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Summary
Algae-inspired microrobots (AIMs) have attracted intense research over the past decade owing to the abundant desired properties of natural microalgae, such as biocompatibility, autofluorescence, and pharmaceutical activity, which make them ideal candidates for biomedical and related applications. With the deepening and widening of applied research, the functions of AIMs have been greatly enriched and enhanced to meet the needs of demanding application scenarios including targeted drug delivery, anticancer/antibacterial therapy, cell stimulation, wound healing, biomolecule sensing, etc. Notwithstanding, multiple challenges remain to be tackled for transformative advances and clinical translation. In this review, we aim to provide a comprehensive survey of representative advances in AIMs accompanied by the underlying biological/technological backgrounds. We also highlight existing issues that need to be overcome in future AIM developments and suggest the directions of future research in this field.

Keywords
Micro/nanorobots, Biological motors, Bio-inspired actuators, Targeted delivery, Nanomedicine, Anticancer/antibacterial therapy, Biomolecule detection, Cell stimulation, Enzyme catalysis

INTRODUCTION
Micro-/nanorobots are miniaturized motors that can be actuated using fuel-driven (*e.g.,* hydrogen peroxide, acid, bromine, and iodine)[1-3] or fuel-free driving methods (*e.g.,* electrical, ultrasound, light, and magnetic fields).[4] In recent years, they have demonstrated superior ability for overcoming biological barriers with precise navigation to hard-to-reach tissues/cavities (some inaccessible to conventional approaches) and are therefore envisioned for advanced biomedical applications such as targeted drug delivery, minimally-invasive surgery, detoxification, biosensing, and so on.[5-9] Notwithstanding, critical challenges still persist for further development of the technology toward clinical translation, primarily arising from the integration and optimization of multiple functions onto biosafe micro-/nanorobots while ensuring the ease of fabrication in bulk at low costs.

Nature provides a wealth of solutions to scientific questions and has indeed inspired novel designs of micro-/nanorobots. One example is from microorganisms in nature, which have gone through centuries of natural selection to evolve diverse structures and functions, *e.g.,* hydrophobicity/hydrophilicity, biodegradability, magnetotaxis, and autofluorescence.[10-15] Microrobots fabricated through modifying natural microorganisms with engineered components can perform highly specific tasks, for which the desired functionality of the microrobots is either intrinsically endowed by properties of the host microorganisms or synthetically enabled by functionalization of the microorganisms through structural design, surface modification, cargo-loading, etc.[16-20] Because a variety of microorganisms are freely available in nature and can be

conveniently cultured under laboratory conditions, cost-effective mass production of micro-/nanorobots for population-level applications through this route is feasible.

As a subset of microorganisms, microalgae have been widely studied and commercialized owing to their abundance of bioactive compounds, equipping them with intriguing features such as phototactic property, pharmaceutical activity, and high-quality nutrients. Furthermore, compared with other microorganisms which may also have some of the above features (e.g., bacteria and paramecia), microalgae are in general free of cytotoxicity concerns and capable of photosynthesis too, making them desirable for food supplements and biomedical exploitations. Accommodating these desired functions in a body size of dozens of micrometers provides useful options for the engineering of algae-inspired microrobots (AIMs).[18-20] Dating back to 2005, the earliest prototype of AIM "microoxen" was demonstrated using *Chlamydomonas reinhardtii*[21] ***(CR***, a model organism in labs; see Fig. 1a*)* as biological motors to transport microparticles with high efficiency.[22] To date, a range of microalga species of distinct morphologies, including *Volvox (**VX***; Fig. 1b*)*,[23] *Eudorina elegans (**EE***; Fig. 1c*)*,[24] *Pandorina morum (**PM***; Fig. 1d*),*[25] *Chlorella (**Ch***; Fig. 1e*)*,[26] *Spirulina platensis (**SP***; Fig. 1f*)*,[27] *Diatom (**DM***; Fig. 1g*)*,[28] and *Tetraselmis subcordiformis (**TS***; Fig. 1h*)*,[29] have been explored by researchers to engineer AIMs for emerging biomedical applications and beyond (Fig. 1i; more examples listed in Table 1).[21-25, 30-44]

Compared with conventional medical tools in clinics, AIMs are capable of precisely delivering therapeutic cargoes to targeted positions and then releasing them in a controlled manner for on-demand tasks (*e.g.,* antibacterial/anticancer therapy) in biofluidic environments, therefore minimizing the drug dosage and associated adverse effects on normal cells.[26, 33, 39, 44] Specific functions can also be integrated into AIMs to enable challenging operations at the single-cell level, such as controlling neural-cell differentiation and inducing muscle-fiber contraction.[45, 46] One particular advantage of AIMs over other types of micro-/nanorobots (e.g., catalytic micromotors relying on toxic fuels and ferromagnetic microrobots made of cytotoxic materials like NeFeB) is their natural biocompatibility and desired biodegradation that helps bridge the overwhelming biosafety gap between current micro-/nanorobotic research and clinical translation, envisioning for accelerated biomedical applications benefiting patients in need. Apart from biomedical applications, AIMs have also been explored for remote sensing,[28] on-cell catalysis,[36] and environmental remediation,[40] manifesting the breadth of their potential in resolving real-world issues. Given the wide commercialization of microalgae products in the agriculture and healthcare sectors, the prospects for continued development and maturation of AIMs toward practical bench-to-bed translation are promising.

In this review, we systematically review recent progress in the fabrication, control, and applications of common AIMs, aimed at informing an atlas of microalgae functionalization and application strategies to accelerate the development of versatile AIMs toward practical applications in clinics. We also discuss the strengths and weaknesses of existing AIMs and provide an outlook for future research directions, including functionality expansion of existing AIMs, inception of new types of AIMs, and innovation of advanced control systems for widening and deepening applications of AIMs in biomedicine and beyond. The scientific rationale, technological basis and cost-effectiveness for pursuing advanced biomedical applications using microalgae-based biorobotics have been introduced in detail by recent reviews,[47-51] and the readers are referred to these works for a broader understanding of the emerging research field.

CLASSIFICATION OF ALGAE-INSPIRED MICROROBOTS (AIMS)

AIMs can be divided into three categories based on whether they retain the microalgae cell activity and whether they preserve the original biological substrate: microalgae-flagellated robots (MFRs), microalgae-hybrid robots (MHRs), and microalgae-templated robots (MTRs).

MFRs are defined as living microalgae deployed as motile microrobots, usually subject to surface decoration with other structures/molecules. Such microalgae usually come with beating

flagella, endowing superior propulsion and sensing capabilities to the as-fabricated microrobots.[52] Their flagella are capable of efficiently converting chemical energy into mechanical work through molecular motors powered by adenosine triphosphate (ATP) hydrolysis. These microalgae have desired features like phototaxis,[53] chemotaxis,[54] and/or electrotaxis,[55] which provide steering mechanisms for motion control. MFRs have been developed for wide-ranging applications, *e.g.,* thrombus clearance,[9] cargo delivery,[43] and environmental remediation.[40, 56] Certain nutrient environments are usually required to ensure the controlled functioning of MFRs, which would otherwise experience flagella inactivation and loss of motility. It is also important to consider their maintained activity and steerability for monitored operations, especially in deep tissues.

MHRs are defined as non-living biohybrid microalgae with optional functional modifications on the algal surface or within the cells. The morphological structure and biological substances of the microalgae will be preserved, but the cell activity is forfeited. With externally coated materials, mostly common magnetic substances like Fe_3O_4 nanoparticles (NPs), it is possible to confer magnetic attributes to the microalgae for remote actuation and steering using magnetic fields. MHRs are versatile in varying scenarios because they can perform complex tasks even in harsh and toxic environments. Additionally, the biological compounds inherited from the microalgae cells can equip MHRs with desired functionalities such as autofluorescence, magnetic resonance (MR) signals, and anticancer activity.[33] Through further integration of additional components, other functions such as photoacoustic (PA) imaging and antibacterial therapy can be achieved, enabled by polydopamine (PDA).[57] Benefiting from diverse functionalization strategies, MHRs can be customized to accommodate a plethora of functions in a single package suiting their applications. When choosing the chemical composition of MHRs for *in vivo* applications, crucial aspects including cytotoxicity, biodegradation, imaging capability and potential immune responses should be considered.

MTRs are defined as synthetic microrobots merely replicating the microalgae morphology, with both the structural matrix and contained compounds removed after fabrication.[31, 41] MTRs rely on an electrochemical deposition-like process in the sense that the microalgae mainly provide a biological template for shaping the microrobots, regardless of their cellular activity and intrinsic functionalities, which are eventually eliminated. Owing to the internal cavity space freed during the fabrication procedures (*e.g.,* annealing treatment), a large quantity of macromolecules or drug payloads can be loaded in MTRs for targeted delivery and controlled release. During the fabrication process, the microalgae structure is susceptible to excessive damage, potentially affecting the locomotion performance of MTRs. Therefore, factors such as temperature and reaction time need to be precisely controlled to preserve the desired microalgae morphology.

MANUFACTURING METHODS

Microalgae-flagellated robots (MFRs)

For fabricating MFRs, there are primarily two requirements for the microalgae candidates. First, they should be able to swim in fluid environments or glide on surfaces, with continuous propulsive forces generated to empower their locomotion. Second, they need to demonstrate a certain tropism for controllable steering of motion toward target locations. Microalgae with flagella or cilia, mostly green algae (*e.g., CR, Volvox, EE, PM*), can intrinsically act as MFRs. For instance, through the phototaxis of *CR and EE*, a novel guiding system was developed for guiding them to traverse crossing channels and transport microscale loads.[24] Similarly, transport of submillimeter-sized cargo has been demonstrated using *Volvox.*[37] *CR* and *PM* were exploited as bio-tweezer systems to manipulate micro-objects,[25] with their performance evaluated considering both hydrodynamics and Brownian motion. Without advanced functional modifications, MFRs in the above studies were mostly suited for relatively simple tasks.

To enable MFRs for designated functionalities, various strategies to modify the microalgae surface with exogenous materials have been proposed. For example, polystyrene (PS) beads

decorated with synthetic 4-hydroxyproline (4-HP) polypeptides were loaded onto the outer surface of *CR* (which is primarily composed of 4-HP-rich glycopeptides) through noncovalent interactions,[22, 58] with the size and number of loaded PS beads controlled by adjusting their relative concentration to that of CR samples. The noncovalent method was also utilized for reversibly anchoring antibiotic vancomycin (Fig. 2a).[59] Electrostatic adsorption, relying on the negative charge of the microalgae cell wall or membrane to deposit positively charged materials, is a widely applied noncovalent method for MFRs (Fig. 2b-d). For instance, chitosan polymer could be attached to the microalgae surface[21, 23] for accommodating biomedical cargo molecules. Santomauro *et al.* proposed MFR magnetization by terbium,[60] which were absorbed onto the algae cell wall due to electrostatic interaction. Negatively charged microbeads could also be converted into positively charged for further electrostatic interaction with the microalgae cell wall, through modification with cationic poly(diallyldimethylammonium chloride) (PDDA),[61] or layer-by-layer deposition of polyelectrolytes PAH/PSS.[34] Typically, noncovalent binding is a simple method with minimal damage to the algal cells, but it suffers from instability and uncertainty.

On the other hand, covalent modification methods have been exploited given their stable bonding. One example was Szponarski *et al.*,[36] where the cell wall of *CR* was decorated with artificial metalloenzyme *via* specific binding of biotin to streptavidin through the reaction of the amino acid residues bearing amino groups with electrophiles such as N-hydroxysuccinimide esters (Fig. 2e). Biorthogonal click chemistry was also introduced to functionalize *CR* with NHS-dibenzocyclooctyne anchor (DBCO), which allowed an azide derivative of the drug to be bonded to the surface (Fig. 2f).[62, 63] Similarly, CR was functionalized by angiotensin-converting enzyme 2 (ACE2) receptor and antibiotic-loaded neutrophil membrane-coated polymeric NPs to remove SARS-CoV-2 spike proteins from aquatic media[40, 56] and to treat acute bacterial pneumonia,[42] respectively.

Microalgae-hybrid robots (MHRs)

MHRs have been fabricated mainly using *SP*, *Ch,* and *Diatom* (occasionally with *CR* and *TS*), which are actuated and controlled by externally applied magnetic fields. Several methods have been employed to magnetize the microalgae, such as dip-coating, electroless deposition/plating, and physical vapor deposition, followed by post-modification functionalities added for designated applications (Fig. 3). Using a facile dip-coating process in Fe_3O_4 NP suspensions, Yan *et al*. developed a multifunctional MHR from *SP* (*CR* and *TS* MHR fabricated too) with intrinsic properties of autofluorescence, biodegradation, and selective cancer-cell cytotoxicity.[33] The superparamagnetic magnetite NPs not only enable magnetic response and magnetic resonance signals for the MHR, but also provide a versatile base for multi-purpose functionalization. To enhance the propulsion efficiency of MHR, Gong *et al*. deposited nickel (Ni) coating onto the surface of *SP* through an electroless plating technique,[35] which achieved a propulsion velocity up to 2613.8 μm/s (about 12 body lengths per second) compared to the Fe_3O_4 NP dip-coated MHRs (average velocity *ca.* 90 μm/s). The swimming performance of Ni-coated diatomite microswimmer under a rotating magnetic field was also characterized by the same group.[64] On the other hand side, electron-beam physical vapor deposition was applied by Guo *et al.* for depositing Ni and Au film on one side of the *Diatom* frustules, and synthesized Ag NPs were uniformly distributed on the surface for SERS detection.[28]

Depending on the target functions for a designated application, the post-modification process can be either directly implemented on the MHRs or through additionally introduced functional substances. Thanks to the porous structure of MHRs, biomacromolecules and small molecules can be encapsulated *via* a simple osmotic diffusion or dehydration/rehydration process of the microalgae cells, which serves as a promising approach for drug loading and targeted delivery.[38] As for exogenous functional substances, PDA (given its strong NIR adsorption and high fluorescence quenching properties) has been coated on MHRs to endow them with photoacoustic

(PA) imaging and photothermal effects,[57] which could also be acquired by loading Pd@Au or CuS NPs in algae cells by electroless deposition.[27, 65] One promising advance is to combine chemotherapeutic drugs with the photothermal effect of MHRs, which can realize dual-model therapy of cancer cells.[27] Another prospect is to modulate tumor hypoxia and release reactive oxygen species through radiotherapy treatment of magnetite-coated microalgae for multimodal tumor inhibition.[30] Apart from anticancer and tumor therapy, magnetic MHRs post-modified with $BaTiO_3$ NPs through electrostatic adsorption have demonstrated the ability to control the differentiation at the single-cell level.[45, 66]

Microalgae-templated robots (MTRs)

Owing to the abundance of microalgae structures in nature, MTRs with various shape options can be conceptualized and fabricated. Compared to microrobots designed by computer programs and manufactured through direct laser writing or template-assisted deposition, MTRs using natural microalgae as biological templates circumvent the cumbersome structure-design process and facile mass-production is possible with well-controlled chemistry in a cost-effective manner. In addition, biological substances of potential toxicity contained in the microalgae can be removed during the fabrication process, which adds to the biocompatibility of MTRs while supporting bulk loading of functional cargos for a variety of biomedical applications.

Among others, *SP*-based MTRs have attracted much attention owing to their inherent helical structure conducive to efficient actuation in viscous biofluids. Experimental procedures have been developed for fabricating porous hollow *SP* MTRs with or without a preserved carbon core (Fig. 4a).[31, 41] In brief, magnetite precursors are first applied to deposit magnetic NPs on the *SP* surface driven by biological extracellular mineralization, during which integrity of the helical structure is maintained throughout. Subsequent annealing treatment (either in vacuum or air) is then employed to remove or transform the *SP* template without altering the deposited coating. Vacuum annealing would result in the acquisition of helical carbon@magnetite core-shell MTRs with the magnetite precursor forming a large quantity of mesopores on the surface, whereas air annealing leads to hematite nanostructured porous hollow micro-helices (Fig. 4b) and further magnetite MTRs after additional reduction reaction. Given the internal cavity structure, MTRs could be exploited to load and release abundant cargos or collect heavy metal ions, serving as a novel platform for the development of biomedical microrobots.

