Enzymatically active microspheres for self-propelled colloidal engines

Jungeun Park
CUNY City College of New York

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Enzymatically active microspheres for self-propelled colloidal engines

Thesis

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Jungeun Park
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Approved:

______________________________
Dr. Charles Maldarelli, Thesis Advisor

______________________________
Dr. Ilona Kretzschmar, Chair
Department of Chemical Engineering
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Abstract

Micro- and nano-motors have attracted numerous attentions from various scientific areas due to their potential applications. Most studies on self-propelled colloidal engines have exploited catalytic decomposition of hydrogen peroxide to drive the motor. Since the hydrogen peroxide is caustic, it is not suitable to use in biological applications, encouraging people to develop “greener” fuels. The aim of this research is to study a new transduction mechanism for self-propulsion not tied to hydrogen peroxide, and which can in particular be used with biological molecules as fuels. In this study, we focus on making particles with enzymatic activity which can effectively decompose biomolecules for self-propulsion. We select elastase as a catalyst and coat it on the surface of polystyrene (PS) particles, and use SucAla$_3$-pNA as a substrate to examine the activity of the elastase-coated particles. We exploit biotin-streptavidin chemistry to couple the elastase on the surface of the PS particles. We confirm that SucAla$_3$-pNA can be effectively decomposed by elastase and elastase-coated particles using spectrophotometric measurement. The results demonstrate that the elastase-coated PS particles are catalytically active, showing great potential to be used in biologically-friendly system.
Acknowledgments

First and foremost, I would like to thank my advisor, Dr. Charles Maldarelli. His guidance and motivation have helped me get through tough life in graduate school and achieve good results more than I expected. His smile and kindness encourage me to research and study harder. Whenever I was in trouble, he believed and supported me. I also appreciate the support and friendship of all members in Charles Maldrelli group. Special thanks to my friends, Dr.Chunqiu Zhang, Dr. Archit Dani, Ali Mozaffari, Hao Zhou and Christiana Oh, for believing in me and teaching experiment skills. I really enjoy working with our group members.

Also, I am grateful to all my friends for encouraging me. Specially, I would like to thank Ankur Jadhav, Fang Liu, Sungyup Jung, and Nelya Akhmetkanova for sharing your knowledge and time for discussions. Without them, I would never have been where I am today. Lastly, I would like to thank my family, my husband, mom, and my younger sister for always believing in me and giving great comfort.
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Chapter 1. Introduction

Micro- and nano-bots, Figure 1a, are ultra-miniaturized, colloid-sized engines which are engineered to react with solute in a solution in which they are immersed, and convert the chemical reaction energy into mechanical self-propulsion without moving parts (for reviews see Howse\textsuperscript{1}, Sen, and Velegol\textsuperscript{2-5}, Wang\textsuperscript{6-10}, Schmidt\textsuperscript{11}, Pumera\textsuperscript{12,13}, Kapral\textsuperscript{14}, and Ozin\textsuperscript{15}). They are designed to transverse liquids in a programmed way along small scale pathways, and their construction is a bottom-up approach made possible by advances in micro- and nano- fabrication. These engines are at the center of a wide range of highly imaginative applications that are on the nanotechnological horizon: In the absence of steering, autonomously moving locomotors more randomly because of (Brownian) thermal fluctuation forces, and this random movement, vigorous because of the onboard locomotion, can be used for micromixing and micropumping\textsuperscript{16}. If the locomotors are configured with chemical receptors, their random motion can be used to bind target molecules for assays, or collect cells. When a steering mechanism is incorporated, the locomotors execute directional motion, and can be used as roving sensors if their chemical propulsion is affected by the presence of solute\textsuperscript{17} or as transporters for ferrying bound molecular cargo\textsuperscript{10,18,19} as for example drugs to selected targets\textsuperscript{20-22}, molecular building blocks to assemble supramolecular structures\textsuperscript{23}, analytes in microfluidic networks for lab on a chip operations\textsuperscript{24-27} or towing biological cells\textsuperscript{28-30}.
Figure 1. (a) Illustration of an autonomous, miniaturized engine. (b) Applications of these engines as roving sensors, scavengers of biomolecules and cells, cargo and cellular transporters, and shuttles in microfluidics.

