Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2020

Electronic Supplementary Information

Supramolecular optimization of the visual contrast in a colorimetric chemosensing assay that releases resorufin dye

Janeala J. Morsby, Madushani Dharmarwardana, Hannah McGarraugh, and Bradley D. Smith *

Department of Chemistry and Biochemistry, 251 Nieuwland Science Hall, University of Notre Dame, Notre Dame, United States.

*Email: smith.115@nd.edu

Materials and Instrumentation

All the solvents and chemicals were purchased from Sigma-Aldrich, Alfa-Aesar, or VWR international and used without further purification unless otherwise stated. ^{1}H and ^{13}C spectra were recorded on Bruker AVANCE III HD 400, 500 MHz spectrometers. Mass spectrometry (MS) was performed using a Bruker microTOF II spectrometer. Synthesized compounds were purified using Biotage flash purification system with SNAP Ultra flash chromatography cartridges.

N-acetyl-β-*D*-glucosaminidase (NAG) Stock Solution

A 0.9 mg/mL solution of NAG enzyme (Sigma-Aldrich) from bovine kidney was prepared in 1 M PBS Buffer + 0.1 M BSA and the vendor's guarantee of enzymatic activity was confirmed using the standard chromogenic substrate p-nitrophenyl-N-acetyl-β-*D*-glucosaminide.

HOCl/ OCl⁻ Stock Solution

Hypochlorite stock solution was prepared using a previously reported method.[1] To a 1500 µL solution of 154 mM NaCl, 250 µL of 10-14% w/w NaOCl was added followed by dropwise addition of 6M HCl to obtain a pH range of 3.92. The concentration of active total chlorine species in solution expressed as $[HOCl]_T$ (where $[HOCl]_T = [HOCl] + [Cl_2] + [Cl_3^-] + [OCl^-]$) in HPLC Grade water was determined by converting all the active chlorine species to OCl^- with 0.1 M NaOH and measuring the concentration of OCl^-. The concentration of OCl^- was determined spectrophotometrically at 292 nm ($\varepsilon = 362\ M^{-1}\ cm^{-1}$) with a UV-visible spectrophotometer. Calculation: $A = \varepsilon cl$; where $l = 1$ cm, $A = 0.6359$, $\varepsilon = 362\ M^{-1}\ cm^{-1}$. Thus, $c = 1.76$ mM HOCl/ OCl^-

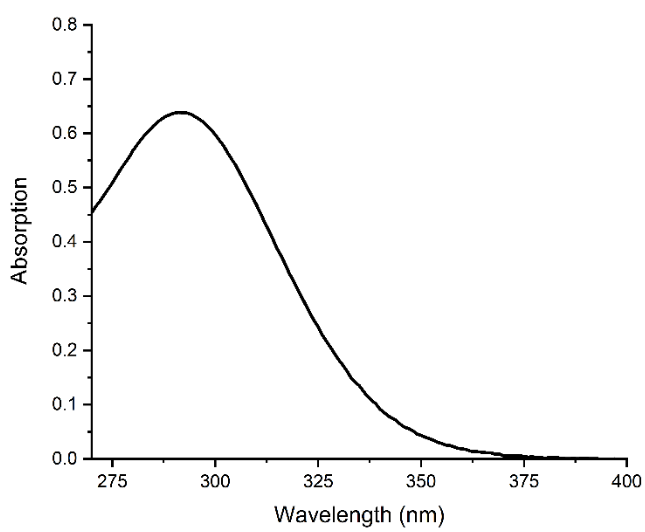


Figure S1: Absorption spectrum of HOCl/ OCl^- for stock solution concentration determination.

Synthesis

The chemosensor **RT-1** and enzyme substrate **NHPO** were synthesized as previously described,[2,3] and the following ^{1}HNMR and HR-MS data demonstrate high purity. Tetralactam macrocycles **M1** and **M2** were synthesized as part of previous studies[4,5] and the purity was confirmed by ^{1}H NMR.

RF-TBA: Resorufin sodium salt (50 mmol, 10.6 mg) and 40 % wt tetrabutylammonium hydroxide solution (50 mmol, 33 µL) were dissolved in 50 mL of PBS. The resulting mixture was extracted with chloroform (3 × 50 mL). The combined chloroform layers were dried under vacuum to obtain pure **RF.TBA** as a dark pink solid.