More recently, Diatom frustules have been exploited as naturally porous hierarchical templates to fabricate motorized microsensors[28] or cargo carriers.[67] Guo *et al.* extracted the diatom frustules from diatomaceous earth powders through dispersion, sonification and filtration, followed by high-temperature calcination to clean the organic residues. A magnetic thin film was then deposited using Ni and Au through electron-beam evaporation, after which plasmonic Ag NPs were synthesized on the surface via catalytic reduction of silver nitrate to enable surface-enhanced Raman scattering spectroscopy for bio-detection (Fig. 4c).[28] Alternatively, Li *et al.* applied acid treatment to remove the calcareous cementitious substances and internal organic matter of the diatoms (Fig. 4d).[67] The obtained diatom frustules were then coated with magnetite particles through electrostatic adsorption to facilitate magnetic actuation and control (Fig. 4e).

ACTUATION AND MOTION CONTROL

Similar to living microorganisms and other micro-/nanorobots, AIMs rely on distinct mechanisms from the commonly encountered strokes at the macroscale for effective propulsion of their miniature body.[5] For AIMs of characteristic length $\boldsymbol{L}$ swimming at a speed of $\boldsymbol{u}$ in a fluid of constant density $\boldsymbol{\rho}$ and dynamic viscosity $\boldsymbol{\mu}$, the Reynolds number (**Re**) defined as

$$\boldsymbol{Re} = \boldsymbol{\rho L u / \mu} \qquad (1)$$

is far below the order of unity, implying vanishing effect of inertia and instantaneous motion/halt in response to propulsion forces. At this **Re** limit, any motion of the microrobot that is time-

reversible and reciprocal (*i.e.,* periodic, symmetric back-forth strokes) does not lead to net movement, which is known as the "scallop theorem." There are two common strategies used by living microalgae to break the scallop theorem, namely flagellar beating and helical rotation,[68, 69] and such propulsion methods can be either directly employed (*e.g.,* for MFRs) or mimicked (*e.g.,* for MHRs and MTRs) by microrobots to achieve locomotion in simple and complex fluid media.[5]

Motion control of AIMs, namely real-time steering of their locomotion, is another crucial factor for on-demand operations, and there are two primary categories of approaches widely employed: optical control methods (mainly for MFRs) and magnetic control methods (mainly for MHRs and MTRs). Optical control methods capitalize on the intrinsic light-sensing ability of living microalgae termed as "phototaxis," where the microalgae demonstrate a tendency of motion toward or against the light gradient. Magnetic control methods rely on the magnetic moment of the magnetic materials (*e.g.,* iron oxide, terbium) integrated on/in the microalgae, based on which magnetic torques or gradient forces are applied to align the magnetized microalgae with the magnetic field direction or gradient direction. In this section, we will discuss in detail the above actuation methods and control methods, together with other available strategies (especially for MFRs) which have also been exploited to achieve designated tasks in complex environments.

MFRs

As previously introduced in Section 2, the locomotion of MFRs relies on their natural motility through flagellar movements. Flagella are long and thin appendages to the cell body that can perform non-reciprocal strokes or collective motion to generate net displacement under low inertia. As *CR* is the predominant species that has been widely developed as MFRs, we take it as an example to elaborate on the mechanism for flagellum-driven propulsion.

CR is a unicellular spheroid about 10 μm in dimension with two anterior flagella sprouting from the cell body (Fig. 5a).[70] The internal motile component within each flagellum is called axoneme (Fig. 5b). The axoneme has a conserved cylindrical architecture consisting of nine outer doublet microtubules and two central microtubules known as the "central pair" (CP), supported by dyneins, radial spokes, nexin for cross-links and regulatory proteins.[71] The dyneins are classified as outer dynein arms providing power output, and inner dynein arms determining the flagellar beating pattern.[72-74] The generated beating motion causes a sliding force between adjacent outer doublet microtubules, which is then converted into bending through the nexin-dynein regulatory complex (N-DRC), a structure interconnecting the outer doublet microtubules and maintaining their alignment. In this manner, dynein efficiently converts the chemical energy *via* ATP hydrolysis into mechanical work. To propel the microalgae body, signals from the CP are transformed through the radical spokes to coordinate the dynein arms for a regular waving pattern and effective propulsion. Axonemes without radical spokes can lead to flagellar paralysis or chaotic beating.[75, 76] Each beating cycle can be divided into two stages in a breaststroke fashion: power stroke and recovery stroke (Fig. 5c).[22] In the former, the flagella flap backward and the cell body moves forward, whereas in the latter the flagella shrink and flap forward pulling the cell body backward to a certain degree. In a full beating cycle, net displacement forward occurs.[9, 61] Different motion control methods have been developed to steer and direct the MFRs for navigation toward designated positions, including optical control, magnetic field, chemoresponses, and electrotaxis, as to be detailed below.

Optical control. Optical control of MFRs usually relies on two approaches: phototaxis and optical tweezers (OT). We take *CR* as an example to explain the first control method, phototaxis. The detection of light by *CR* is through an elaborate subcellular organelle 'eyespot' (an orange spot located near the cell equator about 45° ahead of the flagellar beat plane),[77-79] containing carotenoid-rich granule layers in the chloroplast and the channelrhodopsin photoreceptor proteins ChR1 and ChR2 in the plasma membrane (Fig. 5d).[77] With the eyespot, *CR* presents positive phototaxis

toward low-intensity light and negative phototaxis against high-intensity light, respectively. Because *CR* rotates its body (about twice a second) when swimming forward, the eyespot scans incoming light from different directions.[78, 80-82] A photocurrent influx of Ca^{2+} ions into the flagella can be triggered as the eyespot senses the light information,[83, 84] consequently disrupting the beating balance between the two flagella[78] and causing the eyespot to tilt toward or against the light source depending on the beat amplitude of either flagellum.[82] If the swimming path is parallel to the light direction, the eyespot would perceive a constant illumination signal and keep advancing; otherwise, the flagella would realign the cell. This "tracking signal" pattern forms a closed-loop control to steer the cell toward the right direction.[85] It should be noted that phototaxis is affected by other factors, *e.g.,* the concentration of cations and the temperature of the environment.[55, 78]

In the context of controlled MFR motion using phototaxis, the pioneering work of Weibel *et al.* demonstrated guided transport of microbeads by *CR* cells in a straight channel through their rapid phototactic responses (within 1 s) to the applied LED, with tunable swimming patterns between random, positive, and negative phototaxis under varying light intensities.[22] Similarly, Nagai *et al.* achieved unidirectional transport of a submillimeter block using *Volvox* colonies.[37] Xie *et al.* developed a novel algae guiding system (AGS) to track and steer *EE* cells along arbitrary trajectories, providing a potential pathway to robotize algae cells for targeted delivery or drug screening.[24] Indeed, Shchelik *et al.* realized antibacterial treatment with antibiotics-attached *CR* cells for controlled drug release with robust phototaxis.[62]

The other optical control method widely used for steering MFRs, OT, exerts an optical force on targeted objects with a different refractive index from the surrounding environment. OT is widely used for trapping and manipulation of microparticles or biological materials to measure their physical properties.[86-90] The optical force is generated when light interacts with the object, produced by light reflection, absorption, refraction, or scattering due to changes in its momentum.[89] Xin *et al.* realized controllable locomotion of *CR* cells in a variety of biological media by optical force (Fig. 5e) for indirect manipulation of microparticles and disruption of biological targets such as blood clots.[9] Furthermore, motor arrays of reconfigurable patterns could be formed by *CR* cells to work independently and collaboratively for different tasks, demonstrating the potential of OT-controlled MFRs as a robotic operation platform with high throughput.[9]

Optical control, either through phototaxis or OT, provides extensive opportunities for real-time control of MFRs using light. However, these approaches do have significant limitations. On the one hand, the light signals cannot penetrate sufficient depth in certain environments, and the swimming orientation of MFRs is restricted by the light source position. On the other hand, it can be challenging to integrate a light source into the microscale system and the UV light may cause DNA or protein damage in organisms or humans,[91, 92] which makes potential biomedical translation difficult. Additionally, the focused light may heat the ambient environment outwith the target zone, therefore causing unintended harm to off-target normal cells.[6,18] Therefore, other control methods with desired tissue penetration and biocompatibility are still needed.

Magnetic control. Magnetic fields can wirelessly transmit power with high penetration capacity through the human body in a harmless manner, even with relatively large field strengths.[5, 6, 93, 94] For MFRs subject to magnetization by magnetic particles,[61] Tb^{3+} ions,[60] or treated PS microbeads,[34] a directed magnetic field can be used to control their motion. When exposed to the applied magnetic field, the MFRs would align themselves to the magnetic field direction due to their magnetic moment, thus enabling the steering of MFRs. Indeed, Ng *et al.* and Yasa *et al.* demonstrated that the MFRs showed random helical motion in the absence of magnetic fields, but positive magnetotaxis (artificial) would dominate their motion under low-strength magnetic fields (Fig. 5f).[34, 61]

Chemoresponses. In addition to the optical and magnetic methods introduced above, other strategies have been exploited for controlling the MFRs too, such as chemoresponses.[54] In general, there are two types of chemoresponses from microalgae. The first is chemotaxis, for which the microalgae respond to chemicals without changing their swimming speed and motility, including positive chemotaxis (associated with algae accumulation) and negative chemotaxis (associated with algae repulsion). The other type of chemoresponses is chemokinesis, where the microalgae accumulate as the chemical stimuli affect their motility (causing reduced or halted swimming of the microalgae). Chemotaxis is a promising control method for MFRs without the need for an external control system, with ideal candidates being those naturally produced in the working media (*e.g.,* chemical signals released by cells can be utilized for eliciting a response). However, some drawbacks do exist, such as the poor spatiotemporal resolution of chemical gradients in the environment and the inevitable response delay that may cause real-time control issues. Furthermore, the orientation control using chemotaxis is also subject to a high degree of stochasticity.[95, 96]

Electrotaxis. Electric fields have also been applied to navigate MFRs *via* their electrotaxis behavior (galvanotaxis).[55] One should note the difference between electrotaxis and electrophoresis: the former describes living cells actively swimming toward an electrode in response to electric stimulation, whereas the latter describes objects of net surface charge passively moving under the influence of an electric field.[19, 97] Hayashi *et al.* showed that *Volvox* would only present negative electrotaxis under a wide range of electric field strengths.[55] But if photo- and electric-stimulations were coupled, *Volvox* could perform positive electrotaxis, presumably due to cell polarization. As the strength and direction of electric fields can be easily altered, it serves as a feasible control method for MFRs. Nevertheless, electric control methods might be invasive and could induce electroporation of the microalgae cell membranes.[98] In addition, potential electrolysis may produce toxic gases or change the pH of the working liquid media.[18, 99]

MHRs and MTRs

External fields, such as electric, magnetic, ultrasound, and light,[4, 100-102] have been widely exploited for the control of synthetic microrobots. For the actuation and steering of MHRs and MTRs, magnetic fields are the most used, which can enable navigation in complex three-dimensional environments with superior penetration depth and good biocompatibility under a wide range of magnetic field strengths (*e.g.,* the field strength for MR imaging can be as high as 4T under medical supervision).[5, 6, 93] Typically, an electromagnetic coil system (*e.g.,* tri-axial Helmholtz coils) or permanent magnets are used for generating the magnetic fields needed.[103] Under a magnetic field $\mathbf{B}$ [T], a microrobot with average magnetization $\mathbf{M}$ [m A^{-1}] experiences a magnetic torque $\mathbf{T}$ [N m] (aligning the microrobot with the magnetic field direction) and a magnetic force $\mathbf{F}$ [N] (pulling or repelling the microrobot along the field gradient direction) given by

$$\mathbf{T} = \vartheta\, \mathbf{M} \times \mathbf{B}, \qquad (2)$$

$$\mathbf{F} = \vartheta\, (\mathbf{M} \cdot \nabla)\, \mathbf{B} \qquad (3)$$

where ϑ is the volume of the magnetized microrobot.[103]

Both magnetic forces and magnetic torques have been utilized for the actuation of MHRs and MTRs. The magnetic force can be directed to pull the microrobots toward the region of higher magnetic flux density when the field has a gradient, and the pulling force can be enhanced by increasing the gradient of the applied field. Magnetic torques produced by rotating or oscillating magnetic fields, however, are more commonly adopted due to their precision of control, especially for powering helical microrobots that rely on rotation-translation motion (Fig. 6a).[33] The propulsion direction of the microrobots is approximately perpendicular to the plane of the rotating magnetic field, and either forward or backward translation can be achieved by reversing the magnetic field direction.[104] The rotating motion of MHRs and MTRs should ideally be converted into translation synchronously, and there exists a linear relationship between the increase of propulsion velocity

and the rotating frequency of the imposed magnetic field within a certain range. However, if the rotating frequency is too high, the magnetic torque may fall short to balance the viscous drag in the swimming environment.[105] In this case, the swimming velocity of the microrobot will actually decrease after reaching a critical rotating frequency known as the "step-out" frequency. Additionally, deviation of the swimming path from the magnetic field direction could happen if the microrobot interacts with surfaces or substrates in the ambient environment when its translation control will be affected. Without the need for any potentially hazardous fuels, magnetic fields remain a popular propulsion method for AIMs, and their extensive applications in various medical settings would be conducive to the clinical translation of AIMs too.[104]

A large body of research works in the literature have employed rotating or oscillating magnetic fields to steer MHRs and MTRs for controlled navigation.[27, 31, 33, 35, 38, 41, 45, 57, 64, 66] For magnetization purpose, robust magnetic materials such as Fe_3O_4 and Nickle particles could be deposited on the microalgae, so actuation and steering becomes possible with low-strength magnetic fields for on-demand tasks. Microrobots magnetized from microalgae of various shapes, such as helical and spherical/ellipsoidal ones, have been successfully propelled using magnetic fields (Fig. 6b),[33] achieving predefined paths of complex trajectories such as "staircase" and "circular" (Fig. 6c).[40] In addition to the control of individual microrobots, magnetic fields have the capability to manipulate multiple AIMs (Fig. 6d, e)[44, 67] and propel microrobotic swarms too, where individual microrobots assemble into reconfigurable swarming entities that can be actuated and controlled as a whole subject to altering field frequency or strength for enhanced imaging signals *in vivo* (Fig. 6f).[33] Benefiting from the superior actuation and steering performance, MHRs and MTRs have been exploited for on-demand tasks where complex environments are involved. For instance, *SP* MHRs have been controlled to navigate winding microfluidic channels mimicking the gastrointestinal tract.[38]

Although magnetic field control has been widely applied, it also has limitations. Currently, the magnetic fields applied are mostly generated by permanent magnets or electromagnetic coils. Permanent magnets can generate magnetic fields without the use of electric currents, but the field strength decreases rapidly against the distance between the magnet and the working space of microrobots. Furthermore, the direction of the magnetic field can only be altered by physically changing the magnet position, which may not be practical in complicated application scenarios. In contrast to permanent magnets, electromagnetic coils can precisely modulate the magnetic field direction by controlling the input electric currents. However, the temperature of working coils could increase rapidly because of the resistive heating (especially for large coils designed for sufficient working space), thus limiting the operation time and potentially affecting the biological cells inside the workplace too. It should be noted that for rotating magnetic fields, the timing-varying fields might also risk cardiac fibrillation under certain circumstances due to nerve stimulation. This is because the change in the magnetic field gradient can induce electric fields as a function of $\boldsymbol{d|\mathbf{B}|/d\mathbf{t}}$, with the maximum rate change [T S^{-1}] for controlled operation described as

$$\frac{\boldsymbol{d|\mathbf{B}|}}{\boldsymbol{d\mathbf{t}}} = \mathbf{20}\left(\mathbf{1}+\frac{\mathbf{0.36}}{\boldsymbol{\tau}}\right) \qquad (4)$$

where τ is the period of the monotonically increasing or decreasing gradient in milliseconds.[5]

BIOMEDICAL APPLICATIONS AND BEYOND

Micromanipulation

AIMs have been extensively applied in micromanipulation tasks, where they function as bio-tweezers, micromotors, micropumps, and micromixers, etc. They were demonstrated to perform tasks with precision such as disrupting biological targets,[9] pushing submillimeter objects,[37] manipulating PS microparticles,[25] driving synthetic tools,[106] and so on. For example, *CR*-based living micromotors with multiple functions were controlled using OT to destruct biological aggregates, providing a potential tool for localized therapy of blood clots (Fig. 7a).[9] Shimizu *et al.* innovated a micro pinwheel for trapping four *CR* cells, where the capture components were

designed as two rings (diameters 7 μm and 10 μm) to prevent the CR from reversing their directions or escaping. The pinwheel realized rotation via the *CR*'s propulsion force (Fig. 7b).[106] On the other hand, swarming AIMs have been explored to manipulate larger-scale objects (Fig. 7c, d).[58, 107] Through the phototaxis of AIMs, unidirectional transport of objects can also be achieved with the direction of a light source (Fig. 7e, f).[22, 37]

Apart from using the intact microalgae, their flagella can also be separately exploited for propelling micro-objects. The flagella are easily prepared without complicated purification and reconstitution processes; they tend to attach nonspecifically to surfaces such as a glass tube,[108] and can be reactivated in a controlled manner by adjusting the concentration of ATP in working liquids.[109] Real "artificial flagellates" isolated from *CR* cells have been attached to the surface of microbeads to make them actuators.[30] Biflagellate beads were found capable of moving forward whereas uniflagellate beads only rotated locally, therefore providing general guidelines for designing advanced artificial MFRs.

