Incorporation of tyrosine phosphate into tetraphenylethylene affords an amphiphilic molecule for alkaline phosphatase detection, hydrogelation and calcium mineralization

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Supporting Information

1) Synthesis of fluorogenic probe 1

Synthesis of 4: In brief, under a nitrogen atmosphere, hydroxyl-phenylbenzophenone (2 g, 10 mmol), zinc power (1.44 g, 22 mmol) and 40 mL dried THF were added to a 250 mL three-necked flask equipped with a magnetic stirrer. The solution was cooled to 0 ℃, and $TiCl_4$ (1.3 mL, 12 mmol) was slowly added by using a syringe. The mixture was refluxed overnight. After cooling to room temperature, 40 mL dilute HCl (1 mol L^{-1}) was added to the mixture, which was extracted with DCM. The organic layer was collected and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using 5% methanol in DCM to give compound **4** (1.08 g, 54%) for next reaction. ^1H NMR (400 MHz, DMSO-*d*$_6$): δ 9.03 (s, 2H), 7.36-7.33 (d, 4H), 7.13-7.04 (m, 6H) , 6.94-6.92 (m, 4H), 6.43-6.45(d, 4H). MS: calcd M^+=364.15, obsd $(M-H)^-$ =363.1.

Synthesis of 5: Into a 250 mL three-necked flask, tetrakis (4-hydroxyphenyl) ethane (TPE-OH_2; 1 g, 2.74 mmol), K_2CO_3 (6.08 g, 44 mmol) and 50 mL dry acetone was added under a nitrogen atmosphere. And *tert*-butyl bromoacetate (2.15 g, 11 mmol) was added dropwise. The mixture was stirred for 72 h at reflux under nitrogen. After filtration and removing the solvent, the residues were purified by column chromatography over silica gel using EtOAc/hexane (1:10) as the eluents to afford the compound **5** (0.7, 43.1%) for next reaction. ^1H NMR (400 MHz, DMSO-*d*$_6$): δ 7.60-7.57 (m, 2H), 7.41-7.21 (m, 6H), 7.12-7.02 (m, 4H), 6.84-6.81 (m, 4H), 6.62-6.59 (m, 2H), 4.60-4.47 (m, 4H) and 4.42-4.33 (m, 18H). MS: calcd M^+=592.28, obsd $(M-H)^-$=591.2.

Synthesis of 6: 0.1 mmol of compound **4** (593 mg) was dissolved in 8 mL of 10% trifluoroacetic acid in dry DCM and stirred at room temperature overnight. The reaction mixture was concentrated by vacuum and the white solid was purified by column chromatography over silica gel using EtOAc/hexane (2:1) as the eluents to afford compound **6** (459 mg, 95%) for next reaction. ^1H NMR (400 MHz, DMSO-*d*$_6$): δ 7.58-7.49 (m, 8H), 7.11-7.04 (m, 4H), 6.82-6.80 (m, 4H), 6.61-6.59 (m, 2H) and 4.61-4.46 (m, 4H). MS: calcd M^+=480.16, obsd $(M-H)^-$ =479.1.

