



Recent Progress in Enzyme-Driven Micro/Nanoswimmers: from Fundamentals to Potential Applications

Gerardo Salinas, Seyyed Mohsen Beladi-Mousavi, Alexander Kuhn

► To cite this version:

Gerardo Salinas, Seyyed Mohsen Beladi-Mousavi, Alexander Kuhn. Recent Progress in Enzyme-Driven Micro/Nanoswimmers: from Fundamentals to Potential Applications. *Current Opinion in Electrochemistry*, 2022, 32, pp.100887. 10.1016/j.coelec.2021.100887 . hal-03516094

HAL Id: hal-03516094

<https://cnrs.hal.science/hal-03516094>

Submitted on 7 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Recent Progress in Enzyme-Driven Micro/Nanoswimmers: from Fundamentals to Potential Applications

Gerardo Salinas¹, Seyyed Mohsen Beladi-Mousavi¹, Alexander Kuhn^{1*}

¹Univ. Bordeaux, CNRS, Bordeaux INP, ISM, UMR 5255, F-33607 Pessac, France

*Corresponding author contact: kuhn@enscbp.fr

Abstract

Recent progress in the field of enzyme-powered micro/nanoswimmers is summarized and discussed, covering different theoretical and mechanistic aspects, as well as potential applications. This is motivated by the increasing number of reports focusing on the design of biocompatible systems, able to move in complex environments and their potential use for biomedical applications. Motion is achieved by enzymatic reactions, enabling bubble-propulsion and self-diffusiophoretic or self-electrophoretic displacement. Single- and multiple-enzyme-powered micro/nanoswimmers are presented as interesting and original systems for cargo delivery, the detection of various analytes and the biodegradation of complex organic molecules.

Keywords: microswimmers, enzymes, biocompatibility, biomimetic systems

1. Introduction

Smart biological systems in living organisms can transform chemical energy into mechanical action, and hence carry out precise tasks in confined spaces [1,2]. Inspired by such natural motors, artificial biomimetic systems that can transform different types of energy into mechanical motion, have been developed. These so-called micro/nanoswimmers have gained considerable attention due to their numerous potential applications, e.g., for sensing, cargo delivery, and environmental remediation. However, these artificial devices often suffer from the toxicity of their components (e.g. Al, Ga, Cu) or the employed fuels (e.g. acidic or alkaline solutions, H₂O₂), thus partially preventing

their use for biomedical applications, such as biorecognition, biosensing, imaging and nano-surgery. In order to circumvent these limitations, more recent studies suggested miniaturized engines based on enzymatic catalysts, providing unique characteristics such as biocompatibility of the swimmers and fuels, as well as adaptability to different environments. Such self-propelled enzymatic micro/nanoswimmers, powered by different enzymes, have been proposed for exciting potential applications e.g. active targeted drug delivery [3] or blood-brain barrier crossing [4], which might be important for transporting pharmaceutical compounds toward localized areas inside organs. The rational design of these swimmers, incorporating various enzymes, is a critical factor concerning their performance, in particular the efficiency of their motion in complex environments. Hence, swimmers with different shapes (spherical or tubular) and sizes (from 200 nm to 5 mm), decorated with single or multiple enzymes, have been developed [5•]. Among the different enzymes used to power motion of micro- and nano swimmers, two different enzymatic processes are mostly used. The first category is based on redox reactions like it is the case for glucose oxidase, catalase, bilirubin oxidase or peroxidase, whereas a second category employs hydrolysis reactions e.g. urease and lipase (Table 1). The present review aims to discuss the recent advances concerning the mechanisms of propulsion and the potential applications of single- and multiple-enzyme powered micro/nanoswimmers. In addition, this report especially focuses on devices where motion is achieved by enzymatic process based on redox reactions or fully triggered by electrochemical processes.

Table 1. Representative enzymatic reactions used for the propulsion of micro and nanoswimmers.

Enzymatic processes using redox reactions	
Enzyme	Reaction
Glucose oxidase (GOx)	β -D-Glucose + O ₂ + H ₂ O → H ₂ O ₂ + D-glucono-1,5-lactone
Catalase	H ₂ O ₂ → 2H ₂ O + O ₂

Bilirubin oxidase (BOD)	$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow 2\text{H}_2\text{O}$
Peroxidase	$\text{H}_2\text{O}_2 + \text{AH}_2 \rightarrow 2\text{H}_2\text{O} + \text{A}$
Enzymatic processes using hydrolysis reactions	
Urease	$(\text{NH}_2)_2\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3$
Lipase	$\text{triglyceride} + 3\text{H}_2\text{O} \rightarrow \text{glycerol} + \text{fatty acids}$

