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A Triple-Stimuli Responsive Hormone Delivery System Equipped with Pillararene Magnetic Nanovalves

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Experimental Section

Materials.

Starting materials and reagents were purchased from commercial sources and used without further purification. Seeds of *Arabidopsis thaliana* and cabbages as well as gibberellin acid (GA3) were obtained as gifts from College of Plant Science, Jilin University.

Scanning electron microscopy (SEM) images were collected on a JEOL JSM 6700F instrument. Transmission electron microscopy (TEM) images were obtained on a JEM 2100F instrument at an accelerating voltage of 200 kV. Powder X-ray diffraction (PXRD) measurements were carried out by using a PANalytical B.V. Empyrean powder diffractometer. Thermogravimetric analysis (TGA) was realized by STA 449 Thermal gravimetric analyzer in ambient atmosphere. Fourier transform infrared (FT-IR) spectra were collected on Vertex 80 V spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 ºC on a Bruker AVANCE III 300 MHz, and TMS was used as the internal standard. HRMS (ESI) spectra were analyzed by Bruker HCT. The release experiments were monitored by UV-vis spectroscopy on a Shimadzu UV-1800 spectrophotometer.

Preparation of $sSiO_2$.

The preparation of HMSNs was according to previous methods with slight changes. Firstly, TEOS (2 mL) was added into a mixture solution of ethanol (85.6 mL), distilled water (12 mL) and ammonium aqueous solution (25%-28%, 2 mL). The mixed solution was stirred for 2h under 30 ºC. The $sSiO_2$ nanoparticles were obtained after centrifugation (10000 rpm, 30 min) and washed with distilled water and ethanol for 3 times.

Preparation of $sSiO_2$@CTAB-SiO_2.

$sSiO_2$ nanoparticles were dispersed in distilled water (100 mL) by ultrasound for 30 min. The suspension was then added dropwise into a solution containing CTAB (750 mg), deionized water (150 mL), ethanol (150 mL), and ammonia solution (2.2 mL). The mixture was stirred under room temperature for 1h, and then TEOS (1.25 mL) was quickly added and stirred for 4h. After centrifugation (10000 rpm, 15 min) and washing with distilled water and ethanol for 3 times, the products were obtained.

Preparation of HMSN.

The obtained $sSiO_2$@CTAB-SiO_2 sample (640 mg) was dispersed in water (100 mL), followed by the addition of Na_2CO_3 (2.5 g). After the reaction mixture was heated at 50 ºC under stirring for 24 h, the template containing product was obtained. Then it was dispersed in a mixture solution

containing ethanol (170 mL) and concentrate HCl (36%-38%, 9 mL). After stirring the mixture for 24 h under reflux to remove the CTAB template, final HMSN was obtained.

Synthesis of thiol-modified HMSNs (HMSN-SH).

HMSNs sample (50 mg) was added into a round-bottom flask that contains toluene (30 mL). Then, 3-mercaptopropyltrimethoxysilane (MPTMS, 0.5 mL) was injected into the solution under N_2 protection, the reaction mixture was heated at 80 ºC for 24 h under stirring. The final product was obtained by centrifugation (10000 rpm, 8min).

Synthesis of pyridine-modified HMSNs (HMSN-Py).

HMSN-SH (33 mg) was dispersed in C_2H_5OH (20 mL) and 2,2'-dipyridyl disulfide (Py-ss-Py, 100 mg) was added under N_2 protection. After the reaction mixture was stirred for 30 min, acetic acid (0.33 mL) was added and the reaction mixture was further stirred for another 12 h. At last, the reaction mixture was subjected to centrifugation (10000 rpm, 8 min) to remove the yellow solution and the white precipitate was collected to give HMSN-Py product.

Preparation of ultrasmall superparamagnetic Fe_3O_4 nanoparticles (Fe_3O_4).

Firstly, $NaHCO_3$ (0.378 g) was dissolved in H_2O (10 mL). Secondly, $FeCl_3 \cdot 6H_2O$ (0.541 g) was dissolved in H_2O (10 mL). The $FeCl_3$ solution was added into the $NaHCO_3$ solution dropwise and the obtained mixture was stirred at room temperature for 30 min. Then, vitamin C solution (0.3 M, 10 mL) was added into the above solution, which was allowed to react for 10 min. The obtained solution was transferred into a steel-lined Teflon autoclave and kept at 180 ºC for 8 h.

Preparation of NH_2-Fe_3O_4.

Fe_3O_4 sample (250 mg) was dispersed in toluene (300 mL) which was stirred at 80 ºC under the protection of N_2 for 30 min. Then, 3-aminopropyltriethoxysilane (APTES, 3 mL) was injected into the mixture, followed by a 24 h reaction to obtain NH_2-Fe_3O_4 nanoparticles.

Preparation of WP[5]A-Fe_3O_4.

WP[5]A-Fe_3O_4 was synthesized by conjugating CP[5]A to NH_2-Fe_3O_4 via chemical reaction using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). Briefly, CP[5]A (25 mg, 0.02 mmol) was dissolved in ethanol (60 mL), then NHS (150 mg, 1.30 mmol) and EDC (200 mg, 1.04 mmol) were added successively. The resulting mixture was stirred for 30 min. Then, NH_2-Fe_3O_4 (280 mg) was added and the reaction mixture was stirred overnight at room temperature to get CP[5]-Fe_3O_4. After centrifugation, the as-prepared product was added into mixture solution which contains H_2O (20 mL) and concentrate ammonia water

(25%-28%, 2 mL). The reaction mixture was stirred overnight at room temperature for 12 h and WP[5]A-Fe_3O_4 nanoparticles were achieved with filtration and washing.

Loading of RhB and capping with Fe_3O_4-WP[5]A.

The HMSN-Py (30 mg) was dispersed in RhB (30 mL, 1 mM) aqueous solution followed by stirring at room temperature for 24 h. Then, WP[5]A-Fe_3O_4 sample (10 mg) was added into the reaction mixture followed by another 48 h of stirring. The product was centrifuged (10000 rpm, 8 min) and washed with distilled water twice to obtain RhB-HMSN/Fe_3O_4. A control group without loading anything was also prepared, denoted as HMSN/Fe_3O_4.

Loading of GA3 and capping with WP[5]A-Fe_3O_4.

HMSN-Py sample (30 mg) was dispersed in GA3 (30 mL, 1 mM) aqueous solution, and other procedures were the same as above.

RhB release from RhB-HMSN/Fe_3O_4 nanomaterials.

RhB-HMSN/Fe_3O_4 sample (0.5 mg) was wrapped by a piece of filter membrane, which was placed into phosphate buffer solution (PBS, 3 mL) with different treatment at room temperature. The release amount of RhB was monitered by measuring the solution's UV-vis absorption at the wavelength of 553 nm. The GA3 release curves were obtained as same as the method for collecting RhB release data but by collecting UV-vis data at 253 nm.

Preparation of MS culture medium.

