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Synthesis and Self-Assembly of Oligo(*p*-phenylenevinylene) Peptide Conjugates in Water

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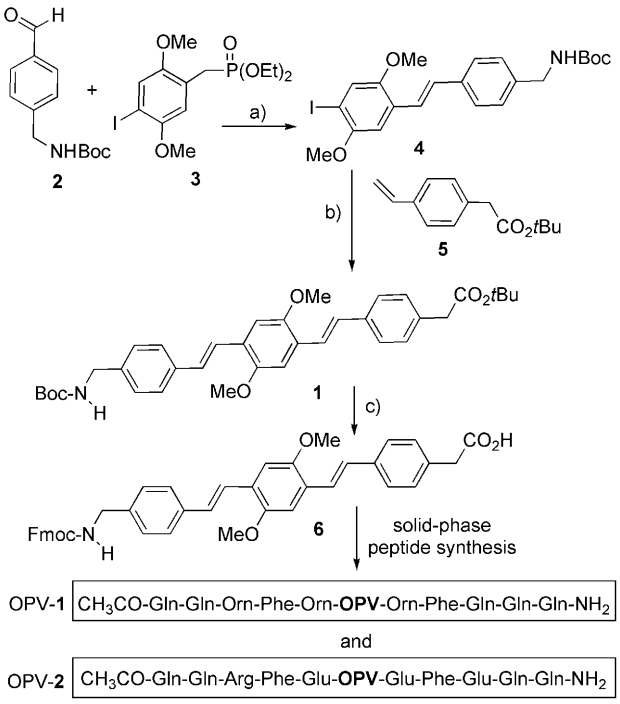
Molecular self-organization is a useful approach to prepare soft and flexible, functional micro- and nanoarchitectures. A practical principle to self-assemble structures is based on simple π–π stacking of π-conjugated oligomers. Self-assembly (SA) of functionalized oligo(*p*-phenylenevinylene)s (OPVs) gives organogels with interesting photophysical properties and potential applications in light-emitting diodes,[1] light-harvesting systems,[2] or thermal imaging.[3] However, organized, robust molecular structures are difficult to obtain by π–π stacking alone.[4] Therefore, a variety of promoters that enable the establishment of additional noncovalent interactions, such as directional hydrogen bonds, have received increased attention.[5] Among them, peptide amphiphiles, made of a π-conjugated unit and hydrophilic peptide sequences, have been considered because of their strong tendency to form well-defined secondary structures and to self-assemble in water.[6,7]

Herein, we report the synthesis and SA characteristics of two OPV peptide conjugates (OPV-**1** and OPV-**2**, Scheme 1) in which a new OPV-based ω-amino acid has been incorporated into two different β-sheet-forming sequences by solid-phase synthesis.[8] In these systems, a reversible SA can be triggered by pH changes. In particular, the ornithine-rich OPV-**1** self-assembles at basic pH, whereas for OPV-**2**, which contains glutamic acid residues, SA occurs at acidic pH.



Scheme 1. Synthesis of peptides OPV-**1** and OPV-**2**. Reagents and conditions: a) NaH, THF, 25 °C, 6 h, 78 %; b) $Pd(OAc)_2$, triethylamine, tri(*o*-tolyl)phosphine, DMF, 90 °C, microwave, 15 min, 63 %; c) trifluoroacetic acid, CH_2Cl_2, 25 °C, 2 h, then trimethylsilyl chloride, diisopropylethyl amine, 9-fluorenylmethylchloroformate, 24 h, 87 %.

The Boc–OPV–O*t*Bu (Boc = *tert*-butyloxycarbonyl) amino acid derivative **1** was synthesized by a two-step procedure as illustrated in Scheme 1. A Horner–Emmons Wittig coupling reaction of *tert*-butyl(4-formylbenzyl)carbamate (**2**)[9] and phosphonate **3** gave the *trans*-stilbene **4** in 78 % yield. In turn, phosphonate **3** was prepared starting from 4-iodo-2,5-dimethoxy benzaldehyde. A subsequent microwave-assisted Heck coupling reaction of **4** with (4-vinylphenyl)acetic acid *tert*-butyl ester (**5**) afforded OPV amino acid **1** in 63 % iso-

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lated yield as a bright yellow solid (see the Supporting Information for synthetic details and characterization of the products). Crystals suitable for X-ray diffraction analysis were grown from an *n*-hexane/ethyl acetate mixture. The 3D structure of **1**, illustrated in Figure 1, confirmed the *trans*–

