

Chapter 1

Introduction

1.1 SOFT BIOMATERIALS FOR HEALTHCARE APPLICATION

Soft biomaterials play a vital role in various biomedical application domains. Materials with dynamic properties, such as the ability to expand and contract, change stiffness, self-heal, or dissolve in response to environmental changes, are highly sought after for various applications including biosensing, drug delivery, soft robotics, and tissue engineering. Rapidly aging civilizations, as well as the need for improved drug delivery, organ functions and tissue repair, have led to the usage of synthetic materials in various regions of the human body. The conventional covalent chemistry has been highly effective in the development of materials. However, the future of soft biomaterials requires the ability to be easily synthesized, have multiple functions, and be effective. Supramolecular biomaterials are a class of advanced materials that are poised to revolutionize the field of biomedicine. These materials are three-dimensional structures formed by crosslinking agents that bond through non-covalent interactions such as hydrogen bonds, π - π stacking, and van der Waals forces. This bonding results in the formation of fibers followed by subsequent crosslinking between the fibers. The properties of these materials are significantly distinct from their covalent counterparts, and this introductory chapter provides a summary of the exciting advancements in this field. However, the range of applications and functional characteristics of these soft biomaterial architectures in the healthcare industry are bit extensive. This thesis study exclusively concentrates on the advancement and utilization of soft self-assembled structures that emerge from the self-assembly of peptides and proteins. Therefore, we confined our discussion to the specific parameters outlined in this thesis.

1.2 SUPRAMOLECULAR ASSEMBLY TO FORM SOFT BIOMATERIAL CONSTRUCT

Supramolecular chemistry, as explained by Nobel Laureate Jean-Marie Lehn, refers to the study of molecular assemblies¹. These assemblies have a specific structure and functioning and are produced through interactions between two or more chemical species². Polymeric biomaterials can be classified in several manners based on the polymer type and their structural attributes. Chemically crosslinked biomaterials are created when polymer chains are permanently connected by non-reversible covalent bonds. As a result, the biomaterials become fragile, occasionally not transparent, and unable to restore themselves when the network is interrupted³. These covalent connections can be formed through a variety of reactions, including Michael type addition, Schiff base creation, thiol-ene photopolymerizations, free radical photopolymerization, enzyme-triggered reactions, and "click" reactions. Chemical crosslinking can be adjusted to effectively alter the mechanical characteristics of biomaterials, and it is commonly employed to create durable and robust one. Supramolecular chemistry diverges from classical chemistry by emphasizing the utilization of non-covalent interactions, which are weaker and reversible, instead of covalent connections⁴. A supramolecular polymer is a structured arrangement of repeating units

composed of monomeric building blocks that are connected by reversible and directed non-covalent interactions. Supramolecular polymers can be created by different types of intermolecular interactions, including hydrogen bonding, metal-ligand complexation, hydrophobic forces, van der Waals forces, π - π interactions, and electrostatic effects. These interactions work together to build the polymers. Supramolecular chemistry has showcased significant concepts such as molecular self-assembly, folding, molecular recognition, host-guest chemistry, mechanically-interlocked molecular structures, and dynamic covalent chemistry⁵⁻⁸. Seemingly basic modular blocks can be used to construct highly intricate functional materials. Supramolecular chemistry enables the formation of complex structures by the self-assembly of modular components. The expansion of these systems beyond the individual molecule level depends on certain crucial non-covalent interactions that result in a controlled self-assembly process. Here, we can see the progression of structures from the initial molecular level to the subsequent polymeric level and then to the interconnected tertiary structure. These materials have the ability to modify their inherent properties and can be used to create new supramolecular structures including hydrogels, micelles, and vesicles.

1.2.1 Peptide Self-assembly as Building Block of Soft Nano-structures.

Amino acids serve as the fundamental constituents of proteins and fulfil many roles such as catalysis, cellular support in the extracellular matrix, DNA replication, and transportation of specific molecules, among others. Since the late 1980s, there has been a growing interest in short peptides derived from native proteins that possess the ability to self-assemble. Following that, a multitude of artificially designed peptides that imitate the structure of naturally occurring self-assembling peptides produced from proteins were created. The assembly process driven by external stimuli was found to be primarily governed by secondary structures, namely β -sheets and α -helices. Ionic strength, pH, and temperature are commonly employed factors for inducing the initiation of supramolecular nanostructure development. The utilization of a bottom-up approach in the development of nanoclusters and nanocomposite hydrogels, which rely on stimuli-responsive behavior, has demonstrated significant potential for biomedical applications. This is primarily attributed to their inherent biocompatibility, biodegradability, and ability to mimic extracellular matrix (ECM)-like microenvironments⁹⁻¹⁴. Furthermore, the many characteristics, such as negative or positive charge, hydrophobicity, hydrophilicity, and polarity, exhibited by the side chains present in amino acids offer extensive opportunities for chemical modification through countless sequence combinations. Moreover, the fundamental configuration of amino acids offers locations for bonding with polymeric substrates. Furthermore, in addition to self-assembly, a range of other features can be easily generated, such as self-healing, shear-thinning, shape memory, and others. Engineered peptides that have been intentionally developed draw inspiration from self-assembling peptides present in natural proteins. These engineered peptides have demonstrated exceptional qualities such as biocompatibility, biodegradability, and the ability to imitate the microenvironments of the extracellular matrix. The assembly of peptides can be induced by external stimuli, including electrolytes, temperature variations, and pH changes. Nanostructures are frequently formed and nanocomposite materials are subsequently generated at circumstances that are characteristic of physiological environments. The distinctive characteristics of side chains present in individual amino acids offer multiple opportunities for chemical modification and conjugation of peptides, facilitating the creation of diverse supramolecular nanostructures and hydrogels with customizable mechanical and physicochemical properties. Furthermore, it is possible to incorporate supplementary capabilities into hydrogels, such as shear-thinning, bioactivity, self-healing, and shape memory. This finding expands the range of potential applications for self-assembling peptide materials. The designed peptides can be efficiently and affordably

manufactured using the automated solid phase peptide synthesizer. Under physiological settings, the formation of nanostructures and subsequent networks created by self-assemble peptides is common. In rare cases, the electrolytes in DMEM media can cause the peptides to assemble, allowing cells to be entrapped within the scaffolds. With customizable mechanical strength and the potential to integrate bioactive peptide motifs, self-assemble peptide materials have exhibited greater efficiency in tissue engineering and regeneration, drug delivery, biosensors, and immunotherapy.

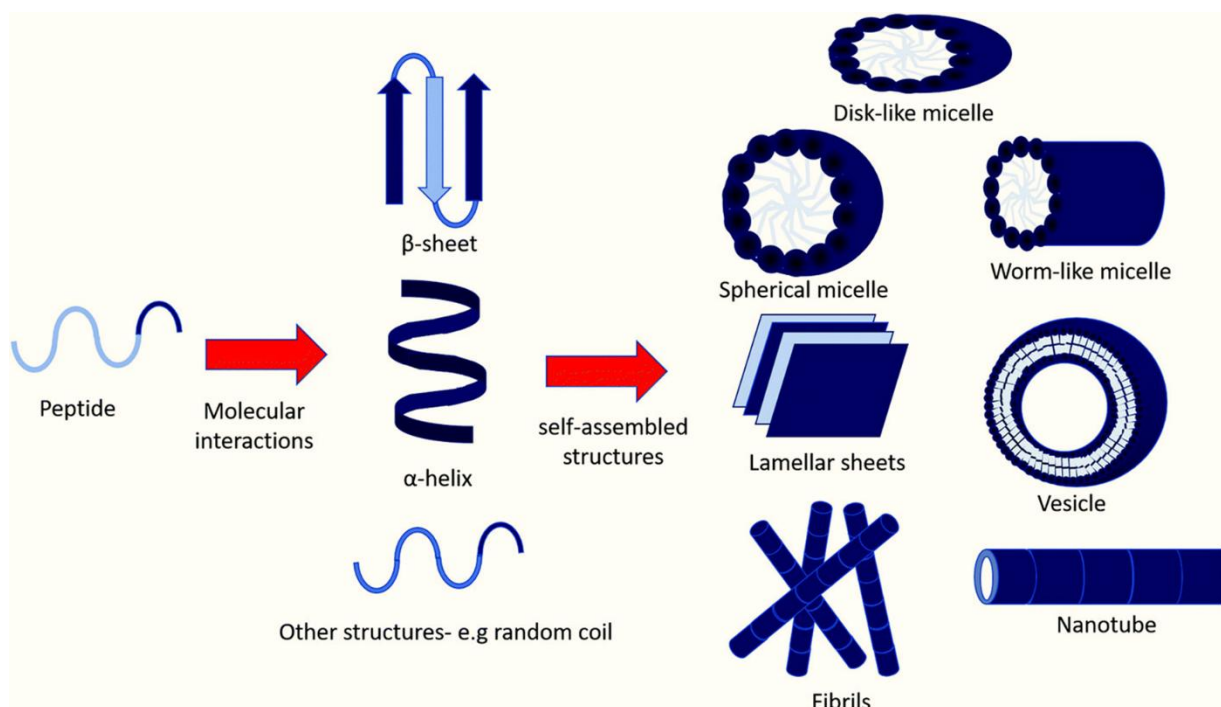


Figure 1.1 Potential configurations formed by peptide amphiphiles during self-assembly. Amphiphilic peptides have the ability to form secondary structures by engaging in both intermolecular and intramolecular interactions, such as electrostatic interactions and hydrogen bonding. These interactions facilitate the aggregation of the peptides into larger self-assembled structures.⁷

