THE MODIFICATION OF GRAPHENE OXIDE AND STUDIES OF THE DETECTION OF NOROVIRUS DNA AND RNA

by

DUY DUC LE

B.S., Cantho University, 2006

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

College of Arts and Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

Major Professor
Dr. Duy H. Hua

ABSTRACT

Graphene oxide (GO) has attracted many researchers in the past years because of its unique electrical and chemical properties which showed the potential applications in many fields such as electronic materials and biology. Increasing research efforts in the biomedical field are bringing to light new discoveries in areas such as drug delivery, treatment of cancers, and biosensors, and are therefore attractive.

The purpose of this work is to prepare GO and modify the surface of GO in order to achieve a new functionalized GO for biosensor applications in the future.

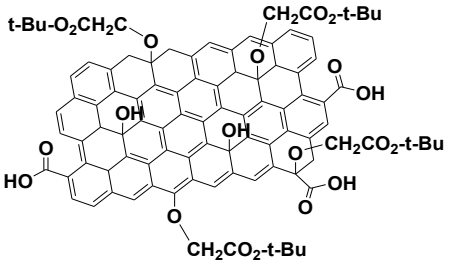
GO was synthesized from the flake graphite by using a modified Hummer's method to achieve higher quality and yield. The flake graphite was first exfoliated by using a microwave reactor. The exfoliated flake graphite then was oxidized by $K_2S_2O_8$, P_2O_5, and $KMnO_4$ under acidic conditions, followed by H_2O_2 to form GO. The following steps were to attach carboxylic acid and benzoic acid groups onto the surface of GO. Atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy were used to identity the modified GO and determine the sizes of the materials after a sequence of reactions. The modified GO will be used in the study of electronic sensing of biomolecules in Hua's laboratory.

TABLE OF CONTENTS

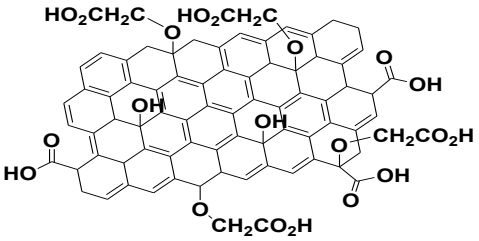
LIST OF FIGURES

LIST OF SCHEMES

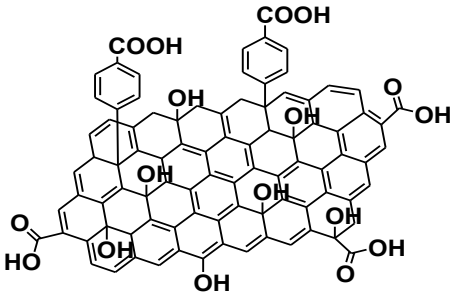
LIST OF STRUCTURE NUMBER CORRELATIONS



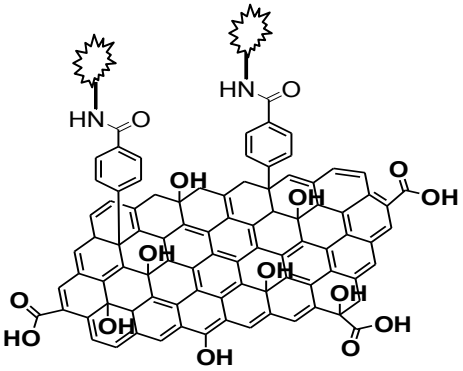
1



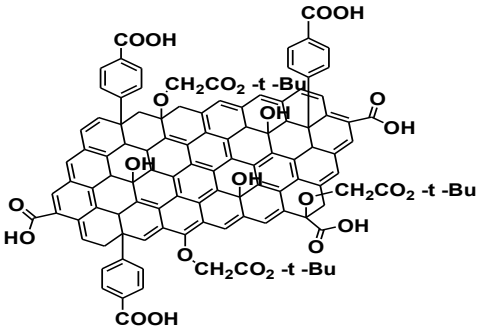
2



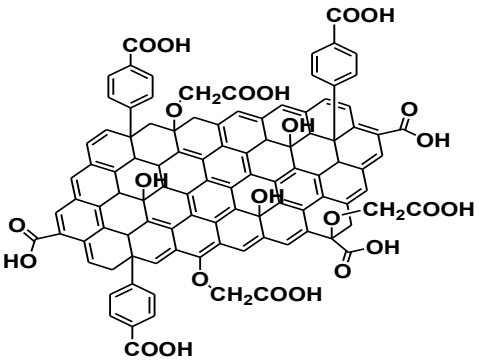
3



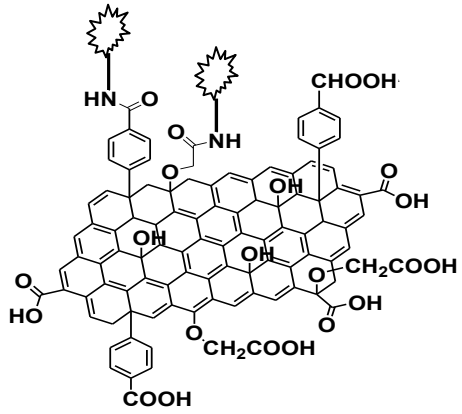
4



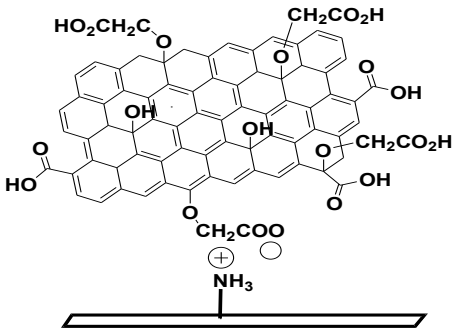
5



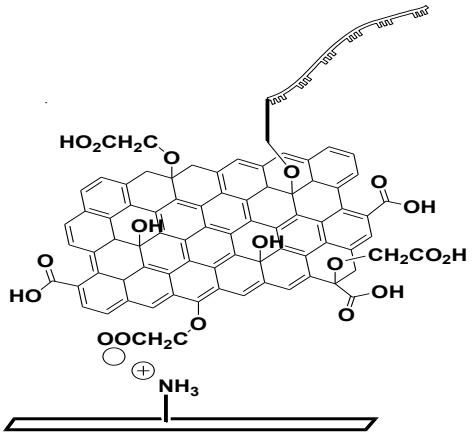
6


HN
O
NH
CHOOH
OH
OH
OH
OH
CH2COOH
HO
OH
CH2COOH
COOH

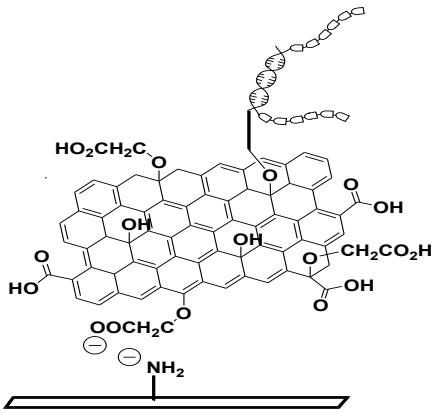
7


HO2CH2C
CH2CO2H
OH
OH
OH
CH2CO2H
HO
OH
CH2COO
NH3

8


HO2CH2C
OH
OH
OH
CH2CO2H
HO
OH
OOCH2C
NH3

9


HO2CH2C
OH
OH
OH
CH2CO2H
HO
OH
OOCH2C
NH2

10

LIST OF ABBREVIATIONS

Aβ	Amyloid-β peptides
AD	Alzheimer's disease
AFM	Atomic force microscopy
CPT	Camptothecin
DDAT-PVK	S-1-dodecyl-S'-(α,α'-dimethyl-α"-acetic acid)trithiocarbonate-polyl(N-vinylcarbazole
DI water	Deionized water
DNA	Deoxyribonucleic acid
DNA-FAM	Deoxyribonucleic acid-carboxyfluorescein
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
FTIR	Fourier transform infrared
GO	Graphene oxide
GO-CS	GO-chitosan
GO-PVK	Poly(N-vinylcarbazole)-covalently functionalized graphene oxide
GO-TDI	Graphene oxide-toluene-2,4-diisocynate
GO-ThS	Graphene oxide-thioflavin-s
RNA	Ribonucleic acid
ssDNA	Single-stranded DNA
THF	Tetrahydrofuran
ThS	Thioflavin-s

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my advisor, Dr. Duy H. Hua, for his generous support and guidance on my academic paths. He gave me the best opportunity to work in his lab in order to achieve a fruitful experience and for my future career.

I would also like to extend my sincere appreciation to Dr. Jun Li and Dr. Stefan Bossmann, my committee members, who gave their best advice for my course study and research.

I also appreciate other faculty and staff members in the Department of Chemistry for all their support during the time I have studied and researched at KSU.

Special thanks to Allan Prior who directed and helped me in my studies and research. I would also like extend my special thanks to Keshar Prasain and Thi Nguyen who helped me in my research. I also appreciate other members in Dr. Hua's lab for their cooperation and their advice during the time I have stayed and worked at KSU.

Finally, I would like to thank Dr. Thanh C. Truong, Department of Chemical Engineering, Cantho University, Vietnam, for his support in my pursuing my goals.

Chapter I

INTRODUCTION

Graphene oxide (GO) is a form of oxidized graphene.[1] The surface of GO has π bond, carboxylic, epoxide, and hydroxyl groups as shown in Figure 1. Many kinds of chemical reactions therefore can take place on the surface of GO. This has led to extremely large potential applications of GO in many fields such as composites, electronic materials, biosensors, and pharmaceuticals in the past years.[2,3,4]

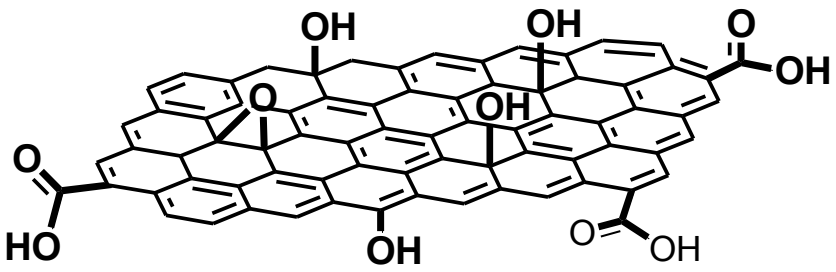


Figure 1. Graphene oxide (GO)

The goal of our study is to use modified GO to apply to biosensors, with a focus on the detection of norovirus.

Norovirus belongs to a family of Caliciviridae viruses and consists of a positive strand RNA genome that causes a certain viral gastroenteritis disease in human.[5] Individuals are infected with norovirus through contact with infected people, water, and foods.[6] In addition, human enteric caliciviruses are responsible for more than 90% of non-bacterial gastroenteritis outbreaks, with as many as 23 million cases occurring in the U.S. each year.[7]

My work was to modify GO so that the modified group can interact with the RNA-virus and be detected by electronic method. The GO was produced from flake graphene by

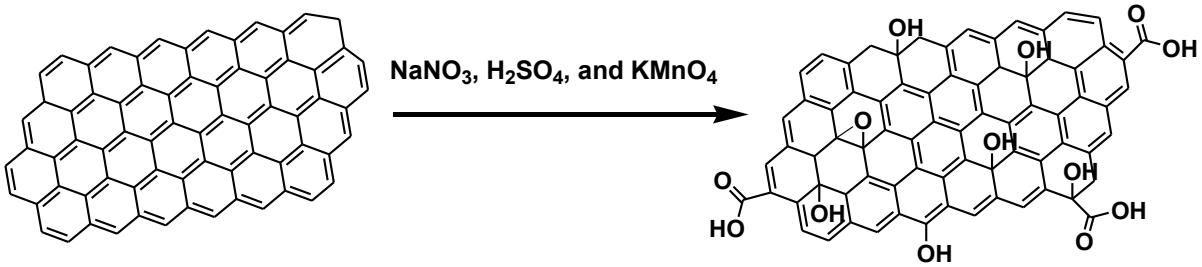
using a modified Hummer's method. The first form of modified GO has the hydroxyl group on the surface of GO. It then covalently attached to oligo-DNA or RNA. When we measured the electronic property of modified GO, we discovered that the non-specific binding of the oligo-RNA also had an important effect on the electronic property of modified GO. We therefore passivated the surface GO by the addition of benzoic acid groups to prevent the non-specific binding.

CHAPTER II

BACKGROUND AND SIGNIFICANCES

II-1. BACKGROUND OF GO

GO is an oxidation form of graphene. When graphene is oxidized to form graphene oxide (GO), the surface of GO contains various functional groups allowing the formation of covalent bonds with numerous reagents. In addition, the surface of GO also contains a lot of double bonds (C=C). We can therefore perform various kinds of reactions on the surface of GO. By adding different kinds of functional groups including epoxide, hydroxyl, and carboxylic acid groups into graphene, the potential applications of GO become extremely large. One of the oxidative methods in producing GO was via Hummers-Offeman's method from graphite flake with sodium nitrate, sulfuric acid, and potassium permanganate, as shown in Scheme 1.[1]



Graphite **Graphene oxide**

Scheme 1. The synthesis of GO

II-2. SIGNIFICANCES OF GO

In recent year, Zhang and coworkers obtained a functionalized GO that is the reaction between GO-toluene-2,4-diisocynate (GO-TDI) and S-1-dodecyl-S'-(α,α'-dimethyl-α"-aceticacid)trithiocarbonate-polyl(N-vinylcarbazole) (DDAT-PVK).[8] The solubility of poly(N-

vinylcarbazole)-covalently functionalized graphene oxide (GO-PVK) was found to be 10 mg/ml in organic solvent, and the energy band-gap 2.02 eV.[8] These results meant that GO-PVK has a potential application in making electronic thin film devices by spin coating.

Another interesting application of GO to treat Alzheimer's disease (AD) was discovered by Li and his coworkers.[9] The deposit of 39-43 amino-acid amyloid-β peptides (Aβ) to form amyloid fibrils may cause AD. Disaggregation of Aβ is therefore a method to prevent AD. Many scientists use hyperthermia (local heat generation) to dissolving Aβ oligomers in order to prevent the formation of Aβ fibrils.[10] Methods of achieving this can include radio frequency, ultrasounds, inductive microwave and antennae, and inductive needles.

These methods of hyperthermia can penetrate deeply into and increase temperature of target tissues.[10] However, the other surrounding tissues are also affected by hyperthermia.[11] In order to decrease an effect of undesirable hyperthermia to healthy tissues, laser irradiation at NIR frequencies was used to kill unhealthy cells.[12] In addition, graphene oxide based materials were discovered that can strongly absorb the irradiation at NIR frequencies.[13] Li examined the binding of Aβ on the surface of GO-thioflavin-s (GO-ThS) that is the product of the reaction between GO and thioflavin-s (ThS).[9] ThS is a dye, and selectively bind to Aβ oligomers. By applying the NIR laser irradiation to dissolve Aβ fibrils -concentrated cerebrospinal fluid of mice - on the surface of GO. The heat was generated selectively to target Aβ oligomers.[9] The surrounding normal tissues therefore are unaffected during treatment with NIR laser irradiation. In addition, the chemicals that bound to GO can go through the blood brain barrier without decomposition as do other drugs and peptides alone.[9] Therefore, this method of modified GO gave the potential application in the treatment of AD.

Another functional GO that has had potential application in medicine is chitosan-functionlized GO (GO-CS).[14] Bao and coworkers modified the surface of GO with chitosan

to form GO-CS.[14] Chitosan was well known about its unique chemical properties such as drug and gene delivery, and antibacterial.[15-18] GO-CS is more stable than CS alone in vivo.[14] Moreover, the surface of GO-CS is the ideal place for the bindings of many kinds of drugs followed by the stacking and hydrophobic interactions.[14] For example, a loading of camptothecin (CPT: anti cancer) into GO-CS to 20 wt.% and a releasing CPT in vitro was 17.5 wt% within 72h at 37^{o}C.[14] GO-CS has opened a new type of nanocomposite for drug delivery.

Many applications that were mentioned above showed the potential application of graphene oxide (GO) in various fields.

Chapter III

RESULTS AND DISCUSSION

Atomic force microscopy (AFM) images were taken by Thi D. T. Nguyen, a graduate student in Dr. Hua's group, using an AFM instrument in the Department of Chemistry. Allan M. Prior, a graduate student in Dr. Hua's group, did the experiment as shown in Scheme 7, instructed me in the synthesis of GO and electrical measurement, and took the fluorescent images (Figure 11) in Dr. Li's laboratory.

