The Pennsylvania State University

The Graduate School

Department of Chemistry

THE CONTROL, ASSEMBLY, SHAPE-DEPENDENT AND MATERIAL-DEPENDENT STUDIES OF ULTRASONICALLY PROPELLED NANO- AND MICROMOTORS

A Dissertation in

Chemistry

by

Suzanne Ahmed

© 2015 Suzanne Ahmed

Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

August 2015

The dissertation of Suzanne Ahmed was reviewed and approved* by the following:

Thomas E. Mallouk Evan Pugh Professor of Materials Chemistry, Physics and Biochemistry Dissertation Advisor Chair of Committee

Ayusman Sen Distinguished Professor of Chemistry

Benjamin Lear Assistant Professor of Chemistry

Tony Jun Huang Professor of Engineering Science and Mechanics

Barbara H. Garrison Shapiro Professor of Chemistry Head of the Department of Chemistry

*Signatures are on file in the Graduate School

ABSTRACT

Fuel free, low power, ultrasonic propulsion of nano- and microscale motors is currently among the leading candidates for use for biomedical applications such as drug delivery and holds potential for use in a variety of other applications. It does not rely on a finite supply of fuel, provides autonomous motion and promises to be biocompatible. In order to realize its potential for use in biological systems as well as other applications it must be, its biocompatibility demonstrated, the assembly behaviors of the propelled nanomotors explored, and the factors affecting motion interrogated. This document aims to shed light on these various aspects of ultrasonic motor propulsion.

An overview of the field of nanomotors, including different propulsion mechanisms, types of motion and the challenges of propulsion at a low Reynolds number are provided in Chapter 1.

In Chapter 2, the steering of nanorod motors in biocompatible media towards living cells was demonstrated. By incorporating a magnetic nickel segment (40 \pm 5nm) within a gold-ruthenium rod that is 300 \pm 30nm in diameter and 4.3 \pm 0.2 μ m long, it is possible to use a small external magnetic field to define the motor path. It is possible to suppress random motion, as quantified by the decrease in the mean displacement angle and rotation diffusion coefficient of the rods, and exert relatively fine control over the steering of acoustically propelled nanomotors using a 40milliTesla external magnetic field. Rods maintain autonomy and can be selectively guided toward single cells with micron level precision. The effect of the exposure to ultrasonic power within the acoustic chamber on living cells in the presence of metallic nanorods was evaluated, and for durations as long as 20 minutes biological cells remain alive.

In Chapter 3, the assembly behavior of magnetic, steerable nanorods is explored. It was found that segmented gold-ruthenium nanorods (300 ± 30 nm in diameter and 2.0 ± 0.2 µm in length) with thin Ni segments at one end assemble into few particle, geometrically regular dimers, trimers and higher multimers while levitated in water by ~4 MHz ultrasound at the midpoint of a cylindrical acoustic cell. The assembly of the nanorods into multimers is controlled by interactions between the ferromagnetic Ni segments. These assemblies are propelled autonomously in fluids by excitation with ultrasound and exhibit several distinct modes of motion. Multimer assembly and disassembly are dynamic in the ultrasonic field. The relative numbers of monomers, dimers, trimers, and higher multimers are dependent upon the number density of particles in the fluid and their speed, which is in turn determined by the ultrasonic power applied. The magnetic binding energy of the multimers estimated from their speed-dependent equilibria is in agreement with the calculated strength of the magnetic dipole interactions. These autonomously propelled multimers can also be steered with an external magnetic field and remain intact after removal from the acoustic chamber for SEM imaging.

In Chapter 4, the shape dependent motion of micromotors in the acoustic chamber is explored. Micromotor structures are designed to interrogate the effect of various levels of shape asymmetry, aspect ratio and rotational asymmetry. Structures of precise shapes and dimensions are fabricated using a combined photolithography and electrodeposition or evaporation approach that allows for the post synthesis release of structures from the substrate for testing within the acoustic chamber. Structures with dimensions larger than 20µm were shown to not undergo random autonomous motion while structures with dimensions smaller than 10µm undergo rotational random autonomous motion. These structures show a consistent polarity of motion indicating that the shape asymmetry of the structure indeed has an effect on its motion. This is in support of the existing consensus that propulsion can be attributed to motor shape asymmetry.

In Chapter 5, various factors that affect nanorod ultrasonic propulsion are explored. The effect of a bimetallic nanorod composition, nanorod size and the location of various acoustic behaviors and their response to changes in the electronic signal are studied. These observations also provide insight into the mechanism of ultrasonic propulsion. Bimetallic nanorod motors, exhibit similar behaviors as their monometallic counter parts including random autonomous motion and nodal patterns. Interestingly, bimetallic rods exhibit a consistent polarity of motion with one metal end leading axial propulsion. While propulsion has been attributed to shape asymmetry, in the presence of material asymmetry, the polarity of motion is determined by material asymmetry in cases where there is a difference in density between the two metal segments. In these cases the material segment with a lower density leads motion. Speed comparisons between rods of different densities have shown that lower density rods travel faster than higher density rods explaining the reason why the lower density segment within a bimetallic rod leads. Where only a small density difference exists between the metal segments in a bimetallic rod, the shape asymmetry at the ends of the rod determines the polarity of motion. Insight into the primary force propelling motors was gained by interrogating the length dependent speeds of rods by varying their lengths. As the rods got longer, their speed was reduced which is in support of a streaming induced drag force dominated motion. This

was further supported by the correlation between the sharp reduction of acoustic streaming, as indicated by the disappearance of spinning chains, and the sharp reduction in axial propulsion speed of random autonomous motors within the levitation plane. The locations of the various nanorod behaviors within the acoustic chamber were noted.

TABLE OF CONTENTS

List of Figures	ix
List of Tables	xvi
Acknowledgements	xvii
Chapter 1 Introduction	1
References	9
Chapter 2 Steering Acoustically Propelled Nanowire Motors towards Cells in a Biologically Compatible Environment using Magnetic Fields	13
2.1 Introduction	13
2.2 Results and Discussion	14
2.2.1.Behavior of Nanomotors in Static Magnetic Fields	14
2.2.2.Magnetic Steering	18
2.2.3.Cell targeting and viability testing	20
2.3 Experimental Details.	22
2.3.1.Nanowire Growth and Characterization	22
2.3.2. Magnetic Field Characterization	23
2.3.3. Acoustic propulsion experiments.	24
2.3.4. HeLa Cell Growth and Viability Testing.	26
2.3.5. Nanowire Motion Tracking.	28
2.4 Conclusions	29
2.5 References	30
Chapter 3 The Self-Assembly of Nanorod Motors into Geometrically Regular	
Multimers and Their Propulsion by Ultrasound	35
3.1 Introduction	35
3.2 Results and Discussion	36
3.2.1 Modes of Motion of Assemblies.	38
3.2.2 Dynamic Equilibrium: Assembly and Disassembly of Multimers	40
3.2.3. Magnetic Manipulation.	47
3.2.4 Isolation and Imaging	48
3.3 Experimental Details	49
3.3.1 Nanowire Synthesis and Characterization	49
3.3.2. Acoustic Experiments	50
3.3.3. Equilibrium expressions for the density of multimers	52
3.3.4. Optical Imaging and Tracking	54
3.4 Conclusions	54

3.5 References	55
Chapter 4 Exploring the Shape Dependence of Acoustic Motor Propulsion	62
4.1 Introduction	62
4.2 Materials and Methods4.2.1 Motor Fabrication: Combined Photolithography and	64
Electrodeposition	67
4.2.2 Motor Fabrication: Combined Photolithography and Evaporation	74
4.3 Acoustic Testing	76
4.4 Electron beam Lithography Trials	81
4.5 Conclusions	82
4.6 References	84
Chapter 5 Experimental Insights into the Mechanism and Factors Affecting Ultrasonic Propulsion of Nanorod Motors	86
5.1 Introduction	86
5.2 Results and Discussion	87
5.2.1 Location of Nanorod Acoustic Behaviors	88
5.2.2 Motion of Bimetallic Rods	89
5.2.3 Length Dependence of Nanorod Speed	94
5.2.4 Controlling the Applied Forces on the Rods via Electronic Signal Manipulation	98
5.3 Experimental Details	101
5.3.1. Nanorod Fabrication, Characterization and Release.	101
5.3.2. Acoustic Chamber.	104
5.3.3. Determining the Width of a Spinning Chain	104
5.3.4. Optical Imaging and Tracking	105
5.4 Conclusions	106
5.5 References	107
Chapter 6 Conclusions and Future Possibilities	111
6.1 Introduction	111
6.2 Future Possibilities	111
6.2.1 Collective Behaviors	111
6.2.2 Biomedical Applications	116
6.3 Conclusions	118
6.4 References	119

LIST OF FIGURES

Figure 1-1 . Biological systems and some artificial motor mimics at different lengths scales. Top row: kinesin on a microtubule (nm), E.coli bacteria (μ m), lady bug (mm), man (m) Bottom row : nanocar (Tour group), nanowire motors (Mallouk group), robotic flies (Wood group) ¹ [reproduced from reference 1]2
Figure 1-2. Schematic diagram of the set up for electrochemical deposition of nanorods within porous alumina templates. Commercial porous alumina templates with a pore size of 300 nm are used. [reproduced from reference 33]4
Figure 1-3. Various motions within the acoustic chamber. A. A zoomed out optical micrograph of various motions of nanorods within the acoustic chamber including random autonomous motion, spinning chains and nodal aggregation. B. Optical micrograph of random autonomous motion with a schematic inset of the nature of the motion of the nanorods imaged
Figure 2-1. A representative FE-SEM image of the Au-Ni-Ru nanowire motors14
Figure 2-2. Representative nanowire tracks plotted from the x and y coordinates obtained from tracking nanowires, the bottom left of the screen is defined as the origin. Arrows on the tracks indicate direction of motion. (A) Representative tracks in the absence and (B) in the presence of a magnetic field; field direction is indicated by the large arrow
Figure 2-3. SQUID magnetometry measurements. (a) The hysteresis curve of a Au-Ni-Ru nanowire sample from -5 to 5T showing the saturation magnetization used to calculate the dipole of the wires. (b) Zoomed in hysteresis curve from -0.25 to 0.25 T showing the ferromagnetism of the nanowires
Figure 2-4. Nanowire motors within an acoustic cell can be confined within acoustic nodal locations eventually forming patterns such as spinning chains. (a) Multiple magnetic nanowire motors forming dynamic spinning chains within nodal lines. (b) Upon the application of an oscillating magnetic field the chain is rapidly disrupted and the nanowire motors follow varying trajectories not confined to the nodal lines. (c, d) Upon the removal of the field the chain re-organizes. Times of frames (a-d) are indicated. The scale bars represent 10µm
Figure 2-5. Top panel: Steered ensemble motion of wires moving at $(25 \pm 5) \mu$ m/s, showing (a) applier and (b) later times in the trajectory. Vallow tracking lines are
showing (a) earlier and (b) later times in the trajectory. Yellow tracking lines are superimposed on the final frame of the video. The wires undergo multiple guided U-turns. Times of frames (a-d) are indicated. Bottom panel: Autonomous steering of motors moving at $(8 \pm 3) \mu m/s$. Times of frames (e-h) are indicated and arrows show initial direction of motion in (e) and consequent guided sharp U-turns (f-h)20
Figure 2-6. Top panel: (a-c) Nanomotor targeting towards a HeLa cell. Nanowires were

guided to make multiple U-turns to demonstrate control before being steered towards the live cell. (see Video S5 of Supporting Information). **Bottom panel:** (d) Nanowires are moving parallel to a group of cells, with their direction indicated by

the arrow. (e) By turning the external field, nanowires are magnetically steered through an approximately 90° turn towards the cells
Figure 2-7. Magnetic field strength as a function of distance from the active area of the acoustic chamber for (A) the static field and (b) the handheld magnet used in steering experiments. Values shown are the average of three measurements. Error bars shown (in cases where they are visible outside the plot symbols) represent one standard deviation
Figure 2-8. Acoustic Chamber. The acoustic chamber was defined as a hole punched into layers of Kapton tape supported on a steel plate. A piezoelectric transducer was affixed to the steel plate on the opposite side. A glass cover slip was placed on top of the acoustic chamber during experiments. The levitation plane is at the midpoint of the height of the cell as shown in the schematic. A stack of magnets is moved in the plane of the microscope stage for steering within the levitation plane
Figure 2-9. A frame sequence of wires (a-e) traveling at 170 μm/sec being collectively steered to trace an arch. Scale bar represents 10 μm
Figure 2-10. Experimental results of the cell viability test. HeLa cells without or with acoustic exposure for 5, 10, or 20 min were cultured for 24 h and their viability was assessed with the WST-1 cell viability test
Figure 3-1. Nanorod monomers and multimers imaged in the levitation plane of an acoustic chamber. (a) Optical image of a monomer. (b) Linear dimer with magnetic segments touching face-to-face. (c) Bent dimer (d) Trigonal planar trimer. (e) Trigonal pyramidal trimer. (f) Tetrahedral tetramer. (g) Trigonal bipyramidal pentamer. (h) Octahedral hexamer. (i) Field Emission Scanning Electron Micrograph (FE-SEM) image of a representative nanorod with an EDS inset with red, green and blue false color representing Ni, Ru and Au. (j) Lower magnification optical image of multimers in the levitation plane of the acoustic cell. Some multimers are pointed out with white arrows. (Density: 0.014 nanorods/ μ m ² , Speed: 30 μ ms ⁻¹). Scale bars for (a)-(h) are 5 μ m. Scale bar for (j) is 20 μ m. Figure 3-8 show additional low-magnification images of multimers at low and high density of nanorods
Figure 3-2. Optical micrographs and schematic drawings of the most common modes of motion of dimers, trimers and tetramers. (a) Linear dimer. Top: one gold end leading, Bottom: rotating while translating. (b) Bent dimer. Top: nickel ends leading, Middle: rotating while translating, Bottom: spinning while translating in a nodal line. (c) Trigonal planar trimer. Top: a gold end leading, Middle: rotating while translating, Bottom: base spinning relative to leading wire of structure. (d) Pyramidal/T-shaped trimer: Top: nickel ends leading, Bottom: rotating while translating with nickel ends leading. (e)Tetrahedral tetramer. Top: a gold end leading, Middle: rotating while translating, Bottom: in a nodal line in an X-shaped structure with its two wire base rotating with respect to its two wire top. (g) Square pyramidal tetramer: Top: nickel ends leading, Bottom: rotating while translating nickel ends leading. The scale bars are 5 μm. Black arrows indicate translational motion and blue arrows represent rotations

Figure 3-3. Representative assembly and disassembly frame sequences of nanorod multimers. (a) Assembly of a trimer from a monomer and a dimer. (b) Disassembly of a trimer into a monomer and a dimer. (c) Assembly of a hexamer from two trimers. (d) Disassembly of a hexamer. White arrows and circles indicate motors involved. Scale bars are 5µm
Figure 3-4. Multimer density as a function of number density of particles in solution at different average monomer speeds. Expressions were derived for the equilibrium concentrations of monomers, dimer and trimers were used to fit the experimental data points. Fit lines are drawn for monomers, dimers and trimers, at (a) 17 ± 5 , (b) 30 ± 8 , and (c) $55\pm7\mu$ m s ⁻¹ . (d) van't Hoff plot of the propulsion energy dependence of the equilibrium constants of dimers and trimers
Figure 3-5. An example of wires interacting to dissociate a dimer. (a) A dimer, with an average internal angle of $83\pm2^{\circ}$, upon approaching an incoming monomer is perturbed as shown in frame B. A slight gap is observed between the dimer rods as the internal angle decreases to $55\pm3^{\circ}$. This distance between the monomer and dimer, shown by the double headed arrow, is approximately $1.4\pm0.1\mu$ m. (c) The dimer dissociates to form two monomers
Figure 3-6. Magnetic steering, manipulation and isolation of assemblies. (a) Optical image frame sequence of the deliberate steering of a dimer through sharp loops using an external magnetic field. Time stamps are indicated and tracking is shown as a yellow line. Scale bar is $10\mu m$. (b) Optical image frame sequence for magnetic manipulation of the mode of motion of a dimer. The dimer is made to go through full inversions as it propels. Scale bar is $5 \mu m$. (c-d) SEM images of a dimer (c) and trimer (d) pipetted out of the acoustic cell and dried on a conductive Si wafer. Scale bars are $1 \mu m$. These assemblies are pointed out with white arrows in lower magnification SEM image (e), scale bar $10 \mu m$
Figure 3-7. Schematic diagram of the acoustic chamber used. (a) The full acoustic chamber. (b) Zoomed in schematic of the acoustic chamber, showing the bulk standing wave that is set up between the base of the cell and the glass cover slip that causes the wires to levitate. Wires are not drawn to scale
Figure 3-8 (a) High density solution of individual nanorods and multimers. Some multimers are pointed out with white arrows. The monomers are moving at an average speed of 23 μ m s ⁻¹ and the density of the suspension is 0.023 rods/ μ m ² . (b) Low density suspension primarily composed of monomers and dimers. The monomers are moving at an average speed of 20 μ ms ⁻¹ and the density of the suspension is 0.0056 rods/ μ m ²
Figure 4-1 . Au nanowires that undergo random autonomous motion. One end is pointed and the other is concave. A. FE-SEM of Au nanowires grown at a current density of -2mA/cm^2 showing the different shapes of the two ends of the wire. One end is concave and the other is pointed. B. Zoomed in FE-SEM image of the concave end of the wire

- Figure 4-3. Mask 1. A. Round "bullet" shape (Dimensions: 20x10µm; 5 µm radius of curvature) B. Sharp "bullet" (Dimensions: 20x10um; 5 um indentation) C. Bullet to test effect of perimeter(Dimensions: 20x10um; 5 µm indentations, 2.5 µm thick wings) D. Bullet to test effect of perimeter 2 (Dimensions: 20x10µm; 2.5 µm indentations, 1.25 µm thick wings) E. Square arrow(Dimensions: 20x10µm; 5 µm height of arrow) F. Inverse Arrow(Dimensions: 20x10µm; 5 µm indentation) G. Equilateral triangle (30 µm side) H. Isosceles right triangle (28 µm sides, 40 µm hypotenuse) I. 30-60-90 right triangle (24 µm,32 µm,40 µm sides) J. 10:1 aspect ratio bullet (Dimensions: 10x1µm; 0.5µm radius of curvature) K. 10:1 aspect ratio bullet (Dimensions: 20x2µm; 1µm radius of curvature) L. 10:1 aspect ratio bullet (Dimensions: 40x4µm; 2µm radius of curvature) M. 10:1 aspect ratio bullet (Dimensions: 50x5µm; 2.5µm radius of curvature) N. 10:1 aspect ratio double concave control (Dimensions: 20x2µm; 1µm radius of curvature) O. Rotor (Dimensions: 20x25µm; 5.1µm indentations, 11 µm and 12 µm cuts on right and bottom) P. Rotor2 (Dimensions: 20x20µm; 5µm indentations, 11 µm and 12 µm cuts

- **Figure 4-6.** Electrochemically deposited motor structures within lithographic resist membranes. Structure dimensions are 100μm x50 μm and were produced by carrying out contact lithography using shapes H and I of mask 2. Structures were deposited anodically in a two electrode cell at -5.65μA/cm² for 45minutes on a silicon cathode. A.20x optical image of inverse arrow B.50x optical image of inverse arrow C. 20x optical image of square arrow D. 50x optical image of square arrow.73
- **Figure 4-7.** Electrochemically deposited motor structures within lithographic resist membranes. Structure is a 10:1 aspect ratio bullet (Dimensions: 50x5μm; 2.5μm radius of curvature) and was produced by carrying out contact lithography using

shape G of mask 2. Structures were deposited anodically in a two electrode cell at at -5.65μ A/cm ² for one hour and thirty minutes on a silver cathode. A. 5x dark field optical micrograph of sample. B. 20x optical micrograph of sample
Figure 4-8. Au right isosceles triangles (28 µm sides, 40 µm hypotenuse) produced by a combined projection photolithography and evaporation approach. A. 5x optical micrograph of structures on wafer after resist removal. B. 20x optical micrograph of structures after release from the wafer
Figure 4-9. A nodal aggregate of the right isosceles triangles (28 μm sides, 40 μm hypotenuse) placed within the acoustic chamber and suspended in the levitation plane
Figure 4-10 . A. Inverse arrow (6 x3 μ m, 2.6 μ m indentation) B. Square arrow (6 x3 μ m, 2.6 μ m protrusion) C. Arrow (6 x3 μ m, 2.6 μ m indentation, 2.6 μ m protrusion) D. Rotor (6 x4 μ m, 1.732 μ m height triangular cut 1 μ m into the structure with 2 μ m sides) E. Inverse arrow(4 x2 μ m, 1.732 μ m indentation) F. 1:1 aspect ratio Inverse arrow (3 x3 μ m, 1.5 μ m indentation) G. Inverse arrow with a smaller indentation than A (6 x3 μ m, 1.5 μ m indentation) H. Rectangular control (6 x3 μ m) I. Square control (3 x3 μ m)
Figure 4-11. A. Fabricated inverse arrow structures ($6 \times 3\mu m$, $2.6 \mu m$ indentation) clockwise: 20x bright field optical micrograph of structures on the wafer, a schematic or the structure, a dark field optical image of the released structure. B. Fabricated square arrow structures ($6 \times 3\mu m$, $2.6 \mu m$ protrusion) clockwise: 20x bright field optical micrograph of structures on the wafer, a schematic or the structure, a dark field optical image of the released structure. These two shapes serve as controls for each other
Figure 4-12. Inverse arrow motion. 20x Optical image frame sequence of inverse arrow motion. Time stamps at bottom of frames
Figure 4-13. The structure on the top is moving a clockwise manner at 63 μm/sec, indented part leading. The structure on the bottom is moving in a counterclockwise manner at 18μm/sec, also the indentation leading, but the motion is less directional. Time stamps at bottom of frames
Figure 4-14. The motion of these two identical shapes is different. The top arrow is moving counterclockwise with the pointed end leading. The bottom arrow is rotating clockwise hinged on one of its flat edges. Time stamps at bottom of frames80
Figure 4-15. The motion of this control arrow is clockwise with the flat end leading. Time stamps at bottom of frames
Figure 4-16. Shapes fabricated used e-beam lithography. Shapes A-C have identical dimensions of $(6x3\mu m)$ but with varying indentations. D. Square arrow control shape $(6x3\mu m)$ E. Smallest fabricated shape $(1x0.5\mu m)$, there is some rounding of the features. All unlabeled scale bars represent 1 μm . Shapes were difficult to release and retrieve

- Figure 5-3. A. Schematic diagram of the shapes at ends of the bimetallic nanorods. The segment deposited first replicates the convex silver sacrificial segment (back dashed circle) and is therefore concave. At the other end Rh, Ru and Pt acquire a flat shape while Au is typically convex. B. FE-SEM (field emission scanning electron microscopy) micrographs of the concave (deposited first) nanorod ends after the dissolution of the sacrificial silver segment and release from the membrane. C. FE-SEM images of bimetallic rods. The different metal segments are labeled. Top row: (metals listed in the order deposited) AuRh (2.3±0.3µm, Au: 1.3±0.2µm, Rh: 1.0±0.2µm); RhAu r (2.1±0.4µm, Rh: 1.04±0.3µm, Au: 1.04±0.1µm); RuRh (1.6±0.2µm, Ru: 1.3±0.1µm, Rh: 0.5±0.1µm); RhRu (2.6±0.1µm, Rh: 0.9±0.1µm, Ru:1.6±0.1µm) A short Au segment was grown in the center of the RhRu rod to allow for controlled Ru growth on top of Rh. In the absence of the Au segment, the Ru segment, deposited at a constant potential, overgrew within seconds on the catalytic Rh metal; Bottom row: (metals listed in the order deposited) AuRu (2.2±0.3µm, Au: 1.0±0.2µm, Ru: 1.0±0.1µm); RuAu (2.3±0.3µm, Ru: 1.2±0.3, Au: 1.1±0.2); AuPt (2.2±0.2µm, Au: 1.2±0.2µm, Pt: 1.0±0.2µm); PtAu (2.0µm±0.1, Pt: 1.0µm±0.1µm, Au: 0.9µm±0.08µm). All scale bars represent 1 µm.......92

- **Figure 5-6.** A. The amplitude (voltage peak to peak) dependence of the speed of autonomous axial motion of nanorod motors. B. The amplitude dependence of the width of a spinning chain. The dashed line marks the complete disbanding of the chain, which corresponds to the plateau in speed of the axially propelled rods. C. Frames showing the disbanding of a spinning chain with decreasing amplitude...100

Figure 5-7. X-Ray diffraction patterns of nanorods within the membrane with indexing. A. Diffraction pattern of AuRu rods. The wide Ru pattern of low intensity indicates the small grain size within the Ru segment. B. X-Ray diffraction pattern of Ru wires. Peaks are indexed. C. X-Ray diffraction pattern of AuRh wires with peaks indexed
Figure 5-8. The plot of the light intensity distribution of a spinning chain (10Vpp) that approximates a bell curve. The spread of the curve is used as the width of the chain. The inset is the zoomed out intensity data
Figure 6-1. Rods of different magnetic character. (yellow represents gold and green represents nickel) A. Gold rod with a magnetic stripe smaller than the diameter of the rod ensuring transverse magnetization. B. Gold rod with a magnetic stripe longer than the 300nm diameter of with a longitudinal magnetization. C. Gold rod containing a polypyrrole polymer segment with embedded Fe3O4 superparamagnetic nanoparticles (white represents polypyrrole, red represents 5nm to 20nm Fe3O4 superparamagnetic nanoparticles)
Figure 6-2. A. FE-SEM image of ferromagnetic transversely magnetizable nanorods. They are made out of gold with three nickel segments. B. TEM image of paramagnetic nanorods. They are made of gold with a polypyrrole segment with iron oxide nanoparticles. C. Zoomed in TEM image of polypyrrole segment containing Fe3O4 nanoparticles
Figure 6-3. Chemical motor motion within a PDMS microchannel network. Panels A-C show nanomotor tracks through the channel, with (a-d) in panel B showing the paths of 4 different nanomotors.[Reproduced from reference 15]117

LIST OF TABLES

Table 3-1. Equilibrium constants (μm^2) for dimer and trimer (reactions 1 and 2) at three
different monomer speeds. Standard deviations are indicated in parentheses43
Table 5-1. Bimetallic nanorods studied and the leading end in autonomous axial motion. The metal segments are listed in the order plated; hence the end of the first metal in the table is concave. *See SI. Due to deposition conditions Ru segments may have a lower density than Rh
segments
Table 5-2. Densities of pure metals 91

ACKNOWLEDGEMENTS

This work would not have been possible without the guidance of my advisor Prof. Tom Mallouk. I would like to thank him for the countless hours of discussion and revision that went into these chapters and the corresponding journal publications. I thank him for the time he invested in teaching me how to do good rigorous science. I thank him for the valuable discussions regarding my professional and career development. I would like to thank the Motors group for the intellectually stimulating questions and meetings that enriched my graduate experience and work. I would especially like to thank Prof. Ayusman Sen for welcoming me into the Motors group. I would like to thank Prof. Tony Huang for his collaboration and Dr. Darrell Velegol for useful discussions. I thank my committee member Prof. Ben Lear. I would like to thank my lab mentor Dr. Wei "Tiger" Wang. I would like to thank the Mallouk Group for their friendship and support. I would like to thank all my collaborators, including but not limited to: Dr. Mauricio Hoyos and Dr. Lamar Mair. This work would not have been possible without the support of the members of the Materials Research Institute (MRI) at Penn State including: Trevor Clark, Michael LaBella, Andrzej Mieckowski and Bangzhi Lui who helped train me on the various instruments that were necessary to complete my work.

