Supporting Information

Fluorescent Receptor-Immobilized Silica-Coated Magnetic nanoparticles as a General Binding Agent for Histidine-Tagged Proteins

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Experimental Section

Materials.

4'-Chloro-2,2':6',2"-terpyridine and 3-(triethoxysilyl)propyl isocyanate were purchased from Aldrich. All other materials were of analytical grade and commercially available, including ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ammonium hydroxide (25% [w/w]), tetraethyl orthosilicate (TEOS), cupper chloride, imidazole, and ethylenediaminetetracetic acid (EDTA).

Instruments.

^{1}H and ^{13}C NMR spectra were measured with a Bruker 300 apparatus and Varian 600 apparatus. IR spectra were obtained for KBr pellets, in the range 400-4000 cm^{-1}, with a Shimadzu FT-IR 8400S instrument, and the MS spectrum was obtained with a JEOL JMS-700mass spectrometer. Field Emission Transmission Electron Microscope (FE-TEM) was measured with a Tecnai F20. High Resolution X-Ray Diffractometer (HR-XRD) was measured with a X'Pert PRO Multi Purpose X-Ray Diffractometer. The fluorescence was measured with a fluorescence spectrometer (RF-5301 fluoremeter). Magnetic properties were measured with a vibrating sample magnetometer (VSM, model-7404, Lakeshore) at room temperature.

Synthesis of Compound 3.

To a stirred suspension of powdered KOH (1.05 g, 18.7 mmol) in dry DMSO (10 mL) at 80°C, the 3-amino-1-propanol (0.28 g, 3.74 mmol) was added. After 30 min, 4'-chloro-2,2':6',2"-terpyridine was added (1.00 g, 3.74 mmol) and the mixture was stirred for 4 h at 80 °C and then poured into 200 mL of distilled water. The aqueous phase was extracted with CH_2Cl_2 (3×200 mL). The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo*, and the residue was recrystalized from ethyl acetate to give 0.68 g (59 %) of **3**. mp 115.8 °C; ^{1}H NMR ($CDCl_3$) δ 8.83 (d, 2H, ArH, J = 4.2 Hz), 8.73 (d, 2H, ArH, J = 8.1 Hz), 8.14 (s, 2H, ArH), 7.99 (t, 2H, ArH, J = 7.5 Hz), 7.47 (m, 2H, ArH), 4.49 (t, 2H, -O-CH_2-CH_2-, J = 6.0 Hz), 3.17 (m, 2H, -CH_2-CH_2-NH_2), 2.24 (m, 2H, -CH_2-CH_2-CH_2-); ^{13}C NMR ($CDCl_3$) δ 167.03 (-C=O), 157.02, 156.03, 148.98, 136.80, 123.82, 121.36, 107.37 (Ar), 66.02, 40.96, 38.76 (-CH_2).

Synthesis of Compound 1.

To a stirred solution 0.60 g (1.96 mmol) of compound **3** in 30 mL of $CHCl_3$, 0.58 g (2.34 mmol) of 3-(triethoxysilyl)propyl isocyanate was added and the reaction mixture was refluxed for 12 h under the nitrogen atmosphere. The solvent was evaporated and then poured into 30 mL of distilled water. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo* to give 0.82 g (76 %) of **1**. mp 91.5 ℃; ^{1}H NMR ($CDCl_3$) δ 8.66 (d, 2H, ArH, J = 3.0 Hz), 8.60 (d, 2H, ArH, J = 8.1 Hz), 7.99 (s, 2H, ArH), 7.85 (t, 2H, ArH, J = 7.5 Hz), 7.33 (q, 2H, ArH, J = 4.8 Hz), 4.97 (t, 1H, -NH, J = 7.5 Hz), 4.88 (t, 1H, -NH, J = 7.5 Hz) 4.30 (t, 2H, -O-CH_2-CH_2-), 3.80 (m, 6H, -CH_2-CH_3), 3.41 (q, 2H, -CH_2-CH_2-NH-, J = 6.3 Hz), 3.16 (m, 2H, -NH-CH_2-CH_2-), 2.06 (m, 2H, -CH_2-CH_2-CH_2-), 1.61 (m, 2H, -CH_2-CH_2-CH_2-), 1.21 (m, 9H, -O-CH_2-CH_3), 0.63 (m, 2H, -CH_2-CH_2-Si-); ^{13}C NMR ($CDCl_3$) δ 166.93 (-C=O), 158.42, 157.05, 155.97, 148.94, 136.84, 123.86, 121.37, 107.33 (Ar), 66.18, 58.38, 42.91, 37.50, 29.49, 23.55 (-CH_2-), 18.23 (-CH_3), 7.54 (-CH_2-Si); IR (KBr) 3236 (NH), 3065 (ArH), 2970-2380 (aliphatic -CH), 1722 (-C=O) 1632 (-CO) 1573 (NH); MS (FAB-MS) m/z 553.73 (calcd 553.27). Elemental analysis: calculated for $C_{28}H_{29}N_5O_5Si$: C, 60.73; H, 7.10; N, 12.65 %. Found C, 60.75; H, 7.09; N, 12.00 %.

