Electronic Supplementary Information

A Multiplex Paper-Based Nanobiocatalytic System for Simultaneous Determination of Glucose and Uric Acid in Whole Blood

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Fig. S1. EDX of (A) GOx&HRP-$Cu_3(PO_4)_2$ HNFs, (B) UAO&HRP-$Cu_3(PO_4)_2$ HNFs.

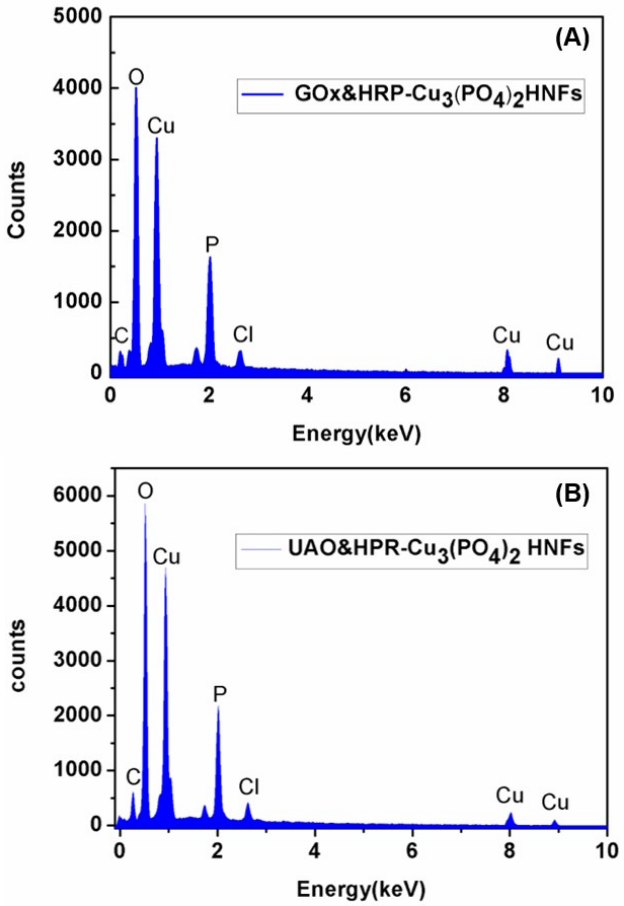


Fig. S2. XRD of (A) $Cu_3(PO_4)$, (B) GOx&HRP-$Cu_3(PO_4)_2$ HNFs, (C) UAO&HRP-$Cu_3(PO_4)_2$ HNFs.

