The Impact of Self-Assembly in Medicine and Pharmacology

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Abstract: The concept, molecular self-assembly, especially considering molecule-molecule interaction as an information-processing phenomenon, have a profoundly novel effect on thoughts and efforts related to Medicine and Pharmacology. This new style of thinking is still too novice to be used solely and independently for explanation of disease mechanisms and appropriate treatment strategies. However it calls for a range of new researches based on new predictions about disease mechanisms (especially Autoimmune diseases, Endocrinopathies, and Neoplasms) and relevant treatment strategies (superstructural drugs).

Keywords: Self-assembly, non-covalent interaction, cell membrane, autoimmunity, anti-viral drugs, sickle cell anemia.

1. INTRODUCTION

The concept of molecular self-assembly deals with chemical, physical, and biological features of chemical species held together and organized by means of intermolecular (noncovalent) binding interactions [1]. In other words self-assembly is a "bottom up approach" in which materials are assembled molecule by molecule to produce supra-molecular structures for appropriate functions [2].

The underlying mechanisms of intermolecular binding interactions include van der Waals interaction between hydrophobic regions (π-π, alkyl-π, alkyl-alkyl), electrostatic interactions (cationic-anionic), hydrogen bonding (donor-acceptor), and complementarity of molecules [3].

These rules of interaction can be unified in terms of information. In other words, instead of considering preconditions based on known phenomena mentioned above for determination of the pattern of self-assembly, we can consider a unified expression of molecular information. Hence by a generalization of our information-centered attitude to biomolecules like DNA and RNA, it is expression of molecular information in a multiple-code programmed system that determines the pattern of self-organization [4]. Fig. (**1**)

Two approaches can be derived from this information-based point of view: first, prediction of characteristics of the self-assembled superstructure of a particular molecule; second, prediction of a chemical formula that can make a particular superstructure with a particular function due to self-assembly.

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1.1 Prediction of Characteristics of the Self-Assembled Superstructure of a Particular Molecule

Thanks to rapid pace of digital computer technology in these decades, new theoretical methodologies are developing to predict characteristics of the self-assembled superstructure of a molecule by extending its single-molecule (gas-phase) calculations to a level at which condensed-phase effects can be calculated [5]. However inclusion of a complete level of present theories (e.g. converging solutions of Schrödinger equations) is still extremely difficult even for chemical systems that appear very simple [6].

One key issue that is not going to be addressed by further advancement in computer technology is the type of solvation model to be employed for the prediction of self-assembled superstructures. In fact water (or any other solvent) has a central role in giving a special order to self-assembling molecules. The main dilemma is whether water (or any other solvent) is considered only as a media, which affects intermolecular interactions of the superstructure (e.g. through its high dielectric constant) or its very molecules take part as an integral component of the resultant superstructure [7]. The ability to predict characteristics of self-assembled superstructures through computer simulation can be further strengthened by attempts aimed at setting theoretical layouts for this purpose [8].

1.2 Prediction of a Chemical Formula that can Make a Particular Superstructure with a Particular Function After Self-Assembly

Considering the difficulties and challenges in predicting self-assembled superstructures of a molecule from its formula, our present knowledge seems quite limited to predict the opposite, i.e. a formula that will give a particular superstructure. This by no means indicates that the efforts being performed today are all based on utter serendipity. In fact there is a synergistic effect between serendipity and rational design in supramolecular chemistry and there are a

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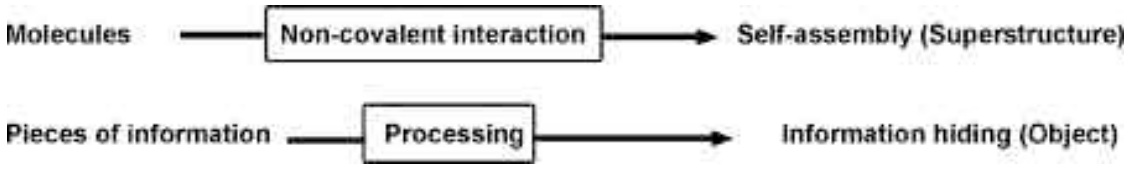

Fig. (1). The analogy between molecule-molecule interaction and information processing.

number of models, each focusing on rather limited chemical systems that give correct answers in an efficient manner [9]. However none of them possess the necessary theoretical rigor to be used in a wide variety of chemical systems.

