. Following enzymatic digestion for 24 h at 37°C, samples receive an equal volume of 20% formic acid, 80% acetonitrile and are allowed to extract for 60 minutes in a water bath sonicator. Using a cut pipette tip, transfer gel slurry digest into the Microcon-SCX unit.

6. Place unit into centrifuge as instructed and centrifuge for 5 minutes at high speed. Remove filtrate, save, and wash membrane with 0.5 ml of 20 mM HCl after vortexing and centrifugation at 13,000 x g for 5 minutes.

7. The digested peptides are recovered by adding 50 ml of 1.4 M NH₄OH in 50% methanol to the Microcon-SCX unit containing a clean collection vial and repeated at least once, using the same vial.

8. Samples can be either directly injected for MS or HPLC analysis or speedvac dried.

Recovery Of Oligonucleotides From Polyacrylamide:

1. Samples are electrophoresed in TBE-urea gels and visualized by UV shadowing while the bands of interest are excised.

2. The gels are cut into 1 mm slices and immersed in 20 mM Tris-HCl, 6 M urea, pH 8 (100-200 µl). Homogenization and extraction of sample from gel are performed as described above.

3. Binding of sample containing slurry to the SCX membrane occurs by lowering sample pH using an equal volume of 0.14 M HCl.

4. Washing and desorption from membrane are performed as described above.

Results

Various peptide standards, protein digests and complex mixtures treated by Microcon-SCX are presented to demonstrate the efficiency of analyte binding, elution and selectivity.

1. Complex mixture of peptides from tryptic cytochrome c (Figure 2) for 6 separate samples show >90% ± 2% recovery to control (untreated).

2. A complex glycopeptide mixture of endo lys c-digested human immunoglobulin heavy chain (hIgG-HC) following SCX is shown in Figure 3 (trace above). Data shown in lower MS spectral scan was used to identify HPLC fractionated peptides in the upper trace in order to confirm peptide and glycopeptide recovery. MS results of SCX-LC samples confirmed that all peptides and glycopeptides were recovered.

3. Following reduction and alkylation, 25 pmol of BSA or hIgG-HC was electrophoresed and prepared for in-gel digestion and SCX desalting. Approximately 40-60% of peptides were recovered.

4. Microcon-SCX can be used to obtain oligonucleotides ranging from 18-45 base pairs and higher that are free of salt or reagents. Overall recovery of different oligomers was determined by reverse phase HPLC and shown to be approximately 90%.

5. DNA functionality of SCX-treated 45-mer was synthesized and gel-purified from a 10% TBE-urea polyacrylamide gel, desalted and annealed to ssM13mp19, extended, and digested with restriction enzymes. The gel-purified-SCX oligo product appears to be a longer extension product in higher yields than the unpurified, native extended product.

Conclusion

The fast kinetics for analyte binding occurs at low pH to allow for a brief 15 second centrifugation. Since the membrane exhibits high selectivity toward protonated biomolecules, most salts and detergents in dilute concentrations are removed during the initial binding step. Desorption of sample is application-specific and performed at either low or high pH with volatile eluants for direct use or speed vac.

Microcon-SCX was affective for removing detergents, chaotropes and polyacrylamide from gel extracted samples. Agressive gel homogenization provides more accessibility of the enzyme to the protein as well as increases sample extraction efficiency in the 40-60% range. While gel remains retained on the membrane, contaminants are removed in a wash step prior to peptide elution. HPLC analysis of in-gel digested BSA demonstrates efficient UV contaminant removal following homogenization, digestion and sample clean-up with Microcon-SCX. Excellent signal to noise ratio, ease of use, convenience and high turnover is achieved while maintaining sample fidelity.

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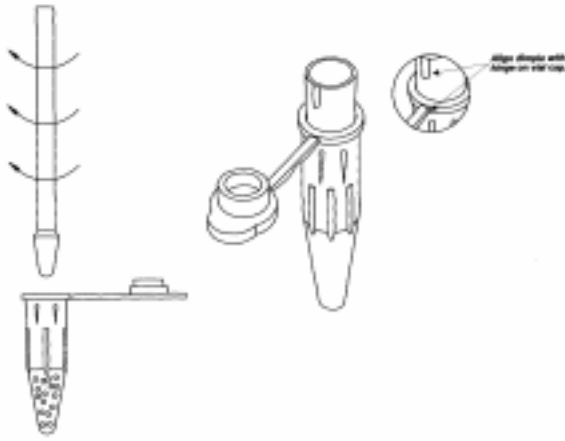


Figure 1. Homogenization and Microcon-SCX Orientation for Sample Binding and Recovery. Eppendorf fitting pestle-homogenizer for aggressive gel homogenization (left). In fixed-angle rotors, optimal recovery of sample in 50–100 μ l requires consistent orientation in the rotor during adsorption and desorption (right).

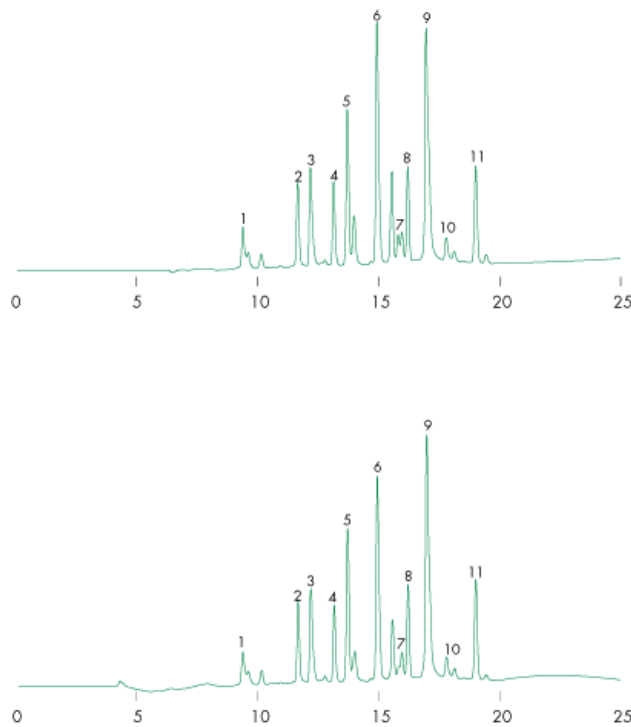


Figure 2. Reversed Phase Chromatography of Trypsinized Cytochrome c Before and After Adsorption to Microcon-SCX. Approximately 250 μ g of digest was diluted to a total volume of 500 μ l and either injected directly onto the column (top) or bound and eluted from Microcon-SCX (below) as described in Methods. Separation was performed with an Amicon, C18-300-10sp (4.6 x 250 mm) column using a linear gradient of 5% ACN to 55% ACN (0.1% TFA in DIW) in 20 minutes at 1 ml/min.

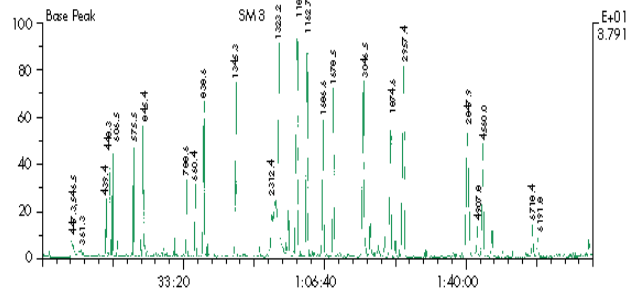
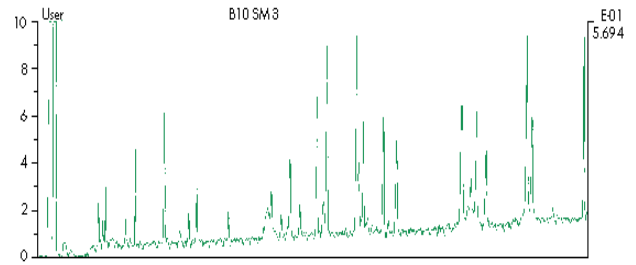


Figure 3. In-Gel Digestion of BSA after Homogenization and Microcon-SCX Preparation. Approximately 10 pmol of BSA was reduced, alkylated and purified by an 8–12% Tris-Glycine acrylamide gel. Samples were prepared for SCX treatment as described in Experimental section. Following SCX elution, samples were speedvac dried to 10 ml, diluted with 40 ml Buffer A (0.1% TFA) and injected onto a 2.1 x 100 mm RP300 (Perkin Elmer) column. Peptides were separated using a 60 min linear gradient of 5–60% acetonitrile in 0.1% TFA. (Upper trace) In-gel digested BSA peptides recovered after homogenization and SCX treatment. (Lower trace) Blank gel digested, homogenized and treated over SCX as experimental.

