Supramolecular Chemistry

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Direct Visualization of Efficient Energy Transfer in Single Oligo(*p*-phenylene vinylene) Vesicles**

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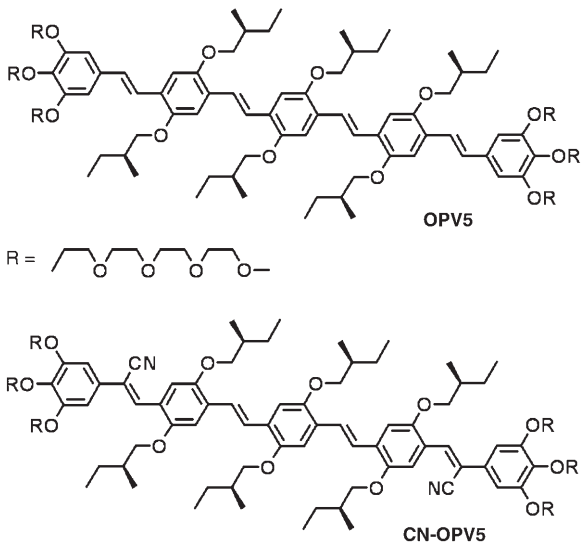
Addressing the properties and interactions of individual self-assembled architectures is of great importance for the actual implementation of such objects in bio- and nanotechnological applications.[1] Supramolecular electronics depends on the ability to control the ordering of π-conjugated systems into well-defined, functional structures.[2] In natural photosynthetic systems, such a controlled organization yields directional energy and electron transfer in an aqueous environment.[3] Remarkably, only a few reports exist on self-assembled π-conjugated systems in water.[4] Our previous work on energy[5] and electron[6] transfer in self-assembled oligo(*p*-phenylene vinylene) (OPV) assemblies,[7] for example, focused on stacked oligomers in apolar solvents.[8] However, the interactions between self-assembled objects in water has become more appealing and challenging through the opportunities provided by scanning confocal microscopy. Herein we report on the use of fluorescence microscopy to characterize the properties and interactions of OPV assemblies in water by monitoring energy transfer.

OPV5 (Scheme 1) was previously synthesized and shown to form chiral assemblies in water, but no further details with regard to the type of architecture was presented.[4b] By using scanning confocal microscopy we now give direct evidence for the formation of OPV vesicles, an arrangement rarely observed with π-conjugated oligomers.[9] In addition we synthesized the analogous cyano-substituted **CN-OPV5**[10] to study energy transfer in mixed donor/acceptor vesicles in bulk solution as well as in single vesicles, immobilized on a glass surface. Furthermore, the exchange of chromophores between vesicles over time could be monitored.



Scheme 1. Chemical structures of the two oligomers used in this study. **OPV5** is used as an energy donor whereas **CN-OPV5** is used as an energy acceptor.

CN-OPV5 was synthesized according to standard literature procedures, and was obtained as a red waxy solid which was fully characterized.[11] **OPV5** was synthesized and characterized as reported previously.[4b] The optical properties of these two compounds were studied in various solvents by using UV/Vis, fluorescence, and circular dichroism (CD) spectroscopy (Figure 1). In general, **CN-OPV5** displays similar properties to **OPV5**: **CN-OPV5** is molecularly dissolved in chloroform, as indicated by a highly symmetrical π-π* transition at $\lambda_{max} = 482$ nm, intense fluorescence at $\lambda_{em} = 568$ nm, and the absence of any Cotton effect. The effect of cyano substitution in **CN-OPV5** on the HOMO–LUMO separation of the oligomer is clearly observed by comparison of the spectra with those of **OPV5**. The π-π* transition of **CN-OPV5** is shifted 32 nm bathochromically in chloroform, a feature which is desired for making it an energy acceptor for **OPV5**.

The absorption maximum of **CN-OPV5** in water[12] displays a modest hypsochromic shift of 9 nm (to $\lambda_{max} = 473$ nm) relative to that in chloroform, and develops a shoulder extending to $\lambda = 600$ nm. Its fluorescence is strongly quenched and red-shifted to $\lambda_{em} = 638$ nm, and concomitantly, a bisignate Cotton effect is observed, which is positive [+] at high energy and negative [−] at low energy ($g_{max[+]} = +4.9 \times 10^{-4}$ and $g_{max[-]} = -6.8 \times 10^{-4}$).[13] The curve crosses the zero line at $\lambda = 477$ nm, which is close to the wavelength of the absorption maximum. These collective findings indicate that **CN-OPV5** forms chiral, H-type aggregates in an aqueous environment through stacking of the π-conjugated backbones, similar to **OPV5**.[4b] Whereas **OPV5** exhibits quantum yields of $\phi = 0.64$ and $\phi = 0.10$ in THF and water, respectively,

