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Abstract

Motion is one of the defining characteristics of life. Inspired by the biological motors at the micron and nanoscale, scientists have developed artificial machines that self propel in solutions and are on the same length scale as biological motors. However, motion at such low length scales is fraught with many challenges. The focus of this thesis is on devising some field free and autonomous strategies for addressing those challenges.

This dissertation starts with a literature review of the field with a discussion about the various self-propelled prevalent in the scientific community. Then the different challenges of motion at low Reynolds number and their applicability to the motors are discussed.

One of the more popular categories of catalytic micromotors is Janus motors which move in a solution of hydrogen peroxide due to the catalytic activity of Platinum. However it is very hard to introduce directionality in these motors due to the Brownian randomizations prevalent in low Reynolds number regime. The first chapter of this thesis introduces a method of overcoming this challenge by exploiting the interaction of Janus micromotors with physical features. It was observed that Janus micromotors could move linearly in geometric "tracks" as long as they have fuel available for their motion, thus overcoming Brownian diffusion rotations.

The second system discussed here takes its inspiration from biological motors and is based on cilia and flagella seen in microorganisms. A design for synthesizing artificial cilia is outlined. This system consists of polymeric rods with a catalytic disk on top which undergo oscillations due to reversible chemical reactions. These synthetic ciliary systems represent an efficient way of mixing fluid at the microscale and overcome the challenges associated with laminar flow at such regimes.

The discussion then moves to the nanoscale with the introduction of enzyme nanomotors in Chapter 4. Enzyme molecules not only show enhanced diffusion in the presence of their substrate such as urea for Urease or hydrogen peroxide for catalase; but they also show chemotactic behavior towards their substrate gradient. This is directly parallel to the chemotactic behavior shown by bacteria. This property is exploited to design a system of separating two enzymes with the same mass and isoelectric point. The separation is observed within a two-inlet, five-outlet microfluidic network, designed to allow mixtures of active (ones
that catalyze substrate turnover) and inactive (ones that do not catalyze substrate turnover) enzymes, labeled with different fluorophores, to flow through one of the inlets. Substrate solution prepared in phosphate buffer was introduced through the other inlet of the device at the same flow rate. In the presence of a substrate concentration gradient, active enzyme molecules migrated preferentially toward the substrate channel leading to separation of the enzymes.

As discussed above enzyme molecules undergo enhanced diffusion in the presence of their substrate. Additionally immobilizing these enzymes on a surface leads to a pump which can pump fluid in all directions in the presence of the respective substrate of the enzyme. This concept has been utilized in Chapter 4 to make a directional microscale pump which can move fluid from one specific point to another. An asymmetrical pattern of enzyme pumps was developed and a substrate gradient was introduced. Due to the asymmetry of the pattern, the fluid being pumped had a particular direction to it which was determined by the direction of gradient. This kind of architecture overcomes the problem of directionality seen in microscale pumps.

Finally, the principle above was adapted for use in nano-biosensors. The above enzyme based pump can be used to drive fluid from point A to point B, thus it can also drive analytes in the system from point A to point B. Therefore the analytes could be concentrated at a particular point increasing the signal at that point.
# Table of Contents

List of Figures ......................................................................................................................... viii
List of Multimedia Files ............................................................................................................. xvi
Acknowledgements .................................................................................................................. xviii
Chapter 1 ................................................................................................................................. 1
  1.1 Introduction ......................................................................................................................... 1
  1.2 Categories of self-propelled motors .................................................................................. 1
    1.2.1: Catalytic Motors ......................................................................................................... 1
    1.2.2: Helical Swimmers ...................................................................................................... 4
  1.3: Considerations for motion .................................................................................................. 4
    1.3.1: Motion at Low Reynolds Number ............................................................................. 4
    1.3.2: Directionality ............................................................................................................ 7
    1.3.3: Chemotaxis ................................................................................................................ 8
  1.4: Conclusions ....................................................................................................................... 8
  1.5: References ......................................................................................................................... 9
Chapter 2 .................................................................................................................................. 14
  2.1: Motivation ......................................................................................................................... 14
  2.2: Janus Motor Synthesis ...................................................................................................... 14
  2.3: Janus micromotors in hydrogen peroxide ......................................................................... 15
  2.3: Brownian rotational quenching near a planar surface .................................................... 17
  2.5: Rotational diffusion quenching near multiple planar surfaces ....................................... 23
  2.5 Mechanism of rotational quenching .................................................................................. 27
  2.6: Conclusion ....................................................................................................................... 31
  2.7: References ......................................................................................................................... 31
  2.8: Appendix .......................................................................................................................... 33
    2.8.1: Rotational Electrophoresis ......................................................................................... 33
    2.8.2: Perturbative analysis ................................................................................................. 35
Chapter 3 .................................................................................................................................. 40
  Fluid Mixing at the Microscale using Cilia ............................................................................. 40
3.1: Introduction .............................................................................................................. 40
3.2: Mechanism of Actuation ....................................................................................... 42
3.3: Design of Cilia ......................................................................................................... 46
3.4: Fluid Mixing Profile .............................................................................................. 51
3.5: Conclusion ............................................................................................................... 56
3.6: References ............................................................................................................... 57
Chapter 4 ....................................................................................................................... 59
Chemotactic Separation of Enzymes ........................................................................ 59
4.1: Motivation ............................................................................................................... 59
4.2: Design of the microfluidic device ........................................................................ 60
4.3: Experimental Set-Up ........................................................................................... 62
  4.3.1: Labeling of Enzymes with Fluorescent Dyes & Preparation of Enzyme Mixtures ..... 62
  4.3.2: Relation between Fluorescence and Concentration of Catalase and Urease Solutions ................................................................................................................... 64
  4.3.3: Proof of Concept Studies using two pairs of enzymes ........................................ 65
  4.3.4: Sensitivity of Separation using the same Enzyme ............................................... 72
4.4: Conclusions ............................................................................................................ 74
4.5: Acknowledgements ............................................................................................... 74
4.6: References .............................................................................................................. 74
4.7: Appendix ................................................................................................................. 77
  4.7.1: Simulations for separation experiments ................................................................. 77
  4.7.2: Flow rate optimization ....................................................................................... 81
Chapter 5 ....................................................................................................................... 83
Convective Transport Using Enzyme Pumps ............................................................. 83
5.1: Introduction ............................................................................................................ 83
5.2: Design of the Experiment .................................................................................... 85
  5.2.1: Fabrication of micropump array patterns ............................................................ 86
  5.2.2: Sample Preparation ......................................................................................... 87
  5.2.3: Enzyme Immobilization ................................................................................... 89
5.3: Convective Pumping observed ............................................................................ 90
5.4: Spatial variations in pumping ............................................................................... 91
5.5: Convection-assisted focusing of analytes ............................................................. 92
5.6: Conclusion ............................................................................................................. 95
5.7: References .................................................................................................................................96
5.8: Appendix .......................................................................................................................................98
  5.8.1: Principles governing the convective transport of microscopic particles ....................98
  5.8.3: Parameters controlling transport properties ........................................................................105
List of Figures

Figure 2.1: Average propulsion velocity of Janus particles of various diameters is plotted as a function of fuel (H$_2$O$_2$) concentration..............................................................16

Figure 2.2: (a) A visual representation of the system (b) Azimuthal angel θ is defined.............17

Figure 2.3: Schematic 3D orientation and experimental trajectories (45 seconds duration, red line) for 2 µm platinum-polymer Janus particle in (a) water settled under gravity against a planar glass substrate; colloid and (b) 10 % hydrogen peroxide solution at the planar surface of a rectangular glass cuvette...........................................................................................................18

Figure 2.4: (Left) Selection of frames from fluorescent microscopy videos (15 µm x 15 µm field of view) for fluorescent platinum-polystyrene (PS) Janus spheres of varying radii, the PS side of the colloid appears bright near to a planar interface in de-ionized (DI) water, and in 10 % aqueous H$_2$O$_2$ solutions. (Right) Azimuthal angle, Θ(t) for typical Janus particles determined from fluorescent microscopy videos (Note the 5 µm particle in water shows strong gravitational alignment constraining Θ close to 0° )...........................................................................................................................18

Figure 2.5 Azimuthal Mean Square Angular Displacement (MSAD) as a function of time for (a) 2 µm Janus particle, (b) 3 µm Janus particle and (c) 5 µm Janus particle.................................................22

Figure 2.6: Particles moving along geometric boundaries, at speeds of up to 10 µm/s. (a-b) schematics of Janus particles encountering multi-planar geometries. Red axis indicates forbidden rotations due to proximity to a plane, green axis indicates unquenched axis of rotation: (a) Janus particle encountering a planar edge while moving along a 2D surface, expected to result in Brownian rotational quenching about two orthogonal axes. (b) Janus particle confined within a square groove; parallel vertical walls confine the rotational diffusion
about one axis; however, if the particle descends to the base of the groove, it is confined about two orthogonal axes.................................................................23

**Figure 2.7:** Overlaid still frames from fluorescence microscopy videos with equal time gaps: yellow line shows complete trajectory, green line shows location of vertical cuvette walls, red arrows indication direction of motion: (a) 3 µm Janus particle (10 % H₂O₂) moving at the bottom of a rectangular glass cuvette a long way away from the edges. (b) 3 µm Janus colloid (10 % H₂O₂) moving along the curved edge of a glass cuvette and reaching the cuvette boundary. (c) 5 µm Janus colloid (10 % H₂O₂) moving along the straight edge of a glass cuvette- left hand inset shows a magnified region, right hand inset shows a “stuck” aligned agglomerate formed at the cuvette boundary..................................................................................................................24

**Figure 2.8:** SEM image of the rectangular grooves........................................................................26

**Figure 2.9:** 5 µm Janus colloid (10 % H₂O₂) moving within a rectangular section groove
(width=8.75 µm).................................................................................................................................27

**Figure 2.10:** Electrophoretic behaviour for Janus colloids..........................................................29

**Figure 2.11:** MSAD data re-scaled to allow comparison with theory...........................................37

**Figure 3.1:** (Left) micrograph image of cilia on the inner surface of Mammalian trachea which shows the morphology along with the structural integrity of this material (Right) SEM image of artificial cilia array designed using Polypyrrole nanorods in this chapter, showing the similarities between the two................................................................................................................................................42

**Figure 3.2:** Schematic of diffusiophoretic forces on a negatively charged particle near a negatively charged wall in an electrolyte..................................................................................................................43
**Figure 3.3:** A schematic of self-diffusiophoresis of AgCl particle in the presence of water and UV
...........................................................................................................................................................................45

**Figure 3.4:** Schematic of diffusiophoresis of silver particle in hydrogen peroxide and HCl in the
presence of UV..................................................................................................................................................................47

**Figure 3.5:** Lithographic process developed for fabricating the cilia.................................................................49

**Figure 3.6:** (Left) PDMS nanorods arranged in an array. The image is taken top down thus the
height of the rods seen in the image is not the true height. The cracks seen on the surface are
the cracks formed in PDMS in response to spin coating resist on them. (Right) SEM image of
the PDMS rods where the substrate is plasma treated to allow smooth coating of resist. Thus
no cracks are visible.........................................................................................................................................................50

**Figure 3.7:** (a) The figure denotes a schematic of how the cilia are expected to move (b) This
figure is a snapshot from the video showing the four cilia pillars arranged in an array and the
tracer particles in the fluid. This snapshot corresponds to PDMS pillars of 5 µm diameter
arranged in a square array where the distance between two cilia is 25 µm.........................................................51

**Figure 3.8:** The mean square displacement of the PDMS rods with respect to time. The mean
square displacements are calculated from the relative displacement of the rod from its center
of mass. The four graphs show the mean square displacement of the four rods seen in Figure
3.7(b). They are arranged in the order they appear in Figure 3.7(b)........................................................................53

**Figure 3.9:** In this figure the total displacement of the rod ($\sqrt{x^2 + y^2}$) where $x$ is the
relative displacement of the one rod from its center of mass in $x$ direction and $y$ is the relative
displacement of the same rod from its center of mass in $y$ direction is measured for the
reaction and for the control. As shown by the figure the total displacement is oscillatory with a
defined period for the reaction........................................................................................................................................54
Figure 3.10: The relative displacements of the rods from their center of mass only in the y-direction. As seen from the graph the rods move in phase thus providing evidence of the synchronization of motion.………………………………………………………………………………………………………54

Figure 3.11: Control experiments (Left) This figure shows the relative displacement of the rods from their center of mass in the y direction vs time in the absence of H$_2$O$_2$. As evident and can be contrasted with Figure 3.8 there is no oscillatory motion and neither is any kind of synchronization present. (Right) This figure shows the relative displacement of the rods from their center of mass in the y direction vs time for the four rods in the absence of UV. As is evident and can be contrasted with (b) there is no oscillatory motion and neither any kind of synchronization present. ……………………………………………………………………………………………………………55

Figure 3.12: Average mean square displacement ($n = 30$) vs time of tracer particles (diameter = 1 µm) in an array of PDMS rods with diameter 5 µm…………………………………………………………………………………………………………56

Figure 4.1: (a) Schematic of the experimental setup used to observe chemotactic separation of enzymes. (b) Dimensions of various sections of the microfluidic separator…………………………………………………………………………………………………………62

Figure 4.2: Correlation between fluorescence and concentration of labeled proteins, measured for enzymes tagged with AF 647 and AF 488 respectively. The profiles are linear at sufficiently dilute enzyme concentrations…………………………………………………………………………………………………………65

Figure 4.3: Intensity profiles of enzyme solutions within the microfluidic separators. The images are from experiments with active and inactive catalases, labeled with AF 647 and AF 488 respectively. The images show smooth flow of liquids near the inlets and the outlets and absence of any undulation and mixing…………………………………………………………………………………………………………69
Figure 4.4: Separation of catalase from urease. Normalized fluorescence intensity profiles of the enzymes in the presence of (A) phosphate buffer and (B) imposed H2O2 concentration gradient. The profiles were recorded within the outlets along a cutline, at a distance of approximately 330 μm away from the split. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. (C) Measured enrichment coefficients of catalase and urease within different outlets of the device. The mean and standard deviations are calculated for three sets of independent observations, each carried out with a newly fabricated device.

Figure 4.5: Separation of urease from β-galactosidase. Normalized fluorescence intensity profiles of the enzymes in the presence of (A) phosphate buffer, (B) imposed urea concentration gradient. The profiles were recorded within the outlets along a cutline, at a distance of approximately 330 μm away from the split. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. (C) Measured enrichment coefficients of urease and β-galactosidase within different outlets of the device. The mean and standard deviations are calculated for three sets of independent observations, each carried out with a newly fabricated device.

Figure 4.6: Separation of active and inactive catalase. Normalized fluorescence intensity profiles of the enzymes in the presence of (A) phosphate buffer, (B) imposed H2O2 concentration gradient. The profiles were recorded within the outlets along a cutline, at a distance of approximately 330 μm away from the split. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. (C) Relative enrichment coefficients of the molecules measured within different outlets of the device. The mean and standard deviations are calculated for three sets of independent observations, each carried out with a newly fabricated device.

Figure 4.7: Simulated separation of enzymes in the presence of substrates. Normalized concentration profiles of catalase and urease in the presence of (A) phosphate buffer, (B)
imposed H$_2$O$_2$ concentration gradient; urease and β-galactosidase in the presence of (C) phosphate buffer, (D) imposed urea concentration gradient; active and inactive catalase in the presence of (E) phosphate buffer, (F) imposed H$_2$O$_2$ concentration gradient. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. The concentration profiles are estimated within the outlets, along a cutline 330 μm away from the outlet splits.

**Figure 5.1:** Schematic of the experimental setup with enzyme micropump array and observations.

**Figure 5.2:** Schematic of the experimental setup. The yellow lines depict the catalytic strips 40 μm wide with a distance of 20 μm between them. The blue square represents the gel soaked with the substrate.

**Figure 5.3:** (Left) A representation of the experimental setup including its length (Right) A still from the videos showing the tracer particles accumulated at the surface.

**Figure 5.4:** Spatial profile of the pumping speed of 4 μm s-PsL particles for (a) platinum and (right) urease systems.

**Figure 5.5:** Density distribution of 4 μm s-PsL particles for 10 nm platinum at (red) 2% H$_2$O$_2$ and (blue) 1% H$_2$O$_2$.

**Figure 5.6:** Density distribution of 4 μm s-PsL particles for (green) 3x10$^{-7}$ M urease and (purple) 3x10$^{-6}$ M urease with 500 mM urea.

**Figure 5.7:** Geometry of the simulation domain. Reagent (released out of gel) enters the domain through the left wall at x=0. Enzyme, coating region A on the bottom, decomposes...
reagent into lighter products. The resulting density variation across the domain generates convective flows, transport tracers.

**Figure 5.8:** Sequential stages of convective transport. (a) Initially, tracers are uniformly dispersed throughout the domain and the hydrogen peroxide concentration (shown by the color bar) is zero. (b) Reagent, diffusing through the left wall, produces a fluid flow that transports tracers. Enzyme at the bottom wall decomposes the reagent, creating a concentration (density) gradient. (c) After reagent is consumed, the flow stops with areal concentration of tracers $n/n_0$ increasing toward the right wall as shown by the red line.

**Figure 5.9:** Formation of maximum in the tracer distribution. (a) Areal tracer concentration $n/n_0$ resulting from the fluid flow shown on the middle and bottom panels. (b) Maximal horizontal velocity goes to zero (blue arrow) around the point where $n/n_0$ is maximal (blue dot). (c) Convective vortex dragging tracers along the bottom and aggregating them into the pile shown on the top panel. Concentration of reagent, consumed by enzyme along the bottom surface, is indicated by the color bar.

**Figure 5.10:** Reaction rate controls position of maximum of tracer distribution. (a) Tracer areal concentration as a function of the position along the channel (b) The low reaction rates provide slower velocities, but enable fluid to flow throughout the entire domain (yellow line) and transport cargo from the left to the right wall. Arrows emphasize the correlation between the maximum values of $n/n_0$ (bold dots) and zero horizontal velocities (bold dots).

**Figure 5.11:** Amount of reagent controls position of maximum of tracer distribution. Increasing amount of reagent (with values $C_0 = 0.05, 0.1, 0.2M$), requires longer time for decomposition during which the fluid flow reaches further along the channel and aggregates tracers into piles.
(characterized by maximum of \( n/n_0 \)) further away from the fuel entrance location at \( x = 0 \).

Shift of \( n/n_0 \)-maximum caused by increasing values \( C_0 = 0.05, 0.1, 0.2 \text{M} \) is demonstrated for the reaction rates: (a) \( k_{cat} = 2.12 \cdot 10^5 \), (b) \( 2.12 \cdot 10^4 \), and (c) \( 2.12 \cdot 10^3 \text{ s}^{-1} \) .................................108
List of Multimedia Files

Video 2.1: 5 µm Janus particle swimming in a 10% H₂O₂ solution

Video 2.2 (a): 2 µm Janus colloid settled at the bottom interface of a glass cuvette in water. Free Brownian rotation and Brownian translational diffusion is observed. (b): 2 µm Janus colloid settled at the bottom interface of a glass cuvette in 10 % H₂O₂ solution. Brownian rotation about the azimuthal axis is quenched, and directed translational propulsion away from the Pt cap is observed.

Video 2.3 (a): 3 µm Janus colloid settled at the bottom interface of a glass cuvette in water. Free Brownian rotation and Brownian translational diffusion is observed. (b): 3 µm Janus colloid settled at the bottom interface of a glass cuvette in 10 % H₂O₂ solution. Brownian rotation about the azimuthal axis is quenched, and directed translational propulsion away from the Pt cap is observed.

Video 2.4 (a): 5 µm Janus colloid settled at the bottom interface of a glass cuvette in water. Brownian rotation is quenched by the mass of the cap to result in near constant bright fluorescence intensity and Brownian translational diffusion is observed. (b): 5 µm Janus colloid settled at the bottom interface of a glass cuvette in 10 % H₂O₂ solution. Brownian rotation about the azimuthal axis is quenched, and directed translational propulsion away from the Pt cap is observed.

Video 2.5: 5 µm Janus colloid settled at the bottom interface of a glass cuvette in 10 % H₂O₂ solution, moving at the junction with a second vertical planar wall of the cuvette. Brownian rotation is quenched about two axis resulting in persistent directed motion. For the 5 µm spheres, the fluorescence images also reveal that the fluorescent Janus colloids in active hemisphere is slightly canted away from the sides of the cuvette as it moves.
**Video 2.6:** Two 5 µm Janus colloids settled at the bottom interface of a glass cuvette in 10% H$_2$O$_2$ solution, moving at the junction with a second vertical planar wall of the cuvette. Brownian rotation is quenched about two axis resulting in persistent directed motion. The two colloids are moving in opposite directions and undergo a collision.

