CHAPTER 1

Engineering Materials from the Bottom Up – Overview

Rudy J. Koopmans[1,*] and **Anton P.J. Middelberg**[2]

Contents

1. INTRODUCTION

Self-assembly is a concept receiving significant attention in a variety of research fields ranging from biology to cybernetics to social sciences. Despite its seemingly self-explanatory simplicity, implying a level of spontaneous assembly or ordering beyond the individual, composing molecules, this process is difficult to formally express in mathematical terms. Still the phenomenon in the natural sciences where atoms and molecules aggregate into higher order structures at various time and length scales is an observable reality. It offers significant potential for designing highly functional and diverse materials once the underlying mechanisms are understood.

Self-organization is often considered as synonymous to self-assembly and for many practical purposes indistinct (Anderson, 2002). However, self-assembly may be taken as the simple collection and aggregation of components into a confined entity, while self-organization can be considered as spontaneous but information-directed generation of organized functional

[1] PolyUrethane R&D, Freienbach, Dow Europe GmbH., Switzerland

[2] The University of Queensland, Centre for Biomolecular Engineering, St Lucia, Australia

[*] Corresponding author.
E-mail address: rjkoopmans@dow.com

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structures in equilibrium conditions (Lehn, 2002). For the purpose of these papers, self-assembly will be used to mean any form of organization that comes about through forces directing hierarchical structure formation from the molecular level up.

At the macroscale, such organized functional structures manifest as physical substances, that is, materials that form the basis for engineer-specified products and applications. For example, wool is a material that can be taken to make yarn for use in cloth making. At the molecular level, wool is a keratin composed of protein molecules with very specific amino acid sequences that have been spontaneously ordered into fibrous structures allowing the mechanical operation of yarn forming (Block, 1939; Corfield and Robson, 1955). However, in contrast to conventional use of either natural or synthetic materials, where a transformation process is needed to shape the product and application, for peptides and proteins the boundary conditions (e.g. temperature, pH, and solvent) direct the aggregation of such molecules into organized structures at various length and time scales, and with differing orders of complexity. These structures are the functional intermediates or potential end products that can be applied in multiple applications for different markets.

The following papers focus mainly on various aspects associated with the self-assembly of peptides. Peptides are relatively short sequences of amino acids, typically less than 50. The limited number of residues brings simplicity but still allows for sufficient differentiation to study self-assembly in its various details. The compositional freedom of the primary molecule allows for a sufficiently rich hierarchical structure creation through aggregation of individual peptides into supramolecular constructs resulting in interesting materials. This chapter looks into relevant patent literature as a reflection of the state of the art of technology in peptide self-assembly.

2. HISTORICAL CONTEXT

Materials define the face of society. Initially, since prehistoric times – and to this day – materials were selected amongst those available in nature. These included, besides stones and metals, basic ingredients obtained from plants, crops, and animals in the form of, for example, wood, flax, wool, and leather. Materials use was a skills-based activity perfected by artists and guild-members handed from one generation to the next.

Not until the late 18th and beginning of the 19th century, commencing with the Industrial Revolution and with an increasing need for natural products, did the search for more and other materials begin. Empire building, commerce, and population growth stimulated investigations into the use of more novel natural products such as rubber and cellulose. Entrepreneurialism combined with scientific understanding and discovery gave rise to new

materials, although initially still based on nature's feedstock such as modified cellulose and coal tar. The prevailing scientific paradigm was analysis, knowing that organic substances obtained from plants and animals could not be created in the laboratory; they could only be isolated and examined, and perhaps broken down into simpler substances. The reverse, the production of the complex from the simple, was, at a scientific level, beyond human competence. It was the work of a creator, or of a life force operating within living systems (Service, 2005). A little more than a half-century later, Archibald Scott Couper (1858) determined the tetra-valence of the carbon atom, and Friedrich August Kekulé (1865) published his paper on the hexagonal ring of the benzene molecule. By now a language for nanoscale objects, that is, atoms and molecules, had been developed that assigned a separate name and compositional diagram to every inorganic and organic compound including composition of natural materials. Suddenly, the science of chemistry became almost unique among the sciences in creating much of the world of materials.