Preparation of Magnetic Nanoparticles (MNPs).

$FeCl_3$•$6H_2O$ (1.0 M) and $FeCl_2$•$4H_2O$ (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 Silica-Coated Magnetic Nanoparticles (SMNPs).

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□ for 24 h and dense silica layer onto magnetite surfaces. Finally, the product was washed with ethanol for 3 times and dried at 60□ for 12 h in vacuum oven.

Preparation of Terpyridine-Immobilized Silica-Coated Magnetic Nanoparticles (TSMNPs).

The particles (1.0 g) were resuspended in toluene (50 mL) under sonication for 30 min. 0.5 g of compound **1** was added and the reaction mixture was refluxed for 24 h. After cooling to room temperature, the particles were filtered and washed with toluene and dried at 60□ for 12 h in vacuum oven.

Immobilizing Cu(II) Ions onto the Surface of TSMNPs.

The nanoparticles (100 mg) were vortex-mixed in aqueous cupper chloride solution (0.1 M, 5 mL) for 1 h. The solution was removed by magnetic separation, and the **TSMNPs-Cu(II)** conjugates were rinsed with deionized water before use. When estimating the binding capacity of Cu(II) ions on the nanoparticles, the **TSMNPs-Cu(II)** in the suspension was collected by magnetic separation .

Protein Assay.

The purified proteins were determined by SDS PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). SDS PAGE was performed on 12% polyacrylamide gels in the presence of sodium dodecyl sulfate, using the PageRuler Unstained Protein Ladder (Fermentas, St. Leon-Rot, Germany) as a molecular weight reference. Electrophoresis was carried out for about 1 h at 80 V (constant voltage) and then for 3-5 h at 120 V (constant voltage) in TGS buffer (25 mM tris base, 192 mM glycine, 0.1% (w/v) SDS).

Separating Protein.

4 mL cell lysate (*BglB*, which have been transfected with plasmid encoding 6xHis-tagged protein) was added **TSMNP-Cu^{2+}** (40 mg) and pipetted with these particles, allowing 5 min for interaction. After applying a magnet, the 6xHis-tagged protein was attracted in several seconds to the wall of tube. After the aqueous phase was removed, these particles were washed with washing buffer (200 mL) to remove the residue of 6xHis-tagged protein and other non-specific bound proteins. Afterwards, the particles were washed in turn by 50 mM, 2500 mM or 500 mM imidazole buffer solutions for testing the binding strength of **TSMNPs-Cu^{2+}**. Denative electrophoresis (SDS-PAGE) was done for each sample obtained, to verify the purity of separated proteins in each step. The imidazole bound particles can be recycled for used after a treatment with EDTA (100 mM).

Amino acid sequence of BglB protein:

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   1 tcatggtttg aatctcttct ctccctcaac cagaaaaata tctctcaacc ttatatccct
  61 cgaagatgca ccgaccctga cctcgtattc tcctgactcg acaacccatt ctttcccatc
 121 gaaactcgca agatctctga gaggaatttc caaggagatt tcttctgatt cacccgggtt
 181 caaaagtttt gttttgtgaa acgctttcag ctcctggaag ggtttgtcta tttttccttt
 241 tggagctttg atgtagacct gtgagacttc ctttccagct ctgtccccag tgtttgtgat
 301 cgtgtacgac actctgagcg tctcaccgtc gatagcgatt tttaaatctt tgtattcaaa
 361 ctttgtgtaa gagaggccgt agccgaattc gtaggcaggt tccacaccga aggtgtcgta
 421 gtacctgtat cccacgtaga tgtcttcctc gtacaccact ctttgcggat tgtcctttgg
 481 ctctcctggg aacgtccagg atggaacgtc cgagtaatcc ttcgggaagg tcgttggaag
 541 ttttccggag ggattaatct ttcccacaag aacatcggcc actattcttc ccatctcctg
 601 tcccgcctgc cagacgagaa gaattccatc cacaaggtct ctccagcttg cgacttcgat
 661 gggacttccg atgttcagaa gaaccacaac tttcttaccc tgatcgtgga attctttcga
 721 gacggttttt atgagttcca gctcgtcatc ggagaggtag aagtcacctt tcaccggctt
 781 tctgtcgtat ccctcaccgg agatcctact gatcacaaca actgcaacat cgtttttctt
 841 tgcagctttc tttatctctt tttctgagag gaaattctct gggagtttcg gttttatgac
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1081 ttcgatttga ccggtgccaa agacggcgac atgggtattt tcatcgaacg gaagaacacc
1141 gttgttctca agaaggacaa caccctccgc acctgcttcg taggcgactt ccgcgtgaga
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1261 tttgagaatg tttctcacac actcatcgag aacctcctca ctcaattttc cctccttcaa
1321 cgcctccatg atttcttcta tttcatctct tctttctgtg ttcacctgat acgctttccc
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1501 ttcgttctgt gaacagtatt ttccattcag tttgttgtaa gcgctcatca cggtccaggg
1561 tcttgctttc ttgacagcaa tttcaaaacc tttcagatat atttctctga gggctcgctc
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1621 ggacacgatc gtgtccacta ccatcctgtt cgtttcctgg ttgttcgcga caaagtgttt
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1921 gggaaatgcc gtcgtgtagt aagtgttttc atcgttttcc cttgtgggat ttattctgag
1981 tcctgcggga ccatctgcca ggacaaacgc aggaattcca agtcttggaa cgggatgtgt
2041 ttctccagcc gcacccgcca ctctggaatg tgggttccca aaaagtcctg gaagaccaac
2101 ccccacaacg agcttcacct tttcctctgt agttaactga gagagaattt catcgatcct
2161 ttccat

