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Molecular machine-based nanomaterials: Recent progress and prospects

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Abstract. Molecular machines are chemical molecules (man-made or natural) which display triggered reversible conformational changes by external stimuli like pH and temperature, followed by observable changes. This property of controllable and coherent motion at molecular level can be observed through the synthesis of macroscopic materials from self-assembly of molecular machines. Due to these properties, molecular machine-based nanomaterials display a wide range of unique characteristics and various useful applications in biomedicine, energy, environmental engineering, etc. This paper reviews recent research articles on some potential applications including biosensing, therapeutics (with a focus on drug delivery system) and catalysis based on MMs and their composites to assess the current practicality of the technology. Although most materials are not ready for real-life applications yet and there is a lack of deep understanding of the efficacy, these nanomaterials display a wide range of very useful and unique characteristics and present a brand-new direction for tackling current challenges.

Keywords: molecular machines, nanomaterial, drug delivery, catalysis.

1. Introduction

This concept of molecular machines (MMs) is known to the public for years in the form of nanobots. However, unlike what people perceive as machinery, which is built from screws and gears, molecular machines are in fact designed molecules whose motions of its components are induced in the form of chemical reactions, if exposed to some external stimuli by the operational environment, like temperature, pH, light, etc. Several important technological advancements opened possibilities for synthesizing molecular-machine-based materials in the 20th century. Benefiting from the advancement of supramolecular chemistry in the 1980s, many of the important prototypes of molecular machines were synthesized. Two famous examples are rotaxane and catenane. They are mechanically interlocked molecules, meaning they are connected as a consequence of their topology, rather than via chemical bonds. Rotaxanes are formed with a ring surrounding a rod with two stoppers, while catenanes are two interlocked rings. As direct synthesis gives very poor yield, the synthesis of such complicated architecture requires the use of the template effect of metal ions to pre-organize the ligands in the correct orientation and achieve self-assembly. And the molecules synthesized kept becoming more complicated structurally through the evolvement of synthesizing methods over the years. Some of them are observed undergoing microscopic motion like molecular rotors and motors [1]. On the other hand, optical microscopes are limited by the resolution calculated by Abbe's formula, starting from the invention of the electron microscope by Ernst Ruska in 1931 (Nobel Prize in 1986), then the invention of STM by

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Heinrich Rohrer and Gerd Binnig in 1981 (Nobel Prize in 1986), and other microscope techniques which "gets around" Abbe limit like STED microscopy (Nobel Prize in 2014), scientists went a long way to make atomic level imaging and manipulation possible. This built the foundation for scientists to "visualise" and explore the world on nanoscale. One important fact which makes nanomaterials desirable is that quantum mechanics starts to take over and dominate the behaviour of nano-sized particles, which leads to very different properties compared to conventional materials. And many nanoparticles have extensive applications in industries such as pharmaceutical, cosmetics and food already [2-4]. In an effort to create ideal materials whose properties are controllable and coherent at nano size, trying to build macroscopic materials based on nano-sized molecular machines in which microscopic changes are predictable seems a good starting point. Methods of interfacing the nano-sized MMs to build bulk materials and an understanding of how much the interfacing affects the properties of MMs measured when isolated in solution are required to amplify the molecular scale motion into a macroscopical level. This field of research started from single molecule devices on a surface, then evolved to 2D self-assembled monolayers and films and eventually to the prototypes of bulk materials: in the form of the polymer matrix, MOFs and between interfaces (most commonly air-water). So, this article aims to examine the applicability of these materials in real life by reviewing research on different branches of their proposed applications and the recent progress.

2. Molecular machines in biosensors

The common method of medical diagnosis nowadays is through the detection of certain biomarkers in biofluids. A biomarker is a biological molecule found in biofluids, which is a sign of an abnormal process like a specific disease. Successful diagnosis at the early stages of diseases is very important in planning the therapy of patients and providing them with a better chance of benefiting from the treatment. And detection of specific biomarkers when they are present in low concentrations is one of the best methods available, which makes more sensitive sensor probes desirable. Inspired by biological systems like Calmodulin (a Ca^{2+} intracellular messenger in nearly all eukaryotic cells), molecular machines which binding to biomarkers can induce conformational changes and therefore changes in physical properties of the material found themselves very useful in the design of chemical sensors.

