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Supplementary Information

Development of a Novel Benzimidazole-Based Probe and Portable Fluorimeter for the Detection of Cysteine in Human Urine

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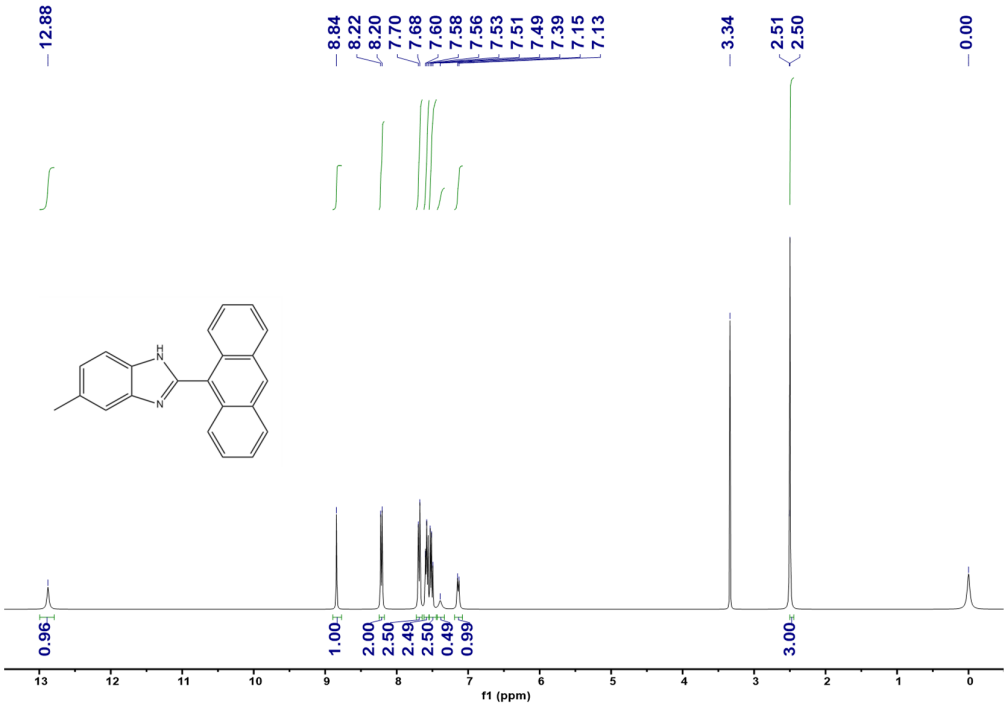


Figure S1. ^{1}H-NMR spectrum of compound **3**.

