

A Supramolecular Approach to Nanoscale Motion: Polymersome-Based Self-Propelled Nanomotors

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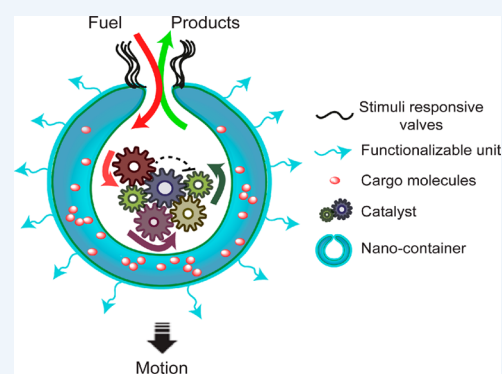
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CONSPECTUS: Autonomous micro- and nanoscale systems have revolutionized the way scientists look into the future, opening up new frontiers to approach and solve problems via a more bioinspired route. However, to achieve systems with higher complexity, superior output control, and multifunctionality, an in-depth study of the different factors that affect micro- and nanomotor behavior is crucial. From a fundamental perspective, the mechanical response of micro- and nanomotors still requires further study in order to have a better understanding of how exactly these systems operate and the different mechanisms of motion that can be combined into one system to achieve an optimal response. From a design engineering point of view, compatibility, degradability, specificity, sensitivity, responsiveness, and efficiency of the active systems fabricated to this point have to be addressed, with respect to the potential of these devices for biomedical applications.

Nonetheless, optimizing the system with regards to all these areas is a challenging task with the micro- and nanomotors studied to date, as most of them consist of materials or designs that are unfavorable for further chemical or physical manipulation.

As this new field of self-powered systems moves forward, the need for motor prototypes with different sizes, shapes, chemical functionalities, and architectures becomes increasingly important and will define not only the way active systems are powered, but also the methods for motor fabrication. Bottom-up supramolecular approaches have recently emerged as great candidates for the development of active structures that allow for chemical or physical functionalization, shape transformation, and compartmentalization, in a structure that provides a soft interface to improve molecular recognition and cell uptake.

Our group pioneers the use of supramolecular structures as catalytically propelled systems via the fabrication of stomatocyte or tubular-shaped motors capable of displaying active motion in a substrate concentration-dependent fashion. This behavior demonstrates the potential of bottom-up assemblies for powering motion at the micro- or nanoscale, with a system that can be readily tuned and controlled at the molecular level. In this Account, we highlight the steps we have taken in order to understand and optimize the design of catalytically powered polymersome-based motors. Our research has been focused on addressing the importance of motor architecture, motion activation, direction control, and biological integration. While our work supports the feasibility of supramolecular structures for the design of active systems, we strongly believe that we are still in the initial stages of unveiling the full potential of supramolecular chemistry in the micro- and nanomotor field. We look forward to using this approach for the development of multifunctional and stimuli-responsive systems in the near future.



INTRODUCTION

Since fabrication of the first catalytically self-powered micrometer scale motor (i.e., micromotor) in 2004,¹ there has been an exponentially increasing interest in the design of “man-made” micro- and nanoscale machines (i.e., micromotor, nanomotor) that could perform specific tasks on demand in a controlled and dynamic fashion. Micro- and nanomotors of different shapes and sizes, with chemically or physically responsive segments with an inherent asymmetric nature, have shown motion, propulsion, collective behavior, and even chemotaxis.² The unique behavior of such systems has enabled these motors to perform a myriad of functions ranging from cargo transport and release to dynamic assembly, transforming the way researchers approach current scientific challenges (e.g.,

drug delivery, quality control, chemical and biological defense).³ However, advancement in this field raises more challenges and limitations, both fundamentally and in terms of motor design, particularly when considering the eventual integration of these artificial systems into *in vivo* scenarios.

Certainly, achieving mobility at such small scales by overcoming the physical forces that dictate particle behavior at the micro- and nanoscale (e.g., viscous forces, Brownian motion), is a significant achievement in the field of material science.⁴ However, the mechanisms involved in the propulsion of micro- and nanomotors are still a topic of debate. The most

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widely studied mechanisms are bubble propulsion, self-diffusiophoresis, self-electrophoresis, and interfacial tension, all of which have been discussed elsewhere.^{2,3,5} The fact that propulsion at the micro- and nanoscale is achievable has led to design of systems that can be controlled externally (magnetic, electric, and acoustic fields) or internally (analyte concentration) to perform explicit tasks, such as material transport and cell penetration.^{6,7} Nevertheless, there are many aspects still to be addressed and explored in order to reach the level of complexity, specificity, space-time output control, organization, and responsiveness of natural systems.

One way to improve motor manipulation and facilitate design optimization involves achieving a better understanding of the mechanical response behind the micro- and nanomotors fabricated to date. Current efforts have addressed the effect of geometry of both the particles and the catalytic segment along with localization of the latter, in dictating the nature of the propelling motion.^{8,9} This design-driven approach has facilitated the in-depth study of different factors that affect motor behavior (e.g., geometry, nature of the catalytic reaction, motion media),^{6,10} while providing new systems that can be further modified and combined to serve different purposes. The techniques employed for the micro- and nanofabrication of such active systems usually involve top-down approaches (e.g., sputtering, lithography), delivering more rigid and inorganic-based systems.⁸ Combinations between top-down techniques and methods based on the assembly of materials (i.e., bottom-up techniques) have also been employed to synthesize micro- and nanoparticles with distinct segments for further functionalization and cargo storage capabilities.^{11–13,8} In this case, incorporation or encapsulation of particles, cells, or molecules in a larger structure or layer-by-layer assembly of polymers over a metal-sputtered surface has led to active systems with higher versatility and responsiveness. The complexity of systems obtained via those techniques has progressively increased, leading to systems that can be multiresponsive and multifunctional.^{7,12} However, there is still a need for fabrication of active systems with high control over architecture (e.g., size, shape, feature size) of the final structure, where significant levels of complexity could be achieved within the same device, without need for time-consuming and expensive processes. Attaining such complexity could lead to micro- and nanomotors with more intricate motion response and with the ability to perform a multitude of functions (e.g., recognition, treatment, delivery, regulation).

