Supporting information for

A "Turn On" Fluorescent Probe for Heparin and Its Oversulfated Chondroitin Sulfate Contaminant

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Contents

Experimental Section

Reagents and instruments. Commercially available solvents and reagents were used as received. Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Val-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH, 1-hydroxybenzotriazole (HOBt), N,N′-diisopropylcarbodiimide, and Rink amide AM were purchased from TianJin NanKai HeCheng. Heparin (Hep) sodium salt from hog intestine was from TCI (Shanghai) Development Co., Ltd. The molecular weight of Hep was defined as 644.2 g/mol according to the major repeating disaccharide unit with sodium as counter ions.[1] Chondroitin sulfate sodium salt from bovine trachea, hyaluronic acid sodium salt from *Streptococcus equi*, and heparinases I, II, III from *Flavobacterium heparinum* were purchased from Sigma-Aldrich. Deuterated solvents for NMR measurements were available from Aldrich. DNA (GTTTCGCCACCTCTGACTTG) and RNA (GUUUCGCCACCUCUGACUUGTT) were purchased from Sangon Biotech (Shanghai). Fluorescence measurements were performed on a HITACHI F-4600 fluorescence spectrophotometer. ^{1}H NMR spectra were obtained using a Bruker AM300 spectrometer with tetramethylsilane (TMS) as internal standard. High resolution mass spectrum (HRMS) of tetraphenylethene fluorogen was measured on a LTQ Orbitrap XL spectrometer, while mass spectrum of **TPE-1** was measured using SHIMADZU LCMS-2020.

Fluorescence measurements. The fluorescence emission spectra of **TPE-1** (10 μM or 1 μM) were measured at 25 °C in mixed solutions of HEPES buffer and DMSO, with excitation wavelength at 340 nm. The slit width was 5 nm, and the PMT voltage was 700 V. Hep samples were prepared in water.

Heparinase Treatment. For the heparinase treatments, cocktail solution of heparinases I, II and III was prepared before use. Stock solutions of heparinases I, II and III were prepared at concentrations of 250 U/mL, 50 U/mL and 25 U/mL. Enzyme cocktail solution was obtained by mixing stock solutions of heparinases I, II and III in a ratio of 1:5:10 (v:v:v), and then diluted for 10 times (~4.7 U/mL total heparinase). Heparinase treatment experiments were performed at 37 °C.

Synthesis of TPE-1. The tetraphenylethene fluorogen, 4-(1,2,2-triphenylethenyl)-benzoic acid (TPE-COOH), was synthesized according to the literature method and fully characterized by ^{1}H NMR and HRMS (Figs. S1 and S2).[2] The tetraphenylethene-peptide conjugate **TPE-1** was synthesized by solid phase peptide synthesis using Fmoc chemistry. The deprotection and cleavage procedures were achieved by treatment with a mixture of TFA/thioanisole/3,6-dioxa-1,8-octanedithiol/H_2O at room temperature overnight. After cleavage from resin, **TPE-1** was purified by preparative-HPLC with a final yield of 33%. The purity of the obtained **TPE-1** was determined to be 99.7% using an analytical HPLC system on a C_{18} column (Fig. S3). MS characterization of **TPE-1** shows a peak at 619.35 $[M+3H]^+$, which matches the calculated value 619.03 very well, indicating the successful synthesis of the product (Fig. S4).

Tables

Table S1. Recently reported detection methods for heparin.