Active drug delivery

Active delivery of therapeutic cargo or drug payloads through biocompatible AIMs to complex locations or narrow regions inside the human body is promising for precise diagnostics and treatment with minimal side effects. Extensive research was dedicated to this blueprint and some promising proofs of concept have been demonstrated, primarily with microalgae species *CR* and *SP*. Several strategies are available for AIMs to load and transport cargo in a controlled manner. *CR* cells could naturally pick up microscale objects they encounter through electrostatic interaction and carry the cargo forward (Fig. 8a),[110] with options to guide the orientation of transportation using a light source.[22] Aided by additional magnetic field control, a highly motile biohybrid *CR* modified by magnetic particles was designed for active cargo delivery and uptake of therapeutics without causing toxic effects (Fig. 8b).[34] More recently, algae motors embedded in pH-sensitive degradable capsules were developed for enhanced delivery of drugs to the narrow intestines,[43] which achieved prolonged retention within the intestinal mucosa through combining the self-propulsion of *CR* cells and the protective function of oral capsules (Fig. 8c).

SP-based AIMs also play a key role in active cargo delivery. For instance, an MTR featuring porous hollow architecture with high specific surface area (SSA) was designed for effective loading and controlled release of nanoparticles and molecules, *e.g.,* Au NPs and RhB (Fig. 8d).[31] The natural dehydration and rehydration process of *SP* cells was found another approach to load molecular cargos (Fig. 8e).[38] The delivered molecules can then be released on the target site from the *SP* cells either through host degradation and/or concentration gradient-driven diffusion, suggesting applications for drug delivery in hard-to-reach regions without invasive operations. Applying this principle, oral delivery of Amifostine for targeted drug accumulation and intestinal protection against cancer radiotherapy was achieved using *SP* AIMs (Fig. 8f).[111] Many other works have reported on-demand delivery of therapeutic cargos through AIMs, manifesting the profound potential of AIMs for targeted therapy that would resolve unmet clinical needs.

Anticancer/Antibacterial Therapy

Anticancer. In recent years, microalgae have been extensively exploited for enhanced cancer therapy, especially in situations where the therapeutic effects are oxygen-dependent, such as radiotherapy (RT) and photodynamic therapy (PDT). This is because oxygen released by live microalgae cells can potentially alleviate hypoxia in the tumor microenvironment. Furthermore, their intrinsic chlorophyll, an imaging agent for fluorescence (FL) and PA imaging,[112, 113] could be utilized for reactive oxygen species (ROS) generation upon laser irradiation.[39, 114] Other bioactive compounds contained by the microalgae cells, such as phycocyanin, were also found capable of selectively killing certain cancer cells.[33] All these desired attributes, taken together, suggest the superiority of microalgae as promising anticancer agents for clinical applications.

A series of photosynthetic biosystems engineered from microalgae have been reported to modulate tumor hypoxia through in situ O_2 generation for imaging-guided PDT or RT therapy.[26,115,116] In these works, *Ch* cells were coated with a protective layer of CaP,[115] SiO_2,[116] or red blood cell membrane,[26] which allowed the microalgae to evade macrophage clearance and reach the tumor sites with maintained photosynthetic activity. To enhance the photodynamic effect, a novel strategy of chlorin e6 (ce6) internalization into *Synechococcus elongatus* has been proposed,[117] where O_2 generated by the hybrid microalgae system could be activated into singlet oxygen and satisfactory therapeutic effects on 4T1 tumor cells and xenografts were achieved.

The above studies rely on passive accumulation or direct injection of microalgae to the tumor site, without actively steering them. Other efforts have been made to guide AIMs to target the tumor site *in vivo* and mitigate tumor hypoxia to enhance FL/PA/MR imaging-guided therapy (Fig. 9a).[39] AIMs combined with photothermal therapy (PTT) and chemotherapy have also been developed for targeted delivery and synergistic cancer therapy.[27] The Pd@Au core-shell NPs endowed the *SP* microrobots with photothermal effects and the Dox loaded further enabled chemotherapy efficacy (Fig. 9b). Such (Pd@Au)/Fe_3O_4@*SP*-DOX microrobots could be actuated and precisely controlled using magnetic fields and the release of delivered drug could be triggered by pH- and NIR-stimuli. As the temperature rises and the microalgae structure degrades, active biocompounds inside the cells could also be released to selectively kill cancer cells,[33] Furthermore, a swarm of such microrobots could be assembled and disassembled in a controlled manner for targeted Dox delivery, therefore holding promise for navigating narrow regions for anticancer treatment (Fig. 9c).[44]

Antibacterial. In parallel with anticancer therapy, advanced antibacterial constructs consisting of microalgae and antibacterial materials have been developed to overcome drug resistance and minimize the adverse effects of antibacterial treatment.[57, 59, 63] This category of AIMs perform antibacterial functions either by releasing loaded antibiotics upon light irradiation or through their photothermal effect endowed by functional coatings. For instance, MFRs for treating infected skin or soft tissue have been facilitated through simplistic chemical surface engineering methods,[59, 63] where the microalgae cells were modified with antibiotics using a photo-cleavable linker while maintaining their viability, motility, and phototaxis (Fig. 9d). Usually an external light source was applied to guide the direction of the AIM for targeted delivery, followed by release of the antibacterial cargo on-site. Strong inhibition of bacterial growth with high-level spatiotemporal precision has been reported,[63] thus verifying the new treatment approach for conventional bacterial infections.

On the other hand, MHRs consisting of SP, Fe_3O_4, and PDA coating were successfully applied for the treatment of multidrug-resistant *Klebsiella pneumoniae* infections in a murine subcutaneous model (Fig. 9e),[57] where the PDA coating of the microrobots not only enhanced their photothermal effect but also enabled robust PA imaging capability. A swarm of such microrobots could be propelled by a rotating magnetic field with real-time motion tracking under the skin through PA imaging. More recently, microalgae integrated (through click chemistry) with neutrophil membrane-coated polymeric nanoparticles loading antibiotics were designed for acute pneumonia treatment,[42] which nicely combined the microalgae motility with multifunctional synthetic components. Uniform distribution, low immune clearance, and superb tissue retention time in deep lung tissues were achieved, effectively reducing the bacterial burden. In the future, more generic antibacterial therapy for the treatment of bacterial infections in narrow and complex tissues or cavities should be envisaged. With such therapies, drug resistance and side effects would considerably decrease given the precise treatment in situ for designated time durations.

Cell stimulation

Cell stimulation is a viable approach for the treatment of nervous system-related diseases, such as Parkinson's disease and myasthenia gravis. The widely applied electrical stimulation has served as

a feasible strategy, but its application is limited by the inhomogeneity of electric fields and the invasiveness of implanted stimulators.[118] Instead, wireless and biocompatible magnetic AIMs hold great promise for precise and efficient neural activation. Recently, Liu *et al.* demonstrated magnetically powered piezoelectric AIMs for precise interactions with neural stem-like cells (Fig. 10a).[45, 66] The tiny AIMs could be accurately guided to a neural stem-like PC12 cell using a rotating magnetic field for the loaded $BaTiO_3$ NPs to convert ultrasonic energy into electrical signals (through their piezoelectric effect) and induce cell differentiation.[66] The neuron stem cell activation is accompanied by a sudden calcium ions influx into the cytosol, which depends on the intensity of the electric field and determines the intensity of activation.[119] Therefore, directed differentiation of the neural stem cell could be achieved by tuning the intensity of input ultrasound for astrocytes, functional neurons, or oligodendrocytes.[45] More recently, *Ch*-based AIMs have also been developed to precisely stimulate skeletal muscle contractions with photothermal effect (Fig. 10b).[46] The superparamagnetic microrobots were magnetically navigated to the muscle injury sites and heated upon NIR irradiation, consequently inducing contraction of the muscle fiber through activation of the actin−myosin interactions. In sum, the AIM platforms, wirelessly operated and minimally invasive, provided a promising interventional tool for the treatment of neural system-related or other cellular-level diseases.

Other applications

In addition to the applications discussed in earlier sections, diverse applications of AIMs are being exploited, *e.g.,* environmental remediation,[40, 41] on-cell catalysis,[36] wound-healing[120] and biomolecule sensing.[28] For instance, an active AIM system consisting of live *CR* cell and an angiotensin-converting enzyme 2 (ACE2) receptor, which is against the SARS-CoV-2 spike proteins (Fig. 10c), has been developed for effective removal of SARS-CoV-2 spike proteins and pseudo-virus from contaminated aquatic media.[40, 56] Owing to the self-propulsion enabled continuous mixing and collision, the developed AIMs achieved high removal efficacy compared to traditional wastewater treatments, thus offering an efficient tool for pathogenic virus removal and overcoming other threats in contaminated water. *SP*-templated porous hollow AIMs have been explored for the adsorption of heavy metal ions, *e.g.,* Chromium ion (Cr^{6+}) for water purification (Fig. 10d).[41] Similar to contaminated water, cell culture media may also contain viruses, pathogenic bacteria, or other nano-biothreats. To tackle that, non-invasive methods are required. One such example was diatombot based on *Phaeodactylum tricornutum Bohlin* combining the wake-riding effect and optical trapping, which demonstrated effective trapping and removal of nanoscale bio-targets such as adenoviruses and pathogenic bacteria.[121]

To enable on-cell catalysis without involving complex transition metal chemistry, artificial metalloenzyme has been grafted on *CR* through surface engineering to provide the cell with versatile chemical capabilities and new reactivity (Fig. 10e).[36] The engineered *CR* cells remained viable and could be guided by light in three-dimensional space for potential medical applications. Oxygen-generating *CR* AIMs coated with heparin have been also developed for accelerating wound healing given their ability of in-depth penetration and enhanced retention in the wounded tissue (Fig. 10f), which substantially improved the cytokine scavenging efficiency and alleviated the hypoxic condition associated with diabetic wounds.[120]

For low-concentration bioanalysis, existing nanosensors with high sensitivity could detect biological molecules of 1 pM-1 fM,[122-125] but at the price of a long testing window, thus hindering practical application in the clinic. Thanks to the advances in micro-and nanorobotics, the detection speed using biological or synthetic micro/nanomotors can be notably shortened.[126-129] However, since the nanoscale sensors could continuously change their position, detecting molecules in real-time or for prolonged duration is difficult, which is commonly required for low-concentration molecule testing. To overcome this issue, *Diatom* frustule-based AIMs have been designed for sensitive, long-time, and position-stable detection of low-concentration DNA molecules in real-

time (Fig. 10g),[28] where the capture speed of molecules was markedly amplified (about four times). The preliminary findings may inspire the rational design of efficient AIMs systems for future sensing of biomarkers.

CONCLUSIONS AND OUTLOOK

Thanks to recent advances in nanotechnology and accumulated foundation knowledge across a range of underpinning disciplines (e.g., applied physics, surface chemistry, synthetic biology, materials sciences), AIMs developed for biomedical applications have witnessed remarkable growth in the past decade. Several microrobotic systems have demonstrated bright prospects for clinical translation. A variety of manufacturing methods, bioengineering techniques and actuation/steering strategies have been exploited to enable biomedical applications of AIMs, depicting an unprecedented landscape of revolutionizing future healthcare with precision medicine strategies such as active drug delivery and targeted therapy. Notwithstanding the fact that multiple AIMs have been assessed with experimental models and evaluated in rodents as well as larger animals, crucial challenges remain to be addressed for steady translation of these microrobots into practical therapies in clinics for benefiting patients. To enlighten solutions for bridging the gap, we provide our perspectives on the current challenges and future directions of AIM research as below.

Current challenges

With substantial technological breakthroughs and diversifying target applications, AIMs have come to a crossroad of challenges and opportunities. Although the volume of AIM research keeps expanding, critical hurdles persist and need to be satisfactorily overcome before bench-to-bed translation is possible. These hurdles include but are not limited to, cost-effectiveness of mass production, safety hazards of human administration, inconsistent precision of untethered control, insufficient accuracy of remote tracking, unclear pathway of in vivo degradation/retrieval and undesired ambiguity of long-term toxicity. The focus of much AIM research remains on exploratory developments in petri dish or incremental understanding of AIM's biophysical/biochemical behavior, rather than enhancing the technological basis of reported studies or advancing their clinical assessment for progressive translation. Whereas such a focus may contribute to novel concepts and more publications in the near future, they may hinder the substantiation and advancements of promising AIMs that have gone through systematic assessment *in vitro*, *ex vivo* or *in vivo*. Furthermore, there is a lack of international standard or unanimous criteria for consistently evaluating and reporting the clinical relevance and potential ethical implications of studied AIMs when certain biomedical applications are claimed or envisioned. The challenges stated above shed light on valuable opportunities and future directions of AIM research that could stimulate the field in significant measures through concerted research efforts, which we present next.

Future directions

There are several key directions of research that can help accelerate the translation of AIMs toward realistic biomedical applications in clinics.

Fabrication. Consolidation and/or innovation of cost-effective methods for manufacturing AIMs out of currently used or newly developed microalgae, which is essential to sustainable fabrication of AIMs with designated maneuverability and functionalities suiting dynamical conditions one would expect in the human body. On the one hand, abundant candidates of microalgae in nature remain to be discovered or mimicked for developing versatile AIMs to meet more demanding scenarios which reflect the multifold uncertainty in a physiological environment, e.g., cellular interactions, biological barriers and immune responses. On the other hand, core functions of existing AIMs (e.g., biocompatibility, degradability, therapeutic activity) should be sufficiently multiplexed through integration of intrinsic microalgae attributes and smart surface engineering.

Navigation. Automated redesign and iterative refinement of AIMs with the aid of artificial intelligence and bio-inspired engineering, preferably incorporating embodied intelligence of microorganisms in nature, which will help advance mature AIMs (e.g., those already evaluated with animal models *in vivo*) toward scalable production and potential commercialization. From a technological perspective, the actuation system and steering methods of AIMs should be rigorously evaluated for well-defined application scenes, primarily meeting three requirements: (i) responsive and intelligent feedback adapting to operator instructions; (ii) robust and precise locomotion accommodating hard-to-reach environments; (iii) reliable and spontaneous imaging equipped for close-loop control.

Translation. Co-design and co-delivery of meaningful AIM proofs of concept with clinicians from early stage to align research with clinical needs and streamline a path to practical therapies or medical treatments. The top-down approach will likely lead to engaging projects and impactful outcomes with clear socioeconomic benefits for facilitating organizational support, industrial engagement and regulatory approval. Such projects require interdisciplinary collaboration and knowledge exchange among researchers from diverse backgrounds (e.g., materials scientists, nanoengineers, chemists, roboticist) and clinical practitioners in the frontline (e.g., physicians, surgeons). One emerging trend in the research field of AIMs is that researchers with less relevant backgrounds (e.g., fluid dynamists, computational modelers) are also growingly involved, who may contribute to fundamental understanding and optimization pathway of AIMs in complex biofluids and physiological environments through mechanistic models and digital twins.

Concluding remarks

To sum up, AIMs are still in their infancy but with transformative promises in shaping precision medicine and stratified healthcare. We hope this article has provided a useful overview of recent progresses on the design and application of algae-inspired microrobots as well as their underlying biological and technological foundations, in the anticipation of fostering greater technological innovation and deeper pre-clinical developments as the stepping stones for clinical translation.

