Electronic Supplementary Material (ESI) for Chemical Communications

Zirconium phosphate nanoplatelets: a novel platform for drug delivery in cancer therapy

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Supporting Information:

Experimental procedure:

Synthesis of *θ*-ZrP. The *θ*-ZrP material was synthesized using the procedure reported by Kijima[23] The typical procedure consists of the dropwise addition of 200 mL of a 0.04 M $ZrOCl_2 \cdot 8H_2O$ aqueous solution to a 200 mL solution of H_3PO_4 (35%). The phosphoric acid solution was preheated in an oil bath at 94 °C in a 500 mL round bottom flask before the addition of the zirconyl chloride. The resulting solution was refluxed with constant stirring at 94 °C for two days. The product is filtered and washed several times with water, obtaining a paste material. This material, as characterized by XRPD, showed an intense peak at low angles (2θ = 8.55°) corresponding to a distance of 10.3 Å, followed by the second order diffraction peak at 5.1 Å.

Intercalation of doxorubicin into *θ*-ZrP. A suspension of *θ*-ZrP was placed in contact with a solution of doxorubicin at 1:3 (DOX: ZrP) molar ratios for five days. In a typical procedure the intercalation process was performed by the batch method, adding the desired quantity of the drug to a water suspension of *θ*-ZrP at the desire molar ratio (loading levels). Then the suspension was stirred for five days at room temperature, monitoring each day the intercalation process by measuring the change in pH and by measuring the UV-vis absorption spectrum of the supernatant of a centrifuged aliquot of the suspension. When the measurements of pH and UV-vis absorption were constant, indicative of the end of the intercalation process, the suspension was centrifuged and then filtered using 0.22 μm filters (Millipore), washed three times with water and lyophilized.

Characterization of the ZrP materials. The complete characterization of the materials was performed using several analytical methods. XRPD experiments were performed from 2 to 40° (2θ-angle) using a Siemens D8 X-Ray diffractometer system with a copper anode source (K_α, λ = 1.5406 Å) with a filtered flat LiF secondary beam monochromator. The divergence, receiver, and detector slits width were 2 mm; the scatter slit width was 0.6 mm. The interlayer distances were determined using the Bragg's Law for the (002) diffraction plane of the diffraction pattern for α-ZrP and *θ*-ZrP, and the (001) diffraction plane of the diffraction pattern for the intercalation products. According to Bragg's Law the distance (d_{hkl}) between planes (*hkl*) is equal to ratio between the wavelength of the source and 2 times the sin of the diffraction angle, or as is expressed in the equation of the Law:

$$d_{hkl} = \frac{\lambda}{2\sin\theta}$$

Thermogravimetry experiments were carried out on a TGA Q500 TA Instrument. The temperature was ramped at 5 °C min^{-1} under a flow of N_2 up to 800 °C. The first weight loss (below 150°C) was attributed to water. The following weight losses were assigned knowing the thermo-decomposition of the intercalated material.

UV-vis absorption spectra were measured with a HP 8453 diode array spectrophotometer. Diffuse reflectance spectra were obtained using a Cary 1E UV-vis spectrophotometer. Luminescence spectroscopy was performed using a SE-900 spectrofluorometer (Photon Technology International, PTI) using a 150 W xenon lamp as the excitation source and a PTI Model 710 photon counting detector with a Hamamatsu R1527P photomultiplier. UV-vis spectrophotometric and steady state

luminescence measurements were performed suspending a determined amount of the probe-exchanged ZrP in water to make a 0.008% (w/v) suspension.

The transmission electron micrographs (TEM) of the samples were acquired using a JEOL 2010 transmission electron microscope at an acceleration voltage of 200 kV. Samples were prepared using copper grids from Ted Pella. Scanning electron microscopy (SEM) images were acquired on a JEOL JSM-7500F (FE-SEM). The Atomic force microscopy (AFM) images for the ZrP nanoplatelets were taken with an Agilent 5500, in contact mode. The AFM image for the DOX:ZrP nanoplatelets were taken with a WITec Alpha300 combined confocal fluorescence/AFM system to allow for sequential confocal fluorescence and AFM imaging of the same area, on AC mode.. The nanoplatelets were deposited on a Si-APTES (3-aminoprpoyltriethoxysilane) substrate by immersion of the freshly cleaned Si-APTES wafer in an ethanol suspension of the nanoparticles (0.008% w/v) for 6 h.

