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**Triphenylamine-embedded copper(II) complex as a “turn-on”
fluorescent probe for the detection of nitric oxide in living animals†**

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Experimental apparatus

¹H NMR and ¹³C NMR spectra were recorded with an AVANCE 400 MHz spectrometer (Bruker) with chemical shifts reported as ppm (in DMSO-*d*₆ and Chloroform-*d*, TMS as an internal standard). Atmospheric pressure ionization (API) mass spectra were recorded on an Agilent 6530 QTOF spectrometer. Absorption spectra were tested with a Perkin Elmer Lambda 900 UV/VIS/NIR spectrophotometer. Fluorescence spectra were recorded with a Spectrofluorometer FS5 luminescence spectrometer. All pH measurements were made with an OHAUS Starter 3100/f meter. Imaging of Cu²⁺ and NO in mice (6 to 8-week-old) were performed on a SPECTRAL Ami Imaging Systems (Spectral Instruments Imaging, LLC, Tucson, AZ) with an excitation filter 465 nm and an emission filter 630 nm. Amiview Analysis software (Version 1.7.06) was used to calculate fluorescence intensity in region of interest (ROI), and values are presented as the mean ± SD for each group of three experiments.

MTT assay of cytotoxicity of the ligand NZ in A549 cells

The cytotoxicity of the ligand NZ to live A549 cells was detected by MTT assays.^{S1} The viability of cell growth was calculated by the following formula:
Viability (%) = (mean of absorbance value of treatment group-blank)/(mean absorbance value of control-blank)×100.

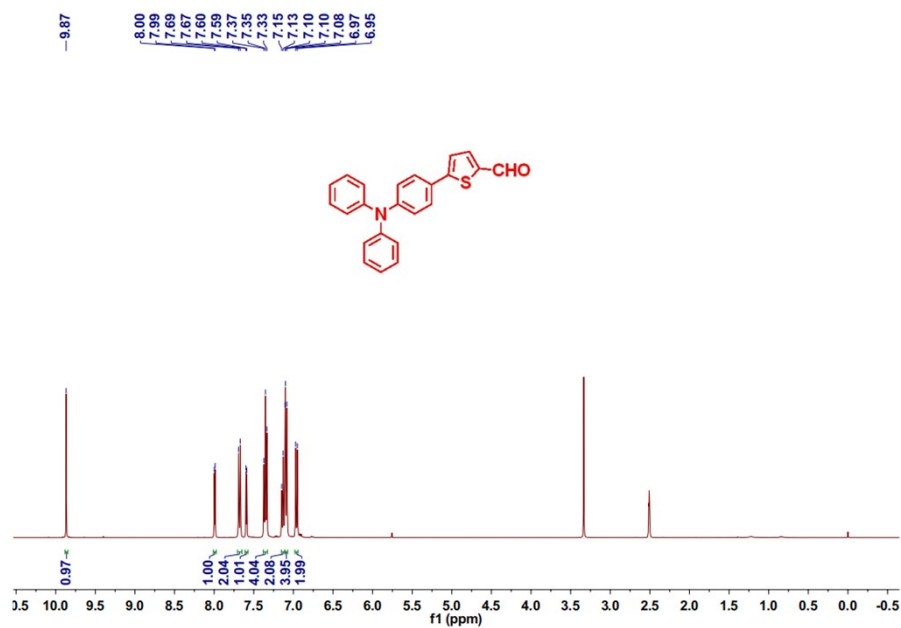


Fig. S1. ¹H NMR of 5-(4'-(diphenylamino)phenyl)thiophene-2-carbaldehyde (**M**) (DMSO-*d*₆, 400 MHz).

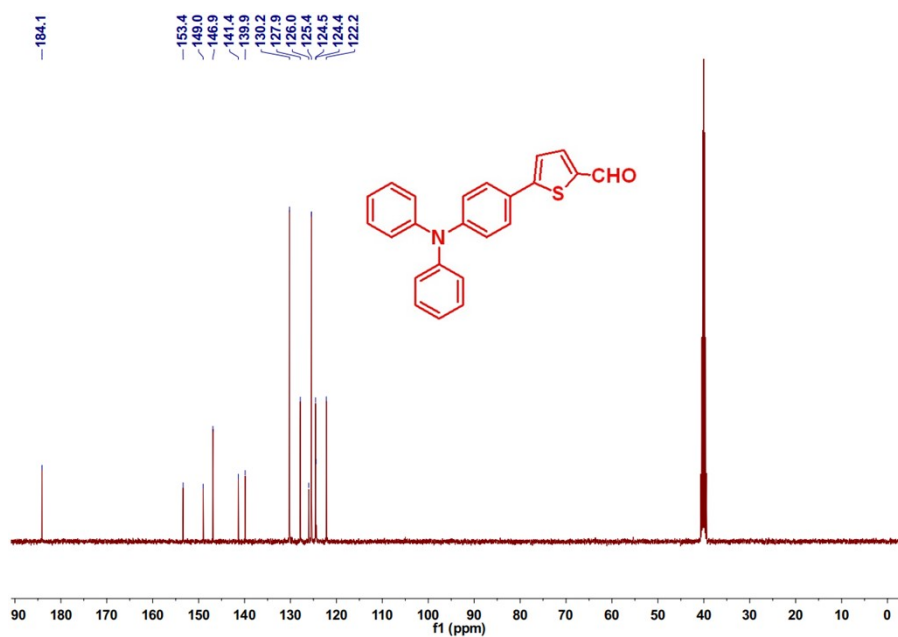


Fig. S2. ¹³C NMR of 5-(4'-(diphenylamino)phenyl)thiophene-2-carbaldehyde (**M**) (DMSO-*d*₆, 101 MHz).

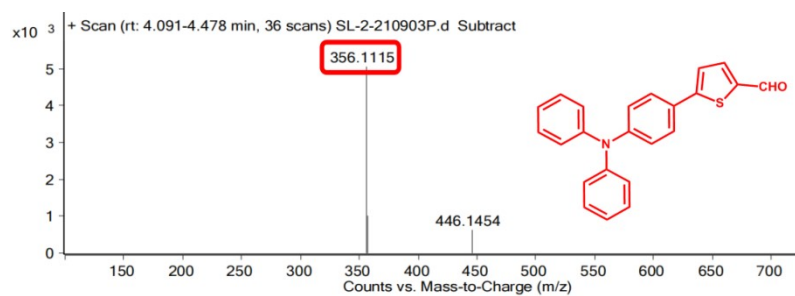


Fig. S3. HR-MS of 5-(4'-(diphenylamino)phenyl)thiophene-2-carbaldehyde (**M**).

