Rapid and Sensitive Determination of Vancomycin by AIE-Active Fluorescent Probe for Clinical Monitoring

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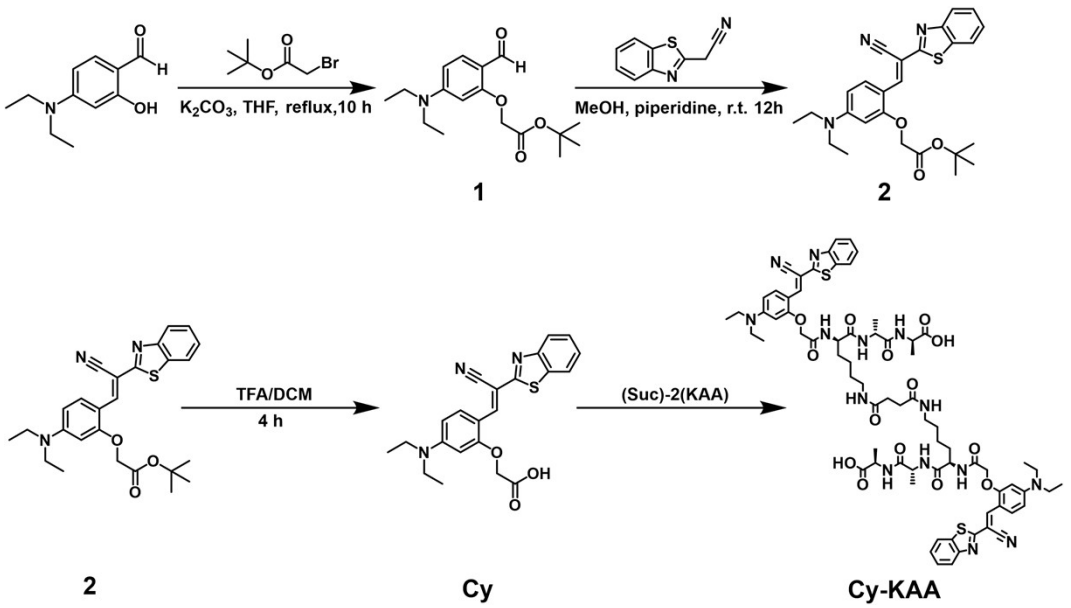
Experimental Section



Scheme S1 Synthesis route of **Cy-KAA**.

Synthesis of 1. The raw material 4-(diethylamino) salicylaldehyde (5 g, 25.87 mmol) was dissolved in dry THF (80 mL) and then K_2CO_3 (7.15 g, 51.75 mmol) was added. Then the solution of tert-butyl bromoacetate (6.24 g, 32.04 mmol) in THF (15 mL) was added dropwise to the reaction mixture under stirring, followed by heating to 75 °C and stirring for overnight. After the reaction was complete, most of the THF was removed by rotational evaporation. Then the residue was extracted twice with EA and washed with saturated brine. The organic layer was combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with PE/EA (3:1, v/v) as eluent, to afford product **1** as a red solid (6.05 g, yield 76.10%). ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ 10.07 (d, J = 0.8 Hz, 1H), 7.52 (d, J = 8.9 Hz, 1H), 6.38 (ddd, J = 9.0, 2.3, 0.8 Hz, 1H), 6.04 (d, J = 2.2 Hz, 1H), 4.83 (s, 2H), 3.42 (q, J = 7.1 Hz, 4H), 1.44 (s, 9H), 1.11 (t, J = 7.0 Hz, 6H).

Synthesis of 2. Benzothiazole-2-acetonitrile (1.63 g, 9.37 mmol) was added to the solution of compound **1** (2.4 g, 7.81 mmol) in dry MeOH (45 mL). Then piperidine (8 mL) was added dropwise to the above mixture, which was stirred at room temperature for overnight. The resulting precipitate was filtered and washed

with cold EtOH, yielding product **2** as an orange solid (2.94 g, 81.30 % yield). ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ 8.54 (s, 1H), 8.26 (d, J = 9.2 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 6.59 – 6.56 (m, 1H), 6.17 – 6.14 (m, 1H), 4.91 (s, 2H), 3.48 (q, J = 7.0 Hz, 4H), 1.46 (s, 9H), 1.14 (t, J = 7.0 Hz, 6H).

