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Supporting Information

# Autonomous Transient pH Flips Shaped by Layered Compartmentalization of Antagonistic Enzymatic Reactions

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# SUPPORTING INFORMATION

## 1. Materials

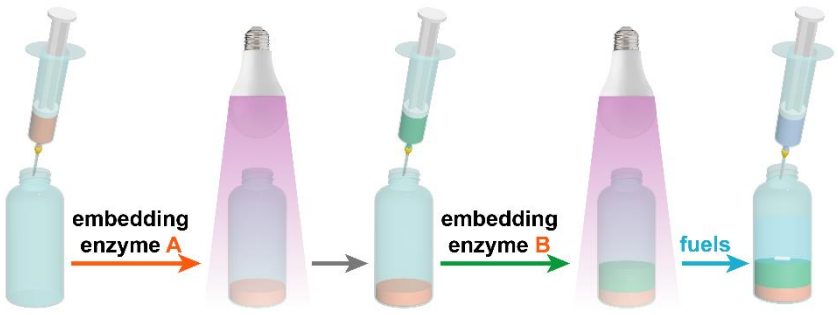
All chemicals were purchased from Sigma-Aldrich unless otherwise stated: polyethylene glycol diacrylate (PEGDA 700, number average molecular weight ($M_n$) 700 g/mol), polyethylene glycol (PEG 1500, $M_n$ 1500 g/mol), PEG 3000 ($M_n$ 3000 g/mol), PEG 6000 ($M_n$ 6000 g/mol), PEG 8000 ($M_n$ 8000 g/mol), acryloyl chloride (97.0%, contains <210 ppm MEHQ as stabilizer), photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, ≥95%), urease from Canavalia ensiformis (Type IX, powder, 50,000-100,000 units/g solid), esterase from porcine liver (lyophilized powder, ≥ 15 units/mg solid), bovine serum albumin (BSA, ≥95%), ethyl acetate (EA, anhydrous, 99.8%), urea (BioXtra), sodium dihydrogen phosphate (BioXtra), disodium hydrogen phosphate (BioXtra), Trizma® base (pH 8.8, $M_w$ 127.2 g/mol). Peptide Fmoc-Leu-Gly-OH was purchased from Bachem AG. 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. Instrumentation

**pH** All pH curves were measured on a 12-channel pH station (EA Instruments) at room temperature. Electrodes were calibrated by calibration buffers (pH=4, 7, 10) each time before using. All pH data are an average of at least three measurements.
**Reflectance** The reflectance of fibrils assembled from peptides in glass vials was recorded on a reflectance spectroscopy setup at 500 nm (Ocean Optics). In order to avoid the stirring magnetic bar to disturb the measurement, the fiber probe was horizontally placed to allow incident light to illuminate on the side face of glass vial. Reflectance from a glass vial containing peptide before fibrillization was remembered as zero, while reflectance from a WS-1 standard white board was referenced as 100%.
**TEM** Self-assembled fibrils were diluted with water and deposited on a glow-discharged carbon-coated cupper grid. After drying, the fibrils were imaged with a FEI Talos 120C at 120 kV operating voltage.

## 3. Experimental protocols

**Synthesis of compartmentalized enzyme hydrogels by layer-by-layer photoinitiated polymerization and recording of the enzymatic pH profiles**
In preliminary screening experiments using different in-house synthesized PEGDA (1500, 3000, 6000, 8000 g/mol)[1], we identified that both enzymes can be efficiently encapsulated without leakage and obvious degradation into PPEGDA hydrogels containing 30 wt% solid content and using LAP as photoinitiator and PEGDA 6000 as monomer. The layered immobilization is proceeded by sequential layer-by-layer photoinitiated polymerization, as summarized as follows for a representative example. Typically, after bubbling for 20 min, a 200 µL solution containing 60 mg PEGDA, 0.96 mg urease, and 0.118 mg LAP was injected in a sealed transparent glass vial with inner diameter of 12.86 mm. The vial was placed on a cooling plate with circulating water running and illuminated for 10 min by a 365 nm 36 W UV lamp. Afterwards, another 800 µL solution containing 240 mg PEGDA, 2.24 mg esterase and 0.472 mg LAP was added on the top of the urease layer and illuminated for 20 min. This led to gel layers of ~1.5 mm and 6 mm, respectively. PBS, BSA solution, urea and EA as chemical fuels were added to the vial to make a total volume of 3200 µL and a height of ~24.5 mm, starting the antagonistic enzymatic reactions with gentle magnetic stir at 20 °C (air conditioned rooms) and the pH profiles were recorded using pH meters while sealing the vial with parafilm (Scheme S1).