This work would not have been possible without the unwavering support of my family. I would like to thank my mother, father and sister for their love, support and encouragement. None of my academic milestones could have been possible without them.

Introduction

Artificial nanoscale and microscale motors, like their macroscale counterparts, convert various forms of energy to mechanical motion. The field of artificial nanoscale and microscale motors has been largely inspired by the desire to mimic the versatility of function found in biological motor systems in different contexts. As such, a number of artificial motors ranging in size from tens of nanometers to microns have been fabricated to mimic biological systems.¹ For example the buckyball-based nanocar is in the same size regime as kinesin motor proteins that "walk" on microtubules within biological cells.² Nanorods, two to three microns in length and ~300 nm wide can consume fuel from their environment to power motion, can exhibit chemotaxis and phototaxis like bacteria. ³These, largely bimetallic motors, also respond to external stimuli, such as light, that can induce collective behaviors as would be seen in swarming bacteria.^{4,5} Recently biological entities, such as enzymes, have been studied in the context of enhanced diffusion in substrate gradients and as fluid pumps when fixed to a surface giving them a new functionality.⁶ In addition to gaining fundamental understanding into the powering and motion of nano and micro objects, while mimicking biology, the field of artificial nano and micromotors has been strongly driven by potential applications. Most prominent among these applications are uses in biomedicine such as drug delivery and microsurgery.^{7–9} Other applications include environmental clean-up, oil recovery, and crack detection and repair.^{10–12} The focus of my work has been on nanorod based motors that are sub-micron in diameter and 2 to 3 μ m in length and hence in the same size

regime as bacteria. Examples of artificial motors and natural motors in the same size regime can be seen in Figure 1-1.



Figure 1-1. Biological systems and some artificial motor mimics at different lengths scales. Top row: kinesin on a microtubule (nm), E.coli bacteria (μ m), lady bug (mm), man (m) Bottom row : nanocar (Tour group), nanowire motors (Mallouk group), robotic flies (Wood group)¹ [reproduced from reference 1]

Nanoscale and microscale motors operate in the low Reynolds number regime (10^{-4}) . The Reynolds number describes the ratio of inertial forces to viscous forces in a system and is expressed using equation 1-1 below, where ρ is the particle density, v is the velocity of the particle, L is the characteristic length of the particle and μ is the dynamic viscosity of the medium. Engineering autonomous motion at a low Reynolds number

presents its own set of challenges. Because viscous forces dominate motion of our rods, constant force is needed to keep them in motion, and sustained motion or "coasting" does not assist. Additionally the scallop theorem applies to our system, meaning that a reversible two-state (i.e., back-and-forth) motor cannot produce a net displacement. It is due to this theorem that most low Reynolds number motors have no moving parts.¹³ In these cases, asymmetry needs to be engineered into the system. Asymmetry is typically incorporated into the motor itself, such as material or shape asymmetry. Brownian motion also becomes apparent at length scales below one micron. Brownian motion tends to reduce the directionality of motor motion. Hence this parasitic effect must be overcome to enable net directional propulsion.

$$Re = \frac{\rho v L}{\mu} \tag{1-1}$$

There are two types of motion that can be engineered into our system, they are: autonomous motion and non-autonomous motion. Autonomous motion as the name suggests refers to motion in which each individual motor is propelled independently of motors in their vicinity, with an independent direction. As such autonomous motion requires the local conversion of fuel or transduction of energy to enable each motor to propel independently of the others. Non-autonomous motion occurs when rods are swept uni-directionally within an external forcing field. Autonomous motion provides the opportunity for the engineering of independent cooperative tasks between multiple motor populations to accomplish a more complex task. 1Autonomously moving motors have been the focus of my work. Many propulsion methods have been developed for nano and microscale motors. Autonomous motion tends to be driven by local gradients generated due to the activity of the asymmetric motor, either catalytic activity or otherwise (self-generated gradients). Examples of autonomously driven motors include those driven by local thermal gradients, self-thermophoretic, those driven by local concentration gradients, self-diffusiophoretic, and those driven by local ionic charge gradients, self-electrophoretic. The first nanorod-based autonomous motor system was a self-electrophoretic system driven by the catalytic decomposition of hydrogen peroxide fueled at the surface of a bimetallic rod. The system was discovered as a result of the collaboration between the Mallouk and Sen groups at Penn State in 2004.³ The nanomotors were fabricated using electrochemical deposition within porous anodic alumina membranes. An evaporated metal film on one side of the membrane served as the cathode within an electrochemical cell. (Figure 1-2) This is the method used for the fabrication of the majority of the motors in the coming chapters.





In order to fulfill their potential for uses in drug delivery and microsurgery nanomotors need to function within biological environments. The above mechanisms for autonomous motion either rely on a toxic fuel source, or they need for a temperature gradient of a few Kelvins that is incompatible with use in a biological system, or they cannot operate in a high ionic strength medium.¹ Hence a new biologically friendly method is needed for the autonomous propulsion of nanomotor systems. Such a method was discovered in 2012 as a result of collaboration between the Mallouk group at Penn State and Dr. Mauricio Hoyos at ESPCI in France. Acoustic waves were used to propel nanomotors. The use of this type of energy is attractive due to its biocompatibility and controllability via the control of the nature of the actuation signal. Acoustophoretic motors also offer the benefit of continuous operation for long durations as they are not reliant on a finite supply of fuel.

Acoustic Propulsion of Nanorods Motors

Acoustic energy can be used to propel metallic nanorods with shape asymmetry. Within an acoustic cylindrical chamber approximately 180 μ m in height and 5 mm in diameter a standing wave is set up at the cell's resonant frequency of approximately 4 MHz with a voltage peak to peak value of 10 V.¹⁴ This acoustic chamber is constructed by affixing a thickness mode piezoelectric transducer to the bottom of the stainless steel plate (or silicon wafer) and attaching 4 layers of Kapton tape with a hole punched in the center on the other side of the plate. A glass cover slip is used as a reflector. A function generator then provides the electronic wave to the piezoelectric at the frequency and

amplitude of choice. In the acoustic chamber, the nanorods are levitated to the approximate midpoint of the cell within the standing node of a vertical standing wave. Within the levitation plane nanorod motors exhibit several behaviors including axial autonomous propulsion, the formation of spinning chains and nodal aggregates. It is the axial autonomous propulsion of these rods that is the focus of my work.

The acoustic manipulation of microparticles has a long history within the field of microfluidics due to its ability to sort and focus a variety of microparticles in a nondestructive way without the need for labeling and other particle modification.^{15–18} Acoustic manipulation has been used for particle separations based on size and material properties, as well "container-less" studies on levitated particles that enable the elimination of substrate and surface effects.^{15,17} Most particle manipulation studies have used symmetric particles, typically of soft polymer or biological materials, such as polystyrene spheres or cells. In contrast, recent results have demonstrated random autonomous axial motion of nanorod particles, and these particles are shape asymmetric and made of metals.¹⁴

The two primary forces acting on particles microns in size in an acoustic field are the primary acoustic radiation force and the streaming induced Stokes drag force. The primary acoustic radiation force dominates microparticle motion for particles larger than 2 μ m in diameter.^{19,20} In addition to its dependence on size, this force depends on the material properties of the microparticle, in particular the density and compressibility of the particle relative to that of the medium. The primary acoustic radiation force is the force primarily responsible for the levitation of particles within a standing wave.¹⁸ The second force responsible for acoustophoretic motion is the streaming-induced Stokes drag force.^{21,22} It is the dominant force resulting in motion for spherical particles below 2 μ m in diameter.^{19,20}

Current Research on Acoustic Nanorod Propulsion

Since its discovery in 2012, fuel free acoustic propulsion of autonomous motors has seen considerable research activity.¹⁴ Effort has been directed at demonstrating acoustically propelled nanomotor biocompatibility and function in biological media (phosphate buffered saline, saliva and serum),^{23,24} targeting and control of their trajectories,^{23–25} actuation within living cells,²⁶ collective behaviors and assembly,^{27–} ²⁹functionalization as well as preliminary results into their drug delivery capabilities³⁰. In addition to research into the random autonomous propulsion of acoustic nanomotors, fundamental research and useful applications are emerging for the patterns attributed to nodal behavior, in particular spinning chains. The fastest rotation observed in a nanorod systems (at 2.5kHz) was observed within the spinning chains in an acoustic chamber similar to ours, and spinning chains have also been proposed for mixing functions within living cells.^{26,31} Figure 1-3 shows a zoomed out view of the various nanorod behaviors that occur within the acoustic chamber including: random autonomous motion and spinning chain formation. Theoretical and experimental explorations into the mechanistic details of ultrasonic propulsion have also been conducted, with acoustic streaming of fluid at the surface of asymmetric nanorod motors identified as the primary candidate for the cause of directional axial propulsion.^{14,24,32}



Figure 1-3. Various motions within the acoustic chamber. A. A zoomed out optical micrograph of various motions of nanorods within the acoustic chamber including random autonomous motion, spinning chains and nodal aggregation. B. Optical micrograph of random autonomous motion with a schematic inset of the nature of the motion of the nanorods imaged.

In this work, I present my contribution to this research effort. In Chapter 2, I present the results of work into the directional control of autonomous ultrasonic motors, their operation in biocompatible media and their effect on living cells while exposed to ultrasonic waves.²³ Chapter 3 presents results on the magnetically mediated self-assembly of ultrasonic motors into "artificial molecules", the unique modes of motion of these molecules and the analysis of the reaction equilibria and kinetics of the association and dissociation these artificial molecules in suspension.²⁷ Chapter 4, the design, fabrication and preliminary evaluation of the structure –function performance of ultrasonically propelled micromotors fabricated by photolithography are presented. In Chapter 5, insights into mechanistic details of ultrasonic propulsion are presented in the context of results regarding the material dependent propulsion of metallic and bimetallic nanorods, the size dependent propulsion as well as details into the location of various behaviors and the effect of actuation signal on these behaviors.

References

(1) Wang, W.; Duan, W.; Ahmed, S.; Mallouk, T. E.; Sen, A. Small Power: Autonomous Nano- and Micromotors Propelled by Self-Generated Gradients. *Nano Today* 2013, *8* (5), 531–534.

(2) Shirai, Y.; Osgood, A. J.; Zhao, Y.; Kelly, K. F.; Tour, J. M. Directional Control in Thermally Driven Single-Molecule Nanocars. *Nano Lett.* **2005**, *5* (11), 2330–2334.

(3) Paxton, W. F.; Kistler, K. C.; Olmeda, C. C.; Sen, A.; St Angelo, S. K.; Cao, Y.;
Mallouk, T. E.; Lammert, P. E.; Crespi, V. H. Catalytic Nanomotors: Autonomous
Movement of Striped Nanorods. *J. Am. Chem. Soc.* 2004, *126* (41), 13424–13431.

(4) Ibele, M.; Mallouk, T. E.; Sen, A. Schooling Behavior of Light-Powered Autonomous Micromotors in Water. *Angew. Chem. Int. Ed.* **2009**, *48*, 3308–3312.

(5) Duan, W.; Liu, R.; Sen, A. Transition between Collective Behaviors of Micromotors in Response to Different Stimuli. *J. Am. Chem. Soc.* **2013**, *135*, 1280–1283.

(6) Sengupta, S.; Spiering, M. M.; Dey, K. K.; Duan, W.; Patra, D.; Butler, P. J.; Astumian, R. D.; Benkovic, S. J.; Sen, A. DNA Polymerase as a Molecular Motor and Pump. *ACS Nano*, **2014**, *8*, 2410–2418.

(7) Mallouk, T.; Sen, A. Powering Nanorobots. *Scientific American* 2009, 300 (5), 72–77.

(8) Gao, W.; Kagan, D.; Pak, O. S.; Clawson, C.; Campuzano, S.; Chuluun-Erdene,
E.; Shipton, E.; Fullerton, E. E.; Zhang, L.; Lauga, E.; Wang, J. Cargo-Towing Fuel-Free
Magnetic Nanoswimmers for Targeted Drug Delivery. *Small* 2012, *8* (3), 460–467.

(9) Sen, A.; Patra, D.; Sengupta, S.; Duan, W.; Zhang, H.; Pavlick, R. Intelligent, Self-Powered, Drug Delivery Systems. *Nanoscale* **2012**, *5*, 1273–1283.

(10) Gao, W.; Wang, J. The Environmental Impact of Micro / Nanomachines: A Review. **2014**, *8* (4), 3170–3180.

(11) Yadav, V.; Pavlick, R. a.; Meckler, S. M.; Sen, A. Triggered Detection and Deposition: Toward the Repair of Microcracks. *Chem. Mater.* **2014**, *26*, 4647–4652.

(12) Kar, A.; Chiang, T.; Rivera, I. O.; Sen, A.; Velegol, D. Enhanced Transport into and out of Dead-End Pores. *ACS Nano* **2015**, *9*, 746–753.

(13) Purcell, E. M. Life at Low Reynolds Number. Am. J. Phys. 1977, 45, 3-11.

(14) Wang, W.; Castro, L. A.; Hoyos, M.; Mallouk, T. E. Autonomous Motion of Metallic Microrods Propelled by Ultrasound. *ACS Nano* **2012**, *6*, 6122-6132.

(15) Bruus, H. Acoustofluidics 1: Governing Equations in Microfluidics. *Lab Chip* **2011**, *11*, 3742.

(16) Friend, J.; Yeo, L. Y. Microscale Acoustofluidics: Microfluidics Driven via Acoustics and Ultrasonics. *Rev. Mod. Phys.* **2011**, *83*, 647–704.

(17) Muller, P. B.; Barnkob, R.; Jensen, M. J. H.; Bruus, H. A Numerical Study of Microparticle Acoustophoresis Driven by Acoustic Radiation Forces and Streaming-Induced Drag Forces. *Lab Chip* **2012**, *12*, 4617.

(18) Barnkob, R.; Augustsson, P.; Laurell, T.; Bruus, H. Acoustic Radiation- and Streaming-Induced Microparticle Velocities Determined by Microparticle Image Velocimetry in an Ultrasound Symmetry Plane. *Phys. Rev. E.* **2012**, *86*, 1–11.

(19) Evander, M.; Nilsson, J. Acoustofluidics 20: Applications in Acoustic Trapping.*Lab Chip* 2012, *12*, 4667.

(20) Ahmed, S.; Wang, W.; Mair, L. O.; Fraleigh, R. D.; Li, S.; Castro, L. A.; Hoyos, M.; Huang, T. J.; Mallouk, T. E. Steering Acoustically Propelled Nanowire Motors toward Cells in a Biologically Compatible Environment Using Magnetic Fields. *Langmuir* 2013, *29*, 16113–16118.

(21) Garcia-Gradilla, V.; Orozco, J.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.; Pourazary, A.; Katzenberg, A.; Gao, W.; Shen, Y.; Wang, J. Functionalized Ultrasound-Propelled Magnetically Guided Nanomotors: Toward Practical Biomedical Applications. *ACS Nano* **2013**, *7* (10), 9232–9240.

(22) Wu, Z.; Li, T.; Li, J.; Gao, W.; Xu, T.; Christianson, C.; Gao, W. Turning Erythrocytes into Functional. *ACS Nano* **2014**, *8*, 12041–12048.

(23) Wang, W.; Li, S.; Mair, L.; Ahmed, S.; Huang, T. J.; Mallouk, T. E. Acoustic Propulsion of Nanorod Motors inside Living Cells. *Angew. Chem. Int. Ed.* **2014**, *53*, 3201–3204.

(24) Ahmed, S.; Gentekos, D. T.; Fink, C. A.; Mallouk, T. E.; Al, A. E. T. Self-Assembly of Nanorod Motors into Geometrically Regular Multimers and Their Propulsion by Ultrasound. *ACS Nano* **2014**, *8*, 11053–11060.

(25) Wang, W.; Duan, W.; Zhang, Z.; Sun, M.; Sen, A.; Mallouk, T. E. A Tale of Two Forces: Simultaneous Chemical and Acoustic Propulsion of Bimetallic Micromotors. *Chem. Commun.* **2015**, *51*, 1020–1023.

(26) Xu, T.; Soto, F.; Gao, W.; Dong, R.; Garcia-Gradilla, V.; Magaña, E.; Zhang, X.; Wang, J. Reversible Swarming and Separation of Self-Propelled Chemically Powered Nanomotors under Acoustic Fields. *J. Am. Chem. Soc.* **2015**, *137*, 2163–2166.

(27) Garcia-Gradilla, V.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.; Yardımcı, C.; Wiitala, D.; Galarnyk, M.; Wang, J. Ultrasound-Propelled Nanoporous Gold Wire for Efficient Drug Loading and Release. *Small* **2014**, *10*, 4154-4159.

(28) Balk, A. L.; Mair, L. O.; Mathai, P. P.; Patrone, P. N.; Wang, W.; Ahmed, S.; Mallouk, T. E.; Liddle, J. A.; Stavis, S. M. Kilohertz Rotation of Nanorods Propelled by Ultrasound, Traced by Microvortex Advection of Nanoparticles. *ACS Nano* **2014**, *8*, 8300–8309.

(29) Nadal, F.; Lauga, E. Asymmetric Steady Streaming as a Mechanism for Acoustic Propulsion of Rigid Bodies. *Phys. Fluids* **2014**, *26*, 082001.

Chapter 2

Steering Acoustically Propelled Nanowire Motors towards Cells in a Biologically Compatible Environment using Magnetic Fields

2.1 Introduction

Research into the propulsion and control of nano- and microscale motors has grown steadily over the past decade. ¹⁻¹⁰ Some of the most interesting potential applications of micromotors are in biosensing and biomedicine, but the most widely studied catalytic propulsion mechanism tend to involve toxic fuels or incompatible with media or high ionic strength.¹¹⁻¹⁶ The recent discovery of the propulsion of nanomotors using acoustic energy has provided a new opportunity to explore their behavior in biocompatible media.¹⁷⁻¹⁸ Self-acoustophoretic nanowire motors are especially attractive as they allow continuous propulsion that is not limited by the supply of fuel. They are operated in a frequency and power range that is biologically safe.^{17,19-20} The power density used in the experiments described in this paper is (13 ± 1) mW/cm², which is well below the FDA limit of 740 mW/cm² for diagnostic ultrasound.¹⁹⁻²⁰ The selfacoustophoretic mechanism also allows one to change the motor speed easily by adjusting the applied power.²¹⁻²⁶ However, in order to take full advantage of ultrasonically propelled motors one must be able to direct and guide their motion towards targets of interest such as cells and tissues. Here we demonstrate that remote steering and targeting of acoustically powered motors is possible by using externally applied magnetic fields. As in previous reports of magnetically oriented autonomous nanomotors, a

magnetic segment was incorporated into the nanowires.²⁷⁻²⁹ A ferromagnetic nickel stripe was electrochemically grown between diamagnetic Au and Ru segments, as shown in Figure 1, and it responded to a weak external magnetic field that could be oriented to define the motor's path in two dimensions.



Figure 1-1. A representative FE-SEM image of the Au-Ni-Ru nanowire motors.

2.2 Results and Discussion

2.2.1. Behavior of Nanomotors in Static Magnetic Fields.

When excited by ultrasonic standing waves near the resonant frequency of the cylindrical cell (3.77 ± 0.01 MHz), nanowires with (40 ± 5) nm thick magnetic stripes exhibited the same behavior as their non-magnetic counterparts, including levitation to the midpoint of the cell, pattern formation into concentric nanowire circles, alignment into spinning chains, and autonomous axial motion.¹⁷ The polar alignment of Au-Ni-Ru wires in spinning chains at the acoustic nodal lines, with the ruthenium end leading, was also consistent with earlier observations with non-magnetic Au-Ru nanorods. The average speed of the wires did not change with the application of a static magnetic field: speeds of (26 ± 12) µm/s and (26 ± 18) µm/s were observed, without and with the field,

respectively (the resulting Reynolds number is approximately $(1 \pm 0.8) \times 10^{-4}$). These values are based on 46 measurements of wires with an applied magnetic field and 120 measurements of wires with no applied field. The positional measurement error was less than 0.3 µm and thus the distribution in speed derives primarily from wire-to-wire speed variation. However, the pattern of movement changed markedly, as shown in Figure 2. In the absence of magnetic field, wire trajectories followed loops (possibly from slight bending of the wires and asymmetry in the wire end shape) and also contained random turns, but in the magnetic field they followed straight line trajectories. In the static field (applied parallel to the y axis in Figure 2-2) the majority of the wires oriented with their long axis within 25 degrees of the direction perpendicular to the field (the x axis in Figure 2-2). This is as expected since the thickness of the magnetic stripe (40 ± 5) nm is smaller than its diameter (300 ± 30) nm; thus, the easy axis of magnetization should lie in the plane of the stripe.^{27,30} The range of angles observed between the direction of movement and the applied field can be explained by the fact that the Ni stripes in the wires in general were only approximately perpendicular to the wire axis (Figure 2-1). These static field experiments were conducted at a constant field of (40 ± 1) mT by placing a Nd-Fe-B magnet (6.60 \pm 0.03) cm from the active area and in the two dimensional plane of the acoustic cell.