Amino acid sequence of Cel5A protein:

1 atgggtgttg atccttttga aaggaacaaa atattgggaa gaggcattaa tataggaaat
61 gcgcttgaag caccaaatga gggagactgg ggagtggtga taaaagatga gttcttcgac
121 attataaaag aagccggttt ctctcatgtt cgaattccaa taagatggag tacgcacgct
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661 aagtggggat ctccagatga tcagaaacat ttgatagaag aattcaattt tatagaagaa
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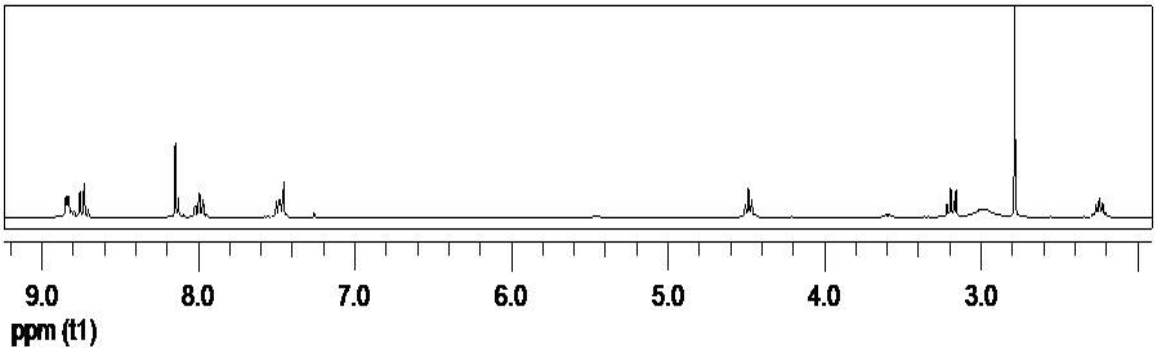

Figure S1. ^{1}H-NMR of compound **3**.

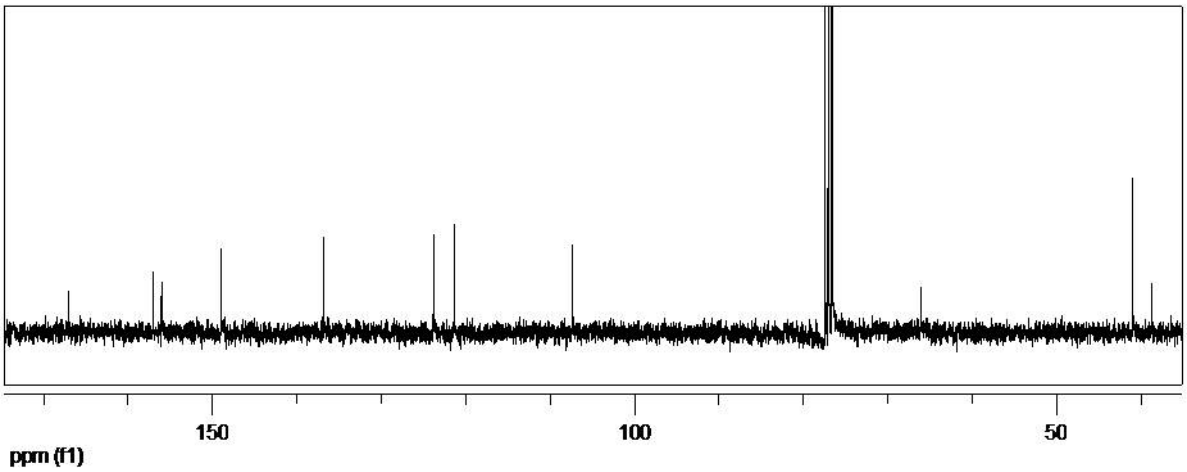

Figure S2. ^{13}C-NMR of compound **3**.