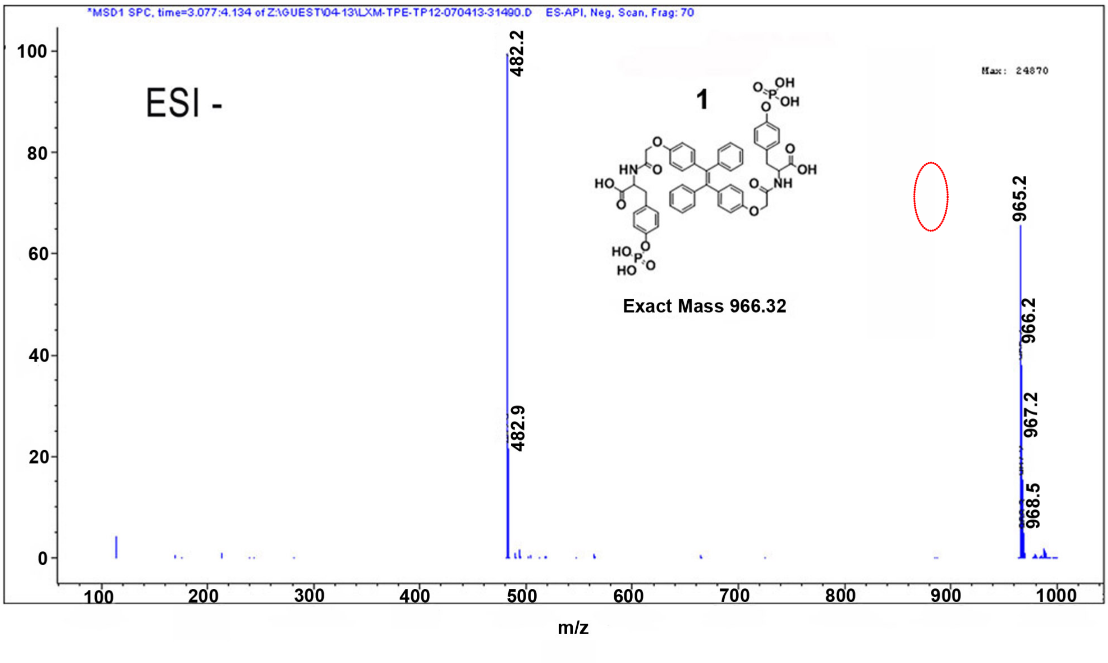

Fig. S1 MS spectra analysis for the generation of **1**.

2) HPLC and LC-Mass analysis of enzymatic conversion of fluorogenic probe of 1 to 2.