Nature can provide inspiration for both fabrication and propulsion of micro- and nanomotors with life-like properties (e.g., flexible, complex, dynamic), thus resembling more closely their biological counterparts. In biological systems, the size, shape, and function are determined from a highly ordered self-assembly of molecules.¹⁴ This bottom-up assembly of small building blocks into complex functional structures has motivated the fabrication of active systems via a pure bottom-up approach. Synthesis of micro- and nanosystems using this methodology is solely based on dynamic assembly of molecules or nanosized building blocks, providing better control over the physical and structural parameters and chemical functionalities in the final system.¹³ The dynamic nature of the final architecture results in structures able to respond to changes in the environment (e.g., pH, temperature, salt concentration). Bottom-up designs include liposomes, colloidosomes, aqueous two-phased systems, and polymerosomes,^{11,15} most of which combine hydrophobic and hydro-

philic sections that facilitate compartmentalization within one system. This increases the viability for incorporation of multiple reactions or functionalities in the same device. Furthermore, the presence of compartments decreases the chances for nonspecific/inhibitory reactions, enhances catalytic performance by physical confinement, and provides catalyst protection.¹⁶ In systems fabricated by a supramolecular approach (e.g., liposomes, polymersomes), the presence of a membrane serves as an interface between inner and bulk environments, controls the passage of molecules, and resembles closely the architecture of cells and organelles in biological systems. For this reason, micro- and nano-architectures made via these supramolecular bottom-up methodologies have usually been used as artificial cells or nanoreactors, that can communicate via chemical signaling to produce specific responses, such as changes in fluorescence, permeability, and cargo release.¹⁶ The motor capabilities of these nanoreactors have not been fully explored, as the majority of the designed structures are spherical, with no net structural asymmetry to achieve mobility. However, this perspective has changed since the demonstration that shape transformation is indeed attainable in supramolecular systems, particularly for those composed of block copolymers with distinct solubility parameters (i.e., polymersomes).

Our group pioneered the fabrication of active systems via a bottom-up approach using polystyrene-*block*-poly(ethylene glycol) (PS-*b*-PEG) block copolymer vesicles that undergo shape transformation to a stomatocyte-like structure, by kinetic manipulation of the hydrophobic portion via osmotic shock.¹⁷ This shape transformation of polymersomes, comprising structural asymmetry, can be used to dynamically encapsulate an inorganic catalyst in the inner compartment of the stomatocytes, thus achieving nanoscale motion in the presence of appropriate fuel. Ongoing research in this topic focuses on not only achieving a fundamental understanding of the shape transformation and manipulation of polymersomes for different applications but also exploring the full potential of stomatocyte-shaped nanomotors, from motion control to drug delivery. The aim of this Account is to provide our own insights in the fabrication of a fully multifunctional and regulated nanomotor system, using the unique structural properties of stomatocytes as inspiration. The work herein highlighted represents our efforts to understand, improve, and fully exploit the versatility of the stomatocyte motors, emphasizing that there are still many questions to be answered, with high scope for optimization. Nevertheless, the use of supramolecular systems to power motion represents a novel platform to design micro- and nanomachines, and we strongly believe in its potential to contribute notably to the future of self-propelled systems.

■ MOTOR ARCHITECTURE

In fluids, motion at the nanoscale is dominated by viscous forces, low Reynolds number, and strong thermal fluctuations. For the system to have sustained motion, it has to be in a nonequilibrium state and requires structural asymmetry or a nonreciprocal recovery mechanism.¹⁸ Hence, the geometry and architecture of the nanomotor plays a crucial role for sustained motion. Generally, active systems have been fabricated using frameworks that dictate the shape and size of nanomotors (Figure 1).^{11,19,20} The techniques employed for motor fabrication usually involve top-down approaches such as electrochemical deposition,¹ rolled up technology,²¹ and

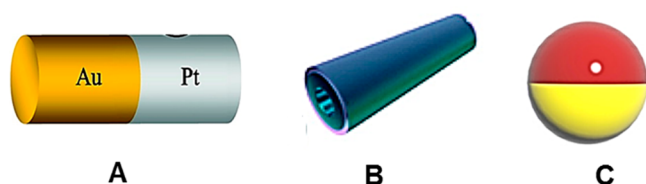


Figure 1. Motor shapes: (A) gold–platinum (Au/Pt) nanorods (Reproduced with permission from ref 1. Copyright 2004 American Chemical Society.); (B) zinc nanotubes (Reproduced with permission from ref 19. Copyright 2012 American Chemical Society.); (C) black titanium dioxide-gold Janus motors (Reproduced with permission from ref 20. Copyright 2017 American Chemical Society.).

physical vapor deposition.²² The major downside for these systems is the use of hard metal surfaces that render them unsuitable for biomedical applications, due to a lack of biocompatibility and biodegradability, and no feasibility for shape transformation.²³ Moreover, these metal-based systems are not capable of encapsulating cargoes and require complex operation to load them and make them suitable for delivery applications.²⁴

To overcome these drawbacks, researchers are exploring methods for micro- and nanofabrication that could offer more design diversity in terms of size, shape, functionality, and scalability, such as bottom-up techniques. Our group was the first to develop a bottom-up approach to form nanomotors from polymersomes, undergoing shape transformation into

bowl-shaped stomatocytes (Figure 2A).¹⁷ Polymersomes are block copolymer vesicles formed by dissolving amphiphilic diblock copolymers, in our case poly(ethylene glycol)-*b*-polystyrene (PEG–PS), in the presence of 1:1 ratio of organic solvent/water. PEG–PS polymersomes consist of a flexible glassy membrane that can fold inward under osmotic pressure due to the presence of plasticizing organic solvent in both inner and outer compartments. These structures provide a soft interface to cells for delivery applications, are easily functionalizable with molecular units,²⁵ and, upon stomatocyte-shape transformation, can facilitate encapsulation of catalytic nanoparticles or enzymes²⁶ to power motion. The geometry of stomatocytes includes a well-defined opening for expulsion of gas molecules and material exchange, and a stomach that provides compartmentalization of molecules, protecting cargoes from the surrounding environment, essential for physiological conditions.

