Preparation and characterization of Ribonucleic acid (RNA)/inorganic materials interfaces using photoemission spectroscopy

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Preparation and Characterization of Ribonucleic Acid (RNA)/Inorganic Materials Interfaces Using Photoemission Spectroscopy

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Electrical Engineering
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Preparation and Characterization of Ribonucleic Acid (RNA)/Inorganic Materials Interfaces Using Photoemission Spectroscopy

Brian Doran

ABSTRACT

The objective of this master’s thesis is the preparation and characterization of ribonucleic acid (RNA)/inorganic material interfaces by electrospray deposition and photoemission spectroscopy. This was done through investigation of the chemical and electronic structure of the surface of Highly Ordered Pyrolytic Graphite (HOPG) and gold before and after multiple steps of RNA thin film deposition by electrospray. Great interest has been shown by researchers into RNA due to its self-assembling ability. A series of experiments was conducted depositing RNA Poly adenosine, RNA Poly cytidine, and (for control purposes) DI water on HOPG. RNA Poly adenosine was also deposited on Gold. Gold is a more practical surface for use with RNA, but HOPG is useful for this study because it allows the precise determination of the density of states (DOS) of RNA. X-ray photoemission spectroscopy (XPS) and ultraviolet photoemission spectroscopy (UPS) were used to characterize the RNA-interfaces. The work function, high binding energy cutoff, and HOMO energies were determined.
The clean, in-vacuum deposition of RNA was carried out using an electrospray thin film deposition device. The HOPG and gold substrates were prepared by in-situ cleavage and sputtering respectively.

The electrospray method can be used for many different types of molecules including Polymers, metal-organics, crystals, and biological materials including RNA or DNA. These measurements provided data that will be helpful in determining the electronic properties of biological and substrate interfaces.
Chapter One

Introduction

It is important to characterize the electronic behavior of materials and material interfaces that are of interest as electronic device components. The properties of the material interfaces are crucial for charge transport, adhesion, and other important factors in the behavior of the material. RNA and DNA are very interesting in this regard because of their self-assembling, and possible conductive characteristics [1-4]. Self-assembly is a great prospect for use in nanotechnology, promising cheap and ubiquitous manufacturing processes. The electronic properties of RNA determine the nature of the chemical bonds and the strength of the material. In recent years, the characterization of organic interfaces by X-Ray Photoemission Spectroscopy (XPS) and Ultraviolet Photoemission Spectroscopy (UPS) has become an active field of research. Previous experiments were reviewed which involved the single-step Ultraviolet Photoemission Spectroscopy (UPS) study of sublimated nucleic acid bases, some in conjunction with the use of molecular orbital calculations [5-10]. XPS measurements have also been made on evaporated nucleic acid base molecules after immersion in solution [11-13].

In the presented work, this method was applied to bio-molecular materials. The following experiments were performed:

- RNA deposition in multiple steps on the surface of Highly Ordered Pyrolytic Graphite (HOPG) via electrospray
- RNA deposition in multiple steps on gold via electrospray
- Electrospray of DI water on HOPG in multiple steps as a control experiment to compare with the deposition experiments.
Our novel approach uses two enabling techniques: photoemission spectroscopy and electrospray deposition. This approach allows the in-situ multi-step deposition and measurement of the Highest Occupied Molecular Orbital (HOMO) spectrum with UPS, enabling the determination of the orbital line-up and electronic structure. It is important to see how the electronic structure changes as the material thickness changes in order to see how the material would behave as a conductor or semiconductor material.

The electrospray method allows deposition of thin films of Polymers, such as RNA and DNA, which due to their large molecular size could not be evaporated in vacuum and deposited directly from solution. In addition to allowing deposition of larger molecules than sublimation is capable of handling, it does not require high temperatures for use. The electrospray system makes deposition and measurement of these materials possible while maintaining the sample in vacuum (in-situ). This presentation will show the mechanism and physical basis for developing interfaces and measuring their electronic characteristics.

Following deposition of material, the electronic characteristics of the material interface can be determined by photoemission spectroscopy. This measurement technique uses the photoelectric effect. It involves emitting a beam of photons at the material surface and determining the chemicals present on the surface based on the kinetic energy of the electrons that are expelled from the surface. Photoemission spectroscopy is a very surface-sensitive method of determining the chemical components present in a sample. The experiments performed in the lab gathered useful data including the high binding energy cutoff, work function, HOMO cutoff energy values, and the composition of the films that formed on the material surface. These results will demonstrate that the electrospray will allow the preparation of thin-films of RNA on inorganic surfaces so that the interface between can then be characterized by photoemission spectroscopy.
Stranski-Krastanov (SK) mode of deposition will be shown to be the preferred deposition mode for RNA Poly ‘A’. This deposition mode is characterized by formation of a thin monolayer followed by clusters of crystallite deposition.
Chapter Two

Background

2.1 Review of DNA Based Self-Assembly Experiments

![Diagram of DNA assembled proteins as links between gold particles to build nanostructures.]

Scientists and engineers have demonstrated an ability to manipulate the self-assembly of biological materials as a means of constructing new structures in the laboratory. Use of DNA in nanotechnology offers the possibility of programmable self-assembly as demonstrated in the experiments cited below.

In Figure 1 above, 16 nm gold particles are modified with DSDA, a gene sequence for the E-coli virus. When streptavidin is added it links with the DSDA forming a new nanostructure. Similarly below, a novel two-component nano-structure is made to form from DNA-directed assembly of gold particles and linking oligonucleotides (a chain of up to 20 nucleotides). Figure 3C shows the particles remaining separate without modification. In Figures 2, 3A and 3B, the 8nm and 31nm gold particles have
been altered by adding complementary strands of thiolated DNA strands to the surface of each. Thiols are sulfur-containing groups that selectively react with gold (Au). Sulfur is chemically attracted to gold. When the complementary strands of DNA link, these structures form. Nano-scale components are assembled in nature using molecular recognition. These structures demonstrate a revolutionary step in the ability for humans to use bio-molecules in the self-assembly of nano-structures.

Figure 2. Controlled Self-Assembly Through DNA Oligomers [15] Example: Use of selectivity of DNA hybridization for controlled assembly of Au clusters. 8 nm Au particles were coated with thiolated DNA strand 1, 31 nm Au particles were coated with thiol modified strand 2. Then linking DNA strands containing complementary sequences for 1 and 2 strands is added to the solution. This causes the two Au cluster types to form ordered structures [16] from [15].
As a sensor, sections of RNA could be used to recognize the presence of the complementary base pairs, as those would be the only molecules with which the strand could bond.

An important development has been made in learning to utilize the ability of RNA and DNA components to only link with their complementary matches. In this way a single base mismatch between two DNA sequences can be detected. It was shown that the sensor application for which this cantilever, shown in Figure 4, would be used would be accurate and sensitive to such a small difference. The ability to detect the degree of mismatch between the complementary pairs was also demonstrated. After binding, the hybridized oligonucleotides were returned to their normal state by application of a solution that broke the hydrogen bonds between complementary bases.

This ability allowed the arrays to be reused at for at least 10 more experiments over several days. This development promises to open a new opportunity for cyclic operation of nano-mechanical devices using a repeatable biomolecular recognition method. These devices could also be triggered by signals from single cells rather than large external controllers. To tap the promise of this and other developments, however, more must be known about the properties of the biological materials involved.
Figure 4. Scheme Illustrating the Hybridization Experiment. Each cantilever is functionalized on one side with a different oligonucleotide base sequence (red or blue). (A) The differential signal is set to zero. (B) After injection of the first complementary oligonucleotide (green), hybridization occurs on the cantilever that provides the matching sequence (red), increasing the differential signal $D_x$. (C) Injection of the second complementary oligonucleotide (yellow) causes the cantilever functionalized with the second oligonucleotide (blue) to bend. [17]

These self-assembly demonstrations provide motivation for the determination of electronic characteristics as performed in our experiments.

2.2 Electrospray (ES) Deposition

The first of two enabling techniques for our experiment was the electrospray method of material deposition. Electrospray (ES) ionization is a method of producing gaseous ionized molecules from a liquid solution by creating a fine spray of droplets in the presence of a strong electric field [18]. This method has been used successfully in the mass spectrometry of biomolecules [19]. Variables that affect the stability of ES operation include the applied voltage, gas flow rate, solution flow rate, viscosity of solution, and the distance between the spray capillary and the counter electrode.
Figures 5 and 6 show the electrospray system ready for use. The solution is pumped through a capillary whose end has a large positive potential applied. The ES chamber itself is electrically grounded. If the applied potential used between the capillary and counter-anode is high enough, the solution will spread into a very fine spray of charged droplets. This process is called 'pneumatic nebulization'. Once charged droplets are discharged from the capillary, they accelerate towards the counter-electrode in a spray pattern that is called the Taylor cone. Under stable spray conditions the Taylor cone will maintain a constant shape, balancing the effects of electric repulsion and surface tension [18, 20, 21].

As the solvent evaporates, the droplet size decreases further and the surface charge concentration of each droplet will increase. Eventually this leads to Coulombic “explosions”: the disintegration of a droplet into smaller droplets from the Coulombic repulsion of like charges. With smaller droplets there is a much larger amount of surface area for each unit of charge. The point where these Coulombic explosions occur is known as the Rayleigh Limit, defined as the where charge $Q$ will overcome the surface tension holding the droplet together. This is defined by:

$$Q^2 = 64 \pi^2 e_o \gamma R^3$$

with $e_o$ as the permittivity of vacuum and $(R)$ is the droplet radius.
The radius (R) of an electrosprayed droplet is determined as follows by:

\[ R \propto (\rho V_f 2\gamma)^{1/3} \]

with fluid density (\( \rho \)), flow rate (\( V_f \)), and surface tension (\( \gamma \)).

Smaller droplets and thus smaller flow rates are preferred, as they are easier to ionize. With this relationship, a high \( V_f \) causes larger initial droplet sizes. Larger droplet sizes cause lower ionization efficiency because their size is further from the Rayleigh limit, which is necessary for further reduction in droplet size and in ionization. A small droplet has a higher surface area per unit volume, meaning that more molecules are ready for ionization. The voltage required for droplets to ionize is higher for liquids of higher surface tension [18], [22, 23].

This shrinking of droplets and Coulombic explosions ideally continues until the solvent is completely removed. Thus, the droplets shrink by two mechanisms, solvent evaporation and disintegration by Coulombic explosions.

The positive ions remaining on the formerly dissolved solute are now distributed on the solute particles as they proceed to the target material. Some of the particles will have multiple charges, as the charges are distributed in a statistical fashion. A drying and nebulizing gas is used to increase the rate of solvent evaporation. Nitrogen is used as this gas in our experiment. This also increases the extent of multiple charging on each particle. Using a smaller diameter capillary and a low flow rate for the solution also creates a higher charge ratio [24].
Solvents are chosen for their polarity and compatibility with the solutes in use. Water was chosen as a solvent in this case due to clogging problems experienced with some other solvents tried, acetyl nitride and methanol. Water has the disadvantage of being harder to remove from the chamber after electrospray. It is also more conductive, so that the electrospray voltage had to be reduced to avoid sparking at the end of the capillary that had caused it to clog. The risk of sparking is greater with higher voltage, but also with lower pressure. UHV conditions are maintained until electrospray begins so that the sample material is not compromised by atmospheric gases. Pressure in the chamber later increased above UHV to just below $10^{-7}$ mbar due to the introduction of the sprayed materials.