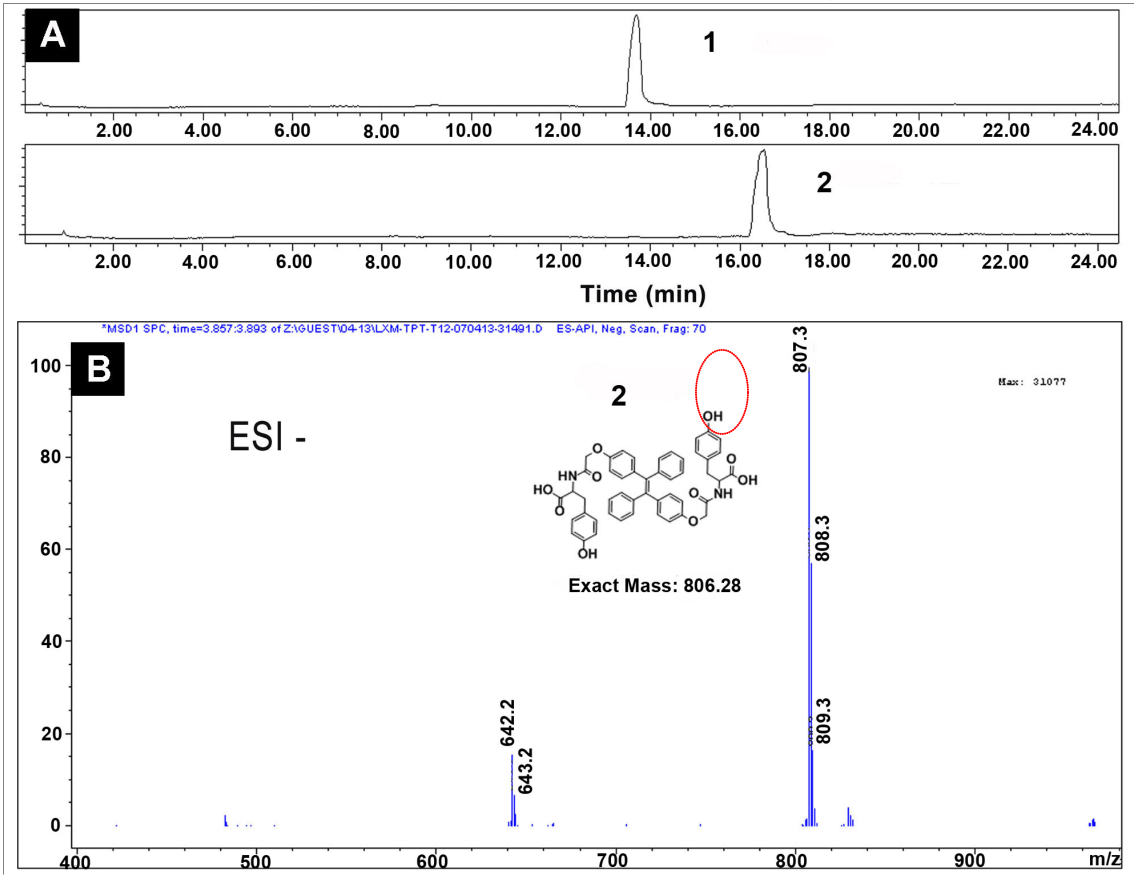

Fig. S2 (A) HPLC chromatograms of **1** (top) and **2** (bottom) before and after enzymatic dephosphorylation by ALP, (B) MS spectra analysis for the generation of **2** through ALP dephosphorylation reaction.

3) Dynamic light scattering analysis

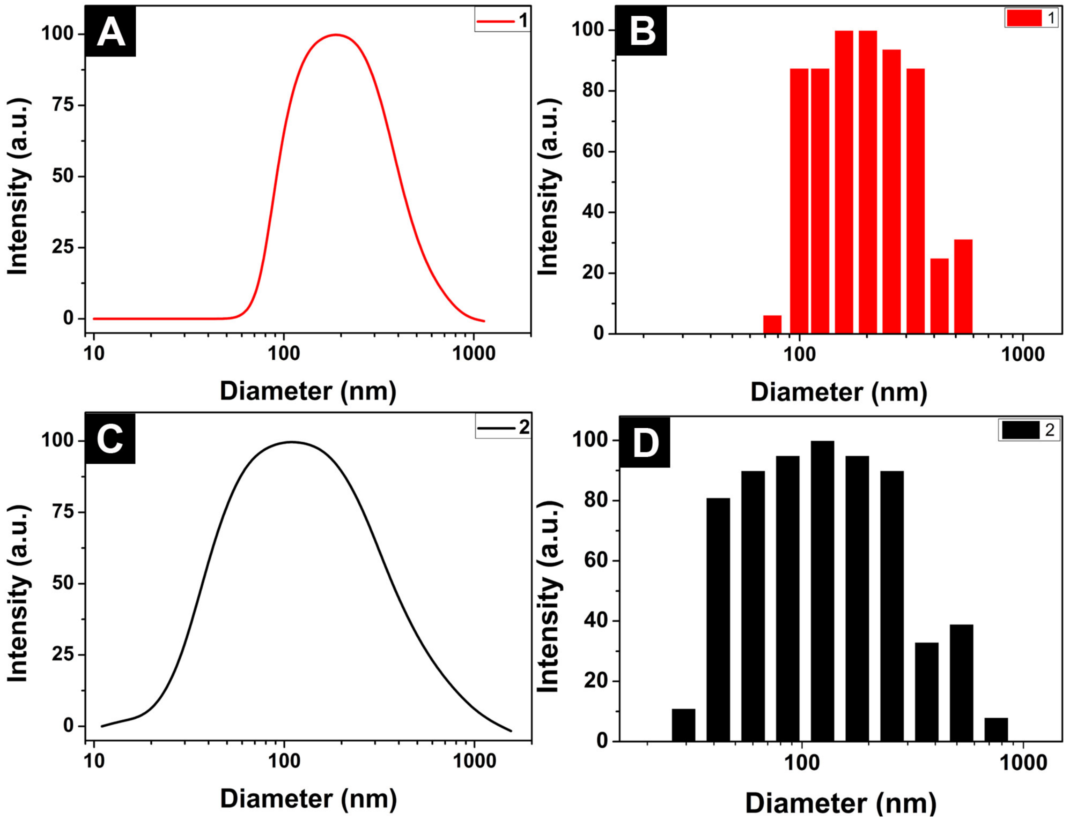

Fig. S3 Dynamic light scattering analysis of the hydrodynamic radius and size distribution of nanostructures formed from **1** solution and **2** suspension as shown in Figure 1.

