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Matrix assisted laser desorption/ionisation (MALDI)-TOF mass spectrometry of supramolecular metalloporphyrin assemblies: a survey †

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Analysis of a representative series of metalloporphyrins and of their supramolecular assemblies using laser desorption/ionisation time of flight mass spectrometry (LDI-TOF MS) revealed that non-covalent metal–ligand interactions in the complexes remain effective in the gas phase. Detectable assemblies range from simple ruthenium(II) porphyrin complexes with N- or P-donor ligands, or tin(IV) and zirconium(IV) porphyrin complexes with O-donor ligands, up to trimeric arrays. Crucial to successful recording of intact complexes is avoiding protic matrices which interfere with Lewis acidic analytes and which may induce ligand exchange reactions to form complexes with the matrix itself. Also described is the *in situ* generation of a series of ruthenium(II) porphyrin dimers *via* laser induced vaporisation of the corresponding monomers and the suppression of dimerisation by nitrogen and phosphorus ligands. Careful adjustment of instrument parameters such as incident laser energy requires special attention. Relative affinities in complexation reactions are found to be consistent with liquid phase chemistry, therefore LDI-MS can provide a fast and simple, yet effective screening method for reactivity exploration. However, it also induces side-reactions such as iodine abstraction/transfer reactions. Furthermore, structurally different porphyrins show variations up to 90% in relative ion formation. Extreme caution is therefore required in the interpretation, and particularly in the quantitation, of such mass spectra.

Introduction

In this report we present a survey covering positive and negative ion time of flight mass spectrometry (TOF-MS) of free base porphyrins and metalloporphyrins embracing a wide range of non-covalent metal–ligand interactions. In the course of investigating supramolecular assemblies, where non-covalent interactions *via* the central metal of the porphyrin form the basic recognition mode, matrix assisted laser desorption ionisation (MALDI) as a soft ionisation technique has proved to be the method of choice for analysing multiporphyrinic assemblies. However, we also indicate some limitations of the technique.

Supramolecular chemistry has attained growing attention over the past decades.[1] The construction of large assemblies *via* non-covalent interactions is probably one of the most promising approaches for the near future in the design of functional entities.[2] Coordination chemistry, with its ability to form spontaneous self-assembled and well defined complex molecular entities in solution, adds to the diversity of architectural motifs available to supramolecular chemistry.[3] In particular, porphyrins which provide a huge structural variety through covalent modification on the periphery combined with complexation chemistry are gaining a key role in supramolecular science.[4] Parallel to the growing complexity of the obtained structures, powerful analytical techniques providing proof of the existence of non-covalent interactions (hydrogen bonds, π–π interactions, coordinate bonds, *etc.*), and thus confirming the existence of the supermolecule, are becoming more and more important.

Mass spectrometry has proven to be a very useful tool in analysis in parallel with classical spectroscopic methods (NMR, IR, UV/Vis spectroscopy). Electrospray ionisation (ESI) and fast atom bombardment (FAB) ionisation are among the most widely used soft ionisation techniques in examining supramolecular assemblies.[5] Limitations, however, arise from incompatibility of the analyte with the matrix or the solvent systems including solubility problems or interference of the protic solvent with hydrogen or coordinating bonds. In aprotic solvent systems, protonation of the analyte is not feasible, and an ion labeling strategy has to be employed.[6] Matrix assisted laser desorption/ionisation (MALDI) is one of the mildest ionisation techniques,[7] providing spectra across an essentially unlimited mass range, and showing little or no analyte fragmentation. Furthermore, the sample preparation could not be simpler: the analyte and the matrix are mixed in solution, spotted on the the probe plate and air dried; in the case of incompatibility with solvents, layered deposition can be used.

The advantage of MALDI-TOF MS in analysis of porphyrins compared to other techniques has been shown in several investigations,[8] and analysis has successfully been applied to covalently linked oligoporphyrins.[9] There have been few reported mass spectrometric sudies of porphyrin based assemblies,[10] and in these non-covalent interactions generally occur at the periphery of the porphyrin rather than *via* a central metal–ligand interaction. A recent study using ES-MS, where iron(III) and manganese(III) porphyrins displayed the most intense species having chloride or hydroxide axially ligated, seems to be the first example of detection of a central metal–ligand interaction,[11] together with earlier reports from our group.[12] The studies described here will aid in exploring appropriate conditions in mass spectral analysis of free base porphyrins and metallated porphyrins and their metal–ligand interactions. We also address the question of the degree of

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† Electronic supplementary information (ESI) available: mass spectrometric data for free base and metallo-porphyrins. See http://www.rsc.org/suppdata/dt/b0/b008638j/

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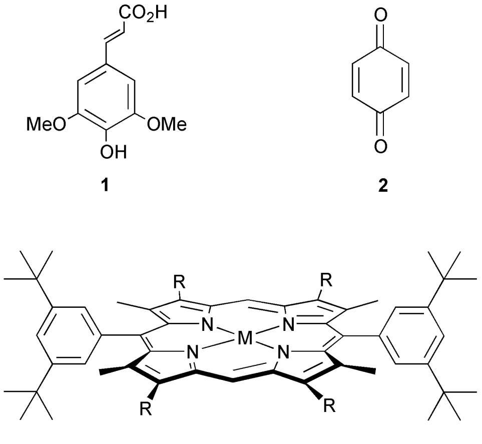
correspondence between the chemistry observed in the MALDI mass spectrometer and in solution.

Results and discussion

Is a matrix required?

The choice of a suitable matrix is generally crucial, and several groups have investigated different types of matrices (acidic, neutral, basic) for the measurement of non-covalent interactions.[9b,13] In some cases, severe side reactions of the analyte with the matrix were detected, *e.g.* imine hydrolysis[13a] and porphyrin demetallation.[8a,9b] Lindsey and co-workers[9b] pointed out that there is no fundamental need to add a matrix when analysing porphyrins, although addition of a neutral quinone matrix seemed to help prevent fragmentation in magnesium porphyrins. The advantage of a porphyrin is its reasonable absorbance in the wavelength of the N_2 laser (337 nm) with which the spectrometer is usually equipped to irradiate the analyte. In this case the analyte absorbs the laser energy sufficiently to give adequate spectra. Of course this feature is not only limited to porphyrins: any analyte absorbing in this wavelength can be measured in the absence of interfering matrices (*e.g.* metal complexes or phenyl substituted organic molecules). Porphyrins can therefore serve as their own matrix, and have themselves been used as matrices in MALDI-TOF MS.[14] Since the analytes are measured in the absence of a matrix the ionisation technique is usually referred to as LDI.‡ We have in some cases added either an acidic matrix (sinapinic acid, sin **1**, Scheme 1) or a neutral matrix (1,4-benzoquinone **2**) to the analyte to determine if there is an influence on the outcome of the spectrum, but in no case have seen any advantage. On the contrary, for oxidatively labile substituents on the porphyrin such as in phosphine **Ni-3**,[15] addition of **2** induced formation of a significant amount of phosphine oxide **Ni-4** upon sample preparation (Fig. 1), together with unknown side-products. With oxophilic metalloporphyrins ligand exchange reactions occurred between the analyte and **1**, giving rise to a molecular ion peak which consisted of the metal–ligand adduct of the porphyrin with the acidic matrix (see below).