III-1 Preparation of graphene oxide and modification of graphene oxide

III-1-1 Preparation of graphene oxide

Graphene oxide was first synthesized by Brodie in 1859.[19] Brodie used potassium chlorate and nitric acids to oxidize graphite. Staudenniaier, Hofmann, and Hamdi later modified Brodie's method by treating graphite with a oxidation mixture of potassium chlorate, concentrated sulfuric acid, and concentrated (63%) nitric acid.[20-21] However, these methods were time consuming and contained large amount of hazardous chemicals. For example, each gram of graphite required 10 g of potassium chlorate to oxidize them.[20-21] In 1958, Hummers and Offeman discovered a new method to synthesize GO used less hazardous chemicals.[1] Moreover, Hummers's method takes place under low temperature (below 45^{o}C).[1] Hummers's method is therefore safer.

In the recent time, many scientists modified Hummers's method to achieve a higher quality of GO and higher yield. In particular, modifying Hummers's method by applying a microwave reactor has been used in the past years.[22-23] Microwave reactors were first used to

exfoliate graphite in order to expose the surface of each graphene sheet for oxidative chemicals to enter the graphite.

In our works to synthesize GO, the flake graphite was firstly exfoliated by using a microwave reactor.[24,25] The exfoliated flake graphite then was treated with $K_2S_2O_8$ and P_2O_5 in concentrated H_2SO_4 at 90°C for four hours. The mixture was then filtered and washed with deionized water (DI water). The residue after filtration was then dried in an incubator. The black material (graphene) was oxidized with concentrated H_2SO_4 and $KMnO_4$ at 40°C. The mixture was cooled to room temperature. The DI water and H_2O_2 were then added to the mixture. After the oxidation step, the black GO bulk solution was centrifuged and the sediment was dialyzed for one week. The process of the synthesis of GO is shown in Figure 2. The thickness of the GO sheet was measured by AFM and was found to be around 1 nm (Figure 3).

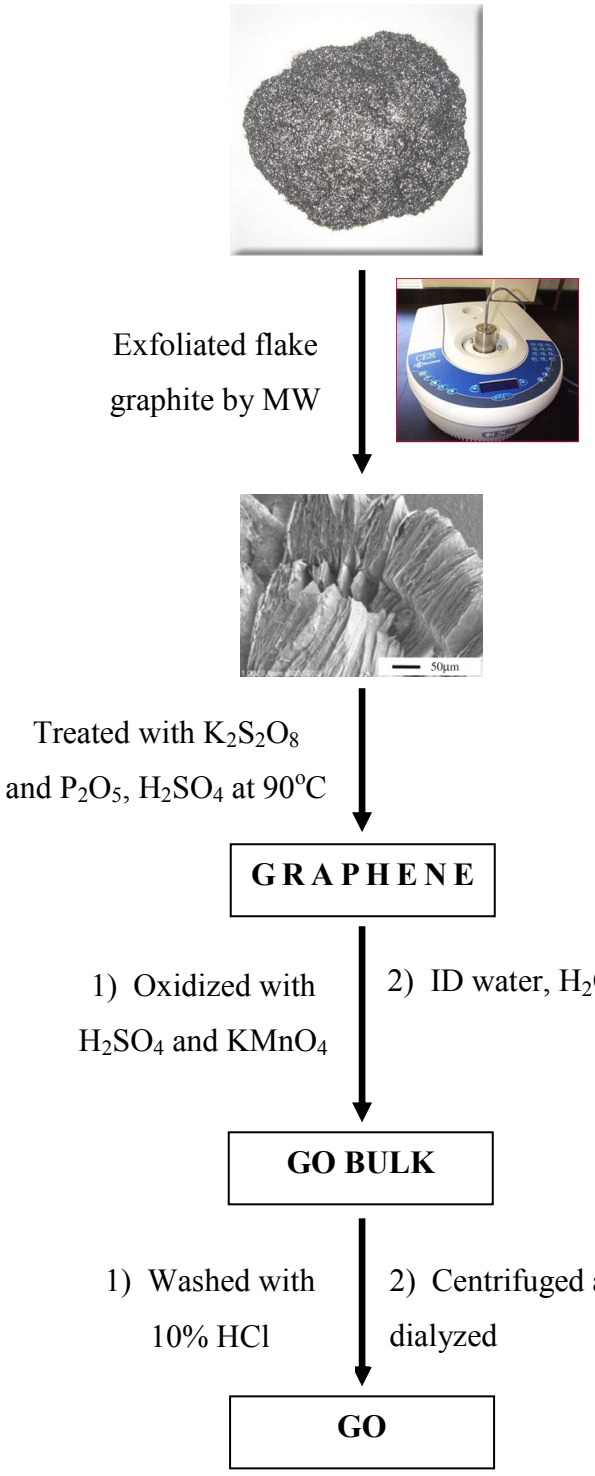


Figure 2. Process of preparation of graphene oxide

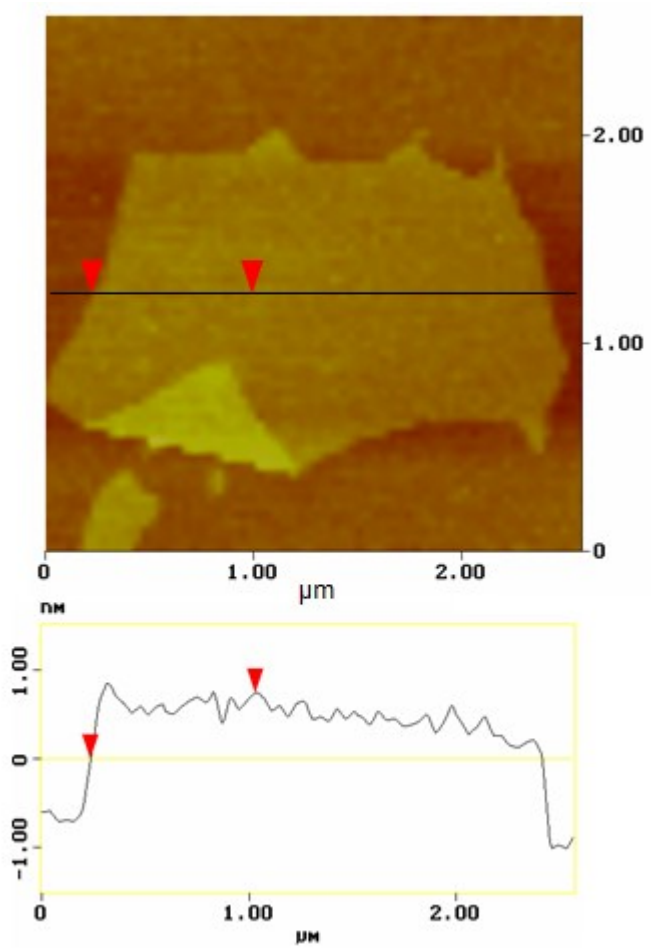
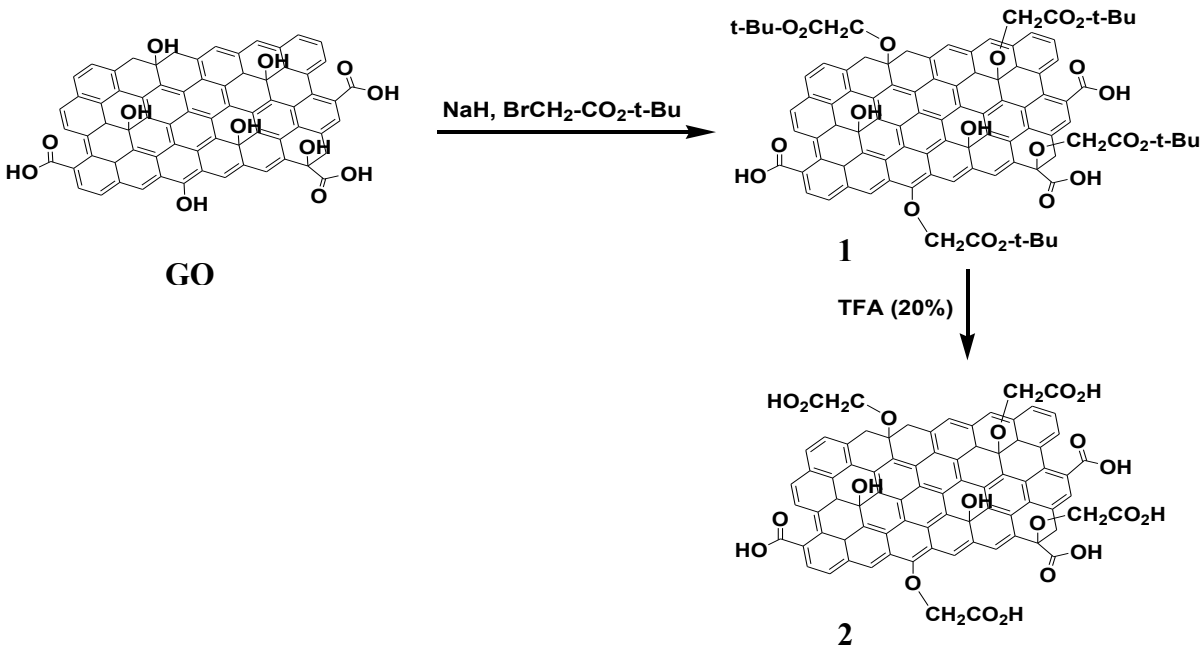


Figure 3. A representative atomic force microscopy (AFM) image of GO

III-1-2 Preparation of GO-acetic acid

For the detection of RNA and DNA, we covalently linked complementary oligo-nucleotide onto GO surface. The surface of GO was therefore functionalized with carboxylic acid groups. The synthesis of GO-acetic acid was shown in Scheme 2. The acetic acid groups were added to GO by the reaction with t-butyl bromoacetate and NaH. Then the t-butyl of GO-t-butyl ester **1** was removed by trifluoroacetic acid (TFA) to form GO-carboxylic acid**.** Fourier transform infrared (FTIR) was used to characterize the GO-acetic acid **2**. IR spectrum of GO-carboxylic acid showed the main carbonyl stretches at around 1686 cm^{-1}



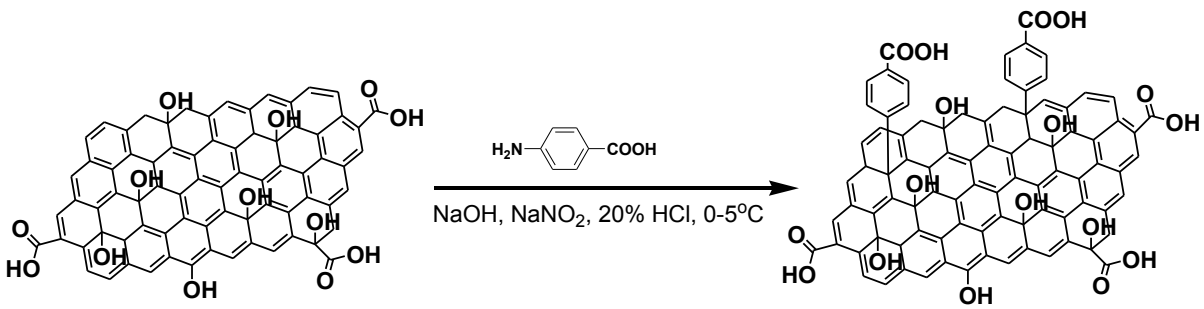
Scheme 2. The synthesis of GO-carboxylic acid

III-1-3 Preparation of GO-benzoic acid

It has been report that when DNA lays flat randomly on the surface of membrane or aligned perpendicular to the membrane, the ion current is also affected.[26] This information made us consider the effect of non-specific binding of RNA and DNA to the electrical current of GO. We therefore modified GO with benzoic acid groups to prevent the non-specific binding of RNA and DNA to GO. The function of benzoic acid groups is to block the empty spaces on the surface of GO which RNA and DNA can bind to. In addition, we used the benzoic acid groups (hindered groups) to prevent the over-reaction on the surface of GO, when we modified the surface of GO. The electrical current of GO will decrease if all of the π electrons of GO react with modified groups. Moreover, benzoic acid and DNA have negative charges that will repel each others. It therefore helps to prevent non-specific

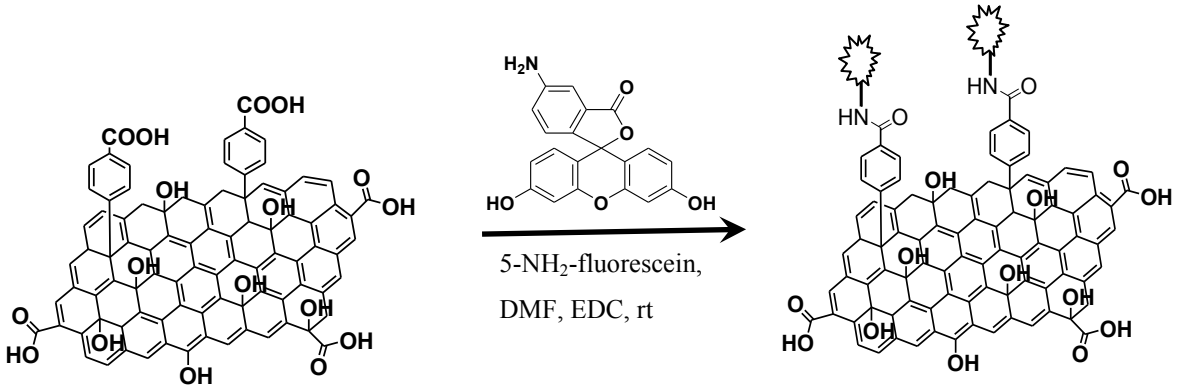
binding. This is one of the methods to prevent the non-specific binding of RNA and DNA to GO.

The synthesis of GO-benzoic acid is shown in Scheme 3. GO reacted with para-diazobenzoic acid under basic condition to form GO-benzoic Acid **3**.[27] In order to confirm the benzoic acid groups attached to GO, we used fluorescent dye (5-aminofluorescein) to react with GO-benzoic acid (Scheme 4). The fluorescence images of GO-benzoic acid-5-aminofluorescein **4** are shown in Figure 4. Some GO had scattered green fluorescent dots. However, the green fluorescent dots appear to be coming from impurities such as dust and physical binding. The fluorescence images therefore do not indicate that the fluorescence groups were attached onto the surface of GO. Fourier transform infrared (FTIR) was then used to indicate carbonyl (COOH at GO edges) and carbonyl (COOH of benzoic acid) of compound **3** stretch at similar frequency around 1725 cm^{-1}. In addition, IR spectrum of compound **4** showed decreasing intensity of the peak of O-H stretch and the carbonyl C=O stretch. The frequency of carbonyl C=O stretch also shifted to 1697 cm^{-1}.



