Supplementary Information for

Mix-and-Read Bioluminescent Copper Detection Platform Using a Caged Coelenterazine Analogue

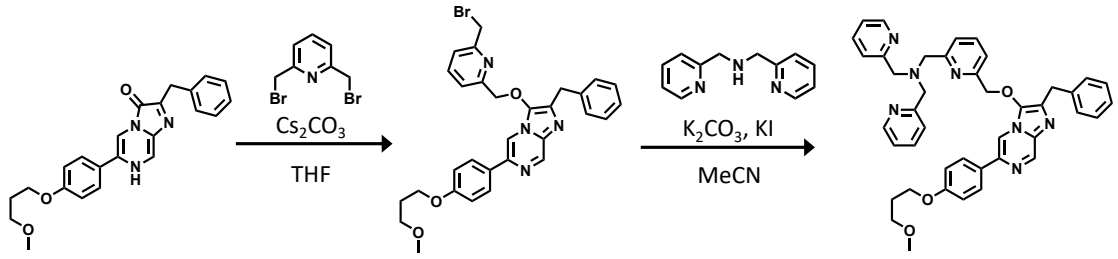
Ryo Nishihara[1,2] and Ryoji Kurita[1,2,3]

1. National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan. 2. DAILAB, DBT-AIST International Centre for Translational and Environmental Research (DAICENTER), National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan. 3. Faculty of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan.

Correspondence should be addressed to
R. Nishihara (r.nishihara@aist.go.jp) and R. Kurita (r.kurita@aist.go.jp)

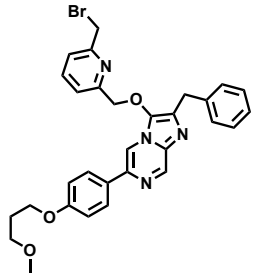
Contents

1. Synthesis



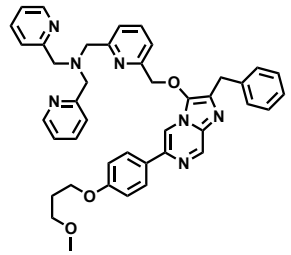
Scheme 1. Synthesis scheme for TPA-H1

2-benzyl-3-((6-(bromomethyl)pyridine-2-yl)methoxy)-6-(4-(3-methoxypropoxy)phenyl)imidazo[1,2-a]pyrazin (1)



To a solution of HuLumino1 (150.0 mg, 0.3 mmol, 1 eq.) and 2,6-bis(bromomethyl)pyridine (204.0 mg, 0.7 mmol, 2 eq.) in THF (16 ml), Cs_2CO_3 (150.0 mg, 0.4 mmol) was added. The mixture was stirred at RT overnight. After filtration, the filtrate was concentrated and purified by flash chromatography (silica gel, eluent composition: n-hexane / ethyl acetate = 50/50), affording 2-benzyl-3-((6-(bromomethyl)pyridine-2-yl)methoxy)-6-(4-(3-methoxypropoxy)phenyl)imidazo[1,2-a]pyrazin (1) as a brown oil (126.6 mg, 57%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.95 (s,1H), 8.23 (s, 1H), 7.83 (d, J = 8.75 Hz, 2H), 7.70 (t, J = 7.72 Hz, 2H), 7.51-7.10 (m, 7H), 7.00 (t, J = 8.80 Hz, 2H), 5.04 (s, 2H), 4.48 (s, 2H), 4.18 (s, 2H), 4.11 (t, J = 6.22 Hz, 2H), 3.58 (t, J = 6.15 Hz, 2H), 3.37 (s, 3H), 2.08 (t, J = 6.24 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 159.71, 157.38, 155.20, 142.29, 138.99, 138.20, 136.79, 134.44, 133.36, 130.41, 129.28, 128.96, 128.77, 128.03, 127.61, 126.65, 123.59, 122.10, 115.01, 109.96, 69.34, 65.11, 58.90, 33.81, 33.40, 29.76. HR-MS: calcd for $C_{30}H_{30}O_3N_4Br$:573.14940 $[M+H]^+$, found m/z 573.14958.

