Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Screening of peptides recognizing simple polycyclic aromatic hydrocarbons

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Detailed experiments

Peptide screening against immobilized naphthalene. 2-Naphthylamine molecules were immobilized on self-assembled monolayers (SAMs) composed of PEG6-NHS ester alkanethiol and PEG3-OH alkanethiol (TOYOBO). The surface coverage of 2-napthylamine molecules was controlled at 10%. Before screening, residual NHS groups on SAMs were reacted using Tris-HCl buffer (50 mM, pH 7.5) for 30 min. A 1.2×10^{10} plaque forming unit (pfu)/ 15 μL aliquot of phage library solution (New England Biolabs) in Tris-buffered saline (TBS, 50 mM Tris, 150 mM NaCl, pH 7.5) was mounted onto the naphthalene-immobilized SAMs, and was incubated for 5 min at ambient temperature. Unbound phages were removed by rinsing the films five times with 30 μL of TBS containing 0.1% Tween20. The bound phages were eluted using 20 μL of the elution buffer (TBS containing 0.5% Tween20) onto the films with stirring at ambient temperature. Tween20 in the eluted phage solution was removed using ultrafiltration (Amicon Ultra-4, 100 kDa molecular weight cut-off). The phages were amplified by infecting to *Escherichia coli* strain ER2738, and purified by a polyethylene glycol/NaCl solution to use in the next round of screening. To remove phages with affinity for OH and ethylene glycol groups, phages were incubated with SAMs without NHS and naphthalene. Five rounds of biopanning were repeated, followed by the cloning and DNA sequencing of the phages.

Phage binding analysis. The binding capabilities of the screened phage clones and wild type (WT) phages were examined by titer count analyses. Each well of 96 well microtiter plates for covalent coupling with amino groups (Immobilized Amino Module and Solid Plates, Nuce) was reacted with 2-napthylamine. The naphthalene-immobilized wells were incubated with phage solution (50 μL, 50 pM in TBS) for 10 min at 20 °C. After rinsing with TBS containing 0.1% Tween 20 (100 μL) five times, the bound phages were eluted using 100 μL of TBS containing 0.5% Tween 20 with stirring at ambient temperature twice. The amount of phage in the eluted phage solution was determined by titer count analysis.

SPR analysis. A Biacore X (GE Healthcare) was used for the SPR analyses, following the approach described in our previous studies[1]. Naphthalene immobilized surfaces were prepared on gold-coated glass slides (Sensor Chip CM5, GE Healthcare) by conventional amine coupling, and the chips were set on the SPR apparatus. HBS-N (10 mM HEPES buffer containing 150 mM NaCl, pH 7.4, GE Healthcare) was flowed at a rate of 20 μL/min at 25 °C during the experiment. After more than 3 h of HBS-N flow, freshly prepared peptide solutions were applied to the naphthalene molecules for 180 sec (association), and then the peptide solutions were exchanged for a peptide-free buffer for 180 sec (dissociation). The resulting sensorgrams at 5 concentrations were analyzed by global fitting using BIAevaluation software version 4.1. This program simultaneously fits all sensorgrams, including association/dissociation processes and responses due to rapid changes in the bulk refractive indices when the association/dissociation of the peptide starts, followed by estimation of the association (k_1) (M^{-1} s^{-1}) and dissociation (k_{-1}) (s^{-1}) rate constants. The binding constant (K_a) (M^{-1}) was calculated by $K_a = k_1/k_{-1}$. The fitting result evaluated by the chi-square (χ^2) value was 6.2 (an index of fitting reliability). Values of less than 10 are considered to be acceptable according to the BIAevaluation handbook. Standard errors were small enough to discuss the values obtained. Benzene, anthracene, and pyrene with lateral amino groups were also immobilized by conventional amine coupling.