One big challenge is its applicability in biofluids, as unlike in simple two-component systems, interactions with other biomolecules present may jeopardize the result. To solve this problem, Krämer et al. developed fluorescent chemosensors based on Cucurbit uril (CB8) rotaxanes which aim to measure tryptophan (Trp) level in human biofluids within the physiological concentration range [5]. The CB8 host forms a complex with a reporter dye and β-cyclodextrin are attached to both ends of the rotaxane as hydrophilic stopper groups to prevent disintegration in biofluids and enable immobilisation on surfaces through covalent interactions. Current measurements for Trp levels are carried out using HPLC (High-performance liquid chromatography), which requires a compulsory deproteinisation step. However, this micro assay can work with untreated blood serum samples at an efficiency of less than 1 minute per assay. The sensing ability for L-Trp was tested for untreated human and bovine blood serum. The fluorescence emission levels upon addition of rotaxane (I_1), and the emission reduction upon subsequent spiking with Trp (I_2) were recorded. Then the excess of indole was added to bind to the rotaxane sensor and quench the emission to a maximum (I_3). And it was found that the quenching efficiency of the assay correlates with the HPLC-measured Trp levels, and samples from healthy and diseased patients with different Trp levels can be distinguished. Moreover, using scanning probe lithography, a micro sensor chip was fabricated by fixing the rotaxane molecules on functionalised glass surfaces, which improves the sensitivity down to submicromolar concentrations and can be a prototype for lab-on-a-chip in the field of microfluidics.

Another important type of detector is built from DNA-based molecular machines, whose structure is tuneable to accommodate different operational environments and stable under physiological conditions. Chandrasekaran et al. designed a DNA nanoswitch to detect the microRNA of Alzheimer's disease (AD) [6]. The binding of the targeted microDNA with modified backbone oligonucleotides caused conformational change between on (looped) and off (linear) states. And the colour came from the Gelred

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dye molecules binding to the nanoswitch, so no extra labelling or amplification of the signal is required. The detector showed high sensitivity with visible detection to 175 fM and lower limit of ~7.7 fM for miR-107 (one example of AD-related microRNA) and high specificity amongst closely related miR mRNAs. And the experiment with real-life RNA samples from healthy and AD-diseased brain tissues further verified the validity of the detector. These DNA nanoswitches can also be assembled to form a scaffold, which allows the testing of multiple biomarkers simultaneously, producing a unique barcode signature [7]. Nanoswitches targeting 6 gene biomarkers for 6 different diseases (smallpox virus, cystic fibrosis, Tay-Sachs disease, breast cancer, human immunodeficiency, Werner syndrome) were synthesised and detection of a single-stranded DNA oligonucleotide from each gene was successful. Furthermore, this design can be extended to different types of biomarkers, namely proteins, antibodies, DNA, and RNA. And the individual measurements in the presence of other biomarkers are consistent with the ones measured with only one type of the biomarker in the solution. This improves the accuracy of diagnosis as more related biomarkers of a single disease can be measured at the same time. The success of this design is that the whole testing can be carried out in hours, and the nanoswitches can be washed and reused, and the interpretation of the results is conveniently through electrophoresis.

3. Molecular machines in therapeutics

Common drug delivery process is very inefficient. Poor targeting of diseased areas not only causes wastage through metabolism, but also induces serious off-target side effects. For example, the therapeutic range of cisplatin (which is an example of anti-cancer drugs) is greatly limited by significant toxicity to healthy tissues. Free circulation around the body only made the side effects worse. Also, its poor oral stability means it needs to be administered by invasive injection. Molecular machines-based materials are promising in designing targeted drug delivery systems, because their properties could be exploited to build stimuli responsive materials which can improve water solubility and control the release of drugs.

