Supporting Information

Co-immobilization of Three Cellulases on Au-Doped Magnetic Silica Nanoparticles for the Degradation of Cellulose

Eun Jin Cho,[a] Sera Jung,[b] Hyun Joo Kim,[b] Yoon Gyo Lee,[b]
Kye Chun Nam,[c] Hong-Joo Lee[d] and Hyeun-Jong Bae*[,a, b, d]

[a] *Bio-energy Research Institute, Chonnam National University, Gwangju, 500-757, S. Korea*
[b] *Department of Wood Science and Landscape Architecture, BK21 Program, Chonnam National University, Gwangju, 500-757, S. Korea*
[c] *Department of Chemistry and Institute of Basic Science, Chonnam National University*
[d] *Department of Bioenergy Science and Technology, Chonnam National University*

Experimental Section

Materials.

Mercaptopropyltriethoxysilane were purchased from Aldrich. All other materials were of analytical grade and commercially available, including ferric chloride hexahydrate ($FeCl_3$•$6H_2O$), ferrous chloride tetrahydrate ($FeCl_2$•$4H_2O$), ammonium hydroxide (25% [w/w]), tetraethyl orthosilicate (TEOS), cupper chloride, imidazole, and ethylenediaminetetracetic acid (EDTA). Bio-Rad reagent for protein assay was obtained from Bio-Rad Laboratories (Hercules, CA). The water used throughout this study was de-ionized and filtered using a U.S. Filter purification system.

Instruments.

IR spectra were obtained for KBr pellets, in the range 400-4000 cm^{-1}, with a Shimadzu FT-IR 8400S instrument. Field Emission Transmission Electron Microscope (FE-TEM) was measured with a Tecnai F20 and JEOL JEM-1400. Field Emission Scanning Elctron Microscope (FE-SEM) was measured with a JEOL JSM-7500F(EDS; Oxford). X-ray Photoelectron Spectroscopy (XPS) was measured with a VG Multilab 2000. High Resolution X-Ray Diffractometer (HR-XRD) was measured with a X'Pert PRO Multi Purpose X-Ray Diffractometer. Magnetic properties were measured with a vibrating sample magnetometer (VSM, model-7404, Lakeshore) at room temperature. High performance liquid chromatography (HPLC) was measured with an RI detector (Waters 2414, USA) using a Rezex RPM column (4.6 x 300 mm; Phenomenex, USA). HPLC-grade water was supplied at a flow rate of 0.6 mL/min as a mobile phase, and temperature was controlled at 80 °C.

Preparation of Magnetic Nanoparticles (MNP).

$FeCl_3 \cdot 6H_2O$ (1.0 M) and $FeCl_2 \cdot 4H_2O$ (0.5 M) were dissolved in aqueous hydrochloric acid (0.4 M, 10 mL) at room temperature under sonication. After the salts were completely dissolved in solution, the mixture was degassed using a pump. Aqueous sodium hydroxide (0.5 M, 100 mL) was slowly added under nitrogen with stirring at room temperature. The mixture was left to react, heated in an oil bath at 80°C for 30 min. After cooling to room temperature, the magnetic nanoparticles were rinsed with aqueous hydrochloric acid (0.1 M) and ethanol to remove any unreacted impurities followed by resuspension in deionized water (40 mL) under sonication for 30 min. The supernatant was collected after centrifugation at 13500 rpm for 30 min at 4°C.

Preparation of Magnetic Silica Nanoparticles (MSNP).

Magnetite nanoparticles in suspension were added to a freshly prepared solution of tetraethyl orthosilicate (TEOS, 98%) in ethanol. The aqueous ammonia solution was dropped until the pH of the mixture raised to 12. Then, the mixture was refluxed at 100°C for 24 h and dense silica layer onto magnetite surfaces. Finally, the product was washed with ethanol for 3 times and dried at 60°C for 12 h in vacuum oven.

Preparation of MSNP with Immobilized Mercaptopropyltriethoxysilane.

Mercaptopropyltriethoxysilane (125 mg) was dissolved in anhydrous toluene (5 mL). The mixture was stirred under nitrogen for 5 min. The **MSNP** (40 mg) was added as solid. The reaction mixture was refluxed and stirred for 24 h. The collected solid was washed copiously with THF to rinse away any surplus mercaptopropyltriethoxysilane and then dried under vacuum.

Preparation of Au Nanoparticles.

Gold nanoparticle seeds were prepared according to the literature. Typically, this involves preparation of a 20 mL aqueous solution containing. To this solution was added 0.6 mL of ice cold 0.1 M $NaBH_4$ with stirring. The solution immediately turned orange-red, indicating formation of gold nanoparticles. The average particle size measured from transmission electron microscopy (TEM) was ~2.0 nm. Citrate serves as a capping agent in this case, and gold particles are stable for a couple of weeks. To the vial was added 9.0 mL of growth solution containing a mixture of 2.5×10^{-4} M $HAuCl_4$ and 0.10 M CTAB solutions. To this solution was added 50 μL of 0.10 M freshly prepared ascorbic acid, and the resulting solutions were stirred gently. Next, 1.0 mL of the seed solution was mixed with vial solution. After 10 s, 1.0 mL was drawn from vial solution.

Preparation of Au-MSNP.

To immobilize gold nanoparticles onto the surface of **MSNP**, the **MSNP** was added to Au nanoparticle solution (2 mL) for 24 h at room temperature. The collected solid was washed copiously with water (20 mL) three times to rinse away any surplus Au nanoparticles and then dried under vacuum.

Immobilization of Cysteine-Containing Cellulases with Au-MSNP or AuNP.

The phosphate buffer solution (PBS/NaCl, KCl, Na_2HPO_4, KH_2PO_4, 200 μL) contains cysteine-containing proteins (50 μg) with added **Au-MSNP** (5 mg) or **AuNP** (1 mL). The mixture was stirred at 0 °C in a darkroom for 1 day. The collected solid was washed

copiously with water (10 mL) to rinse away any surplus cysteine-containing proteins.

Characterization

The size and morphology of iron oxide nanoparticles before and after enzyme immobilization were determined by TEM using a JEOL 100-CX electron microscope. The binding of cellulase to the nanoparticle surface was determined using the Bradford protein assay. The protein found in the removed supernatant was measured by a colorimetric method involving the binding of Coomassie Brilliant Blue G-250 to the protein and then measuring the concentration across a wavelength of 595 nm. Bio-Rad dye reagent was used for the protein assay and bovine serum albumin as the standard. The binding of the enzyme was confirmed by Fourier transform infrared spectroscopy using a Thermo Nicloet Nexus 670 FTIR model and by X-ray photoelectron spectroscopy (XPS) using a Kratos Axis 165 XPS/Auger.

3,5-Dinitrosalicylic acid (DNS) is an aromatic compound which reacts with reducing sugars and other reducing compounds to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm. The DNS method was used in this study to determine the amount of glucose formed as reducing sugars resulting from a reaction with dinitrosalicylic acid reagent. Sugars that are contained in polysaccharides, such as cellulose and starch, or other complex organic compounds, are not accounted for by this method. d-Glucose was used as the standard.

Properties of the Free and Immobilized Cellulase.

1. **Protein assay**

Protein levels were determined using the Bradford assay. [1]

2. **The binding efficiency of enzyme**

The binding efficiency of enzyme to magnetic particles was determined by first evaluating the saturation of cellulase enzyme on the surface of the precipitates. Subsequently, the ideal weight ratio (weight enzyme:weight of nanoparticles) was then determined in order to find

the optimum condition allowing for maximum activity. Weight ratio was determined by measuring the mass of immobilized enzyme as indicated by Bradford and associating this value with the mass of corresponding nanoparticles. The mass of magnetic silica nanoparticles was kept constant at 2 mg.

