Common Self-Assembly Mechanisms and Their Applications in the Medical Field

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Abstract. Due to the different synthesis methods, organic materials made using self-assembly technology have many unique chemical properties compared to other organic materials. These specific chemical properties make these materials important in many fields, especially in the field of drug carrier design and protein structure simulation for pharmaceutical applications. Recently, more self-assembly techniques are being applied in completely new ways at different levels of discipline structure in order to produce results in the medical field. This paper will take the self-assembly method of non-covalent interaction as the starting point, introduce a variety of common mainstream self-assembly technologies from traditional hydrophilic hydrophobic self-assembly, hydrogen bond self-assembly to emerging charge transfer self-assembly, electrostatic self-assembly, and their application in medical treatment from drug transport to pathogenic mechanism simulation, discuss and consider the relationship between existing technological achievements and technical mechanisms themselves, and look forward to the future development and use of this technology and its subordinate categories.

Keywords: Self-assembly; Medical applications; Non-covalent interaction.

1. Introduction

Self-assembly refers to a technique in which basic building blocks (molecules, nanomaterials, microns or larger-scale substances) spontaneously form an ordered structure [1]. In the process of self-assembly, the basic structural elements are spontaneously organized or clustered into a stable, pre-designed regular geometric appearance under pre-covalent bond interactions. As an emerging technology developed with polymer chemistry, self-assembly is low-cost, fast and easy to manufacture in large scale. Self-assembly systems are also of interest to fundamental researchers because they are governed by unique interactions between weak forces, such as hydrogen bonding, electrostatic interaction, coordination, hydrophilic interaction, host-guest interaction, π-π stacking, van der Waals forces, etc., and are able to form self-assembly products of different structures under the control of such weak interactions [2]. As a technology that can provide a variety of materials with rich characteristic structures in the field of degenerative/fatal diseases, self-assembly products have shown extraordinary charm in the medical field, and a variety of research results have emerged [3]. At the same time, they also hint at their great potential for pertinence: the potential of self-assembly materials that have become an emerging focus has expanded from the traditional drug-loaded micelles to a variety of use scenarios such as pathological structure simulation common in degenerative diseases to clinical treatment mostly used for cancer and tumors. This paper reviews different self-assembly mechanisms and related applications, discusses the relationship between existing self-assembly technologies and medical-related applications, and looks forward to the future development of self-assembly in medical treatment.

2. Self-Assembly Mechanism

2.1. Hydrogen Bond Self-Assembly

Hydrogen bond drive is a self-assembly type that takes proton supply and absence as the basic principle. It relies on the induction of the dipole-dipole from the secondary bond as the driving force to form a bond structure [4]. It is characterized by using the natural geometry and spatial orientation

of hydrogen bond formation to ensure that the bonding site and bonding mode of the copolymers are the same as the pre-designed. Typical hydrogen bonds are formed between the element's nitrogen, oxygen, fluorine and hydrogen.

As a class of bonds that are essentially still charging interactions, hydrogen bonds can interact synergistically with a variety of non-covalent forces to apply an electric field or regulate temperature, etc. For example, it can coordinate with π-π bond. They can also be modulated by simpler external conditions, such as applied electric fields or temperature. In block copolymers used for hydrogen bond self-assembly, some hydrogen atoms at the formation site of block copolymers undergo a series of actions, such as Mass effects, to enable them to interact with polar atoms at specific locations on the block of other monomers. When the concentration in the liquid phase system is sufficient, the polarity of the specific part of the block will be due to the electronegativity difference between atoms, which will further lead to the electron cloud distortion around the participating atoms, generate the dipole-dipole induced force with hydrogen bond nature, and finally form the intermolecular hydrogen bond. This shift can be demonstrated by studying very strong hydrogen bonds, which are more similar to three-centered, four-electron covalent bonds than to the electrostatic nature of interactions such as van der Waals forces.

These hydrogen bonds are directional and saturable, and the final structure can be controlled by controlling the site of hydrogen bond formation through different building blocks. As the strongest bond among non-covalent bonds, hydrogen bond assembly can form a wide variety of different structures and has great potential in many fields [5].

2.1.1. Self-assembly of DNA block copolymers

DNA molecules capable of complementary base pairing and preengineered sequences are particularly unique within the broad category of hydrogen bond self-assembly. These preliminary polymers containing matching single-strand DNA blocks can use the dipole-dipole force between single-strand DNA blocks through complementary base pairing to further self-assemble the nanoscale micelles initially assembled by solvophobia into superstructures.

