Biocompetent nanorobots can transfer chemical energy into mechanical work even in ionic environments

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Abstract

Since enzyme-powered micro/nanomotors have demonstrated excellent biocompatibility and bioavailability, as well as the ability to transform chemical energy into mechanical work even in ionic settings, they have sparked considerable interest in the field of hybrid micro/nanomotors. They have enabled active targeting, cargo loading and release, and biosensing, all of which are made feasible by the complex design and functionalization of their devices. Comprehensive understanding of structural complementary domains and synergistic interactions will provide unique characteristics for the bottom-up self-assembling of numerous enzymes in the development of bioinspired biomolecular machines in future concepts, such as the development of bioinspired biomolecular machines. The movement of enzyme-powered nanorobots will be controlled by a concentration gradient in a certain direction. If they are used in medical applications, they will move in reaction to a chemical gradient generated at the site of tissue damage or inflammation. CAT-powered poly (lactic-co-glycolic acid) micromotors that move in response to a hydrogen peroxide concentration gradient for the delivery of a periodontal disease treatment were recently published in a study by Wilson and colleagues. 64 In an in vitro model of inflammatory periodontitis, a phorbol ester activated macrophage cell was employed to investigate the chemotactic self-propulsion of micromotors.

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1 Introduction

Molecular motor proteins (kinesins, dyneins, and myosins) are natural surface walkers in animal cells that use the energy from ATP hydrolysis to travel along polarized cytoskeletal filaments. 1 Scientists have used enzyme biomolecules as catalytic engines to power variably shaped artificial micro-/nanomotors as a result of these motor proteins. 2 Enzymes are proteins that have the catalytic capacity to rapidly transform substrates into products in biological systems. Because of the heat produced during substrate turnover, enzyme macromolecules have increased diffusion at the molecular level. 3 Molecule motility is thought to be caused by local and global heat effects, phoresis, and changes in enzyme conformations, and the processes outside the catalysis–motion connection are currently being debated. 5 Furthermore, when utilizing a microfluidic device with a substrate gradient, enzymatic macromolecules conduct chemotaxis because their increased diffusion occurs in a concentration-dependent manner, and the same behavior has been seen for enzyme-powered micromotors. 7

Enzyme engines have several advantages over conventional engines, including improved performance for hydrogen peroxide decomposition, motion rate sensing of environmental pollutants, and, most importantly, force generation via local chemical conversion of glucose or urea naturally occurring in biofluids. The glucose content in blood serum is typically around 5.5 mm10, while the concentration in saliva is believed to be in the micromolar range (8–210 m11). About 15 years ago, artificial enzyme-powered motors using glucose as the main fuel were created. Carbon-based structures with an immobilized pair of redox enzymes pushed by the impetus of a hydrated proton stream and oxygen bubble generation were created by Mano and Heller12, and Ferringa et al.13, respectively. Urea can be found in human saliva (3–10 mm), human blood (2.6–6.5 mm), and human urine (up to a 50-fold greater content with significant variation).15 Nanomotors that use urea as a fuel have been demonstrated to transport doxorubicin to cancer cells more efficiently than a passive delivery system and to be pH-responsive after being modified with DNA nanoswitches. 17

This is a review of existing enzyme-powered micro/nanomotors as well as recent proof-of-concept applications in this paper. The tubular, spherical, and Janus-like micro/nanomotors, as well as those based on supramolecular stomatocytes and metal–organic frameworks, are grouped together (MOFs).

Micro/Nanomotor Propulsion Using Two Enzymes

Despite the large number of enzymes accessible, only a handful are used as catalytic engines to power micro/nanomotors. They are primarily redox enzymes, and they have attracted a lot of scientific attention as electrocatalytic components of biosensors, bioreactors, and biofuel cells. 22 Micro/nanomotors powered by catalytic hydrogen peroxide reactions have received a lot of attention in the field of micro/nanotechnology.

