Electronic Supplementary Information

Development of small molecule biosensors by coupling the recognition of bacterial allosteric transcription factor with isothermal strand displacement amplification

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Contents of Supporting Information

Materials and Methods

Reagents. The oligonucleotides (Table S1) used in this work were synthesized and purified by Sangon Biotech. Co. Ltd. (Beijing, China). Nicking endonuclease Nb.BbvCI, Klenow fragment (KF), and deoxyribonucleoside 5'-triphosphate mixture (dNTPs) were purchased from New England Biolabs (Beijing, China). Thioflavin T (ThT), H_2O_2, Hemin, and 2,2′-azino-bis(3-ethylbenzothiozoline-6-sulfonicacid) diammonium salt ($ABTS^{2-}$) were obtained from Sigma. All of the solutions were prepared in sterilized milli-Q water (MQ, purified by Millipore). All other chemical reagents used in this work were analytical grade without further purification.

Plasmid construction. The optimized coding sequences (Table S2) of HosA,[1] and HucR[2] were synthesized by Synbio-Tech (Suzhou, China). Then the sequences of these aTFs were cloned into the *NdeI* and *XhoI* restriction sites of pET23b (Novagen). The constructs were confirmed by sequencing.

Expression and purification of recombinant aTFs. All aTFs (HosA and HucR) were expressed in *Escherichia coli* BL21 (DE3). Purification of aTFs were performed as previously described.[3] The concentration of purified proteins was determined by the Bradford method using bovine serum albumin (BSA) as a standard. The purified proteins were analyzed by 18% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Bio-layer interferometry (BLI) assay. Kinetics between aTFs (HosA and HucR) and their target-binding sites were determined by BLI assay using an Octet RED96 system (FortéBIO) as previously described.[4] Briefly, the process consisted of five steps: balance, DNA loading, rebalance, association, and dissociation. The blank tests were carried out using HBS-EP buffer[4] instead of aTFs in the association step, and were used for baseline correction.

Development of a PHBA biosensor by coupling HosA with SDA. To optimize the primer concentration for SDA reaction, 50 μL mixture containing 100 nM T1 and variable amounts (ranging from 0 to 100 nM) of P1 in 1 × NEBuffer 2 was incubated at 95 °C for 5 min. After slowly cooling to 25 °C, 0.4 mM dNTPs, 5 μM ThT, 0.25 U KF, and 0.3 U Nb.BbvCI were added. Total volume of the mixture was supplemented to 100 μL with MQ. Then, the reaction mixture was incubated at 37 °C on the Roche LightCycler® 480II real-time PCR system and real-time fluorescence was monitored at an interval of 1 min. To optimize HosA concentration for the PHBA biosensor, 100 nM T1 and 10 nM P1 mixture was annealed. Next, 0.4 mM dNTPs, 5 μM ThT and variable amounts (ranging from 5 nM to 200 nM) of HosA were added. The SDA reaction was then performed as above using the same real-time PCR system. The proper concentration of HosA for biosensor construction was the minimal amount of HosA that could completely repress the SDA reaction. To characterize the PHBA biosensor, 100 nM T1 and 10 nM P1 mixture was annealed. Then, 0.4 mM dNTPs, 5 μM ThT, 50 nM HosA, and different amounts of PHBA were added. After incubating the mixture at 25 °C for 20 min, the SDA reaction was performed, and real-time fluorescence was monitored. The limit of detection (LOD) was calculated based on 3σ/slope rule, where σ is the standard deviation of three blank samples.[5]