1 **2**

H_2-6	R = ethyl, M = 2H	Mw = 854.00
Zn-7	R = hexyl, M = Zn	Mw = 1140.79
H_2-10	R = hexyl, M = 2H	Mw = 1078.87
Zn-11	R = ethyl, M = Zn	Mw = 916.54
Ni-12	R = hexyl, M = Ni	Mw = 1134.79

Scheme 1

Absence of a matrix, however, may give calibration problems, depending on the type of spectrometer used. For this reason we usually added simple porphyrins (Scheme 1) such as H_2TPP **H_2-5**,[16] H_2DPEP **H_2-6**[17] or ZnDPHP **Zn-7** to the analyte in order to be able to perform internal calibration. These internal calibrants, *i.e.* **H_2-5** and **H_2-6**, lack any functional group and did not interfere with the analyte. Indeed, any porphyrin, once fully characterised, can serve as internal calibrant. Addition of **1** and/or NaCl to a solution of **H_2-5**, **H_2-6** or **Zn-7** prior to sample deposition always showed the molecular ion peak in the positive or negative ion mode; uptake of H^+ or Na^+ (or loss of H^+) was not detected. Therefore we conclude that generally the radical ion $M^{\bullet +}$ or $M^{\bullet -}$ is formed rather than the protonated or deprotonated species.§ This confirms earlier reports,[9] although free base porphyrins were also reported to have been detected in the protonated form.[8a] Generation of the radical ion was observed for most of the compounds (free base and metallated porphyrins) to be discussed throughout this work, except in some cases where functional sidechains are capable of taking up a proton or alkali ions, *i.e.* carboxylic acids or ester functionalities. This is demonstrated in the mass spectrum of the cyclic trimer zinc(II) porphyrin **Zn-8**[19] bearing a total of 12 acid ester sidechains for solubility reasons (Fig. 2). Usually a broad range of mass peaks is detected, consistent with alkaline ion adducts and demetallated species. Addition of 18-crown-6 to the analyte did not suppress salt formation completely, but only one peak with $m/z = [M + 3Na]^{\bullet +}$ was observed instead. No inclusion complex was formed, so this additive did not interfere with the measurement but it simplified the spectrum significantly. Noteworthy is reduced demetallation of the sample upon addition of 18-crown-6, which might be attributed to suppression of traces of acid present in the analyte.

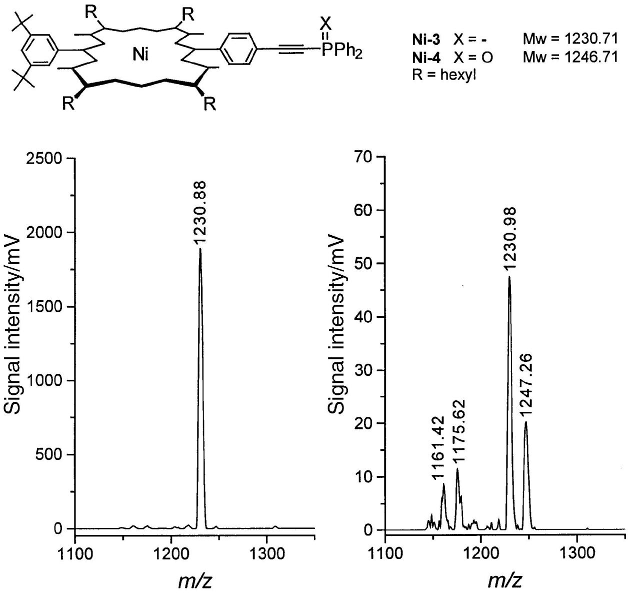


Fig. 1 LDI MS (+ve mode) of compound **Ni-3** as neat sample (left) and with **2** as matrix (right).

Dependence of signal intensity on structure

When measuring single free base porphyrins we observed a strong dependence of signal intensity on the nature of the porphyrin. For example, the relative abundance of positive ions (+ve) for a 1 : 1 mixture of **H_2-5** and **H_2-6** was **H_2-6** ≫ **H_2-5**. Measuring in the negative ion (−ve) mode reversed the relative intensity order. We subjected an equimolar mixture of **H_2-5**,

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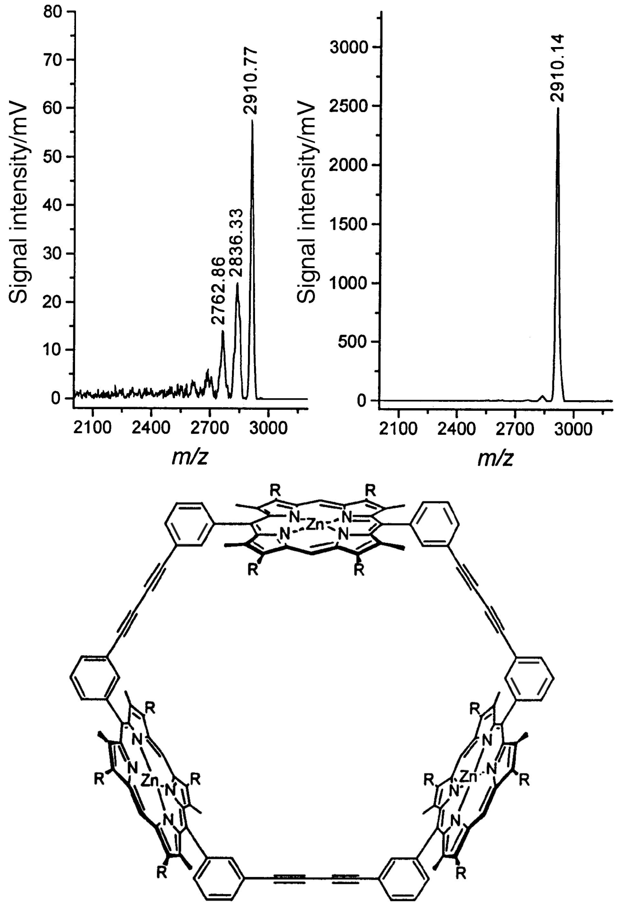
‡ MALDI as a soft ionisation technique usually shows little or no fragmentation of the analytes. The absence of a matrix did not detectably alter the mass spectral behaviour in this respect. We therefore suggest that LDI may also be regarded as being soft.

§ In metal complexes notation of the ionic species as a radical ion raises the question of whether a ligand centred radical ion or an oxidised/reduced metal species is present. Either is possible, depending on the porphyrin substitution pattern, the central metal and on additional ligands.[18] We therefore refer to the mass spectroscopic notation without specifying the location of the charge.



Zn-8 R = $CH_2CH_2CO_2Me$ Mw = 2841.88

Fig. 2 LDI MS (+ve mode) of compound **Zn-8** as neat sample (left) and with 18-crown-6 as additive (right).

H_2-6 and H_2OEP **H_2-9** as well as an equimolar mixture of H_2DPHP **H_2-10** (Scheme 1), ZnDPEP **Zn-11** and NiDPHP **Ni-12** to a positive ion LDI analysis. For the zinc porphyrin we chose the ethyl analogue instead of the hexyl substituted porphyrin (relative intensity difference ≈5%) in order to show a clearer picture of the differences in the three dimensional maps (see below). The positive ion LDI spectrum showed an order of relative intensity of **H_2-9** > **H_2-6** > **H_2-5** (Fig. 3b) and **Zn-11** > **H_2-10** > **Ni-12** (Fig. 4b).

In the case of the free base porphyrins, **H_2-9** showed no molecular ion peak whatsoever; the base peak corresponded to $[M - 56]^{\cdot +}$ indicating loss of a C_3H_7 fragment from the β positions of the porphyrin core. At higher incident laser energy further fragmentation occurred, with subsequent loss of CH_3 or CH_2 groups from the sidechains. This is the only example where we have observed severe fragmentation of a porphyrin at moderate laser powers. The nature of the alkyl sidechain on the β position of the porphyrin (ethyl *vs.* hexyl) of all other porphyrins had only minor influence on the relative amount of ion formation. Both the free base and the zinc porphyrins showed a relative peak ratio of 1:0.95 on changing from the corresponding ethyl to the hexyl sidechain.