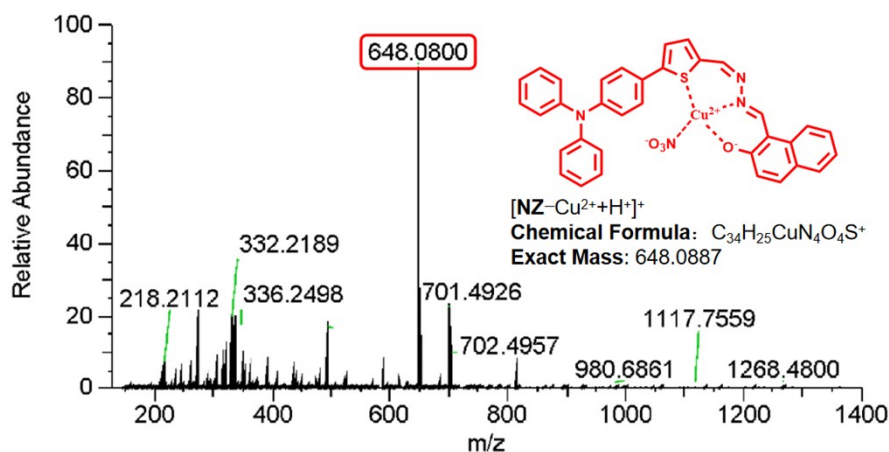


Fig. S7. HR-MS of the probe NZ-Cu²⁺.

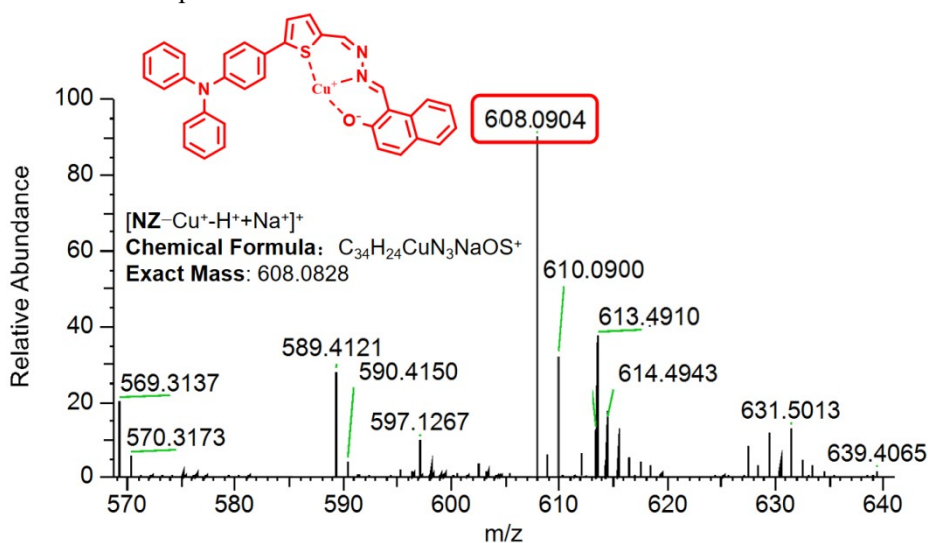


Fig. S8. HR-MS of the probe NZ-Cu²⁺ (10 μ M) in the presence of NO.

Table S1. Cartesian coordinates of NZ in the ground state (S₀).

| Coordinates (ground state S ₀) | | | | |
|--|---------------|-----------------------|--------------|--------------|
| Center Number | Atomic Number | Forces(Hartrees/Bohr) | | |
| | | X | Y | Z |
| 1 | 6 | 0.000000199 | -0.000000789 | 0.000001469 |
| 2 | 6 | -0.000001077 | 0.000001743 | 0.000000126 |
| 3 | 6 | -0.000000699 | -0.000001141 | -0.000001858 |
| 4 | 6 | 0.000002712 | -0.000008434 | 0.000000297 |
| 5 | 6 | -0.000004726 | 0.000005848 | -0.000002285 |
| 6 | 6 | 0.000001525 | -0.000001414 | -0.000001768 |
| 7 | 7 | -0.000007937 | -0.000002374 | 0.000001165 |
| 8 | 6 | -0.000002141 | -0.000003341 | -0.000000762 |
| 9 | 6 | 0.000005223 | 0.000009366 | -0.000001234 |
| 10 | 6 | 0.000000276 | -0.000002104 | 0.000000786 |
| 11 | 6 | 0.000001391 | -0.000000108 | 0.000000262 |
| 12 | 6 | 0.000000328 | 0.000000864 | -0.000000267 |

| | | | | |
|----|----|--------------|--------------|--------------|
| 13 | 6 | -0.000000934 | -0.000000286 | 0.000000154 |
| 14 | 6 | -0.000000513 | -0.000000083 | -0.000000046 |
| 15 | 6 | -0.000000619 | 0.000001398 | 0.000002713 |
| 16 | 6 | 0.000002125 | 0.000002268 | -0.000001779 |
| 17 | 6 | 0.000000818 | -0.000003207 | -0.000001113 |
| 18 | 6 | -0.000003122 | 0.000001673 | 0.000003432 |
| 19 | 6 | 0.000009535 | 0.000001392 | 0.000001089 |
| 20 | 6 | -0.000013016 | 0.000004893 | 0.000007453 |
| 21 | 16 | 0.000010254 | -0.000011684 | -0.000008893 |
| 22 | 6 | 0.000008838 | -0.000002763 | 0.000004989 |
| 23 | 6 | -0.000018611 | 0.000008695 | 0.000006900 |
| 24 | 6 | 0.000017661 | 0.000008725 | -0.000005709 |
| 25 | 6 | -0.000008579 | -0.000009120 | -0.000009541 |
| 26 | 7 | 0.000000407 | 0.000015586 | 0.000005969 |
| 27 | 7 | 0.00000928 | -0.000011605 | -0.000005774 |
| 28 | 6 | 0.000005126 | 0.000001609 | -0.000001049 |
| 29 | 6 | -0.00000100 | 0.000008509 | 0.000002610 |
| 30 | 6 | -0.00000104 | -0.000001194 | -0.000000829 |
| 31 | 6 | 0.000001146 | -0.000001032 | 0.000000323 |
| 32 | 6 | 0.000004817 | 0.000003845 | 0.000000678 |
| 33 | 6 | -0.000005021 | 0.000000541 | 0.000000738 |
| 34 | 6 | -0.000000146 | -0.000011489 | -0.000005069 |
| 35 | 6 | 0.000002355 | 0.000001559 | 0.000000570 |
| 36 | 6 | -0.000003887 | -0.00000068 | 0.000001788 |
| 37 | 6 | 0.000002041 | -0.000003765 | -0.000002223 |
| 38 | 6 | 0.000003101 | 0.000006028 | 0.000001884 |
| 39 | 8 | 0.000008499 | -0.000006037 | -0.000005539 |
| 40 | 1 | -0.000000348 | 0.000000009 | -0.000000046 |
| 41 | 1 | -0.000000234 | 0.000000114 | -0.000000166 |
| 42 | 1 | 0.000000410 | -0.00000126 | 0.000000120 |
| 43 | 1 | -0.000001280 | -0.000000319 | 0.000000590 |
| 44 | 1 | -0.000000283 | 0.000000043 | 0.000000079 |
| 45 | 1 | 0.000001171 | 0.000000844 | -0.000000454 |
| 46 | 1 | -0.000000007 | -0.000000049 | 0.000000042 |
| 47 | 1 | 0.000000031 | -0.000000213 | 0.000000112 |
| 48 | 1 | 0.000000099 | -0.000000138 | 0.000000290 |
| 49 | 1 | 0.000000058 | 0.000001100 | -0.000000475 |
| 50 | 1 | -0.00000285 | 0.000000200 | -0.00000042 |
| 51 | 1 | 0.000000882 | -0.000001219 | 0.000001657 |
| 52 | 1 | -0.000001234 | 0.000001004 | -0.000001605 |
| 53 | 1 | 0.000000896 | -0.000001708 | 0.000000355 |
| 54 | 1 | 0.000003691 | -0.000002977 | -0.000001663 |
| 55 | 1 | -0.000002945 | -0.000002224 | 0.000000589 |
| 56 | 1 | -0.000001637 | 0.000000752 | 0.000002746 |

