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PERSPECTIVE

Artificial Molecular Machines in Nanotheranostics

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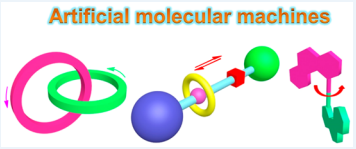
ABSTRACT: **Due to their dynamic nature and excellent stimuli-responsiveness resulting from noncovalent driving forces, artificial molecular machines (AMMs) show great promise in cancer theranostics. In this Perspective, we introduce the potential applications of AMMs in controlled drug delivery, bioorthogonal catalysis, imaging, and cell membrane permeabilization, with the goal of enhancing cancer diagnosis and therapy. We expect this preliminary discussion will garner multidisciplinary interest from scientists to advance AMMs and to expand their future clinical applications.**



The 2016 Nobel Prize in Chemistry was awarded jointly to Profs. Bernard L. Feringa, Jean-Pierre Sauvage, and Sir J. Fraser Stoddart for their pioneering work in the design and development of molecular machines. A molecular machine refers to the assembly of a discrete number of molecular building blocks that produce mechanical motions (output) in response to the appropriate external stimulus (input).[1−4] Molecular machines can be categorized into three broad classes: biological systems, artificial systems, and hybrids of the two. In biological systems, numerous molecular and/or supramolecular machines exist, including antibodies, enzymes, and viruses, which regulate almost every biological process.[5,6] For example, a range of molecular machines in the plasma membranes control the highly selective transport of various (macro)molecules across the phospholipid bilayer, driven by the conformational changes of the motor proteins to build up and to maintain nonequilibrium distributions.[7,8] Moreover, biological molecular machines actively engage in the separation of DNA strands (*e.g.*, helicases) and synthesis of proteins (*e.g.*, ribosome) and facilitate the propulsive movement of organisms (*e.g.*, bacterial flagellar motor).[9] Although biological molecular machines with high complexity are almost unattainable through synthetic preparations, they continuously provide inspiration to scientists to develop artificial systems to mimic their structures and functions.[10,11]

Encouraged by Richard Feynman's famous lecture in 1959, "*There's Plenty of Room at the Bottom*"[12] and subsequent advances, chemists have pursued sophisticated artificial molecular machines (AMMs) through synthesis, aiming to recapitulate the intricacies of natural biomachines.[13,14] Development of smart AMMs with the ability to accomplish the same tasks has been one of the most exciting research areas in supramolecular chemistry over the past several decades.[15,16]

In 1983, Sauvage succeeded in the synthesis of a catenane consisting of two interlocked ring-shaped molecules that are able to move relative to one another.[17] In 1991, Stoddart threaded a molecular wheel onto a dumbbell-type axle to prepare a rotaxane, in which molecular shuttle motion along the axle was realized.[18] After constructing a molecular motor that continuously spun in the same direction in 1999, Feringa utilized a molecular motor to rotate a glass cylinder 10,000 times larger than itself.[19] Fueled by powerful synthetic methodologies, analytical instrumentation, and, most importantly, seemingly unlimited imagination of the scientists, remarkable progress has been made recently showing promising applications in controlled drug/gene delivery, biosensing, and signal transduction.[20,21] Due to their unique topological features, dynamic nature, and abundant stimuli-responsiveness, AMMs possessing compatible size with nucleic acids or proteins in living cells exhibit unique advantages in cancer theranostics, an emerging nanoenabled amalgamation of therapy and diagnosis. In this Perspective, we briefly summarize the recent advances of AMMs focused on interlocked molecular systems in stimuli-responsive drug delivery, bioorthogonal catalysis, imaging, and cell membrane permeabilization, although other molecular machines also have significant potential in theranostics.[22,23]