2 mg of **Au-MSNP** (or 1mL of **AuNP**) was added to 200 μL of phosphate buffer (PBS/NaCl, KCl, Na_2HPO_4, KH_2PO_4). The mixture was sonicated for 15 min and added cysteine-containing proteins from 20 μg to 500 μg. The mixture was stirred at 0 °C in a darkroom for 1 day. The mixture was centrifuged at 14000 rpm for 10 min. The amount of protein in the supernatant was determined by a colorimetric method at 595 nm using the Biorad Protein Assay Reagent Concentrate with bovine serum albumin (BSA) as the protein standard.

3. Effect of pH on cellulases activity

The activity assays were carried out over the pH range 3.0-7.0 to determine the pH profiles for the free and immobilized cellulases. 0.1 M citrate–phosphate buffer was used. Hydrolysis reactions were performed using 10 mg of cellulase-bound nanoparticles, 50 μg of microcrystalline cellulose, and 1 mL buffer. The results of pH are presented in a normalized form, with the highest value of each set being assigned the value of 100% activity. The pH stability of the free and immobilized cellulases was ascertained by the residual activity of the enzyme exposed to 3.0-7.0 under optimal conditions. For pH study, an equal amount of free cellulase enzyme (50 μg), as was immobilized on the **AuNP** and **Au-MSNP**, was tested for activity comparisons.

4. Effect of thermal stability

The thermal stability of the free and immobilized cellulases was measured at pH 4.5 and 80 °C. The enzymes were preincubated in citrate-phosphate buffer under optimal conditions for 0-48 h, and the residual activity was determined using *p*NPG as substrate.

Activity measurements

1. BglB assay

BglB activity was determined by measuring p-nitrophenyl release from p-nitrophenyl β-D-glucopyranoside (pNPG). The assay mixture containing 10 mM pNPG in citrate–phosphate buffer (pH 4.5) was incubated with the enzyme for 15 min at 80 °C in a total volume of 1 mL. The reaction was stopped by adding 1 M Na_2CO_3, and absorbance was measured at 405 nm.

2. CMC assay

CBHII activity was ascertained by measuring the amount of reducing sugars released from a 1% carboxymethyl cellulose (CMC) solution in 100 mM sodium acetate buffer (pH 5.0) for 1 h at 50 °C. One unit of activity was defined as the amount of enzyme that produced 1 M of glucose-reducing sugars per minute. The amount of reducing sugars was measured according to the dinitrosalicylic acid (DNS) method.[2]

3. Filter Paper assay

The filter paper assay was carried out by the method of Mandels' using 2 mg Whatman No. 1 filter paper strips as a substrate. The enzymes were diluted in 0.05M citrate buffer, pH 5.0, with a final volume in the assay of 0.5 mL. All assays were carried out in 18 mm test tubes at 50 °C for 1 h. The amount of reducing sugars was measured according to the DNS method.

Reusability assay

Cellulase enzyme complex (each 50 μg) co-immobilized on 5 mg of **Au-MSNP** were subjected to a hydrolysis reaction with microcrystalline cellulose under optimal conditions. After the specified reaction time, the enzyme-bound nanoparticles were magnetically separated and introduced into solution containing fresh substrate. Activity was determined following each recycle until the activity had fallen below 50%.

Additionally, a series of controls were measured in order to compensate for any interference, one containing enzyme alone and another containing substrate alone. Any responses measured by the spectrophotometer from these controls were subtracted from the primary hydrolysis reaction to aide in increasing accuracy among measurements.

Preparation of cystein-tagged β-glucosidase.

The β-glucosidase gene (*bglB*) of *Thermotoga maritima* encodes 725 amino acids with a predicted molecular weight of 81kDa, and belongs to family 3 glycosyl hydrolases. The recombinant plasmid DNA, pRsetA vector harboring *bglB*, was transferred to *E.coli* BL21 for recombinant protein expression. The protein was purified using affinity chromatography and confirmed by SDS-PAGE and obtains 81kDa single protein band.

Amino acid sequence of BglB protein (Thermotoga maritime) :

1 TCATGGTTTG AATCTCTTCT CTCCCTCAAC CAGAAAAATA
41 TCTCTCAACC TTATATCCCT CGAAGATGCA CCGACCCTGA
81 CCTCGTATTC TCCTGACTCG ACAACCCATT CTTTCCCATC
121 GAAACTCGCA AGATCTCTGA GAGGAATTTC CAAGGAGATT
161 TCTTCTGATT CACCCGGGTT CAAAAGTTTT GTTTTGTGAA
201 ACGCTTTCAG CTCCTGGAAG GGTTTGTCTA TTTTTCCTTT
241 TGGAGCTTTG ATGTAGACCT GTGAGACTTC CTTTCCAGCT
281 CTGTCCCCAG TGTTTGTGAT CGTGTACGAC ACTCTGAGCG
321 TCTCACCGTC GATAGCGATT TTTAAATCTT TGTATTCAAA
361 CTTTGTGTAA GAGAGGCCGT AGCCGAATTC GTAGGCAGGT
401 TCCACACCGA AGGTGTCGTA GTACCTGTAT CCCACGTAGA
441 TGTCTTCCTC GTACACCACT CTTTGCGGAT TGTCCTTTGG
481 CTCTCCTGGG AACGTCCAGG ATGGAACGTC CGAGTAATCC
521 TTCGGGAAGG TCGTTGGAAG TTTTCCGGAG GGATTAATCT
561 TTCCCACAAG AACATCGGCC ACTATTCTTC CCATCTCCTG
601 TCCCGCCTGC CAGACGAGAA GAATTCCATC CACAAGGTCT
641 CTCCAGCTTG CGACTTCGAT GGGACTTCCG ATGTTCAGAA
681 GAACCACAAC TTTCTTACCC TGATCGTGGA ATTCTTTCGA
721 GACGGTTTTT ATGAGTTCCA GCTCGTCATC GGAGAGGTAG
761 AAGTCACCTT TCACCGGCTT TCTGTCGTAT CCCTCACCGG
801 AGATCCTACT GATCACAACA ACTGCAACAT CGTTTTTCTT
841 TGCAGCTTTC TTTATCTCTT TTTCTGAGAG GAAATTCTCT
881 GGGAGTTTCG GTTTTATGAC CGTTCCCCAA GAGTCGGTTC

921 TGGGTTTATA TTCCTCTGTT TCTCTCATCT TTTTTATGTA
961 CTCCTCATAA GTGGAAGCGA GTTCTTCGTC GAACTTCATG
1001 TTTCTTTCTT TTATGCCTTC AAGGATAGAG ATCGTGTATC
1041 TCGGATGGGT GTCTCCACTT CCCGTTCCTC CCTTTATTGT
1081 TTCGATTTGA CCGGTGCCAA AGACGGCGAC ATGGGTATTT
1121 TCATCGAACG GAAGAACACC GTTGTTCTCA AGAAGGACAA
1161 CACCCTCCGC ACCTGCTTCG TAGGCGACTT CCGCGTGAGA
1201 TTCGAGATCC GGCTTGTTTG AGTACCTGTA CCCTTTGAAG
1241 GAAGGCGCGT TCACAAGAAC TTTGAGAATG TTTCTCACAC
1281 ACTCATCGAG AACCTCCTCA CTCAATTTTC CCTCCTTCAA
1321 CGCCTCCATG ATTTCTTCTA TTTCATCTCT TCTTTCTGTG
1361 TTCACCTGAT ACGCTTTCCC AGGCATGATC ATATCGTTTC
1401 CGGCCTTGAG CTGTTCTACA GGGTTGTCTC CCGCGTACCA
1441 GTCGCTCATC ACGAAACCGT CAAATCCCCA TTCTTCCCTG
1481 AGAACCTTCT TCAAAAGCCA TTCGTTCTGT GAACAGTATT
1521 TTCCATTCAG TTTGTTGTAA GCGCTCATCA CGGTCCAGGG
1561 TCTTGCTTTC TTGACAGCAA TTTCAAAACC TTTCAGATAT
1601 ATTTCTCTGA GGGCTCGCTC GGACACGATC GTGTCCACTA
1641 CCATCCTGTT CGTTTCCTGG TTGTTCGCGA CAAAGTGTTT
1681 TATGCAGGCT CCCACCCCTT GAGATTGAAC TCCCTTGACA
1721 AAGGCTGAAG CCATTTCACC GGAAAGGACA GGATCTTCTG
1761 AGTAGTACTC GAAATTCCTT CCACAAAGAG GGTTTCTGTG
1801 AATGTTCATC GCAGGTGCAA GAAGCACATC GACACCGTAT
1841 TCCCTAACTT CTTCTCCCAT GGCTTTTCCC ACTTCTTCCA
1881 GAAGGTCTCT GTTCCAGGTA GAAGCGAGCA TGATTTCAAC
1921 GGGAAATGCC GTCGTGTAGT AAGTGTTTTC ATCGTTTTCC
1961 CTTGTGGGAT TTATTCTGAG TCCTGCGGGA CCATCTGCCA
2001 GGACAAACGC AGGAATTCCA AGTCTTGGAA CGGGATGTGT
2041 TTCTCCAGCC GCACCCGCCA CTCTGGAATG TGGGTTCCCA
2081 AAAAGTCCTG GAAGACCAAC CCCCACAACG AGCTTCACCT
2121 TTTCCTCTGT AGTTAACTGA GAGAGAATTT CATCGATCCT
2161 TTCCATTGCTGCTGC