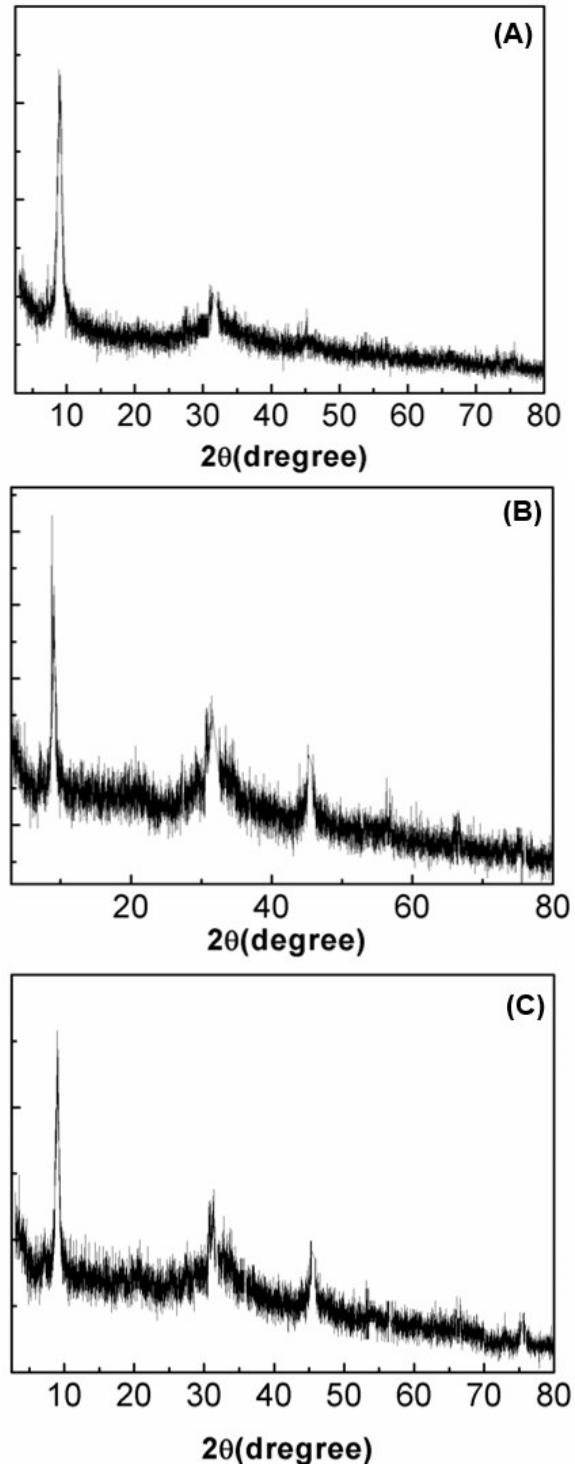


Fig. S3. SEM images of UAO&HRP-$Cu_3(PO_4)_2$ HNFs at different concentration of UAO (A) 0.0 mg·mL^{-1}, (B) 0.5 mg·mL^{-1}, (C) 1.0 mg·mL^{-1} and (D) 2.0 mg ·mL^{-1}.

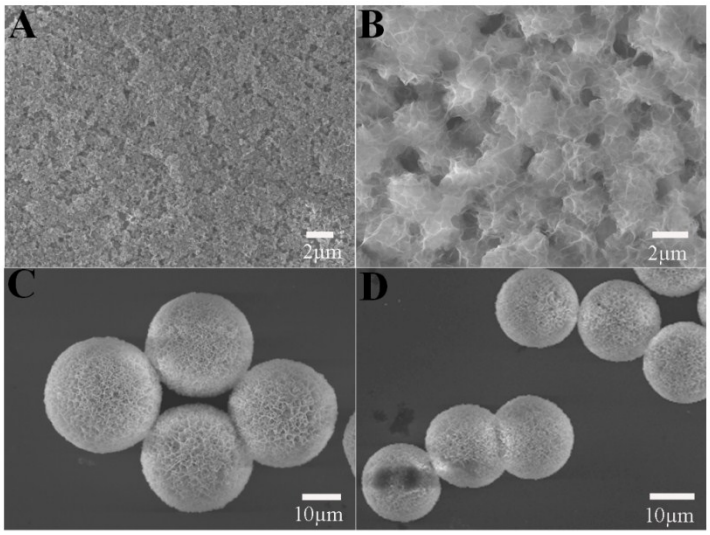


Fig. S4. Growing mechanism of UAO&HRP-$Cu_3(PO_4)_2$ HNFs. (A-F) SEM images of UAO&HRP-$Cu_3(PO_4)_2$ HNFs after growing for (A) 6 h, (B) 12 h, (C) 24 h, (D) 36 h, (E) 48 h and (F) 72 h. The diameter for nanoflowers obtained in different cases is about 4 μm，8 μm，12 μm，16 μm，20 μm and 22 μm, respectively.

