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Understanding Recognition and Self-assembly in Biology using the Chemist´s Toolbox. Insight into Medicinal Chemistry

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Abstract: Medicinal chemistry is intimately connected with basic science such as organic synthesis, chemical biology and biophysical chemistry among other disciplines. The reason of such connections is due to the power of organic synthesis to provide designed molecules; chemical biology to give tools to discover biological and/or pathological pathways and biophysical chemistry which provides the techniques to characterize and the theoretical background to understand molecular behaviour. The present review provides some selective examples of these research areas. Initially, template dsDNA organic synthesis and the spatio-temporal control of transcription are presenting following by the supramolecular entities used in drug delivery, such as liposomes and liquid crystal among others. Finally, peptides and protein self-assembly is connected with biomaterials and as an important event in the balance between health and disease. The final aim of the present review is to show the power of chemical tools not only for the synthesis of new molecules but also to improve our understanding of recognition and self-assembly in the biological context.

Keywords: Biomimetic, conformational diseases, drug delivery, molecular triggers, peptides, protein unfolding, smart vesicles, supramolecular systems.

INTRODUCTION

From biomolecules to complex biological systems Nature reveals to us a complex and sophisticated machinery governed by recognition and self -organization with exquisite spatial and temporal control [1]. Recognition and self-assembly play a pivotal role towards biological activity, together with many other biological processes, including the trafficking of molecules to specific cellular localizations and the regulation of cellular growth [2].

Self-assembly systems [3] are characterized by their complexity, ranging from biomolecular receptor-ligand complex, through the self-assembly of microscopic virus particles [4], up to the formation of natural fibrillar macroscopic structures such as those produced by spiders and worm silk [5]. In addition to proteins, other bioorganic molecules, including lipids and nucleic acids, self-assemble into well-ordered nanostructures such as bilayers and chromatin. In this context, even the simplest organism is the result of small and huge molecules working together in a highly cooperative manner in order to be alive [1].

Medicinal chemistry is related mainly with the application of chemical research techniques to the synthesis of pharmaceuticals. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of bioactive compounds found in plants and animals [6]. Nowadays, the discovery of new bioactive molecules is more related with synthetic organic chemistry, such as combinatorial chemistry. In addition, supramolecular frameworks become to be connected with drug discovery and development, mainly due to their importance in drug transportation, aggregation and bioactivity [7, 8]. In this context, understanding self-organization could provide rational tools in the fighting against diseases, not only to understand them but also to provide effective, selective and specific drugs formulations.

The aim of the present review is to provide some examples showing the power of chemical tools not only for the synthesis of new molecules but also to improve our understanding of recognition and self-assembly in the biological context.

1. MIMICKING RECOGNITION AND SELF-ASSEMBLY BY CHEMICAL BIOLOGY TOOLS

The symphony of life is orchestrated by non-covalent interactions such as electrostatic attractions, van der Waals attractions, hydrogen bonds and hydrophobic effects. This section will provide some selected examples in order to illustrate the power of organic chemistry to mimic recognition and self-assembly with spatial and temporal control.

1.1. Double Stranded DNA as Template for Organic Chemical Reactions

Combinatorial nucleic acid libraries have provided new RNA and DNA molecules that have catalytic properties to template chemical reactions [9]. Mainly, it relies on the use of single stranded DNA sequences to template a variety of synthetically relevant transformations of small organic compounds. [10] The strategy relies on the covalent attachment of the reacting units to appropriate complementary oligonu-

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cleotides that, upon hybridization, increase the effective molarity of the reagents and thereby promote their reaction. Although Nature does not use double stranded DNA (dsDNA) templates for reactivity, the implicit recognition code provided by dsDNA, together with its unique structural features, suggest that it might offer an interesting potential for promoting chemical transformations in a programmed manner. In this context, polyamides composed of three aromatic amino acids, *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and *N*-methyl-3-hydroxypyrrole (Hp), distinguish the four Watson- Crick base pairs by a set of pairing rules [11]. Connecting the two antiparallel strands of aromatic amino acids with a gamma amino butyric acid creates a hairpin motif capable of binding to match dsDNA with increased affinity and sequence specificity [12-14] (Fig. **1**).

