Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supporting information

An efficient detection of bilirubin in human serum through displacement approach

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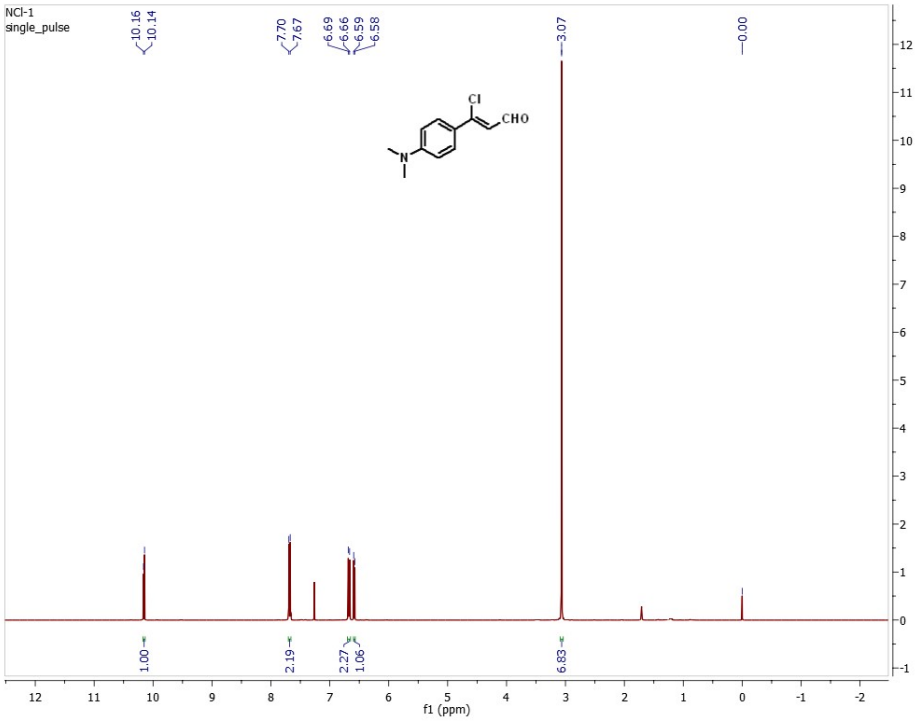


