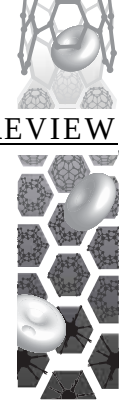
REVIEW



Rise of the nanomachine: the evolution of a revolution in medicine

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Although the current array of nanomachines mostly comprises simple devices (at least from a mechanical viewpoint), the underlying physical and chemical interactions that play key roles in the 'assembly' of these machines have required decades of research to ascertain a fundamental understanding of how such processes can be manipulated at the nanoscale. In this review, we wish to convey a realistic picture of the current developments in the design and implementation of nanomachines, with an emphasis on how these developments are leading to practical applications in medicine, including a sense of how such simple devices are rapidly becoming the building blocks for assembling the nanorobots of tomorrow.

"A machine is a device that applies force, changes the direction of a force, or changes the strength of a force, in order to perform a task, generally involving work done on a load." [1]

Conceptually, the same definition can be applied to nanomachines, except confined to the nanoscale. And who has not read or heard about the fantastic possibilities that exist with the development of nanomachines? An example includes nanorobots that can be injected into the bloodstream, providing desperately needed cures for the most challenging illnesses. One cannot help but hope that the respirocytes (nanorobots that deliver oxygen more efficiently than red blood cells) and microbivores (nanorobots that target microbiological pathogens) found in the writings of Freitas [2–4] are just one research study away from being in the hands of the world's medical professionals. But a quick reality check of research published during the last few years is all that is needed to remind ourselves of just how far we have to go before we will be loading syringes with such sophisticated devices. Yet, the breadth of the current research involving the design of simple nanoscale machines with biocompatible motifs is nothing short of breathtaking. The last 5 years, for example, have seen DNA have a new and vital role as a fundamental building block for the design of simple mechanical devices and structural platforms for highly sophisticated nanomachines. Moreover, nanoparticles (NPs) that were used as contrast agents in medical imaging previously have been transformed into load-bearing drug-delivery systems and remotely controlled targeting agents; both of these tasks are ascribable to simple machines. Furthermore, the nanoscale biological 'machines' of nature have been retrofitted and reprogrammed to perform tasks for scientists, who have envisioned them as the genesis of a new wave of engineered nanorobots. Nevertheless, as with the evolution of any complex machine on the macroscale, the starting point for the development of any nanomachine is from its most fundamental parts. In nature, these parts are nucleic acids and peptides.

Natural building blocks

Nucleic acids

Borrowing from natural architectural motifs generally offers the most probable creation of a biocompatible system and, in nature, the most fundamental of all building blocks are the nucleic acids. Seeman has authored numerous reviews of the initial forays into DNA-based nanotechnology [5–7] and a detailed review by Luo and Li can also be found in the literature [8]. Additionally, a review of research assessing the impact of nanotechnology in living systems, including DNA-based devices, has been compiled [9]. Biocompatibility alone, however, is an insufficient indicator of whether a particular nanomachine will find success in a medical application. Key characteristics required for nanoscale assembly are predictable localized interactions, self-assembly processes subject to methods of environmental control and differentiable components (i.e., unique building blocks) that accommodate functionalization of the system. All of these properties are inherent in DNA nanoscale assembly.

Starting with simple complementary structures, Mao *et al*. used Watson–Crick base-pairing interactions to create DNA 'molecular

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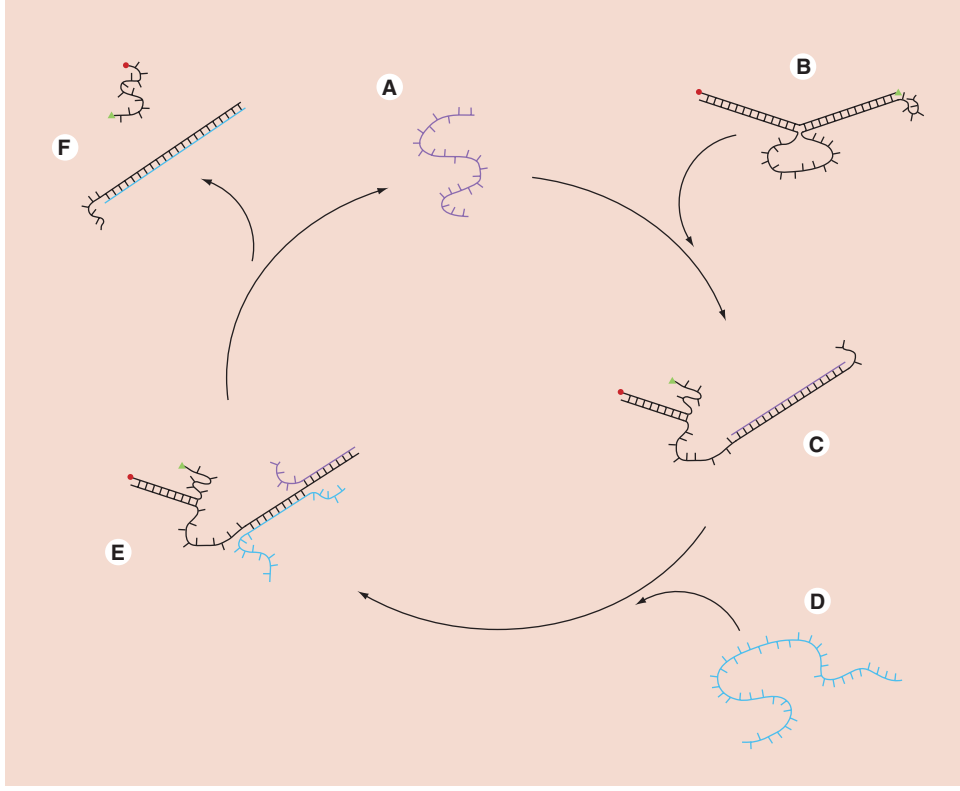
cages' on gold surfaces [10]. In this study, half of a double-stranded DNA (dsDNA) segment is attached to the terminus of a single-stranded DNA (ssDNA) skeleton, which is itself attached to a gold surface through a normal alkanethiol moiety, forming a linearly extended chain. The resulting self-assembled array of these chains on gold encages fluorescent dye molecules reversibly by entrapment beneath a freshly hybridized terminal double-strand. Removal of the complementary strand, also referred to as the 'fuel' strand, from the dsDNA by denaturation releases the encaged molecule. The authors wish to use this type of machine for targeted drug delivery.