The solution is pushed through a 100µm capillary with up to a 5kV charge applied to produce an electric field. Nitrogen is added as an inert transport and drier. Coulombic explosions reduce droplet size and release individual molecules according to the Rayleigh limit.

Atmospheric gases and solvents are removed through three pumping stages, as shown in the Figure 7 below:
The solute molecules are accelerated by differential pumping and electric field forces and deposit a substrate that is then transferred in situ to the analysis chamber. The electrosprayed molecules can be detected with a Faraday cup or other deposition monitor, aiding the adjustment of the electrospray system. A Faraday cup is an electrostatic device that captures charged particles and displays current as measure of their number and charge.

### 2.3 X-Ray Photoemission-Spectroscopy (XPS)

XPS was developed during the mid 1960s by K. Siegbahn and his research group in Sweden. This work was recognized with a Nobel Prize for Physics in 1981. The phenomenon is based on the Einstein’s Theory of the photoelectric effect of 1905 where quantized photons of an EM radiation field (light) strike and cause the ejection of electrons from a surface [25]. The effect was first described by Hertz in 1887.
2.3.1 X-Ray Photoemission Spectroscopy (XPS) Fundamentals

X-Ray Photoemission Spectroscopy (XPS), also known as Electron Spectroscopy for Chemical Analysis (ESCA), as shown in Figure 8 below, is a very surface-sensitive chemical analysis technique used to characterize the surface of solids to a depth of 2-20 atomic layers, (10-100Å, or 5-10nm depth) depending on the material studied [26]. The technique also has several unique factors including its non-destructive nature, the ability to study plastic and organic surfaces, and the ability to operate at moderate vacuum levels. XPS has been successfully used to study a wide range of materials including Polymer surfaces and metal/metal oxide films. While detection limits can approach one atom in 100,000, XPS usually has a detection limit of 0.1%-1.0% of a monolayer.

Figure 8. XPS Interaction with the Surface [27]
2.3.2 Physics of XPS

Photoelectric Effect

\[ BE = h\nu - KE - \Phi, \text{ where } BE = \text{Binding Energy}, \ h\nu = \text{Electron Energy}, \ KE = \text{Kinetic Energy}, \ (\Phi) = \text{Work Function} \]

There is a threshold freq, \( f_0 \), below which the photoelectric effect does not occur.

In XPS, the energy of a photon, as shown in Figure 9, is transferred to the electron in the sample. Electrons released are analyzed for their kinetic energy, which can be used to determine the binding energy level of the electron. The energy of a photon \( E \) is given by Einstein’s equation:

\[ E = h\nu \]

Where \( h = \text{Planck constant} \ (6.626 \times 10^{-34} \text{ J s}) \)

\( \nu = \text{frequency (Hz) of the radiation} \)

E is known because XPS uses a specific x-ray line for excitation. The energy of the photon must exceed the binding energy of the electron in order to make the electron available for detection. When the photon’s energy is greater than the binding energy of the electron, the photoelectron is emitted from the sample [28].
This photoionization process / photoelectric effect is summarized in equations below: An atom (A) is hit by a photon (hν), producing an ionized surface and a free electron.

\[ A + h\nu \rightarrow A^+ + e^- \]

The energy of the atom in the sample material plus that of the photon must equal that of the resulting parts:

\[ E(A) + h\nu = E(A^+) + E(e^-) \]

Rearranging the equation and renaming \( E(e^-) \) as the kinetic energy (KE) of the photoelectron we find:

\[ KE = h\nu - (E(A^+) - E(A)) \]

This equation can be simplified, since we know that the binding energy (BE) of the electron is the difference in energy between the ionized and neutral atoms:

\[ KE = h\nu - BE \]

BE is the amount of energy needed to move the electron from the core levels to the vacuum level. The KE of the electron is equal to the energy of the photon minus the energy required to free the electron.

\[ BE = h\nu - KE \]

By rearranging the equation, the binding energy of an electron level is found by subtracting the kinetic energy of the collected electrons, as measured, from the known energy of the source x-ray beam. Binding energies for each electron orbital level in an element are unique and useful in identifying the elements present [29].

The high degree of surface sensitivity that is characteristic of XPS is due to the very short mean free path of electrons in solids. This is the mean distance traveled by an
electron before undergoing a significant deflection or collision. This distance is dependent on the energy of the electron and of the material itself. The photoelectron will inelastically collide with the lattice structure of the material if it is any greater than about 50Å below the surface when it is first ejected. If this occurs it will not escape the lattice into the vacuum with a unique kinetic energy signal. It will be detected however, if its energy after the collisions still exceeds the binding energy, as this electron and others like it account for the background signal in the XPS spectra [29].

However, the binding energies (BE) in solids are usually measured with respect to the Fermi-level of the solid, instead of the vacuum level as was shown above. To correct this discrepancy with the “BE = \( h\nu - KE \)” equation, a constant is added, accounting for the difference between the Fermi level and vacuum level. This material-dependent constant is known as the work function (\( \Phi \)) of the solid. The work function varies with the composition and chemical environment of the material under investigation.

Thus, \( \text{BE} = h\nu - KE - \Phi \) [28].

![Energy Level Diagram with Photoelectron Being Ejected](image)

**Figure 10. Energy Level Diagram with Photoelectron Being Ejected**
Figure 10 shows an energy level diagram with a photoelectron being ejected as in XPS.

The probability of emitting an electron is dependent on the mean free path and kinetic energy of the electrons in the material. Sensitivity factors have been determined to consider the effects of the mean free path and electron kinetic energy for each element. These allow for the calculation of accurate surface concentration ratios and quantitative analysis by evaluating the peak intensities [26].

![Electron Kinetic Energy, eV](image)

**Figure 11.** Mean Free Path of Electrons in Solids in Dependence with Their Kinetic Energy. Each Point on the Plot Represents an Empirical Measurement in a Different Material with the Curve Fitted to the Data.

The surface sensitivity of XPS is determined by the mean free path in solids, which is in turn determined by the characteristic kinetic energy of the emitted electrons as shown in Figure 11 above. The information depth for a given energy source can be determined from this graph. Knowing that our sources emit photons with 1253.6 eV (Mg Kα) and 1486.6 eV (Al Kα), we can see the information depth to be between 5-10 monolayers. The mean free path is also known as the attenuation length and defined as the average distance traveled by an electron of a given kinetic energy can travel before it loses kinetic energy through inelastic scattering in the material. For this reason, few electrons from further below the surface escape to be detected.
Key Features of XPS:

- The XPS technique is highly surface specific due to the short range of the photoelectrons that are excited from the solid.
- The information depth, resulting from the mean free path of the photoelectrons inside the sample surface, is about 3-5 nm.
- The binding energy of the peaks is characteristic of each element. The peak areas can be used (with appropriate sensitivity factors) to determine the composition of the materials surface.
- The shape of each peak and the binding energy can be slightly altered by the chemical state of the emitting atom. Hence XPS can provide chemical bonding information as well.
- XPS can detect all elements except hydrogen and helium
- generally non-destructive analysis

2.4 Emission Features

![Figure 12. An Example of XPS Survey Spectra. These Spectra are from RNA Poly ‘A’ on HOPG.](image)
As shown in Figure 12, an XPS spectrum consists of a series of peaks corresponding to the binding energies of the photoelectrons that produced these peaks. The binding energies of photoelectrons in a sample are shown above with higher probability for each electron being represented by a larger peak. The Fermi energy level is shown at the zero binding energy position. Auger peaks are discussed in detail in the following section. Also notable, although not shown in the figure above, is the high binding energy cutoff or secondary edge. The spectra would increase in intensity towards higher binding energies with a large spike at energies above approximately 1100-1200 eV. These energies are from electrons that had just enough energy to escape the atom.

The XPS Survey Spectra (using the dual anode x-ray source) provided a snapshot of the elements present near the sample surface. The core level spectra were examined as high-resolution spectra using the monochromator, providing a more detailed view of each chemical peak that was of particular interest. The monochromator’s advantages are that it has a more focused energy resolution, eliminates x-ray satellite peak overlaps resulting from interference by other, non-monochromatic sources, and reduces the amount of damage done to sensitive samples. This allows clearer identification of chemical traces. The disadvantages of the monochromated x-ray source are that it produces lower intensity readings and requires longer measurements in order to achieve a better signal to noise ratio.

It is necessary to interact with the core (inner shell) electrons because the outer electrons are not helpful for indicating what element they came from. Instead, the outer electrons are associated with chemical bonding. These outer electrons are ionized and measured in UPS, which will be discussed later. We can learn the following things about a sample from XPS analysis: The presence and proportions of various elements and how they change over time and with modifications to the sample as well as chemical bonding state and oxidation state information [29].
2.5 Ultraviolet Photoemission Spectroscopy (UPS)

Ultraviolet Photoemission Spectroscopy (UPS) is very similar to XPS except that it uses ultraviolet radiation rather than x-rays. While XPS looks at the core level electrons, UPS looks at the valence states. UPS is especially important for obtaining information about molecules adsorbed on metal surfaces. Much of what we seek to learn about the materials studied by spectroscopy is not simply how the band structure of a material appears by itself, but when interfaced with another material.

In UPS, a noble gas discharge lamp is usually used as the radiation source; in this case a He-discharge lamp emitting He I radiation at 21.21 eV. The low photon energy in UV radiation is only capable of ionizing electrons from the outermost levels of atoms. Thus, only electrons from the valence band (the shallow core levels) are seen by the detector. UPS is advantageous due to the very narrow line width of the radiation and the high flux of photons available from simple discharge sources.

![Figure 13. An Example of UPS Spectra. These Spectra are from RNA Poly ‘A’ on HOPG.](image)
Figure 13 shows an example of UPS spectra in a multi-step deposition series. Notable features present in the UPS spectra are the high binding energy cutoff, work function, HOMO edge, and valence band features.

2.6 Other Spectral Effects

Charging effects are common in insulating samples or if a layer of material has separated from the surface. They are also dependent on the thickness of material deposited on the surface. Charging effects occur when energy directed at the sample results in the top layer of material having a static electrical field. This causes a shift to higher binding energies across the entire photoemission spectrum, but dissipates over time. Similarly, an interface dipole is the formation of a surface charge which results in an electric field (dipole) which shortens the spectrum by shifting the high binding energy cutoff to lower binding energy while not affecting the rest of the spectrum. As a result this also adds to the work function calculation. More detail of this is shown in the Data Evaluation Method section of this document.

