Functional Modification of Graphene Nanoparticles: Covalent Grafting of Peptides and π Bonding for Drug Loading and Delivering

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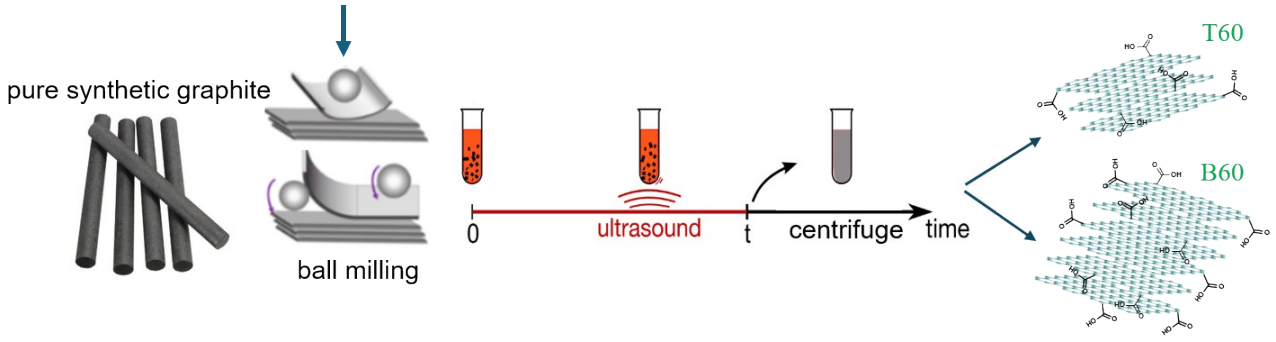
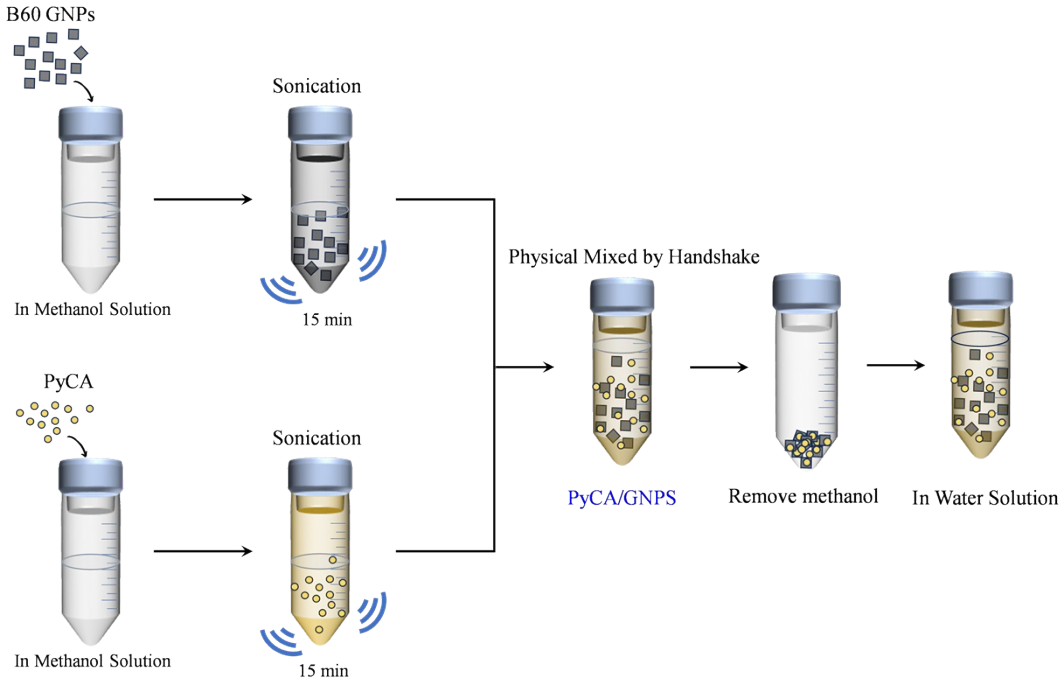
SUPPORTING INFORMATION

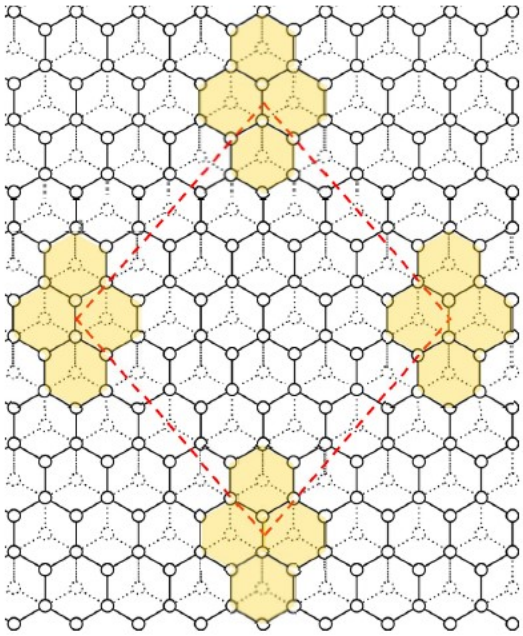


Scheme S1. Diagram of the GNPs preparation process.



Scheme S2. The process of physical mixing for the preparation of PyCA/GNPS samples.

Comment to Scheme S2. A water dispersion containing a mixture of PyCA and B60 GNPs was prepared, for sake of comparison with PyCA@B60. The process of mixing is illustrated in Scheme S2: as in the procedure for the preparation of the conjugates we dissolved PyCA in methanol, but in this case the sonication step is conducted for a shorter time (15 min). A dispersion of the PyCA/GNPS in methanol is obtained by handshaking for 2 min. A water dispersion at the same PyCA/GNPS concentrations as PyCA@B60_0W is then obtained after removal of methanol (by vacuum evaporation) and adding 50 ml of pure water.



Scheme S3. Sketch of a hypothetical regular structure of PyCA monolayer absorbed on the GNP surface. 2D unit cell: broken red lines. Two non-equivalent graphene layers are illustrated according to the AB staking. The figure describe both the AB arrangement of the PyCA (in this case the dotted hexagonal layer represents the top GNP surface) and the AA arrangement of PyCA (in this case the solid-line hexagonal layer represents the top GNP surface). Only the pyrene group of PyCA is represented.

Comment to Scheme S3: calculation of the ideal PyCA loading capacity of B60 GNPs

To evaluate the ideal PyCA loading capacity of our GNPs we adopt a simple model based on the following hypothesis and schematically described in Scheme S3:

(i) PyCA molecules are absorbed on the GNPs surfaces by means of π-π interactions between the graphene surface and the pyrene moiety, namely PyCA molecules stick on the two exposed graphene layers of GNPs.

(ii) The arrangement of PyCA molecules on the graphene layer follows the characteristic AB stacking or the AA stacking, according to the different possible layer stacking in the graphite crystals.

(iii) A regular molecular layer of single PyCA molecules is formed, which allows to describe the graphene top layer decorated by PyCA molecules as a 2-D crystal. A possible dense packing of PyCA is illustrated in Scheme 3, where the distance between first neighboring PyCA is compatible with (ii) and allows the accommodation of the atoms on the periphery of the pyrene group.

(iv) B60 consists of 6 graphene layers, corresponding to the experimental average thickness of B60 nanoparticles, <N> = 6.

(v) the presence of PyCA molecules in the inter-layers regions of B60 is neglected, as well as the presence of PyCA molecules linked on the graphene edges via H-bonding with the native -COOH groups of B60.

