Electronic Supplementary Material (ESI) for RSC Advances.

Supplemental Information

MnO2 nanosheets-based heparin and OSCS fluorescent biosensor with background lowered and hybridization chain reaction amplified

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Content

S1. Experimental part

Chemicals and Apparatus

HP_1, HP_2 and helper DNA and DNA'were synthesized by Shanghai Sangon Biotechnology Co, Ltd. (Shanghai, China) and purified by HPLC. It was dissolved in Tris (hydroxymethyl) aminomethane (Tris)-HCl buffer (pH 7.0) as stock solution. Their sequences are listed in Table 1. Heparin sodium salt (heparin), coralyne chloride hydrate, sodium chloride (NaCl), calcium chloride ($CaCl_2$), ascorbic acid (AA), chondroitin sulfate (ChS), hyaluronic acid (HA), Trypsin, albumin, globulin and fibronectin, SYBR Green I (10 000×), $KMnO_4$ and other reagents were purchased from Shanghai Sangon Biological Technology. Chondroitin sulfate A sodium salt from were purchased from Sigma Aldrich (St. Louis, MO). All the stocks and buffer solutions were prepared by using ultrapure water (PSDK2-10-C, Beijing, China).

Centrifugation was performed using a HERMLEZ 36 HK apparatus (Wehingen, Germany). All pH measurements were measured with a Model pHs-3c meter (Shanghai, China). Fluorescence spectra measurements of SG were obtained using a F-4600 fluorescence spectrophotometer (Hitachi, Japan) equipped with a xenon discharge lamp and 1 cm quartz cells. Both the excitation and emission slit width were set to 5.0 nm. All of the measurements were operated at room temperature of 298 K. Ultraviolet-visible absorption spectra were measured using UV-3010 spectrophotometer (Hitachi, Japan). Characterization of transmission electron microscopy (TEM) was carried out on Tecnai G220 ST (FEI) under the accelerating voltage of 200 kV. The sample for TEM measurement was prepared by the deposition of one drop of aqueous dispersion on a copper grid coated with thin films of carbon, and the solvent was removed by evaporation in air. All pH measurements were measured with a Model pHs-3c meter (Shanghai, China).

Name	sequences (from 5' to 3')
HP_1	TTTTTTTTTTCACGCAGAAACCTACACTCAAAAAAGTGTAGGTTTCTGCGTG
HP_2	AGTGTAGGTTTCTGCGTGGGTTAACACGCAGAAACCTACACTTTTTTT
helper DNA	AAAAAAAAAAAGTGTAGGTTTCTGCGTGAAAAAAAAAA
helper DNA'	ATTAATTATATGTGTAGGTTTCTGCGTGATATTATTAA

Preparation of MnO_2 nanosheet With Atomic Thickness.

MnO_2 nanosheets were synthesized by the Yoshida method(Kai et al., 2008). In brief, 20 mL of a solution containing 0.6 M tetramethylammonium (TMA) and 3% H_2O_2 was added into 10 mL of 0.3 M $MnCl_2$ solution. The resulting suspension was vigorously stirred in air at room

temperature for 12 h, the precipitation was collected and sequentially washed with deionized water and absolute ethanol several times. The final product was dried in a vacuum oven at 60 ^{o}C overnight.

Analytical procedure for heparin.

Firstly, the helper DNA and helper DNA1 (HP_1 and HP_2) solution was heated at 90 ^{o}C for 10 min and gradually cooled to room temperature at a constant rate over the course of 2 h to form the stem-loop structure respectively before use. Then, all the solutions were 600 μL and prepared by mixing substrate solution (100 nM helper DNA1, 10 nM helper DNA, 2 μM coralyne and 500 nM Hg^{2+}) which consisted of 50 mM $MgCl_2$ and 5 mM Tris-HCl with the pH value equal to 7.0. In order to obtain the A_{10}-MB-A_{10}·coralyne probe (probe 1) and helper DNA1, all the samples were incubated at 24 °C for at least 10 min before the experiment. Successively, when the addition of heparin and 2 μM GSH, the solutions was carried out at 45 ^{o}C for 20 min and then SG (260 nM) and100 μg/mL MNS were added with 10 min. Finally, the mixture were used for the determination of fluorescence.

Analytical procedure for OSCS

Firstly, we first prepare the different concentration of OSCS, standard heparin solutions (180 μg/mL) were mixed with a series of OSCS standards from 10^{-10} % to 10 %. Then, the hairpin-like helper DNA and helper DNA1 (HP_1 and HP_2) solution is prepared through the above method. Successively, all the solutions were 600 μL and prepared by mixing 100 nM helper DNA1, 10 nM helper DNA, 2 μM coralyne, 0.2 mM $CaCl_2$ and 500 nM Hg^{2+} in 5 mM Tris-HCl with the pH value equal to 7.0. In order to obtain the A_{10}-MB-A_{10}·coralyne·Ca^{2+} probe (probe 2), all the samples were incubated at 24 °C for at least 10 min before the experiment. Successively, when the addition of OSCS and 2 μM GSH, the solutions was carried out at 45 ^{o}C for 20 min and then SG (260 nM) and100 μg/mL MNS were added with 10 min. Finally, the mixture were used for the determination of fluorescence. As a comparison, the solutions was carried out at 45 ^{o}C for 20 min with addition of heparin.

