Supporting Information

Metal Ions Mediated Structural Evolution of Self-Assembled Peptide-Based Nanoparticles

An-Ping Xu,[a,b] Pei-Pei Yang,[b] Chao Yang,[b] Yu-Juan Gao,[b] Xiao-Xiao Zhao,[b] Qiang Luo,[b] Xiang-Dan Li,[a] Li-Zhong Li,[,a] Lei Wang,[*,b] and Hao Wang,[*,b]*

a. Key Laboratory of Catalysis and Materials Science of the State Ethnic Affairs Commission & Ministry of Education, South-Central University for Nationalities, 182 Minyuan Road , Hongshan District, Wuhan, Hubei Province, China. E-mail: li61071@hotmail.com.

b. CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology (NCNST), No. 11 Beiyitiao, Zhongguancun, Beijing, China. E-mail: wanghao@nanoctr.cn; wanglei@nanoctr.cn.

Experimental Section

1. Materials and Methods

1.1 Materials

Materials: All solvents and reagents for organic synthesis were purchased from commercially available sources and used without further purification. 9-

Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids (Aspartic acid, D; Glycine, G; Arginine, R; Lysine, K; Leucine, L; Valine, V; Phenylalanine, F) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluophosphate (N-HBTU) were obtained from GL Biochem (China). The dimethyl sulfoxide (DMSO; 99.9%, Alfa Asear) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China). 2H-Hexafluoroisopropanol (HFIP) was purchased from Xilong Chemical Co. Ltd. (Guangdong, China). Phosphate-buffered saline (PBS), and fetal bovine serum (FBS) were obtained from HyClone/Thermo fisher (Beijing, China). U87 and MCF-7 cell lines were purchased from Cell Culture Center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Cell counting kit assay (CCK-8) was obtained from Beyotime Institute of Biotechnology (Shanghai, China). Dichloromethane (DCM) and N,N-dimethylformamide (DMF) were distilled over CaH_2 and stored under Ar. Silica gel (200-300 mesh) was used for column chromatography.

1.2 Methods

Mass spectrometry (MS) measurement: Microflex LRF MALDI-TOF was used to determine the molecular weight.

UV-vis spectroscopy: UV-vis absorption spectra were recorded on a Shimadzu 2600 UV/Vis spectrophotometer. Spectroscopic studies of solvents were spectroscopic grade and used as received. The spectra were recorded in quartz glass cuvettes and

according to Lambert-Beer's law the extinction coefficients were calculated.

Fluorescence spectroscopy: A F-280 spectrofluorometer was used for fluorescence spectroscopic studies. These samples (1 mL) were added in a quartz cuvette (1 cm path length) and the fluorescence spectrum was recorded on an F-280 fluorescence spectrophotometer at room temperature. The emission spectra (λ_{ex} = 340 nm) were recorded between 370 and 675 nm.

FT-IR spectroscopy: The FT-IR spectra were detected by the Spectrum One, Perkin Elmer Instruments Co.Ltd.

CD spectroscopy: The CD spectra were recorded by J-810 Circular Dichroism Spectrometer (Jasco, Japan).

Transmission electron microscopy (TEM): The measurements were performed on a Tecnai G2 20 S-TWIN electron microscope operating at an acceleration voltage of 200 kV. For the observation of aggregates, a drop of sample solutions was placed on carbon-coated copper grids. The surface-deposited nanoparticles were negatively stained with 2% uranyl acetate for 40 s before the TEM studies.

Dynamic light scattering (DLS): DLS measurements were performed at room temperature on a Delsa Nano C Particle Analyzer (Beckman Coulter) with 25 mW He-Ne solid state laser (660.0 nm). The above-mentioned samples for TEM were used for DLS measurements. The sizes of samples were calculated by average values of at

least triple measurements. The measurements were performed at 25 °C with a detection angle of 90° and the raw data were subsequently correlated to Z average mean size using a cumulative analysis by the Zetasizer software package.

Scanning electron microscope (SEM): High resolution scanning electron microscopy (SEM) images were acquired on Tecnai G2 F20 U-TWIN under an acceleration voltage of 10.0 kV, a working distance of 5.0 mm.

Cell culture: U87 and MCF-7 cells were cultured in EMEM medium and DMEM medium supplemented with 10% fetal bovine serum and 1% antibiotic solution (penicillin and streptomycin) at 37 °C in the environment containing 5% carbon dioxide.

