Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Monitoring of Yogurt Fermentation Process Based on Rapid Bioluminescent Chiral Pattern Recognition of Amino Acids

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1. Experimental Section

1.1 Solution preparation

10×Spizizen salts solution: 15% $K_2HPO_4 \cdot 3H_2O$, 6% KH_2PO_4, 2% $(NH_4)_2SO_4$, 0.2% $MgSO_4$, and 1% sodium citrate was dissolved in ultrapure water and autoclaved. The solution was stored at room temperature.

GM I solution: 1 mL 10×Spizizen salts solution, 0.1 mL 10% yeast powder, 0.25 mL 20% glucose, and 0.2 mL 1% casein hydrolysate were mixed and the sterilized ultrapure water was added to a total volume of 10 mL.

GM II solution: 1 mL 10×Spizizen salts solution, 0.05 mL 10% yeast powder, 0.25 mL 20% glucose, 0.04 mL 1% casein hydrolysis, 0.05 mL 0.1 M $CaCl_2$, and 1 mL 25 mM $MgCl_2$ were mixed and the sterilized ultrapure water was added to a total volume of 10 mL.

1.2 Plasmid transformation

Transformation of *pBAV1K-T5-Lux* plasmids into *Escherichia coli* BL21 competent cells: Selected a fresh *Escherichia coli* BL21 colony in lysogeny broth (LB) plate, inoculated it in 3–5 mL LB liquid medium, and oscillated at 37°C for about 12 h until the late logarithmic growth phase. Next, inoculated the bacterial suspension in the ratio of 1:50 into a flask containing 100 mL LB liquid medium and shook the flask at 37°C for 2–3 h to make OD_{600} = 0.5. Transferred the bacterial suspension to a centrifuge tube and placed it on ice for 10 min, and then centrifuged it at 5,000 rpm for 10 min at 4°C. Discarded the supernatant, gently suspended the cell pellet with pre-cooled 10 mL of 0.05 M $CaCl_2$ solution and placed the tube on ice for 30 min, and then centrifuged it at 3,000 rpm for 10 min at 4°C. Gently resuspended the cell pellet in 0.05 M $CaCl_2$ solution containing 15% glycerol, and aliquoted 200 μL bacterial suspension (competent cells) into ice-cold tubes and stored them at -80°C for later use. For *pBAV1K-T5-Lux* plasmid transformation, took one tube of competent cells and placed it on ice for a few minutes to melt. Then mixed 10 μL plasmid containing 50 ng DNA with the competent cells, and placed the tube on ice for 30 min and in a 37°C water bath for 5 min consecutively. Added 1 mL LB liquid medium to the tube and shook it at 37°C for 1 h, so that the recombinant luminescent bacteria (EC-*lux*) could return to the normal growth state and expressed the kanamycin resistance gene that encoded by *pBAV1K-T5-Lux* plasmids. Took 100 μL bacterial suspension to coat on the LB plate containing 50 mg/L kanamycin, and let the plate blow-dried and then cultured it at 37°C for 16–24 h.

Transformation of *pBAV1K-T5-Lux* plasmids into *Agrobacterium tumefaciens* C58C1 competent cells: *Agrobacterium tumefaciens* C58C1 strain was isolated with solid YM plate and cultured at 28°C for 48 h. Selected a fresh *Agrobacterium tumefaciens* C58C1 colony in YM plate, inoculated it in 40 mL YM liquid medium, and oscillated (250 rpm) at 28°C for about 12–16 h. Transferred the bacterial suspension to a 50 mL sterile centrifuge tube, and centrifuged it at 8,000 rpm for 8 min at 4°C. Discarded the supernatant, gently suspended the cell pellet with pre-cooled 0.1 M $CaCl_2$ solution and placed the tube on ice for 20 min, and then centrifuged it at 8,000 rpm for 8 min at 4°C. Gently resuspended the cell pellet in 800 μL of 20 mM $CaCl_2$ solution containing 15% glycerol, and aliquoted 100 μL bacterial suspension (competent cells) into ice-cold tubes and stored them at -80°C for later use. For *pBAV1K-T5-Lux* plasmid transformation, took one tube of competent cells and placed it on ice for a few minutes to melt. Then mixed 10 μL plasmid containing 1 μg DNA with the competent cells, and placed the tube on ice for 30 min. After rapid cooling in liquid nitrogen for 1 min, transferred the tube to a 37°C water bath for 5 min. Added 1 mL YB liquid medium to the tube and shook it at 28°C for 2–4 h at 230 rpm, and then centrifuged it at 3,000 rpm for 2 min to collect the recombinant luminescent bacteria (AT-*lux*). The cell pellet was suspended in 100 μL YM liquid medium and then was coated on a YM plate containing 50 mg/L kanamycin. The YM plate was blow-dried and cultured for 28–48 h.

Transformation of *pBAV1K-T5-Lux* plasmids into *Bacillus subtilis* WB600 competent cells: *Bacillus subtilis* WB600 was inoculated on LB plate and cultured at 37°C for 24–48 h. Selected a fresh *Bacillus subtilis* WB600 colony and inoculated it in 5 mL GM I solution, and oscillated at 30°C overnight in a slow shaker (125 rpm). The next day, transferred 2 mL bacterial suspension to a flask containing 18 mL GM I solution and oscillated the flask at 37°C for 3.5 h in a quick shaker (250 rpm). Next, transferred 10 mL bacterial solution to 90 mL GM II solution, and then oscillated at 37°C for 90 min in a slow shaker (125 rpm). After that, the bacterial solution was centrifuged at 5,000 rpm for 10 min and the cell pellet was suspended in 10 mL GM II solution to get competent cells. For *pBAV1K-T5-Lux* plasmid transformation, took one tube of 0.5 mL competent cells and placed it in a 45°C water bath, and added 10 μL plasmid containing 0.5 μg DNA to the competent cells, and then shook the tube at 37°C for 30 min at 200 rpm. Coated the bacterial solution (BS-*lux*) on the LB plate containing 50 mg/L kanamycin and incubated the plate overnight at 37°C.

1.3 MTT Assay

10 μL of MTT reagent (5 mg/mL) was added to the mixture of 100 μL bacteria and 100 μL amino acids

or blank solution per well and incubated at room temperature for 4 h. The bacterial solution was centrifuged at 5,000 rpm for 10 min to remove the supernatant and 200 μL of DMSO was then added to each well. The plate was mixed gently on an orbital shaker for 1h at room temperature before measurement (490 nm).

2. Supplementary Figures

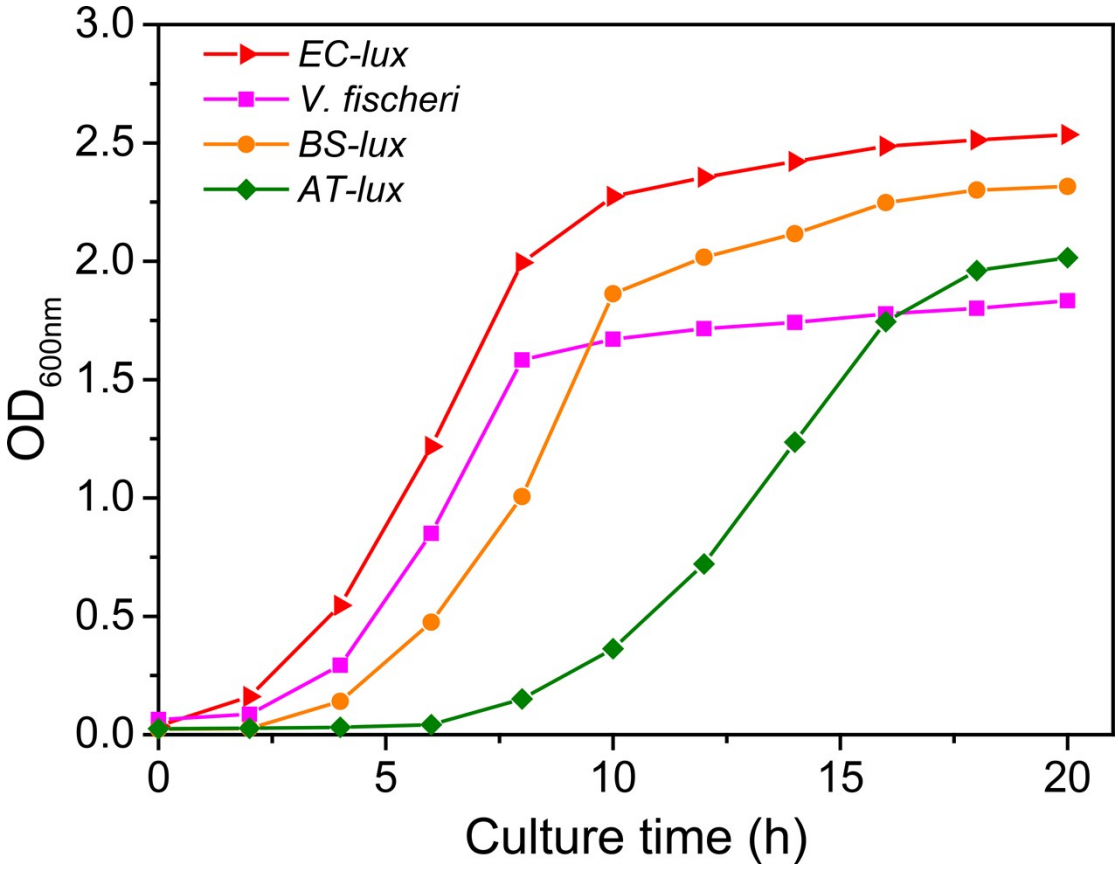

Fig. S1 The growth curves of four bacteria were drawn every two hours at OD_{600nm}.

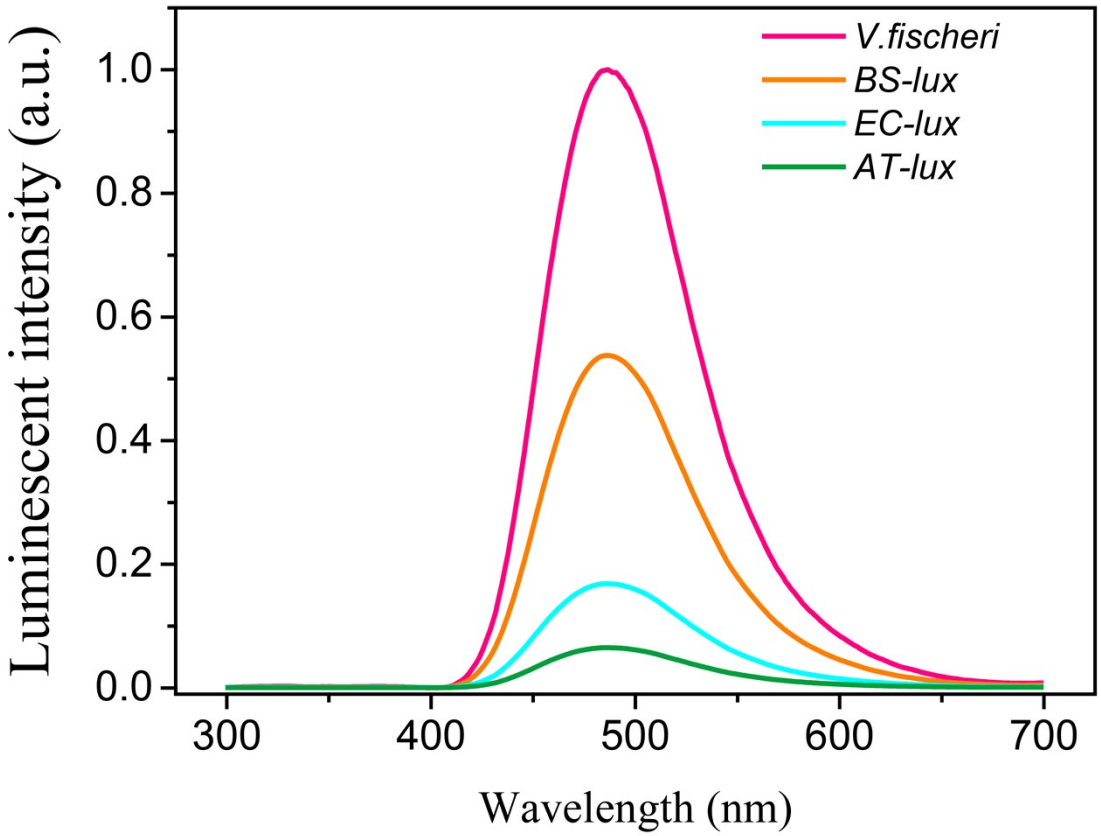

Fig. S2 Bioluminescent spectra of four luminescent bacteria. The bioluminescent spectra were collected with the same cellular concentration (1.3×10^7 cells mL^{-1}) for all these four bacteria.