Hydrogen bonding between base pairs of the double helix is only one feature of the DNA structure that is subject to manipulation. More complex applications of fuel strands in the assembly of simple DNA-based nanomachines can also be found in the literature. One such example is the use of the segment of dsDNA that overhangs its complementary strand and remains unpaired when the two strands are complexed; the overhang is otherwise known as a 'sticky end'. A judicious design of the nucleic acid sequence for such sticky ends can provide a unique bonding site that limits the plethora of possible attachments. A conceptually similar base-pair sequence manipulation between two of the dsDNA segments enables the controlled design of loops in one of the strands or the design of stem-loops. The use of such structures is exemplified in the work of Turberfield *et al.*, in which the formation of a loop is an integral part of a catalytic cycle, enabling the displacement of a strand that is not fully complementary with one that creates a more stable complementary dsDNA pairing over the full length of the strand (Figure 1) [11]. A pragmatic application of such a catalytic process, in which the ultimate objective is the development of a new drug-release platform, can be found in the work of Pei *et al.* [12]. The polycatalytic assemblies examined

Figure 1. Harnessing DNA in a catalytic cycle.



The catalyst strand shown in **(A)** displaces one end of the partially complementary loop, which causes the strand in **(B)** to form the interim product in **(C)**. With the opening of the loop, the fully complementary strand shown in **(D)** is able to bind to the longer loop-forming strand shown in **(B)**, as illustrated in **(E)**. The resulting products are shown in **(F)** with catalyst **(A)** being reformed at the end of the cycle.
Adapted from [11].

by these researchers were designed to diffuse through a hydrogel matrix, with minimal loss from the matrix, cleaving the substrate found on the matrix surface at rates that are controlled by the number of catalytic units incorporated into the assembly and by variation of the recognition regions included in the matrix. Since the nucleic acid catalysts have the appearance of legs extending from the two streptavidin molecules that form the assembly's core (or body), coupled with the fact that these 'legs' are envisioned by the authors as moving through the matrix in a manner resembling the motions of a spider, the research team has dubbed these assemblies 'spider molecules'.

Additional complexity can be introduced to simple DNA-based nanomachines through crossover interactions between separate dsDNA strands, which is exemplified by a typical Holliday junction [13], together with the incorporation of non-nucleic acid structures as attachments to the DNA architecture. An example of the application of such modifications can be found in the work of Shen *et al.*, in which a simple DNA-based nanomachine is used to estimate the binding energy of proteins that are bound to the structure's backbone by monitoring the fluorescence resonance energy transfer (FRET) between a pair of fluorescent dyes incorporated into opposite ends of the structure [14]. The binding energy is estimated by analyzing the results from protein binding to a series of nanomachines, where each structurally unique device has its own cohesive resistance to the type of deformation (Figure 2). The intensity of the signal reflects the relative deformation of the DNA backbone. To enhance the rigidity of the structure, the researchers adjusted the architecture of the DNA strands by including triple crossover motifs between parallel strands.

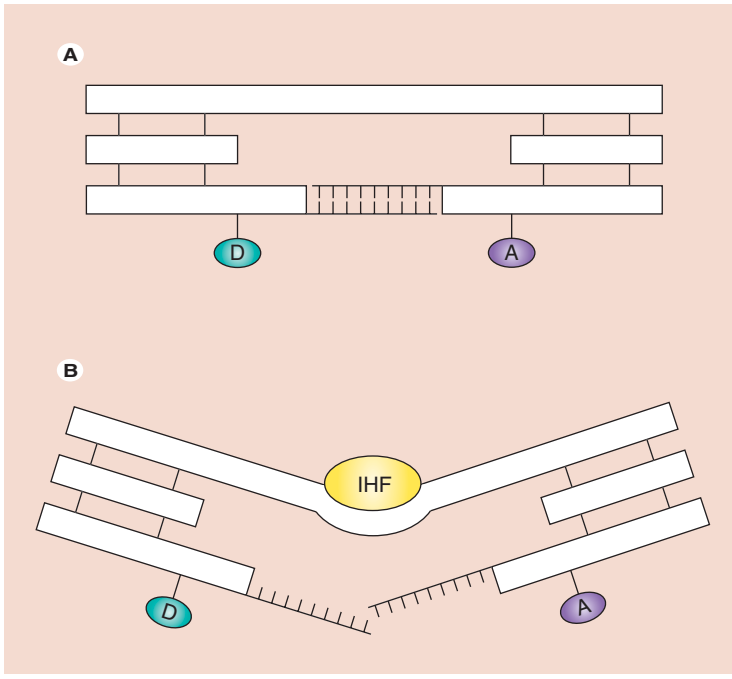
A myriad of additional examples of the increasing sophistication of DNA-based nanomachines can also be found in the literature: devices that open and close [15–17], extend and contract [18–20], move along a track [21–23], form molecular gears [24] and rearrange their structure in response to a stimulus [25–27]. Although these nanomachine-design concepts by themselves are limited in their usefulness, they are finding their way rapidly into more complex systems, including medical devices. A particular development focuses on oligonucleotide-based biosensors that incorporate structures known as molecular beacons (MBs), which are DNA-based probes that provide an optical signal if a specific biological agent is present [28]. These probes include the following design elements: a single strand of sequentially complimentary dsDNA (or RNA) that opens the probe's stem–loop, which is a strand of approximately 15–40 nucleotides; and a fluorescence source that is bound to one end of the probe's DNA strand and separated from a fluorescence quencher that is bound to the other end of the DNA strand, leading to a measurable fluorescence signal upon opening of the stem when hybridizing with the target sequence. These probes can be attached to a surface, such as an optical fiber, through biotin–avidin binding. The key to the success of the probe is the degree to which the approximately six-base-pair stem sequence maintains a closed loop for all agents except the sequence targeted by the MBs.