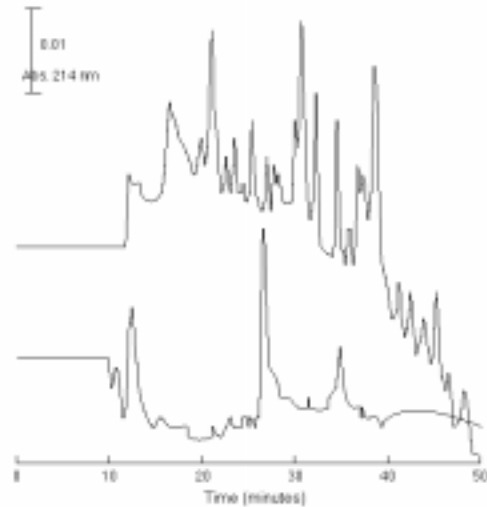


Figure 4. Identification of fragments by LC-MS of Endo Lys c Digested hlgG-HC following Microcon-SCX. Digests were injected into an LC-MS system consisting of an HP1090 plumbed to a Finnigan TSQ7000 with an electrospray source. Approximately 45 mg of digest was loaded on a Nucleosil 300-5 C18 column (0.46 x 25 cm; Macherey-Nagel, Duren, Germany) and eluted with an ACN gradient at 0.7 ml/min; the effluent was analyzed on-line for both UV absorbance (top) and mass without flow splitting (below) (8).

The Influence of Dielectric Constant upon Protein Crystallization by Dynamic Light Scattering Investigations

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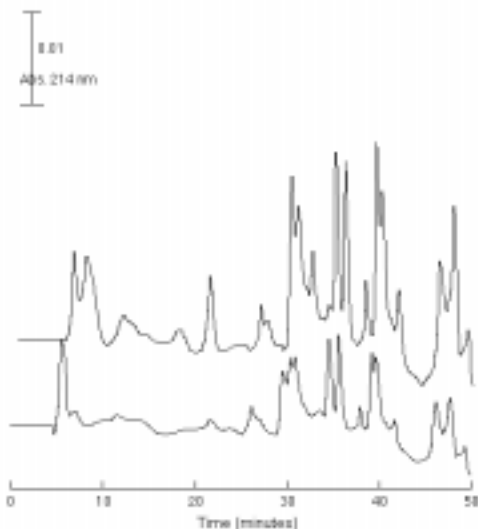


Figure 5. In-gel Digestion of hIgG-HC after Homogenization and Microcon-SCX Preparation. Approximately 25 pmol of hIgG-HC was reduced, alkylated and purified on a Tris-Glycine acrylamide gel (8–12%). Samples were digested with Endo lys c and prepared for SCX treatment as described in the Experimental section. Following SCX elution, samples were speedvac dried to 10 µl, diluted with 40 µl Buffer A (0.1% TFA) and injected onto a 2.1 X 100 mm RP300 (Perkin Elmer) column. Peptides were separated using a 75 min linear gradient of 5–60% ACN in 0.1% TFA after 5 min of 5% ACN. (Upper trace) Approximately 20 pmol of reduced-alkylated Endo lys c-digested HlgG-HC. (Lower trace) HlgG-HC excised from gel, Endo lys c-digested, homogenized and treated over SCX as described.

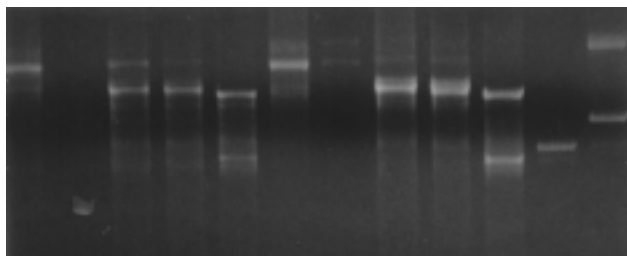


Figure 6. Functionality of Oligonucleotides recovered from Acrylamide gel and Desalted with Microcon-SCX. A 45-mer of M13mp19 primer was either directly annealed or gel purified with Microcon-SCX and then annealed to M13mp19 single-stranded DNA (85°C and cooled to room temperature) (Invitrogen). Respective annealed primers were extended (37°C for 2h with AMV-RT and dNTP's) and digested with three different enzymes as shown in the corresponding lanes: 1) Native oligo (45-mer) annealed and extended, 2) EcoRI-digested native-extended, 3) KpnI-digested native-extended, 4) BsaBI-digested native-extended, 5) SCX gel-purified oligo (45-mer) annealed and extended, 6) EcoRI-digested SCX-purified anealed-extended, 7) KpnI-digested SCX-purified anealed-extended, 8) BsaBI-digested SCX-purified anealed-extended, 9) M13mp19 s.s. 10) M13mp19 d.s. EcoRI and KpnI sites within the M13mp19 priming site harbors one site each for EcoRI and KpnI (one fragment in lanes 2,3,6,7). The M13mp19 priming site harbors one site each for EcoRI and KpnI (one fragment in lanes 2,3,6,7). Two sites are generated in the region extended with AMV-RT (two fragments in lanes 4,8).



Abstract

The influence of ethanol and polyethylene glycols on the aggregation of ribonuclease A, concanavalin A, lipase from wheat germ and bovine serum albumin has been studied in solution using dynamic light scattering methods (DLS). The influence of dielectric constant upon the aggregation behavior of protein solutions has been calculated taking into account the dielectric constant of mixtures between water and ethanol or polyethyleneglycols. Finally, a new device based on the gel acupuncture technique [developed by Garcia-Ruiz and Moreno (see reference in the text)] and on transport phenomena has permitted us to carry out "in situ" investigations of the dielectric influence on the protein aggregation and crystal growth. It is the first time that the effect of the dielectric constant has been evaluated stepwise by using a new method to crystallize proteins in capillary tubes. This calculation has been applied to low and high molecular weight solvents that are useful in protein crystallization.

Introduction

Modern laser technology and the development of dynamic light scattering methods have led to a rebirth of interest in light scattering applications in macromolecular chemistry, biophysical chemistry and biological chemistry. In the case of biological macromolecules, investigations have focused on the crystallization step, which is considered a bottleneck of protein crystallography, not only because of the difficulties found in the search for crystallization conditions, but also because little is known about crystal growth techniques and the solubility behavior of proteins in solution (1, 2). As already mentioned, the protein-solvent system is electrostatically heterogeneous. According to the theoretical interactions between protein and solvent, a description of the physical basis of electrostatic forces in proteins has been reviewed by Nakamura (3). In X-ray crystallography of proteins, a number of water molecules can usually be resolved at the protein surface. Therefore, the protein-solvent interaction and the resident time of water in the local and overall macromolecular surface are important factors to be taken into account to understand the physical and chemical behaviors of hydrophobic protein surfaces in solution (4). The first study that took into account the importance of the dielectric influence of a polyalcohol used as a precipitating agent was done by McPherson in 1976 (5). Several polymeric precipitants for the crystallization of macromolecules have been recently reported, namely nine water soluble polymers that affect the properties and structure of water and protein interactions and solubility (6).

In this article, the influence of mixtures of water and polyethylene glycols (ranging from polyethyleneglycol 400 to polyethyleneglycol 6000) and water-ethanol on the aggregation of ribonuclease A, concanavalin A, lipase from wheat germ and bovine serum albumin have been studied in solution by the use of dynamic light scattering (DLS). It has been possible to distinguish whether the initial formation of clusters and the trend for aggregation are due to either nucleation (crystal formation) or random mechanisms (amorphous precipitation). Finally, it is shown how the experimental predictions are useful in designing new experimental protocols to generate nucleation of a protein, which can subsequently be grown by either macro or microseeding techniques.

Methods

Sample preparation and injection. The following experimental set-up has largely been obtained from the operator's manual supplied by Protein Solutions Co (with permission). The guidelines of this paragraph are focused on how to prepare samples for injection into the DynaPro-801 and are intended as a guide for typical sample preparation. All protein solutions should be made in an appropriate buffer in order to attain the most accurate measurement.

Sample injection.

There are two key ideas to keep in mind when injecting a sample into the DynaPro-801: Always use a filter, and try to eliminate air bubbles entering the sample cell. Following these rules will minimize problems with false readings due to dust, debris and air bubbles.

1. Make sure that all fittings are connected snugly (hand-tight) and that there are no leaks.

2. Take approximately 200 microliters of sample into a 250 microliter syringe. Remove any trapped air bubbles in the syringe by tapping gently with the needle pointing upwards. Slightly working the plunger up and down may help remove bubbles. Make note of how much sample is in the syringe in order to aid sample recovery.

3. Place the appropriate filter on the syringe. With the syringe still pointing upwards, wet the filter by gently pushing the syringe plunger until a slight meniscus is created on the top of the filter. This will remove air from the filter. Now attach the proper needle onto the syringe and you are ready to inject sample into the instrument. All analyses of the data were performed using the AutoPro-801 PC software from Protein Solutions co.

Dynamic light scattering measurements. These were performed using a DynaPro-801 Dynamic Light Scattering Instrument (Protein Solutions, Co.). Samples were injected through Whatman Anotop 10 plus 20 nm filters. Multiple measurements were taken from different samples. Data were collected and analyzed using the AutoPro data software for the DynaPro-801 instrument (Protein Solutions, Co.).

Crystallization. we used several growth techniques, ranging from traditional to more modern techniques such as the gel acupuncture technique designed by Garca-Ruiz and Moreno (7,8). In order to study the "in situ" influence of the dielectric constant on protein crystallization, a new device for crystallization has been recently developed (9), and is shown in Figure 1. All the experiments were carried out at 25°C, leaving the samples undisturbed for at least two days.

Results and Discussion

DLS data were collected using a DynaPro-801 molecular sizing instrument at 90 degrees and 780 nm. The variation of diffusion coefficient (exponential decay, checked by the second Fick's Law versus time) and hydrodynamic radius (exponential growth) versus increments of PEG-400 (for lipase) and PEG-6000 (for concanavalin A) showed a typical aggregation behavior of ordered clustering. In order to gain independent evidence for this assumption, we checked the growth rate. The variation of hydrodynamic radius versus time was proportional to the root square of the time, which demonstrates that the growth of the cluster is diffusion controlled [for further information about transport phenomena in crystal growth see Wilcox et al. (10)].

