Electronic Supplementary Information

Affinity-based release of polymer-binding peptides from hydrogels with their target segments

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Experimental details

Peptide screening. The screening of PPO-binding peptides was performed according to our previously reported protocols.[1-3] Briefly, films with a thickness of approximately 150 nm were prepared from PPO with the number-average molecular weight (M_n) of 7400 and the weight-average molecular weight (M_w)/M_n of 1.07 (Polymer Source) on glass substrates using 20 mg mL^{-1} chloroform solutions. A 1.0 x 10^{10} plaque forming unit (pfu)/30 μL aliquot of phage-displayed 12-mer peptide library solution (New England Biolabs) in Tris-buffered saline (TBS, 50 mM Tris, 150 mM NaCl, pH 7.5) was mounted onto the PPO films and incubated for 30 min at ambient temperature. The unbound phages were removed by rinsing the films five times with 150 μL of TBS containing 0.1% Tween 20 and five times with 400 μL of TBS. The bound phages were eluted by mounting 100 μL of the elution buffer solution (0.5 M glycine-HCl, 1 mg mL^{-1} bovine serum albumin, pH 2.2) onto the films for 15 min at ambient temperature. The eluted phage solution was neutralized with 7.5 μL of the Tris-buffered solution (1 M Tris, pH 9.1). The phages were amplified by infecting *Escherichia coli* strain ER2738 and purified using a polyethylene glycol/NaCl solution for use in the next round of screening. The two rounds of screening were repeated followed by cloning and DNA sequencing of the phages.

Solid-phase peptide synthesis. Peptides with an amidated C-terminus were prepared by solid-phase peptide synthesis using standard 9-fluorenylmethyloxycarbonyl (Fmoc)-based procedures according to a previously reported procedure.[4] Briefly, the peptide chains were assembled on a NovaSyn TGR resin (amino group 0.24 mmol g^{-1}) using Fmoc amino acid derivatives. To cleave the peptide from the resin and remove the side chain protecting groups, the resin was treated with trifluoroacetic acid (TFA)/triisopropylsilane/water (95/2.5/2.5, v/v/v). The crude peptides were purified by reverse-phase high-performance liquid chromatography (ELITE LaChrom, HITACHI High-Technologies) using a C18 column

(COSMOSIL 5C18-AR-300, 20 × 250 mm, Nacalai Tesque) with a linear gradient from 99.9% H_2O/0.1% TFA to 99.9% acetonitrile/0.1% TFA at a flow rate of 6 mL min^{-1}. The peptides were identified by high performance liquid chromatography (Prominence UFLC System, Shimadzu) equipped with electrospray ionization mass spectrometry (MS-2020, Shimadzu) using a 5C15-AR-II column (COSMOSIL, 2. 0 × 150 mm, Nacalai Tesque) with a linear gradient from 99.9% H_2O/0.1% formic acid to 99.9% acetonitrile/0.1% formic acid at a flow rate of 0.2 mL min^{-1}.

For the synthesis of the Dox-conjugated peptide, the PPO-binding peptide with the additional Cys residue at the C-terminus was synthesized on a Fmoc-NH-SAL-PEG resin (amino group 0.19 mmol g^{-1}). The cleavage from the resin and removal of the protecting groups on the side chain was performed in a similar fashion. Dox was dissolved in 5 mL of dimethyl sulfoxide (DMSO) containing 2% triethylamine, and the solution was mixed with *N*-succinimidyl 3-maleimide propionate (linker) dissolved in 3 mL of DMSO (the molar ratio of Dox and the linker was 1.1:1). The mixture was stirred for 2 h at ambient temperature, and formic acid (50 μL) was added to terminate the reaction. Then, the Cys-introduced peptide was added to the solution (the molar ratio of the linker and the peptide was 1.1:1), and the mixture was stirred for 2 h at ambient temperature. The resulting Dox-conjugated peptide was purified and identified in a similar fashion.

Synthesized peptides showed over 95% purities, and the observed mass values agreed with the calculated mass values within a 1.0 mass unit (original peptide: $M_{w\ calcd}$ 1347.7, $m/z_{\ calcd}$ $[M+H]^+$ 1348.7, $m/z_{\ obsd}$ $[M+H]^+$ 1348.7; D1A: $M_{w\ calcd}$ 1304.6, $m/z_{\ calcd}$ $[M+H]^+$ 1305.6, $m/z_{\ obsd}$ $[M+H]^+$ 1305.8; F2A: $M_{w\ calcd}$ 1272.5, $m/z_{\ calcd}$ $[M+H]^+$ 1273.5, $m/z_{\ obsd}$ $[M+H]^+$ 1272.7; N3A: $M_{w\ calcd}$ 1305.5, $m/z_{\ calcd}$ $[M+H]^+$ 1306.5, $m/z_{\ obsd}$ $[M+H]^+$ 1306.7; P4A: $M_{w\ calcd}$ 1322.5, $m/z_{\ calcd}$ $[M+H]^+$ 1323.5, $m/z_{\ obsd}$ $[M+H]^+$ 1322.7; Y5A: $M_{w\ calcd}$ 1256.5, $m/z_{\ calcd}$ $[M+H]^+$ 1257.5, $m/z_{\ obsd}$ $[M+H]^+$ 1256.7; L6A: $M_{w\ calcd}$ 1306.5, $m/z_{\ calcd}$ $[M+H]^+$ 1307.5, $m/z_{\ obsd}$