CD analysis. The CD spectra or the original and the substituted Nap01 peptide solutions with a concentration of 10 μM were recorded on a circular dichroism spectrometer (J-720, JASCO) using a UV cell with 1 cm of optical path length at 25 °C. In the CD experiments, Sodium-phosphate buffer (10 mM, pH 7.5) was used and the CD spectra were co-added for 4 times.

Dispersion of polycyclic aromatic hydrocarbons using peptides. Naphthalene, antharacene, and pyrene were weighed (1.0 ± 0.1 mg) in a microtube and 1 mL of Tris-HCl buffer solution (50 mM, pH 7.5) containing the Nap01 peptide (5 mM) was added. These samples were gently sonicated for 1 h at 25 ± 5 °C in a normal bath-type ultrasonic cleaner (55 W, USK-1R, AS ONE). To remove any insoluble molecules, the samples were centrifuged at 20000 *g* for 60 min at 25 °C. The upper supernatants were used for spectroscopic analyses. The absorption and fluorescence spectra of supernatants were recorded on a UV-vis spectrophotometer (V-670, JASCO) and a fluorophotometer (FL-3500, JASCO), respectively. Naphthalene, anthracene, and pyrene were exited at 270, 350, and 317 nm, respectively. When using naphthalene, the solutions were diluted 10 times, and then the fluorescence spectra were measured because of relatively high solubility in buffer solution compared with anthracene and pyrene. Furthermore, CD spectroscopic analysis of the dispersed naphthalene by the Nap01 peptide was performed using the solution diluted 100 times.

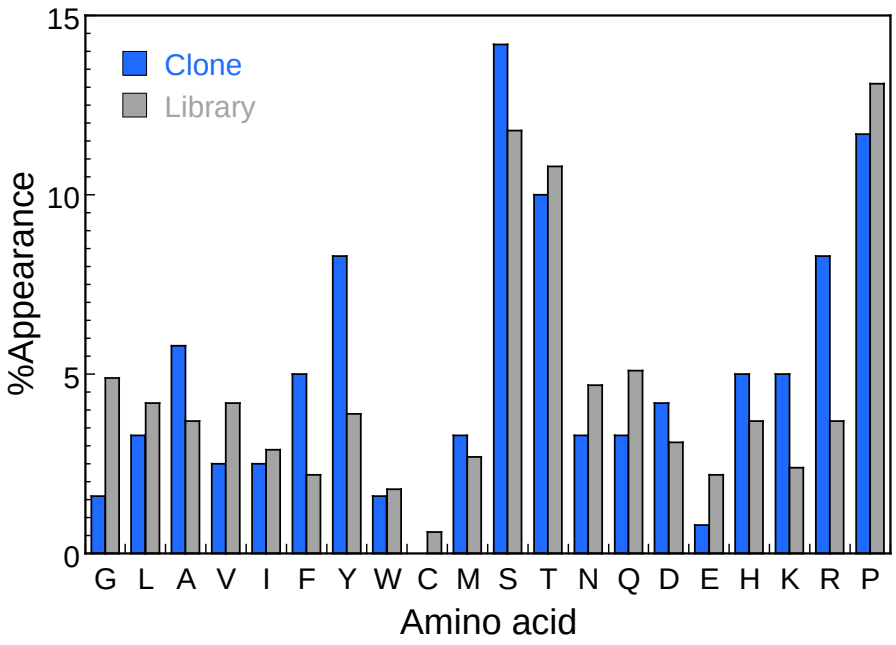

Fig. S1. Percent appearance of amino acids in the peptide sequences.