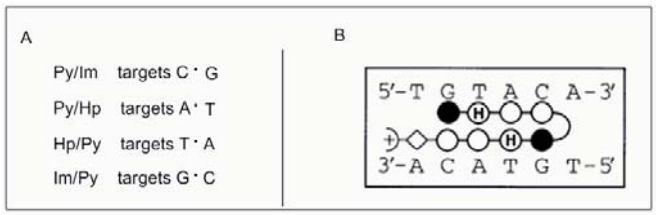

Fig. (1). A. Polyamides which distinguish the four Watson- Crick base pairs by a set of pairing rules. Binding model for the complex formed between ImHpPyPy-g-ImHpPyPy-b-Dp and a 5´-TGTACA-3´sequence. Im is black circles, Py is open circle and Hp is open circle with H.

By this approach, it would possible to accelerate the rate of ligation of two six-ring hairpin polyamides which bound adjacent sites in the minor groove via a 1,3-dipolar cycloaddition to form a tandem dimer. The rate of the reaction is dependent on DNA sequence as well as on the distance between the hairpin-binding sites [15].

Based on Dervan´s work, we focus our attention in Distamycin A, a tripirrolic natural antibiotic which exhibits interesting biological activities owing to its ability to bind AT-rich DNA sequences [16]. Although earlier reports indicated that DNA binding by this polyamide occurs along the floor of the minor groove with a 1:1 stoichiometry, later NMR studies by Pelton and Wemmer have demonstrated that Distamycin may also bind in a 2:1 binding mode [17]. In this case two drug molecules bind side by side to opposite DNA strands and induce a widening of the minor groove. Taking into account this sequence-directed dimerization which allows bringing two molecules into proximity we envisaged that an appropriate functionalization of the tripyrrole with a cysteine and a thioester might afford a dsDNA-promoted native ligation reaction. By selecting specific DNA sequences it was possible not only to increase the rate of the reaction but also to inhibit it depending on DNA sequence [18]. Recently, it has been reported the use of specific dsDNA to control and program the coupling between designed polyamides based on distamycin derivatives. Potential advantages of using dsDNA over ssDNA as reactivity templates stem from the possibility of inducing asymmetric transformations because of the intrinsic chirality of the dsDNA scaffold, the potential applications for encoding synthetic processes in natural settings without the requirement of separating the DNA strands, and the presumably easier implementation of catalytic chemistry [19].

1.2. From Transcription Factors to Designed Sequence-specific DNA-Binding Peptides

Cells receive a wide variety of cellular and environmental signals which must be processed to generate specific and timely genetic responses. The first step in the expression of genetic information, namely the synthesis of RNA from DNA template, is tightly regulated by DNA-binding proteins called transcription factors (TFs). More than 2000 TFs are encoded in the human genome. Such proteins have often been classified according to common structural elements [20]. Frequently, recognition of DNA by many transcription factors relies on short alpha helices that bind the major groove. Most TFs bind specific DNA sequences using dimeric or multimeric motifs, with more or less equal binding energy contributions from each domain in conjunction with cooperativity [21, 22]. Coiled coils in the form of basic leucine zipper (bZIP) domains are common in the TFs that control gene expression in diverse setting. More than 50 members of this family of DNA-binding protein are known in humans. For the bZIP family, TFs are able to recognize two different subdomains in each helix, a C-terminal leucine –rich area, which mediates the dimerization through a parallel coiled-coil and the basic region (BR), located at the terminus of the leucine zipper that is responsible of DNA recognition and binding. These two regions are connected through a spacer of 6 amino acids [23].

The identification of mechanisms to control DNA-binding properties of TFs would permit the regulation of their activity. A major goal was achieved in 1990 when Peter Kim's group reported that it was possible to remove the leucine zipper region of the bZIP protein GNC4, and promoted the dimerization of the remaining basic domain using a covalent disulfide bond. These GCN4 dimers were capable of recognizing and binding with nanomolar affinity to its cognate DNA sequence at low temperatures (4°C) [24]. Since then, many different strategies have been developed to mimic the interaction and recognition of FTs with their target DNA, using the GCN4 model. Some groups have proposed different approaches for covalent and non-covalent dimerization, successfully mimicking the recognition [25], and in some cases improving the selectivity [26]. Also it has been proposed a dual strategy major-minor groove interaction. This system had attached covalently a Distamycin A derivative to the basic region of GNC4 monomer [27]. Through improvements in the linkers between the basic region and the ligand, the affinity and selectivity of the system was increased, obtaining a value similar to the natural protein even at 25 ° C [28]. The major-minor groove strategy has a non-covalent approach using host-guest interaction of the couple cyclodextrin-adamantane to favor dimerization [29].