Mechanically Interlocked Molecules. Generally, AMMs can be prepared from various building blocks, such as inorganic nanoparticles, organic molecules, proteins, and DNA. However, many of the AMMs reported so far depend on mechanically interlocked molecules (MIMs), including rotaxanes and catenanes,[24,25] for the following reasons. First, numerous noncovalent interactions can be utilized for template-directed construction of MIMs with high yield, such as hydrogen bonds, charge-transfer interactions, hydrophobic interactions, electrostatic interactions, $\pi-\pi$ stacking, and metal−ligand coordination.[26,27] Second, molecular mo-

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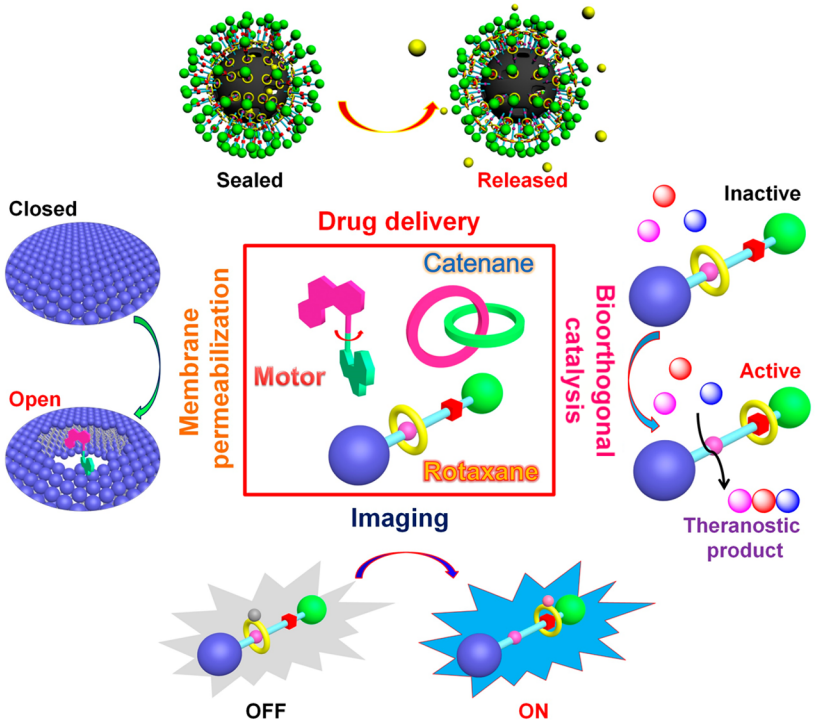

Figure 1. Schematic representations of rotaxane, catenane, molecular motor, and their potential applications in stimuli-responsive drug delivery, bioorthogonal catalysis, imaging, and cell membrane permeabilization.

tions can be triggered in response to certain inputs because noncovalent interactions are modulated by external stimuli due to their dynamic and reversible nature.[28] Rotaxanes are MIMs consisting of at least a dumbbell-like axle threaded by macrocycles (the wheels), terminated by bulky stoppers. Catenanes are MIMs consisting of two or more interlocked macrocycles that cannot be separated unless the covalent bonds of the macrocycles are broken.

Rotaxane-Based Drug-Delivery Systems. It remains a critical challenge for scientists to design and to synthesize a sophisticated molecule possessing multimodal theranostic capability for cancer diagnosis and therapy. Apart from the arbitrary mixing of different molecular building blocks to form nanoparticles (or other assemblies), which can possibly result in confounded theranostic outcomes, it is almost impossible to synthesize a conventional biomaterial with an unambiguous structure that will fulfill theranostic requirements perfectly. However, targeting ligands, therapeutic drugs, and diagnostic agents are capable of facile incorporation into MIMs by modifying the corresponding building blocks separately for theranostic applications, thus avoiding time-consuming and costly covalent syntheses. The sizes of the macrocycles threaded on the axle of rotaxane are relatively small, such as crown ethers, cyclodextrins, cucurbiturils, pillararenes, and cyclophanes.[29] Therefore, targeting groups (*e.g.*, peptides), fluorescent chromophores (*e.g.*, tetraphenylethene, cyanine dyes), photosensitizers (*e.g.*, porphyrins), and anticancer drugs (*e.g.*, doxorubicin, paclitaxel) with large sizes are suitable stoppers. In addition, other functional groups can be introduced into the rotaxanes by modifying the macrocycles, enriching the properties of the resultant theranostic systems. For example, scientists have already designed a biocompatible [2]rotaxane by using the anticancer drug paclitaxel (PTX) as one of the stoppers.[30] Premature drug release in plasma was effectively inhibited due to the presence of the macrocycle, which protected the ester bond between PTX and the axle from being hydrolyzed. Triggered by the overexpression of β-galactosidase in cancer cells, a cascade of reactions took place inside the cells to release the anticancer drugs selectively through the decomposition of the rotaxane, which provided a new strategy to avoid the side effects of chemotherapy in healthy cells.

