Electronic Supplementary Information for

An Inkjet-Printed Bioactive Paper Sensor that Reports ATP through Odour Generation

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1. Materials

Apotryptophanase from *Escherichia coli* (Apo-TPase, EC 4.1.99.1), Kovac's reagent for indoles, Adenosine 5'-triphosphate (ATP) disodium salt, Pyridoxal, L-tryptophan, *S*-methyl-L-cysteine, Triton X-100, Pyridoxal 5'-phosphate (PLP) hydrate, Whatman #1 filter paper, Sodium silicate solution (SS, 14% NaOH, 27% SiO_2) and Dowex 50WX8-100 ion-exchange resin were purchased from Sigma-Aldrich (Oakville, ON). Anhydrous glycerol, Hispur purification column (Product number 89969), Glassine paper and 1-butanol were purchased from Fisher Scientific (Toronto, ON). Nanosep centrifuge devices were purchased from Pall Corporation.

KDP buffer contains 0.2 M potassium phosphate (pH 7.8) and 6.7 mM ammonium sulfate. Apo-TPase reaction solution for indole generation was prepared by dissolving L-tryptophan (10 mM) and PLP (0.2 mM) in KDP buffer.

Bienzyme reaction solution consisted of KDP buffer containing 0.6 mM $MgCl_2$, 10 mM L-tryptophan, 0.2 mM ATP and 0.1 mg/mL pyridoxal, while *S*-methyl-L-cysteine was used as the substrate instead to generate methyl mercaptan.

"Selection" brand Orange punch was purchased at local grocery store.

2. PKase preparation

Pyridoxal kinase (PKase, EC 2.7.1.35) was prepared according to the following method described in the literature.[1] *E. coli* cells carrying the human pdxK gene (pET22-hPLK) were grown in Luria Birtani media at 30 °C, 130 rpm for 8 h, followed by with the addition of 0.5 mM IPTG. Then, cells were cultured for an additional 7 h at 30 °C, 130 rpm before harvesting. The cell pellets were then washed and ruptured. Subsequently, the His-tagged pyridoxal kinase was purified by processing the supernatant with a Hispur purification column and enriched using

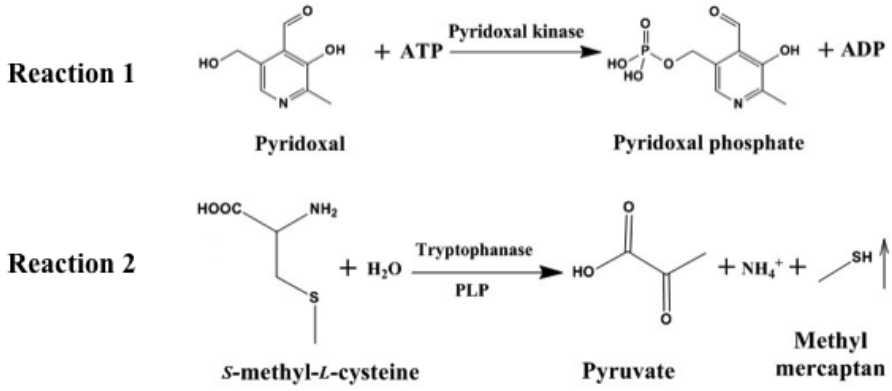
Nanosep centrifuge devices (30 K). The concentration of purified PKase was determined using Bradford reagent.

3. Apo-TPase preparation

Some batches of commercial Apo-TPase may have contained the cofactor, PLP, because they had not been adequately purified. Therefore, purchased TPase was dissolved and tested before use. Purification was sometimes needed to obtain pure Apo-TPase. The purification followed the former reports.[1, 2] Apo-TPase solution at a concentration of 1.5 mg/mL was prepared with KDP buffer, while Apo-TPase-glycerol (ATPG) was obtained by mixing Apo-TPase solution at a concentration of 3 mg/mL with equal volume of anhydrous glycerol. The two solutions at the same concentration (1.5 mg/mL) were used throughout unless otherwise indicated.

4. Mechanism of methyl mercaptan generation

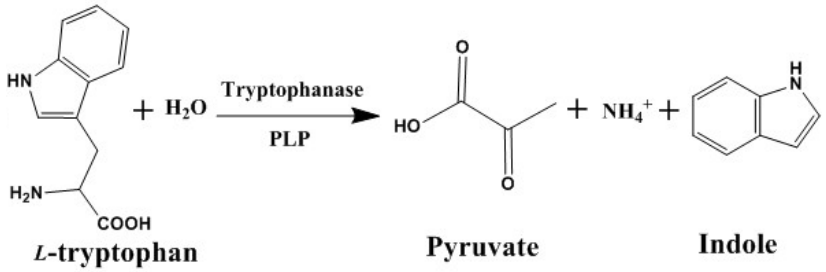
Methyl mercaptan generation can be described by reaction 1 and reaction 2. The two closely related enzymatic reactions are shown below.



5. Indole generation mechanism and determination

Indole generation

Although the reaction mechanism of indole generation is similar to methyl mercaptan generation, the substrate involved in the former is L-tryptophan rather than *S*-methyl-L-cysteine. The indole generation reaction is shown below.



Indole determination

The sample (700 μL) was mixed with 700 μL of water saturated 1-butanol, followed by centrifugation at 1000 g for 30 sec. Then, 400 μL of the supernatant was collected and mixed with 400 μL of Kovac's reagent. After incubating for 5 min, the absorbance of the samples was measured at 570 nm using Beckman Coulter DU800 spectrophotometer. The concentration of indole could be obtained via referring the prepared standard curve.