Figure S1: [1]HNMR spectrum of **NCl-1**

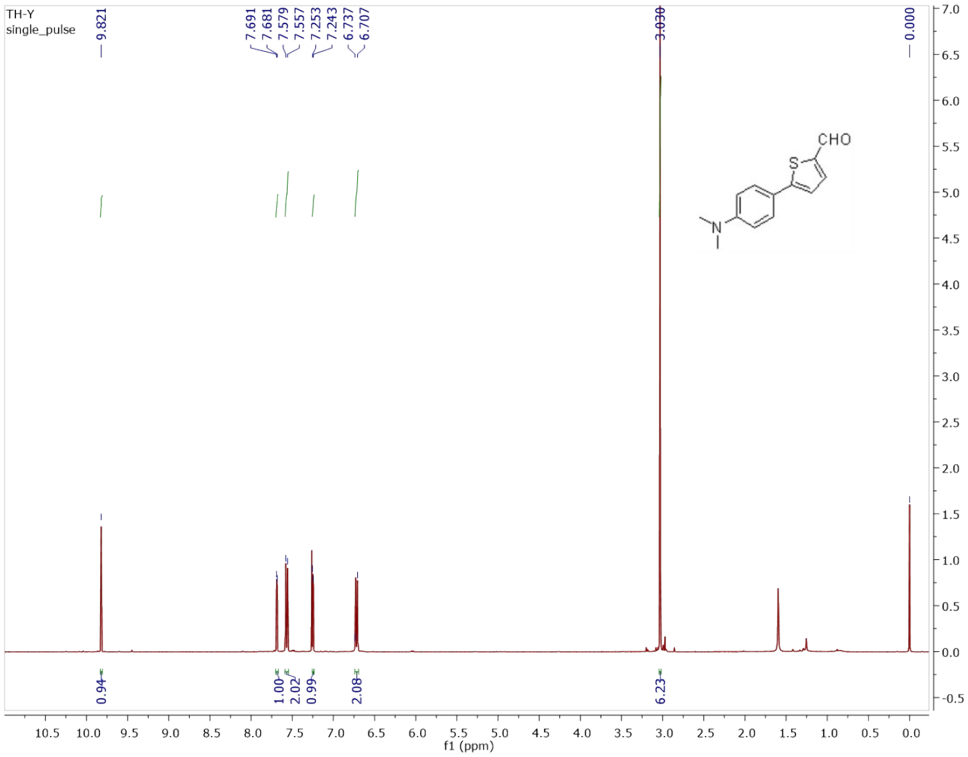


Figure S2: [1]HNMR spectrum of **TH-Y**

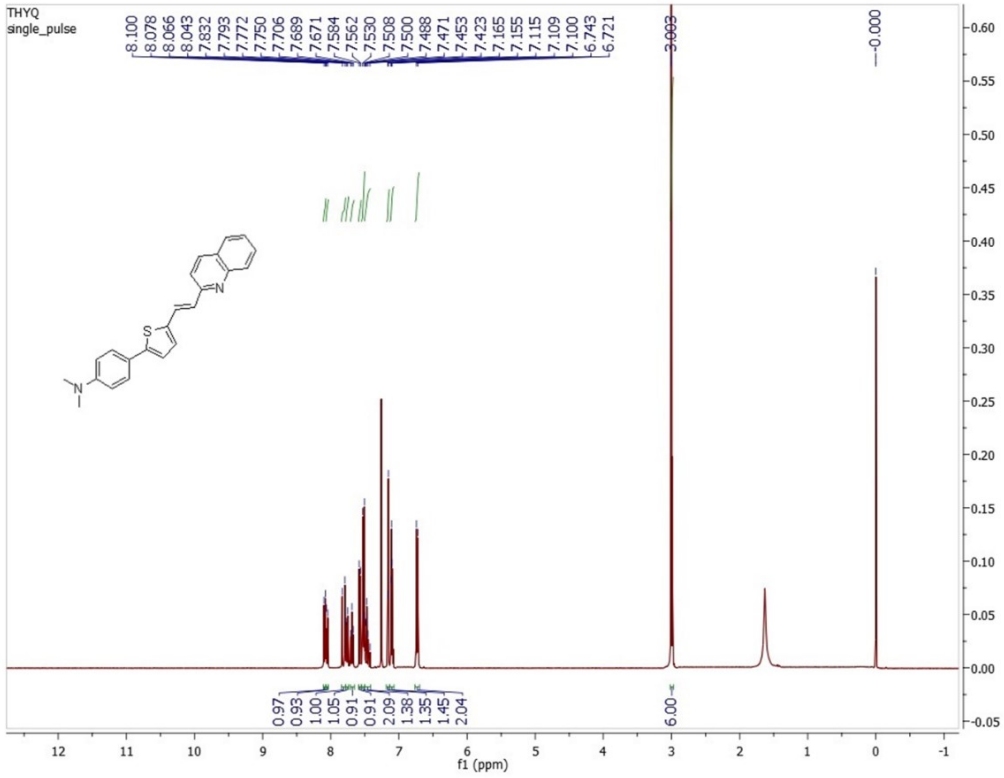


Figure S3: [1]HNMR spectrum of **THYQ**

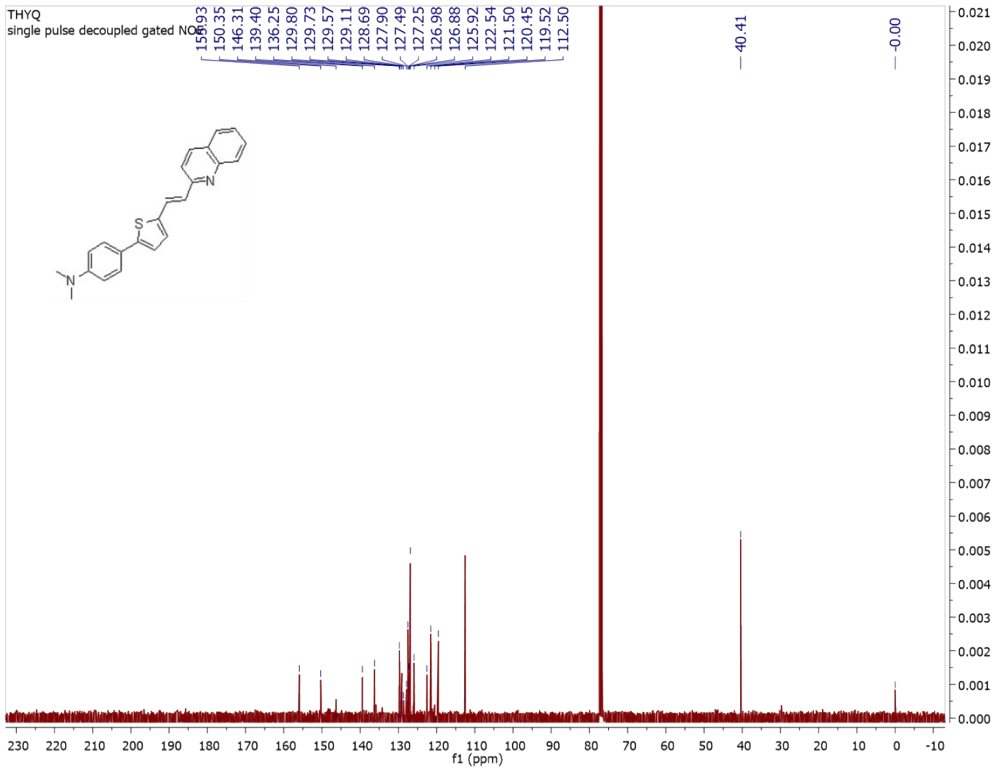


Figure S4: [13]CNMR spectrum of **THYQ**

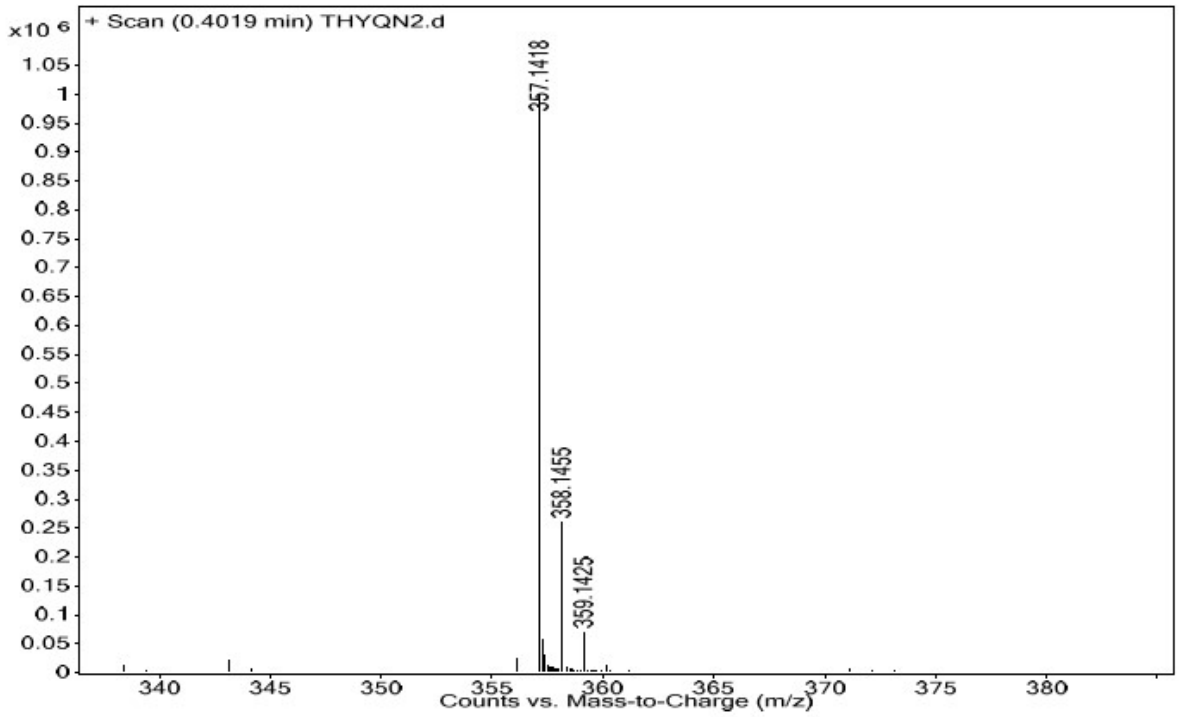


