Scientific Research Journal Vol. 9 No. 1, 43-62, 2012

Factors Affecting Molecular Self-Assembly and Its Mechanism

Hueyling Tan

Faculty of Chemical Engineering,
Universiti Teknologi MARA, 40450 Shah Alam,
Selangor Darul Ehsan, Malaysia
Email:hueyling@salam.uitm.edu.my

ABSTRACT

Molecular self-assembly is ubiquitous in nature and has emerged as a new approach to produce new materials in chemistry, engineering, nanotechnology, polymer science and materials. Molecular self-assembly has been attracting increasing interest from the scientific community in recent years due to its importance in understanding biology and a variety of diseases at the molecular level. In the last few years, considerable advances have been made in the use of peptides as building blocks to produce biological materials for wide range of applications, including fabricating novel supra-molecular structures and scaffolding for tissue repair. The study of biological self-assembly systems represents a significant advancement in molecular engineering and is a rapidly growing scientific and engineering field that crosses the boundaries of existing disciplines. Many self-assembling systems are range from bi- and tri-block copolymers to DNA structures as well as simple and complex proteins and peptides. The ultimate goal is to harness molecular self-assembly such that design and control of bottom-up processes is achieved thereby enabling exploitation of structures developed at the meso- and macro-scopic scale for the purposes of life and non-life science applications. Such aspirations can be achieved through understanding the fundamental principles behind the self-organisation and self-synthesis processes exhibited by biological systems.

Keywords: *peptides, ionic-complementary, self-assembly, electrostatic interactions, amino acid sequence, biomaterials.*

ISSN 1675-7009

Introduction

Molecular self-assembly can be defined as the spontaneous organisation of molecular building blocks into ordered structure due to non-covalent interactions [1]. The evolution and organisation of biological systems is controlled by multiple weak intermolecular interactions such as hydrogen bonds and van der Waals repulsion which, on one hand, can be non-specific but in many cases are highly specific. These weak interactions within biological systems govern and maintain the generation of immensely complex structures as in DNA and proteins. The goal of bio-inspired self-assembly is to develop syntheses of complex molecular systems associated to sophisticated and specific preparation procedures that mimic natural processes. It is only in the last twenty years or so, that chemists and physicists have been able to understand the basic principles of self-organisation [2].

An understanding of the process of peptide self-assembly has a wide variety of biomedical implications, including scaffolding in tissue engineering, controlled drug delivery, surface bioengineering, controlling cell morphology and its functions [24]. Understanding molecular self-assembly is also important in various biological applications and diseases at the molecular level. Self-associating oligopeptides have been used as model systems to study the formation of insoluble macrostructures (fibrils), since they are implicated as the primary cause of several neurodegenerative diseases called amyloid diseases [3].

However, their behaviour and aggregation mechanisms, especially in the presence of some physicochemical factors such as ionic strength, pH and peptide/protein concentration have yet to be fully understood [4]. Thus, the practical application of peptide self-assembly has been hampered due to a lack of control methods for the self-assembly process, which is due to poor understanding of the molecular mechanisms involved [5].

Specific Self-Assemble Systems

Peptide or protein aggregations involved reversible and irreversible phenomena. Isoelectric precipitation and salting-out are most likely inducing reversible aggregation that is start from the native state. Conversely, irreversible aggregation occurs when the hydrophobic surfaces of mis-folded or partially unfolded and unassembled proteins are exposed to extremes of pH or temperature [6].

The insoluble macroscopic membranes of self-assembled oligopeptides have been widely used as a model system to investigate insoluble nanostructures at molecular level [7]. Numerous studies have revealed that a common mechanism of various types protein-related physiological disorder, such as Alzheimer's, Parkinson's, Down's syndrome, Huntington's, prion diseases, and amyloidoses, appears to be related to abnormal protein folding and aggregations [8-10]. *In vivo*, the presence of amyloids has been directly related to considerable tissue damage upon deposition.

One common characteristic of the aforementioned diseases is the presence of amyloid fibrils. Studies have shown that these fibrils have β-sheet structures, of which have the tendency to self-associate into protofibrils [10-12]. In contrast, prion diseases are thought to manifest due to conformational changes arising from the self-assembly of the prion proteins in the brain. These conformational diseases are characterised by the conversion of soluble and functional proteins into insoluble β-sheet rich quaternary structures that are often fibrillar. Such characterization has motivated extensive research into determination of the general mechanism for β-sheet formation and its aggregation or assembly to form fibrillar structures.

