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Enzymatically induced motion at nano- and microscale

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In contrast to adenosine triphosphate (ATP)-dependent motor enzymes, other enzymes are little-known as “motors” or “pumps”, that is, for their ability to induce motion. The enhanced diffusive movement of enzyme molecules, the self-propulsion of enzyme-based nanomotors, and liquid pumping with enzymatic micropumps were indeed only recently reported. Enzymatically induced motion can be achieved in mild conditions and without the use of external fields. It is thus better suited for use in living systems (from single-cell to whole-body) than most other ways to achieve motion at small scale. Enzymatically induced motion is thus not only new but also important. Therefore, the present work reviews the most significant discoveries in enzymatically induced motion. As we will learn, freely diffusing enzymes enhance their diffusive movement by nonreciprocal conformational changes which parallel their catalytic cycles. Meanwhile, enzyme-modified nano- and microobjects turn chemical energy into kinetic energy through mechanisms such as bubble recoil propulsion, self-electrophoresis, and self-diffusiophoresis.

Enzymatically induced motion of small objects ranges from enhanced diffusive movement to directed motion at speeds as high as 1 cm s⁻¹. In spite of the progress made in understanding how the energy of enzyme reactions is turned into motion, most enzymatically powered devices remain inefficient and need improvements before we will witness their application in real world environments.

1. Introduction

The development of “motors” small enough to fit nanometer- and micrometer-sized “vehicles” and of autonomous “pumps” small enough to fit into the tiniest fluid channels is expected to lead to devices with multiple functions which will radically change the way we diagnose, monitor, and cure diseases. Therefore, a significant research effort is currently directed toward developing such motors and pumps. This effort has resulted in a good number of fully synthetic nanomotors which have been nicely reviewed elsewhere. Most synthetic nanomotors induce motion by creating a concentration gradient along their body. Some nanomotors create their local concentration gradient indirectly, for example under the effect of an electric field or by creating first a local temperature gradient. Catalytic nanomotors carry chemically active zones which convert species from their surroundings. They are thus able to produce concentration gradients without any external field. This is very convenient because applying an external field requires additional equipment and is not always possible. Thus, not surprisingly, catalytic nanomotors are among the most popular nanomotors. The recently reported catalytic nanomotors self-propel if surrounded by fuel such as hydrazine, hydrogen peroxide, or bromine (iodine).

In spite of this progress, we are far from using miniaturized motors or pumps to carry out complex studies and interventions in living systems, be it a single-cell or a whole-body. The main reasons of this fact are the size, the inability to deal with real-world samples, and, last but not least, the poor biocompatibility of the great majority of miniaturized motors and pumps developed up to now. This poor biocompatibility made researchers turn their attention to enzymes. Although there is a great diversity of enzymes characterized by amazing turnover numbers and excellent selectivity in physiological conditions, only few motors and pumps based on enzyme catalysis have been developed. These represent a significant step toward devices characterized by biocompatibility and will be reviewed in the present work. Adenosine triphosphate (ATP)-dependent motor proteins (such as kinesin, myosin, and dynein) are intensively studied and used to develop hybrid (bio – non-bio) nanodevices.

2. Motion induced with immobilized enzymes

Listeria monocytogenes moves inside host cells through the polymerization of actin with actin assembly-inducing protein (ActA), a protein that is distributed on the bacterial surface in a polar fashion (i.e. asymmetrically). Aiming for a better understanding of the way this pathogen moves, researchers have created some of the first protein-propelled nanomachines by modifying the surface of polystyrene beads with purified ActA.
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references therein): generated) is given by the following equation (see
diffusiophoresis. The speed (U) of a charged particle subjected to
self-generated concentration gradients was named self-
activation in order to allow the diffusiophoretic mechanism to
operate, and thus the bead to propel itself. When using a 2 µm large bead, this
inhomogeneous distribution of species was achieved only when
their diameter was 0.5 µm (Figure 1). However, when
polystyrene beads as large as 2 µm were taken into work, protein
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order to induce their motion. It is thus clear, that the ActA-
mediated polymerization of actin must produce an
inhomogeneous distribution of species in the vicinity of the bead
in order to allow the diffusiophoretic mechanism to operate, and
thus the bead to propel itself. When using a 2 µm large bead, this
inhomogeneous distribution of species was achieved only when
ActA protein was asymmetrically immobilized onto the bead.

