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TWO-DIMENSIONAL FLUIDIZATION OF NANOMATERIALS VIA BIOMIMETIC MEMBRANES TOWARDS ASSISTED SELF-ASSEMBLY

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Abstract

Materials that take advantage of the exceptional properties of nano-meter sized aggregates of atoms are poised to play an important role in future technologies. Prime examples for such nano-materials that have an extremely large surface to volume ratio and thus are physically determined by surface related effects are quantum dots (qdots) and carbon nanotubes (CNTs). The production of such manmade nano-objects has by now become routine and even commercialized. However, the controlled assembly of individual nano-sized building blocks into larger structures of higher geometric and functional complexity has proven to be much more challenging. Yet, this is exactly what is required for many applications that have transformative potential for new technologies. If the tedious procedure to sequentially position individual nano-objects is to be forgone, the assembly of such objects into larger structures needs to be implicitly encoded and many ways to bestow such self-assembly abilities onto nano objects are being developed. Yet, as overall size and complexity of such self-assembled structures increases, kinetic and geometric frustration begin to prevent the system to achieve the desired configuration. In nature, this problem is solved by relying on guided or forced variants of the self-assembly approach. To translate such concepts into the realm of man-made nano-technology, ways to dynamically manipulate nano-materials need to be devised.

Thus, in the first part of this work, I provide a proof of concept that supported lipid bilayers (SLBs) that exhibit free lateral diffusion of their constituents can be utilized as a two-dimensional platform for active nano-material manipulation. We used streptavidin coated quantum dots (Q-dots) as a model nano-building-block. Q-dots are 0-dimensional nanomaterials engineered to be
fluorescent based solely on their diameter making visualization convenient. Biotinylated lipids were used to tether Q-dots to a SLB and we observed that the 2-dimensional fluidity of the bilayer was translated to the quantum dots as they freely diffused. The quantum dots were visualized using wide-field fluorescent microscopy and single particle tracking techniques were employed to analyze their dynamic behavior. Next, an electric field was applied to the system to induce electroosmotic flow (EOF) which creates a bulk flow of the buffer solution. The quantum dots were again tracked and ballistic motion was observed in the particle tracks due to the electroosmosis in the system. This proved that SLBs could be used as a two-dimensional fluid platform for nanomaterials and electroosmosis can be used to manipulate the motion of the Q-dots once they are tethered to the membrane.

Next, we set out to employ the same technique to carbon nanotubes (CNTs), which are known for their highly versatile mechanical and electrical properties. However, carbon nanotubes are extremely hydrophobic and tend to aggregate in aqueous solutions which negatively impacts the viability of tethering the CNTs to the bilayer, fluorescently staining and then imaging them. First, we had to solubilize the CNTs such that they were monodisperse and characterize the CNT-detergent solutions. We were able to create monodisperse solutions of CNTs such that the detergent levels were low enough that the integrity of the bilayer was intact. We were also able to fluorescently label the CNTs in order to visualize them, and tether them to a SLB using a peptide sequence. Future directions of this project would include employing EOF to mobilize the CNTs and use a more sophisticated single particle tracking software to track individual CNTs and analyze their motion.
TWO-DIMENSIONAL FLUIDIZATION OF NANOMATERIALS VIA BIOMIMETIC MEMBRANES TOWARDS ASSISTED SELF ASSEMBLY

by

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Table of Contents

Abstract

Acknowledgements

Table of Figures

1. Introduction and Background

1.1 Nanomaterials
   1.1.1 High Technology Devices: Nano vs Micro
   1.1.2 What are Nanomaterials and Nanotechnology?
      1.1.2.1 Nanomaterial Manufacturing
      1.1.2.2 Quantum Dots
      1.1.2.3 Carbon Nanotubes
   1.1.3 Current Methods of Nanomaterial Assembly
      1.1.3.1 Self assembly
      1.1.3.2 Templating
      1.1.3.3 Biomolecule-Based Static Organization

1.2 Biomimetic Membranes
   1.2.1 Nature’s building Blocks: Cells
      1.2.1.1 Cell Membranes
      1.2.1.1.1 Lipids and Bilayers
      1.2.1.1.1.1 Model Systems and Solid Supported Lipid Bilayers
      1.2.1.1.1.1.1 Methods of binding particles to SLB

1.3. Motion of Membrane Tethered Particles: Diffusion and Electroosmotic Flow (EOF)
   1.3.1 Brownian Motion
      1.3.1.1 Two-dimensional Random Walks
      1.3.1.1.1 Mean Square Displacement
   1.3.2 Diffusion in Lipid Bilayers
   1.3.3 Electroosmotic Flow (EOF)
      1.3.3.1 Electric Double Layer
      1.3.3.2 EOF
      1.3.3.3 EOF in Bilayers
      1.3.4.1 EOF in SLB’s
      1.3.4.1.1 EOF in SLB’s with proteins

2. Qdot Project

2.1 Concept
   2.1.1 EOF in SLB Augmented with Qdots
   2.1.1.1 Theoretical Drift Velocity Calculation

2.2 Materials and Methods
2.2.1 Materials 49
2.2.2 Small Unilamellar Vesicle Preparation 50
2.2.3 Formation of Supported Lipid Bilayer 51
2.2.4 Custom Flow Chamber 55
  2.2.4.1 Coverslip Sandwich 55
  2.2.4.2 Fully Assembled Flow Chamber 56
2.2.5 Addition of Fluorescent Particles 59
  2.2.5.1 Qdot 655 Streptavidin Conjugate - lipid conjugation 59
  2.2.5.2 Addition of "Flash Red" Fluorescent beads 59
2.2.6 Fluorescence Microscopy and Imaging 60
2.2.7 Single Particle Tracking & Analysis 61

2.3 Results 64
2.3.1 Single particle trajectories 65
  2.3.1.1 Qdots randomly diffusing on SLB (No external field applied) 65
  2.3.1.2 Qdots tethered to SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes 67
  2.3.1.3 Qdots tethered to SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 20 minutes 69
  2.3.1.4 Qdots tethered to SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 30 minutes 70
2.3.2 Fluorescent microspheres (beads) 72
  2.3.2.1 Beads on cover glass (no SLB) just after application of the external field (3.4x10^2 ± 0.3 V/m) 72
  2.3.2.2 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes 73
  2.3.2.3 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 20 minutes 74
  2.3.2.4 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 30 minutes 75
  2.3.2.5 Beads on a SLB (no tethering system) just after application of the external field (3.4x10^2 ± 0.3 V/m) 76
  2.3.2.6 Beads on a SLB (no tethering system) after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes 78
  2.3.2.7 Beads on a SLB (no tethering system) after application of the external field (3.4x10^2 ± 0.3 V/m) for 17 minutes 79
  2.3.2.8 Beads on a SLB (no tethering system) after application of the external field (3.4x10^2 ± 0.3 V/m) for 25 minutes 80
2.3.2 MSD Graphs 81
  2.3.2.1 Qdots 81
    2.3.2.1.1 MSD Plot of Randomly Diffusing Qdots (no field) 81
    2.3.2.1.2 MSD Plot of Qdot Tethered to a SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes 83
    2.3.2.1.3 MSD Plot of Qdot Tethered to a SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 20 minutes 84
    2.3.2.1.4 MSD Plot of Qdot Tethered to a SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 30 minutes 85
  2.3.2.2 Fluorescent Beads 87
    2.3.2.2.1 Beads on cover glass (no SLB) just after application of the external field (3.4x10^2 ± 0.3 V/m) 87
    2.3.2.2.2 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes 88
    2.3.2.2.3 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 20 minutes 89
    2.3.2.2.4 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 30 minutes 90
2.3.2.2.5 Beads on a SLB (no tethering system) just after application of the external field (3.4x10^2 ± 0.3 V/m)

2.3.2.2.6 Beads on a SLB (no tethering system) after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes

2.3.2.2.7 Beads on a SLB (no tethering system) after application of the external field (3.4x10^2 ± 0.3 V/m) for 17 minutes

2.3.2.2.8 Beads on a SLB (no tethering system) after application of the external field (3.4x10^2 ± 0.3 V/m) for 25 minutes

2.3.3 Diffusion Coefficient

2.3.4 Velocity Distribution

3. CNT Project (CNT Organization)

3.1 Concept

3.2 Challenges

3.2.1 Solubility

3.3 Materials and Methods

3.3.1 Materials

3.3.2 Solubilizing CNTs

3.3.3 Characterization of CNT suspension

3.3.4 CNT Binding Peptides

3.3.5 Small Unilamellar Vesicle Preparation

3.3.6 Formation of Supported Lipid Bilayer

3.4 Results

3.4.1 CNT suspension

3.4.1 CNT-SLB Complex

4. Summary & Outlook

4.1 Natural Extensions

4.2 Future Work

5. References
## Table of Figures

Figure 1: Development of number of transistors and other key performance metric between 1986 and 2009 (1). ................................................................. 1

Figure 2: Schematic of the conventional photolithographic process used in the production of semiconductor based electronics (2). ................................................................. 2

Figure 3: Summary of Nanomaterial Properties .......................................................................................................................... 5

Figure 4: Examples of nano-materials. Upper left: TEM image of core-shell qdot particles at 200,000x magnification; scale bar = 20 nm(11), upper right: TEM image of single walled carbon nanotubes produced by chemical vapor deposition, picture below(12): SEM image of gold nanoparticles on a latex bead (13). ..................................................................... 8

Figure 5: Schematic depicting the dependence of the band gap structures and thus their fluorescent wavelength and their size (24). ........................................................................ 11

Figure 6: Creation of the exciton (electron-hole pair) in a qdot due to photon absorption. ...... 12

Figure 7: Schematic of biofunctionalized core-shell quantum dot (26). .................................................... 13

Figure 8: Geometric configuration of carbon nanotubes. (A) shows the chiral vector of carbon nanotubes and (B) illustrates the different configurations of single-walled carbon nanotubes. From left to right: armchair, zigzag, and chiral (33). .............................................................. 14

Figure 9: CNTs incorporated into Kevlar(37) ................................................................................................................ 16

Figure 10: Diagram of self-assembly of colloidal gold nanoparticles during the drying process (41). ..................................................................................................................... 17

Figure 11: Templating of gold nanorods (45). ................................................................................................................. 19

Figure 12: Schematic of a eukaryote cell (58). .................................................................................................................. 22

Figure 13: Left: Cell Membrane. Right: Zoom in on an individual lipid (65). ...................................................... 25

Figure 14: Vesicle fusion process: SUVs fuse into a large vesicle before rupturing onto a solid surface to form a supported lipid bilayer (74). ................................................................. 28

Figure 15: Schematic of a supported lipid bilayer (SLB). Shown is the glass substrate and individual lipid molecules in an aqueous environment ............................................................. 28

Figure 16: Schematic of a His-tagged GFP bound to a solid supported lipid bilayer containing lipids with a Ni-head group (78). .................................................................................. 31

Figure 17: Schematic showing particle trajectories. Left: Brownian Motion, Right: Directed Motion................................................................................................................................. 32

Figure 18: Typical distribution of step sizes of a two-dimensional random walk, generated from numerical simulation (81). ......................................................................................... 33

Figure 19: Mean square displacement as a function of time. A straight line indicates a linear relationship and thus normal Brownian, unobstructed diffusion. .................................................. 34

Figure 20: Characteristic scaling of the mean square displacement with time (85). .............................. 35

Figure 21: Difference in the MSD growth with time for normal diffusion (blue), ballistic transport (red) and a mixed transport with equal times of diffusion and ballistic transport .......... 36
Figure 22: TEM image of core-shell qdot particles at 200,000x magnification; scale bar = 20 nm. Left: Schematic of streptavidin coated Qdot655 (11). ................................................................. 41
Figure 23: Chemical structure of POPC lipid (105) ................................................................. 42
Figure 24: Chemical structure of 18:1 Biotinyl Cap PE (108). .................................................... 43
Figure 25: Chemical structure of β-Bodipy FL C12-HPC (109). ................................................. 44
Figure 26: Diagram representing streptavidin coated Qdot655 being tethered to a solid supported lipid bilayer containing biotinylated lipids. ................................................................. 45
Figure 27: Pictorial representation of the basic scenario in these experiments: streptavidin coated qdots bound to biotinylated lipids embedded in a glass supported lipid bilayer. ..... 45
Figure 28: Diagram showing EOF in the context of membrane attached qdots. ......................... 46
Figure 29: Example of a FRAP experiment. Briefly, a small spot of the bilayer is exposed to high intensity light, effectively photobleaching the region shown on the left. The light is turned off and unbleached lipids diffuse into the region causing the “recovery” as shown on the right. The green trace represents the intensity profile of the figure on the left. The black trace represents the intensity profile of the figure on the right. .............................................. 54
Figure 30: Schematic of the final version of the coverslip sandwich.......................................... 56
Figure 31: Top view of flow chamber ....................................................................................... 57
Figure 32: Side view(left) of flow chamber and cross section (right) of flow chamber .......... 58
Figure 33: Flow chamber (before ends were sealed) ............................................................... 59
Figure 34: Flow chamber mounted on the microscope and connected to a power supply. Orientation of the video microscopy is shown in the plane of the stage................................. 60
Figure 35: Screen shot of tracking GUI input parameters .......................................................... 63
Figure 36: Representative fluorescent image of qdots on a SLB. ............................................. 64
Figure 37: Trajectory for qdot particle 1 undergoing random diffusion. ................................. 66
Figure 38: Trajectory for qdot particle 2 undergoing random diffusion ................................. 67
Figure 39: Trajectory for qdot particle 3 undergoing directed motion. The direction of the field is marked and the qdot travels from left to right. ...................................................... 68
Figure 40: Trajectory for qdot particle 4 undergoing directed motion. The direction of the field is marked and the qdot travels from left to right. ...................................................... 70
Figure 41: Trajectory for qdot particle 5 undergoing directed motion. The direction of the field is marked and the qdot travels from left to right. ...................................................... 71
Figure 42: Trajectory for bead 1 undergoing directed motion. The direction of the field is marked and the bead travels from left to right. .............................................................. 73
Figure 43: Trajectory for bead 2 undergoing directed motion. The direction of the field is marked and the bead travels from left to right. .............................................................. 74
Figure 44: Trajectory for bead 3 undergoing directed motion. The direction of the field is marked and the bead travels from left to right. .............................................................. 75
Figure 45: Trajectory for bead 4 undergoing directed motion. The direction of the field is marked and the bead travels from left to right. .............................................................. 76
Figure 46: Trajectory for bead 5 undergoing directed motion. The direction of the field is marked and the bead travels from left to right. .............................................................. 77
Figure 47: Trajectory for bead 6 undergoing directed motion. The direction of the field is marked and the bead travels from left to right. .............................................................. 78
Figure 48: Trajectory for bead 7 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.