Amino acid sequence of CBHII protein (Neurospora crassa):

1 ATGGCTGCCA AGAAGCTCCT TCTCGCTGCC GCCTTGACGG
41 CCTCTGCCCT CGCCGCTCCC GTCCTCGAGG ATCGTCAGAA
81 CTGCGGCTCT GCCTGGAGCC AGTGTGGTGG CATTGGCTGG
121 TCTGGTGCGA CTTGCTGCTC CTCCGGCAAC TCCTGCGTTG
161 AGATCAACTC TTACTACTCC CAGTGCTTGC CCGGCGCTCA
201 GGTTACCACC ACTGCTGGGG CTTCTTCCAC CAGCCCTACC
241 AGCACCAGCA AGGTCTCTAG CACCACCAGC AAGGTTACCA
281 GTAGCAGCGC TGCCCAGCCC ATCACCACTA CTACCGCTCC
321 TTCGGTGCCC ACCACCACCA TTGCTGGCGG TGCTTCCTCC
361 ACTGCTAGCT TCACTGGCAA CCCCTTCCTT GGTGTTCAGG
401 GCTGGGCCAA CAGCTACTAC TCCTCCGAGA TCTACAACCA
441 TGCCATTCCT TCCATGACTG GCAGCTTGGC TGTTCAGGCG
481 TCTGCCGTTG CCAAGGTCCC CACCTTCCAG TGGCTCGACC
521 GCAACGTCAC CGTGGACACT CTCATGAAGA GCACCCTCGA
561 AGAGATCCGC GCGGCCAACA AGGCCGGTGC CAACCCTCCC
601 TACGCCGCTC ACTTTGTCGT CTACGATCTC CCTGACCGTG
641 ACTGCGCTGC TGCCGCCTCC AACGGCGAGT TCTCCATCGC
681 CAACGGCGGC GTTGCCAACT ACAAGACCTA CATCAACGCC
721 ATCCGCAAGC TCCTGATCGA GTACTCGGAC ATCCGCACCA
761 TTCTCGTCAT CGAGCCCGAC TCGCTTGCCA ACCTCGTCAC
801 CAACACCAAC GTTGCCAAAT GTGCCAACGC CGCCTCCGCC
841 TACAAGGAGT GCACCAACTA CGCCATCACC CAGCTCGACC
881 TTCCCCACGT CGCCCAGTAC CTCGATGCTG GTCACGGTGG
921 CTGGCTCGGC TGGCCTGCTA ACATCCAGCC TGCTGCCACC
961 CTCTTCGCTG ACATCTACAA GGCCGCCGGA AAGCCCAAGT
1001 CCGTCCGTGG TCTCGTCACC AACGTCTCCA ACTACAACGG
1041 CTGGTCACTC TCCTCCGCTC CTTCGTACAC CACGCCCAAC
1081 CCCAACTACG ACGAGAAGAA GTACATTGAG GCTTTCTCCC
1121 CTCTCCTCAA CGCTGCCGGC TTCCCTGCCC AGTTCATCGT
1161 TGACACGGGC CGTTCTGGCA AGCAGCCGAC TGGCCAGATC

1201 GAGCAGGGTG ACTGGTGCAA CGCCATCGGC ACCGGTTTCG
1241 GTGTCCGCCC TACCACCAAC ACTGGCTCCT CGTTGGCTGA
1281 TGCCTTCGTC TGGGTCAAGC CCGGTGGTGA GTCCGATGGT
1321 ACCAGCGACA CCTCGGCTAC CCGCTATGAC TACCACTGCG
1361 GTCTCTCGGA TGCCCTCAAG CCTGCCCCTG AGGCTGGTCA
1401 GTGGTTCCAG GCTTACTTTG AGCAGCTGCT CAAAAACGCT
1441 AACCCCGCTTTCTGA

Amino acid sequence of EGIVCBDII protein (Ruminococcus albus gene for endo-1,4-beta-glucanase):

1 ATGTTGGATA AACTTAAAGT AATAAATGGA AAACTGACAG
41 CCGGTGAGAA ACCAGTAAGA CTTTTCGGGT TATCAACTCA
81 CGGTATAGCC TGGTATCCGG AATATATTTG TGAAGAATCA
121 TTCAATGCCC TAAAAAAAGA CTGGCGTACA AACTGTATAA
161 GGATAGCAAT GTACACAGAC GAATTCAGAG GGTACTGCAA
201 GGACGGCAAC AAACAGCATC TGAAAGAGCT GATCGAGAAA
241 GGCGTTGTTA TTGCAGAAAA ACTGGATATG TATGTAATAG
281 TAGACTGGCA CGTTCTGTGT GATCAGGATC CGATGAAATA
321 TATTGATGAA GCTGAAGAGT TTTTCAGTGA TATGTCAAAG
361 AGGTTCGCCA ATAAAACAAA CGTTATATAC GAGATATGTA
401 ACGAACCTAA CTGCAGCGGC ACATGGGATA AAATAACAGA
441 ATATGCAGAC AGGATAATCC CGATAATCCG CAGTAACTCC
481 CCCGATGCAC TTATTGTCAC AGGAACATCA ACGTGGTCGC
521 AGGACATACA CTGTGCGCTTG AAAAGCCGCT GAAATGGGAC
561 AATGTTATGT ACTCTCTGCA TTTCTATGCT GCTACACAC
601 AAGGGTACA CTGCGCAGCA GACTGGAACG ATGTATTGAA
641 GCCGGGCTTC CGGTTTTTAT CAATGAATTC AATCTGTGTG
681 AAGCTAGCGG TAAGGGCGAT ATCGATATAG ATGAAGCAAA
721 TGCATGGTAT GAGGTAATAG ACAGACTCGG GCTGAGCTGC
761 ATAAGCTGGT GCCTTTCAAA CAGTGGAGAT ACCTGCGGCG
801 TTTTTACCCA AAATTGTACA AAGCTTTCAG GCTGGACGGA
841 TGAAGATATA AAAACATCGG GCAAAATAAT AAAAGGCTGG