4) Kinetic studies of 1 dephosphorylation by ALP hydrolysis

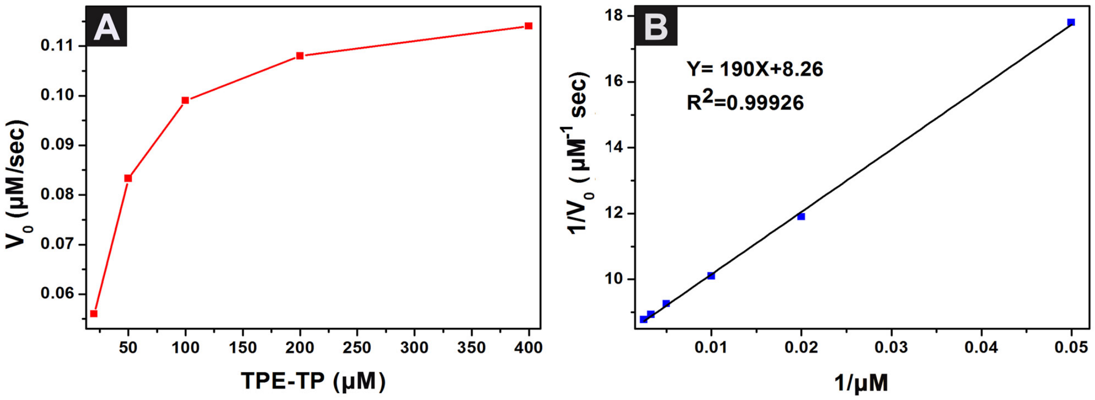

Fig. S4 (A) Michaelis-Menten plot and (B) Lineweaver-Burke plot of the hydrolysis of **1** by ALP in Tris-HCl buffer at 25 ℃.

5) Cell viability measurement with Hela cells

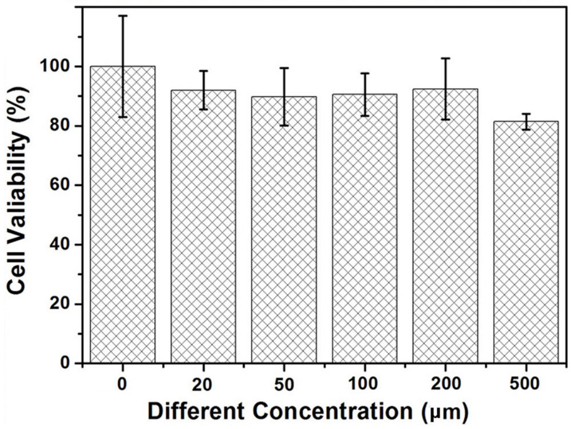

Fig. S5 Cell viability of Hela cells treated with **1** at different concentrations over 24 hr.

6) Fluorescent confocal microscopy analyses of ALP activities in Hela cells by 1.

