

PHOTOIONIZATION OF JET COOLED MOLECULES AND CLUSTERS

Photo-ionization provides a forum of hyphenation for mass spectrometry since one can vary both wavelength and intensity of the light. These parameters afford opportunities for selective ionization, not only off specific compounds or classes of compounds, but off structural isomers. In favorable cases the spectroscopy even distinguishes tautomeric and conformational isomers. Furthermore fragmentation pathways in photo-ionization can differ from those in other ionization methods, providing additional structural information. To fully exploit these features of photo-ionization the vibrational and rotational temperatures should be reduced by entertainment in a supersonic jet. For non-volatile thermally labile compounds this requires a special source that combines laser desorption with a pulsed nozzle.

1. Jet cooling

Expanding a high-pressure gas through a small hole into a low pressure decreases its internal temperature. The translational temperature decreases because the velocity spread (and thus the spread in kinetic energy) narrows, even though the most probable velocity increases (see figure 1). For molecular compounds the increase in average kinetic energy parts it comes at the expense of the vibrational and rotational energy. When a heavy molecule is mixed in low concentration, or seeded, in a light drive gas, the heavier component attains almost the same velocity distribution as the lighter one. The Mach number is inversely proportional to the final temperature and increases with $P_0 d$, the product of stagnation pressure and nozzle diameter. Therefore the lowest temperatures are obtained with the largest nozzle diameters and pressures, and thus with the largest gas load. Thus pumping speed constitutes a limitation, necessitating either differential pumping or reduction of the duty cycle with a pulsed nozzle. The latter solution is quite compatible with laser desorption (1-3).

The advantages of jet cooling are threefold:

1. Cooling improves spectroscopic resolution
2. Cooling reduces fragmentation
3. Cooling provides a pathway for cluster formation

2. Laser desorption

The process of neutral laser desorption is very poorly understood. The general idea is that fragmentation is minimized when the time scale for heating is shortened. The rates required to desorb molecules without fragmentation are of the order of 10^{11} K/sec which corresponds to a 1000 K temperature jump in a typical 10 ns laser pulse. This observation was discussed by Hall in terms of Arrhenius type rates $k = C \cdot P \cdot \exp(-E/RT)$ in which E represents an activation energy, P the pre-exponential factor and C the surface coverage(4). T is the temperature and R the Boltzmann constant. When both E and P are larger for desorption than for thermal chemistry, desorption will dominate at higher

temperatures. Thus fragmentation can be minimized by a high heating rate. Zare and Levine reached a similar conclusion with a “bottleneck” model, in which the laser energy excites the phonons of the surface, which reasonably match the frequency of the bond between the molecules and the surface, whereas the frequencies of the molecular vibrations are much higher (5). Li et al. performed an elegant set of experiments on model surfaces to investigate the effect of the temporal profile of the thermal pulse on desorption (6). Zenobi et al. have studied the role of surface temperatures in laser desorption and the energy distribution of desorbed aniline as a model molecule (7-9).

Combining laser desorption with jet-cooling requires careful optimization of the geometry. One approach is to desorb on the high pressure side of a pulsed valve, for which one can design an extension with a small volume in front of the valve (10, 11). However, desorbing on the low pressure side outside of the valve requires much less material and thus makes the technique much more sensitive. The idea is that the desorbed material must be entrained in the jet as a small cloud, such that lasers downstream can interact with as much of the desorbed material as possible. Arowsmith et al. have optimized the entrainment geometry by mapping laser induced fluorescence profiles of entrained perylene (3). One of the key parameters to optimize is the distance between sample surface and nozzle opening. Meijer et al. explored the characteristics of the technique to optimize cooling and overall efficiency (12). In the most favourable case of unit ionization efficiency one can obtain an overall detection efficiency of 10^{-6} . Jet-cooling takes place within 10 nozzle diameters, so it is important to desorb as close to the nozzle as possible. By analyzing rotational profiles one can show in the case of anthracene entrained in He that a rotational temperature of less than 15 K is attainable. Larger molecules require heavier drive gasses, such as Ar and even Xe, to maximize cooling. Molecular drive gasses, such as CO₂ and SF₆, generally improve cooling but are less desirable because they also tend to promote excessive cluster formation (13).

Ideally the desorption laser wavelength must be chosen to minimize photochemistry of the analyte, while maximizing heating. A good combination is the fundamental of a YAG laser with graphite as a substrate, although UV wavelengths with a metal substrate can be used as well. The sample should be moved to expose fresh sample in subsequent shots. One can use a translating (3) or rotating rod (14) or a rotating wheel (15). (A rotating rod puts most constraints on the geometry). For best shot-to-shot stability - particularly for spectroscopic applications - uniformity of the sample layer is important. Although a matrix is usually not necessary to effect desorption, it is helpful to mix the sample with graphite powder, when using a graphite substrate.

3. Single photon ionization

Single photon ionization requires a photon with an energy larger than the vertical ionization potential (IP). This implies partial selectivity since radiation at frequency ν only ionizes compounds with $IP < h\nu$. Many aromatic compounds have ionization potentials of the order of 7-8 eV, while many aliphatic compounds have ionization potentials of the order of 9-10 eV. This approach thus requires the VUV light. To universally ionize most compounds thus requires about 10 eV photons, corresponding to

approximately 125 nm. A routine technique for this purpose is frequency tripling in Xe of the third harmonic from a Nd:YAG laser, producing light at $1/3 \times 355 = 118$ nm (16). It is common practice to separate the VUV from the near UV by employing a lithium fluoride lens off axis. Such a lens serves triple duty, separating the fundamental from the VUV, focusing the VUV, and separating the Xe cell from the vacuum.

