

# Electronic Supplementary Material

## “Click” Conjugation of Peptide on the Surface of Polymeric Nanoparticles for Targeting Tumor Angiogenesis

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### LIST OF CONTENTS

Ultra-Violet (UV) Spectroscopy-----	2
Nuclear Magnetic Resonance (NMR)-----	2
High-Performance Liquid Chromatography (HPLC)-----	2
Field Emission Scanning Electron Microscope (FESEM)-----	2
Static Light Scattering (SLS)-----	3
Dynamic Light Scattering (DLS)-----	3
Small-Angle Neutron Scattering (SANS)-----	3
Fourier Transform Infrared (FTIR) Spectrometry-----	4
X-Ray Photoelectron Spectroscopy (XPS)-----	5
Fluorescence Spectrometry-----	6
Fluorescence Imaging-----	6

## INSTRUMENTS

**Ultra-Violet (UV) Spectroscopy.** UV-visible spectra were recorded on a U-2010 spectrophotometer (Hitachi) with 1-cm quartz cells (Hellma Cells), between 200 and 900 nm at 25°C. In order to estimate the coupling yields of fluorophores on PVDF NPs, fluorophores molar extinction coefficients were deduced from previously acquired calibration curves.

**Nuclear Magnetic Resonance (NMR).** The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 300 MHz and 75.5 MHz respectively, on a Bruker Avance 300 spectrometer at 298 K for solutions in  $\text{CDCl}_3$  or  $\text{CD}_3\text{OD}$ . Chemical shifts were expressed in part per million (ppm) and  $J$  values were given in Hz.

**High-Performance Liquid Chromatography (HPLC).** The purity of products was determined by analytic reverse-phase HPLC using a VWR Hitachi instrument equipped with an L-2450 autosampler, two L-2130 pumps, a Satisfaction RP18-AE column ( $5\ \mu\text{m}$ ,  $250 \times 4.6\ \text{mm}$ ) and a L-2450 diode array detector, at a flow rate of 1 ml/min. The compounds were purified by preparative reverse-phase HPLC using a VWR LaPrep system consisted of a P202 injector, two P110 pumps, a Satisfaction RP18-AB column ( $5\ \mu\text{m}$ ,  $250 \times 20\ \text{mm}$ ) and a P314 UV detector, at a flow rate of 15 ml/min. The following eluants were used in a gradient mode: (A) 0.1% TFA in  $\text{H}_2\text{O}$  and (B) 0.1% TFA in ACN/ $\text{H}_2\text{O}$  (70/30).

**Field Emission Scanning Electron Microscope (FESEM).** Morphological characterization of nanoparticles was carried out with a FESEM (Hitachi S-4800) equipped with a tip made of Zr monocrystal. This technique allowed us to take nano-scale resolved pictures of the fragile PVDF NPs without metallization using an accelerating voltage as low as 1 kV. The tip current was 10  $\mu\text{A}$  and the working distance was 4 mm.

**Static Light Scattering (SLS).** The radius of gyration of nanoparticles was determined by SLS. SLS measurements were performed with a home-made device at LLB (CEA-Saclay) in water (1 mg/ml) with a wavelength of 647 nm at 25°C and with 20 detection angles.

**Dynamic Light Scattering (DLS).** The average size, size distribution and zeta potential were determined by DLS with a Zetasizer Nano-ZS (Malvern instrument 3000HSA) at LCPO (Bordeaux, France). All DLS measurements were done in water with a wavelength of 633 nm at 25°C with a detection angle of 90°. The samples were diluted to 1 mg/ml.

**Small-Angle Neutron Scattering (SANS).** SANS measurements were performed on PACE spectrometer at LLB (CEA-Saclay). In order to reduce incoherent sample background due to protons, nanoparticle solutions (1 mg/ml) were dialyzed against D2O for one night (11000 +/- 1000 g/mol cutoff) and solutions were poured in 2 mm thick quartz cells. Two spectrometer configurations (sample to detector distance/wavelength) were used: 4.6 m/17 Å and 1.4 m/7 Å. Incoherent scattering was subtracted from the baseline acquired at high scattering vector.

The influence of PAA grafting on the nanoparticles dispersion was investigated by SANS for PVDF-g-PAA nanoparticles at different irradiation doses. Data treatment was performed according to the Brulet *et al.* process (1). Scattered intensity per unit volume,  $I$  (expressed in  $\text{cm}^{-1}$ ), was measured as a function of the scattering vector,  $q = 4\pi\sin(\theta/2)/\lambda$ , with  $\theta$  the scattering angle and  $\lambda$  the wavelength. Quite generally, for spherical particles, the scattered intensity per unit volume can be written as:

$$I(q) = K^2 \phi v P(q) S(q) \quad (1)$$

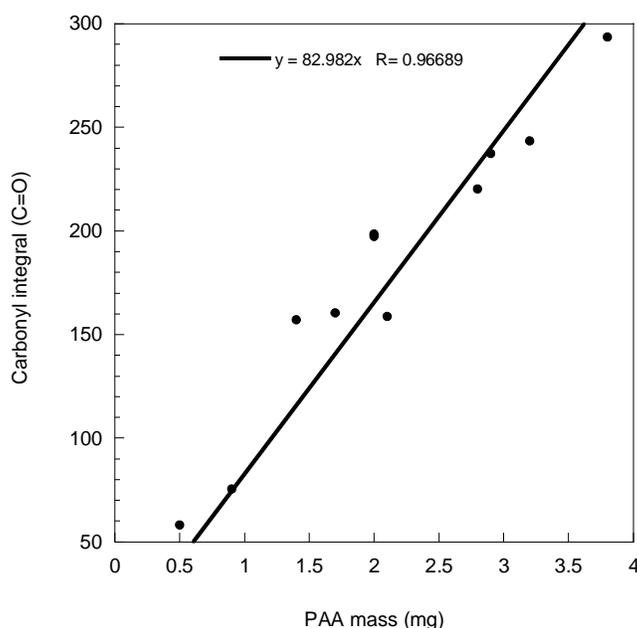
where  $K$  is the contrast factor (the difference of scattering length density between particles and solvent),  $\phi$  the volume fraction of particles,  $v$  the volume of one particle,  $P(q)$  the form

factor of one particle and  $S(q)$  their structure factor. If  $R$  denotes the radius of particles,  $S(q)$  tends to 1 at  $qR > 1$ . As  $q$  tends to 0,  $S(q)$  is proportional to the osmotic compressibility and can be written as a virial expansion at low concentration and low particles interactions:

$$S(0) = 1 - 2a_2C + \dots \quad (2)$$

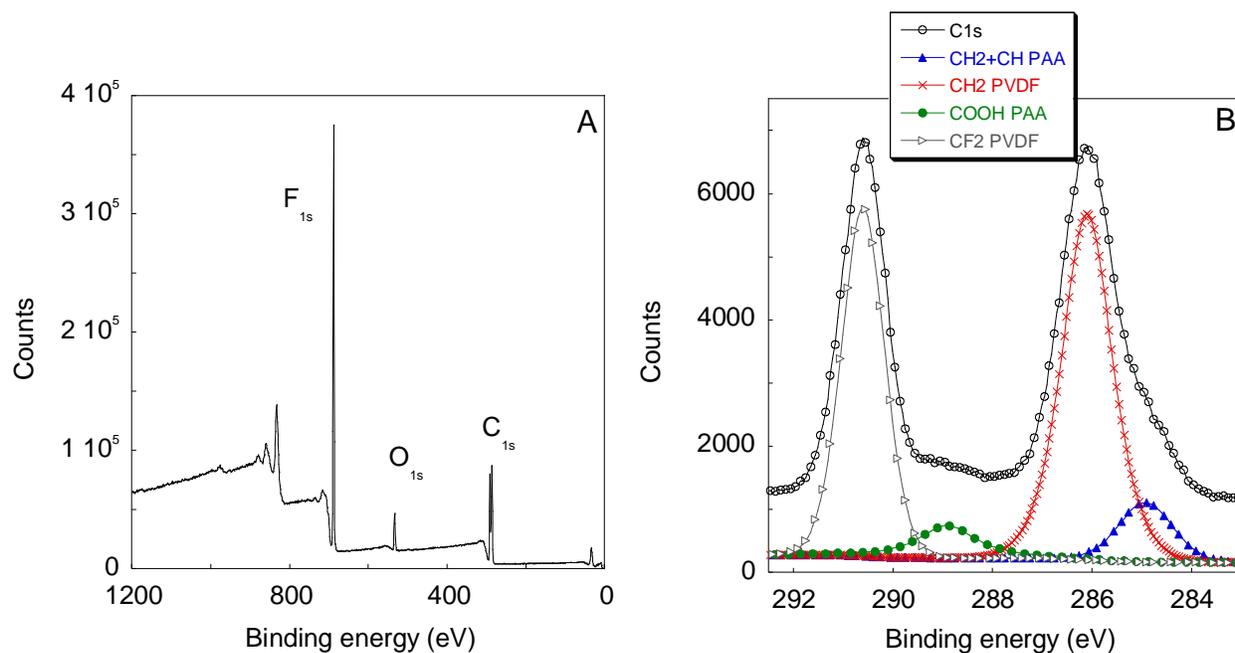
where  $C$  is the concentration of particles expressed in number per unit volume and  $a_2$  the second virial coefficient *i.e.* the two-bodies interaction parameter.

**Fourier Transform Infrared (FTIR) Spectrometry.** Transmission FTIR spectra were recorded on a PerkinElmer Spectrum One spectrometer. Freeze dried PVDF NPs were mixed to KBr powder and shaped into pellets (2% w/w of sample) under pressure. A  $2.0 \text{ cm}^{-1}$  resolution was imposed and 4 scans acquisitions were performed. Grafting yields of PAA on PVDF NPs were evaluated from a standard PAA calibration curve (Figure S1).