Figure S5: HRMS spectrum of **THYQ**

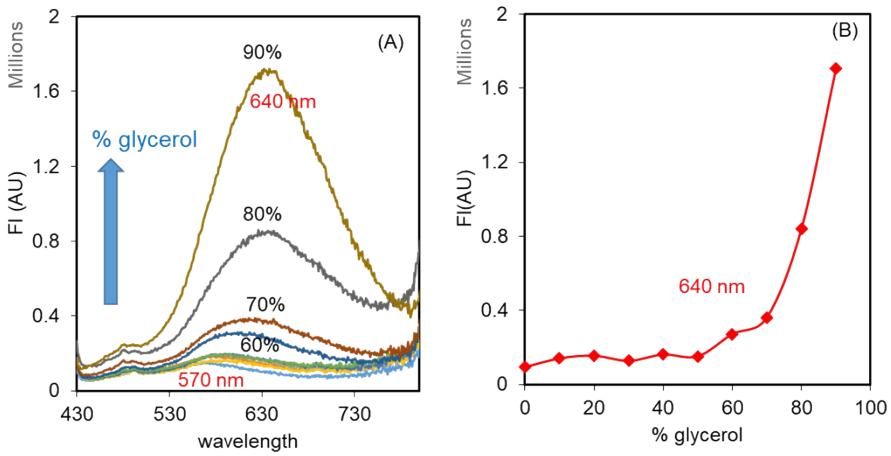


Figure S6: (A) Emission spectra of **THYQ** in water- glycerol binary mixtures (λ_{ex} 420 nm); (B) Line plot of fluorescence intensity at 640 nm v/s the fraction of glycerol

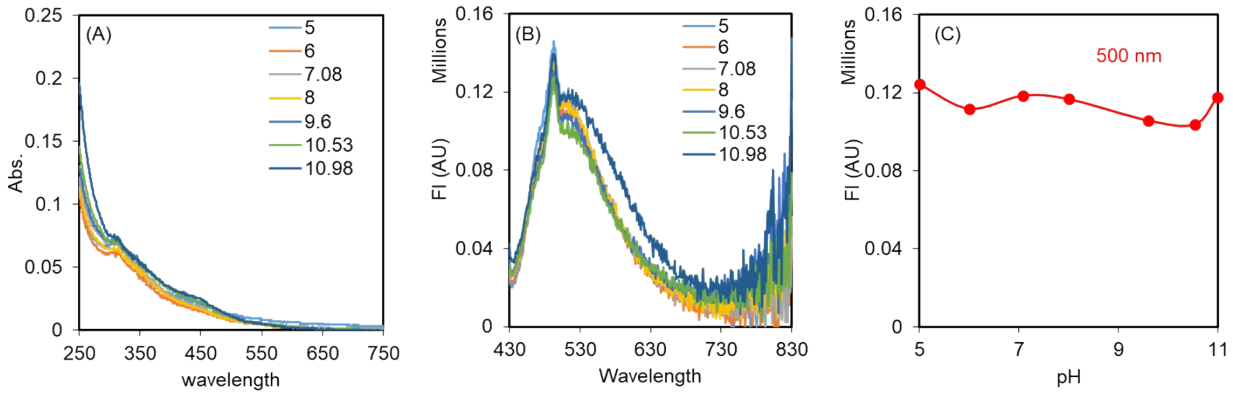


Figure S7: (A) Absorbance and (B) Emission spectra of **THYQ** with the change in pH; (C) The plot of the emission maxima at 500 nm v/s pH

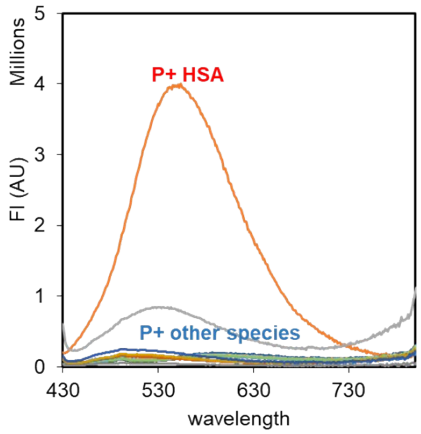


Figure S8: The fluorescence spectra of THYQ (10 μM, HEPES buffer, pH 7.4) in the presence of 5 equivalents each of HSA, anions, thiols, amines and amino acids