Preparation of human serum samples

Human serum samples were kindly provided by the Yi jishan Hospital (Wuhu, China). Human serum samples were centrifuged at 10,000 rpm for 15 min first. Subsequently, the supernatant was diluted with Tris-HCl appropriately before being analyze.

Gel Electrophoresis

Gel electrophoresis was used to confirm the formation of the CHR products. Samples for gel

electrophoresis assays were prepared as follows: (1) probe 1 was used as sample one; (2) probe 1 treated with heparin was used as sample two; (3) probe 2 treated with heparin was used as sample three; (4) probe 2 treated with OSCS was used as sample four. In the gel electrophoresis assay, each prepared sample (8 μL) was put on 5% agarose gels to separate the related substances. The electrophoresis was carried in 0.5 ×TBE buffer (pH 7.9) at 120 V constant voltage for 1 h. After EB staining, the gels were scanned using the Omega 16ic Gel imaging system (ULTRA-LUM, USA).

S2. Compared with other detection assay for Heparin

Table S1. Comparison of different fluorescent sensors for the sensing of heparin.

Method	Linear range(μM)	LOD (S/N =3)	Reference
Electrochemistry	0.5 - 10	Not given	S1
UV-visible spectroscopy	Not given	8.3 × 10^3nM	S2
Colorimetry	0 - 55.8	Not given	S3
Colorimetry	0.14 - 4.8	47 nM	S4
Fluorescence (QD)	0.05-15	12.46nM	S5
Fluorescence (DNA)	0.01- 1	4 nM	S6
Fluorescence (DNA)	0.01 - 4	3 nM	S7
Fluorescence (DNA)	10^{-5}- 10	4 pM	This study

S3. The reproducibility and stability for the detection of heparin

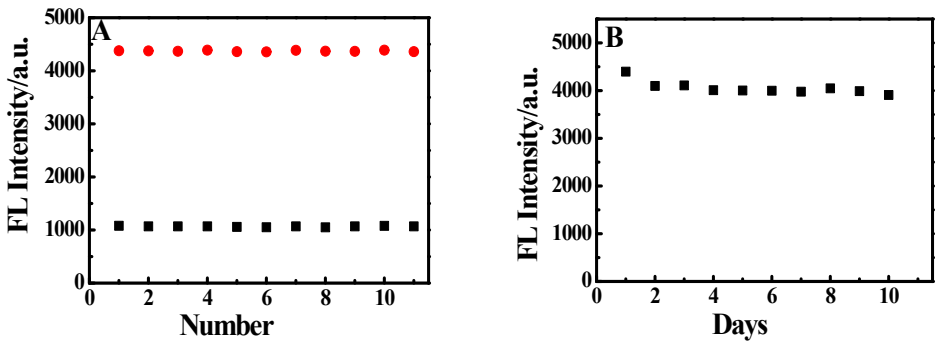

Fig. S1. (A) Fluorescence intensity of eleven independent tests for 200 pM (black) and 1μM (red) heparin used the proposed biosensor. (B) The time dependence of the signal change for 1μM heparin used the proposed biosensor.

S4. The practical analysis for the detection of heparin

Table S2. The results of real sample measurements

Sample	Heparin added	found (n=3)	Recovery (%)	R.S.D(%)
1 % Diluted human Serum	50 pM	51 pM	102	8.25
	500 nM	495 nM	99	7.97
	1 μM	1.01 μM	101	8.12
	2 μM	2.05 μM	103	8.54

S5. Compared with other detection assay for OSCS.

Table S3. Comparison of different method for the sensing of OSCS.

Method	LOD (S/N =3) (w/w)	Reference
strong anion exchange (SAX)-HPLC	0.03%	S8
NMR	0.1%	S9
capillary electrophoresis (CE)	0.1%	S10
polyacrylamide gel electrophoresis (PAGE)	0.2%	S11
near-infrared (NIR)	1%	S12
HNMR		S13
Taq polymerase inhibition	0.16%	S14
Heparin enzyme immunoassay kit	0.1%	S15
Fluorescent assay	10^{-10} %	This study

Table S4. Comparison of different material-based sensors for the sensing of OSCS.