GO **3**

Scheme 3. The synthesis of GO-benzoic acid



3 **4**

Scheme 4. The synthesis of GO-benzoic acid-5- aminofluorescein

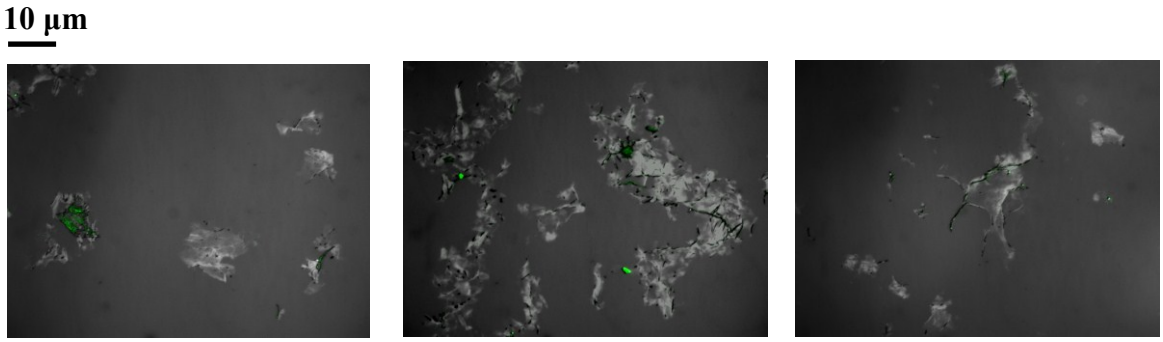


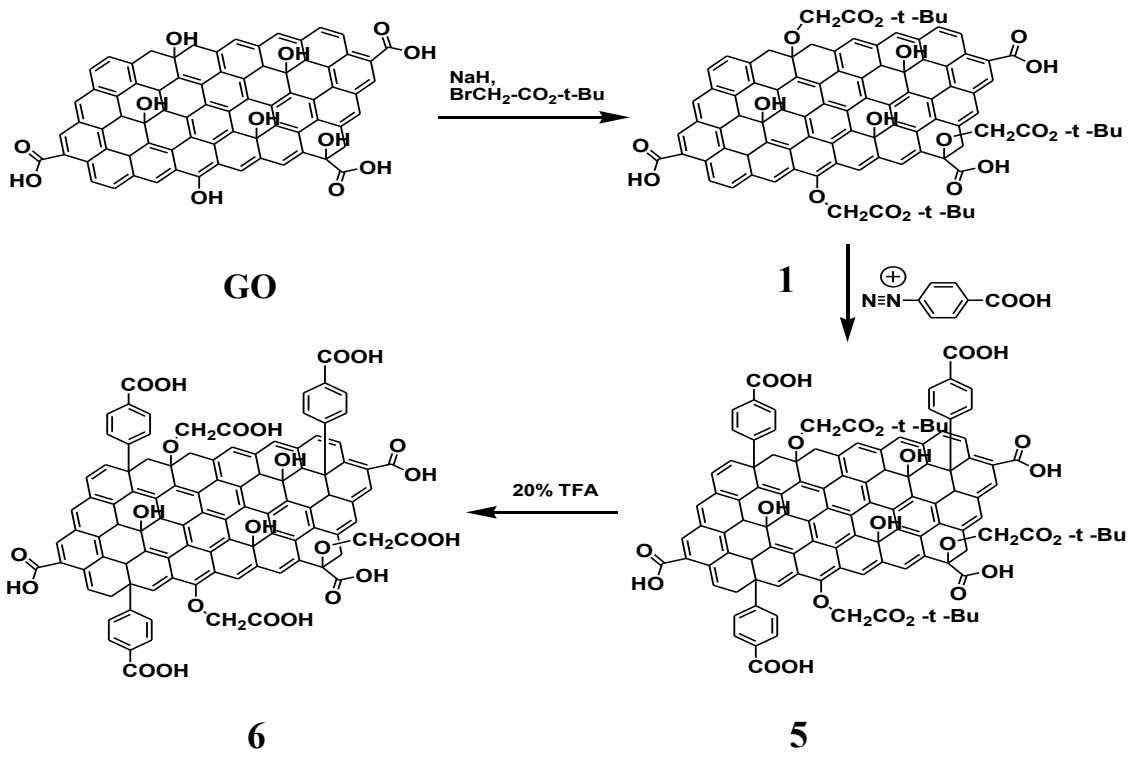
Figure 4. Fluorescence image of GO-benzoic acid-5- aminofluorescein

The IR spectra support the incorporation of benzoic acid group onto the surface of GO. After successful synthesis of GO-benzoic acid, the next step is to synthesize GO-acetic acid-benzoic acid in order to further study the ability to prevent non-specific binding of benzoic acid groups.

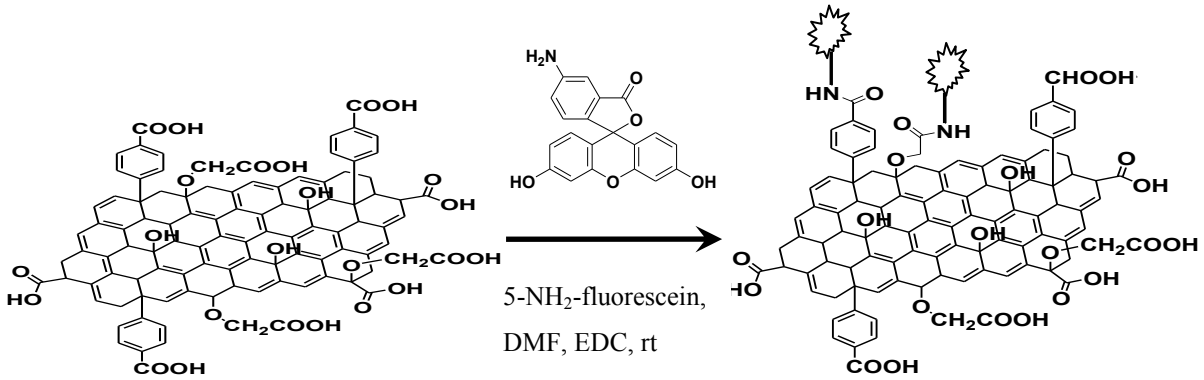
III-1-4 Preparation of GO-acetic acid-benzoic acid

The synthesis of GO-acetic acid-benzoic acid was shown in Scheme 5. GO-t-butyl ester **1** was achieved from GO, t-butyl bromoacetate and NaH. GO-t-butyl ester **1** reacted with para-diazobenzoic acid to form GO-t-butyl ester-benzoic acid **5**. The de-esterification of

GO-t-butyl ester-benzoic acid to form GO-acetic acid-benzoic acid **6** is a fast and mild reaction under acidic conditions. GO-acetic acid-benzoic acid then reacted with fluorescent dye (5-H_2N-fluorescein) in order to detect carboxylic groups on the surface of modified GO (Scheme 6). However, the green fluorescent dots only are onto some GO, as seen in Figure 5. In addition, the green fluorescent dots appear to be coming from impurities such as dust and physical binding of fluorescence dye onto GO surface. The fluorescent method therefore could not prove that the carboxylic acid groups were on the surface of GO. IR spectra of GO-acetic acid-benzoic acid **6** showed the main carbonyl stretches at around 1710 cm^{-1}. In addition, IR spectrum of compound **7** showed changing the shape of the peak of the carbonyl C=O stretch. The frequency of carbonyl C=O stretch also shifted to 1698 cm^{-1}.



Scheme 5. The synthesis of GO-acetic acid-benzoic acid



6 7

Scheme 6. The synthesis of GO-acetic acid-benzoic acid -5- aminofluorescein

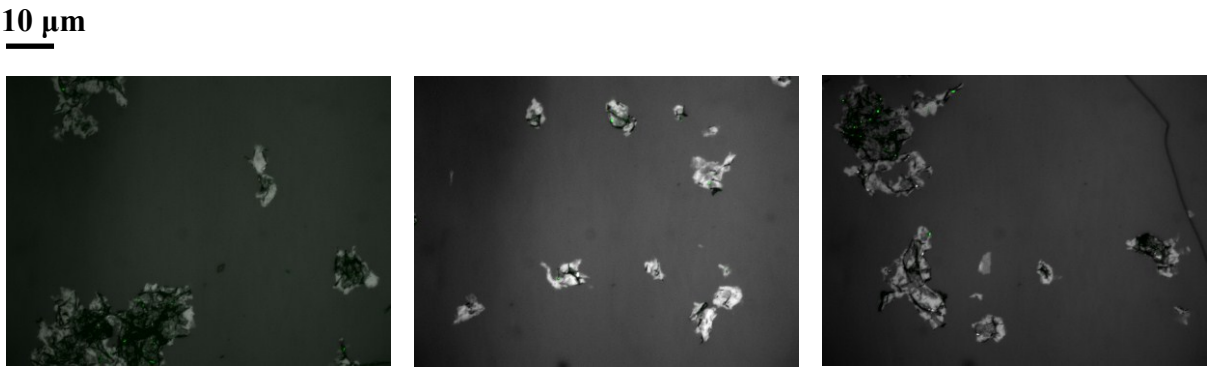


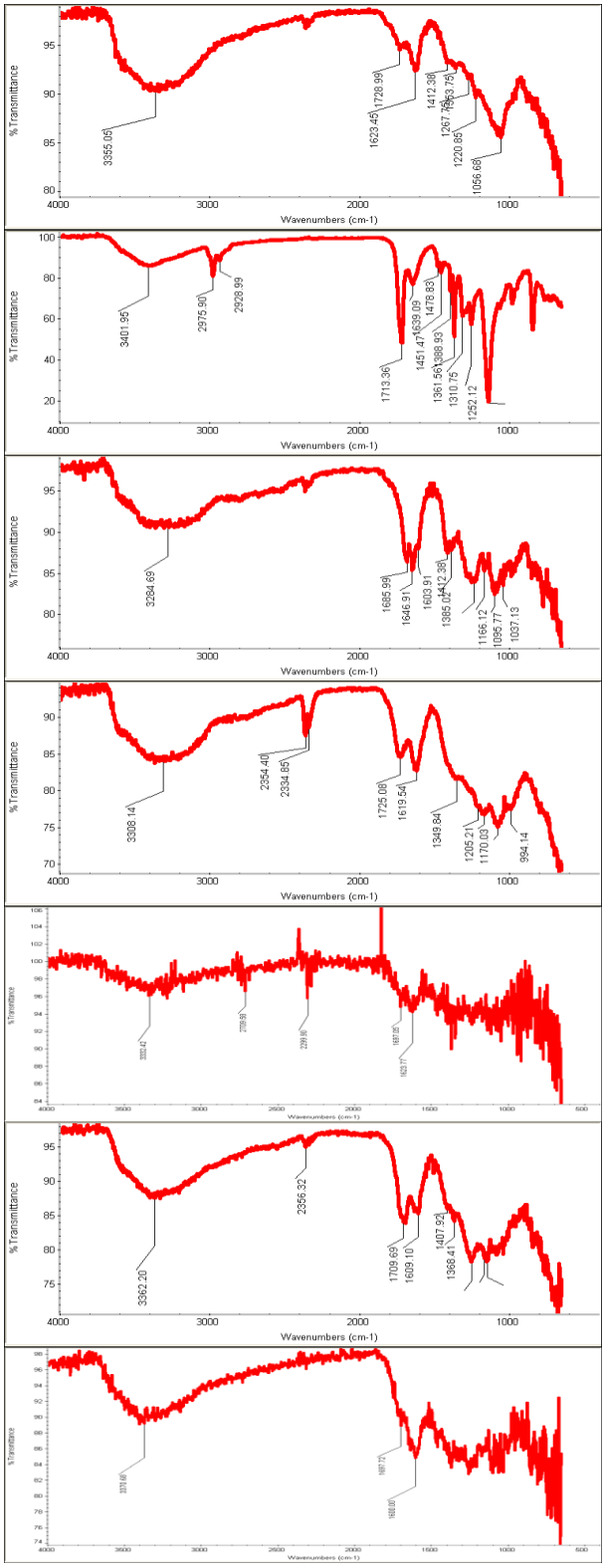
Figure 5. Fluorescence image of GO-carboxylic acid-benzoic acid -5- aminofluorescein

III-2. Characterization of modified GO

The fluorescence method and IR spectroscopy were used to detect the functional groups which were added to the surface of GO. Fluorescence images only showed that fluorescence dyes attached to the GO. However, the fluorescence images from microscope do not confirm that fluorescence dyes attached to GO by chemical reactions with carboxylic acid groups on the surface of GO. We therefore used Fourier transform infrared (FTIR) spectroscopy to support the existence of functional groups on the GO.

By comparing the FTIR spectra of the GO and GO-t-butyl-ester (Figure 6), the FTIR spectrum of GO-t-butyl-ester showed the peaks of t-butyl groups at around 1362 cm^{-1} and

1389 cm^{-1}, and a sp^3 C-H stretching peak at 2929 cm^{-1} and 2976 cm^{-1} as seen in Figure 6b. Two carbonyl stretching frequencies from the COOH groups of GO and t-butyl ester can be seen overlapping at about 1713 cm^{-1} and the C=C peaks at round 1639 cm^{-1} shifted and changed in intensity due to the presence of t-butyl ester groups on the surface of GO. In Figure 6c, after GO-t-butyl ester was transformed to GO-acetic acid, the peaks of t-butyl groups disappeared. In addition, the C=O and C=C bands also changed. As seen in Figure 6d and 6f, when GO was transformed to GO-benzoic acid and GO-acetic acid-benzoic acid, the bands of C=O and C=C also changed slightly in the shape and position. Comparing between Figure 6d and 6e, when 5-amino-fluorescein was added to GO-benzoic acid, the shape and intensity of the O-H stretch peak become smaller. Moreover, the band of C=O moves to shorter wavelength at around 1697 cm^{-1}. In Figure 6g, When 5-amino-fluorescein was added to GO-acetic acid-benzoic acid, the band of C=O also moves to shorter wavelength at around 1697 cm^{-1}.



a) GO

b) GO–O(=O)–O–C(CH3)3

c) GO–O(=O)–OH

d) GO–C6H4–C(=O)–OH

e) GO–C6H4–C(=O)–HN–fluorescein

f) GO–CH2–O(=O)–C6H4–C(=O)–OH

g) HN–fluorescein–O(=O)–CH2–GO–C6H4–C(=O)–OH

Figure 6. FTIR spectra of GO and modified GOs

Raman spectroscopy was used to characterize modified GO, as shown in Figure 7. The Raman spectra of raw graphite flakes and GO have two important visible bands which have been characterized by other researchers, the G and D bands,[28] appearing at 1580 cm^{-1} and 1350 cm^{-1}, respectively. The G band displays in plane symmetric vibration of sp^2-bonded carbon systems. The D band displays the vibration of carbon atoms at the graphite edges. The weak D band of raw graphite flakes indicates the good quality of graphite. When GO was modified, the shape and position of G and D bands change because some carbon atoms change from sp^2 to sp^3 hybridization, and the shape of the graphite edges changes, as showed in Figure 7. The Raman spectra of all samples show that through many steps of modification, they still display the D and G bands like the Raman spectrum of graphene. This information indicated that the modified GOs still maintain the properties of graphene oxide. In summary, we have successfully performed various reactions, such as oxidation of graphite, installing acetic acid and the benzoic acid groups onto graphene oxide to achieve GOs for further application.

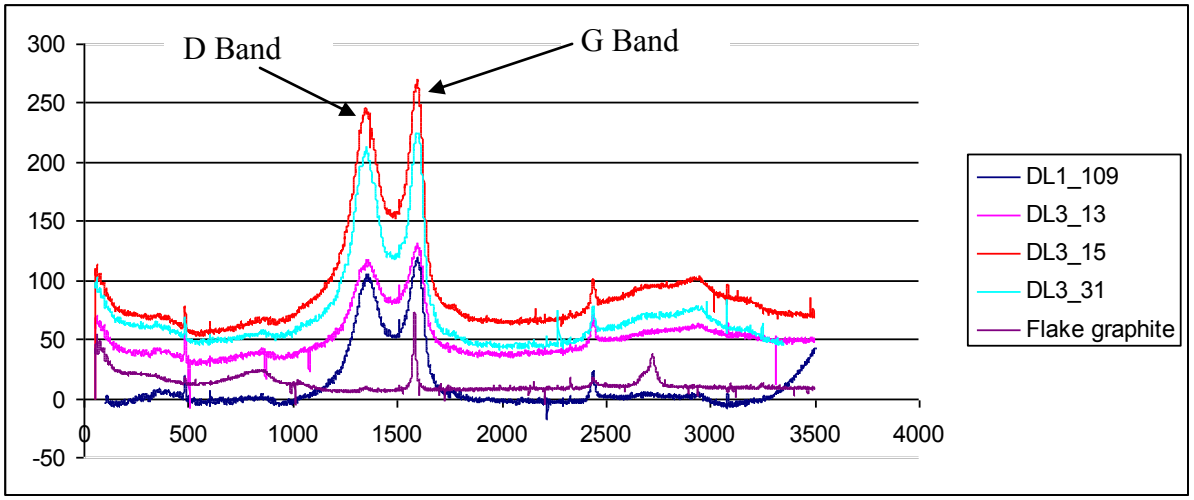
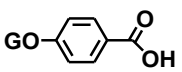
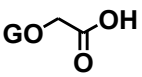
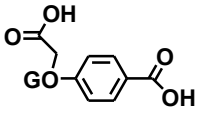


Figure 7. Raman spectra of GO and modified GOs

DL1_109: **GO**

DL3_13: 

DL3_15: 

DL3_31: 

III-3 Studies of the detection of norovirus DNA and RNA

From our research into the application of modified graphene oxide and micro-electronic device, we discovered that the specific binding of norovirus's DNA to modified GO changed the mobility of quasiparticles of modified GO. By measuring the changes of the mobility of electrons inside GO, we can detect the target virus. This is a main purpose of our project.

III-3-1 Microelectronic devices

Commercially available silicon chips were used. The dimension of micro chip is 1 cm^2, and the surface of it is diced with gold, as shown in Figure 8a and 8b. In order to measure the current between two gold electrodes, a manipulator (Figure 8c) was used, which connected to an amplifier (Figure 8d). The electrical current only presents between the two gold electrodes when GO is deposited on top of the two gold electrodes of the micro chip, as shown in Figure 9.