1.2.2 Principles of Self-Assembling Peptide Design and Self-Assembly Process.

Peptides with inherent self-assembly capabilities have been intentionally engineered to give rise to diverse structural aggregates, including nanofibers, nanovesicles, nanobelts, and nanotubes [Figure 1.1]. Over the course of the past few decades, a number of methodologies have been devised with the purpose of designing self-assembling peptides. Various supramolecular nanostructures have been created using synthetic peptides that possess beta sheet, alpha helix, triple helix, ELP-like, or amphiphile structures. These nanostructures were intentionally designed to undergo self-assembly, resulting in the acquisition of specific properties and functionalities¹⁵.

β-sheets Peptides. In recent decades, there has been a proliferation of peptide designs that exhibit β-sheet formation and subsequent self-assembly, drawing inspiration from the amino acid sequence (Ac-(AEAEAKAK)₂-CONH₂) discovered in the yeast protein zuotin. The peptides under consideration consist of a sequence of 12-16 amino acids, with a pattern of alternating hydrophilic and hydrophobic residues. The hydrophilic residues are either R or K, which are positively charged, or E or D, which are negatively charged. On the other hand, the hydrophobic residues are either A or L. This specific arrangement of residues promotes the formation of β-sheet structures in aqueous environments, with one side of the sheet being

hydrophobic and the other side being hydrophilic. The charge distributions on the hydrophilic side exhibit variations across different designs and can be categorized into four commonly employed moduli. Modulus I is represented by the arrangement "- + - + - +", modulus II is represented by "--++--++", modulus III is represented by "---+++", and modulus IV is represented by "----++++". The self-assembly process of peptides is initiated by the introduction of electrolytes, which serves to mitigate the electrostatic repulsions between the individual peptide monomers. Hydrophobic interactions are of significant importance as they effectively exclude the presence of surrounding aqueous medium between the hydrophobic sides of adjacent peptides. Fibrils are formed through the intra and intermolecular hydrogen bonds and electrostatic interactions of peptides that align in a parallel or anti-parallel manner, resulting in β -sheet bilayers. In addition to the initial iteration of self-assembling peptides that form nanofibers, it is possible to engineer various hierarchical structural arrangements such as ribbons, tapes, and fibrils. Furthermore, a number of peptides produced from self-assembling proteins have been discovered to exhibit the ability to arrange themselves into elongated beta strands. The alanine glycine-rich peptide was derived by the hydrolysis process of *Bombyx mori* silk fibroin. This peptide exhibits the creation of cylindrical fibers, which can be attributed to the arrangement of extended beta sheets. The Gelatin laboratory biotinylated peptide sequences that were taken from bone marrow homing peptides, resulting in improved assembly performance and scaffold biomechanics¹⁶. In addition, the incorporation of functional motifs into the peptides was found to enhance cell adhesion, proliferation, and differentiation, while maintaining mechanical characteristics and nanofiber shape that closely resemble physiological conditions.

α -helix. The alpha helix, a fundamental structural motif observed in proteins, has been utilized in the development of many fibrous biomaterials. The α -helix coiled coils are alternatively referred to as hydrogelating self-assembling fibers (hSAF). The fundamental heptad unit for hSAF consists of a sequence of seven residues. The hydrophobic residues at positions a and d, along with the charged residues at locations e and g, play a crucial role in determining the dimer interface. The residues located at locations b, c, and f exhibit surface exposure in the assemblies and demonstrate variability across different designs. The aforementioned locations are frequently held by hydrophobic alanine residues or glutamine residues that exhibit a predisposition for forming hydrogen bonds. The manipulation of coiled coil motifs' configuration can be achieved through alterations in environmental variables, including temperature, pH, and ionic strength. The incorporation of RGD motifs into hSAF peptides has been shown to augment biocompatibility, so rendering them more suitable for several biomedical applications, including tissue engineering and drug delivery. In order to enhance the regulation of assembly, researchers have also made advancements in the development of dual-peptide hSAFs systems.

Peptide Amphiphile. Peptide amphiphiles are composite molecules that consist of a peptide moiety combined with one or more alkyl chains. Both the structural composition and the chemical properties of these peptides exhibit characteristics similar to lipids or surfactants. These molecules have a hydrophobic tail and a hydrophilic head. The utilization of amphiphilic surfactants in conjunction with peptides with self-assembly capability offers a viable approach for manipulating the nanostructure, resulting in the formation of cylindrical or fibril geometries as opposed to micelles. The designed peptide amphiphiles were modified by the inclusion of both negatively charged and hydrophobic amino acid residues. This modification aimed to achieve a balance in charge within the cell culture environment and promote the process of self-assembly. The majority of these substances exhibit distinct critical aggregation concentrations (CAC) and possess the ability to spontaneously organize themselves in aqueous solutions and surfaces, resulting in the formation of highly organized

nanostructures such as peptide bilayers, nanotubes, nanorods, nanovesicles, and micelles¹⁷. The assembly of peptide amphiphiles into nanofibers is primarily driven by hydrogen bonding and beta sheets. The dimensions of these nanofibers, including their diameter and length, may be modulated by factors such as the length of the lipid tail, the number of charged sequences, and the specific conditions under which self-assembly occurs. Additionally, this architecture compels the bioactive motifs that are attached to be displayed on the surface, so facilitating enhanced interactions with protein receptors and cells. The self-assembly of amphiphiles connected to β -sheet peptides can be induced through many methods, including the addition of electrolyte solution, washing with culture media, and pH adjustment. These techniques serve to mitigate the electrostatic repulsion between monomers, particularly those that form beta sheets. Furthermore, the synthesis of collagen-like peptide amphiphiles was achieved through the conjugation of collagen-like peptides, discovered via phage display, to long-chain molecules, resulting in the formation of a wide range of nanofibrous structures that exhibit hierarchical organization.

Elastin-like Polypeptides. Elastin-like polypeptides (ELPs) represent a category of genetically modified peptides that are derived from tropoelastin and possess the ability to undergo self-assembly in specific physiological circumstances. Elastin-like polypeptides (ELPs) consist of recurring "VPGXG" sequences, wherein the polarity of the X position, representing a variable amino acid other than proline, governs the transition temperature of these peptides. The biopolymers exhibit a change from a disordered conformation to cylindrical micelles composed of beta sheets when the temperature surpasses the transition point.¹⁸ The utilization of ELP polypeptides has been observed in several applications involving the development of versatile biomaterials. This is primarily attributed to their inherent characteristics such as rubber-like elasticity, significant extensibility, adaptable deformation, remarkable resilience under stretching, responsiveness to changes in temperature, and ability to undergo biodegradation. The utilization of ELPs matrix as an injectable hydrogel has been documented, wherein the hydrogel undergoes network creation upon reaching body temperature, namely above its transition temperature. The phase behaviour of polypeptides, which is influenced by temperature, has been utilized for drug delivery purposes using both soluble and insoluble phases as carriers.

Collagen-like Peptides. Collagen serves as the principal constituent of the extracellular matrix throughout the human body. Despite the considerable diversity in construction and functionality among the 28 different types of proteins within the collagen family, they all share a common feature of possessing triple helix bundles¹⁹. The collagen triple helix is composed of three polyproline-II type helices, which are connected by periodic inter-chain hydrogen bonding. Each individual strand consists of tripeptide repeats denoted as "G-X-Y," with the X and Y positions commonly occupied by proline and (4R)-hydroxyproline in natural collagens. In light of the constraints associated with the utilization of collagens generated from animals, researchers have turned to collagen-like peptides (CLPs) as a viable alternative. This substitution aims to address issues such as thermal instability and the potential presence of pathogenic substances, thereby enhancing safety and stability. Several collagen-like peptides (CLPs) have been synthesized with the purpose of imitating the process of collagen fibril formation. Additionally, these CLPs have the ability to organize themselves into more complex hierarchical structures through interactions such as π - π stacking, lateral electrostatic interactions, and assembly triggered by metal ions. This allows for the precise control of chemical compositions and molecular structures.