Method	Probes	Response type	Dynamic range	LOD	Ref.
Colorimetric	Au NPs (positively-charged)	Red to blue color change	0.09-3.12 μg/mL	0.03 μg/mL	3
	Au NPs (protamine-coated)	Blue to red color change	0.6-10 μg/mL	0.6 μg/mL	4
	Au NPs/GO/protamine	Red color	0.06-0.36 μg/mL	3 ng/mL	5
	Au NRs/GO/protamine	Turn off	0.02-0.28 μg/mL	5 ng/mL	6
	Cationic polythiophene	Yellow to orange color change	0-56 μM	0.08 μM	7
	Phloxine B/PEI	Purple to red color change	0.01-0.1 U/mL	0.005 U/mL	8
	Mallard blue	Turn off	0-20 μM	N.A.	9
	Tripodal boronic acid/pyrocatechol violet	Turn on	N.A.	N.A.	10
	Polymethinium	Ratiometric	0-64 μM	N.A.	11
Electrochemical	Polyimidazolium	DPV	0.5-10 μM	N.A.	12
	Protamine in organic membrane	Potentiometric	0.01-0.4 U/mL	0.005 U/mL	13
Fluorescent	Silole (silacyclopentadiene) (AIE)	Turn on	23 nM-13 μM	23 nM	14
	Anthracene/TPE	Ratiometric	0-15 μM	20 nM	15
	TPE derivative /GO	Turn on	0-13.2μM	10 nM	16
	Phloxine B/PEI	Turn on	0.05-0.3 U/mL	0.003 U/mL	8
	Pyrene derivative/GO	Turn on	0-1.76 U/mL	0.046 U/mL	17
	Pyrene derivative	Turn on	0.08-1.6 μM	30 nM	18
	Cationic PFBTs	Ratiometric	30 nM-48 μM	30 nM	19
	Si NPs	Turn on	N.A.	2 μM	20
	Alkynylplatinum terpyridyl complex	Turn on	0-8.7 μM		21
	Salicylaldehyde azine derivative (AIE)	Turn on	0.2-14 μg/mL	57.6 ng/mL	22
	Tripodal boronic acid	Turn off	0-3.6 μM	N.A.	23
	Heparin Orange	Turn on	0.1-10 μM	N.A.	24
	Heparin Blue	Turn on	0.1-10 μM	N.A.	24
	FITC-labled protamine	Turn off	0-0.65 U/mL	N.A.	25
	Conjugated polyelectrolyte with glucosamine hydrochloride clusters	Turn off	0-1 μM	0.1 μM	26
	Cationic PFBTs	Turn on	0-72 μM	N.A.	27
	Chromophor-tethered copolymer	Turn off	30 nM-0.22 μM	30 nM	28

Phosphorescent	Mn-doped ZnS quantum dots	Turn on	0-70 μM	0.05 μM	29

Abbreviations: AIE, aggregation induced emission; DPV, differential pulse voltammogram; FITC, fluorescein-4-isothiocyanate; GO, graphene oxide; N.A., not applicable; PEI, polythyleneimine; PFBTs, poly(fluorene-alt-benzothiadiazole)s; Ref., reference; TPE, tetraphenylethene.

Table S2. Recently reported detection methods for OSCS.

Methods	Probes	Response type	LOD (w/w)	Ref.
Fluorescent	Adenosine-repeated molecular beacon	Turn on	0.01%	30
	Gold-heparin-dye	Turn off	10^{-9}%	31
	Polymer-H	Turn on	0.5%	32
	Polymer-H/aXa	Turn on	0.5%	33
NMR	N.A.	N.A.	0.1%	34
	N.A.	N.A.	0.5%	35
	N.A.	N.A.	0.25%	36
SAX-HPLC	N.A.	N.A.	0.03%	37
	N.A.	N.A.	0.02%	38
WAX-HPLC	N.A.	N.A.	0.025%	39
HPLC	N.A.	N.A.	0.06%	40
Capillary electrophoresis	N.A.	N.A.	0.05%	41
	N.A.	N.A.	1%	42
	N.A.	N.A.	0.1%	43
HILIC-MS EIC	N.A.	N.A.	0.1%	44
Pyrolysis MS	N.A.	N.A.	0.1%	45
Raman spectroscopy	N.A.	N.A.	1%	46
Colorimetric microplate	Cationic polythiophene polymer	N.A.	0.003%	47
Heparin enzyme immunoassay	N.A.	N.A.	0.1%	48
Electrochemical	TDMA-doped PVC membrane modified electrode	N.A.	0.005%	49
	TDMA membrane modified electrode	N.A.	0.5%	50
Colorimetric microplate	LPTP/heparinase	N.A.	0.03%	51
Electrokinetic chromatography	N.A.	N.A.	0.07%	52
Taq pol inhibition	N.A.	N.A.	0.07%	53

Abbreviations: EIC, extract ion chromatography; MS, mass spectroscopy; LPTP, 3-(2-(N-(N'-methylimidazole))ethoxy)-4-methylthiophene; N.A., not applicable; PVC, poly(vinyl chloride); SAX, strong anion-exchange column; TDMA, tridodecylmethylammonium; WAX, weak anion-exchange column.

