Supplementary Information

Autocatalytic aptazymes: ligand-dependent exponential amplification of RNA

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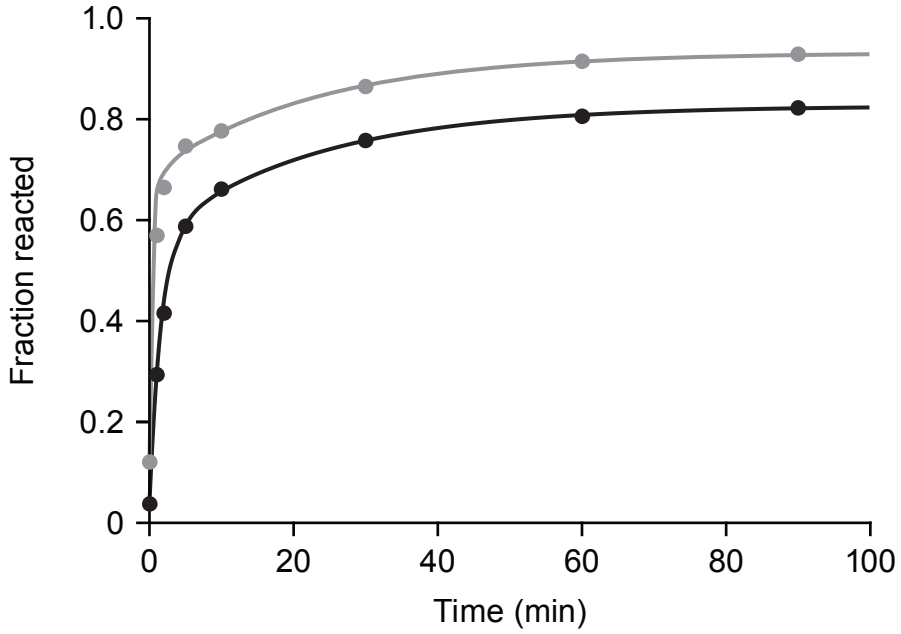

Figure S1. Ligand-dependent RNA-catalyzed ligation of RNA. In the presence of 5 mM theophylline, the aptazyme E_{theo} catalyzed the ligation of A'_{theo} and B′ to form E'_{theo} (gray), and the aptazyme E'_{theo} catalyzed the ligation of A_{theo} and B to form E_{theo} (black). There was no detectable activity in the absence of theophylline or in the presence of 5 mM caffeine. Reaction conditions: 5 μM E_{theo} or E'_{theo}, 0.1 μM [5′-^{32}P]-labeled A'_{theo} or A_{theo}, 6 μM B′ or B, 25 mM $MgCl_2$, and 50 mM EPPS (pH 8.5) at 42 °C.