881 TTTGAGGCAT TTGCAGATGA GGAGAATACA AATGAACAAT

721 GTTTTCGAAG TTCACCAGTG CCTGCACCTG GTGATAACAC

761 AAGAGACGCA TATTCTATCA TTCAGGCCGA GGATTATGAC

801 AGCAGTTATG GTCCCAACCT TCAAATCTTT AGCTTACCAG

841 GTGGTGGCAG CGCCATTGGC TATATTGAAA ATGGTTATTC

881 CACTACCTAT AAAAATATTG ATTTTGGTGA CGGCGCAACG

921 TCCGTAACAG CAAGAGTAGC TACCCAGAAT GCTACTACCA

961 TTCAGGTAAG ATTGGGAAGT CCATCGGGTA CATTACTTGG

1001 AACAATTTAC GTGGGGTCCA CAGGAAGCTT TGATACTTAT

1041 AGGGATGTAT CCGCTACCAT TAGTAATACT GCGGGTGTAA

1081 AAGATATTGT TCTTGTATTC TCAGGTCCTG TTAATGTTGA

1121 CTGGTTTGTA TTCTCAAAAT CAGGAACTA CTAGTGGTAC

1161 CGTCGACTTG TTGCTGTTGC CTCGA

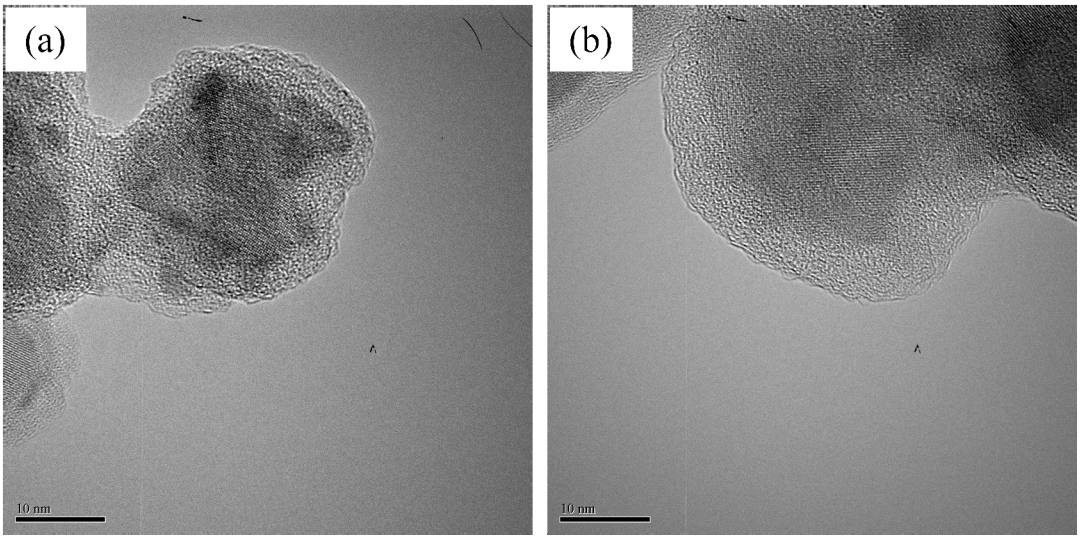


Figure S1. TEM images of **MSNP** (a) before and (b) after functionalization with mercaptopropyltriethoxysilane. The images show nanoparticles with dark Fe_3O_4 cores (a single-crystalline cubic spinel structure**,** about 20 nm in diameter) surrounded by lighter amorphous silica shells about 3–4 nm thick. The diameter of **MSNP** also increased by another 2–3 nm after coating with MPTES.

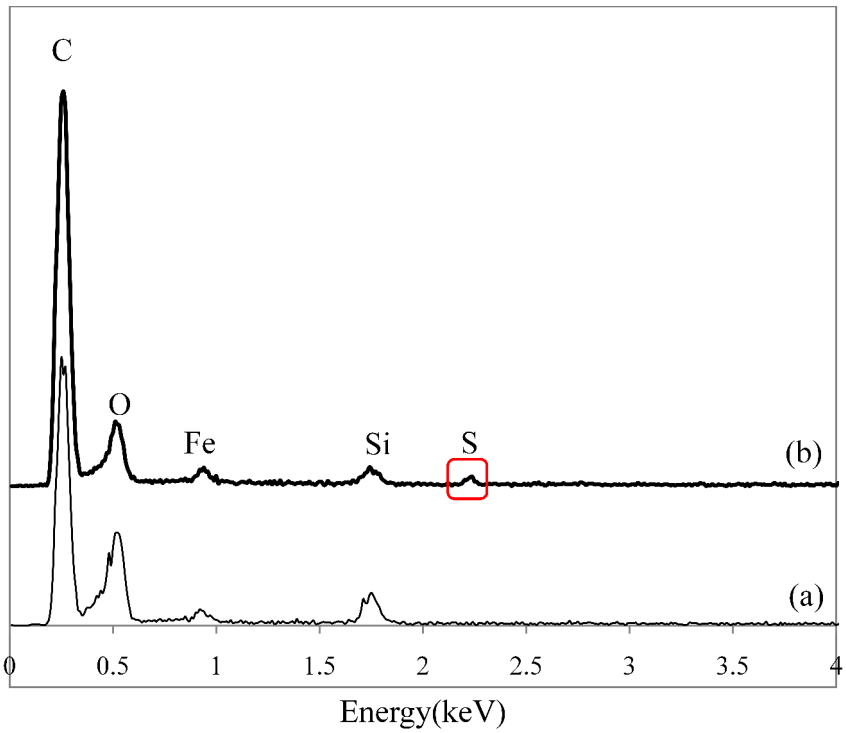


Figure S2. EDX spectrums of **MSNP** (a) before and (b) after functionalization with mercaptopropyltriethoxysilane. EDX measurements confirmed the presence of sulfur atoms.

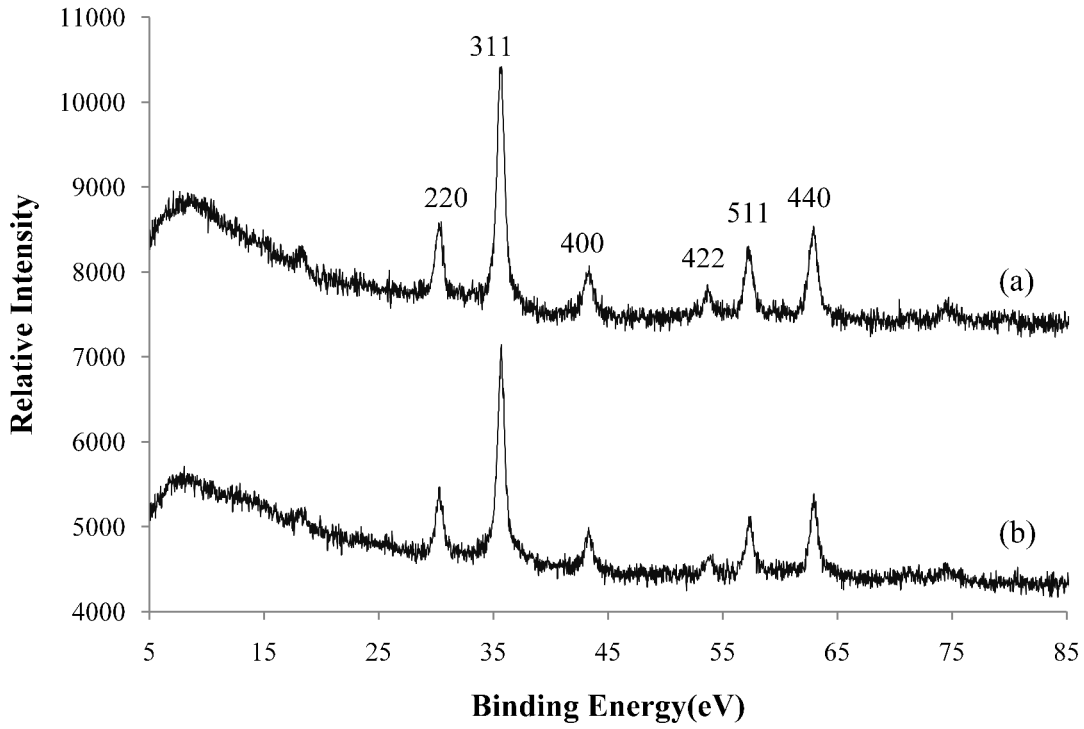


Figure S3. XRD patterns of **MSNP** (a) before and (b) after functionalization with mercaptopropyltriethoxysilane. XRD diffractograms of the **MSNP** exhibited patterns consistent with those of spinel ferrites described in the literature.[3] The diffraction peaks at (220), (311), (400), (422), (511), and (440) are characteristic of magnetite. The same peaks were observed in the diffractograms of **MSNP** with immobilized MPTES.

