Supplementary Information for

Fluorescent recognition of uranyl ions by a phosphorylated cyclic peptide

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I. General data

ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. The precise metal concentrations were measured by Agilent 725 ICP-AES. Fluorescence emission spectra were obtained using LS55 Fluorescence Spectrometer (Perkin Elmer) at 298 K. UV-Vis spectra were obtained using Hitachi U1900 spectrophotometer at 298 K. The metal standard solutions were obtained from Adrich or Acros. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

II. Experimental procedures and characterizations

Peptide synthesis and characterization

Cyclic peptides A and B were synthesized previously using Fmoc solid-phase peptide synthesis (SPPS).[1] The linear precursor with protected side-chains was assembled by SPPS on 2-Chlorotrityl Chloride. Fmoc-amino acids were coupled with the DMF solution containing HBTU (3.6 equiv.), HOBt (4.0 equiv.), DIEA (8.0 equiv.), and Fmoc-amino acid (4.0 equiv.). The Fmoc group was removed by dual treatment (5 and 10 min) with a solution of piperidine in DMF (20%, v/v). After the assembly of the target linear peptide, the resin was washed with DMF and DCM, and dried at room temperature. The peptide was cleaved from the resin by treatment with CH_2Cl_2/TFA(v/v=99/1). The crude peptide was obtained by cold ether precipitation and centrifugation. The linear peptide was then reacted in CH_2Cl_2 solution containing PyBOP (3 equiv.) and DIEA (4 equiv.). The reaction was completed after 3 hours. The solvent was then evaporated. The residue was precipitated with CH_2Cl_2/Et_2O to obtain the cyclic peptide as a powder. The side-chain protections were removed by treatment with the TFA/TIS/H_2O(v/v/v=95/2.5/2.5) solution. After stirring for 2 hours, the solution was concentrated by a N_2 blowing, then precipitated several times with cold ether. The crude peptides were dissolved in water, identified by ESI-MS and purified by semi-preparative HPLC. Finally, the dried peptides were obtained by lyophilization as white powders.

Cyclic peptide A, Analytical HPLC Purity: 97%, Rt=17.4 min. ES-MS: calculated for $C_{56}H_{64}N_{16}O_{14}$, $[M+H]^+$ = 1186.2, found: $[M+H]^+$ = 1186.0, $[M+2H]^{2+}$=593.6.