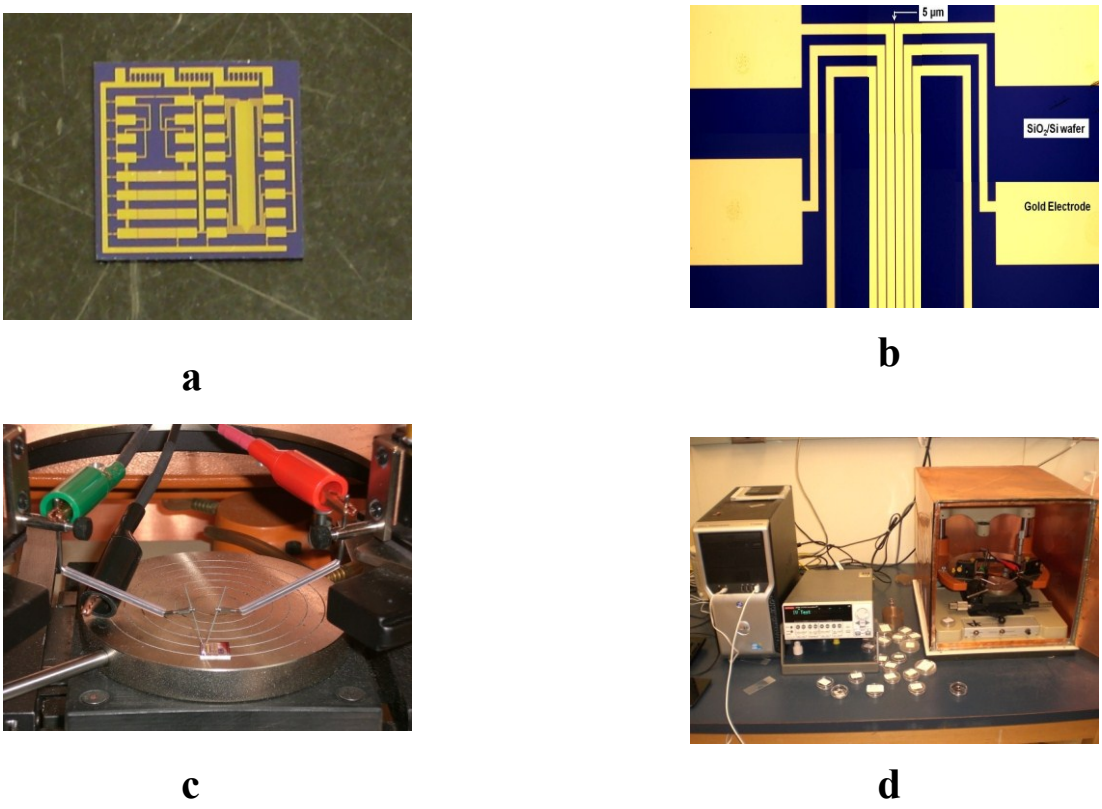


Figure 8. Microelectronic devices

a. Silicon chip **b.** The surface of chip is diced with gold

c. Manipulator **d.** Amplifier system

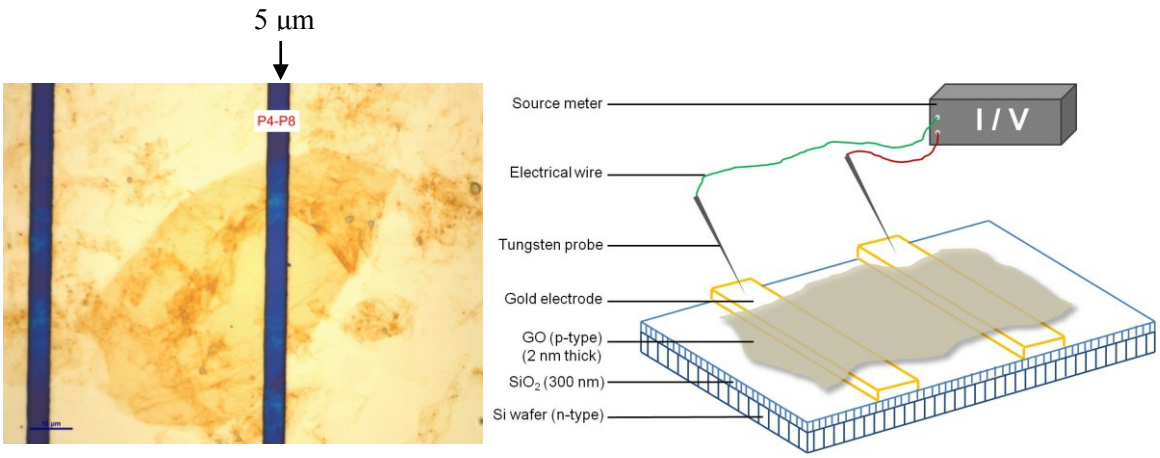


Figure 9. Measurement of the electrical current between two gold electrodes

III-3-2 Deposition of GO onto the silicon microchip

The process of preparation of microchips is shown in Figure 10. The chips were first washed with acetone, isopropanol, and ethanol to remove the thin polymer film layer that covered the chips.[29] The surface of the chips was then cleaned and dried by the oxygen plasma cleaner. In order to make the chips interact with carboxylic groups on the surface of GO through covalent bond or ionic interaction, the surface of the chips was incorporated with amino groups. The chips were therefore silanized with 3-aminopropyltriethoxysilane. Then the chips were washed with ethanol and dried with argon, and were incubated at 120°C for 30 min.

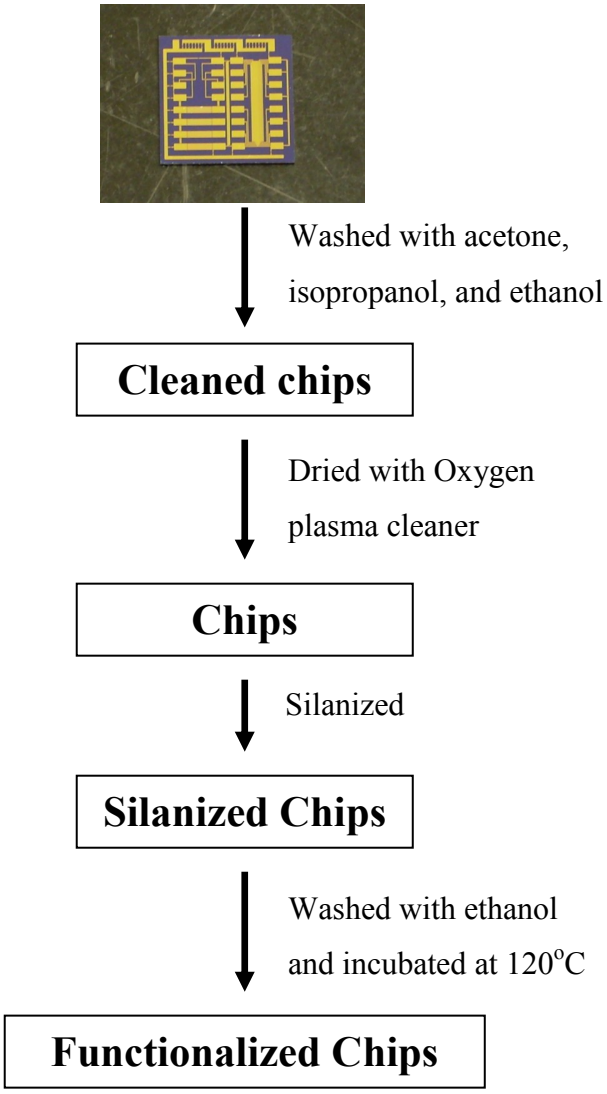
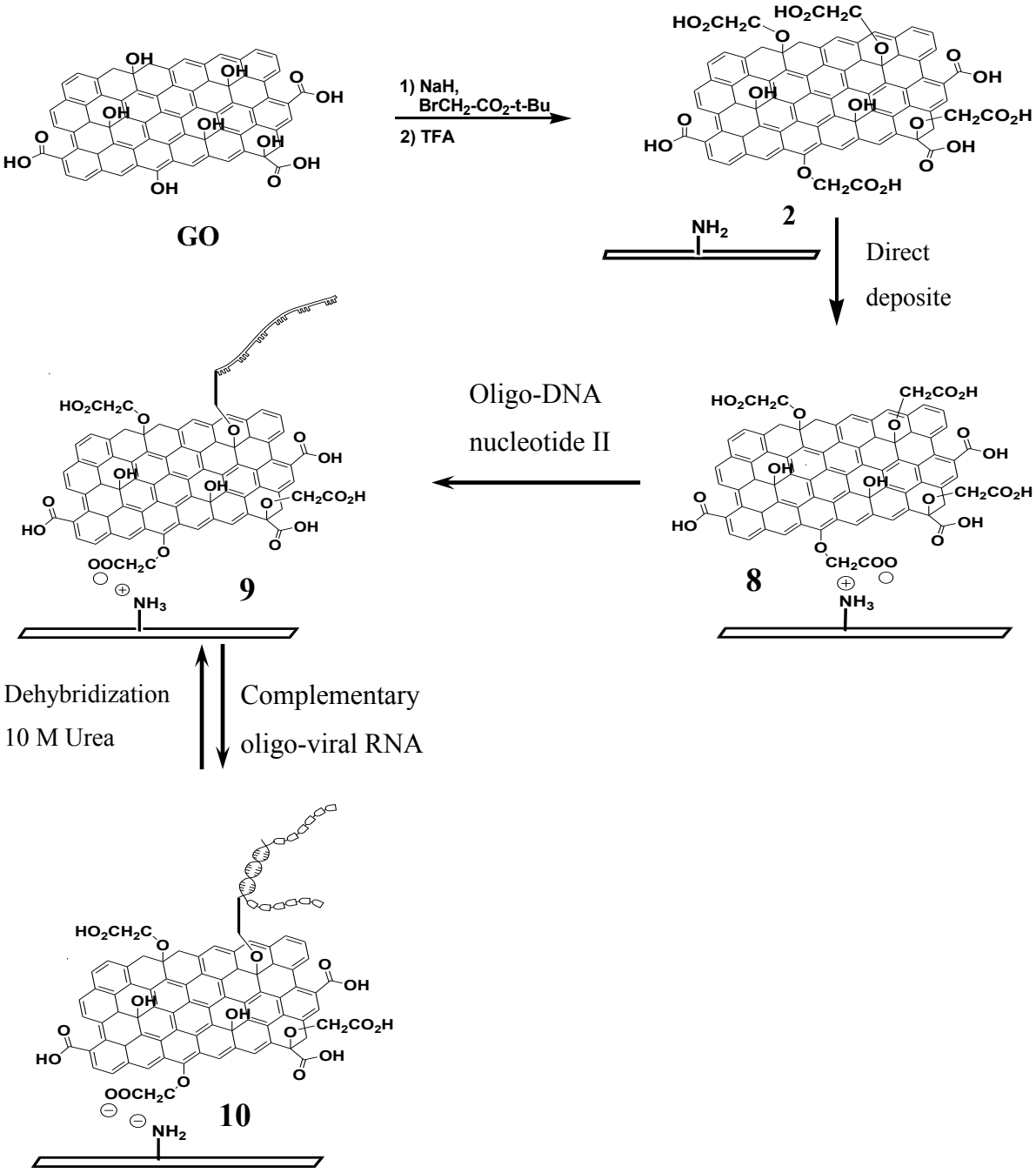


Figure 10. Functionalization of the chips

III-3-3 The attachment of oligo-DNA to modified GO

The process of binding of RNA and DNA (deoxyribonucleic acid) to functionalized GO is shown in Scheme 7. In order to make GO interact with RNA and DNA, the surface of GO was first functionalized with the carboxylic acid groups, followed by the attachment of oligo-nucleotide. Therefore, the carboxylic groups were first added to GO by the reaction with t-butyl bromoacetate and NaH. Then the t-butyl of graphene oxide t-butyl ester was removed by trifluoroacetic acid (TFA) to form modified GO **2**. A dilute suspension of modified GO **2** in water (20 μL) was then added directly onto the P-electrode lane of the chip. The chip was placed in an incubator at 50^{o}C for 30 min. GO bound tightly to the surface of chip through ionic attraction between carboxylic and amino groups. After that a chip containing immobilized GO was allowed to react with oligo-DNA nucleotide II [which has a sequence 5'-/5AmMC12/TGG GAG GGC GAT CGC AATC (MW = 6,461.4 Da)] to form chip **9**. The chip would then be used to detect complimentary RNA-virus.



Scheme 7. Process of depositing GO on chips and hybridization

With norovirus covalently linkage to oligo-nucleotide

III-3-4 The effect of hybridization and dehybridization of oligo-nucleotide II with complementary oligo-RNA and DNA-FAM

Chip **9** reacted with complementary deoxyribonucleic acid-carboxyfluorescein (oligo-DNA-FAM) which has a sequence 5'-/56-FAM/A GATT GCG ATC GCC CTC CCA (MW = 6,575.4 Da). The detection of hybridization and de-hybridization of oligo-nucleotide II with complementary oligo-DNA-FAM is shown in Figure 11. In Figure 11b, we saw the green fluorescent dots on the surface of the modified GO after the hybridization. It showed that oligo-DNA-FAM attached to the surface of GO. As seen in Figure 11c, after the de-hybridization, the green flourescent dots disappeared because oligo-DNA-FAM was washed away. It showed that the hybridization and de-hybridization took place on the surface of modified GO. In addition, the hybridization and de-hybridization of oligo-nucleotide with Complementary oligo-DNA-FAM also affected the current of the chips, as shown in Figure 12, 13, and 14.

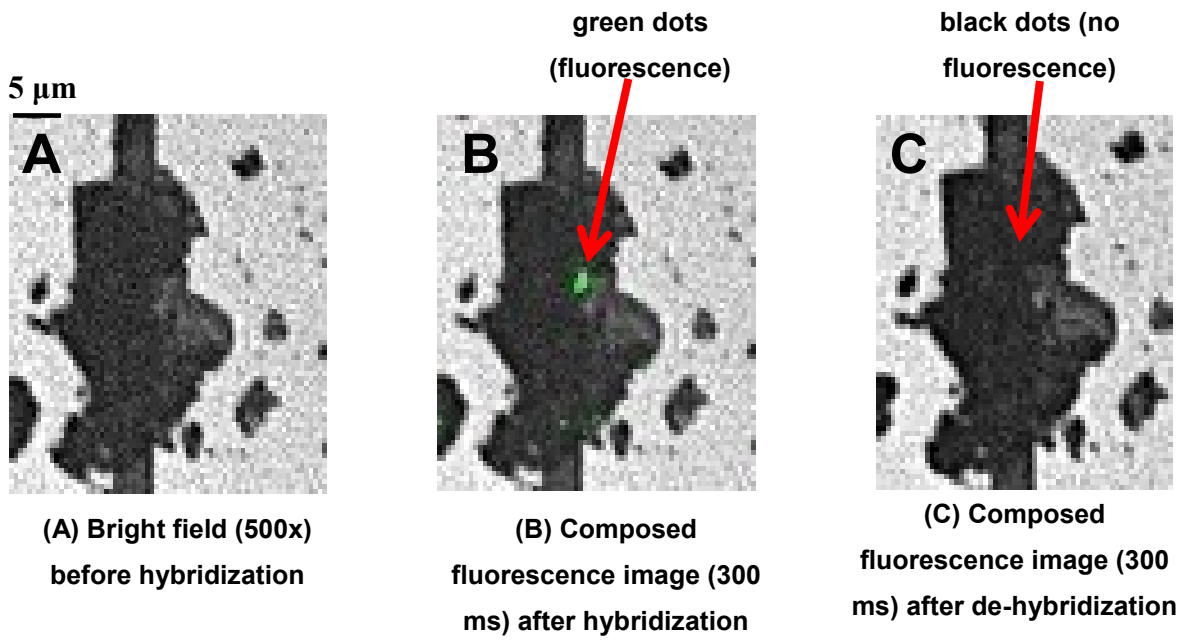
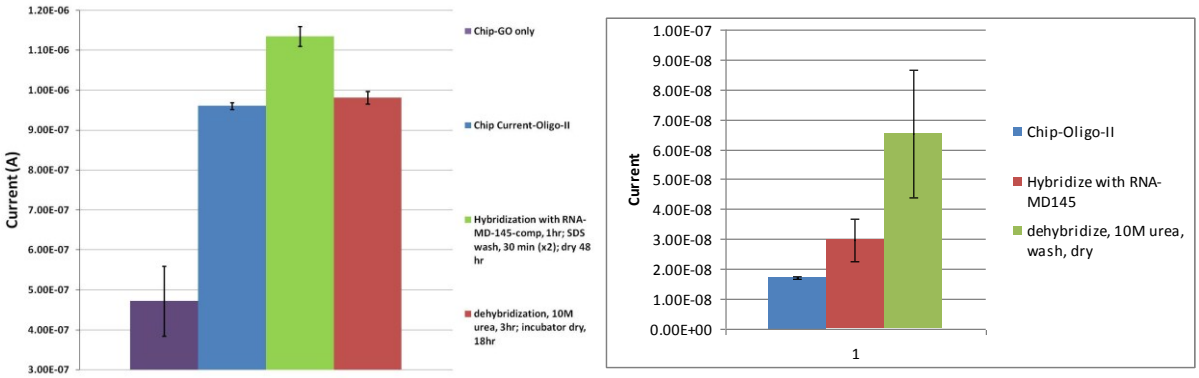