| | | | | |
|----|---|--------------|--------------|--------------|
| 57 | 1 | -0.000001222 | -0.000001604 | -0.000001296 |
| 58 | 1 | -0.000000487 | 0.000000243 | 0.000000148 |
| 59 | 1 | -0.000000879 | -0.000000005 | -0.000001348 |
| 60 | 1 | -0.000000077 | -0.000000251 | -0.000000008 |
| 61 | 1 | 0.000000556 | 0.000000137 | -0.000000329 |
| 62 | 1 | 0.000000032 | 0.000000368 | 0.000000706 |
| 63 | 1 | -0.000001118 | 0.000000643 | 0.000001362 |
| 64 | 1 | -0.000017813 | 0.000005361 | 0.000009368 |

Table S2. Cartesian coordinates of NZ-Cu²⁺ in the ground state (S₀).

| Coordinates (ground state S ₀) | | | | |
|--|---------------|-----------------------|--------------|--------------|
| Center Number | Atomic Number | Forces(Hartrees/Bohr) | | |
| | | X | Y | Z |
| 1 | 6 | -0.000142291 | -0.000130498 | -0.000088202 |
| 2 | 6 | 0.000055027 | -0.000249623 | 0.000125371 |
| 3 | 6 | 0.000154050 | -0.000056231 | 0.000095988 |
| 4 | 6 | -0.000279178 | -0.000093091 | -0.000150361 |
| 5 | 6 | 0.000034761 | 0.000166997 | 0.000004257 |
| 6 | 6 | -0.000125593 | 0.000225798 | -0.000171380 |
| 7 | 7 | 0.000047268 | 0.000006265 | 0.000260620 |
| 8 | 6 | -0.000050420 | -0.000014442 | -0.000007146 |
| 9 | 6 | 0.000179714 | 0.000011445 | -0.000274497 |
| 10 | 6 | -0.000113287 | 0.000170034 | 0.000070198 |
| 11 | 6 | -0.000014730 | 0.000193428 | 0.000166510 |
| 12 | 6 | 0.000108810 | 0.000166541 | -0.000084181 |
| 13 | 6 | 0.000060834 | -0.000218234 | -0.000244604 |
| 14 | 6 | -0.000072433 | -0.000110713 | -0.000000342 |
| 15 | 6 | 0.000245229 | 0.000043289 | 0.000021528 |
| 16 | 6 | 0.000261149 | -0.000037027 | -0.000087028 |
| 17 | 6 | -0.000160698 | 0.000321277 | -0.000138202 |
| 18 | 6 | -0.000132031 | -0.000006582 | 0.000147429 |
| 19 | 6 | -0.000143243 | -0.000103279 | -0.000087503 |
| 20 | 6 | 0.001875335 | -0.002276011 | -0.003408398 |
| 21 | 16 | -0.004246734 | 0.003528124 | 0.005894095 |
| 22 | 6 | 0.000874135 | 0.001053698 | -0.001694075 |
| 23 | 6 | -0.000667698 | -0.000074976 | 0.000212561 |
| 24 | 6 | 0.000750968 | -0.000530990 | 0.000585862 |
| 25 | 6 | 0.000010183 | 0.000143822 | 0.000582982 |
| 26 | 7 | -0.000970110 | -0.002318795 | -0.000623241 |
| 27 | 7 | -0.002003071 | -0.003610292 | 0.000136281 |
| 28 | 6 | 0.000809298 | 0.003714724 | 0.001033208 |
| 29 | 6 | 0.000612927 | 0.001603768 | -0.000421315 |

| | | | | |
|----|----|--------------|--------------|--------------|
| 30 | 6 | -0.000311238 | -0.000132023 | 0.001295105 |
| 31 | 6 | 0.000116384 | -0.000182680 | -0.000442700 |
| 32 | 6 | -0.000038398 | 0.000285587 | 0.000085323 |
| 33 | 6 | 0.000131384 | 0.000011821 | -0.000183262 |
| 34 | 6 | 0.000217054 | -0.000143260 | -0.000538264 |
| 35 | 6 | -0.000020015 | 0.000233990 | 0.000121487 |
| 36 | 6 | 0.000098867 | 0.000339067 | -0.000066295 |
| 37 | 6 | -0.000208604 | -0.000362233 | 0.000285406 |
| 38 | 6 | -0.000120168 | -0.000123773 | 0.000160136 |
| 39 | 8 | -0.000393931 | -0.00116014 | 0.000356056 |
| 40 | 29 | 0.003689497 | -0.000095705 | -0.002349081 |
| 41 | 8 | -0.001344487 | -0.001489315 | -0.00092767 |
| 42 | 7 | 0.000923344 | 0.002038726 | -0.002779035 |
| 43 | 8 | 0.000133433 | -0.002381949 | 0.002301078 |
| 44 | 8 | -0.000304513 | 0.000282159 | 0.000621756 |
| 45 | 1 | 0.000041638 | 0.000036239 | 0.000026304 |
| 46 | 1 | 0.000011669 | 0.000025582 | -0.000007293 |
| 47 | 1 | -0.000013614 | -0.000039121 | 0.000025176 |
| 48 | 1 | -0.000019389 | -0.000001238 | -0.000037379 |
| 49 | 1 | 0.000003658 | -0.000001288 | 0.000019856 |
| 50 | 1 | 0.000035014 | 0.000032592 | -0.000041999 |
| 51 | 1 | -0.000011841 | -0.00004098 | -0.000022644 |
| 52 | 1 | -0.000012709 | -0.000011283 | 0.000003579 |
| 53 | 1 | 0.000010473 | 0.000023922 | 0.000036897 |
| 54 | 1 | -0.000000214 | 0.000034215 | -0.000020921 |
| 55 | 1 | -0.000030308 | -0.000014060 | 0.000079034 |
| 56 | 1 | -0.000110891 | -0.000025333 | -0.000051479 |
| 57 | 1 | 0.000001634 | 0.000025590 | -0.000007993 |
| 58 | 1 | 0.000028926 | -0.000029615 | 0.000024694 |
| 59 | 1 | 0.000072584 | 0.000010483 | 0.000022909 |
| 60 | 1 | 0.000016652 | -0.000029912 | -0.000009644 |
| 61 | 1 | 0.000159725 | 0.000105811 | 0.000040775 |
| 62 | 1 | 0.000246401 | 0.000180674 | 0.000016246 |
| 63 | 1 | 0.000064837 | 0.000075392 | 0.000032067 |
| 64 | 1 | 0.000078373 | -0.000008926 | 0.000011544 |
| 65 | 1 | -0.000011103 | 0.000036600 | 0.000012440 |
| 66 | 1 | 0.000008750 | -0.000020595 | 0.000011915 |
| 67 | 1 | -0.000010973 | 0.000044970 | 0.000009337 |
| 68 | 1 | -0.000017879 | 0.000027370 | -0.000052996 |

