Research Proposal for M.Sc.

Gas Phase Biomolecules: Structural Motifs Revealed by Ionization-Loss Stimulated Raman Spectroscopy

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Introduction

In recent years, much effort has been devoted to the measurement and modelling of the three-dimensional structure and the folding abilities of biologically-relevant molecules, including neurotransmitters [1] and building blocks of deoxyribonucleic acid (DNA) [2]. Previous studies have shown that one can differentiate between the conformers of the same molecule by combing quantum computations with novel laser spectroscopies, employing double resonance or hole-burning techniques, which allow monitoring of electronic and vibrational spectra. The latter were obtained using infrared (IR) sources, using the method of infrared ion dip spectroscopy (IR-IDS) [3] in limited ranges (mainly covering hydride stretch vibrations).

To overcome this problem, our group suggested the measurement of molecular vibrational frequencies, beyond those measured by IR-IDS, by the use of ionization-loss stimulated Raman spectroscopy (ILSRS) [4], coupled with time-of-flight mass spectrometry (TOFMS) [5]. The primary motivation for this innovation is the significantly broader spectral range that can be probed, thus allowing better discrimination between the different conformers, or tautomers, while comparing the measured spectra to results of calculations and thus enabling to pinpoint which conformers (tautomers) are present in the sample. Up to now this method was applied to a variety of neurotransmitters [1], allowing to determine the most stable conformers.

The above mentioned methods, including ILSRS, require the molecules being studied to be isolated, i.e., in gas phase so that their intrinsic properties can be revealed. For some of the molecules being studied this is not a problem, since the vapor pressure of liquids is sufficient to
generate a strong signal through ionization. However, this is not the case for the solid DNA nucleobases which we plan to study. In order to overcome this obstacle, matrix-assisted laser desorption/ionization (MALDI) [6] has been used by other groups to desorb the intended molecules from a matrix (for example, graphite) and to probe the compound in the gas phase. In essence, this method utilizes ultrafast heating of the matrix surface, thus vaporizing the sample (along with the matrix) without significant fragmentation of the sample molecules.

Using the aforementioned MALDI technique and using IR-IDS, work has been done to reveal the structures of the four nucleobases which compose the DNA [2] (guanine - \( \text{C}_5\text{H}_5\text{N}_5\text{O} \), cytosine - \( \text{C}_5\text{H}_5\text{N}_3\text{O} \), thymine - \( \text{C}_5\text{H}_6\text{N}_2\text{O}_2 \) and adenine - \( \text{C}_5\text{H}_5\text{N}_5\text{O} \)). This work has been successful, however, as previously mentioned, IR-IDS has some limits and therefore the spectra of the nucleobases in extended spectral range could not be measured.

**Objectives**

In this project I intend to continue the work done by our group on the structures of the 4-(2-fluoro-phenyl)-ethylamine (F-\( \text{C}_6\text{H}_4\text{–CH}_2\text{–CH}_2\text{–NH}_2 \), 4-FPEA) neurotransmitter in a hydrated environment by the newly developed ILSRS, and to obtain vibrational spectra, including low frequency modes. In the next stage, following the implementation of a MALDI source, the vibrational spectra of nucleobases (guanine to begin with) will be measured. The measured spectra will be interpreted by comparing them to calculated spectra, in order to obtain insight on the available structures.

**Description of the Planned Research**

First and foremost, ILSRS coupled with a TOFMS (see Fig. 1) will be used to probe the sample molecules. The ILSRS uses for that three laser beams [7]. The first one of 269 – 271 nm, is obtained from a UV dye laser pumped by a frequency-tripled Nd:YAG laser at 355 nm (~ 100 mJ/pulse, 10 Hz). This beam is used for preforming the resonant two-photon ionization (R2PI), where the generated ions are detected in the TOFMS. Scanning of the UV laser through the transition related to the different conformers will lead to a R2PI UV spectrum. Another frequency-doubled Nd:YAG laser beam at 532 nm is then split, in a ratio of four to one, so that the former pumps a dye laser, to generate the tunable Stokes beam, \( \omega_S \), and the latter provides the pump beam, \( \omega_p \), at fixed wavelength. In this method, we excite the vibrational energy levels
of the sample, \( \omega_v \), whenever \( \omega_v = \omega_p - \omega_S \), depleting some of the population from the ground state vibrational level and thus reducing the R2PI signal generated by the UV laser. Through this process we will measure the vibrational spectra of a given sample, by using different UV wavelengths to optically select different conformers. The vibrational spectra will then be compared to theoretical calculations of the different conformers (tautomers). For theoretical calculations, we will use a combination of a few methods, including density functional theory (DFT) [8] (for example B3LYP) and \textit{ab initio} methods (for example, MP2) [9].

As previously mentioned, for the solid nucleobases, the TOFMS will be upgraded to include the option of pulsed laser desorption. Instead of the sample vapor being carried into the TOF chamber by argon gas, it will have to be desorbed off from a matrix. In these experiments a mixture of guanine and graphite powder will be deposited on the surface of a solid graphite bar that will be placed directly under the orifice of a pulsed valve (see inset of Fig. 1). After opening the nozzle, a pulsed Nd:YAG laser will desorb sample molecules from the graphite matrix and the desorbed molecules will be entrained in the supersonically expanding carrier gas for ILSRS.

![Fig. 1. Schematic of the experimental system, with an inset showing the laser desorption jet-cooling.](image-url)
Bibliography