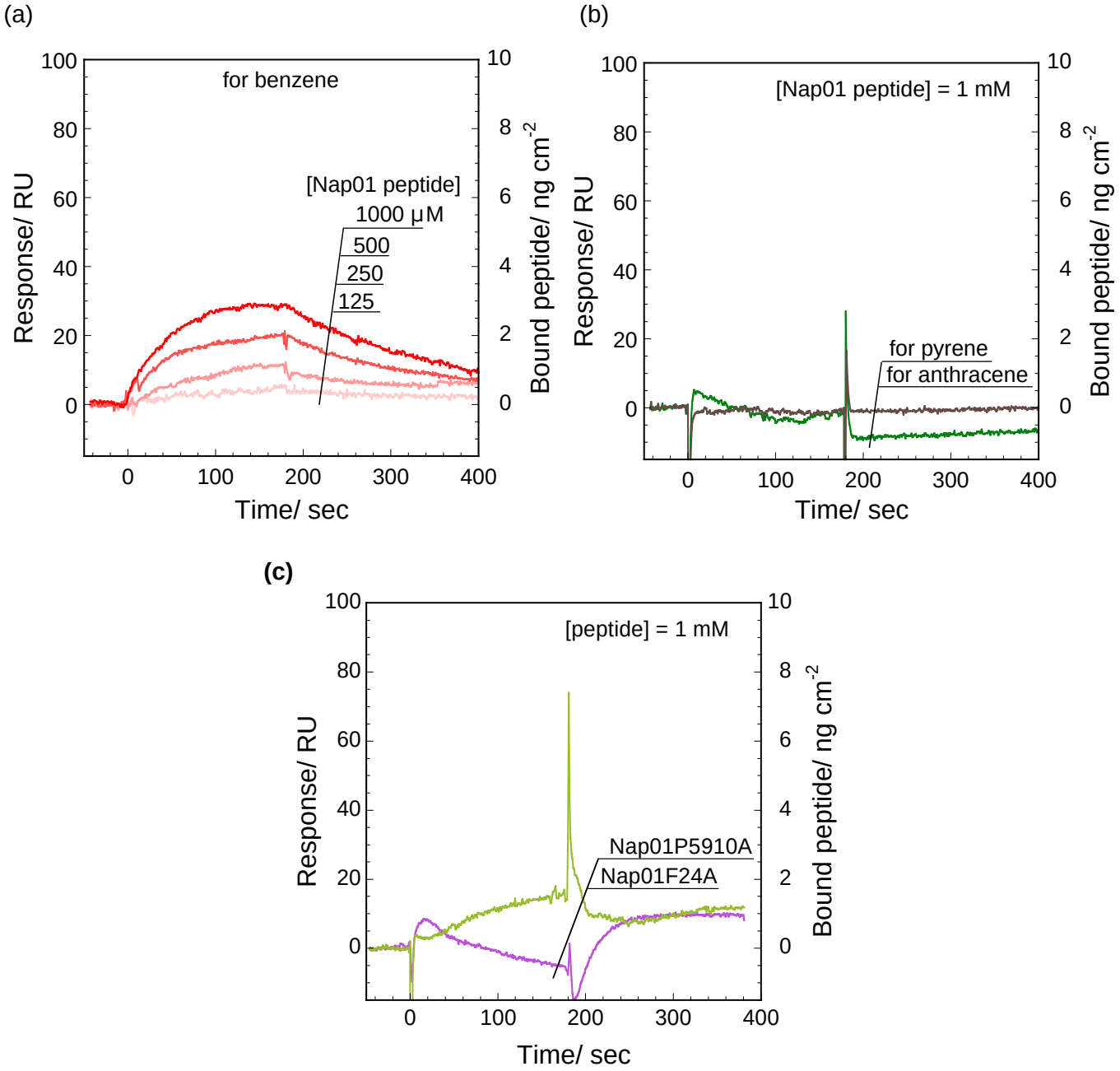

Fig. S2. SPR sensorgrams of (a) the Nap01 peptide against benzene, (b) anthracene and pyrene, (c) Ala-substituted peptide against naphthalene.

