Supporting Information for Inorganic Chemistry

Selective Ion-Exchange Governed by the Irving-Williams Series in $K_2Zn_3[Fe(CN)_6]_2$ Nanoparticles: Toward a Designer Prodrug for Wilson's Disease

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Experimental procedure

Synthesis of the PVP-coated $K_2Zn_3[Fe(CN)_6]_2$ nanoparticles: A solution of 1.0 mM $ZnSO_4$ (75.0 mL) containing 800 mg of PVP (average MW = 40,000) was added slowly to a solution of 1.0 mM $K_4[Fe(CN)_6]$ (50.0 mL) at room temperature to give a clear white to pale-yellow solution. After stirring for 30 minutes, the solution was transferred into a dialysis bag made of regenerated cellulose tubular membrane (MWCO is 3500) and dialyzed against distilled water for two days. The solid product was collected by lyophilization.

Synthesis of the bulk $K_2Zn_3[Fe(CN)_6]_2$ materials: Bulk $K_2Zn_3[Fe(CN)_6]_2$ materials were prepared using solutions of $K_4[Fe(CN)_6]$ and $ZnSO_4$ in the absence of a coating agent. Specifically, 75 mL of aqueous solution of $ZnSO_4$ (1.0 mM) were added to 50 mL of aqueous solution of 50 mL $K_4[Fe(CN)_6]$ (1.0 mM) under vigorous stirring at room temperature. This reaction resulted in a white precipitate in an hour. After stirring for three hours at room temperature, the product was purified by dialysis against distilled water over 48 hours and the product was collected by lyophilization.

Powder X-ray diffraction analyses: The X-ray diffraction (XRD) measurements were recorded for the bulk and nanoparticle samples using a PANanalytical, Inc. X'Pert Pro (MPD) Multi-Purpose Diffractometer with Cu Kα radiation (1.5406 A) at an operating voltage of 45 kV.

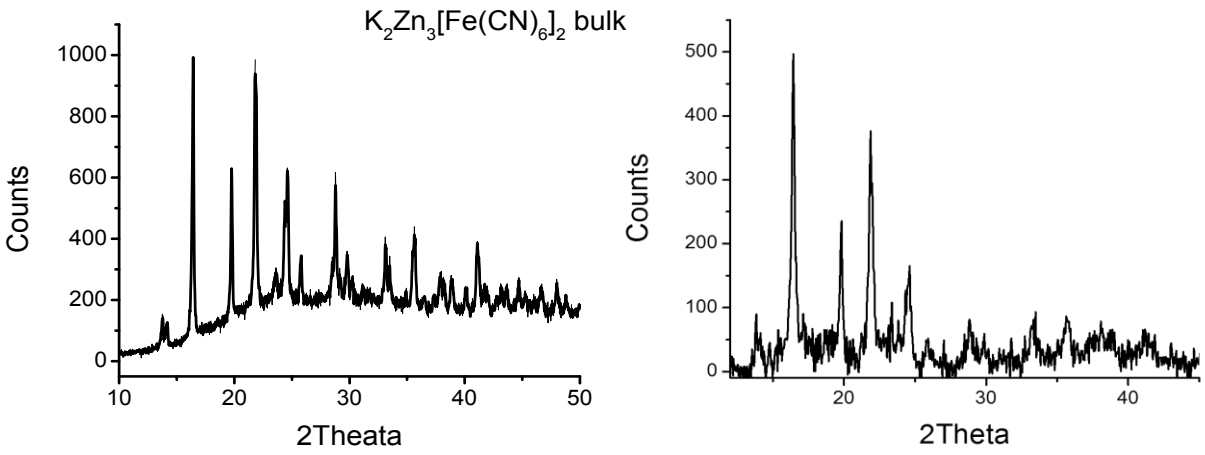

Fig S1 XRD patterns for the bulk $K_2Zn_3[Fe(CN)_6]_2$ (left), and the PVP-coated $K_2Zn_3[Fe(CN)_6]_2$ nanoparticles

