Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Green synthesis of red-emission carbon based dots by microbial fermentation

Qingqing Fang[1a], Yi Guan[1b], Min Wang[a], Linxi Hou[c], Xiancai Jiang[c], Jinlin Long[a], Yuwu Chi[a], Fengfu Fu[a], Yongqiang Dong[a]*

[a]Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, Fujian Key Laboratory of Marine Enzyme Engineering, Fuzhou University.

[b]Key Laboratory of Marine Enzyme Engineering, Fuzhou University.

[c]College of Chemical Engineering, Fuzhou University.

Fujian 350108, China.

E-mail: dongyq@fzu.edu.cn

Materials

3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased byAll chemicals were analytically pure and used as received. Deionized (DI) water was used throughout the experiments. Phosphate buffer solutions (PBS) were prepared by neutralizing 0.100 mol L^{-1} phosphoric acid solution with a concentrated sodium hydroxide solution (5.00 mol L^{-1}) to pH 7.4.

Instrumentation

High-resolution transmission electron microscopy (HRTEM) images were performed on a Tecnai G2 F20S-TWIN electronic microscope at 200KV. Atomic force

microscopy (AFM) images were obtained by tapping-mode on a Nanoscope IIIa Digital Instruments with NSC15 tips (silicon cantilever, MikroMasch). X-ray power diffraction (XRD) patterns were measured with a Japan Rigaku D/max-3C using Cu Kα radiation in the range of 10°to 65°. The UV-Vis, photoluminescence (PL), and Fourier transform infrared (FT-IR) spectrum were recorded by a UV/vis/NIR spectrophotometer (Lambda 750), a FL spectrophotometer（F 4600）with the power of 150 watt Xe lamp, and a FT-IR spectrophotometer (Thermo Nicolet 360), respectively. Elemental analysis was carried out using a Vario MICRO organic elemental analyzer. Raman spectra were measured using a Renishaw 1000 microspectrometer (excitation wavelength of 532 nm).

Synthesis of CDs

The CDs were synthesized by the natural fermentation of leaves at room temperature. In a typical experiment, 5 g well washed green tea leaves were put in a beaker and covered with a layer of plastic wrap, allowing the leaves to be fermented freely at room temperature for two weeks. After that, the obtained materials were well washed with DI water by filtration to remove the microbes. Then 20 mL of 1 mM NaOH solution was added into the washed materials, followed by another filtration to collect the dark brown filtrate. Finally, the filtrate was purified by dialyzing against DI water through a dialysis bag (retained molecular weight: 3.5 kDa). The final production yield was calculated to be about 21%.

Synthesis of B1, B2, B3, F1, F2, F3 and F4: The seven kinds of CDs were synthesized

by fermentation of leaves with different microbe. In brief, 5 g well washed green tea leaves and some microbes (*Aspergillus* sp., *Geotrichum* sp., *Cladosporium* sp., *Bacillus* sp. and *Sphingobacterium* sp. for the synthesis of B2, B3, F1, F2, F3, F4 accordingly) were put in a beaker, allowing the fermentation proceeded at room tempearture for 3 days. The purification operation was same to that mentioned above.

Materials and media for microculture and isolation

Fresh tea-leaves were moisturizing-cultured overnight for microorganisms' growth. Bacteria were isolated on beef-extract peptone medium (3% beer-extract, 10% peptone, 5% NaCl and 2% agar, pH=7.5) and incubated at 37 ºC overnight. Fungi were isolated on SDAY (4% glucose, 1% peptone, 1.5% agar and 1% yeast extract) at 30 ºC for 2.5 d. Single colonies of different microorganisms were selected and preserved at -80 ºC. 16S rDNA of bacteria and ITS (Internal Transcribed Spacer) of fungi were amplified with specific primers (Table S1) and sequenced for verification at Invitrogen (Shanghai, China). The feedback sequences were subjected to NCBI and conducted nucleotide blast with other microbes for DNA similarity assay.

Table S1. Primers for gene cloning and microbial identification.

primers	Sequences (5'-3')	Purpose
16S-F	AGAGTTTGATCCTGGCTCAG	Amplication of bacterial 16S rDNA
16S-R	ACGGCTACCTTGTTACGACTT	
ITS1	TCCGTAGGTGAACCTGCGG	Amplication of fungal ITS
ITS4	TCCTCCGCTTATTGATATGC	

Microorganisms isolated from tea-leaves

In this study, 7 strains were isolated from tea-leaves. The 16S rDNA of bacteria and ITS of fungi amplified from genomes. PCR products were verified by agarose gel electrophoresis and sequenced at Invitrogen. The sequencing results were blast at NCBI and showed that microbes isolated in this study belonged to 2 genera of bacteria and 3 genera of fungi. The sample names and their correspongding genus were shown as followed (Table S2).