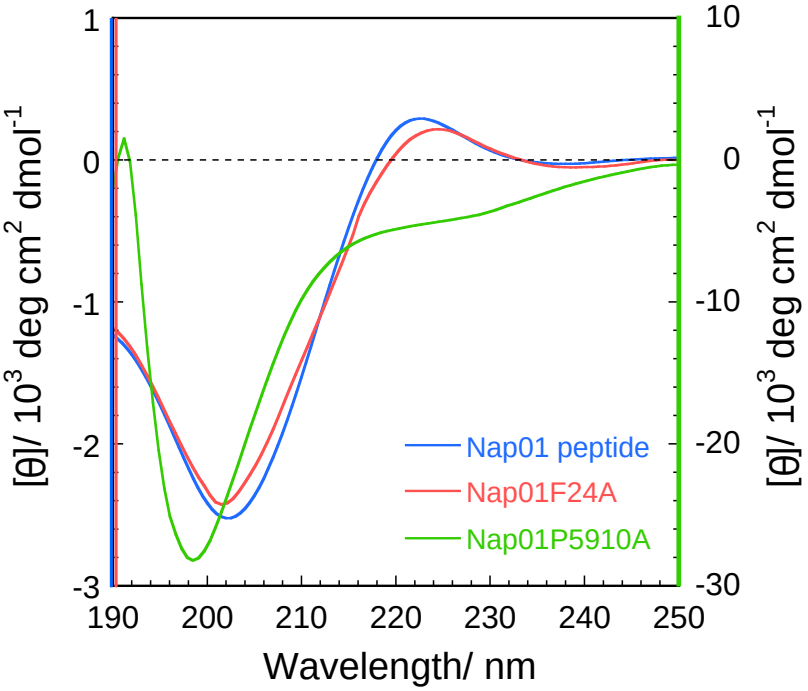

Fig. S3. Circular dichroism secondary structural analyses of the original Nap01 peptide and the Ala substituted peptides. Concentrations of peptide solutions used were 10 μM.

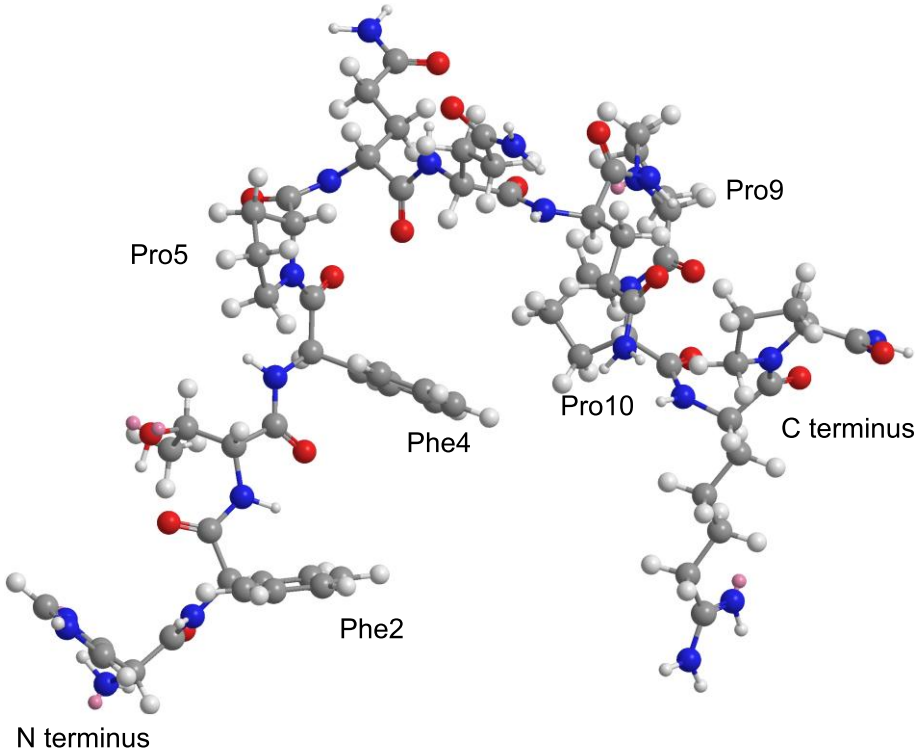

Fig. S4. A possible structure of the Nap01 peptide. Carbon, nitrogen, and oxygen atoms were shown in grey, blue, and red colors, respectively.

