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Functionalization of fullerene at room temperature: toward new carbon vectors with improved physicochemical properties†

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In this work, fullerene has been functionalized with cyanuric chloride at room temperature by a nitrene mediated [2 + 1] cycloaddition reaction. The adduct after functionalization is inherently in the form of azafulleroid and shows broad UV absorption in the wavelength range of 200−800 nm, as well as photothermal conversion and fluorescence with a high quantum yield.

Fullerene (C_{60}) and its functionalized derivatives are interesting candidates for many applications, due to their unique physi-cochemical properties.[1] For example, they can be used as anti-virals, anticancer compounds, antioxidants as well as drug delivery agents in biomedical science.[2,3] Poor solubility, low processability and other drawbacks that hamper applications of fullerene have been mostly addressed by covalent and non-covalent functionalization.[4]

In the past few decades a wide range of functionalization approaches has been reported.[4,5] Nevertheless, a proper tailoring of the distinct physicochemical properties of fullerene remains challenging.[6,7]

UV absorption of fullerene is limited to short wavelengths and therefore it does not show a high photothermal conversion. Furthermore, fullerene and its derivatives exhibit very low fluorescence efficiencies, which render them unavailable for different applications such as bioimaging.[8] These disadvan-tages cause fullerenes to be rather inefficient as compared to their one and two dimensional analogues, namely single walled

carbon nanotubes and graphene oxide. Due to their defined structure which is a clear advantage over other families of carbon based nanomaterials, fullerene derivatives exhibiting comparable optoelectronic properties could be promising alternatives for different applications including bioimaging[9] and photothermal therapy.

Nitrene [2 + 1] cycloaddition reactions and consequently aziridinofullerene-azafulleroid isomerisation is a well-known strategy to obtained nitrogen doped fullerenes with a preserved π-conjugated system.[10] This method allows to effi-ciently functionalize the fullerene cage without affecting the sp^2 carbon network, thus leaving the hybridization of the carbon atoms unchanged upon covalent functionalization.[11] However, the isomerisation reaction often requires relatively high temperatures, which in turn uncompromise the thermal stability of some of the functional groups involved. Recently, we have shown that 2-azido-4,6-dichloro-1,3,5-triazine is a very efficient nitrene precursor and is able to conjugate to carbon based nanomaterials in a [2 + 1] cycloaddition reaction at room temperature. It was observed that aziridine rings are in the open form and therefore the π-conjugated system of those nano-materials is preserved upon functionalization.[12]

In the present work we have successfully extended this strategy for the functionalization of fullerenes. *In situ* reaction between sodium azide, 2,4,6-trichloro-1,3,5-triazine and fullerene at room temperature results in triazine-functionalized fullerene (Full-Trz) in the azafulleroid form with unusual optoelectronic properties (Scheme 1).

The energy path of the cycloaddition reaction shown in Scheme 1 was investigated computationally by means of a CI-NEB[13,14] calculation. The latter study revealed that the reaction lacks of an activation energy, hence confirming that the func-tionalization is energetically favoured and is likely to occur, even at room temperature (Fig. S10†).

The X-ray photoelectron spectroscopy (XPS) spectra of Full-Trz revealed the presence of C, N and Cl atoms, proving the conjugation of triazine groups to fullerene (Fig. S1†). In the C 1s peak spectra, in addition to the peak for carbon atoms of

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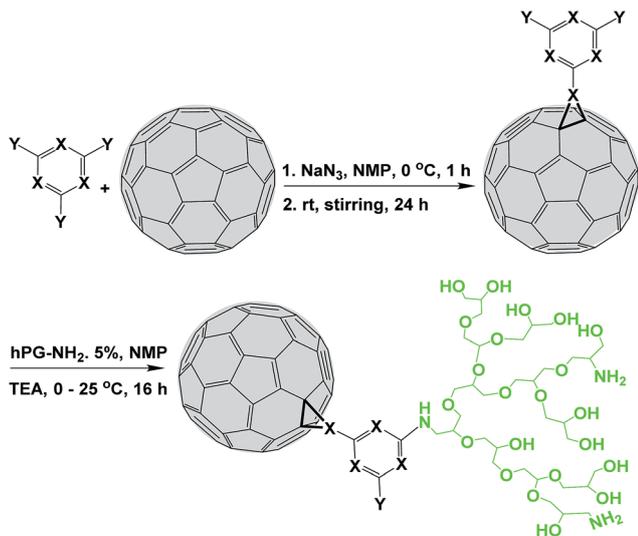
Scheme 1 Functionalization of fullerene by an *in situ* [2 + 1] nitrene cycloaddition reaction at room temperature. Chlorine and nitrogen atoms are symbolized by Y and X, respectively.

fullerene (C_{Full}) at 284.6 eV, an intense peak at 286.3 eV, which is assigned to the carbon atoms of functional groups (C_{Trz}), provides another evidence for conjugation of triazine to the surface of fullerene (Fig. 1a). The peak area ratio of these two types of carbon atoms (C_{Trz}/C_{Full}) is 0.56, confirming that Full-Trz consists of 56% triazine and 44% fullerene moieties (Table S1†). Such ratio indicates conjugation of 5 triazine units to the surface of fullerene.