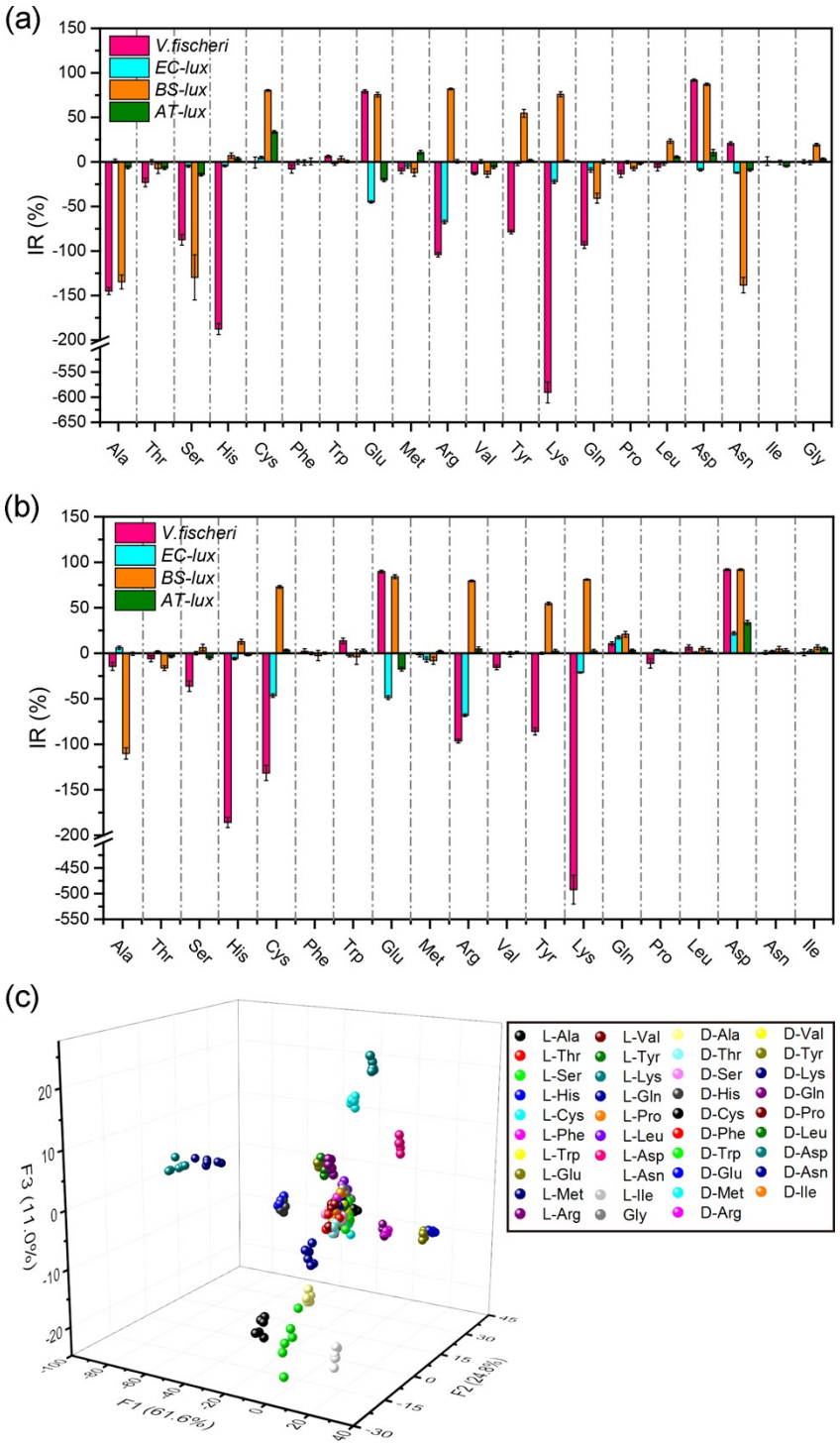

Fig. S3 The luminescence response patterns and their LDA canonical score plot of the sensor array to 39 amino acids. The luminescence response patterns of four biosensors to 19 L-amino acids and Gly (a), and 19 D-amino acids (b) at a concentration of 200 μM. (c) LDA canonical score plot of luminescence response of sensor array to 39 amino acids (19 L-amino acids, 19 D-amino acids, and Gly).

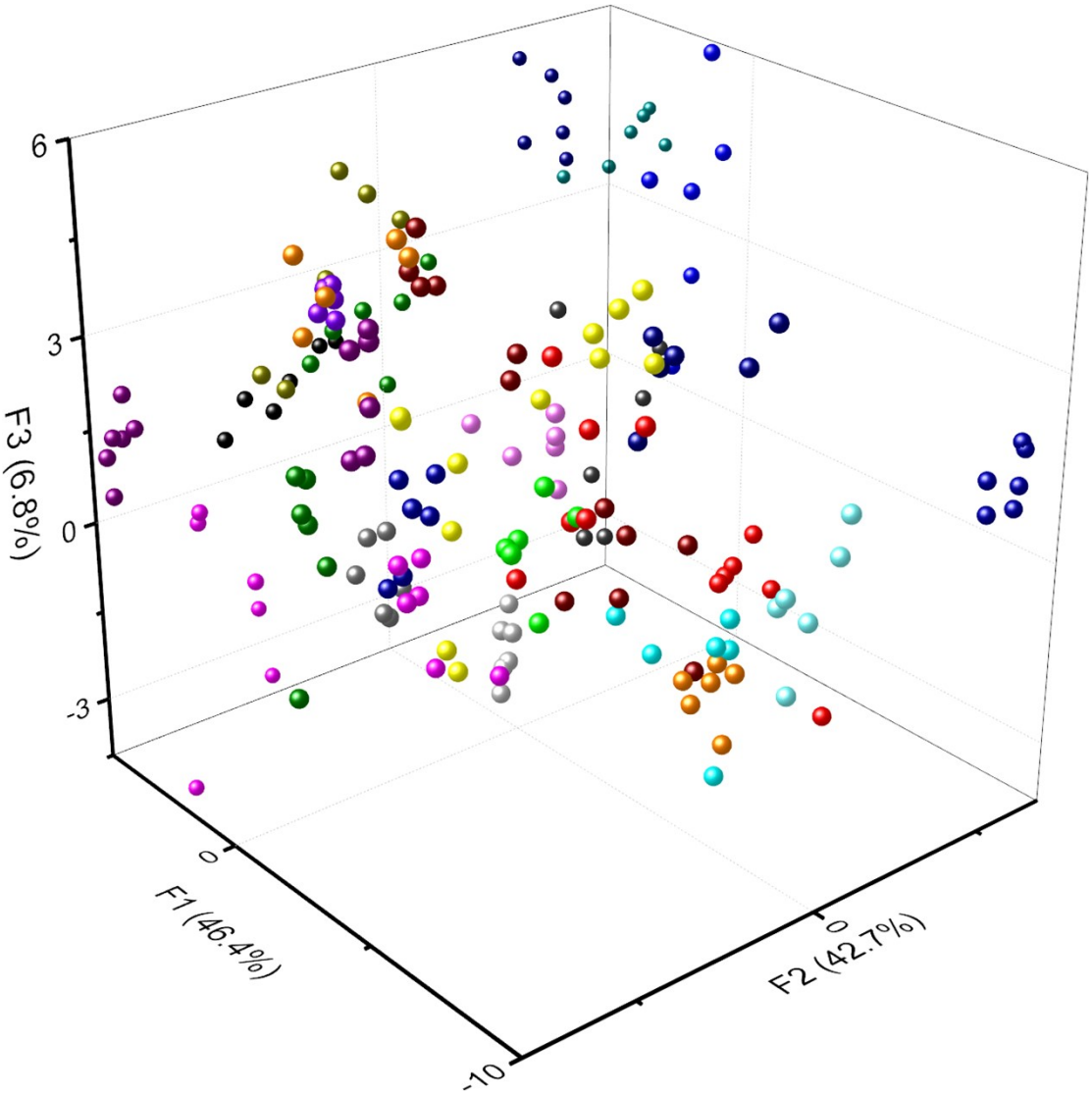

Fig. S4 Local amplification of Figure 2c.

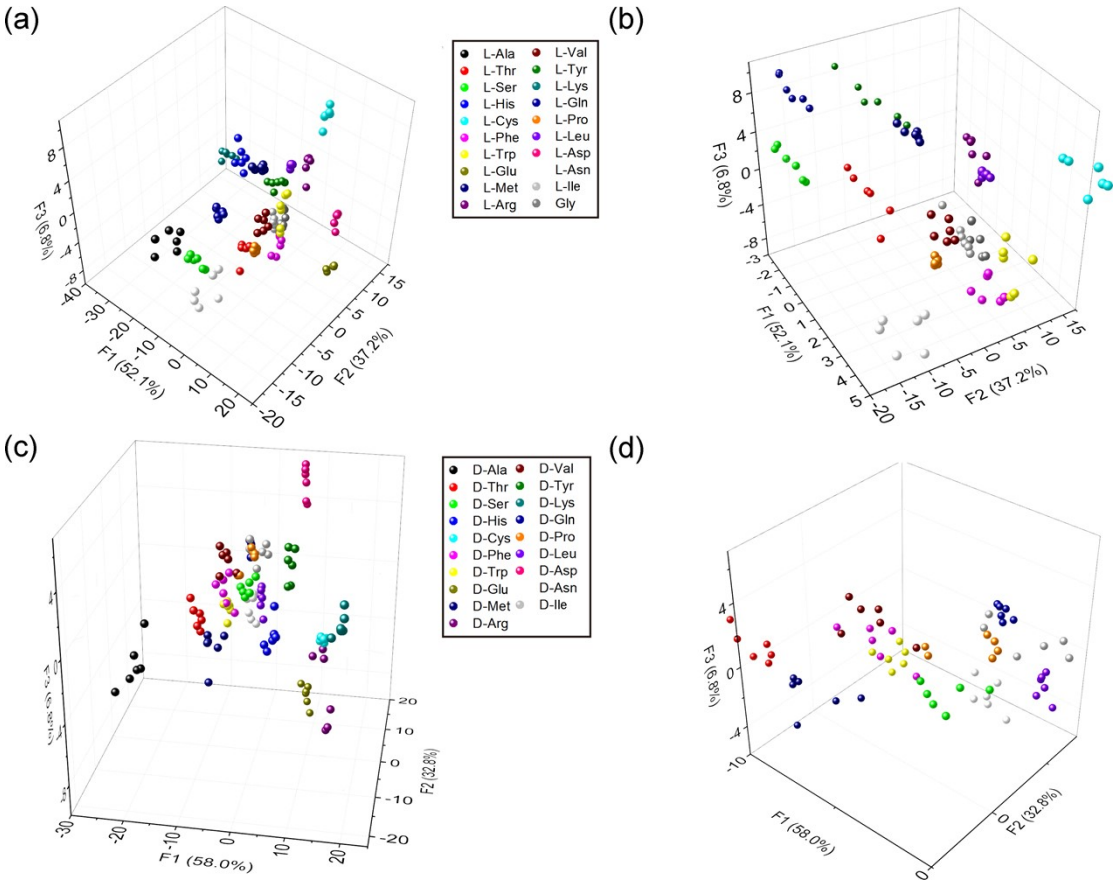

Fig. S5 Amino acids species discrimination by the sensor array. (a) LDA canonical score plot for the luminescence response of the sensor array to 19 L-amino acids and Glycine at a concentration of 400 μM. (b) Local amplification of Figure S5a. (c) LDA canonical score plot for the luminescence response of the sensor array to 19 D-amino acids at a concentration of 400 μM. (d) Local amplification of Figure S5c.

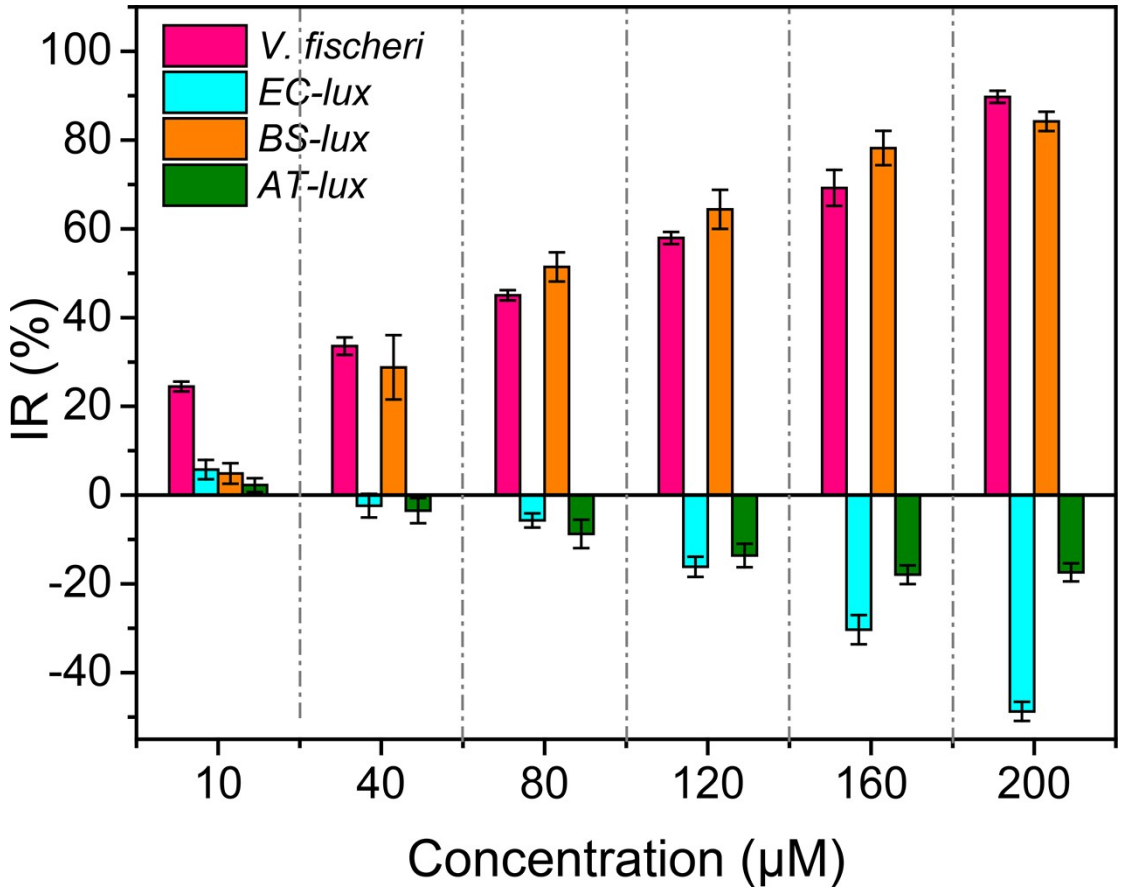

Fig. S6 Luminescence response patterns of sensor array against different concentrations of D-Glutamic acid (Glu).