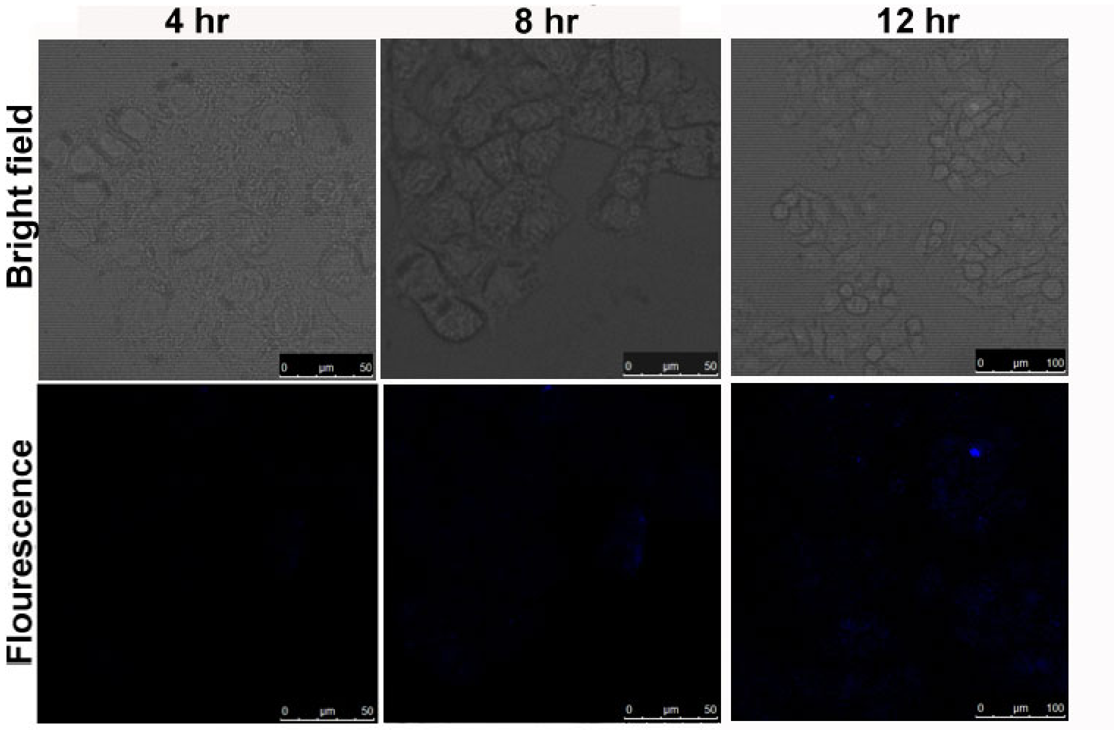

Fig. S6 Fluorescence microscope images of Hela cells for ALP imaging after incubating with **1** at different time-scale in 4 hr, 8 hr and 12 hr.

7）Investigation of fluorescence enhancement upon gelation

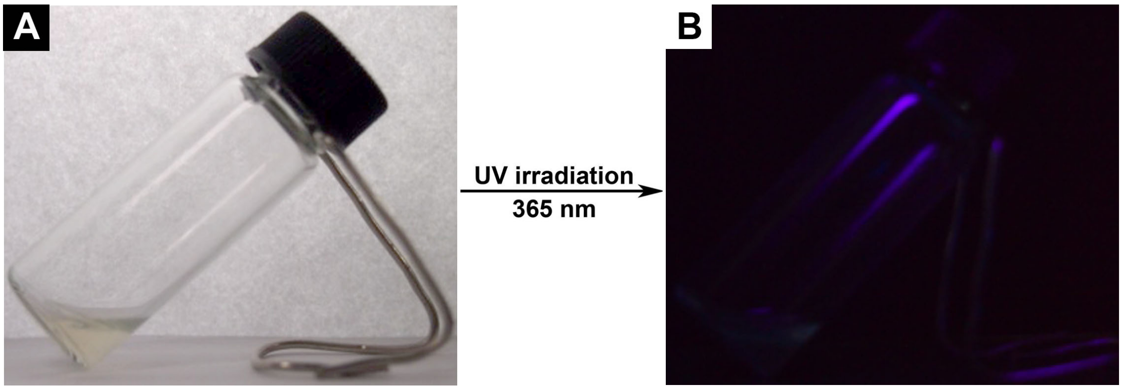

Fig. S7 Optical images of **1** solution (1.8 wt%, pH= 7.4) under (A) visible light and (B) UV irradiation.