Targeting of diseased tissue like tumours is achievable through designing different stimuli for the molecular machine. Usually, it is selected based on the difference in physiological conditions between organs. Yuan et al. reported an example of using Chaperonin-GroEL as a carrier for hydrophobic drug delivery [8]. This natural molecular machine possesses a double layer cage structure, and the hydrophilicity is switchable by Adenosine triphosphate (ATP) binding. Hydrophobic doxorubicin (DOX, an anticancer drug) can be loaded into the cavity and the binding was detectable by UV/vis spectroscopy, which did not lead to large structural change of the protein and made GroEL a perfect “cage” protecting the drug. GroEL can not only release the drug at the tumour site due to the critical ATP concentration near the tumour and improve the solubility of the drug through encapsulation, but also had an affinity for binding with plectin, a protein only expressed on the membrane of tumour cells. This means that GroEL only bound to cell membrane without entering the cell, which reduced its cytotoxicity. The pharmacokinetics showed that GroEL-Cy7 had doubled half-life than free cy-7, showing the increased chemical stability of the encapsulated material. Both in vivo and in vitro tests were conducted to test the targeting of tumours by GroEL. For example, images of stained paraffin sections of different organs ranging from heart to kidney and the tumour of the mice after two weeks of receiving the Gro-EL Dox supported improved effectiveness and delivery of antitumor drugs, and better targeting without many side effects on the major organs.

On the other hand, other conditions can be used to induce the conformational changes. Sun et al. reported an example of applying an external magnetic field to nanomotors to achieve in vivo cell targeting [9]. Carbon nanotubes (CNTs) / Fe_3O_4 nanomotors carrying DOX were synthesised and tested. CNTs were selected for its good biocompatibility and unique helical structures. The pi-pi bonds on the large surface allows for further modification and efficient loading of drugs. On the other hand, the designed stimuli for drug release need to be non-toxic (not inducing irreversible damage to the biological systems) As Fe_3O_4 nanoparticles are ferromagnetic, the motion of the nanomotors can controlled by a remote magnetic field. Varying frequency and voltage of the AC magnetic field is found to be able to change the direction and position of the nanomotors in dispersed solution. And DOX is released upon

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adsorption of near-infrared light by the CNCs. CNCs emit red fluorescence upon excitation of a green laser, and the fluorescence intensity is proportional to the amount of DOX loaded onto the surface of CNCs. And it was observed that the red fluorescence disappears after 180s of irradiation, which supports the idea of the triggered release of DOX. Finally, comparative experiments verifying the targeting ability for HeLa cells were conducted, and as proven by fluorescence images, the nanomotors show a good ability to target and kill HeLa cells in both single cell and cell solution experiments. For example, in a mixed cell solution with the nanomotors, the HeLa cells in the irradiated area died almost completely after 46 minutes, while only a few died in the pure cell solution in the same period.

Recently, there have been attempts to explore the potential of using molecular machines as therapeutic molecules, which may represent a new direction in the field of pharmacology. Santos et al. did experiments on visible light-activated molecular machines (photoisomerization) as an anti-bacterial agent [10]. The effects of MMs are tested with bacteria which showed antibiotic resistance like *A baumannii, E Coli, and P. aeruginosa*. The persister levels were reduced to the limit of detection in minutes, which was faster than most antibiotics, although selectivity for bacteria membrane is an area yet to improve to circumvent the toxicity concern. In the treatment with infected burn wounds, mechanism of the antibacterial activity was discussed. It was shown that the MMs operate with a different mechanism compared with conventional antibiotics, likely through mechanical disruption. Therefore, development of resistance is rather unlikely, which makes it a promising candidate for next generation antibiotics given the huge risk posed to mankind by drug resistant infections.

4. Molecular machine in catalysis

The ability of MMs to undergo controlled molecular motion upon stimuli makes them desirable platforms for catalysts. The conformational changes between “on and off” states result in reversible control of catalytic activity. For example, stimuli-responsive Cu(I)-based catenanes are designed as an on/off catalyst for copper catalysed alkyne azide cycloaddition (CuAAC) reactions [11]. The basic idea of the design is that the encapsulation of Cu(I) within the interlocked structure would hinder its catalytic activity (Off state) and breaking of the mechanical bond upon chemical stimuli would reintroduce the copper catalyst in the system (On state). The Fmoc-protected catenane was synthesised using the Cu(I)-directed template effect and the piperidine (5 equi.) was added to deprotect the Fmoc groups which leads to elimination and decomposition of the macrocycles. The switch in catalytic activity was verified with the fact that no triazole was detected after the reaction between azide and alkyne with the catenane, and it was synthesized with the non-interlocked Cu(I) derivative verified by HPLC of the reaction mixture at different retention times. As cyanide ions are commonly used to remove the Cu(I) metal template, addition of KCN to demetallate the catenane confirmed the inhibition of the Cu(I) activity is via catenand effect. On the other hand, The Alloc-protected catenane was found to be a sensor for Pd(0) in low concentrations, as Pd(0) is required for the activation of the Cu(I) and the following AAC reaction in this case, so triazole formation indicates presence of Pd(0) in the environment.