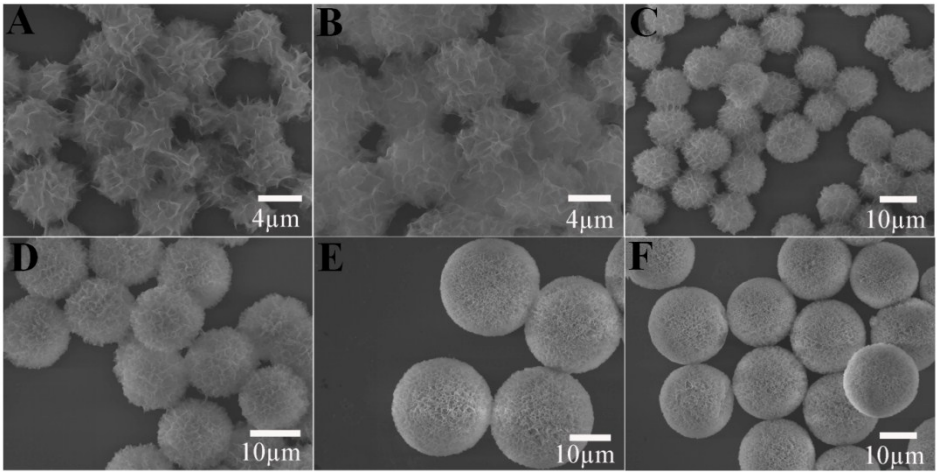


Fig. S5. SEM images of as-prepared UAO&HRP dual-enzyme hybrid nanoflowers in absence of $CuSO_4$.

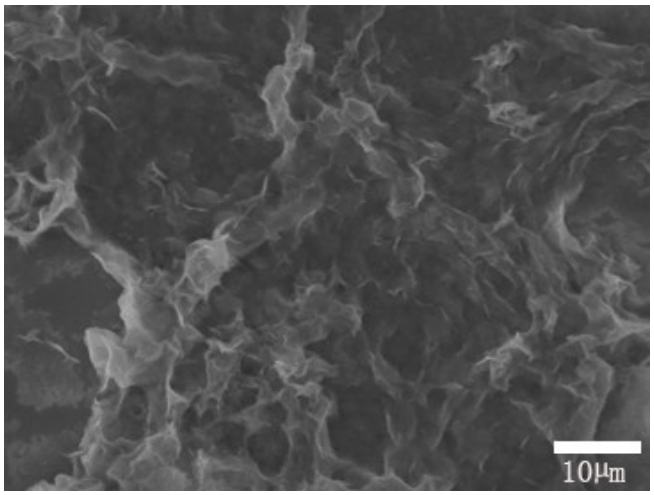


Fig. S6. Optimization of wax melting time of uPADs with duration of 30 s, 60 s, and 120 s for the front and the back.

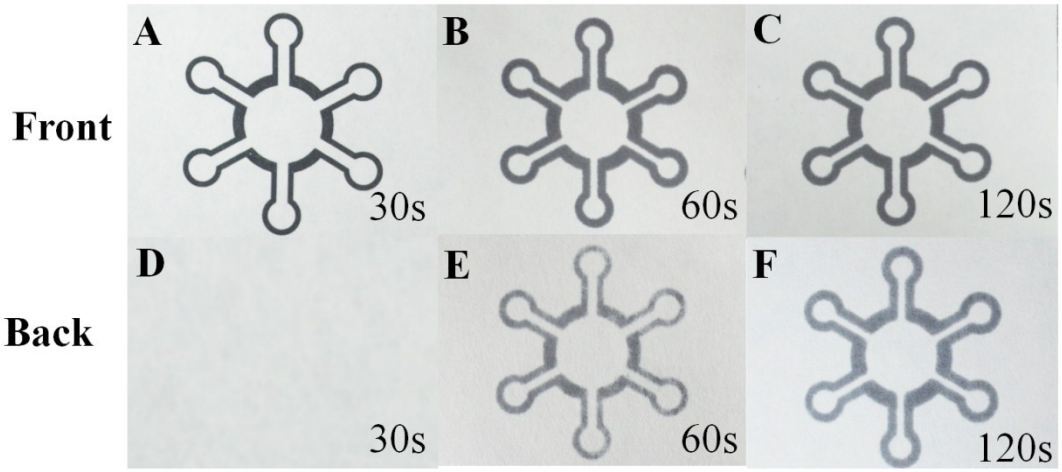


Fig. S7. Optimization of reagent volumes in detecting zone (A) and sample volumes in central zone (B, C, D). All channels were fabricated to be a 6 mm at length and 3 mm at width. And their diameter values for detection zone and central zones were 6 and 15 mm, respectively.