Another interesting strategy was to link these BRs through a rigid photoresponsive device such as an azobenzene moiety, capable of undergoing a substantial geometrical change upon irradiation. The Z-form bound to its cognate DNA with very high affinity, and approximately 60 -70 times more efficiently than E-isomer [30].

Another TF that belongs to bZip family is the cFos/cJun system. It has been described the formation of either Fos/Jun heterodimers or less stable Jun/Jun homodimers which bind

to the specific AP1 DNA-recognition sequences. Coiled coil interactions have been extensively studied as dominant negative peptides that target coiled coils have been designed theoretically and evolved through directed experimental approaches. For example the peptide AFosW, an analogue of FosW, shows an effective negative inhibition heterodimer binding cFos/cJun to DNA. It was possible to synthesize a FosW cross-linked with an azobenzene (XAFosW). Initially, the binding affinity of Z and E isomers was evaluated on AP-1 DNA by EMSA. In addition, the photoswitchable dominant negative XAFosW was tested *in vivo* in 293T cells, showing the possibility to photocontrol the cellular AP-1 activity [31].

Incorporation of azobencene moiety to control TFs-DNA binding was used to basic helix-loop-helix (bHLH), such as MyOD a muscle specific TF [32]. MyOD dimerizes through the HLH domain and contacts the major groove of the DNA target sequences through its N-terminal recognition [33]. The introduction of an azobenzene-moiety into the DNA-recognition helix of MyOD produced a DNA-binding protein which activity could be controlled by light pulses. In its irradiated state, in which the cross linker was predominantly in the Z configuration, significant stabilization of the recognition helix and of the specific DNA complex was observed relative to the dark-adapted state (E-form).

GCN4, Fos/Jun and MyOD are interesting examples showing the importance of specific contacts between the basic region and DNA´s mayor groove and the capability to manipulate such interactions.

Another example taken form biology is the combinatorial control of transcription factors. Considering, the massive complexity of transcription control for the large number of protein-coding genes in a eukaryotic cell presents a fundamental challenge of achieving specificity using a limited number of transcription factors. A solution to this problem has been proposed based on a combinatorial mechanism of transcription control whereby a finite number of transcription factors yield a substantial level of complexity by working in combination [34]. Recently, a peptide analogue of the basic region of the natural GCN4 TF has been obtained equipped with orthogonal functionalities at C- or N- terminal extremity allowing controllable dimerization at both extremes, directing DNA-binding to different sequences depending on dimerization. While the monomeric peptide is not functional, a C-terminal recognizes the natural binding site (5´-ATGAcgTCAT-3') and the N-terminal disulfide dimer binds preferentially to the inverse sequence (5´-TCATcgATGA-3'). These results represent the first proof of concept on the viability of making molecules that can be induced to jump between two different DNA sites, at least, in response to specific external stimuli [35].

2. DESIGN AND EVALUATION OF DRUG DELIVERY SYSTEMS

Organic chemistry has played a pivotal role towards the search for new drugs either by isolating new compounds from biological sources, in the semisynthetic process or in total synthesis of new active compounds. However, in this section it would be stressed the importance of organic amphiphilic molecules, called surfactants, in the design of drug delivery systems (DDS). In the second part, a brief and highly selective overview of selected examples of amphiphilic azobenzene-containing molecules and their use as smart materials is presented.

2.1. Colloids, Liposomes and Liquid Crystals in Drug Delivery Systems

Organic amphiphilic molecules often called surfactants are the principal components of DDS. They are able to form heterogeneous disperse systems useful for pharmaceutical formulations. Within these systems are micelles or associative colloids (supramolecular associations of surfactants), suspensions (surfactants in solid-liquid interface) and emulsions (surfactants in liquid-liquid interface, thermodynamically unstable) [36]. For many years these kinds of systems have been used to develop active molecular formulations for the treatment of human disease [37].