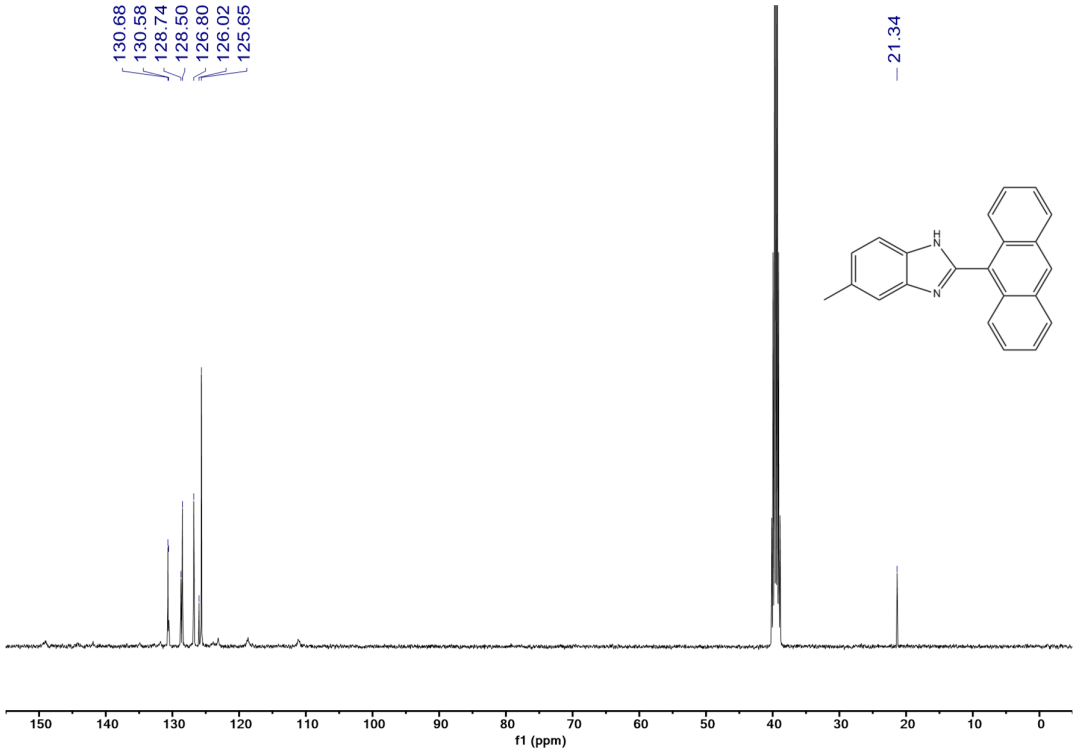


Figure S2. ^{13}C NMR spectrum of compound **3**.

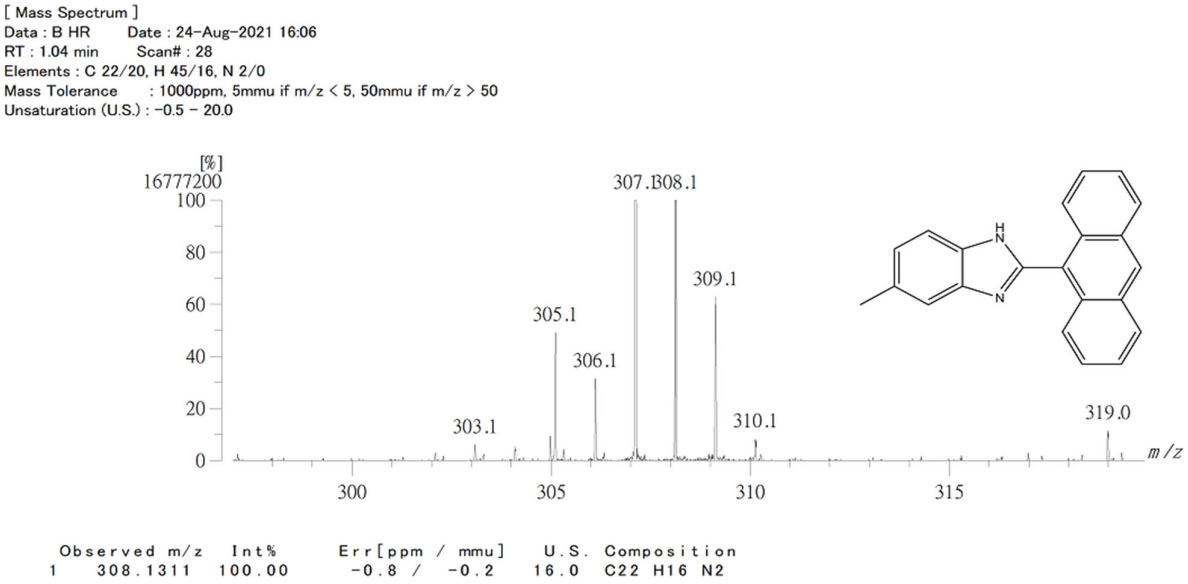


Figure S3. HR-mass spectrum of compound **3**.