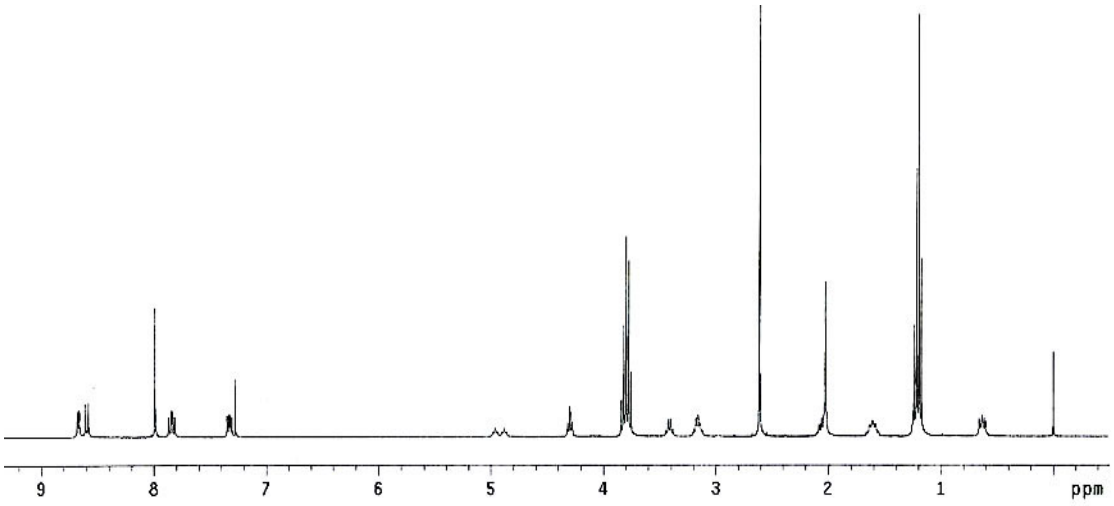

Figure S3. ^{1}H-NMR of compound **1**.

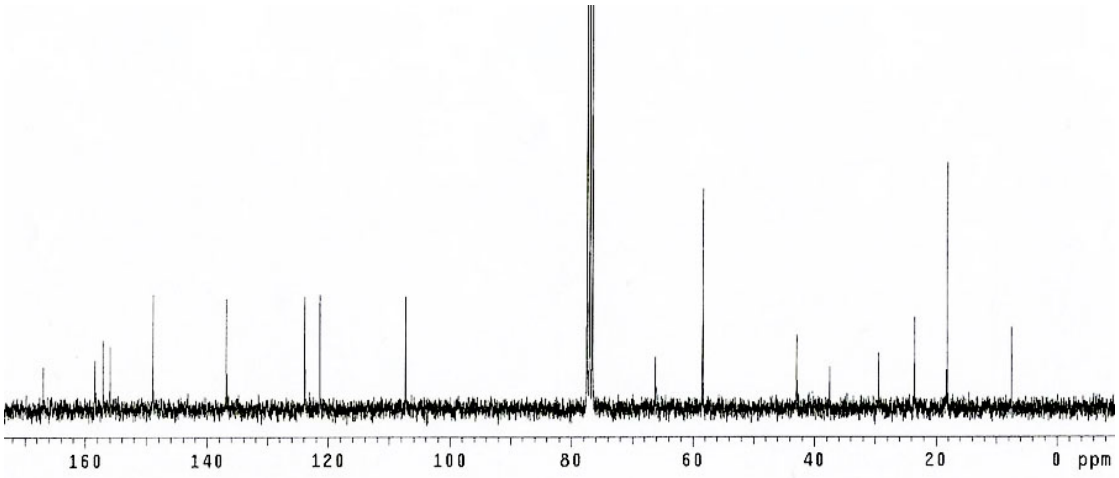

Figure S4. ^{13}C-NMR of compound **1**.