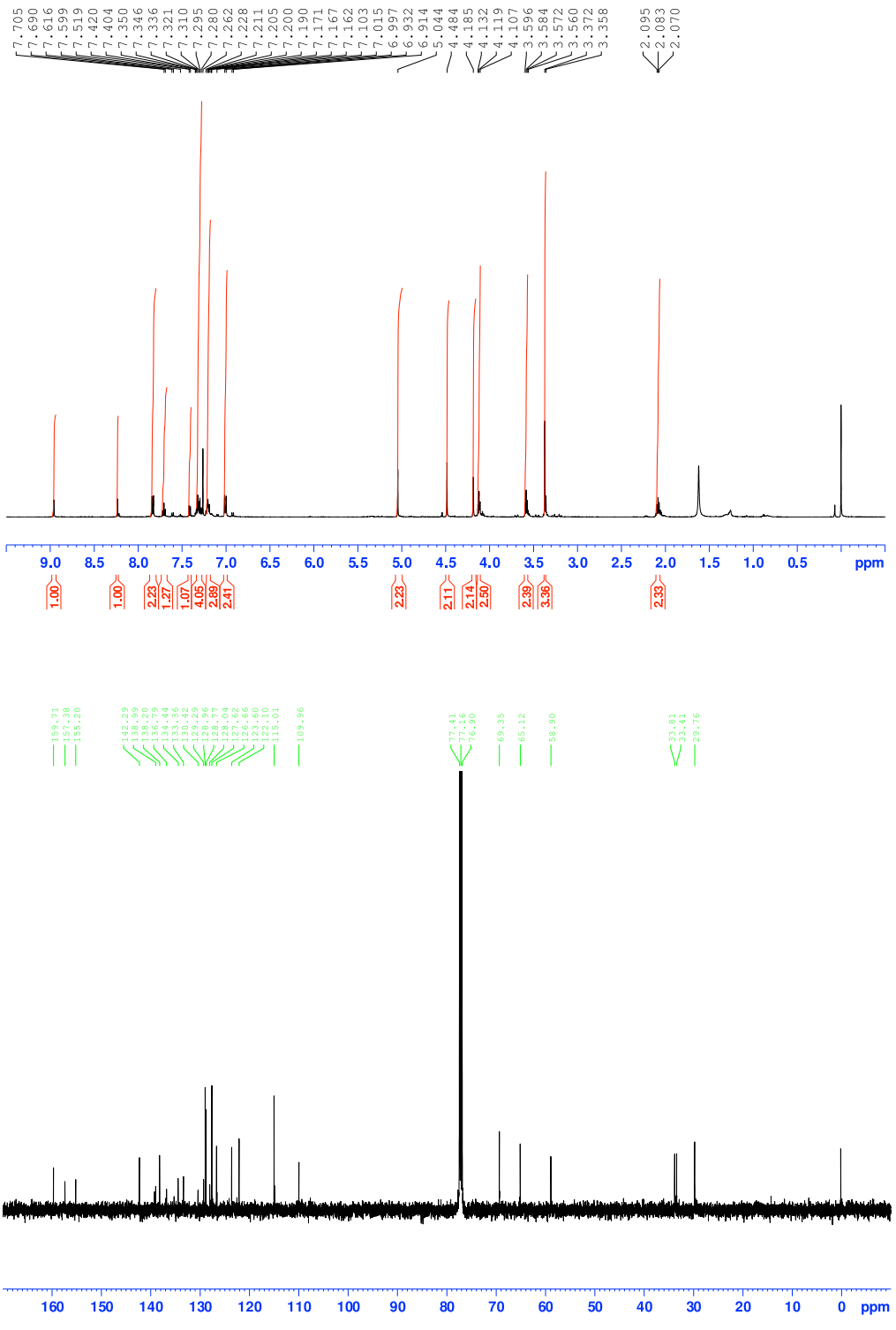
1-(6-(((2-benzyl-6-(4-(3-methoxypropoxy)phenyl)imidazo[1,2-a]pyrazin-3-yl)oxy)methyl)pyridine-2-yl)-N,N-bis(pyridine-2-ylmethyl)methanamine (2) (TPA-H1)