A general review of MB biosensors by Yao *et al.* was published recently and provides an excellent perspective on the status of this field of research [29]. According to Tan and his collaborators, MBs not only have significant potential for real-time bioassays but might also possibly find use in the direct detection of diseases in living systems [30]. However, to use MBs in an inhomogeneous solution environment, such as that encountered with most biological testing, attachment of the MB to a surface is necessary, leading to the development of tethering schemes and other immobilization strategies. A recent article by Zuo *et al.* indicates that the latest research is focused on improving the strength of the signal for surface-immobilized MBs by identifying problems related to the fact that many surface-bound MBs fail to open [31].

The MB format is not the only route to biosensors with mechanical properties. It is also possible to design nucleic acid biosensors that detect analytes based upon their secondary structure rather than their binding sequence. Such nucleic acid structures are known as aptamers and are the subject of a recent thorough review by Yang and Ellington [32]. A mechanistically similar protein-based nanosensor is described in detail here in the following section.

A nanodevice of increasing structural sophistication that executes complex tasks at the molecular level has been described as a nanorobot or, more generically, a nanobot [33] or a nanite [34]. In biological systems, such a device is also sometimes referred to as a biorobot [2] or a biobot [35]. No matter the designation, an example of an existing DNA-based nanomachine that

Figure 2. Measurement of protein-binding energies with a DNA-based nanomachine.



(A) General structure of the device before a protein molecule (IHF) binds to its backbone. Rectangular boxes represent double-helical DNA and the lines connecting the boxes represent crossover motifs. **(B)** Deformation of the device occurs once a binding protein causes the DNA backbone to bend. The IHF protein is an integration host factor from *Escherichia coli.*
A: Acceptor fluorescent dye; D: Donor fluorescent dye.
Adapted from [14].

approaches this added level of sophistication is one in which a stem-loop oligonucleotide is linked covalently to a gold nanocrystal. In this example, the gold nanocrystal acts as a nanoscale antenna that responds to a radiofrequency magnetic field. Inductive coupling of the radiofrequency magnetic field with the nanocrystal increases the local temperature, causing the attached DNA to dehybridize reversibly [36]. The authors note that this concept might be useful in regulating enzymatic activity, gene expression and/or protein function.

Peptides

In a similar approach to that described already for the assembly of DNA-based nanomachines, researchers have used peptides as assembly components. Examples of this work can be found in the writings of Zhao and Zhang regarding designer peptides [37,38]. However, unlike nucleic acids, manipulation of the amino acid sequence in peptide oligomers enables the modular application of hydrophobic and hydrophilic interactions between the peptide 'building blocks', along with a more diverse selection of individual amino acid interactions. The initial self-assembling systems were vesicles and micelles formed by the controlled use of hydrophobic/hydrophilic regions on the individual peptides, creating highly specific attractive interactions between compatibly designed peptides. Possible uses for peptide assemblies include controlled drug release [39] and the *in situ* self assembly of nanoscaffolds for the targeted repair of brain tissue [40].

The use of amino acids in the assembly of simple devices has also taken a more organic route. Specifically, the synthesis of miniature proteins (MPs) either by *de novo* design or by adaptation of existing sequences from larger proteins has developed into a specialization, providing a means of modeling and testing naturally occurring structures and synthesizing biocompatible nanomachines that mimic nature. Nicoli and Allemann have prepared an informative review of MP research [41]. One advantage touted by supporters of this work is that the adopted natural amino acid sequences make MPs more effective biomolecular recognition tools in living systems. There are, however, challenges associated with the approach of creating nanomachines from natural components. For example, research conducted by Künne *et al*. regarding the binding specificity of the transcription factor MyoD found that the binding sequence alone could not account for its specificity for the transcriptional activation of muscle-specific genes and that other factors must be providing cooperative interactions [42].

The mechanistic role of the secondary structure of proteins cannot be overlooked. Adopting pre-existing protein structures from the abundant selection found in nature has its advantages when it comes to creating nanodevices that use structure-related processes. The review by Baltzer *et al*. provides insight into the design challenges of the *de novo* synthesis route for proteins; further, it illustrates why researchers have focused more effort on adapting natural proteins [43]. A pertinent example is provided by the periplasmic-binding proteins (PBPs), in which conformational changes strongly influence ligand binding, providing specific detection of a large variety of molecules. Those PBPs that are not highly target specific can be modified to target a narrower selection. By incorporating PBPs into the structure of a nanomachine, the protein component

traps its target with a hinge-motion mechanism. Sharma *et al*. have comprehensively reviewed this subject [44].

An example of how developing protein-based biodetection technologies are merging with mechanical devices is found in the work of Li *et al*., in which a modified atomic force microscope (AFM) tip is used to create a nanorobotic system that can manipulate objects at the nanoscale [45]. In this example, AFM tips were functionalized with specific antibodies by a direct coating method or an indirect tethering method using a molecular linker. With the biologically functionalized AFM tip, it was possible to identify specific types of receptors on cell membranes. The AFM probe was controlled by a researcher using a 'joystick', viewing real-time AFM images on a monitor. The authors proposed that this new technology illustrates the first concrete steps toward *in situ* imaging, sensing and manipulation at the nanometer scale.

Natural devices

Biological motors

Within all living organisms exists the need to transport matter from one location to another. The biological motors that perform these tasks are in many instances enzymes (complex protein assemblies) that transduce chemical energy supplies into some form of mechanical energy. By harnessing this energy through the incorporation of these motors into nanomechanical devices, researchers can circumvent some of the design challenges associated with assembly at the nanoscale and reduce the number of biocompatibility issues. Two reviews, one by Hess, Banchand and Vogel, and the other by Lee *et al.*, provide useful insight into how these nanomotors can be reoriented from their natural tasks to perform work in nanomachine assemblies [46,47]. In addition, various strategies for using molecular motors as drug-delivery vehicles can be found in an article by Cohen and coworkers [48].