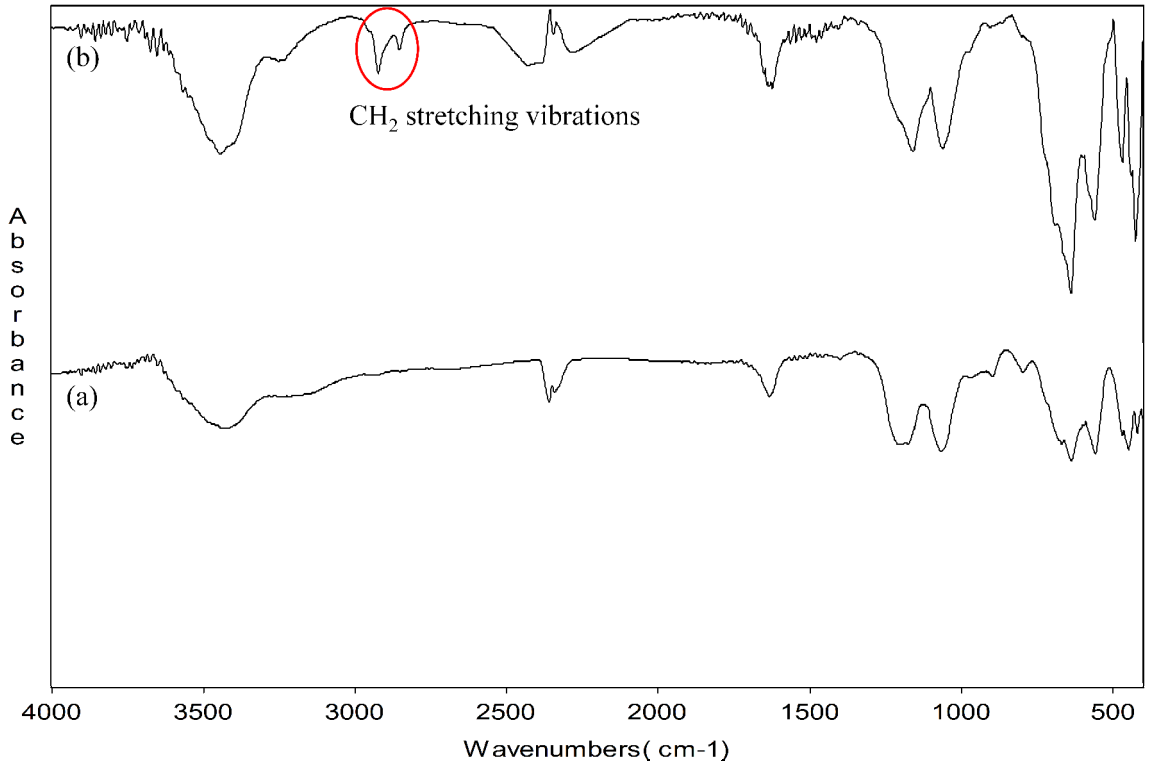


Figure S4. FT-IR spectra of **MSNP** (a) before and (b) after functionalization with mercaptopropyltriethoxysilane: (KBr pellet). Following the attachment of MPTES to **MSNP**, a CH_2 stretching peak appeared at 2920 cm^{-1} in the FT-IR spectrum, strongly indicating covalent attachment of the MPTES group to the surface of the **MSNP**.[4]

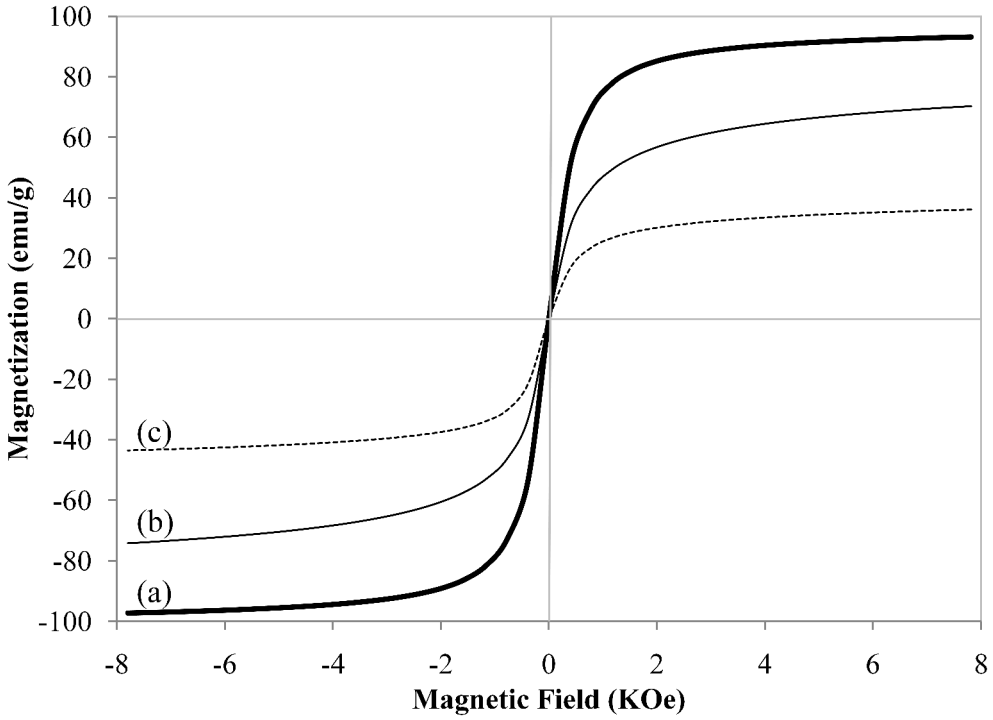


Figure S5. Magnetization curve of (a) magnetic silica nanoparticles(**MSNP**) (saturation magnetization value : 91.1 emu/g), (b) **MSNP** functionalized with mercaptopropyltriethoxysilane (65.6 emu/g), and (c) the gold-doped **MSNP(Au-MSNP)** (33.3 emu/g), obtained by VSM at room temperature.

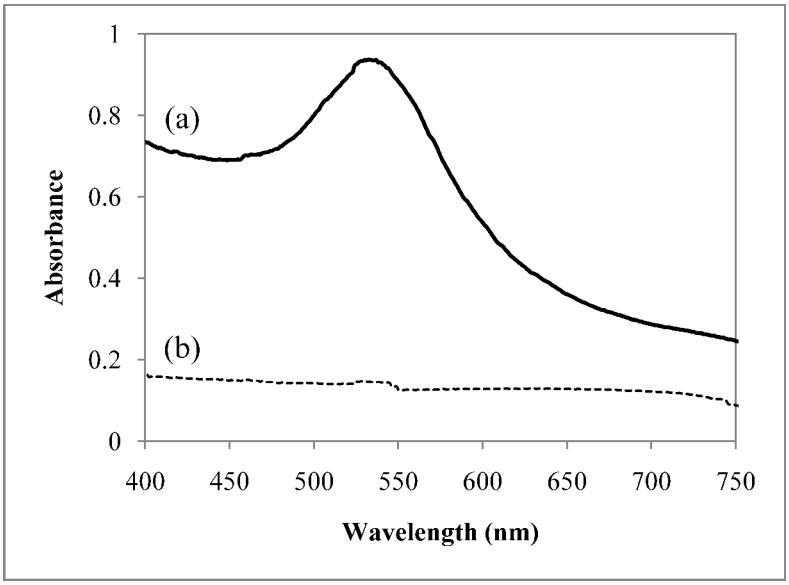


Figure S6. UV-Vis spectrum of (a) colloidal gold solution and (b) after stirring with SH-functionalized **MSNP**. The surface plasmon resonance (SPR) of particles in the colloidal gold solution can be seen at 530 nm.