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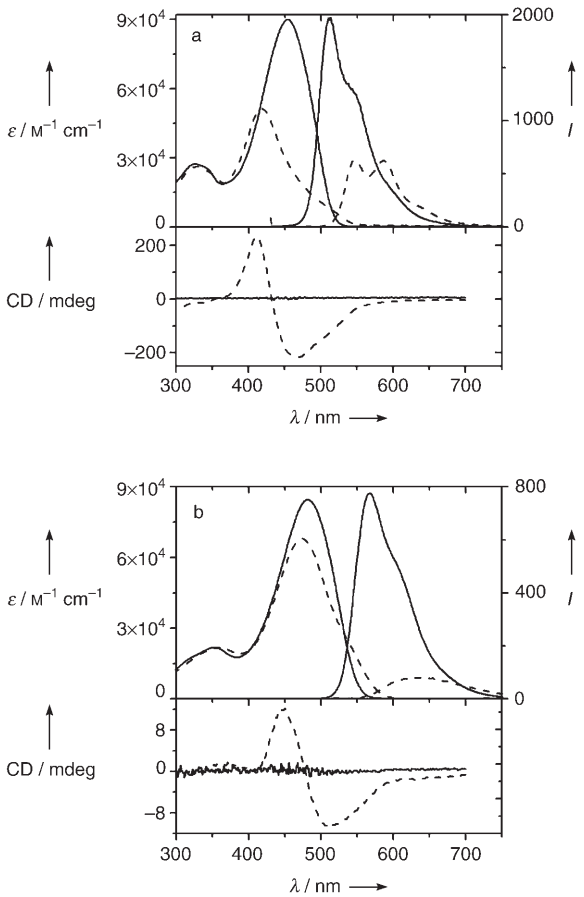

Figure 1. Optical properties of: a) **OPV5** and b) **CN-OPV5** in chloroform (solid line) and water (dashed line) at room temperature as studied by UV/Vis, fluorescence (λ_{exc} = respective λ_{max}), and CD spectroscopy. Concentrations are 1.6×10^{-5} M except for the CD spectroscopic measurements on **CN-OPV5** (7.3×10^{-5} M).

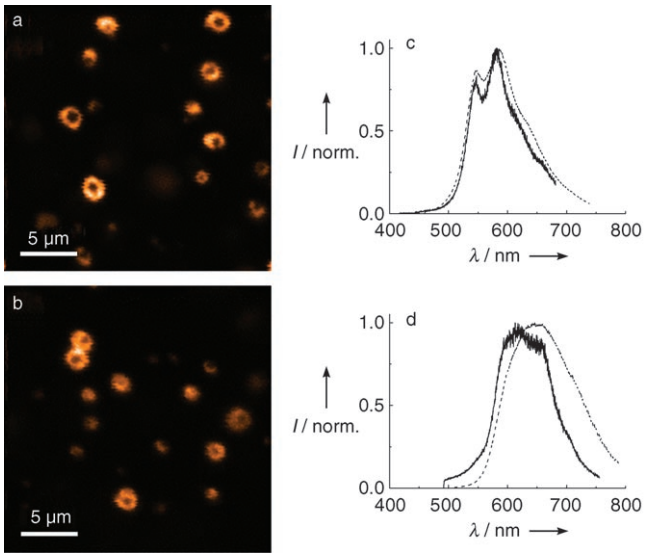

Figure 2. Scanning confocal microscopy images (λ_{exc} = 411 nm) of the vesicles of **OPV5** (a) and **CN-OPV5** (b) and fluorescence of single vesicles (solid lines) of **OPV5** (c) and **CN-OPV5** (d). Solution spectra are shown for comparison (dashed lines).

CN-OPV5 is somewhat less fluorescent, with $\phi = 0.49$ and $\phi = 0.08$ in THF and water, respectively.

To study the stability of the **CN-OPV5** aggregates, temperature-dependent UV/Vis, CD, and fluorescence studies were performed in water.[11] Despite the fact that the Cotton effect gradually disappears, the system is still strongly aggregated at 90 °C. This result is similar to that observed earlier for **OPV5** in water[4b] and was confirmed by dynamic light scattering (DLS) studies.[11] Hence, disorder is introduced at the molecular level, while strong hydrophobic forces hamper vesicle disruption at the microscopic level. The λ_{max} value of **CN-OPV5** recorded at 90 °C displays an additional hypsochromic shift of 35 nm, which suggests that the chromophores in the achiral supramolecular organization at high temperature are more tightly packed.