6. Influence of glycerol for immobilized Apo-TPase activity

In the primary stage, the paper was manually prepared to investigate the feasibility of smell-generating paper. A 15.6 μL droplet of Apo-TPase or ATPG was dropped onto one paper strip with the size of 2.5 cm × 2.5 cm. The resulting strips were allowed to dry for 0.5, 5, 10, 15 or 20 minutes. After the drying, they were placed in the Apo-TPase reaction solution (700 μL) to initiate the enzymatic reaction. The reaction was allowed to proceed for 1 hour and then the amount of indole generated was determined. The experimental results shown in Fig. S1 indicate that no substantial change was detected in the immobilized ATPG activity

(line ATPG) after drying at room temperature for 20 min, compared with the rapid decrease in enzyme activity without glycerol (line Apo-TPase). This makes the preparation of the smell-generating paper successful within an acceptable period of time.

7. Enzyme inks preparation

When preparing enzymes inks, glycerol and Triton X-100 were used to adjust the viscosity and surface tension for the purpose of printing.[3] Thus, ATPG and PKase in glycerol (PKG) were prepared via mixing Apo-TPase or PKase with the same volume of anhydrous glycerol. Then, Triton X-100 was added. The resulting solutions were used as the enzyme inks throughout the experiments unless otherwise indicated. The final concentrations of Apo-TPase, PKase and Triton X-100 were 1.5 mg/mL, 0.5 mg/mL and 0.02 wt%, respectively. The activity of PKase in glycerol (PKG) and influence of Triton X-100 were determined for printing purposes. The results (Fig. S2) show that glycerol and Triton X-100 do not deactivate the enzymes and they can be used to prepare enzyme inks with proper properties for printing,

8. Sol-gel material ink preparation and characterization

Sodium silicate sols were prepared following the procedure described by Brennan's group.[3] Sodium silicate solution (2.9 g) was dissolved in 10 mL of ddH_2O, and then 5 g of Dowex cation exchange resin was added. The mixture was stirred for 2 min to reach a final pH of 4, followed by filtering. The filtrate was further filtered through a 0.45 μm membrane filter. The resulting solution was mixed with glycerol and Triton X-100 to improve printing performance by controlling viscosity and surface tension.

To characterize the coatings on paper substrates, optical profilometry was conducted for paper both with and without the coating of sol-gel material. The photos and peak-to-valley roughness indicate that sol-gel material could make the paper have a uniform surface as shown Fig. S3.

9. Immobilization of enzymes via printing

The thermal inkjet printer was a Model PIXMA MP280 from Canon U.S.A, Inc. and the piezoelectric inkjet printer was a Model DMP-2800 from Fujifilm Dimatix, Inc.

During the printing, enzymes and sol-gel material inks were added into individual cartridges. The coating coverage using the two printers was controlled to be the same (closely). For thermal printing: The amount of printed enzyme ink was controlled by number of layers (via printing more times) and measured by the cartridge weight change. We did that because the cartridge-washing step was avoided during the printing process. The printing amount was also checked with the calculated amount: 14.4 mL/cm^2 per layer. By doing that we could print enzyme / sol-gel material with amounts close to the desired ones. For piezoelectric printing, Enzyme and silica sol inks were added into individual cartridges. The amount of ink printed on the paper was calculated by the drop volume and amount per unit area.

Results in Fig. S4 show a significant enzyme denaturation when using a thermal inkjet printer. When compared with the control, about 90% activity is lost in thermal inkjet printing, while only 50% activity is lost in piezoelectric inkjet printing.

10. Activity tracking of immobilized Apo-TPase

The long term stability of the enzymes in solution and on the paper strip was tested over a period of 16 days during which the enzyme solution and enzyme-contained paper strip were stored at 4 °C. Results are shown in Fig. S5.

11. Strategy for bienzyme system immobilization

The dependence of sensing capability of the paper on the order of enzyme printing was investigated. Fig. S6 shows that the optimal method for immobilization of the bienzyme system is printing the ATPG layer above the PKG layer. This might be because the Apo-TPase is the critical parameter (Fig. S7) in this system, and this enzyme should be kept close to the solution.

12. Activity tracking of bienzyme system in solution during storage

Enzyme inks stored at 4 °C were used here. The activity was determined by concentrations of generated indole. The result is shown in Fig. S8.

13. Influence of sol-gel material for the efficiency of Smell-Tell paper

Paper strips, with immobilized bienzyme system, stored in 4 °C for 12 days were used here. The experiment was set up as shown in Table S1. The reaction solution was stored in a bottle, and the enzymatic reaction was initiated by immersing paper strips into the solution. Then, the bottle was sealed and shook gently. The reaction was allowed to last for 1 hour at room temperature before panelists opened the sealed bottle to smell. The detailed results are shown in Table S2. The results indicate 80% of the samples were identified correctly even after storing for 12 days, and sol-gel-contained papers exhibited higher accuracy (90%) compared with the papers without the sol-gel layers (70%). This result shows that sodium silicate material could be used

to retain the immobilized enzyme activity and improve the human olfactory detection of ATP.

Table S1 Setup of the samples for human smell test

	Set 1			Set 2			Set 3			Set 4		
Paper Type	BE	BE	BE	BE	Plain	Plain	BSES	BSES	BSES	BSES	Plain	Plain
ATP in sample	✓	×	×	✓	✓	✓	✓	×	×	✓	✓	✓
Odor signal	Yes	No	No	Yes	No	No	Yes	No	No	Yes	No	No

BE: paper strips with bienzyme layer printed directly on the paper surface. BSES: paper strips with bienzyme layer printed between the layers of sol-gel on the paper surface. Plain: the filter paper without immobilized enzymes. The tick, '✓', means the presence of 0.2 mM ATP in the bienzyme reaction solution, while the cross, '×', indicates no ATP.

