

Short communication

# Electron transfer, charge stabilization and charge recombination in naphthalenediimide-tryptophan immobilized on silica particles

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## Abstract

*N,N'*-Bis(2-phosphonoethyl)-1,4,5,8-naphthalenediimide (DPN) derivatives containing Ac-Trp (DPN-W) and Ac-Trp-Ala-Ala (DPN-AAW) were prepared on silica-gel solid supports by using a combined zirconium phosphonate and Fmoc-solid phase peptide synthesis strategy. A photophysical study of these particles revealed that the tryptophan (W) residue was subject to less polar surroundings on the DPN-AAW than in the DPN-W particle and a much more intense DPN fluorescence emission was observed on DPN-AAW than on DPN-W. The transient spectra of the particles showed the presence of DPN radical anion and Trp radical cation on the DPN-AAW but no signal was obtained for DPN-W. This finding is indicative of a very fast charge transfer between DPN and the tryptophan moiety that is followed by charge recombination on DPN-W and by charge stabilization on DPN-AAW.

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**Keywords:** Naphthalenediimide; Tryptophan; Charge transfer; Silica particles

## 1. Introduction

The use of 1,4,5,8-naphthalenediimides (NDI) in the development of conducting polymers, field-effect transistors and as model systems for photosynthesis is increasing as a result of their photophysical, photochemical and electrochemical properties [1,2]. The imides and diimides excited molecules generate high yield triplet state that are good electron acceptors, hence these molecules are valuable in photoelectron transfer processes [3–5]. Tryptophan (W) is suggested to play an important role in the photoinduced damage of biomolecules, since W can be converted to a radical cation and/or promoted to its triplet-excited state when photoexcited [6,7]. Information concerning the physical and chemical environments of W has been achieved by fluorescence spectroscopy [8]. The presence of two overlapping  $\pi \rightarrow \pi^*$  electronic transitions, designated as <sup>1</sup>L<sub>a</sub> and <sup>1</sup>L<sub>b</sub> which are very sensitive to polarity, gives information about peptide and protein structures [7,8].

The process of electron transfer between naphthalene-imides and amino acids such as tryptophan has already been investigated in solution [3,9]. The naphthalene-imide triplet states cause photooxidation of W and a charge recombination reaction between the radical species is observed [3,9]. However, reactions between NDI and W immobilized on solid supports had never been studied before. The assembly of structures that join W and NDI's can be very important in obtaining materials that are mimetic of photosynthetic systems and also can show applications as chemical and biochemical sensors by photoinduced electron transfer (PET) processes. For these reasons, in the present work, the syntheses and the basic photophysical and photochemical properties of new photoactive materials on silica-gel particle, designated as DPN-W and DPN-AAW (A is alanine), are described.

## 2. Experimental

### 2.1. Materials

Silica-gel 60 was obtained from Aldrich. *N,N'*-Bis(2-phosphonoethyl)-1,4,5,8-naphthalenediimide (DPN) was prepared as previously described [2]. Fmoc-Ala-OH, Fmoc-

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Trp-OH, 1,3-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt) and piperidine were from Aldrich. All other synthesis materials were from commercially available suppliers.  $\text{POCl}_3$ , DMF and acetonitrile were previously distilled and the other solvents were used as obtained from suppliers.

