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Supporting Information

pH Feedback Lifecycles Programmed by Enzymatic Logic Gates Using Common Foods as Fuels

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1. Materials

All chemicals were purchased from Sigma-Aldrich unless otherwise stated: citric acid monohydrate (CA, BioXtra, ≥99.5%), trisodium citrate dihydrate (Na_3C, BioXtra, ≥99.0%), sodium dihydrogen phosphate (BioXtra, ≥99.0%), disodium hydrogen phosphate (BioXtra, ≥99.0%), tris(hydroxymethyl)aminomethane (Tris, BioXtra, ≥99.9%), hydrochloride acid (36.5-38.0%, bioreagent), sodium carbonate (BioXtra, ≥99.0%), sodium bicarbonate (BioXtra, 99.5%-100.5%), bovine serum albumin (BSA, ≥95%), invertase from baker's yeast (*S. cerevisiae*) (Inv, grade VII, ≥300 units/mg solid), β-galactosidase from Aspergillus oryzae (β-Gal, ≥8.0 units/mg solid), glucose oxidase from Aspergillus niger (GOx, Type VII, lyophilized powder, ≥100,000 units/g solid), catalase from bovine liver (Cat, 2000-5000 units/mg powder), sucrose (≥99%), lactose monohydrate (BioXtra, ≥99%), glucose (BioXtra, ≥99.5%), Trizma® base (pH 8.8, M_w 127.2 g/mol). Activity assay kits of Inv, β-Gal and GOx were bought from BioVision. Baking soda, soda, milk and sugar beet syrup were purchased from local supermarket. Fmoc-ethylenediamine hydrochloride was purchased from Abcr GmbH. All chemicals were used as received without further purification. MilliQ water was used throughout all experiments.

2. Activity assessment of enzymes

2.1. Measurement of Inv activity at different pH values

Inv activity assay kit from BioVision (Catalog #K674-100) was adopted to measure the activity of Inv at different pH values. We follow the protocol of the assay kit but replace the provided buffer with homemade CA/Na_3C (50 mM, pH=3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0), phosphate buffer solution (PBS, 50 mM, pH=6.0, 6.5, 7.0, 7.5, and 8.0), and Tris (50 mM, pH=8.0, 8.5, and 9.0) buffer solutions. After the replacement, the assay kit has following components: Inv hydrolysis buffer (CA/Na_3C, PBS, or Tris), Inv assay buffer (identical to Inv hydrolysis buffer in each measurement), Inv stop solution, OxiRed™ probe in DMSO, Inv enzyme mix, Inv substrate, glucose standard (100 mM), and positive control.

2.1.1. Calibration curves

1 mM glucose standard solution is prepared by diluting 10 µL 100 mM glucose standard with 990 µL MilliQ water. Pipette 0, 0.5, 1.0, 1.5, 2.0, and 2.5 µL 1 mM glucose standard solution to a 384-well plate, Inv hydrolysis buffer is added to adjust the volume to 12.5 µL.

2.5 µL Inv stop solution, 9 µL Inv assay buffer identical to hydrolysis buffer, 0.5 µL Inv enzyme mix, and 0.5 µL probe were added to the glucose solution to make a total volume of 25 µL. Incubate the well plate at 37 °C for 30 min in dark place, then measure the absorbance at 570 nm with a TECAN Spark 10M plate reader. Subtract 0 standard record from all records and plot the calibration curves. Calibration curves measured at different pH values (from 3.0 to 9.0) are shown in Figure S1.

2.1.2. Inv activity measurement

i) Preparation of Inv sample, positive control and background control

Inv sample: pipette 1 µL Inv dissolved in hydrolysis buffer into well plate, and dilute the enzyme solution with 9 µL hydrolysis buffer to a volume of 10 µL. Positive control: dilute 1 µL positive control solution with 9 µL hydrolysis buffer. Background control: mix 1 µL Inv dissolved in hydrolysis buffer and additional 11.5 µL hydrolysis buffer.

ii) Substrate hydrolysis

Add 2.5 µL Inv substrate into wells containing Inv sample and positive control. Then incubate the plate at room temperature (20 °C, same with the temperature that all experiments in the main text were carried out.) for 20 min.

iii) Reaction mix

After the incubation, sequentially add each well including Inv sample, positive control and background control with 2.5 µL Inv stop solution, a mixture of 9 µL assay buffer, 0.5 µL Inv enzyme mix, and 0.5 µL probe. Incubate the well plate at 37 °C for 30 min in dark place, then measure the absorbance at 570 nm with a plate reader.

iv) Calculation

Subtract 0 standard record from all records. And subtract the reading of background control from all records if the background control is significant. Apply the absorbance record of Inv sample to calibration curves to get the amount of glucose generated by sample Inv during the incubation at 20 °C. The activity of sample Inv can be calculated using the following equation:

$$\text{Activity} = n/(m*t)\ \text{mol}/(\text{mg}*\text{min})$$

Where: n is the amount of glucose generated by sample Inv (mol);
m is the amount of sample Inv added into the well plate (mg);
t is the incubation time at 20 °C (min).

Figure 1c in main text presents the activity of Inv used in all our experiments at different pH values.