NaOH solution (1 M) was gradually added into all MS media to adjust the pH value to 7.4. And then BDA (15 μL, 0.15 mmol) was added to per 150 mL medium, leading the BDA concentration to be 1 mM. The media were divided to different groups, each group was treated with different substances. The first group was treated with GA3-HMSN/Fe_3O_4 (3.47 mg) containing ca. 1.08 mg GA3 (3 μmol), which was dispersed in C_2H_5OH (250 μL) and then added into culture medium (150 mL) to result in accumulative concentration of GA3 of 20 μM, denoted as GA3-HMSN/Fe_3O_4. The second group was treated with GA3 (20 μM), where 1.08 mg GA3 (3 μmol) was dissolved in C_2H_5OH (250 μL) and added into MS culture medium (150 mL). The third group is HMSN/Fe_3O_4 group, where 3.47 mg HMSN/Fe_3O_4 without GA3 was dispersed in C_2H_5OH (250 μL) which was then added into the MS medium (150 mL). The final group was set as control group where just ethanol (250 μL) was added into the MS medium (150 mL).

Culture of cabbages.

Healthy seeds were surface sterilized with 10% (v/v) NaClO solution for 10 min, and then washed with sterile water for 3 times. Seeds were sown in MS medium, and each group contains 3 same medium plates. Thirty (30) seeds were sown on each medium plate. Finally, the media were cultured in an incubator with the photoperiods of 14-h light and 10-h dark. The germinations and growth status were calculated at a certain interval of time.

Culture of A. thaliana.

The culture of A. thaliana was similar to the above procedure, and after sown on media, these media were vernalized at 4 ºC for 2 days. After 2 days, these culture media were put into incubators with the photoperiods of 14-h light and 10-h dark. Germination rates were calculated in a certain interval of time and data of stems and roots were collected after being cultured for 16 days.

$$C_{GA3} = C_{RhB} \times \frac{M_{GA3}}{M_{RhB}}$$

Equation S1. Formula to calculate the capacity of HMSN to loading GA3. C_{GA3}= capacity of HMSN for loading GA3; C_{RhB}= capacity of HMSN for loading RhB; M_{GA3} = molar mass of GA3; M_{RhB} = molar mass of RhB.

Table S1. Elemental analysis of HMSN-SH and HMSN-Py.

Samples	C (%)	H (%)	N (%)	S (%)
HMSN-SH	1.29	2.93	0.00	3.72
HMSN-Py	8.43	2.11	1.05	5.19

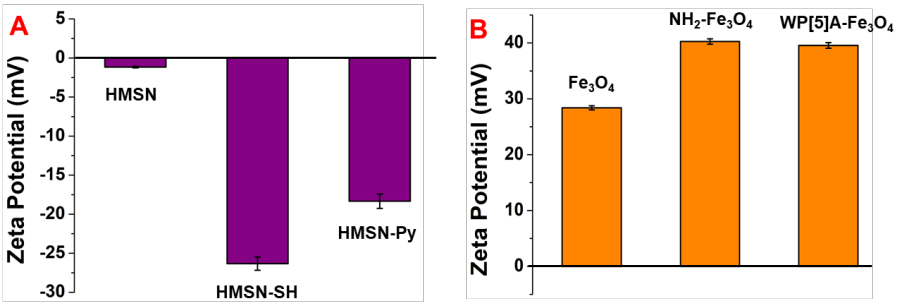

Figure S1. Zeta potentials of (A) HMSN, HMSN-SH, and HMSN-Py; and (B) Fe_3O_4, NH_2-Fe_3O_4, and WP[5]A-Fe_3O_4 in DI water.

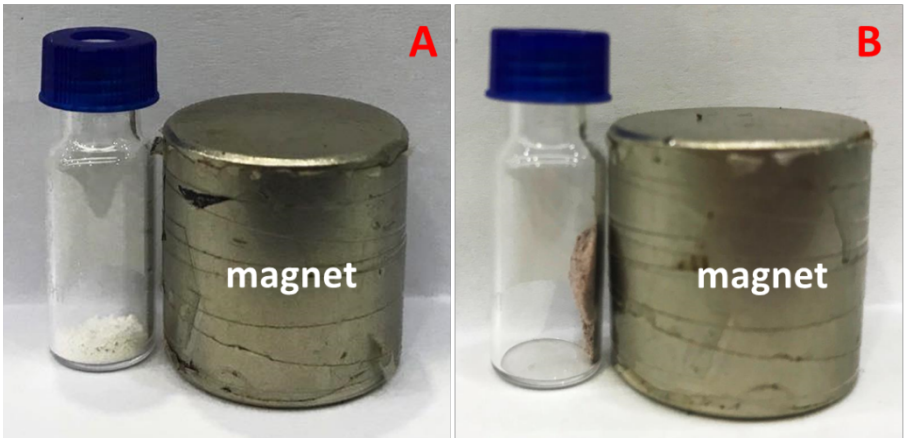

Figure S2. Digital photos of (A) HMSN-Py, and (B) HMSN/Fe_3O_4 in the presence of a magnet.

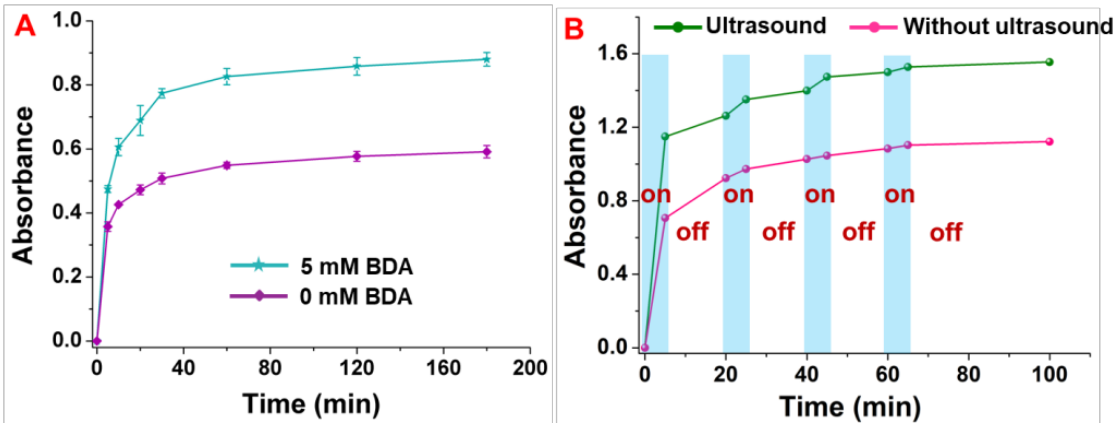

Figure S3. GA3 release curves from GA3-HMSN/Fe_3O_4 triggered by (A) the addition of BDA, and (B) on-off ultrasound.