$[M+H]^+$ 1307.7; G7A: $M_{w\ calcd}$ 1362.6, $m/z_{\ calcd}$ $[M+H]^+$ 1363.6, $m/z_{\ obsd}$ $[M+H]^+$ 1363.0; V8A: $M_{w\ calcd}$ 1320.5, $m/z_{\ calcd}$ $[M+H]^+$ 1321.5, $m/z_{\ obsd}$ $[M+H]^+$ 1321.7; T9A: $M_{w\ calcd}$ 1318.5, $m/z_{\ calcd}$ $[M+H]^+$ 1319.5, $m/z_{\ obsd}$ $[M+H]^+$ 1319.8; P10A: $M_{w\ calcd}$ 1322.5, $m/z_{\ calcd}$ $[M+H]^+$ 1323.5, $m/z_{\ obsd}$ $[M+H]^+$ 1323.7; V11A: $M_{w\ calcd}$ 1320.5, $m/z_{\ calcd}$ $[M+H]^+$ 1321.5, $m/z_{\ obsd}$ $[M+H]^+$ 1321.7; K12A: $M_{w\ calcd}$ 1291.5, $m/z_{\ calcd}$ $[M+H]^+$ 1292.5, $m/z_{\ obsd}$ $[M+H]^+$ 1292.6; Dox-conjugated peptide: $M_{w\ calcd}$ 2146.4, $m/z_{\ calcd}$ $[M+2H]^{2+}$ 1074.2, $m/z_{\ obsd}$ $[M+2H]^{2+}$ 1074.1).

SPR measurements. The same PPO and atactic PMMA with M_n of 12700 and M_w/M_n of 1.03 (Polymer Laboratories) as a reference polymer were applied for SPR measurements. A Biacore X (GE Healthcare) was used for SPR measurements according to our previously published protocols.[1-3] PPO or PMMA films with a thickness of approximately 12 nm were prepared on gold-coated glass slides (SIA Kit Au, GE Healthcare) by spin-casting (2000 rpm) using 2.0 mg mL^{-1} chloroform solutions and placed on the SPR apparatus. HBS-N (10 mM HEPES, 150 mM NaCl, pH 7.4, GE Healthcare) was flowed at a rate of 20 μL min^{-1} at 25 °C during the experiment. After stabilization of the baselines by HBS-N flow, freshly prepared peptide solutions were applied to the films for 180 s (association), and then, the peptide solutions were exchanged for peptide-free HBS-N for 1000 s (dissociation). The resulting sensorgrams at different peptide concentrations were analysed by global fitting using BIAevaluation software version 4.1. The chi-square values as an index of fitting reliability were estimated to 3.9 and 4.6 for the PPO and PMMA films, respectively. It is important to note that values of less than 10 are considered to be acceptable according to the BIAevaluation handbook.

Structural analyses of peptides. The CD spectra of the PPO-binding peptide dissolved in phosphate-buffer solutions (PB, 10 mM phosphate, pH 7.5) or 1/1 (v/v) mixture solutions of PB (5 mM phosphate, pH 7.5) and trifluoroethanol were recorded on a J-725 (JASCO) under

a N_2 atmosphere at 25 °C using a quartz cell with a thickness of 0.2 cm. The data represent the average of 4 scans in the wavelength range of 190-250 nm with a resolution of 0.5 nm and a scanning speed of 50 nm min^{-1}. MM calculations of the PPO-binding peptide were performed using MM2 (PerkinElmer) and were started from the α-helix conformation.