The operating principle for engine self-propulsion is the chemo-mechanical transduction mechanism, which converts the reaction energy into autonomous motion. The first set of synthetic locomotors were bimetallic rods made of platinum and gold (a few microns in length and a few hundred nanometers in diameter) prepared by sequential electrodeposition in a membrane template. A catalytic oxidation of a solute, hydrogen peroxide takes place on the platinum (anode) side of the rod to produce protons in solution, and electrons. The protons migrate to the opposite gold (cathode) side of the rod through the solution, while the electrons conduct through the rod to the gold side. At this cathode end, hydrogen peroxide combines with the released protons and the electrons conducted through the metal to produce water. The accumulation of protons at one end of the bisegmented rod, relative to the opposite end creates an electric dipole field around the rod directed from the anode to the cathode. The field acts on the diffuse layer of positive charge in the water around the cylindrical sides of the particle (which balances the negative charge of the metal) driving fluid from the anode to the cathode and propelling the rod in the opposite direction. A second class of motors utilizes bubble production in a tubular jet configuration for autonomous...
motion (Figure 2b). Using either thin-film roll-up technology \(^{11,38-40}\), or template membrane electrodeposition, conically shaped hollow cylinders are formed tens of microns in length and a few microns in diameter with an inner catalytic platinum surface, which catalyzes the reaction of hydrogen peroxide to water and oxygen on the inside of the cylinder. The buildup of oxygen creates bubbles which are expelled through the wider opening of the cylinder, creating a recoil which propels the object. A second design which uses the expulsion of bubbles produced by the decomposition of hydrogen peroxide on a catalyst to actuate locomotion is shown in Figure 2c. In this case one face of a spherical particle is functionalized with the catalyst, and the particles are propelled by the asymmetric recoil force towards the inert face. If the concentration of hydrogen peroxide is low, then the concentration of oxygen produced at the catalyst surface does not exceed the solubility limit and bubbles are not evident. In this case the particle motion towards the inert face is still observed, but the mechanism is unclear as the motion may be due to micron or nanosized bubbles that are not observable, or the asymmetric distribution of the dissolved oxygen, which produces unbalanced van der Waals interactions which propel the motor (Figure 2d) (see Howse, Golestanian and coworkers and references \(^{1,41-43}\).
Figure 2. (a) Bimetallic rod motors generate self-electrophoretic motion by a redox reaction, (b) tubular engines eject oxygen bubbles from a catalytic reaction of H$_2$O$_2$ on Pt on the inside surface of the tube, leading to motion by recoil, and Janis motors decompose peroxide on the platinum side, ejecting, (c) at high rates oxygen bubbles for self-propulsion by recoil, or (d) at low rates dissolved oxygen, which propels the motor by van der Waals interaction.
Chapter 2. Research Objectives

The current set of autonomous micro or nano engine designs (Figure 2) have in common a reliance on using the decomposition of hydrogen peroxide to drive the motor. Because of its caustic nature, this fuel is not compatible with many of the envisioned biological applications of self-locomotors, and the search for other more biologically compatible, “greener” fuels and their associated paradigms for chemo-mechanical transduction are required for nano and microbot technologies to reach their potential. The aim of this research is to study a new transduction mechanism for self-propulsion not tied to hydrogen peroxide, and which can in particular use biological molecules as fuels. The chemo-mechanical transduction is based on an electrical driving force created by gradients in charged (ionic) species. Figure 3 illustrates how an ionic gradient can propel a particle engine. We consider a concentration gradient of a salt imposed across a channel by flowing streams containing either a fixed concentration of salt ($C_0$) or no salt. Initially, if the cation, for example, is smaller than the anion, its diffusion coefficient ($D_+$) will be larger than the anion coefficient ($D_-$), and the cation will diffuse faster to the opposite end of the channel relative to the anion. This creates an accumulation of positive charge at the interface adjacent to the stream with no salt, giving rise to an electric field directed towards the high-concentration flow channel. This field acts to reduce the flux of cations, and increases the flux of anions, and as a result at steady state the fluxes of each of these species is identical, and the steady field strength is given by