Synthesis of Cy. Compound **2** (1.49 g, 3.21 mmol) was dissolved in DCM (20 mL), and TFA (20 mL) was added dropwise with stirring. The reaction was conducted at room temperature for 4 hours. After completion, the solvent was removed as much as possible by distillation under reduced pressure. The mixture was then washed twice with water, and the organic layer was collected, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was recrystallized from an EtOH/DCM mixture, filtered, and collected as a red solid (**Cy**) on filter paper (1.10 g, 83.99 % yield). ^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ 13.19 (s, 1H), 8.55 (s, 1H), 8.27 (d, J = 9.2 Hz, 1H), 8.10 (dd, J = 8.1, 1.2 Hz, 1H), 7.99 – 7.96 (m, 1H), 7.53 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 7.43 (m, 1H), 6.58 (dd, J = 9.3, 2.4 Hz, 1H), 6.18 (d, J = 2.4 Hz, 1H), 4.94 (s, 2H), 3.49 (q, J = 7.0 Hz, 4H), 1.15 (t, J = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.41, 165.74, 160.27, 153.83, 153.27, 140.95, 134.10, 129.93, 127.25, 125.62, 122.69, 122.57, 118.81, 109.07, 106.31, 94.81, 94.64, 65.61, 44.77, 12.98. HR-MS (m/z) $[M+Na]^+$, calcd 430.4778; found, 430.1198.

Molecular Docking. A semi-flexible molecular docking method was applied. The AutoDock Vina 1.5.6 software (developed by the Scripps Research Institute) was utilized to prepare the 3D structures of Van and **Cy-KAA**, including energy minimization to improve the accuracy and success rates of docking. After setting the docking parameters, a global docking search was conducted. The binding conformation with the highest docking score was chosen for further analysis and visualization using PyMOL.

Molecular Dynamics (MD) Simulation. MD simulations of the **Cy-KAA**–Van complex were carried out using GROMACS 2020.6 for a duration of 80 ns. The AMBER99SB-ILDN force field was applied to generate the topology of Van, while

the Amber20 software and GAFF force field were utilized for the topology of **Cy-KAA**. A truncated octahedral TIP3P water box, with a 10 nm buffer distance, was used, and Na^+/Cl^- ions were added to neutralize the system. Energy minimization was conducted in two stages: 2,500 steps of steepest descent followed by 2,500 steps of conjugate gradient minimization. Following minimization, the system was equilibrated at 300 K with 100 ps of NVT equilibration and 100 ps of NPT equilibration. A 80 ns MD simulation was then performed under periodic boundary conditions (PBC), with trajectory frames recorded every 10 ps. The resulting MD trajectory was analyzed for root mean square deviation (RMSD) and hydrogen bonding interactions to assess the stability of the complex.