To improve the transport and time release of the active principle, it might be interesting to control the size of the system. Therefore, colloidal systems which have sizes between 1 nm and 1 μm are of particular interest as DDS. Among them the most promising are liquid crystals (LC) in their different forms including cubosomes, hexosomas (kind of liquid crystal matrices), liposomes (phospholipid lamellar vesicles), niosomes (lamellar vesicles of nonionic surfactants) and the micro emulsions (surfactants in liquid-liquid interface, thermodynamically stable) [38]. LC systems possess both the three-dimensional order of a crystal and the liquid disorder. LC phases, called mesophases can have positional order in one, two or three dimensions (molecules are oriented forming a lattice) and orientational order [39]. Fundamentally, LC materials are classified as being either thermotropic or lyotropic depending on whether their self-organization occurs only on heating of the pure compounds (thermotropic liquid crystals) or if it is induced by isotropic solvents at different concentrations (lyotropic liquid crystalline phases). Amphiphilic systems due to their nature are able to present both behaviors [40]. Initially, it is possible to retain the drug inside of the lattice and then it can be released from the mesophase when the LC loses its shape in a disassembly process. In fact, the chance that the system could be externally modulable is the main property used for DDS. Such systems are very useful for improving the aqueous solubility of poorly soluble active ingredients or molecules sensitive to external factors such as temperature, pH or enzymatic degradation. Recently, Amar-Yuli *et al.* [41] used hexagonal reverse phase to increase the stability of insulin. Another similar system was developed using a reversed hexagonal mesophase and enhancer amphiphilic peptides to improve transdermal permeation of sodium diclofenac, a non-steroidal anti-inflammatory drug (NSAID). The vehiculization of the active substance was accomplished by loading the cylinders of reverse hexagonal liquid crystal with the drug [42]. Interestingly, hexagonal LC systems formed by: oleyl glycerate (OG), phytanyl glycerate (PG) and glyceryl monooleate (GMO) and water as solvent were employed to transport, and thereby, improved the permeation of diclofenac. In this case, the solubility and protection of the active molecule was enhanced and the release has been controlled from the lyotropic mesophase [43]. Lynch *et al.* [44] reported that when cationic surfactants, as dioctadecyl di-

methyl ammonium chloride and dioctadecyl ammonium chloride, were incorporated to a bicontinuous cubic LC, this particular mesophase could retain anionic active substances such as Ketoprofen, another common NSAID.

In other work, Boyd *et al.* [37] reported a very interesting system made by monounsaturated glycerides. These compounds are used for performing injectable dosage forms. Therefore, the system consisted of a hexagonal mesophase acting as matrix which was able to form nanoparticles. These hexagonal nanoparticles are called hexosomas. Once the nanoparticles were obtained, they were charged with iridiotecan, a drug used in colorectal cancer. This is a good example of the importance of the mesophase´s production in preparing new dosage forms

In this direction, it has been reported the use of cubic LC to obtain cubic nanoparticles employed as DDS, called cubosomes. A variety of active ingredients which exhibited characteristics of low solubility, high degradation or low bioavailability could be incorporated in these new dosage forms [45-47]. LC characterization under well-defined intervals is an important task considering the importance of finding the specific conditions where a mesophase is stable and useful. LC diagrams could be obtained by mean of simple techniques as polarized optic microscopy (POM), dynamic scanning calorimetry (DSC), UV-Vis spectroscopy and conductivity among others. Recently, Benedini *et al.* [48, 49] described ascorbyl palmitate (AP) mesophases evaluation applying these common methodologies. AP is a potent antioxidant compound which can be compared with other natural reducing agents, such as carotenes, polyphenols, and tocopherols. Furthermore, AP was able to develop different LC regions: lamellar, cubic and gel phase depending on concentration and temperature. To corroborate some points of AP´s phase diagram, conductivity measurements were employed. Both, LC and antioxidant behavior become AP an interesting drug carrier due to the antioxidant mesophases formed with the potential protection of the hydrophobic drugs against oxidation LC behavior could be useful not only as carrier systems but also to increase intrinsic drug solubility. One interesting target drug is Amiodarone, a liposoluble drug, useful in cardiac disease. This drug behaves like surfactant presenting problems related to the intravenous administration. Amiodarone develops lamellar mesophase and different kind of aggregates in water solution. In this case, viscosity was employed to recognize self-assembly and dis-assembly of Amiodarone aggregates in water at different temperatures in order to improve its solubility in water [50].