The disproportionation of H2O2 to water and oxygen occurs in the CAT reaction. With the capacity to breakdown more than one million H2O2 molecules per molecule of enzyme, CAT is recognized to have one of the highest enzymatic turnover rates. If H2O2 is accessible as a unique substrate in a neutral solution, HRP catalyzes the H2O2-mediated oxidation of different substrates and produces oxygen in a CAT-like manner. 24

GOx produces H2O2 by oxidizing gluconic acid with glucose. Because of the toxicity of H2O2 and the low activity of GOx, the GOx–CAT pair was utilized to power micro/nanomotors via cascade catalytic processes that started with glucose and ended with oxygen bubbles and water.

A soluble enzyme directly in solution can catalyze the catalytic synthesis of the necessary fuel in situ, in addition to a multienzyme ensemble coupled to micro/nanomotors. This was accomplished by using XOD to catalyze the oxidation of hypoxantine to produce radical anions (2 O2), as well as H2O2. 25

Hydrolytic enzymes, which have been extensively investigated as biochemical catalysts in medicine, biosensing, and bioremediation26, were used to power micro-/nanomotors in order to address the issue of both biocompatible fuel and its turnover products. UR (urea amidohydrolase) is one of them, and it is involved in chemotactic motility. The hydrolysis of urea to generate ammonia and carbamate, which are then decomposed into ammonia and carbon dioxide, is catalyzed by this nickel-dependent metalloenzyme. 27 At 10 mm urea, immobilization of a multilayered assembly of bionylated UR on the micromotors resulted in increased propulsion at speeds of up to (21.50.8) m s1. 28 The fact that ammonia generated during urea conversion has a cytotoxic impact at concentrations higher than 50 mm (total conversion of 25 mm urea) is a significant limitation of UR-powered micromotors. 16

Lipases, as a triglyceride hydrolysis catalyst, can serve a dual purpose in micromotors: 1) they can create thrust by degrading triglyceride-based fuel, and 2) they can decrease high plasma triglyceride levels. 29 Lipases should be chosen for their substrate specificity and positioning preference for building highly effective micro-/nanomotors. One potential drawback of using trypsin as a catalytic component of micro-/nanomotors 30 is its limited selectivity for extracellular proteins, which limits the number of peptide substrates accessible.

3 Micro/Nanomotors Powered by Enzymes

To prevent zero net forces, active self-propulsion of enzyme-powered micro-/nanomotors requires an asymmetric distribution of enzyme biomolecules,31 while an appropriate quantity of immobilized enzyme on the micro-/nanomotors is required to counteract intrinsic Brownian motion. Furthermore, it has been demonstrated that when the same micromotor is fitted with a different enzyme, the resultant motion dynamics are determined by the reaction kinetics of the reaction on a specific substrate.32 Micro/nanomotors powered by enzymes with a high turnover rate exhibit rapid active motion in theory, demonstrating the link between intrinsic enzyme characteristics and propulsion performance. 33

The majority of enzyme-powered micro/nanomotors are spherical capsules, spheres, or tubular shapes. Brownian motion begins to have a major influence on the resultant motion dynamics when the size of artificial motors is reduced to submicron dimensions. As a result of the rapid rotating diffusion, the directionality of motion is lost, and the nanomotors exhibit increased diffusion. 34 The propulsion of enzyme-powered micro-/nanomachines is usually explained by two distinct processes, according to the scientists. 35 First, relatively big particles and tubular geometries frequently use bubble-induced propulsion. It is based on the formation of gas bubbles at the solid–liquid interface, generally with traces of a surfactant present. The motion of micromotors is aided by the emission of gas bubbles, which produce a bubble recoil push. The second process is self-phoresis, in which enzymatic activity generates local concentration 36 or electrical potential gradients over the surface of a motor with submicron or nanoscale dimensions.