**Video 2.7:** 5 µm Janus colloid in 10% H$_2$O$_2$ solution moving along the base of a 8.75 µm square cut groove lithographically manufactured in a silicon substrate.

**Video 3.1:** A solution containing AgCl particles and 1% H$_2$O$_2$ in water under UV illumination. The AgCl particles alternately attract then repel one another. Traveling waves of particle motion can be seen traversing the illuminated area. Synchronization of particle attractions/repulsions is also observed over a variety of length scales. Prior to this movie being recorded and prior to the particles' exposure to H$_2$O$_2$, the particles were placed in pure deionized water and illuminated with UV light for 25 min. H$_2$O$_2$ was then added to the system at a concentration of 1%

**Video 5.1:** Experimental set up of the experiment is shown with the enzyme micropump array with urease as the enzyme. A substrate concentration gradient is established perpendicular to length of the micropump array. The concentration gradient is set up using an agar gel soaked with 500 mM urea and placed perpendicular to the array. The setup is sealed after filling it with buffer solution with 4 µm fluorescent polystyrene particles in them. The fluid flow is realised by observing the trajectories of the 4 µm fluorescent tracer particles at 10x magnification. The videos are real time.

**Video 5.2:** The experimental setup is same as video 5.1, however the video is taken after 60 minutes of reaction time have elapsed. The focusing of the 4 µm fluorescent tracer particles is observed by panning the camera over the entire length of the micropump array at 10x magnification.
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Chapter 1
Microscale Motors

1.1 Introduction
Life is synonymous with motion. Bacteria move in search of food, sperm and algae use flagella or cilia to move in a particular direction and cells use rotating organelles. Transport at the microscale in nature is fraught with a number of challenges as evidenced by the various complex structures developed. Inspired by these biological motors and by the driving need to transport molecules at the small scale, there has been a lot of development in the field of man made micromachines. The last decade has seen tremendous strides in the development of micromachines that convert energy into mechanical motion and a plethora of microdevices has been built powered by different sources of energy like chemical energy, external magnetic fields, electric, acoustic or thermal energy. Two strategies for developing synthetic micromachines have gained prominence. First strategy was mimicking the deformation based motion of biological organelles. Thus colloidal assemblies like a chain of magnetic beads which deform like sperm was developed. Due to non time-reversibility required to drive motion at the microscale, external fields like magnetic or light have been used to drive actuation. Microparticles in these systems are 'driven' colloids. A 'driven colloid' is inherently passive while it's being transported by an external energy source like magnetic, electric or flow fields. In contrast, the second approach is 'active' colloids. Active colloids harness the energy from their surroundings to drive motion and actuation, while modifying it at the same time. This chapter will discuss the various types of active motors and some of the challenges facing motion at the microscale.

1.2 Categories of self-propelled motors

1.2.1: Catalytic Motors

A) Electrophoretic propulsion
An appealing strategy for propelling microscale motors is utilizing chemical reactions. One of the earliest mechanisms for driving motion at the microscale, discovered by Whitesides\textsuperscript{17} and shortly thereafter by Sen and Mallouk\textsuperscript{7} and Ozin\textsuperscript{18}, was the decomposition of hydrogen peroxide by platinum. These micromotors developed by Sen \textit{et al} consisted of bimetallic (Pt and Au) nanorods which autonomously propelled themselves in a solution of hydrogen peroxide. \textsuperscript{19} These bimetallic rods propel themselves through an electrophoretic mechanism where the hydrogen peroxide is oxidized to water and oxygen at the platinum end of the rod. This gives rise to an excess of electrons on the platinum end which travel to the gold end of the rod where they are consumed by the reduction of hydrogen peroxide. The resulting proton ion flux around the rod, in the solution drives its motion relative to the fluid, with the platinum end forward.\textsuperscript{20}

This strategy of catalysis based motors has since proved very popular with a host of other devices being developed. Another example of such motors is spherical Janus particles where the two halves have different chemistries. They are designed by coating one half of a colloid with a catalyst for example platinum which decomposes hydrogen peroxide. This catalytic reaction drives the motion of the particle.\textsuperscript{21} Posner \textit{et al} designed Janus micromotors by evaporating Pt and Au on the two halves of a spherical particle.\textsuperscript{22} Originally the mechanism of motion for Platinum based Janus motors was thought to be self electrophoresis, however recently electrokinetic mechanism has been proved to be the dominant effect.\textsuperscript{23} Fuel sources other than hydrogen peroxide have also been developed. For example Iridium based Janus motors that move autonomously in hydrazine solution or vapor.\textsuperscript{24,25} Sen \textit{et al} have also designed bimetallic rods that move in halogen solutions.\textsuperscript{26}

\textbf{B) Self-Diffusiophoresis}

Self-diffusiophoresis is another mechanism for driving motion at the microscale. Chemical reactions on the motor surface lead to a concentration gradient around the motor which drives its motion. This concentration gradient could be ionic or non-ionic. For motion due to self-diffusiophoresis the concentration gradient of the solute and its interaction with the motor should be strong enough to generate hydrodynamic stress within the double layer of the
colloidal motor. Self diffusiophoretic interactions in silver phosphate, silver chloride and titanium dioxide systems by Sen et al. lead to various collective behavior phenomenons like schooling and predator-prey behavior. Non-ionic self diffusiophoresis based motors are rare outside of biological systems. One of the earliest example was a polymerization powered Janus micromotor. The motor was powered by the catalytic action of Grubbs catalyst deposited on one half of a silica microparticle. Depolymerization of PECA polymer in the presence of base has also been used to develop cone shaped motors that propel due to self diffusiophoresis of neutral species.

C) Bubble Propulsion

For catalytic nanomotors discussed in the previous section, even though the reactions produce gaseous products, there is not a significant amount of bubble formation or release. However, as the size of the catalytic rods and Janus particle increases along and curvature decreases, bubble propulsion is more evident. With the increase in size, bubble nucleation becomes energetically favourable and growth and detachment of bubbles at the surface, propels the motor due to momentum transfer. Wu et al. designed larger Platinum-polystyrene Janus dimers that swim by bubble recoil. Generally the speeds of bubble propelled motors are higher than self-diffusiophoretic motors. Some more examples of spherical motors include Al-Ga colloids half coated with titanium which use water as fuel. Wang group have also designed Mg based Janus colloids that propel through hydrogen bubble generation in seawater and stomach acid. These motors have potential applications in environmental remediation and biological environments. Rolled up microtubes are another major category of bubble propelled micromotors. These tubular microjets sometimes called micro-rockets consist of a catalytic substrate inside a rolled up microtube, which produces bubbles. These bubbles are expelled from one of the two ends of the microtubule thus propelling the motor due to recoil thrust. An example developed by Wang et al. is polymer based microtubules with a Platinum inner layer which can effectively transport colloidal cargo in a solution of hydrogen peroxide.
1.2.2: Helical Swimmers

In nature, most eukaryotic microorganisms and cells use cilia or flagella like structures to move. Cilia and Flagella are structurally similar. There are two types of cilia: motile and non-motile. As the name suggests, non-motile or primary cilia do not undergo any oscillatory motion, while motile cilia beat in a synchronized fashion to create motion. There are three types of flagella: bacterial flagella, archaeal flagella and eukaryotic flagella. Bacterial flagella like the ones found on *E. Coli* are helical filaments which are driven by a rotary motor at the head of the *E.Coli* and generate a screw-like motion. These have served as inspiration for helical swimming devices called "Artificial Bacterial Flagella". Nelson *et al.* have developed these ABFs with a InGaAs/GaAs semiconductor helical tail with a square magnetic head as the rotary motor. In the presence of orthogonal magnetic fields, these swimmers propel themselves in a corkscrew-like fashion analogous to bacterium flagella. Linear chains of magnetic beads can also be used to design artificial flagella. Chains of superparamagnetic beads were anchored on nickel bases. Changing the magnetic field rotated the chains in an asymmetric fashion imitating the motion of flagella. Dreyfus *et al* attached chains of DNA-linked colloidal magnetic particles on surfaces of red blood cells. External magnetic fields could then be used to drive the red blood cells.

1.3: Considerations for motion

1.3.1: Motion at Low Reynolds Number

In spite of the progress made in the field of micromotors, there are several challenges associated with motion at low Reynolds number. Reynolds number (Re) is a dimensionless quantity in fluid mechanics named after Osbourne Reynolds (1842-1912). It is the ratio of inertial forces to viscous forces in a fluid medium and helps to predict the flow patterns in said fluid:

\[
Re = \frac{\text{Intertial Forces}}{\text{Viscous Forces}} = \frac{\rho V^2 l^2}{\mu V l} = \frac{\rho V l}{\mu}
\]  

(1.1)
where $\rho$ is the density of fluid, $V$ is the velocity of the fluid or the object relative to the fluid, $l$ is the characteristic length scale (travelled distance in the fluid, hydraulic diameter etc.) and $\mu$ is the viscosity of the fluid.

For macroscopic objects, Reynolds number is high and the flows are turbulent since the inertial forces dominate. However, for nano- and microscale objects the Reynolds number is low, since the inertial forces (which depend on mass) are low and viscous forces dominate. In such regions, the flows tend to be laminar. This means that if two identical, miscible liquids are flowing parallel to each other, there will be no turbulent mixing and the only mixing would occur due to diffusion across their common interface. This property of motion in low Reynolds number is called microscopic reversibility and is the basis of flow in microfluidic devices.\(^\text{46}\)

Microscopic reversibility or kinematic reversibility means that actions at the microscale are time-independent. Thus any change to the system due to one action can be reversed by performing the same action in reverse. A consequence of this is the 'Scallop theorem'.\(^\text{14}\) A normal scallop moves by opening its shell slowly and closing it quickly. The fast closing of the shell squeezes water out of the shell and the backward momentum of the water thrusts the scallop forward. At low Reynolds number, due to the kinematic reversibility, the act of opening the shell slowly for the second time would undo the original motion and bring the organism back to its original position. Thus for propulsion at low Reynolds number, the swimmer must undergo non-reciprocal motion.

Another challenge to designing motion at the micro and nanoscale is Brownian motion. Every atom or molecule has some intrinsic thermal velocity and moves randomly in three dimensions. This velocity and subsequently kinetic energy depends on the temperature and for ideal gases is given by

$$\text{Kinetic Energy} = \frac{3}{2} k_B T = \frac{m V^2}{2}$$  \hspace{1cm} (1.2)

where $m$ is the mass of the particle, $V$ is the thermal velocity, $T$ is the temperature and $k_B$ is the Boltzmann constant.

Thus any object in a fluid is subject to collisions with the molecules of the fluid and these collisions lead to an exchange of momentum between the object and fluid. At macroscale, due
to the large mass of object, the momentum transfer doesn't affect the particle. Moreover, the collisions of each side of the particles tend to average out and cancel each other. In contrast, for smaller objects, such thermal and random 'bumping' from the solvent molecules is more pronounced in its effect. Due to the small size of the object, there also statistical variations between the collisions on each side of the object and their net effect gives rise to random motion of the particle called 'Brownian Diffusion'. This can also cause random changes in orientations and rotation of the object called 'Brownian Rotational Diffusion'. Brownian motion is an example of continuous-time probabilistic motion and thus, statistics could be used to calculate the average mean square displacement (MSD) of a particle undergoing Brownian diffusion. The mean square displacement of the particle is a function of time interval over which the motion is observed and is given by:

$$MSD = Kt^\alpha$$  \hspace{1cm} (1.3)

where $K$ is a constant whose value depends on the diffusion coefficient of the particle, $t$ is the time interval and $\alpha$ is a constant that depends on the type of diffusion. The most common type of diffusion is where $\alpha = 1$ and the particle is purely diffusive. For such a particle the average root mean square displacement is given by:

$$\text{root MSD} = \sqrt{2nDt}$$  \hspace{1cm} (1.4)

where $n$ is the dimensionality of the system, $t$ is the time interval over which the displacement is measured and $D$ is the diffusion coefficient of the particle.

Particle motion with $\alpha < 1$ is said to be 'subdiffusive' while $\alpha > 1$ is 'superdiffusive' systems.\(^{47}\) Translational diffusion coefficient of a particle undergoing purely diffusive motion can be calculated from Einstein-Smoluchowski equation:

$$D_t = \frac{K_BT}{6\pi\mu r}$$  \hspace{1cm} (1.5)

and the rotational diffusion coefficient is given by:

$$D_r = \frac{K_BT}{8\pi\mu r^3}$$  \hspace{1cm} (1.6)

where $K_B$ is the Boltzmann constant, $T$ is the absolute temperature, $r$ is the radius of the particle, $\mu$ is the viscosity of the fluid in which the particle travels, $D_r$ is the rotational diffusion
coefficient and $D_t$ is the translation diffusion coefficient of the particle. Therefore, any microscopic particle in a solution undergoes has to overcome the constant Brownian randomizations to undergo directed motion.

### 1.3.2: Directionality

As discussed in the section above, an active colloid in a solution tends to undergo Brownian diffusion and subsequently its trajectory gets randomized over measurable intervals of time. For spherical Janus motors, this is of the order of 10 seconds.\textsuperscript{48} Nanorods and microtubules discussed before have asymmetric drag forces; even that is not enough to overcome Brownian randomizations and their motion becomes diffusive over long periods of time. Thus introducing and maintaining directionality in micro-and nanomotors is the subject of a lot of research in the field.

Catalytic motors tend to swim with a certain orientation. If the orientation of the particle could be fixed by preventing Brownian rotation, then the propulsion of the particle will become directional. One of the easiest ways of doing this incorporating a magnetic component in the device and using a magnetic field to orient the device.\textsuperscript{49} Au/Pt carbon nanotubes with Ni incorporated in them by the Wang group could navigate narrow microfluidic channels with the help of an external magnetic field.\textsuperscript{50} As discussed in section 1.2.2, ABF or 'Artificial bacterial Flagellum' is also an example magnetically controlled swimmers.

Another method of controlling the motion of motors is by using ultrasound waves to propel acoustophoretic motors. Bimetallic nanorods in aqueous solutions developed by Mallouk group can rotate, align and assemble in a field of acoustic waves. These rods can be levitated in nodal plains by acoustic waves and show directed propulsion.\textsuperscript{51,52} Moreover Mallouk group has also demonstrated the biological applications of such motors by guiding these nanomotors into cancer cells using ultrasound waves.\textsuperscript{10}

An emerging field of research has tried to overcome this difficulty by taking advantage of the interactions of active swimmers with physical features.\textsuperscript{53} A wall of chevron style obstructions have been shown to guide and sort swimming bacterial cells.\textsuperscript{54} Bimetallic micromotors and Janus swimmers have demonstrated circling behavior around larger immobile colloidal
structures. Similarly tubular micromotors can be concentrated near the cusp of heart shaped structures.

1.3.3: Chemotaxis
The most common example of autonomous directed motion in nature is chemotaxis. Chemotaxis means "motion in response to chemical stimuli." Bacterial cells exhibit chemotaxis when they propel themselves in the direction of food or away from the source of toxins. Bacterial cells can sense temporal changes in the concentration of the desired molecule through the receptors on their surface and can adjust their orientation accordingly. Recently chemotaxis has been observed in some synthetic motors as well. Synthetic micromotors have been shown to propel themselves towards the gradient of their fuel and subsequently collect at the source. Sen et al have demonstrated this behavior with bimetallic nanorods where the rods move towards higher concentrations of hydrogen peroxide, invoking comparisons with motion of E. Coli towards higher concentrations of sugar. Recently, "match-stick" shaped micromotors have also been used to demonstrate the phenomenon. Sophisticated experiments have been carried out by establishing fuel gradients in microfluidic channels, and Janus micromotors and tubular microswimmers were observed moving up the fuel gradient. Interestingly, the mechanism of this behavior is unknown and existing simulations run contrary to the experimental observations. One hypothesis is that such directional migration occurs because with higher diffusivity the motor experiences a higher average displacement so it will continue to move farther as it travels up the gradient. However this is a growing field with a lot of unanswered questions and as of now no conclusions can be drawn.

1.4: Conclusions
This chapter introduces the various synthetic motors in current literature and discusses the difficulties of propulsion at the micro- and nanoscale. The focus of the following chapters would be devising strategies to overcome these limitations of motion at low Reynolds number. We will discuss various strategies for directing the motion and behavior of active swimmers.
1.5: References


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Chapter 2

Directed and Linear Transport of Janus Micromotors

2.1: Motivation
As discussed in Chapter 1, the ability to maneuver a single micromotor and/or steer it along a predetermined path in a liquid medium could have far reaching consequences. Such precise navigation control could see applications in cargo transport, repair and drug delivery within the body, sensing, environmental remediation and even surgery of single cells. This chapter would introduce a new method of steering individual micromotors. Self propelled micromotors which decompose dissolved fuel molecules to generate motion via phoretic phenomena, or bubble detachment are one of the fastest growing fields in material science. Unfortunately directing the motion of such micromotors which include bi-metallic nanorods, nanotubes and Janus spheres is an ongoing struggle. The major challenge in realizing this goal is Brownian motion. Brownian rotations randomize body orientations and the propulsive translation vector of active colloids, leading to long time limit trajectories that exhibit isotropic enhanced diffusion. Current strategies of directing catalytic motors include external magnetic fields, Earth’s gravitational field, electrophoretic traps and artificially patterned microfluidic devices. However these strategies either lack autonomy as in the case of external fields and traps or constrain the trajectories as in the case of gravitational field directions. Chapter 2 will demonstrate a method which realises directed motion of the catalytic motor when it is moving in close proximity to solid surfaces through quenching of its Brownian rotation.

2.2: Janus Motor Synthesis
Our system consists of Janus particles composed of platinum(Pt) capped polystyrene (PS) particles which swim in >1 % H₂O₂fuel due to the catalytic conversion of hydrogen peroxide into...
water and oxygen. Catalytic Janus spheres were prepared by spin coating a 0.1 wt % dispersion of fluorescent polystyrene microspheres (Thermoscientific - a=1 µm, 1.55 µm and 2.4 µm) from ethanol onto freshly cleaned glass microscope slides. A 10 nm thick layer of platinum (>99.9 % Sigma Aldrich) was then evaporated onto one side of the microspheres under vacuum in a Lab18 (Nanofabrication lab, Pennsylvania State University) e-beam evaporator. Low volume fraction solutions of colloids in pure water (Resistivity>15 MΩ) or the specified aqueous concentration of Hydrogen Peroxide (>99.9% Sigma Aldrich) were prepared by removing the colloids from the glass slides. The removal mechanism involved wetting a piece of lens tissue/brush and transferring the colloids to the lens tissue/brush from the glass. Subsequently the lens tissue/brush was put in a solution of 1 mL DI water and agitated gently leading to a solution of Janus particles in water. After centrifuging (1000G, 10 mins) down the solution to 0.1 mL, it was filled up back to 0.5 mL and the process repeated twice, vortexing in between to break up the pellets. Another method of transferring particles is sonicating each of the glass slides in 10 mL DI water for 30 min. The spin-coated platinum coated glass slide covered in Janus colloids prepared as described above, was submerged in 10 mL DI water in a petridish and sonicated for 30 minutes. The resulting dispersion was concentrated to 3 mL by centrifugation at 1000 G in a SorvallBiofuge Primo centrifuge. 50 µL of the concentrated Janus solution was diluted to 600 µL of DI water. For samples with H₂O₂, equal volumes of concentrated Janus solution and 30 % H₂O₂ were sonicated for 5 minutes and left for another 20 minutes to activate the Pt surface before dilution to 5 % H₂O₂.

2.3: Janus micromotors in hydrogen peroxide
Our Janus particles consist of platinum (Pt) capped polystyrene (PS) particles which swim in >1 % H₂O₂ fuel due to the catalytic conversion of hydrogen peroxide into water and oxygen. These Janus particles swim (See video 2.1) with the PS side forward, according to a self-electrophoretic mechanism, wherein ionic currents are set up at the Pt end, between the poles and the equator⁴. The azimuthal angle and in-plane orientation of the particles can be observed under fluorescence microscopy.²⁴ When Janus colloid solution are evenly dispersed into a low volume rectangular section glass cuvette (Suprasil glass cuvettes from Hellma Analytics)
containing water, sedimentation leads to accumulation at the bottom planar surface. However, in the presence of hydrogen peroxide fuel a propulsion velocity due to asymmetrical catalytic fuel decomposition is introduced. With fuel, 2 µm Janus colloids particles now rapidly arrive at both the top and bottom interior planar surfaces of the cuvette; whereas for 3 and 5 µm colloids, accumulation preferentially occurs at the top interface. This biased accumulation for the larger colloids is due to the asymmetrical mass distribution caused by the dense platinum hemisphere resulting in gravitaxis.