Syntheses of CP[5]A and WP[5]A: Dimethoxypillar[5]arene (DMP[5]A), dihydroxypillar[5]arene (DHP[5]A), ethoxycarbonyl substituted pillar[5]arene (EP[5]A) carboxylatopillar[5]arene (CP[5]A) and water-soluble carboxylatopillar[5]arene ammonium salt (WP[5]A) were prepared according to literature reports.[S1,S2]

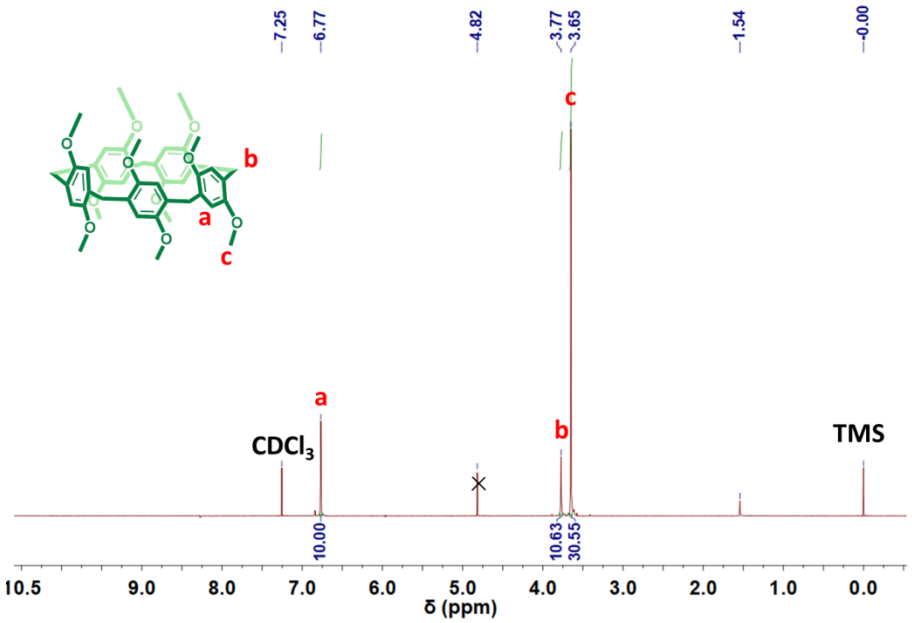

Figure S4. ^{1}H NMR (300 MHz, 298K) spectrum of DMP[5]A in $CDCl_3$.

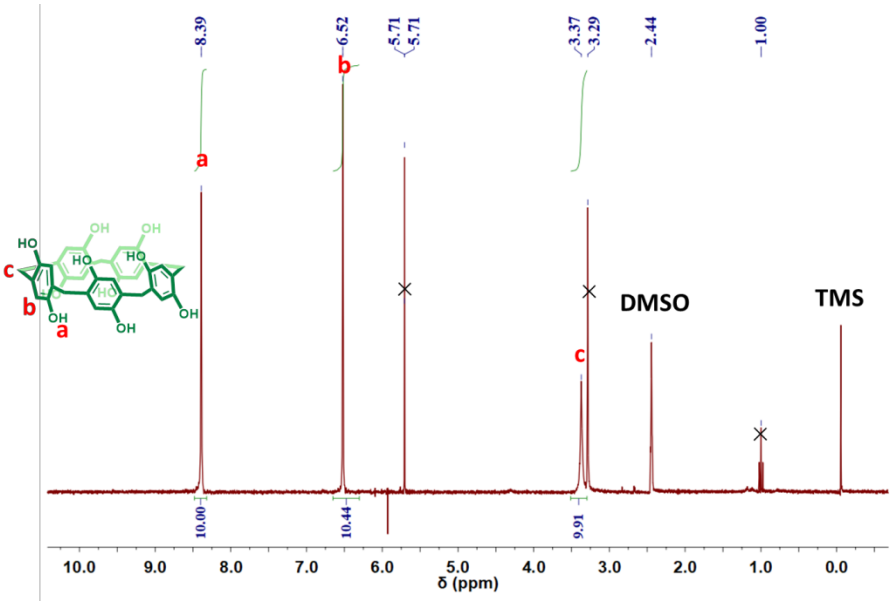

Figure S5. ^{1}H NMR (300 MHz, 298K) spectrum of DHP[5]A in DMSO-d_6.

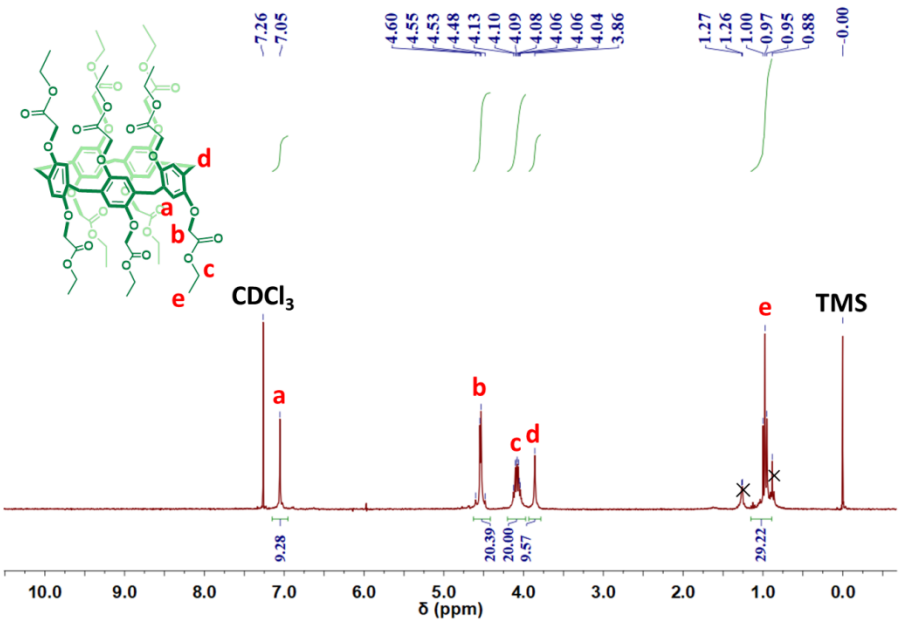

Figure S6. ^{1}H NMR (300 MHz, 298K) spectrum of EP[5]A in $CDCl_3$.

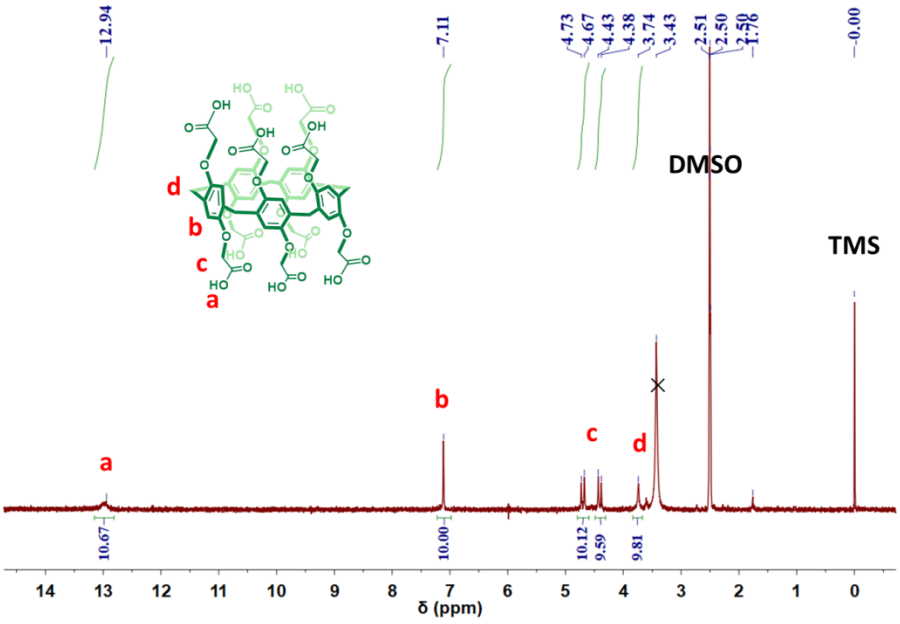

Figure S7. ^{1}H NMR (300 MHz, 298K) spectrum of CP[5]A in DMSO-d_6.