The relative peak intensity is usually strongly dependent on the focus of the laser beam on the analyte (incident laser energy) and on the position on the sample plate.[20] We therefore performed an energy-dependent laser desorption/ionisation (EDLDI) experiment,[21] in which the laser beam was gradually moved over the whole range of the sample area with increasing focus (increasing laser energy) in each step. A three dimensional spectrum gained this way maps the mass spectra (*m*/*z* values and relative ion intensities) as a function of the incident laser energy, and these experiments could be performed reproducibly. Although the absolute ion intensity is highly dependent on the amount of analyte spotted on the sample plate, and on the number of laser shots used to obtain an averaged spectrum, the relative proportions always remained unchanged upon variation of these parameters. Figs. 3(a) and 4(a) show the respective three dimensional maps, and Figs. 3(c) and 4(c) show the graphs (in % of the basis peak intensity) of the *m*/*z* values of interest *vs.* incident laser energy. The relative intensities can directly be compared using the peak maxima of the graphs. In the case of the free-base porphyrins, the intensity of **H_2-5** dropped to about 5% of the peak intensity of **H_2-9**, whereas in the latter case the decrease in intensity on going from zinc to nickel porphyrins is about 40%. As can readily be seen from the graphs, the individual peak maxima are not reached at the same incident laser energy, and recording the spectra at a fixed laser energy leads to different relative intensities, depending on the laser energy chosen.

Limbach and co-workers[22] demonstrated a direct relation between formation of radical molecular cations *via* gas-phase charge-transfer reactions and the difference in ionisation energies between the matrix and the analyte. Porphyrins are known to undergo electron exchange reactions, and excited states can oxidatively or reductively be quenched by appropriate electron acceptors or donors, respectively. The ground state and the excited state redox potentials indicate that **H_2-5** ($E[P^+–{}^1P^*] = -0.67$, $E[P^+–{}^3P^*] = -0.24$, $E[{}^1P^*–P^-] = +1.05$, $E[{}^3P^*–P^-] = +0.62$ eV) could be reduced by **H_2-9** ($E[P^+–{}^1P^*] = -0.95$, $E[P^+–{}^3P^*] = -0.56$, $E[{}^1P^*–P^-] = +0.78$, $E[{}^3P^*–P^-] = +0.39$ eV) as well as by its zinc-analogue ZnTPP ($E[P^+–{}^1P^*] = -1.10$, $E[P^+–{}^3P^*] = -0.64$, $E[{}^1P^*–P^-] = +0.94$, $E[{}^3P^*–P^-] = +0.48$ eV).[18] This would explain the relative ratios observed in the +ve mode, whereas the peak intensity ratio of **H_2-5** to **H_2-9** is reversed in the −ve mode. More detailed investigations will be needed to show whether these results truly can be accounted for by the different redox properties of the excited states of porphyrins, and to what extent LDI-MS can serve to predict photochemical behaviour.

From this we conclude that a mixture of structurally similar porphyrins can directly and reliably be compared by recording an EDLDI mass spectrum. However, as the case of structurally dissimilar porphyrins proves, direct correlations to absolute amounts in the analyte are not possible from simple mass spectra. Comparison of relative intensities without the knowledge of the individual behaviour can strongly be misleading, unless a careful calibration has been undertaken.[23]

Oxophilic zirconium(IV) and tin(IV) metalloporphyrins

As mentioned above, the acidic matrix **1** was found readily to exchange ligands on higher valent, oxophilic metalloporphyrins such as $Sn^{IV}(PhCO_2)_2$(PyHP) **Sn-13**[24] and $Zr^{IV}(AcO)_2$(TPP) **Zr-14**[25] (Scheme 2). Of course, one would like to have information about the additional ligand necessary to neutralise the charge on the central metal. This information can be crucial when investigating differences in reactivity upon ligand exchange on the metal. Many metalloporphyrins cannot readily be analysed by NMR spectroscopy due to the presence of a paramagnetic central metal. Here, mass spectrometry might provide an alternative method for analysis.

The MALDI-TOF mass spectrum of compound **Sn-13**, when measured using **1** as matrix, showed a molecular ion peak at *m*/*z* = 1532.10, consistent with loss of benzoic acid from the complex and formation of the bis-**1** complex $[M - 2\,PhCO_2 + 2\,\mathbf{1}]^{\cdot +}$, together with the base peak identified as the mono-**1** complex. Measuring **Sn-13** as a neat sample in the +ve mode showed only the hydrolysed species $[M - 2PhCO_2 + 2\,OH]^{\cdot +}$ and $[M - 2\,PhCO_2 + OH]^{\cdot +}$, whereas in the −ve mode the molecular ion peak could be detected. Similarly with **Zr-14** the best results were obtained by subjecting a neat sample to LDI-MS (−ve mode). It is noteworthy that for **Zr-14** the molecular ion peak could also be detected in the positive ion mode as

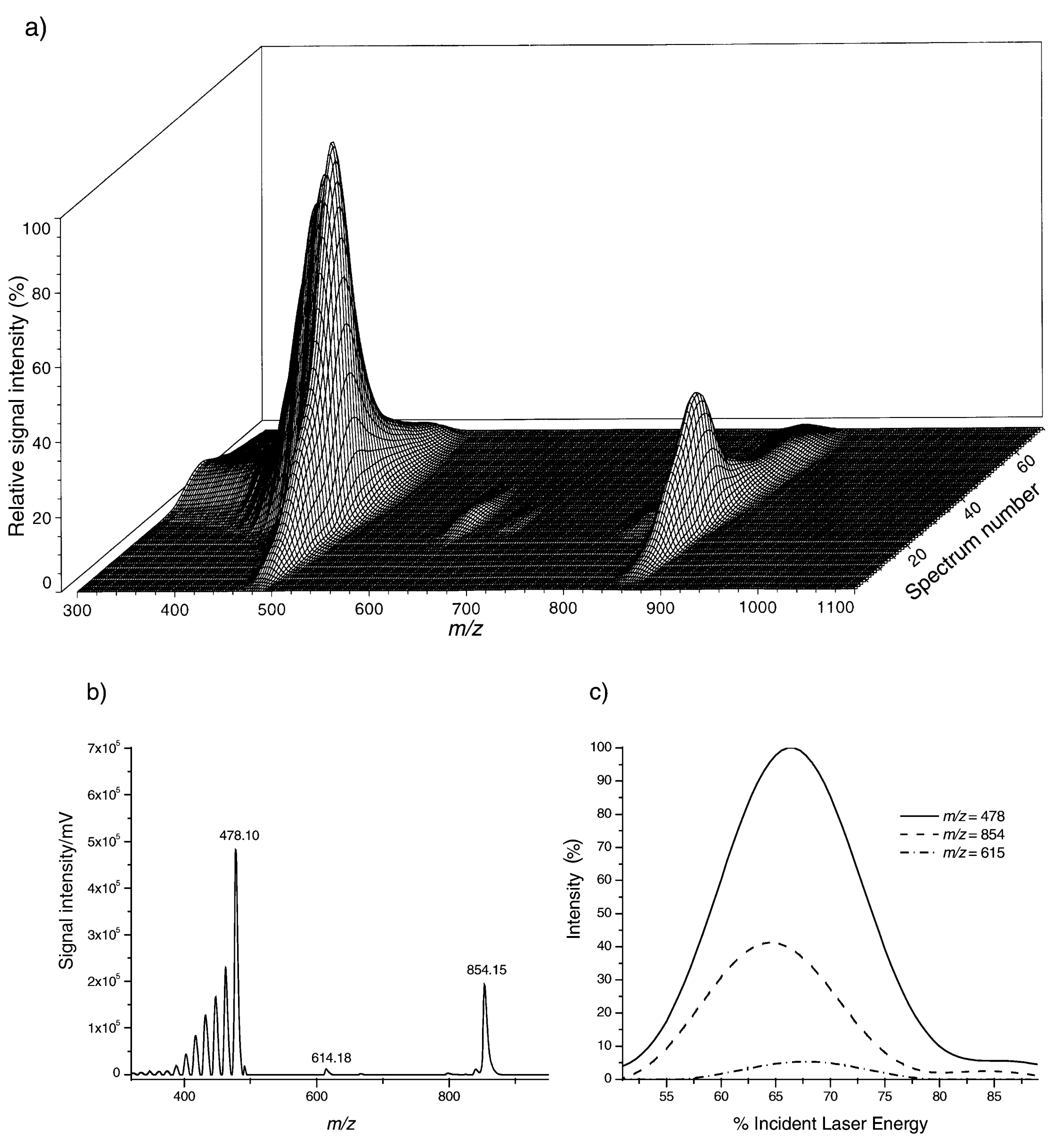
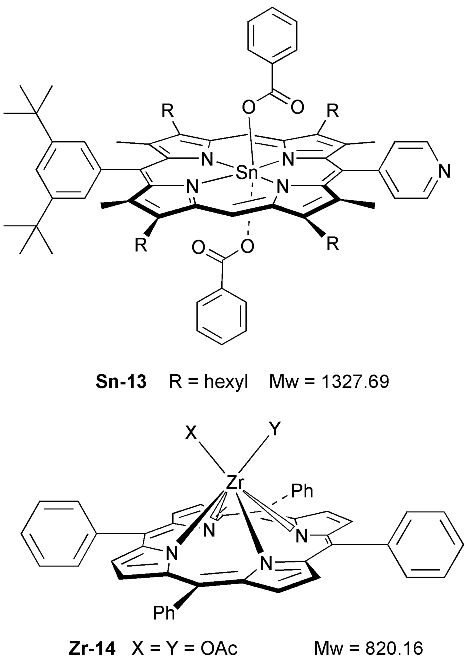