Confocal laser scanning microscope (CLSM): The metal ions induced **BKR** reconstruction on U87 and MCF-7 cell surfaces were investigated on a Zeiss LSM710 confocal laser scanning microscope (Jena, Germany). The U87 cells and MCF-7 cells were cultured for 12 h in glass bottom dishes. The same concentration of **BKR** (3×10^{-5} M) were incubated with U87 cells (10^5 cells/mL) and MCF-7 cells (10^5 cells/mL) in EMEM and DMEM at 37 °C for 1 h, followed by washing with PBS for 3 times. Then, the cells were cultured in DiI solution (5 μM) for 10 min and washed with PBS for 3 times. Finally, the U87 and MCF-7 cells were cultured with EMEM and DMEM for measurements, respectively. The specimens were examined by confocal laser

scanning microscopy with a × 63 oil immersion objective lens using a 405 nm laser for **BKR** and 547 nm laser for DiI.

Cell cytotoxicity assay: U87 cells and MCF-7 cells were used to evaluate the cytotoxicity of **BKR** nanoaggregates by CCK-8 assay. A density of 7×10^3 cells per well were seeded in the 96-well plates. The cells were cultured overnight at 37 °C in a humidified environment containing 5% CO_2. After 24 h incubation, U87 cells and MCF-7 cells were treated with **BKR** nanoaggregates with concentrations (0.03, 0.3, 3, 30 μM) diluted by medium for 24 h. Then the medium was removed and the cells were washed three times with PBS. 10 μL CCK-8 solutions was added to each well and cultured for 4 h. The UV-vis absorptions of each well were measured using a Microplate reader at a test wavelength of 450 nm and a reference wavelength of 690 nm, respectively. Cell viability (%) was calculated and data were presented as mean ± standard deviation (SD) in triplicate.

2. Synthesis and preparation

2.1 The synthesis of BKR molecule. The BP-COOH was synthesized as our previous report. The **BKR** was synthesized by standard solid phase peptide synthesis techniques using Fmoc-coupling chemistry. Asp-resin (loading: 0.32 mM/g) was used to be phase support. Piperidine (20% v/v) in anhydrous DMF was used as deprotection agents to Fmoc group. The complete deprotection was confirmed using the ninhydrin test (ninhydrin, phenol, VC 1:1:1 v/v). Then the activated amino acids (160 mM) by N-HBTU (160 mM) and Hobt was added into NMM (4%) in anhydrous DMF. For peptide synthesis, the process was repeated. After linkage of K amino acid to the resin, the 1, 8-Diazabicyclo[5,4,0]undec-7-ene (2%) in anhydrous DMF was used for deprotection of Fmoc group, and the BP-COOH (30 mM) activated by N-HBTU was linked to the peptide chain. The final production was obtained through deprotectiion of DDE by 2% $N_2H_4 \cdot H_2O$/DMF and cleavage from the resin by the mixture of TFA (95%, volume percentage), TIS (2.5%, volume percentage) and water (2.5%, volume percentage) for 3 h in ice bath. Then the residual TFA was removed by vacuum rotary system. The product was precipitated with cold ether, centrifuged and dried in vacuum oven. The **BKR** molecule was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

2.2 Preparation of BKR nanoaggreates. The **BKR** was dissolved in HFIP to form high concentration solution (1.5×10^{-3} M). The 20 μL of **BKR** solutions were further diluted

with HFIP (680, 580, 480, 380, 280, 0 μL) and mixed with deionized water (300, 400, 500, 600, 700, 980 μL), respectively.

3. Morphological transformation induced by metal ions

3.1 Morphological transformation induced by metal ions in solution by TEM. The **BKR** was dissolved in HFIP with a concentration of 1.5×10^{-3} M. The **BKR** solution (20 μL) was added into 980 μL deionized water. NaCl, $CaCl_2$, $MgCl_2$ and $FeCl_3$ aqueous solutions were further added into the above-mentioned solution with positive charge concentration of 3×10^{-5} M. The resulting samples (at 1 h and 96 h) were drop-coated onto carbon-coated copper grids. After contacting the droplets with copper grids for 5 min, excess droplets were removed using filter papers. The uranyl acetate was drop-coated onto carbon-coated copper grids for 40 s, excess droplets were removed using filter papers. All samples were dried under vacuum before the TEM studies. For the recovery of **BKR** morphology, the EDTA-2Na (3×10^{-4} M) was added to the Ca^{2+} cation induced **BKR** fiber solution. The TEM samples were prepared after 48 h incubation.

