Supporting Information for

Intracellular Mineralization of Gold Nanoparticles Using Gold Ion-Binding Peptides with Cell-Penetrating Ability

Makoto Ozaki, Shuhei Yoshida, Takaaki Tsuruoka and Kenji Usui*

[*] Dr. Kenji Usui, Mr. Makoto Ozaki, Mr. Shuhei Yoshida, Dr. Takaaki Tsuruoka
Faculty of Frontiers of Innovative Research in Science and Technology (FIRST),
Konan University, 6500047, Kobe (Japan)
E-mail: kusui@konan-u.ac.jp (K. Usui)

CONTENTS:

Methods;

S1. General remarks

All chemicals and solvents were of reagent or HPLC grade and were used without further purification. HPLC was performed on a GL-7400 HPLC system (GL Sciences, Tokyo, Japan) using an Inertsil ODS-3 column (10 x 250 mm; GL Sciences) for preparative purification, with a linear acetonitrile/0.1% trifluoroacetic acid (TFA) gradient at a flow rate of 3.0 mL/min. Peptides were analyzed using MALDI-TOF MS on an Autoflex III (Bruker Daltonics, Billerica, MA, USA) mass spectrometer with 3,5-dimethoxy-4-hydroxycinnamic acid as the matrix. Amino acid analysis was conducted using an Inertsil ODS-2 column (4.6 x 200 mm; GL Sciences) after samples were hydrolyzed in 6 M HCl at 110°C for 24 h in a sealed tube and then labeled with phenyl isothiocyanate.

S2. Synthesis of the peptides

Designed peptides were synthesized manually on Fmoc-NH-SAL PEG resin (Watanabe Chemical Industries, Hiroshima, Japan) using Fmoc chemistry[1] with Fmoc-AA-OH (10 eq., Watanabe Chemical Industries) and 5,6-carboxyfluorescein (10 eq., Sigma-aldrich, St. Louis, MO, USA) according to the 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, Watanabe Chemical Industries) method. Side-chain protection was as follows: *t*-butyl (tBu) for Ser and Tyr. R8 was cleaved from the resins and side-chain protection was removed by incubating the peptides for 1 h in TFA/milliQ/triisopropylsilane/thioanisol (47/1/1/1, v/v/v/v). The peptides except for R8 were cleaved from the resins and side-chain protection was removed by incubating the peptides for 1 h in TFA/ethandithiol/thioanisol/m-cresol (43/3/3/1, v/v/v/v). All the peptides were precipitated by the addition of cold diethyl ether, collected by centrifugation, purified by RP-HPLC (Fig. S9a-d), and characterized by amino acid analysis and MALDI-TOF MS (Fig. S9a-d): R8-AuBP, *m/z* 2469.3 ($[M+H]^+$ calcd. 2470.0); R8, *m/z* 1266.6 ($[M+H]^+$ calcd. 1267.6); AuBP, *m/z* 1221.1 ($[M+H]^+$ calcd. 1221.4); Flu-R8-AuBP, m/z 2829.5 ($[M+H]^+$ calcd. 2828.3). Purified peptides were dissolved in MilliQ water to approximately 1 mM, and their concentrations were determined by amino acid analysis. Peptides were stored at 4°C.

S3. Cell culture

Human cervical carcinoma (HeLa) cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. HeLa cells were seeded with 3.0 x 10^5 cells in 6-well plate (Nest Biotechnology Co. Ltd., Wuxi, China) and cultured at 37°C in a 5% CO_2 atmosphere for 24 h.

S4. Gold mineralization in cell using gold ion binding peptide with cell penetrating ability

HeLa cells were seeded with 3.0 x 10^5 cells in a 6-well plate and cultured at 37°C in a 5% CO_2 atmosphere for 24 h. After incubation, the media were removed and the cells were washed three times with 1xPBS. 10 μL of 100 μM R8-AuBP was mixed with 690 μL of MilliQ water, 100 μL of 10xPBS, and 200 μL of 10 mM $HAuCl_4$ incubated for 30 min in test tube. This sample solution (1 mL) was added to cell culture plate. This plate was incubated for 6-48 h at 37°C in 5% CO_2 atmosphere.

