



Nano-powered nanobots could be useful in gene therapy delivery

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Abstract

Rapid improvements in robotic technology have resulted in significant advances in the field of enzyme biotechnology. The development of novel pathways in the field of enzyme engineering has been assisted by advancements in the manufacture of robotic platform technology. Biocompatible fuels may be used to control the movement of nanobots and enzyme-powered nanomotors, which makes them particularly interesting for use in medical applications (e.g., glucose, urea). Technology, regulatory, and commercial barriers continue to stand in the way of the widespread use of nanobots in biotechnology and medicine. Recent developments, on the other hand, have demonstrated proof of concept and shown promise for the future. Nanobots have been shown to be useful in the administration of medication, and it is expected that future developments will change pharmaceutical and medical science.

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Introduction Enzymes and biocatalysts are used in industries for a variety of reasons, with the goal of producing a wide range of sustainable goods (Diefenbach et al. 2018; Sinha and Shukla 2019). Synthetic biology and other molecular biology tools are widely used to engineer enzymes (Labrou 2010; Platis and Labrou 2008; Chronopoulou et al. 2018; Mohammadi et al. 2018; Shukla 2019; Mandeep et al. 2020; Dixit et al. 2020) and fabricate robots (Hägele et al. 2016; Mandeep et al. 2020; Dixit et al. 2020). Robots are rudimentary devices designed to do a certain task. The robotic system's energy is provided directly by batteries or power connectors. Complex laboratory procedures generally demand a lot of time and careful management at every stage. These procedures are time-consuming and need expert assistance. Based on the sensory tools given for the reaction, the robotic platforms enable autonomous handling and accuracy. Life science researchers have been striving to build molecular robots that can respond to external environmental stimuli on their own by interpreting molecular signals, inspired by mechanical engineering (Murata et al. 2013). Enzyme engineering techniques that use recombinant DNA technology improve the utility and applicability of existing enzymes (Basheer and Chellappan 2017; Arbige et al. 2019; Maseko et al. 2019; Mallajosyula et al. 2020). The two most often used techniques in enzyme engineering are rational design and guided evolution (Labrou 2010; Platis and Labrou 2008; Vogel 2019). The need for using high-throughput screening (HTS) techniques for new enzyme discovery or engineering is undeniable (Longwell et al. 2017). As a result, robots speed up the identification of new or innovative enzymes, as well as the development of applications in a variety of sectors (Seo et al. 2018). Another important application area for robots is the optimization of purification methods for protein and enzyme synthesis (Labrou 2003). Robotics has aided in the development of numerous fields, including enzyme screening, biofuel production, and medication delivery, and has put them into practice with great success (Bunzel et al. 2018; Peng et al. 2020; Chen et

al. 2017). The goal of this study is to give an overview of how robots and nanobots are being used in enzyme biotechnology to address the difficulties and dangers created by the inadequacy of current industrial demands and requirements.

An overview of robots' applications in many technical fields.

Various technologies for the discovery or creation of several enzymes and proteins have been developed over the previous decade (Perperopoulou et al. 2018; Labrou 2010). However, as robotics has progressed, new opportunities have arisen (Mazurenko et al. 2019). Robotic systems with high throughput have been developed and are now playing an increasingly significant role in the discovery of new enzymes (Jacques et al. 2017). At the moment, high-throughput robots capable of doing up to 100,000 tests per day are in use (Ye et al. 2018). A robotic handling system, a liquid handling system, a sensor system, and control software are all included in such systems (Fleischer et al. 2018; Haby et al. 2019; Zeng et al. 2020). To improve high-performance screening robotic platforms, fluorescence-activated cell sorting (FACS) and microfluidic droplet devices have been created (Zeng et al. 2020).

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Robotic systems with high throughput require special tools and procedures.

Intensive research has been conducted over the last decade to create new tools and procedures for the design and manufacturing of high-throughput robotic systems. Droplet-based microfluidics have a lot of potential for high-throughput applications since they have better performance, use a lot less assay volume, and take a lot less time and money to analyze than standard high-throughput screening methods. Micro-droplets form when immiscible fluids are mixed in a microfluidics channel (Longwell et al. 2017). Droplet-based microfluidic devices have been created and have become indispensable instruments for assaying numerous enzymes, owing to their better high-throughput capabilities and lower assay volumes (Longwell et al. 2017). Droplet-based microfluidics, in particular, has been successfully utilized to create nano-to-picoliter-scale forms with controlled shape and composition, based on the emulsification of immiscible non-polar solution-like oil in an aqueous solution (Weng and Spoonamore 2019). Droplets are compatible with a wide range of enzymes and test formats, which is a key benefit of droplet-based microfluidics. Droplet-based microfluidics is mostly used to screen enzymes that are presented on the cell surface or enzymes with cell-impermeable substrates (Zeng et al. 2020), allowing for simultaneous quantitative and qualitative evaluations.