3.2 Confocal laser scanning microscopy (CLSM) of BKR aggregates on calcium alginate beads. For calcium alginate beads formation, 50 mL of a 2-2.5% w/v aqueous solution of sodium alginate was introduced dropwise from a glass syringe with a size-22 needle into 100 mL of an aqueous calcium chloride solution being stirred at 400 rpm. The concentration of $CaCl_2$ in the solution ranged from 1% w/v to

3% w/v. The stirring was continued for one hour and the calcium alginate beads were washed with pure water for three times. Then the calcium alginate beads were transferred into glass bottom dishes and incubated with the **BKR** samples (3×10^{-5} M) for 5 h. Last, the calcium alginate beads were washed again with pure water for 3 times.

3.3 Morphological transformation on cell surfaces by SEM. The morphologies of **BKR** interacting with U87 and MCF-7 cells were directly examined using SEM. The SEM samples were prepared by performing immersed **BKR** solution (3.0×10^{-5} M) interacting cells solutions (1 mL) onto silicon wafer. After contacting the solution with silicon wafer for different times (1 and 3 h), an excess amount of solution were removed by filter papers. Subsequently, the cells were solidified with glutaraldehyde (4%) overnight and then coated by gold before scanning for 2 min.

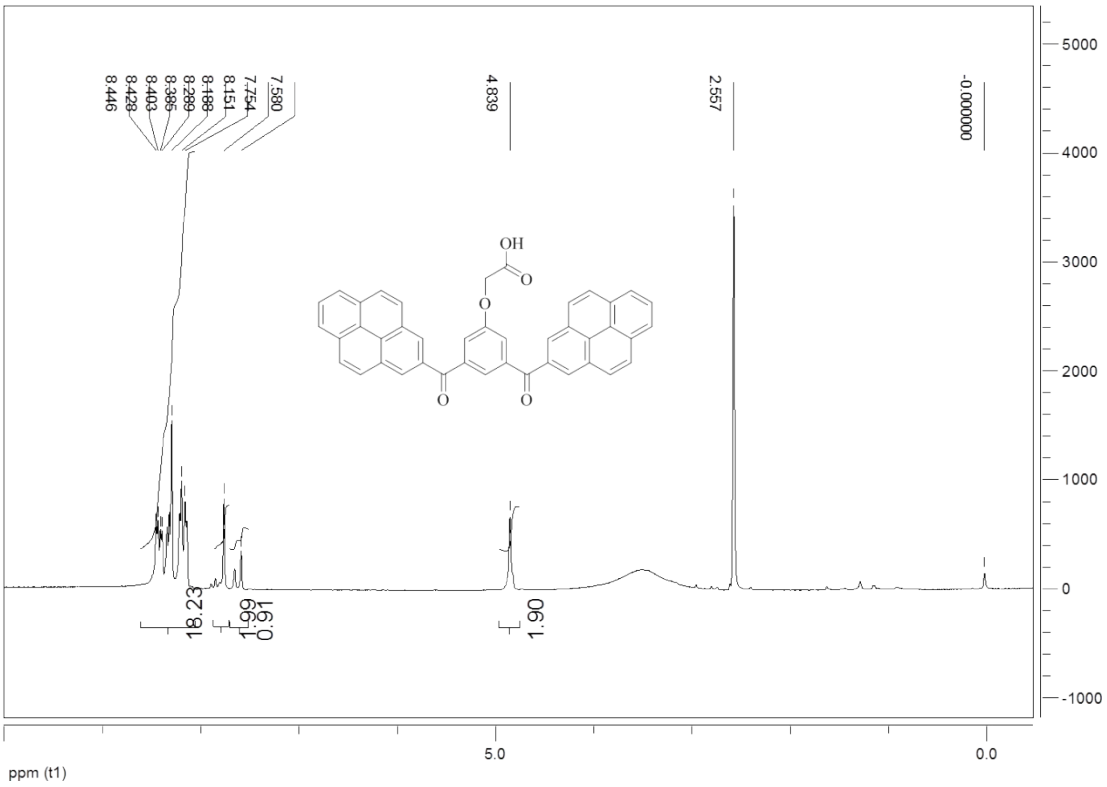

Figure S1. The ^{1}H NMR spectrum of BP-COOH.

