Bioinspired Associative Memory System Based on Enzymatic Cascades

Kevin MacVittie, Jan Halámek, Vladimir Privman, and Evgeny Katz*

a Department of Chemistry & Biomolecular Science, b Department of Physics, Clarkson University, Potsdam, NY 13699, USA

Electronic Supplementary Information (ESI)

Chemicals and Materials. The following enzymes for the biochemical associative memory system were obtained from Sigma-Aldrich and used without further purification: maltose phosphorylase (MPh) from Enterococcus sp. (E.C. 2.4.1.8), glucose dehydrogenase (GDH) from Pseudomonas sp. (E.C. 1.1.1.47), hexokinase (HK) from Saccharomyces cerevisiae (E.C. 2.7.1.1), and glucose-6-phosphate dehydrogenase (G6PDH) from Leuconostoc mesenteroides (E.C. 1.1.1.49). Other chemicals from Sigma-Aldrich used as supplied included: D-(+)-maltose monohydrate, adenosine 5′-triphosphate disodium salt hydrate (ATP), and β-nicotinamide adenine dinucleotide sodium salt (NAD+). Thionine acetate was obtained from Alfa Aesar. Ultrapure water (18.2 MΩ cm) from NANOpure Diamond (Barnstead) source was used in all of the experiments.

Instrumentation and Measurements. A Shimadzu UV-2401PC/2501PC UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) with 1 mL poly(methyl methacrylate) (PMMA) cuvettes was used for all measurements. The halogen bulb lamp (500 W T3 halogen bulb) was purchased from ACE Hardware Corp. During the photochemical reset step, the reaction solution was illuminated with non-filtered polychromatic light from the lamp with the intensity measured with Light Meter LX802 (MN Measurement Instruments).

The system composition and reaction steps. The core “machinery” of the biochemical associative memory system was composed of NAD+ (3 mM), ATP (5 mM), and maltose (22.5 mM) in 0.1 mM phosphate buffer saline (PBS), pH = 7.0 PBS included 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4 and 2 mM KH2PO4 titrated to pH = 7.0. MPh (1 U mL−1) and GDH (2 U mL−1) were used as the “correct” input. The “wrong” input consisted of HK (10 U mL−1) and G6PDH (2 U mL−1). Reactions were carried out in 3 kDa micro-centrifuge tubes (Pall Corporation, Michigan) at ambient temperature 23 °C. Reactions after applying the signals (“correct”, “wrong”, “correct”+“wrong”) in various combinations were allowed to proceed for 10 min (unless stated differently) before the solution was removed by ultrafiltration: by centrifugation at 12,000 rpm in a Microfuge 22R centrifuge (Beckman Coulter, CA). After separation of the supernatant from the used enzyme inputs, the “machinery” components (NAD+, ATP
and maltose) were added to the solution and then the next enzyme input was applied. The “reset” operation returning the NADH output back to NAD⁺ (thus resetting the optical absorbance at λ = 340 nm to its initial value) was performed by the irradiation of the solution with the non-filtered light from the halogen lamp with intensity of 9,000 lux for 5 min in the presence of thionine (250 µM) and oxygen (in equilibrium with air).

Comments on Future Challenges. The present realization of a purely enzymatic associative memory is limited to a rather specific set of conditions. Future work should focus on ideas of improving flexibility and versatility of enzymatic and other bio-inspired memory elements for enabling their networking. As referenced in the main text, networking concepts for useful information processing are rather recent even in electronics, and will have to be developed for biomolecular systems. The “toolbox” of future gates should also include some modularity, i.e., flexibility in which compounds to use and what is their role. For example, in our system the accumulating compound is Glu6P. Changing this, for instance to have glucose accumulate instead, for the memory effect in an otherwise similar system would require complete redesign and use of different operating regimes. The time-evolution of memory systems realized as purely enzymatic biocatalytic cascades should also be studied for kinetic regimes involved. In typical kinetic studies of enzymatic reactions with the system kept under steady state conditions for the time intervals of the experiment. In memory systems, however, accumulation/depletion of intermediate chemical(s) suggests non-steady-state kinetics for both the “learning” and “forgetting” processes.