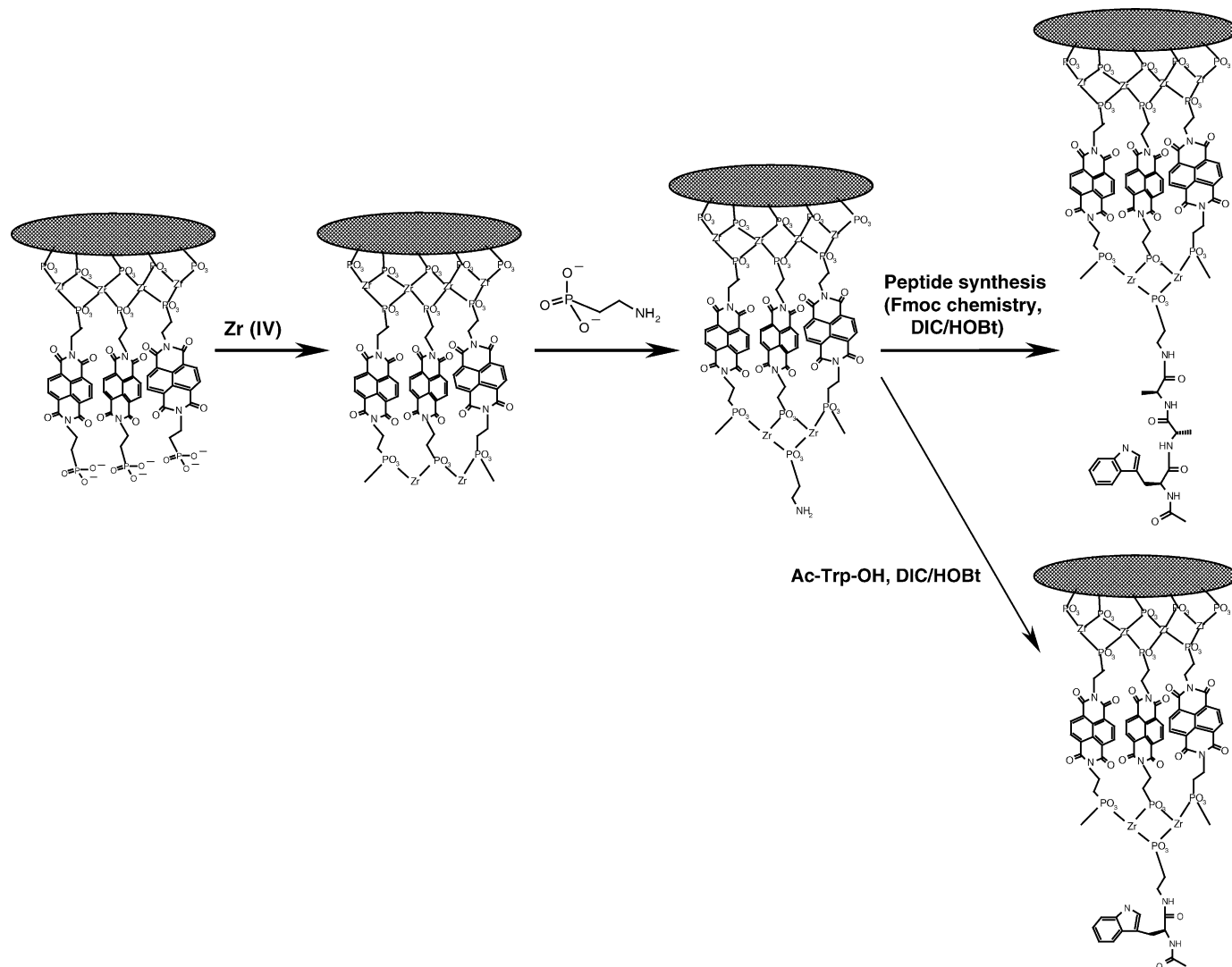
## 2.2. Analytical methods

UV–vis absorbance spectra were recorded using either a Hitachi-U2000 or a Shimadzu UV-2400-PC spectrophotometer. Fluorescence emission spectra were recorded in a SPEX DM 3000-F fluorometer operating with four slits, each having a width of 1 mm (bandwidth  $\sim 13$  nm). Spectral data were manipulated with a 386 Grams software (Galatica, Inc.) and were obtained with 1 cm optical path length quartz cuvettes. Laser flash photolysis (LFP) data were obtained with a system composed of a Nd:YAG laser (Spectron Laser System, England) operating either at 355 or 266 nm, delivering pulses with  $\sim 20$  mJ/20 ns full-width at half maximum (FWHM), a pulsed 150 W Xe lamp, control electronics and a digitizing oscilloscope

(Hewlett-Packard 54510 B) for data capture. Data were stored in a PC compatible microcomputer and analyzed with the software Origin (Microcal) by using a non-linear regression curve fitting. All analytical measurements were conducted with the same particle amount (0.0025 g) suspended in 2.5 mL of water. All experiments were conducted at room temperature ( $\sim 23$  °C).