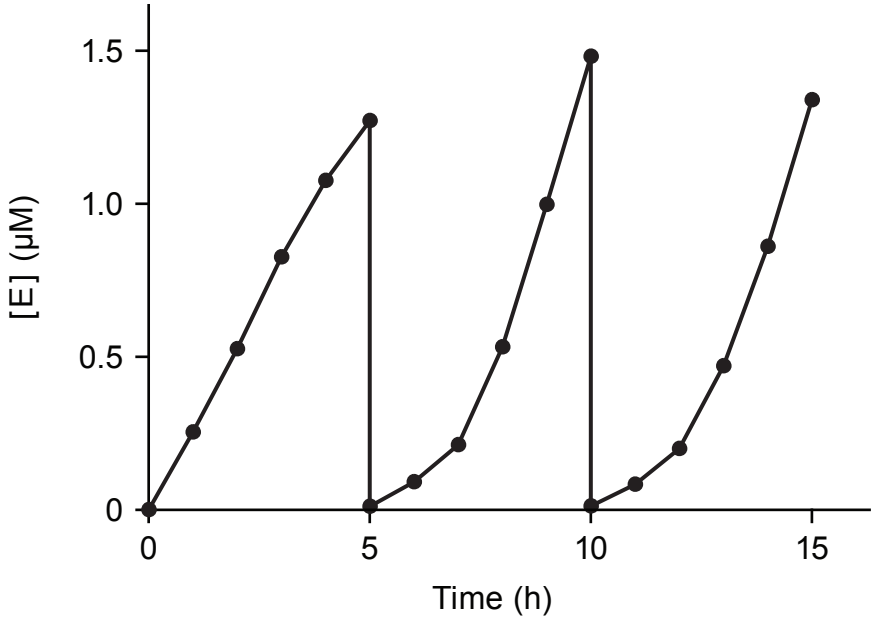

Figure S2. Sustained ligand-dependent exponential amplification of RNA. The theophylline-dependent aptazymes underwent three successive rounds of exponential amplification over 5 h, transferring 1% of the material from a completed round to initiate the next round. Reaction conditions: 0.02 μM E_{theo} and E'_{theo} (first round only), 5 μM A_{theo}, A'_{theo}, B, and B´, 5 mM theophylline, 25 mM $MgCl_2$, and 50 mM EPPS (pH 8.5) at 42 °C.

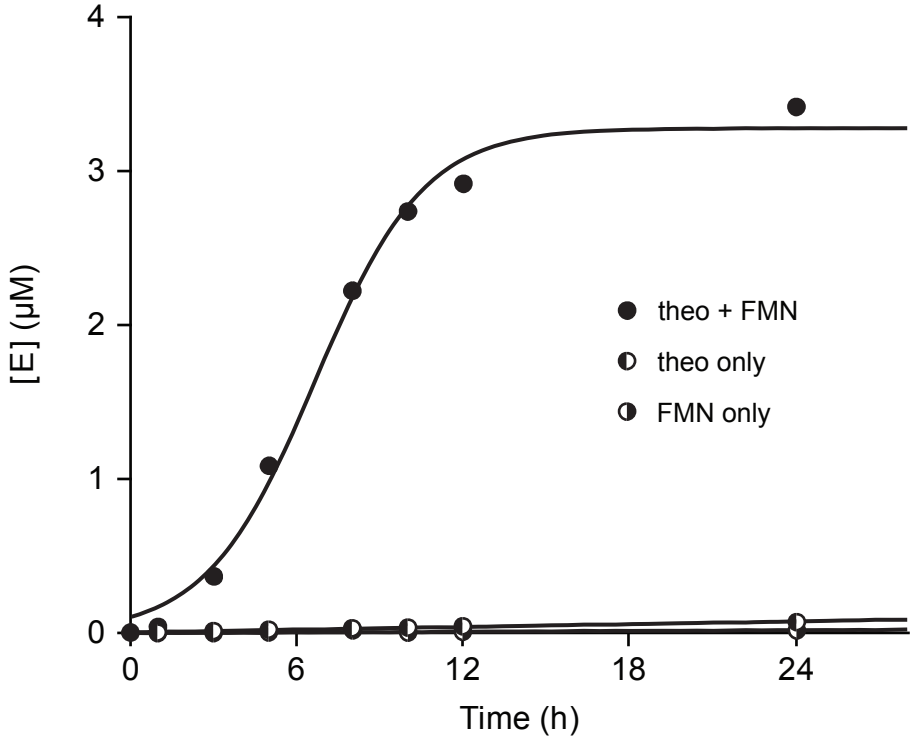

Figure S3. Exponential amplification dependent on the presence of two different ligands. The theophylline aptamer was installed in enzyme E and substrate A, and the FMN aptamer was installed in enzyme E´ and substrate A´. Exponential growth occurred in the presence of both ligands (filled circles), but only linear amplification occurred in the presence of either theophylline or FMN alone (half-filled circles). Similar results were obtained when the theophylline aptamer was installed in E´ and A´ and the FMN aptamer was installed in E and A (data not shown). Reaction conditions: 0.02 μM E_{theo} and E'_{FMN}, 5 μM A_{theo}, A'_{FMN}, B, and B´, 2 mM theophylline and/or 1 mM FMN, 25 mM $MgCl_2$, and 50 mM EPPS (pH 8.5) at 42 °C.