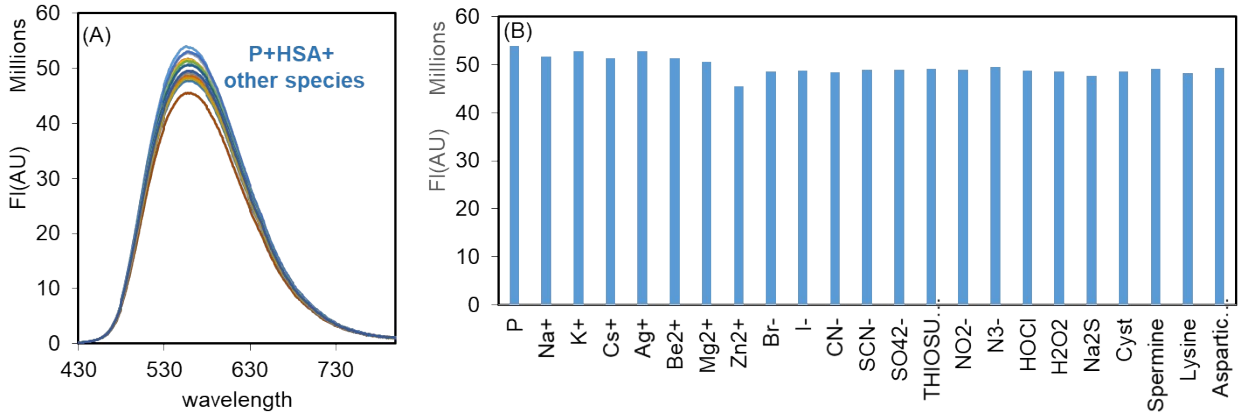


Figure S9: (A) The fluorescence spectra of **THYQ-HSA** complex; (B) Bar graph representing the FI at 555 nm in presence of 5 equivalents each of cations, anions, thiols, amines and amino acids

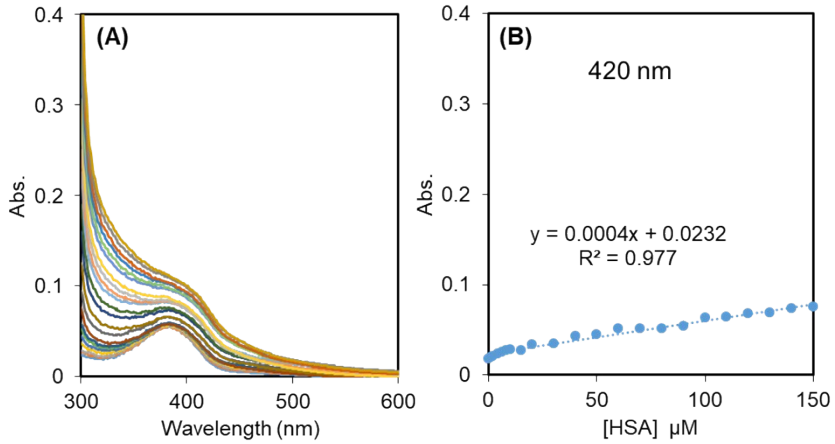


Figure S10: Change in UV-Vis spectrum of THYQ (10 µM, HEPES buffer) on addition of aliquots of HSA

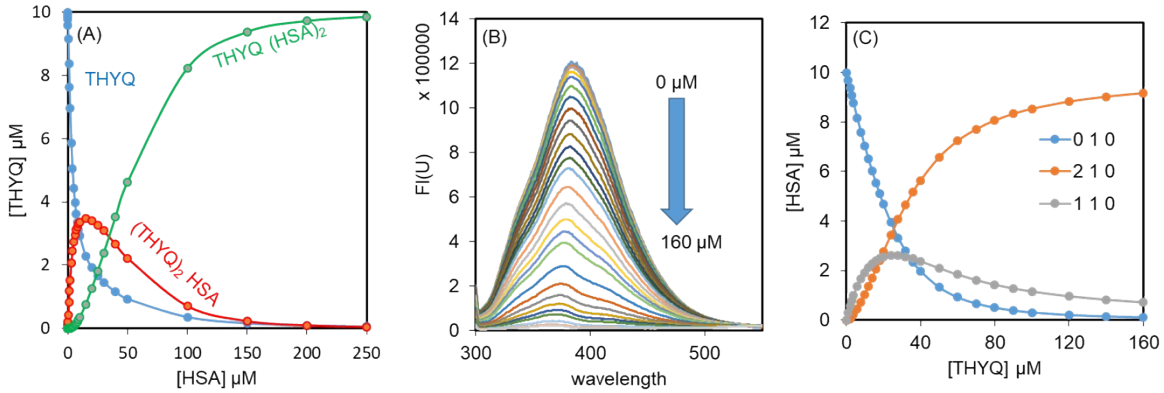


Figure S11: (A) The distribution of different species of complexes of **THYQ** with HSA; (B) The change in emission spectrum of HSA at 345 nm on successive addition of aliquots of probe **THYQ** (λ_{ex} 290 nm); (C) SPECFIT graph showing the distribution of different species of complexes of HSA with THYQ

