

## Supporting Information

### **Redox Supramolecular Self-Assemblies Nonlinearly Enhance Fluorescence to Identify Cancer Cells**

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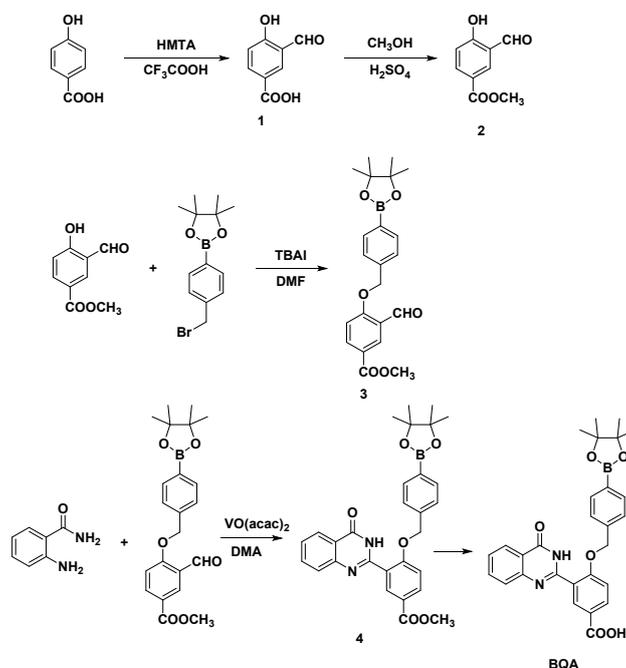
## 1. General Methods

All chemicals were purchased from Sigma-Aldrich and used without further purification unless otherwise stated. NMR spectra were recorded on a Bruker 400 MHz Fourier transform spectrometer. TEM images were obtained on a Tecnai G2 20 S-TWIN transmission electron microscope. High-resolution ESI mass spectra (HRMS) were recorded on a GCMS QP2010 Ultra mass spectrometer. The confocal images were confirmed by confocal microscope (Zeiss 710). The UV spectra were obtained on a Shimadzu UV-2600. The fluorescence spectra were recorded on a F98 fluorometer.

## 2. Chemical Synthesis

### 2.1 Synthesis of the compound BQA

**Scheme S1.** The synthetic route of the compound BQA



#### 2.1.1 The synthesis of 3-formyl-4-hydroxybenzoic acid (1)

The synthesis of compound **1** was referred to the published procedure.<sup>1</sup> 4-Hydroxybenzoic acid (15 g; 108 mmol) was suspended in 40 mL of trifluoroacetic acid. A solution of hexamethylenetetramine (15.3 g; 109 mmol) in 45 mL of trifluoroacetic acid was added dropwise. The resulting mixture was refluxed 2 hours. After cooling to room temperature, the mixture was added to 300 mL of 4 M HCl and stirred for 3 h. The yellow precipitate was then isolated by filtration and abundantly washed with water. The yellow solid was dried under vacuum to yield compound **1** (5.3 g, 30%) without further purification. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 12.84(s, 1H), 11.47(s, 1H), 10.29(s, 1H), 8.24-8.23(d, 1H), 8.05-8.03(dd, 1H), 7.09-7.07(d, 1H).

#### 2.1.2 the synthesis of methyl 3-formyl-4-hydroxybenzoate (2)

Compound **1** (1.66 g; 10 mmol) was suspended in 10 ml of methanol. The resulting mixture was refluxed and then

added into 0.5 ml concentrated sulfuric acid. The resulting mixture was refluxed 3 hours. The mixture was cooled down to room temperature and extracted with EtOAc (3×40 mL). The combined organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> for 2 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hexane:EtOAc =5:1) to give compound **2** (1.69g, 93.8%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ (ppm): 11.56(s, 1H), 10.30(s, 1H), 8.24(d, 1H), 8.07-8.04(dd, 1H), 7.11-7.09(d, 1H), 3.83(s, 3H).

2.1.3 the synthesis of methyl 3-formyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzoate (**3**)  
Compound **2** (900g; 5 mmol), 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.8g; 6 mmol), TBAI (128 mg; 0.35 mmol) and sodium carbonate (1.35 g; 12.7 mmol) were suspended in 10 ml of DMF. The mixture was stirred at 100 °C for 8 h. The mixture was cooled down to room temperature and extracted with EtOAc (3×20 mL). The combined organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> for 2 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hexane:EtOAc =5:1) to give compound **3** (1.76 g, 88.9%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ (ppm): 10.41(s, 1H), 8.27(d, 1H), 8.20-8.17(dd, 1H), 7.72-7.70(d, 2H), 7.54-7.52(d, 2H), 7.44-7.42(d, 1H), 5.42(s, 2H), 3.85(s, 1H), 1.29(s, 12H).

2.1.4 the synthesis of methyl 3-(4-oxo-3,4-dihydroquinazolin-2-yl)-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzoate (**4**)

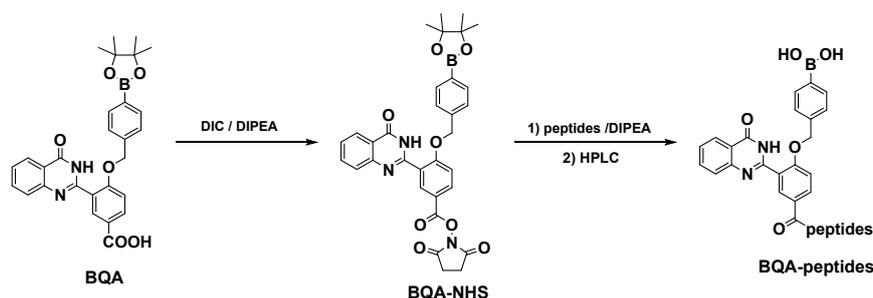
The synthesis of compound **4** was referred to the published procedure.<sup>2</sup> Compound **3** (800 mg; 2 mmol), 2-aminobenzamide (326 mg, 2.4 mmol) and vanadyl acetylacetonate (32 mg; 0.12 mmol) were suspended in 10 ml of N,N-Dimethylacetamide. The mixture was stirred at 120 °C for 12 h. The mixture was cooled down to room temperature and extracted with EtOAc (3×30 mL). The combined organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> for 2 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hexane:EtOAc =3:1) to give compound **4** (282 mg, 27.5%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ (ppm): 12.35(s, 1H), 8.28(d, 1H), 8.16-8.12(m, 2H), 7.86-7.83(t, 1H), 7.76-7.74(d, 1H), 7.65-7.63(d, 1H), 7.57-7.50(m, 3H), 7.41-7.39(d, 1H), 5.32(s, 2H), 3.86(s, 3H), 1.28(s, 12H). ESI MS (m/z): calcd. for C<sub>29</sub>H<sub>29</sub>BN<sub>2</sub>O<sub>6</sub>, 512.3; found [M+H]<sup>+</sup>, 513.3.

2.1.5 the synthesis of 3-(4-oxo-3,4-dihydroquinazolin-2-yl)-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzoic acid (compound BQA)

Compound **4** (300 mg; 0.59 mmol) and NaOH (236 mg; 5.9 mmol) were suspended in 5 ml of ethanol. The mixture was stirred at room temperature for 2 h. And then the mixture was added to about 6 mL 1 M HCl until the pH value to about 7. The white precipitate was then isolated by filtration and abundantly washed with water and ethanol. The white solid was dried under vacuum yielding compound BQA (210 mg, 71.9%) without other purification. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ (ppm): 12.98(s, 1H), 12.32(s, 1H), 8.27-8.26(d, 1H), 8.16-8.14(d, 1H), 8.11-8.09(dd, 1H), 7.87-8-7.83(t, 1H), 7.77-7.74(t, 1H), 7.65-7.63(d, 2H), 7.57-7.50(m, 3H), 7.37-7.35(d, 1H), 5.32(s, 2H), 1.28(s, 12H)

## 2.2 Synthesis of BQA-peptides compounds

**Scheme S2.** The general synthetic route of the BQA-peptide conjugates.



2.2.1 the synthesis of 2,5-dioxopyrrolidin-1-yl 3-(4-oxo-3,4-dihydroquinazolin-2-yl)-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzoate (compound BQA-NHS)

Compound BQA (49.8 mg; 0.1mmol), 1-hydroxypyrrolidine-2,5-dione (23 mg, 0.2 mmol) and N, N'-Diisopropylcarbodiimide (31.2  $\mu$ L; 0.2 mmol) were suspended in 5 mL of DMF. The mixture was stirred at 30 °C for 12 h. The mixture was extracted with EtOAc (3 $\times$ 20 mL). The combined organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> for 2 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hexane:EtOAc =1:1) to give compound BQA-NHS (33.4mg, 56.1%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 12.47(s, 1H), 8.37(d, 1H), 8.29-8.26(dd, 1H), 8.17-8.15(d,1H), 7.87-7.83(t, 1H), 7.77-7.75(d, 1H), 7.66-7.64(d, 1H), 7.58-7.51(m, 4H), 5.38(s, 2H), 2.90(s, 4H), 1.28(s,12H).

2.2.2 the general procedure to synthesize the BQA-peptides

The dipeptides, such as FF, FG, GF, GG were purchased by commercial companies. And GFF, FFG, GGFF were synthesized by standard solid phase peptide synthesis (SPPS).

BQA-NHS (51.2 mg, 0.1 mmol), 3 eq. of peptide and DIPEA (48  $\mu$ L; 6eq) were suspended in 2mL of DMF. The mixture was stirred at 50 °C for 24 h and then subjected to semi preparative HPLC purification to give the white powder. Yield: BQA-GG, 45.6%; BQA-GF, 54.3%; BQA-FG, 50.1%; BQA-FF, 57.2%; BQA-GFF, 68.3%; BQA-FFG, 60.7%; BQA-GGFF, 65.2%.

BQA-GG: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.84-8.81(t, 1H), 8.27-8.22(m, 2H), 8.17-8.15(d, 1H), 8.10-8.07(dd, 1H), 7.87-7.83(t, 1H), 7.77-7.74(m, 2H), 7.57-7.53(t, 1H), 7.46-7.44(d, 1H), 7.39-7.37(d, 1H), 5.29(s, 2H), 3.93-3.91(d, 2H), 3.78-3.77(d, 2H).

BQA-GF: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.76-8.73(t, 1H), 8.25-8.24(d, 1H), 8.19-8.15(t, 2H), 8.08-8.05(dd, 1H), 7.87-7.83(t, 1H), 7.77-7.74(m, 3H), 7.57-7.53(t, 1H), 7.46-7.44(d, 1H), 7.39-7.37(d, 1H), 7.27-7.16(m, 5), 5.29(s, 2H), 4.48-4.43(m, 1H), 3.95-3.81(m, 2H), 3.07-3.03(m, 1H), 2.93-2.87(m, 1H).