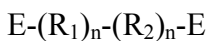
By making the VUV radiation tunable the source becomes selective for IP cutoffs. Among sources for tunable VUV are frequency mixing in mercury vapor (17) and synchrotron sources (18). A typical photo-ionization cross-section is off the order of 10^{-18} cm²; it is largest just above the threshold. A typical flux from a 9th YAG harmonic is of the order of 10^{12} photons/pulse. When focused to 1 mm² of this provides an ionization efficiency of about 10^{-4} , which is competitive with electron impact ionization, especially if the signal is spread over fewer fragments. While lacking the feature of EI of having an extensive library of fragmentation patterns, VUV ionization has the potential advantage of generating parent ions. Single photon ionization it is potentially a soft ionization method. Ionizing close to the threshold minimizes the excess energy, part of which is still carried off by the photoelectron as kinetic energy. Especially when the geometry of the ion is similar to that of the neutral, photo-ionization imparts less than a few tenths of an eV of energy to the ion. Nevertheless larger molecules tend to fragment due to the internal energy of $1/2kT$ per degree of freedom already present in the neutral. Jet cooling removes enough of this energy to stabilize the ion, as shown in the example (19) of figure 2. Panel (a) shows the mass spectrum of squalene, obtained by laser desorption and single photon ionization at 125 nm without any cooling. This mass spectrum is essentially identical to that obtained with electron impact ionization. Panel (b) shows the results with jet cooling. Removal of most of the internal energy suppresses fragmentation entirely. Panel (c) shows the result with partial cooling, leading to non-statistical fragmentation that can give additional information about branching.

The ability to obtain parent masses of large molecules by the combination of single photon ionization and jet cooling is particularly useful in a polymer mass spectrometry for the determination of exact chain length distributions.

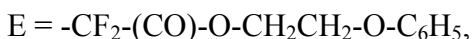
4. Non-resonant two photon ionization

To circumvent the difficulty of generating VUV light, one can employ two photon ionization in the UV region for which commercial lasers are available. This approach requires that the molecule has a real electronic state, S, to absorb the first photon, sufficiently long-lived compared to the laser pulsewidth to be efficiently ionized by a second photon. S should preferably be at an energy of more than half the ionization potential, such that the same laser can be used for both transitions. Convenient wavelengths include 266 nm from a frequency tripled yet laser and 248 nm or 193 nm from an excimer laser. Most purely aliphatic compounds do not absorb in this range, most molecules with an aromatic chromophore do. In the absence of a suitable chromophore it is sometimes possible to label a compound by derivatization.

Figure 3 shows a mass spectrum of a PFPE copolymer of type



with repeat units R and aromatic end groups E as follows:



The spectrum was obtained by laser desorption, jet cooling, and two photon ionization of the end-group at 193 nm (20). There is no fragmentation; each peak in the spectrum 3 represents a parent ion mass, allowing for a complete mapping of the repeat group distribution. Increasing laser flux can cause fragmentation, which then provides additional structural information.

The mass range covered for these polymers extends to about 8000 Da. Comparison of the chain length distributions obtained from these mass spectra with the average distribution obtained from size exclusion chromatography and from NMR comparison of end group and repeat groups shows that for this mass range the photo-ionization efficiency is independent of the mass. Comparison of mass spectra of photophrin polymers, obtained by this technique, with those obtained by SIMS, MALDI, and fast atom bombardment also show a very satisfactory agreement (21).

It appears that the use of femtosecond rather than nanosecond pulses may reduce fragmentation (22). The rationale is that at this timescale absorption of the second photon competes favorably with the intramolecular processes that can lead to dissociation. Furthermore femtosecond pulses may allow ionization via virtual states, thus allowing detection of molecules in the absence of an aromatic chromophore.

5. Resonance enhanced ionization

A. selective ionization

Resonance enhanced two photon ionization (R2PI or REMPI) significantly boosts selectivity. In this approach a photon from a tunable laser resonantly excites vibronic states of the target molecule, usually in the S_1 excited state. A second photon, either from the same laser (one color) or from another laser (two color) ionizes the electronically excited molecules. Two color ionization is more demanding: it requires a second laser and the beams must overlap in space as well as in time. The advantage is twofold: (i) two color ionization allows ionization even when the first photon's energy $h\nu < IP/2$. (ii) by tuning the wavelength of the second photon one may optimize the process. Generally the ionization efficiency is largest just above the threshold. $h\nu_1 + h\nu_2$ can be less than the vertical ionization energy since the Frank-Condon region expands after absorption of the first photon. Since the first transition is resonant and the second one is not there can be typically two orders of magnitude between the laser flux is required to saturate each

transition. This is another reason that two-color ionization can be better optimized. This is especially true if one wants to capitalize on the resonant excitation for its selectivity, because for that purpose it can be advantageous to not saturate the resonance transition. It is then possible to choose a laser flux at which the target molecule is excited at high-efficiency while no other molecule is excited. Such conditions are akin to picking a needle out of a haystack. One does need to know what the needle looks like, in the sense that one needs to know the spectroscopy of the trace compounds one wishes to selectively ionize. Figure 4 shows an example of detection of a trace amount of diphenylamine in a sample of Endoc-C ® grease. At 193 nm ionization in panel (a) all aromatic compounds absorb and ionize, while panel (b) shows the result of resonant ionization at the 0-0 transition of diphenylamine. Because of the resonance enhancement one can use a low power and selectively ionize only the trace compound.

B. isomer distinction

One of the greatest advantages of resonant ionization for mass spectrometry is its potential for isomer distinction. Generally, structural, tautomeric, and even conformational isomers have distinct spectra in which spectral shifts can be from a few to hundreds of wavenumbers. These shifts are usually trivial to distinguish by laser spectroscopy. Notably, in optical spectroscopy a typical resolution is of the order of $E/\Delta E = 30,000 \text{ cm}^{-1}/0.3 \text{ cm}^{-1} = 10^5$ which is almost always orders of magnitude better than resolutions obtained in mass spectrometry. Figure 5, for example, shows the drastically different R2PI spectra of the isomers Ala-Tyr and Tyr-Ala, and Figure 6 shows the R2PI spectra of the PAH isomers (a) triphenylene and (b) chrysene.