The active colloids were observed using the fluorescence mode of a Nikon Eclipse LV100 microscope with illumination through blue excitation band of a Nikon B2A filter cube. Videos were captured using an Andor Neo camera at a frame rate of 33 Hz. Custom software developed using LabView vision was used to identify the x,y coordinate for the centre of each bright particle throughout each video recording, thus generating the trajectory data that was subject to mean-square displacement analysis. Such trajectories can be quantified using Mean-Square Displacement (MSD) versus time analysis to determine propulsion velocity as described in literature.\textsuperscript{16}
**Figure 2.1:** Average propulsion velocity of Janus particles of various diameters is plotted as a function of fuel (H$_2$O$_2$) concentration. As expected the velocity increases with fuel concentration. The top and bottom refers to the velocities of particles swimming at the top of the solution and at the bottom of the cuvette respectively.

**2.3: Brownian rotational quenching near a planar surface**
We aim to study the stochastic trajectories of these Janus colloids when they swim near an interface, so that we can characterise its effect on their active motion.

The azimuthal angle, $\theta$, which probes how the polarity of a Janus particle is oriented with respect to the surface normal vector, and the corresponding in-plane orientation can be observed as 'phases of moon'. In the absence of H$_2$O$_2$, Janus particles sediment in a rectangular cuvette to the bottom wall and are observed to undergo Brownian translational and rotational diffusion about a cap-down equilibrium orientation dictated by gravity, due to the weight of the platinum. But in the presence of H$_2$O$_2$, the Janus particles undergo enhanced diffusion in the bulk and accumulate at both the top and bottom walls, according to the competition between gravitaxis$^5$ and sedimentation. Strikingly, once close to the wall, for sufficiently high H$_2$O$_2$ concentrations, the swimmers maintain an orientation such that the half-moon shape is persistently visible. This suggests that the out-of-plane rotational diffusion is quenched so that the azimuthal angle (defined in Figure 2.2(B)) is $\theta \approx 90^\circ$.
Figure 2.2: (A) A visual representation of the system (B) Azimuthal angel $\theta$ is defined

As seen from the video 2.2, 2.3, and 2.4, our experiments were performed with Janus particles of different sizes (2 $\mu$m, 3 $\mu$m and 5 $\mu$m) in 10% $\text{H}_2\text{O}_2$ and once near the bottom of the cuvette the Janus particles maintain a consistent half moon shape. Figure 2.3 shows a schematic representation of the quenching behavior observed.

![Figure 2.2](image)

Figure 2.3: Schematic 3D orientation and experimental trajectories (45 seconds duration, red line) for 2 $\mu$m platinum-polymer Janus particle in (a) water settled under gravity against a planar glass substrate; colloid and (b) 10% hydrogen peroxide solution at the planar surface of a rectangular glass cuvette.

![Figure 2.3](image)
To describe the time scale and magnitude of the Brownian rotational quenching/alignment phenomena in more detail, image analysis was used to determine the temporal fluctuations in azimuthal angle for each Janus colloid size.

Labview analysis of videos was used to determine the average pixel intensity for the central region of each tracked particle as a function of time. For a given particle size, illumination and camera settings were fixed, to allow quantitative comparison between these intensity values. To convert these relative fluorescence emission values to azimuthal angles, it was necessary to establish the intensities that corresponded to cap down (θ=0 maximum brightness) and cap up (θ=180° minimum brightness) orientations. For 2 µm and 3 µm particles a self-calibration approach was used, where long duration intensity versus time plots were obtained for a number of particles in water, and the average maximum and minimum intensities were assigned to θ=0° and θ=180° azimuthal angles. Linear interpolation between these limits was used to complete the conversion.

Typical graphs comparing θ vs. t for colloids observed both in water and when moving rapidly in high fuel concentrations (10 % w/v) are shown in Figure 2.4. These confirm the variations in θ are quenched to a stable alignment at 90° for high activity, rapidly moving colloids. Figure 2.4 displays still frames from videos (see video 2.2, 2.3 and 2.4) illustrating the observed behaviour. For colloids with 2 µm and 3 µm Brownian rotation about both the azimuthal and in-plane axis occurs. Figure 2.3 schematically summarises this: independent Brownian rotation and translation. In contrast, 5 µm Janus colloids present a nearly constant “full” phase of fluorescence emission while undergoing Brownian diffusion, indicating that the platinum cap is consistently orientated downwards, i.e. azimuthal angle θ close to 0°. This reflects the
increased gravitational energy required to rotate the platinum cap from the lowest energy $\theta = 0^\circ$ position relative to the available thermal energy driving Brownian rotations, resulting from the increased distance of the dense catalyst from the colloids centre of rotation.$^5$

This presents significant challenges in analysing the videos. For 5 $\mu$m Pt-PS Janus particles the gravitational torque makes the probability of rotation to a cap up configuration too low to observe within a reasonable time frame. To overcome this, 5 $\mu$m colloids were frozen within a transparent gellan gum, which allowed the now solid sample to be inverted. The minimum intensity found from observing many of these inverted particles was used as the $\theta=180^\circ$ limit, subsequently allowing conversion to azimuthal angles as before. All presented azimuthal angle data was obtained in this way, however the data collapse shown in Figure 2.5(c) also constrained the short term behaviour for each re-scaled curve to match the theoretical value for the rotational diffusion coefficient in order to further correct for particle to particle variations in fluorescence intensity.

Trajectory analysis confirms the results that propulsion velocity that increases in magnitude with fuel concentration at a given particle size, decreases for larger particle radii at a given fuel concentration, and is little effected by the gravitational direction i.e. cuvette orientation.$^{23,24}$ Most strikingly, it is also apparent that for all particles sizes, with increasing fuel concentration the Janus colloids orientation during motion is increasingly observed as a persistent “half moon” shape, Figure 2.3. This indicates the azimuthal thermal Brownian rotations and gravitational effects observed for the catalytically inactive spheres in the absence of fuel are now dominated by a new surface alignment effect constraining the azimuthal angle, $\Theta$, to remain close to $90^\circ$. This equilibrium configuration for rapidly swimming active colloids is depicted schematically in Figure 2.3, and is qualitatively described as a quenching of Brownian rotation about the azimuthal axis to align the propulsion vector with the planar surface. As illustrated in the trajectory, Brownian rotations about the axis perpendicular to the planar axis are unaffected leading to cross over from ballistic to diffusive transport regimes.

In order to capture the transition between these two behaviours, experiments were also performed at intermediate fuel concentrations allowing observations for colloids over a range
of velocities. Figure 2.5 shows the Mean Square Angular Displacement (MSAD) curves for many Janus particles (n>25 at each condition) arranged by size and binned according to propulsion speeds, along with a comparison to un-fuelled, purely Brownian Janus colloids. For particles undergoing un-quenched Brownian rotation, we have MSAD= $\langle \Delta \theta(t)^2 \rangle = 2D_r \Delta t$. In water, a linear time dependence of the MSAD is observed. However, as Janus sphere velocity is increases the rate of evolution for azimuthal angle incrementally reduces below this upper limit, until at sufficiently high fuel concentrations/velocities the azimuthal angle appears frozen within the experimental ability to resolve fluctuating $\theta$, as seen in Figure 2.5. This data provides a relationship between Janus particle size, activity/velocity and azimuthal angle evolution rate which will is used below to explore the origins for the alignment phenomena. The strength of the orientational quenching is manifestly increased as the propulsion velocity is increased.

Figure 2.5(A): Azimuthal Mean Square Angular Displacement (MSAD) as a function of time for 2 μm Janus particles. The black “Water” line represents the MSAD for Janus particle settled at a planar interface under gravity in water (n>20). The additional curves represents the MSAD for Janus particles with speeds in the defined ranges, at both the top (n>20) and bottom (n>20) planar surfaces of a rectangular cuvette.
Figure 2.5(B): Azimuthal Mean Square Angular Displacement (MSAD) as a function of time for 3 μm Janus particles. The black “Water” line represents the MSAD for Janus particle settled at a planar interface under gravity in water (n>20). The additional curves represent the MSAD for Janus particles with speeds in the defined ranges, at both the top (n>20) and bottom (n>20) planar surfaces of a rectangular cuvette.
Figure 2.5(C): Azimuthal Mean Square Angular Displacement (MSAD) as a function of time for 5 μm Janus particles. The black “Water” line represents the MSAD for Janus particle settled at a planar interface under gravity in water (n>20). The additional curves represents the MSAD for Janus particles with speeds in the defined ranges, at both the top (n>20) and bottom (n>20) planar surfaces of a rectangular cuvette.

2.5: Rotational diffusion quenching near multiple planar surfaces
We then hypothesize that a particle in the proximity of two orthogonal surfaces will have its diffusion quenched along two orthogonal planes (Figure 2.6).

Figure 2.6: Particles moving along geometric boundaries, at speeds of up to 10 µm/s. (a-b) schematics of Janus particles encountering multi-planar geometries. Red axis indicates forbidden rotations due to proximity to a plane, green axis indicates unquenched axis of rotation: (a) Janus particle encountering a planar edge while moving along a 2D surface, expected to result in Brownian rotational quenching about two orthogonal axes. (b) Janus particle confined within a square groove; parallel vertical walls confine the rotational diffusion about one axis; however, if the particle descends to the base of the groove, it is confined about two orthogonal axes.
Indeed we observe that a particle initially undergoing 2-D enhanced diffusion at the surface (Figure 2.7(a)) undergoes persistent linear motion when it reaches the vertical edge of the cuvette (Figure 2.7(b)).

Figure 2.7: Overlaid still frames from fluorescence microscopy videos with equal time gaps: yellow line shows complete trajectory, green line shows location of vertical cuvette walls, red arrows indication direction of motion: (a) 3 µm Janus particle (10 % H₂O₂) moving at the bottom of a rectangular glass cuvette a long way away from the edges. (b) 3 µm Janus colloid (10 % H₂O₂) moving along the curved edge of a glass cuvette and reaching the cuvette boundary. (c) 5 µm Janus colloid (10 % H₂O₂) moving along the straight edge of a glass cuvette - left hand inset shows a magnified region, right hand inset shows a “stuck” aligned agglomerate formed at the cuvette boundary.
Figures 2.7(a) and 2.7(b) show examples of active colloids following curved and straight sections of the boundary for appreciable distances. Figure 2.7(b) also shows the transition from 2-D enhanced diffusion to boundary steering occurring at the moment the colloid reaches the wall. Additionally the left hand inset for Figure 2.7(c) verifies that the colloid equator is aligned at close to 90° as seen above for a single boundary. In fact, over many repeated experiments we observed that all the colloids investigated (which were of different sizes) were directed by the edge of the cuvette for length scales up to several centimetres. Colloidal motion continued around the entire macroscopic cuvette edge, only occasionally stopped by small blemishes (standard laboratory glassware that had not been precision engineered was used), or by encountering other stuck colloids, resulting in pile-ups of aligned colloids as seen in Figure 2.7(c) right hand inset. Due to the build-up of colloids following the edge, sometimes in different directions, collisions between moving colloids were also observed (see video 2.5 and 2.6). Collisions result in stable agglomerates which continued to move along the boundary in a direction determined by the relative propulsive velocities of the colliding components. One example resulted in a period of “jostling” followed by rotation of one Janus colloid to result in a cooperative pair that resumed motion along the edge.

For the 5 µm spheres, the fluorescence images also reveal that the fluorescent Janus colloids in active hemisphere is slightly canted away from the sides of the cuvette as it moves. This should be due to the increase in the gravitational torque and other non-active orientation-sensitive interactions (e.g. electrostatic, vdW, etc) that grow with size (see Appendix), which we have ignored in the above analyses.

The second geometry investigated was an array of lithographically produced deep rectangular linear channels. The rectangular wells were fabricated over silicon wafers in the Nanofabrication Laboratory of Materials Research Institute, Penn State University. Silicon Wafers (4" wafer, 100 prime, 0.1 ohm cm conductivity and 500 µm thick) were cleaned with acetone and air-dried. The wafers were then spin-coated with 1 mL of SPR 955 photoresist (Microposit) at 900 rpm for 10 sec and then at 3000 rpm for 60 sec. This was followed by soft-baking the coated wafers over a hot plate at 95 °C, for 60 sec. The well geometry was modelled in CAD and printed over a chrome-on-glass mask (Nanofabrication Laboratory, Materials
Research Institute, Penn State). For photolithography, the mask was placed in direct contact with the photoresist over the wafers. The resist was then exposed to UV radiation for 12 sec in a Karl Suss MA/BA6 Contact Aligner. The exposed wafers were post-baked for 1 min over a hot plate at 95 °C to cross link the exposed film. MF CD26 developer was used to remove unexposed SPR 955 from the wafers. The mould was developed for 90 sec while being agitated, followed by washing it thoroughly with deionised water. After the wafers were dried with a nitrogen blower, a 30 μm deep master pattern was created on them using deep reactive ion etching to yield the rectangular wells on the silicon surface. The ion etching was done using Alcatel Silicon DRIE in Nanofabrication Laboratory of Materials Research Institute, Penn State and the process used was Low ARDE. After etching the remaining resist was removed from the wafers by agitating the wafers in a solution of NanoRemover PG at 60 °C for 2 hours. After removal, the wafers were cleaned with isopropanol and air dried. To homogenize the surface chemistry, the etched wafers were then treated with oxygen plasma (200 sccm, 400 W) for 30 min.

Figure 2.8: SEM image of the rectangular grooves. A rectangular array of grooves ranging in width from 3 μm to 10 μm was designed with increments of 0.5 μm. The depth of the grooves was 35 μm and their length was 2 mm.
Since the Janus particles now interact with three confining surfaces (two parallel walls and the bottom surface, as seen in Figure 2.6), geometric constraining should lead to a strictly linear motion of the Janus particles in the channels, with few or no “Brownian escapes”. Grooves with a variety of widths were investigated, and the colloids with diameter 5 µm were observed to be rotationally quenched and surface-aligned within channels with widths 7-9 µm. For example, Figure 2.9 depicts an active Janus colloid exhibiting persistent linear motion when confined within a 9 µm wide channel (see video 2.7).

![Figure 2.9: 5 µm Janus colloid (10 % H₂O₂) moving within a rectangular section groove (width=8.75 µm)](image)

### 2.5 Mechanism of rotational quenching

Having determined the phenomenology of rotational quenching experimentally, it was necessary to investigate this effect theoretically, and explore possible mechanisms that can account for these observations. The orientational dynamics of our active Janus particles could be affected by several mechanisms. These include equilibrium effects such as gravitational torque due to inhomogeneous weight distribution in the platinum cap and electrostatic interaction with the surface due to zeta potential difference between the two halves of the Janus particle. There are also non-equilibrium effects such as hydrodynamic coupling between the swimmer and the surface, electroosmotic effects due to the ionic activity of the active Janus colloid, and electrostatic contributions due to additional difference in zeta potential between the two halves as a result of the non-equilibrium catalytic activity on the platinum cap.
There are several observations pointing to the fact that a non-equilibrium effect was observed which is a result of the propulsion mechanism of the particles. Higher concentrations of the fuel, H$_2$O$_2$, result in a greater fraction of time spent by the particle in the half-moon orientation. As expected, higher H$_2$O$_2$ concentrations also result in higher particle speeds both at the surface and in the bulk (Figure 6.1). We know that the gravitational torque is not responsible for the surface aligned Brownian rotation quenching since no surface alignment is observed in the absence of H$_2$O$_2$, and the surface aligned rotational quenching is independent of the direction of gravity, as mentioned above.

To check the electrokinetic effect on the propulsion a solution of 5 μm Janus particles was prepared in 10% H$_2$O$_2$ in 1 mM NaCl. The addition of salt weakens the electrokinetic propulsion$^{25}$ and a decreased quenching effect is also observed.

It is possible that electrostatic colloidal forces could contribute to quenching if the surface potentials changed significantly in presence of the reaction. In order to estimate the contribution of the electrostatic potential term with and without catalytic activity, we measured the zeta potential of each end (Pt and PS) of the Janus particle by carrying out both translational and rotational electrophoresis in 1 mM NaCl (Figure 2.10(a)). As shown in Figure 2.10(b), typical translational electrophoresis motion of a Janus particle (5 μm) in a 2.5 V/cm E-field in the direction of the E-field was observed. The average zeta potential ($\zeta$) was interpreted using the Smoluchowski equation. Averaged over many particles, we found an average of $\zeta = -89 \pm 2$ mV (in 1 mM NaCl) with no fuel present, and $\zeta = -92 \pm 7$ mV with 5 % H$_2$O$_2$ present (Figure 2.10(g)).

We next measured the difference between the Pt and PS sides of the particle. Owing to unequal zeta potentials at each end, the particle undergoes rotational electrophoresis to align the zeta potential dipole with E$_x$ (Figures 2.10 c-f); see Appendix. We tracked particle rotations under fluorescence microscopy and used the measured angular velocity to obtain the zeta potentials for each case (Figures 2.10d-g). The zeta potential of the PS end does not change appreciably upon the addition of fuel. We also find that the zeta potential of the Pt end
stabilizes to a constant value, after about an hour in H₂O₂. While we observe some degree of sample-to-sample variability, based on the above measurements, we can largely conclude that the values of the zeta potentials of the two ends change by a small amount in relative terms due to the non-equilibrium catalytic activity. Hence, we can safely rule out electrostatic interactions as the main cause of rotational quenching.

Figure 2.10: Electrophoretic behaviour for Janus colloids. (a) Typical position vs. time curves obtained by particle tracking a (5 µm) Janus particle in a plane close to a glass interface on application of 2.5 V/cm at 1 mM NaCl. The electric field was in the negative x direction first and then switched every second. The red circles are times when the E field direction was switched (b) Typical φ vs. time curve for rotation of a Janus colloid on application of 2.5 V/cm in a 5 % H₂O₂ solution at 1 mM NaCl. The field direction was flipped after the particle attained a steady
equilibrium orientation aligned with the applied field. (c) See Appendix: \( f(\phi) \) vs. time for the curve shown in (b). (d) Schematic representation of the rotational electrophoresis experiment. Inset shows the relevant physical quantities, a Janus sphere with hemispheres with two different zeta potentials (\( \zeta_{Pt} \) and \( \zeta_{PS} \)) produces a dipole vector \( \hat{e} \). When this vector is not aligned with the applied field vector, \( E \), such that an angle \( \phi \) is subtended between \( E \) and normal to \( \hat{e} \), a torque is generated, resulting in the generation of angular velocity, \( \Omega \). The 3D schematics depict the effect of switching the direction of \( E \). 1. Represents the initial misaligned dipole and applied field orientation immediately after the \( E \) field direction is switched, stages 2-5 show two possible rotations to re-align the dipole with the applied field: on the left hand side about an out of plane axis, with constant azimuthal angle, \( \Theta \), and on the right about an axis parallel to the plane where azimuthal angle changes; at position 5 \( \hat{e} \) reaches the steady state. The black arrows show the direction of translational motion, which is always aligned with the applied field (see a). (e+f) show still frames from a fluorescence microscopy video for a PS-Pt Janus particle rotating about an out of plane axis (e) and about an axis parallel to the plane (f) from the point at which the applied \( E \)-field polarity was reversed to the depicted direction (red arrow). (g) shows the measured zeta potentials for both Pt (blue markers) and PS (red markers) at two time points following sample preparation, each with and without peroxide.

We now consider the most nontrivial aspect of the phenomenon. When a sphere is pulled by a mechanical force near a wall (such as gravity during sedimentation), a coupling between translation and rotation develops that will make the colloid rotate in a sense that is consistent with a rolling tendency along the surface of the wall. A perturbative analysis (See Appendix) of the three effects – electrophoretic, electroosmotic and hydrodynamic was carried out and it was concluded that the unique quenching behaviour is a result of the hydrodynamic interaction of a force-free, self-electrophoretically propelled, particle with the wall and the broken symmetry of the surface flow field. It is the asymmetric slip velocity profile that differentiates these metal-insulator particles from other active particles propelled by self-electrophoresis such as bimetallic Janus particles as well as self-diffusiophoretic Janus spheres, where the slip velocity is symmetric and quenching by this mechanism is not expected. Moreover, if the
surface interactions are modified such that the Janus sphere swims with its platinum cap forward, then this mechanism cannot lead to stable orientational quenching. These spherical Janus swimmers are a special class, in their ability to be steered by boundaries.

2.6: Conclusion
We have shown that geometric constraints can help steer our Janus particles, by lowering the effective dimensionality of the space on their trajectories. One surface constrains the motion in 2D, and two perpendicular surfaces constrain it to 1D. This can be further exploited to create more elaborate constraints. For example, we expect a corner with three perpendicular surfaces to act as a trap and fully constrain the motion of our active colloids.