Sensitivity improvement of the PHBA biosensor by coupling HosA with cross-triggered SDA. To optimize the primer (P1) concentration for cross-triggered SDA system, the mixture containing 100 nM T2 and different concentrations of P1 was annealed. Next, 0.4 mM dNTPs, 5 μM ThT, 100 nM T3, 0.25 U KF and 0.3 U Nb.BbvCI were added to a total volume of 100 μL. Then, the reaction mixture was incubated at 37 °C and real-time fluorescence was monitored. To optimize HosA concentration for cross-triggered SDA system of PHBA biosensor, 100 nM T2 and 200 pM P1 mixture was annealed. Next, 0.4 mM dNTPs, 5 μM ThT, 100 nM T3, and different amounts of HosA were added. The mixture

was incubated at 25 °C for 20 min. Then, 0.25 U KF and 0.3 U Nb.BbvCI were added. The reaction mixture was incubated at 37 °C, and real-time fluorescence was monitored. To characterize the PHBA biosensor, 100 nM T2 and 200 pM P1 mixture were annealed. Then, 0.4 mM dNTPs, 5 μM ThT, 100 nM T3, 10 nM HosA, and different concentrations of PHBA were added. The mixture was incubated at 25 °C for 20 min. Next, 0.25 U KF and 0.3 U Nb.BbvCI were added. The reaction mixture was incubated at 37 °C and real-time fluorescence was monitored. For the construction of the UA biosensor, procedures were the same as those for the configuration of PHBA biosensor.

Colorimetric measurements assay. After SDA reaction, the mixture was incubated with 100 nM hemin in KT buffer (100 mM MES, 50 mM Tris-HCl, 40 mM KCl, 0.05% Triton X-100, 1% DMSO, pH 6.2) for 2 hours at room temperature to allow the DNA strands to form the DNAzymes. To trigger the catalytic oxidation of $ABTS^{2-}$, the solutions of 3.6 mM $ABTS^{2-}$ and 3.6 mM H_2O_2 were simultaneously added to the DNAzymes solution and diluted with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, 0.1 M NaCl, pH 7.8) to a total volume of 100 μL. Data were obtained by recording the absorbance from 380 nm to 500 nm after 5 min of the reaction, and the pictures were taken by Canon G12.

Detection of PHBA and UA by high performance liquid chromatograph (HPLC). For the detection of PHBA in experimental samples, PHBA was prepared in PBS buffer (0.1 mM, pH 7.4) and carefully filtrated through 0.22 μm cellulose acetate membrane. Then, PHBA was analyzed by HPLC-20AT (Shimadzu) equipped with an SPD-20A UV detector. Zorbax SB-Aq (Agilent; 250 mm × 4.6 mm, 5 μm) was used as the analytical column. 0.1% phosphonic acid as the mobile phase A and acetonitrile as the mobile phase B were run at a flow rate of 1.0 mL/min. Injection volume was 20 μL. The detection of PHBA was carried out by UV absorbance at 254 nm. The detection of UA in in

experimental samples and commercial human serum samples using HPLC was performed as previously described.[4]

Supporting Figures

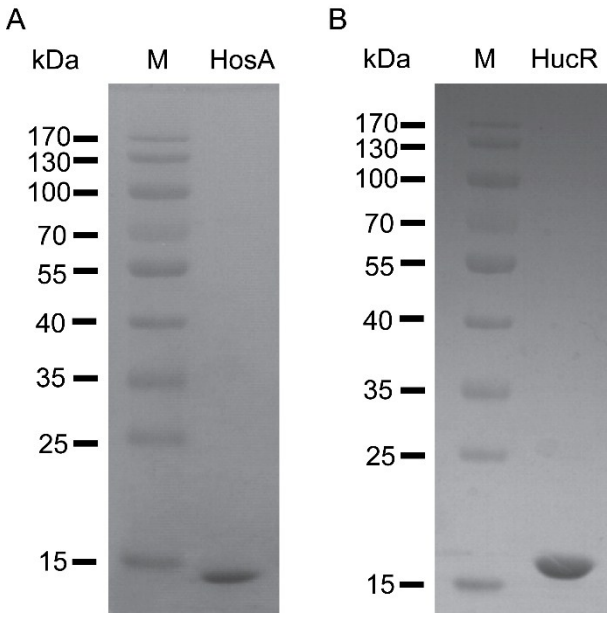

Figure S1 | Purification of recombinant aTFs. (A) Recombinant HosA examined by SDS-PAGE. (B) Recombinant HucR examined by SDS-PAGE. Lane M, protein marker (15-170 kD).