There are three types of peptide systems that can self-assemble. Type I peptides form β–sheet structures in aqueous solution. This class of oligopeptide-based biological materials was discovered by Zhang *et al.* [1] whilst studying the self-assembly of ionic self-complementary oligopeptides (EAK-II). These peptides are structurally unique in that they comprise of alternating hydrophilic and hydrophobic amino acid residues as shown in Figure 1, for which the complementary ionic sides have been classified into several moduli, such as modulus I, II and IV. In this context the Roman numerals indicate the number of the same charges grouped together. Hence, Modulus I have - + - + - + amino acid residues; modulus II, - - + + - - + +, and modulus IV, - - - - + + + +. The size of the resulting interwoven filaments are approximately 10-20 nm in diameter with pores approximately 50-100 nm in diameter [1].

Type 1 peptide is of special interest because they allow the formation of complementary ionic pairs within each chain and/or between different chains. Ionic pairs in the same chain primarily affect single-chain properties, whereas ionic pairs between different chains stabilise aggregates electrostatically. These peptides share some common features of uncharged peptides (e.g. hydrophobicity and hydrogen bonding), as well as possessing

unique charge properties that can sensitively control their aggregation behaviour.

It has been reported that poly(Val-Lys), poly(Leu-Lys), poly(Lys-Phe), poly(Tyr-Lys), poly(Glu-Ala), poly (Glu-Tyr), and oligopeptides (Val-Glu-Val-Lys) and (Val-Glu-Val-Orn) form stable β-sheets in the presence of salt, various pHs and prolonged incubation. It is of significant to note that peptides with similar composition but without an alternating sequence do not form stable β-sheets, instead they tend to form random coils or a stable α-helix as in poly(Leu-Lys-Lys-Leu) [13].

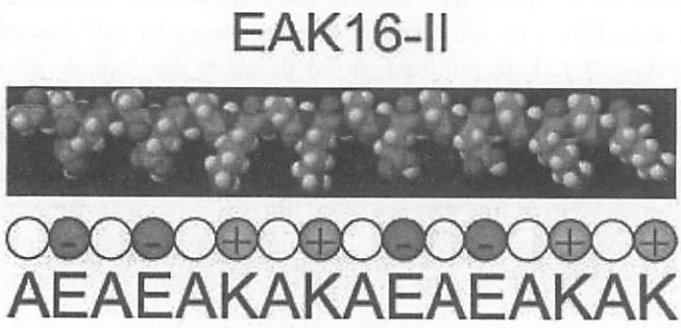

Figure 1: Molecular Model of the Extended Beta-Strand Structure of Individual Molecule is Shown for Type I Peptide of EAK16. The Distance between the Charged Side Chains along the Backbone is Approximately 6.8 Å; the Methyl Groups of Alanines are Found on One Side of the Sheet and the Charged Residues on the other. (Figure 1 is Taken from [8])

Type II peptides were discovered during the analysis of a family of modulus IV. This peptide is observed to undergo structural transformations initiated by changing the solution conditions. For example, DAR 16-IV exhibits a β-sheet circular dichroism spectrum at room temperature, but undergoes an abrupt transition at high temperatures to a stable α-helical spectrum in the absence of a detectable random-coil intermediate. On cooling the α-helical form is retained and takes several weeks at room temperature attain a partial β-sheet structure. Similar structural conformation changes were observed by changing pH. Such evidence suggests that the secondary structures of some sequences, especially segments flanked by clusters of negative charges on the C-terminus and positive charges on the N-terminus, are able to undergo drastic conformational transformations under appropriate conditions [1]. These findings may provide insight into protein-protein interactions during protein folding and the pathogenesis

of some protein conformational diseases, such as scrapie or Alzheimer's. Hong *et al.* [8] recently provided evidence that EAK 16-II and EAK 16-IV are capable of exhibiting distinct nanostructures, even though they possess the same amino acid sequence. According to the observations, EAK 16-II forms fibrillar assemblies regardless of pH values, whereas EAK 16-IV forms globular assemblies at pH values in the range 6.5-7.5 and fibrillar assemblies outside this range. It was consequently proposed that the charge distribution within the sequence is the determining factor in peptide nanostructure formation.

Type III peptides form monolayers on surfaces to produce specific cell patterns or to interact with other molecules. These oligopeptides comprise of three distinct features; namely a ligand, a linker and an anchor, all three of which can be tailored for specific purposes. The terminal ligand segment can be modified to incorporate a variety of functionalities thereby enabling recognition by other molecules or cells. The ligand can be located at either the N- or C-peptide terminus depending on the necessary recognition factors required by other biological moieties, since the peptide exhibits two asymmetric N- and C-termini. The central linker, essentially a variable length spacer, allows for freedom of interaction at a specified distance away from the surface, which is determined by the length of the spacer. The anchor is a chemical group within the peptide structure capable of reacting with a surface and forming a covalent bond.

