Electronic Supplementary Information for

Implementation of Anion-Receptor Macrocycles in Supramolecular Tandem Assays for Enzymes Involving Nucleotides as Substrates, Products, and Cofactors

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1. Additional Experimental Results

Fig. S1 shows the results from the independent monitoring of the enzymatic activity by using ^{31}P NMR. The enzymatic conversion, which transforms ATP into AMP and inorganic orthophosphate resulted in the disappearance of the three ATP ^{31}P NMR signals and the concomitant appearance of two new signals corresponding to the two products.

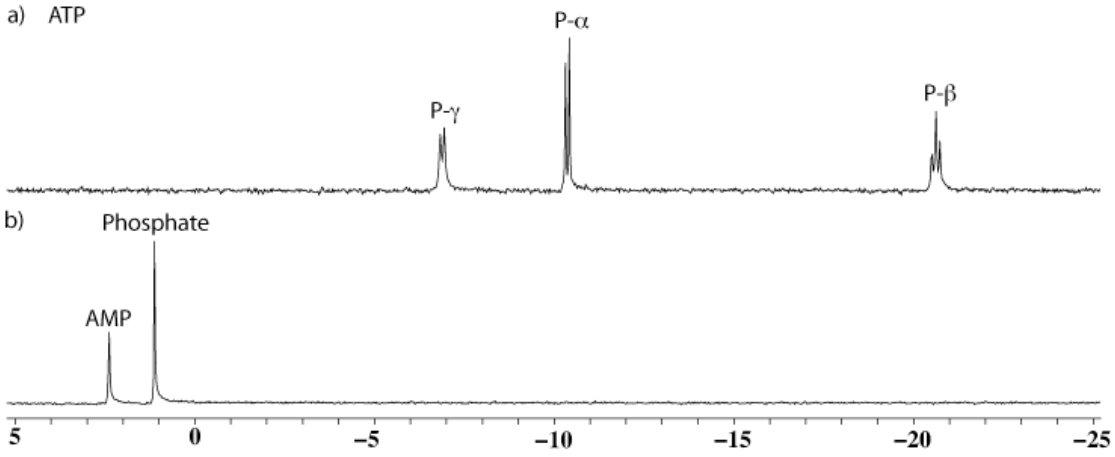

Fig. S1 ^{31}P NMR spectra of 5 mM ATP in D_2O with 1.6 mM $CaCl_2$ a) before and b) after enzymatic conversion to AMP and orthophosphate. The reaction was effected by the addition of 25 μg/mL apyrase and after a reaction time of 120 min.

The dephosphorylation could also be monitored with ADP as substrate, which is shown in Fig. S2.