Peptide release from hydrogels. PEO-PPO-PEO (Pluronic® F127) with M_w of 12600 and a 70 wt% PEO content was kindly donated by BASF Japan and used for the hydrogel components. The 30% (w/w) PEO-PPO-PEO solutions in phosphate-buffered saline (PBS, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) were prepared at 4 °C. The peptides were dissolved into 200 μL of the polymer solution using a glass vial with a diameter of 10 mm. The mixtures were transformed into peptide-containing hydrogels by incubation at 25 °C or 37 °C. Then, 200 μL of PBS was added on the top of the hydrogels for release experiments. For the release of peptides without Dox conjugation, 45 μL of the upper PBS solution was reacted with 15 μL of a fluorescamine solution (3 mg mL^{-1} DMF) for 30 min at 30 °C, and the fluorescence intensity at 475 nm by excitation at 390 nm was measured by a fluorescent spectrophotometer (FP-6500, Jasco) at 25 °C. For the release of the Dox-conjugated peptide or free Dox, 50 μL of the upper PBS solution was taken, and the fluorescence intensity at 594 nm by excitation at 504 nm was measured in a similar fashion. The amounts of released peptides and free Dox were estimated using the corresponding calibration curve.

Cell culture assay. For the preparation of PEO-PPO-PEO hydrogels containing peptides, Dulbecco's PBS was used. The solution containing PEO-PPO-PEO and the peptides (750 μL) that was prepared at 4 °C was added to each well of a 24-well microplate and incubated at 37 °C to achieve hydrogelation. Human cervical cancer cells (HeLa cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS, BioWest), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. The cells were seeded

in a 10-cm diameter Petri dish (Falcon, BD Biosciences) and allowed to grow until approximately 80% confluence. All the incubations were performed at 37 °C with 5% CO_2. The HeLa cells in serum-free medium (100 μL containing 5000 cells) were applied to the Cell desk placed on 24-well microplates and incubated for 2 days. Then, the cell-adhered Cell desk was mounted on the PEO-PPO-PEO hydrogel prepared in the microplate. Fluorescence microscopic observations evaluated alive and dead cells using calcein AM and propidium iodide (Dojindo) on a Ti-U-PH-1 system (Nikon). After the observation, all the cells were collected to quantitate the number of living cells using a cell counting kit (CCK, Dojindo). The viabilities of the cells incubated with peptides were compared to those of cells incubated without peptides (control).

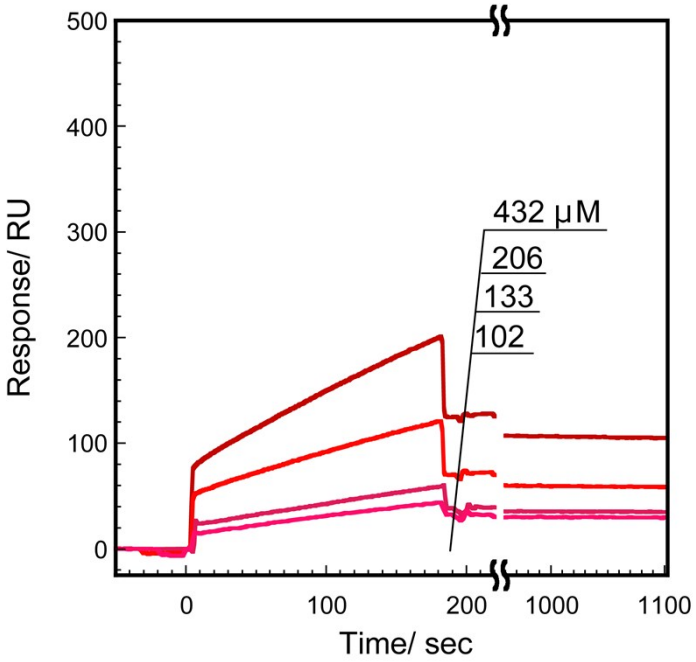

Fig. S1 SPR sensorgrams for the binding of the identified peptide to PMMA films at different concentrations.

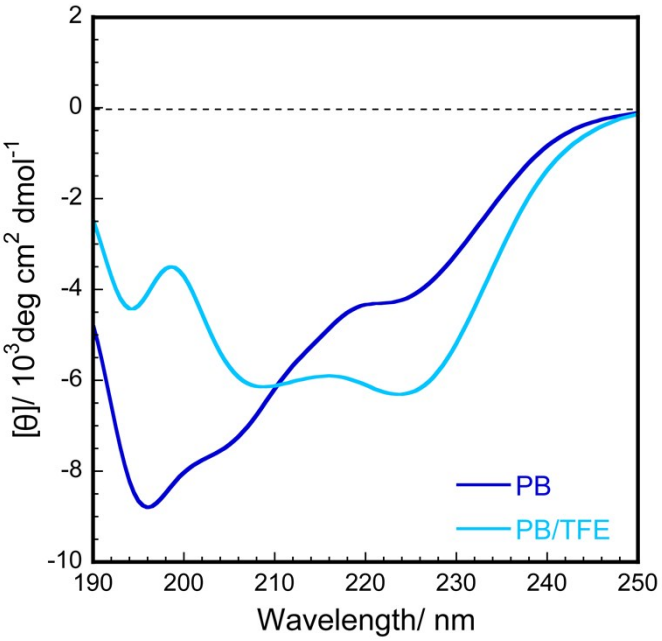

Fig. S2 CD spectra of the PPO-binding peptides with and without trifluoroethanol (TFE).