Date/Time: 2014-11-7,9:41:37
Data File: E:\2014\GD-10(C)-F(416615) 141107.mdy

Analyst: YW
Date/Time: 2014-11-7,9:41:43

Sample Description:
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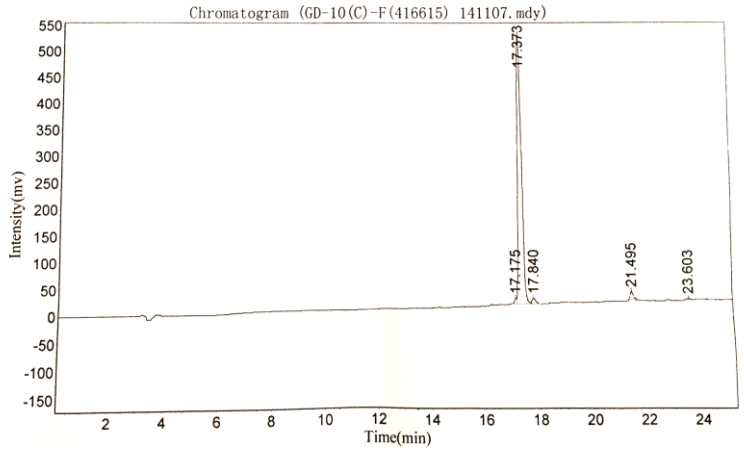
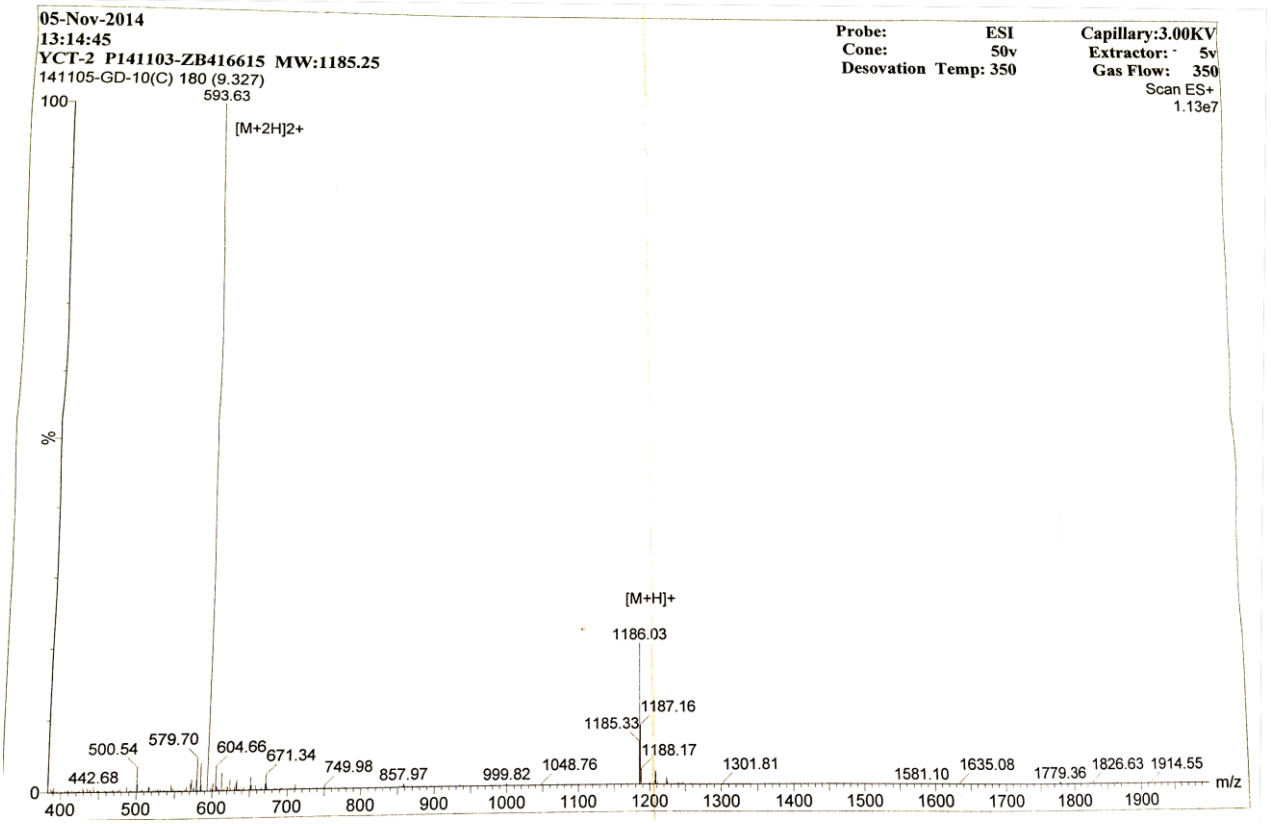
Number : 010250036
Lot No : P141103-ZB416615
Column : 4.6×250mm,Venusil MP C18-5
Solvent A : 0.1% trifluoroacetic in 100% acetonitrile
Solvent B : 0.1% trifluoroacetic in 100% water
Gradient :