Another advantage of our system is shape transformation, which can lead to fabrication of tailor-made architectures, highly desirable for various biomedical applications. Our group has a deep understanding of the controlled shape transformation of polymersomes into spheroids, discs, stomatocytes,²⁷ and tubes.²⁸ Polymersomes obtained from poly(ethylene glycol)-*b*-poly(D,L-lactide) (PEG-*b*-PDLLA) have been observed to undergo osmotically induced shape transformation into polymeric nanotubes upon dialysis under hypertonic conditions²⁹ (Figure 2B). These tubes comprised

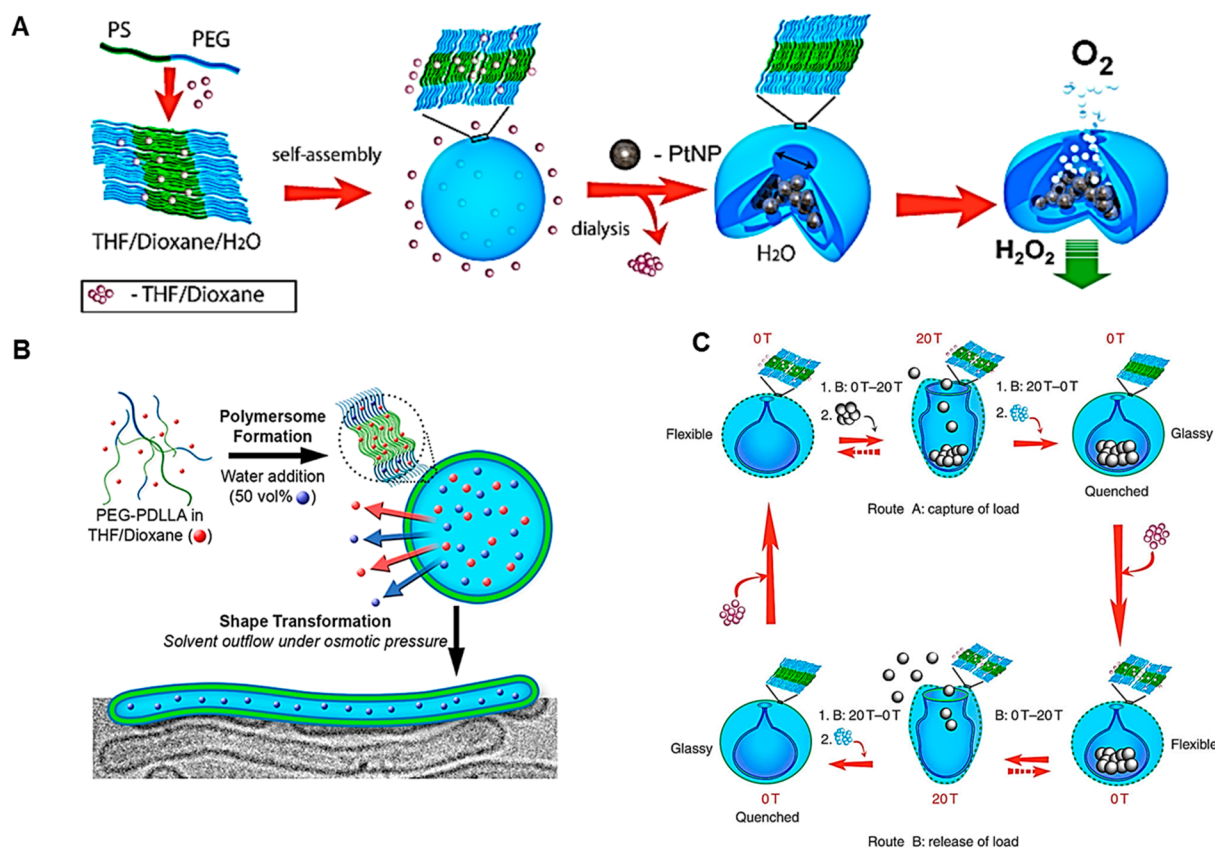


Figure 2. Shape transformation of polymersomes: (A) strategy for the selective and controlled entrapment of PtNPs inside the stomatocytes during shape transformation (Reproduced with permission from ref 17. Copyright 2012 Springer Nature.); (B) the osmotically induced shape transformation of spherical polymersomes into nanotubes (Reproduced with permission from ref 28. Copyright 2016 American Chemical Society.); (C) schematic representation of the reversible and controlled capture and release of particles via deformation of polymeric stomatocytes induced by the magnetic field B at 20 T (Reproduced with permission from ref 30. Copyright 2014 Springer Nature.).