Furthermore, several enzymes have been found to engage in chemotaxis, or directed movement toward areas with high substrate concentrations. 38 Thermodynamically advantageous binding interactions between the active site of an enzyme biomolecule and a substrate are thought to be the source of enzyme chemotaxis. Expulsion of water molecules from enzyme water pockets, in combination with binding and catalysis, causes conformational changes in biomolecules, which result in hydrodynamic fluxes, and therefore enzyme-linked particle propulsion. 38b, 39, 5

Sen et al. recently hypothesized that for liposomes coated with enzymes in microfluidic channels, there was an interaction between enzyme-catalysis-induced positive chemotaxis and solute–phospholipid-based negative chemotaxis. 40 In a gradient using H2O2 as a substrate, CAT-coated liposomes showed positive chemotaxis. In the presence of an ATP gradient, ATPase-linked liposomes displayed positive chemotaxis, but in the presence of the reaction products adenosine diphosphate (ADP) and phosphate ion (Pi), they showed negative chemotaxis. Negative chemotaxis of UR-coated liposomes away from the substrate was observed. Surprisingly, the opposite behavior has been described in the literature for a single UR enzyme, with both chemotaxis toward urea6 and antichemotaxis away from urea. 5 Golestanian et al. sought to explain experimental findings on UR, claiming that enzyme chemotaxis was governed by a struggle between diffusiophoresis and binding-induced changes in the enzyme's diffusion coefficient. 41 Above a threshold substrate concentration, phosis takes precedence, whereas binding-induced changes in diffusion take precedence at lower concentrations.

4 Tubular Micro/Nanomotors Powered by Enzymes

Bubble-driven tubular micromotors collect energy from chemical processes taking place inside a tube or cone's interior cavity. 42 Schmidt's team formed Ti/Au micromotors (2 m long) with CAT immobilized on the inner Au layer in 2010. At 1.5 percent H2O2 concentration, these microjets had a velocity of 226 m s1. 8

Wang et al. formed bilayer PEDOT/Au–CAT microtubes (8–20 m long) using a similar design, which significantly reduced their motion velocity in the presence of enzyme inhibitors like Hg, pesticides, and herbicides, 9, as well as the sarin simulant diethyl chlorophosphate (DCP) in the surrounding environment. 20

Polymer-based tubular micromotors with a thermoresponsive gelatin layer holding Au nanoparticles (NPs), CAT, and doxorubicin were produced to generate a stimulus response for cargo release. At 0.5 percent H2O2 in cell culture conditions and at physiological temperature, the micromotors (20 m long) were bubble driven at a velocity of 4 m s1. The gel–sol phase transition of the gelatin hydrogel caused by the photothermal action of Au NPs under near-infrared (NIR) irradiation triggered doxorubicin release. 19

Wang's group developed fully biological millimeter-sized motors with built-in CAT and peroxidase activities. Motors made from sections of the radish Raphanus sativus push themselves into a solution of H2O2 (5%) while simultaneously decomposing different phenolic compounds as pollutants. 18

Tubular-silica-based nanomotors (200 nm diameter) with UR immobilized within were constructed and demonstrated longitudinal self-propulsion in the presence of urea (100 mm) in addition to CAT/H2O2-based bubble-propelled systems. 21 These nanotube-based motors are said to provide directed motion by using a self-generated concentration gradient. Nanotubes containing UR immobilized on their outer surface were unable to overcome Brownian motion, confirming this hypothesis.

5 Spherical and Janus-like Micro/Nanomotors Powered by Enzymes

5.1 Systems based on mesoporous silicon

Mesoporous materials have garnered a lot of interest in the creation of spherical structures for a variety of applications, ranging from nanocatalysis to energy storage and conversion to healthcare. 43 Spherical mesoporous-silica-based particles, for example, have a limited size distribution, an ordered porous structure, a high pore volume and surface area, and, most importantly, an adjustable surface chemistry that allows biomolecules to be immobilized. 44 The CAT enzyme and single-stranded DNA were chemically conjugated and fixed on opposing sides of mesoporous silica particles (MSPs). The resultant micromotors (500–700 nm) were demonstrated to sense and collect compatible oligonucleotide fragments in biological samples that are self-propelled by bubble thrust at 2.5 wt H2O2. 45 The existence of holes or cavities on the surface of spherical micromotors is ideal for the nucleation of gas molecules. Sanchez and Ma created a Janus mesoporous silica cluster (JMSC) composed of nanoparticles with chemically attached CAT, based on the assumption of a rough surface. 46 Magnetically guided micromotors were produced by half-coating the JMSC with nickel and achieving thrust from oxygen bubbles at 1.5 wt H2O2.