Targeting ligands, therapeutic drugs, and diagnostic agents are capable of facile incorporation into mechanically interlocked molecules by modifying the corresponding building blocks separately for theranostic applications, thus avoiding time-consuming and costly covalent syntheses.

The distance between the stoppers and wheel in the rotaxane falls within the optimal range for Förster resonance energy transfer (FRET), providing an opportunity to monitor drug release in real time.[31,32] A dual-fluorescence-quenched FRET system was constructed using tetraphenylethene (TPE) as the fluorescence donor and doxorubicin (DOX) as the fluorescence acceptor.[33] The energy transfer relay effect mediated by FRET and aggregation-induced quenching was disrupted in the endo/lysosomes due to the release of DOX, recovering the "silenced" fluorescence for *in situ* visualization of the drug-release process. Apart from chemotherapy, rotaxanes are a potential platform for photodynamic therapy using photosensitizers, such as porphyrins and other fluorescent dyes, as stoppers. By conjugating a quencher on the wheel and adjusting the photosensitizer/quencher distance,

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singlet oxygen generation can be controlled by the shuttle-like motion of the macrocycle along the axle in response to a specific stimulus, switching the photodynamic therapy on/off as necessary. Both exogenous and endogenous stimuli, such as light, temperature, pH, redox, and enzymes are able to drive molecular motion. Thus, specific activation of the diagnostic and therapeutic abilities in cancer cells can be realized by taking advantage of these stimuli.

Rotaxane-Based Catalysts. For conventional cancer therapy, anticancer drugs indiscriminately kill cancer cells and normal cells alike, resulting in severe side effects. Selective synthesis of anticancer agents *in situ* in cancer cells from nontoxic components is an ideal choice. If this strategy is realized in the future, the maximum tolerated dose of the reagents can be significantly enhanced to eradicate cancer cells from the body without significant side effects. Within the past decade, bioorthogonal chemistry has emerged as a powerful tool to study biological processes in living systems.[34,35] Bioorthogonal chemistry refers to reactions between abiotic reactants accomplished in living cells (or organisms) without interfering with, or interference from, the other surrounding molecules within a cellular environment.[36,37]

Biology makes use of a wide range of enzyme-based molecular machines to catalyze reactions with high efficiency and selectivity, inspiring the design of rotaxane-based switchable organocatalysts. Because of their unique interlocked topological structures, the catalytic activities can be switched "on" or "off" in response to an analyte or specific stimulus, such as pH, redox, light, ions, electricity, or temperature, which act to move the wheel along the axle to either conceal or reveal the catalytic site.[38] Various catalytic groups can be integrated into rotaxanes to catalyze different reactions, such as Michael addition and Ritter reactions, giving chemists the possibility of assembling common reagents in a controlled manner by switching the catalytic sites "on" and "off" at different steps.[39] More interestingly, artificial rotaxane-based organocatalysts make successive synthesis in one pot possible. The macrocyclic wheel, which contains the catalyst, moves along the axle, picks up the reagents on its path, and links them one by one through successive native chemical ligation reactions to form a final product with a specific sequence.[40] Therapeutic agents, including drugs, biologics, DNA, and RNA may be produced *in situ* from a pool of building blocks by using the states of multiple switchable catalysts, demonstrating potential for applications in chemotherapy and immunotherapy. By grafting targeting ligands on rotaxane, the bioorthogonal catalysis is selectively restricted to cancerous cells, greatly reducing cytotoxicity toward normal cells.