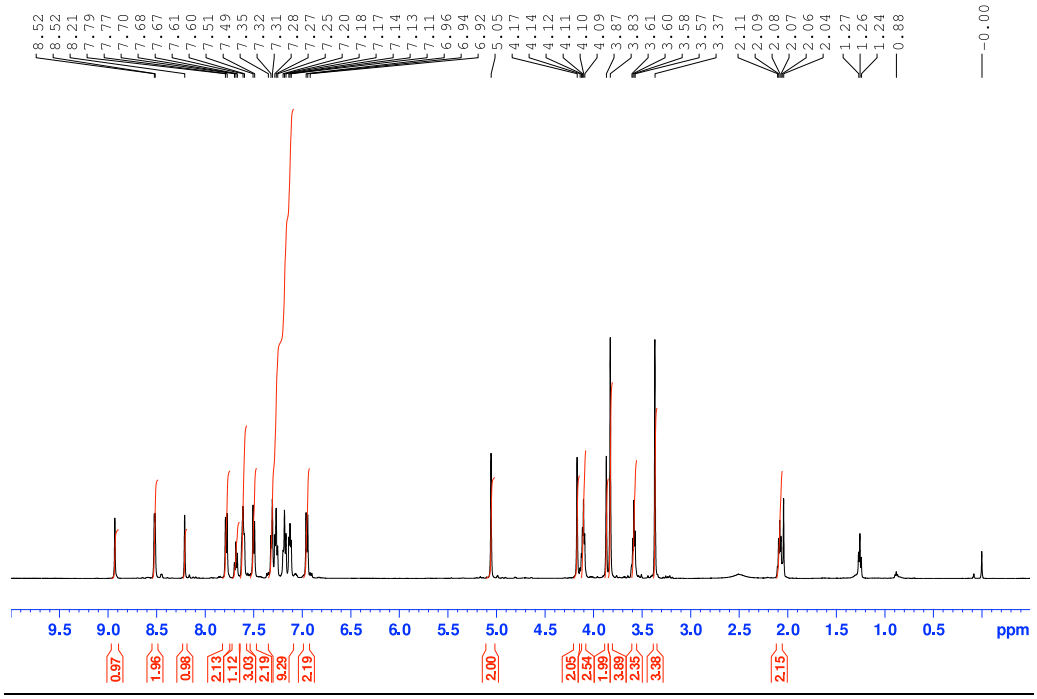
KI (44 mg, 0.2 mmol) and K_2CO_3 (95.2 mg, 0.6 mmol) was added to a solution of 1 (126.6 mg, 0.2 mmol, 1 eq.) and di(2-picolyl)amine (52.6 mg, 0.2 mmol, 1 eq.) in MeCN (17 ml). The mixture was stirred at RT for 6 hours. The mixture was evaporated and extracted with ethyl acetate, and the brown organic phase was washed with water and brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by flash chromatography (basic alumina, eluent composition: n-hexane / ethyl acetate = 50/50), affording 1-(6-(((2-benzyl-6-(4-(3-methoxypropoxy)phenyl)imidazo[1,2-a]pyrazin-3-yl)oxy)methyl)pyridine-2-yl)-N,N-bis(pyridine-2-ylmethyl)methanamine (2) (**TPA-H1**) as a brown oil (72.0 mg, 47%).^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.93 (s, 1H), 8.51 (d, J = 4.20 Hz, 2H), 8.21 (s, 1H), 7.78 (d, J = 8.45 Hz, 2H), 7.68 (t, J = 7.62 Hz, 1H), 7.60 (d, J = 7.30 Hz, 3H), 7.49 (d, J = 7.70 Hz, 2H), 7.34-7.11 (m, 9H), 6.95(d, J =8.50 Hz, 2H), 5.05 (s, 2H), 4.16 (s, 2H), 4.10 (t, J = 5.82 Hz, 2H), 3.86 (s, 2H), 3.82 (s, 4H), 3.58 (t, J =5.96 Hz, 2H), 3.36 (s, 3H), 2.08 (t, J =6.06 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) = 160.08, 159.60, 159.19, 154.48, 149.22, 142.17, 139.03, 138.97, 137.47, 136.85, 136.49, 134.35, 133.26, 129.16, 128.87, 128.67, 127.48, 126.54, 123.00, 122.86, 122.13, 121.01, 114.91, 109.87, 77.63, 69.29, 65.03, 60.18, 60.04, 58.82, 33.69, 29.71. HR-MS: calcd for $C_{42}H_{42}O_3N_7$:692.33406 $[M+H]^+$, found m/z 692.33436.

