Electronic Supplementary Material (ESI) for Lab on a Chip.
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Electronic Supplementary Information (ESI)

Smartphone Surface Plasmon Resonance Imaging for the Simultaneous and Sensitive Detection of Acute Kidney Injury Biomarkers with Noninvasive Urinalysis

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Supplementary Note 1: Materials and instruments

All reagents were used as received without further purification. Retinol-binding protein (RBP), interleukin-18 (IL-18), human neutrophil gelatinase-associated lipocalin (NGAL), monoclonal capture antibody (cAb) and detection antibody (dAb) of human IL-18, NGAL, polyclonal antibody of RBP were all obtained from Beijing Key-Bio Biotechnology (China). Bovine serum albumin (BSA, ≥ 98%) were purchased from Phygene Life Sciences (China). Magnetic nanoparticles (MNPs, fluidMAG-ARA, diameter of 200 nm, with the mass concentration of 25 mg/ml and number of about 2.2×10^{14} particles/g.) with iron oxide core were purchased from Chemicell (Berlin, Germany). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysyccinimide (NHS) were obtained from Sigma-Aldrich (China). Phosphate buffered saline (PBS) was prepared from 140 mM NaCl, 10 mM phosphate, 3 mM KCl, and the pH was adjusted to 7.4. 10 mM acetate buffer (ACT, pH 5.5) was prepared from sodium acetate and acetic acid and the pH was adjusted by HCl and NaOH. PBS Tween (PBST) buffer was prepared by adding Tween 20 (0.05%) to PBS buffer solution. O-(2-Carboxyethyl)-O'-(2-mercaptoethyl)-heptaethylene glycol (thiol-COOH, Catalog No. 37156-0795) was purchased from Polypore (Norway). SH-PEG$_3$-OH (thiol-OH, Catalog No. PS2-OS-3) was obtained from Ponsure Biotechnology (China). Ethanolamine, 1H,1H,2H,2H-tridecafluoro-n-octyltriethoxysilane (TDOS) and ethylene glycol (EG) were purchased from Macklin (China). 2-(N-morpholino)ethanesulfonic acid (MES) was obtained from Sigma-Aldrich. Milli-Q water was used in all experiments. Magnet was purchased from Jianku flagship store

with the diameter of 20 mm and thickness of 10 mm. Urine samples were obtained from the volunteer of our group which were not purified before use. In addition, the urine samples were collected and used under the guideline approved by the Ethic Committee of the Second Affiliated Hospital of Wenzhou Medical University (No. 2021K4202). The healthy urine sample (health 1) was obtained from the healthy volunteer (male) at 25 years old. The patient samples (patient 2 and 3) were provided from the AKI patients at the ages of 32 (male) and 27 (female), respectively.

Size distribution were carried out by Dynamic light scatterer (DLS, Zetasizer Nano ZS NEN3600, Malvern, England). Contact angles were carried out by Contact Angle Meter (model Theta, Biolin Scientific, Sweden). Plasma cleaner (PCE-6, MTI Corporation, Canada) was employed to treat the cover glass hydroxylation.

Supplementary Note 2: Modification of magnetic nanoparticles

The MNP is a sterile, aqueous dispersion of superparamagnetic iron oxide nanoparticles covered by a mixed polysaccharide with the functional subunit glucuronic acid, which is suitable for covalent coupling of biomolecules. MNPs were modified with detection antibodies (dAbs) of AKI biomarkers (NGAL, IL-18, RBP) according to our previous reported protocol, respectively [1]. Briefly, the carboxylic group of MNPs (2 mg) were activated with EDC (1.1 mg/ml) and NHS (1.1 mg/ml) dissolved in 1 ml MES buffer. Then the MNPs were washed with pure MES buffer and incubated with 5 μg dAb of AKI biomarkers for 3 h at room temperature. The unreacted active ester groups on MNPs were blocked by incubation with 1 M ethanolamine at pH 8.5 for 10 min, followed by rinsing with PBST buffer. The sample precipitate was collected with a

magnet. Finally, the MNPs-dAbs were dissolved in PBST buffer and stored at 4 °C for further use. The ratio of dAb to MNP was estimated at 10:1, assuming 90% of dAbs were immobilized on the MNP surface during the labeling process [2].