The trend for aggregation shown in our experiments led us to consider the possibility of whether the size of the polyalcohol was as important as the influence of the dielectric constant on the aggregation behavior of proteins. This was evaluated by mixing ethanol with water and monitoring its influence on protein aggregation. Calculations of the dielectric constant of the mixture were based on the following equation:

$$E(\text{mixture}) = \frac{\% A E(A) + \% B E(B) + \dots + \% Z E(Z)}{100}$$

where:

E is the dielectric constant,
E (A) the dielectric constant of the component A.

Ribonuclease A Crystallization Analysis

The crystallization conditions of ribonuclease A, shown in Fig. 2, were taken from Wlodawer et al. (11) and Srinivasan et al. (12). All data analyses using DLS techniques were compared with the published results of Boyer et al. (13), who studied the precrystallization conditions of ribonuclease A in alcoholic solutions by dynamic light scattering investigations. Neither the dielectric constant considerations nor the analysis of the type of growing cluster has been described elsewhere. When the dielectric constant decreases, the size of the cluster starts to increase, as shown by low diffusion coefficient.

The size continues to increase until a maximum hydrodynamic radius of 6 nm at 43% ethanol (dielectric constant in the plot = 55). These results agree with those of Wlodawer et al. (11). It is important to bear in mind that the aggregation is by a nucleation mechanism (ordered growth of clusters in the biomacromolecular solution) showing exponential growth (Fig. 2 a) or exponential decay (Figs. 2 b and 2 c). Crystals of ribonuclease A were obtained based on these results using the gel acupuncture technique, designed by Garca-Ruiz and Moreno (7, 8). We can conclude from the fitting of the plots that the rapid surface kinetics produced diffusion controlled cluster growth, which may be the only mechanism available to explain this crystal growth.

Experimental conditions for ribonuclease A

The concentration used in the experiments was 0.2 mg/ml in all panels. At the beginning, a stock solution containing ribonuclease A (0.4 mg/ml) was mixed with different concentrations of ethanol solutions. At least two hours were allowed to reach equilibrium before starting the DLS measurements. All experiments were performed at 25°C and protein concentration, volume of solution for analysis and time of equilibrium were held constant, when possible.

Concanavalin Crystallization Analysis

The diffusion coefficient, hydrodynamic radius and estimated molecular weight at different dielectric constant values were evaluated for concanavalin A, based on DLS experiments. A rapid protein aggregation was observed when the dielectric constant value increased, according to diffusion coefficient measurements (Fig. 3a). Consistent with this result, the hydrodynamic radius decreased with decreasing values for the dielectric constant, from 3.0 E8 cm to 2.0 E8 cm (Fig. 3b). In addition, when the protein concentration was approximately 10 mg/ml and PEG 6000 was kept between 2-5% (w/v), crystalline nuclei were formed. An estimation of the size of concanavalin A aggregates can be gained from the estimated molecular weight (Fig. 3c). Taking all of these parameters into account indicates that protein aggregation takes place at high dielectric constant values. It is interesting to note that the behavior observed for this protein is the opposite to that found for BSA, as is shown in the next.

Our results demonstrate that a rational search for crystallization conditions may be carried out if the effect of dielectric constant value on both protein solubility and aggregation is studied by dynamic light scattering techniques and used with the new device employing the gel acupuncture technique (7-9).

Lipase and BSA Analysis

Lipase crystallization conditions. We used PEG-400 as precipitating agent. CaCl₂ at pH 9.0 was added to stabilize the protein in solution. Crystallization trials were performed using the hanging-drop technique. Aliquots of 5 microliters of freshly purified protein solution at a concentration of 5 mg/ml were mixed with 5 microliters of reservoir solution (0.1 M Tris-HCl pH 9.0, 10% (v/v) PEG-400) and 2 microliters of CaCl₂ (0.01M). After 90 days, crystalline nuclei were formed. In the initial crystallization screening an amorphous precipitate was obtained at higher concentrations of PEG. The ordered aggregation behavior shown in Figs. 4a and b, could be explained as a diffusion controlled mechanism of crystal growth.

Several experiments were focused on determining the possible influence of low molecular weight alcohol on protein solubility. The most remarkable results were observed by adding ethanol at pH 9.0, where in the case of lipase, the increased amount of alcohol produced linear aggrega-

tion behavior, resulting in an amorphous precipitate (Figs. 4c and d). This linear behavior of the plot could be explained because of the high rate of aggregates formation, which used to produce amorphous precipitation.

BSA was chosen as an ideal comparative case, because it is well known that this protein is denatured in the presence of ethanol. As shown in Fig. 5, the linear behavior in all plots is a result of a random aggregation of the clusters, provoking amorphous precipitate as the concentration of alcohol is progressively increased.

Conclusions

It is well known that when interface kinetics is controlling, the growth rate increases almost linearly with time or when the precipitating agent increases stepwise. It is important to bear in mind that when the interface kinetics constant is slow, the growth rate depends entirely on the interface kinetics (this is the controlling step in the aggregation reaction).

For rapid interface kinetics the growth is diffusion controlled, as explained in the text. It is possible to say that the linearity obtained in some plots (e.g., ethanol used as a precipitating agent for lipase and BSA), could be approximated by a linear chain to which monomers can be added only at the ends, giving an amorphous precipitate. On the other hand, if the growth is diffusion controlled, this will permit ordered nucleation (e.g., PEG 400 and PEG 6000 used as a precipitating agents for lipase and concanavalin A respectively).

Perspectives

Our next goal will be focused on growing these nuclei until they reach a suitable size for X-ray diffraction analysis. This will be accomplished using the new device that relies on the gel acupuncture technique (9), which will permit us to monitor "in situ" the influence of the dielectric constant on protein crystallization. This method has a simple experimental set-up and permits simultaneous control of several crystal growth parameters, once precrystallization conditions are obtained. It is important to emphasize that with this method, the slow supply of molecules by diffusion causes a reduction of the protein concentration in the vicinity of a rapidly growing crystal. In this way, the reduced protein concentration prevents the formation of new nuclei, and therefore, eliminates unwanted interference between crystals.

Acknowledgments

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CORRESPONDING EDITOR: Gerald M. Carlson

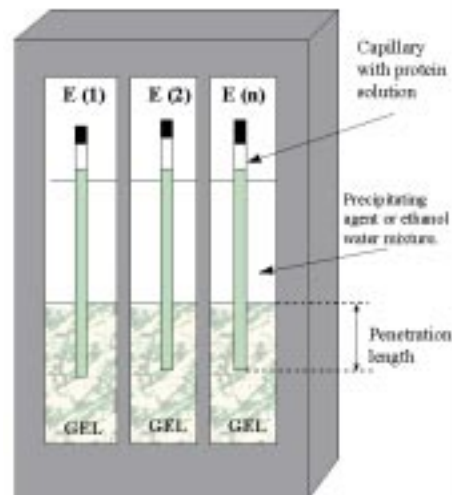
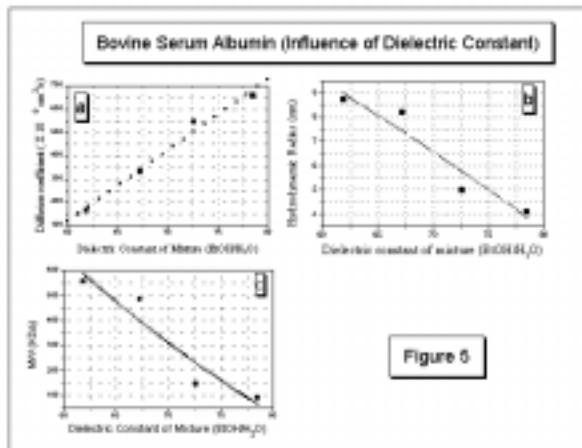
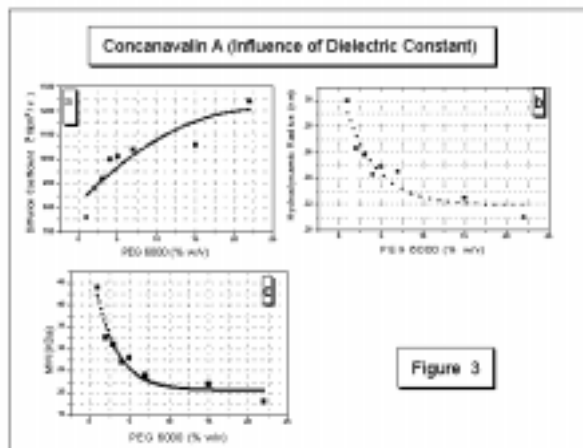
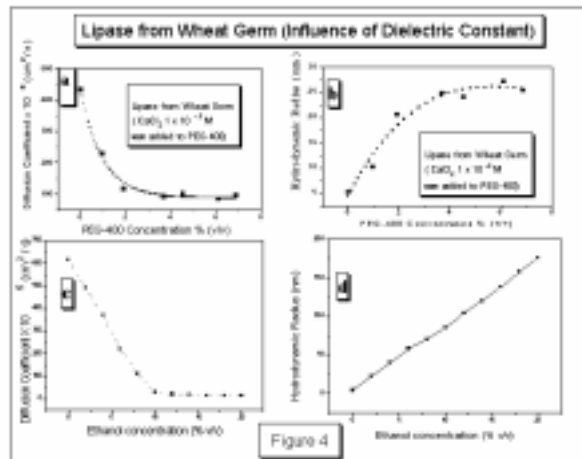
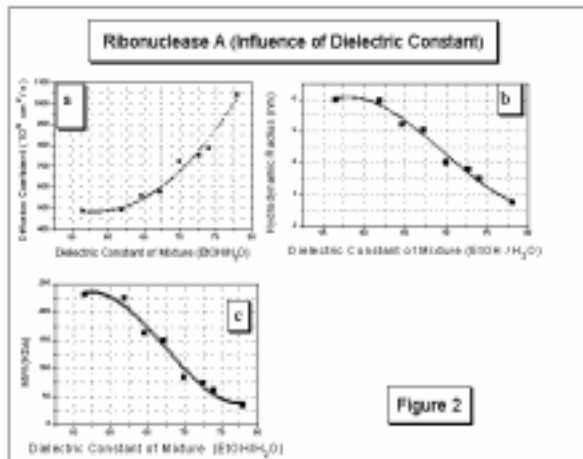