A well-known example of the incorporation of a molecular motor into a nanoscale device is the work of Soong and Montemagno [49], which uses the F_1-ATPase biomolecular motor. With their device, rotary motion in a central subunit of the enzymatic protein is driven by the consumption of ATP-derived energy. Even though this enzyme is only approximately 12 nm in diameter, the researchers managed to attach a nickel rod that was approximately 750 nm in length to the central protein subunit, which was mounted on a nanoscale nickel pedestal. Once the F_1-ATPase was provided fuel from ATP, the researchers witnessed the rotation of this rod, clocking approximately eight rotations per second. Surprisingly, it was possible to observe this rotation with simple optical microscopy. The long-term goals of this research are further detailed in an article by Schmidt *et al*. [50], which include the use of biological motors in the development of injectable mobile systems that can operate *in vivo*, enabling the remote monitoring of biochemicals or the targeted dispensing of drugs.

Another type of biological motor is the cytoplasmic protein kinesin. The gliding motility of kinesin has been particularly well studied with respect to transporting biological and inorganic cargos [51–53]. Another simple nanodevice built upon a biological motor – a molecular actuator – has been considered for a variety of engineering applications, including the nanofluidic transport of macromolecules within living cells [54–56]. Recently, the ability to capture and transport virus particles using the kinesin-driven transport of antibody-functionalized microtubules was reported [57]. These protein-based motors are highly efficient by nature; however, they function only in aqueous solution within a narrow range of temperatures [58].

Man-made structures

Synthetic polymers

Through the use of traditional synthetic organic chemistry, it is possible to prepare key structural components of nanomachines. One such example highlights the need to move objects in a controllable fashion on the nanoscale. To overcome the drawbacks encountered with the aforementioned kinesin-based motor, polymeric systems have been developed to provide nanoscale displacement, as demonstrated by Santer *et al*. [58]. Multicomponent brushes that consist of two or more different polymers (polymer brushes), among which phase separation can occur, were used to move nanometer-sized objects adsorbed on the surface of the polymer film. The authors propose that, together with the phase transition of the polymers, fluctuations in the surface force field occur, leading to competing forces that displace the objects. This system demonstrates a unique strategy by which smart polymer thin-films can be used to transport objects on the nanoscale.

Owing to their large surface area, relatively new polymeric structures known as dendrimers have been explored as possible drug-delivery agents or therapeutic devices. A review by Boas

and Heegaard provides an overview of ongoing drug-related dendrimer research and biocompatibility issues related to these potential nanoscale carriers [59]. A separate article by Baker *et al.* details work with dendrimers as anticancer therapeutic nanodevices [60], which gives the example of a 'dendrimer cluster agent' – a structure that incorporates several types of dendrimeric modules clustered together, with each module addressing a distinct targeted task.

Oligomeric systems

With many of the recent man-made molecular machines, the 'work' involved is related to controlling motions on the molecular scale in response to external stimuli [61–65]. In contrast to the biomolecular nanomotors described earlier, the molecular structure of the 78-atom chemically powered rotating nanomotor synthesized by Kelly *et al.* [66] and the 58-atom motor molecule (which spins when illuminated by solar energy) by Koumura and coworkers [67] are fully characterized structural entities (Figure 3). Complex bonding interactions and steric hindrance have key roles in their operation. Although these 'motors' might be viewed as novelties, they have paved the way for systems of greater complexity.

Recently, interlocked supramolecules, such as catenanes, rotaxanes and polyrotaxanes, have received substantial attention owing to their unique structural features and potential applications in various roles as nanomachines [68–72]. Although reviews of these systems are numerous [73–76], biocompatibility studies are few. Rotaxanes are comprised of a dumbbell-shaped molecule surrounded by a wedding-band-shaped macrocyclic compound called a 'ring'; the dumbbells are sterically bulky groups known as 'stoppers'. Similarly, catenanes are comprised of two interlocked macrocyclic rings with no dumbbell component. Figure 4 provides a conceptual illustration of both species. The movement of these nanomachines can be driven by electromagnetic radiation or chemical stimuli (e.g., pH), entropy or electrochemical interactions that change the relative affinity of the rings for different binding sites on the molecular structure [77].

A light-powered molecular machine in the form of a rotaxane structure has been reported; it consists of the π-electron-donating macrocyclic polyether, bis-*p*-phenylene-34-crown-10 (ring) with a more complex dumbbell-shaped complementary structure (Figure 5) [78]. The dumbbell-shaped component contains the following: a rupolypyridine complex, $[Ru(bpy)3]^{2+}$ (P), as one of its stoppers, a *p*-terphenyl-type ring system as a rigid spacer (S), a 4,4´-bipyridinium segment (A_1) and a 3,3´-dimethyl-4,4´-bipyridinium segment (A_2) as the π-electron-accepting stations, and a tetra-arylmethane group as the second stopper (T). In its resting state, the macrocyclic polyether ring encircles the 4,4´-bipyridinium unit A_1 because this station is a better π-electron acceptor than the other station. Once the photoactive unit P is excited by light, an electron is transferred from the excited P to the A_1 station, leading to the deactivation of that station. As a consequence, the macrocyclic polyether ring is displaced from the reduced station A_1^- and slides to station A_2. When an electron transfers from station A_1^- to P^+, station A_1 regains its electron-acceptor ability, thereby drawing the ring back from station A_2. This system represents only one of the many examples of a functioning molecular shuttle.

By using a variation of this approach, the authors constructed a highly sophisticated two-component molecular device that behaves as a

Figure 3. Detailed chemical structure of (A) a light-driven molecular rotor and (B) a chemically powered rotating nanomotor.