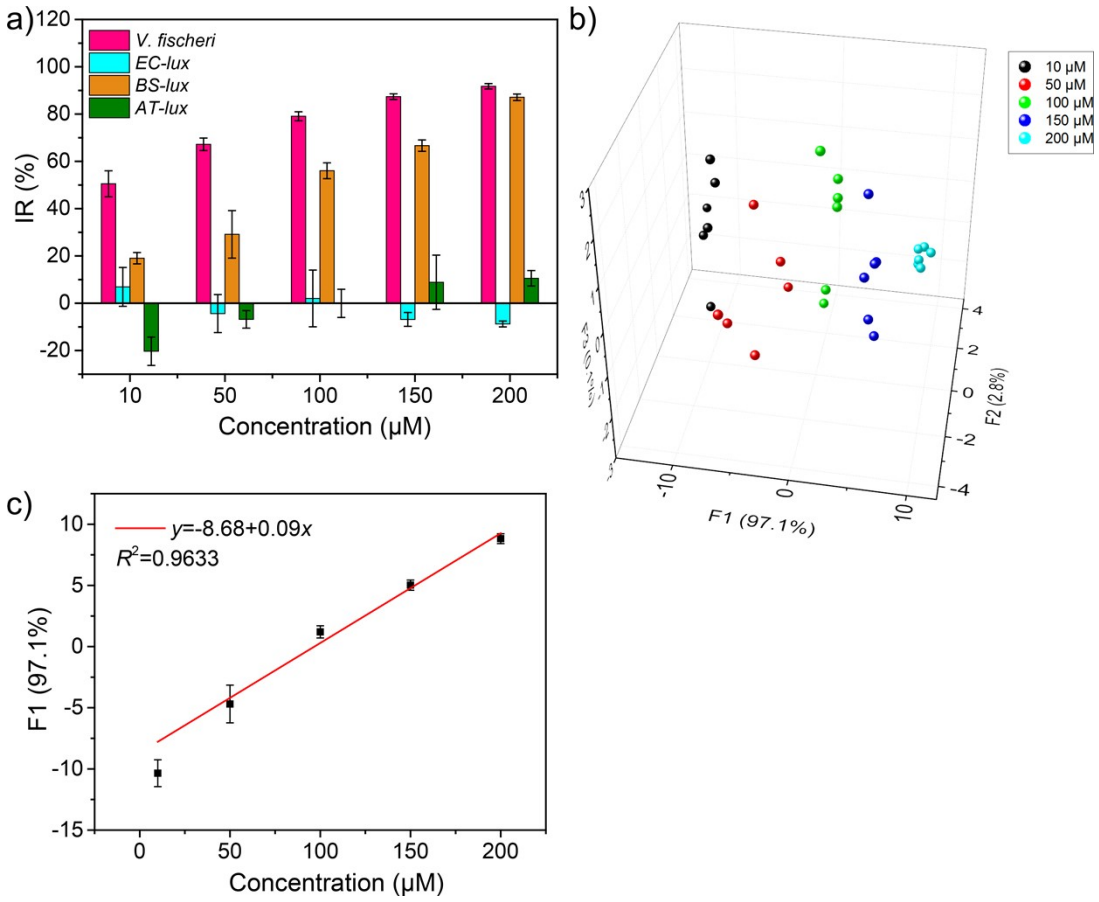

Fig. S7 (a) Luminescence response patterns of sensor array against different concentrations of L-Aspartic acid (L-Asp), (b) LDA canonical score plot of L-Asp at different concentrations and (c) Standard curve and the result of liner fitting between F1(97.1%) and the concentrations of L-Asp.

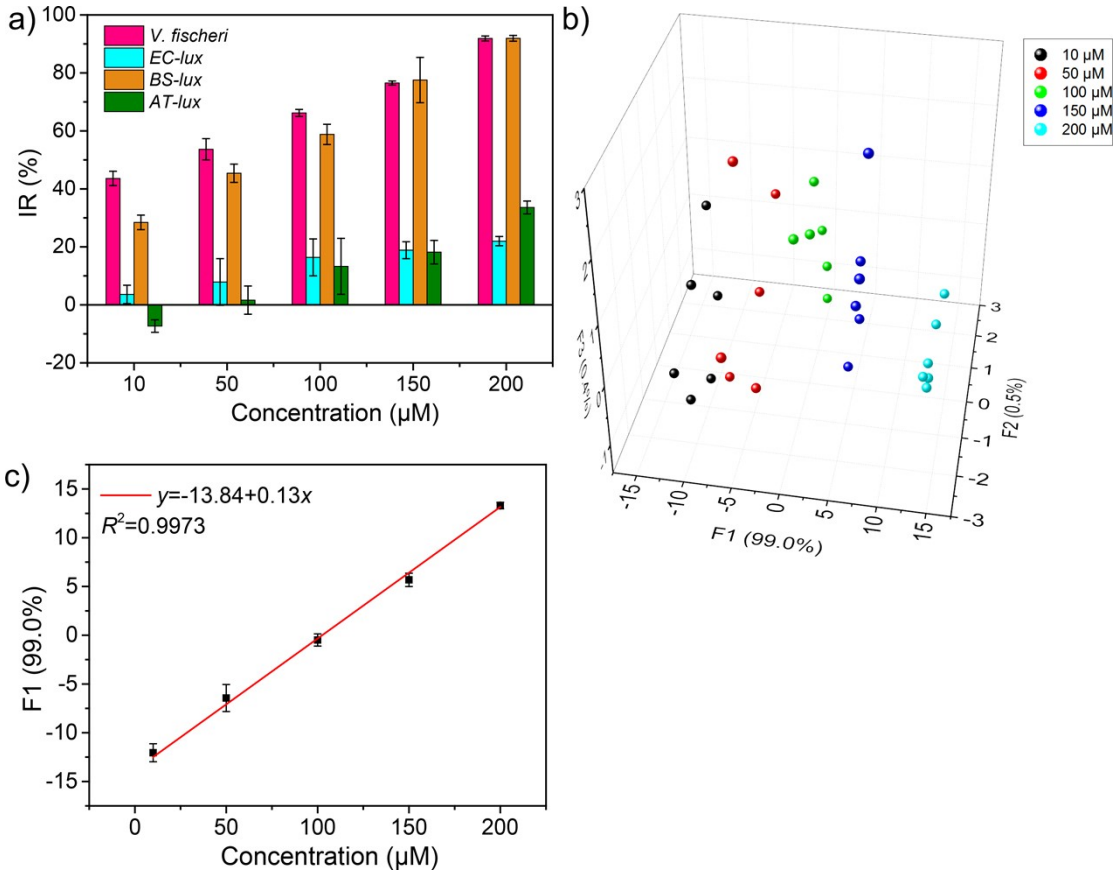

Fig. S8 (a) Luminescence response patterns of sensor array against different concentrations of D-Aspartic acid (D-Asp), (b) LDA canonical score plot of D-Asp at different concentrations and (c) Standard curve and the result of liner fitting between F1(99.0%) and the concentrations of D-Asp.

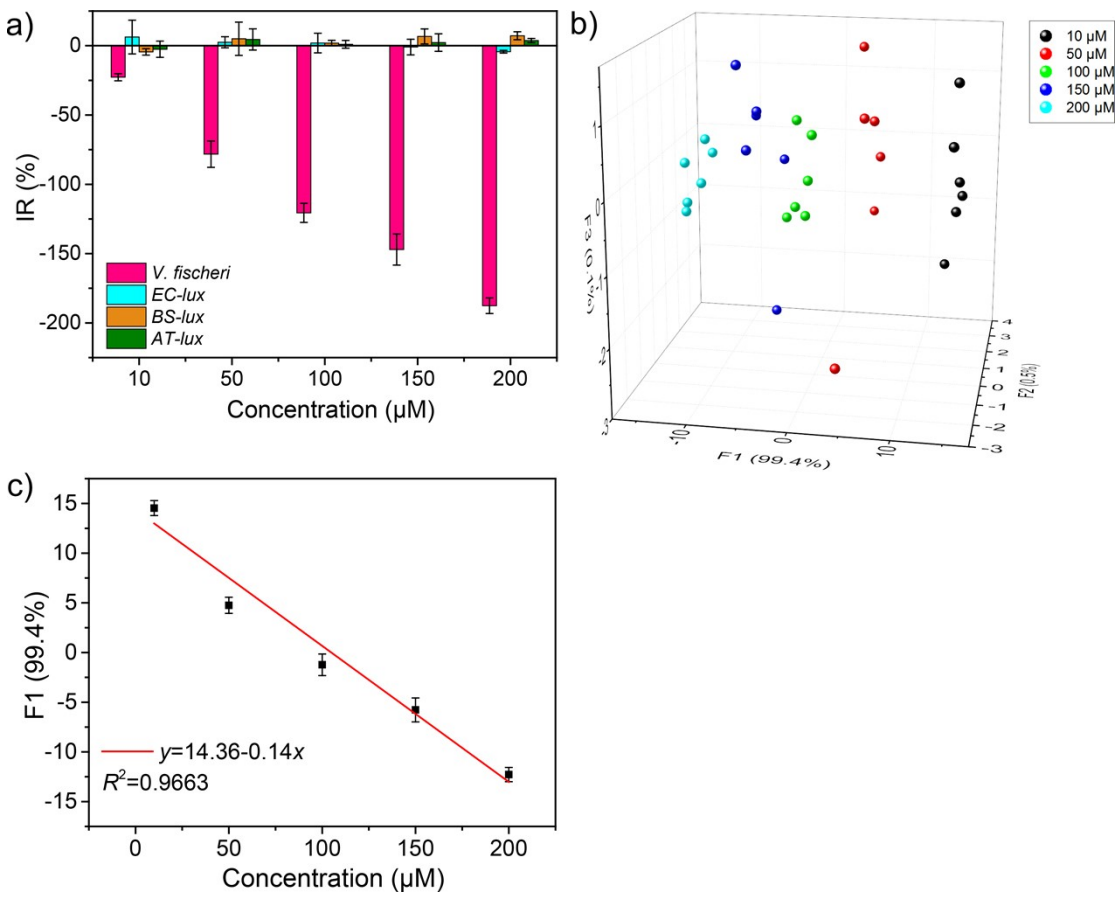

Fig. S9 (a) Luminescence response patterns of sensor array against different concentrations of L-Histidine (L-His), (b) LDA canonical score plot of L-His at different concentrations and (c) Standard curve and the result of liner fitting between F1(99.4%) and the concentrations of L-His.

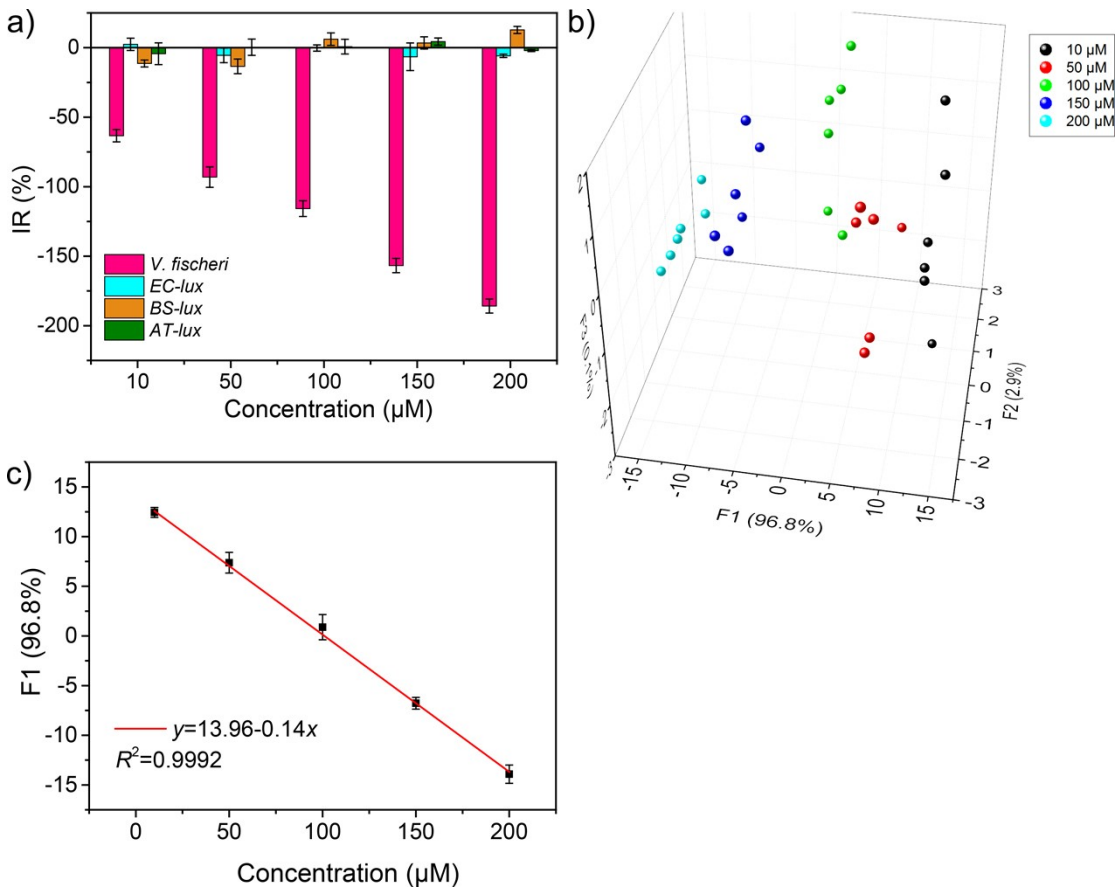

Fig. S10 (a) Luminescence response patterns of sensor array against different concentrations of D-Histidine (D-His), (b) LDA canonical score plot of D-His at different concentrations and (c) Standard curve and the result of liner fitting between F1(96.8%) and the concentrations of D-His.

