Towards the development of biotin carboxylase driven robotic nanoswimmers

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TOWARDS THE DEVELOPMENT OF BIOTIN CARBOXYLASE DRIVEN ROBOTIC NANOSWIMMERS

A Thesis
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in Mechanical Engineering
In The Department of Mechanical Engineering

by
Rachel Allison Yates
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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ ii

ABSTRACT .......................................................................................................................... iv

CHAPTER 1: INTRODUCTION ............................................................................................ 1

1.1 Motivation ..................................................................................................................... 1
1.2 Statement of Work and Thesis Organization ............................................................... 2
1.3 Background and Literature Review ........................................................................... 4 

1.3.1 Janus Particles ........................................................................................................ 4
1.3.2 Biotin Carboxylase ............................................................................................... 5
1.3.3 Biomolecular and Chemical Motors ..................................................................... 7
1.4 Biotin Carboxylase versus Other Motors ................................................................. 9
1.5 Basic Theory ............................................................................................................... 10 

1.5.1 Low Reynolds Number Flow .............................................................................. 10
1.5.2 Stokes Flow .......................................................................................................... 11
1.5.3 Brownian Motion ................................................................................................. 14

CHAPTER 2: NANOSWIMMER FABRICATION ................................................................. 15

2.1 Main Equipment ......................................................................................................... 15
2.2 Silica Nanoparticles ................................................................................................... 16

2.2.1 100 nm Particle Preparation ............................................................................... 16
2.2.2 500 nm Particle Preparation .............................................................................. 20
2.3 Addition of BC to Particles ..................................................................................... 23

CHAPTER 3: ESTIMATION OF NANOSWIMMER PERFORMANCE ............................. 27

3.1 Power Estimation ....................................................................................................... 27
3.2 Enzyme Distribution on Nanosphere ....................................................................... 32

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK ...... 42

4.1 Conclusions ............................................................................................................... 42
4.2 Future Work ............................................................................................................... 43

4.2.1 Experimental Controls ....................................................................................... 43
4.2.2 μPIV .................................................................................................................... 44

REFERENCES .................................................................................................................... 46

VITA ...................................................................................................................................... 50
ABSTRACT

The objective of this research is to take the first step towards demonstrating the use of the enzyme *biotin carboxylase* (BC) as a biomolecular motor. BC is a homodimeric protein involved in fatty biosynthesis in all organisms. The conjecture is that BC can convert chemical energy into useful mechanical work via its conformation change, which acts as a fin or flexible oar producing nonreciprocal motion. To this end, we fabricate a proof-of-concept biomolecular machine driven by BC molecules, viz., a *robotic nanoswimmer*. This machine consists of a Janus-type nanosize silica particle, where one hemisphere is coated with an intermediate layer of chromium and then an outer layer of nickel. Since BC has been engineered to attach to nickel surfaces, this produces an asymmetry on the nanoparticle, which could potentially lead to non-Brownian motion in a low Reynolds number environment. The nanoswimmers have potential applications in drug delivery and transporting cargo in nano and microscale fluidic environments.

The proposed nanoswimmer is fabricated using a 500 nm diameter silica particle. The chromium and nickel coatings on the nanoparticle are created using electron beam evaporation. The presence and activity of the BC molecules on the nickel coating are verified using a Bradford protein assay and a PK/LDH coupled assay.

A theoretical analysis of the drag force on the nanoswimmer, velocity, and mechanical power that the BC molecules can produce is performed based on Stokes law. The analysis shows that as the particle size increases, its expected velocity increases. Further, it shows that BC should be able to produce enough power to overcome the drag force on the nanoswimmer and propel it at velocities in the micrometer per second range.
The random nature of the enzyme location and orientation on the nickel coating is also modeled with the aide of random variables. From this analysis, the direction of net force that the collection of BC molecules can apply to the nanoswimmer is determined. This analysis also allows one to estimate the force efficiency of the nanoswimmer with respect to the random placement of the enzymes.
CHAPTER 1: INTRODUCTION

A biomolecular motor is defined in this dissertation as a biomolecule that converts chemical energy into mechanical work. We call the integration of biomolecular motors with biological, chemical, mechanical, electrical, magnetic, and/or optical components to harness the mechanical work for a particular purpose, a biomolecular machine.

1.1 Motivation

Biological systems have singular specificity, adaptability, and efficiency due to the selective process of evolution over millions of years. It would be desirable to unite these unique biological properties with recent advances in nanofabrication technology to engineer biomolecular machines at the nanoscale. The development of this technology would enable a new generation of integrated devices with important advantages over traditional engineering systems in terms of performance, size, power consumption, efficiency, and ease of fabrication. Biomolecular machines could be designed and programmed to function at the nanoscale like many macroscale devices, e.g., actuators, switches, sensors, cargo shuttles, electrical power sources, robots, and computers (Chirikjian, Kazerounian, & Mavroidis, 2005) (Freitas Jr., 2005) (Månsson, et al., 2005) (Mavroidis, Dubey, & Yarmush, 2004) (Mavroidis C. D., 2003) (Montemagno, 2001). Nanoscale devices are expected to have a revolutionary impact on the future of mankind because of their ability to transport themselves, apply forces, and manipulate objects in nanosize environments (Desviat, Pérez, Pérez-Cerdá, Clavero, & Ugarte, 83). Some scholars believe intelligent biomolecular machines could one day be utilized to cure diseases, clean the environment, and facilitate space travel (Chirikjian, Kazerounian, & Mavroidis, 2005).

Despite their potential, biomolecular machines have seldom been realized beyond very rudimentary devices. A fundamental reason for this is that one is faced with challenges unique to fluid mechanics in small length scales; viz., how to effectively move liquids and particles
through liquids in nano and microscale environments. This difficulty is due to the fact that Reynolds numbers are very low in such scales and as a result viscous forces dominate inertial forces (Purcell, 1977). Under this condition, very high pressures are needed to pump liquids and particles. Micropumps, syringes, and peristaltic pumps are either bulky or too complex, while electrokinetic pumping requires electrodes and remedies for electrolysis problems. These methods are also expensive for integration in disposable biomolecular machines and require an external electrical or mechanical power source. Indeed, as stated in (Mano & Heller, 2005): “The design of man-made machines in which chemical energy is directly converted to mechanical energy, utilized in their propulsion, is one of the challenges of contemporary bioengineering.” Paxton et al. (Paxton, et al., 2004) write: “The creation of miniature ‘engines’ that can convert stored chemical energy to motion is one of the greatest remaining challenges of nanotechnology”. This thesis seeks to contribute to this demand.

1.2 Statement of Work and Thesis Organization

The overarching goal of this research is to introduce a new nanoscale biomolecular motor, viz., the enzyme biotin carboxylase (BC). Biotin carboxylase (BC) is a homodimeric protein (two identical subunits called dimers) involved in fatty biosynthesis in all organisms. As such, BC is an emerging target for drugs against obesity and diabetes (Thoden, Blanchard, Holden, & Waldrop, 2000) and for antibiotics. In fact, Pfizer has included BC in its portfolio of new antibiotic targets. The basic biochemical properties of BC have been fully characterized (see Section 1.3.2 for details). These biochemical properties strongly suggest that BC has the potential to be a valuable addition to the growing toolbox of building blocks for biomolecular machines. That is, two prerequisites must be satisfied for a molecule to be successfully engineered into a biomolecular machine: a) its catalysis should involve a large conformational change (Keller & Bustamante, 2000), and b) it should be biochemically stable and robust
BC has so far satisfied the above two prerequisites with “flying colors.” Most important, its structure and mechanistic properties are simpler than most traditional biomolecular motors, thus facilitating the goal of rationally engineering biomolecular machines driven by BC. The enzyme also has significant potential for nanotechnology applications because its dimensions are less than 10 nm.

In order to evaluate the use of BC as a biomolecular motor, we will engineer a proof-of-concept biomolecular machine—a robotic nanoswimmer. The robotic nanoswimmer consists of a nanoscale sphere half-coated with BC molecules, creating a so-called Janus particle. Our hypothesis is that the conformational change of BC will propel the nanosphere in a non-Brownian manner when placed in a fluidic environment with the enzyme substrates (the machine “fuel”). A conceptual drawing of the robotic nanoswimmer is shown in Figure 1.1.