1.2.3 Peptide Self-Assembly Mechanisms.

Electrostatic Interaction. Electrostatic interactions encompass both attracting and repulsive forces that occur between charged residues derived from amino acids in the process of peptide self-assembly. These interactions also exert significant influence on various other self-assembly phenomena. Peptides having a positive charge can aggregate with peptides or medicines that have a negative charge through electrostatic interactions. Subsequently, they were able to create a durable nanostructure suitable for drug delivery purposes. An example of a multifunctionalized peptide self-assembled nanostructure was created by designing and synthesizing it utilizing cRGD-BSA and KALA cell-penetrating peptides, which interacted electrostatically²⁰. These nanostructures have the potential to be utilized in specific and pH-sensitive delivery of anticancer drugs.

Hydrophobic Interaction. The hydrophobic interaction plays a crucial role in the peptide self-assembly process, being one of the most significant noncovalent interactions. The self-assembly of amphiphilic peptides can be easily achieved through microphase separation, which is driven by thermodynamics due to the presence of both polar and nonpolar areas within the peptide sequences. Under aqueous reaction conditions, the hydrophobic regions of the constituent units will aggregate and form clusters in an attempt to shield them from water. Meanwhile, the polar regions are striving to increase their interaction with water. Amphiphilic pharmaceuticals capable of self-assembling into nanostructures have been created via hydrophobic interactions. The amphiphilic medications consist of a peptide derived from the tau protein, which is linked to a hydrophobic anticancer agent called camptothecin. The materials have the ability to arrange themselves into fibril structures by utilizing hydrophobic contacts and intermolecular hydrogen bonding [Figure 1.2].

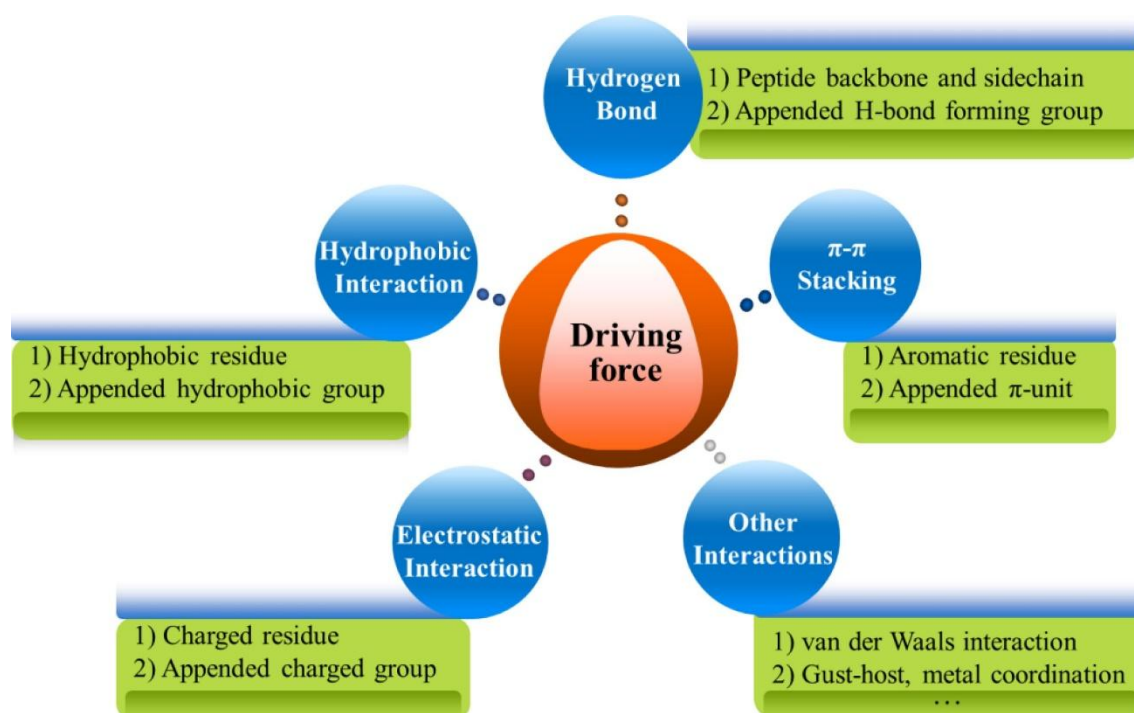


Figure 1.2 The primary “force” factors that propel peptide self-assembly.⁸

Hydrogen Bonding. Peptide sequences are designed to self-assemble into nanostructures by utilizing naturally occurring hydrogen bonding patterns, such as those seen in α -helices, β -sheets, and coiled coils. A hydrogen bond is a strong electrostatic attraction between a hydrogen atom and a neighboring atom that is extremely electronegative, such as nitrogen (N) or oxygen (O). Hydrogen bonding plays a crucial function in both the synthesis and

stabilization of the secondary structure of peptides and the folding of proteins. Hydrogen bonding is the predominant noncovalent interaction in peptide self-assembly. The stabilization of various peptide backbone conformations relies on hydrogen bonding interactions between the amide and carbonyl groups in the backbone. Subsequently, they have the ability to autonomously arrange themselves into β -sheet formations. The structures can be arranged in either parallel or antiparallel configurations, depending on the direction of the peptide sequences. A peptide is usually engineered to consist of recurring amino acid residues that form hydrophobic and hydrophilic areas. Consequently, the portion of the molecule that repels water will be concealed within the self-assembled nanostructure, while the section that attracts water will be exposed to the watery surroundings. Unlike β -sheets, α -helices are created by single peptide chains in which the amide components of the backbone form hydrogen bonds within the molecule. This configuration results in the exposure of amino acid side chains on the outside surface of each helix, making them easily accessible to the surrounding solvent [Figure 1.2].

π - π Stacking. π - π stacking enhances the self-assembly of peptides, particularly those that include aromatic residues. π - π stacking interactions can promote specific growth patterns and are highly stable in water since molecules with aromatic groups have low solubility. The π - π stacking is a particularly prominent driving factor in pure organic solvents, such as toluene and TFA. These solvents can enhance the prominence of π - π stacking compared to other self-assembly phenomena. The self-assembly process of the dipeptide FF is stabilized by π - π stacking between the aromatic groups and hydrogen bonding²¹. These interactions result in the formation of self-assembled FF nanostructures, which have been proven useful for a range of applications, such as drug delivery²² [Figure 1.2].

To summarize, noncovalent interactions are crucial in the processes of peptide self-assembly. External stimuli, such as pH levels, temperature, and solvent polarity, can easily influence noncovalent interactions. These factors have the ability to initiate self-assembly and control the process as well as the resulting nanostructures. For instance, pH values play a crucial role in peptides containing charged amino acids like Glutamic acid (Glu), Aspartic acid (Asp), Lysine (Lys), Histidine (His), and Arginine (Arg). The pH values can significantly influence the status of peptides with negative or positive surface charges, leading to their self-assembly into various nanostructures. There is a strong need to be able to control and manipulate the physical and biological characteristics of peptide self-assembled nanostructures in order to effectively use them in drug delivery applications. When developing peptide self-assembled nanostructures for drug delivery, it is important to carefully consider and strategically apply noncovalent interactions, as well as the types and architectures of the peptides.

1.2.4 Applications of Peptide Self-Assembled Soft-nanostructures as Smart Healthcare Materials.

Drug Delivery. Over the last few decades, researchers have successfully created and employed peptide self-assembled nanostructures of different sizes and shapes for a wide range of biological purposes, including tissue regeneration, biosensors, bioimaging, and drug delivery.^{23,24} This section provides a comprehensive analysis and explanation of peptide self-assembled nanostructures used for delivering anticancer drugs and gene drugs, as well as targeted and stimuli-responsive drug delivery. Biocompatibility, biodegradability, and multifunctionality are the most sought-after characteristics in self-assembled nanostructures for drug delivery applications. Peptide self-assembled nanostructures are more appropriate for drug administration compared to other organic materials because of their inherent physical and biological characteristics.

