Motion Analysis of Biotin Carboxylase-Driven Robotic Nano-swimmers

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MOTION ANALYSIS OF BIOTIN CARBOXYLASE-DRIVEN ROBOTIC NANO-SWIMMERS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
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ABSTRACT

The purpose of this study is to experimentally validate the hypothesis that the enzyme biotin carboxylase (BC) can behave as a bimolecular motor and propel nano-scale particles in fluidic environments. This hypothesis was proposed in the M.S. thesis “Toward the Development of Biotin Carboxylase Driven Robotic Nano-swimmer” by Rachel Yates (Yates, 2012). In (Yates, 2012), the nano-particle (called a *nano-swimmer*) is a Janus particle with BC molecules attached to one hemisphere. The idea is that in the presence of its substrates (i.e., fuel) BC will cause individual nano-particles to undergo non-Brownian motion by virtue of its conformational change.

In order to validate the stated hypothesis, the first step of the present study was to reproduce the nano-swimmer fabrication process introduced in (Yates, 2012) and make necessary improvements. The second step was to analyze the motion of the nano-swimmer in comparison with the control experiments. This step involved developing an experimental setup for tracking individual nano-particles and recording their motion, followed by analyzing the motion of the particles to determine if they are Brownian or not.

Translational and directional diffusion methods were used to analyze the motion of the controls and nano-swimmer. In the translational diffusion method, the experimental mean square displacement versus time data was plotted. The slope of the linear fit of the plot for the nano-swimmers was approximately 1.5, which is an indication of non-Brownian motion. The slope for controls was approximately 1, which is expected for Brownian motion. In the directional diffusion method, the histogram of the directional diffusion angle was generated for both nano-swimmer and controls. The histograms showed that the nano-swimmers were more likely to have small directional diffusion (i.e., difference between the current and previous heading angles was
small) when compared to controls. The overall results of both analyses provide strong indications that the nano-swimmers have non-Brownian motion.
CHAPTER 1 INTRODUCTION

1.1 Motivation

The development of nano-machines has become an important topic of research in recent years because they can be used in broad areas of science and technology. The term “nano-machine” refers to the integration of biological molecules with chemical, mechanical, electrical, magnetic, and/or optical components to form a nano-scale system that converts chemical energy to mechanical work and then harnesses this mechanical work for a particular purpose (Yates, 2012). For instance, nano-machines can potentially be used in drug delivery systems for cancer treatment. In particular, they can transport the drug to defeat a tumor in a specific location in the body without harming other parts of the body and with fewer side effects (Ghalanbor, Marashi, & Rangbar, 2005). Cleaning out clogged arteries, destroying toxic pollutants, and acting as ultra-sensitive disease markers are some other potential applications for nano-machines (Wang, 2009).

“The development of this technology would enable a new generation of devices with several advantages over micro-scale systems in terms of performance, size, power consumption, efficiency, and ease of fabrication” (Yates, 2012).

Biomolecular motors are the key component of nano-machines since they serve as actuators, converting chemical energy to mechanical work. In nature, many living organisms use molecular motors for functions such as cell division and cell shape maintenance (Keller, 2000). The discovery and engineering of biomolecular motors is of great interest to biotechnology researches (Hiratsuka, 2006). A literature review of biomolecular motors is provided in Section 1.4.
1.2 Statement of Work and Thesis Organization

The goal of this study is to test the hypothesis that a nano-scale, Janus-like particle that uses the enzyme biotin carboxylase (BC) as biomolecular motor will undergo non-Brownian motion in a fluidic environment. The biomolecular machine, which is referred to as a nano-swimmer (Yates, 2012), is schematically illustrated in Figure 1-1. The idea is that the nano-swimmer will be propelled in the direction perpendicular to the plane of its hemisphere by the conformational change of the BC molecules. BC is a nano-scale enzyme involved in the metabolism of carbohydrates, fatty acids, and amino acids in all organisms such as animals, plants, fungi, and bacteria (Tong, 2013).

This work involved the following three steps:

1. Fabrication of the nano-swimmers according to the method proposed in (Yates, 2012).
2. Development of an experimental set up and protocol for testing the hypothesis.
3. Analyses of the experimental data to this discern if the motion is Brownian or non-Brownian.

Figure 1-1. Schematic depiction of the nano-swimmer (Yates, 2012).
The thesis is organized as follows. The reminder of Chapter 1 contains background information about the enzyme BC and the theory of Brownian motion, followed by a literature review of biomolecular motors.

Chapter 1 contains background information about the enzyme BC and the theory of Brownian motion, followed by a literature review of biomolecular motors. Chapter 2 explains the nano-swimmer fabrication process. Chapter 3 describes the nano-swimmer experimental set up and presents the results of the motion analysis. Finally, conclusions and suggestions for future works are provided in Chapter 4.

1.3 Background Information

1.3.1 Biotin Carboxylase

Biotin Carboxylase (BC) is one of the components of Acetyl CoA Carboxylase (ACC). ACC is highly involved in fatty acid synthesis, and can be found in animals, plants, bacteria, fungi, etc. ACC utilizes the reaction in two steps:

\[
\begin{align*}
\text{BC} & \quad \text{enzyme-} \text{biotin} + \text{Mg-ATP} + \text{HCO}_3^- & \leftrightarrow & \text{enzyme-} \text{biotin-} \text{Co}_2^+ + \text{MgADP} + \text{Pi} \\
\text{enzyme-} \text{biotin-} \text{Co}_2^+ + \text{acetyl-coA} & \leftrightarrow & \text{malonyl-CoA} + \text{enzyme-} \text{biotin}
\end{align*}
\]

Reaction (1), which is catalyzed by BC, is ATP dependent carboxylation of the vitamin biotin. BC substrates are bicarbonate, ATP, and biotin. In reaction (2) the carboxyl group is transferred to acetyl coenzyme A (acetyl-CoA) to make malonyl-CoA (Broussard, 2013) (Blanchard, Lee, Frantom, & Waldrop, 1999). BC utilizes three substrates: ATP, biotin, and bicarbonate.