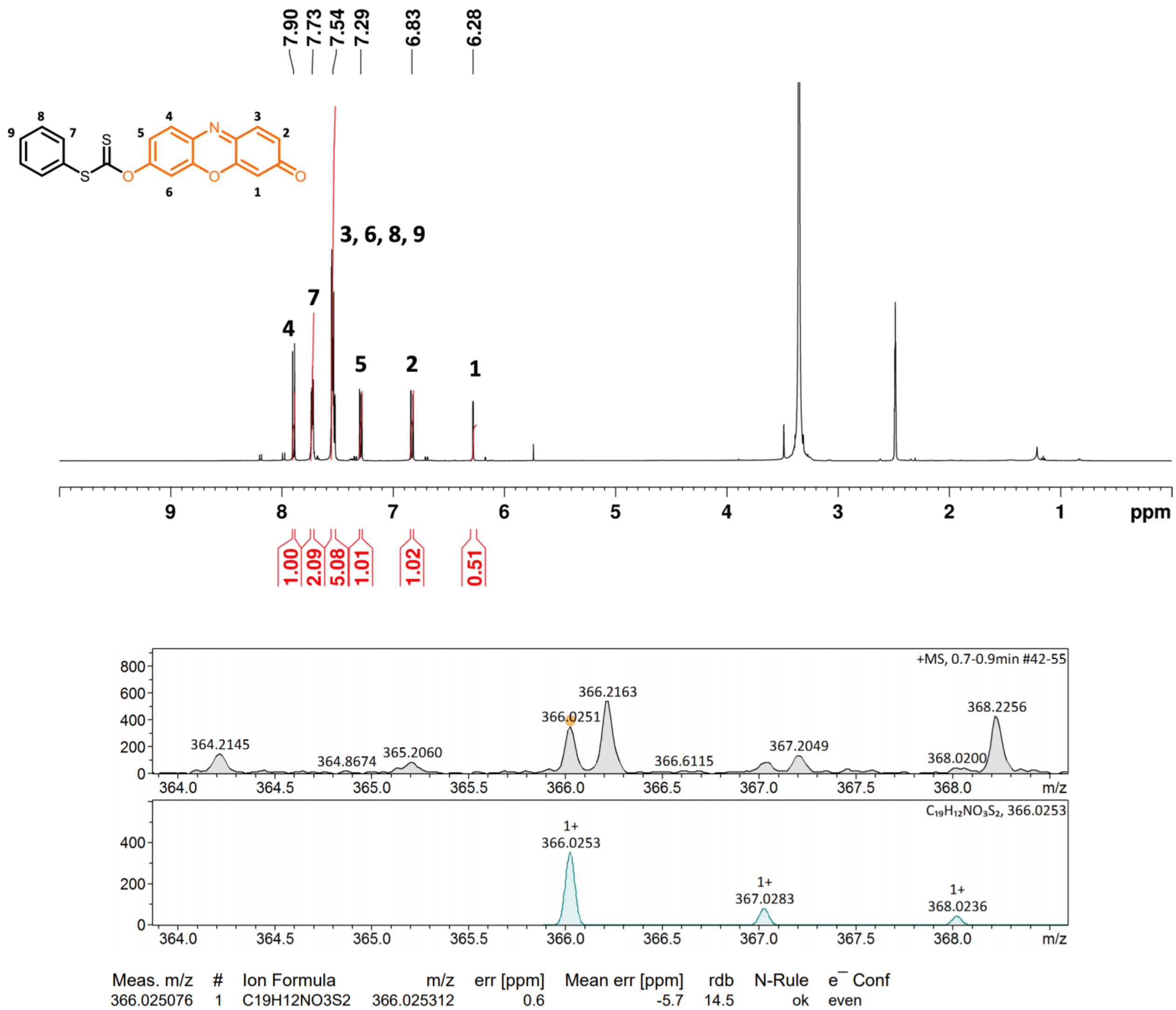


Figure S2. ^{1}H NMR (500 MHz; DMSO-d_6; Me_4Si) and HR-ESI mass spectrum of **RT-1**.

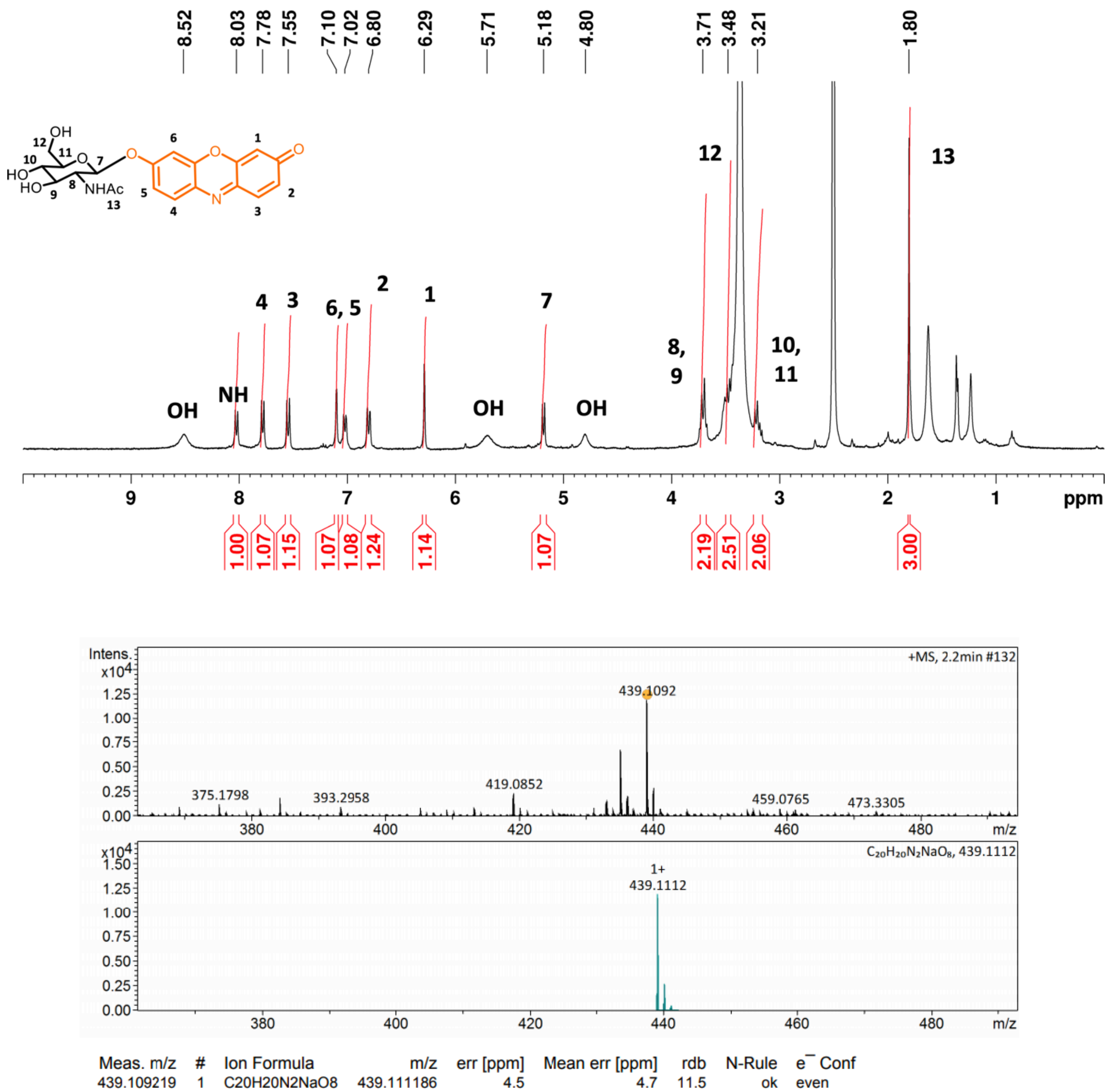


Figure S3. [1]H NMR (400 MHz; DMSO-d_6; Me_4Si) and HR-ESI mass spectrum of **NHPO**. The broad OH peaks in the [1]H NMR spectrum are due to exchange promoted by adventitious water in the DMSO-d_6.

