Enzyme Catalysis To Power Micro/Nanomachines

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ABSTRACT: Enzymes play a crucial role in many biological processes which require harnessing and converting free chemical energy into kinetic forces in order to accomplish tasks. Enzymes are considered to be molecular machines, not only because of their capability of energy conversion in biological systems but also because enzymatic catalysis can result in enhanced diffusion of enzymes at a molecular level. Enlightened by nature’s design of biological machinery, researchers have investigated various types of synthetic micro/nanomachines by using enzymatic reactions to achieve self-propulsion of micro/nanoarchitectures. Yet, the mechanism of motion is still under debate in current literature. Versatile proof-of-concept applications of these enzyme-powered micro/nanodevices have been recently demonstrated. In this review, we focus on discussing enzymes not only as stochastic swimmers but also as nanoengines to power self-propelled synthetic motors. We present an overview on different enzyme-powered micro/nanomachines, the current debate on their motion mechanism, methods to provide motion and speed control, and an outlook of the future potentials of this multidisciplinary field.

KEYWORDS: enzyme catalysis, micro/nanomachines, self-propulsion, nanomotors, synthetic motors

The harnessing of chemically free energy for conversion into mechanical work is ubiquitous in nature and crucial for survival of organisms from all levels of complexity. Tasks such as phagocytosis, vesicle transportation within the cells, locomotion, and cell division are based on mechanical work achieved through surrounding substrate decomposition. In biological systems, enzymes are workhorse proteins that act as catalysts, being able to turnover substrates with high specificity and efficiency that power biological machinery. Examples include synthesis of DNA molecules by DNA polymerase, hydrolysis of proteins by endopeptidase, and using energy by ATP hydrolysis. Effectively, enzymes are themselves considered to be nanomachines because fundamental studies on enzymes revealed that catalytic activity enhances diffusion at the single-molecule level.

Synthetic micro/nanomachines arose from endeavors to mimic biological counterparts abundant in nature, in order to understand its fundamentals and to develop functional and well-controlled tools with applications in a wide range of fields. Synthetic machines controllable at such a tiny scale may power devices for applications in environmental sciences, biomedicine, or diagnostics to name a few.

In this review, we focus on enzymes as molecular machines, as well as their driving force when combined with synthetic micro/nanomachines. These synthetic molecular machines can be based in a myriad of structures. One example is nucleic-acid-based motors, where enzymatic activity controls the hybridization and hydrolysis of DNA and/or RNA strands, promoting motion of structures based in nucleic acids. Recently, enzymatic catalysis was also reported to power the motion of various structures at the micro/nanoscale, such as polymeric and inorganic particles. By coupling enzymes onto the surfaces of these structures, enzymatic turnover of substrates provides necessary energy to overcome random Brownian motion and achieve active motion. Moreover, by functionalizing fixed surfaces with enzymes, the driving force produced by enzymatic catalysis is transferred to the surrounding environment, giving rise to fluid flow (Scheme 1).

Researchers have carried out in-depth studies on enzymes as swimmers and as engines for active synthetic matter. In order to use biocatalytic energy, investigation on the fundamental mechanism of enzymatic reactions has been performed, aiming at understanding the conversion of enzyme catalysis into propulsion power, including on the mechanism of single enzymes as active motors (Scheme 1).

Although this field is still in its infancy, it can have an impact in fields such as smart drug delivery, bio-nanotechnology for...
**Scheme 1. Schematic Illustration of Enzyme-Powered Micro/Nanomachines**