References

1. W. Gao, A. Uygun, J. Wang, Hydrogen-bubble-propelled Zinc-based microrockets in strongly acidic media. *J. Am. Chem. Soc.* **134**, 897-900 (2012).
2. L. Liu, M. Liu, Y. Su, Y. Dong, W. Zhou, L. Zhang, H. Zhang, B. Dong, L. Chi, Tadpole-like artificial micromotor. *Nanoscale* **7**, 2276-2280 (2015).
3. R. Liu, A. Sen, Autonomous nanomotor based on copper-platinum segmented nanobattery. *J. Am. Chem. Soc.* **133**, 20064-20067 (2011).
4. J. Wang, W. Gao, Nano/microscale motors: biomedical opportunities and challenges. *ACS Nano* **6**, 5745-5751 (2012).
5. B. J. Nelson, I. K. Kaliakatsos, J. J. Abbott, Microrobots for minimally invasive medicine. *Annu. Rev. Biomed. Eng.* **12**, 55-85 (2010).
6. M. Sitti, H. Ceylan, W. Hu, J. Giltinan, M. Turan, S. Yim, E. Diller, Biomedical applications of untethered mobile milli/microrobots. *Proc. IEEE* **103**, 205-224 (2015).
7. J. Li, B. Esteban-Fernandez de Avila, W. Gao, L. Zhang, J. Wang, Micro/nanorobots for biomedicine: delivery, surgery, sensing, and detoxification. *Sci. Robot.* **2**, eaam6431 (2017).
8. J. Katuri, X. Ma, M. M. Stanton, S. Sanchez, Designing micro- and nanoswimmers for specific applications. *Acc. Chem. Res.* **50**, 2-11 (2017).
9. H. Xin, N. Zhao, Y. Wang, X. Zhao, T. Pan, Y. Shi, B. Li, Optically controlled living micromotors for the manipulation and disruption of biological targets. *Nano Lett.* **20**, 7177-7185 (2020).
10. J. Christensen, L. Norgaard, R. Bro, S. B. Engelsen, Multivariate autofluorescence of intact food systems. *Chem. Rev.* **106**, 1979-1994 (2006).
11. T.-X. Fan, S.-K. Chow, D. Zhang, Biomorphic mineralization: from biology to materials. *Prog. Mater. Sci.* **54**, 542-659 (2009).
12. B. Metting, J. W. Pyne, Biologically-active compounds from microalgae. *Enzyme Microb. Technol.* **8**,

386-394 (1986).
13. A. R. Parker, H. E. Townley, Biomimetics of photonic nanostructures. *Nat. Nanotechnol.* **2**, 347-353 (2007).
14. P.-Y. Chen, J. McKittrick, M. A. Meyers, Biological materials: functional adaptations and bioinspired designs. *Prog. Mater. Sci.* **57**, 1492-1704 (2012).
15. N. Billinton, A. W. Knight, Seeing the wood through the trees: a review of techniques for distinguishing green fluorescent protein from endogenous autofluorescence. *Anal. Biochem.* **291**, 175-197 (2001).
16. S. Martel, M. Mohammadi, O. Felfoul, Z. Lu, P. Pouponneau, Flagellated magnetotactic bacteria as controlled MRI-trackable propulsion and steering systems for medical nanorobots operating in the human microvasculature. *Int. J. Robot. Res.* **28**, 571-582 (2009).
17. V. Magdanz, S. Sanchez, O. G. Schmidt, Development of a sperm-flagella driven micro-bio-robot. *Adv. Mater.* **25**, 6581-6588 (2013).
18. R. W. Carlsen, M. Sitti, Bio-hybrid cell-based actuators for microsystems. *Small* **10**, 3831-3851 (2014).
19. C. Sanchez, H. Arribart, M. M. G. Guille, Biomimetism and bioinspiration as tools for the design of innovative materials and systems. *Nat. Mater.* **4**, 277-288 (2005).
20. H. Wang, M. Pumera, Fabrication of micro/nanoscale motors. *Chem. Rev.* **115**, 8704-8735 (2015).
21. M. B. Akolpoglu, N. O. Dogan, U. Bozuyuk, H. Ceylan, S. Kizilel, M. Sitti, High-yield production of biohybrid microalgae for on-demand cargo delivery. *Adv. Sci.* **7**, 2001256 (2020).
22. D. B. Weibel, P. Garstecki, D. Ryan, W. R. DiLuzio, M. Mayer, J. E. Seto, G. M. Whitesides, Microoxen: microorganisms to move microscale loads. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 11963-11967 (2005).
23. J. Wang, F. Soto, S. Q. Liu, Q. Q. Yin, E. Purcell, Y. T. Zeng, E. C. Hsu, D. Akin, B. Sinclair, T. Stoyanova, U. Demirci, Volbots: Volvox microalgae-based robots for multimode precision imaging and therapy. *Adv. Funct. Mater.* **32**, 2201800 (2022).
24. S. Xie, N. Jiao, S. Tung, L. Liu, Controlled regular locomotion of algae cell microrobots. *Biomed. Microdevices* **18**, 47 (2016).
25. C. Zhang, S. X. Xie, W. X. Wang, N. Xi, Y. C. Wang, L. Q. Liu, Bio-syncretic tweezers actuated by microorganisms: modeling and analysis. *Soft Matter* **12**, 7485-7494 (2016).
26. Y. Qiao, F. Yang, T. Xie, Z. Du, D. Zhong, Y. Qi, Y. Li, W. Li, Z. Lu, J. Rao, Y. Sun, M. Zhou, Engineered algae: a novel oxygen-generating system for effective treatment of hypoxic cancer. *Sci. Adv.* **6**, eaba5996 (2020).
27. X. Wang, J. Cai, L. Sun, S. Zhang, D. Gong, X. Li, S. Yue, L. Feng, D. Zhang, Facile fabrication of magnetic microrobots based on *Spirulina* templates for targeted delivery and synergistic chemo-photothermal therapy. *ACS Appl. Mater. Interfaces* **11**, 4745-4756 (2019).
28. J. Guo, Z. Liang, Y. Huang, K. Kim, P. Vandeventer, D. Fan, Acceleration of biomolecule enrichment and detection with rotationally motorized opto-plasmonic microsensors and the working mechanism. *ACS Nano* **14**, 15204-15215 (2020).
29. C. Yao, J. Jiang, X. Cao, Y. Liu, S. Xue, Y. Zhang, Phosphorus enhances photosynthetic storage starch production in a green microalga (chlorophyta) *Tetraselmis subcordiformis* in nitrogen starvation conditions. *J. Agric. Food Chem.* **66**, 10777-10787 (2018).
30. N. Mori, K. Kuribayashi, S. Takeuchi, Artificial flagellates: analysis of advancing motions of biflagellate micro-objects. *Appl. Phys. Lett.* **96**, 083701 (2010).
31. X. Yan, Q. Zhou, J. F. Yu, T. T. Xu, Y. Deng, T. Tang, Q. Feng, L. M. Bian, Y. Zhang, A. Ferreira, L. Zhang, Magnetite nanostructured porous hollow helical microswimmers for targeted delivery. *Adv. Funct. Mater.* **25**, 5333-5342 (2015).
32. T. Hatsuzawa, D. Ito, T. Nisisako, Y. Yanagida, Micro-rotary ratchets driven by migratory phytoplankton with phototactic stimulus. *Precis. Eng.* **48**, 107-113 (2017).
33. X. Yan, Q. Zhou, M. Vincent, Y. Deng, J. Yu, J. Xu, T. Xu, T. Tang, L. Bian, Y. J. Wang, K. Kostarelos, L. Zhang, Multifunctional biohybrid magnetite microrobots for imaging-guided therapy. *Sci. Robot.* **2**, eaaq1155 (2017).
34. O. Yasa, P. Erkoc, Y. Alapan, M. Sitti, Microalga-powered microswimmers toward active cargo delivery. *Adv. Mater.* **30**, e1804130 (2018).
35. D. Gong, J. Cai, N. Celi, L. Feng, Y. G. Jiang, D. Zhang, Bio-inspired magnetic helical microswimmers made of nickel-plated *Spirulina* with enhanced propulsion velocity. *J. Magn. Magn. Mater.* **468**, 148-154 (2018).
36. M. Szponarski, F. Schwizer, T. R. Ward, K. Gademann, On-cell catalysis by surface engineering of live cells with an artificial metalloenzyme. *Commun. Chem.* **1**, 84 (2018).
37. M. Nagai, T. Hirano, T. Shibata, Phototactic algae-driven unidirectional transport of submillimeter-sized cargo in a microchannel. *Micromachines* **10**, 130 (2019).

38. X. H. Yan, J. B. Xu, Q. Zhou, D. D. Jin, C. A. Vong, Q. Feng, D. H. L. Ng, L. Bian, L. Zhang, Molecular cargo delivery using multicellular magnetic microswimmers. *Appl. Mater. Today* **15**, 242-251 (2019).
39. D. N. Zhong, W. L. Li, Y. C. Qi, J. He, M. Zhou, Photosynthetic biohybrid nanoswimmers system to alleviate tumor hypoxia for FL/PA/MR imaging-guided enhanced radio-photodynamic synergetic therapy. *Adv. Funct. Mater.* **30**, 1910395 (2020).
40. F. Zhang, Z. Li, L. Yin, Q. Zhang, N. Askarinam, R. Mundaca-Uribe, F. Tehrani, E. Karshalev, W. Gao, L. Zhang, J. Wang, ACE2 receptor-modified algae-based microrobot for removal of SARS-CoV-2 in wastewater. *J. Am. Chem. Soc.* **143**, 12194-12201 (2021).
41. C. Zheng, Z. Li, T. Xu, L. Chen, F. Fang, D. Wang, P. Dai, Q. Wang, X. Wu, X. Yan, *Spirulina*-templated porous hollow carbon@magnetite core-shell microswimmers. *Appl. Mater. Today* **22**, 100962 (2021).
42. F. Zhang, J. Zhuang, Z. Li, H. Gong, B. E. de Avila, Y. Duan, Q. Zhang, J. Zhou, L. Yin, E. Karshalev, W. Gao, V. Nizet, R. Fang, L. Zhang, J. Wang, Nanoparticle-modified microrobots for *in vivo* antibiotic delivery to treat acute bacterial pneumonia. *Nat. Mater.* **21**, 1324-1332 (2022).
43. F. Zhang, Z. Li, Y. Duan, A. Abbas, R. Mundaca-Uribe, L. Yin, H. Luan, W. Gao, R. H. Fang, L. Zhang, J. Wang, Gastrointestinal tract drug delivery using algae motors embedded in a degradable capsule. *Sci. Robot.* **7**, eabo4160 (2022).
44. D. Gong, N. Celi, D. Zhang, J. Cai, Magnetic biohybrid microrobot multimers based on *Chlorella* cells for enhanced targeted drug delivery. *ACS Appl. Mater. Interfaces* **14**, 6320-6330 (2022).
45. L. Liu, J. Wu, S. Wang, K. Liu, J. B. Gao, B. Chen, Y. C. Ye, F. Wang, F. Tong, J. M. Jiang, J. F. Ou, D. A. Wilson, Y. F. Tu, F. Peng, Control the neural stem cell fate with biohybrid piezoelectrical magnetite micromotors. *Nano Lett.* **21**, 9361-9363 (2021).
46. L. Liu, J. Wu, B. Chen, J. Gao, T. Li, Y. Ye, H. Tian, S. Wang, F. Wang, J. Jiang, J. Ou, F. Tong, F. Peng, Y. Tu, Magnetically actuated biohybrid microswimmers for precise photothermal muscle contraction. *ACS Nano* **16**, 6515-6526 (2022).
47. S. Liu, H. Yang, M. Y. Ho, B. Xing, Recent advances of material-decorated photosynthetic microorganisms and their aspects in biomedical applications. *Adv. Opt. Mater.* **11**, 2203038 (2023).
48. D. Gong, L. Sun, X. Li, W. Zhang, D. Zhang, J. Cai, Micro/nanofabrication, assembly, and actuation based on microorganisms: recent advances and perspectives. *Small Struct.* **4**, 2200356 (2023).
49. D. Zhong, Z. Du, M. Zhou, Algae: a natural active material for biomedical applications. *View* **2**, 20200189 (2021).
50. Z. Zhang, Y. Chen, L. H. Klausen, S. A. Skaanvik, D. Wang, J. Chen, M. Dong, The rational design and development of microalgae-based biohybrid materials for biomedical applications. *Engineering* **24**, 102-113 (2023).
51. D. Quashie, Jr., P. Benhal, Z. Chen, Z. Wang, X. Mu, X. Song, T. Jiang, Y. Zhong, U. K. Cheang, J. Ali, Magnetic bio-hybrid micro actuators. *Nanoscale* **14**, 4364-4379 (2022).
52. W. Wang, W. T. Duan, S. Ahmed, T. E. Mallouk, A. Sen, Small power: autonomous nano- and micromotors propelled by self-generated gradients. *Nano Today* **8**, 531-554 (2013).
53. E. H. Harris, Chlamydomonas as a model organism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 363-406 (2001).
54. R. Hirschberg, S. Rodgers, Chemoresponses of Chlamydomonas reinhardtii. *J. Bacteriol.* **134**, 671-673 (1978).
55. Y. Hayashi, K. Sugawara, Simultaneous coupling of phototaxis and electrotaxis in *Volvox algae*. *Phys. Rev. E* **89**, 042714 (2014).
56. J. L. Lai, Q. F. Meng, M. Y. Tian, X. Y. Zhuang, P. Pan, L. A. Du, L. Deng, J. Y. Tang, N. Y. Jin, L. Rao, A decoy microrobot that removes SARS-CoV-2 and its variants in wastewater. *Cell Rep. Phys. Sci.* **3**, 101061 (2022).
57. L. Xie, X. Pang, X. Yan, Q. Dai, H. Lin, J. Ye, Y. Cheng, Q. Zhao, X. Ma, X. Zhang, G. Liu, X. Chen, Photoacoustic imaging-trackable magnetic microswimmers for pathogenic bacterial infection treatment. *ACS Nano* **14**, 2880-2893 (2020).
58. S. X. Xie, L. L. Qin, G. X. Li, N. A. D. Jiao, Robotized algal cells and their multiple functions. *Soft Matter* **17**, 3047-3054 (2021).
59. I. P. Kerschgens, K. Gademann, Antibiotic algae by chemical surface engineering. *Chembiochem* **19**, 439-443 (2018).
60. G. Santomauro, A. V. Singh, B. W. Park, M. Mohammadrahimi, P. Erkoc, E. Goering, G. Schütz, M. Sitti, J. Bill, Incorporation of Terbium into a microalga leads to magnetotactic swimmers. *Adv. Biosyst.* **2**, 1800039 (2018).
61. W. M. Ng, H. X. Che, C. Guo, C. Liu, S. C. Low, D. J. Chieh Chan, R. Mohamud, J. Lim, Artificial magnetotaxis of microbot: magnetophoresis versus self-swimming. *Langmuir* **34**, 7971-7980 (2018).