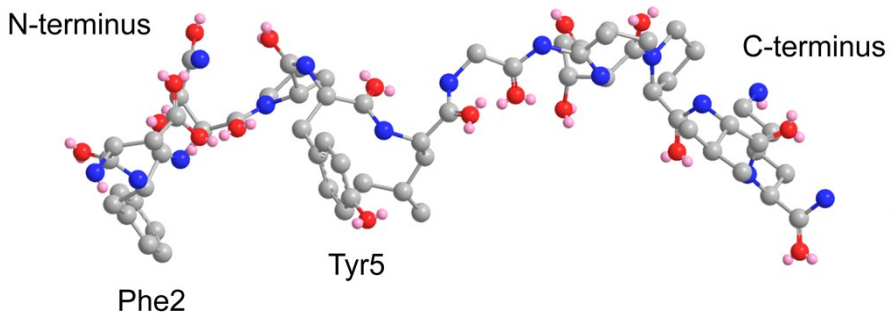

Fig. S3 Structure of the PPO-binding peptide obtained from a MM calculation.

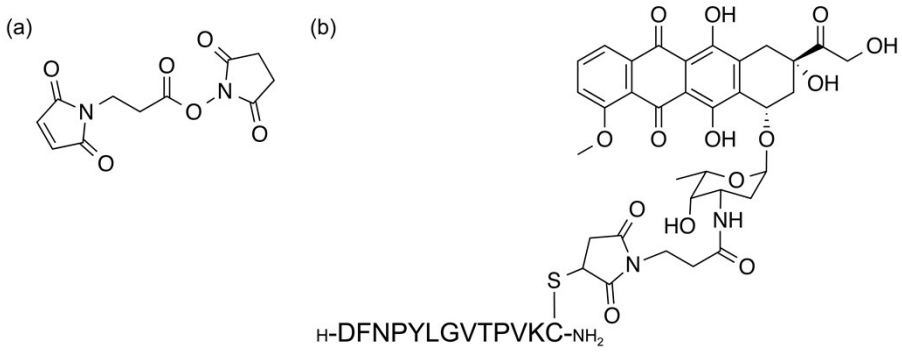

Fig. S4 Chemical structures of (a) the linker for Dox conjugation through the thiol group of Cys and (b) the Dox-conjugated PPO-binding peptide.

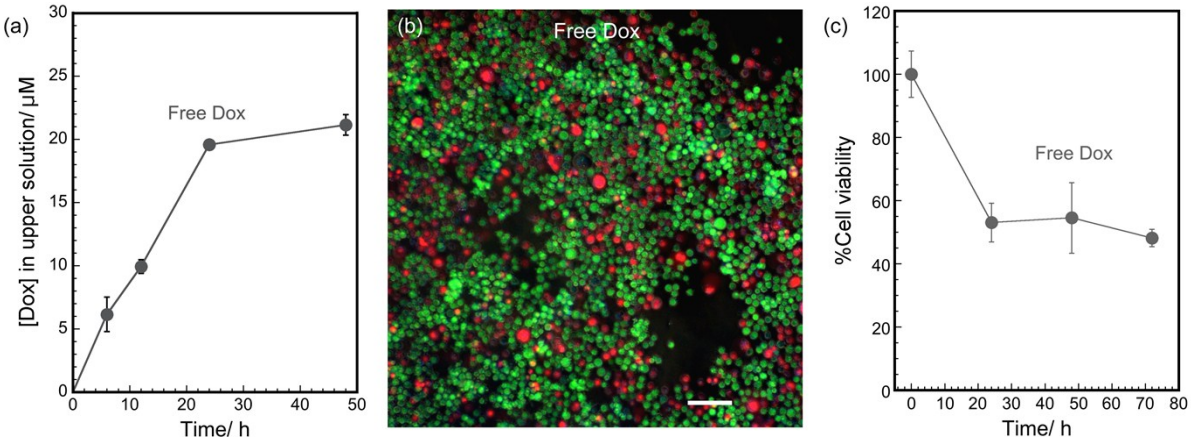

Fig. S5 (a) Time-dependent release of free Dox from PEO-PPO-PEO hydrogels at 37 °C. (b) Fluorescent images of adhered Hela cells stained with green (alive) and red (dead) colors after 72 h incubation with PEO-PPO-PEO hydrogels containing Dox. Scale bar repraesents 100 μm. (c) Quantification of living cells against incubation time.

References

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