Quantitative elemental analysis of $K_2Zn_3[Fe(CN)_6]_2$ nanoparticles: The as-synthesized samples of $K_2Zn_3[Fe(CN)_6]_2$ nanoparticles were first purified by dialyzing against distilled water for two days. The purified samples were then transferred to a crucible and evaporated to dryness. They were heated in an oven at 620 ^oC for 6 hrs to decompose the compound into oxides. The residues obtained were dissolved in the concentrated HNO_3 solution. The solutions were diluted and analyzed by atomic absorption spectroscopy. The metal analysis of nanoparticles gave the molar ratio of K:Zn:Fe to be 0.059/0.074/0.055 while the elemental analysis on C, H and N using the bulk sample showed C%=17.67%, N%=21.41% and H%=0.98%, indicating that the composition of the compound is very close to $K_2Zn_3[Fe^{II}(CN)_6]_2 \cdot 3.7H_2O$.

TEM imaging and EDX measurements: Nanoparticles were first suspended in water by sonication, and then the droplets of the suspension were placed onto the carbon-coated copper TEM grid (400-mesh). The specimens were allowed to air-dry and analyzed at 200 KV using a FEI Tecnai F20 field emission transmission electron microscope (TEM) equipped with an integrated scanning TEM (STEM) unit. The energy dispersive X-ray spectroscopy (EDS) results were obtained with an EDS spectrometer in STEM mode. The spatial resolution is <1 nm through the acquisition of high resolution high-angle angular dark field (HAADF) images, in which the contrast is sensitive to atomic number (Z).

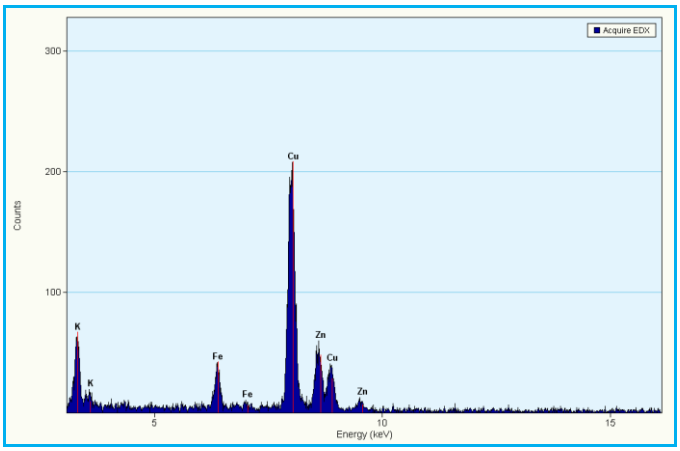

Fig S2 EDX spectrum of a representative $K_2Zn_3[Fe(CN)_6]_2$ nanoparticle

Thermogravimetric analysis: Thermal analysis was conducted on both the bulk and the PVP-coated NP sample using a TA instruments 2950 high-resolution thermogravimetric analyzer (Universal V3.9A) in air from room temperature to 600 °C with a heating rate of 10 °C/min.

