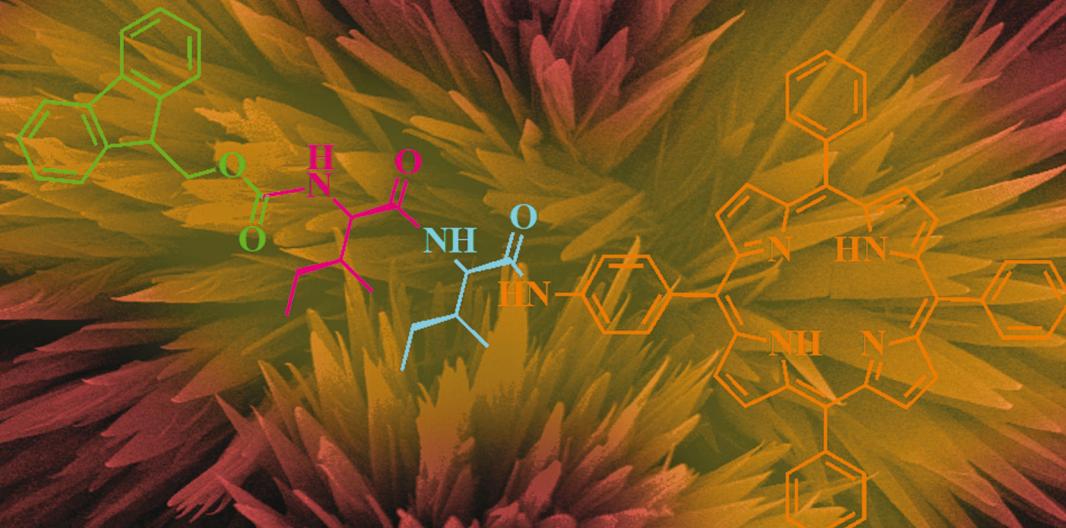


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Self-assembly of aliphatic dipeptides coupled with
porphyrin and BODIPY chromophores



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In this work, the self assembly ability of chromophores covalently linked to aliphatic dipeptides is described. Altering various parameters such as the protecting group, the solvent mixture, the dipeptide and the chromophore resulted in different nanostructures. Interestingly, a peptide–porphyrin hybrid is capable of forming a hydrogel in HFIP–water solvent mixture.

Living natural systems utilize self-assembly in order to fulfil their functions; thus, understanding the basic aspects of this process takes us a step closer to the comprehension of life.¹ Numerous examples in nature have inspired supramolecular scientists to synthesize molecules with self-assembling ability aiming at the construction of materials with improved properties.² Self-assembled architectures that mimic chlorophylls and bacteriochlorophylls in nature are of great interest for light harvesting applications.³ A variety of bioinspired building blocks have been explored for the fabrication of self-assembling molecules including peptides,^{4,5} nucleic acids,^{6,7} peptide nucleic acids,^{8,9} lipids *etc.* Of the small peptides, diphenylalanine (FF), the basic structural motif for the Alzheimer's beta amyloid polypeptide, is an aromatic dipeptide that has been extensively investigated for its self-assembling properties.^{10,11} More specifically, a great number of chromophores such as porphyrins,^{12–15} boron-dipyromethenes,¹⁶ corroles,¹⁷ polyoxometallates¹⁸ and ferrocene¹⁹ have been covalently connected to FF resulting in hybrids that retained the ability to form well-defined nanostructures. In addition, small aliphatic peptides have also been investigated for their self-assembly ability.²⁰ In a recent example, Yan and co-workers reported that a short peptide based on

Self-assembly of aliphatic dipeptides coupled with porphyrin and BODIPY chromophores†

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Isoleucine was employed to induce the self-assembly to hydrophobic porphyrins, which were used as photosensitizers in two-photon PDT.²¹ Light harvesting applications for self-assembled porphyrins have also been reported, such as photocatalytic hydrogen generation in the presence of platinum nanoparticles.²² Moreover, the self-assembling ability of short peptides can lead to the formation of hydrogels, which can improve the properties of chromophores.²³ Hydrogels that contain porphyrin chromophores can be used for hydrogen production,²⁴ water oxidation,²⁵ photodynamic therapy,²⁶ fluorescence-guided monitoring²⁷ and photocurrent generation.²⁸ However, in the above hydrogel systems, the chromophore was not covalently attached to the peptide gelator molecule.