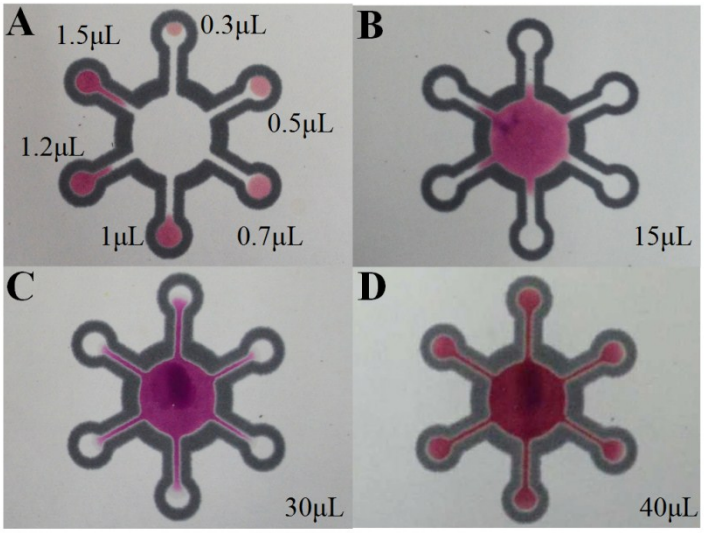


Fig. S8. Steady-state kinetics assay of free enzymes (without copper) and GOx&HRP-$Cu_3(PO_4)_2$ and UAOx&HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers (HNFs). Reaction velocity are plotted with various glucose concentrations (A and C) and various uric acid concentrations (B and D).

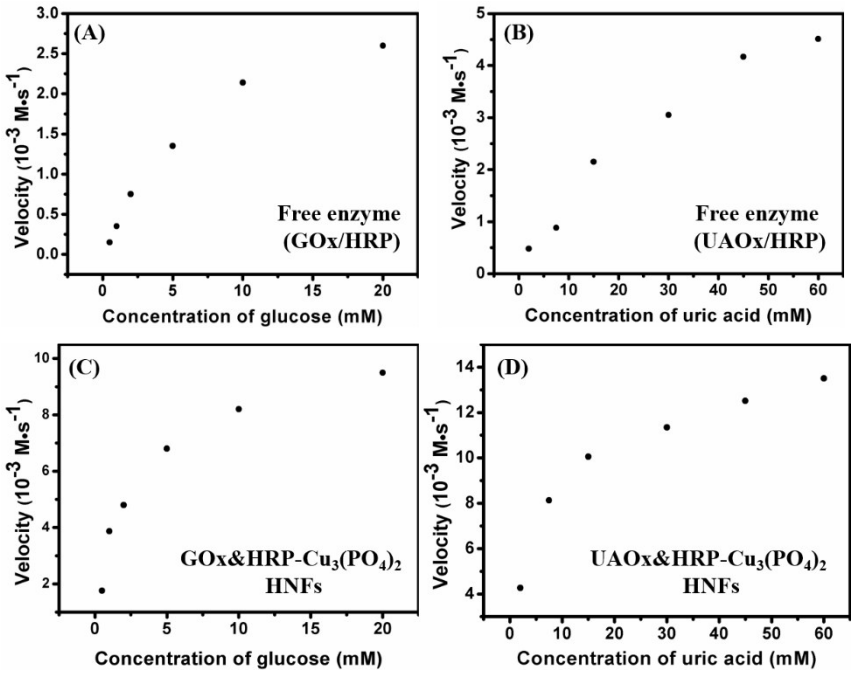


Fig. S9. Photograph of μPADs used for detection of 2 mM glucose and 2 mM uric acid with GOx&HRP-$Cu_3(PO_4)_2$ and UAOx&HRP-$Cu_3(PO_4)_2$ nanoflowers prepared using different reaction times. (A) 24 h, (B) 48 h.