48

49 2. Single-enzyme-powered micro/nanoswimmers

50 In the majority of the literature examples, the motion of single-enzyme-powered swimmers
51 is achieved via bubble-propulsion or self-diffusiophoretic mechanisms. Self-propulsion
52 allows decreasing mass transport limitations and enabling selective directional motion
53 towards specific locations. These swimmers are mostly built by immobilizing various
54 enzymes, such as urease [3,6-8], acetylcholinesterase, glucose oxidase, aldolase [9],
55 catalase [10-12], and lipase [13,14] on a large variety of miniaturized objects with different
56 architectures, by covalent binding, encapsulation, adsorption or drop-casting. For example,
57 Schmidt, et al. designed hybrid Ti/Au-catalase micro-tubes, where motion is based on the
58 enzymatic decomposition of H_2O_2 into water and oxygen (Figure 1a) [15•]. These devices
59 move faster, and with higher efficiency at low H_2O_2 concentrations compared to those
60 comprising a Pt catalyst. Recently, pH-responsive biocatalytic microswimmers were
61 designed by assembling catalase and succinylated β -lactoglobulin in a zeolitic imidazolate
62 framework-L (ZIF-L) (Figure 1b) [16•]. Motion control is achieved due to the pH-induced
63 reversible gelation process of β -lactoglobulin. At neutral pH, β -lactoglobulin is permeable,
64 enabling H_2O_2 to reach the enzymatic sites, causing motion due to a bubble propulsion
65 mechanism. However, at slightly acidic pH, gelation occurs, blocking the access of fuel and
66 stopping the motion. A similar principle was used to produce submarine-like
67 microswimmers that use buoyancy forces to control vertical motion. In this case poly-(2-

diisopropylamino)-ethyl methacrylate (PDPA) and catalase were incorporated into the ZIF-L structure [17]. At neutral pH, the oxygen generated by the enzymatic reaction binds to the hydrophobic PDPA, leading to an ascending motion. In contrast, at slightly acidic pH, PDPA becomes hydrophilic, causing the release of oxygen, and consequently a sinking of the device. In a recent work, protein micro-tubes, functionalized with urease, exhibit self-propulsion in the presence of urea, due to a concentration gradient established by the enzymatic reaction in the inner part of the tube [18] Motion of dissymmetric magnetic microparticles, asymmetrically modified with multilayers of biotinylated urease, has also been reported [19]. Most importantly, these devices move at physiological urea concentrations (10 mM) and in liquids with viscosities four times higher than the viscosity of water, which is important for future *in vivo* applications. Ma et al. synthesized hollow mesoporous SiO₂ microspheres functionalized with urease at the sphere surface [20]. The resulting propulsion is due to the intrinsic asymmetry of the SiO₂ spheres, generating ionic self-diffusiophoresis caused by the enzymatic decomposition of urea. An interesting alternative is the design of systems that can be triggered by different stimuli, thus facilitating motion in complex fluids. For example, lipase-modified dendritic silica/carbon/Pt dissymmetric nanoparticles can move either via self-diffusiophoresis or bubble propulsion, as well as self-thermophoresis (Figure 1c) [21]. In these devices, the Pt nanoparticles catalyze the disproportionation reaction of H₂O₂, the asymmetric photothermal effect of the carbon part causes a thermal gradient when irradiated with near-infrared light, and lipase, loaded in the pores of the dendritic structure, decomposes a triglyceride, causing concentration gradients. Finally, Arnaboldi et al. developed hybrid bioelectrochemical swimmers, for which motion is achieved when coupling the spontaneous reduction of oxygen by bilirubin oxidase (BOD) with the enantioselective

oxidation of 3,4-dihydroxyphenylalanine (L- or D-DOPA) by inherently chiral oligomers ((R)- or (S)-2,2'-bis[2-(5,2'-bithienyl)]-3,3'-bithianaphthene (oligo-BT₂T₄)) (Figure 1d) [22••]. Both, BOD and the enantiopure oligomers are immobilized on opposite extremities of a freestanding miniaturized polypyrrole film, thus allowing electron transfer between the cathodic and anodic sites. This electron transfer across the microswimmer is accompanied by a proton flux, resulting in self-electrophoretic propulsion.