Table S2 Results of determination of smell generating test paper

	Correct Judgments of Panelist				
	1	2	3	4	5
Set 1	1	0	1	1	0
Set 2	1	1	1	0	1
Set 3	1	1	1	1	1
Set 4	1	1	0	1	1

There were three bottles for each set shown in Table S1, among which one bottle can give off the smell while the other two cannot emit the olfactory signal. Five panelists were randomly chosen in our lab to smell the gas from the bottle after the reaction. "0" indicates that the panelist did not correctly select the sample out of the three bottles in the set and "1" indicates that the panelist did correctly.

14. Human smell test for methyl mercaptan generated by different printers printed Smell-Tell paper

Filter paper (Whatman #1) was used as the paper substrate in this work. For immobilization of pure enzymes, 5 μL/cm^2 of 0.5 mg/mL PKG and 2.5 μL/cm^2 of 1.5 mg/mL ATPG were printed directly onto the paper substrate. For immobilization of enzymes within silica materials, the same amount of each enzyme was printed between two layers of 2.5μL/cm^2 sodium silicate material, which was prepared following our previous reports. Paper strips (2.5 cm x 2.5 cm in size) were used here. Control samples contained the same amount of enzymes, which were in solution.

Table S3. Human smell test for control sample

Panelist	Judgments [a]							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	1	1	1	1	1	0.14	-0.84
2	1	1	1	1	1	1	0.03	-1.54
3	1	0	1	1	1	1	0.72	-0.15
4	0	0	1	1	1	1	0.72	-0.15
5	0	1	1	1	1	1	0.14	-0.84
1	0	1	1	1	1	1	0.14	-0.84
2	0	1	1	1	1	1	0.14	-0.84
3	0	0	1	1	1	1	0.72	-0.14
4	0	1	1	1	1	1	0.14	-0.84
5	0	1	1	1	1	1	0.14	-0.84
Group BET = geometric mean, μM ATP							Average of log_{10} = -0.70	
							BET = $10^{-0.70}$ = 0.20	
							Log Stand. Dev. = 0.44	

[a] "0" indicates that the panelist identified the wrong sample in the set; "1" indicates the panelist selected the correct sample.

Table S4. Human smell test for thermal inkjet printed enzyme samples without sol-gel material (TE)

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	1	0	0	0	447.2	2.65
2	1	0	0	0	1	0	447.2	2.65
3	0	0	0	1	1	1	3.58	0.55
4	0	0	0	0	1	1	17.89	1.25
5	0	0	0	0	0	1	89.44	1.95
1	1	0	1	0	0	0	447.2	2.65
2	1	0	1	0	1	1	17.89	1.25
3	1	1	0	1	1	1	3.58	0.55
4	0	0	1	0	1	1	17.89	1.25
5	0	0	0	0	0	1	89.44	1.95
Group BET = geometric mean, μM ATP							Average of log_{10} = 1.67	
							BET = $10^{1.67}$ = 46.98	
							Log Stand. Dev. = 0.82	

Table S5. Human smell test for thermal inkjet printed enzyme samples with sol-gel material (TSES)

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	1	0	1	1	1	3.58	0.55
2	1	0	0	1	1	1	3.58	0.55
3	0	0	0	1	0	1	89.44	1.95
4	0	0	0	0	1	1	17.89	1.25
5	1	1	0	0	0	1	89.44	1.95
1	0	0	1	0	1	0	447.2	2.65
2	0	1	0	1	1	1	3.58	0.55
3	1	0	0	1	0	1	89.44	1.95
4	0	0	0	0	1	1	17.89	1.25
5	0	0	0	0	0	1	89.44	1.95
Group BET = geometric mean, μM ATP							Average of log_{10} = 1.46	
							BET = $10^{1.46}$ = 28.99	
							Log Stand. Dev. = 0.74	

Table S6. Human smell test for piezoelectric inkjet printed enzyme samples without sol-gel material (PE)

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	1	1	1	1	1	0.14	-0.84
2	0	0	0	1	1	1	3.58	0.55
3	1	1	1	1	1	1	0.03	-1.54
4	1	0	1	1	1	1	0.72	-0.15
5	0	0	1	1	1	1	0.72	-0.15
1	0	0	1	1	1	1	0.72	-0.15
2	0	0	1	1	1	1	0.72	-0.15
3	0	1	1	1	1	1	0.14	-0.84
4	0	1	1	1	1	1	0.14	-0.84
5	0	0	1	1	1	1	0.72	-0.14
Group BET = geometric mean, μM ATP							Average of log_{10} = -0.42	
							BET = $10^{-0.42}$ = 0.38	
							Log Stand. Dev. = 0.59	

Table S7. Human smell test for piezoelectric inkjet printed enzyme samples with sol-gel material (PSES)

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	1	0	1	1	1	3.58	0.55
2	0	0	1	1	1	1	0.72	-0.14
3	0	1	1	1	1	1	0.14	-0.84
4	0	1	1	1	1	1	0.14	-0.84
5	1	1	1	1	1	1	0.03	-1.54
1	1	0	1	1	1	1	0.72	-0.15
2	1	0	1	1	1	1	0.72	-0.15
3	0	1	1	1	1	1	0.14	-0.84
4	0	1	1	1	1	1	0.14	-0.84
5	0	1	1	1	1	1	0.14	-0.84
Group BET = geometric mean, μM ATP							Average of log_{10} = -0.56	
							BET = $10^{-0.56}$ = 0.27	
							Log Stand. Dev. = 0.59	

15. Details of calculations of best estimation of threshold (BET)

Here, we take table S3 as an example.