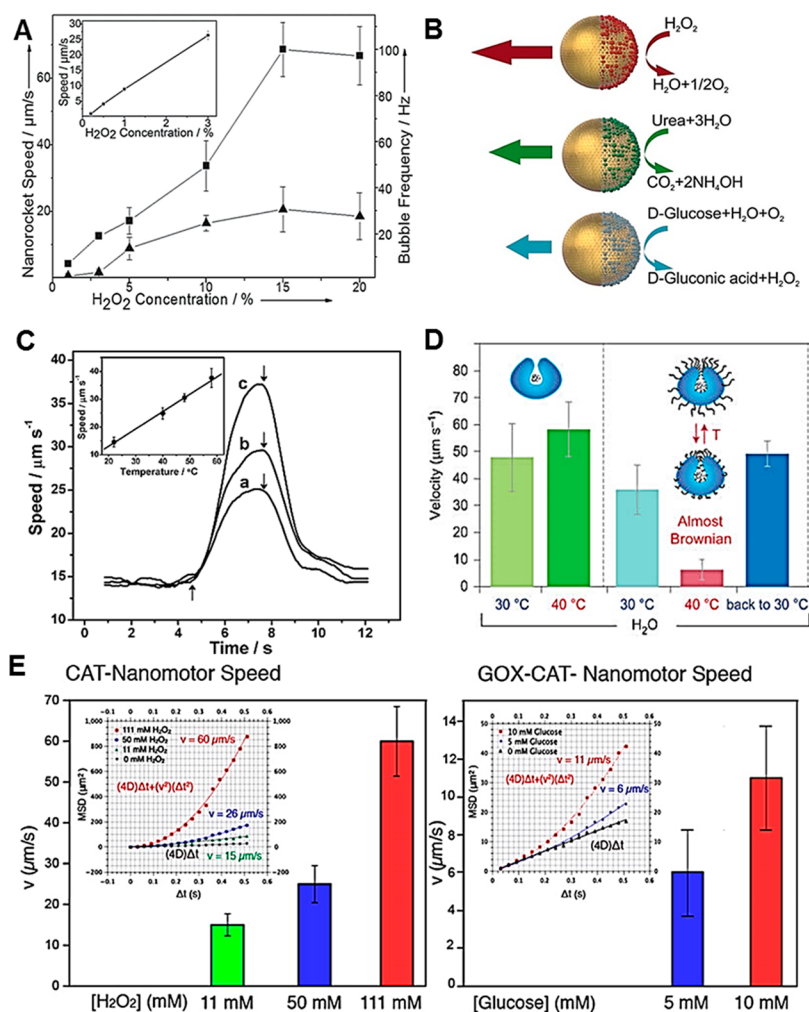


Figure 3. Control over motion: (A) Average speed of polymer based PtNP nanorockets (▲) at different bubble frequencies (■) and H₂O₂ concentrations in the range of 1–20% at 22 °C. Inset shows dependence of the nanorocket speed on low H₂O₂ concentration at 37 °C. Reproduced with permission from ref 31. Copyright 2013 John Wiley and Sons. (B) Enzyme-conjugation onto one face of the Janus nanoparticles via a glutaraldehyde linker molecule. Reproduced with permission from ref 32. Copyright 2015 American Chemical Society. (C) Speed–time profiles of Au–Pt nanomotors during different 3 s heat pulses to 40 (a), 48 (b), and 58 °C (c) in a 5 wt % H₂O₂ solution. The arrows correspond to the time of switching the heating current on and off. Reproduced with permission from ref 33. Copyright 2009 John Wiley and Sons. (D) Velocity of PtNP–stoma–brush/PtNP–stoma in the presence of H₂O₂ at different temperatures. Reproduced with permission from ref 25. Copyright 2016 Springer Nature. (E) Velocity of catalase-filled stomatocytes and GOX–catalase two-enzyme driven nanomotors at different fuel concentrations. Reproduced with permission from ref 26. Copyright 2016 American Chemical Society, respectively.

5% azide handles that were used for catalase immobilization, facilitating motor motion in the presence of hydrogen peroxide (H₂O₂). Replacement of PS with PDLA helps in increasing the biodegradability of the nanomotors when used for biomedical applications. The tube shape provides added advantage in terms of reduced nonspecific adhesion to cells, enhanced uptake, and higher ability to interact with the immune system.²⁸ Apart from this, the amphiphilic block copolymer used in our system has high anisotropic magnetic susceptibility, due to which they can undergo reversible shape transformation under magnetic field resulting in effective capture and release of cargo molecules (Figure 2C).³⁰ These numerous features highlight the potential of our system for the fabrication of versatile and multifunctional nanomotors. Nevertheless, to power and control motion of these structures, factors, like the nature of the catalyst, have been explored and are discussed in the following section.

POWERING MOTION

The major factors that determine the motion and velocity of nanomotors are concentration of fuel, activity of the catalyst and temperature, which have been demonstrated in literature for both top-down and bottom-up systems.¹¹ For instance, polymer-based multilayer nanorockets have shown increased acceleration from 5 to 70 μm/s upon increasing H₂O₂ concentration from 1% to 15%, respectively (Figure 3A).³¹ In another study, Janus nanomotors powered by different enzymes, namely, catalase, urease, and glucose oxidase, showed different mean square displacement values depending on enzymatic activity (Figure 3B).³² Additionally, an increase in speeds from 14 to 45 μm/s has been observed for gold coated Pt nanowires when temperature was increased from 25 to 65 °C, respectively (Figure 3C).³³ In correlation with other nanosystems, the motion of the stomatocytes is also dependent on the above factors.

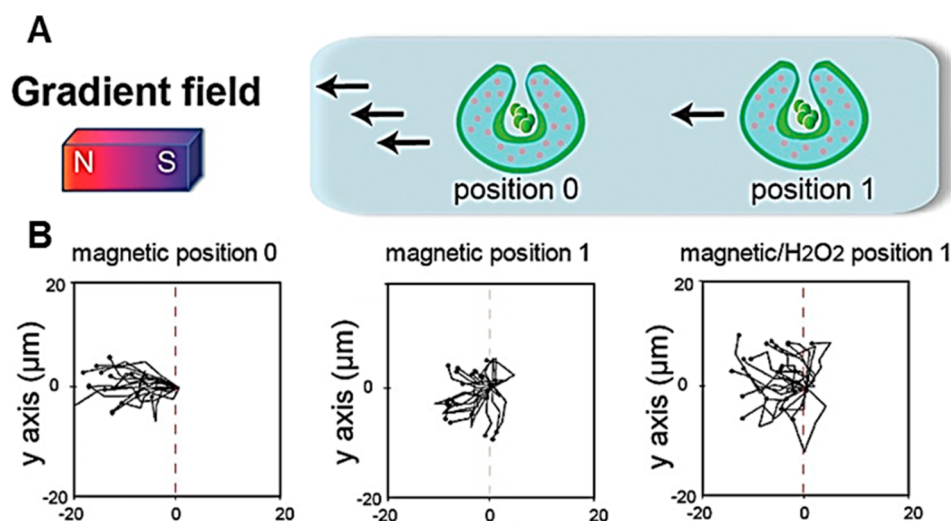


Figure 4. Magnetotactic behavior of stomatocytes: (A) Schematic illustration of the doxorubicin/platinum–nickel-loaded stomatocyte movement to the left toward higher-gradient magnetic field. (B) Moving paths of the doxorubicin/platinum–nickel-loaded stomatocytes over five consecutive frames at position 0 magnet induced, position 1 magnet induced, and position 1 magnet/H₂O₂ induced motion. Reproduced with permission from ref 37. Copyright 2016 John Wiley and Sons.

Enzyme-powered stomatocytes have shown that the motor speed is directly dependent on the concentration of fuel, in our case H₂O₂ and glucose. Catalase-powered stomatocytes attained speeds of 15, 26, and 60 $\mu\text{m/s}$ for 11, 50, and 111 mM concentration of H₂O₂, respectively. We hypothesize that at low H₂O₂ concentrations, propulsion by self-diffusiophoresis is dominant while the effects of bubble-propulsion are weak, but at higher fuel concentrations, bubble propulsion mechanism dominates leading to higher motor speeds. When compared to Pt-loaded stomatocytes, the enzyme-powered prototypes showed three times higher velocities, due to the higher efficiency of catalase over Pt in reducing H₂O₂. In case of the two enzyme system, wherein both glucose oxidase and catalase were encapsulated, the motor speeds reached 6 and 11 $\mu\text{m/s}$ for 5 and 10 mM glucose concentration (Figure 3E).²⁶

With respect to temperature responsiveness, we developed the first nanomotor with controlled speed, employing thermoresponsive polymer brushes on the surface of stomatocytes, without changing the activity or shape of the motor. The poly(*N*-isopropylacrylamide) brushes, due to a lower critical solution temperature (LCST), collapse on the surface of the opening with increasing temperature, preventing access to the fuel for propulsion, thereby decreasing motor speed (Figure 3D).²⁵ The temperature responsive units also provided better control over the “on–off” motion of the motors when subjected to continuous increasing and decreasing temperature cycles.

