Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Fluorescence enhancement and p*Ka* shift of a Rho kinase inhibitor by a synthetic receptor

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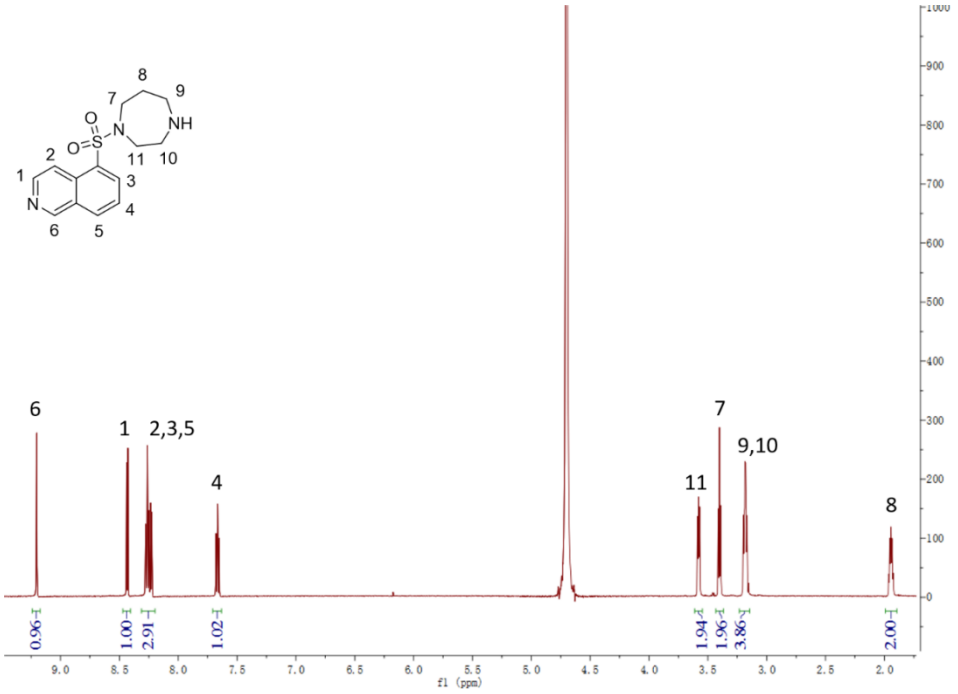


Fig. S1 ^{1}H NMR spectrum of FSD in D_2O.

Details of DFT calculations.

The inclusion complexes (different heights of the guest included in CB[7]) were first constructed using Chem3D before calculations by energy-minimization using DFT (B3LYP/6-31G(d)) thanks to the Gaussian09 Rev.D01 package program. A water continuum (CPCM) model was used to account as best as possible of the solvent in a compromise approach between calculation time and efficiency with respect to a discrete model for water molecules. Frequency calculations were performed to check for true minimas and BSSE corrections applied to account for possible basis set superposition errors.