Figures

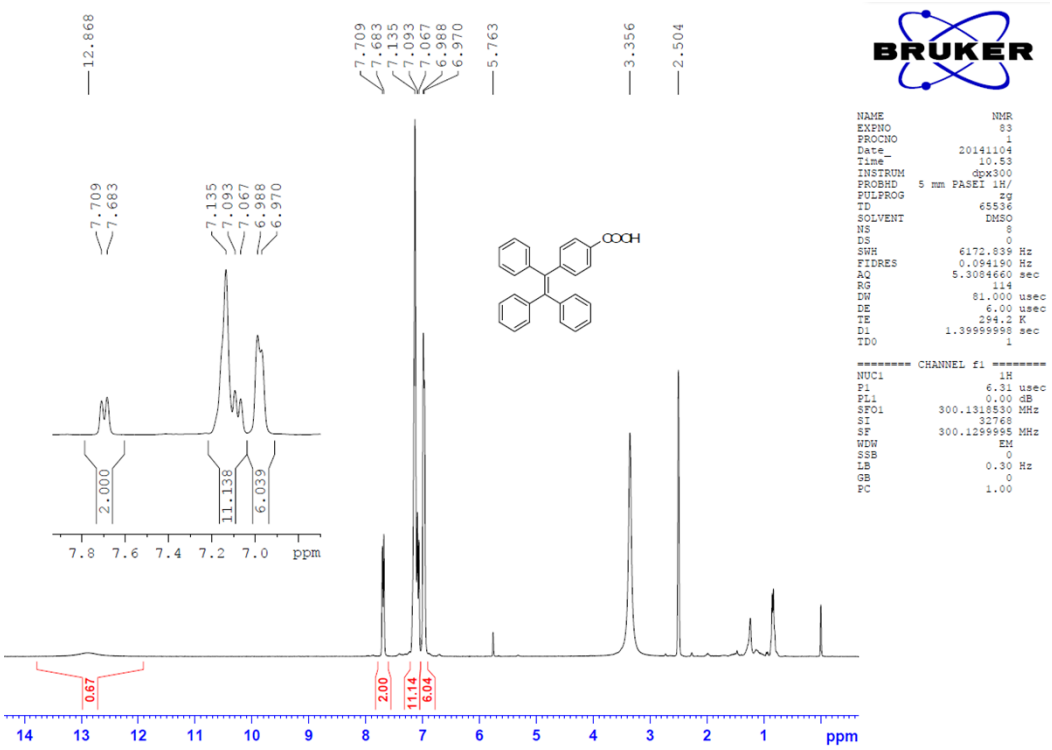


Figure S1. ^{1}H NMR spectrum of TPE-COOH in DMSO-d6.

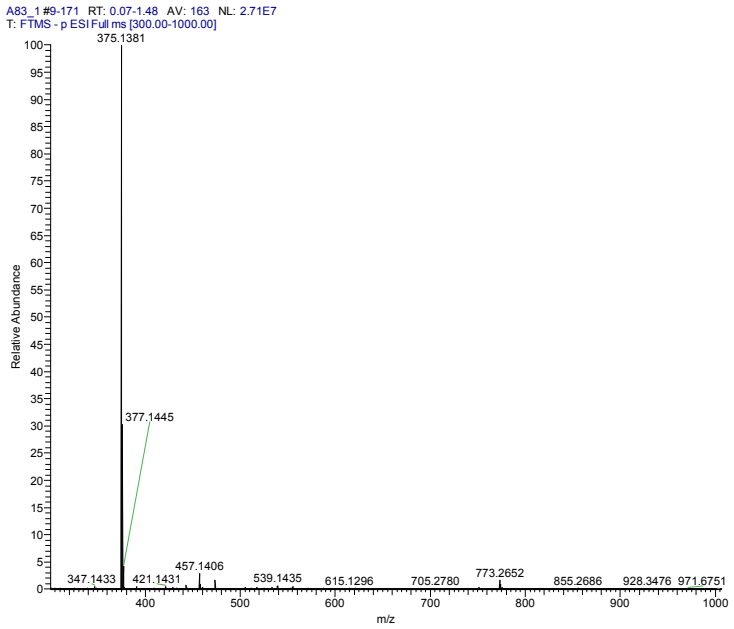
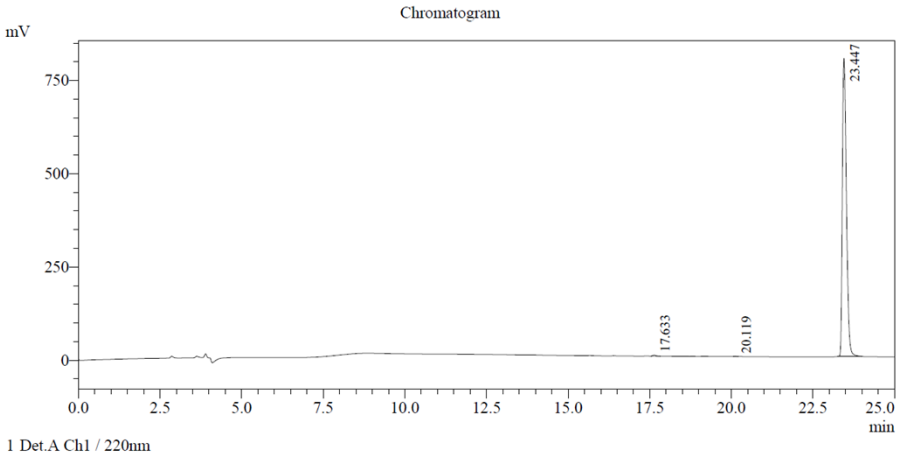


