Electronic Supplementary Information

Multifunctional Nanoparticles with Controllable Dimensions and Tripled Orthogonal Reactivity

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Experimental Details

CVD Copolymerization

A trifunctional copolymer containing ester-alkyne, maleimide, and bromoisobutyrate groups was prepared from starting materials (dimers) of 4-methyl-propiolate-[2,2]paracyclophane, 4-N-maleimidomethyl-[2,2]paracyclophane and 4-methyl-2-bromoisobutyrate-[2.2]paracyclophane via CVD copolymerization using a home-built CVD system reported elsewhere.[1] The synthesis of the three dimers was conducted following reported routes.[2] During the CVD copolymerization process, the dimers were introduced into the system with a controlled molar ratio of 1:1:1 and sublimated under vacuum at approximately 100-130 °C. The vaporized dimers were then transformed into the reactive species (monomers, *p*-quinodimethanes) by pyrolysis, which was kept at 510 °C for 4-methyl-propiolate-[2,2]paracyclophane), 580 °C for 4-N-maleimidomethyl-[2,2]paracyclophane), and 550 °C for 4-methyl-2-bromoisobutyrate-[2.2]paracyclophane). Subsequently, the monomers were transferred into the deposition chamber with a wall temperature of 90 °C, and spontaneous copolymerization formed the trifunctional polymer poly[(4-methyl-propiolate-*p*-xylylene)-*co*-(4-N-maleimidomethyl-*p*-xylylene)-*co*-(4-methyl-2-bromoisobutyrate-*p*-xylylene)-*co*-(*p*-xylylene)] on substrates placed on a rotating and cooled sample holder (15°C) in the deposition chamber. The system pressure was maintained at 75 mTorr during the entire CVD copolymerization process, and the deposition rate was maintained under 0.5 Å/s to ensure the polymer quality.

Nanoparticle Formation

Geometrical control of the trifunctional nanoparticles was achieved using a previously reported double-solution wetting method.[3] For the fabrication of nanospheres, polymer solutions containing the synthesized trifunctional copolymer were prepared in dimethylformamide (DMF) and adjusted to concentrations of 2, 5, 10, and 20 wt%. The anodic aluminum oxide templates (AAO templates; pore diameter ~230 nm, average pore length ~60 μm) were obtained commercially from Whatman, UK and were immersed in the polymer solution for 10 seconds at 20 °C to ensure that the polymer solution filled the pores of the AAO templates by capillary force. The templates were then retrieved from the polymer solution and the excess solution was removed with KimWipes (Kimberly Clark, USA). Subsequently, the filled templates were immersed in a bottle of acetic acid for 30 seconds at 20 °C, after which the samples were again retrieved and dried by a vacuum pump to allow the formation of nanospheres within the nanopores. The AAO templates were then selectively removed using 5 wt% NaOH(aq) to obtain the nanospheres. Finally, the nanospheres were filtered and washed with deionized water using polycarbonate filters (VCTP, pore size: 0.1 μm, obtained commercially from Merck, Germany). For the fabrication of nanotubes, a similar procedure was performed: after the filled AAO templates were retrieved from the polymer solution, the samples were dried with a vacuum pump without immersion in acetic acid. Skipping the immersion in acetic acid should ensure the formation of nanotubes within the nanopores. Finally, the AAO templates were selectively removed using 5 wt% NaOH(aq) to obtain the nanotubes.