Diastereoisomers

Figure 7 shows an interesting application of the isomer selectivity of resonant photoionization. Although enantiomers with a single chiral center have the same R2PI spectrum, that is not necessarily the case for enantiomers with more than one chiral center. Diastereoisomers of the form LL and DD still have the same electronic spectrum but the spectra of homochiral and heterochiral forms differ. Figure 7 shows the R2PI spectra of LL and LD Phe-Phe to illustrate the point that one can use this technique for chiral selectivity. The top traces in each panel determine the number of conformational isomers in each form by double resonant spectroscopy, as outlined in the following section.

6. Double resonant spectroscopy

Double resonant spectroscopy may provide further information from the photo-ionization step (23, 24). In this approach one employs an IR or UV “burn” laser pulse, followed after about 100 nanoseconds by a UV “probe” laser pulse. The latter is tuned to a resonance in the $S_0 \leftarrow S_1$ transition and leads to ion formation after absorption of a second UV photon, either from the same laser (1-color R2PI) or from another laser (2-color R2PI). When the burn laser is tuned to a resonance, it depletes the ground state, which causes a decrease of the ion signal. In this way one can record ground-state IR or

UV spectra of mass selected as well as optically selected species. The optical selection limits the spectra to those of a single isomeric species, allowing tautomer, conformer and structural specificity. The UV-UV combination allows one to determine the number of such isomeric species in a single R2PI spectrum. One may use the IR-UV combination to identify molecules by IR signature, thus providing a unique form of hyphenation between IR spectroscopy and mass spectrometry. Often one can still obtain a sharp IR spectrum, even when the UV absorption is broad.

Figure 7 shows examples of UV-UV double resonance, revealing that the R2PI spectra consist of contributions of 2 isomers for LL-FF and one isomer of DL-FF (25). Figure 8 demonstrates the use of this technique for analysis of a tripeptide, Phe-Gly-Gly (26). The UV-UV data reveal 4 conformational isomers, which are then further analyzed by IR-UV to determine their structures by comparison with quantum chemical calculations. Figure 9 shows the IR-UV fingerprint for three different structural motifs with somewhat larger peptides, including the 15 residue gramicidin A and C. Finally, figure 10 demonstrates the application of jet-cooling mass spectrometry to study clusters, with the example of GC base pairs (27). The capability for soft photo-ionization of clusters, as well as structure determination by their spectroscopy, provides ways to study non-covalent interactions.

Summary

The combination of photoionization and a laser desorption jet-cooling source adds spectroscopic specificity to mass spectrometry. Photoionization provides opportunities for soft ionization, generating parent ions, as well as non statistical fragmentation as a function of laser intensity. Resonant two photon ionization provides very high selectivity in the ionizations step, allowing for isomeric distinction.

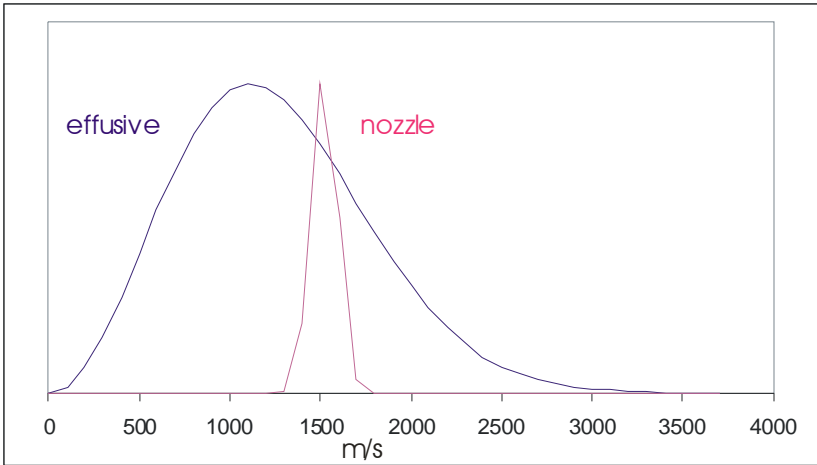


Figure 1. Typical velocity distributions, comparing an effusive beam with a nozzle beam.

