A novel colorimetric "on-off-on" fluorescent probe based on tris(salicylaldehyde) for the sequential recognition of Cu^{2+}/Hcy and its application in bioimaging†

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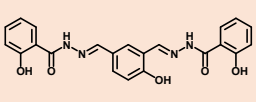
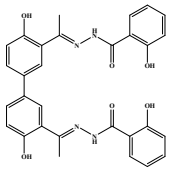
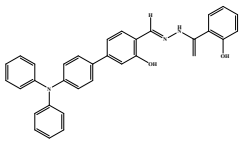
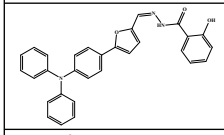
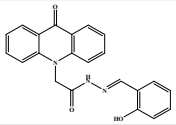
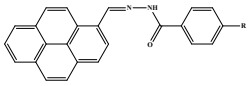
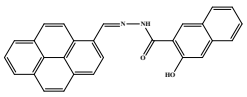
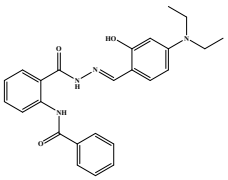
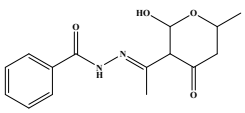
Table S1 Comparison of **DBD** with other representative fluorescence probes

Probe molecule	Test object	Solvent system	Reversibility	LOD	Applications	Ref.
	Cu^{2+} and Hcy	DMSO/PBS (1:1, v/v)	Yes	Cu^{2+}: 7.43 nM Hcy: 54.5 nM	Living Cells Zebrafish Water sample Paper test	This work
	Cu^{2+}	EtOH/HEPES (1:1, v/v)	No	1.54×10^{-8} M	Living Cells	1
	Cu^{2+} and Cys	DMSO/H_2O (7/3, v/v)	Yes	Cu^{2+}: 1.62×10^{-7} M Cys: 1×10^{-4} M	No	2
	Cu^{2+}	EtOH/H_2O (2:3, v/v)	No	Cu^{2+}: 9.8×10^{-8} M	Material	3
	Cu^{2+}	DMSO/H_2O (4:1, v/v)	No	Cu^{2+}: 0.80 μM	No	4
 R=OCH_3/$OCH_2CON(CH_2CH_3)_2$	Cu^{2+}	CH_3CN/CH_2Cl_2 (1000:1, v/v)	No	Cu^{2+}: 8.80×10^{-8}/ 4.94×10^{-7} M	No	5
	Cu^{2+}	DMSO/HEPES（1:1 v/v）	No	Cu^{2+}: 0.26×10^{-6} M	No	6
	Cu^{2+} and Cys	CH_3OH/H_2O（1/1, v/v）	Yes	Cu^{2+}: 9.3×10^{-7}M GSH: 5.86×10^{-6} M	No	7
	Cu^{2+}	H_2O/CH_3CH_2OH（80:20, v/v）	No	Cu^{2+}: 0.156 μM	Water samples	8