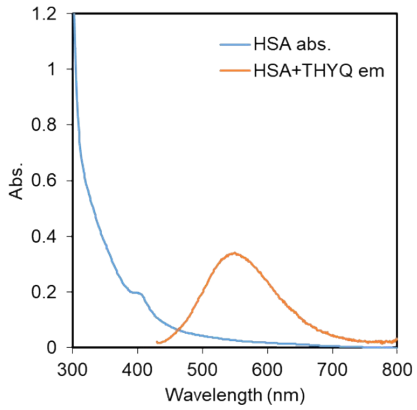


Figure S12: Possibility of FRET mechanism for Bilirubin (BR) detection

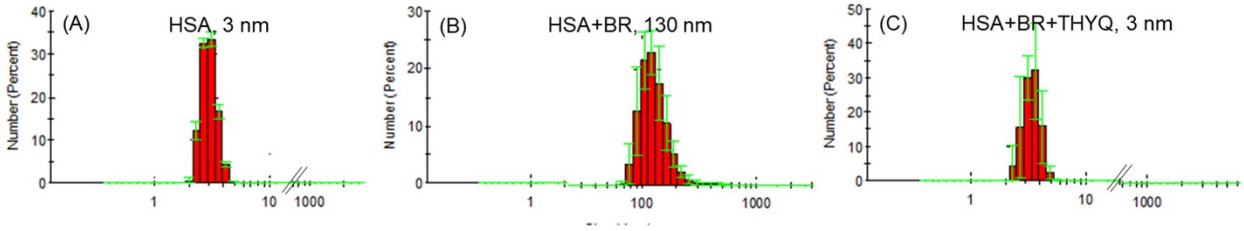


Figure S13: The DLS spectra of THYQ /HSA in the presence of BR

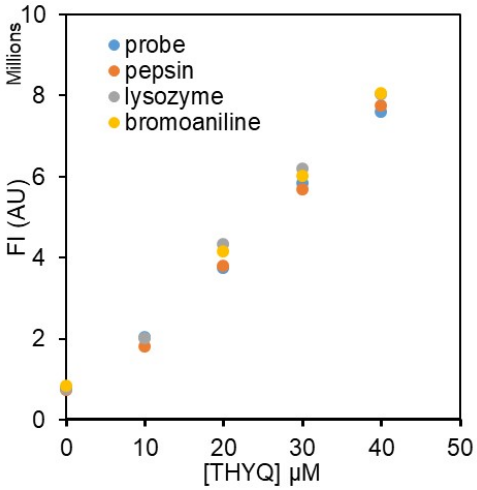


Figure S14: Effect of other proteins in the quantification of BR

Table S1. Photophysical properties of **THYQ** (1µM) in solvents of varied polarities (E_T^{30} Kcal/mol)

Solvent	**E_T^{30} (Kcal/mol)**	**$\lambda_{Abs.}$ Max (nm)**	**$\lambda_{Em.}$ Max (nm)**	**Є (M^{-1} cm^{-1})**	**Φ (At 1 µM)**
cyclohexane	30.9	414	510	11,900	18.38
toulene	33.9	427	527	17,100	20.34
Diethyl ether	34.5	416	538	21,900	29.61
THF	37.4	424	572	25,500	88.12
Ethyl acetate	38.1	415	570	24,700	41.76
CHCl3	39.1	424	555	20,200	---
DCM	40.7	422	581	28,100	52.31
acetone	42.2	427	594	27,800	84.92
DMF	43.2	435	610	29,900	70.87
DMSO	45.1	420,546	622	21,400 and 6,000	67.84
ACN	45.6	421,536	612	19,400 and 13,600	53.18

ethanol	51.9	430,537	626	19,000 and 15,300	7.70
methanol	55.4	---	636	---	7.70
Water	63.1	Broad band	weak band	6100	-

Table S2: Comparison of slopes of E_T^{30} value v/s Emission maxima of **THYQ** with literature reports