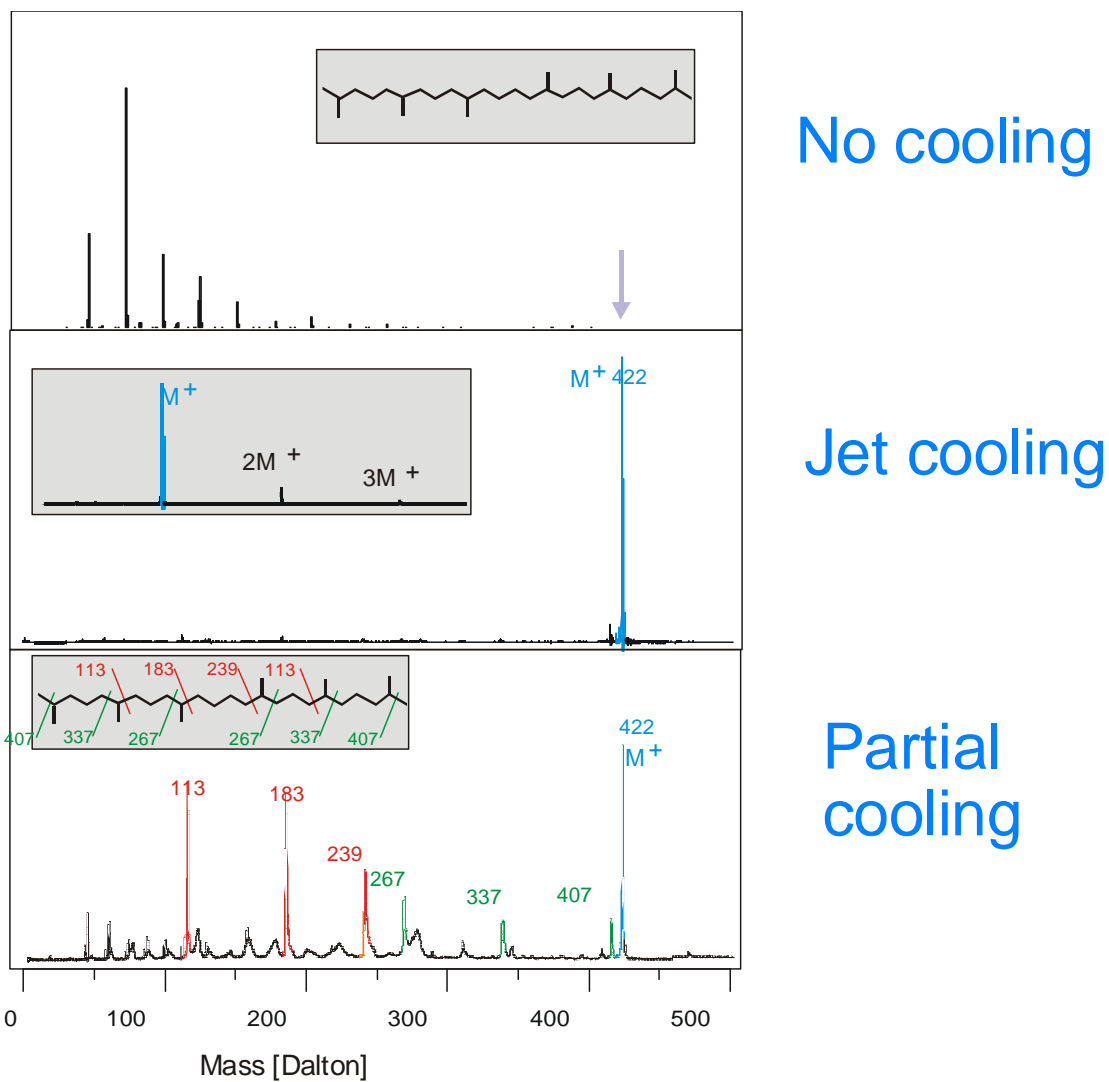


Figure 2: 125 nm single photon ionization of laser desorbed squalene with different degrees of jet-cooling

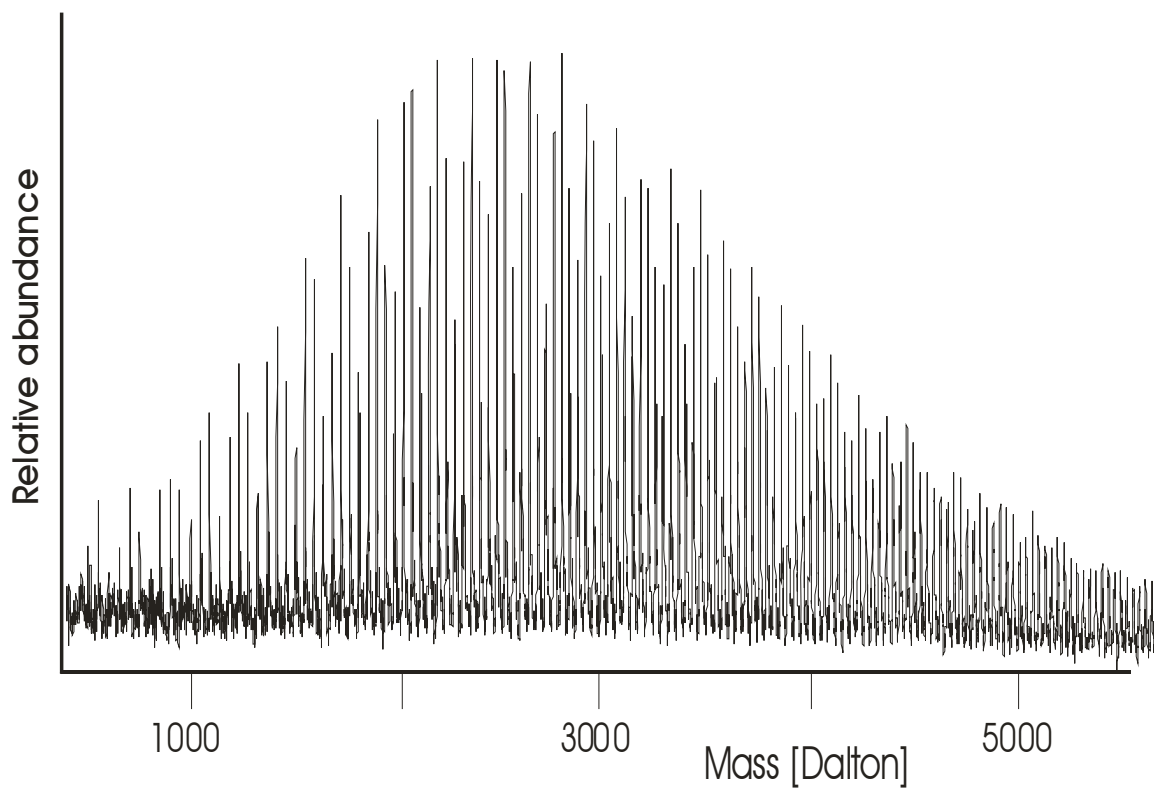


Figure 3: Non-resonant 2 photon ionization mass spectrum at 193 nm of random PFPO polymer. All peaks represent parent masses for different repeat group combinations.