**Figure 1.** (A) ATP synthase 3D structure. Reprinted with permission from ref 31. Copyright 2001 Nature Publishing Group. (B) Direct observation of F1-ATPase rotation movement by coupling a fluorescence actin filament. Reprinted with permission from ref 35. Copyright 1998 American Association for the Advancement of Science. (C) Conformational changes during ATP synthesis. Reprinted with permission from ref 39. Copyright 2013 Nature Publishing Group. (D) Schematic representation of urease self-diffusion enhancement by catalysis and diffusion coefficients of urease when exposed to increasing substrate concentrations. Reprinted from ref 3. Copyright 2010 American Chemical Society. (E) Conformational changes of adenylate cyclase measured by single-molecule force spectroscopy. Reprinted with permission from ref 49. Copyright 2016 Nature Publishing Group. (F) Diffusion coefficient of catalase as a function of the laser power (402 nm) and schematic representation of enzyme motion driven by chemoson acoustic effect. Reprinted with permission from ref 5. Copyright 2014 Nature Publishing Group.
medical purposes, environmental remediation, among others. Therefore, it is important to investigate enzymes, not only understanding the basic knowledge of the biocatalytic process but also carrying out in-depth studies on enzymes as swimmers. Furthermore, it is crucial to unravel the mechanism underlying the motion/swimming when enzymes are conjugated onto more complex structures. Deeper insights on enzymatic propulsion may affect the development of advanced and more versatile types of synthetic micro/nanomachines. Herein, we review the study of enzymes as molecular machines and used as engines to power motion of other structures. We expect that comprehensive studies on this type of propulsion at the micro/nanoscale will help to develop micro/nanomachines, providing insights for future development of this field.

**Enzymes as Motors.** Enzymes are proteins capable of efficiently catalyzing the conversion of a substrate into products, including most forms of biological motion at the cellular level. In this sense, myosins, which move along actin filaments, and kinesins and dyneins, which move along microtubule tracks, are the three main types of molecular motors within the cells. These molecular motors generate energy to move from the hydrolysis of ATP (ATP → ADP + inorganic phosphate (Pi)) by enzymes, e.g., ATPase, with forces that vary between 1 and 10 pN. Other types of intracellular motion can be achieved through single enzymes, as in ATPase rotation. These proteins are motor complexes anchored to organelle membranes and are involved in either the synthesis of ATP coupled to the electrochemical proton gradient formed by electron transfer chains (F-ATPase) (Figure 1A) or the acidification of intracellular compartments (V-ATPase). Although the rotary mechanism of ATPases was hypothesized by Boyer in 1979, it was empirically observed by Noji and collaborators for the first time in 1997, through the conjugation of a fluorescent actin filament to the immobilized enzyme (Figure 1B). The rotation movement of ATPases is triggered by changes in the conformation of the different subunits (Figure 1C) following substrate binding or release. Moreover, in 2002, Montemagno and co-workers discovered that ATPases are capable of generating forces and also move nickel rotors. Apart from these well-known intracellular motion mechanisms, the self-diffusion of cytoplasm-located enzymes has been hypothesized to play a vital role for transduction of intracellular signals. However, there was no empirical demonstration of these nontraditional enzymatic motions until very recently. In this respect, Muddana and co-workers reported in 2010 a catalysis-enhanced diffusion of urease enzyme, which was shown to be highly reliant upon substrate concentration (Figure 1D). The same authors further confirmed these results using both urease and catalase enzymes, where they observed that the diffusion of free urease and catalase enzymes was not only significantly enhanced by the turnover of their substrates \([(\text{NH}_3)_2\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3, \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + 1/2\text{O}_2\text{, respectively}\)] but also displayed preferential movement toward increasing substrate concentrations, which should be regarded as a different form of molecular chemotaxis. These findings have led to the harnessing of chemical energy released by enzymes as a source of power for micro- and nanomotors. Yet, the exact mechanism that underlies enzymatic motion in fluids is not completely understood. Golestanian suggested that the enhanced diffusion of enzymes could be explained by a self-diffusiophoresis mechanism triggered by the asymmetric release of products involved in the catalytic reaction, creating interfacial forces depending on osmotic gradients, charges, or other properties. This theory has been further confirmed by Colberg et al., who reported that self-propulsion forces of angstrom-sized molecules are generated by different interactions of the enzyme with the local gradient of products released. On the other hand, the enhanced diffusion of single enzymes could also be attributed to the conformational changes that play a critical role in catalysis. This phenomenon could result in stochastic swimming. Recently, Pelz and co-workers performed a direct measurement of the energetic drive of substrate-dependent lid closing in the enzyme adenylyl kinase (ATP + AMP → 2ADP) by using a single-molecule force spectroscopy approach based on optical tweezers (Figure 1E). The increase of temperature during catalysis is involved in single-enzyme-enhanced diffusion. In this sense, Riedel et al. recently reported that the enhanced diffusion of enzymes is related to the heat released during substrate turnover. Based on their observations through fluorescence correlation spectroscopy analyzed within the framework of stochastic theory, these researchers proposed a motion mechanism based on the generation of an asymmetric pressure wave by the transient displacement of the center-of-mass of the enzyme (chemoacoustic effect, Figure 1F). However, this is a topic of current debate. In this regard, Golestanian has examined the role of four different mechanisms (i.e., self-thermophoresis, boost in kinetic energy, stochastic swimming, and collective heating) in the temperature-driven enhanced diffusion of enzymes observed by Riedel et al. In this work, it is concluded that there is not enough evidence to assume that either self-thermophoresis or a boost in kinetic energy is responsible for the experimentally obtained values of effective diffusion. As an alternative, he also proposes that the enhanced diffusion of enzymes that catalyze exothermic reactions could be attributed to a combination of (a) global temperature increase in the sample container and (b) enhanced conformational changes that can lead to a hydrodynamic enhancement of effective diffusion coefficient. Although at present there is no conclusive answer to this controversial discussion, fully understanding the fundamental mechanism of the motion of single enzyme is still rather critical for the development of enzyme-powered micro/nanomachines. Sophisticated experimental design will be highly desired in order to distinguish those different effects described before, which will be helpful for the future design and use of these enzymes as “nanoengines” to power artificial systems.