In this communication, we report the self-assembly ability of porphyrin and BODIPY hybrids that are covalently coupled to aliphatic dipeptides bearing various protecting groups (Scheme 1). Namely, Ile-Ile and Ala-Ile dipeptides with Boc-, Fmoc-, Z-, and -OMe protecting groups were investigated using the “good–bad” solvent self-assembling protocol, leading to distinctive supramolecular architectures. One of the hybrids, **Fmoc-Ile-Ile-TPP** formed a hydrogel in HFIP–water solvent mixture. To the best of our knowledge, this is the first example of hydrogel formation by a single hybrid dipeptide–porphyrin molecule and this opens new avenues for possible applications of these self-assembled hybrids.

The synthetic approach for the preparation of **Boc-Ala-Ile-TPP** and **Boc-Ile-Ile-TPP** is illustrated in Scheme S1 (ESI†). The first step is the N-protection of the commercially available dipeptides (Ile-Ile-OH and Ala-Ile-OH) with Boc₂O in the presence of NaHCO₃, followed by the covalent connection with TPP-NH₂ using DCC/HOBt amide coupling reagents. The synthesis of **TPP-Ile-Ile-OMe** begins with the methyl ester-protection of the C-terminus of Ile-Ile-OH dipeptide with SOCl₂ and MeOH followed by an amide coupling reaction with TPP-COOH. In order to successfully prepare the Fmoc protected hybrids, it is necessary to “construct” the dipeptide onto the porphyrin macrocycle starting with an amide coupling reaction between Fmoc-Ile-COOH and TPP-NH₂. Subsequently, we removed the

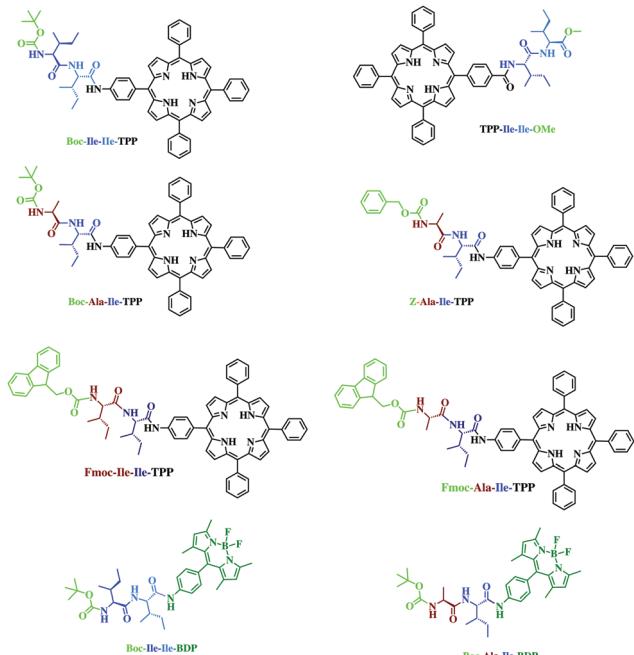
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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9cc06125h





Scheme 1 Structures of **Boc-Ala-Ile-TPP**, **Z-Ala-Ile-TPP**, **Boc-Ile-Ile-TPP**, **TPP-Ile-Ile-OMe**, **Fmoc-Ala-Ile-TPP**, **Fmoc-Ile-Ile-TPP**, **Boc-Ala-Ile-BDP** and **Boc-Ile-Ile-BDP**.

Fmoc protection group using piperidine in DMF yielding the $\text{NH}_2\text{-Ile-TPP}$ hybrid. The final step is the covalent connection of the hybrid *via* amide coupling to the Fmoc-Ala-COOH or Fmoc-Ile-COOH resulting in **Fmoc-Ala-Ile-TPP** or **Fmoc-Ile-Ile-TPP**, respectively (Scheme S2, ESI[†]). In addition, the Boc-protected dipeptides were also coupled with boron-dipyrromethene chromophores following an experimental approach similar to the preparation of the corresponding porphyrin hybrids (Scheme S3, ESI[†]). All the final and intermediate compounds were fully characterized with ^1H and ^{13}C NMR spectroscopies (Fig. S1–S16, ESI[†]).