^{1}H NMR Titration Data

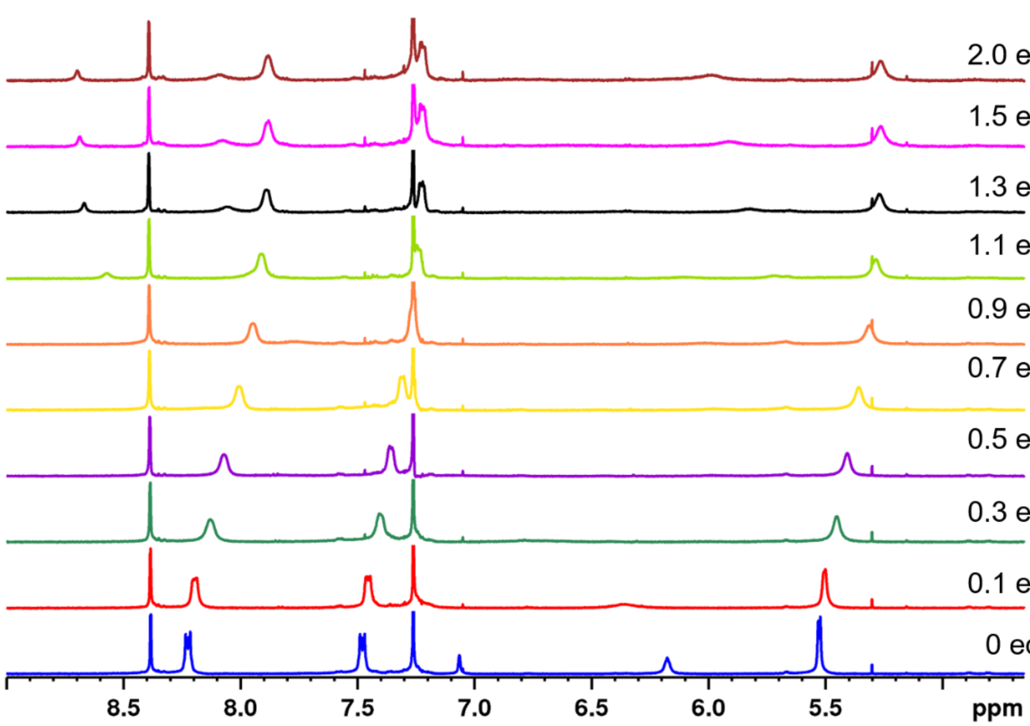


Figure S4. ^{1}H NMR titration (500 MHz, $CDCl_3$, 25° C) that added aliquots from a stock solution containing 10 mM **RF** (tetrabutylammonium salt)/ 0.5 mM **M1** to a solution of **M1** (0.5 mM).

***K_a* Determination by Fluorescence Titration**

Previously described titration method was employed.[6] Stock solutions of the guest, **RF** (1 mM) and host, **M2** (1 mM) were made in pure water. A solution of the guest was placed in a cuvette (10 μM) and aliquots of the host (**M2**) were added fluorescence spectra were acquired (ex: 540 nm, em: 585 nm). The data was plotted and association constant for **RF** binding to **M2** was determined by non-linear squares fitting of the titration points to a model for 1:1 binding within the Origin software.[7]

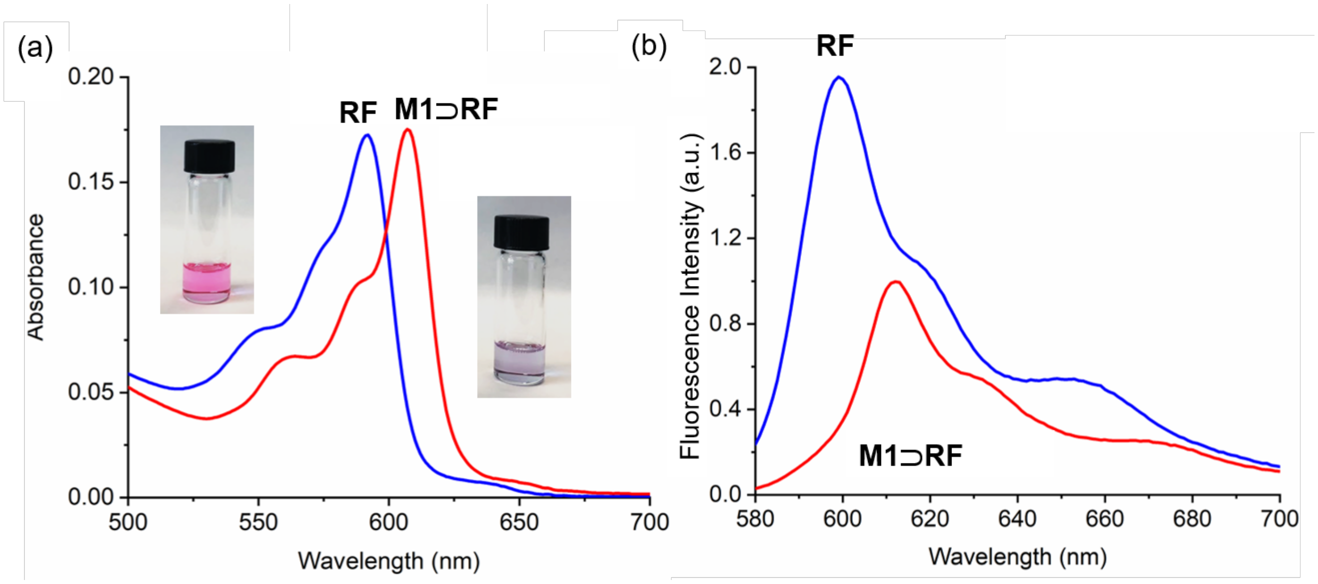


Figure S5. (a) Absorption (b) fluorescence emission of 10 μM **RF** (tetrabutylammonium salt) and **M1⊃RF** in chloroform at 25°C.