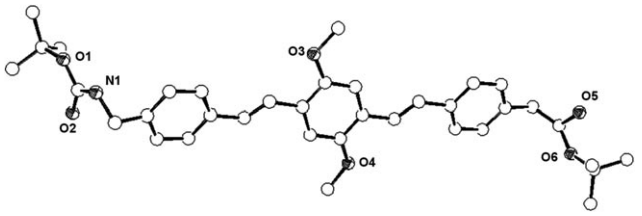

Figure 1. X-ray diffraction structure of the Boc–OPV–O*t*Bu amino acid derivative **1** with heteroatom numbering.

trans configuration of the two OPV C=C bonds.[15] The central phenyl ring of **1** is laterally tilted by 15.2(2) and 12.7(2)° relative to the terminal phenyl rings (which are coplanar within 4.5(2)°). The Boc and O*t*Bu protecting groups lie outside of the plane defined by the OPV moiety. In an antiparallel β-sheet conformation, the intrastrand N(*i*)···N(*i*+5) distance is about 20.8 Å. Because the distance from the N terminus to the C terminus in **1** is 21.2 Å, this OPV amino acid might serve as a replacement for five α-amino acid units in a β strand.

Simultaneous removal of Boc and O*t*Bu groups of **1** with trifluoroacetic acid followed by protection of the N terminus with 9-fluorenylmethoxycarbonyl (Fmoc) gave Fmoc–OPV–OH **6** in 87% yield (Scheme 1). Standard Fmoc-mediated solid-phase syntheses afforded peptides OPV-**1** and OPV-**2** in 49 and 54% isolated yields, respectively, after semipreparative HPLC purifications (see the Supporting Information for details).

A stable hydrogel formed when a 13 mM solution of OPV-**1** was allowed to stand at pH 8 with 1 N aqueous NaOH. Lower OPV-**1** concentrations did not yield any gelation. A similar behavior was observed upon addition of 1 N HCl to a 13 mM solution of OPV-**2**. Although gelation takes place in both cases, gels of OPV-**1** seemed more robust than those obtained from OPV-**2**. The process is fully reversible and original peptide solutions could be restored by changing the pH to acidic and basic values for the OPV-**1** and OPV-**2** gels, respectively.

The FTIR absorption spectra of both gels (see the Supporting Information) show the strong amide I band, typically seen for β-sheet structures, at 1627 and 1614 cm^{-1} for OPV-**1** and OPV-**2**, respectively.[10] On the other hand, both peptide conjugates exhibit a single broad band at 1654 cm^{-1} before gelation. The UV absorption spectrum of OPV-**1** in an acidic aqueous solution (Figure 2A) has two maxima at 264 and 335 nm that redshift, to 292 and 345 nm, respectively, upon gelation. The absorption band of OPV-**2** at 265 nm shows a 30 nm redshift upon gelation. In contrast, no shift was observed for the OPV-**2** absorption band at 335 nm

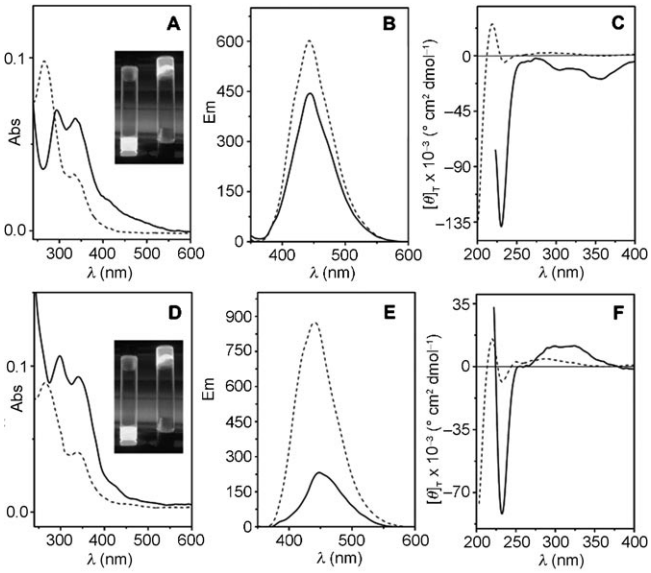

Figure 2. Absorption (A), emission (B), and CD (C) spectra of OPV-**1** in acidic (nonassembled -----) and basic aqueous solutions (assembled ——). Absorption (D), emission (E), and CD (F) spectra of OPV-**2** in basic (nonassembled -----) and acidic (assembled ——) aqueous solutions. Each inset of panels A and D shows a solution (left) and a gel (right) irradiated at 365 nm.

