PEPTIDE NANOSTRUCTURES

Aromatic dipeptides light up

Metal coordination and π–π stacking interactions drive the assembly of dipeptides into nanostructures with superior optical properties.

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Peptides (including dipeptides — those with just two amino acids) are known to contain all the molecular information needed to efficiently and rapidly form ordered assemblies of various architectures at the nanoscale[1]. Dipeptide nanostructures have unique characteristics. They are mechanically rigid, have piezoelectric coefficient values that are comparable to the finest inorganic piezoelectric materials, and have quantum confinement-based blue luminescence[2]. Moreover, they are semiconducting in a quantum-dot organization[3]. Inspired by the spontaneous formation of a high quantum-yield family of fluorophores derived from green fluorescent protein (GFP), writing in *Nature Nanotechnology*, Mingjun Zhang and colleagues at The Ohio State University report a nanoscale dipeptide-based optical detection system[4].

The GFP family is one of the most important systems used for lighting up biological macromolecules in living environments[5,6]. Following the initial structural characterization of the native GFP, many variant proteins have been developed. Through minor modifications — as simple as single amino acid replacements — in the amino acid sequence of GFP (which is composed of 238 residues), the emission spectra of mutant proteins can be altered to form the yellow fluorescent protein, cyan fluorescent protein, and the blue fluorescent protein. Furthermore, other optical parameters of the proteins, such as quantum yield and sensitivity to external stimuli, can be modulated by fine tuning the GFP amino acid sequence.

Motivated by the extensive work on GFP, Zhang and co-workers combined concepts from peptide self-assembly and GFP molecular engineering to create simple dipeptide conjugates that mimic the optical features of GFP-derived proteins. The dipeptide conjugate consists of tryptophan and phenylalanine aromatic amino acids coordinated to Zn(II). This design is similar to another GFP analogue, the zinc ion-binding BFPms1 mutant, whose optical properties are enhanced by the spatial stabilization and rigidification of its excitable electronic system through metal coordination. Zn(II) coordination in the dipeptide conjugates improves the optical features of the conjugates in a similar way. Furthermore, π–π stacking interactions between aromatic moieties like those observed in the yellow fluorescent protein facilitate the self-assembly of these short aromatic peptides into nanoparticles.

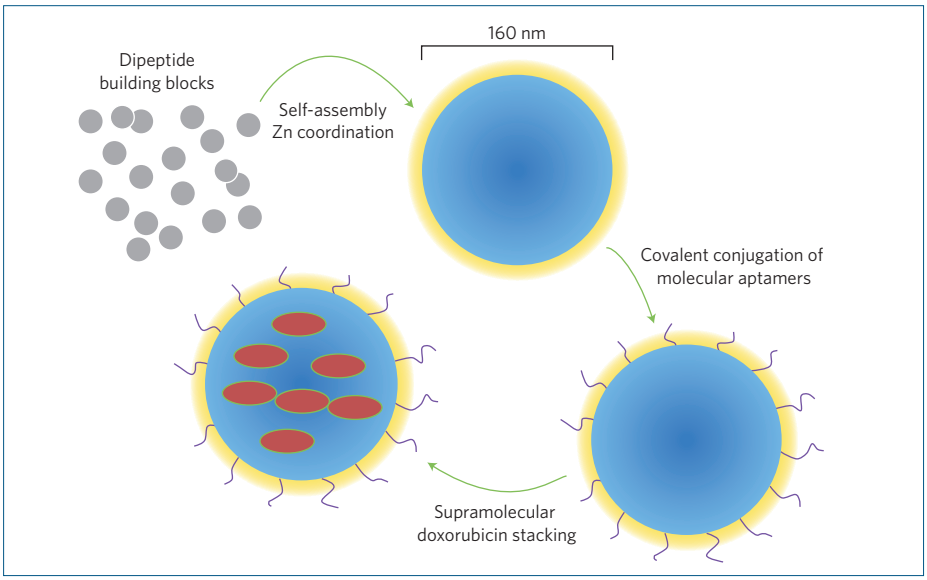

Figure 1 | The assembly of a tri-component system for monitoring cell targeting and drug release. The simple tryptophan–phenylalanine dipeptide (grey circles) can self-assemble to form ordered nanoparticles in the presence of zinc. The stabilization of the peptide with zinc ions results in fluorescent nanoparticles with an average diameter of 160 nm (blue circle). The chemical properties of the peptide nanoparticles allow covalent attachment of aptamers (purple lines) and supramolecular stacking of planar aromatic drugs such as doxorubicin (red ovals).

The newly developed metal-coordinated dipeptide nanoparticles have narrow emission bandwidth and notable photostability. It is suggested that the intense fluorescence is due to a quantum confinement effect similar to other dipeptide assemblies[3,7]. Yet, the improved fluorescence is most likely due to the spatial stabilization of the tryptophan indole group by the zinc ion, as reported for the Zn(II)-stabilized GFP analogue.

Zhang and co-workers showed that these luminescent dipeptide nanoparticles can be used to image the dynamic release of drugs in cancer cells. This is accomplished by covalently attaching the dipeptides to aptamers that specifically recognize the MUC1 cell membrane protein on cancer cells (Fig. 1). Furthermore, the anti-cancer drug doxorubicin is bound to the dipeptides through aromatic stacking interactions. The aptamer targets the dipeptide nano-assemblies to the cancer cell and drug release is monitored by following the changes in fluorescence of both doxorubicin and the dipeptides. The fluorescence of doxorubicin and the dipeptides is quenched after binding to each other, but is re-established when doxorubicin is released from the dipeptides when inside the cells.

Compared with inorganic quantum dot-based structures, the dipeptide conjugates have many advantages. Being composed of two natural amino acids, it is inherently biocompatible. Furthermore, through simple synthetic organic chemistry reactions, the dipeptide nanoparticles can be easily modified for various applications. Moreover, besides doxorubicin, a variety of other

aromatic drug molecules can be loaded into these nanoparticles through aromatic stacking interactions.

This study paves the way for a new front in peptide optics. The ability of very short peptides to form ordered structures at the nanoscale is one of the most studied directions in organic nanotechnology, and a handful of small aromatic peptide building blocks have been identified and characterized[8]. It is likely that a much larger repertoire of building blocks could be exploited. The tryptophan–phenylalanine dipeptide shown by Zhang and co-workers may be the only example of dipeptide that could form GFP-like assemblies. However, similar to the discovery and structural characterization of GFP that led to the development of an entire family of modified fluorescent proteins with different colours and fluorescent properties, it is expected that this new work could be extended to other aromatic building blocks that could lead to the identification of additional fluorescent peptide nanoparticles.

The use of peptides for optical and electro-optical applications is an important direction. For example, it has been shown that dipeptide nanotubes could function as waveguides[9], and the modification of such assemblies could transform them into light-harvesting systems that mimic photosynthesis in plants[10]. The current work provides a new and important tool for such applications, and the conceptual framework of metal coordination of other dipeptide assemblies could significantly extend the scope of such applications. These tools could significantly impact the fields of biotechnology and biomedical imaging, and also non-biological related applications, such as the fabrication of photovoltaic cells and light-emitting devices. ❐

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