Supplementary data

A molecular complex composed of β-cyclodextrin-grafted chitosan and pH-sensitive amphipathic peptide for enhancing cellular cholesterol efflux under acidic pH

Yuki Takechi-Haraya[1,2], *Kento Tanaka*[1], *Kohei Tsuji*[1], *Yasuo Asami*[3], *Hironori Izawa*[2,4], *Akira Shigenaga*[1], *Akira Otaka*[1], *Hiroyuki Saito*[1], *Kohsaku Kawakami*[2,]*

[1]Institute of Health Biosciences, Graduate School of Pharmaceutical Sciences, The University of Tokushima, 1-78-1 Shoumachi, Tokushima 770-8505, Japan

[2]International Center for Materials Nanoarchitectonics, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

[3]TA Instruments Japan, Inc., 5-2-4 Nishi-Gotanda, Shinagawa-ku, Tokyo, 141-0031 Japan

[4]Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, 4-101 Koyama-Minami, Tottori 680-8552, Japan

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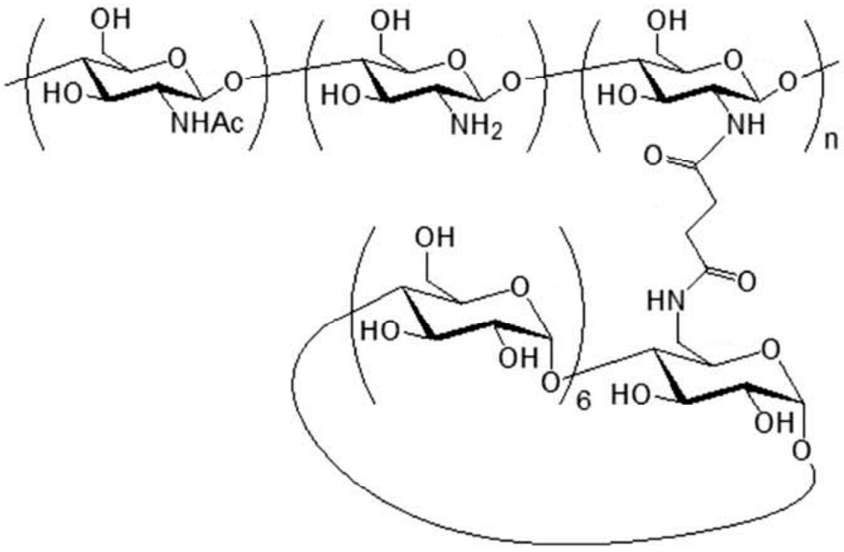


Figure S1. The structure of β-cyclodextrin-grafted chitosan (BCC). The averaged molecular weight was determined from the elemental analysis as 30,800, and β-CyD-content was calculated as 8.0% by NMR, which means β-CyD would be grafted to amino group in chitosan per 10 glucosamine units via a succinic linker.