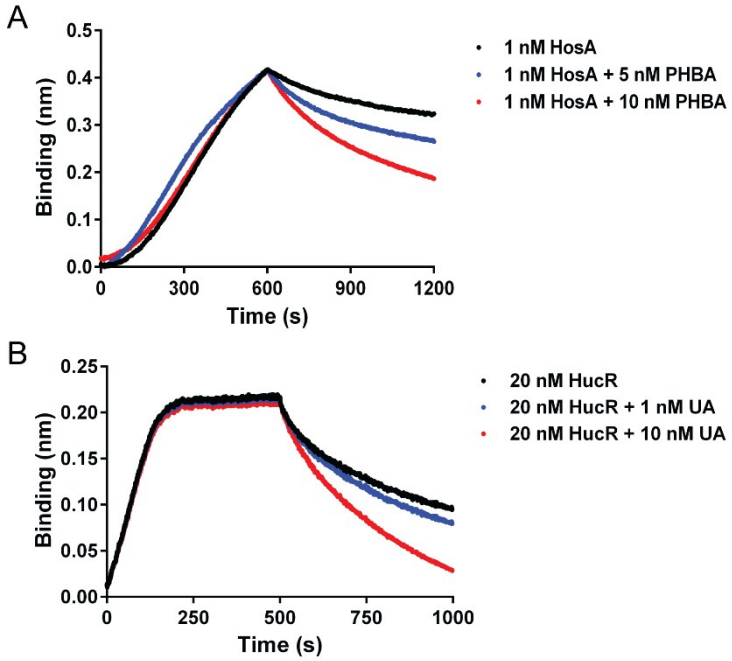

Figure S**2 | Verification of activity of** recombinant **aTFs.** (A) Sensogram of interaction between HosA and its TFBS with or without PHBA by BLI. Biotin labelled DNA containing TFBS of HosA (10 ng/μL) was loaded on the streptavidin sensor, and then interacted with 1.0 nM HosA (black line), 1.0 nM HosA and 5 nM PHBA (blue line), 1.0 nM HosA and 10 nM PHBA (red line). (B) Sensogram of interaction between HucR and its TFBS with or without UA by BLI. Bio-DNA containing TFBS of HucR (10 ng/μL) was loaded on the streptavidin sensor, and then interacted with 1.0 nM HucR (black line), 20 nM HucR and 1 nM UA (blue line), 20 nM HucR and 10 nM UA (red line).