Peptide self-assembled nanostructures have gained significant interest as a potential approach for cancer treatment through the delivery of anticancer drugs. The peptide exhibits the capacity for spontaneous organization into a diverse array of nanostructures, including nanoparticles, nanotubes, nanovesicles, and nanofibers, which subsequently give rise to hydrogels. Each of these methods can be utilized to administer various types of anticancer medications for cancer treatment. For example, the peptide with amphiphilic characteristics has the ability to spontaneously form nanovesicle structures. These structures have been proven to effectively transport hydrophobic anticancer drugs for the purpose of cancer treatment. Additionally, the external layer of these nanostructures can be adjusted to fulfill precise medication delivery objectives. A self-assembled hydrogel composed of peptides, which may be injected, has the potential to directly interact with tumor locations, thereby improving the effectiveness and safety of tumor therapy. Peptide self-assembled nanotubes can be effectively used for cancer therapy by combining them with doxorubicin. Various anticancer drugs, such as doxorubicin, curcumin, fluorouracil, and paclitaxel, have been included into peptide self-assembled nanostructures and studied in preclinical or clinical studies for the treatment of cancer. Peptide self-assembled nanostructures have shown significant advancements in cancer therapy due to their exceptional biodegradability and biocompatibility. Injectable hydrogels composed of self-assembled nanofibers derived from peptides are highly promising materials for delivering anticancer drugs. This is due to their ability to facilitate direct contact between the chemotherapeutic drugs and the specific cancer tissues being targeted, resulting in higher local concentrations of the drugs compared to conventional cancer treatment methods. These peptide hydrogels offer enhanced safety and control as a result of their gradual release rates. For example, hydrogels made from KLD motifs can be utilized as stimuli to control the release of standard cytotoxic anticancer medicines like doxorubicin.²⁵

The self-assembled nanofiber structures formed by the EAK peptides have been proven to effectively transport the anticancer medication ellipticine using an encapsulating technique.²⁶ Two techniques were employed for the examination of the self-assembly and drug delivery applications. The initial method is the UV-based technique. The findings of this study indicate that the bonding between the peptide and the anticancer medication ellipticine is mostly driven by electrostatic interactions. Furthermore, this technique can also be employed to assess the efficacy of drug encapsulation in the peptide self-assembled nanostructures. Another method involves utilizing fluorescence technology, which can be used to monitor the process and effectiveness of conjugation. By monitoring the fluorescence properties, we were able to measure the concentrations of the anticancer medication ellipticine during the entire self-assembly and delivery process. The *in vitro* investigations also showed that the encapsulated anticancer medication ellipticine, when in the self-assembled nanofibers in protonated stage, is more effective for cancer therapy compared to when it is in the crystalline stage. The EAK peptide self-assembled nanostructures and the two encapsulating methods have potential applications in delivering other anticancer medicines for cancer therapy.

The dendrimer tetrapeptide GFLG underwent self-assembly to form tightly packed nanoparticles with negatively charged surfaces following its coupling with PEG and the anticancer medication doxorubicin. The drug loading and releasing experiments have shown a drug loading efficiency of 9.62 wt% and the ability to deliver drugs in response to enzymes. Fluorescent and cellular investigations demonstrated that the cancer treatment with doxorubicin anticancer medicines was both stable and very efficient. Furthermore, this study demonstrated a reduction in the toxicities associated with the doxorubicin anticancer treatment, as well as the absence of observable side effects. Furthermore, researchers have successfully created peptide self-assembled multifunctional nanostructures that incorporate

dual-functional liposomes. These nanostructures have been specifically designed for the purpose of delivering drugs to particular sites in cancer therapy. This approach has the potential to be utilized for the delivery of anticancer drugs by combining them with cell-penetrating peptides and active targeting agents. This study aimed to evaluate the potential of R6H4 as a pH-responsive drug delivery system for anticancer treatment. The R6H4 peptides were coated with hyaluronic acid because of their propensity for fast breakdown. The in vitro and in vivo investigations have shown that these nanocarriers can improve the effectiveness of delivering drugs specifically to tumors in cancer treatment. Peptide-based hybrid nanostructures were also made from PLA and VVVVVVKK (V6K2) peptides. Doxorubicin and paclitaxel could be conjugated with these nanostructures for cancer therapy. The diameter of pure PLA nanoparticles is around 130 nm, while the diameter of PLA-V6K2 self-assembled nanoparticles is only 100 nm. PLA-V6K2 nanoparticles had greater encapsulation and anticancer drug release ratios than pure PLA nanoparticles but slower release. The investigations also showed that PLA-V6K2 nanoparticles conjugated with anticancer medications are more hazardous to cancer cells than free doxorubicin or paclitaxel and pure PLA nanoparticle conjugates. This study showed that PLA-V6K2 nanoparticles are more effective in delivering anticancer drugs, which could aid cancer treatment.²⁷

The peptide self-assembled nanostructures, possessing stimuli-responsive features and precisely controlled releasing functions, have the potential to enhance drug transport efficiency and serve therapeutic objectives. An instance of a hydrogelator system has been constructed with peptides that incorporate the anticancer medication curcumin. The fundamental components consist of curcumin-FFE-ss-ERGD. It has been demonstrated that the peptides FFE and disulfide link had the capacity to create supramolecular hydrogelator structures. The ERGD peptides have the potential to be utilized for the precise targeting of cancer cells. Furthermore, these nanostructures formed by the self-assembly of peptides can also exhibit pH-responsive behavior upon endocytosis, leading to their disintegration into individual molecules. The findings of both in vitro and in vivo experiments have shown that these systems can improve cellular uptake and enable controlled and responsive drug release. Thus, they possess the capacity to impede the growth of cancer cells and tumors by means of responsive drug delivery specifically designed for cancer treatment.

Precise targeting of desired sites is crucial for nanocarriers in drug delivery applications to efficiently deliver or transport drugs. Peptides that form self-assembled nanostructures offer several benefits, including the ability to easily modify their properties and design recognition motifs in a customizable manner. Cell-penetrating peptides, consisting of cationic peptides with fewer than 30 amino acids, can enhance the efficiency of medication or gene delivery by facilitating the penetration of the cell membrane. The self-assembly mechanism plays a crucial role in facilitating improved membrane transport through the use of cell-penetrating peptides. In addition, numerous additional proteins or aptamers can be utilized to augment cell penetration or achieve specialized targeting, particularly for cancer cells or disease areas. An instance of dipeptide WF has been utilized to create self-assembled nanoparticles, which are designed for the specific delivery of drugs to treat cancer.²⁸ The peptide self-assembled nanoparticles exhibit visible fluorescence, in contrast to the intrinsic UV fluorescence of amino acids. The self-assembly and fluorescence generating methods are derived from the green fluorescent protein (GFP) and yellow fluorescent protein (YFP). Consequently, the dipeptide containing tryptophan and phenylalanine has the ability to spontaneously form blue light-emitting nanoparticles by means of π - π stacking and zinc coordination interactions. The experimental results have shown that these nanostructures are both biocompatible and photostable, except for their blue color fluorescent features, which have a narrow emission wavelength. Significantly, these dipeptides spontaneously formed nanoparticles that could

bind with MUC1 aptamer and the anticancer medication doxorubicin, enabling targeted delivery to specific cancer cells and enhancing the effectiveness of cancer treatment. The investigations uncovered the potential and benefits of utilizing peptide self-assembled nanostructures for precise medication delivery applications.