$$\varepsilon = \frac{K_B T}{e} \beta \frac{d \ln C_S}{dz}$$

where $\varepsilon$ is the electric charge, $K_B T$ is the thermal energy, $C_S$ is the salt concentration and $\beta = \frac{D_+ - D_-}{D_+ + D_-}$. If a positive charged particle is immersed in this externally imposed concentration gradient, then the field acts on the diffuse layer of negative charge adjoining the particle surface, causing the electrolyte to move towards the low salt concentration side, and
propelling the fluid towards the high side as self-electrophoresis. In addition, within this diffuse layer, the attractive interaction of the negative charge with the surface is balanced by fluid pressure at the particle surface. As this attraction is larger at the high end of the concentration gradient, a pressure gradient develops along the surface in the diffuse layer creating a slip velocity along the surface towards the low concentration end of the gradient, propelling the particle towards the high concentration end of the gradient (chemophoresis). The combination of these two effects results in a velocity $U$ given by

$$U = \frac{\varepsilon}{8\pi \mu} \left[ \frac{K_BT}{e} \right]^2 \left[ 2\beta \zeta - 4\ln \left( 1 - \tanh \left[ \frac{\zeta}{4} \right] \right) \right] \frac{d\ln[c_s]}{dz}$$

where $\mu$ is the (aqueous) phase viscosity, $\varepsilon$ is the dielectric constant of the aqueous phase and $\zeta$ is the surface (zeta) potential nondimensionalized by $\frac{K_BT}{e}$, with the two terms in brackets representing electrophoresis and chemophoresis, respectively and the entire mechanism referred to as “diffusiophoresis”.

To use diffusiophoresis to power a particle engine, the particle has to carry a surface charge, and the gradient of the ionic species has to be created by the motor itself, rather than being imposed externally as in Figure 3a. The ionic gradient is generated by functionalizing one face of the particle with a catalyst which, in the simplest construction, cleaves a neutral zwitterionic fuel species into positive and negative product molecules (Figure 3b). Ionic gradients caused by the diffusion of the product ions away from the active cap creates the chemphoretic part of the diffusiophoresis motion as the gradient is imposed on the diffuse layer of charge surrounding the motor. In addition, if the zwitterionic fuel is molecularly designed so that upon cleavage one ionic product (for example the cation as in the Figure) is significantly smaller than the other ion (e.g. the anion), the more rapid transport of the smaller ion creates an electric field, which moves the charged particle electrophoretically. As in Figure 3a, if the particle is charged positively, and the
cation produced by the reaction has a smaller diffusion coefficient, then both the electrophoretic and chemophoretic mechanisms will act in concert to increase the magnitude of the self-actuation.

**Figure 3.** Diffusio-electrophoresis: (a) Rectified: An imposed salt gradient of ions with different diffusivities creates an electric field and a slip flow in the diffuse layer of charge which causes the particle to move. (b) Self-diffusio-electrophoresis: Catalyst cleavage of a peptide on one face of the particle produces a pair of oppositely charged products with different sizes, which create ionic gradients and an electric field that self-propels the particle.

Our specific goal is to demonstrate the movement of a colloid engine powered by diffusiophoresis, using a biological fuel and an enzyme catalyst. Rein Ulijn's recent work on aromatic peptides suggests that these molecules have a sufficiently versatile chemical architecture - through the choice of the amino acids and their sequence in the peptide - to produce upon enzymatic cleavage ionic products which are oppositely charged and of different size. In particular, in this initial seed effort we will examine the peptide Fmoc-YDAAR shown in Figure 4. This particular peptide is a zwitterionic species due to the negatively charged aspartic acid and positively charged arginine.
residues, and is neutral at pH 7. The peptide is cleaved by elastase (a serine protease) at the bond between the two alanine residues (Fmoc-YDA⩽AR) resulting in tri- and di-peptide species with -2 (Fmoc-YDA) and +2 (AR) valencies at neutral pH. The important and innovative aspect of this peptide design is the Fmoc (fluorenyl-9-methoxycarbonyl) group at the nitrogen terminus. The group by itself is large so that the cleavage product Fmoc-YDA is larger than the AR dipeptide. If the colloid is positively charged, then both the self-diffusiophoretic and chemophoretic mechanisms act to propel the particle in the direction of the active catalyst side.