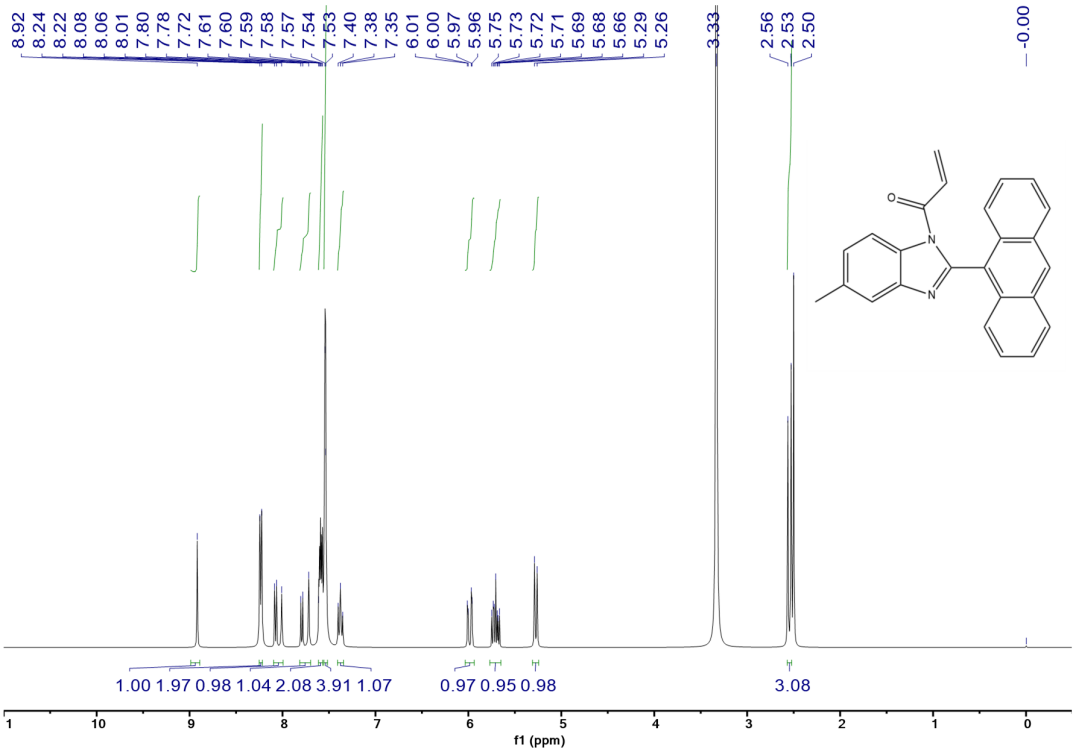


Figure S4. [1]H-NMR spectrum of **ABIA.**

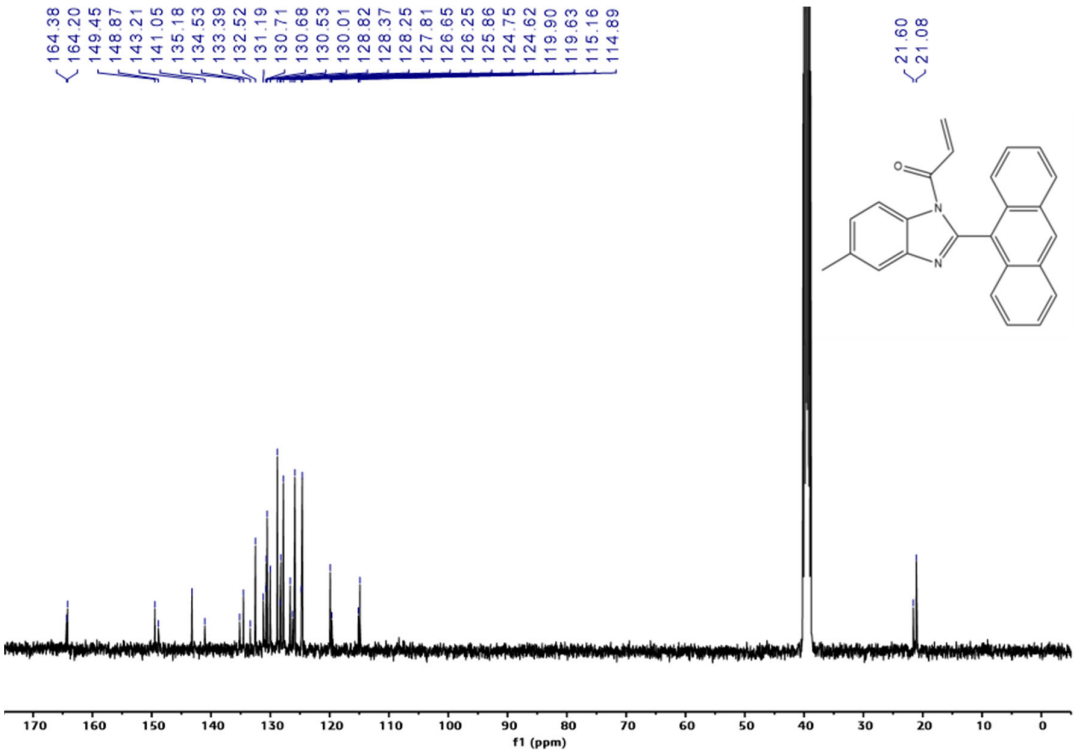