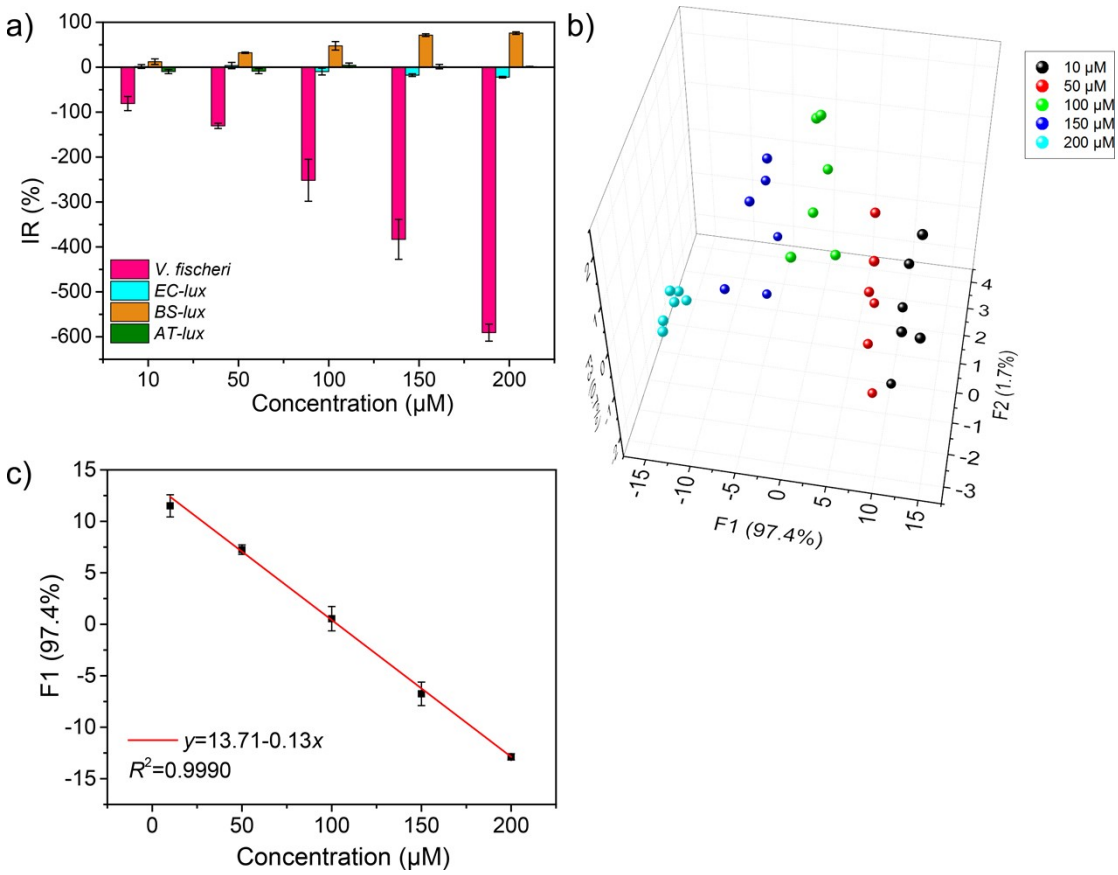

Fig. S11 (a) Luminescence response patterns of sensor array against different concentrations of L-Lysine (L-Lys), (b) LDA canonical score plot of L-Lys at different concentrations and (c) Standard curve and the result of liner fitting between F1(97.4%) and the concentrations of L-Lys.

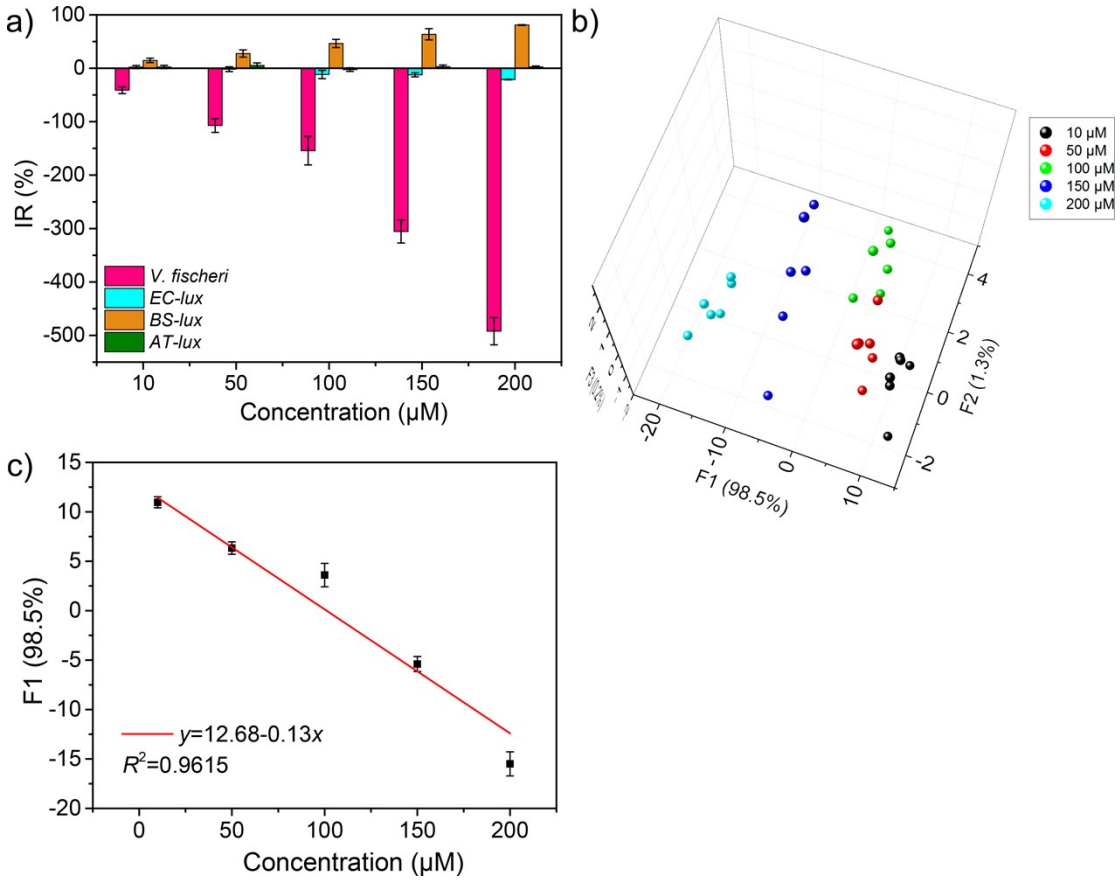

Fig. S12 (a) Luminescence response patterns of sensor array against different concentrations of D-Lysine (D-Lys), (b) LDA canonical score plot of D-Lys at different concentrations and (c) Standard curve and the result of liner fitting between F1(98.5%) and the concentrations of D-Lys.

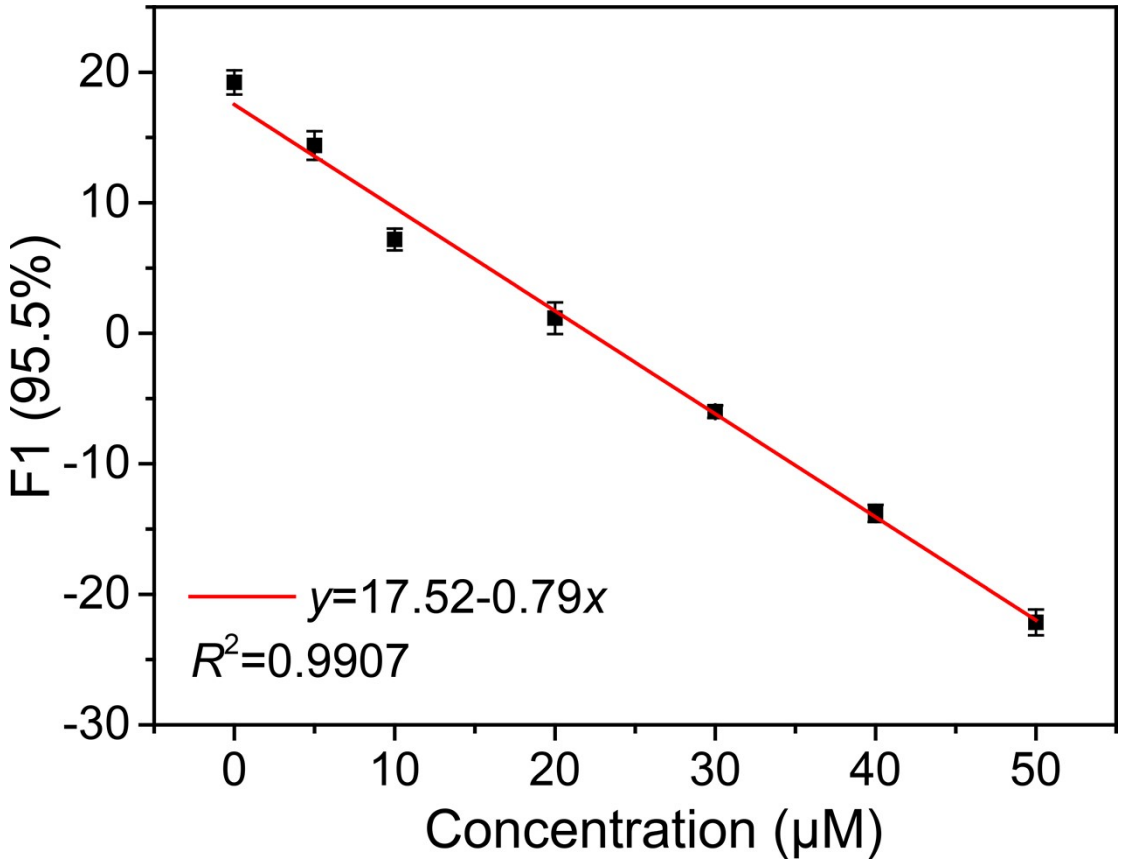

Fig. S13 Standard curve and the result of liner fitting between F1(95.5%) and the concentrations of D-Asp in Fig. 5a.

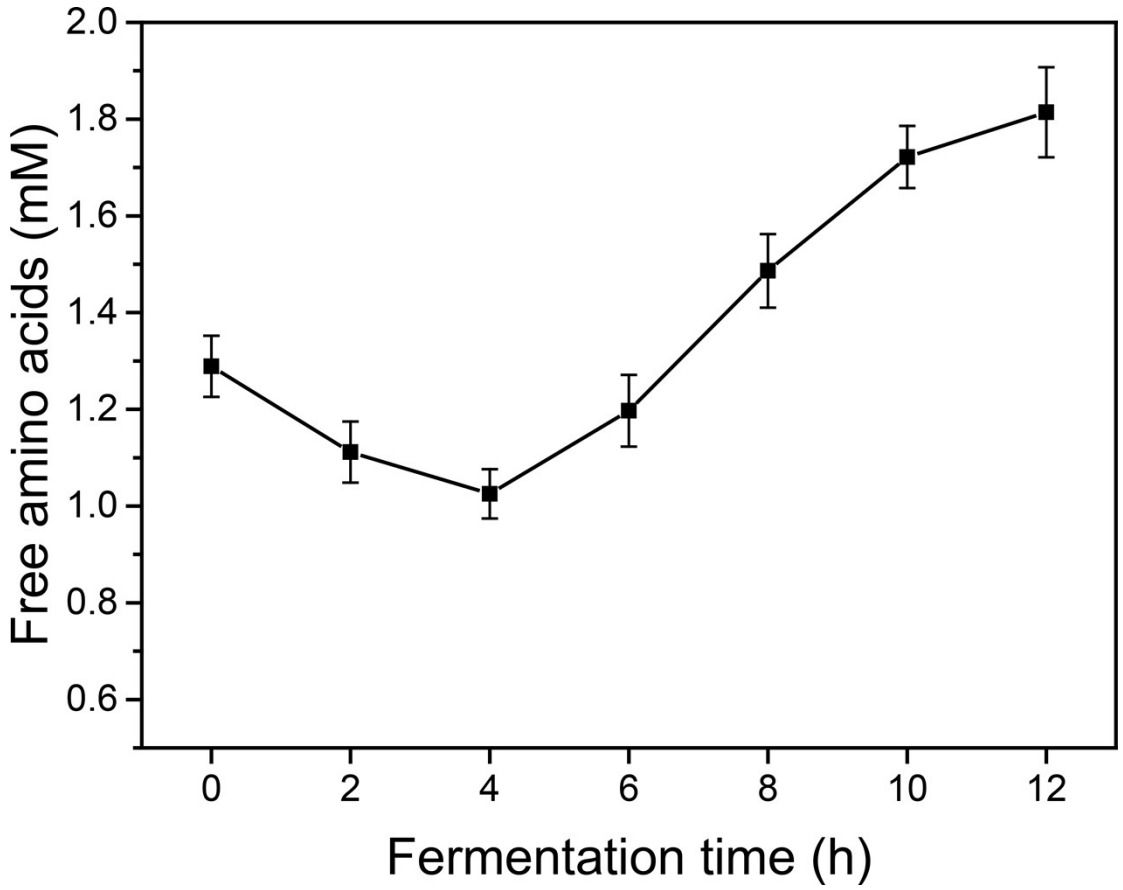

Fig. S14 The concentrations of free amino acids in fermented milk at different stages.