Fig. 3 EDLDI MS of an equimolar mixture of compounds H_2-**5**, H_2-**6** and H_2-**9**; +ve mode. (a) Three dimensional spectrum obtained by recording successive mass spectra with increasing laser energy. (b) Mass spectrum averaged over all data. (c) Extracted intensity profiles as a function of the incident laser energy (in % of maximum laser power).



Sn-13 R = hexyl Mw = 1327.69

Zr-14 X = Y = OAc Mw = 820.16
Zr-16 X + OAc, Y = Cl Mw = 798.39

Scheme 2

a small peak. Tin(IV) porphyrins always showed the most intense mass peak for $[M - L]^{\cdot +}$ in either positive or negative ion mode. It seems most appropriate for these kind of metalloporphyrins to be analysed in the negative ion mode as neat samples.

The ability to detect reactivity in LDI mass spectra prompted us to perform a simple experiment using disodium phenyl phosphate **15** as substrate. Mixing a 1 : 1 molar ratio of compound **Zr-14** with **15** prior to analysis did not alter the mass spectrum. If the monochloride derivative Zr^{IV}(AcO)(Cl)(TPP) **Zr-16** is used instead, formation of mononuclear and dinuclear phosphate complexes (with intact and hydrolysed phosphate ester) is immediate, as depicted in Fig. 5. **Sn-13** (as well as its dihydroxy analogue) did not show formation of any complexes with **15**. The reactivity of **Zr-16** towards **15** is consistent with solution phase chemistry, where the occurrence of mono- and di-nuclear complexes has been demonstrated by ^{31}P NMR spectroscopy and X-ray crystallography; formation of complexes proved to be dependent on the counter ion of the zirconium porphyrin.[25] **Sn-13** showed no affinity towards **15** in solution under identical experimental conditions according to ^{31}P NMR spectroscopy.

Ruthenium(II) porphyrins

For a range of ruthenium(II) porphyrins prepared in this

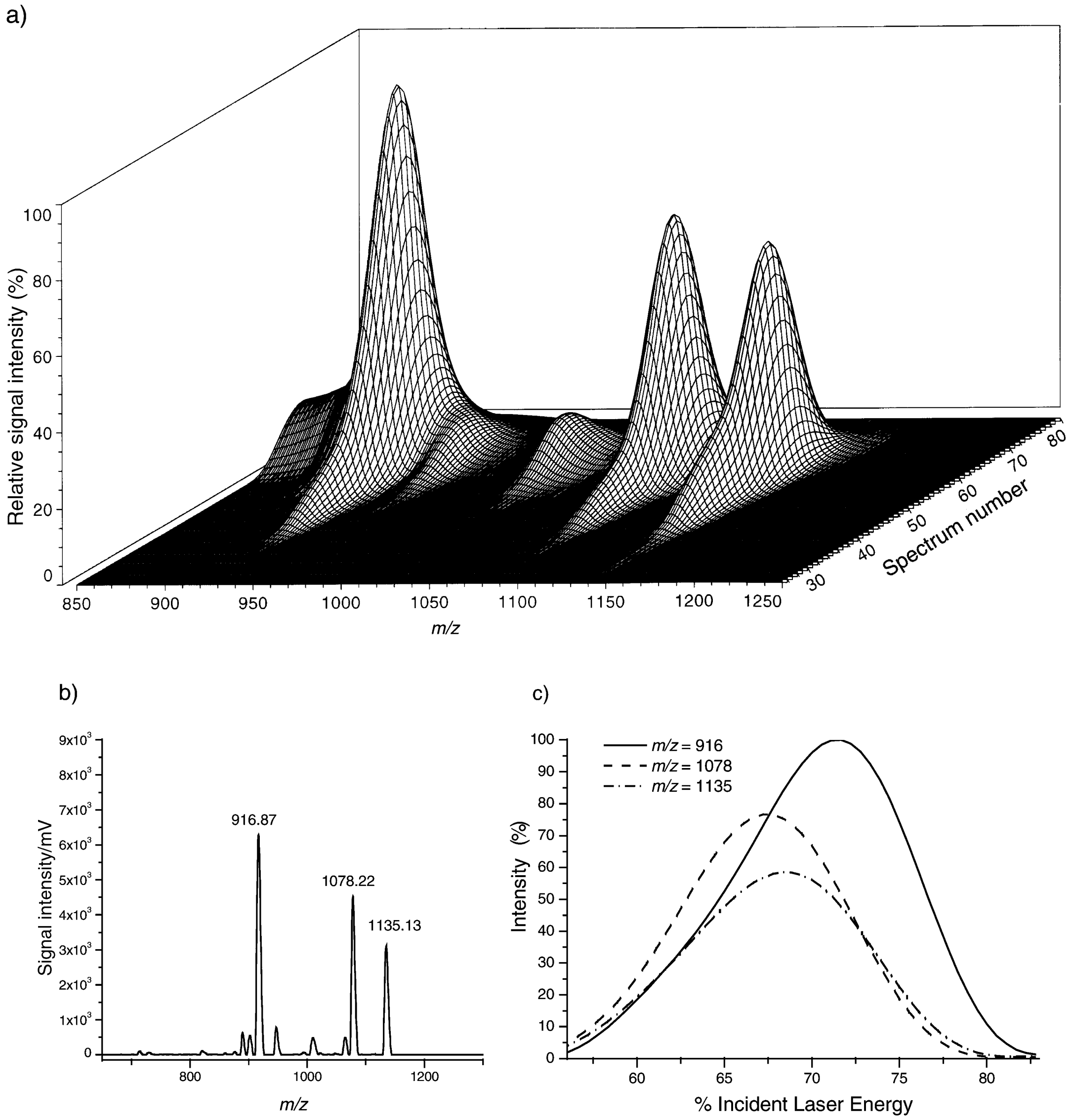


Fig. 4 EDLDI MS of an equimolar mixture of compounds **H_2-10**, **Zn-11** and **Ni-12**; +ve mode. Details as in Fig. 3.