Figure 49: Trajectory for bead 8 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.

Figure 50: Graph of MSD vs time for qdot particle 1 undergoing random diffusion.

Figure 51: Graph of MSD vs time for qdot particle 2 undergoing random diffusion.

Figure 52: Graph of MSD vs time for qdot particle 3 undergoing directed motion.

Figure 53: Graph of MSD vs time for qdot particle 4 undergoing directed motion.

Figure 54: Graph of MSD vs time for qdot particle 5 undergoing directed motion.

Figure 55: Graph of MSD vs time for bead 1 undergoing directed motion.

Figure 56: Graph of MSD vs time for bead 2 undergoing directed motion.

Figure 57: Graph of MSD vs time for bead 3 undergoing directed motion.

Figure 58: Graph of MSD vs time for bead 4 undergoing directed motion.

Figure 59: Graph of MSD vs time for bead 5 undergoing directed motion.

Figure 60: Graph of MSD vs time for bead 6 undergoing directed motion.

Figure 61: Graph of MSD vs time for bead 7 undergoing directed motion.

Figure 62: Graph of MSD vs time for bead 8 undergoing directed motion.

Figure 63: Histogram of drift velocities from beads on a SLB.

Figure 64: Histogram of bead drift velocities on a SLB fitted to a Gaussian.

Figure 65: Graph of bead drift velocities versus the time the electric field was applied.

Figure 66: Graph of qdot velocities versus time field is applied. Average diffusion coefficient for the no field condition is shown on the left axis.

Figure 67: Histogram of qdot drift velocities.

Figure 68: Diagram of peptide bound to CNT. In the upper right the schematic of a carbon nanotube is depicted. In the upper left such a nanotube with a bound peptide is shown. The amino acid sequence of the nanotube binding peptide with its fluorophore attachment.

Figure 69: CNT bound to bilayer.

Figure 70: CNT coated with detergents. Polar head groups are depicted in red (119).

Figure 71: SEM image of dried CNT/detergent/millipore water solution. Dark grey portions of the image show the detergent. CNTs are barely visible within the detergent.

Figure 72: SEM image of dried monodisperse SWCNTs suspended in millipore water.

Figure 73: Fluorescent image of labeled CNT diffusing on a SLB. Images are 10 seconds apart.
1. Introduction and Background

1.1 Nanomaterials

1.1.1 High Technology Devices: Nano vs Micro

Since the mid 1960’s silicon has been the main semiconductor material and thus the darling of high technology devices. In 1975, Gordon E. Moore, co-founder of Intel, predicted that the number of transistors per microchip would double every two years. This trend is shown in Figure 1. This held true up until 2013 where growth began to slow due to material and manufacturing limitations. As of 2015 microchips boast over 5.5 billion transistors per chip (1).

Figure 1: Development of number of transistors and other key performance metric between 1986 and 2009 (1).
Today’s microchips are manufactured in a top-down manner using UV radiation lithography. Top-down manufacturing refers to creating small final products from larger starting material.

Figure 2: Schematic of the conventional photolithographic process used in the production of semiconductor based electronics (2).

In the case of microchips lithographic masks are placed above semiconducting material coated with photosensitive layers that are exposed to light in order to create patterns that protect this layer from subsequent etching procedure (2). In this process depicted in figure 2, there are two key metrics that determine the ability to project patterns with small feature sizes onto the
photoresist covered surface. For one there is the resolution, or the capability to distinguish two nearby features. Secondly, the depth of focus of the light mask projection system determines the precision with which the wafer surface has to be positioned. Both of these system parameters depend on the wavelength \( \lambda \) and the numerical aperture of the optical system. Unfortunately, numerical apertures of optical systems are already optimized and close to their theoretical limits. Thus, for the features of the microchip to shrink further in size, the wavelength of utilized light needs to be reduced in order to minimize refractive blurring of the patterns and thus proper function of the nanostructures. Current lithographic radiation is already in the extreme UV with typical wavelengths around 250 nm. Shorter wavelengths of optical radiation or particle waves (such as electrons or ions) will need to be used to continue to achieve smaller chip features. However, this route poses some practical limitations as shorter wavelength radiation requires not only more safety precautions and expenses but also new materials that can act as photoresist in such technologies. More generally, top-down manufacturing approaches require larger amounts of materials and are constrained either by the particular physical limitation or the scalability or both of the technique used to imprint nano-sized structures onto it.

As of 1999, 95% of all semiconductors were fabricated in silicon. A transistor is a semiconductor device that controls the flow of electricity like a switch. Transistors are also an integral component of logic devices and memory. Silicon has not always been the go-to material when it comes to transistors, in fact, previous generations of transistors had to be reinvented several times to become scalable. Industry competition and market demand are driving the microchip industry to produce smaller and faster microchips but industry leaders are approaching the
physical limitations of the top-down process of microchip manufacturing. In order to move into the next generation of integrated circuits and high technology devices, a new approach must be found. Nanomaterials have the potential to be the foundation of this new approach (3).

With the advent of the buckyball \( \text{(C}_{60} \text{)} \) in 1985 there was a rush to explore and develop nanomaterials that can be integrated into everyday life. Dozens of new nanoparticles were created and characterized; each having their own highly unique set of electrical, optical and physical properties. This new class of materials had properties which were highly tunable almost exclusively based on their size (4). The tunability of material properties is due to two nanoscale effects. The first is the fact that the surface area to volume ratio drastically increases with decreasing size. These surface atoms have far less neighbors leaving many unoccupied bonds. Second, spatial confinement effects become important due to the finite size of the nanostructure (5). The general properties of nanomaterials are summarized in Figure 3.
Integrating this new class of materials into everyday life, for example into electronics or biotechnological applications would revolutionize those spaces. Indeed, we are already seeing the beginnings of this revolution today in products such as sunscreen and Kevlar where nanomaterials have been mixed into the bulk material to enhance and/or compliment their native properties. However, in these cases the nanomaterials are randomly dispersed in a bulk material and only a fraction of the potential of these tunable nano-materials is harnessed. In order to utilize the full potential of nanomaterials we must acquire the ability to assemble those nanometer sized building blocks into organized and functional macro-scale structures. It is only through this route that they can be integrated in the most impactful way. Such organized structures are important for ultimately building, for example, transistors and sensory devices.
which are integrated into electronics (6, 7). These devices are made deliberately from carefully chosen components. With electronic materials where band gaps and conductivity are truly tunable, the potential for fully optimized devices is staggering. Yet, this is precisely where the challenges lay: Assembling nanomaterials into organized structures is entirely non-trivial. The large surface-to-volume ratio leaves unoccupied bonds on the surface which causes nanomaterials to have the tendency to aggregate making it difficult to assemble in deliberate way.

1.1.2 What are Nanomaterials and Nanotechnology?

Nanotechnology is the science of manipulating and controlling matter at the molecular level in order to utilize the structure and size dependent properties which are distinct from those associated with bulk materials. It encompasses matter from several hundred nanometers \((10^{-7} \text{ m})\) down to a single nanometer \((10^{-9} \text{ m})\), approximately 100,000 times smaller than the diameter of a human hair. The International Standard Organization (ISO) describes a nano-object as a material with at least one dimension in the nanoscale \((1-100\text{nm})\). A nanoparticle is an object with all three dimensions in the nanoscale. Graphene which can be tens of microns wide but only 5-10 nm thick is considered a nanomaterial. A quantum dot which has a diameter of tens of nanometers is classified as a nanoparticle. Another distinction can be made between engineered nanomaterials (ENM) and naturally occurring nanomaterials. For example, quantum dots and graphene are engineered nanomaterials, while examples of natural nanomaterials are aquatic colloids such as proteins and peptides. Examples of nanomaterials are shown in Figure 4. Living organisms utilize nanomaterials like proteins to control biological systems and life processes.
Only 50 atoms are needed in the long-chain DNA to store one bit of information about a living cell. A single cell holds all of the information for the organization of a complex organism. Between the size for information storage and the size of a full cell lies the realm of complex biomolecules. Proteins such as insulin (with an approximate diameter of about 5 nm) are by any definition nano-objects and comprise most of the mass of a cell. Thus, life and, as its basic building block, cells are comprised of nanomaterials and have evolved mechanisms that enable the production, transport, control and recycling of nano-objects. In consequence, as we develop tools for the manipulation of nano-objects, cellular life is logical place to look for inspiration and approaches to overcome current technical limitations.

1.1.2.1 Nanomaterial Manufacturing

Nanomaterial Manufacturing has come a long way in the last 30 years. Ideally, we would be able to synthesize nanomaterials with any desired property and to highest purity on a very large scale. While the technology hasn’t come quite that far yet, a significant amount of progress has been made. We are able to consistently synthesize nanomaterials with limited impurities and engineer certain physical and chemical properties by modifying the size and shape of the material or alter their surface chemistry. Manufacturing techniques have also been optimized to improve yield and pave the way for further scale up.

The manufacturing techniques for nanomaterials are as diverse as the materials themselves. Some methods like evaporation or chemical vapor deposition are top-down methods where nanomaterials are synthesized from a larger bulk material (8). Whereas in other bottom-up
approaches like nucleation, nanomaterials are made from smaller molecules or atoms (9).

Carbon nanotubes are most commonly synthesized by the arc discharge method and quantum dots are generally manufactured using wet chemical synthesis or grown by vapor phase epitaxy (8, 10).

Figure 4: Examples of nano-materials. Upper left: TEM image of core-shell qdot particles at 200,000x magnification; scale bar = 20 nm(11), upper right: TEM image of single walled carbon nanotubes produced by chemical vapor deposition, picture below(12): SEM image of gold nanoparticles on a latex bead (13).
1.1.2.2 Quantum Dots

Quantum dots (qdots) are nanometer scale light-emitting semiconductors (14). They are considered zero dimensional semiconducting nanocrystals or nanoparticles relative to bulk semiconductors (15, 16). Qdots are often called “artificial atoms” because they have quantum mechanical wave functions much like atoms and a delta function-like density of states is possible (7, 17). Qdots also stand out from other nanoparticles because their properties are truly tunable based solely on their size (8, 18). Specifically, there are drastic differences in optical absorption, exciton (electron-hole pair) energies and electron-hole pair recombination based on the size of the qdot (8, 19). This phenomenon is largely due to two main factors: the increase in the surface area to volume ratio with decreasing size and the spatial confinement due to the finite size of the nanostructure. Spatial confinement does not affect all materials or their properties equally, but rather it is dependent on the characteristic length scale of the specific property which is determined by the individual material (10, 20). Bulk semiconductors are characterized by the minimum energy necessary to excite an electron in the ground state valance band to the conduction band, this is called the band gap energy (21). When an electron is excited from the valance band to the conduction band, the electron leaves a positively charged orbital hole behind. The negatively charged electron is bound to the positively charged hole forming an electron-hole pair called an exciton (22). In the case of quantum dots, the characteristic length scale is the Bohr radius ($a_0$) of the exciton (electron-hole pair), which is the distance between the electron and the hole and ranges from 2-50 nm depending on the material. As the radius of the quantum dot approaches the Bohr radius, the charge carriers are confined to the spatial
dimension of the quantum dot (8). In qdots quantum confinement occurs when the energy level spacing exceeds the thermal energy $k_B T$ (8, 23).

Qdots can be treated as a spherical potential box. When $r$ (radius of the qdot) < $a_0$ this is called the strong confinement regime. In the strong confinement regime, the Coulomb force between an electron and hole is no longer strong enough to form an exciton, this results in an increase in the band gap energy due to the spatial confinement of the electron and hole. When $3a_0 > r > a_0$ this is called the weak confinement regime. In this case, the Coulomb force is sufficient to form a bound exciton. However, in this regime the exciton is treated as a particle in a spherical potential and there is an increase in the exciton energy due to the quantization of its center of mass movement. Following from the solutions to the Schrödinger equation, the consequences of spatial confinement are a widening of the band gap with decreasing size and emerging of discrete energy levels on the edges of both sides of the band gap. The change from continuous to discrete energy levels with decreasing size is shown in Figure 5. This translates directly to the tunability of the optical band gap by size alone. As the diameter of the qdot decreases, the band gap becomes wider, requiring higher energy photons for excitation and thus effectively blue shifting the luminescence of the quantum dot. This is also depicted in Figure 5.
Figure 5: Schematic depicting the dependence of the band gap structures and thus their fluorescent wavelength and their size (24).

Quantum dots were chosen as the main nanomaterial of interest based on their optical properties, specifically their photoluminescence. When a qdot is excited by a photon from an external light source, an electron from the valence band is promoted to the conduction band, much in the same way as in bulk semiconductors, leaving behind a positively charged hole as seen in Figure 6.
Figure 6: Creation of the exciton (electron-hole pair) in a qdot due to photon absorption.

When the electron and hole recombine, the process can be either radiative or non-radiative. In the case of radiative recombination, a photon is emitted, whose energy is based on the band gap energy. In the case of non-radiative recombination the excess energy is dissipated through heat or other dark transitions and no photon is emitted (25).