The ability to steer Janus motor particles uni-directionally along complicated trajectories by simply following an edge or groove opens the door for many transport and separation tasks such as directed cargo delivery, motility-based sorting, and flow-free microfluidics. Feature-directed steering combined with single-particle\textsuperscript{11} and collective many-body\textsuperscript{12} chemotactic response could provide an ideal toolkit for designing novel strategies to be employed in oil or mineral exploration tasks.\textsuperscript{13} Also as evident from Figure 6.7, this is a useful method for reversible bottom-up assembly of active colloids and gives us a unique advantage in controlling the orientation of the assembled active Janus particles. One further possibility, opened up by understanding the mechanism, is that in addition to “geometric railroad tracks” we could take advantage of chemical patterning of the surface to achieve a higher degree of control when we guide particles along pre-determined paths.

2.7: References
This chapter is adapted from "Das, S. et al. Boundaries can steer active Janus spheres. Nat. Commun. 6, 8999 (2015)"

3. Dunderdale, G., Ebbens, S., Fairclough, P. & Howse, J. Importance of particle tracking and


2.8: Appendix

2.8.1: Rotational Electrophoresis

This analysis was performed by Astha Garg

Standard Dynamic Light Scattering (DLS) equipment is not designed to handle either the material heterogeneity of metal-insulator Janus particles or bubbling at the electrodes in the presence of H₂O₂. Measurement of $\zeta_{\text{Pt}}$ and $\zeta_{\text{PS}}$ using separate homogeneously coated Pt and PS particles is possible, but a constant thickness Pt surface does not undergo the same reaction as a Janus particle with an inhomogeneous coating on the Pt half. Consequently, in order to measure the average colloid zeta potential, $\zeta_J$, we carried out translational electrophoresis in a homemade closed capillary setup.

The spin-coated platinum coated glass slide covered in Janus colloids prepared as described above was submerged in 25 mL DI water in a petridish and sonicated for 30 minutes. The resulting dispersion was concentrated to 3 mL by centrifugation at 1000 g in a SorvallBiofuge Primo centrifuge. 50 µL of the concentrated Janus solution was diluted to 600 µL of 1 mM NaCl solution containing 0.003 % by volume of spell tracers. For samples with H₂O₂, equal volumes of concentrated Janus solution and 30 % H₂O₂ were sonicated for 5 minutes and left for another 20 minutes to activate the Pt surface before dilution to 5 % H₂O₂.

The solution for analysis was fed into a cleaned glass capillary (0.9 mm square cross section, 50 mm long and 0.18 mm from Vitrocom, RCA-I cleaned) and placed on a glass slide making sure there are no bubbles or air gaps. About 1.5 cm long piece of gold wire (0.5 mm, 99.99% purity Alfa Aesar, cleaned using water and ethanol) was inserted at each end and the capillary was sealed off using wax and a UV-curing adhesive. The capillary set up was mounted on the motorized stage of a Nikon TE 300 inverted microscope, so that the capillary surface faced the 20x or 40 x objective. The gold wires were connected to electrodes from a Keithley 2410 source.
meter which was operated in the constant current mode at 3 μA resulting in an electric field of 2.5 V/cm. Measured voltage was much higher than 2.5 V/cm in the presence of H₂O₂ due to increased resistance from bubbles. Videos were recorded at 10-30 fps using a Qimaging QiclickCCD camera in bright field for translational electrophoresis against tracers, and under green fluorescent light for rotational electrophoresis. Electrophoresis experiments were carried out in sealed glass capillaries containing a dilute solution of Janus particles in 1 mM NaCl (99.99% Sigma-Aldrich) with or without 5 % H₂O₂. The motion of particles settled close to the bottom surface was recorded under fluorescence light. The rotation rate due to shear field in the bottom plane of the electrophoresis cell is calculated to be < 1 % of the measured rotation rate due to dipole alignment.

To account for the convection resulting from oxygen bubbles formed by the decomposition of H₂O₂, we measured the speed of Janus particles against sPSL (sulphated polystyrene latex, 1.45 μm radius Life Technologies Batch no. 1212; 1) tracers with a known ζ. ζspsl was measured using DLS (Malvern Nano ZS Zeta Sizer) as well as using our setup, which served to verify that our set-up did not introduce impurities to the system. We measured the velocity of sPSL tracers in at least 7 different planes across the depth of the capillary. We fitted these velocities for ζw and ζspsl to the known parabolic flow profile due to electroosmotic flow in a closed capillary 34. Limited available volume fraction and fast settling did not permit a similar experiment on Janus particles to measure ζj directly.

To determine the zeta potential for each side of the colloid we measured particle orientation changes during field direction reversals in the same set up. Using image analysis software it was possible to directly measure the orientation of the Janus colloid as a function of time for realignments that proceeded via an in plane rotation, Figure 4e. For a spherical Janus particle with potentials ζPt and ζPS on each half, the predicted angular velocity, \( \frac{d\phi}{dt} \) during a rotational event is a function of the angle \( \phi \) between the normal to the dipole moment \( (\zeta_{Pt} - \zeta_{PS}) \hat{e} \) and the electric field \( E_\infty \) 36:

\[
\frac{d\phi}{dt} = \frac{9}{16\eta a} (\zeta_{Pt} - \zeta_{PS}) \hat{e} E_\infty \cos \phi
\]  

(1)
Integrating this equation with respect to \( \phi \) and time (t) yields a function \( f(\phi) \) that varies linearly with t:

\[
f(\phi) = \ln|\sec \phi + \tan \phi| = \frac{9}{16} \eta a (\zeta_{pt} - \zeta_{PS}) E_\infty t + c \tag{2}
\]

Equations 1 and 2 predict that if the particles are aligned anti-parallel to the E-field, the rotation rate will be zero and time will be \( \infty \). This is an unstable equilibrium position. The slightest kick from the ever-present Brownian rotational motion will bring the particle to its stable equilibrium position where the dipole is parallel to the E-field. Thus flipping the polarity of E-field each time the particle reaches its equilibrium allows us to observe the rotation of the particle as it aligns with the E-field.

We do a frame by frame analysis of at least 4 rotations induced by field-reversal within the same field of view and obtain \( \phi \)-t curves (Figure 2.10). Using a least-squares fit of the linear portions of corresponding \( \phi \) vs t curves (Figure 2.10), we calculate the value of \( \zeta_{pt} - \zeta_{PS} \) for each particle observed. Together with the knowledge of \( \zeta_j = (\zeta_{pt} + \zeta_{PS})/2 \), we are able to determine \( \zeta_{pt} \) and \( \zeta_{PS} \) separately (see Supporting Information).

Averages and standard deviations were obtained from the distribution of average ZP for each particle within a trial.

2.8.2: Perturbative analysis

This analysis was carried out by Prof. Ramin Golestanian.

We now consider the most nontrivial aspect of the phenomenon. When a sphere is pulled by a mechanical force near a wall (such as gravity during sedimentation), a coupling between translation and rotation develops that will make the colloid rotate in a sense that is consistent with a rolling tendency along the surface of the wall. We can get an intuitive idea about this coupling by thinking of the singular limit of the problem when the sphere is in no-slip contact with the wall while pulled by an external force; the sphere rolls along the surface. Phoretic transport of colloids with uniform surface properties has been shown to entail an anomalous
anti-rolling behaviour\textsuperscript{14}. In self-phoretic propulsion\textsuperscript{15}, a force-free and torque-free Janus colloid takes advantage of gradients that are generated due to the asymmetric activity across its surface to generate phoretic motion\textsuperscript{16}. When a Janus particle self-propels in the vicinity of a surface, the coupling could lead to a mixture of rolling and anti-rolling tendencies, depending on the geometry and the type of activity\textsuperscript{17,18}. This suggests that the coupling of the phoretically propelled Janus particle with the wall could, in principle, generate the necessary condition for our observed orientational quenching. This can be described via a phenomenological Hookean form for the angular velocity

$$\frac{d\theta}{dt} = -\Gamma(\theta - \theta_s), \quad (1)$$

where $\theta_s \approx \pi/2$ is the stationary orientation, and $\Gamma > 0$ acts as an effective (restoring) elastic constant that ensures rolling and anti-rolling tendencies are invoked below and above the stationary value, correspondingly, to enforce stable quenching. Equation 1 is an approximation that will hold only in the vicinity of $\theta_s$.

Based on the observation that the quenching effect increases with increasing fuel concentration, as well as dimensional grounds, the parameter $\Gamma$ depends on the particle velocity and the radius of the sphere as

$$\Gamma = B \frac{v}{a}, \quad (2)$$

with the dimensionless prefactor $B$ depending solely on the material and geometric parameters, such as zeta potential, height, and radius. Using equation 1, we find the following expression for MSAD (see Theory Supplement S4 for details)

$$\langle \Delta \theta(t)^2 \rangle = \frac{D \varepsilon}{\Gamma} [1 - e^{-2\Gamma t}]. \quad (3)$$

Values of $B$ can be obtained experimentally by plotting the averaged and re-scaled rotational MSAD data as a function of time (Figure 2.11). Data collapsed for a large number of individual trajectories at each particle size gives values of $B$ of order unity.
Using a far-field approximation for the hydrodynamic interaction between a swimmer and a no-slip wall, which has been shown to be fairly accurate even in the vicinity of the surface\textsuperscript{6–8}, we can calculate the angular velocity of the Janus swimmer by prescribing the surface slip velocity profile on its spherical surface. We find that a swimmer with self-phoretic propulsion experiences a stabilising angular velocity described by equation 1.

To have stable orientational quenching (i.e., $\Gamma > 0$) for spherical self-phoretic swimmers, two very specific criteria need to be met. The first criterion comes from symmetry: any surface slip velocity profile that is fore-aft symmetric will result in $\Gamma = 0$. This implies, for example, that the diffusiophoretic component of the surface slip velocity that is fore-aft symmetric cannot lead to the observed quenching of orientation. The second criterion involves the direction of swimming versus the catalytic coating; $\Gamma > 0$ only when the Janus particle swims away from the catalytic patch. The main motility mechanism of our platinum-coated Janus particles involves proton current loops that emanate from the vicinity of the equator and end near the pole\textsuperscript{10}, hence satisfying both of these criteria by serendipity. Based on an example velocity profile, we can estimate the hydrodynamic contribution to the coefficient $B$ as follows.

\begin{figure}[h]
\centering
\includegraphics[width=\linewidth]{figure2.png}
\caption{MSAD data re-scaled to allow comparison with theory, together with fits to equation 3 with estimated values for $B$ (see equation 2).}
\end{figure}
\[ B_h \simeq 0.3 \left( \frac{a}{a+h} \right)^3, \]  

(4)

where \( h \) is the stationary value for closest distance between the colloid and the surface. We expect the prefactor \( B_h \) to be stronger than the above estimate when the colloid is in close proximity of the surface (See Table 1 in Supporting Information). This is consistent with the experimental observations that give out fitted values for \( B \) that are of order unity (see Figure 2.11).

We know that the self-propulsion in our Janus particles is predominantly controlled by electrokinetic effects\(^1\). Therefore, it is natural to expect to have a significant electric field that results from the surface proximity of the proton currents caused by the catalytic activity of the Janus particle; the so-called “image field”. Due to the difference in the zeta potentials between the two halves of the Janus particle, the image field could contribute to the aligning tendency of the Janus particle via an electrophoretic contribution. In the bulk, we can relate the propulsion velocity to the self-generated electric field (caused by proton currents) using the Smoluchowski equation. The presence of the surface will modify the proton currents and the resulting electric field, in a way that could be described by an image distribution which will in-turn result in a torque of the form given in equation 1, with \( \Gamma_{el} \sim e \Delta \zeta E_{image} / \eta a \). This leads to the form given in equation 2 with the following contribution to the coefficient \( B \)

\[ B_{el} \sim \frac{\Delta \zeta}{\zeta} \left( \frac{a}{a+h} \right)^3. \]  

(5)

Therefore, we conclude that our estimate of the electrophoretic contribution \( (B_{el}) \) is smaller than our estimate of the hydrodynamic contribution \( (B_h) \) by a factor of \( \frac{\Delta \zeta}{\zeta} \ll 1 \), based on experimental measurements of the zeta potential (Fig 3g).

Another contribution from the electric field is in the form of electroosmotic flow which feeds back to the hydrodynamic term. We find that our factor for the electroosmotic contribution \( B_{eo} \) is smaller than \( B_h \) by a factor of \( \frac{\zeta w}{\zeta} \left( \frac{a}{a+h} \right)^3 \ll 1 \) within our far-field approximation.

**Translational Diffusion Coefficient Damping:**
To estimate the height of the particles above a fused quartz silica wall we suspended a mixture of fluorescent Janus colloids (2,3,5 μm) in water (ElgaPurelab Option, 15 MΩcm) and allowed them to settle out. With the Janus colloids at their equilibrium height above the bottom wall of the cell videos of 25 individual particles of each size were recorded at a frame rate of 100 Hz. Image analysis algorithms were used to extract 2D trajectories of the particles diffusing in the (x, y) plane and the mean squared displacements $\Delta L^2$ computed A fit to $\Delta L^2$ yielded the hindered diffusion coefficient $D_{||}$. The H$_2$O$_2$ (Sigma Aldrich, 30.0 wt%, pKa = 11.75) that we add to a suspension of Janus sphere swimmer particles to make them swim contains the stabiliser dipicolinic acid at a concentration of 40 mg L$^{-1}$ (pKa = 2.2) giving an estimated ionic strength, $I = 8 \times 10^{-5}$ M, at 10 % wt. We consequently performed diffusion experiments with the Janus colloids dispersed in a KNO$_3$ solution with the same ionic strength ($I = 8 \times 10^{-5}$ M) to estimate colloid height during the swimming experiments.

A suspension of colloidal particles diffusing well away from a wall has a Brownian diffusion coefficient $D_{\infty}$ described by the Stokes-Einstein relation ($D_{\infty} = k_B T / 6 \pi \eta a$). As the colloids settle under gravity and reach an equilibrium height above the bottom surface of their container, their diffusion is hindered by an additional hydrodynamic drag. The hindrance factor for diffusion parallel to a surface is given by

$$\frac{D_{||}}{D_{\infty}} = 1 - \frac{9}{16} \left( \frac{a}{z} \right) + \frac{1}{8} \left( \frac{a}{z} \right)^3 - \frac{45}{256} \left( \frac{a}{z} \right)^4 - \frac{1}{16} \left( \frac{a}{z} \right)^5,$$

where $D_{||}$ is the diffusion coefficient parallel to the surface and the gap height $h$ is contained in the $z = a + h$ term.$^{37,38}$
Chapter 3
Fluid Mixing at the Microscale using Cilia

3.1: Introduction
One of the rapidly growing fields in biotechnology is the use of lab-on-a-chip devices to analyze biofluids. A typical lab-on-a-chip system would consist of a number of microfluidic channels connecting microchambers where dedicated biochemical analysis is carried out. Rapid and often complete mixing is required in many lab-on-a-chip systems for example biochemical or biomolecular detection, drug delivery, nucleic acid sequencing. Many of these systems need homogenizing of reagents and for that to be effective the reaction rate between the biofluid and additional functional material has to be high, thus the interface between the two has to be large. As discussed in chapter 1, at low Reynolds number the flow is laminar and spontaneous mixing happens by diffusion only. Thus special mixing techniques have to be developed to obtain chaotic mixing and subsequent rapid increase in interfacial area for lab-on-a-chip devices.

The two growing methods to achieve this, by using active or passive mixers. Passive mixers involve incorporating different shapes like s-bends or chevron structures into the microfluidic channel to increase the interfacial area between the fluids. Active mixers actively control the fluid and enhance mixing using external forces like pressure, electrokinetic or acoustic disturbances. However biological systems can serve as an inspiration for a new class of active micromixers based on cilia which utilize internal chemical reactions to power the mixing. Cilia are tiny hair-like appendages (with a typical length of 10 µm and a diameter of 250-500 nm) that cover for example the surface of many micro-organisms which helps them move. In human beings, for example, ciliated surfaces are found in the respiratory tract where they sweep mucus (Figure 3.1), or in the Fallopian tubes where they move an ovum to the uterus.
The motion of the fluid above a ciliated surface is generated by periodic beating of cilia. The beating pattern of cilia is asymmetric and composed of two phases: the effective stroke, during which the cilium makes an oar-like motion and propels the fluid, followed by the recovery stroke, when the bent cilium moves backward to return to the original position sweeping along the surface with a frequency between 5 to 30 Hz.\textsuperscript{10,11} Even though each cilium can beat independently, cilia densely covering a surface synchronize their cycles and form metachronal waves, thus increasing their fluid pumping efficiency\textsuperscript{12}. It is theorized that the metachronal waves occur as a result of hydrodynamical interactions between the cilia.\textsuperscript{13,14} Since the ciliary pumping mechanism is highly efficient, same principle can be used for designing artificial cilia that act as microscale and microfluidic pumps and mixer. An important step towards biomimetic cilia was made by Darnton et al., who created a bacterial rug by attaching bacteria to a solid surface and generating fluid flow due to the motion of the flagella.\textsuperscript{15} However the flow patterns were quite complex and ever-changing due to symmetric rotation and weak coordination between bacterial flagella. Experimentally, better control over artificial cilia can be obtained by driving them with external fields, for example, by magnetic or electric fields. The first and the most prolific attempt to fabricate artificial cilia was based on nanorod arrays manufactured from polymeric materials doped with magnetic nanoparticles.\textsuperscript{16} These are an example of helical swimmers discussed in chapter 1. Furthermore, den Toonder et al. have shown that electrostatically driven cilia made of metal-coated polymer films can act as mixers and—outside the low Reynold’s number regime—even as pumps.\textsuperscript{17,18} Light driven actuators have also been manufactured using liquid crystals doped with azobenzene.\textsuperscript{19} Alternatively, lithographically manufactured regular arrays of cilia were also designed that were actuated by an electron beam.\textsuperscript{20} Despite progress in this area, all the mentioned actuators depend upon an external stimuli whether it be light, magnetic energy or pH change. In addition, all the actuators or cilia-like structures have been pretty large compared in dimensions of cilia found in biological especially mammalian systems.
In this chapter, we present an approach for bio-inspired fluid mixing based on nanorods of polymeric polypyrrole (PPy) and PDMS that possess mechanical properties and dimensions close to those of biological tissue (Figure 3.1). We show that the oscillatory and nonreciprocal displacement of the flexible nanorods in response to the chemical stimuli results in uniform fluid motion above the ciliated surface.

![Figure 3.1: (Left) micrograph image of cilia on the inner surface of Mammalian trachea which shows the morphology along with the structural integrity of this material (Right) SEM image of artificial cilia array designed using Polypyrrole nanorods in this chapter, showing the similarities between the two](image)

3.2: Mechanism of Actuation
In 2010 Sen et al. demonstrated that AgCl particles in the presence of hydrogen peroxide (H₂O₂) and UV light exhibit oscillatory motion due to the reversible reduction of silver chloride to silver metal on the particle surface. These oscillations are seen not only in the motion of individual particles but also schools or large collections of such silver chloride particles (see Video 3.1). The mechanism of the motion is electrolyte diffusiophoresis. Electrolyte diffusiophoresis, as outlined in chapter 1 is the motion of particles driven by concentration gradients of the ions. For a particle undergoing electrolyte diffusiophoresis near a charged surface, the velocity is defined by:
\[ U = \frac{\nabla c}{c_0}\left[ \left( \frac{D^+ - D^-}{D^+ + D^-} \right) \left( \frac{K_B T}{e} \right) \frac{\varepsilon (\xi_p - \xi_w)}{\eta} \right] + \frac{\nabla c}{c_0} \left[ \frac{2 \varepsilon T^2 K_B^2}{\eta e^2} \left\{ \ln(1 - \gamma_w^2) - \ln(1 - \gamma_p^2) \right\} \right] \] (3.1)

where \( \nabla c \) is the concentration gradient of the electrolyte, \( D^- \) and \( D^+ \) are the diffusion coefficients of the anion and cation respectively, \( c_0 \) is the bulk concentration of the ions at the particle location, \( K_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \varepsilon \) is the dielectric permittivity of the solution, \( \eta \) is the viscosity of the solution, \( \xi_p \) and \( \xi_w \) are the zeta potential of the wall and particle respectively and \( \gamma = \tanh \frac{Ze \xi}{4kT} \)

Figure 3.2: Schematic of diffusiophoretic forces on a negatively charged particle near a negatively charged wall in an electrolyte.²⁴

The first half of the equation represents electrophoretic motion. Due to the different diffusivities of the cation and anion, an concentration gradient of the ions is set up which generates an electric field. This electric field on the particle can drive its motion due to electrophoresis. The second half is the chemophoretic term. The concentration gradient of the ions also interacts with the ions in the double layer of the wall, leading to a difference in thickness of the double layer. This creates a 'pressure' difference along the wall leading to a
movement of fluid from higher to lower electrolyte concentration. As long as the diffusion coefficients of the cations and anions are different, the chemophoretic effects are negligible.