laboratory, and embracing a wide range of structural features (Scheme 3), we did not observe intact molecular ion peaks in LDI mass spectra. All of these compounds carry a CO molecule to stabilise the low oxidation state *via* π-back bonding and prevent formation of oxo-complexes. It has been noted previously that UV laser irradiation leads to CO ejection, providing mass spectra that consistently exhibit fragment peaks at $m/z = [M - CO]^{\bullet +}$ instead of a molecular ion peak.[26] We have also always observed a major peak at $m/z = [M - CO]_2^{\bullet +}$, which corresponds to the formation of a dimer with loss of CO, and is consistent with direct Ru–Ru bonding. Ruthenium porphyrin dimers containing direct metal–metal double bonds have been synthesized by solid-state vacuum pyrolysis of ruthenium(II) bis(pyridine) porphyrin precursors[27] and by photolytic ejection of CO from $Ru^{II}(CO)$ porphyrins in solution[28] or in Langmuir–Blodgett films.[29] They are extremely sensitive to nucleophiles (to yield axially coordinated monomers) and to molecular oxygen (giving μ-oxo dimers).[27]

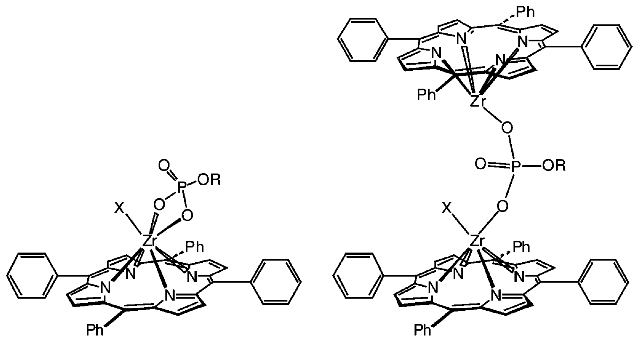
Since ruthenium(II) porphyrins are weakly oxophilic, oxygen-containing functionalities as found in **Ru-17** (Scheme 3) could lead to dimers or higher aggregates *via* intermolecular coordination. However, **Ru-18**–**20** which lack any coordinating functional groups also dimerise under such conditions. Oligomers containing ruthenium(II) porphyrins display the same dimerisation behaviour when subjected to MALDI-TOF analysis; this is a useful tool for molecules which are otherwise difficult to characterise structurally.[30] No dimerisation was observed in control LDI experiments with the zinc(II), nickel(II) or free base analogues of any of these porphyrins.

An EDLDI experiment showed clear correlation between the monomer to dimer ratio and incident laser energy. It did also reveal that the degree of dimerisation is dependent on the porphyrin structure. This is demonstrated in Fig. 6, which shows plots of relative peak intensity *vs.* laser energy for compounds **Ru-18**–**20**. It is surprising that **Ru-20**, which is sterically the most hindered porphyrin, displayed the largest amount of dimer formation. This suggests that not only possible metal–metal bond formation, but also other intermolecular forces such as van der Waals forces (*e.g.* between the *tert*-butyl groups), favour dimerisation in the gas phase. The isotopic pattern of the spectrum of **Ru-18** is consistent with the calculated spectrum for the radical ion of the monomer $[M - CO]^{\bullet +}$ as well as for the dimer $[M - CO]_2^{\bullet +}$ (Fig. 7). It can thus be concluded that no dicationic species $[M - CO]_2^{2+}$ are formed, proving the existence of a monomeric and a dimeric species in the gas phase.

As a control to satisfy the Smith criteria that a mass spectrometric observation is 'real',[31] we examined a mixture of the three porphyrins **Ru-21**–**23** (Scheme 3). The expected three monomer ions were observed and are accompanied by peaks corresponding to the five masses of all possible homo- and hetero-dimers (Fig. 8). This experiment confirms that the species observed are real entities rather than electronic artefacts. Similar arguments have been used to demonstrate the reality of non-covalently assembled dimers observed in electrospray and other mass spectra.[31,32]

Recording the spectra in the reflectron mode using an ion gate set in the dimer region (mass range $\Delta m/z = 50$ for the peak of interest) always also showed peaks for the monomer along with the dimer peak. If the ion gate was set in the monomer region no dimer could be detected. It seems therefore likely that dimerisation occurs in a very early state of vaporisation/ionisation, and that reactions other than disproportionation at a later stage are not probable.

Our studies show no influence on the degree of dimerisation using either an acidic or neutral matrix or measuring neat samples. However, we have found that addition of potential axial ligands to ruthenium(II) porphyrins leads to complex formation and dimer suppression (Fig. 9). This observation was most thoroughly investigated using porphyrin **Ru-18**. Addition of DMAP (4-*N*,*N*-dimethylaminopyridine) to a solution of **Ru-18** resulted in a substantial decrease in the proportion of dimer in the spectra. A minimum of 10 equivalents of DMAP was required to detect two additional peaks which were assigned to mono-DMAP and bis-DMAP complexes (Fig. 9);



Zr-16 - Cl Mw = 761.15
X = - , R = - Mw = 797.09
X = OAc, R = Ph, +Na Mw = 897.12

X = - , R = - Mw = 1499.23
X = OAc, R = Ph, +Na Mw = 1659.27

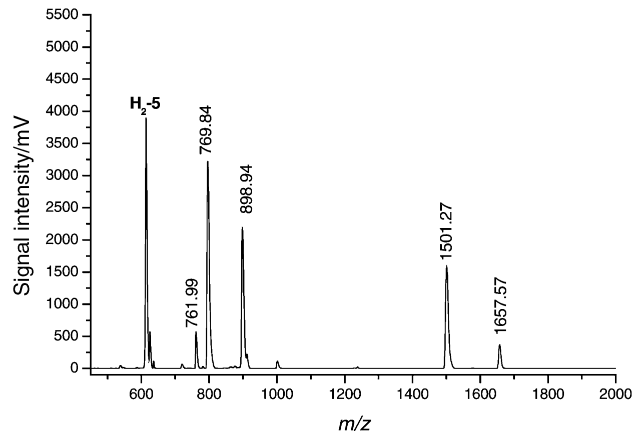
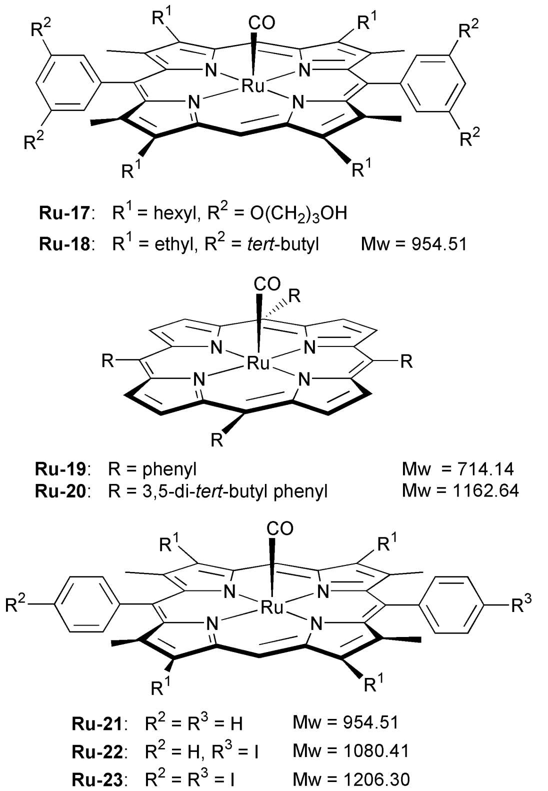


Fig. 5 LDI MS (−ve mode) of compound an equimolar mixture of compound **Zr-16** and disodium phenyl phosphate **15**.