Low intensity XPS (LXPS) measurements are made before and after each UPS measurement. Comparison of the LXPS and UPS measurements allows detection of charging phenomena in the UPS measurements. This allowed for a more accurate determination of work function and the HOMO alignment at the investigated interfaces. Due to the lower energy intensity, LXPS has less charging effects than UPS. Fewer electron-hole pairs are formed, resulting in less charge to be dissipated.

Auger electron peaks manifest themselves when high energy electrons collide with an atom and displace a core level electron. An electron drops from a higher level to fill the hole left by the displaced electron. In order to drop, the higher-level electron surrenders energy to a neighbor, which is then excited and may also be ejected from the atom as an Auger electron. It must first give up some energy to overcome the binding energy of the atom, but the remainder of the excess energy is the kinetic energy of the Auger electron.
There are four Auger series of importance in XPS: KLL, LMM, MNN, and NOO. These series are designated by the electron levels in which the initial and final electron vacancies occur. For instance, the KLL line is formed when a high-energy electron displaces and K shell electron and two vacancies in the L shells result. Auger peaks have energies which are not related to the original incident photon energy[30].

Also notable are shake-up lines, which result from photoelectric effects that lead to an ion that is left in an excited state, a few eV above the normal ground state location. This results in the ejected photoelectron being lower in kinetic energy than normal. The difference is equal to the amount of energy between the ground state and the excited state. This results in a satellite peak that appears a few eV higher in binding energy than the main peak, due to the electron being a few eV lower in kinetic energy. Several of these peaks may occur. The relative intensities and positions of these peaks gives some information about the chemical state of the element [30].

2.7 XPS System Component Overview

The XPS system consists of the following components: an x-ray source, electron energy analyzer, detector, ultra-high vacuum system, and a computer.

2.7.1 X-Ray Source

The x-ray source works by heating accelerating electrons from a heated filament towards a usually aluminum or magnesium anode. When striking the surface material of the anode, the electrons excite inner shell electrons of the atom. Relaxation of these excited electrons results in the emission of x-ray photons at energies characteristic of the anode material. The characteristic x-ray emission lines result in the emission of photons with a constant, known energy, enabling the photoemission spectroscopy measurement. XPS uses “soft x-rays” of 200-2000 eV radiation to examine core-levels [29]. These x-ray photons are emitted from source and focused onto the sample material. In a monochromatic system, the characteristic line is selected by the monochromator.
2.7.2 Concentric Hemispherical Analyzer (CHA)

The electrons are analyzed in the Concentric Hemispherical Analyzer (CHA) to measure the energy distribution of the electrons. The function of the CHA is as shown in Figure 14 below: It consists of two metal hemispheres, with one inside of the other, separated by a small distance. Different voltages are placed on each hemisphere creating an electric field between the two hemispheres. One plate has a negative charge and repels the incoming electrons; the second plate has a positive charge and attracts the incoming electrons. Electrons are injected into the gap between the hemispheres. If the electrons are traveling too fast, they will impinge on the outer hemisphere. If they are traveling too slowly, they will be attracted to the inner hemisphere. Hence only electrons in a narrow energy region (called the pass energy, E_p) succeed in getting all the way round the hemispheres to the electron detector. A series of lenses are placed before the CHA to focus the electrons by the desired amount [31].

Figure 14. Principles of Photoemission Spectroscopy
2.7.3 Ultra High Vacuum (UHV) System

Why UHV is required for XPS measurements:

- Prevents the introduction of atmospheric gases onto the sample, as well as removing some that are already present.
- Prevents the introduction of dust or other contaminants onto the sample during the experiment.
- Increases the mean free path for electrons, ions and photons in the chamber.
- Prevent arcing and ionization of gases which would otherwise be in the chamber during measurements [26].

A UHV environment is considered as one in which pressure is less than $1 \times 10^{-9}$ millibar. In comparison, normal atmospheric pressure is about 1 bar. This shows that atmospheric gas molecules are about 1 trillion ($10^{12}$) times less common within the UHV system than in atmosphere. This is important for experimental purposes due to the way that gases and dust can quickly obscure the needed data in even a freshly cleaved sample. Even at relatively low pressures, such as $1 \times 10^{-6}$ mbar, a monolayer of contaminants from residual gases forms in about 3 seconds [32]. Maintaining UHV conditions staves off this exposure to a great extent, long enough to complete a measurement.

Three rotary vane roughing pumps are used to reduce pressure from atmospheric to approximately $1 \times 10^{-6}$ mbar. A thorough heating of the system with ovens, heating tape, and insulating enclosures, called bakeout, may be necessary to remove adsorbed gases from the chamber walls. Two turbo-pumps then remove remaining atmospheric gases to reduce pressure to approximately $1 \times 10^{-6}$ mbar and below. All of these pumps run continuously during normal operation. The roughing pumps act as priming pumps for the turbopumps, as the turbopumps cannot work versus atmospheric pressure. Ion pumps and titanium sublimation pumps (TSPs) are also used in the operation of the UHV system. Ion pumps do not remove gases from the system but trap them separately. TSPs pump sublimated titanium vapor that combines with residual gas molecules which are
then more easily removed. TSPs are shut off during sputtering or deposition. The base system pressure at which XPS measurements are made is approximately $2 \times 10^{-10}$ mbar. The USF Surface Science Lab UHV equipment is pictured below in Figure 15.

![Photoemission Spectroscopy and UHV Equipment](image)

Figure 15. Photoemission Spectroscopy and UHV Equipment

### 2.8 Materials

#### 2.8.1 Substrate: HOPG

Highly ordered pyrolytic graphite, HOPG, as shown in Figures 16-17, is very useful as a substrate material for depositing material on during XPS experiments or other surface science studies.
Surface scientists find HOPG useful because it cleaves well so that many surface layers can be studied from a small amount of initial material. HOPG is very smooth, if cleaved properly. This should provide for a surface free of defects, except for where grains meet and each atomic step of about 0.3 nm. Cleaving the surface produces a clean surface in UHV.

HOPG has a layered structure, with each layer only weakly bonded to the next by van der Waals forces. It has a planar hexagonal structure with a close packed array of atoms. Each atom is surrounded by its six nearest neighbors, with a distance of about 0.142 nm between each of these and a distance of 0.335 nm between the one layer and the next. Layers are formed because the crystals form in a highly ordered pattern on the horizontal axes, but very disordered in the vertical axis. This layered quality can be seen in the hcp (hexagonal close-packed) crystal structure shown in Figure 17a and b above.
The polycrystalline structure of HOPG has grains of varying size, with the largest at 10 mm across in the highest quality pieces available. The available samples were of high purity and had impurities of 10 ppm or less. By purchasing a higher grade of HOPG, money should be saved because the higher-grade material will have larger, more orderly grains and should cleave into more layers. The degree of order in the crystal is called its mosaic spread angle. This is the angle at which the carbon grains are spread apart from parallel. It is this angle that also separates the high and lower grade materials. Table 1 compares each of the available grades of HOPG, including SPI-1, which is only used for calibration. Grade SPI-3 10x10x4mm or Grade SPI-2 10x10x2mm HOPG blocks were sufficient for this experiment.

Table 1. Comparison of Material Grades of HOPG [33]

<table>
<thead>
<tr>
<th>Material Grade</th>
<th>Mosaic Angle</th>
<th>Grain Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI-1</td>
<td>0.4° +/- 0.1°</td>
<td>10 mm</td>
</tr>
<tr>
<td>SPI-2</td>
<td>0.8° +/- 0.2°</td>
<td>1 mm</td>
</tr>
<tr>
<td>SPI-3</td>
<td>3.5° +/- 1.5°</td>
<td>30-40 nm</td>
</tr>
</tbody>
</table>

Many of HOPG’s characteristics vary with the direction of each grain, such as thermal conductivity, electrical conductivity, physical strength, magnetic properties, and thermal expansion, but are not generally a factor in XPS measurements. HOPG is a zero-bandgap semiconductor, meaning that it has metallic properties. The lack of other emission features near the binding energies of other elements, particularly those present in RNA make HOPG an excellent choice for use in spectroscopy of RNA thin films. The presence of the HOPG substrate in the spectra is easily found, as the only element present is carbon. The thermal conductivity seems to be independent of HOPG grade. The vapor pressure of HOPG is negligible at the temperatures experienced during measurements. HOPG is non-polar and thus chemically inert.
2.8.2 Substrate: Gold

Gold is a metal that is stable in air under normal conditions. It has a ccp (cubic close-packed) crystal structure as shown below in Figure 18. It is unreactive with most materials.

![Gold Crystal Structure](image)

Figure 18. Gold Crystal Structure [35]

The gold photoemission spectrum has features in the valence band, which must be accounted for to determine the deposition-specific results. Many of these valence band features are related to the d-orbitals in the electronic structure of gold. It is the high density of states and position of these orbitals in the valence band that contributes many metallic characteristics for this and other similar elements. Among these characteristics are non-transparency, conductivity, and lustre.
2.8.3 Deposited Material: Ribonucleic Acid (RNA)

Figure 19. RNA and DNA Structure [36]

A ribonucleic acid (RNA) molecule is a self-assembling linear polymer, chemically similar to a single strand of DNA. Some RNA is found in the nucleus, where it is synthesized, and in the cytoplasm, as messenger RNA, transfer RNA or ribosomal RNA. RNA is involved in protein synthesis and other important catalytic steps. The electronic properties and self-assembling nature of RNA are of interest if it can be of use as an electronic device. Some testing has been done which shows some promise for RNA
or DNA as a conductor or semiconductor. However these testing results seem to vary widely.

The chemical backbone of RNA, shown with DNA in Figure 19, is composed of alternating groups of sugars (ribose) and phosphates. Each of these phosphate group monomers is connected with the ringed ribose sugar groups and a nitrogenous base on the side to form the RNA Polymer. There are four nitrogenous base chains in RNA: guanine, C$_5$H$_5$N$_5$O (G), cytosine, C$_4$H$_5$N$_3$O (C), adenine, C$_5$H$_5$N$_5$ (A), and uracil, C$_4$H$_4$N$_2$O$_2$ (U). These bases bond with complementary bases in DNA to form base pairs in the double helix. In RNA, unlike DNA, uracil (U) is substituted for thymine, C$_5$H$_6$N$_2$O$_2$ (T) in the genetic code. Those bases with a single ring of atoms are called pyrimidine; those with two rings are called purine. When each of these is combined with a ribose or deoxyribose sugar and to a phosphate group, they form a nucleotide. Nucleotides form the basic building units of RNA and DNA. RNA delivers DNA's genetic message to the cytoplasm of a cell where proteins are made. Adenosine, C$_{10}$H$_{13}$N$_5$O$_4$, is a nucleoside, a compound that consists of adenine and ribose and is found in DNA or RNA. Similarly, the nucleoside cytidine, C$_9$H$_{13}$N$_3$O$_5$, is a nucleoside that consists of cytosine and ribose.

