Binding of Peptides to Graphene

By

Preeti Pratap
B.E., Anna University, 2011

THESIS

Submitted as partial fulfillment of the requirements for
the degree of Master of Science in Bioengineering
in the Graduate College of the
University of Illinois at Chicago, 2015

Chicago, Illinois

Defense Committee:

Michael Stroscio, Chair and Advisor
Jun Cheng
Mitra Dutta, Electrical and Computer Engineering

ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Dr. Stroscio, for all his encouragement and support throughout this project. The innumerable discussion sessions with him helped me gain a good perspective about my topic of interest and provided the foundation I needed to come up with a suitable project. I sincerely appreciate how he steered me in the right direction towards approaching my thesis, helping me achieve the desired goals. I would like to thank Robin and Xenia, PhD students in Dr. Stroscio's lab, for all their suggestions and guidance. Lastly, I would like to thank my other two thesis committee members, Dr. Mitra Dutta and Dr. Jun Cheng for consenting to be on my committee.

PP

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

LIST OF ABBREVIATIONS

GFP	Green Fluorescent Protein
DNA	Deoxyribonucleic Acid
CT Dyes	Charge Transfer Dyes
CNT	Carbon Nanotube
SWCNT	Single Walled Carbon Nanotube
DWCNT	Double Walled Carbon Nanotube
PZT	Lead Zirconate-Titanate
FTIR	Fourier Transform Infrared Spectroscopy
PES	Polyethersulphone

SUMMARY

Peptides are known to have molecular recognition properties but there has not been a lot of research done based on using peptides in the fabrication of biosensors. In that direction, this thesis aims to explore how peptides can be used to functionalize a graphene substrate. Graphene has excellent electronic mobility, conductivity and strength that make it a suitable choice of substrate for a biosensor. Using a particular sequence of peptide that has been identified by phage display methods to bind to graphene, various modifications are done to the original sequence to study and analyze the selectivity of this peptide in binding. Quantum dots are used to qualitatively verify whether binding occurs or not by imaging the re-emission of incident light under a fluorescence microscope. It has been observed that the peptide sequence is extremely selective and that any modification to its sequence hampers binding. Introducing aromaticity and additional polarity in the sequence does not favor binding. In conclusion, there is a promising future for graphene-based, peptide-functionalized nano biosensors that have improved sensitivity and speeds when compared to sensors available today.

1. INTRODUCTION

1.1 Background

Graphene, in the recent times, has been a preferred choice of material for biological, electrical and optical applications, owing to its excellent transport properties, mechanical strength, ease of functionalization and mass production, high conductivity and commendable electrochemical properties. However, in order for it to be used in electronic applications, suitable functionalization is required due to its zero band gap and inertness to reaction and also to prevent stacking of graphene sheets together due to π-π stacking which makes it hydrophobic, preventing it from being dissolved in common polar solvents. This is one of the reasons why a large number of research projects focus on the functionalization of graphene, its reaction with organic and inorganic molecules and in general, the chemical modification of graphene surfaces. The most important factor to be considered while designing a biosensor based on a carbon-based substrate is the bond between the molecular sensing elements and the substrate, which should not tamper with the transport properties and electronic network [6] of the substrate and in the case of graphene, the extended π-conjugation on its surface. An effective methodology for achieving this, without creating any atomic scale defects is non-covalent functionalization of graphene and this document highlights how functionalization is done using peptides to pave way for sensitive nano biosensors in the field of biotechnology.

1.2 Fluorescence Microscopy and Quantum Dots

Fluorescence microscopy is a very powerful method with high sensitivity and resolution that caters to the needs of various research fields, particularly biology. The fluorophores or fluorochromes or fluorescent probes are chemical compounds that re-emit light upon excitation

of light and are widely used as tracers in fluids, as dyes to stain specific structures but the most important use is tagging them to a macromolecule (such as nucleic acids and proteins) covalently, making them act like markers for bioactive agents such as proteins. These fluorophores impart the above mentioned characteristics to fluorescence microscopy, making it a very reliable and accurate source of extracting information. The versatility, tunability and quantitative capabilities of fluorescent molecules render them highly suitable for application in biological research, among other fields.

Fluorophores have been used in biological research for the last 100 years but advancement in the understanding of fluorescence chemistry has paved the way to a wider selection of fluorophores available today. These have excellent variation, flexibility and performance levels that can be tuned to suit specific needs. In general, fluorophores can be divided into three groups:

a) Biological fluorophores

b) Organic dyes

c) Quantum dots

Biological fluorophores are those available in nature, a good example being *Aequorea Victoria*, [2] a jellyfish from which the luminescent protein *aequorin* and the fluorescent molecule GFP (green fluorescent protein) have been isolated, purified and cloned. It was found that this jellyfish released calcium ions in order to bioluminesce. Blue light is released when the calcium ions bind to *aequorin* and this blue light is absorbed by GFP, which emits green light. GFP is a reporter gene expression protein [3], meaning that they show visible signs of certain

genes being taken up by or expressed by the cell or the organism population. Since the discovery of GFP, many of its variants have been derived including phycobiliproteins (allophycocyanin, phycocyanin, phycoerythrin and phycoerythrocyanin) and many other proteins are being used in many biological research systems for detecting various biological events. The advantages of biological fluorophores are that they can be used to detect and observe a wide range of biological signals and events, introduced into a large number of tissues, easily targeted to subcellular components and photodynamic toxicity is kept at bay [4]. Also these biological fluorophores can be introduced into the system by means of a plasmid (a small DNA molecule which can replicate independent of chromosomal DNA within a cell) to drive the expression of just the fluorophore or combined with a protein and infused in the case of biological events. The disadvantages of these fluorophores, however, is that the entire experimental process is very time consuming and also, using large amounts of light emitting fluorophores for expression might give rise to reactive oxygen species which can further cause toxicity and the size of the fluorescent protein fused can alter the physiology of the cellular protein to which it is fused and hence do not provide with much sensitivity. Also, the excitation spectrum of GFP variants is very narrow and hence, the light emission is over a wide range [5], making it difficult for multiple labeling to occur.