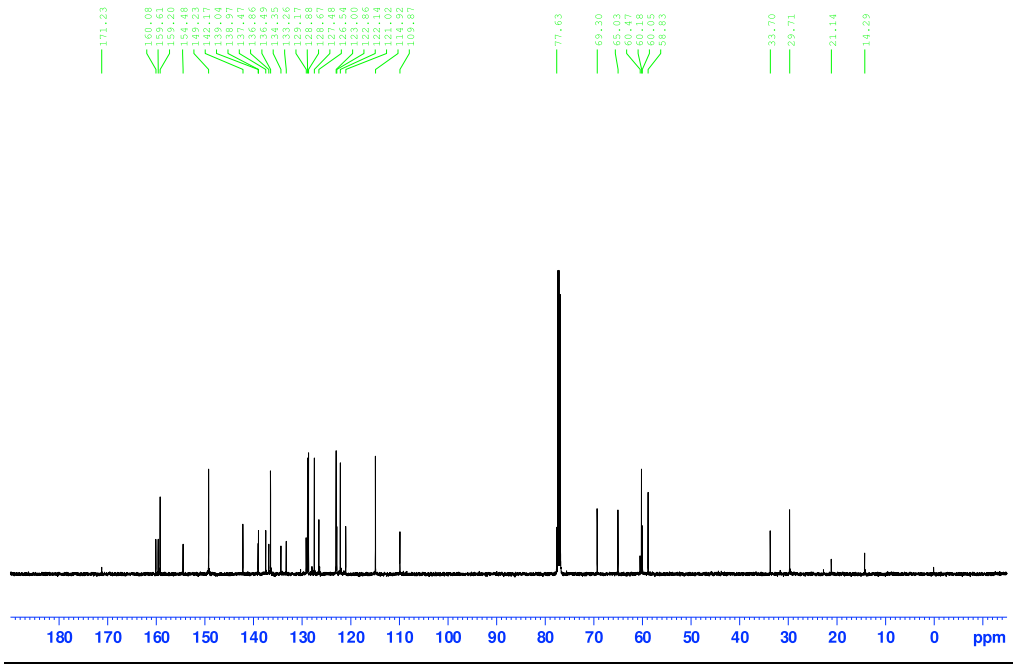
2. NMR spectra

2-benzyl-3-((6-(bromomethyl)pyridine-2-yl)methoxy)-6-(4-(3-methoxypropoxy)phenyl)imidazo[1,2-a]pyrazin (1)



1-(6-(((2-benzyl-6-(4-(3-methoxypropoxy)phenyl)imidazo[1,2-a]pyrazin-3-yl)oxy)methyl)pyridine-2-yl)-N,N-bis(pyridine-2-ylmethyl)methanamine (2) (TPA-H1)





3. Bioluminescence properties

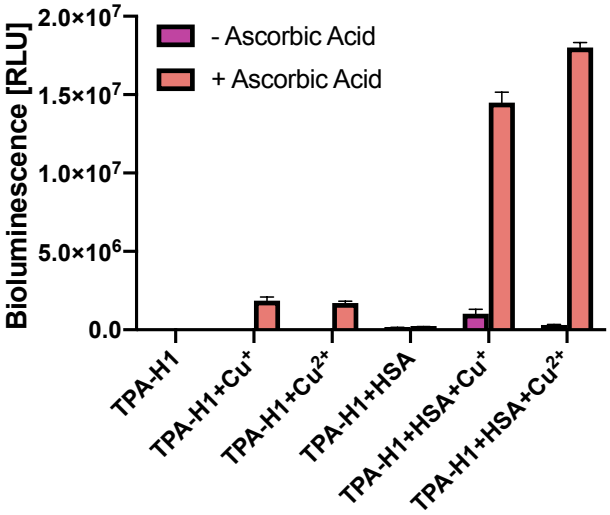


Fig. S1 The response of **TPA-H1** (20 μM) to fatty acid free HSA (20 μM), copper ion (20 μM) in the presence or absence of ascorbic acid (2 mM). Error bars represent the standard deviation of four measurements.

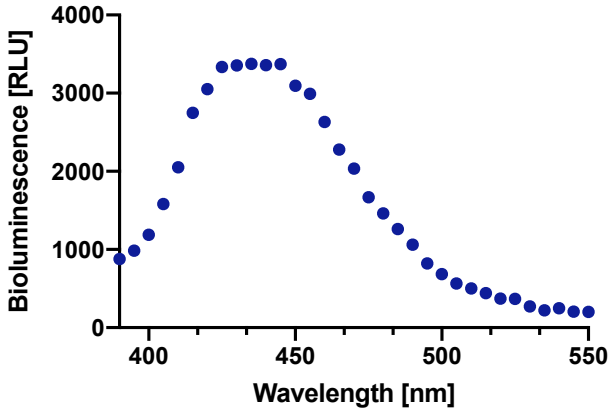


Fig. S2 The bioluminescent spectrum of HuLumino**1**/HSA pair in the buffer solution (HEPES, 10 mM, pH7.4) including 2 mM GSH.