Ru-17: R^1 = hexyl, R^2 = $O(CH_2)_3OH$
Ru-18: R^1 = ethyl, R^2 = *tert*-butyl Mw = 954.51

Ru-19: R = phenyl Mw = 714.14
Ru-20: R = 3,5-di-*tert*-butyl phenyl Mw = 1162.64

Ru-21: $R^2 = R^3$ = H Mw = 954.51
Ru-22: R^2 = H, R^3 = I Mw = 1080.41
Ru-23: $R^2 = R^3$ = I Mw = 1206.30

Scheme 3

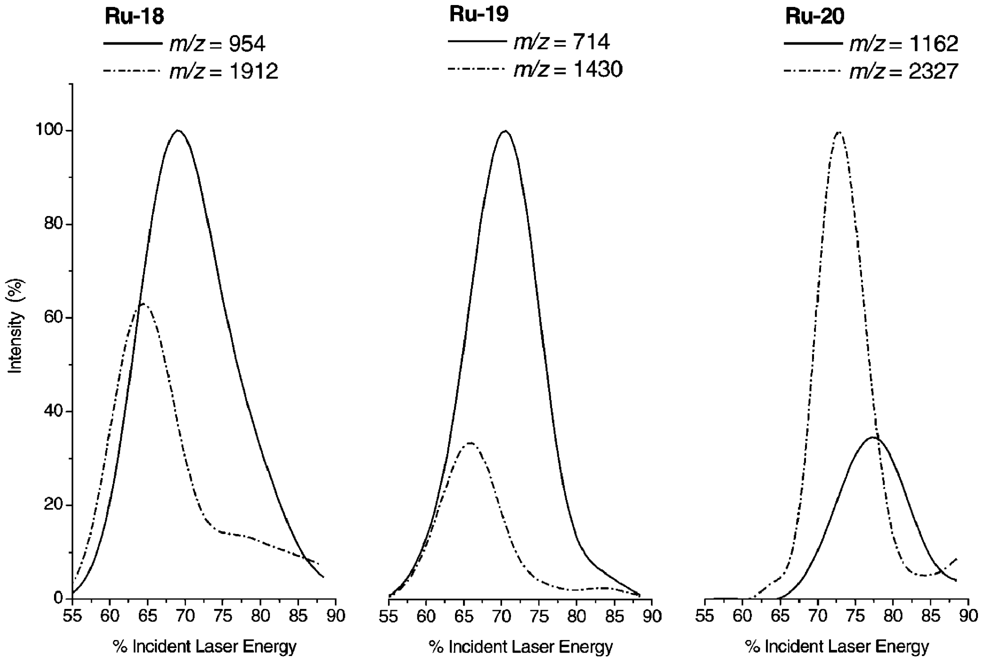


Fig. 6 Relative peak intensity *vs.* incident laser energy (in % of maximum laser power) for ruthenium(II) porphyrins obtained from EDLDI MS; +ve mode. Straight line, monomer; dotted line, dimer. For structures see Scheme 3.

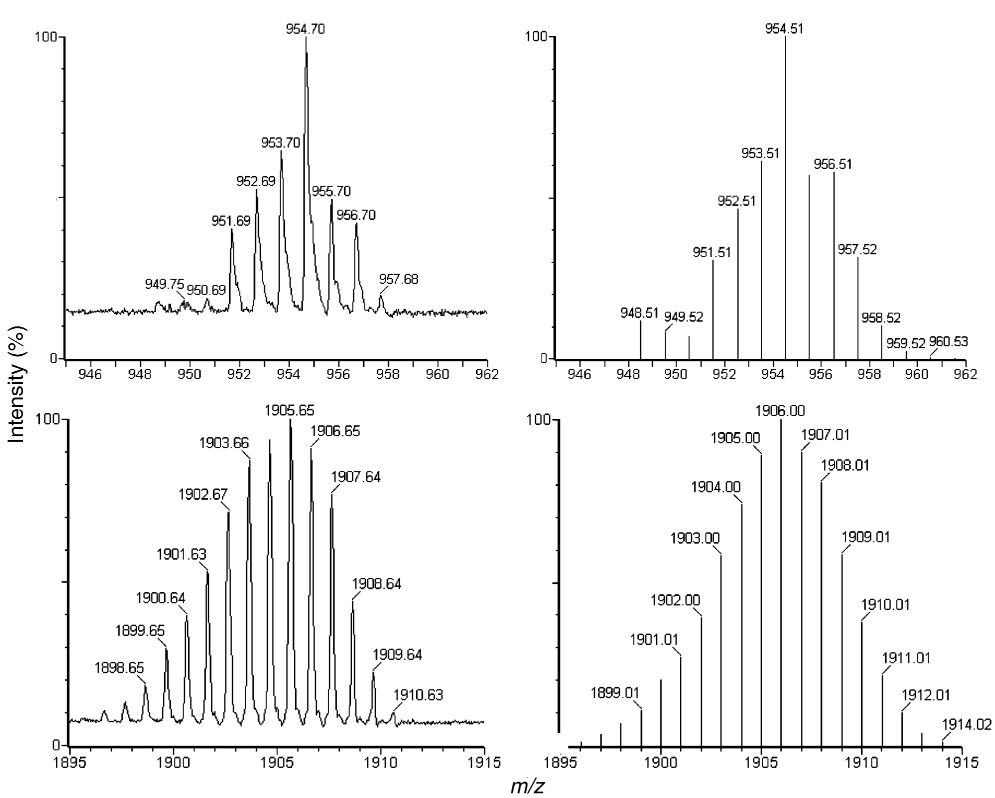


Fig. 7 Left side: isotopic distribution (LDI MS, +ve mode) of compound **Ru-18**. Right side: calculated for $[\mathbf{Ru\text{-}18} - CO]^{\bullet+}$ $C_{60}H_{76}N_4Ru$ (top) and $[\mathbf{Ru\text{-}18} - CO]_2^{\bullet+}$ $C_{120}H_{152}N_8Ru_2$ (bottom).

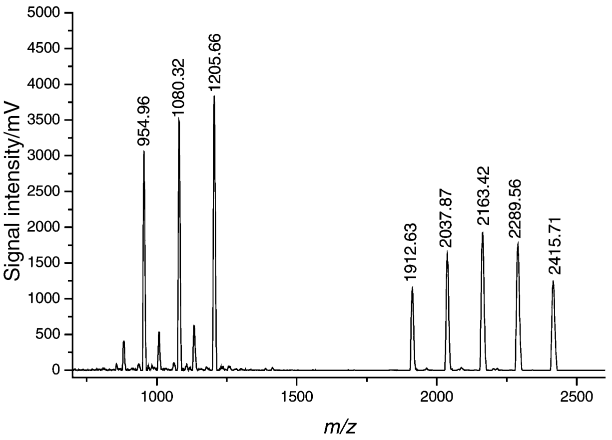


Fig. 8 LDI MS (+ve mode) of an equimolar mixture of compounds **Ru-21**, **Ru-22** and **Ru-23**.

ratios and relative intensities of all signals did not change up to 100 equivalents of DMAP added. With 4,4′-bipy or DABCO no adducts could be detected.

Mixing a solution of compound **Ru-18** with 1, 5 or 10 equivalents PPh_3, PFu_3 (Fu = 2-furyl) or DPPA [1,2-bis(diphenylphosphino)acetylene] prior to recording a spectrum resulted in significant suppression of dimer formation. After addition of 10 equivalents PFu_3 some dimerisation of **Ru-18** was still detectable, whereas addition of 5 equivalents of PPh_3 was sufficient to suppress the dimer almost completely. The PFu_3 complex always showed fragmentation, the base peak corresponding to $[M - 23]^{+\bullet}$ and a small peak at $[M - 47]^{+\bullet}$; these fragments were not identified. One equivalent of DPPA had the same effect as 5 equivalents of PPh_3, and addition of 5 equivalents of DPPA completely suppressed dimerisation at moderate laser powers. Again additional peaks were detected and assigned to ruthenium(II) porphyrin phosphine adducts. A much smaller amount of phosphine ligand is required to suppress dimerisation and formation of detectable adducts compared with DMAP, and the relative amount of complex increased with increasing amount of phosphine added. Despite the fact that DPPA has two potential binding sites, no higher aggregates could be detected. Also, in mixtures containing **Ru-18**, PPh_3 and PFu_3 no mixed-ligand species like $(PPh_3)(PFu_3)$**Ru-18** were detected (Fig. 9, bottom), which is consistent with ^{31}P NMR experiments.[15] Competition experiments using