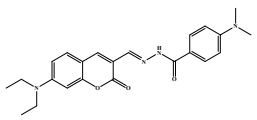
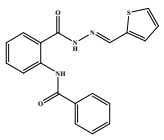
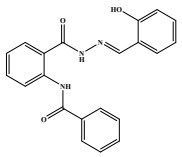
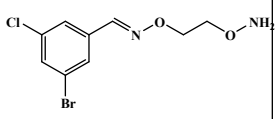
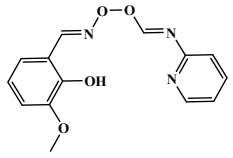
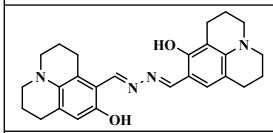
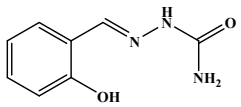
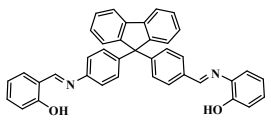
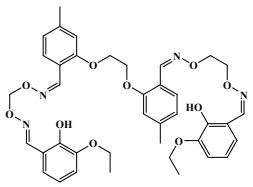
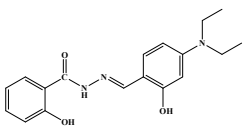
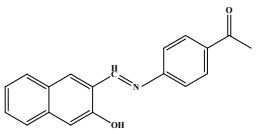
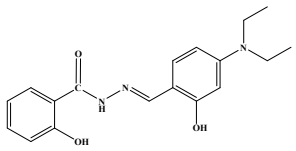
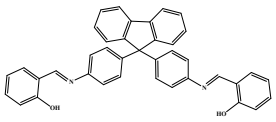
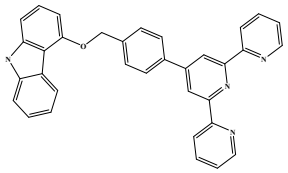
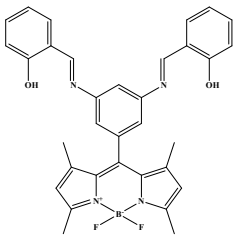
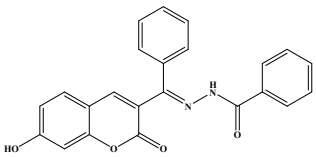
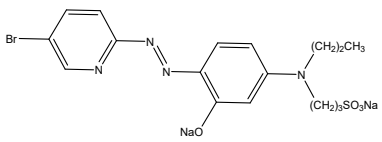
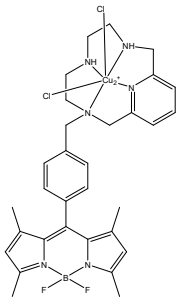
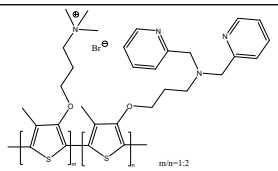
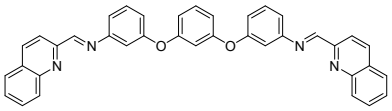
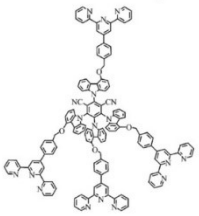
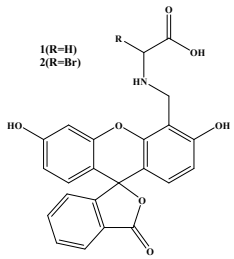
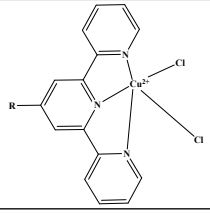
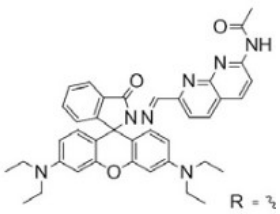
	Cu^{2+} and GSH	CH_3OH/PBS（1/1, v/v）	Yes	Cu^{2+}: 2.40×10^{-8} M GSH:1.29×10^{-7} M	MCF-7cells, Zebrafish	9
	Cu^{2+} and Cys	CH_3OH/Tris (1:1, v/v, pH = 7.2)	Yes	Cu^{2+}: 1.8 μM	Water sample	10
	Cu^{2+}/Ni^{+}	CH_3OH/H_2O (1:1 v/v)	No	Ni^{+}: 1.61×10^{-6} M Cu^{2+}: 2.26×10^{-6} M	Water sample	11
	Cu^{2+} and S^{2-}	HEPES buffer	Yes	Cu^{2+}: 48.59 nM S^{2-}: no	Water sample	12
	Cu^{2+}	DMF:H_2O (9:1, v/v)	No	Cu^{2+}: 8.68×10^{-8} M	Water sample	13
	Cu^{2+}	DMSO:H_2O (1:4, v/v)	No	Cu^{2+}: 0.25 μM	Water sample Paper test	14
	Cu^{2+}/Mg^{2+}	PBS buffer (pH=7.0)	No	Cu^{2+}: 40.5 nM. Mg^{2+}: 9.5 nM	No	15
	Cu^{2+}	DMF/H_2O (2:8, v/v)	No	Cu^{2+}: 2.13 nM	Foods Water samples	16
	Cu^{2+}/Fe^{3+} S^{2-}	CH_3CN:H_2O (9:1, v:v,)	Yes	Fe^{3+}: 1.1×10^{-8} M Cu^{2+} :7.3×10^{-8} M S^{2-}: no	Cells Paper model	17
	Cu^{2+}/Fe^{2+} EDTA	DMSO/H_2O（4:1, v/v）	Yes	Cu^{2+}: 0.18 μM Fe^{2+}: 1.3 μM EDTA: no	Living cells; Logic gate structure	18

Table S2 Comparison of fluorescence probes for sequential recognition of Cu^{2+} and Hcy.

Sensors	Detected ions	LODs	Solvents	Ref.
	Cu^{2+} Cys GSH	Cys: 3.4×10^{-5} M GSH: 2.0×10^{-5} M	C_2H_5OH/H_2O (1:1, v/v)	19
	Cu^{2+} EDTA	Cu^{2+}: 0.18 μM	DMSO/H_2O (4:1, v/v)	20
	Cu^{2+} GSH	Cu^{2+}: 2.13 nM GSH: 2.02 nM	DMF/H_2O （2:8, v/v）	13
	Cu^{2+} Cys Hcy GSH	33.6 nM 0.19 μM 0.21 μM 0.29 μM	CH_3CN/HEPES (1:1, v/v)	21
	Cys Hcy	0.17 μM 0.25 μM	CH_3CN/H_2O (1:1, v/v)	22
	Cu^{2+} Cys Hcy GSH	8 μM 10 μM 10 μM 16 μM	CH_3CN/H_2O (9:1,v/v)	23
	Cu^{2+} Hcy	1.46 μM	HEPES	24
	Cu^{2+} Hcy	116.0 mM	Tris-HCl	25

 PTNPy	Cu^{2+} Hcy	6.79 mM PTNPy	Tris-HCl	26
y-CDs and CuNCs	Cu^{2+} Hcy Cys GSH	0.21 μM 0.46 μM 0.39 μM 0.33 μM	PBS buffer	27
HAS-AuNC@Cu^{2+} complex	Hcy	40 mM	PBS buffer	28
	Cu^{2+} Hcy	1.20 μM 20.2 μM	no	29
	Cu^{2+} Hcy	33.6 mM 0.21 μM	CH_3CN/HEPES (1:1, v/v)	30
	Cu^{2+} Hcy	0.017 ppm 0.036 μM	HEPES buffer	31
	Hcy	0.1 nM	HEPES buffer	32
D-P4 (peptide-based fluorescent probe)	Cu^{2+} Hg^{2+} Cys	105 nM 37 nM 82 nM	HEPES buffer	33
	Cu^{2+} Hcy Cys GSH	NO	EtOH/HEPES (3:7, v/v)	34
A ratiometric nanoplatform based on Eu MOFs and CDs	Cu^{2+} Hcy Cys	0.57 μM 0.14 μM 0.42 μM	HEPES buffer	35