That asymmetry is required for directed motion was subsequently
several times demonstrated with different structures. Because the
movement of particles driven by an applied concentration
gradient is called diffusiophoresis, the movement of particles by
self-generated concentration gradients was named self-
diffusiophoresis. The speed (U) of a charged particle subjected to
an electrolyte concentration gradient (applied externally or self-
generated) is given by the following equation (see 14 and
references therein):

\[
U = \frac{\varepsilon_0 k T V n_0}{4 \pi \eta} \left( \frac{\alpha + \zeta^- \ln \cosh \zeta}{(\alpha - \zeta^-)} \right)
\]

\[
\alpha = \frac{D_2 - D_1}{D_2 + D_1}
\]

\[
\zeta = \frac{Ze \zeta}{4kT}
\]

\[
\zeta = \frac{kT V n_0}{\varepsilon_0 (\alpha - \zeta^-) \ln \cosh \zeta}
\]

where \(\varepsilon_0/4\pi\) is the solution permittivity, \(\zeta^-\) is the zeta potential of the particle, \(\eta\) is the solution viscosity, \(k\) is the Boltzmann constant, \(T\) is the absolute temperature, \(Z\) is the absolute value of the valences of the ions, \(e\) is the charge of a proton, \(Vn^0\) is the concentration gradient, \(n^0(0)\) is the electrolyte concentration at the particle centre in the absence of the particle, and \(D_1\) and \(D_2\) are the diffusion coefficients of the anion and the cation, respectively. While this equation does not apply to all structures presenting diffusiophoresis it gives a good overview of the parameters which impact the phenomenon.

Self-propulsion by decomposition of hydrogen peroxide was first demonstrated in 2002 by the group of G. M. Whitesides from Harvard University. Their 9 mm diameter poly(dimethylsiloxane) plate was carrying a 2 mm x 2 mm piece of platinized porous glass and was moving at the surface of an aqueous solution of 3% hydrogen peroxide. Pt catalyzed the decomposition of hydrogen peroxide (\(2 H_2O_2 \rightarrow O_2 + 2 H_2O\)), and the whole structure moved due to the recoil from the \(O_2\) bubbles departing from the surface of the platinized porous glass. Catalase is the biochemical equivalent of Pt when it comes to decomposition of hydrogen peroxide. This enzyme consists of four subunits (60 kDa each), decomposes hydrogen peroxide at rates approaching the diffusion-controlled limit, and thus is one of the most efficient enzymes known. Therefore is not surprising that, shortly after the publication of Whitesides’s paper, catalase-based biochemical self-propulsion was also reported. First a synthetic catalase was used to modify 80 µm SiO₂ particles and thus achieve their self-propulsion at speeds as high as 35 µm s⁻¹. Natural enzymes lose their activity quite fast once purified. Therefore, synthetic enzymes represent a promising way to gain long-term stability of enzyme-based motors and pumps (given the synthetic enzyme reproduces the selectivity and activity of its natural counterpart). The synthetic catalase-modified SiO₂ particles were shown to self-propel in very high concentrations of hydrogen peroxide (5%) and in organic solvent (acetonitrile). This medium is clearly not something a nanomotor can meet in biological systems.

Non-synthetic catalase-based “microengines”, showing directed motion in hydrogen peroxide solution, were also reported few years later. The microengines were made by rolling up titanium / gold films to obtain 25 µm long microtubes, and by covalently attaching catalase to the golden inner part of...
These microtubes. Due to their complex design, the catalase-based microengines were more efficient than the nanomotors made with synthetic catalase, and moved with an average speed of ~ 200 µm s\(^{-1}\) if dispersed into a solution containing 1.5% hydrogen peroxide. These microengines represent an important progress compared to the nanomotors with synthetic catalase. However, the hydrogen peroxide concentration required for their directed motion is still too high to be compatible with living cells. While catalase-based nanomotors are not yet compatible with living cells, they have found applications in the capture and transport of DNA-modified objects and determination of water quality.

The last application is based on the inhibition of catalase by toxic compounds, inhibition that negatively affects the propulsion speed of catalase-based nanomotors. A little bit better biocompatibility is characterizing the glucose oxidase- and catalase-modified carbon nanotube aggregates and bundles self-propelling in glucose. Glucose oxidase is a dimeric enzyme that converts glucose and oxygen, into glucono lactone and hydrogen peroxide, respectively. Therefore, the self-propulsion of these 200-800 nm long aggregates and bundles in glucose solutions is due to the hydrogen peroxide produced by glucose oxidase and then consumed by catalase with formation of oxygen bubbles. The achieved propulsion rates are again great (0.2 - 0.8 cm s\(^{-1}\)) but the concentration of the fuel needed for propulsion is one which is still difficult to find in biological systems (100 mM glucose). Using poorly defined nanotube aggregates and bundles is also not very advantageous (because they are difficult to reproduce). The reaction cascade self-propelling catalase-modified nano- and microobjects is schematically depicted in Figure 2.

