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.

![31P NMR spectra](image)

**Fig. S1** $^{31}$P NMR spectra of 5 mM ATP in D$_2$O 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 Mn²⁺, λ<sub>exc</sub> = 318 nm, λ<sub>obs</sub> = 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 Ca²⁺, λ<sub>exc</sub> = 403 nm, λ<sub>em</sub> = 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 apyrase 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$^{2+}$, $\lambda_{\text{exc}} = 318$ nm, $\lambda_{\text{obs}} = 462$ 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$^{2+}$, $\lambda_{\text{exc}} = 403$ nm, $\lambda_{\text{obs}} = 512$ nm) and ATP (2.3 mM) as substrate.