Electronic Supplementary Information for

Ultra-small Nd^{3+}-doped Nanoparticles as Near-infrared Luminescent Biolabels of Hemin in Bacteria †

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Experimental Section

Preparation of NPs@hemin complexes

Synthesis of oleic acid-capped $NaGdF_4$:Nd^{3+},Fe^{3+} NPs: Ultra-small $NaGdF_4$:Nd^{3+} NPs codoped with Fe^{3+} (20 mol%) were synthesized via a facile modified co-precipitation route using oleic acid (OA) as the coordinating ligand. Typically, 0.77 mmol of $GdCl_3 \cdot 6H_2O$, 0.03 mmol of $NdCl_3 \cdot 6H_2O$ and 0.20 mmol of $FeCl_3 \cdot 6H_2O$ were mixed with 6 mL of OA and 15 mL of 1-octadecence (ODE) in a 100 mL three-neck round-bottom flask and heated to 150 ºC under N_2 flow for 1h to form a transparent solution. After cooled to room temperature (RT), 10 mL methanol solution containing 6.4 mmol NH_4F and 4.0 mmol NaOH was added to the flask dropwisely. The resulting solution was stirred at RT, heated to 70 and 120 ℃ to entirely evaporate the methanol and water. Thereafter, the mixture was heated at 300 ºC for 1 h and then cooled to RT naturally. The resulting $NaGdF_4$:Nd^{3+},Fe^{3+} NPs were precipitated by addition of ethanol (15 mL), collected via centrifugation, washed with ethanol several times and then dried in a vacuum oven.

Synthesis of ligand-free $NaGdF_4$:Nd^{3+},Fe^{3+} NPs: The ligand-free NPs were obtained by removing the surface oleic acid ligands of the NPs through acid treatment. In a typical process, 20 mg of the as-synthesized NPs were dispersed in a mixture of ethanol (10 mL) and concentrated hydrochloric acid (100 μL) and ultrasonicated for 20 min. After the reaction, the resulting products were collected via centrifugation (20, 000×*g*, 20 min), washed with ethanol and deionized water several times, and finally redispersed in deionized water.

Conjugation of $NaGdF_4$:Nd^{3+},Fe^{3+} NPs with hemin: To conjugate $NaGdF_4$:Nd^{3+},Fe^{3+} NPs with hemin, the NPs in dimethylformamide (DMF) solution (10 mM) were added dropwisely into DMF solution (50 mL) containing hemin (20 mg, purchased from Aladdin Reagent Co., Ltd, Shanghai, China, 98%) and stirred for 24 h. After removing free hemin by centrifugation, the obtained precipitate was washed with DMF and distilled water for several times and resuspended in 1 M KOH solution by sonication for further application.

Conjugation of $NaYF_4:Yb^{3+},Er^{3+}$ UCNPs and $NaYF_4:Ce^{3+},Sm^{3+}$ DCNPs with hemin: For comparison, cubic $NaYF_4:Yb^{3+},Er^{3+}$ UCNPs were synthesized by thermal decomposition of trifluoroacetate precursors in the solvents of 1-octadecene (ODE) and oleic acid (OA). In a typical procedure, CF_3COONa (1 mmol), $Y(CF_3COO)_3$ (0.78 mmol), $Yb(CF_3COO)_3$ (0.2 mmol), and $Er(CF_3COO)_3$ (0.02 mmol) were dissolved in ODE (12 mL) and OA (6 mL) at room temperature (RT) , the obtained mixture was heated under vigorous stirring in N_2 atmosphere to remove water and oxygen (120 ºC, 1 h). Thereafter, the mixture was heated at 290 ºC for 0.5 h and then cooled to RT naturally. The resulting $NaYF_4:Er^{3+},Yb^{3+}$ UCNPs were precipitated by addition of ethanol (20 mL), collected via centrifugation, washed with ethanol several times and then dried in a vacuum oven. The $NaYF_4:Ce^{3+},Sm^{3+}$ NPs were synthesized using the same procedure, except $Y(CF_3COO)_3$ (0.78 mmol), $Yb(CF_3COO)_3$ (0.2 mmol), and $Er(CF_3COO)_3$ (0.02 mmol) were replaced by $Y(CF_3COO)_3$ (0.92 mmol), $Ce(CF_3COO)_3$ (0.05 mmol), and $Sm(CF_3COO)_3$ (0.03 mmol).

The procedures for Synthesis of ligand-free $NaYF_4:Yb^{3+},Er^{3+}$ and $NaYF_4:Ce^{3+},Sm^{3+}$ NPs as well as their conjugation with hemin were similar to that of $NaGdF_4:Nd^{3+},Fe^{3+}$ NPs, except $NaGdF_4:Nd^{3+},Fe^{3+}$ NPs were replaced by $NaYF_4:Yb^{3+},Er^{3+}$ and $NaYF_4:Ce^{3+},Sm^{3+}$ NPs, respectively.

The fermentation experiments

Feedstock and inoculum: Wheat straw was freshly collected from a farm yard in Luhe District, Nanjing, Jiangsu Province, China at the end of May, 2013, and cut into approximate particles of 2-3 mm by using a grinder (Hummer 900). After being air-dried, the straw particles were stored at 4± 0.5 °C until use. The wheat straw particles contained 39.2% cellulose, 28.3% hemicelluloses, 13.2% lignin, and 5.6% ash. The detailed chemical parameters of wheat straw and anaerobic sludge are shown in the following table.