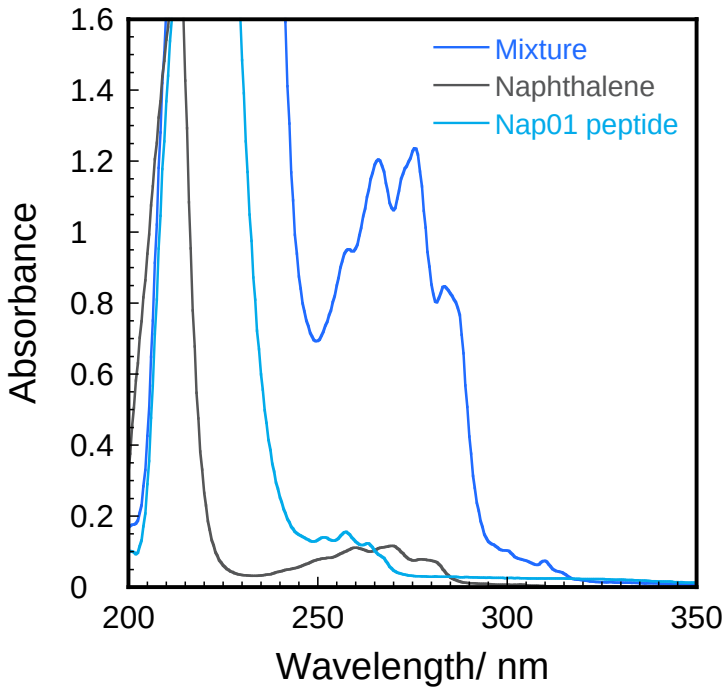

Fig. S5. Absorption spectra of dispersed naphthalene molecules with (mixture) and without the Nap01 peptide, and the Nap 01 peptide. The dispersed solutions were diluted 10 times. Final concentrations of the Nap01 peptide with and without naphthalene were 500 μM.

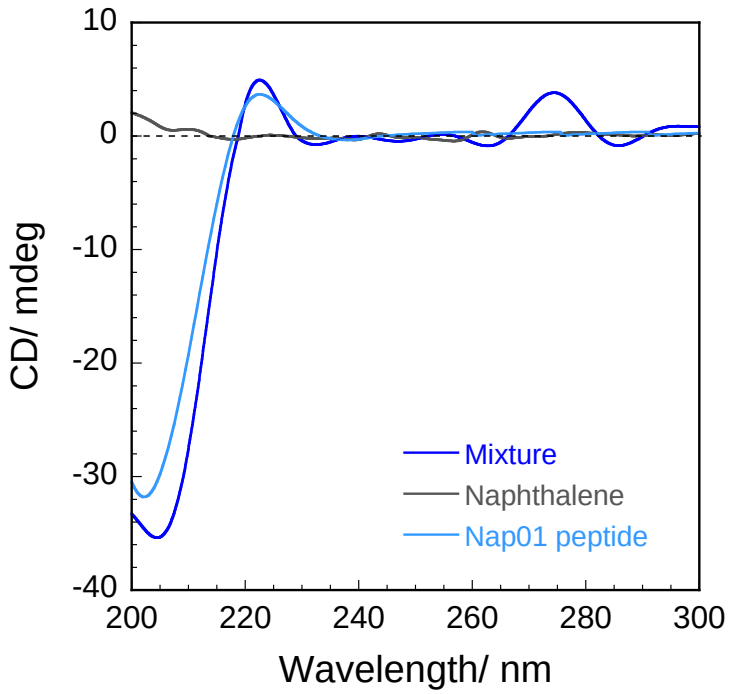

Fig. S6. Circular dichroism spectroscopic analyses of mixtures of the Nap01 peptide with naphthalene, naphthalene, and the Nap01 peptide.