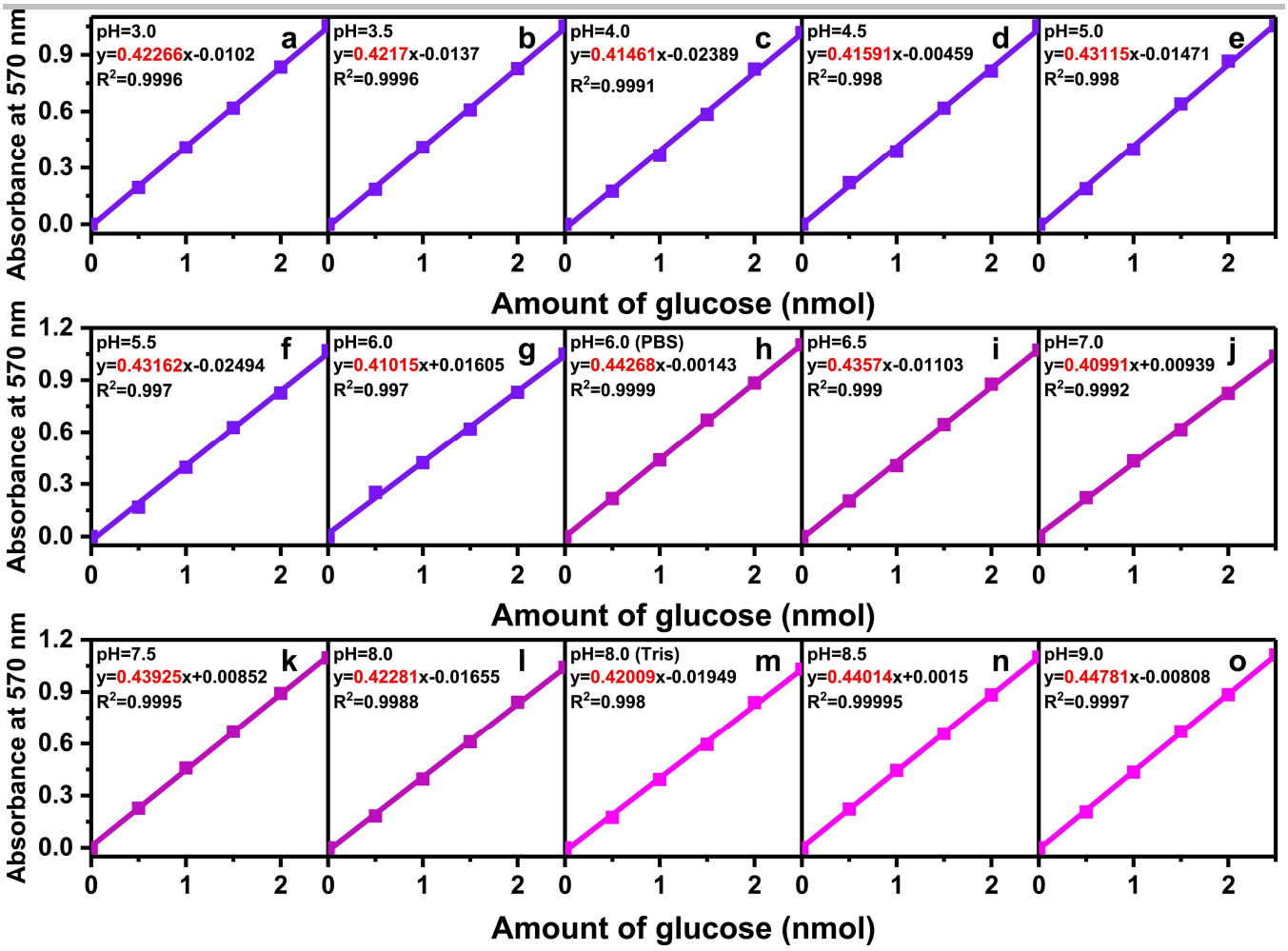


Figure S1. Calibration curves of glucose measured at different pH values: (a-g) pH 3.0-6.0 in CA/Na_3C, (h-l) pH 6.0-8.0 in PBS, and (m-o) pH 8.0-9.0 in Tris.

2.2. Measurement of GOx activity at different pH values

GOx activity colorimetric/fluorometric assay kit from BioVision (Catalog #K788-100) was used to measure the activity of GOx at different pH values. We follow the protocol of the assay kit but replace the provided assay buffer with homemade CA/Na_3C (50 mM, pH=3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0), PBS (50 mM, pH=6.0, 6.5, 7.0, 7.5, and 8.0), and Tris (50 mM, pH=8.0, 8.5, and 9.0) buffer solutions. After the replacement, the assay kit has following components: GOx assay buffer (CA/Na_3C, PBS, or Tris), OxiRed™ probe, GOx substrate, GOx developer, GOx positive control, and H_2O_2 standard (0.88 M).

2.2.1. Calibration curves

Add 0.5 µL 0.88 M H_2O_2 standard to 43.5 µL MilliQ water to make a H_2O_2 solution with a concentration of 10 mM. Then 1 µL 10 mM H_2O_2 solution was diluted with 199 µL assay buffer to a concentration of 50 µM. Pipette 0, 0.5, 1.0, 1.5, 2.0, 2.5 µL 50 µM H_2O_2 standard solution to a 384-well plate, and assay buffer was added to each well to adjust the volume to 12.5 µL.

A mixture of 9 µL assay buffer, 0.5 µL GOx developer, 0.5 µL OxiRed™ probe, and 2.5 µL GOx substrate was added to the H_2O_2 solution and homogeneously mixed. Incubate the plate at 20 °C for 5 min, and measure the absorbance at 486 and 570 nm (Considering that the maximum absorbance of the probe is pH dependent (Figure S2p), we use absorbance at 486 nm when pH≤5.5 and 570 nm when pH>5.5 to plot calibration curves). Subtract the 0 standard record from all records and plot the calibration curve. Calibration curves at different pH values are shown in Figure S2.

2.2.2. GOx activity measurement

i) Preparation of GOx sample, positive control and background control

GOx sample: pipette 0.5 µL GOx dissolved in assay buffer into well plate, and dilute the enzyme solution with 12 µL assay buffer to a volume of 12.5 µL. Positive control: dilute 0.5 µL positive control solution with 12 µL assay buffer. Background control: mix 0.5 µL GOx dissolved in assay buffer and additional 12 µL assay buffer.

ii) Kinetics measurement

A mixture of 9 µL assay buffer, 0.5 µL GOx developer, 0.5 µL OxiRed™ probe, and 2.5 µL GOx substrate was added to GOx sample and positive control, and homogeneously mixed. Another mixture of 11.5 µL assay buffer, 0.5 µL GOx developer, and 0.5 µL OxiRed™ probe was homogeneously mixed with background control. Measure the absorbance of GOx sample, positive control and background control in a kinetic way at 20 °C (Figure S3) and choose the period of linear range to calculate the GOx activity.

iii) Calculation

Subtract the 0 standard record from all records. In a linear range of the kinetic curves of GOx sample, calculate the increasement of absorbance ΔOD in a period, apply the ΔOD to calibration curves to get the amount of H_2O_2 generated by GOx sample during the period. The activity of GOx sample can be calculated using the following equation:

Activity=n/(m*t) mol/(mg*min)

Where: n is the amount of H_2O_2 generated by GOx sample (mol);

m is the amount of GOx sample added into the well plate (mg);

t is the period of ΔOD at 20 °C (min).

Figure 1d in main text presents the activity of GOx used in all our experiments at different pH values.