As a kind of biological macromolecule, the behavior of DNA molecule when it's two hydrogen-bonded single chains is obviously different from that of other organic groups. In the supramolecular self-assembly process of simulated amphiphilic DNA block copolymers reported by Li Xiaoxia et al., this kind of self-assembly has two very clear characteristics: non-disposable hydrogen bonds that are easy to break and different final structures that can be generated by positive and negative connections. Most of the hydrogen bonds formed by specific groups are often disposable, and the hybridization behavior between single DNA strands is thermodynamically reversible, that is, denaturation-renaturation. The effect of this feature has been verified by the unspinning behavior between single DNA strands when the system containing target genes is denaturated and annealed by traditional PCR technology [6].

Therefore, the system temperature can reversibly regulate the self-assembly process of DNA block micelles (i.e., the formation/breaking process of hydrogen bonds), making its conformation easy to be transformed, thus simplifying any structural adjustment for such micelles. The second point is the different structure of the forward and backward connections. Because of complementary base pairing, different DNA single-strand block copolymers self-assemble in different ways to form a variety of structures. The forward complementary chains can bring the solvent-phobic nuclei formed by the initial assembly of copolymers close to each other and then fuse with each other through the hybridization of the double front end. The reverse complementary chain makes the solvent-nuclei far apart, which will lay the foundation for the formation of crosslinked or branched micelles after complete connection [7]. Self-assembly products using DNA base pairing are often easy to degrade and have low toxicity, which makes them have the potential to become responsive release drug-carrying micelles.

2.1.2. Multiple hydrogen bond self-assembly

Among multiple hydrogen bond self-assembly, quadruple hydrogen bond self-assembly has become the most typical self-assembly mechanism, with strong binding force and easy reaction.

In the multi-hydrogen bond self-assembly, quadruple hydrogen bond self-assembly has become the most typical mechanism.

Quadruple hydrogen bonding self-assembly is a type of self-assembly with an even number of hydrogen bonding sites that can form six assembly forms. Because of its specific structure, the products of quadruple hydrogen bond self-assembly have the advantages of including but not limited to strong binding, easy to synthesize, easily modified structure and strong recognition performance, which is an excellent material for making supramolecular [8]. The Quadruple self-assembly of homologous dimers can be divided into four different ways: AADDDAA and DDDAAAA. The typical ones are DADADAD and DDAAAADD. The DADADAD type has a reverse parallel structure. Through interactions between complementary nitrogen and hydrogen atoms, these block copolymers can form a double helix structure at low temperatures similar to the four hydrogen bond connections of DNA double strands. DDAA-AADD, on the other hand, is a hydrogen bond system with stronger bonding forces, and this strength facilitates the formation of highly polymerized polymers in the system. The principle of this system improves the binding force by changing the order of the monomer to reduce the repulsive force of the secondary electrostatic interaction, so that the product is more stable than DADADAD [9].

Quadruple hydrogen bond self-assembly has a special structure and can simulate some life processes that other self-assembly cannot. In the study conducted by Gong Bing et al., the self-assembly of quadruple hydrogen bonds folded by β-like proteins produced a single zipping chain of oligaramide molecules with conformation that could fully simulate the behavior of amino acid polypeptide chains. It can be seen that quadruple hydrogen bond self-assembly will play a potentially important role in exploring the formation mechanism of many major protein-related diseases as a model of protein conformation in future studies [10].

2.2. Crystallization Driving Force

As a non-bonding interaction, crystallization has been used in the preparation of organic liquid crystal materials for its properties of eventually producing stable rod-like micelles. In the process of crystallization in liquid phase environment, the polar crystalline blocks will show weak electrical properties of each part of the block due to the shift of shared electron pairs, and the more symmetrical overall structure makes the induction effect more concentrated. The combined force of the two produces a strong driving force, which acts on these effective functional groups to make the participating block copolymers pack in a specific direction. The initial rod-like micellar structure is formed by the close arrangement around the seed. At this point, adding the same polymer dissolved in good solvent to increase its concentration will speed up further active polymerization: the higher concentration is easy to induce small potential changes in the crystallizable region, which is easier to match the active site at the end of the primary micellar structure and achieve the purpose of lengthening the micellar polymer. This unique crystallinity is largely temperature-dependent and, as a kinetically controlled process, various structures can be generated by controlling the crystallisation pathway [11].

Its actual preparation controllability is exemplified by the final product length distribution observed by Winnik and Mannersin a study of block copolymers using polyferrocenylsilane (PFS) as crystallizable segments [12]. As the amount of copolymer containing PFS increased gradually, the rod-like micelles of polymer in the solution also increased, but it always maintained a positive correlation with the amount of secondary feeding. This property makes the length of the final product driven by this type of crystallization controllable, and the stacking effect only occurs between the same block, which makes it possible to increase the regularity of aggregates by adding other side blocks [13].