Qdots can be specifically engineered to enhance their optical properties. Generally, this is achieved by creating a core-shell qdot. The core of the qdot determines the color of the photoluminescence and the shell enhances the optical properties (26). The shell generally consists of a wider band gap material which confines the exciton by creating a potential barrier around the core (8, 27). A schematic of a core shell qdot is shown in Figure 7. Further the qdots can be simultaneously biofunctionalized and achieve colloidal suspensions by using organic capping agents (8). Organically capped qdots are formed by introducing organic molecules that adsorb onto the qdot surface (28, 29). A schematic depicting organic capping is shown in Figure 7.
1.1.2.3 Carbon Nanotubes

Carbon forms a variety of nanostructured allotropes (0D, 1D, 2D), for example fullerenes (0D), carbon nanotubes (1D) and graphene (2D). Graphene is a single layer sheet of graphite. Carbon nanotubes (CNT) are cylindrical rolled sheets of graphene with a diameter on the order of a few nanometers. They have an exceptionally large length-to-diameter ratio (up to $1.32 \times 10^8$) as they can be up to 18 centimeters in length [30]. Depending on the structure of the CNT, specifically its chirality, they can be either semiconducting or metallic. The chiral vector, $C$

$$C = n_1 a_1 + n_2 a_2,$$  

(1)
where \( n_1 \) and \( n_2 \) are the chiral indices and \( a_1 \) and \( a_2 \) are the lattice vectors of graphene. The chiral vector uniquely describes the nanotube and indicates the rolling up direction and is perpendicular to the tube (31). The indices correspond to graphene unit lattice vectors. The three main structures for CNTs are zigzag, where either \( n_1 \) or \( n_2 = 0 \), armchair, where \( n_1 = n_2 \) and all other values of indices are known as chiral CNTs. Zigzag and armchair nanotubes have shorter unit cells compared to chiral CNTs. CNTs can also be classified by the number of concentric rolled layers. This is represented in Figure 8. Single-walled CNTs (SWCNT) are, as the name suggests, a single cylindrical layer of graphene. Whereas, multi-walled CNTs (MWCNT) consist of two or more concentric rolls of graphene (31, 32).

![Diagram of carbon nanotubes](image)

**Figure 8:** Geometric configuration of carbon nanotubes. (A) shows the chiral vector of carbon nanotubes and (B) illustrates the different configurations of single-walled carbon nanotubes. From left to right: armchair, zigzag, and chiral (33).

Some desirable properties are shared by all CNTs like a large aspect ratio whereas other properties, such as metallic or semiconducting character are dependent on the size and structure of the nanotube. The difference in the band structure of metallic and semiconducting
CNTs comes from the fact that the wave vector along the circumference of the CNT is quantized. This boundary condition is based directly on its size and orientation, so the allowed values of the wave vector are also based on size and orientation (34). If the allowed wave functions cross a corner of the Brillouin zone of the graphene then there is no band gap and the nanotube has metallic character. If the allowed function does not cross a corner of the Brillouin zone then there is an energy band gap resulting in semiconducting nanotubes. Generally, 1/3 of all CNTs are metallic whereas the rest are semiconducting. Armchair CNTs are always metallic. However, zigzag CNTs can be either metallic or semiconducting based on their chirality (34, 35). Further, the band gap of semiconducting CNTs also varies based on the size and orientation of the nanotube. Specifically, in zigzag semiconducting CNTs which range in diameter from 0.8 – 3 nm the band gap ranges from 0.2 -0.9 eV. These semiconducting CNTs have potential applications in field effect transistors and photovoltaics (36).

Metallic CNTs are considered a highly conductive material with a large current carrying capability. They can achieve current densities of over $10^3$ MA/cm$^2$. Uniform current density is observed over SWCNT. Metallic CNTs have potential applications as nanoscale interconnects or electrodes.

1.1.3 Current Methods of Nanomaterial Assembly

Currently, the easiest and most common use of nanomaterials is to simply mix them into the bulk material. Common examples of this are CNT reinforced Kevlar and titanium dioxide nanoparticles in sunscreen (37, 38). Figure 9 shows Kevlar fiber after treatment with a suspension of CNTs.
1.1.3.1 Self assembly

Due to their large surface-to-volume ratio, nanomaterials have much greater reactivity compared to their bulk counterparts (39). This makes them a prime candidate for self-assembly techniques where nanoparticles spontaneously aggregate in order to minimize the free energy of the system (40). For example colloidal gold nanoparticles can be self-assembled into ordered monolayers pictured in Figure 10 (41). Many areas of research focus on finding ways to direct and control the self-assembly of nanomaterials (6, 42). Peptide based organization and assisted self-assembly are discussed in 1.1.3.3. However, this type of self-assembly is prone to defects due to system entrapment in local free-energy minimums that inhibit the system to reach the global free energy minimum (40, 43). In other words, as system size increases self-assembly based on short range interaction such as molecular recognition or binding are prone to kinetic or geometric frustration. This inability to maintain global order leads to an arrest of the assembly.

Figure 9: CNTs incorporated into Kevlar (37)
process before the final structure is completed. In biological systems, such as proteins, this limitation is overcome by employing processes that guide proper protein folding, i.e. the correct assembly of the three-dimensional structure from the native amino acid strand. Such mechanisms, such as the use of chaperone proteins, give rise to the concept of assisted or forced self-assembly. A key prerequisite for such mechanisms is the ability to supply additional energy to overcome local energy barriers. This need gives way to the development of methods that are capable to transfer this concept into the realm of man-made nano-technology. The work described in this thesis, is one such avenue based on the combination of a reconfigurable two-dimensional surface (the cell membrane mimic, here SLB) and means to manipulate nano-sized building blocks attached to that surface.

Figure 10: Diagram of self-assembly of colloidal gold nanoparticles during the drying process (41).
1.1.3.2 Templating

Templating is a method of nanomaterial fabrication that involves a nano-porous template which acts as a scaffold for the desired material. The desired material is formed within the pores of the template and the template is discarded by chemical or other means, where the desired material is formed (44). In the example pictured in Figure 11 an alumina membrane is used as a template on which a silver cap is deposited. Gold nanorods are grown on top of the silver cap, which is then chemically removed, leaving an ordered array of gold nanorods (45). This works well for simple static assemblies of particles but can be time consuming and is a static process, meaning there is no way to dynamically rearrange the nanomaterials. However, such ability is desirable for many applications such as biosensing and smart materials (46). In order to fully realize the potential of nanomaterials as building blocks we would like to be able to dynamically organize them into complex structures.
1.1.3.3 Biomolecule-Based Static Organization

Biomolecules are also being used as “organic” templates. Using biomolecules such as peptides and deoxynucleic acid (DNA) are ideal for organic scaffolding due to their site-specificity and the flexibility to be easily synthesized to different lengths (42, 47). Many groups are using DNA-mediated assembly to create nanodevices from individual nanomaterial building blocks. Contrary to inorganic templates described in the previous section where the template is chemically removed to reveal the desired product, DNA serves as the ‘glue’ which cements the nanostructure together. In general, DNA is adsorbed to the nanomaterial of interest and can specifically bind to a complementary DNA strand connected to another nanoparticle or solid support. This is also called programmed assembly because there is greater control over how the
nanoparticles are assembled. While DNA based assembly does allow for reversibility under specific conditions it does not allow the flexibility of a truly dynamic assembly system.

1.2 Biomimetic Membranes

In the last 30 years, nanomaterial processing technology has exploded, lending itself to a new bottom-up approach for integrated circuits and other high tech device (48, 49). A bottom-up approach uses atomic and/or molecular-sized components to build larger products. This approach can be time consuming but allows for more customizability in devices. In this approach nanomaterials are used as molecular building blocks (MBBs) to build many high tech devices from transistors and logic devices to sensors and light emitting diodes (LEDs) (6, 46). Techniques to manipulate nanomaterials has been a hot area of research. Some of the current methods use interactions between the nanomaterial and an imposed field gradient to exert translational and rotational forces such as optical tweezers, optical spanners and dielectrophoresis (50–52). These techniques have applications in particle separation, trapping and particle transport. For example, diælectrophoretic ratchets can transport particles with velocities of up to 0.2 µm/s (52).

However, these methods generally involve complicated fields and often only act on one particle at a time making them inefficient and not at all practical for building the next generation of logical devices.

Biological systems employ the bottom-up approach and are often used as inspiration for manipulation of engineered nanomaterials (ENMs). Biologically mediated assembly of nanometer and micrometer scale structures have applications in nanoelectronics, materials
synthesis, medical diagnostics and therapeutics, microelectromechanical systems and hybrid sensors. Nanomaterials allow scientists to construct artificial structures that are the same size or smaller than biomolecules. For example, the tip of an atomic force microscope (AFM) is smaller than most viruses and the thickness of a gate insulator of a metal oxide semiconductor (MOS) transistor is smaller than one complete turn of DNA (10, 53). Armed with this nanomaterial tool set, more and more researchers are turning towards nature for inspiration. Nature boasts a variety of complicated molecular systems which can sense and relay information as well as self-assemble into complex structures.

1.2.1 Nature’s building Blocks: Cells

It has been estimated that there are at least 10 million different living species on earth (54). What does it mean to be living? What separates crystal growth and assembly of nanoparticles from tissue growth? Living matter is defined to be anything that exhibits all (or in some cases most) of the life processes (55). The life processes are order, reproduction, growth, energy utilization, response to stimuli, and evolutionary adaptation (56).

The smallest building block of living matter is a cell. In fact, single cellular organisms such as prokaryotes (or single cell eukaryotes), are the most common form of living matter. Bacteria is an example of a prokaryote (54). The average size of a single cellular organism is 1-5 µm. Prokaryotic cells have no defined nucleus. Complex multicellular biological systems are comprised of more complex cells called eukaryotes which have a defined nucleus as well as
other membrane bound organelles. The typical size of a eukaryotic cell is 10-100 µm. Humans are comprised of $10^{13}$ cells (57). All of which were once derived from a single cell.

Figure 12: Schematic of a eukaryote cell (58).

The main components of a eukaryotic cell are the plasma membrane which separates the cell from its environment, the cytoskeleton which helps maintain the cells shape, genetic material i.e. DNA, RNA for information storage, and organelles which specialize in specific vital functions of the cell (59). Eukaryotic organelles include the nucleus which stores DNA, the mitochondria which generates energy, the endoplasmic reticulum which is a transport network, the ribosomes which synthesizes proteins, and lysosomes which dispose of cellular waste. All of the subcellular organelles are also encapsulated by a membrane similar to the cell membrane (54). A pictorial representation of a cell is shown in Figure 12.
1.2.1.1 Cell Membranes

Cell membranes are a fundamental component of all biological systems. The typical diameter of a cell is 10-20 µm whereas the cell membrane is about 5-10 nm thick. Lipids are the basis of the cell membrane and cells use approximately 5% of their genes to synthesize them (60). The membrane is composed of a lipid bilayer in which membrane proteins are embedded. According to the fluid mosaic model of membranes, the membrane can be described as a two-dimensional fluid where lipids and proteins can diffuse easily (61).

The paramount function of cell membranes is to create a boundary between cells and their environment or between organelles within the cell itself (62). This boundary inhibits the interaction of chemical products and processes which are often more efficient when contained in their own isolated environment. The potential for budding, fusion, fission and tabulation is only possible because of lipids. Without these specific conditions and processes in place cell division is not possible. The plasma membrane is also responsible for tightly regulating transport within and across membranes (62). Many biochemical processes take place within the membrane and many cellular processes depend on its selective permeability (62, 63). It also plays an important role in cell signaling and further assembly into tissues and organs, as well as, sensing other external signals.
1.2.1.1 Lipids and Bilayers

Cellular membranes are formed by a lipid bilayer of approximately 5 nm thickness. In a bilayer area of one square micrometer there are about $5 \times 10^6$ individual lipids. Thus, in a typical cell there are about 10 billion individual lipids (64). Lipids are amphipathic molecules with a hydrophilic (polar) head and a hydrophobic (non-polar) tail. This is depicted in Figure 13. Polar molecules dissolve easily in water due to electrostatic interactions with water molecules. Hydrophobic molecules do not have energetically favorable interactions with water molecules which causes them to aggregate in water. The amphipathic nature of lipids allows them to self-assemble in aqueous media with the hydrophobic tails facing each other and the polar heads oriented towards the aqueous media (63). Depending on the length of their fatty acids, lipids generally self-organize into either a spherical micelle with the tails on the inside or a bilayer with the tails sandwiched between head group depending on the shape of the individual lipids. Van der Waals forces in concert with hydrophobic effects act as the main stabilizing agent for lipid bilayers.
There are 3 categories of lipids: phospholipids, cholesterols, and glycolipids. Phospholipids make up most of the membrane lipids (66). They have a polar head group with two hydrocarbon tails. The tails are fatty acid chains which can differ in length and saturation (number of double bonds). Each double bond in the chain creates a “kink” in the tail. The differences in length and tail kinks affect the fluidity of the bilayer and ultimately the diffusion coefficient (60).

Cholesterols enhance the selective permeability of the membrane. They have a rigid ring structure with a short hydrocarbon tail (67). Glycolipids are lipids with a sugar residue. They make up about .5% of the outer lipid leaflet in the bilayer. Glycolipids can form lipid rafts, i.e. membrane micro-domains, due to their tendency to aggregate because of hydrogen bonding between their sugar residues.
At any given time, the lipids in the bilayer are moving in a translational, rotational or transbilayer direction. Lipids are free to rotate around their long molecular axis which is perpendicular to the plane of the bilayer membrane. They do so at frequencies of $10^8$-$10^9$ s$^{-1}$ at physiological temperature (63). Comparatively, this is much faster than membrane proteins which rotate at $10^3$-$10^4$ s$^{-1}$ under the same conditions. Lipids are also free to diffuse along the 2-dimensional plane of the membrane. As such, lipid bilayers can be treated as quasi 2-dimensional fluid where individual lipid molecules freely diffuse within each leaflet. The fluidity of the bilayer depends on the lipid composition and temperature. In the liquid phase lipids exhibit rapid lateral diffusion with a diffusion coefficient on the order of $10^{-8}$ cm$^2$/s. Transbilayer diffusion occurs when a molecule flip-flops from one leaflet of the bilayer to the other. This occurs much less frequently due to the unfavorable energetics of moving a polar part of the molecule through the non-polar bilayer core. In fact, for most lipid species transbilayer flip-flop times range from minutes to hours (68).

