Supporting Information for

Instructed Assembly of Peptides for Intracellular Enzyme Sequestration

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Content

Figure S16. CLSM images of HS-5 cells treated with **Npx-1P** for 1 h and then stained with antibodies of PTP1B (red) and COX-2 (green).

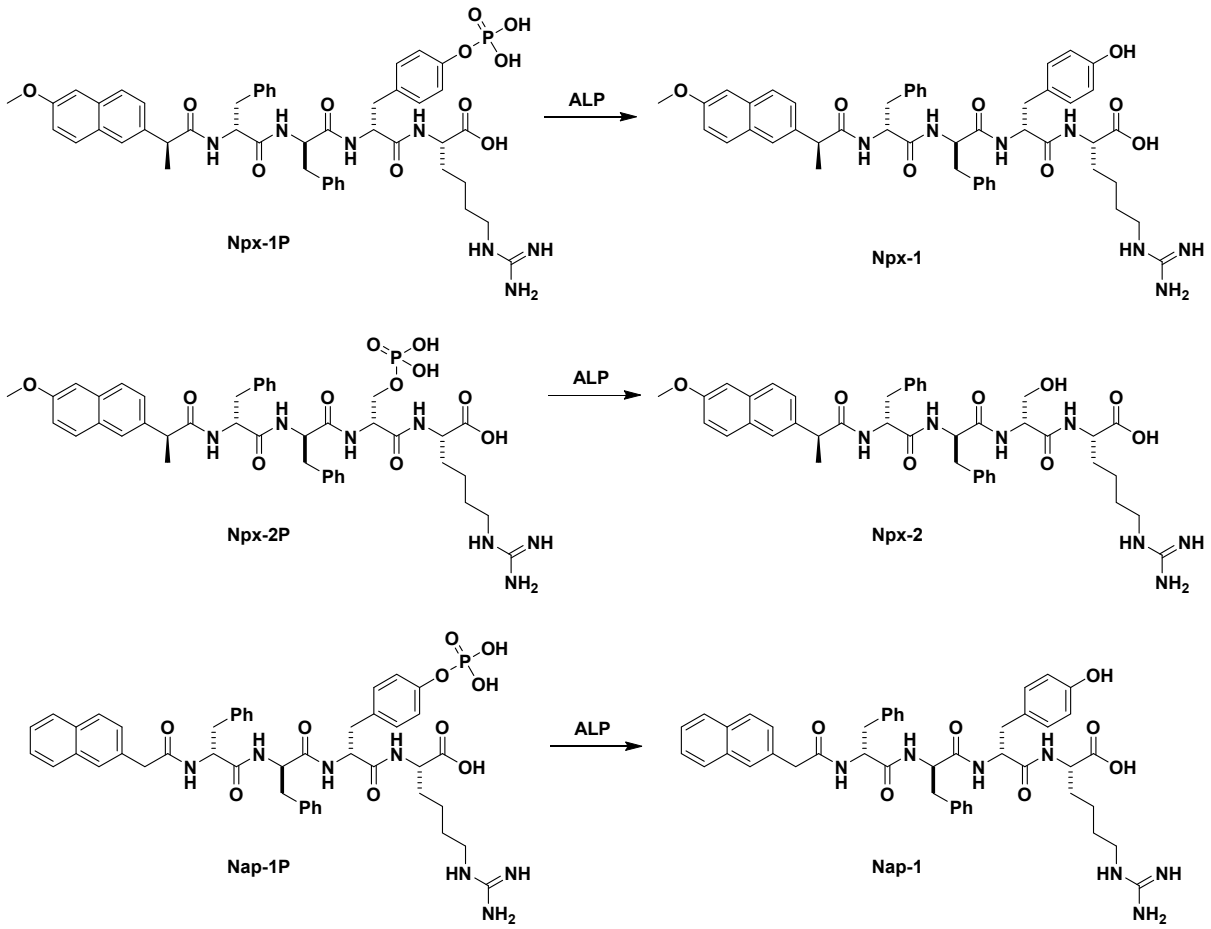
S1. Materials and instruments

2-Cl-trityl chloride resin (1.0-1.2 mmol/g), HBTU, Fmoc-OSu, and other Fmoc-amino acids were purchased from GL Biochem (Shanghai, China). Other chemical reagents and solvents were purchased from Fisher Scientific. Alkaline phosphatase was purchased from Biomatik (Cat. No. A1130, Alkaline Phosphatase [ALP], > 1300 U/mg, in 50% Glycerol.), McCoy's 5A Medium, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and Penicillin-Streptomycin from Gibco by Life Technologies. All precursors were purified with Agilent 1100 Series Liquid Chromatograph system, equipped with an XTerra C18 RP column and Variable Wavelength Detector. The LC-MS spectra were obtained with a Waters Acquity Ultra Performance LC with Waters MICROMASS detector, and ^{1}H-NMR spectra on Varian Unity Inova 400. CLSM images were obtained using Zeiss LSM 880 confocal microscopy at the lens of 63× with oil. The lasers used are 405, 488 and 633 nm.