Organic dyes are the first and the most versatile fluroscent dyes to have been used in biological research. The optical properties of these fluorophores are not highly tunable and even so, in order to improve upon these properties, huge changes in the design strategies have to be done if the structure-property relationship is known [5], since these work on the principle of electronic transitions. There are two types of organic dyes: resonant dyes and CT (Charge

Transfer) dyes based on how the emission occurs. In the case of resonant dyes, the optical transition is completely delocalized over the entire fluorophore while in the case of CT dyes, emission occurs due to intramolecular transitions of charge transfer. Charge transfer bands involve the promotion of an electron from an orbital with metallic characteristics to an orbital with ligand-like characteristics or reverse. These bands have energies that are sensitive to the polarities of the solvents in the microenvironment. Hence, the frequency of the transition depends on the dielectric constant of the surrounding medium and this property can be utilized to design fluorophore probes that can be used for sensing microenvironment polarity. [6] The advantages of organic dyes over quantum dots and biological fluorophores is that their size is small enough to be able to cross-link them to macromolecules for inducing them into biological membranes, without disrupting the physiology of the cell or molecules. Another advantage is that they have a wide range of excitation and emission spectra along with optimal quantum yields that makes them versatile for various fluorescence applications.

Quantum dots are semiconductor nanoparticles of usually chalcogenides (sulphides and selenides), from metals such as zinc and cadmium. These normally comprise anywhere between a few hundred to thousands of atoms and the range of their size varies between 2 and 10 nanometers in diameter (approximately equal to the width of 50 atoms). Recent studies have shown that quantum dots are used as a new type of fluorescent probe, owing to the fact that there are wide applications of semiconductor nanocrystals in the field of biotechnology, especially in studies involving subcellular processes in biology [1].

Quantum dots have properties in between that of bulk semiconductors and discrete molecules. They are small enough so as to be able to exhibit quantum-sized mechanical properties and luminesce by the recombination of excitons (the bound state of an electron hole and an electron held together by Coulomb force). Quantum dots have great advantages over biological fluorophores and organic dyes because of how their emission wavelength can be tuned precisely by varying the size of the quantum dot (as the size decreases, emission wavelength decreases and hence shifts from red to blue). This gives rise to a very symmetric and narrow emission spectra and moreover, a single source of excitation light can be used to give rise to simultaneous excitation of a series of quantum dots varying in the color of emitted light, emitting different and higher wavelength (due to energy loss either by transfer to neighboring molecules or molecular collision) than the excitation source. Also, good quality quantum dots are highly resistant to photobleaching and change in pH and can also undergo multiple cycles of excitation and emission for many hours together. These excellent optical and spectral properties of quantum dots make them highly suitable for a wide variety of multicolor and ultrasensitive bioengineering applications.

1.3 Physics of Quantum Dots

Quantum dots are neither small molecules or bulk materials but are semiconductor particles with radii in the nanometer range. Their small radii has rendered them with excellent, tunable electronic, mechanical and optical properties that have been investigated by research scientists over two decades [7-9].

Consider a semiconductor crystallite (crystals held together by defective boundaries) whose radius is smaller than that of the exciton Bohr radius (most probable distance between an electron and a proton) of the quantum dot. In such a case, the quantum dots exhibit quantum confinement. Due to this, the 'particle in a box' theory can be applied to model the different energy levels, depending on the length of the box. The quantum dot is considered to be 'strongly confined' when the exciton Bohr radius is much smaller than the quantum dot radius while it is considered to be 'weakly confined' if the exciton Bohr radius is around the same value as that of the quantum dot. The advantage of having quantum confinement effects dominating (occurs only when the radius of the quantum dot is small, ideally less than 10 nm) is that the optical properties become highly tunable.

In the perspective of physics, one of the most important qualities of a defect-free, semiconductor is the presence of a band gap, E_{gap}, or a gap in energy where no energy states exist. There exist two bands of energy above and below this band gap known as conduction band and valence band, respectively. Conventionally, the lowest energy of the conduction band is denoted as E_c and the highest energy of the valence band is denoted as E_v.

Now, let us consider an infinitely deep quantum well along a single dimension (along the x axis) where E_c has a value infinity outside of the well in the region $0<x<d$ and a value zero within the well. Quantum mechanics describes that a particle confined in such a well can be described using the time-dependent Schrödinger equation while solving the equation gives the values of the energy that the particle can take, which are always quantized:

$$E_n = \frac{n^2 \pi^2 \hbar^2}{2md^2}, \qquad \text{n=1, 2, 3…} \tag{1}$$

where, E_n gives the values of allowed energies, n is the quantum number, $\hbar$ is the Planck's constant divided by 2 π.

The confinement of an electron or a hole occurs three dimensionally. In order to determine the allowed kinetic energies of an electron or a hole in a dot, all that needs to be done is the substitution of the width of the quantum well, d, with the radius of the quantum dot. When a quantum dot works as a biotag, an electron gets excited from the valence band to the conduction band by an energy equivalent to that of a photon, hν, where h is the Planck's constant while ν is the frequency of the photon. The minimum energy of the incident photon is:

$$h\nu = \frac{h^2}{2m_e a^2} + \frac{h^2}{2m_h a^2} + E_{gap} \tag{2}$$

where, in the right hand side, E_{gap} is the energy band gap between the conduction and the valence band, while the first two terms describe the electron and hole ground state energies with a, the radius of the quantum dot.

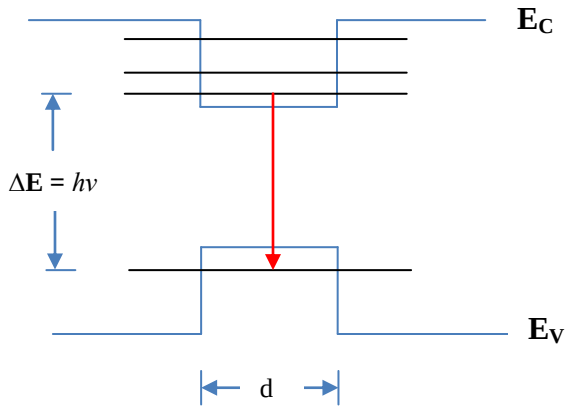


Figure 1. Diagram of bandgap theory and photoluminescence.

When the quantum dot moves from its excited state to the stable, ground state in the valence band, it emits energy either by transitioning between the various energy states in the conduction band or by transferring some of its energy to the surrounding molecules in the lattice. Hence, due to loss of energy, there is an increase in the wavelength of the emitted light. The process of absorption takes is nearly instantaneous (about 10^{-12} to 10^{-15} s) while the fluorescence is much slower, i.e., about 10^{-8} s [10]. Hence, the optical properties of quantum dots are determined by the following factors: 1) the material of which it is made, which decides the band gap energy and the dangling bonds present which are prone to reactivity and 2) the radius or diameter which determine the emission spectrum of the dots, explained pictorially in Figure 2.