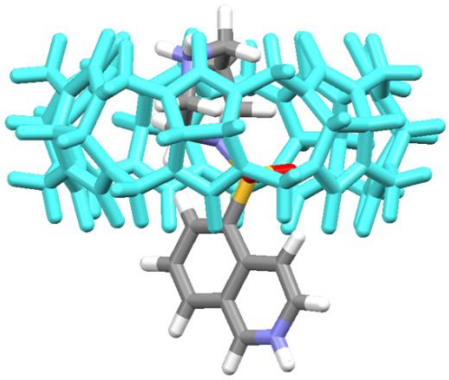
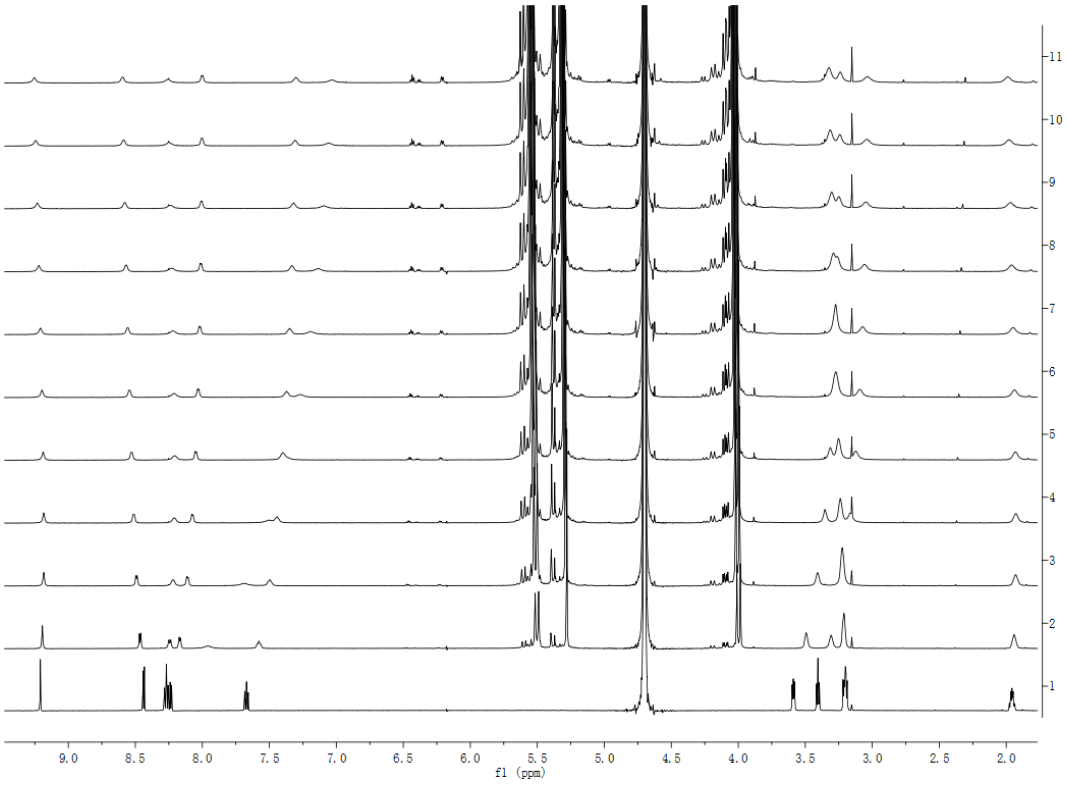
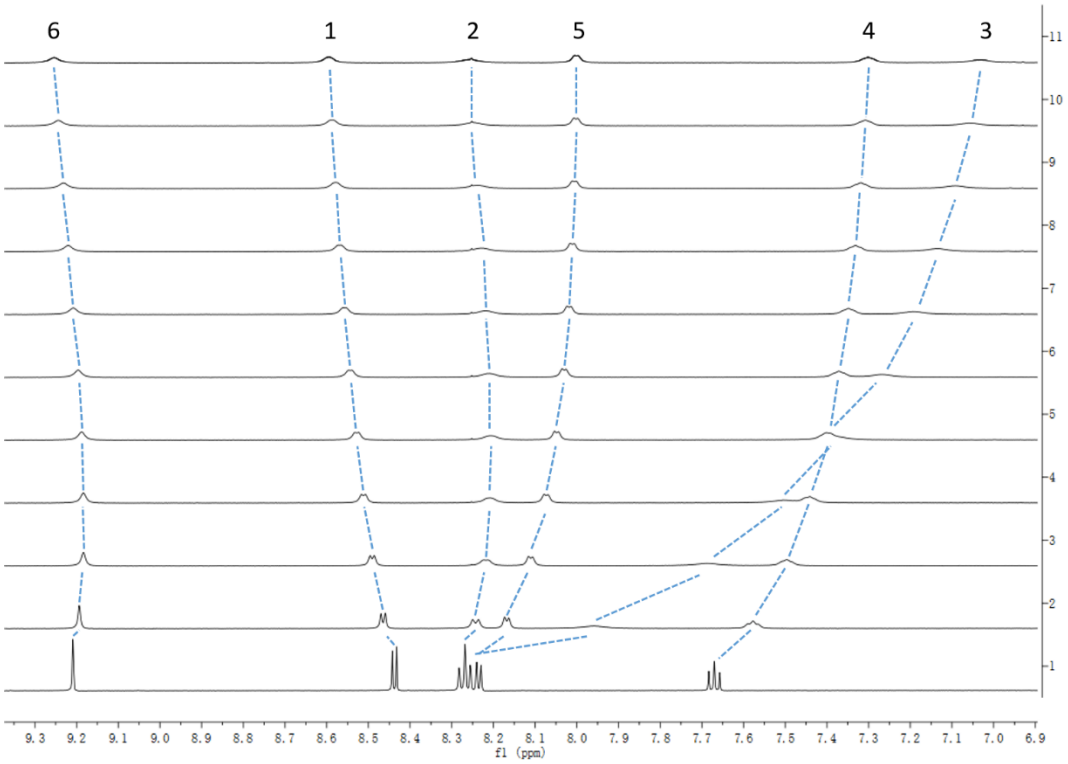