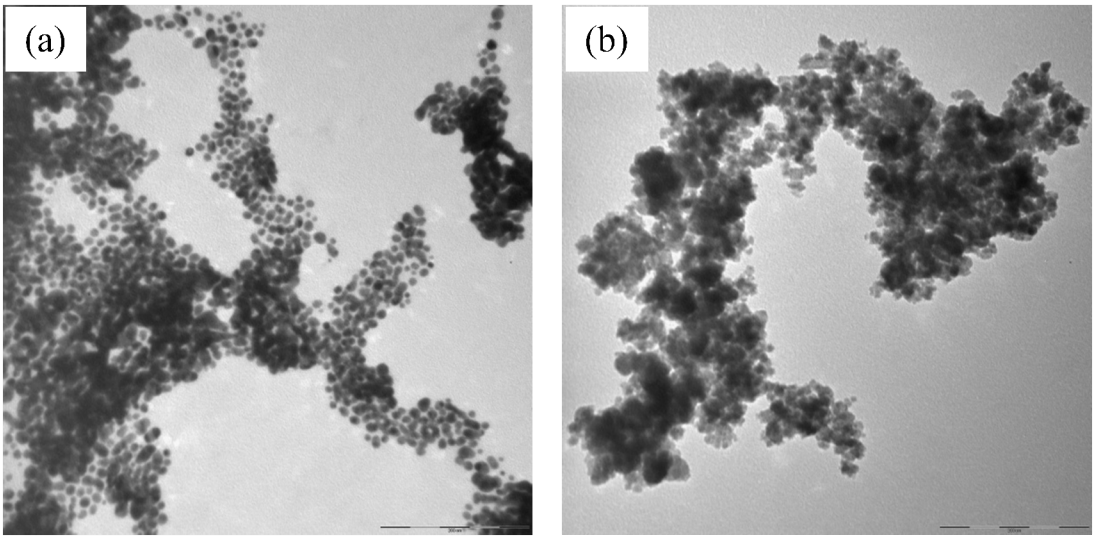


Figure S7. TEM images of (a) **AuNP@cellulase**s and (b) **Au-MSNP@cellulases**.

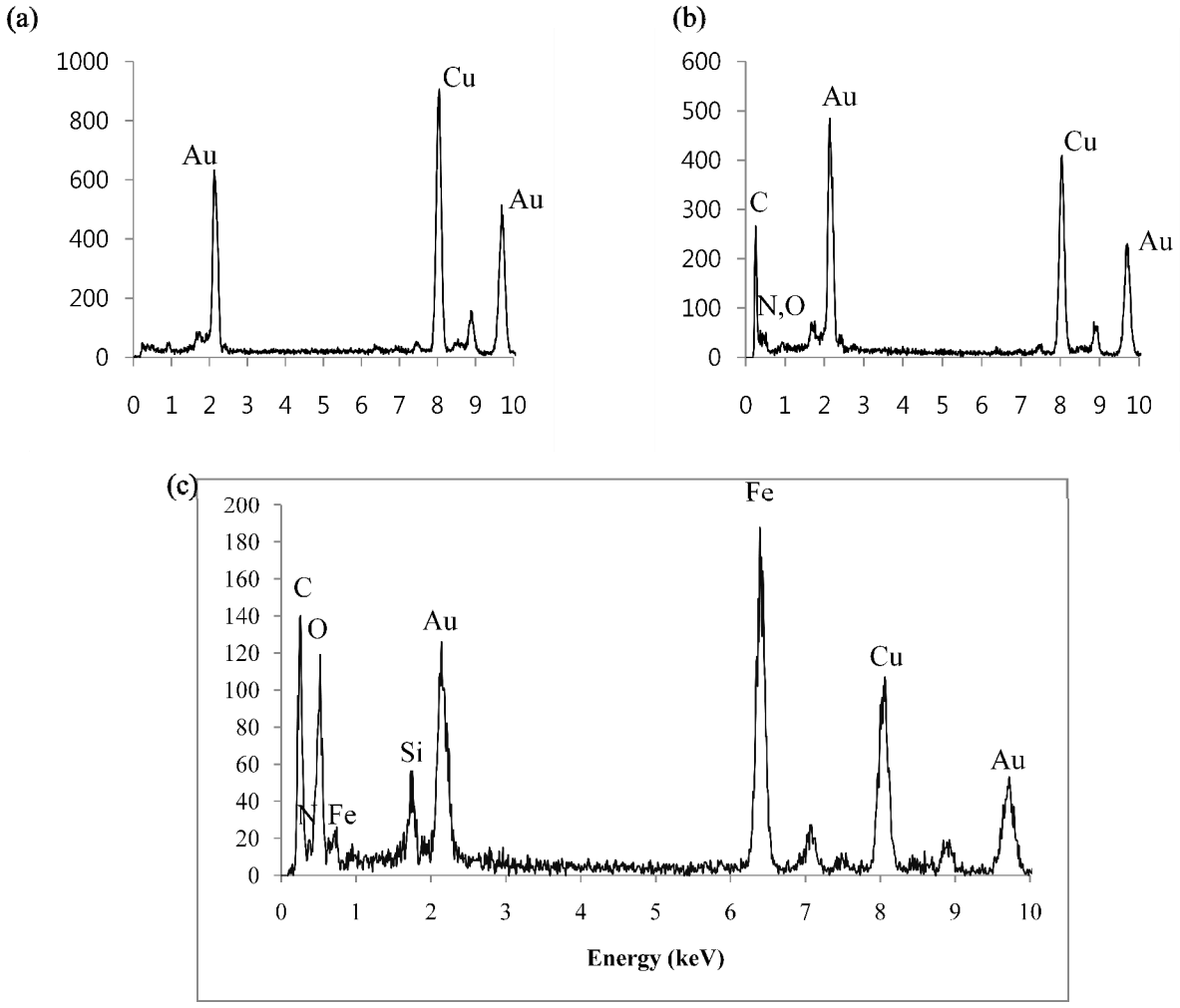


Figure S8. EDX spectrums of (a) **AuNP**, (b) **AuNP@cellulase**s, and (c) **Au-MSNP@Cellulases**.

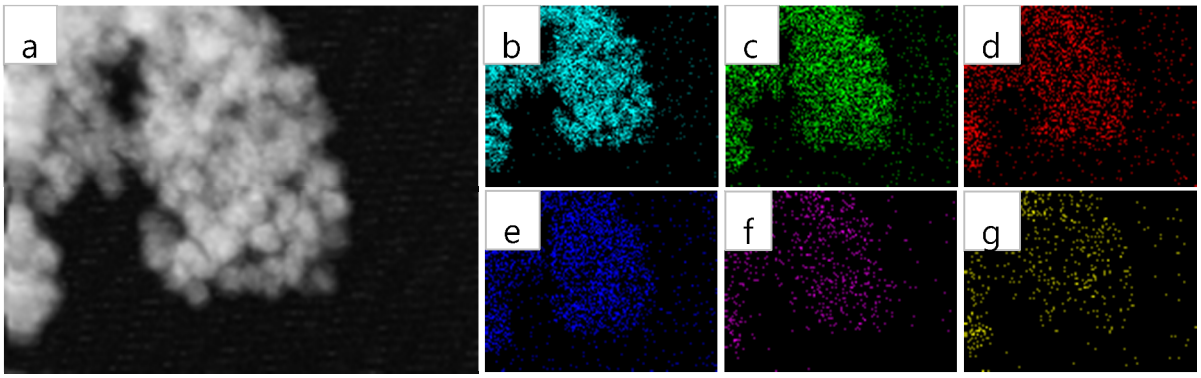


Figure S9. EDX mapping of the cellulases co-immobilized **Au-MSNP.** (a) Zero-loss image, (b) iron component, (c) oxygen component, (d) silicon component, (e) gold component, (f) carbon component and (f) nitrogen component .