In Table S3, six different concentrations of ATP were prepared with a dilution factor of 5. The ATP concentration levels were 0.064 μM, 0.032 μM, 1.6 μM, 8 μM, 40 μM, and 200 μM. For panelist 1 of the first smell test, the best-estimate threshold is $\sqrt{0.32 \times 0.064} = 0.14$ μM ATP. For panelist 2 of the first smell test, he/she could smell the lowest concentration of ATP (0.064 μM). It is assumed that he/she would have been correct at a lower concentration level, where the dilution would have been a factor of 5, which is the space for ATP concentration levels. Consequently, the best-estimate threshold for him/her is $\sqrt{0.064 \times 0.064 / 5} = 0.03$ μM ATP. All other values follow these same calculations and different panelists received different concentration sets. The BET (the panel threshold) is then calculated as the geometric mean of best-estimate thresholds of the individual panelists.

16. Best estimation of threshold (BET) of ATP concentration of stored Smell-Tell paper

BET was calculated following the previous reports.[4] For BET of each panelist, the geometric mean of the highest concentration of ATP that was identified incorrectly and next concentration recognized correctly was calculated. The BET for the entire group (5 people took the tests two times in our case) was then determined as the geometric mean of individual BETs.

Table S8. Determination of BET of ATP concentration after stored for 0 day

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	1	1	1	3.58	0.55
2	0	0	1	1	1	1	0.72	-0.15
3	1	0	1	1	1	1	0.72	-0.15
4	0	1	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
1	0	0	0	0	1	1	17.89	1.25
2	0	0	1	1	1	1	0.72	-0.15
3	1	1	1	1	1	1	0.03	-1.54
4	0	0	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.06	
							BET = $10^{0.06}$ = 1.16	
							Log Stand. Dev. = 0.74	

Table S9. Determination of BET of ATP concentration after stored for 8 days

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	1	0	1	1	1	1	0.72	-0.15
2	0	1	1	1	1	1	0.14	-0.84
3	1	0	0	1	1	1	3.58	0.55
4	0	0	0	1	1	1	3.58	0.55
5	1	1	1	1	1	1	0.03	-1.54
1	0	0	1	0	0	0	447.21	2.65
2	0	0	1	1	1	1	0.72	-0.15
3	0	0	1	1	1	1	0.72	-0.15
4	1	1	0	1	1	1	3.58	0.55
5	1	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.13	
							BET = $10^{0.13}$ = 1.36	
							Log Stand. Dev. = 1.10	

Table S10. Determination of BET of ATP concentration after stored for 16 days

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	1	0	1	0	1	89.44	1.95
2	0	1	1	1	1	1	0.14	-0.84
3	1	1	0	1	1	0	447.21	2.65
4	1	1	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
1	0	1	0	1	0	1	89.44	1.95
2	1	1	0	1	1	1	3.58	0.55
3	0	0	1	1	1	1	0.72	-0.15
4	0	0	0	1	1	1	3.58	0.55
5	0	0	0	1	1	1	3.58	0.55
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.76	
							BET = $10^{0.76}$ = 5.80	
							Log Stand. Dev. = 1.10	

Table S11. Determination of BET of ATP concentration after stored for 24 days

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	1	0	0	1	1	1	3.58	0.55
2	1	0	1	1	1	1	0.72	-0.15
3	0	0	0	1	0	1	89.44	1.95
4	0	1	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
1	0	0	1	1	1	1	0.72	-0.15
2	0	0	1	1	1	0	0.72	-0.15
3	0	1	1	1	1	0	447.21	2.65
4	1	1	0	1	1	1	3.58	0.55
5	1	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.55	
							BET = $10^{0.55}$ = 3.58	
							Log Stand. Dev. = 0.99	

Table S12. Determination of BET of ATP concentration after stored for 32 days

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	0	1	1	17.89	1.25
2	0	0	1	0	1	1	17.89	1.25
3	0	0	0	0	1	1	17.89	1.25
4	0	1	1	1	1	1	0.72	-0.15
5	1	1	0	1	1	1	3.58	0.55
1	0	0	0	0	1	1	17.89	1.25
2	0	0	0	0	1	0	447.21	2.65
3	0	0	0	0	1	1	17.89	1.25
4	0	1	1	1	1	1	0.72	-0.15
5	1	1	0	1	1	1	3.58	0.55
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.83	
							BET = $10^{0.83}$ = 6.81	
							Log Stand. Dev. = 1.05	

Table S13. Determination of BET of ATP concentration after stored for 40 days

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	1	1	1	3.58	0.55
2	0	0	1	0	1	1	17.89	1.25
3	0	0	1	0	1	0	447.21	2.65
4	0	1	1	0	1	1	17.89	1.25
5	0	0	1	1	1	1	0.72	-0.15
1	1	0	0	1	1	1	3.58	0.55
2	0	1	0	1	1	1	3.58	0.55
3	0	0	0	1	0	1	89.44	1.95
4	0	0	0	1	1	1	3.58	0.55
5	0	1	1	1	1	1	0.14	-0.84
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.83	
							BET = $10^{0.83}$ = 6.81	
							Log Stand. Dev. = 1.00	

Table S14. Determination of BET of ATP concentration after stored for 48 days

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	1	0	0	0	0	1	17.88	1.25
2	1	0	0	1	1	1	3.58	0.55
3	0	1	1	1	1	1	0.14	-0.84
4	0	1	0	1	1	1	3.58	0.55
5	1	0	0	1	1	1	3.58	0.55
1	1	0	1	0	0	1	17.89	1.25
2	0	1	0	1	1	1	3.58	0.55
3	1	0	0	1	1	1	3.58	0.55
4	0	0	0	1	1	1	3.58	0.55
5	0	1	1	0	0	1	89.44	1.95
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.69	
							BET = $10^{0.69}$ = 4.93	
							Log Stand. Dev. = 0.72	