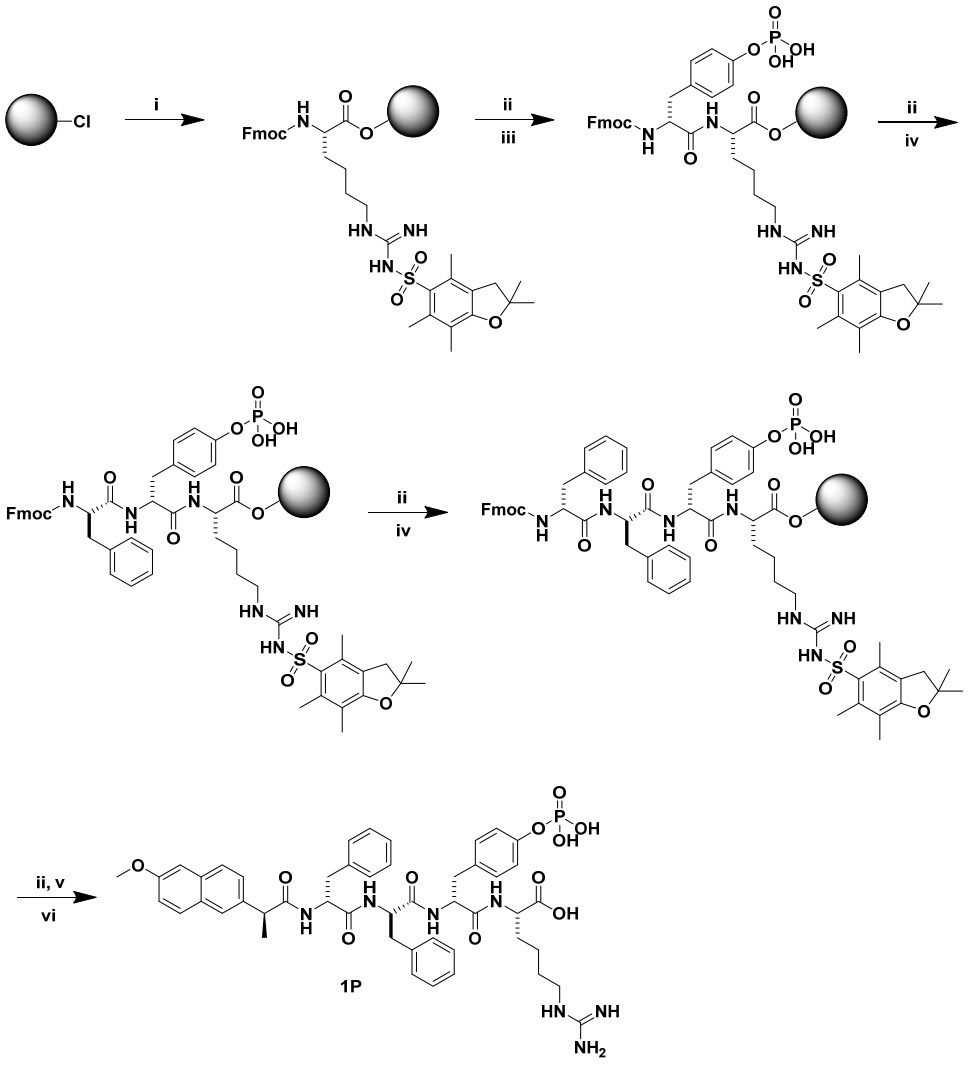
S2. Synthesis and characterization of the precursors

We synthesized Fmoc-Tyr(PO_3H_2)-OH and Fmoc-Ser(PO_3H_2)-OH based on previous work for directly use in solid phase peptide synthesis (SPPS). **Npx-1P**, **Npx-2P**, **Nap-1P** and **Npx-1** (Scheme S1) were synthesized based on reported protocols using SPPS.[1] Scheme S2 shows the synthetic route of **Npx-1P**. The synthetic route of other compounds are the same with **Npx-1P**. All compounds were purified by reverse phase HPLC using HPLC grade acetonitrile and water with

supplement of 0.1% trifluoroacetic acid as the eluents.



Scheme S1. Molecular structures of EISA precursors **Npx-1P**, **Npx-2P**, **Nap-1P** and their corresponding dephosphorylation products **Npx-1**, **Npx-2**, **Nap-1**.



i) Fmoc-HomoArg(Pbf)-OH, DIEA; ii) 20% piperidine; iii) Fmoc-D-Tyr(PO_3H_2)-OH, HBTU, DIEA; iv) Fmoc-D-Phe-OH, HBTU, DIEA; v) Naproxen, HBTU, DIEA; vi) 95%TFA, 2.5% H_2O, 2.5% TIS.

Scheme S2. Synthesis route of **Npx-1P**.

LC-MS (ESI):

Npx-1P (m/z): $C_{48}H_{56}N_7O_{11}P$, calc. 937.38; observed $[M-H]^-$ 936.50.

Npx-1 (m/z): $C_{48}H_{55}N_7O_8$, calc. 857.41; observed $[M-H]^-$ 856.53.

Npx-2P (m/z): $C_{42}H_{52}N_7O_{11}P$, calc. 861.35; observed $[M-H]^-$ 860.43.

Nap-1P (m/z): $C_{46}H_{52}N_7O_{10}P$, calc. 893.35; observed $[M-H]^-$ 892.25.

S3. Sol-gel transition

Npx-1P solutions, from the concentration of 100 to 800 μM, were prepared in pH 7.4 PBS buffer. After adding 1 U/mL ALP for 24 hours, the sol-gel transition was tested.

S4. CMC measurements

A series of **Npx-1P**/ **Npx-1** solutions, from the concentration of 1 μM to 1 mM, were prepared in pH 7.4 PBS buffer. After incubating with Rhodamine 6G (5 μM), we measured the absorbance of above solutions from 520 to 540 nm using a Biotek Synergy 4 hybrid multi-mode microplate reader and determined the λ_{max} for the plotting.

S5. TEM sample preparation

After glowing discharge the 400 mesh copper grids coated with continuous thick carbon film, we placed placing 5 μL samples on the grid, then stained the grid with uranyl acetate and allow to dry in air. TEM images were obtained with Morgagni 268 transmission electron microscope. Specifically, to prepare the TEM samples of nanofibers with proteins, we first added the ALP (1 U/mL) to 150 μL **Npx-1P** (200 μM) solution in pH 7.4 PBS buffer, then incubated 1 μM proteins (i.e., PTP1B, COX-2 or both PTP1B and COX-2) with the solutions for TEM images.

S6. Cell culture

Saos-2 and HS-5 cells were purchased from American Type Culture Collection (ATCC, USA) and maintained at 37 °C in a humidified atmosphere of 5% CO_2. Saos-2 cells were cultured in McCoy's

5A Medium supplemented with 15% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin; HS-5 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) FBS, 100 U/mL penicillin and 100 μg/mL streptomycin.

S7. Pull-down assays

Solutions of **Npx-1P** (200 μM) were prepared in pH 7.4 PBS buffer. 5 μg COX-2 and PTP1B (purchased from abcam) were added and incubated with above solutions upon treating with 1 U/mL ALP for 1 h at room temperature then 4 h at 4 °C, followed by pelleting for 10 min at 12,000 × g at 4 °C. Pellets and supernatants were denatured in Laemmli sample buffer and fractionated by SDS/PAGE, followed by Coomassie staining.