	GSH	0.54 μM		
Eu^{3+}-DTPA-bis(AMC) complex	Cu^{2+} Hcy Cys GSH	0.065 μM 0.05 μM 0.11 μM 0.07 μM	TEAA buffer	36
SiO_2@$COF_{BMTH-HB}$	Cu^{2+} Hcy Cys	 75 nM 104 nM	PBS buffer	37
CuS-Cu hybrid nanostructures	Hcy	1.63 μM	PBS buffer	38

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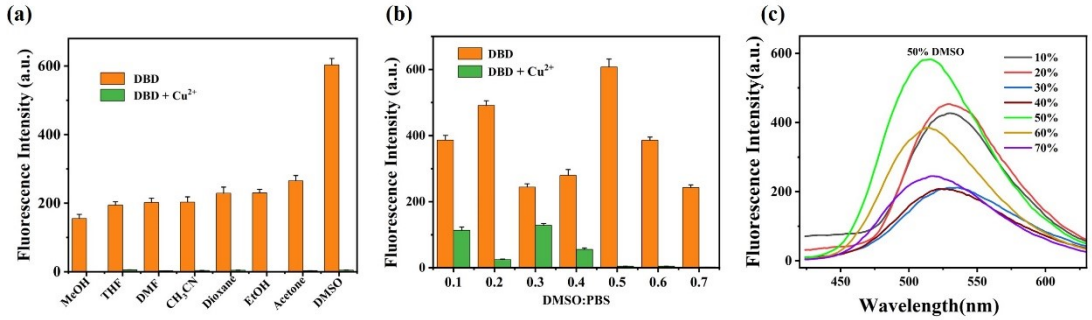

Fig. S1 Fluorescence spectra of **DBD** (10 μM) reaction with Cu^{2+} ions (15 μM) in different solvent (a) or in PBS buffer with different volume of DMSO (b and c).

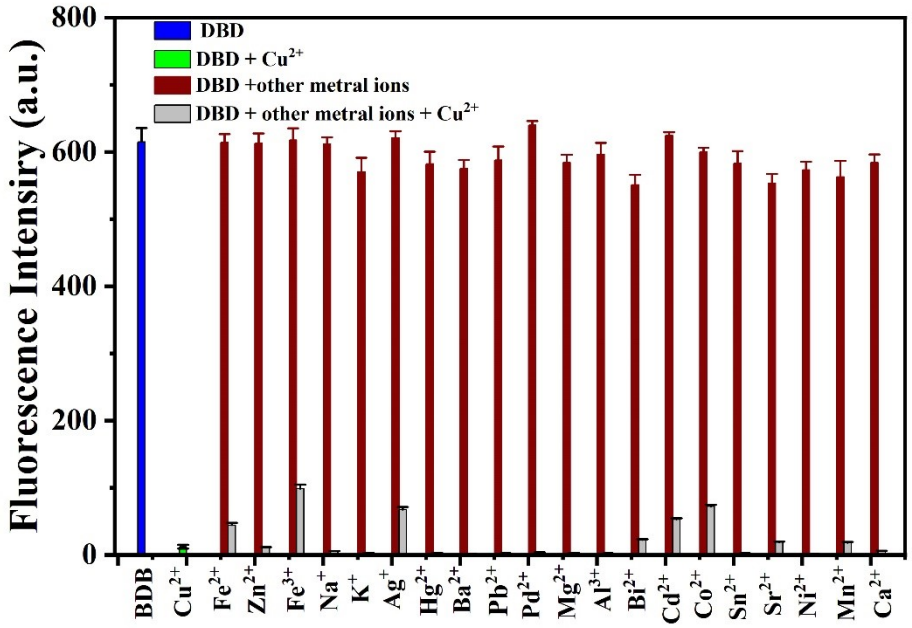

Fig. S2 The fluorescence intensity response of probe **DBD** (10 μM) towards Cu^{2+} ions in the presence of various analytes (15 μM) in DMSO/PBS solution at room

temperature.

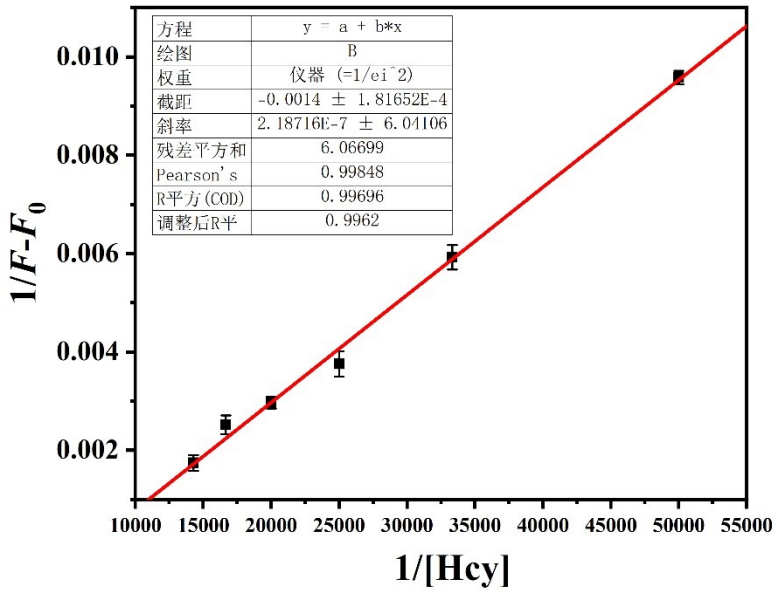

Fig. S3 Benesi-Hildebrand plot analysis of the emission intensity at 515 nm for the complexation between probe **DBD**-Cu^{2+} complex and Hcy (100 μM).