**DNA–Enzyme Motors.** Biological functions are performed by highly complex and hierarchical nanomachineries, namely, motor proteins and nucleic acids. Based on these molecular machines, researchers developed DNA-based motors that can process information and execute transport over considerable distances powered by enzymatic reactions. Typically, these motors consist of single-stranded DNA or RNA that is complementary to domains present along the patterned tracks. The motion is controlled by cyclic reactions of hybridization and hydrolysis between the DNA-based motor and the track, recurring to restriction enzymes that comprehend specific recognition sites in the hybridized motor–track complex. These motors may have applications as cargo transportation devices or biosensors for highly sensitive and sequence-specific nucleic acid assays. Despite their programmability and precise control over the motion along a track, DNA-based
molecular machines’ velocity is limited to rates around 1 nm/min due to compromises between its endurance and speed. To tackle these problems, Salaita and co-workers designed a DNA-based walker that moves through a cog-and-wheel mechanism (Figure 2A), which overcomes trade-off issues of multivalent DNA motors and improves their velocity. Its motion is due to a similar mechanism as referred to above, and it is powered by the addition of RNase H. Its directionality is based on a sequence of reactions of DNA complementary RNA hybridization, hydrolysis by RNase H, and rehybridization with new ssRNA, occurring with consumption of substrate (ssRNA) as the motor rolls upon the track.

Li and co-workers engineered a patterned track and DNA walker conjugated onto the same spherical particle, which increased local effective concentrations of DNA. This motor achieves motion through the hybridization of the walker with the DNA substrate, followed by hydrolysis by a nicking endonuclease.

The majority of DNA-based nanomachines are powered by enzymes with nucleic acid affinity, such as nuclease, ligases, polymerases, or nicking enzymes, but those represent only a small fraction of the enzymes used to catalyze reactions in nature. Recently, Ricci and co-workers employed different classes of non-DNA-recognizing enzymes, namely, proton-producing and proton-consuming enzymes, to control DNA-based nanodevices through pH-dependent DNA reactions. The researchers demonstrated that a DNA switch could be reversibly triggered into opening or closing states by reactions catalyzed by non-DNA-recognizing enzymes. To do so, they used a pH-dependent labeled switch and engineered the protonation and deprotonation of the switch using glutathione transferase (GST) (GSH + CDNB → GS-DNB + HCl) and urease, respectively. Furthermore, they utilized enzymatic reactions as a way to control the load and release of ligands using urease to prompt cargo loading and trigger its release (Figure 2B), proving that enzymes can be a wide toolkit to power biostructures.

Enzyme-Powered Micro/Nanomotors. Enzymes have been used to power the motion of biologically occurring structures. The ability of enzymes to provide sufficient driving force to propel larger synthetic structures has been reported. Sánchez and co-workers fabricated 400 nm diameter Janus hollow mesoporous silica nanoparticles (HMSNPs, Figure 3A(a,b)), by coating either silica or metallic element (Ni) onto one side of a monolayer of the particles through electron beam evaporation (Figure 3A(c)). Three different enzymes, catalase, urease, or glucose oxidase (GOx) ($\beta$-D-glucose + O$_2$ + H$_2$O $\to$ gluconic acid + H$_2$O$_2$), were conjugated onto one face of the particles. Upon addition of corresponding substrates H$_2$O$_2$, urea, or glucose, all nano-
motors exhibited enhanced diffusion that the authors claim to be generated by a chemophoretic mechanism. By utilizing an optical trapping technique (Figure 3A(d)), the authors measured a driving force around 60 fN applied on a catalase-powered nanomotor (Figure 3A(e)).