Table S3. Cartesian coordinates of NZ-Cu⁺ in the ground state (S_0).

| Coordinates (ground state S_0) | | | | |
|-----------------------------------|------------------|------------------------|--------------|--------------|
| Center Number | Atomic Number | Forces (Hartrees/Bohr) | | |
| | | X | Y | Z |
| 1 | 6 | 0.000001016 | -0.000000059 | 0.000000479 |
| 2 | 6 | -0.000000275 | -0.000000393 | 0.000000879 |
| 3 | 6 | -0.000001572 | 0.000003450 | -0.000000249 |
| 4 | 6 | -0.000000394 | 0.000000347 | 0.000000197 |
| 5 | 6 | 0.000001486 | -0.000002188 | 0.000001913 |
| 6 | 6 | -0.000000540 | 0.000000036 | -0.000001038 |
| 7 | 7 | 0.000005512 | 0.000006133 | -0.000011093 |
| 8 | 6 | 0.000003475 | -0.000005014 | 0.000007457 |
| 9 | 6 | -0.000008169 | -0.000003663 | 0.000004736 |
| 10 | 6 | -0.000000797 | 0.000000698 | -0.000003889 |
| 11 | 6 | -0.000001422 | -0.000000954 | 0.000000631 |
| 12 | 6 | 0.000001014 | -0.000000295 | 0.000000367 |
| 13 | 6 | -0.000000643 | 0.000000682 | -0.000000143 |
| 14 | 6 | 0.000001489 | 0.000000924 | 0.000001121 |
| 15 | 6 | -0.000000210 | 0.000001988 | -0.000002887 |
| 16 | 6 | -0.000001510 | -0.000005113 | 0.000006420 |
| 17 | 6 | -0.000002701 | 0.000008134 | -0.000013757 |
| 18 | 6 | 0.000002202 | 0.000002608 | 0.000006723 |
| 19 | 6 | -0.000002832 | -0.000001748 | -0.000002437 |
| 20 | 6 | 0.000013738 | -0.000003599 | 0.000009891 |
| 21 | 16 | -0.000014554 | -0.000031555 | 0.000006528 |
| 22 | 6 | 0.000033891 | 0.000044571 | -0.000002946 |
| 23 | 6 | -0.000012652 | -0.000015218 | -0.000013278 |
| 24 | 6 | -0.000002950 | 0.000002397 | 0.000014832 |
| 25 | 6 | -0.000015511 | -0.000012229 | -0.000022467 |
| 26 | 7 | -0.000005287 | -0.000018387 | 0.000015186 |
| 27 | 7 | 0.000017462 | 0.000014928 | 0.000012834 |
| 28 | 6 | 0.000000890 | -0.00000395 | -0.000016792 |
| 29 | 6 | -0.000001096 | 0.000001184 | 0.000008410 |
| 30 | 6 | -0.000002019 | 0.000002215 | -0.000007653 |
| 31 | 6 | 0.000003355 | 0.000006384 | -0.000000978 |
| 32 | 6 | -0.000002449 | -0.000000344 | -0.000004322 |
| 33 | 6 | 0.000000414 | -0.000003253 | 0.000004226 |
| 34 | 6 | -0.000000297 | 0.000003179 | 0.000000032 |
| 35 | 6 | -0.000001834 | -0.000000296 | -0.000003239 |
| 36 | 6 | 0.000000114 | -0.00000225 | 0.000000219 |
| 37 | 6 | -0.000000498 | 0.000000482 | -0.000000226 |
| 38 | 6 | 0.000001029 | -0.000000432 | 0.000003371 |
| 39 | 8 | -0.000000858 | -0.000024677 | 0.000001863 |

| | | | | |
|----|----|--------------|--------------|--------------|
| 40 | 29 | -0.000013380 | 0.000031611 | 0.000001015 |
| 41 | 1 | 0.000000050 | -0.000000080 | -0.000000006 |
| 42 | 1 | 0.000000096 | -0.000000060 | -0.000000388 |
| 43 | 1 | 0.000001193 | -0.000000347 | 0.000000253 |
| 44 | 1 | 0.000000858 | 0.000001295 | 0.000000883 |
| 45 | 1 | -0.000000293 | -0.000000419 | 0.000000314 |
| 46 | 1 | -0.000000127 | -0.000000813 | 0.000000292 |
| 47 | 1 | 0.000000108 | 0.000000153 | 0.000000117 |
| 48 | 1 | -0.000000254 | 0.000000204 | 0.000000106 |
| 49 | 1 | -0.000000103 | 0.000000024 | -0.000000363 |
| 50 | 1 | -0.000000494 | 0.000000097 | -0.000000556 |
| 51 | 1 | 0.000000295 | -0.000001008 | 0.000000327 |
| 52 | 1 | -0.000000231 | 0.000000579 | -0.000001040 |
| 53 | 1 | 0.000000362 | -0.000000266 | -0.000001330 |
| 54 | 1 | 0.000001011 | 0.000000527 | -0.000000318 |
| 55 | 1 | 0.000001492 | 0.000002548 | 0.000002833 |
| 56 | 1 | 0.000002406 | -0.000000956 | -0.000002797 |
| 57 | 1 | -0.000000328 | 0.000001901 | 0.000000955 |
| 58 | 1 | 0.000000440 | -0.000002572 | -0.000001111 |
| 59 | 1 | 0.000001251 | -0.000000305 | -0.000000433 |
| 60 | 1 | 0.000000020 | 0.000000100 | 0.000000584 |
| 61 | 1 | 0.000000029 | 0.000000125 | 0.000001071 |
| 62 | 1 | -0.000000511 | 0.000000864 | -0.000000216 |
| 63 | 1 | 0.000000518 | 0.000000395 | -0.000000334 |
| 64 | 1 | -0.000000428 | 0.000001679 | -0.000000728 |