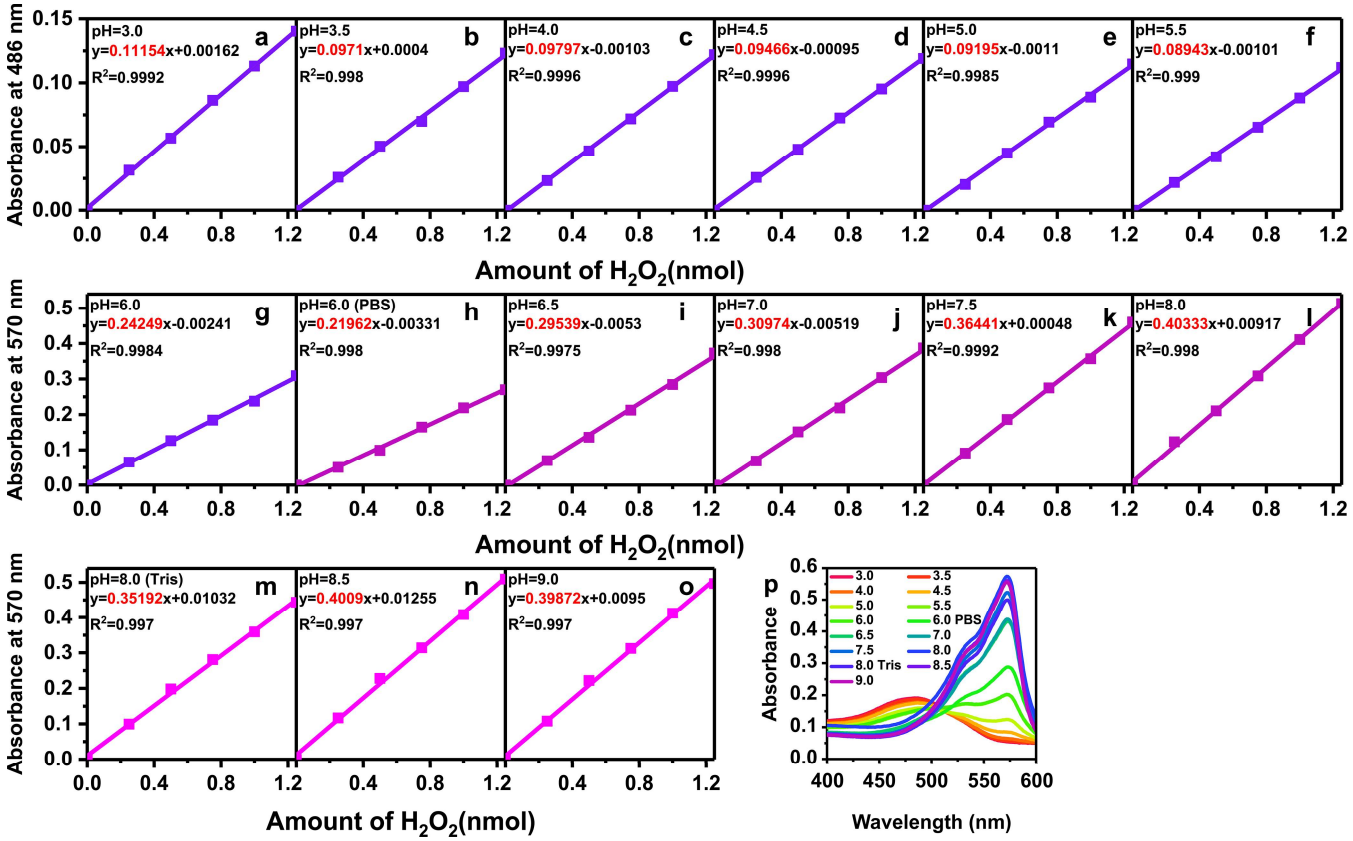


Figure S2. Calibration curves of H_2O_2 measured at different pH values: (a-g) pH 3.0-6.0 in CA/Na_3C, (h-l) pH 6.0-8.0 in PBS, and (m-o) pH 8.0-9.0 in Tris. And (p) the absorbance of probe at different pH values.

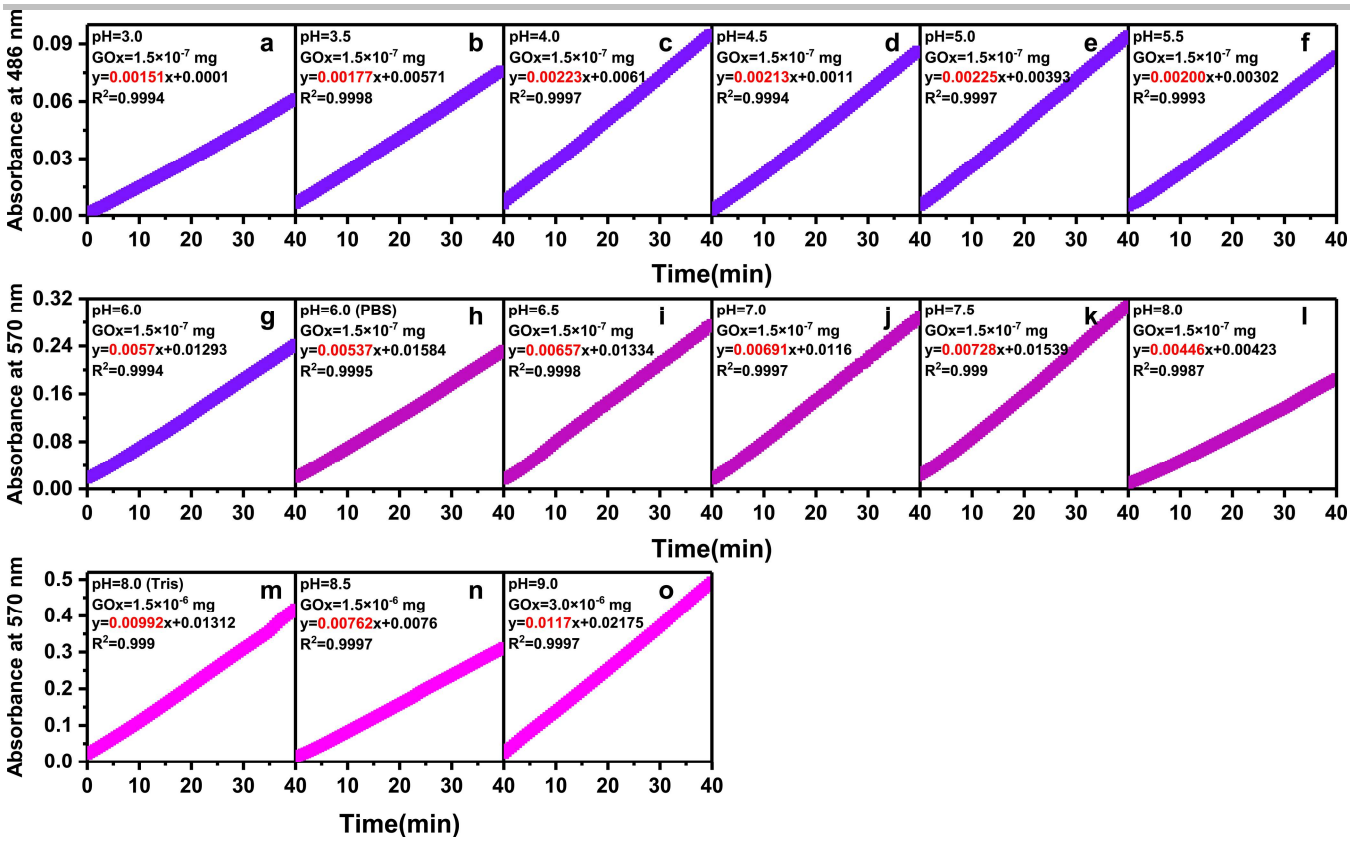


Figure S3. Absorbance kinetics of the hydrolysis of glucose catalyzed by GOx at different pH values: (a-g) pH 3.0-6.0 in CA/Na_3C, (h-l) pH 6.0-8.0 in PBS, and (m-o) pH 8.0-9.0 in Tris.