Figure 1.1: Schematic of the proposed robotic nanoswimmer.

Our choice of the robotic nanoswimmer as a proof-of-concept test bed stems from three reasons. First, the manufacturing of nanoswimmers is relatively simple, unlike nanofluidic devices such as a conveyor belt or mixer where the fabrication of nanoscale topological structures (e.g., nanochannels) is a project in itself. Second, BC’s motility and propulsion properties are readily assessable on the nanoswimmers. For example, the thrust force can be easily estimated using Stokes law. Third, the propulsion of robotic nanoswimmers in fluidic
environments is of fundamental engineering interest due to the low Reynolds number, as well as directly applicable to bioengineering systems that involve the transport of nanoscale cargo; e.g., targeted drug delivery systems, cleaning of clogged arteries, and removal of toxic pollutants (Wang, 2009).

This thesis consists of four chapters and is organized as follows. The reminder of this chapter provides a literature review and background information necessary to understand several aspects of this research. The basic scientific theory needed to justify and analyze the results obtained is also presented. Chapter 2 explains the materials and methods for fabricating the robotic nanoswimmers. It also describes the assays used to validate that the nanoswimmers were successfully fabricated. Chapter 3 details the theoretical analysis for estimating the propulsion characteristics of the nanoswimmer. Finally, Chapter 4 summarizes the results and gives recommendations for future work.

1.3 Background and Literature Review

1.3.1 Janus Particles

“Janus” particles are asymmetric particles that have two different materials on opposite faces (Walther & Müller, 2008). This characteristic makes such particles potentially valuable in systems that involve drug delivery, microfluidic systems, biosensors, and electronic devices (Ye & Carroll, 2010). Janus particles utilize a different metallic coating on each hemisphere or only use one type of coating with the opposite side having the nanoparticle exposed. To date, various research groups have created both spherical and cylindrical Janus particles.

Ke et al. (Ke, Ye, Carroll, & Showalter, 2010) manufactured Janus particles using a 1 µm silica particles with one hemisphere coated in platinum. Howse et al. (Howse, Jones, Ryan, Gough, Vafabakhsh, & Golestanian, 2007) created Janus particles using 1.62 µm polystyrene spheres that were half-coated with a 5.5 nm layer of platinum. Ye and Carroll (Ye & Carroll,
2010) developed a method for creating bimetallic Janus microspheres. Specifically, a coating of one metal was applied to silica microspheres using electron beam evaporation. The microspheres were then inverted coated with a second metal on the opposite face again using electron beam evaporation. Gold, platinum, silver, nickel, cobalt, and aluminum coatings were tested. Titanium was used as an intermediate adhesion layer between gold and silica. All other metals were used without an intermediate coating. The group used 1 µm, 2 µm, and 4 µm silica microspheres.

Paxton et al. (Paxton, et al., 2004) created 370 nm diameter rods with 1 µm segments of platinum (Pt) and gold (Au). The method to manufacture these rods was previously reported by Martin et al. (Martin, et al., 1999).

1.3.2 Biotin Carboxylase

BC is one component of the multi-enzyme complex acetyl-CoA carboxylase (ACC), which catalyzes the first committed step in fatty acid biosynthesis in all animals, plants, and bacteria. As with all biotin-dependent enzymes, ACC utilizes a two-step chemical reaction mechanism. In the first half-reaction, BC catalyzes the adenosine triphosphate (ATP) dependent carboxylation of biotin. In the second reaction, catalyzed by the carboxyltransferase subunit, the carboxyl group is transferred to acetyl-CoA to make malonyl-CoA. The three substrates of biotin carboxylase are ATP, biotin, and bicarbonate. The equations for the chemical reactions are showing in Figure 1.2.

\[
\begin{align*}
\text{enzyme} - \text{biotin} + \text{MgATP} + \text{HCO}_3^- & \rightleftharpoons \text{Mg}^{2+} \text{enzyme} - \text{biotin} - \text{CO}_3^- + \text{MgADP} + \text{P}_i \\
\text{enzyme} - \text{biotin} - \text{CO}_3^- + \text{acetyl} - \text{CoA} & \rightleftharpoons \text{malonyl} - \text{CoA} + \text{enzyme} - \text{biotin}
\end{align*}
\]

Figure 1.2: Acetyl-CoA carboxylase complex.

The E. coli form is a model system for studying structural and functional aspects of ACC because BC and carboxyltransferase retain their catalytic activity when isolated. An important feature of E. coli BC that makes it a paradigm for detailed studies on the mechanism of its
conformational change under this condition is the availability of structural information. The 3-D structure of BC has been determined by x-ray crystallography with a resolution of 1.9 Å (Thoden, Blanchard, Holden, & Waldrop, 2000) (Waldrop, Rayment, & Holden, 1994). In addition, the 3-D structure of BC complex with the substrate ATP has been determined to 2.5 Å resolution (Thoden, Firstine, Nixon, Benkovic, & Holden, 2000). These crystallographic studies confirmed the biochemical studies that BC is a homodimer where each subunit contains a complete active site (Figure 1.3a). The dimensions of each subunit (monomer) are approximately 6.7 nm × 5.2 nm × 4.8 nm. This size makes BC particularly attractive for use in nanoscale machines.

![Figure 1.3: BC structure- (a) dimer and (b) detailed view of monomer.](image)

A detailed analysis of the BC monomer unveiled three distinct domains as shown in Figure 1.3b (Waldrop, Rayment, & Holden, 1994). This structure revealed that binding of ATP resulted in a large conformational change: an approximately 45° hinge-like rotation of the B domain relative to the A and C domains with frequency of about 2 Hz (Thoden, Blanchard, Holden, & Waldrop, 2000); see Figure 1.3b. Since the B domain has a flexible structure, a simple mechanical model for the B domain is a flexible link with a rigid-body motion about the hinge axis. This characteristic makes BC highly qualified for serving as a motor in nanofluidic (low Reynolds number) environments.
While a large conformational change is an important precondition for using BC as a biomolecular motor, there are several other biochemical features that make this enzyme particularly attractive for developing biomolecular machines. The gene coding for the enzyme has been cloned, sequenced, and overexpressed (Cronan Jr. & Waldrop, 2002). This means that the amino acid sequence can be altered by site-directed mutagenesis. In fact, several site-directed mutant forms of BC have already been generated with varying degrees of activity (Blanchard, Lee, Frantom, & Waldrop, 1999) (Levert, Lloyd, & Waldrop, 2000) (Sloane, Blanchard, Guillot, & Waldrop, 2001). Overexpression of the gene coding for BC ensures that a system for large-scale production of both wild-type and mutant forms of BC is available for machine development. Both wild-type and mutant forms of the protein are purified using nickel (Ni)-affinity chromatography via a hexa-histidine tag engineered on the amino terminus of the protein (Blanchard, Lee, Frantom, & Waldrop, 1999). Control experiments have shown attachment of this tag does not affect enzymatic activity. With respect to biomolecular machines, this means that the protein can be attached to other components with Ni chemistry. Finally, BC exhibits a wide temperature range for catalysis with full activity up to 45º C.

1.3.3 Biomolecular and Chemical Motors

Proteins such as myosin, kinesin, dynein, RNA polymerase, and ATP synthase, and bacteria such as bacterial flagellum and mycoplasma mobile have been extensively studied due to their ability to produce linear or rotary motion/propulsion. In addition to these well-known motors, other proteins and bacteria are emerging as potential motors. For example, Knoblauch et al. (Knoblauch, et al., 2003) suggested the use of protein bodies from sieve elements of plants. Sieve tubes are responsible for moving nutrients up and down the plant. The motor named forisome was shown to deform anisotropically when driven by calcium. Mavroidis et al. (Mavroidis, Dubey, & Yarmush, 2004) proposed the use of viral proteins, such as influenza and
HIV, which undergo a conformational change in response to changes in pH. Excellent surveys of biomolecular motors can be found in (Kinbara & Aida, 2005) (Mavroidis, Dubey, & Yarmush, 2004) (Schliwa & Woehlke, 2003) (Schmidt & Montemagno, 2004).