According to the model, the degree of covering of the surface B60 layer can be calculated as follow:

N (C_Py)/N (C_B60 layer) = 16/48 = 0,33.

where:

- N(C_Py) is the number of sp^2 carbon atoms of pyrene in the unit cell: N(C_Py) = 16 since the unit cell contains one only PyCA molecule;
- N(C_B60 layer) = 48 is the number of carbon atoms of the graphene surface belonging to the unit cell.

In order to obtain the ideal loading capability of the GNP we must consider that the particles is formed by of 6 layer and 2 exposed surfaces, so the total amount of GNPs C atoms for each PyCA absorbed molecule is [(48x6)/2] = 144, thus meaning that about 11% GNP Carbon atoms are interacting with PyCA molecules.

The result above can be easily transformed in the weight to weight ratio between absorbed PyCA molecules and GNPs according to:

PyCA loading capacity = weight (PyCA_absorbed) /weight (B60) = $(144 \times M_C / 16 \times M_{PyCA}) = 0.12$

The value obtained indicates that B60 nanoparticles can load (on the graphene surfaces) a PyCA amount of about 12% of their weight.

Interestingly, the loading capacity resulting from the model is compatible with the weight to weight ratio adopted for the preparation of PyCA@B60 conjugates (0.165), indicating that a yield close to 100% could be reached with this formulation. Indeed, the experimental evidences collected on PyCA@B60 samples indicate that a very little amount of B60 is lost during the preparation and washing of PyCA@B60; moreover, some additional amount of PyCA linked to B60 through H bonds and/or sandwiched between graphene layers should be considered by relaxing hypothesis (v).

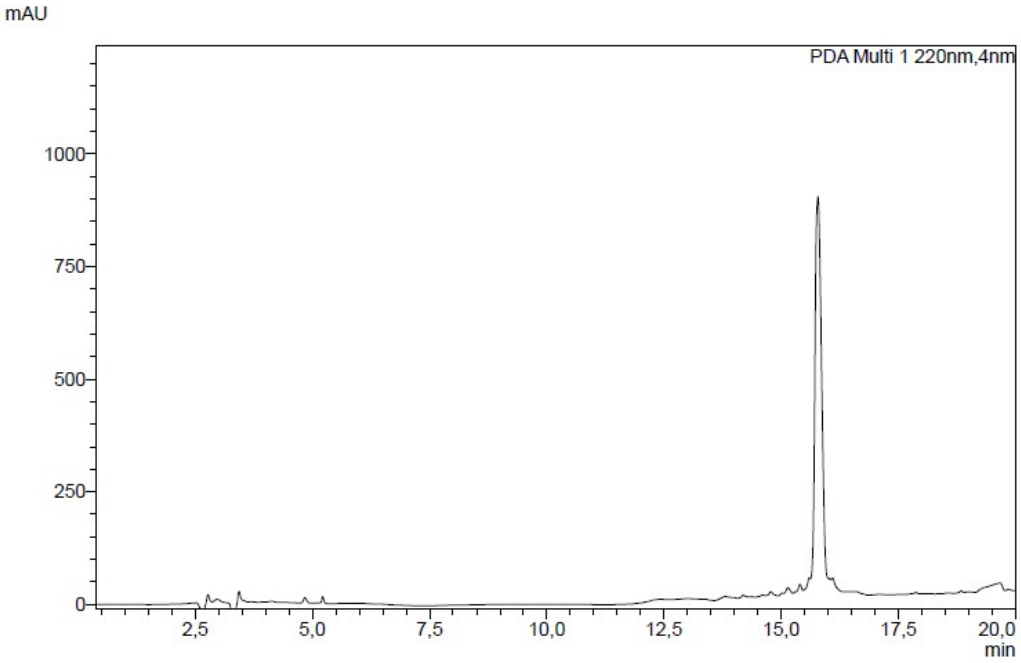


Figure S1. HPLC chromatogram at 220 nm of the peptide R11.

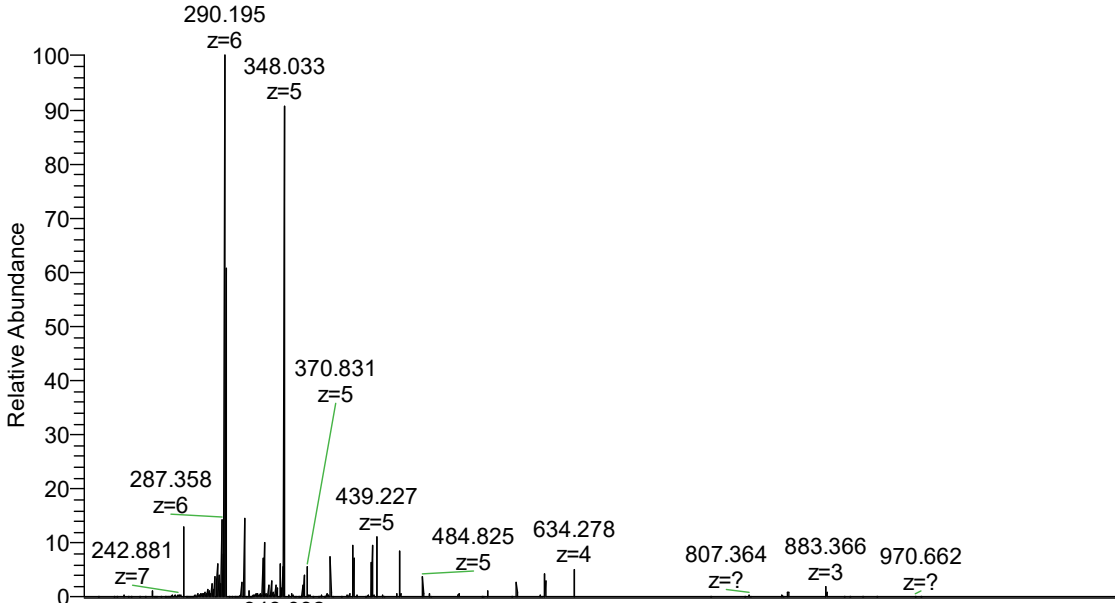


Figure S2. ESI-MS of the peptide R11. $[M+5H]^{5+}$ 348.033, $[M+6H]^{6+}$ 290.195

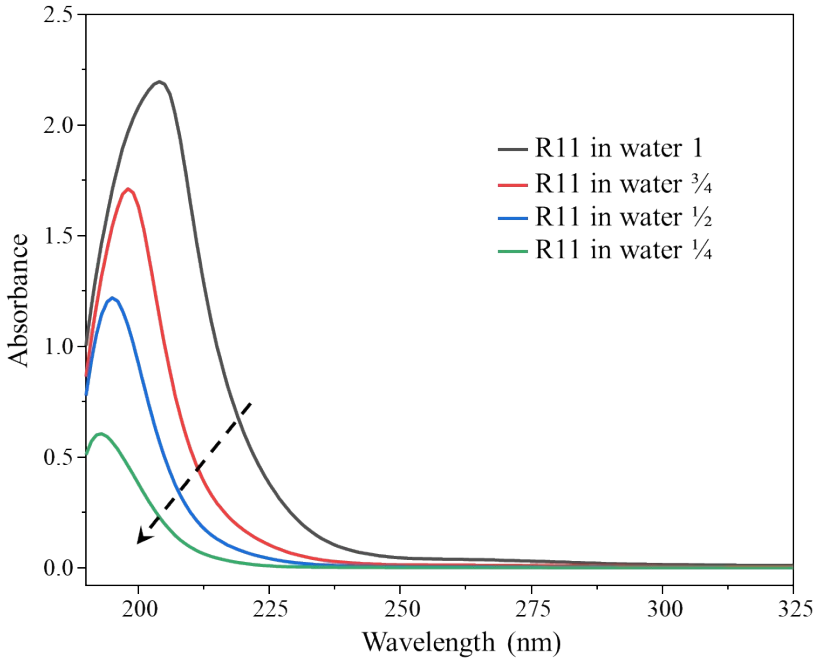


Figure S3.a. UV-vis absorption spectra of R11 water solutions at different concentrations. "R11 in water 1" corresponds to the initial dilution. For the other experiments, the reference sample has been diluted in order to achieve a R11 concentration corresponding to ¾, ½, ¼ of the initial one.