2.3. Measurement of β-Gal activity at different pH values

β-Gal activity fluorometric assay kit from BioVision (Catalog #K821-100) was adopted to measure the activity of β-Gal at different pH values. We follow the protocol of the assay kit but replace the provided assay buffer with homemade CA/Na_3C (50 mM, pH=3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0), PBS (50 mM, pH=6.0, 6.5, 7.0, 7.5, and 8.0), and Tris (50 mM, pH=8.0, 8.5, and 9.0) buffer solutions. After the replacement, the assay kit has following components: β-Gal assay buffer (CA/Na_3C, PBS, or Tris), β-Gal substrate in DMSO, fluorescein standard (1 mM), and β-Gal positive control.

2.3.1. Calibration curves

Add 0.5 μL 1 mM fluorescein standard to 99.5 μL assay buffer, then pipette 0, 0.5, 1.0, 1.5, 2.0, and 2.5 μL diluted fluorescein solution to a 384-well plate, each well was continuously diluted with assay buffer to a volume of 25 μL. Measure emission fluorescence at 520 nm using an excitation wavelength of 480 nm. Subtract the 0 standard record from all records, then plot the fluorescein calibration curve. Calibration curves measured at different pH values are shown in Figure S4.

2.3.2. β-Gal activity measurement

i) Preparation of β-Gal sample, positive control and background control

β-Gal sample: pipette 0.5 μL β-Gal dissolved in assay buffer into well plate, and dilute the enzyme solution with 12 μL assay buffer to a volume of 12.5 μL. Positive control: dilute 0.5 μL positive control solution with 12 μL assay buffer. Background control: mix 0.5 μL β-Gal dissolved in assay buffer and additional 12 μL assay buffer.

ii) Kinetics measurement

A mixture of 12 μL assay buffer, 0.5 μL β-Gal substrate was added to β-Gal sample and positive control, and homogeneously mixed. Another 12.5 μL assay buffer was homogeneously mixed with background control. Then immediately measure the fluorescence of β-Gal sample, positive control and background control in a kinetic way at 20 °C (Figure S5), and choose the period of linear range to calculate the β-Gal activity of the samples.

iii) calculation

Subtract the 0 standard record from all records. In a linear range of the kinetic curves of β-Gal sample, calculate the increasement of fluorescence ΔRFU in a period, apply the ΔRFU to calibration curves to get the amount of fluorescein generated by β-Gal sample during the period. The activity of β-Gal sample can be calculated using the following equation:

$$\text{Activity} = n/(m*t)\ \text{mol}/(\text{mg}*\text{min})$$

Where: n is the amount of fluorescein generated by β-Gal sample (mol);
m is the amount of β-Gal sample added into the well plate (mg);
t is the period of ΔRFU at 20 °C (min).

SUPPORTING INFORMATION

Figure 2a in main text shows the activity of β-Gal used in all our experiments at different pH values.

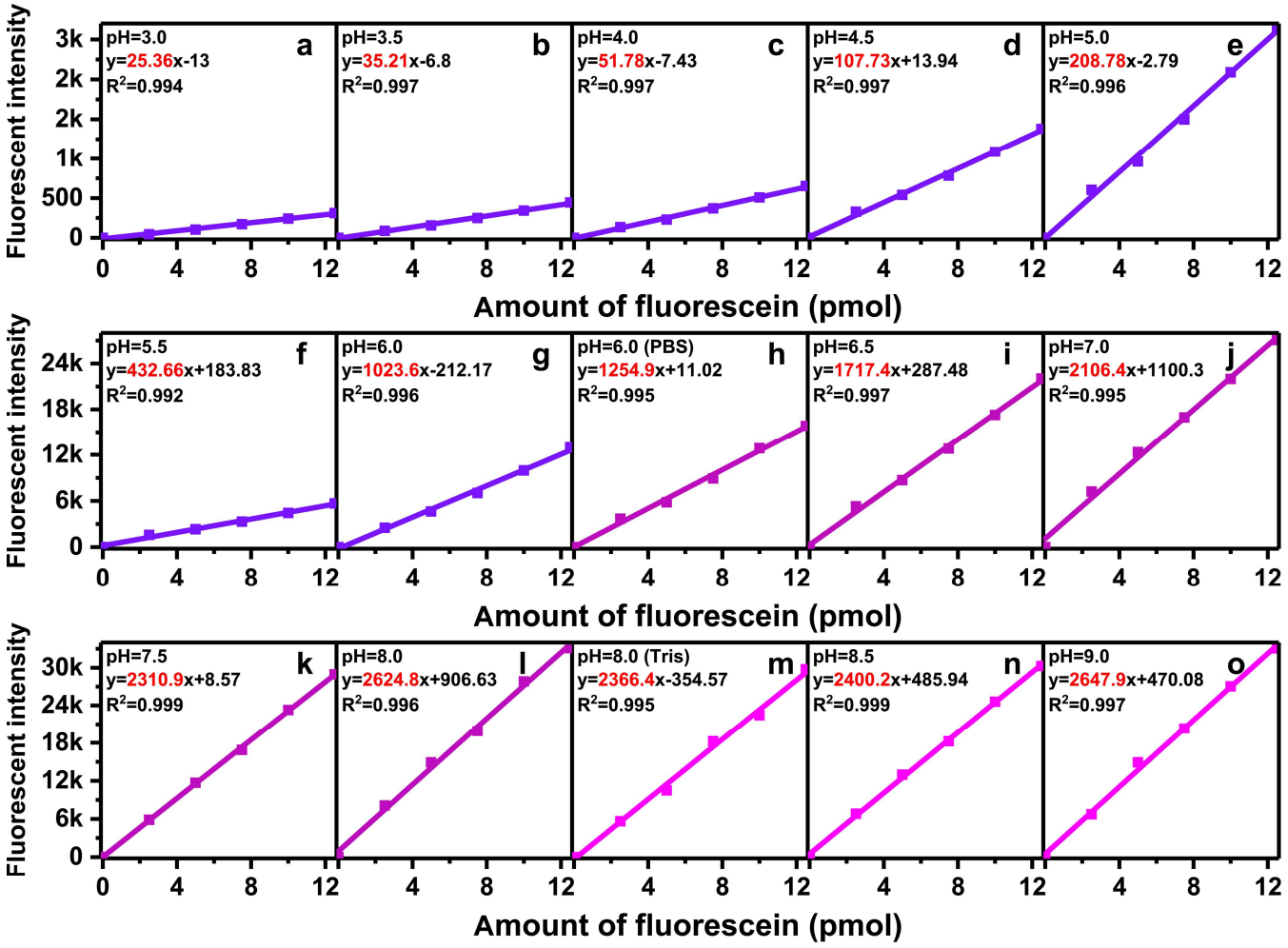


Figure S4. Calibration curves of fluorescein measured at different pH values: (a-g) pH 3.0-6.0 in CA/Na_3C, (h-l) pH 6.0-8.0 in PBS, and (m-o) pH 8.0-9.0 in Tris.