Lipid bilayers have two main phases, a gel or frozen state and a liquid crystal state. When in the liquid crystal phase lipids exhibit both rotational and translational degrees of freedom. However, in the gel state lipids are only free to diffuse laterally (69). The rotational degree of freedom is restricted. Phase transition temperatures of lipids depend heavily on the length of the tails and degree of saturation. Transitions to the gel state are more difficult if the tails are short because they are less likely to interact with each other. Saturated lipids are also resistant to freezing because the kinked tails are hard to pack together and thus resist crystallization (63).
1.2.1.1.1 Model Systems and Solid Supported Lipid Bilayers

Model membranes were created to explore the properties of bilayers and its individual components in a more controlled manner (70). The complexities of bilayer organization, dynamics and function of the bilayer are difficult to explore in vivo. By simplifying and ultimately controlling the composition of the bilayer we can change and explore its intrinsic properties such as diffusion coefficient, lipid phases, etc. We can construct a simplified model by allowing lipids to self-assemble in water (71). Such models still maintain the essence of the lipid bilayer structure while preserving membrane fluidity, i.e. lateral lipid diffusion. In this work we set out to provide evidence that model membranes can also be used to manipulate nanostructures much in the same way that membranes assist in the aggregation of proteins on a cellular level (62).

There are 3 main types of artificial bilayers: liposomes, “black” membranes and solid supported lipid bilayers (SLBs) (62). Liposomes are formed when the bilayer closes in on itself and forms a spherical object similar to a cell (72). Black membranes are formed across a hole in a barrier between two aqueous environments (73). Solid supported lipid bilayers (Figure 15) are planar and are generally formed by vesicle fusion, where artificially prepared liposomes or vesicles meet an appropriate substrate and merge, rupture and fuse together forming a single bilayer or Langmuir transfer to a suitable surface (62, 71, 74). This is illustrated in Figure 14. Many commercially available lipids are dissolved in chloroform and thus the basic steps to prepare small unilamellar vesicles (SUVs) are: solvent evaporation, rehydration, formation of multilamellar vesicles by mixing, and dispersion into SUVs. There are several methods for
dispersion which include extrusion (multilamellar vesicle solution is pushed through a polycarbonate membrane of definite pore size), sonication, and freeze-thawing (71, 75). The preferred method being extrusion because it offers superior control over SUV size and conserves the initial lipid composition.

Figure 14: Vesicle fusion process: SUVs fuse into a large vesicle before rupturing onto a solid surface to form a supported lipid bilayer (74).

Figure 15: Schematic of a supported lipid bilayer (SLB). Shown is the glass substrate and individual lipid molecules in an aqueous environment.
Bilayers deposited onto solid substrates have been one of the most commonly used experimental cell-surface models. Solid supported lipid bilayers can be left to directly interact with their substrate as practiced in this work but are also capable of being tethered to the substrate (76). SLBs planar configuration is highly compatible with the preferred geometric configuration of optical imaging and surface characterization techniques. Thus, compared to other model membrane systems, SLBs are simpler to visualize using fluorescence microscopy, atomic force microscopy (AFM) and other surface sensitive techniques. In addition, SLBs are easy to prepare, their bilayers are mechanically stable, are able to cover large areas of the solid substrate (on the order of cm$^2$), exhibit good fluidity, are compatible with the use of patterned substrate and can be patterned directly (75, 77). In addition, it is straightforward to functionalize SLBs by incorporating proteins or lipids with specific head groups (such as NTA(Ni) or biotin) that have the ability to bind with both organic and inorganic materials (75). Functionalized membranes have the additional advantage that anything tethered to the bilayer will then be constrained to diffuse in two dimensions in a plane parallel to the surface (75). Furthermore, various surface sensitive techniques are available for the characterization of these systems (62). Neutral lipids, when deposited on solid substrates do not form neutral membranes. This is because the substrate itself contributes a negative charge (75).

1.2.1.1.1.1 Methods of binding particles to SLB

One of the main advantages of using a SLB as a model membrane is its planar geometry. However, the ability to functionalize the SLB is a close second. This can be achieved by
controlling the components of the lipid bilayer. In other words, taking advantage of specific chemical interactions and the wide variety of lipid head groups to choose from.

1.2.1.1.1.1.1 Biotin-streptavidin binding

One such important ligand-binder interaction is the biotin-streptavidin system. It is a lock and key type mechanism and is one of the strongest non-covalent bond in biology (78, 79). There are many biotinylated lipids available and there are off the shelf kits to create biotinylated proteins. In addition, incubation times to functionalize the SLB are relatively short, on the order of 15-60 minutes (75).

1.2.1.1.1.1.2 Ni-His tag binding

Another important chemical interaction for functionalizing SLBs is the Ni$^{2+}$-histidine system. Histidine is rich in electrons and can bind to metals such as Ni$^{2+}$ with relatively high affinity (80). Many recombinant proteins can be engineered with a histidine tag. In fact, this is one of the most common ways to purify a protein (81). The protein is expressed with a poly-His tag and a Ni$^{2+}$ column is used to purify the protein. A similar concept can be used to functionalize a SLB using a metal ion chelating lipid and His-tagged proteins (75). The metal ion chelating lipid is simply incorporated into the base lipid mixture and after formation of a SLB the His-tagged protein can be incubated with the bilayer for a relatively short period of time to functionalize the membrane, on the order of 15-60 minutes. This is depicted in Figure 16.
1.3. Motion of Membrane Tethered Particles: Diffusion and Electroosmotic Flow (EOF)

Particles that are bound to a SLB undergo diffusion much like the lipids that make up the bilayer. On first glance this seems surprising, but because the viscosity of the lipid bilayer is about 100 times higher than the one of water, it is the lipids that dominate the motion of the lipid anchored particle. We can track such motion using single particle tracking and characterize it by analysis of its mean square displacement as discussed in the following section.

1.3.1 Brownian Motion

Brownian motion is the random motion of particles in a fluid due to their collisions with the molecules that make up the fluid (82). A pictorial representation is show in Figure 17. Statistically it can be described as a random walk, where the particle trajectory consists of a succession of random steps. Assuming that the impacts with surrounding molecules are distributed normally the probability distribution function (pdf) for the position of the particle can be derived (83).
1.3.1.1 Two-dimensional Random Walks

Two-dimensional random walks can be described by two independent one-dimensional walks in each dimension \( (84) \). The step size distribution is described by a chi distribution and the probability of a particle diffusing a distance \( r \) in time \( \Delta t \) is given by

\[
P(r, \Delta t) = \frac{r}{2D\Delta t} \exp\left(-\frac{r^2}{4D\Delta t}\right),
\]

(2)

where \( D \) is the diffusion coefficient \( (85) \). This equation has been experimentally shown to describe the behavior of lipids in pure lipid bilayers \( (86) \). A typical step size distribution of a two-dimensional random walk is shown in Figure 18.
1.3.1.1 Mean Square Displacement

Because Brownian motion is stochastic and a random walker is equally likely to go in either direction, the average displacement from the origin is zero. Thus, the motion cannot be characterized this way. Instead, this type of motion is characterized using the mean square displacement (MSD) which is found by squaring the displacements and then averaging over the time interval. The MSD is calculated by:

\[
MSD(\Delta t) = \langle (x_{i+n} - x_i)^2 + (y_{i+n} - y_i)^2 \rangle \quad n = 1, 2, 3, \ldots, (N_T - 1).
\]

Where a particle at position \((x_i, y_i)\) moves to position \((x_{i+n}, y_{i+n})\) in time interval \(\Delta t = n \times \text{(video frame time)}\). \(N_T\) is the total number of frames recorded, and for a particular \(n\), \(i\) ranges from 1 to \(N_T - n\). For a randomly diffusing particle in two dimension, the trajectory is described by:
where $D$ is the diffusion coefficient (87). From this equation we know that a particle undergoing pure diffusion should have a linear MSD vs time graph. An example of a typical MSD vs time graph for a particle undergoing pure diffusion is shown in Figure 19.

![Figure 19: Mean square displacement as a function of time. A straight line indicates a linear relationship and thus normal Brownian, unobstructed diffusion.](image)

However, there are other types of motion that can also be characterized by the scaling of their MSD vs time graphs. Examples of several types of diffusion and their scaling factors is shown in Figure 20.
MSD vs time graphs can also be used to decipher ballistic transport vs diffusive motion vs mixed transport. Ballistic transport scales as (88)

$$MSD(\Delta t) \propto (\Delta t)^2.$$  \hspace{1cm} (5)

Mixed transportation is a linear combination of ballistic transport and diffusion. A typical graph comparing these three types of motion is show in Figure 21.
1.3.2 Diffusion in Lipid Bilayers

As discussed in Section 1.2.1.1 Lipids and Bilayers lipids in bilayers are free to diffuse laterally throughout the membrane. Experimentally, this diffusion is usually characterized in fluorescently labeled SLBs by a technique called FRAP (75, 89). FRAP stands for fluorescence recovery after photobleaching. This technique takes advantage of the fact that organic dyes lose their ability to fluoresce after being exposed to high intensity light for some period of time, this is called photobleaching. A small spot on the SLB is photobleached, and is then allowed to “recover” by unbleached lipids diffusing into the spot which had once been bleached. The speed at which the
recovery occurs characterizes the diffusion of the SLB (89, 90). Another method of characterizing diffusion, and the method used in this dissertation is single particle tracking (SPT). SPT generally uses video microscopy to provide measurements for the x and y coordinates of individual particles (91). SPT is used to track individual particles and analysis provides information about the particle instead of the ensemble as in FRAP experiments. The diffusion coefficient can then be calculated by mean squared displacement analysis as discussed in 1.3.1.1.1. It samples a shorter time and smaller area compared to FRAP, which measures an averages diffusion coefficient over many molecules. In cases where colloidal gold probes for fluorescent beads were used to track diffusion of lipids in a supported bilayer, diffusion was 2-4 times smaller compared to FRAP measurements (87). This implies that the measurements are not equivalent. FRAP is most useful for measuring the ensemble average diffusion coefficient where as SPT is a powerful tool to analyze the motion of individual particles.

1.3.3 Electroosmotic Flow (EOF)

1.3.3.1 Electric Double Layer

The electric double layer occurs when a charged surface is exposed to a fluid. Counterions in the fluid are attracted to the charged surface via electrostatic forces forming an electric double layer. This attraction can be described by the Poission equation and their tendency to diffuse away from regions of high concentration is described by the Boltzmann relation. This model assumes that fixed surface charges are smeared uniformly over a plane, that the dielectric constant is uniform within the aqueous phase and that ions in solution act as point charges (92).
1.3.3.2 EOF

Electro-osmosis is a method of moving bulk volumes of liquid, with respect to a charged surface by employing the use of an electric field (88). The movement of bulk liquid in the presence of an external electric field is caused by the electric double layer described above. Counter-ions in the bulk liquid screen the surface charge forming a diffuse cloud of excess charge. An applied electric field exerts a force on the ions and their associated water molecules in the diffuse layer causing fluid flow relative to the surface. The direction of flow is determined by the charge of the layers. The solid supports of SLBs generally have a negative charge (93). In the case of a SLB, the diffuse double layer has an excess of mobile positive ions the electro-osmotic flow will move towards the negative electrode. The drift velocity caused by EOF is given by,

\[ V_D = \frac{E \varepsilon_r \varepsilon_0}{\eta_w} \xi, \]  

(5)

where \( V_D \) is the drift velocity, with \( E \) being the electric field and \( \varepsilon_0 \) and \( \varepsilon_r \) the respective electric permittivities of free space and the dielectric constant of the medium, given the surface zeta potential, \( \xi \), and the viscosity, \( \eta_w \), of the aqueous medium (88). The electro-osmotic velocity is only independent of the distance from the charged surface outside of the debye layer. This results in a plug flow. The bulk flow can then be expected to include freely suspended particles outside of the diffuse double layer (88). The drift velocity of said particles can be determined by MSD analysis of the individual particle trajectories over time. In this case, the MSD is fitted to the equation

\[ MSD = 4D \cdot \frac{1}{2}. \quad (v \cdot t)^2, \]  

(6)

where \( D \) is the diffusion coefficient, \( t \) is time and \( v \) is the velocity of the particle (88).
1.3.3 EOF in Bilayers

Lipid membranes consisting of charged lipid molecules together with water molecules adsorbed to the polar heads and other aqueous ions form a charge distribution at the membrane interface. This results in a potential difference between the membrane surface and bulk electrolyte solution (94). In the presence of an electric field a bulk flow of electrolyte will occur in much the same way as described above, however the lipids in the bilayer will also move with this flow. This was observed experimentally in cell membranes in 1981 by McLaughlin and Poo (95).

1.3.4.1 EOF in SLB’s

If EOF could be used to redistribute lipids in a cell membrane then the natural extension would be to explore this phenomena in a simpler model using a supported lipid bilayer. This was experimentally observed and described by several groups. However, most groups explored the interplay between electroosmosis and electrophoresis because of the charged lipid head groups (88, 96, 96–98). To my knowledge, electroosmosis of neutral lipids has not be explored.

1.3.4.1.1 EOF in SLB’s with proteins

Several groups have used electric fields to manipulate macromolecules tethered to SLBs (88, 99). Most groups have used the interplay between electrophoresis and electroosmosis to direct the motion of charged macromolecules such as proteins. This serves as the foundation for the proposed project discussed below.
2. Qdot Project

2.1 Concept

The inspiration for this project comes from the organization processes of living cells, in which fluid lipid membranes are used to provide a platform for the assembly of larger multi-molecule complexes. However, to take a similar approach to the assembly of nano-objects into larger structures, it is necessary to attach them to membranes in a way that retains lateral mobility of the object. Fortunately, qdots can be purchased already water soluble and biofunctionalized making them the prime candidate for the nano-building block in this proof of concept project. Specifically, Qdot 655 Streptavidin Conjugate consists of streptavidin, a biotin-bind protein, covalently attached to a qdot nanocrystal. Streptavidin has a very high binding affinity for biotin and this system is commonly used for specific detection of a variety of proteins, protein motifs, nucleic acids, and other molecules. The Qdot 655 streptavidin conjugate is ~15-20 nm and thus about the size of a large macromolecule or protein. These qdots are made from a nanosized crystal of a semiconducting material (CdSe), which is coated with an additional semiconductor shell (ZnS) to improve the optical properties of the material. This is shown in Figure 22.
Figure 22: TEM image of core-shell qdot particles at 200,000x magnification; scale bar = 20 nm.

Left: Schematic of streptavidin coated Qdot655 (11).