Supplementary Note 3: Preparation of gold dot array chip

For the preparation of gold dot array chip, the cover glass cleaned with water and ethanol was first treated with plasma cleaner for 10 min, followed by silanization in TDOS solution at 65 °C for 1 h. After that, the cover glass was rinsed with ethanol and ultrapure water, dried with nitrogen gas. Then, the gold dot array chip was prepared by thermal evaporation of gold layer with the thickness of 47 nm on the above cover glass slide, with the gold dot diameter of 1 mm and the period of 2 mm.

Supplementary Note 4: Nonspecific investigation

The nonspecific binding experiments were also implemented as control by investigating the nonspecific binding of MNPs-dAbs on the cAb modified sensor chips. The MNP-anti-NGAL, MNP-anti-IL-18 and MNP-anti-RBP were pumped through the surface in turn for 15 min, followed by rinsing with PBST for 8 min. The SPRi response indicates the amount of nonspecific binding. In addition, the interference interaction between different AKI biomarkers and MNP- dAbs were also investigated in the PBST and urine. Firstly, the gold dot arrays were immobilized with anti-NGAL (400 μg/ml, 0.5 μl), anti-IL-18 (400 μg/ml, 0.5 μl), anti-RBP (400 μg/ml, 0.5 μl) and BSA (1 mg/ml, 0.5 μl) in the 1st line, 2nd line, 3rd line and 4th line in the ACT buffer (pH = 5.5), respectively. Then, the NAGL (1 μg/ml), IL-18 (1 μg/ml) and RBP (1 μg/ml) were pumped through the sensor chip one by one in PBST or urine (V_{urine}:V_{PBST} = 1:1),

respectively. After that, the MNP-anti-NGAL (1 μg/ml, 0.8 ml), MNP-anti-IL-18 (1 μg/ml, 0.8 ml) and MNP-anti-RBP (1 μg/ml, 0.8 ml) were injected into the flow cell successively in PBST.

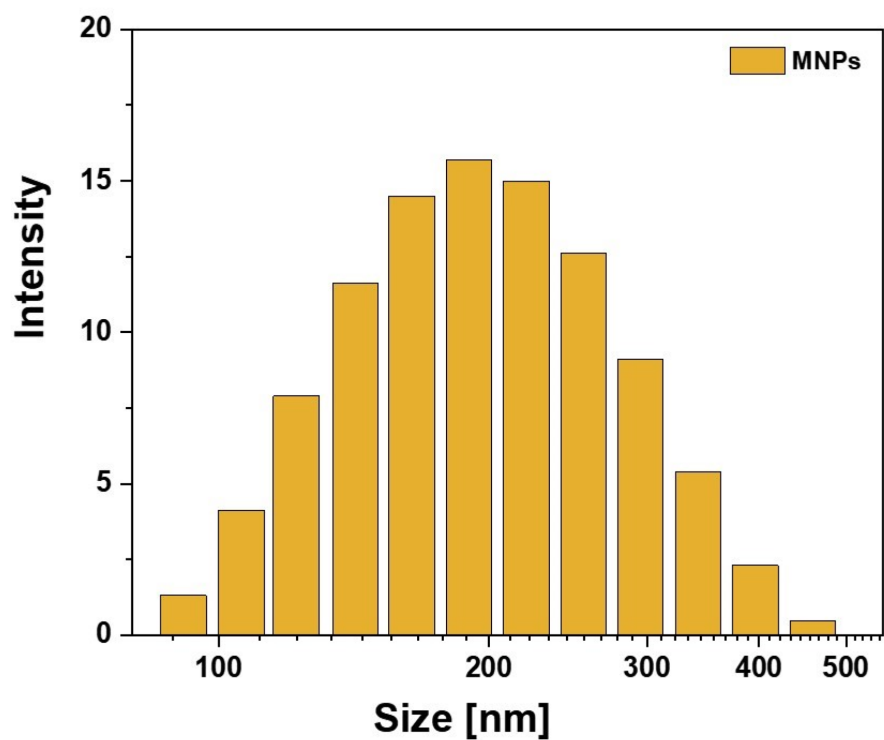
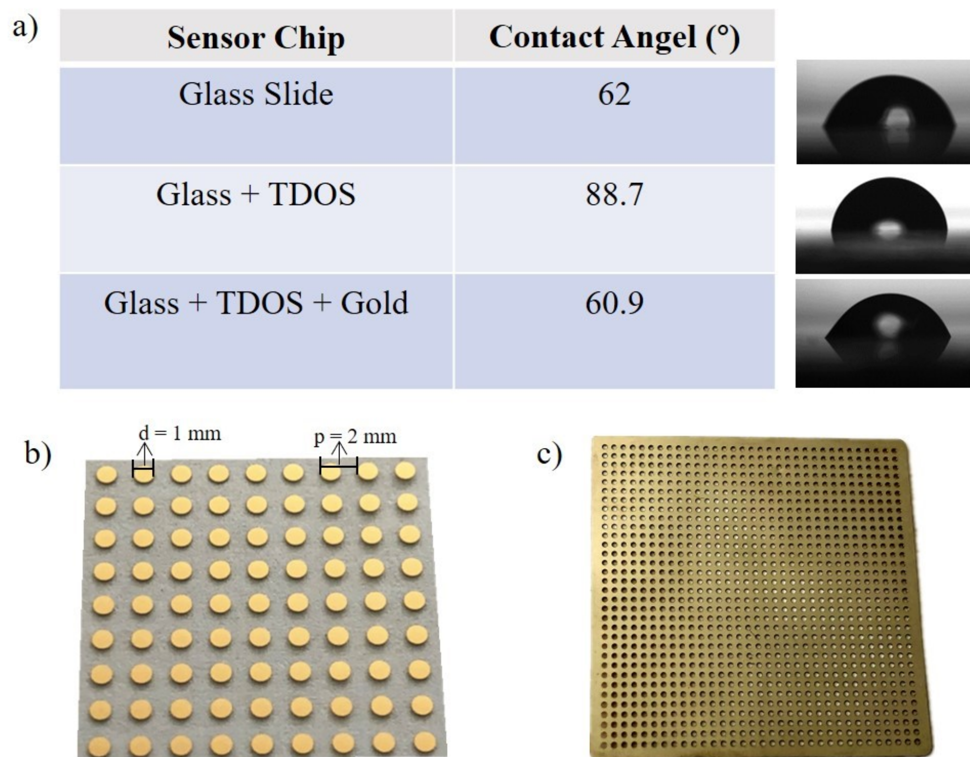