62. I. S. Shchelik, J. V. D. Molino, K. Gademann, Biohybrid microswimmers against bacterial infections. *Acta Biomater.* **136**, 99-110 (2021).
63. I. S. Shchelik, S. Sieber, K. Gademann, Green algae as a drug delivery system for the controlled release of antibiotics. *Chem. Eur. J.* **26**, 16644-16648 (2020).
64. D. Gong, J. Cai, N. Celi, C. Liu, W. Q. Zhang, L. Feng, D. Y. Zhang, Controlled propulsion of wheel-shape flaky microswimmers under rotating magnetic fields. *Appl. Phys. Lett.* **114**, 123701 (2019).
65. D. Gong, N. Celi, L. Xu, D. Zhang, J. Cai, CuS nanodots-loaded biohybrid magnetic helical microrobots with enhanced photothermal performance. *Mater. Today Chem.* **23**, 100694 (2022).
66. L. Liu, B. Chen, K. Liu, J. Gao, Y. Ye, Z. Wang, N. Qin, D. A. Wilson, Y. Tu, F. Peng, Wireless manipulation of magnetic/piezoelectric micromotors for precise neural stem-like cell stimulation. *Adv. Funct. Mater.* **30**, 1910108 (2020).
67. M. Li, J. Wu, D. Lin, J. Yang, N. Jiao, Y. Wang, L. Liu, A diatom-based biohybrid microrobot with a high drug-loading capacity and pH-sensitive drug release for target therapy. *Acta Biomater.* **154**, 443-453 (2022).
68. E. Lauga, T. R. Powers, The hydrodynamics of swimming microorganisms. *Rep. Prog. Phys.* **72**, 096601 (2009).
69. B. Behkam, M. Sitti, Design methodology for biomimetic propulsion of miniature swimming robots. *J. Dyn. Syst-T. Asme.* **128**, 36-43 (2006).
70. X. Garcia, S. Rafaï, P. Peyla, Light control of the flow of phototactic microswimmer suspensions. *Phys. Rev. Lett.* **110**, 138106 (2013).
71. G. J. Pazour, N. Agrin, J. Leszyk, G. B. Witman, Proteomic analysis of a eukaryotic cilium. *J. Cell Biol.* **170**, 103-113 (2005).
72. C. Shingyoji, H. Higuchi, M. Yoshimura, E. Katayama, T. Yanagida, Dynein arms are oscillating force generators. *Nature* **393**, 711-714 (1998).
73. C. J. Brokaw, R. Kamiya, Bending patterns of Chlamydomonas flagella: IV. Mutants with defects in inner and outer dynein arms indicate differences in dynein arm function. *Cell Motil. Cytoskeleton* **8**, 68-75 (1987).
74. R. Ahmad, C. Kleineberg, V. Nasirimarekani, Y. J. Su, S. G. Pozveh, A. Bae, K. Sundmacher, E. Bodenschatz, I. Guido, T. Vidakovic-Koch, A. Gholami, Light-powered reactivation of flagella and contraction of microtubule networks: toward building an artificial cell. *ACS Synth. Biol.* **10**, 1490-1504 (2021).
75. E. F. Smith, W. S. Sale, Regulation of dynein-driven microtubule sliding by the radial spokes in flagella. *Science* **257**, 1557-1559 (1992).
76. D. Luck, G. Piperno, Z. Ramanis, B. Huang, Flagellar mutants of Chlamydomonas: studies of radial spoke-defective strains by dikaryon and revertant analysis. *Proc. Natl. Acad. Sci. U. S. A.* **74**, 3456-3460 (1977).
77. N. Ueki, T. Ide, S. Mochiji, Y. Kobayashi, R. Tokutsu, N. Ohnishi, K. Yamaguchi, S. Shigenobu, K. Tanaka, J. Minagawa, T. Hisabori, M. Hirono, K. Wakabayashi, Eyespot-dependent determination of the phototactic sign in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 5299-5304 (2016).
78. R. R. Bennett, R. Golestanian, A steering mechanism for phototaxis in *Chlamydomonas*. *J. R. Soc. Interface* **12**, 20141164 (2015).
79. O. Tainio, F. Sohrabi, N. Janarek, J. Koivisto, A. Puisto, L. Viitanen, J. V. I. Timonen, M. Alava, Chlamydomonas reinhardtii swimming in the Plateau borders of 2D foams. *Soft Matter* **17**, 145-152 (2021).
80. U. Ruffer, W. Nultsch, High-speed cinematographic analysis of the movement of Chlamydomonas. *Cell Motil. Cytoskeleton* **5**, 251-263 (1985).
81. H. Harz, C. Nonnengasser, P. Hegemann, The photoreceptor current of the green alga Chlamydomonas. *Philos. T. R. Soc. B.* **338**, 39-52 (1992).
82. K. Schaller, R. David, R. Uhl, How Chlamydomonas keeps track of the light once it has reached the right phototactic orientation. *Biophys. J.* **73**, 1562-1572 (1997).
83. H. Harz, P. Hegemann, Rhodopsin-regulated calcium currents in Chlamydomonas. *Nature* **351**, 489-491 (1991).
84. E. M. Holland, F. J. Braun, C. Nonnengasser, H. Harz, P. Hegemann, The nature of rhodopsin-triggered photocurrents in Chlamydomonas. I. Kinetics and influence of divalent ions. *Biophys. J.* **70**, 924-931 (1996).
85. K. W. Foster, R. D. Smyth, Light antennas in phototactic algae. *Microbiol. Rev.* **44**, 572-630 (1980).
86. D. G. Grier, A revolution in optical manipulation. *Nature* **424**, 810-816 (2003).
87. H. Xin, Y. Li, D. Xu, Y. Zhang, C. Chen, B. Li, Single upconversion nanoparticle-bacterium cotrapping

for single-bacterium labeling and analysis. *Small* **13**, 1603418 (2017).
88. K. Norregaard, R. Metzler, C. M. Ritter, K. Berg-Sorensen, L. B. Oddershede, Manipulation and motion of organelles and single molecules in living cells. *Chem. Rev.* **117**, 4342-4375 (2017).
89. J. L. Killian, F. Ye, M. D. Wang, Optical tweezers: a force to be reckoned with. *Cell* **175**, 1445-1448 (2018).
90. H. B. Xin, Y. C. Li, Y. C. Liu, Y. Zhang, Y. F. Xiao, B. J. Li, Optical forces: from fundamental to biological applications. *Adv. Mater.* **32**, 2001994 (2020).
91. R. P. Sinha, D. P. Häder, UV-induced DNA damage and repair: a review. *Photochem. Photobiol. Sci.* **1**, 225-236 (2002).
92. D. I. Pattison, M. J. Davies, Actions of ultraviolet light on cellular structures. In *Cancer: Cell Structures, Carcinogens and Genomic Instability*, Experientia Supplementum, vol 96, pp 131-157. Birkhäuser Basel: Basel, 2006.
93. R. S. M. Rikken, R. J. M. Nolte, J. C. Maan, J. C. M. van Hest, D. A. Wilson, P. C. M. Christianen, Manipulation of micro- and nanostructure motion with magnetic fields. *Soft Matter* **10**, 1295-1308 (2014).
94. S. Martel, Beyond imaging: Macro- and microscale medical robots actuated by clinical MRI scanners. *Sci. Robot.* **2**, eaam8119 (2017).
95. J. Zhuang, M. Sitti, Chemotaxis of bio-hybrid multiple bacteria-driven microswimmers. *Sci. Rep.* **6**, 32135 (2016).
96. D. Kim, A. Liu, E. Diller, M. Sitti, Chemotactic steering of bacteria propelled microbeads. *Biomed. Microdevices* **14**, 1009-1017 (2012).
97. B. Cortese, I. E. Palamà, S. D'Amone, G. Gigli, Influence of electrotaxis on cell behavior. *Integr. Biol.* **6**, 817-830 (2014).
98. T. Y. Tsong, Electroporation of cell-membranes. *Biophys. J.* **60**, 297-306 (1991).
99. T. H. Tran, D. H. Kim, J. Kim, M. J. Kim, D. Byun, Use of an AC electric field in galvanotactic on/off switching of the motion of a microstructure blotted by *Serratia marcescens*. *Appl. Phys. Lett.* **99**, 063702 (2011).
100. S. Tottori, L. Zhang, F. M. Qiu, K. K. Krawczyk, A. Franco-Obregón, B. J. Nelson, Magnetic helical micromachines: fabrication, controlled swimming, and cargo transport. *Adv. Mater.* **24**, 811-816 (2012).
101. W. Wang, L. A. Castro, M. Hoyos, T. E. Mallouk, Autonomous motion of metallic microrods propelled by ultrasound. *ACS Nano* **6**, 6122-6132 (2012).
102. S. Palagi, A. G. Mark, S. Y. Reigh, K. Melde, T. Qiu, H. Zeng, C. Parmeggiani, D. Martella, A. Sanchez-Castillo, N. Kapernaum, F. Giesselmann, D. S. Wiersma, E. Lauga, P. Fischer, Structured light enables biomimetic swimming and versatile locomotion of photoresponsive soft microrobots. *Nat. Mater.* **15**, 647-653 (2016).
103. J. J. Abbott, K. E. Peyer, M. C. Lagomarsino, L. Zhang, L. X. Dong, I. K. Kaliakatsos, B. J. Nelson, How should microrobots swim? *Int. J. Robot. Res.* **28**, 1434-1447 (2009).
104. K. Bente, A. Codutti, F. Bachmann, D. Faivre, Biohybrid and bioinspired magnetic microswimmers. *Small* **14**, 1704374 (2018).
105. K. I. Morozov, Y. Mirzae, O. Kenneth, A. M. Leshansky, Dynamics of arbitrary shaped propellers driven by a rotating magnetic field. *Phys. Rev. Fluids* **2**, 044202 (2017).
106. N. Shimizu, H. Oda, Y. Morimoto, S. Takeuchi, Biohybrid micro pinwheel powered by trapped microalgae. *2021 IEEE 34th International Conference on Micro Electro Mechanical Systems (MEMS), Gainesville, FL, USA, 2021, pp. 533-535. doi: 10.1109/MEMS51782.2021.9375193.*
107. X. D. Wang, N. D. Jiao, S. Tung, L. Q. Liu, Locomotion of microstructures driven by algae cells. *2018 International Conference on Manipulation, Automation and Robotics at Small Scales (MARSS), Nagoya, Japan, 2018, pp. 1-5. doi: 10.1109/MARSS.2018.8481168.*
108. R. Kamiya, G. B. Witman, Submicromolar levels of calcium control the balance of beating between the two flagella in demembranated models of Chlamydomonas. *J. Cell Biol.* **98**, 97-107 (1984).
109. R. Yokokawa, S. Takeuchi, T. Kon, M. Nishiura, R. Ohkura, K. Sutoh, H. Fujita, Hybrid nanotransport system by biomolecular linear motors. *J. Microelectromech. Syst.* **13**, 612-619 (2004).
110. X. J. Teng, W. M. Ng, W. H. Chong, D. J. C. Chan, R. Mohamud, B. S. Ooi, C. Guo, C. Z. Liu, J. Lim, The transport behavior of a biflagellated microswimmer before and after cargo loading. *Langmuir* **37**, 9192-9201 (2021).
111. D. X. Zhang, D. N. Zhong, J. Ouyang, J. He, Y. C. Qi, W. Chen, X. C. Zhang, W. Tao, M. Zhou, Microalgae-based oral microcarriers for gut microbiota homeostasis and intestinal protection in cancer radiotherapy. *Nat. Commun.* **13**, 1413 (2022).
112. R. K. Pandey, L. N. Goswami, Y. H. Chen, A. Gryshuk, J. R. Missert, A. Oseroff, T. J. Dougherty, Nature: A rich source for developing multifunctional agents. tumor-imaging and photodynamic therapy. *Lasers*

Surg. Med. **38**, 445-467 (2006).
113. E. H. Murchie, T. Lawson, Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *J. Exp. Bot.* **64**, 3983-3998 (2013).
114. V. Rizzi, P. Fini, F. Fanelli, T. Placido, P. Semeraro, T. Sibillano, A. Fraix, S. Sortino, A. Agostiano, C. Giannini, P. Cosma, Molecular interactions, characterization and photoactivity of Chlorophyll a/chitosan/2-HP-β-cyclodextrin composite films as functional and active surfaces for ROS production. *Food Hydrocolloids* **58**, 98-112 (2016).
115. D. N. Zhong, W. L. Li, S. Y. Hua, Y. C. Qi, T. T. Xie, Y. Qiao, M. Zhou, Calcium phosphate engineered photosynthetic microalgae to combat hypoxic-tumor by in-situ modulating hypoxia and cascade radio-phototherapy. *Theranostics* **11**, 3580-3594 (2021).
116. W. L. Li, D. N. Zhong, S. Y. Hua, Z. Du, M. Zhou, Biomineralized biohybrid algae for tumor hypoxia modulation and cascade radio-photodynamic therapy. *ACS Appl. Mater. Interfaces* **12**, 44541-44553 (2020).
117. M. F. Huo, L. Y. Wang, L. L. Zhang, C. Y. Wei, Y. Chen, J. L. Shi, Photosynthetic tumor oxygenation by photosensitizer-containing cyanobacteria for enhanced photodynamic therapy. *Angew. Chem.* **59**, 1906-1913 (2020).
118. R. Zhu, Z. Q. Sun, C. P. Li, S. Ramakrishna, K. Chiu, L. M. He, Electrical stimulation affects neural stem cell fate and function in vitro. *Exp. Neurol.* **319**, 112963 (2019).
119. M. D'Ascenzo, R. Piacentini, P. Casalbore, M. Budoni, R. Pallini, G. B. Azzena, C. Grassi, Role of L-type Ca^{2+} channels in neural stem/progenitor cell differentiation. *Eur. J. Neurosci.* **23**, 935-944 (2006).
120. H. Choi, B. Kim, S. H. Jeong, T. Y. Kim, D. P. Kim, Y. K. Oh, S. K. Hahn, Microalgae-based biohybrid microrobot for accelerated diabetic wound healing. *Small* **19**, e2204617 (2023).
121. J. Xiong, Y. Shi, T. Pan, D. Lu, Z. He, D. Wang, X. Li, G. Zhu, B. Li, H. Xin, Wake-riding effect-inspired opto-hydrodynamic Diatombot for non-invasive trapping and removal of nano-biothreats. *Adv. Sci.* **10**, e2301365 (2023).
122. Y. Weizmann, F. Patolsky, I. Willner, Amplified detection of DNA and analysis of single-base mismatches by the catalyzed deposition of gold on Au-nanoparticles. *Analyst* **126**, 1502-1504 (2001).
123. E. D. Goluch, J. M. Nam, D. G. Georganopoulou, T. N. Chiesl, K. A. Shaikh, K. S. Ryu, A. E. Barron, C. A. Mirkin, C. Liu, A bio-barcode assay for on-chip attomolar-sensitivity protein detection. *Lab Chip* **6**, 1293-1299 (2006).
124. P. S. Waggoner, M. Varshney, H. G. Craighead, Detection of prostate specific antigen with nanomechanical resonators. *Lab Chip* **9**, 3095-3099 (2009).
125. G. Zheng, X. P. Gao, C. M. Lieber, Frequency domain detection of biomolecules using silicon nanowire biosensors. *Nano Lett.* **10**, 3179-3183 (2010).
126. S. Schuerle, A. P. Soleimany, T. Yeh, G. M. Anand, M. Haberli, H. E. Fleming, N. Mirkhani, F. Qiu, S. Hauert, X. Wang, B. J. Nelson, S. N. Bhatia, Synthetic and living micropropellers for convection-enhanced nanoparticle transport. *Sci. Adv.* **5**, eaav4803 (2019).
127. Y. Zhang, L. Zhang, L. Yang, C. I. Vong, K. F. Chan, W. K. K. Wu, T. N. Y. Kwong, N. W. S. Lo, M. Ip, S. H. Wong, J. J. Y. Sung, P. W. Y. Chiu, L. Zhang, Real-time tracking of fluorescent magnetic spore-based microrobots for remote detection of *C. diff* toxins. *Sci. Adv.* **5**, eaau9650 (2019).
128. J. Wu, S. Balasubramanian, D. Kagan, K. M. Manesh, S. Campuzano, J. Wang, Motion-based DNA detection using catalytic nanomotors. *Nat. Commun.* **1**, 36 (2010).
129. B. Esteban-Fernandez de Avila, A. Martin, F. Soto, M. A. Lopez-Ramirez, S. Campuzano, G. M. Vasquez-Machado, W. Gao, L. Zhang, J. Wang, Single cell real-time miRNAs sensing based on nanomotors. *ACS Nano* **9**, 6756-6764 (2015).