Supplementary Figures

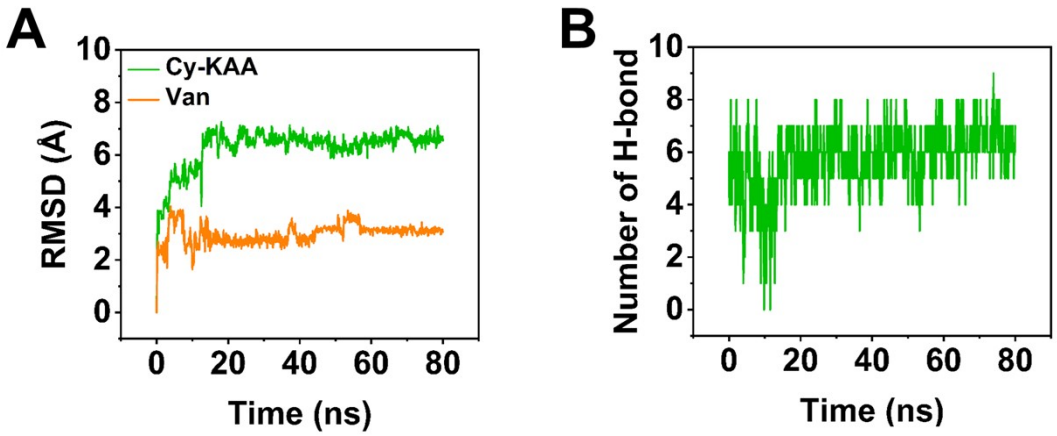


Fig. S1 (A) Root mean square deviation (RMSD) evolution of Cy-KAA and Van over 80 ns molecular dynamics simulation. (B) Number of hydrogen bonds between Cy-KAA and Van over 80 ns molecular dynamics simulation.

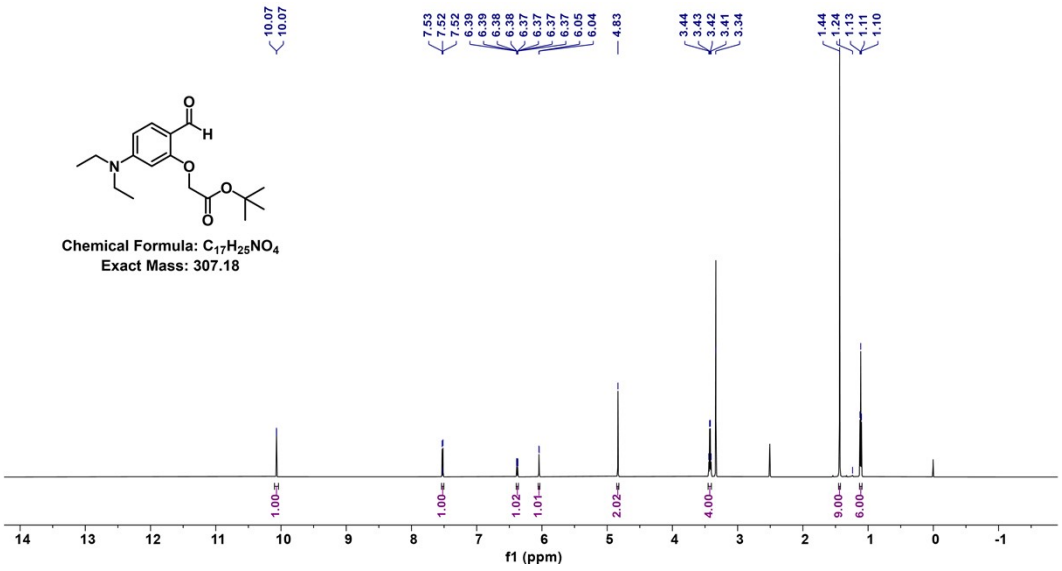


Fig. S2 ^{1}H NMR spectrum of compound **1**.