Since the wall is charged too, the generated electric field can also act electroosmotically on the double layer of the wall. This electroosmotic effect leads to the movement of the solution near the walls. Depending on the charges on the particle and the wall, the direction of the fluid generated due to the two effects could be in the same direction or opposite. The zeta potentials of the wall and particle determine which effect will dominate. The interplay between these forces leads to schooling behavior of the particles. This collective assembly is invariant under time reversal, thus reversing the chemical reactions that lead to motion can generate oscillatory patterns.\textsuperscript{25}

When silver chloride is exposed to water in the absence of hydrogen peroxide it decomposes yielding the following reaction:

\[
4\text{AgCl} + 2\text{H}_2\text{O} \rightarrow 4\text{Ag} + 4\text{H}^+ + 4\text{Cl}^- + \text{O}_2
\]  \( (3.2) \)

In Deionized (DI) water, motion of the AgCl articles is induced due to diffusiophoresis since the ions have different diffusion coefficients. The H\textsuperscript{+} diffuses much faster (D = \( 9.311 \times 10^{-5} \) cm\textsuperscript{2}s\textsuperscript{-1} ) than the Cl\textsuperscript{-} ion (D = \( 2.032 \times 10^{-5} \) cm\textsuperscript{2}s\textsuperscript{-1} ) which leads to an inwardly directed radial electric field around the particle.\textsuperscript{22} Asymmetries in the AgCl particle shape produces imbalances in the angular distributions of the Cl\textsuperscript{-} and H\textsuperscript{+} ions, thus yielding a net electric field at the particle surface to which the particle itself responds.
In addition, silver metal is oxidized to AgCl in the presence of HCl, H$_2$O$_2$, and UV light as shown by the following equation:

$$2\text{Ag} + \text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{Cl}^- \rightarrow 2\text{AgCl} + 2\text{H}_2\text{O} \quad (3.3)$$

By the same principle as above, an electric field is generated. This electric field can induce motion of the Ag particles and this diffusiophoretic motion of the Ag particle can serve as the chemical driving force for the cilia. Since this reaction is reversible, the direction of the electric field oscillates due to time invariance, leading to oscillatory motion of the particle.
3.3: Design of Cilia
To design the cilia, a silver disk would be deposited on top of a flexible rod anchored to a surface, and the electric field acting on the Ag disk would induce an oscillatory motion in the rods as discussed in the previous section.

For designing the nanorods the selection of polymer has to be careful. The polymer chosen had to be bio-compatible, stable at high aspect ratios and also flexible. Two polymers polypyrrole and PEDOT were selected. Both of them are conducting thus a fabrication method combining soft lithography and electrodeposition was developed. PEDOT reacted with the resist SPR 955 used in the fabrication process and thus was rejected, and polypyrrole was finalized. Even though soft lithography had been used for fabrication of the cilia-like rods, the techniques use electron beam and two color process. We developed a lithography technique using a single color process and electrodeposition. However polypyrrole rods had stability problems. For the polymer to be effective as an artificial actuator, the rods have to be both stable and flexible. Both of these characteristics depend on aspect ratios. The aspect ratio range in which
polypyrrole was flexible, the rods undergo breaking at the roots. Table 3.1 gives the comparison of observed stability of polypyrrole rods against lateral collapse due to adhesive forces and the flexibility of movement in solution.

Thus, PDMS (Polydimethylsiloxane) was chosen as the next polymer. For PDMS rods the stability and flexibility of different aspect ratios is given in Table 3.2.

<table>
<thead>
<tr>
<th>Diameter of rods</th>
<th>Height of rods</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µm</td>
<td>5 µm</td>
<td>Not Stable</td>
</tr>
<tr>
<td>1 µm</td>
<td>10 µm</td>
<td>Not Stable</td>
</tr>
<tr>
<td>2 µm</td>
<td>2 µm</td>
<td>Not Flexible</td>
</tr>
<tr>
<td>2 µm</td>
<td>3 µm</td>
<td>Stable/Flexible</td>
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<tr>
<td>2 µm</td>
<td>5 µm</td>
<td>Stable/Flexible</td>
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<tr>
<td>3 µm</td>
<td>3 µm</td>
<td>Not Flexible</td>
</tr>
<tr>
<td>3 µm</td>
<td>5 µm</td>
<td>Not Flexible</td>
</tr>
<tr>
<td>3 µm</td>
<td>10 µm</td>
<td>Stable/Flexible</td>
</tr>
</tbody>
</table>

**Table 3.1:** The viability of polypyrrole rods of various aspect ratios

<table>
<thead>
<tr>
<th>Diameter of rods</th>
<th>Height of rods</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 µm</td>
<td>10 µm</td>
<td>Flexible/Stable</td>
</tr>
<tr>
<td>10 µm</td>
<td>10 µm</td>
<td>Not Flexible</td>
</tr>
<tr>
<td>5 µm</td>
<td>15 µm</td>
<td>Stable/Flexible</td>
</tr>
<tr>
<td>Diameter</td>
<td>Length</td>
<td>Flexibility</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>-----------------</td>
</tr>
<tr>
<td>10 µm</td>
<td>15 µm</td>
<td>Not Flexible</td>
</tr>
<tr>
<td>15 µm</td>
<td>15 µm</td>
<td>Not Flexible</td>
</tr>
<tr>
<td>5 µm</td>
<td>25 µm</td>
<td>Flexible</td>
</tr>
<tr>
<td>10 µm</td>
<td>25 µm</td>
<td>Flexible</td>
</tr>
<tr>
<td>15 µm</td>
<td>25 µm</td>
<td>Not Flexible</td>
</tr>
</tbody>
</table>

**Table 3.2**: The comparison of observed stability of PDMS micropillar arrays against lateral collapse due to adhesive forces and the flexibility of movement in solution

Rods of length 25 µm and diameter 5 µm were selected because they were stable and flexible and also comparable in dimensions to biological cilia. The PDMS rods were fabricated using lift off technique employing silver as the metal mask. Figure 3.5 shows the fabrication process developed. In this process, the etch rate and etching time of PDMS determines the length of the rods.
For fabricating PDMS nanorods Polydimethylsiloxane (SylgardTM 184, Dow Corning) elastomer solution was prepared by mixing prepolymer with a cross-linking agent in weight ratio of 10:1. It was de-gassed in a vacuum dessicator for 2 hours. The solution was then spin coated on glass.
slides (25 x 75 x 0.7 mm) at 3000 rpm for 2 min and baked at 60 °C for 4 hours to yield the desired 25 µm thick PDMS layer. The PDMS layer was oxidized using an O₂ plasma at 150 W for 60 sec and immediately positive resist (Microchem SPR -955) was spin coated on the PDMS layer at 3000 spm for 60 sec. The resist was soft baked at 105 °C for 60 sec. The slide was then exposed to UV through a chrome mask for 11 sec (365 nm, 8 mW/cm²) and developed in Microposit CD-26 (Shipley) for 90 sec to yield the desired pattern. Silver metal is then evaporated on the pattern formed on the resist at a thickness of 100 nm using the Semicore E-gun Evaporator (deposition rate 1-2 Å/s). Lift off of the resist was performed using a NANO™ Remover PG (MicroChem, N-Methyl Pyrrolidinone) bath for 30 min to yield the silver pattern on PDMS. The PDMS was then dry etched for 60 min using standard recipe in the PlasmaTherm 720 with the silver pattern acting as the metal mask to yield 25 µm long pillars. The glass slide was cut into squares of equal sizes for ease of experimentation. All metals were purchased from the Kurt.Lesker company.

Figure 3.6: (Left) PDMS nanorods arranged in an array. The image is taken top down thus the height of the rods seen in the image is not the true height. The cracks seen on the surface are the cracks formed in PDMS in response to spin coating resist on them. (Right) SEM image of the PDMS rods where the substrate is plasma treated to allow smooth coating of resist. Thus no cracks are visible.
3.4: Fluid Mixing Profile
To monitor the generated flow in the fluid, we introduced polystyrene spheres into the sample as tracer particles. The tracers (1 µm in diameter) were much smaller than the diameter of the nanorods and we were able to follow the tracer particles at a height of 50 µm above the ciliated surface.

Figure 3.7: (a) The figure denotes a schematic of how the cilia are expected to move (b) This figure is a snapshot from the video showing the four cilia pillars arranged in an array and the tracer particles in the fluid. This snapshot corresponds to PDMS pillars of 5 µm diameter arranged in a square array where the distance between two cilia is 25 µm.

The experimental setup was build around an inverted microscope (Zeiss AxioVert 200 MAT) equipped with UV light. Circular adhesive wells (Diameter 20 mm, Depth 1.3 mm, “Secure Seal Spacer,” Invitrogen) were added to the surface to act as spacers for the cover-slide. The appropriate solution was added to the well and imaged using a 50x microscope objective. The tracer particles were polystyrene particles (Bangs Labs, diameter 1 µm) and their motion was
recorded using a PointGrey camera. The motion of the particles was tracked using an algorithm written in Labview.

Previous experiments from our group\textsuperscript{26} used silver particles exposed to UV light along with varying concentrations of H\textsubscript{2}O\textsubscript{2} ranging from 1\% to 0.17\% and HCl ranging from 0.17 mM to 0.83 mM. Various experiments were conducted which denoted that the rods would break and disintegrate with higher percentages of H\textsubscript{2}O\textsubscript{2} and HCl.

Through trial and error it was determined that 0.17\% H\textsubscript{2}O\textsubscript{2} in the presence of 0.33 mM HCl would give the best results. When the rods were exposed to UV light in the absence of either H\textsubscript{2}O\textsubscript{2} or HCl no motion was observed. In the presence of UV light with 0.17\% H\textsubscript{2}O\textsubscript{2} and 0.33 mM HCl the center of mass of the rods exhibited an oscillatory motion. The PDMS rods selected were $L = 25 \mu m$ and diameter $= 5 \mu m$ arranged in an array where the distance between any two rods is 25 \mu m. When the solution is exposed to UV light the oscillatory motion of both the rods and tracer particles is seen. In the above case, the oscillatory motion is evidenced in the mean square displacement graphs shown in Figure 3.8(a). The calculated frequency is same for the rods and is 8.5 Hz at $h = 100 \mu m$. The frequency of the artificial cilia is comparable to frequency of cilia in nature (between 5 to 30 Hz).
Figure 3.8: The mean square displacement of the PDMS rods with respect to time. The mean square displacements are calculated from the relative displacement of the rod from its center of mass. The four graphs show the mean square displacement of the four rods seen in Figure 3.7(b). They are arranged in the order they appear in Figure 3.7(b).
Figure 3.9: In this figure the total displacement of the rod \( \sqrt{x^2 + y^2} \) where \( x \) is the relative displacement of the one rod from its center of mass in \( x \) direction and \( y \) is the relative displacement of the same rod from its center of mass in \( y \) direction is measured for the reaction and for the control. As shown by the figure the total displacement is oscillatory with a defined period for the reaction.

The oscillatory motion of the rods is clear when we superimpose the displacements of all four rods. The oscillations of the rods are also synchronized with each other, just like the oscillations of cilia found in biological systems.\(^{12}\)
Figure 3.10: The relative displacements of the rods from their center of mass only in the y-direction. As seen from the graph the rods move in phase thus providing evidence of the synchronization of motion.

For control purposes, we performed the experiments without H$_2$O$_2$ and without UV. In both cases the motion is random which proves that both hydrogen peroxide and UV is needed for the oscillation to happen.
Figure 3.11: **Control experiments (Left)** This figure shows the relative displacement of the rods from their center of mass in the y direction vs time in the absence of H$_2$O$_2$. As evident and can be contrasted with Figure 3.8 there is no oscillatory motion and neither is any kind of synchronization present. **(Right)** This figure shows the relative displacement of the rods from their center of mass in the y direction vs time for the four rods in the absence of UV. As is evident and can be contrasted with (b) there is no oscillatory motion and neither any kind of synchronization present.

3.5: **Conclusion**
In this chapter, we demonstrated a versatile method to make large array of flexible cilia-like structures of tunable dimensions. The technique enables us to use bio-compatible materials facilitating the fabrication of highly responsive sensors and actuators. They are also unique in the sense that these structures are closest to the dimensions of biological cilia. In literature most cilia-like structures are fabricated with much higher dimensions. We have also demonstrated the actuation of these rods and the fluid flow induced by said actuation. We believe structures and control produced by the methods outlined above may be useful in microfluidics, photonic, and sensing applications.
3.6: References


Chapter 4
Chemotactic Separation of Enzymes

4.1: Motivation

In vitro separation and isolation of active biomolecules plays a critical role in biological and biotechnological analyses associated with pathogen detection, cancer cell identification, mRNA isolation, and tissue engineering, among others. Recent advances in device miniaturization have led to the development of integrated lab-on-a-chip devices, offering a variety of simple and efficient separation techniques, dealing mostly with micron-scale particles. Microfluidics has served as a prominent platform for the development of small, inexpensive and efficient diagnostic devices owing to their reduced reagent consumption rate and short sampling-to-result time. Techniques for particle separation in microfluidic systems based on gravitational, magnetic, acoustic, and electrokinetic forces have been demonstrated. Although these protocols often lead to high throughput separation of micron scale particles, their use in biomolecular separations can damage the molecules due to stresses arising from external field-driven sieving and tweezing. Moreover, conventional sorting chips usually involve polymeric gels within the outlets as sieving matrices, which pose difficulties in multistep analysis in molecular separations. Current label-free separation techniques, which rely on the differences in physical properties of particles such as shape, density, adhesion, dielectric constant, or diffusion do not give efficient separation if these differences between the biomolecules in a complex mixture are not high. The challenge therefore remains in harvesting specific biomolecules with very similar physical properties from a complex mixture, and in quantities that are sufficient for downstream sensing and detection.

As discussed in Chapter 1 micro- and nanoscale catalytic motors have recently been the subject of intensive investigations because of their ability to negotiate complex environments. Enzyme molecules also behave as motors due to their ability to power their own motion by
turnover of their respective substrates present in the ambient fluid.\textsuperscript{27–29} Previously our group demonstrated that in the presence of a gradient in substrate concentration, the enzyme molecules migrate toward higher substrate concentration regions, a form of molecular chemotaxis.\textsuperscript{30} We hypothesize that the chemotactic behavior of the enzyme molecules arises from the enhanced diffusion mechanism. The substrate concentration changes continuously as the enzyme diffuses along the gradient. Thus, at every point in space, the diffusivity increases on moving up the gradient and decreases on moving down the gradient. A higher diffusion coefficient leads to a greater spreading of the enzyme molecules on the side of the higher substrate concentration. Thus, the “center of gravity” of the enzyme ensemble moves toward higher substrate concentration. As with any non-equilibrium system, a continuous energy input is required for the directional movement, in this case to maintain the substrate gradient. The proposed mechanism is stochastic in nature and is different from biological chemotaxis, which requires temporal memory of the concentration gradient.

This chapter describes how the \textit{chemotactic} migration of enzymes toward areas of higher substrate concentration can be utilized to separate enzymes from one another in a microfluidic device.

\textbf{4.2: Design of the microfluidic device}

Figure 4.1 shows a schematic diagram of the two-inlet, five outlet microfluidic setup used in experiments. The separation was monitored on-chip with a fluorescence microscope and the separated enzymes were collected through different outlets continuously during the process. Using the microfluidic separator, separation of molecules of catalase from urease, urease from β-galactosidase, and active catalase from its inactive form was demonstrated. The separation efficiency of the device agreed well with finite element simulations of convective diffusion developed using COMSOL Multiphysics software (see Appendix). The proposed separation strategy does not depend on the size or charge of the molecules, and can be carried out under near ambient conditions needed for enzyme activity. Separation based on molecular chemotaxis through substrate turnover should allow enhanced sensitivity for point-of-need assays due to minimum influence of contaminants.
Figure 4.1: (A) Schematic of the experimental setup used to observe chemotactic separation of enzymes. (B) Dimensions of various sections of the microfluidic separator

The microfluidic channels were fabricated following standard soft lithography techniques. Microchannel masters were fabricated over silicon wafers in the Nanofabrication Laboratory of Materials Research Institute, Penn State. Wafers were cleaned using oxygen plasma (350W, 200 sccm) for 60 sec. The wafers were then spin-coated with 2 mL of SPR-955 photoresist (Microposit), using dynamic dispense at 900 rpm for 15 sec and then at 3500 rpm for 50 sec, followed by a de-acceleration step at 900 rpm for 15 sec. This was followed by soft-baking the coated wafers over a hot plate at 95 °C, for 1 min. The microchannel geometry was modeled in L-Edit and printed over a chrome-on-glass mask (Nanofabrication Laboratory, Materials Research Institute, Penn State). For photolithography, the mask was placed in direct contact with the photoresist over the wafers. The resist was then exposed to UV radiation for 8 sec in a Karl Suss MA/BA6 Contact Aligner. The exposed wafers were post-baked for 1 min over a hot plate at 95 °C to cross link the exposed film. MF CD26 developer was used to remove unexposed SPR 955 from the wafers. The mold was developed for 1 min while being agitated, followed by washing it thoroughly with deionized S1 water for 60 sec. After the wafers were dried with a nitrogen blower, a 50 µm deep master pattern was created on them using deep reactive ion etching (17 min, HARLF recipe, Alcatel). The prepared wafer was cleaned for 30 min each using hexane, isopropanol and acetone in that order and air dried. Polydimethylsiloxane
(PDMS, SylgardTM 184, Dow Corning) elastomer solution was prepared by mixing prepolymer with a cross-linking agent in weight ratio of 10:1. The etched wafers were exposed to trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (Sigma Aldrich) for 1 hour in a sealed chamber, to minimize adhesion of PDMS during the peeling step. The PDMS solution was poured on top of the wafers to the desired thickness. Air bubbles in the PDMS mixture were removed by degassing it in a vacuum desiccator for 2 hours. The masters were then cured in an oven at 60 °C overnight. After curing, the PDMS channels were peeled from the mold and inlets/outlets were made using a stainless steel puncher. The devices were sealed to glass coverslips (VWR), by exposing them to oxygen plasma for 2 min, followed by bonding them together manually and baking them on a hot plate at 100 °C for 1 min. For fluid flow through the microchannels, the punched holes in the device were connected with polyethylene tubes (SPC Technology, Internal diameter 0.66 mm). The main channel of the microfluidic chamber has a length of 8 mm, with a depth of 50 μm. Each of the inlets has a width of ∼400 μm while the individual outlets were 120 μm wide. These dimensions ensured minimum interference of signals within adjacent outlets of the device.

4.3: Experimental Set-Up

4.3.1: Labeling of Enzymes with Fluorescent Dyes & Preparation of Enzyme Mixtures

Catalase-Urease: Samples of Bovine liver catalase (Sigma-Aldrich) and Jack bean urease (Sigma-Aldrich) were labeled with amine reactive fluorescent dyes available from Life Technologies as protein labeling kits. Labeling of 8 μM (2 mg/mL) catalase solution with Alexa Fluor 647 (AF 647, ex/em: 650/668) fluorophores was performed in room temperature under gentle stirring of the solution prepared in 100 mM phosphate buffer (pH 7.2) for 2 h. Similarly, 4.17 μM (2 mg/mL) urease was tagged with Alexa Fluor 488 (AF 488, ex/em: 494/519), under the same experimental conditions. As per manufacturer’s instructions, to facilitate the formation of dye-protein conjugate, pH of the protein solutions was raised using 1M sodium bicarbonate S2 before the solutions were put into the fluorophore vials. To reduce the free dye concentrations,
the enzyme-dye complexes were purified using gel filtration kits provided with the dyes. The concentration of the purified enzyme solutions was calculated from UV-vis measurements. Extinction coefficients of catalase and urease at 280 nm were determined separately (230074 M-1 cm-1 for catalase and 75592 M-1 cm-1 for urease) using fluorescence measurements and used in calculations. A mixture of tagged catalase and urease was then prepared while keeping the concentration of each enzyme in the solution fixed at 200 nM.

**Urease -β-Galactosidase**: 4.17 μM (2 mg/mL) buffered solution of Jack bean urease was labeled with AF 647 and 3.70 μM (2 mg/mL) β-Galactosidase (from E. Coli., Sigma Aldrich) was labeled with AF 488 - under gentle stirring in room temperature for about 2 h. The enzyme-dye complexes were purified using the gel filtration kits provided with the dyes and concentrations of the purified solutions were calculated using UV-vis spectrophotometry. Extinction coefficient of β-Galactosidase at 280 nm was taken to be 241,590 M-1 cm-1, as reported in the literature. Mixture of urease and β-Galactosidase was then prepared in phosphate buffer, keeping the concentration of each enzyme in the mixture fixed at 200 nM.