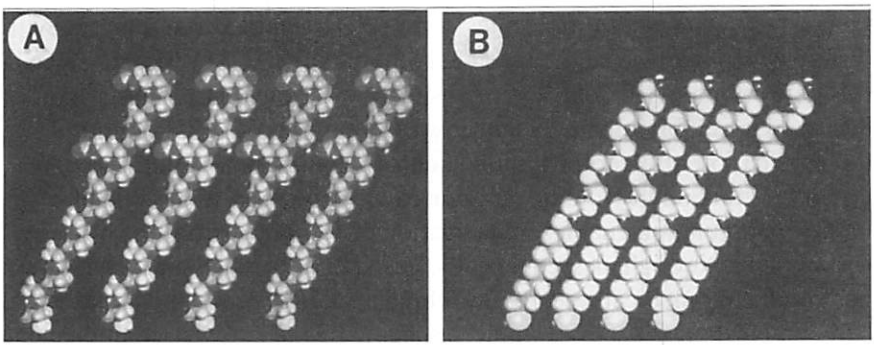

Figure 2: Self-Assembling Type III Peptide used in Biological Surface Engineering; Molecular Models of the Oligopeptide RADSC-14 with the Sequence RADSRADSAAAAAC (A) and Ethylene Glycol Thiolate (EG6SH) (B).
(Figure 2 is taken from [8])

These biomaterials has considerable potential in a number of applications, including platforms or scaffolding for tissue engineering, drug delivery of protein and peptide medicine, as well as biological surface engineering [14]. Furthermore, these peptides are short, simple to design, extremely versatile and easy to synthesise.

Type I self-assembling peptides have been used to design new materials with unique structural properties. This has been achieved by exposing the peptides to salt solution, which causes the formation of matrices comprising of interwoven fibres that can be fabricated in various geometric forms. Several peptide materials have been developed and tested; the results have suggested that the peptide materials can not only support various types of cell attachments, but also allow attached cells to proliferate and differentiate. Type II peptides are able to undergo structural transformation from a β-sheet to an α-helix, which could be exploited with respect to molecular switches that can be regulates by changing temperature or pH. Type III peptides have been developed for application in biological surface engineering. Such simple system is using self-assembling peptides and other substances to modify surfaces. In Figure 2 is shown the molecular models of the oligopeptide RADSC-14 with the sequence RADSRADSAAAAAC (A) and of ethylene glycol thiolate (EG6SH) (B) [8]. The N-terminal segment (RADS) is the ligand used for cell attachment, the five-alanine segment, AAAAA, is the linker, all of which is anchored by cysteine, C, through covalent bonds to gold atoms on the surface. The molecular models show the surface where both molecules form self-assembled monolayers with different heights, where the extended lengths of RADSC-14 and EG6SH are approximately 5 and 4 nm, respectively. Various patterns can be designed in cell biology to dictate how communities of cells communicate; the application of external stimuli such as calcium, potassium, hormones, growth factors and cytotoxic substances to a community of cells through micro-manipulation enables investigation of responses from the other cell communities through messenger cells. Such peptides and their systems may be useful in biomedical research and clinical applications in the form of new detection techniques. For example, a specific ligand known as a 'molecular hook' that interacts with specific molecules on cancer cell surfaces in high affinity would enable the anchoring of cancer cells on specific surfaces thereby enabling detection and consequent treatment.

Factors Controlling Self-Assembling Aggregation

The complexity of protein folding is an inevitable consequence of cellular existence [1] and almost all proteins tend to aggregate, but in different ways and to different extents under different conditions. Molecular self-assembly involves mostly weak and non covalent bonds, such as (i) ionic bonds (electrostatic interactions) (-4 kJ/ mol); (ii) van der Waals interactions (-1 kJ/ mol); (iii) hydrophobic interactions (-10 kJ/ mol); and (iv) hydrogen bonds (-3 kJ/ mol) [7]. Collectively, however, these weak interactions play an important role in all biological structures and their interactions.

Probing the factors affecting molecular behaviour of the peptides in solution is crucial in order to understand the peptide self-assembly mechanism. Some of the physicochemical factors including amino acid sequence, concentration of the peptide, molecular size, pH and ionic strength are discussed in this review paper.

Amino Acid Sequence

Of the aforementioned factors, the effect of the amino acid sequence has been investigated with respect to a number of ionic complementary peptides. For a series of ionic-complementary peptides, self assembly process is dependent on the charge distribution along the peptide backbone. Jun *et al.* [14] performed experimental studies using Atomic Force Microscopy (AFM) and Fourier Transform Infrared (FTIR), as well as theoretical and numerical studies at the molecular-level to investigate the influence of molecular architecture and interactions of the self-assembly of model peptide (EAK16)s of EAK16-I, EAK16-II and EAK16-IV. The AFM results indicate that EAK16-I and EAK16-II form fibrillar assemblies, whereas EAK16-IV forms globular structures. The FTIR spectrum for EAK16-IV indicates the possible formation of a β-turn structure, which is not exhibited by EAK16-I and -II. On the other hand, the theoretical and numerical studies, which used the coarse-grain CWLC (Charged Wormlike Chain) model, suggest that the hairpin structure is energetically stable for EAK16-IV, whereas the chain entropy of EAK16-I and -II favours relatively stretched conformations. In this study, they confirmed that the charge distribution and consequent electrostatic interactions play a critical role in single molecule conformation, which in turn dictates the formation of the resulting nanostructure.