The characteristics of substrate used in the reactors

Parameter	Wheat straw	Anaerobic sludge
Total solids (TS/%)	90.01±2.03	5.11±0.03
Volatile solids (VS/%)	89.26±0.63	68.47±1.44
Total carbon (mg/g-TS)	479.83±0.02	497.63±0.02
Total nitrogen (mg/g-TS)	5.34±0.18	14.25±0.13
Carbohydrate (/TS)	54.62±0.37	NA[b]
Protein (/TS)	3.41±0.32	NA
Cellulose (%)	39.21±0.11	NA
Hemicelluloses (%)	28.32±0.30	NA
Lignin (%)	13.29±0.17	NA
Mg (%, d.b.)	0.83±0.31	0.03±0.01
Ca (%, d.b.)	0.11±0.03	0.15±0.02
Fe (%, d.b.)	0.05±0.01	0.18±0.06
Mn (ppm)	49.02±4.05	26.37±0.12
Zn (ppm)	65.51±11.03	567.68±20.16
Cu (ppm)	—[c]	216.13±22.68
Ni (ppm)	4.05±1.03	19.63±0.21
As (ppm)	—	18.51±2.33
Mo (ppm)	—	1.29±0.07
V (ppm)	7.51±1.53	—
Cd (ppm)	—	—
Sb (ppm)	—	—
Pb (ppm)	—	26.49±3.78

[a] Each value is an average of three parallel replicates and is represented as mean ± standard deviation

[b] No analysis

[c] The concentration lower than the detection limit

The inoculum of anaerobically digested sewage sludge was taken from a wastewater treatment plant in Yangzi Petrochemical Co., Ltd, Nanjing, China. The glucose was fed in the sludge with 1.5 g/L per day at 35±1°C for one month, and then the feeding of glucose was stopped. When no biogas production observed for one week, the seed culture was thoroughly mixed and filtered through a screen with a pore size of 833 μm (20 meshes). This was carried out to ensure the removal of easily degradable organic matter still present in the inoculum and to remove the dissolved methane.

Batch assay methane fermentation setup: All the experiments were conducted in sequencing batch model with total volume of 1000 ml and the active volume of the

reactor was 800 ml. The reactors were fed with wheat straw with anaerobic sludge main containing total solids contents of 4%. The experiments were carried on mesophilic temperature of 35±1°C. After the feedstock was added into the reactors, sealed immediately with butyl rubber stoppers, the batch assay methane fermentation reactors were carefully checked for any leakage and flushed with pure nitrogen (99.9%) for 3 min in order to ensure anaerobic condition. An outlet in the stopper was used for collecting biogas in gas tight glass jars. Each digester of static culture was manually mixed once a day to avoid stratification. The initial carbon-to-nitrogen ratio (C/N) of 30:1 was maintained by the addition of carbamide in the each reactor. For the NPs@hemin group, the NPs@hemin was added in the fermanation system with the concentration of 1.57 mg/L. For comparison, the hemin and hemin-free groups were also performed, with the hemin concentration of 1.0 and 0 mg/L, respectively.

Batch experiments were conducted in triplicate to determine the biogas production rates of wheat straw for 30 d. During anaerobic digestion, the biogas samples were collected daily while the liquid samples were measured at 3-day intervals from the control digester for process stability investigation.

Culturing the bacteria in NPs@hemin medium

For measuring the NIR luminescence of NPs@hemin complexes in bacteria, the three types of bacteria were purified from the microbial communities and then cultured in the medium containing NPs@hemin complexes. The preparation and repackaging of culture medium, inoculation, dilution and enrichment culture of bacteria were performed according to the anaerobic culture technique of Hungate[1] with slight modification. For anaerobic bottle cultivation, the inoculation volume was 15%. Exponentially growing cells were inoculated into 100 mL sealed anaerobic bottles filled with 30 mL of the following medium. The medium (pH 7.0) for culturing the three types bacteria were determined as follows: the medium for fermentative bacteria containing 10.0 g glucose, 5.0 g peptone, 3.1 g NaCl, 3.0 g beef extract, 0.5 g L-cysteine hydrochloride and 1.5 mL resazoin(0.1%) per litre; the