Availability of the structural information of *Escherichia coli* (E-coli) makes it an ideal model to demonstrate the 3 dimensional (3D) structure of ACC (Cronan & Waldrop, 2002). In E-
coli, ACC is made of three polypeptide chains which are referred to as Biotin Carboxylase (BC), Biotin Carboxyl Carrier Protein (BCCP) and Carboxyltransferase (CT) (Thoden, 2000). When isolated from other components, BC from E-coli can retain its enzymatic activity. This characteristic of BC makes it a great model for mechanistic studies. BC can also use free biotin as substrate (Broussard, 2013). The crystallographic of BC confirmed that BC is a homodimer which means it consists of two identical subunits; see Figure 1-2 (a). Each subunit (monomer) of BC is approximately 6.7 nm × 5.2 nm × 4.8 nm and is consisted of 3 domains: A, B and C as shown in Figure 1-2 (b). (Broussard, 2013) (Thoden, 2000) (Thoden J. B., et al., 1969) (Yates, 2012).

![Figure 1-2. BC structure: (a) dimer and (b) detailed view of monomer (Yates, 2012).](image)

One of the characteristics of BC which makes it a candidate to serve as biomolecular motor is that when ATP binds with BC, it goes through a large conformational change, which means a 45° hinge-like rotation of one domain (B domain) relative to other domains (A and C domains) with frequency of 2 Hz (Thoden, Blanchard, Holden, & Waldrop, 2000) (Yates, 2012).

Other than large conformational change of BC, there are some other characteristics that make BC a proper candidate for biomolecular machine fabrication. Gene coding and overexpression of BC enzyme has been done before which means BC can be produced in large quantities
(Broussard, 2013) (Blanchard, Lee, Frantom, & Waldrop, 1999). Previously, to separate the wild type of BC from the mutant form, Blanchard et al., used poly-histidine tag (Blanchard, Lee, Frantom, & Waldrop, 1999). Poly-histidine tag consists of at least six histidine residues fused to N- or C-terminal ends of the enzyme BC (Hayworth). Histidine tag can interact with nickel. Therefore, the histidine tag connects the BC to the nickel. Histidine tagged wild type BC has the potential to be used as the motor for the nano-swimmer which consists of a Janus particle half-coated with nickel with BC enzyme attached to the nickel.

1.3.2 The Theory of Brownian Motion

Brownian motion is the name given to the random movement of small objects in a fluidic environment. It is caused when molecules of water (or any other solution) randomly collide with the objects (Radenovic). The discovery of this phenomenon is credited to Robert Brown as a result of his observations of how pollen behaves in water in 1827 (Powles, 1827). In 1905 Albert Einstein quantitatively analyzed the Brownian motion phenomenon (Einstein, 1905). Einstein proved that the mean square displacement (MSD) of a spherical particle is proportional to time (with the proportionality constant being related to the diffusion coefficient) when Brownian motion is present (Gora, 2006).

A conceptual explanation of Brownian motion can be found in (Bhalerao, 2004). There are a number of important characteristics in Brownian motion (Perrin & Fredrick, 1910):

1. The motion is rotational and irregular.
2. Particles move independently.
3. As the particle size decreases, the motion becomes more active.
4. Density and composition of the particles have no effect on the motion.
5. As the viscosity of the fluid decreases, the motion becomes more active.
6. As temperature increases, motion becomes more active.

7. The motion never stops.

In this thesis, two different methods used to analyze Brownian motion from experimental data. These methods are introduced next.

**Method One: Translational Diffusion**

This method is based directly on Einstein’s theory (Einstein, 1905). Let the discretized, two dimensional (2D) position of particle \(i\) at time \(t\) be given by

\[
q_i(t = k\Delta t) = \begin{bmatrix} x_i(t) \\ y_i(t) \end{bmatrix}
\]

where \(k = 0, 1, \ldots\) are the time samples and \(\Delta t\) is the sampling period. The particle displacement relative to its position at some initial time \(t_0\) is given by

\[
\Delta q_i(t = k\Delta t) = q_i(t_0 + t) - q_i(t_0), \quad k = 1, 2, \ldots
\]

Consider that \(N\) spherical particles are being tracked. The MSD of all particles is defined as

\[
\overline{|\Delta q(t)|^2} = \frac{1}{N} \sum_{i=1}^{N} |\Delta q_i(t)|^2
\]

where \(|\cdot|\) is the Euclidean norm. Einstein showed that if the 2D motion of particles is Brownian, then the following relationship holds (Einstein, 1905)

\[
|\Delta q(t)|^2 = \left(\frac{2kT}{3\pi\mu r}\right) t = 4Dt
\]

where \(K\) is the Boltzmann’s constant, \(T\) is the absolute temperature of the fluid, \(\mu\) is the viscosity of the liquid, \(r\) is the particle radius, and \(D = \frac{kT}{6\pi\mu r}\) is the translational diffusion coefficient for a
spherical particle. That is, a plot of MSD calculated from measured data, versus time is \textit{linear} if the motion is Brownian.

**Method Two: Directional Diffusion**

In this method, the 2D \textit{directional} diffusion of the particle motion is analyzed rather than its translation. To this end, let the displacement of particle $i$ between two consecutive time samples be defined as

$$
\Delta q_i(k) = \begin{bmatrix}
\Delta x_i(k)
\Delta y_i(k)
\end{bmatrix} = \begin{bmatrix}
x_i(k\Delta t) - x_i((k - 1)\Delta t)
y_i(k\Delta t) - y_i((k - 1)\Delta t)
\end{bmatrix}
$$

for $k = 1, 2, \ldots$ and $i = 1, 2, \ldots, N$. Figure 1-2 illustrates the displacement vector in Equation 1-6 along with two angles related to the change in the particle’s direction at each time sample. The angle $\theta_i(k)$ is measured relative to the positive x-axis and is defined by

$$
\theta_i(k) = \text{atan}^{-1}(\Delta y_i(k), \Delta x_i(k))
$$

The angle $\beta_i(k)$ is measured relative to the directional axis at $t = k\Delta t$ (i.e., the direction of the displacement vector $\Delta q_i(k)$) and is defined as follows

$$
\beta_i(k) = \theta_i(k + 1) - \theta_i(k).
$$

When $\beta_i(k) = 0$, the particle does not change direction. When $\beta_i(k) > 0$ ($< 0$, resp.), the particle moves to the left (right, resp.) of the directional axis. When $\beta_i(k) = \pm \pi$, the particle moves backwards along the directional axis. Thus, the angle should be in the interval (-$\pi$, $\pi$]. To this end, the following adjustment was made to the values calculated from Equation 1-7