Characterization
Fourier transform infrared spectroscopy (FT-IR) was performed using an FT-IR 100 spectrometer (PerkinElmer, USA) equipped with a liquid nitrogen-cooled MCT detector. An attenuated total reflectance (ATR) accessory with a diamond/ZnSe crystal (PIKE Technologies, USA) was used for characterization of the PPX nanoparticles, and an advanced grazing angle specular reflectance accessory (AGA, PIKE Technologies, USA) was used to characterize PPX thin films for comparison. The FT-IR spectra were recorded using 128 scans and 4 cm^{-1} resolution from 500 cm^{-1} to 4000 cm^{-1}. The resulting spectra were corrected for any residual baseline shifts. X-ray photoelectron spectroscopy (XPS) data were recorded with a Theta Probe X-ray photoelectron spectrometer (Thermal Scientific, UK) using a monochromatized Al Kα X-ray source. The XPS spectra were recorded using an X-ray power of 150 kW. The survey spectra and the high-resolution C1s elemental spectrum were obtained with pass energies of 200.0 eV and 20.0 eV, respectively. XPS analysis was carried out based on the atomic concentrations (%) and compared to the theoretical values based on the chemical structure. The transmission electron microscopy (TEM) images of the trifunctional nanoparticles were obtained using an H-7650 TEM (Hitachi, Japan) with an accelerating voltage of 75 keV. The samples were prepared by drying a suspension on a carbon film deposited on one side of a copper grid (300 mesh, Electron Microscopy Science, USA). The dimension and shape of the trifunctional nanoparticles was examined using a NovaTM NanoSEM (FEI, USA) that was operated at a primary energy of 5 keV and with a pressure of 5×10^{-6} Torr in the specimen chamber. The samples were prepared by drying the particle solutions on a silicon substrate at 70 °C, and a conductive platinum layer was prepared on the sample prior to examination. Analysis of the particle size distribution was performed using a Zetasizer Nano ZS dynamic light scattering system (DLS, Malvern, UK) at 25 °C with water as the dispersant. The resulting DLS data were calculated using the non-negative least squares (NNLS) algorithm provided by the built-in Zetasizer software.

Conjugations
Alexa Fluor® 555 azides (500 μg/mL, Yao-Hong biotechnology Inc., Taiwan) were used for the detection of the ester-alkyne groups on the trifunctional nanoparticles, and the conjugation was

performed via the azide-alkyne cycloaddition click reaction (without requiring a copper catalyst) for 2 hrs at 20 °C. After the reaction was completed, the resulting solution was centrifuged at 14000 rpm three times using deionized water as a resuspension agent to remove unreacted residues. Similarly, a fluorescent peptide of FITC-RRCC (2 mg/mL, Yao-Hong Biotechnology, Taiwan) in aqueous solution was used for the detection of the maleimide groups on the trifunctional nanoparticles, and the conjugation was achieved via a thiol-maleimide coupling reaction performed at 20 °C for 2 hrs. The unreacted fluorescent molecules were removed using the same aforementioned centrifugal process three times. Examination of the fluorescence signals was performed using fluorescence microscopy (Nikon TE2000-U, Japan). The capability to perform atom transfer radical polymerization (ATRP) was provided by the third functionality, bromoisobutyrate, on the trifunctional nanoparticle. The grafting of poly(ethylene glycol) methyl ether methacrylate (PEGMA) on the particles was conducted via ATRP by mixing 10 mL of a PEGMA solution (50 wt%), 22 mg of CuBr, 10 mg of CuBr2, 58.5 mg of 2,2'-bipyridine (bpy), and 20 mL of deionized water in a Schlenk flask at 20 °C. The resulting solution was degassed using three freeze-pump-thaw cycles before being purged with nitrogen. The mixture was treated with an elevated temperature of 70 °C and was stirred until a homogeneous dark brown solution was obtained. Next, 8 mL of a trifunctional nanoparticle suspension were added into the flask to initiate ATRP for a set reaction time. The growth kinetics of the grafted PEGMA on the particles was observed and analyzed by DLS, TEM, and SEM.