**Scheme S1. Procedure of synthesizing compartmentalized antagonistic enzymes by layer-by-layer photoinitiated polymerization**.

# SUPPORTING INFORMATION

## Supporting Tables and Figures

**Table S1.** Experimental conditions in Scheme 1 in main text

| No. in Scheme 1 | Total volume (mL) | Total height (mm) | $h_{supernatant}$ (mm) | $h_{esterase}$ (mm) | $h_{blank}$ (mm) | $h_{urease}$ (mm) | $c_{esterase}$ (mg/mL) [a] | $c_{urease}$ (mg/mL) [a] | $c_{EA}$ (mM) [a] | $c_{urea}$ (mM) [a] | $c_{PBS}$ (mM) [a] | $c_{BSA}$ (mg/mL) [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| c | 3.2 | ~24.5 | ~24.5 | **all in solution, no hydrogel** | | | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| c | 3.2 | ~24.5 | ~17 | **mix in single gel layer ~7.5** | | | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| d | 3.2 | ~24.5 | ~17 | **~6** | **/** | **~1.5** | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |

[a] All concentrations refer to the total volume including supernatant and gel layers. Variates are marked in bold.

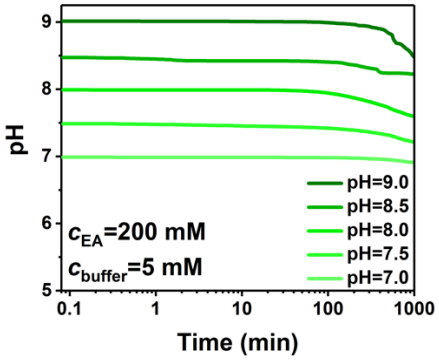



**Figure S1. Hydrolysis of ethyl acetate in buffer solution without esterase at different pH values.**

**Table S2.** Experimental conditions in Figure 1 in main text

| No. in Figure 1 | Total volume (mL) | Total height (mm) | $h_{supernatant}$ (mm) | $h_{esterase}$ (mm) | $h_{blank}$ (mm) | $h_{urease}$ (mm) | $c_{esterase}$ (mg/mL) [a] | $c_{urease}$ (mg/mL) [a] | $c_{EA}$ (mM) [a] | $c_{urea}$ (mM) [a] | $c_{PBS}$ (mM) [a] | $c_{BSA}$ (mg/mL) [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a/a' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | **0.1** | **0.9** | 100 | 100 | 5 | 0.5 |
| a/a' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | **0.3** | **0.7** | 100 | 100 | 5 | 0.5 |
| a/a' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | **0.5** | **0.5** | 100 | 100 | 5 | 0.5 |
| a/a' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | **0.7** | **0.3** | 100 | 100 | 5 | 0.5 |
| a/a' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | **0.9** | **0.1** | 100 | 100 | 5 | 0.5 |
| b/b' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | 20 | 100 | **1** | 0.5 |
| b/b' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | **2** | 0.5 |
| b/b' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | **3** | 0.5 |
| b/b' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | **4** | 0.5 |
| b/b' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | **5** | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **20** | **180** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **40** | **160** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **60** | **140** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **80** | **120** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **100** | **100** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **120** | **80** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **140** | **60** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **160** | **40** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~6 | / | ~1.5 | 0.7 | 0.3 | **180** | **20** | 5 | 0.5 |
| d/d' | 3.2 | ~24.5 | ~17 | **~6** | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| d/d' | 3.2 | ~24.5 | ~18.5 | **~4.5** | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| d/d' | 3.2 | ~24.5 | ~20 | **~3** | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| d/d' | 3.2 | ~24.5 | ~21.5 | **~1.5** | / | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~17 | **~6** | **0** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~17 | **~4.5** | **~1.5** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~17 | **~3** | **~3** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~17 | **~1.5** | **~4.5** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| f/f' | 3.2 | ~24.5 | ~17 | ~6 | **0** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| f/f' | 3.2 | ~24.5 | ~15.5 | ~6 | **~1.5** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| f/f' | 3.2 | ~24.5 | ~14 | ~6 | **~3** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| f/f' | 3.2 | ~24.5 | ~12.5 | ~6 | **~4.5** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |
| f/f' | 3.2 | ~24.5 | ~11 | ~6 | **~6** | ~1.5 | 0.7 | 0.3 | 100 | 100 | 5 | 0.5 |