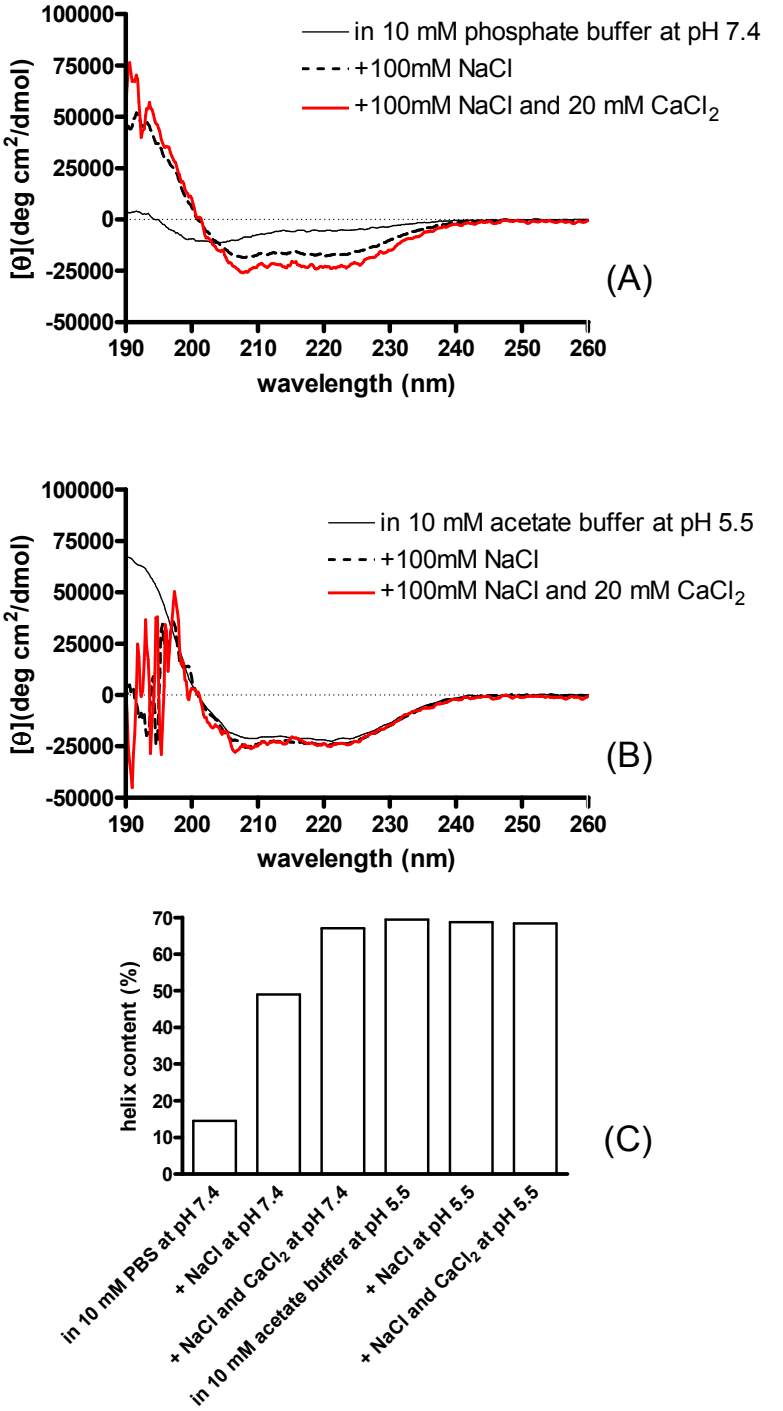


Figure S2. (A) Effects of salt ion on Far-UV CD spectra of CEEP at pH 7.4. (B) Effects of salt ion on Far-UV CD spectra of CEEP at pH 5.5. (C) α-Helix contents of CEEP calculated from $[\theta]_{222}$ at various solvent conditions were shown.