The larger structure of a complete RNA molecule is not a simple helix, though it does have only a single Polynucleotide strand. Due to the presence of large regions of complementary adenine and uracil (AU), or guanine and cytosine (GC) pairs, the molecule folds on itself forming structures called hairpin loops. As seen below in Figure 20, in each of these base pair regions, the RNA molecule forms a partial double helix structure similar to that found in DNA [37]. The formation of these intricate structures is necessary for the RNA to perform its function.
2.9 Thin Film Deposition Modes

2.9.1 Types of Deposition Modes

Previous experiments have shown that photoemission spectroscopy experiments are useful for the study of thin film deposition modes [38]. The deposition modes determined by these measurements are shown below in Figure 21.

Through the study of these AES experiments three primary deposition modes have been found and named after their original investigators. The three main deposition modes are:
- Volmer and Weber or VW mode, which is characterized by 3-D crystal deposition formation prior to any adsorbed monolayer. This mode is also known as a wetting mode. However, the ideal VW mode never really occurs since there is always some finite amount of monolayer formed.

- Stranski-Krastanov or SK mode, which is characterized by formation of a monolayer followed by crystallite deposition.

- Frank-van der Merwe or FM mode, which is characterized by formation of a series of monolayers [38].

The temperature represents the surface energy of the material and determines which thin-film deposition mode will occur. Consider the surface energy of the adsorbate, $\gamma_A$, of the substrate, $\gamma_S$, and of the material interface, $\gamma_I$. When $\gamma_A + \gamma_I < \gamma_S$ the FM mode is preferred. In this case, the substrate has more heat energy than the adsorbate and interface. When $\gamma_A + \gamma_I > \gamma_S$ the SK or VW modes are preferred.

Surface diffusion rates determine when the adsorption equilibrium is reached. The above classifications are based on achieving local surface equilibrium. If the substrate material is more refractive, has temperatures lower than those used as the “isothermal equilibrium temp”, higher deposition rates, or lower surface mobility rates, then the deposition rate may be only marginally stable. This instability gives rise to two additional deposition modes: Simultaneous Multilayer, (SM), and Monolayer plus Simultaneous Monolayer (MSM) deposition modes, shown in Figure 22.

The SM deposition mode arises due to low surface mobility in which each vapor molecule that is deposited on the surface stays where it lands. This low mobility allows layers to be built in a random manner, with a new layer being able to start forming as soon as any of the underlying layers are begun. SM deposition is a less-stable variation of the FM deposition mode and is also called pseudo-FM or Poisson deposition mode.

The MSM deposition mode appears to arise from the change in surface mobility of the deposited adsorbate on the substrate with mobility of the adsorbate on the
underlying deposition layer. In this mode, the first monolayer forms as in FM mode due to high surface mobility of adsorbate on the substrate but with additional layers forming with lower mobility as in SM mode.

![Diagram of deposition modes](image)

**Figure 22.** Additional Deposition Modes Arising from Instability in Surface Deposition Conditions. Simultaneous Monolayer (SM) Mode and Monolayer Plus Simultaneous Monolayer (MSM) Mode.

### 2.9.2 Characterization of Deposition Modes

Deposition thickness vs. time plots for a gas or vapor can show the means of adsorption on the material surface. These plots, as shown in Figure 23, compare the changes in the signals for both the adsorbate and substrate. Breaks or changes in the slope of the curve represent the beginning of a new monolayer. Similar plots were prepared using XPS-derived thickness data in this experiment.

The FM mode has straight-line plots for both the adsorbate and substrate. Changes in the slope of the plots occur when a new monolayer is formed due to attenuation of the signal from the previous layer [38].

The SK mode is characterized by the signal achieving a stable plateau. This is due to the formation of 3-d deposition clusters on the surface after the monolayer. These
clusters cover a relatively small area of the surface but are not easily detected by AES. This characteristic of 3-d clusters is also evident in VW deposition mode spectra.

Thickness monitors are often used to determine the amount of material being deposited. The time-scale isn’t always proportional with the amount of material deposited. The sticking probability, S, changes as new layers are formed. This also may be seen on the thickness graph.

LXPS and XPS, as demonstrated in this series of experiments, have also been used for examining thin-film deposition. LXPS is particularly effective in monitoring FM mode deposition. Changes in the work function, $\phi$, are often related to changes in adsorbed layer structure [38].

![Deposition Thickness Versus Time Plots](image)

Figure 23. Deposition Thickness Versus Time Plots. These Plots Compare the Changes in the Signals for Both the Adsorbate and Substrate to Predict the Deposition Mode on the Material Surface. a) VW Mode, b) SK Mode, c) FM Mode, d) Formation of Monolayer Followed by Mingling of Substrate Molecules into Adsorbed Layers, e) SM Mode, and f) MSM Mode.
Chapter Three

Experimental

3.1 Equipment Used

Equipment used in these measurement series included the UHV system, ES system, XPS and UPS measurement systems, computer, substrate sample materials, and RNA sample materials.

3.2 Sample Preparation

3.2.1 HOPG Sample Preparation

Each sample holder and cleavage foil was cleaned with acetone, isopropanol, and methanol in an ultrasonic bath for 20 minutes successively prior to assembly. It is important that the sample materials be clean and dry before being joined together.

3.2.1.1 Epoxy of HOPG Substrate to Sample Holder

Conductive epoxy was used to bond the sample surface to the sample holder below and to the cleavage foil. A binary component conductive silver epoxy kit from M.E. Taylor Engineering was used for this process. It is important that conductive epoxy is used to avoid sample charging during the measurement. Equal portions of each epoxy component were squeezed out onto a clean surface and mixed thoroughly. A thick coat was applied to the desired bonding surface the other bonding surface was applied to it with light pressure. For this series of experiments Grade SPI-3 10x10x4mm HOPG blocks were originally used but were later upgraded to Grade SPI-2 10x10x2mm blocks.

The epoxy can be cured in four hours at room temperature or in five to ten minutes when heated to 121 °C, although optimum curing is not achieved until 24 hours
have passed. Our samples were heated to 160 °C for one hour and allowed to remain at room temperature for at least a day. This exceeded the curing requirements of the epoxy in all respects while not damaging the material. Properly curing the material was critical, as improper bonding of the epoxy could result in poor cleaving of the material and a failed experiment.

3.2.1.2 HOPG Sample Cleaving

The surface that serves as a substrate should not only be free of surface defects, but impurities from dust, water vapor, and trace atmospheric gases. A cleaving device was used to remove the cleavage foil shown below in Figure 24 and the top layer of sample material. This ensured that a fresh, clean layer of HOPG sample was exposed within the UHV environment and ready for RNA deposition. Cleaving may also be performed by removing a thin layer of the surface by peeling it away by applying a piece of Scotch tape, for example, and pulling it away. This is not practical for our UHV arrangement, however.

![Cleavage Foil](image)

Figure 24. HOPG Sample Cleaving

3.2.2 Gold Sample Preparation

3.2.2.1 Epoxy of Gold Substrate to Sample Holder

A silver epoxy was used to bond the bottom of the sample material to the sample holder below.
3.2.2.2  Sputtering of Gold Sample

Sputtering with an Argon ion gun for 20 minutes at 5kV and pressure at approximately 10⁻⁸ mbar was sufficient to remove ambient contaminants from the gold sample surface as shown below in Figure 25. This is verified by the first measurement prior to deposition of new material.

![Argon Ions](image)

**Figure 25. Gold Sample Sputtered Clean**

3.2.3  RNA Solution Preparation

Small amounts of RNA were carefully weighed and placed in the solution bottle. This was performed by first measuring the weight of the bottle, and then adding the desired amount of RNA solute by measuring the total weight of the bottle with its new RNA contents. The amount of solute present in the bottle is then calculated as the difference between the weight of the bottle with RNA and the weight of the bottle alone. A measured amount of DI water was then placed in the bottle to form the solution. Dissolution of the RNA occurred very quickly. There was no buffer used with the DI water in this experiment. It had been believed that the use of a buffer may complicate clogging problems. The absence of a buffer may allow the RNA to become denatured. The RNA solution was kept available for use from one day but not later than one week after preparation. This was in order to minimize the degradation of the RNA material. The minimization of clogging problems had been of greater concern at the time of the experiment.
The RNA Poly ‘A’ product lot used in this set of experiments had 100-900 nucleotide bases per molecule with a majority of them having 250-400 bases. Unlike naturally occurring RNA structures, this lot does not have other alternating nucleic acid bases, only adenine. As shown below in Figure 26, adenine bonds with a sugar molecule to form adenosine.

![Figure 26. Adenosine Molecule](image)

### 3.3 Measurement Process

Figure 27 shows a simplified representation of the measurement process. A clean sample was introduced into the UHV environment and measured as-is. Some of the RNA film to be measured was deposited on the sample, followed by measurement of the sample again. This sequence is repeated until little or no change is seen. At this point the sample surface will be deeply covered in the new material. In this way, with a multi-step deposition of material, the electronic structure of the sample surface can be characterized as a function of material thickness.
XPS measurements were performed in a commercial UHV multi-chamber system. LXPS spectra and the XPS survey spectra were performed using dual X-ray gun with the Mg anode. LXPS involved using the XPS equipment with the x-ray source in standby rather than in “operate” while measuring at the binding energies normally scanned with UPS. LXPS provides better work function data and is not affected as UPS is by charging. The monochromator was used to measure the core level spectra of XPS with the Al anode. The monochromator provided better resolution for the core level scans, while the dual x-ray anode provided low intensity scans for the survey spectra.

For this work we used the Mg anode for the monochromated core level scans and the Al anode for the survey spectra. MgKα x-rays are emitted at 1253 eV while Kβ x-rays are emitted at 1240 eV. Other energies may occur but these are the most important. The AlKα source photon is emitted at \( h\nu = 1486.6 \text{ eV} \), while the MgKα source radiates at \( h\nu = 1253.6 \text{ eV} \). The base operating conditions were approximately 25°C and 5x10^{-10} mbar.
The Ultra High Vacuum system (UHV) here at the University of South Florida (USF) Surface Science Lab, shown in Figures 15 and 27, is made up of the Molecular Beam Epitaxy (MBE) chamber, Sample Analysis Chamber (SAC), and the Load Lock (LL), as well as the recently added Electrospray (ES) chamber. It is important that XPS analysis be performed in an Ultra-High Vacuum (UHV) environment. This ensures that residual gases in the chamber are at a minimum so that samples remain clean for long periods during measurement [26].

Each of these chambers is important in surface science experiments and has the following purposes: The Load Lock is used as an air lock to introduce samples from atmosphere into the UHV environment. From there a sample will enter the MBE chamber and may be either transferred to the ES chamber or SAC. In the MBE chamber, which is used as a sample preparation area, a sample may be sputtered clean with an ion gun. In the SAC, a sample may be analyzed with either XPS or UPS. Electrospray deposition and cleaving of samples is performed in the ES chamber.

Several experiments were performed in attempts to determine the best method to perform the electrospray with RNA. Methanol and anhydrous acetonitrile were also used as solvents, but due to clogging problems it was determined that DI water was a better choice. RNA dissolves very quickly in water because the positive regions of a water molecule are attracted to the negative portions of RNA and vice-versa. It was decided to use lower concentration solutions to lessen the probability of clogging problems as well. RNA solutions used in these experiments were 1 mg/ml or less. Following preparation of the solution, a syringe with up to 5 ml of the solution was placed in a commercially available Cole Parmer Series 74900 Syringe pump.