The N K-edge NEXAFS spectrum of Full-Trz at the low-energy side (Fig. 1b) reveals two sharp resonances (I, II) that correspond with triazine groups that are attached to fullerene. The low energy resonance (I) is related to the nitrogen atoms in the triazine ring which was found in NEXAFS spectra of triazine in the gas phase.[15,16] Resonance (II) also is characteristic for triazine molecules, coupled to carbon materials and was reported in literature.[13]

In the IR spectra of Full-Trz, two absorbance bands, respectively located at 1181 and 1427 cm^{-1} are the characteristic peaks of C_{60} (Fig. S2a†).[17] Besides, the absorbance bands at 1510 and 1634 cm^{-1} are assigned to C=N and C=C bonds of the triazine

and the fullerene fragments, respectively. In the IR spectra, the characteristic bands of the aziridine rings, created by the [2 + 1] cycloaddition reaction, usually appear around 1000 and 1250 cm^{-1}. Those bands are due to symmetric aziridine ring breathing modes and to the sp^3-hybridized C–N bonds.[18] The absence of such absorbance bands in the IR spectra of Full-Trz could reflect the opening of the aziridine ring and subsequent formation of an azafulleroid structure.

Furthermore, the number of triazine functional groups conjugated to the surface of fullerene was also calculated by thermogravimetric analysis (TGA). Based on the mass loss at 480 °C, we found that the total mass of triazine groups in Full-Trz is 55%. This indicates that 5 triazines conjugate to one C_{60} molecule (Fig. S3†). This result is in good agreement with XPS data. The UV-vis spectrum of Full-Trz shows a broad absorption band in the 200–800 nm range (Fig. 2a). The extension of the UV absorption of fullerene from short wavelengths (200–430 nm) to near-IR is due to the substantial changes in its π-conjugated system.

Usually, covalent functionalization of carbon based nano-materials by [2 + 1] cycloaddition reaction results in sp^3 carbon atoms (at the functionalization site) with consequent disruption of the π-conjugation system.[19] As a result, the useful optoelectronic properties of carbon based nanomaterials will be suppressed, which is a hampering factor for their future applications.[20] In contrast, the present functionalization improve the intrinsic optochemical properties of fullerene, significantly.

The mechanism we propose, as an explanation for these noticeable observations, implies the opening of the aziridine rings followed by sp^3 → sp^2 rehybridization of the carbon atoms (Fig. 3).

This type of isomerisation, usually referred to as aziridinofullerene-azafulleroid interconversion, has been reported for fullerene derivatives previously.[10,11] However, much harder reaction conditions were used.

A similar isomerisation has been observed for single walled carbon nanotubes functionalized by carbon[21,22] or [2 + 1] nitrene cycloaddition reaction.[11] There is a strong correlation between the curvature of tubes and this type of isomerization.[21] Therefore, one would expect that this conversion is more feasible for fullerene as a highly curved aromatic system. Accordingly,
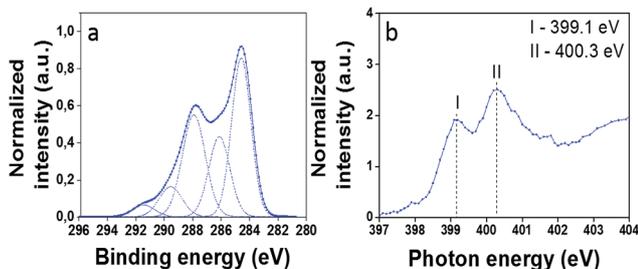