## 3. Results and discussion

The preparation of silica-supported DPN is based on Malouk's zirconium phosphonate chemistry as described elsewhere [10]. DPN-W and DPN-AAW were obtained by depositing a layer of Zr(IV) onto the surface of silica-supported DPN [11]. The particles were then suspended in an aqueous solution containing 3-aminoethylphosphonic acid ( $[\text{APA}] = 0.1 \text{ mol L}^{-1}$ ) for 4 h. For the anchorage of *N*-Ac-W and peptide (Ac-WAA) on the silica particles, 1,3-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) were employed as coupling reagents [12]. Final acetylation was performed with acetic anhydride in  $\text{CH}_2\text{Cl}_2$  (1:1, v/v) for 1 h (Scheme 1). For ana-



Scheme 1. Stepwise synthesis of Ac-Trp-DPN-SiO<sub>2</sub> (DPN-W) and of Ac-Trp-Ala-Ala-SiO<sub>2</sub> (DPN-AAW) particles. Coupling reagents: DIC (1,3-diisopropylcarbodiimide) and HOBt (1-hydroxybenzotriazole).

Table 1  
Substitution degree (mmol g<sup>-1</sup>) of DPN and amino acids on silica particles

	DPN	1st Ala	2nd Ala	W
Ac-Trp-Ala-Ala-DPN-silica-gel	0.25	0.24	0.19	0.13
Ac-Trp-DPN-silica-gel	0.23	–	–	0.16

lytical purposes silica particles were treated with NaF, which cleaves the zirconium phosphonate bond (0.1 mol dm<sup>3</sup>) and releases the material bound to the silica. The extracted residues were then analyzed by reversed phase HPLC followed by ESI-MS. The following products were obtained: Ac-WAA-NH-CH<sub>2</sub>-CH<sub>2</sub>-PO<sub>3</sub>H<sub>2</sub> ([M + H]<sup>+</sup> = 496, theor. 496); Ac-W-NH-CH<sub>2</sub>-CH<sub>2</sub>-PO<sub>3</sub>H<sub>2</sub> ([M + H]<sup>+</sup> = 354, theor. = 354). The number of DPN molecules bound to the silica was also determined by extracting DPN with NaF and determining the absorbance of appropriate aliquots ( $\epsilon_{380} = 26000 \text{ M}^{-1} \text{ cm}^{-1}$ ). Molar absorptivity of the Fmoc-piperidine adduct was determined experimentally ( $8500 \text{ M}^{-1} \text{ cm}^{-1}$ ) and used to quantify the extent of amino acid addition to the silica particles after each coupling step (Table 1). The substitution degree was controlled in the first step, that is, at the phosphorylation level to achieve ~0.2 mmol DPN/g silica. This value is appropriate for growth of peptides in solid phase [12]. A decrease in the substitution degree is observed from DPN-W to DPN-AAW leading to 70 and 52% yield, respectively, but this is expected for non-swelling materials like silica (Table 1).