Other studies have also established the importance of electrostatic interactions in the self-assembly of peptides including Caplan *et al.* [15] who studied the KFE12 system and reported that self assembly is regulated by the superposition of van der Waals attraction and the electrical double-layer repulsion, which was quantified with respect to DLVO theory. Hence, self assembly should occur when an oligopeptide is electrically neutral, otherwise excess charge would cause significant repulsion between the peptides thus preventing them from forming an aggregate. The observations of macroscopic membranes formed spontaneously in phosphate-buffered saline solutions (150 mM Sodium chloride/10 mM sodium phosphate, pH 7.4) assembled from EAK 16-II by Zhang *et al.* [16] suggest that alternating peptides with a tendency to form β-sheets may be able to form larger and more complex structures. The proposed membrane structure has alanines on one side of the β-sheet, which form hydrophobic bonds as in silk fibroin, and glutamic acids and lysines on the other side, which form complementary ionic bonds.

The substitution of a positively charged residue for a negatively charged residue results in the peptide that are no longer capable of undergoing self assembly. Conversely, the substitution of a positive charged residue for another positive charged residue, or negative for another negative, has no significant effect on the self assembly process [1]. It is however of note that the substitution of one hydrophobic residue for another more hydrophobic residue (for example changing alanine for phenylalanine) will impart a greater tendency for a peptide to self-assemble, and form matrices with enhanced strength [1].

Peptides without alternating hydrophilic and hydrophobic amino acid residues tend to form random coils or stable α-helices, examples of which are some short peptides containing alanine (A), glutamic acid (E), and lysine (K) that have been reported to adopt highly stable α-helices in solution. Changing the position of the amino acids affects helical stability as do changes in ionic concentration, temperature and pH, but not the peptide concentration [20]. It has been postulated that peptide helical stability is partly due to the formation of ion pairs or salt bridges between the positively charged lysine and the negatively charged glutamic acids. The most stable α-helix peptide in this class also exhibits the strongest interaction potential within the peptide structure, where the lysine and glutamic acids are separated by three alanines and the glutamic acid is near the N-terminal end.

Effect of Peptide Concentration

Concentration is another important parameter in the self assembly of biomolecules and studies by Shen *et al.* [17] on β-Amyloid protein have shown that fibril formation is strongly dependent on concentration. In the investigation performed they stated that at physiological pH and ionic strength, no fibril formation was observed at a peptide concentration of 0.2 g/L even after 9 days, but upon increasing the concentration to 0.5 g/L or more rapid and immediate fibril formation occurred.

The concentration dependence of self-assembling oligopeptides is expected to be similar to that of biosurfactants, which also comprise of both hydrophobic and hydrophilic components. The analogous nature of oligopeptides to surfactants means that a concentration study may be used to elucidate the Critical Self Assembly Concentration (CSAC) [9]. As in micellar systems, it is believed that oligopeptides are predominantly in monomer form below the CSAC, while peptide aggregation starts to occur at and above the CSAC [7].

Light scattering results on EAK 16-II [9] indicates it has a tendency to form aggregate at concentrations below the estimated CSAC, however an increase in the light scattering intensity above the estimated CSAC does imply that the aggregation rate depends strongly on the CSAC. The peptide concentration has a profound effect on both the size and shape of the resultant agglomerate nanostructures; yet both globular aggregates and thin filaments have been found to exist at concentrations below the CSAC. EAK 16-II forms fibrils at concentrations above the CSAC comprising of of globular aggregates (protofibrils), which align and stack together. The height and diameter of the globular aggregates formed at concentrations above the CSAC are larger than for those formed below the CSAC, which would support the premise that nucleation process is depend on the peptide concentration.

The aggregation behaviour of EAK 16-II is different to that exhibited during normal surfactant aggregation at concentrations above the CSAC. In typical micelle systems, increments in surfactant concentration leads to an increase in micelle concentration above the Critical Micellisation Concentration (CMC), as opposed to an increase in micelle size and shape, which implies a dependence on surfactant packing parameters. In contrast an increase in peptide concentration results in the formation of wider and thicker fibrils, which is evidently a marked distinction from surfactants. In this context once aggregate nuclei form they keep growing via the addition of

further peptide monomers, which is effectively increasing the concentration, culminating in a continuous increase in protofibrils and fibrils size.