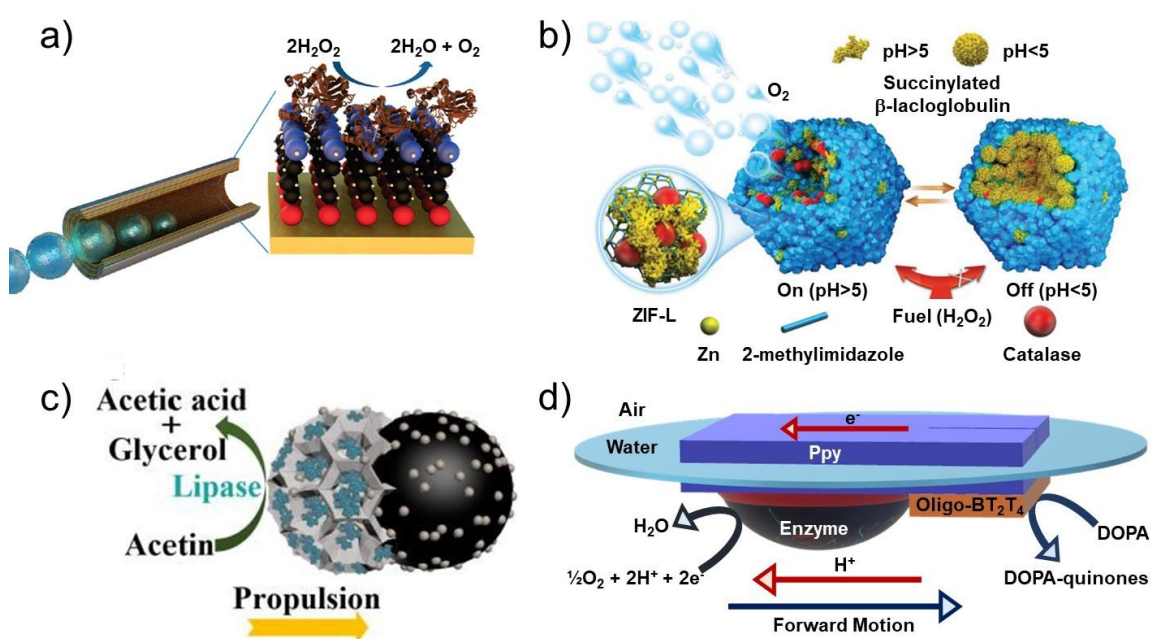


Figure 1. Schematic illustrations of different single-enzyme-powered micro/nanoswimmers. a) Open view of a hybrid biocatalytic micro-tube (left), and surface modification of the inner Au layer allowing the enzymatic decomposition of H₂O₂. Adapted from reference [15•]. b) Structure and propulsion mechanism of a biocatalytic microswimmer with pH-responsive on/off motion. Adapted from reference [16•]. c) Motion of a lipase-modified dendritic silica/carbon/Pt nanoparticles Janus-type swimmer, based on self-diffusiophoresis. Adapted from reference [21] d) Design of a hybrid

bioelectrochemical swimmer in an upside-down configuration at the air/water interface together with a representation of the mechanism of selective motion induced by proton flux. Adapted from reference [22••].

3. Multiple-enzyme-powered micro/nanoswimmers

Multiple-enzyme-powered micro/nanoswimmers have been introduced (i) to achieve higher velocities via combining multiple propulsion mechanisms; (ii) to avoid toxic fuels, particularly H_2O_2 ; (iii) to facilitate the adaption to different environments; and (iv) to generate more functionalities. Hence, swimmers modified with different enzymes, promoting enzymatic domino reactions have been developed. [23•-28]. From a more historical point of view, Mano et al. decorated a carbon fiber with GOx/redox polymer I (RPI) as a glucose oxidizing microanode at one extremity, and BOD/RPII as an O_2 reducing microcathode at the other end (Figure 2a). In the presence of glucose, the electron flow $\text{Glucose} \rightarrow \text{GOx} \rightarrow \text{RPI} \rightarrow \text{fiber} \rightarrow \text{RPII} \rightarrow \text{BOD} \rightarrow \text{O}_2$ is accompanied by an ion flow along the carbon fiber, resulting in its propulsion at the water/oxygen interface [29••]. Feringa, et al. designed swimmers based on multi-wall carbon nanotubes (MWCNTs), decorated with GOx and catalase to transform glucose into H_2O_2 and then to water and oxygen, respectively. Such domino reactions avoid the addition of H_2O_2 and hence lower the biotoxicity [30]. Following a similar elegant principle, Wilson et al. proposed bowl-shaped polymeric systems with a narrow opening containing GOx and catalase enzymes (Figure 2b) [31•]. These systems not only prevent the hydrolysis of the protected enzymes, but also allows generating motion of the biocompatible swimmers in the presence of physiological amounts of glucose (Figures 2b-c) [32]. In another example, Ma et al. assembled a core-shell nanoswimmer utilizing metal-organic frameworks to promote

photodynamic therapy (PDT) and starvation therapy (ST) (Figure 2d). In the involved domino reaction, GOx initially catalyzes the decomposition of intracellular glucose to promote cell starvation and H_2O_2 generation, whereas catalase decomposes the generated H_2O_2 , thus triggering motion and oxygen production, which promotes PDT [33]. An analog enzymatic system was employed by Städler et al., based on Janus microswimmers decorated by enzymes only on one hemisphere (Figure 2e). The diffusion of these biocompatible swimmers is enhanced in the presence of glucose [34]. In a more recent study, He et al. elaborated a nanoswimmer powered by GOx and catalase, trapped in a carbonaceous nanoflask (CNF), moving in a glucose solution via a domino reaction mechanism[35], similar to previous studies (Figure 2f)[30-34]. In this work, it was demonstrated that changes in surface wettability (hydrophobicity or hydrophilicity) of the nanoswimmer can dictate the direction of motion [35]. In another study, a dissymmetric microparticle was proposed where one side included two enzymes, GOx and trypsin, which were working as independent engines using different fuels, namely glucose and bis(benzyloxycarbonyl-L-arginine amide) (Figure 2g). Both enzymes contribute to the enhanced diffusion of swimmers using biocompatible fuels [36]. Finally, Van Hest et al. reported swimmers with a shape similar to the one of Figure 2b, containing multiple enzymes, promoting several feedforward loops using various substrates to produce kinetic energy as the output of the enzymatic network (Figure 2h) [37••].