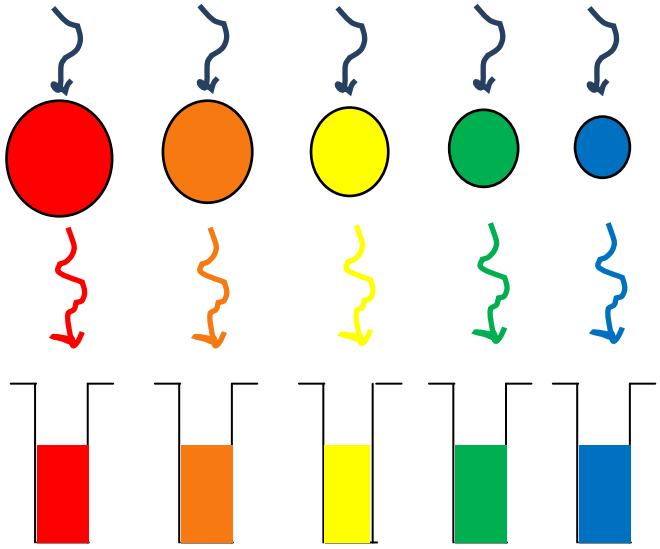


Figure 2. Emission tunability of Quantum Dots based on size.

1.4 Graphene

Graphene is regarded as the "mother of carbon" since it is the building block for various other carbon structures such as Graphite and Carbon Nanotubes (Single Walled (SWCNT) and Double Walled (DWCNT)) [10]. Graphite is layers of stacked graphene while carbon nanotubes are cylindrical, rolled sheets of graphene. Ever since the isolation of graphene was devised using the scotch-tape method in 2004 by Geim in the University of Manchester [11], there has been widespread research surrounding the possibilities of using graphene as a suitable material in the semiconductor industry. Wallace [12] studied the electronic structure of graphene and showed that it exhibits semi-metallic behavior, owing to its zero band gap. The most application-oriented advantage of graphene is that it can be easily integrated into the planar processing system (viewing circuits in a two-dimensional projection and devising individual components of

transistor separately and connecting them together without actual wiring), currently being used in the case of silicon based semiconductor devices and thus making use of its excellent, inherent and tunable properties such as high electron mobility [13] and relativistic carrier properties [14].

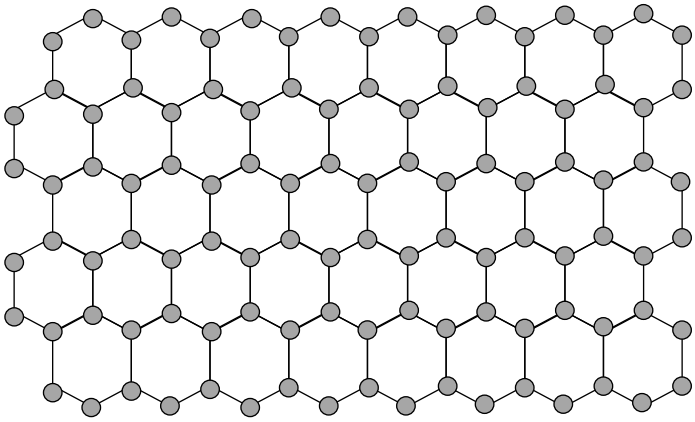


Figure 3. Crystalline, honey-comb structure of graphene.

The unusual electronic behavior that graphene exhibits is due to the massless, two-dimensional Dirac particles. When electronic waves pass through graphene, they undergo interference which causes the unusual behavior. At low energies and low wavelengths, the electrons are no longer governed by mass but by their speed of propagation (Fermi-Dirac velocity), which is around 300 times smaller than that of the speed of light. Also, the electrons obey the relativistic wave equation in two dimensions which is an important factor in terms of theoretical physics. From the point of view of solid state physics, graphene is an interesting material in the sense that it is neither a metal, due to its vanishing density of states at the Dirac point, nor a semiconductor/insulator since it does not have a band gap. Thus, this renders an important property of no threshold for electronic excitations to graphene.

Graphene's ultrahigh current density sustainability and conductivity make it a very promising material for use in the semiconductor industry. It has been found to show intrinsic charge carrier mobility of upto 200,000 cm^2/Vs [13] on a PZT (Lead Zirconate-Titanate) substrate and upto 120,000 cm^2/Vs in the case of suspended graphene at temperature 240 K [15]. There is no presence of backscattering in graphene due to the charge carriers having a pseudo spin (the extra degree of freedom required to explain the presence of additional orbital wave function in the two interpenetrating triangular sublattices) and this leads to a very high mobility [16]. In addition to this, the low electron-phonon coupling and the high sound velocity also contribute to the high mobility of graphene. Although graphene can sustain a current density of upto 5×10^8 A/cm^2, the current on-off ratio is very low making it highly suitable for use in semiconductors. This band gap can be improved upon by disrupting the structure of the sublattices of graphene [17] and ultrafast devices, with processing speeds up to THz, can be made.

Also, graphene has a large, negative thermal expansion coefficient (it contracts when heated and expands while cooled) of -6×10^6 / K [18] due to horizontal displacement caused by flexural or out of plane modes, with little contribution from the in-plane phonons. It also has excellent thermal conductivity (measured at 5000 W/mK, which is higher than the thermal conductivity of diamond. Hence, these novel thermal properties can be made use of in combining with materials having positive thermal expansion coefficient and low conductivity in order to create interesting semiconductor devices that can withstand high temperatures and provide excellent output at room temperature.

Graphene is the strongest known material with a Young's modulus of 1.0 TPa [19], owing to its strong and robust network of sp^2 hybridized atomic orbitals. The one-atom thick layer of graphene is so impermeable to gases and chemically stable that they have been termed as "nano-balloons" [20]. One other important property that graphene possesses is its ability to form ripples when compression and strain are applied [21] which helps in studying about how the electronic properties depend on the morphology [22] and this can be used to device "strain-based" graphene electronic devices. A lot of research has been done on the optical properties of graphene and been made use of in devising optical devices such as solar cells [23].

1.5 Peptide

Graphene-based field effect transistors show good response based on sensitivity towards various analytes [24] but at the same time, it is also non-selective. Selectivity has to be imparted to any such device by means of functionalization. Functionalization can either be covalent or non-covalent but since covalent functionalization leads to the formation of atomic scale defects in graphene, which are highly disruptive [25] and hampers the excellent transport properties (high electron mobility) that are crucial for developing a highly sensitive device, non-covalent functionalization is the key to inducing necessary properties to the sensor without disrupting the electronic network. One of the most promising methodologies of non-covalent functionalization is that of using peptides, utilizing its molecular recognition properties [26] and imparting selectivity to a range of analytes.