Chemical conjugation of CAT, UR, and an ensemble of CAT/GOx or CAT/UR/GOx resulted in a number of MSP-based micro- and nanomotors. 17, 32, and 47. Their half-coating with SiO2, gold, nickel, or iron resulted in a Janus two-faced asymmetric structure of particles, with the last two coatings providing magnetic remote control for micro-/nanomotors. In the presence of fuel, micro-/nanomotors with diameters of 90–800 nm showed increased diffusion, indicating a chemophoretic propulsion mechanism. In terms of micro/nanomotor biocompatibility, those with immobilized GOx and UR were harmless to HeLa cells up to a concentration of 32 g mL1.

Submicron Janus micromotors (800 nm) were made using poly (dopamine) (PDA)-coated silica particles with one hemisphere pegylated with poly (l-lysine)-graft-poly (ethylene glycol) (PLL-g-PEG) for CAT and GOx immobilization utilizing a similar asymmetric structure idea. 48 Depending on the concentration of glucose as a primary fuel, the micromotors showed increased diffusion. It's worth mentioning that the scientists found that the diffusion coefficient in simulated biological media was greater than the diffusion coefficient in the buffer. This is in contrast to previous findings in the micromotor literature, which showed that the velocity of micromotors was reduced in complex biological media compared to buffers. In both phosphate-buffered saline (PBS) and aqueous solutions including urea as a fuel, 49 MSP-based core–shell nanobots (340 nm) with UR immobilized evenly on their surface showed increased diffusion. In the presence of urea, they were demonstrated to load and transport the drug doxorubicin to HeLa cells with enhanced release.16 Furthermore, anti-FGFR3 antibody-conjugated UR-powered MSPs (481 nm) for active self-propulsion in urine and targeting bladder cancer spheroids were created. 50 Passive MSPs without antibody achieved around 14 times greater internalization than actively propelled nanorobots with a targeting moiety. As a result of the nanorobots' generation of ammonia and the action of an anti-FGFR3 antibody inhibiting the fibroblast growth factor (FGF) signaling pathway, spheroid proliferation was reduced.

Covalent immobilization of lipase from Candida rugosa on the surface of MSP-based spheres (430 nm) was used to create hydrolase-powered nanomotors. Lipase has a dual function, generating energy by hydrolyzing water-soluble triacetin (glyceryl triacetate) as a fuel and degrading tributyrin, which is similar to blood lipid. 29

5.2 Polymer-based systems

Antibodies, peptides, and oligonucleotides are known to bind to streptavidin-functionalized polystyrene (PS) microspheres, which have been used in diagnostics and affinity-based purifications. 51 Biocompatible micromotors were made by immobilizing biotinylated CAT or UR evenly on the surface of streptavidin-functionalized PS microspheres (790 nm) based on the high streptavidin affinity for biotin. When the fuel concentration was raised, the micromotors showed greater diffusion (H_2O_2, urea). 7

Another method for making enzyme-powered micro-/nanomotors is layer-by-layer (LbL) self-assembly of polymers into spherical forms with complicated connections. Polyelectrolyte multilayered Janus capsules (8 m) were half-coated with a gold layer for chemical conjugation of CAT and with nickel for magnetic remote control to create soft polymer-based hybrid micromotors with a light-triggered drug release mechanism. 52 At 0.1 percent H_2O_2 and physiological temperature, the resultant micromotors were bubble propelled and followed a circular trajectory with a velocity of 4.2 m s1. Doxorubicin was loaded into anionic poly (styrene sulfonate) and cationic poly (allylamine hydrochloride) multilayers of micromotors and released on-demand by the NIR effect after being loaded into an ethanol solution.