Mechanically Interlocked Molecule-Based Imaging Probes. In order to ascertain tumor location and progression and to gauge the response to therapy, theranostic AMMs with combined diagnostic/imaging capabilities are urgently needed. Various imaging methods are available, including fluorescence imaging, photoacoustic (PA) imaging, X-ray computed tomography (CT), ultrasound (US) imaging, magnetic resonance imaging (MRI), and positron emission tomography (PET)/single-photon emission computed tomography (SPECT).[41] The contrast agents can be integrated into the AMMs as the stopper, the axle, or even the macrocycle.

The microenvironment in and surrounding the tumor is different from normal tissues in terms of pH, enzymatic expression, and hypoxia. These unique environmental conditions trigger the tumor-specific molecular motion of AMMs. As mentioned above, the wheel/stopper distance in the rotaxanes and the relative positions of the locked rings in catenanes are adjustable in response to external stimuli. These conformation changes sometimes alter the optical and magnetic properties of the AMMs, facilitating *in vitro* and *in vivo* imaging. For example, the coordination number of the magnetic resonance contrast agents, such as gadolinium, manganese, and europium, is particularly important for their imaging outcome. The rotaxanes and catenanes developed by Sauvage *et al.* containing bidentate and terdentate ligands are potential chelators for the transition metals for MRI and PET imaging.[42] The relaxivities of the contrasts in AMMs may possibly be influenced by a redox process according to the tumor microenvironment, achieving tumor-specific imaging and diagnosis.

Rotaxane-Based Supramolecular Nanovalve. The past decade has witnessed vigorous development of nanomedicines for the systemic and controlled delivery of diagnostics and therapeutics for the detection, imaging, and treatment of malignancies.[43,44] However, the premature burst release of the encapsulated drugs during circulation and slow release of the drugs in tumor tissue have become the main obstacles to their clinical translation, resulting in increased incidence of side effects to healthy organs and reduced anticancer efficacy.[45] Installation of smart gatekeepers onto the surface of nanocarriers can mitigate this issue, wherein the gatekeepers keep the "doors" closed during systemic cargo transportation and open the "doors" in response to certain external stimuli following uptake by cancer cells for realization of on-demand release of diagnostic probes and therapeutic drugs.[46,47] Rotaxanes and pseudorotaxanes based on host−guest complexes are a natural fit for the requirements of such gatekeepers. They are widely utilized to cap the pore entrances of mesoporous silica nanoparticles (MSNs), playing crucial and prominent roles in realizing switchable drug release. The macrocycles attach to the surface of MSNs to block the exits in the absence of triggers and disengage from the surface to open the "doors" induced by predefined stimuli arising from shuttle-like molecular motions. As discussed above, the conformation changes of the AMMs can activate the catalytic activity of the rotaxanes, whereby the sealed reagents are processed into theranostic compounds when they pass through the "doors". Therefore, these nanoparticulate systems capitalize on bioorthogonal synthesis to release drugs in cancer cells in a controllable manner.

Molecular Motor Permeablizes the Cell Membrane. The development of multidrug resistance (MDR) against a variety of chemotherapeutic agents is one of the major contributors to the failure of chemotherapy-based cancer management. One of the major causes is the overexpression of drug efflux pumps (cellular membrane proteins) that transport anticancer drugs out of the cell.[48] Various strategies have been employed to overcome MDR, such as gene silencing, transcriptional regulation, translation repression, gas therapy, and drug encapsulation. Only a few nontoxic and specific inhibitors have been found, and none of these inhibitors can (yet) be used clinically. Therefore, the discovery of new and efficient strategies to overcome MDR is vital for effective cancer treatment.