S8. Immunofluorescence staining

Immunofluorescence staining was performed using antibodies from abcam and the protocols as following:

1. Seed Saos-2 cells at 1.5×10^5 cells in a 3.5 cm confocal dish for 24 hours to allow attachment.

2. Remove culture medium and incubate the cells with the precursors.

3. Remove the precursors and wash the cells three times with PBS buffer, then fix the cells with 4% formaldehyde for 10 minutes at room temperature.

4. Wash the cells for three times and block with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature.

5. Stain the treated cells with 5 μg/ml primary antibodies (Rabbit polyclonal to COX2 or Mouse monoclonal to PTP1B) overnight at 4°C in PBS containing 1% BSA and 0.1% tween.

6. Incubate the cells with the 2 μg/mL secondary antibody (Alexa Fluor® 647 goat anti-mouse IgG (H+L) or Alexa Fluor® 488 goat anti-rabbit IgG (H+L)) for 1 h.

7. Stain the cell nucleus with Hoechst 33342 and image the cells with Zeiss LSM 880 confocal microscopy at the lens of 63× with oil.

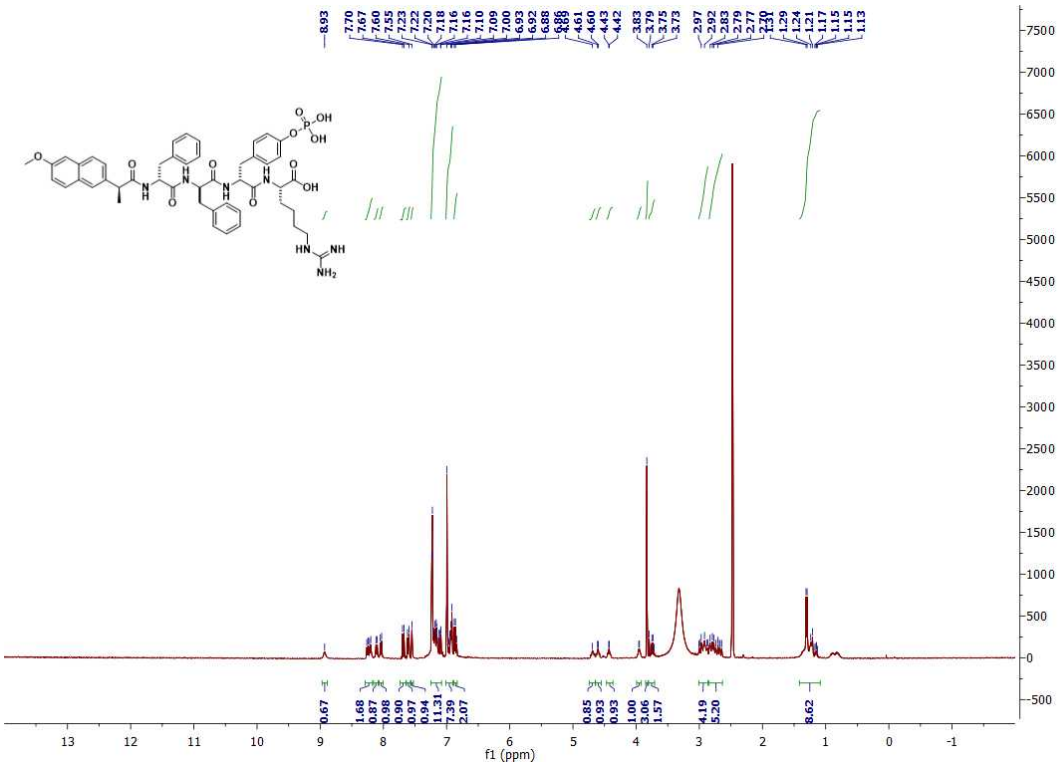


Figure S1. ^{1}H NMR spectrum of **Npx-1P** in DMSO-d_6

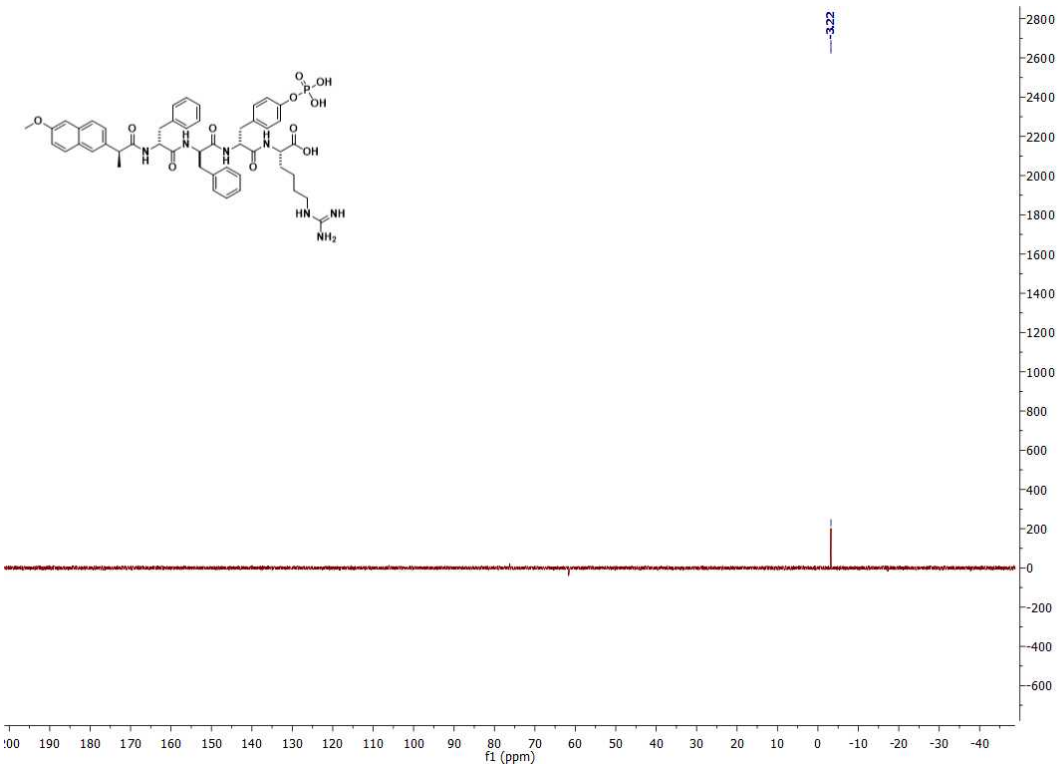


Figure S2. ^{31}P NMR spectrum of **Npx-1P** in DMSO-*d*$_6$

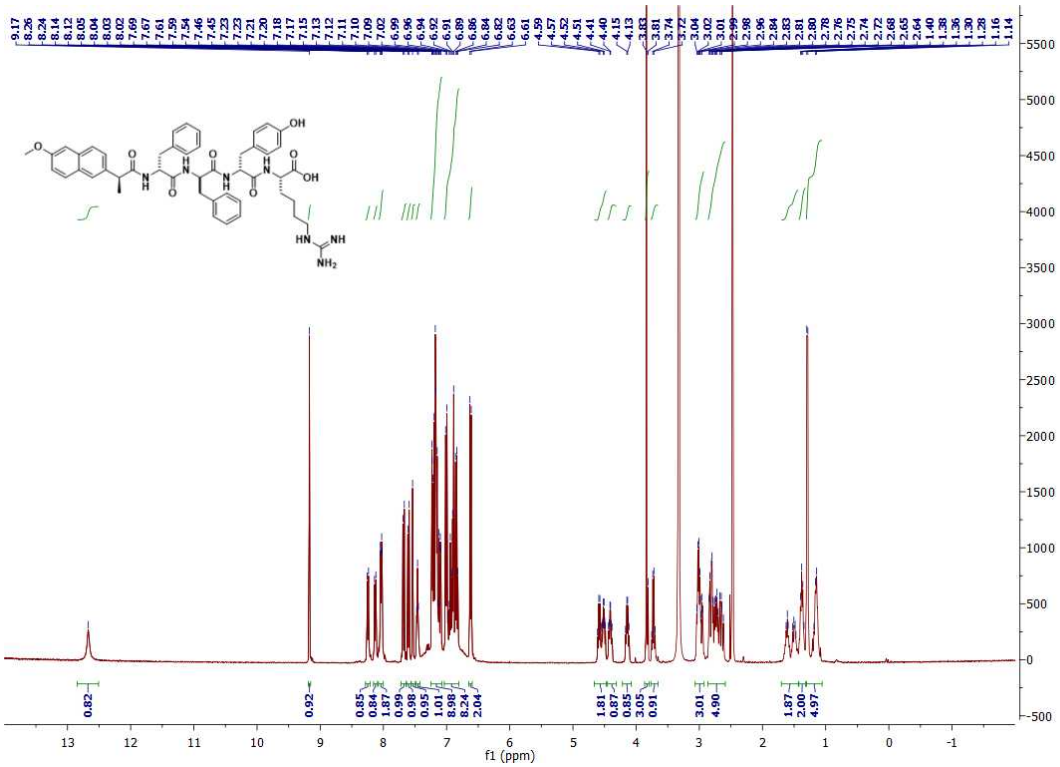


Figure S3. ^{1}H NMR spectrum of **Npx-1** in DMSO-*d*$_6$

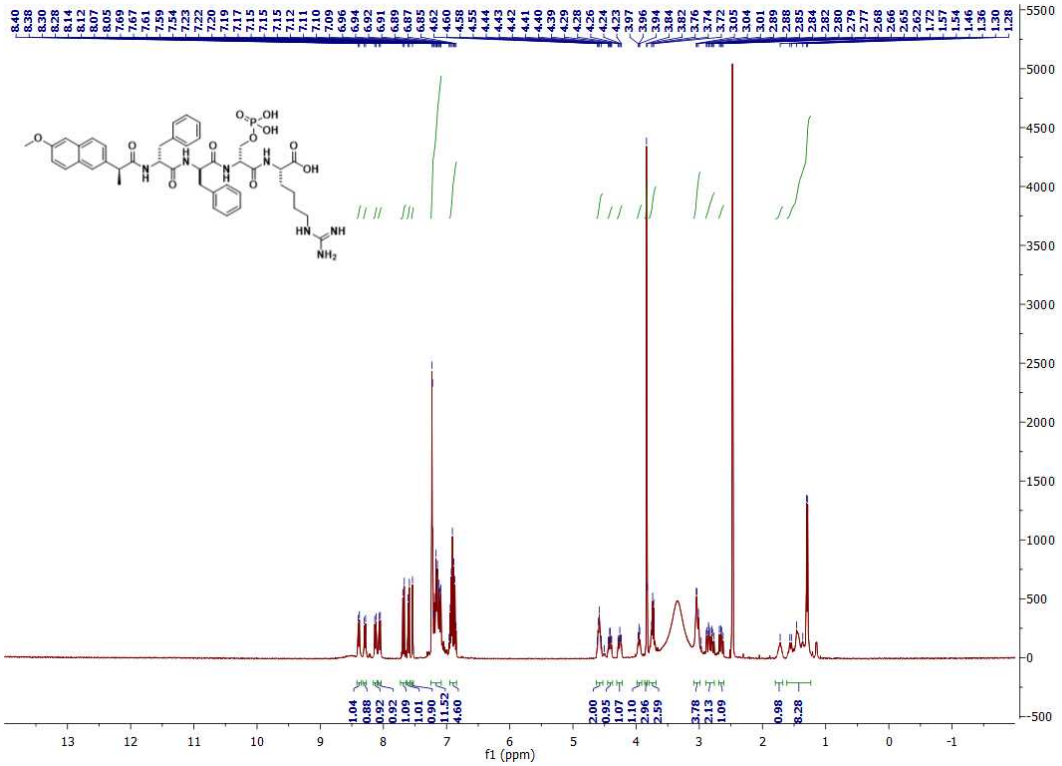


Figure S4. ^{1}H NMR spectrum of **Npx-2P** in DMSO-d_6