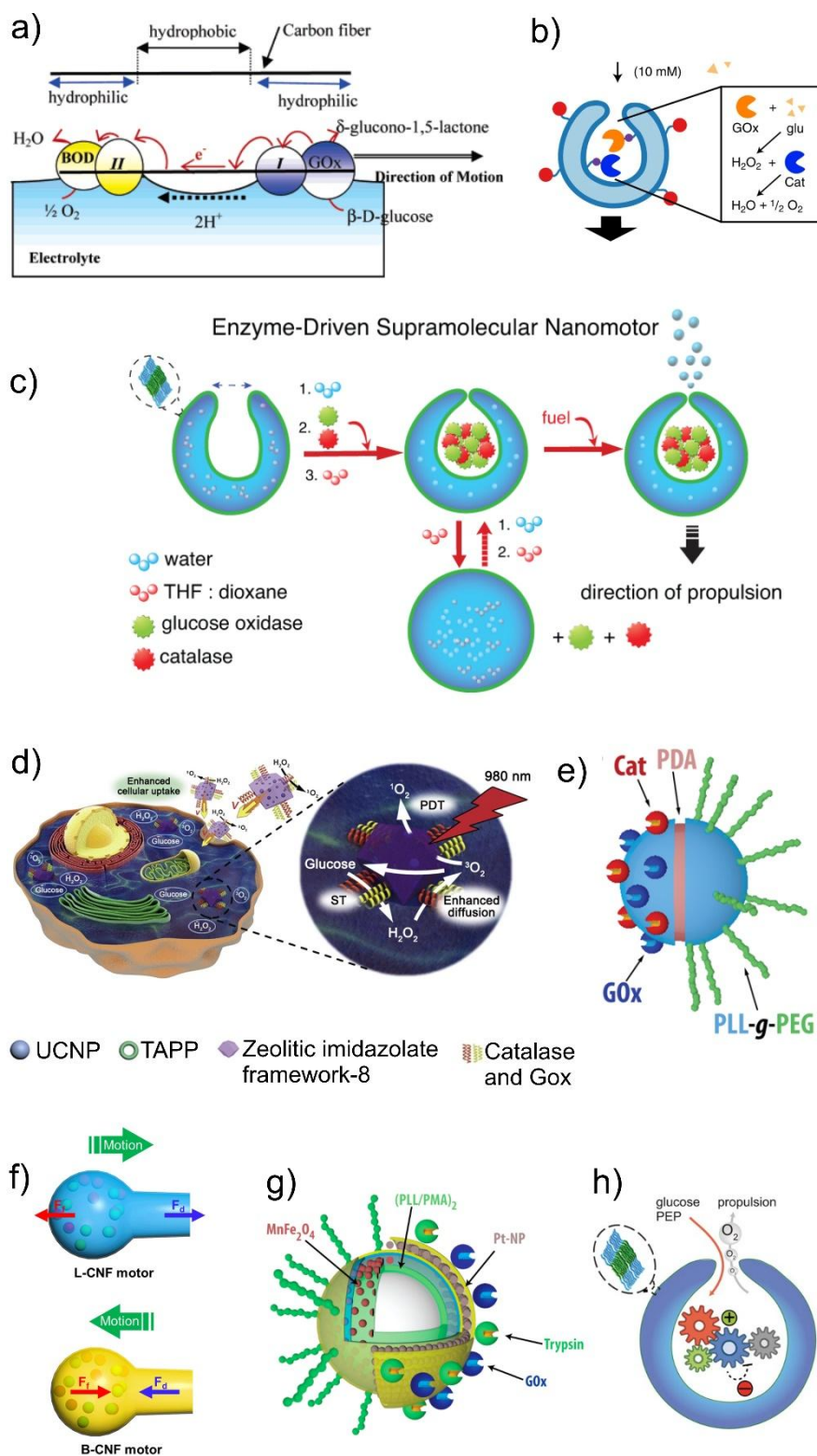


Figure 2. Schematic illustrations of dual/multiple-enzyme-powered micro/nanoswimmers.

a) The structure and propulsion of a carbon fiber decorated with GOx and BOD. Adapted

from reference [29••]. b) Biodegradable polymeric nanoswimmer encapsulating GOx and catalase. Adapted from reference [32]. c) Dual-enzyme (GOx-catalase) loaded supramolecular bowl-shaped nanoswimmer. Adapted from reference [31•]. d) Core-shell enzymatic nanoswimmer for synergetic photodynamic (PDT) and starvation therapy (ST). Adapted from reference [33]. e) Janus microswimmer with one hemisphere decorated with GOx and catalase. Adapted from reference [34]. f) Nanoswimmer powered by GOx and catalase trapped in a carbonaceous nanoflask (CNF). Adapted from reference [35]. g) Janus microswimmer decorated with GOx-Pt and trypsin. Adapted from reference [36]. h) Nanoswimmer containing four enzymatic cycles to convert glucose and phosphoenolpyruvate (PEP) into mechanical energy for the propulsion of the nanoparticle. Adapted from reference [37••].

4. Applications

Enzyme-powered micro/nanoswimmers have gained considerable attention in sensing, cargo delivery [38], and bioremediation [39•]. The enzymatic degradation of azo-dye pollutants by using laccase-based poly-(3,4-ethylenedioxythiophene)-polypyrrole/Pt (PEDOT-PPy-COOH/Pt) tubular microswimmers has been reported [40•]. In this case, disproportionation of H_2O_2 on Pt, present in the inner part of the tubes, is used to produce motion, whereas, laccase is immobilized at the outer part of the swimmer in order to catalyze the oxidation of the azo-dye compounds. An interesting alternative is a design of “2 in 1” systems, where the enzymatic reaction enables the propulsion mechanism and catalyzes the decomposition of organic pollutants. For example, lipase-modified mesoporous silica nanoparticles present random-walk in triglyceride/PBS solutions [41]. As motion is triggered by the catalytic decomposition of triglycerides, causing a