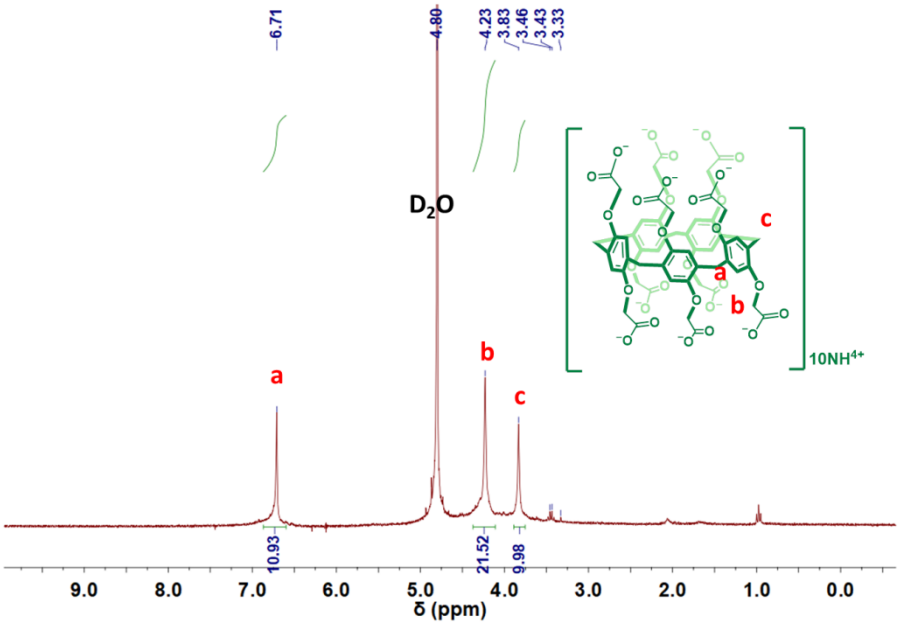

Figure S8. ^{1}H NMR (300 MHz, 298K) spectrum of WP[5]A in DMSO-d_6.

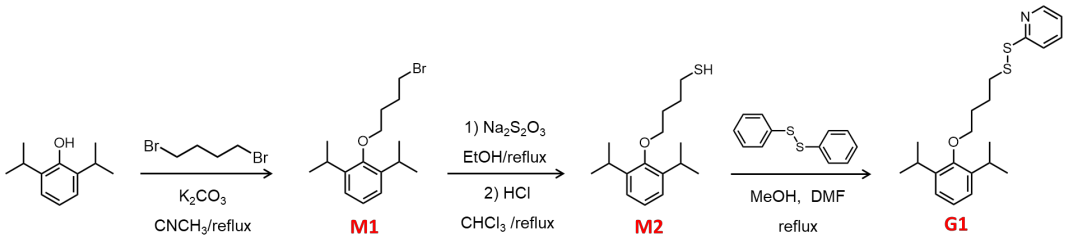

Figure S9. Synthetic routes of G1.

Synthesis of M1: 2,6-Diisopropylphenol (2 mL, 11.2 mmol) was dissolved in MeCN (150 mL), and then K_2CO_3 (2.5g, 18.1 mmol) and KI (50 mg, 0.3 mmol) were added to the solution, followed by stirring under N_2 protection for 30 min. 1,4-Dibromobutane (2.5 mL, 20.9 mmol) was injected to the mixed solution and refluxed for 72 h. The crude product was subject to column chromatography (SiO_2: light petroleum / EtOAc, 20:1 to 10:1) to give the final product M1.[S3] Yield: 1.60 g, 45.8%. ^{1}H NMR (300 MHz, $CDCl_3$, 298K) δ (ppm): 7.10 (s, 3H), 3.75 (t, 2H), 3.51 (t, 2H), 3.25 (m, 2H), 2.12 (m, 2H), 1.95 (m, 2H), 1.22 (d, 12H).

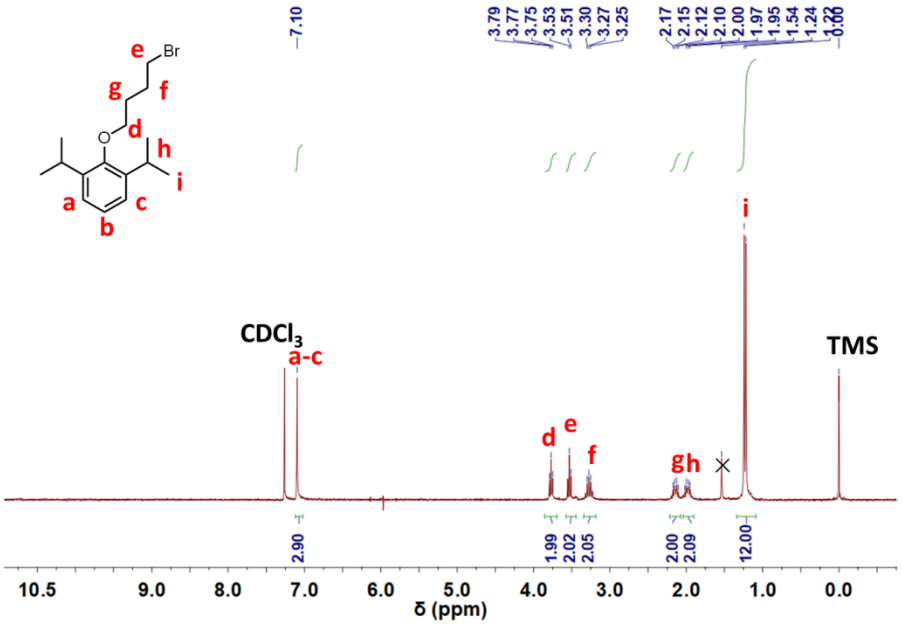

Figure S10. ^{1}H NMR (300 MHz, 298K) spectrum of M1 in $CDCl_3$.

Synthesis of M2: Firstly, the prepared M1 (200 mg, 0.64 mmol) was dissolved in C_2H_5OH. Then, $Na_2S_2O_3$ (203 mg, 1.28 mmol) was dissolved in H_2O (10 mL), which was then gradually added into the M1 solution and refluxed for 24 h. The product was concentrated under reduced pressure, and dispersed into 30 mL $CHCl_3$. HCl (36%-38%, 3 mL) was added into the solution, and the solution color turned into white. Then, the reaction mixture was heated at reflux for another 12h. The organic phase were washed with water for 3 times, and was then concentrated under reduced pressure to get M2. Yield: 140 mg, 87%. ^{1}H NMR (300 MHz, $CDCl_3$, 298K) δ (ppm): 7.09 (s, 3H), 3.75 (t, 2H), 3.29 (m, 2H), 2.66 (t, 2H), 1.89 (m, 4H), 1.24 (s, 1H), 1.22 (d, 12H). ^{13}C NMR (125 MHz, $CDCl_3$, 298K) δ (ppm):153.31, 141.77, 124.47, 123.97, 74.12, 30.74, 29.73, 29.23, 26.49, 24.11. HRMS (ESI): m/z 284.2989 [M+NH^{4+}].