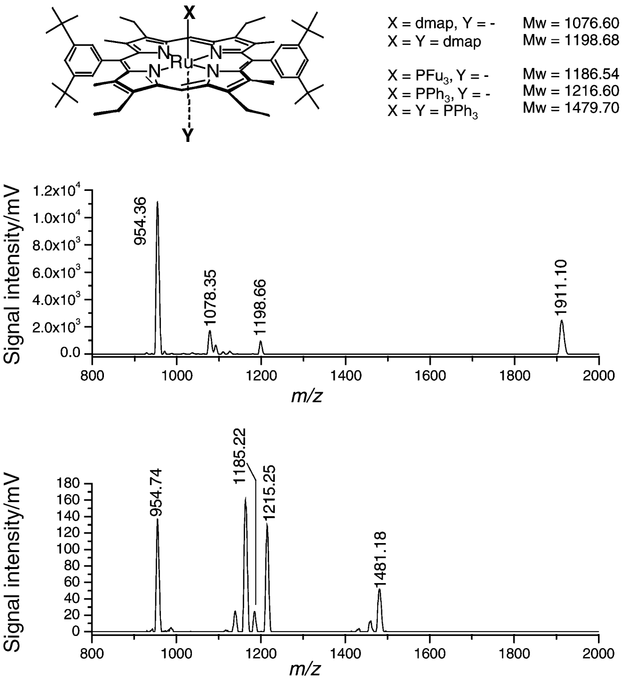


Fig. 9 Top: LDI MS (+ve mode) of a 10 : 1 mixture of DMAP and **Ru-18**. 100 laser shots per signal averaged spectrum at 50% of maximum laser power. Bottom: LDI MS (+ve mode) of a 5 : 5 : 1 mixture of PPh_3, PFu_3 and **Ru-18**. 20 laser shots per signal averaged spectrum at 70% of maximum laser power.

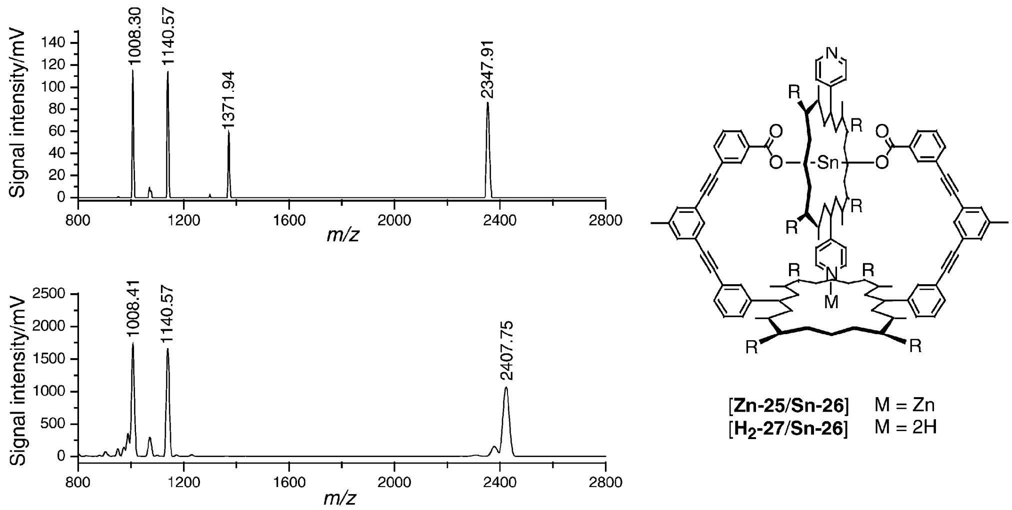


Fig. 10 Top: LDI MS (+ve mode) of a 1.2 : 1 mixture of **Sn-26** ([OH]$_2$**Sn-26**: *M* = 1008.51) and **H$_2$-27** (*M* = 1371.76); **[H$_2$-27/Sn-26]** *M* = 2344.25. Bottom: LDI MS (+ve mode) of a 1.2 : 1 mixture of **Sn-26** and **Zn-25** (*M* = 1433.68), **[Zn-25/Sn-26]** *M* = 2407.15. **Zn-7** (*M* = 1140.79) was added as internal calibrant.

mixtures of DMAP, PPh$_3$, PFu$_3$ and DPPA revealed a relative affinity order DMAP < PFu$_3$ < PPh$_3$ < DPPA in the LDI mass spectra, which also is consistent with the observed affinities in solution.[15] It seems likely that addition of a potential ligand L suppresses the reaction (1) in favour of (2).

$$[\mathrm{Ru(porph)}]^{\bullet+} + [\mathrm{Ru(porph)}] \longrightarrow [\mathrm{Ru(porph)}]_2{}^{\bullet+} \quad (1)$$

$$[\mathrm{Ru(porph)}]^{\bullet+} + \mathrm{L} \longrightarrow [\mathrm{Ru(porph)(L)}]^{\bullet+} \quad (2)$$

Preformation of (dppa)$_2$**Ru-18** showed the occurrence of some Ru–Ru dimer at higher incident laser power; the bisphosphine complex could not be detected. On the other hand, N-donor ligands themselves are not capable of displacing CO from ruthenium in solution, but a bis-DMAP adduct could be observed. This suggests that dimerisation and ligand complexation/decomplexation is a gas phase reaction, presumably taking place in the dense plume of the analyte above the sample plate immediately after desorption/ionisation and CO displacement. Although there is no *direct* evidence for formation of a metal–metal bond in these porphyrin dimers, the results presented here combined with precedent solution-state investigations strongly indicate that such bonds are indeed formed.

Supramolecular assemblies

The observations described so far prompted us to examine the behaviour of non-covalent multiporphyrin arrays under LDI conditions. First, we measured a mixture of the compounds H$_2$TPyP **H$_2$-24** and **Ru-18**. A 1 : 4 ratio of **H$_2$-24** and **Ru-18** showed little formation of a **[H$_2$-24/Ru-18]**$^{\bullet+}$ complex, and no higher aggregates could be detected. This is in contrast to solution chemistry, where this mixture usually leads to formation of a tetrameric complex **[H$_2$-24/4Ru-18]**,[30] but this may reveal the weak nature of the Ru–N bond in the gas phase. A 1 : 1 mixture of **Ru-18** with a phosphine substituted nickel(II) porphyrin **Ni-3** showed clear formation of a 1 : 1 complex of composition **[Ru-18/Ni-3]**$^{\bullet+}$, together with complete suppression of the ruthenium dimer.[15] Analogous to the reactivity of DPPA (see above), no bis-phosphine complex was observed, even at lower **Ru-18** to **Ni-3** ratios.

The successful measurement of the molecular ion peak of the coordination assembly **[Zn-25/Sn-26/Ru-18]** by LDI mass spectrometry has briefly been reported earlier.[12] This complex provides an example of how a mass spectrum is influenced by co-operativity. Fig. 10 shows the LDI mass spectra of the complex **[Zn-25/Sn-26]** and that of the free base analogue **[H$_2$-27/Sn-26]**. In the former case, where three cooperative coordination bonds are present in the complex, no free zinc porphyrin is visible, the assembly remaining intact in the gas phase (free tin porphyrin is due to a 0.2 equivalent excess present in the analyte). With the free base analogue a significant amount of the free base dicarboxylate porphyrin is detected, together with the molecular ion peak of the assembly. Again, the enhanced stability of the zinc containing complex is consistent with ^{1}H NMR studies. Thus it can be shown by LDI mass spectrometry that the overall geometry of the assembly is favourable in terms of providing access to an additional complexation site to **Sn-26**. If the geometry of the **Zn-25** moiety would be such that the pyridyl substituent of the **Sn-26** moiety could not bind to the zinc (either the zinc porphyrin is too far away, or the cavity is too small to allow the pyridyl group to turn towards the zinc porphyrin), no significant influence on the outcome of the mass spectra would be expected.