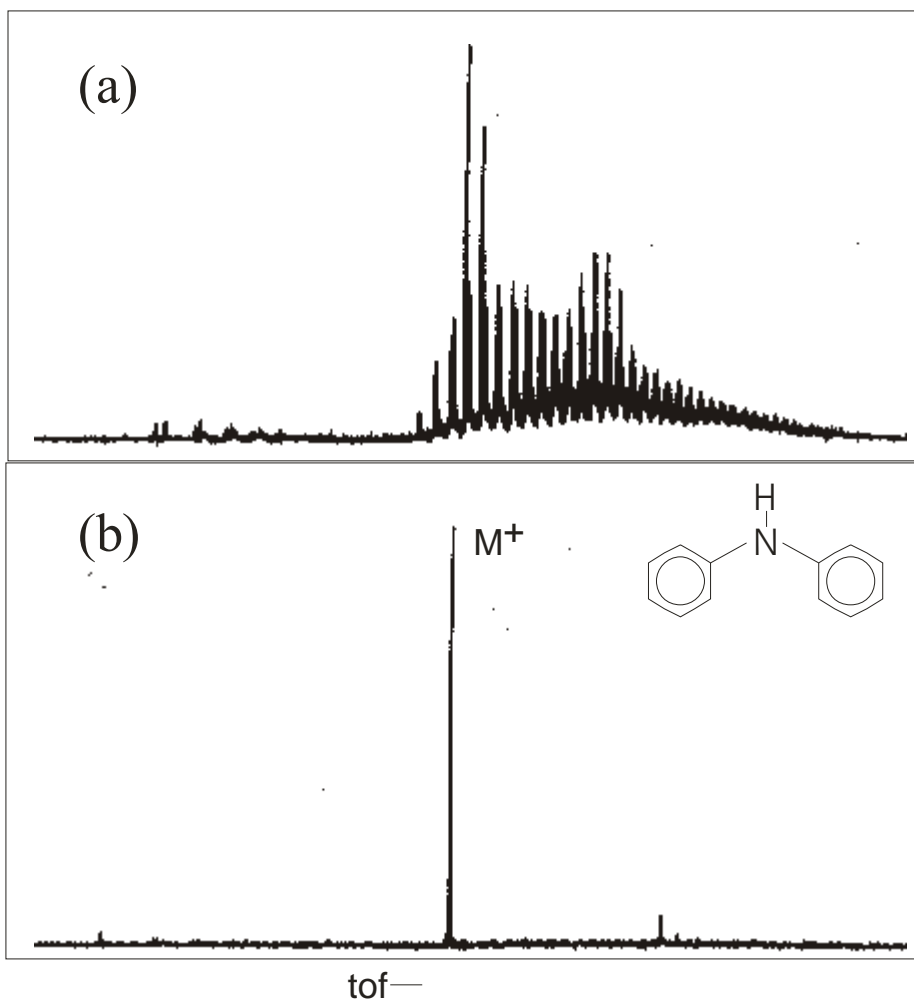


Figure 4: Non-resonant (top) and resonance enhanced 2 photon spectra of laser-desorbed jet-cooled Endoc-C[®] grease, containing a trace of diphenyl amine