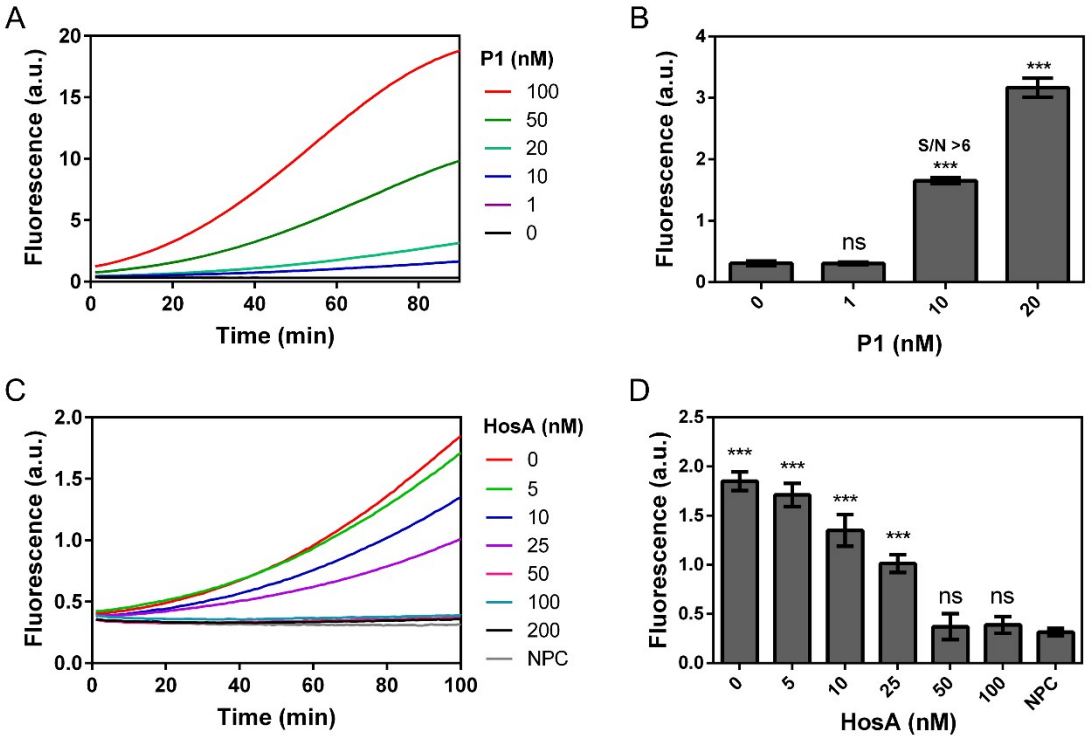

Figure S3 | **Optimizing the concentrations of components for PHBA biosensor coupling SDA reaction.** (A) Fluorescence–time curves for different concentrations of primer P1. (B) Comparison of end-point fluorescence signals of SDA reaction containing different concentrations of primer P1. (C) Fluorescence–time curves for different concentrations of HosA. NPC was the non-primer control. (D) Comparison of end-point fluorescence signals of SDA reaction containing different concentrations of HosA. Significance analysis was conducted by one-way ANOVA. Significance analysis was conducted by one-way ANOVA. Significant difference was labelled with asterisk (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$)，and "ns" indicated no significant difference. For (B) and (D), data were expressed as mean ± standard deviation (SD).

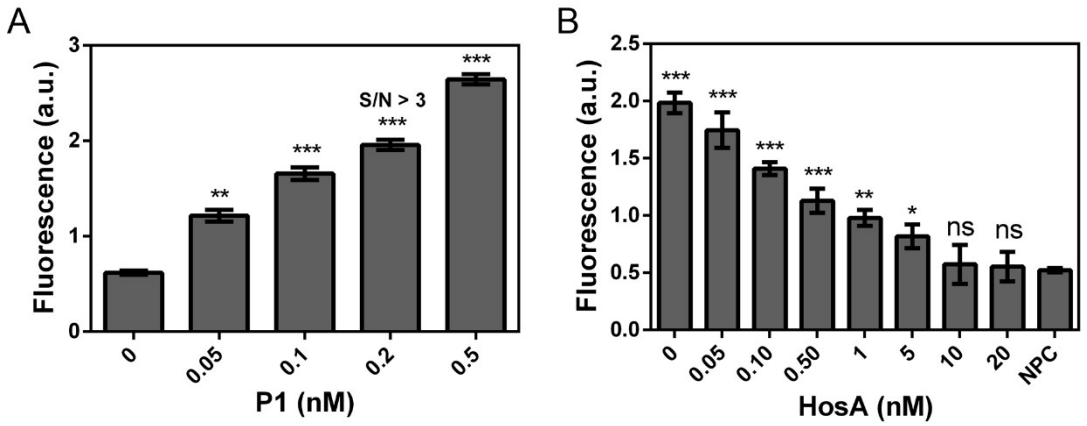

Figure S4 | **Optimizing the concentrations of components for PHBA biosensor coupling cross-triggered SDA reaction.** (A) Comparison of end-point fluorescence signals of cross-triggered SDA reaction containing different concentrations of primer P1. (B) Comparison of end-point fluorescence signals of cross-triggered SDA reaction containing different concentrations of HosA. Results were expressed as mean ± SD. Significance analysis was conducted by one-way ANOVA. Significant difference was labelled with asterisk (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$), and no significant difference was indicated with "ns".