Maintenance of the ion/protein homeostasis of cells is vital to sustain fundamental biological processes. Dysregulation of these homeostasis pathways disturbs a variety of life processes, including energy production, cell proliferation, differentiation,

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metabolism, and apoptosis. For example, multiple previous reports confirmed that various cancer cells display dysregulation of ion concentrations and fluxes, which are partially responsible for the apoptotic resistance and the enhancement in proliferation/migration.[49] Therefore, specific disruption of ion/protein homeostasis in cancer cells is an essential strategy to overcome MDR. However, the hydrophobic lipid bilayers acting as high thermodynamic barriers restrict the free movement of hydrophilic solutes across the cell membrane, thus presenting a formidable challenge for scientists.

Utilization of nanotechnology to disrupt the lipid bilayers of cellular membranes is an alternative approach to destabilizing ion/protein homeostasis, thus possibly overcoming MDR. Indeed, several physical techniques have been employed to open the lipid bilayers of cellular membranes, including electric/magnetic fields, ultrasound, light, and temperature, to induce uncontrolled cell death (necrosis) or programmed cell death (apoptosis). Excitingly, scientists recently used molecular motors to drill through cell membranes *via* nanomechanical action activated by UV irradiation.[50] Various tasks were completed by using this supramolecular method: (a) increased diffusion of molecular motors into and within live cells, (b) delivery of loaded nanocarriers into cells, (c) triggered release of cargoes from synthetic vesicles, and (d) induction of cell apoptosis/necrosis. Furthermore, nanomechanical rotation was conducted selectively at specific recognition sites in the membrane by conjugating peptides onto the molecular motor.

This strategy based on molecular motors provides a distinct approach toward potentially overcoming MDR. The formation of nanopores in cell membranes significantly increases the membrane permeability (including cell membranes and organelles membranes), which dysregulates the ion/protein homeostasis of the cells, thus inducing apoptosis. However, the formed nanopores also enhance the uptake of therapeutic agents by drug-resistant cancer cells through diffusion, thus greatly enhancing anticancer efficacy. In addition, molecular motors are ideal vehicles to carry theranostic agents, in order to improve anticancer therapies guided by imaging technology. In contrast to traditional anticancer drugs, which diffuse nonspecifically during biodistribution, molecular motors are nontoxic and their motions can be selectively switched "on" in cancer cells, avoiding cytotoxicity to healthy cells and tissues.

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CONCLUSIONS AND OUTLOOK

In this Perspective, we summarized the application of AMMs, primarily in the context of MIMs in cancer theranostics. Compared with conventional biomaterials, these AMMs exhibit fantastic properties resulting from their sophisticated topological features and dynamic noncovalent interactions. Diagonostic and therapeutic functional groups can be precisely integrated into one single-molecular platform as the building blocks of MIMs by rational design and synthesis. Although some of the molecular machines run unimpeded by the physiological environment to accomplish interesting tasks, most of them are rarely utilized *in vivo*. Researchers still face many challenges in optimizing the structures and functions of AMMs for better applications in nanotheranostics.

The circulation times of the AMMs need to be effectively prolonged. The sizes of AMMs are typically smaller than 5 nm, unsuitable for passive targeting through the enhanced permeability and retention (EPR) effect. As a result, they are quickly cleared from the body through renal filtration before arriving at their destination, greatly reducing their theranostic performance. Various approaches can be employed to prolong the circulation time of AMMs in the bloodstream. Conjugation of ligands with the ability to bind albumin has been demonstrated to be an effective choice to increase the half-life of the resultant products significantly. Lipids, peptides, and Evans blue derivatives are widely utilized to modify small molecular contrast agents and drugs to pursue better theranostic performances. Functionalization of the AMMs with poly(ethylene glycol) (PEGylation) is another feasible strategy because the formation of a hydration shell around PEG segments effectively hinders opsonization by circulating proteins. Moreover, amphiphilic AMMs can be prepared by using hydrophobic and hydrophilic functional groups as their corresponding building blocks, which self-assemble into nanostructured aggregates with suitable size to take advantage of the EPR effect.

