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MALDI-ToF Mass Spectrometry for Studying Noncovalent Complexes of Biomolecules

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Abstract Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been demonstrated to be a valuable tool to investigate noncovalent interactions of biomolecules. The direct detection of noncovalent assemblies is often more troublesome than with electrospray ionization. Using dedicated sample preparation techniques and carefully optimized instrumental parameters, a number of biomolecule assemblies were successfully analyzed. For complexes dissociating under MALDI conditions, covalent stabilization with chemical cross-linking is a suitable alternative. Indirect methods allow the detection of noncovalent assemblies by monitoring the fading of binding partners or altered H/D exchange patterns.

Keywords Chemical crosslinking · First shot phenomenon · Intensity fading · MALDI matrix · SUPREX

Contents

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Abbreviations

ACN	Acetonitrile
ADP	Adenosine-5′-diphosphate
AMNP	2-Amino-4-methyl-5-nitropyridine
AMP	Adenosine-5′-monophosphate
ANA	2-Aminonicotinic acid
AP	Atmospheric pressure
ATP	Adenosine-5′-triphosphate
ATT	6-Aza-2-thiothymine
CHCA	α-Cyano-4-hydroxycinnamic acid
DHAP	Dihydroxyacetophenone
DHB	Dihydroxybenzoic acid
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
FA	Ferulic acid
hERα LBD	Human estrogen receptor α ligand binding domain
HIV	Human immunodeficiency virus
HPA	3-Hydroxypicolinic acid
IR	Infrared
iTRAQ	Isobaric tag for relative and absolute quantitation
K_a	Association constant
K_d	Dissociation constant
LILBID	Laser induced liquid *beam* or *bead* ionization/desorption
MALDI	Matrix-assisted laser desorption/ionization
MCP	Microchannel plate detector
MS	Mass spectrometry
NHS	*N*-Hydroxysuccinimide
PNA	*p*-Nitroaniline
RNA	Ribonucleic acid
SA	Sinapinic acid
TFA	Trifluoroacetic acid
THAP	Trihydroxyacetophenone
THF	Tetrahydrofuran
ToF	Time-of-flight
TrpR	Tryptophan repressor
UV	Ultraviolet

1 Introduction

Biomolecules that are involved in key cellular processes such as reproduction, growth, and development can communicate via physical interactions. To achieve this, biomolecules specifically recognize their interaction partner. They generally

dock to their partners through short-range biophysical interactions, such as hydrogen bonds, van der Waals forces, and hydrophobic interactions. In other words, intracellular as well as cell–cell communication functions because transient noncovalent interactions are formed between biomolecules. Many such noncovalent interactions take place, including protein–protein, protein–ligand, protein–metal ion, protein–carbohydrate, protein–DNA, DNA–DNA, and DNA/RNA-drug interactions.

Protein–protein interactions are the kernels for the formation of multiprotein complexes, which consist of two or more noncovalently bound proteins. Such multiprotein complexes are often quite large "molecular machines" that perform an equally complex, specific biological function. For instance, the proteasomes are protein complexes for molecular degradation of damaged proteins in all eukaryotes and archaea and in some bacteria [1]. Noncovalent bonds between proteins and between protein and polysaccharides are involved in the recognition of antigens by antibodies [2]. Antibodies, gamma globulin proteins that exist in the blood and other bodily fluids of vertebrates, show a very high binding affinity and specificity for specific antigens. Interactions between proteins and small ligands such as drugs or nucleotides (e.g., adenosine-5′-triphosphate, ATP) are critical for keeping cells fully functional. For instance, ATP plays a fundamental role in signal transduction processes in eukaryotes and many prokaryotes [3]. Proteins can contain one or several metal ions as cofactor, which are generally crucial for proteins to function properly. Hemoglobin, myoglobin, and hemerythrin are important examples of metalloproteins that contain iron and bind oxygen [4]. Noncovalent interactions between proteins and DNA are of paramount importance for the storage and readout of genetic information in living organisms. In eukaryotes, segments of DNA double helix are coiled around a central core formed by eight proteins (histones) to form the so-called nucleosome [5]. Nucleosomes are defined as the basic unit of DNA packaging in eukaryotes. Finally, DNA–DNA interactions are omnipresent [6]: DNA generally forms the well-known DNA double strands that are held tightly together by hydrogen bonds.

A large variety of analytical techniques such as nuclear magnetic resonance, X-ray crystallography, ultracentrifugation, and spectroscopic techniques (fluorescence, circular dichroism, light scattering, or surface plasmon resonance) have been developed to study such interactions (see, for example [7]). Recently, mass spectrometry (MS) has emerged as a powerful tool to investigate noncovalently bound complexes and presents advantages compared to other techniques [8]. Using MS, the molecular weight of intact noncovalent complexes, their stoichiometry, and interactions between subunits can be established. Analysis of biomolecules bound by noncovalent interactions using MS requires only small amounts of sample (on the order of femto- to picomoles), directly provides stoichiometric information and can often be carried out more easily than analyses by other techniques.

Mass spectrometric analysis of noncovalently bound complexes requires a suitable soft ionization method. The two main methods in use are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). MALDI and ESI allow, under appropriate conditions, the preservation of noncovalently

bound complexes in the gas phase (for reviews see [9–14]). ESI-MS is often considered to be the preferred method, although the results are affected by the nature of the intermolecular interaction, by the composition, ionic strength, and pH of the buffer in which the protein complexes are dissolved, and by the voltages and the pressures in the mass spectrometer. However, MALDI-MS is an attractive alternative to ESI-MS because it can overcome some of the difficulties of ESI-MS. Foremost, the mass spectra acquired by MALDI-MS are simpler to interpret than those acquired by ESI-MS due to the predominance of singly charged species. Furthermore, MALDI ionization is more tolerant to the presence of detergents and salts than ESI ionization. To date, ESI analyses are mainly carried out using volatile buffers such as ammonium acetate or ammonium carbonate.

In the current review we illustrate and selectively discuss the major advances in the application of MALDI-MS to study noncovalent complexes. First we describe the challenges in the analysis of noncovalent complexes (Sect. 2). Then we distinguish between two different approaches to analyze noncovalent assemblies: direct methods that allow a direct detection of the noncovalent assembly (Sect. 3) and indirect methods in which the presence of the complex is deduced indirectly, e.g., by the intensity decrease of an interacting partner (Sect. 4). The last section encompasses quantitative strategies to characterize noncovalent interactions (Sect. 5).

2 Challenges for the Detection of Noncovalent Interactions

Detection of noncovalent complexes by MALDI-MS generally involves the co-crystallization of the analyte with a dedicated matrix typically on a stainless steel plate, ionization and desorption of the crystals with a pulsed laser beam, acceleration of ions with an electrical field, their mass analysis, often using time-of-flight (TOF) instruments, and ion detection. This process requires properly adapted instrumental conditions and sample preparation protocols and, for a number of reasons, is not always straightforward.

The most obvious hurdle is that many MALDI matrices present an unnatural, acidic environment, and that they are often used in combination with organic solvents. This environment tends to denature proteins and is certainly not conducive to maintaining noncovalent interactions during sample preparation. Some remedies for preserving noncovalent interactions were proposed. For example, it is possible to carry out the co-crystallization of the sample with the matrix very rapidly, such that there may be insufficient time for complex dissociation. Aqueous solutions can be utilized. The most successful, however, is the use of special, nonacidic matrices for MALDI of weakly bound complexes. The crystalline environment of the matrix itself may cause disruption of complexes. Special sample preparation methods for MALDI-MS of noncovalent complexes will be discussed in detail in Sect. 3.2. It was observed that MALDI mass spectra obtained from a fresh area of the sample by a single laser shot are much more successful for generating intact complex peaks.

This is the so-called "first shot phenomenon" [15] and it is not general, but sample-, preparation-, and matrix-dependent. This will be discussed in detail in Sect. 3.1.3.

Another difficulty in the analysis of noncovalent assemblies is that large, multiprotein complexes generally require high laser pulse energies to be liberated from the condensed phase for the creation of detectable ions. At some point on the molecular weight scale, the required laser pulse energy may be so high that dissociation of the complex is favored over desorption/ablation. In order to stabilize high molecular weight noncovalent assemblies against dissociation, one often resorts to a "trick," chemical crosslinking [16]. Chemical crosslinking will be discussed more extensively in Sect. 3.3.

A third problem is that MALDI produces predominantly singly charged ions, and complexes of very high m/z (over 50,000–100,000 Da) are quite difficult to detect using TOF instruments. The most successful approach to address this problem has been the development of special high-mass detectors, notably cryogenic detectors [17–19] and ion-to-ion conversion dynode detectors [20, 21]. Using these technologies, it is now possible to measure in the hundreds of kDa to MDa range on the m/z scale. For details, see Sect. 3.1.4.