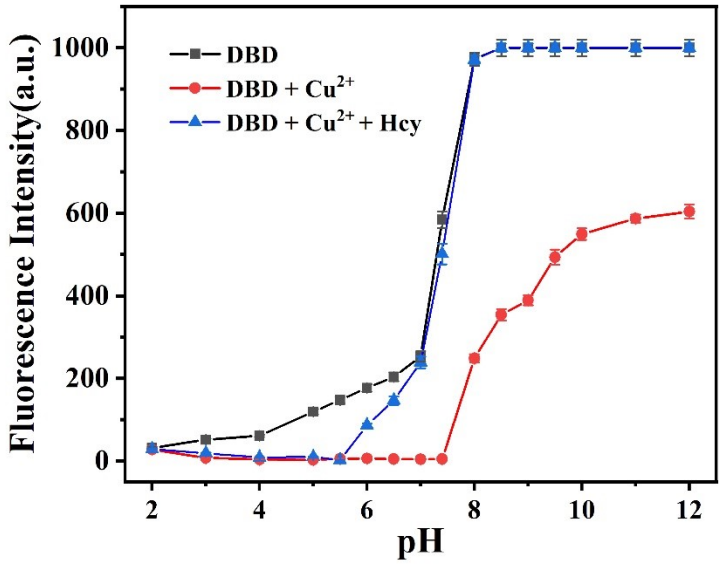

Fig. S4 effect of pH on the fluorescence intensity of **DBD** (10 μM) with and without Cu^{2+} ions (15 μM) and Hcy (100 μM) in different pH solution.