Figure S5. [13]C NMR spectrum of **ABIA.**

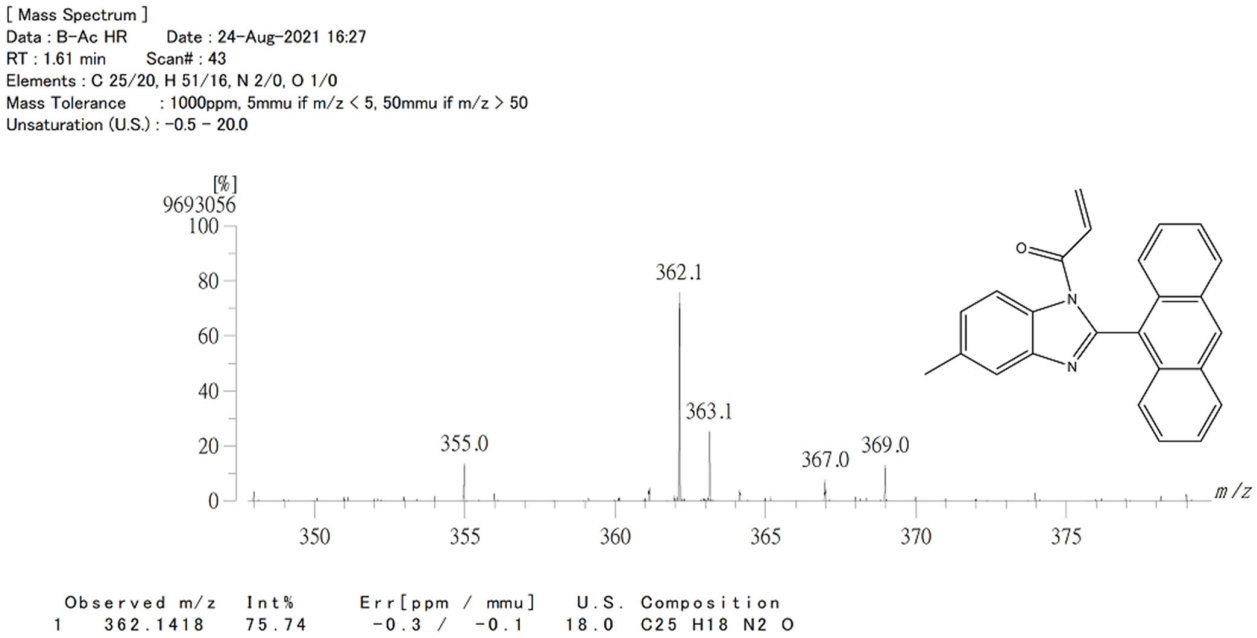


Figure S6. HR-mass spectrum of receptor **ABIA**.