Amongst the biomolecular motors discovered so far, ATP synthase has been the most studied because it is ubiquitous in mammalian cells. ATP synthase is a complex nanosize protein consisting of two domains called $F_0$ and $F_1$ that behave like rotary stepper motors. Montemagno and co-workers were among the first to recognize the potential for using the $F_1$-ATPase motor to engineer biomolecular machines (Liu, et al., 2002) (Montemagno, 2001) (Soong, Bachand, Neves, Olkhovets, Craighead, & Montemagno, 2000). Early work was devoted to establishing several prerequisite technologies necessary for integrating the $F_1$-ATPase motor with nanoelectromechanical systems (NEMS). Later, a simple machine was developed consisting of a 750 to 1400 nm long propeller attached to the $\gamma$ subunit of $F_1$-ATPase.

One of the most interesting biomolecular machines to date was developed by (Tung & Kim, 2006). Bacteria flagella from *E. coli* were tethered to a microchannel to realize a microfluidic pump. The pump’s principle of operation is based on the viscous pumping effect, where continuous rotation of the tethered cells drag fluid from one end of the channel to the other. A similar idea was used where a carpet of *S. marcescens* bacteria was engineered to move fluid in microchannels (Darnton, Turner, Breuer, & Berg, 2004). Another interesting machine was designed in by using *M. mobile* cells moving inside a circular track to pull a microrotor (Hiratsuka, Miyata, & Tada, 2006). More recently, (Behkam & Sitti, 2007) (Behkam & Sitti, 2008) (Behkam & Sitti, 2006) experimentally demonstrated how an array of *S. marcescens* bacteria could be used to propel a 10 $\mu$m polystyrene bead.

A different approach for realizing nano- and microscale motors is based on the principle of catalytic conversion of chemical energy to mechanical work in the form of osmotic or
interfacial forces (Golestanian & Liverpool, 2005). This approach makes use of man-made, non-biological, asymmetric nano- or microscale spheres or rods (see Section 1.3.2), which are propelled by chemical reactions and the corresponding concentration gradients of reactants and products at the surface of the component (Ke, Ye, Carroll, & Showalter, 2010) (Mano & Heller, 2005) (Paxton, et al., 2004) (Wang, 2009). For example, Janus microspheres half-coated with platinum, immersed in a dilute hydrogen peroxide solution, were reported to undergo non-Brownian motion due to the catalytic decomposition of hydrogen peroxide (Howse, Jones, Ryan, Gough, Vafabakhsh, & Golestanian, 2007) (Ke, Ye, Carroll, & Showalter, 2010).

1.4 Biotin Carboxylase versus Other Motors

It is important to compare the properties of BC with those of chemical motors and other biomolecular motors to assess its potential benefits for engineering nanoscale machines. First, chemical motors operate only in very limited synthetic environments (e.g., low ionic strength solutions) with limited fuels (e.g., hydrogen peroxide) (Wang, 2009); thus, precluding them from most in vivo biomedical applications. Biomolecular motors such as myosin, kinesin, and dynein require another large protein for operation (e.g., kinesin, although being of nanosize, generates motion by propagating along microtubules). This prevents their use in nanoscale applications since the machine cannot be any smaller than the size of these large protein complexes. No high-resolution, 3-D structure exists for dynein (Schmidt & Montemagno, 2004), which severely limits the ability to rationally perform any protein engineering necessary for machine development. The advantages of BC over bacterial flagella are the most striking. Bacterial flagella do not function outside of the cell, which means the biomolecular machine is constrained by the requirements for keeping the bacteria viable. Not surprisingly, no biomolecular device utilizing isolated flagella has been described to date. Further, the size of bacteria flagella is in the micron range, which precludes their use in nanoscale machines.
The F₁-ATPase from *E. coli* has been the most studied nanoscale biomolecular motor. Coincidentally, it has many of the same biochemical features as BC; e.g., the gene coding for the subunits have been cloned and overexpressed, and a 3-D structure is available for rational protein engineering. However, F₁-ATPase is more complex, requiring three different protein subunits to make a functional enzyme. BC, on the other hand, only requires one protein subunit. This imposes significant difficulties to deciphering the residue-level behavior of the motor. Further, the enzymatic activity of F₁-ATPase is adversely affected by the addition of histidine tags, which in turn degrades the motor performance when the enzyme is chemically attached to other components. Ekuni et al. reported that F₁-ATPase from *E. coli* with a histidine-tagged γ subunit exhibited only 60% of the wild-type activity (Ekuni, Watanabe, Kuroda, Sawada, Murakami, & Kanazawa, 1998).

### 1.5 Basic Theory

#### 1.5.1 Low Reynolds Number Flow

The Reynolds number, $Re$, compares the inertial forces to the viscous forces in a fluidic system by examining the ratio

$$Re = \frac{\rho lu}{\mu} \quad (1.1)$$

where $\rho$ is the fluid density, $l$ is the characteristic length of the system, $u$ is the velocity, and $\mu$ is the dynamic viscosity of the fluid.

When the Reynolds number is large, inertial forces dominate. Conversely, when the Reynolds number is small, viscous forces dominate. Low Reynolds numbers can occur due to very viscous fluids, characteristic lengths on the order of microns or nanometers, or small velocities. Most micro- and nanoswimmers are suspended in an aqueous solution near room temperature, so the fluid density and viscosity are relatively constant. The characteristic length
scale of micro- and nanoswimmers is typically in the range of $10^{-5}$ to $10^{-9}$ meters. Also the velocities are usually measured in micrometers per second ($\mu$m/s). This results in a system where $Re \ll 1$.

When the Reynolds number is much less than unity, the inertia of an object is almost irrelevant. As Purcell (Purcell, 1977) pointed out, if a 1 $\mu$m object is being pushed at 30 $\mu$m/s in water and then suddenly it is no longer being pushed, it will coast for approximately 0.1 Å before stopping. This example shows that what was happening to the object in the past is nearly irrelevant, and only the forces that are applied at the moment can lead to movement. Because the viscous forces are dominant, it is not possible for objects to “glide” using reciprocal motion, which is motion that involves moving then retracing the same path to return to the original position. For example, the motion of a rigid oar propelling a boat in a lake is reciprocal. Therefore, swimming or gliding in a low Reynolds number environment requires the generation of nonreciprocal motion (Purcell, 1977). One method of achieving this type of motion is via a flexible oar (Purcell, 1977).

1.5.2 Stokes Flow

The non-dimensionalized Navier-Stokes equation is given by (Kirby, 2010)

$$\text{Re} \frac{\partial \mathbf{u}^*}{\partial t^*} + \text{Re} \mathbf{u}^* \cdot \nabla^* \mathbf{u}^* = -\nabla^* p^* + \nabla^2 u^*$$  \hspace{1cm} (1.2)

where $\nabla^* = l \nabla$, $\nabla^2 = l^2 \nabla^2$, $\mathbf{u}^* = \mathbf{u}/U$, $p^* = pl/\mu$, $t^* = t/t_c$, $t_c$ is the characteristic time scale, and $U$ is the characteristic velocity (Childress, 2009). By definition, in Stokes flow, the Reynolds number is small so the unsteady and convective terms (i.e., the terms on the left-hand side of (1.2)) can be neglected. As a result, the Stokes approximation for systems with $Re \ll 1$ is

$$\nabla p = \mu \nabla^2 \mathbf{u}$$  \hspace{1cm} (1.3)
1.5.2.1 Stokes Flow for a Sphere in an Infinite Domain

Consider the axisymmetric flow at velocity $U$ over a sphere of radius $a$ at low Reynolds number in an infinite domain (see Figure 1.4). The solution for the flow can be obtained by assuming that the result can be written in terms of a power series in $a/r$. The flow velocity over the sphere is given by (Kirby, 2010)

$$u(r) = U \left(1 - \frac{3}{2} \frac{a}{r} + \frac{1}{2} \frac{a^3}{r^3}\right) \quad (1.4)$$

where $r$ is the radial distance from the center of the sphere. The variation of the pressure from the free stream value is given by

$$\Delta p = -\frac{3}{2} \mu U \frac{a}{r^2} \cos \theta \quad (1.5)$$

When the pressure and viscous forces are integrated along the surface of the particle as shown below,

$$F_{drag} = 3\pi \mu a U \int_0^\pi \cos^2 \theta \sin \theta \, d\theta + 3\pi \mu a U \int_0^\pi \sin^3 \theta \, d\theta \quad (1.6)$$

where the first integral accounts for the pressure forces and the second integral accounts for the viscous forces (Childress, 2009). When (1.6) is evaluated, the total drag force is found to be

$$F_{drag} = 6\pi \mu U a \quad (1.7)$$
By comparing the characteristic time scale for Stokes particles to equilibrate with their fluid surrounds, \( \tau_p = \frac{2a^2 \rho_p}{9 \mu} \) where \( \rho_p \) is the density of the particles, with the experimental time scale, it can be seen that the steady state results above are applicable even for time-varying \( U \). This is because the flow can be assumed to be in a quasi-steady state even if the flow itself is unsteady.