Generally, however, commercial TOF instruments are not equipped with these special detectors, chemical cross-linking is not widely used, and the temptation has been to work at high sample concentration to increase the chances to observe high-mass complexes by MALDI-MS. However, this leads to another problem, namely unwanted clustering in the expanding MALDI plume. The distinction between a specifically formed noncovalent complex and a nonspecific cluster can become difficult. For example, Chan et al. [22] observed clusters of up to 15-mers of chicken egg white lysozyme when working with a 126-pmol MALDI sample and a liquid matrix, 3-nitrobenzyl alcohol. There are several other examples of clustering in the literature, e.g., clusters between arginine-lysine/oligodeoxyribonucleotide or protein and oligothymidylic acid formed, for instance, by multiple ion-pair interactions [23, 24]. Usually it is impossible to distinguish specifically bound complexes from non-specific complexes in such spectra. Of course, there are also clusters between analyte and matrix, which, given the limited resolution at elevated mass, generally leads to some undesired peak broadening. Occasionally, however, one observes a specific complex peak "sticking out" of an otherwise exponentially decaying nonspecific cluster distribution. This was, for example, the case for avidin [25], where the specific tetramer peak at $\approx$63 kDa was clearly more intense than peaks of the dimer, trimer, pentamer, hexamer, etc. in the spectrum, which were all present due to nonspecific clustering in the plume.

An open question is whether the gas-phase structure of a biomolecule complex is identical to the structure in solution. This may depend on whether the complex is transferred into the gas phase by MALDI (which is somewhat less "soft") or by a very soft "native" ESI method [11, 26]. There are some indirect hints, for example, the charge state distribution in ESI mass spectra or the collision cross section as measured by ion mobility spectroscopy that the gas-phase conformation of such complexes is indeed very close to that in solution phase, but a rigorous proof is still lacking [27].

In conclusion, finding the appropriate strategies for preserving and detecting noncovalent interactions, the distinction between specific and nonspecific complexes, and the resemblance of gas-phase structure to the solution-phase structure are the main challenges during the analysis of noncovalent complexes by MALDI-MS.

3 Approaches for Direct Detection of Specific Complexes by MALDI-MS

The direct detection of noncovalent biomolecular complexes with MALDI-MS was accomplished in the early days after the development of this soft ionization technique. Intact molecular ions of streptavidin [28], glucose isomerase [29], and porin [30] forming quaternary structures with identical subunits could be observed using the water soluble matrix nicotinic acid and minimizing the amount of organic solvents. However, nicotinic acid suffers from several disadvantages, such as low salt tolerance, extensive matrix adduct formation, and lack of compatibility with widespread lasers such as nitrogen lasers or frequency-tripled Nd:YAG lasers with emissions at 337 nm or 355 nm, respectively. Experiments with more typical MALDI matrices (e.g., sinapinic acid) often yielded only signals of subunits rather than of the complex ions. Therefore, dedicated sample procedures were developed to preserve noncovalent biomolecular complexes, often largely dependent on the analyte itself. Thus, several experimental approaches should be tested for each analyte.

Below we describe the instrumental features that should be chosen to detect noncovalent complexes successfully (e.g., source pressure and laser energy). We also discuss how the sample should be prepared prior to the MALDI-MS analysis. Finally, we illustrate the achievements of chemical crosslinking.

3.1 Instrumental Parameters

3.1.1 Laser Pulse Energy and Wavelength

The laser pulse energy strongly influences the presence of the specific intact assembly [31]. In most cases, laser pulse energy values just above the detection threshold value are recommended to maintain reasonable relative intensities of the complex and to minimize the amount of nonspecific clusters formed in the gas-phase (Fig. 1) [32–34].

For the detection of intact double-stranded DNA, desorption with infrared (IR) and ultraviolet (UV) lasers was compared [33]. The IR laser in combination with glycerol/ammonium acetate as matrix was favored for higher numbers of base pairs

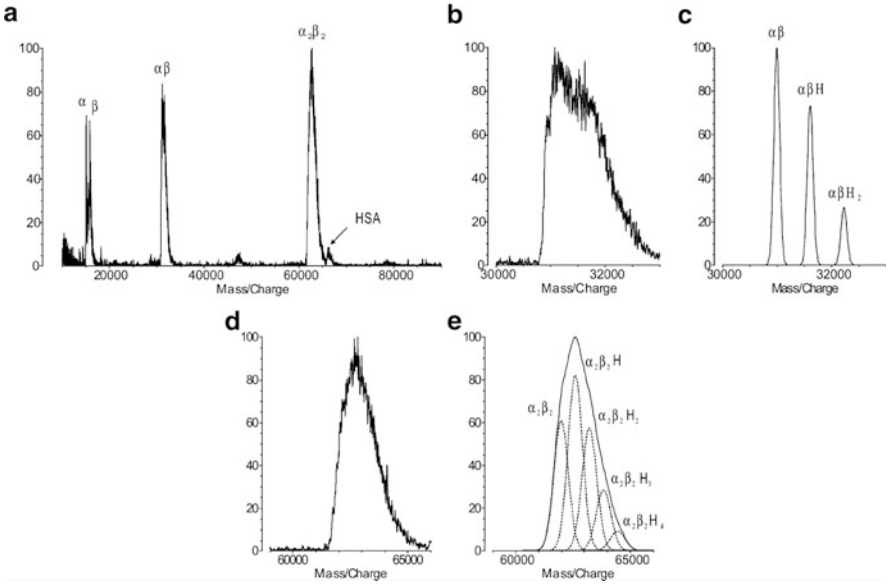

Fig. 1 MALDI-TOF mass spectrum of whole human blood diluted 1:500 in 20 mM ammonium acetate (**a**). In the spectrum the intact hemoglobin $\alpha_2\beta_2$, the hemoglobin α- and β-chains and human serum albumin (*HSA*) are present. The peaks of the heterodimeric subassembly (**b**) and the intact heterotetrameric assembly (**d**) are shown in detail. The corresponding calculated spectra are given (**c**, **e**). *α* indicates the hemoglobin α-chain, *β* the hemoglobin β-chain and *H* the heme b group. A saturated 2,6-dihydroxyacetophenone solution in acetonitrile/20 mM ammonium acetate 1:3 (v/v) was used as matrix. Laser energy values just above the threshold were applied. (Reproduced with permission from [34]. © (2004) John Wiley & Sons, Ltd)

and less fragmentation was observed compared to 6-aza-2-thiothymine (ATT) matrix in the UV range. However, with DNA being smaller than 70 mers, only the UV laser allowed the preservation of the intact assembly.

In contrast, Zehl and Allmaier observed a minor importance of the type of extraction (delayed or continuous), the choice of the acceleration voltage, and the use of reflectron mode concerning the relative intensity of the intact quaternary structure and nonspecific cluster ions, when studying tetrameric avidin [31].

3.1.2 Source Pressure

Few studies presented the utility of ionization at atmospheric pressure (AP). Due to collisions with ambient gases after ionization, AP-MALDI produces ions with lower internal energy than MALDI at ultrahigh vacuum. Thus, weak noncovalent interactions experience less fragmentation. AP-MALDI was successfully used for sugar–sugar [35] and sugar–peptide complexes [36] by IR-AP-MALDI or peptide–peptide complexes by UV-AP-MALDI [37].

3.1.3 The First Shot Phenomenon

In many cases the detection of intact noncovalent assemblies was only possible for the first or the first few laser shots on a non-irradiated sample spot [15, 38–44]. Successive laser shots at the same position mainly yielded monomeric ions (Fig. 2). This observation is generally described as "the first shot phenomenon" and was first investigated by Rosinke et al. for the homotrimeric OmpF porin protein [15]. Cohen et al. suggested segregation or precipitation of the quaternary complex at the crystal surface or dissociation of complexes around the ablation crater induced by laser irradiation [38]. Neither the macroscopic crystal structure, nor the type of substrate, nor the pH stability range of the protein samples had any influence. Detailed investigations of fluorescent or fluorescently labeled protein complexes with confocal laser scanning microscopy measurements confirmed the original assumption of Cohen et al. [38] that size segregation during crystal growth and dissociation of protein complexes in the crystal interior are responsible for the occurrence of the phenomenon [39, 40]. The first shot phenomenon is not generally observed during analysis of noncovalent interactions. Others report the lack of a clear-cut first shot phenomenon for certain analyte-matrix combinations [31, 45]. The extension to types of biomolecular interactions other than protein–protein assemblies has only been reported so far for adenylate kinase with the nucleotides adenosine-5′-monophosphate (AMP), adenosine-5′-diphosphate (ADP), and ATP [44].