Figure 2-2. Representative nanowire tracks plotted from the x and y coordinates obtained from tracking nanowires, the bottom left of the screen is defined as the origin. Arrows on the tracks indicate direction of motion. (A) Representative tracks in the absence and (B) in the presence of a magnetic field; field direction is indicated by the large arrow.

This field strength applied a maximum torque of $(2 \pm 0.5) \times 10^{-16} N \cdot m$ on the nanowires within the cell as calculated from equation (1):

$$\tau = \mu \times B \tag{1}$$

where τ is the torque, μ is the magnetic dipole moment of the wire $(6.5 \pm 0.5) \times 10^{-15} \text{ A} \cdot \text{m}^2$ as determined by ensemble superconducting quantum interference device (SQUID) magnetometry measurements (Figure 2-3), and *B* is the field strength of the aligning magnet. From this value, the energy required to turn the nanowire through 90° (i.e. to align the long axis parallel to the field) is approximately 2×10^{-16} J, which is orders of magnitude larger than the thermal energy $k_{\rm B}T \approx 4 \times 10^{-21}$ J.



Figure 2-3. SQUID magnetometry measurements. (a) The hysteresis curve of a Au-Ni-Ru nanowire sample from -5 to 5T showing the saturation magnetization used to calculate the dipole of the wires. (b) Zoomed in hysteresis curve from -0.25 to 0.25 T showing the ferromagnetism of the nanowires.

In the low Reynolds number limit, the acoustic propulsion force on the wires is equal to the drag force experienced by the wires. The axial propulsion force was determined from the Stokes drag equation (2-2) to be (2 ± 0.1) pN for wires traveling at 25 µm/s.

$$F_{drag} = \frac{2\pi\eta L}{\ln\left(\frac{L}{R}\right) - 0.72} \upsilon \tag{2-2}$$

Here η is the dynamic viscosity of water at 293 K, *L* is the length of the wire, *R* is its radius and *v* its velocity.^{31,32} In contrast, the axial propulsion force on the nanowires due to the magnetic field was negligible as evidenced by the fact that there is no change in the speed of the wires before and after the application of the field. In order to quantify the axial magnetic force, the magnetic field profile was measured as a function of distance (experimental details section) and the gradient at the distance (6.60 ± 0.03) cm between

the sample and the magnet was found to be (-4.02 ± 0.02) mT/cm. From this value the axial magnetic force was calculated to be $(2.6 \pm 0.1) \times 10^{-15}$ N. This is approximately three orders of magnitude smaller than the acoustic propulsion force of (2.0 ± 0.1) pN.

The movement of acoustically propelled wires in the presence and absence of a magnetic field was quantified using two parameters: the rotational diffusion coefficient and the mean displacement angle of the wires. The rotational diffusion coefficient, which is a measure of the random rotation of the wire axis during propulsion, was determined by tracking the location of the head and tail of the wire over 1 s for wires undergoing random autonomous motion both before and after the application of the field.³³ A decrease in the rotational diffusion coefficient from (2.6 ± 1.1) to (0.5 ± 0.2) degrees²/sec was observed, indicating that the magnetic field suppressed rotational diffusion. The mean displacement angle is the angular deviation in a wire path, which quantifies how straight the movement of the wire is.³⁴ This angle was measured at 0.2 sec intervals over a minimum of 3 s for each wire. The mean displacement angle in the absence of the magnetic field was 31.8° ± 9.1 and decreased to 4.4° ± 1.5 in the field.

2.2.2 Magnetic Steering.

Using handheld magnets at a distance of 2.5 ± 0.1 cm, which apply a (45 ± 1) mT field at the sample location, (and hence a gradient of (- 3.80 ± 0.01) mT/cm and an insignificant axial force of 2.4×10^{-15} N), it was possible to reversibly disrupt patterns,

such as spinning chains that are formed in the acoustic field, by reorienting the wires perpendicular to the chain. The disruption of pattern formation can be seen in Figure 2-4.



Figure 2-4. Nanowire motors within an acoustic cell can be confined within acoustic nodal locations eventually forming patterns such as spinning chains. (a) Multiple magnetic nanowire motors forming dynamic spinning chains within nodal lines. (b) Upon the application of an oscillating magnetic field the chain is rapidly disrupted and the nanowire motors follow varying trajectories not confined to the nodal lines. (c, d) Upon the removal of the field the chain reorganizes. Times of frames (a-d) are indicated. The scale bars represent 10µm.

Acoustically propelled motors can travel at axial speeds up to 200 µm/sec.¹⁷

Magnetic steering was demonstrated on acoustically propelled nanowire motors moving at axial speeds ranging from $(8 \pm 3) \mu m/s$ to $(170 \pm 10) \mu m/s$ (experimental details section Figure 2-9). This is illustrated by the ensemble motion of wires, which could be directed to move in concert as shown in Figure 2-5. The autonomous nature of the acoustically propelled motion was retained when the magnetic field was applied. The top panel of Figure 2-5 illustrates the magnetic steering of groups of motors that were initially traveling in approximately the same direction and then taken through a series of turns by re-orienting the external field. The bottom panel of Figure 2-5 shows the effect of the field on wires traveling in opposite directions. These wires are also re-oriented in the field, but in opposite directions, and were also made to take multiple sharp U-turns.


Figure 2-5. Top panel: Steered ensemble motion of wires moving at $(25 \pm 5) \mu m/s$, showing (a) earlier and (b) later times in the trajectory. Yellow tracking lines are superimposed on the final frame of the video. The wires undergo multiple guided U-turns. Times of frames (a-d) are indicated. **Bottom panel:** Autonomous steering of motors moving at $(8 \pm 3) \mu m/s$. Times of frames (e-h) are indicated and arrows show initial direction of motion in (e) and consequent guided sharp U-turns (f-h).

2.2.3. Cell Targeting Experiments.

For possible bioanalytical and biomedical applications, it is important to show that acoustically powered nanomotors can be steered in a biocompatible environment. Here we demonstrate the magnetic steering of nanomotors towards live HeLa cells in an aqueous phosphate buffer. Nanomotors were mixed with HeLa cells and placed into the acoustic cell. Cell viability tests showed no significant degradation in the presence of nanowires after 20 min exposure to acoustic excitation at the power levels used in the steering experiments (see experimental details section). In Figure 2-6 top panel, one nanomotor was selected and guided towards a cell. At the start to demonstrate directional control, the wire was taken through multiple 180° turns before guiding it towards the targeted cell. It was possible to steer nanowire motors towards cells that were adjacent in aggregates and hence only microns apart. Targeting of a single nanowire towards an individual cell, and an approximately 90° turn of a group of nanowires into a group of cells, are illustrated in Figure 2- 6. Nanowire targeting toward cells was successfully repeated multiple times to ensure the robustness of the technique.



Figure 2-6. Top panel: (a-c) Nanomotor targeting towards a HeLa cell. Nanowires were guided to make multiple U-turns to demonstrate control before being steered towards the live cell. (see Video S5 of Supporting Information). **Bottom panel**: (d) Nanowires are moving parallel to a group of cells, with their direction indicated by the arrow. (e) By turning the external field, nanowires are magnetically steered through an approximately 90° turn towards the cells.

These experiments have demonstrated that it is possible to suppress random

motion and exert relatively fine control over the steering of acoustically propelled

nanomotors using a weak external magnetic field. Both the propulsion and steering of

these motors can be carried out in biocompatible buffers, as evidenced by steering of

motors towards live cells in these media.

2.3 Experimental Details.

2.3.1. Nanowire Growth and Characterization.

Bimetallic and trimetallic nanowires were grown electrochemically using commercial anodic aluminum oxide (AAO) membranes (Whatman Inc., nominal pore diameter 200 nm) as templates. Segmented gold-nickel-ruthenium wires were made by sequentially changing the plating solution within an electrochemical cell. A two-electrode electrochemical cell was used for the plating of silver, nickel and gold. ³⁵ A silver layer evaporated onto the AAO membrane served as the cathode and a platinum wire as the anode. Ruthenium was plated in a three electrode cell with an additional Ag/AgCl electrode serving as the reference electrode. A thin gold adhesion layer was deposited between the nickel and ruthenium segments to prevent nanowire fragmentation during release. Gold and ruthenium deposition was conducted under conditions identical to those used previously in order to yield wires with similar morphological and shape asymmetry.¹⁷ Nickel was deposited at a constant current density of 0.7 mA/cm² for 3 min.

The lengths of the nickel segments were (40 ± 5) nm, smaller than a single magnetic domain which is typically ≈ 150 nm, and also smaller than the diameter of the nanorod. This ensured that the easy axis and hence the direction of magnetization was approximately perpendicular to the nanowire long axis.^{27,30,36} In this arrangement the motors can be steered with a magnetic field in the plane of the acoustic cell; the field applies substantial torque but minimal axial force to the nanowire.

2.3.2. Magnetic Field Characterization.

The magnetic properties of the nanowires were characterized using SQUID magnetometry. The magnetic susceptibility of a 7 mm² portion of the template with embedded wires was measured at 5 K from 5 T to -5 T. A background measurement of a control sample containing gold-ruthenium wires grown under identical conditions, but without the nickel segment, was made for subtraction from the sample measurement. The magnetic dipole moment per wire (μ) was determined by dividing the total saturation magnetization of the sample by the number of wires contained within it and was determined to be approximately (6.5 ± 0.5) × 10⁻¹⁵ A[•] m². This value was used to determine the torque on the wires in the applied magnetic fields (Eqn. 1) above.

The magnetic field strength applied to the rods was measured as a function of distance to determine the magnetic field gradient at 6.60 ± 0.3 cm and hence the static field magnetic force on the wires. (Figure 2-7 A) The field strength at this distance was (40 ± 1) mT. The derivative of the best fit polynomial of the points was evaluated at 6.60cm to determine the gradient for magnetic force determination. The gradient was determined to be (-4.02 ± 0.02) mT/cm. The force was evaluated using equation 3:¹

$$F_m = \nabla (m \cdot B) \tag{3}$$

where m is the magnetic dipole moment of the wire and B is the magnetic field. The magnetic force on the wire was thus determined to be $(2.6 \pm 0.1 \times 10^{-15})$ N. The same measurement was conducted to determine magnetic field gradient and axial magnetic force on the wires using the mobile magnets (Figure 2-7 B). The magnetic field strength at (2.50 ± 0.05) cm was (45 ± 1) mT, the gradient was (-3.80 ± 0.01) mT/cm at (2.50 ± 0.05) cm and the axial magnetic force on the wires was $(2.4 \pm 0.1 \times 10^{-15})$ N.



Figure 2-7. Magnetic field strength as a function of distance from the active area of the acoustic chamber for (A) the static field and (b) the handheld magnet used in steering experiments. Values shown are the average of three measurements. Error bars shown (in cases where they are visible outside the plot symbols) represent one standard deviation.

2.3.3. Acoustic propulsion experiments.

The cylindrical acoustic cell with a height of $(180 \pm 10) \mu m$ and a diameter of (5.0 ± 0.1) mm has been described in an earlier work, and was used in all experiments.¹⁷ The solution in the cell was excited by a piezoelectric disc transducer (1 mm thick) affixed to the center of a stainless steel plate (4.2 cm \times 4.2 cm \times 1 mm). Kapton tape was applied to the opposite side of the steel plate, and a hole punched in the center of the tape defined the cell. A glass microscope cover slip was placed on the cell during the experiments as a reflector to set up a standing wave. The resonant frequency of the cell center was (3.77 \pm 0.01) MHz. Experiments were conducted on wires in the levitation plane at the midpoint

of the cell at frequencies close to the resonance frequency. The voltage had a peak to peak value of 10 V and was applied using a waveform generator.

The behavior of the acoustically propelled nanowire motors in the presence of a static magnetic field was studied and compared to their behavior in the absence of a magnetic field. A cylindrical Nd-Fe-B magnet (2.50 ± 0.01) cm in diameter and (7.60 ± 0.01) cm in length was held in the plane of the cell at a fixed distance to maintain a constant field strength at the sample. The strength of the applied magnetic field was measured using a digital DC gaussmeter. The average speed for 46 and 120 wires was determined with the magnetic field on and off, respectively. For steering experiments, hand held magnets were used. The external magnetic field was applied using six rectangular Nd-Fe-B magnets ($2.5 \text{ cm} \times 2.5 \text{ cm} \times 0.625 \text{ cm}$) in the plane of the cell, at a distance of 1 cm to 1.5 cm from the wires. The strength of the applied magnetic field was measured using a digital DC gaussmeter. A schematic of the set up can be seen in Figure 2-8.



Figure 2-8. Acoustic Chamber. The acoustic chamber was defined as a hole punched into layers of Kapton tape supported on a steel plate. A piezoelectric transducer was affixed to the steel plate on the opposite side. A glass cover slip was placed on top of the acoustic chamber during experiments. The levitation plane is at the midpoint of the height of the cell as shown in the schematic. A stack of magnets is moved in the plane of the microscope stage for steering within the levitation plane.

Nanomotors traveling at speeds as high as $(170\pm10) \mu$ m/s were steered. This can be seen in Figure 2-9.



Figure 2-9. A frame sequence of wires (a-e) traveling at 170 μ m/sec being collectively steered to trace an arch. Scale bar represents 10 μ m.

2.3.4. HeLa Cell Growth and Viability Testing.

For cell targeting experiments, living human cervical cancer cells (HeLa cells) were used. The cells were used within one day of culture and stored refrigerated in a phosphate buffer solution that is 0.1 M phosphate, 0.15 M NaCl at a pH of 7.2.

HeLa cells were grown in a cellular nutrient mixture, supplemented with 10 % fetal bovine serum, penicillin (used at an enzyme unit, U, density of 100 mL⁻¹), and 100 μ g/mL streptomycin. They were grown to approximately 50 % confluence. The cells were mixed with gold nanorods, incubated for 24 h, trypsinized, counted with a hemocytometer, and suspended in fresh medium to a cell density of approximately 8 × 10⁶ mL⁻¹.

Cell viability testing was conducted using WST-1 (water soluble tetrazolium-1) cell viability reagent³⁷ (Roche Applied Science) to evaluate the effect of acoustic exposure. We added 20 μ L of the HeLa cell suspensions into the acoustic chamber and treated them under six different conditions: 1) Acoustic power off for 5 min; 2) Acoustic power off for 10 min; 3) Acoustic power off for 20 min; 4) Acoustic power on for 5 min;

5) Acoustic power on for 10 min; and 6) Acoustic power on for 20 min After treatment, the cell suspensions were collected, counted again with a hemocytometer, and diluted to a cell density of approximately 2×10^5 mL⁻¹ with fresh medium. For each of the six conditions, 100 µL of HeLa cell suspension was then seeded into a 96-well black clearbottom plate (approximately 2×10^4 cells/well) with five repeat wells. After seeding, HeLa cells were cultured for 24 h after which we added 10 µL tetrazolium salt WST-1 to each well and incubated for another 2 h. In the case of living cells, the tetrazolium salt WST-1 is cleaved by cellular enzymes to form formazan dye with an absorbance between 420 nm and 480 nm. Thus the level of enzymatic activity results in the formation of formazan dye, the absorbance of which is proportional to the total number of viable (metabolically active) cells in each well.³⁸ Therefore, the cell viability was evaluated by measuring the absorbance of each well at 450 nm and 690 nm (reference wavelength to subtract interference by precipitated proteins, cell debris, or well-to-well variance) with a microplate reader. Similar absorbance levels without and with exposure to acoustic excitation as can be in Figure 2-10, indicating that cell viability is not significantly affected by exposure to acoustic energy at the powers applied in the nanowire propulsion

experiments.



Figure 2-10. Experimental results of the cell viability test. HeLa cells without or with acoustic exposure for 5, 10, or 20 min were cultured for 24 h and their viability was assessed with the WST-1 cell viability test.

2.3.5. Nanowire Motion Tracking.

Nanowire motion was tracked using the open access program Video Spot Tracker (http://cismm.cs.unc.edu/downloads/?dl_cat=3) both in the presence and absence of a magnetic field. Videos of nanomotor motion were taken at $500 \times$ magnification at a frame rate of 30 s⁻¹.

2.4 Conclusion

The recent discovery of fuel-free propulsion of nanomotors using acoustic energy has provided a new avenue for using nanomotors in biocompatible media. Crucial to the application of nanomotors in biosensing and biomedical applications is the ability to remotely control and steer them towards targets of interest such as specific cells and tissues. In this work we demonstrate *in vitro* magnetic steering of acoustically powered nanorod motors in a biologically compatible environment. Steering was accomplished by incorporating (40 ± 5) nm thick nickel stripes into the electrochemically grown nanowires. An external magnetic field of 40 to 45 mT was used to orient the motors, which were acoustically propelled along their long axes. In the absence of a magnetic field, (300 ± 30) nm diameter, (4.3 ± 0.2) µm long nanowires with (40 ± 5) nm thick magnetic stripes exhibit the same self-acoustophoretic behavior, including pattern formation into concentric nanowire circles, aligned spinning chains and autonomous axial motion, as their non-magnetic counterparts. In a magnetic field these wires and their paths are oriented as evidenced by their relatively linear trajectories. Coordinated motion of multiple motors and targeting of individual motors towards HeLa cells with micronlevel precision was demonstrated.

2.5 References

(1) Mallouk, T.; Sen, A. Powering Nanorobots. Scientific American 2009, 300, 72–77.

(2) Ozin, G. A.; Manners, I.; Fournier-Bidoz, S.; Arsenault, A., Dream Nanomachines. *Adv. Mater.* **2005**, *17*, 3011-3018.

(3) Fournier-Bidoz, S.; Arsenault, A. C.; Manners, I.; Ozin, G. A., Synthetic self-propelled nanorotors. *Chem. Commun.* **2005**, 441-443.

(4) Mirkovic, T.; Zacharia, N. S.; Scholes, G. D.; Ozin, G. A., Nanolocomotion - Catalytic Nanomotors and Nanorotors. *Small* **2010**, *6* (2), 159-167.

(5) Gibbs, J. G.; Zhao, Y. P., Design and characterization of rotational multicomponent catalytic nanomotors. *Small* **2009**, *5* (20), 2304-8.

(6) Gibbs, J.; Zhao, Y., Catalytic nanomotors: fabrication, mechanism, and applications. Frontiers of Materials *Science* **2011**, *5* (1), 25-39.

(7) Sengupta, S.; Ibele, M. E.; Sen, A., Fantastic Voyage: Designing Self-Powered Nanorobots. *Angew. Chem. Int. Edit.* **2012**, *51*, 8434-8445.

(8) Fischer, P.; Ghosh, A., Magnetically actuated propulsion at low Reynolds numbers: towards nanoscale control. *Nanoscale* **2011**, *3*, 557-563.

(9) Agarwal, A.; Hess ,H., Molecular motors as components of future medical devices and engineered materials", *Journal of Nanotechnology in Engineering and Medicine* **2009**, *1* (1), 011005.

(10) Wang, J.; Manesh, K. M., Motion control at the nanoscale. *Small* **2010**, *6*, 338-345.

(11) Hess, H.; Jaeger, L., Nanobiotechnology. *Current Opinion in Biotechnology* **2010**, *21*,373-375.

(12) Qin, L. D.; Banholzer, M. J.; Xu, X. Y.; Huang, L.; Mirkin, C. A., Rational design and synthesis of catalytically driven nanorotors. *J. Am. Chem. Soc.* **2007**, *129*, 14870-14871.

(13) Nelson, B. J.; Kaliakatsos, I. K.; Abbott, J. J., Microrobots for Minimally Invasive Medicine. *Annual Review of Biomedical Engineering* **2010**, *12*, 55-85.

(14) Solovev, A. A.; Xi, W.; Gracias, D. H.; Harazim, S. M.; Deneke, C.; Sanchez, S.; Schmidt, O. G., Self-Propelled Nanotools. *ACS Nano* **2012**, *6* (2), 1751-1756.

(15) Sanchez, S.; Pumera, M., Nanorobots: the ultimate wireless self-propelled sensing and actuating devices. *Chem Asian J* **2009**, *4*, 1402-10.

(16) Wang, J.; Gao, W., Nano/Microscale Motors: Biomedical Opportunities and Challenges. *ACS Nano* **2012**, *6*, 5745-5751.

(17) Wang, W.; Castro, L. A.; Hoyos, M.; Mallouk, T. E. Autonomous Motion of Metallic Microrods Propelled by Ultrasound. *ACS Nano* **2012**, *6*, 6122-6132.

(18) Kagan, D.; Benchimol, M. J.; Claussen, J. C.; Chuluun-Erdene, E.; Esener, S.; Wang, J., Acoustic Droplet Vaporization and Propulsion of Perfluorocarbon-Loaded Microbullets for Targeted Tissue Penetration and Deformation. *Angew. Chem. Int. Edit.* **2012**, *51*, 7519-7522.

(19) Barnett, S. B.; Ter Haar, G. R.; Ziskin, M. C.; Rott, H.-D.; Duck, F. A.; Maeda, K., International recommendations and guidelines for the safe use of diagnostic ultrasound in medicine. *Ultrasound in Medicine & Biology* **2000**, *26* (3), 355-366.

(20) Wang, W. Understanding the propulsion and assembly of autonomous nano- and micromotors powered by chemical gradients and ultrasound. Ph.D. thesis. The Pennsylvania State University 2013.

(21) Bruus, H. Acoustofluidics 7: The acoustic radiation force on small particles. *Lab Chip* **2012**, *12*, 1578-86.

(22) Lenshof, A.; Magnusson, C.; Laurell, T., Acoustofluidics 8: Applications of acoustophoresis in continuous flow microsystems. *Lab Chip* **2012**, *12*, 1210-1223.

(23) Friend, J.; Yeo, L. Y., Microscale acoustofluidics: Microfluidics driven via acoustics and ultrasonics. *Rev. Mod. Phys.* **2011**, *83*, 647-704.

(24) Ding, X. Y.; Lin, S. C. S.; Kiraly, B.; Yue, H. J.; Li, S. X.; Chiang, I. K.; Shi, J. J.; Benkovic, S. J.; Huang, T. J., On-chip manipulation of single microparticles, cells, and organisms using surface acoustic waves. *PNAS* **2012**, *109*, 11105-11109.

(25) Shi, J.; Ahmed, D.; Mao, X.; Lin, S.-C. S.; Lawit, A.; Huang, T. J., Acoustic tweezers: patterning cells and microparticles using standing surface acoustic waves (SSAW). *Lab Chip* **2009**, *9* (20), 2890-2895.

(26) Chen, Y.; Ding, X.; Steven Lin, S.-C.; Yang, S.; Huang, P.-H.; Nama, N.; Zhao, Y.; Nawaz, A. A.; Guo, F.; Wang, W.; Gu, Y.; Mallouk, T. E.; Huang, T. J., Tunable Nanowire Patterning Using Standing Surface Acoustic Waves. *ACS Nano* **2013**, *7* (4), 3306-3314.

(27) Kline, T. R.; Paxton, W. F.; Mallouk, T. E.; Sen, A., Catalytic nanomotors: remote-controlled autonomous movement of striped metallic nanorods. *Angew. Chem. Int. Ed.* **2005**, *44*, 744-6.

(28) Solovev, A. A.; Sanchez, S.; Pumera, M.; Mei, Y. F.; Schmidt, O. G., Magnetic Control of Tubular Catalytic Microbots for the Transport, Assembly, and Delivery of Micro-objects. *Adv. Funct. Mater.* **2010**, *20*, 2430-2435.

(29) Garcia-Gradilla,V.; Orozco, J.; Sattayasamitsathit, S.; Soto,F.; Kuralay,F.; Pourazary, A.; Katzenberg, A.; Gao, W.; Shen, Y.; Wang, J. Fucntionalized Ultrasound-Propelled Magnetically Guided Nanomotors: Toward Practical Biomedical Applications. *ACS Nano*, **2013**, *7* (10), 9232–9240.