17. BET of ATP concentration for bienzyme solution and Smell-Tell paper using KDP buffer or orange punch

Table S15. BET of ATP concentration using bienzyme solution with KDP buffer

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	1	1	1	1	0.72	-0.15
2	1	1	1	1	1	1	0.03	-1.54
3	0	1	1	1	1	1	0.14	-0.84
4	0	0	1	1	1	1	0.72	-0.15
5	0	0	1	1	1	1	0.72	-0.15
1	0	0	1	1	1	1	0.03	-1.54
2	0	0	1	1	1	1	0.72	-0.15
3	1	1	1	1	1	1	0.03	-1.54
4	1	1	1	1	1	1	0.03	-1.54
5	0	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = -0.77	
							BET = $10^{-0.77}$ = 0.17	
							Log Stand. Dev. = 0.70	

Table S16. BET of ATP concentration using bienzyme solution with orange punch

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	1	1	1	3.58	0.55
2	1	1	1	1	1	1	0.03	-1.54
3	0	1	1	1	1	1	0.14	-0.84
4	0	1	0	1	1	1	3.58	0.55
5	0	1	1	1	1	1	0.14	-0.84
1	1	0	0	1	1	1	3.58	0.55
2	0	1	1	1	1	1	0.14	-0.84
3	0	1	1	1	1	1	0.14	-0.84
4	1	1	1	1	1	1	0.03	-1.54
5	0	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = -0.49	
							BET = $10^{-0.49}$ = 0.32	
							Log Stand. Dev. = 0.82	

Table S17. BET of ATP concentration using Smell-Tell paper with KDP buffer

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	1	1	1	3.58	0.55
2	0	0	1	1	1	1	0.72	-0.15
3	1	0	1	1	1	1	0.72	-0.15
4	0	1	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
1	0	0	0	0	1	1	17.89	1.25
2	0	0	1	1	1	1	0.72	-0.15
3	1	1	1	1	1	1	0.03	-1.54
4	0	0	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.06	
							BET = $10^{0.06}$ = 1.16	
							Log Stand. Dev. = 0.74	

Table S18. BET of ATP concentration using Smell-Tell paper with orange punch

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	1	1	1	3.58	0.55
2	0	1	1	1	1	1	0.14	-0.84
3	1	0	1	1	1	1	0.72	-0.15
4	0	1	1	1	0	1	89.44	1.95
5	0	0	1	1	1	1	0.72	-0.15
1	0	1	0	0	1	1	17.89	1.25
2	0	0	1	1	1	1	0.72	-0.15
3	1	1	1	1	1	1	0.03	-1.54
4	0	1	0	1	1	1	3.58	0.55
5	0	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.13	
							BET = $10^{0.13}$ = 1.36	
							Log Stand. Dev. = 1.00	

18. Indole determination for odour generating swab-like sensor (Smell-Tell swab) tested in open space

The substrates (70 μg pyridoxal, 420 nmol $MgCl_2$ and 7 μmol L-tryptophan) for the enzymatic reaction were immobilized onto the Smell-Tell swab to attain a one-step detection system. The resulting "all in one" Smell-Tell swab was directly placed onto a wet surface to detect ATP on the surface. After the reaction, reaction solution and the swab were transferred into tubes to extract indole for indole determination. The indole determination results were shown in Fig. S9.

19. BET of ATP concentration for Smell-Tell swab tested in open space

Table S19. BET of ATP concentration using Smell-Tell swab with KDP buffer tested in open space

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	1	1	0	0	1	1	17.88	1.25
2	1	1	1	1	1	1	0.03	-1.54
3	0	0	1	1	1	1	0.72	-0.15
4	1	1	1	1	0	1	89.44	1.95
5	0	0	1	1	1	1	0.72	-0.15
1	0	0	1	0	1	1	17.89	1.25
2	0	0	1	1	1	1	0.72	-0.15
3	0	0	0	1	1	1	3.58	0.55
4	1	0	0	0	0	1	89.44	1.95
5	0	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.48	
							BET = $10^{0.48}$ = 3.05	
							Log Stand. Dev. = 1.11	

Table S20. BET of ATP concentration using Smell-Tell swab with orange punch tested in open space

Panelist	Judgments							
	Concentrations of ATP (μM)						BET	
	0.064	0.32	1.6	8	40	200	Value	Log_{10} of Value
1	0	0	0	1	1	1	3.58	0.55
2	0	1	1	1	1	1	0.14	-0.84
3	0	0	1	1	0	1	89.44	1.95
4	0	0	0	1	1	0	447.21	2.65
5	0	1	0	1	1	1	3.58	0.55
1	1	0	0	0	1	1	17.89	1.25
2	0	1	1	1	1	1	0.14	-0.84
3	0	1	1	0	0	1	89.44	1.95
4	0	0	1	1	1	0	447.21	2.65
5	1	0	1	1	1	1	0.72	-0.15
Group BET = geometric mean, μM ATP							Average of log_{10} = 0.97	
							BET = $10^{0.97}$ = 9.40	
							Log Stand. Dev. = 1.33	