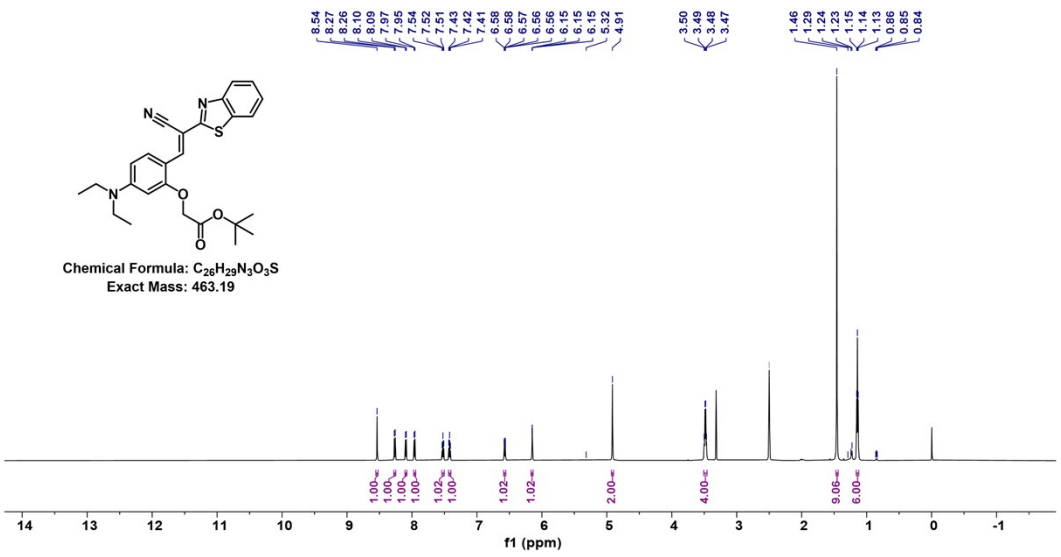


Fig. S3 ^{1}H NMR spectrum of compound **2**.

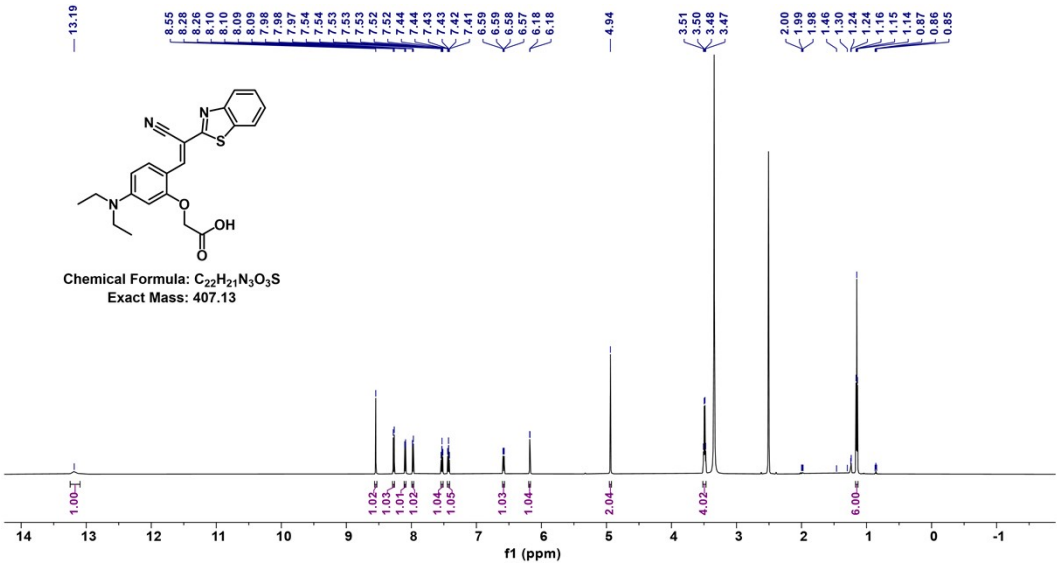


Fig. S4 ^{1}H NMR spectrum of fluorophore **Cy**.