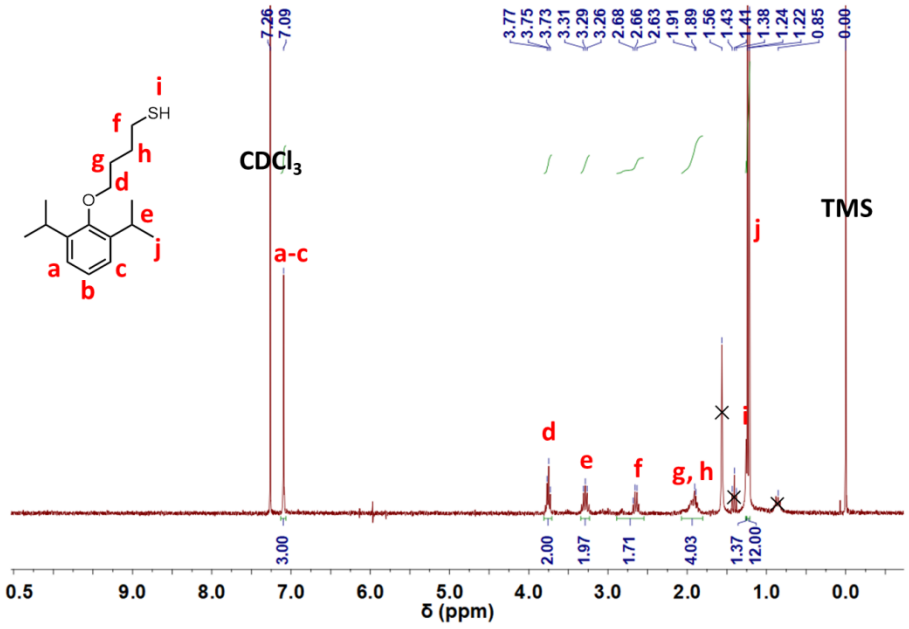

Figure S11. ^{1}H NMR (300 MHz, 298K) spectrum of M2 in $CDCl_3$.

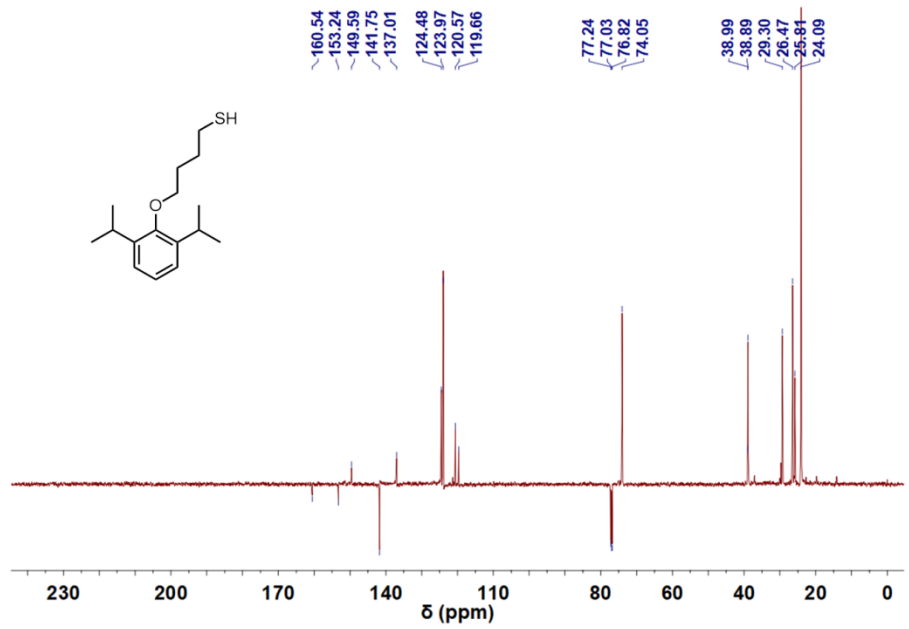

Figure S12. DEPTQ ^{13}C NMR (125 MHz, 298K) spectrum of G1 in $CDCl_3$.

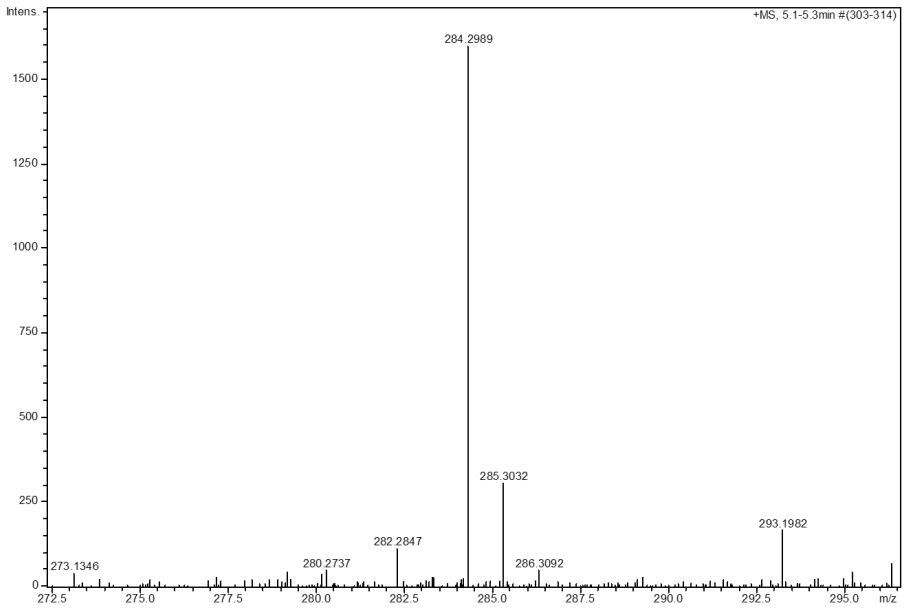

Figure S13. HRMS (ESI) spectrum of M2.

Synthesis of G1: 2,2'-Dithiodipyridine (183.48 mg, 0.83 mmol) was dispersed in a mixed solution (MeOH/DMF, 1:1, 50 mL). The solution was stirred at room temperature under N_2 protection for 30 min. M2 (140 mg, 0.56 mmol) was dissolved in a mixed solvent (MeOH/DMF, 1:1, 8 mL), which was gradually added into the former solution followed by stirring for 24 h at room temperature. Finally, after subjecting to column chromatography (SiO_2: light petroleum / EtOAc, 8:1 to 4:1), a yellow oil product was obtained, which was denoted as G1.[S4] Yield: 174 mg, 83.7%. ^{1}H NMR(300 MHz, $CDCl_3$, 298K) δ (ppm): 8.51 (d, 1H), 7.80 (t, 1H), 7.70 (t, 1H), 7.15 (t, 1H), 7.09 (d, 3H), 3.70 (m, 2H), 3.25 (m, 2H), 2.93 (t, 2H), 1.95 (s, 4H), 1.19 (d, 12H). ^{13}C NMR (125 MHz, $CDCl_3$, 298K) δ (ppm): 160.54, 153.29, 149.59, 141.75, 137.01, 124.48, 123.97, 120.57, 119.66, 74.05, 38.99, 29.30, 26.47, 25.81, 24.09. HRMS (ESI): m/z 376.1818 [M+2H$^+$].