Wilson and co-workers14 loaded enzymes, such as catalase or catalase and GOx combination, into 500 nm supramolecular stomatocytes (Figure 3B), achieving self-propulsion by gas expulsion from a very small opening of these structures. Enzymes were also employed to drive one-dimensional nanoarchitectures. Feringa and co-workers59 claimed bubble propulsion of glucose oxidase/catalase conjugated carbon nanotubes (diameter = 20 nm) with addition of glucose and oxygen. Gáspár and co-workers51,52 conjugated several enzymes, including GOx, glutamate oxidase, xanthine oxidase, horseradish peroxidase, and catalase, onto polypyrrole–gold nanorods whose fuel-dependent enhanced diffusion behavior was explained by self-electrophoresis based on a bioelectro-chemical mechanism ($2H^+ + 2e^- + H_2O_2 \rightarrow 2H_2O; 2O_2 \rightarrow O_2^{2-} + 2e^- $). Such behavior was further utilized for substrate sensing applications.63 Mano and Heller64 coupled another two-enzyme system: glucose oxidase and bilirubin oxidase, onto a macroscale carbon fiber, which moved by bioelectrochemical propulsion at the air−liquid interface when fueled with glucose, resulting in the net bioelectrochemomechanical power-generating reaction ($\beta\text{-D-glucose} + 1/2 O_2 \rightarrow \delta\text{-glucono-1,5-lactone} + H_2O$).

Sen and co-workers16 immobilized two individual enzymes, catalase and urease, onto the whole surface of polystyrene particles. The enhanced diffusion of these particles was explained by a thermal effect due to exothermic enzymatic reactions (Figure 4A(a)). Nevertheless, such a hypothesis needs further investigation as pointed out by the authors. Städl and co-workers65 also immobilized two enzymes, catalase and GOx, onto one face of Janus silica particles, which also showed enhanced diffusion properties (Figure 4A(b)). A long-standing
challenge for utilizing micro/nanomotors as drug delivery carriers is biocompatibility of the whole self-propelled system, which encourages researchers to design enzymatic motors consuming nontoxic fuels.\textsuperscript{14−16,65} Although the above-mentioned works successfully proved the feasibility of using these biocompatible fuels to power micro/nanomotors, the drawback of randomized movement due to Brownian activation makes it hard to meet realistic applications. Very recently, Sánchez and co-workers managed to construct a fully biocompatible microcapsule motor based on Janus hollow mesoporous silica spheres with an average diameter of 2.3 μm (Figure 4B(a)).\textsuperscript{66} The capability of long-range movement (>100 μm), with considerable velocity (>10 μm s\(^{-1}\)) for long time at physiological concentration of urea, makes it a promising candidate for potential biomedical applications.\textsuperscript{66} The urea-powered hollow microcapsule motor demonstrated directional self-propulsion driven by a phoretic mechanism, which provided experimental evidence for the theoretical hypothesis given by Golestanian and co-workers that asymmetric distribution of enzymatic reaction products could lead to phoretic motion (electrophoresis, diffusiophoresis, or osmio- phoresis) of enzyme-conjugated Janus micro/nanoparticles (Figure 4B(b)).\textsuperscript{43} However, for the self-propulsion behavior in the form of enhanced diffusion, in addition to the phoretic mechanism, other effects of enzymatic reactions, such as global temperature increase and conformational changes, might also increase the inherent Brownian motion, leading to enhancement of the effective diffusion coefficient of the motors.