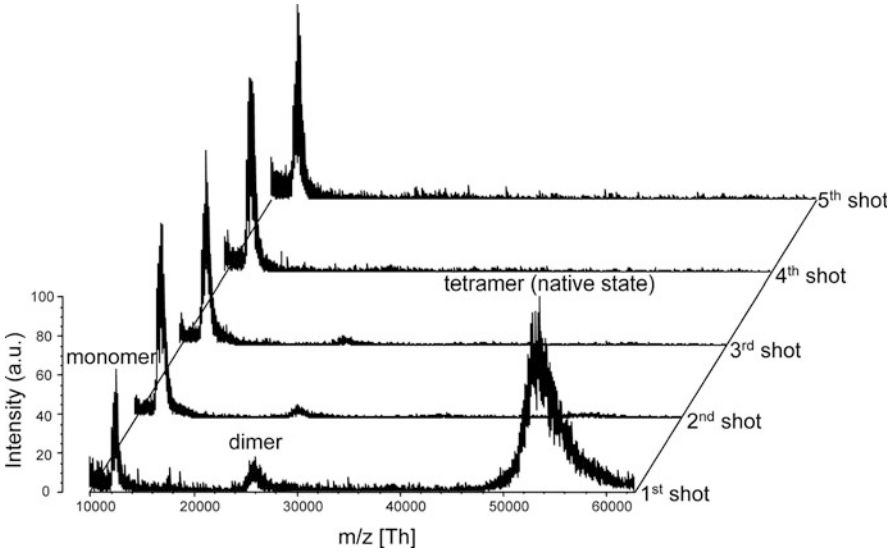

Fig. 2 MALDI mass spectra showing first and subsequent shot data of tetrameric streptavidin. Sinapinic acid was dissolved in triethylammonium bicarbonate at pH 8.5. Sample spots were prepared with a dried droplet preparation using a 1,000:1 matrix-to-analyte ratio. All spectra are normalized to the base peak. (Reproduced with permission from [40]. © (2007) The Royal Society of Chemistry)

3.1.4 High-Mass Detectors

The conventionally used microchannel plate detectors (MCP) suffer from low detection efficiency of high-mass ions due to their low velocity when impinging on the detector surface [46]. Additionally, detector saturation becomes a problem when low-mass ions are present. Ions of lower molecular weight arrive first at the detector. Since the recovery times of detector channels are typically on the order of microseconds [47], the detection sensitivity for high-mass ions with flight times on the order of several hundred microseconds is significantly lower.

Using special high mass detectors, it is possible to measure in the hundreds of kDa to MDa range on the *m*/*z* scale. For so-called superconducting tunnel junction cryodetectors, the impinging analyte ions cause a break-up of Cooper electron pairs under superconducting temperatures. The resulting free electrons can tunnel through a thin oxide layer and thus be detected as excess current [19]. Using this principle, the ion detection is mass-independent and detector saturation effects are avoided due to the fast refresh time of few microseconds. Cryogenic detectors allowed the detection of von Willebrand factor [17] and large molecules such as dendrimers [48]. In ion-to-ion conversion detectors, the analyte ions are converted into secondary ions on a conversion dynode, reaccelerated, and detected with a subsequent secondary electron multiplier [20]. Examples of high-mass MALDI mass spectra with an ion-to-ion conversion detector include the analysis of antibodies [49], PEGylated proteins and glycoproteins [50], and direct profiling and imaging of proteins on tissue [51]. The given examples, however, do not represent noncovalent complexes.

3.1.5 LILBID-MS

Promising results in the analysis of noncovalent interactions were obtained by an alternative ionization technique called laser induced liquid beam ionization/desorption (LILBID) [52]. Instead of a crystallized matrix-analyte mixture, Brutschy et al. used a free liquid beam consisting of a solvent as laser target. After seeding the beam with sample and injecting it into vacuum, partially solvated ions are desorbed by exciting a vibration of the solvent with pulsed infrared laser radiation and mass analyzed with a reflectron TOF tube. The use of an aqueous buffer as liquid beam solvent allowed the conservation of protein–protein interactions, such as hemoglobin [53], ribonuclease S, or calcium-dependent calmodulin/melittin complexes [54]. Shifting the temperature or pH value of the buffer solution to non-native solution-phase conditions induced dissociation into subunits, thus indicating specific complex detection. More recently, the miniaturization of the beam to micro droplets provided higher sensitivity and less sample consumption, extending the application range to more delicate biomolecules of low availability [55]. This variant of LILBID-MS is called laser-induced liquid *bead* ion desorption. Ranging from DNA duplexes to DNA/RNA-ligand complexes, LILBID-MS showed high

potential for the analysis of large macromolecular complexes of nucleic acids in the megadalton mass range [56]. In the case of the human immunodeficiency virus (HIV) Tat:TAR transactivation complex, a quantitative evaluation of the spectra allowed one to draw conclusions on binding specificity, on affinities of different ligands, and on efficiencies of potential inhibitors and the determination of apparent IC_{50} and K_d values [57].

Intact membrane protein complexes could be successfully ionized in the presence of detergent molecules, which partially remained bound to the macromolecule [58, 59]. By increasing the laser intensity, the detergent molecules were stripped off causing disintegration into different oligomeric association states. Being able to detect intact bacterial ATP synthase with a molecular weight of about 540 kDa (Fig. 3) [60], the complex subunit stoichiometries were determined and comparisons between bacterial and eukaryotic species made [61]. In comparison with ESI, LILBID-MS provides simpler spectra due to lower charge states and a higher salt tolerance.

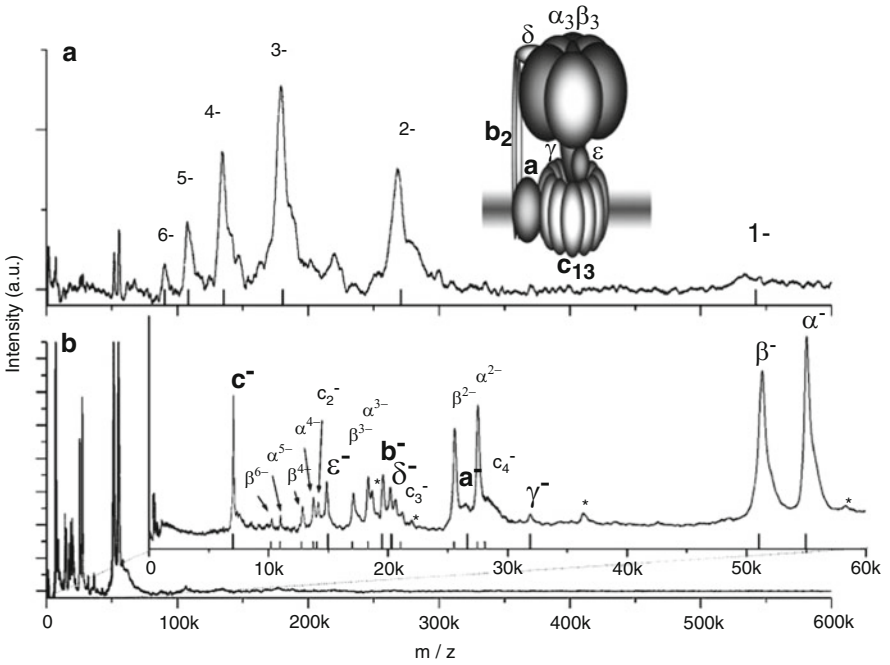

Fig. 3 LILBID anion spectrum of the intact ATP synthase measured under soft conditions (**a**). Using higher laser intensities, all eight subunits are visible (**b**). Additionally, the α and β subunits appear multiply charged and fragments consisting of oligomeric *c* subunits (c_n) are visible as well. Peaks indicated by *asterisks* could not yet be accounted for and probably represent minor impurities from the enzyme preparation. (Reproduced with permission from [60]. © (2008) Elsevier B.V.)

3.2 Dedicated Sample Preparation Procedures

The noncovalent complex has to survive all target preparation and laser desorption steps in order to be detected by MALDI-MS. Several influencing factors that prevent complex disruption could be pointed out. The choice of MALDI matrix [42, 45], the pH of the solution [62–67], the crystal morphology [15, 42], the sample spotting technique, the presence of organic solvent [15], the ionic strength [33, 68], the matrix/analyte ratio [32, 38, 69], and the speed of solvent evaporation [31, 42] are crucial for preserving the noncovalent interaction. Although numerous approaches turned out to be successful for certain complexes, no general protocol applicable for different kinds of biomolecular interactions has been obtained so far.