BQA-FG: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.67-8.65(d, 1H), 8.46-8.43(t, 1H), 8.20-8.15(m, 2H), 8.01-7.98(dd, 1H), 7.87-7.84(t, 1H), 7.76-7.74(d, 3H), 7.57-7.53(t, 1H), 7.44-7.42(d, 2H), 7.35-7.13(m, 5H), 5.26(s, 2H), 4.82-4.76(m, 1H), 3.83-3.80(m, 2H), 3.17-3.12(m, 1H), 3.02-2.96(m, 1H)

BQA-FF: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.61-8.59(d, 1H), 8.33-8.31(d, 1H), 8.17-8.15(m, 2H), 7.98-7.95(dd, 1H), 7.88-7.83(m, 1H), 7.76-7.74(m, 2H), 7.57-7.53(m, 1H), 7.45-7.43(d, 2H), 7.35-7.32(m, 3H), 7.25-7.20(m, 5H), 7.18-7.13(m, 2H), 5.27(s, 2H), 4.80-4.74(m, 1H), 4.51-4.45(m, 1H), 3.12-3.06(m, 2H), 2.99-2.92(m, 2H).

BQA-GFF: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.74-8.71(t, 1H), 8.39-8.37(d, 1H), 8.4-8.23(d, 1H), 8.17-8.15(dd, 1H), 8.06-8.02(m, 2H), 7.87-7.83(m, 1H), 7.46-7.44(d, 1H), 7.38-7.36(d, 1H), 7.28-7.12(m, 9H), 5.28(s, 2H), 4.59-4.54(m, 1H), 4.46-4.41(m, 1H), 3.91-3.86(m, 1H), 3.78-3.72(m, 1H), 3.10-2.89(m, 3H), 2.77-2.71(m, 1H).

BQA-FFG:  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.59-8.57(d, 1H), 8.36-8.33(t, 1H), 8.18-8.13(m, 3H), 7.98-7.95(dd, 1H), 7.88-7.84(m, 1H), 7.77-7.75(d, 3H), 7.58-7.54(t, 1H), 7.45-7.43(d, 2H), 7.35-7.33(d, 1H), 7.32-7.09(m, 10H), 5.27(s, 2H), 4.76-4.70(m, 1H), 4.63-4.57(m, 1H), 3.80-3.78(m, 2H), 3.08-3.08(m, 2H), 3.00-2.81(m, 2H).

BQA-GGFF:  $^1\text{H}$  NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 8.87-8.84(t, 1H), 8.31-8.27(m, 2H), 8.17-8.06(m, 3H), 8.02-8.00(d, 1H), 7.87-7.83(t, 1H), 7.77-7.73(t, 3H), 7.57-7.53(t, 1H), 7.46-7.44(d, 2H), 7.39-7.37(d, 1H), 7.28-7.13(m, 9H), 5.28(s, 2H), 4.57-4.541(m, 1H), 4.44-4.39(m, 1H), 3.89-3.88(d, 2H), 3.75-3.69(m, 1H), 3.62-3.57(m, 1H), 3.08-2.98(m, 2H), 2.98-2.88(m, 1H).  $^{13}\text{C}$  NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  (ppm): 173.14, 171.47, 169.81, 168.82, 166.02, 161.64, 159.03, 138.57, 138.19, 137.87, 135.02, 134.65, 134.09, 132.03, 130.58, 129.64, 129.58, 128.66, 128.47, 127.64, 127.30, 126.90, 126.73, 126.68, 126.34, 123.16, 121.44, 113.24, 70.59, 54.03, 43.24, 42.28, 38.01, 37.12. ESI MS (m/z): calcd. for C<sub>44</sub>H<sub>41</sub>BN<sub>6</sub>O<sub>10</sub>, 824.3; found [M-H]<sup>-</sup>, 823.3.

### 3. The methods of cell experiments

#### 3.1 Cytotoxicity

HeLa cells were maintained in a humidified CO<sub>2</sub> (5%) incubator at 37 °C in DMEM media, supplemented with 10% FBS and 1% Pen Strep. Cells were plated out in flat bottom 96-well plates at a density of 10<sup>3</sup> cells/well and allowed to attach for 24 h. **BQA-GGFF** was dissolved in PBS at 10 mM. After 24 h cell attachment, cell culture medium was replaced by 100  $\mu\text{L}$  pre-warmed media containing the compounds at different concentrations. After 72 h incubation, cell proliferation was assessed by an MTT assay. 10  $\mu\text{L}$  Thiazolyl Blue Tetrazolium Bromide (MTT) (Solarbio) and 90  $\mu\text{L}$  DMEM media were freshly added to each well. After an incubation for 4 h, the media was gently removed. The formed formazan crystals were dissolved in 110  $\mu\text{L}$  DMSO, subsequently the absorbance was measured with a plate reader (Perkin Elmer) at 490 nm.

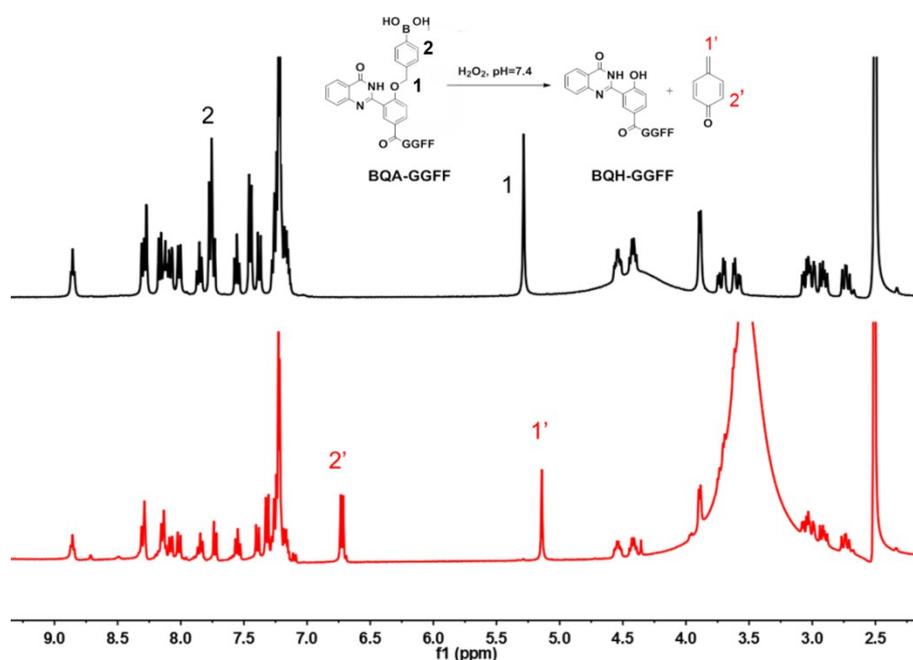
#### 3.2 Living cell imaging

HeLa cells were placed in Glass Chamber and cultured in 2 mL culture medium containing 500  $\mu\text{M}$  of **BQA-GGFF** for different times. Then, remove the medium from the culture dish and wash at least five times with PBS buffer. And then capture the confocal images. After that, it conduct different concentrations of **BQA-GGFF** incubate with HeLa cells and capture images. Then different cell lines incubate with 500  $\mu\text{M}$  of **BQA-GGFF** for 8h and capture the images. At last, using 100 nM of Mito-Tracker™ Red CM-H2XRos dye to incubate with **BQA-GGFF** in HeLa cells to confirm the location.

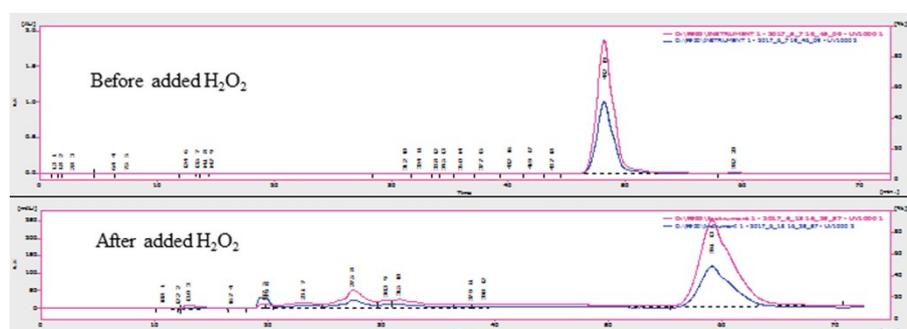
#### 4. Supplementary Results

**Table S1.** Hydrogelation determination of different BQA-peptides reacting to H<sub>2</sub>O<sub>2</sub>. The concentration was 10 mM for all compounds in water.

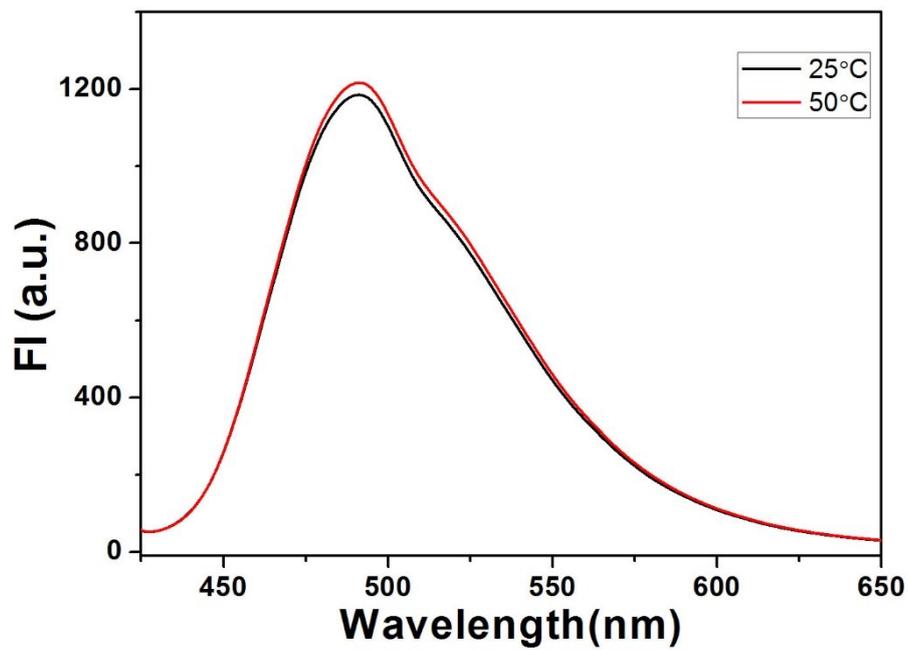
Compound	Before adding H <sub>2</sub> O <sub>2</sub>	After adding H <sub>2</sub> O <sub>2</sub>	Fluorescence	pH
BQA-FF	gel	solution	off	11.2
BQA-FFG	gel	solution	off	10.6
BQA-GFF	gel	gel	on	6.5
BQA-GGFF	solution	gel	on	7.4
BQA-GF	solution	solution	off	7.4
		precipitation	on	4.5
BQA-FG	solution	solution	off	7.4
		precipitation	on	4.7
BQA-GG	solution	solution	off	7.4
		precipitation	on	2.3



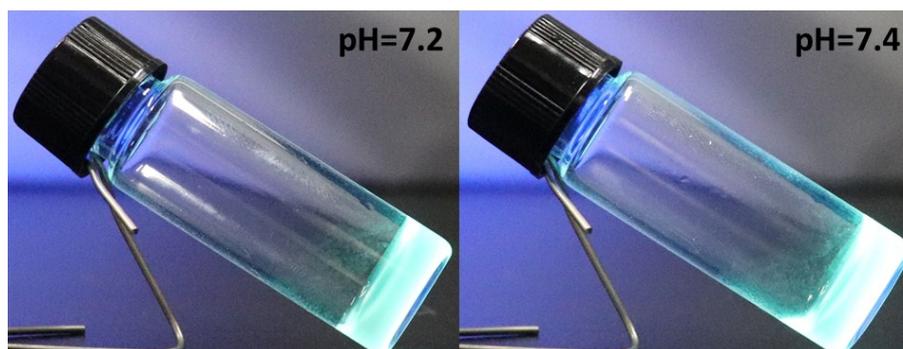
**Figure S1.** The overlap of <sup>1</sup>H NMR spectra of BQA-GGFF and the corresponding products after reaction with H<sub>2</sub>O<sub>2</sub>.