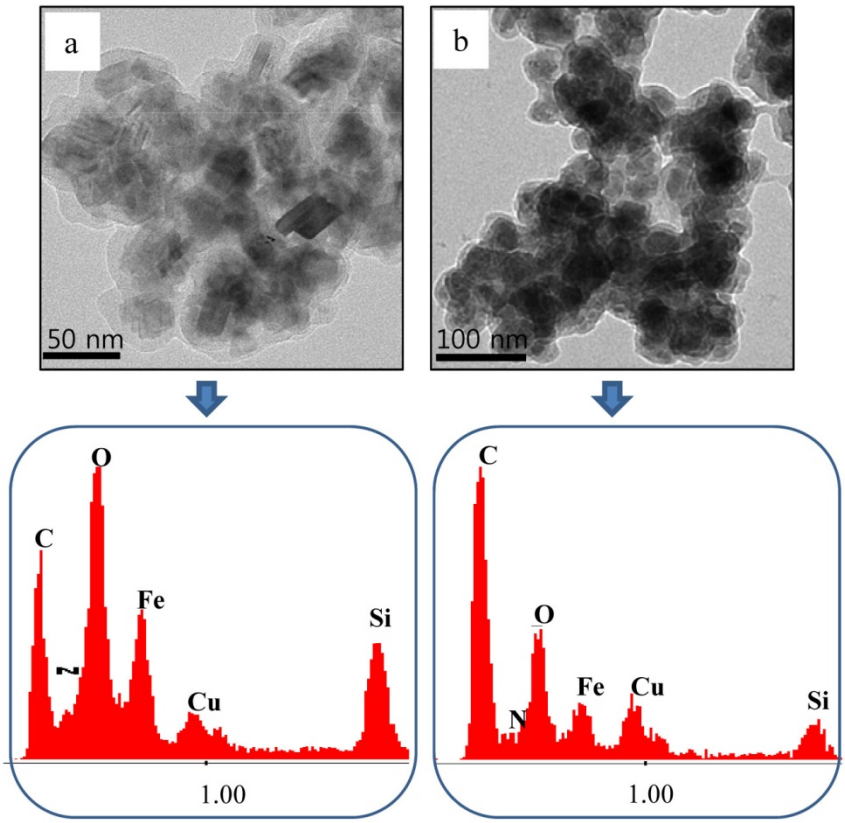

Figure S5. TEM images of **TSMNPs-Cu^{2+}** (a) before and (b) after addition of histidine.

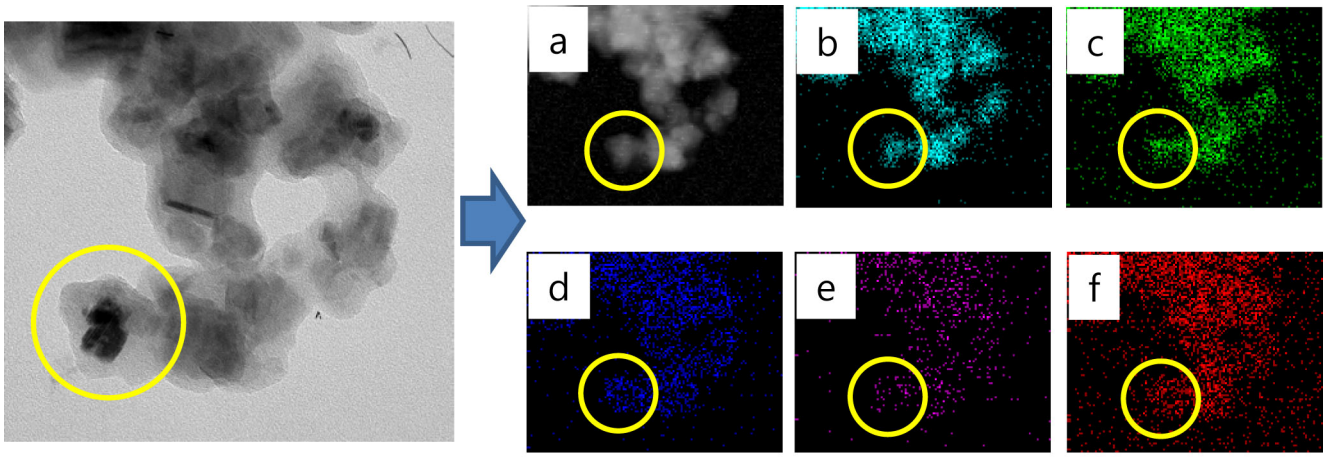

Figure S6. EDX mapping of the **TSMNPs-Cu^{2+}.** (a) Zero-loss image, (b) iron component, (c) oxygen component, (d) silicon component, (e) nitrogen component, and (f) cupper component.