Figure S2. HRMS of TPE-COOH, calculated for $C_{27}H_{19}O_2$ ($[M-H]^-$): 375.1391, observed 375.1381.



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	17.633	15201	2604	0.218
2	20.119	2957	587	0.042
3	23.447	6940901	799104	99.739
Total		6959058	802295	100.000

Figure S3. HPLC profile of **TPE-1**.

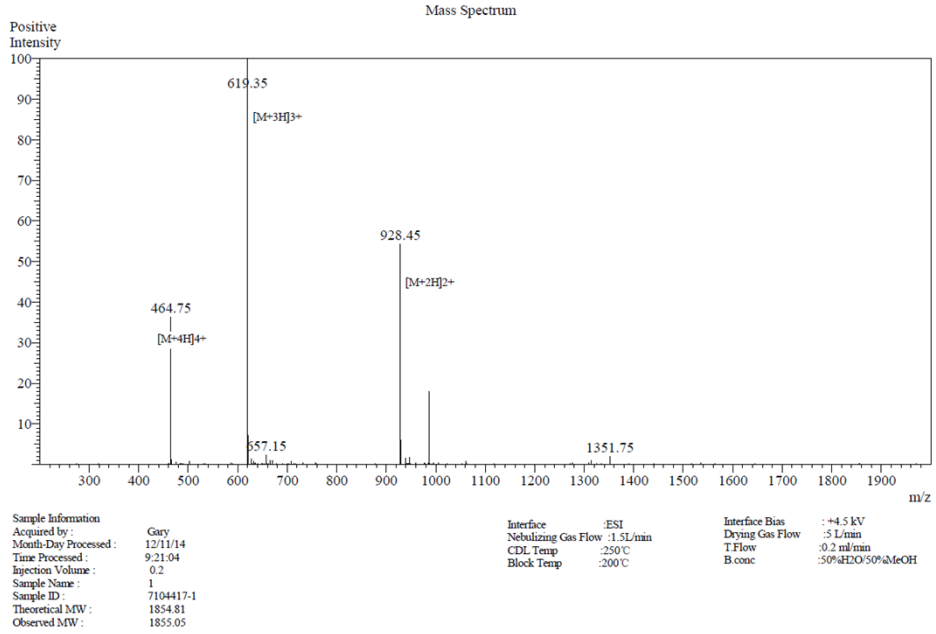


Figure S4. ESI-mass spectrum of **TPE-1**.

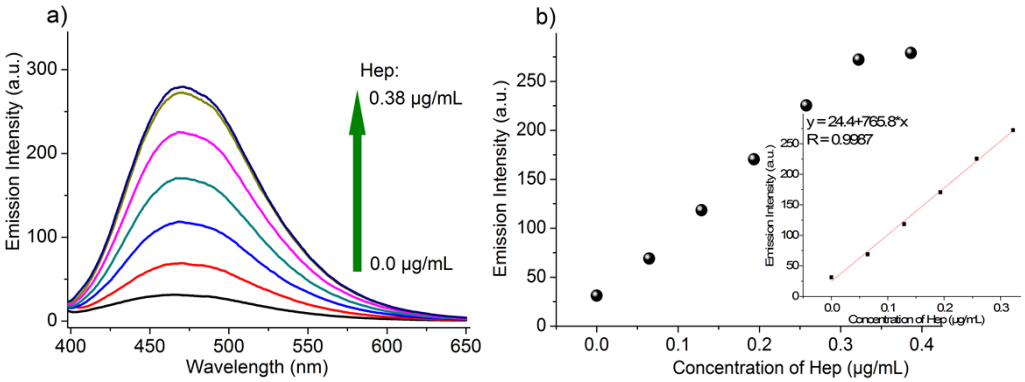


Figure S5. a) Fluorescence titration profile of **TPE-1** (1 μM) with increasing amount of Hep in 10 mM pH 7.4 HEPES/DMSO (95:5, v:v), λ_{ex} = 340 nm. b) Corresponding calibration curve of Hep. Inset: linear response curve of Hep.

