SUPPORTING INFORMATION

Nucleotide-Dependent Bioautocatalytic Timer Reaction

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Additional figures

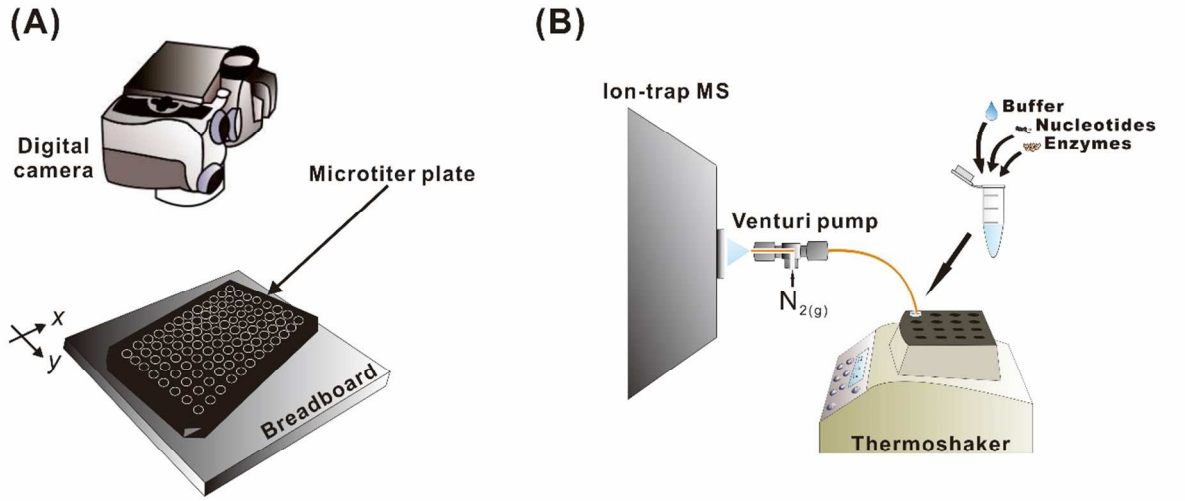


Figure S1. Experimental setup used during the development and monitoring of the tri-enzymatic biochemical timer. (A) Recording luminescence transients of timer reactions using a digital camera. The setup was placed in an incubator to ensure a dark temperature-controlled environment. A humidifier (not shown) was used to prevent evaporation of the reaction aliquots during prolonged monitoring. (B) Mass spectrometry system used to record the signals of ATP, ADP, and AMP at different time points.

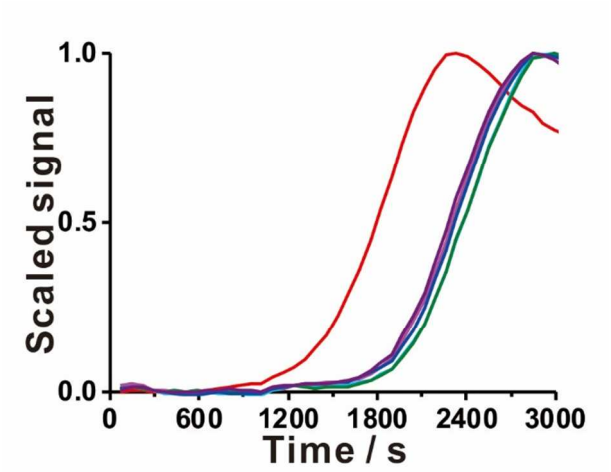


Figure S2. Selectivity of the tri-enzymatic biochemical timer toward different nucleotides (10^{-6} M): red, ATP; light blue, TTP; pink, CTP; green, UTP; dark blue, GTP; and violet, blank. Other conditions are the same as in **Figure 2**. Raise time alignment with respect to the blank measurements has been applied to all the data sets in order to compensate for experimental variability.