	A	B
0.01min	8%	92%
25min	33%	67%
25.1min	100%	0%
30min	STOP	

Flow rate : 1.0 mL/min
Wavelength : 220nm
Volumn : 5ul



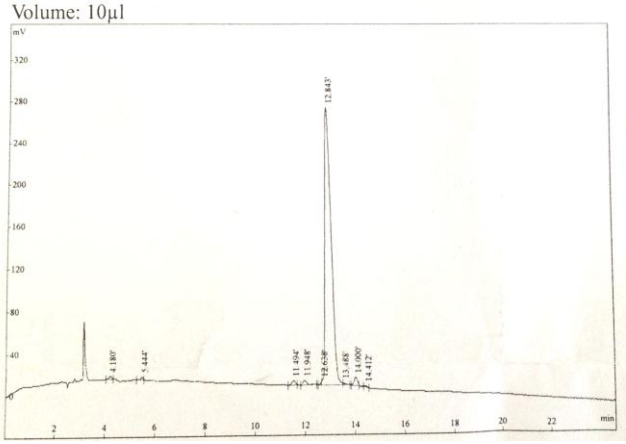


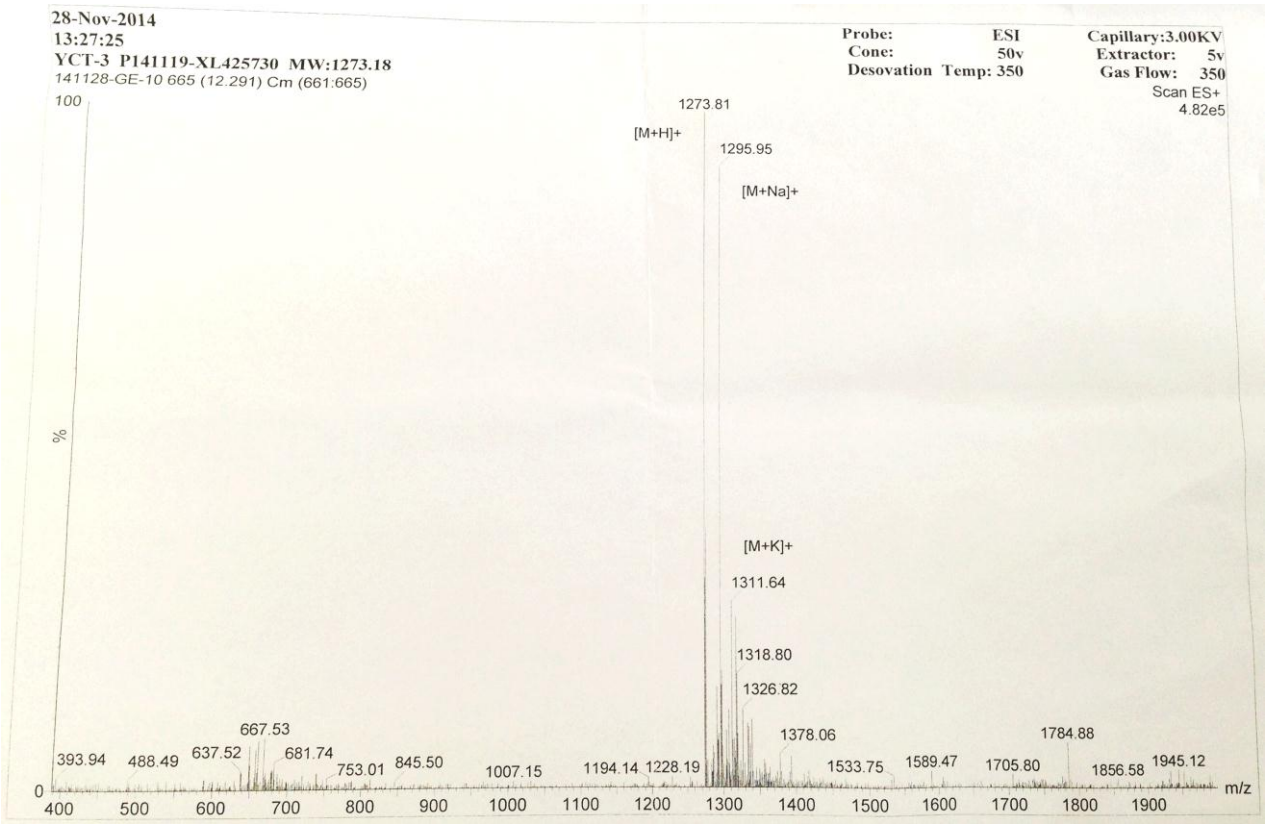
Cyclic peptide B, Analytical HPLC Purity: 97%, Rt=12.8min. ES-MS: calculated for $C_{52}H_{66}N_{12}O_{22}P_2$, $[M+H]^+$ = 1274.1 , found: $[M+H]^+$ = 1273.8, $[M+Na]^+$=1296.0, $[M+K]^+$=1311.6.

Product Name:YCT-3 GE-10
Instrument No: 0200194
Lot No :P141119-XL425730
Column :4.6*250mm, phenomenex C18-5
Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile
Solvent B :0.1%Trifluoroacetic in 100% Water

Gradient :	A	B
0.01min	17%	83%
25in	42%	58%
25.1min	100%	0%
30min	Stop	

Flow rate :1.0ml/min
Wavelength :220nm
Volume: 10μl





Preparation of aqueous solutions

A stock uranyl solution (ca. 0.42 mM) was prepared from uranium standard solution(1000mg U per mL in 1 % HNO_3). The precise uranyl concentration was measured by ICP-AES. Other metal solutions also prepared from standard solution. Peptide solutions (21 μM)were prepared freshly before use. MES buffer was prepared by dissolving solid 2-(N-morpholino)ethanesulfonic acid in H_2O and added NaOH solution (0.1M) to adjust the pH to 6.0.