Cell Culture Experiments. Human Breast Cancer cell line (MCF-7) was grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, and 1% penicillin–streptomicin antibiotics. Cells were maintained at 37° C in a humidified, 5% carbon dioxide atmosphere.

Cellular Uptake Study. 50,000 cells per well were seeded into a 2 well chambered glass slide (Nalge Nunc International, USA) and allow to attach for 24 h. Then cells were exposed to DOX: ZrP nanoplatelets or free DOX at a final DOX concentration 1μM for 4 h at 37 °C. After incubation cells were washed three times with ice cold PBS buffer and fixed with 4 % paraformaldehyde for 30 minutes. After that cell were observed by confocal laser scanning microscopy (Nikon Eclipse *Ti*).

In vitro Cytotoxicity Assay. In vitro cytotoxicity of doxorubicin loaded zirconium phosphate nanoplatelets (DOX: ZrP NP) was determined in MCF-7 cell lines. Briefly, 5000 cell /well were seeded in 96 well plates and incubated for 24 h. The medium was then changed with various concentrations of the Dox, Dox Zr NP and only Zr NP. At 24 h and 48 h, media was removed and 50 μL of (3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide(MTT) Solution (0.5 mg/mL) were added to each well and incubated at 37° C, 5 % CO_2 for 4 hr and then media was removed and 100 μL of dimethylsulfoxide was added to each well to dissolve formazan crystals. The absorbance was measured at 570 nm using micro plate reader (NOVOstar BMG Labtech, USA). The % cell viability was calculated using the following formula: % Cell Viability = (A_{570} treated cells /A_{570} control cells) X 100, where A_{570} is the absorbance value at 570 nm. IC_{50} values were calculated by GraphPad Prism 5.0 (GraphPad Software, Inc. CA, USA).

Figure S1. Idealized structures of the alpha phases of zirconium phosphate: a) α-ZrP showing one of the pseudo-hexagonal zeolite cavities created by the arrangement of the layers; b) α-ZrP showing the unit cell; c) α-ZrP showing a dissection of the layer structure showing their dimension and the spatial arrangement of the layer components.