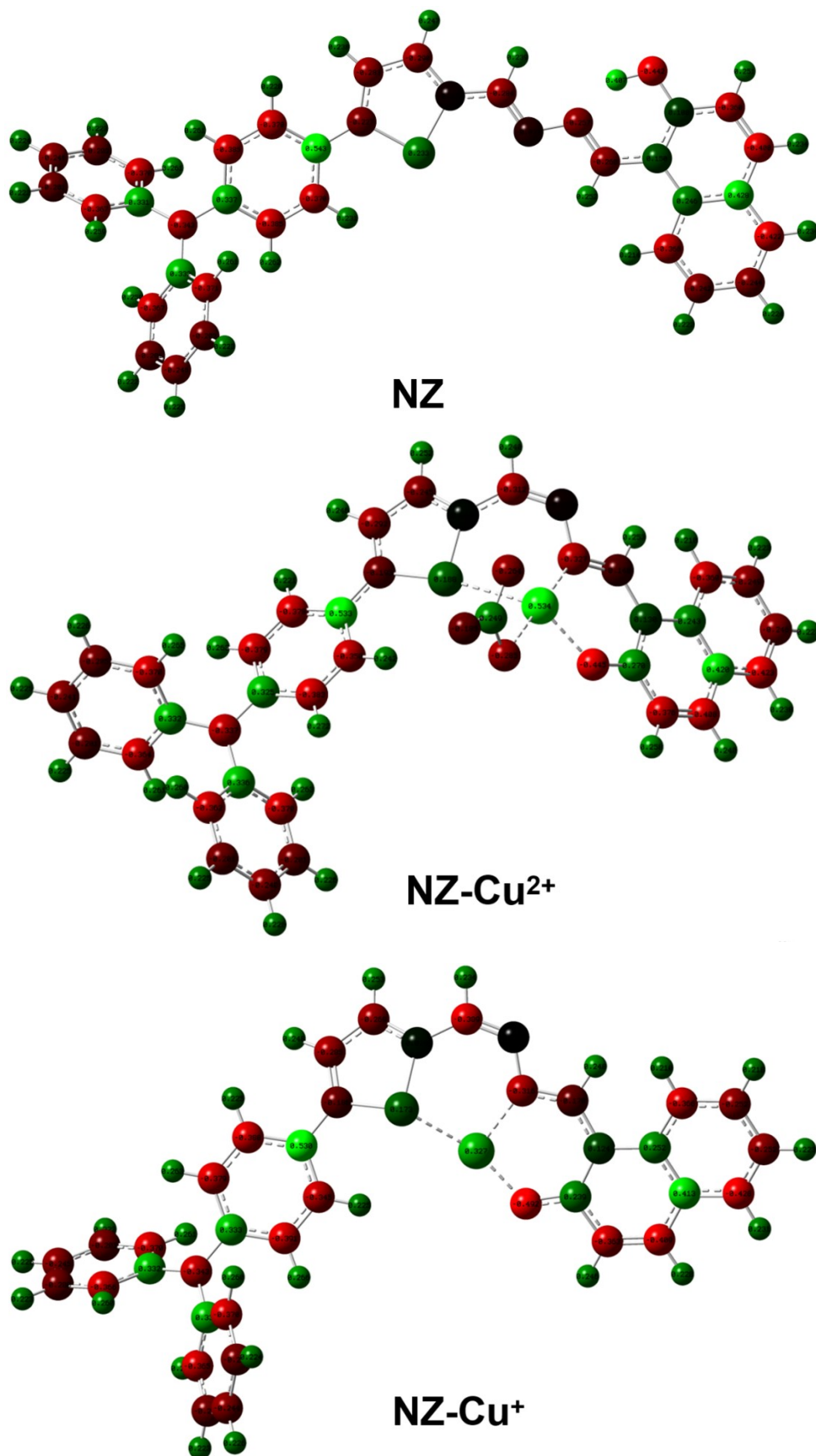


Fig. S9. NBO analyses of NZ, NZ-Cu²⁺ and NZ-Cu⁺.

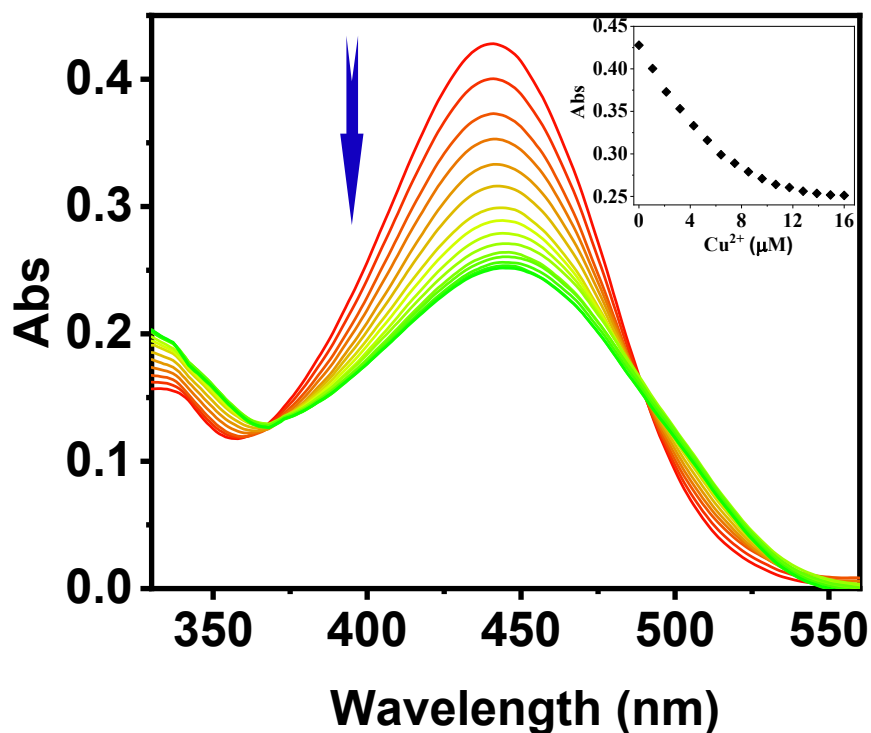


Fig. S10. UV-vis absorption spectra of NZ (10 μM) in the presence of different amounts of Cu^{2+} (0–16 μM) in HEPES aqueous buffer (DMSO:HEPES=6:4, 20 mM, pH=7.4). Inset: absorption of NZ at 439 nm as a function of different Cu^{2+} concentration.