### 1.5.2.2 Stokes Flow for a Sphere in a Finite Domain

The simplest analysis occurs when the particles are assumed to be in an infinite domain. However, as the particles begin to settle to the bottom of a wide channel, it is no longer valid to assume the particles are in an infinite domain. Instead, the particle motion must be analyzed using a finite domain. Due to the no slip condition, the particle motion will be reduced as the particle nears the bottom surface of the channel. For spheres of radius \( a \) that are a distance \( d \) from a surface, the force-velocity relation normal to the surface can be approximated by (Kirby, 2010)

\[
\frac{1}{6 \pi \eta a} \frac{F_{\text{drag}} \cdot \hat{n}}{u \cdot \hat{n}} = 1 + \frac{9}{8} \frac{a}{d} \tag{1.8}
\]

and the force-velocity relation tangent to the surface can be approximated by

\[
\frac{1}{6 \pi \eta a} \frac{F_{\text{drag}} \cdot \hat{t}}{u \cdot \hat{t}} = 1 + \frac{9}{16} \frac{a}{d} \tag{1.9}
\]

where \( \hat{n} \) is the direction normal to the surface and \( \hat{t} \) is the direction tangent to the surface. The effect of the surface becomes negligible at \( d \geq 10a \).

It is assumed that if the surface affects the particle motion, the velocity normal to the surface is negligible and therefore there is only velocity in the tangential direction. In future experiments to determine the particle velocity, the particle motion will be affected by the wall, so (1.9) is necessary to accurately determine the drag force on the particle.
The finite domain may also cause particles to be close together. As a result, drag force on the particles and subsequently the velocity will be affected by the particle-particle interactions. A particle is considered isolated so long as it is more than ten particle diameters away for any other particle (Kirby, 2010).

1.5.3 Brownian Motion

Brownian motion of small particles is the random motion caused by the statistical nature of the fluid forces acting on the particles. As the particles become smaller, the Brownian motion becomes more prominent because the probability of individual particle–fluid collisions becomes larger (Kirby, 2010).

The root-mean-square (RMS) particle image velocimetry measurement error due to Brownian motion can be related to the particle diffusivity $D$ by (Kirby, 2010)

$$\langle \Delta u \rangle^2 = \frac{2D}{\Delta t} \quad (1.10)$$

where $\Delta t$ is the amount time between two images taken by the camera. The diffusion coefficient for particles in Stokes flow is given by the Stokes-Einstein relation (Kirby, 2010)

$$D = \frac{k_B T}{6\pi \mu a} \quad (1.11)$$

where $k_B$ is Boltzmann’s constant and $T$ is temperature. In (1.10), it is assumed that exposures are infinitely fast and determines the errors due to the random velocity fluctuation between exposures. If the exposure is too long, there can be Brownian motion taking place during the time that the camera’s shutter is open to take the image that is not accounted for (Kirby, 2010).
CHAPTER 2: NANOSWIMMER FABRICATION

In this chapter, we describe the design of the proof-of-concept biomolecular machine, which will demonstrate the feasibility of using the large conformational change of BC to propel a nanoscale object. We conjecture that an asymmetrically BC-covered nanospheres will have autonomous, non-Brownian motility in the presence of the enzyme substrates; thus, essentially becoming self-propelled robotic nanoswimmers. This hypothesis is motivated by the belief that the continuous opening and closing of the B domain of BC subunits act as fins or flexible oars (see Section 1.5.1); thus, being capable of producing non-Brownian motion.

2.1 Main Equipment

The main equipment used in fabricating the nanoswimmer is described next. The equipment is housed at LSU’s Center for Advanced Manufacturing (CAMD).

*Electron beam deposition* is a method used to coat a substrate with a thin film of metal. This method leads to line-of-sight deposition, which is ideal for fabricating Janus particles because only half of the particle is coated. The coating process takes place under high vacuum conditions. A beam of high-energy electrons is used to heat a metal sample until it vaporizes. As the metal vaporizes, it will rise until it reaches the substrate where it solidifies. Metals that can be deposited in this manner include chromium, gold, titanium, aluminum, copper, and nickel. The electron beam deposition system used for this project is the Airco Temescal Model BJD-1800. The main components of the system include a stainless steel, high-vacuum chamber with substrate holder designed to hold four 100 mm diameter substrates, a pumping system consisting of a mechanical pump, a diffusion pump, and automated interlocks, and the electron beam source with capability of housing 6 different targets. The process control for the BJD-1800 system is fully automated to run pre-programmed steps.
**Scanning electron microscopy** (SEM) determines information about a sample’s topography by using a beam of high-energy electrons to scan the surface. The clearest SEM images result when the sample is dried on a clean silicon wafer. The SEM used was a Hitachi S-4500 II cold field emission SEM with EDAX. It is has a resolution of 4nm when operated with an accelerating voltage of 1 kV.

### 2.2 Silica Nanoparticles

Silica is a common name for silicon dioxide (SO$_2$). The density of silica nanoparticles is 1.96 g/cm$^3$. The silica nanoparticles used to create the nanoswimmers are approximately 100 nm or 500 nm in diameter and were purchased from Mircospheres-Nanosphere (catalog nos. C-SIO-G0.1 and C-SIO-G0.5, respectively). The particles have a plain surface and are embedded with fluorescent dye. The dye used is fluorescein isothiocyanate (FITC), which has an excitation wavelength of 495 nm and an emission wavelength of 510 nm. The purpose of the fluorescent dye is to allow the particles to be tracked using a micro-particle image velocimetry (µPIV) system. Figure 2.1 shows the nanoparticles on a silicon wafer viewed with SEM.

![SEM Image](image)

**Figure 2.1:** 500 nm silica nanoparticles viewed using SEM.

#### 2.2.1 100 nm Particle Preparation

The 100 nm particles were delivered suspended in water with a concentration of 25 mg/mL. At this concentration, the solution is approximately 1.4% particles by volume. Initially,
the particles were placed on a glass microscope slide that was cleaned with isopropyl alcohol then acetone and dried with nitrogen. The particles were deposited on the glass slide in aliquots of 10 µL, 25 µL, 50 µL, and 100 µL, as shown in Figure 2.2. It was determined that the 100 µL and 50 µL aliquots were too large because the particles started to flake off the slide once it dried.

![Figure 2.2: Glass slide with dried nanoparticle solution.](image)

After the solution had dried, the samples were examined using an optical microscope with a 20x objective. It was found that lines formed after the liquid had evaporated as shown in Figure 2.3 for the 10 µL aliquot of uncoated, dried nanoparticles. Figure 2.4 shows the 100 µL aliquot of dried nanoparticles. The lines formed by the drying solution are larger and the particles started to flake off of the slide.

![Figure 2.3: Uncoated, dried nanoparticles on glass slide, 10µL aliquot, 20x objective.](image)
After examining the samples with SEM, it was determined that there were areas that had more than a single layer of nanoparticles. The concentration of the 1.4% solution was too high to ensure that the particles would form a monolayer when dried on a substrate. Therefore, a 0.75% solution was prepared. The 0.75% solution was deposited on a glass slide, a poly(methyl methacrylate) (PMMA) slide, and a silicon wafer. As shown in Figure 2.5, the solution on the PMMA slide did not dry in a smooth circle for the 25 µL and 10 µL aliquots and it was peeling off the slide surface for the 50 µL aliquot, so PMMA was no longer used as a substrate to dry the nanoparticles.