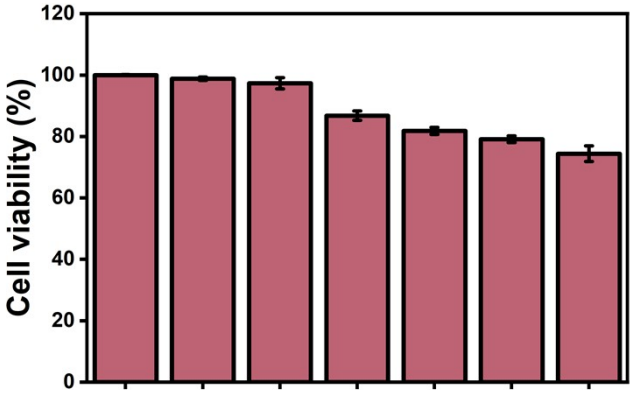

Fig. S5 Viability o

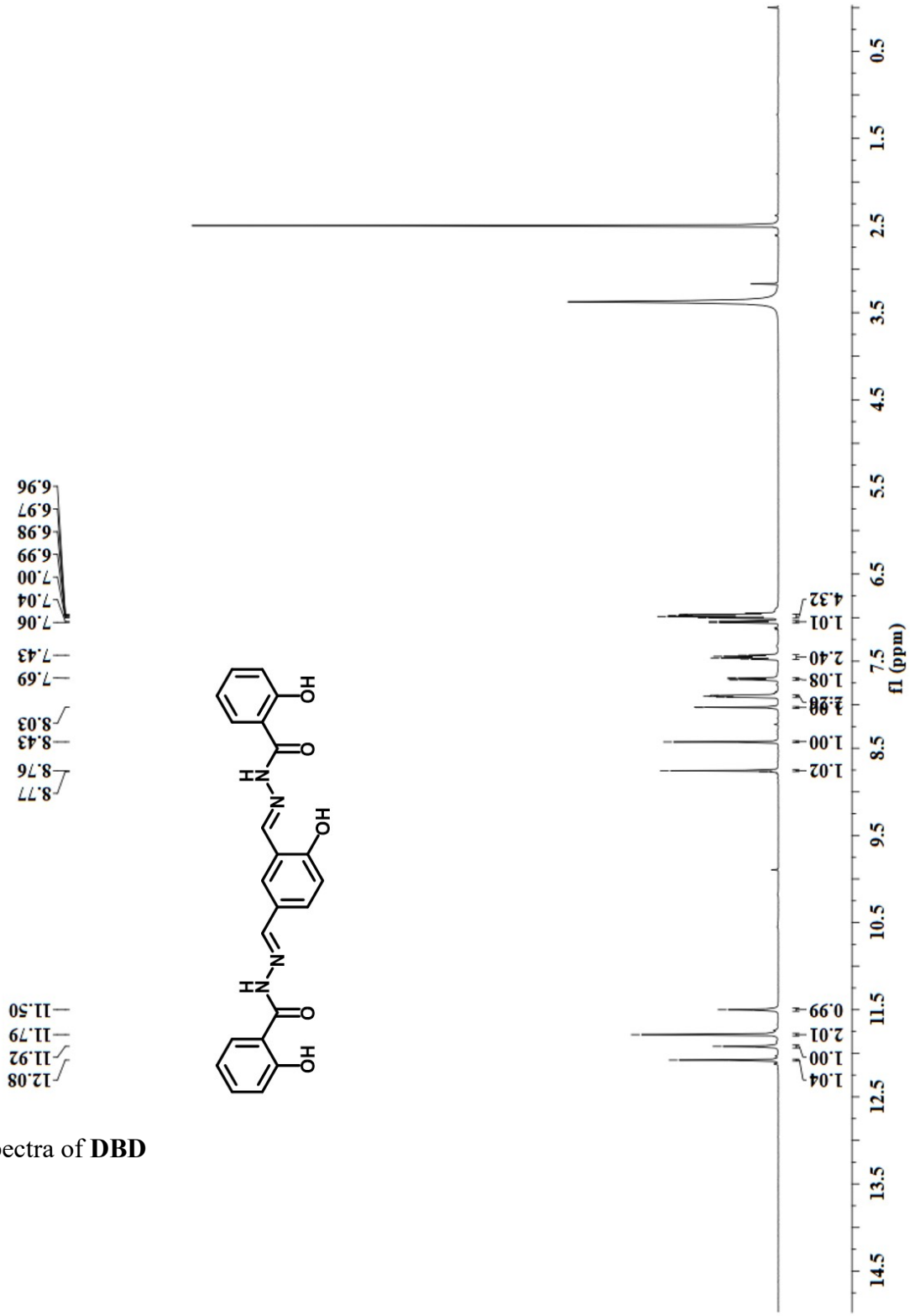

Fig. S6 ^{1}H NMR spectra of **DBD**