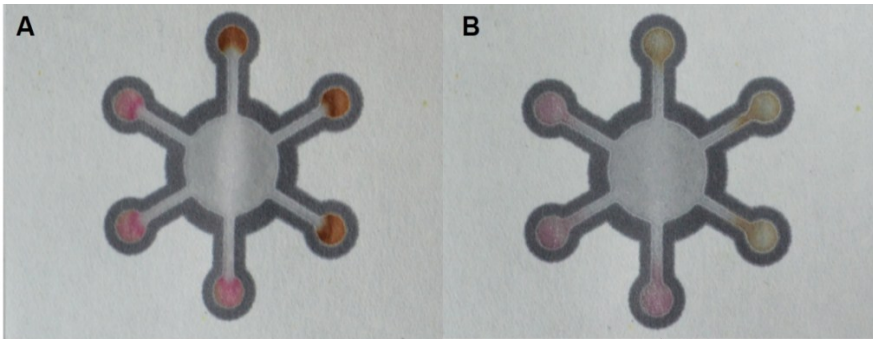


Fig. S10. (A) Effects of pH on the catalytic activities of GOx&HRP-$Cu_3(PO_4)_2$ (black column) and UAOx&HRP-$Cu_3(PO_4)_2$ HNFs (gray column); (B) Effects of different buffers on the catalytic activities of GOx&HRP-$Cu_3(PO_4)_2$ (black column) and UAOx&HRP-$Cu_3(PO_4)_2$ HNFs (gray column).

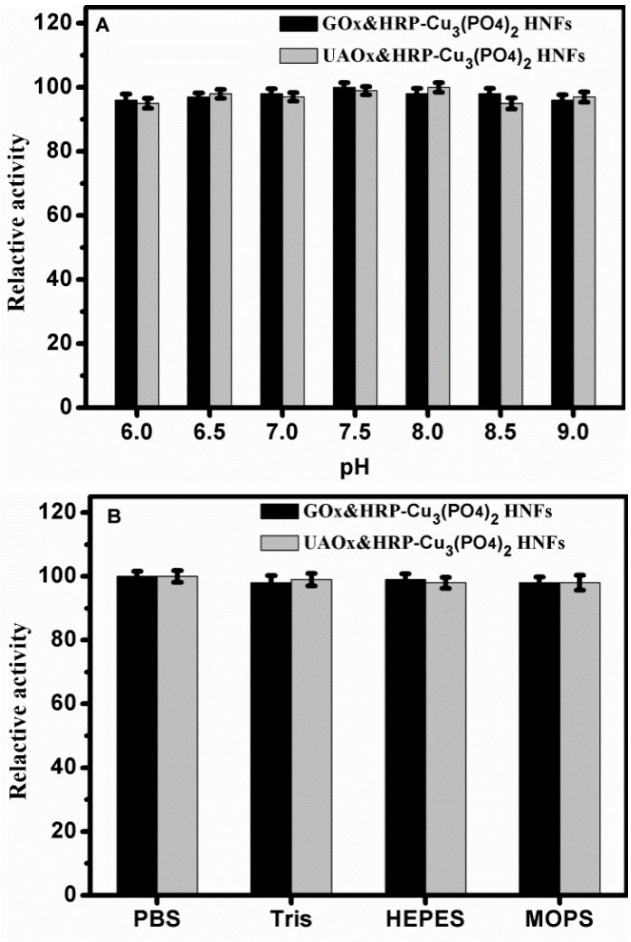


Fig. S11. Image of μPADs used for the detection of glucose and uric acid in the whole blood samples. The whole blood samples all were diluted 5 times for detection of glucose and uric acid.

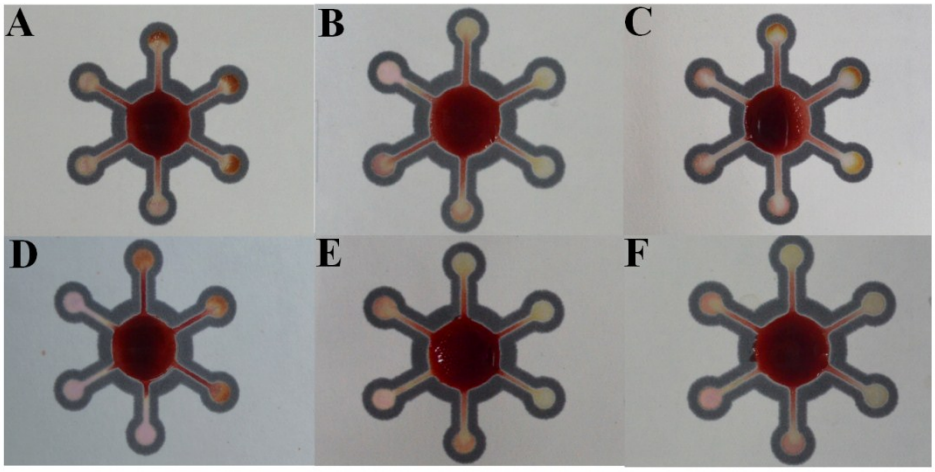


Fig. S12. Comparison of analysis performance of the nanoflower-based μPADs for glucose and uric acid detection in the whole blood (A) and serum samples (B). Before testing glucose and uric acid in serum sample, anticoagulant was first added in the same whole blood to prevent blood clotting and then serum sample was obtained by centrifugation.