(30) Love, J. C.; Urbach, A. R.; Prentiss, M. G.; Whitesides, G. M. Three-dimensional self-assembly of metallic rods with submicron diameters using magnetic interactions. *J. Am. Chem. Soc.* **2003**, *125*, 12696-7.

(31) Happel, J.; Brenner, H. Low Reynolds Number Hydrodynamics; Prentice Hall: Englewood Cliffs, NJ, 1965; eq 5-11.52.

(32) Ui, T.J.; Hussey, R.G. Stokes drag on a cylinder in axial motion. *Phys.Fluids*. **1984**, *27*,787-795.

(33) Paxton, W. F.; Kistler, K. C.; Olmeda, C. C.; Sen, A.; St. Angelo, S. K.; Cao, Y.;
Mallouk, T. E.; Lammert, P.; Crespi, V. H. Autonomous Movement of Striped Nanorods. *J. Am. Chem. Soc.* 2004, *126*, 13424-13431.

(34) Mino, G.; Mallouk, T.E.; Darnige, T.; Hoyos, M.; Dauchet, J.; Dunstan, J.; Soto,
R.; Wang, Y.; Rousselet, A.; Clement, E. Enhanced diffusion due to active swimmers at a solid surface. *Phys. Rev. Lett.* 2011, *106*, 048102/1-4.

(35) Kline, T. R.; Tian, M.; Wang, J.; Sen, A.; Chan, M. W. H.; Mallouk, T. E., Template-Grown Metal Nanowires. *Inorg. Chem.* **2006**, *45*, 7555-7565.

(36) Wei, M.S.; Chou, S.Y. Size effects on switching field of isolated and interactive arrays of nanoscale single-domain Ni bars fabricated using electron beam nanolithography. *J. Appl. Phys.* **1994**, *76*,6679-6681.

(37) Ishiyama, M., Shiga, M., Sasamoto, K., Mizoguchi, M., and He, P. A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye. *Chem. Pharm. Bull.* **1993**, *41*, 1118-1122.

(38) Berridge, M. V. *et al.* The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. *Biochemica*, **1996**, *4*, 15-19.

Chapter 3

Self-Assembly of Nanorod Motors into Geometrically Regular Multimers and their Propulsion by Ultrasound

3.1 Introduction

The ability to controllably assemble materials with complex structures and properties from nanoscale building blocks is the focus of much current research.^{1–6} Controlled assembly of particles can yield colloid "molecules" with emergent properties that derive from their individual building blocks. Particles in such assemblies are also analogous to atoms in crystals, and imaging their interactions provides insight into microscopic phenomena such as crystal nucleation.^{7,8} Many kinds of interparticle interactions have been studied to control nanoparticle assembly. These include DNA base pairing,^{6,9,10} electrostatic,¹¹⁻¹³ hydrophilic-hydrophobic,^{14,15} and magnetic interactions,^{16–28} as well as shape asymmetry.^{29–31}

In parallel, research into the collective interactions of powered microscale objects has been growing steadily and is of interest for understanding the emergent behavior of active matter.³² Most experimental studies of this kind have focused on living microorganisms, or structurally simple objects such as polar nanorods, microspheres, or irregularly shaped colloidal particles.^{33–42} Only a few studies have explored more complex designed shapes such as hinged nanorods⁴³ and self-assembled dimers and trimers.^{44–46} Deliberately assembled particles have the potential to exhibit different kinds of collective behavior and controllable movement, and to incorporate several different

kinds of materials and functions. Here we report the spontaneous assembly of acoustically levitated, dynamically propelled magnetic nanorods into geometrically regular "molecular" dimers, trimers, and higher multimers. The assembly and disassembly of these colloidal molecules occurs in water and in the absence of an applied magnetic field. In these experiments, ultrasonic acoustic power drives the rapid autonomous movement of individual nanorods and multimers. The kinetic energy imparted by ultrasonic excitation is important in the assembly/disassembly process because it can overcome the attractive energy of magnetic and surface interactions. Such interactions can otherwise dominate the assembly of nanoparticles, especially of highly polarizable metals, leading to irreversible aggregation and precipitation.

3.2 Results and Discussion

Segmented Au-Ru-Ni rods were grown by sequential electrodeposition of metals in porous anodic alumina templates from commercial plating solutions as previously described.⁴⁷ The rods were 300 ± 30 nm in diameter and $2.0 \pm 0.3 \mu$ m in length. The Ni, Au, and Ru segments were respectively 80 ± 20 nm, $1.4 \pm 0.2 \mu$ m, and $0.55 \pm 0.09 \mu$ m in length.

The nanorods were placed in a cylindrical acoustic chamber, $(180 \pm 10) \mu m$ height, (5.0 ± 0.1) mm diameter, as previously described.⁴⁸ A schematic of the experimental set up is shown in Figure 3-7 of the experimental section. Upon excitation at the resonant frequency of the cell (~4 MHz), the rods were levitated to the midplane underwent the behaviors previously observed with non-magnetic metallic rods: autonomous axial propulsion and formation of spinning chains and concentric circles of rods at the lateral acoustic nodes. The autonomous axial motion within the levitation plane derives from acoustically-driven fluid streaming around the dense metal rods, which are axially asymmetric because of their concave and convex ends.⁴⁹ Unlike nanorod motors that contained magnetic stripes in the center of the rods, those with Ni stripes at the ends spontaneously assembled into dimers, trimers, and higher multimers depending on the density of the rod suspensions and the speed of their axial movement within the levitation plane.

Interestingly, the rods tended to assemble into regular "molecular" geometries, as shown in Figure 3-1. Earlier experiments have shown that thin Ni segments in Au-Pt nanorods of similar dimensions to those studied here tend to be single-domain ferromagnets, in which the magnetic dipole is oriented in the plane of the Ni disk.⁴⁷ The formation of a linear head-to-head dimer from two Ni-tipped rods can be understood as the minimization of magnetic energy through the interaction of these dipoles. We show below that this energy is on the order of 10⁻¹⁷ J for the assembly of dimers. The formation of regular multimers also clearly results from magnetic interactions, which are the strongest forces in the system, but the details of the orientation and arrangement of magnetic dipoles are not yet understood.

These assemblies are propelled in the acoustic field and exhibit different modes of motion. As they are propelled in the fluid, drag forces tend to break the symmetry of the multimers with the magnetic segments serving as flexible hinges, as shown in Fig. 1c and 1e.



Figure 3-1. Nanorod monomers and multimers imaged in the levitation plane of an acoustic chamber. (a) Optical image of a monomer. (b) Linear dimer with magnetic segments touching face-to-face. (c) Bent dimer (d) Trigonal planar trimer. (e) Trigonal pyramidal trimer. (f) Tetrahedral tetramer. (g) Trigonal bipyramidal pentamer. (h) Octahedral hexamer. (i) Field Emission Scanning Electron Micrograph (FE-SEM) image of a representative nanorod with an EDS inset with red, green and blue false color representing Ni, Ru and Au. (j) Lower magnification optical image of multimers in the levitation plane of the acoustic cell. Some multimers are pointed out with white arrows. (Density: 0.014 nanorods/ μ m², Speed: 30 μ ms⁻¹). Scale bars for (a)-(h) are 5 μ m. Scale bar for (j) is 20 μ m. Figure 3-8 show additional low-magnification images of multimers at low and high density of nanorods.

3.2.1 Modes of Motion of Assemblies.

Various modes of motion were observed for the monomers and multimers.

Monomers exhibit axial autonomous motion with the nickel end leading. Dimers move in

a linear conformation with the gold segment of one of the component rods leading, or in a

bent conformation with the nickel segments leading. The direction of movement in the

bent conformation is consistent with the nickel end leading in the axial propulsion of rod monomers. Trimers have several modes of movement: translation as a trigonal planar structure with one gold end leading, translation with the nickel segments leading in a trigonal pyramidal or T-shaped conformation, in-plane rotation of the complete trigonal planar or trigonal pyramidal structure, and out of plane rotation of the base of the trigonal plane. The latter mode is typically observed within nodal lines. Tetramers tend to translate axially with the gold end of one of its component nanorods leading, as a square pyramid with the nickel segments leading or *via* rotation of the square pyramidal structure. Within the acoustic nodal lines of the cell, tetramers form an X-shaped conformation with its two-wire base rotating with respect to its two-wire top. Pentamers and larger assemblies tend to translate axially as a whole or rotate as a whole. The most prevalent modes of motion are sketched in Figure 3-2.



Figure 3-2. Optical micrographs and schematic drawings of the most common modes of motion of dimers, trimers and tetramers. (a) Linear dimer. Top: one gold end leading, Bottom: rotating while translating. (b) Bent dimer. Top: nickel ends leading, Middle: rotating while translating, Bottom: spinning while translating in a nodal line. (c) Trigonal planar trimer. Top: a gold end leading, Middle: rotating while translating, Bottom: base spinning relative to leading wire of structure. (d) Pyramidal/T-shaped trimer: Top: nickel ends leading, Bottom: rotating while translating (e)Tetrahedral tetramer. Top: a gold end leading, Middle: rotating while translating, leading. (e)Tetrahedral tetramer. Top: a gold end leading, Middle: rotating while translating, Bottom: in a nodal line in an X-shaped structure with its two wire base rotating while translating nickel ends leading. The scale bars are 5 μ m. Black arrows indicate translational motion and blue arrows represent rotations.

3.2.2 Dynamic Equilibrium: Assembly and Disassembly of Multimers.

The assembly of multimers tends to occur through bimolecular collisions. This can occur as a result of the collision of two monomers, a dimer and a monomer, a monomer and a trimer, or two multimers to form a larger assembly. Disassembly occurs spontaneously (presumably when the instantaneous shear force exceeds the magnetic attraction between the component nanorods) or as a result of a collision with another motor that causes fragmentation. Frame sequences of some examples of assembly and disassembly can be seen in Figure 3-3. The relative numbers of monomers, dimers,

trimers, and higher multimers are dependent upon the number density of particles in the fluid and the speed of the motors, which is determined by the ultrasonic power applied. Hence a dynamic equilibrium is established between multimers and the system can be modeled as a set of stepwise association reactions with equilibrium constants for each. The propulsion force is analogous to thermal excitation and thus the equilibrium constants depend on motor speed.



Figure 3-3. Representative assembly and disassembly frame sequences of nanorod multimers. (a) Assembly of a trimer from a monomer and a dimer. (b) Disassembly of a trimer into a monomer and a dimer. (c) Assembly of a hexamer from two trimers. (d) Disassembly of a hexamer. White arrows and circles indicate motors involved. Scale bars are 5μ m.

Expressions for the system of equilibria (1-5) were derived (see experimental

section) and the number density of multimers at varying total concentrations of rods was

measured at three different speeds (as determined from the average speeds of the

		K1		
monomers).	monomer + monomer	↔ di	imer	(1)

$$dimer + monomer \stackrel{K2}{\leftrightarrow} trimer \tag{2}$$

$$trimer + monomer \stackrel{K3}{\leftrightarrow} tetramer \tag{3}$$

$$tetramer + monomer \stackrel{K4}{\leftrightarrow} pentamer \tag{4}$$

$$pentamer + monomer \stackrel{K5}{\leftrightarrow} hexamer \tag{5}$$

At a given speed, equilibrium constants K_1 and K_2 and their standard deviations (Table 3-1) could be obtained from global fits of the measured densities of monomers, dimers, and trimers (Figure 3- 4).



Figure 3-4. Multimer density as a function of number density of particles in solution at different average monomer speeds. Expressions were derived for the equilibrium concentrations of monomers, dimer and trimers were used to fit the experimental data points. Fit lines are drawn for monomers, dimers and trimers, at (a) 17 ± 5 , (b) 30 ± 8 , and (c) $55\pm7\mu$ m s⁻¹. (d) van't Hoff plot of the propulsion energy dependence of the equilibrium constants of dimers and trimers.

Because the ultrasonic standing wave confines the nanorods within a largely 2D

levitation plane, the number density of monomers and multimers was measured per unit area (as opposed to per unit volume). At low concentration, as the number density of rods in the levitation plane increases, so does the number of monomers. The monomer concentration initially increases linearly with total rod density and then grows more slowly as dimers and higher multimers begin to form (Figure 3-4)

The equilibrium distribution of multimers is dependent on the speed of particles, which provides a kind of thermal excitation that balances the attractive magnetic interaction between the ferromagnetic nickel segments. As the speed increases, the distribution shifts towards monomers, consistent with the idea that the association into multimers is exothermic. Pentamers and hexamers appear at lower speeds, and multimers higher than trimers are not present at all at the highest speed sampled in these experiments (55μ m s⁻¹). Equilibrium constants for the dimer and trimer assembly reactions at the three speeds tested are given in Table 3-1.

Speed (µm s⁻¹)	K1	K ₂
17 (5)	36 (13)	63 (22)
30 (7)	16 (6)	50 (16)
55 (8)	13 (6)	38 (11)

Table 3-1. Equilibrium constants (μm^2) for dimer and trimer (reactions 1 and 2) at three different monomer speeds. Standard deviations are indicated in parentheses.

The assembly energy is the attractive magnetic energy of association of the nickel segments of the monomers, plus smaller contributions from van der Waals attractive and electrostatic repulsive energies between the negatively charged rods. A previous study of chemically powered nanorod assembly has established that the van der Waals and electrostatic energies are small compared to the Brownian (thermal) energy in the

absence of fuel.⁴⁵ The magnetic energy between the magnetic dipole of two rods suspended in water can be calculated as:⁵⁰

$$E_m = \frac{\mu m_1 m_2 \cos(\theta)}{4\pi r^3} \tag{6}$$

where E_m is the magnetic potential energy, μ is the permittivity of water (1.26 x 10^{-6} N A^{-2}), and m_1 and m_2 are the magnetic dipole moment of the rods, which was determined to be $2.0 \pm 0.5 \text{ x} 10^{-15} \text{ A.m}^2$ at 298K by using SQUID magnetometry. The distance between the magnetic dipoles at the ends of the rods is r. For dimers moving at 30 μ m s⁻¹, the most common shape was a "V" with an angle θ of approximately 100°. The distance r was taken as 0.15 μ m, which is the center-to-center distance of the magnetic stripes in this geometry. From Eqn. 6, we thus obtain $E_m = 2.0 \pm 0.5 \text{ x} 10^{-17} \text{ J}$.

Because propulsion provides a kind of thermal or kinetic energy that causes dissociation of dimers and higher multimers, the ratio of the magnetic energy E_m to the propulsion energy E_p can be estimated from the rate of dissociation using the Arrhenius equation:

$$k = Aexp(-\frac{E_m}{E_p})$$
⁽⁷⁾

The experimental dissociation rate constants were determined for dimers and trimers moving at speeds of 17, 30, and 55 μ m s⁻¹ to be 0.08, 0.2, and 0.5 s⁻¹ respectively, with corresponding persistence times of 13, 6, and 2 s. We can estimate the attempt frequency A using A= f/2m, where f= F_{drag}/v, F_{drag} is the drag force, v is the velocity, and

m is the mass of the rod.⁵¹ The drag force F_{drag} on a cylinder moving at axial velocity v is given by⁵²:

$$F_{drag} = \frac{2\pi\eta L}{\ln\left(\frac{L}{R}\right) - 0.72}v\tag{8}$$

where L is the length of the rod (2.0 μ m), R is its radius (0.30 μ m), and η is the kinematic viscosity of the water (1.0 x 10⁻³ Pa's). From this calculation we obtain A = 1.4 x10⁶ s⁻¹. Using the E_m value calculated above, we then obtain propulsion energies E_p of 1.2, 1.3, and 1.4 x 10⁻¹⁸ J from Eqn. (7) for wires traveling at 17, 30, and 55 μ m s⁻¹ respectively. Note that this simple calculation ignores the electrostatic and van der Waals components of the interaction energy between rods, which are expected to be small compared to E_m.

Using the values of E_p derived from the Arrhenius equation, we can extract monomer-monomer and dimer-monomer binding energies from the velocity dependence of the equilibrium constant. The van't Hoff equation relates the temperature dependence of the equilibrium constant to the reaction enthalpy:

$$Ln K_{eq} = -\frac{\Delta H}{k_B T} + \frac{\Delta S}{k_B}$$
(9)

Here k_B is Boltzmann's constant and T is the system temperature. Because propulsion provides an average kinetic energy that causes dissociation, we can substitute E_p for k_BT yielding:

$$Ln K_{eq} = -\frac{\Delta H}{E_p} + \frac{\Delta S}{k_B}$$
(10)

46

Thus a van't Hoff plot (Fig. 4d) should have a slope of $-\Delta H$, from which we can calculate a dimer dissociation energy of 1.1×10^{-17} J. This value is in reasonable agreement with the calculated magnetic binding energy ($E_m = 2.0 \times 10^{-17}$ J) and adds further support to the equilibrium model. From the equilibrium data (Fig. 4) and the van't Hoff equation, we can also estimate an energy for the formation of a trimer. An additional stability of 0.6 x 10^{-17} J is gained by forming a trimer from a monomer and a dimer.

We can gain further insight into the balance of forces operating in the system by noting that the propulsion force equals the drag force at low Reynolds number, and is directly proportional to the velocity of the rods (Eqn. 8). F_{drag} for monomers moving axially at 17, 30, and 55 µm s⁻¹ is 1.1, 2.0, and 3.7 x10⁻¹³ N respectively. When aggregates of rods dissociate spontaneously, this force must be comparable to the attractive force between their components. The magnetic component of the attractive force between rods can be obtained by differentiating Eqn. (6) to yield Eqn. (11):

$$F_m = -\frac{3\mu m_1 m_2}{4\pi r^4}$$
(11)

This force is equal to F_{drag} , at r values of 1.8, 1.6, and 1.3 µm for speeds of 17, 30, and 55 µm s⁻¹, respectively. This is consistent with our observations that rods passing within 1-2 µm of each other can be "captured" to form aggregates. Rods passing within

this distance of aggregates can also impart sufficient force to dissociate the aggregates.

An example of such a "fly-by" induced dissociation can be seen in Figure 3-5.



Figure 3-5. An example of wires interacting to dissociate a dimer. (a) A dimer, with an average internal angle of $83\pm2^{\circ}$, upon approaching an incoming monomer is perturbed as shown in frame B. A slight gap is observed between the dimer rods as the internal angle decreases to $55\pm3^{\circ}$. This distance between the monomer and dimer, shown by the double headed arrow, is approximately $1.4\pm0.1\mu$ m. (c) The dimer dissociates to form two monomers.

3.2.3. Magnetic Manipulation.

Magnetic manipulation of colloidal asters has previously been studied, although in much larger (millimeter-scale) assemblies.⁵³ Here we conduct some simple experiments to show that nanorod multimers, like monomers containing magnetic stripes that we reported previously, can be steered in magnetic fields while remaining intact. Using the experimental set up we previously reported, ⁵⁴ a weak magnetic field (on the order of 45 mT) was applied using handheld Nd-Fe-B magnets. The magnets were held in the plane of the acoustic cell at a distance of about 2.5 cm from the sample under observation. The assemblies were reoriented by the magnet and steered to make several loops as shown in Figure 3-6a. Interestingly the mode of motion of an n-mer can also be altered by using a magnetic field. This is because the magnetic components behave as flexible hinges that can allow a variety of motions. A dimer, with application of an external magnetic field, can be driven to make several complete inversions in a "flapping" motion while being acoustically propelled in one direction. This is illustrated in Figure 3-6b



Figure 3-6. Magnetic steering, manipulation and isolation of assemblies. (a) Optical image frame sequence of the deliberate steering of a dimer through sharp loops using an external magnetic field. Time stamps are indicated and tracking is shown as a yellow line. Scale bar is 10μ m. (b) Optical image frame sequence for magnetic manipulation of the mode of motion of a dimer. The dimer is made to go through full inversions as it propels. Scale bar is 5 µm. (c-d) SEM images of a dimer (c) and trimer (d) pipetted out of the acoustic cell and dried on a conductive Si wafer. Scale bars are 1 µm. These assemblies are pointed out with white arrows in lower magnification SEM image (e), scale bar 10 µm.

3.2.4 Isolation and Imaging

Once assembled in water, the rod multimers can be isolated by drying as shown in Figure 3-6. The nanorod suspension in the acoustic chamber was pipetted out, placed on a conductive silicon wafer and dried for FE-SEM imaging. These images show that the

colloidal "molecules" remain largely intact, but are flattened by capillary forces and surface interactions in the drying process. It remains to be seen if suspensions of regular multimers can be reconstituted from dried samples deposited on appropriate substrates.

3.3 Experimental Details

3.3.1 Nanowire Synthesis and Characterization

Nanowires were grown by electrodeposition within the pores of commercial anodic alumina templates (Whatman Inc., nominal pore size 0.2 μ m, actual pore diameter ~0.3 μ m). Au, Ru, and Ni segments were grown sequentially by changing the deposition solution and electroplating conditions. Commercial electroplating solutions (Technic Inc., Au-Orotemp® 24 TRU, Ru-U solution and Ni-Nickel Sulfamate RTU) were used. Gold and nickel were deposited at constant cathodic current densities of 1.17 and 0.7 mA/cm² , for 15 and 6 min., respectively. A layer of evaporated silver on the porous anodic alumina membrane served as the cathode and a Pt wire was used as the anode. Ruthenium was deposited for 30 min. at a constant potential of -0.65 V vs. a Ag/AgCl reference electrode.

The magnetic properties of nanowires were characterized using a SQUID magnetometer from Quantum Design, Inc. Scanning electron microscopy (SEM) images were collected with an FEI Nova NanoSEM 630 FESEM with EDS. A backscatter detector and beam deceleration were used to obtain high elemental contrast images shown in Figure 1.

3.3.2. Acoustic Experiments

Acoustic propulsion experiments were carried out in an acoustic chamber previously described⁴⁸ and shown schematically shown in Figure 7. The chamber was constructed of a stainless steel plate (4.2 cm x 4.2 cm x 1 mm) with a circular piezoelectric transducer attached to it (1mm thickness) on one side and several layers of Kapton tape (height $180 \pm 10 \mu$ m) on the other side. A 5.0 ± 0.1 mm diameter hole was punched into the Kapton tape to define the cell. A glass cover slip was placed on top of the cell and served as a reflector to set up a standing acoustic wave that levitated and propelled the wires. The piezoelectric was actuated at ~4 MHz using a 10 volt peak-topeak signal from a waveform generator. Videos and images were collected 2-3 min after the acoustic power was turned on in order to ensure an equilibrium distribution of multimers. Figure 3-8 shows low-magnification images of multimers at a low and high density of nanorods.



Figure 3-7. Schematic diagram of the acoustic chamber used. (a) The full acoustic chamber. (b) Zoomed in schematic of the acoustic chamber, showing the bulk standing wave that is set up

between the base of the cell and the glass cover slip that causes the wires to levitate. Wires are not drawn to scale.



Figure 3-8 (a) High density solution of individual nanorods and multimers. Some multimers are pointed out with white arrows. The monomers are moving at an average speed of 23 μ m s⁻¹ and

the density of the suspension is $0.023 \text{ rods/}\mu\text{m}^2$. (b) Low density suspension primarily composed of monomers and dimers. The monomers are moving at an average speed of 20 μms^{-1} and the density of the suspension is 0.0056 rods/ μm^2 .

3.3.3. Equilibrium expressions for the density of multimers

The association and dissociation of multimers establishes a dynamic equilibrium between various species. The equilibrium constants for the stepwise association reactions are expressed as:

 $\begin{array}{ll} Monomer + Monomer \leftrightarrow Dimer \ K_1 \\ Dimer + Monomer \ \leftrightarrow Trimer \ K_2 \\ Trimer + Monomer \ \leftrightarrow Tetramer \ K_3 \end{array}$

Where:
$$K_1 = \frac{[Dimer]}{[Monomer]^2}$$
 (1)

and
$$K_2 = \frac{[Trimer]}{[Dimer][Monomer]}$$
 (2)

Hence: $[Dimer] = K_1 [Monomer]^2$ (3)

$$[Trimer] = K_1 K_2 [Monomer]^2 \tag{4}$$

The concentrations are expressed as areal number density (number per μ m²) since the rods are largely confined to the levitation plane of the sample cell.