Figure 1



Message from the Executive Board

The recent ABRF by-laws ballot raised considerable interest among our members, particularly in our electronic discussion group. Of the two provisions on the ballot, that to change the name of the Association for Biomolecular Resource Facilities to Association for Biomolecular Research and Technology was controversial. This was clearly a time for reevaluation of what the ABRF means to each of us. While the vote was in favor of retaining the ABRF name, the approximate 60/40 split in votes underscores that our members join together in their interests in technology, but come from a variety of environments. The ABRF Executive Board wants to emphasize that we will continue to serve all of our members by continuing our mission "to promote and support resource facilities, research laboratories, and individual researchers regarding operation, research, and development in the areas of methods, techniques, and instrumentation relevant to the analysis and synthesis of biomolecules."

Please watch for the call for papers booklet for ABRF '99, which will have several special features of interest to our members. The theme of the meeting is "Bioinformatics and Biomolecular Technologies: Linking Genomes, Proteomes and Biochemistry". Mark Lively and David Landsmann have the majority of an exciting program in place. The abstract deadline will be December 11, so start thinking about presenting your best work. Look for the announcement of the ABRF Travel Awards program, which was very successful last year, and will be sponsored again by PE Biosystems-ABD. For the first time, the ABRF is offering a short course just prior to the meeting. The Mass Spectrometry Research Group has organized an intense course on "Protein and Peptide Sequence Analysis by Tandem Mass Spectrometry" to be taught by Professor Donald F. Hunt and his colleagues. Current ABRF members will be given priority for registration. This is a unique opportunity to learn from a leader in this field. There will be homework! We hope to see you in Durham next March.

This issue of the *Journal of Biomolecular Techniques* is a new step for the ABRF, a merger of the long-standing peer-reviewed newsletter ABRF News and the all-electronic JBT. The merger was done in order to enhance the content of the ABRF News, to provide a permanent, printed version of the electronic JBT, and to consolidate our official publications into a single forum. The proposal for merging the two association publications was prepared by an external taskforce of senior ABRF members, including the parent publication's editors. The proposal was submitted to the Executive Board, along with a detailed cost analysis provided by an outside consultant. The projected cost increases are modest and should be easily recovered from advertising revenues. The Executive Board further

refined and approved the proposed merger on June 23. Efforts are now underway to register the JBT with indexing services, obtain an ISSN number, establish publication and advertising partners and, in general, proceed with the establishment of JBT as a registered periodical. Our intention is not to compete with any other journal, but rather to serve our members by providing a forum for proven techniques and methodology. Accordingly, we will move forward only as needed and as resources allow. We wish Clayton Naeve success in developing this new forum for biomolecular technologies, and encourage you to submit your best work to the JBT.

ABRF News and the Journal of Biomolecular Techniques Merge

Welcome to the first edition of the ABRF's new hardcopy and electronic publication, the *Journal of Biomolecular Techniques*! On June 23 the Executive Board approved the merger of the ABRF's newsletter ABRF News, in its 9th year of production, and the year-old all-electronic *Journal of Biomolecular Techniques*. The combined journal will retain the name *Journal of Biomolecular Techniques* and will incorporate the strengths of both hardcopy and electronic publishing under the direction of Editor-In-Chief, Clayton Naeve. Clive Slaughter and Ken Mitchelhill will serve as Executive Editors assisting with the hardcopy and electronic editions respectively. Current members of both editorial boards were invited to continue their participation in the publication of the new JBT. To those of you who published in the all-electronic JBT this past year, rest assured your contributions will not be lost; all currently accepted JBT articles will remain on the JBT Web server and will be reprinted in the quarterly hardcopy journal. Indeed, the first two JBT articles are reprinted in this issue.

The format of the new publication will incorporate features of both parent publications. The journal will continue to publish Methods and Reviews articles and TIPS articles in the same format used in ABRF News. A new type of article will be added to the format to allow the rapid, electronic dissemination of short methods articles called Rapid Communications. These submissions will typically be no longer than 2-3 pages and incorporate 2-3 figures. They will receive expedited review and, on acceptance, be published immediately on the ABRF's Web server. This will allow us to continue exploiting the advantages of electronic publication; however, the same article will also be reprinted in the next quarterly hardcopy issue and will receive a typical journal citation. This process should alleviate any potential concern regarding the longevity or archiving of electronic publications.

News & Events

Efforts are well underway to move the publication process from a desktop publishing environment to that of a professional publisher, to register the new journal with various indexing services, obtain an ISSN number, etc. It is anticipated that these will be accomplished early in 1999. The financial burden on the organization will be minimized by raising revenues via advertising, the primary source of income for all biomedical journals.

The intent is not to compete with the myriad other journals now being published. Rather, our primary concerns are to move away from labor-intensive desktop publishing and to provide the ABRF and its members with a first-rate, official forum for publication of our research group studies, publication of reviews of developing technologies, and rapid publication of short bioanalytical methods and techniques articles.

Support your association and contribute your next techniques paper to the *Journal of Biomolecular Techniques*!

Joint ABRF/ASBMB Symposium: Present and Future of Biomolecular Mass Spectrometry

R. Niece, Chair
G. Siuzdak, Co-chair

Monday, May 18, 1998, 10:15 AM
Washington, D.C., Convention Center

This was the 10th in a series of symposia jointly sponsored by ABRF and ASBMB designed to bring to researchers in biochemistry and molecular biology the latest information on technologies used in their research fields. In addition to describing emerging technology, practical information on how the new techniques can be accessed and applied to their research problems was presented.

The Symposium was opened by Gary Siuzdak of The Scripps Research Institute. His presentation, "Finding a Mass Spectrometer to Meet Your Needs" described the following desired attributes for mass spectrometry instrumentation: femtomole sensitivity, LC/MS interface, ppm accuracy, high mass range, the ability to analyze small molecules, low cost, and small size. As he pointed out, no one instrument is capable of providing all of these features, however both electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometers are coming close to meeting the ideal system (Table 1).

Suizdak discussed the general utility of MALDI and ESI instruments for peptides and protein analysis. Some of their respective advantages include the ease of interfacing ESI with LC, as well as ESI sample preparation which may be easier for non-specialists to learn. He also discussed

the superior capacity of MALDI to perform whole protein and simultaneous mixture analysis. It was pointed out that while both ESI and MALDI mass spectrometers are becoming easier to use, when buying a system it is important to focus on the strengths of each technique in terms of a lab's individual needs.

The next presentation by Ron Niece, UC Irvine, focused on ABRF mass spectrometry data from member laboratories around the world. The incorporation of mass spectrometry into biomolecular resource laboratories has been dramatic over the course of the last decade because mass spectrometry is a technique that 1) is needed on occasion by many researchers, 2) requires expensive capital equipment, and 3) is expertise-intensive. In 1987, no resource laboratories surveyed (Williams, KR, *et al.*, *FASEB J.* 2: 3124, 1988) indicated that they were offering mass spectrometry. In 1992, nearly one in eight offered the service and in 1996, nearly 50% of ABRF laboratories offered mass spectrometry capabilities (Niece, RL, *et al.*, Association of Biomolecular Resource Facilities, in *Encyclopedia of Bioprocess Technology*, Flickinger, MC, and Drew SW, Eds, Wiley & Sons, in press). Preliminary data from the 1998 Survey committee indicated that MALDI is more readily available than ESI.

In his presentation on what it takes to be successful when analyzing proteins by mass spectrometry, Roland Annan of Smith Kline Beecham Pharmaceuticals discussed capabilities of mass spectrometry as it is used in their laboratory, noting that any well-trained person can obtain the basic information from either type of mass spectrometer, ESI or MALDI. Mass spectrometry is one of the analytical techniques they use because it: 1) provides an intrinsic property (mass-to-charge ratio), 2) furnishes molecular weight information of picomole to femtomole quantities of proteins and peptides, independent of covalent modifications, 3) may do this from mixtures without separation, and 4) permits acquisition of partial to complete sequence data.