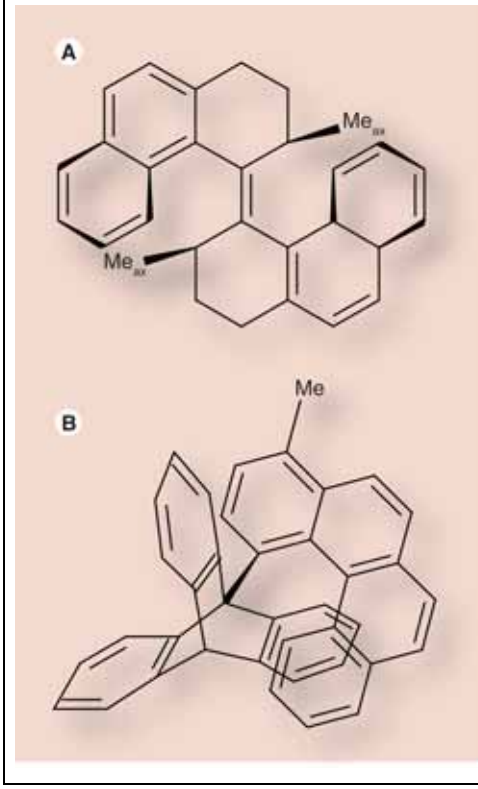
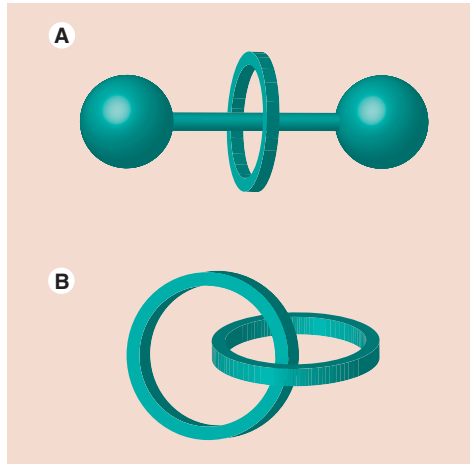


Figure 4. (A) Rotaxanes and (B) catenanes.



nanoscale elevator [79]. This supramolecule consists of three legs (tripod component), each of which contains two different notches: ammonium groups at the top of the legs and 4,4′-bipyridinium at the bottom of the legs (Figure 6). The three legs are interlocked by a central molecule that has three pendant cyclic polyether rings. The mechanical motion of the nanoscale elevator can be controlled simply by adjusting the pH of the solution. At low pH, the ring system resides at the upper level owing to favorable $O\cdots H–N^+$ hydrogen bonding and intermolecular π–π stacking forces. Upon the addition of a base, however, the hydrogen bonding is lost and the ring is electrostatically drawn to the bipyridinium units on the lower level. This particular design is far from biological; however, the authors have suggested that the concept can be adapted to provide a means of controlling the uptake and release of a guest molecule, such as a drug. Likewise, several articles exploring possible drug-delivery schemes based on polyrotaxane systems can be found in the literature [80,81].

Carbon nanotubes

Among the materials used to create nanomachines, carbon nanotubes (CNTs) have received considerable attention, as noted in a recent review by Sinha and Yeow [82]. CNTs possess unique physical properties, including high electrical and thermal conductivity and superior mechanical strength. Nevertheless, CNT-based devices face many challenges. The methods used currently to prepare CNTs are expensive and yield an inconsistent product, requiring a significant investment in purification. Furthermore, CNTs are the subject of much debate regarding their safety, both for the laboratory worker handling the powdered form of CNTs and for the use of CNTs in medical applications [9,82]. Owing to these issues, we have chosen to focus only on research wherein CNTs are used as components of larger assemblies.

Of particular interest, Kim and Lieber created a pair of nanotweezers in which the arms consist of a pair of electrically controlled CNTs [83]. A voltage is applied across the electrodes to operate the tweezers: the applied voltage induces charge separation, causing attractive forces between the arms; when the voltage is reduced, the arms separate. This nanodevice can be used to grasp individual macromolecules in nanorobotic applications. A recent review by Harrison and Atala describes how CNTs can be used to build nanoscaffolds for tissue engineering [84]. Moreover, Fennimore *et al.* recently reported rotational actuators based on CNTs [85]. These researchers constructed an approximately 300-nm actuator on a silicon chip, incorporating a rotatable metal plate with a multiwalled CNT serving as the key motion-enabling element. The speed and position of the actuator can be controlled precisely by an externally applied voltage. Furthermore, the device operates over a wide range of frequencies, temperatures and environmental conditions, unlike chemically driven bio-actuators.

Nanoparticles

Based on the abundance of available literature, the desire to create successful drug-delivery carriers has been one of the strongest motivations behind the development of nanomachines. To this end, a variety of spherical nanostructures show significant potential currently to carry therapeutic agents, such as drugs or DNA, to targeted biological sites. Owing to the extensive interest in this topic, multiple reviews have been published on a variety of NP systems, including silica NPs [86], polymeric spheres [87], colloidal spheres [88], gold NPs [89], metal nanoshells [90] and magnetic NPs [91,92]. Information regarding the efficiency of penetration of biological membranes by NPs and the biocompatibility of NPs can also be found in the literature [9,92,93].

Some of the drug-delivery strategies being pursued through the NP route have organic roots. Liposomes are spherically closed lipid bilayers that are formed through the naturally