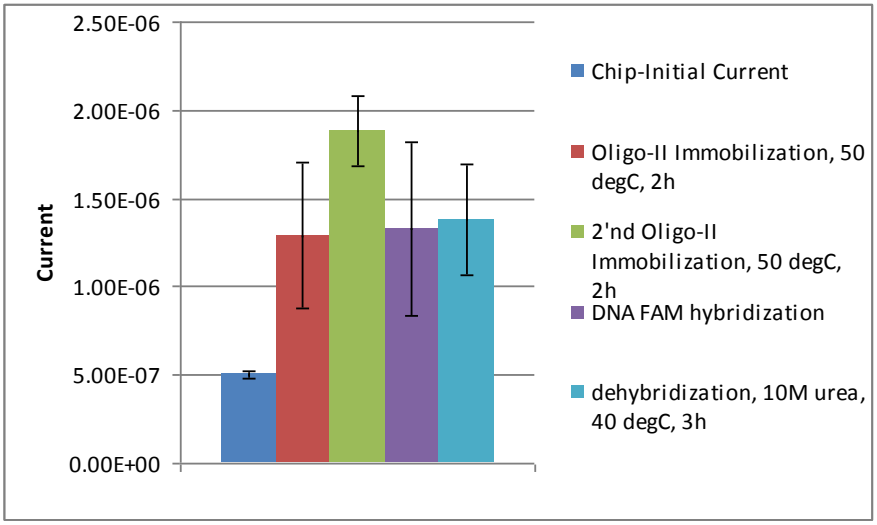
Figure 11. Fluorescence Images: Hybridization and dehybridization of oligo-DNA nucleotide II with Complementary DNA-FAM

In Figure 12a, the electrical current increased when oligo-DNA nucleotide II hybridized with RNA. In addition, the electrical current decreased when the de-hybridization took place. However, in Figure 12b, the electrical current decreased when the DNA-FAM complimented with oligo-DNA nucleotide-II, and after the de-hybridization took place, the electrical current increased. From these results, we noticed that the hybridization and de-hybridization affected the electrical current of GO. However, our data could not tell hybridization increased or decreased the electrical current of GO. When we examined the hybridization and de-hybridization between oligo-DNA nucleotide II and DNA-FAM, we also got the similar results, as shown in Figure 13. Many factors such as non-specific binding and moisture affected the electron current of GO. That was the reason why we got these fluctuated results.

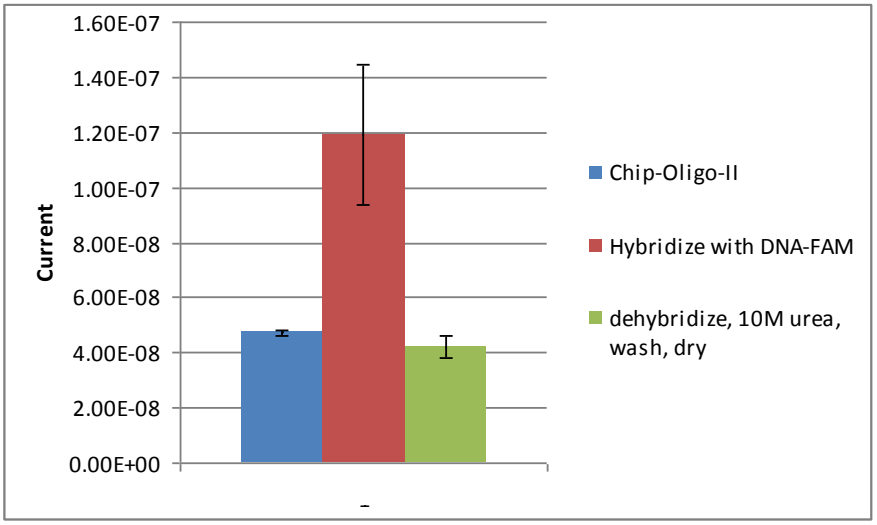


a **b**

Figure 12. The effect of hybridization and dehybridization of oligo-DNA nucleotide II with RNA on the electrical current of chip



a



b

Figure 13. The effect of hybridization and dehybridization of oligo-DNA nucleotide II with DNA-FAM on the electrical current of chip

In order to confirm these different bindings of RNA and DNA on the surface of GO, we examined the reaction between GO and complementary DNA-FAM without coupling reagent HATU. The electrical current still increased when DNA-FAN was added (Figure 14). It indicated that the increases derived from non-specific binding of DNA onto the GO surface. We did another experiment to indicate that the non-specific binding also play an important role on the mobility of electrons of GO. The result is shown in Figure 15. In Figure 15a, after the hybridization between oligo-DNA nucleotide II and complimented norovirus RNA, the electrical current increased lightly. In Figure 15b, even though the non-complimented RNA was incubated with oligo-nucleotide II, the current also increased. The non-specific binding could be therefore directed to increasing mobility electrons of GO.

The non-specific binding of DNA-FAM to GO is difficult to control. Moreover, it could prevent oligo-DNA nucleotide II to hybridize with DNA-FAM. DNA-FAM lays flat on the surface of GO. DNA-FAM therefore can not hybridize with oligo-DNA nucleotide II.

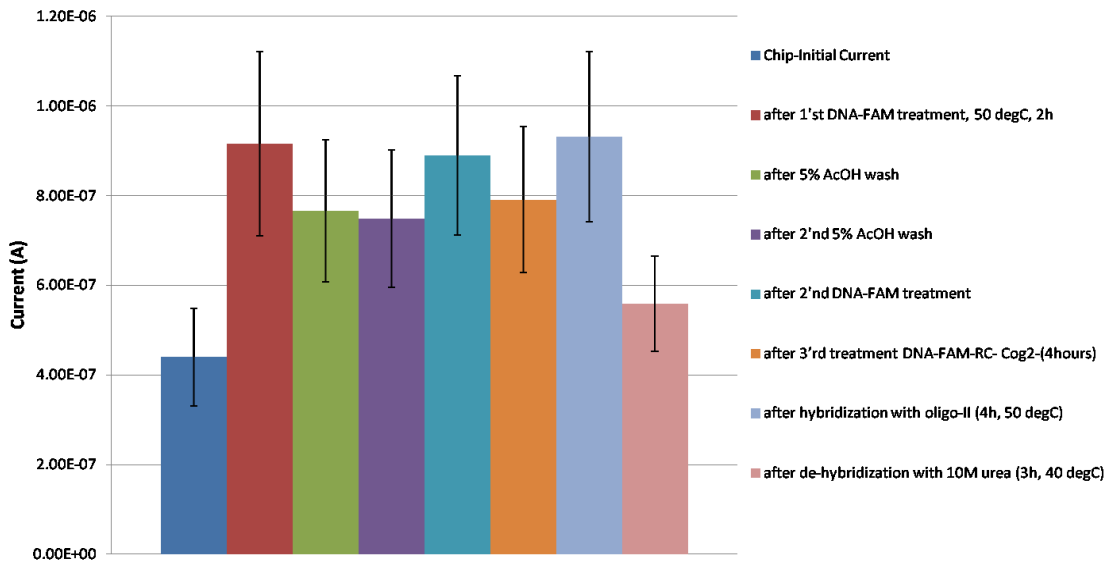


Figure 14. The effect of hybridization and dehybridization of GO with DNA-FAM without HATU on the electrical current of chip

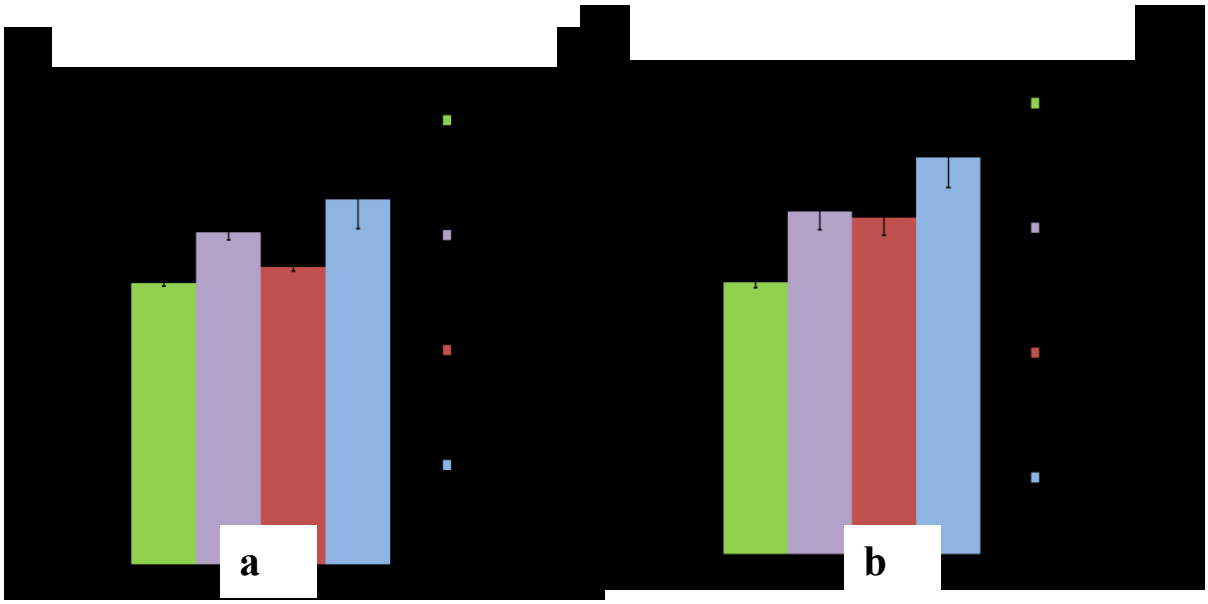


Figure 16.

a) The effect of hybridization of oligo-DNA nucleotide II with complimented RNA and DNA-FAM on the electrical current of chip

b) The effect of hybridization of oligo-DNA nucleotide II with non-complimented RNA and DNA-FAM on the electrical current of chip

CHAPTER VI

CONCLUSION AND FUTURE WORK

The functionalized GOs were successfully prepared by chemical reactions. The methods took place under mild conditions, and they therefore can be applied with large scale preparation. However, this method has some drawbacks. The reaction on the surface of GO is difficult to control, because the type of reactions are heterogeneous reactions which depend on the surface area. GOs break easily into many small sheets when the reactions are stirred under high revolutions per minute (RPM). In order to minimize the defects of surface area, the reaction was stirred at low RPM. Another disadvantage of the use of GO is that the thickness of modified GOs varies in size, such as single layer, double layers, and multi layers. In addition, the modified GO sheets also have many kinds of shapes, such as zigzag and armchair edged, which will affect the electrical properties of GO. These difficulties can be overcome by controlling the shape and size of graphene by multistage cutting and transfer method.[30] Graphene oxide could be made using this method to achieve a better quality of GO.

We were successful in synthesizing the GO-carboxylic acid-benzoic acid compound. The future work is to put the GO-carboxylic acid-benzoic acid compound on the surface of a chip. The current will be measured when the oligo-II on the chip is hybridized with DNA-FAM to test the ability to prevent the non-specific binding of DNA-FAM to the surface of GO.

REFERENCES

1. Hummers, S.W.; Offeman, E.R., *J. Am. Chem. Soc*., 1958, 80, 1339.
2. Li, Y.; Peng, Q.; He, X.; Hu, P.; Wang, C.; Shang, Y.; Wang, R.; Jiao, W.; Lv, H., *J. Mater. Chem*., 2012, 22, 18748.
3. Sun, J.; Meng, D.; Jiang, S.; Wu, G.; Yan, S.; Geng, J.; Huang, Y., *J. Mater. Chem.,* 2012, 22, 18879.
4. Li, S.; Deng, D.; Pang, H.; Liu, L.; Xing, Y.; Liu, S., *J. Solid State Electrochem.,* 2012, 16 2883.
5. Goodgame, R., *J. Current gastroenterology reports,* 2006, 8, 401.
6. Morillo, G. S.; Timenetsky, D. C. S. M., *Rev. Assoc. Med. Bras.,* 2011, 57, 453.
7. Lindesmith, L.; Moe, C.; Marionneau, S.; Ruvoen, N.; Jiang, X.; Lindblad, L.; Stewart, P.; Lependu, J.; Baric, R., *Nat. Med.,* 2003, 9, 548.
8. Zhang, B.; Chen, Y.; Zhuang, X.; Liu, G.; Yu, B.; Kang, E. T.; Zhu, J.; Yongxi Li, Y., *J. Polym. Sci. A Polym. Chem.,* 2010, 48, 2642.
9. Li, M.; Yang, X.; Ren, J.; Qu, K.; Qu, X., *Adv. Mater.,* 2012, 24, 1722.
10. Wust, P.; Hildebrandt, B.; Sreenivasa, G.; Rau, B.; Gellermann, J.; Riess, H.; Felix R.; Schlag, P. M., *Lancet Oncol*., 2002, 3, 487.
11. Huff, T. B.; Tong L.; Zhao, Y.; Hansen, M. N.; Cheng, J.; Wei A., *Nanomedicine*, 2007, 2, 125.
12. Markovic, Z. M.; Harhaji-Trajkovic, L. M.; Todorovic-Markovic, B. M.; Kepic´, D. P.; Arsikin, K. M.; Jovanovi, S. P.; Pantovic, A. C.; Dramic´anin, M. D.; Trajkovic, V. S., *Biomaterials,* 2011, 32, 1121.
13. Kam, N. W. S.; O'Connell M.; Wisdom, J. A.; Dai H., *Proc. Natl. Acad. Sci. USA,* 2005, 102, 11600.

14 Bao, H.; Pan Yo.; Ping, Y.; Sahoo, G. N.; Wu, T.; Li, L.; Li, J.; Gan, H. L., *Small*, 2011, 7,

1569.

15. Kim, J.-H.; Kim, Y.-S.; Park K.; Kang, E.; Lee S.; Nam, H. Y.; Kim, K.; Park, J. H.; Chi, D. Y.; Park, R.-W.; Kim, I.-S.; Choi, K.; Kwon, I. C., *Biomaterials,* 2008, 29, 1920.

16. Mao H.-Q.; Roy, K.; Troung-Le, V. L.; Janes, K. A.; Lin, K. Y.; Wang, Y.; August, J. T.; Leong, K. W., *J. Control. Release*, 2001, 70, 399.

17. Kim, I.-Y.; Seo, S.-J.; Moon, H.-S.; Yoo, M.-K.; Park, I.-Y.; Kim, B.-C.; Cho, C.-S., *Biotechnol. Adv.,* 2008, 26, 1.

18. Säkkinen, M.; Linna, A.; Ojala, S.; Jürjenson, H.; Veski, P.; Marvola, M., *Int. J. Pharmaceut.,* 2003, 250, 227.

19. Brodie, B., *Phil. Trans*., 1859, 149, 249.

20. Hofmann, U.; Frenzel, A., *Bct.*, 1930, 898, 1248.

21. Hamdi, H.; *Kelloid Beihefte*, 1943, 64, 654.

22. Park, S.-H.; Bak, S.-M.; Kim, K.-H.; Jegal, J.-P.; Kim, K.-B., *J. Mater. Chem.,* 2011, 21, 680.

23. Zhu, Y.; Murali, S.; Stoller, D. M.; Velamakanni, A.; Piner, D. R.; Ruoff, S. R., *Carbon*, 2010, 47, 2118.

24 Luo, Z.; Lu Y.; Somers, A. L.; Johnson, A. T. C., *J. Am. Chem. Soc*. 2009, 131, 898.

25. Luo, Z.; Johnson, T. A., *U.S Patent* 61/140,555, 2012.

26 Zeng, J.; Nikolelis, P. D.; Krull J. U., *Electroanalysis,* 1999, 11, 770.

27 Jahan, M.; Bao, Q.; Yang, J.-X.; Loh, P. K., *J. Am. Chem. Soc*. 2010, 132, 14487.

28 Kudin, N. K.; Ozbas, B.; Schniepp, C. H.; Prud'homme, K. R.; Aksay, A. I.; Car, R., *Nano Lett.*, 2008, 8, 36.

29 Riordon, J.; Mirzaei, M.; Godin, M., *Lab Chip*, 2012,12, 3016.

30 Ci, L.; Song, L.; Jariwala, D.; Elıas, L. A.; Gao, W.; Terrones, M.; Ajayan, M. P., *Adv. Mater*., 2009, 21, 1.

APPENDIX A

EXPERIMENTAL SECTION

A. General procedures

DMF was distilled over CaH_2. AFM images were obtained from a NanoScope IIIa using tapping-mode. The fluorescent microscope (Axioskop 2) was used to take fluorescent images. Infrared (IR) spectra were obtained from a NICOLET 380 FT-IR. Raman spectrums were obtained from a DXR Raman Microscope using the wavelength 532 nm in Dr. Jun Li's laboratory.