1.3 The Message from Self-Assembly to BIOMEDICAL Sciences

Apart from the new way that the concept of self-assembly has provided to develop both theories and methods for better prediction as well as understanding of superstructures, the concept of self-assembly itself has much to offer to biology and medicine with its new point of view. From this point of view, biological systems make several functional superstructures like lysosomes, reticulum endoplasmic, Golgi apparatus, mitochondria, and different kinds of supportive and functional filaments all based on self-assembly of biological molecules. Therefore every living system (living cell) must have a corporate mechanism to exert full control on this phenomenon and many if not most diseases must be regarded as an abnormal assembly of molecules. The fact that biological systems do control self-assembly is supported by observations that indicate an asymmetric distribution of membrane lipids in cells [10]. In fact the main duty of many proteins of still unknown function may be predisposition towards desired patterns of self-assembly or deterrence of undesired ones. Likewise the underlying mechanism of many diseases can be regarded as an abnormal pattern of self-assembly and new treatment strategies can be laid out. As the best way of clarification we will bring examples of present challenges of biomedical sciences and then show the new horizons that the concept of self-assembly opens to tackle them.

2. PROTEINS OF UNKNOWN FUNCTIONS

The determination of protein functions is one of the most challenging problems of the post-genomic era. The sequencing of entire genomes and the possibility of accessing co-expression patterns of genes has moved the attention from the study of single proteins or small complexes to that of the entire proteome [11]. Based on the point of view of supramolecular chemistry there must be proteins whose responsibility is controlling self-assembly of other small biomolecules. In other words, like enzymes that control the composition of biomolecules by interference in covalent bonds, there must be proteins whose responsibility is the achievement of desired superstructures by exerting control on noncovalent bonds. There are many proteins known to be associated with particular cellular functions but their role is still unknown. Interestingly most of these proteins like GRIP [12], COP II [13], Trs 20p [14], Yos9 [15], ADP ribosylation factor proteins [16], GP2 [17], TRACP [18], Synaptobrevins [19], etc. are involved in functions such as intracellular transportation, exocrine or endocrine secretion, endocytosis or exocytosis in which reassembly of phospholipid molecules forms vacuoles with especial contents. It is quite likely that at least some of these proteins deal with self-assembly patterns of phospholipid membranes.

Furthermore there are reports of the effect of specific RNAs on membrane permeability, an unexpected role for nucleic acids unless we take the possibility of their role in directing self-assembly of membrane components into consideration [20].

3. PATHOPHYSIOLOGY OF DISEASES

It is very likely that the phenomenon of self-assembly plays a key role in a variety of diseases. Here we will discuss the most likely ones in order to highlight how this new conceptualization can predispose research activities towards new goals.

3.1 Infectious Diseases

There is a huge amount of information about the various microbial and host molecules that contribute to the process of infection and disease. Among several microbial agents viruses, the simplest ones are the most appropriate for our purpose, that is to show how a self-assembly oriented mind would approach present medical challenges. Any virus consists of a nucleic acid surrounded by proteins. These proteins can all be of one single type or of several types. These proteins form a shell called a capsid which encloses nucleic acid. The capsid is simply a superstructure made up of self-assembly of several protein molecules called capsomers.