(Figure 2D). Strong emission was seen for both peptide conjugates (Figure 2B and E) that partially quenches upon gelation. This quenching effect is stronger for assembled OPV-**2** than for OPV-**1**. According to Kashas's exciton theory,[11] the observed redshift in the UV/Vis absorption spectra of peptide gels along with the residual emission may indicate the formation of J-type aggregates of the OPV moiety.

The changes recorded for OPV-**1** and OPV-**2** upon gelation are apparent in the UV absorption spectra (Figure 2A and D, 28 and 30 nm bathochromic shifts of the bands at 264 and 265 nm, respectively) but relatively weak in the case of the emission spectra (Figure 2B and E, 8 nm redshift for OPV-**1** and no shift for OPV-**2**) if compared to previously reported gel-forming OPVs,[12] suggesting a different electronic structure of the aggregates and, therefore, a different geometry.[11] In such cases it has been proposed to address these assemblies as pseudo-J aggregates.[12a]

Circular dichroism (CD) spectra of OPV-**1** and OPV-**2** in water, at acidic and basic pH values, respectively, are almost flat in the OPV absorption region (>250 nm), indicating unspecific structures in solution (Figure 2C and F). Upon pH change, a strong negative band emerges at around 230 nm, for both OPV-**1** and OPV-**2**, along with enhanced CD signals in the region of the OPV absorption that, interestingly, exhibits Cotton effects of opposite signs. Although slightly longer than expected, the 230 nm wavelength indicates a β-sheet structure for the assembled peptides.[13] In both CD spectra the bisignate excitonic bands that coincide with the UV absorption maxima of OPV are lacking. These bands are typical for chiral helical OPV assemblies and the lack of them has been observed for other pseudo-J aggregates.[12,14]

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These findings may indicate the absence of helical structures or the coexistence of different chiral assemblies.

TEM analysis of the OPV-**1** gels (Figure 3A) shows nanotape 1D structures of lengths up to 1 μm and an average width of 7 nm with some interweaving fibers that form left-handed double helices (inset of Figure 3A). In contrast, a spongy network texture was observed for the OPV-**2** gel (Figure 3B).

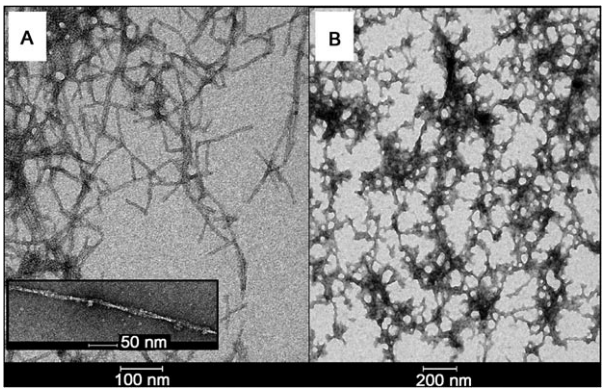

Figure 3. TEM images of self-assembled OPV-**1** (A) and OPV-**2** (B). The inset in A shows two interweaving fibers that form a double helix.

AFM analysis of diluted gel samples of OPV-**1** deposited on silicon chips highlights irregular networks of highly interconnected fibers that are hardly distinguishable. On the other hand, samples of diluted OPV-**2** gels showed left-handed helical fibers with lengths up to micrometers with an uniform helical pitch of 0.15 μm (Figure 4).

In summary, we have reported the synthesis of a new OPV ω-amino acid (**1**) that has been incorporated into two β-sheet-forming sequences through solid-phase protocols. The resulting peptide hybrids are soluble in water and reversibly self-assemble to a stable, fluorescent hydrogel upon pH changes. A detailed analysis of the assemblies revealed complex networks in which helical fibers are present. We are currently exploring the potential of OPV and other π-conjugated oligomer/peptide conjugates to influence the morphology of donor–acceptor systems in photonic devices.

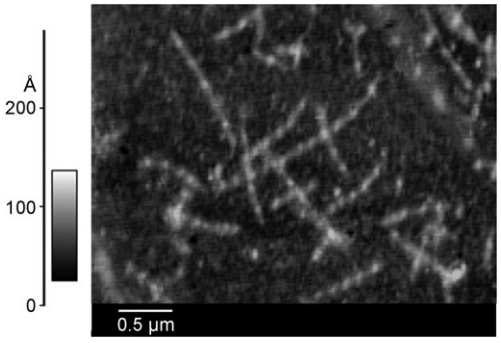

Figure 4. Contact-mode AFM image of self-assembled OPV-**2**.

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Keywords: aggregation · gels · oligo(*p*-phenylenevinylene)s · peptides · self-assembly

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[15] CCDC-791280 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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