Supporting Information for

Enzyme-Instructed Assembly and Disassembly Processes for Targeting Down-Regulation in Cancer Cells

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S1. Experiment materials and instruments

All chemical reagents and solvents were used as receiving from commercial sources without further purification. 2-Cl-trityl chloride resin (1.0-1.2 mmol/g), Fmoc-OSu and other Fmoc-amino acids were obtained from GL Biochem (Shanghai, China). Other chemical reagents and solvents were obtained from Fisher Scientific; alkaline phosphatase was purchased from Biomatik. Minimal Essential Medium (MEM), RPMI 1640 Medium were purchased from ATCC and fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Gibco by life technologies. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from ACROS Organics. All precursors were purified with Water Delta600 HPLC system, equipped with an XTerra C18 RP column and an in-line diode array UV detector. We obtained LC-MS spectrum on Waters Acquity Ultra Performance LC with Waters MICROMASS detector, ultraviolet-visible (UV) spectra on JASCO J-810 spectrophotometer, and $^1$H-NMR spectra on Varian Unity Inova 400, and TEM images on Morgagni 268 transmission electron microscope. MTT assay for cell toxicity test on DTX880 Multimode Detector.

S2. Synthesis and characterization of the precursors

We synthesized Fmoc-Tyr(PO$_3$H$_2$)-OH based on previous work for directly use of solid phase peptide synthesis (SPPS). With SPPS, we synthesized 1-OH-OP based on protocols in published paper.$^2$ The following scheme demonstrate the synthetic route for 1-OH-OP and 1-OMe-OP. The synthetic route of other compounds are the same with 1-OH-OP or 1-OMe-OP. Then, all compounds were purified by reverse phase HPLC using acetonitrile (0.1% TFA) and double-distilled water (0.1% TFA) as the eluents.
Scheme S1. Synthesis route of 1-OH-OP and 1-OMe-OP
Scheme S2. Molecular structure of precursors 1-OMe-OP, 2-(OMe)$_2$-OP and their derivatives

LC-MS (ESI):

1-OMe-OP (m/z): C$_{40}$H$_{40}$N$_3$O$_9$P, calc. 737.25; observed [M-H]$^-$ 736.30.

1-OMe-OH (m/z): C$_{40}$H$_{39}$N$_3$O$_6$, calc. 657.28; observed [M-H]$^-$ 656.67.

1-OH-OP (m/z): C$_{39}$H$_{38}$N$_3$O$_9$P, calc. 723.23; observed [M-H]$^-$ 722.19.

1-OH-OH (m/z): C$_{39}$H$_{37}$N$_3$O$_6$, calc. 643.27; observed [M-H]$^-$ 642.76.

2-(OMe)$_2$-OP (m/z): C$_{46}$H$_{49}$N$_4$O$_{12}$P, calc. 880.31; observed [M-H]$^-$ 879.84.

2-(OMe)$_2$-OH (m/z): C$_{46}$H$_{48}$N$_4$O$_9$, calc. 800.34; observed [M-H]$^-$ 799.24.

2-(OH)$_2$-OP (m/z): C$_{44}$H$_{48}$N$_4$O$_{12}$P, calc. 852.28; observed [M-H]$^-$ 851.69.
2-(OH)$_2$-OH (m/z): C$_{44}$H$_{44}$N$_4$O$_9$, calc. 772.31; observed [M-H]$^-$ 771.34.

**S3. CMC measurements**

A series of precursor/derivative solutions from the concentration of 8 mM to 0.4 µM was prepared in pH 7.4 PBS buffer. After incubating with Rhodamine 6G (5 µM), the $\lambda_{\text{max}}$ was determined by measuring the absorbance from 520 to 540 nm using a Biotek Synergy 4 hybrid multi-mode microplate reader.

**S4. TEM sample preparation**

We first place 5 µL samples (preparation procedure described in S3) on 400 mesh copper grids coated with continuous thick carbon film (~ 35 nm) which is glowed discharged. Then we washed the grid with ddH$_2$O twice. Finally we stained the sample loaded grid with a large drop of the UA (uranyl acetate) and allow to dry in air.