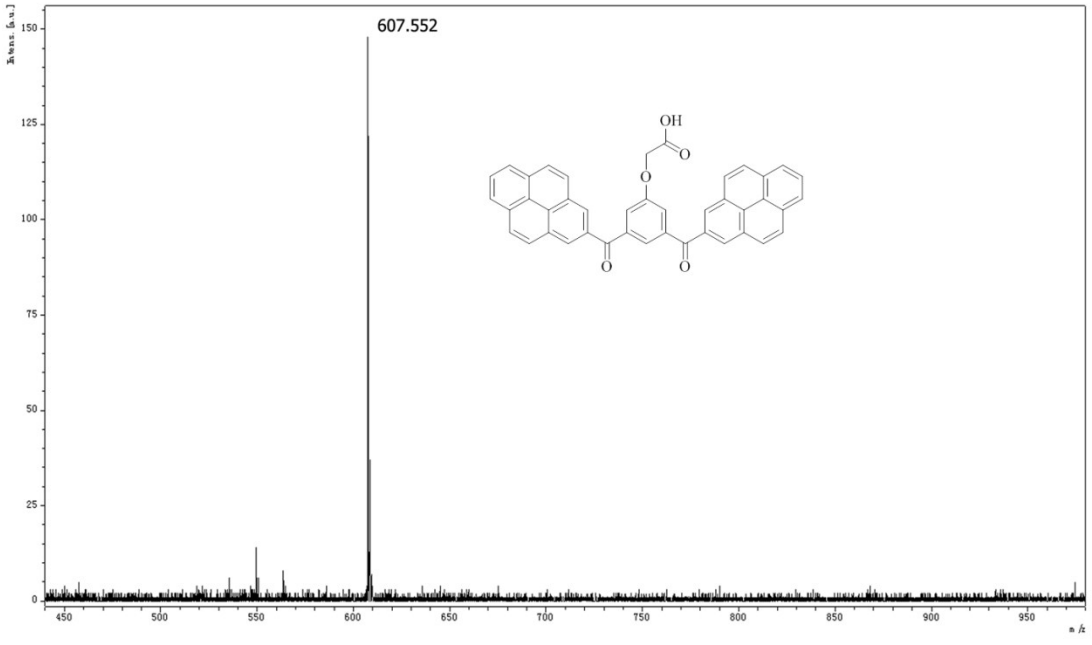

Figure S2. The MALDI-TOF-MS spectrum of BP-COOH.