Liposomes are another big group of self-assembled systems used for drug delivery. These systems also called lipid vesicles are colloidal particles that could be formed naturally or artificially. Liposomes resemble cell membranes in their structure and composition. They are typically made from natural, biodegradable, nontoxic and non-immunogenic lipid molecules, and could encapsulate or bind a variety of drug molecules into or onto their bilayer. Consequently, all these properties become liposomal systems attractive candidates to be used as drug-delivery vehicles for therapeutics purposes [51]. Currently, liposomes are transporters of amphotericin B, used in the treatment of systemic fungal infections and doxorubicin applied in cancer therapy [52]. Additionally, they found to be useful for skin diseases by application of the corticosteroid triamcinolone directly or by pulmonary route. Liposomal stability could be improved by anchoring of polyethylen glycol polymers in their surface [53, 54].

Other interesting vesicles systems are liquid nanovehicles based on microemulsions and emulsified microemulsions [55], novel nanosized self-assembled vehicles [56], novel nanosized self-assembled vehicles and the above mentioned lyotropic LC either cubic or hexagonal mesophases which are mainly used to solubilize non-soluble bioactive compounds. Transport could occur via transdermal or transmembrane pathway.

Finally, niosomes or nonionic surfactant vesicles (NSV's) are an interesting and promising option for developing an oral controlled release system. Niosomes are microscopic lamellar structures obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. Being non-ionic, they are less toxic than ionic ones improving the therapeutic index of the drug by restricting their action to target cells. Recently, Mehta *et al.* delineated the formulation of niosomes from biocompatible surfactant Tyloxapol and their potential use as DDS for anti-tuberculosis drugs [57].

2.2. Photocontrol of Self-assembly by Azobenzene Amphiphiles

The design and synthesis of DDS capable of releasing an active compound in response to the progression of the disease or to certain functions of an organism are particularly attractive. Smart materials can be used for achieving such a goal due to their response to certain physiological variables or external physicochemical stimuli [58]. Among these, light-responsiveness systems are receiving increasing attention because of their sensitivity to electromagnetic radiation mainly in the UV, visible and near-infrared range [59]. Interestingly, light of the specific wavelength can be applied into a precise location of the body during a certain time, thus controlling the dosage in time and space. Some light-responsive DDS are able to undergo reversible structural changes when cycles of light ⁄ dark are applied, behaving as multiswitchable carriers, releasing the drug in a pulsatile manner. To this aim, the azobenzene (AB) moiety has arguably been one of the most promising photoswitches [60].

Light-responsive DDS based AB systems have been focused mainly on colloids such as copolymer micelles and liposomes, although other photoresponsive supramolecular architectures, like LC are also under evaluation [61, 62]. AB are a class of organic compounds that can exist in two forms, namely the cis (Z) and trans (E) isomers, which can interconvert both photochemically and thermally. The photoinduced transformation is followed by a particular molecular movement and a significant geometric and electronic change, which has recognized the azobenzene unit as an excellent candidate for dynamic molecular devices [63].

Many studies have been conducted to prepare artificial photosensitive vesicles, which were also expected to be applied for drug delivery [64], and others have reported changes in the permeability of ions and/or water-soluble compounds across the membrane upon photo-isomerization [65-67].

Kuiper *et al.* [68] have evaluated the behavior of aqueous vesicular dispersions made by double-tailed phosphate azoamphiphiles and phospholipids. Two types of H-aggregates were detected by UV-Vis spectroscopy but only the most loosely packed were able to photoswitch. This study showed how the self-assembly process depended not only on the ionic strength, but also on how the vesicles were obtained. By leakage experiments using calcein as a fluorescent probe, Kupier *et al.* [68] demonstrated that leakage occurred only for mixed vesicles containing more than 20mol % of azobenzenephosphate. This system would be used to photochemical switching with the ultimate aim to steer the opening and closing of mechanosensitive protein channels of large conductance. In this context, we have presented preliminary results in the synthesis of non-ionic azoamphiphiles with potential utility as membrane photoswitch [69]. The new azoamphiphiles presented an interesting thermotropic behaviour which depended on molecular structure and geometry. In general, the smetic polymorphism observed was similar to those of ionic nature. In addition, one of them presented a lamellar mesophase at high temperature. Further, experiments are on-going to evaluate their lyotropism [70].