By the late 19th and early 20th century, chemists discovered and developed a new class of synthetically made materials now known as plastics. In the second half of the 20th century, the perceived abundance of petroleum as cheap feedstock led to an exponential growth through process engineering scale up, establishing plastics as an unsurpassed multipurpose material.

Over these past 150 years, natural and synthetic materials use became much more founded on knowledge of the underlying physical principles. The evermore sophisticated analytical tools that could probe the molecular world provided insight into the hierarchies of organization from the atoms and molecules up to the macroscopic scale of materials. It became clear that there is more to materials than just innovative molecular synthesis.

Although order and the associated structure in a material have long been recognized, it is only in the last few decades that the process of ordering, that is, organizing components and the associated hierarchical structure formation, has received much attention. It is acknowledged that structure can be built up from the nanometer (10^{-9} m) to the macroscopic scale of everyday experience, that is, "from the bottom up" (Whitesides, 2003; Whitesides and Boncheva, 2002). Materials and devices can be built from molecular components that *organize themselves* chemically using principles of molecular recognition. Far more precision and functionality can be achieved than with a "top down" assembly approach where nano-objects are constructed from larger entities without atomic-level control.

The essential realization in this spontaneous ordering process is the importance of noncovalent bonding interaction between molecules, that is, supramolecular chemistry. These conformation-specific interactions are governed by weak forces including hydrogen bonding, metal coordination, van der Waals forces, pi–pi interactions, and electrostatic Coulombic effects. The cooperative action of multiple noncovalent interaction forces is precisely the path nature takes to produce shape and form.

Already in the early 20th century, Nobel Laureate Emil Fischer recognized (Kunz, 2002) in work associated with enzyme-substrate interactions and peptides the importance of supramolecular chemistry. Today, science still tries to understand the fundamental rules of structure and functionality formation in order for technology to apply them. The challenges are legion requiring multidisciplinary approaches as their complexity is substantial. Furthermore, a reductionist approach to materials discovery and invention, as simply defined by the nature of the elemental components, does not apply. A more holistic approach is required for recognizing the importance of environment and boundary conditions on intramolecular and intermolecular forces (Hyde et al., 1997) for structure and functionality development that shape material applications. It indicates the need for appreciating what mathematics, physics, and chemistry have to offer biochemistry, biology, and life processes for understanding and ultimately engineering novel materials and their use.

The search for connection between shape, structure, and function was posed by D'Arcy Thompson in his book *On Growth and Form* first published in 1917 (Thompson, 1992). His book lets one reflect that complex forms or shapes in nature are not solely a consequence of Darwinian natural selection. They can be purely explained on the basis of geometry, physics, mathematics, and engineering and are guided by underlying physicochemical principles that drive organization of molecules to higher order structures (Ball, 1999, 2004).

These principles are best recognized when studying relatively simple molecular systems that have an ability to exploit weak interactions to create structure. Among many, peptides are the perfect choice for such studies considering their versatility in make up given the 20+ natural and synthetic amino acid residues, and their functional diversity. In addition, the amino acid sequence of the primary structure combined with the ability of forming secondary β-sheet or α-helix structures provide substantial room for the creation of hierarchical structures based on weak intermolecular forces, mainly hydrogen bonds. A limited sequence of residues also prevents additional complication from tertiary and quaternary structures as seen with proteins.

In the following papers specifically for peptides, and without claiming comprehensiveness, several research aspects are presented in the drive for innovative and sustainable natural materials.

3. SELF-ASSEMBLING PEPTIDE LITERATURE

Quickly browsing Internet resources with the keyword self-organization (or self-assembly, including the *s* and *z* versions) provides at least five hundred thousand links of varied relevance and quality. Searching the scientific literature for the same topics still provides about a hundred thousands "hits." Specifying further to peptides and proteins reduces the number of

papers to a significant but more manageable number of a few thousand. Irrespective of the content of these papers, it is clear there continues to be a significant number of publications on the subject.

As much of the more relevant scientific literature will be referenced in the subsequent papers, it is worthwhile examining the associated patent literature on peptide self-assembly. Patents are pursued to protect intellectual capital with the aim of developing science and technology into a business proposition. In the last 5 years, about 100 patents have been granted in the peptide self-assembly field with focus on applications in the medical and pharmaceutical markets.