Preliminary scans of the sample were made to achieve a baseline before deposition of RNA on the surface. Measurements were made as close to the same spot on the sample as possible in each measurement step.
During LXPS and UPS measurements, a -5 V bias was applied to the sample to help to shift the high binding energy cutoff position for the sample material away from that of the analyzer. The bias also makes the slope from which the work function is determined somewhat more vertical, simplifying the calculation. To avoid collection of stray electrons from surrounding areas, an elevated sample holder is used, removing the sample from the area of the sample holder assembly [39].

UPS measurements were conducted in order to determine the structure of the valence shell. It was important at the start of each UPS setup procedure that the sample is 3-4 mm away from the measurement position. It has been demonstrated that if the sample is exposed to the Helium lamp when it is first turned on that the work function of the material can be significantly reduced, giving erroneous indications of the nature of the material [40]. This may be due to irradiation causing damage or chemical modification to the structure of the sample, especially biological material such as RNA. Previous experiments have shown that the presence of environmental contaminants, such as water or residual atmospheric gases, may play a key role in these chemical changes. The margin of error for a UPS measurement is regarded to be ±0.1 eV [40]. It should be noted that the UPS measurements were taken with a higher (more closed) aperture setting of 4 (4mm diameter) rather than the XPS aperture setting of 1 (6x20mm slit) for high-resolution core-level spectra or 2 (6mm diameter) in order to compensate for the normally much higher UPS intensity. A pass energy of 0-10 eV was used for small area scans, such as those used in UPS, LXPS, and high-resolution core level spectra. A pass energy of 0-30 eV was used for large area scans, such as those used in XPS survey spectra.

The XPS survey spectra (using the dual anode x-ray source) provided an overview of the elements on the sample surface. The core level spectra, using the monochromator, allowed each element of particular interest to be viewed in greater detail. The monochromated Al source used in measuring the core level spectra has a linewidth of 0.5 eV.
During the electrospray procedure, targeting of the spray was crucial. The beam is very fine and easy to miss. Also, due to the breakup of the solution and the minute volume of material being sprayed, the beam is not visible to the naked eye. Verification of the presence and proper alignment of the spray was determined by several means. The presence of spray could be verified visually inspecting the capillary and nozzle prior to insertion in the vacuum environment, or electronically by the Faraday cup or deposition monitor. Proper alignment of the spray was determined by adjusting the micrometer position of the sample transfer rod and the position of the spray nozzle. A simple crosshair was setup in a viewing port above the sample to ensure that the sample was lined up correctly with the nozzle.

The vacuum pumps were energized and nitrogen gas flow was started. Nitrogen gas pressure was set to 160 kPa (approximately 23 psi). The electrospray flow rate was set to 2 ml per hour and started. The high voltage power supply voltage (set to 1500 V) was applied. It was important to ensure that power did not trip off during the spray process. After starting the syringe pump, the syringe was checked to ensure that it was tightly being depressed. Some looseness between these components was common at the start of an electrospray session. After the syringe became snug and allowing the flow meter to indicate that between 10-20 ml of solution had been sprayed, the gate valve that separated the ES chamber from the spray nozzle was opened. This allowed electrospray to begin to reach its target. The purpose of the 10-20 ml delay in spraying is to ensure that they solution has reached the end of the capillary and actually spraying when the valve is opened.

3.4 Data Evaluation

By comparing the measurements of a sample with some thin film deposited and that of a known sample of the same material, the thickness of the film may be determined. This is done by employing sample standards and correcting the signals with sensitivity factors [31].
The experimental series were evaluated and fitted using the Igor Pro wave analysis, curve-fitting software program from Wavemetrics. Mixed Gaussian-Lorentzian line shapes were used in fitting the high-resolution core level spectra. Each graph has a y-offset in place to avoid overlapping spectra.

The bending in the top of some the UPS spectra for layers with higher thicknesses is not suitable for calculation of the high binding energy cutoff value. The straightest section of the lower portion of the edge was used in the calculation.

Binding energies and work functions were determined for each deposition step for experimental series. The combination of UPS and XPS has been well documented in the study of inorganic interfaces and has proven to be useful with these organic interfaces as well [9-11]. Using UPS alone would limit the results of the experiment, as several phenomena occur simultaneously. Band bending would be the most significant of these phenomena but is not a factor in metallic materials. Band bending would shift the entire photoemission spectrum. An interface dipole shifts only the high binding energy cutoff. This fact is used to distinguish the two effects and to calculate the size of the dipole. The interface dipole is calculated by subtracting the HOMO shift from the total work function change. Determination of the work function and HOMO position from the spectra as a function of film thickness allows further understanding of the interface dipole, orbital alignment, and electronic structure.

The work function is calculated by subtracting the high binding energy cutoff value from the 21.21 eV He I radiation from the UPS lamp and then subtracting a 0.1 eV correction factor. The HOMO cutoff and high binding energy peaks both require this 0.1 eV correction factor for analyzer broadening (including thermal broadening), however the HOMO cutoff is corrected by adding 0.1 eV. The HOMO position may be graphically determined by inspection of the HOMO features on the UPS spectra and fitting straight lines with the HOMO cutoff region to find the intersection of that line with the x-axis. If the substrate material has significant emission in the HOMO region, then
the clean substrate spectrum should be subtracted from subsequent spectra to produce difference spectra for use in HOMO calculations. The effects of interface dipoles and band bending on a typical spectrum are shown below in Figure 28.

Figure 28. Effects of Band Bending and Interface Dipoles on Spectra. a) Normal Hypothetical Band Diagram and Spectrum, b) Band Diagram and Spectrum with Interface Dipole, c) Band Diagram and Spectrum with Band Bending. This figure shows the effects of interface dipoles and band bending on a typical spectrum. The dipole shortens the spectrum by shifting the high binding energy cutoff to a lower binding energy while not affecting the rest of the spectrum. Band bending shifts the entire spectrum to lower binding energy [41].

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It has been shown that with the proper background removal procedure, the properties of the deposited overlayer material can be determined. For the UPS spectra, background removal is only useful in the HOMO peak emission region, while the high binding energy region is distorted.

The onset of charging effects from the photoemission process can also shift the entire photoemission spectrum. Charging occurs when materials with poor conductivity are measured. Charging becomes a greater factor with thicker deposition layers and less time between measurements. Thicker layers of RNA may lead to lower surface conductivity and allow buildup of charge on the surface.

The above formula for measured intensity \( I(E) \) relates the intensity as a function of the binding energy [42]. \( I_0 \) is the maximum intensity of the line, \( E_0 \) is the binding energy of the line, and \( \Gamma \) is the FWHM. Where \( M=G/L \), the Gaussian/Lorentzian ratio, in which \( M=0 \) refers to a pure Gaussian distribution and \( M=1 \) represents a purely Lorentzian distribution. After removal of background noise from spectra, this artificial Gaussian/Lorentzian mixed function was used to simulate spectral components for curve fitting.

\[
I(E) = I_0 \cdot \left[ 1 + 4M \left( \frac{E - E_0}{\Gamma^2} \right)^2 \cdot \exp \left( 1 - M \right) - \frac{4\ln 2 \left( E - E_0 \right)^2}{\Gamma^2} \right]^{-1}
\]

Although the photoelectric spectra and integrated area curves provide a good indicator of the successful deposition of RNA, the application of correction factors dependent on the particular instrument and elements present provides more useful data.

A transmission function (TF) may be used to achieve a better estimate of the elemental concentrations in the sample. The transmission function represents the relationship between the number of electrons that go in the entrance slit of the analyzer and those that come out of the exit and is dependent on the kinetic energy of the electrons. The transmission function is found by the following formula:
\[ 
TF (E_{\text{kin}}) = 1.18 \times 10^6 \times 5.73 \times 10^4 \times E_{\text{pass}} \times (E_{\text{kin}}/E_{\text{pass}})^{-0.66}. 
\]

However, for the purpose of atomic concentration calculations the constants at the front of the equation may be disregarded, so that TF is proportional to \((E_{\text{kin}}/E_{\text{pass}})^{-0.66}\). The exponent, \((-0.66)\), is particular to the dimensions of the aperture used in the experiment. Percentage atomic concentrations for a given element \((C_x)\) are calculated by finding the integrated area or Raw Peak Area (RPA) of each curve, normalizing these numbers with the TF and cross-section factor, and finally finding what percentage that number is out of the total amount of material seen in the scan.

The atomic subshell photoionization cross section is used as a Sensitivity Factor (SF) relating the likelihood of a particular element to absorb a photoelectron. The Normalized Peak Area intensity (NPA) is then found by dividing the RPA by the product of the TF and the SF. The percent concentration of an element is calculated by dividing the NPA for that element by the sum of the NPAs for all other elements measured in the sample and then multiplying by 100.

\[
\text{NPA} = \frac{\text{RPA}}{(\text{TF} \times \text{SF})}, \quad C_x = \frac{\text{NPA}_x}{(\text{NPA}_x + \text{NPA}_y + \text{NPA}_z)} \times 100
\]

Graphs of these concentrations versus volume of RNA solution deposited were developed, as presented in the deposition morphology section of the discussion section for each experiment. The theoretical and experimental values are compared after measurement and evaluation. Additionally these concentrations are presented as ratios of each element to C1s in the discussion section of the experiments.

Possible sources of error for these measurements include the following:
1) Incorrect background removal when calculating peak areas. 2) Incorrect use of sensitivity factors or transmission functions. 3) Instrument error. Also, the area measured may not be representative of the entire surface of interest.
Chapter Four

Results and Discussion

4.1 HOPG/RNA Interfaces

4.1.1 Results for RNA Poly Adenosine on HOPG

A thin film of RNA Poly adenosine was deposited on a freshly cleaved HOPG sample surface. 6.4 ml of a 0.67mg/ml solution were deposited on the surface in 7 steps. The first scan on each experimental series represents the surface material as-is. The last scan should be much more representative of the solute from the solution, RNA. Evidence can be seen of the oxygen, nitrogen, carbon, and phosphorus that are present the RNA. Over time, the process of forming a complete deposition of this new surface can be seen in the UPS and XPS spectra.

The complete series of UP Spectra for the deposition series of RNA Poly adenosine on HOPG is shown in the center of Figure 29 with the high binding energy cutoff shown on the left, and the high binding energy HOMO cutoff position shown on the right. The spectra on the right are shown with the inelastic background removed. The top of high binding area (secondary edge) area of the UPS spectra is shown to distort, while the slope of the line changes as well. The valence band region in the center and left portions of the figure acquires deposition-specific features while those characteristic of the substrate material become less prominent. The center of Figure 30 shows the same UPS spectra featured in Figure 29, but compares it with LXPS spectra from immediately before (left) and after (right) the UPS measurements were performed. A comparison of the change in work function for each of these spectra versus the total volume of material deposited is displayed later in Figure 37.
Figure 29. UPS Spectra with RNA Poly Adenosine on HOPG

Figure 30. High Binding Energy Cutoff with A) LXPS Before UPS, B) UPS, C) LXPS After UPS
Figure 31 shows the XPS survey spectra for this series. Figure 32 features each of the most significant photoelectron lines, O1s, N1s, and P2p, of the major elemental components of RNA which were expected and found on the surface. Figure 33 includes the C1s spectra.