Generally there are three stages for viral infection and hence progression of the viral disease: virus interaction with and entrance into the cell, replication of viral ribonucleic acid and synthesis of capsomers, and finally self-assembly of capsomers together with viral nucleic acid to form several more viruses [21]. Although self-assembly plays a key role in all three stages, its role in the third stage is not only more conspicuous but also simple enough to be subjected to research from the viewpoint of supramolecular chemistry. Here rise some severely under-researched questions that need to be carefully investigated in order to make breakthroughs both in understanding of the basis of viral disease and in developing more effective treatment strategies. Which of the defensive mechanisms of human body is aimed at disruption of proteins/lipid self-assembly in general and capsomers in particular? Do interphones disturb general facilities of cellular proteome self-assembly? What would be a rational molecular design for antiviral drugs that

can easily enter cells but specifically interfere with capsomer self-assembly? Research tools like DSC (Differential Scanning Calorimetery), CD (Circular Dichroism) [22], NOESY (Nuclear overhauser Spectroscopy), AFM (Atomic Force Microscopy), Fluorescence Microscopy, Small Angle X-ray Crystallography [23], etc. that chemists usually use to characterize superstructures can be employed to begin the investigation of the above questions. Mass spectrometric methods have been already used for rapid and quantitative characterization of proteomes [24]. Of course there will be demands for development of new research tools with necessary adjustments to enable them to characterize the dynamics of molecular self-assembly in-vivo. Present efforts to develop antiviral drugs are mainly targeting enzymes, macromolecules and organelles that are responsible for synthesis of viral nucleic acid or proteins. Viruses employ host cell machinery for these purposes. Therefore developing antiviral drugs using these strategies is very difficult because of concomitant toxic effect on all normal cells. Most of the antiviral drugs available today (Ribavirin, Acyclovir, Ganciclovir, Vidarabin, Foscarnet, Cidofovir, etc). are based on interference with synthesis processes of viral nucleic acids or proteins and possess more serious side effects than Amantadine and Rimantadine which interfere with capsomers, a fact that implies possibilities for and advantages of new drugs targeting the assembly of viral capsomers [25].

3.2 Non-infectious Disease

Perhaps the most conspicuous example of human non-infectious disease in which self-assembly plays a central role is Sickle Cell Anemia. In this disease, the sixth amino acid of beta-globin polypeptide chains, which is normally a Glutamate, is replaced with Valine. The abnormal hemoglobin (HbS) has a tendency to self-assemble, when deoxygenated, to form a gelatinous network of fibers. These fibers stiffen the erythrocyte membrane and cause a total deformation of the erythrocyte from its normal biconcave shape to a sickle shape. These sickle cells cannot traverse small capillaries, and this causes both red cell early destruction (Hemolytic Anemia) and several microvascular occlusive consequences. Due to these microvascular occlusions patients not only suffer from frequent pain crises but also a gradual decline in kidney and pulmonary functions that shortens their life span [26].

Generally the most effective treatment for adults with Sickle Cell Anemia is hydroxyurea [27]. Although a number of mechanisms have been mentioned like increase of fetal haemoglobin (HbF), red cell hydration, reduction of red cell-vascular wall adherence, and suppression of granulocyte and reticulocyte count, its indirect effect on interference with the usual pattern of self-assembly of HbS through increasing the level of HbF is likely to be the most important one. It has been shown that addition of only 1% methanol to CCl4 led to a decrease in association constant by a factor of 25 [28].

The strategy of interference with the self-assembly pattern of HbS demands a thorough evaluation of HbS self-assembly mechanisms [29] and development of an appropriate small molecule drug that will directly interfere with HbS assembly [30].

There are other diseases associated with abnormalities in the function of biologic membranes like minimal change nephropathy (MCN) and Acute Respiratory Distress Syndrome (ARDS) whose pathophysiology hasn't been completely understood [31]. Verification of self-assembly patterns of these membranes can add more clues for ascertaining their pathophysiology. For example, DSC (Differential Scanning Calorimetery) profiles of sample tissues from patients with MCN or ARDS may not only shed light on some gaps in understanding of their underlying mechanisms but also prove to be a valuable diagnostic method [32] Fig. (**2**).