Fig. 1 (a) Highly resolved XPS spectra with peak fitting for C 1s Full-Trz, (b) expanded low energy section of N K-edge NEXAFS of Full-Trz. For assignments see Tables S1 and S2.†
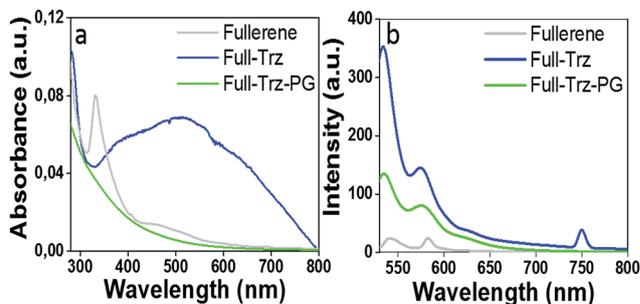

Fig. 2 (a) and (b) UV and fluorescence spectra of 0.1 mg ml^{-1} solution of fullerene (in DMF), Full-Trz (in PBS) and Full-Trz-PG (in PBS), respectively. Spectra are recorded at room temperature.
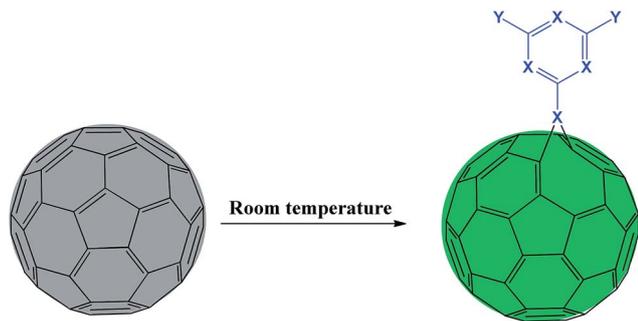
Fig. 3 Aziridinofullerene-azafulleroid interconversion and rehybridization of the carbon atoms of fullerene at ambient condition. X and Y are nitrogen and chlorine atoms, respectively.

computational studies were performed to investigate Full-Trz isomerization.

While aziridinofullerene-azafulleroid interconversion requires heating,[23,24] the functionalization of fullerene by triazine in 5,6/5 and 5,6/6 positions returns preferentially azafulleroid structures (Fig. 4). Moreover, the relative energy of the frontier orbitals of the pristine and functionalized fullerene was calculated. Interestingly, the HOMO–LUMO separation in the azafulleroid form was found to be very close to the one calculated for the pristine C_{60} (Table S3†).

Given the fact that even a very low number of sp^3 carbons would determine a dramatic decrease in the HOMO–LUMO gap of fullerene,[25] the computational results strongly uphold the hypothesis of a retained, homogeneous sp^2 hybridization of all carbon atoms in Full-Trz.

In the open azafulleroid form, the π-conjugated system of fullerene is regenerated and the π-network extends over the triazine rings (Fig. 4). As a result a broad band appears in the absorption spectra of the Full-Trz.

Optical properties of fullerene derivatives were also investigated computationally (ESI page 9 and 10†). When triazine was arranged in a symmetric shell around fullerene, the UV spectra of Full-Trz was very similar to the experimental data (Fig. S6 and S7†). These results suggest not only a core–shell structure for the functionalized fullerene but also prove the influence of the triazine shell on the optical properties of fullerene.
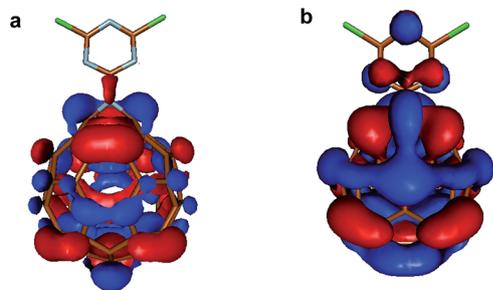
The near-IR absorption suggests that Full-Trz should exhibit unique optical properties that are observed for single walled carbon nanotubes.

Irradiation of a 5 mg ml^{-1} dispersion of Full-Trz in PBS increase the temperature of medium to 53 °C after 60 seconds, while this effect was not observed for fullerene when exposed under the same conditions (Fig. 5).

Due to such high photothermal conversion, Full-Trz could be used for variety of applications such as photothermal therapy. Interestingly, Full-Trz shows a high fluorescence emission upon excitation at different wavelengths (Fig. S4†). Therefore, it could be used as an alternative for single-wall carbon nanotubes and in particular for bioimaging.

However, poor water solubility of Full-Trz hampers such applications. Therefore, polyglycerol with few amino functional groups was conjugated to Full-Trz at room temperature, taking advantage of the high reactivity of triazine groups toward nucleophilic reactions (Scheme 1). IR spectrum of fullerene–polyglycerol conjugate (Full-Trz-PG) shows absorbance bands at 3365, 1652 and 1100 cm^{-1} which are assigned to O–H, C=C and C–O bonds. This result confirms conjugation of polyglycerol to C_{60} (Fig. S2†). Thermogravimetric analysis (TGA) shows that Full-Trz-PG is consisting of 90% polyglycerol and 10% fullerene moieties[5d] (Fig. S3†). This compound shows photothermal and fluorescence properties similar to Full-Trz and it is readily soluble in PBS (Fig. 2, 5 and S8†).