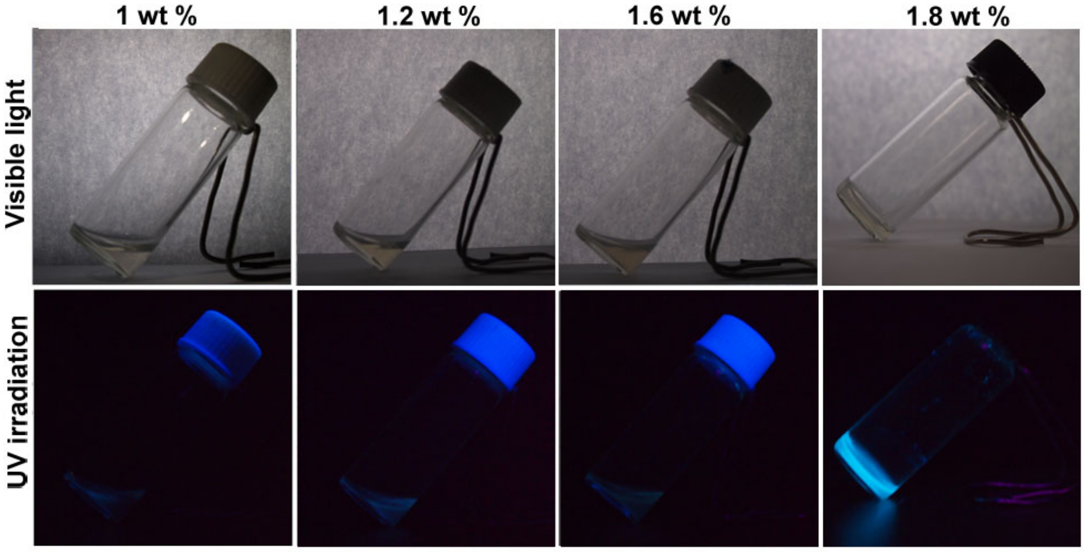

Fig. S8 Optical images of the solutions or hydrogel from **1** at 1.0 wt %, 1.2 wt %, 1.6 wt % and 1.8 wt% under visible light and UV irradiation.

8) EDS profile of calcium minerals on the outer surface of microsphere formed from 1 micelle nanostructure through calcium phosphate mineralization.

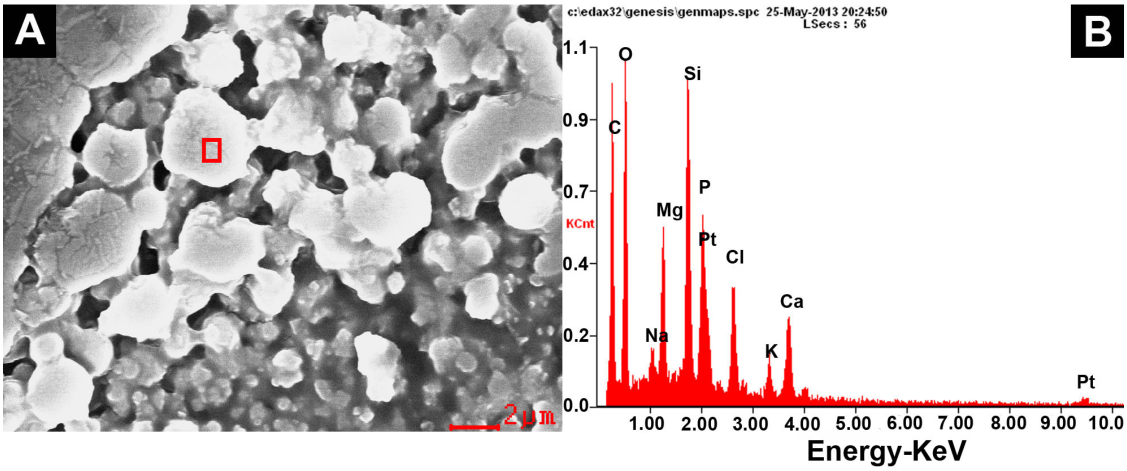

Fig. S9 (A) Scanning electron micrograph of microsphere structure formed by calcium phosphate mineralization; (B) EDS profile of calcium minerals on the outer surface of microsphere through calcium phosphate mineralization after 24 h.