S5. Gold mineralization in cell using endocytosis inhibitor

HeLa cells were seeded with 3.0 x 10^5 cells in a 6-well plate and cultured at 37°C in a 5% CO_2 atmosphere for 24 h. After incubation, the media were removed and the cells were washed three times with 1xPBS. 1 mL of 5 μM cytochalasin D (Fujifilm Wako Pure Chemical Industries, Tokyo, Japan) in D-MEM without fetal bovine serum was added to cell culture plate, and incubated for 15 min at 37°C in 5% CO_2 atmosphere.[2] 10 μL of 100 μM R8-AuBP was mixed with 590 μL of MilliQ water, 100 μL of 10xPBS, and 200 μL of 5 mM $HAuCl_4$ incubated for 30 min in test tube. This sample solution (900 μL) was mixed with 100 μL of 50 μM cytochalasin D, and added to cell culture-plate. This plate was incubated for 48 h at 37°C in 5% CO_2 atmosphere.

S6. Cell lysate

After gold mineralization in cell, the media were removed and the cells were washed three times with 1xPBS. 500 μL of MilliQ was added to cell culture-plate, and the cells were separated cell culture-plate using scraper. The cells were transferred into a micro tube, and were ultrasonicated for 10 min in ice bath using Bioruptor (UCD-250, Cosmo Bio Co. Ltd., Tokyo, Japan). The lysate solution was centrifuged by Capsulefuge PMC-060 (AS ONE, Osaka, Japan), and cell fragments were sedimented.

S7. Phase contrast microscopy

After gold mineralization in cell, gold mineralized cells were observed using phase contrast microscopy (IX 70, Olympus, Tokyo, Japan).

S8. Absorption measurements

The sample solution (350 μL) after cell lysate was transferred into a disposal cell (BR759200, Merck, Darmstadt, Germany) with a 1 cm pathlength. All absorption spectra data were acquired on a UV-1800 spectrometer (Shimadzu, Kyoto, Japan).

S9. Transmission electron microscopy (TEM)

Prior to gold mineralization, the gold mineralization sample solution (20 μL) after cell lysate was incubated for 24-48 h in cell and then placed on a TEM grid (Cu 200 mesh covered with a collodion membrane, Nisshin EM, Tokyo, Japan) for 1 min and dried with filter paper. MilliQ water (20 μL) was then placed on the grid and immediately absorbed with filter paper. This process was repeated three times to remove salts from the sample. In the sample of peptide assembled structures, 20 μL of 2% phosphotungstic acid solution was placed on the grid for 1 min and the MilliQ water washing process was repeated three times. All samples were dried *in vacuo* prior to TEM measurements, which were conducted at an accelerating voltage of 115 kV (JEM-1400, JEOL, Tokyo, Japan).

S10. Confocal microscopy

After gold mineralization for 24 h on plate (Cellview Glass Bottom Dish 4 Compartments No. 627870, Greiner, Stonehouse, UK), the sample solution was removed and the cells were washed three times with 1xHEPES. 200 μL of 1xHEPES was added to cell culture plate, cells were observed using confocal microscopy (LSM-700, ZEISS, Jena, Germany).

S11. Supplemental references

[1] W. C. Chan, P. D. White, Fmoc Solid Phase Peptide Synthesis; Oxford University Press: New York, 2000.

[2] K. Takayama, I. Nakase, H. Michiue, T. Takeuchi, K. Tomizawa, H. Matsui, S. Futaki, *J. Control. Release*, **138**, 128-133 (2009).

Supplementary Figures

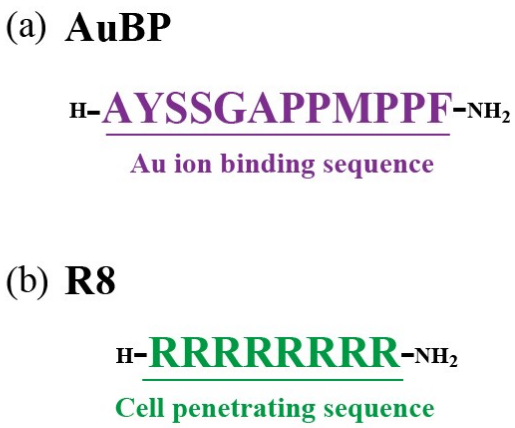

Fig. S1 Sequences of (a) AuBP (Au ion binding peptide without cell penetrating peptide) and (b) R8 (cell penetrating peptide without Au ion binding peptide).