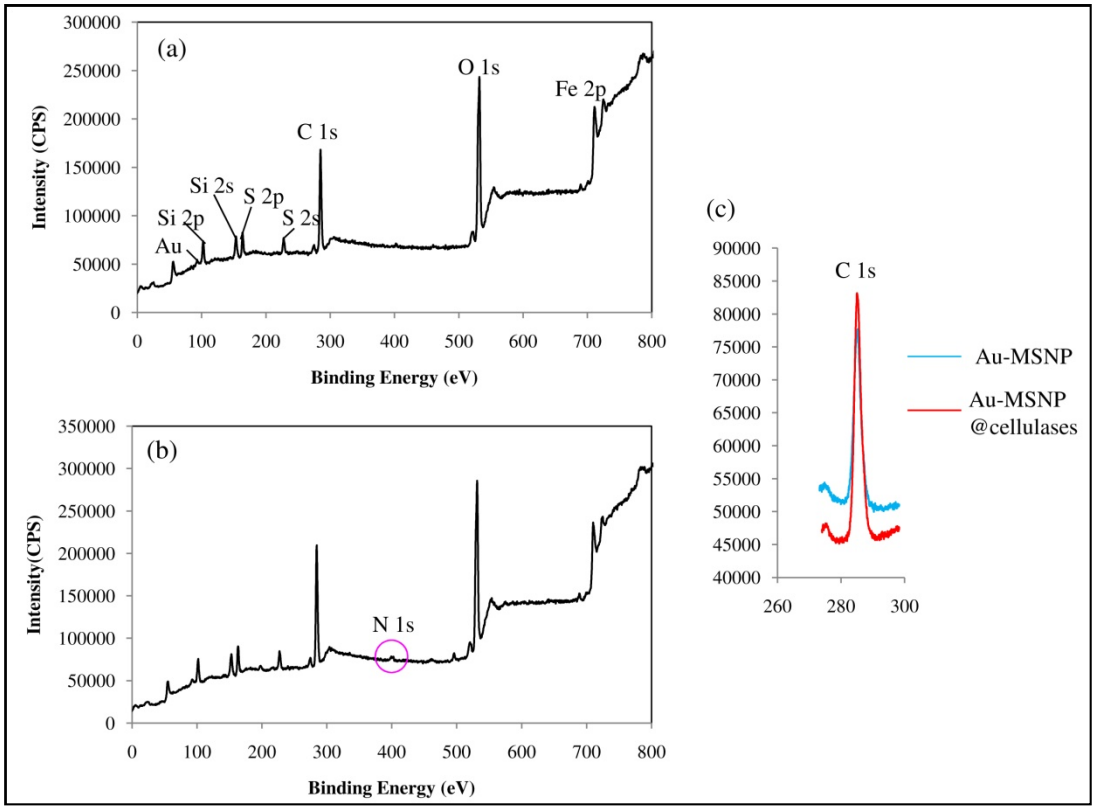


Figure S10. XPS analysis of (a) **Au-MSNP**, and (b) **Au-MSNP@cellulases**. (c) C1s XPS spectra.

Table S1. Elemental analysis of **Au-MSNP** and **Au-MSNP@cellulases** from evaluation by XPS.

	Peak	Position BE(ev)	FWHM (eV)	Raw area (CPS)	Area (N)	SF	Atomic concentration (%)
Au-MSNP	Fe 2p	711.12	5.17	657458.88	5822.15	16.42	5.56
	Si 2p	102.44	2.72	67106.92	11271.75	0.817	10.77
	C 1s	284.98	2.94	338946.93	47173.69	1	45.07
	O 1s	531.66	4.46	712316.35	34622.05	2.93	33.08
	S 2p	163.84	2.65	69924.27	5771.76	1.67	5.51
Au-MSNP @cellulases	Fe 2p	710.9	4.52	475527.26	4210.93	16.42	3.21
	Si 2p	102.19	2.78	81625.15	13709.46	0.817	10.44
	C 1s	284.86	2.95	456887.03	63587.65	1	48.43
	O 1s	531.57	4.29	807561.4	39251.03	2.93	29.89
	S 2p	163.75	2.7	104171.35	8598.56	1.67	6.55
	N 1s	401.69	5.93	24889.91	1944.27	1.8	1.48

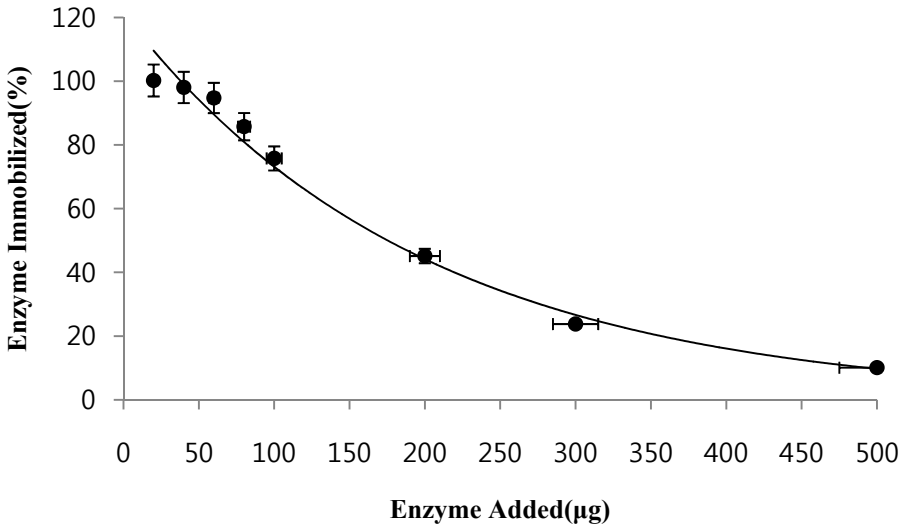


Figure S11. Binding efficiency for varying amounts of cellulases added to 2 mg of **Au-MSNP**.