20. Figures

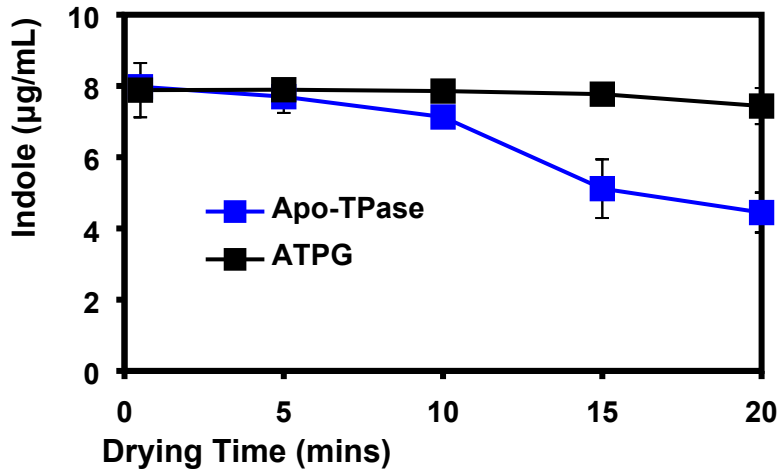


Fig S1. Influence of glycerol on the activity of immobilized Apo-TPase. Two sets of smell-generating papers were prepared via pipetting 20 μL of Apo-TPase or ATPG onto the paper strips (2.5 cm × 2.5 cm). After air-drying at room temperature for a certain period of time, the TPase-immobilized paper strip was placed into 700 μL of Apo-TPase reaction solution. All the reaction tubes were kept in a tube rotator with rotation speed of 18 rpm at room temperature for 1 h before indole determination.

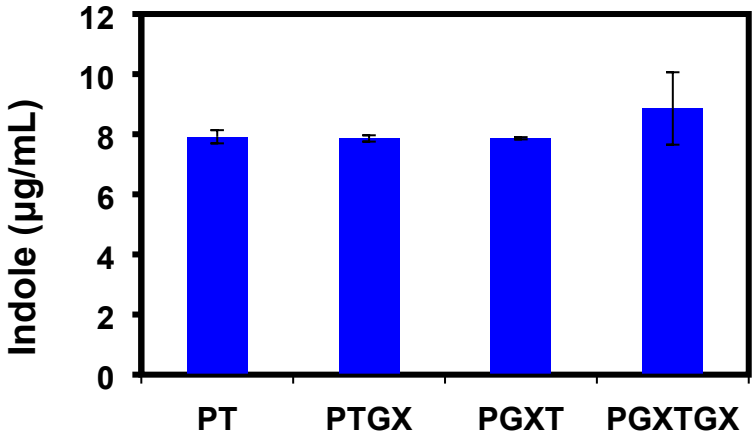


Fig. S2. Influence of glycerol and Triton X-100 on the bioactivity of enzymes. PT: mixture of pure PKase and pure Apo-TPase; PTGX: mixture of pure PKase and Triton X-100-contained ATPG; PGXT: mixture of Triton X-100-contained PKG and pure Apo-TPase; B-4: mixture of Triton X-100-contained PKG and Triton X-100-contained ATPG. The final concentration of Apo-TPase, PKase and Triton X-100 in bienzyme reaction solution (700 μL) were 33 μg/mL, 22 μg/mL and 0.02 wt%. All of the sample tubes were kept in a tube rotator with rotating speed of 18 rpm under room temperature for one hour before indole determination. As shown in the measured data, no significant change in the bioactivity of enzymes was detected in the presence of glycerol and Triton X-100.

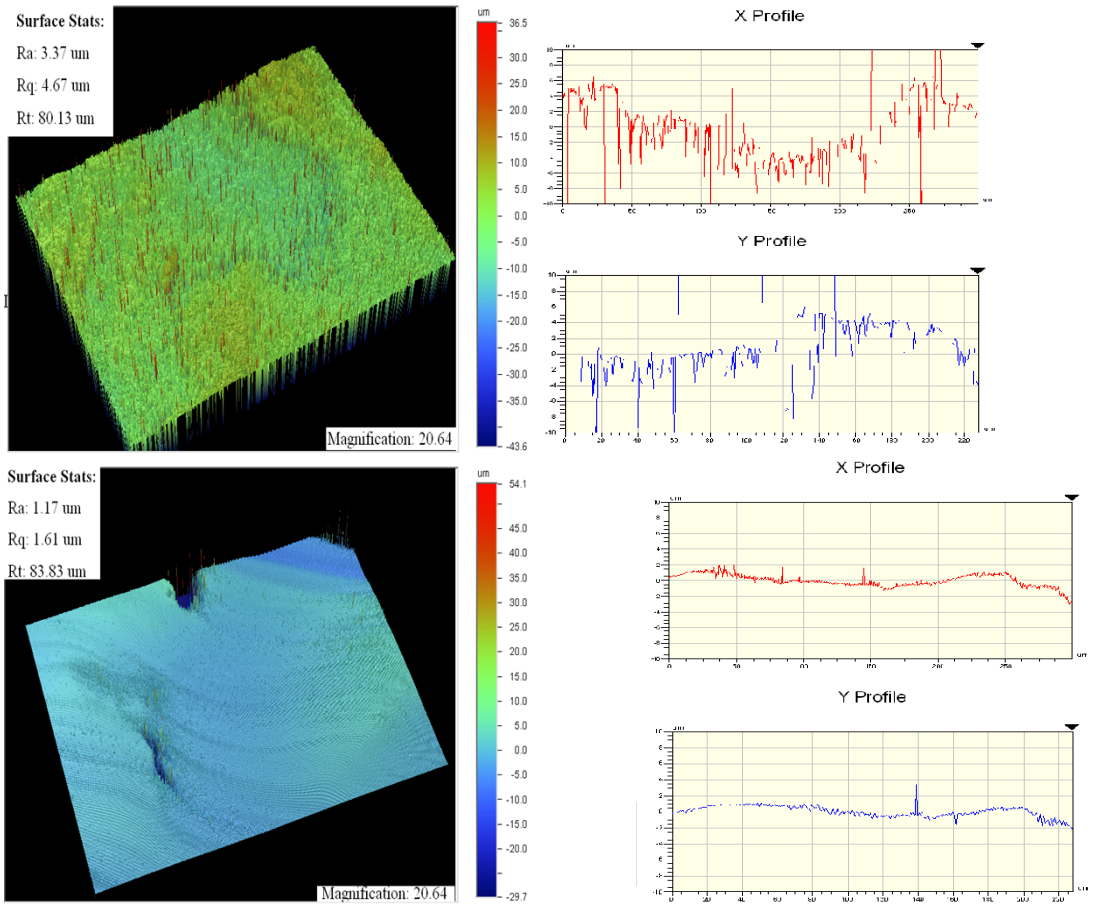


Figure S3. Profilometry images of paper that is uncoated (A) or coated (B) with sol-gel material. Optical profilometry images were obtained with a WYKO Model NT 1100 Optical Profiling System on the basis of the VSI measurement mode and a magnification of 20 ×.