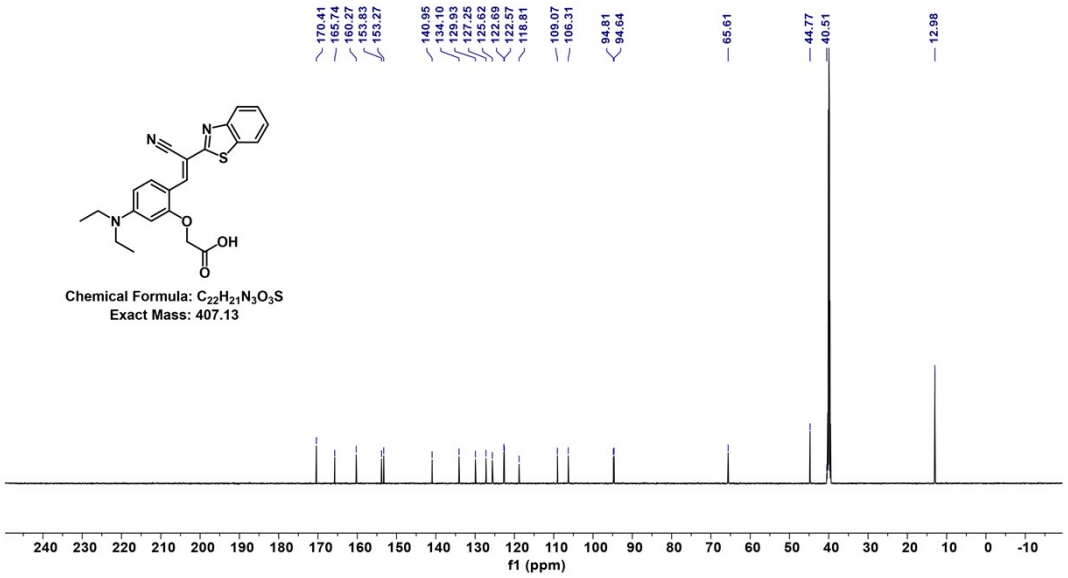
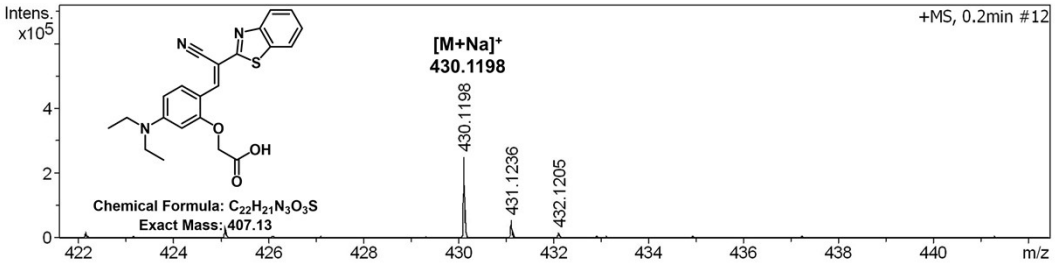


Fig. S5 ^{13}C NMR spectrum of fluorophore **Cy**.



#	m/z	Res.	S/N	I	I %	FWHM
1	430.1198	19976	468.3	249056	100.0	0.0215
2	431.1236	16834	100.8	53604	21.5	0.0256
3	432.1205	11240	26.1	13868	5.6	0.0384