- If $\beta_i(k) > \pi$ then $\beta_i(k) = \beta_i(k) - 2\pi$
- If $\beta_i(k) \leq -\pi$ then $\beta_i(k) = \beta_i(k) + 2\pi$

7
Otherwise, leave angle value as is.

In this method, the directional angle $\beta_i(k)$ for each particle $i = 1, \ldots, N$ are calculated for $k = 1, 2, \ldots, k_{\text{max}}$ and a histogram of all the $Nk_{\text{max}}$ angle values is created. If the angle distribution is approximately flat, showing no directional preference, the motion is Brownian. If the distribution shows preference for small angles, the motion is non-Brownian.

Figure 1-2. Displacement vectors and directional angles. Blue dot indicates the particles position at different time samples.

1.4 Literature Review

1.4.1 Janus Particles

P. G. de Gennes, winner of the Nobel Prize in 1991, was the first to use the name “Janus”, meaning two back-to-back heads in Roman gods (Figure 1-3). This terminology gradually became more common in colloidal particle research. Specifically, Janus is used to describe particles whose hemisphere surfaces are chemically different (Perro, et al., 2005). That
is, a Janus particle is anisotropic in shape and surface chemistry (Hu & al., 2012). These particles are commonly composed of silica particles with different types of metals deposited on either or both hemispheres (Ye, 2011). These particles have been used in different fields such as biomedical, magnetics, drug delivery systems, and microfluidic systems (Yang, 2012) (Ye & Carroll, 2010).

Figure 1-3. Janus god and Janus Particle (Perro, 2005).

To create Janus particles, the particles can be half-coated either with one type of metal or they can be coated in with two different types of metals on each halve. Some of the more common metals used for coating the Janus particles are silver, gold, titanium, nickel, cobalt, and aluminum. Several researchers have fabricated Janus particles. For example, (Ye & Carroll, 2010) and (Ye, 2011) manufactured bimetallic Janus particles. They used silica particles, 4 μm in diameter, coated with gold and platinum. They also successfully coated 2 μm silica particles with gold and silver as well as 4 μm silica particles coated with gold and nickel. In (Howse, 2007) and (Brown & Wilson, 2014), polyesterin particles were half-coated with platinum.
1.4.2 Biomolecular Motors

Different types of proteins such as ATP synthase, myosin, kinesin, and dynin have been widely used as bimolecular motors to produce motion (Yates, 2012). ATP synthase is a ubiquitous protein machine which produces energy in all living cells. ATP synthase is composed of two sectors: F₀ and F₁. These sectors act as rotary stepper motors (Borsch, 2013) (Lu, Lill, & Bald, 2014). These bimolecular motors have been into the interest not only due to their micro/nano-scale size, but also because of their efficiency in converting the chemical energy to mechanical motion (Behkam & Metin, 2007). The high energy is created due to the hydrolyzing of the phosphate bond in ATP, which yields ADP and phosphate (Schmidt & Montemagno, 2004). Ricky Soong and Carlo D. Montemagno have successfully fabricated an organic-inorganic nano-machine which is powered by F₁-ATP synthase (Soong & Montemagno, 2005). Other protein motors such as myosin, kinesin and dynein are also commonly used as nanomotors. Conventional kinesin and muscle myosin are often used in nano-machine fabrication due to their ability of generating force (Agarwal & Hess, 2010). Another interesting bimolecular motor is bacteria flagella. Different types of bacteria use flagella to swim. Flagella can function as a rotary machine which makes it a proper candidate as bimolecular motors (Terashima & al., 2006). In their study Bahareh Behkam and Metin Sittia, used flagella motors inside bacteria named Serratia marcescens as their swimming robot (Behkam & Metin, 2007).

An alternative to biomolecular motors is chemical motors which can be used in nano-machine fabrication. Chemical motors generate force based on chemical reactions to propel small devices. In other words, chemical motors convert chemical energy to useful mechanical work. One of the common examples is the use of half-coated particles with platinum in hydrogen peroxide solution (Howse, 2007). Walter F. Paxton, et al. used 1 micrometer long, 370 nm in
diameter, gold and platinum rad shape particles. The particles move in anon-Brownian manner in 2% and 3% hydrogen peroxide solution by catalyzing the formation of $O_2$ in platinum side of the particles (Paxton, 2004).

Exceptional properties of BC have overcome using other types of motors for the proposed nano-swimmer in this study. Among all bimolecular motors, F$_1$-ATPase has been the most studied. F$_1$-ATPase is similar to BC in biochemical aspect but its structure is more complicated than BC which means F$_1$-ATPase needs three subunits to function properly but BC requires only one subunit to be active. Besides, addition of histidine tag to F$_1$-ATPase affects its enzyme activity. But with BC, adding the histidine tag does not affect the enzymes activity (Ekuni, 1998). Although biomolecules such as myosin and kinesin are commonly used in nano-machine fabrication, they require large protein complexes to function properly. Therefore, the size of the protein would be larger than the nano-machine which is not in interest of nano-scale applications (Yates, 2012) (Schmidt & Montemagno, 2004). Bacteria flagella can be used as bimolecular motor, but there are some limitations that need to be considered. The size of the flagella is in micro scales; therefore it cannot be used in nano-scale machines. Besides, Flagella motors cannot function out of the bacteria cell which is a disadvantage. Hence, flagella motors are not qualified to be used as motors for the purpose of this study (Yates, 2012).

Although chemically propelled motors are commonly used as biomotors, these chemically propelled motors can only operate in synthetic environments. Therefore, they are not a proper choice for some specific purposes such as biomedical applications (Wang, 2009).