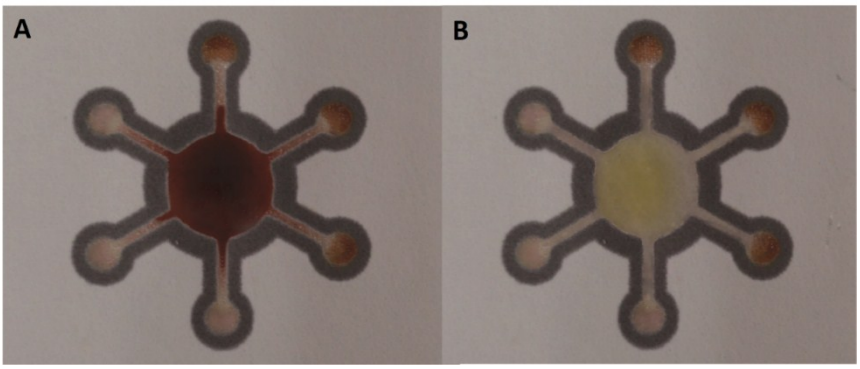


Table S1. Comparison of the apparent Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) of the catalytic reaction between GOx&HRP-$Cu_3(PO_4)_2$ and UAOx&HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers and free enzymes (without copper).

Catalyst	Substrate	K_m (mM)	V_{max} (M/s)
Free enzyme (GOx/HRP)	Glucose	5.5	3.3×10^{-3}
GOx&HRP-$Cu_3(PO_4)_2$ HNFs	Glucose	1.7	9.5×10^{-3}
Free enzyme (UAOx/HRP)	Uric acid	15.1	3.9×10^{-3}
UAOx&HRP-$Cu_3(PO_4)_2$ HNFs	Uric acid	4.2	13.2×10^{-3}

Table S2. Effects of zeta potential on the catalytic activities of GOx&HRP-$Cu_3(PO_4)_2$ and UAOx&HRP-$Cu_3(PO_4)_2$ HNFs. "I" refers to the gray intensity response of the glucose or uric acid; "I_0" refers to the gray intensity response of blank sample. The concentration of glucose and UA are 0.2 and 1mM, respectively.

Dual-enzyme hybrid nanoflowers	Reaction Time (h)	Zeta potential (mV)	Catalytic activity ($(I-I_0)/I_0$)
GOx&HRP-$Cu_3(PO_4)_2$ HNFs	12	-15.2	0.374
	24	-23.4	0.517
	36	-16.72	0.439
	48	-15.97	0.396
UAO&HRP-$Cu_3(PO_4)_2$ HNFs	12	-6.73	0.386
	24	-8.81	0.547
	36	-6.9	0.456
	48	-5.92	0.332

Table S3. Comparison of analytical performance of some assays for glucose and uric acid detection.