**Active and Inactive Catalases**: 8 μM solution of bovine liver catalase was labeled with AF 647, as described previously. The labeled solution is filtered by gel filtration and was used as solution of active catalase. A separate sample of 8 μM catalase was labeled with AF 488 and then incubated with 0.5 M NaCN, prepared in deionized water overnight. Cyanide acts as inhibitor for catalase, without altering any other physical characteristics. The labeled inhibited catalases were separated from unreacted cyanide using membrane dialysis (10 kDa pores; Amicon ultra-4 centrifugal filter unit, Millipore). The concentration of labeled enzymes in the purified solutions was calculated from UV-vis measurements. Mixture of enzymes was then prepared in phosphate buffer, keeping concentration of each enzyme in the solution fixed at 200 nM. The number of dye molecules per enzyme molecule was estimated using concentrations measured by UV-Vis spectroscopy and following the procedure provided with the protein labeling kit. For catalase labeled with AF 647, the calculation showed presence of ~4.5 dye
molecules per molecule of catalase. For urease labeled with AF 488 and AF 647, the degree of labeling was ~1.5 and ~3.7 respectively. For β-Galactosidase and inhibited catalase both tagged with AF 488, the numbers were typically ~2 and ~1 respectively.

The fluorophores used in labeling are selected in a manner to ensure minimal overlap in their spectral profiles and hence possibility of any Fluorescence Resonance Energy Transfer (FRET) between the molecules. The concentrations of the labeled enzymes solutions were calculated using UV-vis measurements. A mixture of these enzymes was prepared in phosphate buffer, keeping their individual concentration fixed at 200 nM.

4.3.2: Relation between Fluorescence and Concentration of Catalase and Urease Solutions
When the enzyme concentrations are sufficiently dilute, the fluorescence of the dye-protein conjugate can be considered proportional to their concentration. The following curves show the variation of fluorescence intensity as a function of the enzyme concentration. The profiles are fairly linear. Thus, with nM solutions of enzymes, their normalized fluorescence intensity profiles recorded at a position within the microchannel can be considered to be proportional to their normalized concentration profile at that position.
Figure 4.2: Correlation between fluorescence and concentration of labeled proteins, measured for enzymes tagged with AF 647 and AF 488 respectively. The profiles are linear at sufficiently dilute enzyme concentrations.

The fluorescence signal from the tagged enzymes was monitored and recorded using highly sensitive fluorescence imaging. The optical setup involved a Nikon Eclipse Ti inverted microscope with a 100 W halogen lamp. Excitation light was passed through the appropriate filter cube (Nikon), depending on the excitation/emission wavelengths of the tagged fluorophores, before it was focused onto the sample through 4x/10x objectives (Plan Apo 4×-0.20/Plan Apo 10×-0.45, Nikon). Fluorescence emission collected by the objective lens, was passed through interference filters, and detected by a sensitive iKon-M 934 CCD camera (Andor Technology) with a resolution of 1024 × 1024 pixels and maximum quantum efficiency of 95%.

4.3.3: Proof of Concept Studies using two pairs of enzymes
We used mixtures of catalase and urease, urease and β-galactosidase, and active and inactive catalase to demonstrate the separation of active biomolecules in the presence of their specific substrates. Each of these pairs of enzymes was carefully chosen to determine the relative merit of chemotactic sorting. For proof of concept studies, the above combinations were selected because of the high substrate turnover rates for catalase ($k_{\text{cat}} = 2.12 \times 10^5 \text{ s}^{-1}$) and urease ($k_{\text{cat}} = 2.34 \times 10^4 \text{ s}^{-1}$). It should be noted that the current method can be employed for any combinations of enzymes or other active molecules exhibiting high catalytic activity. The microfluidic channels described in section 4.1 were used. The dimensions of the channel were chosen through COMSOL simulations (see appendix) to ensure minimum interference of signals within adjacent outlets of the device. Intermediate constrictions consisting of parallel PDMS microchannels, each of width $\sim 100 \mu\text{m}$, as shown in Figure 4.1 were used to regulate the flow of the flow of the liquids. Based on the simulation results, for 200 nM enzyme solutions, we decided to work with flow speeds close to 15 µL/h. It may be mentioned here that obtaining slow flow rates, exhibiting smooth laminar profiles in channels that have dimensions of the order of micrometers is challenging. While a syringe pump could pump fluid slowly (down to
tens of microliters per hour) the flow was not observed to be smooth. As such, we used a gravity-driven flow by placing the enzyme and substrate reservoirs shown in Figure 4.1 at a higher height than the outlets of the microfluidic device. In order to slow the flow rate down further, we placed a second microfluidic device with smaller channel dimensions (~100 µm) between the reservoirs and the separator to act as constrictions. The microfluidic cascade helped to attain smooth gravity driven laminar flow at the inlets at a rate of ~15 µL/h, which was found unattainable using a syringe pump. This flow rate is orders of magnitudes slower than the flow rates typically used in microfluidic experiments. The concentrations of the two enzymes in the mixture were kept the same and they were labeled separately with different fluorescent dyes. When there was no substrate, the flow of phosphate buffer through the other inlet was maintained at a rate equal to that of the enzyme solution. The flow profile was allowed to stabilize and fluorescence profiles of the enzymes were recorded along a cutline, drawn across the outlets 330 µm down from the split (Figure 4.1). The cutline was selected at a position sufficiently away from the split in order to minimize perturbation in fluorescence near the junction of the outlets. For chemotactic separation of enzymes, the phosphate buffer was replaced by a buffered solution of the substrate (corresponding to the active enzyme to be separated) flowing through the same inlet at the same rate. In the given microchannel architecture (Figure 4.1), the interface between the two flows runs through the middle of the third outlet of the device. Hence, the substrate gradient is expected to be maximum between the third and fourth outlets. In the presence of a substrate flow, the intensity profiles were again recorded along the same cutline within the outlets and compared.

**Urease and Catalase**

Before starting the experiments, a buffered solution of bovine serum albumin (BSA, 10 mg/mL) was allowed to flow through the device for about 20 min to minimize sticking of enzymes on the polydimethylsiloxane (PDMS) surface. This was followed by flows of 100 mM phosphate buffer from reservoirs held at approximately 10 cm above the microscope stage through both the inlets. For separation of catalase from urease, a 1:1 mixture of catalase and urease (each 200 nM) was prepared in phosphate buffer, keeping their individual concentration fixed at 200 nM. The separation experiment can be carried out in principle with any concentrations of
enzymes. With the present experimental setup however, concentrations higher than 200 nM were found to saturate the fluorescence detector. Also, separation of enzymes in presence of their substrate concentration gradients is likely to depend on the duration of enzyme-substrate interaction. In other words, more enzyme molecules are expected to get separated out if they are allowed to spend more time between the inlets and the outlets of the microfluidic device. As such, the flow rate of the enzyme and substrate solutions through the microfluidic device should be slow enough to maximize the enzymes' ability to diffuse into the substrate channel.

Keeping this issue in mind, prior to experimental measurements, we optimized the flow rate, for 200 nM enzyme solutions using Multiphysics simulations in COMSOL (see Appendix). Considering a mixture of active and inactive catalase, the excess migration of active catalase was estimated within the substrate outlets, in the presence of its substrate 10 mM H\textsubscript{2}O\textsubscript{2}, for flow speeds ranging from 0.5 µm/s to 3000 µm/s. For the experimentally used microchannel, this corresponded to flow rates ranging from 0.0375 µL/h to 225 µL/h. For very slow flow of liquid (~0.5 µm/s), the simulation resulted hardly any substrate concentration gradient along the interface of the flows. The population of both active and inactive molecules was found to be the same within each of the outlets, resulting in no separation at all. The separation was significant above flow speed of 20 µm/s and increased continuously with the increase in flow rate at the inlets. However, for flow rates higher than 200 µm/s (~15 µL/h), the number of molecules crossing the substrate interface was found to be small for both active and inactive catalase (normalized concentrations ~10\textsuperscript{-7}), which eventually makes the detection of excess migration of the active molecules with respect to the inactives challenging. The details of the simulation results are provided in the Appendix. Thus, for 200 nM concentration a flow rate of 15 µL/hr was chosen. A mixture of the two enzymes was allowed to flow through one of the inlets of the microchannel at 15 µL/h and a flow of phosphate buffer through the other at the same rate was maintained. After the flow had stabilized, the flow profiles of liquids near the inlet and outlets of the microchannel device were checked for uniformity. Typical flow profiles obtained at the inlets and near the outlet splits, for the two tagged enzymes are shown in Figure 4.3.
Figure 4.3: Intensity profiles of enzyme solutions within the microfluidic separators. The images are from experiments with active and inactive catalases, labeled with AF 647 and AF 488 respectively. The images show smooth flow of liquids near the inlets and the outlets and absence of any undulation and mixing.

The fluorescence intensity profiles of the labeled enzymes were recorded within the outlets along a cutline, drawn across the outlets 330 μm down from the splits (Figure 3.1). Next, the flow of phosphate buffer was withdrawn from the channel and buffered solution with 10 mM H$_2$O$_2$ (substrate for catalase) was introduced at the same inlet, keeping the flow rate unaltered. After the flow had stabilized, the fluorescence intensity profiles of the enzymes were recorded along the same cutline. The measured intensity profiles were corrected for vignetting using nonlinear curve fits, adjusted for baselines and normalized before they were compared. In order to quantify the excess migration of catalase in the presence of H$_2$O$_2$, we follow the conventional definition of enrichment coefficient, the normalized concentration ratio of active
to inactive molecules in a particular outlet channel divided by the same ratio at the inlet (= 1 in our setup). For each enzyme, its concentration at the outlet is proportional to the geometrical area under its fluorescence intensity profile (as shown in Figure 4.2). Thus the fluorescence intensity profiles for each enzymes (after normalizing for differences in fluorophore intensities) within different outlets of the microchannel could be calculated.

**Figure 4.4: Separation of catalase from urease.** Normalized fluorescence intensity profiles of the enzymes in the presence of (A) phosphate buffer and (B) imposed H2O2 concentration gradient. The profiles were recorded within the outlets along a cutline, at a distance of approximately 330 μm away from the split. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. (C) Measured enrichment coefficients of catalase and urease within different outlets of the device. The mean and standard deviations are calculated for three sets of independent observations, each carried out with a newly fabricated device.
Figure 4.4 shows normalized intensity profiles for catalase and urease, measured along the specified cutline, in the presence of a flow of pure buffer and one with 10 mM H$_2$O$_2$, respectively. The magnified view of the intensity profiles measured from the middle of the third outlet to the end of the fourth (where the substrate concentration gradient is expected to be the maximum), are shown in the insets. In the absence of H$_2$O$_2$, the area under the intensity profile of catalase was found to be slightly more than that of urease toward the substrate side, starting from the middle of the third outlet and becoming the maximum within the fourth. This difference in migration can be attributed to the normal size-dependent Brownian diffusion of the molecules ($D_{\text{catalase}} = 6.01 \times 10^{-11}$ m$^2$/s, $D_{\text{urease}} = 3.18 \times 10^{-11}$ m$^2$/s). However, in the presence of a substrate concentration gradient, the intensity corresponding to catalase becomes significantly higher than that of urease. The increase in measured enrichment coefficients in absence and presence of H$_2$O$_2$ is shown in Figure 4.4(C).

**Urease and β-galactosidase**

Unlike catalase and urease which are somewhat different in size, a mixture of two enzymes with similar Stokes radii, urease and β-galactosidase ($R_{\text{urease}} = 7$ nm$^{32}$ and $R_{\beta-\text{galactosidase}} = 6.9$ nm$^{34}$) was selected and spontaneous separation of urease in the presence of a steady concentration gradient of urea was realised. After preparing 200 nM protein solutions and washing the microchannels thoroughly with BSA and phosphate buffer as before, the separation experiments were performed with a liquid flow rate of $\sim 15$ μL/h at the inlets. The fluorescence intensity profiles of the enzymes were recorded within the outlets, in the presence and absence of a flow of substrate, which in this case was a buffered solution of 1 M urea. Figure 4.5 show normalized fluorescence intensity profiles of urease and β-galactosidase in absence/presence of a urea gradient, and the measured enrichment coefficients calculated for different outlets of the microfluidic device. The magnified views of the separation profiles are shown in the insets of Figure 4.5 (A, B).
Figure 4.5: Separation of urease from β-galactosidase. Normalized fluorescence intensity profiles of the enzymes in the presence of (A) phosphate buffer, (B) imposed urea concentration gradient. The profiles were recorded within the outlets along a cutline, at a distance of approximately 330 μm away from the split. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. (C) Measured enrichment coefficients of urease and β-galactosidase within different outlets of the device. The mean and standard deviations are calculated for three sets of independent observations, each carried out with a newly fabricated device.

In the absence of urea, both urease and β-galactosidase moved almost identically, the latter migrating a little faster possibly because of its marginally higher Brownian diffusivity. In the presence of substrate, however, the population of urease increased in the direction toward the
substrate side, following the enhanced diffusion of the molecules in the presence of a urea concentration gradient. Figure 4.5(C) shows the relative increase in urease population within different outlet channels of the device, in the presence and absence of urea. The observations clearly establish that by the appropriate choice of substrate, specific enzyme molecules can be chemotactically separated out from a complex mixture, without influencing any of their physical or chemical characteristics.

4.3.4: Sensitivity of Separation using the same Enzyme
Extending the idea of working with molecules of same Stokes radii, we finally probed the sensitivity of the technique in separating out active molecules from their inactive forms, both having the same size and isoelectric point. As discussed earlier, one of the major challenges facing current molecular separation techniques is dealing with proteins with nearly identical physical properties. To demonstrate the unique advantage of chemotactic separation over others, a mixture of active and inactive catalase molecules was chosen and their separation in the presence of an imposed H$_2$O$_2$ gradient was investigated. For this study, molecules of catalase were labeled separately with AF 647 and AF 488 dyes. Enzymes labeled with AF 488 were then inhibited using 0.5 M NaCN solution prepared in deionized water, as described in section 3.2. Solutions of active and inactive catalase were prepared in phosphate buffer keeping their individual concentrations fixed at 200 nM. Newly fabricated microchannels were treated with BSA and washed with flows of phosphate buffer prior to the measurements. The liquid flow rate was maintained at $\sim$15 $\mu$L/h as before to ensure smooth laminar flow inside the main channel. Following the experimental protocol described previously, the intensity profiles of the enzymes, both in presence and absence of the substrate (solution of 10 mM H$_2$O$_2$) were recorded within the outlets along the cutline. Figure 4.6 shows the normalized fluorescence intensity profiles of the enzymes, both in the presence and absence of 10 mM H$_2$O$_2$. As seen in Figure 4.6 (B), spikes in fluorescence signal near the wall of the microchannel could result from accumulation of fluorophores near the wall following no-slip of liquids over them. Figure 4.6(C) shows the measured enrichment coefficients, for different outlets of the device – averaged over three sets of independent measurements. As can be noted, there is a substantial increase
in the population of active catalase within the substrate outlets, following the interaction of active catalase molecules with $\text{H}_2\text{O}_2$. As shown, there is a significant increase in the population of active catalase within the substrate outlets. As expected, the enrichment is observed to be mostly within the region between third and the fourth outlets, where the established substrate concentration gradient is the highest. Clearly, the chemotactic separation technique is sensitive enough to sort out molecules possessing identical physical properties, which cannot otherwise be accomplished using currently known separation techniques.

**Figure 4.6:** Separation of active and inactive catalase. Normalized fluorescence intensity profiles of the enzymes in the presence of (A) phosphate buffer, (B) imposed $\text{H}_2\text{O}_2$ concentration gradient. The profiles were recorded within the outlets along a cutline, at a distance of approximately 330 μm away from the split. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. (C) Relative enrichment
coefficients of the molecules measured within different outlets of the device. The mean and standard deviations are calculated for three sets of independent observations, each carried out with a newly fabricated device.

4.4: Conclusions
In conclusion, this chapter demonstrates a design of a novel microfluidic enzyme separator for inexpensive and stress-free sorting of enzymes from a mixture. This separation depends on the substrate concentration dependent diffusivity of active enzyme molecules and their chemotactic response towards imposed substrate gradients. A variety of enzymes can be separated as evident from the different pairs described in the chapter. Unlike other label free techniques, chemotactic separation does not depend on molecular size and surface charge, and can even be used for molecule with the nearly the same isoelectric point. This has been demonstrated in the chapter with a solution of active an inactive catalase, which to our knowledge, has not been reported previously. This technique can be extended further to separate active inorganic molecules from a mixture. Harnessing the chemotactic response of a particular catalyst toward its substrate, it will be possible to separate out single catalyst molecules from a mixture.

4.5: Acknowledgements
The author acknowledges the role of Dr. Krishna Kanti Dey in spearheading the project and guidance in experiments.

4.6: References
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4.7: Appendix
These multiphysics simulations were carried out by Dr. Krishna Kanti Dey

4.7.1: Simulations for separation experiments
In order to understand the observed chemotactic separation of enzymes, a simple model describing diffusive and convective transport of enzymes in imposed substrate concentration gradients was proposed. The observed enrichment of molecules within the microfluidic channels was simulated in COMSOL multiphysics (v. 4.3), coupling the physics of diffusion of molecular species and principles of laminar flow. The simulation geometry followed the microchannel network used in experiments. The computations were performed in shallow channel approximation, where the simulation contour was assigned a depth of 50 μm, equal to the depth of the microchannels used in experiments. After deciding the domain, one of the inlets of the main channel was assigned a substrate flow rate of 15 μL/h, the same flow rate...
used in experiments. A solution containing a mixture of enzymes was then considered to flow through the other inlet of the microchannel at the same rate. The concentration of each enzyme in the mixture was taken to be 200 nM. This was followed by setting the boundary conditions and initial parameters. The diffusion coefficients of inactive enzymes (enzymes not interacting with substrates) were dictated by their hydrodynamic radii and solution viscosity, and were considered constant in the simulation. The diffusion coefficients of active enzymes were, however, expressed as functions of local substrate concentrations, the functional forms of which were taken from previously reported results and used in simulations as input parameters. After calibrating the simulation mesh for fluid dynamics modeling, the steady-state concentration profiles of the enzymes were measured within the outlets, at a position 330 μm away from the split, along a cutline, drawn across the outlets. Simulations were done for different pairs of enzymes, in the presence and absence of corresponding substrates. Control experiments were simulated by assuming flow of phosphate buffer through the substrate inlet at the rate of ~15 μL/h, and assuming constant diffusivities of the molecules dictated by their hydrodynamic radii. Figure 4.7 summarizes the simulation results obtained for different pairs of enzymes under different experimental conditions.
Figure 4.7: *Simulated separation of enzymes in the presence of substrates.* Normalized concentration profiles of catalase and urease in the presence of (A) phosphate buffer, (B) imposed \( \text{H}_2\text{O}_2 \) concentration gradient; urease and \( \beta \)-galactosidase in the presence of (C) phosphate buffer, (D) imposed urea concentration gradient; active and inactive catalase in the presence of (E) phosphate buffer, (F) imposed \( \text{H}_2\text{O}_2 \) concentration gradient. The images in the insets show the magnified view of the fluorescence profiles near the third and fourth outlets of the device. The concentration profiles are estimated within the outlets, along a cutline 330 \( \mu \)m away from the outlet splits.

For a mixture of catalase and urease, the simulation results show enrichment in catalase population within the outlets, in the presence of the substrate \( \text{H}_2\text{O}_2 \). The simulated enrichment coefficients for catalase within the third and fourth outlets of the microfluidic device are 1.00 and 6.41 in the presence of 10 mM \( \text{H}_2\text{O}_2 \) (compared to 1.01 and 4.04 in phosphate buffer). Interestingly, the values indicate that the enrichment coefficient for catalase/urease system within the fourth outlet increases to 4 even in the absence of the substrate for catalase,
indicating that the molecules can be separated solely based on size. However, addition of the substrate for catalase increases this enrichment coefficient by 58% to 6.41, demonstrating the utility of enhanced separation by molecular chemotaxis. The corresponding experimentally measured values of enrichment coefficients for catalase/urease systems are $1.16 \pm 0.09$ and $2.48 \pm 0.35$ in $\text{H}_2\text{O}_2$ (compared to $1.20 \pm 0.14$ and $1.51 \pm 0.28$ in buffer). Possible factors contributing to the difference in simulated and experimental values are errors involved in the nonlinear fitting of experimental data for substrate dependent diffusivities of the molecules, uncertainties in integrated areas under the fluorescence and concentration profiles, baseline corrections and related processing of measured signals and nonideal behavior of the PDMS microchannels under given experimental conditions. However, for the other pairs of enzymes, the estimated separation coefficients agree well with the experimentally observed values. For example, for urease and β-galactosidase the predicted enrichment coefficients within the third and fourth outlets are 1.00 and 2.04 in the presence of urea (compared to 1.00 and 0.97 in buffer). Experimental enrichment coefficients, averaged over three independent sets of measurements were calculated to be $1.04 \pm 0.04$ and $2.06 \pm 0.07$ in the presence of urea, while those for the controls were $0.92 \pm 0.04$ and $0.96 \pm 0.03$, respectively. For active and inactive catalase, the estimated enrichment coefficients are 1.06 and 1.44 respectively compared to 1.00 and 1.00 in buffer. The experimental values matched well with the estimated ones—the measured values being $1.07 \pm 0.06$ and $1.36 \pm 0.02$ in the presence of 10 mM $\text{H}_2\text{O}_2$ (compared to $1.04 \pm 0.01$ and $1.02 \pm 0.03$ in buffer).