The trade-off between mass accuracy and quantity of material available often determines whether ESI or MALDI is the preferred method for analysis. MALDI is often more sensitive (e.g., picomole amounts of sample at 0.1% accuracy) while ESI often provides better accuracy (e.g., 0.01 % accuracy on 100 picomoles of sample). Differences in mass from expected molecular weights provide information about the identity of synthetic peptides or modifications of proteins. Database searching on masses of peptides following digestion have built-in redundancy; this provides increased confidence of identity because more matches are observed and errors decrease (Table 2).

Catherine Castello of Boston University continued the discussion of proteins, pre and postrationally modified. The focus was on the transthyretin protein in familial amyloidotic polyneuropathy. This protein is usually a covalently modified tetramer. Normal and mutant mono-

News & Events

Table 1. General comparison of ionization sources.

	<i>Electrospray Ionization (ESI)</i>	<i>NanoESI</i>	<i>MALDI</i>
<i>Typical Mass Range</i>	70,000	70,000	300,000
<i>Matrix Interference</i>	none	none	yes
<i>Degradation</i>	none	none	possible photodegradation and matrix reactions
<i>Ability to Analyze Complex Mixtures</i>	somewhat limited	somewhat limited but better than ESI	excellent
<i>LC/MS Capability</i>	excellent	OK, but low flow rates can present a problem	very limited (off-line)
<i>Sensitivity</i>	high femtomole to low picomole	high zeptomole to low femtomole	low to high femtomole
<i>Accuracy and MS/MS capabilities</i>	mass analyzer dependent	mass analyzer dependent	mass analyzer dependent
<i>Salt Tolerance</i>	low (low millimolar)	moderate (low-mid millimolar)	moderate (low-mid millimolar)
<i>Comments</i>	Multiple charging useful. Significant suppression can occur with mixtures. Soft ionization=low fragmentation LC/MS easily automated & quantitative	Multiple charging useful but significant suppression can occur with mixtures. Soft ionization=low fragmentation	Matrix background can be problem for low mass ions. Soft ionization=low fragmentation

Table 2 Comparison of number of peptide matches at various error levels. Database searched: EMBL-NonRedundant Protein Database (>230,000 sequences).

<i>Mass list</i>	<i>Error</i>	<i>Result</i>
1226.680	0.1%	>11,500 proteins
1226.680 1429.756	0.1%	>21,000 have either 1,180 have both
1226.680 1429.756 1786.899	0.1%	>2,985 have two/three 192 have all three
1226.680 1429.756 1786.899 1021.603	0.1% 0.01% 0.001%	38 have all four only 1 has all four only 1 has three/four

mers are cysteinylated or phosphorylated. A mixture of these forms is found when purified from heterozygous individuals. Identifying the many postranslational modifications in collagen indicated several successive levels of modifications, such as glycosylation of modified proline and lysine residues. She also described the use of MALDI with an infrared laser ionization source that allows for deeper pen-

etration into the matrix, less fragmentation, and higher resolution.

Robert McIver of UCI and IonSpec provided a forward-looking discussion on the use of FTMS as an ion detector. Fourier transform mass spectrometry can store ions from an external ion source for as long as hours in some cases. The long time-scale leads to a high signal-to-noise ratio which in turn improves resolution and sensitivity; resolution can exceed 400,000 and internal mass calibrations can lead to errors of only about 1 ppm. The high resolution allows for ESI charge states to be calculated directly from isotope peak spacing. McIver also discussed the utility of ESI FTMS for the analysis of non-covalent interactions.

In general, the applicability of mass spectrometry to biochemical problems is quite extraordinary and what is interesting about the technology is that it has, in many respects, exceeded the needs of biochemists. What we are currently observing is the biochemistry community gradually coming to learn of the potential of this technology. At the same time, mass spectrometry researchers are pushing the limits of the technologies' capabilities even further (MSⁿ, laser photodissociation, hydrogen/deuterium exchange and non-covalent interaction). It is a beautiful time to be in mass spectrometry and an even better time to be a biochemist!

News & Events

Announcement of 1999 Applications for PE Biosystems and ABRF Travel Awards

A program is available to help ABRF members with limited access to travel funds to attend "ABRF '99 *Bioinformatics and Biomolecular Technologies: Linking Genomes, Proteomes, and Biochemistry*" to be held in Durham, NC, March 19-22, 1999. A travel award fund has been established jointly by the **ABRF and PE Biosystems**. The award is intended for both junior and senior scientific staff in resource laboratories. However, facility directors will be considered in special circumstances such as those from very small laboratories or laboratories located outside of North America. Students or postdoctoral fellows directly associated with the mission of member laboratories may also apply. **Applications are encouraged from attendees regardless of travel distances.**

Applicants for this travel award will require a letter of support from their laboratory director or, in the case where a director is applying, a letter of support from a senior colleague or head of department. Letters of support must be emailed to: travelaward@abrf.org

Applicants must complete the form posted at: <http://www.abrf.org/ABRF/ABRFMeetings/ABRF99/travelaward.html>

Letters of recommendation and applications must be submitted **before November 1, 1998** at which time the above addresses will be closed.

Support will be awarded only for transportation expenses with the hope that these funds can be used as a seed to request the remainder of expenses from the home institution. No more than one award will be made to any particular laboratory and 1998 travel award winners will not be eligible for a 1999 award. Successful applicants will be notified by December 1, 1998.

In case an applicant does not have internet access or has questions concerning filing an application, please contact Lowell Ericsson, chairman of the ABRF Travel Award Committee.

1999 ABRF Travel Award Selection Committee

Lowell Ericsson (chair), Karen De Jongh,
Ken Mitchelhill, Len Packman, Alan Smith,
and Tim Schlabach (PE Biosystems representative)

PE Biosystems and ABRF 1998 Travel Awardees

Travel awards to the ABRF '98 meeting were made to 15 recipients, thanks to a generous donation from PE Biosystems. The awardees were:

Yongde Bao - University of Virginia, Charlottesville, VA
Gail L. Drew - Utah State University, Logan, UT
Tony Houthaeve - University of Gent, Gent, Belgium
Frantisek Hubalek - Emory University, Atlanta, GA
Jeffrey Hulmes - Adirondack Biomedical Res. Inst., Lake Placid, NY
Virginia Johnson - Texas A&M University, College Station, TX
Nguyet Le - Albert Einstein College of Medicine, Bronx, NY
John J. Lennon - University of Washington, Seattle, WA
Scott J. Magin - Louisiana State University, New Orleans, LA
Sharad C. Mistry - Leicester University, Leicester, England
Angela Paul - Institute of Cancer Research, London, England
Jose Cesar Rosa - Sao Paulo University, Sao Paulo, Brazil
Steven H. Seeholzer - Fox Chase Cancer Center, Philadelphia, PA
Linda Siconolfi-Baez - Health Science Center, Brooklyn, NY
Glenis Wiebe - University of Calgary, Calgary, Alberta, Canada

1998 ABRF Travel Award Selection Committee:

Lowell Ericsson (chair), Karen De Jongh, Ken Mitchelhill, Len Packman, Laurey Steinke

Research Group/Committee Reports

Education Committee

The Education Committee has been discussing and developing new means to promote the educational needs of the ABRF Research Groups. The committee is seeking the input from all Research Group chairmen; if you have not yet been contacted, please direct your comments and needs to Barbara Merrill (chair).

On a related topic, the Education Committee is soliciting suggestions for training classes to be held in conjunction with the annual meeting. Of particular interest are established courses that could be administered at or near the meeting site. Such courses may not be accessible to ABRF members otherwise, due to scheduling or fiscal concerns. Again, if you have suggestions for the year 2000 meeting and beyond, direct your feedback to the Education Committee chair.

Mass Spectrometry Research Group

Since its inception in 1996, the Mass Spectrometry Research Group (MSRG) has been involved in two successful interlaboratory studies, reported at the 1997 and 1998 annual meetings, and most recently in a short course scheduled to be given immediately prior to the main meeting at ABRF '99. The results for both interlaboratory studies suggested that the comfort level with mass spectrometry varied widely among member labs, and that much work re-

mains to be done in terms of properly integrating this technology into laboratories that are already expert in more established methods of biopolymer analysis. As MS instrumentation continues to become cheaper, smaller and easier to operate and maintain, the demands on the operator will continue to shift more in the direction of sample cleanup, preparation and data interpretation.

The MSRG recognizes that basic instrument operation issues are very much a concern with so many people new to using mass spectrometry. To help with this transition, the MSRG will be putting on a series of short courses that will deal with various topics such as interpretation of peptide fragmentation data, basic instrument operation including tuning and mass calibration, and other topics. We are fortunate to have Professor Donald F. Hunt from the University of Virginia present his short course "Protein and Peptide Sequence Analysis by Tandem Mass Spectrometry" on March 17-19, 1999 in Durham, NC. Fliers for the course are being sent out with announcements for the ABRF '99 meeting. Seating will be available for 100 people, but interested parties are encouraged to sign up early. There will be no on-site registration for this short course. Plans for the Seattle meeting are still being discussed informally, but will likely include a short course dealing with basics of mass spectrometry instrumentation.

The MSRG will also participate in interlaboratory validation studies with other ABRF Research Groups, but hopes to work with these groups to minimize the number of test samples.