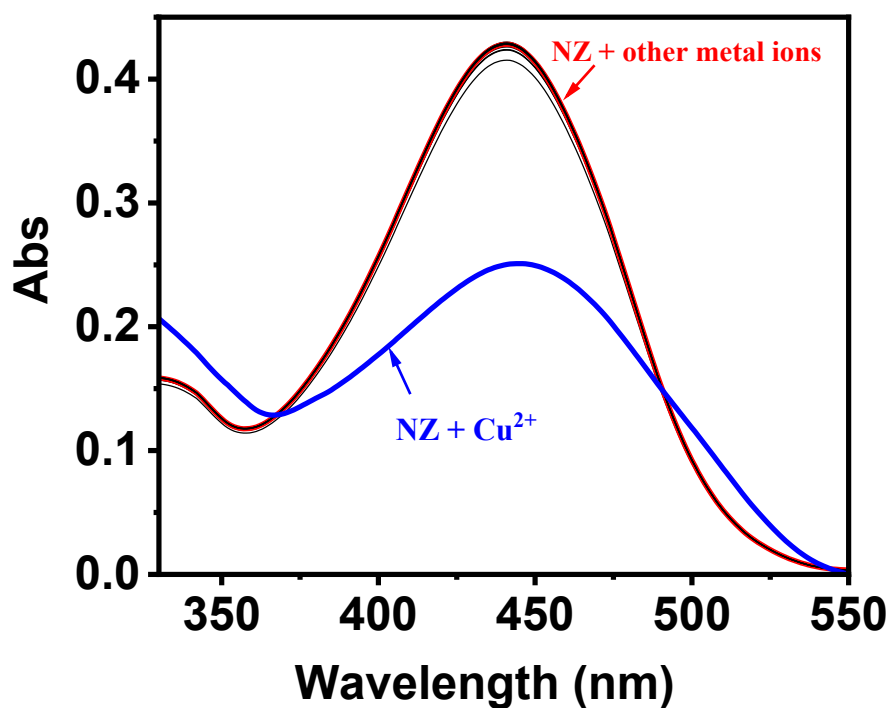


Fig. S11. UV-vis absorption spectra of NZ (10 μM) in HEPES aqueous buffer (DMSO:HEPES=6:4, 20 mM, pH=7.4) upon addition of 16 μM various metal ions: 1. Ca^{2+} , 2. Fe^{3+} , 3. Mg^{2+} , 4. Ni^{2+} , 5. Al^{3+} , 6. Hg^{2+} , 7. Zn^{2+} , 8. Ba^{2+} , 9. Co^{3+} , 10. K^{+} , 11. Na^{+} , 12. Ag^{+} , 13. Cr^{3+} , 14. Cd^{2+} , 15. Li^{+} , 16. Pb^{2+} , 17. Cu^{2+} and 18. blank.

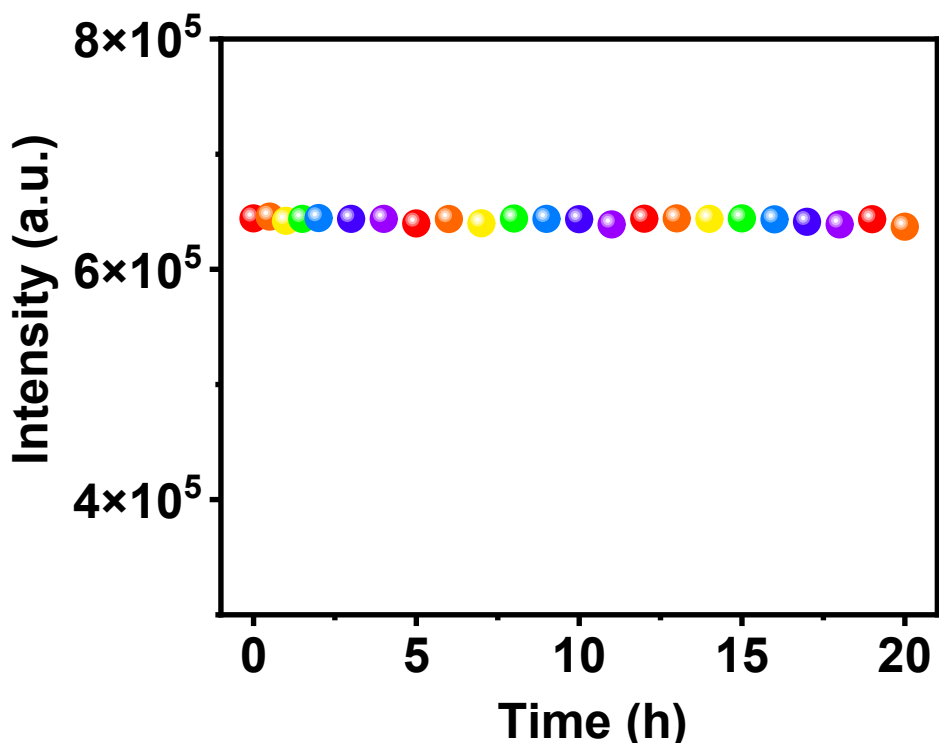


Fig. S12. Fluorescence intensity of NZ (10 μM) at different time in HEPES aqueous buffer (DMSO:HEPES=6:4, 20 mM, pH=7.4) (λ_{ex} =439 nm, λ_{em} =640 nm).

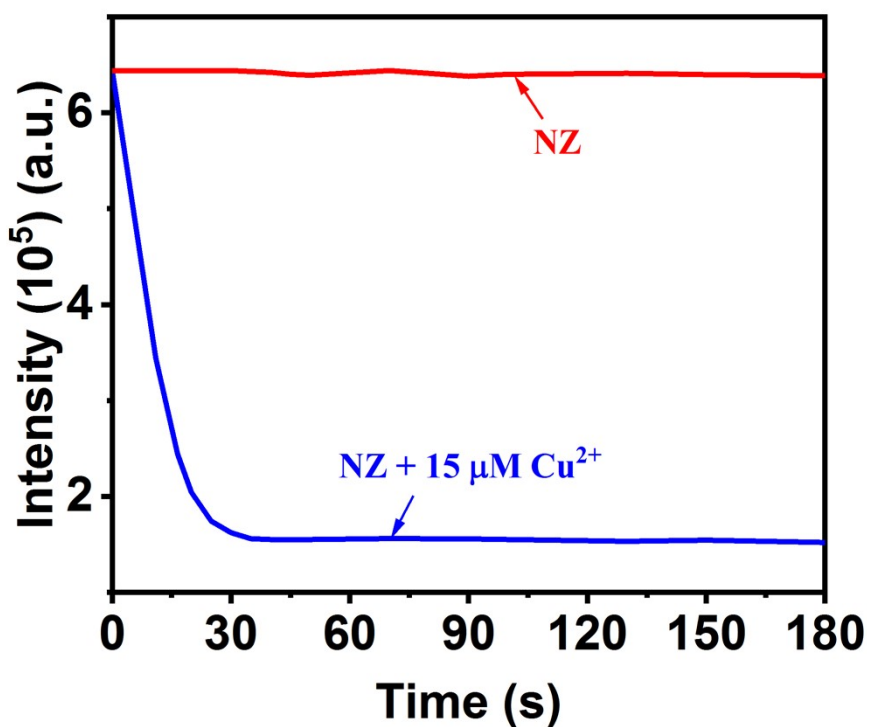


Fig. S13. Time-dependent fluorescence response at 640 nm of NZ in the presence of Cu²⁺ (0 and 15 μM) in HEPES aqueous buffer (DMSO:HEPES=6:4, 20 mM, pH=7.4).