**Figure 4.** The biological fuel Fmoc-YDAAR is zwitterionic, and is cleaved by catalase between the alanine residues into reaction products that are negatively charged (Fmoc-YDA) and positively charged (AR), with the size of the cation much smaller than the anion.
Chapter 3. Research Plan

3.1 Colloid Motor Fabrication

Our strategy for constructing a Janus colloid with elastase bound to one face of the colloid, and with the colloid positively charged, is given in Figure. 5 and involves using the coupling of Streptavidin to its ligand binding partner biotin to attach elastase to the colloid. Streptavidin is a protein with four binding sites for the small molecule ligand biotin. Elastase can be functionalized with biotin by a standard protocol of using NHS-biotin to react with amine groups on the elastase; the NHS group binds to primary amines and functionalizes elastase to bind to Streptavidin. Polystyrene microspheres with the Streptavidin protein conjugated to the surface are commercially available, and can be coated with Au by thermal evaporation to form a Janus particle with one face exposing Streptavidin and one face with gold. After coating, the gold side can be easily functionalized with an amine group by using an amine thiol amphiphile to bind the thiol part of the molecule to the gold, leaving the amine extending from the surface. This renders the gold side positively charged at neutral pH since the primary amine group is protonated. Following this modification, the biotinylated elastase is bound to the uncoated Streptavidin side of the particle to form the motor. Since the elastase and primary amines are positively charged at neutral pH (the pH at which the motor will be operated) the motor surface is entirely positively charged.

3.2 Synthesis of Fuel

The peptide fuel Fmoc-YDAAR is synthesized using standard solid phase peptide synthesis (SPPS) with Fmoc protection so that after synthesis and cleavage from the solid phase resin, the Fmoc group is attached to the amine terminus. The C terminus is then functionalized with a methyl group.
(or alternatively propyl or oxyethylene glycol) to render this end uncharged so the fuel bears no net charge.

**Figure 5.** Colloid motor fabrication: One side of streptavidin coated microbeads, 100 nm-1 µm in diameter, is coated with Au to form a Janus particle. The Au side is functionalized with a self-assembling monolayer of an amine thiol to create a positive surface charge, and biotinylated elastin is bound to the opposite side through linking to the binding sites on surface Streptavidin.
3.3 Measurement of Motor Velocity

The velocity ($U$) of the Janus motors (Figure 5) in the Fmoc-YDAAR fuel will be observed and measured using bright field optical and fluorescence microscopy. The motion will be observed in a microscopy chamber, fitted with a glass slide as a bottom, and filled to a few millimeters in height with the aqueous fuel. Our preliminary work will be with the larger motors ($\approx 1$ µm in diameter) which can easily be observed in bright-field mode at a magnification of 10-20X, using an inverted microscope to focus on the colloids through the bottom glass slide of the chamber. For the smaller particles, visualizing the motion is more easily facilitated using Streptavidin microbeads with an embedded fluorophore (commercially available), and observing the motor fluorophore emission. As the motor diameters are in the range of microns to submicrons, Brownian motion due to thermal fluctuations causes the engines, in the absence of the fuel, to rotate and translate randomly. Two dimensional tracking of the mean square displacement ($\Delta L^2$) of the engine translation (using colloid tracking software) as a function of time $\Delta t$, in the case where there is no fuel, is linear in time with a slope proportional to the motor diffusion coefficient $D_p$, i.e. $\Delta L^2 = 4 D_p \Delta t$ where the Stokes-Einstein relation gives the particle diffusion coefficient as $D_p = \frac{K_B T}{6\pi \mu a}$, where $a$ is the motor radius. This linear relationship, in the absence of fuel, will be verified, and the particle diffusion coefficient will be calculated and compared directly to the theoretical prediction since the diameter of the motors and the viscosity of the suspending phase are known. This will benchmark and validate the tracking procedures. When the fuel is present, the motor motion consists of a forward translation due to the diffusiophoretic driving force. But, concomitant to this motion is the Brownian rotation of the particle, which steers the particle in a random motion. For times $\Delta t$ much larger than the time required for the particle to rotate in a viscous medium due to thermal fluctuations ($T_R = \frac{6\pi \mu a^3}{K_B T}$), the motion is again random, but the apparent diffusion
coefficient ($D_{app}$) is increased from $D_p$ due to the diffusiophoretic motion. $\Delta L^2 = (4D_p + U^2\tau_R) \Delta t$ or $D_{app} = 4D_p + U^2\tau_R$. By plotting the mean square displacement $\Delta L^2$ as a function of $\Delta t$, for $\Delta t >> \tau_R$, a linear dependence should be observed, and the velocity obtained from $D_{app}$. Our aim is to demonstrate velocities of the order of $10 \mu m/sec$, which has been suggested as a viable motility in applications. The dependence of the velocity of the fuel concentration, and the concentration of added electrolyte will be investigated.