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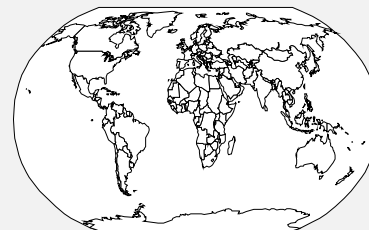
ELECTRONIC COMMUNICATIONS ADDRESSES

Electronic Discussion Group: abrf-request@aecom.yu.edu (*Subscription*)
abrf@aecom.yu.edu (*Correspondence*)
ombudsmn@aecom.yu.edu (*Ombudsman*)

Electronic Discussion Group Archives: <http://www.abrf.org/archives>

WWW Homepage: <http://www.abrf.org>

Journal of Biomolecular Techniques: <http://www.abrf.org/JBT/JBT.html>



Article Watch

This column highlights recently published articles that are of interest to the readership of this publication. We encourage ABRF members to forward information on articles they feel are important and useful to *Clive Slaughter*, HHMI, U.T. Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas TX 75235-9050. Tel: (214) 648-505, Fax: (214) 648-9477, E-mail; slaugh01@utsw.swmed.edu or to any member of the editorial board. Article summaries reflect the reviewers' opinions and not necessarily those of the Association.

Amino Acid Composition and Sequence Analysis

Hsi K-L, O'Neill SA, DuPont DR, Yuan P-M. Visualization of proteins by modification of lysines, cysteines, and phosphorylated serines facilitates sample preparation for microsequencing. *Analytical Chemistry* 1998;258:38-47.

A method is devised for detecting proteins following SDS-PAGE that uses fluorescent or chromogenic probes as tags for certain amino acids. Prior to electrophoresis, proteins are derivatized with dansyl/dabsyl chloride to label lysines, or with iodoacetamidofluorescein (I-15) to label cysteines. I-15 may also be used to tag phosphoserines if ethanedithiol is first employed for β -elimination of the phosphate. Sensitivities of protein detection are comparable to those observed with Coomassie blue staining, but the labelling methods eliminate the need for fixing, staining and destaining. The labels can also be used for selective isolation of lysine-, cysteine- or phosphoserine-containing peptides after enzymatic digestion of the labelled protein.

Hardeman K, Samyn B, van der Eycken J, van Beeumen J. An improved chemical approach toward the C-terminal sequence analysis of proteins containing all natural amino acids. *Protein Science* 1998;7:1593-1602.

A revisit of chemical methods for the serial degradation of peptides from the C-terminus. Conditions are identified that permit the derivatization of all 20 standard amino acids to the corresponding thiohydantoin. These conditions involve activation by acetyl chloride and derivatization with ammonium thiocyanate. C-terminal prolines were derivatized with 30-60% yield. Mass spectrometric data indicate that proline can form an oxazolonium ion that reacts with thiocyanate in the same general way as other amino acids.

Carbohydrates and Glycoproteins

Sheeley DM, Reinhold VN. Structural characterization of carbohydrate sequence, linkage, and branching in a quadrupole ion trap mass spectrometer: neutral oli-

gosaccharides and N-linked glycans. *Analytical Chemistry* 1998;70:3053-3059.

Demonstrates the use of a commercial ion trap mass spectrometer to obtain linear sequence, linkage and branching information for several oligosaccharides and N-linked glycans. The multistage MS/MS (MS^n) capability of the ion trap permitted the assignment of linkages that cannot be made with triple quadrupole mass spectrometers, and the results foreshadow the development of strategies for the complete structural characterization of carbohydrates using a single instrument.

Genes - Cloning, Sequencing and Expression

Berkenkamp S, Kirpekar F, Hillenkamp F. Infrared MALDI mass spectrometry of large nucleic acids. *Science* 1998;281:260-262.

Describes progress in the development of methods for high accuracy mass measurement of DNA fragments obtained by restriction enzyme digestion. An infrared laser is used, in conjunction with liquid glycerol as the MALDI matrix. The conditions permit mass measurement of fragments up to about 700 kDa (2,200 nucleotides) with mass accuracies of 1% or better and resolution values around 50. By contrast, electrophoretic methods are of limited resolution and provide accuracies of only 5-10%. The signals typically represent a composite of the two complementary DNA single strands. Large RNA can also be measured. Sensitivities in the low femtomole range are routine, although a spectrum of a 300 amol sample is also demonstrated.

Mass Spectrometry

Lehmann WD. Single series peptide fragment ion spectra generated by two-stage collision-induced dissociation in a triple quadrupole. *Journal of the American Society for Mass Spectrometry* 1998;9:606-611.

Collision-induced dissociation (CID) of peptides in the source region of a triple quadrupole mass spectrometer, together with precursor ion scanning or neutral loss scanning, is used to provide spectra showing signals of predominantly a single ion series, such as the b or y series. Collisional dissociation induced by adjustment of the skimmer voltage, combined with scanning for neutral loss of 28, generates spectra showing b ions. Skimmer CID of tryptic peptides, combined with scanning for precursors of the y_1 fragment corresponding to the C-terminal arginine (m/z 147), or the y_1 fragment corresponding to the C-terminal lysine (m/z 175), generates spectra showing the y series. Sequence information can be easily extracted from these simplified spectra because the scan mode defines the type of fragments observed.

Zhang X, Herring CJ, Romano PR, Szczepanowska J, Brzeska H, Hinnebusch AG, Qin J. Identification of phosphorylation sites in proteins separated by polyacrylamide gel electrophoresis. *Analytical Chemistry* 1998;70:2050-2059.

A streamlined and sensitive method for identifying phosphorylation sites. The phosphoprotein is subjected to SDS-PAGE, subjected to in-gel digestion with trypsin, and the resulting peptides subjected to MALDI-TOF before and after digestion with a phosphatase to identify phosphopeptides. Peptides are then subjected to on-line LC/MS/MS in an ion-trap mass spectrometer to identify the precise phosphorylation sites. Using this scheme, the authors have assigned 14 phosphorylation sites. Experiments using a gel slice containing as little as 3 pmol of protein have proved sufficient for successful analysis.

Nucleic Acids - Synthesis

Focus on Oligonucleotides. McLuckey SA, (Editor). *Journal of the American Society for Mass Spectrometry* 1998;9:659-691.

A collection of four papers of interest to those wishing to understand current progress and issues in the development of mass spectrometric methods for the characterization of oligonucleotides. The contributions include, firstly, methods for confirming sites of addition, and structural features, of labels incorporated by phosphodiester bonds into the oligonucleotide backbone; secondly, a method that reduces the effects of sodium adducts and diminishes the fragmentation of long oligonucleotides during MALDI; thirdly, methods for determining the site(s) at which carcinogens form adducts to bases; and, fourthly, an evaluation of methods that permit the characterization of mixtures of DNA arising by replication of oligonucleotides damaged by uv light.

Peptides - Synthesis

Miller C, Rivier J. Analysis of synthetic peptides by capillary zone electrophoresis in organic/aqueous buffers. *Journal of Peptide Research* 1998;51:444-451.

Describes mobile phase conditions for assessment of the purity of synthetic peptides by CZE. Triethylammonium phosphate is employed as a modifier, and methanol, acetonitrile and isopropanol are tested at various con-

centrations with significant enhancements of separation efficiency compared to a standard aqueous phosphate buffer.

Jensen KJ, Alsina J, Songster MF, Vágner J, Albericio F, Barany G. Backbone amide linker (BAL) strategy for solid-phase synthesis of C-terminally modified and cyclic peptides. *Journal of the American Chemical Society* 1998;120:5441-5452.

Presents a new, general method for the solid-phase synthesis of peptides in which the carboxy termini are to be modified to other functionalities. Examples described include alcohols, N,N-dialkylamides, aldehydes, esters, and head-to-tail cyclic peptides. The method involves attachment of the initial residue to the polymeric support via a "handle", a bifunctional linker, at one end of which is a group that acts like a smoothly cleavable protecting group, and at the other end of which is a group that permits coupling to the previously functionalized support. The handle used in this case is a novel Backbone Amide Linker (BAL), in which the growing peptide is anchored through a backbone amide nitrogen. This approach allows considerable flexibility in the management of the terminal functionality desired, and avoids often complicated, post-synthetic, solution-phase manipulations.

Proteins – Purification and Characterization

Yan JX, Packer NH, Gooley AA, Williams KL. Protein phosphorylation: technologies for the identification of phosphoamino acids. *Journal of Chromatography A* 1998;808:23-41.

A concise but broad-based methodological review of protein phosphorylation. Lists the known, naturally occurring phosphoamino acids, and phosphorylation sequences that are recognized by various kinases. Summarises methods for detecting phosphate moieties on proteins, including radiolabelling, use of antibodies specific for different phosphoamino acids, and fluorescence labelling. Describes methods for protein hydrolysis, and for identifying phosphoamino acids, including TLC, electrophoresis, HPLC, and mass spectrometry. Describes the β -elimination reactions undergone by Ser(P) and Thr(P), and indicates methods for locating the phosphorylation sites in proteins by Edman degradation and mass spectrometry.



Upcoming Events

Dates to Remember

ABRF members and Corporate Sponsors are encouraged to make announcements about pertinent meetings, workshops, etc. beneficial to our members and open to the public. Send items to be listed to *Daniel J. Strydom*, BioNebraska, Inc. 3820 NW 46th St., Lincoln, NE 68524-1637, Tel: (402) 470-2100, Fax: (402) 470-2345, E-mail: strydom@inetnebr.com.