3.3. Autoimmune Disease, Endocrinopathies, and Cancer

How the immune system distinguishes self from non-self has not been completely understood, although there has been

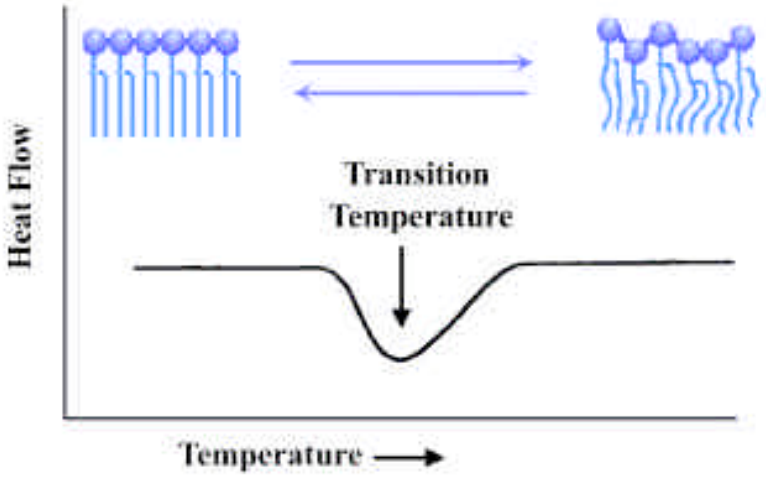

Fig. (2). Diseases with abnormal permeability of a biologic surface (like Minimal change nephropathy and Acute respiratory syndrome) are likely to show characteristic patterns in DSC (Differential Scanning Calorimetery) of a very small tissue sample (less than 50 mg).

much progress during past decades [33]. Whatever those mechanisms are, the fundamental basis of any autoimmune disease is regarded to be non-self assumption of a previously self-assumed antigen. Several complex mechanisms are considered including superantigenic stimulation, epitop spreading, alteration of self antigen, loss of immunologic privilege, cytokin production, increased B cell or T cell function, altered immunoregulation, etc [34]. The concept of self-assembly can tell us a neat and simple story that may turn out to be the fundamental phenomenon behind all those mechanisms. A mixture of two different compounds can make different superstructural features depending on pH, temperature, and their molar ratio [35]. Theoretically when the molar ratio of biomolecules (namely proteins) in a cell changes beyond a specific limit, new superstructures can emerge that are likely to be regarded as unfamiliar antigens. Activation or inactivation of specific metabolic pathways controlled by endocrine systems by adding or removing small functional groups can alter the self-assembly pattern of proteins and further explain the close link between endocrinopathies and autoimmune diseases [36]. In addition, these new protein superstructures may exert an abnormal regulatory feedback on genomes to launch a vicious cycle of further imbalance in gene activity and further changes in superstructural features of intracellular proteomes that eventually end in a cancerous state of totally unregulated cellular activity [37]. Therefore abnormal patterns of self-assembly can be regarded as culprits responsible for autoimmune disease and endocrinopathies as well as for cancer, an important possibility that demands appropriate investigations to determine their actual role.

4. NEW PHARMACEUTICAL SYSTEMS

The concept of self-assembly has inspired drug scientists engaged in research surrounding particulate drug delivery systems for targeting or for sustained release [38]. For targeting, surfactant vesicles are classically loaded with a drug and their surfaces are modified by polymers like PEG (Polyethylenglycol) in a way that protects them from opsonization and macrophageal uptake. Then appropriate ligands like viral proteins, carbohydrates, glycoproteins, hormones, etc. are attached to their surfaces [39]. There are also many other superstructures like ultrathin microcapsules [40], macromolecular nanoshells [41], icosahedrals [42], or even fibers [43] that can either be loaded or embedded with the drug for special targeting or sustained release.

However, the concepts of self-assembly and supramolecular chemistry call for some completely new researches dealing with therapeutic superstructures. In other words there is a vastly under-researched area of superstructural pharmaceuticals in which a specific non-covalent complex of molecules is supposed to play a therapeutic role.