The emission spectra of DPN-AAW and DPN-W particles suspended in water, with excitation wavelengths fixed at 260 and 355 nm, are shown in Figs. 1 and 2, respectively. When the excitation is at 260 nm the emission corresponds mainly to W residues since the DPN has very weak absorption at 260 nm. Resolution of <sup>1</sup>L<sub>a</sub> and <sup>1</sup>L<sub>b</sub> indole transitions was observed only for DPN-AAW particles (Fig. 1). For DPN-W the emission spectrum is the sum of <sup>1</sup>L<sub>a</sub> and <sup>1</sup>L<sub>b</sub> overlapping transitions. The higher contribution of the <sup>1</sup>L<sub>b</sub> transition in the spectrum of DPN-AAW particles indicates that W residues are sensing a more rigid and less polar environment than in the DPN-W particles [6,7]. Another evidence for this proposition is the blue shift (ca. 10 nm) in the tryptophan emission spectrum of DPN-AAW when compared to that of DPN-W particles (Fig. 1).

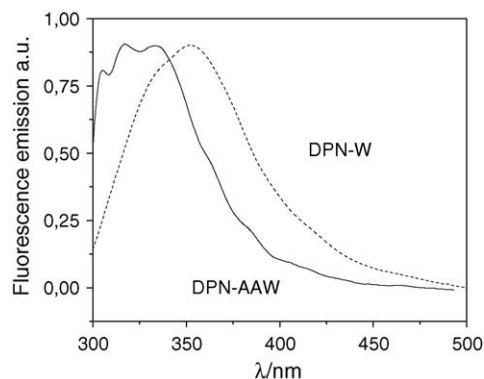


Fig. 1. Fluorescence emission spectra for DPN-AAW (—) and DPN-W (---) obtained at 25 °C in ethylene glycol:water (12.5:1, v/v),  $\lambda_{\text{exc}} = 260 \text{ nm}$ .

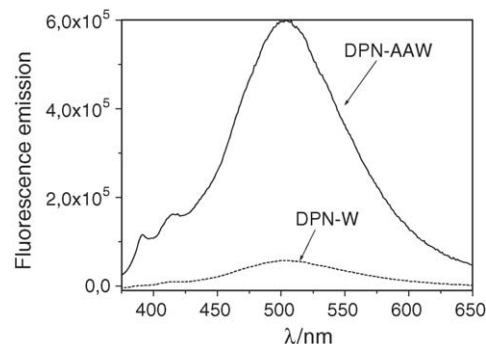


Fig. 2. Fluorescence emission spectra for DPN-AAW (—) and DPN-W (---) obtained at 25 °C in ethylene glycol:water (12.5:1, v/v),  $\lambda_{\text{exc}} = 350 \text{ nm}$ .

Fig. 2 shows the emission spectra of DPN-AAW and DPN-W with the excitation set at 350 nm, where the DPN residues are excited. Both spectra are broad with the maximum around ~500 nm. The small contribution of DPN monomer emission can also be observed on the left edge of the spectra. These features point to an aggregation of DPN residues inside the silica pores. The observed spectra are characteristic of DPN self trapped exciton [13]. Also, maximum emission wavelengths are coincident for both particles indicating no considerable difference in the DPN packing on DPN-W and on DPN-AAW particles. For DPN-AAW, the relative intensity is around 10 times higher than that observed for DPN-W, at the same amount of silica. The DPN fluorescence quenching in DPN-W cannot be attributed to an energy transfer since an uphill process would be necessary [14]. However, a charge transfer from W to DPN singlet state is quite possible due to the strong electron donor character of tryptophan moieties [9]. Also the singlet state of NDI is already known to participate in PET process using other electron donor species [15].

Fig. 3 shows the transient spectra for DPN-W and DPN-AAW obtained by LFP with laser excitation at 355 nm. Although no signal was observed for DPN-W, the observed DPN-AAW signal covers a large range of the spectral region (Fig. 3). The broad band above 500 nm and the peak at 320 nm cannot be attributed to DPN transient species. Studies of flash photolysis with DPN immobilized on the silica-gel particles showed that the DPN radical anion is formed by a reaction between DPN triplet state