Although the multiphysics simulations performed do not take into consideration factors such as backflow of fluids within the outlets and possible mixing near the splits, the results successfully predict the experimental observations and provide a basic model to understand chemotactic separation of enzymes in the presence of imposed substrate concentration gradients. Although a relatively simple microchannel setup was employed, more efficient separation will require a complex tree-like architecture (resulting in higher theoretical plates). Increasing the concentrations of enzymes at the inlets would most likely result in higher number of molecules migrating within the substrate outlets, improving the efficiency of separation even further. With appropriate detection setups, the microchannel geometry may be modified further to sort
out specific molecules at desired locations. The present two-inlet, five-outlet geometry however establishes the principle of chemotactic separation of molecules and demonstrates its applicability in sorting out molecules with identical physical properties. This cannot be achieved with current label-free detection and separation procedures and will provide avenues for efficient chemical and biochemical sorting of active molecules.

4.7.2: Flow rate optimization
Prior to experimental measurements, the flow rate for 200 nM enzyme solutions was optimized based on Multiphysics simulations in COMSOL. Considering a mixture of active and inactive catalase, the excess migration of active catalase was estimated within the substrate outlets, in the presence of its substrate, 10 mM H₂O₂, for flow speeds ranging from 0.5 μm/s (∼0.0375 μL/h) to 3000 μm/s (∼225 μL/h). For very low liquid velocities (0.5 μm/s), the simulation showed no substrate concentration gradient along the interface of the flows; the population of both active and inactive molecules was found to be the same within each of the outlets, resulting in no separation. The separation was significant above a flow speed of 20 μm/s and increased continuously with the increase in flow rate at the inlets. However, for flow rates higher than 200 μm/s (∼15 μL/h), the concentration of enzymes crossing the substrate interface was found to be <10⁻⁴ nM for both active and inactive catalase, making the detection of excess migration of the active molecules with respect to the inactive ones challenging. The detection of chemotactic separation is therefore very sensitive to the flow rate of liquids through the inlets. With more concentrated enzyme solutions, it may be possible to work with an extended range of flow rates, but as mentioned previously, higher concentrations of tagged enzymes saturated the fluorescence detector in the present experimental setup. On the basis of the simulation results, for 200 nM enzyme solutions, we decided to work with flow speeds close to 15 μL/h. However, obtaining slow flow rates exhibiting smooth laminar profiles in channels that have dimensions on the order of micrometers is challenging. While a syringe pump can pump fluid slowly (down to tens of microliters per hour) the observed flow was not smooth. As such, we used a gravity-driven flow by placing the enzyme and substrate reservoirs shown in Figure 3.1 at a higher height than the outlets of the microfluidic device. In order to further slow the flow rate, we placed a second microfluidic device with smaller channel
dimensions (~100 μm) between the reservoirs and the separator to act as constrictions. This microfluidic cascade helped to attain smooth, gravity-driven laminar flow at the inlets at a rate of ~15 μL/h. This flow rate is orders of magnitude smaller than the flow rates typically used in microfluidic experiments.
Chapter 5
Convective Transport Using Enzyme Pumps

5.1: Introduction
The transport of micron-sized particles such as cells, viruses, spores and colloids to a targeted location is essential for a variety of applications. The targeted transport of cells and viruses, for example, has a variety of applications in biosensors and microfluidic systems. The advantages of miniaturizing these sensing systems include speed, sensitivity, and small volume analysis. Concurrently, many micro- and nanosensors have been developed in recent years: for example, microcantilevers that assay antigens, electrochemical sensors, and silicon nanowires to weigh biomolecules, among others. Whatever the sensing mechanism, microfluidic systems are needed to deliver the analyte to the target sensing surface. Unfortunately, performance of sensors at the nano- and microscale is fundamentally limited by insufficient analyte transport. Relying purely on diffusive transport, femtomolar concentrations of 20-base single-stranded DNA molecules require a time scale of minutes to encounter a typical microscale sensor. Detecting much larger, e.g. micron-scale, particles by diffusion would be orders of magnitude slower and, therefore, impractical. Recently, significant progress has been achieved in developing techniques based on different types of gradients to control transport of molecules and nanoparticles. However, with the size of particles increasing up to microns, performance of the techniques based on molecular gradients progressively declines, and alternative approaches should be used. Convection is a mechanism widely used to overcome this problem in sensing devices for transportation of microscale analytes. The directional transportation of the micron-sized analyte immersed in fluid toward to the detecting elements can significantly reduce delivery time and improve the performance of sensing devices.
In this chapter, it is demonstrated that simple chemical reactions can be used to induce unidirectional fluid flows carrying micron-sized particles towards a detecting surface. The study of self-powered nano- and micromotors that convert chemical energy into mechanical energy has become a rapidly growing field in literature. Similarly, a variety of microscale...
pumps have been developed recently\textsuperscript{17, 18, 19, 20, 21, 22, 23, 24} by anchoring these catalytic systems to surfaces, which generates flows in the surrounding fluids. However, a major drawback for these micropumps is the lack of directional pumping. In current micropumps, the convective fluid flow is symmetrical in all directions, thus rendering it ineffective for directed transport of analytes.

One can overcome this problem by introducing a substrate gradient in the self-powered pump systems or by displacing the pump from the center of the chamber to break the symmetry and direct the fluid in a desired manner. Consequently, a battery-like system of these pumps has been designed to increase the efficiency of analyte (mass) transport, thus overcoming the limitations presented in many microscale sensors. The directional pumping approach provides a number of important advantages for sensing applications. The intensity of fluid flows and the time of cargo delivery are controlled by the amount of reagent injected into the system, and thus can be tuned manually. After all the reagent has been consumed, the fluid pump stops and particles sediment on the bottom. The fluid motion can be reignited by injecting another portion of reagent. This process can be repeated until the enzyme is washed away. The approach also provides flexibility with respect to device geometry; the particles do not need to be injected in the immediate vicinity of sensing elements. Moreover, sensors can be deployed in remote locations, remaining dormant until triggered by specific analytes.

Sengupta\textsuperscript{27} et al. have demonstrated that enzyme molecules behave as nanomotors in the presence of their substrate and show concentration-dependent enhanced diffusion\textsuperscript{25, 26}. They have also demonstrated that these molecules, when immobilized on a surface, behave as micropumps and pump fluid and particles autonomously, also with speeds dependent on the concentration of the substrate\textsuperscript{27}. Similarly, immobilized metallic catalysts have also been used to generate fluid flows in autonomous systems\textsuperscript{28}. Using hydrogen peroxide and urea as respective substrates, an experimental system based on platinum and urease as catalytic micropumps is designed, which enables transport of micron-sized particles over a distance on the order of a centimeter. Unlike the diffusive transport of such particles, which requires about $10^7$ s, the convective transport takes only about $10^3$ s (20 minutes). At the end, there is a
discussion on different ways to control the convective transport in millimeter and microscale devices.

5.2: Design of the Experiment
Here we demonstrate two examples of directed transport of microparticles using platinum- and urease-based micropumps. The inorganic catalytic system based on Pt and the biocompatible catalytic system based on urease showcase the broad applicability and robustness of the system. Since the results from the numerical simulations show a dependence of transport efficiency on reaction rate of the catalytic system, we developed a battery-like system of pumps by patterning an array of catalytic regions as a simple means of changing the reaction rate by varying the width of and distance between each thin catalytic pump. Specifically, the actual enzyme concentration on the surface cannot be determined by assay until after the enzyme is attached, and the actual substrate concentration diffusing from the gel in order to create a concentration gradient may differ from the initial soaking concentration. All three methods of changing the reaction rate are described in the paper for completeness, however.

Note also, that spatial period 60 µm with which pumps are assembled into the battery is small enough to break fluid flow along the surface into a system of separate vortexes, and, therefore, is equivalent to the situation with homogeneous bottom surface coverage considered in our simulations.

We begin with a discussion of the design of the experiment in general. Individual pumps, initially 20 µm in width and 2 cm in length with 40 µm spacing between each other, were patterned in a square array on a glass surface (See Fig. 5.1). A cylindrical spacer (20 mm in diameter, 1.3 mm in height) was placed on top of the pattern, and a solution of tracer particles was used to monitor the fluid flow. The system was sealed and observed with a fluorescence microscope. A schematic of the experimental setup is shown in Fig. 5.2 below.
Figure 5.1: Schematic of the experimental setup and observations. Step 1 shows the size and spacing of the catalytic micropump strips and the substrate-soaked gel on a glass slide. For the enzyme-based systems, we follow Steps 1-4. For the metallic catalytic systems, such as platinum, we go right from Step 1 to Step 4. Steps 2 and 3 show the immobilization of enzyme on the gold pattern using a biotin-streptavidin linkage. As shown, the system can use any number of enzymes, though we use urease only. Fluid pumping (shown by the red arrows in Step 4) is observed by tracking the motion of tracer particles within a sealed spacer due to the reaction of the catalytic micropump (Pt or gold-bound urease) and its substrate (H$_2$O$_2$ or urea).

5.2.1: Fabrication of micropump array patterns

The square arrays, consisting of about 200 thin strips of metal spaced evenly, were fabricated over glass slides in the Nanofabrication Laboratory of Materials Research Institute at the Pennsylvania State University using standard lithography and deposition techniques. Glass slides are first cleaned with acetone and air-dried, then spin-coated with 5 mL of SPR-955 photoresist (Microposit) at 500 rpm for 10 sec and then at 3500 rpm for 60 sec. This was followed by soft-baking the coated slides over a hot plate at 95 °C, for 1 min. The array geometry was modeled in CAD and printed over a chrome-on-glass mask (Nanofabrication Laboratory, Materials Research Institute, Penn State). For photolithography, the mask was placed in direct contact with the photoresist over the glass slide. The resist was then exposed to UV radiation for 12 sec in a Karl Suss MA/BA6 Contact Aligner. The exposed wafers were post-
baked for 1 min over a hot plate at 100 °C to cross link the exposed film. MF CD26 developer was used to remove unexposed SPR 955 from the slide. The mold was developed for 2 min while being agitated, followed by washing it thoroughly with deionized water. After the glass slides were dried with a nitrogen blower, a 5 nm thick layer of chromium and either a 50 nm layer of gold or a 10 nm layer of platinum was deposited on them using electron-beam evaporation using the Semicore Evaporator. Once the gold or platinum had been deposited, the glass slide was sonicated in a solution of dimethylsulfoxide (DMSO) to remove the resist layer with gold or platinum on top. This left only the gold or platinum deposited on the glass slides in the desired pattern, shown in Figure 5.1. For enzyme-based micropump arrays, enzyme is attached to the gold stripes, as fabricated above, using a biotin-streptavidin interaction.

5.2.2: Sample Preparation
A 1% agar gel (Alfa Aesar) is prepared and then soaked overnight with substrate. For the platinum micropump arrays, the substrate is hydrogen peroxide (CCI), and this system is analogous to using the enzyme catalase. For the enzyme systems on the gold patterns, the substrate is urea (Sigma Aldrich). The substrate-soaked gel was then placed perpendicularly to the micropump array pattern on the glass slide, at a distance of ~1 mm. The whole system (gel and micropump array pattern) was covered with a secure-seal hybridization chamber (Electron Microscopy Sciences) with dimensions of 20 mm diameter and 1.3 mm height in order to establish a closed system. Care was taken to not damage the gel. To monitor the fluid flow, sulfate-functionalized polystyrene microspheres (Polysciences Inc.), 4 µm in size, were introduced as tracers suspended in deionized water or phosphate buffered solution (PBS). The tracers were fluorescent with excitation of 580 nm and emission of 605 nm. To measure fluid-pumping velocity in each experiment, 30 tracer particles were tracked using the Tracker software (Cabrillo College).
Once the system is closed by the hybridization chamber seal and the tracer solution is introduced, the density difference between the gel’s substrate and the tracer solution causes substrate to begin diffusing from the gel toward the catalytic micropump array. Observation of the system begins after about five minutes of equilibration. An optical and fluorescence
microscope is used to view the system, using a 10x objective. Videos are recorded for a minute at ~8 fps, using an NIS-Elements camera, at different distances from the gel along the micropump pattern in order to determine the relationship between distance and pumping speed (tracer velocities). Videos are also recorded at different times over the course of ~1 hour at these various distances to measure the relationship between time and pumping speed. Finally, after about an hour has passed, tracers begin to sediment to the surface of the glass slide, and a video is recorded to show the particle density distribution at the surface. An increase in particle density near the end of the pattern, with respect to the gel location, is indicative of effective directed transport of particles to a desired location.
Figure 5.2: Schematic of the experimental setup. The yellow lines depict the catalytic strips 40 μm wide with a distance of 20 μm between them. The blue square represents the gel soaked with the substrate.

5.2.3: Enzyme Immobilization

Enzymes were immobilized on the gold strips by using biotin-streptavidin linkage. An enzyme solution (2 mg/mL) was prepared with 100 mM phosphate buffered saline (PBS) as the buffer. Before completing the total volume of the enzyme solution, a certain volume of a 0.1 mM biotin solution was added to get a ratio of 4:1 enzymes:biotin. For urease, the biotin linker used was 3-(N-maleimidylpropionyl)biocytin (Santa Cruz Biotechnology) for targeting the cysteine residues in the enzyme. The mixture was reacted for 2 hrs at room temperature. Dialysis of the final solution was carried out at 4500 rpm for 5 mins, followed by washes with 10mM PBS. The solution obtained at the end was diluted and stored at 4°C until needed. Biotinylation of the Au patterns was achieved by following a procedure reported previously for the biotinylation of Au nanoparticles, through the formation of a self-assembled monolayer (SAM) using a biotin-thiol linker. For the preparation of the linker, a sulfhydryl-reactive biotinylating agent, Biotin HPDP (Apexbio), was reacted with a phosphine compound to form a thiol end group. In specific, Biotin HPDP (1 mg per 2-3 patterns) was dissolved in dimethylformamide (0.13 mg/mL) through sonication for 3 minutes at 45°C, and tri-butylphosphine solution (Sigma) (5 μL per mg of biotin) was added. The reaction mixture was allowed to react for 30 mins at 45°C, after which it was dissolved in a solvent mixture of 1:1 H2O: Ethanol (8 mL of solution per mg of biotin). This was the soaking solution for the Au patterns. The patterns were then incubated overnight at room temperature. After incubation, the patterns were washed several times with deionized water, followed by 10 mM PBS buffer. The SAM-modified surfaces were then incubated in a streptavidin solution (9μM in 10mM PBS) for 3 hrs, after which the surfaces were washed with 10mM PBS buffer. Incubation of Streptavidin-containing surfaces with enzyme-biotin solution was performed for 3-4 hrs prior to the experiments. The enzyme-functionalized surfaces were thoroughly washed with 10mM PBS to remove any unbound enzyme molecules from the surface.
For the enzyme-based micropumps, urease was immobilized on gold patterns, with the same initial dimensions described above. 4 µm sulfate-functionalized fluorescent polystyrene (s-PsL) particles were used to visualize the fluid flow and quantify particle transport. Symmetry in the system was broken by placing a substrate-soaked agar gel near the micropump pattern (Figure 5.2). Substrate diffuses into the surrounding fluid from the gel, resulting in a concentration gradient across the micropump pattern.

5.3: Convective Pumping observed
In the presence of 500 mM substrate (urea), a substrate gradient was set up perpendicular to the array and a unidirectional fluid flow was produced, wherein the tracer particles were pumped away from the gel near the surface of the slide, with a return flow further above the surface to maintain fluid continuity (see video 5.1). The tracer particle speed shows substrate concentration-dependence decreasing from 6.48 ± 0.42 µm/s at 500 mM urea to 2.8 ± 1.13 µm/s at 250 mM urea. This tracer particle speed was measured at the beginning of the pattern, at a distance of 1 mm from the substrate source. In the absence of substrate, minimal fluid flow was observed, with tracer particle speeds in the range of 1.13 ± 0.5 µm/s due to diffusive flows from the gel. These speeds were observed 50 µm above the pattern surface. When viewed from further above the pattern, flow in the opposite direction was observed, preserving fluid continuity.
Figure 5.3: (Left) A representation of the experimental setup including its length (Right) A still from the videos showing the tracer particles accumulated at the surface

For our metallic system, platinum catalyzes the decomposition of hydrogen peroxide ($\text{H}_2\text{O}_2$) into water and oxygen. A gel soaked in 1% $\text{H}_2\text{O}_2$ was used to set up a substrate gradient on one end of the platinum array. Similar behavior to the urease-based system was observed, with a unidirectional fluid flow of the tracer particles being pumped away from the gel. As with urease, the pumping velocity decreases with decreasing substrate concentration, from 9.58 ± 1.08 µm/s at 1% $\text{H}_2\text{O}_2$ to 3.37 ± 0.51 µm/s at 0.5% $\text{H}_2\text{O}_2$. The particle speed was again measured at a distance of 1 mm from the source of hydrogen peroxide. Fluid continuity was also observed in this system.

5.4: Spatial variations in pumping
The fluid pumping speed was investigated across the pattern. An interesting behavior arises when examining the speed of the tracer particles at set distances away from the gel. The tracer particles have a Stokes settling velocity in the fluid, i.e. as we move away from the gel, the tracer particles slow down. Consequently, at a certain distance, the fluid flow stops and tracer particles show Brownian diffusion only. The spatial profile of the tracer velocity for the two different systems is demonstrated in Figure 5.4. The observed distance at which the fluid flow stops and Brownian diffusion dominates depends on the conditions of the system. As shown by the numerical simulations in Appendix, this distance depends on the reaction rate and resulting reagent depletion out of the fluid flow. In regions where the reagent concentration is small, the fluid density variations that generate convective flows are small as well and fluid velocity goes to zero. Simulated reagent concentration with generated fluid flow is shown in Appendix. The figure 5.9 demonstrates correlation between the depletion of the reagent concentration and decreasing horizontal fluid velocity. Systems with lower reaction rates consume reagent slower so that induced convective flows reach further away from the gel. This situation is demonstrated in Fig. 5.10 which shows three representative reaction rates and extent of the corresponding horizontal velocity fields. On the other hand, larger amounts of reagent require
longer times for chemical decomposition what also can produce long-reaching convective flows. These considerations suggest that observed in Figure 5.4 larger extent of the velocity field for urea/urease system is a result of larger amount of the introduced reagent or smaller reaction rate ($k_{cat} = 2.3 \cdot 10^4 \text{ s}^{-1}$) than in case of hydrogen peroxide/platinum system (note that reaction rates for hydrogen peroxide/catalase system is $k_{cat} = 2.12 \cdot 10^5 \text{ s}^{-1}$). For pump arrays with 10 nm Pt deposited on the surface and a substrate gradient generated by 1% $\text{H}_2\text{O}_2$, the distance is 4 mm away from the gel. For an enzyme coverage of $3 \times 10^{-6} \text{ mM}$ urease and 500 mM urea concentration, the distance is 10 mm away from the gel. This behavior is likely a result of a combination of the differences in reaction rates between platinum and urease catalysis and differences in densities and diffusion rates of the chemical species in each system.

![Figure 5.4: Spatial profile of the pumping speed of 4 µm s-PsL particles for (a) platinum and (right) urease systems.](image)

**5.5: Convection-assisted focusing of analytes**

The decrease in tracer speeds across the pattern and subsequent tracer sedimentation could be exploited for focusing of analytes. There exists a uniform distribution of tracers at the start of the experiment. However, at the end of an hour, all the tracers have settled onto the pattern. Moreover, the fluid flow decreases as we move away from the gel. Thus, the tracer particles show a marked increase in concentration as distance from the gel increases at the end of an
hour. Thus s-PsL tracers which serve as analogues for micron-sized analytes, could be focused at desired locations on the surface of the glass slide at the end of an hour (see video 5.2). The concentration of tracer particles was calculated using image analysis software. As seen in Figure 5.5 and 5.6, for urease systems at a distance of 10 mm away from the gel, a 10-fold increase in the concentration of tracer particles was observed. Similarly, for the platinum system, at a distance of 4 mm away from the gel, a 4-fold increase was observed.