Fig. S2 Molecular modeling of $(FSD\text{-}1H)^{+}$@CB[7] calculated by Density Functional Theory (DFT, B3LYP/6-31G(d)).



(a)



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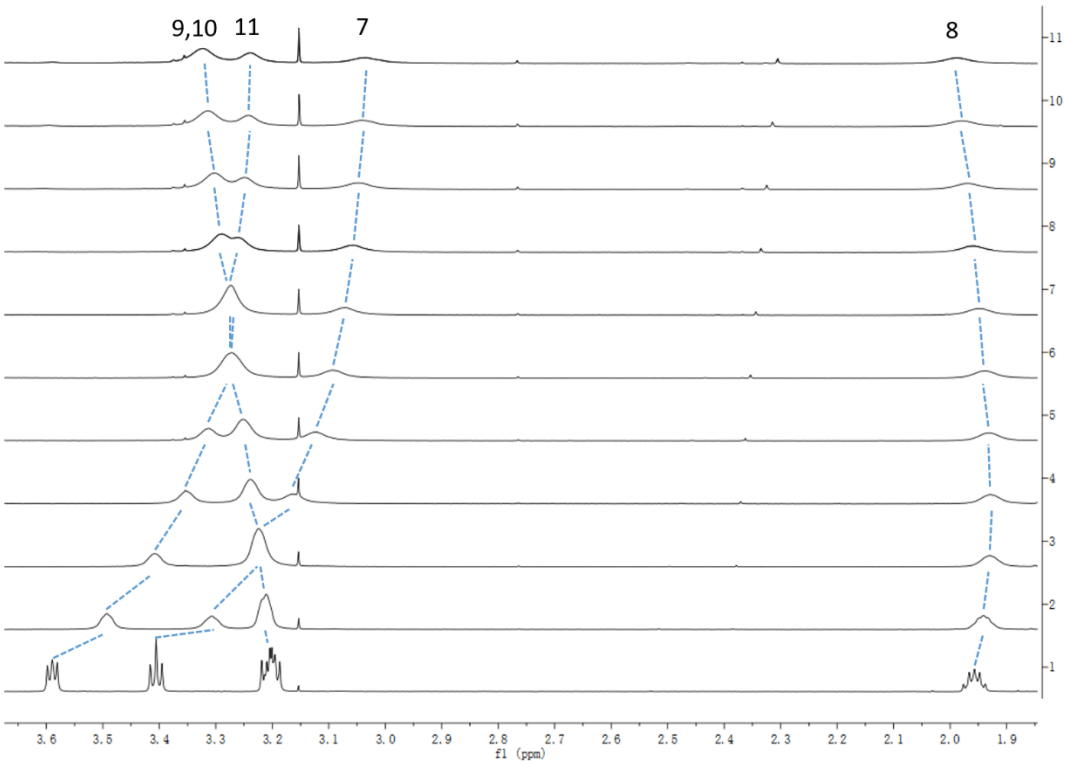


(c)

Fig. S3 ^{1}H NMR stack spectra of NMR titration (with increasing CB[7] concentrations from bottom to top) of FSD@CB[7] (a), downfield of stack spectra (b) and upfield of stack spectra (c).