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Figures and Tables
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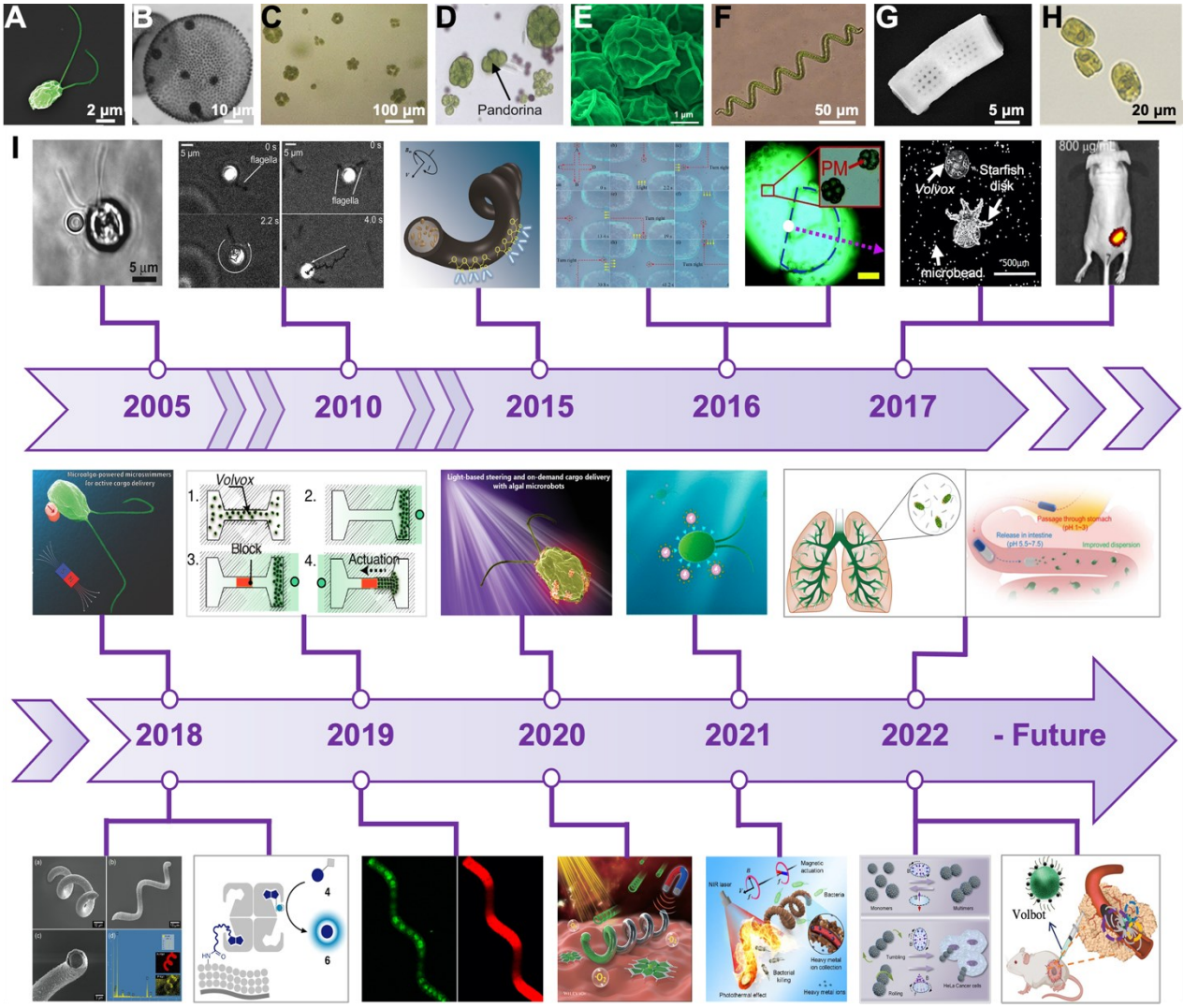

Fig 1. Common microalga species adopted to fabricate microrobots and representative developments of algae-inspired microrobots since 2005. (A) SEM image (pseudocolor) of *Chlamydomonas reinhardtii (CR)*;[21] Copyright, 2020 John Wiley and Sons. **(B)** Light microscopy image of *Volvox (VX)*;[23] Copyright, 2022 John Wiley and Sons. **(C)** Light microscopy image of *Eudorina elegans (EE)*;[24] Copyright, 2016 Springer Nature. **(D)** Light microscopy image of *Pandorina morum (PM)*;[25] Copyright, 2005 Royal Society of Chemistry. **(E)** SEM image (pseudocolor) of *Chlorella (Ch)*;[26] From Qiao et al.[26] Reprinted with permission from AAAS. **(F)** Light microscopy image of *Spirulina platensis (SP)*;[27] Copyright, 2019 American Chemical Society. **(G)** SEM image of *Diatom (DM)*;[28] Copyright, 2020 American Chemical Society. **(H)** Light microscopy image of *Tetraselmis Subcordiformis (TS)*.[29] Copyright, 2018 American Chemical Society. **(I)** Representative AIM developments since its inception in 2005. Controlled microscale cargo transport by *CR* (2005);[22] Copyright, 2005 National Academy of Sciences, U.S.A. Locomotion of flagellated micro-object (2010);[30] Reprinted from Mori et al.[30], with the permission of AIP Publishing. *SP*-templated porous hollow swimmer (2015);[31] Copyright, 2015 John Wiley and Sons. Light-guided motion of robotized *CR* (2016)[24] Copyright, 2016 Springer Nature and Bio-tweezer actuated by *PM* (2016);[25] Copyright, 2005 Royal Society of Chemistry. Manipulation of micro-objects by *VX* (2017)[32] Reprinted from Hatsuzawa et al.[32] and *in vivo* imaging of magnetized *SP* (2017);[33] From Yan et al.[33] Reprinted with permission from AAAS, active cargo delivery by magnetically-guided *CR* (2018),[34] Copyright, 2018 John Wiley and Sons, enhanced propulsion of nickel-plated *SP* (2018)[35] Reprinted from Gong et al.[35] and on-cell catalysis & antibacterial therapy by light-guided *CR* (2018);[36] Copyright, 2018 Springer Nature, submillimeter cargo transport by *VX* (2019)[37] Copyright, 2019 MDPI, and cargo delivery & anticancer therapy by magnetic *SP* (2019);[38] Reprinted from Yan et al.[38], microoperation & cargo delivery by *CR* (2020)[21] Copyright, 2020 John Wiley and Sons, and cell stimulation, anticancer & antibacterial therapy by *SP* (2020);[39] Copyright, 2020 John Wiley and Sons, antibacterial therapy & environment remediation by *CR* (2021)[40] Copyright, 2021 American Chemical Society, and detoxification, anticancer & antibacterial treatment by *SP* (2021);[41] Reprinted from Zheng et al.[41], gastrointestinal drug delivery & acute bacterial pneumonia treatment by *CR* (2022),[42, 43] Copyright, 2022 Springer Nature and Copyright From Zhang et al.[43] Reprinted with permission from AAAS. muscle contraction & anticancer therapy by magnetic *Ch* (2022)[44] Copyright, 2022 American Chemical Society and multimodal imaging & therapy by magnetized *VX* (2022).[23] Copyright, 2022 John Wiley and Sons.

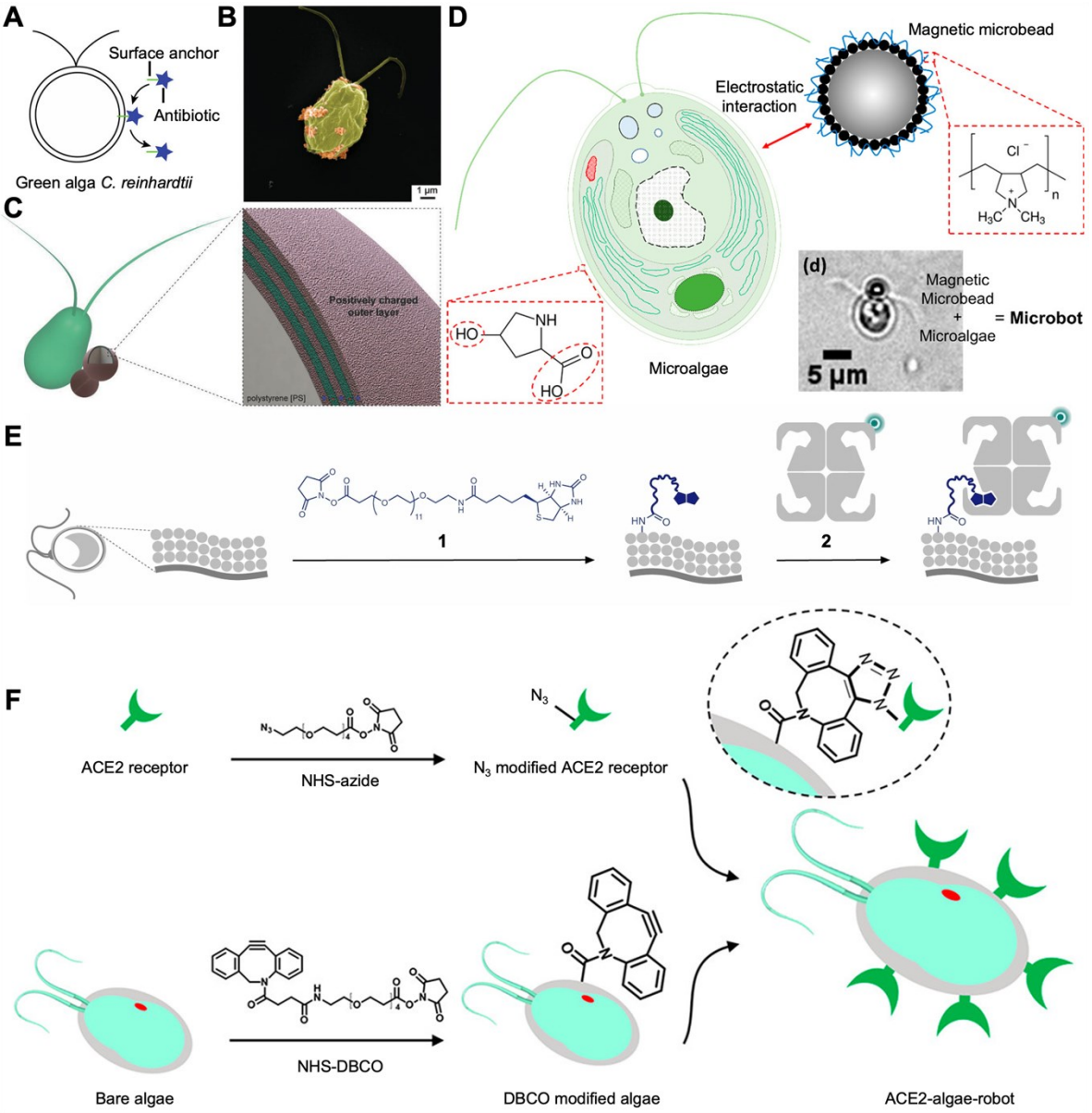

Fig 2. Fabrication methods of MFRs exemplified by *CR*-based microrobots. (A) Illustration of antibiotic vancomycin and 4-hydroxyproline oligomer reversibly attached to the cell wall of a *CR* through noncovalent binding.[59] Copyright, 2018 John Wiley and Sons. **(B)** Chitosan-coated iron oxide nanoparticles coated on a *CR* through electrostatic interaction.[21] Copyright, 2020 John Wiley and Sons. **(C)** Magnetic PS microparticles attached to a *CR* with layer-by-layer polyelectrolyte deposition.[34] Copyright, 2018 John Wiley and Sons. **(D)** A magnetic microbead labeled onto *CR* cell with cationic PDDA modification.[61] Copyright, 2018 American Chemical Society. **(E)** Surface functionalization of *CR* with streptavidin binding.[36] Copyright, 2018 Springer Nature. **(F)** Surface modification of *CR* cells with ACE2 receptor through click chemistry.[40] Copyright, 2021 American Chemical Society.

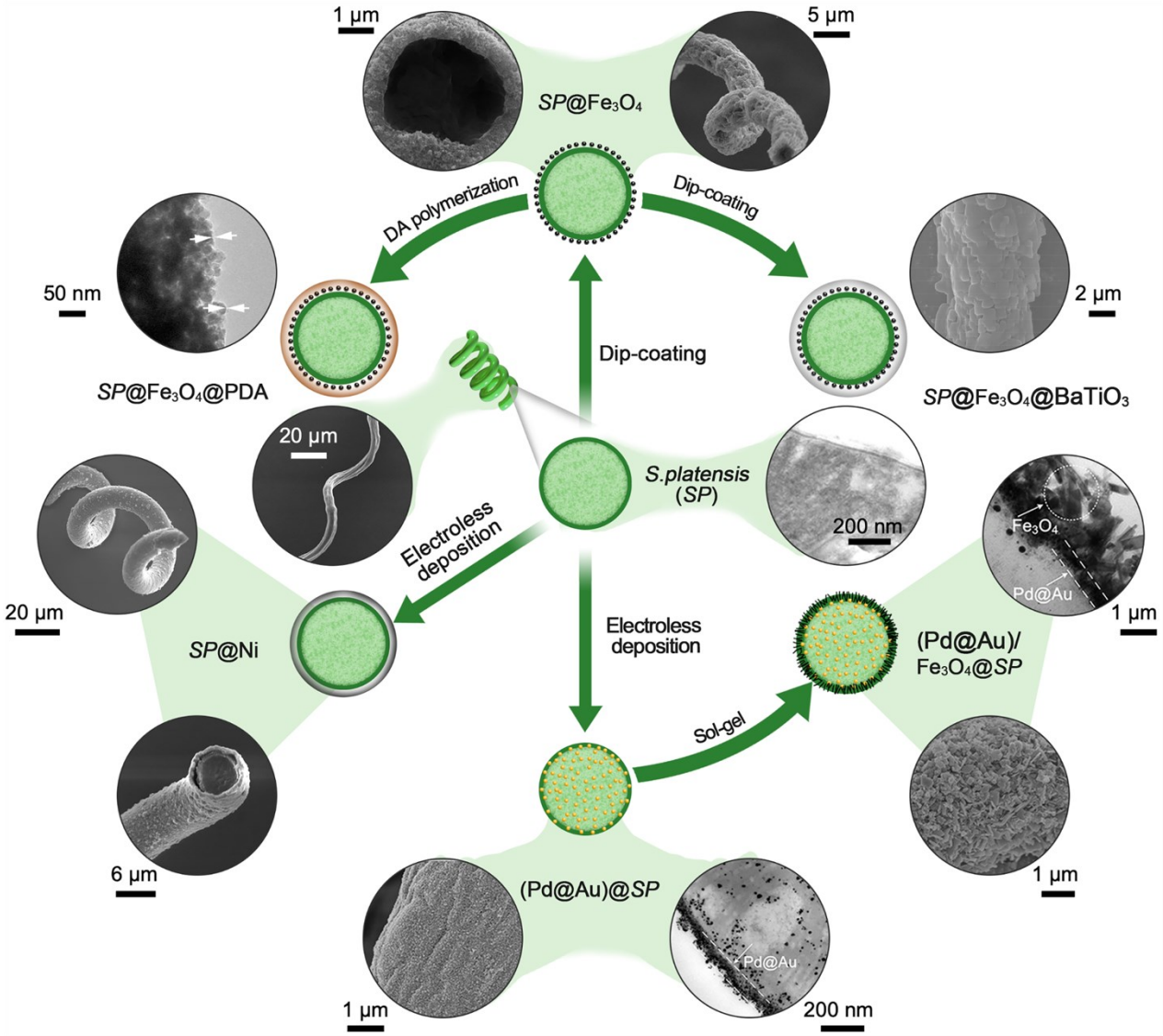

Fig 3. Fabrication methods of MHRs exemplified by *SP*-based microrobots. The multi-layer circular schematic demonstrates how various functional materials can be deposited on or incorporated into the *SP* cells through dip-coating, electroless deposition, sol-gel or polymerization procedures for designated functionalization. *SP*@Fe_3O_4;[33] From Yan et al.[33] Reprinted with permission from AAAS. *SP*@Fe_3O_4@PDA;[57] Copyright, 2020 American Chemical Society. *SP*@Fe_3O_4@$BaTiO_3$;[45] Copyright, 2021 American Chemical Society. *SP*@Ni;[35] Reprinted from Gong et al.[35] (Pd@Au)@*SP*;[27] Copyright, 2019 American Chemical Society. (Pd@Au)/ Fe_3O_4@*SP*.[27] Copyright, 2019 American Chemical Society.