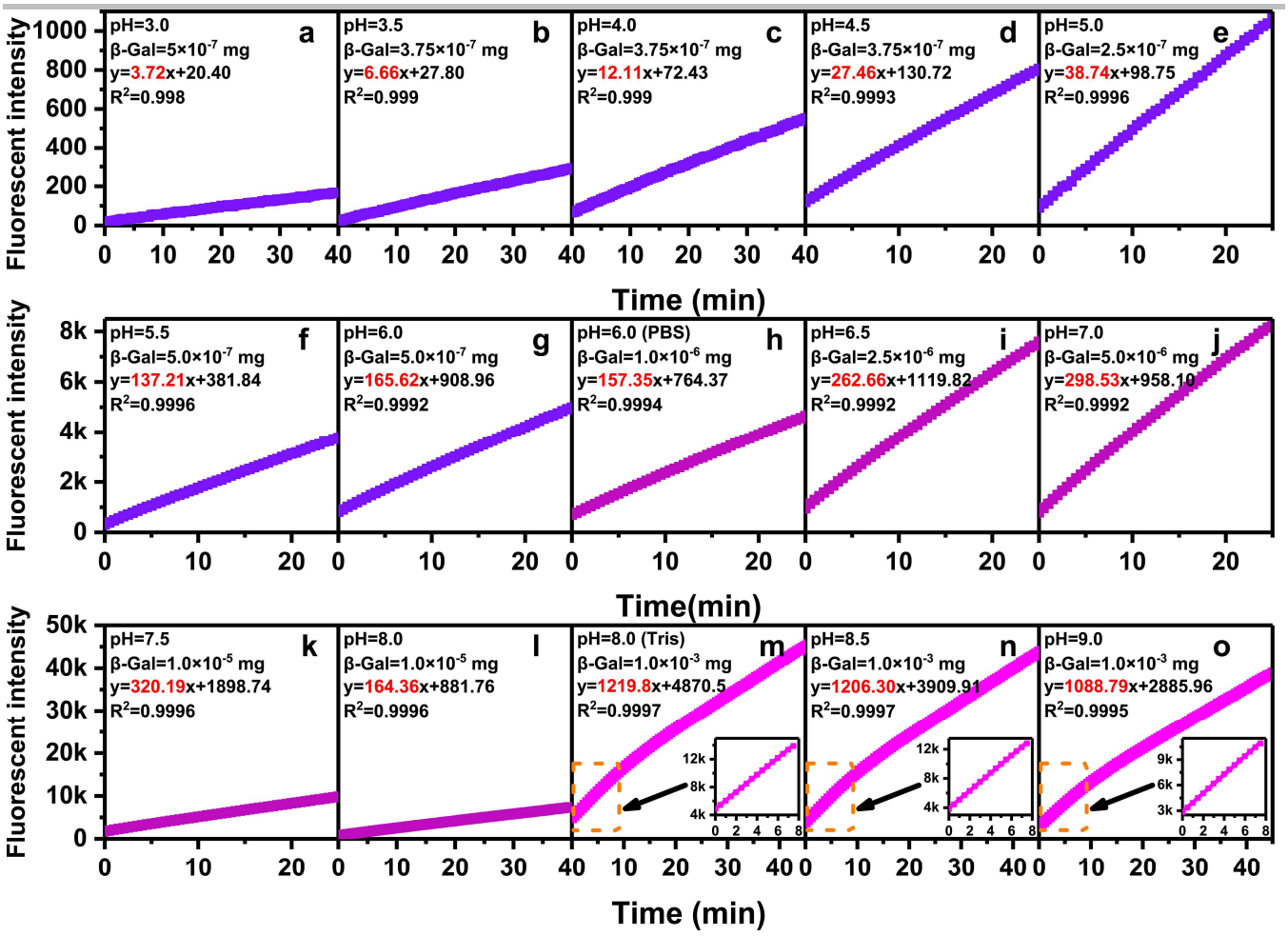


Figure S5. Fluorescence kinetics of the hydrolysis of β-Gal substrate-fluorescein catalyzed by β-Gal at different pH values: (a-g) pH 3.0-6.0 in CA/Na_3C, (h-l) pH 6.0-8.0 in PBS, and (m-o) pH 8.0-9.0 in Tris.

3. Protocol for enzymatic temporal pH feedback system

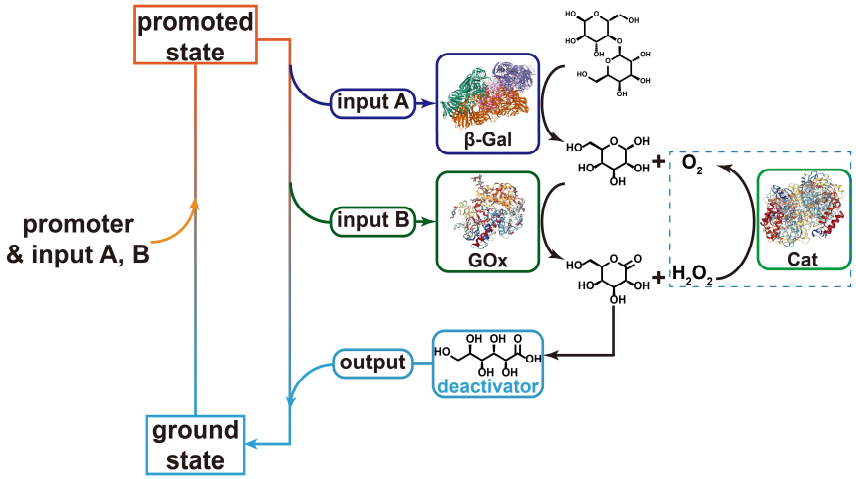
To a stirred solution of calculated amount of sucrose (or lactose) and CA/Na_3C buffer, calculated amount of Inv (or β-Gal), GOx, and Tris buffer loaded in a Hamilton syringe was injected. The pH profiles of the solution were recorded by a 12-channel pH station from EA Instruments. All the pH data are an average of at least three measurements.

In an all-food grade enzymatic temporal pH feedback system, baking soda, soda and sugar beet syrup (or cow milk) were injected instead of Tris and sucrose (or lactose).

4. Component evolution in enzymatic temporal pH feedback system

An Agilent high performance liquid chromatography (HPLC) equipped with a refractive index detector, a column oven working at 85 °C, and a PL1170-6810 column (Hi-Plex Ca, 7.7 x 300 mm, 8 μm) was used to monitor the component evolution during the enzymatic temporal pH feedback. MilliQ water was used as eluent with a flow rate of 0.4 mL·min^{-1}. External standard curves (Figure S7) were plotted to determine the concentration of sucrose (or lactose), glucose and gluconic acid. Before loading to HPLC, all samples were filtered through a 0.22 μm membrane.

5. Supporting Figures



Scheme S1. Schematic of transient alkaline pH profiles programmed by β-Gal-GOx metabolic chain: adding a fast promoter abruptly lifts the system from ground state to promoted state. At promoted state, the dormant deactivator lactose is catalytically hydrolyzed by β-Gal, generating intermediate products glucose and galactose. Glucose is further hydrolyzed to gluconic acid δ-lactone by GOx in the presence of oxygen which migrates from open air, diffuses and dissolves in the solution. The lactone spontaneously hydrolyzes to GLA, driving the system back to ground state.