High on the list of applications claimed are methods and methodologies to prepare and use specific peptide compositions for tissue engineering, drug delivery either via vesicles or hydrogels, and films and membranes in various medical and electronic applications. The novelty of the inventions is closely linked to the self-assembly capacity of the peptides, being a reversible mechanism, controlled by the primary structure and the triggers used for assembly and disassembly. In addition, the primary peptide structure composition and functionality are claimed as options for innovative and highly specific usage in the medical, pharmaceutical, and cosmetic fields.

Professor Sam Stupp and coworkers at NorthWestern University (USA) hold an important patent portfolio aiming at exploiting self-assembling peptides for various medicinal purposes. The approach takes advantage of peptides composed of a hydrophobic alkyl tail and a β-sheet forming hydrophilic head that assembles into a cylindrical fiber with the hydrophilic peptide facing the solvent. The peptide amphiphiles (e.g., palmitoyl—AAAAGGGEIKVAV—COOH or branched versions) can form nanofibers for uses as scaffolds for tissue growth or drug delivery (Hsu and Stupp, 2008; Mata and Stupp, 2008; Stupp and Guler, 2005; Stupp et al., 2003a, b, c, d). These systems can be turned into 2D and 3D structures and possibly cross-linked, forming microtextures with specific function of enhancing neuron growth whether or not combined with actives delivery (Stupp and Kessler, 2006; Stupp et al., 2004).

Although no self-assembly attributes are claimed, similar peptide structures were developed by Sederma SA (France) and Procter & Gamble Inc. (USA). For example, palmitoyl-pentapeptides (pal-KTTKS) are used in cosmetics as skin rejuvenation ingredients claiming no skin irritation as compared to retinol-based ones (Osborne et al., 2005).

Other research groups aim at the same applications of tissue engineering and drug delivery using somewhat different approaches, changing the nature of the primary structure, the methodology of self-assembly, the tissue types targeted, or the actives delivered (Ellis-Behnke et al., 2005; Genove et al., 2005a, b; Horii et al., 2008).

The primary peptide structure brings options for sequence tailoring that generate specific functionalities and self-assembly characteristics. Examples are: amphiphilic dendritic dipeptides making helical pores (Percec, 2006);

cyclic homodetic peptides with repeating D–L chirality for assembling and disassembling molecular tubes that can act as channels to transport ions or glucose across lipid bilayers (Ghadiri, 2003); self-assembling β-sheet – barrel channel – forming peptides for actives delivery (Aggeli et al., 2003).

The primary peptide structure offers sufficient functional groups from selected amino acids that can be linked to actives, which in combination with the self-assembly capacity provide various routes to deliver them to specific receptors or locations difficult to reach (Bhatia et al., 2007; Joyce, 2005; Krafft et al., 2007; Ludwig et al., 2007; Michal et al., 2007; Stupp et al., 2005b, 2006; Zhang and Vauthey, 2003; Zhao and Kessler, 2005).

A very interesting approach exploiting peptide self-assembly is to take advantage of its reversible nature and the various self-assembly triggering mechanisms to form hydrogels. This allows easy transportation of the peptide in solution to a desired place often difficult to reach mechanically and then triggers self-assembly once in place. The hydrogel can function as a structural supporting medium either for cell growth, localizing actives, or inducing templated mineralization (Boden et al., 2004; Lynn et al., 2003, 2006; Narmoneva et al., 2003; Schneider and Pochan, 2006; Semino et al., 2002, 2004; Stupp et al., 2005a).

Alternative to fibers and 2D and 3D woven or nonwoven networks thereof formed as either self-supporting structures or as a hydrogel, it is possible to self-assemble peptides into thin self-assembled monolayers (SAMS) or multilayer structures. Such structures have been reported to act as membranes for controlled diffusion of ions and controlled movement of body fluids and contaminants (Ellis-Behnke et al., 2006, 2007; Holmes et al., 1999). Alternatively, various techniques have been put in place to provide coatings on various substrates ranging from tissue to metals and inorganics, for example, mica (Boden et al., 2002; Haynie, 2005, 2007; Haynie and Zhi, 2007; Yoo et al., 2008).