Effect of Salt

Various salts have been known to affect the solubility, denaturation and association/dissociation of macromolecules, in particular ionic salts are known to exhibit 'salting-in' and 'salting-out' effects on proteins. The formation of surfactant micelles is influenced by the presence of ionic salts, whereby the ionic surfactant CMC often decreases due to charge screening of the hydrophilic heads upon adding salts. The presence of ionic salts is also known to affect biomolecular self-assembly, for example DNA strands which normally exhibit random coil conformations in a phosphate buffer solution will condense and aggregate under physiological conditions due to the presence of multivalent cations [7].

Studies have shown that the presence of sodium chloride in peptide solutions enhances fibril formation and consequent elongation. Lopez *et al.* [18] examined the effect of sodium chloride on amyloid fibril formation from de novo designed KTVIIE-, STVIIE-, KTVIIT-, STVIIT-, KTVLIE-, KTVIVE-, KTVIYE- and STVIYE-hexapeptides, which are form polymeric β-sheet structures arranged in a similar fashion to that believed to be exhibited by amyloid fibrils. At low sodium chloride (< 0.1 M) concentrations the rate of fibril formation increases with ionic strength, however at higher concentrations (> 0.1 M) only short filaments and amorphous aggregates are observed. Hong *et al.*'s [7] investigation of the presence of sodium chloride on EAK 16-II revealed that the dimensions of self-assembled nanofibre morphologies are not strongly dependent on electrostatic interactions. However, the dimensions of self-assembled nanofibres at low peptide concentrations (< 0.1 g/L) exhibit a degree of dependence with respect to sodium chloride concentration, whereby nanofibres increase in size until the sodium chloride concentration reaches approximately 20 mM. At low sodium chloride concentrations (< 0.1 M), surface tension studies indicate that the hydrophobicity of peptides increases (greater surface activity) with increasing sodium chloride concentration. It is believed that the presence of salts also affect the peptide solution CSAC, an example of which is the increase in CSAC value for a EAK 16-I solution from 0.3 to 0.8 g/L as a consequence of the addition of 20 mM of sodium chloride. In this example it is of further note that two structural transitions were exhibited; the second

of which involved a transition from fibrils to fibres and is believed to be related to an increase in the critical concentration due to a reduction in the electrostatic interactions responsible for fibre formation.

Surface active biomolecules exhibit an induction time, which usually manifests in the form of dynamic surface tension; hence when the peptide concentration of peptide is sufficiently low, the surface tension does not change within an initial time period, which is corresponds to an induction time. Miller *et al.* [19] proposed that the induction time represented the time required for the surface monolayer to attain a certain minimum coverage, above which the surface tension is reduced. This proposal is based on a thermodynamic model for the adsorption kinetics of globular proteins at liquid/fluid interfaces, which considers various adsorption states with different molar interfacial area and takes into consideration the transport of molecules in the bulk by diffusion and accounts for transitions between the adsorption states of the protein molecules. MacRitchie and Alexander [20] proposed that the induction period represents a diffusion-controlled adsorption time and that this period ends when the interface is covered with a certain number of proteins and the surface pressure increases only after this certain coverage is reached. Consequently, this adsorbed protein monolayer creates an energy barrier for further adsorption.

A surface tension study by Fung *et al.* [9], which considered EAK 16-II, indicated that the induction time decreases with increasing EAK 16-II concentration. The induction time disappears only at concentrations above the CSAC. It was proposed that this phenomenon is a consequence of diffusion-control and molecular self-rearrangement. Diffusion-control constitutes the diffusion of molecules to a surface, whereas molecular self-arrangement corresponds to the monomers may rearranging themselves on the surface via intermolecular interactions, which could lead to the formation of β-sheet monolayers. This formation provides better surface coverage and hence changes the surface tension significantly. If the concentration is above CSAC then the monomers are able to self-organise and form β-sheets in the bulk prior to diffusion and adsorption onto a surface.

Effect of pH

Various biological environments in the human body are highly pH specific, for example pH 5 in the mouth, 1.5 in the stomach and 7.4 in the blood.

Studies on the effect of pH on nanostructures resulting from the self assembly peptides indicate that certain pH induce conformational transformations. These effects are predominantly observed in the peptides of modulus IV, such as DAR 16-IV, which exhibits a transformation from a β-sheet structure to a stable α-helical structure as pH changes [1]. Another example is the yeast α-agglutinin protein, with the sequence of EYELENAKFFK, which has also been found to undergo similar conformational changes. Hong *et al.* [8] study on EAK 16-IV observed transitions from fibrillar assemblies at pH 4-6.5 to globular nanostructures at pH 6.5-7.5 and then back to fibrillar assemblies above pH 7.5.

It is believed that most secondary protein structures are stable once formed. However, proteins frequently undergo catalysis, transportation and interaction with other substances, consequently structural transformations can be viewed as possible molecular switches that could be used to control or regulate systems with respect pH changes.