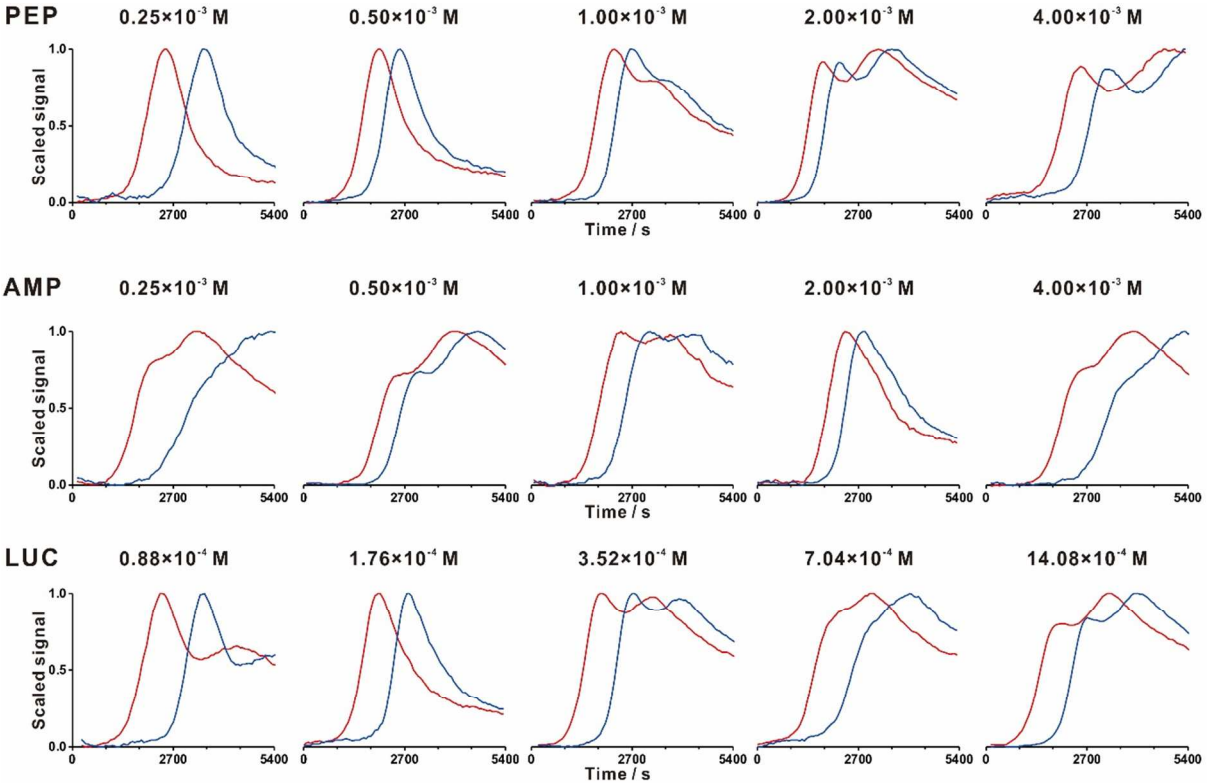


Figure S3. Influence of phosphoenolpyruvate (PEP), adenosine monophosphate (AMP), and luciferin (LUC) concentrations on the luminescence profiles in the biochemical timer. Red lines: solution of 10^{-6} M ATP and 10^{-8} M ADP (mixed together) used to trigger the reaction; blue lines: blank. The central column represents the default conditions (concentrations). Differences among the signal profiles within the central column are due to experimental variability. Differences among the signal profiles within the three rows are due to different concentrations of reagents (indicated in the labels). No raise time alignment has been performed in this set of measurements.

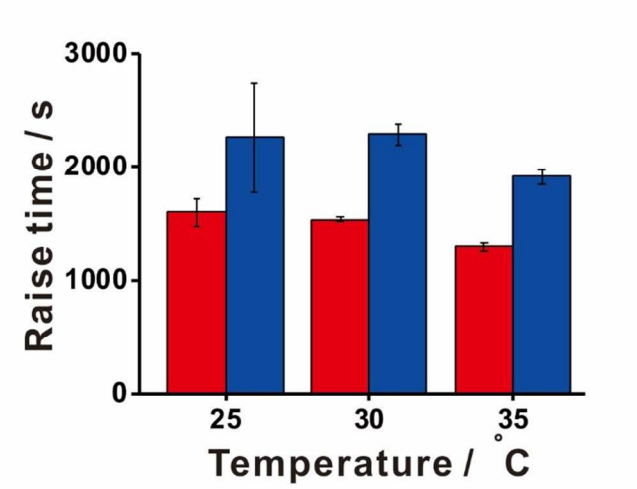


Figure S4. The effect of temperature on the luminescence raise time (at half maximum after scaling) in the biochemical timer. Red bars: 10^{-6} M ATP trigger added; blue bars: blank. Raise time alignment with respect to the blank measurements has been applied to all the data sets in order to compensate for experimental variability. The error bars in (B) represent standard deviations (n = 8).