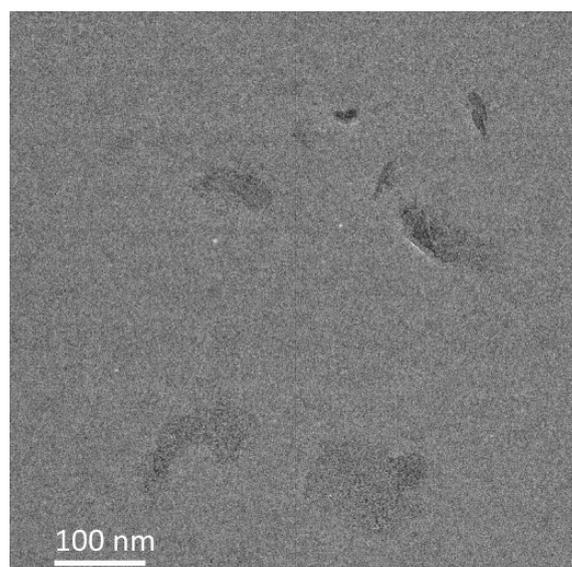
**Figure S2.** The HPLC trace of BQA-GGFF before (above) and after (below) the addition of H<sub>2</sub>O<sub>2</sub>. The HPLC trace monitored at both 254 nm (pink) and 276 nm (blue).



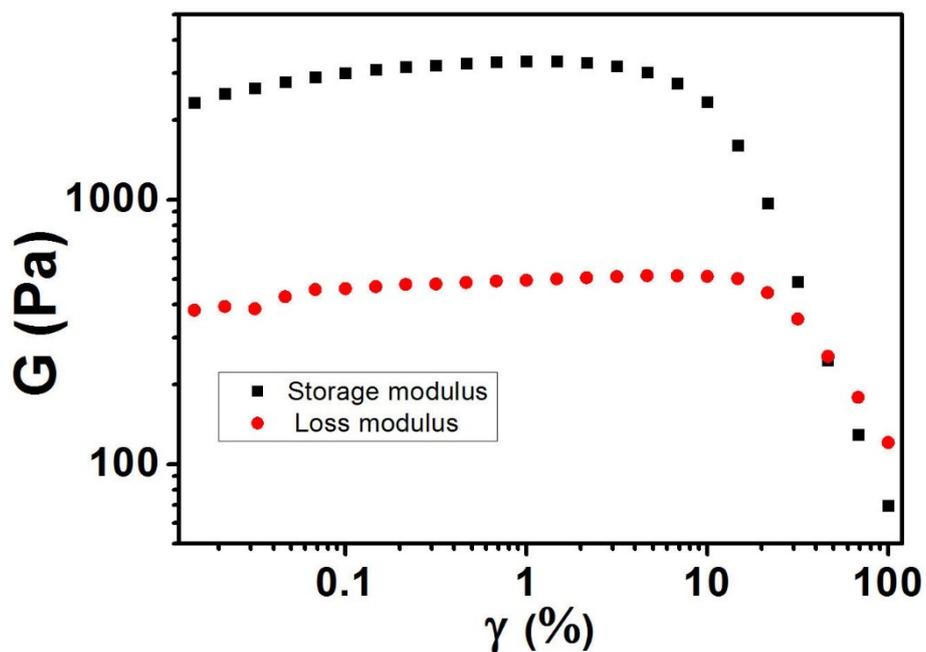
**Figure S3** The fluorescence intensities of hydrogel in 25°C and 50°C.



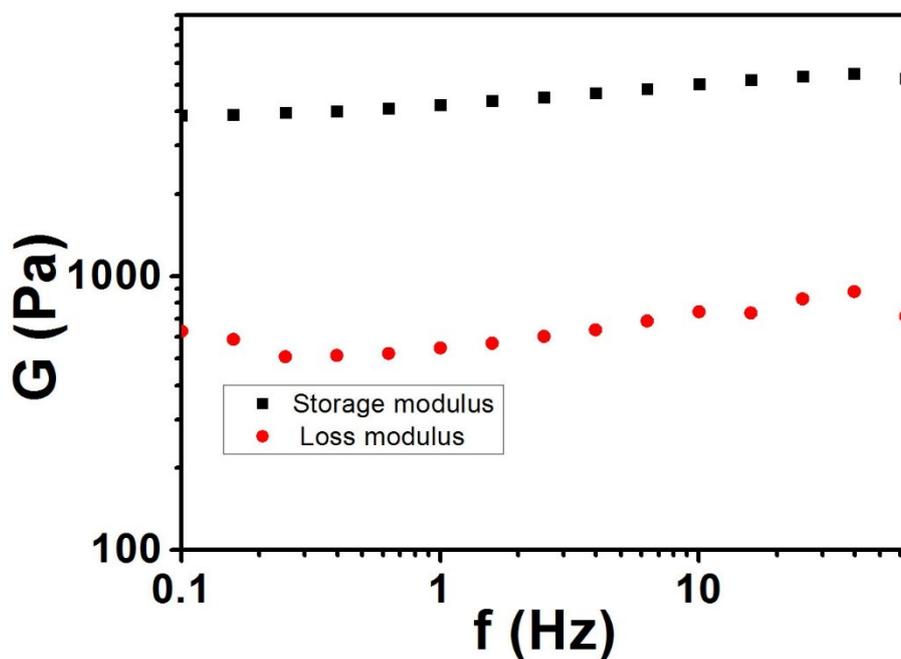
**Figure S4.** The images of the hydrogel of 10 mM BQH-GGFF at pH 7.2 and 7.4 under UV lamp.



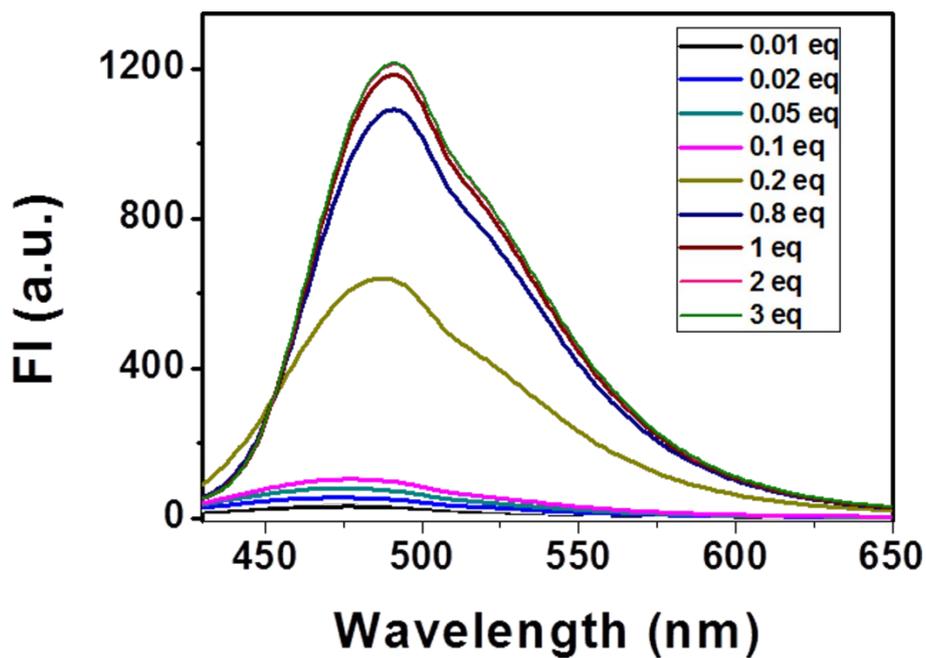
**Figure S5.** The TEM image of 1 mM of BQA-GGFF reacted to 3 eq. of H<sub>2</sub>O<sub>2</sub>.



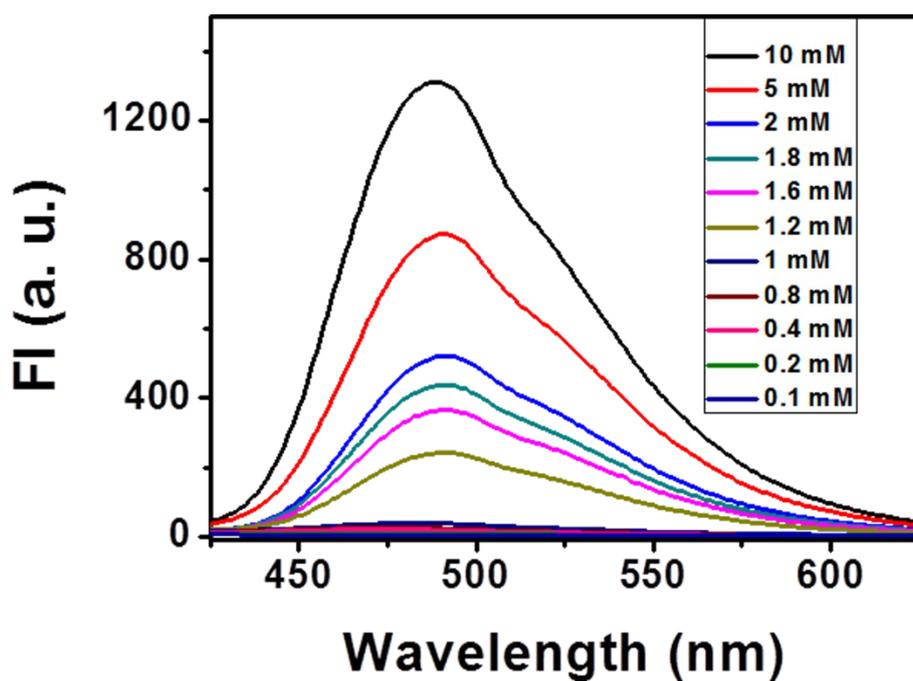
**Figure S6.** Strain sweep of the dynamic storage moduli ( $G'$ ) and the loss moduli ( $G''$ ) of the hydrogel of **BQH-GGFF** (10 mM).