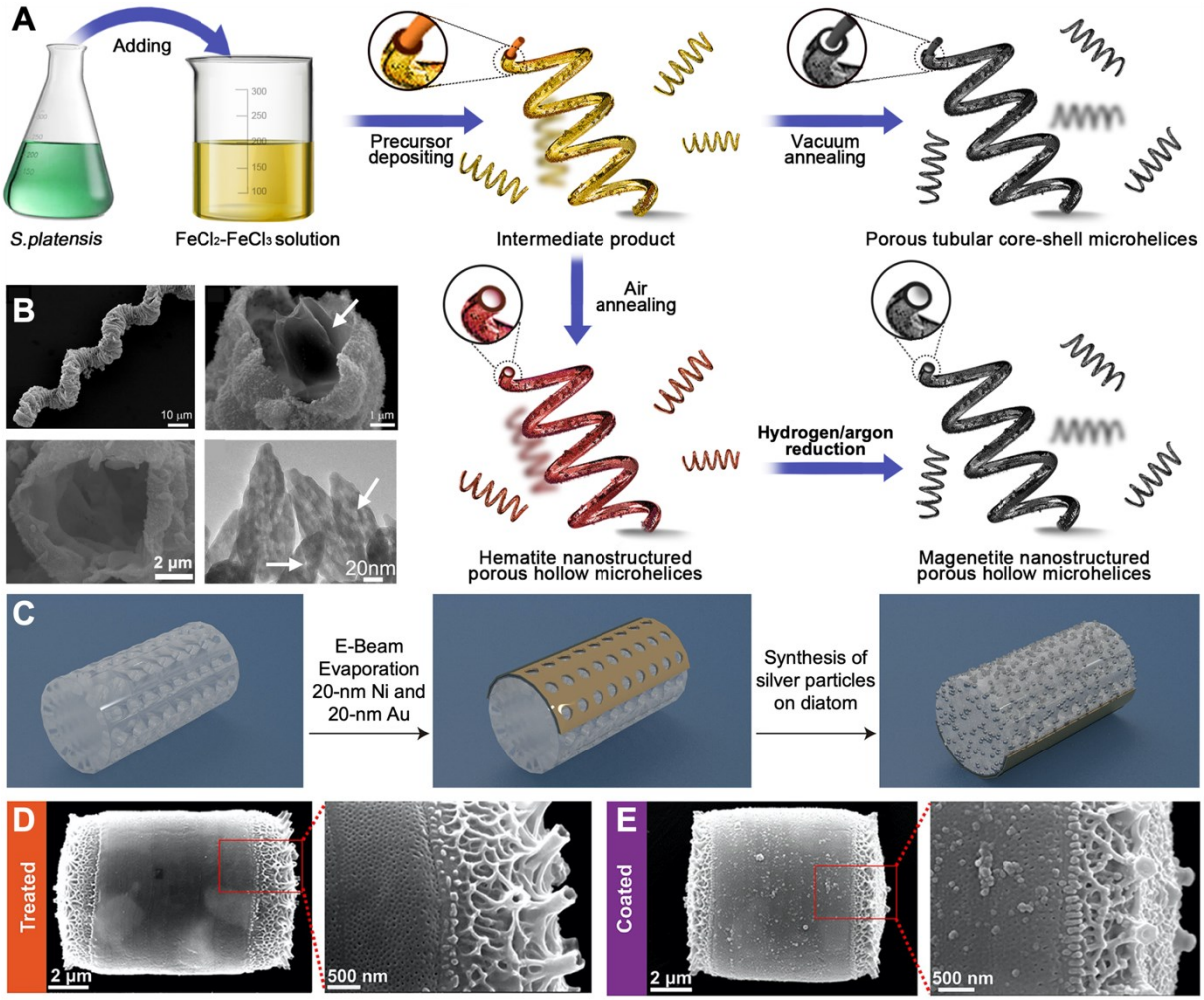

Fig 4. Fabrication methods of MTRs exemplified by *SP*-based and *Diatom*-based microrobots. (A) Schematic of the procedures for fabricating core-shell[41] and porous-hollow[31] *SP* MTRs. Copyright, 2015 John Wiley and Sons. **(B)** SEM images of (top row) a core-shell MTR obtained through precursor deposition plus vacuum annealing of the *SP*[41], Reprinted from Zheng et al.[41], and (bottom row) a porous-hollow MTR obtained through precursor deposition, air annealing plus hydrogen/argon reduction of the *SP*.[31] Copyright, 2015 John Wiley and Sons. The white arrows in the second and fourth panel indicate the carbon core in the middle of the core-shell MTR and the nanoscale mesopores on the shell of the porous-hollow MTR, respectively. **(C)** Schematic of the optoplasmonic MTR fabrication using Diatom frustules.[28] Copyright, 2020 American Chemical Society. **(D-E)** Diatom-based MTR fabricated by hydrochloric acid treatment and coating of Fe_3O_4 nanoparticles.[67] Reprinted from Li et al.[67]

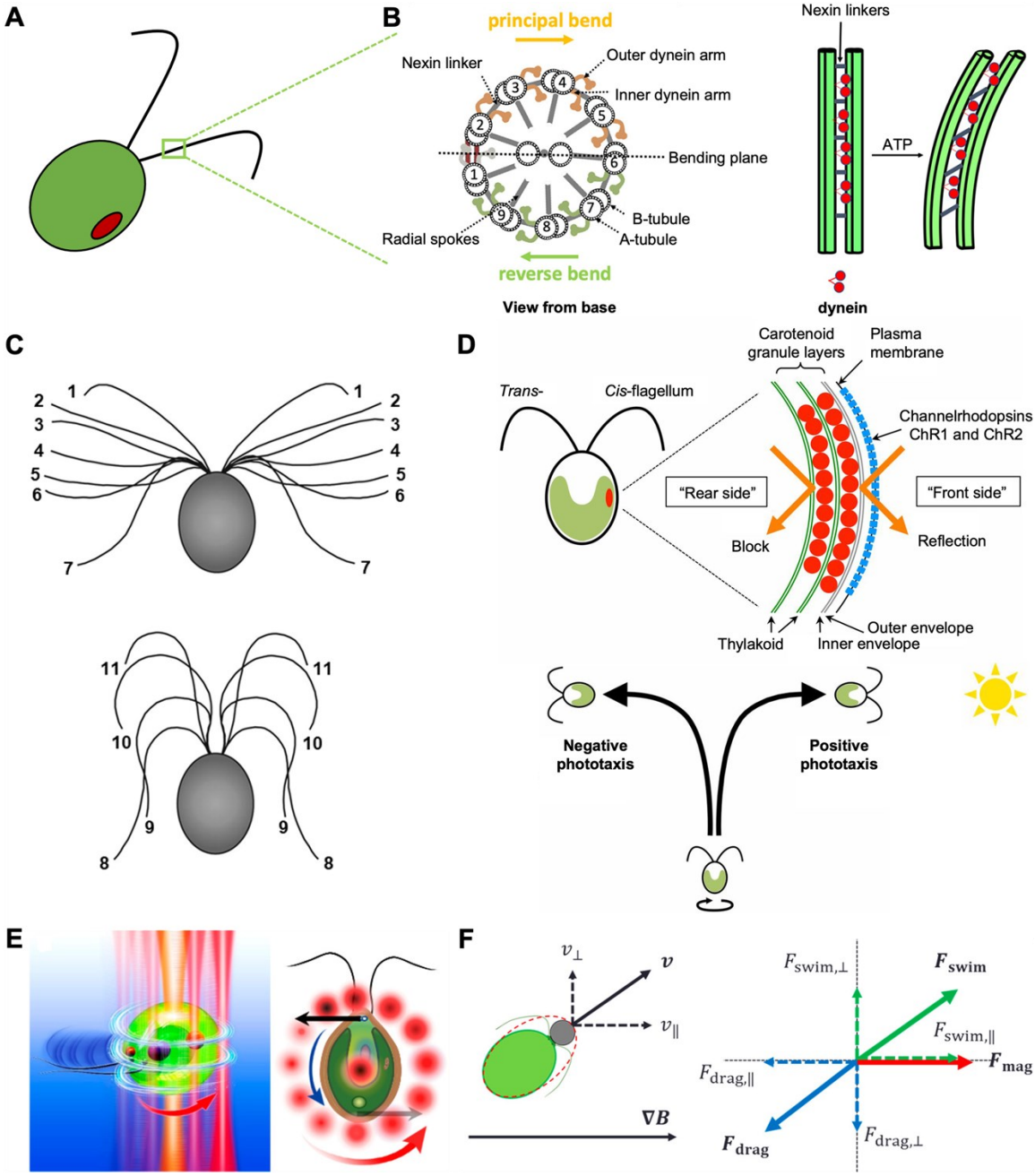

Fig 5. Actuation and steering methods of MFRs illustrated by living *CR* microrobots. (A) Schematic of a Chlamydomonas cell. **(B)** Schematic of the cell's ciliary axonemal cross-section showing how dynein motors convert chemical energy from ATP hydrolysis into mechanical work.[74] Copyright, 2021 American Chemical Society. **(C)** A cartoon illustrating the movement of *CR* flagella during a beating sequence.[22] Copyright, 2005 National Academy of Sciences, U.S.A. **(D)** (top) The eyespot of *CR* located near the cell equator, consisting of carotenoid granule layers (red), photoreceptor proteins, and channelrhodopsins (ChR1 and ChR2; blue). (below) *CR* adjusts the beating balance of its two flagella to exhibit either positive or negative phototaxis.[77] Copyright, 2016 National Academy of Sciences, U.S.A. **(E)** Schematic of the rotating motion of a *CR* cell driven by applied optical force.[9] Copyright, 2020 American Chemical Society. **(F)** Force analysis for a *CR* attached by a magnetic microbead and placed in a low-gradient magnetic field.[61] Copyright, 2018 American Chemical Society.

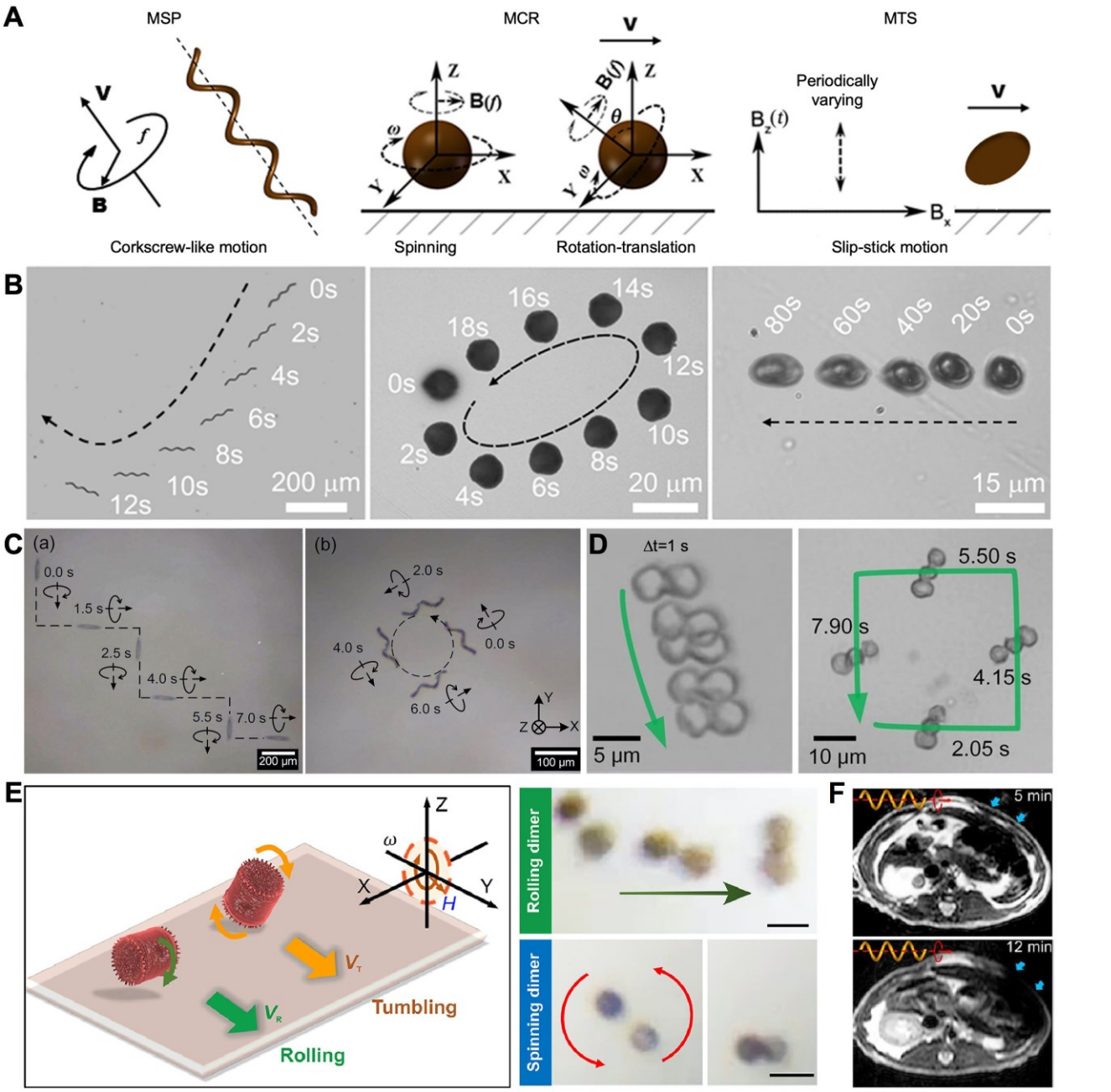

Fig 6. Magnetic actuation and steering of MHRs and MTRs. (A) Schematics of different magnetic actuation strategies for magnetized *SP*, *CR* and *TS*.[33] From Yan et al.[33] Reprinted with permission from AAAS. **(B)** Time-lapse image sequences showing the controlled locomotion of *SP*, *CR* and *TS* MHRs using the actuation methods in (A).[33] From Yan et al.[33] Reprinted with permission from AAAS. **(C)** Time-lapse image sequence of the complex maneuver of a *SP* MHR controlled through a rotating magnetic field.[35] Reprinted from Gong et al.[35] **(D)** Multiple *Ch* MHRs actuated and steered to perform simple and complex locomotion.[44] Copyright, 2022 American Chemical Society. **(E)** Tumbling and rolling motion modes of a Diatom-based MTR under a rotating magnetic field and formation of MTR dimers/trimers.[67] Reprinted from Li et al.[67] **(F)** A swarm of *SP* MHRs remotely actuated in rodent stomach with a rotating magnet and tracked with MR imaging.[33] From Yan et al.[33] Reprinted with permission from AAAS.

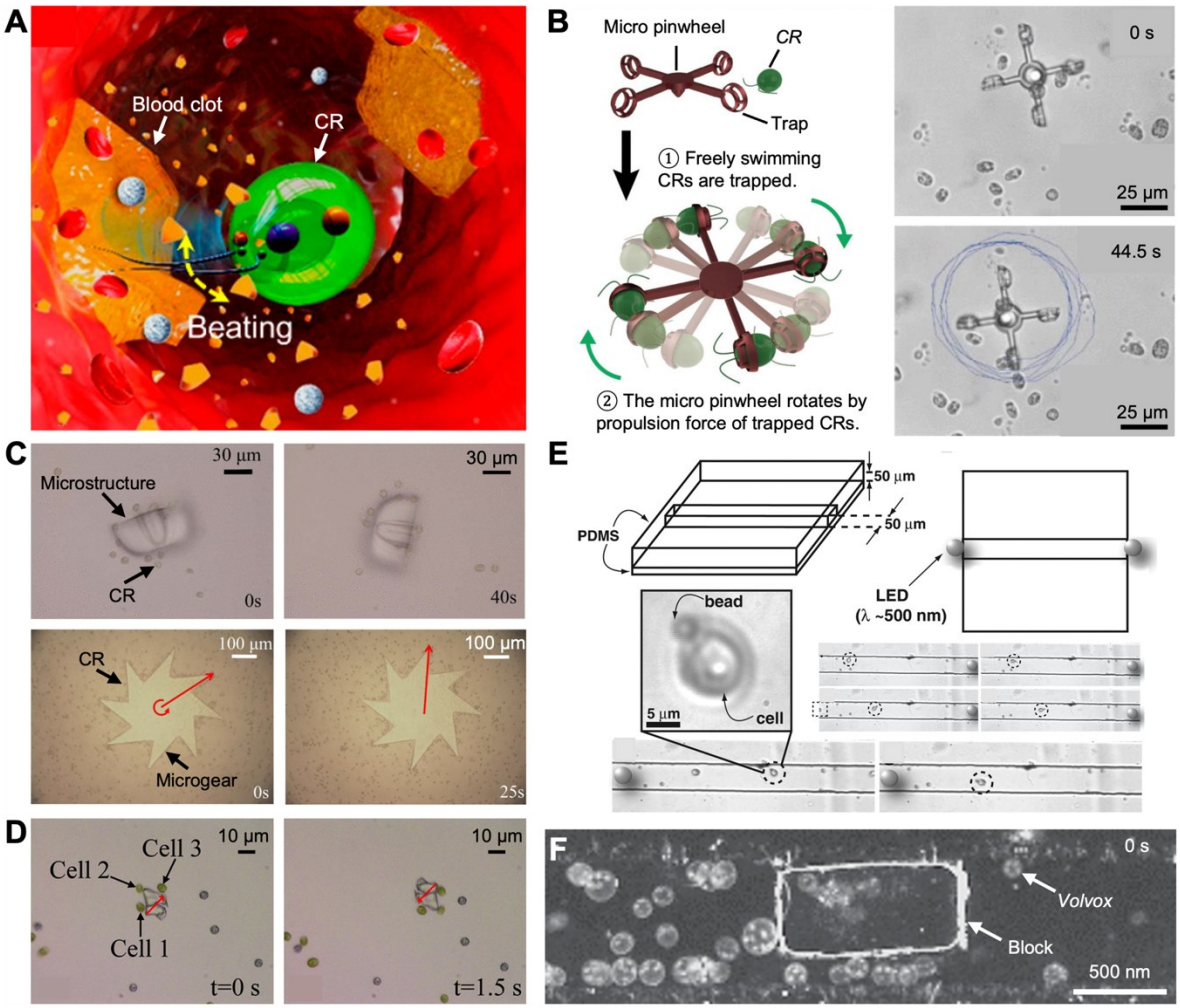

Fig 7. Micromanipulation. (A) Optical force-controlled destruction of bio-aggregates in a capillary-like environment through the beating flagella of *CR*-based MFR.[9] Copyright, 2020 American Chemical Society. **(B)** Rotation of a micro-pinwheel driven by trapped *CR* cells.[106] Copyright, 2021 IEEE. **(C)** Rotation of micro-objects propelled by randomly swimming *CR* cells.[107] Copyright, 2018 IEEE. **(D)** Controlled assembly and optical-guided cooperative motion of *CR* cells for micro-object manipulation.[58] Copyright, 2005 Royal Society of Chemistry. **(E)** Microchannel navigation of *CR* loading a microbead steered through phototaxis.[22] Copyright, 2005 National Academy of Sciences, U.S.A. **(F)** Transport of a submillimeter-sized block in a microchannel by a swarm of *Volvox* through a light irradiation platform.[37] Copyright, 2019 MDPI.