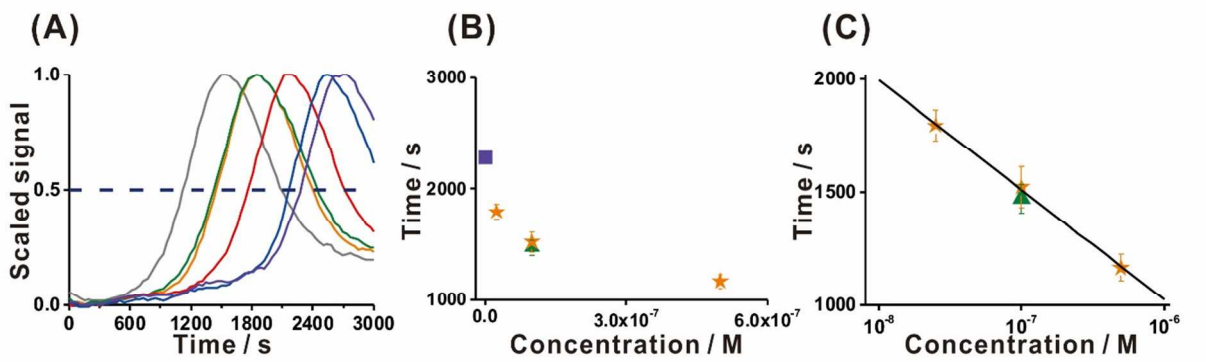


Figure S5. Application of the biochemical timer in quantitative analysis of ATP in a complex matrix (0.05% beef extract; catalogue no. 212610; Becton Dickinson; Bergen, NJ, USA). (A) Temporal traces of luminescence signals measured at different concentrations of ATP: gray, 5.0×10^{-7} M ATP in beef extract; orange, 1.0×10^{-7} M ATP in beef extract; dark green, 1.0×10^{-7} M ATP only; red, 2.5×10^{-8} M ATP in beef extract; blue, beef extract only; and violet, blank (water). (B) Relation between the luminescence *raise time* (*scaled signal* = 0.5) and the ATP concentration: orange stars, ATP with beef extract; violet square, blank (water); and green triangle, ATP only. (C) Relation between the luminescence raise time (*scaled signal* = 0.5) and the ATP concentration (markers are the same as in (B)). The fitted line follows the equation: *raise time* = $-483.83 \times \log(concentration) - 1875.52$ ($R^2 = 0.999$). Raise time alignment with respect to the blank measurements has been applied to all the data sets in order to compensate for experimental variability. Despite that correction, the absolute raise times in this experiment were slightly different than in **Figure 2A**, probably due to unequal activity of the enzyme preparations used and enzyme inactivation during storage.

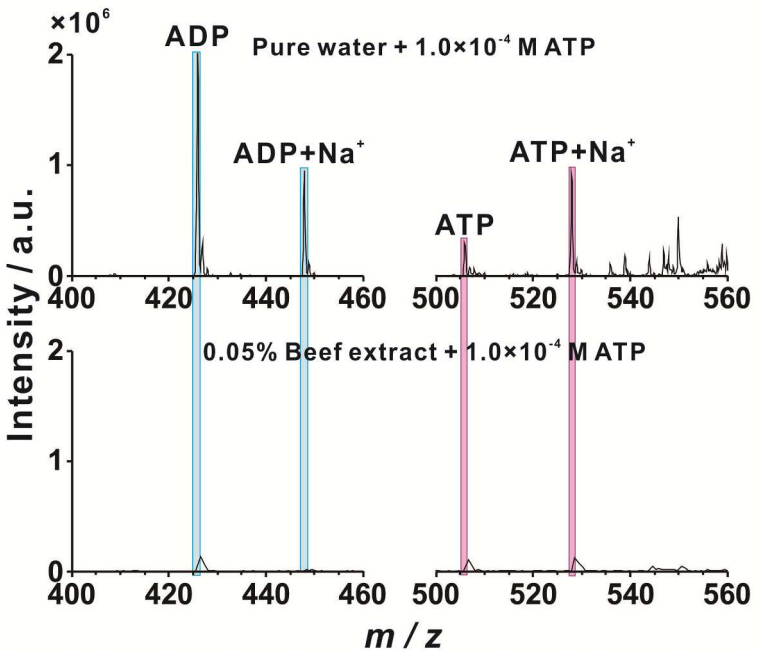
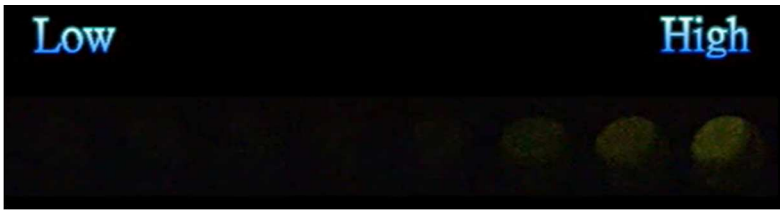


Figure S6. Comparative analysis of ATP in a complex sample (0.05% beef extract) by ESI-MS. Due to the strong ion suppression (related to the complex matrix, beef extract), detection of 1.0×10^{-4} M ATP by ESI-MS is not possible without prior chromatographic separation. Note: ADP signals are due to in-source decay of ATP species.



Movie S1 (video file). Time-lapse video of the microtiter plate containing the tri-enzymatic nucleotide-dependent biochemical timer reaction mixture triggered with ATP: (left = LOW) blank; (right = HIGH) 1.0×10^{-6} M ATP. Speed: ×540.