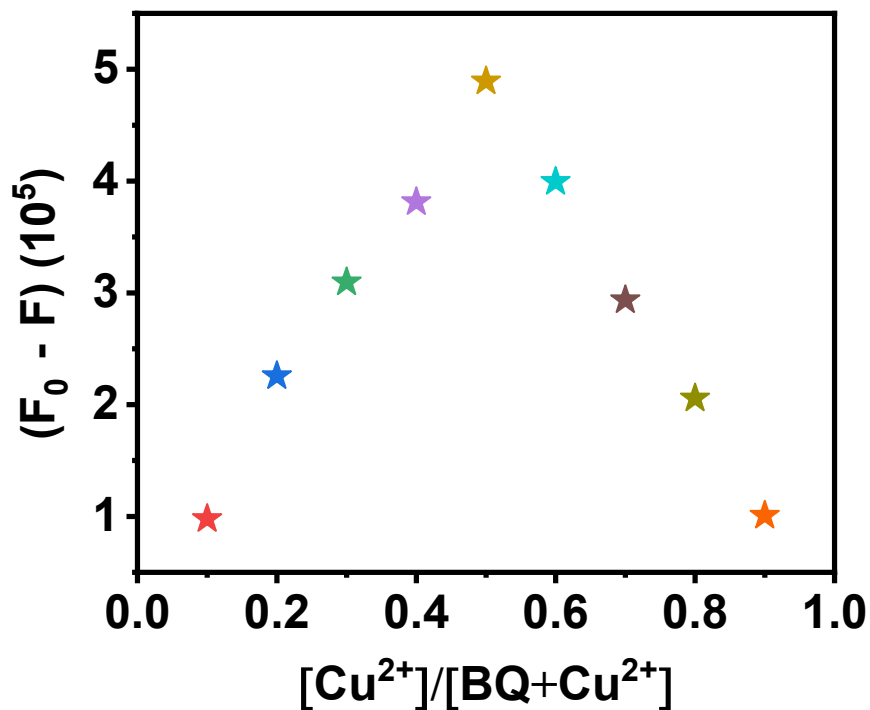


Fig. S14. Job's plot of NZ toward Cu²⁺ in HEPES aqueous buffer solution (DMSO:HEPES=6:4, 20mM, pH=7.4). The total concentration of NZ and Cu²⁺ was 10 μM (λ_{ex} =439 nm, λ_{em} =640 nm).

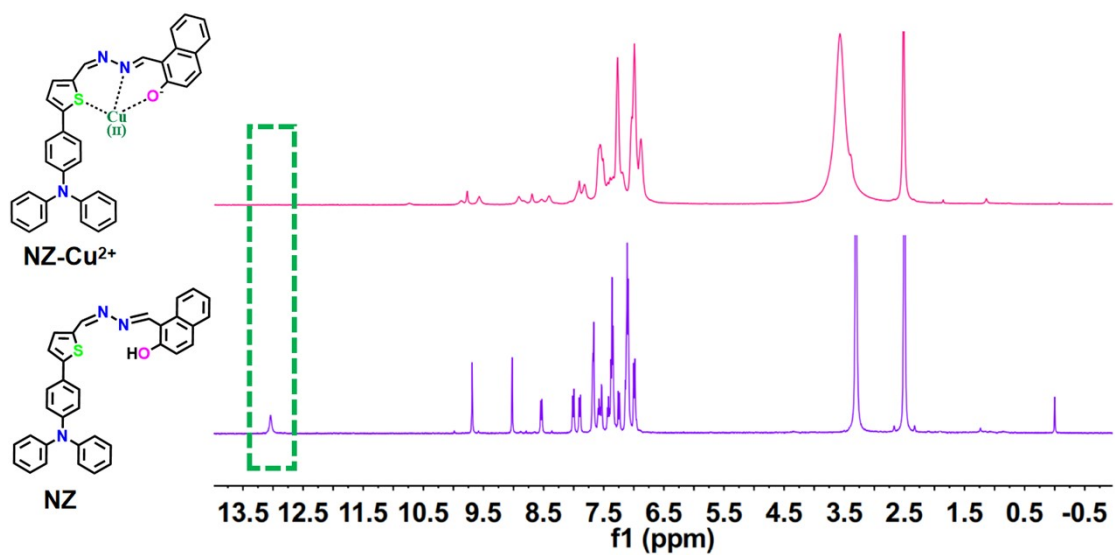


Fig. S15. ¹H NMR of NZ in the absence and presence of Cu(NO₃)₂ in D₂O/DMSO-*d*₆ mixed solution.

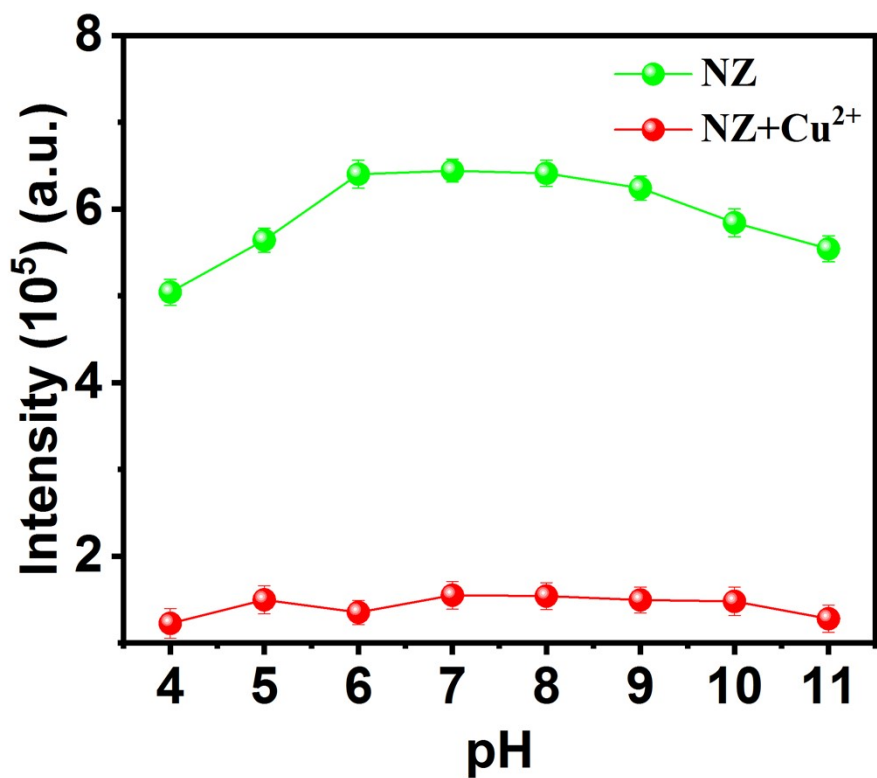


Fig. S16. Influences of pH on the fluorescence intensity of NZ (10 μ M) in the absence and presence of Cu²⁺ (14 μ M), (λ_{ex} =439 nm, λ_{em} =640 nm).