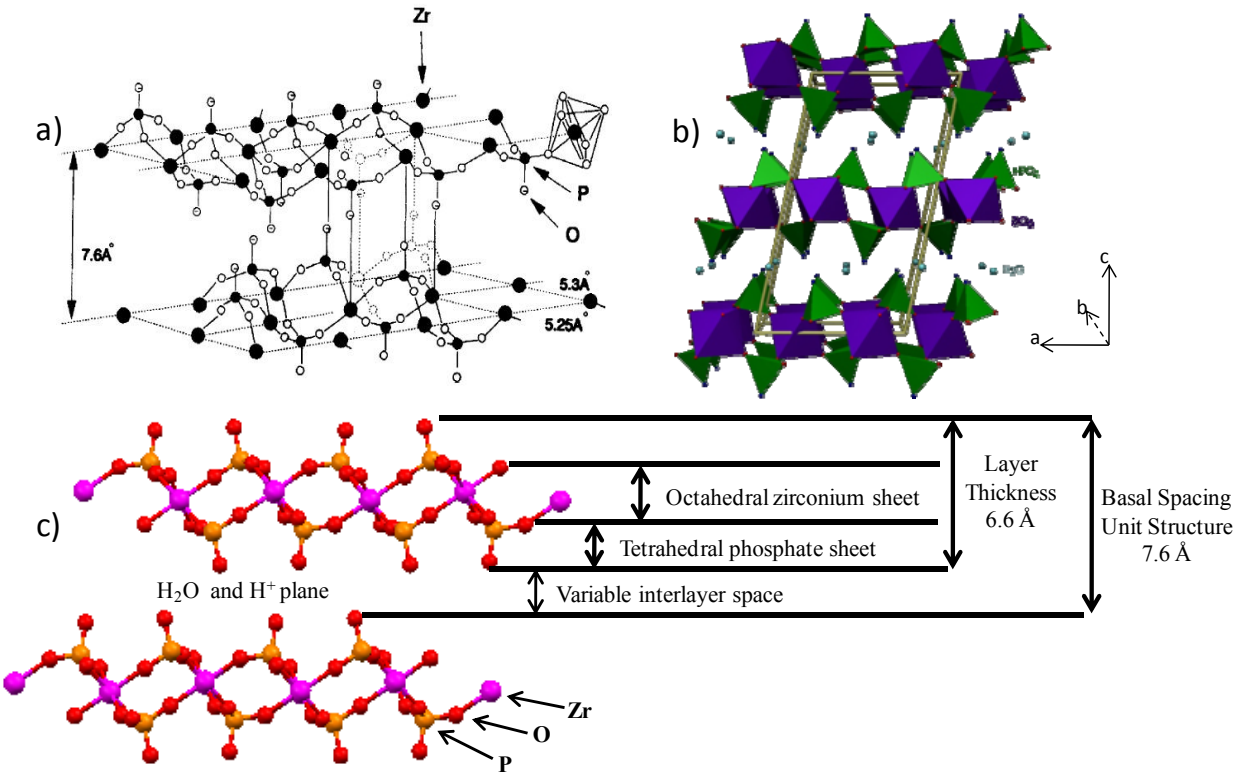

Figure S2. AFM images of ZrP (top) and DOX:ZrP (bottom) nanoplatelets.