[a] All concentrations refer to the total volume including supernatant and gel layers. Variates are marked in bold.

# SUPPORTING INFORMATION

**Table S3.** Experimental conditions in Figure 2 in main text

| No. in Figure 2 | Total volume (mL) | Total height (mm) | $h_{supernatant}$ (mm) | $h_{urease}$ (mm) | $h_{blank}$ (mm) | $h_{esterase}$ (mm) | $c_{urease}$ (mg/mL) [a] | $c_{esterase}$ (mg/mL) [a] | $c_{urea}$ (mM) [a] | $c_{EA}$ (mM) [a] | $c_{PBS}$ (mM) [a] | $c_{BSA}$ (mg/mL) [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 3.2 | ~24.5 | ~17 | **mix in single gel layer ~7.5** | | | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |
| b | 3.2 | ~24.5 | ~17 | **~1.5** | **/** | **~6** | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | **2.5** | **197.5** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | **5** | **195** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | **7.5** | **192.5** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | **10** | **190** | 5 | 0.5 |
| c/c' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | **12.5** | **187.5** | 5 | 0.5 |
| d/d' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | 5 | 195 | **1** | 0.5 |
| d/d' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | 5 | 195 | **2** | 0.5 |
| d/d' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | 5 | 195 | **3** | 0.5 |
| d/d' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | 5 | 195 | **4** | 0.5 |
| d/d' | 3.2 | ~24.5 | ~17 | ~1.5 | / | ~6 | 0.3 | 0.7 | 5 | 195 | **5** | 0.5 |
| e/e' | 3.2 | ~24.5 | ~21.5 | ~1.5 | **0** | ~1.5 | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~20 | ~1.5 | **~1.5** | ~1.5 | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~18.5 | ~1.5 | **~3** | ~1.5 | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~17 | ~1.5 | **~4.5** | ~1.5 | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |
| e/e' | 3.2 | ~24.5 | ~15.5 | ~1.5 | **~6** | ~1.5 | 0.3 | 0.7 | 5 | 195 | 5 | 0.5 |

[a] All concentrations refer to the total volume including supernatant and gel layers. Variates are marked in bold.

**Table S4.** Experimental conditions in Figure 3a-c in main text

| No. in Figure 3 | Total volume (mL) | Total height (mm) | $h_{supernatant}$ (mm) | $h_{esterase}$ (mm) | $h_{urease}$ (mm) | $c_{esterase}$ (mg/mL) [a] | $c_{urease}$ (mg/mL) [a] | $c_{EA}$ (mM) [a] | $c_{urea}$ (mM) [a] | $c_{PBS}$ (mM) [a] | $c_{BSA}$ (mg/mL) [a] | $c_{dipeptide}$ (wt%) [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a-c | 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **60** | **140** | 1 | 0.5 | 0.6 |
| a-c | 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **100** | **100** | 1 | 0.5 | 0.6 |
| a-c | 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **120** | **80** | 1 | 0.5 | 0.6 |

[a] All concentrations refer to the total volume including supernatant and gel layers. Variates are marked in bold.