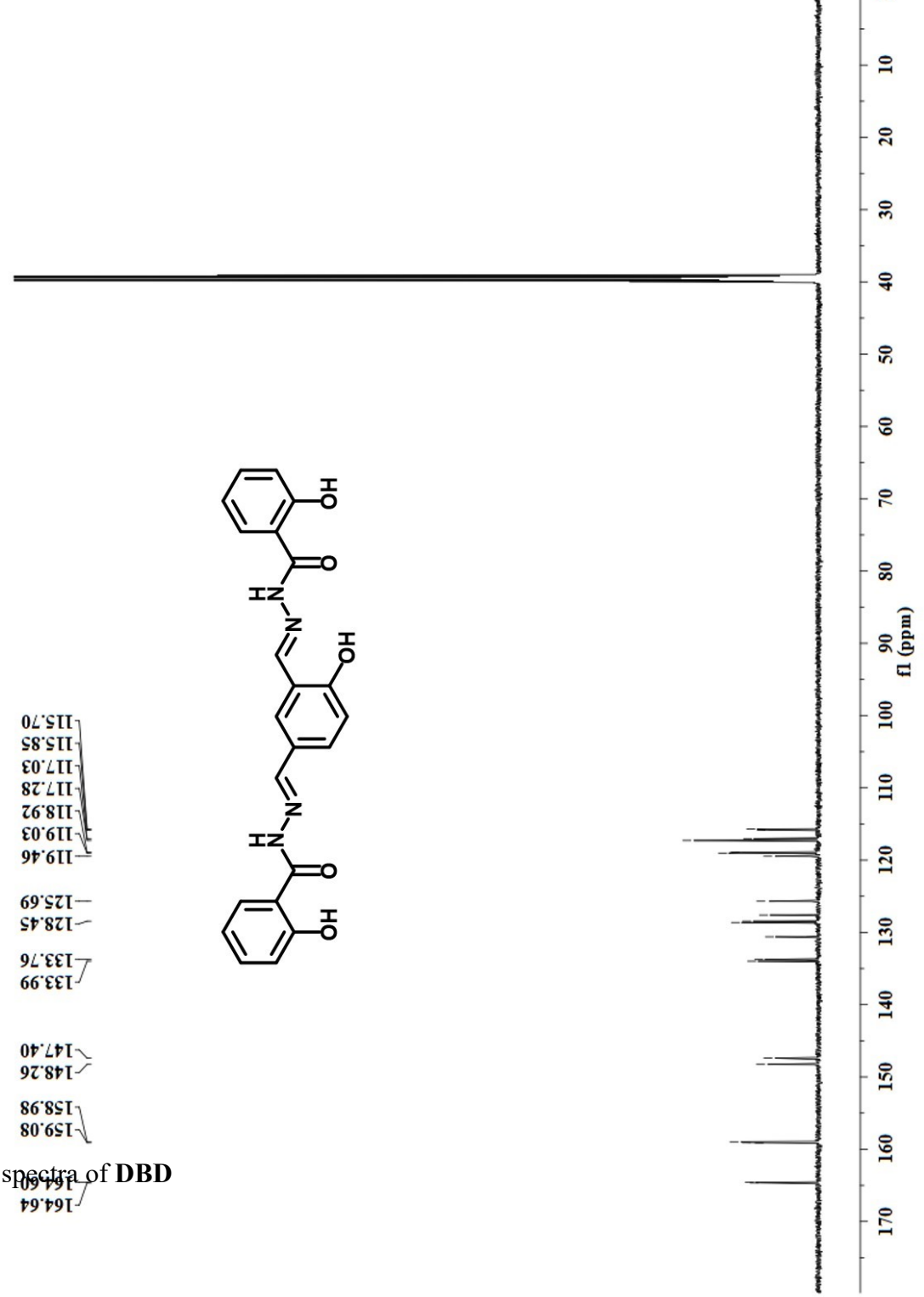

Fig. S7 ^{13}C NMR spectra of **DBD**

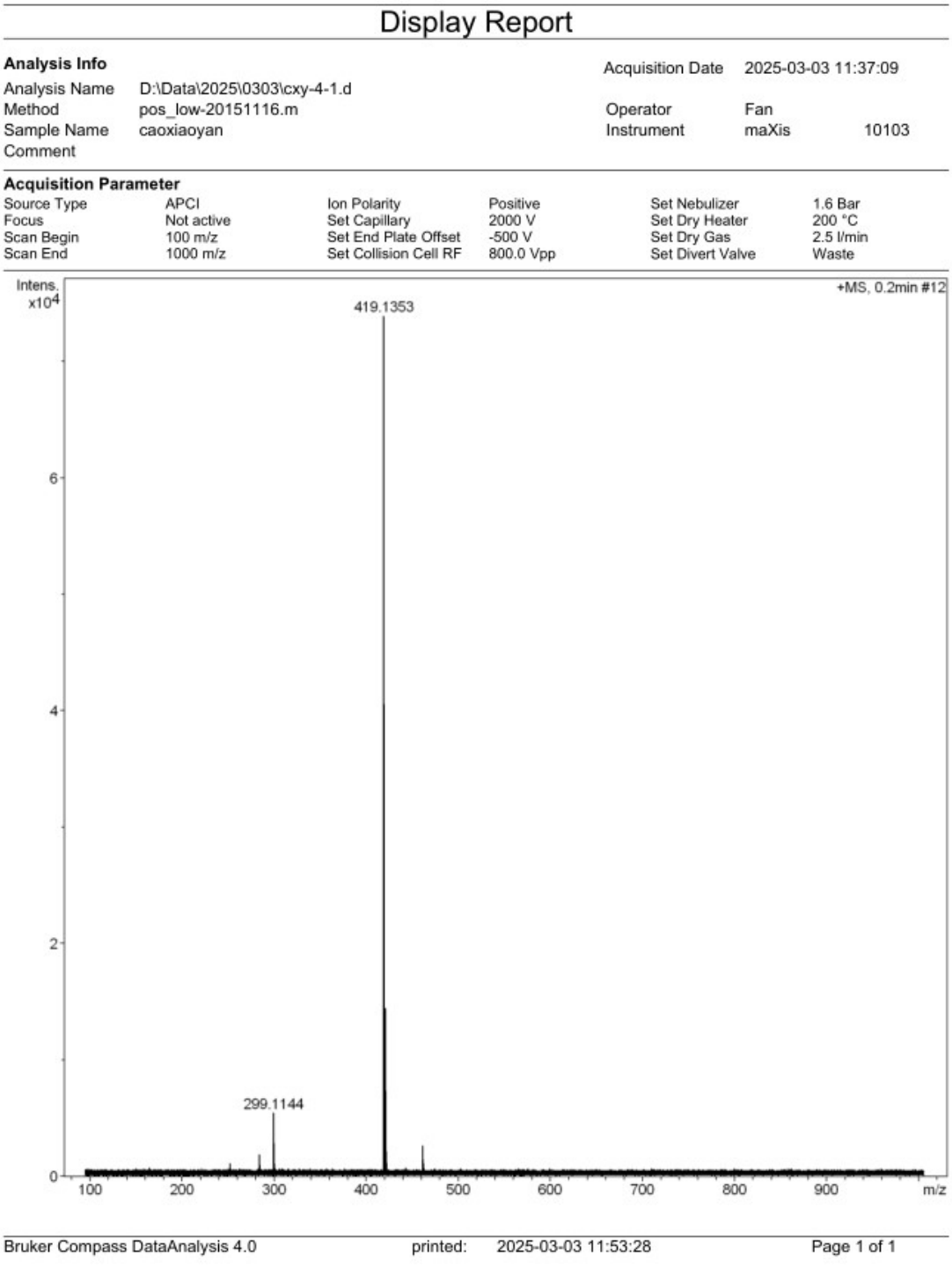

Fig. S8 High resolution mass spectra of **DBD**