A method for fabricating BC-driven nano-swimmers was proposed in (Yates, 2012). BC was successfully attached to the nickel-coated hemisphere of 500 nm silica particles. The location and orientation of the enzymes on the coated half of the particles were statistically
modeled. The theoretical study predicted that BC would be able to convert chemical energy into non-Brownian motion of the nano-swimmers.
CHAPTER 2 NANO-SWIMMER FABRICATION

This chapter describes the two-step process for fabricating the nano-swimmers. The first step involved coating half of the nanoparticles with nickel to create an asymmetry (i.e., a Janus particle). The second step involved attaching the BC molecules to the nickel-coated hemisphere of the particle.

Fabrication of the nano-particles was done at the LSU Center for Advanced Manufacturing (CAMD) and at Chapel Hill Analytical and Nanofabrication Laboratory (CHANL) facility of the University of North Carolina (UNC). All the preparatory procedures were done in a clean room to reduce the risk of contamination.

2.1 Materials and Main Equipment

The nano-particles used in this study were 492 nm Fluorescent-Green SiO$_2$ silica nano-spheres (product line C-SIO-G0.5) (Microspheres-Nanospheres, 2003-2011). The density of the silica particles is 1.96 g/cm$^3$. The particles are embedded with fluorescein isothiocyanate dye with excitation wavelength of 495 nm. The emission wavelength is 510 nm. The purpose of purchasing the fluorescent particles was to enable tracking of the nanoparticles with a micro-particle image velocimetry system. However, a Nikon microscope, which does not require the fluorescent dye, was eventually used to track the nano-particles. Figure 2-1 shows a sample image of the plain 492 nm silica nano-particles.
Figure 2-1. SEM image of 492 nm plain silica particles deposited on a silicon wafer.

Scanning Electron Microscopy (SEM) was used to capture images of the nano-particles. CAMD’s SEM is a Hitachi S-4500 II cold field emission SEM with EDAX. The SEM uses high-energy electrons to scan the particle’s surface.

Transmission Electron Microscopy (TEM) was also used to take high resolution images of the nano-particles. The TEM that was used is a JEOL 2010 F-FasTEM which is a High-Resolution Transmission Electron Microscopy (HRTEM) with a zirconated tungsten thermal field emission tip, and is located at the UNC CHANL facility. The TEM is also equipped with a 2k x 2k Gatan CCD bottom mount camera. All the measurements were performed at an accelerating voltage of 200kV and in bright field mode.

The sputter deposition technique which is a type of vapor deposition (PVD) was used to coat the nano-particles with nickel. In sputtering, the target material (in this case, nickel) is ejected onto a substrate (silicon wafer) in a vacuum chamber. In this technique, the target is bombarded by high energy particles. The momentum transferred from high energy particles to target surface would cause the surface atoms to escape. These atoms can then travel to substrate
creating a thin layer of metal coating (Kurt J. Lesker Company, 1954). The PVD 75 Sputter System at the UNC CHANL facility was used.

2.2 Janus Particle Preparation

2.2.1 Particle Cleaning Procedure

10 ml bottle of 492 nm particles solution was used for this experiment. The particles were suspended in distilled water with concentration of 25 mg/ml. In (Yates, 2012), it was suggested to wash the nano-particles with hydrochloric acid (HCl) before coating them specifically, 1 ml of the nano-particle solution was suspended in 200 ml of 2 N HCl and sonicated for 1 hour. The next step was to let the beaker sit until the particles settled to the bottom of the beaker. Then, HCl was decanted and the beaker was refilled with distilled water. The particles were settled again. The pH level of the particle solution was then measured with a probe. This process was repeated until the pH level was equal to that of distilled water. Finally, the excess water was taken out and the concentrated particle solution was stored in a glass container to be later coated.

The above cleaning procedure was performed on two different sets of particles in two separate beakers. All the steps of cleaning process were the same for both beakers, except that one beaker was placed on a hot plate during the entire procedure while the other was placed on the lab counter at room temperature (Figure 2-2). The hot plate was used as a fast-forward technique in the cleaning procedure.
Figure 2-2. Cleaning procedure of two sets of particles with and without hot plate (hot plate was used to increase the cleaning process speed, but the effect was unknown).

It was observed that by using the hot plate, particles would settle in two hours. Whereas, without the hot plate, it took five hours for the particles to settle. Since the effect of the hot plate on the silica particles was unknown, samples from both beakers (with hot plate and without hot plate) were deposited on two separate silicon wafers. The silicon wafers were first cleaned with acetone, Isopropyl alcohol (IPA), and rinsed with distilled water. The wafers were then dried with air pressure. The wafers were half-covered with a petri dish (to prevent dust contamination) under the hood to dry. After the wafers were completely dried, they were observed under SEM (Figure 2-3).
Figure 2-3. Particles deposited on silicon wafer to be observed under SEM.

Figure 2-4 and Figure 2-5 show the SEM images from the hot plate and room temperature processes, respectively. It was observed that when the particles are exposed to the hot plate, they tend to cluster. On the other hand, the particles that were dried at room temperature are more evenly distributed on the wafer.

Based on this experiment, the use of the hot plate in particle cleaning and drying process was not recommended.
Figure 2-4. SEM image of particles cleaned on the hot plate showing particle clustering.

Figure 2-5. SEM image of particles cleaned without the hot plate showing a more evenly distribution of particles.
Another important observation made during the cleaning procedure was the contamination of the particles. The cleaning procedure was repeated several times with extra caution. The beakers were covered with aluminum foil after each step to exclude dust or any external material from contaminating the beakers. The pH probe was also thoroughly cleaned carefully before taking a measurement. Despite this care, the contamination was still observed. After several attempts, we concluded that the contamination was likely from sonication with HCl. In particular, sonicating glass-type particles may break some of them causing the dye to leak out. Figure 2-6 shows the observed contamination. The dye contamination caused the particles to cluster. After several repetitions of the cleaning procedure yielded the same result, it was decided to bypass the cleaning procedure and directly coat the original particles from the bottle.