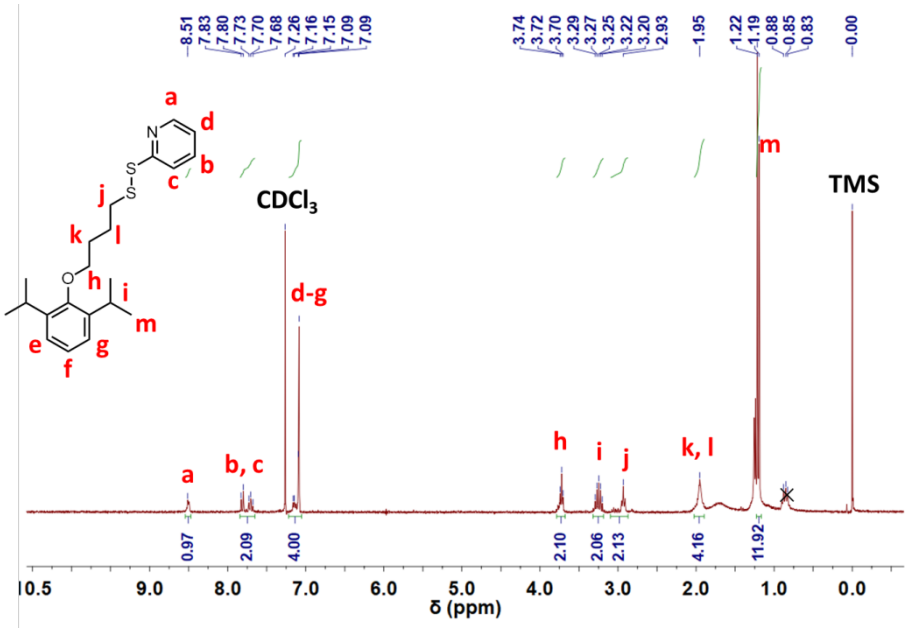

Figure S14. ^{1}H NMR (300 MHz, 298K) spectrum of M2 in $CDCl_3$.

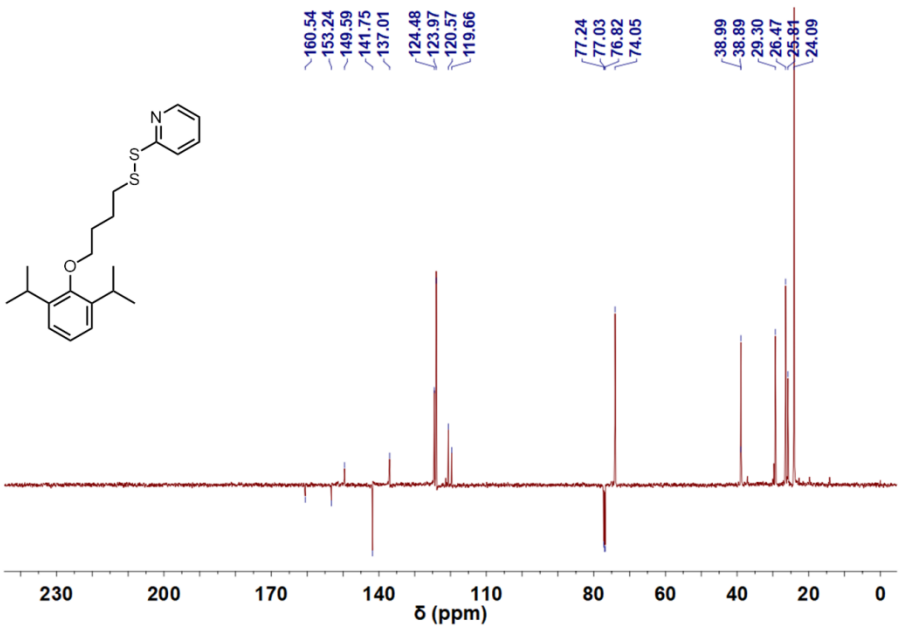

Figure S15. DEPTQ ^{13}C NMR (125 MHz, 298K) spectrum of G1 in $CDCl_3$.

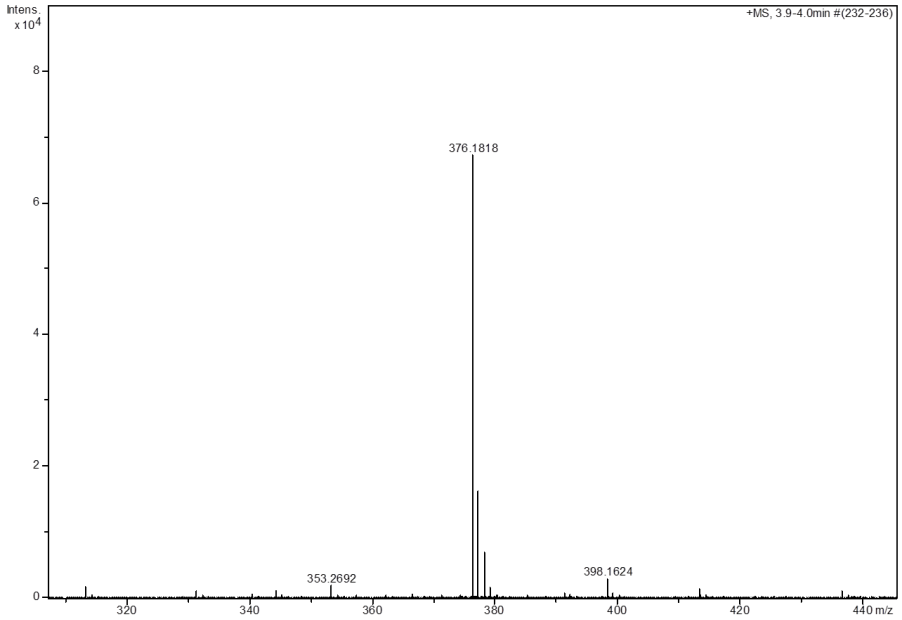

Figure S16. HRMS (ESI) spectrum of G1.

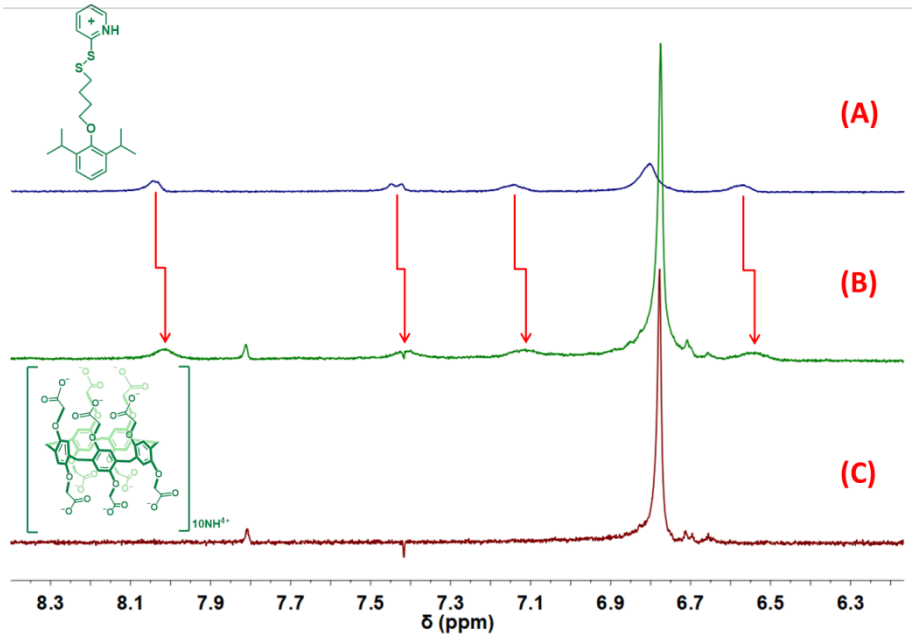

Figure S17. ^{1}H NMR (300 MHz, D_2O, 298K) spectra of (A) G1 (20 mM), (B) G1 (20 mM) and WP[5]A (5 mM) and (C) WP[5]A (5 mM).