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma		Score	rdb	e¯ Conf	N-Rule
430.1198	1	C22H21N3NaO3S	430.1196	-0.5	26.5	2	100.00	13.5	even	ok

Fig. S6 HR-MS spectrum of fluorophore **Cy**.

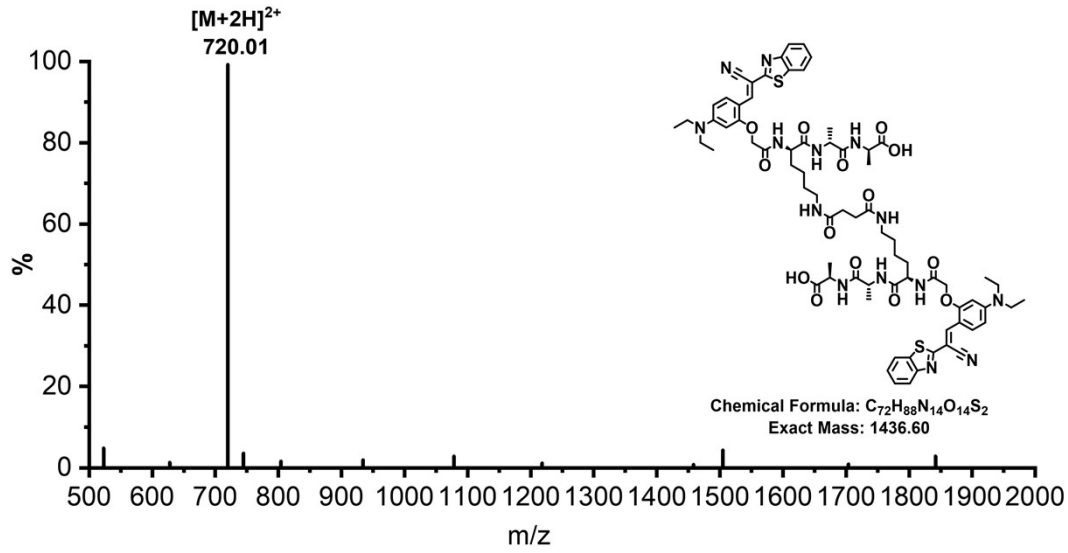
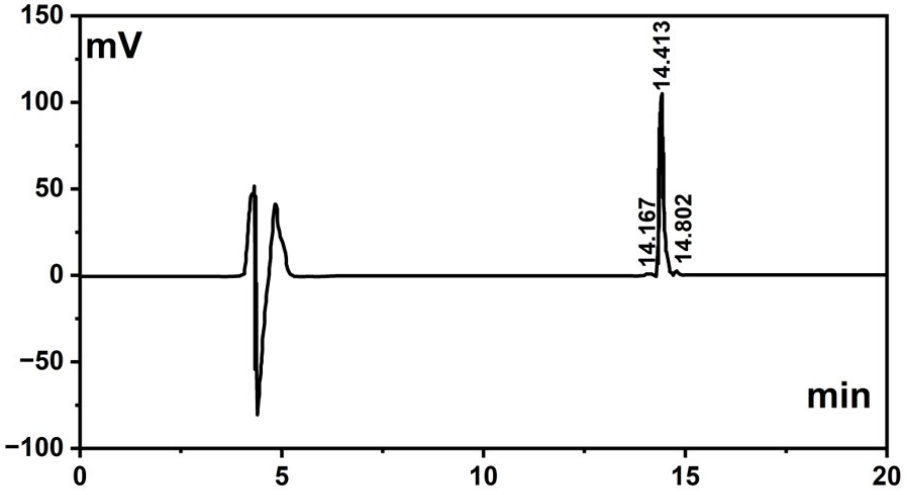