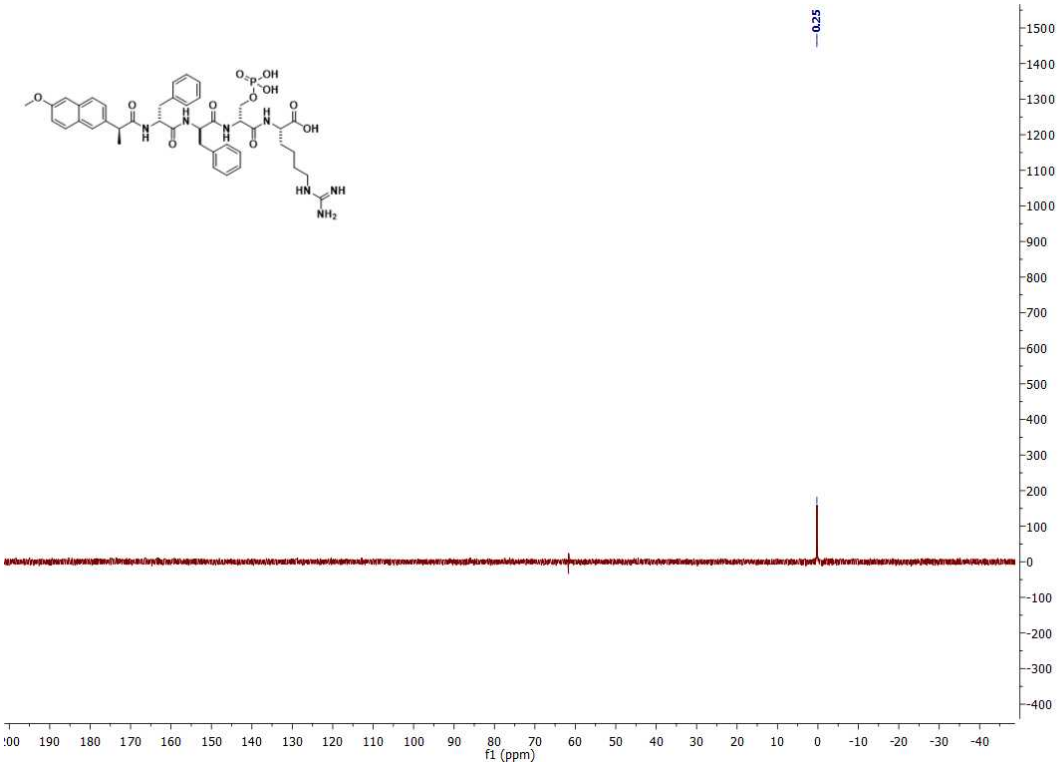


Figure S5. ^{31}P NMR spectrum of **Npx-2P** in DMSO-d_6

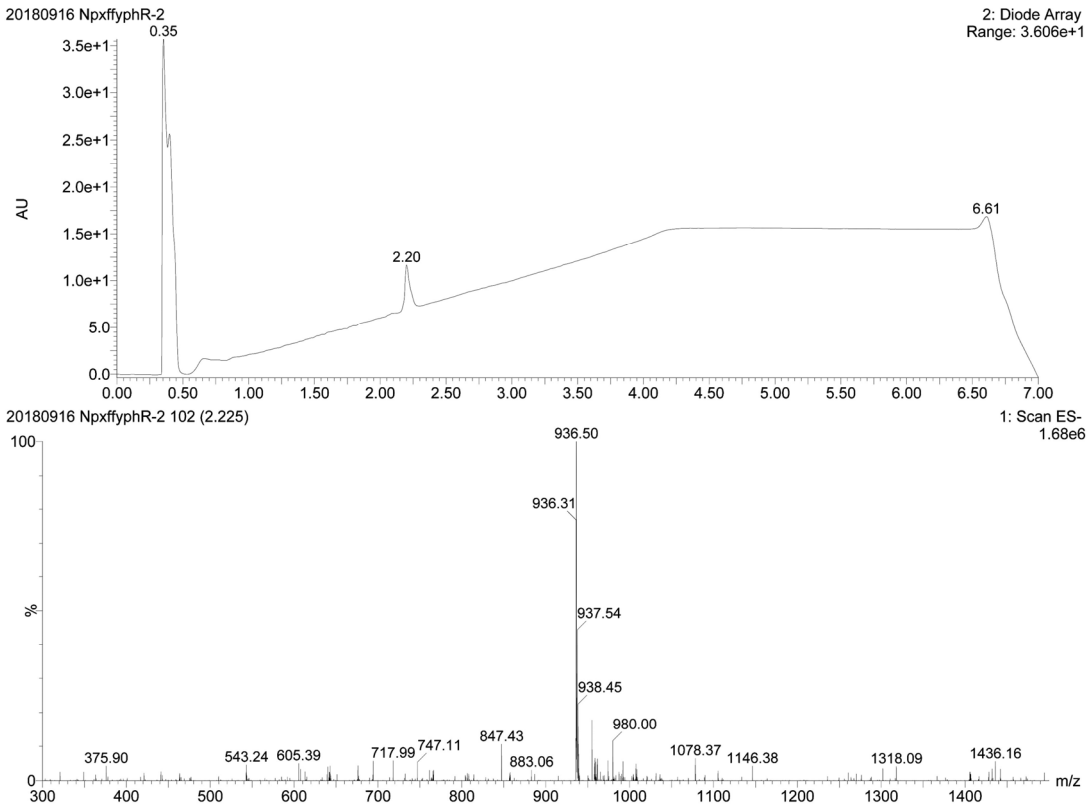


Figure S6. LC-MS spectrum of **Npx-1P**

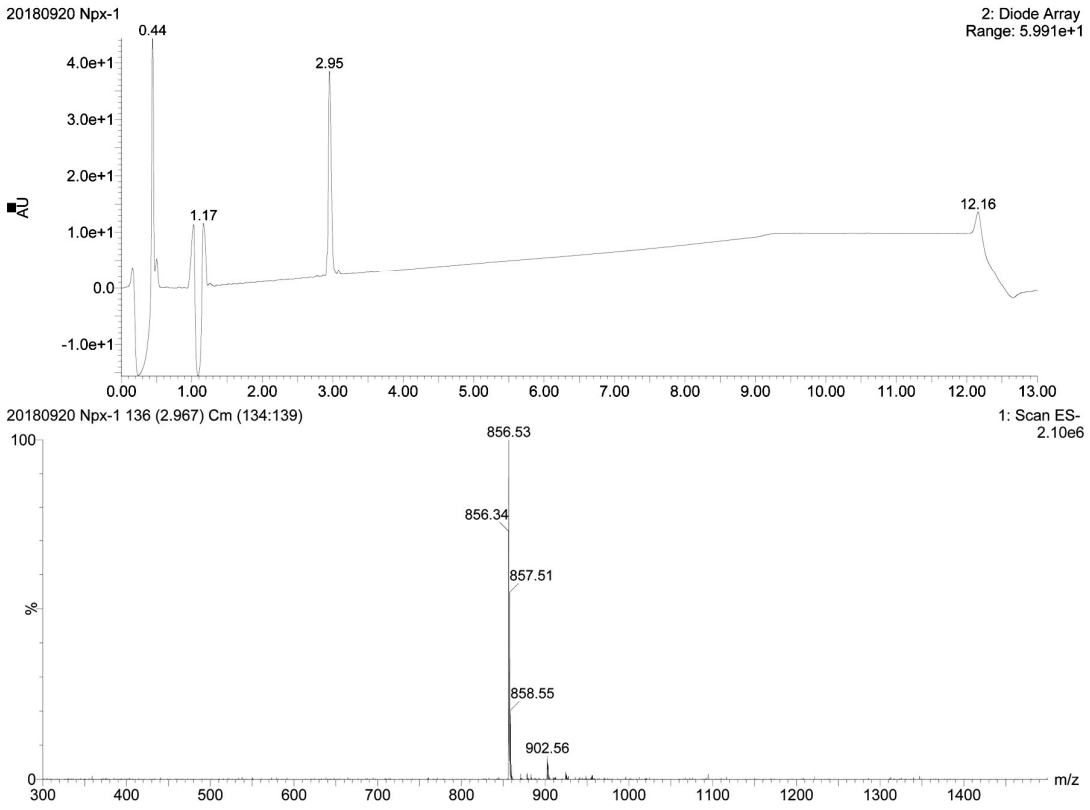


Figure S7. LC-MS spectrum of **Npx-1**

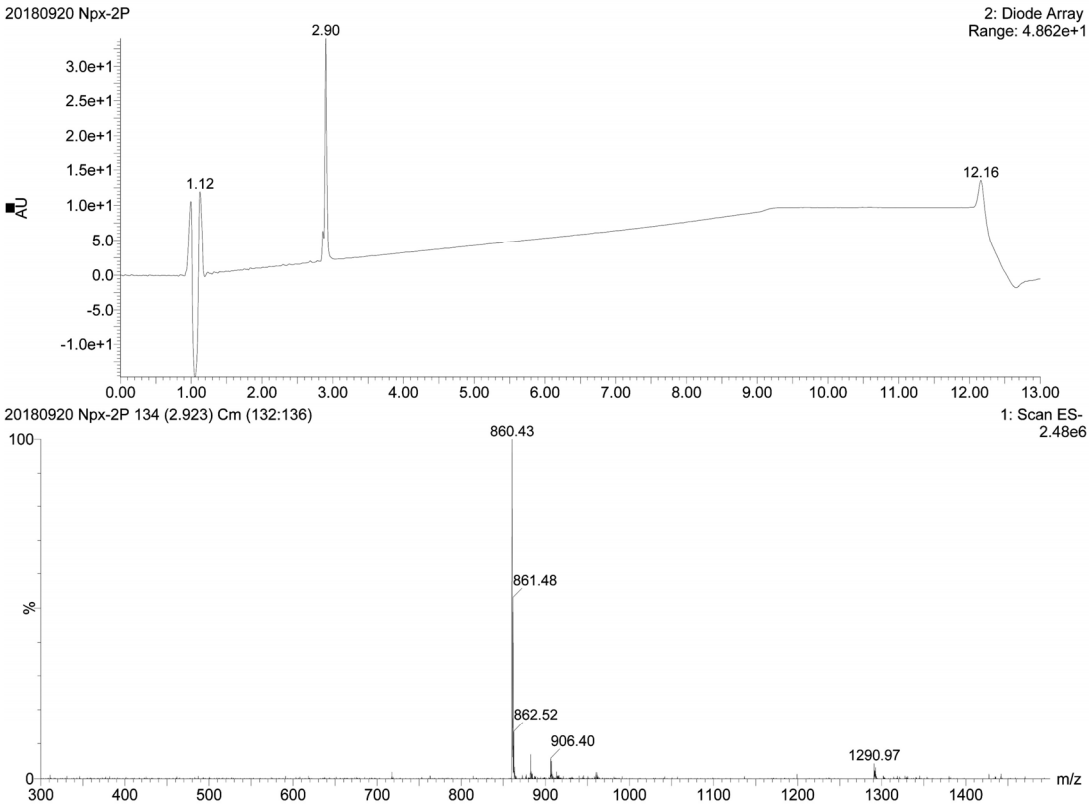


Figure S8. LC-MS spectrum of **Npx-2P**

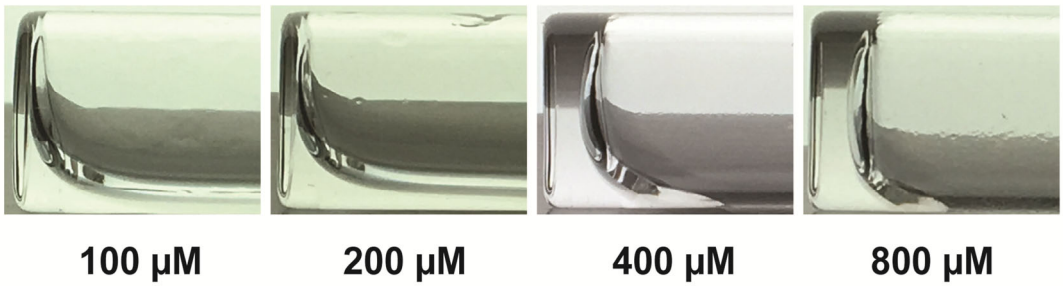


Figure S 9. Optical images of Npx-1P (100-800 μM) after treating with ALP (1 U/mL).