The particles that dried on the silicon wafer and the glass slide were coated with 10 nm of nickel using electron beam deposition. The particles were viewed using the electron microscope, but there was no evidence that the coating had adhered to the particles. The particles had to be
resuspended then dried again to change their orientation in order to view the coating. The first method of resuspension was to add a small amount of water on top of the coated particles and then use a micropipette to rapidly pipet the water up and down to release the particles. In the second method, the particles were released by submerging the slide and wafer in water in a large petri dish and then sonicingating for 3 minutes. Along with releasing some of the particles, the black Sharpie markings used to identify the particles were also released from the glass slide, which contaminated the particles. Due to the size and thickness of the standard microscope slides used, nearly 50 mL of water was needed to cover the slide before sonication, which leads to a very dilute solution once some of the particles were resuspended.

In order to increase the concentration of the coated particle solution, a smaller and thinner substrate was needed. Instead of a standard microscope slide, microscope coverslips were used, which were 18 mm x 18 mm and a fraction of a millimeter thick rather than 3 in. x 1 in. x 1 mm for the microscope slide. Due to the smaller size, a small petri dish could be used to submerge the coverslips and only 3 mL of water was needed to completely cover the coverslip.

Several glass coverslips were cleaned with isopropyl alcohol then acetone. Aliquots of 15 µL of the 0.75% solution and 1:5 and 1:10 dilutions of the 0.75% solution were deposited on the coverslips and allowed to dry. One set was prepared for nickel deposition and one was prepared to be the control to compare with the nickel-coated particles. The particles were coated with 20 nm of nickel using electron beam deposition. After the particles were resuspended and dried on a clean substrate, there was again no evidence to suggest that the coating remained on the particles.

Next, attempts were made to clean the particles with a plasma cleaner and with hydrochloric acid. The cleaned particles along with a set of uncleaned particles were deposited on glass slides, a silicon wafer, and a copper foil. These particles were coated with 20 nm of
chromium then 10 nm of nickel. The chromium was used as an adhesion layer between the silica and the nickel layer. The particles were removed from the copper surface by scraping them off and depositing them on a clean substrate. There were a few isolated particles that appeared to have the coating as shown in Figure 2.6. However, after resuspension using sonication, there was no evidence that the nickel remained on the particles.

![Interface between Ni Coating and Glass Particle](image)

Figure 2.6: SEM image of 100 nm particles with chromium and nickel coating.

### 2.2.2 500 nm Particle Preparation

After numerous failed attempts at coating the 100 nm particles, 500 nm particles were purchased. Other research groups had success coating 1 µm particles (Ke, Ye, Carroll, & Showalter, 2010); however, 1 µm is too large to be used in a nanoscale system, so the 500 nm particles were selected.

First, the solution was diluted with water to create concentrations of 1:1, 1:10, 1:20, and 1:50. The solutions were dried on a silicon wafer that was cleaned with isopropyl alcohol and acetone and then dried with nitrogen. The particles were examined using SEM. As seen in Figure 2.7, there are areas were the particles did not dry in a monolayer with the 1:1 dilution. Therefore, it was determined that the 1:1 dilution was too concentrated to use. Figure 2.8 shows the dried 1:20 dilution. The particles were spread out and there were no areas where particles were stacked.
The 500 nm particles were coated with 10 nm of chromium and 20 nm of nickel using electron beam evaporation. Figure 2.9 shows the successfully-coated particles. The particles were then released from the silicon wafer by sonicating for 2 minutes in water. It was verified that the particles were released by examining the silicon wafer with the SEM. No particles remained on wafer. After the particles were resuspended in water, a sample was deposited onto a
clean silicon wafer. As shown in Figure 2.10, the coating remained on the 500 nm particles after they were resuspended using sonication.

Figure 2.9: 500 nm silica particles with nickel coating on top hemisphere.

Figure 2.10: Resuspended particles with nickel coating.
2.3 Addition of BC to Particles

Once the 500 nm particles were successfully coated and resuspended in water, the enzyme could be added to the nickel coating. Because a relatively small amount of particles were resuspended in 3 mL of water, the concentration of the solution was low. The 3 mL of solution was split into two 1.5 mL samples. One sample contained the solution which would have enzyme added. The other was the control which would not have enzyme added. In order to have a higher concentration, the particles were centrifuged at 2200 relative centrifugal force (rcf) for 15 minutes, causing a pellet of particles to form at the bottom of the tubes. The supernate was removed leaving just the coated particles. Next, 100 µL of wild-type BC at a concentration of 6 µg/mL was added to the first sample and the pellet, and 100 µL of a dialysis buffer without enzyme was added to the control sample. The pellets in both samples were resuspended by repeatedly pipetting the solution. The samples were allowed to incubate for 5 minutes. In order to wash the excess enzyme from the particles, both samples were centrifuged at 2200 rcf for 15 minutes. Again, the supernate was removed, 100 µL of the dialysis buffer was added to each sample, and the pellets were resuspended. The particles were centrifuged at 2200 rcf once more and the particles were resuspended in 50 µL of dialysis buffer.

The first assay performed to determine whether the enzymes were attached to the particles was a Bradford protein assay. A Bradford assay is a spectroscopic procedure used to measure the concentration of a protein based on an absorbance shift of the Coomassie Brilliant Blue G-250 dye in which the dye is converted to a bluer form when it binds to the protein being assayed. To perform the assay, 25 µL of the sample was added to 775 µL of water and 200 µL of Bradford solution. This was done for the sample with enzyme and the control. The solutions were put into cuvettes. The spectrophotometer was zeroed using a sample that contained 800 µL of water and 200 µL of Bradford solution. The absorbance was measured at a wavelength of 595
nm. The absorbance of the control sample was 0.28 AU and the sample with enzyme was 0.45 AU. The higher absorbance of the second sample indicates that the enzyme was present on the particles.

A second type of assay, known as a PK/LDH coupled assay, was run to determine whether the enzyme was present and active on the particles. This assay indirectly measures the production of ADP by BC by using pyruvate kinase (PK) and lactate dehydrogenase (LDH). PK uses ADP and phosphoenolpyruvic acid (PEP) as substrates to generate pyruvate and ATP. The pyruvate generated by PK is a substrate for LDH along with the reduced form of nicotinamide adenine dinucleotide (NADH). NADH reduces the pyruvate to lactate and also produces nicotinamide adenine dinucleotide (NAD). NADH absorbs light at 340 nm while NAD does not absorb light at this wavelength. Therefore, as BC produces ADP, NADH is oxidized to NAD and the absorbance decreases.

Additional particles were coated and resuspended in the same method described above. The solution containing the coated particles was divided into two samples. The two samples were centrifuged at 2,200 rcf and the supernate was removed. In one sample, 300 µL of wild-type BC (4.3 µg/mL) was added and allowed to incubate for 5 minutes. The increased amount of enzyme used was to account for the larger number of particles that were resuspended. In the second sample, 300 µL of dialysis buffer was added. The particles were washed several times to remove any excess enzyme that did not adhere to the nickel coating.

The cocktail in Table 2.1 was prepared for the PK/LDH assay. Three samples were run as shown in Table 2.2. Sample #1 contained enzyme that had not been added to particles to test the activity of the enzyme. Sample #2 was a control that contained coated particles without enzymes. Sample #3 contained 50 µL of the solution containing the particles with enzyme added.
Table 2.1: Cocktail for enzyme assay.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK</td>
<td>17,696 units/mL</td>
<td>0.997</td>
</tr>
<tr>
<td>LDH</td>
<td>12,750 units/mL</td>
<td>0.83</td>
</tr>
<tr>
<td>PEP</td>
<td>20 mM</td>
<td>12.5</td>
</tr>
<tr>
<td>NADH</td>
<td>20 mM</td>
<td>8.75</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>2 M</td>
<td>5.0</td>
</tr>
<tr>
<td>KHCO₃</td>
<td>250 mM</td>
<td>6.5</td>
</tr>
<tr>
<td>HEPES</td>
<td>1 M</td>
<td>50.35</td>
</tr>
<tr>
<td>ATP</td>
<td>8 mM</td>
<td>0.625</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>5.073</td>
</tr>
</tbody>
</table>

Table 2.2: PK/LDH assay samples.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>337.5 µL</td>
<td>297.5 µL</td>
<td>297.5 µL</td>
</tr>
<tr>
<td>Cocktail</td>
<td>90 µL</td>
<td>90 µL</td>
<td>90 µL</td>
</tr>
<tr>
<td>Biotin (400 mM)</td>
<td>62.5 µL</td>
<td>62.5 µL</td>
<td>62.5 µL</td>
</tr>
<tr>
<td>Enzyme Solution</td>
<td>10 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

The data collected for the assay was curve fit with a linear regression. Only the data collected in the first minute was used because the particles began to select after this time and the assay was no longer accurate. The slope of the linear regression indicates the rate of the reaction catalyzed by BC; therefore, a more negative slope is indicative of the presence of more BC molecules in the solution. The data gathered is shown graphically in Figure 2.11: PK/LDH assay results. The slopes and the correlation coefficient for the linear regression of Figure 2.11: PK/LDH assay results, are shown in Table 2.3. The results show that the slope for sample #3 is more than three times larger than the slope for sample #2 and comparable to the one from sample #1. Therefore, it is clear that the enzyme was present on the particles in sample #3, indicating that the nanoswimmer fabrication method for the 500 nm particles was successful.