![Dye contamination](image)

Figure 2-6. SEM image of the dye-contaminated particles due to sonication.
2.2.2 Wafer Preparation

In order to coat the particles with PVD, a *single* layer of particles has to be formed on the wafer. To this end, 25% and 16% diluted particle solutions were prepared and deposited on the silicon wafer. Right after deposition, the solution was spread evenly with the tip of a pipet. The samples were then examined under SEM. It was observed that even with high dilutions, the particles tend to form multi-layers instead of a single layer. If multiple layers of particles were deposited on the wafer, only the top layer would be coated. Figure 2-7 (a) and (b) show 25% and 16% diluted particles deposited on the silicon wafer, respectively. After several attempts the results showed that the dilution technique did not work for our purpose.
The next strategy to create a single layer of particles was to use a spinner to spin the wafer after depositing the particles. 100 μl of the nanoparticles from the original bottle was deposited on the wafer. Then, the wafer was spun for 15 seconds at 500 revolutions per minute (rpm). The dried sample was observed under SEM. Based on SEM results, the spinning technique gave us a single layer of particles on silicon the wafer. Figure 2-8 shows the result after spinning the wafer.

The spinning technique was repeated several times to make sure it gives us the same result every time. This technique was used for depositing the nanoparticles on the wafer in the remainder of the experiments conducted.
Figure 2-8. (a) SEM image showing the spinning technique created a uniform single layer distribution of particles; (b) Amplified view of the deposited particles.
2.2.3 Coating the Nano-Particles

The silica nano-particles were half-coated with nickel at the UNC CHANL facility using the sputtering deposition technique described in section 2.1. After the coating procedure was completed, the wafers were brought back to CAMD for SEM examination. Figure 2-9 shows the top view of the coated particles.

Figure 2-9. Top view of the nickel coated side of the particles.

In order to have a better view of the half-coated particles, a small area was scraped off with a sharp small knife to randomly change the orientation of the half-coated particles on the wafer. The particles were then observed under SEM. Based on the observations; the coating process was successfully done on 492 nm particles. Figure 2-10 (a) shows the side view of the half-coated particle, where the brighter side shows the nickel. Figure 2-10 (b) shows a broader view of half-coated particles after scraping them off the wafer.
Further experiments were done with three coating thicknesses to obtain the optimum thickness.

After each deposition, the particles were scraped off and observed under SEM. The first coating was 32 nm of nickel with 14 nm of chromium as the adhesive layer. Figure 2-11 shows
the SEM image of particles with this coating. Although the coating process was successfully done, some of the nickel coating came off from the particles after they were scrapped off the wafer. Figure 2-12 clearly shows the coating coming off during scrapping process.

![Pealed Coating](image)

Figure 2-11. SEM image of 492 nm particles with 32 nm of nickel and 14 nm chromium.

![Pealed Coating](image)

Figure 2-12. Coating coming off of some 492 nm particles.
The second coating thickness was 20 nm nickel with 9 nm chromium. The image of the particles that were successfully coated is shown in Figure 2-13. Also in this case, it was observed that the coating came off during the scraping process (Figure 2-14).

![SEM image of particles with 20 nm of nickel and 9 nm chromium.](image1)

Figure 2-13. SEM image of particles with 20 nm of nickel and 9 nm chromium.

![SEM image showing the coating coming off from particles with 20 nm nickel and 9 nm chromium.](image2)

Figure 2-14. SEM image showing the coating coming off from particles with 20 nm nickel and 9 nm chromium.
The third coating thickness was 10 nm nickel with 5 nm chromium. A small area was scraped off and then the sample was observed under SEM. Figure 2-15 is the SEM image from 492 nm particles with thinnest metal coating. Higher quality images of the half-coated nanoparticles were obtained using transmission electron microscopy (TEM). Figure 2-16 shows the TEM image of the particles, where the darker shades indicate the nickel coating.

Figure 2-15. SEM image of 492 nm particles with 10 nm of nickel and 5 nm chromium.

Figure 2-16. TEM image of the particles half-coated with nickel.
Based on the SEM and TEM images, the thinnest the coating (10 nm nickel with 5 nm chromium) was least likely to peel off from the silica particles and therefore, was selected for coating the particles prior to the enzyme attachment.

### 2.3 Addition of BC to Nanoparticles

Enzymes attachment was done at the Biochemistry Lab of the LSU Department of Biological Sciences. The coated particles were transferred into two separate micro-tubes: one for experimental control and one for the nano-swimmers. The particles in both micro-tubes were washed by adding 1.5 ml of a second dialysis solution. Second dialysis is a buffer that keeps the protein at its proper pH of 8, so it can function properly. Second dialysis consists of 10 mM hepes and 150 mM KCl. Next, the micro-tubes were vortexed for 2 minutes to re-suspend the particles. The micro-tubes were then placed in a micro-centrifuge and centrifuged for 15 minutes at 2200 relative centrifugal force (rcf), causing the particles to settle at the bottom of the micro-tubes. The supernatant (the excess liquid after centrifugation) was then removed from the tubes. Then, 1.5 ml of BC was added to one of the micro-tubes to create the nano-swimmer tube. In the control micro-tube, 1.5 ml of second dialysis was added. Both micro-tubes were vortexed again to re-suspend the particles. Next, the tubes were incubated for 5 minutes at room temperature. The micro-tubes were then centrifuged again for 15 minutes with 2200 rcf. The supernatant was taken out from both micro-tubes, and 1.5 ml of second dialysis was added again to each micro-tube to wash the particles. The tubes were vortexed again and spun in micro centrifuge for another 15 minutes with 2200 rcf. After 15 minutes, the particles were settled at the bottom of the tubes. The supernatant were then removed from the tubes. 50 µl of second dialysis were then added to each tube. The micro-tubes were vortexed one more time to re-suspend the particles.
The purpose of vortexing in enzyme attachment process was to mix the particles and liquid each time.

At the end of this process, control micro-tube contained half-coated particles without any enzyme attached to them. The nano-swimmer micro-tube contained the half-coated particles with enzyme attached to them.