Table 2. The microbes isolated in this study.

Sample	Genus
T-B1	Bacillus
T-B2	Bacillus
T-B3	Sphingobacterium
T-F1	Aspergillus
T-F2	Aspergillus
T-F3	Geotrichum
T-F4	Cladosporium

> T-B1(Bacillus)
ATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACG
GGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGG
GCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCT
ACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA
AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACA
CGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC
TGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTT
AGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC
GGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAG
AGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGC
GAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGG
TTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTC
GCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAG
> T-B2 (Bacillus)
ATACATGCAAGTCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCGGA

CGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCG
AAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGG
CTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACC
AAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGAC
ACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGT
CTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGT
TAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCC
GGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
ACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAA
AGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGC
GAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGG
TTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTC
GCAAGACTGAAACTCAAGGAATTGACGGGGGCCCGCACAAGC

> T-B3 (Sphingobacterium)

ATACATGCAAGTCGGACGGGATCCATCGGAGAGCTTGCTCGAAGATGGTGAGAGTGG
CGCACGGGTGCGTAACGCGTGAGCAACCTACCTCTATCAGGGGGATAGCCTCTCGAA
AGAGAGATTAACACCGCATAATATAATTTCCCGGCATCGAGGAATTATTAAATATTTA
TAGGATAGAGATGGGCTCGCGTGACATTAGCTAGTTGGTAGGGTAACGGCTTACCAA
GGCGACGATGTCTAGGGGCTCTGAGAGGAGAATCCCCCACACTGGTACTGAGACACG
GACCAGACTCCTACGGGAGGCAGCAGTAAGGAATATTGGTCAATGGGCGGAAGCCTG
AACCAGCCATGCCGCGTGCAGGAAGACTGCCCTATGGGTTGTAAACTGCTTTTGTCCA
GGAATAAACCTCTTTACGAGTAGAGAGCTGAATGTACTGGAAGAATAAGGATCGGCT
AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTTATCCGGATTTATT
GGGTTTAAAGGGTGCGTAGGCGGCCTATTAAGTCAGGGGTGAAATACGGTGGCTCAA
CCATCGCAGTGCCTTTGATACTGATGGGCTTGAATCCATTTGAAGTGGGCGGAATAAG
ACAAGTAGCGGTGAAATGCATAGATATGTCTTAGAACTCCGATTGCGAAGGCAGCTC
ACTAAGCTGGTATTGACGCTGATGCACGAAAGCGTGGGGATCGAACAGGATTAGATA
CCCTGGTAGTCCACGCCCTAAACGATGATAACTCGATGTTGGCGATAGACAGCCAGC
GTCCCAGCGAAAGCGTTAAGTTATCCACCTGGGGAGTACGCCCGCAAGGGTGAAACT
CAAAGGAATTGACGGGGGCCCGCACAAGCGAGGAGCATGTGTTTATTCGATGATACG
CGAGAACCTTACCCGGGCTGAAGTTAGTGA

>T-F1 (Aspergillus)

AATCTTTGGGCCCAACCTCCCATCCGTGTCTATTATACCCTGTTGCTTCGGCGGGCCC
GCCGCTTGTCGGCCGCCGGGGGGGCGCCTTTGCCCCCCGGGCCCGTGCCCGCCGGAG
ACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAG
TTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAA
TGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACAT
TGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGC
CCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAG
CGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGAT
TGGCCGGCGCCTGCCGACGTTTTCCAACCATTTTTTCCAGGTTGACCTCGGATCAGGT
AGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