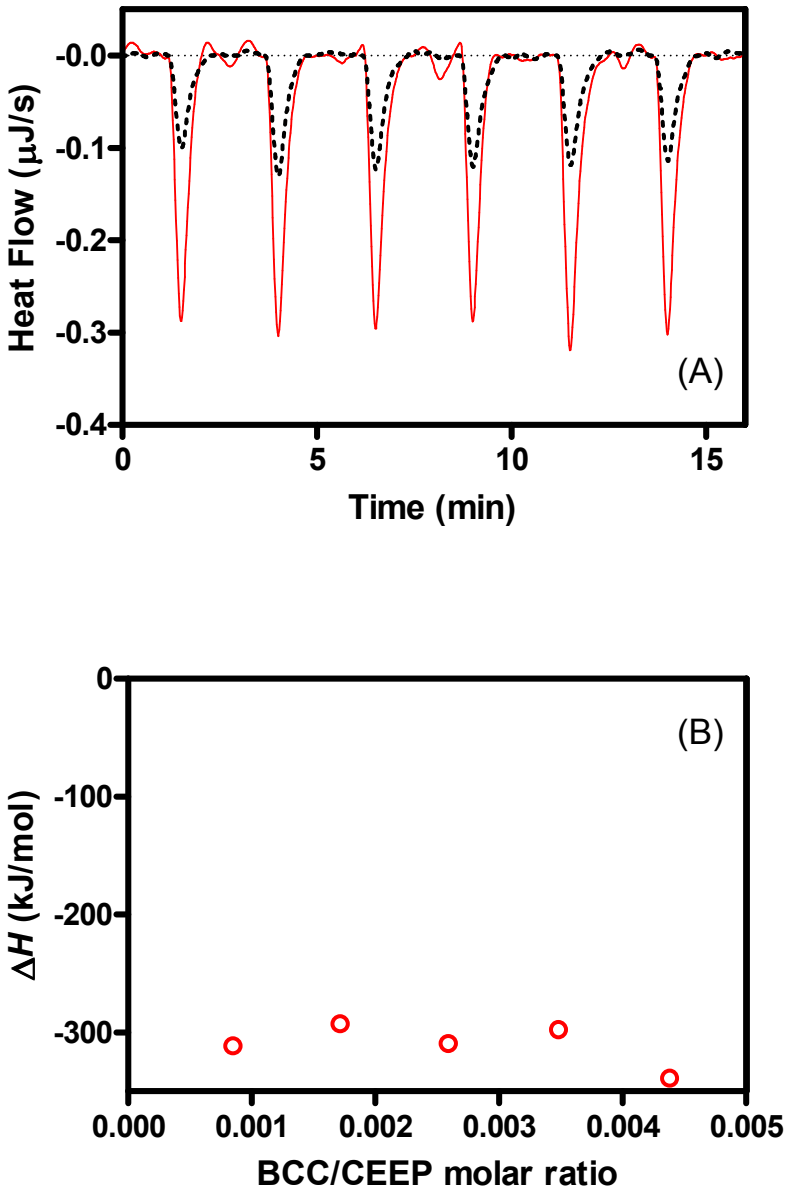


Figure S3. (A) Isothermal titration calorimetry for BCC (6 μM) injected into CEEP (100 μM) at pH 5.5. Each peak in heat flow chart corresponds to the injection of 2.5 μL aliquots of BCC at 25 °C (solid line). The heats of dilution were depicted in control titrations by injecting BCC solution into pure buffer (dotted line). (B) Heats of reaction (integrated from the calorimetric trace) plotted as a function of the BCC/CEEP molar ratio. The heats of dilution were included in the final analysis. The calculated binding enthalpy ΔH° is listed in Table 1.