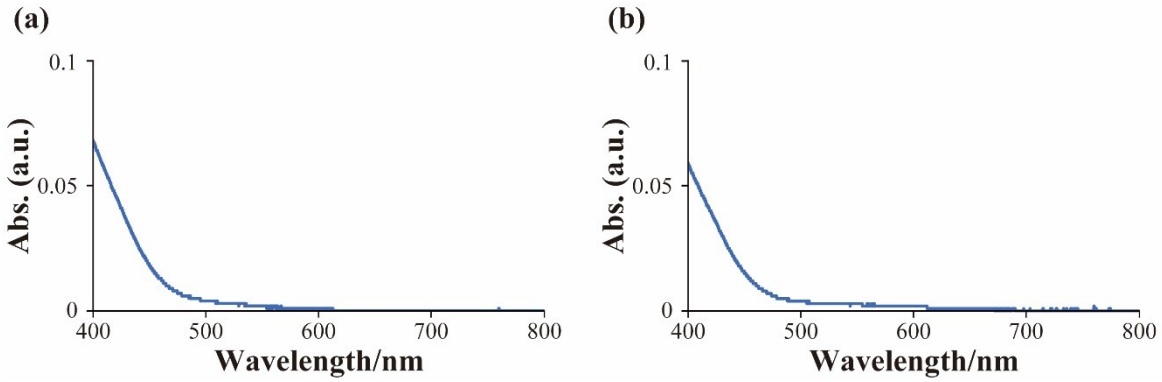

Fig. S2 UV-VIS spectra of the sample after Au mineralization for 24 h using (a) AuBP and (b) R8 in test tubes.

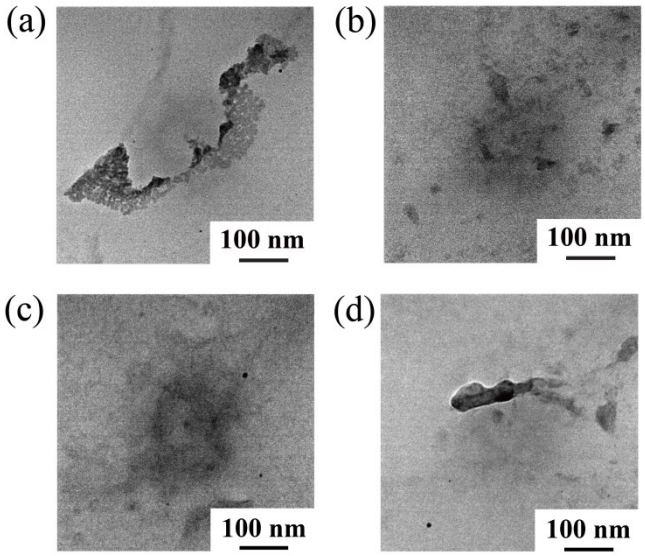

Fig. S3 TEM images of the sample with (a) 1 μM R8-AuBP containing 1 mM $HAuCl_4$ and (b) 1 mM $HAuCl_4$ alone after incubation for 48 h in test tubes. (c) TEM image of the sample with 1 μM R8-AuBP containing 0.5 mM $HAuCl_4$ after incubation for 48 h in a test tube. (d) TEM image of the sample with 10 μM R8-AuBP containing 0.5 mM $HAuCl_4$ after incubation for 48 h in a test tube. All TEM samples were stained with 2% phosphotungstic acid.