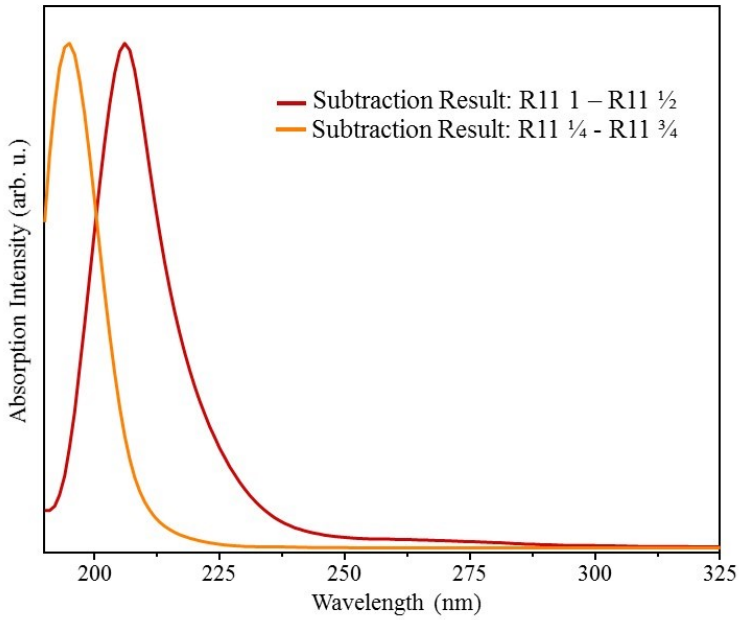


Figure S3.b. Subtraction results of UV-vis spectra of R11 in water. The subtraction spectra are obtained as difference between spectra of the R11 sample at different concentration. The procedure allows to highlight the absorption spectrum of due isolated R11 molecules in water, peaking at lower wavelengths (orange line) and the absorption spectrum of R11 aggregates, peaking at higher wavelengths (red line).

Comment to Figures S3.a – S3.b: UV-vis Spectra of R11 in water.

Water solutions of R11 show a structured absorption band with its maximum ranging from 204 nm to 198 nm, while decreasing the R11 concentration in water. The observed shift of the maximum can be ascribed

to the occurrence of a different relative concentration of isolated peptides (which absorb at shorter wavelengths) and aggregated peptides (which absorb at longer wavelengths) in the samples. Indeed, the subtraction spectra in Figure S3.b, clearly shows that the absorption maximum shifts at shorter wavelengths as far as the peptide concentration lowers, i. e. by reducing the number and the size of the aggregates. According to the above observations, we infer that peptide linked to GNPs in R11@GNPs conjugates (λ_{max}< 200 nm) behave like isolated species.

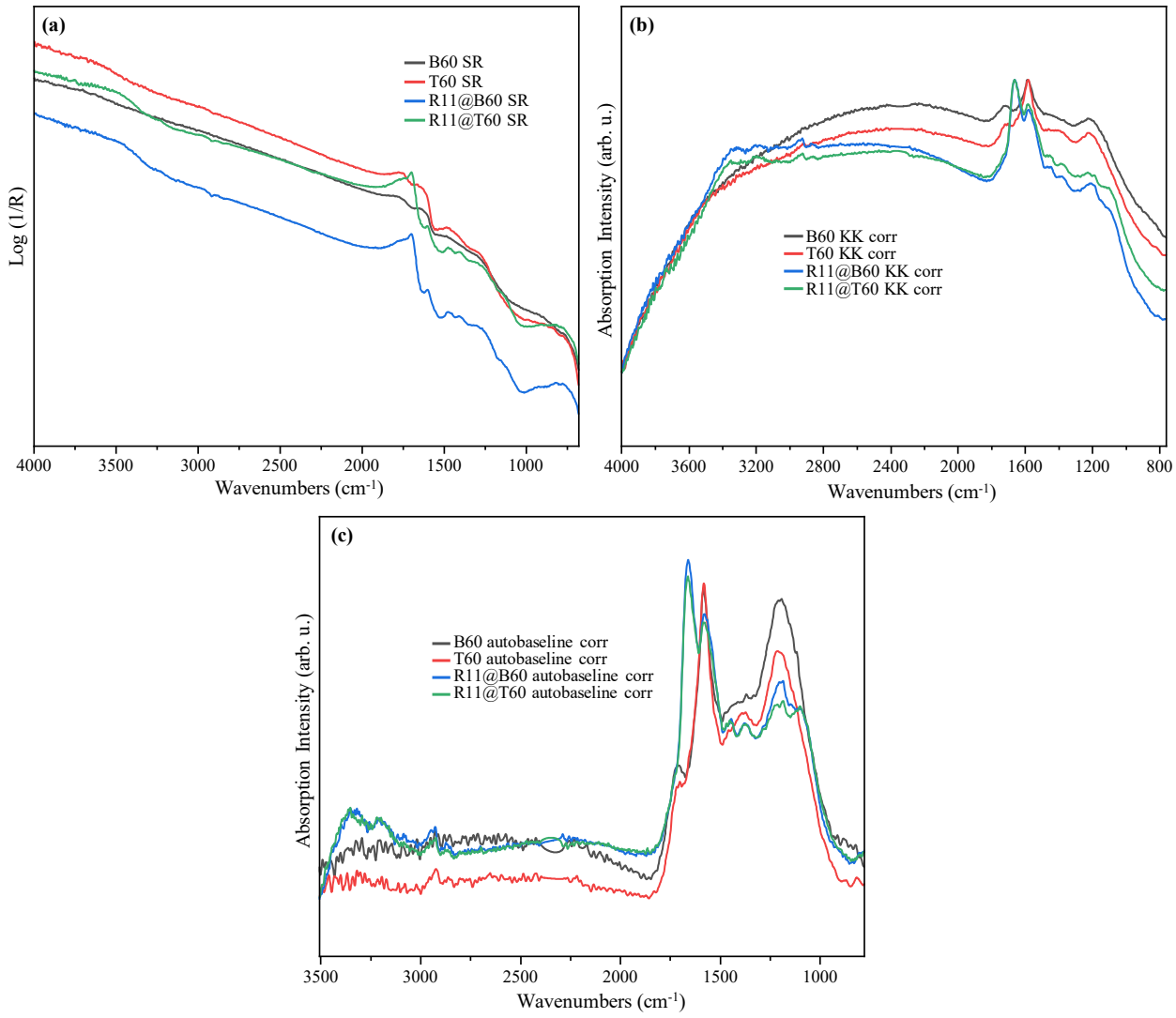


Figure S4. FTIR spectra of T60, B60 GNPs, R11@T60 and R11@B60 conjugates obtained by specular reflection measurements: (a) Raw spectra; (b) spectra after Kramers-Kronig conversion; (c) absorption spectra after automatic baseline correction.