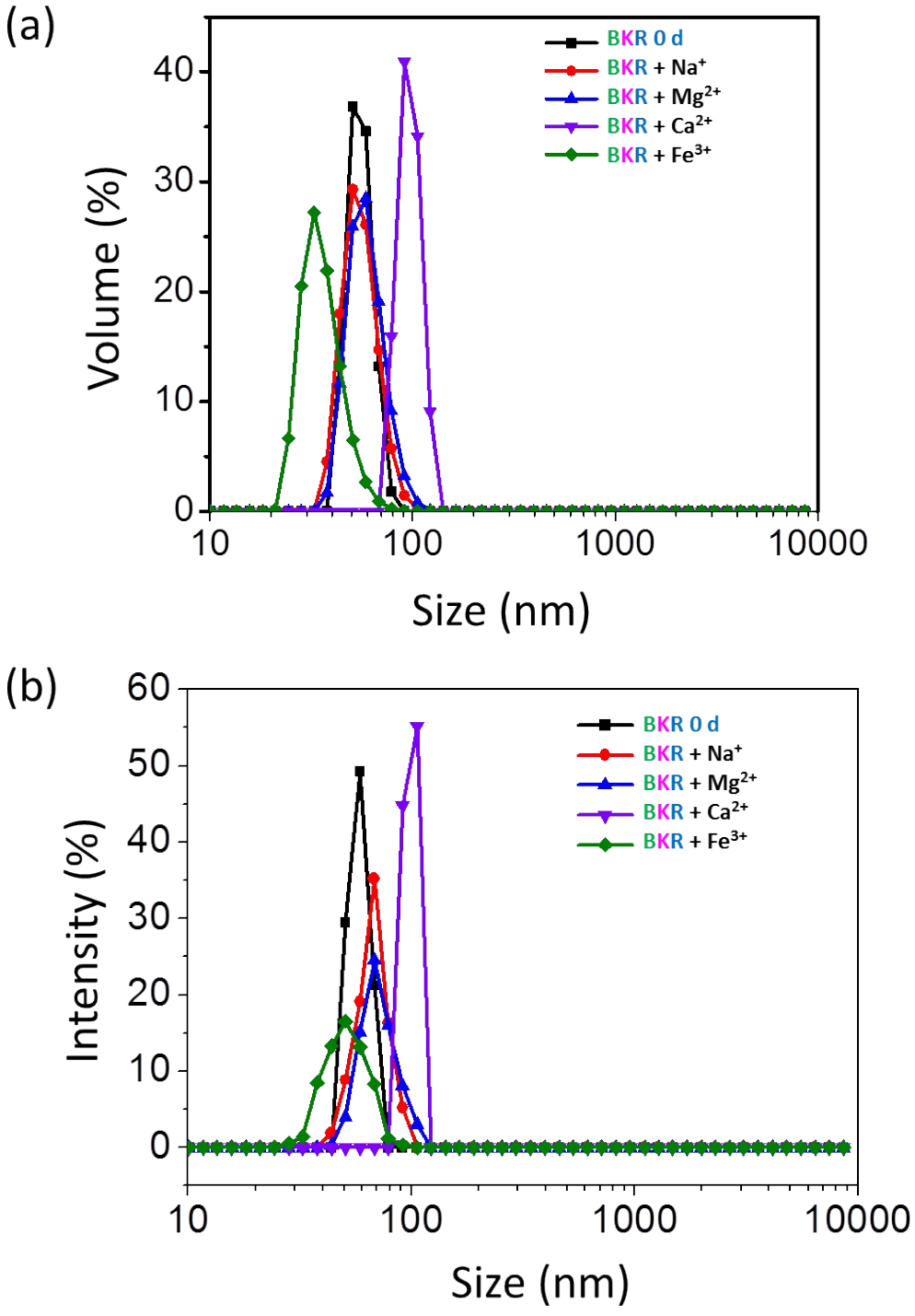

Figure S3. The Dynamic Light Scattering (DLS) characterization of **BKR** in metal ions solution respectively at 0 d. (a) volume vs. size and (b) Intensity vs. size.

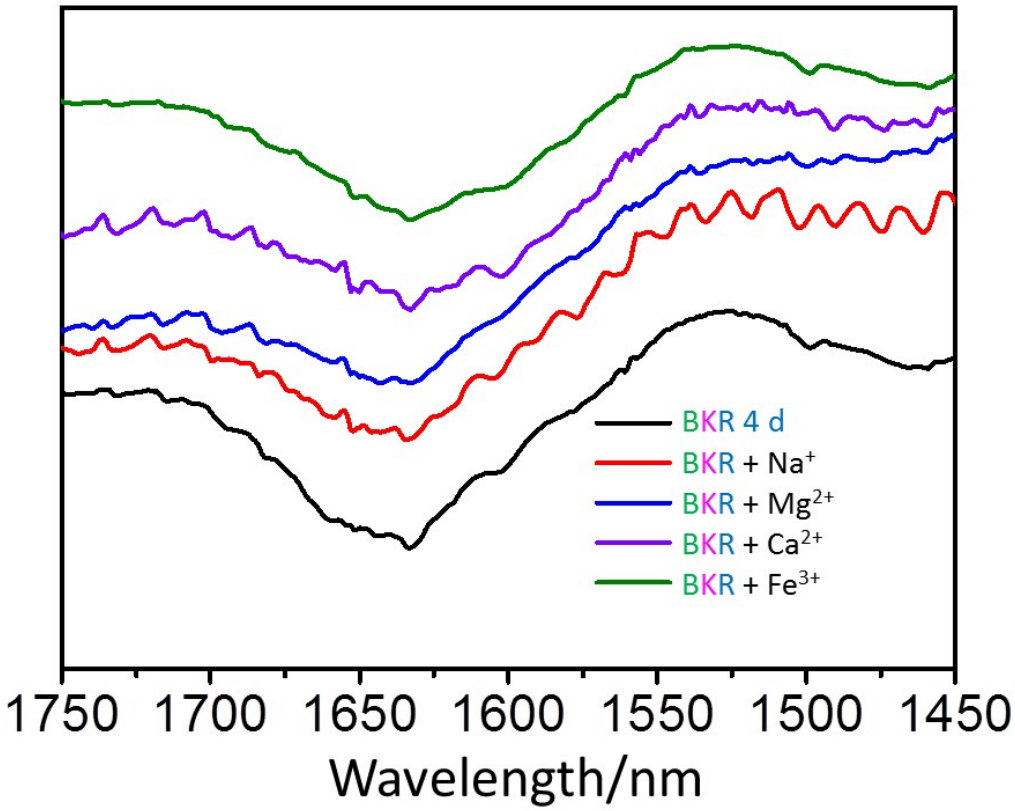

Figure S4. FT-IR spectra of **BKR** in water/HFIP (98/2) and followed by the addition of metal ions after 4 d.