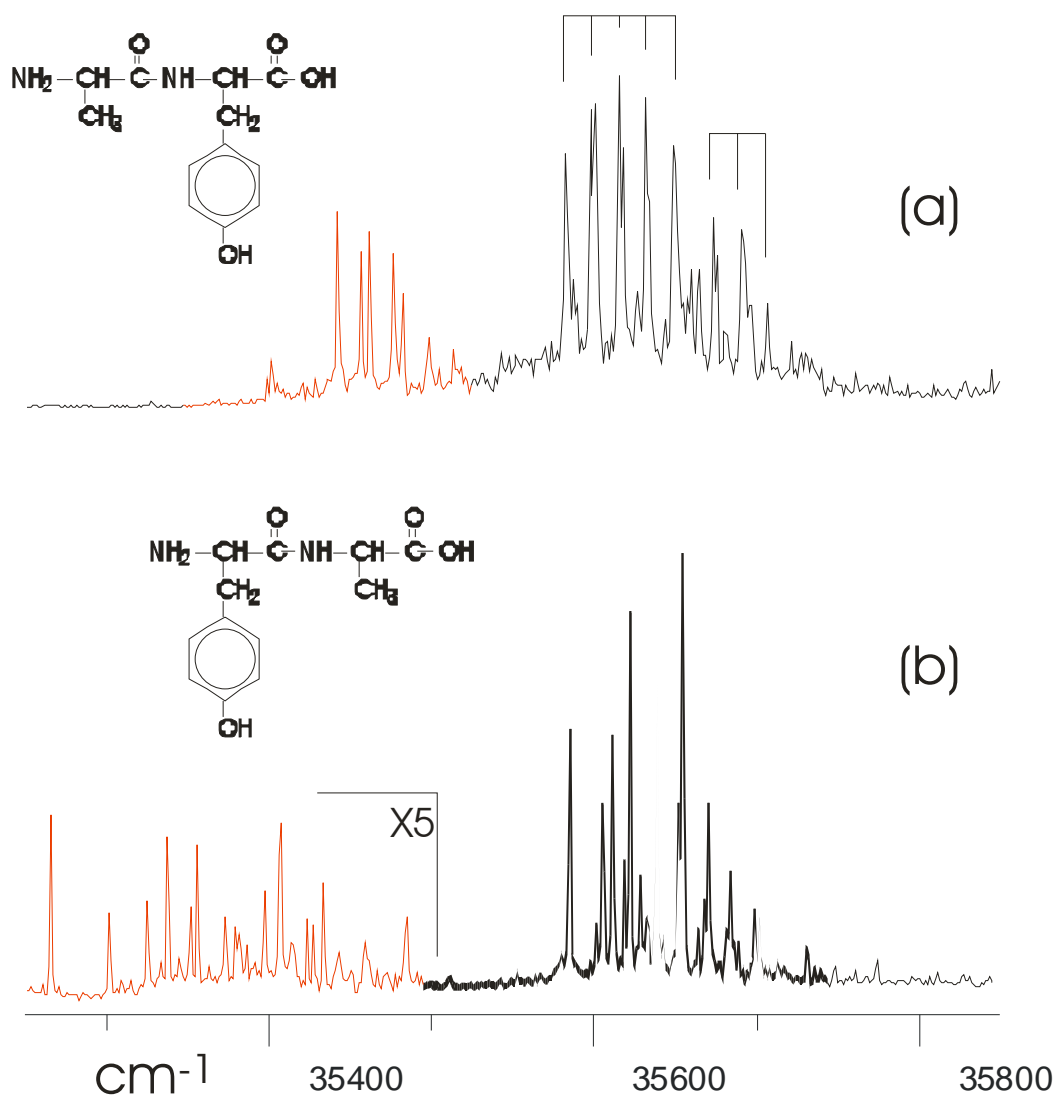


Figure 4: R2PI of (a) Ala-Tyr and (b) Tyr-Ala

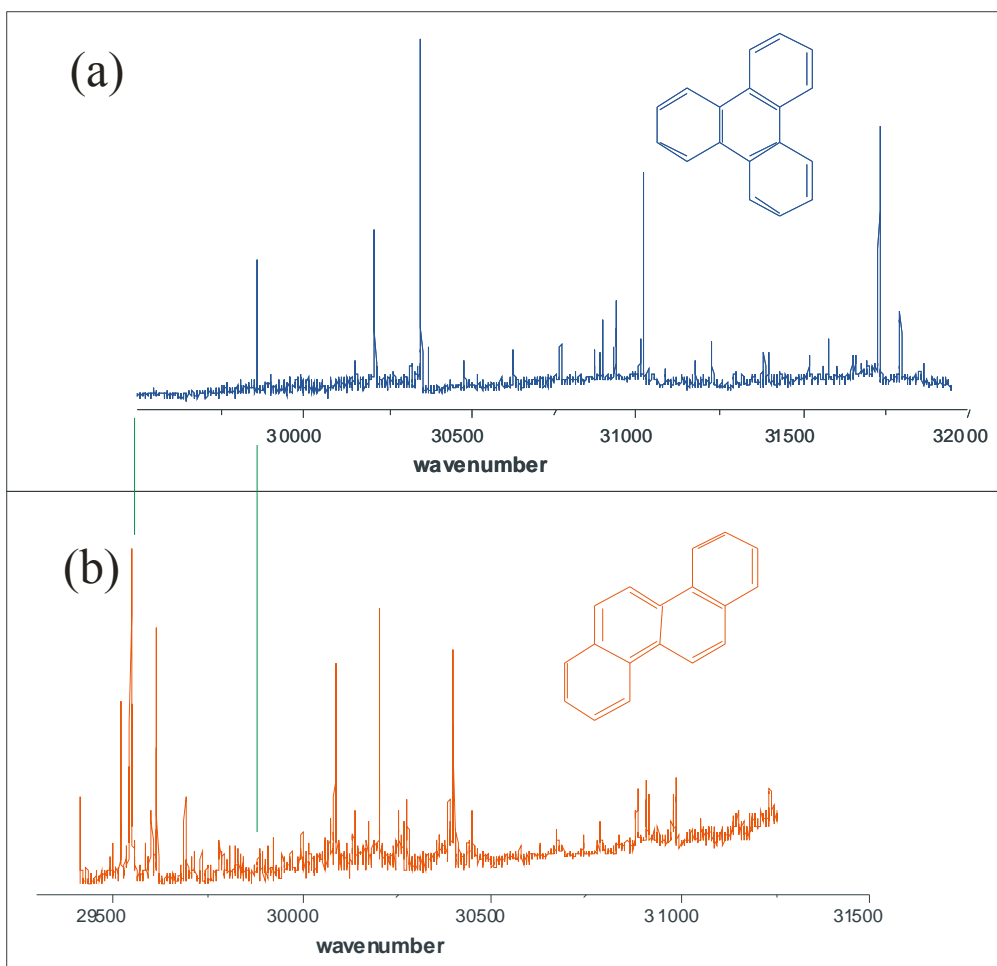


Figure 6: R2PI spectra of the 228 Da isomers (a) triphneylene and (b) chrysene. Green lines indicate examples of some of the many possible wavelengths to selectively ionize one isomer and not the other.