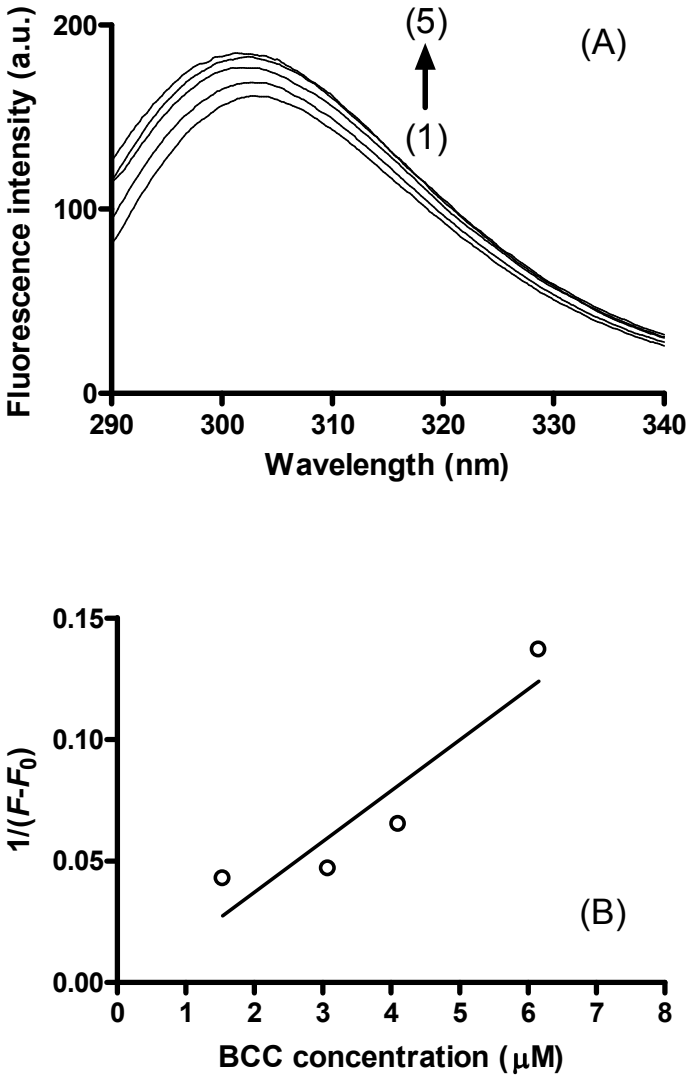


Figure S4**.** (A) Fluorescence spectra of CEEP in the presence of BCC in 50 mM acetate buffer (pH 5.5) at 25 °C. The concentration of CEEP is 1.5×10^{-6} mol/L; BCC: (1) 0. 0, (2) 0.16, (3) 0.24, (4) 0.32 and (5) 0.65×10^{-6} mol/L. (B) Benesi-Hildebrand plot from the result of BCC–CEEP system (Fig. S4A). Binding constant *K* between CEEP and BCC was obtained using Benes-Hildebrand equation: $1/(F - F_0) = 1/Ka[\mathrm{BCC}] + 1/a$, where *F*, F_0 and *a* are the fluorescence of peptide solution in the presence and absence of BCC, and a constant, respectively. The calculated parameters are listed in Table 1. Buffer: 50 mM acetate buffer including 100 mM NaCl at pH 5.5.

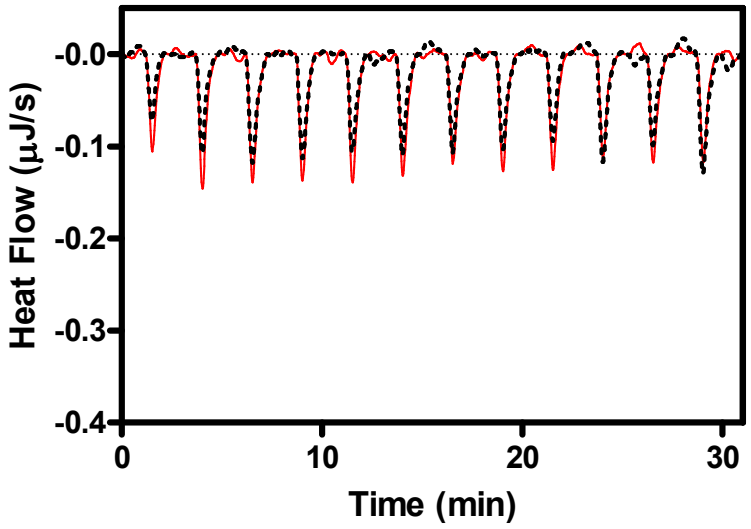
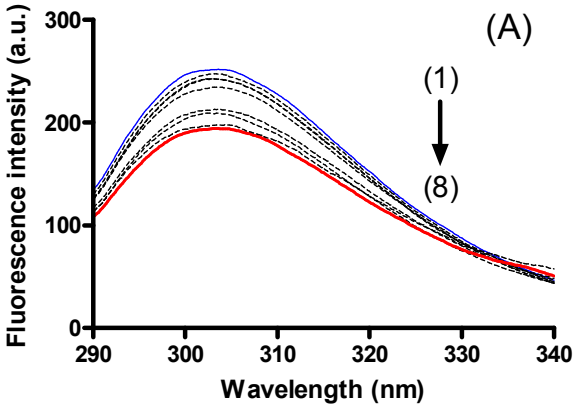
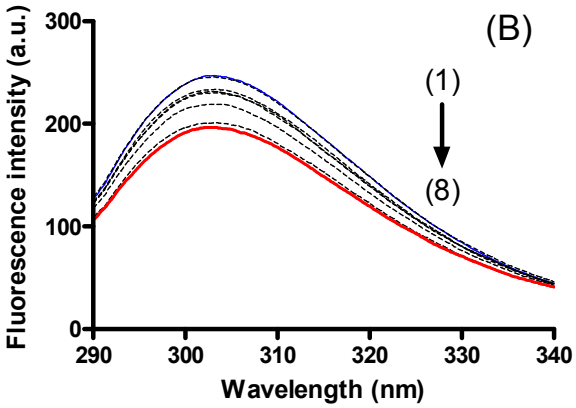


Figure S5. Isothermal titration calorimetry for BCC (6 µM) injected into CEEP (100 µM) at pH 7.4. Each peak in heat flow chart corresponds to the injection of 2.5 µL aliquots of BCC at 25 ℃ (solid line). The heats of dilution were depicted in control titrations by injecting BCC solution into pure buffer (dotted line).