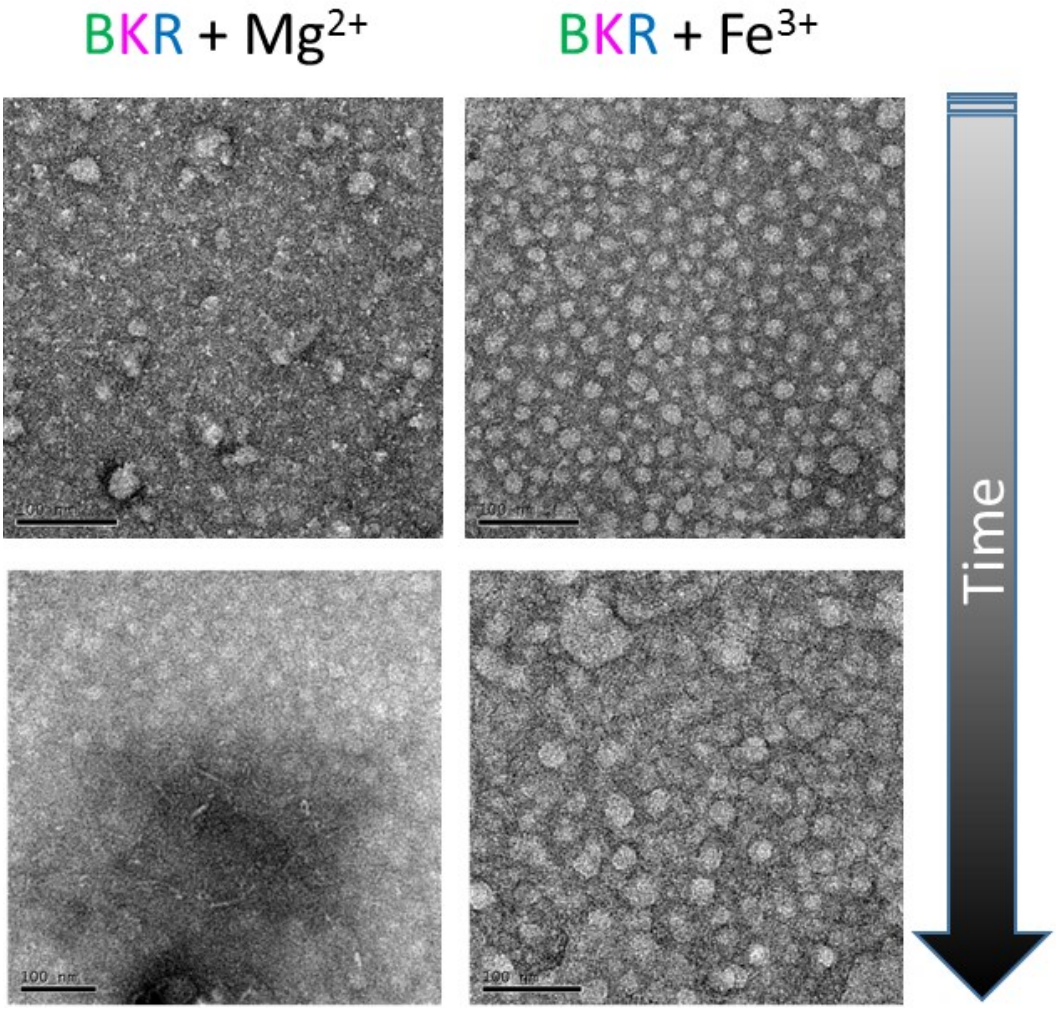

Figure S5. Morphology observation of **BKR** nanoaggregates. TEM images of morphology evolution upon the addition of different Mg^{2+}, Fe^{3+} ions from 0 to 4 d.