A very natural example of therapeutic superstructures is HDL. HDL is a lipoprotein synthesized by the liver and comprised of non-covalent complexation of several molecules including phospholipids, cholesterols (esterified and free), and proteins that is considered an important anti-risk factor for Atherosclevosis (the main cause of infarction and stroke). Considering each single molecule of all molecules that have formed an HDL superstructure, no one of them singly has an anti-risk factor effect (in fact some of them, like cholesterol, are considered an important risk factor per se) but their specific complexation in the form of HDL performs an important therapeutic effect that inhibits progression of atherosclerotic plaques.

Single molecule drugs like antibiotics are a perfect treatment for most infectious diseases but they have not produced successful solutions for non-infectious diseases like cancer and autoimmune disease. Superstructural drugs (a particular complexation of several molecules) may provide a breakthrough in the treatment of many non-infectious diseases. Inspired by these concepts, we have designed and successfully synthesized a lipopeptide molecule (lipid 1) containing a nitrostyrene moiety whose anticancer effect is already known through its anti-telomerase activity [44-45]. The molecule was logically designed in a way that possesses many non-covalent interaction sites for either self-assembly or complexation with natural superstructures like lipoproteins. As shown in Fig. (**3**) the molecule is composed of L-glutamide moiety as a peptide source, double long-chain alkyl groups as lipophilic parts (to help self assembly with hydrophobic moieties of lipoproteins), a nitrostyrene derived group which is supposed to exhibit anti-cancer activity, and a spacer derived from Beta-Alanin to decouple the motion of the nitrostyrene moiety from the rest of the molecule.

It is reported that double chain alkylated L-glutamide moiety is a key unit to create highly-oriented aggregates [46-49]. By changing the spacer, Lipid 1 can be expanded to a class of self-assembling molecules of potential anti-cancer effect. Fig. (**4**) shows another similar compound (lipid 2) where a phosphate-derived spacer is supposed to change the solubility of the molecule.

By employing computational methods based on present theories available today it is quite possible to gradually parameterize a forcefield like OPLS that could eventually deliver molecular simulations consistent with actual experimental findings regarding self assembly and complexation of the above mentioned compounds. After catching customized theories and their related computational tools we can rapidly and reliably probe a library of molecules

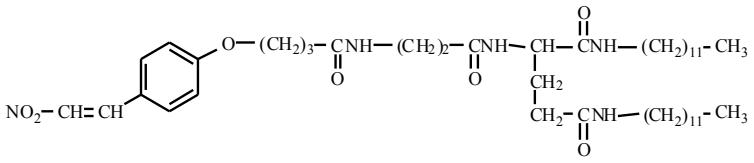

Fig. (3). Lipid 1 composed of L-glutamide moiety, double long-chain alkyl groups, and a nitrostyrene derived Head.

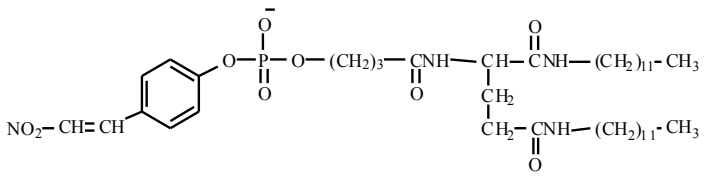

Fig. (4). Lipid 2 composed of L-glutamide moiety, double long-chain alkyl groups, and a nitrostyrene derived Head with a phosphate derived spacer to change the solubility.

close to these structures in order to find appropriate structures for our goal (a superstructural anticancer drug) and some other important applications like development of chirally ordered stationary superstructures for chiral separation. We confess that the credibility of this recent fragment of the paper is very limited nonetheless we present it in order to clarify by a practical example the synergistic effect between serendipity and rational design in supramolecular chemistry and kinds of research works derives from its conceptualization.

CONCLUSION

The concepts of self-assembly and supramolecular chemistry have much to offer to research in the fields of medicine and biology. There are many fundamentally new areas of research inspired by this concept as outlined throughout this article and it is likely that at least some if not many of problems in diagnosis and treatment of patients can be solved through this new approach.

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