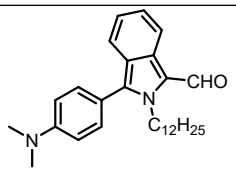
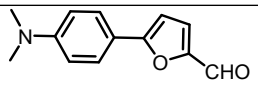
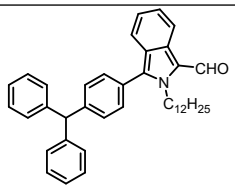
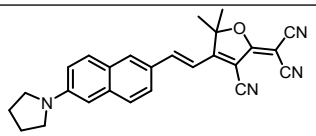
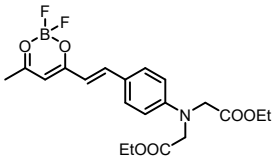
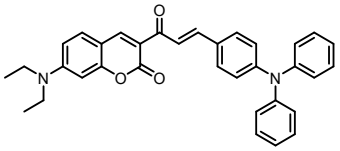
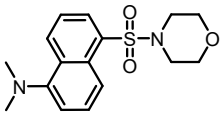
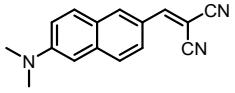
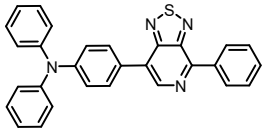
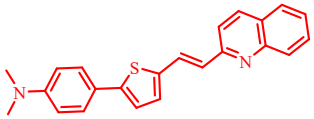
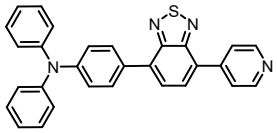
S. No.	**Ref**	**Structure**	**Slope nm /E_T^{30}**	**S. No.**	**Ref**	**Structure**	**Slope nm /E_T^{30}**
1	Ref 1		2.7	**7**	Ref 6		5.7
2	Ref 1		4.6	**8**	Ref 7		7.7
3	Ref 2		3.98	**9**	Ref 8		10.03
4	Ref 3		4.51	**10**	Ref 9		6.14
5	Ref 4		9.32	**11**	**This Work**		7.88
6	Ref 5		9.46				

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Table S3: Comparison of performance of various fluorescence based systems for the detection of bilirubin

Sr. No	**Fluorescence System**	**pH**	**FL ON/OFF**	**Linear range**	**LOD**	**Clinical detection Spiking /direct**	**[Ref], Year**
Organic molecule based probes							
1.	Displacement approach	7.4	**"ON"**	2-80 µM	68 nM	direct	This Work
2.	Imine based	7.4	**"OFF"**	1 pM-500 µM	2.8 pM	spiking	1, 2017
3.	Probe+Fe^{3+} mixture used	7.2	**"ON"**	0–10 µM	76 nM	direct	2, 2020
4.	Probe+Fe^{3+} mixture used	7.4	**"ON"**	0–10 µM	33 nM	direct	3, 2021
5.	Amphiphilic molecule	6.5	**"OFF"**	0–32 µM	330 nM	spiking	4, 2023
6.	imidazole derivative	7.4	**"OFF"**	0–60 µM	11.74 µM	spiking	5, 2023
Nano-materials							
7.	HSA stabilized gold nanoclusters	7.4	**"OFF"**	1–50 µM	248 nM	spiking	6, 2014
8.	Peroxidase method	8.0	**"OFF"**	0.03-5.0 µM	10 nM	spiking	7, 2018
9.	S,N-doped carbon dots + Fe^{3+}	2.5	**"ON"**	0.2 -2.0 nM	0.12 nM	spiking	8, 2018
10.	BSA stabilized copper nanocluster + Fe^{3+}	11.4	**"ON"**	0-70 µM	6.62 nM	spiking	9, 2018
11.	L-cysteine capped Mn doped ZnS_2 quantum dots	4.7	**"OFF"**	10.99 - 63.84 µM	1800 nM	spiking	10, 2019
12.	N doped carbon dots	7.4	**"OFF"**	0-45 µM	89 nM	spiking	11, 2021
13.	glutathione capped copper nanoclusters	ND	**"OFF"**	9-800 µM	148 nM	*ND	[12], 2021
14.	TPE hydrogel	7.4	**"OFF"**	0-8 µM	25 nM	*ND	[13], 2022
15.	competitive binding of Cu^{2+}	5.0	**"ON"**	0.7–3.6 µM	85 nM	spiking	[14], 2022

*ND = not determined

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