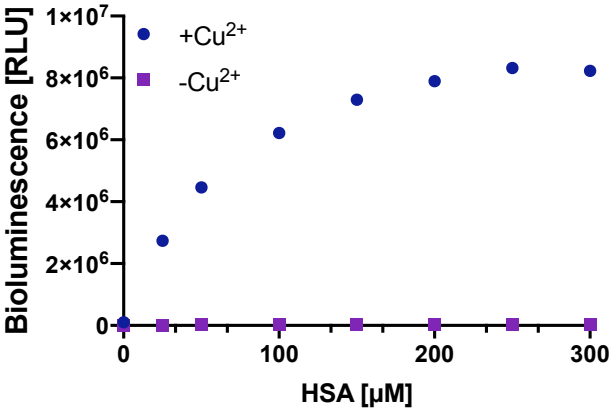


Fig. S3 (a) Bioluminescence intensities of **TPA-H1** containing fatty acid free HSA of various concentration (0-30 μM) in the presence (blue circle) or absence (purple square) of $CuCl_2$ (100 μM) in the buffer solution (HEPES, 10 mM, pH7.4) including 2 mM GSH. Error bars represent the standard deviation of four measurements.

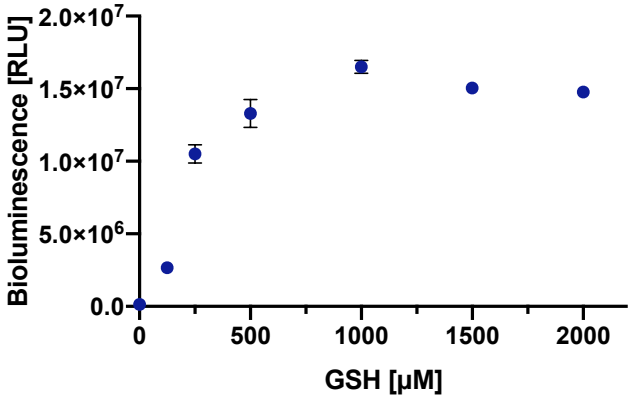


Fig. S4 Bioluminescence intensities of **TPA-H1** containing GSH of various concentration (0-2 mM) in the presence of $CuCl_2$ (100 μM) and fatty acid free HSA (20 μM). Error bars represent the standard deviation of four measurements.

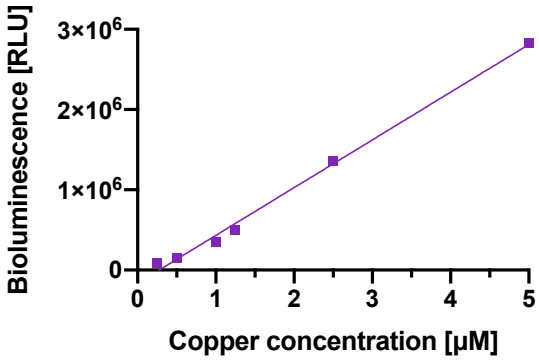


Fig. S5 Bioluminescence intensities of **TPA-H1** (20 μM) to ultrafiltered serum containing various concentration of Cu^{2+} (0.25-5 μM) in the presence of HSA (20 μM) and GSH (2 mM). Error bars represent the standard deviation of three measurements.

Table S1 Absolute bioluminescence intensities of **TPA-H1** for the calculation of copper concentrations in human serum displayed in Table1.

$CuCl_2$ added (μM)	**RLU**
0	$45{,}370 \pm 881$
0.5	$149{,}318 \pm 808$
2.5	$1{,}373{,}448 \pm 33{,}558$
5	$2{,}837{,}528 \pm 33{,}139$

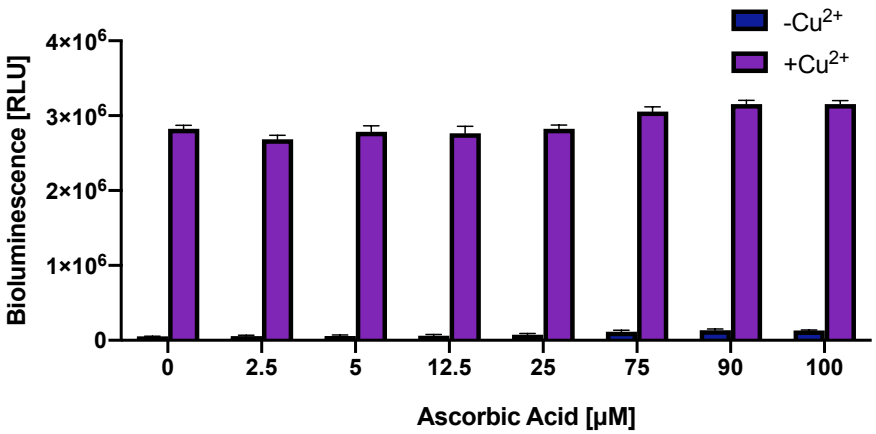


Fig. S6 Bioluminescence intensities of **TPA-H1** containing ascorbic acid of various concentration (0-100 μM), GSH (2 mM), and HSA (20 μM) in the presence or absence of $CuCl_2$ (5 μM). Error bars represent the standard deviation of four measurements.