1998

- Sep 13–18 ISC '98, 22nd International Symposium on Chromatography, Rome, Italy. Contact: F. Dondi, ISC '98, Dip. di Chimica, Università di Ferrara, Via L. Borsari, 46, I-44100 Ferrara, Italy, Tel: +39 (532) 291154, Fax: +39 (532) 240709, E-mail: mo@dns.unife.it, WWW: <http://www.unife.it/isc22>.
- Sep 17–20 10th International Genome Sequencing and Analysis Conference, Miami Beach, Florida. Contact: TIGR Science Education Foundation Conference Office, 9712 Medical Center Drive, Rockville, MD 20850-3319. Tel: (301) 838-3509 or 3515, Fax: (301) 838-0229.
- Sep 23–25 SPICA '98: International Symposium on Preparative and Industrial Chromatography and Allied Techniques, Strasbourg, France. Contact: Mlle Françoise Brionne, ENSIC, 1 rue Grandville-BP 451 F-54001 Nancy Cedex, France. Tel: +(33) 383 17 50 03, Fax: +(33) 383 35 08 11, E-mail: brionne@ensic.u-nancy.fr.
- Sep 28–Oct 1 11th International Ion Chromatography Symposium, IICS '98, Osaka, Japan. Contact: Janet Strimaitis, Century International, P.O. Box 493, Medfield, MA 02052-0493. Tel: (508) 359-8777, Fax: (508) 359-8778, E-mail: century@ixl.net.
- Oct 4–7 International GPC Symposium '98, Phoenix, AZ. Contact: Stacy Rodrigues, Waters Corp., 34 Maple Street, Mailstop MC, Milford, MA 01757, Tel: (508) 482-2993, Fax: (508) 482-2674.
- Oct 8–11 Second Asia-Pacific Symposium on Capillary Electrophoresis and Related

Microscale Techniques, Dalian, China. Contact: Prof. Lin Bingscheng, APCE'98, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 116023 Dalian, China. Tel: +86 411 467 1991, Fax: +86 411 362 2302, E-mail: linbc@dlut.edu.cn.

- Oct 11–16 25th Annual Conference of the Federation of Analytical Chemistry & Spectroscopy Societies, Austin, Texas. Contact: FACSS, 1201 Don Diego Ave., Santa Fe, NM 87505, Tel: (505) 820-1648, Fax: (505) 989-1073.
- Oct 12–14 Analitika '98, Analytical Science into the Next Millennium, Midrand, Gateng, South Africa. Contact: Pat Bayley, P.O. Box 426, Cramerview, 2060 South Africa, Tel: +27 11 465 4015, E-mail: deon@info.mintek.ac.za.
- Oct 19–21 Ninth Annual Frederick Conference on Capillary Electrophoresis, Frederick, MD. Contact: Margaret L. Fanning, SAIC Frederick, NCI-FCRDC, P.O. Box B, Frederick, MD 21702. Tel: (301) 846-5865, Fax: (301) 846-5866, E-mail: fanningm@mail.ncifcrf.gov, WWW: <http://129.43.32.72/cze.htm> or <http://469csal7.ncifcrf.gov/cze.htm>.
- Oct 22–23 Combinatorial Chemistry: Beyond Pharmaceuticals, Newark, DE. Contact: Kathleen Werrell, Engineering Outreach, Univ. of Delaware, Newark, DE 19716-3101. Tel: (302) 831-4863, Fax: (302) 831-8179, E-mail: outreach@mvs.udel.edu.
- Oct 31–Nov 3 2nd Computational Genomics Conference, Reston, VA. Contact: TIGR Science Education Foundation Conference Office, 9712 Medical Center Drive, Rockville, MD 20850-3319. Tel: (301) 838-3509 or 3515, Fax: (301) 838-0229.
- Nov 11-13 15th Montreux Symposium on Liquid Chromatography- Mass Spectrometry, Montreux, Switzerland. Contact: Marianne Frei-Häusler, IAEAC Secretariat, Postfach 46, CH-4123 Allschwil 2, Switzerland, Tel: +41 (61) 481 27 89, Fax: +41 (61) 482 08 05, E-mail: iaeacmfrei@access.ch.

Upcoming Events

- Nov 15–20 Eastern Analytical Symposium, Somerset, NJ. Contact: S. Gold, Eastern Analytical Symp., P.O. Box 633, Montchanin, DE 19710-0633, Tel: (302) 738-6218, Fax: (302) 738-5275.
- 1999**
- Jan 5–8 Third Symposium on the Analysis of Well Characterized Biotechnology Pharmaceuticals, Washington, D.C. Contact: Joan Saluzzi, California Separation Science Society, c/o Rhema Association Management, P.O. Box 411106, San Francisco, CA 94141-1106. Tel: (415) 487-9876, Fax: (415) 487-9875, E-mail: society@hooked.net, WWW: <http://www.casss.org/wcbp>.
- Jan 23–28 HPCE '99, 12th International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques, Palm Springs, California. Contact: Edward S. Yeung, California Separation Science Society, P.O. Box 411106, San Francisco, CA 94141-1106. Tel: (415) 487-9876, Fax: (415) 487-9875, E-mail: hpce@casss.org, WWW: <http://www.casss.org/hpce99>.
- Jan 29–Feb 1 Third Conference on Microbial Genomes: Sequencing, Functional Analysis and Comparative Genomics, Chantilly, VA. Contact: TIGR Science Education Foundation Conference Office, 9712 Medical Center Drive, Rockville, MD 20850-3319. Tel: (301) 838-3509 or 3515, Fax: (301) 838-0229.
- Mar 21–25 217th American Chemical Society National Meeting, Anaheim, California. Contact: American Chemical Society Meetings, 1155 16th Street NW, Washington, DC 20036-4899. Tel: (202) 872-4396, Fax: (202) 872-6128, E-mail: natlmgtgs@acs.org.
- May 9–12 10th International Symposium on Pharmaceutical and Biomedical Analysis, Washington, DC. Contact: Shirley E. Schlessinger, 400 East Randolph Drive, Suite 1015, Chicago, IL 60601, WWW: <http://www.cc.ukans.edu/~pbasymp>.
- May 30–Jun 4 HPLC '99: 23rd International Symposium on High Performance Liquid Phase Separations and Related Techniques, Granada, Spain. Contact: Ana Costejà, Palacio de Congresos, Departamento de Convenciones, Avenida Reina Ma Cristina, s/n, 08004 Barcelona, Spain. Tel: +34 (3) 233 23 77, Fax: +34 (3) 426 28 45, E-mail: hplc99@website.es, WWW: <http://www.website.es/hplc99>.
- Aug 31–Sep 4 6th International Symposium Solid Phase Synthesis & Combinatorial Chemical Libraries, York, England. Contact: Prof Roger Epton, Mayflower Worldwide Ltd, P.O. Box 13, Kingswinford, West Midlands, DY6 0HR, England, UK. Tel: +44 (0) 1384 279324; Fax: +44 (0) 1384 294463; E-mail: r.epton@mayflower.demon.co.uk.
- Sep 18–21 Eleventh International Genome Sequencing and Analysis Conference, Miami Beach, FL. Contact: TIGR Science Education Foundation Conference Office, 9712 Medical Center Drive, Rockville, MD 20850-3319. Tel: (301) 838-3509 or 3515, Fax: (301) 838-0229.
- 2000**
- Jul 11–14 Protein 2000: The Proteome and Protein Engineering into the Next Millennium, London, England. Contact: Prof. Roger Epton, Mayflower Worldwide Ltd, P.O. Box 13, Kingswinford, West Midlands, DY6 0HR, England, UK. Tel: +44 (0) 1384 279324, Fax: +44 (0) 1384 294463, E-mail: r.epton@mayflower.demon.co.uk.



Upcoming Events

Training & Workshop Courses

ABRF members and corporate sponsors are encouraged to contribute to this column, sponsored by the ABRF Education Committee, by sending announcements of new courses to *Nili Leffers*, Macromolecular Synthesis and Sequencing Shared Resource, Georgetown University, E401 Research Bldg., 3970 Reservoir Rd., Washington, D.C. 20007, Tel: (202) 687-6466, Fax: (202) 687-7505, E-mail: leffersn@gunet.georgetown.edu. This list of courses may also be found on the ABRF Web site.

Oct 5–7 Mass Spectral Interpretation, Waters Corp., Contact: Maryann Stern, Tel: (800) 252-4752 Ext. 8502, Fax: (508) 482-8701, E-mail: maryann_stern@waters.com, WWW: <http://www.waters.com>. Other dates and courses also available.

Oct 5–8 Hybridoma Technology and Monoclonal Antibody Product Development, American Type Culture Collection, in Rockville, MD. Contact: Tel: (800) 359-7370, E-mail: workshops@atcc.org, WWW: <http://www.atcc.org/workshops/workshop.html>.

Oct 5–9 Recombinant DNA Methodology, Exon-Intron, Inc., in Columbia, MD. Tel: (410) 730-3984 or (800) 407-6546, Fax: (410) 730-3983, E-mail: workshop@dnatech.com. Other dates and courses also available.

Oct. 6–7 Method Validation for HPLC, Waters Corp., Contact: See previous listing for Oct 5-7. Other dates and courses also available.

Oct 8–9 Chemistry of Resolution, Waters Corp., Contact: See previous listing for Oct 5-7. Other dates and courses also available.

Oct 13–16 Microbial DNA Fingerprinting, American Type Culture Collection, in Rockville, MD. Contact: See previous listing for Oct 5-8.