Fluorescence titrations

The measurements were performed at 298 K after 2 minutes' stirring. Trp fluorescence titrations were performed with 285 nm excitation (excitation slit: 15 nm, emission slit: 10 nm). The volt was adjusted (650~800) to avoid signal saturation. Experiments were repeated to ensure reproducibility.

III. Supplementary Fluorescence and UV Spectra

Figure S1. Fluorescence responses of cyclic peptide A (21 μM in 20 mM MES buffer solution, pH = 6.0) toward UO_2^{2+} (0 to 20 equiv, λexc = 285 nm, slits: 15 nm/10 nm).

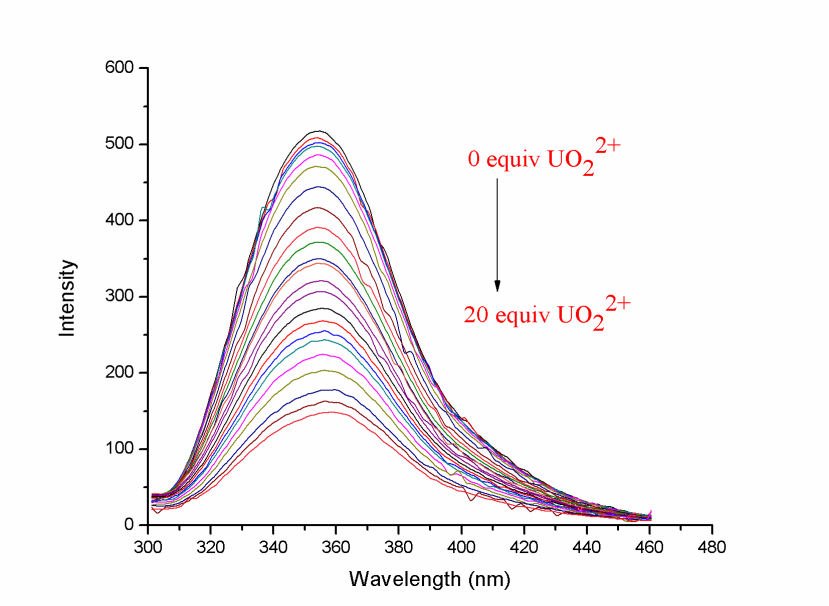
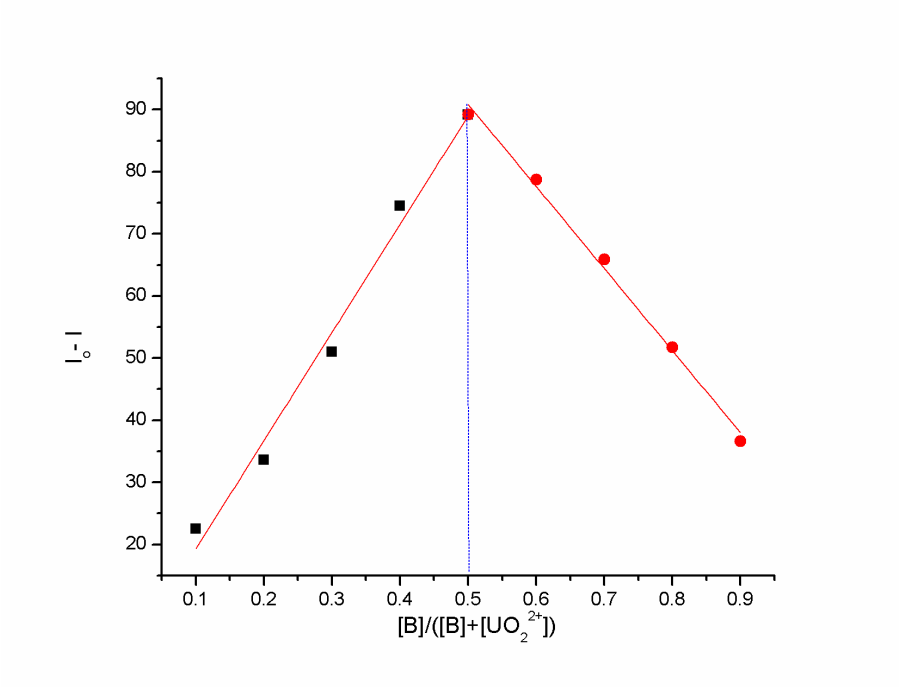