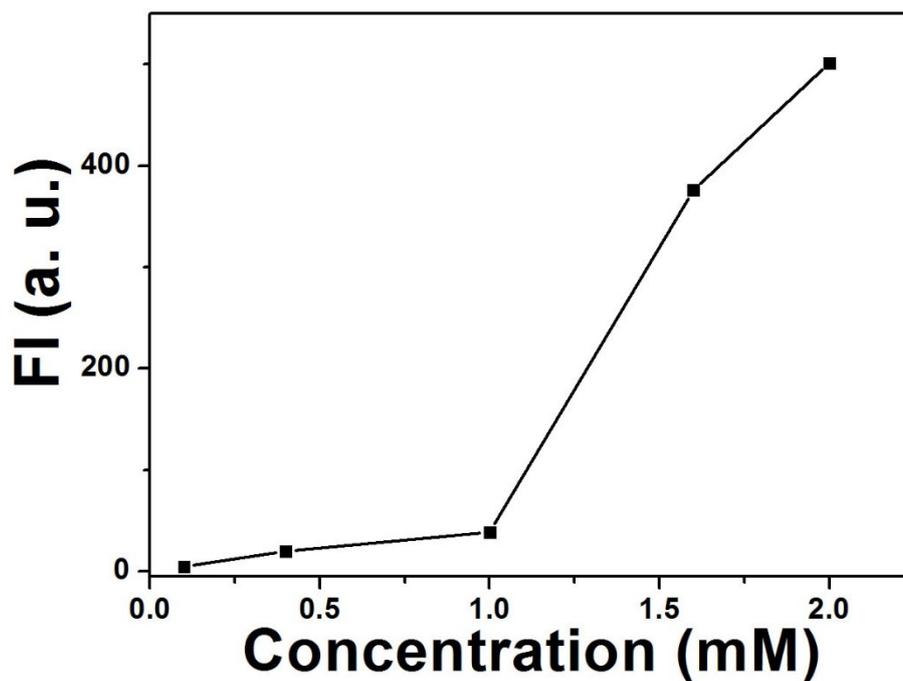
**Figure S7.** Frequency sweep of the dynamic storage moduli ( $G'$ ) and the loss moduli ( $G''$ ) of the hydrogel of **BQH-GGFF** (10 mM).



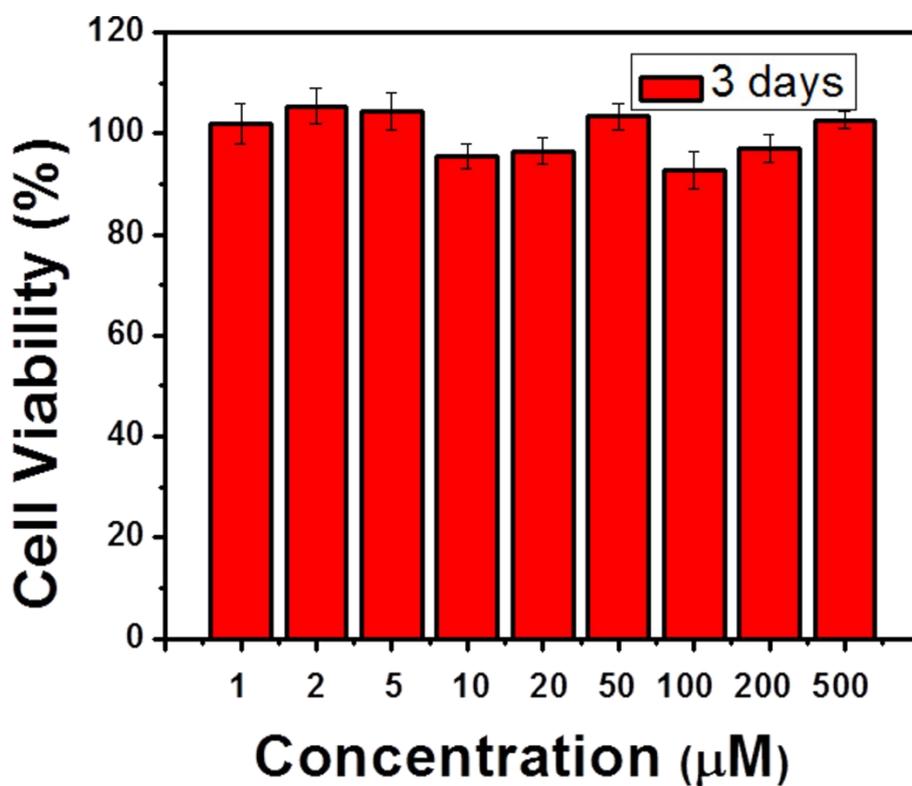
**Figure S8.** The fluorescence spectrum of different concentration (0.01-3 eq.) of H<sub>2</sub>O<sub>2</sub> reacted to 10 mM of BQA-GGFF.



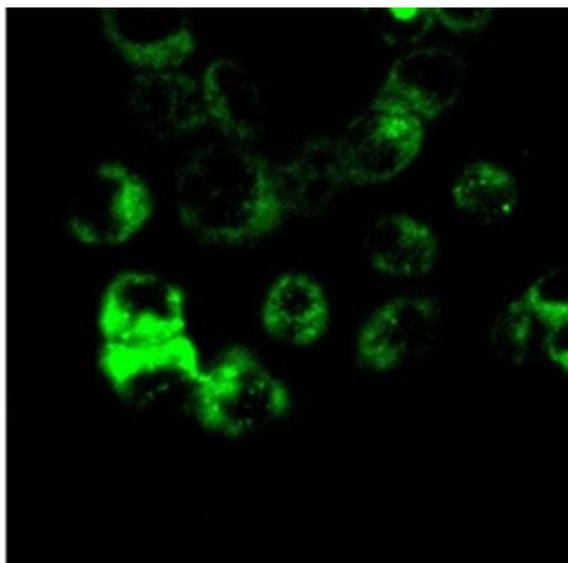
**Figure S9.** The fluorescence spectrum of different concentration (0.1-10 mM) of BQA-GGFF reacted to 3eq H<sub>2</sub>O<sub>2</sub>.



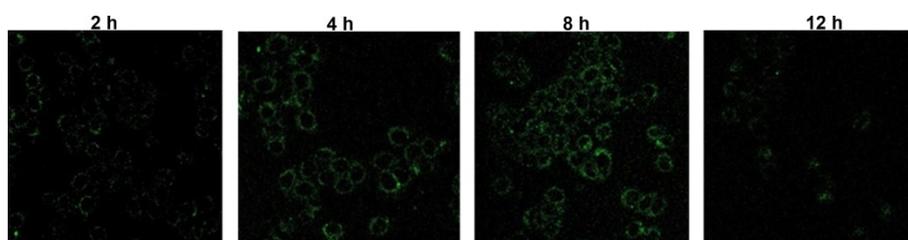
**Figure S10.** The fluorescence intensities (emission at 490 nm) of a series of concentrations (0.1-2 mM) of BQH-GGFF.



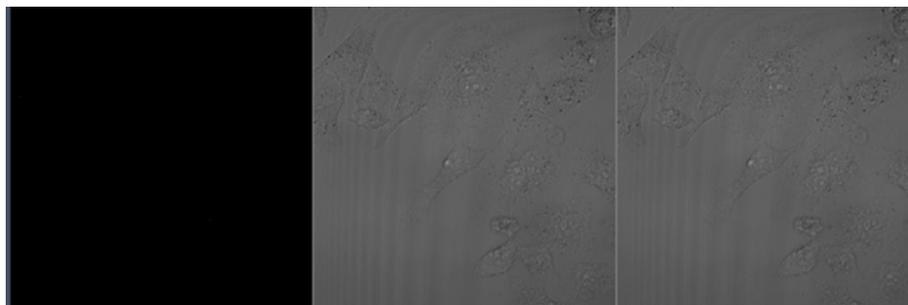
**Figure S11.** 72 h cell viability test of BQA-GGFF against HeLa cells.



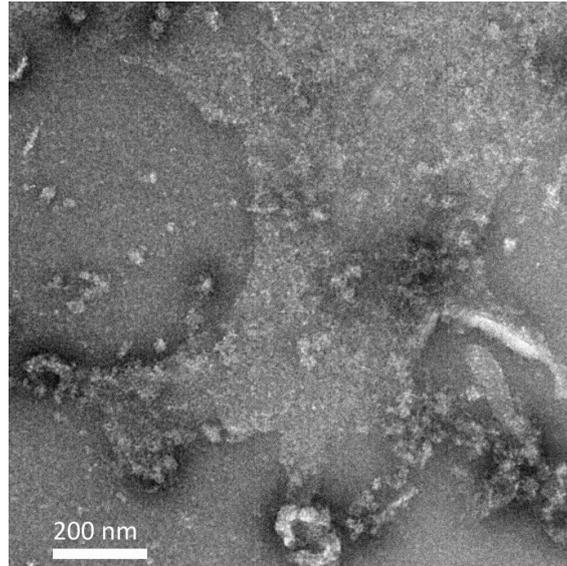
**Figure S12.** The confocal image of HeLa cells incubated with 2 mM of **BQA-GGFF** for 8 h.



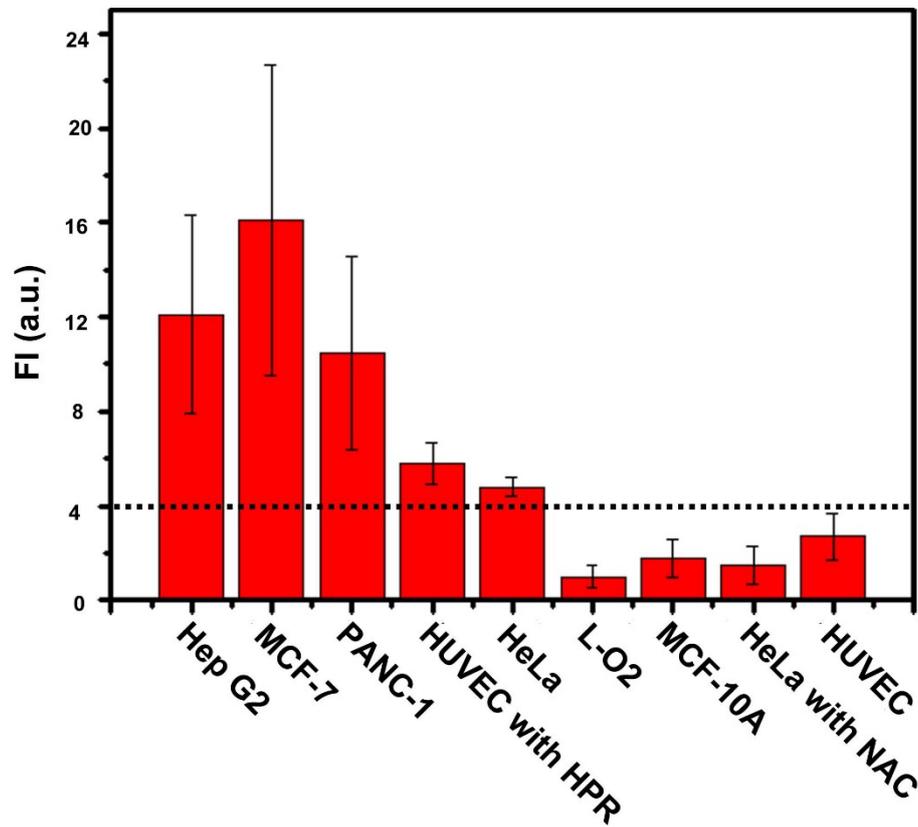
**Figure S13.** The confocal images of HeLa cells incubated with 500  $\mu$ M of **BQA-GGFF** captured at different time points.