Fig. S1. The size distribution of MNPs measured by DLS.



Sensor Chip	Contact Angel (°)
Glass Slide	62
Glass + TDOS	88.7
Glass + TDOS + Gold	60.9

Fig. S2. a) Contact angle experiments of different chips, including cover glass, glass modified with TDOS, and gold dot array with 47 nm gold film on the glass slide modified with TDOS modification. The photograph of b) gold dot array chip and c) the copper mask.

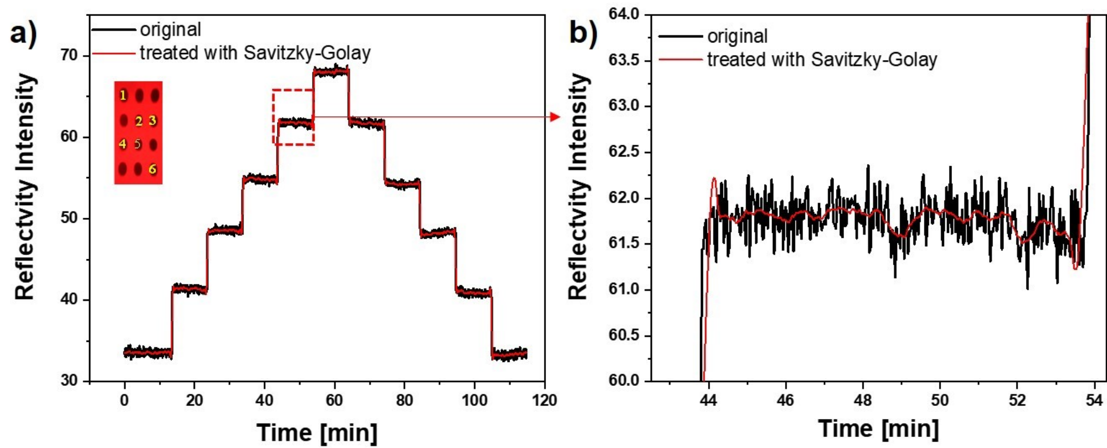


Fig. S3. a) Reflectivity dynamic curves (black line) obtained from the smartphone SPRi upon the injection of different concentration of EG in water (0 ~ 10%), measured at the corresponding 6 different gold dots (numbered 2) as shown in the inset. And the corresponding smoothed curve (red line) treated with Savitzky-Golay method, polynomial order of 2. b) The corresponding zoom-in part as labeled in a).