In 2020, Tao Daliao et al. investigated seed-growing self-assembly by self-seeding with live crystal bundles (live CDsas) [14]. In this study, a homogeneous fibrous micellar 5-b-P2VP44 used as an oral poliomyelitis vaccine was used as a crystal seeding system for reaction, during which the reaction was characterized by transmission electron microscopy. The characterization results showed that obvious growth occurred in the initial sample crystal during the reaction process, and both ends of the gradually formed chain structure still had the activity that could continue to extend. This crystal-driven self-assembly structure can be used to prepare a variety of functional liquid crystalline micelles by adding more different side chains, functional ions or using different processes for additional adjustment and modification, and then be applied in optical anti-counterfeiting and biological staining. For example, in a strategy reported by Qin Lang et al. to replace traditional fluorescent dyes with structural colors of liquid crystal photonic crystals, quantum dots are added to ordinary liquid crystal micelles to prepare two-color liquid crystal microspheres that can reflect different colors under different colors of light and can be used as anti-counterfeiting inks [15].

2.3. Charge Transfer

Through the interaction of electron donor and electron acceptor (electron-rich units and electron-deficient units), the preliminary polymer without liquid crystal properties is further self-assembled into a columnar superstructure, while exhibiting liquid crystal properties due to the presence of electric charge.

In 2005, Chao Wang et al. used 1-[11-oxy-11 - (pyrene - 1-methoxy) -undecylalkyl] pyridine bromide (PYR) containing an electron-rich pyrene group as an electron donor and two electron-deficient dinitrobenzene units in ethane -1, 2-diyl bis (3, 5-dinitrobenzoate) (DNB) as an electron acceptor [16]. A series of supramolecular polymeric micelles with an adjusted structure were successfully co-assembled. The results were characterized by solubility changes, absorption spectrum and confocal scanning microscopy, which confirmed that charge transfer did occur during the self-assembly of PYR and DNB. Clear tubular micelles were observed, which are the final product of typical self-assembly of charge transfer.

This type of technology also has medical potential. In the studies of Pablo Escribá et al., many cellular functions of the plasma membrane occur in or around the membrane, suggesting that changes in membrane composition and structure may be related to the normal function of cells [17]. A large number of different kinds of lipids can be found in the plasma membrane, some of which have electron-rich groups that can interact with charge-deficient amino acids in proteins. These polar structures are known as polar heads, and lipid structures in the plasma membrane have potentially important implications for cell signaling [18]. This response regulation in turn is very similar to the conformational conversion using electron-rich groups and electron-deficient ions in charge transfer self-assembly. This suggests the feasibility of charge transfer assembly to prepare deformable biofilm-like structures, and this feasibility offers the prospect of charge-transfer self-assembly products that can be converted from tubes to vesicles as specific drug-carrying micelles.

2.3.1. Ion interaction assembly

Using charge position transferrings, ion interaction assembly is a freer type of self-assembly than the typical charge transfer self-assembly. At the connection sites of the two heteromonomers, ions of specific structure are combined with each other, so that the two associated monomers enter the state of relatively electron rich and relatively electron deficient respectively. When the two kinds of raw materials are placed in a specific concentration of organic solvent, the ionic end will be inclined to match the other ionic end due to the interaction of the non-polar solvent, so as to complete the assembly. Based on this principle, different bonding sites can produce different structures. For example, side chain interaction can produce network or single arranged side chain, while main chain interaction is more inclined to produce products with linear structure. Its unique properties are similar to those of typical charge transfer self-assembly.

This technique can simplify the preparation of non-polar organic compounds. For example, using ion self-assembly can simplify the polymerization process of peptides and proteins. In the study of

Zhang Shuguang et al. on peptide and protein, sites of ions, semiconductors and metals were researched with bonding and coating to prepare raw materials [19]. Then block copolymers were obtained from these raw materials through self-assembly. Experiments have shown that different coating materials and different ground and unblocked sites can plant a wide variety of state structures, and these platform structures can be used to prepare a large number of products with important medical applications, from vascular stents to nanostructured tubes. At the same time, the synergistic self-assembly of ions and peptides is similar to that of amyloidosis in many protein-conformation diseases, thus providing new opportunities for revealing specific pathological phenomena [20].

2.4. Hydrophilic and Hydrophobic Self-Assembly

As a kind of self-assembly mechanism with good dismemberability and the ability to add multiple groups to achieve different drug release responses, hydrophilic and hydrophobic self-assembly is the most commonly used self-assembly for the preparation of drug-carrying micelles? Using the recognition of hydrophilic and hydrophobic molecules, the pre-synthesized amphiphilic block copolymer molecules will automatically aggregate into spherical micellar structures with hydrophilic end outward and hydrophobic end inward in aqueous solution system. By supplementing the side chains, columnar, network and tubular micelles can also be formed. Since such self-assembled materials have chain segments with different affinity, this leads to the thermodynamic incompatibility of the block copolymer itself, which leads to the short chain segment breaking from the long chain segment to form a microphase with regular structure and uniform dispersion, which results in microphase separation, and finally produces self-assembled products with different structure [21,22].