Note that the binding strength of a noncovalent interaction is not necessarily a good indicator for whether or not an intact complex will be successfully detected by MALDI-MS. For example, to the best of the authors' knowledge, no biotin-streptavidin complex, which represents one of the strongest known noncovalent interactions, has ever been measured in its assembled form so far by MALDI-MS.

3.2.1 Choice of MALDI Matrix

MALDI matrices enhance desorption and ionization of the analyte molecules by absorbing the laser light and are typically small organic molecules that have a high absorption at the corresponding laser wavelengths. The physicochemical nature of the MALDI matrix is one of the key factors for detecting intact noncovalent complexes. Depending on the solvent, the crystal morphology and the analyte incorporation change. For example, the intact porin trimer was only detectable in sufficient amounts of ferulic acid (FA) in tetrahydrofuran (THF), but not with the same matrix dissolved in other solvents, such as acetonitrile (ACN), ACN:0.1% trifluoroacetic acid (TFA) = 1:2 (v/v) or acetone [15]. Other tested matrices such as a 2,5-dihydroxybenzoic acid (DHB):2-hydroxy-5-methoxy benzoic acid = 9:1 (v/v) mixture dissolved in acetonitrile:0.1% TFA = 1:2 (v/v) did not provide any signal of intact trimers. For the ternary system porin/FA/THF, only the finely structured, microcrystalline areas of the sample spots yielded the trimer. In addition to FA [15, 38, 42], several other matrices allowed the detection of reasonable amounts of oligomeric species for certain protein quaternary structures. One of the more common ones are the less acidic hydroxyacetophenone derivatives such as isomers of dihydroxyacetophenone (DHAP) [31, 34, 38, 40, 42, 44] or trihydroxyacetophenones (THAP) [38], 3-hydroxypicolinic acid (HPA) [45], and ATT [39, 43, 69] dissolved in various solvent mixtures, preferably aqueous buffers containing ammonium salts. The latter also represents a favored matrix for biomolecular interactions of low-molecular weight complexes, such as those involving oligonucleotides [33, 41, 70–77], peptide–peptide [37, 67, 78–81], peptide-amino acid [82], or peptide–metal ion complexes [64, 66]. Additionally, other matrices have been used: *p*-nitroaniline (PNA) [64, 83–87] and 2-amino-4-methyl-5-

nitropyridine (AMNP) [65, 88] for the analysis of peptide–metal ion/ligand complexes, HPA for double-stranded DNA [89], and DHAP to preserve DNA-peptide interactions [68]. In a few cases, highly acidic matrices such as sinapinic acid (SA) [25, 32, 40, 62, 63, 90, 91] or α-cyano-4-hydroxycinnamic acid (CHCA) [92] showed promising results.

3.2.2 pH Value of the Matrix Solution

One would expect that acid-sensitive complexes tend to dissociate at low pH values when acidic matrices are used. Thus, raising the pH of the matrix solution to physiological values can facilitate the observation of oligomeric species [62, 69]. In the case of several enzyme-substrate complexes, Woods et al. tested SA as a matrix dissolved in ethanol:1 M ammonium citrate = 1:1 (v/v) or ethanol:water = 1:1 (v/v, pH < 2). Ions for the enzyme-substrate complex were only observed under the first conditions [62]. However, the stability gain of noncovalent complexes with increased pH values is often counterbalanced by a loss in ionization efficiency [93].

As demonstrated by Jespersen et al., the physicochemical properties of the matrix seem to have stronger influence than the acidity or basicity of the matrix itself [45]. Investigating glutathione-*S*-transferase and streptavidin with different acidic or basic matrices in a pH range of 2–7.1, species related to the protein quaternary structures were only detected using HPA (pH 3.8) dissolved in water, but not with DHB, CHCA, or basic matrices such as 2-aminonicotinic acid (ANA), PNA, and AMNP (Fig. 4). Matrices were dissolved in water except for CHCA, which was dissolved in ACN:water = 30:70.

In addition to ammonium salts, additives such as methylene blue, peptides, or spermine can stabilize a noncovalent assembly during crystal growth and/or desorption/ionization and thus facilitate its detection by MALDI-MS (Fig. 5) [76].

3.2.3 Sample Preparation

Different sample preparations have been used ranging from variations of the dried-droplet method [15, 25, 31–34, 38–40, 42–45, 62–70, 72–84, 87–89, 91] to layer techniques [37, 40–42, 92] such as thin layer or sandwich preparations. For the dried droplet method [94], the analyte is directly mixed with the matrix solution either on the MALDI plate or in an Eppendorf tube and dried under room temperature. The thin layer technique [95] utilizes a thin layer substrate of matrix crystals as a seeding ground for subsequent cocrystallization of the analyte solution. For the sandwich method [95], a second layer of matrix is added on top of the sample prepared by the thin layer technique. However, the dried-droplet method is most widely applied. The sandwich method is compatible with the matrixes CHCA and SA and has slightly higher tolerance to sample impurities. Fast sample spot drying under reduced pressure turned out to be more successful than slow drying [31, 42].

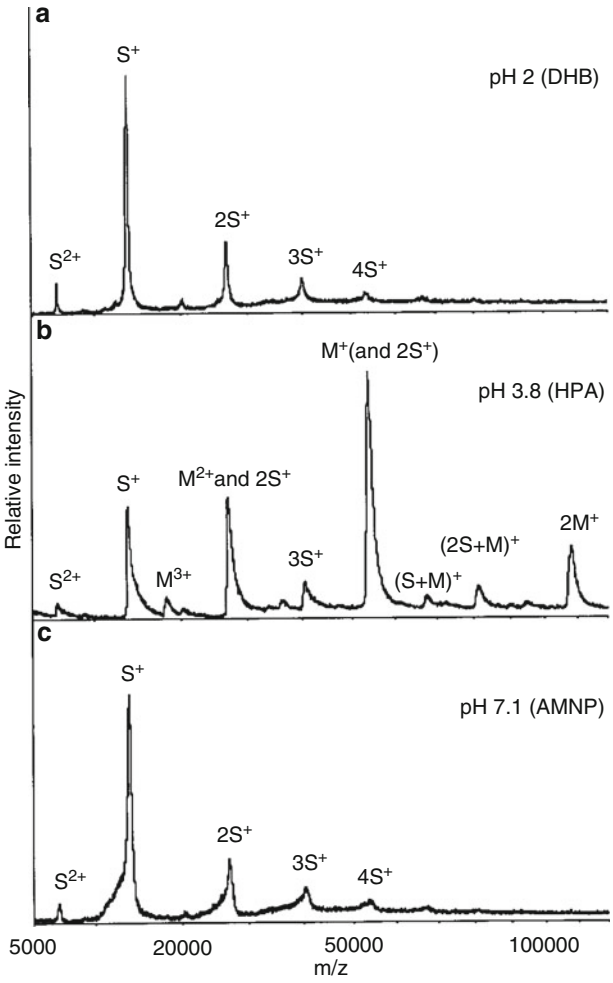

Fig. 4 Positive ion mode MALDI mass spectra of recombinant streptavidin obtained at different pH values with three different matrix compounds: (**a**) DHB in water (pH 2), (**b**) HPA in water (pH 3.8), and (**c**) AMNP in water (pH 7.1). Peaks corresponding to the dissociated and undissociated subunits are indicated as "S" and "M," respectively. (Reproduced with permission from [45]. © (1998) John Wiley & Sons, Ltd)

3.2.4 Sample Concentration

The detection of intact assemblies is often only possible for a limited concentration range. On one hand, a sufficient amount of matrix is required for isolating sample molecules from each other; on the other, the sample concentration should not be too low compared to the matrix concentration. Cohen et al. found for the homomeric protein complex streptavidin a valid range between 0.05 and 10 g/L (3.8–770 pmol/μL) and for alcohol dehydrogenase between 0.1 and 0.5 g/L

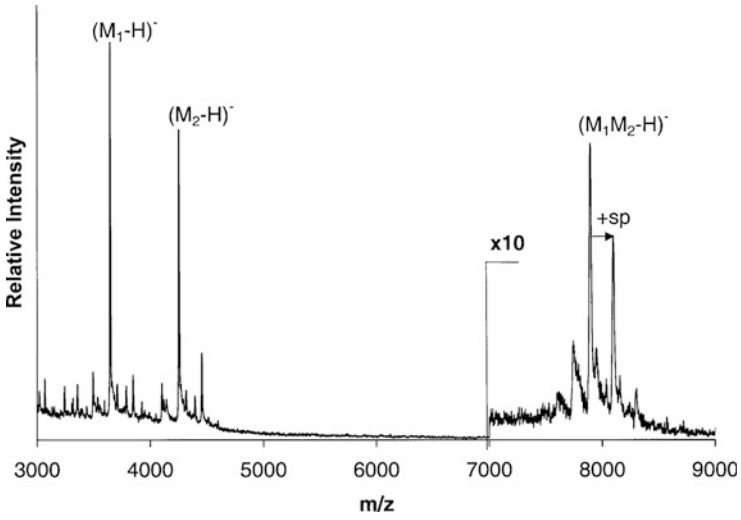

Fig. 5 Negative ion mode MALDI mass spectrum of a duplex oligonucleotide with the peptide β-melanocyte stimulating hormone as additive. The peptide:duplex ratio was 1:250. The mixture of 6-aza-2-thiothymine and spermine (*sp*) was used as matrix. (Reproduced with permission from [76]. © (2002) Elsevier B.V.)