Gene Delivery. Progress in biotechnology and other industries has changed the diagnosis and treatment of many diseases by better understanding their gene-level pathological mechanisms. Scientists have utilized recombinant plasmid DNA as a therapeutic agent to transport genes to a particular target for the purpose of gene therapy. By utilizing the functional proteins derived from the corresponding gene encoding, it is possible to administer therapeutic treatment to patients. Efficient and affordable techniques, as well as noninvasive procedures, are required for the administration of gene-based drugs in the context of this particular genetic illness treatment.²⁹ Despite the increasing focus on gene therapy, significant improvement is still required for the investigation of nonviral gene drug delivery platforms at present. To enhance the nanocarriers used for gene medication delivery, various aspects need to be addressed, such as toxicity, immunogenic reaction, and inadequate cellular and nuclear absorption. Hence, it is crucial to focus on enhancing cellular delivery, achieving targeted delivery, and improving loading efficacy when designing and fabricating nanostructures for gene medication delivery. Cationic nanostructures have undergone extensive research and application due to their enhanced ability to enter cells and their great capacity to carry nucleic acids. Peptide self-assembled nanostructures offer a highly promising and effective technique for delivering gene drugs. This is primarily owing to their inherent features and the ability to precisely control their production methods. Peptide self-assembled nanotubes can also be utilized for gene medication delivery by changing the nanotube structures into nanovesicles during the endocytosis process. Consequently, numerous variations of gene medications and peptide self-assembled nanostructures have been recently created for gene drug delivery systems. The surfactant peptide has the ability to spontaneously arrange itself into nanotubes or nanovesicles that have a diameter of around 50 nm. This surfactant peptide was specifically engineered using cationic lipid systems to enhance its effectiveness in delivering gene drugs. The length of the peptide monomers is around 2 nm. The N-terminus of the peptide is positively charged and hydrophilic, including one or two lysine and histidine amino acids. Subsequently, six amino acids, namely Ala, Val, and Leu, combine to create the peptide tail, which exhibits hydrophobic characteristics. If the pH values exceed the isoelectric point (pI), the nanostructures have the potential to undergo additional self-assembly and form nanosheet structures. Due to their distinctive self-assembly and charge characteristics, these cationic peptides self-assembled nanostructures have the potential to be highly beneficial in linking negatively charged DNA and RNA for effective gene medication delivery purposes. A novel peptide consisting of four segments has recently been discovered for the purpose of delivering gene medicines in gene therapy applications. This peptide is coupled with lipopeptides to enhance its transfection capabilities. The peptide consists of cysteine, lysine, histidine residues, and alkyl chains. The self-assembly of these nanostructures facilitated the transport of gene medicines into cells via histidine residues. The transportation of gene medicines into the nucleus was facilitated by the lysine residues possessing charges at pH values that are neither acidic nor basic. A novel targeting peptide, GE11, has been synthesized and utilized in the formation of nanostructures alongside other components for gene drug delivery. These peptide-based nanostructures consist of the GE11 targeting peptide, branched polyethyleneimine, S-S bond, and polyethylene glycol. The experimental results have shown that both GE11 and branching GE11 self-assembled nanostructures had effective capacity for gene condensation and transfection. Furthermore, they exhibit minimal toxicity and enhanced targeting capabilities. Significantly, when compared to the self-assembled nanostructures of GE11, the branching nanostructures exhibit

a superior ability to specifically target cancer cells that have an overexpression of EGFR. Thus, this study has shown that the utilization of peptide self-assembled nanostructures holds great potential for delivering gene drugs in gene therapy. A crucial characteristic of peptide self-assembled nanostructures for gene medication delivery is the binding between these nanostructures and DNA. Furthermore, because to the enhanced ability to modify and adjust the peptide building blocks, these self-assembled nanostructures made of peptides could further enhance the uptake of DNA into the cell membrane and nucleus. In addition, they possess the capacity to regulate the release of gene drugs and augment gene expression. Consequently, researchers can prioritize the development of vectors that provide enhanced efficacy, safety, and specificity. Despite the existence of several studies utilizing peptide self-assembled nanostructures for gene medication delivery, it remains far from meeting the actual requirement.

Antibacterial Agent. Peptide-based antibacterial medicines have distinct advantages compared to traditional antibiotics. Due to their distinctive way of killing by disrupting the membrane, they have the ability to impede the development of resistance and attack germs that are resistant to many drugs. In addition, antimicrobial peptides exhibit a wide range of antimicrobial properties. The antibacterial properties of bulk materials can be modified by altering the peptide sequence at the molecular level, enabling the straightforward identification of the structure/chirality-encoded antimicrobial activity. Specifically, the process of engineering molecular self-assembly in antimicrobial peptides has been effectively shown to enhance their antibacterial efficacy while simultaneously decreasing hemolysis and allergic reactions. Meanwhile, the peptidyl nano-assemblies enhance the accumulation process of individual peptides and increase their binding affinity with the cell membrane. Based on the formulations, self-assembled peptide antimicrobial units can be classified into two types: amphipathic antimicrobial peptides and short amyloid-related peptides. Amphipathic antimicrobial peptides possess amphiphilicity, a characteristic that allows them to interact with biological membranes and undergo molecular self-assembly through certain secondary structures. Typically, these peptides consist of a head that carries an electric charge and a tail that repels water, or they may have repetitive sections of both water-repelling and electrically charged amino acids. Surfactant-like peptides serve as a prime example, and their nano-assemblies have been extensively suggested for the purpose of inhibiting microorganisms. Chen and colleagues conducted a study on the antibacterial effects of amphiphilic peptides A3K, A6K, and A9K.³⁰ They discovered that the effectiveness of these peptides against both gram-positive and gram-negative bacteria notably improved as the length of the hydrophobic tail grew.³⁰ The enhanced antibacterial capability was attributed to their heightened hydrophobicity, resulting in a stronger hydrophobic attraction to the bacterial membrane and consequently a more efficient disruption of membrane integrity. Additionally, the heightened hydrophobicity can enhance the strength of hydrophobic contact during self-assembly, leading to the formation of smaller self-assemblies. Nanostructures of lower dimensions can more easily traverse the bacterial membrane envelope and reach the cell membrane surface to cause damage.

Cai group discovered that including unsaturated motifs into antimicrobial peptides might greatly reduce their hemolytic activity.³¹ This characteristic addressed the issue of how increasing the lipophilicity of saturated fatty acid inside amphipathic peptides might both enhance the antibacterial activity and cause hemolysis. In order to comprehend the antimicrobial mechanism of self-assembled lipopeptides, a range of lipopeptides were created. These lipopeptides had the sequence of Cx-G(IKK)yI-NH₂, where CxGy represents a variable sequence with x ranging from 4 to 12 and y = 2. The design involved adding various lipid chains to the N-terminus of peptides that form α -helices. It was discovered that when

the carbon number in the lipid chain (represented by x) rose (up to a maximum of 10), both the critical aggregation concentrations (CACs) and minimum inhibitory concentration (MIC) decreased. This indicates that there was an increase in hydrophobicity and an improvement in antimicrobial activity. Notably, the minimum inhibitory concentration (MIC) values were considerably lower than the corresponding critical aggregation concentration (CAC), suggesting that the disruption of the bacterial membrane was caused by a combination of lipid dissolution and membrane-induced aggregation. In addition to lipid chains, aromatic groups have been included to serve as the hydrophobic tail at the N-terminal.³²

Based on the discovery that certain conventional amyloid proteins and peptides possess antibacterial properties, it has been suggested that amyloid-related short peptides could be utilized to combat bacterial infections. These antimicrobial peptides include a significant amount of aromatic phenylalanine, which is considered to have a crucial role in speeding up and stabilizing the formation of amyloidogenic assemblies. Gazit's group was the first to develop and use the self-assembled diphenylalanine (FF) motif as an antibacterial agent. When comparing diglycine (GG), which cannot self-assemble, with individual FF, it has been discovered that self-assembled FF is highly effective in inhibiting bacterial growth.^{33,34}

One significant advantage of hydrogel-based antibacterial medicines is their capacity to incorporate other agents for combined therapy. Wang and his colleagues have created a supramolecular hydrogel with antibacterial properties that can be switched on and off by changing the pH. The hydrogel is formed through self-assembly of Ac-Leu-Lys-Phe-Gln-Phe-His-Phe-Asp-NH₂. This hydrogel, which is compatible with living organisms, demonstrated a wide-ranging antibacterial action that is triggered by acidic conditions. The antimicrobial hydrogel, after incorporating cypate (a photothermal agent) to reduce excessive inflammation and proline (a procollagen component) to enhance cellular proliferation, effectively eliminated integrated biofilms and promoted healing in chronic wounds. Yan, Wang, and their colleagues discovered a naturally occurring antimicrobial peptide called Pro-Phe-Lys-Leu-Ser-Leu-His-Leu-NH₂. This peptide has the ability to assemble itself in a solution containing adenosine diphosphate (ADP), which can induce platelet aggregation. The researchers used this peptide to create an injectable hydrogel that has both anti-infection and hemostatic properties. The hydrogel produced in a rat model with a defect in the lateral wall of the cecum shown outstanding effectiveness in preventing adhesion.