>T-F2（Aspergillus）
GTGGGGTCGAGTCGGGGTCTTTGGGCCAACCTCCCATCCGTGTCTATTGTACCCTGTT
GCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCC
CGTGCCCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGA
TTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAA
GAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGT
CTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCAT
TGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGG
GCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCAC
ATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
>T-F3 (Geotrichum)
TGTGAATTTACACAGCAAACAATAATTTTATAAGTCAAAACAAAAATAATCAAAACT
TTTAACAATGGATCTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATATT
TATTGTGAATTGCAGAAGTGAATCATCAGTTTTTGAACGCACATTGCACTTTGGGGTA
TCCCCCAAAGTATACTTGTTTGAGCGTTGTTTCTCTCTTGGAATTGCATTGCTTTTCTA
AAAATTCGAATCAAATTCGTTTGAAAAACAACACTATTCAACCTCAGATCAAGTAGG
ATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
>T-F4 (Cladosporium)
GTCTTACCACCGGGATGTTCATAACCCTTTGTTGTCCGACTCTGTTGCCTCCGGGGCG
ACCCTGCCTTCGGGCGGGGGCTCCGGGTGGACACTTCAAACTCTTGCGTAACTTTGCA
GTCTGAGTAAACTTAATTAATAAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGG
CATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTT
CGAGCGTCATTTCACCACTCAAGCCTCGCTTGGTATTGGGCAACGCGGTCCGCCGCGT
GCCTCAAATCGACCGGCTGGGTCTTCTGTCCCCTAAGCGTTGTGGAAACTATTCGCTA
AAGGGTGTTCGGGAGGCTACGCCGTAAAACAACCCCATTTCTAAGGTTGACCTCGGA
TCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

Cell Viability Assay: The viability and proliferation of cells in the presence of F2 were evaluated using MTT assay. In brief, MCF-7 cells were seeded into 96-well plates at a density of 1×10^4 per well in 200 μL of media and grown overnight. The cells were then incubated with various concentrations of F2 sample for 24 h. Following the incubation, cells were incubated in media containing 0.5 mg/mL of MTT for 4 h. Finally, the MTT solution was removed and the precipitated violet crystals were dissolved in 200 μL of DMSO. The absorbance was measured at 570

nm using a BioTek microplate reader.

Cell imaging

Cells were seeded at a density of 1 × 10^4 cells/cm^2 onto poly-L-lysine (0.1 mg/mL) coated coverslips for cell attachment overnight. The cells were then incubated with 500 ug/mL F2 sample. After 4 hours, the cells were washed three times with PBS and the PL images were acquired by confocal laser scanning microscopy.

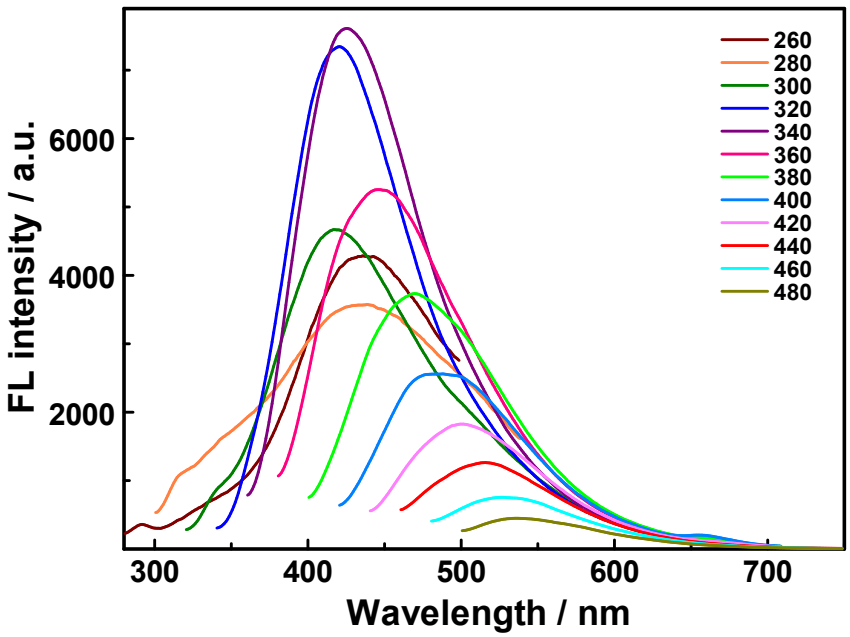

Figure S1. FL spectra of the reduced CDs obtained from the natural fermentation of tea leaves.

Figure S2. Photograph of the tea leaves after the fermentation.