The enzyme substrates were prepared separately. The substrates consisted of 1 ml of 800 mM biotin, 1 ml of 2 M magnesium chloride, 1 ml of 492 mM bicarbonate, and 600 μl of 0.2 mM ATP. Although magnesium chloride is not a direct substrate for BC, it binds with ATP. ATP was kept in a separate micro-tube and was added to the samples when needed. After the enzyme attachment, a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) test was run. Sodium dodecyl sulfate (SDS) is a detergent that denatures the protein so the protein retains its primary structure. SDS also creates a negative charge on the protein. The sample was also heated to 60°C for further denaturation. To separate different protein sizes with different moving rates, discontinuous polyacrylamide gel was used. An electric field was applied on the gel so the negatively-charged proteins were attracted toward the anode. When the current was turned off, the gel was suspended in a dye solution so the proteins become visible. In presence of the enzyme, a blue band appears on the gel. The band corresponds to the standard ladder which is a guide for the test (Caprette, 1996) (SDS-PAGE (PolyAcrylamide Gel Electrophoresis), 2001).

BC’s molecular weight is about 50 KDa. If BC is attached to the particles, a band should appear at 50 KDa. For this experiment, SDS-PAGE was run for both the control and the nano-swimmer in separate lanes on the gel. The SDS-page results were also run for the uncoated particles as a benchmark. Figure 2-17 shows that the enzyme was present in the nano-swimmer tube and therefore was attached to the nickel coating.
Figure 2-17. SDS-PAGE results showing successful enzyme attachment to the nano-particles.

To make sure the enzyme was active after attachment; an assay was run on the uncoated particles, coated particles without enzyme, and nano-swimmers in the presence of enzyme substrates. The assay shows the enzyme activity per minute. The activity of the enzyme is determined by measuring the rate of ATP that is hydrolyzed. Table 1 shows the enzyme activity for two analyses. A bar chart of the results is shown in Figure 2-18. The enzyme activity for the uncoated and half-coated particles were similar and in the same range. The slight difference was due to instability of ATP. The enzyme activity for the half-coated with enzyme (nano-swimmer) was more than double than that of the control particles, indicating the BC was active while attached to the nano-swimmer.
Table 1: Comparison of Enzyme Activity

<table>
<thead>
<tr>
<th>Controls</th>
<th>First Measurement (Activity/min)</th>
<th>Second Measurement (Activity/min)</th>
<th>Average (Activity/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>0.008098296</td>
<td>0.008848296</td>
<td>0.008473296</td>
</tr>
<tr>
<td>Half-Coated</td>
<td>0.008829454</td>
<td>0.0121049</td>
<td>0.010467177</td>
</tr>
<tr>
<td>Half-Coated+Enzyme</td>
<td>0.01677103</td>
<td>0.02633346</td>
<td>0.021552245</td>
</tr>
</tbody>
</table>

Figure 2-18 Enzyme activity measurements.
CHAPTER 3  NANO-SWIMMER EXPERIMENTATION

This chapter describes the experimental procedure used to test the hypothesis that the nano-swimmer undergoes non-Brownian motion in the presence of the enzyme substrates. The following experiments were conducted immediately after the enzyme attachment procedure was completed to ensure the BC molecules on the nano-swimmers were still alive.

3.1 Experimental Setup

A Nikon Microscope with 100x lens was used to observe the movement of the nano-swimmers and the controls. A TUCSEN TrueChrome II camera was attached to the microscope to capture and save the videos at 30 frames per second on a SD card.

In order to observe the nano-particles under the microscope, a viewing chamber was fabricated (Yates, 2012). Its purpose is to enclose the observation area and avoid any external factors from biasing the motion of the nano-particles.

The viewing chamber was made of two 18×18 mm micro-glasses (catalog number 48366045) (VWR International-Chemicals and Laboratory Scientific Supplies). A 9 mm diameter, 0.12 mm deep secure-seal spacer (Invitrogen, S24737) (Secure-Seal™ Spacer) was used in between the micro-glasses where the sample drop was placed. A schematic of the viewing chamber is given in Figure 3-1 (Yates, 2012).

The Video Spot Tracker v08.01 software (CISMM, 2013) was used to track and record the 2D position of the nano-particles over time. Specifically, the software outputs the $x(t)$ and $y(t)$ coordinates of the particles in a CSV file for post-analysis.
3.2 Experimental Controls

The nano-swimmers consisted of particles half-coated with nickel with BC molecules attach to the nickel surface. In order to activate the enzyme, the fluidic environment contained the enzyme substrates (biotin, bicarbonate, and ATP). The following three controls were also used in the experiments.

1. Uncoated particles.
2. Particles half-coated with nickel but \textit{without} BC attached to the nickel surface. Henceforth, this control is referred to as \textit{half-coated particles}.
3. Particles half-coated with nickel with BC attached to the nickel surface but \textit{not exposed} to all the enzyme substrates. Specifically, ATP was not present in the solution, making the enzyme inactive. Henceforth, this control is referred to as \textit{inactive nano-swimmers}.

Figure 3-1. Schematic representation of the viewing chamber for observing the particles under the microscope (Yates, 2012).
3.3 Experimental Protocol

The protocol followed for observing the uncoated particles, was the following. One drop of the uncoated particles and one drop of the BC substrates were added to a separate micro tube and were mixed by using a pipet tip. Then one drop of the mixture was placed at the center of the secure seal which was stuck on a micro-glass. Immediately after adding the drop, a second micro-glass was placed over the drop such that the drop was stuck in the viewing chamber. The viewing chamber was placed under the microscope to observe and record the movement of the particles. In order to prevent any transient bias from the addition of the particles and substrate mixture to the micro-slide, a 40 second gap was given to the sample before initiating the video capture. The motion of the particles was then captured with the camera and was saved on a SD card for further analysis. The captured video for each particle had 360 frames \( (k_{\text{max}} = 360) \) with time increments of 1/30 s. Therefore, each particle was tracked for 12 s. Since the particles drop out of the focus of the lens after 12 seconds, this was the maximum time that a single particle could be tracked.

The same protocol was followed for the half-coated particles, inactive nano-swimmers, and nano-swimmers, except that for the inactive nano-swimmer, ATP was not added to the particle mixture.

3.4 Analysis and Results

Figure 3-2 shows the trajectory of the particles for the three controls and nano-swimmer from the video spot tracker software. The yellow line represents the trajectory of the particles in the time interval \([0,12]\) s. Two distinct particles are shown for each case. The trajectories in Figure 3-2 (A,B,C,D,E,F) suggest that the motions of the controls are likely Brownian since the particle displacements appear random and confined to the neighborhood of their position at \(t=0\).
This is in sharp contrast to the trajectories of the nano-swimmer, which are more directed and move away from the initial position. That is, the nano-swimmer motility appears to be non-Brownian.