Dynamic light scattering studies indicated the presence of spheres of several hundreds of nanometers and micrometers in size for **CN-OPV5** and **OPV5**, respectively.[11] Scanning confocal microscopy was used to determine whether these spheres were hollow or solid (Figure 2). For these experiments, the solutions were gelated using 1 mg mL^{-1} gelatine[14] after which they were dropcast onto a glass surface and allowed to dry. It should be noted that the fluorescence spectrum of the parent solution, the undried gel, and the dried gel are similar, thus implying that the confocal images are representative of the solution conditions. Spheres as well as rings of micrometer size could be observed on a glass surface for both **OPV5** and **CN-OPV5**, thus suggesting the presence of vesicles. Slices could be made through the *z*-direction of a single vesicle by adjusting the focal plane of the microscope, which revealed a transition from a solid sphere via a ring back to a solid sphere. The photoluminescence spectra of these separate vesicles (Figure 2, λ_{exc} = 411 nm) were essentially identical to those obtained in solution, thus yielding definitive proof of the formation of OPV vesicles in water. Since supramolecular chirality is expressed in these structures, the vesicles are presumably composed of domains of helical OPV aggregates, as observed before for thiophene vesicles.[9b]

Energy-transfer experiments on **CN-OPV5** and **OPV5** were performed in water (Figure 3), with the aim of generating mixed vesicles and subsequent energy transfer from the **OPV5** donors to the **CN-OPV5** acceptors. Mixtures with various donor/acceptor ratios of were prepared in THF and subsequently injected into water to yield mixed vesicles. These mixtures were first studied in bulk solutions, followed by measurements at the single vesicle level.

Fluorescence data on the mixed samples show a strong decrease of host luminescence at λ_{em} = 546 nm as the acceptor content increases. At 1.6 mol % acceptor, the photoluminescence spectrum is almost completely dominated by **CN-OPV5** as a consequence of efficient energy transfer from **OPV5**. The luminescence of the sensitized acceptor at λ_{em} = 589 and λ_{em} = 634 nm is characteristic of molecularly dissolved species and indicates that **CN-OPV5** exists as isolated chromophores inside the donor vesicles. Increasing the amount of **CN-OPV5** to 31 mol %[11] induces acceptor aggregation, which is expressed in diminished and bathochromically shifted acceptor fluorescence. Time-resolved single-

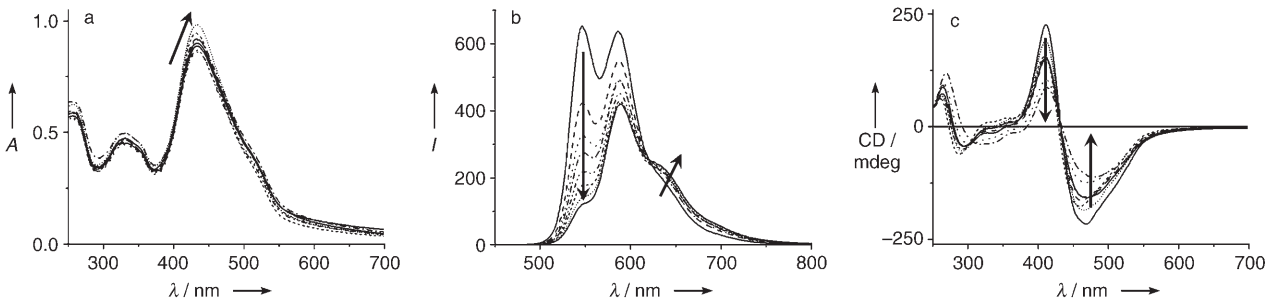

Figure 3. Mixtures containing 0–1.6 mol% **CN-OPV5** in **OPV5** in water as studied by: a) UV/Vis, b) fluorescence ($\lambda_{exc} = 419$ nm), and c) CD spectroscopy ([**OPV5**] $= 1.6 \times 10^{-5}$ M).

photon counting (TCSPC) measurements ($\lambda_{exc} = 400$ nm) were performed on **OPV5** doped with **CN-OPV5** and on both pure oligomers,[11] the latter showing a fluorescence lifetime increase upon cyano substitution of the backbone. Mixed vesicles show a sharp decrease in the lifetime of **OPV5** at $\lambda = 546$ nm, as a consequence of rapid depletion of its first excited state by energy transfer to **CN-OPV5**. The contribution of the longer-lived **CN-OPV5** luminescence at this wavelength becomes more dominant upon increasing the **CN-OPV5** content.