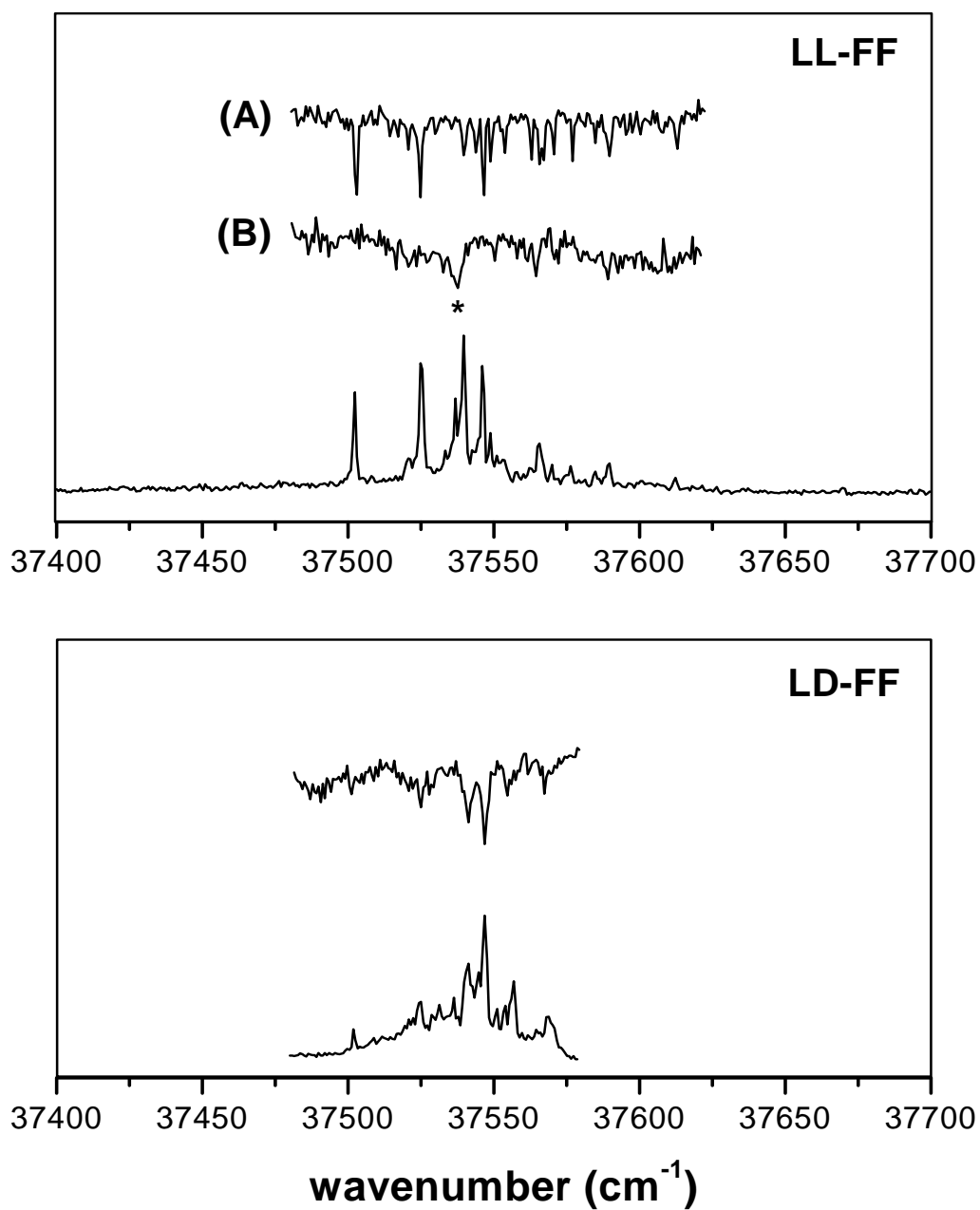


Figure 7: R2PI spectra (bottom traces in each panel) and UV-UV double resonant spectra (top traces in each panel) of the two Diastereoisomers of Phe-Phe.

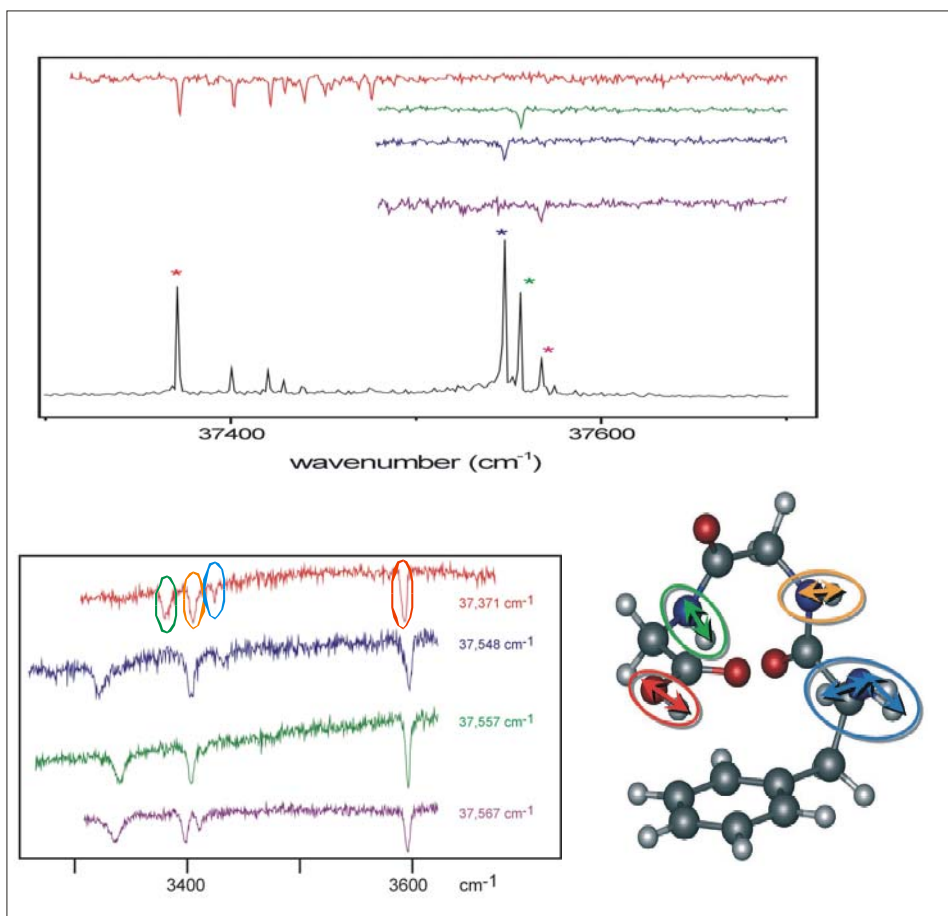


Figure 8: UV-UV (top) and IR-UV (bottom) double resonance spectra of Phe-Gly-Gly. The structure corresponds to the conformation with the red-most origin. The local modes corresponding to the peaks in the top IR-UV trace are indicated with color coding.

