Electronic Supplementary Information

pH Oscillating System for Molecular Computation as Chemical Turing Machine

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S1. Alternative Area Definition

An alternative definition of the Area also provides a highly effective means of discerning between in-language (IL) and out-of-language (OoL) words. The main definition (ACSTR), shown in equation (S1), is the difference between the two measured Areas. The alternative definition (ACSTR') instead utilizes the ratio between the same two measured Areas. The plot resulting from calculating the Area using equation (S2) can be seen in figure S1. It can be seen that ACSTR' is at least as effective as ACSTR at discerning IL and OoL words. The relationship between ACSTR and ACSTR' can be further examined in figure S2, where it can be seen that the two are linearly related, with an R^2 of 0.99999.

\[ ACSTR = \left( \int_{t_{s_2}}^{t_{s_2}+\tau} pH_{osc}(t) \, dt - pH_{min} \cdot \tau \right) - \left( \int_{t_{s_1}}^{t_{s_1}+\tau} pH_{osc}(t) \, dt - pH_{min} \cdot \tau \right) \]  
(S1)

\[ ACSTR' = \left( \frac{\int_{t_{s_2}}^{t_{s_2}+\tau} pH_{osc}(t) \, dt - pH_{min} \cdot \tau}{\int_{t_{s_1}}^{t_{s_1}+\tau} pH_{osc}(t) \, dt - pH_{min} \cdot \tau} \right) \]  
(S2)
Figure S1: A plot of the output Area ratios for multiple tested words in the pH oscillator chemical Turing machine. Points are the arithmetic means of three experiments and error bars are the standard deviation. The red curved line is a parabola fit of the IL words with equation $f(x): (2.36 \pm 0.049) \times 10^{-3}x^2 + (0.928 \pm 0.0015)$ and an $R^2$ of 0.9996. The black dotted line is a linear fit of the IL words, with equation $f(x): (2.54 \pm 0.369) \times 10^{-3}x + (0.870 \pm 0.0175)$ and an $R^2$ of 0.9794.

Figure S2: A plot of the Area ratios (A) against the Area differences (A) for in-language words, in the pH oscillator chemical Turing machine. The linear fit has the equation $f(x): (91.8 \pm 10.13)x - (91.5 \pm 10.37)$ and an $R^2$ of 0.99999.
S2. Extended Methods and Materials

Sodium sulfite, sodium carbonate, and hydrogen peroxide (30%) were purchased from Sigma-Aldrich. Sulfuric acid (10 N) was purchased from Fisher Scientific. Copper sulfate pentahydrate was purchased from Honeywell. Iodine (0.1 N standard solution) was purchased from Acros. High-purity water was purchased from VWR. The hydrogen peroxide concentration was confirmed by titrating against potassium permanganate, which was itself titrated against sodium oxalate. All reagents were purchased as high-purity and used without further purification.

Stock solutions for the aliquot additions were generated by dilution and dissolution to the concentrations shown in table S1. All solutions are aqueous.

| Symbol | Chemical  | Concentration / mM | Volume / µL |
|--------|-----------|--------------------|-------------|
| #      | H₂O₂      | 585                | 300         |
| a      | CuSO₄     | 1.25               | 20          |
| b      | I₂        | 50.0               | 8           |
| c      | Na₂CO₃    | 6.0                | 80          |

Table S1: Aliquots used as symbols to influence the reaction pathway of the pH oscillating chemical Turing machine.

Solutions for the base CSTR oscillating reaction were freshly prepared each day. Two base solutions were made using nitrogen-degassed high-purity water and excluding air through headspace purging, according to the concentrations shown in table S2. The sodium carbonate and sodium sulfite solutions were additionally sonicated for 10 minutes. The solutions were then drawn up into 60 mL syringes, sealed with parafilm, and stored at 4 °C for 20 hours before use. These pre-prepared syringes were then directly used in the experiment.

| Solution | Chemical  | Concentration / mM | Concentration in Reactor / mM |
|----------|-----------|--------------------|-------------------------------|
| 1        | H₂O₂      | 133                | 66.5                          |
| 1        | H₂SO₄     | 0.8                | 0.4                           |
| 2        | Na₂SO₃    | 12.0               | 6.0                           |
| 2        | Na₂CO₃    | 1.2                | 0.6                           |

Table S2: The make-up of the two CSTR aqueous solutions used as the base oscillating pH reaction. Each solution is comprised of two chemical species, and is pumped into the reactor at the same rate. Therefore, the chemical concentrations in the reactor are half that of the solution concentrations.

The reactor consisted of a 10 mL beaker nested inside a 50 mL water-jacketed beaker, held in place using custom 3D printed spacer rings. The space between the 10 mL beaker and the 50 mL beaker was filled with distilled water to allow for thermal transfer. A custom 3D printed raised cap held the required tubing and probe in place. The inlets were situated 10 mm from the base of the beaker and located at opposite positions. The pH probe (Mettler Toledo InLab Micro Combination, connected to a NI SensorDAQ and recorded using NI LabView 2020) was positioned 18 mm from the base of the beaker, centered, and orientated such that the reference junction was equidistance between the two inlets. The reactor was held at (20.0 ± 0.1) °C and constantly stirred at 870 rpm with a CAT MCS67 stirrer plate and a 20 mm × 8.5 mm magnetic stirrer bar. The pH probe was calibrated using two data points (pH 4.01 and 7.0), daily.