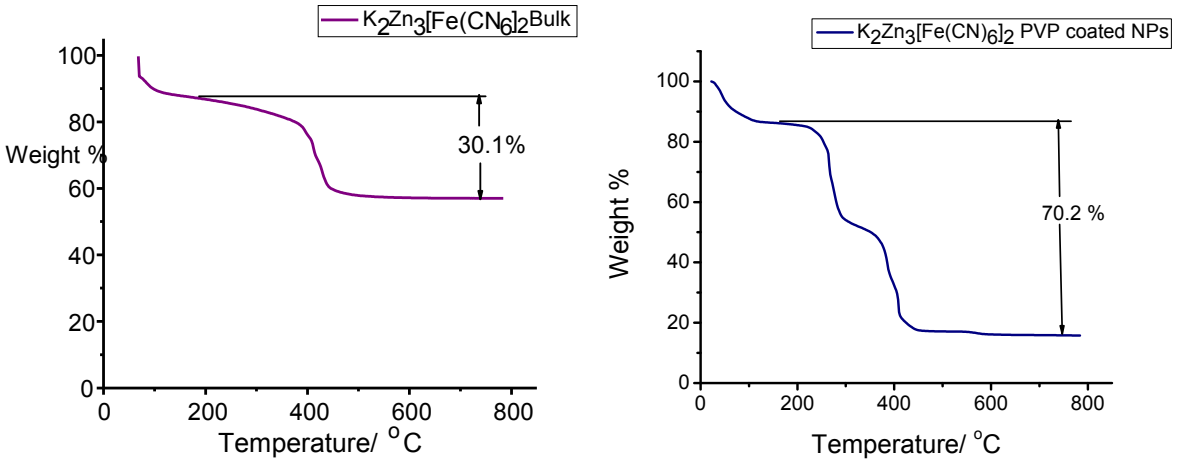

Fig S3 The TGA curves of the bulk $K_2Zn_3[Fe(CN)_6]_2$ sample (right), and the PVP-coated $K_2Zn_3[Fe(CN)_6]_2$ NPs (left)

Fourier transform infrared spectroscopy (FTIR): The FTIR data were obtained on a Bruker Tensor 27 Instrument using KBr matrix.

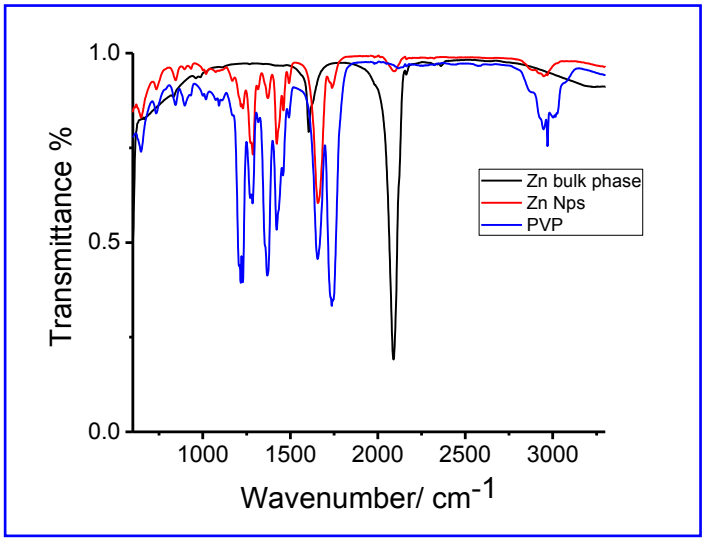

Fig S4 The IR spectra of the bulk $K_2Zn_3[Fe(CN)_6]_2$ sample and the PVP-coated $K_2Zn_3[Fe(CN)_6]_2$ NPs in comparison to that of a PVP sample

Studies of ion-exchange kinetics, capacity and selectivity in aqueous solution: The kinetics of copper removal was done by using a solution containing 100 ppm of $CuCl_2$ (50 mL). A water dispersion of ZnPB NPs (6.7 mM and 10 mL) was sealed in a dialysis bag (MWCO-3,500) which was brought in contact with the above copper solution. The copper concentrations of the solution outside the dialysis bag were periodically analyzed by AA. The kinetics data of the ion-exchange reaction could be fitted into two separate rate laws. A *pseudo* first-order up to the reaction time point of ~7 hours with a rate constant of k_1=5.7×10^{-5} s^{-1} and a half-life of $t_{1/2}$ = 202 min (See **Fig S5**). The second part can be fitted to a second order rate law with a rate constant of k_2 = 4.02×10^{-2} M^{-1}s^{-1} (See **Fig S6**).

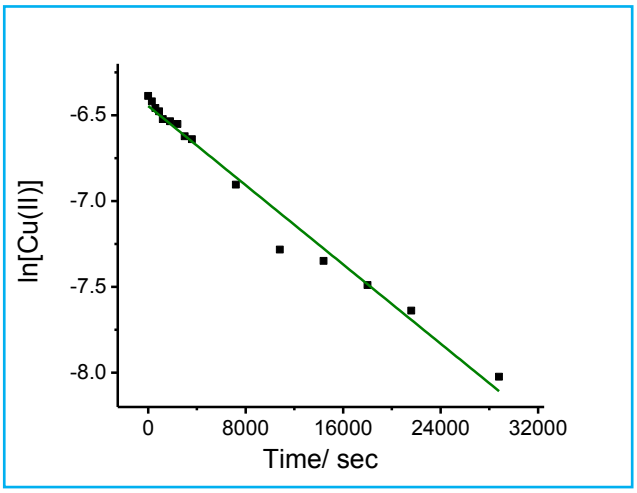

Fig S5 The kinetic data fitting curve to a *pseudo* first-order reaction for the ion-exchange between $K_2Zn_3[Fe(CN)_6]_2$ NPs and Cu^{2+} ions in aqueous solution