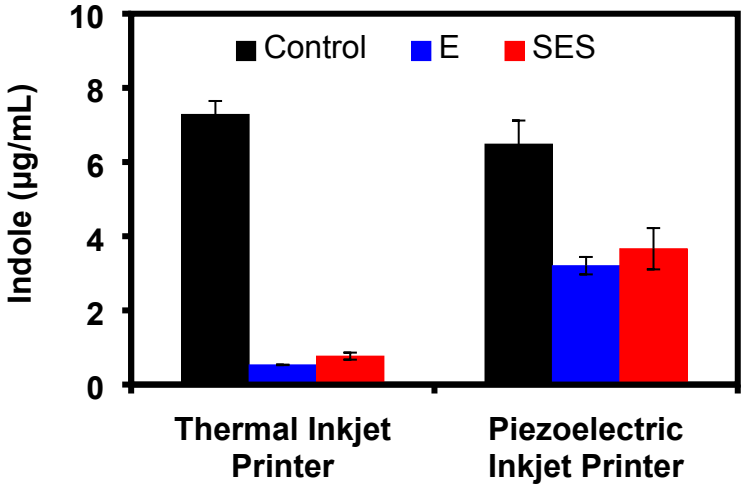


Figure S4. The activity of printed enzymes using different printers based on indole determination. E: pure enzyme immobilization; SES: enzymes immobilized in a sandwich format using sodium silicate material.

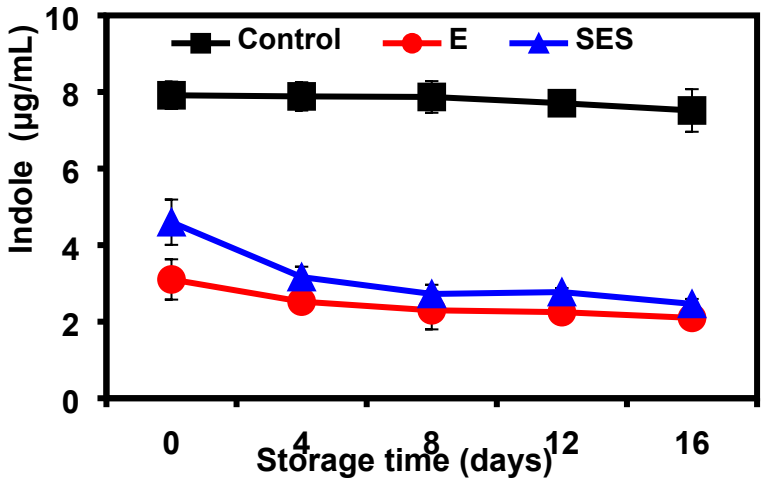


Figure S5. Storage Activities of immobilized Apo-TPase. E: 2.5 μL/cm^2 of ATPG was printed directly onto the filter paper. SES: ATPG was printed between the two layers of 2.5 μL/cm^2 of sol-gel material on the filter paper. After being printed, the enzyme paper was stored at 4°C. Every 4 days, 2.5 cm × 2.5 cm paper strips were cut and placed into Apo-TPase reaction solution. Control: ATPG stored in solution at 4°C. Every 4 days, 15.6 μL control samples were taken out and placed into Apo-TPase reaction solution. The enzyme bioactivity of papers was evaluated using the same method as described in Figure S1.

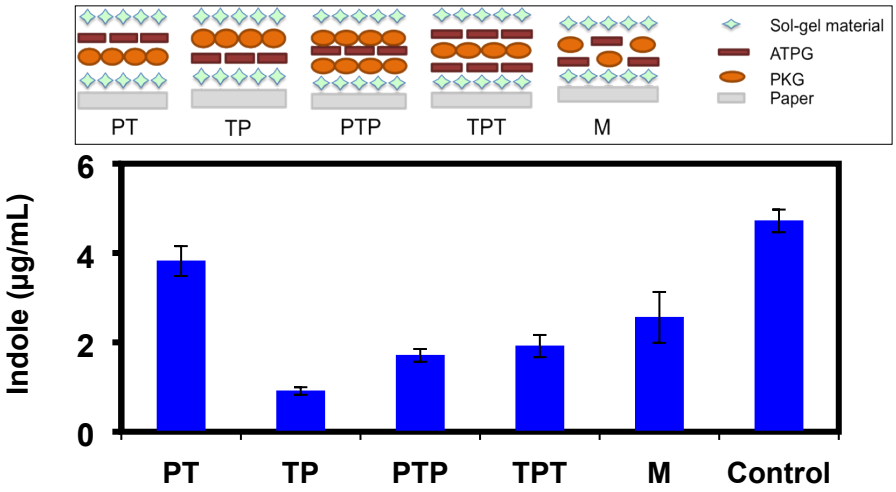


Figure S6. Immobilized bienzyme activities on paper with different enzyme distributing strategies. Different orders to print bienzyme are shown in the upper box (PT: printing PKG layer first and then ATPG layer; TP: printing ATPG layer first and then PKG layer; PTP: printing ATPG layer between two layers of PKG; TPT: printing PKG layer between two layers of ATPG; M: printing the mixture of ATPG and PKG.) Besides the similar sol-gel material layers on the bottom and top, on one paper, the same amount of each enzyme is involved in different layers if the enzyme is printed two times. Moreover, all of papers have the same total amount of enzymes (2.5 μL/cm^2 of ATPG and 5 μL/cm^2 of PKG). Control: 15.6 μL of ATPG and 31.2 μL of PKG solutions mixed with the bienzyme reaction solution.