Hamada *et al.* [71] have studied the mechanism of morphological switching in synthetic cell-sized vesicles with a photosensitive amphiphilic molecule containing azobenzene, leading to the development of new ways to control large-scale changes in vesicles using light. They demonstrated that the photo-isomerization at the molecular level could switch the morphology when a vesicle exhibited suitable asymmetry. The mechanism of the transformation could be understood in terms of the change in effective cross-sectional area of the photosensitive molecule. Li *et al*. [72] performed the photoisomerization of an amphiphilic azobenzene incorporated in a random copolymer. The photoresponsive uniform colloidal spheres were constructed through gradual hydrophobic aggregation of the polymeric chains in THF-H_2O dispersion media. Jiang *et al.* [73] designed an azoamphiphile containing three alkyl chains composed of eighteen methylene units each which had been capped by addition of cyclodextrin (CD) through the inclusion complex formation. As the amphiphilic compound self-assembles into vesicles in water, they have studied the optical switching of the assembly and disassembly through the isomerization of the azo groups, thus affecting the complex formation with the CDs.

3. PEPTIDE AND PROTEIN SELF-ASSEMBLY SYSTEMS

Many of the naturally existing assemblies found in biological systems are constituted by proteins folded into their three-dimensional structures, highlighting the relevance of biological self-assembly for the correct function of the cell [74]. Protein folding can be produced as a co-translational process, initiated before the completion of protein synthesis. However, it can also be produced in the cytoplasm after the protein is released from the ribosome, and also in specific compartments such as mitochondria during the intracellular trafficking [75]. In this section, the use of peptide nanostructures is highlighted in technology in conjunction with the importance of protein self-assembly in the balance between health and disease.

3.1. Peptides as Biomaterials Scaffolds

During the last several years, there has been growing interest in understanding peptide self-assembly in order to translate this knowledge into biomedical and pharmaceutical applications [76]. Consequently, there has been a clear focus among scientists to design and assemble of bioinspired materials according to the "bottom-up" strategy [77-79].

A key initial application was based upon the synthesis of nanotubes via cyclic peptide self-assembly [80]. Drug delivery was also proposed as a potential use given that the nanotubes can serve as nanocontainers. Later, immobilization of peptidic nanotubes onto Au electrodes allows their utilization in nanoelectronics [81]. More recently, it has been described that cyclic peptides containing γ-amino acids were able to form transmembrane nanotubes in lipid bilayers and selectively transport alkaline ions [82].

Nowadays a growing number of peptide folding motifs are becoming understood, which mainly include the α-helical coiled coils and the β-structured amyloid-like assemblies. The α-helix is formed by winding the polypeptide backbone into a right-handed helix which is stabilized by internal backbone hydrogen bonding. But because this bonding is not enough to completely stabilize the structure, α-helices usually pack together and gain additional stabilization by hydrophobic effect and van der Waals' forces. Then it is possible that two or more of these amphipathic helices can pack together. The α-helical coil represent an extreme of this type of helix-helix packing characterized by a tandem heptad sequence that is repeated [83]. Thus, the design of α-helical coiled coils can range from simple naturally observed structures including parallel and antiparallel dimers to more complex structures such a probe that binds a cancer-associated coiled-coil protein or coiled coils that switch conformational state [77]. Several works have described for example fibrous biomaterials based on the α-helical coiled, such as the called self-assembling fibers (SAF), designed with a α-helical dimer with complementary sticky ends, that promote longitudinal assembly into α-helical coiled coils [84-86]. Recently, it has been described the utilization of two designed stranded coiled coils to target a tumor-suppressor protein implicated in colorectal cancers [87].

The other more common peptide folding motif is the β-structured amyloid-like. The amyloid fibers are large and highly ordered self-organized structures, ordered in bundles. The secondary structure of the fibers is also very uniform, mainly consisting of a cross β-sheet [88]. It was recently reported that the self-assembly of aromatic dipeptides of the core recognition motif of Alzeimer's β-amyloid polypeptide to produce well-ordered peptide nanotubes [89, 90]. The simplicity of the self-assembly into supramolecular structures was in agreement with previous works, suggesting the key role of aromatic stacking interactions in many cases of ordered amyloid fibril formation [91]. The authors proposed that geometrically restricted interactions between aromatic moieties may provide and energetic contribution and the directionality needed for the formation of well-ordered amyloid fibrils, in a process called one-dimension crystallization.