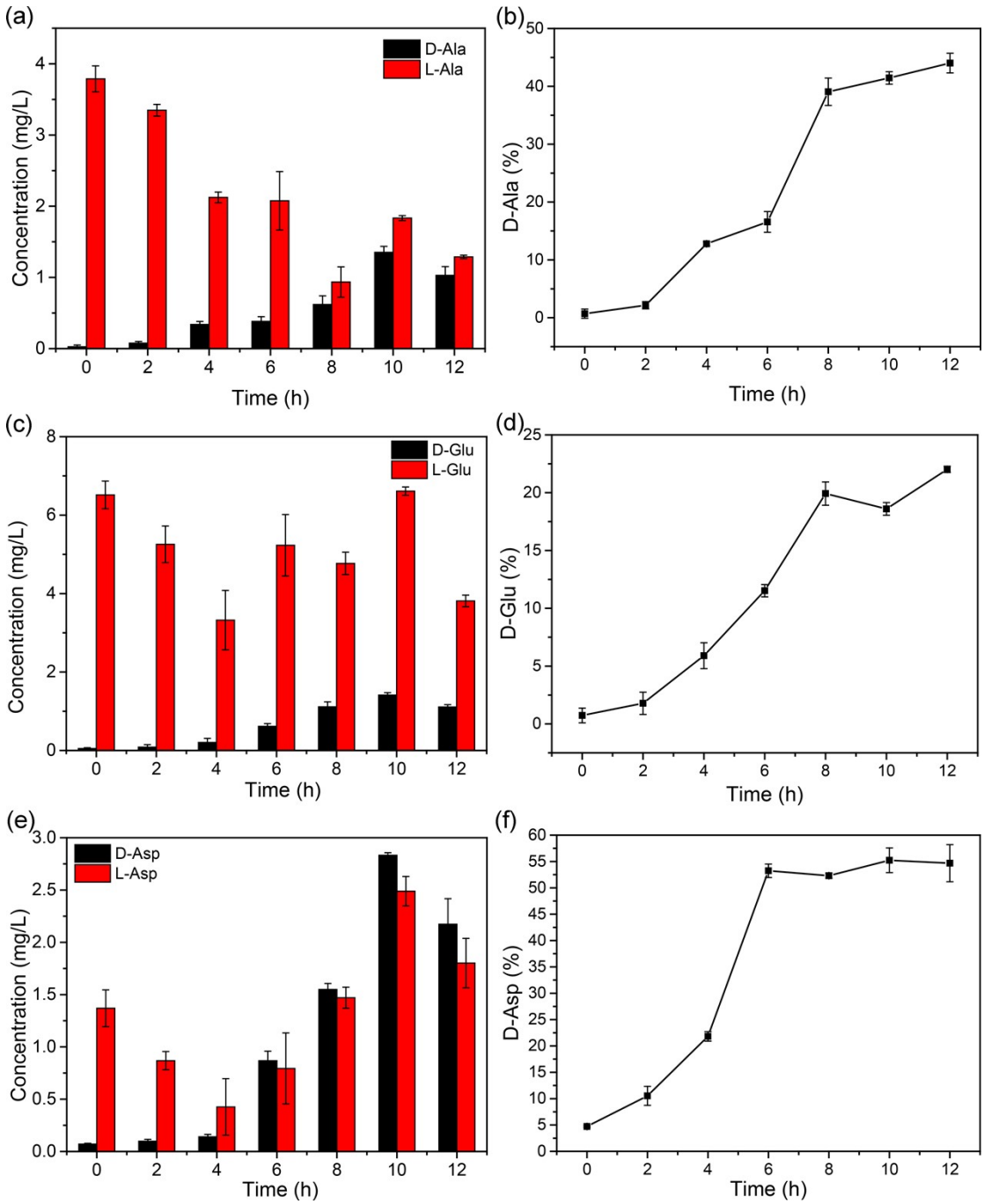

Fig. S15 The concentrations of D/L-Alanine (Ala) (a), D/L-Glu (c) and D/L-Aspartic acid (Asp) (e) and the percentages of D-Ala (b), D-Glu (d), and D-Asp (f) in their enantiomer mixture during the fermentation process obtained from the result of high performance liquid chromatographic analysis.

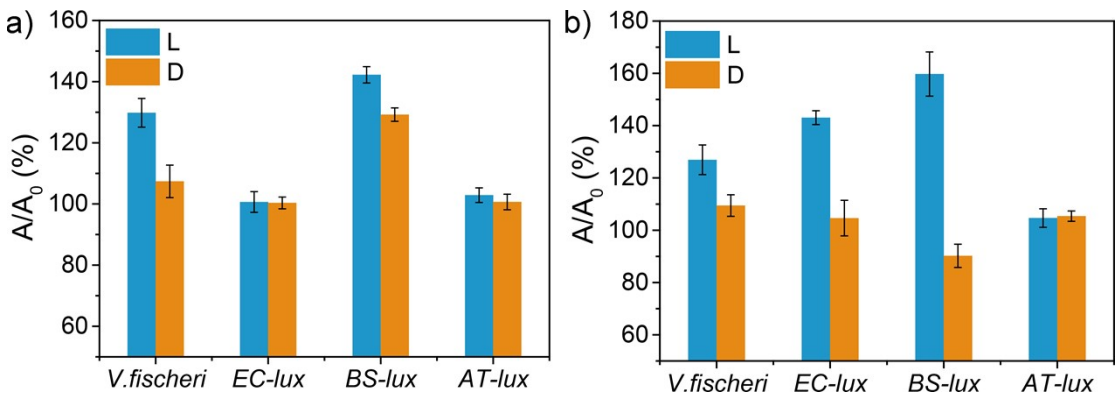

Fig. S16 The MTT analysis results of luminescent bacteria incubated with a) Ala and b) Ser.

3. Supplementary Tables

Table S1 Comparison of array methods for identifying chiral amino acids.

Analytes	Number of sensing elements	Number of parameters	Misclassifications	Accuracy	References
2 amino acids+2 thiols	3	3	0/90	100.0%	[1]
10 amino acids	6	6	0/30	100.0%	[2]
4 amino acids+4 amino alcohols+8 amines	3	6	0/340	100.0%	[3]
39 amino acids	5	5	3/234	98.7%	[4]
37 amino acids	12	36	13/185	92.9%	[5]
39 amino acids	4	4	2/234	99.1%	This work

Table S2 The limit of detection (LOD) values of amino acids.

Amino acids	D-Glutamic acid	L-Asp	D-Asp	L-His	D-His	L-Lys	D-Lys
LODs (μM)	2.25	3.17	4.64	1.56	2.12	8.26	2.04

Raw data of array sensing

Table S3 Matrix of the response patterns against amino acids at 200 μM.

Amino acids	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
L-Alanine	-137.57	2.02	-131.91	-4.72
L-Alanine	-143.93	0.37	-123.68	-4.66
L-Alanine	-145.79	3.54	-146.69	-5.13
L-Alanine	-147.39	-2.81	-137.87	-8.91
L-Alanine	-145.99	1.45	-140.13	-7.03
L-Alanine	-149.01	1.32	-127.38	-5.94
L-Threonine	-16.45	-2.5	-8.07	-5.27
L-Threonine	-19.8	-3.04	-13.28	-5.61
L-Threonine	-21.9	-0.87	-8.26	-5.58
L-Threonine	-30.93	-0.01	-8.5	-9.34
L-Threonine	-24.29	2.63	-13.99	-8.39
L-Threonine	-24.32	3.38	1.04	-7.21
L-Serine	-77.4	-4.19	-172.09	-13.87
L-Serine	-89.33	-2.42	-144.19	-15.61

L-Serine	-86.76	-6.09	-90.53	-12.82
L-Serine	-96.02	-4.56	-135.13	-12.37
L-Serine	-86.55	-6.1	-120.11	-16.33
L-Serine	-88.02	-4.06	-115.46	-14.56
L-Histidine	-179.33	-4.02	11.89	6.58
L-Histidine	-192.45	-4.88	4.73	3.95
L-Histidine	-184.17	-3.22	5.69	3.24
L-Histidine	-193.06	-4.59	3.9	4.08
L-Histidine	-182.99	-3.18	9.98	2.97
L-Histidine	-193.43	-6.01	6.88	1.59
L-Cysteine	5.26	5.77	81.84	36.21
L-Cysteine	2.38	5.91	80.68	32.37
L-Cysteine	-2.13	6.16	80.41	33.5
L-Cysteine	3.36	5.42	80.26	33.34
L-Cysteine	-1.27	2.69	78.92	32.11
L-Cysteine	-12.01	3.45	79.55	34.41
L-Phenylalanine	0.72	4.72	-1.3	8.24
L-Phenylalanine	-6.54	0.53	-1.42	-0.56
L-Phenylalanine	-9.72	-2.25	1.2	-1.57
L-Phenylalanine	-12.47	-1.71	2.41	-1.36
L-Phenylalanine	-11.03	-1.49	-5.97	-1.85
L-Phenylalanine	-8.29	-0.64	1.08	0.79
L-Tryptophan	8.12	2.24	5.94	1.28
L-Tryptophan	6.43	-1.36	-0.5	-1.12
L-Tryptophan	6.68	-1.5	5	-0.93
L-Tryptophan	4.49	-2.79	6.51	-0.36
L-Tryptophan	5.44	-4.15	4.26	0.74
L-Tryptophan	6.61	-2.51	1.2	2.35
L-Glutamic acid	82.82	-43.05	73.31	-18.36
L-Glutamic acid	78.47	-46.53	75.36	-19.44
L-Glutamic acid	77.88	-45.46	77.62	-22.87
L-Glutamic acid	77.99	-45.59	76.28	-21.17
L-Glutamic acid	78.77	-44.59	71.35	-21.33
L-Glutamic acid	79.44	-44.34	78.86	-18.3
L-Methionine	-4.78	-0.31	-11.08	13.89
L-Methionine	-13.04	-5.28	-9.98	8.51
L-Methionine	-12.42	-6.79	-6.03	11.77
L-Methionine	-9.69	-6.5	-10.01	10.5
L-Methionine	-10.86	-5.31	-18.46	8.23
L-Methionine	-9.53	-3.74	-16.43	11.94
L-Arginine	-99.24	-63.79	83.61	3.95
L-Arginine	-106.22	-69.05	81.83	-0.64
L-Arginine	-103.98	-68.49	81.6	0.01

L-Arginine	-106.09	-69.09	81.12	-0.47
L-Arginine	-103.59	-67.69	80.69	-0.91
L-Arginine	-105.48	-66.13	82.22	2.7
L-Valine	-11.35	3.98	-20.85	-2.23
L-Valine	-13.14	1.23	-13.06	-7.18
L-Valine	-12.65	-0.88	-12.95	-6.47
L-Valine	-15.32	-1.82	-13.73	-5.7
L-Valine	-12.56	-0.97	-10.08	-6.57
L-Valine	-13.12	1.06	-11.67	-6.42
L-Tyrosine	-76.55	2.97	57.18	1.19
L-Tyrosine	-79.86	-2.09	53.17	-0.34
L-Tyrosine	-76.42	-2.22	56.2	2.73
L-Tyrosine	-81.64	-3.74	45.56	2.28
L-Tyrosine	-79.53	-3.53	56.79	2.47
L-Tyrosine	-77.3	-1.04	58.95	2.61
L-Lysine	-584.54	-18.61	79.09	2.99
L-Lysine	-614.36	-22.65	70.99	0.22
L-Lysine	-561.5	-21.06	77.19	0.17
L-Lysine	-614.2	-23.66	73.57	0.65
L-Lysine	-590.39	-23.73	76.82	0.84
L-Lysine	-578.46	-23.25	78.02	1.38
L-Glutamine	-95.3	-5.93	-44.04	3.65
L-Glutamine	-91.27	-10.66	-46.42	-2.51
L-Glutamine	-91.13	-12.42	-42.48	-1.74
L-Glutamine	-95.38	-8.65	-37.91	0.89
L-Glutamine	-98.55	-9.7	-43.82	0.09
L-Glutamine	-87.89	-7.5	-29.65	1.62
L-Proline	-7.95	2.52	-2.77	-0.15
L-Proline	-16.23	-2.15	-8.43	-2.15
L-Proline	-12.35	-1.48	-7.9	-3.9
L-Proline	-9.76	-0.64	-8.29	-0.61
L-Proline	-17.24	0.46	-8.99	-1.8
L-Proline	-16.63	-0.41	-8.66	-1.67
L-Leucine	-5.75	0.53	26.88	7.32
L-Leucine	-3.92	-1.92	22.64	5.27
L-Leucine	-2.46	-2.38	22.68	6.58
L-Leucine	-4.64	-4.67	19.41	4.63
L-Leucine	-13.22	-2.3	25.4	3.8
L-Leucine	-7.02	1.24	23.1	5.88
L-Aspartic acid	93.91	-6.74	89.6	14.72
L-Aspartic acid	92.07	-9.01	86.01	13.38
L-Aspartic acid	90.88	-8.41	86.36	11.54
L-Aspartic acid	91.46	-10.24	85.86	4.61

L-Aspartic acid	90.23	-10.21	86.52	9.59
L-Aspartic acid	91.94	-7.89	88.32	9.48
L-Asparagine	22.78	-12.86	-153.8	-7.65
L-Asparagine	22.71	-12.14	-141.57	-8.66
L-Asparagine	21.16	-11	-130.75	-8.01
L-Asparagine	18.7	-12.06	-135.24	-10.63
L-Asparagine	18.57	-12.89	-141.56	-8.34
L-Asparagine	20.41	-12.24	-127.02	-10.77
L-Isoleucine	-0.86	-0.61	3.06	-3.63
L-Isoleucine	3.46	-0.44	-3.39	-4.78
L-Isoleucine	1.43	-0.17	-1.42	-4.88
L-Isoleucine	4.39	-0.21	-1.81	-5.98
L-Isoleucine	-8.19	-0.68	-2.02	-4.84
L-Isoleucine	4.81	-0.12	2.62	-4.61
Glycine	-2.08	-0.01	22.12	3.35
Glycine	-0.78	2.12	18.61	4.79
Glycine	3.19	-0.04	17.78	3.53
Glycine	-1.66	-0.87	20.69	2.74
Glycine	1.04	-0.8	17.83	1.66
Glycine	2.58	-4.85	18.74	4.27
D-Alanine	-19.9	6.2	-98.91	-1.92
D-Alanine	-18.56	3.78	-108.15	-2.93
D-Alanine	-14.42	6.14	-117.51	1.52
D-Alanine	-12.91	6.11	-111.97	-0.76
D-Alanine	-7.27	9.21	-116.06	0.21
D-Alanine	-13.18	5.23	-108.15	0.9
D-Threonine	-2.53	3.31	-17.08	-4.09
D-Threonine	-8.42	-0.04	-17.6	-4.03
D-Threonine	-10.48	0.54	-19.84	-4.92
D-Threonine	-7.37	1.54	-16.01	-3.45
D-Threonine	-4.89	2.07	-15.35	-1.34
D-Threonine	-2.58	2.13	-11.26	-3.4
D-Serine	-35.3	2.28	0.74	-5.99
D-Serine	-41.43	0.62	6.03	-7.85
D-Serine	-35.48	-3.1	5.5	-3.47
D-Serine	-32.72	0.86	8.01	-5.34
D-Serine	-43.72	-0.77	4.18	-4.48
D-Serine	-28.02	0.54	12.76	-3.07
D-Histidine	-177.11	-5.08	13.42	-2.56
D-Histidine	-192.1	-5.84	9.98	-2.49
D-Histidine	-186.63	-5.94	11.17	-1.33
D-Histidine	-190.76	-8.23	11.71	-3.07
D-Histidine	-186.32	-5.96	12.23	-1.28