The self-assembly behavior of the synthesized bio-conjugates was examined through Scanning Electron Microscopy (SEM). For the preparation of the samples, the corresponding compounds were dissolved in a chaotropic “good” solvent, namely 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), and subsequently a “bad” solvent was introduced such as ethanol (EtOH), methanol (MeOH) or water (H_2O). Noteworthily, the “bad” solvents were utilized in order to induce the self-assembly process. We investigated the effect of various parameters on the formation of the self-assembly, such as the protecting group, the nature of the dipeptide, the solvent mixture as well as the ratio of the “good”/“bad” solvent.

As it is illustrated in Fig. 1a, the **Boc-Ile-Ile-TPP** hybrid assembled into uniform spiky spheres by using the HFIP–MeOH 2 : 8 solvent system at 1 mM final concentration. The supramolecular architectures were formed within an hour after the addition of the bad solvent and did not evolve either for the next 24 h or the following week, proving the inherent stability of the system (Fig. S17, ESI[†]). By simply changing the “bad” solvent from MeOH to EtOH different morphologies on the self-assembled structures were obtained. To be more specific, **Boc-Ile-Ile-TPP** gave rise to flake – shape structures in HFIP–EtOH 2 : 8 mixed solvent system

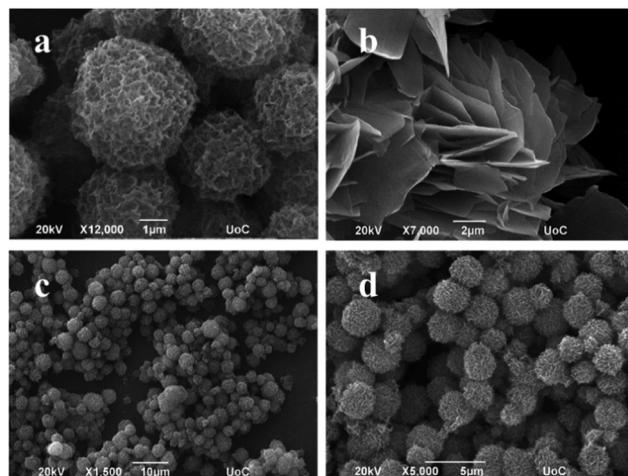


Fig. 1 Self-assembly behavior of (a) **Boc-Ile-Ile-TPP** in HFIP–MeOH 2 : 8, (b) **Boc-Ile-Ile-TPP** in HFIP–EtOH 2 : 8, (c) **Boc-Ala-Ile-TPP** in HFIP–MeOH 2 : 8 and (d) **Boc-Ala-Ile-TPP** in HFIP–EtOH 2 : 8.

at 1 mM final concentration after 24 hours (Fig. 1b). Notably, the flakes were not observed after one hour, and thus their formation needs more time than the spiky spheres, but remained stable also after one week (Fig. S18, ESI[†]). Well-dispersed spherical assemblies with uniform size were observed by using water as a “bad” solvent. Interestingly, the size of these spheres was larger than the spheres obtained with MeOH, but lacked the spiky surface (Fig. S19, ESI[†]).

We replaced one of the two isoleucine amino acids with an alanine and the **Boc-Ala-Ile-TPP** hybrid assembled into uniform spiky spheres by using HFIP–MeOH and HFIP–EtOH 2 : 8 solvent systems (Fig. 1c and d) while the use of water did not result in defined self-assemblies (Fig. S20, ESI[†]). The replacement of Boc- protecting group with the Z-(benzyl carbamate) **Z-Ala-Ile-TPP** showed also uniform spheres with spiky surfaces in the same examined conditions (Fig. 2 and Fig. S21, ESI[†]).