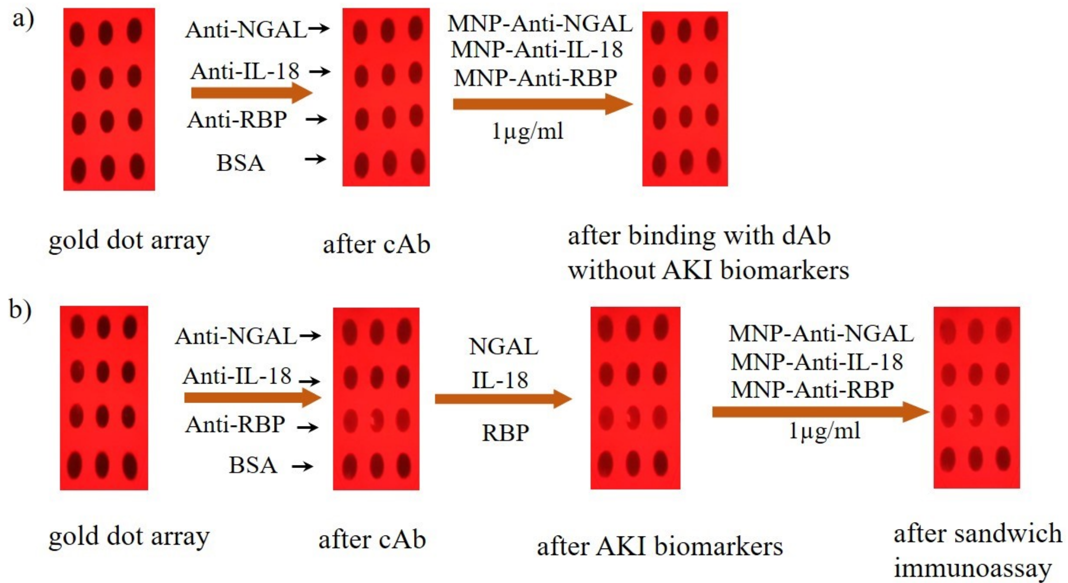


Fig. S4. The smartphone based SPRi images, (a) gold dot array before and after the immobilization of capture anti-NGAL, anti-IL-18, anti-RBP and BSA on different lines, and followed with incubation of MNP-anti-NGAL, MNP-anti-IL-18 and MNP-anti-RBP without AKI biomarkers, respectively; (b) on the gold dot array before and after immobilization with capture antibody (cAb) of AKI biomarkers, and incubation with NGAL, IL-18 and RBP, followed with the incubation of MNP-anti-NGAL, MNP-anti-IL-18 and MNP-anti-RBP in PBST.

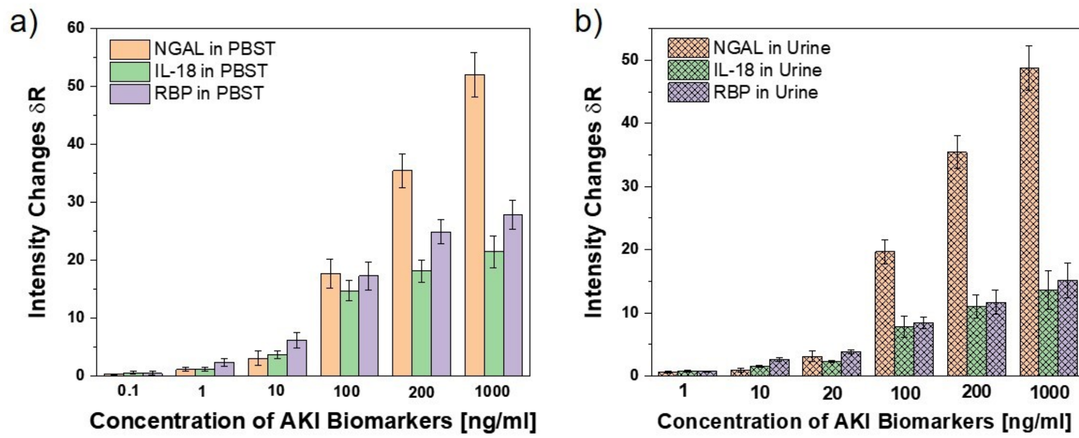


Fig. S5. The histograms of reflectivity intensity changes with different concentration of NGAL, IL-18, and RBP in PBST (a) and urine (b) by sandwich immunoassay according to the dynamic curves in Figure 4 (a-f), respectively.