Additionally, the relationship between motor geometry and orientation for propulsion of the active systems fabricated in our group has also been explored via fabrication of microscale model systems.³⁴ Spherical asymmetric hydrogel microparticles consisting of poly(ethylene glycol) diacrylate (PEGDA) and dextran, containing an opening with a high degree of surface roughness, were synthesized using a microfluidic chip. Incorporation of catalase in the PEGDA phase resulted in bubble propulsion in the presence of H₂O₂, with circular or linear trajectories depending on how bubbles were pinned. The orientation of the motor opening was observed to be opposite to the direction of propulsion. This behavior could be extrapolated to the bubble-propelled nanomotors studied in

our group, for which motor orientation cannot be determined via current motion-analysis techniques.

■ CONTROL OVER DIRECTIONALITY

In order to have a highly efficient motor, it is necessary to have a control over both motion and directionality. Generally, direction of motor propulsion can be rectified by using electric fields, light, magnetic fields, or chemical gradients to achieve motor-taxis. Their working principles have been discussed previously.³⁵ With the stomatocyte motors developed in our group, we have explored mostly their magnetotactic and chemotactic capabilities for directed motion.

In comparison to other means of controlling directionality, magnetic fields have higher biocompatibility and flexibility and allow safe maneuvering in biological systems.³⁶ For this purpose, we fabricated stomatocytes containing Pt–nickel nanoparticles, which allowed for both motor activation via chemical inputs and direction control via magnetic manipulation. The nanomotors could readily sense the change in magnet position and reorient their motion toward the magnet (Figure 4).³⁷ These motors were also observed to have magnetotactic behavior in a collagen gel tissue-like model guiding the motion for longer distances. The magnets not only provided directionality but also assisted motor motion, as increased velocity was observed with decreased distance between magnet and stomatocytes.

Certainly, in biological systems it would be more beneficial to move the nanomotors autonomously toward a signaling compound, rather than using external magnetic or electrical fields. This could be achieved by the use of chemical gradients to rectify motor motion via chemotaxis. Even though there has been reports showing chemotactic behavior of Janus motors³⁸ and tubular microjets,³⁹ their size and limited cargo loading capacity constitutes limitations in their applications. To overcome this, we fabricated Pt loaded stomatocytes that showed chemotactic behavior in static and dynamic systems together with *in vitro* conditions.⁴⁰ The stomatocytes exhibited substrate concentration dependent directional movement when studied using glass channels and microfluidic flow, along with directional movement when tested with activated

neutrophils that produce H_2O_2 gradient *in situ*. The nanomotor speed gradually increased along their migration path toward the activated neutrophils (Figure 5).

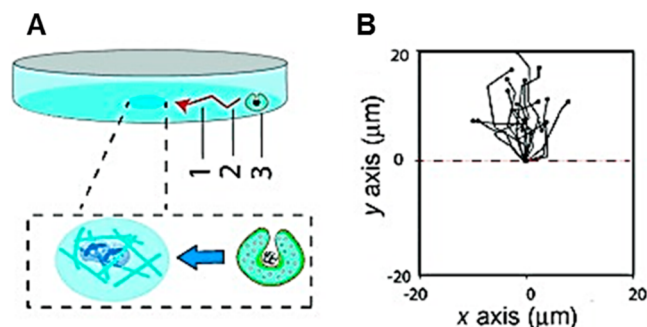


Figure 5. Chemotactic behavior of stomatocytes: (A) Chemotaxis evaluation with activated neutrophils as H_2O_2 source and Dox-PtNP loaded stomatocytes in the solution. (B) Tracking paths over 5 consecutive frames of the Dox-PtNP loaded nanomotors moving directionally toward the neutrophils. Reproduced with permission from ref 40. Copyright 2015 John Wiley and Sons.

BIOLOGICAL INTEGRATION

Up to this point, it is clear that we can achieve motion with stomatocyte motors, control and direct them to specific targets, and even change the final shape of our system to suit different functions. However, our main goal is to be able to demonstrate the potential of these supramolecular systems for applications in the biomedical field. Many examples in the literature have shown the remarkable capabilities of micro- and nanoparticle systems that combine motion with cargo loading and targeted delivery. Microtubes, rockets, and Janus capsules or spheres have been chemically or magnetically manipulated to reach a cell target and used to deliver cargo by scaffold degradation or near-infrared stimulation.^{7,35} Others systems have stepped further in achieving membrane attachment and penetration without disrupting the integrity of the cell, using magnetic and acoustic fields for motion control.^{12,41} Sustained mobility in biological fluids has also been demonstrated for bubble propelled and magnetically guided micro- and nanomotors, as well as for biohybrid systems.^{7,12} Similarly, a great part of our work has been dedicated to explore the feasibility of catalytically powered stomatocytes for biomedical applications, particularly drug delivery.