Proposed Mechanisms of Peptide Aggregation

Most of the proteins that characterise pathologies, such as Alzheimer's, type II diabetes and Huntington's disease do not appear to be similar, either in sequence or in function, and there is no direct evidence pertaining to the mechanisms involved in cellular death. Among the hypothetical mechanisms proposed, the disruption of the cellular membrane by oligomeric precursors prior to full fibre precipitation appears to be most widely accepted [8,10].

It is increasingly accepted that amyloid self-organisation implies some degree of structural complexity, which involves processes that go beyond a non-specific aggregation of a preformed β-sheet. Recent studies have shown that β-sheet fibres usually manifest via various intermediates, such as helical ribbons, globular aggregates or toroids. The structure and properties of such intermediates are important in understanding the process of self-assembly and more over the intermediate, rather than the final fibril, may be the pathogenic species responsible in the case of amyloidoses [21].

Self assembly is an intrinsic property of peptide molecules due to their common peptide backbone [25]. Biological peptides and protein molecules are complex systems in terms of their molecular structure, as well as in terms of their folding and self-assembly properties, whereby each protein may consist of a mixture of α-helices, β-sheets, turns, loops,

and flexible random coil segments. From a physicochemical perspective, there are three major inter- and intra-molecular forces responsible for molecular self-assembly, namely hydrogen bonding, hydrophobic forces and electrostatic interactions [8]. Hydrogen bonding is thought to be the primary determining factor in the formation of protein secondary structures, whereas protein folding and molecular recognition processes in aqueous solution strongly rely on hydrophobic forces. Electrostatic interactions are able to induce different protein conformations by changing the ionic state of the amino acid components, thereby altering close proximity attractive and repulsive forces.

The folding process of a monomeric protein molecule is presented in Figure 3. This process starts from its least ordered state (U) and evolves through a series of partially folded states (I) on route to the fully native state (N). There is the possibility that the native state (N) will form ordered aggregated species (crystals and fibers) and that the unfolded and partially folded states degrade in the cell or form aggregated species. Such aggregates are frequently disordered or, under the appropriate conditions, highly ordered in amyloid fibrils. In living systems there tend to be transitions between the different states, which are highly regulated by the environment and by the presence of molecular chaperones, proteolytic enzymes and other factors.

Despite the lack of correlation among the proteins that form amyloid fibres *in vivo*, the physical process of formation and the final organisation of these structures do share some general characteristics. These features include common fibre morphology, which has been observed by using electron microscopy (EM). Results revealed that this polypeptide has a kinetic pattern very similar to that observed in nucleated polymerisation. In other words, polypeptide chain has an inherent sequence-dependent capability to form fibres under the right physico-chemical conditions as well as the presence of certain types of amino acids in the sequences. Such polypeptide exhibits the highest rates of amyloid fibril formation, i.e. the fastest kinetics of aggregation [10].

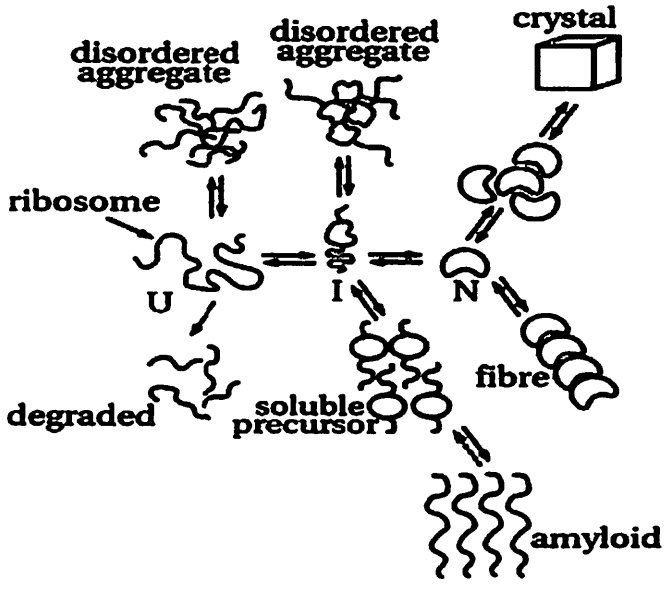

Figure 3: Some of the States Accessible to a Polypeptide Chain following its Biosynthesis. (Figure 3 is taken from [26])

An investigation of amyloid fibril formation from the synthetic peptide β(1-28), derived from β-Amyloid peptide, by Shen *et al.* [22] identified that fibril formation and elongation are the dominant processes before fibril-fibril interactions impose any significant effect. The results suggested that fibril formation is rapid and immediate if there is any lag time prior to inter-fibrillary aggregation. An initial rapid increase in size has been attributed to the formation of fibril networks, which is primarily a consequence of protofibrils coalescing to form fibrils. A second rapid size increase is believed to be due to the formation of fibril bundles. This work indicated that the aggregation mechanism was kinetically, as opposed to thermodynamically, limited and hence several factors, such as sample preparation, initial peptide concentration, pH, ionic strength and time significantly affected resultant aggregate concentration, size and morphology.