Inspired by the idea of the regulatory mechanism of enzymes responding to the changes in the external environment, Zubi et al. achieved regulation of propel ligopeptidase (POP) enzyme activity by incorporation of DNA metal-responsive nanoswitches [12]. This design requires the enzyme to have two conformations with different catalytic activities, like the POP protein has “open” state (active) and “off” state (inactive). Then activation/deactivation of the linking groups (LG) genetically encoded into the enzyme would favour/disfavour one state over the other, leading to a reversible control over the enzyme. Structural and Molecular dynamics (MD) simulations were used to help to locate the residues which would allow the covalent bonding formation of LG in one conformation and keeping two LGs apart in the other. And mutants of the POP protein were selected based on appropriate coordination sites for BpyAla LG pairs. The BpyAla LG switches are activated through the addition of metal ions, which inhibits the enzyme. And all nanoswitches were tested for screening from divalent metal ions including Ni(II), Cu(II), Zn(II) and Fe(II). Although the extent of inhibition of different variants by different metal ions was different, reflecting the difference in coordination geometries and binding affinities, presence of metal ions can lead to the inactivation of catalysts. UV-vis spectroscopy supports the formation of

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linkage between BpyAla LG pairs with the strong MLCT emission at around 520nm with Fe(II). The reversible control was further verified by the addition of a competitive metal chelator ethylenediaminetetraacetic acid (EDTA), which led to recovery of catalytic activities except for Fe(II) ones, which could be because of poorer affinity of EDTA with Fe(II)).

Some molecular machines also show potential for stereoselective catalysis. Dommaschk et al. reported a rotaxane-based asymmetric catalyst which controls the chirality of the product of conjugate addition of aldehydes and vinyl sulfones [13]. The non-interlocked substrate is a 2,5 substituted pyrrolidine with pyridyl-acyl hydrazine and glycyl amide groups, which are possible binding sites for benzylic amide macrocycles. And the substrate was tested as a potential organocatalyst, which gives very poor enantiomeric excess (ee) of either S or R product. The stereoselectivity of the enamine catalysts comes from steric bulk on the substituted pyrrolidine disfavours one direction of approach of the electrophile over the other. In this case, the substrate possesses pseudo-meso symmetry, which results in hardly any bias for different reaction pathways for different stereoisomers. However, binding of the macrocycle through clipping reactions onto the pyrrolidine catalyst breaks this local symmetry, introduces additional steric bulk and possible non-covalent interactions to left or right side of the catalyst and therefore produces enhanced (R) or (S) catalysts. Moreover, the reversible interconversion of configuration was achievable through exposure to UV light ((R) to (S)) and treatment with acid respectively and verified through ^{1}H NMR. The ee of the interlocked catalyst was still very modest (ranged from 20% to 40%, highest was 40% ee for (S)), but much improved from the non-interlocked substrate.

5. Conclusion

In summary, this article has mainly discussed three branches of applications of molecular-machine-based nanomaterials: biosensing, therapeutics and catalysis. The scope of the applications is very wide due to the various types of MMs available and the potential to modify and functionalise the MMs accordingly. Both synthesized and natural molecular machines can act as nano-capsules for hydrophobic drugs and the unique advantage over conventional material is the release of drugs only under the physiological conditions of the environment around the diseased organs. Similarly, targeted binding of biomarkers leads to a change in the colour of the MM based sensor and the detection limit of the sensors is much improved due to their small sizes. Finally, different conformations of MMs can be exploited as on/off states of a catalyst of which catalytic activity can be regulated.

Despite the Trp chemical sensors which can operate in real biofluids, most of the applications discussed above are only valid for scientists for whom expertise lies in these fields now and more attention could be focused on achieving industrial-level synthesis of such materials.

Also, there could be more discussion viewing molecular machines as a potential nanomaterial. With the current mainstream bottom-up approach through supramolecular synthesis, the development of such new materials would benefit from the additional approach of top-down approaches from engineering and material science. There is still a large gap between the types and complexity of molecular machines synthesized and the investigations of how to utilize their properties at a macroscopic level. Finally, due to the limited scope of this article, there are many other promising applications like anticounterfeiting by responsive luminescent MOFs and molecular shuttles, which would allow for more complete discussions for the future of this technology.

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