1.6 Structure of Peptide Adsorbed on Graphene

Naik et al. [27] has resolved the structure of a peptide that binds to graphene, the structure of which was previously simulated by Molecular Dynamics methods [28]. When FTIR (Fourier Transform Infrared) spectroscopy is performed on the powdered form of GBP (Graphene Binding Peptide), it displays broad amide I and amide II bands at respective wavenumbers (between 1660-1650 cm^{-1} and 1530 cm^{-1} respectively), pointers to the fact that the structure is that of a secondary α-helix [29]. However, when the GBP is dissolved in D_2O, amide II band is no longer visible due to the conversion N-H (Nitrogen-Hydrogen) bond to N-D (Nitrogen-Deuterium) bond while the frequency of amide I band is increased. The strong amide I band presence suggests that the structure of the peptide changes from an α-helix to a distorted helical structure on exposure to an aqueous medium.

The thickness of peptide adsorbed on graphene is about 1 nm thick, similar to when adsorbed on a carbon nanotube [30]. The substrate is that of graphene on silicon dioxide and the important point is that when the graphene binding peptide is incubated in such a substrate, the silicon dioxide layer seems to be undisturbed. Also, the electronic network of graphene seems to be undisturbed, as expected of non-covalent functionalization.

2. REVIEW OF LITERATURE

2.1 Graphene-based Sensors

Graphene-based field-effect transistors have been devised by Xu et al. [31]. They have demonstrated that when graphene is non-covalently functionalized using a pyrene moiety (1-pyrenebutanoic acid, succinimidyl ester), bound to an aptamer (short DNA or RNA sequences capable of binding to specific targets, in this case a cocaine aptamer) with its one end attached to methylene blue, an electron donor, the aptamer bends on detecting cocaine, reducing the distance between the methylene blue molecule and the graphene surface. This results in a change in the I-V (current vs voltage) characteristics of the graphene FET. The pyrene moiety present in the linker molecule has side groups with overlapping π-bonds and hence binds well to the basal plane of graphene by π-stacking. Aptamers are a better choice than antibodies for this application since their length is within the Debye length, necessary for detection within the net charge carrier's effect in solution.

2.2 Peptide Sequences that Bind to Graphene-based Substrates

A peptide of sequence EPLQLKM has been shown to bind to the edges of a specific type of graphene (SLP 30 potato-shape graphite family) [32]. This peptide has been found to bind to graphene owing to its E (Glutamic acid) residue, which is negatively polarized and the positively charged hydrogen terminations in the graphene plane. The reason for specific binding of the peptide to the edges has been attributed to π-π interactions or electrostatic interactions that take place, giving rise to good mechanical stability and electronic properties. Another peptide of the sequence, HSSYWYAFNNKT, has been found to decorate the graphene surface but with the

formation of pores of depth ~20-90 nm, which was unexpected since this peptide binds to CNTs and both CNT and graphene have similar atomically exposed π-π bonds.

Pyrene moiety (1-pyrene butyric acid) is attached to the N-terminus of a peptide that binds to a graphene oxide substrate, having a sequence of GGGRKRIHIGPGPAFYTT, in order to study protein-peptide interactions [34]. Pyrene binds to graphene oxide non-covalently and thus does not disrupt the electronic network of graphene. Also, pyrene-based fluorophores are excellent optical contrast agents in molecular beacons [33] and re-emit light on excitation, with many events per photon absorbed. Pyrene's fluorescence is quenched due to the proximity of the graphene oxide layer but when the peptide sequence bound to pyrene detects the target protein, desorption followed by dequenching of pyrene occurs due to competition between the pyrene molecule and the target protein for the graphene oxide substrate.

One self-assembling, peptide characterized as wild-type of sequence – IMVTESSDYSSY is selected from among the biocombinatorially selected peptide sequences. There is incredible symmetry associated with the formation of these peptides on the surface owing to the hexagonal structure of the graphene layer. A huge correlation between the formation of peptide and its sequence has been observed along with a transition from its normal amorphous state to orderly state, when it assembles on graphene. Different parts of the peptide have been associated with different factors that contribute to binding. The aromatic domain influences the binding while the hydrophilic and hydrophobic domains influence the diffusion and self-assembly of the peptide [35].

Katoch et al. studied the structure of a dodecameric peptide that binds to graphene. The peptide of sequence – GAMHLPWHMGTL was previously found to bind to high ordered pyrolytic graphene by means of phage display [36]. It was demonstrated that the structure of this peptide was very different from its original α-helical structure in its powder form, when it binds to graphene. It takes the shape of a distorted helix. Also, binding occurs by the alignment of the peptides aromatic residues – tryptophan and histidine along the graphene nanosheet. Importance of tryptophan binding to graphene was shown by comparing the binding energies of mutant peptides with tryptophan replaced with alanine. Course-grained simulation studies were performed to study the importance of Van der Waals forces, π-stacking and hydrophobic interactions. Density functional studies were carried out to determine how the density of states of graphene were affected by biomolecular adsorption [27].

3. METHODS AND EXPERIMENTS

3.1 Conceptual Framework

As discussed previously, the basic idea of this thesis is to bind peptides to graphene, in order to be able to devise a biosensor in the future, making use of the excellent transport and electronic properties of graphene as well as the molecular recognition and excellent selective properties that a peptide can induce to graphene by non-covalent functionalization. Also, studies are done to determine the selectivity of the peptide used in the experiment by replacing a number of amino acids in the sequence at a time and determining the importance of specific amino acids or groups of amino acids in binding to the graphene substrate.

3.2 Materials Used

97% single layered graphene on silicon dioxide substrate, synthesized by the Army Research Laboratory, MD, United States using electron vapor deposition methods is used in this project.

CdSe quantum dots with an emission spectra of 525 nm have been used in this experiment. The quantum dots were synthesized by Life Technologies (Catalog number: Q21341MP) and are carboxylate functionalized, i.e., can be easily coupled with peptide sequences with amine endings. The advantage of these quantum dots when compared to amine functionalized ones is that they have more binding sites. 8 μM solution of this quantum dot was purchased.

Peptide sequence – GAMHLPWHMGTLGGGGGGG-NH_2 is used as the base sequence for testing of binding to graphene. This peptide has been found to bind to graphene by phage display methods, as explained earlier. A seven glycine (G) amino acid chain has been used as a linker to enable the quantum dot to effectively bind to the peptide, without disrupting the peptide's linkage to the graphene surface. This peptide was manufactured by the Protein Research Lab at the Research Resources Center, University of Illinois at Chicago and a purity of 99% was verified by mass spectrometry.