Lomakin *et al.* [23] proposed a model to describe the aggregation mechanism of Aβ in a similar self-assembly system. In their model, fibrillogenesis is initiated by the presence of nuclei in solution from either

seeding or micellisation, depending on the concentration of Aβ. The nuclei provide a core about which the Aβ molecules can arrange themselves into fibrils and fibril elongation, in this context, occurs by irreversible binding of Aβ monomers to the fibril ends. The derivation of association kinetics has been achieved with respect to whether the bulk concentration of Aβ is above or below a critical concentration.

Zhang *et al.* [13] hypothesised that individual peptides arrange themselves into β-sheets initially, then the β-sheets aggregate into filaments and further into membranes. This proposed mechanism was later supported by Fung *et al.* [9] in their study on EAK 16-II in which they suggested that peptide molecules aggregate to form fibrils in two stages. In the first stage the peptide monomers self-associate into protofibrils via nucleation and/or seeding depending on the solution concentration; below the CSAC the peptide monomers self-assemble to form nuclei, which initiate the aggregation of the protofibrils. The resultant protofibrils adopt one of two different nanostructures, namely globular aggregates or filaments. Both structures comprise of β-sheets held together predominantly by hydrogen bonding. The second stage comprises of the protofibrils interacting with each other and forming fibrils; one hypothetical pathway for which is that the globular aggregates align with the filaments.

Conclusion

Self-Assembly offers endless opportunities and has a significant future in various fields. Recent studies have identified new challenges and directions, which should be investigated in order to understand peptide self-assembly at the molecular level. It has been demonstrated that peptides have the potential to be used in various ways to engineer complex nanostructures suitable for different applications, such as molecular electronics, tissue engineering and drug delivery. Irrespective of the possibilities, in order to realise the true potential of molecular level engineering in biological systems, there is a necessity to achieve consistent and reproducible peptide self-assembly process. Once this has been resolved numerous possibilities for nano-technological applications will be attainable.

It is of note that biological on the other hand, a sensible selection of molecules and nano-clusters that function as conductors, semiconductors or insulators and have been "engineered" and have the potential to be used in developing novel applications and achieving device realisation

for use in micro/optoelectronics, catalysis, energy/magnetic storage and biotechnology. An understanding of effective cell signalling could be used to approach comprehension of more complex signalling pathways in stem cells and cancer cells, as well as the formulation of hybrid systems with macromolecules and/or inorganic components, hence enabling directed drug delivery with improved functionality.

Investigating multi-component systems comprising of several different nano-sized building blocks that are structurally and chemically compatible has the potential to enable the development of novel materials, with structural, functional and highly dynamic properties that are able to adapt to their environment. Considerable challenges still lie ahead, especially in terms of understanding the self-assembly mechanism, the design and preparation of three-dimensional structures of precisely controlled geometries culminating in tailored applications. It is anticipated that in the near future what was achieved at the macro-scale can be translated to the micro- and nano-scale opening up a plethora of exciting possibilities.

References

[1] S.G Zhang., Altman M., 1999. Peptide Self-assembly in Functional Polymer Science and Engineering, *Reactive and Functional Polymers*, Vol. 41, pp. 91-102.

[2] Editorial Overview, 2003. Self-Assemby: Weak and Specific Intermolecular Interactions at Work, *Current Opinion in Colloid and Interface Science;* Vol. 8, pp. 1-4.

[3] Davies R. P. W., Beevers A. A., Boden N., Carrick L. M., Fishwick C. W. G., McLeish T. C. B., Nyrkova I., Semenov A. N., 2006. Self-Assembling β-Sheet Tape Forming Peptides, *Supramolecular Chemistry,* Vol. 18(5), pp. 435-443.

[4] Hwang W., Marini D. M., Kamm R. D., Zhang S. G., 2003. Supramolecular Structure of Helical Ribbons Self-Assembled From A β-Sheet Peptide, *Journal of Chemical Physics*, Vol. 118(1), pp. 389-397.

[5] Zhang S.G., Altman M., 1999. Peptide Self-assembly in Functional Polymer Science and Engineering, *Reactive and Functional Polymers*, Vol. 41, pp. 91-102.

[6] Majhi P. R., Ganta R. R., Vanam R. P., Seyrek E., Giger K., Dubin P. L., 2006. Electrostatically Driven Protein Aggregation: *β-Lactoglobulin* at Low Ionic Strength, *Langmuir*, Vol. 22, pp. 9150-9159.

[7] Reed W. F., Ghosh S. *et al.*, 1991. Dependence of Polyelectrolyte Apparent Persistence Lengths, Voscosity, and Diffusion on Ionic Strength and Linear Charge Density, *Macromolecules*, Vol. 24, pp. 6189-6198.