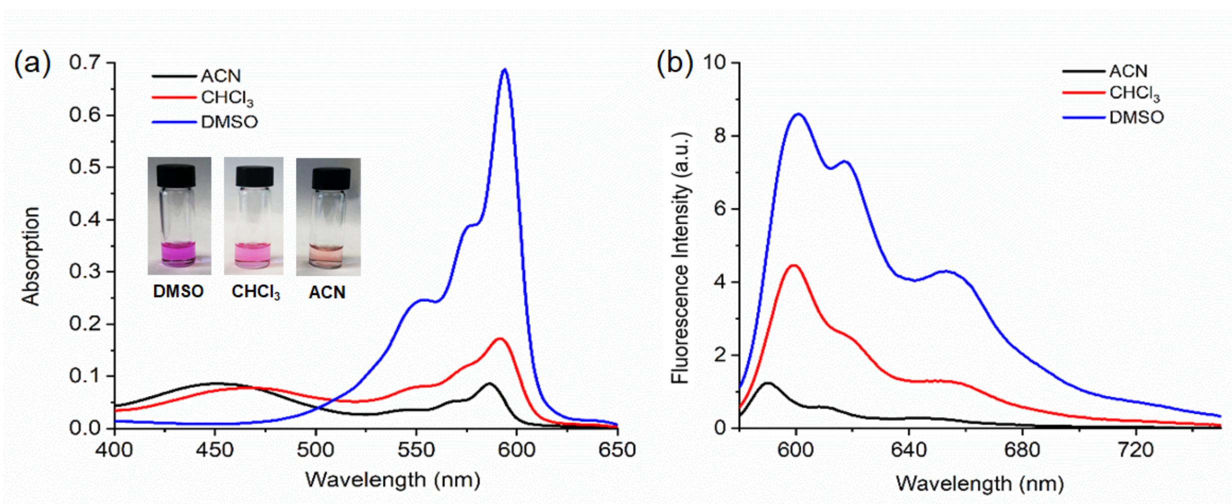


Figure S6. Absorption and emission spectra of 10 μM **RF** (tetrabutylammonium salt) in different organic solvents at 25°C, along with photographs of the solutions.

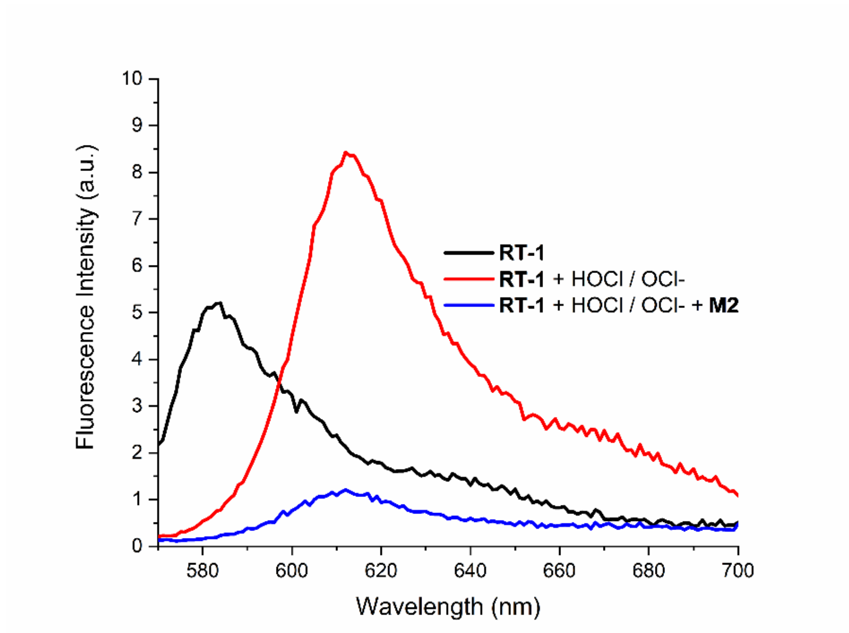


Figure S7. Fluorescence spectra (λ_{ex} = 550 nm) of a sample, initially containing **RT-1** (50 µM, black line), and 3 minutes after addition of HOCl/OCl$^-$ (5 µM, red line), or 3 minutes after a two-step addition sequence of HOCl/OCl$^-$ (5 µM) and then **M2** (500 µM) (blue line). In 200 mM PBS, pH 7.4 at 25°C.

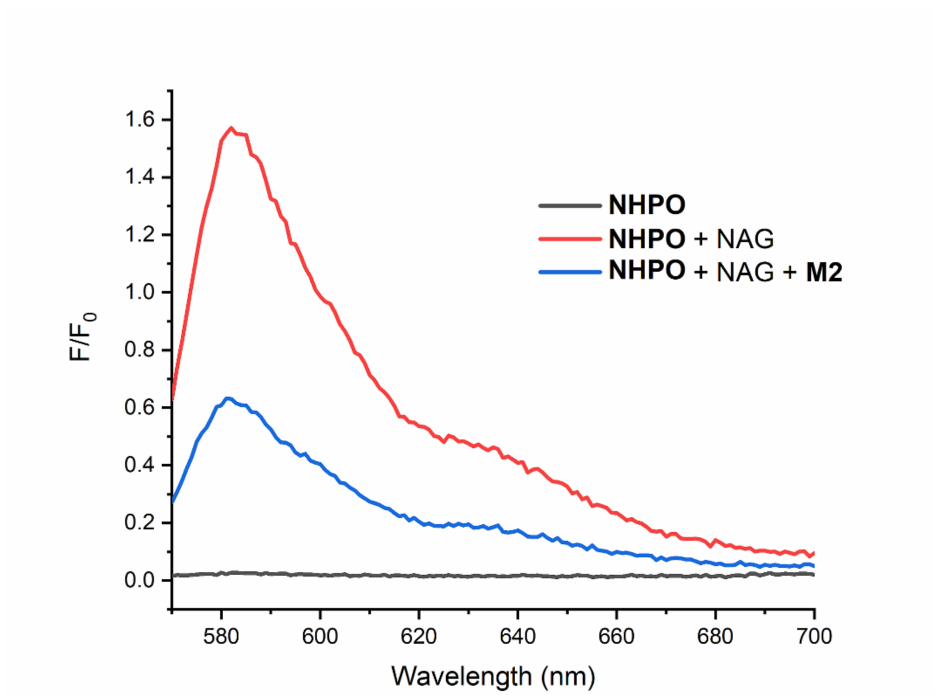


Figure S8. Fluorescence spectra (λ_{ex} = 550 nm) of a sample initially containing **NHPO** (50 µM, black line), 30 minutes after addition of 0.9 µg/mL NAG (red line), or 45 minutes after a two-step addition sequence of 0.9 µg/mL NAG and then **M2** (500 µM) (blue line). In 100 mM PBS + 100 µM BSA, pH 7.4 at 25°C.