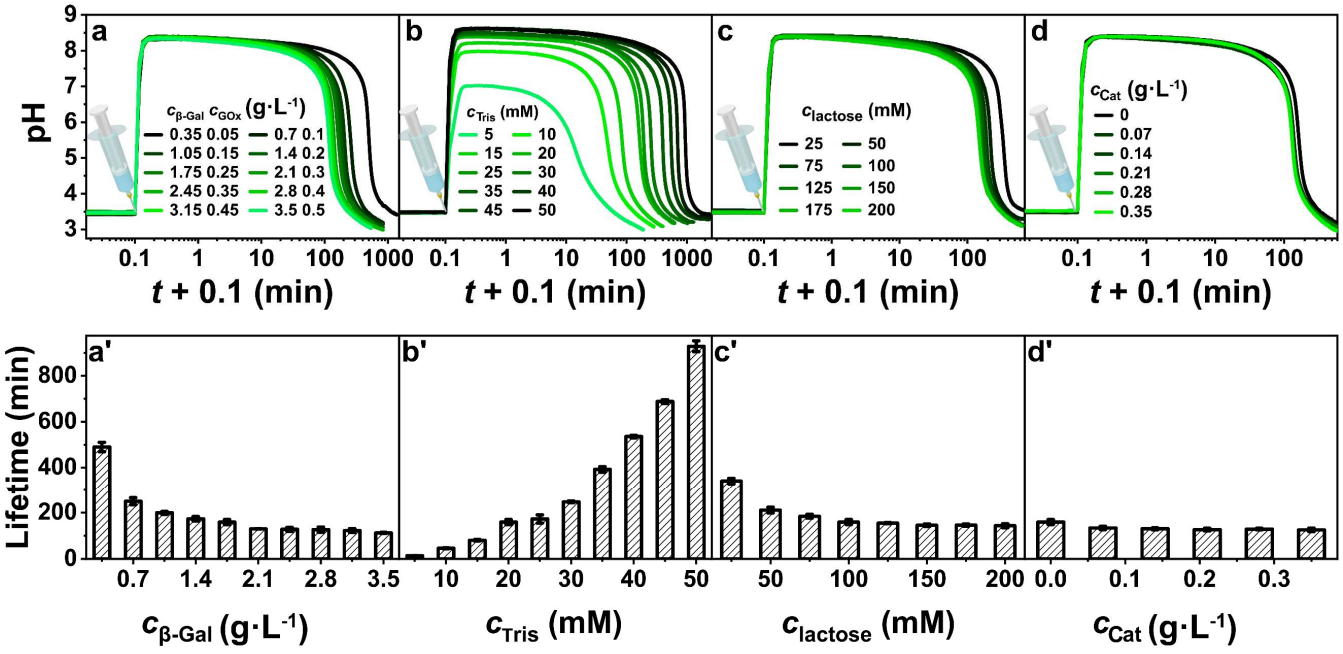


Figure S6. (a) The t_{lf} monotonically decreases when feeding more Inv and GOx. Conditions: $c_{β\text{-}Gal}/c_{GOx}$ = 7/1, 100 mM lactose, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8). (b) The t_{lf} of the transient alkaline state is effectively controlled by the c_{Tris}. Conditions: 2.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 100 mM lactose, and 1.5 mM CA/Na_3C (pH = 3.0). (c) The concentration of chemical fuel (lactose) moderately controls the t_{lf}. Conditions: 2.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8). (d) Adding Cat into the metabolic chain shortens t_{lf}. Conditions: Cat from 0 to 0.35 g·L^{-1}, 2.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 100 mM lactose, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8). (a'-d') The corresponding t_{lf} of the transient alkaline states in figure (a-d). All the data are an average of three measurements.

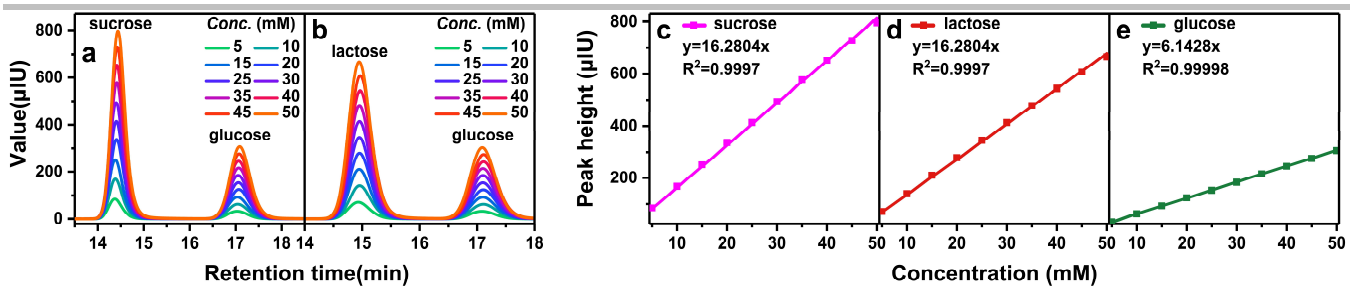


Figure S7. (a-b) HPLC traces of sucrose, lactose, and glucose with different concentrations, and (c-e) calibration curves of sucrose, lactose and glucose.

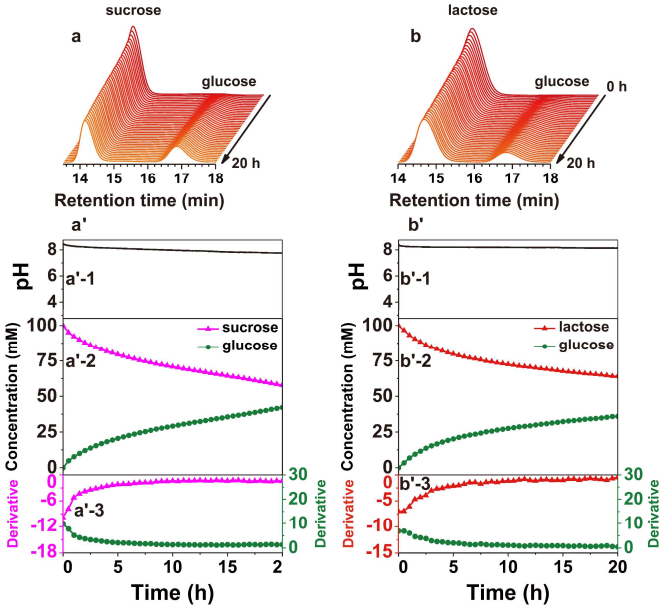


Figure S8. (a and b) HPLC curves and (a' and b') component evolution of temporal pH feedback system programmed by (a and a') Inv, and (b and b') β-Gal with 100 mM chemical fuels. Experiment condition: (a and a') 0.50 g·L^{-1} Inv, 100 mM sucrose, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8); (b and b') 2.00 g·L^{-1} β-Gal, 100 mM lactose, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8). The decreasing of substrates and increasing of products processes move relatively smooth (a'-2 and b'-2) and do not show obvious feedback (a'-3 and b'-3).