At the single-cell level, methods based on fluorescence-activated cell sorting (FACS) have been developed for screening desired target cells (Zeng et al. 2020). Microfluidic FACS, which combines microfluidics and cell sorting, can help to overcome the limits of traditional FACS. Enzyme microchambers have also been developed and tested. These microchambers may separate each enzyme variation into its own vessel, allowing the microtiter plate format to be miniaturized. Enzyme engineering and strain screening have both benefited from the development of microcapillary and microwell arrays. In 96-well microtiter plates, high-throughput culture analysis may be performed (Regnault et al. 2018), providing for quick and efficient strain and growth condition screening. Dorr and colleagues, for example, created a high-throughput enzyme screening robotic device in 2016. In comparison to the microfluidic FACS-based screening technology, this robotic platform has been shown to be more efficient. This technology, in particular, was able to screen huge mutant libraries and give consistent enzyme screening and selection findings. This platform was also utilized to monitor and analyze different microorganism growth circumstances (Dörr et al. 2016). Haby and colleagues have also created a high-throughput robotic micro bioreactor. This technology combines the trade-off between experimental throughput and process control, allowing online monitoring of both cultivation conditions and process parameters at the same time (Haby et al. 2019).

Enzyme screening and microbial engineering robots

The development and fabrication of fully automated robotic systems for high-throughput enzyme screening is currently underway. Several kinds of enzymes, including hydrolases, Baeyer village monooxygenase, dehalogenase, transaminase, and acylase, have been effectively screened using such platforms. The expression of cellulose cel5A in *K. lactis*, for example, was studied utilizing the fully automated robotic system RoboLector (Mühlmann et al. 2017). In another work, the RoboLector system was used to do automatic high-throughput screening using multi-well filter plates with micro and ultra-filtration stages. This robotic system effectively completed a combined screening of both upstream (medium preparation, inoculation, culture) and downstream processing (enzyme extraction and analysis) parameters (Mühlmann et al. 2017). A library of mutant glucose oxidases exhibited on the surface of yeast cells was successfully screened utilizing a flow cytometry high-throughput screening technique in another work. The research led to the discovery of two mutants with 1.3 and 2.3 times higher V_{max} values than the wild-type enzyme (Kovaevi et al. 2019). In another study, Leferink and colleagues created an automated approach for screening plant monoterpene cyclases/synthases (mTC/S) produced in *E. coli* libraries. The screening system looked into robotic liquid handling systems with GC-MS capabilities (Leferink et al. 2019). Diverse machine learning technologies, including artificial neural networks (ANN), genetic algorithm-artificial neural networks (GA-ANN), and other comparable tools, have recently been coupled with high-throughput screening approaches and investigated in various enzyme engineering procedures (Kumar et al. 2019; Mandeep et al. 2021; Saini et al. 2020).

Beneyton and colleagues released another fascinating paper in which they employed a droplet-based microfluidic technology to evaluate designed enzymes. The platform was created by utilizing the yeast *Yarrowia lipolytica*'s superior secretion capability. Five enzymes from *Aspergillus niger* were chosen, overexpressed, and secreted in active forms in the crude supernatant of *Yarrowia lipolytica* (endo-1,4-xylanase B and C; 1,4-cellobiohydrolase A; endoglucanase A; aspartic protease). A droplet-based microfluidic device was used to screen the enzymes (Beneyton et al. 2017).

Michael and colleagues (Michael et al. 2008) created a quantitative high-throughput screening (qHTS) system that can give quantitative measurements at various concentrations of chemicals, allowing the creation of concentration-response curves and an inclusive data set for each experiment. In comparison to traditional screening approaches, the robotic platform was able to perform several tests in the same set of circumstances (Michael et al. 2008).