The use of self-assembling peptides is also explored beyond the medical, pharmaceutical, or cosmetics industry. Areas of interest are among others functional foods, electronics, functional coatings, and catalysis (but different from enzyme research). As an example, peptides can be designed to switch from a random coil-like primary structure organization into an α-helix or β-sheet secondary structure with unique properties. Short peptides align to form β-sheet tapes with different functionalities, for example, hydrophilic and hydrophobic on either side of the tape to form monolayer coatings (Boden et al., 1996).

While the aforementioned technologies focus either on bulk or on solid-phase assembly, a more recent innovation in peptide self-assembly has targeted the soft or fluid–fluid interface. Designer peptides of 7 amino acid "heptad repeats" can be reversibly triggered to fold into an α-helix conformation to organize both hydrophilic and hydrophobic faces, thus inducing surfactant-like structure. These peptide surfactants, however, interact with each other in the interfacial plane, through reversible metal–ion coordination

at the fluid–fluid interface, to give a gel-like film that has switchable rheology. This switchable character enables rapid and reversible "on demand" phase coalescence, thus differentiating the performance of these "Pepfactants" from nonswitchable conventional surfactants (Dexter and Middelberg, 2007; Malcolm et al., 2006). Importantly, the inherent design capability embedded in the amino acid code allows the engineering of application-specific function. It includes the ability to turn-off bulk self-assembly, thus maximizing interfacial availability and mass transfer rates, as well as allowing the interfacial activity and trigger conditions to be tuned for specific applications targeting phase separation and foam stability.

4. A FUTURE OF CHALLENGES AND OPPORTUNITIES

In subsequent papers, a nonexhaustive discussion is presented that covers several topics associated with the use of peptides and proteins as component molecules to develop structure and novel materials through self-assembly. First, a paper is devoted to the underlying scientific principles of peptide self-assembly. It is important to understand which amino acid sequences will trigger self-assembly under which conditions of temperature, concentration, pH, and solvent type. Also, it is essential to know what kind of structures can be expected in order for a rational molecular design to target specific applications. The experimental data should form a good basis for testing the validity of theoretical models discussed in the second paper. The state of the art in capturing the self-assembly process into a mathematical framework that accurately simulates hierarchical structure formation is an extremely challenging subject of research. Still for peptides to be useful, it is one thing to be able to have tailored species with well-defined structures created in the laboratory at mg scale but it is another, particularly an engineering feat, to produce peptides in sufficient quantities of sufficient purity at a reasonable cost. Therefore, a third paper considers what the options and challenges are of producing peptides. Subsequently there are three papers devoted to the potential applications. One explores the space of natural and artificial silk, another examines biomedical applications associated with tissue engineering. A third paper considers alternative applications where peptides are combined with polymers to form hybrid A-B block copolymers, to steer novel structure formation guided by the self-assembly of the peptide.

This series of papers aim to demonstrate the fascinating science and technology that self-assembling peptides bring. Self-assembly can be considered as the "how" to build novel materials for the future using peptides as building blocks. With nature as a reference, being a source of inspiration, the type of materials that potentially can be shaped seems infinite. Today, the research into this field has barely begun with few but very active academic groups from around the world exploring the possibilities. Medical, pharmaceutical,

cosmetic, and personal care companies are the most likely early adopters. Self-assembling peptides are very feasible solutions to important health and wellness challenges particularly in view of aspects such as biocompatibility with living organisms, highly functionally specific and controlled action, scar-free tissue engineering and repair, and agent delivery options. The key challenge here is successful clinical trial results to forge a market position of sufficient size that justifies the cost of the required research and development. In other sectors of industry, there may be additional hurdles related to regulatory legislation and socioeconomic acceptance. A major breakthrough will be needed in terms of economically providing designers peptide that perform under real-life conditions.

How long all this will take depends on the science and technology breakthroughs and the associated meme. Therefore, writing on the engineering perspectives of self-assembling peptides is by default limited to the imagination of today's scientists as reported in literature. It just remains a nonexhaustive attempt to inspire researchers, engineers, and any other interested party to discover a path of learning and applying the lessons of nature. Still the research efforts are fast paced and growing as can be gathered from the subsequent contributions. Accordingly, engineering innovative products using self-assembling peptides will become a discipline intimately linked to scientific understanding of multiple research fields, with applications in areas and markets we have just begun to imagine.

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