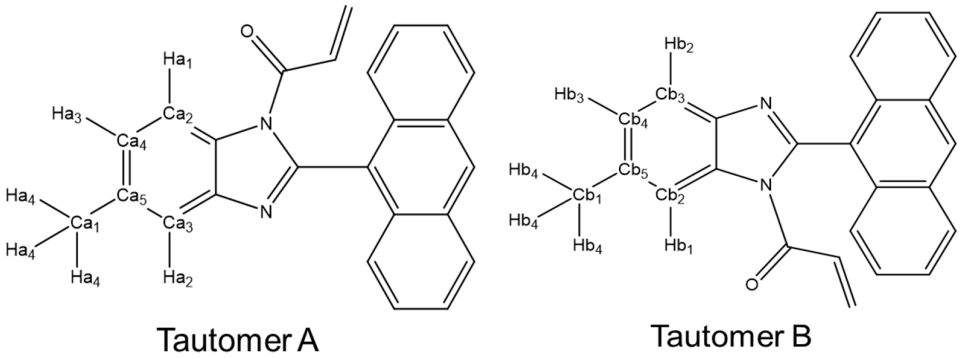


Figure S7. Structures of the probe **ABIA** tautomer's **A** and **B.**

Table S1. ^{1}H NMR and ^{13}C NMR chemical shifts (δ ppm) of the **ABIA** tautomer's **A** and **B.**

Compound	**ABIA**			
	^{1}H NMR		**^{13}C NMR**	
Proton or Caron Label	Tautomer A	Tautomer B	Tautomer A	Tautomer B
H[a,b]$_1$ / C[a,b]$_1$	8.06	7.80	130.71	130.68
H[a,b]$_2$ / C[a,b]$_2$	8.01	7.72	124.75	124.62
H[a,b]$_3$ / C[a,b]$_3$	7.40	7.35	119.90	119.63
H[a,b]$_4$ / C[a,b]$_4$	2.56	2.53	115.16	114.89
C[a,b]$_5$	-	-	21.60	21.08

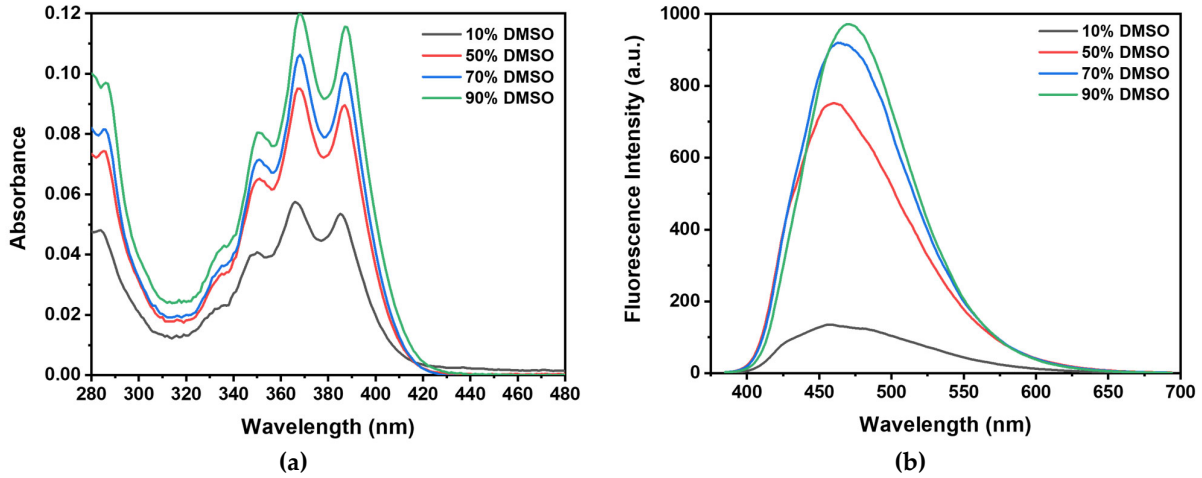


Figure S8. (**a**) UV-vis absorbance spectra, (**b**) Fluorescence spectra of compound **3** in 10, 50, 70, and 90% DMSO:0.01 M HEPES buffer solution (λ_{ex} = 368 nm).