(2.7–13.5 pmol/μL) [38]. For aerolysin, Moniatte et al. could measure the heptameric complex only in a concentration window of 0.5–1.5 pmol/μL [32]. In order to avoid suppression of analyte signals, matrix/analyte ratios as well as mixing ratios between different analytes in heteromeric complexes have to be carefully optimized. Testing several analyte/matrix ratios by preparing analyte solutions ranging from 0.1 to 10 pmol/μL was recommended.

3.2.5 Successful Examples

The direct detection of specific noncovalent complexes by MALDI-MS was accomplished for homomeric or heteromeric protein–protein [15, 25, 31, 32, 34, 38–45, 63, 69, 90, 91] (Fig. 1), protein–peptide [62, 69, 92], peptide–peptide [37, 67, 78–81], protein/peptide–ligand [44, 84–87], protein/peptide–metal ion complexes [62, 64–66, 83, 88], peptide-amino acid [82], oligonucleotide double strands [33, 68, 70, 74–76, 89] (Fig. 5), and protein/peptide/guanidinium derivatives-oligonucleotide interactions [41, 65, 68, 71–73, 77].

3.3 Chemical Crosslinking

Chemical crosslinking has been extensively used in the past to determine the stoichiometry of noncovalent complexes in combination with gel electrophoresis [96].

For this, biomolecule assemblies were covalently stabilized with homobifunctional reagents such as imidoesters. Starting in the early 1990s, chemical crosslinking has been successfully applied in order to prevent complex dissociation of protein quaternary structures during MALDI-MS analysis. In the first experiments, glutaraldehyde was selected as crosslinker [16]. Due to its polymerization reaction in solution, several lengths of bifunctional crosslinkers are generated that can react with ε-amino groups of lysines and α-amino groups of the N-termini. Thus, a variety of distances between the subunits can be bridged. Using this approach, Caprioli and Farmer detected dimeric and tetrameric complexes such as avidin and yeast alcohol dehydrogenase (Fig. 6) [16]. Only a few studies demonstrated the feasibility of this approach [97, 98]. One reason may be the high amount of generated polymerization products that deteriorates the spectra quality significantly.

Since the early days, numerous hetero- or homobifunctional linkers have been developed and applied to study biomolecular noncovalent interactions. In order to identify interaction partners and to determine the stoichiometry of complexes, nonselective photoreagents, as well as site-specific linkers reacting with a limited number of amino acid side chains, were examined. The underlying chemistry and applied functional groups are summarized in the literature [99, 100] and commercial product catalogs [101]. Among the photoreactive linkers, azido groups are particularly noteworthy. If higher selectivity is needed, *N*-hydroxy-succinimide (NHS) esters are often chosen as reactive end groups. The main targets of these

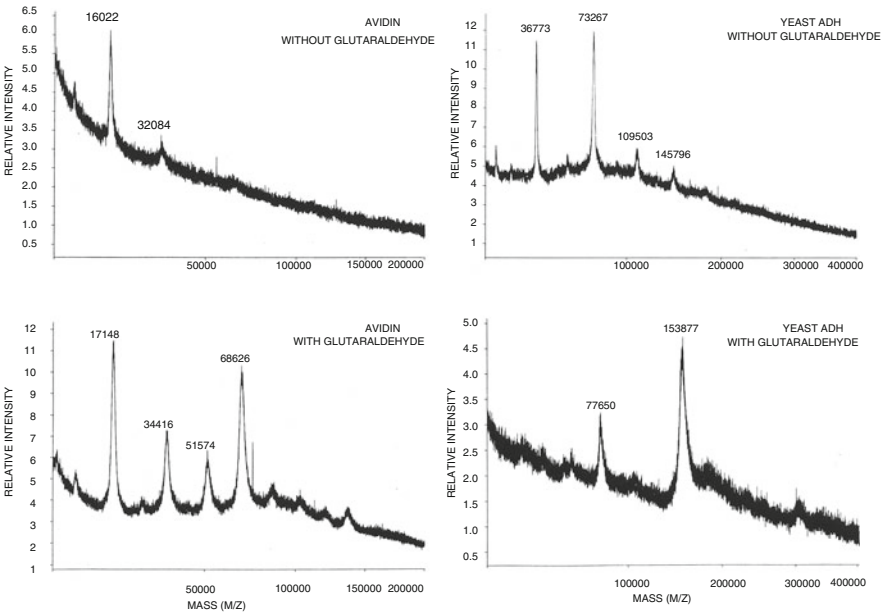

Fig. 6 MALDI mass spectra of avidin and yeast alcohol dehydrogenase before and after crosslinking with glutaraldehyde. (Reproduced with permission from [16]. © (1991) John Wiley & Sons)

esters are primary or secondary amines. However, side reactions with hydroxyl groups of serine, threonine and tyrosine have been reported as well [102, 103].

As an example, a heterobifunctional linker bearing both reactive groups, i.e., sulfosuccinimido-2-(7-azido-4-methylcoumarin-3-acetamido)ethyl-1,3′dithiopropionate, was used to confirm the 1:1 stoichiometry of the gp120 (HIV-1 virus) interaction with the CD4 receptor of T lymphocytes [104].

In combination with previously described high-mass detectors, the analysis of biomolecular complexes up to several MDa became possible. The application of high-mass MALDI and crosslinking for epitope mapping, kinetic studies, sandwich assays for immunocomplexes [49, 105], monitoring of ligand regulation mechanisms (Fig. 7) [106], screening of protease inhibitors [107], and determining association states [108] was demonstrated using NHS esters as crosslinkers.

In comparison with ESI analysis of noncovalent interactions under native conditions or direct MALDI-MS analysis, chemical crosslinking has a remarkable advantage. Since the stabilization of the complex is performed under solution conditions, gas-phase labile complexes (e.g., bound by the hydrophobic effect) are easily preserved during the analysis [109].

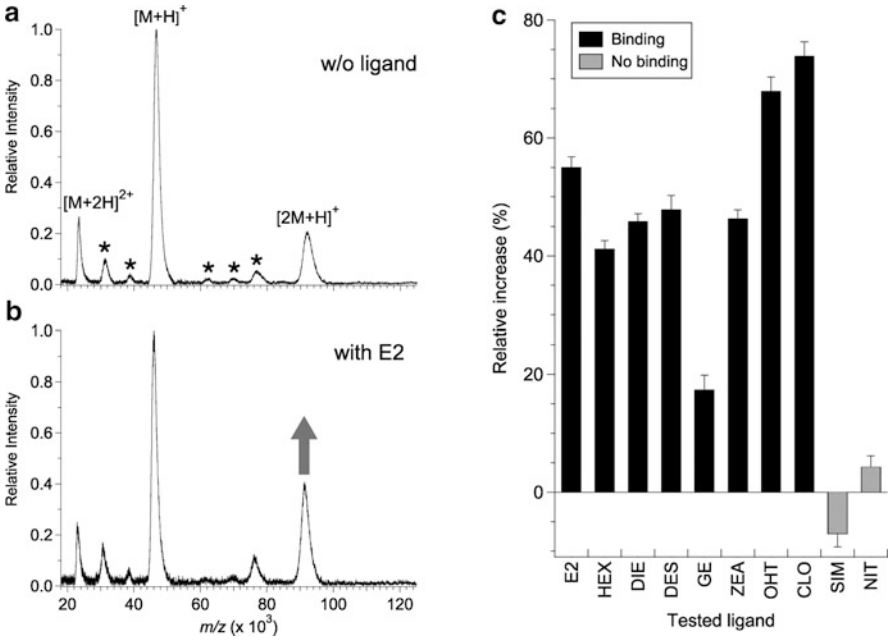

Fig. 7 High-mass MALDI-MS showing a ligand-dependent dimerization in solution for the mutant human estrogen receptor α ligand binding domain (hERα LBD) after chemical crosslinking with NHS esters. MALDI mass spectrum of crosslinked hERα LBD without ligand (**a**) and after adding the ligand E2 (**b**). After incubation with E2 and crosslinking, the MALDI mass spectrum clearly shows an increase of the homodimer, which is labeled $[2M + H]^+$. After incubation with different test compounds and crosslinking (**c**), only the ligands SIM and NIT did not increase the homodimer abundance relative to the aporeceptor significantly. The *asterisks* indicate sample impurities. (Reproduced with permission from [106]. © (2008) American Chemical Society)

Combining chemical crosslinking with electrophoretic separation, digestion and subsequent MALDI-MS of the excised bands to perform peptide mapping allowed the identification of several interacting partners [110, 111]. For example, experiments in living cells with paraformaldehyde revealed interactions of adhesin proteins with other membrane proteins [110]. Applying a heterobifunctional crosslinker comprising azido and an NHS ester functionalities allowed the identification of bacterial surface adhesins with a carbohydrate-containing crosslinking probe [111]. Potential candidates of triadic proteins interacting with RyR1 or TRPC3 in skeletal muscles were established using a maleimide crosslinker [112].