**Figure S14.** The confocal images of HeLa cells incubated with 500  $\mu$ M of **BQA-GG**, in which no fluorescence generated in cells.



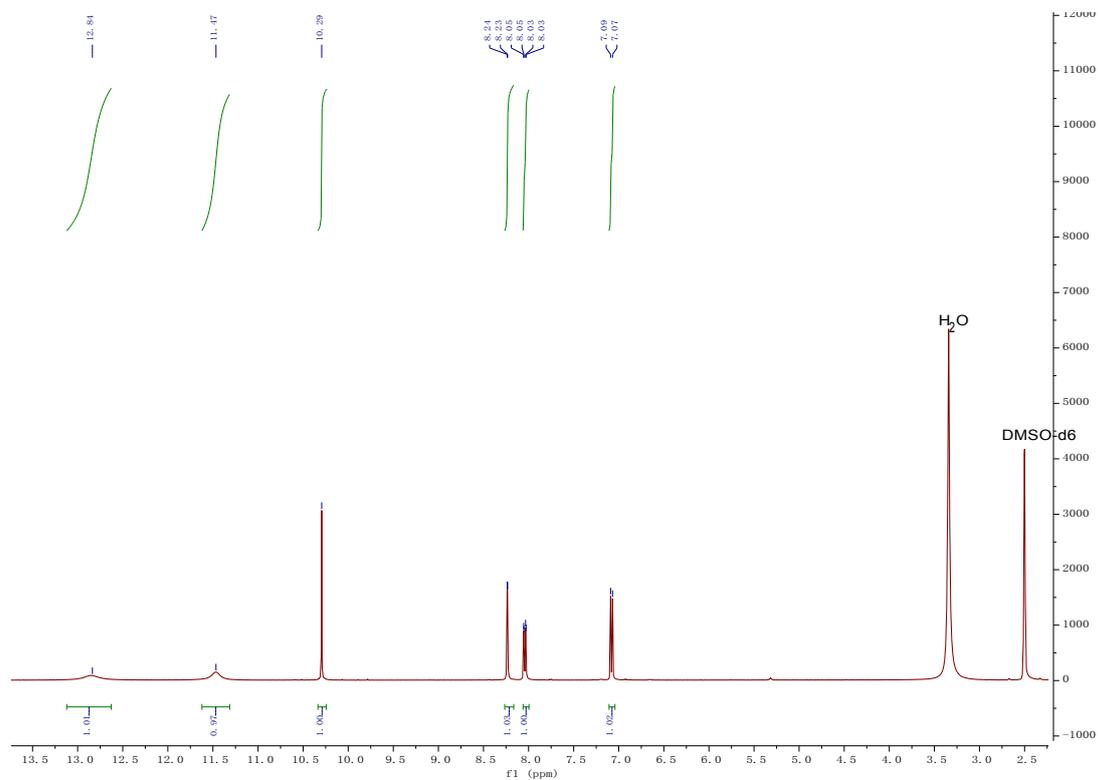
**Figure S15.** A TEM image of the mitochondria fraction from HUVEC cells pre-incubated with 500  $\mu\text{M}$  BQA-GGFF.



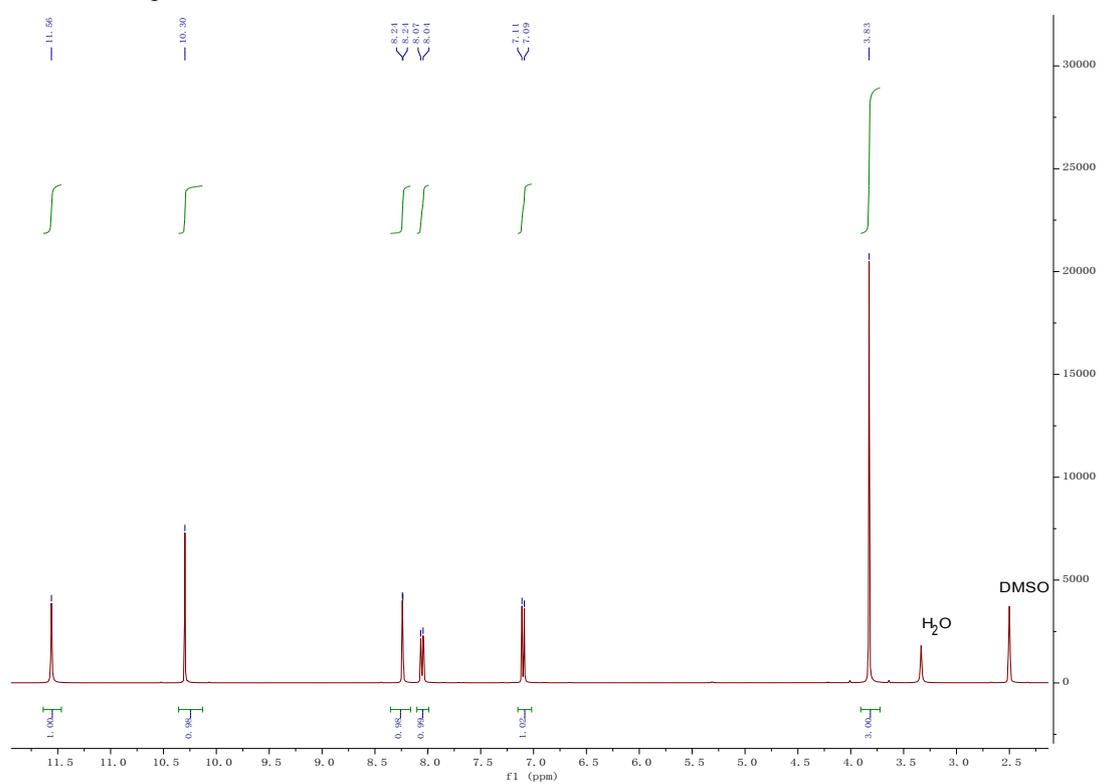
**Figure S16.** The average fluorescence intensity in individual cell among various cancerous and normal cell lines, calculated by image J. HPR: 4-hydroxyphenylretinamide; NAC: N-Acetyl-cysteine.

## 5. NMR spectra

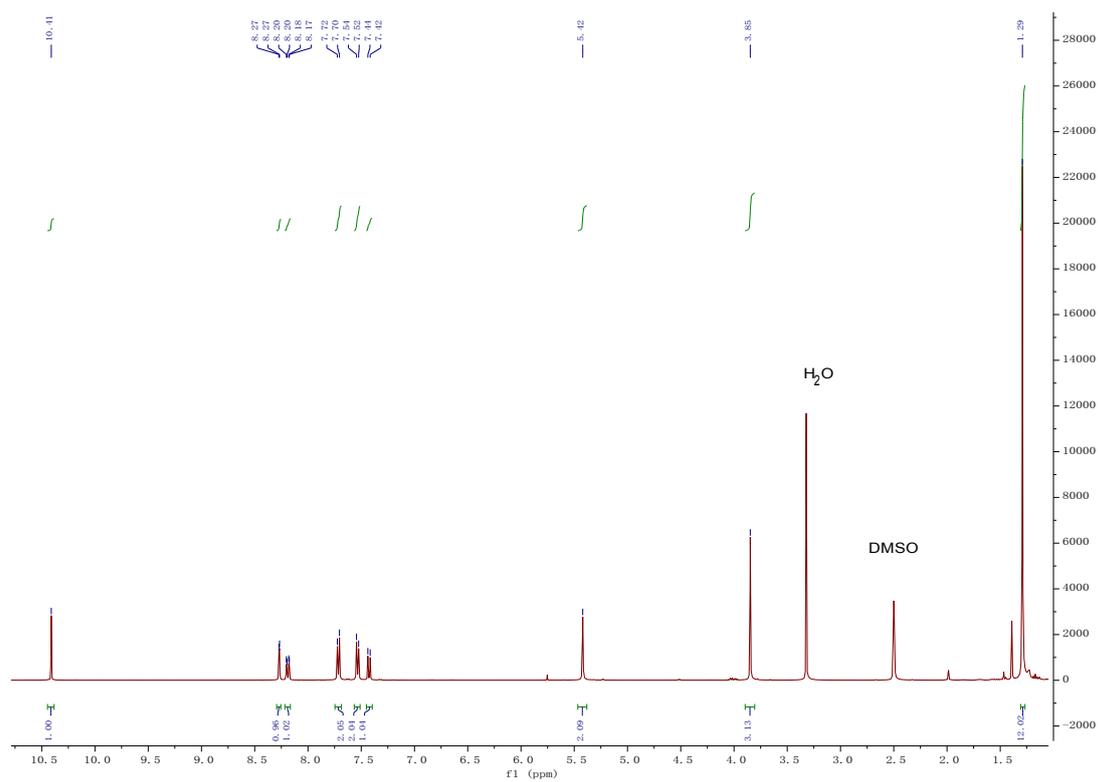
### The $^1\text{H}$ NMR of compound **1**



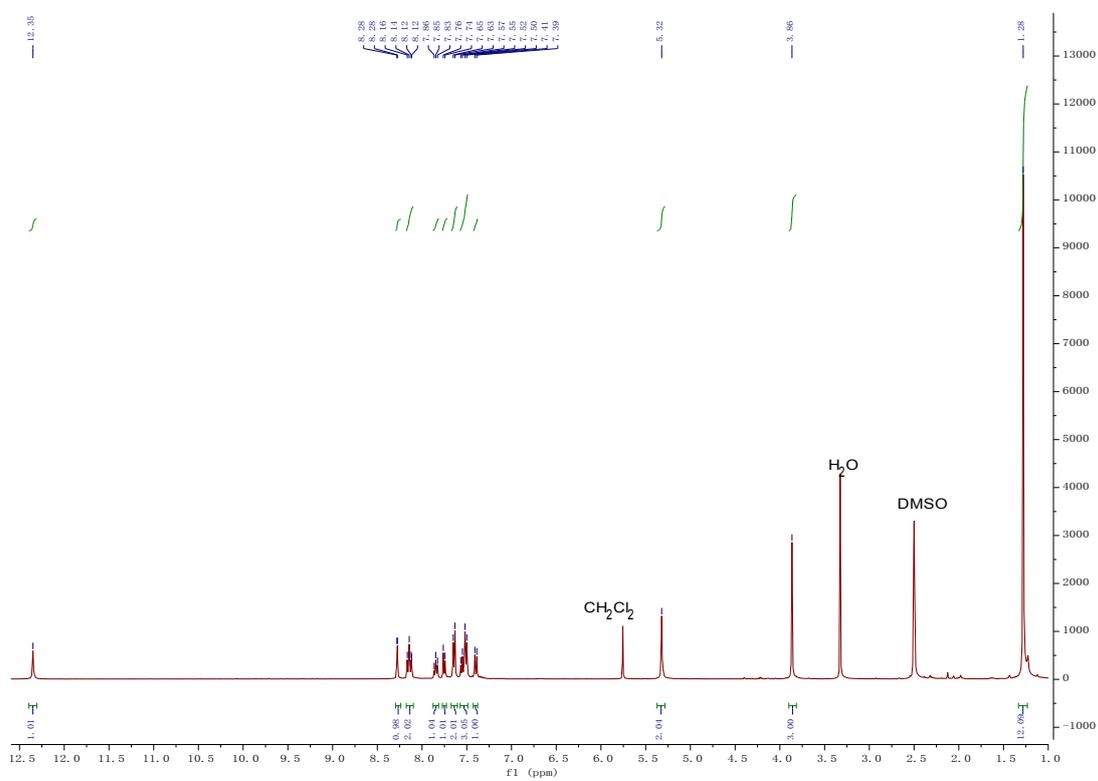
### The $^1\text{H}$ NMR of compound **2**