Table S2. Photophysical properties of compound **3** (10×10^{-6} M) in varying percentages of DMSO:0.01 M HEPES buffer solution.

DMSO Percentage (%)	λ_{max} (nm)	$\varepsilon\ 10^4$ [$M^{-1}\,cm^{-1}$]	λ_{em} (nm)	Stoke's shift (nm)
10%	368	0.56	458	90
50%	368	0.95	460	92
70%	368	1.06	462	94
90%	368	1.20	470	102

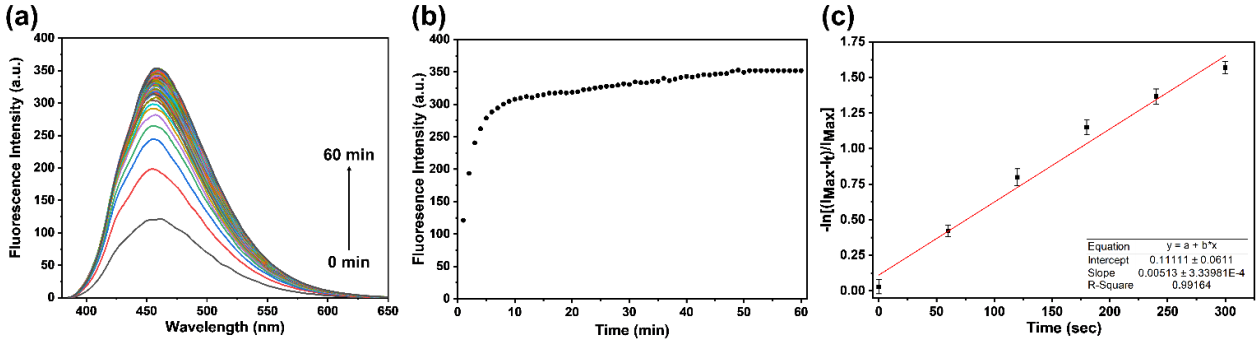


Figure S9. (**a**), (**b**)Time-dependent fluorescence response and (**c**) pseudo-first-order kinetic plots of **ABIA** (10×10^{-6} M) reaction with Cys (50×10^{-6} M) in 90% DMSO:0.01 M HEPES buffer solution (λ_{ex} = 368 nm, λ_{em} = 455 nm).

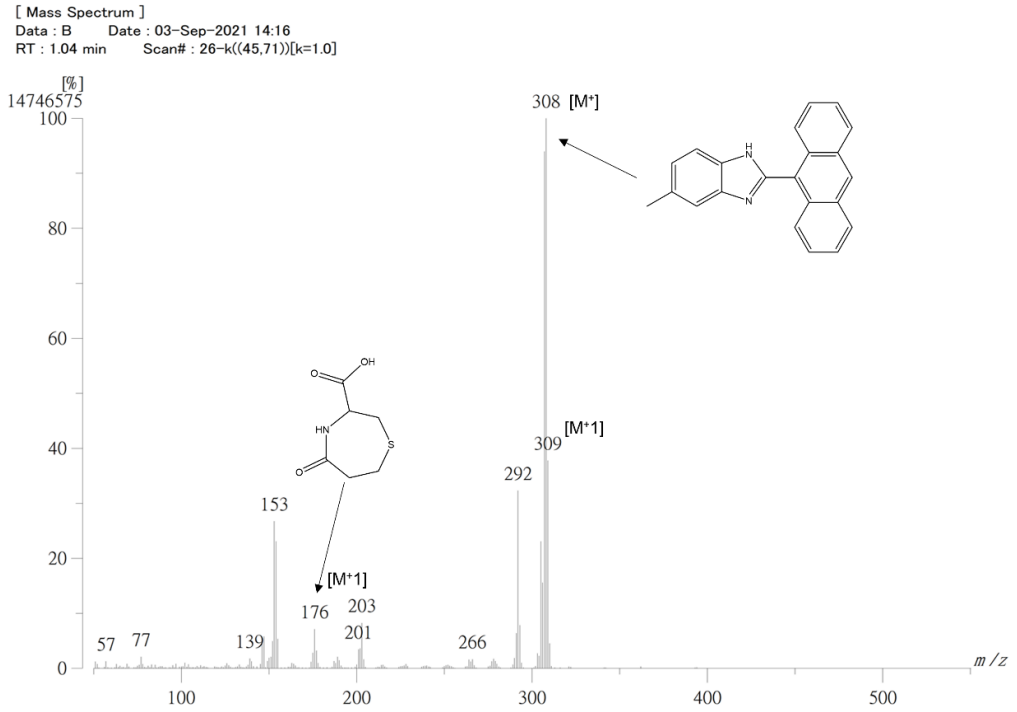
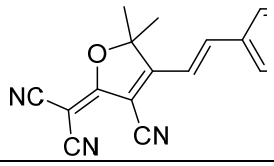
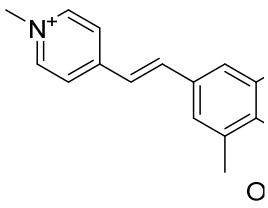
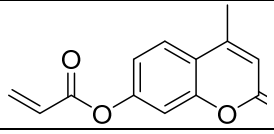
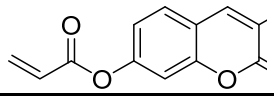