The crosslinker arm can also act as a ruler to map spatial proximities of amino acids in proteins and protein complexes [113]. Typical "bottom–up" protocols for the analysis of protein assemblies include the crosslinking reaction of the interacting proteins, purification of reaction products, and their digestion. The generated peptides are chromatographically separated and subjected to MS and tandem MS analysis. The data analysis using dedicated software [114, 115] allows the identification of intra-molecularly or inter-molecularly crosslinked peptides and modified amino acids. The derived distance constraints yield low-resolution tertiary structures of proteins. Using an NHS ester crosslinking agent and MALDI-post source decay analysis, the bovine basic fibroblast growth factor FGF-2 was identified as a member of the β-trefoil family [113]. The same methodology showed promising results for mapping binding interfaces of noncovalent protein–protein interactions [116–122]. Utilizing different crosslinkers, several subunits of the ATP synthase from *Saccharomyces cerevisiae* were investigated and their role in this yeast machinery was deduced [117–119]. With the same protocol, topology and spatial organization models of several multiprotein complexes were proposed [120]. Since the number of unmodified peptides or fragments by far exceeds the number of modified ones, data analysis is often the crucial step in this method. With the use of cleavable crosslinkers, affinity tags or isotopic labeling, improvements in the detection of intra-molecularly or inter-molecularly crosslinked species were obtained [123–135]. Recently, several crosslinkers have been optimized for MALDI-MS conditions. On one hand, this optimization was accomplished by inserting photolabile groups that give characteristic fragmentation patterns induced by the UV laser pulse in the mass spectrometer [136]. On the other hand, specific signal enhancement of peptides modified with crosslinker molecules was accomplished by incorporating a CHCA moiety in the linker [137].

In order to study protein-oligonucleotide binding interfaces, several crosslinking approaches were tested in combination with MALDI-MS. Most of them incorporate photochemical reactions, which are reviewed in [138]. A strategy avoids the use of any crosslinker molecules and relies on the natural UV reactivity of the nucleobases. Upon UV radiation of protein-oligonucleotide complexes, covalent linkages between the interaction partners are formed at the binding interface (Fig. 8). The resulting species can be analyzed directly by MALDI-MS to find interaction partners [139] or can be subjected to digestion procedures and MS [140–143] or tandem MS [144, 145] analysis to identify binding sites. Since direct UV-induced crosslinking often suffers from low yields of crosslinked products,

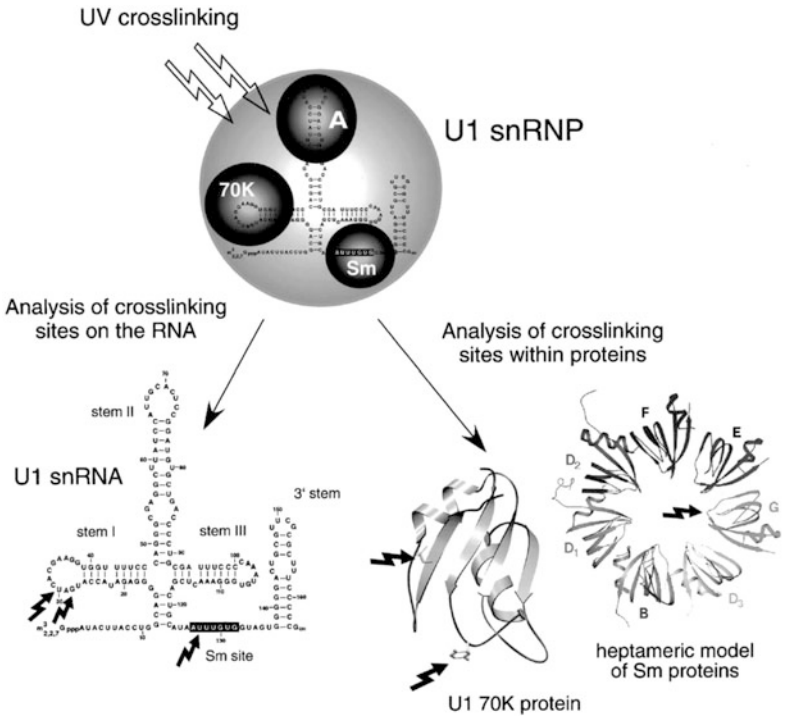

Fig. 8 Complementary strategy for identification of protein–RNA crosslinking sites in native ribonucleoprotein (RNP) particles as outlined for UV-irradiated U1 small nuclear (sn)RNPs. Arrows in the U1 snRNA secondary structure indicate the crosslinking sites on the RNA as identified by a immunoprecipitation/primer-extension method. Arrows at the 3D protein models of the U1 70K protein and of the heptameric Sm protein ring show the corresponding crosslinking sites within the protein as identified by Edman degradation combined with MALDI-MS. (Reproduced with permission from [143]. © (2002) Elsevier Science (USA))

dedicated enrichment protocols are preferable [146–148]. Alternatively, the oligonucleotides are chemically modified with reactive groups forming either specific [149, 150] or nonspecific photo-induced [139, 151] covalent linkages to the protein. Using this approach, amino acids in close proximity to these modifications in RNA/DNA-binding domains of proteins could be identified. The main challenges for the analysis of protein-oligonucleotide complexes are conflicting conditions for an optimum ionization of the interacting molecules. Thus, sample preparation conditions should be carefully chosen [140, 152].

In recent studies, MALDI-MS has been mainly used as a fast and sensitive tool to monitor the crosslinking step and to optimize its reaction conditions prior to ESI analysis [132, 153–155] or as a supportive tool to complement ESI data [156].

In order to avoid the detection of nonspecific assemblies, the concentration levels of biomolecules should be kept as low as possible. Control experiments using the same crosslinking conditions, but nonbinding biomolecules, are strongly

recommended. A study focusing on the application range of chemical crosslinking has been published recently. Mädler et al. pointed out that complexes with lower affinity than a K_d of 25 μM cannot be analyzed using standard protocols [157].

4 Indirect Methods to Detect Specific Complexes by MALDI-MS

In contrast to the described direct methodologies, alternative approaches have been developed to detect the presence of noncovalent complexes by the appearance or fading of certain binding partners in the mass spectra. In this review, these methods will be referred as "indirect methods."

4.1 Intensity Fading

In the so-called intensity fading approach, the formation of a complex between a target biomolecule and its ligands is monitored in the presence and absence of the biomolecule. When the target biomolecule is present, the relative intensity of a ligand decreases (i.e., fades), if compared to control mixtures where no target molecule is present.

In early immunoassay studies, proteolysis products of antigens after the reaction with a monoclonal antibody were analyzed by MALDI-MS and their relative intensities were compared to reaction mixtures where no antibody was present [158–160]. An intensity decrease for certain peptides revealed their participation in the epitope. This approach was also used for high-throughput screening [161, 162] and in combination with dedicated software [163].

More recently, the intensity fading of intact binding partners has been monitored. A non-binding molecule, with similar mass and ion intensity as the interacting partner, was used as internal control. Utilizing this strategy, complexes between proteases and their corresponding inhibitors, as well as protein-nucleic acid complexes, were successfully analyzed [164, 165]. In order to preserve the noncovalent interaction during crystallization, DHAP in ammonium buffers with low acetonitrile content was applied as matrix. Immobilizing the protein target on microbeads and incorporating prefractionation steps dramatically increased the efficiency of this approach [166]. For instance, 16 protein inhibitors of serine proteases among a complexity of nearly 2,000 molecular species were identified in the saliva of the leech *Hirudo medicinalis*. The validity of the intensity fading methodology was confirmed with high-mass MALDI measurements after chemical crosslinking [107]. However, the mechanism is still not fully understood. A detailed study on experimental conditions, which are necessary to observe intensity fading, revealed the necessity of sub-μM concentrations of the binding partners and the presence of several non-binding compounds [167].