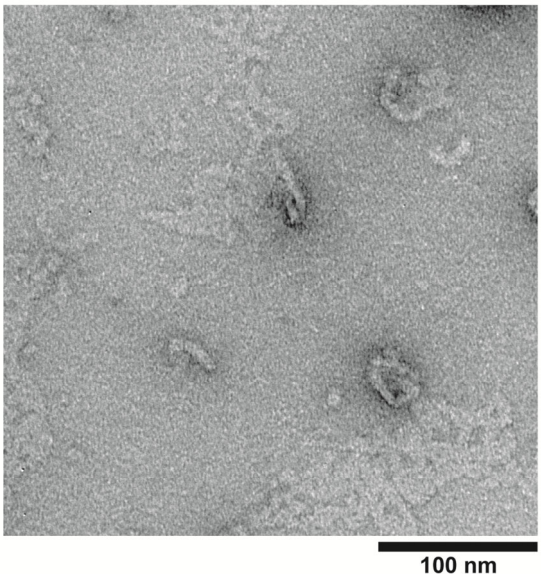


Figure S10. TEM images of the solution of **Npx-1P** (12.5 μM) in PBS buffer (pH 7.4).

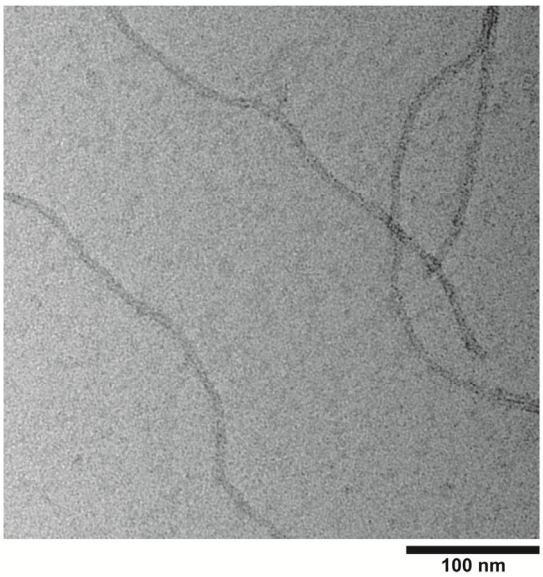


Figure S11. TEM images of the solution of **Npx-1P** (0.5%wt) in PBS buffer (pH 7.4).

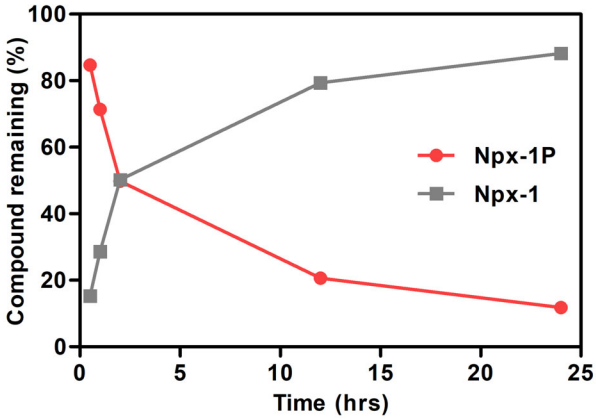


Figure S12. Dephosphorylation of **Npx-1P** (12.5 μM) after incubating with 0.1 U/mL ALP at different time.

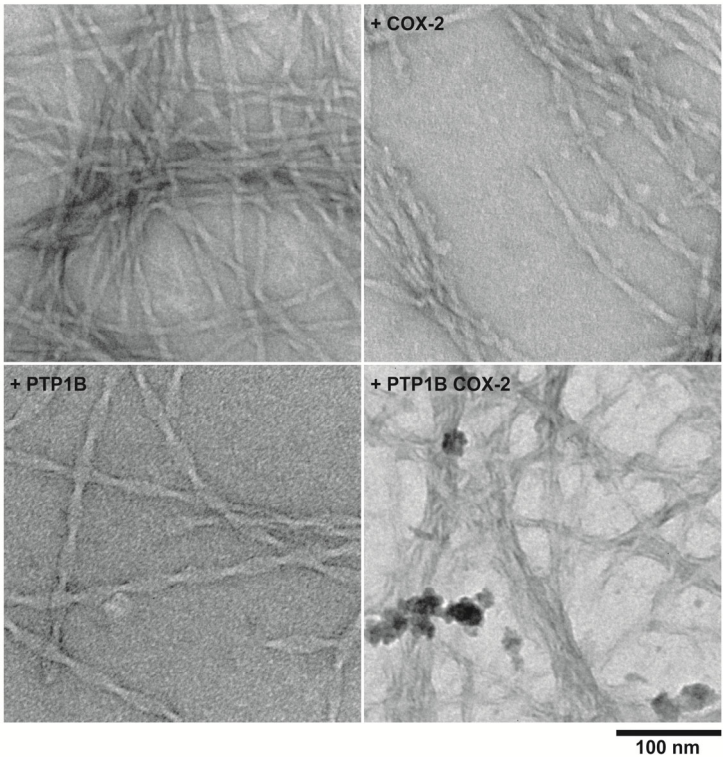


Figure S13. TEM images of nanostructures formed by treating **Npx-1P** (200 μM) with 1 U/mL ALP, without or with the co-incubation of PTP1B, COX-2, or both PTP1B and COX-2, in PBS (pH 7.4); scale bar = 100 nm.

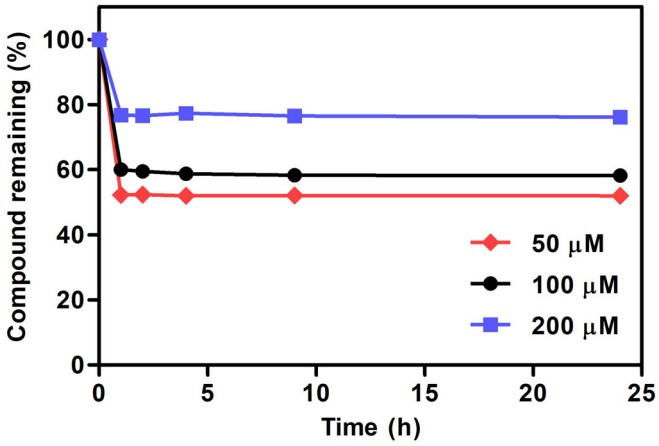


Figure S14. Dephosphorylation of **Npx-1P** at different concentrations after incubating with 45 U/mL PTP1B at different time in 10 mM Tris buffer (pH 7.5) with 50 mM NaCl and 1 mM $MnCl_2$.

