Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares

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Abstract: The self-assembly of peptides and proteins frequently shows structural plasticity depending on subtle alterations. The acquisition of structural data of these peptide assemblies with atomic level precision will be crucial to understand and predict assembled morphologies.

Despite recent important advances in the field, the prediction of the folding and aggregation of peptides and proteins remains to be a fundamental challenge shared by chemists, material scientists and crystallographers. From a joint perspective, all are interested in how the molecular information — by amino acid sequence or molecular structure — defines a network of cooperative forces that drives the system towards the assembly of functional structures. However, even simple synthetic peptides, which can be programmed for precise folding, can challenge the actual state of the art in assembly prediction. General predictive strategies capitalizing on the preorganization of the

peptide backbone have been developed such as for the Phe-Phe motif.[1] Still, fluctuation between polymorphic structures can be observed, generally after small environmental changes such as heat or concentration of salt or protons.[2] Such structural plasticity suggests that even simpler peptides can show a complex energy landscape, and a potential extrapolation as simplified aggregation models might imply certain inaccuracy. Therefore, the elucidation of the molecular mechanisms that govern morphological transitions requires a detailed picture of the peptide folding and the corresponding non-covalent interaction network. Even for certain short peptides, only qualitative assembly models are possible due to the complexity of the potential aggregation trajectories.[3]

The complex energy landscape of peptide and protein assembly can be shaped by different local minima that coexist within the global potential well, and subtle alterations can lead to completely different assembly modes.[4] This situation can trigger peptide and protein misfolding and aggregation, which can have dramatic consequences, as it has been found for polymorphic amyloid fibers. The wrong interpretation of low-resolution structural data for native and synthetic protein filaments can frequently lead to incomplete or even erroneous models.[5] Such potential misinterpretations can affect the mechanistic understanding of biomolecular self-assembly, which could also have important implications in pathogenic phenomena. For example, detailed studies of purified fibrils obtained from Alzehimer's brain tissue revealed that different polymorphs can be obtained by different face-to-face electrostatic interactions of similar protofilaments.[6] All these recent discoveries highlight the clear necessity of acquiring precise atomic structural information of the assembled states of peptide and proteins monomers to achieve robust mechanistic insights that would allow supramolecular rational design.

Acquiring high resolution structural information for both complex and simplified molecular assemblies is a key aid to understand how the molecular structure determines the aggregation

morphology. Traditionally, X-ray crystallography and nuclear magnetic resonance (NMR) have been widely employed in the structural determination of protein and peptide assemblies. Nevertheless, limitations such as the complex crystallization of certain molecules for X-Ray or the need of high concentrations and the limited long-range distance information for the NMR, hinders the precise and fast structural elucidation of peptide and protein supramolecular systems.[7] Recent achievements in cryogenic electron microscopy (Cryo-EM) — recognized with the 2017 Nobel Prize in chemistry to Jacques Dubochet, Joachim Frank, and Richard Henderson — have shaken the field of structural characterization.[6,8,9] Cryo-EM allows the acquisition of the different folded and even functional states of newly discovered peptides and proteins at atomic resolution, which facilitates mechanistic elucidation and accelerates potential therapeutic development. Cryo-EM structural reconstruction can also help in resolving the conformation of natural and *de novo* peptide supramolecular structures, which would then provide the critical feedback required to match structural design and assembly assessment.[8]

In the article published by Wang *et al*,[9] a step forward is taken towards detailed understanding of the self-assembly of an amphiphilic octapeptide monomer (Ac-FKFEFKFE-NH_2, KFE8). Surprisingly, KFE8 exhibits a much more complex organization pattern than could be *a priori* predicted. Previously reported molecular dynamics models postulated a preferential helical ribbon assembly for the KFE8 peptide, composed of double anti-parallel β-sheets with inner and outer layers and having similar H-bonding pattern.[10] Intriguingly, Cryo-EM reconstruction with atomic resolution of the nanotubes formed by KFE8 peptides revealed a double-walled structure composed of outer anti-parallel and inner parallel peptide stacks. Cryo-EM confirmed the presence of two different nanotube morphologies — namely thinner and wider helical nanoribbons — with distinctive structural symmetry. The Cryo-EM reconstruction also allowed an accurate description

of the peptide monomeric interactions in different organization patterns within the same supramolecular structure, which suggested the emergence of chaotic nucleation events that would depend on subtle external factors. This unambiguous experimental finding opens an intriguing dilemma for supramolecular chemistry. If the evolution of simple supramolecular systems can be subjected to chaotic monomer nucleation, would then precise deterministic prediction be possible for complex supramolecular systems?

Supramolecular assemblies are integrated by molecular elements that should interact with each other according to certain defined rules. However, once even the simplest rules are established, highly complex assemblies can emerge that would depend on all the different molecular and physical players contributing to the system free energy interplay. As chemists and materials scientists, we intend to understand molecular assembly by mastering the entropic pathways towards synthetic supramolecular surrogates of nature's functional structures. However, robust design premises are required for synthetic peptide supramolecular systems in order to reveal nature's assembly rules and thus enable a superior predictive control. Accurate description of nucleation and amplification process of polymorphs as well as the reconstruction of their free energy rugged landscape are essential not only to understand the underlying assembly principles, but to provide the opportunity to predict the supramolecular assembly of synthetic and natural systems. The article by Wang and coworkers constitutes one step ahead in the understanding and future design of peptide complex assemblies. We expect that the observation of supramolecular peptide structures under the new light of Cryo-EM will provide more exciting surprises and eventually, the keys to understand diversity as represented in polymorphism of peptide and protein supramolecular assemblies.

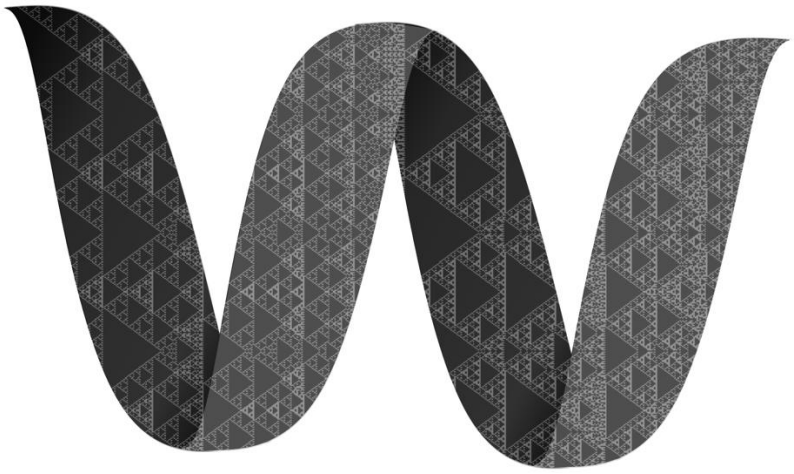

Figure 1. Emergence of chaotic assembly patterns in supramolecular peptide nanostructures.

Acknowledgment. We thank the AEI [PCI2019-103400, PID2020-117143RB-I00], the ERC (DYNAP-677786, TraffikGene-838002), the HFSP (RGY0066/2017) and the Oportunius (Gain).

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