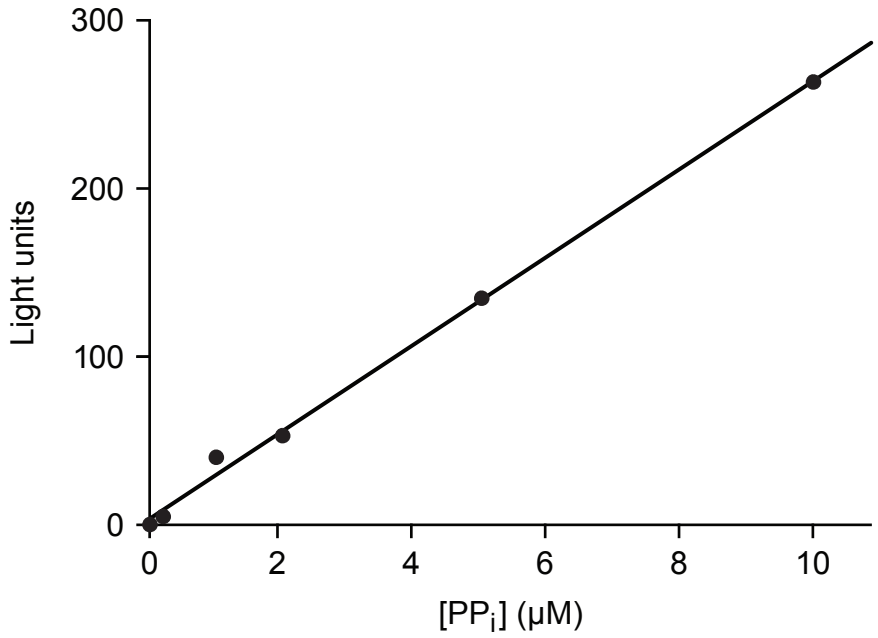

Figure S4. Calibration of pyrophosphate-dependent luminescent signal in the ATP-regenerative assay based on analysis of standard concentrations of inorganic pyrophosphate. The best-fit line had a slope of 260 light units per μM pyrophosphate ($r = 0.999$).

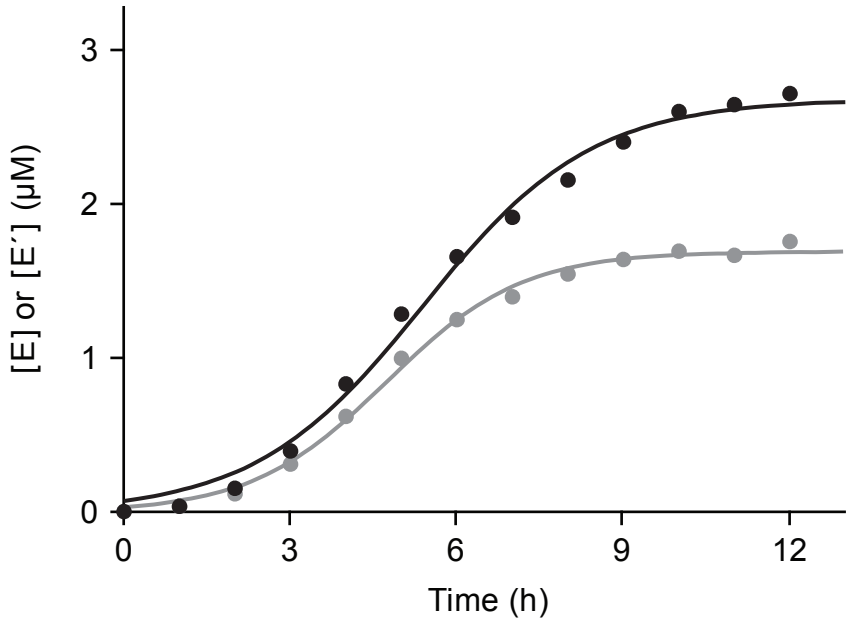

Figure S5. Ligand-dependent exponential amplification of RNA in the presence of deproteinized bovine calf serum. The theophylline-dependent aptazymes, E_{theo} (black) and E'_{theo} (gray), exhibited exponential growth rates of 0.97 and 0.82 h^{-1}, respectively, similar to their behavior in the absence of calf serum (**Fig. 2a**). Reaction conditions: 0.02 μM E_{theo} and E'_{theo}, 5 μM A_{theo}, A'_{theo}, B, and B´, 5 mM theophylline, 25 mM $MgCl_2$, 50 mM EPPS (pH 8.5), 1 U/μL Superasin, and 10% phenol-extracted bovine calf serum at 42 °C.