medium for acetogenic bacteria containing 10.0 g CH_3CH_2COONa, 3.0 g NaCl, 0.1 g $MgCl_2$, 1.0 g $CaCl_2$, 0.75 g K_2HPO_4, 0.75 g KH_2PO_4, 1.0 g NH_4Cl, 1.0 g yeast extract, 1.0 g peptone, 10 mL trace element solution and 10 mL compound vitamin liquid per litre; the medium for methanoges bacteria containing 1.0 g NH_4Cl, 0.1 g $MgCl_2 \cdot 6H_2O$, 0.4 g $K_2HPO_4 \cdot 3H_2O$, 0.2 g $KH_2PO_4 \cdot 3H_2O$, 1.0 g yeast extract, 0.1 g peptone, 2.0 g $CH_3COOHNa$, 2.0 g HCOONa, 0.5 g L-cysteine hydrochloride, 0.2 g $Na_2S \cdot 9H_2O$, 2.0 g $NaHCO_3$, 1.0 mL resazoin(0.1%), 10 mL trace element solution and 10 mL compound vitamin liquid per litre. The screening agar plate medium was prepared by dispersing the powdered agar in the above medium with the concentration of 20 g/L. These medium was purged with nitrogen gas to remove dissolved oxygen and then heat sterilised at 121 ºC for 30 min. The three types of bacteria were purified using their respective screening agar plate medium via a continuous selection method and then transferred to the anaerobic bottle for further enrichment culture. The concentrations of these bacteria were monitored by measuring the optical absorption at 600 nm using a standard ultraviolet/visible spectrophotometer (Lambda 35, Perkin Eimer). When the optical density at 600 nm reached 0.7-0.8, these bacteria were collected by centrifugation (8, 000×*g*) at 4 ºC and dispersed in 5 mL of physiological saline solution with the cell density of 2×10^6 cells/mL. Then, these bacteria were cultured in their respective culturing medium containing NPs@hemin complexes (96 mg/L, 30 mL). After incubation at 35 ± 1 ºC on a shaker for the log phase (16, 24, and 96 h for fermentative bacteria, acetogenic bacteria and methanoges bacteria, respectively) for uptaking the NPs@hemin complexes, the bacteria were harvested by centrifugation (8000 g, 10 min at 4 ºC), washed three times with sterile water, and finally resuspended in physiological saline solution (2×10^6 cells/mL) for further NIR luminescence measurements.

For comparing the activity of the cytochrome P450 enzymes, these bacteria were also incubated in the medium without NPs@hemin for the log phase with the same procedure as that of containing NPs@hemin.

Cell disruption with ultrasonication

After 30-d anaerobic digestion, the culture (2L, collected from 5 reactors) obtained was thoroughly mixed and then filtered through a screen with a pore size of 44 μm (100 meshes). The cells were harvested from the fermentation filtrate by centrifugation, washed and dispersed in 100 mL physiological saline solution. The cell disruptions were performed using a Vibra-Cell™ ultrasonicator (Model VCX134PB, Sonics & Materials, Inc.). For cell disruption, the ultrasonication probe was immersed into the cell solution and the ultrasonication was conducted with a frequency of 40 kHz and the switched powers of 32.5 and 130 W at ~ 20 ºC. Cell debris was harvested by centrifugation at 10, 000×*g* for 15 min at 4 ºC, and washed three times, and dispersed in 100 mL physiological saline solution. Finally, the solutions of cell supernatant and debris were used directly for the NIR luminescence and optical absorption measurements. For the XRD measurement, the NPs@hemin powders were collected from the cell supernatant by centrifugation at 20, 000×*g*, washed with deionized water several times and dried in a vacuum oven.

The the similar ultrasonication method was also applied for disrupting the bacteria cultured for the log phase in NPs@hemin medium, except that the culture was 200 mL, collected from sealed anaerobic bottles.

Characterization

Characterization of biogas: The daily biogas production was directly obtained from the volume of displaced saturated $NaHCO_3$ solution in the graduated cylinder after the mixture being stirred manually. Methane concentration in the biogas was analyzed using a gas chromatograph (GC 9890A, Renhua, China) equipped with a TCD, a TDC-01column (Φ4mm×1 m, Shimadzu, Japan) and hydrogen as the carrier gas. The injector, oven and detector temperatures were 100, 150 and 120°C, respectively. Flow rate of carrier gas was 50 ml/min, and the injection volume of sample was 0.5 ml.

Chemical composition analyses: The parameters of total solid (TS), volatile solid (VS) and volatile suspended solids (VSS) were performed in accordance with standard methods of APHA (American Public Health Association, 1998). Total carbon (TC) and total nitrogen (TN) were analyzed by the CHN (carbon, hydrogen,

nitrogen) analyzer vario EL (Perkin Elmer, USA made).The protein content was calculated with a conversion factor of 6.25. Carbohydrate was calculated as the fraction of VS remaining after the subtraction of protein and lipids.[2] The pH value was directly measured from liquid samples with a digital pH meter (FE20K, Mettler-Toledo, Switzerland). Lipids were determined by a Soxhlet system at 65°C with more than 60 circulations using methylene dichloride as an extractive reagent.[3] The sample weights before and after extraction were used to calculate the lipids content. For the determination of major and trace metalelements dried samples were pretreated with a mixture of $HNO_3/H_2O_2/HF$ followed by neutralization with H_3BO_3 and the resulting clear solution was analyzed by inductively coupled plasma atomic spectrometry (ICP-OES,ThermoFisheriCAP 6200) according to standard procedures. Content of cellulose, hemicellulose and lignin were determined by sequential fiber analysis using Goehring and Van Soest's method with FIWE Cellulose Analyzer (Velp Scientifica Company, Italy).[4]