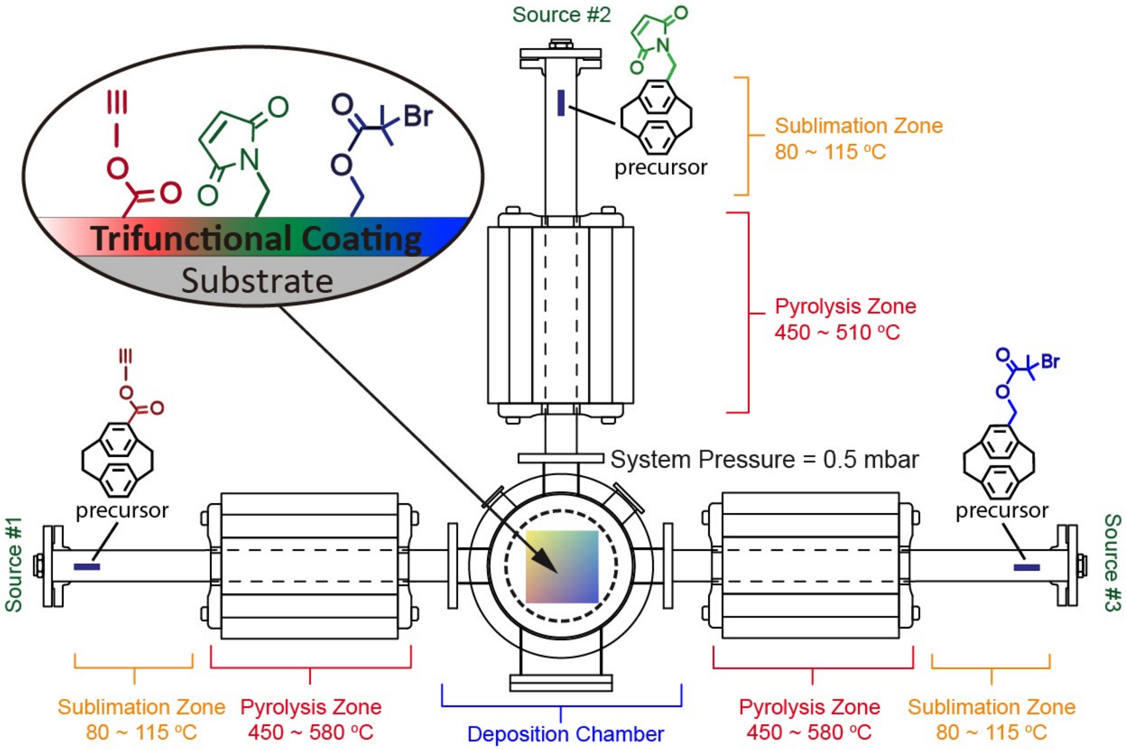


Figure S1. Schematic illustration of a three-source chemical vapor deposition (CVD) copolymerization system. The CVD system is equipped with three inlet sources that each contain an independent sublimation zone and pyrolysis zone, and the three sources are connected to a center deposition chamber. During the CVD copolymerization process, sublimation occurred for the starting precursors (dimers) ester-alkyne-[2.2]paracyclophane, maleimide-[2.2]paracyclophane, and bromoisobutyrate-[2.2]paracyclophane at approximately 80 - 115°C. These sublimed paracyclophanes with a 1:1:1 molar ratio were simultaneously transferred from each source to the pyrolysis zone and exposed to elevated temperatures of 580°C, 510°C, and 580°C, respectively, to thermally convert them into the corresponding quinodimethanes (monomers). Finally, the quinodimethanes spontaneously copolymerized upon condensation on a cooled (approximately 20°C) substrate to generate the trifunctional poly-para-xylylene polymer.