It was noted that the system is directly affected by ambient air flow in the lab, from sources ranging from the building’s AC turning on to a colleague walking past. Therefore, all experiments were conducted in a cabinet with the sash down and the airflow off. This was found to be necessary to enable high repeatability.
During experiments, the 10 mL reactor was permitted to overflow into the larger 50 mL beaker, thereby maintaining a constant volume in the 10 mL reactor. The overflow waste was permitted to mix with the water already present in the 50 mL, and was pumped out using a peristaltic pump (LongerPump BT100-2J) once the level was too high. In order to ensure there was complete disconnect between the active 10 mL reactor and the surrounding waste in the 50 mL beaker, the outer side of the 10 mL beaker was hydrophobized. This was achieved by submerging the outside of the freshly and thoroughly cleaned beaker in a 10 % w/v dichlorodimethylsilane in toluene solution for 20 minutes, before rinsing in distilled water and drying at 90 °C for 4 hours.

To initiate the reaction, the two solutions were infused into the beaker using a syringe pump (New Era NE-4000 or NE-1600) at a flow rate of 1.65 mL min\(^{-1}\) solution\(^{-1}\). This gave a total inflow rate of 3.3 mL min\(^{-1}\), a residence time of 182 s, and \(k_0 = 5.49 \times 10^{-3} \) s\(^{-1}\). The outflow was conducted using a peristaltic pump at a constant higher flow rate. The reactor was both filled and operated at the same flow rate.

Once initiated, the pH response of the probe was monitored immediately, and recording started as soon as a single oscillation was observed – this was generally when approximately 90 % of the beaker had filled. The reactor was then allowed to stabilize for 800 s. At 800 s the first aliquot symbol (#) was added, after a fixed time-interval of 130 s had passed the next aliquot symbol was added, and onwards until the final aliquot symbol (another #) was added. Aliquot additions to the reactor were done by hand using air-displacement pipettes. To ensure repeatability, the pipette tip was always located away from the pH reference junction and at a depth of approximately 10 mm.

After the experiment, the data (recorded in mV) was converted to pH using the calibration data and processed in MatLab R2020b. The reactor and all tubing were cleaned after each run using a minimum of three rinses of distilled water (which was found to be sufficient, due to the lack of any precipitate or inorganic residue).

**S3. Example pH Traces**

Included are some example experimental pH traces from a selection of in-language (IL) and out-of-language (OoL) words, including the data from \(t = 0\) s. In all cases, the end Area is shaded red and the start Area is shaded green, and the end \(pH_{\text{min}}\) is indicated by the red line whilst the start \(pH_{\text{min}}\) is indicated by the green line.
Figure S3: A pH trace of IL word $abc$ being run using the pH oscillator chemical Turing machine in CSTR. The input sequence is $\#abc\#$, with appropriate aliquot additions at the marked timepoints. The output Area is derived using equation (S2); i.e., by subtracting the green shaded region from the red shaded region.

Figure S4: A pH trace of IL word $a^3b^3c^3$ being run using the pH oscillator chemical Turing machine in CSTR. The input sequence is $\#aabbccccc\#$, with appropriate aliquot additions at the marked timepoints. The output Area is derived using equation (S2); i.e., by subtracting the green shaded region from the red shaded region.
Figure S5: A pH trace of OoL word $a^2bc$ being run using the pH oscillator chemical Turing machine in CSTR. The input sequence is #aabc#, with appropriate aliquot additions at the marked timepoints. The output Area is derived using equation (S2); i.e., by subtracting the green shaded region from the red shaded region.

Figure S6: A pH trace of OoL word $ab^2c$ being run using the pH oscillator chemical Turing machine in CSTR. The input sequence is #abbc#, with appropriate aliquot additions at the marked timepoints. The output Area is derived using equation (S2); i.e., by subtracting the green shaded region from the red shaded region.
**Figure S7:** A pH trace of OoL word $abc^l$ being run using the pH oscillator chemical Turing machine in CSTR. The input sequence is $#abccc#$, with appropriate aliquot additions at the marked timepoints. The output Area is derived using equation (S2); i.e., by subtracting the green shaded region from the red shaded region.

**Figure S8:** A pH trace of OoL word $b^2a^2c^2$ being run using the pH oscillator chemical Turing machine in CSTR. The input sequence is $#bbaacc#$, with appropriate aliquot additions at the marked timepoints. The output Area is derived using equation (S2); i.e., by subtracting the green shaded region from the red shaded region.
S4. System Arrangement

**Figure S9:** (a) A photograph of the pH oscillator chemical Turing machine in CSTR setup. The bright orange item is the custom 3D printed raised cap. (b) A cartoon of the experimental setup, showing a cross-section of the reactor. The dark blue is the recirculating water in the 50 mL beaker, the medium blue is the overflow water, and the light blue is the reactor mixture.