Laser-induced iodine transfer

An analogous assembly to the one just described is the monocarboxylate zinc porphyrin–tin porphyrin couple **[Zn-28/Sn-26]**,[12] where the zinc moiety bears an iodoaryl substituent. Recording an LDI spectrum of **[Zn-28/Sn-26]** (*M* = 2275.00) showed the expected molecular ion peak at *m*/*z* = 2274.53 (Fig. 11), along with a peak at *m*/*z* = 2148.72 corresponding to the fragment with loss of one iodine [(**Zn-28** − I)/**Sn-26**]$^{\bullet+}$ (M = 2148.09); formation of trimeric arrays was not observed. This is by itself not striking, since the aryl–iodine bond is known to be cleavable by photolysis;[33] this is also visible from the fragment peak of the zinc moiety with loss of iodine. What was more surprising was the observation of a peak at *m*/*z* = 2406.88 corresponding to [(**Zn-28/Sn-26**)+I]$^{\bullet+}$ (*M* = 2402.74) where uptake of an additional iodine occurred at the tin moiety. This is concluded from the observation of a strong peak at *m*/*z* = 1101.07, corresponding to the iodotin porphyrin complex (*M* = 1101.40). If the free base–Sn couple **[H$_2$-29/Sn-26]** (*M* = 2213.08) is subjected to an LDI measurement instead, almost no uptake of iodine is observed in the array, as displayed in Fig. 11. Also notable is the relative amount of dissociation compared to that of **[Zn-28/Sn-26]**. Performing LDI experiments using the tin moiety together with either a diiodo free base porphyrin or with its zinc metallated analogue revealed no iodine transfer in the former case, but significant iodine transfer in the latter. These experiments allow us to conclude that, upon desorption/ionisation of the analyte, iodine is easily lost by laser irradiation, and, if the iodine porphyrin is in close

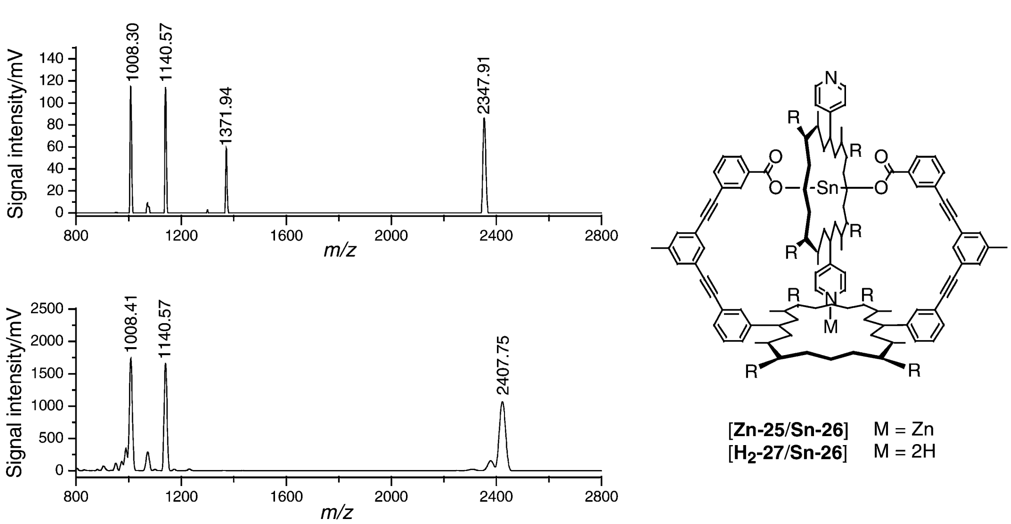


Fig. 11 LDI MS (+ve mode) of iodine containing assemblies. Top: **[Zn-28/Sn-26]**. Bottom: **[H_2-29/Sn-26]**. $(OH)_2$**Sn-26**: $M = 1008.51$, **Zn-28**, $M = 1300.50$, **H_2-29**, $M = 1238.59$. For peak assignments see text.

proximity to the tin porphyrin, this iodine is transferred to the Sn in an intramolecular process. Complexation *via* zinc seems to be essential, since with the free base analogues iodination of Sn is observed to a much lesser extent, and in the uncomplexed assembly is not observed at all. Additional uptake of iodine in **[Zn-28/Sn-26]** can therefore only be explained by a dissociation/reassociation process of the assembly in the gas phase after iodine transfer. Whether these observations may lead to an useful synthetic application needs to be demonstrated.

Conclusion

In this report we have demonstrated that LDI-TOF MS provides a useful tool for analysing non-covalent bonds in supramolecular metalloporphyrin arrays. Careful adjustment of the instrument parameters such as incident laser energy, positive or negative ion detection and the number of laser shots to obtain an averaged spectrum allows detection of labile metal–ligand complexes, and the gas phase affinities compare well to those of solution phase chemistry. We believe that this technique will not only be extremely versatile in studying porphyrinic arrays, but will be expanded to a wider range of supramolecular assemblies with the aim of obtaining preliminary results in the exploration of reactivity. Addition of interfering matrices will not be necessary since metal complexes usually show strong absorbance in the wavelength of the laser used; on the other hand, easily accessible simple porphyrins such as as H_2TPP **H_2-5** can be used as a matrix themselves. It is also possible to obtain structural information from the mass spectra, but these should be compared with spectroscopic data, whenever possible, to be conclusive.

Experimental

Materials

Sinapinic acid **1**, 1,4-benzoquinone **2** and disodium phenyl phosphate **15** were obtained from Aldrich and used without further purification. H_2OEP **H_2-9** was purchased from Strem Chemicals. The syntheses of the porphyrins were reported earlier; references are given in the text.

Sample preparation and mass spectra recording

All porphyrin solutions were prepared using freshly distilled acid free $CHCl_3$ (stored over Na_2CO_3). Reference solution for internal calibration: 2.0 mg **H_2-5** + 0.5 mg **H_2-6** or **Zn-7** in 2.0 ml $CHCl_3$.

Analyte preparation. The porphyrin samples were prepared as a 1 mM solution in $CHCl_3$. 2 to 5 µl of a 1 : 1 mixture of sample solution and reference solution were subsequently deposited on the sample plate surface and air-dried. Results from 20, 50 or 100 laser shots were applied to one target area to give one spectrum; 50 to 80 individual spectra, recorded from different areas of the analyte deposition sphere, were signal averaged to obtain a final spectrum. The incident laser energy and the amounts of laser shots per spectrum were individually adjusted for each system.

Instrumentation

LDI-TOF mass spectra were recorded on a Kompact MALDI 4 mass spectrometer (Kratos Analytical Ltd). A nitrogen laser (337 nm, maximum output 6 mW, 3 ns pulse width, target area diameter ≈100 µm) was used to desorb the sample ions. Incident laser energy was varied using the internal optical lens system. The instrument was operated in linear time of flight mode (unless otherwise stated) with an accelerating potential of 20 kV. Calibration was performed after final averaging of the spectra using the peaks corresponding to Na^+, K^+, **[H_2-5]**$^{\cdot +}$ ($C_{44}H_{30}N_4$) and **[H_2-6]**$^{\cdot +}$ ($C_{60}H_{78}N_4$) or **[Zn-7]**$^{\cdot +}$ for +ve recording, or **[H_2-5]**$^{\cdot -}$ ($C_{44}H_{30}N_4$) and **[H_2-6]**$^{\cdot -}$ ($C_{60}H_{78}N_4$) for −ve recording.

The isotopic resolution LDI MS of compound **Ru-18** was recorded using a Micromass (Wythenshawe, Manchester, UK) TofSpec-2E mass spectrometer, fitted with a time-lag focusing source. Data were generated in the reflectron mode of operation at an accelerating voltage of 20 kV. A mixture of three peptides (Bradykinin, Angiotensin and Adrenocorticotropic hormone) was employed for calibration of the instrument.

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