Modeled after the cellular membranes of living cells, the self-assembly properties of amphipathic lipid molecules in aqueous solution can be explored to create artificial quasi-two-dimensional membrane surfaces. Besides closed surfaces such as vesicles and liposomes, lipids can also form planar bilayers on solid supports such as glass (100). Since the bilayer structure is suspended on a thin water film on the glass, lipids and other molecules in the membrane retain the freedom to move laterally within the plane of the membrane. Thus, this liquid-like surface allows for dynamic rearrangements of its structure and content. While many forms of these bio-mimetics have been recently exploited for biomedical and basic science research, their potential to act as organizing surfaces of non-biological materials has so far been mostly neglected (101–104).
POPC is typically considered one of the best model lipids for biophysical experiments. The fatty acid composition, i.e., saturated chain in the sn-1 position and unsaturated chain in the sn-2 position, mimics mammalian phospholipid composition (105). POPC is also neutral and fluid at 37°C being that its transition temperature is -2°C (105, 106).

![Chemical structure of POPC lipid](image)

Figure 23: Chemical structure of POPC lipid (105)

18:1 Biotinyl Cap PE is a biotinylated lipid. However, instead of the biotin being directly attached to the PE lipid, there is a six-carbon spacer between the biotin and the PE. The spacer arm extends the biotinyl group out from the membrane surface making it an ideal candidate for a linker for the qdots since they will be well within the bulk flow of liquid (107). A figure showing the biotinylated PE with the six-carbon spacer is shown Figure 24.
Figure 24: Chemical structure of 18:1 Biotinyl Cap PE (108).

β-BODIPY® FL C12-HPC is a neutral phospholipid with BODIPY dye-labeled acyl chains. The wavelength of its excitation maximum is 500 nm and its emission maximum is 510 nm. BODIPY dye was selected to avoid spectral overlap with the particles to be tracked (either Qdot 655 Streptavidin Conjugate which can be excited at 415-415 nm and emits at 655 nm or Flash Red Microspheres which absorbs at 660 nm and emits at 690 nm).
Combining ability to create bilayers with biotinylated lipids and the fact that the qdots are biofunctionalized with streptavidin we can study the behavior of membrane bound qdots that are laterally mobile. This constitutes a new, approach that might ultimately enable dynamic 2D patterning and assembly techniques of nanomaterials not available with the current solid and static methods. However, simply mobilizing the qdots on a lipid bilayer is not enough to influence the assembly of larger structures. We must be able to direct the motion of the qdots in a controllable way. This is where EOF comes into play. Using the lipid bilayer as a two-dimensional fluid platform, an electric field will be applied to induce electroosomotic flow to direct the motion of the qdots on the bilayer. In our system, the top of the tethered qdots protrude ~15-20 nm, which is well beyond the electrical double layer (~1nm), meaning
theoretically, the qdots should move with the bulk flow of liquid. A schematic of the linkage system is shown in Figure 26 and an expanded schematic of the qdot-SLB complex is presented in Figure 27.

Figure 26: Diagram representing streptavidin coated Qdot655 being tethered to a solid supported lipid bilayer containing biotinylated lipids.

Figure 27: Pictorial representation of the basic scenario in these experiments: streptavidin coated qdots bound to biotinylated lipids embedded in a glass supported lipid bilayer.
2.1.1 EOF in SLB Augmented with Qdots

Electroosmosis is the motion of bulk liquid in relation to a stationary charged surface in an electric field. In our experiments the cover glass acquires a negative charge which is translated to the neutral bilayer (neutral lipids were used) and the electroosmotic flow of bulk fluid is induced toward the negative electrode due to the excess of positive ions in the diffuse double layer (88). This is shown in Figure 28.

Figure 28: Diagram showing EOF in the context of membrane attached qdots.
2.1.1.1 Theoretical Drift Velocity Calculation

The electro-osmotic velocity, \( \nu_D \), given the surface zeta potential, \( \xi \), and the viscosity, \( \eta_w \), of the aqueous medium is given by

\[
V_D = \frac{E \varepsilon_r \varepsilon_0}{\eta_w} \xi, \tag{7}
\]

with \( E \) being the electric field and \( \varepsilon_0 \) and \( \varepsilon_r \) the respective electric permittivities of free space and the dielectric constant of the medium (88). An important characteristic of electrolyte solutions is that they ‘screen’, i.e. lessen, the effect of an electric charge by forming around it a cloud of counter-ions. The Debye length is the resulting characteristic decay length of an electric field created by an electric charge in such an environment. In monovalent salt solutions, like the sodium chloride solution used for the experiments, it is defined to be \( \kappa^{-1} \), given by

\[
\kappa^{-1} = \frac{\varepsilon_r \varepsilon_0 k_B T}{2 N_A e^2 c}. \tag{8}
\]

In this equation \( N_A \) is Avogadro’s number, \( e \) is an elementary charge, \( k_B T \) the thermal energy and \( c \) the concentration of the salt (88). Under our buffer conditions (\( c/(NaCl)= 100\text{mM} \), \( \varepsilon_0 = 8.85 \times 10^{-12} \text{F} \cdot \text{m}^{-1} \), \( \varepsilon_r = 80 \), \( N_A = 6.022 \times 10^{23} \text{mol}^{-1} \), \( k_B T = 4.11 \times 10^{-21} \text{J} \), \( e = 1.60 \times 10^{-19} \text{C} \)) the Debye length is approximately 1 nm. With a diameter 15-20 nm functionalized Qdots’ size is well above the Debye length. To calculate the electroosmotic velocity, we need to know the zeta potential of the surface. At low zeta potentials, it can be calculated using

\[
\xi_{surface} = \frac{\kappa^{-1} \sigma}{\varepsilon_0 \varepsilon_r \kappa}. \tag{9}
\]
where $\sigma$ is the surface charge, $\kappa^{-1}$ is the Debye length, and $\epsilon_0$ is again the permittivity of free space and $\epsilon_r$ the dielectric constant for water (110). Combining equation (8) and equation (10) we get for the electro osmotic velocity:

$$V_D = \frac{E \kappa^{-1} \sigma}{\eta_w}, \quad (10)$$

where $E$ is the electric field given by the voltage, $V$, between the electrodes divided by the distance, $d$, between them:

$$E = \frac{V}{d}. \quad (11)$$

For our experimental set-up the voltage was typically $23.5 \pm 0.5$ V and the distance between electrodes $70 \pm 5$ mm. Thus, the corresponding field calculates to $3.4 \times 10^2 \pm 0.3$ V/m.

For this calculation of the electro-osmotic flow, we will assume that the viscosity of the phosphate buffered saline (PBS) solution is the same as the viscosity of water. Further, we will assume that the surface charge density of the cover glass is $-0.25$ C/m$^2$ based on the charge density expected on a silicon oxide surface at pH 8 and ionic strength (88). Typical membrane formation conditions for this experiment were pH 7.4 ionic strength of 100 mM making the surface charge density a ballpark figure.

Further we will assume that because the lipids that form the bilayer are neutral that the surface charge of the substrate is fully seen on the bilayer surface. In reality there will be a screening effect from the bilayer. Yet to date, there have not been any good attempts to characterize this
screening effect either numerically or experimentally and we proceed with this ‘worst-case’ scenario. Plugging in all the values we obtain a theoretic electro-osmotic velocity of

\[ V_D = 84 \pm 8 \mu m/s. \]

It should be clear that this number is at best an upper bond or rough ball park figure as it is based on many assumptions that leave much room for discussion. Thus, we expect the actual drift velocity of the Qdots to be less due to the errors introduced by not accounting for the charge shielding effect from the bilayer as well as the drag force on the Qdot due to being directly tethered to the lipid bilayer. Furthermore, the bilayer presence – like any surface – affects both the dielectric constant and effective water viscosity close by. Finally, it is possible that Qdots are linked to more than one biotinylated lipid as they are conjugated to 5-10 streptavidins per particle.

2.2 Materials and Methods

2.2.1 Materials

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt) (18:1 Biotinyl Cap PE) were obtained from Avanti Polar Lipids (Alabaster, AL). All lipids were dissolved in chloroform and stored at -20°C. \( \beta \)-BODIPY® FL C_{12}-HPC (2-(4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Dodecanoyl)-1-Hexadecanoyl-sn-Glycero-3-Phosphocholine) was obtained from Life Technologies (Grand Island, NY). Qdot 655 Streptavidin Conjugate was obtained from Life Technologies (Grand Island, NY). Flash Red fluorescent microspheres were obtained from Bang Laboratories (Fishers, Indiana). All HPLC grade organic solvents were obtained from Sigma-
Aldrich (St. Louis, MO) and used without further purification. Bovine Serum Albumin (BSA) were also purchased from Sigma-Aldrich (St. Louis, MO). All aqueous solutions (buffers etc.) were prepared using Millipore water (double-deionized, organic-free) of high specific resistivity (approx. 18.0 mΩ/cm).

2.2.2 Small Unilamellar Vesicle Preparation

The supported lipid bilayers used in the experiments were formed by rupture and fusion of small unilamellar vesicles (SUVs) with a mean diameter of about 100 nm as depicted in Figure 14 (111). Thus, the first step is the production of such SUVs whose composition mirrors the desired ratio of different lipids in the final bilayer. Starting point for this procedure are lipids stored in organic solvent (typically chloroform) at various concentrations (typically 0.1 – 10 mg/mL). The desired mol% ratio of lipids in the bilayer is determined and the appropriate volume of each lipid stock solution is calculated. In a clean round bottom flask (piranha etched and dried at 80°C overnight and then rinsed with chloroform several times before use) appropriate amounts of POPC, BODIPY and 18:1 Biotinyl Cap PE were added and agitated for approximately 30 seconds to ensure thorough mixing. The round bottomed flask is then attached to a roto-vap and the chloroform is evaporated off while the flask rotates in a warm water bath above the transition temperature of the lipids (>2°C) for approximately 30 minutes. This results in a lipid multilayer on the interior of the flask. The lipid film is then rehydrated with Milipore water to a final concentration of 2 mg/ml causing the spontaneous formation of lipid multilayer liposomes. The liposome solution is first homogenized by vigorous pipetting before the next step of high pressure extrusion produces SUV. Prior to use the extruder (LIPIEX 1.5 ml extruder from
Transferra Nanoscience Inc., Burnaby, Canada) was cleaned according to manufacturer's instructions. Briefly, each component was thoroughly rinsed with isopropanol to ensure there is no cross contamination between lipid preparations. The extruder was then assembled and heated to 50°C and 5 mL of milli-pore water was run through the apparatus to wet the polycarbonate membrane and rinse any remaining isopropanol.

The lipid solution is then extruded with a high-pressure extruder (LIPEX™ extruder, Northern Lipids Inc., Burnaby, BC, Canada) through a polycarbonate filters with pores of 100 nm diameter. Ten sequential extrusion cycles are sufficient to produce a clear suspension, indicating monodisperse, unilamellar vesicles. The resulting SUV solution was stored at 4°C for no longer than 3 days but most often used immediately.

2.2.3 Formation of Supported Lipid Bilayer

The formation of a supported lipid bilayer on glass was performed via the following procedure: No. 1 (170 µm thickness) rectangular glass cover slides with dimensions of 22 x 70 mm (Fisher Scientific International Inc., Hampton, NH) held in a glass slide holder, were prepared by first ultrasonically cleaning them in isopropanol using a Fisher Scientific Digital Ultrasonic Cleaner FS30D (Fisher Scientific, Pittsburgh, PA). The first step is a 5-minute degassing cycle which consists of mostly low pressure sound waves causing cavitation of the liquid forming millions of microscopic bubbles. This is followed by a 15-minute sonication phase which uses high pressure sound waves to collapse the bubbles releasing energy. These implosions act like scrubbers and release adhered particles from the surface of the coverslip (112). This is followed by extensive rinsing in Millipore water in preparation for the Piranha etching step. Piranha etch reacts violently with organic solvents and extreme care must be taken to ensure that all surfaces are
properly rinsed and proper personal protective equipment (PPE) is used. After extensive rinsing, a 5 minute etch in Piranha solution (25% hydrogen peroxide, 75% sulphuric acid) is performed with proper PPE (inside a designative chemical safety hood, using rubber gloves and apron as well as a face shield). Due to the strong dehydrating power of sulfuric acid, addition of hydrogen peroxide is highly exothermic. Atomic oxygen, an extremely reactive free-radical is generated. The intense heat and strong oxidative reagents rapidly oxidize organic compounds and thus remove them from the glass surface. Furthermore, atomic oxygen increases the number of silanol groups on the glass surface which form hydrogen bonds with water molecules and promote surface hydrophilicity. At neutral pH silanol groups deprotonate (SiO\(^-\) + H\(^+\)) and the glass surface becomes negatively charged (75, 93). The cleanliness of glass substrates is extremely important to the successful formation of supported membranes (75). Piranha etching is followed by an intense rinsing in Millipore water (typically 10 rinses). The vesicle solution is next diluted 1:1 with buffer solution (PBS- 130mM NaCl, 7mM sodium phosphate dibasic, 3mM sodium phosphate monobasic) a drop of this mixture (90 µL for a 22 x 70 rectangular cover glass) is placed on a sterile plastic petri dish. A freshly etched (less than 24 hours) cover glass is then dried under an anhydrous nitrogen stream before being placed on top of the vesicle containing droplet. The formation of the bilayer is effectively instantaneous although certain lipids can benefit from a 3-5 minute incubation period at this point. The petri-dish with the cover glass is next submerged in a large pyrex crystallization dish containing 1 L of PBS buffer that was adjusted to a pH of 7.4 using 0.1 molar NaOH. The cover glass containing the bilayer is then carefully pried off of the petri dish. Utmost care must be taken at this point to ensure that the newly formed bilayer has no contact with the air, any loss of water contact will result in the
immediate degradation of the bilayer. The cover glass is gently swirled in the buffer to remove unfused lipid vesicles before being placed in a custom flow chamber (described below) while ensuring a constant aqueous environment. Finally, the flow chamber and an additional cover slide are assembled into a “cover glass sandwich” described in more detail on 55 pp.