### 3.4 Specific Objectives

Our specific goal in this thesis is to make particles with enzymatic activity which can cleave the peptides (fuels) for self-propulsion. First, we develop an efficient way to prepare elastase-coated particles. To achieve this, we functionalize the elastase with biotin functional groups and coat the biotinylated elastase on the surface of the streptavidin-coated PS particles using biotin-streptavidin chemistry. Then, we measure the catalytic activity of the elastase-coated PS particles. To ensure the reliability of the experiments, we measure the extinction coefficient of our model molecule (pNA) and compare it with previously reported values. Then, we measure the catalytic activity of the elastase to confirm if it can efficiently cleave the substrate molecule (SucAla$_3$-pNA) in various conditions such as storage time, concentration, etc. Lastly, we measure the catalytic activity of elastase-coated particles to confirm if it can be used as a colloidal engine.
Chapter 4. Experimental Procedures

4.1 Materials

Streptavidin coated polystyrene microspheres with a diameter of 1 µm were purchased from Bangs Laboratories. EZ-Link Micro NHS-PEG₄-Biotinylation kit was purchased from Thermo Scientific. Elastase from porcine pancreas (Type IV, protein 50-90%, Iyophilized powder), N-succinyl-Ala-Ala-Ala-Ala-p-nitroanilide (SucAla₃-pNA), and 4-nitroaniline were purchased from Sigma-Aldrich. Deionized water was used. Figure 6 shows a structure of elastase. Four amine functional groups (three lysine groups and one N-terminus) are used for the biotinylation reaction. Catalytic triad (aspartic acid, serine, and histidine) shown in the Figure 6 is the active site for the catalytic reaction.

![Figure 6. Structure of elastase from Protein Data Bank. (PDB ID: 3EST)](image-url)
4.2 Biotinylation of elastase

1 mg of elastase was dissolved in 2 ml of PBS buffer (pH 7.2), used as a stock solution. 2 mg of NHS-PEG₄-biotin was dissolved in 170 µl of PBS buffer to prepare 20 mM biotin solution. 300 µl of elastase solution was mixed with 14 µl of biotin solution and the mixture was incubated at room temperature for 60 min. The amine group of the elastase reacts with the NHS functional group (leaving group) of the NHS-PEG₄-biotin, forming an amide bond (Figure 7). The solution was occasionally shaken by hand. After the reaction, biotinylated elastase was separated using a desalting column.

![Diagram of Biotinylation of Elastase](image)

**Figure 7.** Biotinylation of elastase.
4.3 Elastase coating onto polystyrene particles

50 µl of streptavidin-coated particles were washed with PBS buffer three times using centrifugation at 12,000 rpm for 5 min and redispersed in 500 µl of PBS buffer. The particle suspension was added with 314 µl of biotinylated elastase solution. The mixture was stirred at room temperature for one hour. The elastase-coated particles were washed three times with PBS buffer using centrifugation at 12,000 rpm for 5 min (Figure 8). Finally, the elastase-coated particles were resuspended in 200 µl of PBS buffer.

Figure 8. Fabrication of elastase-coated PS particles using streptavidin-biotin chemistry.
4.4 Activity measurement

2 mg of SucAla<sub>3</sub>-pNA was dissolved in 1 ml of PBS buffer, containing around 0.4 unit per ml of elastase in buffer. Spectrophotometer (BioMate 3 series, Thermo Fisher Scientific) with a wavelength of 400 nm was used to measure the absorption intensity of pNA, a result of catalytic activity by elastase. We used a constant concentration of the substrate (SucAla<sub>3</sub>-pNA) and varied the concentration of enzyme. Table 1 shows the detailed conditions for each sample. The concentration of elastase on the particles were calculated based on the assumption that one biotinylated elastase can bind with one streptavidin on the surface of particles. Figure 9 shows the hydrolysis of SucAla<sub>3</sub>-pNA catalyzed by elastase.

