Electronic Supplementary Material (ESI) for Chemical Communications

Enzyme-Based D-Flip-Flop Memory System

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Electronic supplementary information (ESI)

Chemicals and Materials: The following enzymes for the biochemical D-flip-flop system were obtained from Sigma-Aldrich and used without further purification: L-lactate dehydrogenase (LDH) from porcine heart (E.C. 1.1.1.27) and alcohol dehydrogenase (AlcDH) from *S. cerevisiae* (E.C. 1.1.1.1). Other chemicals from Sigma-Aldrich include: pyruvic acid, ethanol, glutamate, oxaloacetate, butyramide, β-nicotinamide adenine dinucleotide sodium salt (NAD^+), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH) and other standard organic/inorganic compounds. Water used in all of the experiments was ultra pure (18.2 MΩ·cm) from a NANOpure Diamond (Barnstead) source.

Composition and Performance: The core "machinery" of the D-flip-flop system was composed of LDH (0.1 U/mL) and AlcDH (16.5 U/mL). NAD^+ (120 μM), and NADH (120 μM) were used as the initial logic states of **0** and **1** respectively. In the presence of ethanol, AlcDH reduced NAD^+, setting the system to the logic value **1**. Conversely, in the presence of pyruvate, LDH oxidized NADH to NAD^+ setting the system to the logic value **0**.

The logic value **0** of the **Data** input was defined as pyruvate (2.4 mM), while the logic **1** value of the **Data** input was defined as ethanol (42 mM), operating with LDH and AlcDH, respectively. The activities of the enzymes and the concentrations of the inputs were optimized to result in the full transition of the system to the **0** or **1** state upon the **Data** input signals. The **Clock** input of **0** was realized by the addition of the inhibitors oxaloacetate (8 mM), glutamate (6 mM) and butyramide (20 mM). The **Clock** input of **1** was realized as the absence of these inhibitors. All reactions were performed in 0.1 mM phosphate buffer, pH 7.2, at ambient temperature 23 °C.

The serial steps of the input system were performed using a 30-kDa microfiltration tube as a reaction vessel. After 5 minutes the reaction solution was filtered, leaving the "machinery" in the upper chamber of the microfiltration tube. The absorbance of the filtrate was measured to check

the state of the system, and then re-added to the microfiltration tube to continue the steps of the experiment.

Optical Measurements: Absorbance measurements were performed using a UV-2401PC/2501PC UV-visible spectrophotometer (Shimadzu, Tokyo, Japan) at 23 °C. The optical measurements took place in a 1 mL poly(methyl methacrylate) (PMMA) cuvette. The change of state between NADH and NAD^+ was monitored at λ = 340 nm after 5 min of reacting with the respective input signals. The absorbance below 0.3, corresponding to the absence of NADH, was defined as logic **0** state of the system, while the absorbance above 0.3, corresponding to the presence of NADH, was defined as logic **1** state of the system.

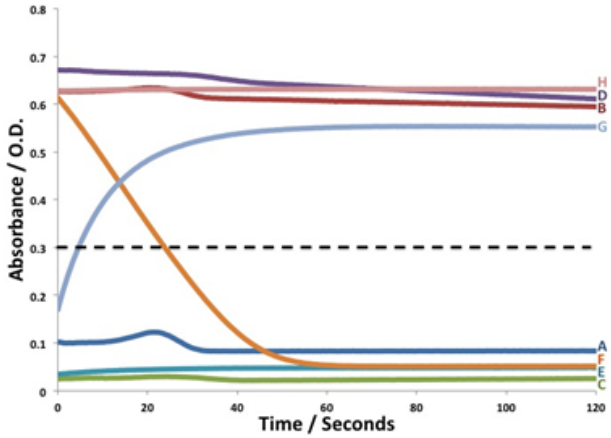

Figure ESI1. Kinetics of the system transitions between different states upon application of different combinations of the **Data** and **Clock** inputs corresponding to the reactions shown in Table 1.

Additional references reflecting recent developments in the area of enzyme-based information processing systems:

1. M. Zhou and J. Wang, *Electroanalysis*, 2012, **24**, 197–209.
2. M. Zhou and S. Dong, *Acc. Chem. Res.*, 2011, **44**, 1232–1243.
3. M. Zhou, F. Wang and S. Dong, *Electrochim. Acta*, 2011, **56**, 4112–4118.
4. M. Zhou, Y. Du, C. Chen, B. Li, D. Wen, S. Dong and E. Wang, *J. Am. Chem. Soc.*, 2010, **132**, 2172–2174.
5. M. Zhou, F. Kuralay, J. R. Windmiller and J. Wang, *Chem. Commun.*, 2012, **48**, 3815–3817.
6. M. Zhou, N. Zhou, F. Kuralay, J. R. Windmiller, S. Parkhomovsky, G. Valdés-Ramírez, E. Katz and J. Wang, *Angew. Chem. Int. Ed.*, 2012, **51**, 2686–2689.