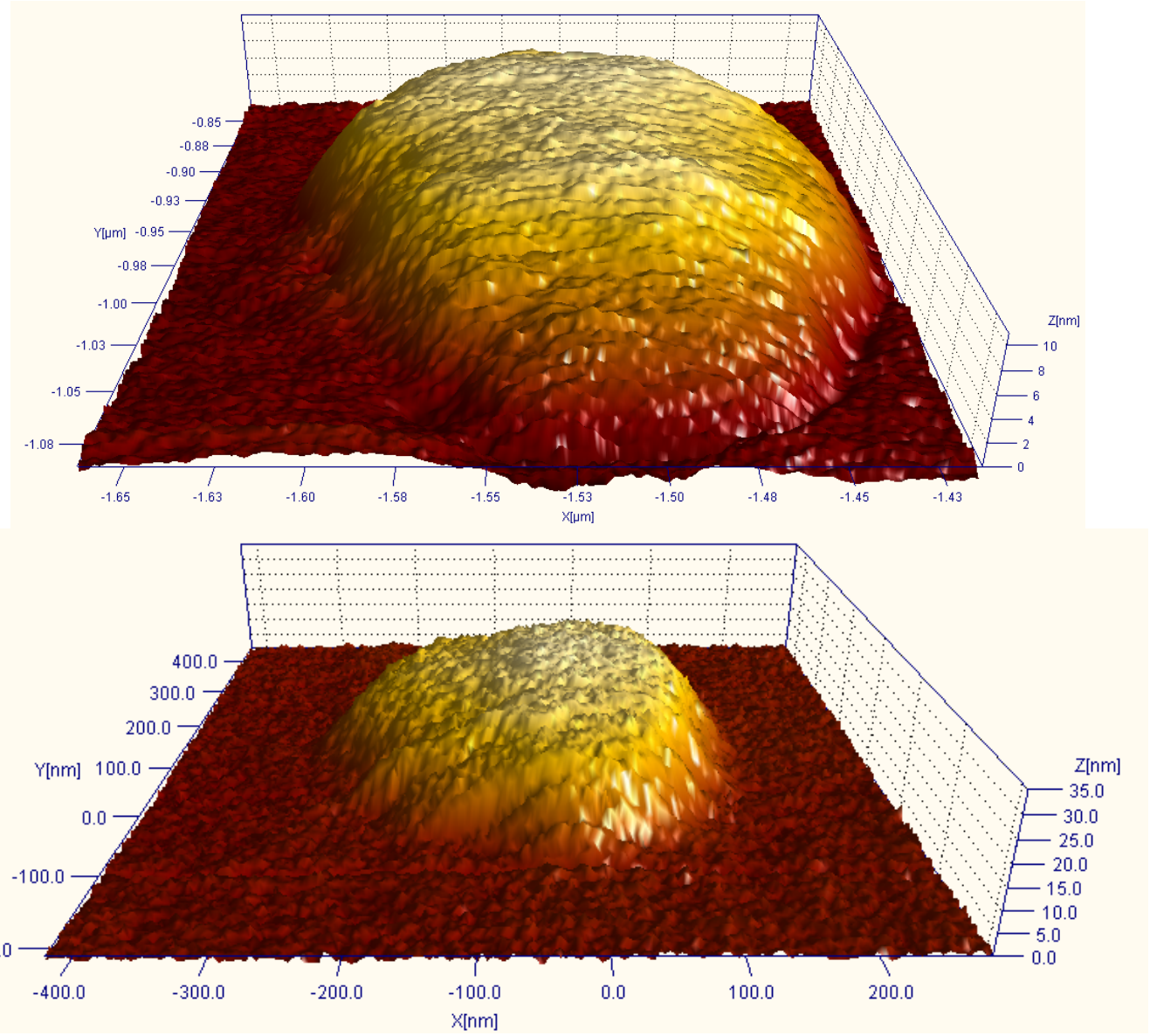

Figure S3. TGA of DOX:ZrP

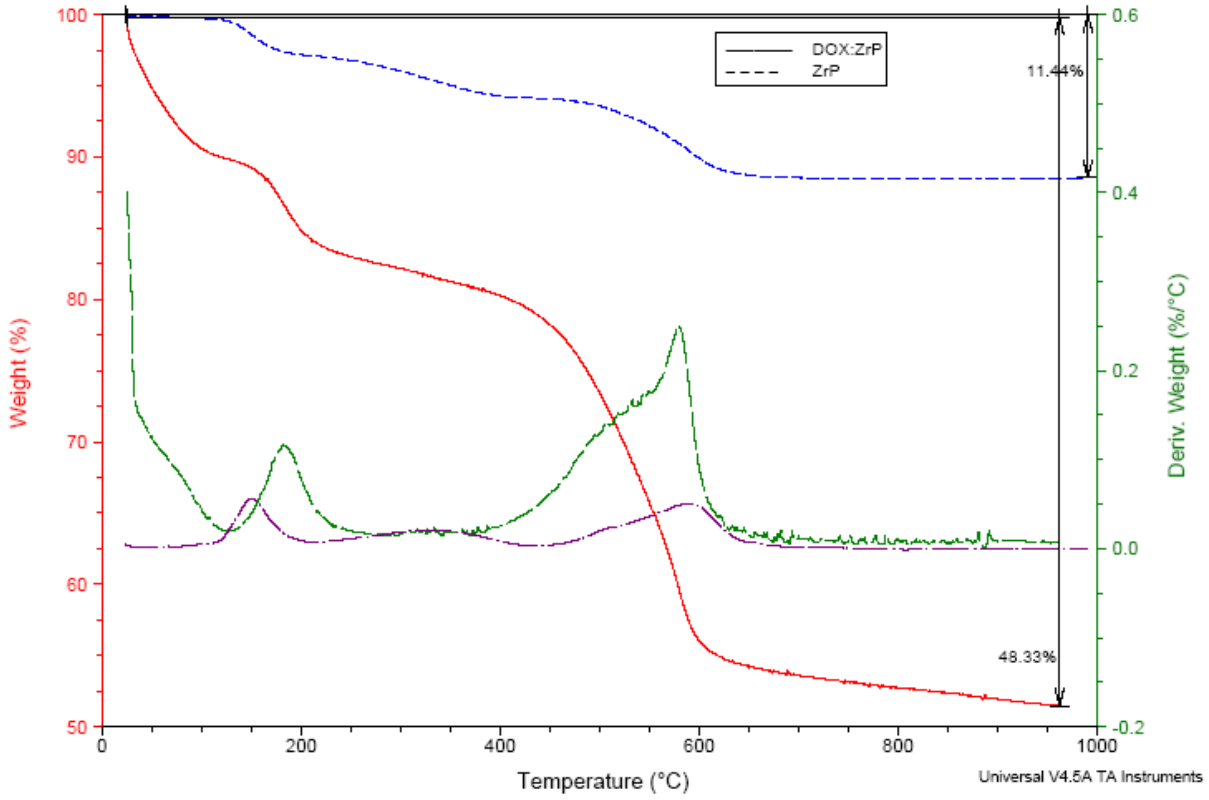

Figure S4. Effect of blank ZrP nano-platelets on cell viability of MCF-7 cells