Fig. S7 ESI-MS spectrum of probe **Cy-KAA**.



Rank	Time	Conc.	Area
1	14.167	0.9069	6666
2	14.413	97.02	713042
3	14.802	2.076	15259
Total		100	734967

Fig. S8 HPLC chromatogram of probe **Cy-KAA**.

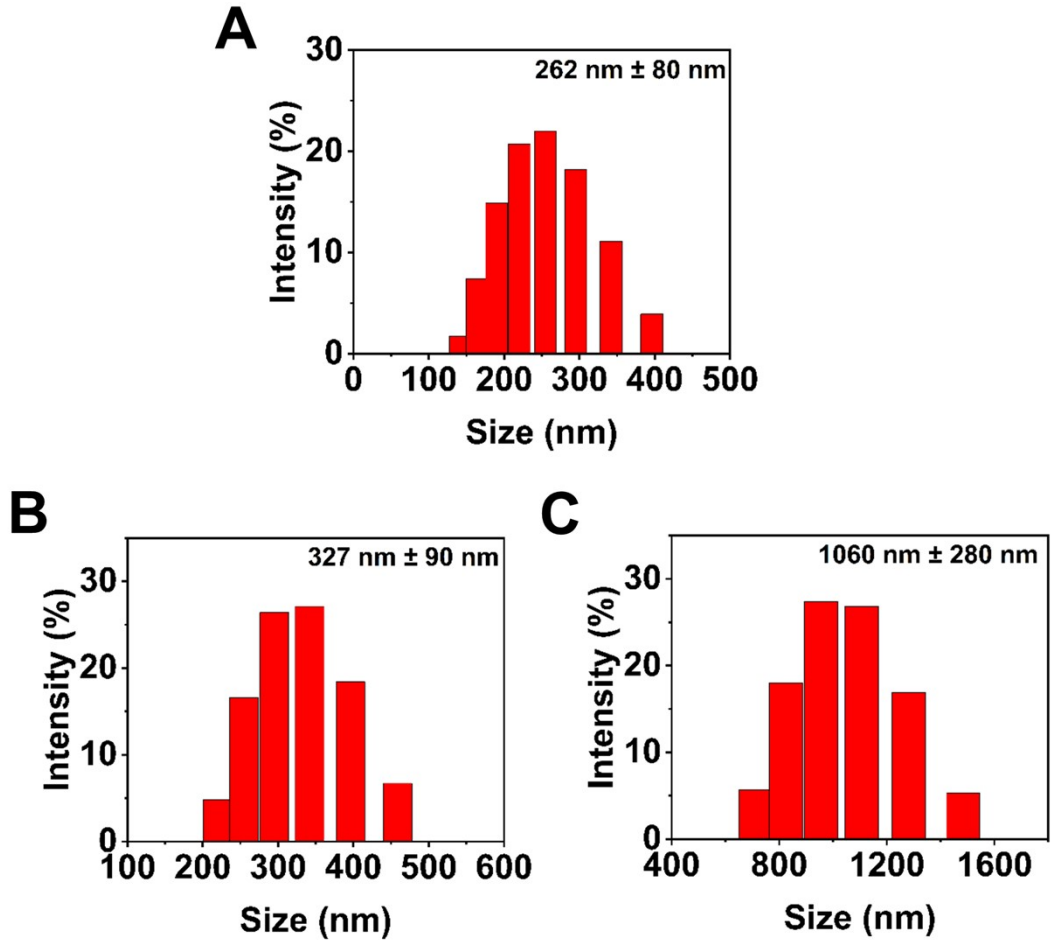


Fig. S9 Dynamic light scattering (DLS) analysis of 20 μM **Cy-KAA** aggregation in the presence of Van (A) 0, (B) 20, (C) 60 μM.

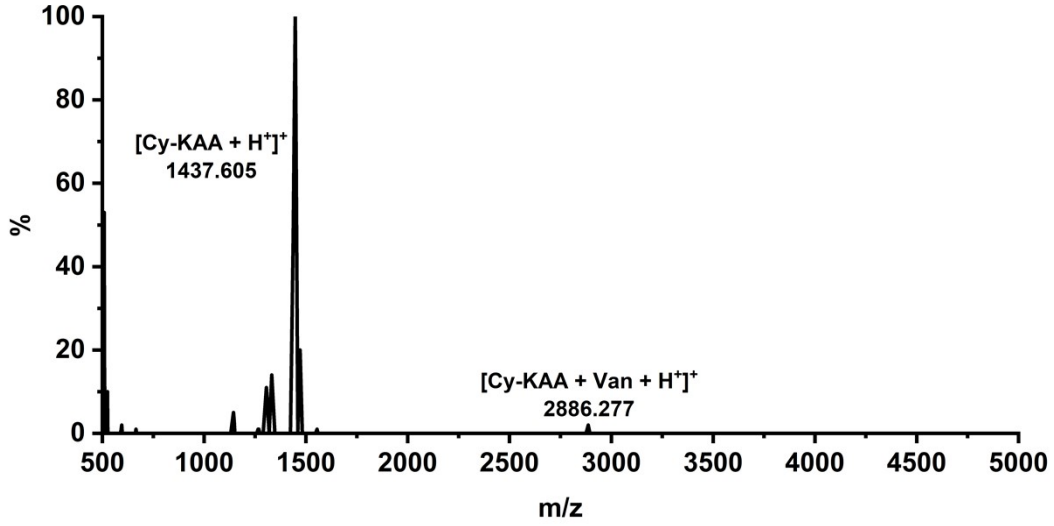


Fig. S10 The spectrum of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) of **Cy-KAA** (20 μM) in the presence of Van (200 μM).