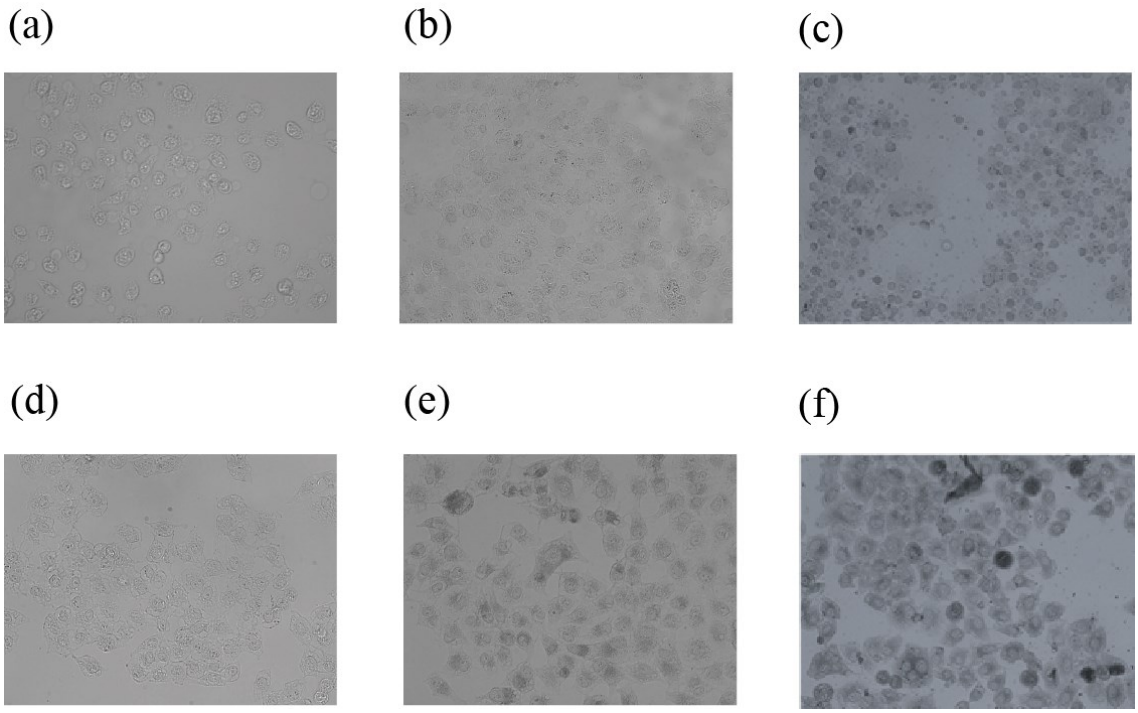

Fig. S4 Phase contrast microscopic images of the cells after Au mineralization for (a) 6 h, (b) 24 h, and (c) 48 h without the peptide. Phase contrast microscopic images of the cells after Au mineralization for (d) 6 h, (e) 24 h, and (f) 48 h with R8-AuBP.