![Chemical structure](image)

**Figure 9.** Hydrolysis of SucAla<sub>3</sub>-pNA catalyzed by elastase.
**Table 1.** Different concentration of samples for activity measurement. (unit: µl)

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Elastase 1</th>
<th>Elastase 2</th>
<th>Elastase-coated particle 1</th>
<th>Elastase-coated particle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS buffer</td>
<td>186.7</td>
<td>184.7</td>
<td>180.7</td>
<td>184.7</td>
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<tr>
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<td>13.3</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Enzyme Sol.</td>
<td>0</td>
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<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>
Chapter 5. Results and Discussion

5.1 Measurement of extinction coefficient of Product pNA

We measured an absorbance of the pNA at 400 nm of wavelength as a function of concentration to measure the extinction coefficient of pNA. We use pNA as an indicating material which is generated after SucAla3-pNA is cleaved by the enzymatic activity. Figure 10 shows a plot for the absorbance of pNA as a function of concentration. More detailed values are in the Table 2. It shows that the absorbance linearly increases as the concentration of pNA increases. It agrees with the Lambert-Beer’s law, \( A = \varepsilon c d \), where \( A \) is an absorbance, \( \varepsilon \) is an extinction coefficient (\( M^{-1} cm^{-1} \)), and \( C \) is a concentration (M), and \( d \) is a path length, in which the absorbance is proportional to the concentration of the solute. From the slope of the plot, we obtained the extinction coefficient of 9200 \( M^{-1} cm^{-1} \), close to the values obtained from other literatures, 9450 \( M^{-1} cm^{-1} \) (405 nm) and 8800 \( M^{-1} cm^{-1} \) (410 nm)\textsuperscript{47,48}.

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
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<th>30</th>
<th>50</th>
<th>70</th>
<th>100</th>
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<tbody>
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<td>Absorbance</td>
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<td>0.0102</td>
<td>0.277</td>
<td>0.445</td>
<td>0.615</td>
<td>0.886</td>
</tr>
</tbody>
</table>

Table 2. Absorbance of pNA at different concentrations
5.2 Enzymatic Activity of Elastase in solution

It has been known that the activity of enzyme is highly sensitive on storage period and condition as well as the types of enzymes. Generally, it decreases as the storage time increases. It is essential to know the maximum storage time and the activity changes of elastase since it can significantly affect the final activity of the elastase-coated particles. We measured the activity of three elastases, fresh one, stored for 5 days, and 11 days, as a function of time (Figure 11). It shows that all elastases have activities to cleave the substrate, SucAla3-pNA, but the degree of activity significantly decreases as the storage period increases. This finding indicates that freshly prepared elastase can more effectively cleave the substrate than others. It suggests that it is crucial to use a
fresh elastase for higher intensity and to use an elastase stored in the same conditions for consistent results.

![Graph showing activity measurements for elastase as a function of reaction time. Freshly prepared elastase for red line, elastase stored for 5 and 11 days for blue and black lines, respectively.]

**Figure 11.** Activity measurements for elastase as a function of reaction time. Freshly prepared elastase for red line, elastase stored for 5 and 11 days for blue and black lines, respectively.

To confirm if the concentration of elastase can affect the cleavage rate of substrate, SucAla3-pNA, we varied the concentration of elastase and measured the absorbance changes of pNA. Figure 12 demonstrates that the cleavage rate is significantly affected by the concentration of elastase and proportionally increases. It implies that higher density of elastase on particles would hydrolyze the substrate more effectively.
Figure 12. Activity measurements for various concentration of elastase as a function of reaction time. 2 µl of elastase for blue line, 3 µl for red, and 5 µl for black.

From the results, we obtained reaction rates depending on the concentration of elastase (Table 3). We calculate the concentration changes of pNA over reaction time using Lambert-Beer’s law with the extinction coefficient obtained above (9200 M\(^{-1}\)cm\(^{-1}\)). Figure 13 shows the reaction rates at different concentration of elastase, demonstrating that the reaction rate proportionally increases as the concentration of elastase increases. As the reaction continues the concentration of the substrate gradually decreases, while the concentration of pNA increases. Finally, the \(\frac{d[pNA]}{dt}\) will become zero once the substrate is fully consumed. Based on the Michaelis-Menten kinetics mechanism, we think that the reaction rate we obtained above is the initial velocity of the reaction. The Michaelis-Menten equation is as follows; \(V_i = \frac{d[P]}{dt} = \frac{v_{max}[S]}{K_m + [S]} = \frac{K_{cat}[E][S]}{K_m + [S]}\), where \(V_i\) is the
initial velocity, $V_{\text{max}}$ is the maximum velocity, $K_{\text{cat}}$ is the catalytic constant, $K_m$ is the Michaelis constant ($K_m = \frac{K_2}{K_1}$), and the [Et] is the total active site concentration (enzyme concentration).