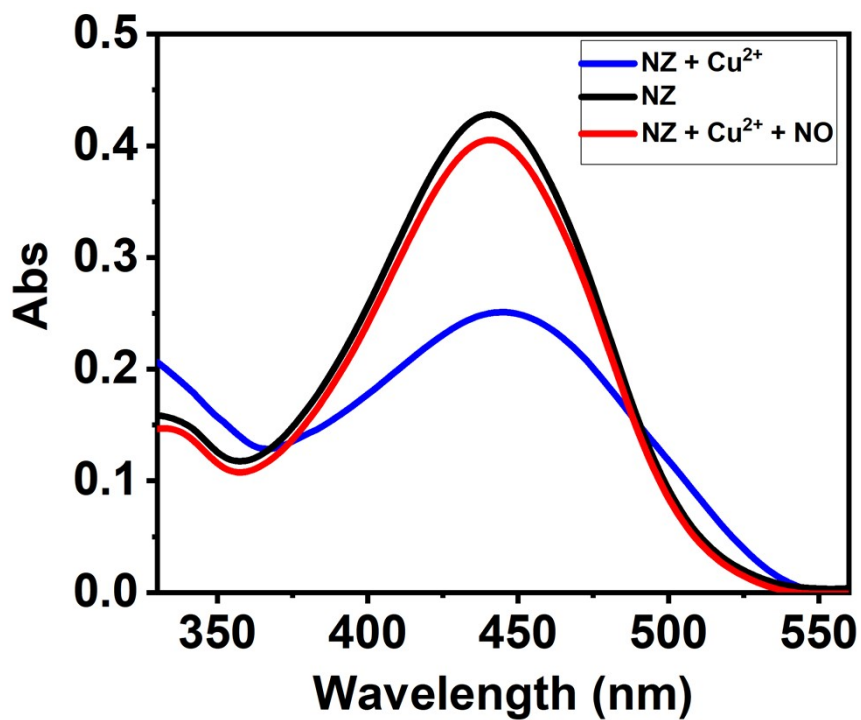


Fig. S17. UV-vis absorption spectra of NZ, NZ-Cu²⁺ and NZ-Cu²⁺ with NO (10.5 μ M) in DMSO/HEPES (6:4, v/v, pH=7.4, 20 mM).

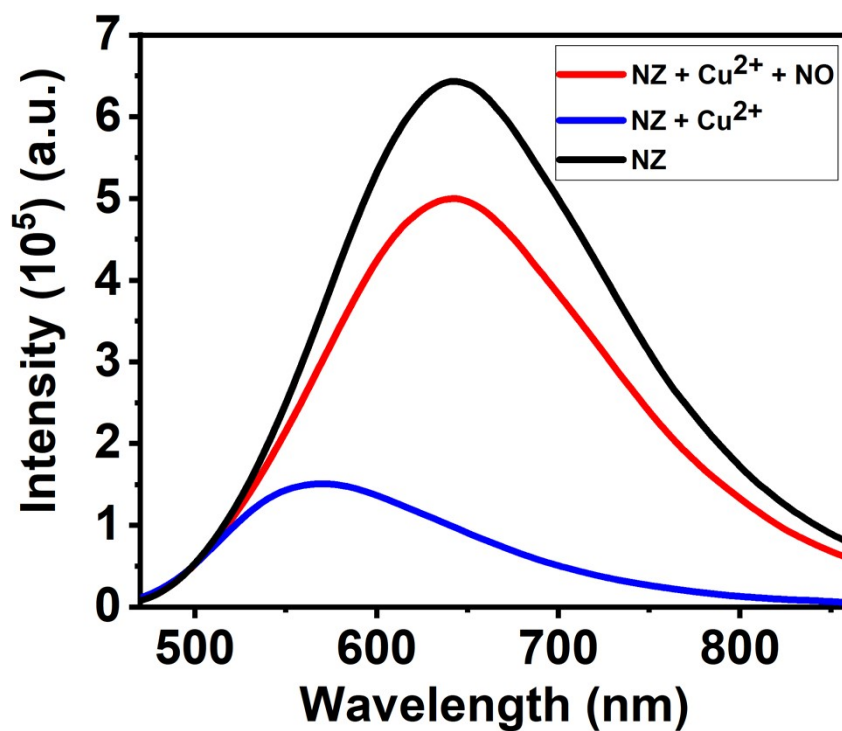


Fig. S18. Fluorescence spectra of NZ, NZ-Cu²⁺ and NZ-Cu²⁺ with NO (10.5 μM) in DMSO/HEPES (6:4, v/v, pH=7.4, 20 mM).

Table S4. Comparison of probe NZ-Cu²⁺ with reported NO fluorescent probes.

| Probes | Emission wavelength | Detection limit | Response time | Detection of NO in live animals | Ref. |
|--------------------------|---------------------|-----------------|---------------|---------------------------------|------|
| 1-copper(II) | 534 nm | --- | 7.9 min | No | S2 |
| L2 | 532 nm | 1.6 nM | --- | No | S3 |
| RB-TP | 580 nm | 5 nM | 20 min | No | S4 |
| RB-Py | 590 nm | 20 nM | 20 min | No | S4 |
| LJ-1 | 365 nm | 10 nM | 2 h | No | S5 |
| FL3A-P ₉ K-CC | 520 nm | 82 nM | 120 s | No | S6 |
| FL3A-P _{pz} -CC | 520 nm | 21 nM | 150 s | No | S6 |
| TRP | 580 nm | 1.8 μM | 50 min | No | S7 |
| PIP | 560 nm | --- | 700 s | No | S8 |
| 1 | 580 nm | 0.18 μM | 10 min | No | S9 |

| | | | | | |
|---|--------|---------|--------|-------|------------------|
| A6 | 533 nm | 24 nM | 10 min | No | S10 |
| PYNO | 481 nm | 163 nM | 15 s | Mouse | S11 |
| PYSNO | 548 nm | 242 nM | 15 s | Mouse | S11 |
| 1 | 558 nm | 31 nM | 30 min | No | S12 |
| PyDA-NP | 523 nm | 2.13 pM | --- | No | S13 |
| FP-H₂O₂-NO | 581 nm | --- | 15 min | NO | S14 |
| NZ | 640 nm | 12.9 nM | 60 s | Mouse | This work |

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