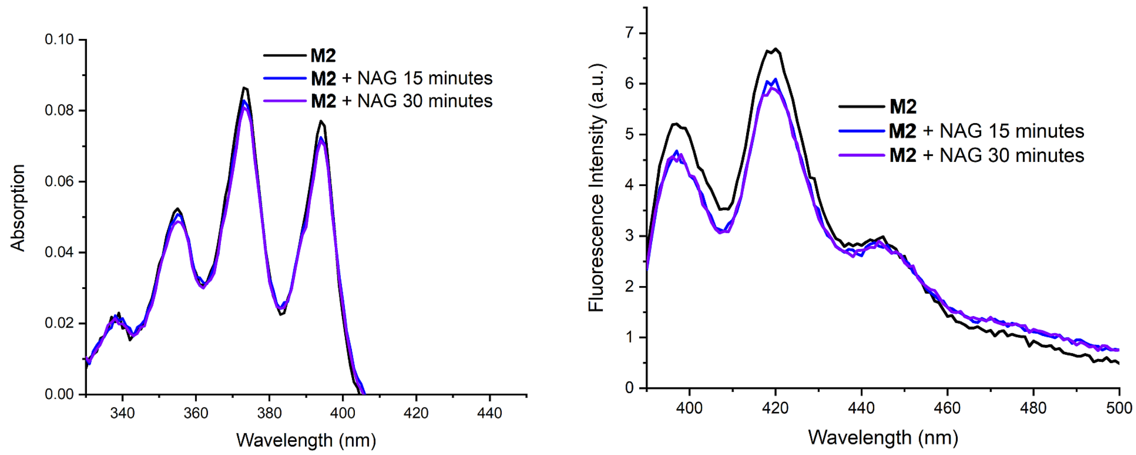


Figure S9. Absorption and fluorescence emission (ex: 370 nm, em: 390 nm) of a solution containing 15 µM **M2** in the presence and absence of (0.1 µg/mL NAG enzyme plus ~10 µM BSA), in water and 25°C. The very small intensity decrease upon protein addition is due to sample dilution, and it appears there is negligible interaction of NAG or BSA with **M2**.

Molecular Modeling

The semiempirical PM7 method was employed within the MOPAC program. (J. J. P. Stewart, MOPAC; Stewart Computational Chemistry: Colorado Springs, CO, 2008.) The dielectric constant of the solvent was set at 78.4 for water and 25 °C. Solubilizing groups are shortened to hydrogens.

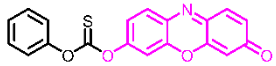
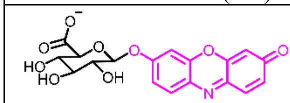
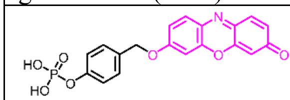
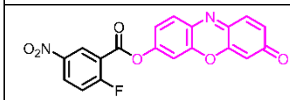
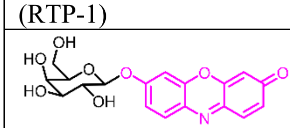
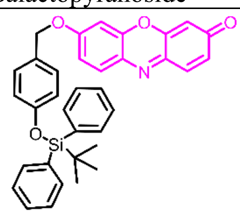
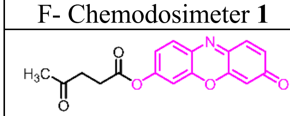
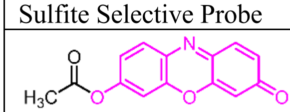
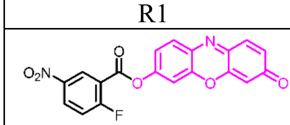
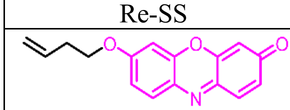
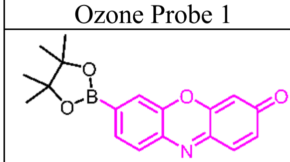
Cartesian Coordinates at the PM7 Level