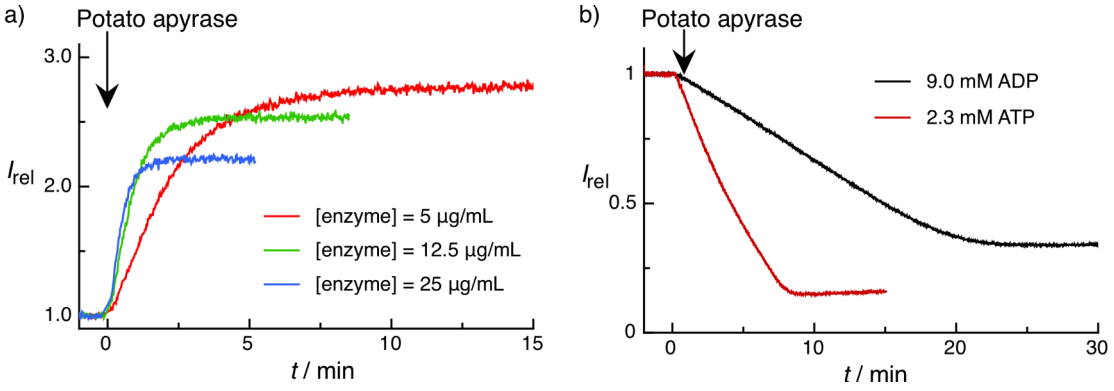

Fig. S2 a) Changes in normalized fluorescence intensity of the **1**/ANS reporter pair (both 25 µM, in NaOAc buffer, pH 5.5, with 1.8 mM Mn^{2+}, λ_{exc} = 318 nm, λ_{obs} = 462 nm) monitoring potato apyrase activity with ADP as substrate; assays were initiated by addition of different concentrations of potato apyrase to 25 µM ADP. b) Changes in normalized fluorescence intensity of the **2**/HPTS reporter pair (both 6.3 µM, in sodium succinate buffer, pH 6.5, with 1.6 mM Ca^{2+}, λ_{exc} = 403 nm, λ_{em} = 512 nm) monitoring potato apyrase activity with ADP versus ATP as substrate; assays were initiated by addition of 100 µg/ml potato apyrase to 9.0 mM ADP (black) and 2.3 mM ATP (red).

Fig. S3 shows the evolution of steady-state fluorescence intensity with time monitoring potato apyarse activity at different enzyme concentrations. The initial rates, v_0, obtained by linear fits of the normalized intensities (assuming a conversion linear with the fluorescence intensity and full conversion at the plateau region) increased approximately linearly with the enzyme concentration. The v_0 values were as follows: $v_{0,\ 50\ \mu g/ml}$ = 0.23 a.u./min, $v_{0,\ 25\ \mu g/ml}$ = 0.15 a.u./min, $v_{0,\ 12.5\ \mu g/ml}$ = 0.07 a.u./min, and $v_{0,\ 5\ \mu g/ml}$ = 0.02 a.u./min for the **1**/ANS reporter pair (Fig. S3a), and $v_{0,\ 100\ \mu g/ml}$ = 0.17 a.u./min, $v_{0,\ 50\ \mu g/ml}$ = 0.09 a.u./min, and $v_{0,\ 25\ \mu g/ml}$ = 0.04 a.u./min with the **2**/HPTS reporter pair (Fig. S3b). Note that the absolute rates are not directly comparable between the two reporter pairs due to the use of arbitrary units.

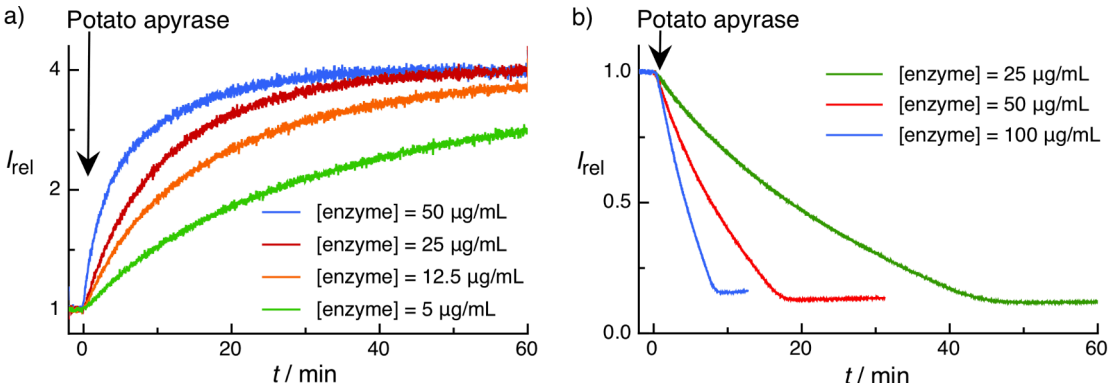

Fig. S3 Evolution of normalized fluorescence intensity monitoring potato apyrase activity at different enzyme concentrations a) with the **1**/ANS reporter pair (both 25 µM, in NaOAc buffer, pH 5.5, with 1.8 mM Ca^{2+}, λ_{exc} = 318 nm, λ_{obs} = 462 nm) and ATP (25 µM) as substrate, and b) with the **2**/HPTS reporter pair (both 6.3 µM, in sodium succinate buffer, pH 6.5, with 1.6 mM Ca^{2+}, λ_{exc} = 403 nm, λ_{obs} = 512 nm) and ATP (2.3 mM) as substrate.