The XPS survey spectrum in Figure 31 and the core level survey spectra in Figures 32 and 33 revealed four elements present on the sample surface in significant amounts. These were oxygen, nitrogen, phosphorous, and carbon. For carbon, the C1s line at 285 eV and a KVV line at 1223eV were noted. Shake-up peaks were noted in the binding energies just above the primary C1s line. These peaks became more pronounced as the primary C1s line became weaker with successive layers of deposition. The P2p group is the most distinctive and important for phosphorous [30].
Figure 32 shows high resolution spectra of the most significant peaks except carbon. The most prominent O1s emission peak, in the left portion of Figure 32, occurred at 533.3 eV with a smaller intensity peak at 531.3 eV. The large and small oxygen peaks may be due to the presence of non-bridging and bridging oxygen bonded with phosphorus in a phosphite group (PO₃), although the smaller peak may also have a contribution from a hydroxyl group, OH. These peaks shifted by approximately 0.5 eV to higher binding energies from the beginning to end of the experiment as the overlayer grew thicker. These shifts are likely a result of developing charging effects as the film grows thicker. The next-to-last layer showed a full wave half maximum (FWHM) of 2.2 for the primary peak, and 1.3 for the smaller peak. The final layer is not used for this measurement due to possible charging effects. The intensity of the second peak is approximately 40% of the primary peak and remained so for the duration of the experimental series.

![Figure 32. O1s, N1s, and P2p Spectra with RNA Poly Adenosine, Background Removed](Image)

**Figure 32.** O1s, N1s, and P2p Spectra with RNA Poly Adenosine, Background Removed
The most prominent N1s emission peak, shown in the center graph of Figure 32, occurred at 399.4 eV with a smaller intensity peak at 400.9 eV. The peak shifted by approximately 0.5 eV to higher binding energies as the overlayer grew thicker. The smaller peak is not distinct and forms a shoulder on the left of the larger peak. The next-to-last layer showed a FWHM of 1.4 for the primary peak, and 2.4 for the smaller intensity peak. The intensity of the second peak is approximately 68% of the primary peak. The large peak is likely due to the presence of nitrogen in unsaturated chemical bonds, while the lesser peak blended into the shoulder is likely due to a carbon-nitrogen (C-N) bond.

The most prominent P2p emission peak, as shown on the right in Figure 32, occurred at 134.1 eV. This peak was expected to have a smaller intensity doublet peak due to orbital splitting, but the second peak was too close to the first to be resolved in this measurement. The peak shifted by approximately 0.5 eV to higher binding energies as the overlayer grew thicker. The next-to-last layer showed a FWHM of 1.6 for the primary peak. The location of the peak is indicative of metaphosphates such as HPO₃.
The C1s spectra, displayed in Figure 33, initially show a very large primary peak at 284.2 eV and a small set of shake-up peaks with the most distinct one at 291.0 eV arising from the $\pi$ to $\pi^*$ transition. The primary peak is characteristic of graphitic carbon. These diminish as the RNA layer grows, and at least four smaller deposition peaks rise at higher binding energies, with the strongest at approximately 287.0 eV. The primary peak was shifted by approximately 0.5 eV to higher binding energies as the overlayer grew thicker. The primary peak became less intense, while the deposition peaks became more intense with increasing layer thickness. These shifts occurred due to changes in the chemical composition of the surface. The largest of the deposition peaks appears to be consistent with a C-N or C-O bond, such as in CO$_x$ or HCO$_x$. The next-to-last layer
showed a FWHM of 0.7 eV for the primary peak, and 2.6 eV for the smaller peak. The intensity of the shake-up peak grew to approximately 40% of the primary peak for the thickest layer.

4.1.2 Discussion

4.1.2.1 Deposition Morphology

The findings relating to deposition of RNA Poly adenosine on HOPG show successful deposition of material. This deposition appears in a steady linear fashion, although the amount of spray was doubled after each run. This deposition behavior depends on the deposition mode [38, 44]. This would seem to suggest that the surface accepts a layer of material and may then become somewhat resistant to accepting more material. Of course, once the surface material becomes thick enough, the lower layers will no longer be fully seen by XPS and UPS. It was expected that the amount of each major element present in RNA Poly ‘A’ would be properly proportionally represented in the measured sample. This would not be altered by any degree to which each RNA chain may have been broken or damaged in processing or in measurement. Atomic Force Microscopy (AFM) has verified the presence of string-like structures forming in ring patterns on the surface of the sample after deposition. Percentage atomic concentrations for each element were also used as indicators for deposition rates.

AFM scans of HOPG with and without deposition are shown below in Figure 34. In the plain HOPG, shown on the left, the thin layered nature of HOPG can be seen. The right portion of the figure shows droplets of RNA deposited on the surface. The portions of the rings which are shown in red are at the high end of the measurement scale used in this figure. These areas account for 5.6% of the total area being measured. The majority of the remaining surface is covered in a series of overlapping droplets.
Figure 34. AFM Study of a Clean Sample of HOPG in 100 µm² and RNA Poly ‘A’ on HOPG in 25 µm². Although These Are Not of the Same Area of HOPG, They Do Provide Insight Into the Changes in Surface Properties.

The integrated areas for each thickness layer were calculated versus the amount of solution electrosprayed. These values were used in calculating the amount of material present. The final atomic concentrations of the O1s, N1s, P2p and C1s traces above background were approximately 14.10%, 12.08%, 3.84%, and 69.98%, respectively. Consider that the chemical composition of Poly adenosine is (C_{10}H_{11}N_{5}O_{6}P)_{x}. The theoretical atomic concentrations should be 27.27%, 22.73%, 4.55%, and 45.46%, respectively, using a simple ratio of each element out of the total, neglecting hydrogen. Presented as ratios of each element to C1s carbon the results were approximately 0.20, 0.17, and 0.06 : 1 respectively, while the theoretical atomic concentrations should be 0.60, 0.50, and 0.10 respectively, while neglecting hydrogen.

The carbon concentration is expected to be higher than the presence of RNA would account for due to the deposition not completely covering the HOPG surface in sufficient thickness. The peak HOPG intensity was reduced 86% from its original values. Other atomic concentrations are lowered accordingly due to lack of coverage of the HOPG surface to a sufficient depth to eliminate the contribution of the substrate to the spectra. As shown earlier, Figure 33 shows the characteristics of the C1s spectra changing as the deposition continues. The intensity of the main peak is reduced, as
expected, but the intensity of peaks at higher BE’s increases. This indicates a change in the source of the carbon on the surface. The carbon from RNA is becoming dominant in these spectra. The percentage atomic concentrations used in Table 2 and Figure 35 were calculated as described in the deposition characterization section. These results are within an acceptable margin of error for measurements of this type. The data for these results was calculated from the XPS high resolution spectra for each element using the appropriate sensitivity factors and using consistent measurement parameters throughout.

<table>
<thead>
<tr>
<th>Volume of RNA Poly’A’ (ml)</th>
<th>O1s</th>
<th>N1s</th>
<th>P2p</th>
<th>C1s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.04</td>
<td>0.14</td>
<td>0.02</td>
<td>99.80</td>
</tr>
<tr>
<td>0.05</td>
<td>1.01</td>
<td>0.63</td>
<td>0.24</td>
<td>98.13</td>
</tr>
<tr>
<td>0.2</td>
<td>2.19</td>
<td>1.87</td>
<td>0.53</td>
<td>95.41</td>
</tr>
<tr>
<td>0.4</td>
<td>4.05</td>
<td>3.11</td>
<td>1.10</td>
<td>91.73</td>
</tr>
<tr>
<td>0.8</td>
<td>6.84</td>
<td>5.63</td>
<td>1.68</td>
<td>85.85</td>
</tr>
<tr>
<td>1.6</td>
<td>9.07</td>
<td>7.45</td>
<td>2.37</td>
<td>81.11</td>
</tr>
<tr>
<td>3.2</td>
<td>11.93</td>
<td>9.82</td>
<td>2.92</td>
<td>75.33</td>
</tr>
<tr>
<td>6.4</td>
<td>14.10</td>
<td>12.08</td>
<td>3.84</td>
<td>69.98</td>
</tr>
</tbody>
</table>
The deposition mode of RNA ‘A’ on HOPG appears to be consistent with the SK or VW mode of deposition, when comparing the deposition observed in the experiment, shown in Figure 35, with the deposition mode analysis for typical SK deposition mode, shown in Figure 23 [38]. However, rather than crystal deposition, as a Polymer RNA is more likely to form clumps of RNA strands. Further study may be required to determine if a monolayer is formed early in the deposition of RNA on the surface. More data points would be useful for a conclusive determination of deposition mode.

4.1.2.2 Electronic Structure

Table 3 shows the photoelectron lines which are expected to appear in the XPS spectra, including Auger peaks. The more significant lines are annotated with an asterisk (*).
Table 3. Expected Spectral Lines for RNA on HOPG. The Lines Which Are Marked with ‘*’ Are Particularly Important and Most Prominent

<table>
<thead>
<tr>
<th>Photoelectron Lines</th>
<th>Auger peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td></td>
</tr>
<tr>
<td>*1s line at 531eV</td>
<td>KLL lines at 978, 999, and 1013eV</td>
</tr>
<tr>
<td>2s line at 23eV</td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
</tr>
<tr>
<td>*1s line at 398eV</td>
<td>KLL line at 1107eV.</td>
</tr>
<tr>
<td>Phosphorous</td>
<td></td>
</tr>
<tr>
<td>2s line at 188eV</td>
<td>LMM line at 1367eV</td>
</tr>
<tr>
<td>*P2p1/2 line at 131eV</td>
<td></td>
</tr>
<tr>
<td>*P2p3/2 line at 130eV</td>
<td></td>
</tr>
<tr>
<td>3s line at 14eV</td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td></td>
</tr>
<tr>
<td>*1s line at 285eV</td>
<td>KVV line at 1223eV</td>
</tr>
</tbody>
</table>

The data for the electronic structure of RNA Poly ‘A’ on HOPG was derived from the UPS spectra in Figure 29 and the LXPS ‘A’ spectra in Figure 30. Data was acquired for this experimental series on the high binding energy cutoff (SE), work function, and HOMO cutoff energy values for each thickness layer in this experiment and are displayed below in Table 4. High binding energy cutoff values for RNA Poly ‘A’ were determined using both UPS and LXPS. The values derived from UPS were believed to be deceptive due to charging effects. Values derived from LXPS ‘A’, seemed more reliable and remained nearly constant throughout the measurement series. The values for the 1.6 ml deposition step were typical of those for the series. The high binding energy cutoff, work function, and HOMO cutoff energy values were 16.82 eV, 4.38 eV, and 2.4 eV respectively. Figure 36, below, illustrates the method for determining the HOMO high binding energy cutoff. The LXPS ‘A’ data also suggests that there is no significant interface dipole present.
Table 4. High binding energy cutoff, Work Function, and HOMO Cutoff Energy Values for RNA Poly ‘A’ Derived from Figure 29. HOMO values were derived as in Figure 36 below. High Binding Energy Cutoff (SE) Values Were Derived from the LXPS Curves Rather than UPS to Avoid Charging Effects.