[8] Hong Y. S., Legge R. L., Zhang S., 2003. Effect of Amino Acid Sequence and pH on Nanofiber Formation of Self-Assembling Peptides EAK16-II and EAK16-IV, *Biomacromolecules*, Vol. 4(5), pp. 1433-1442.

[9] Fung S. Y., Keyes C., Duhamel J. *et al.*, 2003. Concentration Effect on the Aggregation of a Self Assembling Oligopeptide, *Biophysical Journal*, Vol. 85, pp. 537-548.

[10] Zanuy D., Haspel N., Tsai H. H., 2004. Side Chain Interactions Determine the Amyloid Organization: A Single Layer β-sheet Molecular Structure of the Calcitonin Peptide Segment 15-19, *Physical Biology I*, pp. 89-99.

[11] Hwang W., Marini D. M., Kamm R. D., Zhang S. G., 2003. Supramolecular Structure of Helical Ribbons Self-Assembled From A β-Sheet Peptide, *Journal of Chemical Physics*, Vol. 118(1), pp. 389-397.

[12] Grigsby J.J., Blanch H. W., Prausnitz J, M., 2002. Effect of Secondary Srtucture on the Potential of Mean Force for Poly-L-Lysine in the α-Helix and β-Sheet Conformations, *Biophysical Chemistry*, Vol. 99, pp. 107-116.

[13] Zhang S., Lockshin C., Cook R., Rich A., 1994. Unusually Stable β-Sheet Formation in an Ionic Self-Complementary Oligopeptide, *Biopolymers*, Vol. 34, pp. 663-672.

[14] Jun S., Hong. Y., Imamura H., 2004. Self-Assembly of the Ionic Peptide EAK16: The Effect of Charge Distribution on Self-Assembly, *Biophysical Journal,* Vol. 87, pp. 1249-1259.

[15] Harrington S., Poole P. H., Sciortino F., Stanley H. E., 1997. Equation of State of Supercooled Water Simulated Using the Extended Simple Point Charge Intermolecular Potential, *Journal Chemical Physics,* Vol. 107(18), pp. 7443-7449.

[16] Zhang S. G., Holmes T., Lockshin C., Rich A., 1993. Spontaneous Assembly of A Self-Complementary Oligopeptide to Form a Stable Macroscopic Membrane, *Proc. Natl. Acad. Sci. USA (Chemistry),* Vol. 90, pp. 3334-3338.

[17] Shen C. L., Scott G. L., Merchant F., Murphy R. M., 1993. Light Scattering Analysis of Fibril Growth From the *Amino-Terminal Fragment β*(1-28) of *β-Amyloid Peptide*, *Biophysical Journal*, Vol. 65, pp. 2383-2395.

[18] Lopez de la Paz M., Goldie K., Zurdo J., Lacroix E., 2002. Dobson C. M., De novo Designed Peptide-Based Amyloid Fibrils, *Proc. Natl. Acad. Sci. USA*, Vol. 99, pp. 16052-16057.

[19] Miller R. E., Aksenenko V., Fainerman V. B., Pison U., 2001. Kinetics of Adsorption of Globular Proteins at Liquid/Fluid Interfaces, *Colloids Surfaces A: Physicochemical and Engineering Aspects,* Vol. 183-185, pp. 381-390.

[20] MacRitchie F., Alexander A. E., 1963. Kinetics of Adsorption of Proteins at Interfaces. Part I. The Role of Bulk Diffusion in Adsorption, *Journal of Colloid Science*, Vol. 18, pp. 453-457.

[21] Haile J.M., 1997. *Molecular Dynamic Simulation: Elementary Methods*, John Wiley & Sons.

[22] Shen C. L., Scott G. L., Merchant F., Murphy R. M., 1993. Light Scattering Analysis of Fibril Growth From the *Amino-Terminal Fragment β*(1-28) of *β-Amyloid Peptide*, *Biophysical Journal*, Vol. 65, pp. 2383-2395.

[23] Lomakin A., Teplow D. B., Kirschner D. A., Benedek G., 1997. Kinetic Theory of Fibrillogenesis of Amyloid β-Protein, *Proc. Natl. Acad. Sci. USA (Biophysics),* Vol. 94, pp. 7942-7947.

[24] Hong Y. S., Pritzker M. D., Legge R. L., 2005. Effect of Sodium chloride and Peptide Concentration on the Self-Assembly of an Ionic-Complementary Peptide EAK16-II, *Colloids and Surfaces B: Biointerfaces,* Vol. 46, pp. 152-161.

[25] Costenaro L., Ebel C., 2002. Thermodynamic Relationships Between Protein-Solvent and Protein-Protein Interactions, *Acta Cryst.*, Vol. D58, pp 1554-1559.

[26] Dobson, C.M., 2001. The Structural Basis of Protein Folding and Its Links with Human Disease, *Phil. Trans. R. Soc. Lond. B*, Vol. 356, pp. 133.