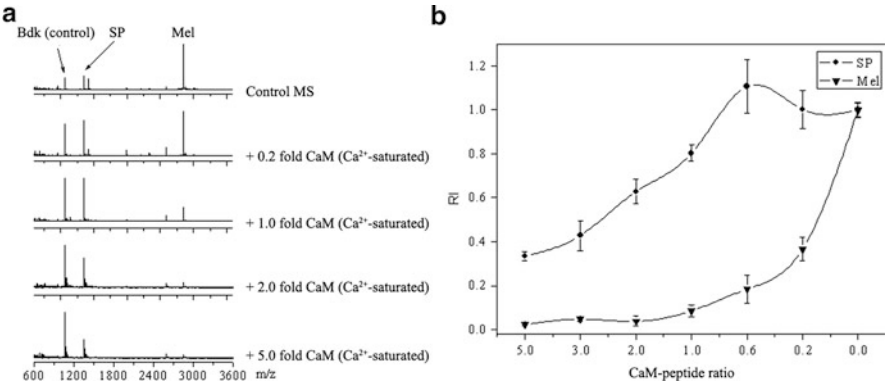

Fig. 9 MALDI mass spectra of mixtures of calmodulin-binding peptides melittin (*Mel*), substance P (*SP*), and a nonbinding control (bradykinin, *BDK*) after the addition of different concentrations of calcium-saturated calmodulin (**a**). Plot of the relative intensities (*RI*) of both melittin and substance P (corrected with the RI of the control) after the addition of calcium-saturated calmodulin with different concentrations (**b**). THAP was used as the matrix. (Reprinted with permission from [170]. © (2009) Elsevier Inc.)

Attempts to use the "intensity fading" strategy to gain quantitative information on a noncovalent complex were published for the interaction between the protease papain and its inhibitor cystatin [168]. In this study, the number of binding sites of cystatin for papain was determined by a modified Scatchard analysis. In order to draw the Scatchard plot, the relative intensity decrease of free cystatin was monitored. However, in the age of computer-based curve fitting, the linearization provided by the Scatchard plot is not really necessary, and it is somewhat problematic because the ordinate and abscissa are not independent. Today, nonlinear least squares fits can easily be used instead of linearization. More recent approaches demonstrated the feasibility of the "intensity fading" approach for studying metallopeptidase-inhibitor complexes [169], calcium-ion dependent calmodulin-peptide interactions (Fig. 9) [170], complexes between proteases and inhibitors from the plant *Capsicum annuum* [171], small molecules inhibiting the formation of a dimeric kinase [172], and RNA-polypeptide interactions [173].

4.2 Identification of Affinity-Separated Interaction Partners

Since its early days, MALDI-MS has served as a tool for the study of immunocomplexes. Instead of detecting the intact immunocomplexes, an indirect approach based on affinity-capture of the antigen on immobilized antibodies has often been applied [174]. In order to identify the antibody recognition site of the antigen, a proteolytic protection assay was used [175–178]. The antigen was captured by antibodies immobilized on beads, subsequently digested, and the

unbound peptides were washed off. Due to the high stability of the antibody to proteolysis, the epitope remained noncovalently attached to the antibody and was directly analyzed on the beads by MALDI-MS. This approach is generally termed as "epitope excision." If the antigen is proteolytically cleaved before binding to the antibody, "epitope extraction" is the term of choice. With the use of magnetic beads, the separation efficiency from unbound material is increased [179–182].

Competition assays allow the quantitation of binding efficiencies of different ligands [179, 180]. However, the immobilization step of one partner can alter the binding affinity due to covalent modifications. Binding the antibody to an immobilized protein, such as protein G, circumvents this problem [183, 184]. The same approach of noncovalent immobilization of an antibody was applied for immunoassays performed on self-assembled monolayers on a gold surface [185]. Using a porous gold layer, MALDI imaging techniques provided high-throughput and high sensitivity analysis [186]. A less costly alternative to antibody immobilization is the use of silica surfaces with covalently bound specific peptides [187].

MALDI-MS coupled with surface plasmon resonance (SPR) allows the simultaneous determination of binding kinetics during affinity separation, as reviewed in [188] (Fig. 10). In this method, a chip, which is directly analyzed by MS, replaces the beads [189]. An immobilized compound is used as a hook to fish unknown ligands from a complex biological sample [190]. MALDI-MS analysis again serves as a tool to identify the bound ligands and to map the recognition sites of the immobilized compound [191, 192]. High-throughput measurements can be easily carried out, when different target molecules are immobilized in distinct areas of the chip [193]. Moreover, the use of reflectometric interference spectroscopy was suggested as an alternative technique to SPR to investigate quantitative and qualitative binding of mixtures of ligands to target biomolecules [194].

MALDI-MS commonly serves as a tool to identify complex partners in diverse biological samples after electrophoretic separations [195] or tandem-affinity purification [196]. However, co-purified, nonspecific interactors are not easily distinguishable from specific ones. Additionally, changes in the complex composition can occur during sample preparation. In order to overcome these difficulties, a four-channel iTRAQ (isobaric tag for relative and absolute quantitation) approach provides in a single liquid chromatography-MALDI-TOF/TOF analysis the identification of genuine partners of the bait and the detection of variations in complex composition [197].

4.3 Hydrogen/Deuterium Exchange

MALDI-MS is used to study biomolecular interactions with hydrogen/deuterium (H/D) exchange strategies, as reviewed in [198]. In brief, deuterium atoms are integrated in interaction partners of interest by replacing backbone amide hydrogen atoms. As hydrogen is exchanged for deuterium, the increase in mass is

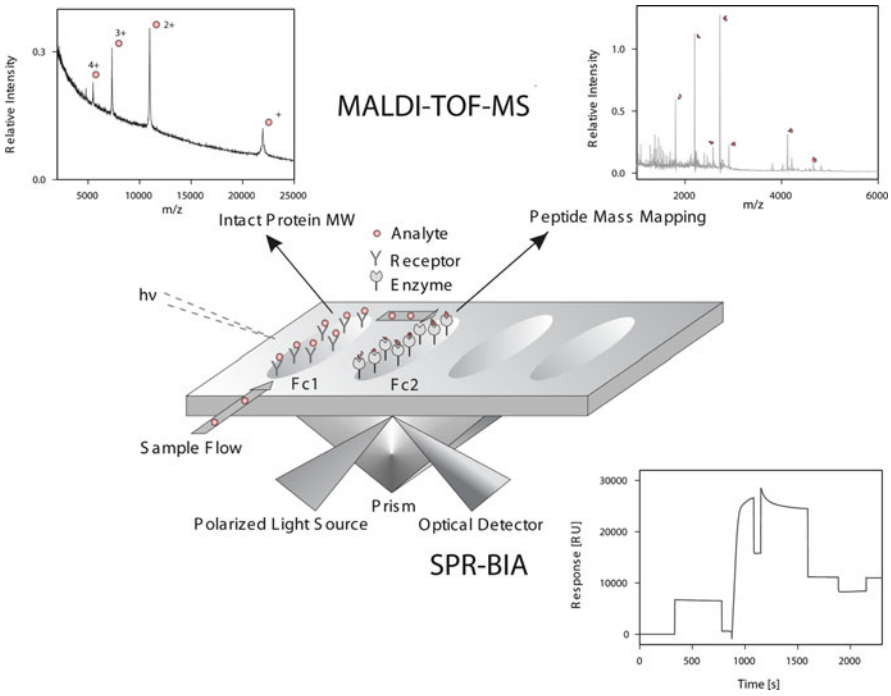

Fig. 10 Overview of MS coupled with SPR with on-chip incorporated proteolytic digestion. A receptor is covalently immobilized on the surface of the first flow cell (FC1). A second flow cell (FC2) is derivatized with a proteolytic enzyme. The analyte (ligand)-containing solution is routed through FC1 where the component of interest is affinity-captured. Following washing of non-specifically retained components, the ligand is eluted/routed from FC1 into FC2, where time for digestion is allowed. MALDI-MS analysis performed on the surface of FC2 yields accurate masses of the proteolytic peptide fragments that can be used for in-depth protein characterization. MALDI-MS performed on the surface of FC1 yields the mass of the intact protein. (Reprinted with permission from [188]. © (2000) WILEY-VCH Verlag GmbH)

monitored by MS. The exchange rates depend on the extent of inter- and intramolecular hydrogen bonding and solvent accessibility. Upon binding of an interaction partner, the H/D exchange rate at the binding site is altered. Komives and coworkers demonstrated the feasibility of MALDI-MS analysis to identify binding sites on a protein–protein interface. They studied the cyclic-AMP-dependent protein kinase complex with a kinase inhibitor and ATP [199] and the thrombin-thrombomodulin fragment complex [200]. Additionally, investigations on epitope mapping [201], assembly of viral capsids [202], conformational changes induced by aggregation [203–206], structural changes upon complex association (Fig. 11) [207–209], and the topology of supramolecular protein complexes [210] were conducted.