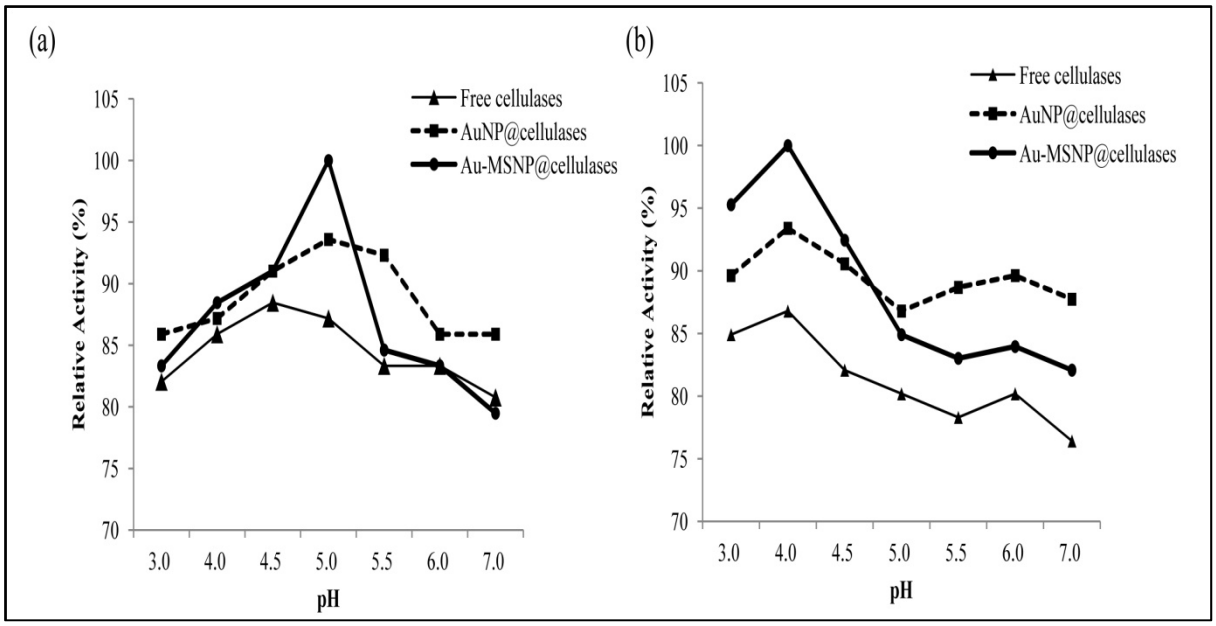
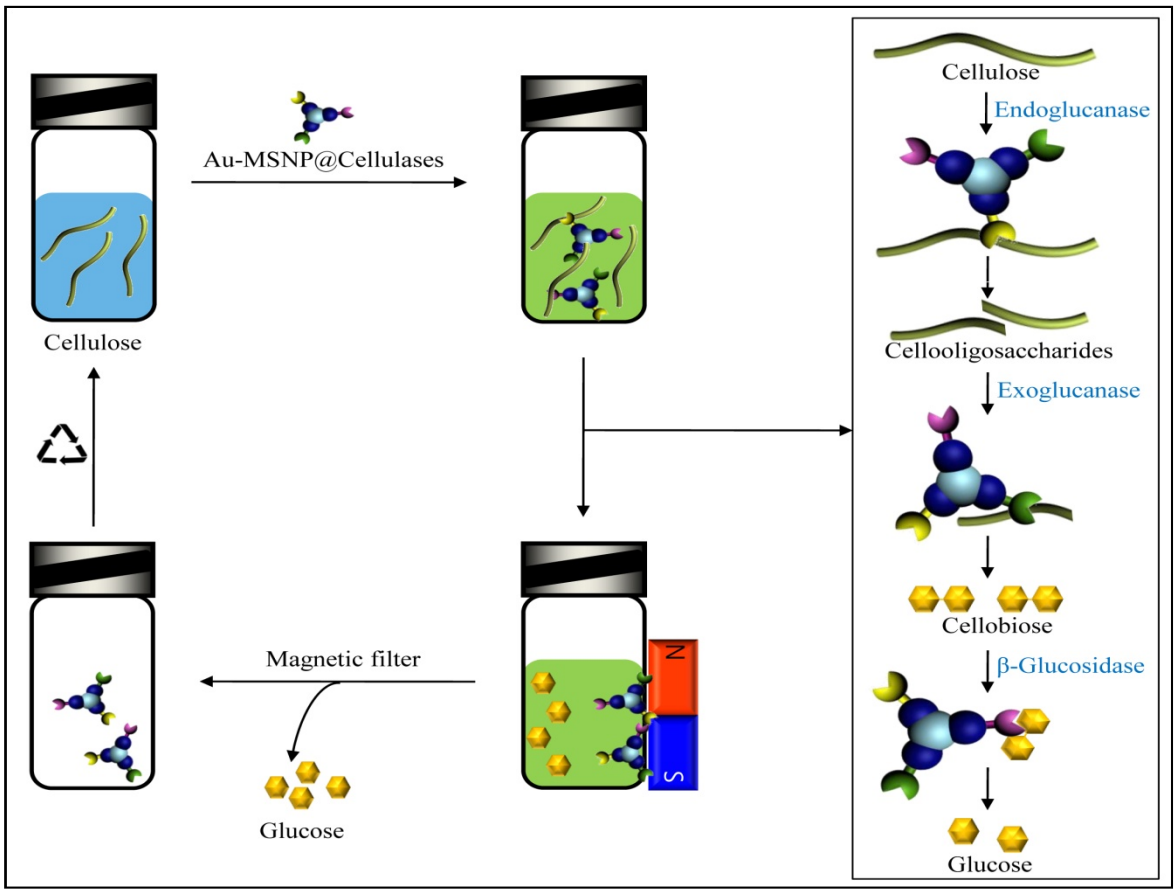


Figure S12. Effect of pH on free and co-immobilized cellulases activity using (a) CMC (at 50 ºC) and (b) filter paper assays (at 50 ºC).



Scheme S1. Illustration of the mechanism if enzymatic cellulose degradation.

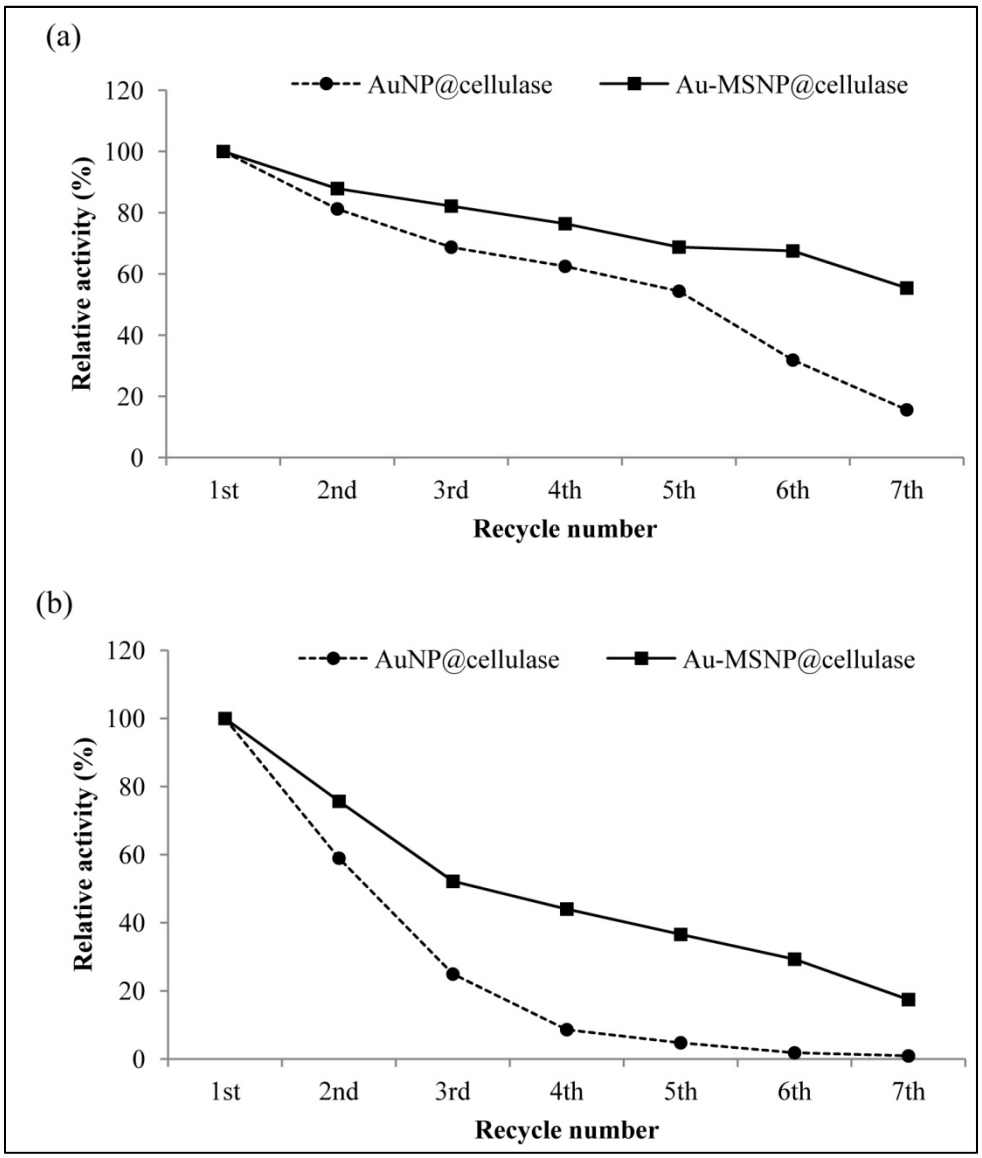


Figure S13. Activity loss over 7 reuses of cellulase enzymes immobilized on **AuNP** and **Au-MSNP**.; (a) Enzymatic activity on CMC, (b) Enzymatic activity on filter paper.

References.

1. M. Bradford, *Anal. Biochem*., 1976, **72**, 248.
2. (a) J. L. Miller, R. Blum, W. E. Glennon, and A. L. Burton, *Anal. Biochem*., 1960, **1**, 127. (b) G. L. Miller, *Anal. Chem*., 1969, **31**, 426.
3. J. H. Chang, K. H. Kang, J. Choi, and Y. K. Jeong, *Superlattices Microstruct*. 2008, **44**, 442.
4. D. R. Bae, S. J. Lee, S. W. Han, J. M. Lim, D. Kang, J. H. Jung, *Chem. Mater*. 2008, **20**, 3809.