The composition of the bilayer for my experiments was chosen such that it provided a number of different functionalities. Firstly, the bilayer should be chemically inert and neutral, to this end the main lipidic constituent of the system was selected to be POPC, a chemically inert neutral lipid and resulting in lipid bilayers with excellent fluidity at 37°C. Unsaturated lipids like POPC are commonly used as the main lipid species for most applications in biological sciences (75). Secondly, it was desirable to be able to independently confirm the integrity of the bilayer, thus 0.25 mol% of a fluorescently labeled lipid Bodipy was included. Bodipy is a bright, stable, high quantum yield with narrow spectral bandwidth. To ensure the neutrality of the bilayer, fatty acid (tail) labeled Bodipy was used. This allowed us to easily monitor proper bilayer deposition and to visualize potential bilayer defects such as holes and confirm the fluidity of the bilayer using Fluorescence recovery after photobleaching (FRAP)(89). FRAP is a well-established method to assess motion of fluorescently labeled molecules in a plane. In short, by prolonged exposure of a restricted area in the bilayer by high intensity light (here achieved by closing an aperture down to limit illumination of the bilayer by the excitation light to only a part of the bilayer) leads to irreversible destruction of the fluorophore on those molecules. One can now over time image the recovery of the fluorescence signal in that bleached spot. Of course, this only occurs if molecules with functioning and destroyed fluorophores are able to diffuse in the plane. Thus,
this procedure is a very straightforward way to verify that after the deposition of the bilayer and assembly of the observation chamber the lipid membrane is still fluid.

Figure 29: Example of a FRAP experiment. Briefly, a small spot of the bilayer is exposed to high intensity light, effectively photobleaching the region shown on the left. The light is turned off and unbleached lipids diffuse into the region causing the “recovery” as shown on the right. The green trace represents the intensity profile of the figure on the left. The black trace represents the intensity profile of the figure on the right.

It is performed before any single particle tracking experiments in the presence of a bilayer. The bilayer was partially photobleached (dark area in the left most image). The recovery of the fluorescence can be seen in the right image and is quantified by normalized intensity profiles along the red lines (graph in the middle). The recovery of the fluorescence signal established that the bilayer is fluid and thus its lipids are capable of two-dimensional in-plane diffusion.
2.2.4 Custom Flow Chamber

2.2.4.1 Coverslip Sandwich

Several iterations in the design of the coverslip sandwich and the flow chamber as a whole were necessary before a solution was created that enabled the proper execution of the experiments. We started simple and tried to use two coverslips assembled into a sandwich. The idea was to deposit the bilayer as previously described and then assemble the sandwich as depicted in Figure 30. Capillary action would then be used to flow through the blocking solution and the Qdot solution. However, this ultimately did not work as there was not enough space in between the coverslips. Building upon this we tried to use Teflon tape as a spacer but the tape would not stay in place which made the sandwich hard to assemble. Further using a combination of Teflon tape and vacuum grease made the pliable tape too sticky and did not work either. After consulting the machine shop we decided to cut a 100 µM Mylar strips as a spacer. Mylar is used in food packaging and is chemically inert which is ideal for our experiments. Mylar is similar in weight and rigidity to a thin sheet of plastic. It does not lose its shape like Teflon tape but is flexible enough to be compressed by the flow of the flow chamber to form a tight seal with vacuum grease. 100 µM was deemed to be the best size for the cross section of the chamber as it balances the need for narrow confinement, ease of operation and handling, and minimizing of optical artifacts due to the top glass slide. The first iterations of this mylar spaced coverslip sandwich used a coverslip as the top portion and hence the name “coverslip sandwich.” However capillary action alone was not sufficient to flow fluids through the chamber in order to block the bilayer and incubate with the Qdot solution. We decided to use syringe needles to inject fluids into the system. This required a few things, one of which was holes in the top of the
sandwich. Being 170 µm thin, the coverslip is too small to drill holes into, and we used a standard microscopy glass slide instead. (I will, however, still refer to the glass slide-coverslip sandwich simply by coverslip sandwich.) The final iteration of the coverslip sandwich is shown schematically in below Figure 30.

![Schematic of the final version of the coverslip sandwich](image-url)

**Figure 30:** Schematic of the final version of the coverslip sandwich

### 2.2.4.2 Fully Assembled Flow Chamber

In order to successfully establish EOF between the membrane and the upper microscope slide of the cover slip sandwich and to observe the membrane and quantum dots, a buffer reservoir, a viewing window for the inverted microscope, electrodes to apply a field to the system and a way to flow fluids through the system are needed. The first problem was solved by creating a holder for the coverslip sandwich made of Lexan, as seen in Figure 31. The holder is closed on all four sides but has a cut-out on the bottom for mounting on the microscope. The holder also has a top. Next an inlet and an outlet for fluids was added. The inlet is a luer locking needle connected to a syringe and the outlet is a luer locking needle connected to a small piece of tubing. The
ends of the needles were cut off resulting in a blunt end ensuring that the flow of liquid through it would apply even pressure to the coverslip.

![Top view of flow chamber](image)

**Figure 31:** Top view of flow chamber

The top of the sample serves to apply pressure to the coverslip sandwich to form a seal so that the only direction the fluid can flow is parallel to the bilayer. This is illustrated in the right side of drawing Figure 32.
Screws are used to tighten and vacuum grease is applied to fill in the gaps. The coverslip sandwich together with the sample holder forms two buffer reservoirs where the coverslip sandwich acts as the bridge between them. This design is based on the flow chamber used by Groves and Boxer (1995) (97). The assembled product, before the end caps were put on is pictured in Figure 33. The cut out on the bottom of the sample holder needed to be graded several times to allow room for the 60x WI objective lens to properly fit underneath.
2.2.5 Addition of Fluorescent Particles

2.2.5.1 Qdot 655 Streptavidin Conjugate - lipid conjugation

Qdot 655 streptavidin conjugate stock solution is supplied as a 1 µM solution. It is then diluted in 6% BSA in PBS to a final concentration of $1.0 \times 10^{-8}$ µM. The bilayer is first blocked for 30 minutes with 200 µL 0.5% BSA. Then 200 µL of $1.0 \times 10^{-8}$ µM Qdot 655 Streptavidin Conjugate solution is added to the flow chamber. It is incubated for 30 minutes before several rinses with PBS are executed.

2.2.5.1 Addition of “Flash Red” Fluorescent beads

In addition to the streptavidin modified qdots, also experiments with larger (~8 µm) polystyrene beads containing a red fluorophore (Flash Red) were conducted (Bang Laboratories, Inc., Fisher, IN). There were several reasons for it. Firstly, as they are easier to visualize, there were helpful to establish that the basic setup works. In addition, using them with and without supported
membranes enabled us to establish that the presence of the membrane indeed impacts EOF.

Flash Red beads are supplied as a 0.5% stock solution. They are diluted to a final concentration of 0.0005% with PBS. The bilayer is first blocked for 30 minutes with 200 µL 0.5% BSA. Then 200 µL of 0.0005% Flash Red solution is added to the flow chamber and used immediately. For experiments without a bilayer the cover glass were incubated with 200 µL 0.5% BSA. Then 200 µL of 0.0005% Flash Red solution was added to the chamber and used immediately.

2.2.6 Fluorescence Microscopy and Imaging

Figure 34: Flow chamber mounted on the microscope and connected to a power supply. Orientation of the video microscopy is shown in the plane of the stage.

Standard epifluorescence imaging was undertaken using the built-in imaging pathways on the microscope (Nikon Ti-Eclipse, Nikon, Tokyo, Japan). Fluorophore specific filter cubes were used
for the two imaging channels (green channel for the bilayer and red channel for the particles). A 60x, 1.2NA, water immersion objective (Nikon Corp., Tokyo, Japan) was used. Videos were taken of particles at 75-80 ms/frame for up to 1,400 frames. Particle trajectories were analyzed using a particle tracking algorithm and custom matlab discussed below. The electric field was applied continuously for 30 minutes. Video microscopy was performed before the field was applied and then approximately every 10 minutes until the field was turned off. The experimental set-up is shown in Figure 34.

2.2.7 Single Particle Tracking & Analysis

For the analysis of all experimental data, stationary particles were excluded, the total number of frames for each qdot was greater than 60 and the least-squares fit to the data was performed over 50% of the steps. For example, if a trajectory was 100 frames, the fitting algorithm was applied to frames 1-50. Stationary particles are attributed to microscopic defects in the SLB that allows access to the glass that was not blocked by BSA. For a randomly diffusing particle in two dimensions the MSD is described by (4. However, when the field is turned on and EOF is induced the MSD in two dimension is described by (5.

The open source code, TrackingGUI_rp.m for Matlab produced by the Parthasarathy Lab at University of Oregon was used to track individual particles. Briefly the TrackingGUI_rp.m uses an algorithm that exploits the radial symmetry of particles and offers an algebraic solution instead of an iterative solution like the Gaussian fitting via non-linear least squares method or Gaussian fitting via maximum-likelihood estimation. The algorithm determines the point of maximum
radial symmetry to determine the location of the particle’s center. It exploits the fact that for a radially symmetric distribution, a line drawn parallel to the gradient through any point will intersect the particle’s center. However, in a noisy image such as the ones that are produced by real experiments, lines do not precisely intersect the particle’s center. The algorithm then estimates the particle’s center by finding the point that minimizes the distances between itself and all such lines. This allows the algorithm to determine particle centers much more quickly than an iterative approach while still being just as accurate as the Gaussian methods which were previously the standard (113).

The input parameters for the tracking GUI are threshold intensity, localization method, link max step\(^2\) and link memory. The threshold intensity sets a threshold for the local intensity maxima, keeping all points above the threshold*100 percentile. The localization method sets the localization algorithm. The link max step\(^2\) parameter sets the maximum step size that a particle can have between frames. Meaning that if the distance-squared is greater than the value the objects are considered different particles. The link memory sets the maximum number of frames an object can be absent and still maintain the same particle ID number. A screenshot of the input parameters is shown in Figure 35.

For all experiments the tracking parameters were:

Threshold intensity = 0.999; This means that only the top 0.1% of pixel intensities were considered.

Particle localization = Radial symmetry; This is the quickest algorithm and is discussed on pp 56.
Link MaxStep^2 = 10 for Brownian motion and 20 for ballistic motion; Particles undergoing ballistic motion move much faster than those undergoing diffusive motion so their max step size is larger.

Link Memory = 5; Qdots often “blink” during the experiment and are not fluorescing in all frames.

Figure 35: Screen shot of tracking GUI input parameters

In order to select particles that were in the field of view for more than 60 steps and thus could be reliably used to characterize the motion, I wrote a Matlab (MathWorks, Natick, MA) script that accomplished this selection and calculated the corresponding mean squared displacement.
for each time point in the track based on Equation 3. The MSDs were graphed and fitted to appropriate equations using Igor Pro (WaveMetrics, Inc., Lake Oswego, OR) which utilizes a least-squares algorithm for curve fitting (114). The error bars represent the standard error.

2.3 Results

Qdots were successfully tethered to a SLB containing biotinylated lipids. The qdots were freely diffusing throughout the bilayer. A representative fluorescence image is shown in Figure 36.

Figure 36: Representative fluorescent image of qdots on a SLB.

The trajectories taken by four typical qdots are shown in figures Figure 37, Figure 38, Figure 39, Figure 40, and Figure 41; their corresponding mean square displacement versus time plots, analyzed using unweighted internal averaging over all time pairs is shown in figures Figure 50, Figure 51, Figure 52, Figure 53, and Figure 54. As expected in the 0-field and thus no-flow
situation, a linear relationship is observed for most particles. In these cases their diffusion coefficient can be extracted from the slope of the linear MSD growth with time.

2.3.1 Single particle trajectories

2.3.1.1 Qdots randomly diffusing on SLB (No external field applied)

Trajectories of individual qdots were reconstructed using the SPT measurements for the x and y coordinates. Representative trajectory plots for freely diffusing particles (no external field) are shown in figures Figure 37 and Figure 38. The trajectory appears to be random diffusion but MSD analysis is necessary to draw any definitive conclusions. The corresponding MSD graphs are shown in figures Figure 50 and Figure 51.
Figure 37: Trajectory for a qdot particle 1 undergoing random diffusion.
2.3.1.2 Qdots tethered to SLB after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 10 minutes

The external field was continuously applied to the qdot-SLB complex for 30 minutes. Videos were taken approximately every 10 minutes until the field was turned off. A Representative trajectory of qdots exposed to an external field for 10 minutes is shown in Figure 39. The trajectory shows a clear coupling with the flow which we expect to flow in the same direction as the field. This
was also validated by a build-up of fluid at the negative electrode buffer reservoir. The corresponding MSD graphs are shown in Figure 52.

Figure 39: Trajectory for qdot particle 3 undergoing directed motion. The direction of the field is marked and the qdot travels from left to right.
2.3.1.3 Qdots tethered to SLB after application of the external field (3.4x10^2 ±0.3 V/m) for 20 minutes

A Representative trajectory of qdots exposed to an external field for 20 minutes is shown in Figure 40. The trajectory still shows a clear coupling with the flow which we expect to flow in the same direction as the field. The particle appears to travel further in less steps than the qdots exposed to the field for 10 minutes. Further MSD analysis and determination of average particle velocity is discussed in Sections 2.3.2 MSD Graphs and 2.3.4 Velocity Distribution. The corresponding MSD graphs is shown in Figure 53.
2.3.1.4 Qdots tethered to SLB after application of the external field ($3.4 \times 10^2 \pm 0.3$ V/m) for 30 minutes

A representative trajectory of qdots exposed to an external field for 30 minutes is shown in Figure 41. The trajectory still shows a clear coupling with the flow which we expect to flow in the same direction as the field. No conclusions can be drawn from the particle trajectory alone,
further MSD analysis and determination of average particle velocity is discussed in Sections 2.3.2 MSD Graphs and 2.3.4 Velocity Distribution. The corresponding MSD graph is shown in Figure 54.

Figure 41: Trajectory for qdot particle 5 undergoing directed motion. The direction of the field is marked and the qdot travels from left to right.
2.3.2 Fluorescent microspheres (beads)

To better understand how external fields effect our system, fluorescent beads (diameter ~8 µm) were used to map the flow patterns caused by EOF. The beads are an order of magnitude larger than the qdots and have nothing to tether them to the bilayer, thus allowing them to move freely with the bulk fluid flow. Beads were used to map the flow field in the chamber both with and without a SLB present. As discussed in 2.1.1.1, the bilayer should screen the surface charge from the glass support effectively altering the flow field as compared to the flow field caused just by the glass support.