Analysis methods	Object	Sensing time	Sensing range	LOD	References
Electrochemical analysis	glucose	Not given	Not given	100 nM	S1
	uric acid	Not given	Not given	100 nM	
Electrochemical analysis	glucose	Not given	0.08–5 mM	0.03 mM	S2
	H_2O_2	Not given	0.075–10 mM	0.041 mM	
Electrochemical analysis	glucose	3−4 s	2.25−30 mM	2.25 mM	S3
	uric acid	4−5 s	400–930 μM	400 μM	
Electrochemical analysis	glucose	Not given	0−1 mM	0.18 mM	S4
	uric acid	Not given	0−1 mM	0.11 mM	
Electrochemical analysis	glucose	Not given	0.42−50 mM	0.14 mM	S5
	uric acid	Not given	1.4−47 mM	0.52 mM	
Colorimetry analysis	glucose	Not given	4.5−5.8 mM	23 μM	S6
	uric acid	Not given	130−380 μM	37 μM	
Colorimetry analysis	glucose	30 min	0.3−1.0 mM	0.213 mM	S7
	uric acid	30 min	0.3−1.0 mM	0.287 mM	
Colorimetry analysis	glucose	Not given	0−12 mM	0.7 mM	S8
	uric acid	Not given	0−5 mM	0.3 mM	
Colorimetry analysis	glucose	10 min	0.5–20 mM	0.5 mM	S9
	uric acid	10 min	0.1–7 mM	0.1 mM	
FRET probes	glucose	10 min	0.1−1.0 μM	0.05 mM	S10
	uric acid	10 min	25–500 nM	0.025 mM	
GQDs-based fluorescent probe	glucose	60 min	0.1−30 μM	0.021 μM	S11
	uric acid	60 min	0.1−45 μM	0.026 μM	
Microfluidic thread-based analytical device	glucose	10 min	1.0−100 μM	0.1 μM	S12
	uric acid	10 min	10−100 μM	3 μM	
Paper-based nanobiocatalytic system	glucose	5 min	0.1−2 mM	60 μM	*This work*
	uric acid	5 min	0.1−10 mM	25 μM	

Table S4. Stability of our devices was kept at room temperature (25 ◦C). Background signals were obtained by spotting 0.1 M phosphate buffer solution while standard test signals were obtained by spotting 2 mM glucose and 8 mM uric acid. The grayscale values were used to analyze the color intensity and gradient by Image J software.

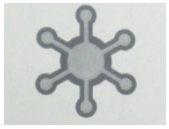
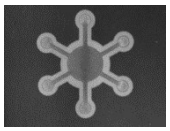
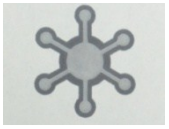
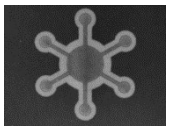
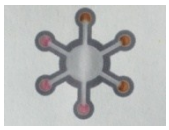
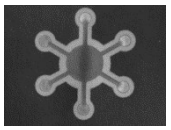
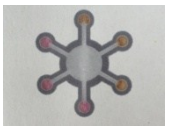
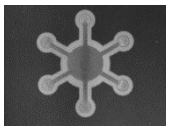
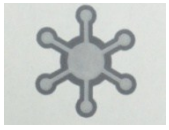
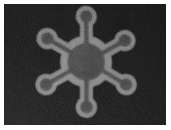
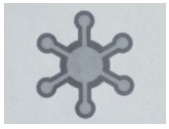
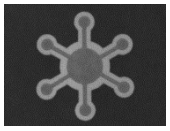
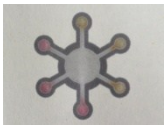
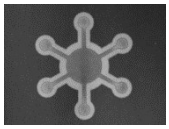
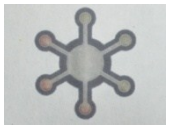
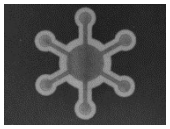
Standard test	Storage for 8 days at room temperature (25 ℃)		Storage for 12 days at room temperature (25 ℃)	
	Image of μPADs	Grayscale image	Image of μPADs	Grayscale image
Standard sample I				
Hybird nanoflowers				
Standard sample II				
Free GOx and HRP				

Table S5. The simultaneous detection of glucose and uric acid in human whole blood.

Samples		Certified concentration (mM)	Our propsed method (mM)	RSD (n=3, %)
Blood sample A	glucose	9.87	9.81	3.1
	uric acid	0.23	0.19	3.5
Blood sample B	glucose	3.50	3.22	4.1
	uric acid	0.46	0.41	3.6
Blood sample C	glucose	6.42	6.34	3.4
	uric acid	0.35	0.38	3.0
Blood sample D	glucose	7.23	7.15	3.6
	uric acid	0.29	0.25	4.5
Blood sample E	glucose	2.30	2.92	3.3
	uric acid	0.33	0.34	3.5
Blood sample F	glucose	3.50	3.18	4.2
	uric acid	0.58	0.54	3.2

Notes and references

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