Self-assembly

https://doi.org/10.1038/s44160-023-00345-z

Synthetic pathway dictates supramolecular structure

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Supramolecular assemblies of peptides with differing morphologies and properties are formed by varying the synthetic route to the fibrous assemblies.

In the synthesis of molecules, one product usually has only one set of properties. When molecular self-assembly of a product occurs, however, this scenario can be more complex.

In self-assembly, non-covalent bonds like hydrogen bonds, van der Waals interactions, and the hydrophobic effect combine molecules into supramolecular constructs. The interplay between these supramolecular interactions can lead to hysteresis effects, resulting in a scenario where one molecule assembles into various morphologies. Specifically, one molecule can occupy different thermodynamic minima within an energy landscape depending on its supramolecular configuration. These minima, each with unique structural and material properties, can be accessible through different assembly pathways. Now, writing in *Nature Synthesis*, Weil, Ng and co-workers report a self-assembling disulfide peptide with varying properties depending on its synthetic pathway[1]. This finding implies that both molecular design and synthetic pathways should be considered when developing supramolecular materials.

Nature already uses such pathway complexity to diversify its product library without changing the chemical identity. Protein folding is a perfect example of such pathway complexity. Within the folding funnel, numerous minima exist that should be accessible by different synthetic pathways that include the order of the folding steps, the role of chaperones, the environment of the protein transcription, and post-translational modifications. Each minimum is associated with a unique secondary and tertiary structure of the same protein with vastly different properties. With the increasing complexity of supramolecular building blocks, pathway complexity has also been identified as critical in non-biological systems. For example, for building blocks that simultaneously undergo multiple strong non-covalent interactions, molecules can remain trapped in local minima in the energy landscape that are not necessarily the global minimum[2–4]. Thus, the preparative pathway of these assemblies affects their morphology and, with that, the resulting material properties. While such systems have been reported[2–4], the interplay between covalent and non-covalent interactions and their influence on pathway complexity has yet to be explored in detail.

Weil, Ng and co-workers report an elegant reaction cascade that displays pathway complexity in a system where both covalent and non-covalent interactions play a role. The four-step cascade is initiated by the photolytic removal of a protecting group from a peptide which liberates an amine and a nitroso remnant of the protecting group (Fig. 1). The amine undergoes a native chemical ligation reaction with a neighbouring thioester, forming a peptide bond and a thiol. In the third step, two thiol-containing peptides oxidize by reacting with the nitroso remnant to form their corresponding disulfide. This synthetic pathway is referred to as the cascade pathway. In the last step, the disulfide peptide assembles and fibres form. Kinetic analysis reveals that the chemical reaction cascade occurs in situ and quantitatively yields the disulfide-containing product after 24 hours. In addition, transmission electron microscopy shows that the disulfide-containing peptide produced by the reaction cascade assembles into fragmented fibres, with a strong tendency to form localized clusters among a few mature fibres.

The synthetic pathway can be modified slightly by introducing dithiothreitol (DTT). Whilst this pathway yields the same peptide, the presence of DTT initiates redox cycling between the thiol-containing peptide and the disulfide-containing product, thus delaying the conversion into the final disulfide-containing product: this pathway is the cycled-cascade pathway. Given that the product of the cycled-cascade pathway is the same as the cascade pathway, it is expected that these would be the same self-assembled product. Yet, the cycled-cascade pathway yields fibres with a greater persistence length than the fibres formed by the cascade pathway (Fig. 1). Besides the difference in the hierarchical morphology, the supramolecular interactions between the peptides differ too, which were quantified by spectroscopic assays.

The two synthetic pathways yield the same disulfide peptide product but with vastly different morphology and likely differing material properties. The differing morphologies of the cycled-cascade pathway compared to the cascade pathway is likely a result of the delayed production of the disulfide peptide. Yet, the underlying mechanism explaining how each synthetic pathway yields a unique morphology is not fully understood. These findings demonstrate that the chosen pathway determines the supramolecular structure and hierarchical morphology. In other words, different hierarchical structures can be obtained from the same chemical identity. Unlike recent works in which concentrations, solvents or temperature dictate the assembly pathway[5–7], Weil, Ng and co-workers use synthetic pathways, more specifically, reaction cascades, to dictate and control structural order. Here, in situ, controlled, and reversible production of assembly intermediates guides structure formation. Given the broad range of chemical reaction networks, such pathways offer more versatility to obtain different hierarchical structures and materials without changing the overall environmental conditions such as temperature, solvent or concentration.