There are very few nano- or microobjects with motion induced through the use of other enzymes than catalase. Probably the most notorious of these objects are the carbon fibers, with one end modified with glucose oxidase and the other end modified with bilirubin oxidase, which show self-propulsion at the surface of an aqueous solution of glucose (2 - 32 mM)\(^23\). The speed of the self-propulsion of these fibers is impressive (1 cm s\(^{-1}\)) but the fibers are large (0.5 – 1 cm), manually modified with enzymes, and their movement is restricted to 3 minutes and to the surface of the glucose solution. All these features make the structure a poor candidate for biological applications. The structure is still of great importance because of its mechanism of self-propulsion that is completely different from the bubble recoil mechanism powering catalase-based structures (and from the self-diffusiophoresis powering the early ActA-based motors described above). Glucose oxidase- and bilirubin oxidase-modified carbon fibers gain kinetic energy by a mechanism most often called self-electrophoresis. Movement of charged particles in a solution, driven by an applied electric field is called electrophoresis. Self-electrophoresis is thus the movement of charged particles driven by a self-generated electric field. The speed (U) of a charged particle presenting electrophoresis (or self-electrophoresis) is given by the following equation (see \(^14\) and references therein):

\[
U = \frac{\varepsilon \xi}{4 \pi \eta} E
\]

where \(\varepsilon/4\pi\) is the solution permittivity, \(\xi\) is the zeta potential of the particle, \(\eta\) is the solution viscosity, and \(E\) is the applied (or self-generated) electric field. While this equation does not apply to all structures presenting electrophoresis it gives a good overview of the parameters which impact the phenomenon. Self-electrophoresis of the above enzyme-modified fiber involves i.) an oxidation reaction occurring at one end of the fiber (i.e. oxidation of glucose by glucose oxidase), ii.) a reduction reaction occurring at the other end of the fiber (i.e. reduction of oxygen by bilirubin oxidase), and iii.) a current flow through the fiber (from glucose oxidase to bilirubin oxidase). As a result of these three processes, protons travel in the electrical double layer of the fiber, from the glucose oxidase-modified end of the fiber to the bilirubin oxidase modified end, dragging water molecules with them. Motion of the liquid adjacent to the nanomotor propels the nanomotor in the opposite direction. The reaction cascade self-propelling this glucose oxidase- and bilirubin oxidase-based nanomotor through self-electrophoresis is schematically depicted in Figure 3.
Nanoscale

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Fig. 5 Fluid pumping using a small immobilized enzyme spot developed inside the fluidic channel. Important to note: Such autonomous micropumps will turn ON only in the presence of the substrate of the immobilized enzyme. Moreover, their pumping speed will be advantageously dependent on the concentrations of the enzyme substrate. (Reprinted by permission from S. Sengupta, D. Patra, I. Ortiz-Rivera, A. Agrawal, S. Shklyaev, K. K. Dey, U. Córdova-Figueroa, T. E. Mallouk, and A. Sen, Nat. Chem., 2014, 6, 415–422. Copyright 2014 Macmillan Publishers Ltd).