Table 2.3: PK/LDH assay results

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope/Rate (AU/min)</td>
<td>-0.0201</td>
<td>-0.0059</td>
<td>-0.0184</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9709</td>
<td>0.4991</td>
<td>0.8938</td>
</tr>
</tbody>
</table>
After the assays were conducted, an aliquot of sample #2 was viewed using the SEM. It was confirmed that the nickel coating remained on the particle even after they were centrifuged several times. The results are shown in Figure 2.12.

Figure 2.12: Coating remained on particles after centrifugation.
CHAPTER 3: ESTIMATION OF NANOSWIMMER PERFORMANCE

In this chapter, we present a theoretical analysis of the propulsion characteristics of the BC-driven nanoswimmers.

3.1 Power Estimation

The values of the various material properties needed in the following calculations are given in Table 3.1.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of water</td>
<td>998.2 kg/m$^3$</td>
</tr>
<tr>
<td>Density of silica</td>
<td>1.96 g/cm</td>
</tr>
<tr>
<td>Viscosity of water</td>
<td>1.002 \times 10^{-3} Ns/m$^2$</td>
</tr>
<tr>
<td>Boltzmann Constant</td>
<td>1.381 \times 10^{-23} m$^2$kg/s$^2$K</td>
</tr>
<tr>
<td>Temperature</td>
<td>20ºC</td>
</tr>
</tbody>
</table>

Recall from Figure 1.2 that in the first half-reaction BC catalyzes the ATP dependent carboxylation of biotin. When one of the phosphate groups is removed from an ATP molecule, it becomes adenosine diphosphate (ADP). For this decomposition, the change in Gibbs free energy is $-30.5$ kJ/mole (Berg, Tymoczko, & Stryer, 2007). Based on Avogadro’s number ($6.022 \times 10^{23}$), the change in Gibbs free energy per molecule is $-5.065 \times 10^{-20}$ J. Due to the fact that the chemical reaction that is catalyzed by BC produces one molecule of ADP and the enzymatic frequency is approximately 2 Hz, each BC enzyme is capable, under 100% efficiency, of producing $1.013 \times 10^{-19}$ Watts.

It is assumed that half of the surface area of the particle is coated in nickel and that each enzyme has a footprint of 8 nm x 8 nm. In order to avoid steric interference and hydrodynamic interaction between molecules of BC, the density of BC molecules on each nanoswimmer will be limited. Therefore, we assume that only 66.7% of the coated surface area is covered with BC molecules. Figure 3.1 shows the number of enzymes expected to adhere to the nickel surface.
based on the particle diameter. For a 100 nm particle, it is expected that 164 enzymes will attach to the particle. For a 500 nm particle, it is expected that 4093 enzymes will attach to the particle.

![Graph showing number of enzymes attached to nickel surface based on particle diameter.](image)

Figure 3.1: Number of enzymes attached to nickel surface based on particle diameter.

The drag force on a nanoswimmer in an infinite domain can be determined using Stokes law in (1.7). First, however, the maximum velocity for which Stokes law is still applicable must be determined. Using (1.1), Figure 3.2 shows the maximum velocity that results when $Re = 0.01$ for particles up to 2 µm in diameter. The maximum velocity is 10.03 cm/s and 2.01 cm/s for 100 nm and 500 nm particles, respectively. Figure 3.3 shows the drag force on a 100 nm and 500 nm spheres for various velocities calculated from (1.7).

![Graph showing maximum velocity for Stokes flow.](image)

Figure 3.2: Maximum velocity for Stokes flow.
Using the drag force previously determined, the power required from the BC molecules to overcome the drag force is given by

\[ P = F_{\text{drag}} \cdot u \]  

(3.1)

where \( u \) is the particle velocity. Figure 3.4 depicts the power required to overcome the drag force, calculated from (1.7) and (3.1), as a function of the particle velocity for the 100 nm and 500 nm particles. Figure 3.3 and Figure 3.4 indicate that for the same amount of force or power, the larger particle will have a larger velocity.
By looking at the efficiency of other biomolecular motors, an expected efficiency range for BC can be predicted. The maximum efficiency of F\textsubscript{1}-ATPase is at least 80% while kinesin is believed to have a maximum efficiency between 30% and 60% (Schmidt & Montemagno, 2004). The efficiency of bacterial flagella in swimming cells is approximately 5% (Meister, Lowe, & Berg, 1987). Since BC is more similar to F\textsubscript{1}-ATPase and kinesin than bacterial flagella so, we conjecture that BC has efficiency between 30% and 80%.

Now, based on the values given in Section 3.1, we can estimate the power of the BC-driven nanoswimmer as follows

\[ P_{NS} = \eta N P_{BC} \]

where \( P_{NS} \) is the nanoswimmer power, \( \eta \) is the efficiency, \( N \) is the number of enzymes attached to the particle, and \( P_{BC} \) is the maximum power produced by a single molecule of BC (1.013 x 10\textsuperscript{-19} Watts). Figure 3.5 and Figure 3.6 show a plot of (3.2) for three efficiency values relative to (3.1) for the 100 nm and 500 nm particles, respectively. From the plots, we can estimate the velocity that the nanoswimmer will have when placed in an infinite domain, low Reynolds number, fluidic environment. For example, from Figure 3.6, we can see that the 500 nm particle will be propelled at about 115 \( \mu \text{m/s} \) when \( \eta = 0.3 \). For comparison purposes, the 1 \( \mu \text{m} \) platinum/silica chemical motor developed by Howse et al. has a maximum velocity of 3 \( \mu \text{m/s} \) while the 370 nm diameter by 2\( \mu \text{m} \) nanorods developed by Paxton et al. have a maximum velocity of 6.6 \( \mu \text{m/s} \) (Ebbens & Howse, 2010). From this analysis, it is clear that even if the nanoswimmers had an efficiency of unity, the velocity is significantly less than the maximum velocity for which Stokes flow is a valid assumption.
Figure 3.5: Nanoswimmer power and power from drag force at various efficiencies for a 100 nm particle.

Figure 3.6: Nanoswimmer power and power from drag force at various efficiencies for a 500 nm particle.
3.2 Enzyme Distribution on Nanosphere

The efficiency parameter $\eta$ in (3.2) accounts for the efficiency of the chemical reactions in Figure 1.2 as well as the efficiency of the nanoswimmer itself. More precisely, if $\eta_c$ is the chemical efficiency and $\eta_m$ is the mechanical efficiency, then $\eta = \eta_c \eta_m$. The mechanical efficiency is affected by several factors, including the manner in which the nanoswimmer is fabricated. Specifically, the location and direction of the propulsion force of each BC molecule will be random due to nanoswimmer fabrication process described in Chapter 2. Thus, we write $\eta_m = \eta_r \eta_o$, where $\eta_r$ accounts for the random nature of the propulsion force and $\eta_o$ accounts for other mechanical-related factors. The following simulation attempts to model and quantify the effect of this random process on the nanoswimmer propulsion.