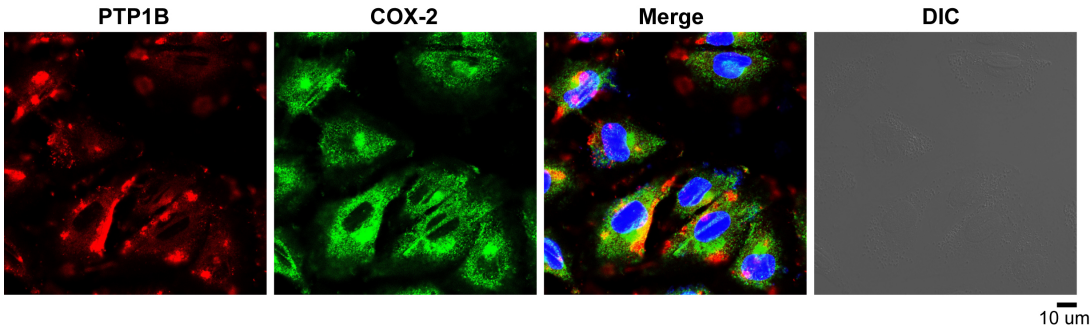


Figure S15. CLSM images of Saos-2 cells treated with **Npx-1P** (12.5 μM) for 0.5 h and then stained with antibodies of PTP1B (red) and COX-2 (green).

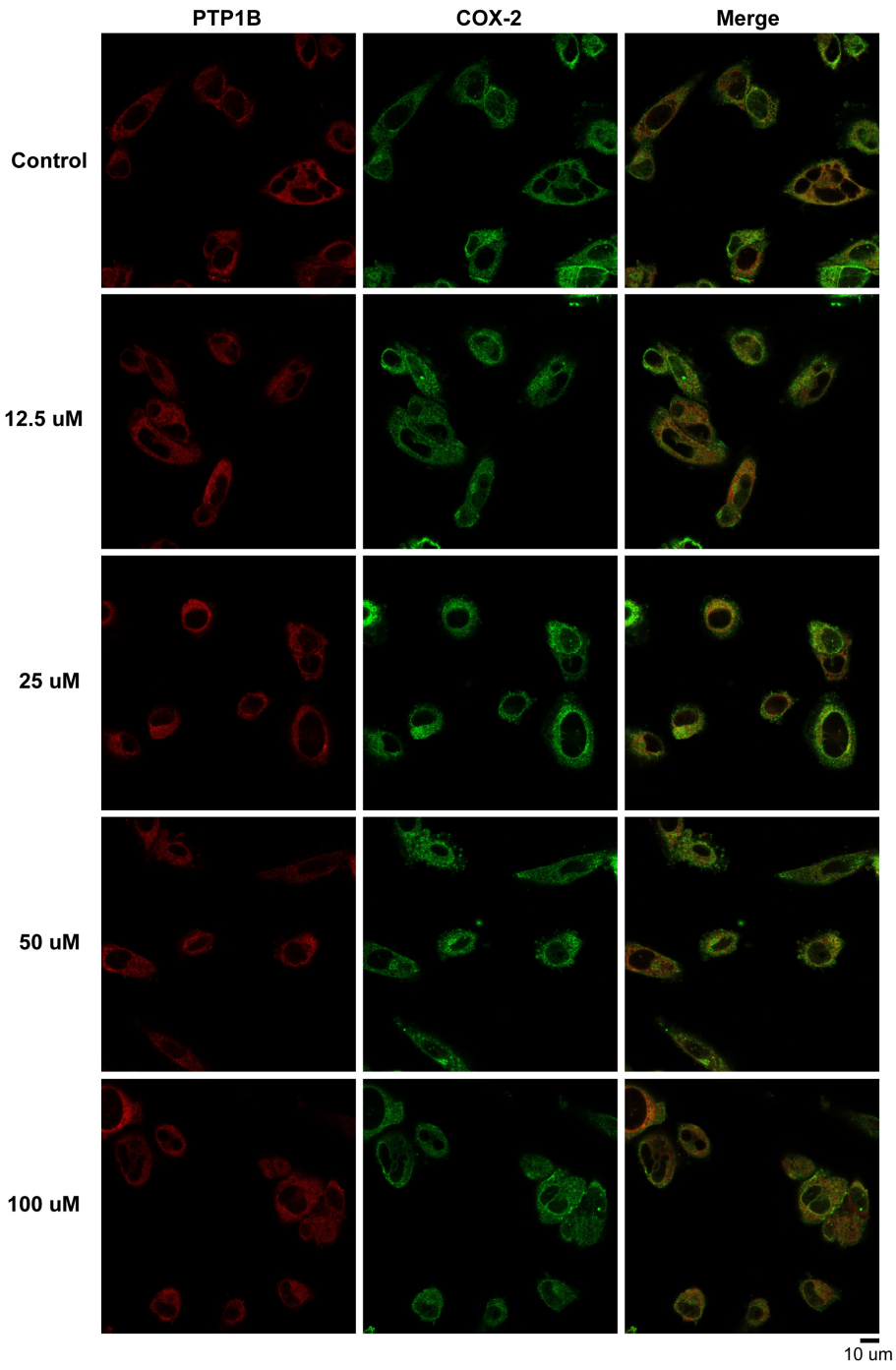


Figure S16. CLSM images of HS-5 cells treated with **Npx-1P** for 1 h and then stained with antibodies of PTP1B (red) and COX-2 (green).

Reference

(1) Shi, J.; Du, X.; Yuan, D.; Zhou, J.; Zhou, N.; Huang, Y.; Xu, B. *Biomacromolecules* **2014**, *15*, 3559; Ottinger, E. A.; Shekels, L. L.; Bernlohr, D. A.; Barany, G. *Biochemistry* **1993**, *32*, 4354.