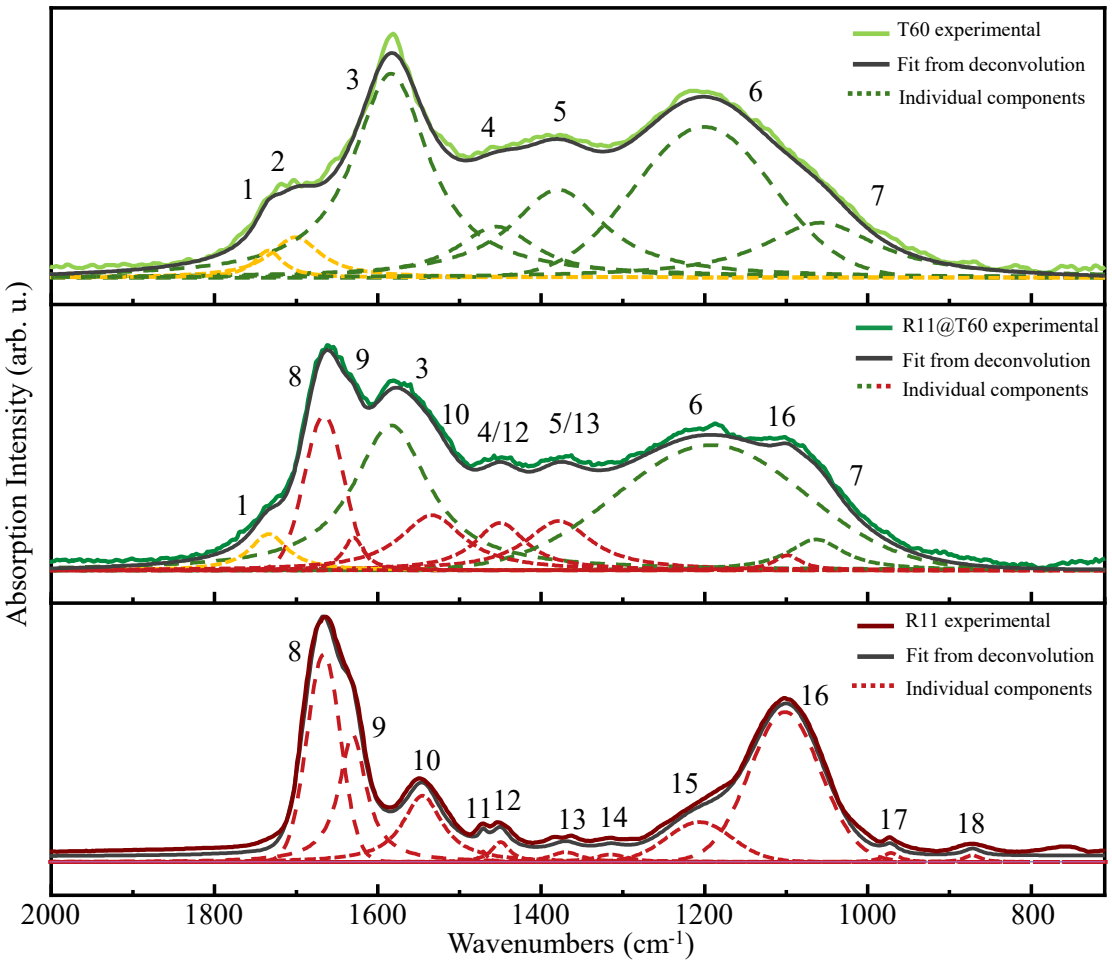


Figure S5. Deconvolution of FTIR spectra of T60, R11 and R11@BT60.

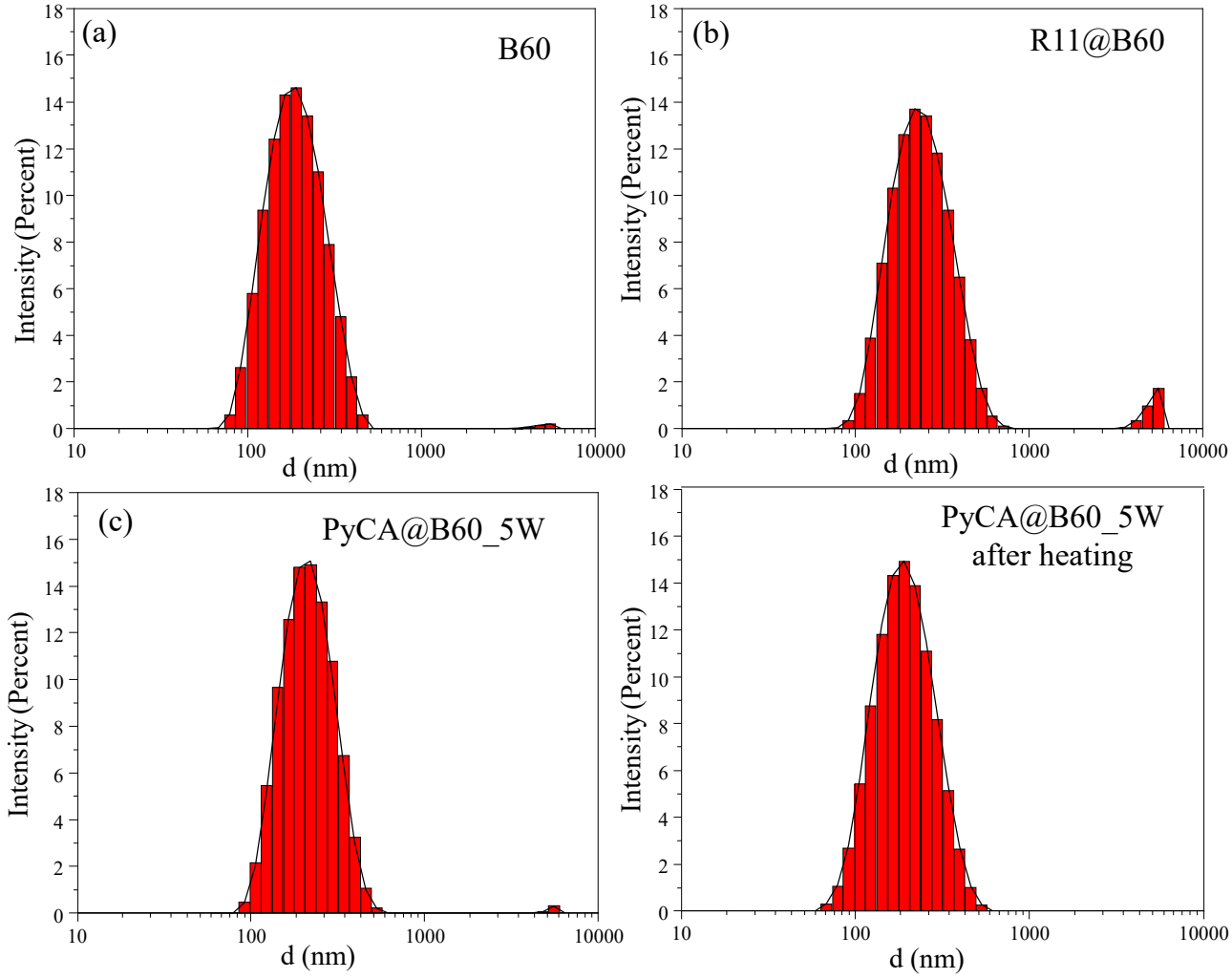


Figure S6. Diameters distributions from Dynamic Light Scattering (DLS) experiments for different GNPs water dispersions: (a) B60, (b) R11@B60, (c) PyCA@B60_5W and (d) PyCA@B60_5W after heating at T = 40 °C for 55 min.