Fluorescence microscope is used for studying the optical signal from the quantum dots under Bright Field settings. A typical setup of a fluorescence microscope consists of a light source (usually mercury vapor lamp), an excitation filter, a dichroic mirror and an emission filter. A source of light is used to illuminate the specimen, which is absorbed by the fluorophores, which re-emit light of lower energy and higher wavelength. This re-emitted light is filtered by the use of a specific spectral emission filter.

3.3 Experiment Procedure

The protocol provided by Invitrogen (Life Technologies) is used as the framework for carrying out the procedure of binding quantum dots to peptide and to graphene, there on.

Steps followed:

1) Out of the total 2 nmol concentration of quantum dots provided, $1/20^{th}$ of its concentration is used (0.1 nmol). Since the concentration is 8 μM, the volume required is 12.5 μL (moles/concentration = volume).

2) Take a small glass vial with a stirbar and dilute 12.5 μL of 8 μM Qdot solution to 8 times its value, i.e., 1×10^{-4} mL with 10 mM borate buffer at pH 7.4 (Refer Table I). Stir and mix well.

TABLE I

INITIAL SOLUTIONS PREPARATION

Moles of Quantum Dot	**Concentration**	**Volume of Qdot solution (L)**	**Volume of Qdot solution (mL)**	**Total Volume after Dilution (mL)**	**Volume of Borate Buffer (mL)**
1×10^{-9}	0.000008	0.0000125	12.5 uL	0.0001	0.0000875

3) With a quantum dot:peptide ratio of 1:40, number of moles of peptide taken is 40 nM. Using the molecular weight of each peptide provided by the Protein Research Laboratory, the mass of each peptide is calculated (Mass = No. of moles of peptide × molecular weight).

4. The concentration of peptide in aqueous buffer, Tris-HCl (pH: 7.5, 100 Mm), is 200 μg/ml [27]. Using this, the total volume of solution needed is back-calculated, since the mass of the peptide that needs to be dissolved is known.

5. This procedure is done for variations of the GBP (Graphene Binding Peptide), to result in 4 other peptides which will be tested for binding to graphene (Table II). Hence, this will determine the selectivity of the peptide binding to graphene.

TABLE II

PEPTIDE SOLUTIONS PREPARATION

Peptide Sequence[a]	**No. of nmoles**	**M.W**[b] **g/mol**	**Mass (g)**	**Mass (µg)**	**No. of g/solution: 200 µg/L**	**Volume of Solution Needed (µL)**
GAMHLPWHMGTL	4	1749	0.000006996	6.99	0.002	34.98
GAMHLPGHMGTL	4	1620	0.00000648	6.48	0.002	32.4
DAMNFEWNMGTF	4	1861	0.000007444	7.44	0.002	37.22
WGAMHLPWHMGTL	4	1935	0.00000774	7.74	0.002	38.7
GNMHQPWHMRTL	4	1906	0.000007624	7.624	0.002	38.12

[a] All peptide sequences have the following spacer component: GGGGGGG attached to an amine terminal (NH_2)

[b] M.W - Molecular Weight

6. Using the ratio QD:EDC as 1:1500, the mass of EDC required for the entire experiment is calculated.

TABLE III

EDC Mass Calculation

No. of moles of EDC	**M.W.**	**Mass of EDC (g)**	**Mass of EDC (μg)**
0.00000015	191.7	0.000028755	0.028 mg = 30 μg

7. So, for every peptide, the required volumes of each component have been calculated. Therefore, the quantum dot, the borate buffer (pH: 7.4), EDC (600 μg is dissolved in 1 ml of deionized water and $1/20^{th}$ of it (50 μl = 30 μg required) is used. Add the required amount of peptide and make up the solution to the calculated value from Table III.

7a) For the peptide GAMHLPWHMGTLGGGGGGG-NH_2:

1) Add 12.5 μL of Qdot along with 87.5 μL of buffer;

2) Take 2 mg of peptide and dissolve in 10 ml of 100 Mm Tris-HCl;

3) Dissolve 600 μg of EDC in 1 ml of deionized H_2O and use $1/20^{th}$ of 1 ml (30 μg EDC);

4) Take 6.99 μg of peptide and make up the entire solution to 35 μL of stock solution.

8. Follow the above procedure for all the peptides to prepare the stock solutions and stir all the solutions at room temperature for 2 hours for conjugation to occur.

9. To remove large aggregates, filter the conjugate solution with a 0.2 μm PES syringe and transfer the entire solution to a clean ultrafiltration centrifugal unit with a 100 kD cutoff.

10. To remove any unbound, excess protein, centrifuge at the recommended speed for the ultrafiltration unit (12 rpm for 5 minutes) at least for 5 exchanges using 50 mM borate buffer at pH: 8.3. Ensure that the volume is at least greater than 10 times the initial volume of the solution started with.

11. Store the quantum dot conjugate solution at 4°C. Do not freeze the solution.

12. Immerse a 1 mm × 1mm graphene sample in the quantum dot solution for 10 minutes and wash with deionized water. Repeat this cycle several times to wash off any unbound protein.

13. With fluorescence microscope in bright field settings and with the lights in the room powered down, view the sample under the microscope making use of suitable optical filter to detect the signals.

TABLE IV

Optimal Filter Sets

Name of Filter	**Filter Type**
Exciter 1 filter	425DF45
Exciter 2 filter [c]	415WB100
Dichroic filter	475DCLP
Emitter filter	525WB20

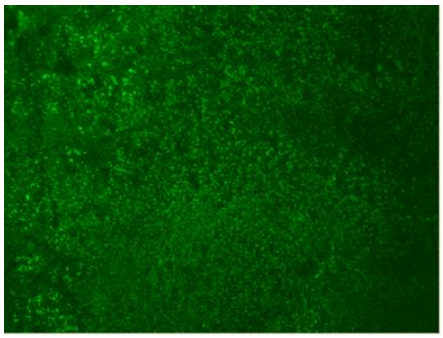
c – Use either exciter 1 or 2

4. RESULTS AND DISCUSSION

4.1 Selectivity of Peptide

The results obtained during this experiment verify that the peptide sequence of GAMHLPWHMGTL (Peptide 1), determined by phage display techniques has been found to bind to graphene, evidenced by the presence of strong fluorescence from the quantum dots.