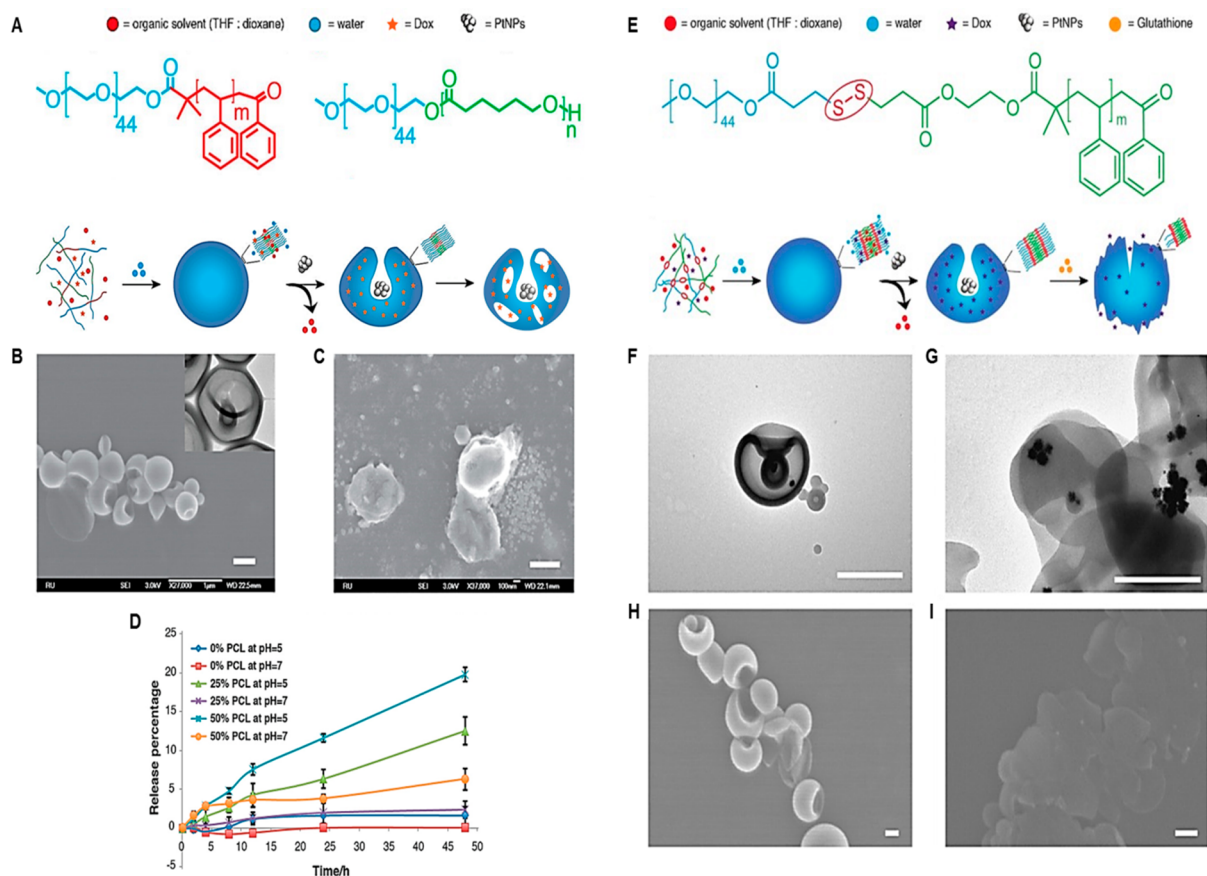


Figure 6. Stimuli-responsive drug release: (A) Drug release by partial structure degradation and schematic for self-assembly of Dox-loaded hybrid stomatocyte nanomotor. (B) SEM image of stomatocytes with 50% PEG-*b*-PCL before acidic degradation (inset, TEM image of a single stomatocyte). (C) SEM image of stomatocytes with 50% PEG-*b*-PCL after acidic degradation. Scale bars: 400 nm. (D) Release of Dox from a stomatocyte with different percentages of PCL at different pH. (E) Drug release by complete structure degradation and schematic of stomatocyte formation using a redox-responsive block copolymer PEG-SS-PS, followed by glutathione-triggered disassembly of the redox-sensitive nanomotor. (F, G) TEM images of a stomatocyte nanomotor (F) before and (G) after exposure to glutathione. (H, I) SEM images of a stomatocyte nanomotor (H) before and (I) after treatment with glutathione. Scale bars: 200 nm. Panels A–D reproduced with permission from ref 42. Copyright 2017 American Chemical Society. Panels E–I reproduced with permission from ref 43. Copyright 2017 John Wiley and Sons, respectively.

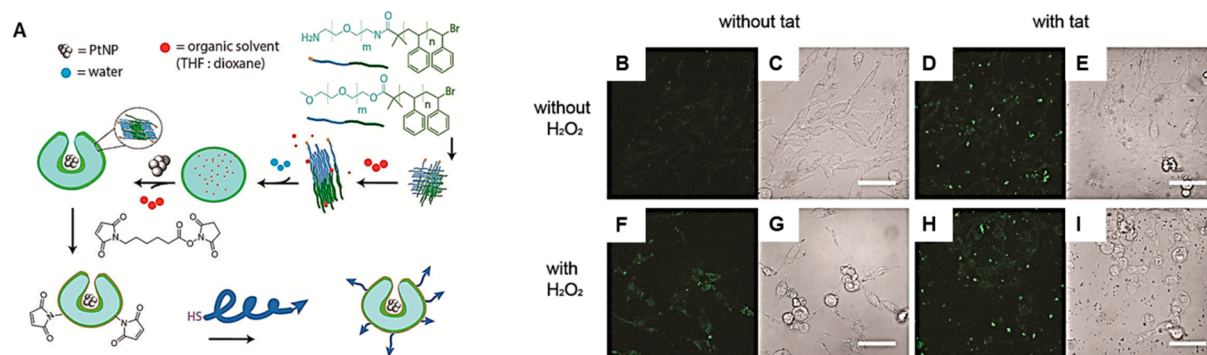


Figure 7. Stomatocyte uptake by cells: (A) Schematic for fabrication of peptide-functionalized stomatocyte nanomotors. (B–I) Characterization of dye-tagged stomatocyte uptake under different conditions: (B, F) confocal fluorescence image and (C, G) confocal bright field image of human cervical cancer (HeLa) cells incubated with Pt-stomatocytes for 6 h (B, C) without and (F, G) with 0.015% H₂O₂, (D, H) confocal fluorescence image and (E, I) confocal bright field image of HeLa cells incubated with peptide-tagged Pt-stomatocytes for 6 h (D, E) without and (H, I) with 0.015% H₂O₂. Scale bar: 60 nm. Reproduced with permission from ref 44. Copyright 2017 Royal Society of Chemistry.