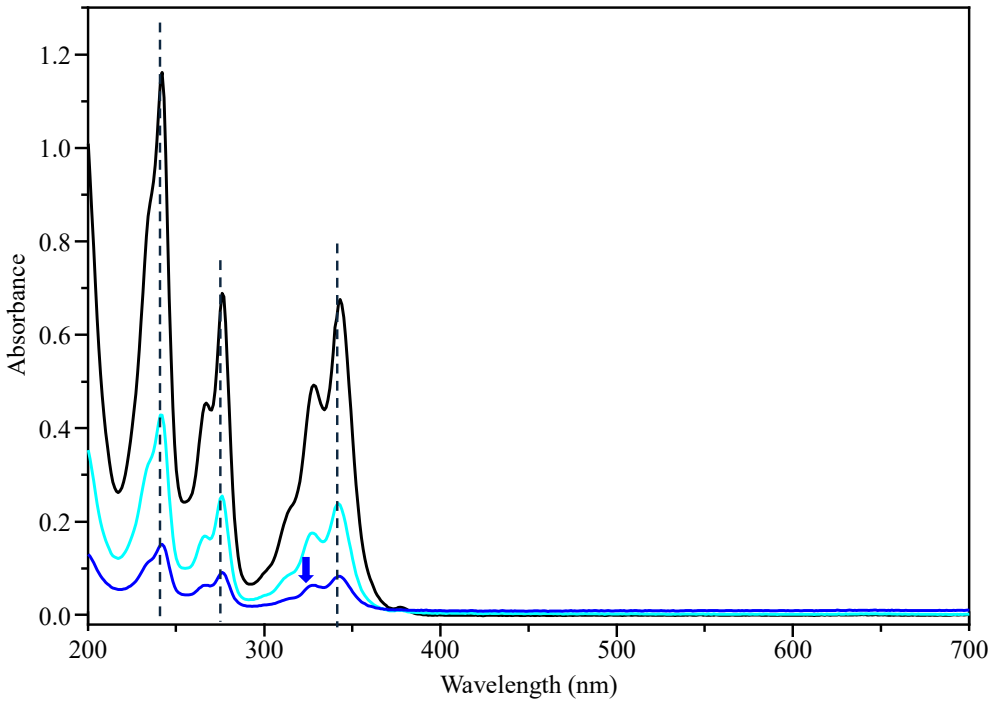


Figure S7. UV-vis absorption spectra of PyCA in (4:1) water/methanol mixture (black line), and water (blue lines) at the same PyCA concentration (0.005 mg/ml). The light blue spectrum and the dark blue spectrum correspond to UV-vis measurements at different times: immediately after preparation of the water solution and after few minutes, respectively.

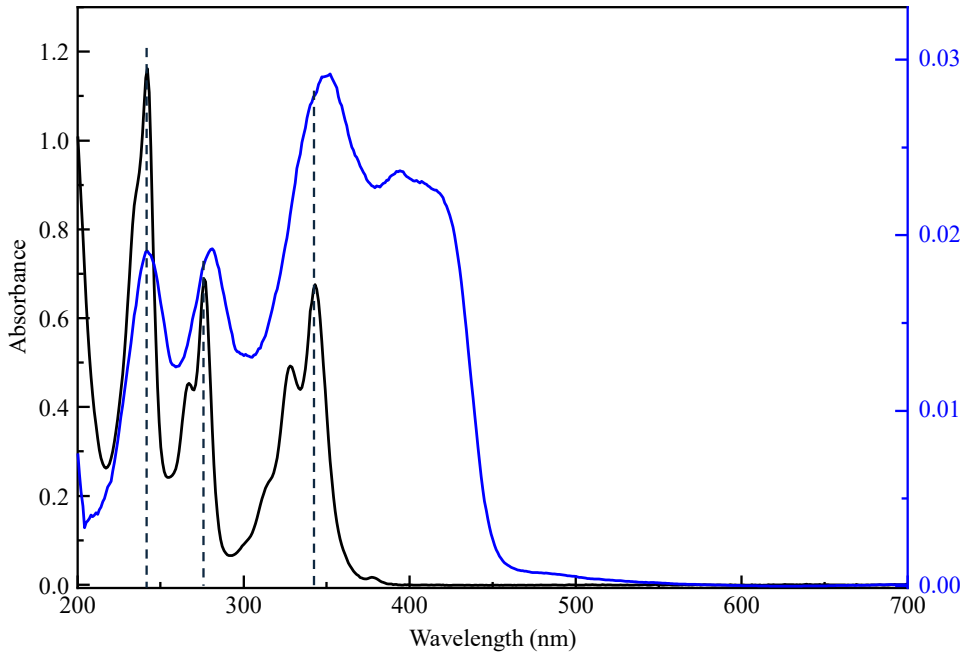


Figure S8. UV-vis absorption spectrum (blue line) of a solid PyCA sample (PyCA powder mixed with $BaSO_4$). The spectrum has been recorded in diffuse reflectance and corrected by Kubelka-Munk transformation. The spectrum of PyCA in water/methanol solution (black line) is shown for sake of comparison.

Comment to Figures S7 – S8. UV-vis absorption spectra of PyCA.

The spectra of pure PyCA in water (0.005 mg/ml), Figure S7, shows a remarkable decrease of the absorption intensity of PyCA at different times after the preparation of the solution, due to PyCA precipitation. Interestingly, the absorption profile (peaks position and bands shapes) does not change during time, thus suggesting that the observed absorption features come from isolated PyCA molecules in water, while mostly PyCA aggregates precipitate. The above interpretation is supported by the UV-vis spectrum of PyCA dissolved in a 4:1 water/methanol mixture, which is a very good solvent of PyCA (Figure S7, black line). The spectrum of PyCA in water/methanol solution is strong and its absorption profile and intensity are stable over time; moreover, the spectral pattern coincides to that obtained for PyCA water solution. The above observation indicates that – in solution – the environment has a negligible effect on the three structured absorption features ascribed to the pyrene moiety in the region 220 - 400 nm, which instead are sensitive to solid-state interactions in the PyCA crystal. The solid state spectrum shows broadening of the three main bands – without appreciable shift of their maxima – and the appearance of a large absorption feature at longer wavelengths (onset at λ = 450 nm), shown in Figure S8.

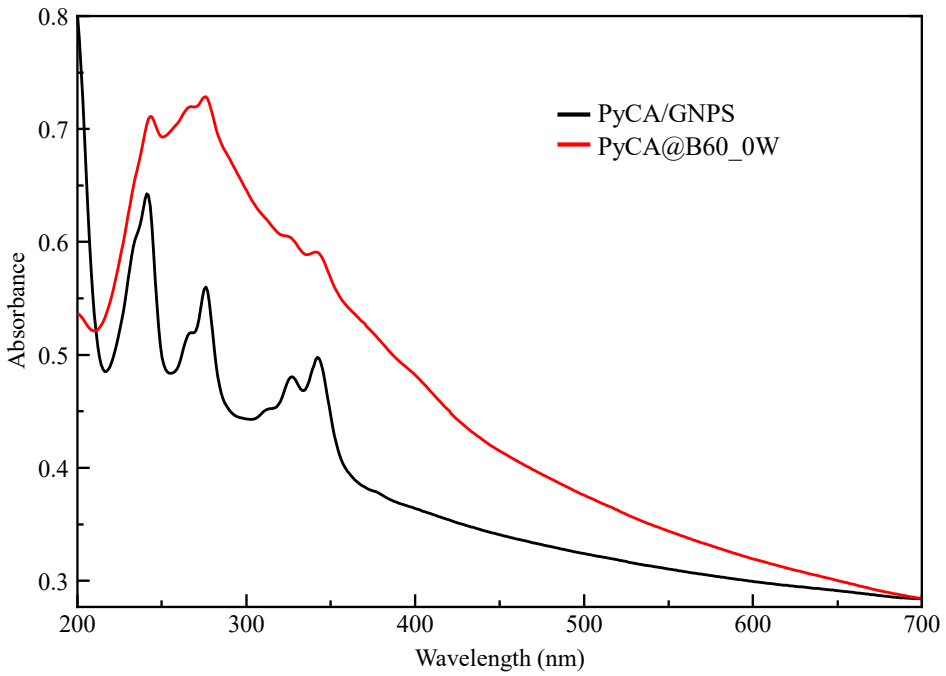


Figure S9. UV-vis absorption spectra of PyCA@B60_0W conjugate (red line) and PyCA/GNPS physical mixture (black line) at the same PyCA concentration.