To directly prove the presence of mixed vesicles, a solution of **OPV5** containing 2 mol% **CN-OPV5** was prepared and deposited on a glass surface. The observed size of the vesicles decreased (Figure 4a); this effect was also observed in DLS studies on a similar mixture of the two oligomers.[11] More interestingly, the fluorescence spectra indicated almost complete energy transfer to and subsequent emission from **CN-OPV5** for all vesicles. In agreement with the solution data, the shape of the fluorescence spectrum proved the existence of isolated **CN-OPV5** in the donor **OPV5** vesicles. Furthermore, the sensitized **CN-OPV5** emission was bleached after about 50 s of excitation ($\lambda_{exc} =$ 411 nm) of a single vesicle.[11] As a result, the energy-transfer process was terminated and the fluorescence of the donor oligomer at $\lambda_{exc} = 546$ nm was restored. The luminescence of the donors started to bleach upon prolonged illumination. Interestingly, the bleaching of **CN-OPV5** in a mixed donor/acceptor vesicle occurred much faster than the bleaching of pure **CN-OPV5** vesicles, thus suggesting that **CN-OPV5** is in a state of enhanced excitation, because of light-harvesting from **OPV5**.

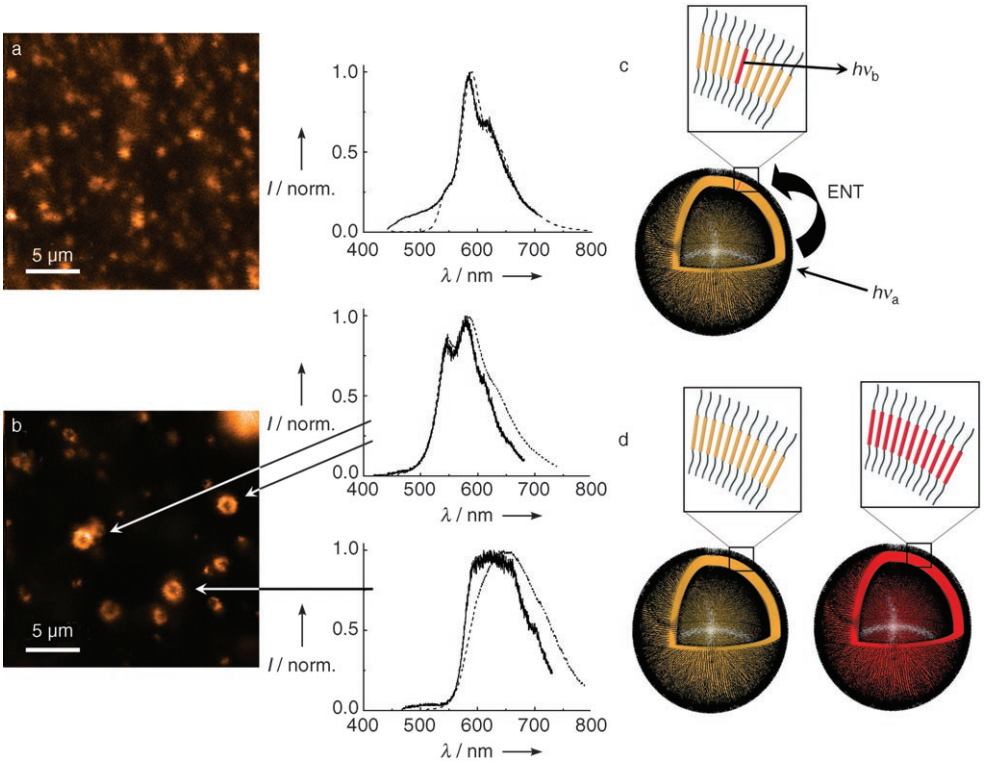

Figure 4. Scanning confocal microscopy images and resulting fluorescence spectra ($\lambda_{exc} = 411$ nm) of mixtures of **OPV5** and **CN-OPV5**: a) with premixing in THF (2 mol% **CN-OPV5**) and b) without premixing in THF (9 mol% **CN-OPV5**). The fluorescence of these single vesicles (solid lines) is compared to the corresponding solution spectra (dashed lines). c), d) Schematic representations of the vesicles formed in (a) and (b), respectively. ENT = energy transfer.