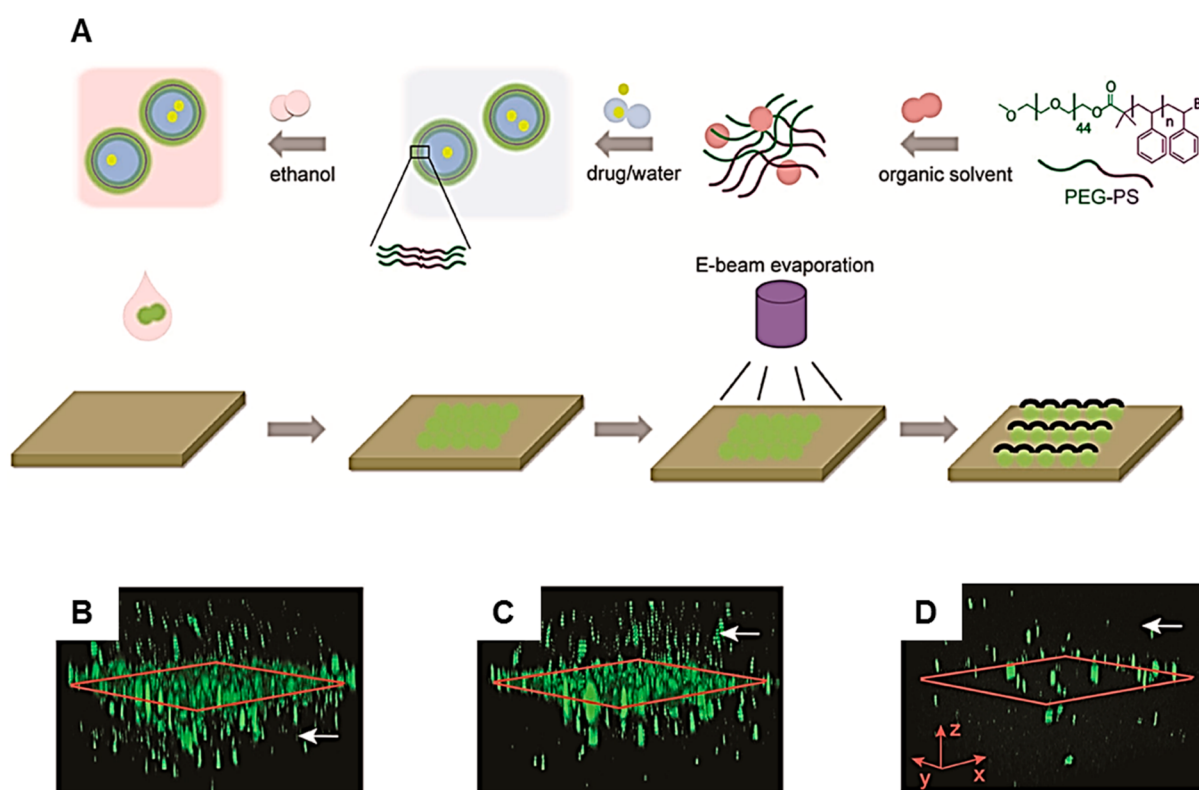


Figure 8. Enhanced EPR by active transport: (A) Schematic for fabrication of PEG-*b*-PS polymersome-based Janus nanomotors. (B–D) Fluorescence Janus-motor distribution for different populations: (B) Janus-motor/H₂O₂ group; (C) Janus-motor/H₂O control group; (D) nonmotor/H₂O₂ control group. The white arrow points to the particle enriched top chamber. Reproduced with permission from ref 45. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA.

We started with studying the drug loading capability of stomatocyte motors. This was demonstrated with both chemically and magnetically guided Pt- or Pt/nickel-loaded stomatocyte motors assembled from PEG-PS block copolymer (Figures 4 and 5), with doxorubicin loaded in the lumen of the structures.^{37,40} Drug release was also achieved via partial or complete degradation of the stomatocyte structure. In the former, degradability of polymer domains in a drug-loaded Pt-stomatocyte was achieved by the incorporation of block copolymer chains containing poly(ϵ -caprolactone) (PCL), a biodegradable polymer, and PEG, in the PEG-PS stomatocyte framework (Figure 6A).⁴² During stomatocyte formation, the

PCL-PEG chains dynamically arranged in domains, causing pore formation and drug release in acidic media (Figure 6B–D). Stimuli-responsive release of cargo by complete scaffold degradation was attained with drug-loaded stomatocytes fabricated with disulfide bonds integrated within the block copolymer chain (PEG-S-S-PS) (Figure 6E).⁴³ Exposure to physiological redox conditions led to the cleavage of the disulfide bonds, leading to stomatocyte degradation and drug release (Figure 6F–I).

Further studies to explore the potential of stomatocyte motors for cargo delivery *in vivo* involved improving cell-particle interactions to facilitate uptake and exploring the effect