Table S1. Binding affinities of FSD@CB[7] calculated by each proton according to the ^{1}H NMR stack spectra of NMR titration.

Proton	K_a (M^{-1})
H(1)	483.7±34.9
H(2)	NA
H(3)	1098.4±55.3
H(4)	1165.8±34.2
H(5)	1674.5±99.7
H(6)	NA
H(7)	1358.8±83.9
H(8)	NA
H(9)	147.8±27.2
H(10)	147.8±27.2
H(11)	1647.7±156.3

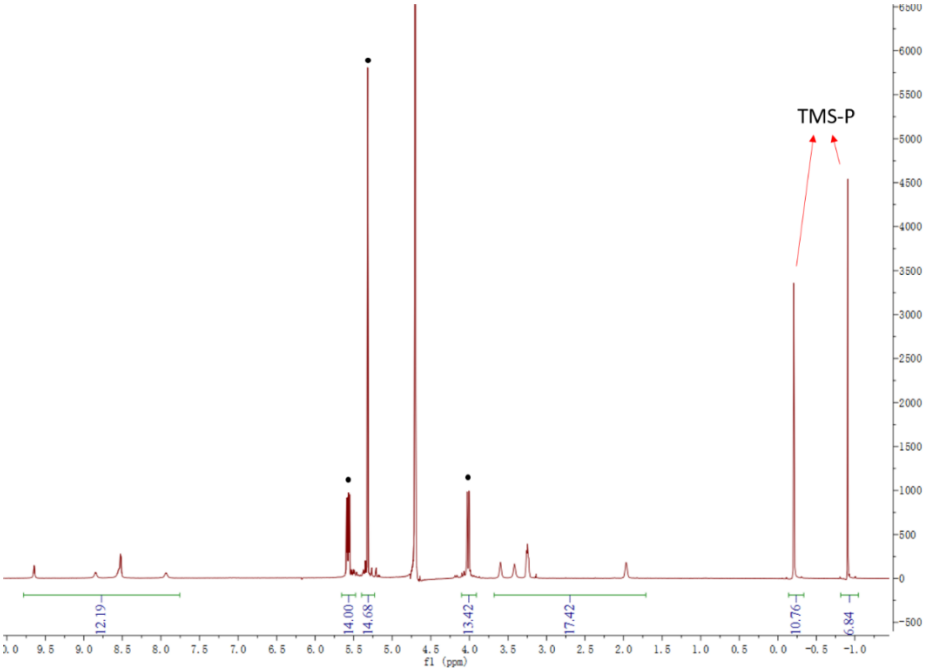


Fig. S4 ^{1}H NMR spectrum of competitive binding between FSD and TMS-P to CB[7] in acidic condition (p*D* = 2.0).

Binding constant of {FSD•CB[7]}

$[TMSP]_{bound}$ = 6.84/9 = 0.76 mM;

$[TMSP]_{free}$ = 10.76/9 = 1.20 mM;

$[FSD]_{total}$ = 29.61/16 = 1.85 mM;

$[CB[7]]_{total}$ = 1 mM;

Therefore, $[FSD]_{bound} = [CB[7]]_{total} - [TMSP]_{bound}$ = 1 – 0.76 = 0.24 mM;

$[FSD]_{free}$ = 1.85 - 0.24 mM = 1.61 mM;

As $K_{FSD•CB[7]}/K_{TMSP•CB[7]} = [FSD•CB[7]]_{complex} \times [TMSP]_{free}/([TMSP•CB[7]]_{complex} \times [FSD]_{free})$ = 0.24 × 1.20/(0.76 × 1.61) = 0.235 and $K_{TMSP•CB[7]} = (1.82 \pm 0.22) \times 10^7\ M^{-1}$;

Therefore, $K_{IC8•CB[7]} = (4.28\pm0.21) \times 10^6\ M^{-1}$.