D-Histidine	-182.23	-4.23	18.03	-1.86
D-Cysteine	-134.22	-45.62	73.27	3.44
D-Cysteine	-139.88	-47.72	74.41	3.39
D-Cysteine	-141.17	-49.37	74.69	3.19
D-Cysteine	-126.14	-43.59	72.5	1.78
D-Cysteine	-120.38	-46.35	70.2	4.76
D-Cysteine	-127.81	-46.34	72.3	4.19
D- Phenylalanine	4.03	1.08	4.16	2.14
D- Phenylalanine	-0.15	-0.93	2.29	-1.2
D- Phenylalanine	-0.3	-1.44	0.5	-0.38
D- Phenylalanine	0.96	-1.23	-2.77	-0.25
D- Phenylalanine	1.97	-0.11	-7.88	-0.22
D- Phenylalanine	6.83	2.27	-11.52	0.94
D-Tryptophan	15.32	-0.07	5.84	6.11
D-Tryptophan	10.26	-2.52	-3.11	2.8
D-Tryptophan	11.55	-2.75	1.38	1.17
D-Tryptophan	11.55	-4.69	-2.49	1.07
D-Tryptophan	14.79	-3.09	-4.5	2.98
D-Tryptophan	18.17	-1.19	-20.61	2.22
D-Glutamic acid	88.4	-47.25	82.33	-17.49
D-Glutamic acid	89.05	-45.14	83.78	-20.09
D-Glutamic acid	89.25	-50.1	81.08	-14.11
D-Glutamic acid	89.43	-49.41	85	-18.45
D-Glutamic acid	90.01	-49.5	85.03	-16.27
D-Glutamic acid	92.29	-50.97	87.93	-18.06
D-Methionine	-1.52	-4.86	-2.2	0.81
D-Methionine	-3.85	-5.76	-10.44	1.56
D-Methionine	-1.12	-8.07	-3.9	2.65
D-Methionine	-2.67	-6.41	-7.83	1.03
D-Methionine	-2.98	-11.78	-10.58	2.45
D-Methionine	2.84	-3.91	-13.51	3.97
D-Arginine	-94.08	-66.01	77.64	4.61
D-Arginine	-97.75	-67.02	79.54	1.25
D-Arginine	-99.11	-68.36	79.65	3.27
D-Arginine	-97.59	-69.73	79.94	4.49
D-Arginine	-96.19	-68.94	79.67	7.56
D-Arginine	-93.58	-68.97	80.47	6.78
D-Valine	-18.26	1.18	-1.59	0.91
D-Valine	-12.19	0.01	0.27	-1.03
D-Valine	-18.64	0.24	-4.52	2.67
D-Valine	-15.7	-0.1	1.86	1.06
D-Valine	-14.31	0.52	-4.84	0.67
D-Valine	-13.98	1.63	2.24	1.39

D-Tyrosine	-85.42	2.21	51.77	1.4
D-Tyrosine	-85.71	-0.22	55.4	3.17
D-Tyrosine	-85.94	-0.97	57.09	0.89
D-Tyrosine	-93.23	-1.19	54.93	-0.56
D-Tyrosine	-82.05	-0.03	54.37	5.28
D-Tyrosine	-82.83	0.05	54.46	3.41
D-Lysine	-456.37	-21.52	80.42	3.05
D-Lysine	-503.35	-21.98	81.37	-0.56
D-Lysine	-463.41	-20.43	81.74	1.61
D-Lysine	-531.81	-20.31	80.96	4.06
D-Lysine	-497.54	-21.07	79.83	2.54
D-Lysine	-500.3	-21.43	81.44	4.29
D-Glutamine	11.8	14.66	17.52	1.7
D-Glutamine	13.97	17.26	18.53	3.43
D-Glutamine	9.58	18.9	20.14	2.95
D-Glutamine	9.44	16.49	19.18	2.59
D-Glutamine	9.89	17.99	27.05	3.35
D-Glutamine	8.49	19.37	22.6	5.47
D-Proline	-7.7	2.18	2.71	0.05
D-Proline	-7.63	3.85	4.91	1.31
D-Proline	-16.29	3.36	0.26	-0.92
D-Proline	-17.39	3.53	1.72	1.18
D-Proline	-12.9	3.32	0.68	-0.23
D-Proline	-4.66	4.67	0.95	0.84
D-Leucine	2.55	1.04	7.36	0.11
D-Leucine	4.9	0.88	7.24	-0.65
D-Leucine	6.32	0.5	2.75	2.58
D-Leucine	8.35	0.98	6.12	2.16
D-Leucine	7.12	2.06	5.03	3.93
D-Leucine	9.84	-0.09	2.51	6.91
D-Aspartic acid	90.86	21.73	90.3	30.34
D-Aspartic acid	91.4	21.42	91.54	32.81
D-Aspartic acid	91.5	20.36	91.88	32.47
D-Aspartic acid	91.69	20.82	91.87	32.98
D-Aspartic acid	92.17	22.31	92.27	36.4
D-Aspartic acid	93.59	25.24	93.68	36.57
D-Asparagine	0.53	2.67	2.92	1.01
D-Asparagine	-2.69	0.97	8.4	0.24
D-Asparagine	3.08	2.63	-0.37	3.53
D-Asparagine	2.86	2.5	8.1	2.41
D-Asparagine	1.32	0.7	4.66	4.52
D-Asparagine	0.45	3.02	3.35	6.08
D-Isoleucine	0.97	3.33	9.86	3.45

D-Isoleucine	1.77	1.57	8.91	6.27
D-Isoleucine	-4.38	1.32	2.28	5.45
D-Isoleucine	-0.32	0.06	4.24	4.54
D-Isoleucine	1.41	2.91	7.83	5.53
D-Isoleucine	7.02	4.86	6.03	7.18

Table S4 Matrix of the response patterns against amino acids at 400 μM.

Amino acids	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
L-Alanine	-161.71	16.57	-124.31	-9.59
L-Alanine	-175.94	16.18	-169.67	-21.04
L-Alanine	-181.92	7.81	-123.27	-17.16
L-Alanine	-139.03	15.58	-140.98	-18.7
L-Alanine	-153.55	14.29	-179.69	-6.08
L-Alanine	-154.77	17.04	-151.21	-4.49
L-Threonine	-88.22	-0.26	-25.42	-28.24
L-Threonine	-98.31	8.37	-17.87	-28.32
L-Threonine	-80.31	10.51	-18.16	-29.71
L-Threonine	-78.39	8.37	-17.35	-29.55
L-Threonine	-59.01	10.31	-21.62	-26.1
L-Threonine	-55.19	6.9	-41.91	-40.55
L-Serine	-63.63	-7.64	-140.86	-16.39
L-Serine	-71.37	-29.5	-159.2	-13.63
L-Serine	-75.27	-5.26	-148.95	-13.45
L-Serine	-70.08	-8.49	-144.99	-17.14
L-Serine	-70.81	-12.62	-119.27	-27.54
L-Serine	-69.52	-6.76	-124.58	-26.31
L-Histidine	-210.11	-15.9	11.59	34.35
L-Histidine	-198.09	-62.62	7.65	29.13
L-Histidine	-186.53	-22.98	19.43	22.23
L-Histidine	-184.8	-60.57	10.23	15.32
L-Histidine	-185.46	-20.82	10.2	20.12
L-Histidine	-218	-17.77	12.86	17.82
L-Cysteine	14.32	26.15	83.93	39.78
L-Cysteine	15.99	26.01	91.22	55.66
L-Cysteine	16.24	35.97	95.46	48.89
L-Cysteine	21.33	32.04	89.73	50.99
L-Cysteine	-2.3	7.29	91.21	48.55
L-Cysteine	1.23	8.73	90.21	49.38
L-Phenylalanine	-13.91	11.89	23.76	-22.45
L-Phenylalanine	-12.93	18.64	23.2	-20.52
L-Phenylalanine	-4.6	28.82	26.49	-25.69
L-Phenylalanine	-6.67	24.99	25.82	-29.37

L-Phenylalanine	-18.05	17.75	24.83	-37.86
L-Phenylalanine	-18.15	13.57	11.17	-34.8
L-Tryptophan	1.4	11.99	16.91	7.22
L-Tryptophan	8.59	1.63	8.85	-16.33
L-Tryptophan	13.4	-8.73	3.41	-11.38
L-Tryptophan	5.27	2.83	6.52	-0.64
L-Tryptophan	5.36	14.83	5.94	1.8
L-Tryptophan	20.19	16.88	10.45	9.93
L-Glutamic acid	94.62	-47.06	78.8	-43.58
L-Glutamic acid	98.78	-50.45	78.78	-45.29
L-Glutamic acid	98.67	-45.57	97.36	-43.45
L-Glutamic acid	96.25	-48.61	95.67	-43.95
L-Glutamic acid	99.77	-46.41	96.58	-43.19
L-Glutamic acid	99.71	-47.97	95.96	-43.96
L-Methionine	-30.7	-14.58	-27.6	27.38
L-Methionine	-33.58	-14.57	-25.91	30.82
L-Methionine	-33.37	-36.08	-25.26	28.87
L-Methionine	-32.19	-16.16	-30.4	29.91
L-Methionine	-31.73	-16.52	-44.57	30.04
L-Methionine	-34.9	-9.69	-48.48	31.7
L-Arginine	-49.69	-57.34	92.27	21.83
L-Arginine	-65.39	-60.09	95.11	22.32
L-Arginine	-62.13	-59.39	95.91	22.72
L-Arginine	-62.05	-44.88	94.33	21.29
L-Arginine	-59.5	-43.62	91.6	16.03
L-Arginine	-59.67	-39.24	99.74	1.55
L-Valine	-15.29	5.96	-19.3	-5.35
L-Valine	-19.16	-4.6	-6.54	-11.23
L-Valine	-19.17	1.41	-16.24	-13.07
L-Valine	-18.88	-15.99	-32.97	-11.46
L-Valine	-18.29	3.16	-15.11	-1.1
L-Valine	-22.34	-0.97	-29.94	-3.22
L-Tyrosine	-98.46	-19.22	69.96	4.92
L-Tyrosine	-104.84	-13.5	67.4	5.53
L-Tyrosine	-122.47	-11.65	67.45	3.67
L-Tyrosine	-164.66	-10.15	67.53	1.42
L-Tyrosine	-139.07	-13.37	64.37	2.67
L-Tyrosine	-135.2	-24.84	66.63	-3.29
L-Lysine	-383.22	-70.86	97.59	5.46
L-Lysine	-374.4	-69.48	81.68	9.79
L-Lysine	-405.62	-66.91	85.26	8.65
L-Lysine	-439.05	-70.42	89.28	8.86
L-Lysine	-404.28	-63.38	81.46	10.35

L-Lysine	-419.67	-66.08	80.32	3.19
L-Glutamine	-126.42	-24.69	-73.34	4.67
L-Glutamine	-126.46	-24.73	-73.87	3.63
L-Glutamine	-118.96	-23.21	-73.67	-1.11
L-Glutamine	-113.26	-23.79	-73.82	-2.84
L-Glutamine	-103.84	-22.2	-70.75	2.54
L-Glutamine	-96.17	-25.87	-72.86	1.69
L-Proline	-17.96	3.33	-34.15	-20.95
L-Proline	-16.2	6.99	-37.17	-23.71
L-Proline	-16.76	1.35	-33.36	-22.41
L-Proline	-20.1	0.82	-27.01	-23.63
L-Proline	-16.93	-9.45	-36.53	-25.01
L-Proline	-19.07	-1.16	-28.79	-25.47
L-Leucine	-21.74	3.36	36.68	23.21
L-Leucine	-17.98	-2.66	36.06	24.3
L-Leucine	-20.06	0.09	35.52	24.39
L-Leucine	-22.79	-0.43	35.08	20.98
L-Leucine	-22.72	0.37	34.94	23.47
L-Leucine	-23.03	2.51	35.52	24.06
L-Aspartic acid	90.81	-35.9	85.4	-12.14
L-Aspartic acid	95.59	-41.87	88.83	-18.38
L-Aspartic acid	89.27	-38.71	86.88	-12.41
L-Aspartic acid	94.44	-13.11	89.59	-8.24
L-Aspartic acid	93.2	-34.05	90.56	-6.34
L-Aspartic acid	96.61	-20.09	88.24	-7.57
L-Asparagine	42.42	-43.99	-152.82	-12.71
L-Asparagine	44.96	-44.46	-165.52	-14.48
L-Asparagine	42.33	-44.95	-199.54	-19.95
L-Asparagine	38.3	-44.94	-147.23	-33.56
L-Asparagine	45.78	-42.25	-190.89	-32.12
L-Asparagine	39.08	-41.54	-197.98	-26.59
L-Isoleucine	-34.66	-29.77	5.9	-2.82
L-Isoleucine	-21.17	-23.64	4.11	-6.49
L-Isoleucine	-14.8	-23.26	2.35	-10.25
L-Isoleucine	-13.97	-25.28	2.73	-12.81
L-Isoleucine	-16.96	-23.01	1.99	-10.01
L-Isoleucine	-19.31	-19.05	2.07	-8.7
Glycine	-17.82	-9.23	29.37	-14.57
Glycine	-25.51	-10.6	37.21	-11.01
Glycine	-30.48	-12.23	35.48	-5.55
Glycine	-27.36	-11.52	30.14	-3.54
Glycine	-28.22	-8.01	29.29	-14.27
Glycine	-20.07	-7.8	29.52	-16.63