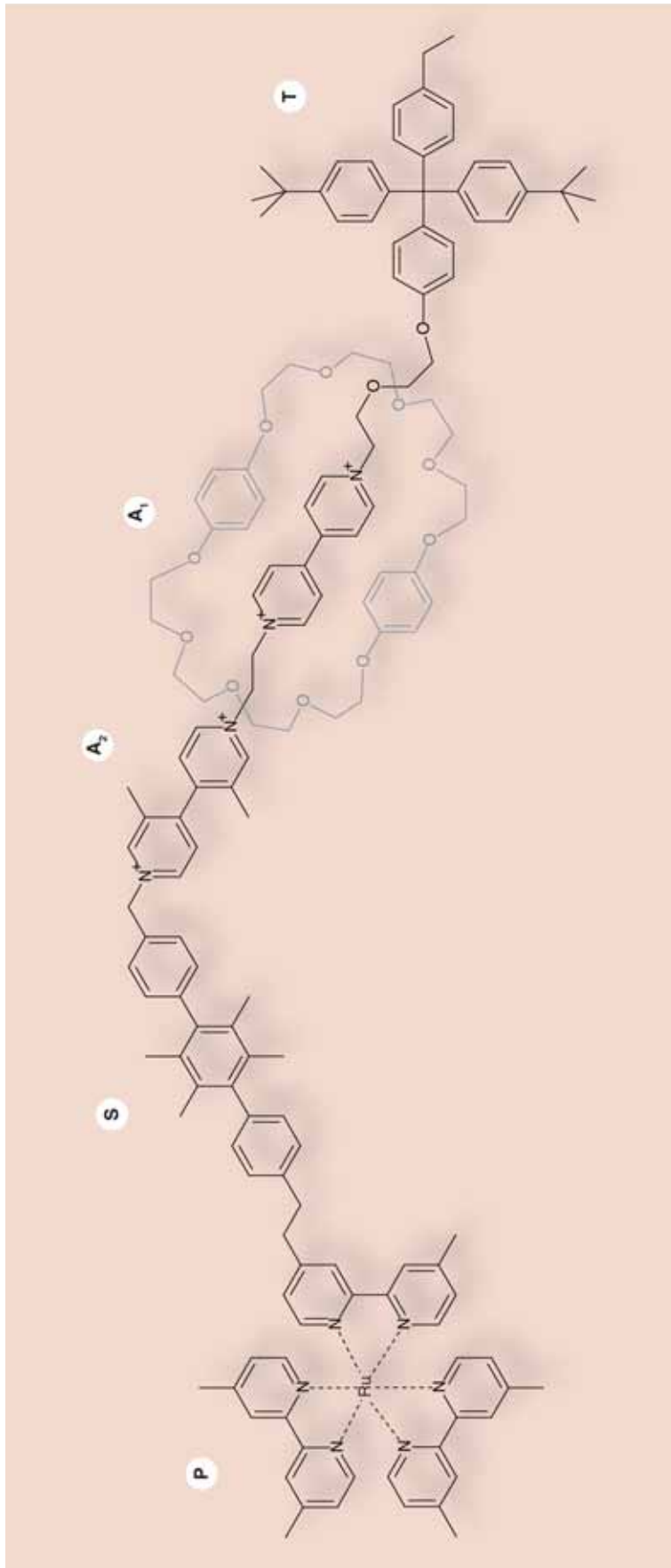
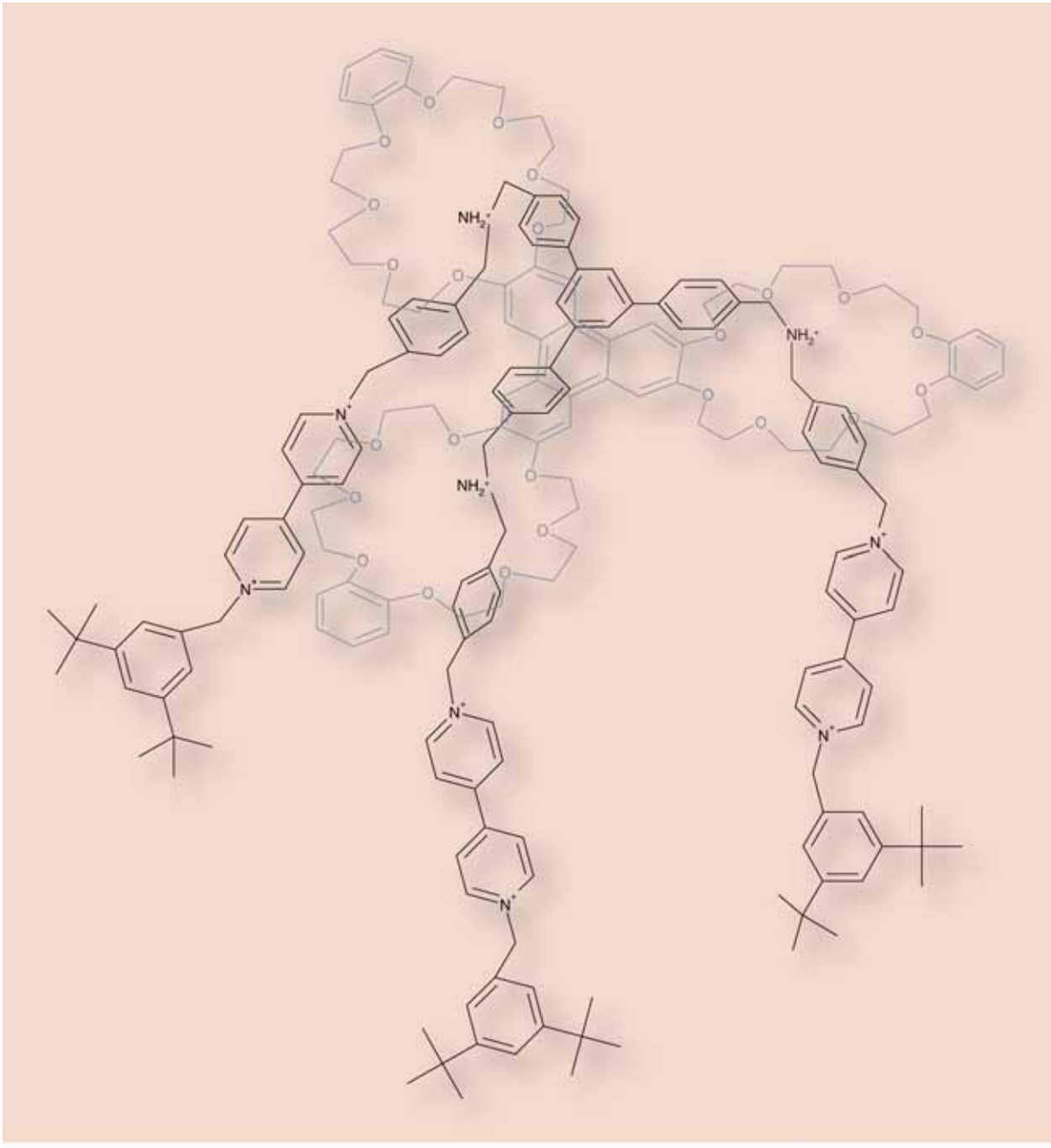


Figure 5. Detailed chemical structure of the rotaxane.