However, MALDI-MS analysis coupled with H/D exchange suffers from several disadvantages. One major problem is the back-exchange effect occurring during

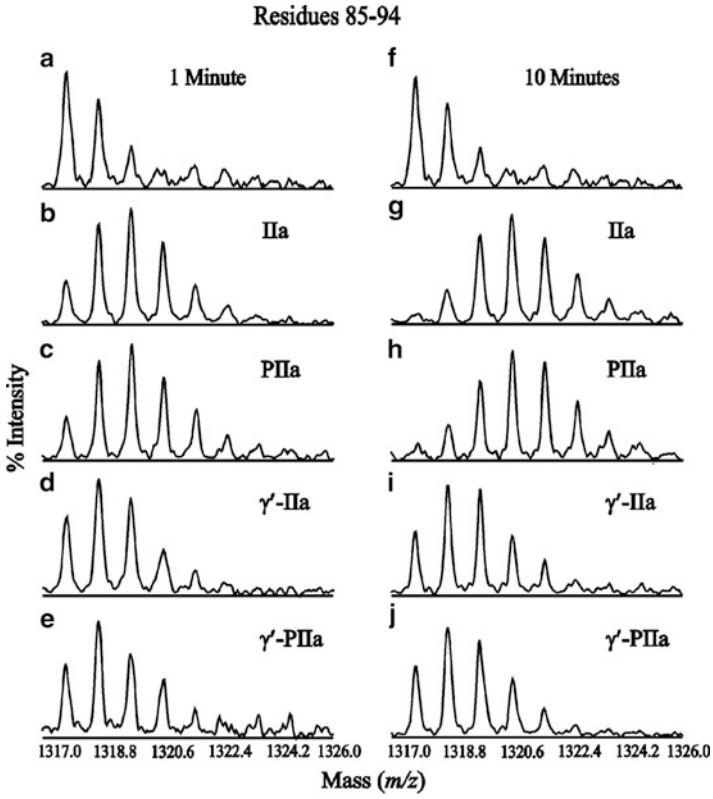

Fig. 11 Conformational analysis of γ′ peptide (410–427) interactions with thrombin anion binding exosite II (ABE-II). MALDI mass spectra representing residues 85–94 of ABE-II (*m/z* 1317.73) after 1 and 10 min of deuteration and subsequent proteolysis: (**a**, **f**) undeuterated peak cluster, (**b**, **g**) thrombin spectrum in the absence of ligands, (**c**, **h**) inhibited thrombin, (**d**, **i**) γ′ peptide bound to thrombin, (**e**, **j**) γ′ peptide bound to inhibited thrombin. This peak cluster contains the ABE-II residue R^{93} and experiences a significant degree of protection from deuterium in the presence of the γ′ peptide. Since this protection is maintained over 10 min, the HDX data present evidence that the γ′ peptide is interacting with R^{93}. (Reprinted with permission from [209]. © (2006) American Chemical Society)

analysis which is more prominent than in ESI experiments, although protocols have been developed to reduce [211] or to quantify its importance [212]. The application of collisional-induced dissociation to obtain site-specific information about the incorporation of deuterium into peptides and proteins is problematic due to "scrambling" of the deuterium position [213]. In contrast, in-source decay fragmentation induced less scrambling [214]. Although the higher salt tolerance and the simplified spectra are a strong advantage of MALDI and can often lead to abandonment of chromatographic purification, ESI is still the main ionization technique used for H/D exchange studies of biomolecular complexes.

5 Quantitative Characterization of Biomolecular Interactions

Noncovalently bound complexes are composed of interacting molecules (e.g., A and B) and it is possible to determine their binding affinities by MALDI-MS. The propensity of a noncovalently bound complex (e.g., AB) to dissociate into its components can be quantified by calculating an equilibrium constant named dissociation constant. The dissociation constant is usually indicated by K_d and is the inverse of the association constant K_a:

$$A + B \rightleftharpoons AB \qquad \frac{[A][B]}{[AB]} = K_d = \frac{1}{K_a}.$$

The method SUPREX (stability of unpurified proteins from rates of H/D exchange) was developed by Fitzgerald's group to investigate the strength of protein–ligand binding interactions in solution using H/D exchange and MALDI-MS [215]. The SUPREX protocol for the determination of K_d values includes an initial H/D exchange by adding a tenfold excess of deuterated exchange buffer to the target protein, usually at physiological pH. The exchange buffers contain varying concentrations of a chemical denaturant such as guanidinium chloride and urea. The denaturant leads to unfolding of the protein and to an enhancement of the H/D exchange rate, thus increasing the rate of deuterium incorporation into the protein. At a given exchange time, a small aliquot of the reaction mixture is added to the matrix (sinapinic acid) solution in a tenfold excess and the sample is subjected to MALDI analysis. The change in mass relative to the fully protonated sample (Δmass) in the spectra is plotted as a function of denaturant concentration, and the data is fitted to a sigmoidal function to obtain a transition mid-point. Solution-phase folding free energies (ΔG°f) in the presence and the absence of ligand can be calculated. The folding free energies ΔG°f of a protein and a protein–ligand complex are different, and this difference, ΔΔG°f, can be used to determine the protein–ligand binding constant. SUPREX works in a high-throughput automated fashion, requires only minute amounts of sample, and is applicable to purified as well as unpurified protein–ligand complexes. A prerequisite for SUPREX is that the protein must unfold in a two-state manner, i.e., only the fully folded and the fully unfolded states of the protein should be populated at equilibrium, thus posing a limit on its application.

As an example, the ternary protein–DNA complex formed of tryptophan repressor (TrpR), two molecules of L-tryptophan, and a 25-base pair duplex of DNA containing TrpR's cognate DNA sequence was analyzed by SUPREX [216]. The K_d values of the complexes, investigated in this study, were in agreement with previously established K_d values.

SUPREX was also used to measure quantitatively the stability of unpurified proteins in complex biological matrices [217]. Experiments of Fitzgerald's group in this field led to some excellent studies where SUPREX was applied to measure the thermodynamic stability of proteins both in vitro and in vivo with good accuracy and high precision [218, 219].

Related to SUPREX, Fitzgerald's group developed a technique called SPROX (stability of proteins from rates of oxidation) to determine the thermodynamic stability of proteins and protein–ligand complexes [220]. Proteins are oxidized with hydrogen peroxide in the presence of increasing concentrations of a denaturant (e.g., guanidine hydrochloride). Using MALDI-MS, the degree of oxidation is established at each oxidation time as a function of the denaturant concentration. By correlating denaturant concentration and oxidation rate, a folding free energy (ΔGf) and m value (δΔGf/δ[denaturant]) are measured during protein unfolding. If ΔGf and m values of the proteins are measured in the presence and absence of ligands, it is possible to evaluate protein–ligand affinities (e.g., ΔΔGf and K_d values). The main advantage of SPROX over SUPREX is the use of irreversible oxidation. The chemical stability of the oxidized proteins enables the manipulation of the modified proteins after oxidation. As an example, oxidized methionine amino acids were used to probe the solvent accessibility of these amino acidic residues as a function of temperature in order to construct thermal denaturation curves [221].

The Wanner group described a novel MALDI-based binding assay to determine affinity constants between small ligands and proteins in saturation and competition experiments, showing an excellent agreement between the K_d values determined by the two methods. A known ligand of the protein was used as an internal standard to generate a calibration function. They compared MS binding assays based on MALDI-MS/MS and those based on LC-ESI-MS/MS quantification. The instrument they used was a MALDI-triple quadrupole with high speed in the analysis of small molecules, commercialized as "FlashQuant system" [222].

6 Conclusions

In this review, we described the major applications of MALDI-MS to investigate noncovalent complexes of biomolecules. We highlighted the strengths and the limitations of the MALDI-based approaches to detect and quantify noncovalent complexes. We described not only the experimental approaches to detect complexes directly, but we also explained what the critical instrumental parameters are, and how intact complexes can be preserved by chemical cross-linking. Finally, we illustrated the possibility of detecting noncovalent complexes indirectly.

Overall, MALDI-MS represents an excellent method for studying noncovalent interactions. The substantial advantages of MALDI-MS over other techniques are sensitivity, speed, a higher salt tolerance, and the possibility to obtain precise molecular weights and stoichiometric information.

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