Nanorods with a polypyrrole segment and a gold segment made by template assisted electrodeposition were recently modified with either one or two of the following hemeproteins: horseradish peroxidase, catalase, and cytochrome c. Modification with a given hemeprotein was always restricted to one of the two segments. The used hemeproteins are all able to convert hydrogen peroxide by either peroxidase-like or catalase-like activity, depending on the experimental conditions. Because of this ability of the hemeproteins, the hemeprotein-modified nanorods were characterized by diffusion coefficients which increased with the hydrogen peroxide concentration (up to 10 mM hydrogen peroxide at least). For example, the nanorod with its polypyrrole segment modified with horseradish peroxidase has enhanced its diffusion coefficient with 16% in 10 mM hydrogen peroxide as compared to its coefficient in water. Unmodified nanorods, and nanorods symmetrically modified with only one hemeprotein, do not show such behavior. Interestingly, the results also show that the enhanced diffusive movement of the hemeprotein-modified nanorods is most probably due to self-diffusiophoresis in the case of the nanorod carrying horseradish peroxidase on its polypyrrole segment, and due to self-electrophoresis in the case of the other investigated nanorods. However, the observed self-electrophoresis was lacking the support of a significant current through the hemeprotein-modified nanorod. This situation was partially rectified when the horseradish peroxidase- and cytochrome c-modified nanorods were suspended in mixtures of superoxide and hydrogen peroxide. Superoxide and hydrogen peroxide are two biologically important reactive oxygen species which were produced with xanthine oxidase and hypoxanthine in order to test the nanorods. In the presence of superoxide and hydrogen peroxide the horseradish peroxidase- and cytochrome c-modified nanorods combine an oxidation reaction (occurring on the peroxidase-modified segment), a reduction reaction (occurring on the cytochrome c-modified segment), and an electron transfer in between their two segments (just as shown in Figure 3 for glucose oxidase and bilirubin oxidase). This cascade of events increased the diffusion coefficient of the hemeprotein-modified nanorods with 22% in a solution with 300 mU mL$^{-1}$ xanthine oxidase and 200 µM hypoxanthine as compared to the diffusion coefficient in water. The increase of the diffusion coefficient was sensitive to the substrate of xanthine oxidase used to generate the reactive oxygen species (hypoxanthine or xanthine) as well as to an inhibitor of xanthine oxidase (quercetin) (Figure 4). Moreover, the diffusion coefficient increased with 30% (over the value observed in water) when the nanorod was made electrically more conductive leaving thus no doubt that enzymatically-induced self-electrophoresis is involved. Such peroxidase- and cytochrome c-modified nanorods represent an important step toward nanomotors which are able to rush to cells in oxidative stress and neutralize the reactive oxygen species causing problems.

Some of the above enzyme-based nanomotors are, in principle at least, able to pump liquid if they are immobilized into a fluid channel (because moving the liquid adjacent to their body remains the only option once they cannot move). However, turning an enzyme-based nanomotor into a pump was seldom carried out. Deoxyribonucleic acid (DNA) template - T4 DNA polymerase complexes show enhanced diffusive movement in the presence of their substrate, deoxy 2′-adenosine triphosphate, dATP (as detailed in the next section). When immobilized onto a spot of a fluidic channel, instead of moving they pump liquid at linear speed of up to 1.4 µm s$^{-1}$ in the presence of the mentioned substrate. The ability of a spot of immobilized DNA polymerase complex to act as “pump” was explained by a density gradient that is established around the spot following the catalytic substrate conversion.
Similar enzymatic micropumps were very recently built with catalase, lipase, urease and glucose oxidase (Figure 5). Due to the very high activity of catalase, catalase-based micropumps provided one of the highest pumping speeds (4.51 \( \mu \text{m s}^{-1} \)), which was observed at hydrogen peroxide concentrations as high as 0.1 M. The ability of these pumps to turn ON when a given compound is present was also a little bit explored and exploited by building, an autonomous, glucose oxidase-powered pump that delivers insulin in response to glucose. In this pump, glucose oxidase and insulin were both immobilized into a hydrogel which was releasing insulin at higher speeds when glucose was available in higher concentrations.

Modification with enzymes is clearly a promising way both to achieve the autonomous motion of small objects and to pump liquid through microfluidic channels while maintaining increased compatibility with living systems. Interestingly, modification with enzymes also reproduces most of the mechanisms responsible for the self-propulsion of catalytic (i.e. non-enzymatic) nano- and micromotors.

3. Self-propulsion of freely-diffusing enzymes

Enzymatically induced motion was revealed by an unintentional top-down approach, that is, the self-propulsion of enzyme-modified nano- and microparticles was reported before the enhanced diffusive movement of enzyme-DNA complexes and of enzyme molecules. The enzymatically induced motion of nano- and microobjects was just reviewed in the previous section. This section will review the enhanced diffusive movement of freely-diffusing enzyme-DNA complexes and enzymes.

DNA-T7 RNA polymerase complexes were shown to enhance their diffusion coefficient in the presence of nucleoside triphosphates. The enhanced diffusive movement of such complexes turns into directed motion (chemotaxis) when a gradient of nucleoside triphosphates appears. As shown in Figure 6, DNA template-T4 DNA polymerase complexes also increase their diffusion coefficient in the presence of dATP and Mg\(^{2+}\). The ability of the DNA polymerase to act as “motor” was explained by the cycles of nonreciprocal conformational changes suffered by enzyme during catalytic cycles. Taking into account the large size of T4 DNA polymerase (898 amino acids, 103 kDa), the conformational changes of T4 DNA polymerase during a catalytic cycle can indeed be quite significant. However, one can expect that the effectiveness of swimming by this mechanism is significantly reduced when the enzyme is small.