concentration gradient, these devices can act as active cleaners of fully and partially soluble oils. In a recent study, PEDOT-Au/peroxidase microswimmers were used as dynamic catalytic systems for the removal of phenolic compounds, such as phenol, bisphenol A, guaiacol, pyrogallol and catechol [39•]. In these devices the immobilized peroxidase not only catalyzes the decomposition of H_2O_2 to form oxygen (bubble propulsion), but also degrades the phenolic compounds (Figure 3a). Different urease-powered pH-responsive swimmers have been designed for the delivery of chemotherapeutic agents and bioimaging probes [3,42,43]. Drug-loaded catalase-powered nanoswimmers have been used for local drug delivery against inflammations [44]. These devices present positive chemotaxis towards H_2O_2 concentration gradients, produced by phorbol ester-stimulated macrophages. Recently, catalase-powered Au-mesoporous silica nanoparticles, functionalized with disulfide-linked oligo-(ethylene glycol) chains, acting as Janus gatekeepers have been reported [45]. These devices exhibit motion via the catalytic decomposition of H_2O_2 and deliver their cargo upon recognition of a reducing agent (glutathione). Finally, enzyme-powered swimmers, equipped with different transducers for pH, DNA, and biomarkers, have been used for sensing [46-48]. An interesting alternative is to take advantage of the *on-off* mode of light emission in the presence of a fluorescent molecule. For example, catalase-powered dissymmetric rods, functionalized with tetraphenylethene (TPE) derivatives and fluorescein isothiocyanate (FITC) as fluorophores, exhibit self-propulsion due to the enzymatic decomposition of H_2O_2 , and fluorescence changes from blue to green after the capture of tumor cells [49]. Another approach is the use of the changes in the swimming behavior, *i.e.* trajectory or speed, to detect species of interest. For example, PEDOT/Au-catalase micro-tubes exhibit biocatalytic inhibition of the enzyme in the presence of chemical stress (*e.g.*, heavy metals, pesticides and herbicides), which results in

a decrease of bubble production, directly detectable via their swimming behavior (Figure 3b) [50•]. Recently, the directional control of self-electrophoretic BOD/Ppy/oligo-BT₂T₄ swimmers has been demonstrated [22••]. Enantioselective clockwise or anti-clockwise motion is caused in this case by a site-specific proton flux associated with the oxidation of an enantiomer present in the solution (Figure 3c). In addition, the curvature of the track can be used for the direct visualization of the degree of enantiomeric excess in the solution.

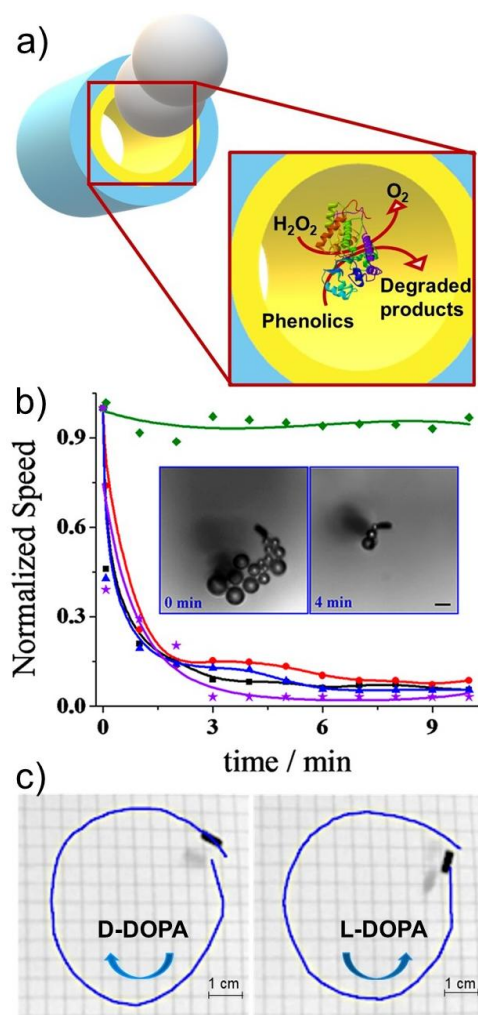


Figure 3. a) Scheme of a “2 in 1” peroxide-driven micromotor for bioremediation of phenolic pollutants. The active inner part of the swimmer catalyzes hydrogen peroxide

decomposition (propulsion) and the degradation of phenolic compounds by the immobilized peroxidase Adapted from reference [39•]. b) Normalized speed as a function of time for a PEDOT/Au-catalase micro-tube in the presence of: 100 μ M Hg (black square), 0.6 mM Cu (purple stars), 25 μ M sodium azide (red circle), 625 mM aminotriazole (blue triangle), and a control experiment without the toxins (green diamond). Inset: time-lapse images of the microswimmer recorded after 0 and 4 min swimming in a 100 μ M Hg solution. Scale bar, 6.0 μ m. Adapted from reference [50•]. c) Macroscopic enantiospecific motion of hybrid bioelectrochemical swimmers at the air/water interface of a solution containing 5 mM of D-DOPA (left) and 5 mM of L-DOPA (right). Adapted from reference [22••].