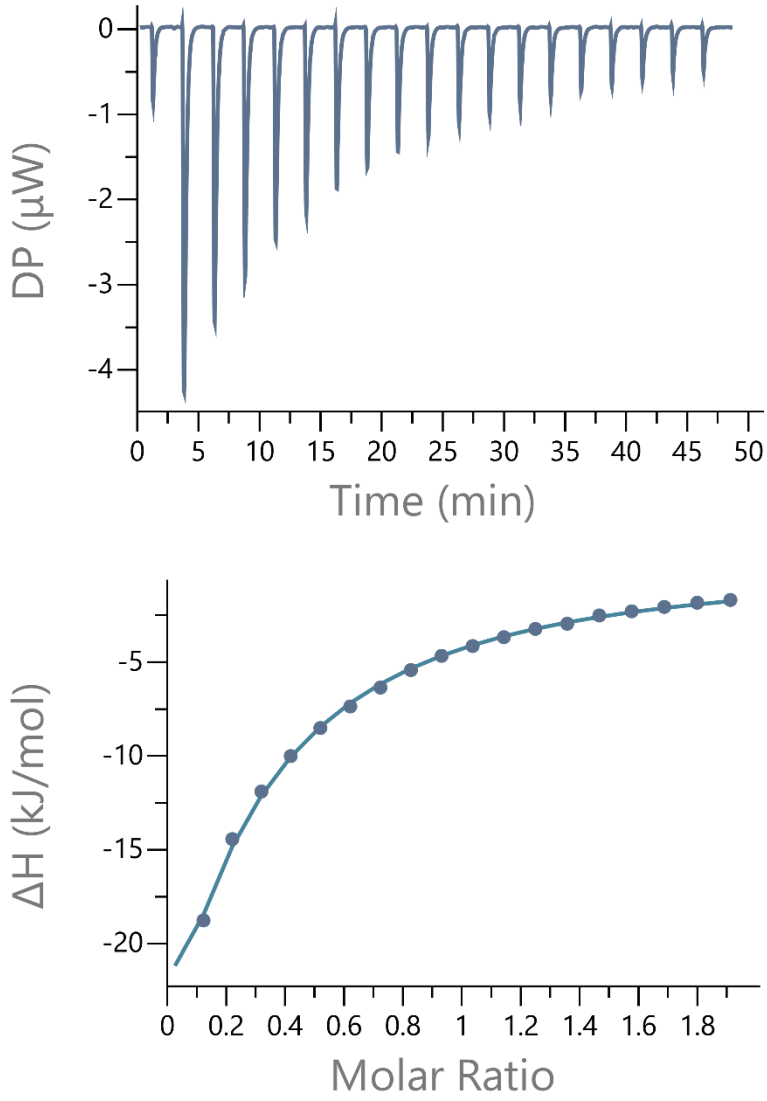


Fig. S5 Raw plot and integrated heat plot of FSD at syringe with CB[7] at cell in neutral condition (pH = 7.0).

Table S2. ITC results of FSD with CB[7] in neutral condition (pH = 7.0).

Syringe	Cell	[Syr] (M)	[Cell] (M)	K_a (M^{-1})	ΔH (kJ/mol)	ΔG (kJ/mol)	$T\Delta S$ (kJ/mol)
CB[7]	FSD	2×10^{-3}	1×10^{-4}	$6.62(\pm1.22)\times10^{3}$	-335	-21.8	-313
FSD	CB[7]	2×10^{-3}	2×10^{-4}	$6.66(\pm1.62)\times10^{3}$	-335	-21.9	-313