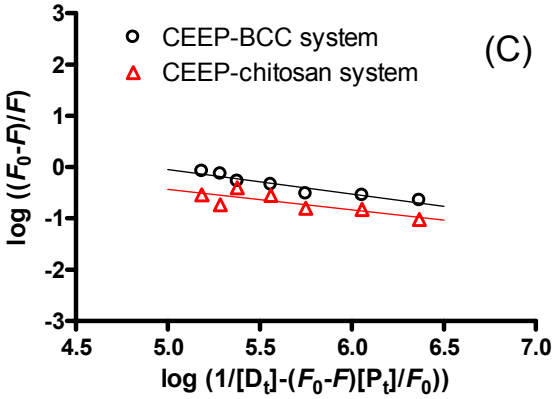


Figure S6**.** Fluorescence spectra of CEEP in the presence of BCC (A) or chitosan (B) in 50 mM phosphate buffer (pH 7.4) at 25 °C. The concentration of CEEP is 1.5×10^{-6} mol/L; BCC or chiosan: (1) 0.0, (2) 0.6, (3) 1.2, (4) 2.5, (5) 4.3, (6) 8.0, (7) 10.4 and (8) 11.7×10^{-6} mol/L. (C) The plots of $\log((F_0 - F)/F)$ vs. $\log(1/([D_t] - (F_0 - F)/F_0))$ from the result of BCC–CEEP (Fig. S6A) or Chitosan-CEEP (Fig. S6B) system. The binding constant K, $\Delta G°$ and stoichiometry of BCC-CEEP system are listed in Table 1. Buffer: 50 mM phosphate buffer including 100 mM NaCl at pH 7.4.