The distance at which the analytes are concentrated depends on the reaction rate and conditions of the system as described in Appendix. In general, decreasing reaction rate without further changes to the type of system leads to a decrease in the distance between the substrate (fuel) source and point of the concentration maximum, as well as a decrease in the maximum itself. As seen from Figures 5.5 and 5.6, the maximum of the density distribution graph shifts and changes in height as the reaction rate is changed. The reaction rate was controlled using two different parameters depending on the system. For the inorganic platinum-based array, the thickness of the catalyst deposited on the strip was constant, while the substrate concentration was changed in order to change the reaction rate. Enzyme-based micropump array had the same substrate concentration, while the concentration of urease was changed, changing the reaction rate. The first way of controlling the reaction rate by the amount of injected reagent was numerically explored in Appendix. In particular, Figure 5.11 demonstrates how increase in the amount of reagent shifts maximums of the tracer density distribution further away from the point of entrance. Similar shift further away from the gel is observed in Fig. 8 under increase of the hydrogen peroxide concentration from 1 to 2%.
Figure 5.5: Density distribution of 4 µm s-PsL particles for 10 nm platinum at (red) 2% H$_2$O$_2$ and (blue) 1% H$_2$O$_2$. The number of particles settled on the surface are determined by image analysis software for ~0.5 mm$^2$ areas along the center of the pattern, increasing in distance from the substrate. As seen from the graph a 3.5-fold concentration increase for the tracer particles at a distance of ~8 mm from the substrate is observed for 2% H$_2$O$_2$. In contrast a 7-fold increase in concentration is seen for 1% H$_2$O$_2$ and the location of the maximum has changed to ~4 mm. The observed shift in maximum of the tracer density distribution qualitatively agrees with simulated shifts presented in Figure 5.11 under increasing amount of reagent.
Figure 5.6: Density distribution of 4 µm s-PsL particles for (green) $3 \times 10^{-7}$ M urease and (purple) $3 \times 10^{-6}$ M urease with 500 mM urea. The number of particles settled on the surface are determined by image analysis software for ~0.5 mm$^2$ areas along the center of the pattern, increasing in distance from the substrate. For $3 \times 10^{-7}$ M urease concentration a 5-fold increase in tracer concentration was observed at a distance of ~5 mm away from the substrate. (b) The area occupied by the particles settled on the array was calculated, which is proportional to the number of particles on the array. Increasing the enzyme concentration to $3 \times 10^{-6}$ M changed the maximum to ~10 mm away from the substrate, resulting in a 10-fold number concentration increase.

5.6: Conclusion
This chapter has demonstrated the suitability of catalytic micropumps in sensing and lab-on-a-chip devices. One of the problems of fluid flow at low Reynolds number is the generation of directional fluid flow without the use of external fields. Inorganic (metal-based) and biological (enzyme-based) micropumps can both be used to generate long distance directional convective fluid flow as demonstrated above. This convective fluid flow could also be exploited for focusing and concentration of passive analytes giving rise to numerous applications in biosensors.
5.7: References


10. X, Y, Z. No Title. Placeholder 1


5.8: Appendix

These simulations were performed by Dr. Oleg Shkhlyaev and Dr. Henry Shum

5.8.1: Principles governing the convective transport of microscopic particles

The mechanism of transducing chemical energy into fluid motion demonstrated by Sengupta et al.\textsuperscript{29} can be utilized for on-demand transportation of micron-sized cargo suspended in the fluid. This mechanism is based on the chemical decomposition of reagent by surface-bound enzymes into products that generate local density variations, which in turn give rise to fluid flows. The density variation of an aqueous solution, comprising solvent of density $\rho_0$ with $N^c$ chemical solutes characterized by corresponding concentrations $C^j$, can be approximated as

$$\rho = \rho_0 \left(1 + \sum_{j=1}^{N^c} \beta_C^j C^j + \beta_T(T - T_0) \right) \quad \text{(1)}$$

This expression also accounts for possible variations due to temperature change $T - T_0$ (measured with respect to reference value $T_0$) resulting from exothermic chemical reactions. The expansion coefficients $\beta_C^j$ and $\beta_T$ characterize the magnitude of density variations in response to changes in solute concentrations $C^j$ and temperature $T - T_0$.

To demonstrate the physical mechanisms of convective transport, we choose a particular realizable system. In particular, we consider convection in the long, narrow channel shown in Fig. 1 and, as a representative example of a catalytic chemical reaction, we choose the
well-studied decomposition of an aqueous solution of hydrogen peroxide by catalase to form oxygen and water: \( 2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \). At small hydrogen peroxide concentrations, \( C_{H_2O_2} \approx 0.1 \text{ mol/L} \), the chemical reaction is accompanied by production of small concentrations of oxygen, \( C_{O_2} \approx 0.1 \text{ mol/L} \), and a temperature increase of \( T - T_0 \approx 0.1 - 1 \text{ K} \). These values, combined with hydrogen peroxide \( \beta_{C_{H_2O_2}} \approx 0.011 \text{ L/mol} \) and oxygen \( \beta_{C_{O_2}} \approx 0.0014 \text{ L/mol} \) solutal expansion coefficients, and the thermal expansion coefficient for water \( \beta_T \approx 10^{-4} \text{ K}^{-1} \), reveal that the solution density variation due to hydrogen peroxide concentration, \( \beta_{C_{H_2O_2}} C_{H_2O_2} \approx 10^{-3} \), is approximately an order of magnitude larger than the contribution due to the oxygen concentration, \( \beta_{C_{O_2}} C_{O_2} \approx 10^{-4} \), and thermal effect \( \beta_T (T - T_0) \approx 10^{-4} \). We, therefore, simplify the density expression in eq. (1) to \( \rho = \rho_0 (1 + \beta_{C_{H_2O_2}} C_{H_2O_2}) \).

Cargo immersed in the fluid is modeled as polystyrene-sulfate spherical tracers of radius \( R = 2 \mu m \) and density \( \rho_t = 1.05 \text{ g/cm}^3 \). Assuming the regime of slow fluid velocities, in which particles do not interact with either the fluid flow or each other, the motion of each tracer is determined by the superposition of advection with the local fluid flow \( \mathbf{u} \), the sedimentation velocity \( V = \frac{2(\rho_t - \rho_0)}{9 \eta} R^2 g \), and Brownian motion, characterized by the tracer diffusion coefficient \( D_t = \frac{k_BT}{6\pi\eta R} = 1.3 \cdot 10^{-13} \text{ m}^2/\text{s} \). Here \( g \) is the gravity, \( \eta \) is the dynamic viscosity of the host fluid, \( k_B \) is Boltzmann constant, and \( T \) is the absolute temperature. Tracers are prevented from escaping the computational domain by including repulsive interactions with the walls as discussed below. We ignore that friction between the tracers and the wall that potentially slows down the dynamics.
Figure 5.7: Geometry of the simulation domain. Reagent (released out of gel) enters the domain through the left wall at x=0. Enzyme, coating region A on the bottom, decomposes reagent into lighter products. The resulting density variation across the domain generates convective flows, transport tracers.

For simplicity, we perform simulations in a two-dimensional rectangular domain \{(x, y) : 0 \leq x \leq L, 0 \leq y \leq H\}, which has length \(L\) and height \(H\). The dimensions of the domain (see Fig.1) match the experimental setup described further below. The domain is filled with water of density \(\rho_0 = 10^3 \text{kg/m}^3\) and kinematic viscosity \(\nu = 10^{-6} \text{m}^2/\text{s}\). Hydrogen peroxide, characterized by diffusivity \(D = 10^{-9} \text{m}^2/\text{s}\), diffuses into the domain through the left wall. The fluid motion is described by velocity \(u = (u_x, u_y)\) and pressure \(p\), the reagent concentration field is given as \(C\), and position of \(N\) tracers is specified by vectors \(r_i(t) = (x_i(t), y_i(t))\). Below, the respective governing equations are: the continuity, Navier-Stokes (in the Boussinesq approximation), reagent diffusion, and Langevin equation in the overdamped limit:

\[
\nabla \cdot u = 0 \tag{2}
\]

\[
\frac{\partial u}{\partial t} + (u \cdot \nabla)u = -\frac{1}{\rho_0} \nabla p + \nu \nabla^2 u - e g \beta C \tag{3}
\]

\[
\frac{\partial C}{\partial t} + (u \cdot \nabla)C = D \nabla^2 C \tag{4}
\]

\[
\frac{\partial r_i}{\partial t} = u + e V + \mu \sum_{i=1}^{N} F(r_i - r_i^{\text{eq}}) + \sqrt{2D_i \xi_i(t)}, \quad 1 \leq i \leq N, \tag{5}
\]
where $\nabla$ is the spatial gradient operator and $e = (0,1)$ is a vector specifying the direction of the gravitational force. Stochastic fluctuations $\xi_i = (\xi^x_i, \xi^y_i)$ are assumed to be Gaussian white noise,

$$\left\langle \xi_i^x(t) \right\rangle = 0, \quad \left\langle \xi_i^x(t_1) \xi_i^y(t_2) \right\rangle = \delta_{t_1} \delta_{t_2} \delta(t_1 - t_2)$$

with $1 \leq i, j \leq N$, $\alpha, \beta = x, y$. In eq. (2), $\mu = (6\pi \eta R)^{-1}$ is the capsule mobility, $r_i - r^w_i$ specifies the vector from the tracer to the closest point on the wall, and $F(r) = -\frac{\partial U(r)}{\partial r}$ is the repulsive force between tracers and walls, modeled via Morse potentials

$$U(r) = \varepsilon \left(1 - \exp[-\omega(r - R)]\right)^2 \quad \text{for } r < R.$$ 

Parameters $\varepsilon$ and $\omega$ characterize the potential strength and width, respectively.

Decomposition of an aqueous solution of hydrogen peroxide by catalase, into oxygen and water occurs over a region $A$ of the bottom surface, $y = 0$, and is modeled via the Michaelis-Menten relation with the reaction rate

$$r = \frac{r_{\text{max}} C}{K_M + C}.$$  

(6)

Here, the maximal reaction rate $r_{\text{max}} = M k_{\text{cat}} [E]$ incorporates the number $M = 4$ of active sites per enzyme molecule, the reaction rate per catalase molecule $k_{\text{cat}} = 2.12 \cdot 10^5 \text{ s}^{-1}$, and areal enzyme concentration $[E] = 2 \cdot 10^{-8} \text{ mol m}^{-2}$ (with assumed radius of catalase molecule to be $R_{\text{cat}} = 5.1 \cdot 10^{-9} \text{ m}$) in moles per unit area. $K_M = 0.093$ is the substrate concentration at which the reaction rate is half of $r_{\text{max}}$.

Using eq. (3) and the diffusion flux $J = -D \nabla C$, the reagent decomposition in region $A$ can be described by

$$-D \frac{\partial C}{\partial y} = \frac{r_{\text{max}} C}{(K_M + C)}.$$ 

To model the experimental situation where a finite amount of reagent is introduced into the microchannel (by releasing substrate from agar gel previously soaked with reagent as described in the experimental section), we specify that reagent concentration decays with time as $C(x = 0, t) = C_0 \exp(-\lambda t)$ along the left wall $x = 0$. To match experimental observations, the characteristic time of fuel depletion from the gel was set $\lambda^{-1} = 70 \text{ min}$ and the initial gel concentration was chosen $C_0 = 0.1 \text{ M}$. Other walls are
assumed to be impermeable to substrate. The requirement of zero velocity at the walls, combined with the conditions on the concentration field, yields the full set of boundary conditions:

\[ \begin{align*}
  x = 0 & : \ u = 0, \quad C = C_0 \exp(-\lambda t), \\
  x = L & : \ u = \frac{\partial C}{\partial x} = 0;
\end{align*} \]

\[ y = 0 : \ u = 0, \quad \frac{\partial C}{\partial y} = \begin{cases} 0 & x \in B, \\
  \frac{r_{\text{max}} C}{D(K_M + C)} & x \in A,
\end{cases} \]

\[ y = H : \ u = \frac{\partial C}{\partial y} = 0, \quad (7) \]

where \( A \) is the enzyme-covered region of the bottom surface and \( B \) is the uncovered part.

Equations (2)-(5), along with the corresponding boundary conditions, eq. (7), were solved as follows. First, a lattice Boltzmann algorithm\(^{29}\) was applied to simulate the continuity (2) and Navier-Stokes (3) eqs. Then a second order finite difference scheme was used to solve the diffusion terms in eq. (4). At each time step, the computed flow field \( u \) is used for the chemical advection term in eq. (4). The updated concentration field is then used for the buoyancy forces in eq. (3) to advance the lattice Boltzmann scheme to the next time step. Finally, for known \( u \), a first order time integration was applied to solve the equation for tracer motion, eq. (5). Initially, \( N = 500 \) tracers are randomly distributed throughout the domain and the initial reagent concentration is set to \( C(t = 0) = 0 \).
Figure 5.8: Sequential stages of convective transport. (a) Initially, tracers are uniformly dispersed throughout the domain and the hydrogen peroxide concentration (shown by the color bar) is zero. (b) Reagent, diffusing through the left wall, produces a fluid flow that transports tracers. Enzyme at the bottom wall decomposes the reagent, creating a concentration (density) gradient. (c) After reagent is consumed, the flow stops with areal concentration of tracers $n/n_0$ increasing toward the right wall as shown by the red line. Black line shows averaged value of $n/n_0$. Simulations were performed for $C_0 = 0.1M$ and $k_{cat} = 2.12 \cdot 10^2 \text{ s}^{-1}$.

5.8.2: Physical mechanism of convective particle transport

We first consider a situation when the reaction rate is relatively small, $k_{cat} = 2.12 \cdot 10^2 \text{ s}^{-1}$. The initial homogeneous distribution of spherical polystyrene tracers in the domain with zero substrate concentration is shown in Fig. 2a. Hydrogen peroxide entering the domain increases the solution density relative to pure solvent and thus, generates downward convective flow, which spreads out horizontally along the bottom surface. The decomposition of substrate along the bottom plane results in the reagent concentration decreasing from the left to the right wall.
as shown by the color map in Fig. 5.8b. The corresponding density variations generate the fluid flow indicated by the arrows in Fig. 5.8b; this flow carries particles toward the right wall along the bottom plane. At the right boundary, the less dense fluid rises up and, by continuity, returns along the top plane back to the left boundary. The positions of propagating tracers are shown with gray markers in Fig. 5.8 for three successive time instances.

The tracer sedimentation velocity $V = 0.44 \, \mu m/s$, in the absence of convective flow, implies that in the domain with $H = 1.3$ mm all tracers should reach the bottom within 50 min. This value roughly sets the time during which tracers are carried by the flow. Sedimentation ensures that fluid flowing to the right in the lower half of the microchannel contains more tracers than returning flow carrying tracers to the left in the upper half. This asymmetry creates a net transport of tracers in the direction away from the entrance point of the reagent. Since we neglect friction between particles and domain walls, tracers that have already reached the bottom continue to move with the local fluid velocity $u(x, R, t)$.

Figure 5.9: Formation of maximum in the tracer distribution. (a) Areal tracer concentration $n/n_0$ resulting from the fluid flow shown on the middle and bottom panels. (b) Maximal horizontal velocity goes to zero (blue arrow) around the point where $n/n_0$ is maximal (blue dot). (c) Convective vortex dragging tracers along the bottom and aggregating them into the pile shown on the top panel. Concentration of reagent, consumed by enzyme along the bottom
surface, is indicated by the color bar. Simulations were performed for \( C_0 = 0.1M \) and \( k_{cat} = 2.12 \cdot 10^5 \text{ s}^{-1} \).

The efficiency of the convective transportation can be characterized by a local areal concentration \( n = m / (\Delta x \Delta z) \), defined as a number of tracers, \( m \), in a region of area \( \Delta x \Delta z \) in the bottom plane. Here, \( \Delta z \) represents a small thickness of the computational domain (in the direction perpendicular to \( x \) and \( y \)) where the system can be considered to be uniform in the \( z \) direction. (Recall that the simulations are performed in 2D, implying the system is uniform in \( z \).) Relative to the initial uniform distribution \( n_0 = N / (L \Delta z) \), the areal concentration

\[
\frac{n}{n_0} = \frac{m / dx}{N / L}
\]

of tracers is plotted with the red lines in Fig. 5.8. (Note that \( n / n_0 \) depends on the size of the bin \( \Delta x \) and is limited from above by values \( n / n_0 = L / \Delta x \), describing the situation when all tracers are found in one bin of length \( \Delta x \).) With time, the tracer distribution \( n / n_0 \) increases toward the right wall. After the reagent has been consumed, the fluid flow stops, and yields the tracer distribution shown in Fig. 5.8.

### 5.8.3: Parameters controlling transport properties

When the reaction rate is relatively large (\( k_{cat} = 2.12 \cdot 10^5 \text{ s}^{-1} \)), the areal concentration of tracers \( n / n_0 \) demonstrates a pronounced peak (blue dot in Fig. 5.9a) that results from the structure of the convective vortex, which is marked in yellow in Fig. 5.9c. The fast depletion of reagent in the solution above the bottom surface limits the spatial extent of the density variations and the resulting convective flows. Thus, the suspended tracers are transported by the flow to the edge of the convective vortex, where the horizontal component of the velocity reaches zero (blue dot in Fig. 5.9b). Here the tracers have aggregated into a pile. The correlation between the peak in \( n / n_0 \) (blue dot in Fig. 5.9) and zero maximal horizontal velocity (blue dot in Fig. 5.9) is emphasized by the blue arrow.

When reaction rate is decreased, the depletion of the reagent in the solution above the bottom surface become slower; this allows the substrate concentration and the generated flow to reach further along the channel. The more elongated convective vortexes, with horizontal
velocity shown in Fig. 5.11, form piles of tracers further from the left wall. Figure 5.10a depicts the shift of the maximum (bold dots) of the areal concentration $n/n_0$ for a decreasing sequence of three representative reaction rates: $k_{cat} = 2.12 \cdot 10^5$, $2.12 \cdot 10^3$, and $2.12 \cdot 10^2$ s$^{-1}$. To indicate the influence of the convective vortex on the spatial distribution of the tracers, we draw arrows between the position of maxima in $n/n_0$ and corresponding zeros in the horizontal velocity field (which mark the edge of this vortex). The high reaction rates provide high velocity flow in a restricted area next to the left wall, while the fluid in the right part of the domain mostly remains motionless. The low reaction rates provide slower velocities, but enable fluid to flow throughout the entire domain and transport cargo from the left to the right wall.

Keeping the reaction rate fixed, the position of maximum of the areal tracer concentration $n/n_0$ can also be shifted by changing the amount of the fuel injected in the microchannel. Controlling the amount of fuel by fixing the reagent concentration at the left boundary, we plot in Fig. 5.10 the areal tracer concentrations $n/n_0$ corresponding to values $C_0 = 0.05$, $C_0 = 0.1$, and $C_0 = 0.2$ M for the reaction rates $k_{cat} = 2.12 \cdot 10^5$, $2.12 \cdot 10^4$, and $2.12 \cdot 10^3$ s$^{-1}$ shown in Fig. 5.11a, b, and c, respectively. Consumption of larger amounts of reagent takes longer and, therefore, the fluid flow reaches further along the channel, shifting the maximum of $n/n_0$ away from the fuel entrance location at $x = 0$. 


Figure 5.10. *Reaction rate controls position of maximum of tracer distribution.* (a) Tracer areal concentration $n/n_0$ as a function of the position along the channel. Smaller $k_{cat}$ shift maximum values of $n/n_0$ (bold dots) to the right. (b) The low reaction rates provide slower velocities, but enable fluid to flow throughout the entire domain (yellow line) and transport cargo from the left to the right wall. Arrows emphasize the correlation between the maximum values of $n/n_0$ (bold dots) and zero horizontal velocities (bold dots). The latter dots indicate the edge of the convective vortex that drives the aggregation of the tracers into a pile.
Figure 5.11. *Amount of reagent controls position of maximum of tracer distribution.*

Increasing amount of reagent (with values $C_0 = 0.05, 0.1, 0.2M$), requires longer time for decomposition during which the fluid flow reaches further along the channel and aggregates tracers into piles (characterized by maximum of $n/n_0$) further away from the fuel entrance location at $x = 0$. Shift of $n/n_0$-maximum caused by increasing values $C_0 = 0.05, 0.1, 0.2M$ is demonstrated for the reaction rates: (a) $k_{cat} = 2.12 \cdot 10^5$, (b) $2.12 \cdot 10^4$, and (c) $2.12 \cdot 10^3 \text{ s}^{-1}$. 
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