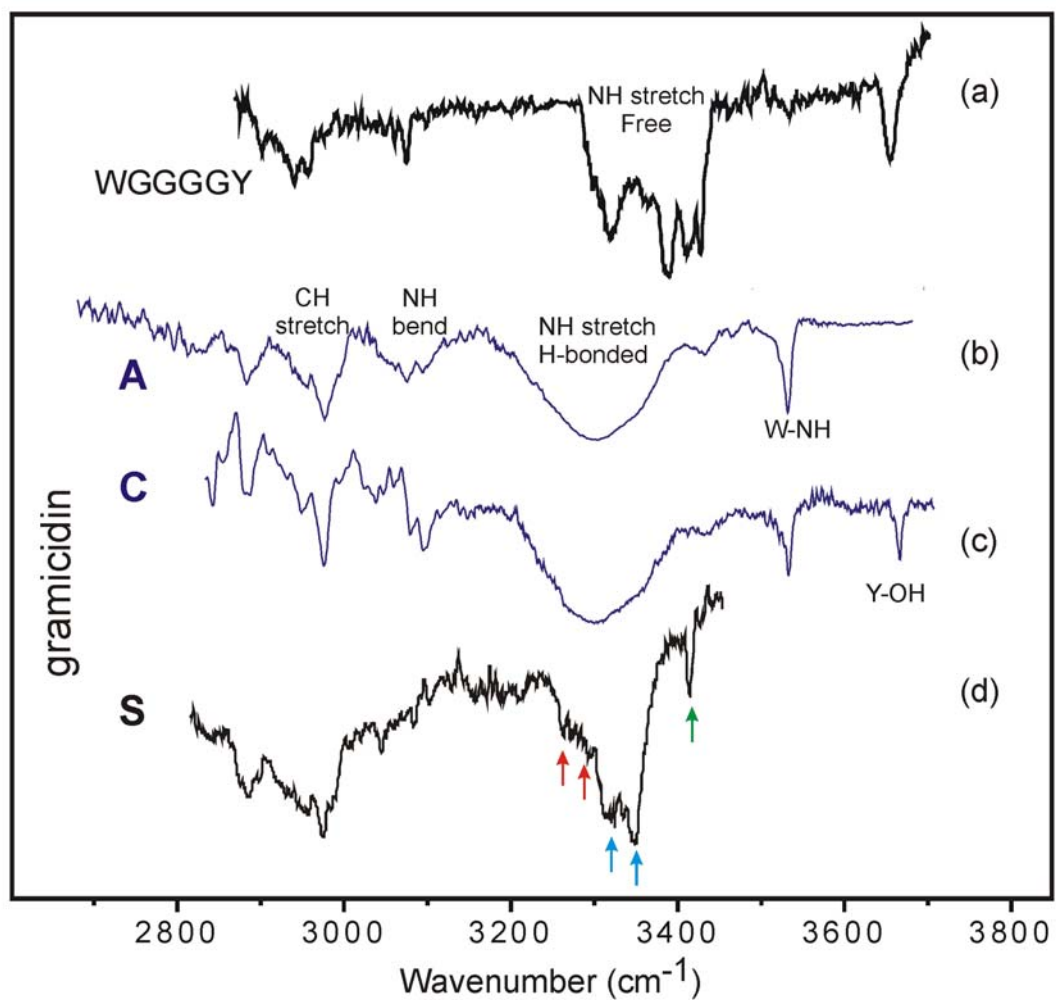


Figure 9: IR-UV double resonance spectra of a series of small peptides. The colored arrows indicate NH_2 stretches (green), free NH stretches (blue) and hydrogen bound NH stretches (red) in gramicidin-S.

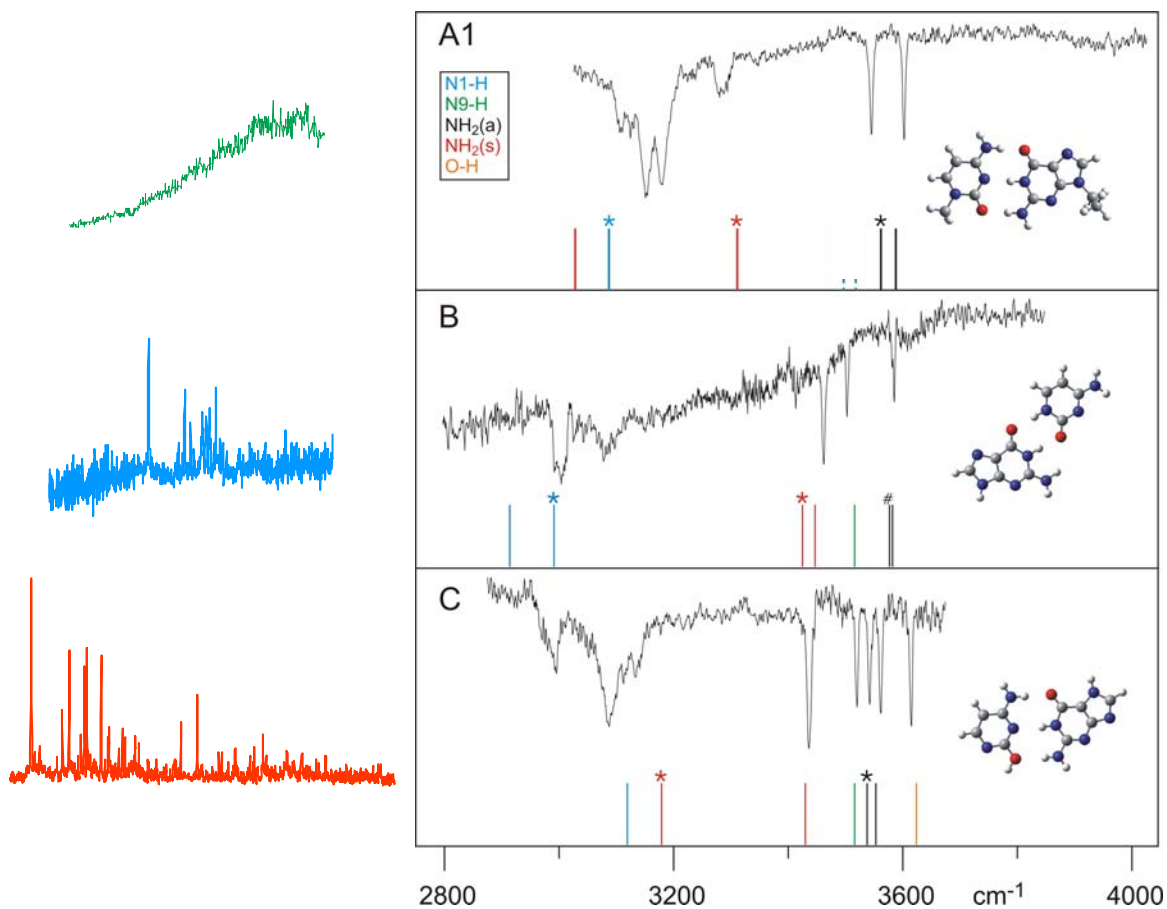


Figure 10: UV-UV (left) and IR-UV (right) double resonance spectra of different structures of GC base pair clusters. The stick spectra are from *ab initio* calculations with color coding to indicate the modes, as listed in the key in top panel. Asterisks denote mode on the guanine.

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