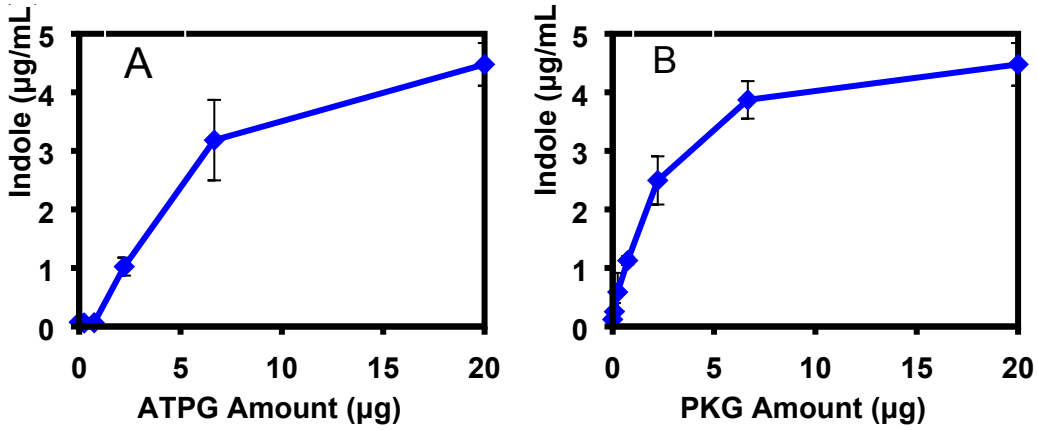


Figure S7. The dependence of bienzyme system efficiency on the amounts of ATPG and PKG. In bienzyme reaction solution (700 μL), when investigating the influence of ATPG concentration, 20 μg of PKase was used, while 20 μg of ATPG was involved to evaluate PKG concentration influence. Indole generated was determined after the enzymatic reaction was allowed to proceed for 1 hour. As shown in section A, the low concentration of ATPG cannot induce the generation of enough indole, and no detectable signal could be collected. Accordingly, the apparent catalytic ability of paper is deemed to be strongly dependent on the Apo-TPase activity.

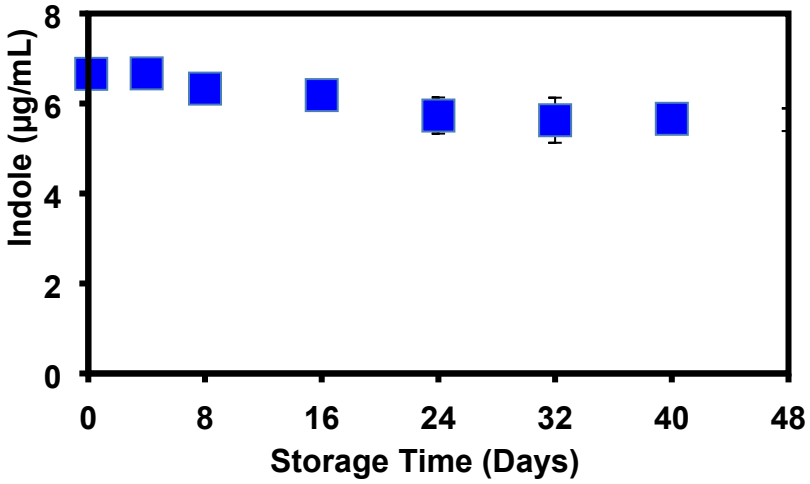


Figure S8. Activity of bienzyme system in solution during storage. Enzyme solutions were stored at 4°C. 15.6 μL of ATPG and 31.2 μL of PKG solutions were used.

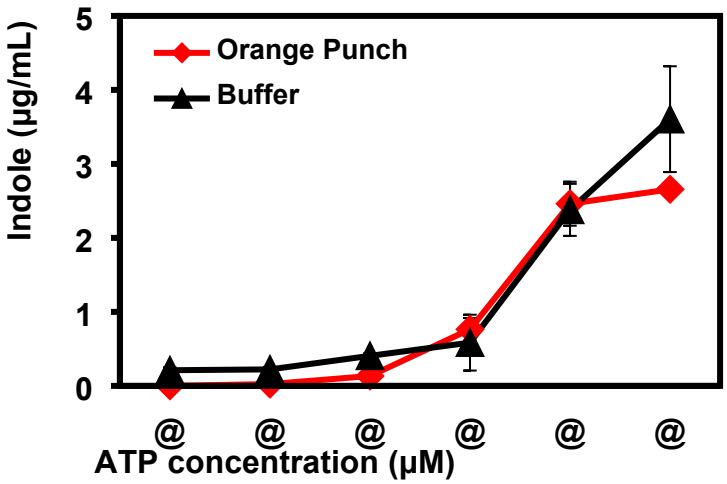


Figure S9. Indole generated by swab with performance in Orange Punch and buffer. The swab contained all of the components of the assay (pyridoxal, Mg^{2+}, and L-tryptophan PKase and Apo-TPase).

Reference

1. M. L. di Salvo, S. Hunt and V. Schirch, *Protein Expression Purif.*, 2004, **36**, 300-306.
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