The total density of rods in solution is:

$$C_T = [Monomer] + 2[Dimer] + 3[Trimer] + \dots$$
(5)

Substituting equations S-3 and S-4 into S-5 we obtain:

$$C_T = [Monomer] + 2K_1[Monomer]^2 + 3K_1K_2[Monomer]^3 + \cdots$$
(6)

where (...) represents terms for higher multimers (4[*Tetramer*], etc.). We can eliminate these higher terms by defining a concentration C* that is the total number of rods found in monomers, dimers, and trimers:

$$C^* = [Monomer] + 2[Dimer] + 3[Trimer]$$

$$C^* = [Monomer] + 2K_1[Monomer]^2 + 3K_1K_2[Monomer]^3$$
(8)

C* can be obtained in any video frame from Eqn. 7 by counting the numbers of monomers, dimers and trimers.

In order to plot the simulation curves shown in Fig. 4, values of K_1 and K_2 were chosen and the monomer concentration was calculated for a range of C* values as the real positive root of cubic equation 8, using the cubic formula:

$$x = \{q + [q^2 + (r - p^2)^3]^{1/2}\}^{1/3} + \{q - [q^2 + (r - p^2)^3]^{1/2}\}^{1/3} + p$$
(9)
and $p = -b/(3a), q = p^3 + (bc - 3ad)/(6a^2), r = c/(3a)$ (10)

In equations 9 and 10, x represents [monomer], $b=2K_1$, $c=3K_1K_2$, and $d=-C^*$. The concentration of dimers and trimers was then calculated for each value of C* from equations 3 and 4.

In order to obtain best fit values of K_1 and K_2 to the experimental data, a residual function was defined as the sum of the squares of the differences between the experimental and calculated densities of monomers, dimers, and trimers:

$$R = \sum (m_i - m_{calc})^2 + \sum (d_i - d_{calc})^2 + \sum (t_i - t_{calc})^2$$
(11)

where m, d and t represent monomer, dimer, and trimer densities respectively, with the subscripts "*i*" and "*calc*" representing experimental and calculated values.

This residual function was minimized for each monomer speed to give the K_1 and K_2 values and their standard deviations shown in Table 1.

3.3.4. Optical Imaging and Tracking

Videos were recorded using an Olympus BX60 microscope at 500x magnification at a frame rate of 30s⁻¹ using the video software Dazzle Video Creator Plus. Nanowire tracking was carried out using the open access Video Spot Tracker software (<u>http://cismm.cs.unc.edu/downloads/?dl_cat=3</u>).

3.4 Conclusions

Metal nanorods tipped with ferromagnetic segments assemble into n-mer "molecules" with regular geometries while levitated in a fluid acoustic cell. Levitation with ultrasound allows us to eliminate surface effects and sedimentation, enabling assembly in the absence of an applied magnetic field and in an ordinary fluid (water). The relative concentrations multimers are described by a set of stepwise equilibrium constants and the kinetics of their dissociation obey the Arrhenius law, both of which are modulated by an effective temperature that is controlled by adjusting the acoustic power. These assemblies are dynamic motors that exhibit various modes of motion. They can be steered with a weak applied magnetic field, their modes of motion can be altered by the magnetic field, and they can be isolated while remaining intact.

3.5 References

(1) Whitesides, G. M.; Grzybowski, B. Self-assembly at All Scales. *Science* 2002, 295, 2418–2421.

(2) Li, F.; Josephson, D. P.; Stein, A. Colloidal Assembly: The Road from Particles to Colloidal Molecules and Crystals. *Angew. Chem. Int. Ed.* **2011**, *50*, 360–388.

(3) Löwen, H. Colloidal Dispersions in External Fields: Recent Developments. J. *Phys: Cond. Matter* **2008**, *20*, 404201.

(4) Shevchenko, E. V; Talapin, D. V; Kotov, N. A; O'Brien, S.; Murray, C. B. Structural Diversity in Binary Nanoparticle Superlattices. *Nature* **2006**, *439*, 55–59.

(5) Damasceno, P. F.; Engel, M.; Glotzer, S. C. Predictive Self-Assembly of Polyhedra into Complex Structures. *Science* **2012**, *337*, 453–457.

(6) Grzybowski, B.; Jiang, X.; Stone, H.; Whitesides, G. Dynamic, Self-Assembled Aggregates of Magnetized, Millimeter-Sized Objects Rotating at the Liquid-Air Interface: Macroscopic, Two-Dimensional Classical Artificial Atoms and Molecules. *Phys. Rev. E* **2001**, *64*, 011603.

(7) Schade, N. B.; Holmes-Cerfon, M. C.; Chen, E. R.; Aronzon, D.; Collins, J. W.;
Fan, J. A.; Capasso, F.; Manoharan, V. N. Tetrahedral Colloidal Clusters from Random
Packing of Bidisperse Spheres. *Phys. Rev. Lett.* **2013**, *110*, 148303.

(8) Arkus, N.; Manoharan, V.; Brenner, M. Minimal Energy Clusters of Hard Spheres with Short Range Attractions. *Phys. Rev. Lett.* **2009**, *103*, 118303.
(9) Mastroianni, A. J.; Claridge, S. A.; Alivisatos, A. P. Pyramidal and Chiral Groupings of Gold Nanocrystals Assembled Using DNA Scaffolds. *J. Am. Chem. Soc.* **2009**, *131*, 8455–8459.

(10) Xu, X.; Rosi, N. L.; Wang, Y.; Huo, F.; Mirkin, C. A. Asymmetric Functionalization of Gold Nanoparticles with Oligonucleotides. *J. Am. Chem. Soc.* 2006, *128*, 9286–9287.

(11) Gangwal, S.; Cayre, O. J.; Velev, O. D. Dielectrophoretic Assembly of Metallodielectric Janus Particles in AC Electric Fields. *Langmuir* **2008**, *24*, 13312–13320.

(12) Ma, F.; Wu, D. T.; Wu, N. Formation of Colloidal Molecules Induced by Alternating-Current Electric Fields. J. Am. Chem. Soc. 2013, 135, 7839–7842.

(13) Chaudhary, K.; Chen, Q.; Juárez, J. J.; Granick, S.; Lewis, J. A. Janus Colloidal Matchsticks. *J. Am. Chem. Soc.* **2012**, *134*, 12901–120903.

(14) Chen, Q.; Bae, S. C.; Granick, S. Directed Self-assembly of a Colloidal Kagome Lattice. *Nature* **2011**, *469*, 381–384.

(15) Gao, W.; Pei, A.; Feng, X.; Hennessy, C.; Wang, J. Organized Self-Assembly of Janus Micromotors with Hydrophobic Hemispheres. *J. Am. Chem. Soc.* **2013**, *135*, 998–1001.

(16) Yan, J.; Chaudhary, K.; Chul Bae, S.; Lewis, J. A.; Granick, S. Colloidal Ribbons and Rings from Janus Magnetic Rods. *Nature Comm.* **2013**, *4*, 1516.

(17) Erb, R. M.; Son, H. S.; Samanta, B.; Rotello, V. M.; Yellen, B. B. Magnetic Assembly of Colloidal Superstructures with Multipole Symmetry. *Nature* **2009**, *457*, 999–1002.

(18) Goubault, C.; Leal-Calderon, F.; Viovy, J.-L.; Bibette, J. Self-assembled
Magnetic Nanowires Made Irreversible by Polymer Bridging. *Langmuir* 2005, *21*, 3725–3729.

(19) Helseth, L. E. Self-assembly of Colloidal Pyramids in Magnetic Fields. *Langmuir*2005, *21*, 7276–7279.

(20) Ilievski, F.; Mani, M.; Whitesides, G. M.; Brenner, M. P. Self-assembly of Magnetically Interacting Cubes by a Turbulent Fluid Flow. *Phys. Rev. E* 2011, *83*, 017301.

(21) Martínez Pedrero, F.; Tirado Miranda, M.; Schmitt, a; Callejas Fernández, J. Forming Chainlike Filaments of Magnetic Colloids: The Role of the Relative Strength of Isotropic and Anisotropic Particle Interactions. *J. Chem. Phys.* **2006**, *125*, 084706.

(22) Ooi, C.; Erb, R. M.; Yellen, B. B. On the Controllability of Nanorod Alignment in Magnetic Fluids. *J. Appl. Phys.* **2008**, *103*, 07E910.

(23) Piet, D. L.; Straube, A. V.; Snezhko, A.; Aranson, I. S. Viscosity Control of the Dynamic Self-Assembly in Ferromagnetic Suspensions. *Phys. Rev. Lett.* **2013**, *110*, 198001.

(24) Rybczynski, J.; Ebels, U.; Giersig, M. Large-Scale, 2D Arrays of Magnetic Nanoparticles. *Coll. Surf. A* 2003, *219*, 1–6.

(25) Smoukov, S. K.; Gangwal, S.; Marquez, M.; Velev, O. D. Reconfigurable Responsive Structures Assembled from Magnetic Janus Particles. *Soft Matter* **2009**, *5*, 1285.

(26) Snezhko, A.; Aranson, I.; Kwok, W.-K. Dynamic Self-Assembly of Magnetic Particles on the Fluid Interface: Surface-Wave-Mediated Effective Magnetic Exchange. *Phys. Rev. E* 2006, *73*, 041306.

(27) Yang, Y.; Gao, L.; Lopez, G. P.; Yellen, B. B. Tunable Assembly of Colloidal Crystal Alloys Using Magnetic Nanoparticle Fluids. *ACS Nano* **2013**, *7*, 2705–2716.

(28) Piet, D. L.; Straube, A. V; Snezhko, A.; Aranson, I. S. Model of Dynamic Selfassembly in Ferromagnetic Suspensions at Liquid Interfaces. *Phys. Rev. E* 2013, *88*, 033024.

(29) Sacanna, S.; Irvine, W. T. M.; Chaikin, P. M.; Pine, D. J. Lock and Key Colloids. *Nature* **2010**, *464*, 575–578.

(30) Walker, D. A; Leitsch, E. K.; Nap, R. J.; Szleifer, I.; Grzybowski, B. A. Geometric Curvature Controls the Chemical Patchiness and Self-Assembly of Nanoparticles. *Nature Nanotechnol.* **2013**, *8*, 676–681.

(31) Velev, O. D. Patchy Nanoparticles: Curvature Makes a Difference. *Nature Nanotechnol.* **2013**, *8*, 620-621.

(32) Marchetti, M. C.; Joanny, J. F.; Ramaswamy, S.; Liverpool, T. B.; Prost, J.; Rao,
M.; Simha, R. A. Hydrodynamics of Soft Active Matter. *Rev. Mod. Phys.* 2013, *85*, 1143–1189.

(33) Surrey, T.; Nedelec, F.; Leibler, S.; Karsenti, E. Physical Properties Determining Self-Organization of Motors and Microtubules. *Science* **2001**, *292*, 1167–1171.

(34) Ibele, M.; Mallouk, T. E.; Sen, A. Schooling Behavior of Light-Powered Autonomous Micromotors in Water. *Angew. Chem. Int. Ed.* **2009**, *48*, 3308–3312.

(35) Dombrowski, C.; Cisneros, L.; Chatkaew, S.; Goldstein, R. E.; Kessler, J. O. Self-Concentration and Large-Scale Coherence in Bacterial Dynamics. *Phys. Rev. Lett.* **2004**, *93*, 098103.

(36) Bendix, P. M.; Koenderink, G. H.; Cuvelier, D.; Dogic, Z.; Koeleman, B. N.; Brieher, W. M.; Field, C. M.; Mahadevan, L.; Weitz, D. a. A Quantitative Analysis of Contractility in Active Cytoskeletal Protein Networks. *J. Biophys.* **2008**, *94*, 3126–3136.

(37) Wilson, D. A., Nolte, R.J.M., Van Hest, J.C.M. Autonomous Movement of Platinum-Loaded Stomatocytes. *Nature Chem.* **2012**, *4*, 268-274.

(38) Cates, M. E.; Marenduzzo, D.; Pagonabarraga, I.; Tailleur, J. Arrested Phase Separation in Reproducing Bacteria Creates a Generic Route to Pattern Formation. *Proc. Natl. Acad. USA* **2010**, *107*, 11715–11720.

(39) Kagan, D.; Balasubramanian, S.; Wang, J. Chemically Triggered Swarming of Gold Microparticles. *Angew. Chem. Int. Ed.* **2011**, *50*, 503–506.

(40) Miño, G.; Mallouk, T. E.; Darnige, T.; Hoyos, M.; Dauchet, J.; Dunstan, J.; Soto,
R.; Wang, Y.; Rousselet, A.; Clement, E. Enhanced Diffusion Due to Active Swimmers at a Solid Surface. *Phys. Rev. Lett.* 2011, *106*, 048102.

(41) Palacci, J.; Sacanna, S.; Steinberg, A. P.; Pine, D. J.; Chaikin, P. M. Living Crystals of Light-Activated Colloidal Surfers. *Science* **2013**, *339*, 936–940.

(42) Saintillan, D.; Shelley, M. J. Active Suspensions and Their Nonlinear Models. *Comptes Rend. Phys.* **2013**, *14*, 497–517.

(43) Gao, W.; Sattayasamitsathit, S.; Manesh, K. M.; Weihs, D.; Wang, J. Magnetically Powered Flexible Metal Nanowire Motors. *J. Am. Chem. Soc.* 2010, *132*, 14403–14405.

(44) Gibbs, J. G.; Zhao, Y. Self-organized Multiconstituent Catalytic Nanomotors. *Small* **2010**, *6*, 1656–1662.

(45) Wang, W.; Duan, W.; Sen, A.; Mallouk, T. E. Catalytically Powered Dynamic Assembly of Rod-Shaped Nanomotors and Passive Tracer Particles. *Proc. Natl. Acad. USA* **2013**, *110*, 17744–17749.

(46) Dong, B.; Zhou, T.; Zhang, H.; Li, C. Y. Directed Self-Assembly of Nanoparticles for Nanomotors. *ACS Nano* **2013**, *7*, 5192–5198.

(47) Kline, T. R.; Tian, M.; Wang, J.; Sen, A.; Chan, M. W. H.; Mallouk, T. E. Template-Grown Metal Nanowires. *Inorg. Chem.* **2006**, *45*, 7555–7565.

(48) Wang, W.; Castro, L. A.; Hoyos, M.; Mallouk, T. E. Autonomous Motion of Metallic Microrods Propelled by Ultrasound. *ACS Nano* **2012**, *6*, 6122–6132.

(49) Nadal, F.; Lauga, E. Asymmetric Steady Streaming as a Mechanism for Acoustic Propulsion of Rigid Bodies. *Phys. Fluids* **2014**, *26*, 082001.

(50) Khalil, K. S.; Sagastegui, A.; Li, Y.; Tahir, M. a; Socolar, J. E. S.; Wiley, B. J.; Yellen, B. B. Binary Colloidal Structures Assembled Through Ising Interactions. *Nature Comm.* **2012**, *3*, 794.

(51) Li, T.; Kheifets, S.; Medellin, D.; Raizen, M. G. Measurement of the Instantaneous Velocity of a Brownian Particle. *Science* **2010**, *328*, 1673–1675.

(52) Happel, J.; Brenner, H. Low Reynolds Number Hydrodynamics; 1965; p. 229.

(53) Snezhko, A.; Aranson, I. S. Magnetic Manipulation of Self-Assembled Colloidal Asters. *Nature Mater.* **2011**, *10*, 698–703.

(54) Ahmed, S.; Wang, W.; Mair, L. O.; Fraleigh, R. D.; Li, S.; Castro, L. A.; Hoyos,
M.; Huang, T. J.; Mallouk, T. E. Steering Acoustically Propelled Nanowire Motors
Toward Cells in a Biologically Compatible Environment Using Magnetic Fields. *Langmuir* 2013, 29, 16113–16118.

Chapter 4

Exploring the Shape Dependence of Acoustic Motor Propulsion

4.1 Introduction

Acoustic energy has recently been harnessed for the fuel free propulsion of autonomously propelled nano and microparticle motors.¹ The axial directional motion of these particles is unique and has been attributed to the shape asymmetry of the particles.^{1–} ³ Nanorod particles that undergo random autonomous motion have shape asymmetry due to their deposition conditions.¹ The end that is deposited first and comes into contact with the sacrificial silver cathode tends to be concave while the other end tends to either be pointed, flat or convex depending on the material being deposited. Figure 4-1. Reports have attributed the axial motion of nanord particles to this shape asymmetry, where differential interaction of the edges with the acoustic field and the flow generated as a result of the acoustic field generates a net propulsive force on the particle. Of yet little systematic experimental tests of this hypothesis exist. In this chapter we hope to shed further light on the effect of particle shape on motion within the levitation plane of an acoustic chamber applying a bulk acoustic wave. The chamber has a resonant frequency of 3.77MHz. The set up of the acoustic chamber have been described in detail in previous chapters.



Figure 4-1. Au nanowires that undergo random autonomous motion. One end is pointed and the other is concave. A. FE-SEM of Au nanowires grown at a current density of $-2mA/cm^2$ showing the different shapes of the two ends of the wire. One end is concave and the other is pointed. B. Zoomed in FE-SEM image of the concave end of the wire.

The manipulation and separation of particles using acoustic energy has a long history in the field of microfluidics.^{4–6} Yet despite this, little work has been done to evaluate the effect of particle shape on acoustophoretic motion, regardless of the overall size of the particle. In the cases where shape has been studied, only particles large enough to neglect acoustic streaming, greater than 2µm in diameter, have studied. These efforts have been targeted at biological separations, such as blood fractionation based on the shape of different types of blood cells, such as red blood cells from other blood cells.^{7–11} Much work has been done to explore the effect of vessel shape on particle manipulation within microfluidic channels with more recent work exploring the effect of vessel shape on acoustic streaming patterns and hence sub-2 µm particle motion.⁶ To my knowledge no systematic work has looked into the effect of particle shape on particle motion where the streaming-induced drag force is a dominant, non-negligible force.

In this work we take a preliminary look into the effect of motor shape on the motion of particles in the size range of 1 to $50\mu m$. In these cases both radiation forces

and streaming-induced Stokes drag force effect motion. We first present a fabrication method for the "mass" fabrication of thousands of motor particles of various sizes and shapes that are uniform. We then test the acoustophoretic behavior of these particles in an acoustic chamber at resonance. Hence this work begins to address a void in the literature by looking into the effect of shape on the acoustophoretic motion of particles, with a particular emphasis on random autonomous directional axial motion.

4.2 Materials and Methods

While electrochemical deposition into porous alumina membranes yields nanorods with shape asymmetry at the ends, this asymmetry tend to be difficult to control and varies from wire to wire. Additionally, given that the diameter of the wire is 300nm \pm 30nm it is impossible to resolve the two ends of the wire optically. In order to systematically test the effect of motor shape on motion in an acoustic field the shape of the motors must be uniform and have dimensions observable with an optical microscope (>0.7 µm). These two factors can be afforded using photolithography. Photolithography can yield precisely defined structures based on a mask with submicron resolution (0.7 µm) in the case of projection photolithography and 1 µm resolution , with 2 µm obtained more reliably; using contact photolithography. Hence an approach combining photolithography and electrochemical deposition or evaporation was adopted.

Micromotors were designed in order to test the effect of various factors on motion within an acoustic field. Shapes of varying sizes, symmetries, expected drag "profiles", aspect ratios and rotational symmetries were designed. The desired shapes were drawn in the L-edit software program and exported as a GDS II file in order to be made into a lithographic mask. Both contact and projection photolithography masks were tried. The contact mask is made out of soda lime while the stepper mask is made out of Quartz. Projection lithography masks made from quartz can be used in both the projection lithography stepper tool and the contact lithography tool, but it must be noted that the size of the shapes on a projection mask are usually five times larger than the desired final shaped due to a 5x reduction lens in the stepper photolithography tool. While both contact lithography and projection lithography were attempted it was found that there was little difference between exposure using the two methods and since projection lithography offers better resolution, more flexibility in density of shapes exposed on a wafer, more flexibility in the size of the wafer used and allows more shape designs (die) on a single mask it was used primarily.

Custom made electrochemical deposition or evaporation templates were fabricated using electrochemical deposition-compatible photoresists or lift off resists respectively. Custom made templates were synthesized on conductive silicon wafers (B doped) after a layer of Ag (70nm) was evaporated on them. This Ag layer is a sacrificial layer that is dissolved after the deposition of the structures to yield free floating structures in the both the electrodeposition and evaporation processes. It also served as a cathode for the electrochemical deposition of structures in some cases. Following evaporation of the Ag sacrificial layer the appropriate positive photoresist was spun on to the wafer and baked. A dose array on the stepper projection lithography tool will be carried out to determine which focus and exposure time was most suitable for each shape. Post exposure the positive photoresist was developed. This removes the resist from the solubilized regions defining the motor shape exposing the Ag sacrificial layer for deposition. Post deposition the resist was stripped in an organic solvent, and Ag layer was selectively dissolved yielding free floating structures. The structures were then resuspended in water and placed in the acoustic chamber for testing. The fabrication process is depicted schematically in Figure 4-2.



Figure 4-2. A. Fabrication of motors using a combined photolithography and electrodeposition process. B. Fabrication of motors using a combined photolithography and evaporation process.

Electrochemical deposition was attempted first as it would yield thicker structures and hence a larger aspect ratio in terms of thickness to the length and width of the shape.

4.2.1 Motor Fabrication: Combined Photolithography and Electrodeposition

A positive electrodeposition compatible photoresist, SPR 955-2.1, was spun onto conductive wafers with a Ag sacrificial layer. A double layer of this resist, was used to allow for the electrochemical synthesis of thick structures. An HMDS adhesion layer was first spun to promote adhesion of the resist to the wafer. It was spun using a dynamic dispense procedure at 1200rpm for 35sec (using the D09 12/35 recipe as saved on the automated spin coaters). It was baked at 110°C for 30sec and allowed to cool to room temperature. The first layer of SPR 955-2.1 was then spun onto the wafer using identical conditions. It was baked for 2 minutes at 105°C and allowed to cool to room temperature. The thickness of this layer is about 3.35µm. An additional layer of SPR955-2.1 was spun on to the wafer (without the HMDS adhesion layer the second time) and allowed to cool to room temperature. The thickness of a SPR 955-2.1 double layer was measured to be 7.1 μ m. For contact photolithography attempts the MABA6 tool was used. The sample was exposed for 25 seconds under hard contact conditions. The sample was then developed in CD26 developer for 5minutes. For the use of the stepper projection lithography tool a dose array was done to pinpoint the exposure time and focus for the shape being exposed prior to each exposure. The best values for the shapes in mask 1 (Figure 2) were found to be 0 focus offset at a 0.22sec exposure. It must be noted that these conditions may vary from instrument to instrument.

The projection lithography mask contained 16 dies each with a different shape, and corresponding controls, in order to test out a number of different variables including: level of shape asymmetry for shapes of equivalent size, surface perimeter, area, level of expected drag of a shape including sharpness of edges and aspect ratio. Additionally shapes that contain rotational asymmetry were designed in order to potentially design rotors. The initial size range under investigation was in the tens of microns range. This mask can be seen in Figure 4-3.