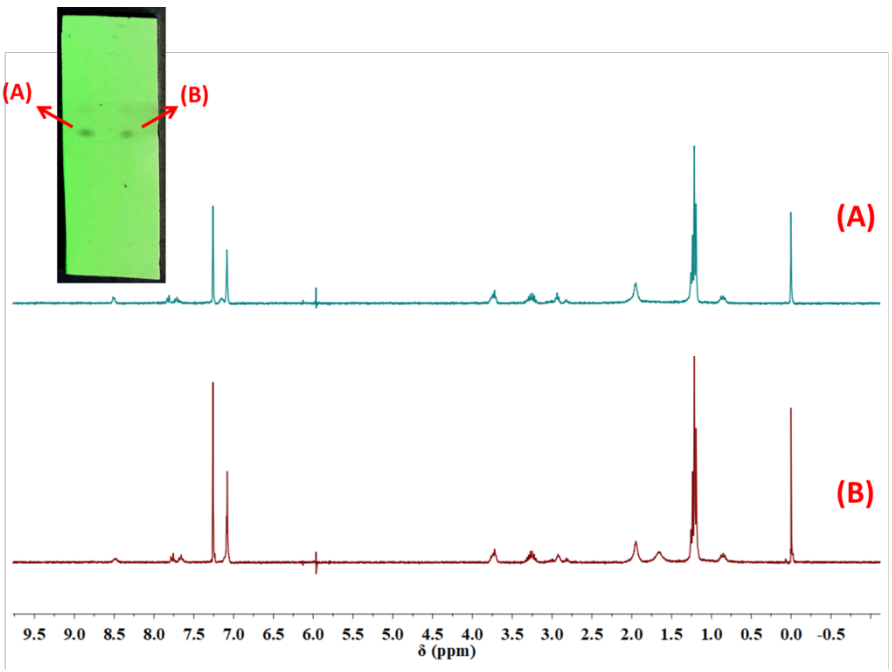

Figure S18. ^{1}H NMR (300 MHz, $CDCl_3$, 298K) of G1 (A) after being treated with ultrasound for 30 min, (B) without any treatment. Inset shows the positions of A and B in a thin-layer chromatography with the developing solvent of light petroleum: EtOAc = 4 : 1 (v/v).

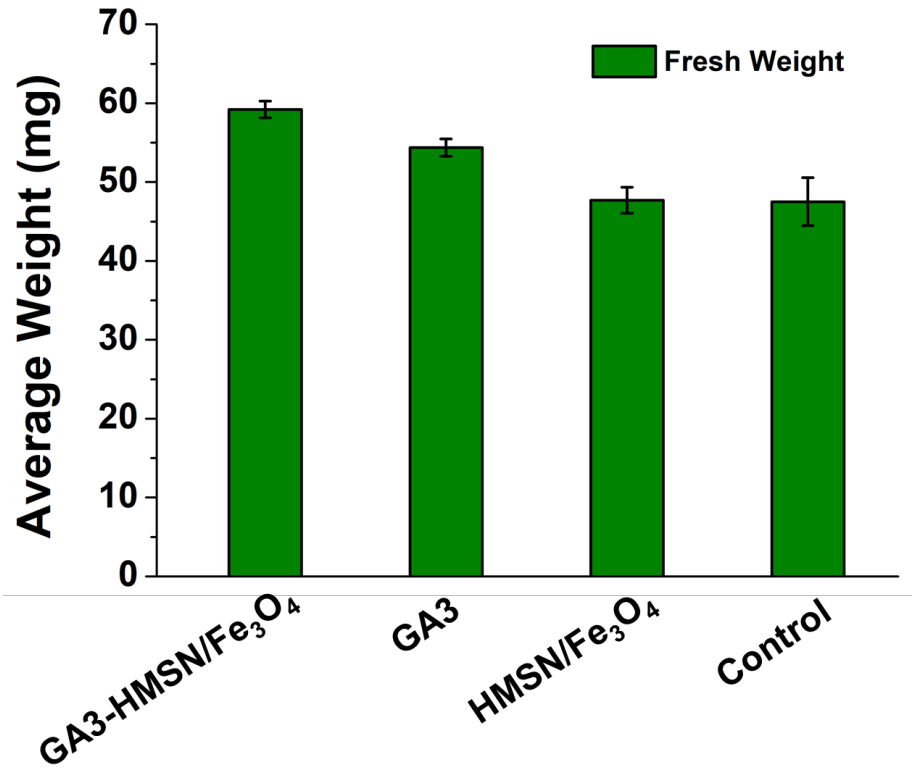

Figure S19. Biomass diagrams of cabbages cultivated for 5 days in different conditions.

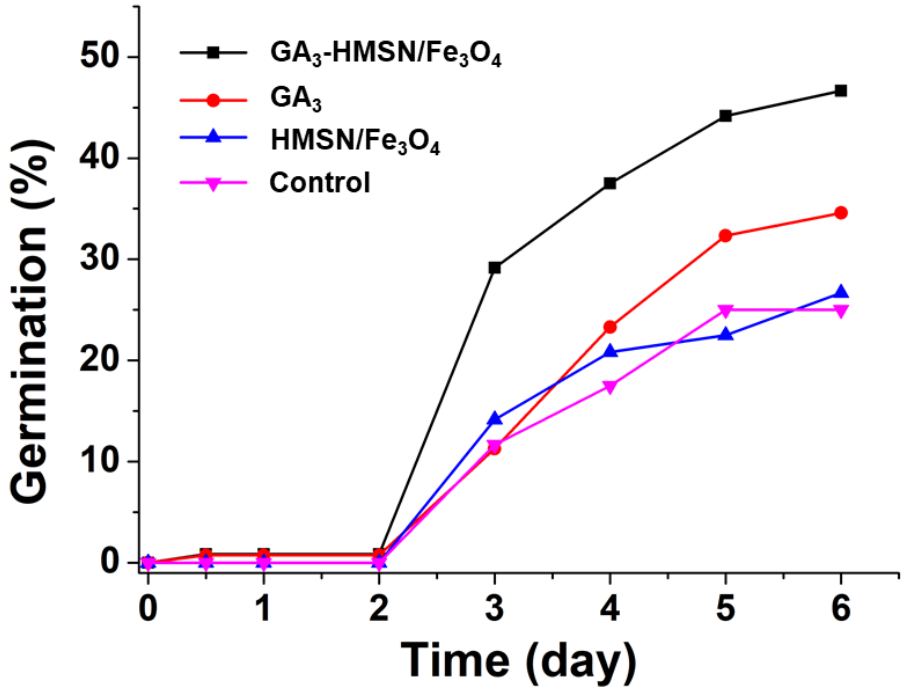

Figure S20. Germination curves of *A. thaliana* after cultured in different conditions.