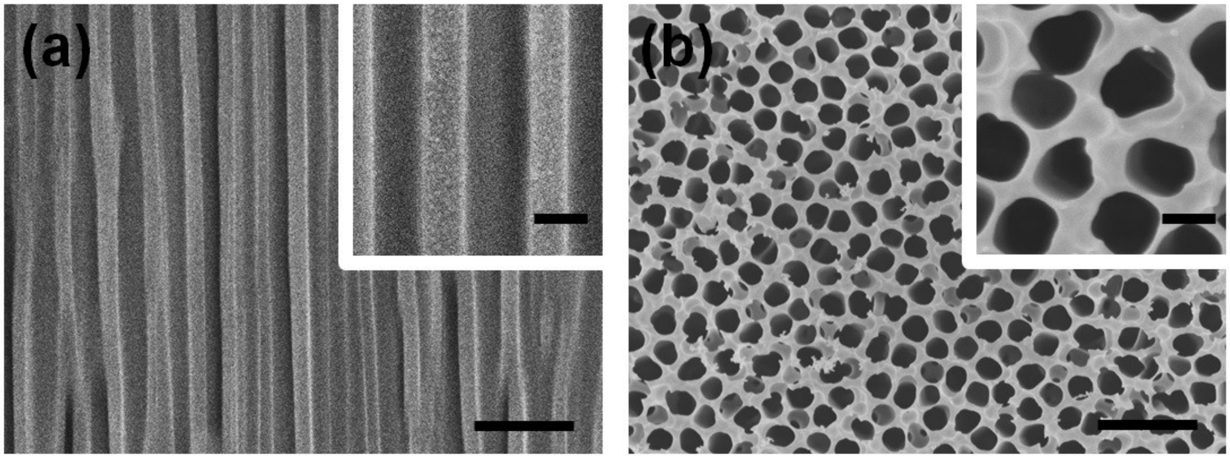
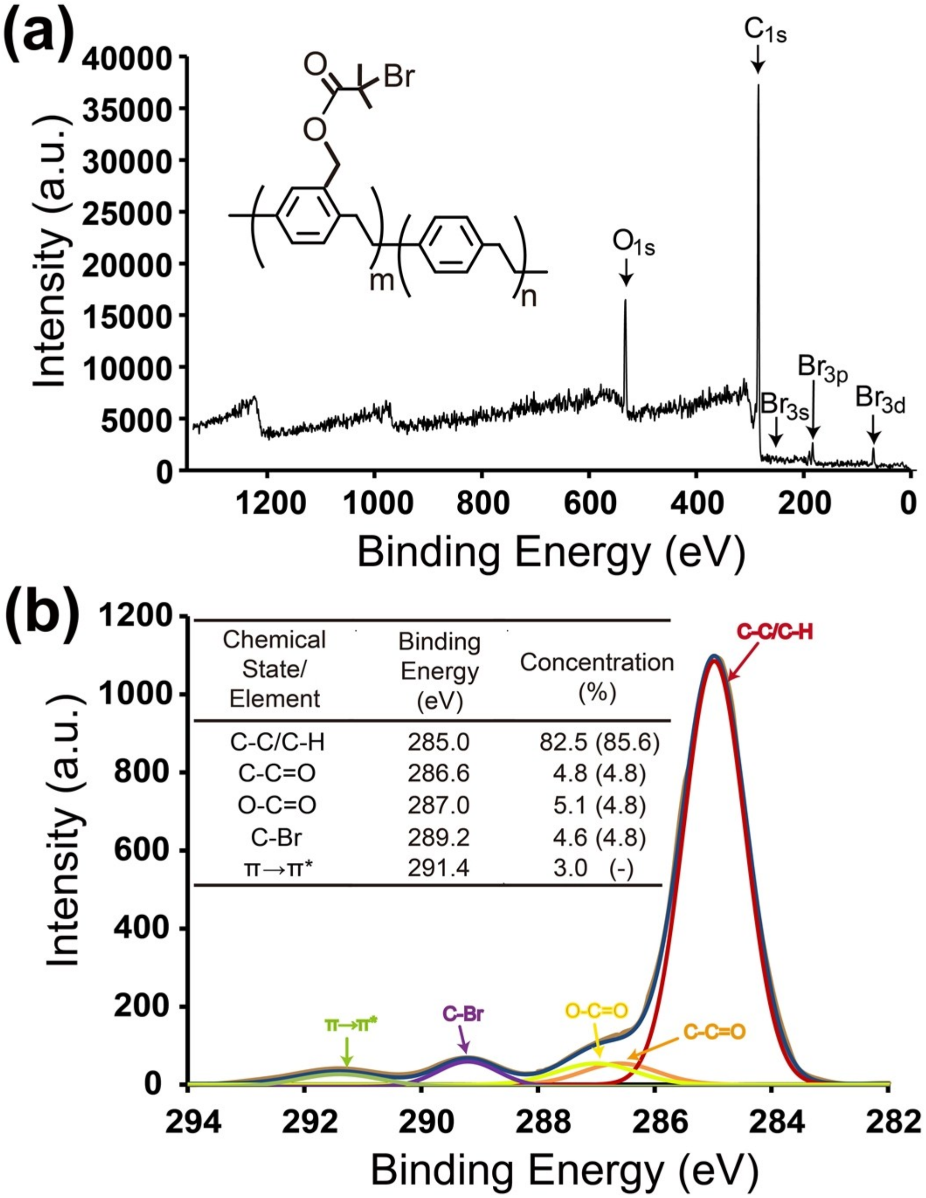


Figure S2. SEM images of the porous AAO templates viewed from the side (a) and the top (b). Scale bars: 1 μm for the main images and 200 nm for the insets.



Chemical State/ Element	Binding Energy (eV)	Concentration (%)
C-C/C-H	285.0	82.5 (85.6)
C-C=O	286.6	4.8 (4.8)
O-C=O	287.0	5.1 (4.8)
C-Br	289.2	4.6 (4.8)
$\pi \rightarrow \pi^*$	291.4	3.0 (-)

Figure S3. Characterization of the chemical composition of the bare ATRP-ester bromide PPX nanoparticles. (a) XPS survey spectrum and (b) XPS high-resolution C1s spectrum of the bare ATRP-ester bromide PPX nanoparticles. The table insert shows the experimental values for each chemical binding state compared to the theoretical values (in parentheses).

References

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