Figure 4-3. Mask 1. A. Round "bullet" shape (Dimensions: $20x10\mu$ m; 5 µm radius of curvature) B. Sharp "bullet" (Dimensions: $20x10\mu$ m; 5 µm indentation) C. Bullet to test effect of perimeter(Dimensions: $20x10\mu$ m; 5 µm indentations, 2.5 µm thick wings) D. Bullet to test effect of perimeter 2 (Dimensions: $20x10\mu$ m; 2.5 µm indentations, 1.25 µm thick wings) E. Square arrow(Dimensions: $20x10\mu$ m; 5 µm height of arrow) F. Inverse Arrow(Dimensions: $20x10\mu$ m; 5 µm indentation) G. Equilateral triangle (30 µm side) H. Isosceles right triangle (28 µm sides, 40 µm hypotenuse) I. 30-60-90 right triangle (24 µm,32 µm,40 µm sides) J. 10:1 aspect ratio bullet (Dimensions: $10x1\mu$ m; 0.5µm radius of curvature) K. 10:1 aspect ratio bullet (Dimensions: $20x2\mu$ m; 1µm radius of curvature) L. 10:1 aspect ratio bullet (Dimensions: $40x4\mu$ m; 2µm radius of curvature) M. 10:1 aspect ratio bullet (Dimensions: $50x5\mu$ m; 2.5µm radius of curvature) N. 10:1 aspect ratio double concave control (Dimensions: $20x2\mu$ m; 1µm radius of curvature) O. Rotor (Dimensions: $20x25\mu$ m; 5.1µm indentations, 11 µm and 12 µm cuts on right and bottom) P. Rotor2 (Dimensions: $20x20\mu$ m; 5µm indentations, 11 µm and 12 µm cuts on right and bottom)

Several shapes were designed to test multiple factors. Shapes M, J,K and L are identical with a 10:1 aspect ratio as with our alumina membrane deposited wires with one end concave and the other convex but with different dimensions being 50:5 μ m, 10:1 μ m, 20:2 µm and 40:4 µm respectively. It must be noted that projection lithography mask shapes are five times larger than the target size due to the presence of a 5x size reduction length in the exposure instrument. These structures were designed to determine the effect of size on the presence and strength of directional axial propulsion. Shape N is a symmetric control shape for these structures, and in particular shape K, with both ends being identical, concave. Shapes G,H and I are equilateral, right isosceles and 30-60-90 triangles respectively with equal areas and approximately equal surface perimeters. Shapes O and P are a rotationally asymmetric shape and a rotationally symmetric shape that serves as a control. Shapes A and B are of similar dimensions of the same motif with shape A having curved edges and shape B having sharp pointed edges. Shapes B, C and D are of the same dimensions but have differing surface perimeters and aim to test the effect of scattering surface area and perimeter on motion. Shapes E and F have identical areas and perimeters but are of complementary shapes with E expected to experience less drag than F.

A wafer with a single shape was exposed using a stepper, developed and assembled within an electrochemical cell. A two electrode constant current setup would was used where the conductive silicon wafer with the silver seed layer served as the cathode and a platinum wire served as the counter and pseudo-reference electrode. Gold was electrodeposited and within seconds the resist delaminated. The photoresist rapidly peeled off the wafer and hence electrochemical deposition of the desired shapes was not possible. It was determined that the distance between adjacent shapes was too small and hence adhesion of the resist to the wafer in the deposition solution and during deposition when there is gas production was poor. Additionally, potentially poor adhesion of the photoresist to the silver seed layer used as the cathode for the deposition may also be a factor. Hence a second mask with larger inter-motor spaces (30µm) of a selection of the shapes of mask 1 was made. (Figure 4-4) A contact mask of shape B of mask 2 was also made (with 30 µm inter-motor spaces) to determine if better results could be obtained with contact lithography. It should be noted that mask 2, although designed as a projection lithography mask, can also be used for contact lithography but to make structures five times larger than those made using projection lithography with the same mask.



Figure 4-4. Mask 2. A. Equilateral triangle (30 μ m side) B. Isosceles right triangle (28 μ m sides, 40 μ m hypotenuse) C. 30-60-90 right triangle (24 μ m,32 μ m,40 μ m sides) D. 10:1 aspect ratio

bullet (Dimensions: $40x4\mu$ m; 2μ m radius of curvature) E. 10:1 aspect ratio bullet (Dimensions: $20x2\mu$ m; 1μ m radius of curvature) F. 10:1 aspect ratio double concave control (Dimensions: $20x2\mu$ m; 1μ m radius of curvature) G. 10:1 aspect ratio bullet (Dimensions: $50x5\mu$ m; 2.5μ m radius of curvature) H. Square arrow(Dimensions: $20x10\mu$ m; 5μ m height of arrow) I. Inverse Arrow(Dimensions: $20x10\mu$ m; 5μ m indentation)

New samples were made with mask 2 The process was repeated and indeed there

was better photoresist adhesion during the electrodeposition step. It was difficult to

control the growth across the wafer and initial trials yielded much overgrowth. This can

be seen in Figure 4-5. Electrodeposition of Au was done at a constant current density -

 $280\mu A/ cm^{2}$.



Figure 4-5. Electrochemically deposited motor structures within lithographic resist membranes. Structure is a right isosceles triangle (28 μ m sides, 40 μ m hypotenuse) and were produced by carrying out contact lithography using a separate soda lime mask of shape B in mask 2. Structures were deposited anodically in a two electrode cell at -280 μ A/cm² for 45minutes on a silicon substrate. A. 50x dark field optical micrograph of structures. Overgrowth beyond the structure's boundary is clear. B. 20x dark field optical micrograph of structures. C. 5x dark field optical micrograph of structures.

In an attempt to improve photoresist adhesion, the adhesion layer and photoresist

were spun directly onto the conductive silicon wafer without the silver sacrificial layer.

This was done because these adhesion layers are primarily designed to promote resist adhesion to silicon. To get free structures the removal of the native oxide layer in HF acid would be done. The process yielded deposited structures as with a silver cathode but there was no measurable enhancement to the amount of time a deposition could be conducted before delamination. Another attempt was made to improve silver and the resist adhesion by using an alternate adhesion layer, Omnicoat® instead of HMDS. This only made a marginal impact on the adhesion time of the photoresist during depositon.

Multiple electrochemical deposition attempts were made with several of the shapes, yet growth was non-uniform across the wafer and there was considerable variation among shapes. Better uniformity across a wafer was achieved with lower current density but there was a limitation on time of deposition as photoresist adhesion was compromised beyond 2 hours. One of the better trials can be seen in Figure 6. There is less overgrowth but there still isn't any shape to shape uniformity. Electrodepositon was done at -5.65μ A/cm² for a total time of 45minutes for the samples in Figure 4-6.



Figure 4-6. Electrochemically deposited motor structures within lithographic resist membranes. Structure dimensions are 100 μ m x50 μ m and were produced by carrying out contact lithography using shapes H and I of mask 2. Structures were deposited anodically in a two electrode cell at - 5.65 μ A/cm² for 45minutes on a silicon cathode. A.20x optical image of inverse arrow B.50x optical image of inverse arrow C. 20x optical image of square arrow D. 50x optical image of square arrow

Growth was fairly uneven, occurring in patches across the exposed area of the

wafer. The photoresist was then cleanly removed using PG remover (photoresist stripper) after the deposition and thickness profilometer measurement was taken. It was found that even within a single motor structure there was variability in the structure height. For the structures shown in Figure 4-7, the height of the structure at the sides was taller (70nm) than in the center (40nm) resulted in a "winged" structure. Hence due to the non-uniformity of the growth across a wafer and the variation of height within a single motor electrodeposition was abandoned as the way of synthesizing high aspect ratio motor structures.



Figure 4-7. Electrochemically deposited motor structures within lithographic resist membranes. Structure is a 10:1 aspect ratio bullet (Dimensions: $50x5\mu$ m; 2.5 μ m radius of curvature) and was produced by carrying out contact lithography using shape G of mask 2. Structures were deposited anodically in a two electrode cell at at -5.65 μ A/cm² for one hour and thirty minutes on a silver cathode. A. 5x dark field optical micrograph of sample. B. 20x optical micrograph of sample.

Evaporation in combination with photolithography was then adopted for the synthesis of more uniform structures.

4.2.2 Motor Fabrication: Combined photolithography and evaporation

We moved from electrodeposition to evaporation to get more uniform fabrication of motors across a wafer and more uniform and controlled height within a single motor. A liftoff process, which combines photolithography and metal evaporation, was used in order to synthesize the desired structures. As previously done for samples intended for photolithography followed by electrodeposition substrates with an evaporated sacrificial layer for post-synthesis release from the substrate were used. The same masks described above were used.

A liftoff process was found to be the most successful. The resists used were LOR 5A (Lift Off Resist 5A) which was spun using the recipe D09-40-45 yielding a thickness of approximately 1µm and the 3012 resist on top of that with a thickness of 0.5 µm, spun

under the same conditions. The resists were baked at 160 degrees and 180 degrees for 1 min and 3 min respectively. The maximum metal thickness that could be evaporated is 75% of the thickness of the 3012 resist which is a maximum thickness of 375nm. A dose array was carried out using a stepper to determine the best exposure and focus conditions for the particular shape being synthesized and an 11×11 matrix of the dies previously described was used to populate a 2 inch B doped Si wafer. Post exposure, development was done in the commercial solution CD-26 for one minute, and rinsed in di H_2O followed by drying with a nitrogen gun. Approximately 150nm of Au was then evaporated on to the structures and lift off was done using the commercial polymer stripper PGremover. The wafers were then soaked in 1:1 solution of concentrated nitric acid and diH₂O to remove the Ag evaporated sacrificial layer and generate free floating structures. Repeated rinses followed by centrifugation were then done in order to suspend the structures in distilled water for acoustic testing. The approximate density of the structures was 400,000 structures/mL. Fabrication of shape B in mask 2 (Figure 4-3) of a right isosceles triangle (28 µm sides, 40 µm hypotenuse) was carried out. The fabrication was successful and the resultant structures can be seen in Figure 4-8.



Figure 4-8. Au right isosceles triangles (28 μ m sides, 40 μ m hypotenuse) produced by a combined projection photolithography and evaporation approach. A. 5x optical micrograph of structures on wafer after resist removal. B. 20x optical micrograph of structures after release from the wafer.

4.3 Acoustic Testing

The right isosceles triangles were placed in the acoustic chamber where, within the levitation plane, they rapidly aggregated into nodal locations. They did not display random autonomous motion and in their "focusing" to nodal regions their motion did not display any consistent directionality or polarity of motion. An image of the structures aggregated in a node can be seen in Figure 4-9.



Figure 4-9. A nodal aggregate of the right isosceles triangles (28 μ m sides, 40 μ m hypotenuse) placed within the acoustic chamber and suspended in the levitation plane.

These structures do not exhibit random autonomous motion as their motion is dominated by the acoustic radiation force. Therefore structures with only much smaller sizes need to be made to observe random autonomous motion. W mask containing structures that are smaller in size and hence closer to actual nanomotors dimensions was made. Figure 4-10. As projection photolithography offers better resolution and allows the synthesis of multiple shapes on a single mask this technique was selected and contact lithography abandoned.



Figure 4-10. A. Inverse arrow (6 x3µm, 2.6 µm indentation) B. Square arrow (6 x3µm, 2.6 µm protrusion) C. Arrow (6 x3µm, 2.6 µm indentation, 2.6 µm protrusion) D. Rotor (6 x4µm, 1.732 µm height triangular cut 1 µm into the structure with 2 µm sides) E. Inverse arrow(4 x2µm, 1.732 µm indentation) F. 1:1 aspect ratio Inverse arrow (3 x3µm, 1.5 µm indentation) G. Inverse arrow with a smaller indentation than A (6 x3µm, 1.5 µm indentation) H. Rectangular control (6 x3µm) I. Square control (3 x3µm)

All structures on mask 3 had dimensions smaller than 8 μ m. Structures A and B are complementary shapes as previously described in Mask 1 and 2 in this case they are 4 μ m by 2 μ m in size. C is of the same dimensions and motif as shapes A and B but has both an indented and pointed end. Shape D is designed to be a rotor. Shapes A and E are identical but of different sizes with shape E being 2 μ m by 1 μ m in size. Shapes A and G are similar but with different size indentations to test the effect of the level of shape asymmetry on directional random autonomous propulsion with shape H serving as a control. Shaped F and I are to test the effect of the aspect ratio of the structure with as well shape asymmetry as both shapes have a 1:1 aspect ratio. The first experiments focused on the synthesis of shapes A and B following the method outlined in Figure 2B. The synthesis was successful yielding uniform structures across multiple wafers with little variation from shape to shape as can see in Figure 4-11 below.



Figure 4-11. A. Fabricated inverse arrow structures ($6 \times 3\mu m$, $2.6 \mu m$ indentation) clockwise: 20x bright field optical micrograph of structures on the wafer, a schematic or the structure, a dark field optical image of the released structure. B. Fabricated square arrow structures ($6 \times 3\mu m$, $2.6 \mu m$ protrusion) clockwise: 20x bright field optical micrograph of structures on the wafer, a schematic or the structure, a dark field optical image of the released structure. These two shapes serve as controls for each other.

Upon levitation the inverse arrow motor exhibited primarily random autonomous

rotational motion. Approximately 50 separate microstructures were observed and their

mode of motion noted. Interestingly, unlike what was hypothesized based on expected

pressure differences or expected drag force profiles on each side of the structure,

approximately 90% of structures (43 out of 48) moved in the reverse direction as would

be expected with the edge with the indentation leading. Figure 4-12.



Figure 4-12. Inverse arrow motion. 20x Optical image frame sequence of inverse arrow motion. Time stamps at bottom of frames.

In order to decipher the mechanism of motion, further experiments are needed. Shapes similar to the inverse arrow synthesized above but with varying indentations can be made and the relative speeds of these shapes determined systematically. It is assumed that due to level of shape asymmetry the speeds of these structures should differ, with the ones with larger indentations moving faster. Shape G in mask three is similar to that of a described above, also an inverse arrow, with a smaller indentation placing these two shapes simultaneously within the acoustic chamber would be informative.

A preliminary observation of shapes with different indentations (Figure 4-13)indeed shows that this could be true. The speed of rotation of the shape with a larger indentation is faster at 63 μ m/sec, while the speed of rotation of the shape with a smaller indentation, and hence smaller shape asymmetry, is 18 μ m/sec. In addition to the movement being slower the movement is also less directional. More systematic experiments are due.



Figure 4-13. The structure on the top is moving a clockwise manner at **63 \mum/sec**, indented part leading. The structure on the bottom is moving in a counterclockwise manner at **18\mum/sec**, also the indentation leading, but the motion is less directional. Time stamps at bottom of frames.

A control structure (mask 3, shape B), a regular arrow with identical perimeter and surface area, was fabricated and preliminary observations made. Preliminary observations seem to indicate that the orientation of motion is not as consistent from motor to motor as the inverse arrow. Shapes were observed to move in 3 separate orientations. Rotation occurred with the pointed end forward, the flat end forward and sideways. (Figures 4-14 & 4-15)



Figure 4-14. The motion of these two identical shapes is different. The top arrow is moving counterclockwise with the pointed end leading. The bottom arrow is rotating clockwise hinged on one of its flat edges. Time stamps at bottom of frames.



Figure 4-15. The motion of this control arrow is clockwise with the flat end leading. Time stamps at bottom of frames.

In addition to the level of shape asymmetry as indicated above different sizes of

the structure can also be made. 2 different sizes of the inverse arrow can be made, one

that is slightly larger and the other smaller. The limit on how small a shape can be made

will depend on the resolution of the optical microscope, as the two sides of the motor

must be distinguishable in order to determine orientation of motion.

4.4 Electron beam Lithography Trials

The motion of the structures discussed so far have been primarily rotational random autonomous motion and the desired axial motion is not observed. This may be due to either a slight asymmetry in the motor in the axis perpendicular to the direction of motion or that the structures are large and hence more susceptible to deviation from the axial motion observed in nanorods. In order to address these two issues e-beam lithography was attempted. A PMMA-MMA resist double layer was used to allow for lift off following evaporation. The MMA resist was spun at 3000 rpm and baked at 150°C for 3min and allowed to cool to room temperature. The PMMA resist was spun on top of that at 4000 rpm and baked at180°C for 3min and allowed to cool to room temperature. A dose array of a selection of the shapes was tried to ensure symmetry where desired for larger shapes and to achieve smaller structures while keeping the size large enough for optical distinction of each edge. The resist was developed in a 1:1 mix of MIBK and isopropyl alcohol for 1 minute followed by isopropyl alcohol bath for 30 seconds and then rinsed with distilled water. The smallest structure targeted was an inverse arrow 1.0µm x 0.5 µm in size. The array was developed and gold was evaporated onto it. Figure 4-16. Optically it was possible, but fairly difficult, to distinguish the two ends of the small structure while still attached to the wafer. Release of these structures was attempted and it was found they were hard to retrieve. Hence beyond this trial it was determined that e-beam lithography would not yield a significant advantage over photolithography.



Figure 4-16. Shapes fabricated used e-beam lithography. Shapes A-C have identical dimensions of $(6x3\mu m)$ but with varying indentations. D. Square arrow control shape $(6x3\mu m)$ E. Smallest fabricated shape $(1x0.5\mu m)$, there is some rounding of the features. All unlabeled scale bars represent 1 μm . Shapes were difficult to release and retrieve.

4.5 Conclusions

From these experiments it seems clear that the shape of the motor does have an effect on polarity of acoustic propulsion. Yet the overall sizes of these structures are large enough such that axial random autonomous motion is not observed. Attempts were made at reducing the size of the structures further but there is a limit on how small structures can get as the ends need to be distinguished in an optical microscope. E-beam attempts did not prove promising. The approach they can be taken to ensure motor structure is large enough for optical distinction of its edges while reducing the effect of the radiation force a size comparable to that on the nanorod motor traveling in water, as by changing experimental medium. Reports have demonstrated that acoustic propulsion is possible in various media. Reports have also shown that it is possible to alter the characteristic particle length below which radiation forces no longer become dominance in the motion of the particle by altering the fluid medium in which a particle is suspended. ^{12,13} Hence

doing these experiments with the photolithography defined structures described above, but in a different medium, would yield more informative results regarding axial random autonomous motion.

4.6 References

(1) Wang, W.; Castro, L. A.; Hoyos, M.; Mallouk, T. E. Autonomous Motion of Metallic Microrods Propelled by Ultrasound. *ACS Nano* **2012**, *6*, 6122-6132.

(2) Garcia-Gradilla, V.; Orozco, J.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.;
Pourazary, A.; Katzenberg, A.; Gao, W.; Shen, Y.; Wang, J. Functionalized UltrasoundPropelled Magnetically Guided Nanomotors: Toward Practical Biomedical Applications. *ACS Nano* 2013, 7 (10), 9232–9240.

(3) Nadal, F.; Lauga, E. Asymmetric Steady Streaming as a Mechanism for Acoustic Propulsion of Rigid Bodies. *Phys. Fluids* **2014**, *26*, 082001.

(4) Friend, J.; Yeo, L. Y. Microscale Acoustofluidics: Microfluidics Driven via Acoustics and Ultrasonics. *Rev. Mod. Phys.* **2011**, *83*, 647–704.

(5) Leong, T.; Johansson, L.; Juliano, P.; McArthur, S. L.; Manasseh, R. Ultrasonic Separation of Particulate Fluids in Small and Large Scale Systems: A Review. *Ind. Eng. Chem. Res.* **2013**, *52*, 16555–16576.

(6) Bruus, H. Acoustofluidics 1: Governing Equations in Microfluidics. *Lab Chip* **2011**, *11*, 3742.

(7) Ranjan, S.; Zeming, K. K.; Jureen, R.; Fisher, D.; Zhang, Y. DLD Pillar Shape Design for Efficient Separation of Spherical and Non-Spherical Bioparticles. *Lab Chip* **2014**, *14*, 4250–4262.

(8) Beech, J. P.; Holm, S. H.; Adolfsson, K.; Tegenfeldt, J. O. Sorting Cells by Size, Shape and Deformability. *Lab Chip* **2012**, *12*, 1048.

(9) Jakobsson, O.; Antfolk, M.; Laurell, T. Continuous Flow Two-Dimensional Acoustic Orientation of Nonspherical Cells. *Anal. Chem.* **2014**, *86* (1), 6111–6114.

(10) Masaeli, M.; Sollier, E.; Amini, H.; Mao, W.; Camacho, K.; Doshi, N.; Mitragotri, S.; Alexeev, A.; Di Carlo, D. Continuous Inertial Focusing and Separation of Particles by Shape. *Phys. Rev. X* **2012**, *2*, 1–13.

(11) Zeming, K. K.; Ranjan, S.; Zhang, Y. Rotational Separation of Non-Spherical Bioparticles Using I-Shaped Pillar Arrays in a Microfluidic Device. *Nat. Commun.* 2013, *4*, 1625.

(12) Barnkob, R.; Augustsson, P.; Laurell, T.; Bruus, H. Acoustic Radiation- and Streaming-Induced Microparticle Velocities Determined by Microparticle Image Velocimetry in an Ultrasound Symmetry Plane. *Phys. Rev. E* **2012**, *86*, 1–11.

(13) Settnes, M.; Bruus, H. Forces Acting on a Small Particle in an Acoustical Field in a Viscous Fluid. *Phys. Rev. E* **2012**, *85*, 1–12.

Chapter 5

Experimental Insights into the Mechanism and Factors Affecting Ultrasonic Propulsion of Nanorod Motors

5.1 Introduction

Artificial nano- and micro-swimmers are of growing interest because they provide insight into the emergent and collective behavior of motile living systems, as well as new functionality and applications in biomedicine, analysis, separations, environmental chemistry, and materials science.¹⁻⁴ The discovery of biocompatible, fuel free ultrasonic propulsion of nanomotors presents some promise for use in biomedical applications.⁵ Recent reports of acoustic nanomotors have demonstrated their control in biocompatible environments, their actuation within living cells, and as their use as drug delivery agents.^{6–8} Their collective behavior and assembly have also been explored.^{9–11} In order to design better acoustic motors that can carry out useful functions, it is important to understand the mechanism of their propulsion as well as the factors that affect their propulsion. Experimental and more recently theoretical research have proposed mechanisms in which the axial asymmetry of the rods results in motion perpendicular to the direction of acoustic excitation.^{5,12,13} A recent paper by Nadal and Lauga provides a coherent and detailed theoretical proposal that the propulsive force is a net hydrodynamic drag force arising from oscillatory fluid flow along the axis of the nanorods. In this model, the rods are treated as rigid bodies, and because their density is higher than that of the surrounding fluid, their inertia becomes significant at high driving frequency.¹³ In this work, we consider the additional effect of material asymmetry of a nanorod motor, and also measure the effects of size and acoustic power. We find that bimetallic rods exhibit a consistent and predictable polarity in their axial motion, with the lighter end leading. Both the length- and power-dependence of nanorod speed are consistent with an acoustic streaming-induced drag force dominated mechanism of propulsion, largely in agreement with the model proposed by Nadal and Lauga.

5.2 Results and Discussion

Single metal and bimetallic nanorods containing Au, Rh, Pd, Ag, Pt and Ru segments were fabricated by electrochemical deposition within the pores commercial porous anodic alumina templates (diameter 300±30nm) using commercial plating solutions. ¹⁴A metal film (typically silver) evaporated onto one side of the alumina membrane served as the cathode of the electrochemical cell, and a sacrificial silver segment several microns long was first plated into the pores. Bimetallic rods were fabricated by sequentially changing the deposition solution in the cell. The silver backing layer and sacrificial segment were dissolved in nitric acid and the rods were then released from the membrane by dissolution in strong base. The sacrificial silver segment typically has a convex tip, resulting in a concave end of the first metal plated. Depending on the surface interaction between the second metal and the pore walls, the second metal segment can have a flat tip, or more typically a convex tip, as described in more detail below.

Acoustic excitation of nanorod motors was carried out in a cylindrical acoustic chamber (with a height of 180µm and diameter of 5mm) operated at a resonant frequency

of ca. 4 MHz as previously described. ⁵ At resonance a bulk acoustic standing wave is set up and the rods are levitated to the midpoint plane of the chamber. In this plane, rods undergo random autonomous propulsion as well as aggregation within lateral nodes, where polar spinning chains are formed.⁵ The behavior of interest for the present study is the random autonomous motion that occurs far from the lateral acoustic nodes in the levitation plane.

5.2.1 Locations of Nanorod Acoustic Behaviors

Within the levitation plane of the acoustic chamber there are different regions in which spinning chains of rods, aggregates, and rods moving autonomously in random directions are observed. The location of these various behaviors can be seen in Figure 5-1. Random autonomous motion occurs in several regions: 1) near the cell boundary, 2) At the intersection of the outspray of a nodal aggregate and a spinning chain, and 3) at the intersection between the outspray of two or more spinning chains. The presence of random autonomous motion near the boundary of the cell is consistent with streaming induced propulsion as this propulsion is most likely a result of boundary streaming. Random autonomous motion on the outskirts of spinning chains is also consistent with streaming induced propulsion.



Figure 5-1. Locations for nanorod acoustic behaviors. A. Optical micrograph that points out the location of patterns such as spinning chains and nodal aggregates and the location of random autonomous motion relative to these structures. Axial motion occurs at the intersection of the outsprays of nodal structures. B. The location of random autonomous motion at the "outspray" of nodal spinning chains. C. The location of random autonomous motion near the boundary of the acoustic chamber. A zoomed in micrograph of rods undergoing axial motion.