<table>
<thead>
<tr>
<th>Volume of RNA Poly ‘A’ (ml)</th>
<th>SE</th>
<th>WF</th>
<th>HOMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOPG as is</td>
<td>16.87</td>
<td>4.44</td>
<td>n/a</td>
</tr>
<tr>
<td>0.05</td>
<td>16.79</td>
<td>4.52</td>
<td>2.1</td>
</tr>
<tr>
<td>0.2</td>
<td>16.89</td>
<td>4.42</td>
<td>2.2</td>
</tr>
<tr>
<td>0.4</td>
<td>16.87</td>
<td>4.44</td>
<td>2.2</td>
</tr>
<tr>
<td>0.8</td>
<td>16.84</td>
<td>4.46</td>
<td>2.2</td>
</tr>
<tr>
<td>1.6</td>
<td>16.82</td>
<td>4.48</td>
<td>2.4</td>
</tr>
<tr>
<td>3.2</td>
<td>16.84</td>
<td>4.46</td>
<td>2.5</td>
</tr>
<tr>
<td>6.4</td>
<td>16.83</td>
<td>4.46</td>
<td>2.9</td>
</tr>
</tbody>
</table>

![Graph showing HOMO cutoff calculation method](image-url)

Figure 36. Demonstration of HOMO Cutoff Calculation Method at the 1.6ml Deposition Step
Figure 37. The Change in Work Function as a Function of Material Deposited, Measured with UPS and LXPS Before UPS, and After UPS. The Results from LXPS Before UPS (LXPS‘A’) Suggest That There is Little Change in the Work Function as a Function of Layer Thickness. UPS Results Suggest That the UV Light Charges the Sample, although it only seems to effect the work function measurement. LXPS After UPS (LXPS‘B’) indicate that the Changes in the Work Function from the UPS Are Reversible and Thus Are Not Likely to be from an irreversible a Chemical Reaction.

Figure 37, above shows how the work function changes as the layer thickness grows for each method of measurement, UPS, LXPS before UPS (LXPS ‘A’), and after UPS (LXPS ‘B’). The work function as measured by UPS becomes higher with increasing layer thickness. These effects are not seen in the LXPS prior to UPS, and minimized in the LXPS afterward. This suggests that the sample is becoming charged during each exposure to UV light. The effect appears to be reversible and diminishes over time, as is evident from the UPS and LXPS ‘B’ results. The LXPS ‘B’ results are close to those measured in LXPS ‘A’ before UPS.
The band diagram for RNA Poly ‘A’ on HOPG, above in Figure 38, is derived from the data in Table 4. The energy levels for the plain HOPG surface are displayed on the left and RNA Poly A on the right. Using the LXPS data, no dipole is formed and energy values do not change significantly. Any band bending and dipole region would be shown at the material interface in the center of the diagram. The data used in the figure above was taken from the pre-deposition, as-is measurement step for the HOPG area and the 1.6ml deposition step for the deposited RNA. The data from later deposition steps is not represented here due to the onset of charging effects.

4.2 DI Water/HOPG Interface

4.2.1 Results for DI Water on HOPG

This experimental series was conducted in order to compare the results of electrospraying RNA onto HOPG with the effect of electrospraying the solvent by itself. 3.6 ml of the solvent were sprayed onto a clean sample surface in 4 steps as a control experiment.

Figure 39 shows the UPS spectra from the DI water on HOPG series. The center of the graph shows the entire UPS spectra, while the high binding energy cutoff is on the left, and the valence band region is shown with background removed on the right. In the
UPS spectra, a peak at approximately 13.5 eV, characteristic of graphite, shows little change in intensity as the spray process continues. High binding energy cutoff calculations were made using the straightest section of the graph on the left. Each graph has a y-offset in place to avoid overlapping spectra. Changes are visible in the valence band of the UPS spectra and may be due to dissociation of water molecules or atmospheric contamination.

Figure 39. UPS Spectra with DI Water on HOPG

Figure 40 shows the XPS survey spectra for the DI water on HOPG series. With the exception of carbon, no strong photoelectron lines are present. Figure 41 shows binding energies in which the most significant photoelectron lines of the major elemental components of RNA, O1s, N1s, and P2p would appear. In contrast with the findings for the RNA deposition series, the core level spectra for oxygen, nitrogen, and phosphorous in Figure 41, do not show any significant signal above noise level. The O1s spectra show a very small peak beginning to form at 532.5 eV. The C1s spectra shown in Figure 42, display a strong peak at 284.3 eV and several small shake-up peaks at approximately 291.0 eV arising from the $\pi$ to $\pi^*$ transition. These are characteristic of the carbon that
makes up the HOPG sample. These shake-up peaks occurred in binding energies just above the primary C1s line.

![Figure 40. XPS Survey Spectra with DI Water on HOPG](image)

![Figure 41. O1s, N1s, and P2p Spectra with DI Water on HOPG, Background Removed](image)
4.2.2 Discussion

4.2.2.1 Deposition Morphology

No significant deposition of material was noted, as was the ideal expectation. An analysis of the spectra and the integrated areas of the major elemental components of RNA was made, but is not presented here due to the minimal changes in deposition. The A small amount of deposition of oxygen was noted as the results showed only a slight O1s peak developing. This may have been due to a small amount of atmospheric gases leaking in during the electrospray process or due to the accumulation of the oxygen from
the water on the surface. The intensity of the carbon peak had diminished only 9%, during the experimental run. A lack of material deposition was demonstrated here, as was expected.

### 4.2.2.2 Electronic Structure

There were notable changes in the UPS spectra. While not extreme these changes may be due to repeated exposure to the UV light, as discussed earlier. Some decrease in work function was noted in the data, but this was not within the significant figures used in this measurement. The 0.1 ml deposition step was typical of the results. The high binding energy cutoff, work function, and HOMO cutoff energy values were 16.8 eV, 4.4 eV, and 3.5 eV respectively. These were derived from the UPS Spectra, but showed no significant change or charging effects.

### 4.3 Gold/RNA Interfaces

This experimental series was conducted in order to determine the electronic structure of the Au/RNA interface. HOPG is useful because of the “flat” UPS spectra that can give us the position of the RNA HOMO peak without complicated fitting procedures. However, gold is the preferred contact material in conductivity related experiments on DNA [15]. Therefore, the Au/RNA interface was investigated to determine its electronic properties. In particular, determination of the injection barriers was the main objective of the experiments.

#### 4.3.1 Results for RNA Poly Adenosine on Gold

A thin film of RNA Poly adenosine was deposited on a clean, freshly sputtered gold sample surface. There were 2.4 ml of a 0.67mg/ml solution deposited on the surface in 4 steps.
The complete UPS spectra for the deposition series of RNA Poly adenosine on gold is shown in the center of Figure 43 with the high binding energy cutoff shown on the left, and the high binding energy HOMO spectral region shown on the right. The HOMO spectra are shown with their backgrounds removed. The features in this area of the graph are characteristic of gold [30] and are notably different from the earlier series on HOPG. D-orbital related peaks appear at approximately 6.25, 4.25, and 2.75 eV, and become less intense as the layer thickness increases. The spectra shown in the figures have been shifted by a y-offset to avoid overlapping traces. The straightest vertical section of each high binding energy cutoff spectrum was used for calculation of the work function. Unfortunately LXPS data is not included in this series as it was not part of the measurement procedure at the time of these measurements.

Figure 43. UPS Spectra RNA Poly Adenosine on Gold

Figure 44 shows the XPS survey spectra for the deposition of RNA Poly ‘A’ on gold. The photoelectron lines present in the as-is measurement step are characteristic of gold and become less intense as layer thickness increases. Figure 45 features high-
resolution spectra of the most significant emission lines of RNA, O1s, N1s, and P2p. Figure 46 shows the C1s spectra.

Each of these elements, oxygen, nitrogen, phosphorous, and carbon, were found in significant amounts in the core level survey spectra in Figures 45 and 46. The most prominent O1s emission peak, shown in Figure 45, occurred at 533.1 eV with a smaller intensity peak at 531.2 eV. Both of these oxygen peaks may be an indication of oxygen in bridging and non-bridging phosphite groups. The smaller peak may also be due to the presence of a hydroxyl group, OH, as in the RNA on HOPG experiment. No peak shifting or band bending was noted. The final layer showed a full width at half maximum (FWHM) of 1.5 eV for the primary peak, and 1.4 eV for the smaller peak. The intensity of the second peak is approximately 59% of the primary peak and remained near this value for so for the majority of the experimental series.

Figure 44. XPS Survey Spectra RNA Poly ‘A’ on Gold
Figure 45. O1s, N1s, and P2p Spectra with RNA Poly ‘A’ on Gold, Background Removed

The most prominent N1s emission peak, shown in the center graph of Figure 45, occurred at 399.3 eV with a smaller intensity peak at 400.8 eV. The primary peak was shifted by approximately 0.5 eV to higher binding energies as the overlayer grew thicker. However, the smaller peak was not distinct and blended into the shoulder of the large peak. The large nitrogen peak is likely due to the presence of nitrogen in unsaturated chemical bonds. The smaller peak at the left shoulder is likely due to a carbon-nitrogen (C-N) bond. The final layer showed a FWHM of 1.4 for the primary peak, and 1.7 for the smaller intensity peak. The intensity of the second peak is approximately 49% of the primary peak in the last measurement step.

The most prominent P2p emission peak, as shown on the right in Figure 45, occurred at 133.8 eV with a smaller intensity, doublet peak at 134.7 eV. The doublet peak is not distinct and blends into the shoulder on the left of the larger peak and is only distinguishable from the noise level for the last two measurement steps. This doublet
peak is likely due to the presence of a phosphate group. The signal to noise ratio was relatively low for the P2p spectra in general for this experimental series. The final layer showed a FWHM of 0.8 for the primary peak and doublet. The intensity of the second peak is approximately 50% of the primary peak.

The C1s spectra displayed in Figure 46, features its most prominent peak at 286.8 eV and a small set of $\pi$ to $\pi^*$ transition shake-up peaks with the most distinct one at approximately 6.7 eV higher in BE. Unlike in the RNA on HOPG experiment, the carbon present in these spectra is due to the deposition alone. The largest deposition peak here corresponds to the 2nd largest peak in the RNA on HOPG experiment. This large peak is likely due to a C-N bond. This peak and the shake-up peaks develop more as the RNA overlayer grows thicker. The final layer showed a FWHM of 1.8 eV for the primary peak, and 1.3 eV and 1.2 eV for the smaller peaks, respectively. The intensities of the shake-up peaks were approximately 36% and 19% of that of the primary peak.