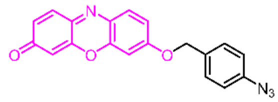
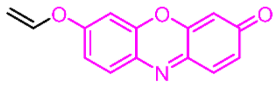
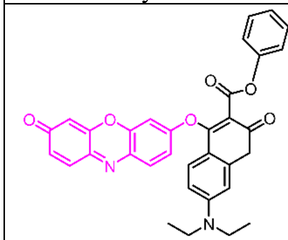
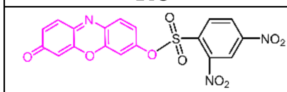
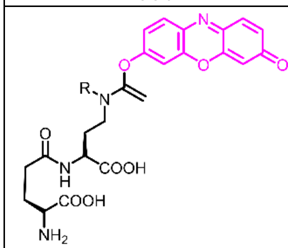
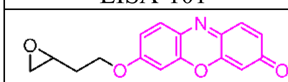
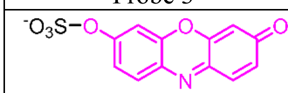
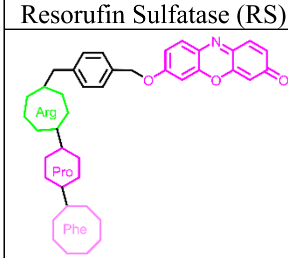
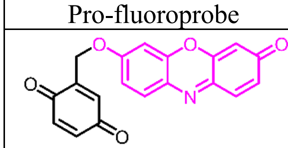
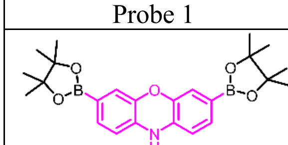
```
TOTAL ENERGY            =     -10938.84941 EV
FINAL GEOMETRY OBTAINED
 EPS=78.4 PM7 CHARGE=-1 EF xyz GNORM=0.100 SHIFT=80

  C     3.81380829 +1   1.30462607 +1   2.77072192 +1
  C     4.46630897 +1   1.72282102 +1   3.97923758 +1
  C     3.85445875 +1  -0.07469538 +1   2.41744974 +1
  C     4.52278289 +1  -0.98838362 +1   3.30059312 +1
  C     5.10257652 +1   0.82824274 +1   4.78463703 +1
  C     5.12626829 +1  -0.55492481 +1   4.44154131 +1
  C     9.41399981 +1   3.11447739 +1  -2.24281169 +1
  C    10.04647500 +1   3.20811044 +1  -0.99419112 +1
  C     9.29500666 +1   1.84768291 +1  -2.88538464 +1
  C     9.85732272 +1   0.71176655 +1  -2.28633684 +1
  C    10.54072507 +1   2.05956950 +1  -0.36123024 +1
  C    10.48068323 +1   0.80420943 +1  -1.03341507 +1
  C    11.07067419 +1  -0.34128367 +1  -0.39753859 +1
  C    11.11361398 +1   2.10070945 +1   0.95557584 +1
  C    11.63809947 +1   0.98753886 +1   1.53732985 +1
  C    11.63203900 +1  -0.25491023 +1   0.83943026 +1
  C     8.60147273 +1   1.78196920 +1  -4.14153263 +1
  C     8.08799392 +1   2.89726345 +1  -4.72912531 +1
  C     8.23196843 +1   4.16783564 +1  -4.10018097 +1
  C     8.87252893 +1   4.26962074 +1  -2.90343033 +1
  C     3.25327707 +1  -0.50384341 +1   1.22532922 +1
  C     2.69733434 +1   0.42381134 +1   0.33341905 +1
  C     2.64242909 +1   1.80101397 +1   0.69491638 +1
  C     3.15193730 +1   2.21368170 +1   1.93383405 +1
  C     2.18031863 +1   0.03179460 +1  -0.94739029 +1
  C     1.64134407 +1   0.94355754 +1  -1.80277847 +1
  C     1.58561273 +1   2.32119784 +1  -1.44026884 +1
  C     2.07068204 +1   2.73223286 +1  -0.23651708 +1
  C     2.97568267 +1   3.64315633 +1   2.37838983 +1
  N     4.10172557 +1   4.45187396 +1   1.88602183 +1
  C     3.98824233 +1   5.80990099 +1   1.87460416 +1
  O     2.96967542 +1   6.34369128 +1   2.30922113 +1
  C     5.10337542 +1   6.62404164 +1   1.32444276 +1
  C     6.38485448 +1   6.10926719 +1   1.12902971 +1
  C     7.36039780 +1   6.90974041 +1   0.53609494 +1
  C     7.07784819 +1   8.24076581 +1   0.21022773 +1
  C     5.81018581 +1   8.76272941 +1   0.45002614 +1
  C     4.81776714 +1   7.95278454 +1   0.99423942 +1
```

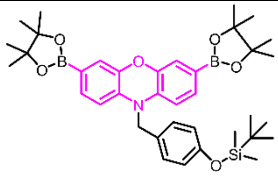
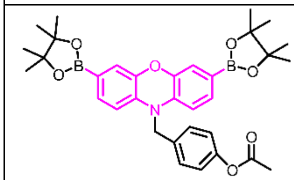
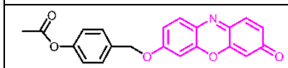
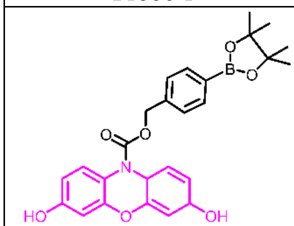
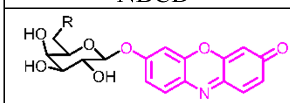
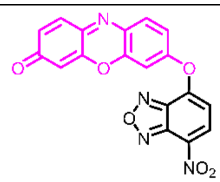
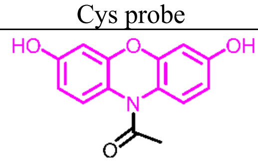
C	8.71547027	+1	6.40633728	+1	0.19078891	+1
O	9.62541231	+1	7.18453767	+1	-0.08450807	+1
N	8.94079512	+1	5.05990192	+1	0.15666159	+1
C	10.22853927	+1	4.56094241	+1	-0.35167652	+1
C	9.81440175	+1	-0.62152972	+1	-2.98973330	+1
N	8.86679137	+1	-1.51732854	+1	-2.30813718	+1
C	8.83586574	+1	-2.83672581	+1	-2.63377849	+1
C	7.92036886	+1	-3.72709992	+1	-1.87215821	+1
C	6.70340644	+1	-3.27154521	+1	-1.36823457	+1
C	5.89690175	+1	-4.14725979	+1	-0.64300816	+1
C	6.28730303	+1	-5.47429457	+1	-0.44640047	+1
C	7.49606565	+1	-5.92644182	+1	-0.96994227	+1
C	8.31574334	+1	-5.05362756	+1	-1.68115676	+1
C	3.19119740	+1	-1.97800281	+1	0.91807835	+1
N	4.45861722	+1	-2.41980201	+1	0.31691706	+1
C	4.59556303	+1	-3.71530687	+1	-0.06988925	+1
O	3.66530444	+1	-4.51044116	+1	0.07215575	+1
O	9.56540770	+1	-3.27990411	+1	-3.51908982	+1
C	6.08430783	+1	0.95544590	+1	-1.20747864	+1
N	6.66002226	+1	0.04065272	+1	-0.42496370	+1
C	6.00918089	+1	2.34672912	+1	-0.83380284	+1
O	6.53805765	+1	2.75599878	+1	0.37023477	+1
C	7.18921373	+1	0.44149613	+1	0.73424741	+1
C	7.14156687	+1	1.81290239	+1	1.16304041	+1
C	7.67159566	+1	2.26693632	+1	2.33190105	+1
C	7.84407762	+1	-0.50023230	+1	1.60726775	+1
C	8.38501465	+1	-0.09210632	+1	2.77501733	+1
C	8.32636327	+1	1.31144390	+1	3.19984307	+1
C	5.51597253	+1	0.58717751	+1	-2.47873205	+1
C	4.94527984	+1	1.51739875	+1	-3.27221356	+1
C	4.87300455	+1	2.92795791	+1	-2.87563148	+1
C	5.44012539	+1	3.31441643	+1	-1.59684750	+1
O	4.34109097	+1	3.75401182	+1	-3.61738014	+1
O	8.82536173	+1	1.65456233	+1	4.27007678	+1
H	4.46014140	+1	2.78371788	+1	4.23936159	+1
H	4.55184649	+1	-2.04663149	+1	3.03732730	+1
H	5.61015438	+1	1.14825144	+1	5.69459216	+1
H	5.64279222	+1	-1.24860737	+1	5.10493309	+1
H	11.06509406	+1	-1.29519247	+1	-0.92472535	+1
H	11.10797265	+1	3.04860425	+1	1.49986932	+1
H	12.06288670	+1	1.02023999	+1	2.54033571	+1
H	12.07757238	+1	-1.12666835	+1	1.31866048	+1
H	8.48078401	+1	0.80821591	+1	-4.62198552	+1
H	7.55675086	+1	2.84170275	+1	-5.67916012	+1
H	7.81422108	+1	5.04548630	+1	-4.59408984	+1
H	8.97856143	+1	5.24510619	+1	-2.42709583	+1
H	2.23997220	+1	-1.01948292	+1	-1.23589298	+1
H	1.25520253	+1	0.64476097	+1	-2.77698197	+1
H	1.16035048	+1	3.03050105	+1	-2.15043929	+1
H	2.04594398	+1	3.79118971	+1	0.02934829	+1
H	2.90765132	+1	3.72837743	+1	3.49225535	+1
H	2.00585196	+1	4.06961559	+1	2.01658031	+1