Following the classic self-propulsion system based on Pt/\textsubscript{Sal}\textsubscript{2}O\textsubscript{4}, researchers initially used catalase to replace Pt. For instance, Sánchez and co-workers first immobilized catalase into the tubular micromotor (length of 25 μm) by covalent linkage and achieved ultrafast movement by bubble propulsion (Figure 4C(a)).\textsuperscript{67} They improved self-propulsion efficiency by utilizing enzymatic reactions compared to Pt/H\textsubscript{2}O\textsubscript{2} system. Similar strategy was employed by other research groups, including He and Wang, to fabricate bubble propulsion tubular micro-motors,\textsuperscript{68−71} where they demonstrated proof-of-concept applications, including active drug delivery toward cells,\textsuperscript{68} water quality testing,\textsuperscript{69} toxin sensing,\textsuperscript{70} and decontamination.\textsuperscript{71}
applications. Besides tubular micromotors, which can generate bubbles through one-dimensional confinement, catalase was conjugated onto one side of Janus particles, as well. With a rough surface (Figure 4C(b)) or a relatively large size (>10 μm) (Figure 4C(c)) at the biocatalytic face, oxygen bubbles could generate quickly and push the motors toward the non-enzyme side. Catalase-based enzymatic motors by a bubble propulsion mechanism can achieve directional movement with extremely high velocity up to hundreds of micrometers per second, more than 10 times higher than the phoretic motion of micromotors such as urea-powered microcapsule motors, but biotoxicity and high oxidative activity of H₂O₂ fuel limited these motors’ realistic applications, especially in the biomedical field.

Enzyme-based nano/micromotors have shown, for catalase enzyme, high efficiency compared to that with Pt-based counterparts. That effect was observed in tubular microjets and in stomatocyte nanocapsules. The high efficiency of enzyme-based micro/nanomotors could be attributed to the high catalytic rate of the catalase enzyme and the fact that enzymes were confined into the cavities of the micro/nanomotors, where products accumulate and are thereafter expelled through the openings of the motors as nozzles or jets.

To achieve external control on the movement of micro/nanomotors, Sen and co-workers demonstrated one-dimensional guidance of single-enzyme motors (catalase, urease) and enzyme-conjugated micromotors in a microfluidic setup (Figure 5A). The enzymatic motors prefer to move toward the high concentration region of the substrate through collective behavior of chemotaxis. Furthermore, computational models have been developed using surface-bound enzymatic reactions to organize structures in solution. Another common strategy is remote magnetic guidance. Researchers accomplished directional guidance by incorporating magnetic element, such as Fe or Ni, into the motors’ structure. Remote control on the orientation of the enzymatic micromotors was readily available by applying a magnetic field (Figure 5B-
In addition to directional guidance, Sánchez and co-workers realized velocity manipulation by tuning the enzymatic activity of urease with addition of enzyme inhibitors, such as Hg$^{2+}$ or Ag$^+$ ions (Figure 5B(a)). Enzyme inhibition property was also utilized for water quality sensing through direct observation of the inhibited motion behavior of bubble propulsion microtubular motors.

**Enzymatic Micropumps.** Nonmechanical micropumps that can function without the need for an external power source have great potential as active biosystems, but the use of nonbiocompatible fuels hinders their applicability. Sánchez and co-workers developed a nonmechanical, tunable, catalytic micropump that operates by decomposing low concentrations of hydrogen peroxide into water and oxygen, generating bubbles that provoke fluid flow. A myriad of catalysts can trigger hydrogen peroxide decomposition, among which are most transition metals and enzyme catalase. However, it is a toxic fuel, hindering this micropump’s applicability.

As discussed previously, single enzymes’ diffusion increases in a concentration-dependent manner. Tethering enzymes to a fixed surface permits the transfer of this force to the surrounding environment, moving fluid as well as particles in a directed fashion. In addition, these micropumps are activated by the presence of specific compounds, such as substrate molecules and cofactors, thus enabling the use of such devices both as sensors and triggered micropumps.