Tissue Engineering. Self-assemble peptide hydrogels are extensively utilized in tissue engineering because to their ability to create a supportive three-dimensional milieu for cellular adhesion, infiltration, migration, and proliferation.³⁵⁻³⁸ In addition, the integrated bioactive patterns in the matrix can determine the biological activities and functions of cells. According to the findings, human normal dermal fibroblasts that were cultivated in RADA16-I nanofiber hydrogels had the ability to develop into cartilage cells, as evidenced by a substantial increase in the expression of the proteoglycan aggrecan. Furthermore, RADA hydrogels were employed to enhance the development of neurites and facilitate the differentiation of murine pluripotent stem cells by offering a support system similar to the extracellular matrix (ECM). The utility of FKFE scaffolds in tissue engineering was also investigated. The FKFE-II hydrogel was employed as a carrier for cells, possessing mechanical qualities that were tailored to closely resemble those of the natural nucleus pulposus. Following the 3D cultivation of nucleus pulposus cells within peptide scaffolds, the increased expression of KRT8, KRT18, and FOXF1 suggests effective regeneration with a high level of cell viability. Furthermore, there was an apparent augmentation in the accumulation of extracellular matrix (ECM) components, including aggrecan and type II collagen, in the nucleus pulposus. In addition, KLDL12 self-assemble peptides were utilized to fill polylactic acid/beta-tricalcium phosphate scaffolds in order to improve bone regeneration without the

need for cell transplantation. Sieminski conducted a study on the adhesion of human umbilical vein endothelial cells to four peptide scaffolds that create beta sheets.³⁹⁻⁴⁰ The results indicate that the cells have a preference for expanding into RADA scaffolds compared to KLDL and FKFE hydrogels, perhaps due to the favorable stiffness of the RADA material. In addition to investigating peptides that form beta sheets, researchers also examined hydrogels composed of alpha helix peptides. The researchers utilized a peptide scaffold that was functionalized with RGD in order to enhance the proliferative activity of mouse embryonic neural stem cells. Electrophysiological studies revealed the presence of mature neuron-like behavior, suggesting that functional peptides may have the potential to address the difficult problem of repairing nerve tissue. The application potential of self-assembling peptide amphiphiles was also investigated. Motamed created a hydrogel at physiological circumstances by developing an amphiphile that is connected to beta sheet peptides. It enhances the attachment and growth of brain cells by creating a favorable microenvironment.

As previously said, functional peptide sequences can be utilized to adorn the self-assembling peptides in order to facilitate the biological activities of cells. The LLVFGAKMLPHHGA sequence was utilized to replicate the mineralization functional motif of hydroxyapatite in order to facilitate bone tissue engineering. The LLVFGAD sequence is accountable for the process of self-assembly, while the remaining residues facilitate mineralization by offering a multitude of functional groups such as hydroxyapatite. The bioactive motifs SNV, KPS, and KAI from BMP-7 were utilized to adorn RADA16-I peptides for the purpose of regenerating the nucleus pulposus. The findings indicate that the SNV and KPS functionalized nanofiber hydrogels promote the growth, movement, and extracellular matrix secretion of human degenerated nucleus pulposus cells, in comparison to the non-decorated and KAI-decorated RADA hydrogels. Cakmak devised a peptide amphiphile hydrogel with many layers to cultivate human articular chondrocytes and bone marrow mesenchymal stem cells in an osteochondral cocktail medium. Cartilage and bone tissue were regenerated successfully without the need for any growth factor.⁴¹⁻⁴²

1.2.5 Future of Peptide based Supramolecular Assembly as Biomaterials.

Peptides that can assemble themselves have been intentionally designed to create nanostructures at the supramolecular level, possessing appropriate characteristics. The amino acid build-blocks of these materials have not only demonstrated biocompatibility, biodegradability, and low toxicity, but they have also shown the potential to induce self-healing, shape-memory, and shear-thinning capabilities by chemical modification. As a result, these materials have garnered significant attention in recent decades. The ability of peptides to undergo stimuli-responsive gelation or transition allows for their use in programmed drug administration, which optimizes therapeutic treatment while minimizing side effects. Furthermore, the combination of self-healing and shear-thinning properties makes it an excellent choice for injectable hydrogels and bioinks. The cells and bioactive motifs within the carriers can be safeguarded by the inherent or altered capabilities of the self-assembled network. Although the use of self-assemble peptide scaffolds in bioprinting is currently not well-researched, it shows promise as a means of creating complex 3D microenvironments that can enclose numerous cell types and bioactive chemicals with exact distribution. The potential inclusion of shape-memory functionality allows for its use in minimally invasive procedures. The prepared scaffolds can be folded and implanted through a small incision, then regain their intended shape at the target site. Moreover, self-assemble peptides can readily include functional peptide motifs obtained from ECM molecules and bioactive growth factors, thanks to their inherent amino acid components. The functional peptide sequence, when induced, can work similarly by facilitating intracellular signaling pathways with diminished immune response and lower cost. Proof-of-concept demonstrations have successfully shown improved

performance of self-assemble peptide materials in 3D cell culture, tissue engineering, and drug delivery. Nevertheless, there exist other obstacles that hinder the practical use of self-assembling peptides in therapeutic settings. Enhancements in the stability and uniformity of nanostructures produced using functionalized peptides are required. The effect of self-assembling peptide sequences on the immune system ranges from not causing an immunological response to enhancing the immune response, depending on the specific design. Further investigation is required to elucidate the immunogenic process of peptides in various types. In addition, it is necessary to systematically control external factors, such as pH, temperature, ionic strength, and other variables, in order to optimize the stability of nanostructure creation in various batches. Further investigation is necessary to fully understand the functioning of nanocomposite hydrogels. Although there are still obstacles to overcome, self-assemble peptides have significant potential for integrating several functionalities and features into a single system. Furthermore, we can anticipate further biomedical applications for these peptides.

1.2.6 Protein Self-Assembly to Form Soft Biomaterials and its Healthcare Application.

Protein self-assembly is an essential biological process in which proteins spontaneously arrange themselves into intricate and useful structures without any external guidance. This process is essential for the development of many biological functions.⁴³⁻⁴⁴ Nevertheless, in cases when protein self-assembly is wrong, it has the potential to initiate the onset of several illnesses, highlighting the crucial significance of comprehending this phenomenon. Historically, protein self-assembly has been associated only with biological processes, both normal and abnormal. However, in the last ten to twenty years, it has also been discovered to have significant promise for creating materials used in biomedical applications [Figure 1.3]. Consequently, it is imperative and appropriate to summarize the fundamental elements of protein self-assembly: the ways in which protein structure and self-assembly circumstances (such as chemical environments, kinetics, and the physicochemical properties of protein complexes) can be employed for the purpose of designing biomaterials.⁴⁵ Utilizing self-assembly offers a significant advantage in creating materials with precise control over their structures and functions. By designing the primary sequence of proteins, it becomes possible to regulate the self-assembly process and manufacture biomaterials with customized properties, including state, size, shape, and thus influence important characteristics such as mechanical strength and durability. Additionally, these self-assembled materials can be engineered to imitate the properties of natural tissues, such as elasticity, stiffness, and biodegradability.

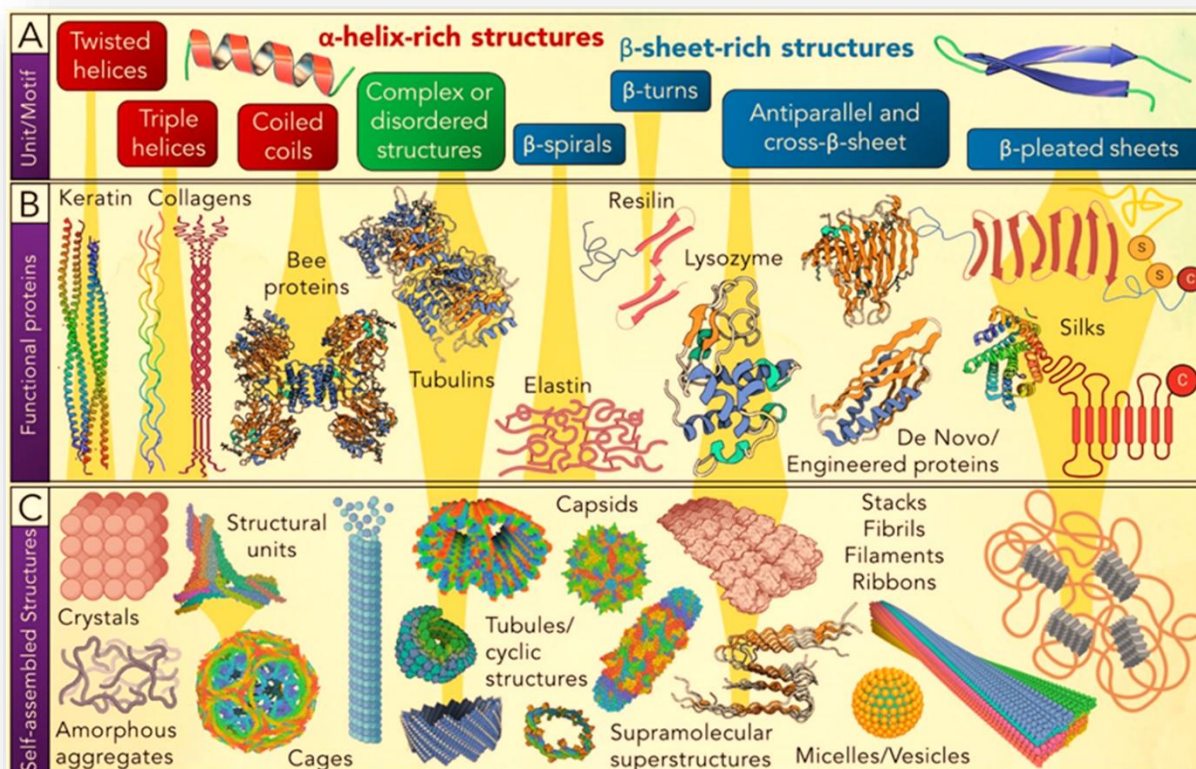


Figure 1.3 The structural hierarchy of protein self-assembly encompasses the arrangement of building blocks and structural motifs, leading to the formation of self-assembled structures.⁴⁵