H	4.88030841	+1	3.94571460	+1	1.48305548	+1
H	6.62622777	+1	5.09489682	+1	1.45217784	+1
H	7.85352421	+1	8.87211527	+1	-0.23473706	+1
H	5.59220871	+1	9.80464951	+1	0.20547888	+1
H	3.81447609	+1	8.35585677	+1	1.16485293	+1
H	8.22454506	+1	4.36372020	+1	0.32136289	+1
H	10.96703491	+1	4.54264697	+1	0.48862585	+1
H	10.66878882	+1	5.28384485	+1	-1.08673499	+1
H	10.83345957	+1	-1.08709250	+1	-3.01392265	+1
H	9.52843975	+1	-0.53460490	+1	-4.06752197	+1
H	8.27262727	+1	-1.10292388	+1	-1.59182849	+1
H	6.38000558	+1	-2.24100260	+1	-1.54902425	+1
H	5.64420075	+1	-6.15715447	+1	0.11590198	+1
H	7.80038316	+1	-6.96446016	+1	-0.82338215	+1
H	9.26662705	+1	-5.40591135	+1	-2.09040512	+1
H	2.34140368	+1	-2.23234920	+1	0.23727619	+1
H	2.98204237	+1	-2.57440552	+1	1.84340073	+1
H	5.19314717	+1	-1.72297880	+1	0.21319645	+1
H	7.62270163	+1	3.29617288	+1	2.65361514	+1
H	7.88608749	+1	-1.54385129	+1	1.28306589	+1
H	8.88914855	+1	-0.77683924	+1	3.44729399	+1
H	5.56946817	+1	-0.46567424	+1	-2.77011967	+1
H	4.51120938	+1	1.26814086	+1	-4.23614250	+1
H	5.39113145	+1	4.35269899	+1	-1.31209333	+1

Table S1: Abridged collection of enzyme substrates and chemosensors that release resorufin (RF).

Probe	Analyte	Method of Detection	Reference Number
 Resorufin Thionocarbonate (RT)	Mercury Hg^{2+}	Chromogenic	8
 Resorufin β-D-glucuronide (REG)	E. coli	Chromogenic	9
 Novel Probe 1	Alkaline Phosphatase (ALP)	Fluorescence	10
 Resorufin turn on Probe (RTP-1)	Hydrazine (N_2H_4)	Fluorescence	11
 Resorufin-*β*-D-Galactopyranoside	Biotinylated DNA	Fluorescence	12
 F- Chemodosimeter **1**	Fluorine (F^-)	Chromogenic/ Fluorescence	13
 Sulfite Selective Probe	Sulfite (SO_3^{2-})	Chromogenic/ Fluorescence	14
 R1	Perborate (BO_3^-) /Hydrazine (N_2H_4)	Chromogenic/ Fluorescence	15,16
 Re-SS	Polysulfides	Fluorescence	17
 Ozone Probe 1	Ozone (O_3)	Chromogenic/ Fluorescence	18
 PC1	Hydrogen Peroxide (H_2O_2)	Fluorescence	19

 ABR	Hydrogen Sulfide (H_2S)	Fluorescence	20
 Mercury Probe 1	Mercury (Hg^{2+})	Chromogenic/Fluore scence	21
 RC	Hydrogen sulfide (H_2S), Cysteine (Cys), Homocysteine (Hcy), Glutathione (GSH)	Fluorescence	22
 Probe 1	Acetyl-cholinesterase	Fluorescence	23
 LISA-101	γ-glutamyl cyclotransferase	Fluorescence	24
 Probe 3	Epoxy-hydrolase	Fluorescence	25
 Resorufin Sulfatase (RS)	Sulfatase	Fluorescence	26
 Pro-fluoroprobe	Thrombin protease	Fluorescence	27
 Probe 1	Glucose	Fluorescence	28
 PR1	Reactive Oxygen, Nitrogen ($ONOO^-$)	Fluorescence and Colorimetric	29