Aqueous solutions of individual vesicles of **OPV5** and **CN-OPV5** were added to study vesicle–vesicle interactions. Since no initial intermixing is possible in this way, the confocal image (Figure 4b) merely shows the presence of either pure **OPV5** or pure **CN-OPV5** vesicles as separate objects, which is concluded on the basis of their fluorescence spectra ($\lambda_{exc} = 411$ nm). The stability of the system containing separate vesicles in solution was examined over time by heating the solution at 35 °C for 48 h (Figure 5). Samples taken over this period were gelated and subsequently studied by confocal microscopy. After 48 h the system showed exclusively mixed vesicles, as characterized by efficient energy transfer from **OPV5** to **CN-OPV5**. Since **OPV5** vesicles could no longer be observed, we attribute this phenomenon to exchange between separate donor and acceptor vesicles. After 2 h, the ongoing exchange process could already be observed

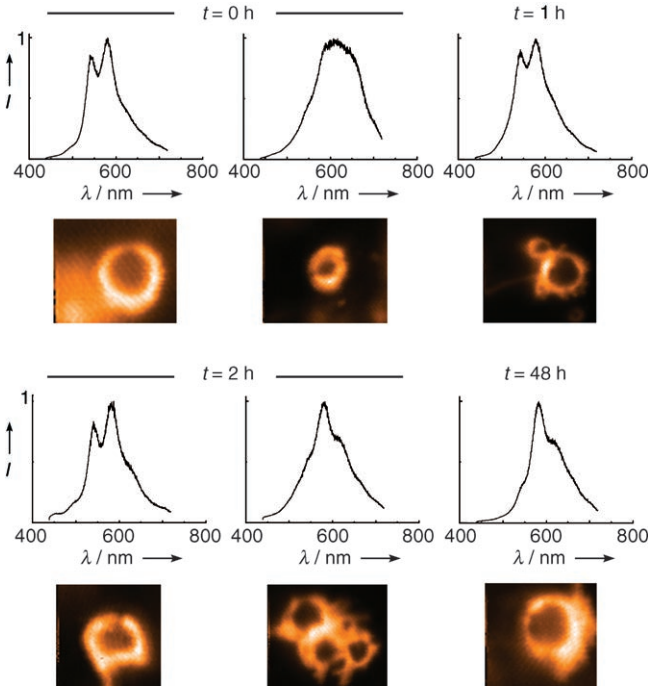

Figure 5. Scanning confocal microscopy images and normalized fluorescence (λ_{exc} = 411 nm) of single vesicles showing the transition from nonmixed to mixed vesicles on prolonged heating of a 2 mol% solution of **CN-OPV5** at 35 °C. At t = 0 h (and t = 1 h), **OPV5** vesicles and some **CN-OPV5** vesicles predominate. At t = 2 h some vesicles show energy transfer. After 48 h all the vesicles are mixed and show almost exclusive **CN-OPV5** luminescence (image sizes are 6×5, 10×9, 10×10, 8×8, 9×9, and 7×6 μm^2 respectively).

for some selected vesicles by scanning confocal microscopy, while the solution spectra[11] still showed an average situation that lacked energy transfer (Figure 5). Energy transfer in those vesicles resulted from the doping of **OPV5** vesicles with small amounts of **CN-OPV5** by the proposed exchange mechanism. These experiments show qualitatively that the OPV vesicles have individual properties which differ from the average solution data.

In conclusion, we have demonstrated the formation of π-conjugated OPV vesicles by using optical studies and scanning confocal microscopy. The synthesis of a cyano-substituted acceptor oligomer enabled us to study energy transfer in doped vesicles in water. Mixed vesicles were visualized on a glass surface by scanning confocal microscopy and thus the energy-transfer process could be studied at the single vesicle level. Moreover, probing the ongoing exchange process between separate donor and acceptor vesicles over time proved that the properties of individual vesicles were different from those of the bulk solution. These data convey the message that the study of single, self-assembled objects and the interaction between such objects yields important and detailed information on their behavior at the molecular level.

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[11] See the Supporting Information.

[12] In general, OPV aggregates are prepared in water by injecting a concentrated solution of OPV (4×10^{-4} M) in THF into water, followed by removal of the THF at 90 °C.

[13] The less intense CD signal relative to that of **OPV5** is probably a consequence of detrimental steric and electronic effects that accompany close packing between nitrile-substituted OPVs.

[14] Solutions containing OPV vesicles had to be gelated with gelatin prior to the confocal microscopy measurements, since otherwise the vesicles did not stick to the glass surface.