Figure S2. Job's plot of cyclic peptide B with UO_2^{2+} in in 20 mM MES buffer solution(pH = 6.0) representing stoichiometry 1:1.[2]



X = Mole fraction of cyclic peptide B

Figure S3a. The comparison of fluorescence responses of cyclic peptide A and B (21 μM in 20 mM MES buffer solution, pH = 6.0) toward UO_2^{2+}.

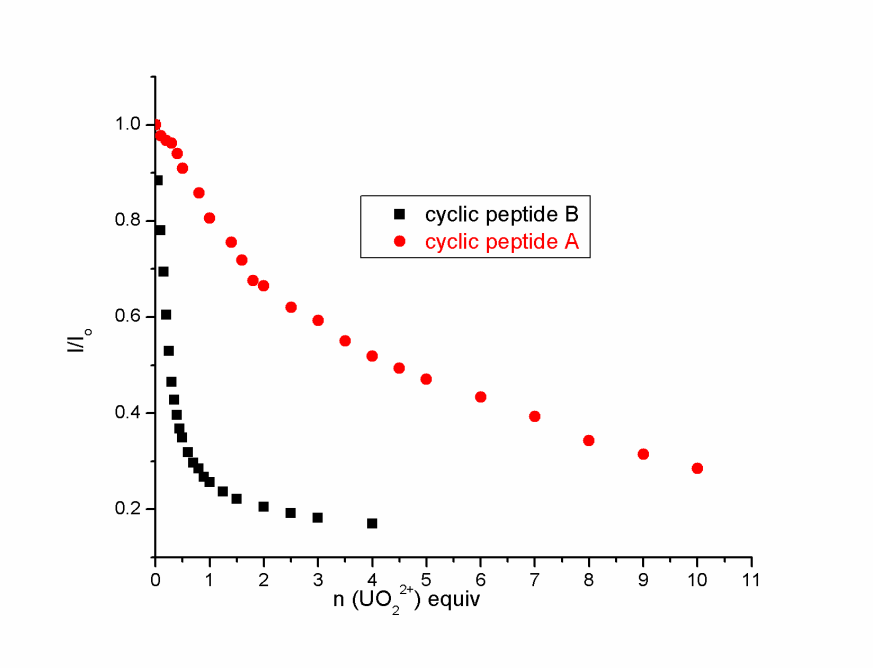
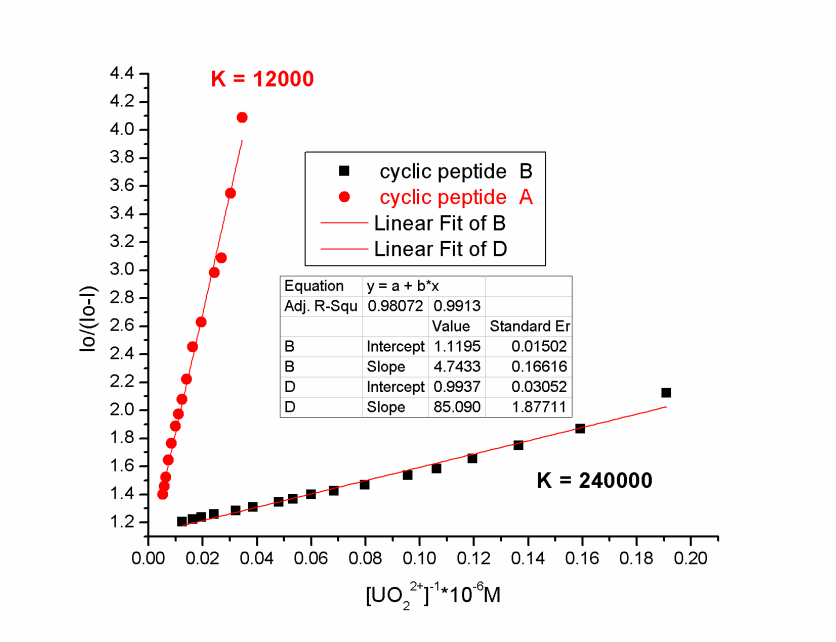


Figure S3b. The linear Benesi-Hilderand expression of cyclic peptide A and B (21 μM in 20 mM MES buffer solution, pH = 6.0) toward UO_2^{2+}.