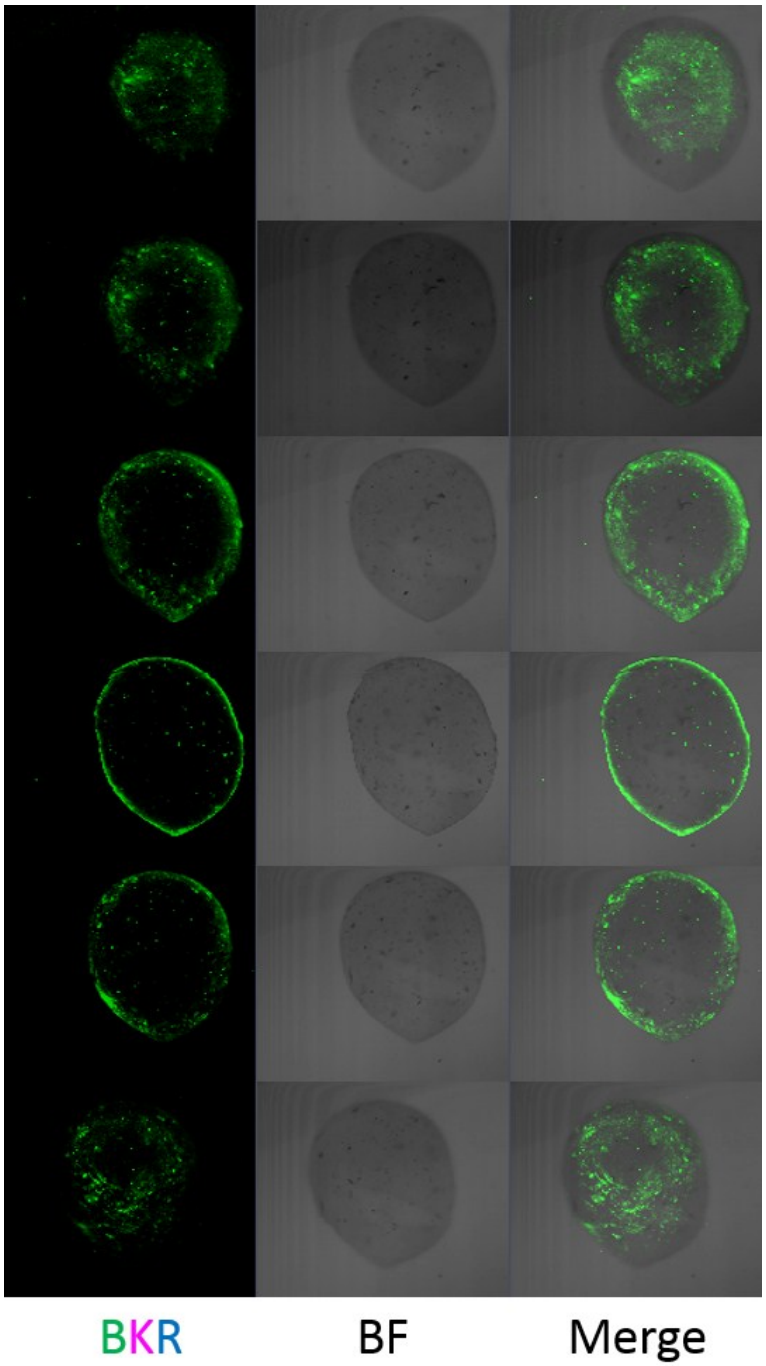

Figure S6. Application in phantom confocal microscope of layer scanning of the surface assembly of **BKR** on phantom at other layers.

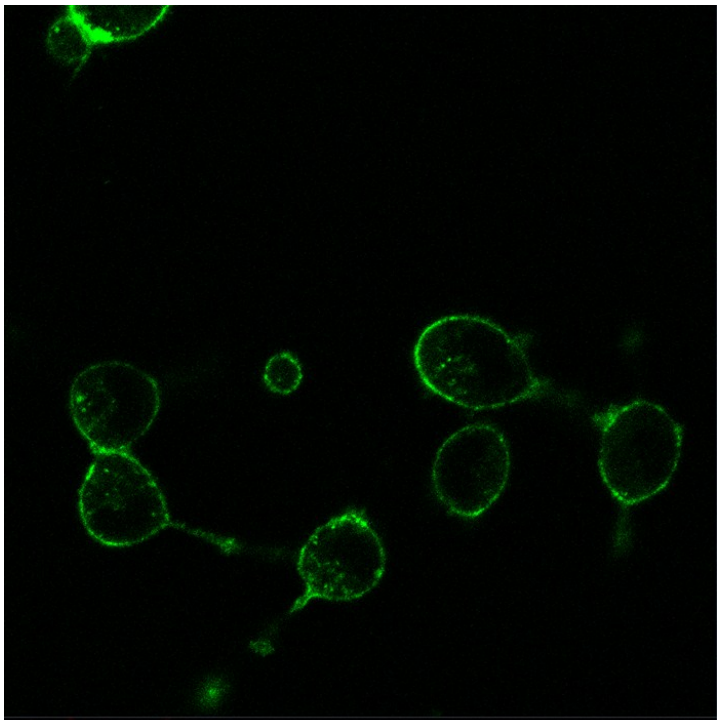

Figure S7. CLSM image of U87 cells in green channel, which were pre-treated and EDTA-2Na for 15 min, followed by incubation with **BKR** nanoparticles for 2 h and DiI track for 10 min. The green channel CLSM images clearly showed some BKR nanoparticles inside the EDTA-2Na pre-treated U87 cells in 2 h.