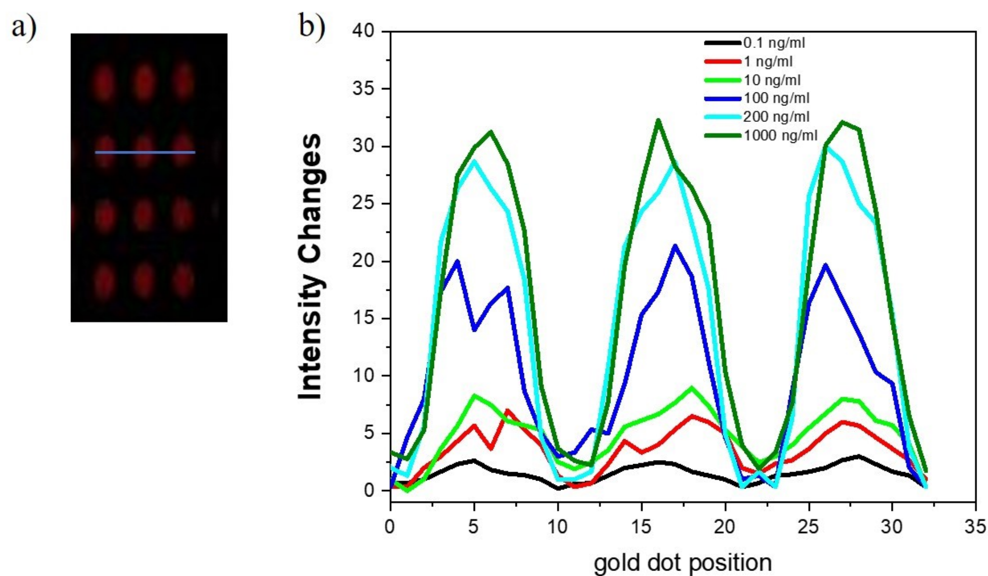


Fig. S6. a) The SPRi photo after sandwich immunoassay of RBP. b) Intensity changes curves on the gold dots after sandwich immunoassay of RBP from 0.1 ng/ml to 1 μg/ml in PBST at the labeled blue line in a).

Table S1 The recoveries of the 3 urine samples were analyzed by smartphone SPRi biosensor for the spiked AKI biomarkers in urine, respectively.

Sample Number	AKI Biomarkers	Spiked Concentration (ng/ml)	Detected Concentration (ng/ml)	Recovery (%)	Average Recovery (%)
1	NGAL	150	161	107.3	99.86 ± 5.12
	IL-18	300	310	103.3	
	RBP	140	138	98.6	
2	NGAL	90	84	93.3	
	IL-18	250	244	97.6	
	RBP	100	97	97	
3	NGAL	100	108	108	
	IL-18	160	155	96.9	
	RBP	600	580	96.7	

Table S2 The concentrations of AKI biomarkers (NGAL, IL-18 and RBP) from 3 real urine samples measured with the smartphone SPRi system.

Sample Number	Sex	Age	NGAL (ng/ml)	IL-18 (ng/ml)	RBP (ng/ml)
Health 1	Male	25	---	---	181,1.22±0.28
Patient 2	Male	32	33.2±1.9	6.7±0.7	38.2±2.15
Patient 3	Female	27	31.7±2.3	13.5±1.8	22.8±1.5

Table S3 The performances comparison with other smartphone based plasmonic biosensors.