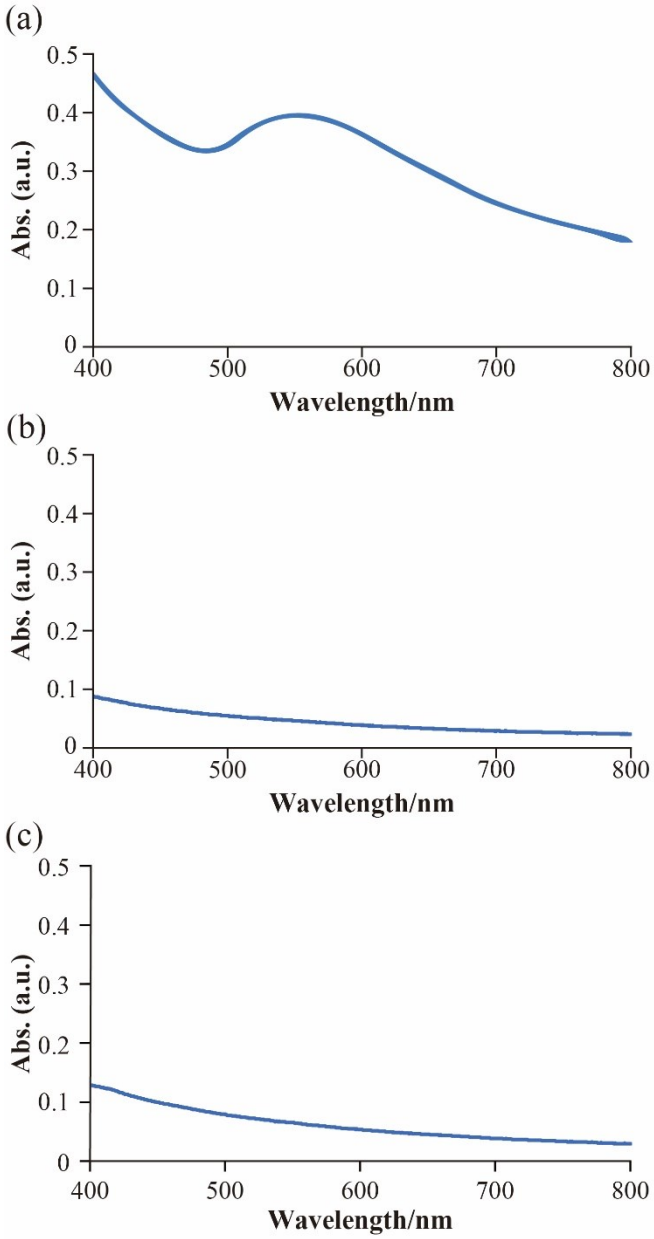

Fig. S5 (a) UV-VIS spectrum of the sample after Au mineralization for 48 h without the peptide. (b) UV-VIS spectrum of lysate solution of the cells after 48 h incubation using R8-AuBP without $HAuCl_4$. (c) UV-VIS spectrum of lysate solution of the cells after 48 h incubation without the peptide and $HAuCl_4$.

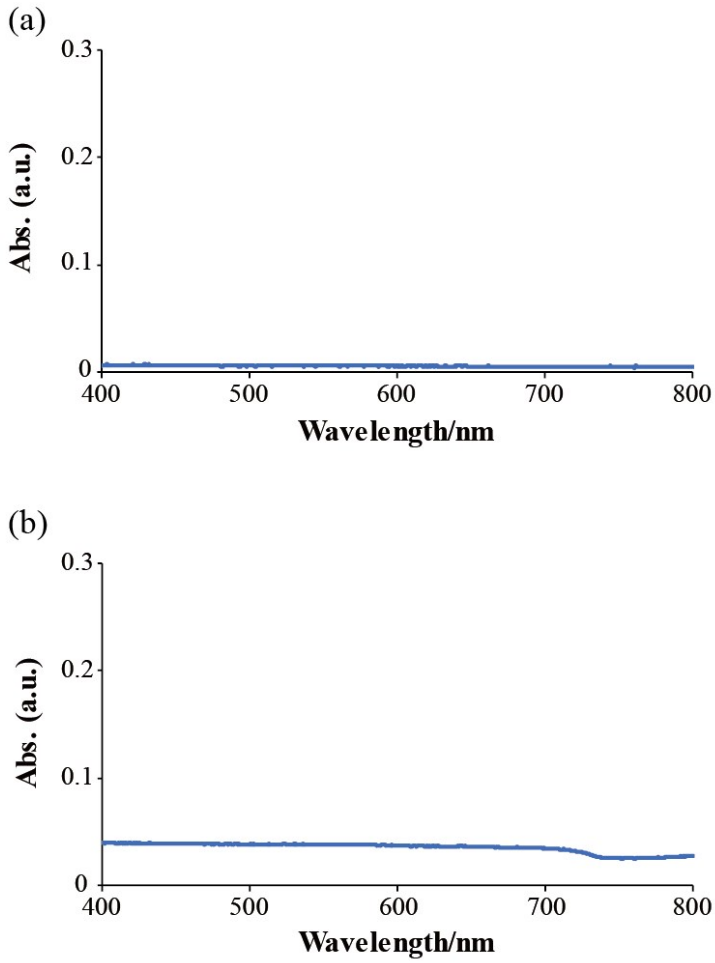

Fig. S6 UV-VIS spectra of washing PBS solution after (a) the first and (b) the second rinse of the incubated cells for the mineralization.