5.2.2. Motion of Bimetallic Rods

The bimetallic rods fabricated for this study are listed in Table 5-1. These

particular combinations were selected because the two segments were distinguishable by

color in the optical microscope, and hence the direction of movement could be

determined. Bimetallic rods (AuRu, AuRh, AuPt, AuPd, AuAg and RuRh) were

propelled with the lower density metal segment leading, those segments being Ru, Rh,

Au, Pd, Ag and Ru respectively. Figure 5-2 shows examples of polar axial propulsion of the bimetallic rods as frame sequences and videos, respectively. The densities of the pure metals are listed in Table 5-2.

Bimetallic Rod	Lower Density End	Leading End
AuRu	Ru	Ru
RuAu	Ru	Ru
AuRh	Rh	Rh
RhAu	Rh	Rh
AuPt	Au	Au
PtAu	Au	Pt
AuPd	Pd	Pd
AuAg	Ag	Ag
RhRu	Ru*	Ru
RuRh	Ru*	Ru

Table 5-1. Bimetallic nanorods studied and the leading end in autonomous axial motion. The metal segments are listed in the order plated; hence the end of the first metal in the table is concave. *See SI. Due to deposition conditions Ru segments may have a lower density than Rh segments.



Figure 5-2. Top row: Frame sequence of AuRh wires propelled with Rh end leading. (Au segment deposited first) Time stamps are indicated. **Bottom row:** Frame sequence of AuPt wires propelled with Au end leading. (Au segment deposited first) Time stamps indicated.

Metal	Density (g/cm3)
Au	19.32
Ru	12.41
Rh	12.41
Pt	21.45
Ag	10.5
Pd	12.02

Table 5-2. Densities of pure metals.

Bimetallic rods are asymmetric, as noted above, because the end plated first is convex. In order to deconvolute the effects of material and shape asymmetry, the order of electrodeposition was reversed, and the shape and direction of movement were determined. AuRu, AuRh, RuRh and AuPt rods were fabricated in reverse order: RuAu, RhAu, RhRu and PtAu. As shown in Figure 5-3, the segment plated first always has a concave end, and the second segment has a flat (Pt, Rh, Ru) or a convex end (Au). The direction of motion (Table 5-1) was towards the lower density end, i.e., RuAu, RhAu and RhRu rods with Ru, Rh and Ru leading, respectively. In the case of RhRu rods, where the densities of the two pure metals are similar, X-ray powder diffraction patterns show that the Ru segment consists of smaller scattering domains (details in experimental details section), which suggests that it may be porous. In any case, the leading end of both RuRh and RhRu rods is towards the Ru end, indicating that in this case the effect of material asymmetry is greater than that of shape asymmetry. In the other case (PtAu, AuPt) in which the densities of the two metals are very similar, the direction of motion is reversed by reversing the order of electrodeposition. Thus in this case, shape asymmetry appears to determine the direction of motion – with the concave end leading - and in all other cases the material asymmetry determines the direction. Movement towards the concave end is predicted by calculations on axisymmetric near-spheres that have opposing convex and concave sides,¹³ consistent with our observations. We note that our own earlier model of pressure differences
generated by scattering of acoustic energy from the convex and concave ends made the opposite prediction,⁵ and is thus inconsistent with the data.



Figure 5-3. A. Schematic diagram of the shapes at ends of the bimetallic nanorods. The segment deposited first replicates the convex silver sacrificial segment (back dashed circle) and is therefore concave. At the other end Rh, Ru and Pt acquire a flat shape while Au is typically convex. B. FE-SEM (field emission scanning electron microscopy) micrographs of the concave (deposited first) nanorod ends after the dissolution of the sacrificial silver segment and release from the membrane. C. FE-SEM images of bimetallic rods. The different metal segments are labeled. **Top row:** (metals listed in the order deposited) AuRh ($2.3\pm0.3\mu$ m, Au: $1.3\pm0.2\mu$ m, Rh: $1.0\pm0.2\mu$ m); RhAu r ($2.1\pm0.4\mu$ m, Rh: $1.04\pm0.3\mu$ m, Au: $1.04\pm0.1\mu$ m); RuRh ($1.6\pm0.2\mu$ m, Ru: $1.3\pm0.1\mu$ m, Rh: $0.5\pm0.1\mu$ m); RhRu ($2.6\pm0.1\mu$ m, Rh: $0.9\pm0.1\mu$ m, Ru: $1.6\pm0.1\mu$ m) A short Au segment was grown in the center of the RhRu rod to allow for controlled Ru growth on top of Rh. In the absence of the Au segment, the Ru segment, deposited at a constant potential, overgrew within seconds on the catalytic Rh metal; **Bottom row:** (metals listed in the order deposited) AuRu ($2.2\pm0.3\mu$ m, Au: $1.0\pm0.2\mu$ m, Ru: $1.0\pm0.1\mu$ m); RuAu ($2.2\pm0.3\mu$ m, Au: $1.0\pm0.2\mu$ m, Ru: $1.0\pm0.1\mu$ m); RuAu (2.0μ m ±0.1 , Pt: 1.0μ . (2.0μ m ±0.1 , Pt: 1.0μ .) Au: $1.0\pm0.1\mu$ m). All scale bars represent 1 μ m.

The effect of different metals on rods speed was also evaluated. Bimetallic RhAu rods $(2.1\pm0.1\mu m, 1.0\pm0.1\mu m Rh segment, 1.0\pm0.1\mu m Au segment)$ and single metal Au rods of the same total length $(2.0\pm0.2\mu m)$ were mixed together in equal amounts and the sample was placed in the acoustic chamber. The autonomous motion of the Au and RhAu rods was recorded at a frequency of 3.77MHz and amplitude of 10Vpp in the same location of the chamber. The speed of each type of rod was determined independently. This was possible due to the optical contrast between Rh and Au. Care was taken to ensure that the shape asymmetry was similar for the two kinds of rods used in these experiments. Rh was deposited first in the case of RhAu and hence the concave shape it acquired was similar to that of the single element Au rods. The other end for both bimetallic RhAu and the single metal Au rods was Au, which forms the same convex tip shape for any Au segment. The RhAu rods were 25% faster than Au rods of the same length, $49\pm6 \,\mu\text{m}$ vs. $40\pm7 \,\mu\text{m}$ s⁻¹. In contrast, when single metal Rh and Au rods were compared in the same way, their speeds were $63\pm14 \ \mu m \ s^{-1}$ and $31\pm2 \ \mu m \ s^{-1}$, respectively. The speed of single element Rh rods was double that of single element Au. This is close to the ratio of the densities of the two metals: 19.32 g/cm^3 for Au and 12.41g/cm³ for Rh. The same experiment was done with a mixture of RuAu and Au rods yielding speeds of $29\pm6\mu m$ and $20\pm9\mu m s^{-1}$, again with the rod containing the lower density metal moving at a faster speed, and leading with its lighter end. (Figure 5-4). These comparisons indicate that lighter metal rods move faster, and that shape asymmetry alone can lead to relatively fast movement. However, the effects of shape and material asymmetry do not appear to be additive.



Figure 5-4. Speed comparison for bimetallic and monometallic nanorods of equal lengths. A. Au and Rh $(1.4\pm0.2\mu m)$ B. RhAu and Au $(2.0\pm0.2\mu m)$ C. RuAu and Au $(2.3\pm0.3\mu m)$ D. Optical micrograph representing the method used to obtain the data in A-B where nanorods were mixed and optical contrast was used to track each type separately. A mixture of Au and Rh nanorods is shown.

5.2.3 Length Dependence of Nanorod Speeds.

Two predominant forces are exerted on particles suspended in fluids an acoustic field. They are the primary acoustic radiation force and the acoustic streaming-induced drag force. Typically the primary radiation force is dominant on particles of diameters larger than about 2 μ m, whereas the motion of smaller particles is dominated by the streaming-induced drag force.^{15,16} Most research in this field has focused on spherical

polymer (e.g., polystyrene) particles and thus the applicability of these critical diameters to cylindrical metal rods with concave and convex ends is unknown.

In order to determine which force(s) dominate the motion of metallic nanorods, the length dependence of the speed was measured. Au nanorods 300 ± 30 nm in diameter and 1.4 ± 0.1 , 2.4 ± 0.1 , and $4.7 \pm 0.1 \mu$ m in length grown by varying the deposition time (14, 20, and 70 min., respectively, at a current density of -2.0 mA/cm^2). A 1:1:1 mixture of these nanorods in water was prepared and care was taken to monitor their movement at the same location within the acoustic chamber, in regions where the predominant mode of motion was random, autonomous propulsion along their axis. The axial velocity of these nanorods is plotted as a function of length in Figure 4. The resulting experimental trend was evaluated in terms of equations describing the size-dependent streaming-induced force and the primary acoustic radiation force.

Equation (5-1) describes the drag force on a cylindrical particle (approximated as a prolate ellipsoid) of length L and radius R moving at velocity v, and conversely equation (5-2) expresses the velocity as a function of the drag force.

$$F_{drag} = \frac{2\pi\eta L}{\ln\left(\frac{L}{R}\right) - 0.72} v \tag{5-1}$$
$$v = F_{drag} \frac{\ln\left(\frac{L}{R}\right) - 0.72}{2\pi\eta L} \tag{5-2}$$

The acoustic power density at a particular frequency and amplitude should be roughly constant over the small observation volumes we used to compare the speeds of rod mixtures. A detailed theoretical treatment of an axisymmetric near-sphere shows that the streaming-induced drag force is proportional to the radius R_0 of the sphere. ¹³ Here, we keep two dimensions of the cylindrical rod constant and vary only the length, so we expect the force to vary weakly with L. Since the velocity at a given force scales roughly as 1/L according to Equation 2, a longer rod would move at a slower speed than a shorter one if the motion is driven by streaming-induced drag. This is indeed what is observed (see Figure 5-5B).

The primary acoustic radiation force on a spherical particle can be described using equation 5-3:

$$F_{rad}(x) = 4\pi R^3 k \Phi E_{ac} \sin(2kx) \tag{5-3}$$

Where F_{rad} is the primary acoustic radiation force on a particle, R is the radius of the particle, E_{ac} is the acoustic energy density which is in the range of 10-100 J/m³ for operation in the MHz range at voltages less than or equal to 10 Volts peak to peak.¹⁷ k is the wavenumber, x is the distance from the node. Since the particles are at the node in the z direction, x represents the distance from a lateral node in the acoustic levitation plane (x-y plane) and $\boldsymbol{\Phi}$ is the acoustic contrast factor, which is related to the ratio of the density of the particle to that of the medium. The acoustic contrast factor is described by equation 5-4¹⁷:

$$\Phi = \frac{1}{3} \left[\frac{5\rho_p - 2\rho_m}{2\rho_p + \rho_m} - \frac{\beta_p}{\beta_m} \right]$$
(5-4)

where ρ_p is the density of the particle, in this case the density of a metal such as gold, and ρ_m is the density of the medium, in this case water. β_p and β_m are the compressibility's of the particle and medium respectively expressed by equation 5-5 where

$$\beta_p = \frac{1}{\rho_p c_p^2} \tag{5-5}$$

The corresponding size dependence of the velocity of the particle in an acoustic field due to the radiation force can be derived using the drag force on a spherical particle such that $v_{rad} = F_{rad}/6\pi\eta R$ where η is the dynamic viscosity of water $1 \times 10^{-3} \text{ kg} \cdot \text{s}^{-1} \text{m}^{-1}$.¹⁶ This is expressed by equation 5- 6:

$$v_p(x) = \frac{2\pi\Phi kR^2 E_{ac} \sin(2kx)}{3\pi\eta} \tag{5-6}$$

The equivalent volume sphere to nanorod cylinders of various lengths can be used to describe our particles. As the velocity is related to the radius of the particle squared (equation 5-6), as the particle size increases so would the velocity of the particle. In our system we observe the opposite trend. Hence it is clear that the trend in the experimental data is not due to the primary radiation force on the system. The data supports the streaming-induced drag model.



Figure 5-5. A. Experimental data with a fit using drag force equation (2). B. Theoretical speed dependence on rod length for radiation force dominated motion (red) and streaming induced force dominated motion (black).

5.2.4 Controlling the Applied Forces on the Rods via Electronic Signal Manipulation

The acoustic levitation and propulsion of nanorods results primarily from two forces: the acoustic radiation force and the streaming-induced drag force. The acoustic radiation force is responsible for the levitation of the particles, and the lateral component of acoustic radiation force is responsible for the formation of nodes within the levitation plane.^{18,19} The streaming induced drag force tends to form vortical patterns within the fluid and contributes to the organization of spinning chains or spindles in the system.^{20–22} One can control the relative contribution of each type of force active in the system by controlling the power input.²³ The acoustic power density can be decreased in the system by reducing the amplitude of the actuating wave to the piezoelectric transducer. Because the acoustic radiation force in the vertical direction is much stronger than the acoustic streaming-induced drag force, by gradually reducing the amplitude it is possible to sharply reduce acoustic streaming while keeping the nanorods in the levitation plane. It is thus possible to monitor these forces separately by monitoring their effects. The presence of the acoustic radiation force can be monitored by noting the levitation of the particles as well as the presence of lateral nodes within the levitation plane. In the absence of spinning chains, acoustic streaming can be considered no longer a dominant factor in the particle motion in the levitation plane. Simultaneously monitoring the presence of random autonomous motion, speed of nanorod propulsion as well as other patterns in the chamber one can gain insight into the forces responsible for propulsion.

Gold nanorod motors $(1.4\pm0.1\,\mu\text{m} \log)$ were placed within the acoustic chamber operated at resonance (3.77MHz) and at 10V peak-to-peak (pp) driving amplitude. The rods were suspended within the levitation plane and after 30 s, random autonomous motion, spinning chains and nodal aggregates were established. While monitoring the levitation of the rods, the presence of spinning and random autonomous motion the amplitude of the driving voltage was reduced by 1Vpp every 10 seconds. By monitoring the speed of rods undergoing random autonomous motion and the width of a spinning chain, it was found that there was an approximate correspondence between the disappearance of spinning chains and the sharp reduction of the speed of random autonomous propulsion, as shown in Figure 5. This observation provides further support that the streaming-induced drag force is the primary driving the autonomous axial motion of nanorods in regions far from the lateral nodes in the cell. We found that the speed of the random autonomous propulsion of the wires is proportional to the square of the amplitude of the driving voltage.



Figure 5-6. A. The amplitude (voltage peak to peak) dependence of the speed of autonomous axial motion of nanorod motors. B. The amplitude dependence of the width of a spinning chain. The dashed line marks the complete disbanding of the chain, which corresponds to the plateau in speed of the axially propelled rods. C. Frames showing the disbanding of a spinning chain with decreasing amplitude.

5.3 Experimental Details

5.3.1. Nanorod Fabrication, Characterization and Release.

The metals Au, Rh, Pd, Ag, Pt and Ru were electrochemically deposited from commercial plating solutions within porous anodic alumina templates obtained from Whatman. The nominal pore diameter of the membranes was $0.2 \,\mu m$, but the crosssectional diameter through the bulk of the membrane was 300 ± 30 nm. A 350 nm layer of Ag evaporated on one side of the alumina template served as the cathode. A 350nm evaporated Cu layer was the cathode for the AuAg sample containing Ag, in order to selectively dissolve the cathode layer without etching away the Ag segment of the wire. The metals Au, Rh, Pd, Ag and Pt were deposited using a two electrode cell under constant current conditions with a Pt coil serving as the pseudoreference/counter electrode. Au was deposited from Au-Orotemp24 RTU (Technic Inc.) at a cathodic current of 1.24 mA/cm² with a deposition time of 13 min. yielding a 1µm segment. Rh, Pd, Ag and Pt were deposited from Techni Rhodium RTU, Pallaspeed VHS-RTU, 1025 RTU and Platinum TP RTU (Technic Inc.) at constant current densities of 1.76mA/cm², 0.88mA/cm²,2.21 mA/cm² and 1.76 mA/cm². Under these conditions for Rh, Pd, Ag and Pt deposition times of 120, 20, 8, and 40 min, respectively yielded 1µm segments of the metal. Ru metal was deposited at a constant potential of -0.650V in a three electrode cell with a Ag/AgCl reference electrode and a Pt coil counter electrode from Ru-U solution (Technic Inc.). A 25 min. depositon yielded a 1µm segment of Ru. In all cases a sacrificial Ag segment (Cu segment in the case of electrodeposited Ag) was plated directly onto the evaporated Ag cathode (Cu cathode in

the case of Ag) prior to the deposition of the metal of interest. The length of the sacrificial segment in all cases was approximately 10µm. Cu was deposited from a 0.1 M copper sulfate solution at a constant current density of 0.88 mA/cm² with a Pt coil counter electrode for one hour to obtain a 10 µm sacrificial segment. Bimetallic wires were fabricated by changing the deposition solution within the cell. After deposition, the cathode material and sacrificial segment were selectively dissolved. The silver cathode and sacrificial segment were dissolved in 1:1 water: concentrated nitric acid solution by soaking the membrane for 20 minutes. The copper cathode and sacrificial segment were dissolved in a proprietary copper etchant solution Copper Etch BTP (Technic Inc.) by soaking for one hour. The alumina membrane was then dissolved by soaking it in 2M aqueous NaOH overnight. Multiple rinsing steps were then done, involving repeated centrifugation, removal of the supernatant and filling the centrifuge tube with water, to suspend the wires in water.

FESEM images of nanorod samples were obtained to determine the length of the rods and the length of each metal segment. At least 21 rods were measured per sample. X-ray powder diffraction patterns of nanorod samples, still embedded in the membrane, after removal of the Ag or Cu sacrificial layer, were obtained over a range of 10 to 90 20 angles. Typically a quarter of a membrane, which had an area of 0.44cm², was used in X-ray diffraction experiments. Typically a quarter of a membranes was used at an area of 0.44cm². X-Ray diffraction results reveal a AgCl phase within the Ru containing wires. As AgCl has a density of 5.56g/cm³ which is lower than the density of Rh and Ru (12.41 g/cm³) its presence in the Ru segment of the wire results in an overall reduction of density of that segment of the wire. Additionally scherrer analysis of the X-Ray diffraction patterns reveal that the average grain size within the Ru metals segments is 4.3nm while it is 10.7nm, more than double the value for Rh, hence the packing of the Rh

gains within the segment is more compact than that for Ru which may result in a slight reduction of the density of the Ru segment relative to Rh. The X-Ray diffraction patterns of the samples can be seen in Figure 5-7.



Figure 5-7. X-Ray diffraction patterns of nanorods within the membrane with indexing. A. Diffraction pattern of AuRu rods. The wide Ru pattern of low intensity indicates the small grain size within the Ru segment. B. X-Ray diffraction pattern of Ru wires. Peaks are indexed. C. X-Ray diffraction pattern of AuRh wires with peaks indexed.

5.3.2. Acoustic Chamber.

The cylindrical acoustic chamber consists of a stainless steel plate (4.2 cm x 4.2 cm x 1mm), a few layers of kapton tape (180µm height) with a 5mm hole punched in the center defining the chamber and a thickness mode piezoelectric transducer (1mm thick, PZ26 Ferroperm, Kvistgard, Denmark) attached to the other side of the stainless steel plate (epoxy, Chemtronics, ITW, Kennesaw, GA, USA). The center of the cell has a resonance frequency (levitation frequency) of 3.77MHz. An amplitude of 10 volts peak to peak was used. To set up the standing wave within the chamber a glass coverslip was used as a reflector.

5.3.3 Determining the Width of a Spinning Chain.

The width of the spinning chain in Figure 5 was quantified by first selecting a frame from the frame sequences representing a particular amplitude (voltage peak to peak value). The image was imported into ImageJ an open access software program for image processing and analysis developed by the National Institutes of Health. A rectangular section 134 μ m in width by 1 μ m in length was selected and using the "Plot Profile" function within the "Analyze" menu the brightness intensity distribution within the selected area was produced. For a chain this approximated a bell curve, the spread of which was the width of the spinning chain. This process was repeated three times for each voltage peak to peak plotted. An example of the method used can be seen in Figure 5-8.



Figure 5-8. The plot of the light intensity distribution of a spinning chain (10Vpp) that approximates a bell curve. The spread of the curve is used as the width of the chain. The inset is the zoomed out intensity data.

5.3.4. Optical Imaging and Tracking.

Optical imaging was done with an Olympus BX60 light microscope. Video capture was done in the range of 25 to 60 frames per second. Open access video tracking software Video Spot Tracker (<u>http://cismm.cs.unc.edu/downloads/?dl_cat=3</u>) was used to track the movement of individual nanorods.

5.4 Conclusion

Bimetallic rods axially propel in a polar fashion with the lower density metal segment leading. This can be attributed to lower density wires moving at faster speeds. When the densities of the two metal segments are close, shape asymmetry determines polarity with motion occurring towards the concave end. Nanorod speed decreases with increasing length, in support of a streaming induced drag force dominated motion. There is also a correlation between the reduction to elimination of spinning chains and random autonomous motion and a correspondence in their further supporting a streaming induced drag force dominant motion. The results help shed light on the mechanism of propulsion as well as provide additional detail in the material dependent behavior of ultrasonically propelled motors that can incorporated into existing theoretical models aiming to explain propulsion.

5.5 References

(1) Wang, W.; Duan, W.; Ahmed, S.; Mallouk, T. E.; Sen, A. Small Power: Autonomous Nano- and Micromotors Propelled by Self-Generated Gradients. *Nano Today* 2013, 8 (5), 531–534.

(2) Mallouk, T.; Sen, A. Powering Nanorobots. *Scientific American* **2009**, *300* (5), 72–77.

(3) Yadav, V.; Pavlick, R. a.; Meckler, S. M.; Sen, A. Triggered Detection and Deposition: Toward the Repair of Microcracks. *Chem. Mater.* **2014**, *26*, 4647–4652.

(4) Kar, A.; Chiang, T.; Rivera, I. O.; Sen, A.; Velegol, D. Enhanced Transport into and out of Dead-End Pores. *ACS Nano* **2015**, *9*, 746–753.

(5) Wang, W.; Castro, L. A.; Hoyos, M.; Mallouk, T. E. Autonomous Motion of Metallic Microrods Propelled by Ultrasound. *ACS Nano* **2012**, *6*, 6122-6132.

(6) Ahmed, S.; Wang, W.; Mair, L. O.; Fraleigh, R. D.; Li, S.; Castro, L. A.; Hoyos, M.; Huang, T. J.; Mallouk, T. E. Steering Acoustically Propelled Nanowire Motors toward Cells in a Biologically Compatible Environment Using Magnetic Fields. *Langmuir* 2013, *29*, 16113–16118.

(7) Wang, W.; Li, S.; Mair, L.; Ahmed, S.; Huang, T. J.; Mallouk, T. E. Acoustic Propulsion of Nanorod Motors inside Living Cells. *Angew.Chem.Int. Ed.* 2014, *53*, 3201– 3204. (8) Garcia-Gradilla, V.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.; Yardımcı, C.; Wiitala, D.; Galarnyk, M.; Wang, J. Ultrasound-Propelled Nanoporous Gold Wire for Efficient Drug Loading and Release. *Small* **2014**, *10*, 4154-4159.

(9) Ahmed, S.; Gentekos, D. T.; Fink, C. A.; Mallouk, T. E.; Al, A. E. T. Self-Assembly of Nanorod Motors into Geometrically Regular Multimers and Their Propulsion by Ultrasound. *ACS Nano*, **2014**, *8*, 11053–11060.

(10) Wang, W.; Duan, W.; Zhang, Z.; Sun, M.; Sen, A.; Mallouk, T. E. A Tale of Two Forces: Simultaneous Chemical and Acoustic Propulsion of Bimetallic Micromotors. *Chem. Commun.* **2015**, *51*, 1020–1023.

(11) Xu, T.; Soto, F.; Gao, W.; Dong, R.; Garcia-Gradilla, V.; Magaña, E.; Zhang, X.; Wang, J. Reversible Swarming and Separation of Self-Propelled Chemically Powered Nanomotors under Acoustic Fields. *J. Am. Chem. Soc.* **2015**, *137*, 2163–2166.