Enzyme activity assays: The total dehydrogenase activity assay was based on the reduction rate of triphenyltetrazolium chloride (TTC) to triphenyl formazan.[5] The protease activity was analyzed by a Folin-phenol Reagent method.[6] The acetate kinase was determined spectrophotometrically based on Mu's method.[7] For the coenzyme F_{420} assay, a sample of 2 ml from the digester was first disintegrated and suspended in a Bead Bug (D 1030, Benchmark,USA) at 500 oscillations/min for 100 s, then was centrifuged at 10,000 rpm and 4 °C for 15 min to remove the waste debris. The suspensions were measured using a Fluorescence/Luminescence Spectrometer (LS 55, Paekin Elmer, USA) under the synchronous scan regime. The difference between excitation and emission wavelengths was 20 nm. The excitation wavelength was changed from 360 nm to 480 nm and the emission wavelength was synchronously changed from 380 nm to 500 nm. Fluorescence at 420 nm was used in the experiments.[8] The enzyme activity of cytochrome P450 assay was based on the methoed of UV-visible absorption spectrum.[9] The intracellular concentrations of NADH and NAD^+ were assayed using a cycling method.[10]

Characterization of the nanoparticles: Powder X-ray diffraction (XRD) patterns were

collected using a BRUKER D8 Discover power diffractometer with Cu-Ka1 radiation (λ=0.154 nm). The transmission electron microscopy (TEM) measurements were performed using a Tecnai G^2 F20 S-Twin field-emission TEM (FEI, U.S.A.) operated at 200 kV. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a Nicolet 6700 spectrometer. The visible luminescence spectra were recorded on a Princeton Instruments (SP-2500i) spectrofluorimeter equipped with a 3W excitation laser source of 980 nm (Inter-Diff Optoelectronics Technology Co., Ltd, Shanghai). Dynamic light scattering (DLS) experiments were carried out on an ALV-5000 spectrometer-goniometer 5 equipped with an ALV/LSE-5004 light scattering electronic and multiple tau digital correlator and a JDS Uniphase He-Ne laser (632.8 nm) with an output power of 22 mW. The NIR luminescence spectra were collected on an Applied NanoFluorescence spectrometer with an excitation laser source of 785 nm. TGA were performed with TG/DTA 6200 (SII EXSTAR 6000 of Seiko Instrument) under N_2 atmosphere at a rate of 5 °C min^{-1}. Absorption spectra were recorded using PerkinElmer's Lamda 25 UV/vis spectrophotometer. The photos of the $NaGdF_4:Nd^{3+},Fe^{3+}$ NPs and NPs@hemin complexes were obtained digitally on a Nikon multiple CCD Camera.

Table

Table S1 Anaerobic digester performance evaluation in the reactors[a]

Item Unit	Methane contents (%)	Methane yield (mL/g-TS_{added})	Residual content of cellulose (TS/%)	Residual content of hemicellulose (TS/%)	Residual content of lignin (TS/%)
hemin-free	53.2±0.6	209.3±2.3	25.6±0.3	26.4±1.2	21.8±0.1
hemin	60.3±0.6	257.4±2.3	23.6±0.7	24.7±0.6	26.6±0.6
NPs@hemin	59.9±1.2	250.7±1.1	22.3±0.5	25.2±0.4	25.9±0.3

[a] Each value is an average of three parallel replicates and is represented as mean ± standard deviation

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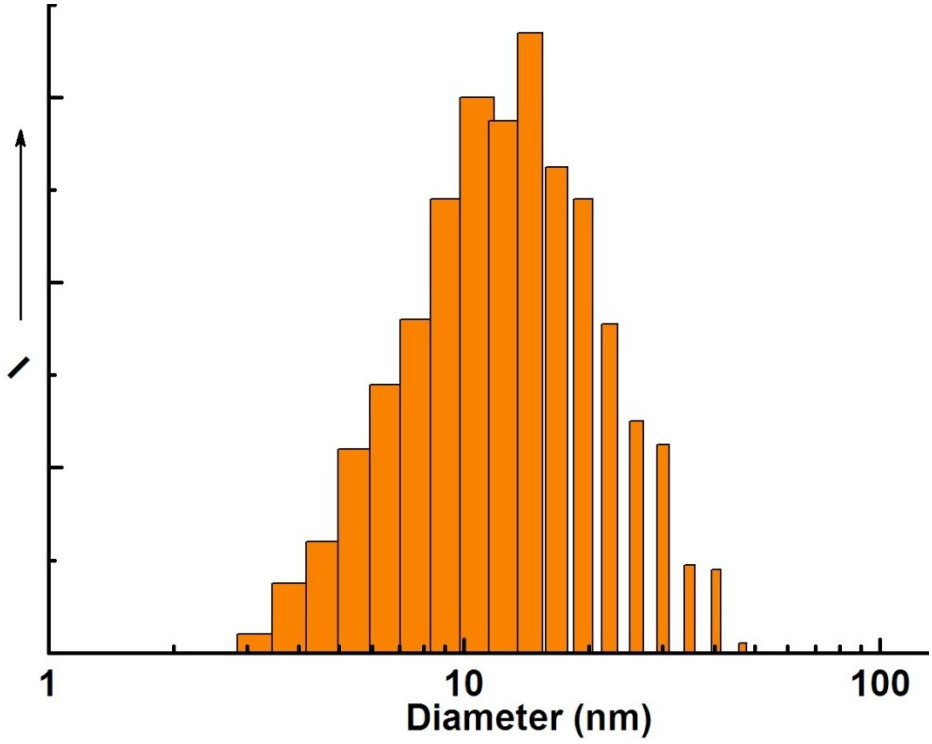

Fig. S1. Size distributions the NPs@hemin complexes determined by DLS.