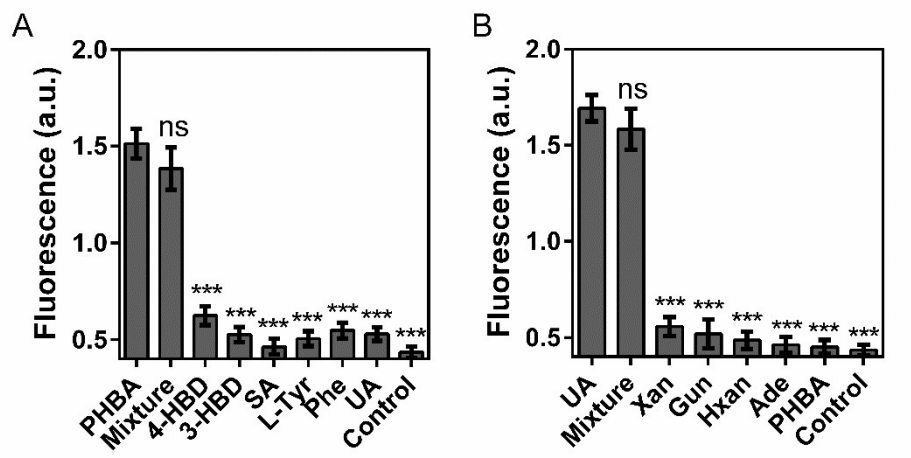

Figure S5 | Evaluation of the specificity of our developed biosensors. Here biosensors coupling cross-triggered SDA reaction with fluorescence output was used for the test. (A) Specificity of the PHBA biosensor. PHBA, *p*-hydroxybenzoic acid. 4-HBD, 4-hydroxybenzaldehyde. 3-HBD, 3-hydroxybenzaldehyde. SA, salicylic acid. L-Tyr, L-tyrosine. Phe, phenylalanine. UA, uric acid. For each chemical, 100 nM was used. Mixture contained 100 nM each chemical of 4-HBA, 4-HBD, 3-HBD, SA, L-Tyr and Phe. No analyte was used as the control. (B) Specificity of UA biosensor. UA, uric acid. Xan, Xanthine. Gua, Guanine. Hxan, hypoxanthine. Ade, Adenine. PHBA, *p*-hydroxybenzoic acid. For each chemical, 200 nM was used. Mixture contained 200 nM each chemicals of UA, Xan, Gua, Hxan, and Ade. No analyte was used as the control. All the data were expressed as mean ± SD. Significance analysis was conducted by one-way ANOVA. Significant difference was labelled with asterisk (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$), and no significant difference was indicated with "ns".

Supporting Tables

Table S1 Primers and oligonucleotides used in this work.

Primer		Sequence[a] (5′ - 3′)
Usage	Name	
Preparation of the DNA_{TFBS} of HosA	DNA_{TFBS}-F1	Biotin-CAGAGGCGTATTTTAATAATAACGTTCGTATACGAACAATTAAGAGAATAAACATGAG
	DNA_{TFBS}-R1	CTCATTGTTTATTCTCTTAATTGTTCGTATACGAACGTTATTATTAAAATACGCCTCTG
Preparation of the DNA_{TFBS} of HucR	DNA_{TFBS}-F2	Biotin-CAGAGGCGTATTTTAATAATAACTACTTAGATGTCTACCTAAATTAAGAGAATAAACATGAG
	DNA_{TFBS}-R2	CTCATTGTTTATTCTCTTAATTTAGGTAGACATCTAAGTAGTTATTATTAAAATACGCCTCTG
SDA	T1	AACCCAACCCGCCCTACCCAA***CCTCAGC***GTTCGTATACGAACACAACG-P
	T2	AACCCAACCCGCCCTACCCAA***CCTCAGC***ACAACGCTACAA***CCTCAGC***AGTTCGTATACGAACACAACG-P
	T3	AACCCAACCCGCCCTACCCAA***CCTCAGC***AGTTCGTATACGA***CCTCAGC***ACAACGCTACAA-P
	P1	CGTTGTGTTCGTATACGAAC
	HucR-T2	AACCCAACCCGCCCTACCCAA***CCTCAGC***ACAACGCTACAA***CCTCAGC***ATACTTAGATGTCTACCTAACA-P
	HucR-T3	AACCCAACCCGCCCTACCCAA***CCTCAGC***ATACTTAGATGTC***CCTCAGC***ACAACGCTACAA-P
	HucR-P	TGTTAGGTAGACATCTAAGTA