(12) Garcia-Gradilla, V.; Orozco, J.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.;
Pourazary, A.; Katzenberg, A.; Gao, W.; Shen, Y.; Wang, J. Functionalized UltrasoundPropelled Magnetically Guided Nanomotors: Toward Practical Biomedical Applications. *ACS Nano* 2013, 7 (10), 9232–9240.

(13) Nadal, F.; Lauga, E. Asymmetric Steady Streaming as a Mechanism for Acoustic Propulsion of Rigid Bodies. *Phys. Fluids* **2014**, *26*, 082001.

(14) Wang, Y.; Hernandez, R. M.; Bartlett, D. J.; Bingham, J. M.; Kline, T. R.; Sen,
A.; Mallouk, T. E. Bipolar Electrochemical Mechanism for the Propulsion of Catalytic
Nanomotors in Hydrogen Peroxide Solutions. *Langmuir* 2006, *22* (25), 10451–10456.

(15) Muller, P. B.; Barnkob, R.; Jensen, M. J. H.; Bruus, H. A Numerical Study of Microparticle Acoustophoresis Driven by Acoustic Radiation Forces and Streaming-Induced Drag Forces. *Lab Chip* **2012**, *12*, 4617.

(16) Barnkob, R.; Augustsson, P.; Laurell, T.; Bruus, H. Acoustic Radiation- and Streaming-Induced Microparticle Velocities Determined by Microparticle Image Velocimetry in an Ultrasound Symmetry Plane. *Phys. Rev. E* **2012**, *86*, 1–11.

(17) Bruus, H. Acoustofluidics 7: The Acoustic Radiation Force on Small Particles.*Lab Chip* 2012, *12*, 1014.

(18) Evander, M.; Nilsson, J. Acoustofluidics 20: Applications in Acoustic Trapping. *Lab Chip* **2012**, *12*, 4667.

(19) Woodside, S. M.; Bowen, B. D.; Piret, J. M. Measurement of Ultrasonic Forces for Particle–liquid Separations. *AIChE J.* **1997**, *43*, 1727–1736.

(20) Aktas, M. K.; Farouk, B. Numerical Simulation of Acoustic Streaming Generated by Finite-Amplitude Resonant Oscillations in an Enclosure. *J. Acoust. Soc. Am.* 2004, *116*, 2822.

(21) Sadhal, S. S. Acoustofluidics 13: Analysis of Acoustic Streaming by Perturbation Methods. *Lab Chip* **2012**, *12*, 2292.

(22) Wiklund, M.; Green, R.; Ohlin, M. Acoustofluidics 14: Applications of Acoustic Streaming in Microfluidic Devices. *Lab Chip* **2012**, *12*, 2438.

(23) Hoyos, M.; Castro, A. Controlling the Acoustic Streaming by Pulsed Ultrasounds. *Ultrasonics* **2013**, *53* (1), 70–76.

Chapter 6

Conclusions and Future Possibilities

6.1 Introduction

Since their discovery in 2004, artificial nanomotors have garnered considerable research attention and have been studies for use in a number of potential applications.^{1–5} In this work we have accomplished several goals towards moving ultrasonic motors into the applied realm including controlling their motion, verifying their biocompatibility, understanding their assembly and looking into some of the factors that affect their motion including shape and material. In this chapter I outline some of the thrusts that could be explored to help move this field into a more applied realm.

6.2 Future Possibilities

6.2.1. Collective Behaviors

Among the promise of autonomous motors is the possibility of having independent populations of nanomotors carrying out coordinated and collaborative tasks. ^{2,6,7} In order to achieve collective behaviors between nanomotors, whether or not they are identical or different, communication or signaling between motors is necessary. Biological organisms, such as ants, leave a chemical trace for other organisms to detect in order to coordinate tasks. Chemical signaling between motors that results in clusters larger than a few particles have been demonstrated, these motors are typically light powered.^{8–10} These reports have focused on collective behaviors between identical particles. Only in a few cases, where passive silica particles are mixed with light activated AgCl and Ag ₃PO₄ particles, are between different populations of particles reported.^{9,10} Electrostatic interactions between chemically powered bimetallic rods and passive tracer particles has also been demonstrated as the consumption of hydrogen peroxide fuel leads to differential local concentrations of ions on each end of the rod.¹¹ In all of these cases, whether the signaling method is chemical or electrostatic, it has relied on the presence of ions within the media. These requirements disqualify chemically mediated collective behaviors for a variety of applications including biomedical applications where there is a high ionic strength medium.

Hence there is a need for a non-chemical method of achieving collective behaviors for nanomotors. Enabling the collective behaviors between fuel free ultrasonically propelled rods through physical interactions is desirable. Preliminary reports of few particle assemblies of ultrasonic motors using magnetic interactions presents a starting point for the engineering of collective behaviors between different populations of ultrasonic motors using magnetic interactions. ¹² By varying the magnetic character of different populations of nanorods it should be possible to engineer specific inter-motor interactions. The behavior of chemical motors of different magnetic character including: ferromagnetic with transverse and longitudinal magnetization directions and paramagnetic rods have been studied when placed on a magnetic garnet film.¹³ The ability to selectively control magnetic nanorods within a mixture containing paramagnetic beads by controlling the size of the magnetic field has been demonstrated. Magnetic rods were steered through a mixture containing paramagnetic beads using an external magnetic field and the rods attracted the beads behaving like a "vacuum cleaner". ¹⁴

When traveling in groups, biological organisms thed to follow different patterns. Birds tend to have multiple leaders when flying in flocks whereas packs of wolves have a structure where there are leaders and followers. In order to achieve leader-follower interactions that are magnetically mediated, one set of wires (leaders) should be responsive to an external magnetic field, while another set of wires (followers) are not responsive to the external magnetic field yet responsive to the local magnetic field (magnetic signals) of the leader wires by virtue of their proximity to them. To achieve this one may control either the magnetization direction of the two populations of the wires or the magnetic character of the two populations of wires. Ferromagnetic wires with a transverse magnetization (length of magnetic segment smaller than diameter of the rod) are steered using magnetic fields in the plane (xy direction) of the set-up, while those with a longitudinal magnetization need a magnetic component in the z direction.^{15–17} This could be used to steer one group of wires and not the other all while magnetic interactions between rods can serve to coordinate their motion making for leader-follower wires. Alternatively, ferromagnetic wires that are steerable using small external magnetic fields (milliTesla) may serve as leaders while paramagnetic wires as followers. To make paramagnetic wires, a polymer segment with paramagnetic iron oxide nanoparticles incorporated into it, is grown within the gold nanorods. Schematics of the wires to be used can be seen in Figure 6-1.



Figure 6-1. Rods of different magnetic character. (yellow represents gold and green represents nickel) A. Gold rod with a magnetic stripe smaller than the diameter of the rod ensuring transverse magnetization. B. Gold rod with a magnetic stripe longer than the 300nm diameter of with a longitudinal magnetization. C. Gold rod containing a polypyrrole polymer segment with embedded Fe_3O_4 superparamagnetic nanoparticles (white represents polypyrrole, red represents 5nm to 20nm Fe_3O_4 superparamagnetic nanoparticles)

Ferromagnetic, nickel containing, nanorods were made with transverse

magnetization. Ferromagnetic rods containing a single magnetic stripe 80nm in length and 300nm in diameter moving at approximately 17 μ m/sec have an interaction distance of 1.8 μ m/sec, this is smaller than the body length of the rod. ¹² To increase the interaction distance two additional nickel segments were grown within the gold rod. Figure 6-2 Hence three nickel segments approximately 40 nm, 100 nm and 100 nm in length were electrochemically deposited in a three electrode cell as previously described.¹² Paramagnetic rods were also made. They were gold with a polypyrrole segment with embedded superparamagnetic Fe₃O₄ nanoparticles. Polypyrrole was electrodeposited at 0.75V vs Ag/AgCl with a platinum couter electrode from distilled pyrrole. Iron oxide nanoparticles were synthesized using a standard co-precipitation technique.¹⁸ The particles were embedded into the polymer segment by mixing them in the pyrrole deposition monomer solution.¹³These rods were released from the membrane and TEM images were captures. Figure 6-2.



Figure 6-2. A. FE-SEM image of ferromagnetic transversely magnetizable nanorods. They are made out of gold with three nickel segments. B. TEM image of paramagnetic nanorods. They are made of gold with a polypyrrole segment with iron oxide nanoparticles. C. Zoomed in TEM image of polypyrrole segment containing Fe_3O_4 nanoparticles.

Squid magnetometry would be needed to determine the magnetic dipole moments of the rods and hence the maximum approach distance needed for one rod to detect the other at varying speeds in order to determine the optimal nanorod density and speed for inter-wire interaction. Preliminary trials in the acoustic chamber show that both rods types propel strongly. The ferromagnetic wires are steerable using a milliTesla magnetic field, similar to the single magnetic stripe rods, while the paramagnetic rods are not responsive to the magnetic field, as desired. Further experiments are needed to determine the appropriate conditions for inter-wire communication and quantify these interactions.

6.2.2. Biomedical Applications

Acoustic Chamber Design

Acoustic propulsion of nanorod motors has so far been done exclusively in an acoustic chamber of dimensions far from those that the rod would encounter in a biological system. It remains to be seen how acoustic propulsion would translate in vessels whose dimensions and aspect ratios more closely approximate that of the arteries (0.1-10mm diameters), veins and capillaries (5µm diameter) in a biological body. These structures do not have strict 3D confinement, hence levitation would be less likely to occur or the attainment of a resonance frequency of a vessel. Recent reports seem to demonstrate that levitation at a resonance frequency, although useful for fundamental studies as it eliminates the occurrence of sedimentation, is not crucial for the observation of acoustic propulsion as propulsion was observed at the bottom of the acoustic chamber while rods were not in levitation.^{19,20} It is not clear as well how the behavior of the rods will change, including the maximum speed they can attain, as they propel within a capillary of varying sizes where the effect of the boundaries is more pronounced. ²¹Acoustic streaming seems to be the crucial factor in inducing acoustic propulsion, and boundary streaming is always present, and its patterns will be altered. ^{22–24} Hence systematic experiments are called for to explore the effect of chamber design on acoustic propulsion and the forces that can be attained from acoustic propulsion. Additionally, as capillaries and arteries are usually organized into networks it would be informative for a variety of applications to see how they move or select their paths within a capillary network.²¹ (Figure 6-3) Nodal behavior at this point seems to be largely either nonessential or parasitic, further study into this may be useful. Exploring fluids of varying viscosities or fluids that are biologically relevant, as well as capillary walls that may be more accurate mimics of biological blood vessels in terms of softness are also crucial for applications in biological systems.



Figure 6-3. Chemical motor motion within a PDMS microchannel network. Panels A-C show nanomotor tracks through the channel, with (a-d) in panel B showing the paths of 4 different nanomotors. [Reproduced from reference 15.]

Biological Cells

The interaction of acoustically propelled motors and biological cells has been the topic of several recent reports. The use of red blood cells as ultrasonically propelled motors, targeting and delivery of drugs towards cells, incorporation and actuation of acoustic motors within cells and the evaluation of the biocompatibility of cells mixed with motors for varying amount of time have been reported.^{15,25–28} The motivation behind the majority of these reports has been the elimination of cancerous or diseased cells. Yet in all of the above examples there has been no attempt to mechanically puncture the cell wall either to deliver cargo or to attempt to kill the cell. Reports of drug delivery using acoustically powered rods have only talked about the release of cargo in the vicinity of cells, not within them .²⁷ Hence the ability of motors to "enter" a cell is important as it

would allow drug delivery or microsurgery within the cell. Magnetic mircodrillers have been demonstrated and provide a road map of what can potentially be achieved with acoustic motors.²⁹ The rapid rotation of rods within spinning chains provides another avenue for using them as microdrillers.³⁰It would also be interesting to determine the viability of the cell after puncture. Once inside a cell, the ability to target the motor to different organelles or to interrogate the conditions within the cell is crucial. In order to do this, one must stain separate organelles in order to explore the effect of the presence and motion of the motor on them. Mitochondrial staining and targeting would be a good place to start as it is the energy producing center of the cell and its function may be indicative of disease. ³¹ Full organismal studies would also be useful to more closely evaluate the effect of the presence of motors on the organism's health and life expectancy. Although initial reports have been made on nanomotors inside full organisms (rat), the rats were sacrificed within hours and there is no discussion of the effect of the motors on the liver or if there was any immune response.³²

6.3 Conclusions

Much progress has been made in the field of nanomotors in the past decades including the discovery of a variety of propulsion mechanisms and much effort geared towards applications. Ultrasonic motors in particular have shown promise for biomedical applications. In this work we are hopeful we contributed this field forward towards applications.

6.4 References

Paxton, W. F.; Kistler, K. C.; Olmeda, C. C.; Sen, A.; St Angelo, S. K.; Cao, Y.;
 Mallouk, T. E.; Lammert, P. E.; Crespi, V. H. Catalytic Nanomotors: Autonomous
 Movement of Striped Nanorods. *J. Am. Chem. Soc.* 2004, *126* (41), 13424–13431.

(2) Wang, W.; Duan, W.; Ahmed, S.; Mallouk, T. E.; Sen, A. Small Power: Autonomous Nano- and Micromotors Propelled by Self-Generated Gradients. *Nano Today* **2013**, *8* (5), 531–534.

(3) Sen, A.; Patra, D.; Sengupta, S.; Duan, W.; Zhang, H.; Pavlick, R. Intelligent,
 Self-Powered, Drug Delivery Systems. *Nanoscale* 2012, *5*, 1273–1283.

(4) Abdelmohsen, L. K. E. a.; Peng, F.; Tu, Y.; Wilson, D. a. Micro- and Nano-Motors for Biomedical Applications. *J. Mater. Chem. B* **2014**, *2*, 2395–2408.

(5) Gao, W.; Wang, J. The Environmental Impact of Micro / Nanomachines : A Review. *ACS Nano*, **2014**, *8* (4), 3170–3180.

(6) Wang, W.; Duan, W.; Ahmed, S.; Sen, A.; Mallouk, T. E. From One to Many: Dynamic Assembly and Collective Behavior of Self-Propelled Colloidal Motors. *Acc. Chem. Res.* **2015**, **DOI:** 10.1021/acs.accounts.5b00025.

(7) Solovev, A. a; Sanchez, S.; Schmidt, O. G. Collective Behaviour of Self-Propelled Catalytic Micromotors. *Nanoscale* **2013**, *5*, 1284–1293.

(8) Ibele, M.; Mallouk, T. E.; Sen, A. Schooling Behavior of Light-Powered Autonomous Micromotors in Water. *Angew. Chem. Int. Ed.* **2009**, *48*, 3308–3312.

(9) Ibele, M. E.; Lammert, P. E.; Crespi, V. H.; Sen, A. Emergent, Collective Oscillations of Self-Mobile Particles and Patterned Surfaces under Redox Conditions. *ACS Nano* **2010**, *4* (8), 4845–4851.

(10) Duan, W.; Liu, R.; Sen, A. Transition between Collective Behaviors of Micromotors in Response to Different Stimuli. *J. Am. Chem. Soc.* **2013**, *135*, 1280–1283.

(11) Wang, W.; Duan, W.; Sen, A.; Mallouk, T. E. Catalytically Powered Dynamic Assembly of Rod-Shaped Nanomotors and Passive Tracer Particles. *PNAS* **2013**, *110*, 17744–17749.

(12) Ahmed, S.; Gentekos, D. T.; Fink, C. A.; Mallouk, T. E.; Al, A. E. T. Self-Assembly of Nanorod Motors into Geometrically Regular Multimers and Their Propulsion by Ultrasound. *ACS Nano* **2014**, *8*, 11053–11060.

(13) Dhar, P.; Cao, Y.; Kline, T.; Pal, P.; Swayne, C.; Fischer, T. M.; Miller, B.;
Mallouk, T. E.; Sen, A.; Johansen, T. H. Autonomously Moving Local Nanoprobes in
Heterogeneous Magnetic Fields. *J. Phys. Chem. C* 2007, *111*, 3607–3613.

(14) Zhao, G.; Wang, H.; Sanchez, S.; Schmidt, O. G.; Pumera, M. Artificial Micro-Cinderella Based on Self-Propelled Micromagnets for the Active Separation of Paramagnetic Particles. *Chem. Comm.* **2013**, *49*, 5147–5149.

(15) Ahmed, S.; Wang, W.; Mair, L. O.; Fraleigh, R. D.; Li, S.; Castro, L. A.; Hoyos, M.; Huang, T. J.; Mallouk, T. E. Steering Acoustically Propelled Nanowire Motors toward Cells in a Biologically Compatible Environment Using Magnetic Fields. *Langmuir* 2013, *29*, 16113–16118.

(16) Kline, T. R.; Paxton, W. F.; Mallouk, T. E.; Sen, A. Catalytic Nanomotors: Remote-Controlled Autonomous Movement of Striped Metallic Nanorods. *Angew. Chem. Int. Ed.* **2005**, *44* (5), 744–746.

(17) Love, J. C.; Urbach, A. R.; Prentiss, M. G.; Whitesides, G. M. Three-Dimensional Self-Assembly of Metallic Rods with Submicron Diameters Using Magnetic Interactions. *J. Am. Chem. Soc.* 2003, *125* (42), 12696–12697.

(18) Sahoo, Y.; Goodarzi, A.; Swihart, M. T.; Ohulchanskyy, T. Y.; Kaur, N.; Furlani,
E. P.; Prasad, P. N. Aqueous Ferrofluid of Magnetite Nanoparticles: Fluorescence
Labeling and Magnetophoretic Control. *J. Phys. Chem. B* 2005, *109*, 3879–3885.

(19) Wang, W.; Duan, W.; Zhang, Z.; Sun, M.; Sen, A.; Mallouk, T. E. A Tale of Two Forces: Simultaneous Chemical and Acoustic Propulsion of Bimetallic Micromotors. *Chem. Commun.* **2015**, *51*, 1020–1023.

(20) Xu, T.; Soto, F.; Gao, W.; Dong, R.; Garcia-Gradilla, V.; Magaña, E.; Zhang, X.; Wang, J. Reversible Swarming and Separation of Self-Propelled Chemically Powered Nanomotors under Acoustic Fields. *J. Am. Chem. Soc.* **2015**, *137*, 2163–2166.

(21) Burdick, J.; Laocharoensuk, R.; Wheat, P. M.; Posner, J. D.; Wang, J. Synthetic Nanomotors in Microchannel Networks: Directional Microchip Motion and Controlled Manipulation of Cargo. *J. Am. Chem. Soc.* **2008**, *130* (26), 8164–8165.

(22) Wiklund, M.; Green, R.; Ohlin, M. Acoustofluidics 14: Applications of Acoustic Streaming in Microfluidic Devices. *Lab Chip* **2012**, *12*, 2438.

(23) Sadhal, S. S. Acoustofluidics 13: Analysis of Acoustic Streaming by Perturbation Methods. *Lab Chip* **2012**, *12*, 2292.

(24) Nadal, F.; Lauga, E. Asymmetric Steady Streaming as a Mechanism for Acoustic Propulsion of Rigid Bodies. *Phys. Fluids* **2014**, *26*, 082001.

(25) Wang, W.; Li, S.; Mair, L.; Ahmed, S.; Huang, T. J.; Mallouk, T. E. Acoustic Propulsion of Nanorod Motors inside Living Cells. *Angew. Chem. Int. Ed.* **2014**, *53*, 3201–3204.

(26) Wu, Z.; Li, T.; Li, J.; Gao, W.; Xu, T.; Christianson, C.; Gao, W. Turning Erythrocytes into Functional. *ACS Nano* **2014**, *8*, 12041–12048.

(27) Garcia-Gradilla, V.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.; Yardımcı, C.; Wiitala, D.; Galarnyk, M.; Wang, J. Ultrasound-Propelled Nanoporous Gold Wire for Efficient Drug Loading and Release. *Small* **2014**, *10*, 4154-4159.

(28) Garcia-Gradilla, V.; Orozco, J.; Sattayasamitsathit, S.; Soto, F.; Kuralay, F.; Pourazary, A.; Katzenberg, A.; Gao, W.; Shen, Y.; Wang, J. Functionalized Ultrasound-Propelled Magnetically Guided Nanomotors: Toward Practical Biomedical Applications. *ACS Nano* **2013**, *7* (10), 9232–9240.

(29) Xi, W.; Solovev, A.; Ananth, A. N.; Gracias, D.; Sanchez, S.; Schmidt, O. G. Rolled-up Magnetic Microdrillers: Towards Remotely Controlled Minimally Invasive Surgery. *Nanoscale* **2012**, *5* (4), 1294-1297.

(30) Balk, A. L.; Mair, L. O.; Mathai, P. P.; Patrone, P. N.; Wang, W.; Ahmed, S.; Mallouk, T. E.; Liddle, J. A.; Stavis, S. M. Kilohertz Rotation of Nanorods Propelled by Ultrasound, Traced by Microvortex Advection of Nanoparticles. *ACS Nano* **2014**, *8*, 8300–8309.

(31) Zhou, F.; Wu, S.; Yuan, Y.; Chen, W. R.; Xing, D. Mitochondria-Targeting Photoacoustic Therapy Using Single-Walled Carbon Nanotubes. *Small* **2012**, *8*, 1543–1550.

(32) Gao, W.; Dong, R.; Thamphiwatana, S.; Li, J.; Gao, W.; Zhang, L.; Wang, J. Arti Fi Cial Micromotors in the Mouse's Stomach : A Step toward in Vivo Use of Synthetic Motors. *ACS Nano* **2015**, *9*, 117–123.

VITA Suzanne Ahmed

Education

The Pennsylvania State University Ph.D. Chemistry, August 2010- August 2015, GPA: 3.930 Dissertation Title: The Control, Assembly, Shape-Dependent and Material-Dependent Studies of Ultrasonically Propelled Nano and Micromotors Adviser: Thomas E. Mallouk, Ph.D.

University of California, Berkeley M.S. Chemistry, May 2009, GPA: 3.904

University of California, Riverside B.S. Biochemistry with an emphasis in Chemistry, June 2006, GPA: 3.961 with Highest Honors

Publications

- 1. **S.Ahmed**, W. Wang, M. Hoyos and T.E. Mallouk, "Shape and Density Effects in the Acoustic Propulsion of Bimetallic Nanorod Motors," in preparation.
- 2. S. Ahmed, D. T. Gentekos, C. Fink and T. E. Mallouk, "Dynamic self-assembly of nanowire motors into regular geometric multimers," ACS Nano, 8, 11053-11060 (2014).
- S. Ahmed, W. Wang, L. O. Mair, R. D. Fraleigh, S. Li, L. A. Castro, M. Hoyos, T. J. Huang, and T. E. Mallouk, "Steering acoustically propelled nanowire motors towards cells in a biologically compatible environment using magnetic fields," Langmuir, 29, 16113-16118 (2013).
- 4. W. Wang, S. Li, L. Mair, **S. Ahmed**, T. J. Huang, and T. E. Mallouk, "Acoustic propulsion of nanorod motors inside living cells," Angew. Chem. Int. Ed., 53, 3201-3204 (2014).
- 5. W. Wang, W. Duan, **S. Ahmed**, T.E. Mallouk, and A. Sen, "Small power: autonomous nano- and micromotors propelled by self-generated gradients," Nano Today, 8, 531-554 (2013).
- A. L. Balk, L. O. Mair, P. P. Mathai, P. N. Patrone, W. Wang, S. Ahmed, T. E. Mallouk, J. A. Liddle, and S. M. Stavis, Kilohertz Rotation of Ultrasonically Propelled Nanomotors Traced by Microvortex Advection of Nanoparticles. ACS Nano, 8, 8300-8309 (2014).
- 7. W. Wang, W. Duan, **S. Ahmed**, A. Sen, and T. E. Mallouk, "From one to many: dynamic assembly and collective behavior of self-propelled colloidal motors," Acc. Chem. Res.in press

Teaching Experience

University of Cincinnati, Raymond Walters College Blue Ash, Ohio January 2010 - June 2010 Adjunct Chemistry Lecturer University of California, Berkeley Department of Chemistry Fall semesters of 2006, 2007, 2008 and Spring semester 2009 General Chemistry Lab Instructor