2.3.2.1 Beads on cover glass (no SLB) just after application of the external field (3.4x10² ± 0.3 V/m)

Beads were introduced to the flow chamber in the same way as the qdots. However, in this first scenario no bilayer was present on the cover glass but the cover glass was cleaned as described in Section 2.2.3 Formation of Supported Lipid Bilayer. In the absence of an external field, the beads remained stationary on the glass. Figure 42 is a representative trajectory of a bead just after the external field is applied. This is contrary to the qdot system where there is a time lag between the time the field is applied and when the qdots start exhibiting ballistic motion.
2.3.2.2 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes

Figure 43 is a representative trajectory of a bead after the external field is applied for 10 minutes. The corresponding MSD graph is shown in Figure 56.
Figure 43: Trajectory for bead 2 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.

2.3.2.3 Beads on cover glass (no SLB) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 20 minutes

Figure 44 is a representative trajectory of a bead after the external field is applied for 20 minutes. The corresponding MSD graph is shown in Figure 57.
Figure 44: Trajectory for bead 3 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.

2.3.2.4 Beads on cover glass (no SLB) after application of the external field ($3.4 \times 10^2 \pm 0.3$ V/m) for 30 minutes

Figure 45 is a representative trajectory of a bead after the external field is applied for 30 minutes. The corresponding MSD graph is shown in Figure 58.
Figure 45: Trajectory for bead 4 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.

2.3.2.5 Beads on a SLB (no tethering system) just after application of the external field (3.4x10^2 ± 0.3 V/m)

In this second bead scenario, a SLB of the same composition as used in the qdot trials was formed on the cover glass. Being that the beads are not tethered to the bilayer in the absence of an external field the beads remain stationary. The beads do not exhibit any diffusive motion like the qdots did in the no field condition. This provides further evidence that the qdots were in fact tethered to the bilayer and the bilayers two-dimensional fluidity was transferred to the qdots.
Figure 46 is a representative trajectory of a bead just after the external field is applied. This is contrary to the qdot system where there is a time lag between the time the field is applied and when the qdots start exhibiting ballistic motion. The corresponding MSD graph is shown in Figure 59.

Figure 46: Trajectory for bead 5 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.
2.3.2.6 Beads on a SLB (no tethering system) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 10 minutes

Figure 47 is a representative trajectory of a bead after the external field is applied for 10 minutes. The corresponding MSD graph is shown in Figure 60.

Figure 47: Trajectory for bead 6 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.
2.3.2.7 Beads on a SLB (no tethering system) after application of the external field \((3.4\times10^2 \pm 0.3 \text{ V/m})\) for 17 minutes

Figure 48 is a representative trajectory of a bead after the external field is applied for 17 minutes. The corresponding MSD graph is shown in Figure 61.

Figure 48: Trajectory for bead 7 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.
2.3.2.8 Beads on a SLB (no tethering system) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 25 minutes

Figure 49 is a representative trajectory of a bead after the external field is applied for 25 minutes. The corresponding MSD graph is shown in Figure 62.

![Figure 49: Trajectory for bead 8 undergoing directed motion. The direction of the field is marked and the bead travels from left to right.](image-url)
2.3.2 MSD Graphs

The MSD of each particles trajectory was calculated as described in Section 2.3.1 Single particle trajectories. The MSD plots were used to extract information regarding the diffusion coefficient for randomly diffusing qdots and the velocities of particles undergoing ballistic motion. Qdots tethered to a SLB under the no field condition have linear MSD plots that suggest the qdots are freely diffusing within the bilayer. Qdots tethered to a SLB have quadratic MSD plots suggesting ballistic motion after application of an external field for at least 10 minutes. Beads tracking the flow under the influence of an external field are static in the absence of the field and immediately show ballistic motion (quadratic MSD plots) as soon as the field is applied.

2.3.2.1 Qdots

2.3.2.1.1 MSD Plot of Randomly Diffusing Qdots (no field)

Figure 50 and Figure 51 are representative MSD plots of qdots tethered to a SLB when no external field is applied. The plots are approximately linear implying that the qdots are freely diffusing throughout the bilayer. Physically, the diffusion coefficient is proportional to the slope and the offset represents the localization uncertainty (115). The corresponding particle trajectories are shown in Figure 37 and Figure 38.
Figure 50: Graph of MSD vs time for qdot particle 1 undergoing random diffusion.

Mean Squared Displacement (um$^2$)

$MSD = bt + a$

$a = -1.2 \pm 0.1$

$b = 2.2 \pm 0.1$
2.3.2.1.2 MSD Plot of Qdot Tethered to a SLB after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes

Figure 52 is a representative MSD plot of a qdot tethered to a SLB when an external field is applied for 10 minutes. The plot is approximately quadratic implying that the qdot is exhibiting ballistic or directed motion. This is consistent with the qdot being coupled to the flow field. The corresponding particle trajectory is shown in Figure 39. Physically, K2 is proportional to the
square of the particle velocity, \( K_1 \) is proportional to the diffusion coefficient (this is explicitly set to zero because ballistic motion is the dominant term), and \( K_0 \) represents the localization uncertainty (88, 115).

Figure 52: Graph of MSD vs time for qdot particle 3 undergoing directed motion.

2.3.2.1.3 MSD Plot of Qdot Tethered to a SLB after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 20 minutes

Figure 53 is a representative MSD plot of a qdot tethered to a SLB when an external field is applied for 20 minutes. The plot is approximately quadratic implying that the qdot is exhibiting
ballistic or directed motion. The corresponding particle trajectory is shown in Figure 40. Note the K2 coefficient which is equal to $v^2$ is increasing meaning the particle velocity has increased the longer the field is applied. (These are not the same particles but are representative of their group).

Figure 53: Graph of MSD vs time for qdot particle 4 undergoing directed motion.

2.3.2.1.4 MSD Plot of Qdot Tethered to a SLB after application of the external field ($3.4 \times 10^2 \pm 0.3$ V/m) for 30 minutes

Figure 54 is a representative MSD plot of a qdot tethered to a SLB when an external field is applied for 30 minutes. The plot is approximately quadratic implying that the qdot is exhibiting...
ballistic or directed motion. The corresponding particle trajectory is shown in Figure 41. Note the K2 coefficient which is equal to $v^2$ is no longer increasing meaning the particle velocity appears to have leveled out. (These are not the same particles but are representative of their group).

Figure 54: Graph of MSD vs time for qdot particle 5 undergoing directed motion.

\[ \text{MSD} = K_0 + K_1t + K_2t^2 \]

- $K_0 = 13.65 \pm 1.17$
- $K_1 = 0 \pm 0$
- $K_2 = 14.189 \pm 0.0716$
2.3.2.2 Fluorescent Beads

2.3.2.2.1 Beads on cover glass (no SLB) just after application of the external field (3.4x10² ± 0.3 V/m)

Figure 55 is a representative MSD plot of a bead just after an external field is applied to the system. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 42.

![Graph of MSD vs time for bead 1 undergoing directed motion.](image)

\[ \text{MSD} = K_0 + K_1t + K_2t^2 \]

- \( K_0 = -0.6 \pm 0.2 \)
- \( K_1 = 0 \pm 0 \)
- \( K_2 = 25.6 \pm 0.1 \)
2.3.2.2.2 Beads on cover glass (no SLB) after application of the external field (3.4x10^2 ± 0.3 V/m) for 10 minutes

Figure 56 is a representative MSD plot of a bead after an external field is applied to the system for 10 minutes. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. The corresponding particle trajectory is shown in Figure 43.

\[ \text{MSD} = K_0 + K_1 t + K_2 t^2 \]

- \( K_0 = -0.6 ± 0.3 \)
- \( K_1 = 0 ± 0 \)
- \( K_2 = 14.5 ± 0.1 \)

Figure 56: Graph of MSD vs time for bead 2 undergoing directed motion.
2.3.2.2.3 Beads on cover glass (no SLB) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 20 minutes

Figure 57 is a representative MSD plot of a bead after an external field is applied to the system for 20 minutes. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 44.

Figure 57: Graph of MSD vs time for bead 3 undergoing directed motion.

\[
\text{MSD} = K_0 + K_1 t + K_2 t^2
\]

- \(K_0 = 1.2 \pm 0.2\)
- \(K_1 = 0 \pm 0\)
- \(K_2 = 4.6 \pm 0.1\)
2.3.2.2.4 Beads on cover glass (no SLB) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 30 minutes

Figure 58 is a representative MSD plot of a bead after an external field is applied to the system for 30 minutes. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 45.

\[
\text{MSD} = K_0 + K_1 t + K_2 t^2
\]

\[
\begin{align*}
K_0 &= 0.5 \pm 0.1 \\
K_1 &= 0 \pm 0 \\
K_2 &= 3.0 \pm 0.1
\end{align*}
\]

Figure 58: Graph of MSD vs time for bead 4 undergoing directed motion.
2.3.2.2.5 Beads on a SLB (no tethering system) just after application of the external field (3.4x10^2 ± 0.3 V/m)

Figure 59 is a representative MSD plot of a bead on a SLB just after an external field is applied to the system. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 46.

Figure 59: Graph of MSD vs time for bead 5 undergoing directed motion.

\[
\text{MSD} = K_0 + K_1t + K_2t^2
\]

| \(K_0\)  | =1.8 ± 0.1 |
| \(K_1\)  | =0 ± 0    |
| \(K_2\)  | =4.0 ± 0.1|
2.3.2.2.6 Beads on a SLB (no tethering system) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 10 minutes

Figure 60 is a representative MSD plot of a bead on a SLB after an external field is applied to the system for 10 minutes. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 47.

![Graph of MSD vs time for bead 6 undergoing directed motion.](image)

\[
\text{MSD} = K_0 + K_1t + K_2t^2
\]

- \(K_0 = 0.67 \pm 0.2\)
- \(K_1 = 0 \pm 0\)
- \(K_2 = 2.8 \pm 0.1\)
2.3.2.2.7 Beads on a SLB (no tethering system) after application of the external field \(3.4 \times 10^2 \pm 0.3\) V/m for 17 minutes

Figure 61 is a representative MSD plot of a bead on a SLB after an external field is applied to the system for 17 minutes. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 48.

\[
\text{MSD} = K_0 + K_1 t + K_2 t^2
\]

\[
K_0 = 1.5 \pm 0.2
\]

\[
K_1 = 0 \pm 0
\]

\[
K_2 = 4.1 \pm 0.1
\]

Figure 61: Graph of MSD vs time for bead 7 undergoing directed motion.
2.3.2.2.8 Beads on a SLB (no tethering system) after application of the external field \((3.4 \times 10^2 \pm 0.3 \text{ V/m})\) for 25 minutes

Figure 62 is a representative MSD plot of a bead on a SLB after an external field is applied to the system for 25 minutes. The plot is approximately quadratic implying that the bead is exhibiting ballistic or directed motion. This is consistent with the bead being coupled to the flow field. The corresponding particle trajectory is shown in Figure 49.

![Graph of MSD vs time for bead 8 undergoing directed motion.](image)

\[
\text{MSD} = K_0 + K_1 t + K_2 t^2
\]

\[
K_0 = 2.0 \pm 0.2
\]

\[
K_1 = 0 \pm 0
\]

\[
K_2 = 3.6 \pm 0.1
\]
2.3.3 Diffusion Coefficient

The mean diffusion coefficient for qdots tethered to the SLB with no external field is 0.49 ± 0.1 µm\(^2\)/s. The diffusion coefficient for a planar membrane made up purely of POPC determined by FRAP measurements is 6.1 µm\(^2\)/s (89). This is consistent with SPT measurements for diffusion coefficients being 2-4 times smaller than FRAP (116). The potential reason for the discrepancy between the different measurements is the fact that FRAP measures the average diffusion coefficient of the ensemble, whereas SPT measure the average diffusion coefficient of a single particle (87).

2.3.4 Velocity Distribution

Figure 63 is a graph of the velocity distribution of all beads on a SLB in the presence of the field. For the case of beads on a SLB the bead velocity seems to be independent of the time the field is applied. A graph of velocity versus time field is applied as shown in Figure 65. The distribution is approximately normal and Gaussian fitting was applied. The spread is likely a convolution of the spread due to the finite number of measurement, as well as, experimental variations (88).
Figure 63: Histogram of drift velocities from beads on a SLB

Figure 64 shows the bead (on a SLB) velocity distribution fitted to a Gaussian curve. The mean is 1.7 ± 0.1 μm/s with the width being 0.27 ± 0.15 μm/s. This type of fitting allows us to determine the expected values of the mean and standard deviation (117).
Figure 64: Histogram of bead drift velocities on a SLB fitted to a Gaussian

Figure 65 shows a graph of the bead velocities versus time of applied field. Beads are immobile before field is applied. As discussed above the bead velocities in the bilayer scenario are approximately independent of the time of applied field. However, the beads in the no bilayer scenario appear to vary with the length of time the field is applied.
Figure 65: Graph of bead drift velocities versus the time the electric field was applied

Based on this graph the actual EOF velocity on a similarly sized particle would be \(~3.5 \mu m/s\) on etched cover glass and \(~1.8 \mu m/s\) in the presence of a bilayer. From the graph two things are apparent: Firstly, actual drift velocities of object in the electro osmotic flow are much lower than the theoretically calculated one as calculated in 2.1.1.1. Since the beads are essentially like ships on a rapid flowing river, they should have the same velocity as the flow itself (i.e. the relative velocity between beads and water flow should be zero after some initial acceleration.) So that
means that actual electro-osmotic flow close to the membrane (where the observation is made) is much slower than the theoretical calculation would predict. However, we know if we assume no-slip boundary conditions, that any flow has a parabolic velocity profile, starting with zero velocity right at the boundary. It stands to reason that due to the proximity of the beads to the bilayer (otherwise we would not be able to optically observe them) they are located in a part of the flow that is retarded. This is in addition to the state assumptions, which might be oversimplifications of the actual charge and flow scenario.