D-Alanine	-36.78	20.76	-114.45	-6.92
D-Alanine	-36.86	5.01	-122.4	-29.39
D-Alanine	-36.84	-3.53	-128.12	-17.17
D-Alanine	-37.08	0.74	-130.48	-6.82
D-Alanine	-37.58	-5	-137.25	-20.81
D-Alanine	-39.92	-1.28	-154.82	-32.65
D-Threonine	-16.85	26.29	-56.99	-10.2
D-Threonine	-22.3	18.07	-54.61	-12.97
D-Threonine	-17.13	6.06	-51.68	-14.24
D-Threonine	-16.32	8.98	-45.79	-14.32
D-Threonine	-13.42	15.79	-46.96	-16.09
D-Threonine	-12.54	9.01	-47.27	-25.72
D-Serine	-42.21	9.65	2.15	0.72
D-Serine	-49	8.67	4.77	-3.96
D-Serine	-52.37	12.13	7.34	-14.92
D-Serine	-67.45	12.55	12.34	-13.19
D-Serine	-43.77	20.18	14.31	-13.42
D-Serine	-44.57	19.49	22.08	-9.35
D-Histidine	-154.98	-14.88	34.18	1.75
D-Histidine	-219.07	-25.62	30.51	-12.35
D-Histidine	-199.51	-16.72	30.99	-21.85
D-Histidine	-160.74	-21.18	31.1	-22.92
D-Histidine	-149.11	-24.1	24.94	-28.06
D-Histidine	-128.3	-21.9	22.89	-23.85
D-Cysteine	-136.37	-68.31	95.02	16.75
D-Cysteine	-118.62	-71.42	93.04	16.08
D-Cysteine	-132.58	-67.47	86.74	15.93
D-Cysteine	-176.62	-66.68	87.53	17.28
D-Cysteine	-143.93	-64.73	87.08	16.68
D-Cysteine	-172.51	-67.46	89.94	17.61
D- Phenylalanine	0.14	-0.28	-15.47	3.78
D- Phenylalanine	-1.51	16.53	-16.92	6.25
D- Phenylalanine	-7.1	12.4	-10.17	16.47
D- Phenylalanine	-5.09	14.31	-26.65	8.19
D- Phenylalanine	3.54	12.58	-17.12	-2.28
D- Phenylalanine	1.43	-6.91	-5.37	-1.32
D-Tryptophan	4.68	-2.83	-18.81	7.5
D-Tryptophan	14.35	-15.03	-15.01	1.89
D-Tryptophan	20.66	-7.58	-11.25	9.15
D-Tryptophan	26.38	-1.47	-13.1	6.54
D-Tryptophan	10.84	-12.18	-10.95	10.44
D-Tryptophan	4.63	-12.42	-14.9	16.81
D-Glutamic acid	98.05	-67.63	82.99	-36.37

D-Glutamic acid	98.55	-76.07	88.78	-38.76
D-Glutamic acid	97.6	-71.16	92.24	-39.06
D-Glutamic acid	99.1	-73.74	89.55	-40.13
D-Glutamic acid	99.04	-81.73	90.29	-41.64
D-Glutamic acid	99.44	-92.39	93.23	-42.27
D-Methionine	-14.23	-21.08	-21.21	-0.61
D-Methionine	-15.69	-11.98	-41.05	-4.21
D-Methionine	-13.36	-17.54	-42.22	-4.67
D-Methionine	-8.58	-49.95	-44.9	-2.99
D-Methionine	-10.59	-28.61	-30.19	0.3
D-Methionine	-9.26	-17.85	-41.09	-3.08
D-Arginine	-141.91	-125.15	91.32	-0.95
D-Arginine	-93.24	-125.08	93.61	-8.12
D-Arginine	-99.41	-70.59	93.47	7.95
D-Arginine	-95.8	-61.73	82	-6.76
D-Arginine	-101.81	-80.91	93.72	11.49
D-Arginine	-145.19	-116.04	95.35	4.88
D-Valine	-17.71	33.65	-15.9	8.27
D-Valine	-21.7	20.89	-0.41	1.14
D-Valine	-23.01	35.93	-9.23	-1.54
D-Valine	-15.38	38.71	-10.54	1.99
D-Valine	-19.13	43.73	-18.17	4.63
D-Valine	-24.92	18.51	-22.07	8.98
D-Tyrosine	-104.73	7.57	64.92	17.69
D-Tyrosine	-50.64	-15.26	61.57	12.83
D-Tyrosine	-52.92	-11.91	58.19	8.5
D-Tyrosine	-86.13	3.4	62.68	7.99
D-Tyrosine	-113.16	7.94	56.08	9.17
D-Tyrosine	-98.88	15.44	58.49	11.22
D-Lysine	-358.73	-52.87	91.54	8.62
D-Lysine	-335.31	-58.41	82.29	12.28
D-Lysine	-339.83	-57.17	95.14	12.68
D-Lysine	-342.48	-46.01	97.08	21.2
D-Lysine	-362.69	-52.67	94.52	18.48
D-Lysine	-362.11	-58.08	90.2	18.01
D-Glutamine	15.93	35.85	19.44	8.36
D-Glutamine	14.54	36.78	20.36	5.76
D-Glutamine	16.43	35.8	20.81	-1.98
D-Glutamine	19.74	31.74	21.21	8.73
D-Glutamine	20.64	38.05	24.35	-9.81
D-Glutamine	20.12	32.68	20.86	-5.72
D-Proline	-12.79	11.25	0.05	11.54
D-Proline	-17.7	16.48	17.72	26.24

D-Proline	-16.79	17.08	19.86	19.99
D-Proline	-32.45	16.78	20.79	14.74
D-Proline	-25.92	19.3	21.84	14.4
D-Proline	-24.01	9.19	1.91	13.65
D-Leucine	10.03	-13.25	27.64	2.39
D-Leucine	12.12	-11.99	29.66	7.92
D-Leucine	16.04	-6.63	29.96	12.59
D-Leucine	13.27	-9.77	28.7	13.49
D-Leucine	18.3	-12.09	28.03	11.27
D-Leucine	21.91	-13.82	31.9	-13.81
D-Aspartic acid	99	29.16	98.66	28.12
D-Aspartic acid	97.09	33.15	97.62	26.11
D-Aspartic acid	98.8	55.11	98.8	24.59
D-Aspartic acid	98.94	56.92	99.27	32.34
D-Aspartic acid	92.08	55.64	98.1	28.64
D-Aspartic acid	95.66	47.81	99.04	25.52
D-Asparagine	8.27	-8.54	10.58	9.46
D-Asparagine	2.46	-23.88	13.76	6.86
D-Asparagine	-5.2	-5.3	18.47	7.32
D-Asparagine	-20.93	-6.14	16.87	4.88
D-Asparagine	-4.5	-26.98	19.44	4.28
D-Asparagine	-6.02	-21.7	10.54	12.44
D-Isoleucine	-2.77	12.33	24.82	7.64
D-Isoleucine	-5.56	14.33	31.96	19.52
D-Isoleucine	-8.95	16.49	39.27	11.21
D-Isoleucine	-4.38	15.38	36.9	24.31
D-Isoleucine	-7.39	18.18	16.1	23.61
D-Isoleucine	-2.72	26.45	17.69	21.86

Table S5 Matrix of the response patterns against glutamine (Gln) with different enantiomeric purity.

%D-Gln	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
0	-95.3	-5.93	-44.04	3.65
0	-91.27	-10.66	-46.42	-2.51
0	-91.13	-12.42	-42.48	-1.74
0	-95.38	-8.65	-37.91	0.89
0	-98.55	-9.7	-43.82	0.09
0	-87.89	-7.5	-29.65	1.62
20%	-67.57	-8	-28.71	0.86
20%	-57.02	-8.05	-27.93	-2.69
20%	-44.36	-13.88	-29.16	-0.61
20%	-56.88	-9.97	-30.64	-3.06
20%	-56.97	-7.57	-30.63	-1.32

20%	-67.84	-7.2	-39.99	1.47
40%	-33.99	-1.71	-17.68	-6.86
40%	-34.45	-4.63	-15.97	-6.86
40%	-36.27	-3.94	-12.74	-7.19
40%	-23.37	-4.03	-14.5	-6.49
40%	-23.51	-5.02	-25.35	-5.82
40%	-34.04	-2.85	-11.47	-3.37
60%	-28.74	-1.32	-5.5	-0.28
60%	-25.4	1.03	-9.27	2.17
60%	-22.98	-4.11	-6.27	1.93
60%	-22.98	-1.16	-6.3	-2.6
60%	-19.14	-3.29	-4.89	-1.65
60%	-15.64	1.06	0.65	-2.69
80%	0.34	12.5	3.92	-6.64
80%	13.41	14.13	6.21	-0.58
80%	2.05	12.73	2.41	7.65
80%	19.13	13.41	8.35	2.91
80%	0.66	13.07	5.48	7.99
80%	23.22	4.41	11.9	3.61
100%	11.8	14.66	17.52	1.7
100%	13.97	17.26	18.53	3.43
100%	9.58	18.9	20.14	2.95
100%	9.44	16.49	19.18	2.59
100%	9.89	17.99	27.05	3.35
100%	8.49	19.37	22.6	5.47

Table S6 Matrix of the response patterns against D-Glutamic acid with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	25.46	8.18	6.65	0.16
10	24.61	2.99	7.94	1.24
10	23.39	4.19	5.22	2.99
10	23.09	6.71	4.29	1.91
10	24.25	4.54	3.79	4.61
10	25.91	8.01	1.29	2.7
40	34.96	0.61	28.61	-5.37
40	32.48	-0.37	40.07	-3.46
40	32.31	-0.51	27.82	-2.54
40	32.28	-3.19	17.25	-4.26
40	32.41	-5.29	29.97	-6.8
40	36.97	-5.52	28.96	1.43
80	46.76	-2.71	48.28	-2.35
80	44.51	-7.24	54.12	-9.5

80	45.06	-6.12	53.99	-10.24
80	43.28	-6.04	53.88	-10.97
80	45.48	-6.78	46.52	-9.89
80	45.1	-5.29	51.82	-9.5
120	58.12	-11.98	63.99	-17.32
120	57.07	-18.19	65.59	-13.89
120	60.61	-17.75	57.9	-11.6
120	57.26	-16.39	71.5	-10.52
120	57.56	-17.24	64.53	-15.96
120	56.97	-15.35	62.88	-12.33
160	70.31	-24.45	82.11	-19.83
160	71.78	-30.61	75.75	-14.65
160	73.01	-32.35	73.18	-18.28
160	61.55	-32.46	75.71	-17.55
160	70.05	-33.26	79.8	-20.44
160	68.64	-28.72	82.64	-16.82
200	88.4	-47.25	82.33	-17.49
200	89.05	-45.14	83.78	-20.09
200	89.25	-50.1	81.08	-14.11
200	89.43	-49.41	85	-18.45
200	90.01	-49.5	85.03	-16.27
200	92.29	-50.97	87.93	-18.06

Table S7 Matrix of the response patterns against L-Asp with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	39.92	8.33	20.88	-26.01
10	53.67	11.7	22.16	-21.37
10	55.53	13.31	20.74	-25.49
10	54.22	10.82	17.97	-9.8
10	46.81	8.46	17.35	-14.8
10	53.01	-11.03	15.24	-24.25
50	63.53	12.66	33.42	-3.18
50	68.66	-4.46	15.08	-6.39
50	70.25	-12.48	26.13	-1.26
50	66.89	-8.06	42.41	-7.86
50	63.99	-8.65	18.8	-10.09
50	69.89	-5.2	38.94	-12.02
100	80.11	7.46	58.74	6.32
100	80.16	7.26	59.18	3.34
100	79.64	11.22	60.13	4.75
100	80.88	15.28	53.04	1.67
100	75.05	-14.96	52.88	-7.05
100	78.53	-14.01	52.2	-9.32

150	87.57	-8.81	66.43	24.75
150	85.03	-6.16	71.03	6.92
150	88.72	-10.71	67.48	-1.13
150	86.77	-6.3	64.87	22.78
150	87.94	-7.71	63.25	6.32
150	88.15	-1.28	66.83	-6.45
200	93.91	-6.74	89.6	14.72
200	92.07	-9.01	86.01	13.38
200	90.88	-8.41	86.36	11.54
200	91.46	-10.24	85.86	4.61
200	90.23	-10.21	86.52	9.59
200	91.94	-7.89	88.32	9.48