Oct. 13–16 Troubleshooting and Performance Maintenance, Waters Corp., Contact: See previous listing for Oct 5-7. Other dates and courses also available.

Oct 14 Differential Display PCR, Exon-Intron, Inc., in Columbia, MD. Contact: See

previous listing for Oct 5-9. Also available Dec 2.

Oct 19–21 Laboratory Safety and Health, American Chemical Society, in Somerset, NJ. Tel: (800) 227-5558, Fax: (202) 872-6336, E-mail: shortcourses@acs.org.

Oct 19–23 Quantitative RNA Techniques, Exon-Intron, Inc., in Columbia, MD. See previous listing for Oct 5-9. Other courses and dates also available.

Oct. 20–23 Developing HPLC Separations, Waters Corp., Contact: See previous listing for Oct 5-7. Other dates and courses also available.

Oct 21–23 Downstream Processing, Recovery and Purification of Proteins, American Type Culture Collection, in Rockville, MD. Contact: See previous listing for Oct 5-8.

Oct 28–30 DNA Typing RFLP & PCR-based Systems, Exon-Intron, Inc., in Columbia, MD. Contact: See previous listing for Oct 5-9. Other courses also available.

Nov 3–5 Advanced Interpretation of Mass Spectra, Hewlett-Packard, in Montreal, QC. Tel: (800) 227-9770 in the U.S., (800) 265-7768 in Canada. Other dates and locations also available.

Nov 3–6 Theory and Practice of Gel Permeation Chromatography, Waters Corp., Contact: See previous listing for Oct 5-7. Other dates and courses also available.

Nov 7–12 Computational Genomics, Cold Springs Harbor Laboratory, in Cold Spring Harbor, NY. Contact: See previous listing for Oct 14-27. Other courses also available.

Nov 9–13 Recombinant DNA: Techniques and Applications, American Type Culture Collection, in Rockville, MD. Contact: See previous listing for Oct 5-8.

Nov 9–13 PCR Methodology, Exon-Intron, Inc., in Columbia, MD. Contact: See previous listing for Oct 5-9.

Upcoming Events

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| <p>Nov 16–17 Modern Techniques in Gas Chromatography, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> <p>Nov 16–17 Long Range PCR, Exon-Intron, Inc., in Columbia, MD. Contact: See previous listing for Oct 5-9.</p> <p>Nov 17–20 Polymerase Chain Reaction (PCR) Applications/Cycle DNA Sequencing, American Type Culture Collection, in Rockville, MD. Contact: See previous listing for Oct 5-8.</p> <p>Nov 18–20 Interpretation of IR Spectra, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> <p>Nov 18 Laboratory Information Management Systems, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> | <p>Nov 19–20 Fundamentals of HPLC, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> <p>Nov 19–20 Interpretation of Mass Spectra, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> <p>Nov 19–20 Quality Assurance/Quality Control in the Analytical Testing Laboratory, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> <p>Nov 19–20 Technical Writing Workshop, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 19-21.</p> <p>Nov 20–21 Quality Assurance/Quality Control in the Analytical Testing Laboratory, American Chemical Society, in Somerset, NJ. Contact: See previous listing for Oct 30-31.</p> |
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Mission Statement from the By-Laws of the Association of Biomolecular Resource Facilities

The ASSOCIATION OF BIOMOLECULAR RESOURCE FACILITIES has been organized for the following purposes:

- A. To promote and support resource facilities, research laboratories, and individual researchers regarding operation, research, and development in the areas of methods, techniques, and instrumentation relevant to the analysis and synthesis of biomolecules.
- B. To provide mechanisms for the self-evaluation and improvement of procedural and operational accuracy, precision, and efficiency in resource facilities and research laboratories.
- C. To provide a mechanism for the education of resource facility and research laboratory staff, users, administrators, and interested members of the scientific community.

Suggested Subject Headings for Messages Posted to the Electronic Discussion Group

Heading	Description
AAA	Amino acid analysis
Comp	Computer analysis or database searches
CE	Capillary electrophoresis
CHO	Carbohydrate analysis
Digest	Enzymatic or chemical digestion conditions
DNASeq	DNA sequencing
DNASyn	DNA synthesis (oligonucleotides)
EBlot	Electroblotting
GLP/GMP	Compliance and accreditation matters
HPLC	HPLC peptide/protein purification
MolBio	Miscellaneous molecular biology matters
MS	Mass spectrometry
PCR	PCR
PepSyn	Peptide synthesis
ProtSeq	Protein sequencing
P2D	Protein purification by 2-dimensional gels
EqpNeed	Equipment needed
EqpSale	Equipment for sale
JobNeed	Those looking for positions vacant
JobOpen	Those with vacancies to fill
Nostalgia	Just for graybeards or whiteheads
Misc	None of the above

JOURNAL OF BIOMOLECULAR TECHNIQUES

Editorial Policy and Call for Articles

The *Journal of Biomolecular Techniques* (JBT) is a peer-reviewed publication issued quarterly by the Association of Biomolecular Resource Facilities. It was established to promote the central role biotechnology plays in contemporary research activities, to disseminate information among biomolecular resource facilities, and to communicate the biotechnology research conducted by the Association's Research Groups, Association members, and interested investigators.

The publication has an international audience and is intended for professionals engaged in biotechnology research and service. We welcome contributions from specialists in protein and nucleic acid chemistry, mass spectrometry, bioinformatics, biomolecular resource facilities operations and management, and related areas.

To keep the publication focused on topics of interest, articles are often solicited by the Editorial Board. Direct submissions or inquiries about the suitability of proposed articles may be directed to the Editor, Clayton W. Naeve, Ph.D., Center for Biotechnology, St. Jude Children's Research Hospital, Memphis, TN, 38101, Tel: (901) 495-3861, Fax: (901) 495-2945, E-mail: clayton.naeve@stjude.org.

JBT is published in English and features three types of articles: Methods & Reviews, Tips Articles and Rapid Communications. The purpose and requirements of all three types of articles are discussed in the "Instructions to Authors". The newsletter is also published electronically on the Association's Web site at <http://www.abrf.org>.

Instructions to Authors

General Instructions

Manuscripts should be prepared according to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" [Ann. Intern Med 1997: 126:36-47]. A digital version is available on various Web sites including <http://www.acponline.org/journals/resource/unifreq.htm>.

All articles are reviewed by at least two independent reviewers. The editor reserves the right to edit articles for clarity and to modify the format to fit the publication style of the *Journal of Biomolecular Techniques*.

Manuscripts should be sent in both hardcopy and electronic form (on diskette or by e-mail). Most electronic formats can be handled; however, we prefer that text be sent in plain text (ASCII), MS Word, or WordPerfect formats for the PC.

Submit photographs as glossy, camera-ready photographic prints. Electronic submission of graphics are accepted and most formats can be handled; however, we prefer that graphics be sent in .TIF, .GIF, or .EPS format for the PC. The preferred font for graphics is Times New Roman. If possible, convert Macintosh graphics files to any of the suggested formats. Resolutions to 1200 dpi are supported.

References should be numbered consecutively in the order in which they are first mentioned in the text. Use the styles for articles in journals, books, electronic material, etc as illustrated in the "Uniform Requirements for Manuscripts..." cited above. The titles of journals should be abbreviated according to the style used in *Index Medicus*. This list can be obtained from the NLM or obtained through the library's Web site at <http://www.nlm.nih.gov>.

Send four complete copies of all manuscripts to the Editor at the address above. Invited articles may be submitted directly to the Associate Editor coordinating the review.

Methods & Reviews and Tips manuscripts may be submitted at any time. Content destined for a specific issue (e.g. News & Events items) is typically submitted in camera-ready form one month prior to publication; i.e. Feb. 1, May 1, Aug. 1, and Nov. 1.

Methods & Reviews

Methods & Reviews articles provide reviews of existing or emerging technologies or comprehensive descriptions of

bioanalytical techniques. Methods & Reviews serve to keep ABRF members apprised of new technologies and to aid in planning. Although largely solicited by the Editorial Board, any review article on an appropriate subject will be considered for publication. Methods & Reviews also serve to disseminate the results of ABRF Research Group studies.

Begin each of the following sections on a new page and in this order: title and authors, abstract, text, acknowledgements, references, each table, figure legends, and each figure.

Methods & Reviews articles are typically 5000 words in length and include 8-10 figures or tables.

TIPS Articles

TIPS articles are shorter articles focusing on bioanalytical techniques or provide detailed descriptions of new methodologies or new modifications to techniques that improve the performance of the method. TIPS articles help readers improve their current capabilities.

Begin each of the following sections on a new page and in this order: title and authors, text, acknowledgements, references, each table, figure legends, and each figure.

TIPS articles typically do not exceed 1000 words and contain 6-7 figures and tables.

Rapid Communications

Rapid Communications articles are typically very short, 750 words and 2-3 figures. They are intended to foster the rapid communication of new developments in bioanalytical methodology to ABRF membership and the research community at large. They may consist of new techniques, improvements to methods, solutions to common problems, etc. Rapid Communications are submitted by email, receive expedited on-line review and, upon acceptance, are immediately posted on the ABRF Web server. Rapid Communications are reprinted in the quarterly hardcopy edition of the journal. Detailed instructions for Rapid Communications can be obtained from the ABRF Web server at <http://www.abrf.org>.