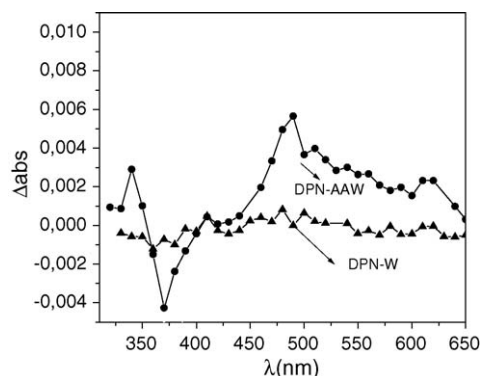


Fig. 3. Transients spectra of DPN-AAW (●) and DPN-W (▲) from LFP with laser excitation at 355 nm.

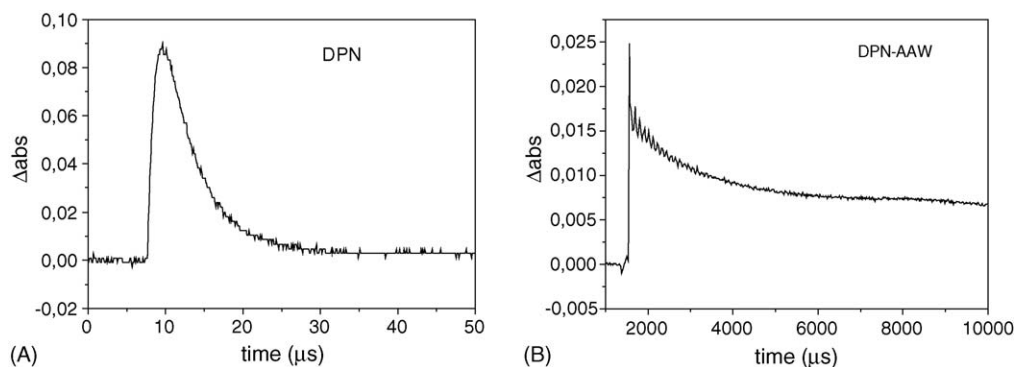


Fig. 4. Transients decays at 480 nm of DPN (A) and DPN-AAW (B) with laser excitation at 355 nm and 10 ms/pulse.

and their molecules in the ground state [3], with the radical anion characterized by an intense and sharp peak at 480 nm [10]. On the other hand, the tryptophan transient spectrum (cation and neutral radical) contains a broad band from 470 nm up to 600 nm and a peak at 320 nm [14] as shown in Fig. 3. This result indicates that the DPN molecules are photosensitizing the W transient species on the DPN-AAW particle. Photochemical and photophysical studies of imides and W in solution have shown the presence of an electron transfer from tryptophan to imides singlet and triplet state, which extinguishes the transient signal [3]. This finding is in accordance with the absence of the signals attributed to DPN-W in the fluorescence emission and transient absorbance spectra as well. The electrons could be transferred from W to DPN transient species by a very fast process followed by charge recombination. The most remarkable result in this work is the charge stabilization on DPN-AAW (Fig. 4). The lifetimes of the DPN radical anion (Fig. 4A) and of the W radical cation (data not shown) in solution are in the microsecond order whereas on the DPN-AAW particles it is in the millisecond range (Fig. 4B). In PET process, the reorganization energy depends mainly on the solvent, on the distance between the photoactive molecules and on the nature of the spacers [15,16]. The solvent should not be the cause of the lower reorganization energy observed for DPN-AAW than for DPN-W particles since the W moieties are sensing a less polar milieu on DPN-AAW than on DPN-W particle as stated before, but a more polar solvent is required for charge stabilization. The only significant spacer is the alanine residue that is not photoactive. Thus, the spacer length can be attributed as the main factor. However, molecular modeling and photochemistry studies are being conducted to elucidate this point.

The present findings show a convenient way of generating silica particles modified with photoactive molecules, whose photochemical activity can be altered by the presence of a spacer.

This material exhibits potential for technological applications such as sensors and photosynthetic systems, especially because of their ability to significantly stabilize transient species.

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