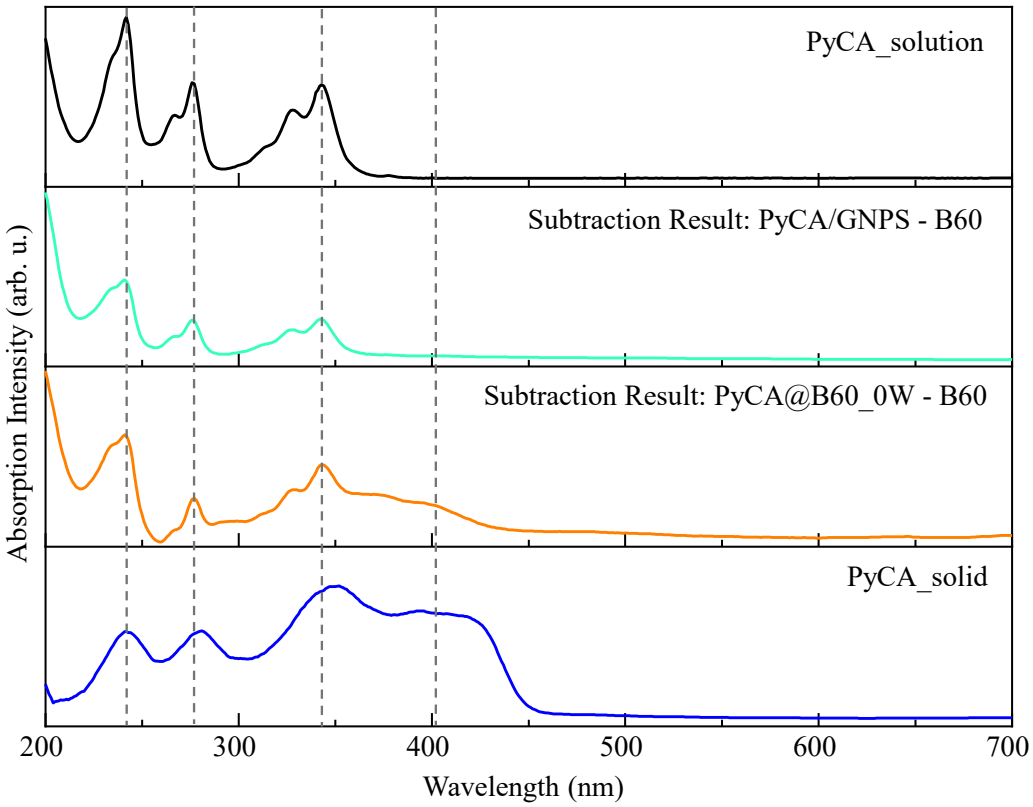


Figure S10. Difference spectra obtained by subtracting the spectrum of B60 water dispersion from that of PyCA@B60_0W (orange line) and from that of PyCA/GNPS (green line). The spectrum of solid PyCA (blue line) and of PyCA in solution (black line) are reported for sake of comparison.

Comment to Figures S9-S10. UV-vis absorption spectrum of PyCA/GNPs physical mixture vs PyCA@B60_0W.

The pattern of PyCA@B60_0W spectrum is different from that of the sample of the physical mixture PyCA/GNPS, as shown in Figure S9. The comparison is made between the two samples PyCA@B60 and PyCA/GNPS, before washing to guarantee that the two water dispersions contain PyCA molecules at the same concentration. However, the empirical evidence that GNPs precipitate during the measurement of the PyCA/GNPS sample could explain why in the PyCA/GNPS spectrum the signals of PyCA are strong compared to the contribution from GNPs, while the rather weak absorption peaks of PyCA float on the broad absorption feature of B60 in the case of PyCA@B60_0W. Interestingly, in the range 200 - 350 nm, the subtraction of the spectrum of B60 from that of PyCA/GNPS (Figure S10), show the same absorption bands of PyCA observed in the case of PyCA@B60, while, in the region 350 - 450 nm, there is a clear difference. Indeed, the physical mixture does not show any absorption feature below 350 nm, while PyCA@B60 shows a broad absorption which can be ascribed to those PyCA molecules experiencing a strong π-π interactions with GNPs and it is possibly related to PyCA sandwiched between graphene layers.

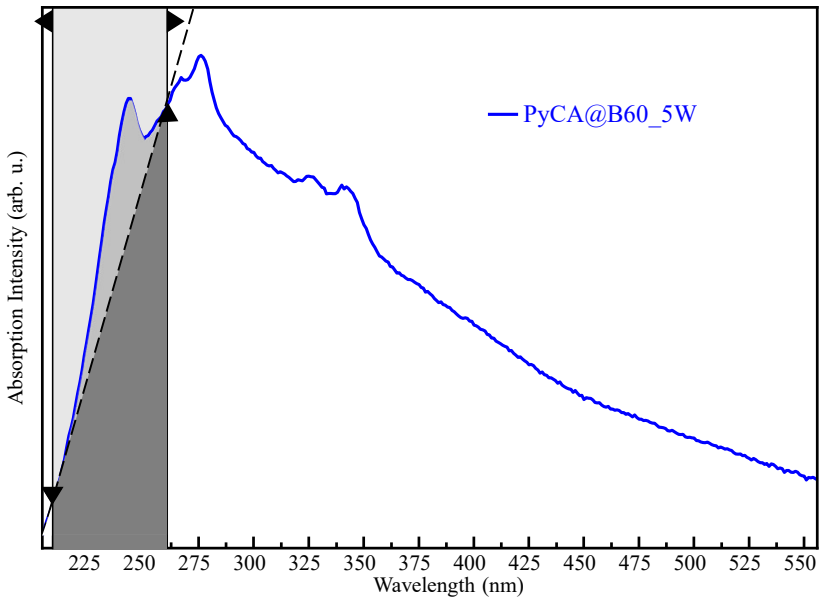


Figure S11. The figure illustrates the procedure adopted for the determination of the parameter R, which is a proxy of the relative contribution of PyCA to the UV-vis absorption of the sample PyCA@B60_5W. On the plot, the dashed segment corresponds to the baseline selected for the measurement of the contribution of PyCA absorption (medium grey area between the spectrum - blue line - and the dashed segment). The whole absorption intensity coming from both GNPs and PyCA contributions is described by the total area under the spectrum – blue line – in the range between 210 and 260 nm (medium grey + dark grey areas).