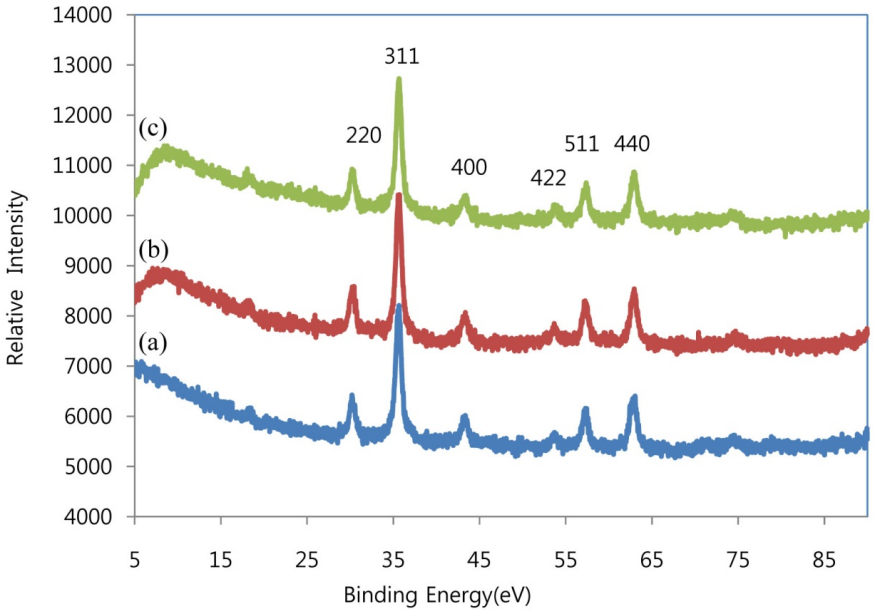

Figure S7. XRD patterns of (a) **MNPs**, (b) **SMNPs**, and (c) **TSMNPs**.

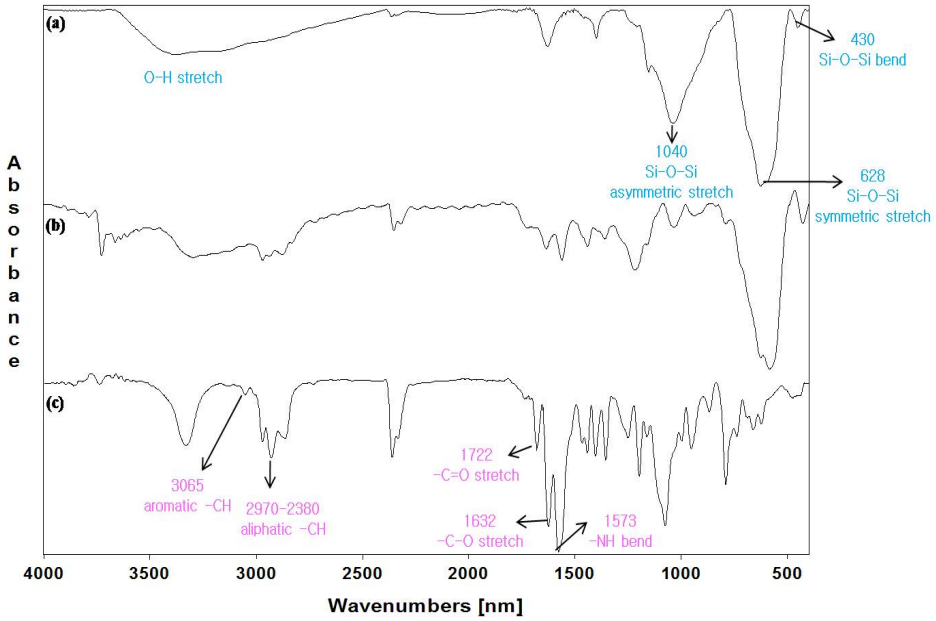

Figure S8. FT-IR spectra of (a) **SMNPs**, (b) **TSMNPs**, and (c) receptor **1**.