The stimuli need to be optimized. On the one hand, the stimuli used to induce molecular motions currently rely on pH changes, light, and electrochemistry. Searching for new and effective stimuli will enrich the choices for chemists to develop powerful AMMs possessing interesting properties. On the other hand, two or more "locks" need to be installed onto one AMM in order to improve its selectivity to cancer cells, where the AND logic gate should be simultaneously opened by two "keys".[51] For most AMMs, the intracellular differences in pH or redox potential are too subtle to distinguish cancer cells from the large population of surrounding normal cells. In sharp contrast with other stimuli, light as a noninvasive trigger has attracted special interest because it is easily localized to a specific site, leading to *in situ* activation of the AMMs. However, almost all light-responsive AMMs employ UV light with poor tissue penetration, severely limiting their *in vivo* application. Fabrication of novel, NIR light-responsive AMMs will pave the way to solve this obstacle by using two-photon active groups or upconversion nanoparticles as the components of the AMMs.[52] Moreover, AMMs responsive to other stimuli with excellent penetration ability will become advantageous in this field.

Delivery and catalytic efficiencies need to be improved. Although imaginative nanovalves that are responsive to predefined stimuli have been developed, the sealed drugs are released from the containers primarily through passive diffusion. Consequently, the drug concentration in cells is relatively low, significantly reducing their therapeutic outcomes. "Molecular pumps" are needed for the gated materials to push the theranostic species from porous carriers after the "gates" are open.[53] For example, gold nanomaterials (*e.g.*, nanoparticles, nanorods, cubes, rings, disks) and magnetic nanoparticles (*e.g.*, Fe_3O_4) in the core of the hybrid materials can accelerate drug release rates by taking advantage of

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photothermal and magnetothermal effects. Additional photothermal therapy and magnetic hyperthermia are able to ablate primary tumors, and the chemotherapy effectively kills metastasized cancer cells, thus resulting in a comprehensive therapy to prevent tumor recurrence. Interestingly, these inorganic cores are excellent photoacoustic and MRI contrast agents, which are capable of image-guided cancer theranostics. Mineralized nanoparticles (*e.g.*, $CaCO_3$, $Ca_3(PO_4)_2$) are excellent candidates; they are stable during the delivery process, minimizing undesirable side effects, yet completely release the encapsulated drugs due to the decomposition of the supported solid carriers in the endo/lysosomes under acidic conditions.[54]

In order to achieve superior therapeutic results, intracellular bioorthogonal reactions generally require reasonable yield and rapid kinetics in the presence of abundant interferent molecules, including biological electrophiles, nucleophiles, and reactive oxygen species. The reported reactions catalyzed by rotaxane-based organocatalysts so far are relatively simple, barely achieving *in situ* synthesis of theranostic species inside cancer cells. It is necessary to introduce other catalytic sites in the AMMs as components, such as transition metal catalysts. At the same time, the selectivity and efficiency of the reaction should be guaranteed in a complex biological environment inside cells. Apart from the catalysts, the reactions also need to be rationally designed. The products produced within cancer cells should be highly potent to ensure their anticancer efficacy. Ideally, the half maximal inhibitory concentrations (IC_{50}) of the products should be in the nanomolar range because the concentrations of the drug at the target site are often low.

Although a series of exciting achievements for AMMs in cancer theranostics have been realized, this field remains in its infancy. As this field forms at the nexus between chemistry, materials science, nanotechnology, biology, pharmacology, and oncology, closer collaborations are required between experts in different fields for better applications of AMMs in precise diagnosis and therapy. By leveraging their unparalleled topological features, stimuli-responsiveness, and nanoscale properties, AMMs will undoubtedly play integral roles in the fight against cancer. In view of great efforts being dedicated to cancer nanotheranostics, we firmly believe that supramolecular nanomedicines fabricated from AMMs will provide new hope for patients.

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Notes

The authors declare no competing financial interest.

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