The other interesting insight presented in the Figure 65 is that the presence of the bilayer has an obvious effect on the electro osmotic drift velocity, since the bilayer itself is rather thin (~10 nm) it stands to reason that its presence does indeed modulate the surface charge and thus the effective zeta potential at the membrane surface.

Figure 66 is a graph of the qdot velocity versus time of applied field. The qdot velocity increases with the length the field is applied and then appears to level out at ~ 3.0 µm/s. This is consistent with the time delay between the field being turned on and qdots coupling with the flow observed experimentally. This is behavior that is clearly unique to the qdots and not observed in either bead scenario.
Figure 66: Graph of qdot velocities versus time field is applied. Average diffusion coefficient for the no field condition is shown on the left axis.

Streptavidin has 4 binding sites and may introduce artificial cross linking to multiple biotinylated lipids which could slow diffusion and drift rates. Other experimental sources of error are mechanical stability of the apparatus, ability of the particle tracking algorithm to accurately track vesicles with a changing intensity profile due to the blinking of the qdots. Multiple tethering can also occur and would reduce the diffusion coefficient. Video microscopy of diffusing qdots shows that some vesicles appear to become temporarily immobilized. Yoshina-Ishii et al observed
similar results in their work with tethered vesicles (87). They showed that so-called sticky defects, where defects in the supported bilayer act as weak binding sites, would not be reflected in the MSD plot (87).

Figure 67 shows the velocity distribution of qdots after an external field has been applied for at least 10 minutes and no longer than 30 minutes. The histogram shows that the distribution is approximately normal. The spread is likely a convolution of the spread due to the finite number of measurement, as well as, experimental variations (88).

Figure 67: Histogram of qdot drift velocities

Overall the project was successful in that the qdots were successfully tethered to the SLB and were able to exhibit free diffusion in the absence of a field. Further, in the presence of an external field, the qdots coupled into the flow created by EOF and have the potential to be
directed and further controlled into complex assemblies. Additionally, the behavior of the qdots in the system were unique in that there was a time delay between the time the field was applied and when the qdots exhibit directed motion. Further, there was a time dependence of the velocity after the qdots had coupled with the flow. This behavior is distinctly different than freely floating beads that were introduced to the system to map the flow created by EOF. All the data suggests that the flow starts as soon as the field is turned on.
3. CNT Project (CNT Organization)

3.1 Concept

This is a natural extension of the Qdot project. Qdots are the simplest nanomaterial to use for a proof-of-concept, being that they are engineered to be fluorescent and can be purchased already bio-functionalized but their applications compared to CNTs are limited. A viable way to bio-functionalize CNTs has recently been opened with the discovery of short peptide sequences that specifically bind to single walled CNTs. Using in vitro selection via phage display technique, Wang et al. found that the sequence WPHHPHAAHTIR is specific to CNTs even compared to graphite surfaces (118). Using a similar approach, another peptide sequence (LLADTTHHRPWT) has been determined more recently that shows an even higher binding affinity to single walled carbon nanotube (119, 120). The ability to utilize peptides to bind to CNTs, opens up the expansive toolbox of protein engineering to functionalize lipid membranes with CNTs.

With the discovery of a peptide sequence that selectively binds to CNT’s a new world of possibilities opens for bio-functionalizing CNTS. Peptides can be engineered to include a histidine tag very easily. This histidine tag can be used to tether the His-tag to the bilayer. This functionally attaches the CNT to the bilayer, thus confining the CNT to move in 2-dimensions as well as imparting the fluidity of the bilayer to the CNT. In this case, the bilayer acts as a 2-dimensional fluid scaffolding for the movement of the CNTs and the peptide with the His-tag acts as the glue. Unlike qdots, CNTs are not luminescent and therefore must be explicitly labeled to view and track them. This is also accomplished with the selective peptide sequence. A cysteine is included
in the sequence to allow for a fluorophore attachment. A diagram of the peptide sequence bound to a CNT is shown in Figure 68.

Figure 68: Diagram of peptide bound to CNT. In the upper right the schematic of a carbon nanotube is depicted. In the upper left such a nanotube with a bound peptide is shown. The amino acid sequence of the nanotube binding peptide with its fluorophore attachment

Combining the results of the work on the CNT specific peptide binding motif and the ability to attach peptides to membrane surfaces, we can study the behavior of membrane bound CNTs that are laterally mobile. A diagram of the proposed experimental set up is shown in Figure 69.
Figure 69: CNT bound to bilayer

3.2 Challenges

3.2.1 Solubility

CNTs are extremely hydrophobic and tend to aggregate in water. However, in order to properly bio-functionalize and integrate the CNTs into the bilayer complex they must first be solubilized. This is done accomplished using detergents. Much like lipids, they are amphipathic molecules with a polar head group which is attracted to water and a hydrophobic chain. When detergents are dispersed in aqueous solutions they spontaneously form spherical micelles (121). The CNTs are soluble once the detergents start to coat the CNT. The polar head groups face the aqueous
solution while the hydrophobic chains face the CNT as shown in Figure 70 below.

Figure 70: CNT coated with detergents. Polar head groups are depicted in red (122).

3.3 Materials and Methods

3.3.1 Materials

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid)succiny] (nickel salt) (DGS-NTA(Ni)) were obtained from Avanti Polar Lipids (Alabaster, AL). All lipids were dissolved in chloroform and stored at -20°C. β-BODIPY® FL C12-HPC (2-(4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Dodecanoyl)-1-Hexadecanoyl-sn-Glycero-3-Phosphocholine) was obtained from Life Technologies (Grand Island, NY). Single walled carbon nanotubes were obtained from Nano-lab (Waltham, MA). Solubilizing agent Nanosperse Aq was also obtained from Nano-lab (Waltham, MA). All HPLC grade organic solvents were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. Bovine Serum Albumin (BSA) were also purchased from Sigma-Aldrich (St. Louis, MO). All aqueous solutions (buffers etc.) were prepared using Millipore water (double-deionized, organic-free) of high specific resistivity (approx. 18.0 mΩ·cm).
3.3.2 Solubilizing CNTs

CNTs were first solubilized per manufacturer’s instructions. Briefly, 8 drops of nanosphere AQ was gently mixed into 10 mL Millipore water. Next 10 mg of CNT powder was carefully stirred and then sonicated using a tip sonicator for 20 minutes at 10% amplification. However, this was not sufficient for a monodispersed CNT suspension. The solution had visible CNT aggregates.

A larger amount of nanosphere AQ was used in order to create a monodisperse solution. However, this led to an abundance of detergent in the solution, so much so, that the CNTs were masked by the detergent in SEM images. One such image is shown in Figure 71. The black spots in the image are detergent. We used 25 kDa dialysis tubes to remove the excess detergent. The CNTs were then ready for fluorophore labeling and binding to the bilayer.

Figure 71: SEM image of dried CNT/detergent/millipore water solution. Dark grey portions of the image show the detergent. CNTs are barely visible within the detergent.
3.3.3 Characterization of CNT suspension

In order to confirm that the suspensions of CNTs were monodisperse we employed scanning electron microscopy (SEM). Briefly, 10 µL droplets of the CNT suspension were allowed to dry overnight on a silicon wafer. After samples were completely dried microscopy was performed on a JEOL JSM-6060.

3.3.4 CNT Binding Peptides

Two kinds of peptides based on the LLADTTHHRPWT binding motif by Su were custom synthesized by GL Biochem (Boston, MA) (119). The shorter one has the sequence CGGGGLLADTTHHRPWT, i.e. a N-terminal cysteine for fluorescent labeling with thiol-reactive maleimides derivatives of the series of Alexa fluorophores (Invitrogen, Carlsbad, CA) is connected to the binding motif via a poly-glycine linker. Also, a longer version with the sequence HHHHHHHHHHHC GGGLLADTTHHRPWT was contracted. It is identical to the short binding peptide, save the His\textsubscript{10} tag with which it can bind to Ni\textsuperscript{2+} containing entities.

3.3.5 Small Unilamellar Vesicle Preparation

SUVs were prepared as previous described in 2.2.2 Small Unilamellar Vesicle Preparation. Briefly, appropriate amounts of POPC, BODIPY and DGS-NTA(Ni) were added and agitated for approximately 30 seconds to ensure thorough mixing. The chloroform is evaporated off and the lipid film is resuspended in Millipore water to a final concentration of 2 mg/ml. The lipid solution is then extruded with a high-pressure extruder (LIPEX\textsuperscript{TM} extruder, Northern Lipids Inc., Burnaby,
BC, Canada) through a polycarbonate filters with pores of 100 nm diameter. The resulting SUV solution was stored at 4°C for no longer than 3 days but most often used immediately.

3.3.6 Formation of Supported Lipid Bilayer

The formation of a supported lipid bilayer was performed as previous described in 2.2.3 Formation of Supported Lipid Bilayer. Briefly, the SUV solution is diluted 1:1 in PBS solution at pH 7.4. A 90 µL droplet is placed on a sterile petri dish and a clean glass coverslip is dropped on top. The coverslip is thoroughly rinsed in PBS and assembled into the holder. A FRAP experiment is performed to ensure the integrity of the bilayer.

3.4 Results

3.4.1 CNT suspension

SEM images show dried H₂O/detergent/CNT solution. The solubilization procedure leads to individual and separated carbon nanotubes. This is shown in Figure 72.

Figure 72: SEM image of dried monodisperse SWCNTs suspended in millipore water
3.4.1 CNT-SLB Complex

The images show in Figure 73, show the successful translation and rotation of a fluorescently labeled carbon nanotube. Since the tube does not move out of focus during the time for observation (~30 s) it can be safely assumed that it attached successfully to the fluid lipid membrane and freely diffuses throughout the bilayer.

Figure 73: Fluorescent image of labeled CNT diffusing on a SLB. Images are 10 seconds apart.
4. Summary & Outlook

At this point we provide proof-of-concept experiments that demonstrate that fluid lipid bilayers can be successfully used to mobilize nano materials such as quantum dots and CNTs. Further we demonstrated that qdots can be coupled to EOF and have the potential to be controlled. Others have demonstrated that lipid bilayers and EOF can be used to move proteins around and man-made objects have been used for the labeling of bio-molecules, but this is the first time that EOF has been used to manipulate man-made nano-objects.

4.1 Natural Extensions

The most obvious extension would be to investigate the possibility of separation of qdots based on their size. In addition, after having determined the basic behavior of qdots under external forcing, more complex flow fields that can vary in time and space will be used to study how membrane bound qdots can be manipulated to arrange into structures of higher geometric complexity. More complex flow patterns could be investigated using four electrodes to achieve a two-dimensional flow pattern. If the fields are turned on at different times how would that affect the system? Lastly, the CNT-SLB complex needs to be further characterized. The diffusion coefficient of CNTs diffusing on a SLB needs to be determined and compared to the results from the qdot project.

4.2 Future Work

In order to direct lasting assemblies of nanomaterials into larger structures, they need to be interconnected. To create well defined geometric assemblies of CNTs, qdots or both, location
specific conjugation sites are an important prerequisite. Many possible avenues seem possible. For example, exploitation of light activated chemistry to direct ligation of DNA based CNT-CNT connections or the use of frayed DNA structures that would allow the attachment of 3 CNTs via their endpoints, thus allowing for more complex 2D structures. Also, questions regarding the geometric constraints of membrane bound CNTs (for example by binding them to large vesicles) are attractive future directions. In summary, it appears the directions and possibilities of the general concept of membrane attached nanomaterials are seemingly endless.
5. References


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• Research and estimate project budgets.
• Collaborate with legal team on IP research.
• Develop methods, create work instructions, and training modules.
• Prepare presentations and reports for internal and third party clients and investors.
• Collaborate with QA team to write SOPs in compliance with cGLP standards.

BIOMATERIALS RESEARCH EXPERIENCE
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Researcher

Two-Dimensional Fluidization of Quantum Dots by Biomimetic Membranes

• Prepare vesicle solutions from stock using roto-vap and extrusion.
• Create lipid bilayers by vesicle fusion method.
• Characterize the membrane dynamics using FRAP.
• Create model system by tethering quantum dots to membrane by biotin-streptavidin bond.
• Collaborate with machine shop to design and build a flow chamber using SketchUp.
• Employ wide field fluorescent microscopy to monitor dynamics of the q-dot-membrane system.
• Induce electro-osmotic flow in the model system and monitor the dynamics with fluorescence microscopy.
• Analyze images and track q-dot trajectories using single particle tracking software.
• Write Matlab code to analyze trajectory data.
• Design and implement proper control experiments to further test findings.
• Create graphs, tables, figures and videos to display results.
• Manage inventory and prepare order forms.

**Two-Dimensional Fluidization of Single Walled Carbon Nanotubes by Biomimetic Membranes**

• Solubilized SWCNT in water using sonication.
• Characterized SWCNT-water system using SEM and AFM microscopy.
• Purified fluorescently labeled peptides using spin columns.
• Fluorescently labeled SWCNT using specific binding peptides.
• Purified labeled SWCNT using dialysis.
• Tethered SWCNT to membrane using Ni-histidine interactions.
• Used wide field fluorescent microscopy to observe SWCNT-membrane dynamics.

**Crystallization of SWCNT-detergent networks and SWCNT-peptide networks**

• Formed SWCNT-detergent and SWCNT-peptide crystals.
• Characterized crystals using SEM and AFM microscopy techniques.

**MATERIALS SCIENCE RESEARCH EXPERIENCE**
Binghamton University, College of Arts and Sciences, Binghamton, NY

**Research Assistant** 2008-2009

**Characterization of Zinc Oxide Thin Films for Use in Transparent Thin Film Transistors**

• Created thin zinc oxide and multilayered films using sputtering techniques.
• Characterized surface topology using SEM and AFM microscopy techniques.
• Characterized crystal spacing and chemical composition using XRD and ellipsometry.
• Collaborated with shop to create an ‘H’-shaped film to enable electrical testing.
• Wrote formal operation procedures for XRD.

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**Instructor- Experimental Physics I&II** Fall 2015

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Teaching Assistant- Experimental Physics I&II (8 sections) 2009-2015
- Collaborated on curriculum and grading criteria
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CUWiP 2015- Graduate Program Recruiter for SU Physics
CUWiP 2014- Graduate Program Recruiter for SU Physics
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WISE Associate
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