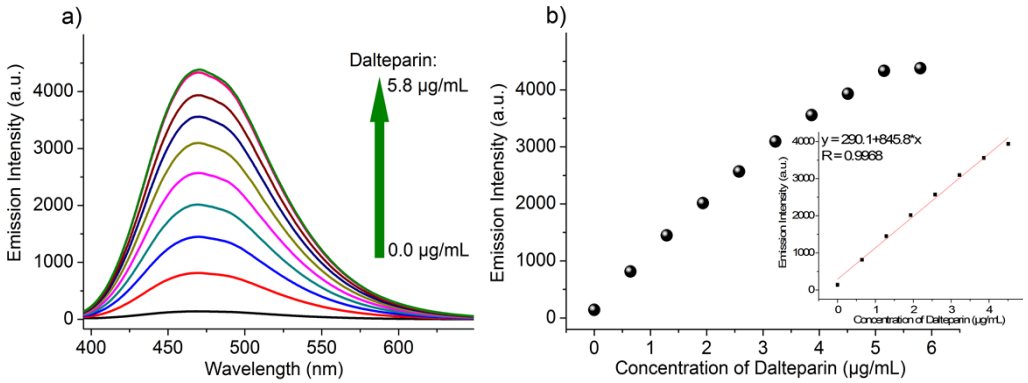


Figure S6. Dalteparin detection with the **TPE-1** probe. a) Fluorescence titration profile of **TPE-1** (10 μM) with increasing amount of Dalteparin in 10 mM pH 7.4 HEPES/DMSO (95:5, v:v). λ_{ex} = 340 nm. b) Corresponding calibration curve of Dalteparin. Inset: linear response curve of Dalteparin.

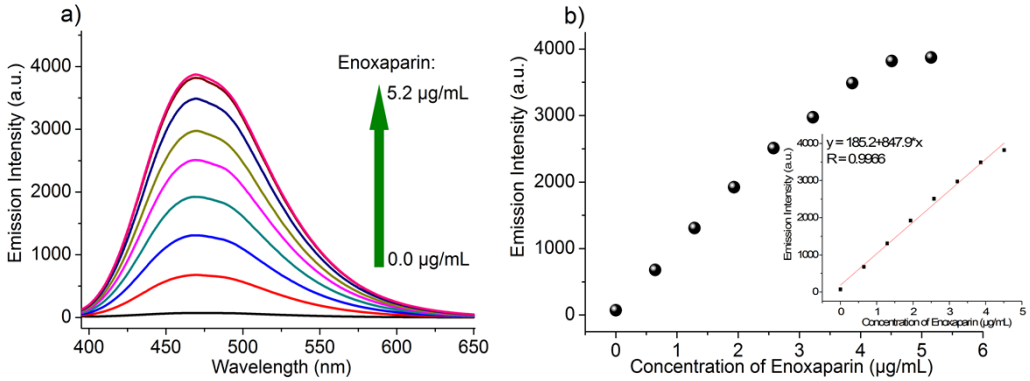


Figure S7. Enoxaparin detection with the **TPE-1** probe. a) Fluorescence titration profile of **TPE-1** (10 μM) with increasing amount of Enoxaparin in 10 mM pH 7.4 HEPES/DMSO (95:5, v:v). λ_{ex} = 340 nm. b) Corresponding calibration curve of Enoxaparin. Inset: linear response curve of Enoxaparin.