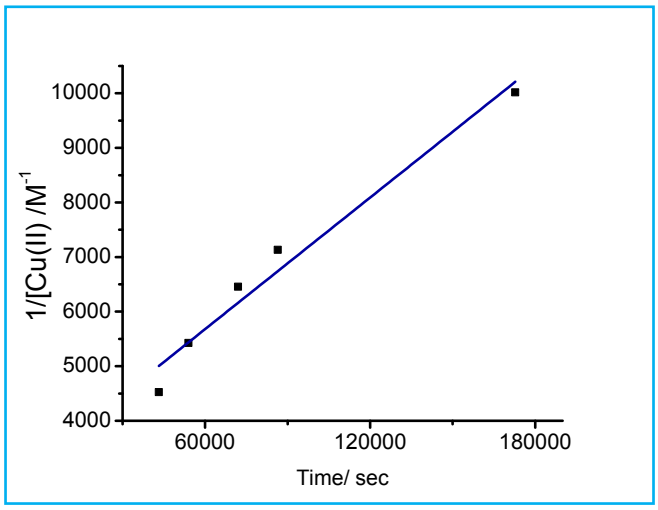

Fig S6 The kinetic data fitting curve to a second-order reaction for the ion-exchange between $K_2Zn_3[Fe(CN)_6]_2$ NPs and Cu^{2+} ions in aqueous solution

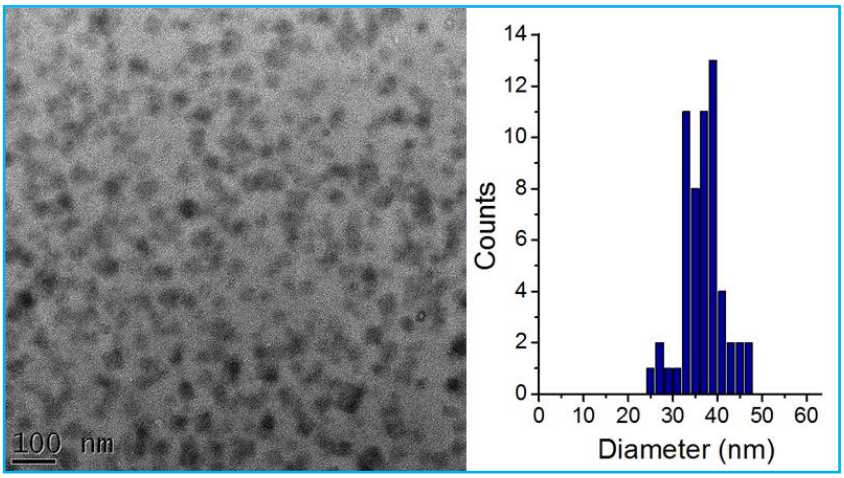

Fig S7 The TEM image (left) and histogram (right) of the ion-exchanged product between $K_2Zn_3[Fe(CN)_6]$ and Cu^{2+} ion with a composition $K_2Cu_xZn_{3-x}[Fe(CN)_6]$ determined by AA. The range of *x* is from 0.07 to 0.11 depending on the extent of the ion-exchange

Selectivity studies were performed by soaking the dialysis bag containing 5 mL of nanoparticles (10 mM) in a solution of copper(II), manganese(II), iron(II), and calcium(II). The concentration of each metal in the competitive solution was ~100 mg/L. After 24 hours, an aliquot of solution was taken out and diluted with 2% HNO_3 acid and analyzed for each metal ion using AA. In a separate batch reaction, the same amount of ZnPB NPs was broght in contact with Zn^{2+} ions at the same concentration for 24 hours. The zinc concentration was analysized by AA to show that there was no ion-exchhange.