Researchers at The Pennsylvania State University were first to show that urease and catalase are also characterized by larger diffusion coefficients in the presence of their substrates than in the absence of these substrates. Urease, the first ever enzyme crystallized, is another large enzyme made up of six subunits with \( \sim 90 \text{ kDa} \) each. It increases its diffusion coefficient in 100 mM urea with 28% as compared to the coefficient in the absence of urea. Catalase increases its diffusion coefficient in 100 mM hydrogen peroxide with 45% as compared to the coefficient in the absence of hydrogen peroxide. Interestingly, this increase is similar to the increase observed above for the DNA polymerase complex in 5 mM dATP. Moreover, just as the DNA-enzyme complexes, urease and catalase also “swim” toward regions with higher substrate concentrations. The microfluidic channel for observing this chemotaxis-like behavior of enzymes is schematically depicted in Figure 7. The exact mechanism causing enzymes to self-propel is still unclear. Changes in solution viscosity, temperature, and pH, and formation of bubbles due to the enzyme reaction were ruled out as possible causes for the self-propulsion of the enzymes. Both phoretic mechanism and nonreciprocal conformational changes occurring during the catalytic cycle were considered as the most plausible cause of the self-propulsion. Important to note, urease and catalase, just as...
T4 DNA polymerase, are large enzymes. It can thus be expected 
that their conformational changes during catalytic cycles are 
indeed significant.

Self-propulsion is clearly a property of both enzyme-modified small objects and freely diffusing enzyme molecules exposed to 
an enzyme substrate concentration gradient. It is to be discovered 
if the mechanisms of self-propulsion of freely diffusing enzymes play a role also in the self-propulsion of enzyme-modified small objects.

4. Conclusions

Enzymatically induced motion (with enzymes which are not 
ATP-dependent motor proteins) was demonstrated at both single 
and enzyme-modified nano- and microobject level. DNA-enzyme complexes and enzymes were shown to enhance their 
diffusive movement in the presence of their substrates. Enzyme-modified nano- and microobjects were shown to present not only enhanced diffusive movement but also directed motion 
at speeds ranging from 0.119 µm s\(^{-1}\) to 1 cm s\(^{-1}\). These propulsion speeds were achieved by a variety of mechanisms including self-diffusiophoresis, recoil from bubbles, and self-electrophoresis. Using enzymes to develop an autonomous micropump able to move liquid at 4.51 µm \(s^{-1}\) linear speed was also demonstrated.

On the negative side we can notice that the enzyme-based nanomotors still require high fuel concentrations compared to the concentrations of these fuels in biological systems, move for a limited time, and/or their motion is restricted to the air-solution interface. We have definitively got closer to having tiny motors and pumps able to perform in living systems but we are not yet there.

How can we take enzymatically induced motion closer to applications (in biology)? A better understanding of the mechanism behind the enzymatically induced motion is definitively required. Engineering enzymes to show superior activity and better stability could also help. Enzyme-based nanomotors and micropumps developed up to now are based on few, relatively simple enzyme immobilization methods. These methods are easy to implement but they also tend to denaturate enzymes (negatively affecting their activity). Therefore, screening methods to immobilize different enzymes onto different locations of the same nano- or microobject, while maintaining enzyme activity, can also be part of the answer to the above question. Nanomotors moving through self-electrophoresis in fact small short circuited batteries (fuel cells). Enzyme-based biofuel cells made already their way into the living body where they were able to perform for 110 days with no signs of rejection or inflammation. Therefore, using some of (or getting inspiration from) the technical solutions developed for enzyme-based biofuel cells could also help to develop better enzyme-based nanomotors.

What can we expect from enzymatically induced motion in the close future? The ultrasonic propulsion of rod-shaped Au nanomotors inside living cells was recently demonstrated. Taking into account this achievement and those described above, enzyme-based nanomotors will certainly be deployed in the extra- and intracellular space of living cells. The motion of enzyme-based nanomotors is by default sensitive to biologically relevant molecules. Therefore, the easiest to imagine is that the motion of enzyme-modified nanorods will be used to monitor the concentration of such molecules inside and/or outside living cells with good spatiotemporal resolution.

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Notes and references

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