The Au4f spectra shown in Figure 47 are composed of a pair of peaks at 84.0 eV and 87.6 eV. The second, higher binding energy peak is at approximately 78% of the intensity of the primary peak and remains near this value for the duration of the experiment. Each peak is well above the noise level, as should be expected with gold as the sample material. No peak shifting was noted. Band bending is not possible in metals due to the inability to form depletion areas. The final layer showed a full wave half maximum (FWHM) of 0.8 for the primary peak.
Figure 46. C1s Spectra with RNA Poly ‘A’ on Gold, Background Removed

Figure 47. Au4f Spectra with RNA Poly ‘A’ on Gold, Background Removed
Figure 48. UPS Difference Spectra

The UPS difference spectra displayed in Figure 48 were determined using the RNA on Au spectra from the right portion of Figure 43 and subtracting the normalized Au signature form the spectra. The resulting difference spectra represent the RNA emission characteristics alone, similar to those achieved when measuring with RNA on HOPG in the right portion of Figure 29.

4.3.2 Discussion

4.3.2.1 Deposition Morphology

The findings relating to deposition of RNA Poly Adenosine on gold show successful deposition of material on the sample surface. The results closely resemble those of the electrospray deposition of RNA Poly ‘A’ on HOPG. The peak locations are very close to those in the previous experiment, indicating the same chemical species
being present on the material surface. This deposition appears in a steady linear fashion, although the amount of spray was increased steadily after each run. As in other deposition steps the surface seems to have become more resistant to accepting more material. It was expected that the amounts of each major element present in RNA Poly ‘A’ would be properly proportionally represented in the measured sample.

A deposition mode study was performed using percent atomic concentrations for each element studied. This study is aimed at finding the comparative deposition rates of each component of RNA on the surface. The percentage atomic concentrations are presented in Table 5 and Figure 49 and were calculated as described in the deposition characterization section. The final atomic concentrations of the O1s, N1s, P2p, C1s and Au4f traces after background removal were approximately 11.95%, 10.95%, 2.63%, 32.72%, and 41.75%, respectively. Knowing that the chemical composition of Poly adenosine is (C_{10}H_{11}N_{5}O_{6}P), theoretical atomic concentrations should be 27.27%, 22.73%, 4.55%, 45.46%, and 0%, respectively, using a simple ratio of each element out of the total, neglecting hydrogen. Presented as ratios of atomic concentrations of each element to C1s carbon the results were approximately 0.37, 0.33, 0.08, and 1.28 : 1 respectively, while the theoretical atomic concentrations should be 0.60, 0.50, and 0.10 respectively, while neglecting hydrogen.

These results approach the theoretical values, are in correct proportion for RNA Poly ‘A’, and are within an acceptable margin of error for measurements of this type.

The maximum gold peak intensity was reduced by 64% from its original value, however. This suggests that the electrospray did achieve significant coverage of the material surface, or at least of the surface being scanned [45]. Since carbon was not the background material in this experiment, it is interesting to compare the carbon traces with those of the HOPG series. This series on gold allows a determination of how much carbon is seen from deposition by electrospray, where this was more difficult with an HOPG background.
The deposition mode of RNA ‘A’ on gold, as with HOPG in the previously discussed measurement, appears to be consistent with the SK mode of deposition. Comparison of the deposition rates achieved in the experiment, shown in Figure 49, with the deposition mode analysis for typical SK deposition mode, shown in Figure 23 suggests that the SK deposition mode is preferred in these circumstances. However, rather than forming crystals as is familiar in SK deposition mode, the RNA Polymer is more likely to form clumps of RNA strands. Further study may be required to determine if a monolayer is formed early in the deposition of RNA on the surface. More data points would be useful for a conclusive determination of deposition mode. AFM studies of RNA on gold have not been helpful so far due to the roughness of the gold surface at this small scale being measured.

Table 5. Atomic Concentration (%) for Various Elements in Deposition of RNA Poly ‘A’ on Gold

<table>
<thead>
<tr>
<th>Volume of RNA Poly‘A’ (ml)</th>
<th>O1s</th>
<th>N1s</th>
<th>P2p</th>
<th>C1s</th>
<th>Au4f</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.63</td>
<td>1.14</td>
<td>0.77</td>
<td>0.95</td>
<td>96.51</td>
</tr>
<tr>
<td>0.2</td>
<td>3.64</td>
<td>2.43</td>
<td>0.56</td>
<td>14.35</td>
<td>79.01</td>
</tr>
<tr>
<td>0.4</td>
<td>3.93</td>
<td>2.66</td>
<td>0.67</td>
<td>23.16</td>
<td>69.58</td>
</tr>
<tr>
<td>1.4</td>
<td>10.64</td>
<td>9.52</td>
<td>2.71</td>
<td>33.39</td>
<td>43.73</td>
</tr>
<tr>
<td>2.4</td>
<td>11.95</td>
<td>10.95</td>
<td>2.63</td>
<td>32.72</td>
<td>41.75</td>
</tr>
</tbody>
</table>
Figure 49. Atomic Concentrations of Elements on Surface Versus Volume of RNA Poly ‘A’ on Gold
4.3.2.2 Electronic Structure

The photoelectron lines, including Auger peaks that are expected to appear in the XPS spectra for RNA on gold are shown in Table 9 below. The most significant lines are annotated with an asterisk (*).

<table>
<thead>
<tr>
<th>Photoelectron Lines</th>
<th>Auger peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen *1s line at 531eV</td>
<td>KLL lines at 978, 999,</td>
</tr>
<tr>
<td>*1s line at 398eV</td>
<td>KLL line at 1107eV.</td>
</tr>
<tr>
<td>Phosphorous 2s line at 188eV, *P2p_{1/2} line at 131eV</td>
<td>LMM line at 1367eV</td>
</tr>
<tr>
<td>*1s line at 285eV</td>
<td>KVV line at 1223eV</td>
</tr>
<tr>
<td>Carbon 4s line at 763eV</td>
<td>NOO line at 1416eV</td>
</tr>
<tr>
<td>*1s line at 4d_{5/2} line at 335eV</td>
<td>NNN line at 1342eV.</td>
</tr>
<tr>
<td>*1s line at 5p_{1/2} line at 74eV</td>
<td>NNN line at 1324eV</td>
</tr>
</tbody>
</table>

The data for the electronic structure of RNA Poly ‘A’ on gold was derived from the UPS spectra in Figure 43. The high binding energy cutoff, work function, and HOMO cutoff energy values versus electrospray deposition thickness are displayed below in Table 7. The values derived from UPS were believed to be deceptive due to charging effects. HOMO values were derived as in Figure 50 below. The shift in some of these values may be due to charging effects.
Table 7. High binding energy cutoff, Work Function, and HOMO Cutoff Energy Values for RNA Poly ‘A’ on Gold Derived from UPS Spectra in Figure 43 and Difference Spectra in Figure 46. HOMO values were calculated as demonstrated in Figure 50.

<table>
<thead>
<tr>
<th>Volume of RNA Poly‘A’ (ml)</th>
<th>SE</th>
<th>WF</th>
<th>HOMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>sputtered Gold</td>
<td>16.17</td>
<td>5.14</td>
<td>n/a</td>
</tr>
<tr>
<td>0.2</td>
<td>16.45</td>
<td>4.86</td>
<td>2.30</td>
</tr>
<tr>
<td>0.4</td>
<td>16.57</td>
<td>4.74</td>
<td>2.30</td>
</tr>
<tr>
<td>1.4</td>
<td>16.92</td>
<td>4.39</td>
<td>2.40</td>
</tr>
<tr>
<td>2.4</td>
<td>17.00</td>
<td>4.31</td>
<td>2.40</td>
</tr>
</tbody>
</table>

Figure 50. Demonstration of HOMO Calculation Using Difference Spectrum for Gold from the 2.4 ml Deposition Step

The band diagram in Figure 51 is derived from the data in Table 7. The energy levels for the plain Au surface are displayed on the left, RNA Poly A on the right, and the material interface in the middle. In an interface with band bending, the injection barrier would be the distance from HOMO to the Fermi edge minus the band bending amount. Due to the lack of any band bending, the injection barrier is the same as that of the HOMO value.
Figure 51. Band Diagram with RNA Poly ‘A’ on Gold
Chapter Five

Summary

The energy level alignment at the interfaces between RNA with highly oriented pyrolytic graphite (HOPG) and RNA with gold (Au) were determined using in situ thin film deposition in combination with x-ray photoelectron spectroscopy (XPS) and ultraviolet photoemission spectroscopy (UPS) measurements. The organic thin films were grown in multiple steps by electrospray deposition, then sequentially characterized in situ after each deposition step. The data acquired in the preceding measurements will further the knowledge about thin films of RNA and may assist in its possible usefulness as an electronic component. Deposition mode analysis of RNA seems to indicate that it upon deposition it follows the Stranski-Krastanov (SK) mode of deposition. This deposition mode is characterized by formation of a thin monolayer followed by clusters of crystallite deposition.

The use of electrospray has given the opportunity for deposition of thin films of Polymers, such as RNA Poly ‘A’, to be ionized and deposited. Information on the electronic structure and chemical state of materials from these experiments is presented within and summarized below.

X-Ray Photoemission Spectroscopy (XPS) has given much information about the composition and stoichiometry of the thin film deposited. Useful data gathered in these experiments include the work function and composition of the films that formed on the surface of HOPG and gold following deposition of RNA. A control experiment consisting of an electrospray series of DI water on HOPG was also performed.

Although useful for valence band region data, the high binding energy cutoff values derived from Ultraviolet Photoemission Spectroscopy (UPS) were believed to be
deceptive due to charging effects in the later deposition steps of each experiment. High binding energy cutoff values derived from Low Intensity X-ray Photoemission Spectroscopy (LXPS) seemed more reliable and remained nearly constant throughout the measurement series.

The high binding energy cutoff, work function, and HOMO cutoff energy values were determined versus volume of electrospray deposited for each experiment. The measurement of HOPG as-is yielded high binding energy cutoff and work function values of 16.8 eV and 4.44 eV. After deposition of 1.6 ml of RNA Poly ‘A’ solution on HOPG these values were 16.82 eV and 4.48 eV. Measurement of clean, sputtered gold yielded high binding energy cutoff and work function values of 16.17 eV and 5.14 eV. After deposition of 1.4 ml of RNA Poly ‘A’ solution, these values became 16.92 eV and 4.39 eV. These values as well as Highest Occupied Molecular Orbital (HOMO) cutoff values had converged to similar values. While the surface of each substrate began the experiment with distinctly different electronic characteristics, both experiments concluded with similar electronic surface properties. It is reasonable to conclude that these properties are those of RNA Poly ‘A’. These results demonstrate that RNA can be successfully deposited in-situ for thin film deposition by electrospray.
References


Bibliography


7. [http://augustus.scs.uiuc.edu/nuzzogroup/PPT/XPS%20Class%2099.PPT]