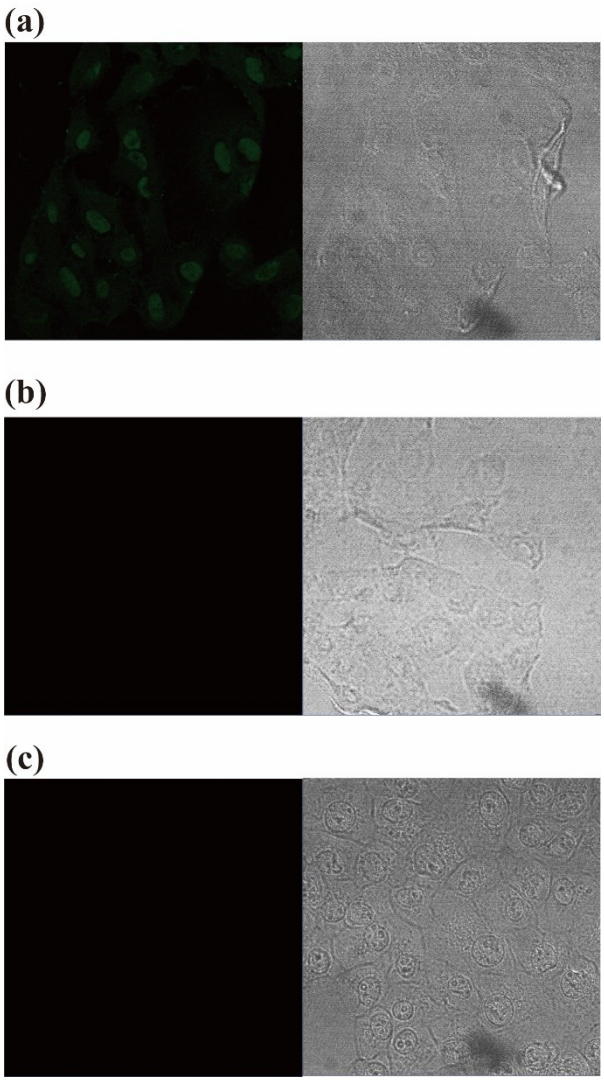

Fig. S7 (a) Confocal microscopic images of the cells after incubation for 24 h using Flu-R8-AuBP without $HAuCl_4$. (b) Confocal microscopic images of the cells after incubation for 24 h without the peptide and $HAuCl_4$. (c) Confocal microscopic images of the sample after incubation for 24 h with $HAuCl_4$ alone.