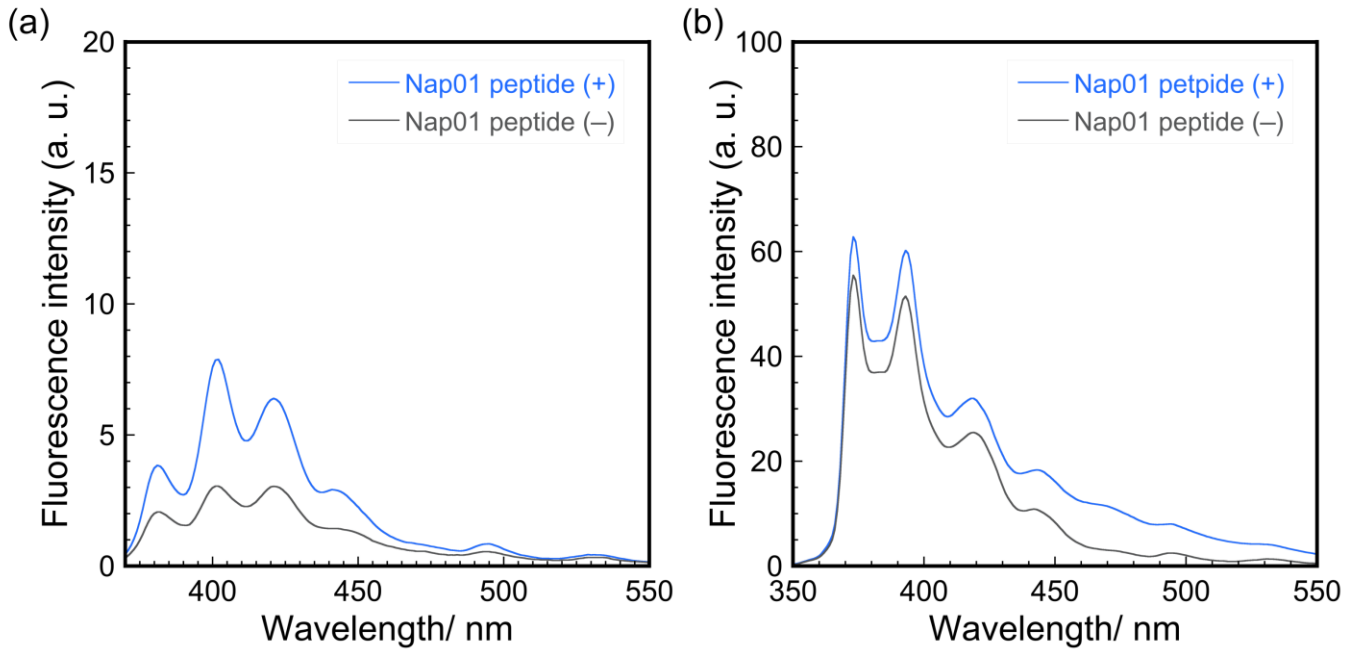

Fig. S7. Fluorescence spectra of dispersed (a) anthracene and (b) pyrene molecules with (blue) and without (gray) the Nap01 peptide in buffer solution. Anthracence and pyrene were excited 350 nm and 317 nm, respectively.

References

1. (a) T. Serizawa, T. Sawada and H. Matsuno, *Langmuir*, 2007, **23**, 11127; (b) H. Matsuno, J. Sekine, H. Yajima and T. Serizawa, *Langmuir*, 2008, **24**, 6399; (c) T. Date, J. Sekine, H. Matsuno and T. Serizawa, *ACS Appl. Mater. Interfaces*, 2011, **3**, 351; (d) T. Sawada, K. Ishiguro, T. Takahashi and H. Mihara, *Mol. BioSyst.*, 2011, **7**, 2558.