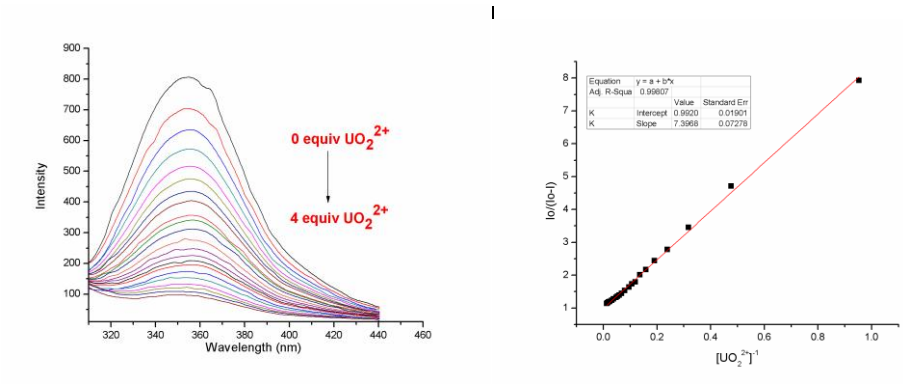
We calculated the binding constant of cyclic peptides with UO_2^{2+} by using the linear Benesi-Hilderand expression[3]:

$$\frac{I_0}{I - I_0} = \frac{b}{a - b}\{\frac{1}{K[M]} + 1\}$$

$$\mathbf{1/K_A = 85.09*10^{-6}/0.9937,\ 1/K_B = 4.74*10^{-6}/1.12}$$

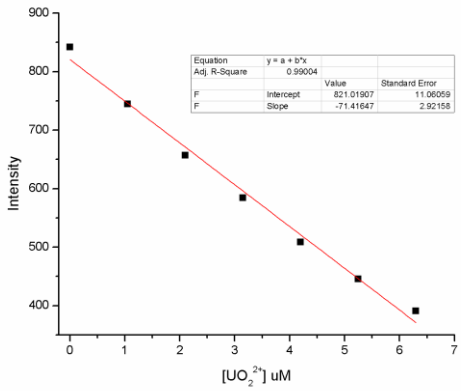
The binding constants of cyclic peptide A and B with UO_2^{2+} are 1.2×10^4, 2.4×10^5 at pH 6.0, respectively.

Figure S4. Fluorescence responses of cyclic peptide B (21 μM in 20 mM MES buffer solution, pH = 4.0) toward UO_2^{2+} (0 to 4 equiv, λexc = 285 nm, slits: 15 nm/10 nm).



The binding constant of cyclic peptide B with UO_2^{2+} is 1.3×10^5 at pH 4.0, respectively.

Figure S5. Fluorescence intensity at 285nm of cyclic peptide B upon addition of UO_2^{2+} (0–7 μM)



The detection limit was calculated with the equation: detection limit = $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity versus sample concentration.[4] The detection limit was measured to be 0.36 μM at S/N = 3 (signal-to-noise ratio of 3:1).

Figure S6. UV spectra of cyclic peptide B (0.1 mM in 50 mM MES buffer solution, pH = 6.0) toward UO_2^{2+} (0 to 10 equiv).