Table S8 Matrix of the response patterns against D-Asp with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	42.44	3.15	31.74	-7.23
10	46.47	7.59	29.25	-5.72
10	47.21	4.04	30.33	-6.39
10	42.22	7.31	28.3	-5.39
10	43.16	-0.76	27.14	-7.22
10	40.12	0.34	23.85	-11.87
50	47.96	-6.39	39.08	10.26
50	55.35	9	44.88	4.25
50	57.45	17.95	45.19	2.26
50	51.41	14.65	47.74	-5.23
50	58.26	9.99	46.54	-1.2
50	51.5	2.22	48.93	-0.54
100	67.3	12.36	59.27	20.92
100	64.41	9.69	66.27	17.97
100	65.4	12.84	57.41	20.38
100	68.08	12.87	56.95	19.66
100	65.82	25.93	57.06	-4.85
100	66.14	24.51	55.83	5.61
150	75.42	16.88	88.87	24.52
150	76.12	14.25	85.31	21.9
150	76.32	17.35	77.88	18.24
150	76.58	21.18	65.73	12.19
150	77.09	22.56	75.9	15.6
150	77.55	20.89	71.6	16.65
200	90.86	21.73	90.3	30.34
200	91.4	21.42	91.54	32.81
200	91.5	20.36	91.88	32.47
200	91.69	20.82	91.87	32.98

200	92.17	22.31	92.27	36.4
200	93.59	25.24	93.68	36.57

Table S9 Matrix of the response patterns against L-His with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	-23.57	22.6	-5	0.67
10	-24.96	14.04	-7.19	-1.22
10	-24.25	16.07	-5.7	-2.59
10	-25.07	1.93	-5.83	-8.52
10	-20.3	-7.05	-2.92	7.04
10	-18.32	-10.12	-0.23	-10.65
50	-83.39	10.08	1.95	-3.59
50	-74.12	3.86	6.93	1.45
50	-66.29	-2.83	9.77	7.91
50	-69.68	-0.04	13.93	19.59
50	-81.12	1.32	16.91	3.13
50	-94.81	2.45	-19.61	-1.53
100	-111.92	-1.83	2.33	-3.75
100	-126.32	7.77	5.93	-0.57
100	-119.46	11.62	-0.36	0.02
100	-123.15	0.29	0.69	2.53
100	-112.24	-10.45	1.14	2.87
100	-130.49	4.04	1.43	4.93
150	-150.23	-9.43	2.85	-3.55
150	-135.2	0.18	-2.45	11.11
150	-151.19	-2.58	14.17	5.7
150	-130.66	-4.88	11.06	7.62
150	-150.35	2.39	7.7	-0.97
150	-164.67	8.5	6.88	-6.34
200	-179.33	-4.02	11.89	6.58
200	-192.45	-4.88	4.73	3.95
200	-184.17	-3.22	5.69	3.24
200	-193.06	-4.59	3.9	4.08
200	-182.99	-3.18	9.98	2.97
200	-193.43	-6.01	6.88	1.59

Table S10 Matrix of the response patterns against D-His with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	-67.84	-2.71	-9.46	-6.49
10	-67.85	-2.17	-11.75	-6.98
10	-66.08	-0.5	-7.61	6.38
10	-60.17	4.64	-12.92	3.26
10	-55.75	6.38	-11.13	-17.86

10	-62.25	8.82	-15.38	-4.41
50	-97.68	-14.77	-13.97	4.57
50	-79.29	1.2	-4.05	-7.05
50	-90.71	-3.31	-19.05	-3.59
50	-96.65	-1.04	-16.83	10.18
50	-102.8	-7.52	-9.14	1.87
50	-91.33	-7.66	-17.63	-4
100	-123.1	-3.14	11	1.39
100	-118.13	-0.53	7.18	-7.92
100	-119.47	-0.13	6.26	1.65
100	-117.13	-2.61	8.98	3.14
100	-110.52	3.54	6.48	9.21
100	-106.28	1.28	-3.26	-2.98
150	-147.78	5.77	8.99	4.23
150	-162.07	-8.91	-2.03	5.99
150	-160.7	-19.04	-1.99	2.69
150	-153.46	4.36	6.83	-0.27
150	-161.17	-18.7	6.78	7.62
150	-155.21	-2.72	1.8	6.03
200	-177.11	-5.08	13.42	-2.56
200	-192.1	-5.84	9.98	-2.49
200	-186.63	-5.94	11.17	-1.33
200	-190.76	-8.23	11.71	-3.07
200	-186.32	-5.96	12.23	-1.28
200	-182.23	-4.23	18.03	-1.86

Table S11 Matrix of the response patterns against L-Lys with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	-71.24	5.16	20.3	-10.12
10	-102.07	4.02	18.29	-17.98
10	-103.67	3.72	10.57	-9.71
10	-72.95	4.5	3.16	-10.53
10	-69.42	-3.1	15.46	-6.77
10	-65.95	-5.64	6.15	-3.13
50	-121.41	14.99	33.17	-17.48
50	-136.51	4.94	33.71	-7.05
50	-136.44	8.37	33.78	-12.42
50	-129.06	-6.8	31.38	-1.3
50	-134.47	2.77	29.63	-3.6
50	-125.04	-2.3	31.43	-10.88
100	-248.78	-1.83	54.47	2.76
100	-233.91	-6.15	56.53	10.56
100	-223.74	-4.58	55.41	10.85

100	-227.22	-8.13	36.53	-2.19
100	-222.34	-12.01	50.06	4.28
100	-354.06	-25.01	32.57	0.01
150	-404.3	-20.04	68.88	5
150	-354.21	-18.51	69.35	7.75
150	-352.63	-16.04	76.9	5.11
150	-343.2	-21.97	71.87	-2.89
150	-371.67	-12.67	68.75	-5.64
150	-471.97	-16.81	72.61	-1.37
200	-584.54	-18.61	79.09	2.99
200	-614.36	-22.65	70.99	0.22
200	-561.5	-21.06	77.19	0.17
200	-614.2	-23.66	73.57	0.65
200	-590.39	-23.73	76.82	0.84
200	-578.46	-23.25	78.02	1.38

Table S12 Matrix of the response patterns against D-Lys with different concentrations.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
10	-45.84	5.11	18.2	4.44
10	-44.87	-0.57	11.31	4.84
10	-42.43	-2.6	15.59	-3.28
10	-30.99	3.34	17.8	4.45
10	-34.31	2.3	17.96	4.45
10	-48.11	5.33	7.19	-0.24
50	-119.11	-11.33	21.29	7.93
50	-97.58	-0.87	27.4	5.88
50	-111.31	1.5	41.17	2.22
50	-118.42	2.09	25.02	1.29
50	-82.96	-1.97	21.37	14.03
50	-113.15	-0.04	29.41	0.36
100	-143.9	-18.44	33.12	-2.07
100	-185.29	-23.19	52.36	-8.8
100	-196.95	-2.69	53.78	-3.2
100	-131.03	-7.93	49.65	-2.04
100	-136.91	-2.79	50.6	2.89
100	-132.03	-15.22	38.69	-1.61
150	-324.44	-9.73	45.08	4.02
150	-295.05	-9.78	67.08	0.47
150	-264.2	-8.3	69.61	7.38
150	-325.75	-11.09	61.21	1.98
150	-319.45	-17.16	79.08	-1.97
150	-304.04	-17.86	59.41	5.62
200	-456.37	-21.52	80.42	3.05

200	-503.35	-21.98	81.37	-0.56
200	-463.41	-20.43	81.74	1.61
200	-531.81	-20.31	80.96	4.06
200	-497.54	-21.07	79.83	2.54
200	-500.3	-21.43	81.44	4.29

Table S13 Matrix of the response patterns against the different concentrations of D-Aspartic acid in the mixture.

Concentration (μM)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
0	-25.93	-13.54	-51.27	4.59
0	-29.19	-22.95	-47.62	3.56
0	-26.85	-18.78	-51.5	1.17
0	-28	-10.59	-58.38	-1.88
0	-26.05	-15.85	-48.58	-0.69
0	-24.35	-16.34	-51.5	-4.2
5	-27.99	-25.56	-60.11	-2.18
5	-25.59	-27.2	-67.52	-3.02
5	-29.15	-23.71	-70.08	-3.35
5	-28.93	-30.05	-61.54	-8.41
5	-26.07	-28.32	-63.63	-9.51
5	-26.64	-28.41	-60.25	-7.27
10	-32.06	-41.24	-69.43	-18.93
10	-27.39	-39.64	-66.6	-21.44
10	-27.97	-44.58	-66.8	-17.24
10	-28.57	-39.26	-70.41	-27.95
10	-29.68	-39.85	-71.18	-23.16
10	-28.91	-41.43	-65.21	-17.88
20	-21.59	-52.11	-67.99	-30.14
20	-13.64	-57.57	-78.47	-29.1
20	-19.55	-45.91	-73.98	-30.47
20	-24.3	-52.05	-72.86	-31.66
20	-26.21	-51.22	-68.95	-29.27
20	-25.51	-52.31	-62.51	-32.9
30	-2.56	-68.25	-76.98	-33.07
30	-3.95	-67.58	-69.41	-36.78
30	-6.01	-70.58	-71.35	-35.54
30	-9.24	-63.62	-71.95	-42.27
30	-13.35	-68.2	-70.15	-40.1
30	-8.09	-58.55	-82.76	-38.32
40	18.1	-83.24	-79.76	-41.6
40	18.85	-82.9	-75.26	-42.75
40	17.5	-82.61	-65.81	-42.12
40	9.47	-82.71	-74.58	-40.54

40	22.19	-78.54	-75.02	-41.36
40	20.89	-78.96	-79.83	-40.1
50	34.69	-97.58	-75.11	-49.29
50	35.75	-100.01	-90.77	-45.61
50	34.42	-99.36	-82.52	-47.35
50	25.27	-103.15	-84.48	-47.88
50	23.08	-103.63	-76.91	-45.27
50	28.6	-115.82	-76.91	-44.41

Table S14 Matrix of the response patterns against the different concentrations of D-Aspartic acid and L-Alanine in the mixture.

Groups	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
A	-24.91	-32.54	-80.47	-10.59
A	-21.57	-32.78	-83.68	-14.05
A	-21.7	-39.1	-72.46	-9.76
A	-22.47	-41.15	-79.63	-10.33
A	-24.15	-33.18	-79.14	-8.39
A	-20.65	-29.33	-77.58	-9.59
B	-21.94	-42.64	-73.25	-17.18
B	-22.4	-35.63	-63.87	-18.95
B	-20.89	-37.68	-63.36	-20.55
B	-22.05	-39.95	-60.6	-10.09
B	-23.04	-43.88	-71.25	-12.64
B	-21.47	-44.74	-60.66	-17.85
C	-20.4	-77.62	-49.99	-28.32
C	-18.69	-73.85	-52.87	-26.12
C	-18.33	-64.93	-58.74	-24.34
C	-19.82	-58.6	-48.43	-16.93
C	-16.97	-69.43	-56.47	-17.41
C	-20.34	-65.66	-47.52	-22.63
D	-15.76	-95.55	-48.01	-29.69
D	-14.39	-97.05	-50.79	-36.09
D	-12.3	-92.98	-51.21	-32.59
D	-13.3	-103.25	-54.4	-30.91
D	-13.74	-98.68	-63.79	-31.11
D	-14.82	-88.12	-47.08	-29
E	-4.01	-104.81	-50.85	-40.13
E	-0.72	-106.04	-53.62	-45.62
E	-1.37	-104.52	-52.71	-38.25
E	0.36	-115.94	-47.8	-31.56
E	4.22	-109.83	-57.9	-34.72
E	-3	-112.88	-52.35	-36.51
F	16.65	-128.71	-61.23	-63.71

F	15.28	-119.41	-55.98	-62.69
F	12.68	-116.72	-63	-60.39
F	22.82	-122.85	-64.22	-50.69
F	22.23	-119.41	-61.09	-51.42
F	12.68	-126.63	-55.98	-47.17
G	29.56	-131.17	-69.12	-71.93
G	33.41	-138.65	-62.94	-69.34
G	28.53	-131.95	-66.14	-73.91
G	29.38	-147.87	-70.05	-59.84
G	27.92	-147.05	-63.79	-64.31
G	25.6	-142.91	-59.17	-64.15

Table S15 Matrix of the response patterns against free amino acids of fermented milk at different stages.

Fermented time (h)	*V. fischeri*	*EC-lux*	*BS-lux*	*AT-lux*
0	81.4	-109.08	26.39	11.95
0	78.69	-106.75	22.11	13.36
0	78.13	-108.79	28.36	14.9
0	78.03	-110.3	31.77	11.41
0	78.17	-106.07	26.42	12.45
0	80.86	-107.33	26.87	16.39
2	87.16	-90.36	50.24	15.09
2	86.14	-104.01	40.73	14.36
2	85.51	-111.96	42.73	17.57
2	85.93	-103.96	47.9	17.38
2	86.1	-98.15	46.09	21.01
2	88.07	-99.47	45.85	20.2
4	89.64	-92.88	68.61	20.7
4	88.95	-90.03	65.05	23.36
4	88.19	-94.04	62.28	21.07
4	89.85	-95.07	63.07	22.83
4	89.5	-90.68	64.14	24.03
4	90	-83.28	65.24	25.27
6	80.52	-98.94	28.59	6.4
6	79.7	-101.35	22.09	3.52
6	77.36	-101.22	29.62	3.26
6	81.43	-99.7	21.29	1.49
6	82.32	-96.76	19.74	13.29
6	82.16	-93.51	23.09	9
8	21.42	-112.51	-36.72	-10.02
8	20.11	-124.22	-54.8	-7.56
8	20.22	-126.87	-60.64	-5.15
8	24.51	-125.6	-31.55	-6.7

8	19.12	-120.39	-61.33	-4.94
8	28.39	-110.7	-53.27	-7.21
10	-46.46	-131.51	-63.52	-15.54
10	-42.7	-131.76	-78.59	-13.57
10	-42	-130.57	-74.9	-12
10	-44.24	-133.57	-88.9	-13.37
10	-45.72	-136.11	-78.52	-11.87
10	-42.41	-124.89	-83.71	-11.51
12	-37.37	-130.4	-63.77	-16.38
12	-38.1	-142.09	-76.68	-19.11
12	-40.28	-140.03	-74.64	-17.71
12	-38.15	-143.29	-80.56	-19.54
12	-37.73	-131.47	-78.03	-23.87
12	-39.89	-128.34	-76.88	-21.19

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