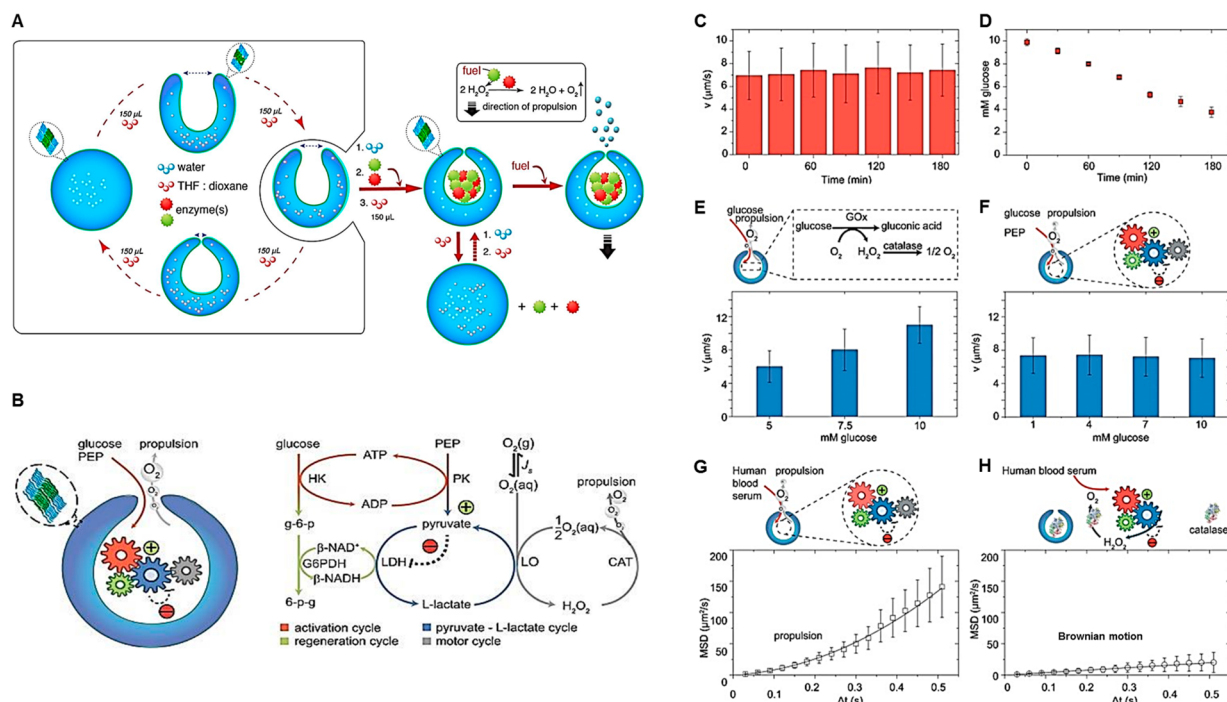


Figure 9. Enzyme-powered stomatocytes: (A) Supramolecular assembly of catalase- or catalase/glucose-oxidase driven nanomotors. Motion analysis of these motors can be found in Figure 3. Reproduced with permission from ref 26. Copyright 2016 American Chemical Society. (B) Schematic representation for assembly of the nanomotor with an enzyme-network entrapped inside the structure. (C–H) Multienzyme nanomotor analysis: (C) average speeds of enzyme motors in the presence of 10 mM glucose; (D) experimental determination of glucose concentrations, showing that glucose depletion does not influence nanomotor speed (panel C); average initial speeds of (E) catalase/glucose-oxidase motors and (F) nanomotors containing an enzymatic network fueled with different glucose concentrations; (G) multienzyme nanomotor movement in human serum with the full network compartmentalized; (H) motion of enzyme-motors loaded with catalase only; the rest of the network is added to the serum. Reproduced with permission from ref 46. Copyright 2016 American Chemical Society.

of active transport in the enhanced permeation and retention (EPR) effect for drug delivery. To achieve this, trans-activator of transcription (tat) peptides were attached onto the surface of stomatocyte motors (Figure 7A), and their chemotactic and cell-penetrating capabilities were tested by incubation with human cervical cancer cells in the presence and absence of H_2O_2 (Figure 7B–I).⁴⁴ Stomatocyte uptake by cells, without disruption of the cell membrane and nucleus, was achieved in both conditions, with higher motor-uptake in samples exposed to H_2O_2 . For active versus passive drug transport, Pt-capped cargo-loaded Janus polymersomes (Figure 8A) were studied using a tumor vasculature model.⁴⁵ Enhanced EPR effect was observed for cargo-loaded polymersome motors, compared to passive cargo delivery, demonstrating the advantages of active diffusion for delivery applications (Figure 8B–D).

The chemotactic, cargo-loading and -release, and cell uptake properties of Pt-stomatocyte motors demonstrated the feasibility of these systems for applications *in vitro*, but for *in vivo* scenarios the use of biocompatible catalysts and fuels is preferable. For this, enzyme-powered systems were fabricated by incorporation of catalase- or catalase/glucose-oxidase combination into the stomach of stomatocytes to achieve motion in the presence of H_2O_2 or glucose, respectively (Figure 9A).²⁶ In both cases, motor propulsion was observed in the presence of fuel following a substrate concentration dependent trend (Figure 3E). For catalase/glucose-oxidase stomatocyte motors, motor propulsion was achieved using significantly lower glucose concentrations (5 mM) than previously reported systems employing the same enzyme combination.²⁶ Incorporation of these motors into solutions

containing proteolytic enzymes did not translate into motor inactivation, demonstrating the protective capabilities of catalyst compartmentalization in stomatocytes.

Sustained autonomous motion of enzyme-powered stomatocytes has also been achieved by enclosing a more complex enzyme reaction network in the catalyst compartment. For this purpose, a non-natural regulatory metabolic network composed of an activation cycle, a regeneration cycle and a motor cycle was employed in order to control motor response via the regulation of fuel consumption (Figure 9B).⁴⁶ Motor propulsion of these enzyme-powered stomatocytes was independent of the substrate concentration, giving a constant motion output even after hours from initial input (Figure 9C–F). This behavior was also observed with motors propelling through human blood serum, reinforcing the fact that stomatocyte motors can be indeed used in crowded environments, without catalyst inactivation (Figure 9G,H).⁴⁶ The biocompatibility of the scaffold used for stomatocyte formation has also been recently addressed in our systems through the analysis of shape transformation of biodegradable polymersomes fabricated with the block copolymer PEG–PDLLA (poly(D,L -lactide)), briefly discussed in “Motor Architecture” (Figure 2B). This fully biodegradable system will allow us to fabricate and test other motor shapes, using enzymatic reactions to power motion.²⁸

■ FUTURE PERSPECTIVES

Our work to date has indeed demonstrated the potential of supramolecular structures for fabrication of next-generation active systems. However, the research is still in its infancy and

requires thorough and in-depth understanding of nanomotor design engineering, propulsion mechanisms, and motion regulation in *in vivo* conditions. We look forward to designing multifunctional micro- and nanomotors that harness different energy sources in the same system, rendering them ideal for recognition, treatment, soft robotics, chemical sensors, formulation of self-healing materials, and therapeutics. For these reasons, we are currently focusing on the study of (1) alternative energy sources for powering motion, (2) size limitations in supramolecular assemblies for cargo-delivery applications, (3) effect of shape in motor efficiency, (4) effect of confinement (e.g., macromolecular crowding effect) in enzyme-powered systems, (5) the potential of supramolecular structures as nanoreactors, and (6) the use of different fuels and catalysts to achieve propulsion via different mechanisms. We recognize that the field is evolving at a fast pace, with innovative systems constantly being developed. Nonetheless, minimal emphasis has been given to the fundamental principles dictating motor behavior, which would pave the way for higher motor regulation, control, and performance. The systematic study of different factors and features that play a role in motor functionality will be crucial in order to better understand the behavior of more complex systems and be able to explore other nature-inspired characteristics, such as adaptability, communication, responsiveness, and selectivity. Future research should emphasize the fact that in nature “structure determines function”; likewise, in active systems the architectural features of the motor will dictate its behavior, especially in crowded environments. We should also learn to extrapolate the knowledge obtained from nonbiodegradable or nonbiocompatible systems to fabricate biofriendly analogues that will serve as a platform for the design of stimuli-responsive dynamic materials. Last but not least, understanding individual motor response will expand the scope of developing systems with better environmental adaptability, transport, and delivery capabilities via concerted processes and selective activation through multiple stimuli. We believe that bottom-up supramolecular assemblies will be of significant importance to achieve these goals with autonomous systems, providing a multitude of opportunities for mimicking biological systems and potentially helping us to attain levels of complexity closer to those observed in nature.

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Notes

The authors declare no competing financial interest.

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Daniela A. Wilson received her Ph.D. degree (2007) “summa cum laude” from “Gh. Asachi” Technical University of Iasi, Romania. She is currently full professor at the Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands. Her research interests focus on the design of intelligent, self-propelled, and self-guided supramolecular assemblies and their communication and interaction as next generation nanoengineered delivery systems.

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