Figure 6. Detailed chemical structure of the molecular elevator.



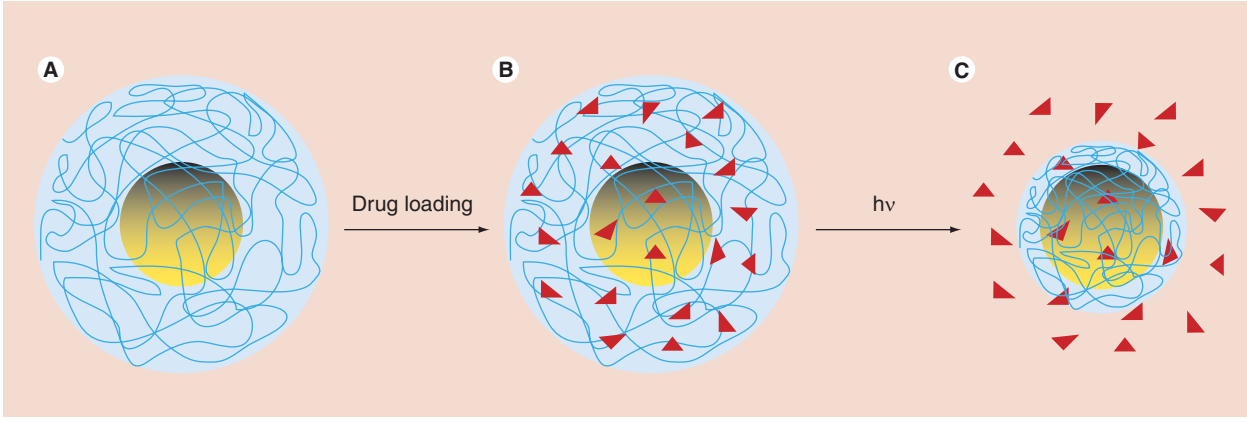
occurring self-assembly of phospholipids. Since they are composed of materials found in living systems, liposomes can be loaded with drug molecules and delivered into the bloodstream without adverse effects arising from the liposomes themselves [94]. Although this field is relatively well developed compared with other NP systems, current research continues to explore the use of liposomes or liposome-like vesicles for the delivery of pharmaceuticals (e.g., anticancer drugs) [95]. Furthermore, in a recent biosensing application, Ma *et al*. prepared vesicles from 2,4-tricosadiynoic acid as the lipid matrix and dioctadecyl glyceryl ether-β-glycoside as the receptor to detect *Escherichia coli* [96]. As a whole, however, applications involving liposomes have

been limited owing to their inherent colloidal and biological instability, leading to short lifetimes in the bloodstream [97]. Efforts to address these concerns have included strategies to adjust the size, composition and surface characteristics of these drug-delivery agents. One particularly successful example of the modifications under study is the incorporation of polyethylene glycol (PEG) into the liposome matrix, forming a 'stealth liposome' that increases liposome lifetime *in vivo* and provides insight on how other nanomachines might be modified to improve biocompatibility. A critical analysis of stealth liposomes by Moghimi and coworkers provides additional insight [98]. Furthermore, Immordino *et al.* have compiled detailed information regarding liposome technology, including a comprehensive list of liposome-based drug systems [99].

To overcome the drawbacks associated with vesicle lifetimes *in vivo*, substantial effort has focused on the development of stable polymeric NPs that are biocompatible [100–102]. In ideal cases, these polymer particles can be filled with anticancer drugs, with the aim of achieving targeted and/or controlled drug delivery. Reis *et al.* have described various polymer systems and associated drugs therapies that have been developed or are under study currently [103]. Recently, amphoteric polymeric particles were prepared by an interfacial polyaddition reaction of ethylene glycol diglycidyl ether and L-lysine in a water-in-oil emulsion system [104]. The pH-responsive amphoteric NPs were used to separate and deliver ssDNA, in which the ssDNA was 'trapped' on the surface of the NPs through the formation of ion-complexes at pH 7. When the trapped ssDNA was exposed to a solution of pH 11, the ssDNA was almost fully released from the surface. Such amphoteric particles could be used to deliver or purify a variety of biomolecules, although the system of interest must be amenable to highly basic conditions. Folic acid-decorated thermo-responsive hydrogel NPs have also been used for the specific targeting of cancer cells [105]. These hydrogel NPs are comprised of a poly(*N*-isopropyl acrylamide) (poly[NIPAM]) core with the surface decorated with folic acid moieties; in these studies, the folate receptors are overexpressed in human tumors [106]. Through the use of fluorescence labeling, the authors showed that the NPs were delivered efficiently to the cytosol and not retained in the endosomes, consistent with their potential use in the delivery of drugs to specific types of cells.

Other spherical structures that are being tested as nanoscale therapeutic agents or drug-delivery systems include silica NPs, metal NPs and magnetic NPs [86,107–110]. Priyabrata *et al.* showed that gold NPs can be made to be bifunctional nanomachines, holding both an antiangiogenic and anticancer agent simultaneously [107]. As highlighted in a recent review [90], others have used gold nanoshell particles to deliver heat photolytically to tissue and/or tumor cells. In separate work by Maxwell *et al.* [111], gold NPs were combined with nucleic acid-based FRET sensors to create DNA probes. For this

Figure 7. Photothermal drug delivery using hydrogel-coated nanoparticles.



(A) Hydrogel-coated gold nanoparticle before drug loading. **(B)** The same nanoparticle after drug loading; the red triangles represent the drug molecules. **(C)** Exposure to near-infrared light activates the plasmon resonance of the core, heating the system and causing the hydrogel to collapse and release the drug.
Adapted from [113].

particular device, the signal of the sensor is quenched when the unhybridized oligonucleotide chain brings the fluorophore in contact with the gold surface. Hybridization with the target DNA causes the fluorescent dye to separate from the gold surface, producing a fluorescent signal. In studies with magnetic NPs, Hilger and coworkers used a rapidly oscillating magnetic field to induce the thermal ablation of breast cancer tumors. In this approach, the magnetic NPs accumulate spontaneously in or near the tumors; receptor-specific targeting can be used to enhance the accumulation [112].