5. Conclusions and perspectives

In this review, we have examined recent work in the field of enzyme-driven micro/nanoswimmers based on different propulsion mechanisms. In the case of single-enzyme-powered swimmers, the enzymatic reaction triggers either the asymmetric release of bubbles or the self-generation of chemical or electric gradients, causing the propulsion of the devices. In contrast to this, in multiple-enzyme swimmers, a cascade of enzymatic reactions can generate motion based on bubble propulsion or self-electrophoresis. In addition, different applications of these devices are presented, where the enzymatic reaction is used either for the degradation of organic molecules or as analytical tools. These devices allow biomimetic self-propulsion, associated with improved biocompatibility, and efficient energy conversion. Their size and controlled trajectories might enable their use in different organs and cells. Nevertheless, there are still many issues to address, e.g. the toxicity of some fuels, the easy and direct visualization, the efficient biodegradability and the limited

life-time of the devices (recyclability). However, different approaches have been proposed to solve these problems, like modifying the shape, increasing the catalytic performance of the enzymes or using cascade reactions, and employing materials able to be digested completely by enzymes (e.g., proteases) or in biological media. Finally, the enzyme-based nanoswimmers, in particular those using redox reactions, have shown promises for biomedical applications. However, in order to develop drug delivery systems for “real world” *in vivo* applications, tiny robotic systems inspired by biological reactions in which robots and fuels are entirely biocompatible are mandatory and deserve additional research efforts. Furthermore, many other features of the robots, most importantly stability, biocompatibility, selectivity, directionality and the velocity in different biological environments need to be significantly improved prior to their commercialization.

6. Acknowledgements

The work has been funded by the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement n° 741251, ERC Advanced grant ELECTRA).

7. References and recommended reading

•Paper of special interest.

••Paper of outstanding interest.

1. Gao C, Wang Y, Ye Z, Lin Z, Ma X, He Q: **Biomedical micro-/nanomotors: From overcoming biological barriers to in vivo imaging.** *Adv Mater* 2020, 2000512.

251 2. Schwarz L, Medina-Sanchez M, Schmidt OG: **Hybrid biomicromotors**. *Appl Phys Rev*
252 2017, **4**:031301.

253 3. Tang S, Zhang F, Gong H, Wei F, Zhuang J, Karshalev E, Esteban-Fernández de Ávila
254 B, Huang C, Zhou Z, Li Z, Yin L, Dong H, Fang RH, Zhang X, Zhang L, Wang J:
255 **Enzyme-powered Janus platelet cell robots for active and targeted drug delivery**. *Sci*
256 *Robot* 2020, **5**:eaba6137.

257 4. Joseph A, Contini C, Cecchin D, Nyberg S, Ruiz-Perez L, Gaitzsch J, Fullstone G, Tian
258 X, Azizi J, Preston J, Volpe G, Battaglia G: **Chemotactic synthetic vesicles: Design and**
259 **applications in blood-brain barrier crossing**. *Sci Adv* 2017, **3**:e1700362.

260 5. Yuan H, Liu X, Wang L, Ma X: **Fundamentals and applications of enzyme powered**
261 **micro/nano-motors**. *Bioact Mater* 2021, **6**:1727-1749.

262 •Different enzyme-powered micro/nanoswimmers are discussed in detail.

263 6. Feng Y, Yuan Y, Wan J, Yang C, Hao X, Gao Z, Luo M, Guan J: **Self-adaptive**
264 **enzyme-powered micromotors with switchable propulsion mechanism and motion**
265 **directionality**. *Appl Phys Rev* 2021, **8**:011406.

266 7. Arque X, Andres X, Mestre R, Ciraulo B, Ortega-Arroyo J, Quidant R, Patino T,
267 Sanchez S: **Ionic species affect the self-propulsion of urease-powered micromotors**.
268 *Research* 2020, 2424972.

269 8. Hortelao AC, Patino T, Perez-Jimenez A, Blanco A, Sanchez S: **Enzyme-powered**
270 **nanobots enhance anticancer drug delivery**. *Adv Funct Mater* 2018, **28**:1705086.

- 271 9. Arque X, Romero-Rivera A, Feixas F, Patino T, Osuna S, Sanchez S: **Intrinsic**
272 **enzymatic properties modulate the self-propulsion of micromotors.** *Nat Commun* 2019,
273 **10**:2826.
- 274 10. Somasundar A, Ghosh S, Mohajerani F, Massenburg LN, Yang T, Cremer PS, Velegol,
275 D, Sen A: **Positive and negative chemotaxis of enzyme-coated liposome motors.** *Nat*
276 *Nanotech* 2019, 14:1129-1134.
- 277 11. Sun J, Mathesh M, Li W, Wilson DA: **Enzyme-powered nanomotors with controlled**
278 **size for biomedical applications.** *ACS Nano* 2019, **13**:10191-10200.
- 279 12. Chen C, He Z, Wu J, Zhang X, Xia Q, Ju H: **Motion of enzyme powered microshell**
280 **motors.** *Chem Asian J* 2019, **14**:2491-2496.
- 281 13. Hu Y, Sun Y: **Autonomous motion of immobilized enzyme on Janus particles**
282 **significantly facilitates enzymatic reactions.** *Biochem Eng J* 2019, **149**:107242.
- 283 14. Wang L, Marciello M, Estevez-Gay M, Soto Rodriguez PED, Luengo Morato Y,
284 Iglesias-Fernandez J, Huang X, Osuna S, Filice M, Sanchez S: **Enzyme conformation**
285 **influences the performance of lipasepowered nanomotors.** *Angew Chem Int Ed* 2020,
286 **59**:21080-21087.
- 287 15. Sanchez S, Solovev AA, Mei Y, Schmidt OG: **Dynamics of biocatalytic microengines**
288 **mediated by variable friction control.** *J Am Chem Soc* 2010, **132**:13144-13145.
- 289 •First report on the efficient use of enzymes as catalysts in self-propelled microswimmers.