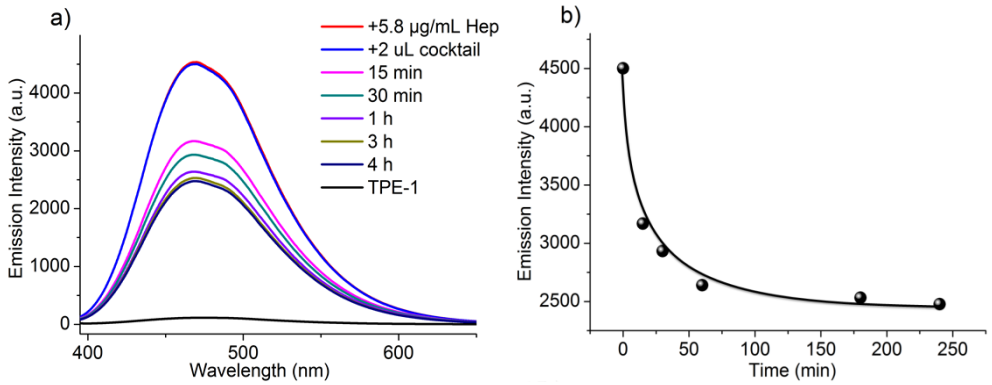


Figure S8. Heparinase induced fluorescence quenching. a) Time course fluorescence spectra of a mixture of **TPE-1** (10 µM) and Hep (5.8 µg/mL) upon addition of heparinase cocktail. b) Corresponding emission intensity at 470 nm as a function of time.

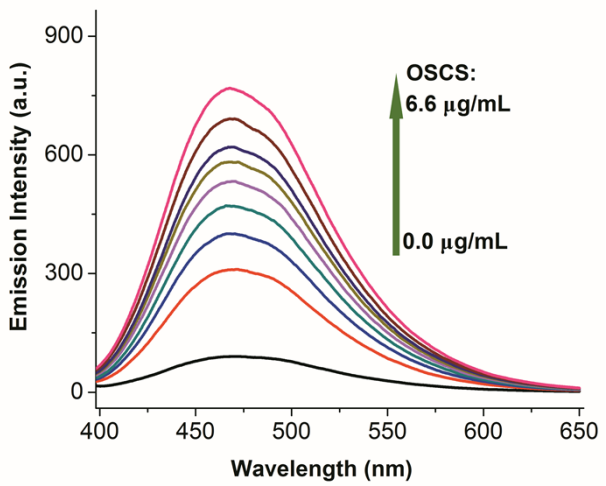


Figure S9. Fluorescence titration profile of **TPE-1** (10 μM) with increasing amount of OSCS in 10 mM HEPES/DMSO (95:5, v:v, pH 7.4). λ_{ex} = 340 nm.

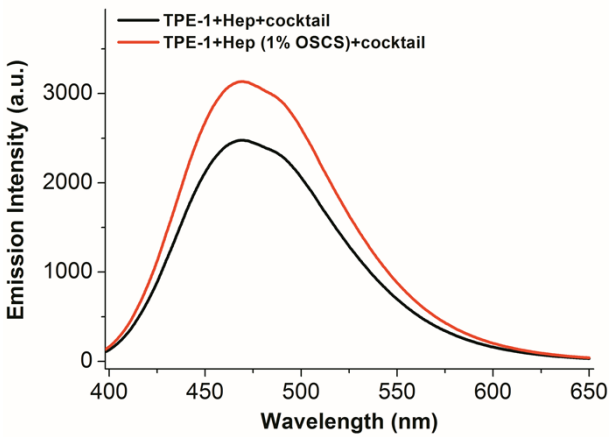


Figure S10. The presence of OSCS in Hep enhanced the fluorescence of **TPE-1** (10 μM) after heparinase cocktail treatment.

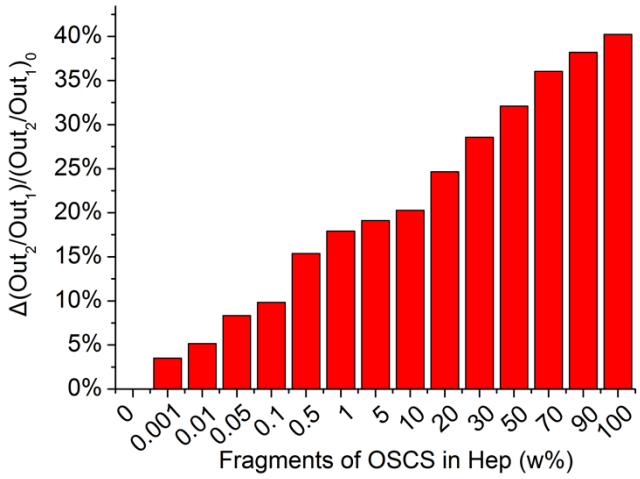


Figure S11. Changes in Out_2/Out_1 values of **TPE-1** versus different fragments of (w%) of OSCS in Hep, showing that 0.001% OSCS (w/w) in Hep can be detected. $(Out_2/Out_1)_0$: the initial value of Out_2/Out_1 in the absence of OSCS.

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