Sen and co-workers designed multiple triggered micropumps with the flow rate tunable by analyte concentration. First, they used the wild-type of the enzyme T4 DNA polymerase, which can switch the mode of action from polymerase to exonuclease. In the case of the presence of a single nucleotide, T4 DNA polymerase action is restricted and the primer strand is shifted back and forth while the enzyme incorporates and removes nucleotides. By immobilizing this enzyme onto a self-assembled monolayer (SAM), the researchers developed a micropump with energy conversion efficiency comparable to that in previously reported synthetic systems. Later, they made use of a similar approach with ATP-independent enzymes of distinct classes—catalase, urease, lipase, and GOx, demonstrating the first examples of ATP-independent enzyme-based pumps. The pumping ability of each enzyme was assessed by injecting a substrate solution in a sealed system containing tracer particles (Figure 6 C), which were used to monitor the speed and directionality of fluid flow. Interestingly, the convective flow in urease micropump is reversed, contrary to that in the other micropumps tested. Researchers pointed out that urea decomposition products by urease catalysis are ionic, which can increase the density of the fluid near the patterned surface, causing it to spread along the glass and driving it away from the pattern. They hypothesize catalysis-induced density-driven convective flow as a mechanism for the directional fluid pumping (Figure 6B). Furthermore, the same group proved the applicability of such pumps as biomedical devices, demonstrating the triggered release of insulin in response to glucose (Figure 6A).

The applications of a self-powered enzyme-based micropump go beyond the biomedical field. Recently, Sen’s group demonstrated the use of urease and catalase pumps as sensors for toxic substances. Enzymatic activity can be severely affected in the presence of sufficient concentration of inhibitors, which in enzyme-based micropumps affects the fluid flow, thus translating into a signal of contamination. This demonstrates...
the possibility of using these devices not only as drug delivery systems but also as sensors and actuators for bioremediation.\(^6\)

**CONCLUSIONS AND OUTLOOK**

Enzymes are naturally presented as biological “engines” of molecular machines in biological systems, which convert chemical energy into mechanical motion in order to accomplish different kinds of biofunctions. Enzymatic bioconversion plays a critical role in the energy conversion process, and therefore, researchers have explored the fundamental mechanism of these bioconversion reactions and contributed considerable efforts to unveil the motion mechanism of enzyme-powered molecular motors. Recent results suggest that single enzymes have been investigated as nanomotors exhibiting enhanced diffusion by turning over corresponding substrate, but debate on their motion mechanism is still under discussion. Through combination with biological molecules (e.g., DNA) or organic and inorganic micro/nanoarchitectures (e.g., silica particles, carbon fibers, metallic nanorods, microfluidic setup, etc.), enzymes have been utilized to power micro/nanosystems as self-propelled motors or pumps. Apart from catalase/H\(_2\)O\(_2\)-based bubble propulsion, there is still a scientific need for understanding the motion mechanism of enzyme-powered synthetic micro/nanomotors, in particular, enhanced diffusion of nanomotors and directional/phoretic motion of micro-motors. Current achievements of enzyme-powered micro/nanomachines, both biological and synthetic, are summarized in Table 1.

At present, enzyme-powered micro/nanomachines have been proven to be useful tools in various proof-of-concept applications, presenting possible solutions for many engineering problems from different fields, such as environmental protection, biosensing, and nanomedicine. Compared to conventional inorganic catalyst-based catalytic motors, micro/nanomotors powered by enzyme-based biocatalytic reactions are advantageous considering the biocompatibility of enzymes as well as versatile choices of enzymes/fuels in nature, which allows for future development of biocompatible propulsion micro/nanomachines. Especially, the recent achievement of biocompatible fuel-powered micro/nanomotors has aroused significant attention for the potential of using natural substrate-powered micro/nanomotors as active drug delivery systems in physiological conditions. However, the stability and sensitivity of enzymes toward the environment conditions, such as pH, temperature, and poisonous chemicals, are disadvantages for enzyme-powered micro/nanomotors. Moreover, other chal-

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**Table 1. Summary of Enzymatic Micro/Nanomachines**