- 290 16. Gao S, Hou J, Zeng J, Richardson JJ, Gu Z, Gao X, Li D, Gao M, Wang DW, Chen P,
291 Chen V, Liang K, Zhao D, Kong B: **Superassembled biocatalytic porous framework**
292 **micromotors with reversible and sensitive pH-speed regulation at ultralow**
293 **physiological H₂O₂ concentration.** *Adv Funct Mater* 2019, 1808900.
- 294 •Motion control due to a reversible pH-induced gelation process of β -lactoglobulin.
- 295 17. Gao Z, Wang T, Rawal A, Hou J, Cao Z, Zhang H, Xu J, Gu Z, Chen V, Liang K:
296 **Biocatalytic self-propelled submarine-like metal-organic framework microparticles**
297 **with pH-triggered buoyancy control for directional vertical motion.** *Mater Today* 2019,
298 **28:10-16.**
- 299 18. Sugai N, Morita Y, Komatsu T: **Nonbubble-propelled biodegradable microtube**
300 **motors consisting only of protein.** *Chem Asian J* 2019, **12:2953-2957.**
- 301 19. Luo Ming, Li S, Wan J, Yang C, Chen B, Guan J: **Enhanced propulsion of urease-**
302 **powered micromotors by multilayered assembly of ureases on Janus magnetic**
303 **microparticles.** *Langmuir* 2020, **36:7005-7013.**
- 304 20. Xu D, Zhou C, Zhan C, Wang Y, You Y, Pan Xi, Jiao J, Zhang R, Dong Z, Wang W,
305 Ma X: **Enzymatic micromotors as a mobile photosensitizer platform for highly**
306 **efficient on-chip targeted antibacteria photodynamic therapy.** *Adv Funct Mater* 2019,
307 **1807727.**
- 308 21. Xing Y, Du X, Xu T, Zhang X: **Janus dendritic silica/carbon@Pt nanomotors with**
309 **multiengines for H₂O₂, near-infrared light and lipase powered propulsion.** *Soft Matter*
310 **2020, 16:9553-9558.**

311 22. Arnaboldi S, Salinas G, Karajic A, Garrigue P, Benincori T, Bonetti G, Cirilli R,
 312 Bichon S, Gounel S, Mano N, Kuhn A: **Direct dynamic readout of molecular chirality**
 313 **with autonomous enzyme driven swimmers.** *Nat Chem* 2021,
 314 <https://doi.org/10.1038/s41557-021-00798-9>.

315 •• First example of enantioselective trajectory control.

316 23. Ma X, Hortelão AC, Patiño T, Sánchez S: **Enzyme catalysis to power**
 317 **micro/nanomachines.** *ACS Nano* 2016, 10:9111-9122.

318 •Different enzyme-powered micro/nanoswimmers are discussed in detail.

319 24. Mathesh M, Sun J, Wilson DA: **Enzyme catalysis powered micro/nanomotors for**
 320 **biomedical applications.** *J Mater Chem B* 2020, 8:7319-7334.

321 25. Zhao X, Gentile K, Mohajerani F, Sen A: **Powering motion with enzymes.** *Acc Chem*
 322 *Res* 2018, **51**:2373-2381.

323 •Comprehensive review covering the field of enzyme powered swimmers.

324 26. Patiño T, Arqué X, Mestre R, Palacios L, Sánchez S: **Fundamental aspects of**
 325 **enzyme-powered micro- and nanoswimmers.** *Acc Chem Res* 2018, 51:2662-2671.

326 27. Ou J, Liu K, Jiang J, Wilson DA, Liu L, Wang F, Wang S, Tu Y, Peng F: **Micro-**
 327 **/nanomotors toward biomedical applications: The recent progress in biocompatibility.**
 328 *Small* 2020, **16**:1906184.

329 28. Vilela D, Blanco-Cabra N, Eguskiza A, Hortelao AC, Torrents E, Sanchez S: **Drug-**
330 **free enzyme-based bactericidal nanomotors against pathogenic bacteria.** *ACS Appl*
331 *Mater Interfaces* 2021, **13**:14964-14973.

332 29. Mano N, Heller A: **Bioelectrochemical propulsion.** *J Am Chem Soc* 2005, **127**:11574-
333 11575.

334 •• First example of a bioelectrochemical swimmer.

335 30. Pantarotto D, Browne WR, Feringa BL: **Autonomous propulsion of carbon**
336 **nanotubes powered by a multienzyme ensemble.** *Chem Commun* 2008, **13**:1533-1535.

337 31. Abdelmohsen LKEA, Nijemeisland M, Pawar GM, Janssen, G-JA, Nolte RJM, van
338 Hest JCM, Wilson DA: **Dynamic loading and unloading of proteins in polymeric**
339 **stomatocytes: Formation of an enzyme-loaded supramolecular nanomotor.** *ACS Nano*
340 2016, **10**:2652-2660.

341 •An enzyme-driven swimmer elaborated with biocompatible organic polymers.

342 32. Toebes BJ, Cao F, Wilson DA: **Spatial control over catalyst positioning on**
343 **biodegradable polymeric nanomotors.** *Nat Commun* 2019, **10**:5308.