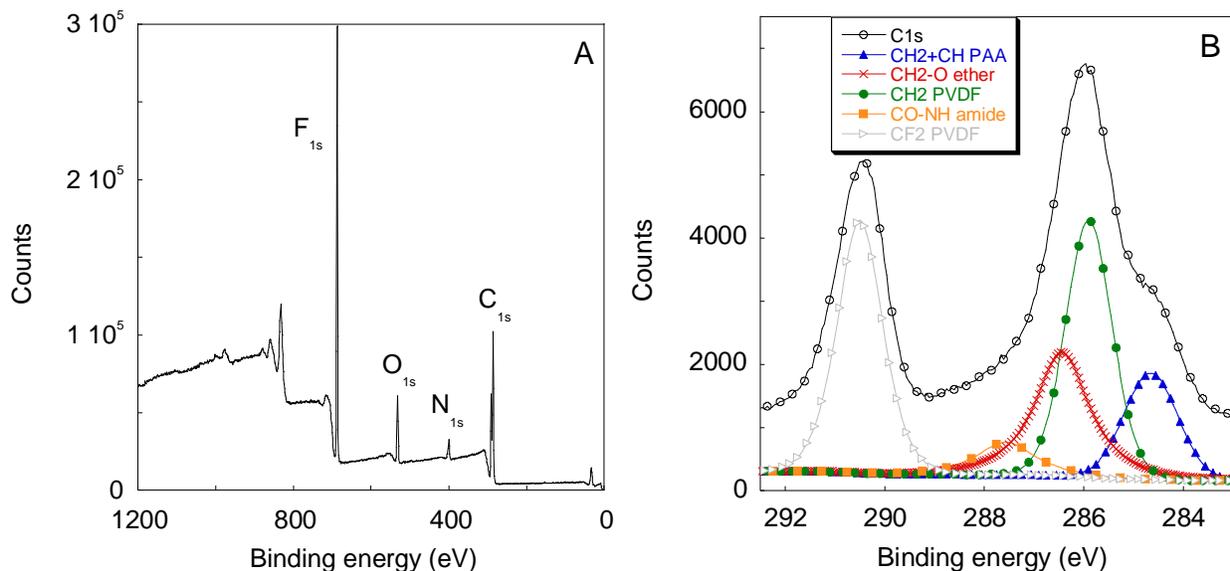


**Figure S1.** Calibration curve done by integration of the C=O stretching band in FTIR from known amounts of a standard PAA in KBr pellets.

**X-Ray Photoelectron Spectroscopy (XPS).** XPS spectra were recorded on a Kratos Axis-Ultra DLD spectrometer using a monochromatic Al K $\alpha$  X-ray source (1486.6 eV) with a power source equals to 150 W at the SPCSI Laboratory (CEA–Saclay, France). The concentric hemispherical electron energy analyzer was equipped with a multichannel detector operating at a constant energy analyzer mode at electron take-off angle of 90°. The use of a charge compensator permitted to reduce charge effects. The pass energy of 20 eV was used for both the survey and core level scans. The resolution was around 0.3 eV. The energy scale of the instrument was calibrated by setting Au 4f7/2 = 83.70 eV. Binding energies were determined by reference to the C<sub>1s</sub> component due to carbon bond only to carbon and hydrogen, set at 285 eV. Shirley baseline for background subtraction and Gaussian functions were used for peak fitting. Atomic percentages were determined from peak areas by using Scofield factors (C<sub>1s</sub> = 1, F<sub>1s</sub> = 4.43, O<sub>1s</sub> = 2.93, N<sub>1s</sub> = 1.8). The XPS spectra of PVDF-g-PAA and PVDF-g-PAA-mTEG nanoparticles are shown in Figure S2 and Figure S3 respectively.



**Figure S2.** A/ XPS survey spectrum of PVDF-g-PAA and B/ XPS C<sub>1s</sub> spectra and deconvoluted peaks. Nanoparticles were irradiated at 2.5 kGy.



**Figure S3.** A/ XPS survey spectrum of PVDF-g-PAA-mTEG nanoparticles and B/ XPS C<sub>1s</sub> spectra and deconvoluted peaks. Nanoparticles were irradiated at 2.5 kGy.

**Fluorescence Spectrometry.** Emission and excitation spectra of functionalized nanoparticles were recorded between 500 and 900 nm on a photon counting Edinburgh FLS920 spectrofluorimeter equipped with a Xe lamp and using a 715-nm filter in order to decrease the light scattering. The excitation wavelength was set at 690 nm while the emission wavelength was set at 820 nm. All measurements were performed in 3 ml cuvettes containing 2 ml of functionalized PVDF NPs aqueous suspension stirred at 25°C, in presence of a 2 μM fluorophore.

**Fluorescence Imaging.** Images of functionalized PVDF NPs were performed in 0.5-cm cell from an Odyssey MousePOD™ infrared imaging system (LI-COR Biosciences), using one emission channel (800 nm), with a 0.5 intensity and high quality mode. For this experiment, 1.7 mg/ml functionalized PVDF NPs suspensions were prepared corresponding to 60 μM NIR dye.

1. Brulet A, Lairez D, Lapp A, Cotton J-P. Improvement of data treatment in small-angle neutron scattering. *J Appl Crystallogr.* 2007;40:165-77.