| Material (Size)                      | Enzymes                                    | Mechanism                        | Ref.   |
|-------------------------------------|--------------------------------------------|----------------------------------|--------|
| **Single-enzyme motors**            |                                            |                                  |        |
| NA                                  | ATPase                                     | Rotation induced by conformational changes | 36-38,40 |
|                                     | urease                                     | Catalysis-enhanced diffusion by phoretic mechanism (plausible) | 3      |
|                                     | urease, catalase                           | Chemoacoustic effect by exothermic catalytic reactions | 4      |
|                                     | catalase, urease, alkaline phosphatase, and triose phosphate isomerase | | 5      |
|                                     | glutathione transferase/urease             | pH-sensitive switch activated by proton-producing/proton-consuming enzymes | 55     |
| **DNA–enzyme nanomachines/motors** |                                            |                                  |        |
| ssDNA                               | Restriction enzyme (Nt.AlwI)               | Hybridization/cleavage cycles    | 52     |
| DNA template                        | T4 DNA polymerase (wild-type)              | Nonreciprocal conformational changes | 78     |
| DNA origami tile (100 nm × 70 nm) + ssDNA | Restriction enzyme Nt.BbvCl                | Hybridization/cleavage cycles    | 12     |
| Gold nanoparticle coated with ssDNA | Restriction enzyme Nb.BvCl                | Hybridization/cleavage cycles    | 13     |
| DNA-coated spherical particle (Ø = 5 or 0.5 μm) | RNase H                                    | Hybridization/hydrosolysis cycles | 15     |
| Janus HMSNP (389 nm)               | Catalase/urease/GOx                        | Phoretic mechanism               | 14     |
| Supramolecular stomatocytes (500 nm) | Catalase/catalase+GOx                     | Gas expelling                     | 59     |
| MWCNT (20 nm × 1 μm)               | Catalase+GOx                               | Self-electrophoresis             | 61-63  |
| Polyppyrolyle–gold (PPy–Au; 200 nm × 1.5–2 μm) nanorods | GOx, glutamate oxidase (GluOx), xanthine oxidase (XOD); horseradish peroxidase (HRP) + catalase; HRP | |        |
| **Enzyme-powered micromotors**     |                                            |                                  |        |
| Polystyrene particles (0.79 μm)    | Catalase/urease                            | Collective heating                | 16     |
| Janus silica particles (0.8 μm)    | Catalase+GOx                               | Buoyancy effect (Archimedes law)  | 65     |
| Janus mesoporous silica microcapsule (2.3 μm) | Urease                                    | Phoretic mechanism               | 66     |
| Rolling up microtube (Au/NI, 3 × 25 μm) | Catalase                                   | Bubble propulsion                 | 67     |
| Bovine serum albumin/poly-L-lysine (PLL/BSA) | Catalase                                   | Bubble propulsion                 | 68     |
| Multilayer tube (5 μm × 20 μm)     | Catalase                                   | Bubble propulsion                 | 69,70  |
| PEDOT/Au tube (2 μm × 20 μm)       | Catalase                                   | Bubble propulsion                 | 72     |
| Janus poly(styrene sulfonate)/poly(diallylamino hydrochloride) (PSS/PAH) polymer capsule (8 μm) | Catalase+peroxidase                       | Bubble propulsion                | 73     |
| Janus silica particles              | Catalase                                   | Bubble propulsion                 | 71     |
| Plant (radish) tissue tube (1 mm × 7 mm) | Catalase+peroxidase                      | Bioelectrochemical propulsion     | 64     |
| Carbon fiber (7 μm × 0.5–1 cm)      | Catalase/urease/lipase/GOx                 | Catalysis-induced density-driven convective flow | 17     |
| **Enzyme-powered macromotors**     |                                            |                                  |        |
| SAM/gold pattern in PEG-coated glass surface (Ø = 6 mm) | Catalase                                  | Nonreciprocal conformational changes | 78     |
| **Enzyme-powered micropumps**      |                                            |                                  |        |
| SAM/gold pattern in PEG-coated glass surface (Ø = 6 mm) | T4 DNA polymerase (wild-type) | Nonreciprocal conformational changes | 78     |
| **SAM/gold pattern in PEG-coated glass surface (Ø = 6 mm) | Catalase/urease | Catalysis-induced density-driven convective flow | 81     |
angen in a biological environment, including high viscosity, strong flow, and component complexity of biological fluids, need to be overcome in the near future. Nevertheless, the field of enzyme-powered micro/nanomachines has been undergoing a quick growth and attracted increasing interests, wherein further advancement requires collaboration from multiple disciplines, including physics, biology, chemistry, and engineering.

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Notes
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VOCABULARY

synthetic micro/nanomachines, man-made micro- and nanoscale devices capable of performing assigned tasks; molecular machines, molecular components assembled to produce mechanical work in response to specific stimuli; biocatalytic energy, energy obtained through biological conversion of chemically free energy; propulsion, force that provokes motion; enzymatic catalysis, increase on the rate of a given reaction caused by the active site of a protein; micro/nanomotors, micro- and nanoscale devices capable of converting energy into active motion

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