Protein folding and aggregation are opposing processes in the energy landscape, resulting in the creation of various aggregates like amorphous aggregates, β -sheet-rich oligomers, amyloid fibrils, and more intricate structures. For instance, it has been demonstrated that amyloid fibrils are highly stable in the thermodynamic state of the energy landscape. Nevertheless, it is important to note that the final structures can be metastable due to external factors, which ultimately leads to the polymorphism of the resulting structures. Proteins, unlike peptides, cannot be manufactured on solid support, making integrating unnatural functionality more challenging. Nevertheless, the combination of recombinant protein production and genetic engineering frequently allows for the creation of the necessary amounts of proteins with satisfactory efficiency and cost. Proteins, like peptides, are generally biocompatible and degradable. However, proteins often have more functionality than peptides because of their increased complexity, which allows them to self-assemble and have a bigger biological effect. Proteins, such as collagen, fibronectin, and laminin, are a suitable option for various biomaterials applications due to their self-assembling nature inside the extracellular matrix. Furthermore, the properties and geometries of these proteins can exhibit significant variation due to their intricate structure, which distinguishes them from predominantly linear peptides. Nature encompasses a diverse array of proteins, offering abundant inspiration and a rich reservoir of raw materials. Several proteins found in nature, such as collagen, elastin, and spider silk, have the ability to self-assemble in their natural environments. Many research labs have utilized these proteins to create biomaterials. Tirrell and his colleagues aimed to methodically develop a hydrogel material. To achieve this, they created a triblock polypeptide consisting of leucine zipper motifs that were interspersed with

an unstructured amino acid sequence ((AG)3PEG)₁₀.⁴⁴ Leucine zippers are composed of alpha helical segments that have a repeating pattern of seven amino acids. These segments come together to form a complex by interactions between hydrophobic and charged amino acid side chains. They recombinantly expressed a polypeptide gene in *E. coli* to create a hydrogel at greater concentrations. The gel might be dissolved to a viscous liquid by increasing the temperature, denaturing the leucine zipper structure, or decreasing the pH, protonating acidic residues needed to stabilize the alpha helix. These gels' breakdown rates may be modified over two orders of magnitude by designing the leucine zipper sequence to favor inter- vs. intra-molecular interaction, showing the power of genetic engineering to modify protein-based materials. Research on protein fibrils has taken many paths, used many methods, and examined many proteins. Amyloid-like assemblages have been studied in relation to external factors like sound energy. Inspired by natural substances, Romano et al. investigated synthetic bovine alpha-lactalbumin amyloid-like fibrils for delivering capsaicin (CAP), known for its health advantages.⁴⁴ Capsaicin accelerated fibril production, stability, and digestive breakdown, which may affect consumer health. One notable recent discovery is the development of asymmetric tubular microgels made from reconstituted silk fibroin. These microgels have a liquid core and a protein fibrillation network on the surface. These particles are anticipated to be appropriate for holding physiologically active cargo molecules, such as antibodies. This makes them an appealing delivery mechanism for biomedical and pharmacological purposes.

1.2.7 Future Prospect.

Without a doubt, the quantity of novel protein-based materials is increasing annually, often surpassing our comprehension of the methods to get them and our capacity to articulate their characteristics. Furthermore, there are numerous aspects that go beyond the scope of the present study, nevertheless they are equally captivating and necessitate a distinct discourse. In this summary, we have outlined recent advancements in the molecular design of novel protein-based biomaterials. These breakthroughs encompass self-assemblies at various organizational levels, including fibrillar assemblies, nanoparticles, vesicles, capsids, capsules, as well as nano- and microgels. Furthermore, we have briefly discussed the diverse applications of these biomaterials. Given the current advancements in machine learning and the existing knowledge, it is now feasible to utilize these algorithms to forecast the characteristics of protein-based supramolecular assemblies. These assemblies have the potential to be promising options for the development of biomaterials.

1.3 REFERENCES

1. Lehn, J.-M. Supramolecular Chemistry—Scope and Perspectives Molecules, Supramolecules, and Molecular Devices (Nobel Lecture). *Angew. Chem., Int. Ed. Engl.* **1988**, 27, 89– 112.
2. Lehn, J.-M. Toward Self-Organization and Complex Matter. *Science* **2002**, 295, 2400– 2403.
3. Whitesides, G. M.; Mathias, J. P.; Seto, C. T. Molecular Self-Assembly and Nanochemistry: A Chemical Strategy for the Synthesis of Nanostructures. *Science* **1991**, 254, 1312– 1319.
4. Whitesides, G. M.; Grzybowski, B. Self-Assembly at All Scales. *Science* **2002**, 295, 2418– 2421.
5. Pochan, D. J.; Schneider, J. P.; Kretsinger, J.; Ozbas, B.; Rajagopal, K.; Haines, L. Thermally Reversible Hydrogels via Intramolecular Folding and Consequent Self-Assembly of a de Novo Designed Peptide. *J. Am. Chem. Soc.* **2003**, 125, 11802– 11803.
6. Gazit, E. Self-assembly of Short Aromatic Peptides: From Amyloid Disease to Nanotechnology. *NanoBiotechnology* **2005**, 1 (3), 286–288.
7. Edwards-Gayle, C. J. C.; Hamley, I. W. Self-Assembly of Bioactive Peptides, Peptide Conjugates, and Peptide Mimetic Materials. *Org. Biomol. Chem.* **2017**, 15 (28), 5867– 5876,