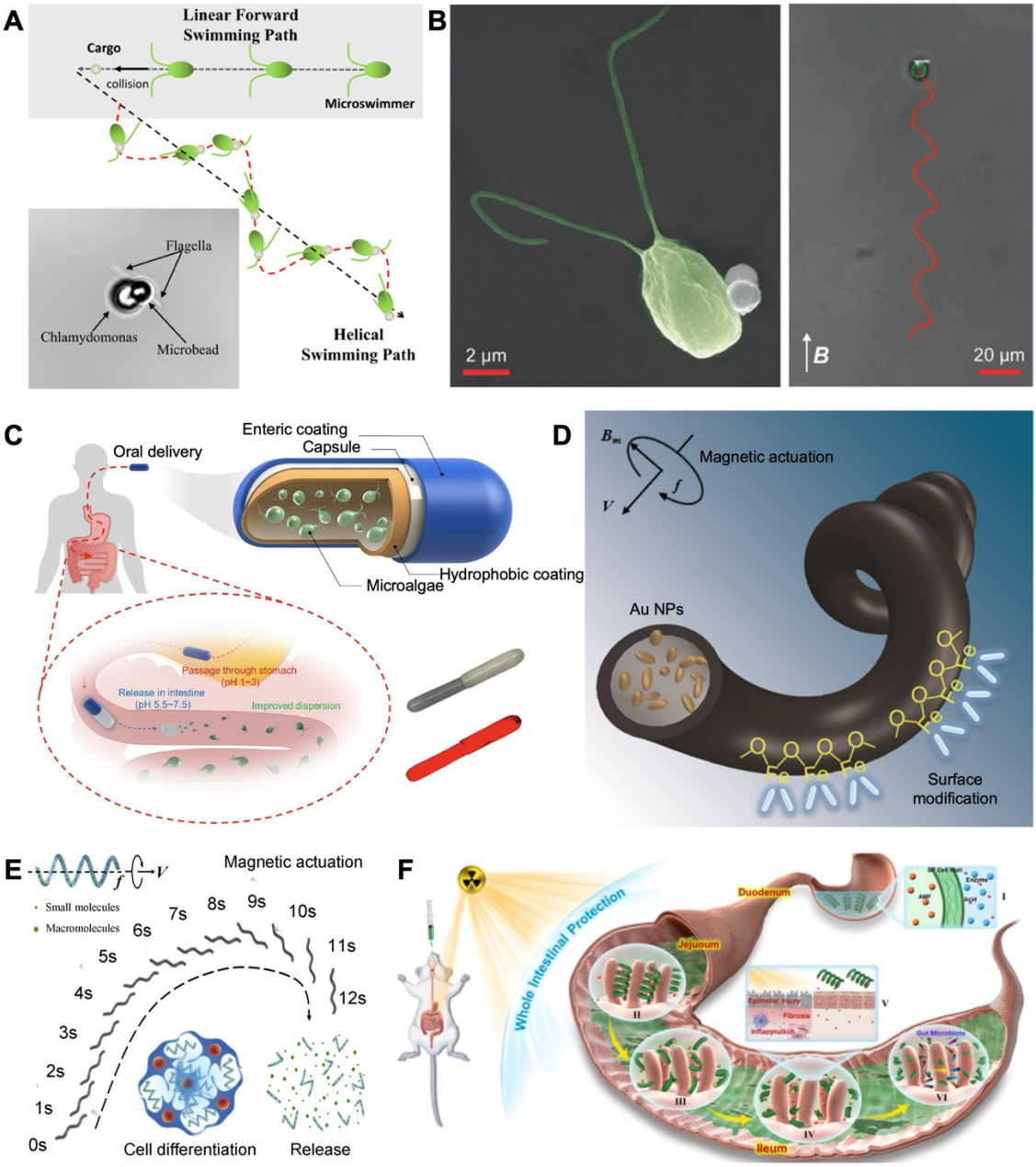

Fig 8. Active delivery. (A) Cargo loading and transport with a freely swimming *CR*.[110] Copyright, 2021 American Chemical Society. **(B)** Magnetically-assisted delivery with *CR* attaching a polyelectrolyte-functionalized magnetic cargo.[34] Copyright, 2018 John Wiley and Sons. **(C)** Gastrointestinal tract delivery and retention of therapeutic drugs loaded by *CR* cells encapsulated in a capsule.[43] From Zhang et al.[43] Reprinted with permission from AAAS. **(D)** Loading and release of Au NPs inside *SP*-based MTR.[31] Copyright, 2015 John Wiley and Sons. **(E)** Delivery and release of macromolecules for triggering cell differentiation with *SP*-based MHR.[38] Reprinted from Yan et al.[38] **(F)** Oral delivery of Amifostine-loaded *SP* MHR for drug accumulation and intestinal protection against cancer radiotherapy.[111] Copyright, 2010 Nature Publishing Group.

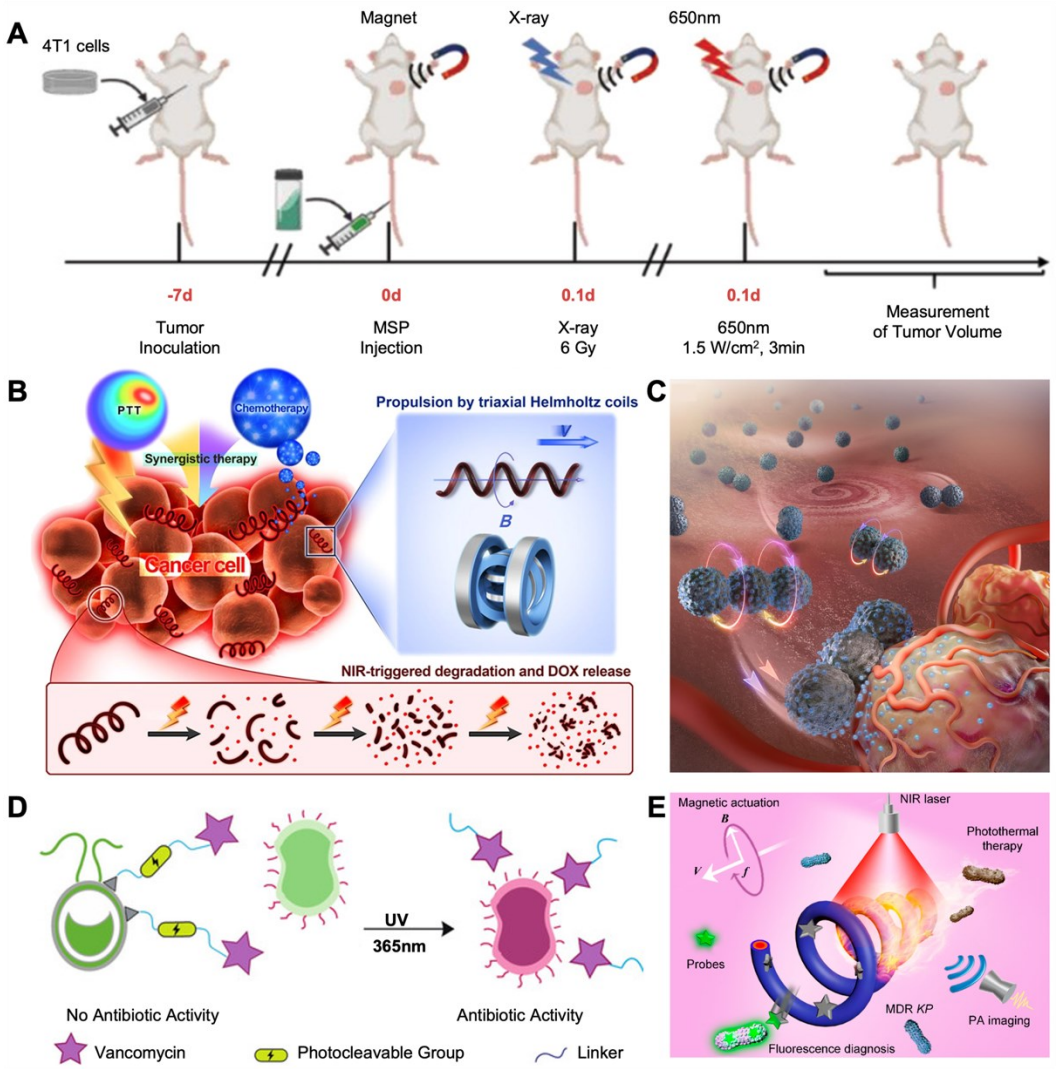

Fig 9. Anticancer and antibacterial therapy. (A) Combined PDT and RT therapy of solid tumor with *SP*-based magnetic microrobots in a breast tumor model.[39] Copyright, 2020 John Wiley and Sons. **(B)** Synergistic chemo-photothermal treatment of cancer cells (EC109 and 769-P) with DOX-loaded *SP* MHRs.[27] Copyright, 2019 American Chemical Society. **(C)** Chemotherapy of HeLa cells with *Ch*-based magnetic microrobots loaded with DOX.[44] Copyright, 2022 American Chemical Society. **(D)** Controlled release of antibiotic vancomycin with *CR* cell as living drug carrier.[63] Copyright, 2020 John Wiley and Sons. **(E)** Treatment of pathogenic bacterial infection using a swarm of PDA-coated magnetic *SP* MHRs.[57] Copyright, 2020 American Chemical Society.

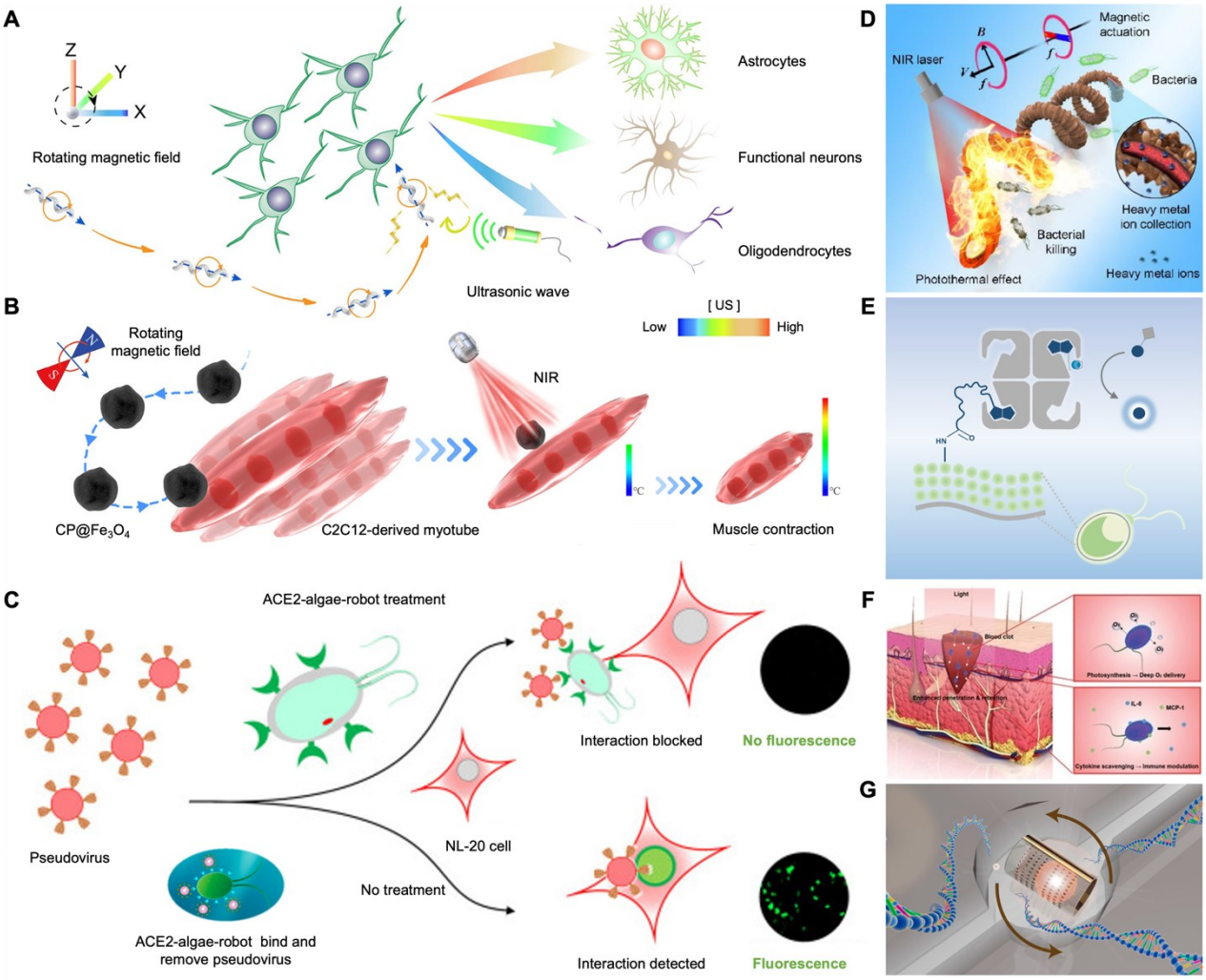

Fig 10. Cell stimulation and other applications. (A) Differentiation of neural stem cells triggered by *SP*@Fe_3O_4@BaTiO3 MHRs through converting ultrasound waves into electrical signals.[45] Copyright, 2021 American Chemical Society. **(B)** Muscle activation through targeted myotube contraction induced by the photothermal effect of *Ch*-based MHR coated with Fe_3O_4 nanoparticles.[46] Copyright, 2022 American Chemical Society. **(C)** *Ch*-based MFR for removal of SARS-CoV-2 pseudovirus in water.[40] Copyright, 2021 American Chemical Society. **(D)** *SP*-based core-shell MTRs for heavy metal adsorption.[41] Reprinted from Zheng et al.[41] **(E)** On-cell catalysis through surface functionalization of *CR* with artificial metalloenzyme.[36] Copyright, 2018 Springer Nature. **(F)** Acceleration of wound healing in diabetic mice through enhanced oxygen delivery and cytokine scavenging with *CR*-based microrobots.[120] Copyright, 2022 John Wiley and Sons. **(G)** Accelerated DNA enrichment and detection by Diatom-based optoplasmonic micromotor-sensors.[28] Copyright, 2020 American Chemical Society.

Table 1. Representative applications of algae-inspired microrobots.

Microrobots	Microalgae	Manufacturing	Functions	Applications	References
Microalgae Flagellated Robots (MFRs)	*Chlamydomonas reinhardtii, Eudorina elegans, Pandorina morum, Volvox*	Biotin streptavidin binding, electrostatic interaction, click chemistry	Phototaxis/magnetotaxis, photodynamic/photothermal, biocompatibility, degradability, oxygen generation, immunoregulation	Micromanipulation Active drug delivery Anticancer therapy Antibacterial therapy Biological detoxification On-cell enzyme catalysis Wound healing	9, 22, 25, 37, 58, 106, 107 21, 22, 34, 43, 110 23 42, 59, 63 40, 56 36 120
Microalgae Hybrid Robots (MHRs)	*Spirulina platensis, Chlorella, Diatom, Chlamydomonas reinhardtii, Tetraselmis subcordiformis*	Dip-coating, sol-gel, electroless deposition, surface polymerization, electrostatic interaction	Drug loading/release, off-on fluorescence sensing, enhanced photothermal, selective cytotoxicity, tunable biodegradability, piezoelectric effect, opto-hydrodynamic effect	Active drug delivery Anticancer therapy Antibacterial therapy Disease diagnosis Cell stimulation Biological detoxification	38, 111 27, 33, 39, 44 57, 65 33, 57 45, 46, 66 121
Microalgae Templated Robots (MTRs)	*Spirulina platensis, Diatom*	Biomineralization, annealing treatment, physical vapor deposition, electrostatic interaction	High payload capacity, photothermal effect, remote biosensing, heavy metal ion adsorption	Active drug delivery Antibacterial therapy Biological detoxification Biomolecule detection	31, 67 41 41 28