The capacity of ion-exchange (q, mg/g) was determined using the mass balance equation given below, where C_o is the initial metal concentration in mg/L, C_t is the metal concentration mg/L in solution after time t in min, V is the volume of metal solution in L, and W is the weight of NPs in g. We performed these experiments using our NPs by a batch reaction method. The copper removal capacity was found to be 88 mg/g as shown in **Fig S8**.

$$q = \frac{(C_o - C_t)V}{W}$$

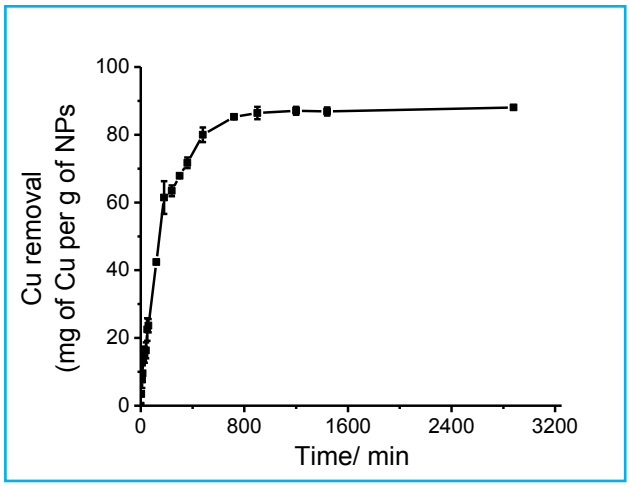

Fig S8 Copper removal capacity of $K_2Zn_3[Fe(CN)_6]_2$ NPs

Determination of the K_{sp} values (solubility products) for $K_2Zn_3[Fe(CN)_6]_2$ and $K_2Cu_3Fe(CN)_6]_2$: The two analogous compounds are assumed to dissociate according to the following scheme:

$$K_2M_3[Fe(CN)_6]_2(s) \longrightarrow 2K^+(aq) + 3M^{2+}(aq) + 2[Fe(CN)_6]^{4-}(aq) \quad (M=Zn \text{ or } Cu) \quad [1]$$

Hence, $K_{sp} = [K^+]^2 \times [M^{2+}]^3 \times [Fe(CN)_6^{4-}]^2$

The K_{sp} values for the above two compounds were determined by a static method. Specifically, about 800 mg of each compound was allowed to equilibrate with 1000 mL of deionized water at 25 ^oC under stirring for 48 hrs. The volume of each solution was then reduced to a smaller volume and transferred into a porcelain crucible. The solution was then further heated to dryness. The content in the crucible was heated at 630 ^oC for 6 hours. A small amount of concentrated nitric acid was added to dissolve the metal oxides obtained from the above decomposition reaction. The final metal concentrations were determined by AA for each of solution. The determinations of solubility product by static method gave the K_{sp} value of $3.8 \pm 1\times10^{-46}$ mol^7dm^{-21} for $K_2Cu_3[Fe(CN)_6]_2$, and $1.1 \pm 2\times10^{-38}$ mol^7dm^{-21} for $K_2Zn_3[Fe(CN)_6]_2$, respectively.

Ion-exchange reaction between $K_2Zn_3[Fe(CN)_6]_2$ NPs and Cu(I) ions in aqueous solution: First, a 0.3 mM Cu^+ solution was prepared by dissolving CuCl in a small amount of concentrated HCl and diluted to the intended concentration. A proper amount of $K_2Zn_3[Fe(CN)_6]_2$ NPs was added to this solution to reach the dispersion level of ~0.6 mM. The solution was quickly sonicated and transferred to a quartz cuvette. The ion-exchange was monitored using a UV-vis spectrophotometer over a period of 4 hours. This reaction showed the identical spectroscopic feature as the ion-exchange reaction between $K_2Zn_3[Fe(CN)_6]_2$ NPs and the Cu(II) ion.