Biological robots are utilized for enzyme screening in the same way as mechanical robots are. A biological robot, for example, was created to screen aspartate kinase III variations of the bacterial phage M13. By inserting the plasmid AP-Lys-B into *E. coli* XL-Blue cells, the biological robot was created. On the basis of cell-phage interactions, parallel and high-throughput screening was realized. The sensitivity of this cell-phage screening method was excellent in a high-throughput, low-cost configuration (Song and Zeng 2017).

The use of robotic systems in biofuel production

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Microorganisms' capacity to release biofuels at a quick rate and at a low cost on an industrial scale is critical to their effectiveness in converting biomass into biofuels (Koppolu and Vasigala 2016). Microorganisms have been manipulated at the gene level to increase their production capacities thanks to genetic alterations (Tiwari et al. 2018; Singhal et al. 2020). Metabolic engineering, like genetic engineering, is a common strategy for transferring biofuel-producing pathways into microbial hosts, allowing for increased biofuel generation, quality, and yield. Using high-throughput robotic platforms, many microorganisms, such as bacteria, cyanobacteria, microalgae, and yeasts, have been extensively produced and tested (Nabil-Adam et al. 2020; Arnone 2020). *Mesorhizobium loti* was shown to have a very effective carbonic anhydrase. *Chlorella vulgaris* and *Chlorella sorokiniana* were electroporated with the carbonic anhydrase gene cloned into an expression vector (Lin et al. 2018). These transgenic strains were cultured in a two-layer photo bioreactor (TPR). Transgenic microalgae produced more biomass, had a higher protein content, and accumulated more lipids. The transformants were able to produce an increase in lipid accumulation of up to 1.1 g/L. (Lin et al. 2018). Furthermore, microalgae genetic engineering has been widely used for the production of biofuel precursors such as triacylglycerol and polysaccharide starch, which may be converted into biodiesel and bioethanol. *C. reinhardtii* transgenic cells overexpressing acetyl-CoA synthetase, which catalyzes the conversion of acetate to acetyl-CoA, for example, demonstrated a 2-fold increase in starch content and a 60% rise in acyl-CoA pool (Rengel et al. 2018). Other species, such as *E. coli*, have also been studied as potential hosts for biofuel generation. Majidian and colleagues, for example, engineered the *Escherichia coli* KO11 strain by adding the pyruvate decarboxylase and alcohol dehydrogenase genes from *Zymomonas mobilis* and deleting the fumarate reductase gene at the same time. The modified strain was successfully cultivated at a 3.5 percent (v/v) ethanol

concentration using sophisticated nutritional additives (Majidian et al. 2018). Another study used the alcohol dehydrogenase and aldehyde dehydrogenase genes from the fungus *Aspergillus nidulans* to create a genetically modified *Escherichia coli* strain that uses ethanol as a carbon source. Mevalonic acid was biosynthesised using the ethanol generated (Cao et al. 2020).

Through metabolic engineering, robot-assisted techniques have improved the generation of biofuels. Various types of robotic platforms have been developed for single-cell analysis and have been shown to be effective bioethanol production equipment (Chang et al. 2013). High-throughput robotic systems for fast cloning, expression, and screening of heterologous genes in bacteria and yeast strains have been created (Hughes et al. 2011). Researchers used the high-throughput robotic platform RoboLector to screen cellulases from *Trichoderma reesei* (Mühlmann et al. 2017). Another research created a high-throughput robotic device for semi-quantitative monitoring of alcohol production in *C. acetobutylicum* microtiter cells. The use of this test permitted the discovery of butanol-tolerant strains with a 420 percent higher butanol production potential than the wild-type strain. The semi-quantitative screening technique was shown to be suitable, offering new possibilities for combinatorial methods to enhance solventogenic *Clostridia* (Scheel and Lütke-Eversloh 2013).

For the development of efficient medication delivery systems, robotic systems are being used.