344 33. You Y, Xu D, Pan X, Ma X: **Self-propelled enzymatic nanomotors for enhancing**
345 **synergetic photodynamic and starvation therapy by self-accelerated cascade reactions.**
346 *Appl Mater Today* 2019, **16**:508-517.

347 34. Schattling P, Thingholm B, Städler B: **Enhanced diffusion of glucose-fueled Janus**
348 **particles.** *Chem Mater* 2015, **27**:7412-7418.

- 349 35. Gao C, Zhou C, Lin Z, Yang M, He Q: **Surface wettability-directed propulsion of**
350 **glucose-powered nanoflask motors.** *ACS Nano* 2019, **13**:12758-12766.
- 351 36. Schattling PS, Ramos-Docampo MA, Salgueiriño V, Städler B: **Double-fueled Janus**
352 **swimmers with magnetotactic behavior.** *ACS Nano* 2017, **11**:3973-3983.
- 353 37. Nijemeisland M, Abdelmohsen LKEA, Huck WTS, Wilson DA, van Hest JCM: **A**
354 **compartmentalized out-of-equilibrium enzymatic reaction network for sustained**
355 **autonomous movement.** *ACS Cent Sci* 2016, **2**:843-849.
- 356 •• First example of multiple-enzyme-driven swimmers promoting several feedforward
357 loops.
- 358 38. Choi H, Cho SH, Hahn SK: **Urease-powered polydopamine nanomotors for**
359 **intravesical therapy of bladder diseases.** *ACS Nano* 2020, **14**:6683-6692.
- 360 39. Bayraktaroglu M, Jurado-Sanchez B, Uygun M: **Peroxidase driven micromotors for**
361 **dynamic bioremediation.** *J Hazard Mater* 2021, 418:126268.
- 362 •”2 in 1” system where the enzymatic reaction produces motion and simultaneously
363 degradation of organic pollutants.
- 364 40. Uygun M, Asuncion-Nadal V, Evli S, Uygun DA, Jurado-Sanchez B, Escarpa A: **Dye**
365 **removal by laccase-functionalized micromotors.** *Appl Mater Today* 2021, **23**:101045.
- 366 • Enzymatic degradation of azo-dye pollutants by using laccase-based swimmers

- 367 41. Wang Lei, Hortelao AC, Huang X, Sanchez S: **Lipase-powered mesoporous silica**
368 **nanomotors for triglyceride degradation.** *Angew Chem Int Ed* 2019, **58**:7992-7996.
- 369 42. Chen Z, Xia T, Zhang Z, Xie S, Wang T, Li X: **Enzyme-powered Janus nanomotors**
370 **launched from intratumoral depots to address drug delivery barriers.** *Chem Eng*
371 *Technol* 2019, **375**:122109.
- 372 43. Llopis-Lorente A, Garcia-Fernandez A, Murillo-Cremaes N, Hortelao AC, Patino T,
373 Villalonga R, Sancenon F, Martinez-Manez R, Sanchez S: **Enzyme-powered gated**
374 **mesoporous silica nanomotors for on-command intracellular payload delivery.** *ACS*
375 *Nano* 2019, **13**:12171-12183.
- 376 44. Wang J, Jelle Toebes B, Plachokova AS, Liu Q, Deng D, Jansen JA, Yang F, Wilson
377 DA: **Self-propelled PLGA micromotor with chemotactic response to inflammation.**
378 *Adv Healthcare Mater* 2020, **9**:1901710.
- 379 45. Llopis-Lorente A, Garcia-Fernandez A, Lucena-Sanchez E, Diez P, Sancenon F,
380 Villalonga R, Wilson DA, Martinez-Manez R: **Stimulus-responsive nanomotors based on**
381 **gated enzyme-powered Janus Au–mesoporous silica nanoparticles for enhanced cargo**
382 **delivery.** *Chem Commun* 2019, **55**:13164.
- 383 46. Russell SM, Alba-Patino A, Borges M, de la Rica R: **Multifunctional motion-to-color**
384 **Janus transducers for the rapid detection of sepsis biomarkers in whole blood.** *Biosens*
385 *Bioelectron* 2019, **140**:111346.
- 386 47. Zhang X, Chen C, Wu J, Ju H: **Bubble-propelled Jellyfish-like micromotors for**
387 **DNA sensing.** *ACS Appl Mater Interfaces* 2019, **11**:13581-13588.

388 48. Patino T, Porchetta A, Jannasch A, Llando A, Stumpp T, Shcaffer E, Ricc F, Sanchez
389 S: **Self-sensing enzyme-powered micromotors equipped with pH responsive DNA**
390 **nanoswitches**. *Nano Lett* 2019, **19**:3440-3447

391 49. Zhao L, Liu Y, Xie S, Ran P, Wei J, Liu Q, Li X: **Janus micromotors for motion-**
392 **capture-ratiometric fluorescence detection of circulating tumor cells**. *Chem Eng*
393 *Technol* 2020, **382**:123041.

394 50. Orozco J, Garcia-Gradilla V, D'Agostino M, Gao W, Cortes A, Wang J: **Artificial**
395 **Enzyme-Powered Microfish for Water-Quality Testing**. *ACS Nano* 2013, **7**:818-824.
396 •Inhibition of the enzyme due to chemical stress can be directly correlated with changes in
397 the swimming behavior.

398