**Table 3.** Absorbance and pNA concentration rate changes at different elastase concentrations

<table>
<thead>
<tr>
<th>Elastase concentration (µl)</th>
<th>dA/dt(sec(^{-1}))</th>
<th>d[pNA]/dt (µMsec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.0003</td>
<td>0.033</td>
</tr>
<tr>
<td>3</td>
<td>0.0009</td>
<td>0.097</td>
</tr>
<tr>
<td>5</td>
<td>0.0015</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Figure 13.** Reaction rate as a function of elastase concentrations.
5.3 Activity of Elastase on Bead Surface

We coated the particles with the elastase and measured their activity. We measured the activity of pure elastase and elastase-coated particles to compare their differences. The concentration of elastase in the solution or on the particles is adjusted to 0.039 unit/ml. Figure 14 shows the catalytic activity of pure elastase. As can be seen in Figure 14, the absorbance of pNA gradually increases over the reaction time and reaches to 0.87 after 5 min of reaction.

![Figure 14](image)

**Figure 14.** Activity measurements with 6 µl of elastase as a function of reaction time.

We attempted to measure the catalytic activity of the elastase-coated particles without removing them from solution, but we could not get correct results due to the uneven light scattering or absorption by the particles. To minimize the optical interference by the particles, we first reacted the substrate with the elastase-coated particles for five minutes, removed the particles from the
solution by centrifugation, and then measured the absorbance (Figure 15). As shown in the Figure 15, the initial absorbance begins around 0.67 since it was measured after reaction and removal process. It clearly indicates that the elastase-coated particles can effectively cleave the substrate. We noticed that the activity of the elastase-coated particles is lower than that of the elastase in solution. We believe that there are two reasons for that. First, the elastases are immobilized on the surface of the particles so that the diffusion of them are limited compared with the pure elastase dissolved in the medium. Second, the concentration of the elastases on the particles could be lower than we expected. We calculated the number of elastases on the particles based on our assumption that the yield of all the binding processes is 100%.

![Figure 15. Activity measurements for elastase-coated particles as a function of reaction time after filtration of the particles.](image)
Chapter 6. Summary

We studied how to fabricate elastase-coated PS particles and measured catalytic activity of pure elastase and elastase-coated PS particles. First, we prepared biotinylated-elastase and then they are coated on the surface of PS particles using streptavidin-biotin chemistry. Prior to measuring the catalytic activity of elastase or elastase-coated particles, we confirmed the reliability of spectrophotometer by measuring absorbance of various concentrations of pNA at a wavelength of 400 nm. It gave an extinction coefficient of 9200 M⁻¹cm⁻¹, which agrees with the values reported in other literatures.

First, we measured activity of elastase with different storage time to see their activity changes. It showed that the activity significantly decreased as the storage time increased. It suggests that using freshly prepared elastase solution is essential to obtain higher activity. Next, we measured the absorbance changes of pNA in the presence of various concentrations of elastase. The result demonstrated that the substrate (SucAla₃pNA) is more effectively cleaved when a higher concentration of elastase is introduced. Then, we measured the activity of the elastase and elastase-coated particles. We observed that the activity of pure elastase is higher than that of elastase-coated particles. We suspect there are two reasons for that. One is the diffusion of the elastase is much faster than the elastase coated on the particles. The other is that we may have less concentration of elastase than the numbers we calculated. To eliminate optical interferences by elastase-coated particles while we measure their activity, we first reacted the substrate with the elastase-coated particles and then separated the particles from the solution and measured the absorbance of the solution. With this manner, we were able to avoid any optical interferences by the particles. These results offer useful information for further research and demonstrate that the elastase-coated particles are enzymatically active and can be used as self-propelled colloidal engines.
Future research will be to fabricate Janus particles with a half sphere coated with gold by thermal evaporation and with the other half coated with elastase. Due to the anisotropy of the Janus particle, we could make efficient colloidal motors that can be used in biological conditions. We also plan to synthesize effectively designed peptide to use as biological fuels.
References