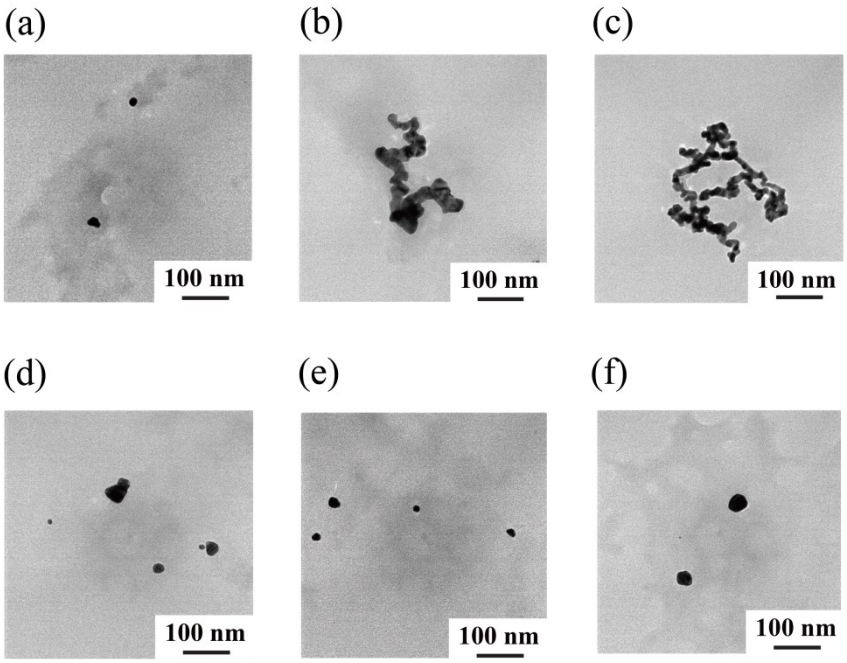

Fig. S8 (a) TEM image of the sample with $HAuCl_4$ alone after Au mineralization for 48 h in cells without the peptide. TEM images of the sample with R8-AuBP after Au mineralization for (b) 24 h and (c) 48 h in cells. (d) TEM image of the sample with R8-AuBP after Au mineralization for 48 h using cytochalasin D. TEM images of the sample with (e) R8 and (f) AuBP after Au mineralization for 48 h in cells.

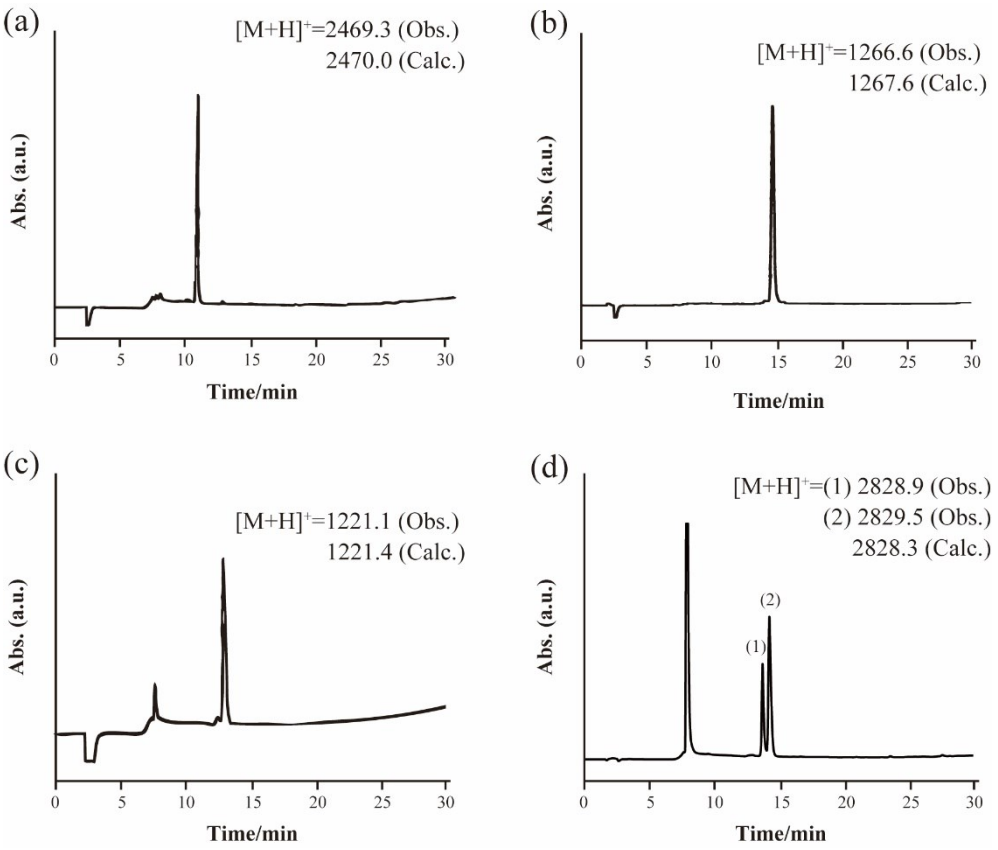

Fig. S9 HPLC charts for purified (a) R8-AuBP, (b) R8, (c) AuBP, and (d) Flu-R8-AuBP separated on an ODS columun (150×4.6 mm, 5 μm) with MilliQ water (containing 0.1% TFA) using a gradient from (a, c, and d) 0% to 100% or (b) 0% to 30% acetonitrile (containing 0.08% TFA) over 30 min, 1.0 mL/min; detection time at 220 nm.