Planar Graphene under White Light



Planar Graphene under White Light

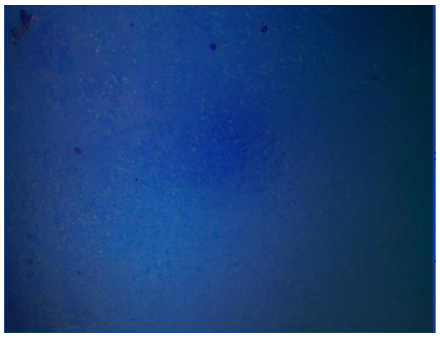


Figure 4. Fluorescence microscope image of peptide 1 on planar graphene.

Another interesting observation is that the peptide shows preferential binding to the graphene edges when compared to the planes. This can be attributed to the π-π interactions between the basal plane of graphene and the aromatic residues (Tryptophan (T) and Histidine (H)) present in the peptide which yield to binding.

Graphene Edge under White Light

Graphene Edge under Mercury Vapor Lamp Illumination

Figure 5. Fluorescence microscope image of peptide 1 on graphene edge.

Specific number of amino acid replacements were done to this peptide to result in slightly modified peptide sequences compared to the base peptide used.

The changes made were:

1) One replacement is made to peptide 1's sequence. Tryptophan is replaced with glycine resulting in Peptide 2.
2) Three replacements were made to the sequence of peptide 1. Glycine is replaced with arginine, leucine with glutamine and alanine with asparagine, resulting in Peptide 3.
3) Five replacements were made to peptide 1's sequence. Glycine is replaced with aspartic acid, two histidines with asparagines, leucine with phenyalanine and proline with glutamic acid.

These changes resulted in three new peptides, namely:

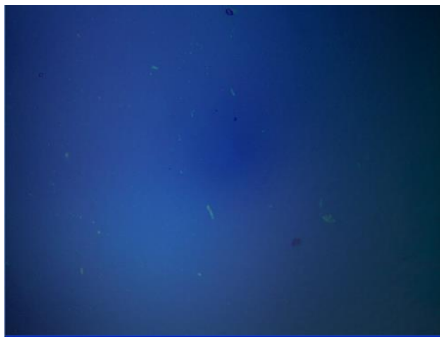
Peptide 2: GAMHLPGHMGTLGGGGGGG-NH_2

Peptide 3: GNMHQPWHMRTLGGGGGGG-NH_2

Peptide 4: DAMNFEWNMGTFGGGGGGG-NH_2

One incubating graphene in all of the above peptides, no fluorescence of the quantum dots were observed and hence it can be concluded that no binding occurs.

Planar Graphene under White Light



Planar Graphene under Mercury Vapor Lamp Illumination

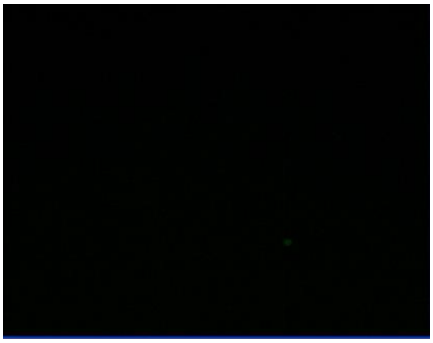


Figure 6. Fluorescence microscope image of peptide 2 on graphene planar surface.

Graphene Edge under White Light

Graphene Edge under Mercury Vapor Lamp Illumination

Figure 7. Fluorescence microscope image of peptide 2 on graphene edge.

Planar Graphene under White Light

Planar Graphene under Mercury Vapor Lamp Illumination

Figure 8. Fluorescence microscope image of peptide 3 on planar graphene.

Graphene Edge under White Light

Graphene Edge under Mercury Vapor Lamp Illumination

Figure 9. Fluorescence microscope image of peptide 3 on graphene edge.

Planar Graphene under White Light

Planar Graphene under Mercury Vapor Lamp Illumination

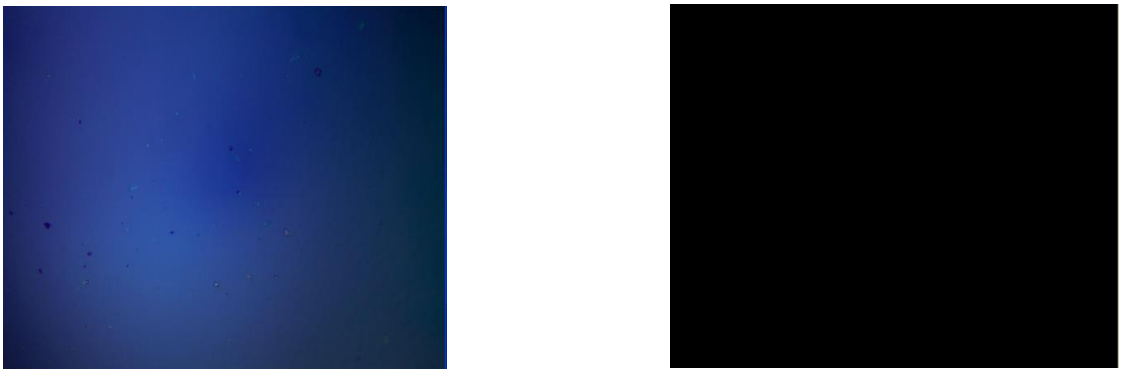


Figure 10. Fluorescence microscope image of peptide 4 on planar graphene.

Graphene Edge under White Light

Graphene Edge Mercury Vapor Lamp Illumination

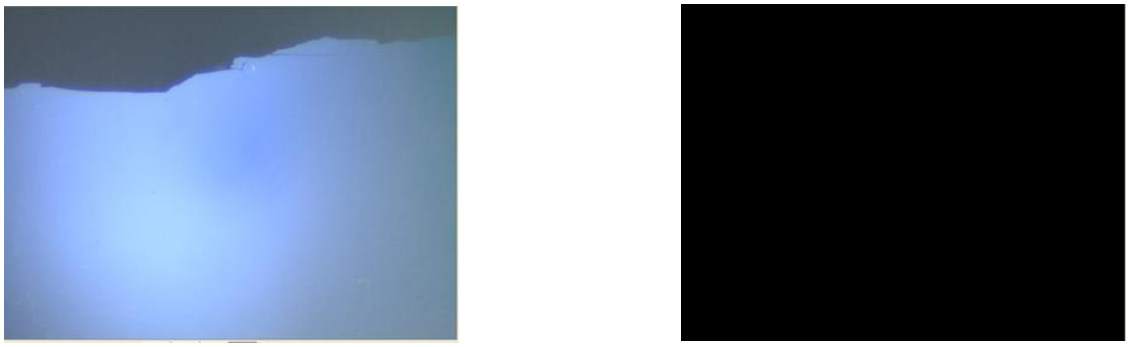
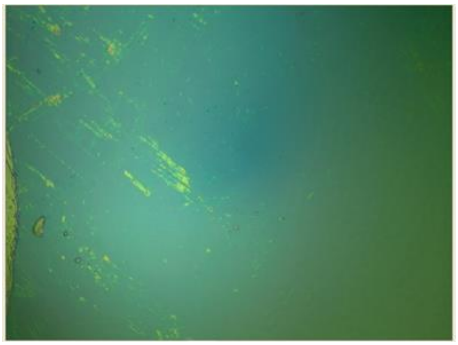


Figure 11. Fluorescence microscope image of peptide 4 on graphene edge.