Method	LOD (S/N =3)	Reference	Time	Enzyme treatment
Potentiometric Polyanion Sensors	0.5%	S16	10min	No
Disposable striptype electrochemical polyion sensor	0.05%	S17	120s	No
Watersoluble cationic polythiophene polymer	0.1%	S18	30min	Yes
Polymer H sensor	0.5%	S19	110min	Yes
Gold Nanoparticle-based fluorescent sensor	10^{-9}%	S20	30 min	Yes
Molecular Beacon-Based Fluorescent Assay	0.01%	S21	5 min	No
DNA-Based Fluorescent Assay	10^{-10} %	This study	10 min	No

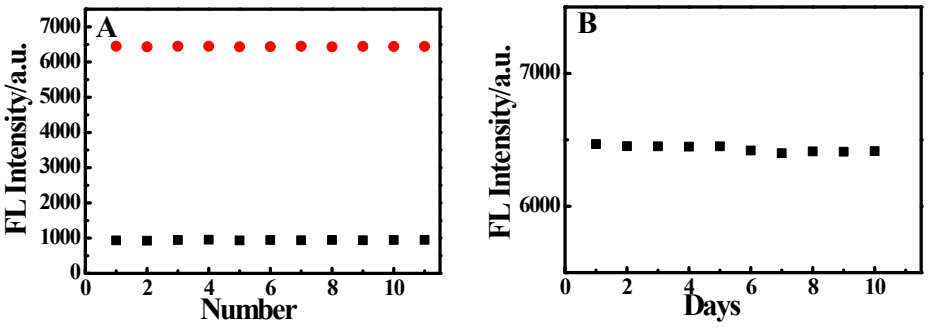

Fig S2. (A) Fluorescence intensity of eleven independent tests for (10^{-7}, 10^{-4}, and 10% w/w OSCS in heparin) used the proposed biosensor. (B) The time dependence of the signal change for 10% w/w OSCS used the proposed biosensor.

S6. References

S1 H. T. Qi, Z. Li, L. F. Yang, P. Yu, L. Q. Mao, *Anal. Chem*. 2013, **85**, 3439-3445.

S2 S. M. Bromfield, A. Barnard, P. Posocco, M. Fermeglia, S. Pricl and D. K. Smith, *J. Am. Chem. Soc*. 2013, **135**, 2911-2914.

S3 R. Y. Zhan, Z. Fang and B. Liu, *Anal. Chem*.2010, **82**, 1326-1333.

S4 R.Cao and B. X. Li, *Chem. Commun*. 2011, **47**, 2865-2867.

S5 Z. P. Liu, Q. Ma, X. Y. Wang, Z. H. Lin, H. Zhang, L. L. Liu and X. G. Su, *Biosens. Bioelectron*. 2014, **54**, 617-622.

S6 S. Y.Hung and W. L. Tseng, *Biosens. Bioelectron*. 2014, **57**, 186-191.

S7 C. Y. Kuo and W. L. Tseng, *Chem. Commun*. 2013, **49**, 4607- 4609.

S8 M. L. Trehy, J. C. Reepmeyer, R. E. Kolinski, B. J. Westenberger and L. F. Buhse, *J. Pharm. Biomed. Anal.* 2009, **49**, 670-673.

S9 I. McEwen, B. Mulloy, E. Hellwig, L. Kozerski, T. Beyer, U. Holzgrabe, R. Wanko, J. M. Spieser and A. Rodomonte, *Pharmeuropa Bio.* 2008, **2008**, 31-39.

S10 N. Volpi, F. Maccari and R. J. Linhardt, *Anal. Biochem.* 2009, **388**, 140-45.

S11 Z. Zhang, B. Li, J. Suwan, F. Zhang, Z. Wang, H. Liu, B. Mulloy and R. J. Linhardt, *J. Pharm. Sci.* 2009, **98**, 4017-4026.

S12 J. A. Spencer, J. F. Kauman, J. C. Reepmeyer, C. M. Gryniewicz, W. Ye, D. Y. Toler, L. F. Buhse and B. J. Westenberger, *J. Pharm. Sci.* 2009, **98**, 3540-3547.

S13 D. J. Langeslay, C. N. Beecher, A. Naggi, M. Guerrini, G. Torri and C. K. Larive, *Anal. Chem*. 2013, **85**, 1247−1255.

S14 C. Tami, M. Puig, J. C. Reepmeyer, H. Ye, D. A. D'Avignon, L. Buhse and D. Verthelyi, *Biomaterials.* 2008, **29**, 4808-4814.

S15 S. Bairstow, J. McKee, M. Nordhaus and R. Johnson, *Anal. Biochem*. 2009, **388**, 317321.

S16 L. Wang, S. Buchanan and M. E. Meyerhoff, *Anal. Chem.* 2008, **80**, 9845-9847.

S17 Y. Kang, K. Gwon, J. H. Shin, H. Nam, M. E. Meyerhoff and G. S. Cha, *Anal. Chem*. 2011, **83**, 3957-3962.

S18 C. D. Sommers, D. J. Mans, L. C. Mecker and D. A. Keire, *Anal. Chem*. 2011, **83**, 3422-3430.

S19 S.Lühn, S. Schiemann and S. Alban, *Anal. Bioanal. Chem*. 2011, **399**, 673-680.

S20 C. Y. Lee and W. L.Tseng, *Anal. Chem*. 2015, **87**, 5031-5035.

S21 M. Kalita, S. Balivada, V. P. Swarup, C. Mencio, K. Raman, U. R. Desai, D. Troyer and B. Kuberan, *J. Am. Chem. Soc.* 2013, **136**, 554-557.