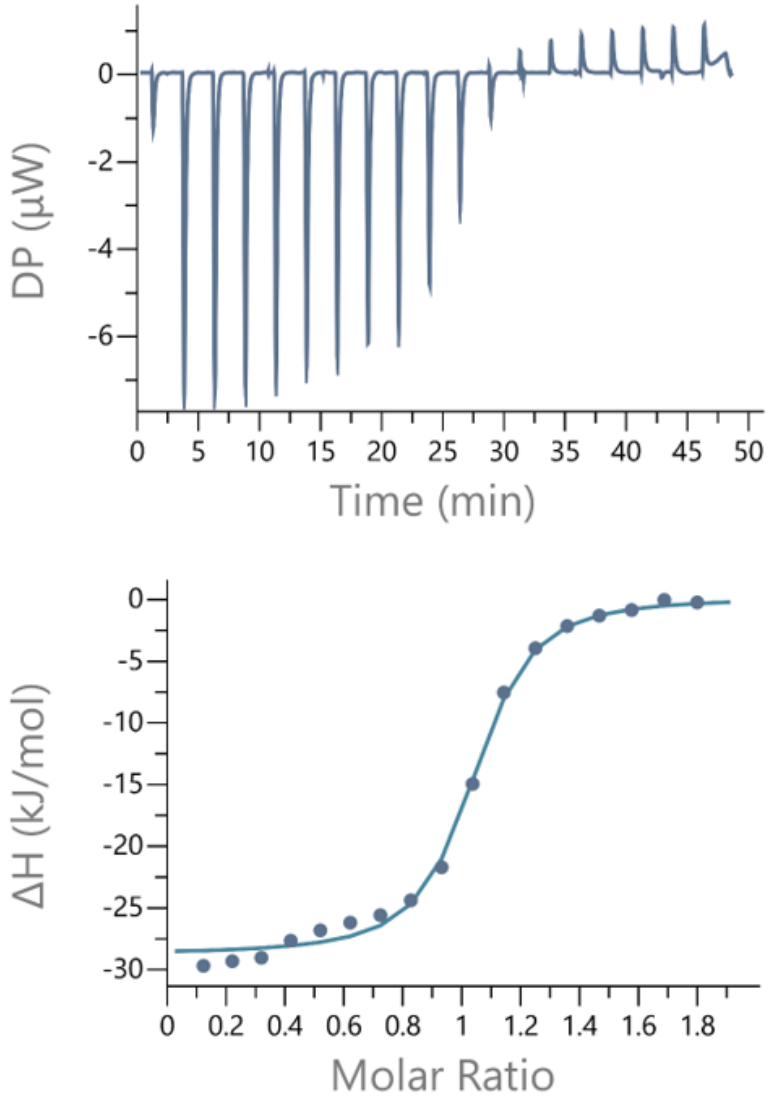
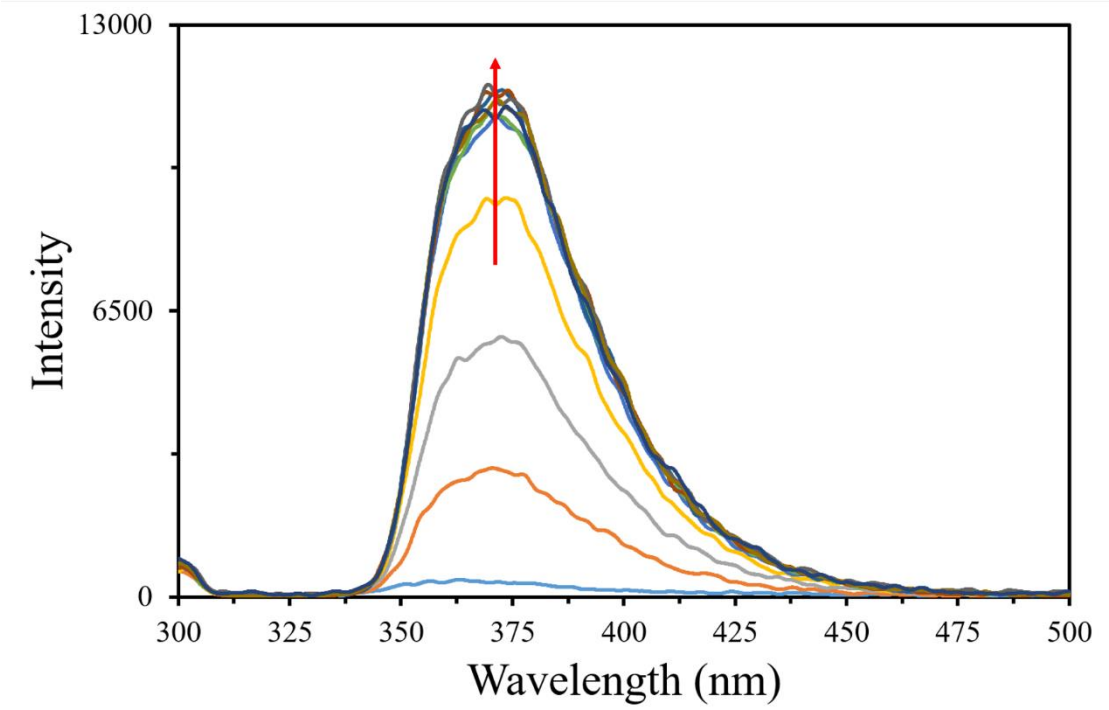