While the fullerene content of Full-Trz-PG is low (10%), its fluorescence is remarkable. Therefore it can be considered as a promising vector with the unique optoelectronic properties for bioimaging (Fig. S9†).

Quantum yields of Full-Trz and Full-Trz-PG are much higher than those reported for fullerene (Table S4†).

In order to investigate the efficiency of Full-Trz and Full-Trz-PG as fluorescence probes, they were incubated with the A549 cells and then imaged by confocal laser scanning microscopy (CLSM). Fig. 6 show confocal images of A549 cells incubated with these compounds after 4 hours. Uptake of both Full-Trz
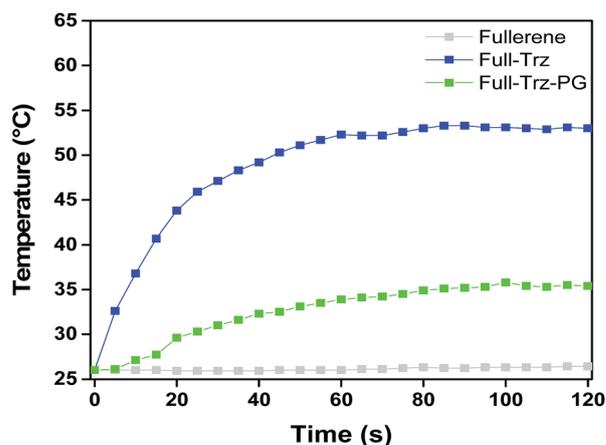

Fig. 4 Representation of the HOMO of (a) 5,6/5-Full-Trz and (b) 5,6/6-Full-Trz calculated at the M06-2X/def2-SVP level of theory, using an isosurface value of 0.02 e Å^{-3}.

Fig. 5 Evaluation of photothermal conversion of fullerene, Full-Trz, and Full-Trz-PG dispersions in PBS (5 mg ml^{-1}) over a period of 2 min under NIR laser irradiation (785 nm, 0.5 W cm^{-2}).
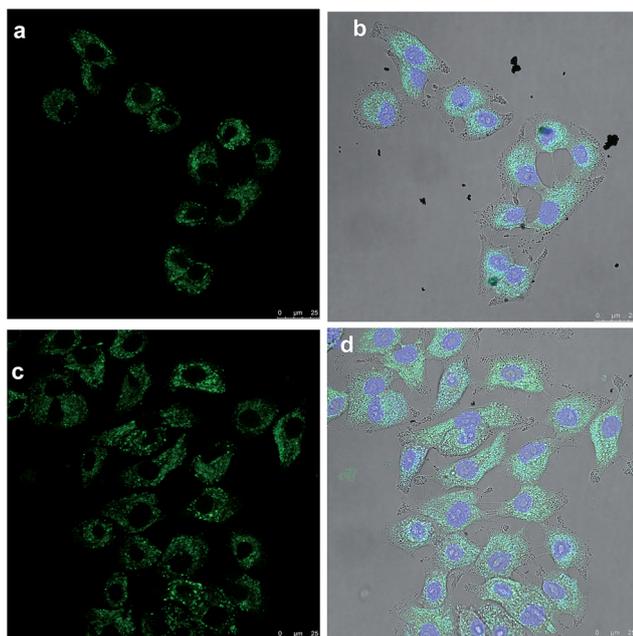
Fig. 6 The CLSM images of A549 cells incubated with 30 µg ml^{-1} of Full-Trz (a, b) and Full-Trz-PG (c, d) after 4 hours. Left column is fluorescence images of Full-Trz (up) and Full-Trz-PG (down) and right column is the related overlay images.

and Full-Trz-PG can be followed through their fluorescence. However, intensity of fluorescence in the case of Full-Trz-PG is higher, considering 10% Full-Trz content for this compound. Due to the higher solubility of Full-Trz-PG and less aggregation in buffer lower quenching of fluorescence occurs.

Taking advantage of this new functionalization method, fullerene derivatives with the unique optoelectronic and physicochemical properties for a variety of applications could be synthesized. Due to its well-defined structure, Full-Trz is good alternative to nanomaterials with less-defined structures such as single walled carbon nanotubes and quantum dots in bioimaging.

Acknowledgements

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