The number of enzymes excepted to adhere to a particle based on its diameter can be determined from Figure 3.1. One would expect the enzymes to be randomly placed on the nickel-coated hemisphere of the nanosphere. To simulate the placement of each enzyme on the nanosphere, two random variables varying from 0 to 1, $R_1$ and $R_2$, were used to determine the polar angle $\theta$ and the azimuthal angle $\phi$ using the following two equations

$$\theta = 2\pi R_1 \quad (3.3)$$
$$\phi = \cos^{-1}(1 - 2R_2). \quad (3.4)$$

For a nanosphere with a diameter of 150 nm, 368 enzymes are expected to adhere to the nickel hemisphere according to Figure 3.1. Figure 3.7 shows the results from the simulation for a 150 nm nanoswimmer, where each point represents an enzyme molecule. This particle diameter was chosen for illustration purposes only because it has enough enzymes to show the distribution on the sphere surface without overcrowding the figure. Note that in the following plots the $z$ axis is normal to the plane of the coated hemisphere.
Figure 3.7: Randomly placed enzymes for a 150 nm particle.

The case where $\eta_r = 1$ would correspond to the idealized nanoswimmer shown in Figure 3.8, where each enzyme produces a force along the $z$ axis. Unfortunately, the enzymes are not expected to all be aligned in the $z$ direction. Thus, the direction of the propulsion force of each enzyme will not be as in Figure 3.8.

Figure 3.8: Idealized nanoswimmer with enzymes oriented in the $z$ direction.

Figure 3.9 displays an axis along the radial direction of each molecule location shown in Figure 3.7. However, the enzymes will also not be exactly oriented in the radial direction because the histidine tag will form a flexible connection between the nickel coating and the enzyme.
To account for this, two additional random variables varying from 0 to 1, $R_3$ and $R_4$, were used to determine the orientation of the enzyme from the radial direction. To accomplish this, a local coordinate system $[x^*, y^*, z^*]$ was placed at each enzyme location such that the $z^*$ axis coincided with the outward normal. Using the inverse of the cumulative distribution function of a normal distribution, the angle of rotation about the $x^*$ and $y^*$ axes was determined using $R_3$ and $R_4$, respectively. A normal distribution with mean $\theta = 0$ and variance $\sigma^2 = 0.03$ was used for this purpose. The probability density function for this normal distribution is shown in Figure 3.10. Figure 3.11 shows the results of randomly orienting each enzyme on a 150 nm nanoswimmer.

Figure 3.9: Randomly placed enzymes with normal at each enzyme location for a 150 nm particle.

Figure 3.10: Normal distribution used to orient each enzyme, $\sigma^2 = 0.03$. 
Figure 3.11: Randomly located and oriented enzymes for a 150 nm particle, \( \sigma^2 = 0.03 \).

To allow for the enzymes to rotate further from the radial axis, the analysis was carried out two more times using a normal distribution with \( \theta = 0 \) and variances \( \sigma^2 = 0.065 \) and \( \sigma^2 = 0.135 \). The distributions are shown in Figure 3.12 and Figure 3.14. Figure 3.13 and Figure 3.15 show the randomly oriented enzymes on a 150 nm nanoswimmer when the angles are determined using these normal distributions. Comparing Figure 3.11, Figure 3.13, and Figure 3.15, we can see that as the variance of the normal distribution increase, the orientation of the enzymes expectably becomes more erratic.

Figure 3.12: Normal distribution used to angles to orient each enzyme, \( \sigma^2 = 0.065 \).
Figure 3.13: Randomly located and oriented enzymes for a 150 nm particle, $\sigma^2 = 0.065$.

Figure 3.14: Normal distribution used to angles to orient each enzyme, $\sigma^2 = 0.135$.

Figure 3.15: Randomly located and oriented enzymes for a 150 nm particle, $\sigma^2 = 0.135$. 

36
From the placement and orientation of each enzyme, the net force produced by the collection of enzymes on the nanosphere can be estimated. To this end, each enzyme is assumed to produce one unit of force in the direction it is oriented. To produce the results that follow, the location and orientation of the enzymes were randomly determined as previously described.

Let $i = 1, 2, \ldots, M$ be the index for the iterations (i.e., number of times the random procedure was run). Let $j = 1, 2, \ldots, N$ be the index for the enzymes, where $N$ is the total number of enzymes on the coated hemisphere. Let $F_j^i = [F_{jx}^i F_{jy}^i F_{jz}^i]$ be the unit force vector for enzyme $j$ in iteration $i$, i.e.,

$$
\|F_j^i\| = \sqrt{(F_{jx}^i)^2 + (F_{jy}^i)^2 + (F_{jz}^i)^2} = 1. \quad (3.5)
$$

The total force applied by the $N$ enzymes on the nanosphere for each iteration is given by

$$
F^i = [F_{x}^i F_{y}^i F_{z}^i] = \sum_{j=1}^{N} F_j^i = \left[ \sum_{j=1}^{N} F_{jx}^i \sum_{j=1}^{N} F_{jy}^i \sum_{j=1}^{N} F_{jz}^i \right], i = 1, \ldots, M. \quad (3.6)
$$

The average of the $M$ total force vectors is then

$$
\bar{F} = [\bar{F}_{x} \bar{F}_{y} \bar{F}_{z}] = \frac{1}{M} \sum_{i=1}^{M} F^i = \left[ \frac{1}{M} \sum_{i=1}^{M} F_{x}^i \frac{1}{M} \sum_{i=1}^{M} F_{y}^i \frac{1}{M} \sum_{i=1}^{M} F_{z}^i \right]. \quad (3.7)
$$

Based on the above equations, a simulation was carried out for nanoswimmers of 100 nm and 500 nm with normal distribution variances of 0.03, 0.065 and 0.135, and $M = 500$. The results are shown in Table 3.2 and Table 3.3.

**Table 3.3.**

<table>
<thead>
<tr>
<th>Mean</th>
<th>100 nm $\sigma^2 = 0.03$</th>
<th>100 nm $\sigma^2 = 0.065$</th>
<th>100 nm $\sigma^2 = 0.135$</th>
<th>500 nm $\sigma^2 = 0.03$</th>
<th>500 nm $\sigma^2 = 0.065$</th>
<th>500 nm $\sigma^2 = 0.135$</th>
</tr>
</thead>
<tbody>
<tr>
<td>x-direction</td>
<td>-0.2837</td>
<td>-0.5053</td>
<td>0.7472</td>
<td>-0.4735</td>
<td>-2.3152</td>
<td>0.1236</td>
</tr>
<tr>
<td>y-direction</td>
<td>0.1528</td>
<td>0.0465</td>
<td>-0.4093</td>
<td>1.0627</td>
<td>-1.6012</td>
<td>0.2277</td>
</tr>
<tr>
<td>z-direction</td>
<td>79.5088</td>
<td>76.9779</td>
<td>71.5433</td>
<td>1987.7</td>
<td>1917.2</td>
<td>1789.1</td>
</tr>
</tbody>
</table>
Table 3.3: Standard deviation of the average total force.

<table>
<thead>
<tr>
<th>Standard Deviation</th>
<th>100 nm $\sigma^2 = 0.03$</th>
<th>100 nm $\sigma^2 = 0.065$</th>
<th>100 nm $\sigma^2 = 0.135$</th>
<th>500 nm $\sigma^2 = 0.03$</th>
<th>500 nm $\sigma^2 = 0.065$</th>
<th>500 nm $\sigma^2 = 0.135$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x$-direction</td>
<td>7.1673</td>
<td>7.2879</td>
<td>7.0868</td>
<td>34.9279</td>
<td>35.5042</td>
<td>36.9527</td>
</tr>
<tr>
<td>$y$-direction</td>
<td>7.5255</td>
<td>7.7278</td>
<td>7.6323</td>
<td>36.4594</td>
<td>39.5682</td>
<td>37.8933</td>
</tr>
</tbody>
</table>

From the simulation results, as expected, the force on the 500 nm particle is significantly higher than that for the 100 nm particle. This is due to the larger number of enzymes expected to adhere to the larger nanoswimmer. There is no discernible trend for the total force in the $x$ and $y$ directions for the different variances for each size particle. However, the force in the $z$ direction decreases as the variance increases.