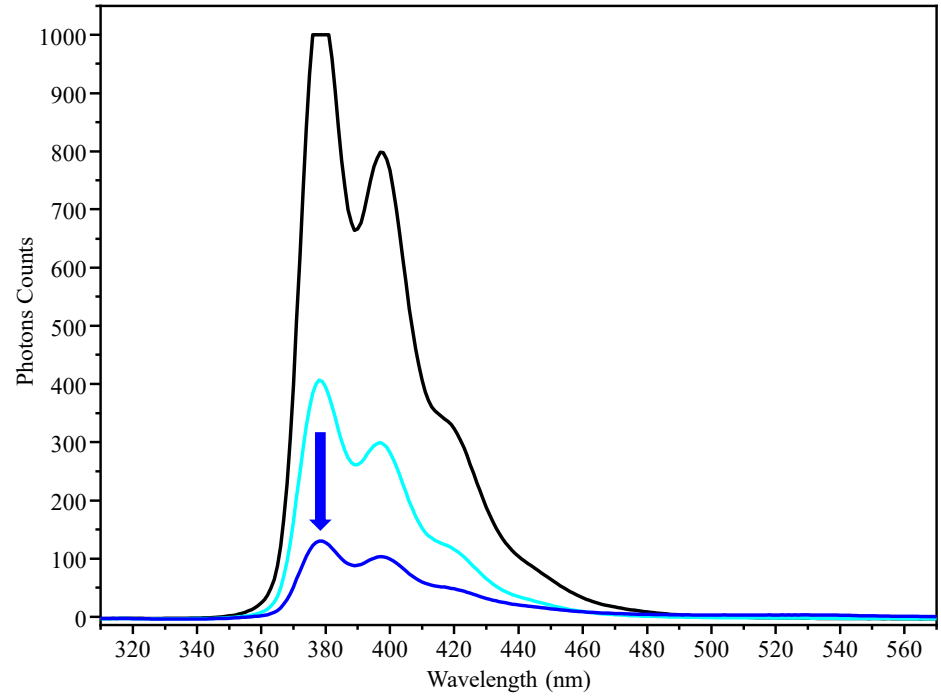


Figure S12. Fluorescence spectra of PyCA in water (light and dark blue lines: spectra recorded at different times after the preparation of the solution) and in water/methanol solution (black line). The strongest fluorescence peak of PyCA in water/methanol solution shows saturation of the signal. The PyCA concentration is the same for the three samples.

Comment to Figure S12– Fluorescence spectra of PyCA solutions.

Figure S12 report fluorescence spectra of PyCA recorded in water and water/methanol mixtures. The PyCA concentration and the parameters adopted for the measurement set-up are the same for the two solutions, so that the strength of the fluorescence signal of the spectra can be compared. The spectrum profile of PyCA in water is practically superimposable to the one recorded in the water/methanol solution, but the intensity of the signal is lower, and it decreases in intensity over time. Instead, in a mixed solution of water and methanol (4:1), the fluorescence intensity is stable. This confirms the conclusions reached by means of UV-vis spectroscopy, namely that PyCA molecules in water are likely to form aggregates and are prone to precipitate. The remarkable decrease of the fluorescence signal from water/methanol to water solution could be ascribed either to fluorescence quenching in the aggregates, or to their precipitation. As in the case of the UV-vis absorption spectrum, we take the fluorescence spectrum in water (or in water/methanol) as representative fluorescence pattern of the isolated PyCA molecules.

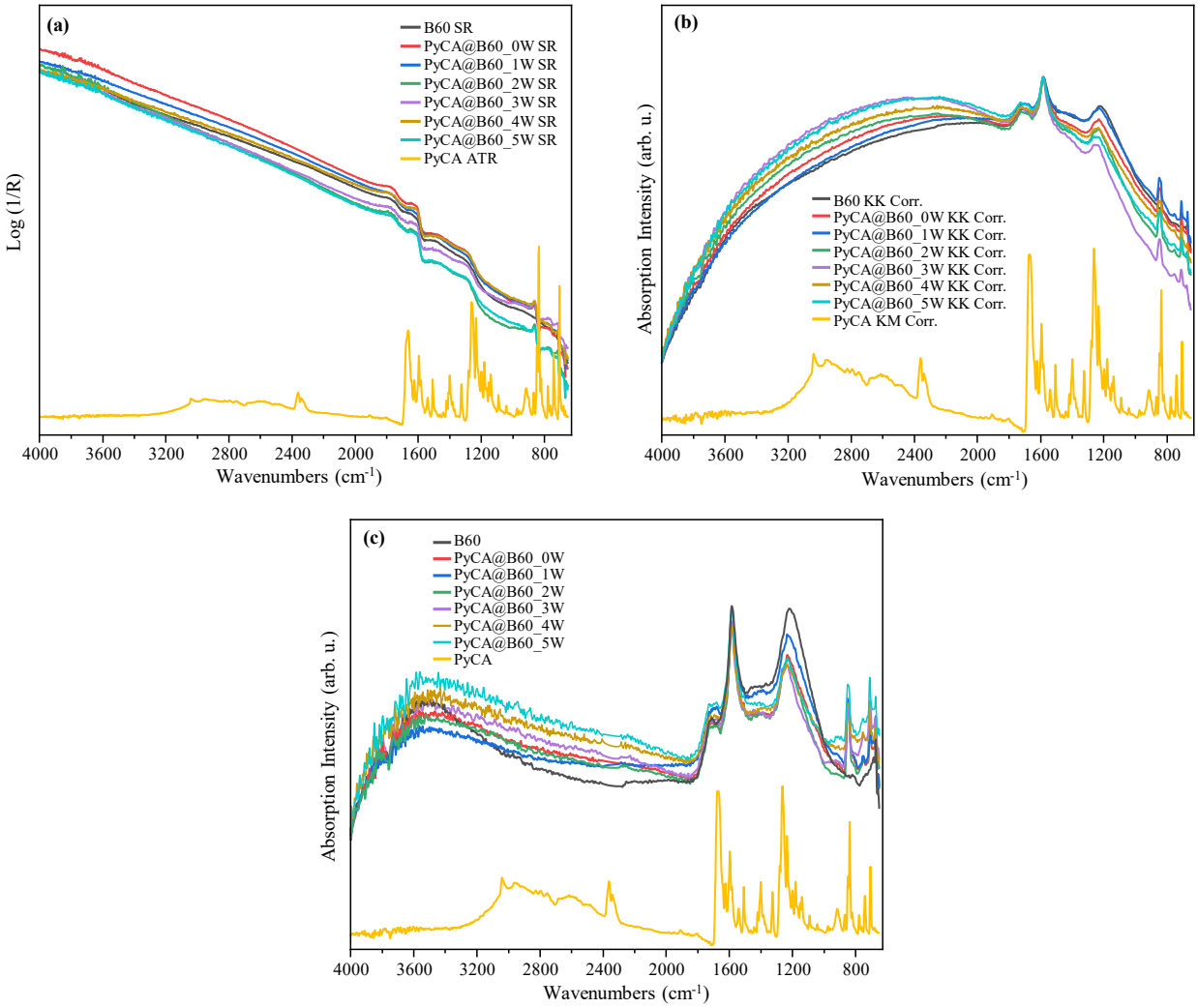


Figure S13. FTIR spectra of B60 GNPs, PyCA@B60_MW (M = 0 - 5) obtained by specular reflection measurements. FTIR spectrum of powder of PyCA, from ATR measurement: (a) Raw experimental spectra; (b) Experimental spectra after Kramers-Kronig conversion (Kubelka-Munk correction for PyCA spectrum); (c) Experimental absorption spectra after automatic baseline correction.