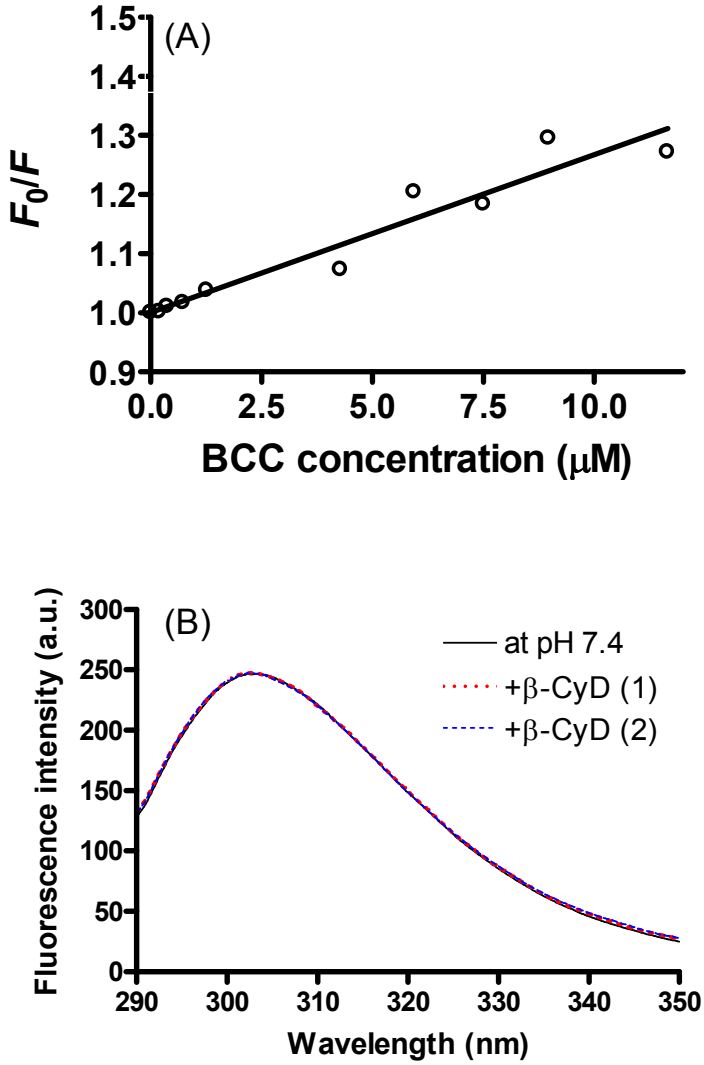


Figure S7. (A) Stern–Volmer plot of the BCC–CEEP system at pH 7.4. Each value of the plot was calculated from the Figure S6A. Stern-Volmer equation: $F_0/F = K[\mathrm{BCC}]+1$, where F and F_0 are the fluorescence of peptide solution in the presence and absence of BCC, respectively. (B) Fluorescence spectra of CEEP in the absence or presence of β-CyD at pH 7.4. The concentration of CEEP was 1.5×10^{-6} mol/L; + β-CyD (1): 40 and (2) 80×10^{-6} mol/L. No change in the fluorescence spectra was observed regardless of the addition of excess β-CyD. Buffer: 50 mM phosphate buffer including 100 mM NaCl at pH 7.4.

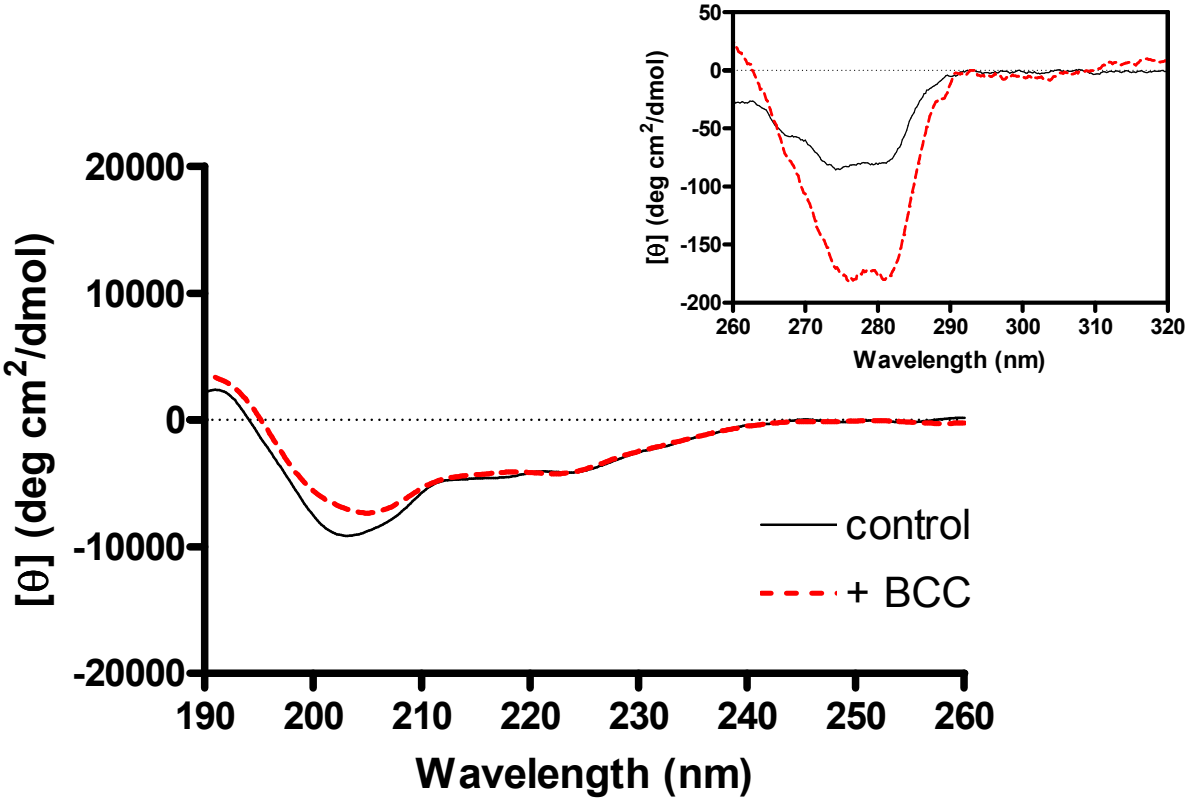


Figure S8. Far-UV CD spectra of CEEP in the presence or absence of BCC at pH 7.4. The concentration of CEEP and BCC were 50 μg/ml and 300 μg/ml, respectively. The inset shows the near-UV CD spectra of CEEP in the presence or absence of BCC at pH 7.4. The concentration of CEEP and BCC were 300 μg/ml and 2 mg/ml, respectively.