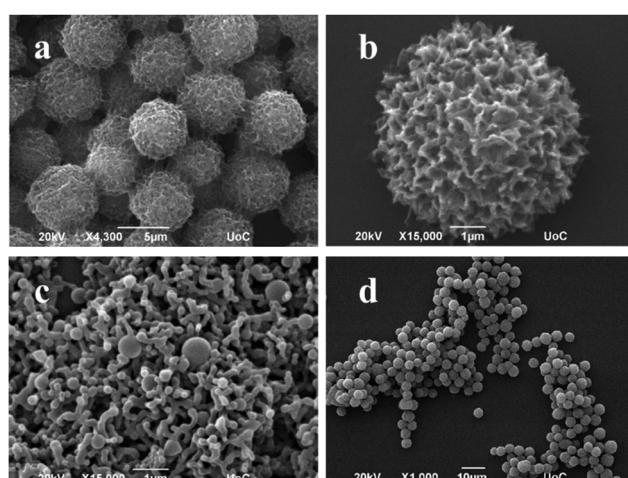


Fig. 2 Self-assembly behavior of (a) **Z-Ala-Ile-TPP** in HFIP–MeOH 2 : 8, (b) **Z-Ala-Ile-TPP** in HFIP–EtOH 2 : 8, (c) **Fmoc-Ala-Ile-TPP** in HFIP–MeOH 2 : 8 and (d) **Fmoc-Ala-Ile-TPP** in HFIP–EtOH 2 : 8.



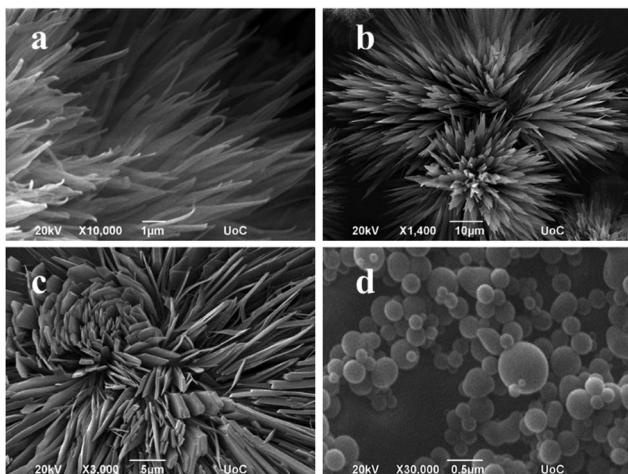


Fig. 3 Self-assembly behavior of (a) **Fmoc-Ile-Ile-TPP** in HFIP-MeOH 2:8 and (b) **Fmoc-Ile-Ile-TPP** in HFIP-EtOH 2:8, (c) **TPP-Ile-Ile-OMe** in HFIP-MeOH 2:8 and (d) **TPP-Ile-Ile-OMe** in HFIP-H₂O 2:8.

The influence of another aromatic protecting group, Fmoc, was investigated as well. The **Fmoc-Ala-Ile-TPP** hybrid (Fig. 2 and Fig. S22, ESI[†]) was able to form spherical architectures in HFIP-MeOH and HFIP-EtOH 2:8 solvent conditions. In the above cases of Ala-Ile dipeptide hybrids, no flakes were obtained and water was not an appropriate solvent for the formation of self-assembled nanostructures.

A completely different supramolecular architecture was observed in the case of the **Fmoc-Ile-Ile-TPP** hybrid (Fig. 3a, b and Fig. S23, ESI[†]). It was able to form spiky nano-assemblies, which is not common in these kinds of systems, after dissolving in HFIP and using MeOH or EtOH as a bad solvent. Surprisingly, by using water as a “bad” solvent, hydrogels were formed (Fig. 4a, b and Fig. S24, ESI[†]). Hydrogels consist of an interconnected fibrillar network with high water content (Fig. 4b) and can be used in many biomedical applications. This was an unexpected result since, as far as we know, it is the first chromophore molecule that forms a hydrogel in the absence of any other gelator molecules. Moreover, we examined the effect of protecting the carboxyl terminus of the dipeptide, as far as it may influence the self-assembly process. More specifically, the **TPP-Ile-Ile-OMe** hybrid was studied in HFIP-MeOH 2:8 and HFIP-EtOH 2:8 and formed flakes by applying MeOH or spheres when EtOH was used as a “bad” solvent (Fig. 3c and d).

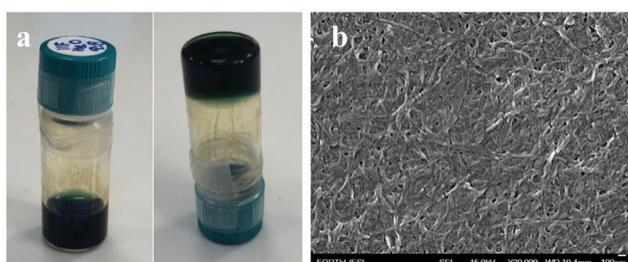


Fig. 4 (a) Photographs of the hydrogel formed from the **Fmoc-Ile-Ile-TPP** hybrid in HFIP-H₂O 2:8 solvent mixture at 1 mM final concentration. (b) FESEM image of the formed hydrogel.