When comparing the standard deviations, the results in Table 3.3 can be deceptive. It shows that the standard deviation is much smaller for the 100 nm nanoswimmers. A normalized standard deviation $\sigma^*$ was determined by

$$\sigma^* = \frac{\sigma}{\|\mathbf{F}\|} \quad (3.8)$$

where $\mathbf{F}$ was defined in (3.7). This equation was then multiplied by 100 to show the results as a percentage of the norm of the total force. The results are given in Table 3.4. As expected, the normalized standard deviation increases as the variance increases. The normalized standard deviation in the $x$ and $y$ directions have a similar magnitude for each case. However, the normalized standard deviation in the $z$ direction is roughly 60% of that for the $x$ and $y$ directions. The normalized standard deviation for the 500 nm nanoswimmers is about 20% of the value for the corresponding 100 nm nanoswimmer. Due to the substantially larger number of enzymes on the larger nanoswimmer, it is expected that the total force will be less affected by the random nature of the enzyme placement and orientation, which accounts for the smaller normalized standard deviation in the 500 nm nanoswimmer.
Table 3.4: Standard deviation of the average total force normalized using the average total force.

|                | 100 nm $\sigma^2 = 0.03$ | 100 nm $\sigma^2 = 0.065$ | 100 nm $\sigma^2 = 0.135$ | 500 nm $\sigma^2 = 0.03$ | 500 nm $\sigma^2 = 0.065$ | 500 nm $\sigma^2 = 0.135$
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>y-direction</td>
<td>9.4649</td>
<td>10.0388</td>
<td>10.6673</td>
<td>1.8343</td>
<td>2.0639</td>
<td>2.1180</td>
</tr>
<tr>
<td>z-direction</td>
<td>4.8968</td>
<td>5.2759</td>
<td>6.0291</td>
<td>0.9731</td>
<td>1.0454</td>
<td>1.2575</td>
</tr>
</tbody>
</table>

Figure 3.16 and Figure 3.17 show the direction of the total force vector from the enzymes on a 100 nm and 500 nm particle, respectively, with a variance of 0.135. Note that the length of the arrow is not indicative of the magnitude of the force vector. It is evident from the figures that the net force produced by the collection of enzymes on the coated hemisphere is virtually aligned with the $z$ axis. That is, the simulation indicates that nanoswimmer will be propelled by the enzymes in the direction normal to the plane of the coated hemisphere.

Figure 3.16: Direction of resulting force on the 100 nm particle, $\sigma^2 = 0.135$. 

![Diagram showing direction of force](image-url)
Figure 3.17: Direction of resulting force on the 500 nm particle, $\sigma^2 = 0.135$.

Next, the random-related mechanical efficiency of the nanoswimmer $\eta_r$ was estimated by dividing the total force by the number of enzymes on the nanoswimmer, i.e.,

$$\eta_r = \frac{F}{N}. \tag{3.9}$$

In (3.9), we used the fact that, due to (3.5), the total force of the idealized nanoswimmer in Figure 3.8 is equal to $N$. The random efficiency was determined for 100 nm and 500 nm particles with variances of 0.03, 0.065, and 0.135. The results are given in Table 3.5. The random efficiency decreases as the variance increased. However, it appears to be independent of the size of the nanoswimmer. The values in Table 3.5, although only accounting for effects from the random location and orientation of the enzymes on the nanosphere, are within the expected efficiency range suggested in Section 3.1.
### Table 3.5: Estimated value for $\eta_r$.

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>100 nm</th>
<th>500 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma^2 = 0.03$</td>
<td>0.4853</td>
<td>0.4855</td>
</tr>
<tr>
<td>$\sigma^2 = 0.065$</td>
<td>0.4689</td>
<td>0.4682</td>
</tr>
<tr>
<td>$\sigma^2 = 0.135$</td>
<td>0.4373</td>
<td>0.4367</td>
</tr>
</tbody>
</table>
CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 Conclusions

A method to fabricate the proposed BC-driven robotic nanoswimmers has been determined. After several failed attempts to use 100 nm silica nanoparticles, 500 nm silica particles were used. Using a 10 nm layer of chromium as an intermediate adhesion layer, the particles were successfully coated with 20 nm layer of nickel with electron beam evaporation. Wild-type BC was attached to the nickel coating. The presence and activity of the enzymes attached to the particles were confirmed using Bradford and PK/LDH coupled assays. Thus, the nanoswimmer was successfully fabricated.

An analysis was performed to first determine the drag force on a nanoswimmer then to establish whether the enzymes would produce enough power to overcome this drag force. From this analysis, estimations were made about the expected velocity of the nanoswimmers based on its diameter. Using a set of random variables, the location and orientation of the enzymes on the nickel coating was modeled. Several different normal distributions were used in the simulation to understand the effect of the enzyme orientation. As the enzymes were allowed to rotate further from the normal direction, the total force decreased. Additionally, the normalized standard deviation of the total force decreased with a larger particle size.

The analysis showed that a 500 nm nanoswimmer would have a velocity of approximately 115 \( \mu \text{m/s} \) assuming the total efficiency is 30%. The 100 nm nanoswimmer is expected to have a velocity of approximately 50 \( \mu \text{m/s} \) with the same efficiency. The components of the force due to the enzymes in the \( x \) and \( y \) directions are expected to be negligible and therefore, the resulting force will be in the \( z \) direction. The mechanical efficiency of the
nanoswimmer due to the random placement of the enzymes is expected to be between 43.5% and 48.5% depending on the extent to which the enzymes deviate from the radial direction.

The theoretical random-related mechanical efficiency and the expected chemical efficiency based on the efficiency of other biomolecular motors indicates that BC will be able to convert the chemical energy released from the oxidation of ATP to ADP into non-Brownian motion of the nanoswimmer. Furthermore, the analysis shows that the BC driven nanoswimmers will have a velocity that is much greater than other microscale chemical motors.

4.2 Future Work

The next step of this project is to experimentally determine the non-Brownian motility properties of the nanoswimmer. The fluorescent dye in the silica nanoparticles can be used to track the nanoswimmer motion with a μPIV.

4.2.1 Experimental Controls

Two control experiments should be executed to compare with the experimental sample. The first control experiment would use silica nanoparticles that have been half-coated with nickel, but do not have BC attached to the nickel coating. The nanoparticle solution should include the three substrates of BC: biotin, ATP, and bicarbonate.

The second control experiment would contain silica nanoparticles that have been half-coated with nickel and have BC attached to the nickel coating. However, one of the enzyme’s substrates would not be present in the solution, so that BC will not be active.

The experimental sample would contain silica nanoparticles that have been half-coated with nickel and have biotin carboxylase attached to the nickel coating. The solution would include all three of the enzyme’s substrates. It is expected that this sample will lead to non-Brownian motion of the nanoswimmers.
4.2.2 μPIV

When the particles are in solution, the movement can be viewed using a μPIV. For this experiment, the μPIV system consists of a microscope, a camera, and a dichromatic filter. The microscope uses a dichromatic filter that is compatible with the excitation and emission wavelengths of FITC fluorescent dye in the nanoparticles. The particles can be viewed using objectives ranging from 20x to 80x.

The microscope used in the μPIV system is an Olympus UIS2 series microscope, while the fluorescence mirror unit is an Olympus U-MNIB3 unit. The excitation filter (BP470-495) transmits light between 470 nm and 495 nm. The emission filter (BA510F) transmits light with a wavelength greater than 510 nm. The dichromatic mirror (DM505) transmits light with a wavelength greater than 505 nm and reflects light with a wavelength less than 505 nm.

In order to view the movement of the nanoswimmers, a viewing chamber has to be designed to use with the μPIV. This viewing chamber is placed on the microscope where a typical sample would be placed. The viewing chamber could be made out of two glass microscope coverslips with a 9mm diameter, 0.12 mm deep Secure-Seal™ spacer (Invitrogen, Catalog No. S-24737). A diagram of the viewing chamber is shown in Figure 4.1. The particles are viewed normal to the circle in the Secure-Seal Spacer. For the 500 nm nanoswimmers, the spacers could be stacked in order to create a deeper well for the nanoswimmers to move.
Figure 4.1: Diagram of the viewing chamber.
REFERENCES


VITA

Rachel Yates was born in 1989 in Metairie, LA. She attended Academy of the Sacred Heart in New Orleans, LA for high school. She graduated Cum Laude with a Bachelor of Science in Mechanical Engineering from Louisiana State University in May 2011. She participated in the department’s Accelerated Masters Program. She is a candidate for a Master of Science in Mechanical Engineering to be awarded in August 2012.