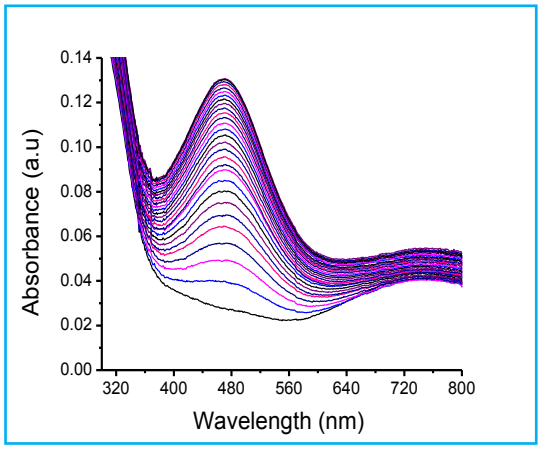

Fig S9 UV-vis spectroscopic scanning results for ion-exchange between $K_2Zn_3[Fe(CN)_6]_2$ NPs and the Cu(I) ion

Confirmation of ion-exchange between the K^+ ion in NPs and the Ca^{2+} ion in aqueous solution and the suppression of this reaction: A water dispersion of as-synthesized ZnPB NPs (5 mM and 2 mL) was sealed in a dialysis bag (MWCO-3,500) which was immersed in an aqueous solution containing about 100 ppm of $CaCl_2$. After stirring for 4 hours, the concentrations of both calcium and potassium of the outside solution were analyzed by AA. The results showed that there is an ion-exchange reaction between the K^+ ion in the NPs and the Ca^{2+} ion from the aqueous solution with a molar ratio between K and Ca close to 1:2. In a separate experiment, a similar amount of ZnPB NPs was soaked overnight in an aqueous solution containing ~0.1 M. After centrifugation and washing with distilled water 3 times, the NPs were sealed in a dialysis bag and submerged in another aqueous solution containing about 100 ppm of $CaCl_2$ for 18 hours. The elemental analysis of the solution for both calcium and potassium showed that there is essentially no change in their concentrations due to ion-exchange.

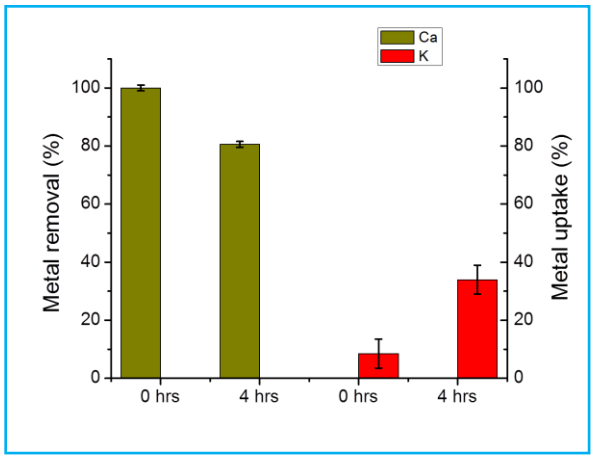

Fig S10 Changes in the calcium and potassium concentrations of the aqueous solutions indicating the ion-exchange reaction between the K^+ ion in the NPs and the Ca^{2+} ion from the aqueous solution

Copper removal in the presence of De-PEN: In this study, we reacted the ZnPB NPs separately with an aqueous solution containing 100-pm Cu^{2+} ions and with another aqueous solution containing D-PEN complexes of copper (pH = 5.6) at the same cocentration. The copper removal was measured in eachh solution by AA. The results showed that the ZnPB NPs can effectively compete against D-PEN for copper ions from aqueous solution (see **Fig S11**).