Supramolecular materials — defined as materials in which the self-assembly of molecules dictates function — have become widely applied. Supramolecular polymers in self-healing rubbers, materials for regenerative medicine, and liquid crystals in liquid-crystal displays are only a few examples of successful supramolecular materials[8–10]. For such materials, it is centrally believed that one product forms one assembly, thus one material. However, recent studies[2–4] and the study of Weil, Ng and co-workers challenge this central belief. The findings of the latter study reveal that the synthetic pathway towards a self-assembled product should be carefully considered when designing

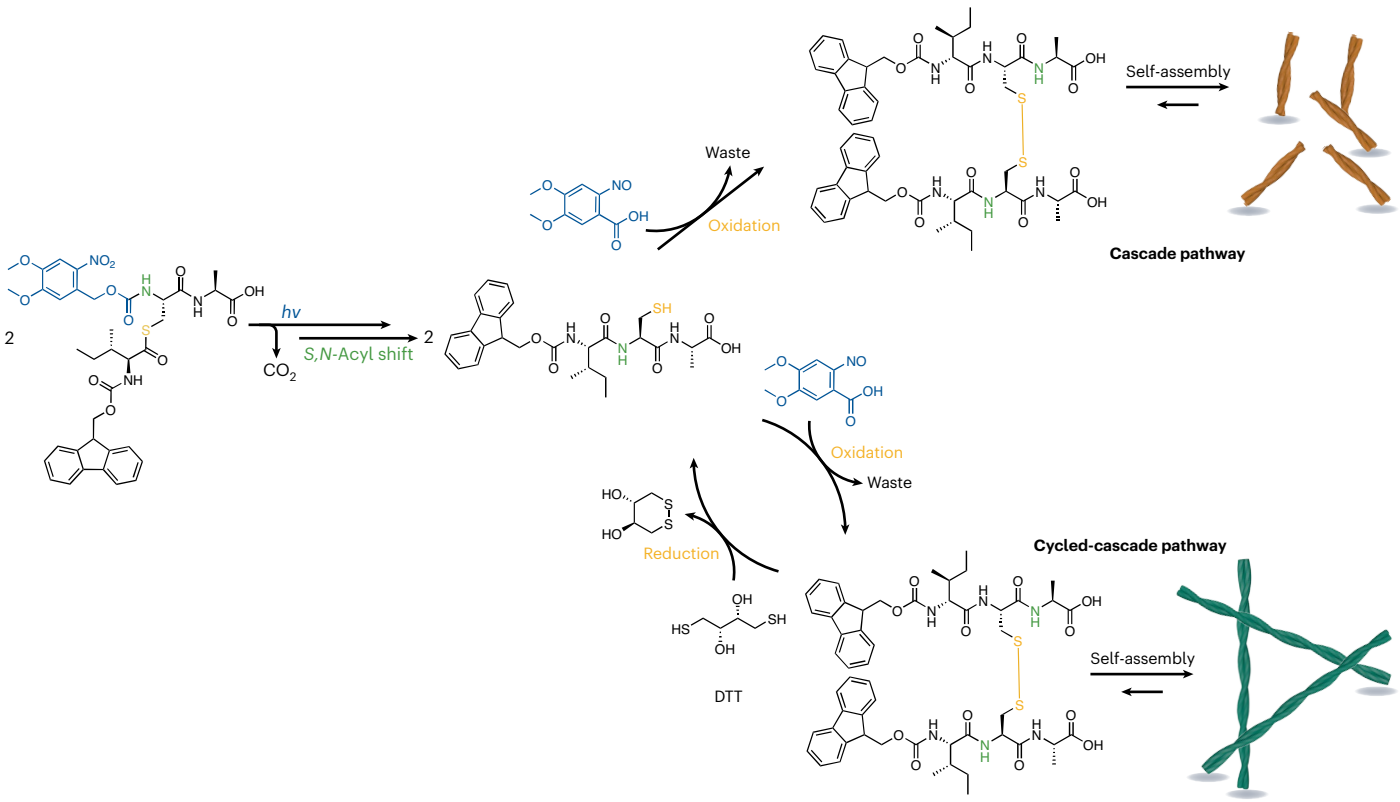

Fig. 1 | The synthetic pathway guides the supramolecular structure and hierarchical morphology. The photolytic removal of a protecting group from a peptide initiates a four-step cascade that yields a thiol-containing peptide via a *S*,*N*-acyl shift. Two thiol-containing peptides oxidize by reacting with the nitroso by-product of the photolytic cleavage to form the corresponding disulfide. In the last step, the disulfide peptide assembles (the cascade pathway). The presence of reducing agent dithiothreitol (DTT) initiates redox cycling between the thiol-containing peptide and the disulfide product, thus delaying the conversion into the disulfide product (the cycled-cascade pathway). Although both products are chemically identical, they assemble in different morphologies, directed by the synthetic pathway.

new materials. In the future, studies to understand the mechanisms in the described pathway complexity are warranted to incorporate this feature into supramolecular materials. For example, how the reaction dynamics influence the coassembly of the thiol and disulfide product could lead to new insight into the mechanisms at play. Moreover, the combination of photochemistry, native-chemical ligation, and redox cycling could be designed to yield products beyond fibre-forming peptides; for example, micelles, vesicles, or liquid crystals. Such assemblies and their pathway complexity can lead to appealing functional materials. For example, peptide fibres have been explored for their ability to form hydrogels which, in turn, can support the growth of cells. Synthetic pathway complexity thus offers a useful toolbox for developing supramolecular materials in future.

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Published online: 8 June 2023

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Competing interests

The authors declare no competing interests.