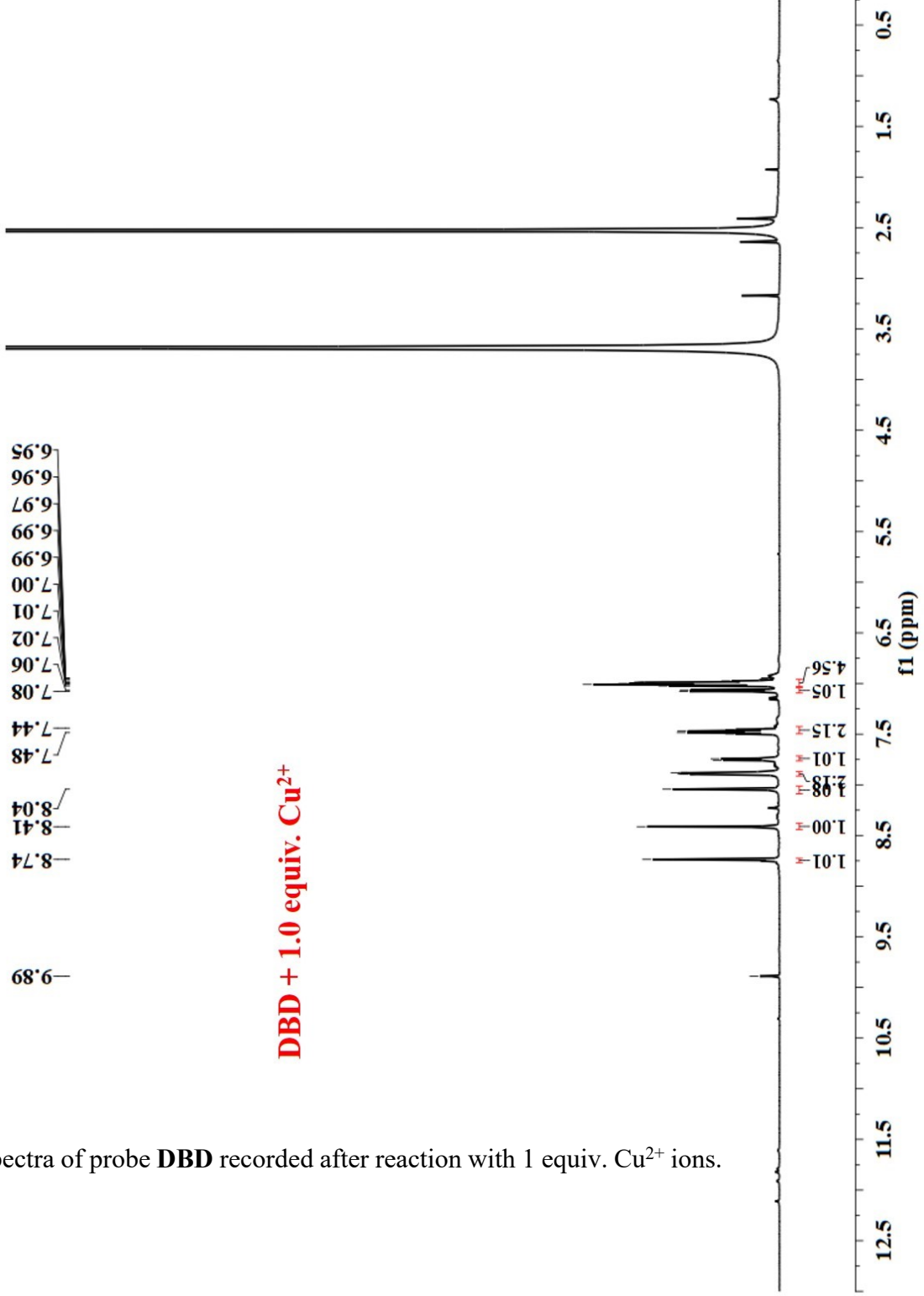

Fig. S9 ^{1}H NMR spectra of probe **DBD** recorded after reaction with 1 equiv. Cu^{2+} ions.