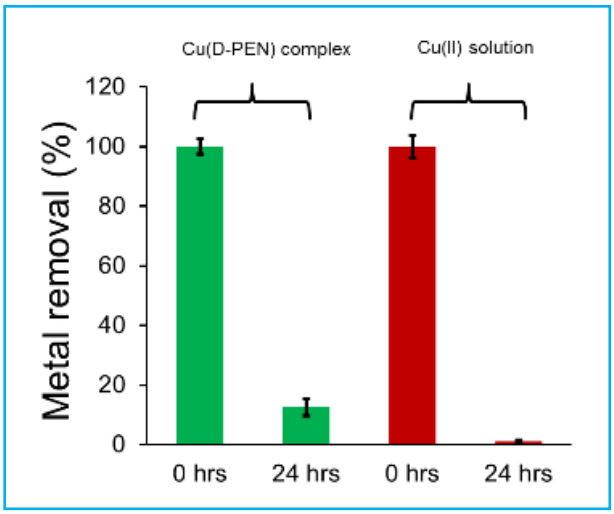

Fig S11 Competetion for copper by ZnPB NPs over D-PEN

Cell viability assay: Cytotoxicity studies were performed using the MTT assay. HeLa cells were seeded in a 96-well plate at a density of 2×10^4 cells per well with the DMEM low glucose medium and incubated for 24 hrs at 37 °C in an atmosphere of 5% CO_2 and 95% air to allow cells to attach to the surface. Cells in each well were then treated with 100 µL of fresh medium containing varying concentrations of the nanoparticles and then incubated for 24 hrs. Control wells contained the same medium without nanoparticles. After 24 hours, DMEM medium containing nanoparticles was removed and then, the cells were incubated with fresh DMEM media containing MTT reagent 10 µl, 1% (w/v) for 4 hours. After the MTT solution was removed, the precipitated violet crystals were dissolved in 100 µL of detergent. The absorbance was measured at 560 and 630 nm using a microplate reader. Each concentration was tested in replicates of three. The assay results are presented as percent viable cells.

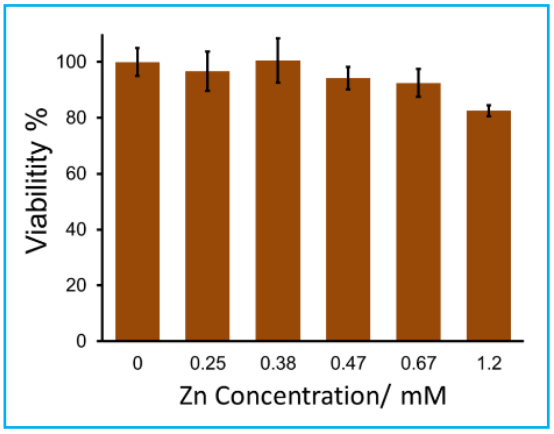

Fig S12 The cell viability curve of PVP-coated ZnPB NPs

Surface functionalization of $K_2Zn_3[Fe(CN)_6]$ NPs by fluorescence dye molecules and cell uptake studies: An aqueous Zn^{2+} solution (1 mM, 75 mL) containing PVP (average MW=40000, ~ 800 mg) and ~2.0 mL of 0.5mM of ethylenediamine was slowly added to an aqueous $K_4[Fe(CN)_6]$ (1 mM, 50 mL) solution under vigorous stirring. The resulting solution was further stirred at room temperature for *ca.* 5 hrs. These NPs have terminal $–NH_2$ groups. After dialyzed for 24 hours, the NPs were concentrated to ~2.5 mM. Then EDC (i.e. N-(3-dimethylaminopropyl)-N-(ethylcarbodiimide hydrochloride, 0.0018g) and 6-carboxyfluorescene dye (0.0030 g) were allowed to react to form the activated 6-carboxyfluorescene dye. The final fluorescence dye concentration was adjusted to ~0.8 mM. The fluorescence dye (~ 1mL) was added to the NPs (10 mL) and stirred overnight to allow the coupling reaction to occur. The reaction mixture was then dialyzed for 2 days to remove unbound dye molecules using the regenerated cellulose tubular membrane (MWCO is 12000) against distilled water. The covalent binding of fluorescence dye molecules to the surface of the NPs was confirmed by fluorescence spectroscopic measurements (see **Fig. S13**).

The fluorescence dye-labeled NPs were then incubated with HeLa cells to visualize the cellular uptake of these NPs by confocal microscopy. HeLa cells were seeded in an 8 well chamber at a density of approximately 1.0×10^5 cells per well for 24 hrs at 37 ^oC. The cells were then incubated with dye-labeled NPs for 4 hrs at 37 ^oC and washed thoroughly with PBS buffer before they were viewed under a confocal microscope. The cells were then imaged using a confocal microscope with 488 nm excitation wavelength.