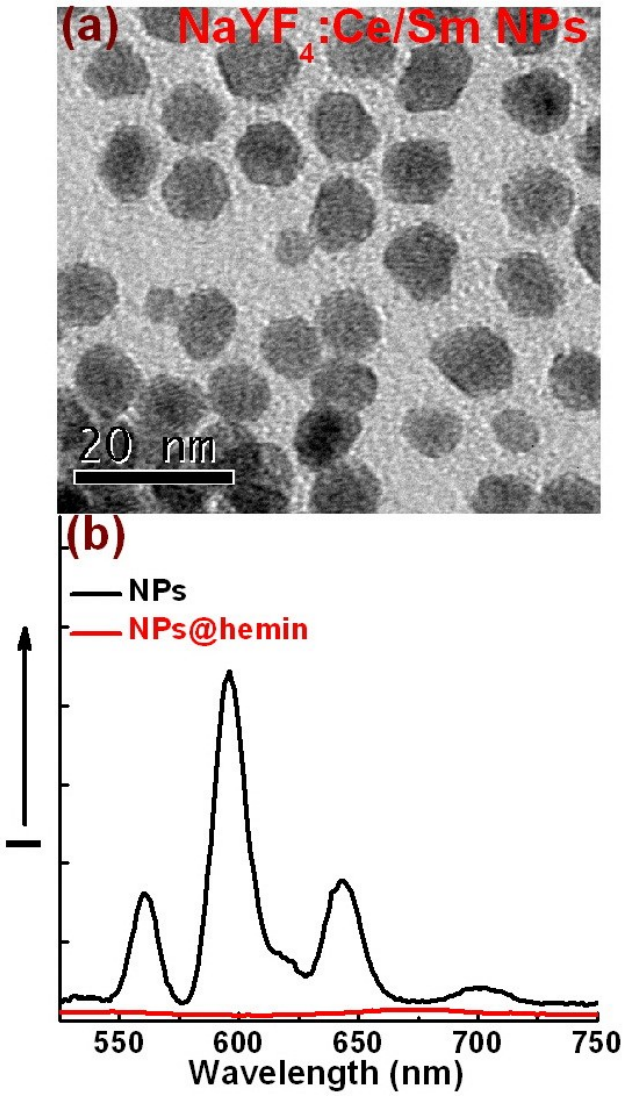

Fig. S2. (a) TEM image of $NaYF_4:Ce^{3+},Sm^{3+}$ NPs and (b) the visible emission spectra for $NaYF_4:Ce^{3+},Sm^{3+}$ NPs and their corresponding NPs@hemin complexes upon excitation at 258 nm.

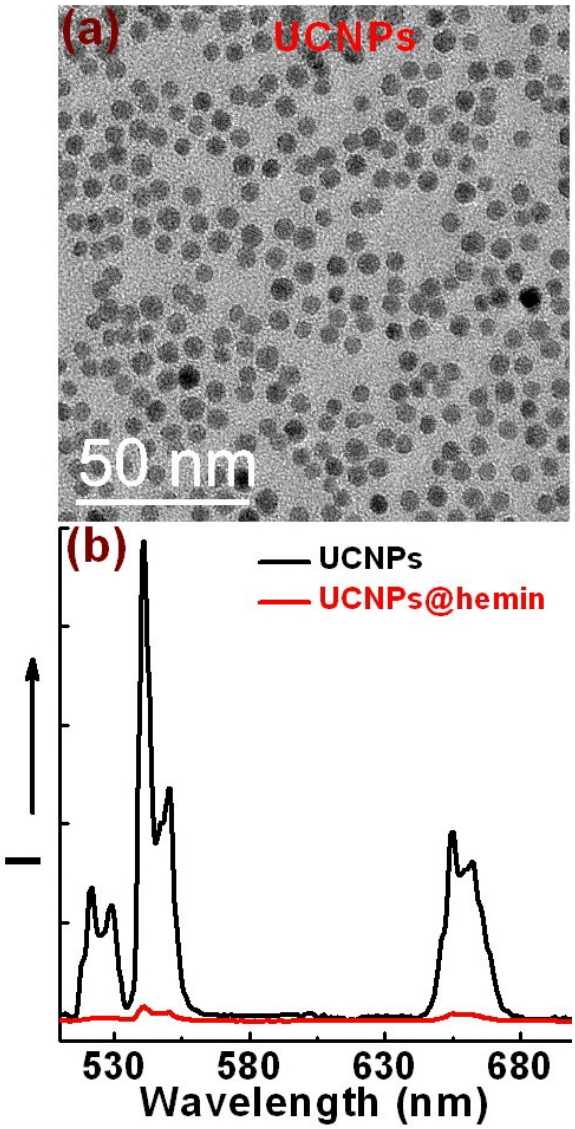

Fig. S3. (a) TEM image of $NaYF_4$:Yb^{3+},Er^{3+} UCNPs and (b) UC emission spectra for the $NaYF_4$:Yb^{3+},Er^{3+} UCNPs and their corresponding UCNPs@hemin complexes upon excitation at 980 nm.