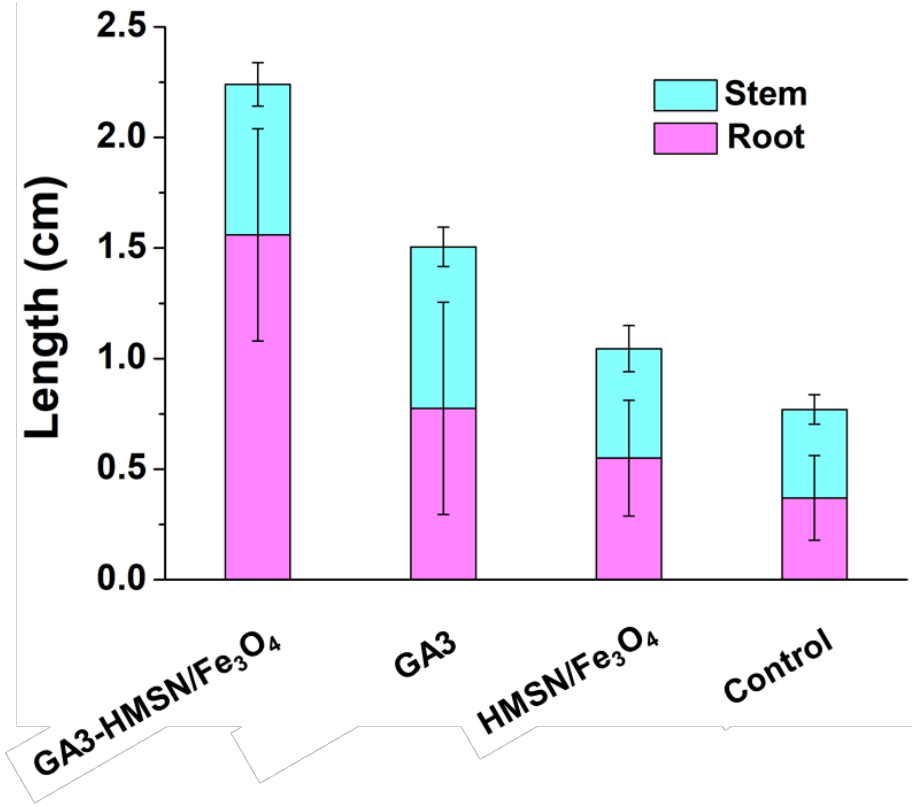

Figure S21. Lengths of *A. thaliana* after cultured in different conditions for 16 days.

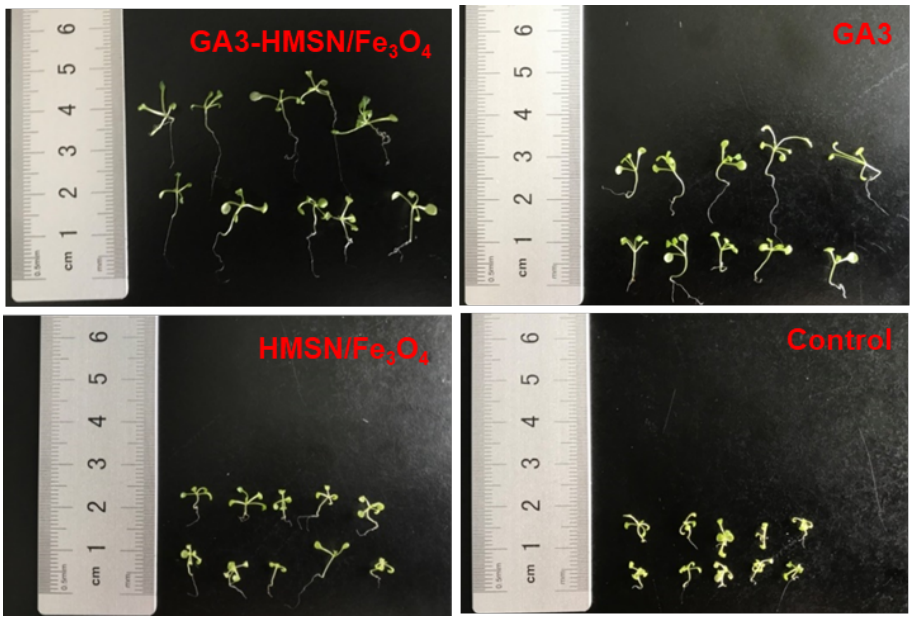

Figure S22. Digital photos of *A. thaliana* after cultured in different condition for 16 day.

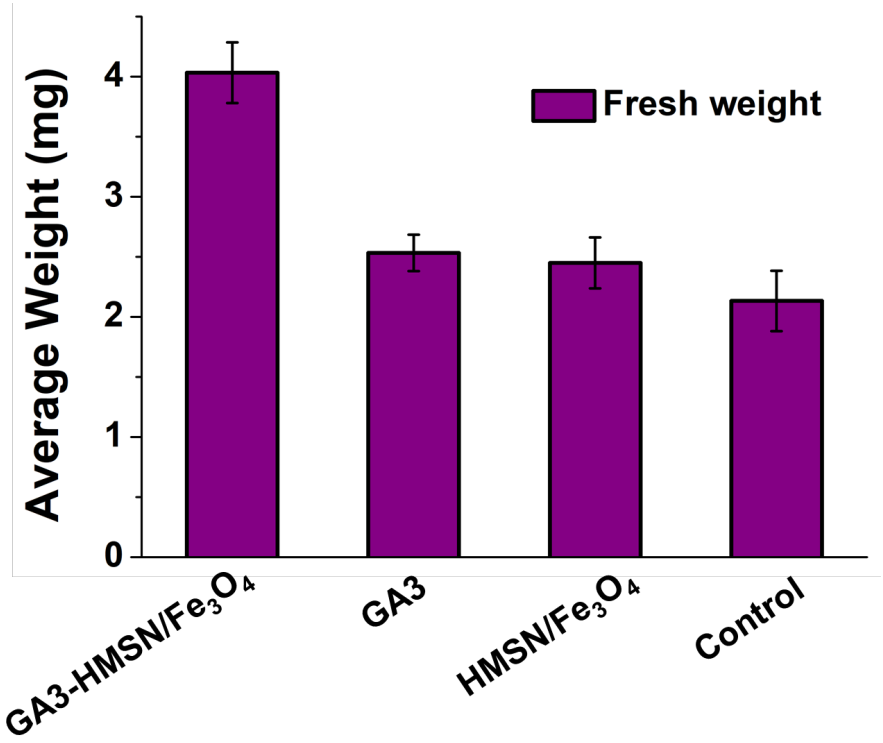

Figure S23. Biomass diagrams of *A. thaliana* after cultured in different conditions for 16 days.

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