Figure S10. Mass spectra of a reaction product obtained by reacting equimolar **ABIA** and Cys for 60 min at 25 °C.

Table S3. A comparison of response time for cysteine detection by various methods.

Sr. No.	Probe	Response Time (min)	Rate Constant (s^{-1})	Ref.
1		90	8.16×10^{-4}	[1]
2		80	7.16×10^{-4}	[2]
3		30	2.70×10^{-3}	[3]
4		20	1.1×10^{-2}	[4]

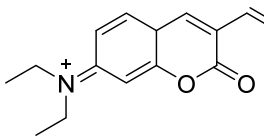
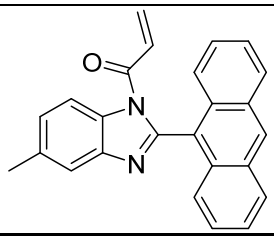
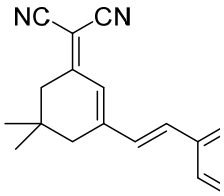
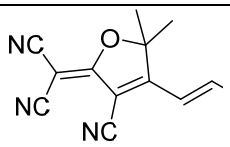
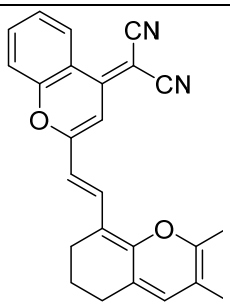
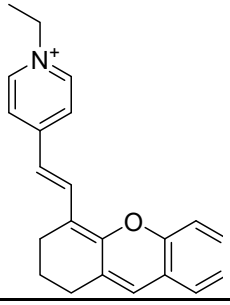
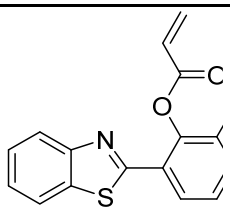
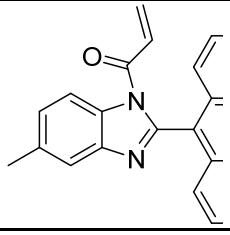
5		15	2.3×10^{-3}	[5]
ABIA		10	5.13×10^{-3}	**This Work**

Table S4. A comparison of different probes used for cysteine detection.

Sr. No.	Probe	LOD (nM)	Urine Test	Live Cell Imaging	Ref.
1		260	No	Yes	[6]
2		40	No	Yes	[7]
3		48	No	Yes	[8]
4		48.9	Yes	Yes	[9]

5		110	No	Yes	[10]
ABIA		16.3	Yes	Yes	**This Work**

[a]–not reported.

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