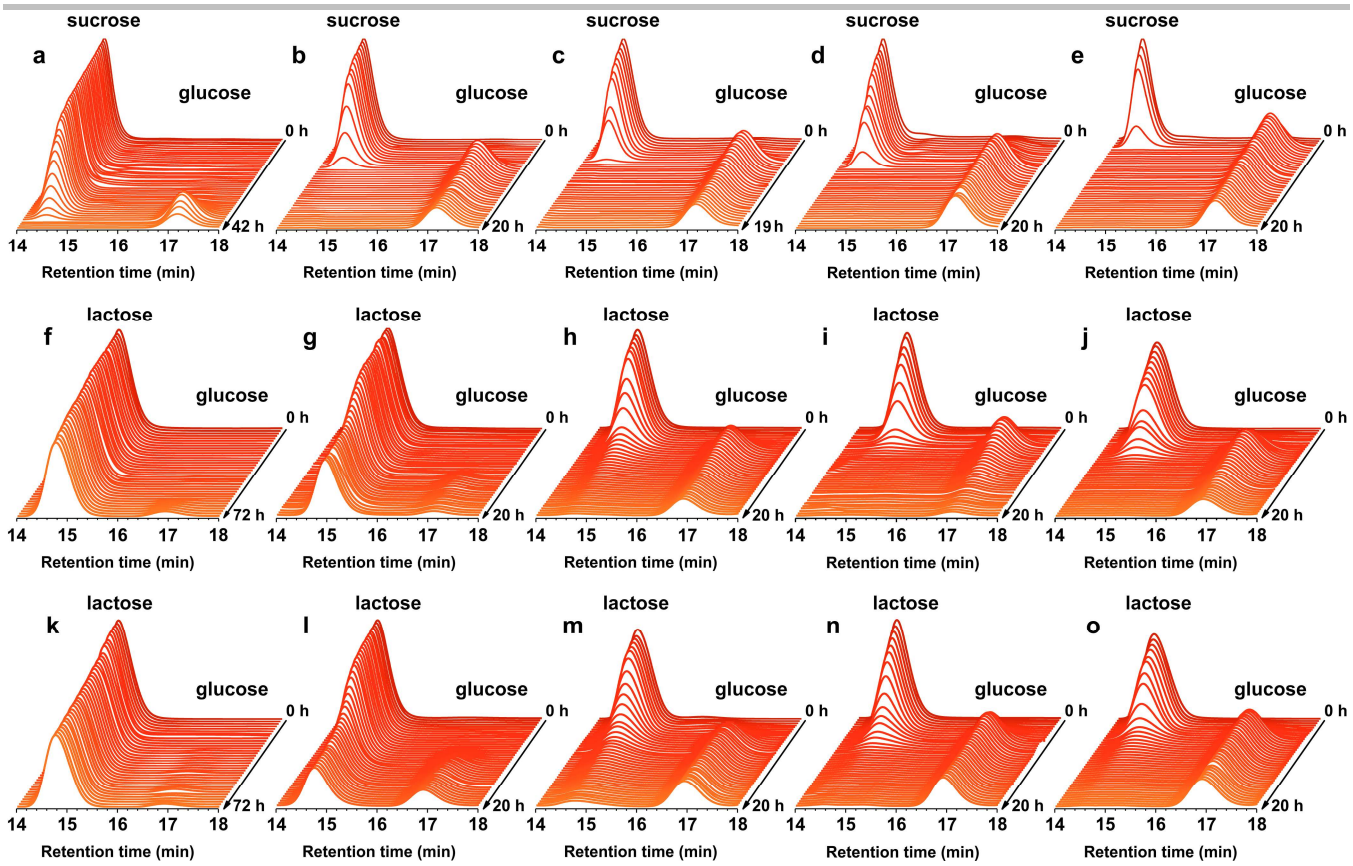


Figure S9. HPLC curves of temporal pH feedback system programmed by (a-e) Inv-GOx and (f-o) β-Gal-GOx ECs. Experiment condition: (a) 0.01 g·L^{-1} Inv, 0.49 g·L^{-1} GOx, 100 mM sucrose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (b) 0.05 g·L^{-1} Inv, 0.45 g·L^{-1} GOx, 100 mM sucrose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (c) 0.15 g·L^{-1} Inv, 0.35 g·L^{-1} GOx, 100 mM sucrose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (d) 0.45 g·L^{-1} Inv, 0.05 g·L^{-1} GOx, 100 mM sucrose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (e) 0.15 g·L^{-1} Inv, 0.35 g·L^{-1} GOx, 100 mM sucrose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 10 mM Tris (pH = 8.8); (f) 0.05 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 100 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (g) 0.25 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 100 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (h) 1.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 100 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (i) 2.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 100 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (j) 2.00 g·L^{-1} β-Gal, 0.05 g·L^{-1} GOx, 100 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (k) 0.05 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 200 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (l) 0.25 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 200 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (m) 1.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 200 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (n) 2.00 g·L^{-1} β-Gal, 0.25 g·L^{-1} GOx, 200 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8); (o) 2.00 g·L^{-1} β-Gal, 0.05 g·L^{-1} GOx, 200 mM lactose, 1.5 mM CA/Na$_3$C (pH = 3.0), and 20 mM Tris (pH = 8.8).

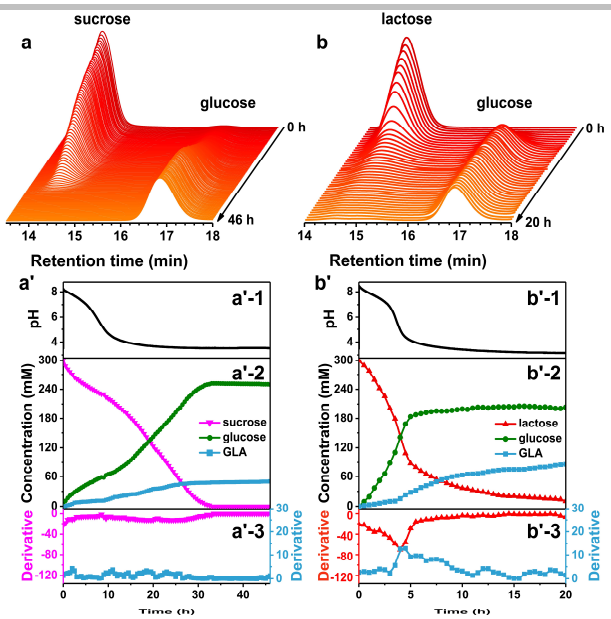


Figure S10. (a and b) HPLC curves and (a' and b') components evolutions of temporal pH feedback system programmed by (a and a') Inv-GOx and (b and b') β-Gal-GOx ECs with 300 mM chemical fuels. Experiment condition: (a and a') 0.45 g·L^{-1} Inv, 0.05 g·L^{-1} GOx, 300 mM sucrose, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8); The decreasing of $c_{sucrose}$ and increasing of c_{GLA} processes move relatively smooth (a'-2), $c_{glucose}$ was gradually lifted beyond K_m of GOx. (b and b') 2.00 g·L^{-1} β-Gal, 0.05 g·L^{-1} GOx, 300 mM lactose, 1.5 mM CA/Na_3C (pH = 3.0), and 20 mM Tris (pH = 8.8). The decreasing of $c_{lactose}$ and increasing of c_{GLA} display a maximal rate around 4.0 h in derivatives (b'-3), then turn to slow decreasing and increasing, $c_{glucose}$ rapidly climbs above K_m of GOx (b'-2).