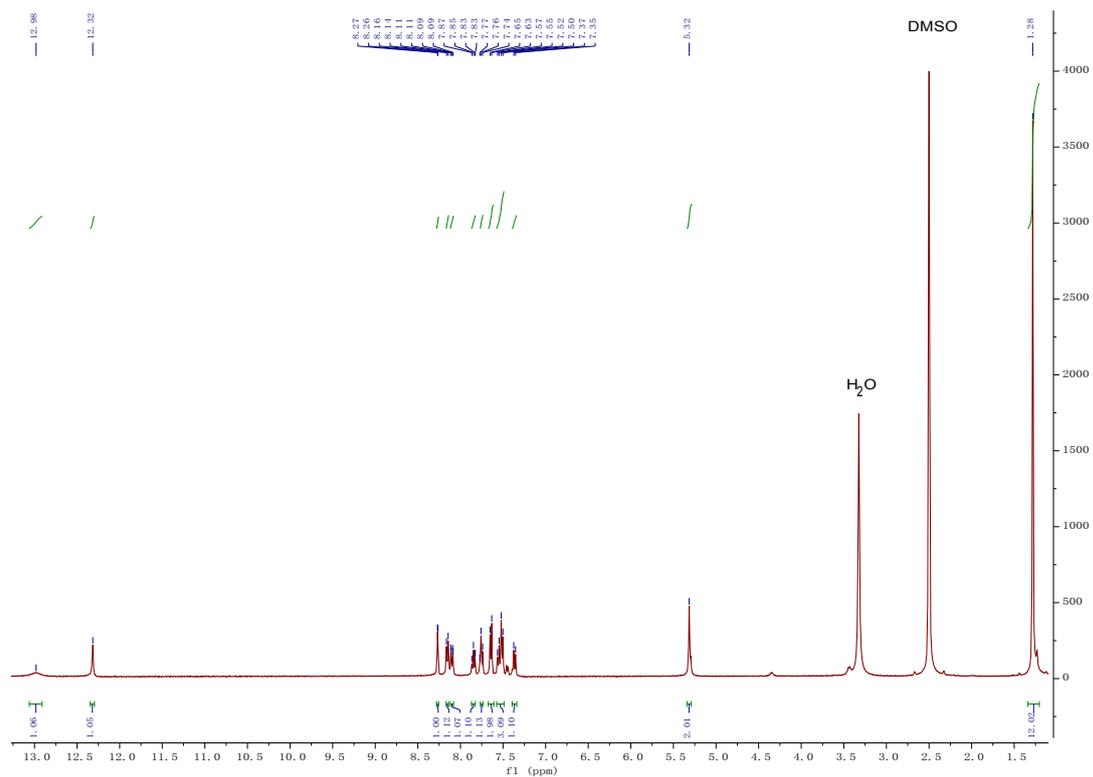
The  $^1\text{H}$  NMR of compound 3



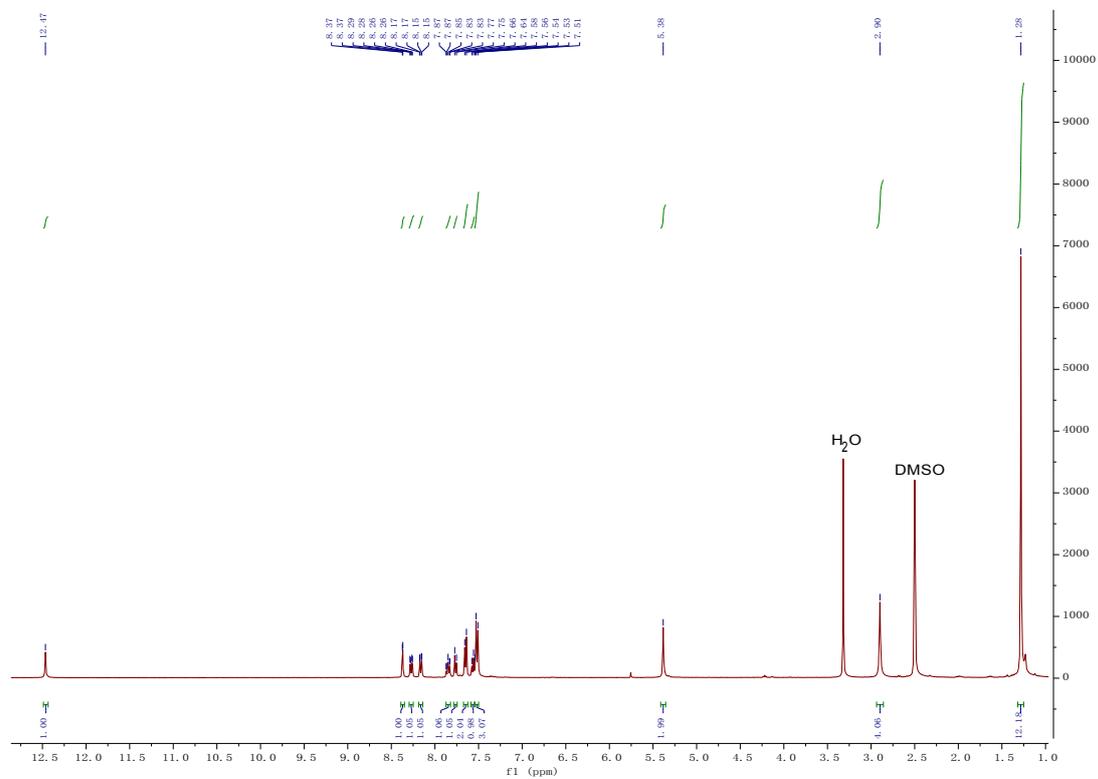
The  $^1\text{H}$  NMR of compound 4



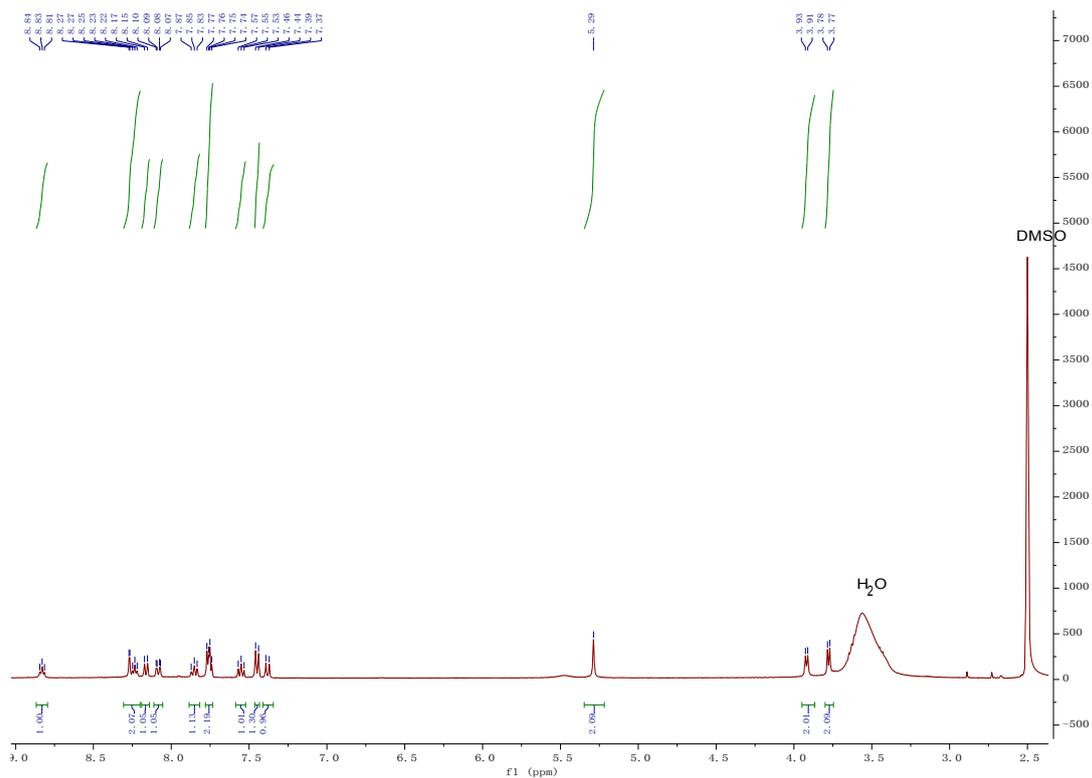
### The <sup>1</sup>H NMR of BQA



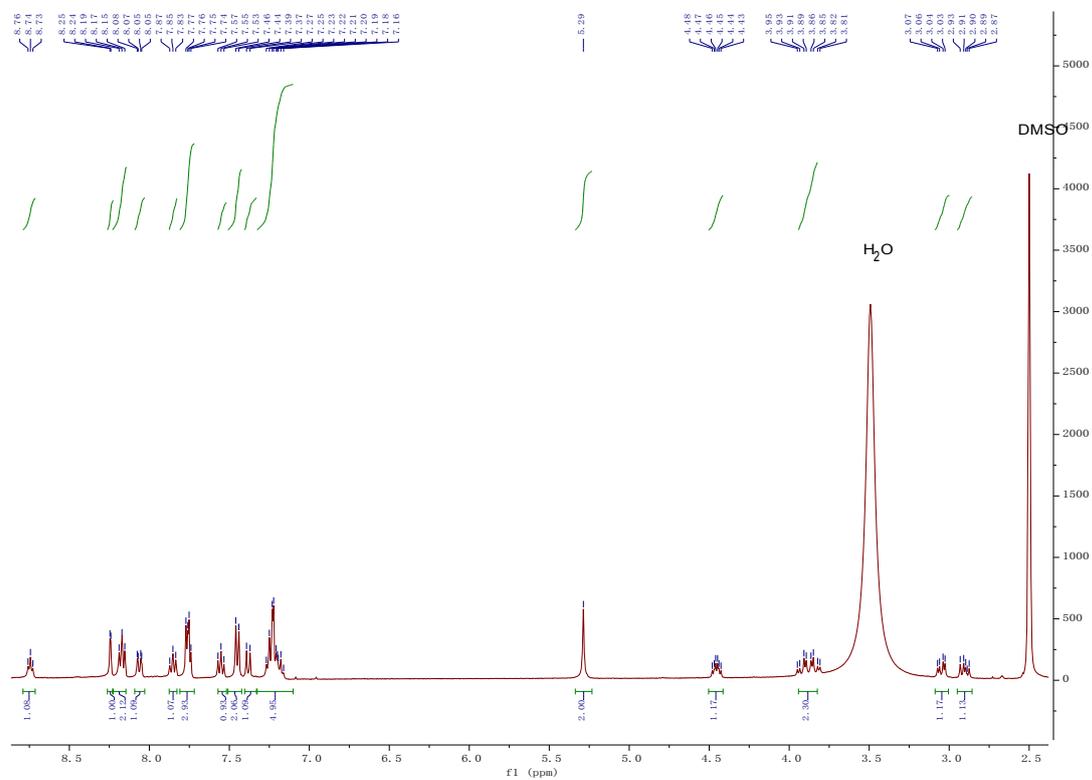
### The <sup>1</sup>H NMR of BQA-NHS



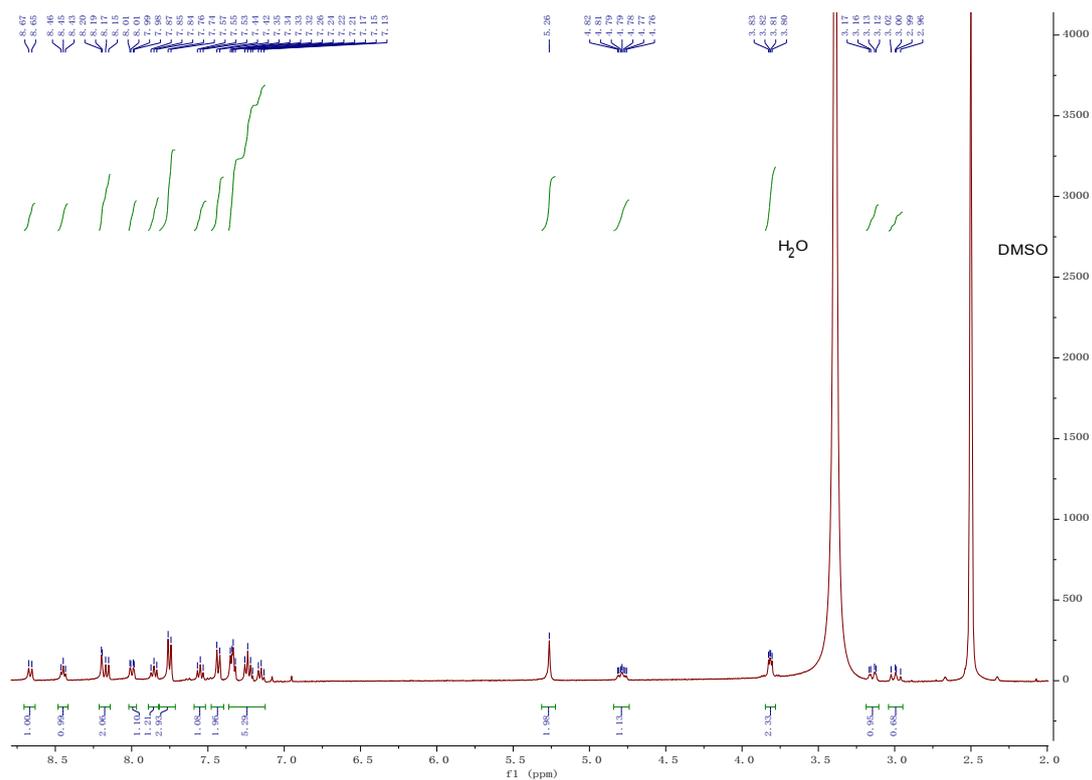
### The <sup>1</sup>H NMR of BQA-GG



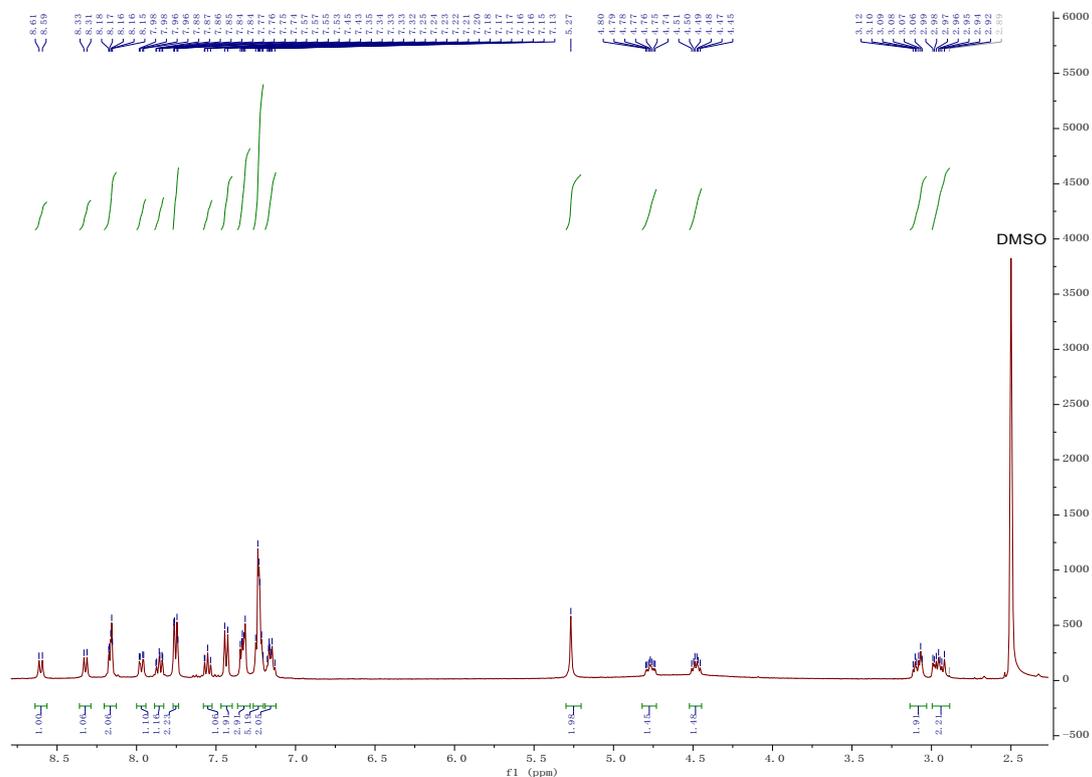
### The <sup>1</sup>H NMR of BQA-GF



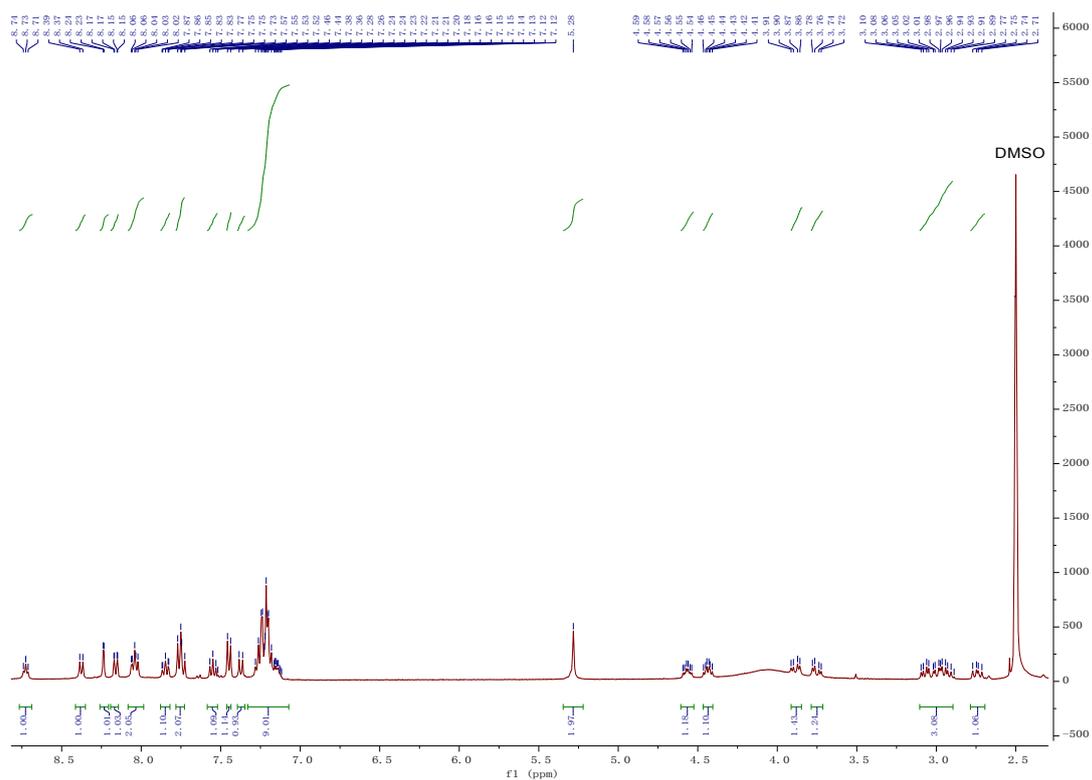
### The <sup>1</sup>H NMR of BQA-FG



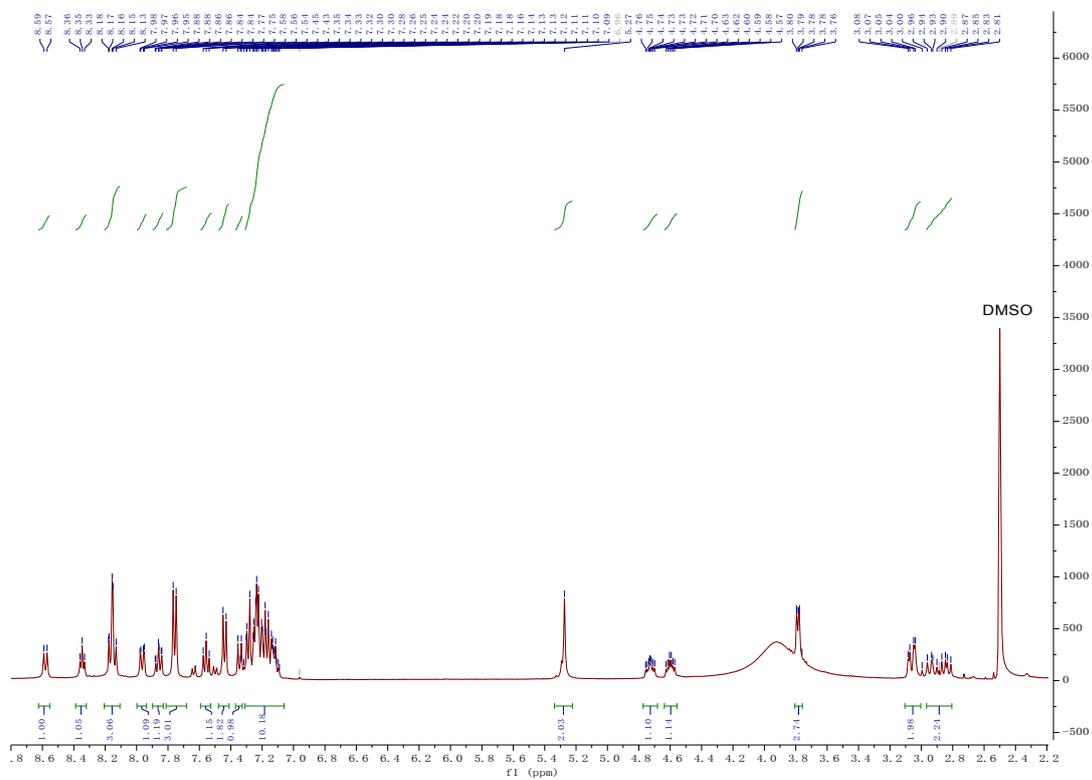
### The <sup>1</sup>H NMR of BQA-FF



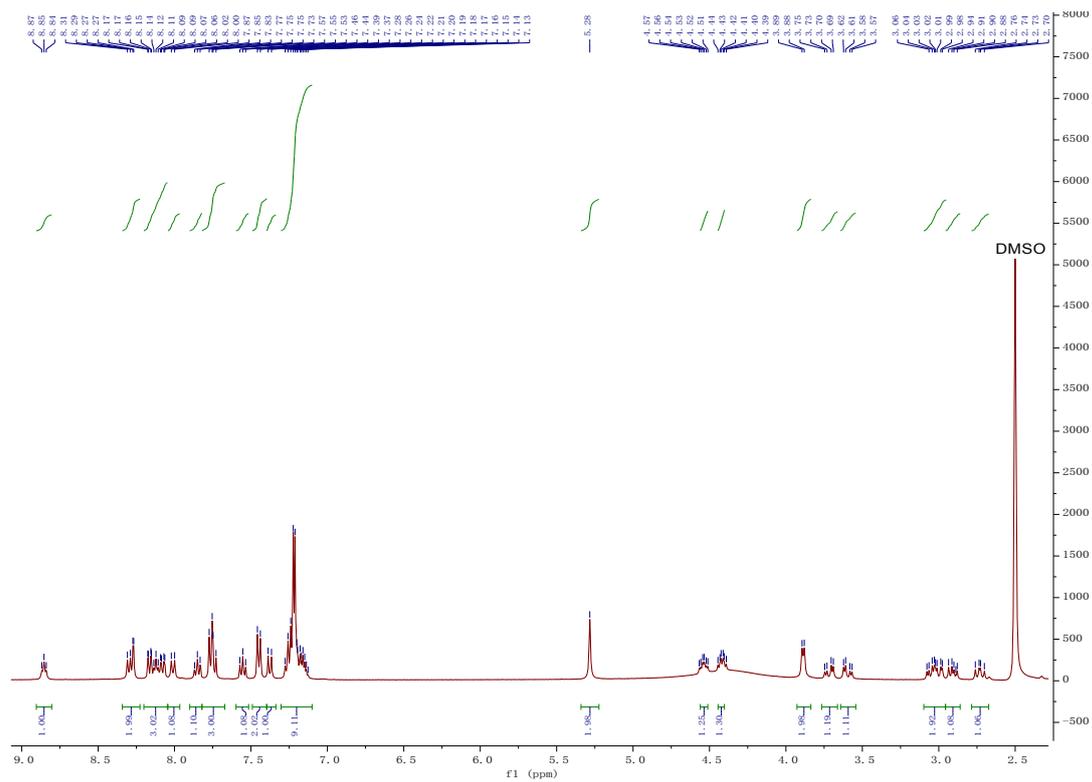
### The <sup>1</sup>H NMR of BQA-GFF



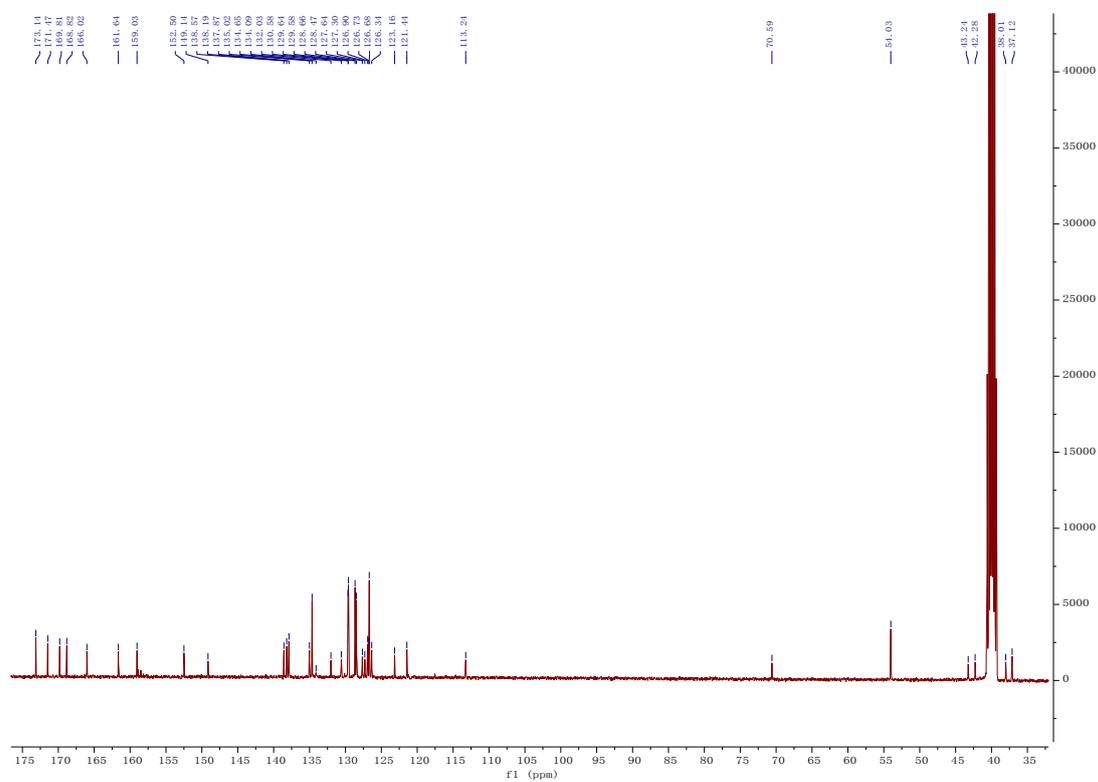