Lee and coworkers demonstrated a unique approach recently for controlled drug delivery using NPs by coating gold NPs with the pH- and temperature-responsive hydrogel derived from the copolymerization of NIPAM and acrylic acid [108,113]. These nontoxic composite NPs can be loaded with drug molecules and the NP core can be activated photothermally, which collapses the hydrogel coating and releases the drug molecules (Figure 7). By using metal nanoshells as the core, tissue-transparent near-infrared light can be used for *in vivo* delivery. As a brief summary, Table 1 provides selected highlights of NP-based machines that are being explored for use in diagnostic, therapeutic and/or drug-delivery applications.

Conclusion

The purpose of this review is to provide a brief summary of the various nanomachines that have been developed to date, with an emphasis on those with medicinal relevance. At this point in time, these developments range from systems that are composed of and effected by purely natural agents, to those in which the natural agents have been harnessed and/or 'tuned' by artificial modification, to those in which the agents are entirely artificial or 'man made'. Many of the current nanomachines are relatively simple devices that are now beginning to find their way into more complex structures and applications. The examples highlighted in this review might appear to be merely small advances in a world littered with expectations abundantly fertilized by science fiction; however, they nevertheless provide clear indications that a nano revolution in medicine has begun.

Future perspective

A broad array of nanomechanical biosensor technologies is emerging that will provide faster and cheaper technologies for real-time diagnostics and therapeutics. Although many of these new approaches still require fine-tuning, the near-term impact for medical professionals should be the awareness of new methodologies that operate on the nanometer scale. Furthermore, with multiple

Table 1. Selected examples of nanoparticle-based nanomachines with medicinal relevance.

First author (year)	Particle type	Size (nm)	Action	Ref.
Hofheinz R-D (2005)	Liposomes		Cancer targeting	[95]
Immordino ML (2006)	Stealth liposomes		Drug delivery	[99]
Leamon CP (2003)	Surface-modified liposomes	~10	Cancer targeting	[106]
Ma Q (2001)	Shell-crosslinked vesicles	35	Biosensing	[96]
Reis CP (2006)	Polymer NPs		Drug delivery	[114]
Avgoustakis K (2002)	Copolymer NPs	~150	Drug delivery	[101]
Mosqueira VCF (2001)	Modified nanocapsules	~240	Drug delivery	[102]
Taira S (2005)	Amphoteric polymer NPs	~200	Drug delivery	[104]
Du Y-Z (2004)	Bifunctional latex NPs	~200	Targeted binding	[115]
Nayak S (2004)	Hydrogel NPs	~270	Cancer targeting	[105]
Tan W (2004)	Silica NPs	5–400	Biosensing	[86]
Maxwell DJ (2002)	Modified gold NPs	2.5	Biosensing	[111]
Mukherjee P (2005)	Bifunctional gold NPs	~5	Cancer targeting	[107]
Kim J-H (2004)	Hydrogel-coated gold NPs	~150	Drug delivery	[108]
Kim J-H (2006)	Hydrogel-coated gold NPs	~150	Drug delivery	[113]
Bergey EJ (2002)	Magnetic NPs	20–50	Magnetocytolysis	[109]
Hilger I (2005)	Magnetic NPs		Magnetocytolysis	[112]

NP: Nanoparticle.

laboratories exploring possible new treatments that use nanoscale drug-delivery elements, both diagnostic and therapeutic applications involving nanoscale materials will continue to be highly visible in professional journals for many years to come. The accompanying funding and, hopefully, stories of successful nanomachine-based treatments will inevitably drive diversification of this particular technological platform. And, as with previous leading-edge technologies, the desire of the curious researcher to add new features will inevitably lead to nanomachines that will satisfy the general public's thirst for respirocytes and microbivores.

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Executive summary

Introduction

- A nanomachine is a device that performs a task at the nanoscale. The development of nanomachine technology for medical applications is best understood by reviewing the evolution of simple nanomachines.

Natural building blocks: nucleic acids

- Nucleic acids offer natural architectural motifs for the assembly of simple nanomachines, providing for the creation of devices with a higher likelihood of being biocompatible.
- Researchers have used hydrogen bonding, 'sticky ends', carefully planned nucleic acid sequencing, Holliday junctions and other methods of structural control to create nanodevices from nucleic acids; an example being that of the molecular beacon biosensor.

Natural building blocks: peptides

- Amino acid assemblies in the form of designer peptides, miniature proteins, synthetically produced proteins and modified natural proteins have all been used in the development of nanomachines for medical applications.

Natural devices: biological motors

- Natural biological motors, such as the enzyme F_1-ATPase, have been incorporated into nanomachines to provide a source of mechanical energy.

Man-made structures: synthetic polymers

- Synthetic polymers, including dendrimers, have found roles in the development of nanoscale medicinal applications, most notably as drug carriers.

Man-made structures: oligomeric systems

- Although lacking practical applications in medicine, nanomachines created through organic synthetic methods (e.g., catenanes and rotaxanes) have provided researchers with useful insight into the design of environmentally controlled mechanical motion at the nanoscale.

Man-made structures: carbon nanotubes

- Carbon nanotubes have received considerable attention in the development of nanodevices but continue to be plagued with cost and safety concerns.

Man-made structures: nanoparticles

- The need for methods of efficient therapeutics and drug delivery have led to the creation of a large array of nanoparticle (NP) systems, including liposomal and polymeric nanocapsules used as transport vesicles, magnetic NPs in which thermal ablation is effected by an oscillating magnetic field and a variety of metal NPs and shell–core NPs that deliver agents in response to changes in pH, temperature, photolysis and/or receptor binding.

Conclusion

- Overall, recent nanomachine technology shows a growing sophistication in the structure and function of the nanodevices that are available for medical applications.

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