In order to corroborate the visual, qualitative results from the videos, a quantitative analysis was performed on the \((x(t), y(t))\) data of each particle. To this end, the two methods describe in section 1.3.2 were applied to the experimental data to compare the translational and directional diffusion of the recorded particle motions.

Figure 3-2: Single particle trajectory. A and B: Uncoated particles, C and D: Half-coated particles, E and F: Inactive nano-swimmers, G and H: Nano-swimmer (each tick mark interval equals 500 nm).
3.4.1 Translational Diffusion Analysis

Recall the translational diffusion equation for Brownian motion given by Equation 1-4. This equation indicates that the MSD of Brownian motion is linear in time. To facilitate the evaluation of this relationship, the experimental MSD versus time was plotted in log-log scale. Notice that in a log-log scale, Equation 1-4 would result in a line with slope of one since

\[
\log |\Delta q(t)|^2 = \log (4Dt) = \log (t) + \log (4D). \tag{3-1}
\]

That is, the line fit to the log-log plot of the experimental data will have slope of approximately one if the motion is Brownian and slope greater than one if the motion is non-Brownian.

For this analysis, 15 particles were tracked for each control and the nano-swimmer. The particles were tracked for 12 seconds (360 frames). The displacement in Equation 1-2 was
calculated for each particle by varying $t_0$ and averaging over several equal values of $t \in \left[ \Delta t, \left( \frac{k_{max}}{2} \right) \Delta t \right]$ where $k_{max} = 360$. For example, to compute $\Delta q(t = \Delta t)$, we calculated the displacement vector from time samples 0 to 1, time samples 1 and 2, and so on, and took their average. To compute $\Delta q(t = 180\Delta t)$, we averaged over the displacement vectors from time samples 0 to 180, time samples 1 and 181, and so on. A second average was then taken over all particles ($N = 15$) to compute the MSD in Equation 1-3 for each control and the nano-swimmer.

Figure 3-3 to Figure 3-6 show the log-log plots of the MSD versus time for the uncoated particle, half-coated particles, inactive nano-swimmers, and nano-swimmers. A linear fit, $y = ax + b$, was applied to each data set using the “polyfit” function in MATLAB.

The slopes for the resulting linear fits are summarized in Table 2. The 95% confidence intervals ($\alpha = 0.05$) on slopes are calculated as $S_a \times t_{\alpha/2,\infty}$, where $S_a$ is the standard error of the slope (Equation 3-3) and $t_{\alpha/2,\infty}$ equals 1.96 (Freund, 2003).

Table 2: Comparison of slopes of MSD linear fit.

<table>
<thead>
<tr>
<th>Case</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated Particles</td>
<td>0.956 ± 0.028</td>
</tr>
<tr>
<td>Half-Coated Particles</td>
<td>0.920 ± 0.113</td>
</tr>
<tr>
<td>Inactive Nano-Swimmers</td>
<td>0.975 ± 0.039</td>
</tr>
<tr>
<td>Nano-Swimmers</td>
<td>1.472 ± 0.047</td>
</tr>
</tbody>
</table>
Figure 3-3. Log-log plot of MSD versus time for uncoated particles.

Figure 3-4. Log-log plot of MSD versus time for half-coated particles.
Figure 3-5. Log-log plot of MSD versus time for inactive nano-swimmers.

y = 0.975 x + 1.373

Figure 3-6. Log-log plot of MSD versus time for nano-swimmer.

y = 1.472 x + 0.972
In order to check the statistical significance \((\alpha = 0.05)\) of the difference between the calculated slope values against 1, t-test has been applied to the Table 2 results. The null hypothesis \((H_0)\) is that the slope of the fitted line equals 1, and the alternative hypothesis \((H_1)\) is that the slope of the fitted line is different from 1. The t-value for one sample t-test is calculated as:

\[
t = \frac{a-a_0}{s_a}
\]

where \(a\) is the slope of the fitted line, \(a_0\) is 1, and \(s_a\) is the standard error of the slope of the fitted line. To calculate \(s_a\), the following formula was used

\[
s_a = \sqrt{\frac{s}{n-2}\sum_{i=1}^{n}(x_i-x)^2}
\]

where

\[
s = \sum_{i=1}^{n}(ax_i + b - y_i)^2
\]

Here, \(x_i = \log(t_i)\), \(y_i = \log(MSD(t_i))\), and \(n = 160 \times 15\), where 160 corresponds to the number of fitted data points in Figure 3-3 to Figure 3-6 and 15 corresponds to the number of particles.

The t-test results are represented in Table 3. The calculated t-value in Table 3 was compared to \(t_{0.025} = 1.96\). If the calculated t-value is less than 1.96, the null hypothesis will not be rejected. If the calculated t-value is greater than 1.96, the null hypothesis will be rejected and the slope is significantly different from 1, corresponding to non-Brownian motion. When compared to the critical t-value (1.96), half-coated particles and inactive nano-swimmers were not significantly different from 1. In the case of uncoated particles, although 3.03 is greater than...
1.96, the difference is not strong. The t-value of the nano-swimmers is 38.69 which is significantly different from 1.

Table 3: t-values for three controls and nano-swimmer.

<table>
<thead>
<tr>
<th>Case</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated Particles</td>
<td>3.03</td>
</tr>
<tr>
<td>Half-Coated Particles</td>
<td>1.36</td>
</tr>
<tr>
<td>Inactive Nano-Swimmers</td>
<td>1.25</td>
</tr>
<tr>
<td>Nano-Swimmers</td>
<td>38.69</td>
</tr>
</tbody>
</table>

The slope values for the experimental controls indicate that their motion was Brownian as expected. The slope for the nano-swimmer was significantly higher than the controls which is indicative of non-Brownian behavior.