Prepare silicon chip for GO immobilization

A silicon chip was washed with a strong jet of acetone (10 sec), iso-propanol (10 sec) and ethanol (10 sec) and this was repeated once more. After drying the chips with a stream of argon gas the chips was placed into oxygen plasma for 45 min using the high setting. These chips was placed into a solution of 3-aminopropyltrioxysilane in ethanol (10 μL of -aminopropyltrioxysilane/10 mL ethanol/chip) for 30 min at room temperature. The chips were washed with a strong jet of ethanol for 10 sec and dried with stream of argon gas. The chip was incubated at 120^{o}C for 30 min and used immediately for GO immobilization.

Measure electrical current of the chips

Electrical conductivity measurements were taken using a Keithley source meter. TSP express program was used to measure the electrical current of the chips. The program which was used to record I/V scans is shown below.

Channel A IV test-4V-v-step0.04.tsp

-BASIC I VS V CONSTANTS

-- Use these to control the range and resolution of each IV measurement

numaverages=500 --filter averages the measurement over 5 readings

vstart=-4

vstop=4

vstep=0.04

--ENDCONSTANTS

--IV LOOPING CONSTANTS

-- Use these to set how many IVs you want to take and how long to wait in between them

numberloops=15

time_between_loops=20/60 --in minutes

--END LOOPING CONSTANTS

Washing the chips after DNA-FAM or RNA treatment

Chips were rinsed with 5% acetic acid in DI water (10 sec) and DI water (10 sec). Chips were then dried with a stream of argon for 30 sec.

B. Experimental section for synthesis

Graphene oxide (GO)

Graphite flakes (2 x 250 mg) were expanded by heating in a microwave reactor (30 W, 4 min) under argon atmosphere. The two batches of expanded graphite flakes were combined and added to a stirring solution of $K_2S_2O_8$ (15g) and P_2O_5 (15g) in 90 mL concentrated HCl at 90^{o}C. Contents stirred at 85^{o}C for 4 hours. The mixture was cooled to 10^{o}C and added slowly to ice cold deionized water (750 mL). The mixture was filtered through a medium gauge glass frit funnel and the black residue was washed with deionized

water (3 L). The black residue was transferred to a beaker and dried in an incubator at 60^{o}C overnight to yield a black fluffy solid (550 mg). The black fluffy solid was transferred to a 500 mL Erlenmeyer flask to which H_2SO_4 (80 mL) was added at 0^{o}C. $KMnO_4$ was added and the mixture was heated to 35-40^{o}C with stirring for 2 hours. The mixture was cooled to room temperature and added to cold deionized water (500 mL) followed to stirring for 2 hours. H_2O_2 (30%, 20 mL) was added drop wise at 0^{o}C via a dropping funnel. A yellow mixture formed which was left to sit at room temperature for 2 days allowing the graphene oxide to settle to the bottom. The clear supernatant was decanted and the yellow graphene oxide suspension was transferred to 15 mL centrifuge tubes. The tubes were centrifuged at 2500 rpm for 2 hours. The clear supernatant was decanted and the graphene oxide residue was washed with 10% HCl (10 mL each). The tubes were centrifuged and the clear supernatant was decanted. The graphene oxide was further washed three times with deionized water (10 mL each) using centrifugation method. The washed graphene oxide was transferred into three dialysis bags (12000 Mw cut off) and dialyzed for 1 week changing the dialysis water daily. The graphene oxide was lyophilized to provide a puffy light brown solid. **IR** (O-H stretch) 3355 cm^{-1}, (C=O stretch) 1729 cm^{-1}, and (C=C stretch) 1623 cm^{-1}. **Raman** (G band) 1600 cm^{-1}, (D band) 1360 cm^{-1}.

GO-benzoic acid (3)

p-Amino benzoic acid (480 mg) and NaOH (140 mg) were added to DI water (40 mL). The mixture was then cooled down to 0-5^{o}C. Sodium nitrite (264 mg) was added slowly into the mixture at 0-5^{o}C. 20% HCl in DI water (4 mL) was added quickly and stirred for 45 min. The solution turned into pale yellow. This solution was then poured into GO (60 mg). The mixture was at 0-5^{o}C about 4 hr. The mixture was then shaken in a shaker for 2 days at room temperature. GO was washed with DI water by using the centrifugation

technique at 2500 rpm for 1 hour (repeated 4 times). The remaining solid was lyophilized to give GO-benzoic acid (45 mg). **IR** (O-H stretch) 3308 cm^{-1}, (C=O stretch) 1725 cm^{-1}, and (C=C stretch) 1619 cm^{-1}. **Raman** (G band) 1600 cm^{-1}, (D band) 1360 cm^{-1}.

GO-benzoic acid-5-aminofluorescein (4)

GO-benzoic acid (3.5 mg) was suspended in dry DMF (2.5 mL) in a round-bottom flask. The mixture was shaken in a shaker for 30 min. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (2.5 mg) and 5-H_2N-fluorescein (4.5 mg) were then added into the mixture. The round-bottom flask was capped and covered by aluminum foil, and was put in a shaker for 2 days at room temperature. The resulting GO-benzoic acid-5-aminofluorescein was then kept in the dark under argon. GO-benzoic acid-5-aminofluorescein was washed with DI water by using the centrifugation technique at 2500 rpm for 1 hour (repeated 7 times). **IR** (O-H stretch) 3332 cm^{-1}, (C=O stretch) 1697 cm^{-1}, and (C=C stretch) 1624 cm^{-1}.

GO-acetic acid (2)

Trifluoroacetic acid (4 mL) was added to a suspension of graphene t-butyl ester (45 mg) in DI water (14 mL). The suspension was shaken in a shaker for 1 hr. The reaction was centrifuged at 2500 rpm for 35 min and supernatant was decanted. The GO-acetic acid was washed twice with deionized water using the centrifugation technique at 2500 rpm for 1 hour (repeated 4 times). The remaining solid was lyophilized to give GO-acetic acid as a brown solid (32 mg). **IR** (O-H stretch) 3285 cm^{-1}, (C=O stretch) 1686 cm^{-1}, and (C=C stretch) 1646 cm^{-1}. **Raman** (G band) 1600 cm^{-1}, (D band) 1360 cm^{-1}.

GO-acetic acid-benzoic acid (6)

Trifluoroacetic acid (4 mL) was added to a suspension of GO-t-butyl ester-benzoic acid (48 mg) in DI water (14 mL). The suspension was shaken in a shaker for 1 hr. The reaction was centrifuged at 2500 rpm for 35 min and supernatant was decanted. The GO-acetic acid-benzoic acid was washed twice with deionized water using the centrifugation technique at 2500 rpm for 1 hour (repeated 4 times). The remaining solid was lyophilized to give GO-acetic acid-benzoic acid as a brown solid (35 mg). **IR** (O-H stretch) 3362 cm^{-1}, (C=O stretch) 1710 cm^{-1}, and (C=C stretch) 1609 cm^{-1}. **Raman** (G band) 1600 cm^{-1}, (D band) 1360 cm^{-1}.

GO-carboxylic acid-benzoic acid-5-aminofluorescein (7)

GO-carboxylic acid-benzoic acid (3.5 mg) was expanded in dry DMF (2.5 mL) in a round-bottom flask. The mixture was shaken in a shaker for 30 min. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (2.5 mg) and 5-H_2N-fluorescein (4.5 mg) were then added into the mixture. The round-bottom flask was capped and covered by aluminum foil, and was put in a shaker for 2 days at room temperature. GO-carboxylic acid-benzoic acid-5-aminofluorescein was then kept in the dark under argon. GO-carboxylic acid-benzoic acid-5-aminofluorescein was washed with DI water by using the centrifugation technique at 2500 rpm for 1 hour (repeated 7 times). **IR** (O-H stretch) 3371 cm^{-1}, (C=O stretch) 1697 cm^{-1}, and (C=C stretch) 1600 cm^{-1}.

GO t-butyl ester (1)

Dry DMF (40 mL) was added to a mixture of graphene oxide (100 mg) and diethyl ether washed 60% NaH (6.6 mmol) under argon atmosphere. The mixture was shaken in a

shaker for 1 hr. t-Butyl bromoacetate (1.287 mg, 6.6 mmol) was added and the mixture was shaken in a shaker for 2 days. The reaction mixture was centrifuged at 2500 rpm for 30 min and the supernatant was decanted. The graphene oxide t-butyl ester was washed with water (10 mL) followed by centrifugation at 2500 rpm for 1 hour. The supernatant was removed and the graphene oxide t-butyl ester was washed twice with deionized water using centrifugation method. The remaining solid was lyophilized to give graphene oxide t-butyl ester as a brown fluffy solid (90 mg). **IR** (O-H stretch) 3402 cm^{-1}, (CH_3 stretch) 2976 cm^{-1} and 2929 cm^{-1}, (t-butyl stretching vibrations) 1362 cm^{-1} and 1389 cm^{-1}, (C=O stretch) 1713 cm^{-1}, and (C=C stretch) 1639 cm^{-1}. **Raman** (G band) 1600 cm^{-1}, (D band) 1360 cm^{-1}.

GO-t-butyl ester-benzoic acid (6)

p-Amino benzoic acid (480 mg) and NaOH (140 mg) were added to DI water (40 mL). The mixture was then cooled down to 0-5^{o}C. Sodium nitrite (264 mg) was added slowly into the mixture at 0-5^{o}C. 20% HCl in DI water (4 mL) was added quickly and stirred for 45min. The solution turned into pale yellow. This solution was then poured into GO-t-butyl ester (60 mg). The mixture was at 0-5^{0}C about 4 hr. The mixture was then shaken in a shaker for 2 days at room temperature. GO was washed with DI water by using the centrifugation technique at 2500 rpm for 1 hour (repeated 4 times). The remaining solid was lyophilized to give GO-t-butyl ester-benzoic acid (48 mg).

Attachment of single stranded DNA (Oligo-II) to GO without HATU (9)

100 μL oligo-DNA nucleotide II (0.011 μmol/mL) and a chip containing GO were placed into a well dish. Deionized water (400 μL) was added to the well so as to submerge the chip. Deionized water was added to surrounding empty wells to help create a humid atmosphere inside the dish slowing the rate of evaporation of water in the well containing the

chip. The dish was covered and sealed and incubated at 50°C for 2 hrs. The chip was removed and washed with a strong jet of deionized water for 1 min and dried with a stream of argon for 30 sec. The chip was dried in the incubator at 35°C overnight. Electrical conductivity measurements were taken using a Keithley source meter, and data are shown in Figure 15.

Attachment of single stranded DNA (Oligo-II) to GO (9)

100 μL oligo-DNA nucleotide II (0.011 μmol/mL) and a chip containing GO were placed into a well dish. Deionized water (400 μL) was added to the well so as to submerge the chip. Deionized water was added to surrounding empty wells to help create a humid atmosphere inside the dish slowing the rate of evaporation. An HATU solution (100 μL; 2 mg/mL) was added to the well containing the chip. The dish was covered and sealed and incubated at 50°C for 2 hrs. The chip was removed and washed with a strong jet of deionized water for 1 min and dried with a stream of argon for 30 sec. The chip was dried in the incubator at 35°C overnight.

Dehybridization (From compound 10 to compound 9)

The chip was placed into a solution of 10 M urea (5 mL) and incubated with gentle shaking for 3 hrs at 40°C. Chip was washed with a strong jet of deionized water for 1 min dried with a stream of argon for 30 sec. Microscope images were taken to observe GO deposit and electrical conductivity measurements were taken using a Keithley source meter, and data are shown in Figure 13,14, and 15.

Hybridization with complementary norovirus RNA (10)

A solution of complementary norovirus RNA (100 μL; 0.011 umol/mL) (obtained from Dr. Kyeong-Ok Chang) was added to Ultrahyb-Ambion Northern Max hybridization buffer (100 μL) and the resulting solution was heated at 90^{o}C for 30 sec using a water bath to disaggregate self-double helix. 40 μL of the complementary norovirus RNA solution was added to the P-electrode lane of the chip containing oligo-II. The chip was placed in a well chamber and deionized water was put into the surrounding wells to create a humid atmosphere preventing evaporation. The container was covered and sealed and placed in an incubator at 40^{o}C for 2 hrs. The chip was taken out and washed with a strong jet of deionized water (1 min) and dried with a stream of argon gas. Electrical conductivity measurements were taken using a Keithley source meter, and data are shown in Figure 13.

Hybridization with DNA-FAM-RC-Cog2 (10)

A solution of DNA-FAM-RC-Cog2 (100 μL; 0.011 μmol/mL) obtained from Intergrated DNA Technologies was added to Ultrahyb-Ambion Northern Max hybridization buffer (100 μL) and the resulting solution was heated at 90^{o}C for 30 sec using a water bath to disaggregate double helix. 40 μL of the DNA-FAM-RC-Cog2 solution was added to the P-electrode lane of the chip containing oligo-II. The chip was placed in a well chamber and deionized water was put into the surrounding wells to create a humid atmosphere preventing evaporation. The container was covered and sealed and placed in an incubator at 40^{o}C for 2 hrs. The chip was taken out and washed with a strong jet of deionized water (1 min) and dried with a stream of argon for 30 sec. Electrical conductivity measurements were taken using a Keithley source meter, and data are shown in Figure 14. Fluorescent images were recorded to observe DNA-FAM-RC-Cog2 attachment, as shown in Figure 12.

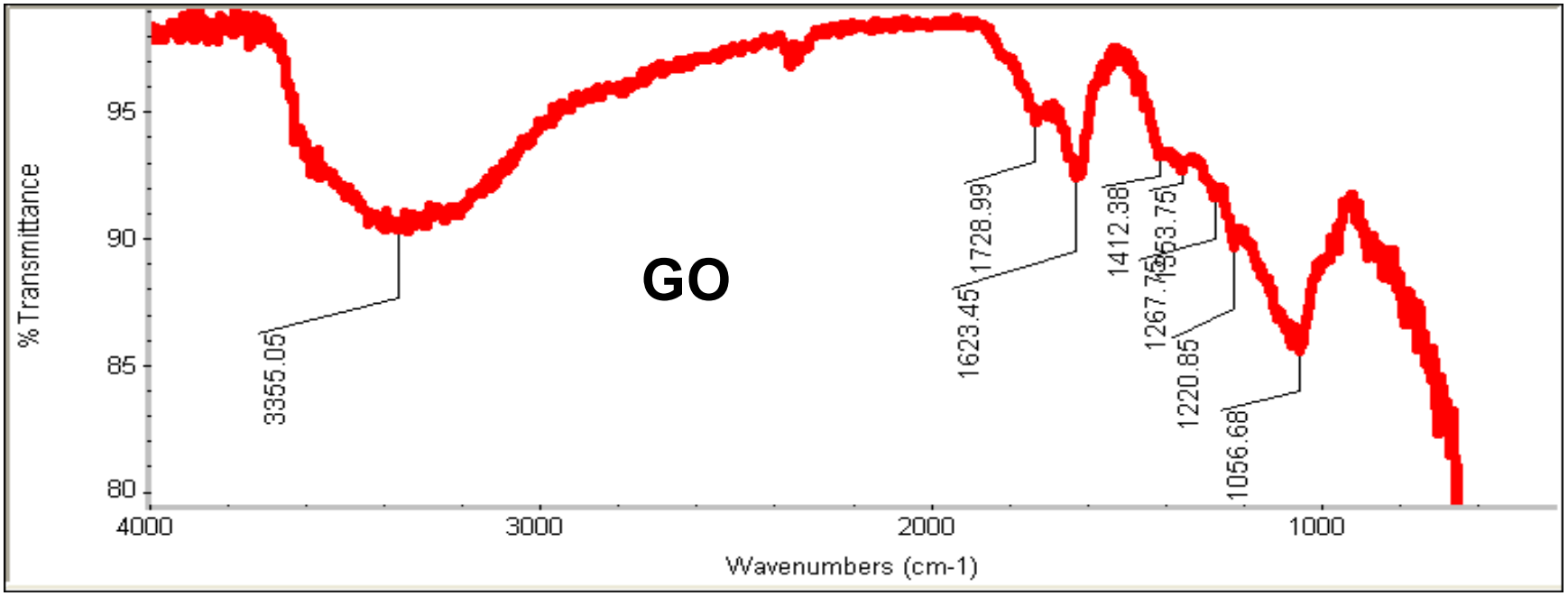
Hybridization with non-complementary norovirus RNA (10)

A solution of non-complementary norovirus RNA (100 μL; 0.011 umol/mL) (obtained from Dr. Kyeong-Ok Chang) was added to Ultrahyb-Ambion Northern Max hybridization buffer (100 μL) and the resulting solution was heated at 90^{o}C for 30 sec using a water bath to disaggregate self-dimmers. 40 μL of the non-complementary norovirus RNA solution was added to the P-electrode lane of the chip containing oligo-II. The chip was placed in a well chamber and deionized water was put into the surrounding wells to create a humid atmosphere preventing evaporation. The container was covered and sealed and placed in an incubator at 40^{o}C for 2hrs. The chip was taken out and washed with a strong jet of deionized water (1 min). Electrical conductivity measurements were taken using a Keithley source meter, and data are shown in Figure 16b.