Platform	Detection Method	Detection Range	LOD	Buffer	Sample	Year	Ref.
SPR sensing on cell phones	sandwich immunoassay	0.132 μg/mL~ 1.32 μg/mL	0.1 μg/mL	HEPES	β2-microglobulin	2012	[3]
Grating-coupled SPR smartphone biosensor	direct assay	10 ng/mL ~10 μg/mL	32.5 ng/mL	PBS	lipopolysaccharides	2018	[4]
Nanoplasmonic biochips with a smartphone	indirect competitive immunoassay	0.2 ppb ~ 10 ppm	1 ppb	HEPES	Imidacloprid pesticides	2016	[5]
SPR based smartphone platform	direct immunoassay	67 nM ~ 1 μ M	47.4 nM	PBS	IgG	2015	[6]
Patterned plasmonic gradient biosensor with smartphone	direct assay	1 nM ~ 15 nM	1 nM	PBS	streptavidin	2019	[7]
Smartphone SPRi	sandwich immunoassay	0.1~1000 ng/ml in PBS, 1~1000 ng/ml in urine	NGAL 0.19 ng/ml, IL-18 0.51 ng/ml, RBP 0.7 ng/ml in urine	PBS and urine	NGAL IL-18 RBP	---	this work

Table S4 The performances comparison with other methods for the detection of AKI biomarkers.

Method	Detection AKI Biomarker	Detection Range	LOD	Sample	Year	Ref.
Electrochemical immunosensor	NGAL	50~500 ng/mL	21.1 ng/ml in PBS	Human urine	2017	[8]
Electrochemical aptamer-based sensor (EAB)	NGAL	2 ~ 32 nM	2 nM in urine	Human urine	2020	[9]
Enzyme-linked immunosorbent assay (ELISA)	NGAL	20 ~ 5000 pg/ml	25.5 pg/ml in buffer	Human urine	2021	[10]
Ag@BSA core/Shell microspheres based electrochemical immunosensor	RBP	50 ~ 4500 ng/ml	18 ng/ml in PBS	Human urine	2012	[11]
ECL immunosensor based MWCNTs and Ru-Nafion@SiO_2	RBP	78 ~ 5000 ng/ml	---	Human urine	2013	[12]
Smartphone SPRi sandwich immunoassay	NGAL, IL-18, RBP	0.1~1000 ng/ml	NGAL 0.19 ng/ml, IL-18 0.51 ng/ml, RBP 0.7 ng/ml in urine	Human urine	---	This work

References

1. Y. Wang, J. Dostalek and W. Knoll, *Anal. Chem.*, 2011, **83**, 6202-6207.
2. I. Koh, X. Wang, B. Varughese, L. Isaacs, S. H. Ehrman and D. S. English, *J. Phys. Chem. B*, 2006, **110**, 1553-1558.
3. P. Preechaburana, M. C. Gonzalez, A. Suska and D. Filippini, *Angew. Chem. Int. Edit.*, 2012, **51**, 11585-11588.
4. J. Zhang, I. Khan, Q. Zhang, X. Liu, J. Dostalek, B. Liedberg and Y. Wang, *Biosens. Bioelectron.*, 2018, **99**, 312-317.
5. K.-L. Lee, M.-L. You, C.-H. Tsai, E.-H. Lin, S.-Y. Hsieh, M.-H. Ho, J.-C. Hsu and P.-K. Wei, *Biosens. Bioelectron.*, 2016, **75**, 88-95.
6. Y. Liu, Q. Liu, S. Chen, F. Cheng, H. Wang and W. Peng, *Sci. Rep.-UK*, 2015, **5**, 12864.
7. J. Bian, X. Xing, S. Zhou, Z. Man, Z. Lu and W. Zhang, *Nanoscale*, 2019, **11**, 12471-12476.
8. J. Yukird, T. Wongtangprasert, R. Rangkupan, O. Chailapakul, T. Pisitkun and N. Rodthongkum, *Biosens. Bioelectron.*, 2017, **87**, 249-255.
9. C. Parolo, A. Idili, G. Ortega, A. Csordas, A. Hsu, N. Arroyo-Curras, Q. Yang, B. S. Ferguson, J. Wang and K. W. Plaxco, *ACS Sensors*, 2020, **5**, 1877-1881.
10. L. Kang, S. Smith and C. Wang, *J. Colloid Interface Sci.*, 2021, **588**, 101-109.
11. C. Hu, D.-P. Yang, K. Xu, H. Cao, B. Wu, D. Cui and N. Jia, *Anal. Chem.*, 2012, **84**, 10324-10331.
12. B. Wu, C. Hu, X. Hu, H. Cao, C. Huang, H. Shen and N. Jia, *Biosens. Bioelectron.*, 2013, **50**, 300-304.