The coupling of microfluidics with polymer hydrogel is regarded as a cutting-edge technological platform, with applications in cell culture, biosensing, sensing, and separation, the creation of improved microfluidic devices53, and the construction of artificial micro-/nanomotors. 54 Biocompatible hydrogel-based micromotors were created using a microfluidic chip and a bottom-up construction approach. Asymmetric motors (20 m) were made from cross-linked PEG diacrylate with entrapped CAT and dextran and bubble propelled at 2–4 percent H_2O_2, usually in a circular trajectory. 55

5.3 Metal–polymer hybrid systems

The symmetric and asymmetric immobilization of CAT, HRP, and cytochrome c (Cyt c) on opposite ends of nanorods made of polypyrrole (PPy) and Au segments resulted in a series of hemeproteinase-driven nanomotors (200 nm1.5–2.5 m). As a result of the enzymes being at different ends of the nanorods, gradients of reactants and products form along the nanorods, allowing self-propulsion via diffusiophoresis. 56 Electrophoresis was assumed for the self-propulsion mechanism of (HRP) PPy–Au (Cyt c) nanorods, since both enzymes were capable of transporting electrons to and from solid substrates. 37 In situ in the solution of nanomotors, XOD-catalyzed hypoxanthine turnover provided superoxide anion radicals and hydrogen peroxide.

6 Supramolecular Assemblies Powered by Enzymes

6.1 Polymer stomatocytes

Nanomotors (400 nm) with specific stomatocyte shapes were constructed using biocompatible supramolecular assemblies based on amphiphilic copolymers of PEG and PS. 57 The trapping of both CAT and a combination of CAT and GOx (1:3) in the "stoma" of stomatocytes resulted in nanomotors that were self-propelled in a solution of glucose (5 mm) by expelling oxygen molecules. It's worth mentioning that self-diffusiophoresis and bubble propulsion were found to govern the movement of similar platinum-based stomatocyte nanomotors in response to H_2O_2 concentration. 58

PEG-b-PS-based polymersomes were extruded over a membrane, resulting in their reassembly into 150 nm-sized polymersomes, which were tiny enough for internalization by the cells. The generated osmotic pressure shock 59 resulted in a stomatocyte form with encapsulated CAT with the addition of PEG as a fusogen of the polymersome membrane. 60 HeLa cells were able to pick up the enzyme-driven stomatocyte nanomotors and penetrate a monolayer of pulmonary artery endothelial cells. Biodegradable PEG-b-PDLLA-based stomatocytes were created by replacing the PS block with poly (d, l-lactide) (PDLLA). Covalent bonding of enzymatic biomolecules and surface functionalization of the resultant stomatocytes were made possible by using PEG ends with azide groups. Other functionalized molecules were connected to modified groups on the stomatocytes' outer surface, whilst CAT and GOx were covalently bonded inside the stoma of the stomatocytes. 61

6.2 MOFs

Two-dimensional MOFs are an interesting material platform for immobilizing molecular catalysts due to their large pore volume and specific surface area. 62 Kong et al. recently developed pH-responsive, CAT-powered micromotors by enclosing CAT and succinylated—lactoglobulin in a zeolitic imidazolate framework-L. (ZIF-L). 63 The self-propulsion of the CAT-immobilized porous framework particles with a cruciate flowerlike shape was dependent on the H_2O_2 concentration at pH 7. In an acidic environment (pH 5), succinylated-lactoglobulin experiences a sol–gel transition, preventing H_2O_2 access and resulting in random Brownian motion in the micromotors.

7 Conclusion

Because of their biocompatibility, bioavailability, and capacity to convert chemical energy into mechanical work even in ionic environments, enzyme-powered micro/nanomotors have attracted a lot of interest in the field of hybrid micro/nanomotors. Active targeting, cargo loading and release, and biosensing have all been made possible because of their sophisticated architecture and functionalization. A thorough knowledge of structural complimentary domains and synergistic interactions will give unique features for the bottom-up self-assembly of numerous enzymes in the development of bioinspired biomolecular machines in future concepts. 71-348

A concentration gradient will control the movement of enzyme-powered nanorobots in a certain direction. They will travel in medical applications in response to a gradient of chemicals released at areas of tissue injury or inflammation. Wilson et al. recently published a paper on CAT-powered biodegradable poly (lactic-co-glycolic acid) micromotors that moved in response to a hydrogen peroxide concentration gradient for the administration of a periodontal disease medication. 64 A phorbol ester activated macrophage cell was used to study the chemotactic self-propulsion of micromotors in an in vitro model of inflammatory periodontitis.

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