One of the main properties of supramolecular peptide assemblies to be highlighted is the ability to change the con-

formations in response to external stimuli such as temperature, pH and presence of specific small molecules [92]. By exploiting these immeasurable characteristics, natural and designed peptides can be used to engineer stimulus-responsive system, with incredible potential applications in research areas such as biomaterials, tissue engineering and drug delivery.

3.2. Protein Folding and Misfolding in Health and Disease

Since the evident importance of protein folding in nature, it is not surprising that the failure of proteins to correctly fold or to remain properly folded is the origin of a wide variety of pathological conditions. These conditions are generally called protein misfolding diseases or protein conformational diseases. They include states in which the impaired folding of a protein result in a reduction in the quantity of the protein available to play certain role. This reduction can be caused by post-translational processes such as increased degradation as a result of a quality control system, as occurs in the cystic fibrosis [93]. However, the most significant and largest group of misfolding diseases is associated with the conversion of certain peptides from the soluble functional state into highly organized aggregates, generally formed by β-sheet structures [94].

Protein conformational diseases can be classified into three groups: a) neurodegenerative conditions, in which aggregates are formed in the brain; b) non-neuropathic localized amyloidosis, in which the aggregates are formed in only one tissue but other than brain; and c) no-neuropathic systemic amyloidosis, in which the aggregates occurs in multiples organs [95]. One of the most significant cases of neurodegenerative condition associated to self-organized structures is the Alzheimer's disease, characterized by extracellular deposition of amyloid-β protein in the brain, in the form of fibrils. Other example is Parkinson's disease, where the synaptic protein α-synuclein accumulates in neural bodies and axons; and also Huntington's disease where proteins accumulate in cellular nucleus and cytoplasm [96]. All of them tend to form fibrillar or amorphous aggregates known as amyloid deposits [97].

Intrinsically disorder proteins (IDPs) and proteins with intrinsically disorder regions (IDRs) are biologically active and yet fail to form specific 3D structure, existing instead as collapsed or extended dynamically mobile conformational ensembles [98]. Proteins as α-synuclein, transactivation domain of p53 and the repeat domain of the microtubule-associated protein tau [99] are able to change their disorder structure to a β- sheet conformation when they are exposed to different environments such as temperature, ionic strength and the presence of different ligands, generating protein fibrils and inclusion bodies, generally referred as amyloid fibrils [100, 101]. Currently, a few aggregation mechanisms of the mentioned proteins have been described. One of them is the nucleation-polymerization process in which firstly, monomers associate into oligomers. These oligomers are the seeds that allow the growth, elongation and the formation of self-propagating mature amyloid fibrils and their ultimate entangled aggregates. This mechanism has been observed in alpha- synuclein [102], β- Amyloid peptide [103], and proteins implicated in cancer as p53 [104].

Inhibition of protein–protein interactions (PPIs) using small molecules were exquisite reviewed by Wilson, *et al*. [105]. A rational approach to inhibitor design was presented based on evaluation of the secondary structure at the interface, thus different classes of designed ligands which disrupt PPI selectively have emerged, including constrained peptides, foldamers and proteomimetic-derived ligands. By this approach, *in vivo* activation of the p53 pathway by small-molecule antagonists of Mouse double minute 2 homolog (MDM2), was presented. Overexpression of MDM2, an important negative regulator of p53, is found in many human tumors, effectively impairs p53 function. Inhibition of MDM2-p53 interaction by a designed small molecule stabilized p53 offering a novel strategy for cancer therapy [106].

Evidence from the last decade indicates that oligomers might be the most toxic species in the misfolding aggregation pathway, although there is also evidence about the toxicity of protofibrils. Amyloid fibers can also elicit cellular damage, but usually at much higher concentration [107-109]. Additionally, it has been described the occurrence of a novel 0.3–0.6 micrometer molecular assembly for alpha synuclein. This new stage denoted as acuna, served as nucleation, expansion and liberation of filamentous fuzzy fibre which were the precursors of mature amyloid fibrils. Cryo-electron tomography provided evidence of the acuna inner structure, which was observed as a scaffold of highly condensed colloidal masses interlinked by thin beaded threads [110].