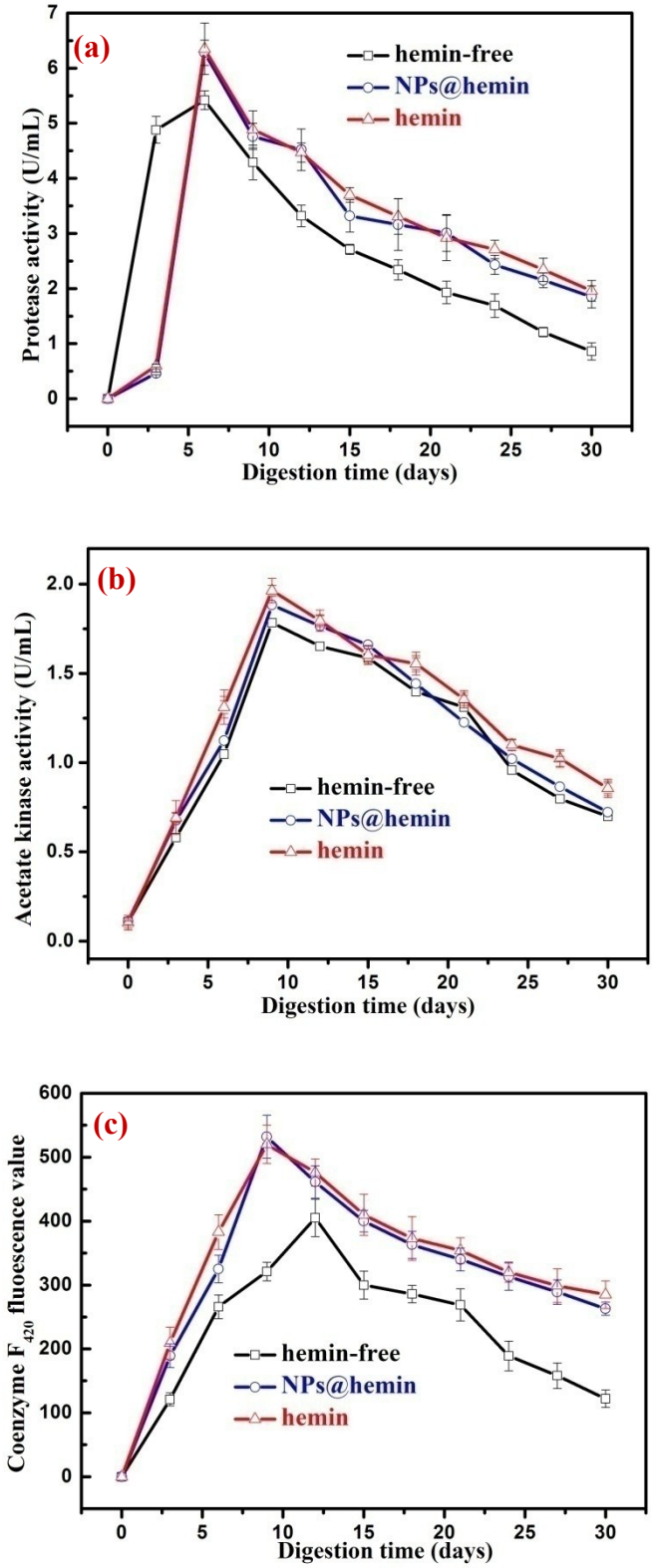

Fig. S4. The effects of different exogenous electron carriers on the enzymes activities of protease, acetate kinase and coenzyme F_{420} during anaerobic digestion. The plotted data were the averages from parallel experiments.