[a]The TFBS of aTFs is indicated in red. The complementary sequence of G-quadruplex is indicated in blue. The italic and black bold letters are the restriction site of Nb.BbvCI.

Table S2 The optimized coding sequence of aTFs used in this work.

aTFs	Coding sequence (5′ - 3′)
hosA (444 bp)	ATGATCGCAGCAGATAGCCTGCCGGGTGTTTATATGGCACTGCGTAATAAAGCATTT CATCAGCTGCGTCAGCTGTTTCAGCAGCATACCGCACGTTGGCAGCATGAACTGCCG GATCTGACCAAACCGCAGTATGCAGTTATGCGTGCAATTGCAGATAAACCGGGTATT GAACAGGTTGCACTGATTGAAGCAGCAGTTAGCACCAAAGCAACCCTGGCAGAAAT GCTGGCACGTATGGAAAATCGTGGTCTGGTTCGTCGTGAACATGATGCAGCAGATAA ACGTCGTCGTTTTGTTTGGCTGACCGCAGAAGGTGAAAAAGTTCTGGCAGCAGCAAT TCCGATTGGTGATAGCGTTGATGCAGAATTTCTGGGTCGTCTGAGCGGTGAAGAACA GGAACTGTTTATGCAGCTGGTTCGTAAAATGATGAGCAAACTCGAGTGA
hucR (543 bp)	ATGAGCGCGCGCATGGATAACGATACTGCTGCGCTGCTGGAACGTATTCGTAGCGAC TGGGCACGCCTGAACCATGGTCAAGGTCCGGATTCCGATGGTCTGACCCCAAGCGCT GGCCCGATGCTGACCCTGCTGCTGCTGGAGCGTCTGCACGCTGCACTGGGTCGTGAA ATTGAACGTACTTATGCGGCATCTGGCCTGAACGCTGCGGGTTGGGACCTGCTGCTG ACCCTGTACCGTTCTGCTCCGCCGGAAGGTCTGCGCCCGACCGAACTGTCCGCCCTGG CGGCGATCAGCGGCCCGTCCACTTCTAACCGCATTGTTCGTCTTCTGGAAAAAGGTCT GATTGAACGCCGCGAAGATGAGCGCGACCGCCGTTCTGCAAGCATCCGTCTGACTCC GCAGGGCCGTGCGCTGGTTACTCATCTGCTGCCAGCTCACCTGGCGACCACTCAGCG TGTGCTGGCGCCGCTGTCTGCGCAGGAACAGCGTACTCTGGAAGAACTGGCTGGTCG TATGCTGGCGGGCCTCGAACAGGGTGTTTGA

Table S3 Comparison with previously reported UA biosensors.