Even when there were no replacements done to the existing dodecameric peptide and only tryptophan, the most important amino acid required for binding is appended to the beginning of the sequence to generate a new peptide (Peptide 5), binding did not occur. No fluorescence was observed even in the edges of the graphene.

Graphene Edge under White Light

Graphene Edge under Mercury Vapor Lamp Illumination



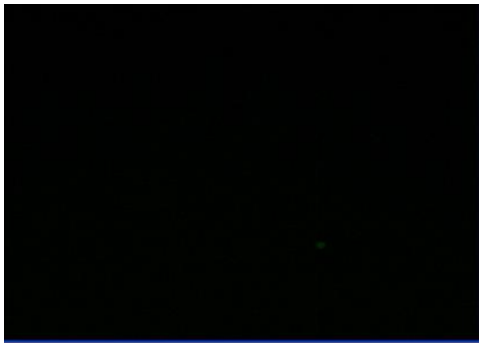


Figure 12. Fluorescence microscope image of peptide 5 on graphene edge.

4.2 Theory and Observation

The replacement of a number of peptides in the original sequence was based on the fact that when peptides with no aromatic rings were replaced with those having an aromatic ring or polarity, there will be increased binding affinity due to π - π interactions between those amino acid residues and the graphene edges. No binding was observed with the change in aromaticity and polarity of the peptide used hence, the aromaticity of a singular amino acid in the sequence does not influence binding. It can be inferred that small motifs within the peptide itself have to have affinity towards each other in order to induce stability to the peptide and result in binding. The most interesting result, however, is that addition of a tryptophan, which is required for binding changes the structure of the peptide in such a way that binding does not occur. This concludes that suitable combination of the amino acids alone does not suffice for binding but this exact sequence cannot be modified for binding to occur, or in other words, this peptide is highly selective towards graphene surfaces.

4.3 Future Work

Binding of peptides to graphene can be very useful in devising novel biosensors with very high sensitivity. A possible extension of this project would be to introduce an aptamer (a short DNA sequence with high selectivity towards specific molecules) bound to an electron donor, methylene blue and create a graphene based FET. Previously, only pyrene moieties have been used as linkers but using this peptide as the linker in future graphene-based biosensors can improve the sensitivity of the device greatly, owing to the high molecular recognition abilities of proteins and also increase the biocompatibility and decrease toxicity levels, which will make them ideal for use in the field of medicine.

CITED LITERATURE

1. Jovin, T.M.: Quantum dots finally come of age. *Nat. Biotechnol*., 21: 32-33, 2003.

2. Shimomura, O.: A Short Story of Aequorin. *Biol. Bull*., 189: 1-5, 1995.

3. Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. Green fluorescent protein as a marker for gene expression. *Science*., 263: 802-5, 1994.

4. Zhang, J., Campbell, R. E., Ting, A. Y., and Tsien, R.Y.: Creating new fluorescent probes forcell biology. *Nat. Rev. Mol Cell Biol*., 3(12): 906-918, 2002.

5. Kasten, F. H.: Introduction to fluorescent probes: properties, history and application. In: *Fluorescent and luminescent probes for biological activity*., 2nd ed. W.T. Mason (ed.), pp. 17-38. San Diego, London, Academic Press, 1999.

6. Karousis, N., Tagmatarchis, N., Tasis, D.: Current Progress on the Chemical Modification of Carbon Nanotubes. *Chem. Rev.*, 110(9): 5366-5397, 2010.

7. Chen, H.M., Huang, X.F., Xu, L., Chen, K.J., and Feng D.: Self-assembly and photoluminescence of CdS-mercaptoacetic clusters with internal structures. *Superlattice Microst.* 27(1): 1-5, 2000.

8. Kuno, M., Fromm, D.P., Hamann, H.F., Gallagher, A., and Nesbitt, D.J.: Nonexponential "blinking" kinetics of single CdSe quantum dots: a universal power law behavior. *J. Chem. Phys.*, 112(7): 3117-3120, 2000.

9. Empedocles, E., Norris, D.J., and Bawendi, M.G.: Photoluminescence spectroscopy of single CdSe nanocrystallite quantum dots. *Phy. Rev. Lett*., 77: 3873-3876, 1996.

10. Geim, A.K. and Novoselov, K.S.: The rise of graphene. *Nat. Mater.*, 6(3): 183-191, 2007.

11. Novoselov, K.S., Geim, A.K., Morozov, S.V., Jiang, D., Zhang, Y., Dubonos, S.V., Grigorieva, I.V., and Firsov, A.A.: Electric field effect in atomically thin carbon films. *Science*, 306(5296): 666-669, 2004.

12. Wallace, P.R.: The Band Theory of Graphite. *Phys. Rev*., 71(9): 622, 1947.

13. Chen, J.-H., Jang, C., Xiao, S. Ishigami, M., and Fuhrer, M.S.: Intrinsic and extrinsic performance limits of graphene devices on SiO2. *Nat Nano*, 3(4): 206-209, 2008.

14. Novoselov, K.S., Geim, A.K., Morozov, S.V., Jiang, D., Katsnelson, M.I., Grigorieva, I.V., Dubanos, S.V., and Firsov, A.A. Two-dimensional gas of massless Dirac fermions in graphene. *Nature*, 438(7065): 197-200, 2005.

15. Bolotin, K.I., Sikes, K.J., Jiang, Z., Klima, M., Fudenberg, G. Hone, J., Kim, P., and Stormer, H.L. Ultrahigh electron mobility in suspended graphene. *Solid State Commun.*, 146(9-10): 351-355, 2008.

16. Ando, T., Nakanishi, T., and Satio, R. Berry's phase and absence of back scattering in carbon nanotubes. *J. Phys. Soc. Jpn.*, 67(8): 2857-2862, 1998.

17. Zhou, S.Y., Gweon, G.H., Fedorov, A.V., First, P.N., de Heer, W.A., Lee, D.H., Guinea, F., Castro Neto, A.H., and Lanzara, A. Substrate-induces bandgap opening in epitaxial graphene. *Nat. Mater*., 6(10): 770-775, 2007.