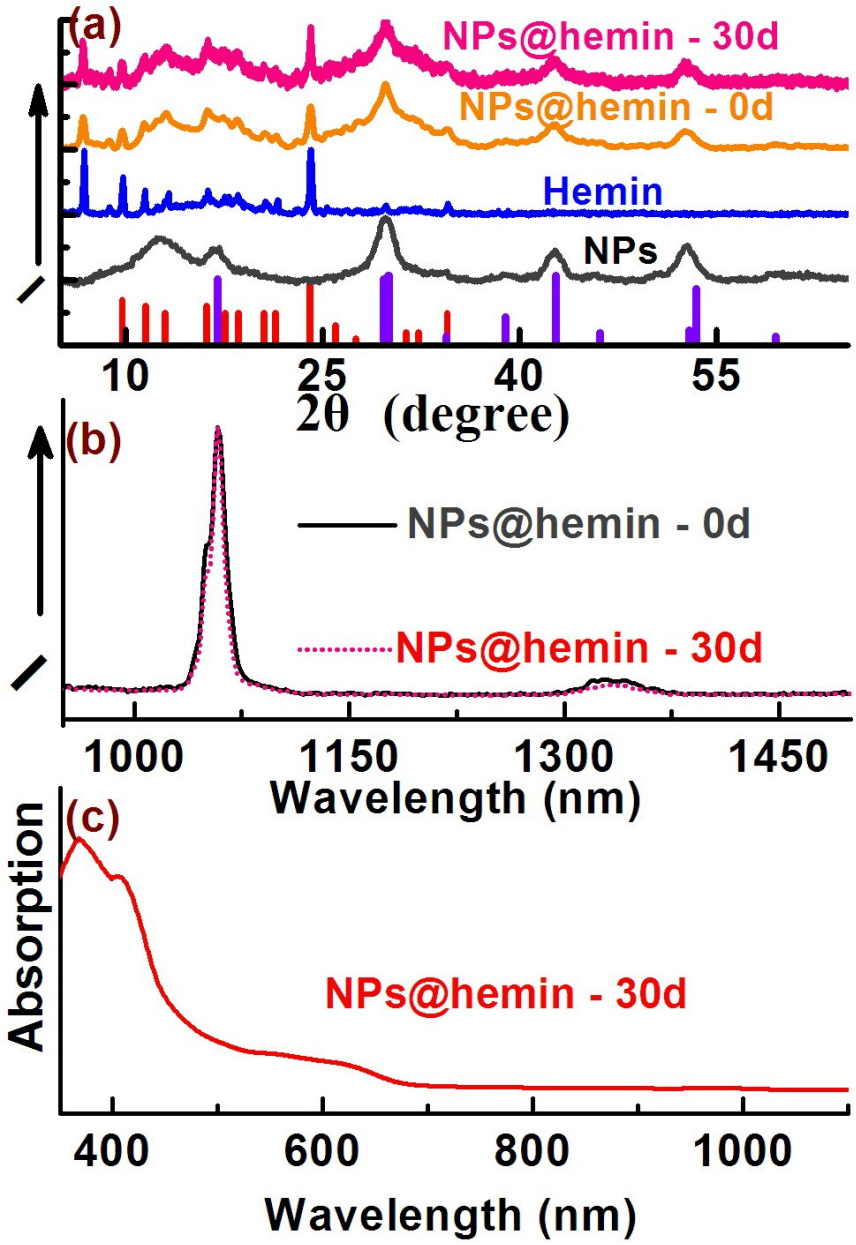

Fig. S5. XRD, NIR luminescence, and optical absorption of the NPs@hemin before (NPs@hemin -0d) and after ((NPs@hemin -30d) the digestion processes. For comparision, the XRD patterns for hemin and the $NaGdF_4:Nd^{3+},Fe^{3+}$ NPs, standard data for hemin (red columns) and $NaGdF_4$ (violet columns) were also presented in S4a.

The XRD pattern of hemin shows mainly the pattern of hemin (JCPDS card No. 08-0761) and an impurity peak at 6.8° possibly due to the relatively low purity of our hemin samples (98%, from Aladdin Reagent Co., Ltd), which can be assigned to the main diffraction peak of the allotropic substances of hemin such as 3-Iron oxychloride-1-trimethylamine (JCPDS card No. 20-1725) or iron oxide chloride benzamidine (JCPDS card No.35-1844). After conjugated with $NaGdF_4:Nd^{3+},Fe^{3+}$ NPs, the impurity peak at 6.8° can also be seen in the XRD pattern of NPs@hemin samples, which may imply that the impurities could be also adhered on the NPs, either via direct conjugation with $NaGdF_4:Nd^{3+},Fe^{3+}$ NPs or absorped on hemin molecules.

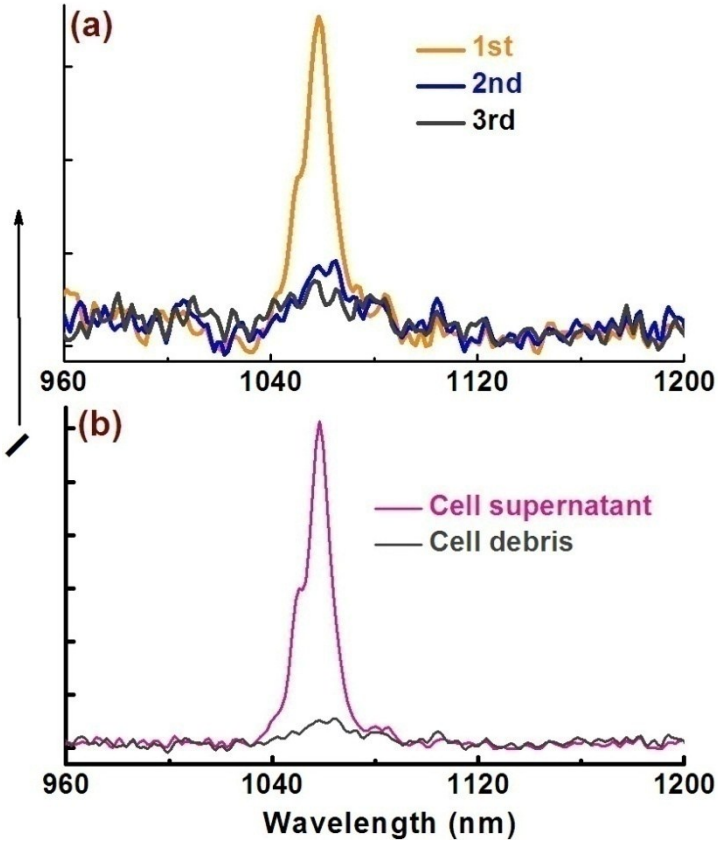

Figure S6. The NIR luminescence of (a) the supernatant after washing the bacteria for different times and (b) the cell supernatant and debris after cell disruption with ultrasonication for the fermentative bacteria cultured in the NPs@hemin medium