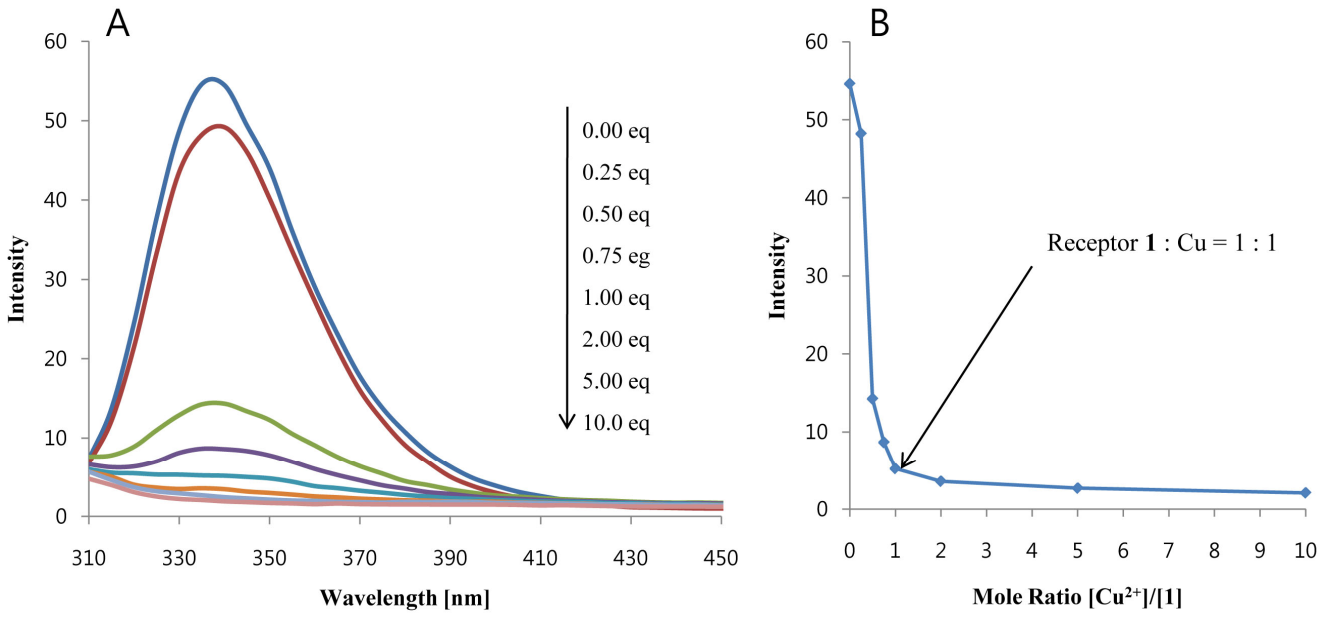

Figure S9. (a) Fluorescence titration spectra of receptor **1** (3 μM) upon addition of various concentration of $CuCl_2$ in CH_3CN : water = 9 : 1 at room temperature. The excitation wavelength is 280 nm. (b) The binding isotherm at 335 nm.

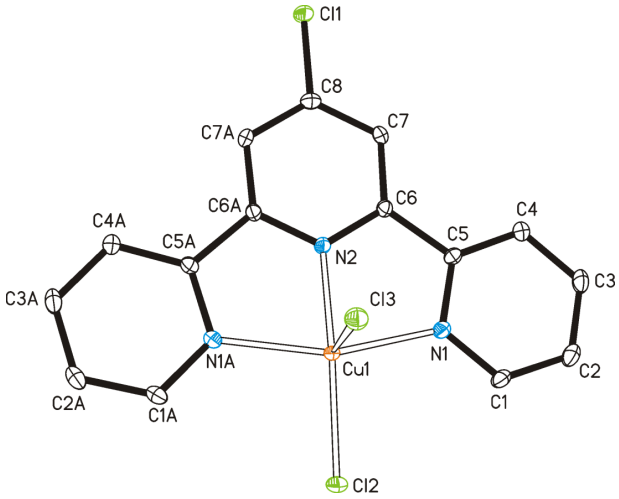

Figure S10. Crystal structure of compound **2** with $CuCl_2$.

Table S1. Crystal data and structure refinement for compound **2** with $CuCl_2$.

Empirical formula	C15 H10 Cl3 Cu N3 O2	
Formula weight	434.15	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/m	
Unit cell dimensions	a = 10.3495(7) Å	α= 90°.
	b = 13.5576(9) Å	β= 111.5980(10)°.
	c = 12.7601(9) Å	γ = 90°.
Volume	1664.7(2) $Å^3$	
Z	4	
Density (calculated)	1.732 Mg/m^3	
Absorption coefficient	1.806 mm^{-1}	
F(000)	868	
Crystal size	0.30 x 0.10 x 0.10 mm^3	
Theta range for data collection	1.72 to 28.28°.	
Index ranges	-13<=h<=13, -17<=k<=18, -16<=l<=16	
Reflections collected	7471	
Independent reflections	2055 [R(int) = 0.0853]	
Completeness to theta = 28.28°	95.2 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2055 / 0 / 118	
Goodness-of-fit on F^2	1.121	
Final R indices [I>2sigma(I)]	R1 = 0.0459, wR2 = 0.1025	
R indices (all data)	R1 = 0.0547, wR2 = 0.1060	
Largest diff. peak and hole	0.796 and -0.753 $e.Å^{-3}$	