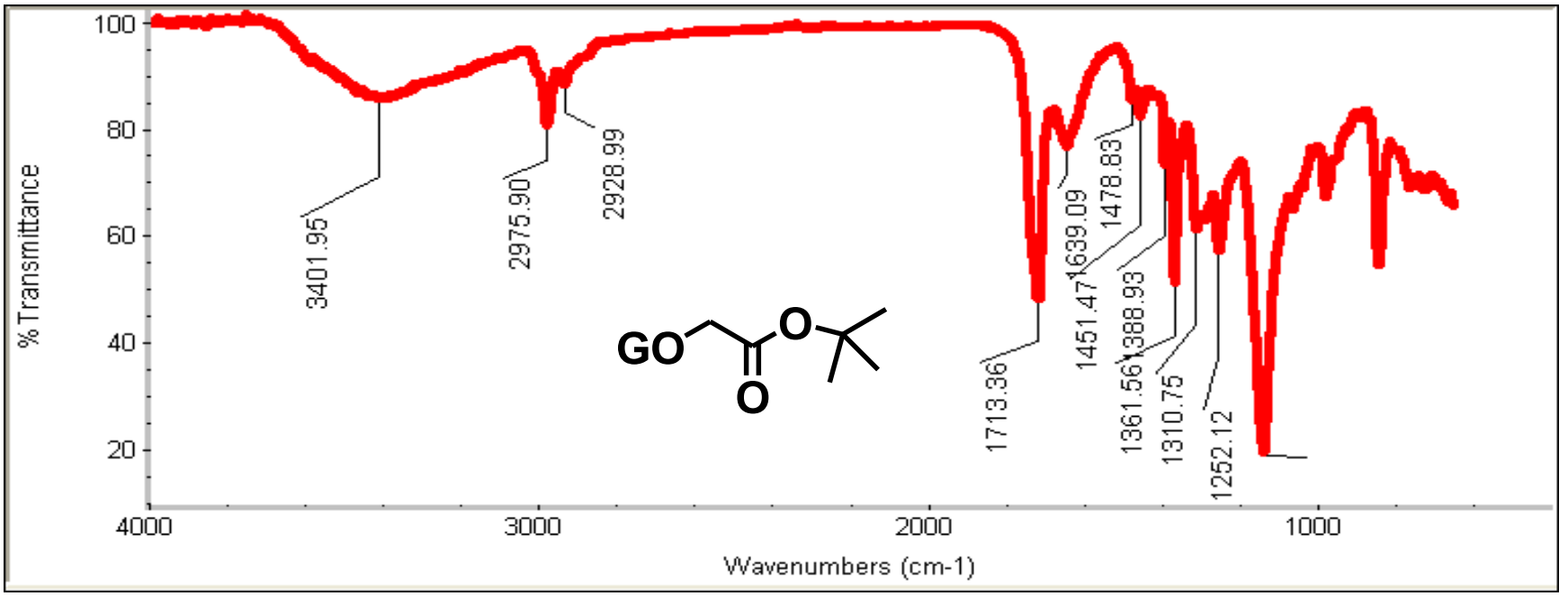
APPENDIX B:

FTIR AND IR SPECTRA

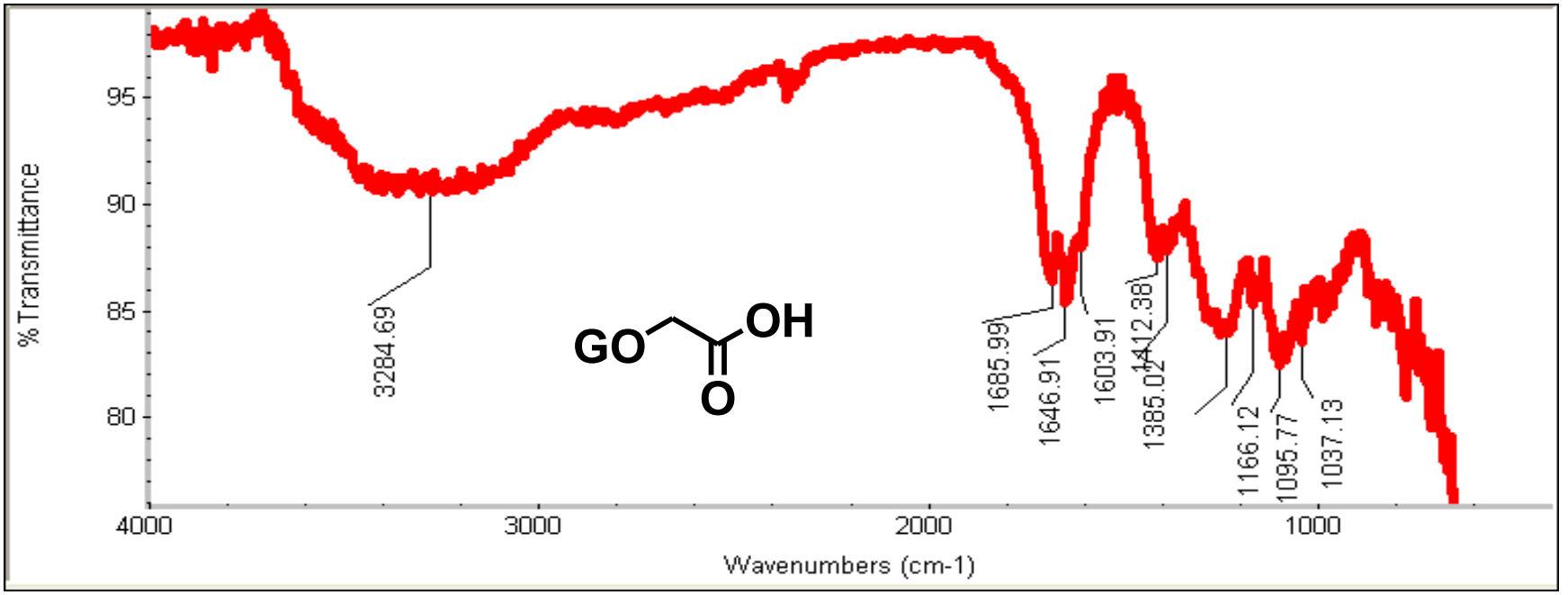
FTIR of GO



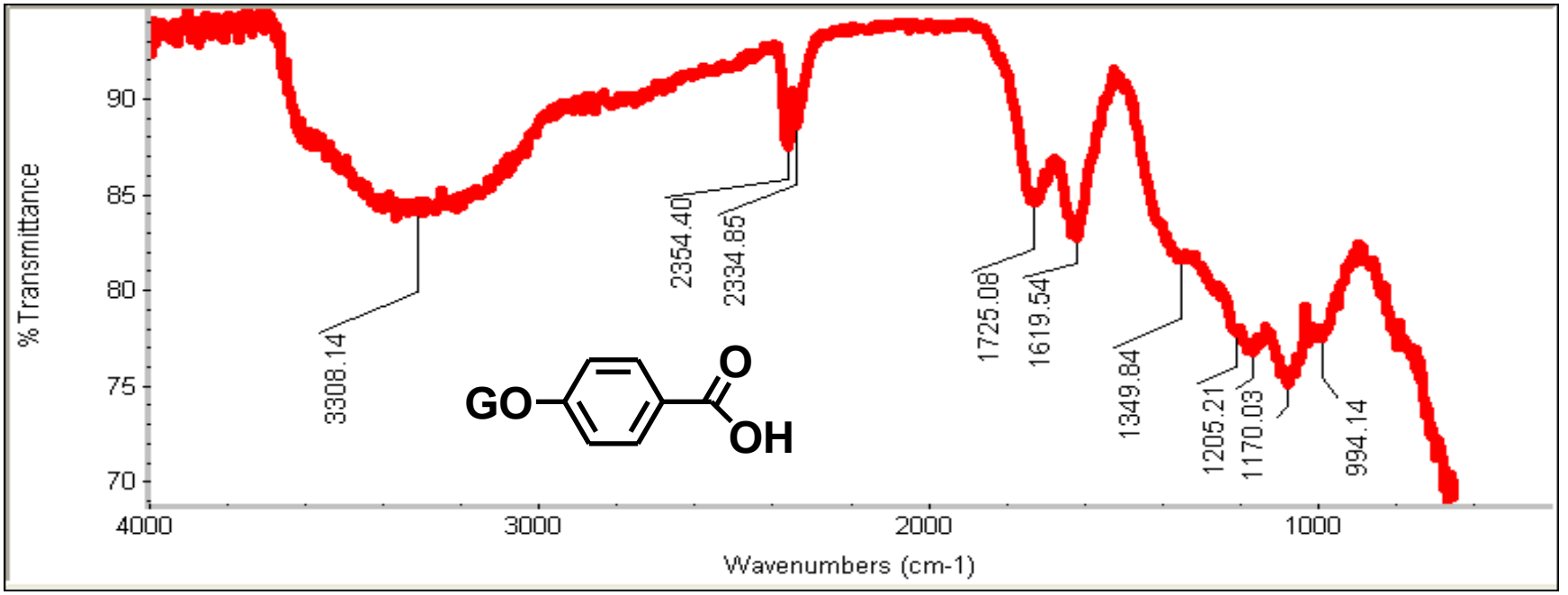
FTIR of GO-t-butyl ester



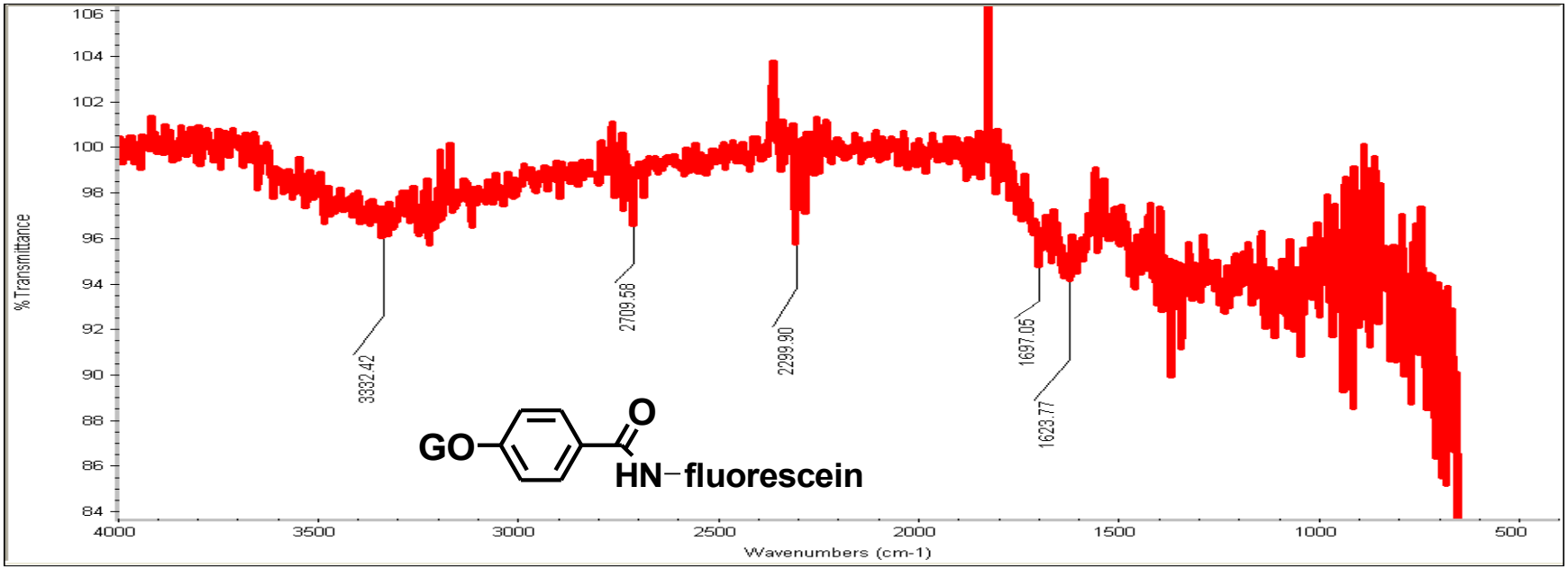
FTIR of GO-carboxylic acid



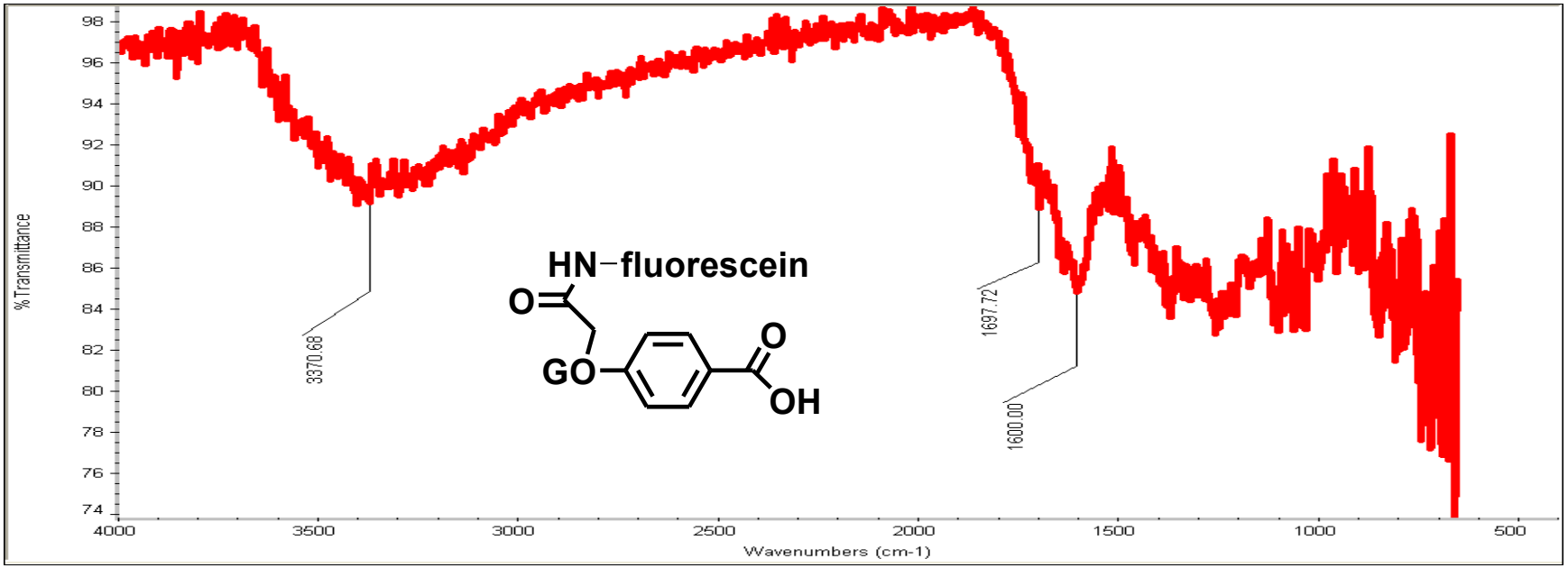
FTIR of GO-benzoic acid



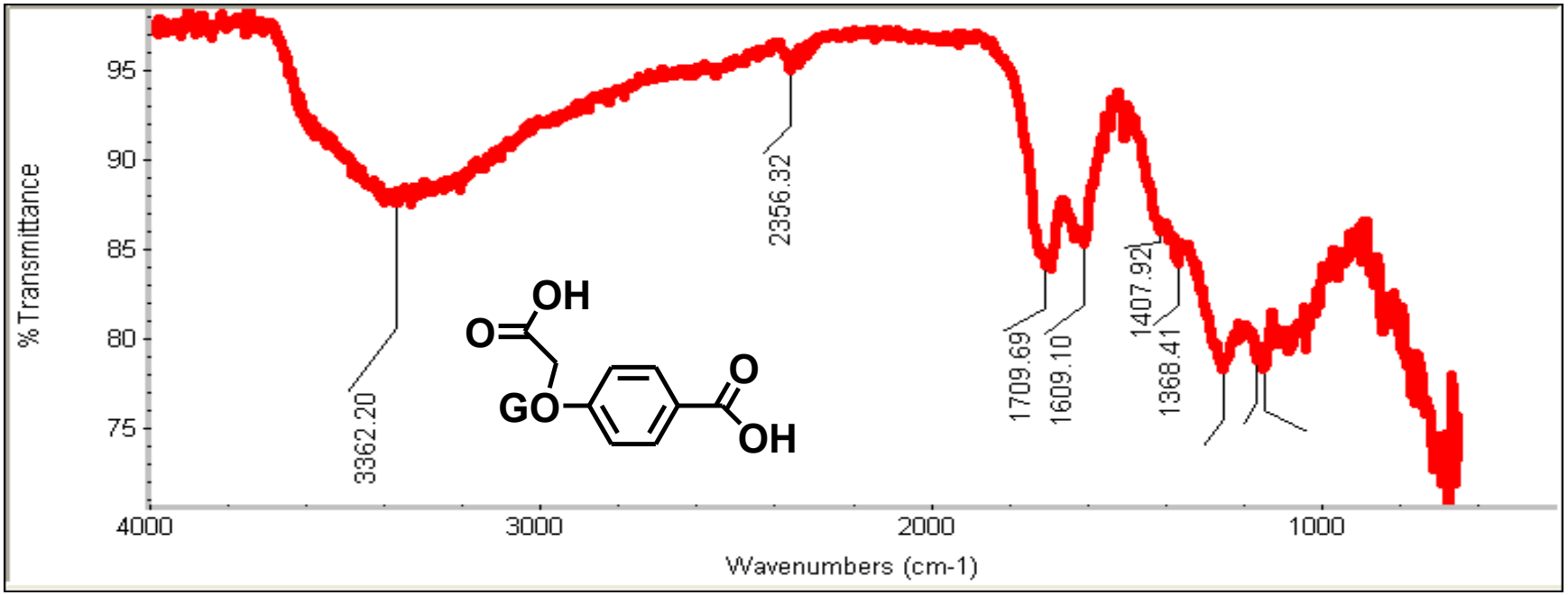
FTIR of GO-benzoic acid-5-aminofluorescein



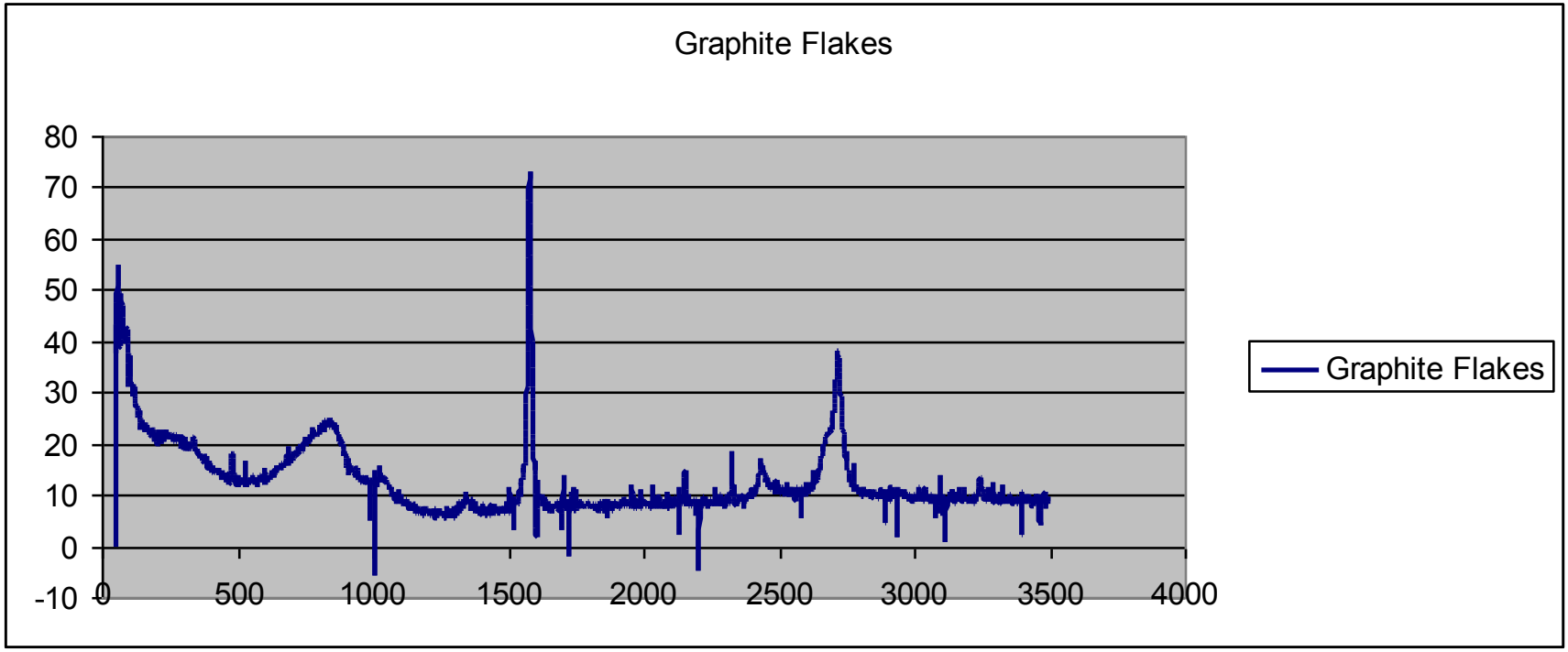
FTIR of GO-carboxylic-benzoic acid-5-aminofluorescein



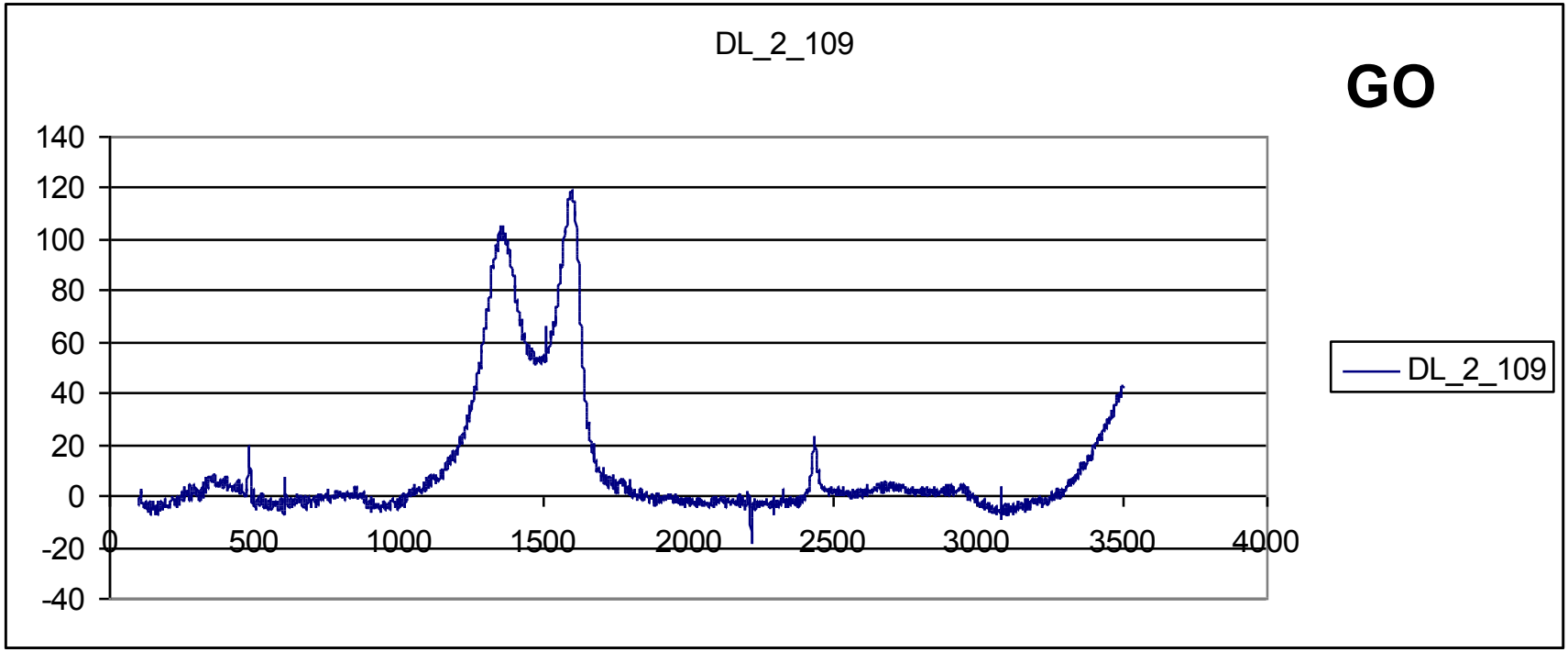
FTIR of GO-carboxylic acid-benzoic acid



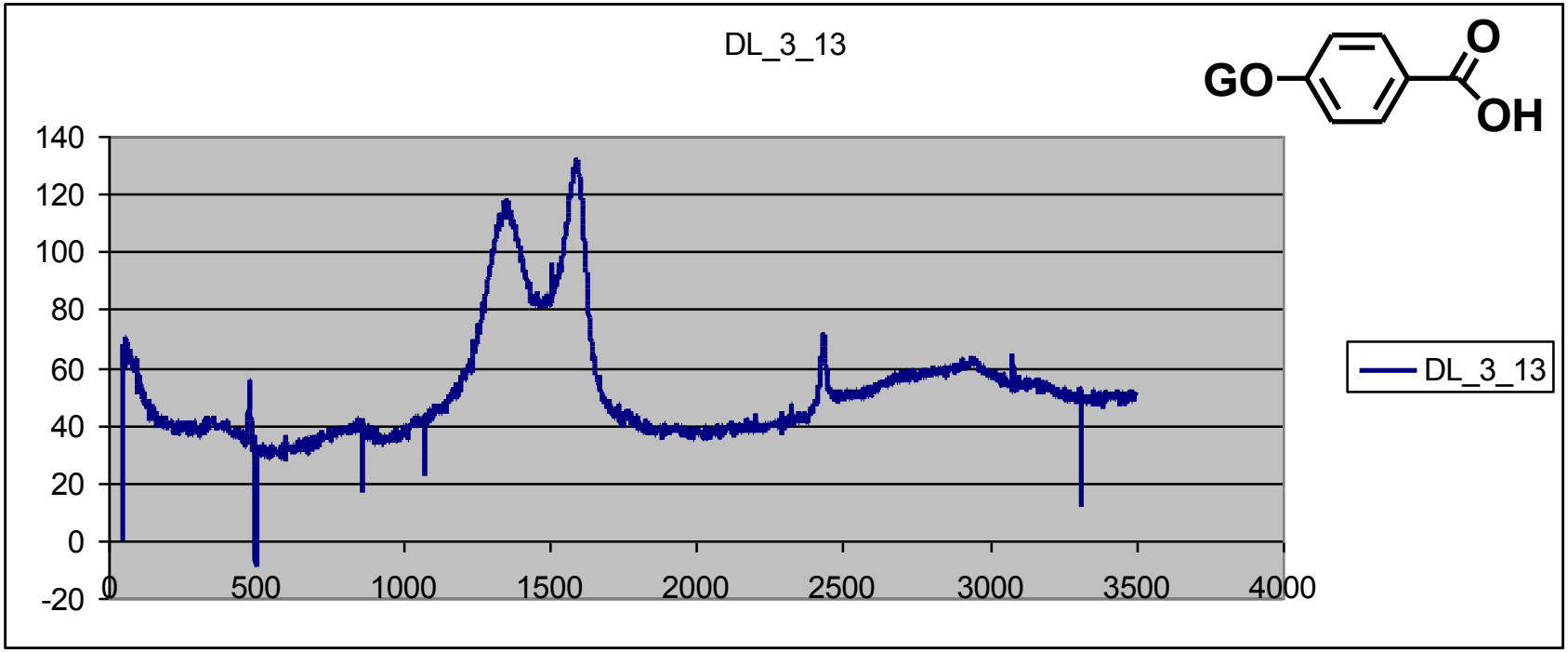
Raman spectrum of Graphite Flake



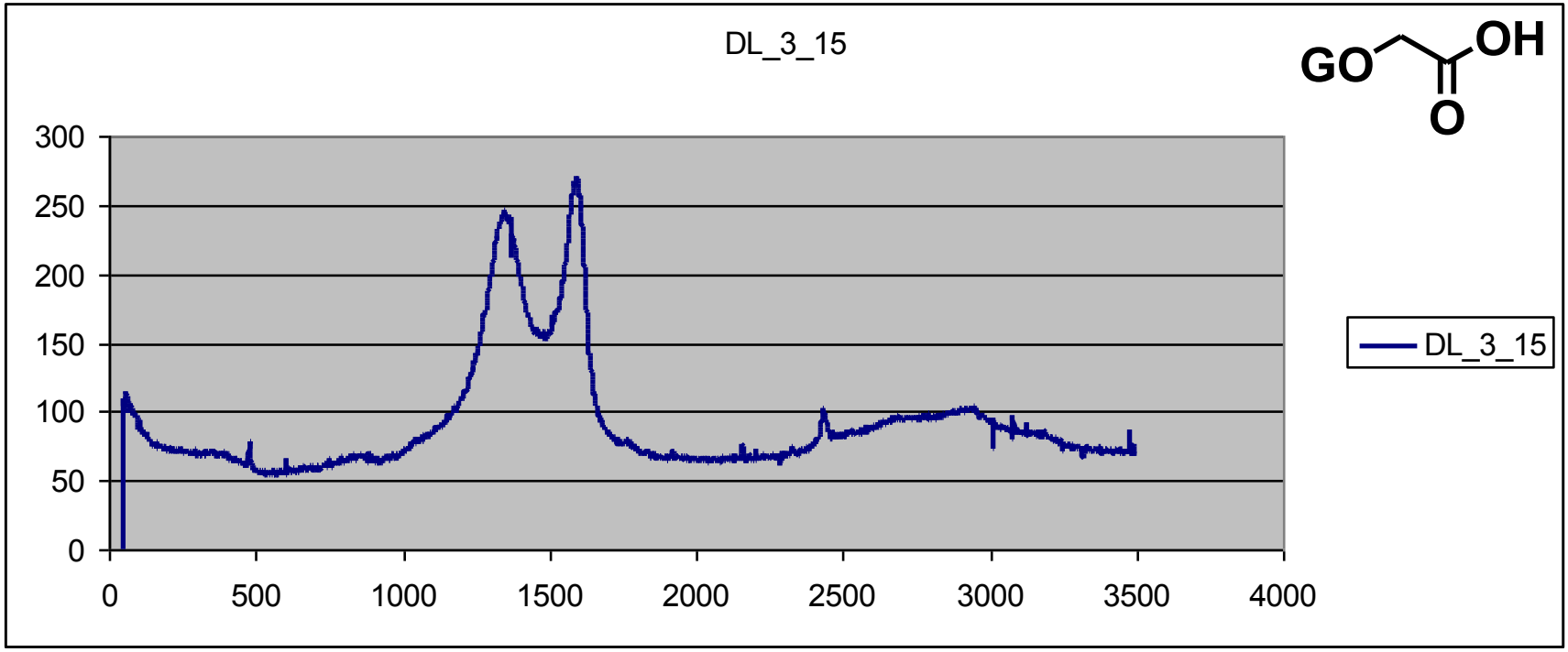
Raman spectrum of GO



Raman spectrum of GO-benzoic acid



Raman spectrum of GO-carboxylic acid



Raman spectrum of GO-carboxylic acid-benzoic acid