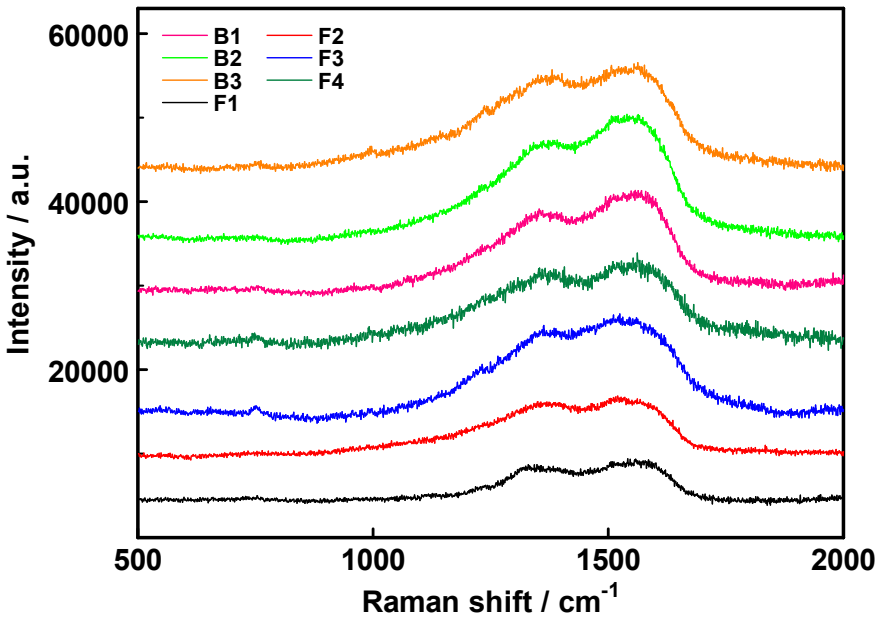

Figure S3. Raman spectra of the seven kinds of CDs obtained by the fermentation of tea leaves with different microbes.

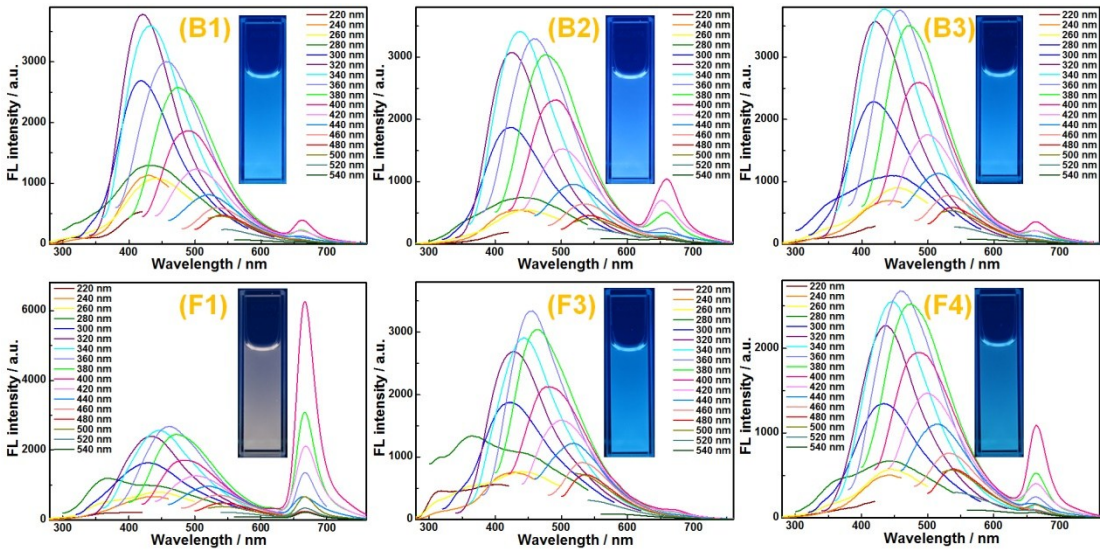

Figure S4. FL spectra of the B1, B2, B3, F1, F3 and F4. Insets in the figures are the photographs of the corresponding CD solution under illumination of 365 nm UV light.