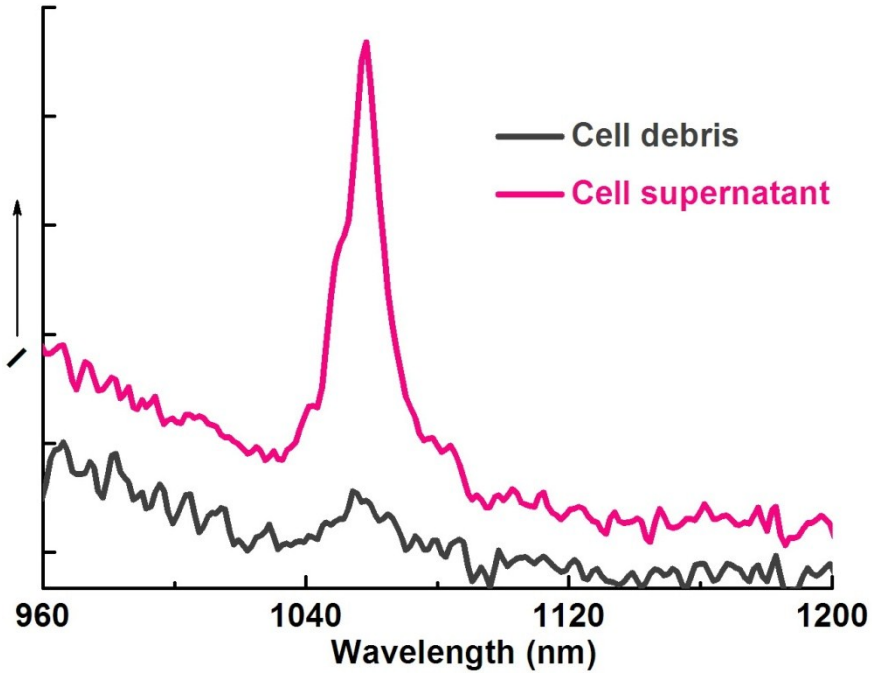

Figure S7. The NIR luminescence of the cell supernatant and debris after cell disruption with ultrasonication for the methanoges cultured in the NPs@hemin medium

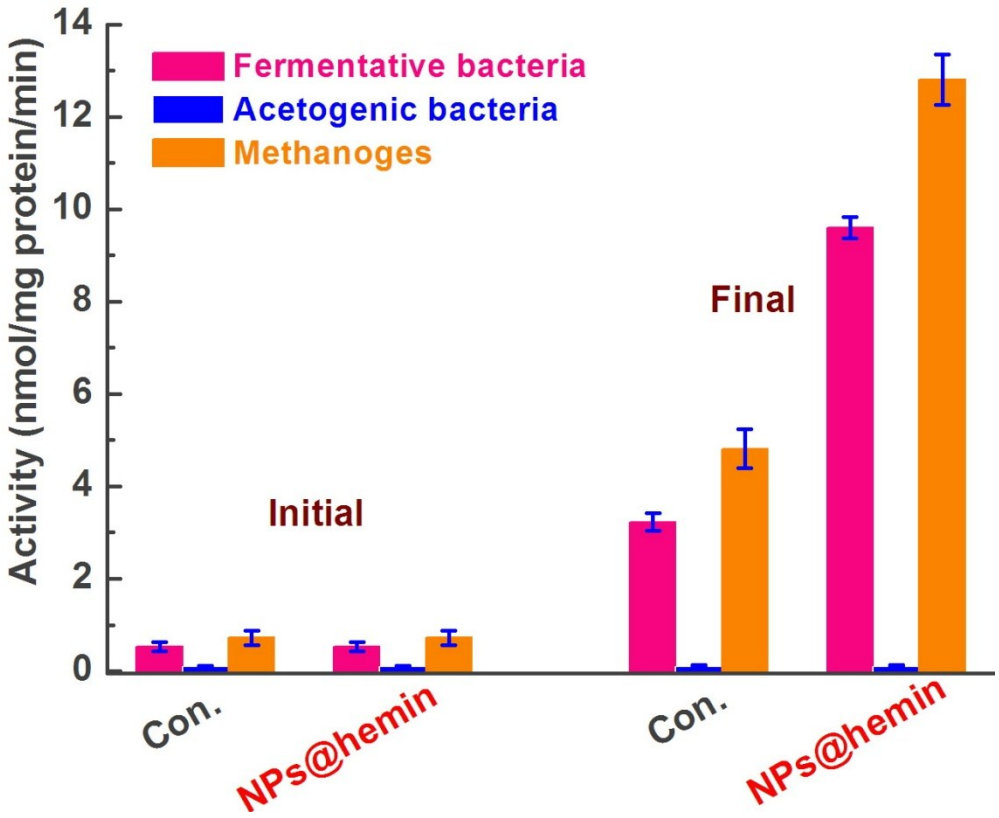

Fig. S8. The effects of exogenous electron carrier on the activity of cytochrome P450 enzymes in bactreia with and without NPs@hemin in the culture medium.The plotted data were the averages from parallel experiments. The group **Initial** is the activities for bactreia before culture, and the group **Final** is that cultured for the log phase in medium.

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