8. Qin, S.-Y.; Feng, J.-Q.; Cheng, Y.-J.; Liu, W.-L.; Zhang, A.-Q.; Wang, L.; Wang, H.; Zhang, X. Z. A Comprehensive Review on Peptide-Bearing Biomaterials: From Ex Situ to In Situ Self-Assembly. *Coord. Chem. Rev.* **2024**, *502*, 215600.
9. Yuan, C.; Ji, W.; Xing, R.; Li, J.; Gazit, E.; Yan, X. Hierarchically oriented organization in supramolecular peptide crystals. *Nat. Rev. Chem.* **2019**, *3*, 567–588.
10. Adler-Abramovich, L.; Gazit, E. The physical properties of supramolecular peptide assemblies: from building block association to technological applications. *Chem. Soc. Rev.* **2014**, *43*, 6881–6893.
11. Li, Y.; Xue, B.; Cao, Y. 100th Anniversary of Macromolecular Science Viewpoint: Synthetic Protein Hydrogels. *ACS Macro Lett.* **2020**, *9* (4), 512–524.
12. Wei, G.; Su, Z.; Reynolds, N. P.; Arosio, P.; Hamley, I. W.; Gazit, E.; Mezzenga, R. Self-assembling Peptide and Protein Amyloids: From Structure to Tailored Function in Nanotechnology. *Chem. Soc. Rev.* **2017**, *46*, 4661–4708.
13. Das, S.; Jacob, R. S.; Patel, K.; Singh, N.; Maji, S. K. Amyloid Fibrils: Versatile Biomaterials for Cell Adhesion and Tissue Engineering Applications. *Biomacromolecules* **2018**, *19* (6), 1826–1839.
14. Knowles, T. P.; Mezzenga, R. Amyloid Fibrils as Building Blocks for Natural and Artificial Functional Materials. *Adv. Mater.* **2016**, *28*, 6546–6561.
15. Zhao, X.; Zhang, S. Designer self-assembling peptide materials. *Macromolecular bioscience* **2007**, *7*(1), pp.13–22.
16. Gelain, F.; Silva, D.; Caprini, A.; Taraballi, F.; Natalello, A.; Villa, O.; Nam, K. T.; Zuckermann, R. N.; Doglia, S. M.; Vescovi, A. BMHP1-Derived Self-Assembling Peptides: Hierarchically Assembled Structures with Self-Healing Propensity and Potential for Tissue Engineering Applications. *ACS Nano* **2011**, *5*, 1845–1859.
17. Hendricks, M. P.; Sato, K.; Palmer, L. C.; Stupp, S. I. Supramolecular Assembly of Peptide Amphiphiles. *Acc. Chem. Res.* **2017**, *50*, 2440–2448.
18. Choi, J.-W.; Choi, S.-H.; Won, J.-I. Self-assembly Behavior of Elastin-Like Polypeptide Diblock Copolymers Containing a Charged Moiety. *Biomacromolecules* **2021**, *22*, 2604–2613.
19. Boudko, S. P.; Bächinger, H. P. Structural Insight for Chain Selection and Stagger Control in Collagen. *Sci. Rep* **2016**, *6* (1), 37831.
20. Chen, B.; He, X. Y.; Yi, X. Q.; Zhuo, R. X.; Cheng, S. X. Dual-Peptide-Functionalized Albumin-Based Nanoparticles with pH-Dependent Self-Assembly Behavior for Drug Delivery. *ACS Appl. Mater. Inter.* **2015**, *7*, 15148–15153.
21. Basavalingappa, V.; Bera, S.; Xue, B.; O'Donnell, J.; Guerin, S.; Cazade, P.-A.; Yuan, H.; Haq, E. U.; Silien, C.; Tao, K.; Shimon, L. J. W.; Tofail, S. A. M.; Thompson, D.; Kolusheva, S.; Yang, R.; Cao, Y.; Gazit, E. Diphenylalanine-Derivative Peptide Assemblies with Increased Aromaticity Exhibit Metal-like Rigidity and High Piezoelectricity. *ACS Nano* **2020**, *14* (6), 7025–7037.
22. Yan, X.; Zhu, P.; Li, J. Self-Assembly and Application of Diphenylalanine-Based Nanostructures. *Chem. Soc. Rev.* **2010**, *39* (6), 1877.
23. Lu, H.; Wang, J.; Wang, T.; Zhong, J.; Bao, Y.; Hao, H. Recent Progress on Nanostructures for Drug Delivery Applications, *Journal of Nanomaterials* **2016**, *1*, P5762431.
24. Leite, D.M.; Barbu, E.; Pilkington, G.J.; Lalatsa, A. Peptide Self-Assemblies for Drug Delivery. *Curr Top Med Chem.* **2015**, *15*(22), 2277–89.
25. Yishay-Safranchik, E.; Golan, M.; David, A. Controlled Release of Doxorubicin and Smac-Derived Pro-Apoptotic Peptide From Self-Assembled KLD-Based Peptide Hydrogels. *Polym. Adv. Technol.* **2014**, *25*, 539–544.
26. Wu, Y.; Sadatmousavi, P.; Wang, R.; Yuan, Y.; Chen, P. Self-Assembling Peptide-Based Nanoparticles Enhance Anticancer Effect of Ellipticine in vitro and in vivo. *Int J Nanomedicine.* **2012**, *7*, 3221–3233.
27. Lee, S.; Trinh, T.H.T.; Yoo, M.; Shin, J.; Lee, H.; Kim, J.; Hwang, E.; Lim, Y.B.; Ryou, C. Self-Assembling Peptides and Their Application in the Treatment of Diseases. *Int J Mol Sci.* **2019**, *20*(23):5850.
28. Fan, Z.; Sun, L.; Huang, Y.; Wang, Y.; Zhang, M. Bioinspired Fluorescent Dipeptide Nanoparticles for Targeted Cancer Cell Imaging and Real-time Monitoring of Drug Release, *Nature Nanotechnology.* **2016**, *11*, 388–394, 2-s2.0-84954545923.
29. Jeong, S.G.; Ryu, Y.C.; Hwang, B.H. Synergistic gene delivery by self-assembled nanocomplexes using fusion peptide and calcium phosphate. *Journal of Controlled Release* **2021**, *338*, pp.284–294.
30. Chen, C.; Pan, F.; Zhang, S.; Hu, J.; Cao, M.; Wang, J.; Xu, H.; Zhao, X.; Lu, J. R. Antibacterial Activities of Short Designer Peptides: A Link between Propensity for Nanostructuring and Capacity for Membrane Destabilization. *Biomacromolecules* **2010**, *11*, 402–411.

31. Niu, Y.; Padhee, S.; Wu, H.; Bai, G.; Qiao, Q.; Hu, Y.; Harrington, L.; Burda, W. N.; Shaw, L. N.; Cao, C.; Cai, J. Lipo-gamma-AApeptides as a new class of potent and broad-spectrum antimicrobial agents. *J. Med. Chem.* **2012**, *55* (8), 4003.
32. Mukherjee, N.; Ghosh, S.; Sarkar, J.; Roy, R.; Nandi, D.; Ghosh, S. Amyloid-Inspired Engineered Multidomain Amphiphilic Injectable Peptide Hydrogel—An Excellent Antibacterial, Angiogenic, and Biocompatible Wound Healing Material. *ACS Appl. Mater. Interfaces* **2023**, *15*, 28, 33457–33479.
33. Hu, B.; Shen, Y.; Adamcik, J.; Fischer, P.; Schneider, M.; Loessner, M. J.; Mezzenga, R. Polyphenol-Binding Amyloid Fibrils Self-Assemble into Reversible Hydrogels with Antibacterial Activity. *ACS Nano* **2018**, *12*, 3385–3396.
34. Hu, B.; Yu, S.; Shi, C.; Gu, J.; Shao, Y.; Chen, Q.; Li, Y.; Mezzenga, R. Amyloid-Polyphenol Hybrid Nanofilaments Mitigate Colitis and Regulate Gut Microbial Dysbiosis. *ACS Nano* **2020**, *14*, 2760–2776.
35. Tao, Y.; Xia, W.; Zhao, Q.; Xiang, H.; Han, C.; Zhang, S.; Gu, W.; Tang, W.; Li, Y.; Tan, L.; Li, D.; Liu, C. Structural mechanism for specific binding of chemical compounds to amyloid fibrils. *Nature Chemical Biology* **2023**, *19*(10), 1235–1245.
36. Mukherjee, N.; Adak, A.; Ghosh, S. Recent Trends in the Development of Peptide and Protein-Based Hydrogel Therapeutics for the Healing of CNS injury. *Soft Matter* **2020**, *16*(44), 10046–10064.
37. Mukherjee, N.; Ghosh, S. Substance P-Derived Extracellular-Matrix-Mimicking Peptide Hydrogel as a Cytocompatible Biomaterial Platform. *Chembiochem: A European journal of chemical biology* **2023**, *24*, e202300286.
38. Cui, H.; Webber, M. J.; Stupp, S. I. Self-assembly of Peptide Amphiphiles: From Molecules to Nanostructures to Biomaterials. *Biopolymers* **2010**, *94*, pp. 1–18.
39. Panda, J. J.; Chauhan, V. S. Short Peptide Based Self-Assembled Nanostructures: Implications in Drug Delivery and Tissue Engineering. *Polymer Chemistry* **2014**, *5*, pp. 4418–4436.
40. Adler-Abramovich, L.; Reches, M.; Sedman, V.; Allen, S.; Tendler, S.; Gazit, E. Thermal and Chemical Stability of Diphenylalanine Peptide Nanotubes: Implications for Nanotechnological Applications. *Langmuir* **2006**, *22*, 1313–1320.
41. Vauthey, S.; Santoso, S.; Gong, H.; Watson, N.; Zhang, S. Molecular Self-assembly of Surfactant-like Peptides to form Nanotubes and Nanovesicles. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5355–5360.
42. Zhao, X.; Pan, F.; Xu, H.; Yaseen, M.; Shan, H.; Hauser, C. A.; Zhang, S.; Lu, J. R. Molecular self-assembly and applications of designer peptide amphiphiles. *Chem. Soc. Rev.* **2010**, *39* (9) 3480–3498.
43. Shimanovich, U.; Efimov, I.; Mason, T. O.; Flagmeier, P.; Buell, A. K.; Gedanken, A.; Linse, S.; Akerfeldt, K. S.; Dobson, C. M.; Weitz, D. A.; Knowles, T. P. J. Protein Microgels from Amyloid Fibril Networks. *ACS Nano* **2015**, *9*, 43–51.
44. Stephanopoulos, N.; Ortony, J.H.; Stupp, S.I. Self-Assembly for the Synthesis of Functional Biomaterials. *Acta Mater.* **2013**, *61*(3), 912–930.
45. Solomonov, A.; Kozell, A.; Shimanovich, U. Designing Multifunctional Biomaterials via Protein Self-Assembly. *Angew Chem Int Ed Engl.* **2024**, *63*(14): e202318365.