The above results provide real evidence that the choice of the protecting group, the nature of the dipeptide and the solvent mixture can lead to distinctive supramolecular architectures. One can conclude that the Ile-Ile dipeptide is able to induce more distinctive architectures, namely spheres, flakes and spikes, while Ala-Ile can lead only to spherical assemblies.

The self-assembling behavior of another chromophore, namely boron-dipyrromethene, was also investigated in this work. Therefore we synthesized hybrids **Boc-Ala-Ile-BDP** and **Boc-Ile-Ile-BDP** and tested their behavior in the same solvent systems as the corresponding porphyrin hybrids. Interestingly, no self-assembling ability was observed in HFIP-EtOH and HFIP-MeOH mixtures (Fig. S25, ESI[†]). This can be attributed to the partial solubility of BODIPY molecules in ethanol and methanol. Since BDP-dipeptide hybrids are quite soluble in EtOH and MeOH, there is not an actual “bad” solvent to induce self-assembly in these two systems. On the other hand, when water was used as a “bad” solvent, well defined spherical nanostructures with a uniform size were formed (Fig. 5).

The synthesized hybrids were studied with UV-vis absorption spectroscopy in solution and in the solid state. The broadening and red shifts of all bands of the chromophore (and especially the Soret band) observed in all porphyrin hybrids in relation to the respective bands of the compounds in solution (DCM) indicate the formation of J-aggregates (side-by-side) of the porphyrin moiety in the assemblies (Fig. S26-S29 and Table S1, ESI[†]). Equivalent observations were made during the fluorescence studies, where the observed red shift supports the formation of J-aggregates (Fig. S30, S31 and Table S2, ESI[†]).

In conclusion, this work describes the synthesis and characterization of porphyrin and BODIPY hybrids coupled with aliphatic peptides that are able to self-assemble in mixed solvent systems. More specifically, two dipeptides namely Ile-Ile and Ile-Ala bearing a series of protecting groups (Fmoc-, Boc-, -Z, -OCH₃) were connected with porphyrin and BODIPY chromophores. To the best of our knowledge, this is the first example where Ile-Ile and Ala-Ile dipeptides are covalently attached to chromophores in order to transfer their self-assembling properties. The resulting conjugates were able to form spherical and spiky nanostructures depending on the protecting group, the solvent mixture and the nature of the dipeptide and the chromophore. Moreover, the **Fmoc-Ile-Ile-TPP** hybrid was able to form hydrogels in HFIP-H₂O 2:8 solvent mixture at 1 mM final concentration. The BODIPY hybrids were

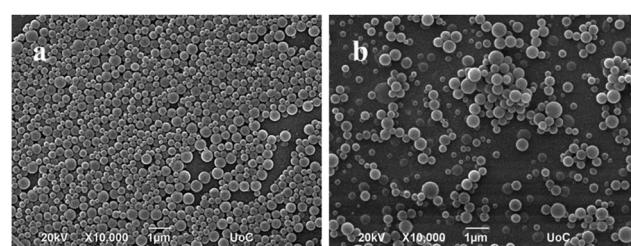


Fig. 5 Self-assembly behavior of (a) **Boc-Ala-Ile-BDP** in HFIP-H₂O 2:8, and (b) **Boc-Ile-Ile-BDP** in HFIP-H₂O 2:8.



not able to self-assemble when MeOH and EtOH were used as “bad” solvents, probably due to their limited solubility in these solvents, however they assembled into uniform nanometric spheres in the HFIP-H₂O solvent system. The various self-assembled configurations that can be adopted depending on the conditions described above leave “plenty of room” for a variety of applications. In particular, rough and “spiky” spheres could be used as catalysts due to their larger surface.²⁹ Moreover, “smooth spheres” with nanometric dimensions can easily enter cells and be applied in drug delivery and photodynamic therapy.³⁰ Finally, hydrogels can be utilized for biomedical applications.³¹

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Conflicts of interest

There are no conflicts to declare.

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