 Pinkment-OTBS	($ONOO^-$ and F^-)	Fluorescence and Colorimetric	29
 Pinkment-OAC	Esterase and H_2O_2	Fluorescence and Colorimetric	29
 Probe 1	Carboxylesterase	Fluorescence	30
 NBCD	H_2O_2	Fluorescence	31
 Res-GlcGcase	β-Glucocerebrosi dase	Fluorescence	32
 Cys probe	Cysteine	Fluorescence	33
 AR	Phosphite and Nickel oxide	Fluorescence	34

References:

(1) M. C. Robson, W. G. Payne, F.Ko, M. Mentis, G. Donati, S. M. Shafii, S. Culverhouse, L. Wang, B. Khosrovi, R. Najafi,; et al., *J. Burns Wounds,* 2007, **6**, 80-90.
(2) M. G. Choi, Y. J. Lee, K. M. Lee, K. Y. Park, T. J. Park and S. Chang, *Analyst,* 2019, **144**, 7263–7269.
(3) F. Yan, X. Tian, Z. Luan, L. Feng, X. Ma and T. D. James, *Chem. Commun.,* 2019, **55**, 1955–1958.
(4) J. J. Gassensmith, E. Arunkumar, L. Barr, J. M. Baumes, K. M. Divittorio, J. R. Johnson, B. C. Noll and B. D. Smith, *J. Am. Chem. Soc.,* 2007, **129**, 15054–15059.
(5) C. F. A. Gómez-Durán, W. Liu, D. Lourdes and B. D. Smith, *J. Org. Chem.,* 2017, **82**, 8334–8341.
(6) W. Liu, E. M. Peck, K. D. Hendzel and B. D. Smith, *Org. Lett.,* 2015, **17**, 5268–5271.
(7) E. M. Peck, W. Liu, G. T. Spence, S. K. Shaw, A. P. Davis, H. Destecroix and B. D. Smith, *J. Am. Chem. Soc.,* 2015, **137**, 6–9.
(8) M. G. Choi, S. Y. Park, K. Y.Park and S. K. Chang, *Sci. Rep.* **2019**, *9*, 1–8.
(9) G. Magro, R. E. S. Bain, C. A. Woodall, R. L. Matthews, S. W. Gundry and A. P. Davis, *Environ. Sci. Technol.,* 2014, **48**, 9624–9631.
(10) H. Zhang, C. Xu, J. Liu, X. Li, L. Guo and X. Li, *Chem. Commun.,* 2015, **51**, 7031–7034.
(11) T. Tang, Y. Q. Chen, B. S. Fu, Z. Y. He, H. Xiao, F. Wu, J. Q. Wang, S. R. Wang and X. Zhou, *Chinese Chem. Lett.,* 2016, **27**, 540–544.
(12) Z. Li, R. B. Hayman and D. R. Walt, *J. Am. Chem. Soc.,* 2008, **130**, 12622–12623.
(13) S. Y. Kim and J. I. Hong, *Org. Lett.,* 2007, **9**, 3109–3112.
(14) M. G. Choi, J. Hwang, S. Eor and S. K. Chang, *Org. Lett.,* 2010, **12**, 5624–5627.
(15) M. G. Choi, J. O. Moon, J. Bae, J. W. Lee and S. K. Chang, *Org. Biomol. Chem.,* 2013, **11**, 2961–2965.
(16) M. G. Choi, S. Cha, J. E. Park, H. Lee, H. L. Jeon and S. K. Chang, *Org. Lett.,* 2010, **12**, 1468–1471.
(17) J. Liu and Z. Yin, *Analyst,* 2019, **144**, 3221–3225.
(18) Y. Zhang, W. Shi, X. Li and H. Ma, *Sci. Rep.,* 2013, **3,** 2–7.
(19) E.W. Miller, O. Tulyanthan, E.Y. Isacoff and C. Chang, J. *Nat. Chem. Biol.,* 2007, **3**, 263–267.
(20) M. Kim, Y. H. Seo, Y. Kim, J. Heo, W. D. Jang, S. J. Sim and S. Kim, *Chem. Commun.,* 2017, **53**, 2275–2278.
(21) M. S. Thakare and P. M. Yeole, *J. Biol. Chem. Chron.,*2018, **4**, 48–53.
(22) H. Zhang, L. Xu, W. Chen, J. Huang, C. Huang, J. Sheng and X. Song, *Anal. Chem.,* 2019, **91**, 1904–1911.
(23) K. Cui, Z. Chen, Z. Wang, G. Zhang and D. Zhang, *Analyst,* 2011, **136**, 191–195.
(24) T. Yoshiya, H. Ii, S. Tsuda, S. Kageyama, T. Yoshiki and Y. Nishiuchi, *Org. Biomol. Chem.,* 2015, **13**, 3182–3185.
(25) M. L. S. O. Lima, M. R. B. Chaves, R. M. C. Do Nascimento, C. C. S. Gonçalves and A. J. *J.* Marsaioli, *Braz. Chem. Soc.,* 2018, **29**, 1149–1156.
(26) E. L. Smith, C. R. Bertozzi and K. E. Beatty, *ChemBioChem.,* 2014, **15**, 1101–1105.
(27) D. Arian, J. Harenberg and R. Krämer, *J. Med. Chem.,* 2016, **59**, 7576–7583.
(28) X. Gao, X. Li, Q. Wan, Z. Li and H. Ma, *Talanta,* 2014, **120**, 456–461.
(29) L. Wu, A. C. Sedgwick, X. Sun, S. D. Bull, X. P. He and T. D. James, *Acc. Chem. Res.,* 2019, **52**, 2582–2597.
(30) Y. Zhang, W. Chen, D. Feng, W. Shi, X. Li, and H. Ma, *Analyst* **2012**, *137*, 716–721.
(31) Z. Han, , X. Liang, X. Ren, L. Shang and Z. Y, *Chem. Asian J.* **2016**, *11*, 818–822.
(32) M. C. Deen, C. Proceviat, X. Shan, L. Wu, D. L. Shen, G. J. Davies, and D. J. Vocadlo, *ACS Chem. Biol.* **2020**, *12*, 824–829.
(33) J. Zhang, Y. Miao, Z. Cheng, L. Liang and C. Liu, *Analyst* **2020**, *145*, 1878–1884.
(34) Y. Chang, M. Liu and J. Liu, *Anal. Chem.* **2020**, *92*, 3118–3124