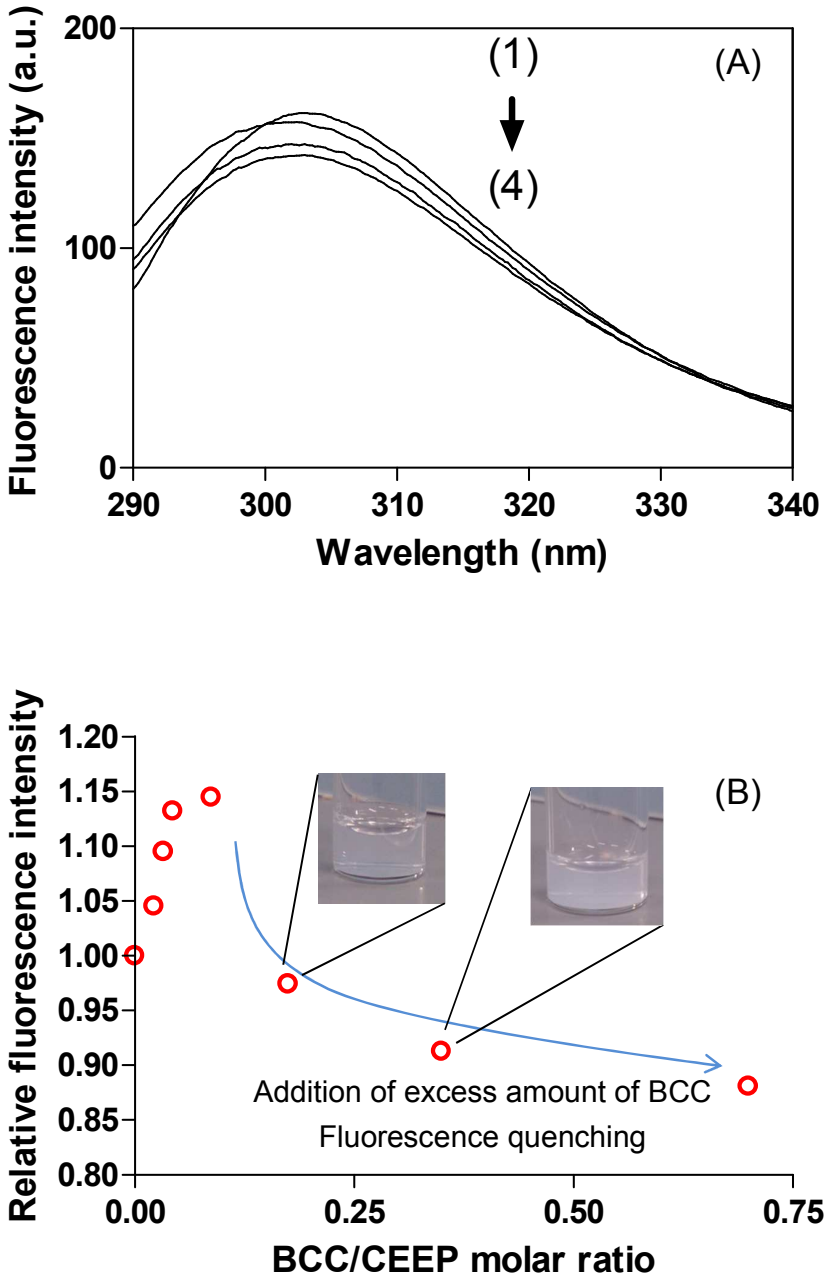


Figure S9. (A) Fluorescence spectra of CEEP in the presence of excess BCC at pH 5.5. The concentration of CEEP is 1.5×10^{-6} mol/L; BCC: (1) 0.0, (2) 1.3, (3) 2.6 and (4) 5.2×10^{-6} mol/L. (B) Relative fluorescence intensity of CEEP as a function of BCC/CEEP molar ratio. The inset shows a photograph of cloudy BCC-CEEP mixture at a BCC/CEEP molar ratio. Note that BCC concentration in photograph was fixed at 32×10^{-6} mol/L and not the sample for the fluorescence measurement. Buffer: 50 mM acetate buffer including 100 mM NaCl at pH 5.5.