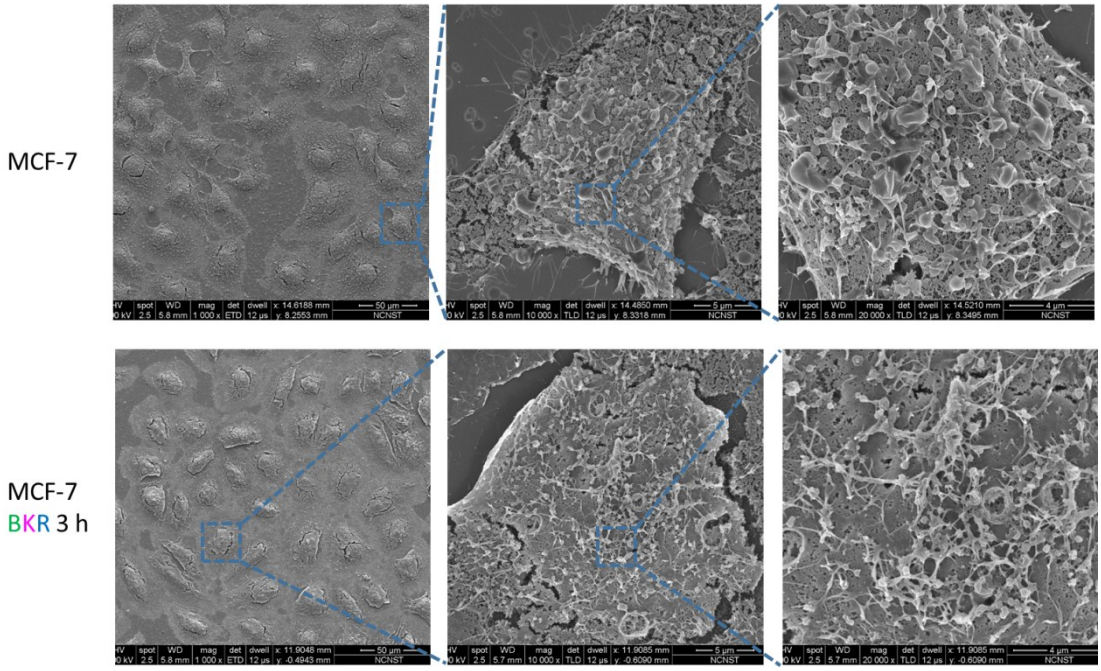

Figure S8. SEM images show the morphology of control group MCF-7 and interactions between **BKR** and MCF-7 cells membrane after 3 h.

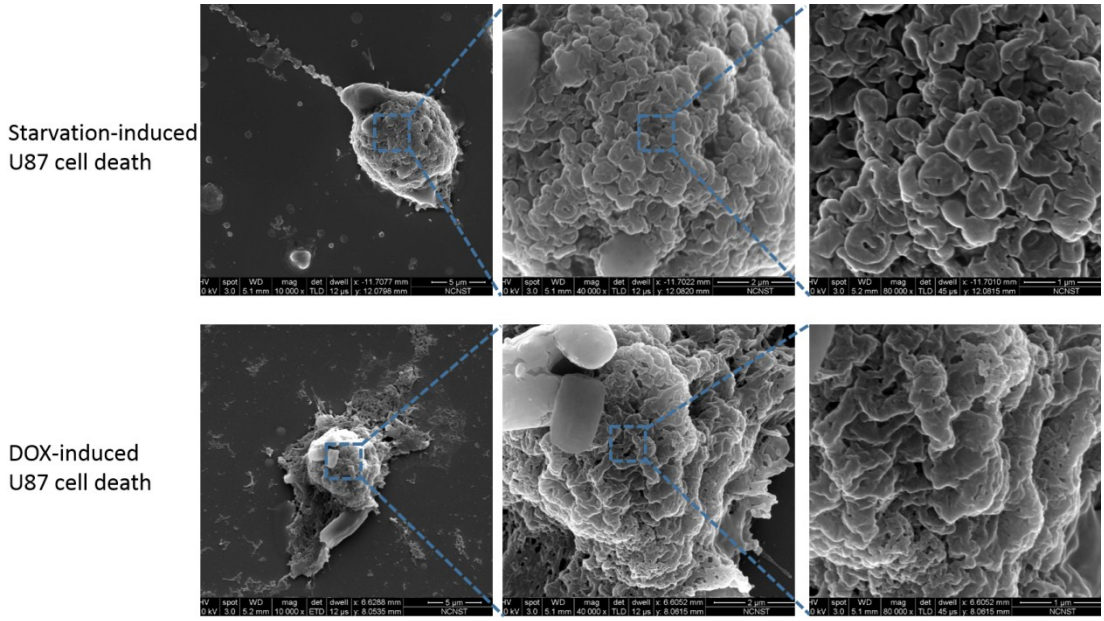

Figure S9. The SEM of cells membrane morphology of starving the living cancer cells U87 to death and DOX killing U87 cells membrane.