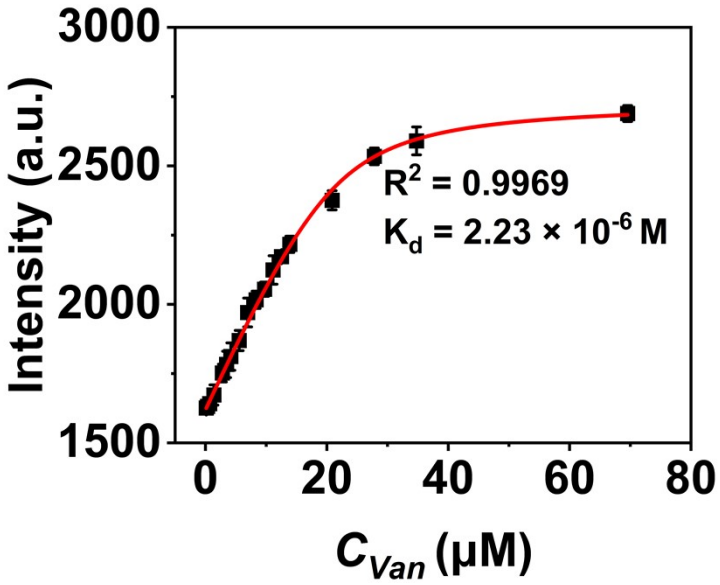


Fig. S11 Non-linear least square fitting of fluorescence intensity as a function of Van by a 1:1 binding model. (λ_{ex} = 460 nm, λ_{em} = 634 nm)

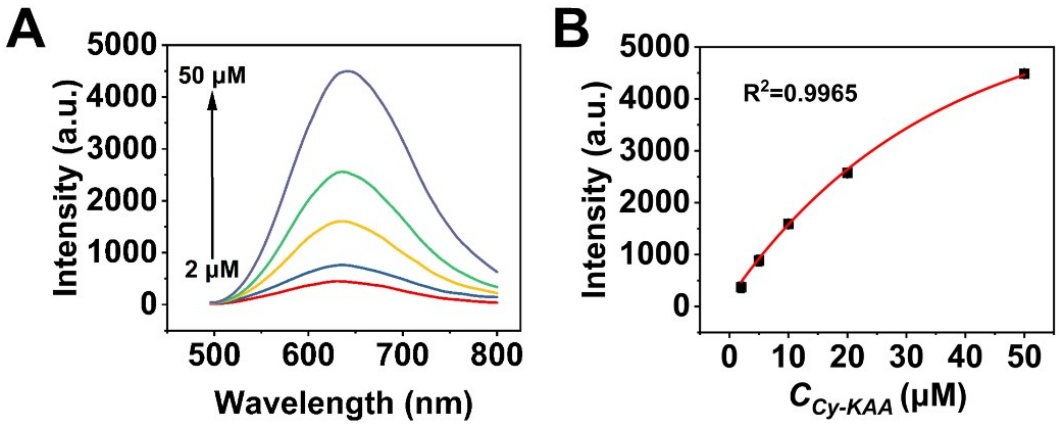


Fig. S12 (A) Fluorescence emission spectra of **Cy-KAA** at 2, 5, 10, 20, 50 μM in water. (B) Plot of fluorescence intensity versus **Cy-KAA** concentration. (λ_{ex} = 460 nm, λ_{em} = 634 nm)

Supplementary Tables

Table S1 Comparison of **Cy-KAA** and other existing detection methods.

Ref.	Method	Sample Type	Ease of use (Pretreatment)	LOD ($\mu g\ mL^{-1}$)	Quantitative range ($\mu g\ mL^{-1}$)
19	HPLC	buffer	extraction	Not given	25-175
23	Chemiluminescence	human serum	deproteinization	2.03	5.80-1449.25
30	FICA	human serum (patient)	25 times dilution	0.81	1.25-50
31	Fluorescence	human serum (healthy volunteers)	20 times dilution	0.26	0.5-50
32	Fluorescence	rat serum	2 times dilution	Not given	1.45-144.92
33	Fluorescence	spiked human plasma or urine	deproteinization	0.29×10^{-3}	0.001-0.036
34	Fluorescence	commercial human serum	4 times dilution	0.13	5.79-43.47
35	Fluorescence	spiked human plasma	deproteinization	0.31×10^{-3}	0-0.12
This work	Fluorescence	human serum (patient & healthy volunteers)	10 times dilution	2.48×10^{-3}	0.1-100

Table S2 Contrast of fluorescence lifetime of **Cy-KAA** with the addition of Van

	Cy-KAA (in water)	**Cy-KAA + Van (in water)**	**Cy-KAA (in 10％ serum)**	**Cy-KAA + Van (in 10％ serum)**
τ (ns)	2.2820	2.4217	3.2756	2.3118