3.4.2 Directional Diffusion Analysis

In order to have a second form of validation, the directional diffusion parameter defined in Equations 1-7 and 1-8, was applied to the experimental data. Here again, 15 particles were tracked. Figure 3-7 to Figure 3-10 show the histograms of the directional angles for the controls and nano-swimmer. The abscissa of the histogram gives the angles in the interval \((-\pi, \pi]\) with bin sizes of \(\frac{2\pi}{59}\). The bin size was calculated using the following formula (Doebelin, 1975):

\[
\text{Number of Bins} = 1.87 (n - 1)^{0.4} + 1
\]

where \(n\) represents the total number of samples. In this case, \(n = 358 \times 15\), where 358 is the number of \(\beta\) angles for each particle and 15 is the number of tracked particles. The ordinate of histogram is the frequency of occurrence of the range of angles within each bin.
Notice from Figure 3-7 to Figure 3-9 that the angle frequency distribution for the controls shows no strong directional preference, which is consistent with Brownian motion. On the other hand, the angle frequency distribution for the nano-swimmer in Figure 3-10 has two larger peaks in the neighborhood of $\beta = 0$. This indicates that the nano-swimmers have less of a tendency to change their direction while moving in the fluid and are more likely to move in the direction that the BC “motors” are propelling them (perpendicular to the plain of the Janus particle hemisphere). Thus, their motion is non-Brownian.

Figure 3-7. Directional diffusion histogram for uncoated particles.
Figure 3-8. Directional diffusion histogram for half-coated particles.

Figure 3-9. Directional diffusion histogram for inactive nano-swimmer.
The histograms of the directional angles for the controls and nano-swimmer was also plotted for $\beta_i(k) = \theta_i(k + 2) - \theta_i(k)$ and $\beta_i(k) = \theta_i(k + 4) - \theta_i(k)$, but the nature of the histograms remained the same.

In order to test the equality of two probability distributions, Kolmogorov-Smirnov (K-S) test was applied to all histogram pairs (Pollard, 1979). The K-S test was applied to the $[-0.36\pi, +0.36\pi]$ region of the histograms because the $[-\pi, -0.36\pi]$ and $[0.36\pi, \pi]$ regions in the histograms are similar. The null hypothesis here is that the two compared histograms are statistically indifferent. If the p-value from K-S test was less than 0.05, the null hypothesis will be rejected and the histograms are significantly different. Otherwise, the p-value from K-S test was greater than 0.05, the histograms are not significantly different. The results of the K-S tests are summarized in Table 4. Based on the K-S test P-values ($\alpha = 0.05$), the controls are not significantly different from each other as expected. The nano-swimmers are significantly
different from uncoated particles and half-coated particles. The test did not show a significant difference between inactive nano-swimmers and nano-swimmers which may be due to not having enough data points.

Table 4: K-S Test Results

<table>
<thead>
<tr>
<th>Case</th>
<th>P-value of K-S Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated vs. Inactive Nano-swimmers</td>
<td>0.154</td>
</tr>
<tr>
<td>Uncoated vs. Half-Coated Nano-swimmers</td>
<td>0.304</td>
</tr>
<tr>
<td>Half-Coated vs. Inactive Nano-swimmers</td>
<td>0.154</td>
</tr>
<tr>
<td>Uncoated Particles vs. Nano-swimmers</td>
<td>0.029</td>
</tr>
<tr>
<td>Half-Coated Particles vs. Nano-swimmers</td>
<td>0.004</td>
</tr>
<tr>
<td>Inactive Nano-Swimmers vs. Nano-Swimmers</td>
<td>0.154</td>
</tr>
</tbody>
</table>
CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 Conclusions

This thesis presented results that strongly suggest that the enzyme biotin carboxylase (BC) can serve as a biomolecular motor, converting chemical energy into useful mechanical work. This was demonstrated by covering the nickel-coated hemisphere of a Janus-like, 492 nm particle with BC molecules. Nickel was used because BC can be attached to it via a histidine tag. It was shown that BC can propel the nano-particles in a non-Brownian manner in comparison to three control experiments when the particles are immersed in a fluidic environment with the enzyme substrates. For this demonstration, videos of the nano-swimmers and controls were recorded, and the motion of 15 individual particles was tracked via a free software.

Videos showed the nano-swimmers moving in directed manner and away from their initial position, whereas motion of the controls was random and in the proximity of their initial position. Translational and directional diffusion analyses were performed on the experimental data of the nano-swimmer and controls. The slope of the linear fit for the mean square displacement versus time plot of the nano-swimmers was approximately 1.5, indicating non-Brownian motion according to Einstein’s theory (the slope of the controls was approximately one, which is indicative of Brownian motion). Further, the histogram of the directional diffusion angle of the nano-swimmers was less flat than those of the controls and showed higher probability for small angles. This means the nano-swimmer are more likely to move in the direction in which they are propelled by the BC motors.

4.2 Recommendations for Future Work

In order to produce better nickel-coated particles, it is recommended that a more efficient way to remove the half-coated particles from silicon be developed. The method used in this work
involved scraping the particles off the wafer, which can damage some of them. An alternative method is to use a wafer with a layer of salt on it. To remove the half-coated particles safely, the wafer can be submerged in the salt solution so the particles would come off the wafer. A nano-size mesh can be used to remove the particles from the solution. Other than using the wafer with a layer of salt, using surfactants and adding charge are also other options to optimize the removing procedure of the half-coated particles from the wafer.

It is also recommended to reduce the size of the nano-particles to 300 nm or 100 nm when fabricating and testing the nano-swimmers. The non-Brownian motion of the nano-swimmers will likely be more noticeable as the particles size decreases.

Preliminary step toward this recommendation was taken by half-coating 300 nm particles using the procedure described in this thesis. The 300 nm particles were observed under SEM, showing that the coating successfully adhered to the particle. Enzyme attachment and testing of the 300 nm nano-swimmers was not performed though. Coating of the 100 nm particles might require a different procedure that the one used here.
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VITA

Sima Hannani was born in 1987 in Sari, Iran. She graduated from Islamic Azad University North-Branch of Tehran with a Bachelor in Science in Natural Resources Engineering in July 2009. She came to the United States in August 2010 and attended a Master’s degree program in Civil Engineering department at Louisiana State University in August 2012. She is a candidate for a Master of Science in Civil Engineering to be awarded in May 2015.