**S5. Static light scattering measurement**

We performed static light scattering on using an ALV (Langen, Germany) goniometer and correlator system with a 22 mW HeNe ($\lambda = 633$ nm) laser and an avalanche photodiode detector. We prepared the samples of 1-OMe-OP at the concentration of 20 µM, in pH 7.4 PBS buffer. After addition of 1 U/mL ALP or 1 U/mL CES or both, we incubated the tubes for 24 hours and test the static light scattering at degree of 30°. The resulting intensity ratios are proportional to the amount of aggregates in the samples.
S6. Cell culture and MTT assay

Cell culture: HepG2 cells were purchased from American-type Culture Collection (ATCC, USA). OVSAHO cells were given by Dr. Daniela Dinulescu group. OVSAHO cells were cultured in RPMI 1640 Medium supplemented with 10% v/v fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin; HepG2 cells were cultured in Minimal Essential Medium (MEM) supplemented with 10% v fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO2.

MTT assay: The cytotoxicity was determined by the viability of cells with MTT assay. OVSAHO cells lines or HepG2 cells were seeded in 96-well plates at 1×10^5 cells/well for 24 followed by culture medium removal and subsequently addition of culture medium containing different concentration of the precursors (immediately diluted from fresh prepared DMSO stock solution of 100 mM). After 24/48/72 hours, 10 μL MTT solution (5 mg/mL) was added to each well and incubated at 37°C for another 4 h, and then 100 μL of SDS-HCl solution was added to stop the reduction reaction and dissolve the purple formazan. The absorbance of each well at 595 nm was measured by a multimode microplate reader. The results were expressed as cell viability percentage relative to untreated cells. The cytotoxicity assay was performed three times and the average value of the three measurements was taken.

S7. Enzyme expression profile

We retrieved Affymetrix mRNA expression levels from CCLE database in gctx file format (downloaded for the latest curated version as Nov. 11, 2016). Among 1651 genes and 1037 cancer
cell lines, we targeted several alkaline phosphatases and esterases for their expression levels in HepG2 and OVSAHO cancer cell lines while the value for each probe set is median-centered and divided by the MAD (robust z-score).

S8. Relative secreted esterase activity measurements

1. Seed HepG2 and OVSAHO cells in 96-well plates at 2×10^5 cells/well for 24 hours to allow attachment.

2. Remove the medium and subsequently add 100 µL/well fresh medium (FBS free).

3. Incubate for 2, 4, 8 or 24 hours and take out 80 µL conditioned medium (medium incubated with cells) per well

4. Add 6-carboxyfluorescein diacetate (final concentration as 25 µM) into above conditioned medium or fresh medium (control) and incubate at 37 ºC in dark for 30 minutes.

5. Determine the fluorescence intensity of each well using microplate reader equipped with 485 nm excitation and 535 nm emission filter.

6. Calculate the relative secreted esterase activity with equation:

\[
\text{relative secreted esterase activity} = \frac{\text{fluorescence of conditioned medium} - \text{fluorescence of fresh medium}}{\text{fluorescence of fresh medium}}
\]

(Equation S1)

S9. Confocal microscopy

OVSAHO/HepG2 cells were seeded in 3.5 cm confocal dish at 1.5×10^5 cells for 24 followed by culture medium removal and subsequently addition of fresh culture medium containing 500 µM of 1-OMe-OP. After 2 h of incubation, cells were washed with live cell image solution (Life
Technologies A14291DJ) for 3 or 1 times and stained with 0.1 mg/mL Congo red at 37 °C in dark for 30 minutes. Followed by three or one-time wash with live cell image solution, cells were stained with 1.0 μg/ml Hochst 33342 for 10 min at 37 °C in dark. Finally, the cells were rinsed three or one times, and then kept in the live cell imaging solution for imaging.

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![Bar plot of mRNA expression levels of phosphatases ALPL and esterases CES1 in HepG2 and OVSAHO (obtained from the Cancer Cell Line Encyclopedia (CCLE)^1).](image)

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