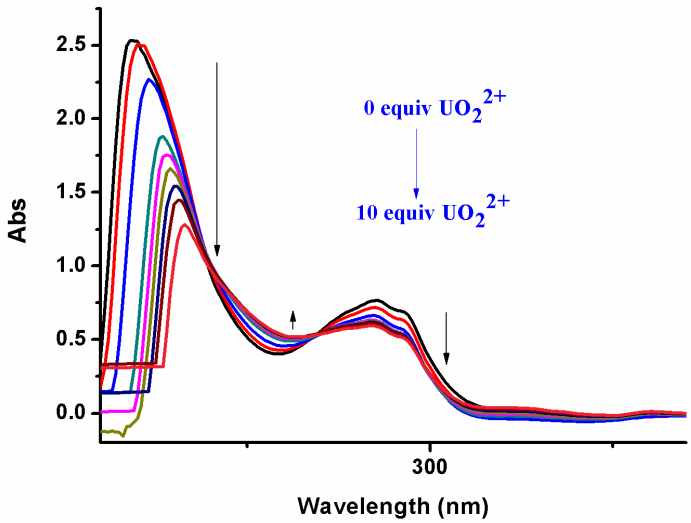
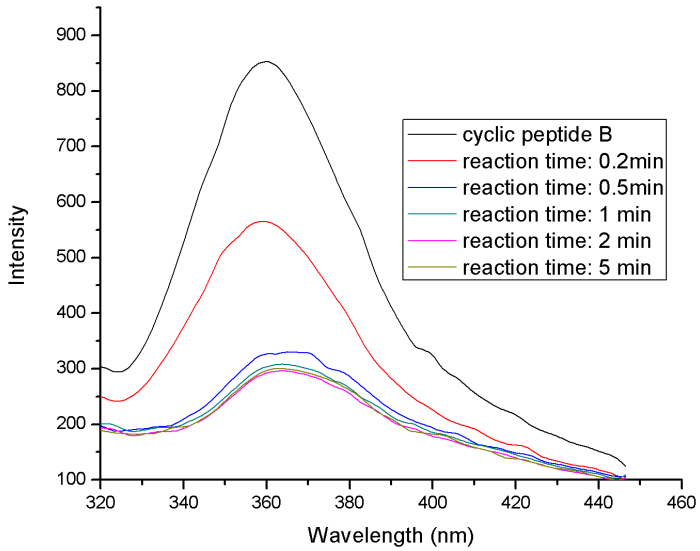


Figure S7. The optimization of reaction time of cyclic peptide B (21 μM in 20 mM MES buffer solution, pH = 6.0) toward UO_2^{2+} (0.5 equiv, λexc = 285 nm, slits: 15 nm/10 nm).



Reaction time	0.2 min	0.5 min	1 min	2 min	5 min
Intensity at 360 nm	564.37	326.84	303.95	292.26	297.02

IV. Supplementary Calculating Data

Computation Details

All calculations were conducted with Gaussian 09 program.[5] Geometry optimization was performed with LC-BLYP method,[6] because this method has been found to be excellent in reproducing the U-X(X=O, N, Cl etc) bond distances of uranyl complexes.[7] The pseudo potential of SDD is used for U, and 6-311G(d,p) basis set was used for all the other atoms.

Cartesian coordinate of the DFT predicted UO^{2+}-cyclic peptide B structure

N	-0.59383400	1.78638400	-1.75849500
C	-1.83228400	1.86966100	-2.47310100
C	-3.04213100	1.59406200	-1.60598800
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C	-1.83821900	0.88084400	-3.62004900
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C	-6.73335900	0.11156200	0.77927300
C	-5.92597300	-0.30978800	1.99208600
O	-6.48482700	-0.69793400	2.98874400
N	-4.58960400	-0.20252900	1.87192800
C	-3.76004400	-0.40335200	3.04157300
C	-2.55514500	0.51263100	3.12268900
O	-2.08826200	0.78407600	4.20557700
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N	-1.98321900	0.93415100	1.97102800
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C	0.28556000	1.72948600	2.53783100
O	1.11175400	2.60931600	2.74819000
C	-1.67959400	3.34252600	2.52365700
N	0.58663200	0.44134700	2.70890200
C	1.86348200	0.04966900	3.21302400
C	2.96542600	0.33917500	2.20601200
O	2.72691900	0.48663400	1.01976000
C	1.83848600	-1.42997500	3.53434200
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C	5.30469100	0.38400800	1.69936300
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O	4.87274900	-1.94464300	1.54537200
C	6.54192200	0.61025100	2.56248100
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C	4.69681500	0.33965500	4.03903400
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C	2.72018300	1.98703600	-2.62334200
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O	1.74007200	-3.05100200	-2.09123600
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C	4.51290400	4.39099900	-0.53827700
C	3.64484700	3.67459700	1.45651300
C	5.49082600	4.22261000	0.46977700
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H	-2.78393400	-4.33655400	-0.21475000
H	-3.14331100	-3.46373000	-1.50289000

V. References

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