### The <sup>1</sup>H NMR of BQA-FFG



### The <sup>1</sup>H NMR of BQA-GGFF



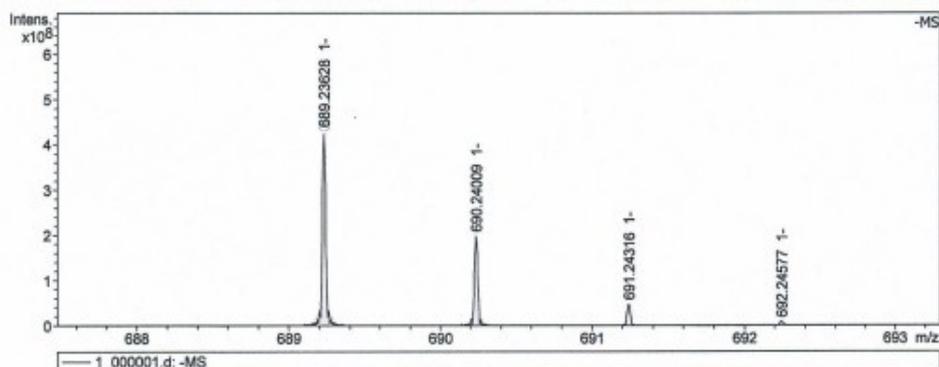
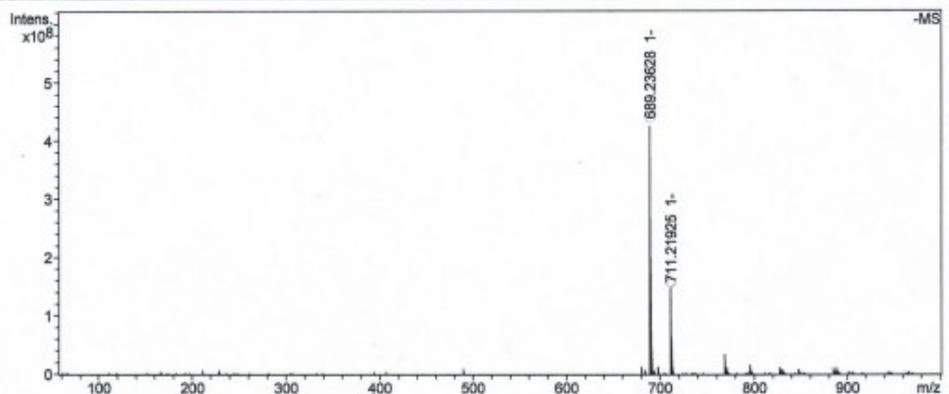
### The <sup>13</sup>C NMR of BQA-GGFF



# ESI(N),1,20180312

Analysis Info Acquisition Date 3/12/2018 9:56:02 AM  
 Analysis Name D:\Data\ESI\2018\2018-03\0312\1\_000001.d  
 Sample Name 1 Instrument solarix

Acquisition Parameter  
 Acquisition Mode Single MS Acquired Scans 10 Calibration Date Mon Mar 12 09:51:03  
 Polarity Negative No. of Cell Fills 1 Data Acquisition Size 2048578  
 Broadband Low Mass 57.7 m/z Source Accumulation 0.001 sec Data Processing Size 2097152  
 Broadband High Mass 1000.0 m/z Ion Accumulation Time 0.100 sec Apodization Sine-Bell Multiplication



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e <sup>-</sup> Conf	N-Rule
689.236279	1	C37H33N6O8	100.00	689.236536	0.4	-0.1	16.4	24.5	even	ok
711.219252	1	C35H27N12O6	69.90	711.218200	1.5	-1.9	5.2	28.5	even	ok
	2	C36H23N18O2	100.00	711.219538	0.4	-0.2	7.1	33.5	even	ok
	3	C38H35N2O12	96.98	711.219548	0.4	0.3	8.6	22.5	even	ok
	1	C37H32N8NaO8	94.50	711.218480	-1.1	-1.4	5.8	24.5	even	ok
	2	C38H28N10NaO4	100.00	711.219817	-0.6	0.4	8.5	29.5	even	ok

## 6. Reference

- [1] V. Bereau, V. Jubera, P. Arnaud, A. Kaiba, P. Guionneau, J. P. Sutter, *Dalton T.*, 2010, **39**, 2070-2077.
- [2] Z. X. Bie, G. S. Li, L. Y. Wang, Y. Lv, J. Y. Niu, S. Gao, *Tetrahedron Lett.*, 2016, **57**, 4935-4938.