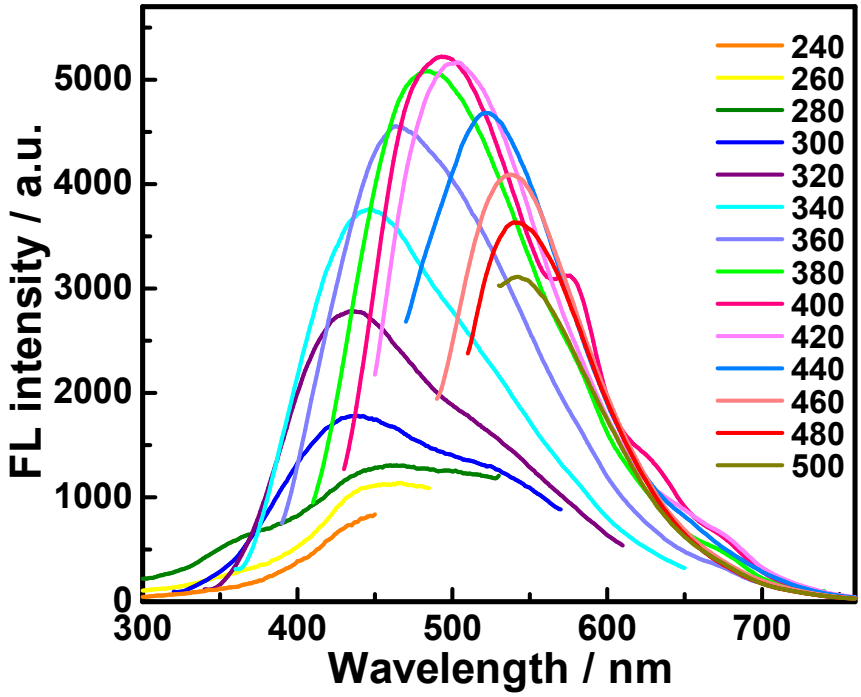

Figure S5. FL spectra of the CDs obtained from the natural fermentation of magnolia tree leaves.

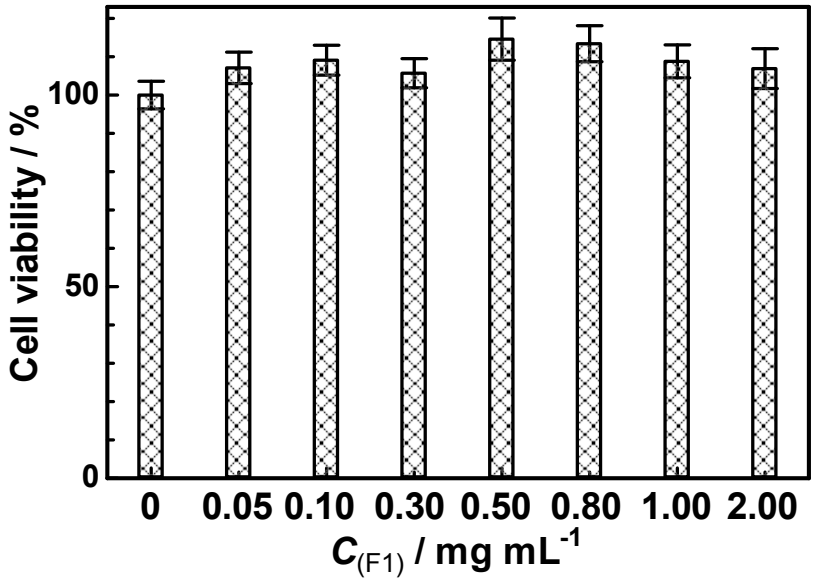

Figure S6. Cell viability assay with human breast cancer MCF-7 cell treated with different concentration of F1.

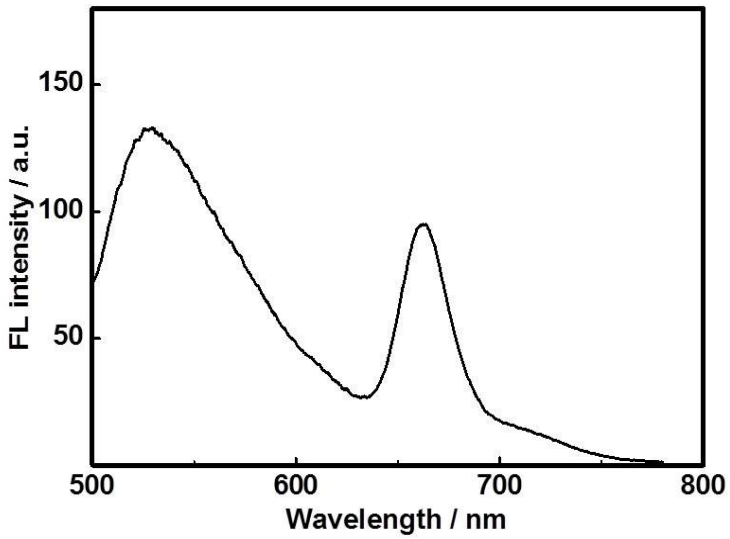

Figure S7. FL spectrum of F2 (500 ug/mL) excited at 488 nm.