18. Bao, W., Miao, F., Chen, Z., Zhang, H., Jang, W., Dames, C., and Lau, C.N. Controlled ripple texturing of suspended graphene and ultrathin graphite membranes. *Nat. Nano*, 4(9): 562-566, 2009.

19. Booth, T.J., Blake, P., Nair, R.R., Jiang, D., Hill, E.W., Bangert, U., Bleloch, A., Gass, M., Novoselov, K.S., Katsnelson, M.I., and Geim, A.K. Macroscopic Graphene Membranes and Their Extraordinary Stiffness. *Nano Lett.*, 8(8): 2442-2446, 2008.

20. Bunch, J.S., Verbridge, S.S., Alden, J.S., van der Zande, A.M., Parpia, J.M., Craighead, H.G., and McEuen, P.L. Impermeable Atomic Membranes from Graphene Sheets. *Nano Lett*., 8(8): 2458-2462, 2008.

21. Katsnelson, M.I. and Geim, A.K. Electron scattering on microscopic corrugations in graphene. *Phil. Trans. R. Soc. A*: Mathematical, Physical and Engineering Sciences, 366(1863): 195-204, 2008.

22. Kim, E.A., and Castro Neto, A.H. Graphene as an electronic membrane. *Europhys. Lett.*, 84(5): 57007, 2008.

23. Wang, X., Zhi, L., and Mullen, K. Transparent, Conductive Graphene Electrodes for Dye-Sensitized Solar Cells. *Nano Lett*., 8(1): 323-327, 2007.

24. Dan, Y.O., Lu, Y., Kybert, N.J., Luo, Z.T., Johnson, A.T.C. Intrinsic response of Graphene Vapor Sensors. Nano Lett., 9(4): 1472-1475, 2009.

25. Ostrovsky, P.M., Gornyi, I.V., Mirlin, A.D. Electron Transport in Disordered Graphene. *Phys. Rev. B.*, 74: 235443, 2006.

26. Sarikaya, M., Tamerler, C., Jen, A.K.Y., Schulten, K., Baneyx, F. Molecular Biomimetics: Nanotechnology through Biology. *Nat. Mater*., 2(9): 577-585, 2003.

27. Katoch, J., Kim, S.N., Kuang, Z., Farmer, B.L., Naik, R.R., Tatulian S.A., and Ishigami, M. Structure of a Peptide Adsorbed on Graphene and Graphite. *Nano Lett*., 12: 2342-2346, 2012.

28. Cui, Y., Kim, S.N., Jones, S.E., Wissler, L.L., Naik R.R., and McAlpine, M.C. Chemical Functionalization of Graphene Enabled by Phage Displayed Peptides. *Nano Lett*., 10(11): 4559-4565, 2010.

29. Tatulian, S.A. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy: A Method of Choice for Studying Membrane Proteins and Lipids. Biochemistry. 42: 11898-11907, 2003.

30. Kuang, Z., Kim, S.N., Crookes-Goodson, W.J., Farmer, B.L., Naik, R.R. Biomimetic Chemosensor: Designing Peptide Recognition Elements for Surface Functionalization of Carbon Nanotube Field Effect Transistors. *ACS Nano*, 4(1):452, 2009.

31. Radi, A.E., Acero Sánchez, J.L., Baldrich, E., O'Sullivan, C.K. *J. Am. Chem. Soc.*, 128: 117-124, 2006.

32. Kim, S.N., Kuang, Z., Slocik, J.M., Jones, S.E., Cui, Y., Farmer, B.L., McAlpine M.C., and Naik, R.R. Preferential Binding of Peptides to Graphene Edges and Planes. *J. Am. Chem. Soc*., 133(37): 14480-14483, 2011.

33. Oh, K.J., Cash K.J., and Plaxco, K.W. Excimer-Based Peptide Beacons: A Convenient Experimental Approach for Monitoring Polypeptide-Protein and Polypeptide-Oligonucleotide Interactions. *J. Am. Chem. Soc*., 128(143): 14018-14019, 2006.

34. Lu, C.H., Li, J., Zhang, X.L., Zheng, A.X., Yang, H.H., Chen, X., and Chen, G.N. General Approach for Monitoring Peptide-Protein Interactions Based on Graphene-Peptide Complex. *Anal. Chem*., 83: 7276-7282, 2011.

35. So, C.R., Hayamizu, Y., Yazici, H., Gresswell, C., Khatayevich, D., Tamerler, C., and Sarikaya, M. Controlling Self-Assembly of Engineered Peptides on Graphite by Rational Mutation. *ACS Nano*., 6(2): 1648-1656, 2012.

36. Cui, Y., Kim, S.N., Jones, S.E., Wissler, L.L., Naik, R.R., McAlpine, M.C. Chemical Functionalization of Graphene Enabled by Phage Displayed Peptides. *Nano Lett*. 11: 4559-4565, 2010.

BIBLIOGRAPHY

Design and Applications of Nanomaterial-based and Biomolecule-based Nanodevices and Nanosensors for the Design and Applications of Nanomaterials for Devices and Sensors" within the Springer "Challenges and Advances in computational Chemistry and Physics" series by Ke Xu, Mohsen Purahmed, Kimber Brenneman, Xenia Meshik, Sidra Farid, Shripriya Poduri, **Preeti Pratap**, Justin Abell, Yiping Zhao, Barbara Nichols, Eugene Zakar, Michael Stroscio, and Mitra Dutta, Vol 16, May 2014 (In Press)

Applications of Physics in Nanoscience and Nanoelectronics, Michael A. Stroscio, Mitra Dutta, Sicheng Liao, Banani Sen, Jun Qian, Robin Xu, Nanzhu Zhang, Min Choi, Yi Lan, Kimber Brenneman, Xenia Meshik, **Preeti Pratap**, and Donna Wu, Seminar at Ball State University, March 28, 2013.

Design and Applications of Graphene-based and Biomolecule-based Nanosensors and Nanodevices for the CRC Press “Graphene Science Handbook” Vol.6 “Applications and Industrialization”, by Ke Xu, **Preeti Pratap**, Mitra Dutta and Michael Stroscio (Submitted)

VITA

Name: Preeti Pratap

Education: B.E., Biomedical Engineering, Anna University, Chennai, India, 2011
M.S.,Bioengineering, University of Illinois at Chicago, IL, 2015

Research: Research project with Dr. Michael Stroscio, 2012-2014 working with graphene and peptides using the technique of quantum dot conjugation