Biomolecular engineering techniques have made considerable progress in the creation of effective medication delivery systems (Gupta et al. 2017; Li et al. 2019; Nabil-Adam et al. 2020; Irais et al. 2020). A nanorobot is a molecule with specific characteristics that aid in the completion of a certain task. As medication delivery vehicles, effective microrobots and nanobots have recently been created. Self-contained electrical, electric, or mechanical characteristics are encoded into such molecular structures. Nanobots having self-propelled nanomotors, for example, and the capacity to carry and deliver cargo (e.g., medication) to particular target locations (e.g., sick cells) are critical. Chemotaxis is the capacity of nanobots to migrate towards their target locations (Plutnar and Pumera 2019). Nanobot design and fabrication is a challenging process. This is because nanorobotics encompasses a wide range of disciplines, including nanofabrication techniques for nanoactuators, nanomotors, and nanosensors. To transport and deliver medicines to the targeted locations, various nanomaterials such as liposomes, polymersomes, metal nanoparticles, mesoporous silica nanoparticles, dendrimers, and magnetic nanoparticles are used (Luo et al. 2018). Many methods are being used to achieve motions in desired directions at the nano-microscale, including magnetic fields, ultrasonic waves, and light (Luo et al. 2018). The nanobots are made in two ways: bottom-up and top-down (Wang and Pumera 2015). Several studies have been published that show how to leverage enzymes' controlled self-assembly capabilities to create purpose-designed nanomachines for a variety of purposes. Enzyme-powered nanomachines use enzyme-triggered chemical changes to release energy stored in a substrate's chemical bonds, allowing them to actuate themselves into active motion. Enzymes play a crucial part in many biological processes, turning chemical energy into kinetic energy and completing numerous tasks. The ability to reduce random Brownian motion and create active movement is provided by substrate turnover (Ma et al. 2016; Shahriari et al. 2019). Using enzymatic processes to create

self-propulsion, researchers have lately studied several synthetic micro/nanobots (Halder and Sun 2019). Many enzymes are used as nanobot power sources, including glucose oxidase, catalase, acetylcholinesterase, trypsin, and urease (Jimenez-Falcao et al. 2019; Abdelmohsen et al. 2016; Zhao et al. 2018; Schattling et al. 2017; Luo et al. 2020). Hortelo et al. (2018) created nanobots made of silica nanoparticles with a solid silica core and a mesoporous silica shell. The modified Stöber technique was used to make these nanobots, which included the use of cetyltrimethyl ammonium bromide as a porogenic agent and triethanolamine as a base catalyst. 3-aminopropyltriethoxysilane, which supplied amino groups on the surface of nanoparticles, was used to further modify them. Using glutaraldehyde as a linker molecule, these amino groups were covalently attached to urease. In urea-containing ionic solutions, urease coated in a mesoporous silica shell was able to convert chemical energy into kinetic energy (Hortelo et al. 2018).

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Because of urease, such shells may convert urea to carbon dioxide. Because the shells always have minute asymmetries, the carbon dioxide creates a current in the fluid that propels them out of the shell in a jet-like fashion. Another research developed ultra-small stomatocyte nanobots with a size of about 150 nm. The inner compartments of stomatocytes were changed into catalase in order to produce such stomatocyte nanomachines. In a related study, nanobots based on Janus Au–mesoporous silica nanoparticles with self-propulsion and controlled drug release capabilities were produced with the breakdown of H₂O₂ to O₂ causing movement of stomatocyte nanobots in an O₂ concentration-dependent manner (Sun et al. 2019). Nanobots were created using the Pickering emulsion technique. The resultant nanostructures were functionalized with disulfide-associated gatekeepers on the mesoporous side and catalase on the gold side (Llopis-Lorente et al. 2019). Jana and colleagues created enzyme-based nanoparticles with an average size of 193.3 nm for insulin delivery in a more recent study. Hyaluronic acid and 2-nitroimidazole were used in an ionic gelation method to create the nanoparticles. To provide self-propulsion capabilities, the nanoparticles were loaded with insulin and connected to glucose oxidase. These nanoparticles may release the encapsulated insulin at high glucose concentrations (hyperglycemic state), indicating that enzyme-dependent nanoparticles could be used as biomimetic devices for regulated insulin release (Jana et al. 2020).

Conclusion

Rapid advancements in robotic technology have resulted in substantial gains in enzyme biotechnology. The creation of new paths in the field of enzyme engineering has been aided by improvements in robotic platform manufacturing. The movement of nanobots and enzyme-powered nanomotors may be controlled using biocompatible fuels, which makes them particularly appealing (e.g., glucose, urea). Nanobots in biotechnology and medicine continue to encounter technological, regulatory, and commercial hurdles. Recent advancements, however, have proven proof of concept and indicated future promise. Nanobots have been shown to be beneficial in medication delivery, and further advancements are predicted to transform pharmaceutical and medical science (Moataz Dowaidar. 2008-2023).

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