Structural evidence has been supported by ATR-FTIR studies involving alpha-synuclein and amyloid beta peptide [111, 112]. Both, systems showed a secondary structure change when fibrillation process occurred. Prefibrillar toxic oligomers adopt an antiparallel β-sheet structure, whereas fibrils adopt a parallel arrangement.

Recently, Gustot *et al*. [113] evaluated human lysozyme aggregation which is associated with severe systemic amyloidosis. It could be possible to obtain the protein in the following states: monomer, spherical oligomers and fibrils. Interestingly, only fibrils which possess cross-β structure were able to trigger cytokine secretion. These findings suggest that cross-β form could be recognized as a generic danger signal by the immune system.

Amyloid fibrils are recognized as important entities in biological systems, however their unique physical properties and architectural complexity, represent a challenge for structure determination at atomic resolution. In this context, covalent cross-linking and mass spectrometry are appealing methods, mainly that a large amount of information can be extracted from a small sample in a single experiment. Recently, a pH-switchable system (Aβ16–22, a sequence from the amyloid-β peptide) was used to examine cross-linking chemistry in morphologically distinct supramolecular structures containing, or entirely composed of, diazirine-functionalized peptides [114].

Another interesting type of self assembly in proteins is the formation of coacervates. Ostuni, A *et al.* [115] described that natural stable proteins as elastin, make aggregates that are generated by coacervation. This self-assembly process represents that the protein comes out of solution as a second phase on an increase in solution temperature. The ability to

undergo coacervation is related to the predominantly nonpolar character of the protein. A similar behaviour was observed in gliadin but here the coacervation process was dependent of pH [116].

Conformational changes and autoaggregation ability have been also observed in extrinsic proteins that we incorporate in the diet which can lead to inflammation and activate the autoimmune response. The most important is Creutzfeldt–Jakob disease, a human protein infection caused by the ingestion of infected meat. This disease is characterized by brain deposition of an insoluble, protease-resistant isoform of the host-encoded cellular prion protein (PrPC), named PrPSc, which generates amylogenic depositions that are observed with congo red in histological brain preparations [117]. Another alimentary disease is related with gluten present mainly in wheat, barley and rye. The ingestion of these protein complex, mainly gliadin and glutenin, induce alimentary disorders such as celiac disease (CD), allergy (WA) and, gliadin sensibilty (GS) [118]. CD and WA result from innate and adaptive immune system dysregulation due to gliadin protein. Besides CD and WA there are cases of gluten reactions in which neither allergic nor autoimmune mechanisms are involved, those are clasified as GS. Despite the immunological responses, the three disorders are connected at early stages, when gliadin protein or its proteolytically resistant fragments [119] are able to reach the intestinal epithelium. One of them is 33 mer which is considered the most immunogenic derived by gliadin protein [120]. Recently, it has demostrated that 33-mer gliadin peptide is able to self- assemble into nanospheres which align into fractal linear arrays above a certain concentration under physiological conditions. Moreover, the 33-mer fragment adopts a polyproline II (PPII) structure at a concentration of 100 μM while further increase of the peptide concentration to 600 μM led to a conformational transition to β-turn structures. An initial self-assembly model based on classical electrostatics and molecular dynamics calculations has been proposed [121]. Currently, our efforts are directed toward understanding the role of the supramolecular assemblies in gliadin related disorders.

Another system which aggregates posssess PPII structure is Amelogenin, a protein present in dental enamel. This protein is able to generate nanospheres and fibrils which are essential in their functional behaivor [122]. Recently, Beniash *et al*. [123] demostrated by FT-IR that amelogenin aggregates contain large portions of extended intramolecular beta-sheet structure and PPII.

CONCLUSION

Chemistry is the discipline which posseses the methods and knowledge to connect biology with physics. In this context, understanding recogntion and self-assembly in biology can open new oportunities to manipulate biological or even better pathological events externally, with the potential impact to mantain or restore health.

Currently, epigenetics research [124] has opened new avenues towards a best comprehension of how external signals are involved in the fine balance between health and disease. In this new scenario, as Nature does, medicinal chemistry needs to work in a highly cooperative manner by using the newest molecular tools directed towards translational research in order to contribute to society's well-being.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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