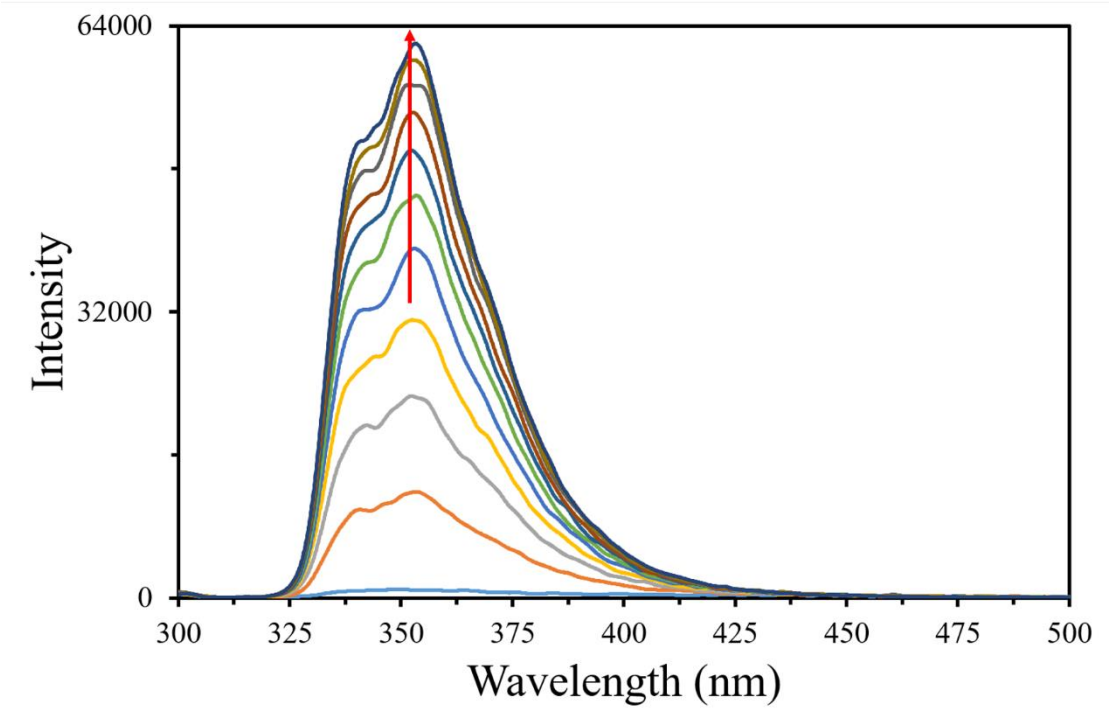
Fig. S6 Raw plot and integrated heat plot of CB[7] at syringe with FSD at cell in neutral condition (pH = 2.0).

Table S3. ITC results of FSD with CB[7] in acidic condition (pH = 2.0).

Syringe	Cell	[Syr] (M)	[Cell] (M)	K_a (M^{-1})	ΔH (kJ/mol)	ΔG (kJ/mol)	$T\Delta S$ (kJ/mol)
CB[7]	FSD	2×10^{-3}	2×10^{-4}	$5.26(\pm0.73)\times10^{5}$	-28.9	-32.7	3.74
FSD	CB[7]	1×10^{-3}	8×10^{-5}	$1.61(\pm0.11)\times10^{6}$	-35.2	-35.5	0.268



(a)



(b)

Fig. S7 Fluorescence spectrum of FSD in the absence and in the presence of CB[7] (up to 3.0 equivalent) in acidic condition (a) and in neutral condition (b).