a nutrients in sugar beet syrup

nutrient	content (%)
fat	0.5
saturated fatty acids	0.5
carbohydrates	69
of which sugar	66
protein	2.3
salt	0.03

b nutrients in milk

nutrient	content (%)
fat	2
saturated fatty acids	5
carbohydrates	2
sugar	5
protein	7
salt	2

Figure S11. Weight percentage of nutrients in (a) sugar beet syrup and (b) milk purchased in local supermarket, 66 wt% of sugar in syrup and 5 wt% sugar in milk were adopted to calculate the molar concentration of sucrose and lactose injected to all-food grade temporal pH feedback systems.

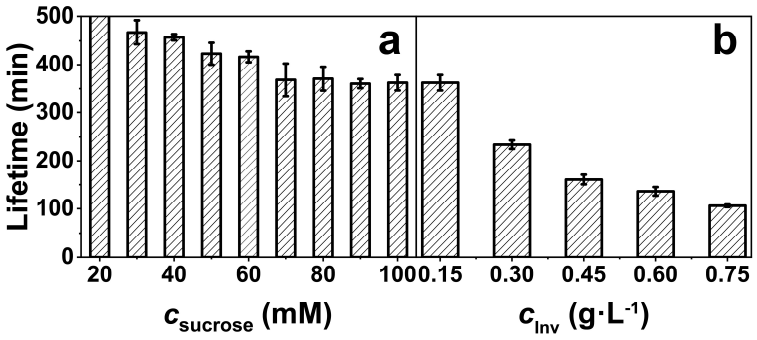


Figure S12. The corresponding t_{lf} of the transient alkaline states in (a) Figure 5a and (b) Figure 5b. All data are an average of three measurements.

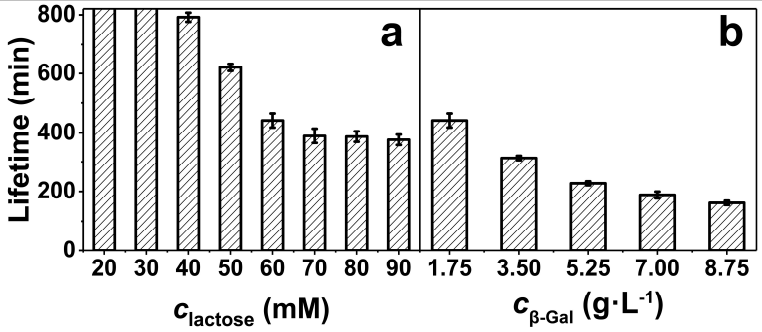


Figure S13. The corresponding t_{lf} of the transient alkaline states in (a) Figure 5e and (b) Figure 5f. All data are an average of three measurements.

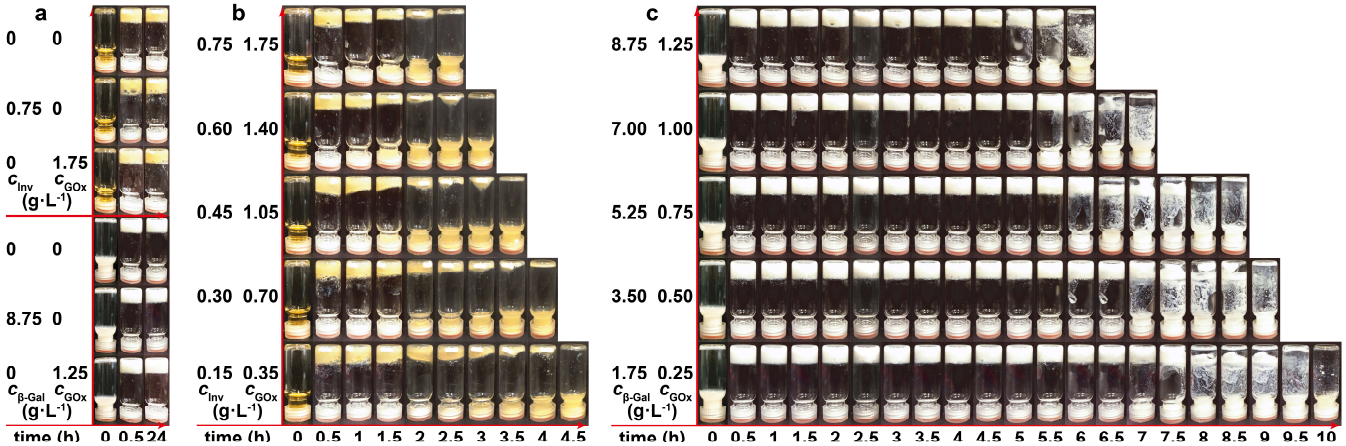


Figure S14. Snapshots of so-to-gel-to-sol transition of 1 wt% Fmoc-ethylenediamine programmed by all-food grade robust temporal pH feedback system. (a) Truth table of Inv-GOx (upper part) and β-Gal-GOx (bottom part) ECs programming self-assembly of peptide, we define hydrogel state and sol state as output signal **1** and **0**, respectively. The output signal stays at **1** even after 24 h in the cases where input signals are in the absence of both Inv (or β-Gal) and GOx (**0**,**0**), in the presence of Inv (or β-Gal) but in the absence of GOx (**1**,**0**), and in the absence of Inv (or β-Gal) but in the presence of GOx (**0**,**1**). Experiment condition: For Inv-GOx logic gate, 100 mM sucrose (calculated from sugar content in sugar beet syrup), 1.5 mM CA/Na_3C (pH = 3.0), and $c(CO_3^{2-}) = c(HCO_3^-)$ = 10 mM; For β-Gal-GOx logic gate, 60 mM sucrose (calculated from sugar content in milk), 1.5 mM CA/Na_3C (pH = 3.0), and $c(CO_3^{2-}) = c(HCO_3^-)$ = 10 mM. (b) Snapshots of transient output signal **1** programmed by Inv-GOx EC with different enzyme concentrations, where the input signal (**1**,**1**) means that both Inv and GOx are present, experiment condition: c_{Inv}/c_{GOx} = 3/7, 100 mM sucrose, 1.5 mM CA/Na_3C (pH = 3.0), and $c(CO_3^{2-}) = c(HCO_3^-)$ = 10 mM. (c) Snapshots of lifetime adjustable output signal **1** programmed by β-Gal-GOx NAND gate with different enzyme concentrations, experiment condition: $c_{β\text{-}Gal}/c_{GOx}$ = 7/1, 60 mM lactose, 1.5 mM CA/Na_3C (pH = 3.0), and $c(CO_3^{2-}) = c(HCO_3^-)$ = 10 mM.