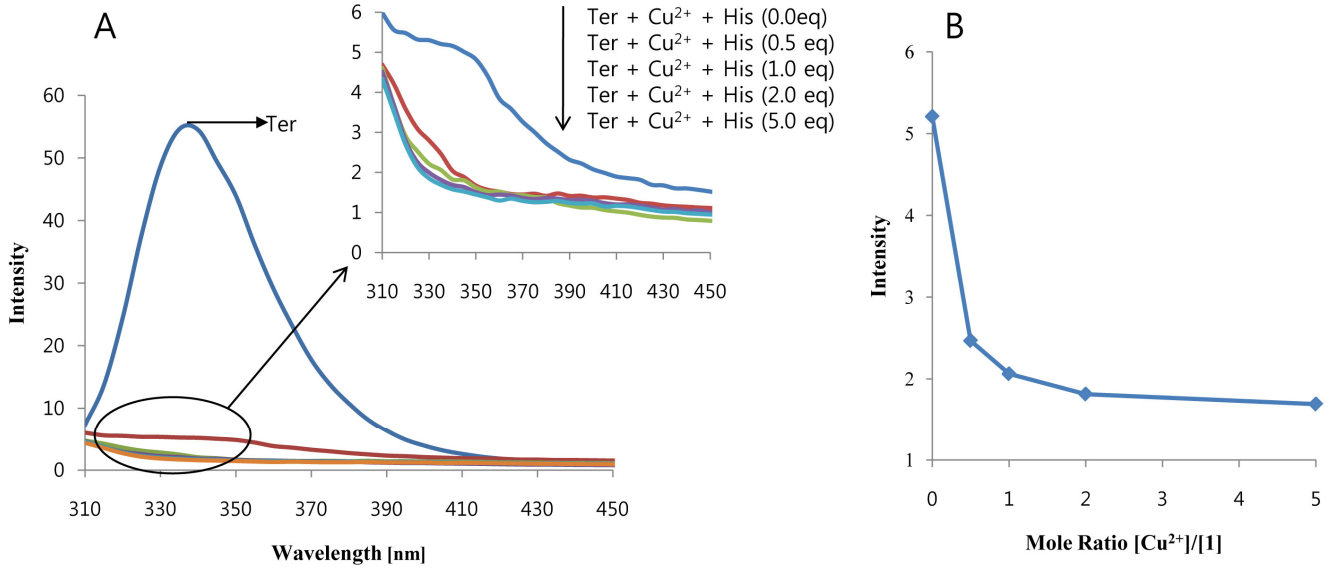

Figure S11. (a) Fluorescence titration spectra of receptor **1** (3 μM in CH_3CN : water = 9 : 1) upon addition of various concentration of histidine in the presence of Cu^{2+} (1.0 eq) at room temperature. The excitation wavelength is 280 nm. (b) The binding isotherm at 335 nm.

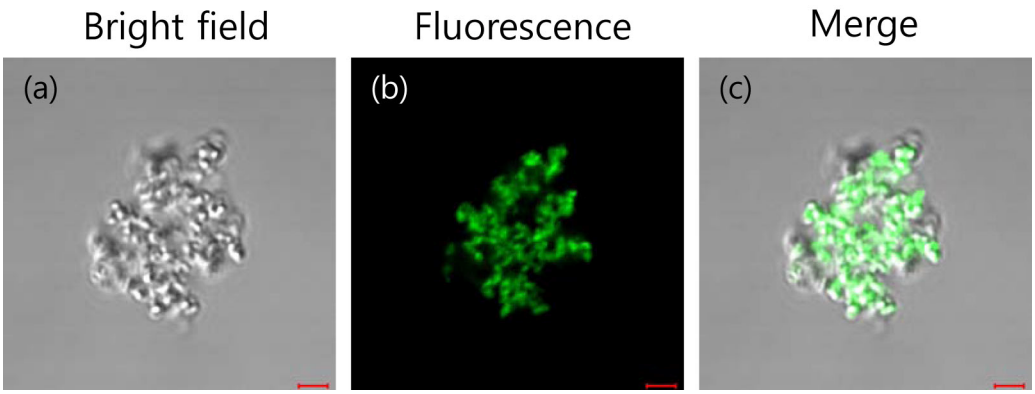

Figure S12. Confocal images of TSMNPs with GST protein containing histidine residues: (a) bright field, (b) fluorescence, and (c) merge images.

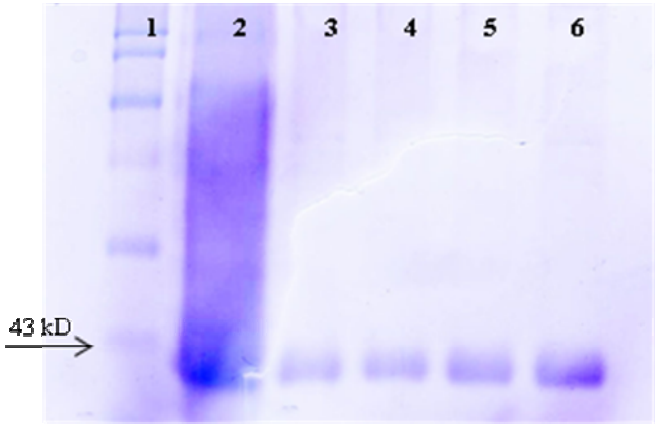

Figure S13. SDS/PAGE analysis of the purity of the His-tagged Cel5A protein. Lane 1: molecular weight marker; lane 2: cell lysate; lane 3: the fraction washed off commercial Ni^{2+}–NTA column; lanes 4-6: The fractions washed off the **TSMNPs** using imidazole solutions (50, 250, and 500 mM).