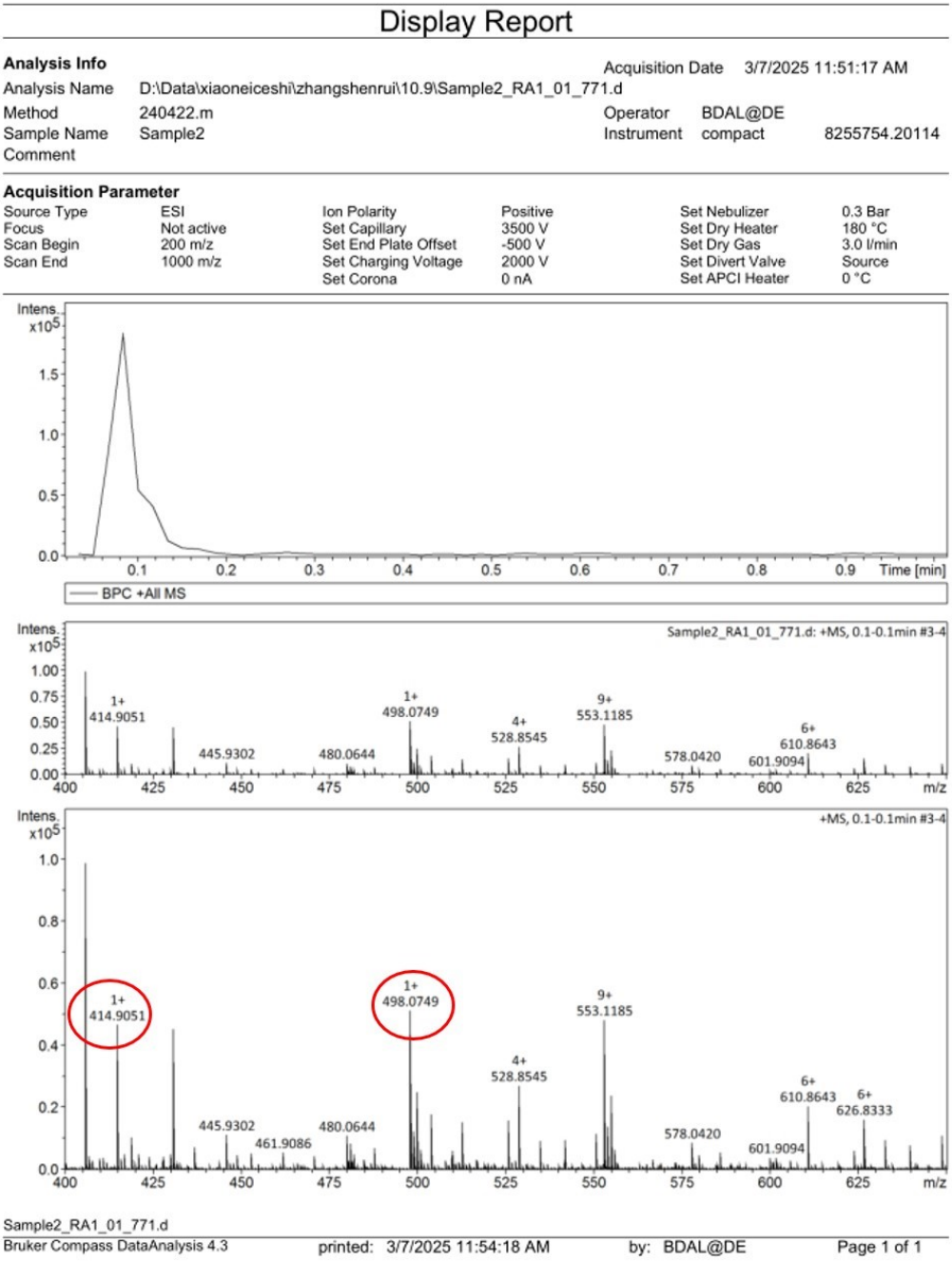

Fig. S10 High resolution mass spectra of probe **DBD** recorded after reaction with 1.5 equiv. Cu^{2+} ions.

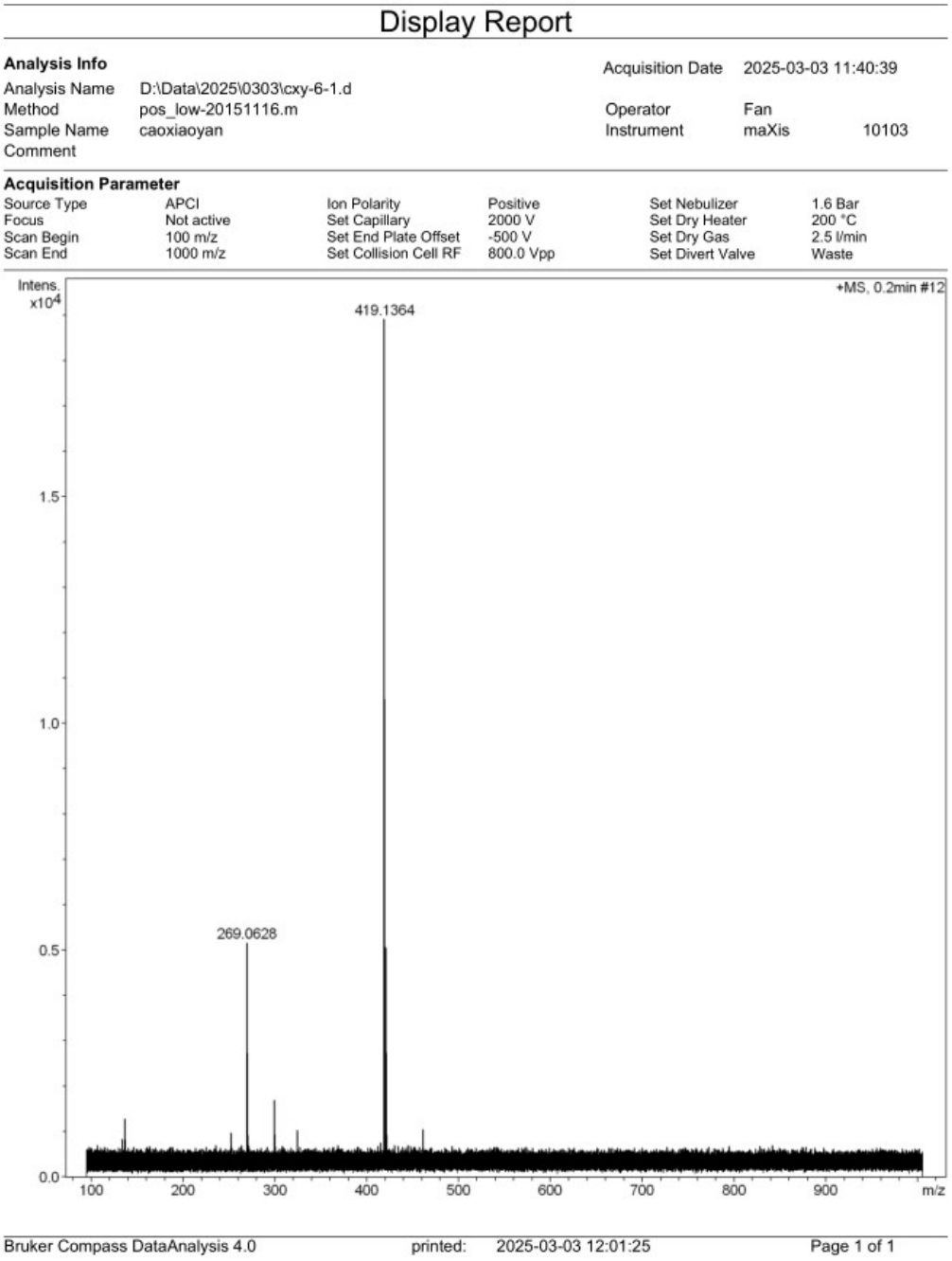

Fig. S11 High resolution mass spectra of **DBD-Cu^{2+}** complex recorded after reaction with 10 equiv. Hcy.