This amphiphilic copolymer, due to its outstanding degradation ability and lipid-water separation ability, has the advantages of increasing the solubility of drugs, ensuring the effectiveness of targeting lesions and reducing the harm of drugs to other organs and so on, which is suitable to be used as drug carrier in vivo. These advantages are lack of traditional tumor targeting drugs, which are prone to cause adverse reactions due to their cytotoxicity and damage to multiple organs and are often composed of fat-soluble substances and glycoproteins. The use of amphiphilic drug-carrying micelles with response groups added to achieve targeted drug release can ensure the effectiveness of the drug and a certain cell transfection rate. At the same time, the degradation products are non-toxic (or tumor toxic), which can significantly reduce the occurrence of side effects while ensuring the therapeutic effect [23].

For example, in the study of Hongli Zhou et al., specific self-assembled drug-loaded micelles prepared by adding Golgi targeted blocks and characterized by the drug release process using fluorescence tracing and electron microscopy [24]. After such drug-loaded micelles are actively transported into cells through energy-dependent pathways, their blocks targeting the Golgi apparatus can accurately regulate the disintegration of vesicle micelles at the Golgi apparatus. After disintegration, the micelles release fat-soluble drugs whose killing activity is well preserved by the hydrophobic end, and complete the killing of the Golgi apparatus, an organelle that undertakes a large number of important protein packaging work [25]. Precise interference with Golgi apparatus can minimize side effects while ensuring the blocking effect of tumor cell metastasis; At the same time, this drug-loaded micelle also exhibits a higher transfection rate as described above, increasing the clearance and inhibition efficiency of lesion cells in a single administration.

2.5. Electrostatic Actuated Self-Assembly

Electrostatic self-assembly refers to the technique of producing multilayer ultra-thin films by alternately depositing polyelectrolytes with opposite charges in an aqueous solution onto a sheet of adherable material. The core principle is the interaction of electrostatic repulsion and electrostatic attraction between polar molecules, which is commonly seen in ionic surfactants and ionic liquids. Because the forces are strong enough, the self-assembly can be carried out in simple liquid-phase systems, thus eliminating the need for harsh reaction conditions or sophisticated reaction equipment, greatly reducing the difficulty of synthesis.

Electrostatic self-assembly technology with the above advantages has been developed greatly in recent years. During a period of development, such driving force extended from the initial electrostatic force to a variety of non-covalent forces, such as hydrophilic and hydrophobic forces. The components used for assembly also extended from traditional polyelectrolytes to non-living small molecules with multi-functional groups, colloids and living DNA, proteins, etc. [26]. At the same time, by using multi-layer self-assembly technology, various functional materials can be assembled outside the colloidal particles, and new nano- or micron-level materials with a variety of structures from core shell to hollow and multiple response modes from optical to redox can be obtained [27].

In addition to forming massive micelles, a variety of liquid crystals and hydrogels, the structure of DNA surfactant complexes has a number of unique features such as a specific distribution of electrical conductivity and remarkable dielectric properties [28]. These characteristics enable them to be used as electron blockers and hole transport layers in lasers and nonlinear semiconductor optics, such as Light Emitting Diode (LED) or photovoltaic devices. The unique fluorescence signal amplification effect reported in Yutakak et al.'s study also stems from the dielectric properties of this complex [29]. At the same time, due to good biocompatibility and degradability, DNA surfactant complexes also have excellent gene and drug delivery capabilities. These two advantages indicate that if the block copolymer material can be properly prepared and then reasonably self-assembled by electrostatic driving, the DNA surfactant complex as the product can form a new kind of medically available liquid crystal materials, such as harmless, strong color tracer reagents.

3. Conclusion

In conclusion, the achievements and potential of self-assembly technology in the medical field cover many fields from structural simulation of pathogenic mechanism research, cell tracing to clinical practical treatment of drug target vectors and drug supply systems, etc., and almost complete systems from research to diagnosis and treatment. As a class of technologies that consume less energy and are generally simpler to prepare, self-assembly that relies on different basic mechanisms and forces is becoming a new approach to addressing medical issues. In the future, more in-depth research should be carried out on existing and possible self-assembly technologies, so that the existing technical deficiencies, such as insufficient strength, low purity, and long preparation cycle, can be solved. At the same time, the preparation of new self-assembled medical products should fully consider their degradability, toxicity and preparation difficulty, so as to maximize the benefits of protecting the environment and providing new materials, so as to achieve sustainable development and excellent medical effects.

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