**Table S5.** Experimental conditions in Figure 3d-f in main text

| No. in Figure 3 | Total volume (mL) | Total height (mm) | $h_{supernatant}$ (mm) | $h_{urease}$ (mm) | $h_{esterase}$ (mm) | $c_{urease}$ (mg/mL) [a] | $c_{esterase}$ (mg/mL) [a] | $c_{urea}$ (mM) [a] | $c_{EA}$ (mM) [a] | $c_{PBS}$ (mM) [a] | $c_{BSA}$ (mg/mL) [a] | $c_{peptide}$ (wt%) [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| d-f | 3.2 | ~24.5 | ~17 | ~1.5 | ~6 | 0.3 | 0.7 | **5** | **195** | 1 | 0.5 | 0.3 |
| d-f | 3.2 | ~24.5 | ~17 | ~1.5 | ~6 | 0.3 | 0.7 | **10** | **190** | 1 | 0.5 | 0.3 |
| d-f | 3.2 | ~24.5 | ~17 | ~1.5 | ~6 | 0.3 | 0.7 | **12.5** | **187.5** | 1 | 0.5 | 0.3 |

[a] All concentrations refer to the total volume including supernatant and gel layers. Variates are marked in bold.

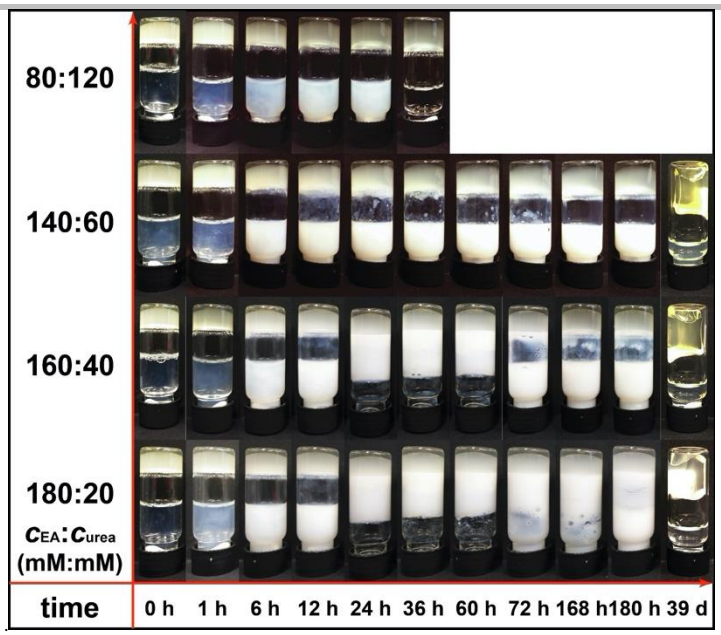



**Figure S2**. **Snapshots of transient programmed Fmoc-Leu-Gly-OH with different ratio of EA and urea**, conditions: 0.7 mg/mL esterase, 0.3 mg/mL urease, $h_{esterase}$ : $h_{urease}$ = 4:1 (total height = ~7.5 mm), 1 mM PBS buffer (pH = 7.0; 0.5 mg/mL BSA), and 0.6 wt% Fmoc-Leu-Gly-OH. Detailed experimental conditions are listed in Table S6.

**Table S6.** Experimental conditions in Figure S1.

| Total volume (mL) | Total height (mm) | $h_{supernatant}$ (mm) | $h_{esterase}$ (mm) | $h_{urease}$ (mm) | $c_{esterase}$ (mg/mL) [a] | $c_{urease}$ (mg/mL) [a] | $c_{EA}$ (mM) [a] | $c_{urea}$ (mM) [a] | $c_{PBS}$ (mM) [a] | $c_{BSA}$ (mg/mL) [a] | $c_{dipeptide}$ (wt%) [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **80** | **120** | 1 | 0.5 | 0.6 |
| 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **140** | **60** | 1 | 0.5 | 0.6 |
| 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **160** | **40** | 1 | 0.5 | 0.6 |
| 3.2 | ~24.5 | ~17 | ~6 | ~1.5 | 0.7 | 0.3 | **180** | **20** | 1 | 0.5 | 0.6 |

[a] All concentrations refer to the total volume including supernatant and gel layers. Variates are marked in bold.

## References

[1] G. M. Cruise, D. S. Scharp, J. A. Hubbell, *Biomaterials* **1998**, *19*, 1287-1294.