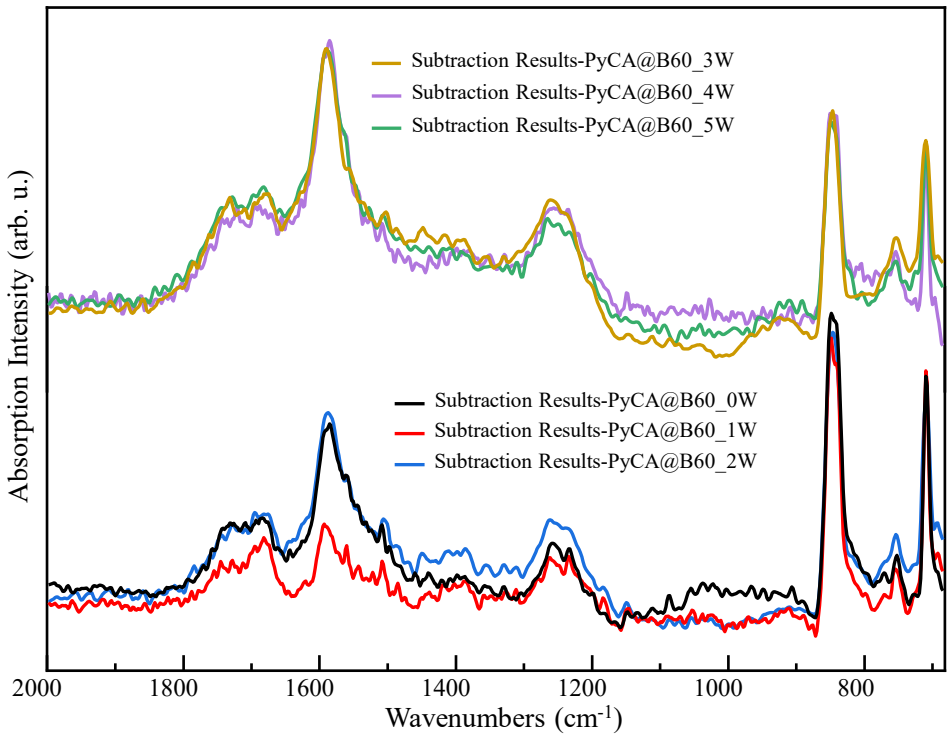


Figure S14. FTIR spectra obtained by subtracting the spectrum of B60 from those of PyCA@B60_MW conjugates (M= 0-5).

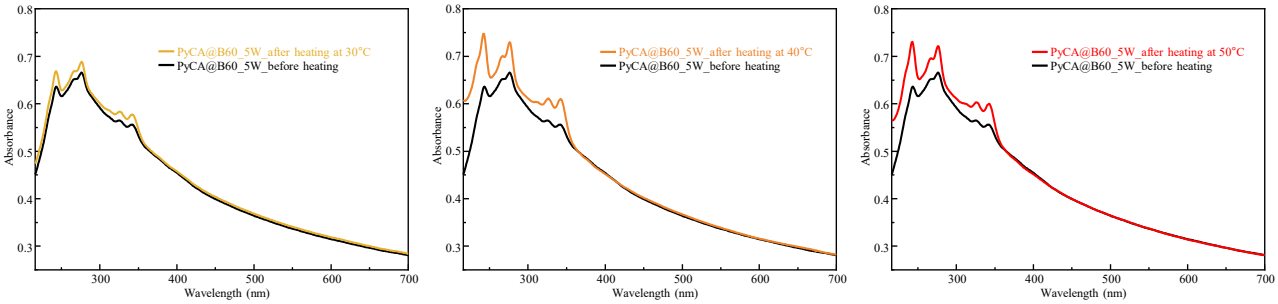


Figure S15. UV-vis absorption spectra of PyCA@B60_5W in water dispersion before heating and after local heating T= 30 °C, for 25 min, at T= 40 °C for 55 min, and at T= 50 °C for 55 min, respectively.

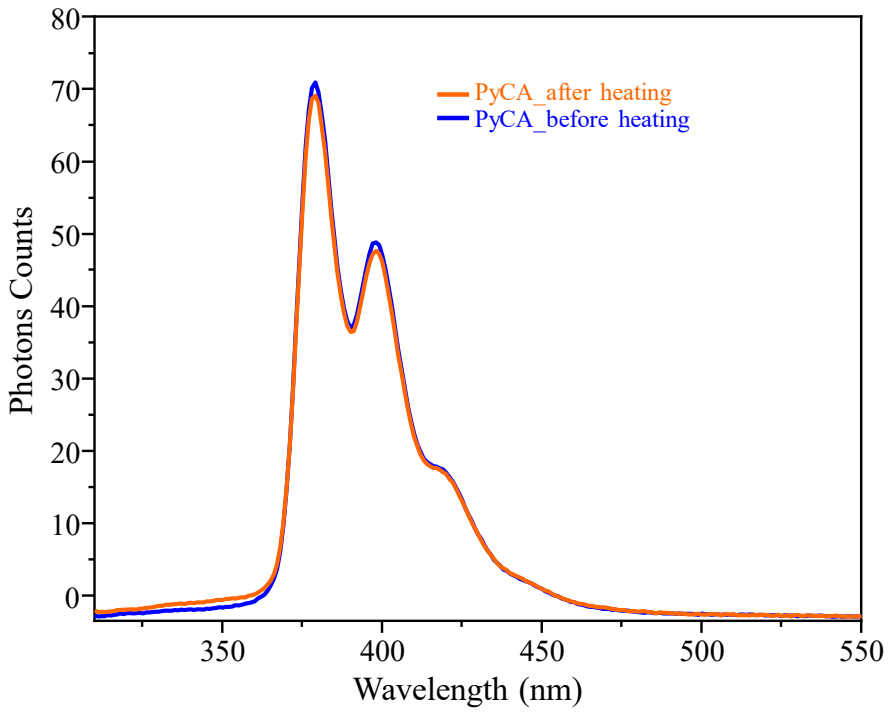


Figure S16. Fluorescence spectra of pure PyCA in water solution before and after heating.

Table S1. Functions adopted for fitting the individual components in the deconvolution of FTIR spectra of T60, B60, R11@T60, and R11@B60.

Samples	Fitting Components	Type of Peak
T60	1734; 1701; 1584; 1455; 1380; 1058	Lorentzian
	1201	Voigt
B60	1734; 1701; 1583; 1453; 1371; 1056	Lorentzian
	1196	Voigt
R11@T60	1734; 1630; 1584; 1534; 1450; 1379; 1098; 1064	Lorentzian
	1666; 1192	Voigt
R11@B60	1734; 1630; 1583; 1536; 1451; 1374; 1095; 1050	Lorentzian
	1666; 1201	Voigt
R11	1631; 1546; 1471; 1450; 1370; 1315; 972; 872	Lorentzian
	1670; 1207; 1099	Voigt

Table S2. Functions adopted for fitting the individual components in the deconvolution of FTIR spectra of B60 and PyCA@B60.

Samples	Fitting Components	Type of Peak
B60	1734; 1701; 1583; 1453; 1371; 1056	Lorentzian
	1196	Voigt
PyCA@B60_5W	1735; 1687; 1586; 1583; 1506; 1453; 1412; 1371; 1242; 1056; 917; 846; 757; 709	Lorentzian
	1196	Voigt

Supervised fitting procedure of PyCA@B60_5W spectrum

We performed a supervised fitting of the PyCA@B60_5W spectrum (15 components) under the following constraints:

(i) All the B60 components have been introduced (bands #1-#7); peaks frequencies of bands #3 -#7 have been fixed to the values obtained by fitting the B60 spectrum. Bandwidths of the components #3, #6, and #7 have been fixed to the values from the fitting of the B60 spectrum.

(ii) 6 additional components (#3', #6', #8, #9, #10, #11) – from PyCA - have introduced at frequency values of the peaks of the difference spectrum (see Figure 12 and Figure 13).

(iii) No further components in the C=O stretching region (namely #1' and #2') have been added to the fitting of the PyCA@B60_5W spectrum. We indeed obtained several different but equally good solutions in the attempt to fit the C=O stretching region with more than two components. For this reason, we decided to introduce only two components (#1 and #2, namely the two higher frequency bands from B60 spectrum) and to allow adjustment of their peaks frequency/bandwidth during the fitting. These components will be interpreted as sum of the contributions from COOH groups of B60 and of PyCA.

(iv) Two additional components (#4' and #5') in the region between bands #3 and #6 are needed to achieve a good fitting. Even if the subtraction spectrum is noisy in the range 1500 - 1300 cm^{-1} and does not help in the identification of these components, our choice can be justified because of the presence of absorption features of PyCA in that region.