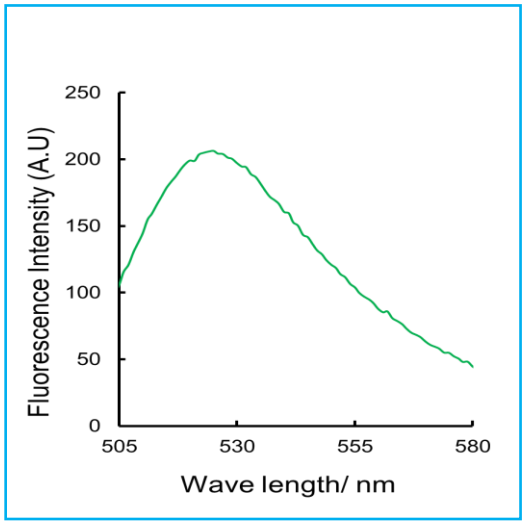

Fig S13 Fluorescence spectrum of the dye-labeled ZnPB NPs.

Leaching of CN^- and Zn^{2+} ions from the nanoparticles under various conditions: About 200 mg of $K_2Zn_3[Fe(CN)_6]$ NPs were sealed in a membrane dialysis bag (MWCO=3,500). This dialysis bag was gently stirred in 25 mL of distilled water at neutral pH, with PBS buffer and with HCl to obtain the pH = 1.0 for 24 hours. The resulting solutions were analyzed for free CN^- ions released from the NPs by a fluorometric method using the cyanide test kit developed by LaMotte Co. (Chestertown, Maryland; Code 7387-01). The calibration curve was established using the standard KCN solutions with the concentrations at the ppm level (see **Fig S14**).

Intracellular copper removal by ZnPB NPs: The elevated copper level in HeLa cells was induced by incubating the cells with a 50 μM $CuCl_2$ solution for 12 hours. The cells were washed three times with PBS and then incubated with the culture medium containing the NPs (50 μM) for 4 hours at 37 °C. After 4 hours incubation period, the cells were washed three times with PBS to remove the non-internalized NPs and further incubated with fresh culture medium for another 2, 4 and 8 hours. The cells grown in separate flasks were then trypsinized, centrifuged, re-suspended in PBS, and counted using a hemocytometer for 2, 4 and 8 hours, respectively. The cells were then collected by centrifugation and lysed with concentrated nitric acid. The lysates were diluted to 5.00 mL with deionized water and filtered through a 200 μm filter. The copper contents in the lysates were analyzed by AA.

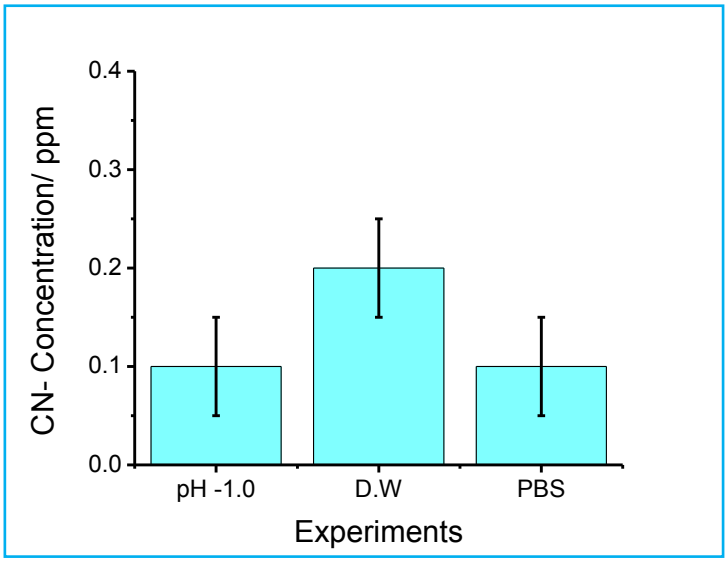

Fig S14 Results of CN^- leaching from $K_2Zn_3[Fe(CN)_6]_2$ NPs under different conditions