Recognition elements	Transducing elemets	Linear range (μM)	LOD (μM)	Time and references
Uricase	UCNPs	20–850	6.7	2016[6]
Uricase	PdAg NFs/rGO/GCE	1–150	0.081	2015[7]
Uricase	PTH/CNT/CFE	2–100	0.08	2014[8]
Uricase	GSH-capped CdTe NPs	0.22–6	0.1	2016[9]
Uricase	Naf/Fc/GCE	0.5–600	0.23	2015[10]
Uricase	ZnO:N	50–1000	40	2013[11]
Uricase	CuO/Pt/glass	50–1000	140	2012[12]
Uricase	Ni/NiO/ITO/glass	50–1000	30	2014[13]
Uricase	Graphite/$Ru(bpy)_3^{2+}$	10–1000	3.1	2013[14]
Uricase	Chi-CNTsNF/AgNPs/Au	1–400	1	2014[15]
Uricase	NiO/Pt/Ti/glass	50–1000	40	2014[16]
Uricase	HRP-CdS quantum dots	125–1000	125	2015[17]
Uricase	PANI-Ppy/Au	2.5–85	1	2008[18]
Uricase	PPy-Fc/Pt	1–50	0.5	2006[19]
Uricase	c-MWCNT/GEL/PVF/GCE	0.2–710	0.023	2015[20]
Uricase	CS/HRP–PD/Au_{plate}/Au	1–1250	0.1	2016[21]
Uricase	UCNPs/4-AAP/TOPS/$ZnFe_2O_4$	10–1000	2.86	2016[22]
Uricase	S, N co-doped C-dots	0.08–10	0.07	2016[23]
Uricase	BSA–AuNCs	10–800	6.6	2016[24]
Uricase	Nafon/Uricase-ZNRs/Ag/glass	10–4560	0.005	2017[25]
Uricase	TOPS/4-AAP	2.0–40	0.5	2017[26]
Uricase	G-quadruplex/DNAzyme/GODs	2–300	0.5	2017[27]
Uricase	CeO2-x/C/rGO nanocomposites	49.8–1050.0	2	2018[28]
Uricase	Mn^{2+}-doped $NaYF_4$:Yb,Er UCNPs	0.004–10	0.0019	2018[29]
Uricase	4-AAP/TOPS	10–1000	2	2018[30]
aTF HucR	Alpha technology	0.001–30	0.001	2016[4]
aTF HucR	SDA reaction	0.020–1	0.00565	This work

Table S4 Evaluation of the performance of PHBA and UA biosensors in experimental samples.

Approach	Accuracy (%)						precision (RSD%)	recovery (%)
	PHBA samples (nM)			UA samples (nM)				
	5	50	200	50	200	800		
HPLC	100	100	100	100	100	100	/	/
PHBA biosensor	106.23	94.77	104.52	/	/	/	5.04	103.8
UA biosensor	/	/	/	95.17	97.25	107.34	5.32	98.2

Note: Here PHBA biosensor coupling cross-triggered SDA reaction with DNAzyme output was used for the test. Accuracy was determined by comparing the concentration determined by biosensors with that obtained by HPLC, which was fixed to 100%. For each biosensor, precision was the relative standard derivation (RSD) of accuracies generated from four different samples. Recovery tests were implemented by using 300 nM of PHBA or 1 μM of UA. For each sample, measurement was repeated for six time.

Table S5 Evaluation of the performance of UA biosensor in human serum.

Approach	Concentration (μM)		Accuracy (%)		Precision (RSD%)	Recovery (%)
	Sample 1	Sample 2	Sample 1	Sample 2		
HPLC	93.35 ± 2.47	120.37 ± 6.34	100	100	/	/
UA biosensor	88.72 ± 4.03	118.51 ± 8.61	95.04	98.45	3.52	97.91

Note: Human serum with different brands (Solarbio, sample 1; LabLead, sample 2) were purchased from different suppliers (Beijing Solarbio Science & Technology Co., Ltd. and Beijing LabLead Biotech Co., Ltd.). Concentration was the average value and standard derivation of three independent tests. Accuracy was determined by comparing the concentration determined by UA biosensor with that obtained by HPLC, which was fixed to 100%. For UA biosensor, precision was the relative standard derivation (RSD) of accuracies generated from two different samples. Recovery tests were implemented by using 1 μM of UA. For each sample, measurement was repeated for six time.

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